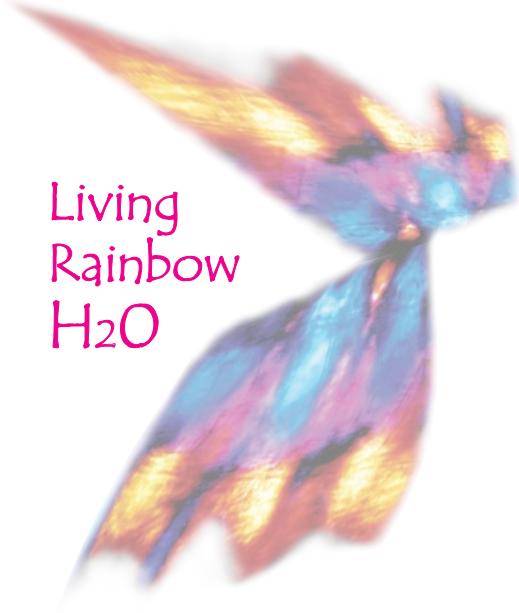


Living Rainbow H_2O

Mae-Wan Ho

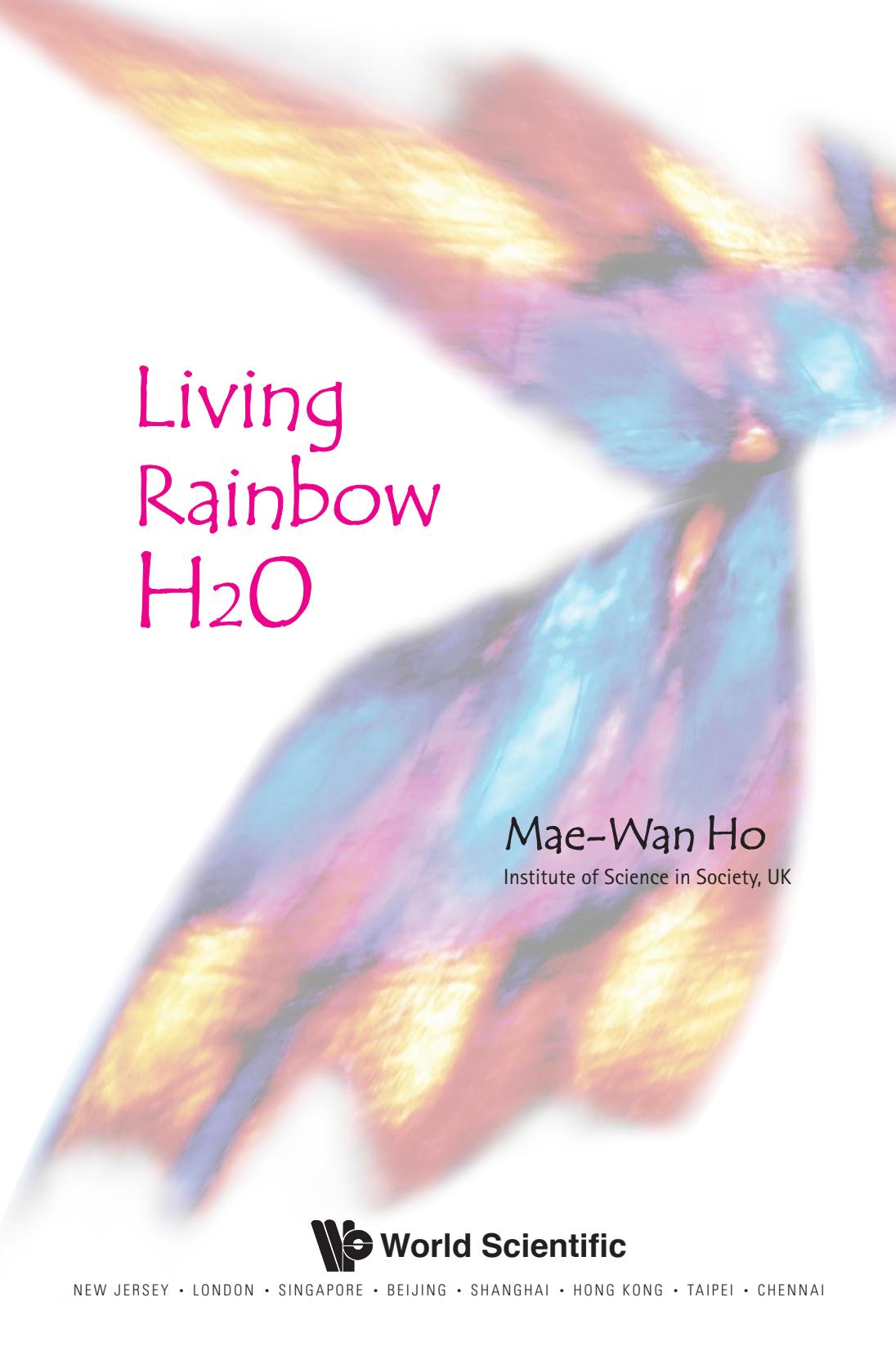


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Living Rainbow H_2O

Mae-Wan Ho

Institute of Science in Society, UK



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LIVING RAINBOW H₂O

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Preface

Chasing rainbows is just what people do, especially when gazing into the clear blue sky. I found mine 20 years ago while peering down a microscope. This rainbow was dancing inside a tiny fruit fly larva freshly hatched from its egg. It told me that all organisms and cells are liquid crystalline and coherent to a high degree, even quantum coherent, thanks to the water in the living matrix that creates the dancing rainbow of life.

So inspired was I that the first draft of *The Rainbow and the Worm: The Physics of Organisms* (of which the present book is a sequel) was finished in a month. I dared not pause as ideas tumbled out from a melee of swirling imagery and extrasensory perceptions faster than I could write on my computer. And indeed, I drew pictures before I could find the words. It was an exhilarating experience. The big question — what is life — that had preoccupied me for the best part of my career up to then was being answered, if ever so tentatively; many old puzzles fell into place. Nature was speaking to me directly; or so I imagined.

The first edition of that book was published in 1993. By 1996, I found most of the solution to the circular thermodynamics of organisms, which led to the 1998 second edition. Since then, I have discovered the work of Gilbert Ling, Gerald Pollack, Martin Chaplin, and others on water at interfaces, inside the cell, and in the bulk. When my publisher, World Scientific, requested a sixth reprint of the second edition, I realized it was time to do the third. The 2008

much enlarged third edition completed the circular thermodynamics of organisms and sustainable systems, thanks to inspiration from environmental engineer George Chan, whose integrated food and waste management system was a living example of how to integrate oneself into the circular thermodynamics (or economy) of nature. The third edition also contained the contributions of those remarkable scientists who reignited my long love affair with water. I coined the term “liquid crystalline water” for the highly ordered and polarized water of the living rainbow in organisms.

But the beauty and perfection of the living rainbow still eluded me. Despite the major insights offered by the best water scientists in the world, the full significance of liquid crystalline water did not present itself. The experimental findings were amazing, often contradictory, and subject to conflicting interpretations. It really needed a coherent narrative all its own, building on the insights of my first book.

It was late August 2011 when epiphany struck. Shortly before then, I had finished reviewing the latest literature on a range of topics that was unusually wide even by my standards, which probably put my mind in a heightened state of awareness. It included a remarkable theory of everything that claims to relate *all* the forces of nature; followed by cold (nuclear) fusion in desktop devices; non-thermal biological effects of very weak electromagnetic fields; and membrane potential changes in development, regeneration, and cell proliferation. With those out of the way, I devoted my entire attention to water research in preparation for the summer school I was invited to teach by Djuro Koruga, distinguished Serbian scientist and pioneer of nanomedicine, who is very interested in how water is involved in the function of molecular machines. (Djuro’s laboratory is one of the few in the world that take nanotoxicity very seriously, so safety assessment is a key part of the research of his team.)

Peter Saunders, my husband, and I met Djuro for the first time in 2009 when he invited us to speak at a conference on evolution celebrating the 200th anniversary of Darwin’s birth, and I was also invited to speak about *Rainbow Worm* to his students in the engineering

faculty and the Serbian Medical Association. It was then that we discovered our shared passion for water and many other big questions in science. Djuro is a remarkable Serbian academician, whose knowledge and expertise range widely from astronomy and mathematics to engineering and nanotechnology. He was the one who alerted me to the golden mean — a classical concept of beauty and harmony — which is turning up everywhere in contemporary chemistry and biology, in quasicrystals that are finding many applications in photonics and electronics, and in proteins and DNA.

In the course of preparing my lectures, I rediscovered the work of my old friend Emilio Del Giudice, whom I met in 1986 at a conference organized by Clive Kilmister (then Professor Emeritus of Mathematics at King's College London, who sadly died in May 2010). It was on that same occasion that I met quantum biophysicist Fritz Popp, then at the University of Kaiserslautern in Germany. Fritz and Emilio changed my life forever, although I understood not a single thing that either of them had said. I did, however, get the message that quantum coherence was very important for understanding living systems, and they were right, as my *Rainbow Worm* bears full witness.

I lost touch with Emilio when he became deeply involved in cold fusion research since the late 1990s, a controversial area that I only caught up to in 2006. By then, he had moved on to the quantum coherence of water, among other things.

While absorbed in the writings of Emilio and colleagues, I re-read Gilbert Ling's latest book *Life at the Cell and Below-Cell Level* and more recent papers that he kindly sent to me. I also revisited the exquisite experiments of Ludwig Edelmann at Saarland University in Germany, which corroborated Gilbert's hypothesis that the cytoplasm binds potassium ions in preference to sodium. The reason potassium concentration is much higher inside the cell than outside also explains why cell water naturally excludes solutes in the resting state, even in the absence of the cell membrane. Thus, those properties conventionally attributed to the cell membrane are largely due to the equilibrium resting state of the cytoplasm that requires very little energy to maintain.

As I was writing all that down, a sudden illumination flooded my mind. A great calm took hold, and the turbulence that had obscured my view crystallized into a limpid pool. Water *is* the medium, the message, and the means; it is literally the mother of life, as Szent-Györgyi told us more than 50 years ago. It is the rainbow within that mirrors the one in the sky. The veil is lifted, and a dazzling pot of gold appears at this end of the rainbow. Nature is speaking once more, and I must transcribe as quickly and clearly as I can.

*Mae-Wan Ho
January 2012*

Acknowledgements

This book was inspired by the many dedicated and brilliant scientists whose work I shall be describing.

I am especially indebted to those already mentioned in the Preface for the genesis of the present book; among them, Djuro Koruga, who subsequently also read the penultimate draft of the entire manuscript and responded with unreserved approval.

Many thanks are due to Gilbert Ling, Philippa Wiggins, Gerald Pollack, Martin Chaplin, Emilio Del Giudice, Mishra Bonn, Hongfei Wang, Heather C. Allen, and Norio Ise for e-mail exchanges that clarified my understanding of their works, even though they may not agree with my interpretation.

I am particularly grateful to Frank Mayer, James Clegg, Rickey Welch, Veljko Veljkovic, Tapash Chakraborty, and Yutaka Maniwa, for reading entire drafts of the manuscript and for their incisive comments. Individual chapters were also read and commented on by Gary Fullerton and Stephan Förster. Ivan Cameron sent me an important paper about to be published as this book was going to press.

Special thanks to Bill Stranger for reading an early draft; it was his enthusiastic response that convinced me to devote extra effort into making this book accessible to the wider public. I am also grateful to my good friend Jackie Lambert, who responded very positively as a non-scientist to a later draft.

This book was a joy to write, in large measure due to my colleagues at the Institute of Science in Society (ISIS), who worked

cheerfully around my schedule and provided support whenever I needed it. Joe Cummins, a dear friend and comrade-in-arms in reclaiming science for the public good, has been tireless in keeping me updated with new developments in many subjects including water. Julian Haffegee, a founding and key member of ISIS, made valuable contributions to *Living Rainbow*, including the image of zebrafish trunk muscles used in the cover design. And ISIS would not have existed but for the financial and moral support of Third World Network and Salvia Foundation. Much of the work described in this book is based on articles published in ISIS' trend-setting quarterly magazine, *Science in Society* (details on ISIS' website www.i-sis.org.uk).

My son Adrian took time off his punishing schedule to read the first and final drafts and gave me invaluable feedback and encouragement.

To my husband Peter Saunders and lifelong travelling companion in science without frontiers, I owe far more than I can say: for constant inspiration and support, for filling my days with laughter, my evenings with good food and wine and great dinner conversation, and much else besides. He has read successive drafts and given me praise, criticism, and advice in just the right places and the right measure.

How to Read this Book

This book is a unique synthesis of the latest findings in the quantum physics and chemistry of water that will tell you why it is so remarkably fit for life. It is the story of water in living cells and organisms as told by many scientists carrying out theoretical and empirical studies published in the scientific literature, especially within the past five years. It starts from first principles, building up the narrative by degrees, from simple to increasingly complex. My hope is to take the most naïve readers step by step towards the secret of the living rainbow within, which I can reveal to be the special water in living cells and organisms that makes life possible.

I have tried to reduce scientific jargon to a minimum in the main narrative, and to define all the terms used, which are also presented in a glossary at the end, to be consulted with ease whenever required. The more technical details on concepts and techniques are largely confined to boxes or footnotes for the benefit of advanced readers up to and including research scientists, and others not afraid to venture more deeply into the subject. They are there also to reassure readers that the book is based strictly on good science, and *can be ignored without losing the plot*, so please do not let them hold you back. Another reason for including the technical details is to show how indirect and theory-laden many empirical scientific observations are. The more sophisticated the technique, the more it is dependent on interpretation based on a model of how molecules interact with the probe and with other molecules in the system. The data are only as good as the model is

accurate, which is why coherence and convergence among different sets of data are so important.

This book is by no means a compendium of all research on water. On the contrary, I have concentrated on studies that are informative (and interesting), leaving out others either because the time scale of the observations, or the systems or models used are not appropriate for the questions asked. (I apologize in advance to those scientists whose relevant work I have failed to cite. It is mainly due to ignorance on my part, which you can remedy by sending me the key publications. As journal articles are very expensive to obtain, I have also been limited by lack of access.)

More important, even the work I have cited will not represent the scientists' full story. My overriding concern in this book is to bring out the convergences among different investigators, rather than their disagreements with one another, because most of the conflicts are due to the distinctive model systems used and therefore quite irrelevant to the plot.

For example, static *in vitro* systems favoured by physicists can never compare directly with the incredibly dynamic situation inside the living cell and organism, just as the behaviour of water in bulk is demonstrably different from water under confinement, as you shall see for yourself. Nevertheless there are common threads that we shall be following in our quest for a coherent narrative.

This book will not give you the definitive answer to the mystery of life. It will give you a new vision on what is important to know about.

Rainbow Dancing in the Worm

Love of the Rainbow Worm

A rainbow danced in a little fruit fly larva freshly hatched from its egg, and took my breath away.

It was summer 1992. I was peering down a *polarized light microscope*,¹ which geologists and others use to look at inanimate samples of rock or *liquid crystals*² that appear in brilliant *interference colours*,³ literally the colours of the rainbow. But this worm was alive, and all its molecules were engaged in transforming energy as it crawled around exploring its new environment (Fig. 1.1).

Later, the true meaning of this vision dawned on me. I was seeing life in its true colours in more than one sense. The brilliant interference colours are typical of liquid crystals, thereby revealing the organism as being truly composed of liquid crystals. Moreover, the organism was alive, revealing itself in a true-to-life state, as opposed to the usual dead, fixed specimen that passes for life. (This speaks volumes in favour of non-invasive, non-destructive techniques in studying organisms and cells.) Furthermore, this image would not have been possible unless *all* the molecules in the cells and tissues within its body were aligned as liquid crystals, as liquid crystals are usually aligned, and, not so usually, *moving coherently together*; all including the water molecules that form dynamically coherent units with the macromolecules embedded in it. That was the hallmark of life and living organization.

Because light vibrates that much faster than molecules move, the molecules appear aligned and static to the light passing through

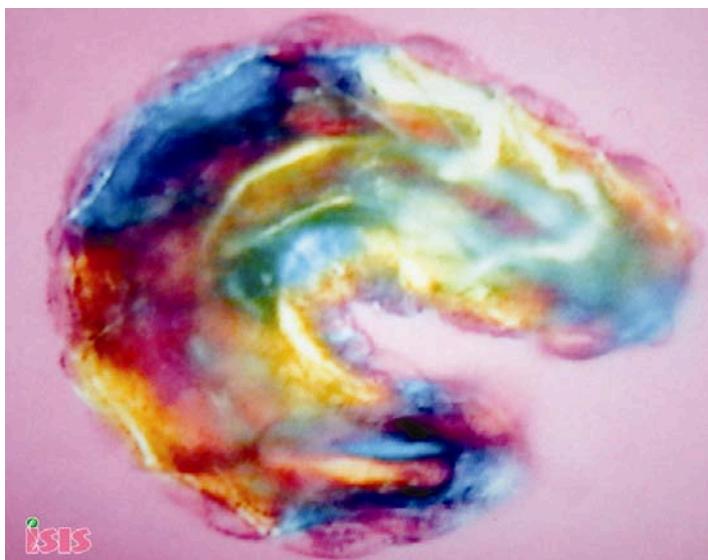


Figure 1.1 The rainbow in the worm.

at any instant, so long as the molecular motions are coherent. In confirmation of that, the most active parts of the organism are also the most brightly coloured, as indicative of the highest degree of coherence; the colours fade when the organism dies, when incoherent thermal motions take over.

The fruit fly larva is not unique; all living cells and organisms display themselves like that under the polarized light microscope. They are coherent liquid crystalline phases through and through. The liquid crystalline matrix is polarized and aligned along the anterior-posterior axis in bilaterally symmetric organisms as well as single-celled organisms. It is a quantum coherent electrodynamical field, with the water, which I call liquid crystalline water, an intrinsic part of the quantum molecular machines that transfer energy at the molecular level to power cells and organisms at close to 100% efficiency. If not for that, life would be impossible; it would literally burn out from all the excess waste heat generated, as typical of classical machines such as the steam engine, or the motor-car.

My colleague Michael Lawrence and I had inadvertently discovered a new setting for the polarizing microscope that's particularly good for seeing biological liquid crystals,⁴ and what revelations! It opened a unique window on life.

In *the Rainbow and the Worm: The Physics of Organisms*, first published in 1993 and now in its third, much enlarged 2008 edition (referred to as *Rainbow Worm* hereafter),⁵ I presented theoretical arguments and empirical evidence for two new ideas: a circular, zero-entropy thermodynamics of organisms, and by analogy sustainable systems; and the quantum coherence of organisms, thanks to the liquid crystalline water matrix of quantum molecular machines working seamlessly together to transform material and energy without loss or dissipation. The two aspects are of course completely interlinked. (Like Erwin Schrödinger's book, *What is Life?*⁶ which the first edition of *Rainbow Worm* was patterned after, the latter elaborated on consciousness and the meaning of life, especially in the light of the new knowledge presented.)

Water is central to the action of quantum molecular machines, the archetype of which is the enzyme. Enzymes carry out all the chemical reactions necessary for life, and are known to speed up chemical reactions by a factor of 10^{10} to 10^{23} (see Chapter 10), although conventional cell biology and biochemistry still fail to recognize the primary role of water in making this happen. Thermodynamically, water enables the organism to function in the zero-entropy circular mode, which takes us right back to quantum coherence.

The Quantum Jazz Dancer

As explained in *Rainbow Worm* and elsewhere,⁷ quantum coherence is a sublime state of wholeness; a quantum superposition of coherent activities over all space-times, constituting a pure dynamic state towards which the system tends to return. The organism is a macroscopic quantum being, and has a wave function that never ceases to evolve by entangling other quantum organisms in its environment.

The organism is thick with coherent activities on every scale, from the macroscopic down to the molecular and below. I call the totality of these activities “quantum jazz” to highlight the immense diversity and multiplicity of players, the complexity and coherence of the performance, and above all, the freedom and spontaneity.⁸ The quantum coherence of organisms is the biology of free will.⁹ In liberating herself from the laws of mechanical physics, from mechanical determinism and mechanistic control, the organism becomes a sentient, coherent being that is free, from moment to moment, to explore and create her possible futures.

The quantum coherent organism plays quantum jazz to create and recreate herself from moment to moment. Quantum jazz is the music of the organism dancing life into being.¹⁰ It is played out by the whole organism, in every nerve and sinew, every muscle, every single cell, molecule, atom, and elementary particle, a light and sound show that spans 70 octaves in all the colours of the rainbow.

There is no conductor or choreographer. Quantum jazz is written as it is performed; each gesture, each phrase is new, shaped by what has gone before, though not quite. The organism never ceases to experience her environment, taking it in (entangling it) for future reference, modifying her liquid crystalline matrix and neural circuits, recoding and rewriting her genes, as I have described elsewhere in connection with the fluid genome¹¹ of the new genetics.¹²

The quantum jazz dancer lives strictly in the now, the ever-present overarching the future and the past, composing and rewriting her life history as she goes along, never quite finishing until she dies. But her script is passed on to the next generation; not just her biological offspring, but the species as a whole. Each generation rewrites, edits, and adds to the score, making it unique.

Intercommunication is the Key

Intercommunication is the key to quantum jazz. It is done to such sublime perfection that each molecule is effectively intercommunicating

with every other, so each is as much in control as it is sensitive and responsive. And intercommunication is predominantly electronic and electromagnetic, thanks to liquid crystalline water, as I shall elaborate in the course of the present book. (That is why we *are* sensitive to the very weak electromagnetic fields of the mobile phone and wireless networks saturating our environment, and not just us, but all organisms, birds and bees.¹³ The evidence linking the use of mobile phones to brain cancer has considerably strengthened recently.¹⁴)

Quantum coherence is the “I” in everyone that gives unity to conscious experience, as Erwin Schrödinger (1887–1961) pointed out,¹⁵ and as I argued in detail in an article “Quantum Coherence and Conscious Experience” published in 1997.¹⁶ This ideal coherent whole, I suggest, is also the ideal of health. The coherent organism is a unity of brain and body, heart and mind, an undivided bundle of intellect and passion, flesh, blood, and sinew that lives life to the full, freely and spontaneously, attuned not just to the immediate environment, but the universe at large.

Water is the Means, Medium, and Message

Quantum coherence and quantum jazz are possible because of the 70% by weight of liquid crystalline water that makes up the organism. Quantum jazz is diverse multiplicities of molecules dancing to the tunes of liquid crystalline water. Water is the means, medium, and message of life. It is the dancing rainbow within, to which this book is dedicated.

Notes

1. A polarized light microscope uses *polarized light* to examine objects; in this case, plane-polarized light that has its electric field oriented perpendicular to the light wave’s direction of travel.
2. Liquid crystals are a fourth state of matter between liquid and solid, with long-range orientational order imposed by molecular anisotropy (e.g., long thin molecules) and electrical dipole interactions. This long-range orientational order defines their special

optical property (e.g., birefringence), as well as mechanical, electrical, and magnetic properties, making them responsive to electric and magnetic fields, with many applications in liquid crystal displays (LCDs) for televisions and computers, digital watches, calculators, etc. (see Ruiz-Bermejo, 2010).

3. Interference colours are produced by subtraction (destructive interference) of certain frequencies in the spectrum of white light. Thus liquid crystals as well as rock crystals such as quartz, which are *birefringent*, split light into two waves that are reflected and transmitted at different rates and interfere with each other when recombined, generating interference colours (for more details see Ho *et al.*, 2006).
4. Ho and Lawrence (1993); Ho and Saunders (1994); Newton *et al.* (1995); Ho *et al.* (1996); Ross *et al.* (1997); Zhou (2000); Ho *et al.* (2006).
5. Ho (1993, 1998, 2008).
6. Schrödinger (1944).
7. Ho (1997).
8. Ho (2006a).
9. Ho (1996).
10. Ho (2006a, 2007e).
11. Ho (2003a).
12. Ho (2004j, 2009e).
13. Ho (2007b,c,d,f).
14. Ho (2011h).
15. Schrödinger (1944).
16. Ho (1997).

2

Weird and Wonderful Water

Strangely Fit for Life

There is no simpler compound than water. Its chemical formula H₂O is almost the first thing in chemistry that one learns in school. It is also the most abundant substance on the surface of the Earth, and life as we know it is impossible without water. So much so that water means life, which is why the discovery of water on Mars has been greeted with such excitement.¹

But water is by no means simple; it has the most complex properties and baffling anomalies. Generations of the best scientists have pitched their wits (and sophisticated instrumentation) at water, only to have it slip gracefully through their fingers. But we persist, so great is our passion for unveiling the secrets of weird and wonderful water.

As American chemist and natural theologian Lawrence J. Henderson (1878–1942) pointed out at length in his book *The Fitness of the Environment*, published in 1913,² the strangeness of water consists of precisely those properties that make water fit for life on Earth or elsewhere in the universe.

Compared with its neighbours in the periodic table of chemical compounds, water is definitely out of line (see Table 2.1).³ Hydrides (compounds with hydrogen) in the first row are all gases at ambient temperatures (above 20 °C) and, except for HF, boil well below 0 °C, the freezing point of water. As one moves up the column for Group 6a hydrides from Te (tellurium) to S (sulphur), their boiling points progressively decrease. Extrapolating these boiling points to

Table 2.1 Water stands out from its neighbours: comparing boiling points

Group	3a	4a	5a	6a	7a
	B_2H_6 -92.5 °C	CH_4 -164.0 °C	NH_3 -33.4 °C	H_2O +100.0 °C	HF +19.5 °C
				H_2S -60.7 °C	
				H_2Se -41.5 °C	
				H_2Te -2.0 °C	

the molecular weight of water gives an expected boiling point of about -75 °C instead of 100 °C. Water should be a gas at ordinary temperatures and pressures, not a liquid.

As a liquid, it behaves contrary to ordinary liquids. For example, water frozen into solid ice floats on the cold liquid, and the volume occupied by the ice contracts when it melts into liquid. That means cold liquid water is denser than its solid phase, which is the opposite of what happens when other liquids turn into solids: they become denser as the molecules are more restricted in motion and packed closer together.

That water is a liquid at ordinary temperatures and pressures is very good for the fishes and other aquatic life; as is ice being lighter so it floats on water, allowing aquatic life to survive the wintry months in the warmer sheltered water beneath the ice.

Liquid water can be supercooled (cooled below its freezing point without freezing); but on heating, the supercooled liquid does not expand as expected.³ Instead it shrinks until a maximum density is reached at about 4 °C. Under pressure, both the melting point and the maximum density point shift to lower temperatures. With ordinary liquids, however, pressure promotes freezing and consequently shifts the freezing point to higher temperatures.

Water becomes less compressible with increasing temperature, reaching a minimum near 46.5 °C, whereas warmer liquids are usually more compressible. At sufficiently high pressures, this anomalous volume and temperature behaviour disappears.

Another surprise is that at ordinary temperatures below 35 °C, increasing the pressure results in lower viscosity, again the opposite of what happens in other liquids.

Water Loves Bonding

The strangeness of water and its fitness for life lie in its penchant for bonding. The water molecule is a permanent *electric dipole* in which positive and negative charges are separated, with the two hydrogen atoms at the positive pole and the oxygen atom at the negative pole (see Fig. 2.1). Like other dipoles, water molecules can stack together in dipole interactions with alternating positive and negative poles next to each other. They can also engage in electrostatic interactions with charged ions and other dipoles dissolved in water.

In addition, the water molecule likes to form hydrogen bonds with other water molecules (Fig. 2.2), and with molecules and ions dissolved in water. A *hydrogen bond* consists of hydrogen shared between two electronegative atoms, such as oxygen or nitrogen. The compound or group that donates the hydrogen is the hydrogen donor, while the compound or group that accepts the hydrogen is the hydrogen acceptor. Water is both a hydrogen donor and acceptor; it can donate two hydrogen atoms and its oxygen can accept two other hydrogen atoms to form the hydrogen bonds. The water molecule is generally represented as a tetrahedron with four arms — two hydrogen donors and two hydrogen acceptors — pointing at the vertices. This tetrahedral structure is typical of ordinary ice, where all the water molecules are cross-linked in a crystalline, hexagonal array (see later).

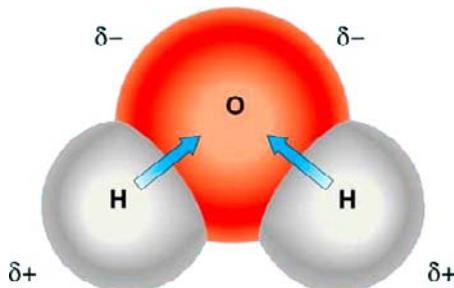


Figure 2.1 The water molecule is a dipole with separated positive and negative charges.

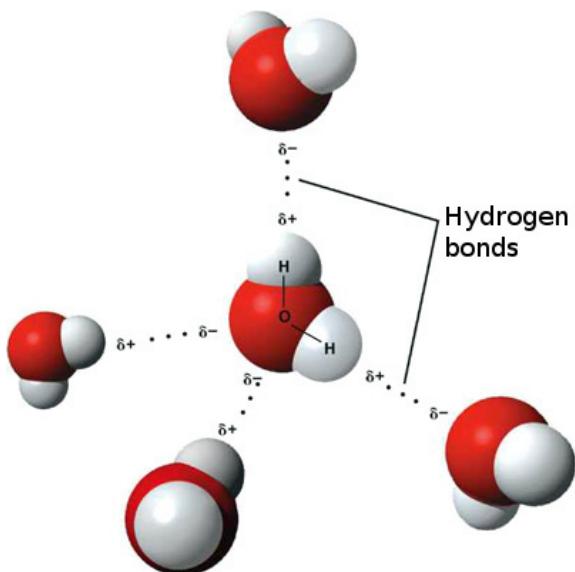


Figure 2.2 Hydrogen-bonded water molecules.

The dipolar nature of water and its propensity for hydrogen bonding account for its unusually high dielectric constant of ~78 at room temperature, making it the most important polar solvent in chemistry and biology, which also means it is easily polarized by an electric field. The dielectric constant, or relative static permittivity, is a measure of the extent to which it concentrates electrostatic lines of flux relative to a vacuum. Researchers led by Manu Sharma at Princeton University have shown by molecular dynamics computer simulations from first principles that the high dielectric constant of water is due to two effects of the hydrogen bonds contributing in almost equal measure.⁴ The hydrogen-bonding serves to align the dipoles and, at the same time, pull away positive and negative charges within a molecule, enhancing the average molecular polarization.

Huge Diversity of Supramolecular Structures

The propensity of water molecules to form hydrogen bonds and the flexibility of the molecule and the bonds make for a huge diversity of possible supramolecular structures.

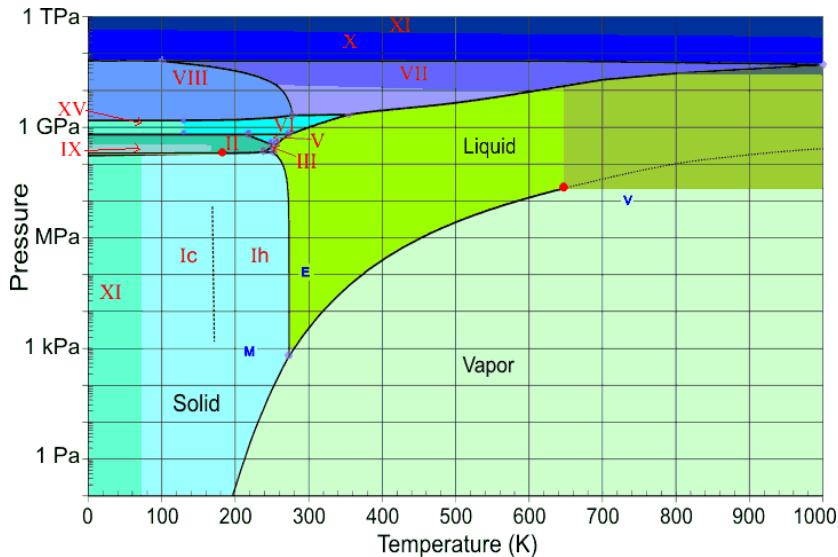


Figure 2.3 Phase diagram of water.⁶

We can already see that in the different forms of ice (frozen structures) encountered in nature: snowflakes and hailstones falling from the sky, icicles hanging from the eaves or branches of trees, glaciers over land, packed ice and ice caps in the polar regions of the Earth. Snowflakes, in particular, are so diverse that each one is unique. That has inspired Masaru Emoto in Tokyo to read “messages in water” by crystallization, to acclaim and controversy in equal measure.⁵

Within the laboratory, there are 15 known crystalline forms of ice that appear under different temperatures and pressures (see Fig. 2.3). And there are amorphous non-crystalline ices, as well as glass-like ices (transparent, but non-crystalline).

But it is liquid water that stretches our imagination.

Water, Water in Every Guise

Liquid water is perfectly transparent and has no colour, except when light shines through it or reflects from it, creating brilliant blue skies and emerald green oceans; and especially when fine droplets refract

sunlight into the dazzling spectrum of the rainbow. Water has no shape other than that of the containing vessel, no sound, no movement, and little resistance; except when coaxed by the summer breeze into smiling ripples, or tickled into undulating waves lapping like laughter. Or else when whipped up by hurricanes into howling surges that hurl ships into the air, or heaved by submarine earthquakes into rumbling tsunamis that deluge shore and land.

The dramatically different, infinitely varied colours, forms, and moods of water are the stuff of life, if not also great art. Indeed, a good scientific theory needs to capture the art, to explain the long-range cohesion coalescing massive volumes into gigantic whirlpools, and at the same time bespeak the endless diversity in molecular structures that perhaps encode memories of dissolved substances in homeopathic preparations⁷ and, equivalently, makes every snowflake a unique event in the history of the universe. But I am rushing too far ahead. Let me concentrate on what has been found for liquid water, strictly scientifically, for now.

Decades of research have resulted in a near-consensus that water at ambient temperatures and pressures exists as a dynamic network of supramolecular clusters where a proportion of the molecules are linked together by flickering hydrogen bonds, similar to those in ordinary ice. It is also widely acknowledged that the hydrogen-bonded network of liquid water accounts for most, if not all, of its anomalous properties, as described earlier. Beyond that, there is no agreement over the exact proportion of molecules linked by tetrahedral ice-like bonds, the precise structure and size of the clusters, how freely the molecules can move around, and especially whether interactions are strictly local with the nearest neighbour or much more global in extent.⁸

Within the past decade, substantial evidence has emerged for cooperative interactions between water molecules that result in remarkable long-range coherence in liquid water under ordinary conditions.

Notes

1. Batty (2011).
2. Henderson (1913).

3. Vedamuthu *et al.* (1994).
4. Sharma *et al.* (2007).
5. Emoto (1999); Ho (2002a).
6. Chaplin (2011).
7. See Ho (2011d).
8. Ho (2010b).

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3

Cooperative Coherent Water

Cooperativity through Hydrogen Bonds

Cooperativity in chemistry refers to the tendency of individual chemical interactions to influence each other, so that local actions can have global effects, and vice versa; the whole being greater than and not predictable from the sum of the parts. That's how organisms work, and why water is so vital for life, as I stressed in *Rainbow Worm* and elsewhere.¹

Different forms of ice are to a large extent variations on the theme of tetrahedral molecules arranged mostly in the form of hexagons, or six-membered rings, as determined by X-ray and neutron diffraction.² Figure 3.1 depicts the structure of ordinary ice (ice Ih).

In gas clathrates — where gases such as methane are caged in ice at the bottom of the Arctic Ocean — the water molecules tend to form pentagonal arrays of cages fused along the edges.

Quantum chemist Roger A. Klein at Bonn University found evidence of cooperativity from electronic and quantum chemical computations in the formation of the six- and five-membered rings.⁴ There is greater stabilizing energy and higher electron density at the bond critical point (i.e., bond strength) for each hydrogen bond in the five- and six-membered clusters compared to a single hydrogen bond between two water molecules (dimers). The greater bond strength comes from the hydrogen bond shortening within the cluster compared with that in a dimer (Fig. 3.2).

Increasing cooperativity is more due to a greater proportion of tetrahedral ice-like bonds in water clusters than cluster size alone,

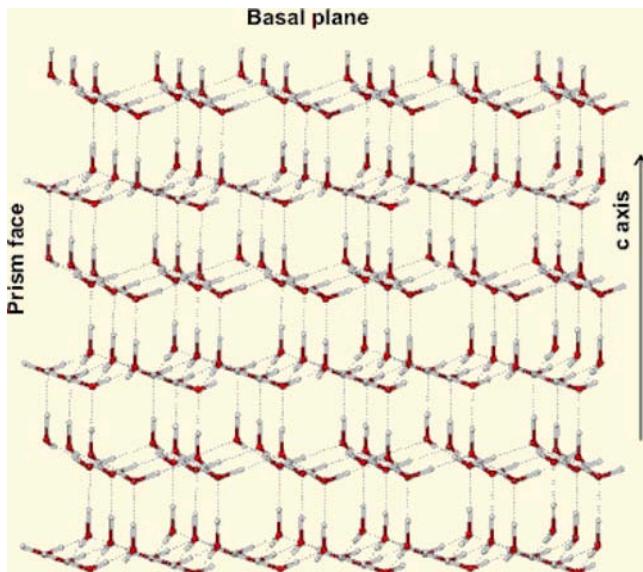


Figure 3.1 Structure of ice Ih.³

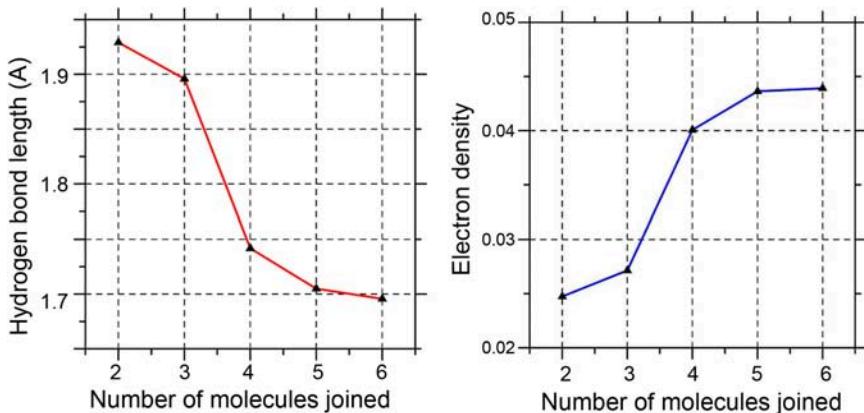


Figure 3.2 Cooperativity shortens (left) and strengthens (right) the hydrogen bonds; redrawn after Klein.⁴

once an optimal ring size of five or six water molecules has been reached. Thus, it is no accident that the hexagonal motif is common in liquid water and ice, and the edge-fused pentagonal motif in gas clathrates,⁵ for both are optimized for cooperative interactions,

which in turn makes it likely for large clusters to form. There is evidence that large clusters do exist in liquid water under ordinary conditions, enabling coherent energy transfer to take place.

Resonant Energy Transfer through Hydrogen-bonded Networks

At a temperature of 300 K ($\equiv 27^\circ\text{C}$), $\sim 90\%$ of water molecules are hydrogen-bonded, a picture supported by molecular dynamics simulations⁶ and a large body of experimental evidence.⁷ The structure of the hydrogen-bonded network fluctuates on time scales ranging between 10 fs (femtosecond, 10^{-15} s) and ~ 10 ps (picosecond, 10^{-12} s), and includes changes of molecular orientations and distances, hydrogen bonds breaking and reforming, and slower rotational motions.

The O-H bond stretching vibration exhibits a 270 cm^{-1} wide band (in *wavenumber*, an alternative way of expressing frequency) centred at $\sim 3400\text{ cm}^{-1}$ in the infrared region (but see later chapters for more details). As the temperature decreases, the maximum of the band shifts to lower frequencies and the envelope changes shape, indicating an overall enhancement of hydrogen-bonding. The O-H bond stretching vibration is a sensitive indicator of structural and dynamical fluctuations in the extended hydrogen-bonded network.

Two-dimensional infrared photon echo (2DIR-PE) spectroscopy has been added recently to the armoury of techniques for probing water. The technique depends on exciting (pumping) the O-H bond stretching vibration with infrared light, and looking for the response (echoes) in the infrared spectrum.

Using 2DIR-PE spectroscopy, researchers at the University of Toronto and the Max Born Institute for Nonlinear Optics and Short-Pulse Spectroscopy discovered ultrafast resonant transfer of excitation energy between water molecules that takes place via dipole coupling in the hydrogen-bonded network.⁸ The energy transfer time of ~ 80 fs is unaffected by temperature from 340 to 274 K. This transfer time in pure water is substantially faster than the 700 fs previously measured in a mixture of heavy water (deuterium oxide)

with water ($D_2O:H_2O$), where resonant energy transfer is obstructed, and is also much shorter than the average hydrogen bond lifetime of ~ 1 ps.

The experiment carried out at different temperatures showed that frequency correlations (between excitation and echo) due to local structures are lost in about 50 fs at high temperatures (hinting at a much more flexible hydrogen-bonded network).⁹ But at lower temperatures these correlations are longer-lived. At 274 K (1 K above freezing), a dramatic change takes place, and the frequency correlations persist beyond ~ 200 fs, indicating that the hydrogen-bonded supramolecular structures are stabilized.

Two-state Water

Liquid water at ambient temperatures is traditionally regarded as a homogeneous phase, with the same average structure everywhere. However, some scientists, beginning with Wilhelm Conrad Röntgen (1845–1923) who won the 1901 Nobel Prize for the discovery of X-rays, believed that the anomalies of water are best explained if liquid water is an equilibrium mixture of two states that differ in density.¹⁰

Wilse Robinson (1924–2000) and his group at Texas Tech University revived the two-state theory of water in the 1980s. By the early 1990s, they were able to show that the experimental measurements of the density of water over the range of -30°C (in the supercooled region) to 70°C could be accurately fitted to the two-state model.¹¹ They envisaged low-density regions in the liquid with intermolecular hydrogen bonds like that of ordinary ice Ih (see Fig. 3.1) intermixed with high-density regions with compact bonding similar to that of ice II. As the temperature approaches 225 K (-48°C) in the deep supercooled regime, only ice I-type bonding is present.

X-ray diffraction data on liquid water and other structural data on different forms of ice indicate that high-density and low-density water (HDW and LDW) differ in bonding and exist in larger water structures.

At ordinary temperatures, liquid water consists of rapidly inter-converting LDW clusters where the second neighbour intermolecular O–O distance is 0.45 nm, and compact HDW clusters where the corresponding O–O distance is 0.35 nm. In supercooled water, LDW clusters predominate, and hence its volume shrinks when heated, as more and more HDW clusters form until 4 °C, when practically all the clusters are HDW and the water is at its maximum density.

The X-ray diffraction data contain a lot of information, especially in the *radial distribution function* that gives the probability of finding a second molecule at a distance r from the first. This consists of a series of peaks and troughs, including one at 0.35 nm (second-neighbour O–O distance of HDW) that has never been adequately explained on a structural basis.

The two-state network model of Robinson and colleagues leaves some questions unanswered. For instance, considerable pressure would be required for the extensive formation of ice II clusters (from ice Ih) to get the density of water right, as well as the required number of non-bonded close contacts between water molecules.

While the molecular movements in liquid water require constant breaking and reorganization of individual hydrogen bonds on a picosecond time scale, it is thought that at any instant, the degree of hydrogen-bonding is very high, greater than 95%.

The Chaplin Model

Martin Chaplin at London South Bank University has been studying the chemistry of water for years. His web resource “Water Structure and Science”¹² contains everything you could know about water, and is world famous for the quality of information it provides. Chaplin became impressed by the many pieces of evidence pointing towards a large extent of order in the hydrogen-bonded network of water molecules in the liquid state. These include the fine structure in the X-ray diffraction data, vibration spectra that indicate the presence of big clusters, and the formation and properties of low-density water

in gels (see later). A random network has been described for LDW and supercooled water, but the entropy (a measure of disorder) of LDW is much lower than that of a random network.

Although several workers including Robinson have suggested that the structure of liquid water should be related to ice forms (see earlier), the structures in liquid water must be significantly different from that in ice, because water supercools quite easily and releases a high heat of fusion when it freezes, which means that a large amount of energy is lost when water turns from liquid to solid.

In an ingenious feat of molecular modelling, Chaplin proposed that the fluctuating network of molecules in liquid water is organized locally into an icosahedron (a regular icosahedron is a three-dimensional shape with 20 faces made of equilateral triangles) consisting of 280 fully hydrogen-bonded molecules. Each icosahedron is formed by the regular arrangement of 20 identical units of 14 water molecules.¹³

The icosahedral structure explains many anomalies of water, including its temperature-density and pressure-viscosity behaviour, its radial distribution function, the presence of pentamers and hexamers of water molecules, the change in properties, and the two-state model on supercooling.

Further on, it also explains the interactions of ions, hydrophobic molecules, carbohydrates, and macromolecules with water, which I shall describe in a later chapter.

Chaplin developed the model by assembling alternating sheets of boat-form and chair-form water hexamers (literally six-membered rings in the shape of a boat and a chair) from the lattices of hexagonal and cubic ice respectively, using the software package HyperChem. The resulting icosahedron contains 280 water molecules, all (except for those in the outer ring) completely hydrogen-bonded, two as donors and two as acceptors. It has large pores capable of partial collapse due to interactions between hydrogen-bonded partners competing with interactions between non-hydrogen-bonded neighbours. It also has cavities capable of enclosing small solutes.

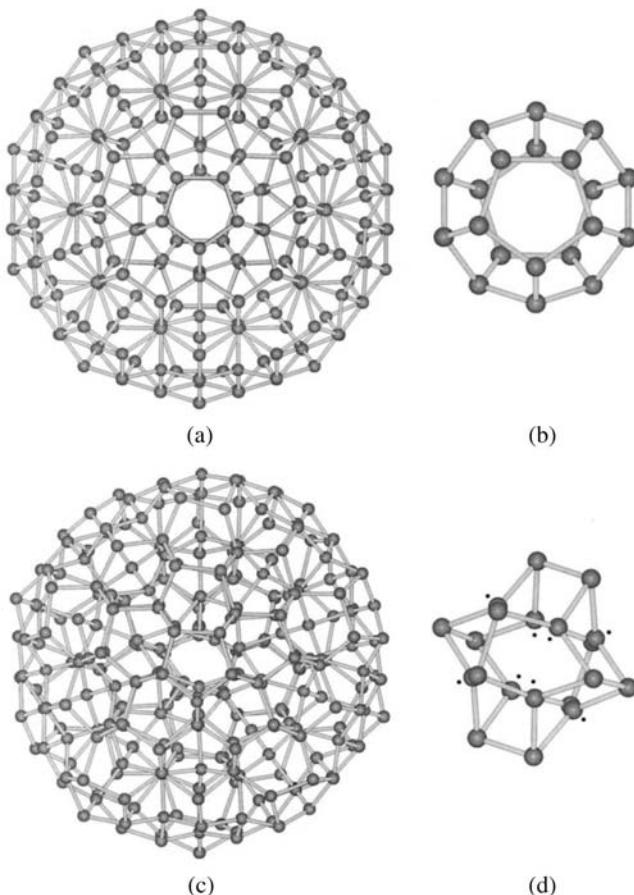


Figure 3.3 Chaplin's two-state model of a highly ordered icosahedral structure interconverts between a fully expanded low-density form (a) and a collapsed high-density form (c); their respective central 20 molecules (dodecahedral) units (b) and (d).¹³

The 280-molecule icosahedron measures 3 nm in diameter in its fully expanded form. The beauty of the model is that it can convert between a low-density fully expanded structure (ES) and a high-density collapsed structure (CS) without hydrogen bonds breaking; they only need to bend (Fig. 3.3), with bond strengths about 1% weaker in the CS.

Model Fits Well with the Data

Chaplin's model explains an impressive list of observations. The density of the ES is 0.94 g cm^{-3} and that of the CS 1.00 g cm^{-3} . The density of the ES is comparable to that of low-density water at 0.96 g cm^{-3} found around macromolecules, and that of supercooled water (-45°C) and low-density amorphous (non-crystalline) ice, both at 0.94 g cm^{-3} . The density of the CS, on the other hand, compares with that of water at 0°C , also 1.00 g cm^{-3} . The CS is capable of further collapse with weaker hydrogen-bonding or greater pressure. Collapse of all dodecahedral structures gives a density of 1.18 g cm^{-3} , similar to the density of 1.17 g cm^{-3} of high-density amorphous ice.

The strongest evidence for Chaplin's model is the agreement with the radial distribution function. The CS model was used to generate a radial distribution function of the intermolecular O–O distances, which was compared with that from X-ray data at 4°C . The peaks are in the same positions, albeit less distinct in the X-ray data because of the relative movements expected in a real liquid. There are 16 peaks plus a further 34 blips, using the first derivative (change of probability as a function of r), which make a total of 50 positions. All 50 positions except for two show correspondence between the X-ray data and those predicted from the CS. There is also good correspondence with the radial distribution function derived from neutron diffraction data, although those show less fine structure than the X-ray data.

Support for the structure of the ES comes from its agreement with radial distribution functions of solutions, supercooled water, and low-density amorphous ice. The cavity-cavity peak at 0.55 nm of supercooled water is close to the cavity-cavity peak predicted from the ES at 0.54 nm .

Since Chaplin proposed his model, the inner four shells of the ES (Fig. 3.3), consisting of 160 water molecules ($20 \text{ H}_2\text{O}$ at 0.39 nm ; $20 \text{ H}_2\text{O}$ at 0.66 nm ; $60 \text{ H}_2\text{O}$ at 0.79 nm ; $60 \text{ H}_2\text{O}$ at 1.06 nm), have been found in almost identical positions and orientations within a nanodroplet of water encapsulated in polyoxomolybdate.¹⁴

“The evidence for my model has accumulated since, and there is no counter-evidence,” Chaplin says.¹⁵ But he cautions against



Figure 3.4 2011 Nobel laureate in Chemistry Daniel Shechtman with a quasicrystal icosahedron.

the idea that the icosahedral structures are permanent or even complete. “The molecules are in constant flux but the structuring remains. Consider the analogy with a wave at sea where the structure of the wave continues across an ocean but the water molecules come and go.”

Water Quasicrystals and the Golden Mean

Chaplin’s water icosahedron belongs to a new class of ordered structures called quasicrystals, the discovery of which by Daniel Shechtman at the Israel Institute of Technology (Fig. 3.4) was honoured with the 2011 Nobel Prize in Chemistry.¹⁶

A quasicrystal is an ordered structure that is not periodic. It can continuously fill all available space, but it lacks translational

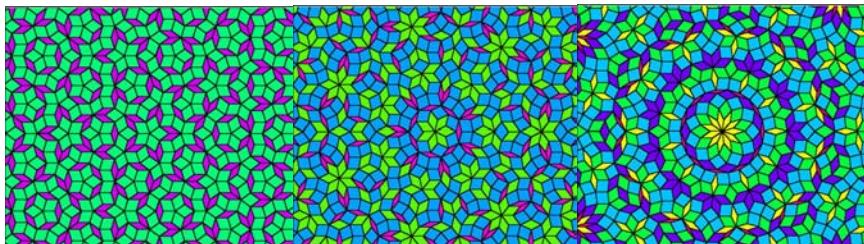


Figure 3.5 Penrose tilings from Paul Steinhardt with five-, seven- and eleven-fold symmetries (left to right).¹⁷

symmetry, which means that an arbitrary part of it cannot be shifted from its original position to another without destroying the symmetry. While crystals, according to the classical crystallographic restriction theorem, can possess only two-, three-, four-, and six-fold symmetries, quasicrystals show other symmetry orders, for instance five-fold, which is “forbidden”.

Paul J. Steinhardt, Professor of Physics at Princeton University defines quasicrystals more precisely as quasiperiodic structures with forbidden symmetries that can be reduced to a finite number of repeating units.¹⁷ Quasicrystal structures were inspired by Penrose tilings in two dimensions.

Aperiodic tilings were discovered by mathematicians in the early 1960s. In 1972, world-famous mathematical cosmologist Roger Penrose (now Emeritus Rouse Ball Professor of Mathematics at the Mathematical Institute at Oxford University) created a two-dimensional tiling pattern with only two different pieces that was not periodic and had a five-fold symmetry (see Fig. 3.5). Quasicrystals are higher-dimensional Penrose tilings (and obey rules other than what Penrose had discovered). They are a new class of solids not just with five-fold symmetry, but any symmetry in any number of dimensions.

An intriguing feature of quasicrystals is that the mathematical irrational constant — irrational because it cannot be expressed as a fraction — known as the Greek letter φ , or the golden ratio, is embedded in the structure,¹⁸ which in turn underlies a number sequence worked out by Fibonacci in the 13th century, where each number is the sum of the preceding two.

Two quantities are in the golden ratio if the ratio of the sum of the quantities to the larger quantity is equal to the ratio of the larger quantity to the smaller one. The golden ratio is approximately 1.61803398874989. Other names frequently used for the golden ratio are the golden proportion, the golden section, or the golden mean.

The Fibonacci sequence is a sequence of numbers, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, 144, ..., where the ratio of a number in the sequence to the previous approaches φ asymptotically, i.e., more and more exactly as the numbers get larger and larger. The icosahedron and the *dodecahedron* (with 12 pentagonal faces) are the last two of the five *Platonic solids* (the first three being the tetrahedron, the cube, and the octahedron) and the only ones with φ appearing in their dimensions. Interestingly, Plato associated the icosahedron with water, and the dodecahedron with the universe.

The golden mean was the Greek ideal of beauty and harmony, and had a tremendous influence on architecture, art, and design. It is significant that quasicrystals do represent minimum energy structures,¹⁹ and hence a kind of equilibrium between harmony and tension in just the right degree, which may be where beauty lies.

Two-state Model Confirmed

The two-state model of water did not gain acceptance for quite a long time. But recently, researchers at Stanford University have provided fresh evidence that liquid water is indeed inhomogeneous over a length scale of about 1 nm.²⁰ Combining small-angle X-ray scattering, which gives information on the size of supramolecular structures, with X-ray emission and X-ray Raman scattering, which yield information on hydrogen-bonding, the researchers were able to show that water at ordinary temperatures exists in two distinct states (with no intermediates): low-density water (LDW), in which the molecules are hydrogen-bonded in ice-like tetrahedral bonds, and high-density water (HDW), in which the molecules are linked by more distorted hydrogen bonds. Hence, density fluctuations occur over the length scale of ~1.2 nm.

The length scale of ~1.2 nm encompasses about 160 molecules of water. It is tempting to equate that with the inner core of Martin Chaplin's icosahedron of 280 molecules; the same 160-molecule structure was previously found as a nanodroplet inside polyoxomolybdate (see earlier).

At 24 °C, the proportion of LDW with ice-like tetrahedral bonds is about 28.6%, and only disappears completely at the boiling point.

In the next chapter, we shall look at the long-range order that can appear in colloid particles dispersed in water, which gives us important insights as to how water may be crucial for the self-assembly of macromolecules that make cells and extracellular matrices.

Notes

1. Riley *et al.* (2010).
2. X-ray diffraction is the scattering of X-rays by the atoms of a crystal, resulting in a diffraction pattern from which the structure of the crystal can be determined; neutron diffraction similarly uses the scattering of neutrons to determine the atomic or magnetic structure of a material.
3. Chaplin (2011).
4. Klein (2006).
5. A clathrate is a compound in which molecules of one component are trapped inside the crystal structure of another.
6. Molecular dynamics is a form of computer simulation in which atoms and molecules interact for a period of time according to known physics and chemistry, in order to predict or explain certain experimental results.
7. Klein (2006).
8. Cowan *et al.* (2005); Kraemer *et al.* (2008).
9. Kraemer *et al.* (2008).
10. See Ho (2006c).
11. Urquidi *et al.* (1999).
12. Chaplin (2011).
13. Chaplin (1999); Ho (2006c).

14. Müller *et al.* (2003).
15. Chaplin, personal communication (2004).
16. Ho (2011g).
17. Steinhardt (2011).
18. Chaplin (2011), “Platonic Solids”.
19. Steinhardt (2011); Chaplin (2011), “Platonic Solids”.
20. Huang *et al.* (2009).

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4

Water and Colloid Crystals: The New Age of Alchemy

Colloid Crystal Diversity Defies Description

Colloids are nanoparticles of dimensions ranging from nanometres (10^{-9} m) up to several micrometres suspended in water or other solvents. And colloid crystals are literally crystals made of colloid particles arranged in orderly fashion, like atoms in ordinary crystals. Also like ordinary crystals, and macromolecules in living organisms, colloid crystals self-assemble, that is, they form spontaneously when precipitated or evaporated from suspension onto a substrate such as a carbon or silicon-oxide film. The same colloids can self-assemble into a variety of crystals according to the conditions of crystallization, for example temperature, pH, ionic and other additives. These three-dimensional colloid crystals are finding important applications as electronic and photonic devices.¹ The formation of colloid crystals should offer crucial insights into the self-assembly processes that create liquid crystalline structures inside the cell and in the extracellular matrix.

Significantly, mixtures of colloid particles can crystallize into new and exotic structures. Colloid crystallization is more an art than a science, as the process is far from being understood.

Researchers at IBM, Columbia University, and the University of Michigan created more than 15 different binary nanoparticle super-lattice (BNSL) structures by combining semi-conducting, metallic, and magnetic nanoparticles; at least ten of the structures were new.² In many cases, several BNSL structures form simultaneously

on the same substrate under identical experimental conditions. The same nanoparticle mixture can also assemble into BNSLs differing in proportion of the two particles and packing arrangement. For example, 11 distinct BNSL structures were prepared from the same batches of 6.2 nm PbSe and 3.0 nm Pd nanoparticles. The structural diversity of BNSLs “defies traditional expectations”, the researchers wrote, “and shows the great potential of modular self-assembly at the nanoscale”.

The New Age of Alchemy in Water

The amazing array of colloid crystals that can be fabricated is the age-old alchemist’s dream come true: base materials are being transformed into an endless variety of exotic electronic and photonic chips worth many times their weight in gold. The history of colloid crystals is no less remarkable, and it is still unfolding.

Theoretical and experimental work started in the 1960s showed that latex particles of a uniform size suspended in water spontaneously undergo phase transition from a disordered to an ordered state, changing their appearance from milky white to iridescent, reminiscent of opals made of colloidal silica. Japanese researchers Sei Hachisu and Shigekuni Yoshimura at the University of Tsukuba were the first to demonstrate the formation of a variety of colloid crystals using mixtures of uniform-sized latex particles.³

Norio Ise and his team in Osaka, Japan, using the same digital video-recording system, studied the formation of colloid crystals in a suspension of latex particles 160 nm in diameter.⁴ To their delight, they were able to demonstrate that the two ordered and disordered regimes existed side by side, as reported previously by Hachisu and Yoshimura (Fig. 4.1).

The diagram is a composite of a video sequence lasting one second with a frame taken every 1/30 of a second. The average position of the centre of each particle was computed from frames 11 to 20, and the trajectory of the particles during the first ten frames and the final ten frames were plotted from that position. As can be seen, the particles hardly moved in the upper-right half of the field. It was an

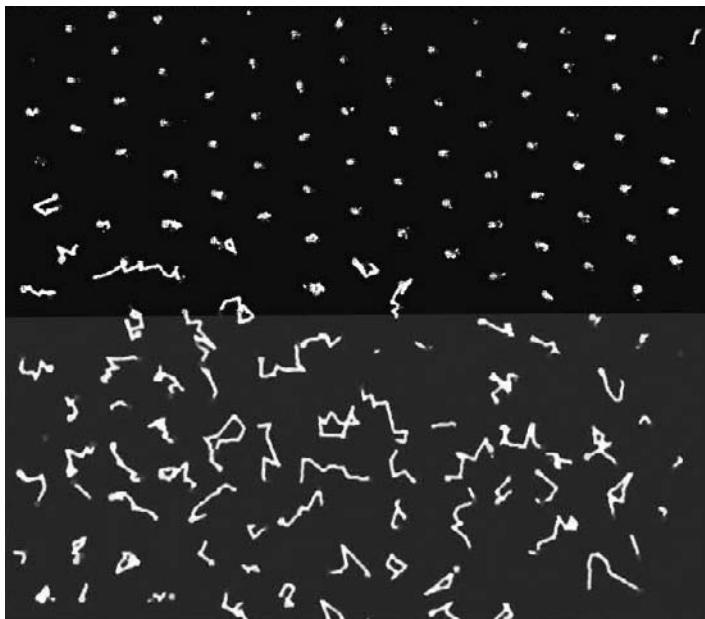


Figure 4.1 Trajectories of latex particles in the disordered (bottom) and ordered (top) regimes during one second.⁵

incredibly ordered regime, against all conventional expectations. The particles in the lower-left half were in the expected random Brownian motion, as described in textbooks. The ordered regime was a molecular supercrystal formed in the water, and such crystals are huge.

Ise and colleagues filled a capillary tube about 50 mm long and 2 mm in diameter with the latex dispersion, and were able to show by means of ultra-small-angle X-ray scattering in two dimensions that the entire volume was filled with a single crystal.

It is tempting to equate the ordered regime with the tetrahedrally hydrogen-bonded molecules that are like crystalline ice, or as envisioned by Martin Chaplin, where the water molecules form ordered supramolecular clusters or quasicrystals. The two states — ordered and disordered — also anticipate the quantum coherent domains and incoherent domains predicted for liquid water under ambient conditions in a quantum electrodynamics theory (Chapters 6 to 8).

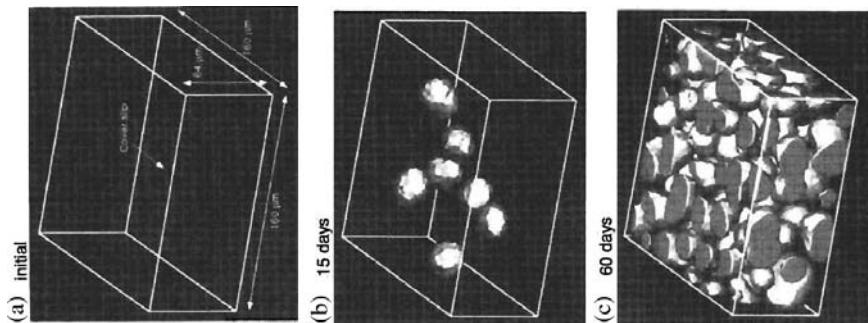


Figure 4.2 Formation of void regimes in a dispersion of polystyrene latex beads; the voids are represented as bright blobs in the diagram.⁵

In the experiment of Ise's team, there was a third regime, a void, that contained no particles altogether. This arises naturally in dilute solutions when many ordered regimes or crystals form, and the distances between particles are at their closest, about 260 nm (with particles of diameter 112 nm).

Using a confocal laser-scanning microscope, Ise and colleagues were able to follow the formation of void regimes in a dilute dispersion of polystyrene-based latex particles over the course of 60 days (Fig. 4.2).

Ise and colleagues postulated a new long-range attractive force between particles with the same electrical charge in the formation of colloid crystals, due to the existence of counter-ions (ions of opposite charge from those on the colloids). This was considered unheard of, not just in colloid science, but in the foundations of chemistry. Like charges repel; only opposite charges attract. The conventional wisdom in colloid science is that a few static charges on the colloid particles' surfaces can cause repulsion strong enough to keep them stably separated, and that's why the particles stay dispersed. Actually, particles with the same charge (negative charges in the case of the latex particles) can only repel one another at short range (nanometres); otherwise, they are shielded from each other's repulsive influences by counter-ions, so the interaction drops off exponentially with distance. (Ise's

recent rebuttal to his critics provides calculations showing that, indeed, the existence of counter-ions can result in long-range attraction.⁶⁾

The experimental observations suggested there was an attractive force between like charges at distances of 5 to 50 µm, which could be demonstrated also between the negatively charged latex particles and the negatively charged glass wall that contained the latex dispersion. And the more highly charged the particles, the stronger the attraction.

Can Like Charges Attract?

At first the results from Ise's laboratory were treated with utter scepticism by the scientific establishment, and attributed to artefacts such as unclean glassware. But other laboratories have repeated the results since.

David Grier and colleagues at the University of Chicago used optical tweezers (laser light) to position two polystyrene beads of radius 482 nm in deionized water, and measured the interaction between the pair by tracking their motions with digital video microscopy.⁷ The interaction potential between the pair of charged colloidal spheres was purely repulsive (see Fig. 4.3a). But when the same pair of spheres was confined between parallel glass walls separated by 3.5 µm, an attractive (negative energy) minimum developed in the interaction potential at a separation distance of about 2 µm (Fig. 4.3b). Attractions of about the same range and strength were thought to be involved in the formation of superheated colloidal crystals (Fig. 4.3c).

Doubt arose again later when the attractive force was only found in particles narrowly confined between glass walls that were thought to be largely responsible for the attractive forces. Explanations proposed to account for the effect include a strong nonlinear coupling between the colloid ions and the simple counter-ions shielding them from one another, so that electrical neutrality is no longer satisfied, and a residual electrostatic attraction develops between the particles.⁸

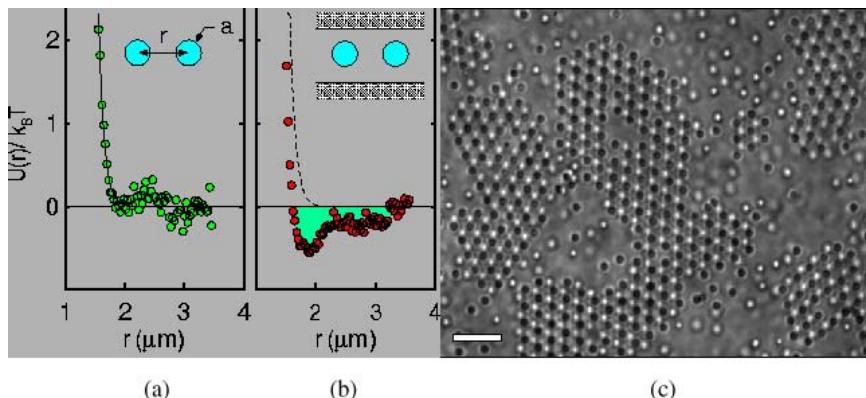


Figure 4.3 Interaction potential between two polystyrene spheres in free solution (a) and confined between glass plates (b). Superheated colloid crystals showing disordered and void regimes, scale bar 20 mm (c).⁷

One aspect largely ignored is the role of water. Work done by Kinoshita and colleagues at Kyoto University showed that including a dipolar solvent such as water results in an effective attraction between the colloid particles, and when the size of the counterions is sufficiently large, and the ionic concentration sufficiently high, the interaction between highly like-charged colloid particles can be strongly attractive.⁹

But simple attraction could result in the colloid particles aggregating and precipitating out. It still does not explain why the particles should form these extraordinary large crystals in the water.

We shall see in Chapter 12 that the explanation lies in the special water structured on solid interfaces.

Colloid Quasicrystals Self-assemble in Water

Excitement mounted another notch when colloid monolayers with quasicrystal structures were reported in 2008. Jules Mikhael and colleagues at the University of Stuttgart grew the quasicrystal using five laser beams to form an interference pattern that confers a ten-fold symmetry to the layer.¹⁰ The interference pattern interacts with the colloid particles, causing them to arrange themselves into pentagons.

By tuning the strength of the surface potential, the growth of the structures can be controlled. Regular crystals form when particle-particle interactions dominate, and quasicrystals form when particle-surface interactions dominate. The quasicrystals showed rings of ten particles surrounding a central particle.

In 2011, an international team led by Stephan Förster at the University of Bayreuth discovered colloid quasicrystals that form spontaneously through self-assembly.¹¹ The colloid particles are micelles, tiny vesicles formed by *amphiphilic* molecules with both water-loving (hydrophilic) and water-fearing (hydrophobic) tendencies, so that the water-loving end faces outward to interact with water, and the water-fearing end is hidden away inside. The particular micelles used were formed by the block co-polymer poly(isoprene- β -ethylene oxide) (P_n -PE_m), where the hydrophobic block polymer is linked to the hydrophilic block polymer, and the degree of polymerization n or m can be varied. The co-polymer forms micelles in water, with the hydrophilic block surrounding the hydrophobic block. Above a concentration of about 10%, these micelles undergo a phase transition from disordered to ordered state, and self-assemble into liquid crystalline structures.

The team took a closer look at the disorder-order transition of the PI₃₀-PEO₁₂₀ micelles using small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS), and discovered quasicrystalline 12-fold (Q₁₂) and 18-fold (Q₁₈) symmetries (see Fig. 4.4).

At a temperature of 20°C, Q₁₂ symmetry appeared as the micelles underwent phase transition from disordered to ordered state at 13%, remaining stable until 18% micelle concentration, and changing to the ordinary six-fold crystal at higher concentrations. Q₁₈ symmetry appeared at a lower temperature of 10°C.

The existence of colloid quasicrystals has been predicted for many years, and is confirmed by the new experiments. Quasicrystals with 18-fold symmetry have never been reported previously.

The unusual symmetry of quasicrystals is particularly useful for making photonic devices that prevent light within a range of wavelengths from propagating. The ease with which colloidal quasicrystals

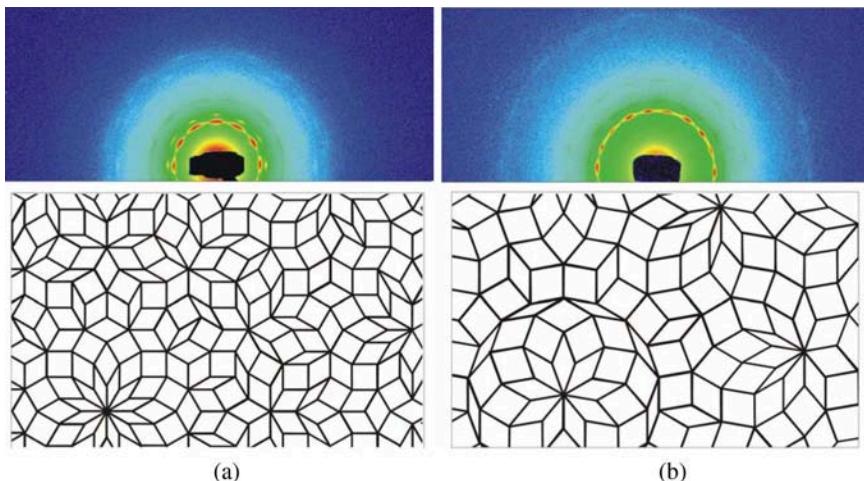


Figure 4.4 Colloid quasicrystals self-assembled with 12-fold (left) and 18-fold (right) symmetries.¹¹

can be fabricated suggests to the team that water-based wet chemistry is the best way ahead.

These experiments tell us that many intricate structures inside and outside the cell, such as membrane stacks, muscles fibres, vesicles, organelles, as well as extracellular structures like collagen fibres and even beautifully sculpted diatom shells and skeletons could have arisen by similar water-based self-assembly processes. Quasicrystalline and crystalline nanostructures are spontaneously formed, without detailed specific instructions from DNA or genes, although the latter can fine-tune or modify the self-assembly process. It is the natural tendency of water itself to form crystalline and quasicrystalline structures that sets the stage for the self-assembly of colloids and macromolecules.

Notes

1. Allard *et al.* (2004).
2. Shevchenko *et al.* (2006).
3. Hachisu and Yoshimura (1980).

4. Ise *et al.* (1999).
5. Norio Ise informed me in an e-mail dated 5 December 2011 that his team was not the first to demonstrate the formation of colloid crystals. He wrote: “Colloid crystals were found by scientists at BASF and at DuPont. Confirmation by microscope was first done by Sei Hachisu, Tsukuba University, Japan, who introduced me to the technique. We are the first to notice the attraction, which was not at all mentioned by others.”
6. Ise (2010).
7. Grier (1998).
8. Grier and Han (2004); Hansen and Lowen (2000).
9. Kinoshita *et al.* (1996).
10. Mikhael *et al.* (2008).
11. Fischer *et al.* (2011).

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5

Quantum Coherent Water

Quantum Effects

By now, you have probably gathered that studying water is not easy. The many kinds of spectroscopic methods used to probe the structure of water give different squiggly lines that need to be interpreted, and in order to help make sense of the results, a number of models are proposed and molecular dynamics simulations are carried out to reproduce the experimental results. If the models are correct, the computer simulations will match the experimental results quite closely. However, if the models are wrong, the simulations will differ substantially from the experimental observations. And even when the model matches the experimental observations, there is still no guarantee that the explanation is correct.

Numerous experiments and molecular dynamics simulations later, more and more researchers are coming around to the view that quantum effects need to be included in the simulations in order to reproduce and interpret a range of experimental results.¹ For example, Kim Hyeon-Deuk and Koji Ando at Kyoto University believe that quantum effects play a key role in the dynamics of the hydrogen-bonded network, and are therefore essential for understanding the anomalous properties of water.²

Nuclear quantum effects — effects due to atomic nuclei of different mass — have a significant impact on the behaviour of water. This is evident in the large changes observed in many properties of

water when hydrogen (H) is substituted by its heavier isotopes³ deuterium (D) and tritium (T). For example, the melting point of D₂O is 3.82 K higher than that of ordinary water, and the effect is even more pronounced in T₂O. These differences would not exist in classical physics.

Researchers at several institutes in Madrid, Spain, demonstrated that nuclear quantum effects have to be included in order to reproduce experimental heat capacity measurements over a wide range of temperatures encompassing ice and liquid water. They regard the heat capacity of water as “a signature of nuclear quantum effects”.⁴

Francesco Paesani and colleagues at the University of California, San Diego, investigated the molecular reorientation associated with the dynamics of the hydrogen-bonded network in liquid water using quantum molecular dynamics simulations.⁵ They found the calculations in excellent agreement with the corresponding experimental results obtained at different temperatures with *polarization-resolved femtosecond infrared spectroscopy*. Comparison with the results obtained using classical molecular dynamics simulations clearly indicated that the explicit inclusion of nuclear quantum effects is critical for reproducing the experimental results.

What is Quantum Coherence?

Water not only possesses quantum properties; it may actually be *quantum coherent*. Quantum coherence is such an important concept that we must deal with it here before proceeding further. What exactly is quantum coherence? And is it different from simply quantum effects?

Coherence is generally understood as wholeness, a correlation over space and time. Atoms vibrating in phase, teams rowing in synchrony in a boat race, choirs singing in harmony, troupes dancing in exquisite formations — all these conform to our ordinary notion of coherence.

Quantum coherence implies all that and more. It is incredibly dynamic, involving astronomical numbers of the most diverse players, all doing their own thing with maximum spontaneity and freedom. I described it in Chapter 1 as the most sublime state of wholeness that maximizes both local freedom and global cohesion, and an apt metaphor is quantum jazz with a dynamic range of some 70 octaves, where each individual player is freely improvising from moment to moment, yet keeping in tune and in step with the whole.

I have also defined quantum coherence quite precisely since the 1993 first edition of *Rainbow Worm* in terms of *factorizability* after Roy Glauber,⁶ who was awarded the 2005 Nobel Prize for his theory of optical quantum coherence.⁷ It goes like this: a system is quantum coherent if its parts are so perfectly correlated that their cross-correlations factorize exactly as the product of the individual self-correlations, so that each appears paradoxically as though totally uncorrelated with the rest.⁸ Coherence can exist to different degrees, or orders, according to the number of parts exhibiting factorizable cross-correlations.

Quantum coherent systems are characterized by a wave function with complex quantum phases, and some people would regard *any* quantum system as quantum coherent.⁹ Therefore, quantum effects are by definition quantum coherent effects.

Quantum Coherence from NMR

Evidence for quantum coherence in water has come from unexpected observations in nuclear magnetic resonance (NMR) experiments (see Box 5.1) over the past 20 years. These are intermolecular multiple quantum coherence signals generated by the collective effects of the magnetic dipole coupling of the spin of each molecule with that of every other in the sample up to 1 mm apart.¹⁰ This implies that all the spins of the molecules in the sample are correlated, and driven by the same (electromagnetic) field to oscillate in phase.

Box 5.1

Nuclear Magnetic Resonance and NMR Spectroscopy¹¹

NMR spectroscopy is a technique that exploits the magnetic properties of certain atomic nuclei to determine the physical and chemical properties of atoms or molecules containing them. These atomic nuclei possess spin, such as protons ^1H and ^{13}C . All stable isotopes that contain an odd number of protons and/or neutrons have an intrinsic magnetic moment and angular momentum or spin, while all nuclei with even numbers of both have spin 0.

When placed in a magnetic field NMR active nuclei absorb at a characteristic frequency, depending on the strength of the magnetic field. For example, in a 21 tesla magnetic field, protons resonate at 900 MHz. In the Earth's magnetic field, these nuclei resonate at audio frequencies (20 to 20 000 Hz). The effect is exploited in the Earth's field NMR spectrometers and other instruments that are portable and inexpensive, and often used for teaching or field work.

Depending on the local chemical environment, different protons in a molecule resonate at slightly different frequencies. As both the frequency shift and the fundamental resonant frequency are directly proportional to the strength of the magnetic field, the shift is converted into a field-independent dimensionless value known as the chemical shift, measured from some reference resonance frequency. For the nuclei ^1H , ^{13}C , and ^{29}Si , tetramethylsilane (TMS) is commonly used. This difference between the frequency of the signal and the frequency of the reference is divided by the frequency of the reference signal to give the chemical shift. As the shift is generally very small, it is expressed in parts per million (ppm).

In NMR spectroscopy, a magnetic field gradient is used, and the resonance signal depends on where in the gradient field the sample is located.

The magnetic nuclear spins of the sample are first aligned in a constant magnetic field. Then the alignment is perturbed by a radio frequency (RF) pulse. The two fields are chosen to be perpendicular to each other as this maximizes the NMR strength. The process called population relaxation refers to nuclei that return to the thermodynamic state in

(Continued)

Box 5.1 (*Continued*)

the magnetic field. This is the t_1 , spin lattice or longitudinal magnetic relaxation, t_1 being the mean time for an individual nucleus to return to its thermal equilibrium state of the spins. Once the nuclear spin population is relaxed, it can be probed again with another RF pulse. The perturbed nuclei begin to spin at the resonant frequency, and precess (gyrate) until they fall out of alignment with one another and stop producing a signal. This is called t_2 or the transverse relaxation time. t_1 is always longer than t_2 because of smaller dipole-dipole interaction effects. In practice, the value of t_{2*} is the actually observed decay time of the NMR signal.

Most applications of NMR involve full NMR spectra. Short square pulses of a given carrier frequency contain a range of frequencies centred about the carrier frequency, with the range of excitation (bandwidth) being inversely proportional to the pulse duration. Applying such a pulse to a set of nuclear spins simultaneously excites all the single-quantum NMR transitions, causing precession about the external magnetic field vector at the NMR frequency of the spins. This oscillating magnetization vector induces a current in a nearby pickup coil, creating an electrical signal oscillating at the NMR frequency. This signal is known as the free induction decay and contains the vector sum of the NMR responses from all the spins. Fourier transformation of the signal gives the frequency spectrum.

Using pulses of different shapes, frequencies, and durations in specifically designed patterns or pulse sequences allows the scientist to extract many different types of information about the molecules. Multidimensional NMR spectroscopy involves at least two pulses, and as the experiment is repeated, the pulse sequence is varied.

Warren and collaborators discovered in the 1990s that it was possible to observe intermolecular multiple-quantum effects in simple liquids using pulse sequences that involve as few as two RF pulses. High orders of quantum coherence (up to the tenth) in water have been detected in simple experiments. In one of these, a 90° RF pulse is followed immediately by a gradient pulse (see Box 5.1) to code the induced coherences; after a delay Δ of 150 ms, a second 90° RF pulse

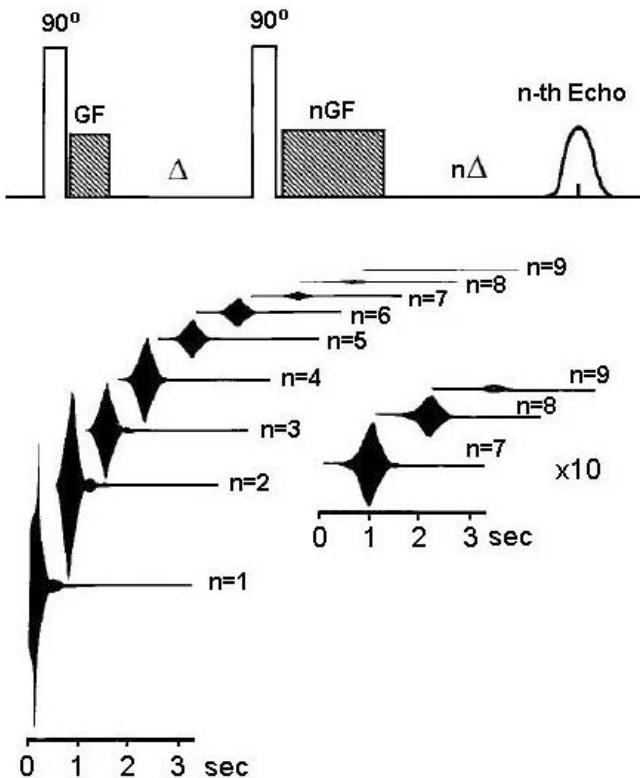


Figure 5.1 High-order intermolecular quantum coherence in water detected by NMR; redrawn after Zhang et al.¹⁴

is applied to make the coherence observable, and the coherence of order n is selected with a second gradient pulse of the same amplitude lasting n times as long as the first (see Fig. 5.1).¹²

These remarkable findings have resulted in significant contrast enhancement in magnetic resonance imaging and functional imaging, and many other applications.¹³ But their fundamental significance — that water can be quantum coherent to a high degree — has remained unappreciated by the general scientific community.

The possibility that naturally occurring water may be quantum coherent should be considered in the light of the ambient magnetic field of the Earth, to which water is constantly exposed. At the strength of the Earth's field, nuclei including protons would

resonate at audio frequencies (see Box 5.1). Could it be that these natural nuclear magnetic resonances have vital biological consequences, including the possibility of homeopathy? I am rushing ahead of myself, again.

Quantum Magnetic Signatures

Djuro Koruga, Professor of Mechanical Engineering at Belgrade University heads the NanoLab he set up in 1983 with an interdisciplinary team that reflects the extraordinary scope of his research interests, spanning mathematics, physics, and biology.

Koruga recognizes the overriding importance of understanding the mechanism of molecular recognition in water and the structure of water for drug design. He and his team have several inventions, including the opto-magnetic fingerprint (OMF), a potentially powerful non-invasive analytical technique based on the magnetic properties of water and biological tissues.¹⁵

A conventional optical microscope gives a picture of a sample based on ordinary light. The OMF gives a spectrum of the sample computed from the difference between diffuse white light and reflected polarized light produced when the source of diffuse white light irradiates the surface of the sample at Brewster's angle. Brewster's angle (also known as the polarization angle), named after Scottish physicist Sir David Brewster (1781–1868), is an angle of incidence at which non-polarized (diffuse) light reflected from the surface is perfectly polarized. For water, this angle is $\sim 53^\circ$.

The OMF device is represented in Fig. 5.2. The reflected light is polarized perpendicularly to the plane of incidence, so only the electric component of the light is detected by the digital camera. The magnetic component transmitted by the sample can thus be estimated by taking the difference between the incident white light (top) and the reflected polarized light (bottom). A spectrum is obtained by exploiting the RGB (red, green, and blue) system of the digital camera, which records the intensity of the colours in three separate channels. By subtracting the B intensity values (400 to 500 nm) from the R values (600 to 700 nm) over a 100 nm bandwidth for white diffuse

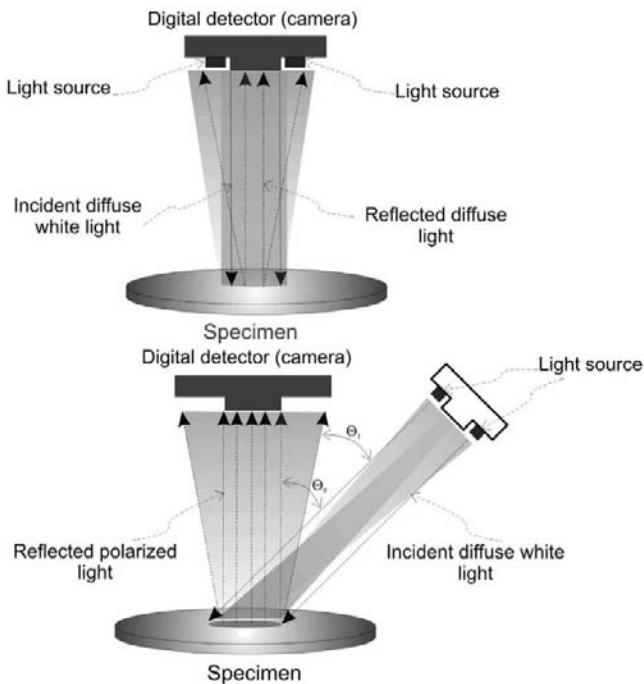


Figure 5.2 The opto-magnetic fingerprint device in direct diffuse white light (top) and reflected polarized light (bottom).¹⁵

light (W) and reflected polarized light (P), a unique spectrum or fingerprint is obtained for the sample.

The first results on pure water showed that the measurements were highly reproducible. In 24 identical experiments, the standard deviation of the wavelength difference was ± 0.14 nm, while that of the intensity difference was ± 0.0032 standardized unit signal.

The opto-magnetic and electric fingerprints of pure water at different temperatures are presented in Fig. 5.3. It can be seen that distinctive magnetic fingerprints are produced. Below freezing and at room temperatures, two paramagnetic (positive) and diamagnetic (negative) peaks are seen; but at higher temperatures, only one peak of each is evident and at different positions. Of note is that the peaks are strongest at 25°C, at which nuclear magnetic

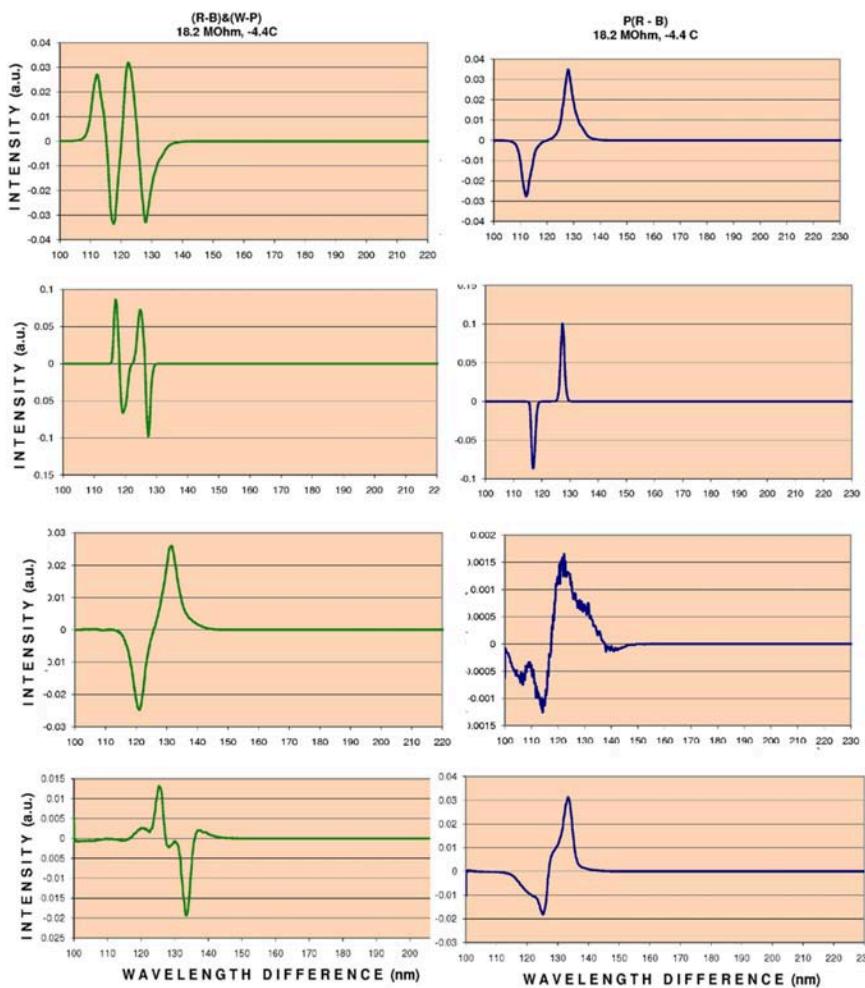


Figure 5.3 The opto-magnetic (left) and electric (right) fingerprints of pure water at (top to bottom) -4.4°C , 25°C , 50°C , and 91.2°C .

resonance experiments detected intermolecular multiple quantum coherence due to dipole coupling of nuclear spins (see earlier). Koruga considers the double peaks in water at 25°C resembling those of ice as evidence of quantum properties.

The technique is showing considerable promise as a quick method for detecting viruses in blood samples.¹⁶

Quantum Coherence in Symphonic Structures

For me, among the most convincing evidence of water's coherence has come from an extraordinary and brilliant artist, Michel Kappeli in Switzerland, who wrested the hidden symphonic structures of water flowing in his local river. His method was simply to allow water to speak directly, unmediated and unobstructed by opaque instrumentation. These coherent structures are macroscopic (centimetres in dimension).

Kappeli said, "To capture a purest possible image of the flux, it was most important to reduce technical aids as far as possible."¹⁷ But with lots of aesthetic imagination, he might have added.

Independent evidence of quantum coherent water under ambient conditions has come from quantum field theory considerations, which I shall describe in the next chapter.

Notes

1. Morrone and Car (2008).
2. Hyeon-Deuk and Ando (2010).
3. Isotopes are different forms of the same element, i.e., with the same *atomic number*, but different *atomic weight*. The atomic number of an element is the number of protons (positively charged elementary particles) it has in the atomic nucleus, which is equal to the number of electrons (negatively charged elementary particles) in the atomic orbit(s). The atomic weight is determined by the number of protons and neutrons (elementary particles without electric charge) in the nucleus. Thus, the atomic number of H and its atomic weight are both 1, as it has only one proton. But deuterium, D, has one neutron in addition, and hence has an atomic weight of 2, while tritium, T, has two neutrons, and hence an atomic weight of 3.
4. Vega et al. (2010).
5. Paesani et al. (2010).
6. Glauber (1969).
7. "Roy J. Glauber", Wikipedia, 20 November 2011, http://en.wikipedia.org/wiki/Roy_J._Glauber.

8. I am indebted to my teacher and friend Fritz-Albert Popp for this insight.
9. I discussed quantum phases and quantum coherence in Ho (2004c).
10. See Ho (2010b).
11. Based on “Nuclear Magnetic Resonance”, Wikipedia, 19 November 2011, http://en.wikipedia.org/wiki/Nuclear_magnetic_resonance.
12. Richter and Warren (2000); Warren *et al.* (2002).
13. Richter *et al.* (2000); Galiana *et al.* (2009).
14. Zhang *et al.* (2001).
15. Koruga *et al.* (2010).
16. Papić-Obradović *et al.* (2010).
17. Kappeli (2010, 2011).

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6

QED Water I

Coupling Matter to Electromagnetic Field

Perhaps the most significant discovery within the past 30 years is that water has quantum properties under ambient conditions, and may even be quantum coherent, as revealed by nuclear magnetic resonance and other measurements (see Chapter 5).

However, neither classical nor standard quantum theory predicts quantum coherence for water, first of all, largely because they ignore quantum fluctuations and the interaction between matter and electromagnetic fields, which is taken into account in *quantum electrodynamics (QED) field theory*.

Quantum fluctuations and the coupling between matter and electromagnetic fields in QED indeed predict quantum coherence for liquid water even under ordinary temperatures and pressures, according to Emilio Del Giudice and his colleagues at Milan University, who have been researching this problem since the 1990s. Their theory suggests that the interaction between an electromagnetic field and liquid water induces the formation of large, stable coherent domains (CDs) of about 100 nm in diameter at ambient conditions, and these CDs may be responsible for all the special properties of water, including life itself.¹

QED of Condensed Matter

Quantum field theory explicitly recognizes an extended vacuum field — zero-point field — interacting with matter, as well as quantum

fluctuations whereby energy in the form of photons in the vacuum field can be captured by matter. Quantum field theory combines Heisenberg's uncertainty principle in quantum mechanics with the energy-matter equivalence of Einstein's special relativity.² Two formulations of Heisenberg's uncertainty principle apply to quantum systems. The first states that the position, x , and momentum, p , of a particle cannot both be known precisely, $\Delta x \Delta p \geq h/4\pi$ (where h is Planck's constant, the smallest unit of action), and the second, the energy of a system, E , can fluctuate temporarily within time Δt , without violating the conservation of energy so long as $\Delta t \Delta E \geq h/4\pi$. Einstein's energy-matter equivalence is his famous equation $E = mc^2$, where energy, E , is equated to mass, m , times the square of the velocity of light, c .

Quantum field theory began in the 1920s and 1930s with the work of German physicists Max Born (1882–1970) and Werner Heisenberg (1901–1976), English physicist Paul Dirac (1902–1984) and others, and later, Richard Feynman (1918–1988) and Freeman Dyson in the United States. But standard quantum field theory still does not explain water adequately.

In standard quantum field theory, the energy levels of material systems are shifted by their interaction with the fluctuations of the electromagnetic (EM) fields in the vacuum. The first clear example was the *Lamb shift*, the energy of an electron surrounding the proton in a hydrogen atom being slightly different from the value calculated from atomic theory based on purely static forces. Although this shift is very small, it provides evidence of the quantum vacuum fluctuation that has to be understood within the framework of quantum electrodynamics. In the case of the hydrogen atom, the effect is due to the interactions between the electric current of the electron orbiting the nucleus and the fluctuating electromagnetic field.

For a collection of particles, the usual approach is to apply the Lamb shift to each particle separately. While this is correct for very low-density systems like gases, where the distance between any two particles is larger than the wavelength of the relevant fluctuating fields coupled to the systems, dense systems — condensed matter or liquids and solids — show an entirely different behaviour.

When energy is absorbed from the vacuum field, the particles will begin to oscillate between two configurations, a ground state

and an excited state. In particular, all particles coupled to the same wavelength of the fluctuations will oscillate in phase with the EM field, that is, they will be coherent with the EM field. The total energy of the system, E_{tot} , is a combination of the energy of the fluctuating EM field, E_{fl} , and the energy of excitation of the particles shifted from their ground state to the excited configuration, E_{exc} , plus the E_{int} of the Lamb-like shift:

$$E_{\text{tot}} = E_{\text{fl}} + E_{\text{exc}} + E_{\text{int}}. \quad (6.1)$$

While E_{fl} and E_{exc} are positive, E_{int} is negative. As shown by Giuliano Preparata (1942–2000) in 1995,³ E_{fl} and E_{exc} are proportional to the number N of particles in a coherence domain (CD), but E_{int} is proportional to N^2/N . Consequently, there is a critical number of particles N_{crit} enclosed in a CD for which $E_{\text{tot}} = 0$. At that point, a phase transition occurs. The coherent oscillations of the particles in the CD no longer require any external supply of energy, and the oscillation is stabilized. Moreover, the CDs will begin to attract more molecules and attract each other, thereby turning gas into liquid in a change of state. With a further increase in density, the system becomes a net exporter of energy because the stabilized coherent state has a *lower* energy than the incoherent ground state (see later).

(This explains why a large amount of heat (heat of condensation) is released when water vapour turns into liquid, so rain falling from the skies actually warms the air; conversely, liquid water needs to absorb a large amount of latent heat in order to vaporize, which is why evaporation from the surface of oceans and lakes, as well as transpiration from forest trees and other plants, cools the air. These properties of water are very important for distributing heat and water across the globe and between oceans and landmasses,⁴ which is yet another example of why water is so fit for life.)

The size of the CD is just the wavelength λ of the trapped EMF. The wavelength λ of the trapped EMF depends on the excitation energy E according to the equation

$$\lambda = hc/E_{\text{exc}}. \quad (6.2)$$

The CD is a self-produced cavity for the EMF; the photon of the trapped EMF acquires an imaginary mass, and is therefore unable to leave the CD. Because of this self-trapping of the EMF, the frequency of the CD EMF becomes much smaller than the frequency of the free field having the same wavelength. This result so far applies to all liquids.

Coherent Water as a Source of Almost-free Electrons

The special thing about water is that the coherent oscillation occurs between the ground state and an excited state at 12.06 eV, which is just below the first ionizing threshold of water at 12.60 eV, when $\text{H}_2\text{O} \rightarrow \text{H}^+ + \text{OH}^-$. In liquid water, the CD is about 100 nm in diameter, according to Equation 6.2. Such a CD contains millions of water molecules, and includes an ensemble (or plasma) of millions of almost-free electrons that can be donated readily to electron-acceptor solutes. And this is possibly the most significant property of quantum coherent water, as we shall see later.

Calculations carried out by Del Giudice and colleagues showed that the CD is a quantum superposition of the ground coherent state and excited state in the proportion of 0.87 and 0.13, giving an average energy of excitation per molecule of 1.56 eV. This is combined with the energy of the fluctuating electromagnetic field of 3.52 eV per molecule, and the interaction energy of -5.34 eV per molecule, according to Equation 6.1, thus resulting in a negative energy of -0.26 eV per molecule. The renormalized (physically observable) frequency of the trapped EMF in the CD corresponding to 0.26 eV is 6.24×10^{13} Hz in the infrared region.⁵

Liquid water is therefore a two-fluid system⁶ (in analogy with superfluid helium), consisting of a coherent phase — about 40% of the total volume at room temperature — and an incoherent phase. In the coherent phase, the water molecules oscillate between two electronic configurations in phase with a resonating EMF. The two phases have widely different dielectric constants: that of

the coherent phase is 160, due to the high polarizability of the coherently aligned water molecules that are oscillating in concert; while the dielectric constant of the incoherent state is about 15.

This picture of liquid water, according to Del Giudice and colleagues, is reflected in the many observations supporting a two-state model of water,⁷ in which a substantial fraction of the molecules exist in a hydrogen-bonded state resembling that of ordinary ice (see Chapter 3). In fact, the hydrogen bonds — short-range interactions — are the consequence of the induced coherence in the coherence domains. But there is a rapid interchange of molecules between the CDs and the incoherent phase, hence it is impossible to detect CDs when the detection time is longer than the period of the oscillations, which is less than 10^{-13} s. (This highlights the difficulty of acquiring information about the behaviour and structure of water, and of the living system that it makes.)

One remarkable phenomenon that can be explained by the QED theory of Del Giudice and colleagues is how water can *burn*.

Burning Water

Water-powered cars and welding torches that burn water had been ricocheting around the Web for some years, but were impossible to pin down as they typically depended on a proprietary or otherwise ill-defined process.

A report finally caught my eye in 2009, as the experimental details were quite well described and documented.⁸ Retired broadcast engineer and inventor John Kanzius was trying to find a cure for cancer when he stumbled upon a way of setting fire to water. He had made a radio frequency generator, intending to kill cancer cells loaded up with metal nanoparticles. When he aimed the radio waves at a test tube of salt water, presumably as a control, it produced an unexpected spark. Kanzius put a lighted match to the top of the water, and the water ignited and kept on burning for as long as it remained in the radio frequency field. The phenomenon was reproduced on YouTube for the benefit of the local TV station.

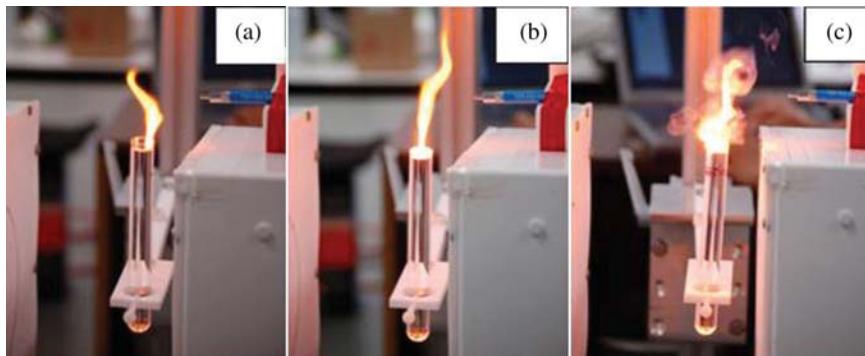


Figure 6.1 Burning water at different NaCl concentrations: (a) 0.3%, (b) 3.0%, (c) 30%.⁹

Independent witnesses verified that the flame was burning at $1\,500^{\circ}\text{C}$, and the heat was strong enough to run a small Stirling engine.

The YouTube video attracted the attention of Rustum Roy (1924–2010) — Distinguished Professor of Materials at Arizona State University and Professor of the Solid State and of Geochemistry at Pennsylvania State University — who followed up the research and held a public demonstration in September 2007, which was reported in *National Geographic News*. Further details appeared in a preliminary report in the March 2008 issue of *Materials Research Innovations*, a journal then edited by Roy (see Fig. 6. 1).⁹

The maximum power for most experiments was about 300 W and the frequency of the polarized radio frequency beam was ~ 13.56 MHz.

The radio wave was aimed at Pyrex test tubes containing solutions of 0.1 to 30% salt (NaCl). The solutions typically sustained a continuous flame till the water was exhausted. Immediately after the power was turned on, the flammable gas could be ignited, and it extinguished instantly when the power was turned off. The smallest flame sustained was at 1% NaCl. So salt was crucial.

The phenomenon is real, but the mechanism entirely unknown. Roy and colleagues suggested specific resonant coupling of the RF radiation to the structure of water in the presence of NaCl, causing it to split into “intimately mixed” hydrogen and oxygen. When ignited, the hydrogen burns, regenerating water or steam.

Electrolytic splitting of water is well known. But, as first demonstrated by British physicist Michael Faraday (1791–1861), it takes >1.23 V to split water into hydrogen and oxygen. The 13.56 MHz polarized RF beam delivers at most 10^{-8} of the energy required, so the answer has to be in the structure of water, more specifically, coherent water.

As Del Giudice and colleagues have shown, coherent water, which can be stabilized on the hydrophilic surfaces of the glass tube (see next section), is already highly excited to the verge of ionization (see earlier). The RF radiation may well trigger a resonant effect, resulting in water splitting into hydrogen and oxygen as proposed.

Del Giudice and colleagues¹⁰ indeed argue that water CDs can be easily excited, and are able to collect small excitations to produce single coherent vortices whose energy is the sum of all the small excitation energies, turning the originally high entropy energy into low entropy coherent energy that is trapped stably in the water CDs. This coherent energy in turn enables selective coherent energy transfer to take place. All molecules have their own spectrum of vibrational frequencies. If the molecule's spectrum contains a frequency matching that of the water CD, it will be attracted to the CD, become a guest participant in the CD's coherent oscillation, and settle on the CD's surface. Furthermore, the CD's excitation energy will become available to the guest molecules as activation energy for chemical reactions to take place.

One possible explanation for the phenomenon of burning water, I suggest, is that the protons as hydronium ions hovering just outside the CD (see next section) could be resonating to the RF frequency trapped by the CD, thereby becoming attracted to the CD surface to combine with the almost-free electrons to form hydrogen gas, and in the process, triggering the simultaneous evolution of oxygen. The requirement for NaCl is unclear. NaCl is expected to ionize into Na^+ and Cl^- when dissolved in water. Ions increase the electrical conductivity of water, and are also known to affect water structure (see Chapter 9).

Burning water is just a particularly dramatic oxidation of water that is at the heart of life on Earth, and quantum coherent water is what makes it possible.

Quantum Coherent Water and Life

Oxidation and reduction or redox reactions are central to energy transformation in living organisms. It involves the transfer of electrons from one substance (donor) to another (acceptor) to power all living activities, the electron coming ultimately from splitting water in photosynthesis by green plants, algae, and cyanobacteria. We shall deal with the redox dynamo that powers life in Chapters 21 and 22. Here, we address a more fundamental question of how water can be split at all.

As we have seen, it takes 12.60 eV to split water, an energy corresponding to soft X-rays, which is not what the green plants, algae, and cyanobacteria use.

Some 60 years ago, the father of biochemistry, Hungarian-born US scientist Albert Szent-Györgyi (1893–1986), already highlighted the importance of water for life, and proposed that organized water close to surfaces such as cell membranes is able to induce a very long-lasting electronic excitation of the different molecular species present, thereby activating them and enabling their mutual attraction for reactions to take place.¹¹ He proposed that water in living organisms existed in two states: the ground state and the excited state, and that water at interfaces such as membranes existed in the excited state, which requires considerably less energy to split.

A sign of excited water, according to Szent-Györgyi, is that a voltage should appear at the boundary between interfacial water and bulk water. That was indeed observed. This property of water enables energy transfer to take place in living organisms, ensuring long-lasting electronic excitations. Szent-Györgyi's ideas were largely ignored by the scientific mainstream that became obsessed instead with molecular genetics.

The anomalous water at interfaces has been the subject of numerous research papers and reviews¹² and was already known in the late 1940s, as Del Giudice and colleagues point out. Most if not all water in living organisms is interfacial water, as it is almost never further away from surfaces such as membranes or macromolecules than a fraction of a micrometre.

A vivid demonstration of interfacial water was achieved by Gerald Pollack's research team at the University of Washington.¹³ Using a hydrophilic gel and a suspension of microspheres just visible to the eye, they showed that interfacial water apparently tens of micrometres or even hundreds of micrometres thick forms on the surface of the gel, which excludes the microspheres as well as other solutes such as proteins and dyes, and hence referred to as an exclusion zone (EZ). Formation of the EZ depends on fixed charges on the gel. When negatively charged gels were used, a potential difference of -150 mV was measured, in line with Szent-Györgyi's prediction, and protons were also excluded, becoming concentrated just outside the EZ, giving a low pH there. We shall look at this phenomenon in greater detail in Chapter 12.

Del Giudice and colleagues¹⁴ suggest that EZ water is in fact a giant coherence domain stabilized on the surface of the hydrophilic gel. Inside the cell, the EZ would form on surfaces of membranes and macromolecules, as envisaged by Szent-Györgyi. Because coherent water is excited water with a plasma of almost-free electrons, it can easily transfer electrons to molecules on its surface. The interface between fully coherent interfacial water and normal bulk water becomes a redox pile. In line with this proposal, EZ water does indeed act as a battery, as Pollack's research team demonstrated.¹⁵

The selectivity of CDs in chemical reactions may be why out of 100 different amino acids only 20 have been selected for making proteins in living organisms.¹⁶

There is independent evidence that molecules taking part in a biochemical reaction do share a common frequency, which is how they attract each other, essentially by resonating to the same frequency.¹⁷ So it is likely that the reactants are attracted to the surface of the same water CD, where the reaction takes place, greatly facilitated by the excitation energy of the water CD. After the reaction, the energy released can also be absorbed by the water CD, shifting the CD's oscillation frequency, and hence changing the molecular species that become attracted to it, thereby in principle facilitating the next reaction to take place in a chemical pathway.

Quantum coherent water underlies other strange phenomena, such as the formation of a stiff water bridge floating in space just above two beakers of water placed next to each other and subjected to a strong electric field, as explained by Del Giudice and colleagues elsewhere,¹⁸ which we shall reluctantly pass over.

However, the relationship of quantum electrodynamics to non-thermal electromagnetic field effects on biological systems, and its possible link to homeopathy are too important to miss, and will be dealt with in the next chapters.

Notes

1. Arani *et al.* (1995); Del Giudice (2007); Del Giudice *et al.* (2010b, 2002); Ho (2011c).
2. Zee (2004).
3. Preparata (1995).
4. “Water Cycle”, Wikipedia, 29 December 2011, http://en.wikipedia.org/wiki/Water_cycle.
5. Del Giudice (2007); Del Giudice *et al.* (2010b).
6. Del Giudice *et al.* (2002).
7. Ho (2006c, 2010a).
8. The account is based on Ho (2009c).
9. Roy *et al.* (2008).
10. Del Giudice *et al.* (2010b).
11. Szent-Györgyi (1960, 1961).
12. Clegg (1984).
13. Del Giudice *et al.* (2010b).
14. Zheng and Pollack (2003); see Ho (2004d).
15. See Ho (2008).
16. Del Giudice *et al.* (2010b).
17. See Ho (2007a); Veljkovic *et al.* (2007); Veljkovic *et al.* (2011).
18. Del Giudice *et al.* (2010a).

QED Water II: Non-thermal EMF Effects

Debate over Non-thermal EMF Effects¹

Non-thermal electromagnetic field (EMF) effects are by definition those due to very low-intensity fields that do not heat up the cells or tissues of organisms sufficiently for a rise in temperature to be detected. These effects lie at the heart of the persistent debate over the health hazards of mobile phones, which is a continuation of the debate on the safety of EMFs in the entire frequency spectrum (see Box 7.1) from the extremely low-frequency domestic electricity supply to the radio waves and microwaves used in telecommunication that began in the 1950s.²

Box 7.1

Electromagnetic Waves and the Electromagnetic Spectrum

Electromagnetic waves propagate through empty space at the speed of light, 300 000 km/s, and include the light that enables us to see, which vibrate at frequencies of about 10^{14} Hz (hertz, cycle per second, named after Heinrich Hertz (1857–1894), the German physicist who discovered electromagnetic waves in 1888). They have both an electrical component and a magnetic component vibrating at right angles to each other.

(Continued)

Box 7.1 (Continued)

The entire electromagnetic spectrum is extremely wide, ranging from waves that vibrate at less than 1 Hz to 10^{24} Hz. The corresponding range of wavelengths (speed/frequency) is 3×10^8 m to 3×10^{-15} m.

Above the visible spectrum are the ultraviolet rays, X-rays, and γ -rays, the ionizing radiation that breaks molecules up into electrically charged entities and can damage DNA, causing harmful mutations.

Below the visible range is the non-ionizing electromagnetic radiation, the safety of which has been hotly debated for well over half a century.

The “Thermal Threshold” Fallacy

According to our regulators to this day, there is no conceivable mechanism whereby the very low-intensity EMFs emitted by mobile phones and base stations or high-tension power lines could have any biological effects, because the energy involved is below that of the random molecular motions of a system at thermodynamic equilibrium. I add the emphasis because everyone who has studied physics or chemistry at school will have recognized that organisms are anything but “systems at thermodynamic equilibrium”, so anybody using that argument is grossly, if not wilfully, ignorant, and should be immediately disqualified as a public menace.

In conventional (equilibrium) thermodynamics, the energy of a system is nkT , where n is the number of molecules in the system, k is Boltzmann’s constant (1.3807×10^{-23} joules per kelvin), and T the absolute temperature in kelvin; this random thermal energy is evenly distributed throughout the system and unavailable for doing work. So, any incoming energy less than kT — the kinetic energy of an individual molecule — is below the thermal threshold at which useful work can be done, and hence can have no effect.

The thermal threshold is a fallacy arising from assuming that living organisms can be described in terms of conventional equilibrium thermodynamics, whereas by general consensus they are open systems meticulously organized and maintained far away

from thermodynamic equilibrium. Useful work is done everywhere within the system because *coherent* energy is being mobilized for growth and development and for the myriad activities that life entails. In such systems, extremely weak electromagnetic fields with energies below the thermal threshold can indeed have macroscopic effects because these fields can *affect an astronomical number of molecules simultaneously engaged in the same activity*, typically 10^{17} to 10^{20} molecules for a human weighing 70 kg.

Organisms are indeed coherent to a high degree, or quantum coherent, and liquid crystalline, as described in *Rainbow Worm* and further elaborated in this book. Organisms and cells, as well as molecules, rely on electric and electromagnetic fields for intercommunication;³ and that is how living systems from bacteria to whales can function as perfectly coordinated and coherent wholes.

Specificity of Non-thermal Effects

There is abundant evidence of non-thermal biological effects going back decades. However, the picture is clouded by apparently conflicting results due to a failure to take into account the fact that EMF effects are often frequency-specific as well as specific to developmental stage, as we demonstrated with distinctive helical transformations of the normal segmental pattern in fruit fly larvae emerging from eggs briefly exposed to very weak static magnetic fields (many orders of magnitude below the thermal threshold) for 30 minutes during the first two hours of development.⁴ Exposing the developing eggs to the magnetic field outside this time window had little or no effect. And exposing the eggs to alternating electromagnetic fields produced effects other than the characteristic helical transformations.

Most intriguingly, the EMF effects often deviate from classical dose-response behaviour; in other words, they depart from the usual assumption that the effect should go up linearly with field intensity until a point of saturation. Instead, some effects can only be observed in a specific range of intensities, and disappear at both higher and lower levels. These intensity and frequency windows have simply stretched the imagination of many in the scientific

community, and so the tendency is to dismiss those effects altogether, even by some of those, like myself, who did experiments on non-thermal EMF effects.

Ion Cyclotron Resonance

The archetypal example of non-thermal EMF effects that exhibit both frequency and intensity windows was discovered in the laboratories of Carl F. Blackman,⁵ Research Scientist in the Environmental Carcinogenesis Division of the US Environmental Protection Agency (EPA), and Abraham R. Liboff⁶ at Florida Atlantic University during the 1980s. (Blackman has been scrupulously careful to state that his work on non-thermal EMF effects is not done as an EPA employee, and his views do not represent those of the EPA.) In their experiments, they combined a static (DC) and an alternating (AC) magnetic field, which caused an increase in the concentration of free calcium ions in nervous tissues in a very narrow resonance window of the AC magnetic field, with the maximum corresponding to the cyclotron frequency of Ca^{2+} ions.

Ions in a static and uniform magnetic field will typically move in a circle with a cyclotron frequency f_c determined by its charge q , mass m , and the strength of the magnetic field B :

$$f_c = (qB)/(2\pi m). \quad (7.1)$$

These ion cyclotron resonance effects were extensively investigated in a number of laboratories. The calcium cyclotron frequency was found to affect the calmodulin (calcium-binding protein) regulation of calcium ion concentration in solution and a host of biological functions: the motility of diatoms, the rate of cell proliferation in culture, melatonin synthesis in the pineal gland, calcium concentration in lymphocytes and thymus cells, the germination and growth of seeds, etc.

Ion cyclotron resonance was extended to other ions such as potassium on the rate of cell proliferation, and lithium and magnesium on animal behaviour.

Mikhail Zhadin's research team at the Institute of Cell Biophysics of the Russian Academy of Sciences attracted serious attention to the phenomenon when they showed that the ion cyclotron resonance could be demonstrated for a simple amino acid dissolved in water.⁷

Ion Cyclotron Resonance for Amino Acids

Zhadin's team used a solution of glutamic acid (0.33 g/L) in water adjusted to pH 2.85 with dilute acid in an electrolytic cell. A cubic cell ($2 \times 2 \times 2$ cm, 8 ml in volume) was filled with the solution. Gold electrodes with an area of 2 cm^2 were placed in the cell 1 cm apart, and the potential difference between the two electrodes was adjusted to 80 mV with an external power supply. The cell was placed within two coils, with one coil located inside the other, the axes of the coils coinciding with each other. The outer coil created the DC magnetic field, B , and the inner coil made the AC field. The electric field between electrodes was perpendicular to the coils' axes. The coils were located within a Permalloy chamber that shielded them from all external fields. The DC magnetic field was $40 \mu\text{T}$ (microtesla), about the same as Earth's magnetic field. The sinusoidal current through the inner coil generated the AC magnetic field of amplitude $0.02 \mu\text{T}$. The AC field frequency was scanned in the range of 1 to 10 Hz with a speed of 0.05 Hz/s. A baseline steady current of a few nA (nanoampere) was recorded under non-resonant conditions. At resonance, a transient sharp increase in current was found.

Initially, in order to find a minimum of the AC field at which an effect could be detected, the amplitude was increased in small steps starting with 10 nT (nanotesla). To their great surprise, a "quite prominent" brief peak of current through the solution was already found at 20 nT. There was only one peak, coinciding with the calculated cyclotron frequency of glutamic acid ion of 4.18 Hz. The peak of the current was 10 to 80 nT, and the rise time typically 0.5 s, while the decay time was 15 to 20 s. There was also an amplitude window, above or below which the effect was not detectable.

This striking effect has been reproduced by different laboratories, including that of Emilio Del Giudice at the University of Milan.⁸

Initially, the success rate for producing the effect was about 20%, but increased to 70% in the most recent experiments, where it was confirmed to be a field effect, and could be produced even when the electrodes were placed outside the electrolytic cell.⁹

QED Explanation Required

There is only one problem. The effect of apparent ion cyclotron resonance cannot be explained in terms of classical physics. Not only is the energy involved in the AC magnetic field some 11 orders of magnitude smaller than the thermal threshold of thermal noise, the calculated radius of the circular path taken by the ion at resonant frequency is in metres, much larger than the experimental cell in which the observations were made.

In order to explain this phenomenon, a quantum electrodynamics field theory was needed, as described in the previous chapter, originally proposed by Giuliano Preparata and elaborated by Del Giudice and other colleagues (after Preparata's untimely death in 2000).

Quantum field theory predicts that liquids, being condensed matter with high density, are not governed by purely static local interactions such as hydrogen bonds and dipoles. On the contrary, the hydrogen-bonding is induced by radiated long-range electromagnetic fields. A collection of molecules interacting with the EMF above a density threshold and below a critical temperature acquires a new minimum energy state different from the conventional where the oscillations of individual molecules are uncorrelated and the EMF is nearly zero. The new minimum energy state is a coherence domain (CD) that oscillates in unison and in tune with an EMF trapped within it. For water, the CD is about 100 nm in diameter (see Chapter 6).

How Ion Cyclotron Resonance Could be Explained

Recall that according to the QED theory, water is a two-fluid system consisting of a coherent phase (about 40% of volume at room temperature) and an incoherent phase (Fig. 7.1). The two phases have

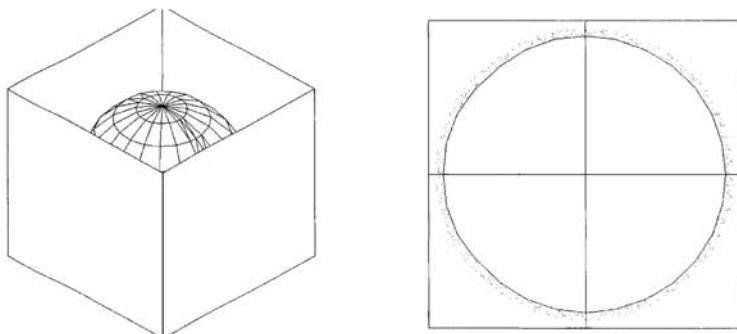


Figure 7.1 Coherence domain in water surrounded by incoherent phase.¹⁰

widely different dielectric constants: that of the coherent phase is 160, highly polarizable due to the coherently aligned water molecules that are oscillating in concert; while the dielectric constant of the incoherent state is about 15. The externally applied electric fields are therefore only felt in the non-coherent phase.

The electrolyte (dissolved ions) forms a coherent system within the non-coherent phase of the solvent. The ions oscillate in their respective Debye–Hückel (DH) cages (water molecules surrounding the ions due to dipole interactions). And these DH oscillations satisfy the quantum electrodynamic condition for coherence at all accessible concentrations. A major effect of the ion coherence is the elimination of interionic collisions, because all ions oscillate with the same frequency. So kT thermal noise is irrelevant. The enormous radius attributed to the cyclotron orbit is also irrelevant, as it only applies to the high speeds attainable in the gas-like phase.

The ions are driven into stable circular orbits around the CD equatorial plane so as to minimize their energy; the cyclotron frequency is $f_c = qB/2\pi m$ (Equation 7.1), the radius of the orbits being that of the water CD.

If now a weak AC magnetic field is superimposed, B_{ac} of frequency ω , sidebands are added to the fundamental cyclotron frequency, $f_s = f_c - n\omega$, with intensity proportional to B_{ac} , so long as B_{ac} approaches zero (being very small). When $\omega = f_c/n$, at resonance, f_s vanishes, and this zero frequency becomes a translational movement out of the

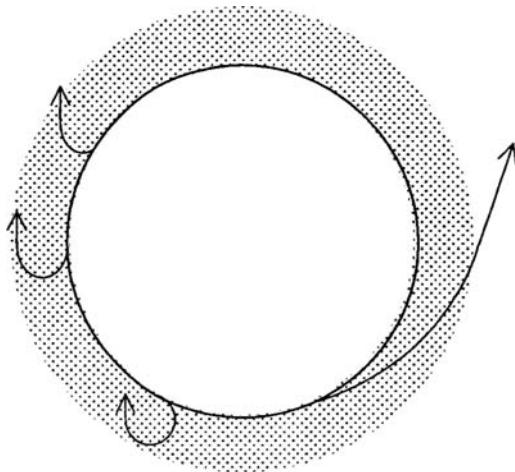


Figure 7.2 Escape of ions from orbit around the CD at cyclotron resonance.¹⁰

orbit, thereby contributing to an increase in electrical current, as observed in the experiment (Fig. 7.2).¹⁰

This explanation based on QED and quantum coherent water accounts well in general for the ion cyclotron resonance of electrolytes and small molecules. But many details remain unclear, especially the precise manner in which inorganic ions and amino acids, not to mention peptides, proteins, and nucleic acids, interact with water. These will be addressed in later chapters.

Most important, water within living organisms is different from bulk water, as will become clear in the course of this book, so how does ion cyclotron resonance work in that context? These are wide-open questions. In the next chapter, we examine how QED might explain homeopathy, as promised.

Notes

1. This chapter is based on Ho (2011d).
2. Ho (2003b,c).
3. Ho (2007a).
4. Ho *et al.* (1992).

5. Blackman *et al.* (1985).
6. Liboff (1985).
7. Zhadin and Giuliani (2006); Zhadin *et al.* (1998).
8. Comisso *et al.* (2006).
9. Giuliani *et al.* (2008).
10. Del Giudice *et al.* (2002).

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8

QED Water III: Homeopathy

Homeopathy and the Memory of Water

The issues of homeopathy and the memory of water have been hotly debated for well over a century with no sign of resolution. Peter Fisher, Clinical Director and Director of Research at the Royal London Hospital for Integrated Medicine and Physician to Her Majesty the Queen, has presented a comprehensive review of the biological effects of highly dilute homeopathic preparations in a wide range of cells and organisms, together with physicochemical measurements indicative of structural anomalies in such preparations.¹ Yet, for most scientists in the conventional research community, “memory of water” and “homeopathy” are both terms of abuse and derision, synonymous with quackery or any improbable cure.

Prominent water research chemist Martin Chaplin at London South Bank University described many examples in conventional chemistry where, indeed, water appears to have a memory.² A well-known example is associated with the formation of clathrate hydrate, a cage-like structure of water around small molecules such as methane gas (see Chapter 3). A water sample that has been crystallized into a gas clathrate under pressure and then melted will more quickly re-form the clathrate hydrate when mixed with the gas and pressurized, compared with water that has not experienced the hydrate state. Considering the extensive ice crystalline structure in the clathrate hydrate, which easily translates into corresponding liquid crystalline hydrogen-bonded supramolecular

structures on melting (Chapter 3), it is perhaps not so surprising that such a memory effect is observed.

The issue took a very exciting turn recently. French scientist Luc Montagnier at the Pasteur Institute, who was awarded a Nobel Prize for discovering human immunodeficiency virus (HIV), announced that certain bacterial and viral DNA sequences dissolved in water cause electromagnetic signals to be emitted at high dilutions.³ That was bad enough as far as the medical/scientific establishment was concerned; but at least such EM signals could be the beginning of the science of homeopathy, as it would be possible to correlate them with biological effects.⁴

Since then, new results from his laboratory have appeared to show that the DNA sequence itself can be reconstituted from the EM signal.⁵ That has so stunned the scientific community that one prominent supporter and practitioner of homeopathy was nonetheless moved to remark: “Luc is either a genius or he is mad!”

But Del Giudice’s team are taking that very seriously, and have linked Montagnier’s findings to their QED theory of water. So let’s see what Montagnier and his team have found.

DNA Emits EM Signals at High Dilution

Montagnier’s journey began ten years ago when he discovered the strange behaviour of a small bacterium, *Mycoplasma pirum*, a frequent companion of HIV infection that, like HIV, has special affinity for the human lymphocytes (white blood cells). He was trying to separate the bacterium of about 300 nm from the virus particles of about 120 nm, using filters of pore size 100 nm and 20 nm, starting with pure cultures of the bacterium on lymphocytes.

The filtrate (solution that went through the filter) was sterile, and no bacterium grew in a rich culture medium that would normally support its growth. Furthermore, polymerase chain reactions (PCRs) based on primers (short starting sequences) derived from adhesin, a gene of the bacterium that had been cloned and sequenced, failed to detect any DNA in the filtrate.

But, to Montagnier’s amazement, when the filtrate was incubated with lymphocytes that were not infected with *Mycoplasma*

(according to the most stringent tests), the bacterium was regularly recovered.

So, was there some information in the filtrate responsible for directing the synthesis of the bacterium? That was the beginning of a long series of investigations on how DNA behaves in water, which led to the discovery that the *M. pirum* DNA was emitting low-frequency electromagnetic waves in some diluted solutions of the filtrate in water, and this property of *M. pirum* DNA was soon extended to other bacterial and viral DNA.

The instrument picking up the EM signals consists of a solenoid (a coil of wire) that detects the magnetic component of the waves produced by the DNA solution in a plastic tube as it induces an electric current in the wire. This current is amplified and analysed on a laptop computer with special software, and the resultant signals plotted out on the computer screen.

In summary, ultra-low-frequency (500 to 3 000 Hz) electromagnetic signals were detected in certain dilutions of the filtrate from cultures of micro-organisms (virus, bacteria) or from the plasma of humans infected with the same agents. The same results were obtained from their extracted DNA. The EM signals were not linearly correlated with the initial number of bacterial cells before filtration. In one experiment, the EM signals were similar in suspensions of *Escherichia coli* cells varying from 10^9 down to 10. It is an “all or none” phenomenon. The EM signals were detected only in some high water dilutions of the filtrates; for example, from 10^{-9} to 10^{-18} in some preparations.

In *M. pirum*, an isolated single gene, adhesin (previously cloned and sequenced), could induce the EM signal, suggesting that a short DNA sequence is sufficient to induce the signal. Similarly, a short HIV DNA sequence of 104 base pairs is enough to induce the EM signal.

Certain bacteria do not produce EM signals (at least in the range detected by the instrument), for example probiotic bacteria such as *Lactobacillus* and some lab strains of *E. coli*.

EM signals were detected from some retroviruses (HIV, FeLV), hepatitis viruses (HBV, HCV), and influenza A cultures. In general, EM signals were produced by 20 nm filtrates of viral suspensions or

from the extracted DNA. In HIV, the virus RNA was not a source of EM signals, but the provirus DNA present in infected cells. In bacteria, the EM signals were produced by 100 nm filtrates, and not by the 20 nm filtrates. This suggested to Montagnier's team that nanostructures of water are carriers of the information. Although highly purified water was used, the presence of trace contaminants forming nanostructures (such as silica from the glass tubes and bottles used) cannot be ruled out. The production of EM signals is resistant to treatment with RNase, DNase, protease, or detergent. However, it is sensitive to heat over 70 °C and freezing at –80 °C. This sensitivity is reduced when purified short DNA sequences are used. To produce the EM signals, succussion (vigorous shaking) is necessary, as well as stimulation by an electromagnetic background of very low frequency, either from natural sources (the Schumann resonances, which start at 7.83 Hz) or from artificial sources, such as the mains.

DNA Sequence Recreated from Its EM Signature in Pure Water

In the new experiments of Montagnier's team, a fragment of HIV DNA was taken from its long terminal repeat (a stretch containing repeated sequences at one end of the HIV genome) to generate EM signals. This fragment was amplified by PCR to a 487 bp sequence and a 104 bp sequence. Dilutions of the DNA were made and the characteristic EM signals produced and detected under the ambient electromagnetic background.

Then, one of the diluted solutions (say, 10^{-6}) which gave a positive signal was placed in a container shielded by 1 mm thick mu-metal (an alloy that absorbs EM waves). Close to it, another tube containing pure water was put in place. The water content of each tube had gone through 450 nm and 20 nm filters and had been diluted from 10^{-2} to 10^{-15} , as for the DNA solution. A copper solenoid was placed around the tubes and a low-intensity electric current oscillating at 7 Hz was passed through the solenoid by an external generator to produce the AC magnetic field, which was maintained for 18 hours at room temperature. EM signals were then recorded

from each tube. At that point, the tube containing pure water also emitted EM signals at the dilutions corresponding to those giving positive EM signals in the original DNA tube. This result showed that the EM signals carried by the nanostructures in the water originating from the DNA had been transmitted to the pure water in 18 hours. No such transfer of EM signals was achieved when the time of exposure was less than 16 to 18 hours, or when the coil was absent, or when the generator of the magnetic field was turned off, or when the frequency of excitation was less than 7 Hz, or when DNA was absent in the donor tube.

Now for the most crucial test: could the EM signals transmitted to the pure water that had never had DNA in it provide sufficient information to recreate the DNA sequence? For the test, all the ingredients necessary to synthesize the DNA by the polymerase chain reaction—nucleotides, primers, DNA polymerase enzyme—were added to the tube with the pure water that had gained the EM signals. The reaction was done under ordinary conditions, and the DNA produced was then run through an agarose gel electrophoresis to separate out the DNA molecules into bands according to size.

A single DNA band of the expected size was found. It was 98% identical to the sequence of DNA from which the EM signals originated (only 2 out of 104 nucleotides were different).

The experiment was highly reproducible, 12 out of 12 times, and was also repeated with another DNA sequence from the bacterium *Borrelia burgdorferi*, the agent of Lyme disease.

Bringing Bacterium to Life from Its DNA Signals?

The startling results suggest an explanation for Montagnier's original observation that the bacterium could be reconstituted from a sterile filtrate incubated with human lymphocytes. The EM signals of all the bacterium's DNA were in the sterile filtrate. The nanostructures induced by *M. pirum* DNA in the filtrate carried information representing different segments of its genomic DNA. Each nanostructure, when in contact with the human lymphocytes, directs the synthesis

of the corresponding DNA by the DNA polymerases in the cell. There is then a certain probability that each piece of DNA recombines within the cell to reconstruct the whole DNA genome of *Mycoplasma*. From there, the synthesis of the rest of the bacterium — membrane lipids, ribosomes, and proteins — can take place, thanks to the synthetic machinery of the cells. One single reconstituted *Mycoplasma* is sufficient to generate the whole infection of lymphocytes. The report stated: “All the steps assumed in the regeneration from water can be analysed and open to verification.”⁵

Improbable as the findings may seem, Montagnier and colleagues reminded us that human genome sequencer Craig Venter, who now runs an institute named after himself, had claimed to have created life by first reassembling an entire *Mycoplasma* genome from pieces of DNA bought off the shelf.⁶ So at least that step is not impossible.

The finding also dovetails with evidence that molecules inter-communicate by electromagnetic signals, which bring them together for biochemical reactions in a very crowded cell.⁷

However, it raises several fundamental questions:

1. What is the nature of EM signals emitted by the DNA?
2. How can water store and receive EM signals?
3. How can the EM signals provide information of such precision that the DNA sequence can be recreated from the EM signals without a template, which is how it is normally done within the cell?

Some of the crucial answers take us back to QED.

DNA EM Signals from Earth's Field NMR

The answer to the first question is that the EM signals emitted by DNA are very likely the result of Earth's field NMR. As described in Chapter 5, magnetically active nuclei of atoms in molecules exposed to a static magnetic field tend to align along the field, and when simultaneously stimulated by an AC magnetic field, exhibit resonance at certain frequencies. At the strength of Earth's field, of the order of

50 μT , the resonance frequencies are expected to be in the audio range (20 to 20 000 Hz). The EM signals detected by Montagnier and colleagues were 500 to 3 000 Hz, well within the expected range. The observation that producing signals from the DNA required exposure to Earth's field as well as the ambient EM field of Schumann resonances or equivalent is consistent with this hypothesis.

It is significant that Earth, the only planet in our solar system to support life, is also unique in having a magnetic field, which envelopes the planet like angel wings to protect it against the fierce solar winds.⁸ Perhaps the magnetic field is also needed for producing the NMR of natural molecules that may be crucial for the evolution of life. After all, molecules need to intercommunicate via EM signals for the right chemistry to happen. If they resonate to the same EM frequencies they can find each other for vital reactions or co-assembly into primitive protoplasts...

QED and Homeopathy

What Montagnier's team claim to have discovered is this: the information in DNA, detectable as EM signals imprinted in water in highly dilute solutions, can be transferred to pure water that has never been exposed to the DNA, and furthermore, this information can instruct the recreation of the DNA.

As described earlier, it is quite plausible that DNA can emit EM signals due to natural nuclear magnetic resonance. But how it imprints water, how that information is stored, transferred to pure water, and then used to instruct the recreation of the DNA sequence are not at all clear. That's where QED comes in.

The coherent domains (CDs) of water, as we have seen in Chapter 6, trap EMFs inside and produce coherent oscillations. So it is not unlikely that the CDs are imprinted by solutes like DNA that are emitting EMFs. In fact, the CDs store externally supplied energy in the form of "coherent vortices [that] are long lasting because of coherence, so that a permanent inflow of energy produces a pile up of vortices; they sum up to give rise to a unique vortex whose energy is the sum of the partial energies of the excitations which

have been summed up".⁹ That is how EM signals from DNA dissolved in water can be stored in the water, constituting the memory of water. I have suggested that contaminants such as silica may also be responsible for the memory of water observed by Montagnier's team;¹⁰ its role is to stabilize coherent domains (and specific excitations) as interfacial water.

In the same way, the EM signals now radiated from the imprinted water can be transferred to the CDs of pure water that had never been exposed to the DNA. Del Giudice and colleagues further suggest that the surface of the CDs in water may be where such instructed synthesis occurs.

The CDs oscillate at a frequency common to the EMFs trapped in it and the water molecules in the CD, and this common frequency changes when further energy is stored in the CD. The water CD effectively traps energy and exports it. When the oscillation frequency of the CD matches the oscillation frequency of some non-aqueous solute molecules present on the CD boundaries, these guest molecules become members of the CD and are able to access the entire stored energy, which becomes activation energy for the guest molecules to engage in chemical reactions. Proteins and nucleic acids that share common functions or reactions do share a common EM frequency, as do enzymes and substrates.¹¹ For molecules to find each other in the cell is like you trying to find your dance partner in the dark across a very crowded room: you simply have to call to each other.

Albert Szent-Györgyi proposed more than 50 years ago that water surrounding biomolecules is the source of the excitation energy responsible for chemical reactions.¹² So, if the ensemble of frequencies in the CD is able to attract the component monomer building blocks (nucleotides) of a polymer (DNA), the polymer will be created from the monomers attracted to the CD. Thus, it is possible to induce the polymerization of monomers by supplying the monomers when the EMFs have the relevant frequencies (the EM information).

The role played by the background of low-frequency EMF is to provide a resonant alternating magnetic field in order to load

energy into the water CDs.¹³ In higher organisms, such as humans, the researchers suggest, the resonant AC magnetic field is produced by the nervous system. Organisms such as bacteria may use the naturally occurring Schumann resonances of the geomagnetic field. However, it is entirely possible that even higher organisms are sensitive to the Schumann resonances. These are the stationary modes (standing waves) produced in the cavity bounded by the surface of the Earth and the conductive ionosphere, which should have a frequency $\nu_s(n)$:

$$\nu_s(n) = \frac{c}{2\pi R} \sqrt{n(n+1)}, \quad (8.1)$$

where R is the radius of the Earth. The real Earth-ionosphere cavity is not an ideal one, so the real frequencies are a bit lower than the values given by the equation. The peaks experimentally found are 7.83, 14.3, 20.8, 27.3, and 33.8 Hz.

Consequently, in order to produce the energy loading of CDs, the biological system should select ions having a q/m ratio that, given the local magnitude of the static magnetic field B in the organism, fits in with one of the Schumann resonances. The local value of B is expected to be not much different from the Earth's magnetic field of $\sim 50 \mu\text{T}$.

The Schumann mode of 7.83 Hz appears resonant for the DNA signal.

Quantum Biology and Consciousness Arriving

The explanation is incomplete in many respects; what has been proposed is a bare outline of possibilities. We do not know how DNA molecules (and counter-ions, which must be present, as DNA is a polyelectrolyte with many charged groups) interact with the water CD. Studies on DNA and protein hydration reveal dynamic coherence between hydration water and macromolecules (see Chapters 10 and 11). However, it is far from clear whether studies on macromolecules in solution can tell us how they behave inside the living cell (see Chapter 18).

Nevertheless, the QED theory of water provides a useful framework for further investigations that decisively move biology away from classical towards quantum physics and the quantum coherence of organisms, which will also provide a much better understanding of non-thermal EMF effects as well as homeopathy.

When I first proposed that the organism is quantum coherent in the early 1990s, only a handful of exceptional scientists thought quantum theory had anything to do with biology at all. The situation has greatly changed since then. Google ran a workshop on quantum biology at the end of 2010,¹⁴ where various scientists spoke about quantum coherence at the micrometre scale for photosynthesis, and “collapse of the quantum wave function” as the basis of consciousness in microtubules of brain cells, and quantum computing in the brain. But they have yet to catch up with the quantum coherent organism, *brain and body together*, or mind and body undivided.

Quantum coherence is the prerequisite for conscious experience, as I argued in an important paper published in 1997.¹⁵ It is the “I” in every one of us, despite the fact that we are made of 100 trillion cells, each with 100 trillion atoms, the vast majority of which are in water. There is now good evidence that water is quantum coherent under ambient conditions. It should not surprise anyone, therefore, that cells and organisms are quantum coherent.

I shall flesh out the picture by degrees, starting with how liquid water dances with ions and macromolecules, individually and in concert, at interfaces outside and inside the cell. I shall show how water, ions, and macromolecules animate and energize cells and organisms, regulates and coordinates the diverse wet chemistry that fills life with feelings, passions, and excitement, which elude any electronic artificial life.¹⁶

Most of all, the quantum coherent organism is a macroscopic quantum being, with a unique, evolving wave function spread ultimately throughout the entire universe, entangling the wave functions, or consciousness, of all other quantum beings. Perhaps we are already in touch with extraterrestrial life without our being overtly conscious of it; we are almost certainly in touch with all life

on Earth, from bacteria to whales. The scope for quantum jazz is the entire universe of entangled organisms, all dancing one another into being...

Notes

1. Fisher (2011).
2. Chaplin (2007).
3. Montagnier *et al.* (2009a,b); Ho (2010f,g).
4. Ho (2010g).
5. Montagnier *et al.* (2011).
6. See Ho (2010a).
7. See Ho (2007a).
8. See Ho (2011m).
9. Montagnier *et al.* (2011).
10. Ho (2010f).
11. Ho (2007a).
12. Szent-Györgyi (1957).
13. Montagnier *et al.* (2011).
14. Google Workshop on Quantum Biology, 22 October 2010, <http://sitescontent.google.com/google-workshop-on-quantum-biology/>.
15. Ho (1997).
16. Ho (2002).

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9

Dancing with Ions

Love of Water

Water is a very good solvent for many substances that are hydrophilic (water-loving), such as acids, alcohols, and salts, as opposed to those that do not dissolve in it, like fats and oils, which are hydrophobic (water-fearing). Hydrophilic and hydrophobic interactions are very important for the self-organization of living systems. Thus, lipids that are *amphiphilic*, i.e., water-loving at one end and fat-loving at the other, spontaneously form the bilayer membranes that enclose the cell as well as many organelles within the cell, with the fat-loving ends inside the bilayer and the water-loving ends facing into the water on either side of the membrane.

Water is a good solvent because it is a dipole with separated positive and negative charges in the same molecule (see Chapter 2). When an *ionic* substance (one that dissociates into positive and negative charged species or *ions*) or another polar substance enters water, the molecule becomes hydrated, i.e., surrounded by water molecules. The positive end of the dipole, or positively charged ions, is attracted to the negative end of the water molecule, and *vice versa*, the negative end of the dipole, or negatively charged ions, is attracted to the positive end of the water molecule.

Acid and Base

Chemically, water is also *amphoteric*, which means it can act as either an *acid* or a *base* in chemical reactions.¹ An acid is a substance that

donates a proton (H^+ , a hydrogen ion) in a reaction, and a base is one that accepts a proton. For example, when hydrochloric acid (HCl) dissolves in water, water acts as a base and accepts a proton from HCl to form a positively charged hydronium ion (hydrated proton) and a negatively charged chloride ion (which also gets hydrated):



When ammonia gas (NH_3) dissolves in water, however, water acts as an acid and donates a proton to it to form a positively charged ammonium ion and a negatively charged hydroxyl ion:



Pure water itself also ionizes to a very small extent to give a positive hydronium ion and a negative hydroxyl ion:



The concentration of hydrogen ions, H^+ , is very important to chemistry inside and outside the body. It is expressed in terms of pH, the negative logarithm to the base 10 of the molar concentration of the hydronium ion, H_3O^+ (a proton associated with a water molecule), and is a measure of the acidity or basicity of an aqueous solution. Pure water is said to be neutral with a pH close to 7.0 at 25 °C, which represents 10^{-7} M of H^+ or H_3O^+ .

The strength of an acid is measured by its acid dissociation constant, K_a , which characterizes the ionization of the acid into positively charged proton and a negatively charged species in terms of the product of the molar equilibrium concentrations of the dissociated ions relative to the concentration of unionized acid.² Thus, in the ionization reaction



$$K_a = [H^+] [A^-] / [HA] \quad (9.5)$$

The values of K_a span an enormous range over many orders of magnitude, as in the case of H^+ concentration, so the same convention is used, and the values are expressed as pK_a , which is the negative logarithm to the base 10 of K_a . Thus, strong acids will have a

very low value of pK_a , 1 to 3, while weak acids have intermediate ones of 4 to 6, and bases will have pK_a values higher than 7.

The values are very important for predicting how chemical groups react under different pH conditions. Thus, at low pH, only strong acids will ionize, while at high pH, most of the acid groups will be ionized.

The Importance of Ions

Ions are atoms or groups of atoms with an electric charge, by losing or gaining one or more electrons. Cations are positively charged, having lost electron(s), while anions are negatively charged, having gained electron(s). Because water is such a good solvent, ions are ubiquitous in water both inside and outside the cell and the organism, in streams, rivers, lakes, and seas that support all manner of life.

Within the body, the major ions in the intracellular and extracellular fluids, including the blood plasma, are also known as electrolytes, and a balance of electrolytes in the extracellular and intracellular fluids is crucial for life.³ Nine electrolytes are essential in relatively large quantities (grams to tens of milligrams a day required), of which five are cations: Na^+ (sodium), K^+ (potassium), Ca^{2+} (calcium), Mg^{2+} (magnesium), and H^+ or H_3O^+ ; and four are anions: Cl^- , HCO_3^- (bicarbonate), PO_4^{3-} (phosphate), and OH^- .

In addition, many other minerals are required in smaller or trace quantities: iron, manganese, zinc, copper, chromium, molybdenum, selenium, sulphur, and iodine. They serve as cofactors of enzymes or transport proteins needed for growth and development and other vital functions, and in the general maintenance of health.⁴

All electrolytes and essential minerals are normally acquired in a healthy, balanced diet of fresh vegetables, meat, fish, and fruits; and in addition, adequate intake of water, without which the ions cannot work their magic. So let's see how ions interact with water.

To Bond or Not to Bond

The interaction of water with ions has been investigated for over a century, but much remains unclear. In general, ions are classified into two groups — *kosmotropes* and *chaotropes* — according to whether they induce order or disorder in water.⁵

Thus, Li^+ (lithium ion) dissolving in water results in a large negative entropy change (entropy being a measure of disorder, so negative entropy indicates an increase in order), as typical of a kosmotrope, while Cs^+ (caesium ion) dissolving is accompanied by a positive entropy change, as appropriate for a chaotrope. Another measure is viscosity (the resistance to being deformed or reluctance to flow), which increases with lithium and decreases with caesium.

Both types of ions are generally surrounded by water molecules forming a solvation shell or hydration shell that shields their charges from other ions, and water is very good at doing that, with its high dielectric constant (see Chapter 2). The strength with which the ions bind to their solvation shell is expressed in terms of activation energy, the energy needed to strip a water molecule away from the ion, relative to that needed to strip a water molecule from another water molecule. Kosmotropes are strongly bound to their neighbouring water, and therefore have large positive activation energies, as distinct from chaotropes that are more weakly bound.

In general, the solvation shell consists of a single layer of water molecules hydrogen-bonded to the ion. A strong kosmotrope can have up to five or six water molecules in its solvation shell. A strong chaotrope, on the other hand, can bind a single water molecule or none at all, as it approaches the limit of a nonpolar solute, where the surrounding water molecules only form hydrogen bonds with one another, enclosing the solute in a cage-like clathrate structure (see Chapter 3).⁶

The Jumping Bond

How rigid is the solvation shell around the solute? Does the hydrogen bond — which breaks and reforms within a picosecond (10^{-12} s) — readily change partners or reorientate itself?

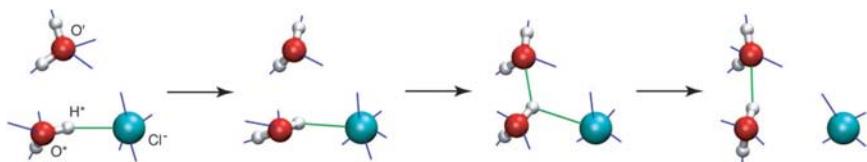


Figure 9.1 A water molecule switching partners in hydrogen-bonding between the ion and another water molecule, redrawn after Laage and Hynes.⁷

Molecular dynamics simulations carried out on NaCl solutions by researchers at l’École Normale Supérieure and the University of Colorado at Boulder showed that the hydration shell around chloride ions is labile, consistent with previous assessments of chloride as a weak chaotrope.⁷ A molecule in the first solvation shell can switch partners by a large reorientation — an angular jump of some 60 to 70° — after which the water molecule involved leaves the anion’s solvation shell and moves to the second shell (Fig. 9.1).

This will no doubt contribute to the challenging study of water dynamics in the hydration of biological molecules such as proteins and nucleic acids, where both experiments and simulations have already pointed to a dynamic regime quite different from bulk water. In particular, it suggests that the first hydration shell is very labile, exchanging rapidly with the second shell, and not rigid as previously thought.

Researchers led by K.J. Gaffney at Stanford University and Stockholm University decided to put the simulation result to experimental test.⁸ They devised a variant of two-dimensional infrared spectroscopy to investigate this jump in orientation that occurs on switching partners in hydrogen-bonding. The results confirmed that a water molecule can shift its donated hydrogen bond between water and perchlorate acceptors by large angular jumps of $49 \pm 4^\circ$ on average.

Actually, changing partners involves the water molecule doing a swivel dance in two steps: a really fast swivel of 40° in about 50 fs, followed by a slower rotation of 27° in about 1 ps.

Dissolving sodium chlorate (NaClO_4) in water mixed with heavy isotope tracer deuterium (D_2O) makes it possible to distinguish two kinds of O–D bonds by the way they stretch when excited by

infrared light. The OD group acting as donor to another water molecule (OD_w) absorbs at 2 534 cm⁻¹, whereas the OD group donating to a perchlorate anion (OD_p) absorbs at 2 633 cm⁻¹. Thus, one can track hydrogen-bond exchange in two-dimensional infrared spectroscopy. In addition, the orientation jump angle can be measured using polarized light probes that make the signal from the exchanged hydrogen bond highly dependent on the polarization direction. That was how the researchers managed to catch this exotic dance step in water's quantum jazz.

Dancing with Multiple Partners

What happens when water is dancing with two oppositely charged ions, as is most likely the case in real life? It depends on whether the ion pairs are both kosmotropes that bind strongly to water.

Researchers at the Institute for Atomic and Molecular Physics in Amsterdam used a combination of terahertz dielectric spectroscopy and femtosecond (ultrafast) infrared (fs-IR) spectroscopy to study water dynamics around different ions: magnesium, lithium, sodium, and caesium cations, as well as sulphate, chloride, iodide, and perchlorate anions.⁹ They found that the effect of ions and counter-ions (ions of the opposite charge) on water can be strongly interdependent and non-additive, and may extend well beyond the first solvation shell, especially when both ions are strong kosmotropes; then many more water molecules are bound than the sum of the two kosmotrope ions individually. This is because the water molecules are pinned by both the dipole interactions and hydrogen bond interactions (recall that water is both a permanent dipole with separated positive and negative charges, and also has the capacity to form hydrogen bonds, as described in Chapter 2).

Terahertz dielectric spectroscopy involves characterizing the propagation of terahertz (10^{12} Hz) pulses, each lasting \sim 1 ps, through the salt solution. The pulses are delayed by refraction (bending of light or electromagnetic waves) and diminished by absorption. In water, a marked frequency dependence of refraction and absorption arises when the dipoles of water molecules fail

to keep up by reorienting with the externally applied oscillating field. This leads to a large absorption peak at GHz (10^9 Hz) and a smaller one around 0.6 THz for pure water at room temperature. These peaks are attributed respectively to the collective reorientation of water molecules (relaxation time, or time for the disturbance to subside, $\tau_D \sim 8$ ps), and the reorientation of partly hydrogen-bonded individual water molecules (relaxation time $\tau_2 \sim 250$ fs). As a result of interaction with solvated ions, the reorientation of water molecules around ions slows down, shifting the absorption peak to lower frequencies and reducing the absorption at THz frequencies. This is referred to as depolarization. The hydration number N_p is the number of moles of slow water dipole per mole of dissolved salt and is directly proportional to the slopes of the lines plotting depolarization on terahertz dielectric spectroscopy against concentration.

Fs-IR spectroscopy probes the reorientation dynamics of individual water molecules through the O-H bond stretch, with high time resolution. In these experiments, water is doped with 4% of D₂O. The O-D bond stretching vibration is excited, and molecules with their OD group preferentially aligned along the polarization axis of the excitation pulse are most efficiently tagged. By using a second laser probe pulse to interrogate the number of tagged molecules parallel and perpendicular to the excitation axis, the rotation of tagged molecules can be tracked, and the amount of slowed water and hence the number of water molecules bound to ions worked out.

The researchers found an apparent discrepancy between the dielectric measurements and the fs-IR measurements. For hydrated cations, the two methods give different results: Mg²⁺ and Li⁺ show large immobilized fractions when measured with dielectric spectroscopy but the fs-IR measurements of Mg(ClO₄)₂ and LiI show almost complete reorientation, with a negligible slow fraction.

The differences between the results of dielectric relaxation and fs-IR spectroscopies can be understood by noting the different vectors that the two techniques probe: the permanent dipole moment of water p in the case of dielectric relaxation, and the O-D stretch

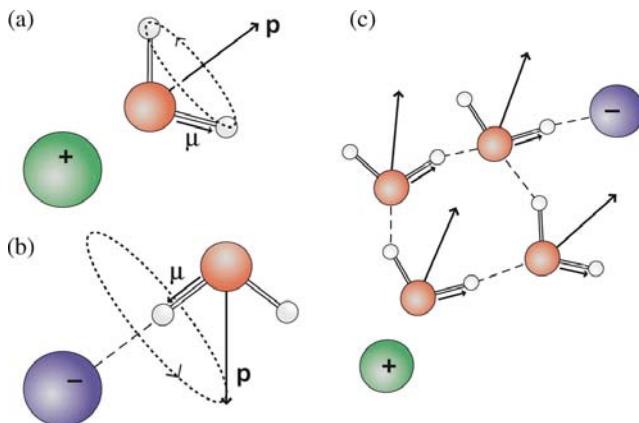


Figure 9.2 Cooperative dynamics of water induced by ions, redrawn after Tielrooij *et al.*⁹

transition dipole moment μ due to the hydrogen bond in the case of fs-IR spectroscopy (Fig. 9.2).

The local electric field around the ions causes the dipole vector p of water molecules in the solvation shell of a cation to point away from the cation (Fig. 9.2a), whereas for an anion, one of the OH groups of a hydrogen-bonded water molecule points linearly towards the anion (Fig. 9.2b). For cations, the rotational motion of water molecules that changes the dipole vector μ in the solvation does not lead to reorientation of the vector p . In the case of anions, the reverse effect occurs; the motion of p is unrestricted within a cone with fixed axis μ . That explains the insensitivity of dielectric spectroscopy to anionic hydration, and of fs-IR spectroscopy to cationic hydration. However, when the paired ions are both kosmotropes, the ions are essentially pinned by both hydrogen-bond and permanent dipole interactions to many more molecules of water (Fig. 9.2c).

These findings have large implications for water dynamics within the living cell that contains an enormous variety of molecules and ions all participating in water's quantum jazz. They also warn against basing explanations on a single method of measurement. Different methods probe distinctive, different interactions, which

occur over an enormous range of time scales. We shall need to look at many more things before venturing into the cell. The next stop is to see how macromolecules dance with water.

Notes

1. “Properties of Water”, Wikipedia, 11 September 2011, http://en.wikipedia.org/wiki/Properties_of_water#Water_as_a_solvent.
2. “Acid Dissociation Constant”, Wikipedia, 1 September 2011, http://en.wikipedia.org/wiki/Acid_dissociation_constant.
3. Rayat (2008).
4. See “Minerals and Their Function”, AltMedAngel, <http://altmedangel.com/mineral.htm>.
5. This chapter is modified from a previously published article with the same title, Ho (2010c).
6. See Ho (2010b).
7. Laage and Hynes (2007).
8. Ji *et al.* (2010).
9. Tielrooij *et al.* (2010).

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10

Dancing with Proteins

The Importance of Proteins

Proteins make up 17% of the body mass of an ideal lean man weighing 70 kg¹ and are responsible for practically all his vital functions. Proteins are large molecules up to a few million Da (dalton, a unit of atomic weight approximately equal to that of one hydrogen atom), consisting of one or more polypeptides. A polypeptide is a chain-like molecule made of units called *amino acids* joined end to end in peptide bonds.

Amino acids, or α -amino acids (in which the amino group $-\text{NH}_2$ is joined to the carbon atom of the carboxyl acid group ($-\text{COOH}$)), have the general chemical formula RNH_2COOH , where R represents 20 different substitutions which life has selected for making natural proteins. The peptide bond is formed between the $-\text{NH}_2$ group of one amino acid and the $-\text{COOH}$ of another by eliminating a water molecule, which makes it a dehydration reaction:



The structure of amino acids and the formation of a peptide bond are illustrated in Fig. 10.1.

The amino acid is a zwitterion, in that it can become positively charged through the $-\text{NH}_2$ group acquiring a proton, or negatively charged through the $-\text{COOH}$ group losing a proton, depending on the pH of the medium. This property extends to the polypeptides

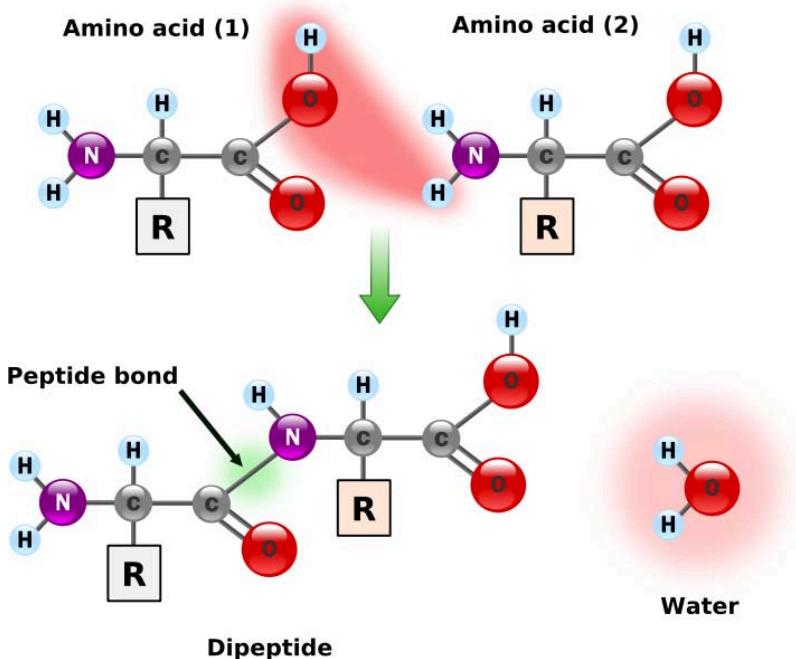


Figure 10.1 Formation of a peptide bond from two amino acids with the elimination of a water molecule, by Yassine Mrabet, Wikimedia.²

and the proteins themselves. Proteins differ from one another in the amino acid sequence of its polypeptide chain(s). The sequence of the 20 amino acids commonly found in natural proteins is specified by the genetic code consisting of triplets of four bases in the base sequence of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), which are the other classes of important polymeric macromolecules in cells and organisms that we shall look at in the next chapter.

The 20 amino acids fall into several groups according to their side chain R. For example, some have electrically charged side chains: aspartic acid ($R = \text{CH}_2-\text{COO}^-$) and glutamic acid ($R = \text{CH}_2-\text{CH}_2-\text{COO}^-$) are negatively charged; arginine ($R = (\text{CH}_2)_3-\text{NH}-\text{(NH}_2)^+$) and lysine ($R = (\text{CH}_2)_4-\text{NH}_3^+$) are positively charged. These charged amino acids will interact with ions and water. Some amino acids have polar side

chains — such as serine ($R = \text{CH}_2\text{--OH}$), threonine ($R = \text{CH}(\text{OH})\text{--CH}_3$), asparagine ($R = \text{CH}_2\text{CONH}_2$), and glutamine ($R = \text{CH}_2\text{--CH}_2\text{--CONH}_2$) — which will also interact with water. Amino acids with hydrophobic side chains, some of them aromatic (ring structures), are not expected to interact with water, and are usually considered to be buried inside the folded globular protein (see later). But it has become clear that water can end up in very hydrophobic confined spaces, as we shall see in a later chapter, and can form cages around nonpolar groups.

The amino acid sequence of a polypeptide is its primary structure. There is often a secondary structure stabilized by hydrogen bonds between peptide bonds along the backbone of the polypeptide chain. The most common are the α -helix and β -pleated sheets (see Fig. 10.2). In addition, polypeptides may have tertiary folded globular structures and quaternary structures in which different folded polypeptides aggregate together to form a complex. Proteins are responsible for practically all living functions, especially in their role as enzymes, and molecular motors.

The Importance of Enzymes

Organisms have an enormous repertoire of chemical reactions that enable them to transform energy and materials for growth, development, and to do all that's required for being alive. Perhaps most remarkably, these chemical reactions are catalysed by specific enzyme proteins that accelerate the reaction rates by a factor of 10^{10} to 10^{23} . But the question of how enzymes work remains unanswered to this day,⁴ the key role of water still having eluded most enzymologists.

It is well known that enzymes and other macromolecules, DNA and RNA, need a minimum amount of water in order to work at all, and much more to work efficiently. It is not accidental that cells are loaded with water, some 70% by weight. In terms of number of molecules, water far outnumbers all other chemical species — ions, small organic molecules, and macromolecules — added together.

In the first instance, water is needed for macromolecules to become flexible, so they can dance freely to water's quantum jazz.

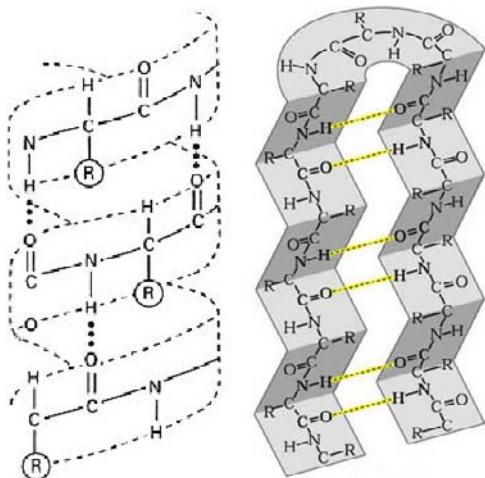


Figure 10.2 Diagram of the α -helix (left) and β -pleated sheet (right) stabilized by hydrogen bonds between CO and NH groups of the peptide bonds in the polypeptide backbone.³

But in order to understand how they accomplish their otherwise impossible task of making sluggish chemical reactions happen spontaneously and effortlessly, enzymologists need to look into the possibility that the necessary catalytic power in terms of specificity and activation energy may be provided by the quantum coherent liquid crystalline water at the interface (Chapters 6 to 8).

Many scientists are indeed coming around to the view that proteins act quantum mechanically, or, as I suggested some years ago in the first edition of *Rainbow Worm*, enzymes are the archetypal quantum molecular energy machines (see Chapter 1) that transform and transfer energy and material at close to 100% efficiency, and water plays an essential part in this remarkable feat.⁵ But we must backtrack to flesh out some details.

Supercool Hydration Water

Within the past 20 years, water has gained some recognition as an active constituent of cell biochemistry and not just an inert solvent.⁶ Water has a special relationship with proteins in the proteins' hydration shells.

The hydration shell can be defined as water associated with the protein at the hydration end point, when further addition of water produces no change in its essential properties; in the case of an enzyme, this would be its enzyme activity. This hydration shell is a single layer of water molecules covering the protein surface.⁷ Water outside the monolayer is perturbed to a significantly smaller extent, typically not detected by measurements of properties such as heat capacity, volume, or heat content (though it is increasingly recognized by newer measurement techniques, see later). The hydration shell is about 0.2 g/g dry protein. The activity of lysozyme, for example, closely parallels the development of surface motion as hydration increases, which is thus responsible for the function of the protein.

In contrast to bulk water, protein hydration water does not freeze at 0 °C, but can be supercooled right down to a glass transition at $T_g \sim 170$ K ($\equiv -103^\circ\text{C}$), and this is reflected in discontinuities in the specific heat and thermal expansion coefficient of the hydration water.⁸ Below that temperature, the hydration water freezes into a glass, a non-crystalline transparent solid that does not have a defined molecular order. Near T_g , the movement of water is arrested.

The protein, however, has its own dynamic transition, an abrupt onset of atomic motions that can be detected by neutron scattering, and occurs at $T_\Delta \sim 225 \pm 5$ K. This is a generic property of hydrated proteins, and is absent in dehydrated systems. It is therefore related to the dynamics of the hydration shell. Some researchers regard the protein dynamic transition as the “microscopic manifestation” of the glass transition of the hydration shell. Others, however, interpret it as a liquid-to-liquid transition, from a fluid, high-density liquid to a less fluid, low-density liquid, or supercooled water.

Similar behaviour was found for confined water in various biological and non-biological environments, and a range of 18 solutions including low-molecular-weight organic glass formers, polymers, sugars, as well as protein and DNA were probed with broadband dielectric spectroscopy (see Box 10.1) between 10^{-2} and 10^7 Hz.⁹ Universal features in the dynamics of water appeared both above and below the glass transition, which were similar to that of supercooled

Box 10.1

Dielectric Spectroscopy

A dielectric is a molecule such as water that is polarized — with separated positive and negative charges — or can be polarized by an applied electric field. Dielectric spectroscopy measures the dielectric response of a sample to an external electromagnetic field applied over a range of frequencies. The dielectric property of a material is expressed as a complex number (see Glossary for a definition of complex numbers) known as permittivity. The real part of permittivity measures the energy stored, while the imaginary part measures the energy loss and is called the dielectric loss. When the external field is applied, dipoles in the sample will orient with the applied field. At low frequencies, they can follow the polarization of the applied field perfectly, resulting in maximum permittivity and minimum dielectric loss. As the frequency increases, the molecules can no longer keep up with the changing electric field, which results in less energy stored and higher losses. At even higher frequencies, the molecules no longer respond to the applied field, and the molecules come apart. For example, water molecules are stretched at frequencies above 10^{11} Hz, and beyond that, they break apart.

The dielectric relaxation time is a measure of the time it takes for separated charges in a dielectric to become neutralized by the conduction process.

water and supercooled confined water respectively. However, the glass transition temperature spans a rather broad range from 165 to 220 K. We shall look at water confined in nanospace in greater detail in Chapter 19.

More Hydration Shells Revealed

Within the past several years, a new terahertz (10^{12} Hz) spectroscopy has created a stir in the protein hydration community. Martina Havenith and colleagues at Ruhr University Bochum have been using this new “tabletop” technology to reveal global properties of

proteins and their hydration shells,¹⁰ prompting a number of water scientists to rethink protein hydration.

Many sophisticated techniques have been used to probe the dynamics of proteins and hydration water over a wide range of time and space. For example, NMR relaxation spectroscopy resolves water dynamics from nanoseconds to seconds whereas X-ray crystallography reveals fixed protein structures and bound water molecules in the protein interior and vicinity. Both techniques provide information on the short-range hydration up to 3 Å (angstrom, 10^{-10} m) from the protein surface, which corresponds to one hydration layer. Traditional dielectric spectroscopy probes dynamics from 100 s down to 100 ps. Terahertz dielectric spectroscopy extends the time scale down to the ps range. Neutron scattering resolves ps dynamics. On the fastest time scales, two-dimensional infrared spectroscopy probes processes that take place in femtoseconds. And the remarkable picture emerging is that protein and hydration dynamics may be correlated over all time scales, as indeed they would be in quantum coherent systems (see Rainbow Worm).

Terahertz absorption spectroscopy opens up a new window between microwaves and infrared to peer into the global interactions of water with proteins that are fully dissolved (in excess water).

The absorption of the solvated protein increases linearly with frequency in a narrow frequency range of 2.25 to 2.55 THz. Concentrating on measurements at a single frequency, the researchers found that the absorption coefficient of the solution relative to bulk water increases with protein concentration before dropping and decreasing almost linearly at higher concentrations. The concentration dependence of the THz absorption gives similar curves at three different temperatures: 15, 20, and 22°C.

At 0.5 to 1 mM protein concentration, when the absorption coefficient peaks and begins to fall again, the volume of water displaced by the protein molecules is negligible at around 1%. Consequently, the hydration water around the protein must be contributing substantially to the total THz absorption.

The researchers resorted to molecular dynamics simulations to compute the absorbance of the protein and the first hydration

layer as a function of the distance between protein surfaces, which would depend on the protein concentration. In agreement with experiment observations at concentrations beyond the peak of absorbance, the distance between the proteins significantly influences the absorbance of the protein and its first hydration layer and layers beyond. The absorbance decreases as the proteins are brought closer together from 24 to 18 Å, then flattens for the shorter distances, changing little with distance between proteins.

Further molecular dynamics simulations revealed that the hydrogen bonds between water molecules in hydration layers up to about 10 Å survive longer than those in bulk water. The experimental data indicated an average separation of >20 Å when absorbance peaked, spanning some seven to eight layers of water.

The initial findings were made in a genetically engineered protein λ^* ₆₋₈₅, but similar results were obtained with natural lysozyme and myoglobin. At 23 g water per g protein of lysozyme, absorbance surged upwards with increased structural flexibility of the protein. Myoglobin, similarly, exhibited a maximum at 98% by weight of water (24 g/g).

While bulk water and its absorption decrease with increasing protein concentration, this is more than offset by the absorption of hydration layers and the hydrated protein, which becomes a dynamically coherent unit.

Ferroelectric Hydration Water

The unexpected findings in terahertz dielectric absorption spectroscopy have stimulated researchers to examine the electrostatic properties of the hydration water. According to David LeBard and Dmitry Matyushov at Arizona State University, those observations require a very large “effective dipole moment of the protein and its hydration shell, much exceeding the dipole moment of the protein itself”.¹¹

LeBard and Matyushov showed by means of numerical simulations that protein hydration waters are polarized into a ferroelectric shell some three to five water molecules thick with a large average dipole moment. (A *ferroelectric* is a crystalline dielectric

with permanent electric polarization that varies in strength according to the applied electric field.) Moreover, the dipole moment of the hydration shell fluctuates with large amplitude, much bigger than those in bulk water, and far exceeding the magnitude prescribed by the usual linear response theory, as also demonstrated in real measurements. These large fluctuations are dominated by slow nanosecond dynamics probably associated with the collective conformational movements of the protein.

These findings are just what is expected from the dynamic liquid crystalline nature of living organisms discovered in my laboratory in 1992 implying that water associated with macromolecules is also polarized and moving coherently with the macromolecules (see *Rainbow Worm*). In Chapter 20, we shall see that measurements carried out in model systems closer to what the protein's environment would be like in the cell corroborate these new results.

Is there any evidence that the protein-hydration system is quantum coherent?

Quantum Dance of Proteins

The possibility of quantum tunnelling has been invoked in enzyme catalysis. Quantum tunnelling is a quantum mechanical effect in which particles go under an energy barrier to achieve a chemical reaction that's impossible in classical chemistry. Quantum tunnelling is already well accepted in electron transfer and frequently observed for proton transfer.

Recent computational and experimental studies have clearly shown that the flexibility of the proteins induced by water is important in reducing the free energy barrier between reactants and products. The flexing of proteins increases the probability of quantum tunnelling between reactants and products by a transient compression of the energy barrier. (Obviously, in the case of proton or electron transfer, quantum tunnelling will only take place if the actions of donor and acceptor are also correlated or coherent, so coherence must extend to the ensemble of donor and acceptor proteins.) The same studies indicate that the dynamics of proteins

is closely tied to that of water. Some scientists would go as far as to say it is mainly the mobility of water that determines the magnitude of protein fluctuations, not only at the protein surface, but also in the protein core. But then the collective conformational movements of proteins would feedback to influence the hydration water coherently (see earlier).

One sign of the quantum effect is the difference in behaviour observed when hydrogen (H) is substituted with its heavy isotope deuterium (D), as already clearly indicated for water itself (see Chapters 4 and 5).

Fabio Bruni and colleagues at Roma Tre University used dielectric spectroscopy to compare the dielectric relaxation of lysozyme in ordinary water (H_2O) and in heavy water (D_2O) in the frequency range of 10^{-2} to 10^7 Hz, and over the temperature range of 210 to 330 K.¹²

If the effect were classical, there should be no difference between the samples in H_2O and D_2O . However, large differences were found. In particular, the dielectric relaxation times of H_2O were completely different from those measured with D_2O over the entire temperature range (Fig. 10.3).

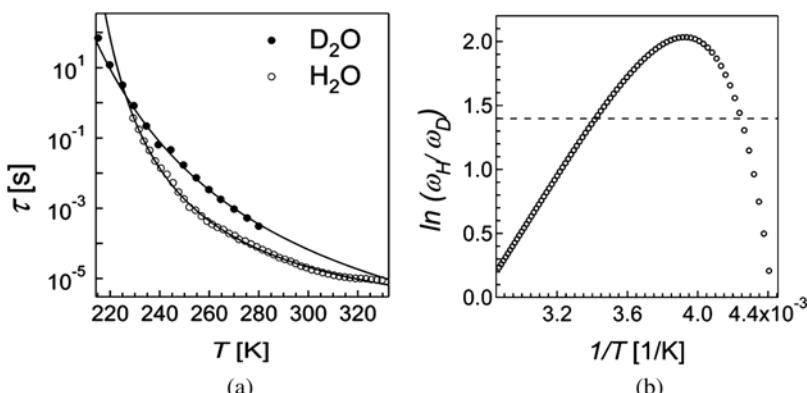


Figure 10.3 Dielectric relaxation time τ vs temperature T (a); natural logarithm of the reciprocal of relaxation times ratio τ_D/τ_H ($\equiv \omega_H/\omega_D$) vs reciprocal of temperature $1/T$ (b); the horizontal dotted line marks the value 1.4, which should hold for all temperatures if there were no quantum effects.¹²

These observations were in close agreement with the results of a sophisticated theoretical study using quantum mechanical methods from first principles.

In addition, the researchers reanalysed data from a deep inelastic neutron scattering experiment, which measured the momentum distribution of protons, $n(p)$, and the mean kinetic energy for a lysozyme sample prepared the same way as for the dielectric spectroscopy experiment. Measurements were made both above and below the protein's dynamic transition temperature (see earlier) at 290 K and 180 K respectively. They computed the minimum fraction of protons required to produce the observed difference in the distribution of momentum between the two temperatures, assuming that the mean kinetic energies of the proton are independent of temperature except for the small kT (Boltzmann, background) contribution, and that the momentum distribution at 290 K arises from a population of protons with the same $n(p)$ as that at 180 K plus a contribution due to an unknown fraction of protons showing a characteristic quantum mechanical behaviour. This fraction of protons that showed quantum behaviour turned out to be 0.29, precisely the fraction of protons present in the sample that belonged to the hydration water.

Notes

1. “Body Composition, Fact Sheet 1”, Rowett Research Institute, http://www.rowett.ac.uk/edu_web/sec_pup/body_comp.pdf.
2. “Amino Acid”, Wikipedia, accessed 12 September 2011, http://en.wikipedia.org/wiki/Amino_acid.
3. “Alpha Helix”, accessed 13 September 2011, <http://withfriendship.com/user/crook/alpha-helix.php>; “ β -pleated Sheet”, accessed 1 December 2011, <http://www.bio.miami.edu/~cmallery/150/protein/sf3x10b2.jpg>.
4. Kraut *et al.* (2003).
5. Ball (2009).
6. Chen *et al.* (2010a).
7. Doster *et al.* (2010).

8. Cerveny *et al.* (2008).
9. Ebbinghaus *et al.* (2007).
10. Born and Harenith (2009).
11. LeBard and Matyushov (2010).
12. Pagnotta *et al.* (2009).

Dancing with DNA

Glorious DNA

The cell biology community can be forgiven for its excessive emphasis on DNA and the molecular basis of heredity in view of much contrary evidence indicating that genomic DNA can be marked and changed by environmental influences (see Chapter 1). A typical description goes as follows: “Deoxyribonucleic acid (DNA) ... contains the genetic instructions used in the development and functioning of all known living organisms (with the exception of RNA viruses). The DNA segments that carry this genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information. Along with RNA and proteins, DNA is one of the three major macromolecules that are essential for all known forms of life.”¹

In Chapter 8, we saw how DNA emits electromagnetic signals that can be imprinted in water, transmitted via radiation to pure water that has never been exposed to the DNA, which then instructs the recreation of the DNA. In the course of this chapter, we shall see that DNA may indeed have other fascinating functions than coding for the amino acid sequence in proteins, thanks to its hydration water.

Nucleic acids include both DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), long polymers of building blocks called nucleotides, and therefore also referred to as polynucleotides. A nucleotide consists of an organic base joined to a sugar molecule joined in turn to one or more phosphate groups. There are four nucleotides in DNA, differing only in one of four nitrogenous bases: *adenine*

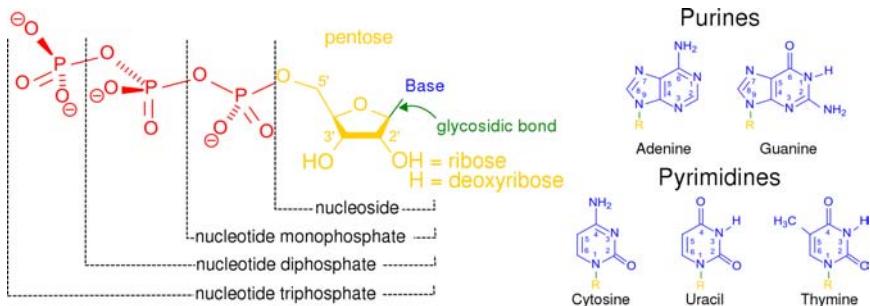


Figure 11.1 Chemical structures of a nucleotide and the five bases, by Boris, Wikimedia.²

and guanine are purines; cytosine and thymine are pyrimidines. In RNA, instead of thymine, the base uracil is present. It is the base sequence that gives individual DNA and RNA molecules their distinctive characteristics, not least in the segments that code for the amino acid sequence of proteins. The code is a triplet code: a sequence of three bases serves as a codon for 1 of 20 amino acids, plus a stop codon to tell the polypeptide chain where to end.² Four bases give $4^3 = 64$ possible triplets, so the code is redundant, and some amino acids have more than one codon. The chemical formula of a nucleotide and the four bases are shown in Fig. 11.1.

In the polynucleotide chain, alternating sugar and phosphate groups form the backbone with the bases sticking out more or less flat at 90° to the backbone. The crowning glory of DNA was when its double-helix structure and specific base-pairing between two DNA strands (Fig. 11.2) were discovered in 1953 by US biologist James D. Watson and English biophysicist Francis Crick (1916–2004), then at the Cavendish Laboratory of Cambridge University.³ The base-pairing between two strands of DNA gives a ready template mechanism for heredity. That is because the base sequence of one strand is complementary to the other: A (adenine) always pairs with T (thymine), and G (guanine) always with C (cytosine); the pairing being stabilized by hydrogen bonds. As can be seen from Fig. 11.2, the paired bases are stacked flat between the double helix, and this has important electronic consequences (see later).

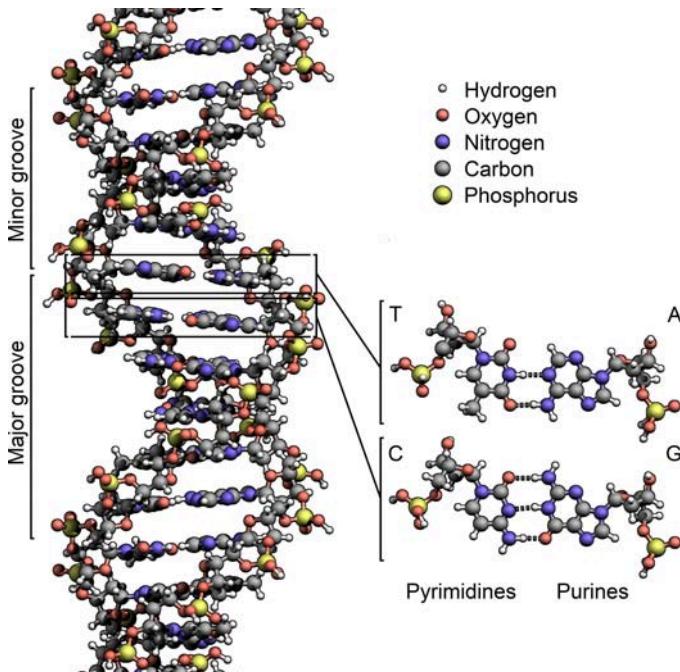


Figure 11.2 The DNA double helix, by Zephyris, Wikimedia.⁴

Wetting DNA

The story of DNA and water is just beginning. Hydrating shells of DNA share many of the properties of hydration shells of proteins. In addition, water turns DNA into an electrical conductor and gives it magnetic properties; and scientists are looking into exploiting synthetic DNA in new molecular electronic devices.

The hydration water of DNA appears to have quite unusual dynamics as measured by time-resolved Stokes shift (TRSS) spectroscopy and confirmed by molecular dynamics simulation. In the TRSS experiments on a 17-base-pair oligonucleotide, light excites the DNA and is re-emitted as fluorescence, shifted to lower energy compared to the light absorbed (Stokes shift). The mean fluorescence frequency is measured as a function of time after excitation, covering six orders of magnitude from 40 fs to 40 ns.

Most unexpectedly, the relaxation (decay of fluorescence) did not show distinctive time scales that could be easily attributed to multiple independent processes. Rather it fitted a single power law (see Glossary), suggesting a high degree of correlation over all time scales.

Researchers led by Mark Berg at the University of South Carolina used a polarization model based on the idea that the coupling between components in the system is due to polarization of one component by another, which not only fitted the experimental data well, but also enabled them to assign the TRSS response to different components of the system: the water, the counter-ions of DNA, or the DNA molecule itself.⁵ The results showed that water is the dominant contributor to the TRSS response at all times. Its relaxation spans the entire measured time range and accounts for the power law behaviour. Counter-ions have a secondary, but non-negligible contribution with a well-defined relaxation time of about 200 ps. The DNA relaxation time is near 30 ps, but the amplitude is very small.

Water Electrifies DNA

The ability of DNA to conduct electricity remained controversial for years, until the decisive role of water was revealed. Christophe Yamahata and colleagues at Tokyo University and Kagawa University convincingly demonstrated that the ability of DNA to transport electrical charge depends on water.⁶

DNA bundles were suspended in air between nano-tweezers (literally tweezers at the nanometre scale) (see Fig. 11.3) and the current passing through was measured as voltage was increased.

The electric current, and hence conductivity, was found to increase exponentially up to 10^6 -fold as the relative humidity (r.h.) of the air increased from 0 to 55% and the voltage increased from 0 to 10 V. The results obtained for 55% r.h. are shown in Fig. 11.4 for DNA bundles varying in thickness from 100 nm to 400 nm or more. The rise in conductivity was attributed to an increase in the concentration of charge carriers as DNA hydration went up with relative humidity, the charge carriers being H^+ and OH^- , suggesting

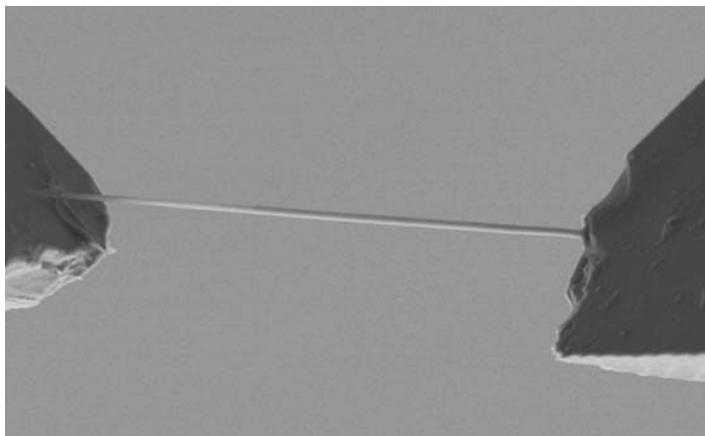


Figure 11.3 DNA suspended in air with nano-tweezers.⁶

that proton (positive electricity) jump conduction could be involved as well as electron conduction. (See Chapter 13 for proton conduction in water.)

Quantum Chemistry and DNA Conductance

It is not clear exactly how water electrifies DNA. But an important finding by other researchers is that the overlapping π molecular orbitals of the stacked base pairs in the core of the DNA double helix (see Fig. 11.2) can create a pathway for charge transfer (conduction).⁷ A molecular orbital is a waveform describing the distribution and energy of a pair of electrons, and is most commonly represented as a linear combination of atomic orbitals of atoms forming a covalent bond in the molecule. The molecular orbital of a π bond involves two lobes of the atomic orbitals overlapping and joining up. The halves of a molecule joined by a π bond cannot rotate about that bond without breaking it. Carbon and nitrogen compounds form π bonds when they engage in multiple bonding, as in C=C and N=N. In ring compounds such as benzene and bases in DNA, where double and single bonds alternate, the π bond electrons are *delocalized*, or spread over the entire ring structure, thereby stabilizing it.

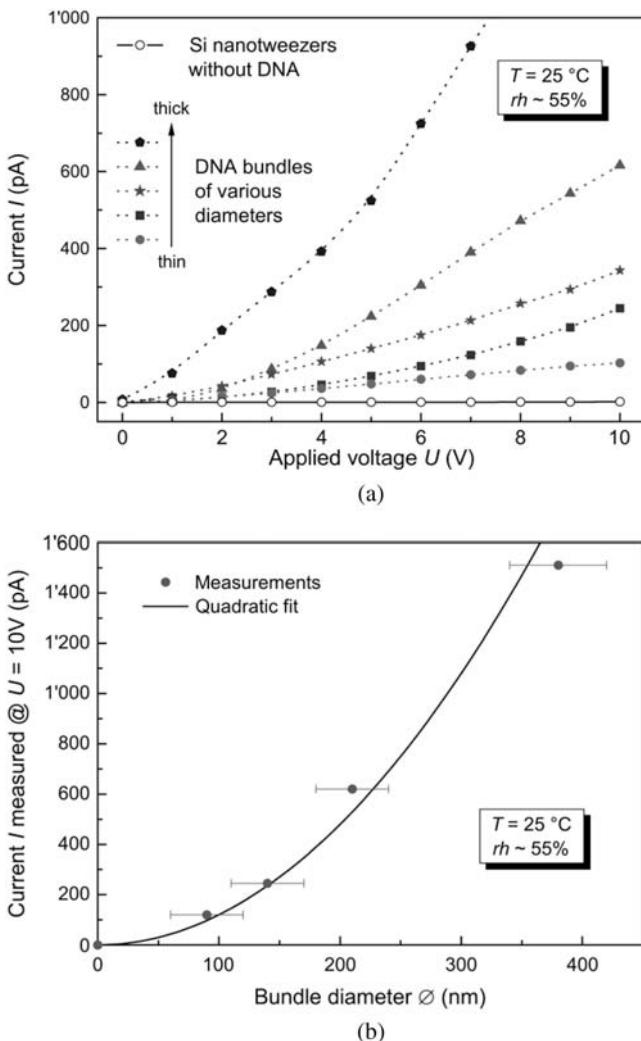


Figure 11.4 DNA conductivity measured as current increase with applied voltage (a), and with different thickness of DNA (b).⁶

Julia Berashevich and Tapash Chakraborty at the University of Manitoba have shown that the bases form hydrogen bonds with water molecules, which results in a significant change in the π bonds, reducing the energy barrier for a positive charge (hole) to tunnel between successive base pairs; and that a reason for an

increase in DNA conductivity with rising humidity is the occurrence of unbound π electrons.⁸ The unbound π electrons result in breaking π bonds, because of the redistribution of the electron density from the π bonds towards the nitrogen atoms engaged in hydrogen-bonding with water molecules. The increase in charge transfer between successive base pairs due to the contribution of such unbound electrons was up to 250 times, but still much less than found in the experiments (Fig. 11.4). Berashevich and Chakraborty suggested that was because the transformation of DNA structure had not been taken into account, specifically from A (dehydrated) to B (hydrated) DNA, B DNA being the form normally found in the cell. They decided to carry out quantum chemical molecular dynamics simulations to find out what happens when DNA is hydrated, including the transformation from A to B DNA.

First, they obtained the crystal structures of the hydrated AT and GC pairs (Fig. 11.5).

They then built up polymers $(AT)_n$ and $(GC)_n$, and the mixed sequence $(AT)(GC)$, optimized the geometries of the B and A forms of DNA, and analysed the interaction of the π molecular orbitals of

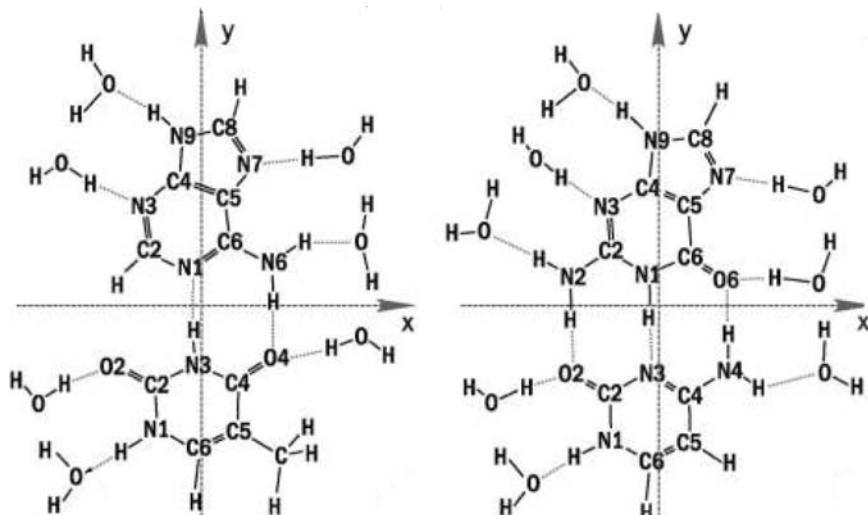


Figure 11.5 Water binding to DNA base pairs: (left) AT, (right) GC.⁷

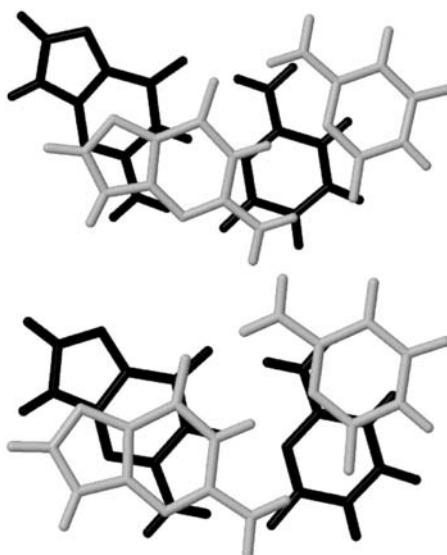


Figure 11.6 A DNA (top) changing to B DNA (bottom) on hydration leads to greater overlap of π orbitals in successive bases of the same strand, as well as increased electronic coupling between the base pairs $(GC)_2$.⁸

the stacked base pairs in the two forms of DNA. The results for $(GC)_2$ are presented in Fig. 11.6, showing a greater overlap in the π orbitals of the bases in the same strand as well as increased electronic coupling between the base pairs, resulting in increased charge conduction.

The results for $(AT)_2$ are somewhat different at low temperatures, but at room temperature, charge transfer with the participation of unbound electrons in hydrated B DNA is possible for all sequences up to 10^3 -fold that of A DNA.

Berashevich and Chakraborty have not taken into account hydration of the DNA surface around the backbone sugar, and in particular the phosphate groups, which could also make a significant contribution to electrical conductivity. It has been estimated that the shell of tightly bound water at the DNA surface is about two layers thick.⁹ Numerous studies on DNA hydration have shown that both bases and phosphate groups have significantly organized

hydration shells, the extent of hydration being larger around phosphates, but water being more organized around the bases.¹⁰

DNA Magnetism

Berashevich and Chakraborty also showed that although dry DNA is always diamagnetic, because it has no unbound electrons, wet DNA should be paramagnetic at low temperatures, as discovered by other researchers, and the strength of paramagnetism should be dependent on the DNA sequences.¹¹ (Paramagnetic material is magnetized in the direction of the external magnetic field, and proportional to the magnetic field. It depends on the material having unpaired electronic spins.)

In the case of wet DNA, only neighbouring unbound electrons with parallel spins are paramagnetic. At low temperatures, the efficiency of charge transfer is determined by the spin interaction of two unbound electrons located on neighbouring bases of the same strand. Exchange is allowed only when the electron spins are antiparallel (in opposite directions), that is, when the DNA is not paramagnetic. Hence the conductance of DNA can be controlled by a magnetic field. This gives the potential for developing nanoscale spintronic devices based on the DNA molecule, where the efficiency of the spin interaction will be determined by the DNA sequence.

Regardless of the practical applications, the findings have profound implications for the biological functions of DNA, apart from serving as a linear code for the sequence of amino acids in proteins. We are only touching the tip of a very large iceberg.

Notes

1. “DNA”, Wikipedia, 11 September 2011, <http://en.wikipedia.org/wiki/DNA>.
2. “Nucleotide”, Wikipedia, 1 September 2011, <http://en.wikipedia.org/wiki/Nucleotide>.
3. Watson and Crick’s discovery was based, at least in part, on the X-ray crystallography data of biophysicist Rosalind Franklin (1920–1958), then at King’s College London. Franklin’s crucial

X-ray diffraction picture was shown to Watson and Crick by her colleague Maurice Wilkins (1916–2004), without her knowledge or consent. Wilkins later shared a Nobel Prize with Watson and Crick in 1962 (see Note 1).

4. “DNA”, Wikipedia, 11 September 2011, <http://en.wikipedia.org/wiki/DNA>.
5. Sen *et al.* (2009).
6. Yamahata *et al.* (2008).
7. Chalikian *et al.* (1994a).
8. Berashevich and Chakraborty (2008).
9. Chalikian *et al.* (1994b).
10. Schneider *et al.* (1998).
11. Berashevich and Chakraborty (2008).

12

Water at Solid Interfaces

Life at the Interface

Life exists at the interface, or more precisely, in interfacial water. Organisms are crowded with convoluted surfaces inside cells and in the extracellular matrix, so much so that the 70% by weight of the water they contain is rarely more than a micrometre away from the surface of a membrane, a protein, or other macromolecules such as nucleic acids and polysaccharides.

Numerous studies over the past 50 years have documented that interfacial water has special properties different from those of water in the bulk, as we have already seen in Chapters 10 and 11, for the hydration of proteins and DNA. However, researchers cannot agree on how many layers of water molecules are altered. The majority believe that interfacial water is no more than one to at most a few layers deep. But many observations on water at solid interfaces challenge this assumption.

Interfacial Water in the Ideal

Research on water adjacent to solid surfaces has been going on for more than 100 years, and many reports indicate that this interfacial water is structurally altered, and over far greater distances, from 10 to 10 000 molecules thick.¹

Table 12.1 Properties of interfacial water on macromolecules and solid surfaces³

Property	Bulk water	Interfacial water
Density (g cm^{-3})	1.00	$0.96\text{--}0.97$
Specific heat (cal kg^{-1})	1.00	1.25
Thermal expansion coefficient ($^{\circ}\text{C}^{-1}$)	250.10^{-6}	$300\text{--}700.10^{-6}$
Adiabatic compressibility coefficient (atm^{-1})	7.10^{-17}	35.10^{-17}
Heat conductivity ($\text{cal sec}^{-1} ^{\circ}\text{C}^{-1} \text{cm}^{-1}$)	0.0014	0.010–0.050
Viscosity (cP)	0.89	2–10
Dielectric relaxation frequency (Hz)	19.10^9	2.10^9

Walter Drost-Hansen, now in Williamsburg, Virginia, USA, first suggested in the 1980s that macromolecules in aqueous solution are hydrated, and that this hydration water is identical to the hydration water of solid surfaces.² Table 12.1 lists some physical properties of interfacial water compared with bulk water.

As can be seen, interfacial water differs remarkably from bulk water. It is two to ten times more viscous, seven to thirty-five times as heat conductive, and five times as compressible. The almost ten-fold reduction in dielectric relaxation frequency indicates that the water is in a collective dielectric mode, as consistent with a high degree of order. And it is independent of the nature of the surface, indicating that the overriding influence on interfacial water comes down to the unique hydrogen-bonding system between water molecules, according to Drost-Hansen.

Although interfacial water next to different surfaces share many characteristics, they also differ in detail, as would be expected purely on the basis of the flexibility of H-bonds in water, and the strength with which different chemical groups interact with water molecules, as seen in earlier chapters of this book. In my opinion, the set of properties listed in Table 12.1 may well represent the ideal, unconstrained interfacial water where, for example, the perfect tetrahedral hydrogen-bonding is permitted. At the local level of confined nanospaces typical of the cell and extracellular matrices, the properties of interfacial water will deviate more or less from that unconstrained ideal (see Chapter 19).

Probing the Interface Directly

The introduction of nonlinear optical techniques has made it possible to probe the interfaces directly in an attempt to find out how water molecules structure themselves and interact with different surfaces. Two techniques, in particular, are *sum frequency* (SF) spectroscopy, which depends on two laser beams mixing at a surface and generating an output beam with a frequency equal to the sum of the two input frequencies, and *second harmonic* (SH) spectroscopy, a special case of SF spectroscopy in which the photons interacting at the surface have the same frequency and are combined to form new photons with twice the energy, and therefore twice the frequency and half the wavelength. These have enabled scientists to study not just interfacial water next to solid surfaces, but also air-water interfaces that we shall look at in later chapters.

In an SF vibrational spectroscopy (SFVS) experiment, the interface is probed via the water molecules' O–H bond stretching vibrations. An infrared and a visible laser pulse are mixed on the water surface and the sum frequency of the two laser beams generated selectively at the surface is detected. If the infrared light is resonant with the OH stretching vibration of the surface water, this process is resonantly enhanced, and the vibrational spectrum is obtained in terms of the nonlinear electric susceptibility (ease of polarization) of the surface at different frequencies (expressed usually as wavenumbers).⁴

For water next to hydrophilic surfaces such as quartz, the SF vibrational spectra generally show a peak at $\sim 3\,400\text{ cm}^{-1}$ assigned to the liquid-like hydrogen-bonded O–H stretch, and a peak at $\sim 3\,200\text{ cm}^{-1}$ assigned to the ice-like hydrogen-bonded O–H stretch. A characteristic peak at $\sim 3\,700\text{ cm}^{-1}$, normally present at hydrophobic interfaces, is assigned to the free dangling O–H that is not hydrogen-bonded. Although the spectra obtained by different groups are similar, there is substantial disagreement over the precise interpretation of the spectra. One main problem is that the usual spectrum does not include phase information, indicating whether the O–H bond points towards the interface or into

the water, whereas such information can be extracted easily from the data. It is the imaginary part of the complex (number) nonlinear electric susceptibility measurement.

Researchers led by Yuen-Ron Shen at the University of California, Berkeley, obtained more informative data on the water-quartz interface at different pH values using phase-sensitive SFVS.⁵ Figure 12.1 plots the spectra given by the imaginary part of the nonlinear electric susceptibility over a range of pH values that is expected to affect the ionization of the charged groups on the quartz surface.⁶ The solid line is a fit to the data based on a liquid-like peak (dashed line) and an ice-like peak (dotted line). The liquid-like peak is a single entity at $\sim 3400\text{ cm}^{-1}$. The ice-like peak is further separated into two sub-peaks: a negative one centred at $\sim 3000\text{ cm}^{-1}$ and a positive one centred at $\sim 3200\text{ cm}^{-1}$. Negative in this case means the O of the O–H is next to the quartz, and positive means the H is towards the quartz. So how can one explain these results?

At first glance, it seems that there are two different bonding sites for water molecules on the quartz surface. Those contributing to the liquid-like peak are deprotonated (losing a proton and becoming negatively charged) and saturate at around pH 7, but remain so even at very low pH (high concentrations of H⁺), when they still attract the H of the O–H bond. Those contributing to the ice-like peak, on the other hand, are less easily deprotonated, and tend to hydrogen-bond to the O of the water molecule, and start to deprotonate only above pH 4.5, saturating at pH 11.5.

However, a much more elegant explanation was proposed previously by other researchers based on molecular dynamics simulations.⁷ Just one kind of site, –SiOH, occurs on the quartz surface. The O can bind two hydrogen-donor water molecules, while a third water molecule is bound as acceptor of the H (see Fig. 12.2, top left). If the site is deprotonated, all three water molecules are bound as hydrogen donors (Fig. 12.2, top right). Then, it can be shown that for interfacial water with a more or less ice-like structure, there are always more H-bonds with H facing the surface (see Fig. 12.2, bottom).

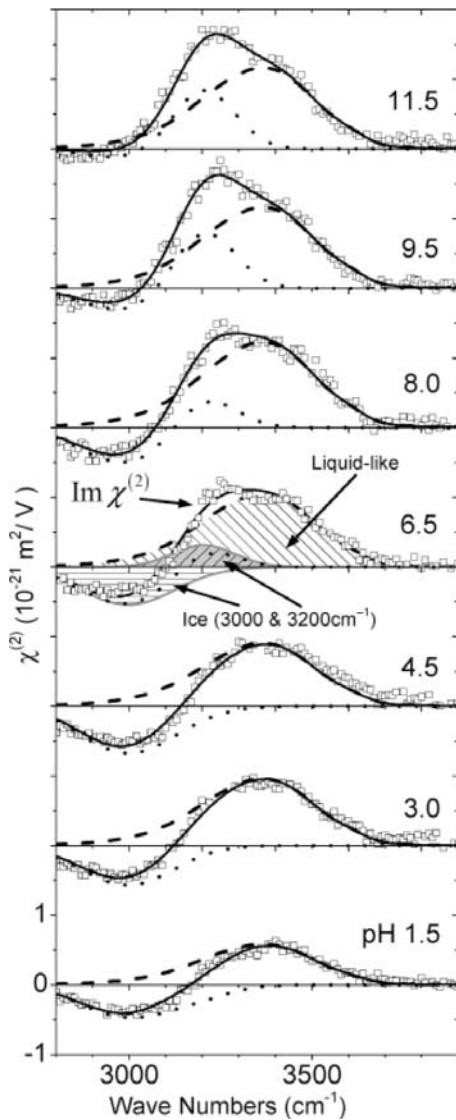


Figure 12.1 Phase-sensitive SFV spectra⁶ (see text).

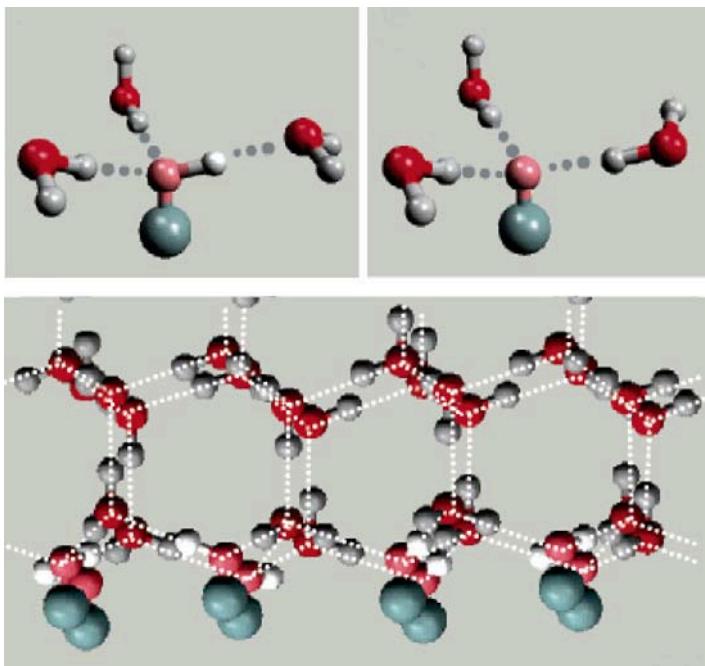


Figure 12.2 Water at the quartz surface, after Shen and Ostroverkhov.⁸

These elegant results hardly prepare us for the surprises springing from a very different experimental setup producing results that can be seen with the naked eye, and without having to interpret spectra indirectly.

Water Forms Massive Exclusion Zones

Pre-eminent water researcher Gerald Pollack and his team at Washington University created a sensation when they unveiled their new results on interfacial water.⁹

Pollack and his student Jian-Ming Zheng decided to do some simple experiments to find out exactly how far interfacial water can extend from hydrophilic surfaces. They used as solute microspheres 0.5 to $2\ \mu\text{m}$ in diameter, which are just visible under the ordinary light microscope. For hydrophilic surfaces, they found several common hydrogels known to interact strongly with water.

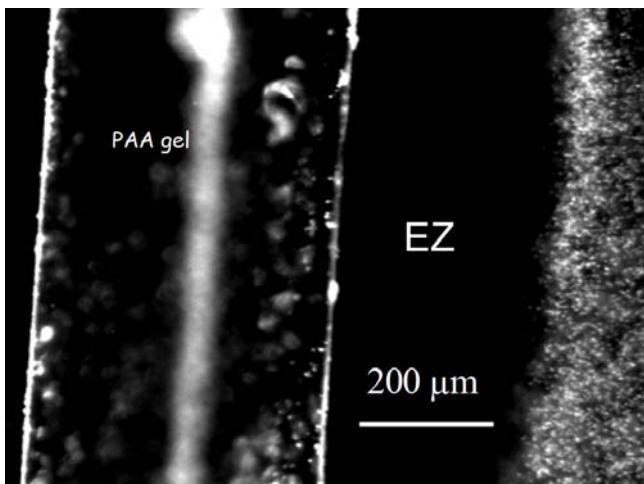


Figure 12.3 Interfacial water next to the gel surface forms a massive exclusion zone (EZ).⁹

In the first experiment they put a small gel sample between two large glass cover slips, and filled the space to either side with a suspension of the microspheres, then sealed the chamber. The whole assembly was placed on the stage of a microscope fitted with a camera to follow what happened.

In a second experiment, the gel was formed around a small glass capillary tube, which was withdrawn after the gel was formed, leaving a channel 1 mm in diameter, which was then filled with the suspension of microspheres and placed under the microscope.

To their amazement, they found that the microspheres were excluded from the gel surfaces in both experiments over distances of tens of micrometres, and in extreme cases, up to $250\text{ }\mu\text{m}$ or more. Such massive exclusion zones were totally unexpected (Fig. 12.3).

Microspheres are almost completely absent from the exclusion zone, and the boundary between exclusion and non-exclusion is rather sharp, on the order of 10% of the width of the exclusion zone. The zone forms quite quickly, and appears 80% complete after 60 s. The microspheres migrate away from the gel surface at a velocity of about $1.5\text{ }\mu\text{m/s}$, and microspheres near the boundary migrate at

the same speed as those far away from it. Once formed, the exclusion zone remains stable for days afterwards.

Could this be an artefact? For example, could there be some invisible threads sticking out from the gel surface to push the microspheres away? Zheng and Pollack tested that by using the atomic force microscope and other sensitive probes to detect such strands, but no protruding strands were detectable. Yet the exclusion zone persisted even after they fixed and cross-linked the gel, and washed it extensively, so no loose strands could ever leak out.

Could it be that the gel was in fact shrinking away from the surface and extruding water, and therefore squirting the microspheres away? But no such shrinkage was detectable; the boundary did not shift appreciably as the microspheres migrated away from it. Over a period of 120 minutes, the diameter of the cylindrical hollow in the gel changed by less than $2 \mu\text{m}$. Thus, in the two-minute period during which the exclusion zone was formed, shrinkage was insignificant.

Could it be that polymers were leaking out into the exclusion zone and pushing away the microspheres? They added a polymer to the microsphere suspension, but this only narrowed the exclusion zone.

Yet another test was to continuously infuse the microsphere suspension into the cylindrical hollow in the gel under pressure at a speed of about 100 mm/s, so that any suspended invisible solutes would be washed out. But the exclusion zone persisted, virtually unchanged even at the highest speeds.

The exclusion zones were not a quirk due to the particular gel used. Polyvinyl alcohol gels, polyacrylamide gels, polyacrylic acid gels, and even a bundle of rabbit muscle all gave similar results (though in the case of the rabbit muscle, the exclusion of microspheres was not complete, and some isolated particles were left in the EZ). In fact, a single layer of hydrophilic charged groups coated on any surface was sufficient to give an exclusion zone.¹⁰ All that was necessary was to have chemical groups that could form hydrogen bonds with water molecules (as in the experiment with quartz described earlier). Similarly, solutes did not have to be microspheres, they could be red blood cells, bacteria, colloidal gold,

and even molecules such as serum albumin labelled with a fluorescent dye, and fluorescent dye molecules themselves as small as 200 to 300 Da.¹¹ All of these were excluded from EZ water. Microspheres of different dimensions, coated with chemicals of opposite charge, nevertheless resulted in exclusion zones.

Thus, exclusion zones appear to be a general, if not universal, feature of hydrophilic surfaces.

Exclusion was most profound when the microspheres were most highly charged, so negatively charged microspheres gave maximum exclusion at high pH, whereas positively charged microspheres gave maximum exclusion at low pH. The presence of salt tended to decrease the size of the exclusion zone somewhat. The size of the exclusion zone also went up with the diameter of the microspheres.

Millions of Layers Thick

After ruling out several trivial explanations, Zheng and Pollack considered whether it could be due to layers of water molecules growing in an organized manner from the gel surface and extending outwards, pushing the microspheres out at the same time. That would seem consistent with the observation that the speed of migration of the microspheres is constant regardless of distance from the boundary.

The increase in exclusion zone with charge, too, is consistent with their water-structuring hypothesis, as a higher surface charge is known to be associated with a larger extent of water structuring. But, as they remark, “While these several observations fit the water-structure mechanism, no reports we know of confirm any more than several hundred layers of water structure at the extreme, and not the 10^6 solvent layers implied here.”¹²

More Revelations

In a prestigious public lecture,¹³ Pollack made further startling revelations. EZ water was also found at the air-water interface. It is highly viscous, like interfacial water next to solids, and that is why insects like the water boatman (and even a species of lizards dubbed

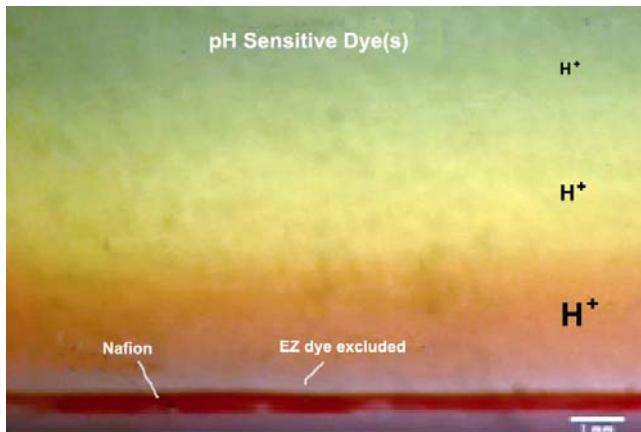


Figure 12.4 Proton-rich zone above the EZ grading upward into higher-pH regions, revealed by using pH-sensitive dye.¹⁵

the Jesus lizard, as Pollack mentioned in a more recent version of the lecture)¹⁴ can literally walk on water. It also explains why a stiff water bridge can form between two beakers of water, filled nearly to the brim, placed next to each other when a high voltage is passed from one beaker to the other. The water bridge is so stiff that the beakers can be pulled apart slowly up to several centimetres while the water bridge stretches and remains straight and intact over the gap.

Interfacial water at the air-water boundary is possibly a generalization of Drost-Hansen's proposal that interfacial water is independent of substrate (see earlier). But we shall consider that in more detail in later chapters.

When pH-sensitive dyes were used as solutes to see if they, too, were excluded from the EZ, Pollack's team found that indeed they were, and there also showed up a zone of unusually low pH right above the EZ (see Fig. 12.4).¹⁵

The excess of protons above the EZ suggests that charge separation has taken place as follows:



But where did the negatively charged OH^- ions go? Measurements of electrical potential show that away from the EZ, the bulk solution

had the same electrical potential everywhere. However, as soon as the measuring electrode entered the EZ, the electrical potential began to fall, slowly at first, and much more steeply as it approached the gel, dropping to -120 mV or more, depending on the type of gel, and remaining at that level well into the gel itself (see Fig. 12.5, top). Adding low concentrations of chloride solutions with different cations significantly changed the potential profile, so that the potential dropped more gradually within the exclusion zone, with K^+ ions having the greatest effect, followed by Na^+ and Li^+ . Interestingly, Ca^{2+} most resembled distilled water (DW) and even surpassed it (Fig. 12.5, bottom).¹⁶

The macroscopic separation of charges associated with the EZ is stable, as is the EZ itself. It is in fact a water battery. And like any other battery, it can be used, in principle, to power light bulbs or laptops. But what charges up the water battery? It takes energy to separate the charges, so where does the energy come from?

Light Charges Up Water, QED

It turns out that water is sensitive to light, as revealed by the EZ next to a gel. It thickens on being exposed to light, which means that light appears to enhance the formation of interfacial water. The entire spectrum of sunlight is effective, but the peaks are in the visible blue and especially the invisible near-infrared ($3\,000\,\text{nm}$) regions. A mere five-minute exposure to the infrared light will cause the EZ to thicken several-fold. And if you connect up the EZ and the bulk water above to an external circuit, there is a measurable current which lasts for a considerable time after the infrared light is turned off.

The $3\,000\,\text{nm}$ region ($3\,333\,\text{cm}^{-1}$ wave-number) falls between the $3\,200$ and $3\,400\,\text{cm}^{-1}$ peaks identified on VSFS for the ice-like hydrogen-bonded O–H stretch of interfacial water next to quartz (see earlier), so there may be some common thread here.

The findings are also in line with the predictions of Del Giudice and colleagues, based on quantum electrodynamics (QED) considerations, that water forms quantum coherent domains (CDs) by absorbing electromagnetic radiation from the environment, which

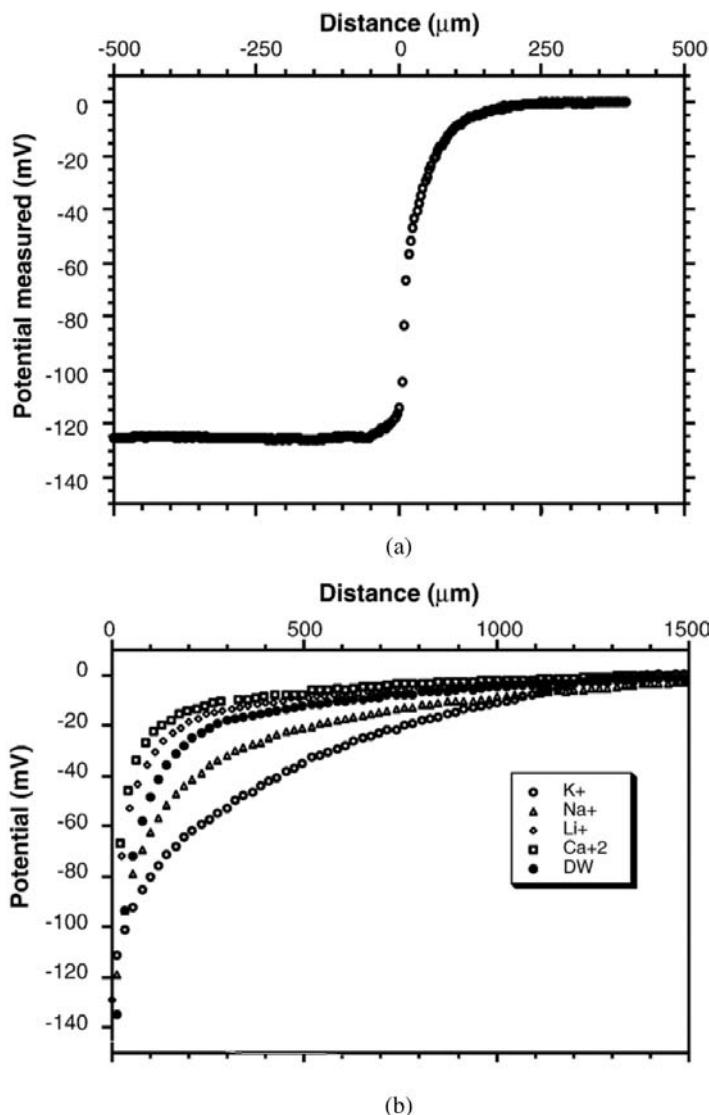


Figure 12.5 Electrical potential as a function of distance from the polyacrylic acid gel surface: in distilled water (top) and in 1 mM chloride solutions with different cations (bottom).¹⁶

physicists like to refer to as “the vacuum”, to emphasize the fact that electromagnetic radiation can travel through absolutely empty space (see Chapter 6). They suggested that EZ water is just a giant coherent domain stabilized at the vast area of interface available. The CDs are predicted to have plasmas of almost-free electrons, which would be consistent with the positively charged protons swarming outside the EZ. And furthermore, it is this excited water at the interface that makes photosynthesis possible.

Green plants and especially blue-green bacteria have been splitting water for billions of years, in order to obtain energy from the sun, and in the process fixing carbon dioxide to make carbohydrates and other macromolecules to feed practically the entire biosphere. The separation of charges in the formation of the EZ gives life the head start it needs (as explained in Chapter 6, more in Chapters 21 and 22).

Interfacial Water is Liquid Crystalline Water

As EZ water can be produced in bulk, it is easy to demonstrate other altered properties. NMR measurements confirmed that it is associated with decreased mobility (increased ordering) relative to bulk water. It also has a distinctive absorption peak at 270 nm in the ultraviolet region, which is not present in bulk water.¹⁶

Under the ordinary polarized light microscope, the EZ appears as a bright, birefringent band next to the gel, precisely as a biological liquid crystal would. Certainly, this justifies calling interfacial EZ water “liquid crystalline water”.

Pollack suspects that the EZ is intermediate between ice and water, and consistent with the hypothesis, the characteristic absorption peak at 270 nm appears transiently as ice melts.¹⁷

Of Colloid Crystals and Protein Folding

EZ water easily explains a host of puzzling phenomena,¹⁸ among these the formation of colloid crystals in water (Chapter 4), which are finding many applications in electronics and photonics.

Norio Ise and his colleagues in Osaka, Japan, discovered colloid crystals forming in water more than 20 years ago, and explained the phenomenon in terms of a long-range attraction between the colloid particles, though the precise mechanism has remained elusive. The major difficulty is that the colloid particles have the same charge and it is impossible, according to conventional theory, for like charges to attract one another.

The findings of Pollack's group provide just the mechanism required. Colloid particles and microspheres are like the hydrophilic gel surfaces that form layers of liquid crystalline water or the EZ. In the case of the gel, the EZ has an excess of negative charges with excess positive charges in the region outside (see Fig. 12.4). In the case of the microspheres and colloid particles, each is enclosed in its own shell of EZ water with excess negative charges, while the positive charges are also driven outside (see Fig. 12.6). The repulsion between the negatively charged particles is exactly balanced by the attraction to the positive charges in between. In the space between two particles, there will be an excess of positive charges compared to elsewhere, which is why the particles end up being attracted to one another.

This is simply a special case of charge shielding through counter-ions in water that prevents like charges from repelling each other, and is expected to be quite common within the hydrated cell.

Researchers on protein folding have acknowledged for quite some time the importance of water, but only in a negative role, in that it is the *avoidance* of water in hydrophobic interactions that determines how the chain folds up. Until quite recently, most computer algorithms for predicting the folded structure of proteins did not take water into account.

But a team led by Peter Wolynes at the University of California, San Diego, and the University of Illinois at Urbana-Champaign thought to include water molecules appropriately in their computer simulation, so that charged groups could make contact via water molecules.¹⁸

The results were quite unexpected. It seems that highly charged amino acids don't like to be in direct contact, and such contacts are unstable, whereas the contact is stabilized if mediated by water.

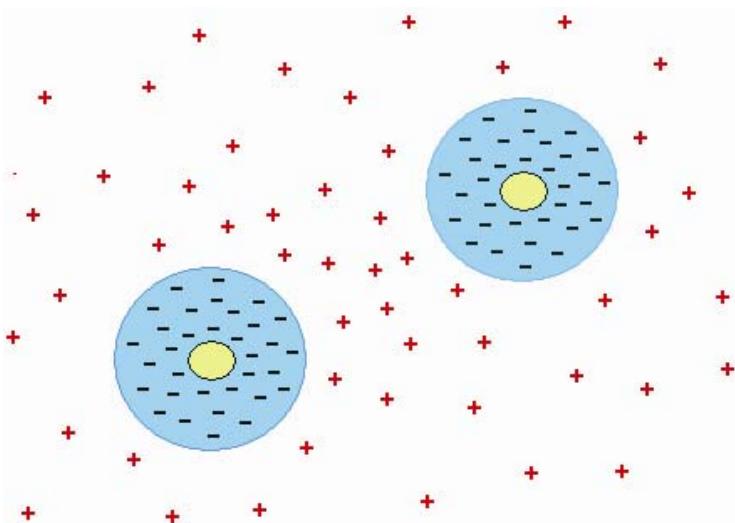


Figure 12.6 How like attracts like.¹⁹

That is because a lot of energy has to be spent getting the hydrophilic groups to let go of water molecules they are already bound to, so they tend to contact other highly charged groups via one or two water intermediates. Even more interesting is that not only oppositely charged residues attract each other through water, but so do groups with the same charge, which suggests that one or the other of the groups in contact must be changing its sign, or else the contact partners fluctuate coherently together and take turns being the charged or uncharged one, rather like dancing rapidly back and forth perfectly in step.

Including water significantly improved the fit to the native folded protein; some of the improvements were very striking, especially for proteins larger than 115 amino acid residues. The results showed that water not only induces protein folding, but also actively participates in linking charged groups by forming water chains. Adding water may well improve protein docking with each other in protein-protein complexes, and in drug design strategies.

The long-range attraction between like particles is the major mechanism for self-assembly processes inside cells. It is the organizing

principle that has long eluded biology, or as Albert Szent-Györgyi said, “Life is water dancing to the tune of molecules.”²⁰

Perhaps it is the other way round as well: life is molecules dancing to the tune of water. There are more intricate dances at different kinds of interfaces to look into before we get closer to the rainbow inside.

Notes

1. See Drost-Hansen (2006).
2. Etzler and Drost-Hansen (1983).
3. Drost-Hansen (2006).
4. “Second-harmonic Generation”, Wikipedia, 30 August 2011, http://en.wikipedia.org/wiki/Second-harmonic_generation; Zhang *et al.* (2008).
5. Ostroverkhov *et al.* (2005).
6. Shen and Ostroverkhov (2006).
7. Lee and Rossky (1994), cited in Shen and Ostroverkhov (2006).
8. Shen and Ostroverkhov (2006).
9. Zheng and Pollack (2003); see Ho (2004d).
10. Zheng *et al.* (2006).
11. Pollack (2008); Ho (2008); Zheng and Pollack (2006).
12. Zheng and Pollack (2003).
13. Pollack (2008); Ho (2008).
14. Pollack (2010).
15. Pollack (2008); Ho (2008).
16. Zheng *et al.* (2006).
17. So *et al.* (2011).
18. Papoian *et al.* (2004); see Ho (2005d).
19. Pollack (2008); Ho (2008).
20. Cited by Pollack (2008).

13

Water Electric

Hopping Down a Daisy Chain

The interfacial water aligned on the vast expanse of interfaces inside the cell and in the extracellular matrix works like magic when it comes to conducting electricity, and it depends on the hydrogen bonds of successive molecules forming a kind of daisy chain. This is yet another example of the cooperativity in water described in Chapter 3.

The electricity conducted is the positive charge associated with “jumping” protons, a special semi-conduction comparable to the migration of positively charged “holes” in solid-state materials. The proton doesn’t actually move or jump; instead it passes rapidly down the chain by relay (see Fig. 13.1).¹

The free proton at one end takes over bonding with the oxygen of the first water molecule of the chain, creating a second free proton that displaces its neighbour down the line until the last proton comes off at the other end (Fig. 13.1, I to III). (Note that an electron moves in the opposite direction.) Jump conduction is much faster than ordinary electricity passing through a metal wire, which involves electrons actually moving; and much, much faster than conduction by charged ions diffusing through water. But it needs to have chains of water in a sufficiently ordered state and, as we shall see in chapters to come, proteins and membrane surfaces may impose that kind of order on water, particularly in the confined spaces inside the cell and in the extracellular matrix.

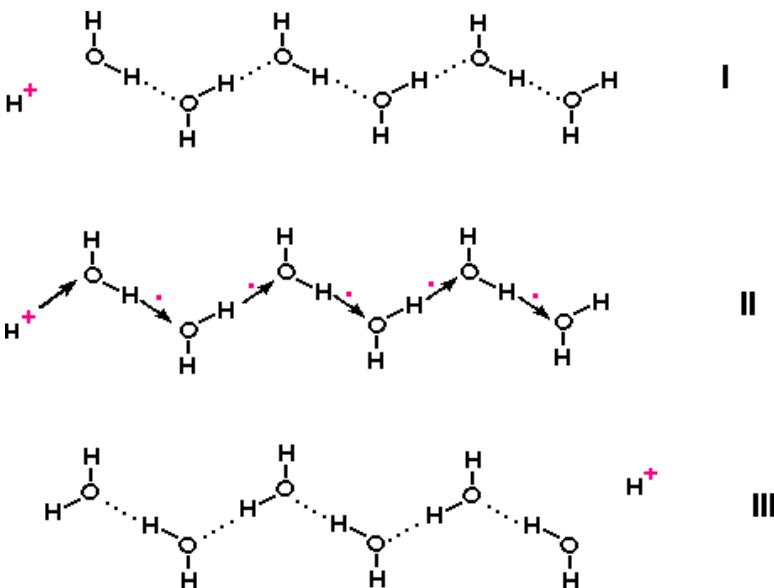


Figure 13.1 Proton jump conduction down a chain of hydrogen-bonded water molecules.²

The evidence for jump conduction of protons via daisy chains of water molecules has come from several sources.

Charging Up the Batteries of Life

Most good biochemistry textbooks — and you will need a good one — will tell you that living organisms are charged up predominantly by accumulating protons on one side of a membrane, and discharged by protons flowing back down to the other side. Protons are transported across biological membranes by special membrane proteins called “proton pumps”. The protons are pumped uphill (to a higher energy state) using an external source of energy such as the oxidation of foodstuff in animals like us, or the absorption of sunlight in the case of green plants, algae, and cyanobacteria. They are then returned downhill via another enzyme, ATP synthase embedded in the same membrane, which uses the energy to make ATP, the universal energy transduction intermediate that powers all living activities.

This is the gist of the chemiosmotic hypothesis that won British biochemist Peter Mitchell (1920–1992) the 1978 Nobel Prize for Chemistry. The protons are supposed to exist in bulk solution on either side of the membrane, and it is the difference in concentration between the two compartments separated by the membrane that drives the synthesis of ATP.

Structural studies on proton pumps² show that they form a channel through the cell membrane, which is threaded by a chain of hydrogen-bonded water molecules from one side of the membrane to the other. The best-known example of these proteins is bacteriorhodopsin, the light-harvesting pigment of the purple membranes belonging to Archaea such as *Halobacteria*.³ Bacteriorhodopsin is an integral membrane protein usually found in crystalline patches known as “purple membrane” that can occupy up to 50% of the surface area of the cell. The repeating unit in the hexagonal crystalline lattice is composed of three identical protein chains, each rotated 120° relative to the others. Each chain has seven transmembrane α -helices and contains one molecule of retinal buried deep inside the helices. The absorption of one photon by the retinal leads to a change in conformation, triggering a cycle of six intermediates before returning to the resting state, with the transfer of one proton from the cytoplasmic side to the extracellular side.⁴ Another example is the cytochrome c oxidase that catalyses the last stage in the oxidation of foodstuffs in the membrane of the mitochondria, in which oxygen is reduced to water by combining with protons and electrons.⁵

Proton Conduction Along Biological Membranes

However, biochemists have noticed that the rate of some proton pumps, such as the cytochrome c oxidase, which pumps more than 10^3 protons per second, is higher than the rate at which protons can be supplied to the proton-conducting channel via the bulk diffusion rate.⁶ And since the chemiosmotic hypothesis was first proposed, chemist R.J.P Williams at Oxford University⁷ and others subsequently (see Rainbow Worm) have suggested that the protons,

rather than accumulating in the bulk solution of the cell compartment, actually diffuse along the membrane surface, perhaps directly from proton pumps such as the cytochrome c oxidase enzyme to the ATP synthase embedded in the same membrane.

Experimental findings have indicated that proton conduction can indeed take place along the surface of both natural and artificial membranes at the interface with water, and more specifically in the water layer(s) immediately next to the membrane surface.⁸ Long-distance migration of protons along membranes has been observed in purple membrane and reconstituted bacteriorhodopsin. There is a high rate of proton diffusion along the membrane surface and a tendency for protons to remain on the membrane surface as opposed to going into the bulk of the cell compartment, or into solution outside the cell.

When protons diffuse along the surface of membranes instead of through the bulk, the rates of proton transport processes are significantly increased. That is due to a fundamental difference between diffusion in two and three dimensions. In three dimensions, a proton far away from its target, say, the entrance to an ATP synthase embedded in a membrane, will have a very small probability of being caught by the target. But in two dimensions, the probability of the proton being caught is exactly equal to 1; in other words, it will be caught sooner or later. And if instead of random diffusion, protons are jump-conducted along chains of interfacial water molecules aligned by hydrogen bonds, proton transport can indeed be quite fast.

Researchers at the Max Planck Institute of Biochemistry first showed that very thin films of water (down to about one layer) adsorbed onto a solid surface exhibit a “surprisingly high conductivity”, using a *scanning tunnelling microscope*.⁹ A tunnelling microscope depends on the flow of an electrical current and thus cannot be used to directly image insulating material. But in a humid atmosphere, a thin film of water settles on the surface of the material and is sufficient to provide the conductivity needed for imaging at currents below 1 pA (picoampere, 10^{-12} ampere).

New evidence for proton migration along interfacial water has been obtained in a study headed by Peter Pohl at Johannes Kepler

University of Linz.¹⁰ Membrane-bound “caged” (chemically trapped) protons were released by flashes of UV light, and their arrival at distant sites ($70 \mu\text{m}$ away) monitored by a pH-sensitive fluorescent dye. Proton diffusion along the membrane was fast. The rate was independent of the membrane lipids present, even one that lacked any ionizable groups, and only slowed down considerably when D_2O was substituted for H_2O (another example of a nuclear quantum effect, see Chapter 5). The apparent diffusion coefficient of proton migration estimated was $5 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, some seven orders of magnitude faster than could be accounted for by proton-hopping between charged groups on membrane lipids. The researchers concluded that proton migration takes place along interfacial water on the membrane.

Nanotubes, Water Transport, and Proton Wires

A concrete model of a proton-conducting water chain or proton wire has come from a further unexpected source: studies in carbon nanotubes. A carbon nanotube is a new form of carbon discovered in 1991, in which carbon atoms are joined up into the shape of a long thin tube. Such tubes are typically nanometres in diameter, and can be micrometres in length. Although predominantly hydrophobic, these nanotubes are found to interact substantially with water.

Carbon nanotubes belong to the family of carbon molecules known as *fullerenes*, after US systems theorist and inventor Buckminster Fuller (1895–1983). The 60-carbon spherical fullerene, also called a buckyball, has the shape of a truncated icosahedron with 32 faces: 20 hexagons and 12 pentagons. The relationship between the buckyball and the carbon nanotube is shown in Fig. 13.2.

Scientists at the National Institutes of Health and the University of Maine simulated some experimental results on the computer.¹¹ They showed that a single-walled nanotube 1.34 nm long and 0.81 nm in diameter rapidly filled up with water from the surrounding reservoir, and remained occupied by a chain of about five water molecules on average during the entire 66 ns of simulation. A nano-second or 10^{-9} s is a long time in the life of a molecule.

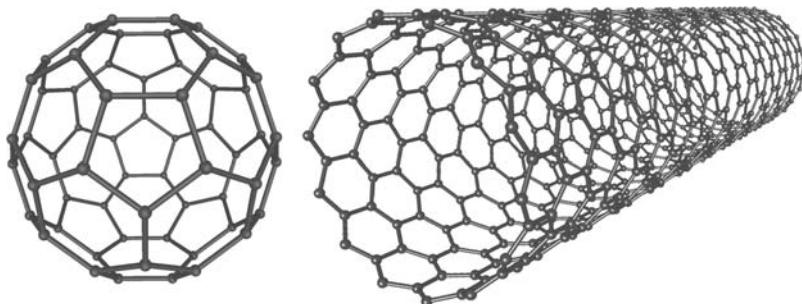


Figure 13.2 Buckyball (left) and single-walled carbon nanotube.

This result was surprising first of all because carbon does not have a high affinity for water. But it seems that getting into tight places restricts the distribution of energies in the water molecules, so they end up with a lower average energy than in bulk water, and hence it becomes energetically favourable for the water to enter the nanotubes. This is like getting into a crowded underground carriage, where people's movements are restricted, and hence the range of energy distribution is narrowed towards the lower end of the scale.

Hydrogen bonds between water molecules inside the nanotube are shielded from fluctuations in the environment, and hence much more stable. Within the nanotube, only 0.02% of pairs of water molecules in contact distance (0.35 nm) are unbound, compared with 15% in bulk water. H-bonds in the nanotube are highly oriented, with less than 15% of the H–O \cdots H angles between adjacent water molecules exceeding 30°, compared to 37% in bulk water. The average life time of the H-bond inside the nanotube is 5.6 ps, compared with 1.0 ps in bulk water. The H-bonds are nearly aligned with the nanotube axis, collectively flipping direction from one side to the other every 2 to 3 ns on average.

Water molecules not only penetrate and stay within nanotubes, but are also conducted through them.¹² During the 66 ns of simulation, 1 119 molecules of water entered the nanotube on one side and left on the other side, about 17 molecules per ns. This rate is comparable to that measured through the twice-as-long channel of the transmembrane water-conducting protein aquaporin-1.¹³ Water

conduction occurs in pulses, peaking at about 30 molecules per ns, again reminiscent of single ion channel activity in the cell, and is a consequence of the tight H-bonding inside the tube.

There is a weak attractive force between the water molecules and the carbon atoms, through *van der Waals* force (due to permanent or transient dipole interactions), which is 0.114 kcal/mol. Reducing this by 0.05 kcal/mol (less than 5%) turns out to drastically change the number of water molecules inside the nanotube, which fluctuates in sharp transitions between empty states (with zero water molecules) and filled states. This suggests that changing the conformation of channel proteins may control the transport of water from one side to another in the cell membrane.

Do such water-filled channels conduct protons? Yes, they do. If there is an excess of protons on one side of the channel, positive electricity will spirit down fast, in less than a picosecond, some 40 times faster than similar conduction of protons in bulk water, according to Gerhard Hummer of the National Institutes of Health in the United States, who led the team that carried out the nanotube simulation studies.¹⁴

If the nanotubes, instead of swimming in free water solution, were immobilized in membranes, they could be used for all kinds of applications, including light sensing, field-effect transistors for proton currents, and desalination of seawater, the researchers suggested.

We shall be looking at water confined in nanotubes again in Chapter 19.

Notes

1. Pomes and Roux (1998); Ho (2005d).
2. Riistama *et al.* (1997).
3. “Bacteriorhodopsin”, Wikipedia, 14 July 2011, <http://en.wikipedia.org/wiki/Bacteriorhodopsin>.
4. Bondar *et al.* (2004).
5. Riistama *et al.* (1997); Ho (2005c).
6. Georgievskii *et al.* (2002).

7. Williams (1993).
8. Gabriel and Teissie (1996).
9. Guckenberger *et al.* (1994).
10. Springer *et al.* (2011).
11. Hummer *et al.* (2001).
12. Hummer (2003).
13. Aquaporins are integral membrane proteins forming pores in the membrane of special cells that transport water very rapidly. The aquaporin monomer is made up of six transmembrane α -helices surrounding a water channel, with the amino and the carboxyl termini both located on the cytoplasmic side of the membrane. Aquaporins form tetramers in the cell membrane with each monomer acting as a water channel (see “Aquaporin”, Wikipedia, 27 July 2011, <http://en.wikipedia.org/wiki/Aquaporin>).
14. Hummer (2003).

14

Water + Air = Life

Special Chemistry

Water covers most of the Earth's surface: 71% in oceans alone, not counting numerous lakes, rivers, streams, and ponds. In addition, a vast expanse of air-water interfaces is to be found in *aerosols*, micrometre-sized solid or liquid droplets suspended in the atmosphere. Within the past 15 years, air-water interfaces have become the centre of attention for climate scientists, chemists, and, not least, those passionate about the possibility of extraterrestrial life, on Mars or any other heavenly body that has a whiff of ice or water on it. And it is appearing more likely than ever that life may have originated on Earth and elsewhere at different air-water interfaces.¹ Very special chemistry happens when water meets air.

It is now known that all surfaces of marine and fresh water are covered by an organic film, or surface microlayer, 1 to 1 000 μm thick (see Fig. 14.1). Chemical analysis of the organic film at the sea surface revealed the presence of amphiphiles derived from marine organisms. These include fatty acids, fatty alcohols, sterols, and amines. The amphiphile layer can act as both source and sink for a range of pollutants, and it is indeed highly contaminated in many parts of the world. The unique environment of the microlayer gives rise to specialized resident micro-organisms, or neustons. This layer is important, not only because it governs gas exchange between the water and the atmosphere, but also because the chemical reactions that can take place in the layer are legion.

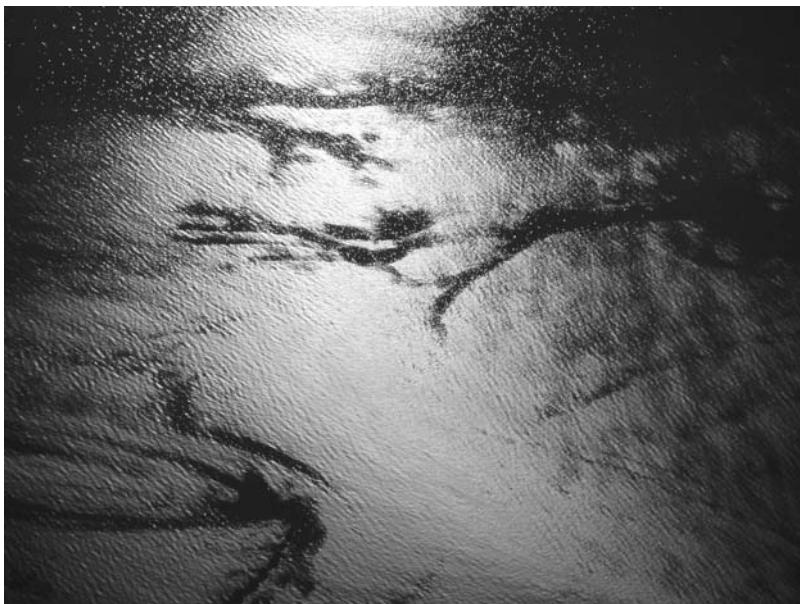


Figure 14.1 Surface microlayer on the Mediterranean near Naples, Italy.

For example, light-catalysed oxidations, or photo-oxidations, can take place readily in such films, which have twice the concentration of the photosynthetic pigment *chlorophyll* as bulk seawater.² Chlorophyll itself is photo-oxidized by oxygen with the help of water. Chlorophyll also oxidizes halides from sea salts, especially bromide and chloride into bromine and chlorine, again with the help of water, which then forms oxidation products with the halide.

Reaction rates are greatly enhanced due to the high concentrations of reactants and rapid diffusion attainable in two dimensions, plus special catalytic possibilities in molecular recognition, patterning, and self-assembly.³ Molecular recognition through hydrogen-bonding takes place efficiently at air-water interfaces, with rate constants comparable to those in biological systems between receptors and ligands, enzymes and substrates. This makes it possible to fabricate patterns with precise molecular-level interactions. Specific two-dimensional patterns can be assembled, which are

then transferred onto a mica surface. It is a veritable nano-lab-bench with a molecularly flat environment, a boundary region between two phases with very different dielectric constants (2 in air vs 80 in water), and a highly dynamic medium within the plane of the interface providing access points between hydrophobic compounds and hydrophilic compounds. These interfaces are very much like the phospholipid membrane surfaces that enclose and fill living cells. In effect, they allow chemists to mimic biochemical reactions taking place inside the cell. Some chemists have made use of the air-water interface to synthesize complex polymers such as polypeptides,⁴ and were even able to control whether the polypeptide adopts α -helical or β -pleated sheet conformation (see Chapter 10).

Origin of Life at Air-Water Interfaces

Among the most exciting reasons for studying water interfaces is that they may provide just the right alchemy for the origin of life. Life has the most complex chemistry imaginable, and where better to look for the origins of biological building blocks such as amino acids, polycyclic compounds, nitrogenous bases, and sugars than at air-water interfaces?

Prebiotic chemistry is the synthesis of the building blocks of living organisms, starting from simple inorganic molecules such as CH_4 , CO_2 , N_2 , and NH_3 . The building blocks — carboxylic acids, amino acids, bases, sugars, etc. — then underwent further reactions to make biologically functional molecules such as proteins, lipids, nucleic acids, and so on. Water is an essential ingredient in all of that.

Numerous experiments have been carried out in bulk water and gases in the laboratory, which were energized with electrical discharge to simulate prebiotic conditions on Earth, and simple amino acids were successfully generated. The experiments were done using different gas mixtures, sources of energy, as well as temperatures and pressures. However, the diversity of the compounds obtained has been rather limited, and without the typical chirality

(handedness) of biological molecules. In particular, nucleic acid bases and short carboxylic acids have only been detected in a few experiments. This has led some chemists to investigate the chemical possibilities of water at interfaces.⁵

The huge water surfaces that cover the Earth are connected by a cycle of evaporation, condensation, and rain. In addition, organic and inorganic surface products of the oceans can be transported within aerosols to the atmosphere above, where they are exposed to radiation and electrical discharges in the form of lightning. Some scientists have proposed that the cycle of natural aerosols from the ocean water pool to the atmosphere and back to the ocean may have played an important role in the synthesis of organic molecules that generated the first self-organized proto-life systems (see Fig. 14.2).

Aerosols could have played a most important role as prebiotic microreactors, creating a wide range of building blocks in the favourable chemical environment of the air-water interface, and also introducing the handedness typical of biological molecules.⁶ Aerosols are already rich in products of the ocean's surface microlayer, but the continuous recycling process of aerosols forming and bursting would have further enhanced the prodigious diversity of chemical reactions and reactants available. The formation of aerosols requires only a liquid water-air interface and a physical mechanism that ejects bubbles into the atmosphere, such as wind, sea waves, or shock waves.

Recreating Life in the Lab

Researchers led by Marta Ruiz-Bermejo at the Astrobiology Centre of the Spanish National Aerospace Laboratory (CSIC-INTA) decided to carry out laboratory experiments on prebiotic synthesis in a flask with water and electrical discharge, with and without a sonic device that generates aerosols.⁷ In addition, they investigated the potential of other water-air interfaces, such as those associated with the melting and freezing cycles of ice. These are particularly relevant for the origin of life in a range of different environments. For example, it is

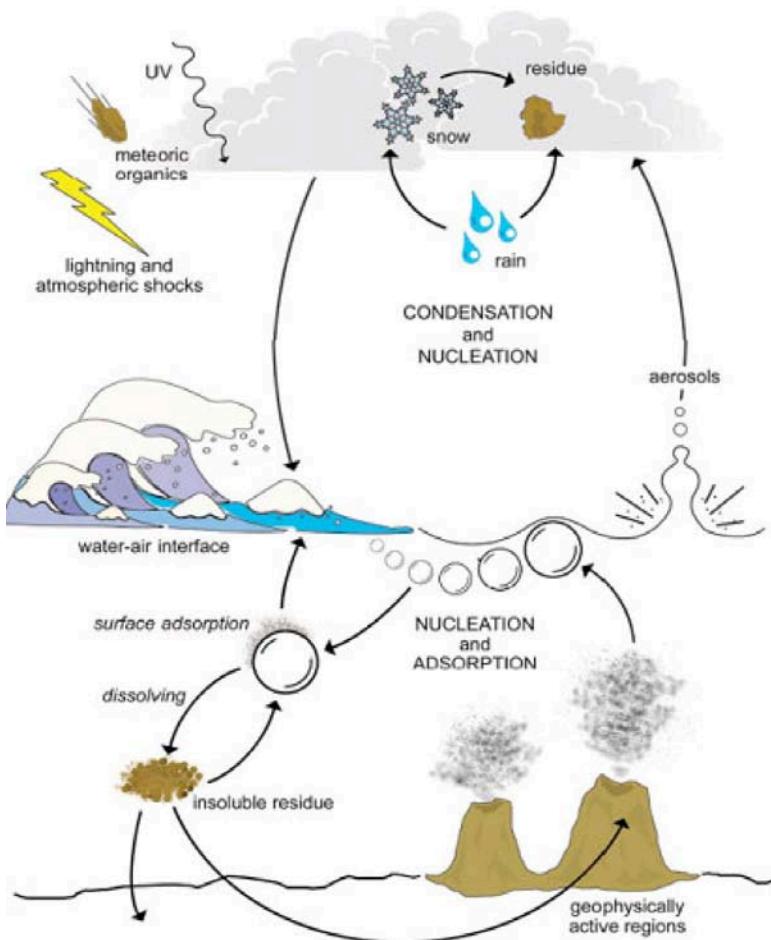


Figure 14.2 Aerosols and the water cycle in the prebiotic origin of life; redrawn from Lerman, L., *Lunar and Planetary Science* 2006.⁶

now clear that Mars has water in its polar ice-caps, and the existence of water is suspected on the icy moons of Saturn.

Sure enough, the researchers found a significant increase (37%) in the organic molecules obtained in the flask with aerosols. Aerosols resulted in a wider range of amino acids, as well as carboxylic acids, hydroxy acids, and heterocyclic (ring structures consisting of carbon, nitrogen, and/or oxygen) compounds, including the

bases adenine (occurring in DNA and RNA) and diaminopurine, which were almost entirely absent in the flask without aerosols.

The researchers also investigated the effects of salt in the presence of aerosols, as it has been suggested that the salinity of ancient oceans was 1.5 to 2 times higher than now, and that significant quantities of dissolved iron could have been present. They found that a high-salinity solution with 1.5 times the salt concentration of sea water further increased the amount and number of amino acids formed ($39.77 \mu\text{mol}$ compared with $23.64 \mu\text{mol}$, a 68.2% increase). The amounts of carboxylic and hydroxy acids were also increased. While the presence of FeS decreased the amount of amino acids synthesized, it led to the formation of sulphur-containing amino acids such as cysteine.

Polycyclic aromatic hydrocarbons (PAHs) are thought to be key molecules in prebiotic synthesis. Because of their photochemical properties, they could have played the role of a primitive light-absorbing pigment that drove the synthesis of amphiphilic compounds. Amphiphilic PAHs are capable of self-assembly and may form bilayer structures similar to cell membranes. Low temperatures favour the formation of acetylene, the main precursor of PAHs. In an experiment simulating the freeze-melt cycle of ice energized by spark discharges at temperatures between -5 and $+5$ °C, the researchers succeeded in producing a range of PAHs from a mixture of $\text{CH}_4/\text{N}_2/\text{H}_2$. Significantly, no PAHs were synthesized in a control experiment at room temperature, which yielded a range of non-cyclic alcohols instead. This shows how important low-temperature chemistry is for life.

Adding 0.1 M urea to the water in the freeze-melt cycle experiment resulted in diverse products, including high yields of cyanuric acid (7.1% of introduced urea converted) and the base cytosine (4.2%) plus uracil and a small amount of adenine, all of them in DNA or RNA. Again, a control experiment carried out at room temperature gave different products that did not include any pyrimidine (cytosine and uracil) or purine (adenine).

Water at the interface may indeed have played an essential role in the origin of life. Interfacial chemistry is rich beyond our

imagination. One main reason it is so favourable for the origin of life may be interfacial coherent water itself acting like a redox pile (see Chapters 6 to 8), not to mention proton migration along interfacial water on primitive membranes (Chapter 13). We need to get back to some basics on interfacial water at the air-water boundary.

Notes

1. Donaldson and Vaida (2006).
2. Reeser *et al.* (2009).
3. Donaldson and Vaida (2006).
4. Ariga and Hill (2011).
5. Fukuda *et al.* (2000).
6. Donaldson and Vaida (2006).
7. Ruiz-Bermejo *et al.* (2010).

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15

Water Meets Air

Walking on Water

The most distinctive property of the water surface next to the air is its high *surface tension* (72.8 mN/m at 20°C), the highest of the non-metal liquids. That is why objects heavy enough to sink in water, such as a small metal coin or a metal paper clip, can nevertheless sit on the surface supported by an invisible, deformable film.¹ Even more remarkably, over 1 200 species of insects, spiders, birds, fish, reptiles, and mammals have the ability to walk on water.²

The usual explanation of surface tension is the cohesive forces among the liquid molecules. In the bulk of the liquid, each molecule is pulled equally in all directions by neighbouring molecules, resulting in a net force of zero. At the surface, however, the molecules no longer have molecules all around them, and are therefore pulled inward towards the bulk of the liquid (see Fig. 15.1).³ This makes liquid surfaces contract to the minimal area, which is why water forms spherical drops in air.

It is generally believed that the unusually high surface tension of water is due to the propensity of water molecules to form hydrogen bonds, and the hydrogen-bonding may be more stable or pronounced at the surface,⁴ as they appear to be next to solid surfaces (see Chapter 11).

The relatively recent introduction of appropriate nonlinear optical techniques such as sum frequency (SF) spectroscopy and second harmonic (SH) spectroscopy has made it possible to probe the interface directly⁵ (see Chapter 12).

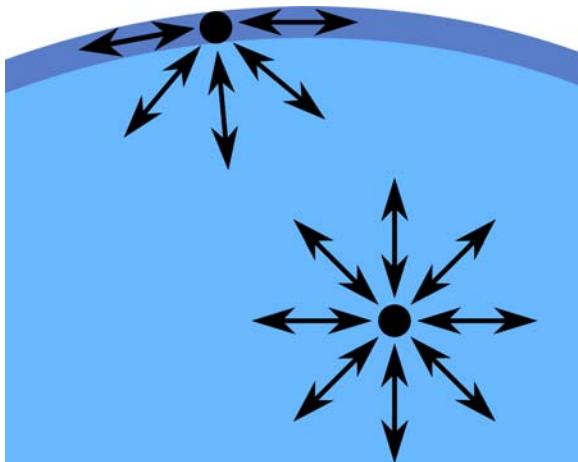


Figure 15.1 Water molecules at the surface experience unequal intermolecular forces, giving rise to surface tension, by Füsiahh, Wikimedia.¹

Investigations using SF and SH spectroscopies, together with molecular dynamics (MD) simulations, showed that the air-water interface is about 1 nm deep (three to four water molecules), and contrary to expectation, 20 to 25% of the surface is covered with non-hydrogen-bonded, free, or dangling –OH groups sticking into the air,⁶ which is rather like a soft hydrophobic interface.

When the usual SF vibrational spectroscopy (SFVS) experiment is used to probe the surface via the O–H bond vibrations (see Chapter 12), the air-water interface spectrum typically contains a broad peak 2800 to 3600 cm⁻¹ from interfacial OH groups that are hydrogen-bonded, and in addition, a narrow peak at 3700 cm⁻¹ from the non-hydrogen-bonded dangling OH groups.

The spectra obtained by different research groups are quite similar, but there is no agreement over how the spectra are to be interpreted. A phase-sensitive SFVS developed more recently measures both the amplitude and the phase of the resonant OH vibrations, which indicates whether the OH group is pointing up into the air (positive phase) or down into the bulk water (negative phase) (see Chapter 12). This has greatly improved the interpretation of spectra and enabled researchers to distinguish between spectra that

appear at first sight to be identical. But it has not solved the problem entirely, for that requires a theory of molecular structure at the water-air interface, which is still missing.⁷

The research team led by Mischa Bonn at the FOM Institute, Amsterdam, used a new version of sum frequency generation (SFG) that gives ultrafast time- and polarization-resolved spectroscopy to probe the vibrational and reorientation relaxation of the non-hydrogen-bonded –OH groups.⁸ They found that the dangling –OH group is oriented at an average angle of 55° to the normal (perpendicular) of the air-water interface, and its rate of reorientation in the plane of the interface is some three times faster than water in the bulk. These results were confirmed by molecular dynamics simulations.

The faster relaxation rate means that the molecules with the dangling –OH are less constrained by hydrogen-bonding than in the bulk, suggesting that the remaining –OH in these molecules is more weakly hydrogen-bonded. That has turned out to be the case, as shown in a subsequent publication.⁹ These results make the high surface tension of water even more difficult to understand. As Bonn admits, “SF and SH studies have not shed much light on the surface tension.”¹⁰

This illustrates how the interpretation of spectroscopic data is heavily dependent on theory. An erroneous theory will give correspondingly wrong or misleading conclusions despite reliable and reproducible data.

Ions at the Interface

It has been assumed for a long time that the high surface tension of water means that ions are essentially excluded from the surface layers. The study of the effect of ions on air-water interfaces dates back to the early 1900s when German scientist Adolf Heydweiller measured surface tension for a series of salt solutions. He found that adding simple salts raises the surface tension of water; while cations show little specificity, anions are specific, and follow the *Hofmeister series* (see Chapter 17), with strongly hydrated anions increasing the surface tension more than weakly hydrated ones.¹¹

In the 1930s, Norwegian-born American physicist Lars Onsager (1903–1976) and his colleague Nicholas Samaras described this effect in terms of the repulsion of ions from the air-water interface by electrostatic *image forces*, forces generated by induced polarization of the surface. So it became conventional wisdom that surfaces of aqueous solutions of simple salts are formed by an ion-free water layer.¹¹ But that picture turned out not to be correct. Experiments and MD simulations in the 1990s showed ion specificities for both alkali cations (Li^+ , Na^+ , K^+ , Rb^+ , Cs^+) and halide anions (F^- , Cl^- , Br^- , I^-) in water clusters. While small alkali cations and fluoride are surrounded by hydration shells of 10 to 100 molecules, heavier halides tend to reside on the surface of water clusters.

An international research team led by Douglas Tobias at the University of California, Irvine, carried out an MD simulation, crucially with a *polarizable force field*, a mathematical function describing the potential energy of the system that includes induced dipole interactions of both water and ions.¹²

They found that while the kosmotropes Na^+ and F^- with high surface charges are repelled from the air-water interface by image forces, the heavier halides (chaotropes) move to the surface, with Cl^- spreading right up to the interface, and Br^- and I^- even peaking in density at the surface. For I^- and Br^- , accumulation at the surface is accompanied by depletion from the subsurface, where Na^+ peaks. As a result, a negative electric potential develops at the interface, and the surface tension increases in agreement with the experimental results obtained, which follow the rank order $\text{F}^- > \text{Cl}^- > \text{Br}^- > \text{I}^-$ of increasing ion size and hence decreasing surface charge.

Thus, an increase in surface tension with salt concentration does not rule out the presence of dissolved ions at the interface. The polarizable force field is essential in giving the correct results. Judging by the rank order, surface tension appears proportional to the strength of polarization.

Ion-specific Effects

As mentioned earlier, it has been well established that the SF spectrum for the neat water-air interface has two prominent features.

One is the narrow peak centred at 3700 cm^{-1} and the other a fairly broad band from 3000 to 3600 cm^{-1} . The former is the signature of the non-hydrogen-bonded –OH group that sticks out of the liquid phase (free –OH), while the latter is the signature of the O–H stretching vibrations of differently H-bonded water molecules on the liquid side of the interface.

The research team at Beijing National Laboratory for Molecular Sciences, Chinese Academy of Sciences, led by Hongfei Wang obtained SFG vibrational spectra of the water molecules at the NaF and KF aqueous solution surfaces, and found significant differences between them.¹³ The results are presented in Fig. 15.2.

Unlike the NaF solution surfaces, KF solution surfaces at comparable (low) concentrations remained almost unchanged from neat water in the entire range of 3000 to 3800 cm^{-1} (Fig. 15.2, middle panel), which is consistent with the low water-binding tendency of K^+ (see Chapter 9).

However, at high KF concentrations of 2.0 M and 6.0 M (only attainable with KF, not NaF), the spectral intensity of the broad 3400 cm^{-1} band (liquid-like structure) dropped significantly, while the intensity of the broad ice-like band below 3200 cm^{-1} increased well above that in neat water (Fig. 15.2, bottom panel). The band below 3200 cm^{-1} represents symmetric four-coordinate DDAA (D, donor; A, acceptor) water molecules. Thus, there is much more ordered H-bonded water at the interface at high concentrations of KF.

In contrast, the whole broad band in the region of 3000 to 3600 cm^{-1} for NaF solution surfaces decreased significantly with the increase in NaF concentration (Fig. 15.2, top panel), indicating that Na^+ disturbed water hydrogen-bonding with other water molecules, as consistent with its ability to bind to water molecules.

The team also developed and used the surface-sensitive non-resonant SH technique to measure the polarization-dependent response from interfacial water molecules.¹⁴ They looked at KF salt solutions in addition to NaF, because they could extend the bulk concentration range of the KF to 15.4 M compared to only 0.98 M for NaF. Surprisingly, they not only confirmed the F^- effect observed by Tobias' team (see earlier), but also found that KX (X for F, Cl, or Br) solutions caused the average tilt of the non-straddled (non-dangling)

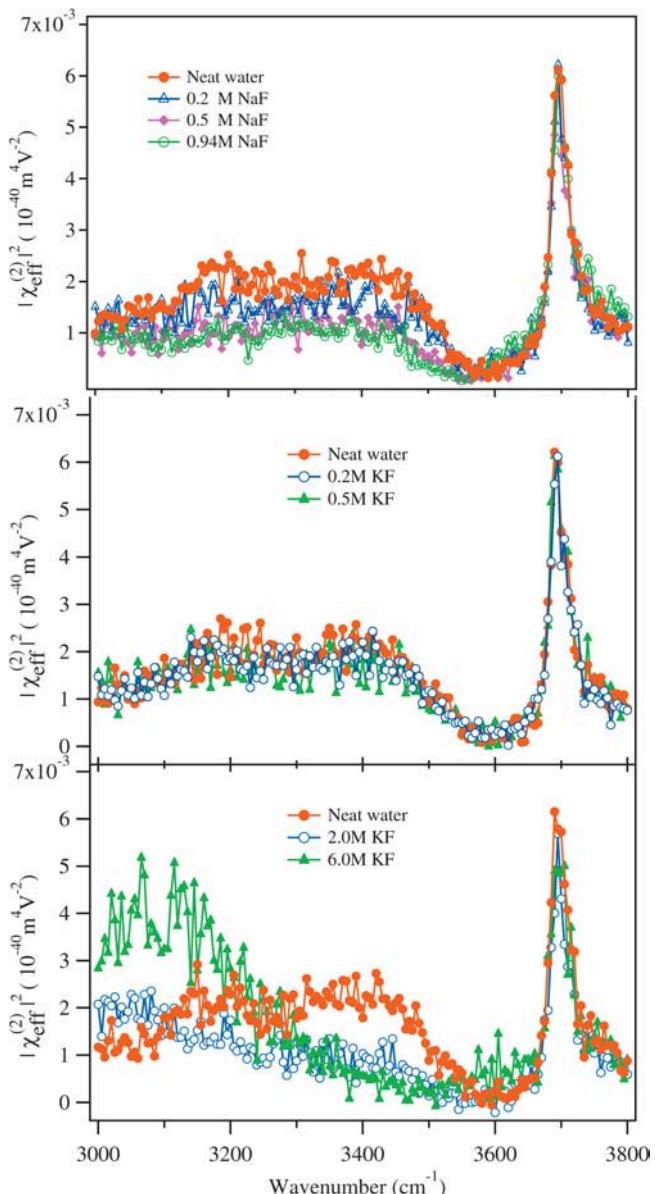


Figure 15.2 SFG vibrational spectra of NaF and KF.¹³

OH bond in the interfacial water molecules to go in the opposite direction from that of the NaX solutions as the concentrations increased. KX caused the tilt angle to become slightly larger than the 40.1° of neat water, while NaF caused the tilt to become slightly smaller.

Also, the thickness of the interfacial water layer at the surfaces of all the six salt solutions increased with bulk concentration. The abilities to increase the thickness of the interfacial water layer were found to be in this order: $\text{KBr} > \text{NaBr} > \text{KCl} > \text{NaCl} \sim \text{NaF} > \text{KF}$. Thus, there were specific cation effects, Na^+ vs K^+ , as well as specific anion effects, F^- vs Cl^- vs Br^- . Note that the rank order for the increase in thickness is in the opposite direction to the increase in surface tension reported by Tobias' team, suggesting that the thickness of the interfacial layer is not correlated with surface tension. Instead, induced polarization in the interfacial region may be more correlated with surface tension.

According to Wang and his team, polarization-dependent, non-resonant SH spectroscopy, unlike SFVS, which measures specific chemical bond stretching vibrations, measures the overall response from the whole interfacial region. In the non-resonant mode, the contribution to the SH signal from the interfacial ions can be neglected, as the hydrated ions can be assumed to be centrosymmetric, so non-resonant SH is the direct measure of the water species in the whole interfacial region—from the hydrogen-bonded water molecules below the topmost layer—because the topmost layer lies almost parallel to the plane of the interface, and their contribution to the measured signal has to be negligible.¹⁵ This is consistent with the SFVS results showing that the narrow free OH peak around 3700 cm^{-1} belonging to water molecules in the topmost layer remained almost unperturbed with the addition of salts (see Fig. 15.2). The interfacial thickness for the NaBr solution increased almost linearly by about 30% as the NaBr concentration increased from 1 to 5.0 M. For the NaCl solution, it increased by about 10%, while for NaF there was about 3% increase as the bulk concentration went to 0.9 M saturated solution. The interface Br^- concentration increased to 1.29 times that of the bulk concentration.

While SH probes the second-order electronic polarizability of water molecules in the interfacial region, the SFVS also probes the second-order vibrational or infrared polarizability, thereby giving different results.

However, there are still disagreements and inconsistencies between experimental measurements from different laboratories as well as in the theoretical and computational results that are being addressed.¹⁶

The interaction of ions with the air-water interface is complicated enough, and the picture not at all clear, even though recent findings have overturned some old assumptions that almost everyone had been taking for granted, such as the lack of ions at the interface. Air-water interfaces are not directly relevant to conditions inside cells though very relevant to the *alveoli*, tiny air sacs lined with fine capillaries that terminate the respiratory tree of the lungs where gas exchange takes place. In the next chapter we shall see what happens when amphiphilic lipids or surfactant molecules are added to air-water interfaces forming model membranes that enclose cells and fill up most of their interior.

Notes

1. “Surface Tension”, Wikipedia, 6 October 2011, http://en.wikipedia.org/wiki/Surface_tension.
2. Bush and Hu (2006).
3. “Surface Tension”, Wikipedia, 6 October 2011, http://en.wikipedia.org/wiki/Surface_tension.
4. See “Water”, Wikipedia, 29 September 2011, <http://en.wikipedia.org/wiki/Water>.
5. “Second-harmonic Generation”, Wikipedia, 30 August 2011, http://en.wikipedia.org/wiki/Second-harmonic_generation; Zhang et al. (2008).
6. Hsieh et al. (2011).
7. Shen and Ostroverkhov (2006).
8. Hsieh et al. (2011).
9. Zhang et al. (2011).

10. Mischa Bonn, e-mail to me, 2 October 2011.
11. Heydweiller, cited in Vrbka *et al.* (2004).
12. Vrbka *et al.* (2004).
13. Feng *et al.* (2009).
14. Bian *et al.* (2009).
15. Bian *et al.* (2008).
16. Feng *et al.* (2011).

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16

Water Meets Membranes

Membranes: the Vital Interface

Membranes not only surround cells, they fill up much of the interior of cells. Cell biology textbooks will tell you that membranes are important because they separate the inside of the cell from the outside, and divide the cell into small compartments. Dividing the cell into small compartments is very important, as I stressed in *Rainbow Worm* and will elaborate further in later chapters of this book. One thing to remember from *Rainbow Worm* is that compartments are dynamic as much as physical, and can exist in the absence of physical confinement.

More important is that life is lived at the interface and depends on it in so many different ways, not least interfacial water. Membranes serve to increase the area of the vital interface enormously.

The eukaryotic cell (belonging to all higher organisms and having a membrane-bound nucleus) is filled with many convoluted, membrane-bound organelles: *mitochondria*, “powerhouses” of the cell where foodstuff is oxidized; *chloroplasts* in green plants and algae, which capture sunlight to reduce carbon dioxide into carbohydrates and split water to release oxygen for air-breathing organisms, including us; the *endoplasmic reticulum* (ER), where proteins, lipids, and steroids are synthesized and other metabolic reactions are carried out; the *Golgi body*, where proteins are processed and packaged for secretion from the cell; not to mention *lysosomes*, in

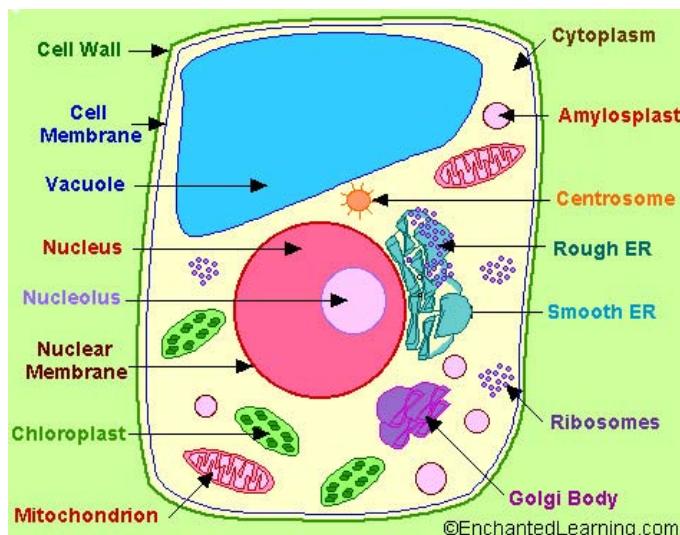


Figure 16.1 Diagram of a plant cell.¹

which waste materials and other cellular debris are broken down for recycling; and other specialized vesicles (see Fig. 16.1).¹

The internal membrane surface area far exceeds the area of the plasma membrane. It has been estimated that in liver cells, the surface area of intracellular membranes is 50 times that of the plasma membrane.² (Figure 16.1 has greatly minimized the extents of the different membrane compartments for clarity of representation.) The proliferation of membranes not only organizes the cell's interior, but also puts the cell's entire interior readily in touch with the outside. That's because the membrane-bound spaces inside are interconnected, and some of the membranes are continuous with the cell membrane, so that membrane-bound spaces inside the cell open ultimately to the outside. Most important of all, the vast surface area of intracellular membranes increases the interfacial liquid crystalline water, along with additional enormous surface areas associated with the cytoskeleton that has the same effect. We shall see what the cell is really like in Chapter 18. For now, let's look at lipid membranes in contact with water as models of biological membranes with interfacial water in the cell.

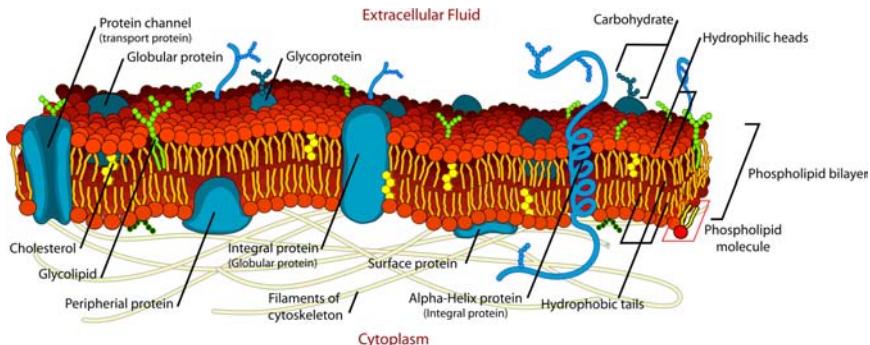


Figure 16.2 Diagram of the cell membrane, by Mariano Ruiz, Wikimedia.³

Biological Membranes

Biological membranes typically consist of a lipid bilayer with proteins embedded in it, and enclose spaces or compartments that differ from the outside.³ Three major classes of lipids make up biological membranes: phospholipids, glycolipids, and cholesterol. Figure 16.2 is a diagram of the cell membrane. Different membranes have distinctly different compositions of lipids as well as embedded or associated proteins.

Phospholipids and glycolipids are amphiphiles with two long hydrophobic hydrocarbon chains linked to a hydrophilic head group. The head group is phosphorylated (with an inorganic phosphate group attached), and consists of glycerol (hence the name phosphoglycerides), or sphingosine, which gives sphingomyelin (see Fig. 16.3). Glycolipids all have sphingosine in the head group with one or more sugar units attached to it. The simplest member is glucocerebroside, with glucose attached to the sphingosine. More complex members, gangliosides, have more than one sugar attached. As can be seen, the hydrophobic chains belong either to two fatty acids in the case of phosphoglycerides, or one fatty acid, and the hydrocarbon tail of sphingosine in the case of sphingomyelin and the glycolipids. The fatty acids in phospholipids and glycolipids usually contain an even number of carbon atoms typically between 14 and 24, 16 and 18 being the most common. They can be

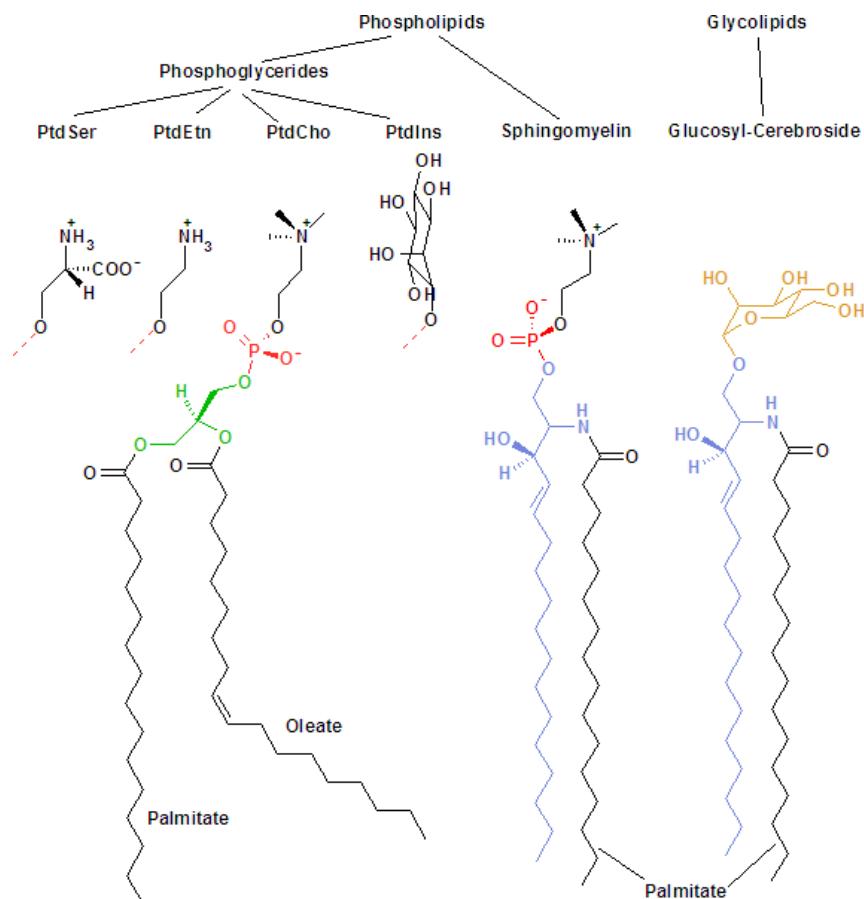


Figure 16.3 Phospholipids and glycolipids; the phospholipids are phosphatidylserine (PtdSer), phosphatidylethanolamine (PtdEtn), phosphatidylcholine (PtdCho), phosphatidylinositol (PtdIns), and sphingomyelin; the glycolipid is glucocerebroside, the simplest member; by BorisTM, Wikimedia.³

saturated or unsaturated, according to whether the fatty acid chain is fully hydrogenated or not. The length and the degree of unsaturation of the fatty acids have an important effect on the fluidity of the membrane, which in turn is critical for its function.

Cholesterol, a polycyclic steroid with a hydrophilic –OH group (Fig. 16.4),⁴ is preferentially associated with sphingolipids in cholesterol-rich lipid-raft areas of membranes, and is important for

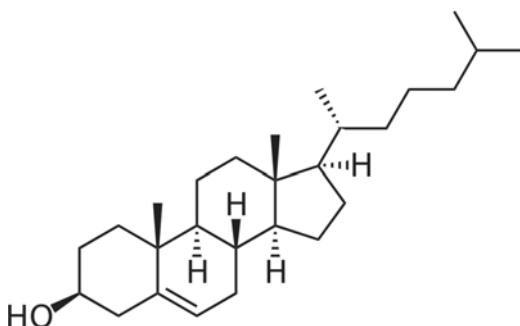


Figure 16.4 Cholesterol, by BorisTM, Wikimedia.⁴

maintaining the integrity of cell membranes, preventing them from becoming overly fluid.

In connection with membrane fluidity, it is important to note that in unsaturated fat or fatty acids, double bonds exist between some neighbouring carbon atoms in the long carbon chain. This gives rise to two alternative configurations: *cis*, where the carbon chain continues on the same side as the segment before the double bond (Fig. 16.3), and *trans*, where the carbon chain continues on the opposite side. Natural unsaturated fats are nearly all in *cis* configuration which increases membrane fluidity, whereas artificial hydrogenation of oil (to make margarine) creates large amounts of *trans* fats that are generally considered bad for health, as they have a tendency to solidify and clog up arteries, increasing the risk of heart disease and stroke.⁵ The US Food and Drug Administration is requiring nutritional labels on foods to contain information on the amount of *trans* fats present, but it is still not required in the UK. Best to avoid margarine and anything that has hydrogenated fats in it.

Wetting Membranes

Hydration of membrane lipids has been studied with nuclear magnetic resonance (NMR), X-ray and neutron scattering, as well as infrared vibrational spectroscopy. The findings reveal that water molecules near the head groups of phospholipids are hydrogen donors and

form strong hydrogen bonds with the lipid phosphate and carbonyl groups, and the electrostatic potential due to the charges on the head group leads to ordering of water dipoles and results in stronger hydrogen bonds between neighbouring water molecules. Molecular dynamics (MD) studies suggest that water forms stronger hydrogen bonds with the lipid phosphate groups than the carbonyl ($-\text{C}=\text{O}$) groups, and the most probable sites to accept a hydrogen bond are the double-bonded oxygen atoms connected to the phosphorus atom. Moreover, at the phospholipid-water interface, water is thought to be perturbed up to 1 nm away by the dipole potential of the head group. However, direct probing of water at the biological membrane interface has been relatively rare.

Sum frequency vibrational spectroscopy (SFVS) allows scientists to do just that, as we have seen in earlier chapters. It has been used to study water orientation at the phospholipid-water interface directly. By now, you will be well aware that in pure water, the dangling (free) $-\text{OH}$ groups of surface water molecules give a peak at 3700 cm^{-1} while hydrogen-bonded O-H modes give a broad peak from 3000 to 3600 cm^{-1} .

When the water surface is covered with a phospholipid monolayer, the dangling $-\text{OH}$ groups decrease significantly, but still persist at the surface with a red-shifted (lower) frequency. However, the signal in the H-bonded region increases by many times in the presence of all kinds of phospholipids, providing evidence of an ordering of water molecules by the phospholipid head groups. The question is: do the water dipoles point towards the lipid tails sticking up into the air, or into the bulk water?

Well, it so happens that you can tell, in phase-sensitive SFVS. The technique gives the imaginary part of the complex nonlinear second-order electric susceptibility — the ability of a material to polarize in response to the field inside the material — which indicates its orientation (see Chapter 14).

Researchers at the Ohio State University, led by Heather C. Allen, carried out a study using a series of phospholipid monolayers created at the air-water interface.⁶ The five phospholipids included in the study have different head groups and charges but the same

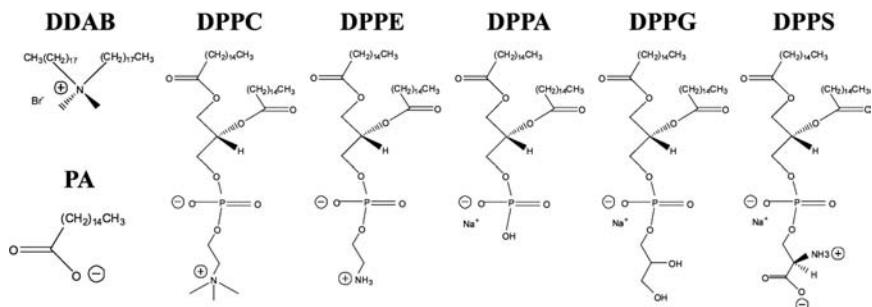


Figure 16.5 Phospholipids and simpler derivatives used in water ordering study.⁵

fatty acid tails: dipalmitoyl phosphatidylcholine, DPPC; dipalmityl phosphatidylethanolamine, DPPE; dipalmitoyl phosphate acid, DPPA; dipalmitoyl phosphatidylglycerol, DPPG; and dipalmitoyl phosphatidylserine, DPPS. In addition, the water orientation by simpler molecules — palmitic acid, PA, negatively charged at pH 13, and dimethyldioctadecylammonium bromide, DDAB, positively charged — was investigated to check the phase determination (see Fig. 16.5).

The first experiments were carried out with pure (neat) water, and water on which the simple derivatives, PA and DDAB, were spread in a monolayer. The results and the interpretations are given in Fig. 16.6.

The intensity spectrum of pure water shows two characteristic overlapping peaks at 3200 and 3400 cm^{-1} , commonly referred to as ice-like and liquid-like water structures (Fig. 16.6a, bottom panel). The phase spectra (Fig. 16.6a, middle panel), according to previous information, assign the donor (D) O-H bond stretches to the negative region (3450 to 3600 cm^{-1}) of three-coordinate water molecules, DDA and DAA (A, acceptor), in the topmost layer; the negative region 3200 to 3450 cm^{-1} has been assigned to asymmetrically donor-bonded four-coordinate DDAA molecules, while the positive region from 3000 to 3200 cm^{-1} was mainly attributed to symmetrically donor-bonded DDAA molecules. The assigned regions overlap, leading to a crossover point around 3200 cm^{-1} .

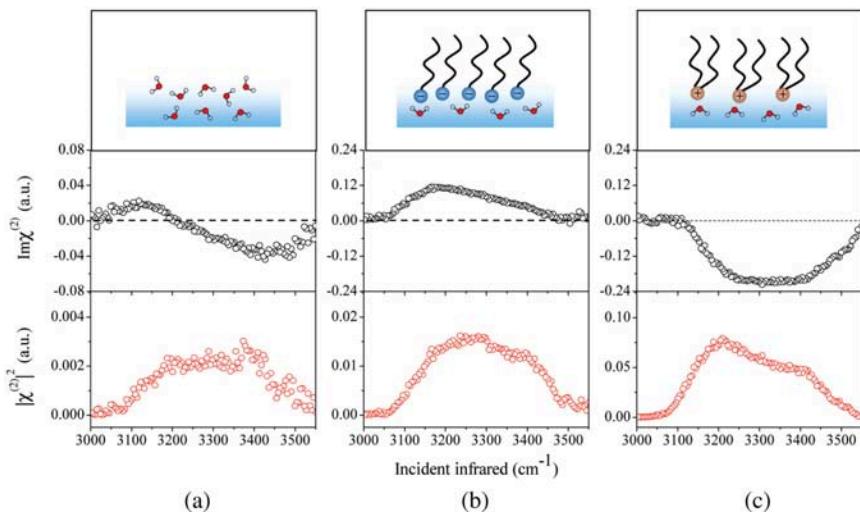


Figure 16.6 Phase-sensitive SFVS of neat water (a), PA (b), and DDAB (c).⁵

In the experimental setup, the negative parts of the spectrum correspond to hydrogen pointing down, whereas the positive part corresponds to hydrogen pointing up (Fig. 16.6a, top panel). As expected, PA gives positive peaks with hydrogen pointing up (Fig. 16.6b), and DDAB gives negative peaks (Fig. 16.6c) in the entire region with hydrogen pointing down, in accord with the neat water data.

With the negatively charged phospholipids, DPPA, DPPG, and DPPS, the conventional SFVS spectra agree generally with published results (see Fig. 16.7a, bottom panel): DPPS gives the lowest intensity, followed by DPPA and DPPG.⁴ The phase-sensitive spectrum indicates that all the peaks are positive, with the hydrogen atoms pointing up. The absolute intensities of the signal are about five- to ten-fold that of neat water, indicating greater polarization, though not necessarily that more layers of water are ordered. All negatively charged phospholipids order the water molecules with hydrogen pointing up, similar to PA. This indicates that the phospholipid head groups determine the net interfacial water orientation despite the difference in functional groups and head group

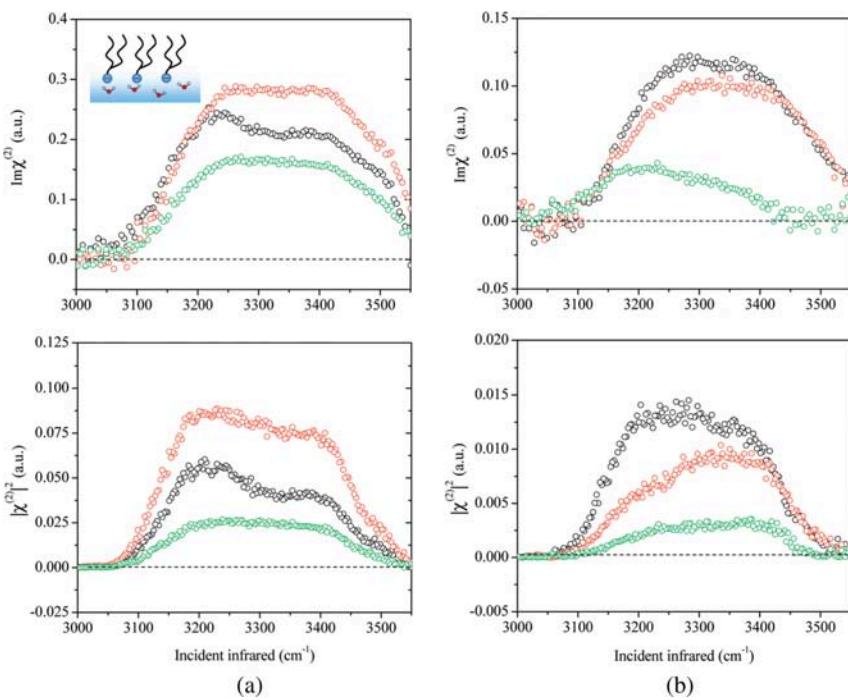


Figure 16.7 Spectra from negatively charged phospholipids (a): DPPG (red), DPPA (black), DPPS (green); and neutral phospholipids (b): DPPE (red), DPPC (black), DPPC (green), with 0.4 M CaCl_2 .⁴

orientation. These phospholipids together with their sodium counterion form an electric double layer in the interfacial region.

Phospholipids with zwitterionic (net neutral) head groups such as DPPC and DPPE are common in biological membranes, and hence most studied. As seen in Fig. 16.7b, these phospholipids give positive phase signals over the whole range of the hydrogen-bonded O-H stretch region, indicating that interfacial water molecules are oriented on average with hydrogen atoms pointed up towards the surface, similar to the negatively charged monolayers, although the lower relative intensity suggests a weaker orienting effect. These results agree with recent MD simulations suggesting that the charge on a phosphatidylcholine head group is regarded as negative by interfacial water molecules despite its zwitterionic

nature. Adding 0.4 M CaCl_2 to the water beneath DPPC greatly decreased the intensity of the signal without changing the phase.

Ca^{2+} Dehydrates Phosphate Groups

Cations are attracted to both the carbonyl and phosphate groups. Owing to the greater negative charge of the phosphate group, it is reasonable to suppose that Ca^{2+} ions favour binding to the phosphate rather than the carbonyl group.

Looking at a different part of the SFVS spectra, two informative peaks were found at ~ 1070 and $\sim 1100 \text{ cm}^{-1}$ (Fig. 16.8, left): the first was assigned previously to the stretch of the P–O ester bond (attaching the phosphorus atom to the fatty tail in the phospholipid) and the second to the symmetric stretch of PO_2^{2-} (the two single-bonded oxygen atoms). The second peak is known to be sensitive to the hydration state of the phosphate group, and blue shifts (to higher wavenumber) upon dehydration.

As can be seen, adding Ca^{2+} blue-shifted the PO_2^{2-} peak to $\sim 1109 \text{ cm}^{-1}$ (Fig. 16.8, left), suggesting that calcium-binding dehydrated the

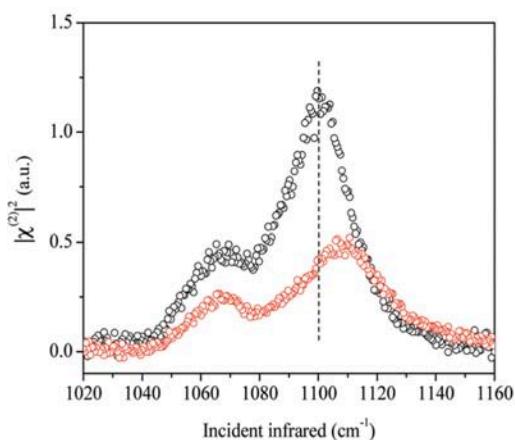


Figure 16.8 Effect of CaCl_2 on interfacial water; left: spectra of DPPC on neat water (black) and on 0.4 M CaCl_2 (red); right: diagram of DPPC monolayer with negatively charged phosphate group in blue and positively charged choline group in brown, Ca^{2+} as green spheres and Cl^- as purple spheres.⁴

phosphate group, making the interfacial water less ordered (Fig. 16.8, right).

The PO_4^{2-} peak remains nearly unchanged with 0.5 M NaCl, suggesting that the electrostatic interaction between sodium ions and the phosphate is relatively weak and does not affect the hydration shell of the phosphate. At the Ca^{2+} concentration used, interfacial water molecules still experience a net negative charge from the DPPC so the phase spectrum is still positive.

The effect of Ca^{2+} in reducing the polar ordering of interfacial water was confirmed in a subsequent study using a monolayer of PA.⁷ Ca^{2+} interacted strongly with the $-\text{COO}^-$ group of PA, much more so than Mg^{2+} also included in the study.

Ca^{2+} plays a key role in the ubiquitous *signal transduction cascades* that amplify an initial signal or stimulus into a change in activity of an entire cell or tissue.⁸ However, its mechanism of action is still poorly understood.

Like Na^+ , Ca^{2+} is a weak kosmotrope (see Chapter 9), and most likely acts by binding to $-\text{COO}^-$ and phosphate groups of protein as well as membrane lipids, perturbing interfacial water and changing the conformation of the protein or membrane lipid as well as the local cellular environment. We shall return to this important topic in the next chapter, and at the end of this book.

As you may have noticed, studies on model systems are still fragmentary and tend to concentrate on one type of molecule at a time. This has the advantage of starting from the simplest system, in the hope that complex systems can be understood by adding components to the simple system one at a time. But the biggest pitfall of the reductionist analytic approach is that complex systems are, as a rule, not merely the sums of their parts. In the next chapter, we shall examine what happens when proteins and ions meet water together.

Notes

1. “Plant Cell Anatomy”, Enchanted Learning, <http://www.enchantedlearning.com/subjects/plants/cell/>.

2. Alberts *et al.* (1994).
3. “Membrane Lipids”, Wikipedia, 9 January 2011, http://en.wikipedia.org/wiki/Membrane_lipids.
4. “Cholesterol”, Wikipedia, 12 October 2011, <http://en.wikipedia.org/wiki/Cholesterol>.
5. “Trans Fats 101”, University of Maryland Medical Center, accessed 3 November 2010, <http://www.umm.edu/features/transfats.htm>.
6. Chen *et al.* (2010b).
7. Tang *et al.* (2011).
8. “Calcium Signaling”, Wikipedia, 28 November 2011, http://en.wikipedia.org/wiki/Calcium_signaling.

The Rainbow Ensemble

How Proteins and Ions Do Water's Quantum Jazz

Apart from water, the cell is crowded with molecules of the most diverse kinds, the most abundant of which, in terms of atomic and molecular diversity, are proteins that make structures, transport ions and metabolites, and catalyse the thousands of chemical reactions that enable organisms to live life to the full. You already know that proteins can't do anything without water, and ions play a major role in how water dances with membranes and proteins. So that's where we start in getting acquainted with the incredible rainbow ensemble of molecules that dance life into being.¹

Salt Out If You Must

The interaction of charged ions with water and proteins is at the heart of numerous signal transduction processes in the cell. Enzymes and cofactors are highly specific in their requirements for metal ions, while the addition of a phosphate group to proteins and metabolites — phosphorylation — is widely involved in activating enzyme pathways of biosynthesis and energy metabolism. What is the origin of these ion-specific effects? You will not be surprised to know that water holds the key. You have seen already that Ca^{2+} , which plays a key role in many signal transduction cascades, has a great fondness for phosphate groups and carboxylate groups, and its binding introduces considerable disorder into interfacial water.

The first question we need to ask is: why do different salts vary so much in their ability to dissolve in water? And why do some salts precipitate proteins from solution more so than others?

As described in Chapter 9, small ions of high surface charge density are kosmotropes (order-inducing) and bind water molecules strongly, while large ions of low surface charge density are chaotropes (disorder-inducing) and bind water molecules weakly relative to the strength with which water molecules form hydrogen bonds with one another. You will have noticed by now that the terms “order-inducing” and “disorder-inducing” cannot be taken literally, as the net effect of the given ion could be just the opposite of what it is supposed to be, especially when other molecules are present besides water.

Kosmotropes tend to attract a solvation shell with a large number of molecules, while chaotropes have small solvation shells with fewer water molecules. But that is only half the story. The other half of the story is how the ions interact with proteins in the presence of water.

Czech scientist Franz Hofmeister (1850–1922) discovered that some salts helped egg white proteins to dissolve in water, while others caused the proteins to precipitate out, and there were those that had effects in between. He ranked the ions according to their ability to “salt out” and “salt in”, which resulted in the *Hofmeister series*. The Hofmeister series is also correlated with the ability of the ions to induce protein unfolding, coalescence of bubbles, and many other phenomena, though there has never been a satisfactory explanation.²

Kim Collins at the University of Maryland Medical School may have found the answer, and it is related to the ions’ affinity for water relative to that of the ion-binding groups on proteins, the most important of which are the carboxylate side chains of aspartic and glutamic acids.³

When pairs of oppositely charged ions have similar affinities for water, something special happens: they come out of their solvation shells, join up, and neutralize each other. That’s because they can just as easily form intimate partners with each other as with water molecules; exchanging water molecules for the counter-ion does not cost anything in energetic terms. This law of matching water

affinities appears to explain why certain salts are less soluble than others, and why some salts precipitate proteins out of solution while others help them dissolve. The answer is that only neutral molecules precipitate (or crystallize) out of solution; neutral molecules have a much lower solubility.

More specifically, according to Collins, a radius of 1.06 Å separates small monovalent cations from large ones, and a radius of 1.78 Å separates small monovalent anions from large ones. Small monovalent ions are strongly hydrated, while large monovalent ions are weakly hydrated (see Fig. 17.1). For example, LiF contains small monovalent ions that readily come out of their hydration shells to pair up as contact ion pairs; it has a solubility of only 0.1 M. In contrast, CsF has a large cation and a small anion that do not pair up in solution; it has a solubility of 24.2 M. In the same way, NaF saturates at 0.94 M, while KF saturates at 15.4 M.

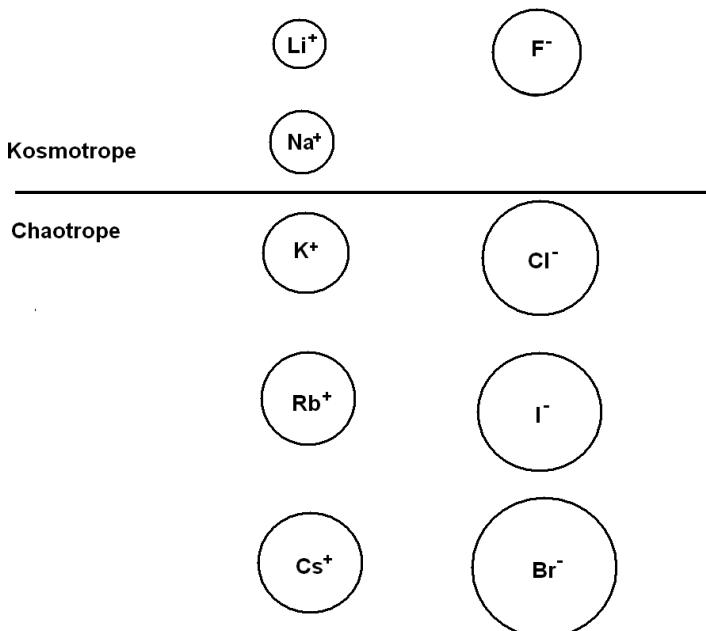


Figure 17.1 Monovalent kosmotropes and chaotropes ordered by size; the size of their hydrated ion is in inverse order.³

Proteins have strong negatively charged, ionized carboxylate groups ($-COO^-$) on their side chains that pair up well with kosmotrope cations, so Na^+ salts out proteins, while a chaotrope such as Cs^+ salts them in. Similarly, Ca^{2+} is well matched to carboxylate in water affinity and will also salt out proteins.

Support for the Law of Matching Water Affinities

Collins' theory has received strong support from other scientists. Pavel Jungwirth and colleagues at the Czech Republic Academy of Sciences have quantified the higher affinity of Na^+ over K^+ for proteins by means of molecular dynamics simulations and conductivity measurements.⁴ Both methods showed that sodium binds at least twice as strongly to the protein surface than potassium, and it is the same for all proteins studied. The carboxylate groups of aspartate and glutamate side chains (see Chapter 10) played a major role. This same selective preference of the carboxylate groups for Na^+ can be demonstrated in the isolated amino acids, glutamic acid and aspartic acid, as well as in short peptides, and even in the simplest carboxylic acids, formate or acetate, as shown by molecular dynamics and quantum chemical calculations.

The research team led by Richard Saykally at the US Department of Energy's Lawrence Berkeley National Laboratory provided further support to Collins' theory using the laboratory's sophisticated X-ray probes of molecular bonds in samples ejected as microjets.⁵ The selective binding of Na^+ was demonstrated for acetate, though not for formate.

However, Berk Hess and Nico van der Vegt at the Max Planck Institute for Polymer Research showed that Collins' law fails to describe the interaction of cations with carboxylate on the surface of many proteins.⁶ Their molecular simulation results showed that the order of increasing binding affinity with carboxylate, $K^+ < Na^+ < Li^+$, is caused by a stronger preference for forming weak solvent-shared ion pairs — ion pairs that retain their solvation shells — rather than contact ion pairs that join up directly with each other. Contact pair interactions of these cations with protein surfaces, according to their simulations, are insignificant, indicating that the thermodynamic

Table 17.1 Ionic composition of intracellular and extracellular fluids (mM)

Ion	Intracellular	Extracellular	
		Plasma	Interstitial
Cations			
Potassium	160	4	4
Sodium	10	142	145
Calcium	2*	5	5
Magnesium	26	2	2
Total cations	198	153	156
Anions			
Chloride	3	101	114
Bicarbonate	10	27	31
Phosphate	100**	2	2
Sulphate	20	1	1
Organic acid	0	6	7
Protein	65	16	1
Total anions	198	153	156

*Almost all intracellular calcium is bound, with only 0.0001 mM existing as free cation.

**Free phosphate only 5 to 8 mM.

stability and interactions between proteins in alkali salt solutions are predominantly through hydration water molecules. But this turns out not to be true, as we shall see in Chapter 23.

Why Quantum Jazz is Possible

The most important explanation offered by Collins' theory is of why the ions present inside cells are so different from those outside, which has long puzzled biologists (see Table 17.1).⁷ Intracellular fluid has high concentrations of potassium and magnesium cations and phosphate and sulphate anions, and very low concentrations of sodium and chloride. The converse is true of extracellular fluid: low in potassium, magnesium, phosphate, and sulphate, and high in

sodium and chloride. While there appears to be not much difference between extracellular and intracellular calcium, most of the intracellular calcium is bound, with only 10^{-7} M free Ca^{2+} most of the time, except for very transient, local increases associated with signal transduction.

Apart from the inorganic ions, there are some 6.5 mM of proteins present in the cytoplasm rich in carboxylate anions in their side chains (equivalent to ten times in ionic concentration). As Collins pointed out,⁸ the intracellular ions are optimized for mismatches in water affinities, so as to maintain high solubility of the proteins and other constituents of the cytoplasm at all times. That's why quantum jazz of the rainbow ensemble is possible.

Increasingly, protein-folding disorders are being identified, including Alzheimer's disease, Parkinson's disease, transmissible spongiform encephalopathies (mad cow disease), Huntington's disease, and type II diabetes, which have been linked to ligand binding and hydration.⁹ In all likelihood, these diseases represent different failures in keeping almost all the molecular participants in cellular biochemistry dancing with water at any one time, so some of them end up salting out at inappropriate places. (That's why the structure of water is so important, and everyone should be reading this book!)

In view of the high affinity of sodium ions for carboxylate, the intracellular concentration of sodium is kept very low, and, it is generally believed, by an Na^+/K^+ -ATPase that pumps sodium out of the cell in exchange for potassium. RNA and DNA and membrane phospholipids are phosphate diesters built upon the phosphate anion. Phosphate and carboxylate anions are the fundamental ions of the cell. Phosphate is also important in metabolism, where many small molecules are phosphorylated to keep them in the cell and to provide a "handle" for enzymes to bind onto (very likely mediated by water). The nucleotide triphosphates (adenosine triphosphate, ATP, and others) play an apparently critical and essential role in energy metabolism. (We shall see how critical ATP is in Chapter 23.) Phosphate functions as a reversible marker in signal transduction, with phosphorylation activating or deactivating proteins. As seen in

Chapter 16, phosphate groups bind strongly to water as hydrogen acceptors, and Ca^{2+} , which is well matched to carboxylate, as well as to phosphate, acts as a dehydrating agent. This suggests that hydration and dehydration of proteins and metabolites may be one important avenue to signal transduction.

Ca^{2+} readily pairs with carbonates and inorganic phosphates, forming insoluble complexes that organisms need, *but only in the right place and at the right time*. Calcium carbonate in eggshells and oyster shells is highly insoluble, calcium oxalate (kidney stones) even more so, and calcium hydroxyphosphate in bones and teeth is the most insoluble of all.

So, while the intracellular concentration of free phosphate is about 5 to 8 mM, the concentration of free calcium is 10^{-7} M. To maintain this low concentration, calcium is pumped out of the cell or returned to intracellular stores, of which a major one is the endoplasmic reticulum.¹⁰

The intracellular high concentration of potassium is well suited to keeping phosphate in solution; the solubility of K_2HPO_4 is about 8.6 M, while that of Na_2HPO_4 is 0.93 M. K^+ is also mismatched to carboxylates compared with Na^+ as indicated by *in vitro* experiments mentioned earlier. Nevertheless, K^+ may still form pairs with carboxylates under certain circumstances, as it is only a weak chaotrope (see Fig. 17.1).¹¹ We shall see how proteins in the cytoplasm actually prefer K^+ over Na^+ in Chapter 23.

In contrast to K^+ , which has a water affinity apparently lower than carboxylate and phosphate, that of Mg^{2+} is higher; which makes Mg^{2+} suitable as an intracellular ion. It predominantly interacts with proteins and nucleic acids through shells of water molecules. ATP, the main energy intermediate in organisms, must be bound to a magnesium ion in order to be biologically active. What is called ATP is actually Mg-ATP. Similarly, magnesium plays a role in the stability of all polyphosphate compounds in the cells, including those associated with DNA and RNA synthesis. Magnesium is involved in the folding and stabilization of RNA, both in a “diffuse” form without a specific binding site and by binding tightly at discrete sites, not mediated by water.

The Big Mystery Remains

But the big mystery remains. How has the cell managed to have just the right combination of ions inside, which is completely the opposite of what's on the outside? According to the membrane theory of the cell, generally accepted almost without question today, it is the armoury of channels and pumps located in the cell membrane that keeps the intracellular ion concentrations so distinctly different from that outside. But how could the cell have evolved before it acquired all the complicated pumps and channels? This key question will pre-occupy us from this point on, for it will lead to the new cell biology we are after.

Let's begin with the work of Philippa Wiggins, a pioneering water researcher now at Mairangi Bay, Auckland, New Zealand.

Water's Effortless Action through Ions

Wiggins was among the first to take seriously the idea that water exists in two states under ambient conditions — low-density water (LDW) and high-density water (HDW) — which is now finally gaining acceptance within the conventional community (see Chapter 3). She has further suggested that the spontaneous interconversion of these two forms of water may be what gives life its seemingly boundless "free energy".¹²

Bioenergetics is the big problem of how living organisms capture and transform energy for growth, development, and all that life entails, and it fills many pages in standard biochemistry textbooks.

In a nutshell, green plants capture energy from the sun in photosynthesis to make simple carbohydrates (the building blocks for everything else) from carbon dioxide and water. In respiration, carbohydrates are broken down ultimately back into carbon dioxide and water, and along the way, energy is abstracted to make ATP. ATP is also made in photosynthesis, but the most important job of photosynthesis is capturing carbon for making carbohydrates, and amino acids, proteins, nucleotides, nucleic acids, membrane lipids, etc., down the line.

According to conventional wisdom, ATP is the “high-energy intermediate” or “energy currency” that fuels all living processes: synthesizing proteins, DNA, RNA, carbohydrates, and fats, operating molecular motors, ion pumps, and so on. The free energy of hydrolysis of ATP is transferred to the enzyme involved, which then uses the energy to perform the work of transport, or sliding filaments, or synthesis of peptides, polynucleotides, etc.

But that’s a myth, says Philippa Wiggins, who has spent nearly 40 years investigating the role of water in living processes.

Wiggins does not question the observation that ATP hydrolysis — (reaction with water) into ADP (adenosine diphosphate) and Pi (inorganic phosphate) — is an essential part of many reactions in living organisms. She questions the central idea that ATP hydrolysis is necessary and sufficient for transforming energy, while the role of water as solvent and organizing medium for the reactions is completely ignored.

Many of the chemical reactions taking place in living organisms require extraordinarily harsh conditions if done in a test tube without enzymes. So the story goes that enzymes can work miracles by “conformational” (shape) changes, again ignoring the role of water.

For example, to break peptide bonds in proteins or nucleotide bonds in DNA or RNA would require boiling in strong (6 M) hydrochloric acid, whereas with the enzymes — protease, DNase, and RNase — the bonds are readily broken at neutral pH and ambient temperature. Similarly, our bodies make bones (consisting largely of calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$) continuously (and break them down continuously too) from very dilute solutions of calcium and phosphate at pH 7.4 and 38 °C. The salts crystallize out from water in conjunction with specialized bone-forming cells, the osteoblasts. To make similar ceramics requires a temperature of 1200 °C. Wiggins showed how it is more likely the change in physical chemical properties of water that enables enzymes to work and salts to crystallize out from dilute solution precisely where it is appropriate.

Wiggins is not alone in thinking that water is the lead player in living processes. Gilbert Ling¹³ has been criticizing the conventional account of energy transformation since the 1950s and

proposed a comprehensive alternative theory that I shall describe in Chapter 23.

Water in Confined Spaces

The two-state model of water explains many seemingly baffling and contradictory observations that Wiggins and her colleagues and a handful of other researchers have made over the years, and more important, tell a much more coherent story of how water, by changing its physical and chemical properties, can make apparently impossible chemical reactions happen effortlessly in living organisms.

Wiggins and colleagues began experimenting in the 1980s with cellulose acetate films that have small pores about 2 nm in diameter. By soaking the films in aqueous solutions containing different salts, they discovered how different ions completely changed the physical and chemical properties of water.¹⁴

They found that the water in the pores of films soaked in water, NaCl, LiCl, or MgCl₂ solutions had the infrared spectrum of ice Ih, like LDW, whereas films soaked in solutions of KCl or CsCl had the spectrum of liquid-like water.

The water in the pores was also highly selective to ions. KCl and CsCl were accumulated if the external concentration was small, and as the external concentration increased, the concentration in the pores became equal to the external. NaCl, LiCl, HCl, CaCl₂, and MgCl₂ were increasingly excluded from the pores; and their degree of exclusion increased with the external concentration. The selectivity to K⁺ vs Na⁺ is similar to that of the cell, which has high concentrations of K⁺ inside and low concentrations of Na⁺, the reverse of the situation in blood and extracellular fluids. Wiggins and colleagues concluded that KCl and CsCl were selectively accumulated into the ice-like LDW in the pore, but that at high concentrations, made LDW revert to normal water. NaCl, LiCl, and MgCl₂, on the other hand, were selectively excluded (the exclusion of Mg²⁺ does not occur in the cell, so cellulose acetate pores are not exactly comparable to the cell), and imposed an osmotic pressure gradient on the pore water, causing it to decrease in density and increasing the exclusion of the ions.

What's the evidence that an osmotic gradient due to excluded ions is involved? Adding an excluded salt such as $MgCl_2$ to the external solution to balance out the osmotic pressure gradient increases the accumulation of KCl in the pore water.

After many experiments of a similar nature, not only with cellulose acetate films, but also with microporous polyamide beads and hydrophobic glass beads, and investigations on the changes in viscosity of water with different solutes, Wiggins proposed a dynamic theory of phase changes in water induced by ions and other small molecules.

Wiggins' Theory

The two-state model of water is represented as domains of HDW mixed with LDW, as opposed to the single-state homogenous water (see Fig. 17.2).

A solute in single-state water will experience a single environment, whereas in two-state water, the solute will partition differently in the two kinds of domains according to its preference for the different domains. Consequently, instantaneous gradients will be set up between neighbouring unlike domains. In Fig. 17.3, the solute has preference for LDW; this sets up local gradients in osmotic pressure which are eliminated by the transformation of LDW into HDW.

Conversely, a solute that partitions preferentially in HDW will create local gradients that are eliminated by conversion of HDW into LDW.

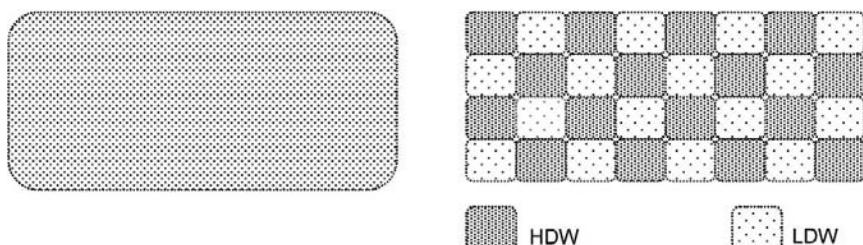


Figure 17.2 Diagrammatic representation of single-state homogeneous water (left) and two-state water with mixed domains of HDW and LDW.¹⁴

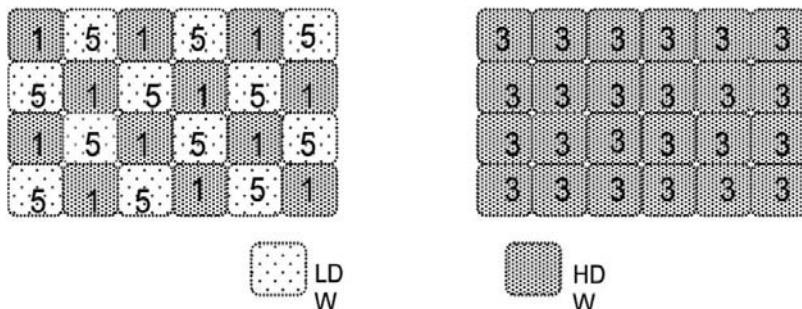


Figure 17.3 A solute that partitions preferentially into LDW domains (left) creates local gradients in osmotic pressure that are eliminated by conversion of LDW domains into HDW domains.¹⁴

In other words, there are two classes of solutes, those that prefer LDW and others that prefer HDW, and once they partition into their preferred domain, they tend to transform it into the other due to the local osmotic gradient created. And that's how a dynamic cycle can be kept going. It is really quite ingenious, and can explain many of the miraculous things that enzymes and ATP are supposed to do.

The two classes of solutes also happen to correspond, roughly, to the traditional distinction between chaotropes (K^+ , NH_4^+ , Cl^- , Br^- , HCO_3^- , HSO_4^- , H_2PO_4^-), that decrease the structure of water (by inducing HDW), and kosmotropes (H^+ , Li^+ , Na^+ , Ca^{2+} , Mg^{2+}), that increase the structure of water (by inducing LDW, which has lower entropy than HDW). Note that this scheme differs from the usual description of chaotropes and kosmotropes (see Chapter 9).

Making ATP Without Enzymes

One of the most convincing pieces of evidence for water's effortless action — which requires no energy input — is the synthesis of ATP from ADP in the LDW within the pores of cellulose acetate films. In the experiment, ADP was converted to ATP in the presence of nothing but potassium phosphate (K_3PO_4) to provide inorganic phosphate. It was a spontaneous reaction in LDW, and the K^+ -induced transformation of the LDW to HDW released the ATP (and associated K^+) to the

external solution so the dynamic cycle could be repeated. When the external solution contained NaCl or MgCl₂, however, no ATP was detected. That was because the ATP formed in LDW in the pores was trapped inside, and could not be released because NaCl and MgCl₂ stabilizes LDW, and the dynamic cycle was interrupted.

The cell makes ATP by the membrane-bound enzyme ATP synthase, requiring a flux of H⁺ through the enzyme, and down a concentration gradient (see Chapters 21 and 22). The concentration gradient across the membrane is built up by abstracting energy from food (in respiration) or from the sun (in photosynthesis). (As described in Chapter 13, the existence of a concentration gradient in the bulk phase is hotly disputed. There is now ample evidence that H⁺ ions simply migrate along the interfacial water on the membrane to the ATP synthase.) According to Wiggins, the flux of H⁺ ions is required, not to provide energy for ATP synthesis, but to break down the LDW formed within the cavity containing the active site of the enzyme, allowing the release of spontaneously synthesized ATP.

Enzyme Action Depends on Two-state Water

How does the hydrolysis of ATP, proteins, nucleic acids, etc., by enzymes take place?

Water is a very weak acid and base (see Chapter 9). The concentrations of H⁺ and OH⁻ ions are each at 10⁻⁷ M at 25 °C. This again is unusual because oxygen-containing acids of neighbouring elements in the periodic table are often very strong, such as H₂SO₄ and H₃PO₄. The reason is that H⁺ is the most powerful kosmotrope of all univalent cations, and the OH⁻ ion is one of the very few kosmotropes among univalent anions (negatively charged ions). Water ionizes in HDW, but not in LDW; however, the immediate consequence of ionizing in HDW is to convert the HDW to LDW to eliminate the steep osmotic pressure gradient created between neighbouring domains, so liquid water is largely unionized.

Strong acids such as H₃PO₄ ionize first to produce the powerful kosmotrope H⁺ and the powerful chaotrope H₂PO₄⁻, so there is practically no osmotic imbalance created, and hence little

conversion of LDW into HDW domains or vice versa. In this case, water remains ionized in the HDW domains.

In order to carry out hydrolytic reactions, there must be sufficient ionized H^+ and OH^- ions present.

This suggests how enzymes that carry out hydrolytic reactions might work. Proteases, for example, often require Ca^{2+} , which reinforces the ionization of water, so water surrounding the peptide bond can act as a strong acid and strong base to break it. The same would apply in the case of DNase and RNase that break polynucleotide bonds.

Similarly, in the operation of the sodium pump, Na^+/K^+ -ATPase, which pumps Na^+ out of the cell in exchange for K^+ , it is the change in water structure that provides the decisive, effortless action, rather than the “free energy” from the hydrolysis of ATP into ADP.

Figure 17.4 shows how the enzyme might work: (a) the Na^+/K^+ -ATPase cavity with the active site of the enzyme in its resting state contains HDW with Na^+ as counter-ion to the aspartic acid residue that's to be phosphorylated, and to two negative sites near the top of the cavity; (b) Mg-ATP phosphorylates the aspartic acid residue, Mg^{2+} remaining as counter-ion, and LDW is induced and moves up the cavity, pushing Na^+ out ahead of it through the open channel as K^+ enters selectively into the LDW; (c) the K^+ ions induce HDW to balance the osmotic gradient created and ionizes water, so the phosphate is hydrolysed off; K^+ , Mg^{2+} , phosphate, and ADP

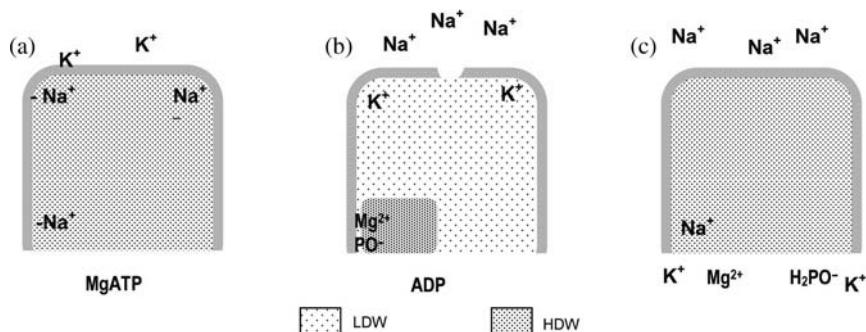


Figure 17.4 How the Na^+/K^+ -ATPase enzyme works.¹⁴

diffuse out and Na^+ re-enters to neutralize the aspartic acid residue. Two more ions enter in exchange for K^+ and the cavity reverts to its resting state (a).

Wiggins deals with many more examples in her paper, which is aptly titled “Life Depends upon Two Kinds of Water”.¹⁵ Reading it will upset, if not overturn, previously held beliefs and convictions about biochemistry and bioenergetics. Wiggins may be wrong on details, but the big picture has a lot going for it. The interconversion between two states of water is the key. Wiggins’ findings already have numerous applications in the preservation of blood cells and frozen cells.

We don’t have all the answers yet, but water is beginning to appear as both concertmistress and lead player in the quantum jazz of life.

Notes

1. Ho (2010e).
2. Preuss (2008).
3. Collins (1997, 2006).
4. Vrbka *et al.* (2006).
5. Uejio *et al.* (2008).
6. Hess and van der Vegt (2009).
7. Patlak, “Ionic Composition of Body Fluids”, accessed 19 August 2010, <http://physioweb.med.uvm.edu/bodyfluids/ionic.htm>.
8. Collins (2006).
9. Silva *et al.* (2010).
10. “Calcium Signaling”, Wikipedia, 28 November 2011, http://en.wikipedia.org/wiki/Calcium_signaling.
11. Collins (2006).
12. I first wrote about Wiggins’ work in Ho (2006e); Wiggins’ paper, first released on the web in 2003 (<http://www.lsbu.ac.uk/water/contr2.html>), was republished as Wiggins (2008).
13. Ling (2001).
14. The account is based on Ho (2006e).
15. Wiggins (2008).

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18

True Portrait of the Cell

What's Wrong with Our Picture of the Cell?

We now know that a typical cell is filled with membranes and organelles, as well as an extensive cytoskeleton (see Chapter 16 for a diagram of a cell). But for far too long, the cell has been regarded as a membrane enclosing an otherwise featureless cytoplasm consisting of proteins dissolved in water. Although no one would subscribe to that “bag of enzymes” view today, the dominant theory of how enzymes work, and how energy and materials are transformed in living systems, is still based on bulk-phase chemistry of random diffusion and chance collision between reactants in dilute solution. In other words, it is based on an implicit picture of a disorganized cell not much more than a bag of enzymes in water. Correspondingly, a key role is credited to the cell membrane in preventing the free diffusion and loss of cell contents while protecting the cell against noxious substances in the environment (see Chapter 17).

Before going further, let us retrace our steps to see how we got here, so we can leap over the present impasse to understand life at a time when we have the most powerful analytical and theoretical tools at our disposal. So, a galloping digression into the history of cell is in order.

Protoplasm vs Cell

Joseph Needham (1900–1995), distinguished British chemical embryologist/physical biochemist and historian of Chinese science,¹ was

also one of the most trenchant critics of the prevailing view of the disorganized cell. His book *Order and Life*, first published in 1936,² drew attention to the remarkable physicochemical properties of living protoplasm that are completely different from proteins in aqueous solutions, and presented evidence, even then, for the meticulous molecular organization that exists inside the cell. In the same book, Needham also anticipated the discovery of the liquid crystalline organism in my laboratory by 56 years, proposing that organisms are polyphasic liquid crystals. He was clearly way ahead of his time.

Thus, the transparent sea-urchin eggs have a light absorption spectrum distinctly different from that of crushed eggs, or eggs burst open in hypotonic solution, both of which resemble the absorption profile of a solution of egg albumin instead. The study of living organization at the time focused on protoplasm as the unit of life, and not the cell.

The single-celled *Amoeba* was a favourite experimental animal. When amoebae were immersed in noxious solutions of narcotics or organic solvents such as ethyl alcohol, chlorethane, chloroform, or ether, their lipid-soluble cell membrane gave them little protection, and the poor creatures soon succumbed. But microinjecting the noxious solutions or solvents directly into the protoplasm inside the membrane had no effect at all. Similarly, high concentrations of picric acid that would have coagulated proteins in solution, and were very toxic when applied directly to the external surface of the amoebae, were well tolerated when microinjected into the protoplasm.

Simultaneously, evidence was emerging that the newly discovered metabolic pathways for the oxidation of glucose were exquisitely organized in the protoplasm, so much so that the product of one enzyme appeared directly shunted on as substrate to the next enzyme in the pathway, with little or no free diffusion involved.

The importance of protoplasm rather than cell is the thesis of a recent extended essay³ by Rickey Welch, now at Cambridge University, and James Clegg at the University of California, Davis, Bodega Marine Laboratory, both leading champions of the organized cytoplasm, and I have featured their work prominently in *Rainbow Worm*. Welch and Clegg argue that the origin of the systems view of the cell dates back 150 years to the birth of the protoplasm

concept, and it is the protoplasmic theory, not the cell theory, that set the course for the development of modern cell biology. The emphasis on cell over protoplasm is hence entirely misplaced.

From Proto-life to Cell and Protoplasm

According to Welch and Clegg, the protoplasmic theory reached its high point 150 years ago, but its influence lasted only 50 years or so thereafter, waning early in the 20th century as the cell theory took over along with the reductionism of molecular biology (although, as we have seen, the protoplasmic theory of life was still being vigorously defended by people like Joseph Needham).

The notion of “proto-matter” that underlies life originated in ancient Greece, and the establishment of Newtonian mechanics rekindled interest in the role of proto-matter in animate systems during the 18th century. The cell theory was conceived in the 19th century, largely due to the work of three Germans: physiologist Theodor Schwann (1810–1882), botanist Matthias Jacob Schleiden (1804–1881), and physician Rudolf Virchow (1821–1902); although it also owed much to the invention of the compound microscope in the mid-17th century, and scientists who made use of it and improved it.⁴ English philosopher and polymath Robert Hooke (1635–1703) first used the term “cell” to describe the walled compartments of dead cells he saw in a slice of cork under the microscope. Dutch tradesman and scientist Antonie van Leeuwenhoek (1632–1723) was the first to see and describe the living cell of the green alga *Spirogyra*. The idea that cells in multicellular organisms could be separated into individual units was proposed by German botanists Ludolph Christian Treviranus (1779–1864) and Johann Jacob Paul Moldenhawer (1766–1827), which led French physician, botanist, and physiologist Henri Dutrochet (1776–1847) to declare that “the cell is the fundamental element of organization”.

In its fully developed form, the cell theory stated that all living organisms are constructed of units or cells, the cell being a “bladderlike structure with membrane contents and nucleus”,⁴ thereby giving primary emphasis to the nucleus and the cell wall, or membrane, while relegating cellular contents to a secondary role.

The cell theory came under widespread attack in the mid-19th century. French zoologist Felix Dujardin (1802–1860) described the physicochemical properties of the living substance inside the cell membrane and German botanist Hugo von Mohl (1805–1872) was credited with the first use of the term “protoplasm” to describe what was inside the plant cell. Subsequently, German biologist Ferdinand Cohn (1828–1898) in 1850 unified plants and animals not only in their being constructed from cells, but also, at a most fundamental level, sharing “a common substance, protoplasm, filling the cavities of those cells”.

Max Schultze (1825–1874), a German microscopic anatomist, was responsible for the conviction that the true basis of life is to be found in the study of protoplasm, and not the cell. His paper on the study of the muscle syncytium was a milestone, as US historian of science Gerald L. Geison (1943–2001) commented: “The publication of this paper, more than any other single event, marked the birth of the protoplasm theory of life. On physiological rather than structural grounds, and with special emphasis on the properties of contractility and irritability, Schultze demonstrated that a single substance, called protoplasm, was the substratum of vital activity in the tissues of all living organisms, however simple or complex.”⁵

Swedish historian Erik Nordenskiöld (1832–1901) went further. His assessment in 1928 was that Schultze’s work “laid the foundation on which cell research has since been built” and “marks a new era in the science of cytology”.⁶

Extending Schultze’s ideas, German physician and physiologist Ernst von Brücke (1819–1892) wrote an influential paper published in 1861 suggesting that protoplasm must have a complex infrastructure like an “elementary organism”, which provides the seat of all cellular activities.⁷

On 8 November 1868, British biologist Thomas H. Huxley (1825–1895) (“Darwin’s bulldog”) gave a public lecture in Edinburgh titled “On the Physical Basis of Life”, which was published in the popular journal *The Fortnightly Review* in 1869. The lecture literally made “protoplasm” a household word, as well as its perceived role as the “locus of life”.

Closer to our time, US historian Thomas S. Hall at Washington University in St. Louis remarked that the protoplasm theory engendered “the final repudiation of the tradition comparing living units to utricles, sacks, boxes, bubbles, or any other sort of envelope or container”. As it gained momentum, some biologists even argued that the term “cell” should be dropped. “Thus some 150 years ago, protoplasm had come to signify ‘matter possessing a certain molecular constitution permitting it to manifest life’.”⁸

By the late 19th century, however, biology, like physics, was heading inexorably towards atomism, and hence speculations on the smallest living unit of protoplasm soon focused on “living molecules”. With the rise of biochemistry, the metaphor of the cell as a “chemical factory” took over, as did analogies with the division of labour in industrial factories.⁹

Enzymes became the centre of attention. As Welch and Clegg remarked: “Throughout the history of biochemistry, it has been taken as a matter of course that most metabolic activity of the cell results from the superposition of the action of individual enzymes dissolved in an aqueous phase, with the dynamics being governed by simple mass-action laws and random thermal motions of metabolite molecules in weak electrolyte solution. Despite the ‘structural’ and ‘organizational’ view of the cell coming from protoplasmic theorists, the newfound gas-phase kinetics and the *in vitro* chemical laws in the later 19th century were grafted on to the analysis of cellular metabolism.”¹⁰

A number of key observations fostered this view. In Germany, botanist Wilhelm Pfeffer (1845–1920) reported in 1877 that the cell behaved as if a semi-permeable plasma membrane separated two ordinary aqueous solutions, one inside and the other outside the cell. Later, chemist Eduard Buchner (1860–1917) won the 1907 Nobel Prize in Chemistry on the strength of his fermentation studies in cell-free yeast extracts, which gave full credence to the view that the cell is merely a bag of enzymes, setting the stage for the development of biochemistry during the next 50 years.¹¹

The rediscovery of the work of Austrian monk Gregor Mendel (1822–1884) on particulate inheritance at the beginning of the

20th century gave just the right impetus to the great hunt for the molecular basis of the gene that culminated in the discovery of DNA and its double helical structure, initiating us to the present era of genetic engineering.¹² “Protoplasm” was soon relegated to a distant relic of a bygone age, as Welch and Clegg lamented.

The Skeleton in the Cell

But protoplasm did not go away. It is the skeleton in the cell that turned up in the latter half of the 20th century.

It was the invention of the microscope that put the cell centre stage in biology. However, as microscopic techniques became more and more powerful — with sophisticated light microscopy, confocal microscopy, fluorescence techniques, antibody staining, *in situ* hybridization, electron microscopy (including high-voltage electron microscopy) — the interior of the cell revealed itself to be highly complex, not only stuffed with membranes and organelles, but also a cytomatrix¹³ in the spaces remaining, which has been assumed to be proteins in free solution, and hence referred to as “cytosol”.

Canadian-born US cell biologist Keith R. Porter (1912–1997), whose work began in the 1940s, believed that the cytomatrix consisted of organized macromolecules, and was convinced this would be revealed with improved methods of microscopy and preparation of specimens. In the early 1970s, Porter played a key role in installing a high-voltage electron microscope at the University of Colorado. Using this microscope, he obtained images of what he called the “microtrabecular lattice”, an extensive network of *trabeculae* (cylinders 7 nm in diameter and of variable length) that ramified throughout the cell (see Fig. 18.1).¹⁴ Porter also stated that the volume between the formed elements in the microtrabecular network was the “water-rich” space.

There is no doubt that Porter’s “microtrabecular lattice” corresponds largely to what we now know as the cytoskeleton, pictures of which fill entire scientific journals.¹⁵ It is about time Porter is given credit for the discovery. A recent opinion paper presented abundant evidence that the spatial organization and regulation of protein synthesis in eukaryotic cells are due to the cytoskeleton,

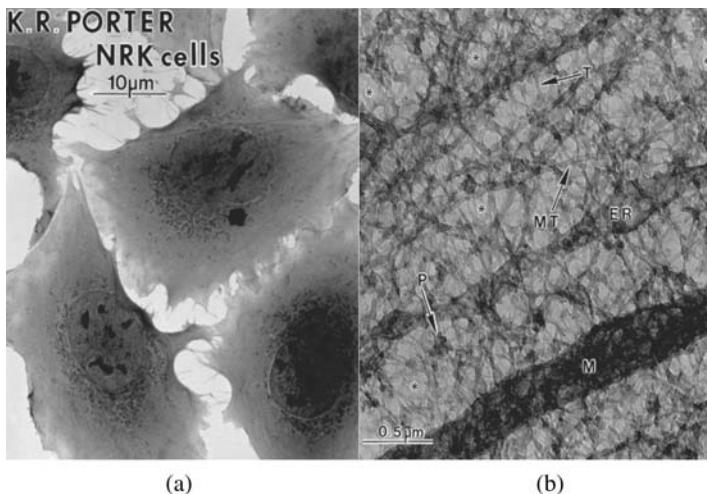


Figure 18.1 High-voltage electron micrograph of whole cultured newborn rat kidney cells (low power, right), magnified (left) to show the microtrabecular lattice; M, mitochondria; ER, endoplasmic reticulum; T, trabeculum; courtesy of Rick Welch and Jim Clegg.

notably actin microfilaments. It stated: “The cytoskeleton has evolved as a scaffold that supports diverse biochemical pathways”,¹⁶ which is precisely what Porter had proposed for the microtrabecular lattice, as Welch and Clegg reminded us.

Clegg cautions against the idea that the microtrabecular lattice is a fixed scaffold. Instead, it is a dynamic structure consisting of cytoplasmic proteins of both signalling pathways and much of intermediary metabolism binding and unbinding on time scales of perhaps seconds.¹⁷

The discovery of the cytoskeleton was a complex history of conjectures, inferences, and piecing together of fragmentary observations, described as a “300-year epic” involving numerous scientists across several continents.¹⁸ Only when fluorescent antibodies became available to stain cytoskeletal proteins was the “panoramic” view of the entire cytoskeleton unveiled. Figure 18.2 is a state-of-the-art digital image of a live cultured cell stained with fluorescent antibodies for actin and tubulin, proteins representing the two major cytoskeletal networks (see later).

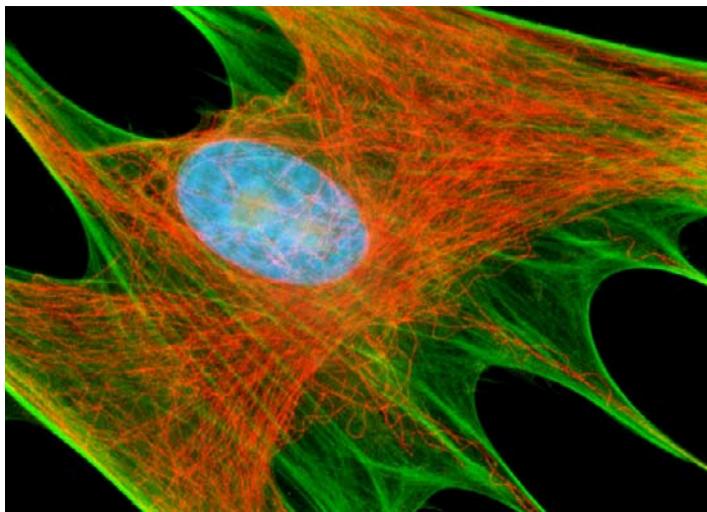


Figure 18.2 African water mongoose skin fibroblast cell (A.P. Mongoose line), stained with antibodies against tubulin (red) and actin (green); the nucleus appears in light blue.¹⁹

The Cytoskeleton in all Cells

The cytoskeleton is present in *all* cells, both eukaryotes (higher organisms) and prokaryotes (bacteria), where it was a more recent discovery. The cytoskeleton is a dynamic structure that serves to maintain cell shape, enables the cell to move and to divide, and provides tracks for intracellular transport of vesicles and organelles. It also offers an enormous surface area for anchoring enzymes in the cytoplasm, and most of all for interfacial liquid crystalline water. In eukaryotes, the cytoskeletal filaments are formed from proteins of three superfamilies: actin, tubulin, and intermediate filaments. Actin and tubulin form dynamic actin filaments and microtubules respectively. The intermediate filaments consist of more diverse proteins, and form relatively more static structures (see Box 18.1). Together, the three kinds of cytoskeletal filaments link up into an intricate web that criss-crosses the entire interior of the cell, but is constantly changing according to the activities of the cell.

Box 18.1

The Cytoskeletal Proteins²⁰

In eukaryotes, the cytoskeletal filaments are formed from proteins of three superfamilies: actin, tubulin, and intermediate filaments (IFs, which include the keratins, lamins, and other specialized proteins).

There are several tubulin proteins; the best characterized are α - and β -tubulin, the main components of microtubules. Microtubules make up the mitotic spindle, a special structure for separating daughter chromatids (newly duplicated chromosomes) during mitosis (cell division). The microtubules are attached to an organelle, the centrosome located near the centre of the cell, from which the filaments radiate out like a starburst. Microtubules also provide tracks for molecular motors to transport molecules, vesicles, or organelles. Microtubules are made up of 13 filaments. Each filament consists of longitudinally associated heterodimers of $\alpha\beta$ -tubulin, and 13 filaments laterally associate into a tube-like structure.

A guanosine triphosphate (GTP) binding site is at the N-terminus of the protein (with the $-\text{NH}_2$ group of the first amino acid in the polypeptide chain), while the C-terminal domain (with the $-\text{COOH}$ group of the last amino acid in the polypeptide chain) carries some of the catalytic residues for GTP hydrolysis. The tubulin proteins are GTPases (enzymes that break down GTP by hydrolysis into GDP and Pi), with the active site formed at the interface between subunits. Thus, GTPase activity only occurs when two or more subunits associate. There is a distinct polarity in the filaments with α and β subunits alternating. The β -tubulin is at the plus end while the α -tubulin is at the minus end. Microtubules exhibit dynamic instability, where the filaments may grow or shrink rapidly. Subunit exchange cannot occur within the filament, so the filaments can only grow or shrink from the ends, though growth is preferentially at the plus end. The nucleotide-binding pocket between subunits of tubulin is occluded, so nucleotide exchange within the filament is also prohibited. GTP hydrolysis induces a destabilizing conformational change within the filament, causing a bent or curved morphology. The GDP bent form of the filament is unstable, and if

(Continued)

Box 18.1 (Continued)

unrestrained, the filament disassembles rapidly. The ends of the microtubules are often capped with GTP. If the GTP-cap is hydrolysed, the filaments are free to spontaneously disassemble.

Eukaryotic actin is a highly abundant protein. It forms a dynamic network for motor proteins to transport cargo around the cell. Actin filaments are nucleated near the cell membrane and form a cortex beneath the membrane. In some cells, actin is largely responsible for determining cell shape and enabling cell locomotion. For example, actin filaments are directly involved in the formation of *pseudopodia* that enable amoebae to crawl. Actin and myosin form the core part of muscle cells.

Actin has two domains, I and II, each with two subdomains, A and B. The larger two of these, IA and IIA, comprise a five-stranded β -sheet enclosed by three α -helices. IB and IIB, the smaller subdomains, show variation in both size and structure across the actin family. Between the two domains lies a highly conserved ATP-binding pocket containing essential aspartate residues that, together with either Mg^{2+} or Ca^{2+} , bind and hydrolyse ATP. Hydrolysis of ATP is central to the disassembly of polymerized F-actin (see later). Actin shows cooperative assembly kinetics, with a slow nucleation step. The actin subunits assemble into dynamic helical polymers known as F-actin, which consists of two helical strands wrapped around each other. After polymerization, each actin subunit undergoes structural changes facilitated in part by the rotation of domains I and II with respect to each other. Actin is asymmetric, and the ends of filaments have different biochemical properties. Actin displays *tread-milling* as the two ends have different propensities for polymerization. Actin filaments preferentially assemble at the barbed end, and after ATP hydrolysis and phosphate release, subunits dissociate from the pointed end. This leads to a net movement of subunits through the filament, or tread-milling.

Intermediate filament proteins form filaments with a diameter between that of F-actin and microtubules. They are abundant, relatively stable filaments that provide mechanical support in a wide range of eukaryotic cell types. Intermediate filaments form a layer just

(Continued)

Box 18.1 (Continued)

beneath the nuclear membrane. They have no role in cell motility, and are not able to undergo tread-milling. IF proteins are fibrous monomers extremely α -helical in structure, but otherwise very diverse.

The three kinds of filaments form networks that ramify throughout the entire cell and are cross-linked or stabilized by associated proteins.

All three families of eukaryotic cytoskeletal proteins have counterparts in bacteria.²¹

The FtsZ protein forms a contractile ring structure that mediates cell division, and is the prokaryotic counterpart of tubulin. MreB forms a helical cytoskeletal element that functions in a number of processes, including morphogenesis and segregation of chromosomes; it is a homologue of actin. Crescentin resembles intermediate filament protein in that it polymerizes into long filaments *in vitro*, and acts as a determinant of cell curvature in the crescent-shaped bacterium *Caulobacter crescentus*. In addition, there are other proteins such as MinD and ParA that have no homologue in eukaryotes.

EF-Tu, a bacterial protein well known as an elongation factor in protein synthesis, is actually a major protein of the prokaryote cytoskeleton, as discovered by Frank Mayer at Georg August University, Göttingen.²² EF-Tu is the main constituent of a cytoskeletal web located close to the inner surface of the cytoplasmic membrane, enclosing the entire cell cytoplasm and exhibiting additional fibres crossing the cytoplasm. This protein has been known to form filaments and bundles *in vitro*. Mayer's team found non-random distribution of EF-Tu; the protein appeared to be aligned in rows and networks. A bacterium with a truncated gene for EF-Tu inserted in addition to the wild-type gene led to the dissolution of the cytoskeletal web, resulting in the lysis of the cell. *In vitro*, the truncated form of the protein inhibited self-assembly of the protein into protofilaments. EF-Tu can make up 9% of total bacterial cell protein, sufficient for forming a cytoskeleton, more so than Mbl, and a related protein Mreb. Mayer suggested that EF-Tu might have been a cell-stabilizing (cytoskeletal) protein first, and the elongation factor function might have evolved later.

Metabolic Channelling in the Cytomatrix

While the various subcellular structures were being discovered in the course of the 20th century, the major metabolic pathways were also being charted out. Electron microscopy and cell-fractionation techniques began to show that certain enzymes are associated with specific organelles, and since then, more refined microscopy, *in situ* analyses, and extraction procedures have provided increasing evidence that a multitude of intermediary metabolic reactions are located on the surfaces of intracellular membranes and cytomatrix structures, including signal transduction, intracellular trafficking, protein synthesis, and DNA/RNA processing.²³ For example, enzymes of the glycolytic pathway were once thought to be classic examples of soluble proteins, but we now know that many of them, possibly all, are not free to diffuse. Not only the enzyme proteins localized to the surfaces of intracellular membranes and the cytoskeleton, but also the substrates or metabolic intermediates, are much restricted in their diffusion. Renewed attention has been given to the role of localized heterogeneous micro-environments, or nanospaces,²⁴ in defining the true kinetic and thermodynamic nature of biochemistry in the living cell. In *Rainbow Worm*, I elaborated on how the nested (fractal) organic space-time optimizes both the thermodynamic efficiency and kinetics of living processes. Nanospaces represent the microscopic end of spatial organization in the cell, but they do not define the time dimensions. The astonishingly fine differentiation of time even within a nanospace will be revealed in later chapters. Here I shall describe evidence for kinetic optimization of enzyme reactions.

Metabolic channelling refers to the direct transfer of metabolic intermediates from one enzyme to the next without free diffusion.²⁵ Such enhanced probability for intermediates to be transferred from one active site to another requires stable or transient association of the relevant enzymes in multienzyme complexes, or metabolons. Metabolons, therefore, contain enzymes of a part of or the whole metabolic pathway. In other words, metabolism depends on the structural organization of enzymes and substrates in microcompartments or nanospaces.

The metabolon was discovered by US scientist Paul Srere (1925–1999), who isolated it from mitochondria by gentle sonication, and found that it contains all the Krebs (tricarboxylic acid) cycle enzymes, resulting in high metabolic flux.²⁶ An early observation that led to the recognition of metabolons was that the average concentration of oxaloacetate in the mitochondrial matrix is too low to sustain the Krebs cycle at its measured rate. Therefore, a high local concentration must exist in the microenvironment (microcompartment or nanospace) of citrate synthase.²⁷

Due to metabolic channelling, the rate of a biochemical reaction is faster than free diffusion by a factor of 1 000. The classic example reported in 1970 is the *E. coli lac* repressor, which finds the operator site on the DNA at a rate 1 000-fold the upper limit of the diffusion rate for macromolecules.²⁸

We must remember that the cell is extremely crowded with solute, macromolecules, and membranes. The cytoplasm has a protein concentration of some 200 to 300 mg/ml. In the mitochondria, enzymes and other proteins constitute more than 60% of the matrix volume. The high protein density results in a gel-like structure. Srere once remarked that the mean distance between enzymes with an average molecular mass of 100 000 Da is well below the diameter of an average tetrameric protein molecule;²⁹ hence associations of proteins are very likely. In Chapter 20, we shall see how enzymes entrapped in nanospace can exhibit precisely the “superactivity” observed *in vivo*, even without metabolic channelling from non-random association of enzymes.

Electromagnetic Signalling and Assembly of Metabolons

More to the point, unless there is non-random association of enzymes into metabolons and metabolic channelling, there is no way for enzymes and substrates to find each other by free diffusion in such a crowded solution. What none of the proponents of the organized cytomatrix and metabolic channelling has tried to explain is why

that should happen. Why do enzymes that share a function or related function associate, and how do substrates home in on the enzyme?

The answer is: they do it by electromagnetic signalling. Enzymes and substrates share a common frequency, i.e., they resonate to each other and hence attract each other. In the same way, enzymes that share a pathway may also have a common resonating frequency. I have argued for this since the first edition of *Rainbow Worm*, and there has been fresh evidence since, as mentioned in Chapters 6 to 8.

We are now getting a much better picture of what the cell and cytoplasm are really like, an extremely crowded, yet meticulously organized, gel-like sponge with numerous microcompartments or nanospaces filled with water; but nevertheless, extremely dynamic and responsive. It is clear that any attempt to model the cytomatrix and the associated liquid crystalline water as static systems is beset with difficulties, simply because of the hive of activities that take place inside the cell.

Systems such as the massive exclusion zone of liquid crystalline water next to surfaces of gels, or those next to quartz, are the ideal, static, equilibrium models (see Chapter 12). That's perhaps what a totally resting, quiescent living cell approaches. Any activity will take it away from that state. Furthermore, the organization into microcompartments or nanospaces effectively isolates local action from the bulk phase, so that many different local equilibrium or non-equilibrium states can co-exist in the same cell, a thesis that I have developed at length in *Rainbow Worm*. To complete this chapter, let us look at some new mathematical models of what the cell is really like.

The Fractal Cell

A *fractal* is a physical or dynamical structure that has fractional dimensions instead of the usual one, two, or three dimensions. It is defined as a “rough or fragmented geometric shape that can be split into parts, each of which is (at least approximately) a reduced-size

copy of the whole, a property called self-similarity".³⁰ Although the mathematics of fractals goes back to the 17th century, the term "fractal" was coined by French-American mathematician Benoit Mandelbrot (1924–2010) in 1975. Since then, fractal geometry has been found to describe practically all natural processes from coast-lines, branching of trees and blood vessels, to allometric scaling of organisms³¹ and the healthy heart beat.³² Indeed, some physicists have argued that space-time itself is fractal, and I subscribed enthusiastically to that idea in *Rainbow Worm*.

It has not escaped the notice of some biologists that the complex structure of the cytomatrix is fractal. And fractal dynamics also provides the proper theoretical principles for understanding enzyme kinetics, mass transport, and thermodynamic flow-force relationships in the cellular microenvironments, as Welch and Clegg pointed out.

In a paper published in 1994, Miguel Aon and Sonia Cortassa at Universidad Nacional de Tucumán proposed that the cytoplasm is fractal in structure. By quantifying the fractal dimension in micrographs of quick-frozen, deep-etched electron micrographs of the cytoskeleton, they showed that the cytoplasm can be described as a percolation cluster or random fractal.³³

A percolation cluster is the ensemble of holes or sites in a lattice connected to a chosen centre to which a fluid is injected, so that the fluid will percolate to those sites. The most remarkable feature of percolation processes is the existence of a percolation threshold, below which the spreading process is confined to a finite region. The percolation probability $P(p)$ is the probability that a fluid injected at a site chosen at random will wet infinitely many sites. Below the percolation threshold, the cluster behaves as locally connected while above it the connection extends indefinitely. Near the critical probability P_c , as the number of holes p is increased, the percolation process undergoes a transition from a state of local connectedness to one where the connections extend indefinitely.

Consequently, local cytoplasmic fluctuations or perturbations may spread to remote regions in the cell. This implies that above a

critical threshold, cytoplasmic activities may show global coherent behaviour.

This is exactly the kind of dynamics needed for the most efficient and responsive utilization of energy and material resources that maximizes both global cohesion and local autonomy, as argued in *Rainbow Worm*.

A similar proposal was made by Bichu Wu and colleagues at the Institute of High Energy Physics, Academia Sinica, Beijing, in a paper also published in 1994.³⁴ Using an optical microscope with video-recording to track the movement of particles in a living cell of the water mould in culture, they discovered three modes of movement: local mode, in which the particle is confined in a limited region (radius $0.64 \mu\text{m}$); extended mode, in which the particle has an extended trajectory (radius $2.73 \mu\text{m}$); and mixed mode, in which some parts of the trajectory are local while other parts are extended (radius $2.28 \mu\text{m}$).

The tracks were analysed with the line-segment method. Beginning with one end of the track, a circle of radius r is drawn, and a line is drawn between the starting point and the point where the circle intersects the track. The intersecting point is then used as the new starting point and the procedure is repeated until it comes to the other end of the track, and the number of line segments $N(r)$ recorded. The entire procedure is repeated with another value of r . A log-log plot results in a straight line, which is consistent with fractals or self-similarity. The fractal dimensions are obtained from the slope of the fitted curve.

A total of 25 tracks were analysed and the fractal dimensions varied from 2.04 to 1.35, and were independent of the size of the particles.

One apt model of the fractal cytoplasm is the Sierpinski sponge (Fig. 18.3). The self-similar structure at many scales is well displayed. Spaces connect to smaller spaces and so on, leading to the smallest nanospaces with quantum molecular energy machines embedded in its liquid crystalline water, freely improvising its part in the grand quantum jazz of life.

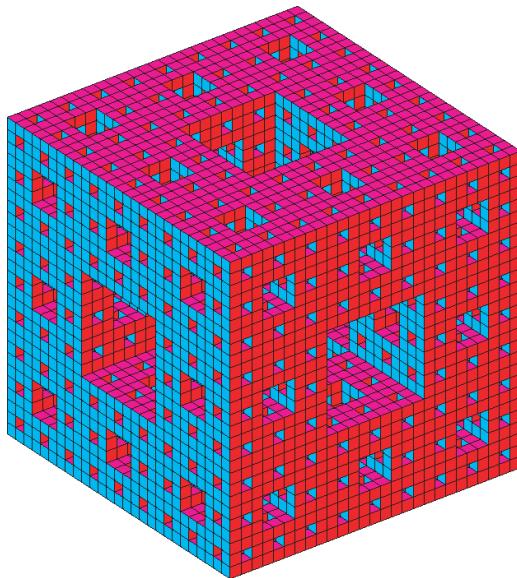


Figure 18.3 The Sierpinski sponge by Moses Boone³⁵ as a model of the cytoplasm.

Notes

1. “Joseph Needham”, Wikipedia, 3 October 2011, http://en.wikipedia.org/wiki/Joseph_Needham.
2. Needham (1936).
3. Welch and Clegg (2010).
4. “Cell Theory”, Wikipedia, 17 October 2011, http://en.wikipedia.org/wiki/Cell_theory.
5. Geison (1969).
6. Nordenskiöld (1928); von Brucke (1861).
7. Welch (1995).
8. Hall (1969).
9. Reynolds (2007).
10. Welch and Clegg (2010), p. C1282.
11. Kohler (1972).
12. See Ho (1998, 1999, 2007).

13. The term “cytomatrix” was used in an influential article by Clegg (1984).
14. Porter (1984).
15. “Cytoskeleton”, *Traffic, The International Journal of Intracellular Transport*, http://www.traffic.dk/virtual_issue_page.asp?id=8.
16. Kim and Coulombe (2010).
17. Clegg (2010).
18. Frixione (2000).
19. From “Small World Competition: Gallery”, Nikon MicroscopyU, <http://www.microscopyu.com/smallworld/gallery/index.html>.
20. See Alberts et al. (2008).
21. Monahan and Harry (2009).
22. Mayer (2003).
23. Clegg et al. (2001).
24. Mayer (2003).
25. Clegg et al. (2001).
26. Ovádi and Saks (2004).
27. Srere (1985).
28. Riggs et al. (1970).
29. Srere, cited in Ovádi and Saks (2004).
30. “Fractal”, Wikipedia, 13 October 2011, <http://en.wikipedia.org/wiki/Fractal>.
31. See Ho (2004a,b).
32. See Ho (2007g).
33. Aon and Cortassa (1994).
34. Tang et al. (1994).
35. “Sierpinski Sponge”, by Moses Boone, 30 May 2003, <http://www.mathworks.com/matlabcentral/fileexchange/3524>.

Water in Nanospace

Structured Water at £1.50 a Bottle¹

Structured water is serious science, as you must have realized by now. Generations of big instruments have been deployed to detect it, among the latest being ultrafast electron crystallography.² Scientists have dedicated over a decade of neutron scattering, X-ray diffraction, nuclear magnetic resonance, dielectric and infrared spectroscopy, etc., not to mention the herculean number-crunching to extract information from the observations, and hours upon hours of computer simulations that go into reproducing the data according to models that may or may not be correct.

The best that the armoury of high-power instruments can produce are ghostly diffraction patterns, and bumps on squiggly-lined spectra that only the specialists running these scientific séances can decipher. Nevertheless, structured water has captured the public imagination, and enterprising companies have made it a selling point for water-purification devices, in one case for bottled water selling at £1.50 a time (though it went off the market rather quickly).

Up until 2004, no one had actually seen structured water itself, which makes it much more elusive than unidentified flying objects or poltergeists. But it was finally caught on camera; not an ordinary camera, admittedly.

First Sighting of Structured Water

Researchers at the A.J. Drexel Nanotechnology Institute at Drexel University, the University of Illinois at Chicago, and Tokyo Institute of Technology produced stunning high-resolution transmission electron micrographs of carbon nanotubes of different sizes with water trapped inside.³

Carbon nanotubes, a new form of carbon discovered in 1991, are long thin tubes either single-walled or multi-walled, and can be closed or open at the ends, depending on how they are made. (See Fig. 13.2 in Chapter 13 for a diagram of a single-walled carbon nanotube.) Yury Gogotsi, Haihui Ye, and colleagues previously found that autoclaving multi-walled nanotubes with closed ends and inner diameters ranging from 2 nm to 200 nm caused water to enter through defects in the walls and remain trapped inside the hollow tube. This provided a great opportunity for investigating the properties of water at different scales of confinement. There has been a spate of discoveries suggesting that water and other fluids in confined spaces (of nanometre dimension) have strange properties, exhibiting new phases and behaviour not observed in bulk.

Theoretical studies had predicted new phases of ice inside carbon nanotubes, but not all scientists agreed. So there is nothing like real data to help settle the issue.

The nanotubes were sealed with water in a gold capsule, which was then treated in the autoclave at 300 to 650°C under 20 to 80 MPa pressure (1 MPa, megapascal = 1 000 000 pascals ~10 atmospheres). This treatment caused the nanotubes to fill up with water, ready to have their pictures taken by a high-resolution transmission electron microscope (TEM). Accompanying the high-resolution imaging was a parallel modelling of the molecular arrangements using a computer software package, HyperChem. As a result, corresponding HyperChem snapshots were obtained, showing models of water molecules inside nanotubes being observed under the electron microscope.

The Devil in Small Nanotubes

The researchers discovered that in large-diameter carbon nanotubes (10 to 200 nm), water behaves fairly conventionally, much as it would in an ordinary glass capillary tube. The liquid water inside the hollow tube showed up in low contrast, and at the boundary between liquid and gas phases, a typical meniscus (concave surface) was observed (Fig. 19.1a). Such a curvature indicates strong interaction between water molecules and the inner wall of the nanotube.

Pictures of small-diameter nanotubes (2 to 5 nm) were something else. The liquid water showed up in high contrast, giving a bright beady appearance (Fig. 19.1b), quite unlike the water trapped in the bigger nanotubes. Also contrary to large nanotubes, there was no meniscus separating the liquid from the gas phase. In fact, the water molecules appeared not to interact with the wall of the nanotube at all, but were concentrating their interactions with one another, leaving a gap between the liquid water and the wall.

And now comes the bit of science that helps to convince someone like me that the bright and beady appearance of the water is really structured water. The researchers turned on HyperChem to simulate the molecular arrangements, and used the molecular arrangements to simulate what it would look like under the electron microscope at different degrees of focus.

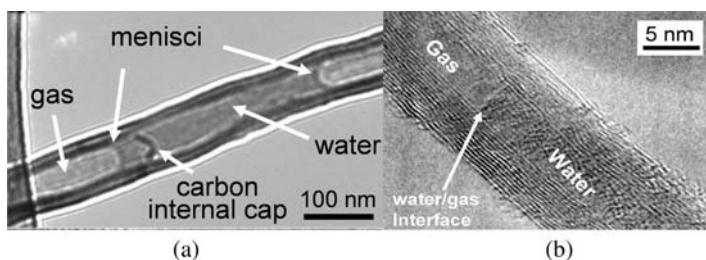


Figure 19.1 High-resolution TEM of big nanotube (a) and small nanotube (b) with water trapped inside.³

And they got back the bright, beady appearance, in which the “beads” themselves could be identified as clusters of a few molecules of water.

The individual water molecules were sufficiently ordered (structured) and restricted in motion to be captured on film. This structured water was a cylindrical lattice-work some ten layers of molecules across for a 4 nm diameter nanotube. In some pictures, such as that of the nanotube filled with heavy water (D_2O) (Fig. 19.2), the liquid water phase inside the small nanotube looked rather like a twisted multi-strand pearl necklace, suggesting the formation of strings of hydrogen-bonded water molecules.

That was the start of a fascinating journey taken on by many other laboratories. Confined water has relevance not only for biology but also for geology, where it is responsible for fracturing rocks and soil, and the swelling of clay minerals. Water and carbon nanotube interactions are important for developing nano-devices in aqueous environments, for filtration, desalination, water pumping, power cells, and so on.

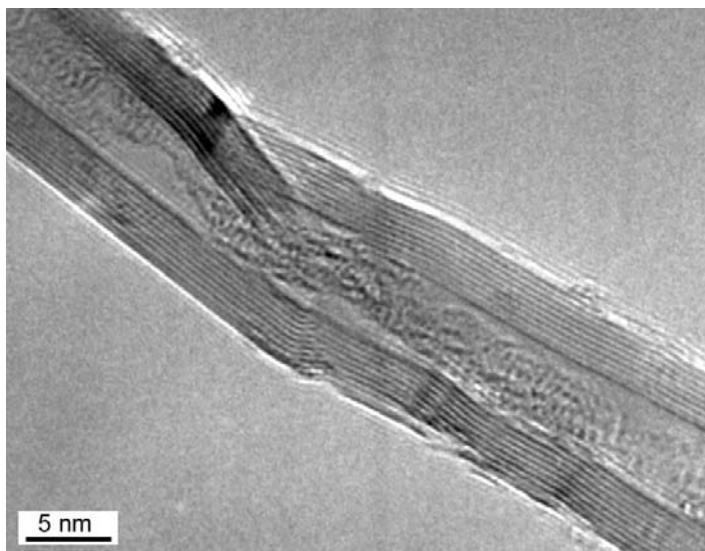


Figure 19.2 Nanotube with heavy water.³

Yutaka Maniwa and colleagues at Tokyo Metropolitan University have been collaborating with researchers at several other Japanese universities and research institutes to find out more precisely how confinement affects the phase and structure of water; specifically, how the diameter of carbon nanotubes interacts with temperature in the transition between liquid and solid phases and the possible liquid crystalline structures adopted by water (although the researchers did not describe their ordered water as “liquid crystalline”).⁴

Defining Phases and Structures under Confinement

Using a combination of X-ray diffraction, nuclear magnetic resonance, and electrical resistance measurements, the research team found that water inside single-walled carbon nanotubes (SWCNTs), manufactured to high precision standards with bore diameters between 1.68 and 2.40 nm, undergoes a wet-dry transition as the temperature is lowered. Below the transition temperature T_{wd} , the ice slips out of the carbon nanotube, leaving it in a dry state. T_{wd} increases with increasing diameter, from 218 K to 237 K.

Molecular dynamics simulations showed that in small-diameter SWCNTs (bore diameter < 1.02 nm), water molecules form six-membered hollow ice nanotubes regardless of water content. With larger diameters (bore diameter > 1.10 nm), water nanotubes with filled centres were formed even at low water content, which grew to fill the entire tube as the water content increased. In SWCNTs with bore diameters between 1.02 and 1.10 nm, hollow water nanotubes appeared at low water content until the entire SWCNT was filled; further increase in water content led to additional water filling the centre. Measurements on SWCNTs with a mean bore diameter of 1.46 nm strongly suggested that such filled water nanotube structures exist.

Phase Diagram of Confined Water

Diameter has a strong effect on phase transition and water structure. Maniwa’s team produced the first phase diagram of confined water

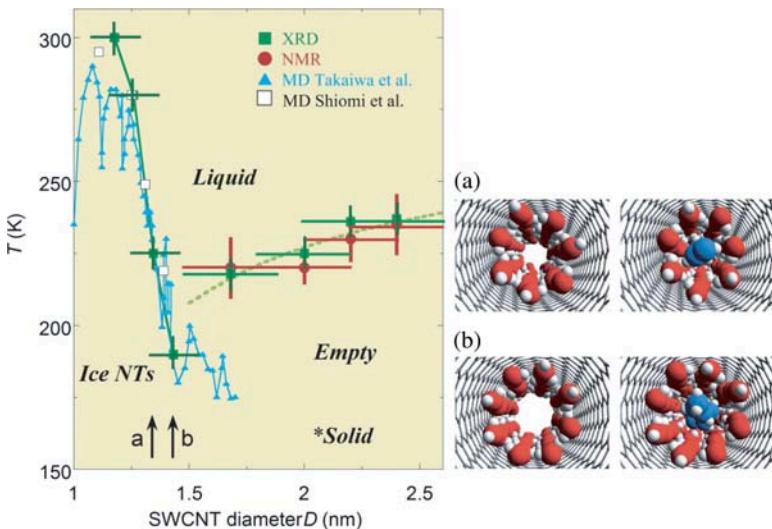


Figure 19.3 Temperature-diameter phase diagram of water inside SWCNTs (left); XRD, X-ray diffraction data; NMR, nuclear magnetic resonance data; MD, molecular dynamics simulation; dotted line represents melting point of bulk water in a capillary tube; * indicates solid phase obtained when rapidly quenched from high temperature; arrows a and b mark the diameters to either side of 1.4 nm where structures of water are presented (right) based on calculations, as hollow tubes when water content is low, and filled tubes when water content is high.⁴

in SWCNTs, including both their own experimental research results as well as molecular dynamics simulation results obtained by other researchers (Fig. 19.3).

As can be seen, at diameters ~ 1.6 nm, the phase transition curve is rather similar to that of water in an ordinary capillary tube, increasing with diameter. However, at diameters ~ 1.4 nm, the phase transition curve goes dramatically in the opposite direction. The transition temperature rises sharply from ~ 170 K to 300 K as diameter decreases from ~ 1.4 nm to 1.17 nm (Fig. 19.3, left).

A Diversity of Liquid Crystalline Structures

Water in confinement forms a diversity of liquid crystalline (ordered) structures. At diameters around 1.4 nm, seven- and eight-membered

nanotubes of water are filled with a chain of water molecules at the centre (Fig. 19.3, right). At diameters ~ 1.5 nm, other researchers have proposed double- and triple-walled water nanotube structures. For bore diameters smaller than about 1.02 nm, six-membered ice nanotubes are formed, irrespective of the water content; larger than that, and water chains fill the hollow interior.

This research raises a host of interesting questions. What is responsible for the paradoxical phase transition behaviour under confinement, which at first lowers the solid-liquid phase transition temperature with moderate degrees of confinement — at diameters between 2.40 and 1.68 nm — then sharply increases it as confinement becomes more restricted at diameters less than 1.4 nm? Considering the large variety of ordered structures that can be created under both high and moderate degrees of confinement, what kind of proton-conduction properties would these water nanotubes have? Judging from the observations presented in Chapter 13, they may be something like a superconducting proton cable. Such superconducting water nanotubes may well exist in the extracellular matrix of animals in association with collagen, as we shall see in the next chapter.

To complete our survey of water in nanospace, we look at other model systems.

Water in Extended Nanospace

Takehiko Kitamori and colleagues at the University of Tokyo and Tokyo Institute of Technology carried out NMR studies on water confined in extended nanospaces 295 to 5 000 nm in diameter created in amorphous silicon on a glass chip under carefully controlled conditions.⁵ These are not unlike the cavities created by the fractal cytoskeleton in the cell (see Chapter 18).

The researchers found that the water molecules kept the four-coordinate H-bonded structure seen in bulk water. However, the full line width at half-height broadened with a decrease in the size of the space, indicating a distortion from the tetrahedral structure as the space became smaller.

The spin-lattice relaxation rate increased sharply below 800 nm and remained constant for space sizes 800 to 5 000 nm. The $1/T_1$

value of 1.62 s^{-1} for a 320 nm space was five times that of the bulk value 0.32 s^{-1} . This was due to reduced translational movement, together with enhanced proton conduction along linear O...H-O hydrogen-bonded chains of water molecules. There was also a chemical exchange of protons between water molecules adsorbed on the surface and water away from the surface.

Thus, protons migrate through water-water and/or water-surface hydrogen-bonded networks. The results are best explained in terms of a model that includes an adsorption layer 0.3 nm thick immediately next to the surface, above which is a proton-conducting phase 50 nm thick, while the rest is the bulk water phase (Fig. 19.4).

Interestingly, when the unmodified hydrophilic surface with -OH groups was compared with a modified hydrophobic surface that

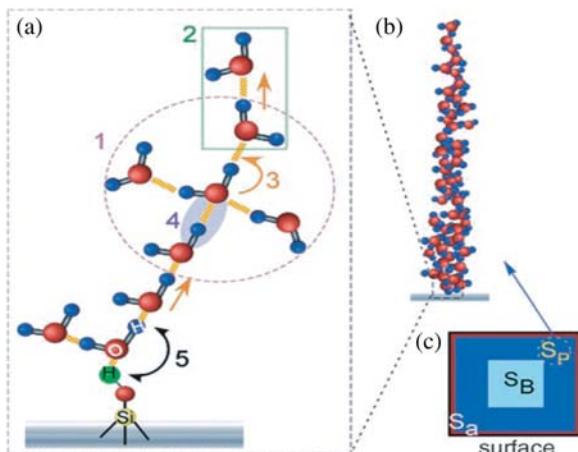


Figure 19.4 Water in extended nanospace on a glass chip;⁵ (a) (1) the O-O distance between water molecules in bulk water is retained; (2) the intermolecular translational motions are restricted; (3) protons migrate from one water molecule to another through hydrogen-bonding networks; (4) proton-charge distribution is localized along its linear O...H-O hydrogen-bonding chains; (5) chemical exchange of protons between water and the silanyl group on the surface enhances proton mobility of water. (b) Loosely coupled water within 10 to 100 nm of glass-water interface. (c) Diagram of the three phases of water in extended nanospace: SB, bulk phase; Sp, proton-conducting phase; and Sa, the adsorbed water phase.⁵

included $-\text{CH}_3$ groups, the effects of confinement appeared at a larger size. The $1/T_1$ values began to increase at 1000 nm instead of 800 nm. The interaction of water molecules with $-\text{CH}_3$ was much weaker than with $-\text{OH}$. The stronger confinement effect in hydrophobic nanospaces is thought to be due to hydrophobic hydration that surrounds the $-\text{CH}_3$ groups (rather than binding to it), resulting in highly directional hydrogen-bonding networks with more coupled water molecules.

Reverse Micelles for "Superactivity"

A micelle is an aggregate of surfactant (amphiphilic) molecules dispersed in water or a hydrophilic solution, such that the hydrophilic ends of the surfactant face out into the solution. A reverse micelle is the same aggregate of surfactant molecules dispersed in a hydrophobic solvent, so that the hydrophobic ends of the molecules face outward, and a droplet of water is trapped inside (Fig. 19.5). Reverse micelles were pioneered in the late 1970s by Karel Martinek and colleagues at the Czechoslovak Academy of Sciences as a branch of applied enzymology.⁶ Specifically, they were investigating the activity of enzymes trapped in reversed micelles, recognizing that the model system more reliably resembles the microenvironment that enzymes find within the cell (see Chapter 18). Indeed, studies on the conformational properties of peptides and proteins, protein-folding, enzyme activities and specificity, proteolysis, DNA splicing by restriction enzymes, and so on have yielded substantially different results from those obtained in an aqueous medium.⁷

One of the most striking effects is the greatly enhanced activity, or "superactivity", of the entrapped enzymes, which is also found *in vivo* (see Chapter 18). For example, the activity of peroxidase entrapped in a range of different surfactants was more than 100 times that of its activity in water.⁸ Other examples of enhanced activity in reverse micelles include acid phosphatase, trypsin,⁹ and dihydrofolate reductase.¹⁰

Reverse micelles were characterized using a range of physical methods, principally light-scattering spectroscopy and small-angle

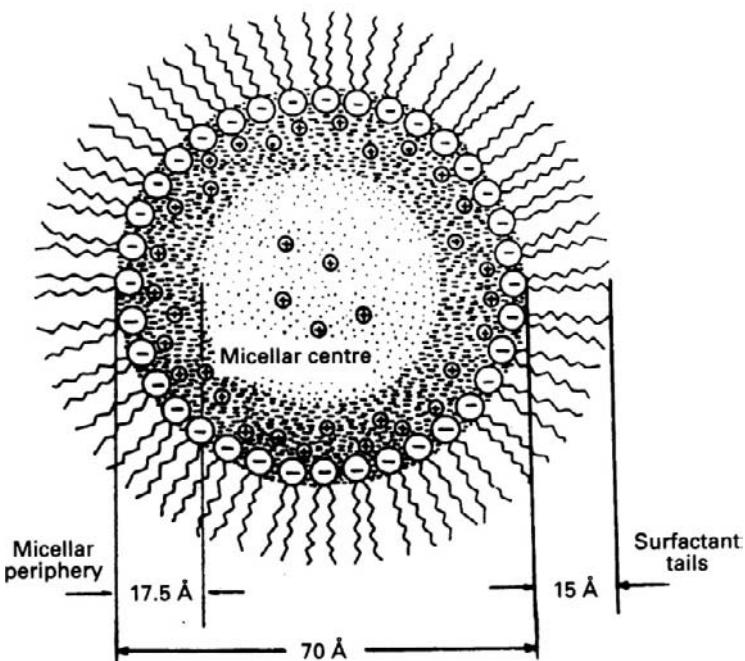


Figure 19.5 Diagram of reverse micelle.¹¹

neutron and X-ray scattering.¹¹ It is widely accepted that reverse micelles consist of spherical nanometre-sized water droplets coated with a close-packed surface monolayer of surfactant molecules, oriented in such a way that the surfactant head groups are hydrated at the surface of the water droplet, with the apolar tails protruding out into the organic solvent. The size distribution of a population is nearly uniform, according to the ratio of water to surfactant, $\omega_o = [\text{water}]/[\text{surfactant}]$. The radius of the water droplet $r_w = 0.175 \omega_o \text{ nm}$. Thus, the size of the droplets can vary from zero to several tens of nanometres, although the upper limit depends on the system being studied. The most widely used system consists of water, octane, and the surfactant dioctyl sodium sulphosuccinate (AOT, for aerosol OT, a trademark substance from American Cyanamid that contains dioctyl sodium sulphosuccinate as a surface active agent).

When water and surfactant are varied simultaneously at the same ω_0 , the reverse micelles vary in concentration but not in size.

Reverse micelles are extremely dynamic, and exchange their contents very rapidly through a transient fusion of two micelles, which then split up again. Hence the rate of exchange is independent of the size of solubilized ions in the water phase. The rate constant for the entire process is $\sim 10^6$ to $10^8 \text{ M}^{-1} \text{ s}^{-1}$, depending on the reverse micelle system, indicating that one collision in 1000 to 10 000 results in exchange of content. If the solubilized molecule is not completely confined to the water pool, but can partition into the interface or the organic solvent, a different exchange mechanism can operate. Mere contact will suffice for exchange at the interface; and if the molecules are distributed in both water and organic solvent, then transfer is more rapid, as the micelles do not need to be in contact at all.

It was noted from the beginning that the reaction rate was higher in reverse micelles than in bulk water, despite the fact that the overall concentrations of enzyme and substrate were the same. Likewise, the pH-independent k_{cat} (catalytic constant, or the number of reactions per unit time) was shown to be greater in reverse micelles. The term “superactivity” was used for enzymes that display a greater substrate and pH-independent catalytic constant in reverse micelles than in bulk water.

It has been difficult to interpret the kinetic behaviour of enzymes in reverse micelles. In the usual bulk water system, the K_m (Michaelis constant, the concentration of substrate at half the maximum reaction velocity) for two different substrates simply reflects the microscopic affinity of the enzyme for each one, but in reverse micelles, the observed K_m also reflects the interaction of each substrate with the surrounding medium. Thus, enzymes in reverse micelles might be regarded as a simplified version of what may occur in a living cell. Every enzyme exists at high concentration locally, and activity is controlled by the limited supply of substrate, not by its abundance.

Water Dynamics in Reverse Micelles

The water confined within reversed micelles has been the subject of intensive physicochemical study.¹¹ Anomalous water at low hydration resembles the water next to biological membranes. Infrared data have provided direct evidence for the existence of different populations of water. Bulk water exhibits a band at 1670 nm, while water not involved in a tetrahedral array of H-bonds absorbs at ~1400 nm. At ω_o of 1.5, only the 1400 nm band is seen, shifting to longer wavelengths as ω_o increases. The 1670 nm band is first clearly present at ω_o of 8.3. These findings have led to the proposal of a layer of structured water of reduced motion of about ten water molecules per surfactant polar head group.

Recent studies have confirmed this general picture. Michael Fayer's team at Stanford University investigated the dynamics of water trapped in AOT reverse micelles from a diameter of 1.6 to 20 nm with ultrafast infrared spectroscopy. In large micelles, the dynamics of water can be separated into two ensembles: slow interfacial water and bulk-like water in the core. As the micelle size decreases, the interfacial water and the collective nature of water reorientation begin to slow down the core water molecules, until these effects dominate and all the water molecules have the same slow reorientation. The crossover between two-ensemble and collective reorientation occurs near a micelle diameter of 4 nm. Smaller than that, and the water molecules behave as a collective.

Thus, the two-ensemble dynamics is clearly seen in reverse micelles with $\omega_o \geq 16.5$, while the collective long time reorientation dynamics takes over at $\omega_o \leq 5$. At $\omega_o = 10$, however, the two-ensemble dynamics remains, but both are slowed down by interactions between the two populations of interfacial and core water.

Because of extensive hydrogen-bonding among water molecules, reorientation of even a single molecule requires the concerted rearrangement of its first and second hydration shells involving some 16 molecules. If one or more of those hydration

molecules are close to interfacial water, as is more likely in smaller micelles, it will affect the dynamics of *all* its neighbours. Another important factor is the change in structure of the micelle interface as the diameter decreases. In large micelles, the curvature of the interface is relatively gentle and close to flat. But below $\omega_0 = 16.5$, important structural changes in the interface occur. Both molecular dynamics simulations and infrared spectroscopy of the head group of AOT show that there is increased association between the sulphonate head groups and sodium counter-ions at lower hydration levels. This leads to a rigid structure of ionic interactions with water molecules trapped between sodium ions and sulphonate head groups. At $\omega_0 = 10$, there is a small amount of ion pairing that becomes increasingly important below $\omega_0 = 6$. Very small reverse micelles have low surface area per AOT and significant surface curvature so that water molecules may interact with multiple sulphonate groups simultaneously (see Fig. 19.6).

The graph in Fig. 19.6 may explain why micelles of $\omega_0 \geq 16.5$ do not change much in the two-ensemble dynamics of water, while $\omega_0 = 5$ is the transition point to collective dynamics. We shall see what the entrapped protein does to the water dynamics in the next chapter.

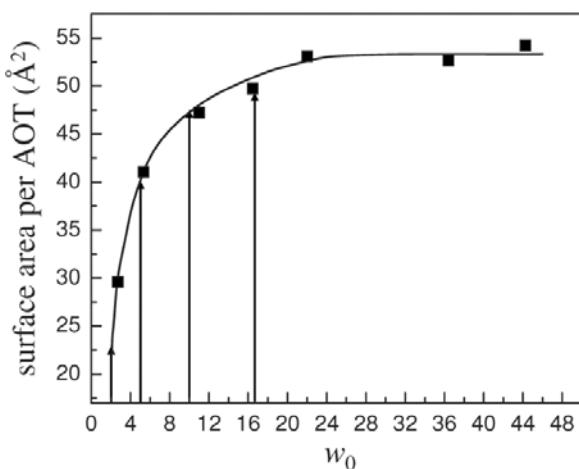


Figure 19.6 Surface area per AOT vs ω_0 .¹²

Notes

1. This first section of this chapter is based on Ho (2005b).
2. Ruan *et al.* (2004); Zubavicus and Grunze (2004).
3. Ye *et al.* (2004); Naguib *et al.* (2004).
4. Kyakuno *et al.* (2011).
5. Tsukahara *et al.* (2007).
6. Martinek *et al.* (1987).
7. Bru *et al.* (1995).
8. Martinek *et al.* (1987); Hoppert *et al.* (1994).
9. Fadnavis *et al.* (1993).
10. Katiyar *et al.* (1989).
11. Bru *et al.* (1995).
12. Moilanen *et al.* (2009).

Protein and Water in Nanospace

Water Nanotubes, Collagen, Acupuncture, and Energy Medicine

In the previous chapter, we saw that water forms nanotubes inside single-walled carbon nanotubes. Those water nanotubes could be relevant to a very common nanospace in living organisms, specifically that associated with collagen, a protein that makes up the bulk of the extracellular matrix of higher animals. Collagen is the main protein in connective tissues and the most abundant protein in mammals. Connective tissues also contain a lot of water; soft connective tissues (all apart from bones and cartilage) are typically 60 to 70% water by weight. The proteins and water constitute the liquid crystalline matrix in which every single cell in the body is embedded, making connective tissues the ideal medium for intercommunication, as suggested in *Rainbow Worm*.

In traditional Chinese medicine, a system of acupuncture meridians is supposed to transport *qi* or living energy to every part of the body, but all attempts to locate the meridians to anatomical structures have failed. In 1998, David Knight, then at King Alfred's College, Winchester, and I proposed that the acupuncture meridians may correspond to the structured water aligned with collagen in the connective tissues,¹ and *qi*, the positive electric currents carried by jump conduction of protons through the hydrogen-bonded water molecules aligned along the collagen fibres. Evidence for the idea has been accumulating since.²

A number of observations and molecular dynamics simulations confirm that water molecules organized in hydrogen-bonded chains can act as “proton wires” to support jump conduction of protons much faster than the ordinary flow of electrons through wires, and protons can migrate rapidly along interfacial water (see Chapter 13). The importance of the liquid crystalline matrix is not restricted to acupuncture; it is relevant to all forms of energy medicine, subtle or otherwise, as already pointed out in *Rainbow Worm*.

Jim Oschman, cell biologist turned world expert on complementary medicine, has recognized the importance of the living matrix for massage and other healing practices for years. He pioneered the field of energy medicine, putting it firmly on a scientific footing,³ and now teaches the subject at Energy Medicine University, accredited in the State of California in the US.

I should mention that liquid crystals are piezoelectric, or flexoelectric,⁴ in that they interconvert mechanical energy and electrical polarization. They are also sensitive to temperature and pH. In other words, liquid crystals integrate and interconvert *all* forms of energy within the organism. On that basis alone, they are ideal for intercommunication, and hence for making organisms.

The structure of water associated with collagen has been yielding to X-ray diffraction studies on both native and synthetic collagens. But the precise organization of the water molecules around the protein molecules remained unknown, until fairly recently.

Gary Fullerton, Ivan Cameron, and colleagues at the University of Texas at San Antonio have produced a model based on experimental results suggesting that the water associated with collagen is ordered to a high degree.⁵ The water appears to be structured in regular chains in the form of water nanotubes along the collagen microfibrils, which would indeed facilitate the proton jump conduction that enables every part of the body — from molecules to cells and tissues — to intercommunicate for perfect coordination, or quantum jazz (see Chapter 1).

The importance of protons for intercommunication has been stressed in *Rainbow Worm*. Rickey Welch referred to proton transport within the cell as a proton-neural network.⁶ If proton conduction is

body-wide, so is the proton-neural network. Travelling protons and associated electrons are the basis of reduction-oxidation reactions that underlie energy metabolism in living organisms. They feed the metabolic pathways that branch out into the rich wet chemistry that makes life exciting (more in the next chapters).

First Calculations

Tendons have high concentrations of type I collagen, in some instances approaching 100% of dry weight, which makes it easy to purify and crystallize for structural studies, and a great deal is already known.

The collagen molecule is a rod about 300 nm long and 1.5 nm in diameter, made up of three polypeptide subunits wrapped around one another in a triple helix. Collagen molecules spontaneously self-assemble end to end into long microfibrils, microfibrils aggregate into fibrils, fibrils into fibres, and fibres into larger bundles or sheets.

A distinctive feature of collagen is the repeat of three amino acid subunits along the polypeptide chain Gly–X–Y, where X is frequently proline and Y hydroxyproline, which accounts for the tendency of three polypeptide strands to form a triple helix, with glycine in the interior of the helix, and the rings of proline and hydroxyproline stacked and pointing outward.

Type I collagen has a mean molecular weight per amino acid residue of 91.2 Da (a dalton is a unit of mass equivalent to 1 g/mol), calculated from the amino acid sequences of the polypeptide chains. This allows accurate estimates of the number of water molecules associated with the protein molecule.

High-resolution X-ray diffraction studies on both native and synthetic collagens reveal an extensive water bridge network surrounding the collagen molecule. These and other structural studies have provided a molecular model of collagen with at least two categories of water. The most tightly bound consists of one highly immobilized water bridge for every three amino acid residues (0.0658 g water/g protein). A second less immobilized water

fraction consists of three additional water molecules per tripeptide unit, residing in the three groove-like depressions between the peptide chains of the triple helix ($0.197 \text{ g water/g protein}$). The tightly bound and three cleft waters together complete a chain of four water molecules per tripeptide, forming a triple helix of water in the three clefts between the protein chains that make up the triple helix. This implies a chain of water in each of the three grooves requiring a water content of $0.0658 + 0.197 = 0.263 \text{ g water/g protein}$.

Fullerton and Maxwell Amurao first produced detailed calculations based on existing data and their own careful measurements of tendon diameter at different degrees of dehydration to convince themselves that the remaining water on native collagen (other than the first two categories of bound water) is in the first monolayer covering the entire surface of the collagen molecule ($1.315 \text{ water/g protein}$),⁷ and not, as others have suggested, in multiple layers. This category of water is less immobilized than the first two categories, but still restricted in motion relative to bulk water.

They noted that fully hydrated bovine tendon in the native state has $1.62 \text{ g water/g protein}$. This is equivalent to $1.62/0.263 = 6.2$ chains of water molecules per chain in the triple helix, or about 18 chains of water molecules per triple helix. This works out to be just sufficient to form a monolayer network over native tendon while maintaining the minimum spacing necessary to accommodate hexagonal water chains possessing the four hydrogen bonds per molecule as in bulk water. The arrangement of the 18 water chains around each triple-helix molecule is shown in Fig. 20.1. This makes three six-membered water nanotubes per triple helix, one in each groove.

Armed with this model, Fullerton and colleagues carried out measurements of nuclear magnetic resonance (NMR) relaxation times of water associated with native tendon at different dehydration levels.

The NMR spin-lattice relaxation time is the time taken for the spin induced in magnetic nuclei (such as that of hydrogen) to relax back to equilibrium with its surroundings (the lattice), and is

inversely correlated with how restricted its motion is; the more constrained its motion, the higher the rate of relaxation.

Fast Proton Exchange Between All Structured Water

Fullerton and colleagues used a model in which all the categories of structured water exchanged protons rapidly even though they had different energy levels: the lowest being the most immobilized water molecules, and the highest the least immobilized, but still below that of bulk water. Using this model, they were able to identify categorical changes in water motion at critical hydration levels (g water/g collagen) corresponding to 1, 4, and 24 water molecules per tripeptide unit of collagen, at 0.0658, 0.264, and 1.584 g water/g collagen respectively. This was precisely predicted by the model based on a monolayer of water covering the entire collagen molecule, consisting of six water chains in each cleft of the triple helix (see Fig. 20.1).

The relaxation rates of the bulk water (reciprocal of relaxation time) were 0.347 s^{-1} for bulk water, 33.1 s^{-1} for the cleft water (the

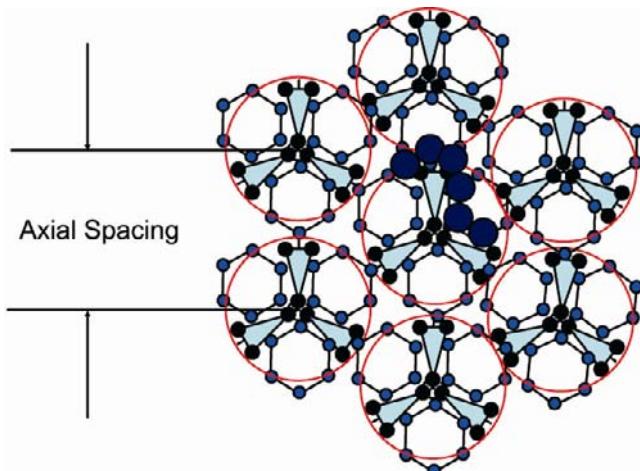


Figure 20.1 Cross-section of a collagen fibril consisting of seven triple helices, each of which has $3 \times 6 = 18$ water chains covering it completely.⁷

three water chains covering the deepest part of the clefts in the triple helix), and 1.351 s^{-1} for monolayer water (the remaining 15 water chains covering the rest of the collagen molecule (Fig. 20.1).

Phase Transitions of Water Structure

Coincidentally, in collaboration with Franco Musumeci and his team at the University of Catania, we investigated the delayed luminescence of bovine tendon at different stages of dehydration. We found dramatic and abrupt changes in the delayed luminescence of bovine tendon at critical hydration levels, two of which were close to those identified by Fullerton's group. Although our critical hydration values were less precise, the discontinuities observed were more like phase transitions. Four different phases were identified, separated by rather sharp transitions at 1.52, 0.53, and 0.26 g water/g collagen.⁸

These observations suggest that the structured water associated with collagen is highly ordered as a single phase at any time, and that at critical points of hydration, abrupt phase transition occurs to a different state of order, in analogy with ice structures. The fast exchange of protons in the structured water is also consistent with proton jump conduction extending throughout the structured water (see Chapter 13), while the collagen water chains are reminiscent of the six-membered water nanotubes seen in carbon nanotubes 1.02 nm in diameter (Chapter 19).

As this book was going to press, Cameron, Fullerton, and Anthony Lanctot presented a molecular stoichiometric hydration model for calculating the size of hydration fractions that is applicable not just to collagen, but also to globular proteins and cells.⁹ The water fractions differ in molecular motion and other physical properties due to electrostatic interactions of polar water molecules with electric fields generated by covalently bound pairs of opposite partial charge on the protein backbone. This has led to the identification of at least four hydration fractions that complete a monolayer coverage: single water bridges (between opposite charges),

double water bridges, dielectric water clusters over polar-hydrophilic surfaces, and water clusters over hydrophobic surfaces. Additional water with non-bulk characteristics is found in cavities or further surface layers, or when proteins adopt extended conformations (see Chapter 23).

Protein's Secret Water Music in Nanospace¹⁰

The hydration of proteins has been carried out typically either in solution, or as freeze-dried powder in a humid atmosphere, environments that are very different from those they would experience in the cell (see Chapter 18). Those studies could be giving an incomplete, if not downright misleading, picture. That is very much the message of a report of a study using a combination of perhaps more appropriate techniques.¹¹

Researchers at the University of Pennsylvania, led by Joshua Wand, used reverse micelles, vesicles of surfactants enclosing nano-sized droplets of water dispersed in an organic solvent (described in detail in Chapter 19), to entrap single protein molecules. In this state, it becomes much easier to probe the relationship between the protein and its hydration water — the water molecules immediately surrounding the protein — using sophisticated NMR measurements. As a result, they found a remarkably diverse range of water molecules associated with the protein molecules that move in concert with different parts of the protein itself, down to individual amino acids, which has never been seen before, overturning a number of previous misconceptions.

More Like Nanospaces in a Living Cell and More Precise Measurements

As pointed out in the previous chapter, reverse micelles create conditions much more like the situation inside a living cell, as pioneers of the technique for researching protein hydration recognized.¹² Frank

Mayer and his team at Göttingen University showed how encapsulating enzymes in reversed micelles increased their specific activity two-to ten-fold, and at the same time greatly improved their thermostability.¹³

The technique also increases the precision of NMR measurements in focusing on signals from the water immediately around the protein, without being contaminated by the bulk solvent outside, which is an organic solvent, not water.

The confinement in the nano-volume has the effect of vastly lengthening the residence time of water molecules on the protein (probably by restricting the motion of the protein itself), while decreasing general hydrogen exchange with bulk water, as well as any contribution of long-range coupling to bulk solvent water. This allowed the first detailed, site-specific analysis of relative hydration water mobility across an entire protein surface.¹⁴

The protein used in the study was ubiquitin, a small 8.5 kDa polypeptide of 76 amino acids expressed in all eukaryotic cells (cells with nuclei, in higher organisms). Its main function is in the turnover of proteins.¹⁵ Ubiquitylation is an ATP-dependent proteolytic process, in which proteins marked with one or more ubiquitin molecules are rapidly degraded with the release of free ubiquitin.

The reverse micelles were made of the surfactant bis(2-ethylhexyl) sulphosuccinate (AOT), and contained an average of 1 000 molecules, including the single protein molecule. The AOT molecules have their hydrophilic acid head groups facing inside the micelle, paired with Na^+ , rather like membrane lipids in the cell. The reorientation of the water confined in the reverse micelle is typically an order of magnitude slower. In order to probe the interaction between protein and water, the protein was heavily labelled with the ^{15}N isotope in its amide groups ($-\text{NH}$), and the cross peaks (cross-magnetization, cross-correlation) between the amide ^1H and ^1H of water molecules analysed by three-dimensional NMR, NOE, and ROE spectroscopy (see Box 20.1).

Box 20.1

NMR, NOE, and ROE Spectroscopy¹⁶

When placed in a magnetic field, the NMR active nuclei of atoms (usually with an odd number of nucleons, such as ¹H, ¹³C, and ¹⁵N) absorb electromagnetic radiation, and precess (rotate) at a frequency characteristic of the nuclei. A radio frequency (RF) pulse at 90° to the magnetic field is sent into the sample, and the emission from the sample is monitored. An NMR spectrum is acquired by varying the frequency of the RF radiation, or alternatively, varying the magnetic field. The resonant frequency and the strength of the signal are proportional to the strength of the magnetic field. For example, in a 21 tesla magnetic field, protons resonate at 900 MHz; in weaker fields, the resonance frequency decreases accordingly.

Depending on the local chemical environment, different protons in a molecule resonate at slightly different frequencies. Both this frequency shift and the fundamental resonant frequency are directly proportional to the strength of the magnetic field.

A two-dimensional NMR experiment consists of a sequence of two RF pulses with a delay period in between. It is the timing, frequency, and intensity of these pulses that distinguish one experiment from another. A typical two-dimensional experiment consists of a preparation period where a magnetization coherence is created through a set of RF pulses, p_1 , followed by the evolution period, t_1 , when the nuclear spins are allowed to freely precess, then a second set of pulses, p_2 , followed by a mixing period where the coherence is manipulated by the pulse to give an observable signal, and the detection period, t_2 , in which the signal from the sample is registered as a function of time, in a manner identical to one-dimensional NMR. In three-dimensional NMR, three RF pulses are used, giving two sets of mixing periods. In four dimensions, four RF pulses are used, with three sets of mixing periods, and so on. Higher-dimension NMR is used when large molecules, such as proteins, are analysed, as they are crowded with signals and spreading them out in more dimensions prevents overlap.

The two dimensions of two-dimensional NMR are two frequency axes representing a chemical shift. Each frequency axis is associated

(Continued)

Box 20.1 (*Continued*)

with one of the two time variables, the length of the evolution period, t_1 , and the time elapse during the detection period (detection time). These are each converted from a time series to a frequency series by two-dimensional Fourier transform (a mathematical technique). Thus, a two-dimensional NMR experiment is generated as a series of one-dimensional experiments by varying the specific evolution time t_1 . The end result is a two-dimensional plot showing an intensity value for each pair of frequency variables. For three-dimensional NMR, the additional dimension is the second mixing period between t_1 and t_2 , and the results are represented as a cube.

A multidimensional NMR spectrum shows two kinds of peaks. Diagonal peaks have the same frequency coordinate on each axis, while cross peaks have different values for each frequency coordinate and appear off the diagonal. Diagonal peaks correspond to the peaks in a one-dimensional NMR experiment, while the cross peaks indicate couplings between pairs of nuclei, due to magnetization transfer, either between two nuclei in the same molecule (connected by chemical bonds) or else in different molecules nearby, as in nuclear Overhauser effect (NOE) spectroscopy (NOESY).

Rotating-frame nuclear Overhauser effect (ROE) spectroscopy (ROESY) is similar to NOESY except that the initial state is different. Instead of observing cross peaks from an initial state of z-magnetization (the usual direction), the equilibrium magnetization is rotated 90° onto the x-axis and then spin-locked by an external magnetic field so that it cannot precess.

A Diversity of Correlated Protein–Water Dynamics

The NMR spectra of the confined protein in reverse micelles showed dozens of cross peaks representing correlations between specific amino acid amide groups and hydration water. This was in contrast to the relatively few peaks identified in ordinary solution NMR of the labelled protein, practically all of which were due to H-exchange with nearby labile side-chain hydrogen.

Within the reverse micelles, researchers discovered a diversity of correlated protein-water dynamics, with water rearrangement

rates varying by a factor of more than 10^{10} from one region of the protein surface to another.¹⁷

The majority of detected hydration sites showed substantial motion or shorter residence time. Approximately three quarters of the amide hydrogen within the NOE detection distance limit (4.3 Å) of solvent showed measurable cross peaks to water. No cross peaks to water from the buried amide hydrogen were detected.

A group of very slow and spatially restricted water molecules is evident along the C-terminal of the protein, and a cluster of very fast water molecules is found on the surface of the α -helix. Clusters with intermediate dynamics are the mixed β -sheet. The slowest hydration sites have a residence time of ~10 ns or longer.

The water molecules in the network of water around the protein seem to act cooperatively locally yet independently of the behaviour of the bulk solvent and of the other regions. Water molecules that have similar hydration dynamics (similar residence times) form clusters across the protein surface.

These results show that the hydrogen-bonded network of water is much more flexible than previously believed, as already hinted at in recent findings on how ions interact with water (Chapter 9).

Crystallographic Structures are Misleading

Until now, much work on site-resolved hydration has relied on crystallographic data. As most protein crystal structures are heavily hydrated, the general view seems to be that protein crystal structures represent the native protein in crowded conditions in the living cell, and hence crystallographic water should also be representative of the native protein in the cell. Extensive analysis of crystallographic water has been combined with molecular dynamics simulation data to build up a picture of the protein hydration layer. Ubiquitin is a case in point.¹⁸

Two crystal structures for wild-type human ubiquitin have been proposed; only about half of the water molecules identified

in the two structures are within 1 Å of each other, even though the proteins were crystallized under very similar conditions. There is little correlation between the waters detected by solution NMR and the crystallographic waters. Only ~60% of the crystallographic waters common to both crystal structures appear within the 4.3 Å NOE distance of sites where the waters are long-lived. Over half of the long-lived protein-water interactions detected on NMR involve amide hydrogen atoms outside NOE distance from the nearest crystallographic water molecule common to both crystal structures.

Implications for Living Cells and Recapitulation

Cell biologists are coming round to the view that cells are packed with macromolecular surfaces that confine proteins to nanometre volumes, and confinement can potentially change the fundamental properties of proteins and associated liquid crystalline water, much as the pioneers Frank Mayer and Jim Clegg, for example, have been telling us for years (see Chapter 18).

I hope that by now you will have seen that static *in vitro* systems investigated by physicists — such as those for bulk water in Chapters 2 to 8; for ions, proteins, and DNA in bulk solution in Chapters 9 to 11; and also for macroscopic interfaces in Chapters 12, 14, 15, and 16 — can never compare directly with the incredibly dynamic situation inside the living cell, whose portrait we began to paint in Chapter 18. You also saw how the behaviour of water in bulk is demonstrably different from that under different confined conditions: within pores of cellulose acetate (Chapter 17) and inside carbon nanotubes and other nanospaces closer to what the intracellular and extracellular milieu is like (Chapters 13, 19, and 20).

Nevertheless, some common threads run through all the systems: the tendency of water molecules to exist in two states, one of which adopting the ice-like crystalline tetrahedral hydrogen-bonded structure wherever possible, even within nanospaces, this being a consequence of quantum coherence

established in water as predicted by quantum electrodynamics field theory (Chapters 6 to 8). And the ease with which alternative bonding structures are adopted based on collective constraints so that collective dynamics dominate at sufficiently small dimensions of reverse micelles, for example, as described in this chapter.

The second common thread is that water is almost infinitely flexible, which is why it has a dazzling array of crystalline and quasicrystalline structures, as seen within single-walled carbon nanotubes (Chapter 19), and lest we forget, in snowflakes, each of which is unique (Chapter 2). Water's ability to form a variety of crystalline and quasicrystalline structures is most probably the reason it can help colloid crystals and quasicrystals self-assemble, accounting incidentally for their diversity under different conditions (Chapter 4).

Water's prodigious flexibility makes it the perfect medium for quantum jazz (Chapter 1). Like the ideal manager, its overriding message is for everyone to perform their best on their own terms, while providing them with the conditions for doing so. Water enables all molecules, small and large, to carry out their specific vital functions at all times, and in perfect coordination with the whole. Water enables all performers to express their full degrees of freedom (which may span ten orders of magnitude in tempo for a macromolecule, as we have seen in this chapter), while providing, at all times, the ultra-sensitive intercommunication channels that may trigger an abrupt change of phase at the global level. In that way, water is not only the medium, but also both the message and messenger of life.

This brings us to the third common thread: water's role as supreme catalyst, the super-facilitator of chemical reactions, whether as a redox pile in the form of liquid crystalline coherent water that also offers activation energy and specific attraction between enzyme and substrate (Chapters 6 to 8), or as the result of dynamic switching between two states of water, according to Philippa Wiggins (Chapter 17). I emphasize that the two are not

mutually exclusive alternatives. And this is where water surpasses itself as medium and message.

So far, the focus has been on water as the medium and message of life, rather neglecting its essential role as the *means* of life on Earth; the next two chapters will be dedicated to that important task. The final chapter after that will provide a synthesis towards the new cell biology.

Notes

1. Ho and Knight (1998).
2. Ho (2005a).
3. Oschman (2000, 2003, 2011).
4. Krekhov *et al.* (2011).
5. Welch (1985).
6. Fullerton and Amurao (2006); Fullerton *et al.* (2006); Ho (2006b).
7. Fullerton and Amurao (2006).
8. Ho *et al.* (2003, 2006).
9. Cameron *et al.* (2011).
10. Ho (2011i).
11. Nucci *et al.* (2011).
12. Ho (2004h).
13. Hoppert and Mayer (1999).
14. Nucci *et al.* (2011).
15. “Ubiquitin”, Wikipedia, 1 September 2011, <http://en.wikipedia.org/wiki/Ubiquitin>.
16. Rewritten from the following sources: “Nuclear Magnetic Resonance Spectroscopy”, <http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/nmr/nmr1.htm>; “Nuclear Magnetic Resonance Spectroscopy”, Wikipedia, 8 September 2011, http://en.wikipedia.org/wiki/Nuclear_magnetic_resonance_spectroscopy; “Nuclear Magnetic Resonance”, Wikipedia, 31 August 2011, http://en.wikipedia.org/wiki/Nuclear_magnetic_resonance#Multi-dimensional_NMR_Spectroscopy; “Nuclear Overhauser Effect”, Wikipedia, 25 September 2011, http://en.wikipedia.org/wiki/Nuclear_Overhauser_effect;

- “Two-dimensional Nuclear Magnetic Resonance Spectroscopy”, Wikipedia, 10 September 2011, http://en.wikipedia.org/wiki/Two-dimensional_nuclear_magnetic_resonance_spectroscopy.
17. Hilser (2011).
 18. Nucci *et al.* (2011).

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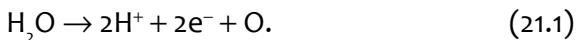
21

Fire and Water

Water and Redox Chemistry of Life

I have been concentrating almost exclusively on the physics of water up to now — or water as medium and message — rather neglecting its chemistry that provides the very means of life. This chapter and the next are dedicated to water’s central position in the fundamental chemistry of life, which consists of reduction and oxidation reactions, or redox reactions. (Water has other important chemical reactions such as acid/base and hydrolytic/dehydration reactions, which I have mentioned in Chapters 9 and 17.) Redox reactions are the stuff of energy transduction in living systems. They involve the transfer of electrons from one substance (donor) to another (acceptor) in accordance with their relative reduction potential, or their affinity for electrons compared with hydrogen (see Box 21.1).

Where do the electrons come from? They come from splitting water, making use of energy trapped from sunlight, which yields electrons and associated protons, and oxygen (Equation 21.1):



The electrons (and associated protons) go to reduce carbon dioxide into carbohydrates, and the oxygen enables air-breathing (aerobic) organisms to survive. The entire process is *photosynthesis*, carried out by green plants, algae, and blue-green bacteria.

Box 21.1

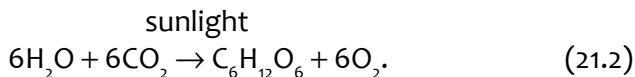
Reduction Potential

The reduction potential (also reduction-oxidation potential or redox potential) is the affinity of a substance for electrons. The value for each substance is compared to that of hydrogen, which is set arbitrarily to zero, at standard conditions of 25 °C, 1 atmosphere, and 1M concentration.

Substances that have positive redox potentials accept electrons from hydrogen, becoming reduced, while substances that have negative redox potentials donate electrons to hydrogen, becoming oxidized. Oxidation and reduction always go together.

The redox potential is also the same as the electrochemical potential and the Fermi level used in solid-state physics.¹

Equation 21.2 gives a summary of the process (the carbohydrate C₆H₁₂O₆ is glucose):



The carbohydrate feeds the photosynthetic organisms, providing the carbon skeleton for making amino acids and proteins, bases and nucleic acids, enabling them to grow and multiply. The photosynthetic organisms in turn provide food and energy for most of the rest of the biosphere that feed on them directly or indirectly. The carbohydrate contains chemical energy (reduced potential) that can be released for use in all living activities through oxidation reactions during the process of respiration, which, in summary, is just the reverse of photosynthesis (Equation 21.3).



Thus, photosynthesis and respiration together make a grand redox cycle or dynamo, the magic roundabout that draws on the energy of the sun to create the marvellous diversity of life on Earth, *all out of non-living simple chemical compounds and elements*.

In respiration, every molecule of glucose generates 24 electrons that travel down the respiratory *electron transport chain* to the final acceptor, oxygen (to give carbon dioxide). (The electron transport chain, which will be described in detail later, is a group of compounds that pass electrons from one to another via redox reactions coupled with the transfer of protons across a membrane to drive adenosine triphosphate (ATP) synthesis.) The carbon in glucose has a redox potential of -0.42 V , whereas oxygen, the most electronegative substance in the reaction, has the largest redox potential of $+0.82\text{ V}$. The difference in potential is -1.24 V . The energy in kcal released when one mole of electrons passes down a potential drop of 1 V is -23.062 , and the total energy generated is thus $24 \times (-23.062) \times 1.24 = -686\text{ kcal} = -686 \times 4.184 = -2870.2\text{ kJ}$.

To synthesize a molecule of glucose by photosynthesis, 24 electrons must be removed from water molecules where they are held by the redox potential of oxygen ($+0.82\text{ V}$) and pumped “uphill” to carbon atoms that are partially reduced to carbohydrate with a redox potential of -0.42 V . The difference in potential is $+1.24\text{ V}$. The change in free energy is in the positive direction, as electrons are moving against the gradient, to put energy into the glucose, so it is $+686\text{ kcal} = +2870.2\text{ kJ}$.

Oxygen from Water Pivotal for Life on Earth²

Tapping into water for electrons was an ingenious invention in evolution, because water is the most abundant substance on the surface of Earth, and the liberation of oxygen also enabled air-breathing organisms to evolve, and greatly enlarged the range of energy accessible to the biosphere. It was the blue-green bacteria (cyanobacteria) (Fig. 21.1) that invented photosynthesis and initiated the Great Transformation some 2.5 billion years ago.

Earth’s reducing atmosphere, hitherto only fit for anaerobic (non-oxygen-requiring) organisms, slowly became an oxidizing one suitable for aerobic metabolism, paving the way for the evolution of diverse, complex life forms.

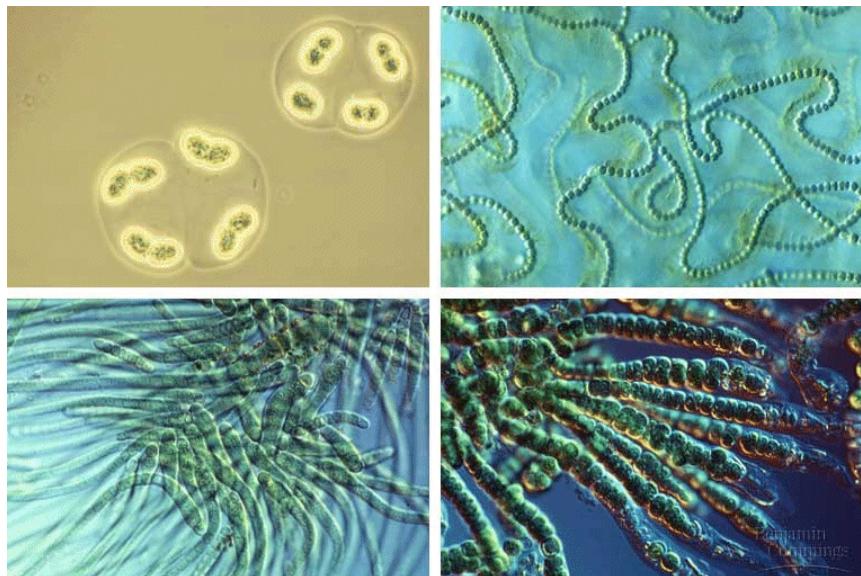


Figure 21.1 Living cyanobacteria, Courtesy of Bellarmine University.

The creation of the photosynthetic apparatus gave access to water, a new, unlimited source of electrons and protons, thereby greatly expanding the thermodynamic range of energy available to life.³ Up until then, some bacteria had carried out a form of photosynthesis that does not produce oxygen and depends on the limited availability of chemical substances that can act as electron donors.

The primitive photosynthetic pigment bacteriochlorophyll *a* (BChl *a*), found in green non-sulphur bacteria, when excited by sunlight, has a standard electrochemical potential of 0.55 V, which is sufficient for using ferrous iron, carbon, and sulphur substrates as electron donors. To use water as electron donor, and hence to produce oxygen, requires the creation of the chlorophyll *a* in cyanobacteria and green plants and algae that can be boosted to a higher electrochemical potential of 0.82 V, thereby giving life access to a higher energy level and a practically unlimited energy source. This event literally changed the face of Earth. The accumulation of

oxygen in the atmosphere paved the way for aerobic respiration, a much more efficient transformation of energy that generates 18 times more molecules of ATP from one sugar molecule than anaerobic respiration. ATP is the universal intermediate for energy transduction in living organisms, and is therefore a convenient measure of useful energy (but don't mistake it for a high-energy intermediate!). Aerobic metabolism, in turn, enabled the evolution of complex, multicellular, energy-efficient eukaryotic organisms, as Lauren Koch and Steven Britton at the University of Michigan argue in a comprehensive review published in the *Journal of Physiology*.⁴

Photosynthesis as we know it today is responsible not only for the vital task of taking carbon dioxide out of the atmosphere, but also for replenishing the atmosphere and the oceans with oxygen that's essential for sustaining all aerobic life.

Scientists monitoring climate change and policy-makers have been focusing almost exclusively on the rapid increase in carbon dioxide in the atmosphere due to excessive burning of fossil fuels. But recent research has shown that oxygen in the atmosphere is depleting faster than carbon dioxide is rising.⁵ It is becoming clear that reducing carbon dioxide emissions is not enough; replenishing oxygen is just as important, if not more so. Deforestation and destruction of natural ecosystems can create extra oxygen sinks that prevent oxygen getting back into the atmosphere. Some ecosystems tie up more oxygen than others. Lignins in forest trees are more reduced (contain less oxygen) than cellulose and starch in crop plants. The synthesis and use of large amounts of chemical fertilizers, similarly, could tie up extra oxygen in nitrates.

Water Crisis

Even though water appears unlimited as a source of electrons and protons, it is not an unlimited resource. That's because human beings, along with land plants and animals as well as freshwater organisms, depend on freshwater to survive. The amount of water on Earth is enormous: 70.9% of Earth's surface is covered by water, its total volume $\sim 1\,338\,000\,000\text{ km}^3$. Most of that, 96.5%, is found in oceans, 1.7%

in glaciers and ice caps, 1.7% in groundwater, the remaining in large bodies of water, and 0.001% in air as water vapour and precipitation. Only 2.5% of Earth's water is fresh; 98.8% of that is stored in ice and groundwater; and less than 0.3% of freshwater is in rivers, lakes, and the atmosphere.⁶

Freshwater is widely regarded as the world's most essential natural resource, underpinning human life and economic development and the survival of countless organisms ranging from microscopic life to fish, amphibians, birds, and terrestrial animals of all kinds. Unfortunately, this precious and vital life resource is being depleted and degraded at alarming rates.

The world's rivers, the single largest water resource for humans and a crucible of aquatic biodiversity, are in crisis, according to a global analysis published in 2010, the first to simultaneously account for the effects of pollution, dam-building, agricultural runoff, the conversion of wetlands, and the introduction of exotic species on the health of the world's rivers.⁷ Among the startling findings is that rivers in the developed world, including much of the United States and Western Europe, are under severe threat despite decades of pollution control and investments in environmental protection. Huge investments in water technology and treatment reduce threats to humans, but mainly in developed nations, and leave biodiversity in both developed and developing countries under high levels of threat.

In the major croplands of the world—China, India, and US—with half the world's population, industrial farming practices have severely depleted underground water, dried out rivers and lakes, eroded top-soil, and decimated wildlife with fertilizer and pesticide runoffs.⁸

A proper understanding and appreciation of water is a matter of the utmost urgency, so that we can stop the degradation and devote major efforts to conserving and restoring this essential life resource.

Water has its own cycle of generation and regeneration in nature, which is essential for the health of the planet, as Austrian forester and pioneer water scientist Viktor Schauberger (1885–1958) discovered (See Alick Bartholomew's Story of Water⁹).

Oxygen and the Evolution of Complex Life Forms

Life on Earth is generally believed to have originated 3.9 billion years ago, though some scientists have put it back as far as 4.38 billion years ago on the basis that all the necessary conditions for life — liquid water, energy sources, and chemical building blocks — were already present.¹⁰ The atmosphere was devoid of oxygen, and hence the primordial organisms were solely dependent on getting electrons from organic compounds like sugar or inorganic molecules such as hydrogen, sulphide/sulphur, ammonia, and metal/metal ions. Anaerobic respiration cannot make much energy available to support many activities. Consequently, no multicellular complex organisms are exclusively anaerobic.

Bacteria capable of harvesting light energy in a primitive photosynthetic process that does not produce oxygen appeared about 3.3 billion years ago. Oxygen-producing photosynthesis only became established around 2.5 billion years ago. That initiated the Great Oxidation Event to build up oxygen in the atmosphere to 2% by 2 billion years ago. The oxygen level remained static for the next billion years when aerobic respiration evolved and small multicellular organisms became widespread. Between 1.0 and 0.5 billion years ago, oxygen rose sharply to its current level of 20%. This increase is widely believed to have fuelled the Cambrian explosion, the rapid evolution of all major animal phyla on Earth between 540 and 490 million years ago.¹¹ The oxygen level then took a steep dive to below 15% before rising sharply to 35% 300 million years ago, coincident with the evolution of large woody land plants with very active photosynthesis.¹² This period saw the emergence of gigantic insects, as the high atmospheric oxygen concentration lifted the constraint on its availability to tissues and cells supplied through the insects' tracheal system.¹³ The subsequent drop in oxygen concentration to more or less the present 20% about 260 to 234 million years ago is believed to be due to a substantial reduction in lowland forests and swamps.

A rough correlation is found between increasing oxygen levels and the rise in the number of different cell types estimated from

the protein sequence data using molecular clock methods (which are not reliable, however¹⁴). Organisms with two to three cell types appeared shortly after the initial increase in atmospheric oxygen to 2% 2 billion years ago, with further increases occurring only when oxygen started to rise again at 1 billion years ago, up to 120 cell types by 0.5 billion years ago when the oxygen level reached 20%.

Oxygen is Stable, Abundant, and Fit for Life

Life is created out of the 85 stable elements; and 7 of the most abundant 10 elements in the universe — hydrogen, helium, oxygen, carbon, neon, nitrogen, magnesium, silicon, sulphur, and iron — are represented in organisms. So, it is very probable that life anywhere else in the universe will also be similar to that on Earth.

Oxygen is the third most abundant element and has special features that make it most fit for life. First, the molecules of life are carbon-based, and among the elements, only nine are more electronegative than carbon and therefore able to serve as an acceptor of electrons from carbon-based substrates. Of the nine — selenium, sulphur, iodine, krypton, bromine, nitrogen, chlorine, oxygen, and fluorine — oxygen ranks second only to fluorine in electronegativity, and the other elements are either a solid, highly reactive, less abundant, or substantially less electronegative. Thus, reduction of oxygen (burning, transferring electrons to oxygen) provides close to the largest possible transfer of energy for each electron. Another advantage of oxygen is that it exists typically as a stable triplet molecule consisting of two atoms (O_2),¹⁵ which is also a freely diffusible gas, and hence available to all life forms, in the air, on land, and in water. Furthermore, oxygen gas is much more easily transported to tissues and cells inside the organism.

Oxygen in Action, Past and Present

Large evolutionary events are associated with oxygen in the past (see earlier). But the evidence is also all around us today.¹⁶

For example, the trend for animals to be larger at higher latitudes — polar gigantism — is best correlated with oxygen. A measurement of 1853 bottom-dwelling amphipod crustaceans from 12 sites worldwide that included polar, tropical, marine, and freshwater environments revealed a strong correlation between maximum body length and oxygen content ($r^2 = 0.98$, $p < 0.0001$). In the laboratory, a reduction of oxygen level by 10% decreases the body mass of fruit flies, while an increase in the oxygen level by 40% increases body mass. The atmospheric oxygen burst due to land plants that boosted the oxygen level in the Earth's atmosphere to 35% 300 million years ago and its subsequent decline to present levels were indeed accompanied by a dramatic rise and fall in the size of insects.

The fossil record shows that vertebrates started to come on land about 415 million years ago. Thereafter, the record disappeared for the interval 360 to 345 million years ago. This 15-million-year lull in the fossil record of vertebrates' colonization of land, known as Romer's Gap, remained a complete mystery until a research team tested the hypothesis that environmental factors might be responsible. They looked at the ranges of terrestrial arthropods over the same time period, and found a pattern similar to that of vertebrates; that is, few new groups evolved for both vertebrates and arthropods during Romer's Gap. They suggested that it coincided with and is explained by the low atmospheric oxygen at the time, less than 15% at its lowest.

The current global warming is particularly challenging for fish because warming of the oceans results in less dissolved oxygen while pushing up the metabolic rate at the same time. A study of eelpouts from the North and Baltic Seas revealed that thermally limited oxygen delivery closely matches environmental temperatures, and there is a threshold beyond which growth and abundance decrease. Consequently, warming of the seas will be the first process to cause extinction or relocation of organisms to cooler waters.

Actually, warming seas hit photosynthetic plankton first, and have already done so. Plankton is the major food source that supports marine life; it also replenishes oxygen in seawater.¹⁷ The

amount of plankton biomass created is the difference between the photosynthetic rate and the respiration rate. As temperature rises, both the photosynthetic and respiration rates go up. Unfortunately, respiration goes up faster, resulting in less plankton growth. The warming oceans are already showing signs of being starved of oxygen, probably linked to phytoplankton decline.¹⁸ Phytoplankton are the fastest-growing photosynthetic organisms on Earth, and are literally the lifeline of the entire biosphere, both on land and in the sea, in terms of regenerating oxygen. When phytoplankton goes, we can expect anoxic oceans and mass extinctions of air-breathing animals, as had happened at the end of the Permian 251 million years ago, which is thought to be caused by low oxygen levels from global warming.¹⁹ This time round, the human species may be among the first to go.

Oxygen and the Complexity of the Metabolic Network

Oxygen makes more energy available and at a greater efficiency; at the same time, it increases the complexity of metabolic networks.

The core metabolic network connects major chemical species (metabolites) that are transformed by biochemical reactions involved in energy transduction and mobilization. It is a very complicated branching network that offers infinite possibilities for channelling and diverting metabolites to different ends for maximum effect.²⁰

The connectivity of the networks of 43 different organisms in all three domains of life from bacteria to higher organisms was investigated using the tools of graph theory and statistical mechanics.²¹ The analysis revealed that the metabolites are not randomly connected. Instead the number of connections per node (metabolite) approximates a power law, $P(c) \sim c^{-\gamma}$, where c is the number of connections, and $P(c)$ is the probability of finding a node with c connections. In other words, most metabolites have one or a few connections, and the number of nodes with many connections

drops off rapidly. Significantly, the most highly connected metabolites were those associated directly with energy transfer, with water at the top of the list.

This scale-free or fractal network has been found to describe biological structures and living processes in general,²² including the cytoskeleton (see Chapter 18); it also applies to the World Wide Web, Internet, and social networks.

A direct investigation on how oxygen availability changed the architecture of metabolic networks was carried out by “seeding” with a pre-specified set of metabolites that were allowed to react according to known enzymatic reaction rules.²³ Once all possible reactions had been carried out, the products joined the seed metabolites, potentially allowing new reactions to occur. This process was reiterated until no new products were generated. This separated four discrete groups of networks of increasing complexity, with transitions between groups contingent on the presence of the key metabolites: NAD⁺, S-adenosyl methionine, coenzyme A, ATP, oxygen, carbon dioxide, ammonia, pyruvate, and 2-oxoglutarate. The most complex group IV reactions were associated almost exclusively with the presence of oxygen, and had as many as 1 000 reactions more than those of the largest networks achieved without oxygen.

Notes

1. Reiss (1985).
2. The rest of this chapter is based on Ho (2009b).
3. Dismukes *et al.* (2001).
4. Koch and Britton (2008).
5. Ho (2009e).
6. “Water”, Wikipedia, 12 December 2011, <http://en.wikipedia.org/wiki/Water>.
7. Vörösmarty *et al.* (2010).
8. Brown (2009).
9. Bartholomew (2010).

10. “Scientists Set Back Clock on Origin of Life”, AFP, 20 May 2009, <http://www.google.com/hostednews/afp/article/ALeqM5jQtza4JldbayIBiYWnolxNkl6TEQ>.
11. Valentine (2004).
12. Bemer *et al.* (2007).
13. Koch and Britton (2008).
14. See Ho (2009a).
15. See Glossary for a definition of the triplet, an electronic state of an atom or molecule. In the case of the oxygen molecule, the triplet state is stable, whereas the alternative singlet state is highly reactive, and is usually generated by sunlight, accounting for sunlight’s damaging effects on many organic materials (see Ho, 2009c, for further details).
16. Koch and Britton (2008).
17. See Ho (2006f,g).
18. See Ho (2009f).
19. “Permian-Triassic Extinction Event”, Wikipedia, 27 November 2011, http://en.wikipedia.org/wiki/Permian-Triassic_extinction_event; Ho (2009g).
20. Ho (1995).
21. Jeong *et al.* (2000).
22. Ho (2004b, 2007g,h).
23. Jeong *et al.* (2000).

Water Fuels the Dynamo of Life

Water and Energy Metabolism

Water is indeed central to the energy metabolism that powers all living processes. The redox dynamo of life is fuelled, lubricated, and super-facilitated by water. As we saw in the previous chapter, the redox cycle of the biosphere consists of photosynthesis in one direction and respiration in the other. In this chapter, we shall look at the two processes in more detail.

Respiration

Respiration occurs in two phases, one anaerobic and the other aerobic (thanks to oxygen made available by photosynthesis). The anaerobic phase is *glycolysis*, which splits glucose, a six-carbon molecule, into two molecules of pyruvic acid, a three-carbon compound. The aerobic phase, the *citric acid cycle*, oxidizes pyruvic acid completely to carbon dioxide and water, and takes place in the mitochondria, special membrane-bound organelles serving as “powerhouses” in the cell (see Fig. 22.1), along with the oxidative electron transport chain that makes ATP.¹

The number of mitochondria in a cell varies from a few hundred to thousands in very active cells. Their main function is to convert energy extracted from food into ATP. A mitochondrion has an outer membrane enclosing the entire structure, and a much-folded inner membrane that encloses a matrix, projecting numerous thin plate-like folds or *cristae* into it. Between the two membranes is a

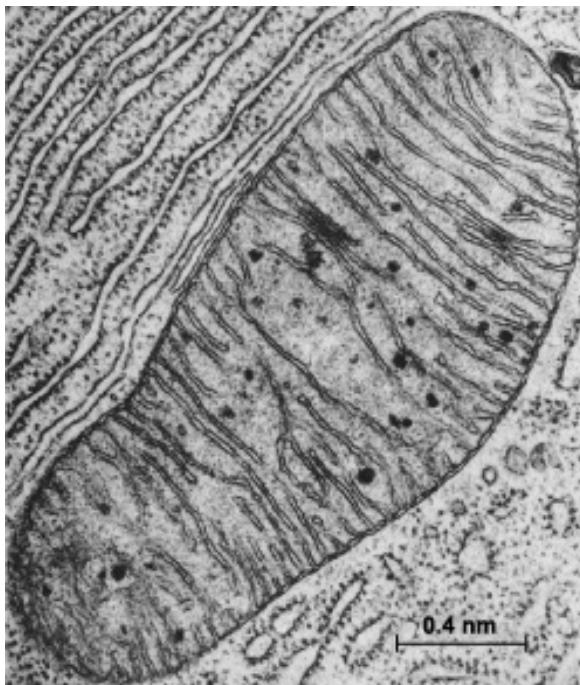


Figure 22.1 Electron micrograph of a mitochondrion in a cell of the bat pancreas, by Keith Porter.²

labyrinthine intermembrane space. Each mitochondrion also has five to ten circular molecules of mitochondrial DNA that are replicated and inherited independently of the cell's genome.³

The outer membrane of the mitochondrion contains many complexes of integral membrane proteins that form channels through the membrane, where a variety of molecules can move in and out of the mitochondrion. The inner membrane contains five complexes of integral membrane proteins of the oxidative electron transport chain: nicotinamide adenine dinucleotide (NADH) dehydrogenase (Complex I), succinate dehydrogenase (Complex II), cytochrome c reductase (Complex III), cytochrome oxidase (Complex IV), and ATP synthase (Complex V).

The matrix of the mitochondrion contains a mixture of 'soluble' enzymes that catalyse the oxidation of pyruvic acid in the citric acid

cycle (also called the Krebs cycle), the main product of which is reduced (hydrogenated) NADH, a redox carrier for transferring protons and electrons between electron donors and acceptors.⁴ Recall that in Chapter 18, evidence was presented that the matrix enzymes in the mitochondria are not at all soluble, but associated into multi-enzyme metabolons that channel metabolites from one enzyme to the next in sequence.

A summary of the component processes of respiration is depicted in Fig. 22.2.

Glycolysis takes place in the cytoplasm, and does not require oxygen, so it can occur anaerobically. It converts each molecule of glucose into two molecules of pyruvate and generates two net molecules of ATP. Four molecules of ATP per glucose are actually produced, but two are consumed in the initial reactions, one to phosphorylate glucose, so it can be cleaved into two, and another

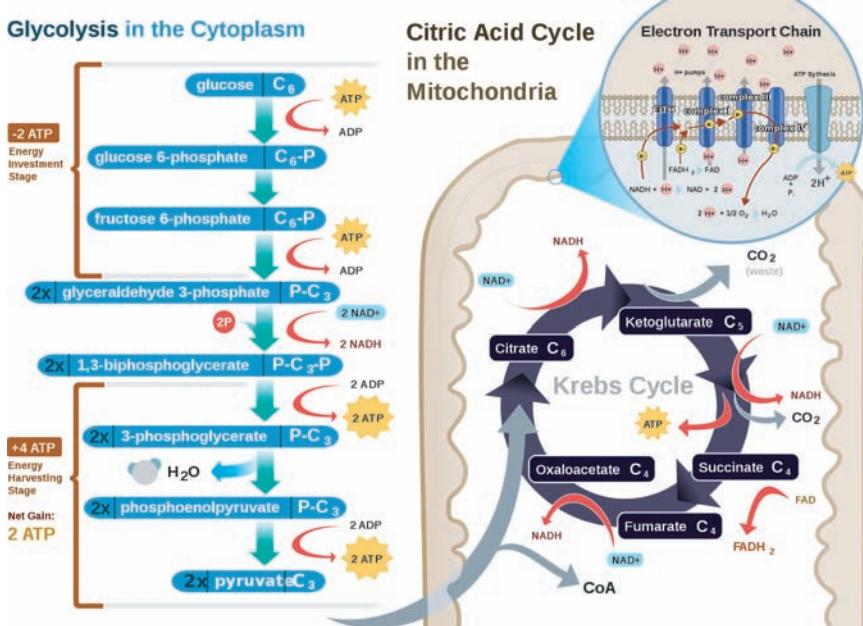
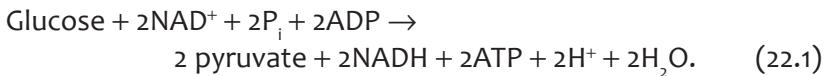


Figure 22.2 The component processes of respiration, by RegisFrey, Wikimedia.⁵

to make two phosphorylated three-carbon molecules. During subsequent reactions, four ATP and two NADH are produced ending in pyruvate. The overall reaction is as follows:



The two ATPs consumed in glycolysis are for activating the substrates, and more examples will turn up in the Calvin cycle of photosynthesis later.

The next aerobic phase requires oxygen, and takes place in the matrix of the mitochondrion. Pyruvate is oxidized to a two-carbon fragment joined to co-enzyme A to form acetyl-CoA with the release of one molecule of CO_2 and generation of one molecule of NADH by the pyruvate dehydrogenase complex (PDC), which contains multiple copies of three enzymes. This step is also known as the *link reaction*, as it links glycolysis and the Krebs cycle, also known as the tricarboxylic acid (TCA) cycle, or the citric acid cycle.

The citric acid cycle consists of eight reactions (Fig. 22.2). Acetyl-CoA (two-carbon) joins up with oxaloacetate (four-carbon) to form citrate (six-carbon), which is rearranged into a more reactive form called isocitrate. Isocitrate is oxidized, losing one carbon to CO_2 and generating one NADH to become α -ketoglutarate (five-carbon), which is activated to succinyl-CoA, resulting in the loss of one carbon to CO_2 , generating another NADH as well as one guanosine triphosphate (GTP, subsequently converted to ATP) as succinate is released from CoA. Succinate is oxidized further to fumarate, generating one molecule of FADH_2 (reduced flavin adenine dinucleotide). Fumarate is oxidized to malate, and finally, to oxaloacetate with the generation of a third molecule of NADH. The net energy gain from one cycle is three NADH, one FADH_2 , and one ATP. Thus, the total energy yield from one glucose molecule (two pyruvate molecules) is six NADH, two FADH_2 , and two ATP.

The third component is the oxidative electron transport chain, which couples electron transfer between an electron donor (such as NADH) and an electron acceptor (such as O_2) with the transfer of

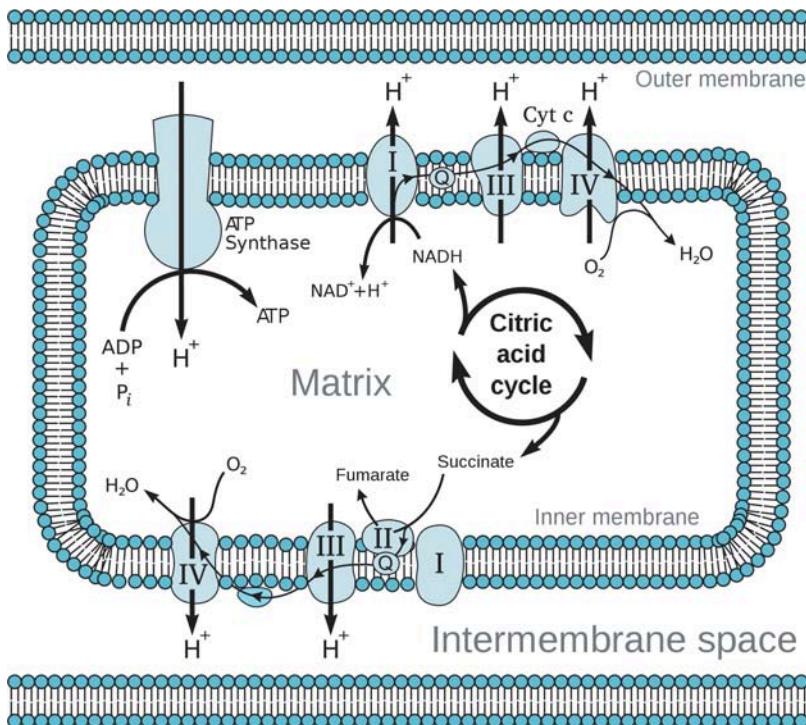


Figure 22.3 Oxidative electron transport chain in the mitochondrion, by Fvasconcellos, Wikimedia.⁶

H^+ ions (protons) across a membrane. The proton is used to generate ATP. Electron transport chains are present in photosynthesis as well as in respiration. The electron transport chain of the mitochondrion is depicted in Fig. 22.3.

There is good evidence that protons actually migrate through liquid crystalline water on biological membranes to the ATP synthase without entering into the bulk phase to set up a proton gradient (see Chapter 13).

Photosynthesis

Photosynthesis is the conversion of carbon dioxide into organic compounds, using the energy from sunlight to split water into hydrogen

(and electrons) for reducing carbon dioxide into acids and sugars, and oxygen, which makes oxidative metabolism possible (see Chapter 21).

Photosynthesis is carried out by green plants and algae, and many species of bacteria, but not Archaea. In addition to maintaining normal levels of oxygen in the atmosphere, photosynthesis is the source of energy for nearly all life on Earth, either directly, through producing food plants, or indirectly, as the source of their food web. The exceptions are chemoautotrophs that live in rocks or around deep sea hydrothermal vents that use chemical energy from simple compounds.

The rate of energy capture by photosynthesis is immense, approximately 100 trillion watts (1 trillion watts = 1 terawatt, or TW), about ten times the current power consumption of the human species. In all, photosynthetic organisms convert around 100 to 115 petagrams ($1 \text{ Pg} = 10^{15} \text{ g}$) of carbon into biomass per year.⁷

Photosynthesis begins when light is absorbed by chlorophyll in the photosystem consisting of clusters of special proteins. In plants and algae, these proteins are present in special organelles inside cells, the chloroplasts. In bacteria, the photosynthetic proteins are embedded in the plasma membrane. The energy absorbed from sunlight is used directly to synthesize ATP and to split water by removing electrons (and protons) from it to fix carbon dioxide, liberating oxygen to the atmosphere. The overall process is depicted in Fig. 22.4.

Chloroplasts perform photosynthesis during daylight hours, and photosynthetic cells use the immediate products of photosynthesis, reduced nicotinamide adenine diphosphate (NADPH) and ATP, to make many organic molecules such as simple sugars that are exported to other non-photosynthetic cells. An electron micrograph of a plant chloroplast is presented in Fig. 22.5.

Chloroplasts are like mitochondria, and couple electron transport to proton transfer across a membrane to generate ATP. Structurally, they are much larger, and have an extra compartment. They are enclosed by a highly permeable outer membrane, and a much less permeable inner membrane in which membrane transport proteins

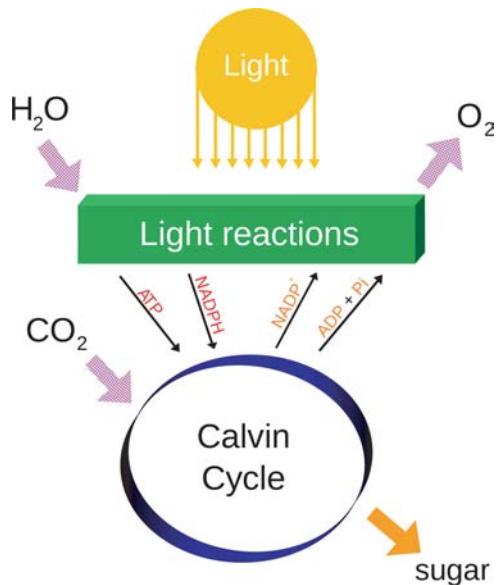


Figure 22.4 Overview of photosynthesis by Daniel Mayer, Wikimedia.⁷

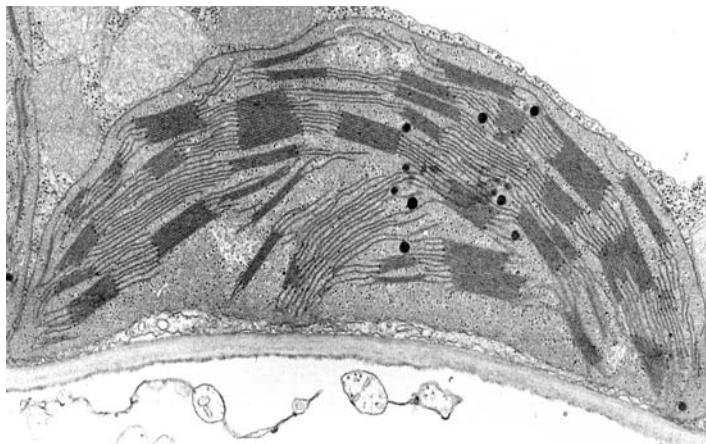


Figure 22.5 Electron micrograph of a chloroplast, courtesy of University of Wisconsin, Botany Department.

are embedded, with a very narrow intermembrane space in between. The inner membrane encloses a space called the stroma, analogous to the mitochondrial matrix, and contains many metabolic enzymes. Like the mitochondrion, the chloroplast also has its own genome, with transcription and translational machinery. Unlike the mitochondrion, the inner membrane of the chloroplast is not folded up into cristae, and does not contain electron transport chains. Instead, the electron transport chain, together with the ATP synthase and the photosynthetic light-capturing system, are all embedded in the *thylakoid membrane* that forms a series of flattened sacs, the thylakoids that constitute a third compartment, the thylakoid space, which is thought to be connected with and separate from the stroma surrounding it. The thylakoid membrane also forms local stacks called *grana*.⁶

The many reactions of photosynthesis can be grouped into two categories: the *light reactions*, or photosynthetic electron transfer, and the *dark reactions*, or carbon fixation. The light reactions depend on the presence of light, in which the energy from sunlight excites an electron in the chlorophyll to move along an electron transport chain in the thylakoid membrane, the electron coming ultimately from water. This splits the water, producing O₂. Electron transport is coupled to the movement of H⁺ across the thylakoid membrane, which in turn drives the synthesis of ATP in the stroma via ATP synthase. As the final step of electron transport, the high-energy electrons are loaded together with H⁺ onto NADP to make NADPH.

In the dark reactions, the NADPH and ATP produced in the light reactions provide reducing power and energy for fixing CO₂. The carbon-fixing reactions begin in the stroma of the chloroplast and continue in the cytoplasm, producing sucrose and other organic compounds in the plant.

Carbon capture occurs either via C₃ or C₄ pathways. C₃ carbon fixation occurs in 95% of green plants. It converts CO₂ and ribulose-biphosphate, a five-carbon sugar, into 3-phosphoglycerate, which is reduced to glyceraldehyde 3-phosphate by NADPH, thereafter going through many reactions ending with the regeneration of ribulose 5-phosphate and ribulose 1,5-biphosphate (see Fig. 22.6).

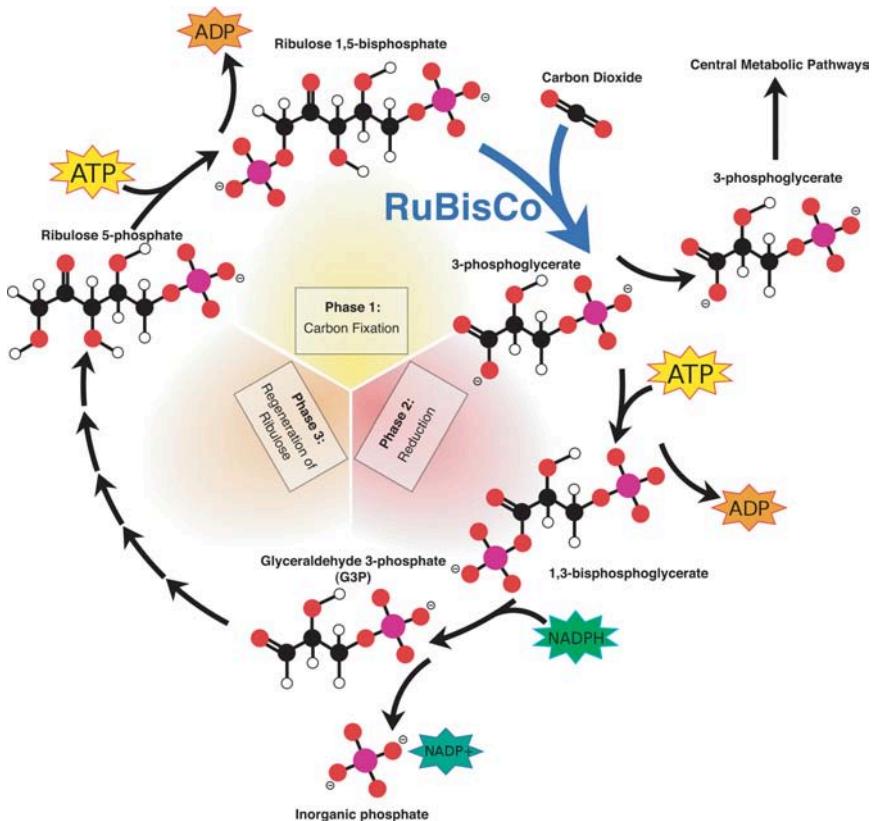


Figure 22.6 Carbon fixation through the Calvin–Benson cycle in photosynthesis, by Mike Jones, Wikimedia.⁷

In C₄ plants, CO₂ is drawn from malate, a four-carbon compound, before it enters the Calvin cycle, rather than directly from the air. Crassulacean acid metabolism (CAM) occurs in plants growing in arid conditions. CO₂ enters through pores (stomata) on stems and leaves during the cool night, and is converted into organic acids that release CO₂ for use in the Calvin cycle during the day when the stomata are closed to prevent loss of water through transpiration. The jade plant *Crassula ovata* and cacti are typical of CAM plants.

The light reactions are initiated in the chloroplast by chlorophyll. When a quantum of light is absorbed by the chlorophyll, an electron

is excited from its ground state to a higher energy state. An excited electron is unstable, and tends to return to its ground state by one of three ways: radiating the energy away as heat, transferring it to neighbouring chlorophyll molecules by resonance energy transfer, or by transferring the electron to an electron acceptor nearby and replacing it with a low-energy electron from an electron donor.⁸

In photosynthesis, the two latter alternatives are greatly enhanced by two different protein complexes: resonance energy transfer by an *antenna complex* and high-energy electron transfer by a *photochemical reaction centre*. The antenna complex and the linked photochemical reaction centre together make up a *photosystem (PS)*.

The antenna complex consists of transmembrane proteins (light-harvesting complexes) bound to a large set of pigment molecules; several hundred chlorophyll molecules plus accessory pigments, carotenoids that protect the chlorophyll from oxidation, and help collect light of other wavelengths. When light excites a chlorophyll molecule in the antenna complex, the energy is rapidly delocalized over all the pigment molecules by resonance energy transfer until it reaches a special pair of chlorophyll molecules in the photochemical reaction centre. Each antenna complex essentially acts as a collecting funnel to channel light energy to specific sites where it can be used effectively.

A photochemical reaction centre is a transmembrane pigment-protein complex. The special pair of chlorophyll molecules acts as an irreversible trap for the excitation energy, so that an excited electron from splitting water is immediately passed along to the nearby electron transport chain; electron transport is coupled to proton transport and photophosphorylation takes place to make ATP, as in oxidative phosphorylation (see Fig. 22.7).

Note that in green plants, two photosystems (PSII and PSI, Fig. 22.7) work in relay to boost electrons up the necessary redox potential to reduce NADPH.

Within the past several years, researchers have discovered that light-absorbing molecules in photosynthetic proteins capture and transfer energy quantum-mechanically, with long-range quantum coherence between molecules previously thought impossible;¹⁰ this

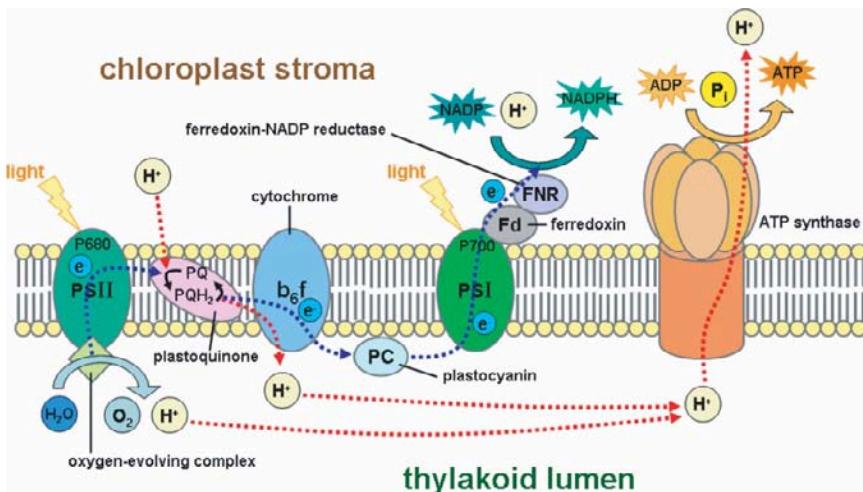


Figure 22.7 Photophosphorylation coupled to electron transport in the photosynthetic electron transport chain,
Tameeria, Wikimedia.⁹

“long-range” quantum coherence being no more than the 5 nm distance across proteins.

Sooner or later, they too will discover that the entire organism is quantum coherent.

Notes

1. “Carbon Fixation”, Wikipedia, 27 September 2011, http://en.wikipedia.org/wiki/Carbon_fixation.
2. Obtained from Answers.com.
3. “Cellular Respiration”, Wikipedia, 10 September 2011, http://en.wikipedia.org/wiki/Cellular_respiration.
4. “Electron Transport Chain”, Wikipedia, 12 September 2011, http://en.wikipedia.org/wiki/Electron_transport_chain.
5. “Cellular Respiration”, Wikipedia, 10 September 2011, http://en.wikipedia.org/wiki/Cellular_respiration.
6. “Electron Transport Chain”, Wikipedia, 12 September 2011, http://en.wikipedia.org/wiki/Electron_transport_chain.

7. "Photosynthesis", Wikipedia, 15 September 2011, <http://en.wikipedia.org/wiki/Photosynthesis>.
8. See Alberts *et al.* (2008).
9. "Photophosphorylation", Wikipedia, 2 December 2011, <http://en.wikipedia.org/wiki/Photophosphorylation>.
10. Law *et al.* (2004); Collini *et al.* (2010).

Electronic Induction Animates Life

Sweeping Away Old and New Cobwebs

We are now ready to draw all the strands together towards a new cell biology that will take us beyond the clutter of molecular nuts and bolts that fill the usual textbooks. You may have noticed that, so far, I have not found it necessary to disagree substantially with the conventional account of cell biology. That's because most of this book is on what has been *left out*, even though almost all the scientific work I have described is drawn from the published scientific literature.

To clear the path, we must sweep away some deeply entrenched conventional wisdom, especially about the cell membrane, which has already been criticized in Chapter 18; and the best way to proceed is to consider the *membrane potential*. The membrane potential has been gaining a lot of attention lately on account of its central involvement in determining the vital states of cells and tissues, a sign of the fundamental electrodynamical nature of life.¹ Yet very few people really understand what makes the membrane potential.

What's Really Responsible for the Membrane Potential^[2]

All cells are enclosed in a plasma membrane, and an electrical potential difference can be measured by inserting a microelectrode through the membrane into the cell. A cell at rest has an average

membrane potential of -50 mV, but this can change a lot when the cell is active. Changes in membrane potential determine the cell's vital states, from the specification of body axis during development and regeneration, to cell proliferation, differentiation, and cancer.² But what exactly is the membrane potential?

A typical account goes like this: "The membrane potential arises from the net actions of ion channels and ion pumps (such as the sodium-potassium ATPase) embedded in the membrane, which produce different concentrations of ions (and therefore electrical charge) on the intracellular and extracellular sides of the membrane. The ion channels, when active, partially discharge the membrane potential, while the ion pumps restore and maintain it."³

That account leaves a lot unexplained. What exactly determines which ions end up inside or outside the cell? What determines the correct concentration differences inside and outside the cell?

The best (conventional) description I could find says that the membrane potential is generated by the asymmetric ion distribution across the membrane and selective ion channels, mostly K^+ and Na^+ channels.⁴ The "simplified" explanation goes as follows.

There is a difference in the concentration of potassium across the membrane, being a lot higher inside at about 150 mM than the 5 mM outside. The intracellular K^+ is nearly balanced by the negative charges on proteins. The membrane is highly permeable to potassium, as there are many potassium channels in the cell membrane; however, the proteins cannot get out as the membrane is not permeable to proteins.

The reason the cell is slightly negative on the inside is that K^+ tends to diffuse out, down its concentration gradient. However, as the K^+ leaves the cell, the charge-balancing proteins cannot follow, so the movement of K^+ out of the cell leads to the net transfer of positive charge to the outside, and consequently a small amount of unbalanced negative charge inside the cell, generating the resting membrane potential.

But why doesn't all the K^+ leave the cell? The negative charge generated inside holds them in, balancing the chemical gradient that makes them diffuse out. This is a point of equilibrium at which there is no net movement of K^+ into or out of the cell.

The situation is a bit more complicated. There is a concentration gradient of Na^+ , much higher outside the cell at about 150 mM than the 10 mM inside. This concentration gradient and the negative membrane potential both tend to send Na^+ inside the cell. The membrane also contains a small number of Na^+ channels. So there is a very slow leak of sodium into the cell. Every time an Na^+ ion leaks into the cell, a K^+ ion leaves the cell to maintain the electrical balance. If this were to continue, all of the K^+ concentration gradient would be dissipated. The cell's defence against that is the Na^+/K^+ -ATPase — the sodium-potassium exchange pump, or the sodium pump — which pumps sodium out of the cell in exchange for potassium, three Na^+ for two K^+ ions, for every ATP hydrolysed to adenosine diphosphate (ADP) and inorganic phosphate (Pi). (See Chapter 17 for an alternative explanation of how a change in water in the nanospace of the enzyme active site is involved in the working of the pump.)

Sepehr Eskandari at California State Polytechnic University, Pomona, rightly cautions that the Na^+/K^+ -ATPase is not directly responsible for generating the resting membrane potential, only for maintaining it, pointing out that one can poison the sodium-potassium exchange pump with ouabain, and the cell will continue to have a resting potential for hours.

What Eskandari has failed to explain is why K^+ rather than Na^+ should exist at high concentration inside the cell. He has also failed to say that the reason K^+ does not leave the cell is that it is not free to do so. Instead, most, if not all of it, is selectively bound to the carboxylate ($-\text{COO}^-$) groups of amino acid side-chains on proteins. Chinese-born US physiologist Gilbert Ling has been saying that for more than half a century, based on extensive experimental and theoretical investigations.⁵ Yet the membrane theory still holds sway. As explained in Chapter 18, the ascendancy of the membrane theory is closely tied to the triumph of cell over protoplasm in being regarded as the unit of life.

The Membrane Theory

According to the membrane theory, the cytoplasm (apart from organelles) is essentially an aqueous solution of proteins, ions, and other

molecules, surrounded by a membrane that restricts free diffusion and served by special ion pumps working ceaselessly to pump unwanted ions out and the right kinds of ions in, against numerous concentration gradients across the membrane. The main pump is the Na^+/K^+ -ATPase. In addition, active transport proteins ferry metabolites such as glucose into the cell. Above all, the water in the cell is no different from bulk water, except perhaps for the single layer around macromolecules.

The membrane theory has encountered many difficulties since it was proposed. First, pumps and transport proteins all require ATP, which many believe to be the “energy currency” of the cell. ATP was initially thought to possess high-energy phosphate bonds. But that was decisively disproved in 1956 by direct measurement of the energy released in the hydrolysis of ATP, which was found to be no different from any ordinary phosphate bond.⁶ Second, Ling estimated conservatively that the minimum energy needed to operate just the sodium pump in frog muscle was at least 15 to 30 times the maximum available energy.⁷ Indeed, the combined action of metabolic poison and low temperature that inhibited ATP synthesis completely nevertheless failed to change the concentration of K^+ inside the cell after five hours.

In a span of nearly 60 years, Ling and co-workers have shown time and again how his *association-induction* (AI) hypothesis (see later) can explain all the characteristics attributed to the cell membrane. The first part of the AI hypothesis is that all K^+ in the cytoplasm is selectively bound.

Potassium Bound to the Cytoplasm

When Ling proposed that practically all K^+ in the cytoplasm is bound to the carboxylate groups of proteins, the cytoskeleton that pervades the cell, which we now take for granted, was not yet known (see Chapter 18). But there was already considerable evidence that the cytoplasm is in an organized gel-like state, rather than a disorganized thick soup of protein dissolved in water.

The evidence that K^+ is bound or adsorbed, presented in detail in Ling’s book, includes the following.⁸ There is low intracellular

electrical conductance inside the cell despite the high concentration of K⁺, which indicates that most of the intracellular K⁺ is bound. Intracellular K⁺ mobility is strongly reduced compared to K⁺ outside the cell, again indicating that most of it is bound. There is a one-to-one correspondence between K⁺ ions and intracellular carboxylate groups. The intracellular K⁺ ion absorption sites were identified as the aspartate and glutamate side chains of intracellular proteins. (To refresh your knowledge of proteins, see Chapter 10.)

Among the strongest evidence that K⁺ is adsorbed to the cytoplasmic proteins is that the intracellular concentration of K⁺ remains high for hours after the cell membrane is cut or made permeable with detergent, or indeed, when the sodium-potassium exchange pump is poisoned with ouabain.

Potassium Bound *Selectively* Inside the Cell

Ling's theory links selective K⁺ adsorption to a special state of intracellular proteins and especially cell water (see later). Ludwig Edelmann at Saarland University, a biophysicist who took Ling's ideas very seriously, succeeded in obtaining the most true-to-life and beautiful electron micrographs of cells without the harsh dehydrating treatments that rob cells of their associated liquid crystalline water,⁹ proving that K⁺ is selectively adsorbed to carboxylate groups of proteins in preference to Na⁺; and incidentally also why "beauty is truth and truth beauty".¹⁰ The non-dehydrated cell has many more structures, and is hence most likely to be much closer to what the living cell is like; it was also unanimously judged to be by far more beautiful than an electron micrograph of the same cell prepared by state-of-the-art conventional methods involving dehydration (see Fig. 23.1).

In one of many experiments, Edelmann incubated cryo-fixed and embedded thin sections of frog muscle in solutions containing LiCl, NaCl, KCl, RbCl, and CsCl (pH 7.0 Tris buffer) at concentrations of 50 mM, 50 mM, 10 mM, 10 mM, and 10 mM respectively, and carried out micro mass-spectroscopy to determine the amount of the different ions bound to the muscle section. He found the following

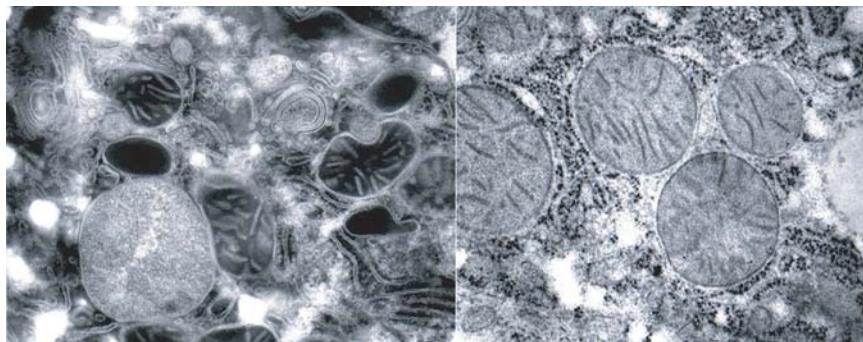


Figure 23.1 Which is the more beautiful?

order of selective ion uptake: $\text{Li}^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+$, or $\text{Cs}^+ > \text{Li}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+$.¹¹ Thus, K^+ was invariably preferred over Na^+ , despite the five-fold preponderance of Na^+ over K^+ in the solution.

The preferred adsorption of K^+ by the cytoplasm is the equilibrium resting state, which gives rise to the potential difference between the cell and the extracellular medium, even in the absence of the cell membrane. That is why the cell does not need the constant action of the Na^+/K^+ -ATPase to pump Na^+ out in exchange for K^+ . That pump is only ever activated to restore small deviations from a previous equilibrium; and we shall see how small and local these deviations are.

So long as the cytoplasm selectively binds K^+ , it will give the apparent membrane potential measured with a microelectrode stuck into the cell. In fact, Ling's absorption equation, presented in his book, has the same form as the usual Goldman–Hodgkin–Katz equation for the membrane potential based on the difference in concentration of ions outside and inside the cell.¹² But contrary to the conventional assumption that the potential difference applies to the inside of the cell as a whole compared to the outside, Ling assumes that the potential difference is strictly local, applying to the bit of cytoplasm containing the tip of the microelectrode and the external medium.¹³ By the same token, the change in the absolute number of K^+ ions that brings about a local alteration in membrane potential, as in an action potential, can be extremely small, and is readily restored to equilibrium by local pumps. This is

consistent with the fractal nature of the cytoplasm as described in Chapter 18; the nanospaces form dynamically autonomous micro-domains, unless the local stimulus exceeds a percolation threshold allowing it to spread globally. It is the intricate space-time differentiation that enables living systems to optimize both the kinetics of living processes as well as their thermodynamic efficiency (see Rainbow Worm).

So far so good; but when proteins are tested in solution outside the cell, they almost invariably prefer to bind Na^+ over K^+ (see Chapter 17), which led to a lot of scepticism of Ling's theory, as Ling himself recognized.

Both Na^+ and K^+ ions are hydrated (surrounded by water molecules). As pointed out by Martin Chaplin at London South Bank University — another scientist who takes Ling very seriously — Na^+ , being a smaller ion than K^+ , has a greater net charge on its surface than K^+ , and hence tends to form hydrogen bonds with water molecules, resulting in a large hydration shell (see Chapter 9). K^+ , being a bigger ion, has a smaller surface charge density and correspondingly weaker attraction for water; so water molecules preserve their hydrogen-bonding to one another and form a cage around the K^+ ion instead, rather than binding to it directly. Consequently, K^+ tends to pair *directly* with the carboxylate group ($-\text{COO}^-$) with no water of hydration in between. Na^+ , on the other hand, tends to form water-mediated pairing with the carboxylate group.¹⁴ The two ions, therefore, bind very differently, with the K^+ ion preferring stronger acids, i.e., groups with high electron-withdrawing (or low electron-donating) tendencies, whereas Na^+ prefers weaker acids, i.e., groups with low electron-withdrawing (or high electron-donating) tendencies, which happen to be the carboxylate groups of proteins, at least outside the cell. So how can one explain the selective binding of K^+ inside the cell?

Ling's Association-Induction Hypothesis

Ling's answer is that the proteins are in a very different state inside the cell, and so is the water; and the main reason is the ubiquitous

presence of ATP.¹⁵ Pioneers of the protoplasmic theory of life had indeed found that to be the case, as described by Joseph Needham (see Chapter 18).

Cells go to extraordinary lengths to keep the ATP concentration constant within the cell, replenishing it immediately with a reserve of creatine phosphate. And the sole function of creatine phosphate appears to be to resynthesize ATP from ADP under anaerobic conditions, which is particularly important for muscle cells in athletes.¹⁶

Proteins are long linear polymers of amino acids joined end to end in a peptide bond ($-\text{CONH}-$) (see Chapter 10). Peptide bonds on the same chain can form hydrogen bonds with one another, giving rise to secondary structures of α -helices or β -pleated sheets. Most proteins in solution are also folded up in further globular tertiary structures. That's the conventional textbook story.

That story does not apply to the cytoplasm, according to Ling. In his fully developed association-induction hypothesis, he proposed that the major components of living protoplasm — water, proteins, and K^+ — exist in a closely associated, high-energy state at rest. Within the resting cell, most if not all proteins are fully extended (instead of being bound up in α -helix or β -pleated sheet secondary structure) so that the peptide bonds along their polypeptide backbone are free to interact with water molecules to form polarized multilayers of aligned water molecules, while the carboxylate side chains preferentially bind K^+ over Na^+ . Both are due to the ubiquitous presence of ATP in living cells. And that is perhaps why ATP is maintained at a constant concentration in living cells.

In the absence of ATP, proteins tend to adopt secondary structures — α -helix or β -pleated sheet — as hydrogen bonds form between peptide bonds in the same chain or between different chains, and so they don't interact with water (Fig. 23.2, left). In this state, the carboxylate and amino side chains are also unavailable for binding ions, as they can pair up in a *salt linkage* (combination of basic and acidic groups) with each other. And the water next to the protein is not too different from the bulk phase outside the cell.

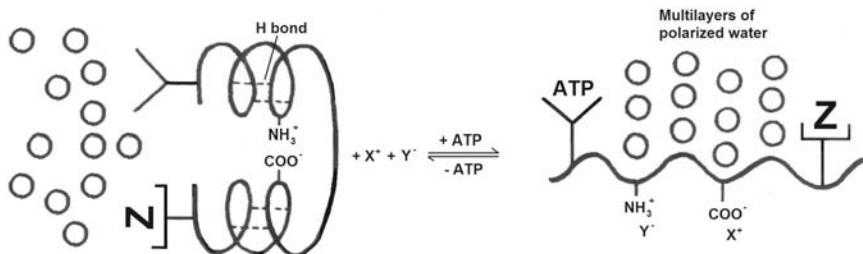


Figure 23.2 Gilbert Ling's elemental living machine.¹⁷

However, when ATP is bound to the cardinal site of the protein, it withdraws electrons away from the protein chain, thereby inducing the hydrogen bonds to open up, unfolding the chain, exposing the peptide bonds on the backbone, and enabling them to interact with water to form polarized multilayers (PM) (Fig. 23.2, right). At the same time, the carboxylate and amino side chains are opened up to interact with the appropriate inorganic cation X^+ and anion Y^- . The protein "helper" Z bound to the polypeptide chain is now also fully exposed. In muscle, the polypeptide chain binding ATP is myosin, and Z could well be actin. The cation X^+ is K^+ in preference to Na^+ , because ATP binding turns the carboxylate group into a strong acid that prefers K^+ over Na^+ . When ATP is split into ADP and Pi, and detaches from the protein, the reverse change takes place, the protein reforms its secondary structure and expels the PM water. Notice that the state change involves a major change in the water between an ordered PM to a relatively disordered state. (Something like this could well be the basis of how actin and myosin function in muscle contraction, and how molecular motors function in other cells.)

The switching between states is the elemental "living machine". It is what animates and energizes the living cell. According to Ling, this is also the unit protoplasm, the basis of life. Thus, protoplasm is almost infinitely divisible, as discovered by the pioneers of cell biology. In its resting state, the unit protoplasm consists of protein, ATP, K^+ , and PM water. Ling uses the red blood cell to illustrate the principle of the unit living protoplasm.

The Unit Protoplasm

A mature red blood cell (rbc) is 65% water; of the remaining 35% dry matter, 97% is a single protein haemoglobin, 1% is 100 mM K⁺, and the rest, organic products of energy metabolism, including ATP and 2,3-diphosphoglycerate, which are essential for the oxygen transport function of haemoglobin. The rbc appears homogeneous under the electron microscope, rather like a uniform gel phase (see Fig. 23.3). It is very stable; a mature rbc can be cut into the tiniest pieces without liberating its haemoglobin.

Based on the composition of mature rbc, Ling suggested a formula of individual elements referred to as nano-protoplasm in the red blood cell: Hb₁(H₂O)₇₀₀₀ K⁺₂₀ ATP₁.¹⁸ The molecular weight of haemoglobin is 68 000 Da, and 34% of the weight of mature rbc is haemoglobin, so there is 340 g/L, or 340 000/68 000 = 5 mM of haemoglobin, or 5 mM of the nano-protoplasm. Assuming a spherical shape, the nano-protoplasm would be 8.6 nm in diameter, rather close to the dimension of the nanospace predicted in the cell.

Physiologically active haemoglobin has 11% of its amino acid residues carrying fixed anions and an approximately equal percentage of fixed cations. The average amino acid residue weight is estimated to be 114. Dividing into 340 gives 2.98 to 3 mol of amino acid residues per litre of fresh rbc. As 11% carry β- or γ-carboxyl groups (available for binding to K⁺), the total concentration of those groups is 328 mmol/L. As the concentration of K⁺ is about 100 mmol,

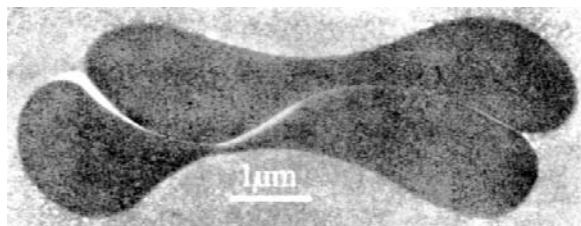


Figure 23.3 Electron micrograph of the red blood cell, by Ludwig Edelmann.

virtually all K^+ is adsorbed, leaving 228 mmol of carboxyl groups free to form salt linkages with fixed cations within the same haemoglobin molecules or with other haemoglobin and non-haemoglobin molecules nearby. Ling suggested that the gel state is one in which these salt linkages are formed, as opposed to the sol state, where no such linkages are formed.

Ling's Hypothesis and the Liquid Crystalline Cell

The interaction of unfolded protein chains with water is particularly significant. When protein chains are unfolded, their peptide bonds ($-\text{CONH}-$) become exposed, forming an alternating chain of negative ($-\text{CO}^-$) and positive (NH^+) fixed charges that is very good at attracting polarized multilayers of oriented water molecules (see Fig. 23.4). For one thing, fixed changes increase the probability of binding, just as someone standing still is much easier to catch hold of. I referred to this water as “liquid crystalline water” in the third edition of *Rainbow Worm*, both on the grounds that it forms dynamically quantum coherent units with the macromolecules (enabling them to transfer and transform energy seamlessly with close to 100% efficiency), and the individual water molecules are aligned with an order close to crystalline. It is this liquid crystalline

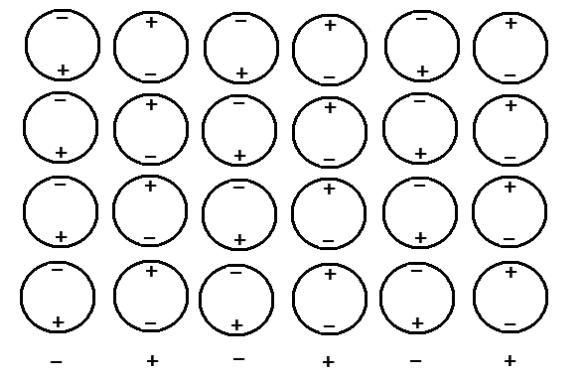


Figure 23.4 Polarized multilayers of water molecules form on the extended polypeptide chain.

water that gives the cell all its distinctive vital qualities. Though not mentioned by Ling, PM water is expected to be extremely good at resonant energy transfer over long distances; and at conducting positive electricity by jump conduction of protons (see Chapter 13). It has been estimated that the rate of proton migration along an ordered hydrogen-bonded chain of water is 40 times that of bulk water.

Ling pointed out that investigations on synthetic polypeptides in the 1950s clearly showed that when dissolved in solution, each polypeptide assumes only one or the other of two alternative conformations: in an α -helix where the imino ($-\text{NH}$) and carbonyl ($-\text{CO}$) groups are oriented in the same direction as the polypeptide axis, or else in a fully extended state where the groups are perpendicular to the polypeptide axis, which cannot be the “random coil” conformation generally assumed. It is also well known that the $-\text{CO}$ group in the peptide bond is highly polarizable, and the $-\text{NH}$ group much less so. Consequently, the effective electron density of the carbonyl oxygen atom of an amino acid residue determines that residue’s α -helical potential, i.e., its propensity to form an α -helix secondary structure.

Ling emphasized that the polarized multilayers of water molecules are also highly oriented, and referred to them recently as polarized oriented multilayers (POM) instead.¹⁸ The POM theory proposes a checkerboard of alternating positive and negative (NP) sites each uniformly 3.1 Å apart. Calculations show that as the distance from the idealized NP surface increases, the water-to-water interaction energy does not fall to zero, but approaches a constant value.

A diagram of the POM in a matrix of fully extended protein chains in the cell is given in Fig. 23.5.

POM water molecules are highly polarized and oriented. According to Ling, they are restricted in motion, and have shortened nuclear magnetic resonance relaxation times. (That is the basis of nuclear magnetic imaging that detects cancerous tissues by their longer relaxation times, as indicative of less structured water.) POM water does not freeze at the temperature of liquid

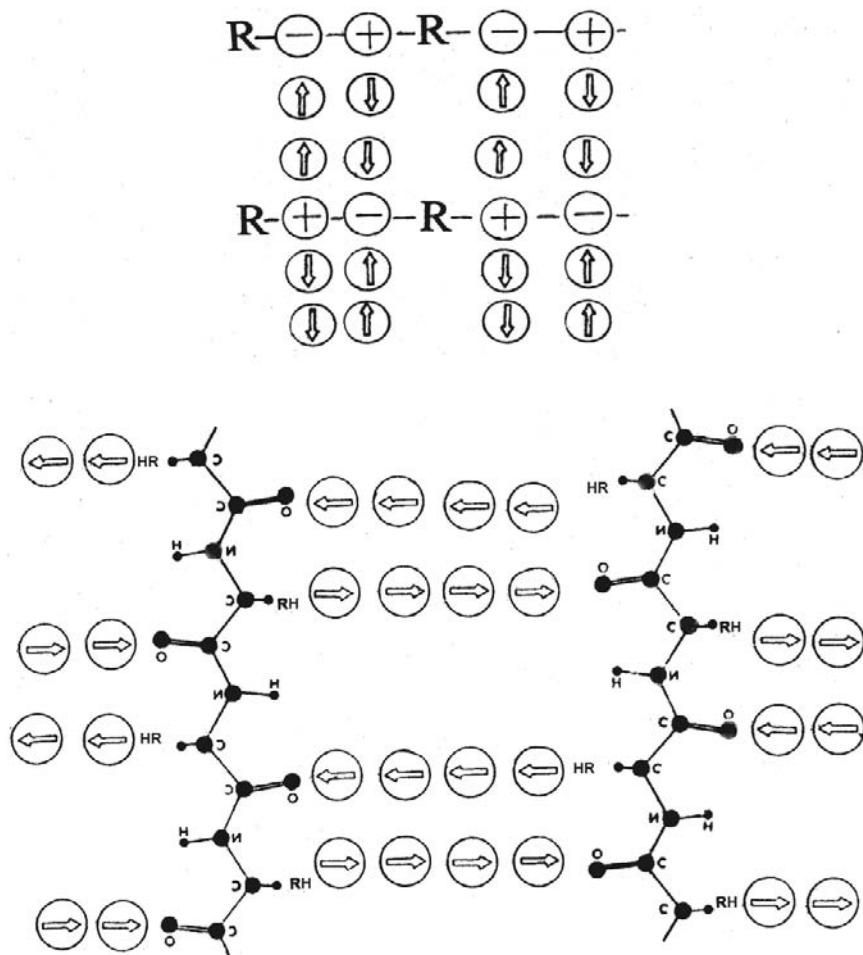


Figure 23.5 Ling's polarized oriented multilayers of water in a matrix of fully extended proteins: (top) side view; (bottom) view from surface; redrawn after Ling.¹⁸

nitrogen, and tends to exclude solutes, which accounts for the apparent diffusion barrier for many molecules erroneously attributed to the cell membrane. In fact, the cell membrane offers very limited restriction to diffusion, and it is the POM water that excludes them.

POM and the Exclusion of Solutes

A solute in a living cell can exist in two forms: adsorbed or bound to macromolecules, mostly proteins; or dissolved in cell water. Dividing the total concentration of the solute in the cell by the equilibrium concentration of the solute in the surrounding medium gives the ρ -value of the solute, or its apparent equilibrium distribution coefficient. Solutes with $\rho > 1$ are adsorbed; solutes with $\rho \sim 1$ or below are mostly dissolved in the intracellular cell water. The ρ -value of solutes can be obtained by plotting the intracellular concentration of the solute against its corresponding equilibrium concentration in the bathing medium. If the plot appears as a straight line over a substantial concentration range, it suggests that all or virtually all the intracellular solute is dissolved in the intracellular water. If the plot is not a straight line, most likely part of the solute is adsorbed.

However, even though the solute is dissolved in water, it is to varying extents excluded from POM water, according to the size rule, discovered by Ling and his colleagues. And this can be demonstrated quite simply with a 30% native bovine haemoglobin solution inside a dialysis bag that is allowed to come to equilibrium with a buffered solution containing different concentrations of non-electrolyte solutes of different sizes. These included, in increasing size, ethylene glycol, glycerol, erythritol, mannitol, sucrose, trehalose, raffinose, insulin, and polyethylene glycol. A straight line was obtained with a slope of about 1, indicating that there was no exclusion from the haemoglobin solution inside the dialysis bag. When the haemoglobin inside the dialysis bag was denatured (unfolded) with NaOH to bring the pH to ~ 12.0 , the solutes gave slopes substantially different from 1: the larger the solute, the smaller the slope, i.e., the more they are excluded by the POM formed on the surface of the extended haemoglobin chain.

The experiment would have been more convincing if ATP were included within the dialysis bag with haemoglobin, together with the other major constituent, K^+ , of the nanoplasma, $Hb_1(H_2O)_{7000}K^+_{20}ATP_1$. But that has not yet been done.¹⁹ The nanoplasma itself would also be an extremely interesting object for study, entrapped in a reverse micelle (see Chapter 20).

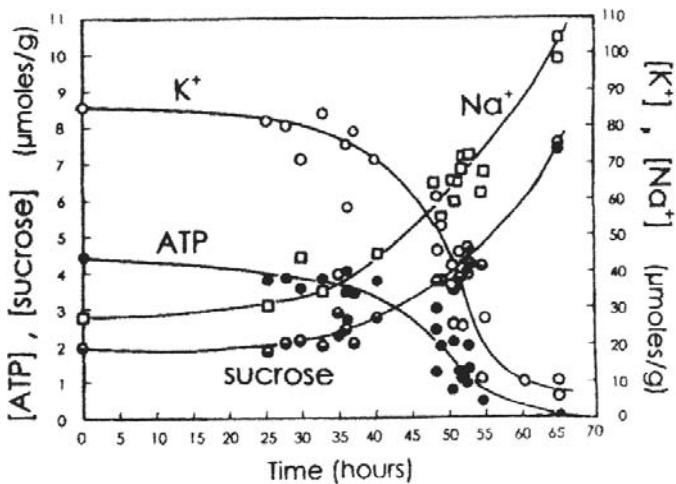


Figure 23.6 Time course of intracellular concentrations of K^+ and ATP decreasing in dying frog muscle cell as Na^+ and sucrose increase in anti-parallel fashion; redrawn after Ling.²⁰

When frog muscle was poisoned with iodoacetate, the dying cells showed characteristic parallel changes in K^+ and ATP mirrored by a precisely anti-parallel time course in the changes of Na^+ and sucrose (see Fig. 23.6). This was as predicted by Ling's AI hypothesis. As ATP depleted, the proteins in the cell regained their secondary structure and folded up, losing K^+ and gaining Na^+ ; at the same time, the loss of POM coincided with the cessation of sucrose being excluded from the cell.

Many recent findings — already described in earlier chapters — lend support to Ling's hypothesis and my idea of the liquid crystalline cell. I shall recapitulate these in the next section.

Support for the Liquid Crystalline Cell and Ling's AI Hypothesis

POM, or liquid crystalline water, resembles the supercooled hydration water of proteins identified by numerous methods recently, though the precise thickness of the layers varies from a single layer to four layers (see Chapter 10). A similar phase of water has been

found on hydrophilic solid surfaces, especially hydrophilic gels where the number of layers runs into millions, forming a giant exclusion zone that indeed excluded all solutes tested, including albumin and pH-sensitive dyes (Chapter 12).

Significantly, quantum electrodynamic considerations strongly suggest that under ambient conditions, water forms quantum coherent domains about 100 nm in diameter, and that the giant exclusion zones may represent macroscopic coherent domains stabilized on the surface of hydrophilic gels (Chapter 6).

In Chapter 2, I mentioned resonant energy transfer over long distances in bulk water under ambient conditions, with time constants of 80 fs, which is much shorter than the time constant of 1 ps for hydrogen bonds in the hydrogen-bonded network. As POM water is highly ordered and stabilized compared with bulk water, resonant energy transfer is expected to be even faster in the liquid crystalline water that permeates the cell and organism; although no direct measurement has yet been made. In *Rainbow Worm*, I pointed out how remarkably rapidly the body can react in ordinary people during an emergency, or in trained athletes during crucial moments in a match, which far exceeds the speed of nerve conduction; and suggested the possibility of intercommunication via resonance energy transfer through the liquid crystalline water matrix that permeates the entire body and into the interior of every single cell.

Liquid crystalline water offers yet another channel for rapid intercommunication: jump conduction of protons, which is much faster than conduction of electrons through metal wires. Jump conduction is described in Chapter 13, where evidence is presented for its occurrence in liquid crystalline water along biological membranes and in water trapped within narrow carbon nanotubes, which conducts protons 40 times as fast as bulk water. Proton jump conduction is also inferred for liquid crystalline water within extended nanospaces that mimic those in the cell (Chapter 19), as well as in water chains along collagen fibrils in the extracellular matrix (Chapter 20).

A cell with 80% water content would have POM water some four molecules thick that anastomose and surround the abundant

cytoplasmic proteins of the ubiquitous cytoskeleton as well as intracellular membranes. Chapter 18 presents a true portrait of the cell, where the cytoskeleton is shown to have a fractal structure, which is important both in ensuring that local compartments can form dynamically autonomous domains under normal conditions, and can percolate through, under critical conditions, ultimately to all cellular compartments and perhaps even beyond, to the extracellular matrix where all other cells are embedded. It is of interest that the highly polarized water around proteins identified in terahertz absorption spectroscopy within the past several years is also four molecules thick (Chapter 10).

Is there evidence that proteins inside cells are in extended conformation, rather than the conventional picture of hydrogen-bonded folded conformation? An opinion review article published in 2005 stated: “Recent progress in predicting protein structures has revealed an abundance of proteins that are significantly unfolded under physiological conditions. Unstructured, flexible polypeptide are likely to be functionally important and may cause local cytoplasmic regions to become gel-like.”²¹ This is another indication that Ling may well be right.

Indeed, the major cytoskeletal proteins — actin, tubulin, and intermediate filament proteins — polymerize into extended fibrous networks throughout the cell in the presence of ATP (guanosine triphosphate, GTP, in the case of tubulin) (see Chapter 18). The cytoskeletal proteins are also all highly acidic, with glutamate and aspartate carboxylate side chains and termini exposed and organized in clusters that are expected to show considerable preference for binding K⁺ over Na⁺ in the resting polymerized state.²² When stimulated into activity, the depolymerization of the cytoskeletal proteins would release ATP or bound ADP and Pi, thereby bringing about a change in protein conformations that also alters the state of cell water, and with that, membrane depolarization and new chemistry due to the influx of previously excluded solutes and ions.²³

Before leaving POM water, we might ask what its structure is. Ling himself argues for dipole stacking, while most other

researchers favour a hydrogen-bonded structure that in the ideal resembles ordinary ice. But as we have seen, there are many possible ice-like structures with different bond angles, very much dependent on the degree of confinement, and almost certainly also the presence of specific ions. Water is nothing if not flexible; and as pointed out by Manu Sharma and colleagues (see Chapter 2), hydrogen-bonding serves to align the dipole as well as to strengthen it, and hence should not be seen as a competing structure. It is entirely possible that dipole interactions are more important under certain conditions, as for example, at air-water interfaces, where hydrogen bonds are notably weaker than in the bulk phase (see Chapter 15), while hydrogen-bonding may be more prominent in other circumstances. Consequently, I don't think this is an important question, and certainly not one worth arguing about.

Ling's AI Hypothesis in Contemporary Cell Biology

Gilbert Ling's AI hypothesis has many points of contact with today's cell biology, and goes beyond it in important ways (see Box 23.1 for my perspective, which differs somewhat from Ling's own). However, we must guard against a too literal application of Ling's AI hypothesis. Rather, we should take it as a somewhat idealized picture of how water is centrally involved in all energy transformation and transduction reactions.

Box 23.1

Gilbert Ling in Today's Cell Biology

Ling's AI hypothesis

Cardinal sites

Cardinal adsorbents

Electronic induction

*Informed energy

Current cell biology

Receptor/cofactor-binding sites

Ligands (hormones, drugs, Ca^{2+} , etc.)

Signal transduction

*Information separate from energy

The role of ATP as a universal energy intermediate is generally recognized (see Chapter 22) although its mechanism of action is far from being understood. It is almost certainly much broader than envisaged in Ling's AI hypothesis. ATP is involved in activating substrates and enzymes by transferring phosphate groups to them. As mentioned in Chapter 17, phosphate and carboxylate anions are the fundamental ions of the cell, and it is thought that many small molecules are phosphorylated to keep them in the cell and to provide a "handle" for enzymes to bind to. Phosphate functions as a reversible marker in signal transduction, with phosphorylation (via ATP and protein kinases) activating or deactivating proteins. As seen in Chapter 16, phosphate groups, at least in membrane phospholipids, bind strongly to water as hydrogen acceptors. This suggests that the hydration and dehydration of proteins and metabolites *per se* may be important aspects of activation control and deactivation.

Binding of ATP and its equivalent, GTP, controls the polymerization of cytoskeletal proteins, whereas hydrolysis of ATP and GTP is associated with depolymerization (see Chapter 18). The cytoskeleton, as you know, is very dynamic and has the important tasks of maintaining cell shape, while enabling the cell to move, to divide, and provides tracks for intracellular transport of vesicles and organelles. Here, ATP- and GTP-binding clearly do not result in the complete extension of the proteins involved, but possibly in more localized changes in conformation that favour polymerization. On the other hand, the molecular motors transporting vesicles and organelles, which also depend on ATP-binding and subsequent hydrolysis into ADP and Pi, are more likely to involve the kind of changes proposed in Ling's AI hypothesis.

Ling rightly sees his proposed cardinal sites on proteins to include the ubiquitous receptor sites of cell biology, and, going beyond them, to include cofactor binding sites.²⁴ For example, both ATP and 2,3-diphosphoglycerate (2,3-DPG) are essential for the action of haemoglobin, the iron-containing oxygen-carrier protein in red blood cells.²⁵ Binding of ATP and 2,3-DPG reduces the affinity of haemoglobin for oxygen, so that haemoglobin can deliver the oxygen to the lungs. ATP is therefore not the only cardinal adsorbent.

Drugs, hormones, 2,3-DPG, Ca^{2+} , and other potent agents at very low concentrations may interact with cardinal sites to maintain the resting living state of the protoplasm or to bring about change.²⁶

Ouabain is a water-soluble poisonous *cardiac glycoside*, one of several compounds derived from plants that stimulates the contraction of heart muscle, and is believed to poison the membrane Na^+/K^+ -ATPase, or the sodium pump. At extremely low concentrations, ouabain dramatically increases the level of Na^+ in living cells. Ouabain acts as an electron-donating cardinal adsorbent. When it binds to a cardinal site, it induces an across-the-board rise of the β - and γ -carboxyl affinity for H^+ , making them weak acids, and hence more attractive to Na^+ than K^+ . It is estimated that a single ouabain molecule occupying a cardinal site controls roughly 1 042 β - and γ -carboxyl groups.

Another example is the action of insulin on glucose uptake. D-glucose is excluded by the normal muscle cell water, and the ratio of concentration in the cell to concentration in the external medium is 0.227. So how can the muscle cell get enough energy needed to fight or flee during intense activities? The answer is insulin. Ling and colleagues discovered that isolated frog muscle can take in D-glucose at 0°C, but cannot metabolize it. So it is possible to study the mechanism of D-glucose accumulation independent of metabolism. Unfortunately, low temperatures also stop insulin activity in promoting the entry of D-glucose into muscle cells.

So they first exposed muscle cells to insulin at 25°C together with glucose for six hours, before transferring to cold buffer solution containing different concentrations of labelled glucose for 16 hours, and compared the results with cells pre-incubated in the same buffer but without insulin or glucose. In the absence of both insulin and D-glucose in the pre-incubation, the labelled D-glucose is taken up at low levels in subsequent incubation at 0°C, with a concentration ratio inside/outside <0.25. However, the presence of insulin in the pre-incubation medium substantially increased the uptake of labelled glucose, and this increase was attributed to specific adsorption sites that had been opened up by insulin. (However,

another interpretation could be an increase in bulk cell water at the expense of POM, which therefore let in more glucose.)

Ca^{2+} is the most common ion involved in signalling, functioning as a ubiquitous intracellular second messenger;²⁷ its resting concentration in the cytoplasm is maintained in the range of 10 to 100 nM, most of it being stored in the endoplasmic reticulum or mitochondria. Signalling occurs when the cell is stimulated to release Ca^{2+} from intracellular stores, and when it enters the cell through ion channels in the cell membrane. Ca^{2+} signalling is involved in numerous processes such as muscle contraction, neuronal transmission, cell motility, fertilization, cell growth, or proliferation. Various Ca^{2+} -binding proteins help to relay the Ca^{2+} signal inside the cell, the most important being calmodulin, found in all eukaryote cells and reaching concentrations as high as 1%. It functions as an all-purpose intracellular Ca^{2+} receptor.

Calmodulin is a small acid protein approximately 148 amino acids long. It has a helix-loop-helix structural domain known as the EF hand in which the Ca^{2+} ions are bound, and is found in a large family of calcium-binding proteins. After binding calcium, the hydrophobic methyl groups from methionine residues become exposed on the protein, which can in turn bind to complementary hydrophobic regions on target proteins. This is consistent with the dehydrating role of Ca^{2+} on binding to phosphate groups or carboxylate groups in lipid monolayers, described in Chapter 16.

Clearly, the relevant observations are isolated and fragmentary, because insufficient attention has been paid to the central role of liquid crystalline water in orchestrating, facilitating, and energizing living processes.

Electronic Induction Animates the Cell

The big message from Ling is that *electronic induction* is the mode of action in the cell;²⁸ it is what animates life. The cardinal adsorbents are electron-donating or electron-withdrawing. This thesis dovetails with the quantum electrodynamic nature of life and living processes (see *Rainbow Worm*).

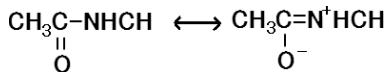


Figure 23.7 The resonating peptide bond.

Ling sees induction happening via the polypeptide chain, which possesses a partially resonating structure as the peptide bond is 40% double-bond and 60% single-bond (see Fig. 23.7),²⁹ and is therefore highly polarizable, enabling it to transfer energy and information over long distances.

I would only add that POM water, highly ordered and polarized if not quantum coherent, is equally good at resonant energy and information transfer, if not more so, and over the widely anastomosing networks that connect up the whole cell *via* the cytoskeleton; and beyond that, the extracellular matrix that connects up the entire body.

Significantly, as this book is going to press, evidence is emerging that cancer is fundamentally a disease of electronic or redox imbalance, which has large implications for cancer therapy and prevention.³⁰

The New Cell Biology

We have reached the end of a wide-ranging survey on the quantum physics and chemistry of water that tells us why it is so remarkably fit for life. Water is truly the means, medium, and message of life. Yet nearly everything we have learned about water in this book has been left out of the conventional account of cell biology and biochemistry, even though practically all of it is in the published scientific literature.

What would the new cell biology be like?

The starting point of the new cell biology is the quantum electro-dynamical nature of life, based on quantum coherent water created by fluctuations of the ambient electromagnetic field (zero-point field or vacuum field) and stabilized on interfaces as liquid crystalline water. Liquid crystalline water not only provides the excited electrons and protons to fuel the redox dynamo that generates life,

but also the activation energy and specific resonances that super-facilitate the numerous chemical reactions that life entails. Such liquid crystalline water interfaces could well have been responsible for the origin of life itself, by synthesizing the simple building blocks and polymers that created the first protoplast by molecular resonances and self-assembly.

Because life is energized by the movement of protons and electrons — the essence of redox reactions — electronic induction is not just limited locally to the nanoplasm. On the contrary, it can cause the movement of protons and electrons that connect up the entire cell, and ultimately all the cells and tissues of the organism via the extracellular matrix, to deliver the relevant chemistry as appropriate, by changing the state of liquid crystalline water.

What is important to know about in the new cell biology?

Instead of concentrating exclusively on describing the molecular nuts and bolts, the new cell biology would dedicate major effort to the quantum physics and chemistry of common molecules. Do enzymes and substrates find each other by resonating to the same frequencies emitted by liquid crystalline interfacial water? Does ATP binding to protein favour the extended conformation for forming liquid crystalline water and selective binding of K^+ over Na^+ ? What is the effect of general signal transduction processes such as phosphorylation on proteins and their hydration? Which ligands are electron-donating or electron-withdrawing? What triggers amyloid precipitation that's associated with an increasing number of common diseases?

While much effort has been dedicated to “creating life” by synthesizing and manipulating genomes, genomes fall considerably short of what we recognize as life. To come anywhere near to creating life, we need to understand the nature of protoplasm, and perhaps to create and study model nanoplasm inside reverse micelles. Also needed are more studies on resonant energy transfer and proton conduction in liquid crystalline interfacial water.

Does that mean we should ignore exotic chemistry? Not at all.

Redox reactions provide the core metabolism, which branches out profusely to link up with a complex metabolic network that

synthesizes an enormous diversity of “luxuriant” chemical species, not just to protect us from harm, but especially to make life exciting. They are the stuff of our most exquisite sensations, feelings, and passions, out of which come the truly remarkable creativity of organisms. That is ultimately why computers can never be like real organisms.³¹

That wet and wonderful chemistry is the rich tapestry of life, the dancing rainbow within that mirrors the one in the sky.

Suddenly, a whole new vista has opened up. The coming decades could be the most exciting in the history of cell biology.

Notes

1. Ho (2011e).
2. The following account is based on Ho (2011f).
3. “Membrane Potential”, Wikipedia, 4 July 2011, http://en.wikipedia.org/wiki/Membrane_potential; “Calmodulin”, Wikipedia, 21 October, 2011, <http://en.wikipedia.org/wiki/Calmodulin>.
4. Eskandari (2011).
5. Ling (2001); Ho (2004f).
6. Podolsky and Morales (1956).
7. Ling (2001).
8. Chaplin (2006).
9. Edelmann (2002); Ho (2004g).
10. Ho (2011b).
11. Edelmann (1981).
12. “Goldman Equation”, Wikipedia, 23 June 2011, http://en.wikipedia.org/wiki/Goldman_equation; “Goldman—Hodgkin—Katz Equation”, interactive Java applet, accessed 27 July 2011, <http://thevirtualheart.org/GHKindex.html>.
13. Ling (2007).
14. Ho (2011a).
15. Ling (2001).
16. “Phosphocreatine”, Wikipedia, 14 November 2011, <http://en.wikipedia.org/wiki/Phosphocreatine>.
17. Ling (2001).

18. Ling (2007).
19. Ling, personal communication (2011).
20. Ling (2001).
21. Bray (2005).
22. Chaplin (2006).
23. Chaplin (2004).
24. Ling (2007).
25. Benesch *et al.* (1969); Garby *et al.* (1969).
26. Ling (2007).
27. Alberts *et al.* (2008); “Calcium Signaling”, Wikipedia, 28 November 2011, <http://en.wikipedia.org/wiki/Calmodulin>.
28. Ling (2001, 2007).
29. Mizushima *et al.* (1950).
30. Ho (2012).
31. Ho (2002b).

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Glossary

Adiabatic compressibility coefficient	Degree of volume change in response to pressure in the absence of heat exchange with the environment.
Aerosol	Micrometre-sized solid or liquid droplets suspended in the atmosphere.
Ampere (A)	A unit of electric current, named after André-Marie Ampère (1775–1836), a French physicist considered the father of electrodynamics . It is equal to one coulomb (or 6.241×10^{15} electrons) per second.
Amino acid	Also α -amino acid, in which the amino group – NH ₂ is joined to the carbon atom of the carboxyl acid group (–COOH), and has the general chemical formula RNH ₂ COOH, where R represents 20 different substitutions in organisms.
Amphiphilic	Of molecules that are water-loving at one end and water-fearing at the other.
Amphoteric	Of molecules that can act as acid or base depending on the pH ; an acid is a substance that donates a proton (H ⁺ , a hydrogen ion) in a reaction, and a base is one that accepts a proton.

Atmosphere (atm) A unit of pressure due to Earth's atmosphere at sea level, about one kilogram per square centimetre.

Atom An atom is the smallest unit of a chemical element (of which there are 118 as of 2011) and consists of a central nucleus surrounded by one or more electrons. Each electron carries a negative charge, while the nucleus contains one or more relatively heavy particles known as protons and neutrons. Each proton carries a positive charge that balances the negative charge of the electron. The number of protons in the nucleus of an atom is the *atomic number* for the chemical element, and is equal to the number of electrons in orbit. A neutron is electrically neutral. The *atomic weight* of the element is the sum of the number of protons and neutrons.

Base sequence This refers to **nucleic acids** that are long chain-like molecules made up of building blocks differing in the organic bases, of which there are four: adenine, thymine (uracil in RNA), guanine, and cytosine. The specific sequence in which the bases occur defines the nucleic acid molecule's genetic function.

Birefringence Also known as double refraction, the ability of certain anisotropic crystalline material such as quartz to split light into two unequally reflected or transmitted waves which interfere with each other, generating interference colours if white light is used.

Brewster's angle Also known as the polarization angle, named after Scottish physicist Sir David Brewster (1781–1868), it is an angle of incidence at which non-polarized (diffuse) light reflected from the surface is perfectly polarized.

Cardiac glycoside	One of several compounds containing carbohydrates from plant sources such as the foxglove, used medicinally to increase the force of contraction of heart muscle and to regulate heartbeats.
Chaotropes	Ions or substances that cause disorder in water.
Chirality	The property of certain chemical molecules that exist in two forms — the D- and L-enantiomers (for right- and left-handed), which are mirror images of each other — and is a hallmark of biological molecules that only one of the two forms is represented; for example, natural amino acids are exclusively in the L-form, whereas natural sugars are exclusively in the D-form.
Chlorophyll	Green pigment responsible for absorbing sunlight for photosynthesis in green plants, algae, and cyanobacteria.
Chloroplast	An organelle (intracellular organ) in green plants and algae containing chlorophyll and other pigments that can capture sunlight to excite electrons to reduce carbon dioxide into carbohydrates and split water to release oxygen for air-breathing organisms.
Clathrate	A compound in which molecules of one component are trapped inside the crystal structure of another.
Colloids	Colloids are nanoparticles of dimensions ranging from nanometres (10^{-9} m) up to several μm suspended in water or other solvents.
Colloid crystals	Crystals made of colloid particles arranged in orderly fashion, like atoms in ordinary crystals.
Complex number	A number written in the form $a + ib$, where a and b are real numbers and i is $\sqrt{-1}$; a is referred to as the real part and ib the imaginary part.

	In electrodynamics, a represents the amplitude and ib the phase of the electromagnetic field.
Cooperativity	Cooperativity in chemistry refers to the tendency of individual chemical interactions to influence each other, so that local actions can have global effects, and vice versa, the whole being greater than and not predictable from the sum of the parts.
Coulomb (C)	A unit of electric charge equal to 6.241×10^{15} electrons.
Crystal	A homogeneous solid formed by a repeating, three-dimensional pattern of atoms, ions, or molecules and having fixed distances between constituent parts.
Dielectric	A non-conductor of electricity that supports an electrostatic field (becomes polarized) efficiently.
Dielectric constant	Of a material, the extent to which electrostatic lines of flux are concentrated relative to the vacuum; a measure of its polarizability.
Dielectric spectroscopy	A technique for characterizing molecules by its impedance or resistance to alternating current at different frequencies.
Dodecahedron	A Platonic solid with 12 pentagonal faces.
Electric dipole	A molecule with separated positive and negative charges (see Polar).
Electric susceptibility	The ability of a material to polarize in response to the field inside the material.
Electron transfer chain	A group of compounds that pass electrons from one to another via redox reactions coupled with the transfer of protons across a membrane to drive ATP synthesis.
Electron volt (eV)	A unit of energy equal to approximately 1.602×10^{-19} joules ; the amount of energy gained by a single unbound electron when it accelerates through an electric potential difference of one volt .

Energy-matter equivalence	This refers to the famous equation by Einstein, equating energy and mass: $E = mc^2$.
Endoplasmic reticulum	A pervasive system of convoluted membranes inside the cell, where proteins, lipids, and steroids are synthesized and other metabolic reactions are carried out.
Entropy	Disordered or degraded incoherent energy that is unavailable for work; also a measure of disorder (see <i>Rainbow Worm</i> for a complete, comprehensible description of this deep concept).
Ferroelectric	A crystalline dielectric with permanent electric polarization that varies in strength according to the applied electric field.
Fibonacci sequence	A sequence of numbers, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, 144, ... , where the ratio of a number in the sequence to the previous approaches φ (the golden ratio) asymptotically, i.e., more and more exactly as the numbers get larger and larger.
Fourier transformation	A mathematical operation that decomposes a function (or a sequence of signals) into its constituent frequencies, resulting in a frequency spectrum.
Fractals	Physical or dynamical structures with fractional dimensions instead of the usual one, two, or three; rough or fragmented geometric shapes that can be split into parts, each of which is, at least approximately, a reduced size copy of the whole, a property called self-similarity .
Frequency	The number of waves or oscillations per unit time, usually a second.
Genome	The totality of the genetic material characteristic of a species, present in practically every cell of the organism.

Glass	A non-crystalline solid that does not have a defined molecular order.
Golden ratio	Two quantities are in the golden ratio if the ratio of the sum of the quantities to the larger quantity is equal to the ratio of the larger quantity to the smaller one. The golden ratio is approximately 1.61803398874989. Other names frequently used for the golden ratio are the golden proportion, the golden section, or the golden mean.
Golgi body	The special membrane stack where proteins are processed and packaged for secretion from the cell.
Halides	Compounds of halogens (fluorine, chlorine, bromine, and iodine) with hydrogen.
Heisenberg's uncertainty principle	There are two formulations of the principle, which applies to quantum systems. The first states that the position x and momentum p of a particle cannot both be known precisely, $\Delta x \Delta p \geq h/4\pi$, and the second, the energy E of a system can fluctuate temporarily within a time period Δt without violating the conservation of energy, $\Delta \tau \Delta E \geq h/4\pi$.
Hofmeister series	An ordering of ions based on their ability to precipitate proteins out of solution, based on their effects in modifying the structure of water, due to Czech scientist Franz Hofmeister (1850–1922).
Hydrogen bond	A chemical bond consisting of hydrogen shared between two electronegative atoms such as oxygen or nitrogen; one being the hydrogen donor, and the other the hydrogen acceptor.
Hydrophilic	Water-loving.
Hydrophobic	Water-fearing.

Icosahedron	A Platonic solid with 20 faces of equilateral triangles (triangles with three equal sides).
Image forces	Forces induced by polarization, due to charged ions.
Infrared spectroscopy	A technique that uses the absorption of infrared light at specific frequencies by molecules to determine their structure; two-dimensional infrared spectroscopy examines the response of the system at different frequencies of the infrared spectrum as a function of the probe frequency; polarization-resolved femtosecond infrared spectroscopy uses polarized light and a very fast, femtosecond probe to study fast reorientation of molecules.
Interference colours	Colours produced by subtraction (destructive interference) of certain frequencies in the spectrum of white light (see birefringence).
Ionization	The dissociation of a molecule into ions, atoms or groups of atoms with an electric charge, due to the loss or gain of one or more electrons.
Ion cyclotron resonance	A phenomenon involving the circular (cyclotron) movement of an ion in a static magnetic field, the angular frequency of this cyclotron motion depending on the strength of the magnetic field, as well as the mass and charge of the ion; an electromagnetic signal having the same frequency will resonate with this cyclotron motion.
Isotopes	Different forms of the same element, i.e., with the same <i>atomic number</i> (number of protons) but different <i>atomic weight</i> because of additional neutrons (see Atom). Thus, the atomic number of H and its atomic weight are both 1, as it has only one proton. But deuterium, D,

Joule (J)	has one neutron in addition, and hence has an atomic weight of 2, while tritium, T, has two neutrons, and hence an atomic weight of 3. A unit of energy equal to a force of one newton through a distance of one metre, or the work required to move an electric charge of one coulomb through an electrical potential difference of one volt.
Kosmotropes	Ions or substances that increase order in water by binding to it.
Lamb shift	The small difference in energy between two states of the hydrogen atom, detected by US physicist Willis Eugene Lamb (1913-2008).
Liquid crystal	A fourth state of matter between liquid and solid, with long-range orientational order imposed by molecular anisotropy (e.g., long thin molecules) and electrical dipole interactions. The long-range orientational order defines special optical properties (such as birefringence), making liquid crystals responsive to electric and magnetic fields, with many applications in liquid crystal displays (LCDs) for televisions and computers, digital watches, calculators, etc.
Lysosome	A membrane-bound vesicle in which waste materials are broken down for recycling.
Magnetic dipole	An object or molecule that generates a magnetic field from two opposite poles.
Membrane potential	The electrical potential difference across the cell membrane.
Metabolic channelling	The direct transfer of metabolic intermediates from one enzyme to the next in a metabolic pathway without free diffusion, from which one can infer the detailed supramolecular organization that exists in the cytoplasm.

Metabolon	A stable or transient association of the enzymes of a metabolic pathway in a multi-enzyme complex for metabolic channelling .
Micelles	Vesicles formed by amphiphilic molecules so that the hydrophilic ends face outward towards the aqueous solvent and the hydrophobic ends are hidden inside.
Mitochondrion	A “powerhouse” organelle inside the cell where foodstuff is oxidized to release energy that can be used for all living activities.
Molar solution (M)	A solution containing one mole of the substance dissolved in one litre of the solvent, usually water at standard temperature and pressure (25°C , 1 atmosphere).
Mole (mol)	A unit of measurement that contains the molecular weight of a substance in grams; it contains about 6.022×10^{23} molecules of the substance.
Molecular dynamics simulation	A computer simulation to study the motion of molecules, usually in response to given stimuli.
Molecular orbital	A waveform describing the distribution and energy of a pair of electrons, most commonly represented as a linear combination of atomic orbitals of atoms forming a covalent bond in the molecule. A π molecular orbital involves two lobes of the atomic orbitals overlapping and joining up. The halves of a molecule joined by a π bond cannot rotate about that bond without breaking it; carbon and nitrogen compounds form π bonds when they engage in multiple bonding, as in C=C and N=N.
Neutron diffraction	A technique for determining the atomic structure of a material by scattering neutrons (0.1 nm wavelength) from it.

Newton (N)	A unit of force, named after Isaac Newton, required to accelerate a one-kilogram mass at a rate of one metre per second squared.
Nuclear magnetic resonance	A physical phenomenon in which magnetic nuclei in a magnetic field absorb and re-emit electromagnetic radiation at a specific resonance frequency depending on the strength of the magnetic field and the magnetic properties of the nuclei.
Nuclear magnetic resonance spectroscopy	A technique that exploits the magnetic properties of certain atomic nuclei possessing magnetic spin, such as protons ^1H and ^{13}C , that show nuclear magnetic resonance to determine the physical and chemical properties of atoms or molecules containing them.
Nucleic acid	Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), which form the hereditary material of organisms and viruses.
Nucleotide	A molecule consisting of an organic base joined to a sugar joined in turn to one or more phosphate groups; nucleotides are building blocks of nucleic acids , also called polynucleotides.
Pascal (Pa)	A unit of pressure equal to one newton per square metre and to $\sim 9.9 \times 10^{-6}$ atmospheres .
Percolation cluster	The ensemble of holes or sites in a lattice connected to a chosen centre to which a fluid is injected, so that the fluid will percolate to those sites.
pH	The negative logarithm to the base 10 of the molar concentration of hydrogen ions, a measure of acidity or basicity.
Phase transition	The transformation from one state to another, as solid to liquid and vice versa; also any abrupt change applying to all the

	molecules in a system, as from a disordered to an ordered state, or vice versa.
Photosynthesis	The process whereby green plants, algae, and cyanobacteria absorb sunlight to reduce carbon dioxide into carbohydrates, and split water to release oxygen.
pK_a	The negative logarithm to the base 10 of the acid dissociation constant, K_a , which characterizes the propensity of the acid to dissociate into a positively charged proton and a negatively charged ion in terms of the product of the equilibrium concentrations of the dissociation ions relative to the concentration of the unionized acid.
Platonic solids	Five regular solids with all sides the same length — tetrahedron, cube, octahedron, dodecahedron , icosahedron — named in honour of ancient Greek philosopher Plato who theorized that the classical elements (Earth, Water, Air, Fire) were constructed from regular solids.
Planck's constant	A fundamental constant due to German physicist Max Planck (1858–1947), the father of quantum mechanics, giving the ratio of the energy of a quantum of radiation to its frequency, and has a value $\sim 6.626 \times 10^{-34}$ joule seconds. It is also the smallest unit (quantum) of action.
Polar	Of molecules with separated positive and negative electric charges. To polarize is to strengthen or increase the separation of positive and negative electric charges.
Polarizable force field	A mathematical function describing the potential energy of the system that includes induced dipole interactions.

Polarized light

A plane-polarized light has its electric field oriented perpendicular to the light wave's direction of travel. If the electric field rotates as the wave travels, it is circular or elliptical polarization; in the latter case, the oscillations can rotate either towards the right or towards the left in the direction of travel, which is the wave's chirality or handedness.

Polarized light microscope

A microscope using polarized light to examine objects.

Polymerase chain reaction (PCR)

A technique using the enzyme DNA polymerase to make many copies of a specific DNA sequence, starting from short primer sequences that bind to complementary sequences at both ends, so the specific DNA sequence can be readily detected (by running the products through a gel by electrophoresis and staining it), and its **base sequence** determined.

Polypeptide

A chain-like molecule made of units called amino acids joined end to end in *peptide bonds*.

Power law

A distribution of a variable x that scales approximately as $x^{-\alpha}$, a signature of long-range correlation.

Provirus

The DNA sequence or the complementary DNA sequence of a virus, usually integrated into the host **genome**.

Quantum coherence

Quantum coherence is a special kind of coherence that maximizes both local freedom and global cohesion. It is technically defined in quantum optics as *factorizability* (see *Rainbow Worm*). A system is quantum coherent if its parts are so perfectly correlated that their cross-correlations factorize exactly as the product of the

individual self-correlations, so that each appears as though totally uncorrelated with the rest.

Quantum coherent systems are characterized by a wave function with complex quantum phases, and some would consider quantum coherence a property of all quantum systems (see Ho, 2004c).

**Quantum
electrodynamics**

A quantum theory that deals with the interaction between electrically charged particles and the electromagnetic field.

**Quantum field
theory**

A quantum theory that treats fields as primary, rather than particles.

**Quantum
fluctuation**

Temporary changes in the energy of a quantum system due to **Heisenberg's uncertainty principle**, $\Delta t \Delta E \geq h/4\pi$, which means the conservation of energy can be violated temporarily.

**Quantum
tunnelling**

A reaction going under an energy barrier via quantum effects.

Quasicrystal

An ordered structure that is not periodic, or a quasiperiodic structure with forbidden symmetry.

**Radial distribution
function**

A mathematical function describing the probability of finding a second molecule at a distance r from the first, used in analysis of **X-ray diffraction** and **neutron diffraction** data.

Raman scattering

An inelastic scattering of light from atoms in which energy is absorbed, so that the re-emitted light is either red-shifted to a lower frequency (Stokes effect) or blue-shifted to a higher frequency (anti-Stokes effect).

Redox reaction

A reduction-oxidation reaction involving the transfer of electrons from one substance (electron donor) to another (electron acceptor).

Resonance

Resonance occurs between oscillating or vibrating objects or charges when they share the same frequency, in which case the amplitude of the oscillation increases, the archetypal example being the shattering of a crystal wineglass when exposed to a musical tone of the right pitch, the proverbial perfect soprano. Resonating molecules are able to attract one another (when out of phase), and there is evidence that they depend on this to find one another in the cell for necessary reactions or binding interactions.

Resonant energy transfer

The transfer of energy between objects or molecules vibrating to the same frequency.

Respiration

The process whereby carbohydrates are oxidized into carbon dioxide and water to release energy for all living activities.

Salt linkage

The linkage between an acidic and a basic group on the same or different polypeptide chains due to electrostatic interaction or hydrogen-bonding.

Scanning tunnelling microscope

A microscope that scans the surface of a sample with a tiny sharp probe that has a small voltage applied, causing electrons to tunnel between tip and surface, out of which a three-dimensional image of the surface is reconstructed.

Schumann resonances

Peaks in the extremely low-frequency range of electromagnetic fields produced as standing waves in the giant cavity formed between Earth's surface and the ionosphere, naturally excited by electric currents in lightning discharges; they are the principal background electromagnetic spectrum of Earth. The fundamental frequency is 7.83, whose wavelength is equal to the circumference of Earth.

Second harmonic generation spectroscopy	A special case of sum frequency spectroscopy in which the two laser beams mixing at a surface have the same frequency and are combined to form new photons with twice the energy.
Second messenger	A molecule that relays an original stimulus (signal) to downstream processes in a signal transduction cascade .
Self-similarity	The property of having a similar structure over many scales (see Fractals).
Signal transduction	A mechanism that converts a chemical, mechanical, or electrical stimulus into a specific cellular response.
Signal transduction cascade	A series of interactions that amplifies an initial stimulus (signal) into a large effect, such as the activity of the entire cell or tissue.
Specific heat	The amount of heat required to raise the temperature of a unit mass by one degree Celsius.
Sum frequency spectroscopy	A spectroscopic technique for studying surfaces (and interfaces), that depends on two laser beams mixing at the surface and generating an output beam with a frequency equal to the sum of the two input frequencies.
Surface tension	The tension of the surface film of a liquid due to the attraction of particles in the surface layer by the bulk of the liquid.
Tesla (T)	A unit of magnetic field (also known as magnetic flux density) equal to one weber per square metre, named in honour of Serbian inventor physicist and electrical engineer Nikola Tesla (1856–1943).
Thermal expansion coefficient	The degree of expansion in volume in response to an increase in temperature.
Thermodynamics	The science of energy transformation.

Thermodynamic equilibrium	A state at which there is no net flow of energy or matter within the system, it being everywhere uniform.
Tread-milling	The net movement of subunits along a cytoskeletal filament consisting of protein subunits due to assembly at one end and disassembly at the other.
Triplet state	The electronic state of an atom or molecule that splits the energy level into three in the presence of a magnetic field.
Two-dimensional infrared photon echo spectroscopy	A technique that depends on exciting (pumping) the hydrogen-bond stretching vibration with infrared light, and looking for the response (echoes) in the infrared spectrum.
Van der Waals force	A weak attractive force between nonpolar molecules due to permanent or transient dipole interactions.
Virus	An infective agent consisting of a nucleic acid molecule in a protein coat too small to be seen with an ordinary light microscope, and needs a host cell to replicate itself.
Viscosity	The resistance of a fluid to being deformed, or its inability to flow.
Volt (V)	A unit of electric potential named after Alessandro Volta (1745–1827), such that an electric current of one ampere dissipates one watt of power, or the electric potential difference between two points one metre apart in an electric field of one newton per coulomb .
Wavenumber	The number of wavelengths per unit distance, usually one centimetre.
Weber (Wb)	A unit of magnetic flux which, linking a circuit of one turn, would produce in it an electromotive force of one volt if it were reduced to zero at a uniform rate in one second; named

after German physicist Wilhelm Eduard Weber (1804–1891) who co-invented the first electromagnetic telegraph.

X-ray diffraction

A technique for determining crystal structure by scattering X-rays (0.1 to 10 nm wavelength) from the regularly spaced atoms.

Zero-point field

The lowest possible energy that a quantum mechanical system may have, or its ground state; on account of **quantum fluctuations**, all quantum mechanical systems have an associated zero-point energy.

Zwitterion

A molecule that can become positively charged or negatively charged depending on the pH.

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References

- Alberts, B., Bray, D., Lewis, J., Raff, M., Raff, M., Roberts, K. and Watson, J.D. *Molecular Biology of the Cell*, 3rd edition. Garland Publishing, Inc., New York, 1994.
- Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P. *Molecular Biology of the Cell*, 5th edition. Garland Publishing, Inc., New York, 2008.
- Allard, M., Sargent, E.H., Lewis, P.C. and Kumacheva, E. “Colloidal Crystals Grown on Patterned Surfaces”. *Adv Mater* 15 (2004): 1361–1364.
- Aon, M. and Cortassa, S. “Hypothesis on the Fractal Nature of Cytoplasm”. *FEBS Lett* 344 (1994): 1–4.
- Arani, R., Bono, I., Del Guidice, E. and Preparata, G. “QED Coherence and the Thermodynamics of the Water”. *Int J Mod Phys B* 9 (1995): 1813–1841.
- Ariga, K. and Hill, J.P. “Monolayers at Air-Water Interfaces: From Origins-of-Life to Nanotechnology”. *Chem Rec* 11 (2011): 199–211.
- Ball, P. “Water as a Biomolecule”. *ChemPhysChem* 9 (2009): 2677–2685.
- Bartholomew, A. *Story of Water*. Floris Books, London, 2010.
- Batty, D. “Strongest Evidence Yet for Water on Mars”. *The Guardian*, 4 August 2011, <http://www.guardian.co.uk/science/2011/aug/04/strongest-evidence-yet-water-mars>.
- Bemer, R.A., VandenBrooks, J.M. and Ward, P.D. “Oxygen and Evolution”. *Science* 316 (2007): 557–558.
- Benesch, R.E., Benesch, R. and Yu, C.I. “The Oxygenation of Haemoglobin in the Presence of 2,3-Diphosphoglycerate. Effect of Temperature, pH, Ionic Strength, and Haemoglobin Concentration”. *Biochemistry* 8 (1969): 2567–2571.

- Berashevich, J. and Chakraborty, T. "How the Surrounding Water Changes the Electronic and Magnetic Properties of DNA". *J Phys Chem B* 112 (2008): 14083–14089.
- Bian, H.T., Feng, R.R., Xu, Y.Y., Guo, Y. and Wang, H.F. "Increased Interfacial Thickness of the NaF, NaCl and NaBr Salt Aqueous Solutions Probed with Non-resonant Surface Second Harmonic Generation (SHG)". *Phys Chem Chem Phys* 10 (2008): 4920–4931.
- Bian, H.T., Feng, R.R., Guo, Y. and Wang, H.F. "Specific Na⁺ and K⁺ Cation Effects on the Interfacial Water Molecules at the Air/Aqueous Salt Solution Interfaces Probed with Nonresonant Second Harmonic Generation". *J Chem Phys* 130 (2009): 134709.
- Blackman, C.F., Benane, S.G., Rabinowitz, J.R., House, D.E. and Joines, W.T. "A Role for the Magnetic Field in the Radiation-Induced Efflux of Calcium Ions from Brain Tissue *in vitro*". *Bioelectromagnetics* 6 (1985): 327–337.
- Bondar, A.-N., Elstner, M., Suhai, S., Smith, J.C. and Fischer, S. "Mechanism of Primary Proton Transfer in Bacteriorhodopsin". *Structure* 12 (2004): 1281–1288.
- Born, B. and Havenith, M. "Terahertz Dance of Proteins and Sugars with Water". *J Infrared Milli Terahz Waves* 30 (2009): 1245–1254.
- Bray, D. "Flexible Peptides and Cytoplasmic Gels". *Genome Biol* 6 (2005): 106.
- Brown, L. "The Geopolitics of Food Scarcity". *Spiegel Online International*, 2 November 2009, <http://www.spiegel.de/international/world/0,1518,606937,00.html>.
- Bru, R., Sanchez-Ferrer, A. and Garcia-Carmona, F. "Kinetic Models in Reverse Micelles". *Biochem J* 310 (1995): 721–739.
- Bush, J.W.M. and Hu, D.L. "Walk on Water: Biocomotion at the Interface". *Ann Rev Fluid Mech* 38 (2006): 339–369.
- Cameron, I.L., Lanctot, A.C. and Fullerton, G.D. "The Molecular Stoichiometric Hydration Model (SHM) as Applied to Tendon/Collagen, Globular Proteins and Cells". *Cell Biol Int* 35 (2011): 1205–1215.
- Cerveny, S., Alegria, A. and Colmenero, J. "Universal Features of Water Dynamics in Solutions of Hydrophilic Polymers, Biopolymers and Small Glass-forming Materials". *Phys Rev E* 77 (2008): 031803.
- Chalikian, T.V., Sarvazyan, A.P. and Breslauer, K.H. "Hydration and Partial Compressibility of Biological Compounds". *Biophys Chem* 51 (1994a): 89–107.

- Chalikian, T.V., Sarvazyan, A.P., Plum, G.E. and Breslauer, K.H. "Influence of Base Composition, Base Sequence, and Duplex Structure on DNA Hydration: Apparent Molar Volumes and Apparent Molar Adiabatic Compressibilities of Synthetic and Natural DNA Duplexes at 25 Degrees C". *Biochemistry* 188 (1994b): 2394–2401.
- Chaplin, M.F. "A Proposal for the Structuring of Water". *Biophys Chem* 83 (1999): 211–221.
- Chaplin, M.F. "The Importance of Cell Water". *Science in Society* 24 (2004): 42–45.
- Chaplin, M.F. "Information Exchange within Intracellular Water". In *Water and the Cell* (G.H. Pollack, I.L. Cameron and D.N. Wheatley, eds.), pp. 113–124, Springer, The Netherlands, 2006.
- Chaplin, M.F. "The Memory of Water: An Overview". *Homeopathy* 96 (2007): 143–150.
- Chaplin, M.F. "Water Structure and Science". 26 July 2011, <http://www.btinternet.com/~martin.chaplin/ice1h.html>.
- Chen, S.H., Lagi, M., Chu, X., Zhang, Y., Kim, C., Faraone, A., Fratini, E. and Baglioni, P. "Dynamics of a Globular Protein and Its Hydration Water Studied by Neutron Scattering and MD Simulations". *Spectroscopy* 24 (2010a): 1–24.
- Chen, X., Hua, W., Huang, Z. and Allen, H.C. "Interfacial Water Structure Associated with Phospholipid Membranes Studied by Phase-sensitive Vibrational Sum Frequency Generation Spectroscopy". *JACS* 132 (2010b): 11336–11342.
- Clegg, J.S. "Properties and Metabolism of the Aqueous Cytoplasm and Its Boundaries". *Am J Physiol* 246 (1984): R133–R151.
- Clegg, J.S. "Re-visiting the Microtrabecular Lattice". *Cell Biol Int* 34 (2010): 1105–1107.
- Clegg, J.S., Kell, D., Knull, H., Welch, G.R. and Wilson, J. "Macromolecular Interactions: Tracing the Roots". *Trends Biochem Sci* 26 (2001): 91.
- Collini, E., Wong, C.Y., Wilk, K.E., Curmi, P.M.G., Brumer, P. and Scholes, G.D. "Coherently Wired Light-harvesting in Photosynthetic Marine Algae at Ambient Temperature". *Nature* 463 (2010): 644–647.
- Collins, K.D. "Charge Density-dependent Strength of Hydration and Biological Structure". *Biophys J* 72 (1997): 65–78.
- Collins, K.D. "Ion Hydration: Implications for Cellular Function, Polyelectrolytes, and Protein Crystallization". *Biophys Chem* 119 (2006): 271–281.

- Comisso, N., Del Giudice, E., De Ninno, A., Fleischmann, M., Giuliani, L., Mengoli, G., Merlo, F. and Talpo, G. "Dynamics of the Ion Cyclotron Resonance Effect on Amino Acids Adsorbed at the Interfaces". *Bioelectromagnetics* 27 (2006): 16–26.
- Cowan, M.L., Bruner, B.D., Huse, N., Dwyer, J.R., Chugh, B., Nibbering, E.T.J., Elsaesser, T. and Miller, R.J. "Ultrafast Memory Loss and Energy Redistribution in the Hydrogen Bond Network of Liquid H₂O". *Nature* 434 (2005): 199–202.
- Del Giudice, E. "Old and New Views on the Structure of Matter and the Special Case of Living Matter". *J Phys: Conf Ser* 67 (2007): 012006.
- Del Giudice, E., Fleischmann, M., Preparata, G. and Talpo, G. "On the "Unreasonable" Effects of ELF Magnetic Fields upon a System of Ions". *Bioelectromagnetics* 23 (2002): 52–60.
- Del Giudice, E., Fuchs, E.C. and Vitiello, G. "Collective Molecular Dynamics of a Floating Water Bridge". *Water* 2 (2010a): 69–82.
- Del Giudice, E., Spinetti, P.R. and Tedeschi, A. "Water Dynamics at the Root of Metamorphosis in Living Organisms". *Water* 2 (2010b): 566–586.
- Dismukes, G.C., Klimov, V.V., Baranov, S.V., Kozlov, Y.N., DasGupta, J. and Tyryshkin, A. "The Origin of Atmospheric Oxygen on Earth: the Innovation of Oxygenic Photosynthesis". *Proc Natl Acad Sci USA* 98 (2001): 2170–2175.
- Donaldson, D.J. and Vaida, V. "The Influence of Organic Films at the Air-Aqueous Boundary on Atmospheric Processes". *Chem Rev* 106 (2006): 1445–1461.
- Doster, W., Busch, S., Gaspar, A.M., Appavou, M.-S., Wuttke, J. and Scheer, H. "Dynamical Transition of Protein-Hydration Water". *Phys Rev Letts* 104 (2010): 098101.
- Drost-Hansen, W. "Vicinal Hydration of Biopolymers: Cell Biological Consequences". In *Water and the Cell* (G. Pollack, I.L. Cameron and D.N. Wheatley, eds.), pp. 175–217, Springer-Verlag, Dordrecht, 2006.
- Ebbinghaus, S., Kim, S.J., Heyden, S.M., Yu, X., Heugen, U., Gruebele, M., Leitner, D.M. and Havenith, M. "An Extended Dynamical Hydration Shell Around Proteins". *Proc Natl Acad Sci USA* 104 (2007): 20749–20752.
- Edelmann, L. "Selective Accumulation of Li⁺, Na⁺, K⁺, Rb⁺ and Cs⁺ at Protein Sites of Freeze-dried Embedded Muscle Detected by LAMMA". *Fresenius Z Anal Chem* 308 (1981): 218–220.

- Edelmann, L. "Freeze-Dried and Resin-Embedded Biological Material is Well Suited for Ultrastructure Research". *J Microsc* 207 (2002): 5–26.
- Emoto, M. *Messages from Water*. Hado Publishing, Tokyo, 1999.
- Eskandari, S. "The Resting Membrane Potential (Simplified)". Eskandari Lecture Notes on Physiology, accessed 26 July 2011, <http://www.colorado.edu/che/courses/chen3838/foundations/Reading%20Assignments/The%20Membrane%20Potential%20-%20Simplified.pdf>.
- Etzler, F.M. and Drost-Hansen, W. "Recent Thermodynamic Data on Vicinal Water and a Model for Their Interpretation". *Croat Chem Acta* 56 (1983): 563–592.
- Fadnavis, N.S., Chandraprakash, Y. and Deshpande, A. "Protein-Protein Interactions in Reverse Micelles: Trypsin Shows Superactivity towards a Protein Substrate α -Chymotrypsinogen A in Reverse Micelles of Sodium Bis(2-ethylhexyl)sulfosuccinate (AOT) in Isooctane". *Biochimie* 75 (1993): 995–999.
- Feng, R.R., Bian, H.T., Guo, Y. and Wang, H.F. "Spectroscopic Evidence for the Specific Na^+ and K^+ Interactions with the Hydrogen-bonded Water Molecules at the Electrolyte Aqueous Solution Surfaces". *J Chem Phys* 130 (2009): 134710.
- Feng, R.R., Guo, Y., Lü, R., Velarde, L. and Wang, H.F. "Consistency in the Sum Frequency Generation Intensity and Phase Vibrational Spectra of the Air/Neat Water Interface". *J Phys Chem A* 115 (2011): 6015–6027.
- Fischer, S., Exner, A., Zielske, K., Perlich, J., Deloudi, S., Steurer, W., Lindner, P. and Förster, S. "Colloidal Quasicrystals with 12-fold and 18-fold Diffraction Symmetry". *Proc Natl Acad Sci USA* 108 (2011): 1810–1814.
- Fisher, P. "Homeopathy, the Art and Science of Healing Water". In *Quantum Jazz Biology*Medicine*Art*, (M.W. Ho, ed.), pp. 44–49, ISIS, London, 2011.
- Frixione, E. "Recurring Views on the Structure and Function of the Cytoskeleton". *Cell Motil Cytoskeleton* 46 (2000): 73–94.
- Fukuda, K. Shibusaki, Y., Nakahara, H. and Liu, M.-H. "Spontaneous Formation of Polypeptides in the Interfacial Thin Films of Amphiphilic Amino Acid Esters: Acceleration of the Polycondensation and Control of the Structure of Resultant Polymers". *Adv Colloid Interface Sci* 87 (2000): 113–145.
- Fullerton, G.D. and Amurao, M.R. "Evidence that Collagen and Tendon Have Monolayer Water Coverage in the Native State". *Cell Biol Int* 30 (2006): 56–65.

- Fullerton, G.D., Nes, E., Amurao, M., Rahal, A., Krasnosselskaia, L. and Cameron, I. "An NMR Method to Characterize Multiple Water Compartments on Mammalian Collagen". *Cell Biol Int* 30 (2006): 66–73.
- Gabriel, B. and Teissie, J. "Proton Long-range Migration Along Protein Monolayers and Its Consequences on Membrane Coupling". *Proc Natl Acad Sci USA* 93 (1996): 14521–14525.
- Galiana, G., Branca, R.T., Jenista, E.R. and Warren, W.S. "Accurate Temperature Imaging Based on Intermolecular Coherences in Magnetic Resonance". *Science* 322 (2009): 421–424.
- Garby, L., Gerber, G. and de Verdier, C.H. "Binding of 2,3-Diphosphoglycerate and Adenosine Triphosphate to Human Haemoglobin A". *Eur J Biochem* 10 (1969): 110–115.
- Georgievskii, Y., Medvedev, E.S. and Stuchebrukhov, A.A. "Proton Transport via Coupled Surface and Bulk Diffusion". *J Chem Phys* 116 (2002): 1692–1699.
- Geison, G.L. "The Protoplasmic Theory of Life and the Vitalist-Mechanist Debate". *Isis* 60 (1969): 272–292.
- Giuliani, L., Grimaldi, S., Lisi, A., D'Emilia, E., Bobkova, N. and Zhadin, M. "Action of Combined Magnetic Fields on Aqueous Solution of Glutamic Acid: The Further Development of Investigations". *Biomagn Res Technol* 6 (2008): 1–10.
- Glauber, R.J. "Coherence and Quantum Detection". In *Quantum Optics* (R.J. Glauber, ed.), Academic Press, New York, 1969.
- Grier, D.G. "Colloids: A Surprisingly Attractive Couple". 6 September 1998, <http://www.physics.nyu.edu/~dg86/bowen2b/>.
- Grier, D.G. and Han, Y. "Anomalous Interactions in Confined Charge-stabilized Colloid". *J Phys Condens Matter* 16 (2004): S4145.
- Guckenberger, R., Heim, M., Cecv, G., Knapp, H.F., Wiegrabe, W. and Hillebrand, A. "Scanning Tunnelling Microscopy of Insulators and Biological Specimens Based on Lateral Conductivity of Ultrathin Water Films". *Science* 266 (1994): 1538–1540.
- Hachisu, S. and Yoshimura, S. "Optical Demonstration of Crystalline Superstructures in Binary Mixtures of Latex Globules". *Nature* 283 (1980): 188–189.
- Hall, T.S. *Ideas of Life and Matter: Studies in the History of General Physiology* (2 vols.). University of Chicago Press, Chicago, 1969.

- Hansen, J.-P. and Lowen, H. "Effective Interactions between Electric Double-layers". *Annu Rev Phys Chem* 51 (2000): 209–242.
- Henderson, L.J. *The Fitness of the Environment*. Macmillan, New York, 1913.
- Hess, B. and van der Vegt, N.F.A. "Cation Specific Binding with Protein Surface Charges". *Proc Natl Acad Sci USA* 300 (2009): 13296–13300.
- Hilser, V.J. "Finding the Wet Spots". *Nature* 469 (2011): 166–167.
- Ho, M.W. *The Rainbow and the Worm: The Physics of Organisms*. World Scientific, Singapore, London, 1993, 1998 (2nd ed.), 2008 (3rd ed.).
- Ho, M.W. "Choreographer and Dancer". In *Bioenergetics, Living Processes S327 Book 2* (M.W. Ho, ed.), pp. 117–138, Open University Press, Milton Keynes, 1995.
- Ho, M.W. "The Biology of Free Will". *J Conscious Stud* 3 (1996): 231–244.
- Ho, M.W. "Quantum Coherence and Conscious Experience". *Kybernetes* 15 (1997): 263–274.
- Ho, M.W. *Genetic Engineering Dream or Nightmare? The Brave New World of Bad Science and Big Business*. Third World Network, Gateway Books, Macmillan, Continuum, Penang, Malaysia, Bath, Dublin, Ireland, New York, 1998, 1999, 2007 (reprint with extended Introduction).
- Ho, M.W. "Crystal Clear — Messages from Water." *Sicence in Society* 15 (2002a): 25–26.
- Ho, M.W. "Will Computers Become Super-Human?" *Science in Society* 16 (2002b): 38–41.
- Ho, M.W. *Living with the Fluid Genome*. Third World Network and ISIS, Penang and London, 2003a.
- Ho, M.W. "Non-Thermal Effects". *Science in Society* 17 (2003b): 12–13 + 43.
- Ho, M.W. "The Excluded Biology". *Science in Society* 17 (2003c): 14–15.
- Ho, M.W. "Biology of Least Action". *Science in Society* 18 (2003d): 38–39.
- Ho, M.W. "No System in Systems Biology". *Science in Society* 21 (2004a): 46.
- Ho, M.W. "Biology's Theory of Everything". *Science in Society* 21 (2004b): 46.
- Ho, M.W. "Quantum Phases and Quantum Coherence". *Science in Society* 22 (2004c): 6–7.
- Ho, M.W. "Is Water Special"? *Science in Society* 23 (2004d): 47–48.
- Ho, M.W. "Water Forms Massive Exclusion Zones". *Science in Society* 23 (2004e): 50–51.

- Ho, M.W. "Strong Medicine for Cell Biology". *Science in Society* 24 (2004f): 32–33.
- Ho, M.W. "What's the Cell Really Like?" *Science in Society* 24 (2004g): 46–47.
- Ho, M.W. "What's the Bacterium Really Like?" *Science in Society* 24 (2004h): 48–49.
- Ho, M.W. "Mobile Phones and Brain Damage". *Science in Society* 24 (2004i): 50–51.
- Ho, M.W. "Life after the Central Dogma Series". *Science in Society* 24 (2004j): 4–13.
- Ho, M.W. "Acupuncture, Coherent Energy and the Liquid Crystalline Organism". Plenary Lecture, Second International Congress on Acupuncture, 2–5 June 2005a, Barcelona, Spain, <http://www.isis.org.uk/onlinestore/papers1.php#section4>.
- Ho, M.W. "First Sighting of Structured Water". *Science in Society* 28 (2005b): 47–48.
- Ho, M.W. "Positive Electricity Zaps Through Water Chains". *Science in Society* 28 (2005c): 49–50.
- Ho, M.W. "Water Smoothing Protein Relationships". *Science in Society* 28 (2005d): 51.
- Ho, M.W. "Quantum Jazz. The Meaning of Life, the Universe and Everything". *Science in Society* 32 (2006a): 11–14.
- Ho, M.W. "Collagen Water Structure Revealed". *Science in Society* 32 (2006b): 15–16.
- Ho, M.W. "Two-States Water Explains All?" *Science in Society* 32 (2006c): 17–18.
- Ho, M.W. "Water and Colloid Crystals". *Science in Society* 32 (2006d): 19–20.
- Ho, M.W. "Water's Effortless Action at a Distance". *Science in Society* 32 (2006e): 21–23.
- Ho, M.W. "Shutting Down the Oceans Act II. Abrupt Plankton Shifts". *Science in Society* 31 (2006f): 18–19.
- Ho, M.W. "Shutting Down the Oceans Act III. Global Warming and Plankton, Snuffing Out the Green Fuse". *Science in Society* 31 (2006g): 20–21.
- Ho, M.W. "The Real Bioinformatics Revolution. Proteins and Nucleic Acids Singing to One Another?" *Science in Society* 33 (2007a): 42–45.
- Ho, M.W. "Drowning in a Sea of Microwaves". *Science in Society* 34 (2007b): 11–13.

- Ho, M.W. "Cancer Risks from Microwaves Confirmed". *Science in Society* 34 (2007c): 14–15.
- Ho, M.W. "Mobile Phones and Vanishing Birds". *Science in Society* 34 (2007d): 16.
- Ho, M.W. "Quantum Jazz, the Tao of Biology". *Science in Society* 34 (2007e): 17–21.
- Ho, M.W. "Mobile Phones and Vanishing Bees". *Science in Society* 34 (2007f): 34.
- Ho, M.W. "The Heartbeat of Health". *Science in Society* 35 (2007g): 10–13.
- Ho, M.W. "Happiness is a Heartbeat Away". *Science in Society* 35 (2007h): 14–18.
- Ho, M.W. "Liquid Crystalline Water at the Interface, Just Add Sunlight for Energy and Life". *Science in Society* 38 (2008): 36–39.
- Ho, M.W. "Development and Evolution Revisited". In *Handbook of Developmental Science, Behavior and Genetics* (K. Hood, C. Halpern, G. Greenberg and R. Lerner, eds.), Blackwell Publishing, New York, 2009a.
- Ho, M.W. "Living with Oxygen". *Science in Society* 43 (2009b): 9–12.
- Ho, M.W. "Can Water Burn?" *Science in Society* 43 (2009c): 12–13.
- Ho, M.W. "The Body Does Burn Water". *Science in Society* 43 (2009d): 14–16.
- Ho, M.W. "Epigenetics & Beyond" series, *Science in Society* 41 (2009e): 4–19.
- Ho, M.W. "O₂ Dropping Faster than CO₂ Rising". *Science in Society* 44 (2009f): 8–10.
- Ho, M.W. "Warming Oceans Starved of Oxygen". *Science in Society* 44 (2009g): 11 + 17.
- Ho, M.W. "Synthetic Life? Not by a Long Shot". *Science in Society* 47 (2010a): 16–17.
- Ho, M.W. "Cooperative and Coherent Water". *Science in Society* 48 (2010b): 6–9.
- Ho, M.W. "Dancing with Ions". *Science in Society* 48 (2010c): 10–11 + 15.
- Ho, M.W. "Dancing with Macromolecules". *Science in Society* 48 (2010d): 12–15.
- Ho, M.W. "The Rainbow Ensemble". *Science in Society* 48 (2010e): 16–19.
- Ho, M.W. "Homeopathic' Signals from DNA". *Science in Society* 48 (2010f): 36–39.
- Ho, M.W. "Electromagnetic Signals from HIV". *Science in Society* 48 (2010g): 40–43.

- Ho, M.W. "Life is Water's Quantum Jazz". In *Celebrating ISIS, Quantum Jazz Biology*Medicine*Art*, ISIS, London, 2011a, <http://www.i-sis.org.uk/pdf/QJBpreview.pdf>.
- Ho, M.W. "Why Beauty is Truth and Truth Beauty". *Science in Society* 50 (2011b): 32–37.
- Ho, M.W. "Quantum Coherent Water and Life". *Science in Society* 51 (2011c): 26–29.
- Ho, M.W. "Quantum Coherent Water, Non-thermal EMF Effects, and Homeopathy". *Science in Society* 51 (2011d): 30–34.
- Ho, M.W. "Membrane Potential Rules". *Science in Society* 52 (2011e): 12–15.
- Ho, M.W. "Electronic Induction Animates the Cell". *Science in Society* 52 (2011f): 22–25.
- Ho, M.W. "Golden Mean Wins Nobel Prize in Chemistry". *Science in Society* 52 (2011g): 10–11.
- Ho, M.W. "Wireless Phones and Brain Cancer". *Science in Society* 51 (2011h): 10–11.
- Ho, M.W. "Protein's Secret Water Music in Nanospace". *Science in Society* 52 (2011i): 26–27.
- Ho, M.W. "A Scientist's Earth Music". *Science in Society* 52 (2011j): 9.
- Ho, M.W. "Cancer a Redox Disease". *Science in Society* 54 (2012), in press.
- Ho, M.W., Haffejee, J., Newton, R., Zhou, Y.M., Bolton, J.S. and Ross, S. "Organisms as Polyphasic Liquid Crystals". *Bioelectrochem Bioenerg* 41 (1996): 81–91.
- Ho, M.W., Haffejee, J.P., Privitera, G., Scordino, A., Triglia, A. and Musumeci, F. "Delayed Luminescence and Biological Water in Collagen Liquid Crystalline Mesophases". Unpublished manuscript, 2003.
- Ho, M.W. and Knight, D. "The Acupuncture System and the Liquid Crystalline Collagen Fibers of the Connective Tissue". *Am J Chinese Med* 26 (1998): 251–263.
- Ho, M.W. and Lawrence, M. "Interference Colour Vital Imaging: A Novel Noninvasive Technique". *Microscopy and Analysis* September (1993): 26.
- Ho, M.W. and Saunders, P.T. "Liquid Crystalline Mesophases in Living Organisms". In *Bioelectrodynamics and Biocommunication* (M.W. Ho, F.A. Popp and U. Warnke, eds.), pp. 213–227, World Scientific, Singapore, 1994.
- Ho, M.W., Stone, T.A., Jerman, I., Bolton, J., Bolton, H., Goodwin, B.C., Saunders, P.T. and Robertson, F. "Brief Exposure to Weak Static Magnetic

- Fields During Early Embryogenesis Cause Cuticular Pattern Abnormalities in *Drosophila* Larvae". *Phys Med Biol* 37 (1992): 1171–1179.
- Ho, M.W., Zhou, Y.-M., Haffegee, J., Watton, A., Musumeci, F., Privitera, G., Scordino, A. and Triglia, A. "The Liquid Crystalline Organism and Biological Water". In *Water in Cell Biology* (G. Pollack, ed.), Springer, Dordrecht, 2006.
- Hoppert, M., Braks, I.J. and Mayer, F. "Stability and Activity of Hydrogenases of *Methanobacterium thermoautotrophicum* and *Alcaligenes eutrophus* in Reverse Micellar Systems". *SPIE Proceed* 3111 (1994): 501–509.
- Hoppert, M. and Mayer, F. "Prokaryotes". *Am Sci* 87 (1999): 518–525.
- Hsieh, C.S., Campen, R.K., Verde, A.C.V., Bolhuis, P., Nienhuys, H.-K. and Bonn, M. "Ultrafast Reorientation of Dangling OH Groups at the Air-Water Interface Using Femtosecond Vibrational Spectroscopy." *Phys Rev Lett* 107 (2011): 116102.
- Huang, C., Wikfeldt, K.T., Tokushima, T. and Nilsson, A. "The Inhomogeneous Structure of Water at Ambient Conditions". *Proc Natl Acad Sci USA* 106 (2009): 15214–15218.
- Hummer, G. "Water and Proton Conduction through Carbon Nanotubes". Banff, April 2003, <http://www.pims.math.ca/birs/workshops/2003/03w5039/contrib/Hummer.pdf>.
- Hummer, G., Rasalah, J.C. and Noworyta, J.P. "Water Conduction through the Hydrophobic Channel of a Carbon Nanotube". *Nature* 414 (2001): 188–190.
- Hyeon-Deuk, K. and Ando, K. "Quantum Effects of Hydrogen Atoms on the Dynamical Rearrangement of Hydrogen-bond Networks in Liquid Water". *J Chem Phys* 132 (2010): 164507.
- Ise, N. "Like Likes Like: Counterion-mediated Attraction in Macroionic and Colloidal Interactions". *Phys Chem Chem Phys* 12 (2010): 10279–10287.
- Ise, N., Konishi, T. and Tata, B.V.R. "How Homogeneous are "Homogenous Dispersions"? Counterion-mediated Attraction between Like-charged Species". *Langmuir* 15 (1999): 4176–4184.
- Jeong, H., Tombor, B., Albert, R., Oltvai, Z.N. and Barabasi, A.L "The Large-scale Organization of Metabolic Networks". *Nature* 407 (2000): 651–654.
- Ji, M., Odelius, M. and Gaffney, K.J. "Large Angular Jump Mechanism Observed for Hydrogen Bond Exchange in Aqueous Perchlorate Solution". *Science* 328 (2010): 1003.

- Kappeli, M. "Water, Uncharted Spaces — the Body of the Transitory". *Science in Society* 48 (2010): 20–21.
- Kappeli, M. "Symphonies of Water". In *Celebrating ISIS, Quantum Jazz Biology*Medicine*Art* (M.W. Ho, ed.), pp. 30–36, ISIS, London, 2011.
- Katiyar, S.S., Kumar, A. and Kumar, A. "The Phenomenon of Super Activity in Dihydrofolate Reductase Inside Reverse Micelles in Apolar Solvents". *Biochem Int* 19 (1989): 547–552.
- Kim, S. and Coulombe, P.A. "Emerging Role for the Cytoskeleton as an Organizer and Regulator of Translation". *Nature Rev Mol Cell Biol* 11 (2010): 75–78.
- Kinoshita, M., Iba, S. and Harada, M. "Interaction between Macroparticles in Aqueous Electrolytes". *J Chem Phys* 105 (1996): 2487.
- Klein, R.A. "Cooperativity in Large Water Clusters. Liquid Water, Ice and Clathrates". NIC Symposium 2006 (G. Munster, D. Wolf and M. Kremer, eds.), pp. 65–74, John von Neumann Institute for Computing, Jülich, 2006.
- Koch, L.G. and Britton, S.L. "Aerobic Metabolism Underlies Complexity and Capacity". *J Physiol* 586 (2008): 83–95.
- Kohler, R. "The Reception of Eduard Buchner's Discovery of Cell-Free Fermentation". *J Hist Biol* 5 (1972): 327–353.
- Koruga, D., Miljkovic, S., Ribar, S., Matija, L. and Kojic, D. "Water Hydrogen Bonds Study by Opto-Magnetic Fingerprint Technique". *Acta Physica Pol A* 117 (2010): 777–781.
- Kraemer, D., Cowan, M.L., Paarmann, A., Huse, N., Nibbering, E.T.J., Elsaesser, T. and Miller, R.J. "Temperature-dependence of the Two-dimensional Infrared Spectrum of Liquid H₂O". *Proc Natl Acad Sci USA* 105 (2008): 437–442.
- Kraut, D.A., Carroll, K.S. and Herschlag, D. "Challenges in Enzyme Mechanism and Energetics". *Ann Rev Biochem* 72 (2003): 517–571.
- Krekhov, A., Pesch, W. and Buka, A. "Flexoelectricity and Pattern Formation in Nematic Liquid Crystals". *Phys Rev E* 83 (2011): 051706.
- Yakuno, H., Matsuda, K., Yahiro, H., Inami, Y., Fukuoka, T., Miyata, Y., Yanagi, K., Maniwa, Y., Kataura, H., Saito, T., Yumura, M. and Iijima, S. "Confined Water Inside Single-walled Carbon Nanotubes: Global Phase Diagram and Effect of Finite Length". *J Chem Phys* 134 (2011): 244501.

- Laage, D. and Hynes, J.T. "Reorientational Dynamics of Water Molecules in Anionic Hydration Shells". *Proc Natl Acad Sci USA* 104 (2007): 11167–11172.
- Law, C.J., Roszak, A.W., Southall, J., Gardiner, A.T., Isaacs, N.W. and Cogdell, R.J. "The Structure and Function of Bacterial Light-harvesting Complexes (Review)". *Mol Membr Biol* 21 (2004): 183–191.
- LeBard, D.N. and Matyushov, D.V. "Ferroelectric Hydration Shells around Proteins: Electrostatics of the Protein-Water Interface". *J Phys Chem B* 114 (2010): 9246–9258.
- Liboff, A.R. "Cyclotron Resonance in Membrane Transport". In *Interactions Between Electromagnetic Fields and Cells* (A. Chiabrera, C. Nicolini and H.P. Schwan, eds.), pp. 281–296, Plenum Press, New York, 1985.
- Ling, G.N. *Life at the Cell and Below-Cell Level*. Pacific Press, New York, 2001.
- Ling, G. "Nano-protoplasm: The Ultimate Unit of Life". *Physiol Chem Phys Med NMR* 39 (2007): 111–234.
- Martinek, K., Berezin, I.V., Khmelnitski, Y.L., Klyachko, N.L. and Levashov, A.V. "Enzymes Entrapped into Reversed Micelles of Surfactants in Organic Solvents". *Biocatalysis* 1 (1987): 9–15.
- Mayer, F. "Cytoskeletons in Prokaryotes — Status Report and Hypothesis". *Cell Biol Int* 27 (2003): 429–438.
- Mikhael, J., Roth, J., Helden, L. and Bechinger, C. "Archimedean-like Tiling on Decagonal Quasicrystalline Surfaces". *Nature* 454 (2008): 501–504.
- Mizushima, S., Simanouti, T., Nagakura, S., Kuratani, K., Tsuboi, M., Baba, H. and Fujioka, O. "The Molecular Structure of N-Methylacetamide". *J Am Chem Soc* 72 (1950): 3490–3494.
- Moilanen, D.E., Fenn, E.E., Wong, D. and Fayer, M.D. "Water Dynamics in Large and Small Reverse Micelles: From Two Ensemble to Collective Behavior". *J Chem Phys* 131 (2009): 014704.
- Monahan, L. and Harry, E. "The Bacterial Cytoskeleton". *Australian Biochemist* 40 (2009): 4–8.
- Montagnier, L., Aïssa, J., Ferris, S., Montagnier, J.-L. and Lavallée, C. "Electromagnetic Signals are Produced by Aqueous Nanostructure Derived from Bacterial DNA Sequences". *Interdiscip Sci* 1 (2009a): 80–91.
- Montagnier, L., Aïssa, J., Lavallée, C., Mbamy, M., Varon, J. and Chenal, H. "Electromagnetic Detection of HIV DNA in the Blood of Patients Treated by Antiretroviral Therapy". *Interdiscip Sci* 1 (2009b): 245–253.

- Montagnier, L., Aissa, J., Del Giudice, E.D., Lavallee, C., Tdeschi, A. and Vitiello, G. "DNA Waves and Water". *J Phys Conf Ser*, 2011, in press, arXiv: 1012.5166Ms.
- Morrone, J.A. and Car, R. "Nuclear Quantum Effects in Water". *Phys Rev Lett* 101 (2009): 017801.
- Müller, A., Bögge, H. and Diemann, E. "Structure of a Cavity-encapsulated Nanodrop of Water". *Inorg Chem Commun* 6 (2003): 329.
- Naguib, N., Ye, H., Gogotsi, Y., Yazicioglu, A.G., Megaridis, C.M. and Yoshimura, M. "Observation of Water Confined in Nanometer Channels of Closed Carbon Nanotubes". *Nano Lett* 4 (2004): 2237–2243.
- Needham, J. *Order and Life*. Yale University Press, New Haven, 1936.
- Newton, R.H., Haffejee, J. and Ho, M.W. "Colour-Contrast in Polarized Light Microscopy of Weakly Birefringent Biological Specimens". *J Microscopy* 180 (1995): 127–130.
- Nordenskiöld, E. *The History of Biology*. Knopf, New York, 1928.
- Nucci, N.V., Pometun, M.S. and Wand, A.J. "Site-resolved Measurement of Water-Protein Interaction by Solution NMR". *Nat Struct Mol Biol* 18 (2011): 245–250.
- Oschman, J.L. *Energy Medicine*. Churchill Livingston, Edinburgh, 2000.
- Oschman, J.L. *Energy Medicine, Therapeutics and Human Performance*. Harcourt Health Sciences/Butterworth Heineman, Oxford, 2003.
- Oschman, J. "Energy Medicine & Quantum Jazz". In *Quantum Jazz Biology*Medicine*Art* (M.W. Ho, ed.), pp. 36–39, ISIS, London, 2011.
- Ostroverkhov, V., Waychunas, G.A., and Shen, Y.R. "New Information on Water Interfacial Structure Revealed by Phase-Sensitive Surface Spectroscopy". *Phys Rev Lett* 94 (2005): 046102.
- Ovádi, J. and Saks, V. "On the Origin of Intracellular Compartmentation and Organized Metabolic Systems". *Mol Cell Biochem* 256/257 (2004): 5–12.
- Paesani, F., Yoo, S., Bakker, H.J. and Xantheas, S.S. "Nuclear Quantum Effects in the Reorientation of Water". *J Phys Chem Lett* 1 (2010): 2316–2321.
- Pagnotta, S.E., Bruni, F., Senesi, R. and Pietropaolo, A. "Quantum Behaviour of Water Protons in Protein Hydration Shell". *Biophys J* 96 (2009): 1939–1943.
- Papić-Obradović, M., Kojic, D. and Matlja, A.L. "Opto-magnetic Method for Epstein-Barr Virus and Cytomegalovirus Detection in Blood Plasma Samples". *Acta Physica Pol A* 117 (2010): 782–785.

- Papoian, G.A., Ulander, J., Eastwood, M.P., Luthey-Schulten, Z. and Wolynes, P.G. "Water in Protein Structure Predictions". *Proc Natl Acad Sci USA* 101 (2004): 3352–3357.
- Podolsky, R.J. and Morales, M.G. "The Enthalpy Change of Adenosine Triphosphate Hydrolysis". *J Biol Chem* 218 (1956): 945–959.
- Pollack, G.H. "Water, Energy and Life: Fresh Views from the Water's Edge". 32nd Annual Faculty Lecture, University of Washington at Seattle, Washington, USA, January 2008, <http://uwtv.org/programs/displayevent.aspx?rID=22222>.
- Pomes, R. and Roux, B. "Free Energy Profiles for H⁺ Conduction along Hydrogen-bonded Chains of Water Molecules". *Biophys J* 75 (1998): 33–40.
- Porter, K.R., ed. "The Cytoplasmic Matrix and the Integration of Cellular Function: Proceedings of a Conference. Fogarty International Center, National Institutes of Health, 17–20 October 1993". *J Cell Biol* 99 (1984): 1–2485.
- Preparata, G. *QED Coherence in Matter*. World Scientific, Singapore, 1995.
- Preuss, P. "New Clues to How Proteins Dissolve and Crystallize". Research News, Berkeley Lab, 12 May 2008, <http://www.lbl.gov/Science-Articles/Archive/CSD-protein-clues.html>.
- Rayat, C.S. "Essential Ions of Our Body for Sustaining Life". Human Anatomy & Physiology and Renal Disorders, 21 November 2008, <http://renaldiseorders.blogspot.com/2008/11/essential-ions-of-our-body-for.html>.
- Reeser, D.I., George, C. and Donaldson D.J. "Photooxidation of Halides by Chlorophyll at the Air-Salt Water Interface". *J Phys Chem* 113 (2009): 8591–8595.
- Reiss, H. "The Fermi Level and the Redox Potential". *J Phys Chem* 89 (1985): 3783–3791.
- Reynolds, A. "The Cell's Journey: From Metaphorical to Literal Factory". *Endeavour* 31 (2007): 65–70.
- Richter, W. and Warren, W.S. "Intermolecular Multiple Quantum Coherences in Liquids". *Concept Magnetic Res* 12 (2000): 396–409.
- Richter, W., Richter, M., Warren, W.S., Merkle, H., Anderson, P., Adriany, G. and Ugurbil, K. "Functional Magnetic Resonance Imaging with Intermolecular Multiple-quantum Coherences". *Magn Reson Imaging* 18 (2000): 489–494.

- Riggs, A.D., Bourgeois, S. and Cohn, M. "The Lac Repressor-Operator Interaction". *J Mol Biol* 53 (1970): 401–417.
- Riistama, S., Hummer, G., Puustinen, A., Dyer, R.B., Woodruff, W.H. and Sikstrom, M. "Bound Water in the Proton Translocation Mechanism of the Haem-copper Oxidases". *FEBS Lett* 414 (1997): 275–280.
- Riley, D., McCraty, R. and Snyder, S. "Quantum Jazz Biology. Interview with Mae-Wan Ho". *Science in Society* 47 (2010): 4–9.
- Ross, S. Newton, R.H., Zhou, Y.M., Haffejee, J., Ho, M.W., Botton, J. and Knight, D. "Quantitative Image Analysis of Birefringent Biological Materials". *J Microscopy* 187 (1997): 62–67.
- Roy, R., Rao, M.L. and Kanzius, J. "Observations of Polarised RF Radiation Catalysis of Dissociation of H₂O-NaCl Solutions". *Mater Res Innov* 12 (2008): 3–6.
- Ruan, C.Y., Lobastov, V.A., Vigliotti, F., Chen, S. and Sewail, A.H. "Ultrafast Electron Crystallography of Interfacial Water". *Science* 304 (2004): 80–84.
- Ruiz-Bermejo, M., Menor-Salván, C., Zorzano, M.-P., El-Hachemi, Z., Osuna-Esteban, S. and Sanyuk, B. "Liquid Crystals: Simple View on a Complex Matter". Kent State University, accessed 18 September 2010, <http://dept.kent.edu/spie/liquidcrystals/index.html>.
- Schneider, B., Patel, K. and Berman, H.M. "Hydration of the Phosphate Group in Double-Helical DNA". *Biophys J* 75 (1998): 2422–2434.
- Schrödinger, E. *What is Life?* Cambridge University Press, Cambridge, 1944.
- Sen, S., Andreatta, D., Ponomarev, S.Y., Beveridge, D.L. and Berg, M.A. "Dynamics of Water and Ions Near DNA: Comparison of Simulation to Time-resolved Stokes-shift Experiments". *J Am Chem Soc* 131 (2009): 1724–1735.
- Sharma, M., Resta, R. and Car, R. "Dipolar Correlations and the Dielectric Permittivity of Water. *Phys Rev Lett* 98 (2007): 247401.
- Shen, Y.R. and Ostroverkhov, V. "Sum-frequency Vibrational Spectroscopy on Water Interfaces: Polar Orientation of Water Molecules at Interfaces". *Chem Rev* 106 (2006): 1140–1154.
- Shevchenko, E.V., Talapin, D.V., Kotov, N.A., O'Brien, S. and Murray, C.B. "Structural Diversity in Binary Nanoparticle Superlattices". *Nature* 439 (2006): 55–59.
- Silva, J.L., Vieira, T.C.R.G., Gomes, M.P.B., Bom, A.P.A., Lima, L.M.T.R., Freitas, M.S., Ishimaru, D., Cordeiro, Y. and Foguel, D. "Ligand-binding and Protein

- Misfolding: Insights from Studies of Prion and p⁵³ Tumor Suppressor Proteins". *Acc Chem Res* 43 (2010): 271–279.
- So, E., Stahlberg, R. and Pollack, G.H. "Exclusion Zone as Intermediate between Ice and Water". *WIT Trans Ecol Envir* 153 (2011), online, doi.10.2495/WS110011.
- Springer, A., Hägen, V., Cherepanov, D.A., Antonenko, Y.N. and Pohl, P. "Protons Migrate along Interfacial Water without Significant Contributions from Jumps between Ionizable Groups on the Membrane Surface". *Proc Natl Acad Sci USA* 108 (2011): 14461–14466.
- Srere, P.A. "The Metabolon". *Trends Biochem Sci* 10 (1985): 109–110.
- Steinhardt, P.J. "What are Quasicrystals?" Accessed 23 October 2011, www.physics.princeton.edu/~steinh/QuasiIntro.ppt.
- Szent-Györgyi, A. *Bioenergetics*. Academic Press, New York, 1957.
- Szent-Györgyi, A. *Introduction to a Submolecular Biology*. Academic Press, New York, 1960.
- Szent-Györgyi, A. "Introductory Comments". In *Light and Life* (W.D. McElroy and B. Glass, eds.), pp. 7–10, Johns Hopkins Press, Baltimore, 1961.
- Tang, C.Y., Huang, Z. and Allen, H.C. "Interfacial Water Structure and Effects of Mg²⁺ and Ca²⁺ Binding to the COOH Headgroup of a Palmitic Acid Monolayer Studied by Sum Frequency Spectroscopy". *J Phys Chem B* 115 (2011): 34–40.
- Tang, X.W., Liu, J.B. and Wu, B.C. "Fractal Property of the Cytoskeleton in the Living Cell". *Chin Phys Lett* 11 (1994): 522–525.
- Tielrooij, K.J., Garcia-Araez, N., Bonn, M. and Bakker, H.J. "Cooperativity in Ion Hydration". *Science* 328 (2010): 1006–1009.
- Tsukahara, T., Hibara, A., Ikeda, Y. and Kitamori, T. "NMR Study of Water Molecules Confined in Extended Nanospaces". *Angew Chem Int Ed Engl* 119 (2007): 1199–1200.
- Uejio, J.S., Schwartz, C.P., Duffin, A.M., Drisdell, W.S., Cohen, R.C. and Saykally, R.J. "Characterization of Selective Binding of Alkali Cations with Carboxylate by X-ray Absorption Spectroscopy of Liquid Microjets". *Proc Natl Acad Sci USA* 105 (2008): 6800–6812.
- Urquidi, J., Robinson, G.W., Cho, C.H., Xiao, B. and Singh, S. "Explicit Outer Bonding Transformations in Liquid Water. The Key to Its Understanding". 1999, <http://www.phys.ttu.edu/~dujcb/ECCC5/index.html>.
- Valentine, J.W. *On the Origin of Phyla*. University of Chicago Press, Chicago and London, 2004.

- Vedamuthu, M., Singh, S. and Robinson, G.W. "Properties of Liquid Water: Origin of the Density Anomalies". *J Phys Chem* 98 (1994): 2222–2230.
- Vega, C., Conde, M.M., McBride, C., Abascal, J.L.F., Noya, E.F., Ramirez, R. and Sesé, L.M. "Heat Capacity of Water: A Signature of Nuclear Quantum Effects". *J Chem Phys* 132 (2010): 046101.
- Veljkovic, N., Gilsic, S., Perovic, V. and Veljkovic, V. "The Role of Long-range Intermolecular Interactions in Discovery of New Drugs". *Informa Healthcare* 6 (2011): 1263–1270, doi:10.1517/17460441.2012.638280.
- Veljkovic, V., Veljkovic, N., Esté, J.A., Hüther, A. and Dietrich, U. "Application of the EIIP/ISM Bioinformatics Concept in Development of New Drugs". *Curr Med Chem* 14 (2007): 133–155.
- Von Brücke, E. "Die Elementarorganismen". *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften zu Wien. Mathematisch-Naturwissenschaftlichen Classe* 44 (1861): 381–406.
- Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R. and Davies, P.M. "Global Threats to Human Water Security and River Biodiversity". *Nature* 467 (2010): 555–561.
- Vrbka, L., Mucha, M., Minofar, B., Jungwirth, P., Brown, E.C. and Tobias, D.J. "Propensity of Soft Ions for the Air/Water Interface." *Curr Opin Colloid Interface Sci* 9 (2004): 67–73.
- Vrbka, L., Vondrášek, J., Jagoda-Cwiklik, B., Vacha, R. and Jungwirth, P. "Quantification and Rationalization of the Higher Affinity of Sodium over Potassium to Protein Surfaces". *Cell* 103 (2006): 15440–15444.
- Warren, W.S., Huang, S.Y., Ahn, S. and Lin, Y.Y. "Understanding Third-order Dipolar Effects in Solution Nuclear Magnetic Resonance: Hanh Echo Decays and Intermolecular Triple-Quantum Coherences". *J Chem Phys* 116 (2002): 2075–2084.
- Welch, G.R., ed. *Organized Multienzyme Systems*. Academic Press, Orlando, 1985.
- Welch, G.R. "T. H. Huxley and the "Protoplasmic Theory of Life": 100 Years Later". *Trends Biochem Sci* 20 (1995): 481–485.
- Welch, G.R. and Clegg, J.S. "From Protoplasmic Theory to Cellular Systems Biology: A 150-Year Reflection". *Am J Physiol Cell Physiol* 298 (2010): C1280–C1290.
- Wiggins, P. "Life Depends upon Two Kinds of Water". 2003, <http://www.lsbu.ac.uk/water/contr2.html>.

- Wiggins, P. "Life Depends upon Two Kinds of Water". *PLoS ONE* 1 (2008): e1406.
- Williams, R.J.P. "The History of Proton-driven ATP Formation". *Biosci Rep* 13 (1993): 191–212.
- Yamahata, C., Collard, D., Takekawa, T., Kumemura, M., Hashiguchi, G. and Fujita, H. "Humidity Dependence of Charge Transport through DNA Revealed by Silicon-based Nanotweezers Manipulation". *Biophys J* 94 (2008): 63–70.
- Ye, H., Naguib, N. and Gogotsi, Y. "TEM Study of Water in Carbon Nanotubes". *JEOL News* 39 (2004): 2–7.
- Zee, A. "Quantum Field Theory". 14 January 2004, http://v.youku.com/v_show/id_XMTEoOTI4Nzgo.html.
- Zhadin, M. and Giuliani, L. "Some Problems in Modern Bioelectromagnetics". *Electromagn Biol Med* 25 (2006): 227–243.
- Zhadin, M., Novikov, V.V., Barnes, F.S. and Pergola, N.F. "Combined Action of Static and Alternating Magnetic Fields on Ionic Current in Aqueous Glutamic Acid Solution". *Bioelectromagnetics* 19 (1998): 41–45.
- Zhang, H., Lizitsa, N., Bryant, R.G. and Warren, W.S. "Experimental Characterization of Intermolecular Multiple-Quantum Coherence Pumping Efficiency in Solution NMR". *J Magn Reson* 148 (2001): 200–208.
- Zhang, W.K., Zheng, D.S., Xu, Y.Y., Bian, H.T., Guo, Y. and Wang, H.F. "Reconsideration of Second-Harmonic Generation from Isotropic Liquid Interface: Broken Kleinman Symmetry of Neat Air/Water Interface from Dipolar Contribution." *J Chem Phys* 123 (2005): 224713.
- Zhang, Z., Piatkow, L., Bakker, H.J. and Bonn, M. "Ultrafast Vibrational Energy Transfer at the Water/Air Interface Revealed by Two-dimensional Surface Vibrational Spectroscopy". *Nat Chem* 3 (2011): 888–893.
- Zheng, J.-M. and Pollack, G.H. "Long-range Forces Extending from Polymer-gel Surfaces". *Phys Rev E* 68 (2003): 314–318.
- Zheng, J.-M., Chin, W.-C., Khijniak, E., Khijniak, E. Jr. and Pollack, G.H. "Surfaces and Interfacial Water: Evidence that Hydrophilic Surfaces Have Long-range Impact". *Adv Colloid Interface Sci* 127 (2006): 19–27.
- Zhou, Y.-M. "Optical Properties of Living Organisms". Ph.D. Thesis, Open University, UK, 2000.
- Zubavicus, Y. and Grunze, M. "New Insights into the Structure of Water with Ultrafast Probes". *Science* 304 (2004): 974–976.

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