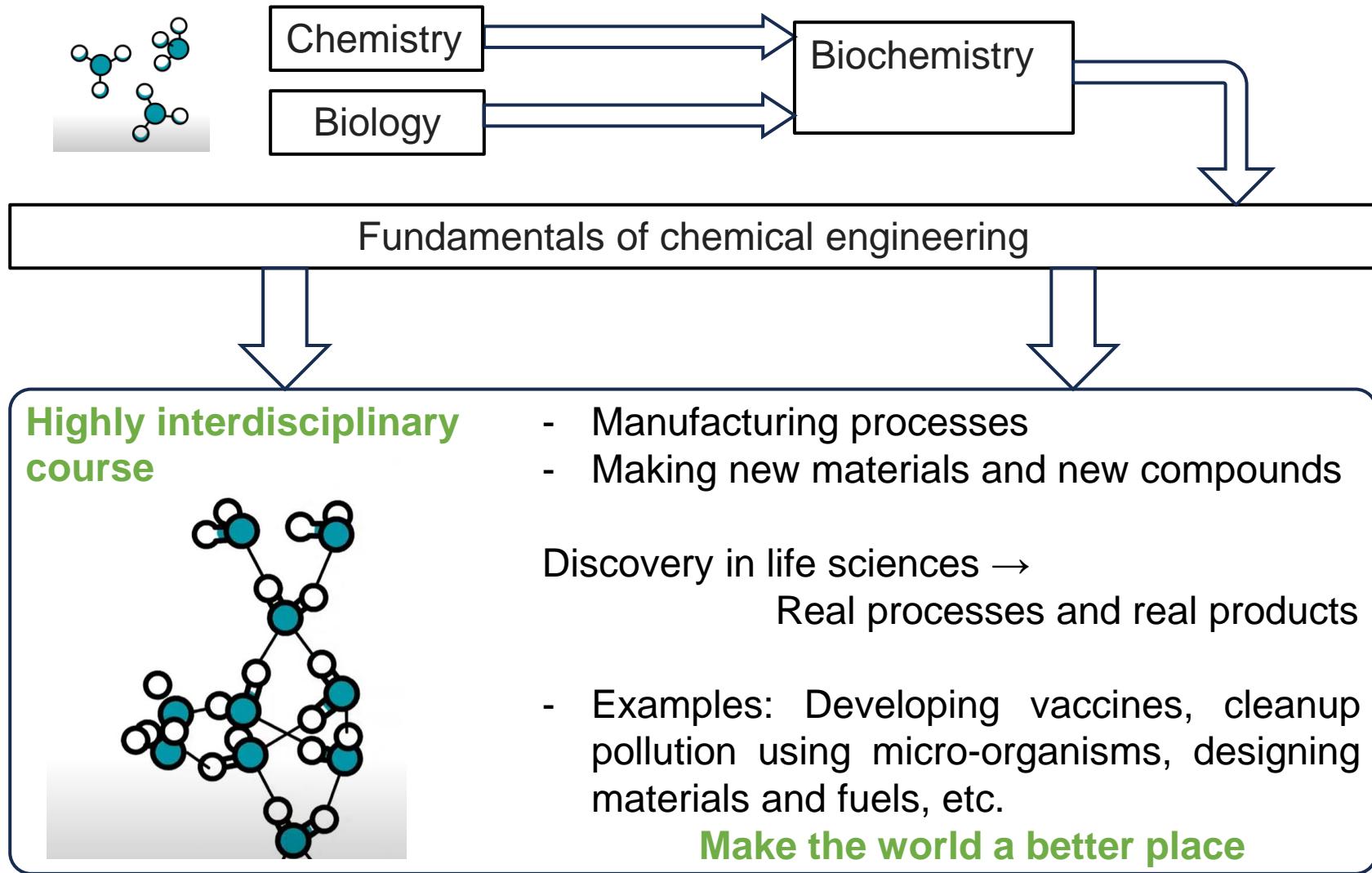


1. What is Biochemical Engineering?



What is Biochemical Engineering?

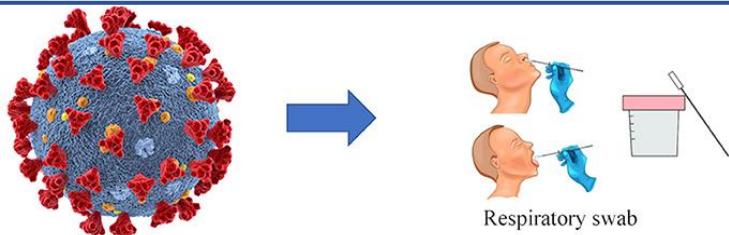
Biochemical Engineering: mixing Biology and Chemistry into Chemical Engineering



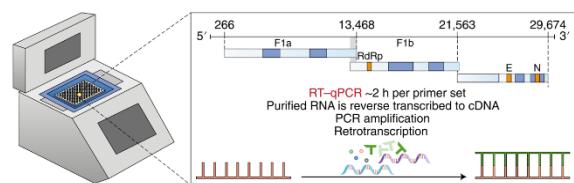
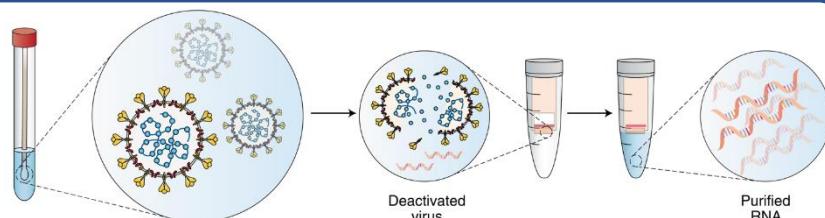
What we will learn in the course

Biochemistry

COVID-19 diagnostics



Sample travels to the lab

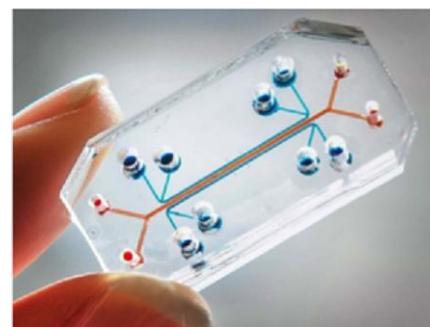


Takes 1~2 days for complete analysis

Importance of Biochemical Engineering in real-world problems

Biochemical Engineering

Point-of-care (POC) Diagnostics
On-site is possible, no need to carry to lab



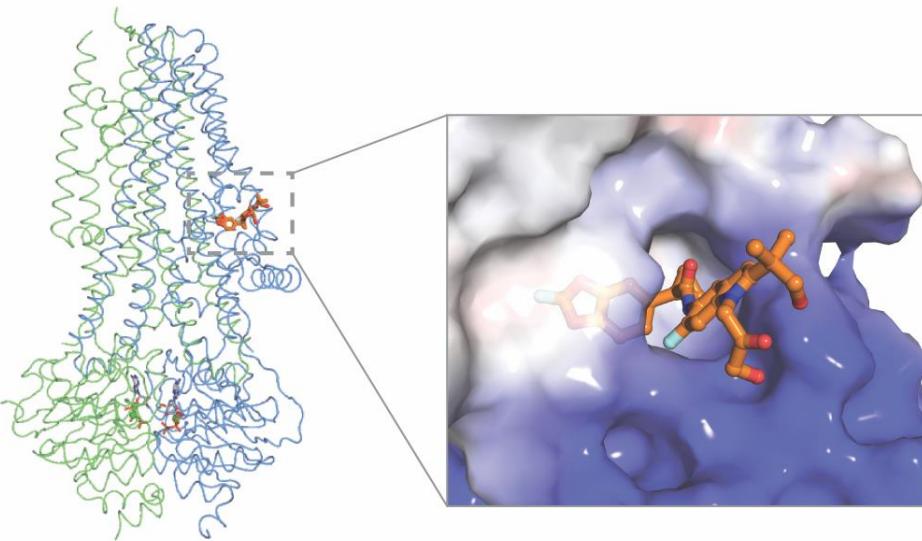
The whole system can be squeezed into a single microchannel

Takes less than 2h for complete analysis

What we will learn in the course

Biochemistry

Biological revolution: Medicines
Ongoing exciting drug development research



From “painless” to “pain”
Cystic Fibrosis – genetic disorder
Cancer: Breast cancer, Skin cancer etc.
High cholesterol

Importance of Biochemical Engineering in real-world problems

Biochemical Engineering

Converts technology to a product



What we will learn in the course

Biochemistry

Forensic research

Ongoing exciting DNA research

Genetics in the news

After searching for more than 40 years, authorities say an ex-cop is the Golden State Killer

By Ray Sanchez, Elizabeth I. Johnson, Steve Almasy and Alanne Orjoux, CNN
© Updated 10:44 AM ET, Fri April 27, 2018



Break in Golden State Killer case came from DNA on genealogy website

Christal Hayes, USA TODAY Published 8:14 p.m. ET April 26, 2018 | Updated 1:18 p.m. ET April 27, 2018

Justice system
History

Genetic expression

Importance of Biochemical Engineering in real-world problems

Biochemical Engineering

Engineering portable analyzer



What we will NOT learn in the course



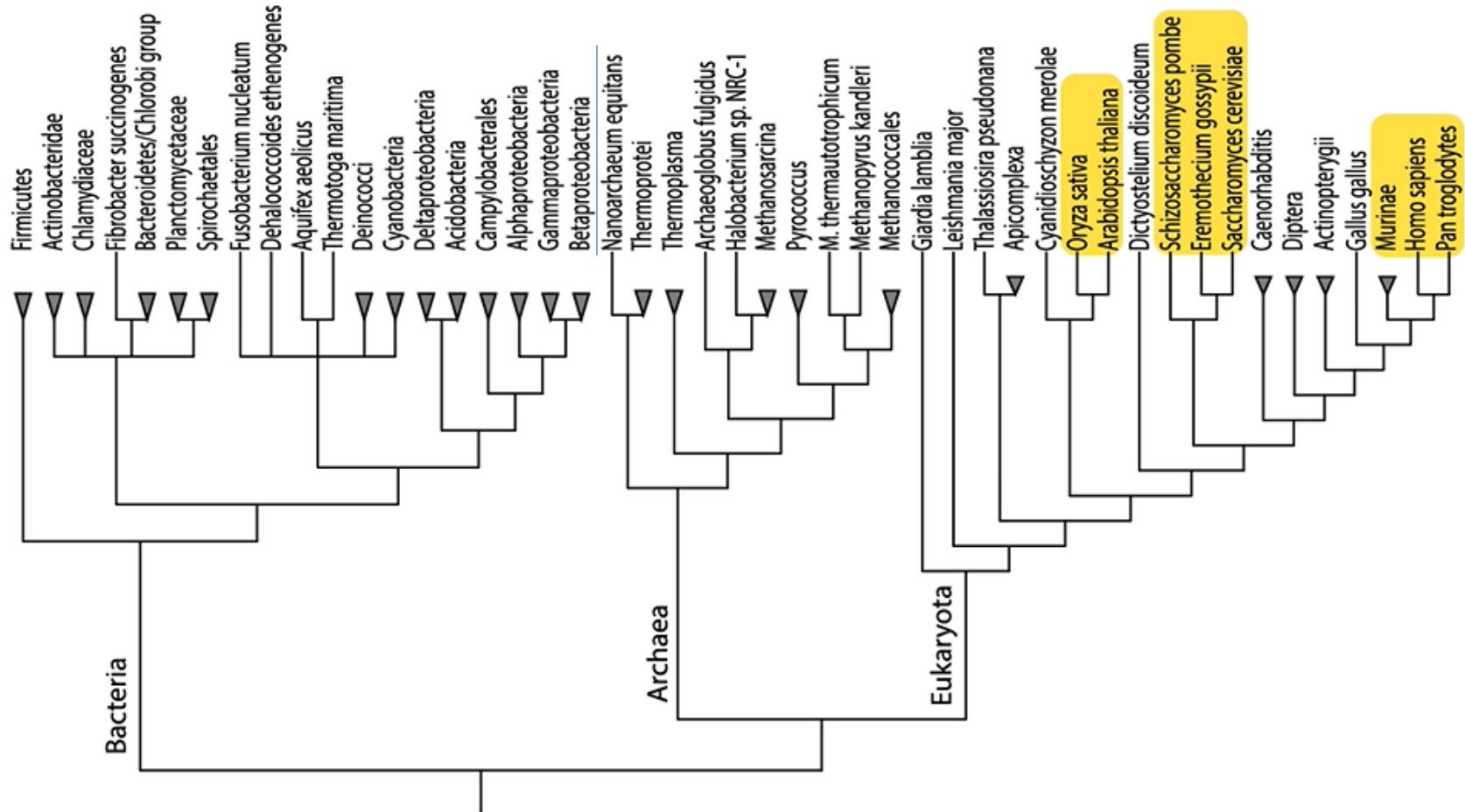
How arc reactor energy source interacts with the biological human body to become Iron man!



We will not study- Diversity of life

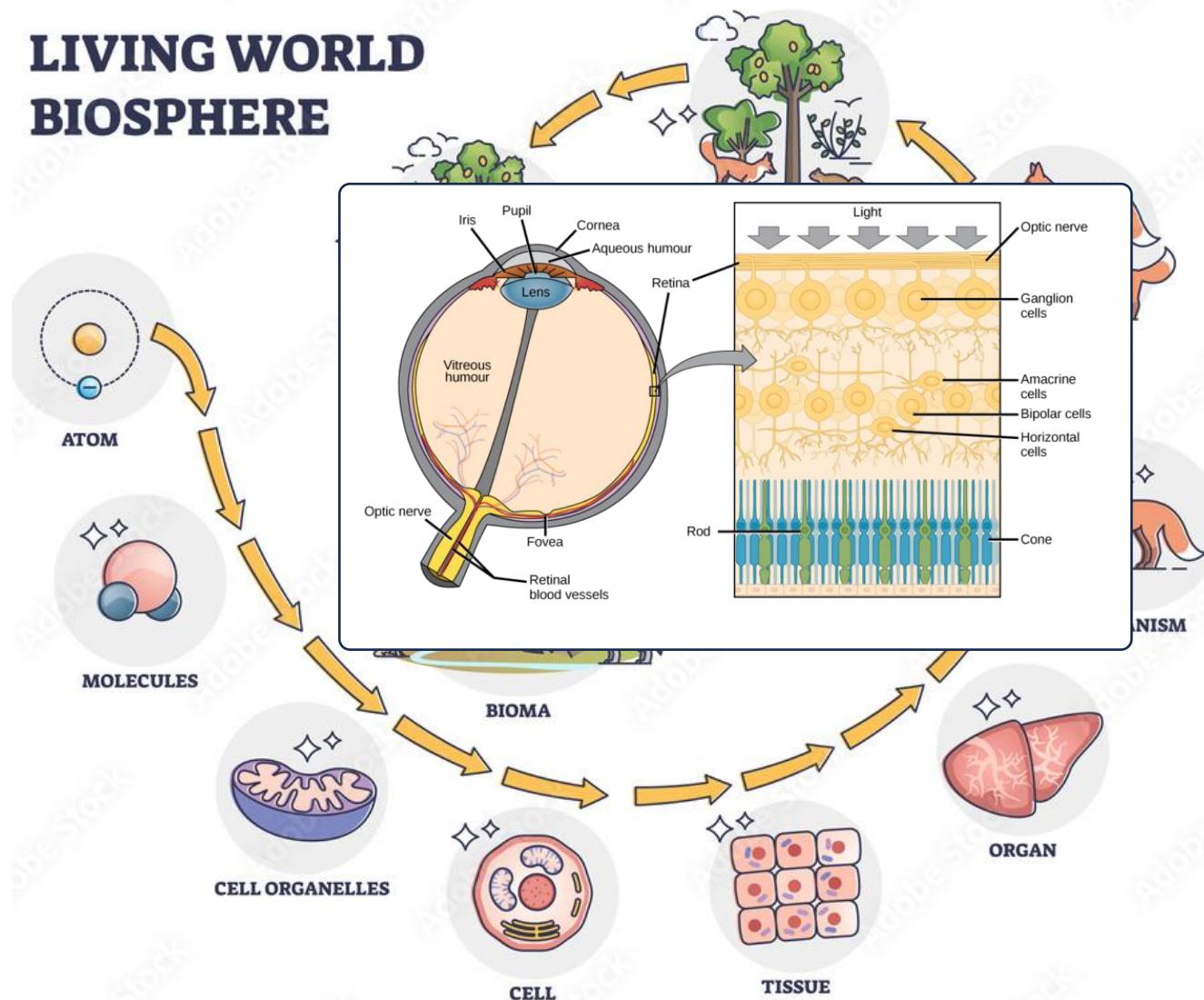


We will not study- Evolution of life



We will not study - Diversity of life

LIVING WORLD BIOSPHERE



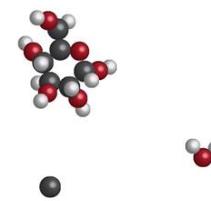
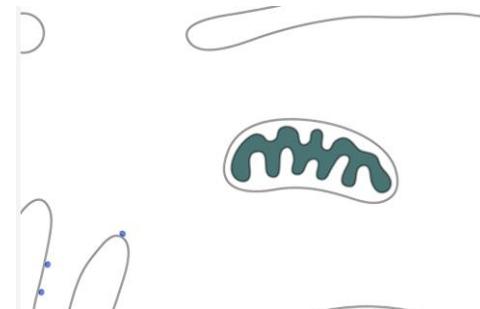
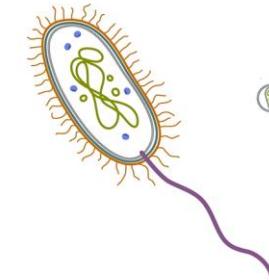
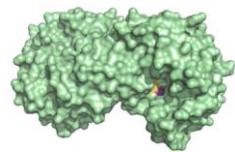
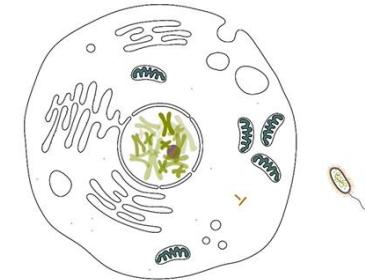
We will study- Commonality of life



1 px = 100 um

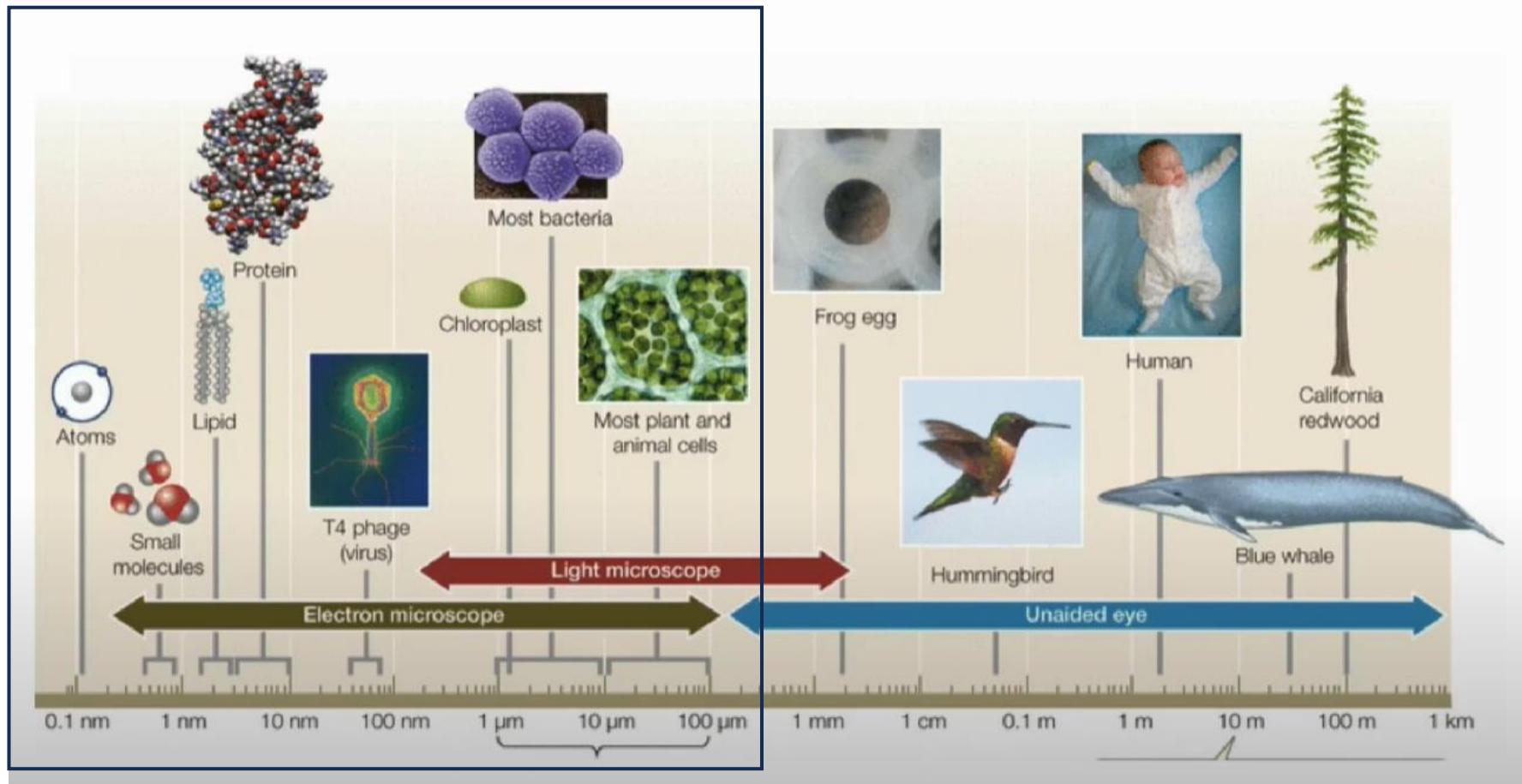


100 px = 1 um
1 px = 10 nm

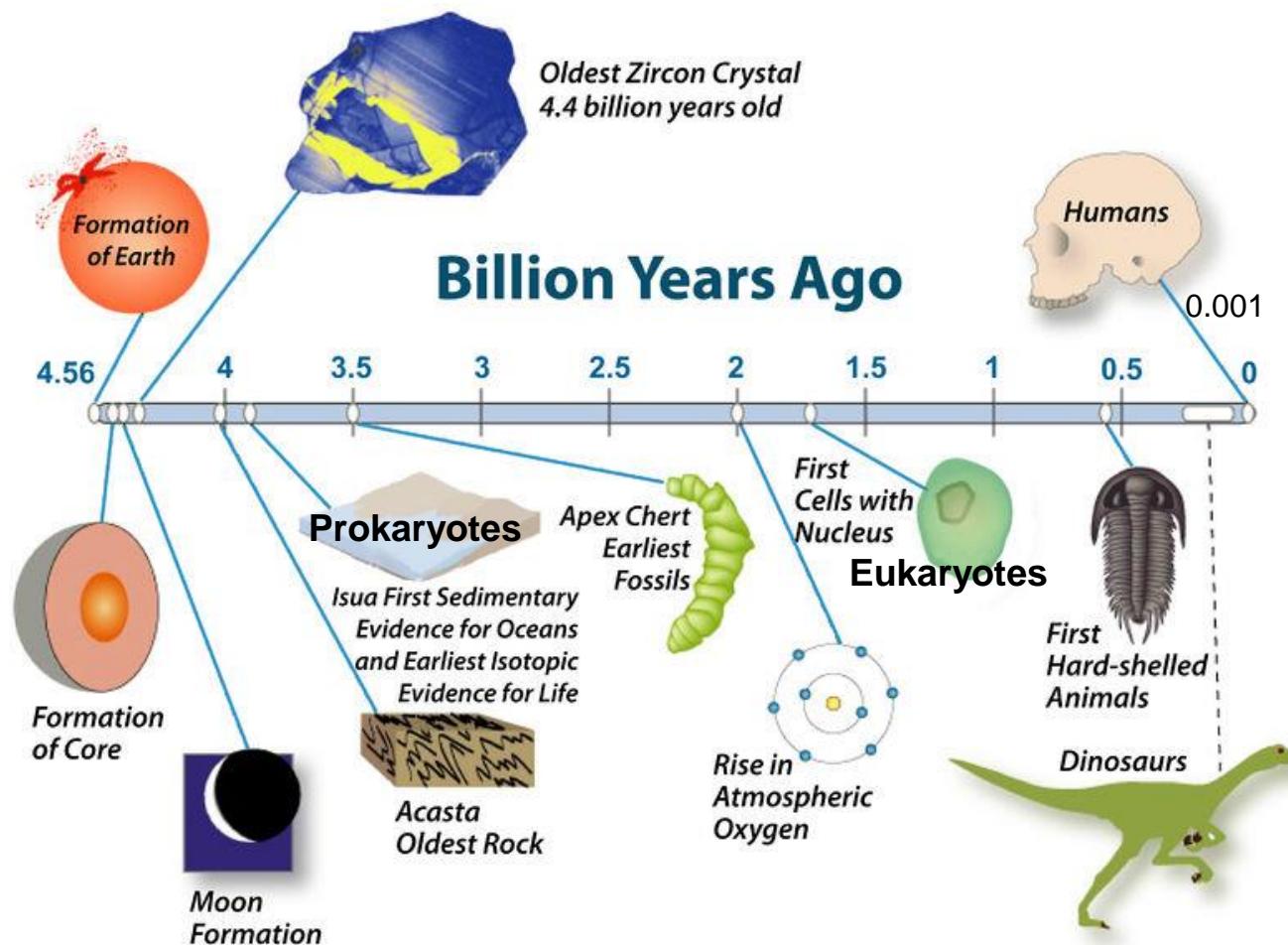


[Interactive tool](#)

Commonality of life- we will study

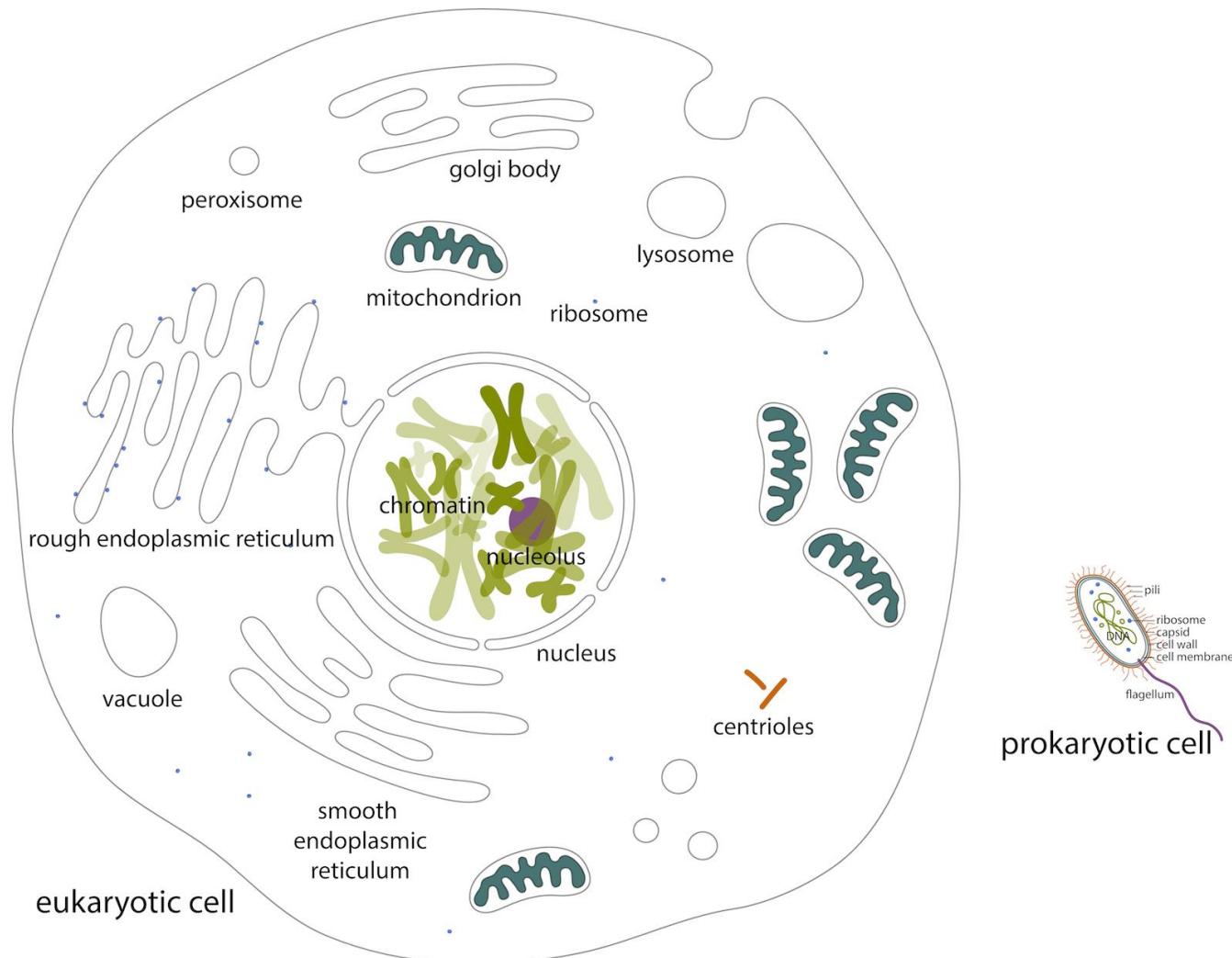


Evolution of life- some important dates

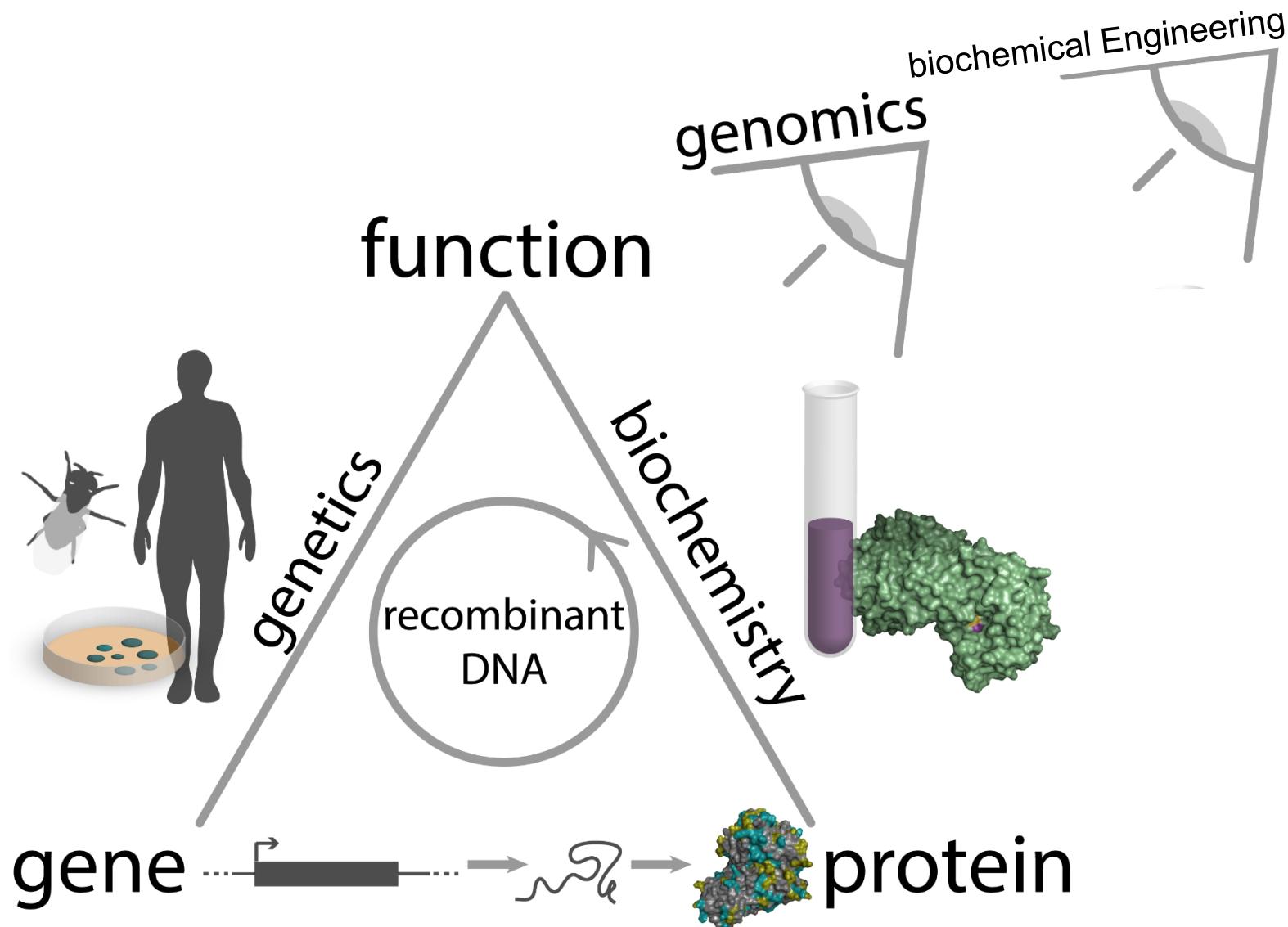


0.00000007 Billion Years Ago- IIT Kharagpur was founded!

Cell structure and classification

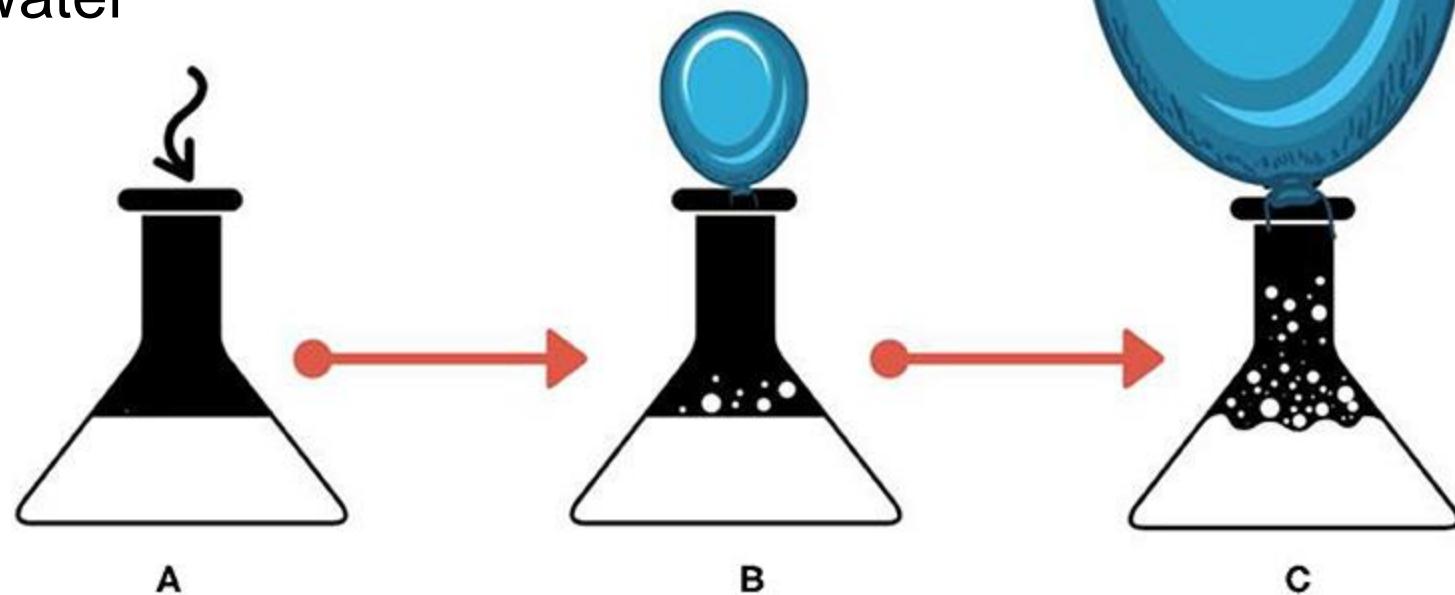


Secret of Life

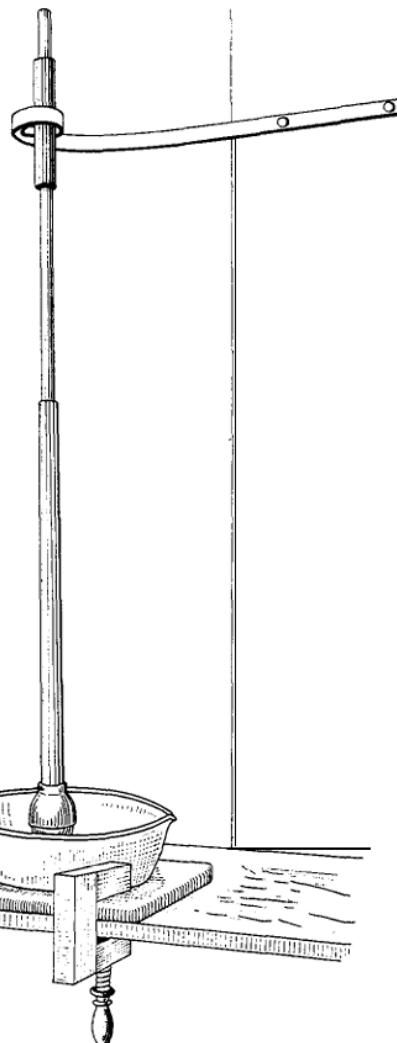


Fermentation and vitalism!

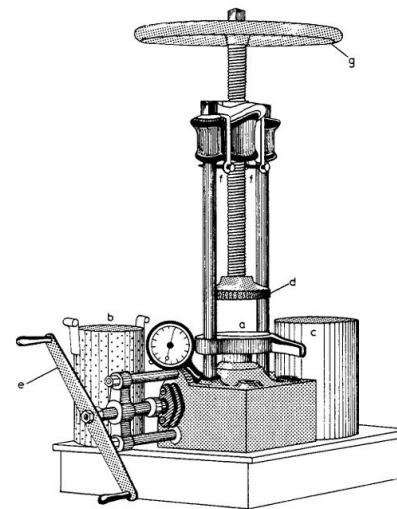
Sugar water or
fruit juice in
water



Buchner and the death of vitalism!



Eduard Buchner
(1860-1917)
1897 found fermentation in
broken yeast cells
1907 Nobel Prize in Chemistry



→ Yeast juice
“zyme”!

Buchner and fermentation

"Distinguished audience, as you'll see, we are still far from an understanding of the processes involved in alcohol fermentation of sugar, as well as from a more detailed description of the nature of the zymes. Quite the contrary, every step we take the present leads to fresh complications. We must be thankful, however, if the increasingly narrow and steep paths do not end in an unclimbable cliff.

Nevertheless, there is no cause for discouragement. The progress made in the field of fermentation processes is clearly revealed when you compare our present knowledge with that of just a few decades ago. The differences between the vitalistic view and the enzyme theory have been reconciled.

The fermentation process becomes comprehensible to us now that it is possible to separate it from the rest of the processes of life. Just as the first step toward the explanation of the phenomenon of combustion rested on the fact that it was possible to separate the generation of light and heat from the processes of oxidation.

We are seeing the cells of plants and animals more and more clearly as chemical factories where the various products are manufactured in separate workshops.

The enzymes act as the overseers. Our acquaintance with these most important agents of living things is constantly increasing. Even though we may still be a long way from our goal, we are approaching it step by step. Everything is justifying our hopes. We must never therefore let ourselves fall into the way of thinking ignorabimus, we shall never know. But we must have every confidence that the day will dawn with even those processes of life, which are still a puzzle today, will cease to be inaccessible to us natural scientists."



Eduard Buchner
(1860-1917)
1897 found fermentation in
broken yeast cells
1907 Nobel Prize in Chemistry

We understand now what was unimaginable then.

Apply Buchner's philosophy to all the things you do not understand today!

Questions that arises

- What are those enzymes?
- Can we understand these amazing properties of life?
- Properties of being able to carry out chemical transformations
- Properties of being able to do even more simple things.
- Having cells in the first place.
- Membranes around cells- How in the world do you do that?
- What makes a membrane divides a cell's inside from its outside?
- How do you do other sorts of things?

- Can we understand all of those things in terms of fundamental chemistry?

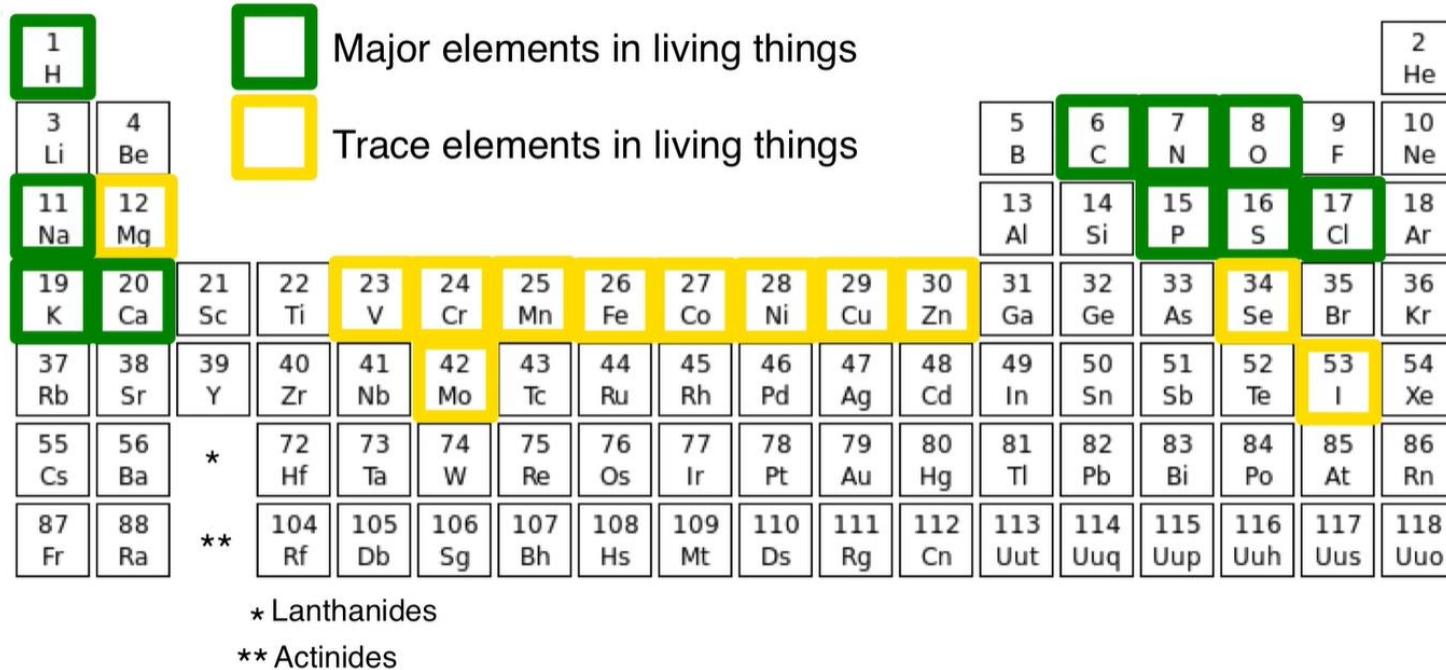
WHAAAAA?!?!



That's what biochemistry's asking us here.

Fractionating life biochemistry style!

Atomic composition in a human body

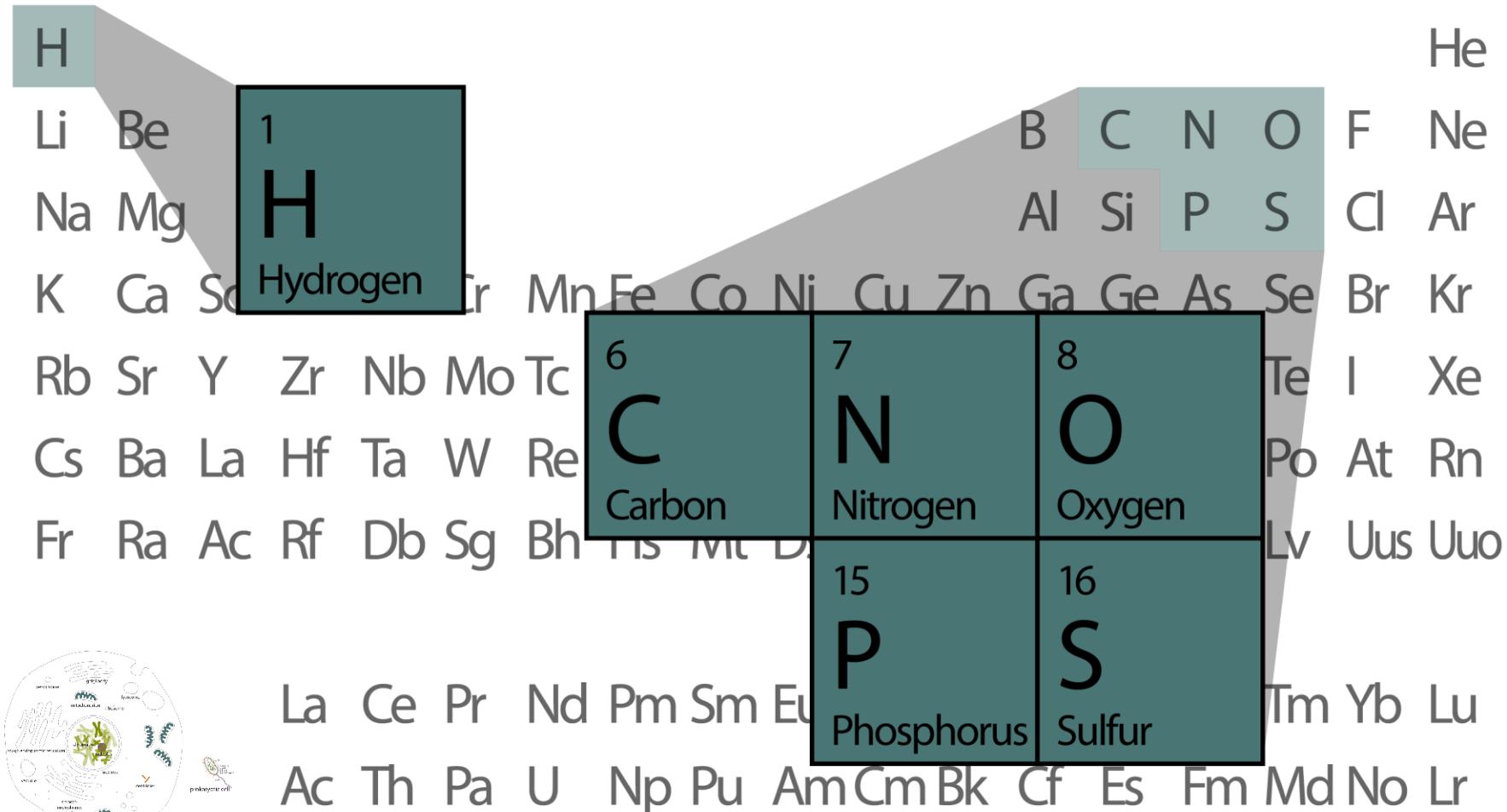


Fractionating life biochemistry style!

Atomic composition in a human body

Elements	Human Body	Earth's Crust
Oxygen	65%	49%
Carbon	18%	<1%
Hydrogen	10%	<1%
Nitrogen	3%	trace
Calcium	2%	3%
Iron	<0.05%	5%
Aluminum	<0.001%	8%
Silicon	trace	26%

Atomic composition in a cell



NOT SO MUCH FUN!!

Molecular composition in a living being

IIT Kharagpur student



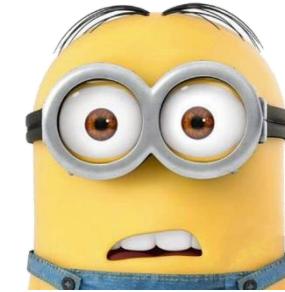
Component	Wt %
Water	80
Others	20

Other	Dry Wt %
Proteins	50
Carbohydrates	15
Lipids	10
Fats	10
Nucleic Acids	15

NOT SO MUCH FUN!!

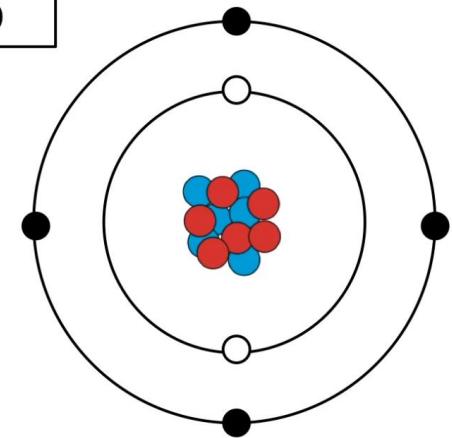
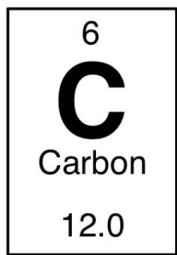
We want more interesting descriptions!

- ❑ We want to actually know, what are those molecules there.
- ❑ What do they do? **WHAAAT?**
- ❑ What are these proteins about?
- ❑ What are these carbohydrates about?
- ❑ What are these lipids about?
- ❑ And how do their properties account for the amazing properties of cells?
- ❑ We want to understand the **chemistry** and how the chemistry gives rise to the properties.

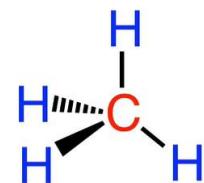
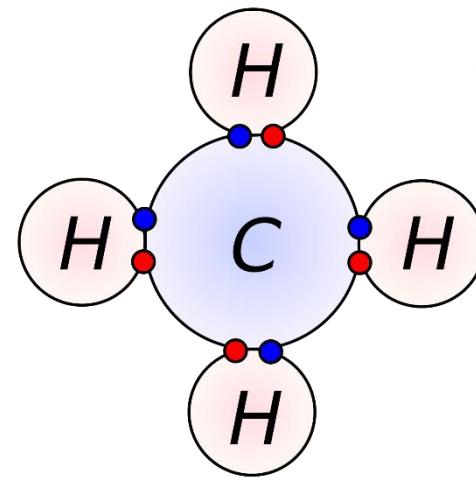
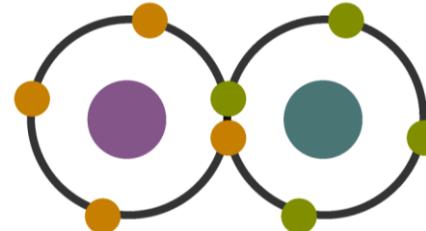
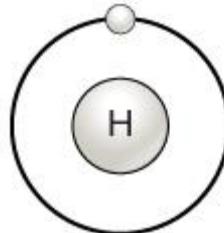


Covalent bonds: Sharing is caring!

Covalent bonds



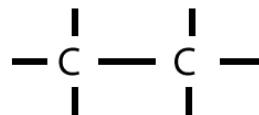
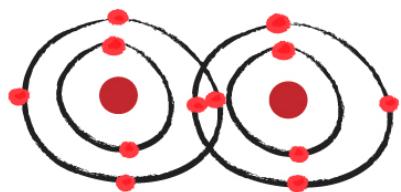
H



- Shared pair of electrons
- Strong

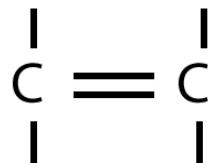
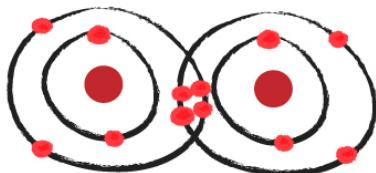
● Electron from hydrogen
● Electron from carbon

Shared pairs

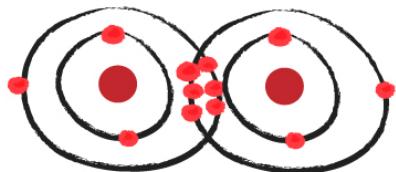


SINGLE BOND

80 kcal/mol



DOUBLE BOND

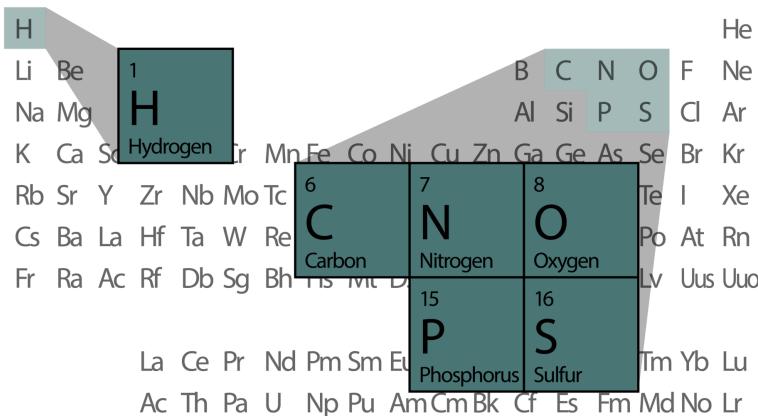


TRIPLE BOND

Random fluctuations at room temperature for a molecule: 0.6 kcal/mol

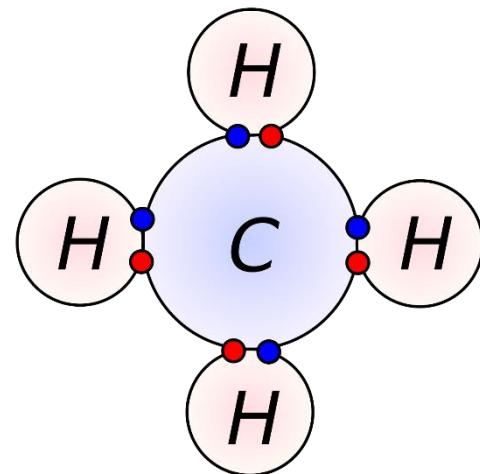
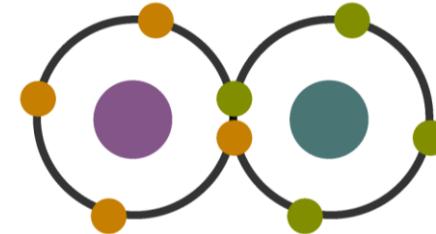
Strength is defined by the energy needed to break the bond
Covalent bonds are extremely stable

Maximum number of bonds



Our Favorite Atoms	Max number of bonds
H	1
O	2
N	3 (4)
C	4
P	5
S	6

Covalent bonds



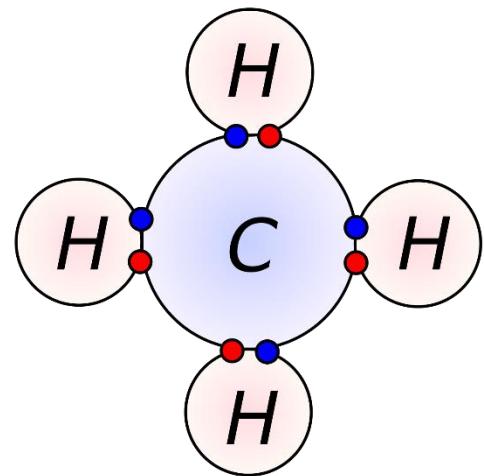
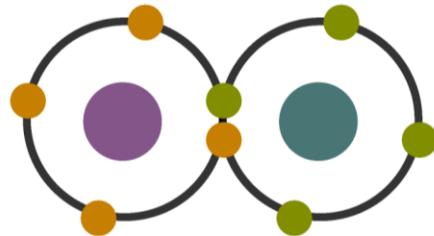
- Electron from hydrogen
- Electron from carbon

Sharing !

Happy Utopia where sharing is equal!



Covalent bonds



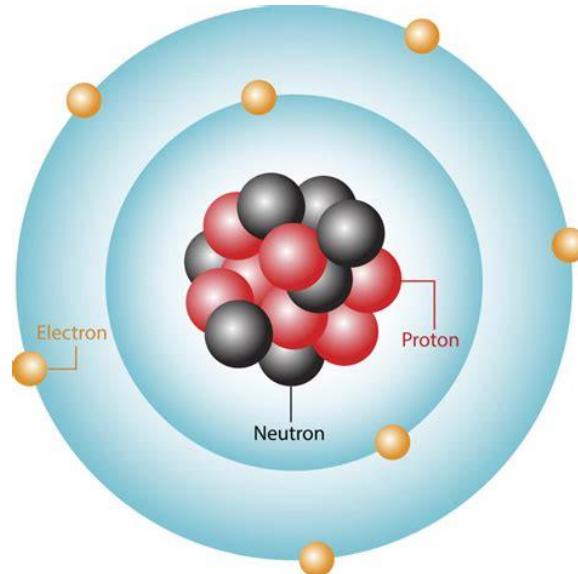
Sharing is usually never equal!
Also true at atomic level!

- Electron from hydrogen
- Electron from carbon

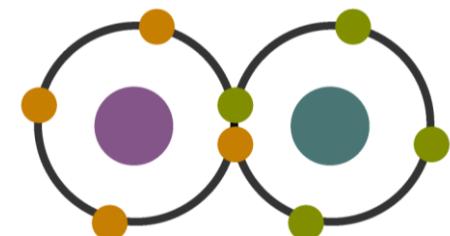
Sharing !

Some atoms are greedier than the others!

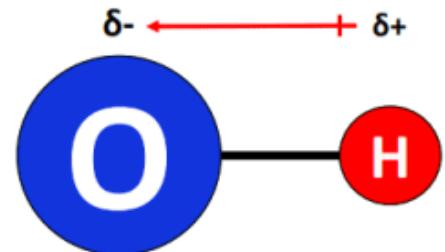
- Protons
- Distance



Non-polar



Polar



Electronegativity
O, N >> C, H

Take-Away Menu

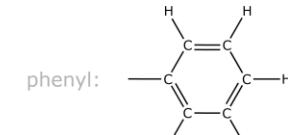
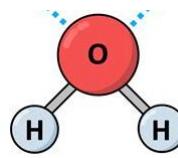
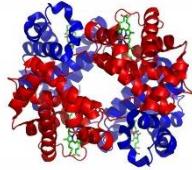
- Covalent bonds
- They're really strong.
- They don't break at random.
- We have unequal sharing.
- Some bonds are polar.
- Some bonds are not.



What are the consequences?

- ❑ Now let's go on and ask, what are the consequences of this all?
This business of unequal sharing?
- ❑ It turns out that these covalent bonds are the backbones of molecules.
- ❑ Big molecules are collections of atoms held together by covalent bonds.

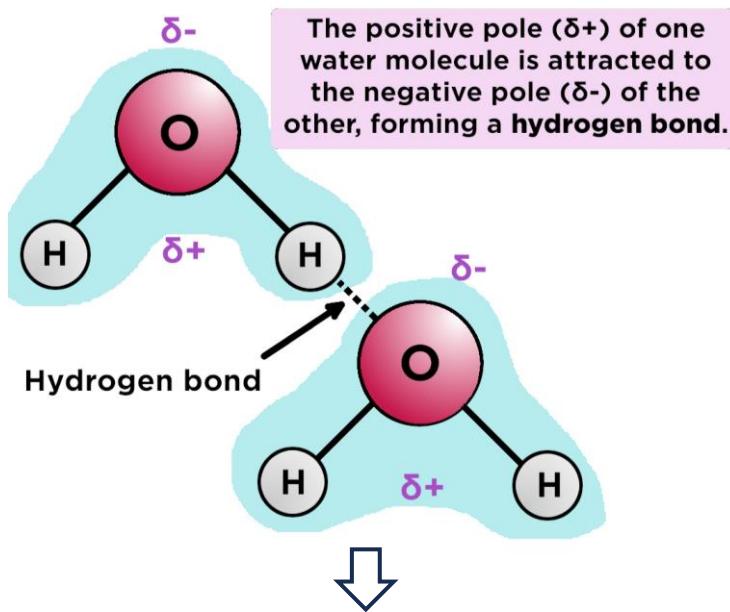
❑ Structures?



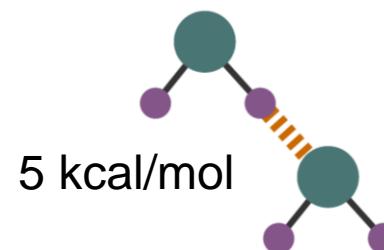
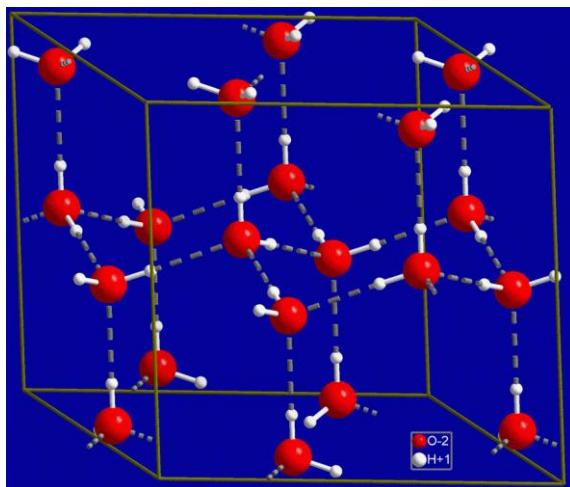
functional groups

- ❑ Understanding biological properties require non-covalent bonds and other funny forces.

Hydrogen Bonds



Highly
structured
Strength in
numbers!



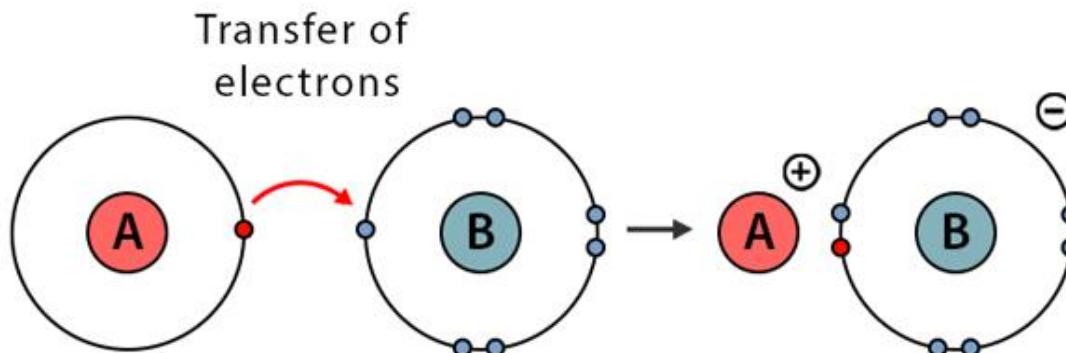
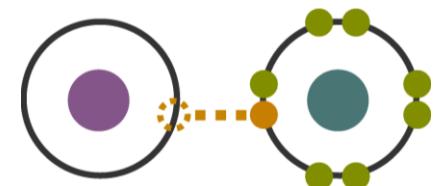
Hydrogen bonds are built for spiders!



Spiderman fails chemistry!

Ionic Bonds

No sharing!



Net charges are created!

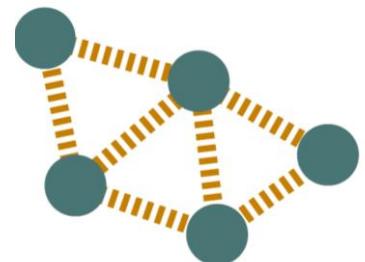
Van Der Waals forces

---- C – H

Non-polar

H – C ----

Non-polar

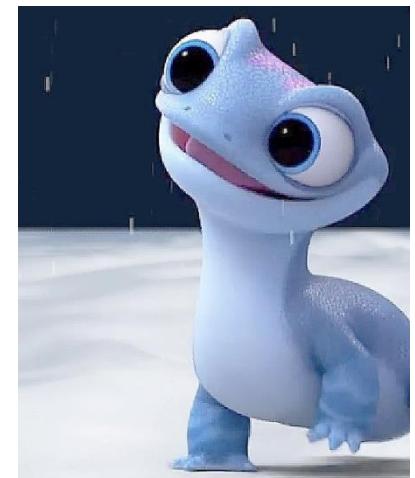
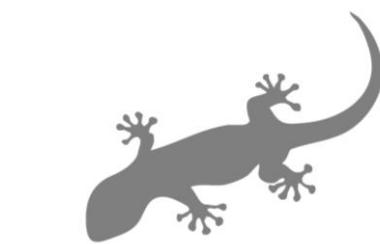


- Not completely non-polar
- Negative charge keep fluctuating a bit



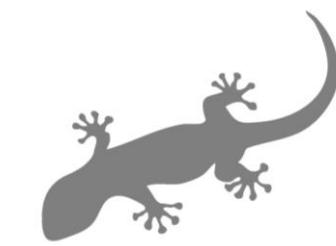
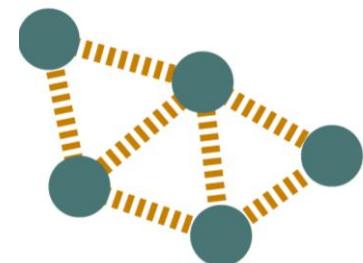
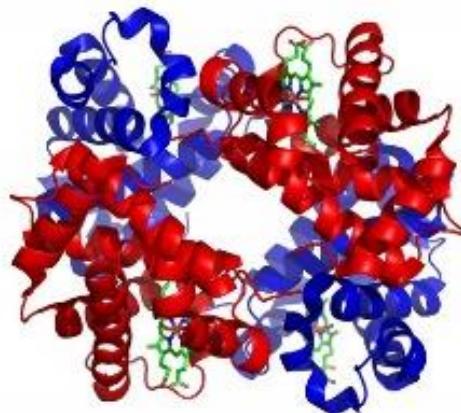
1 kcal/mol
Puny!

Not very impressive, right?

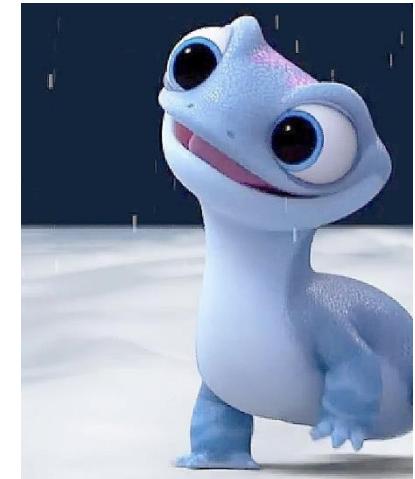


Strength in numbers

- Individually the non-covalent bonds look small
- Imagine a big molecule- like a protein

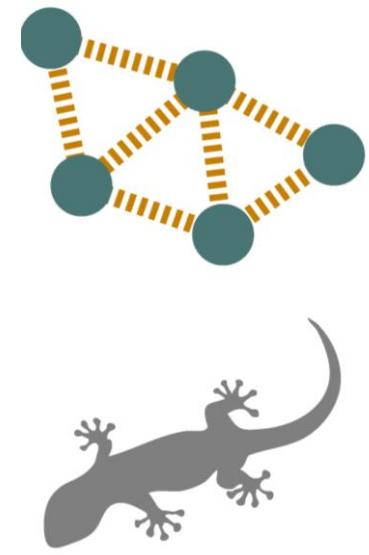


- Even though covalent bonds hold them,
- **Non-covalent bonds determine the shape they take up**



Strength in numbers

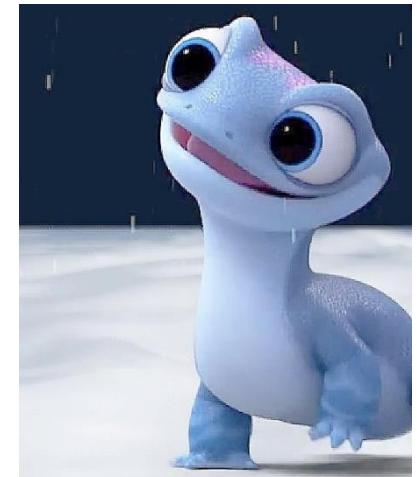
- Let me show you how powerful these forces can be



Flaps make so much contact- keeps it from falling from the ceiling

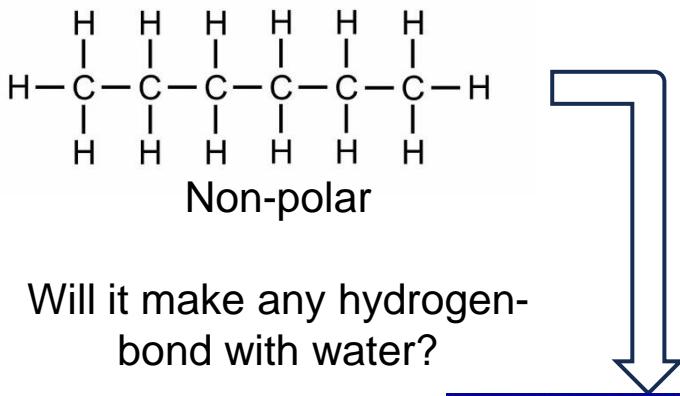
Strength in numbers!

Do not try to climb the ceiling at home!

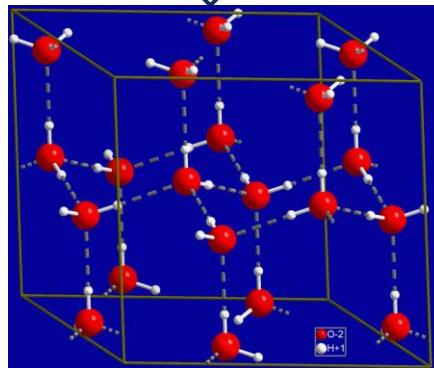


Hydrophobic forces

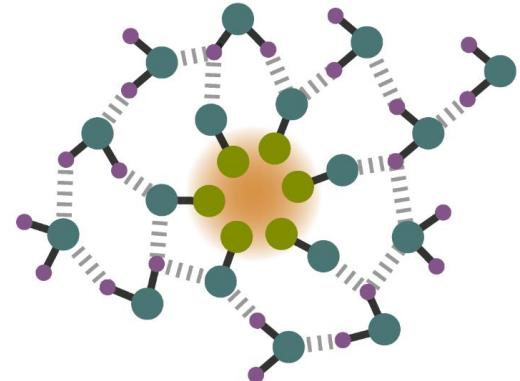
It is not technically a “bond” or a “force”!



Will it make any hydrogen-bond with water?



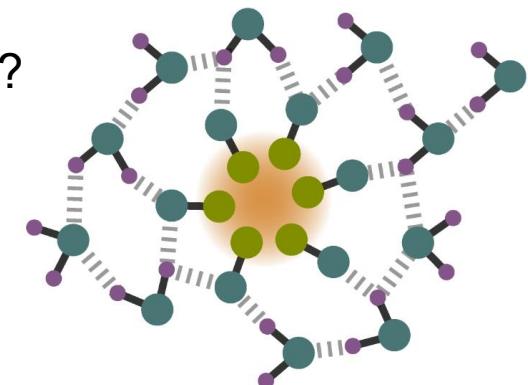
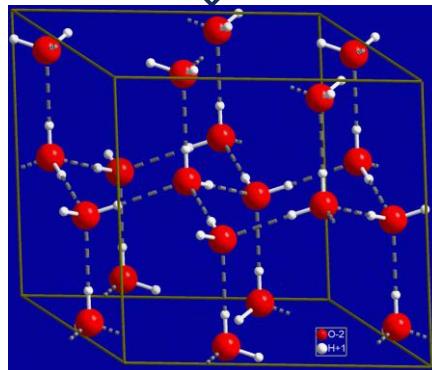
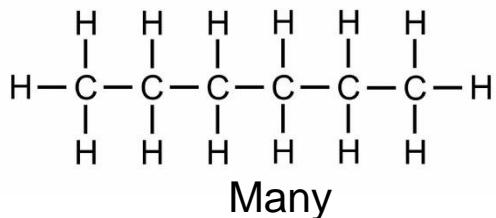
Bull in a China shop!



- Disrupting hydrogen bonds
- Breaking favorable bonds.
- Energetically unfavorable
- It is breaking apart hydrogen bonds.
- Big energetic cost.

Hydrophobic forces

What if we throw many such “non-polar” molecules into water?



- Everyone disrupting hydrogen bonds
- Other hydrogen bonding with each other try to push out the interlopers, these non-polar molecules
- They are going to separate



Non-polar bonds
can't hydrogen bond

Oil in water

This will be the launchpad into **biochemistry**:
lipids and membranes

Some deep-dive into the concepts we have learned so far before we go to the next part

Some more quick basics of chemistry before we jump on to the fun stuff!

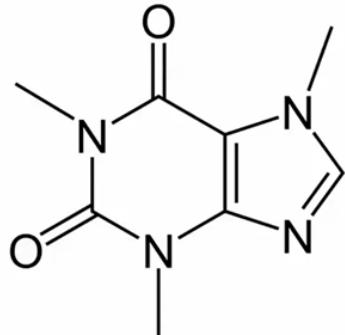
- How to read a chemical structure
- Functional groups and polarity
- Intermolecular bonding

Also aims to help you to start seeing our everyday products in terms of molecules!

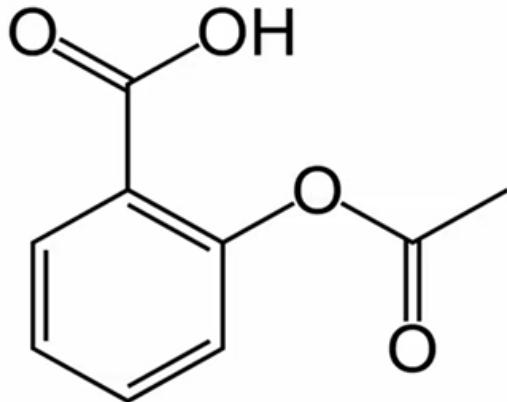
Coffee!



- Carbon at corners
- Hydrogen bonded to carbon atoms are implied
- Chemical formula:



Headaches



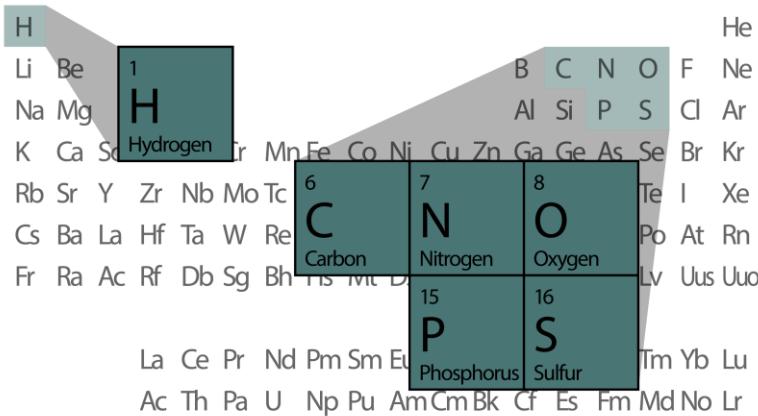
- Carbon at corners
- Hydrogen bonded to carbon atoms are implied
- Chemical formula:



Some more quick basics of chemistry before we jump on to the fun stuff!

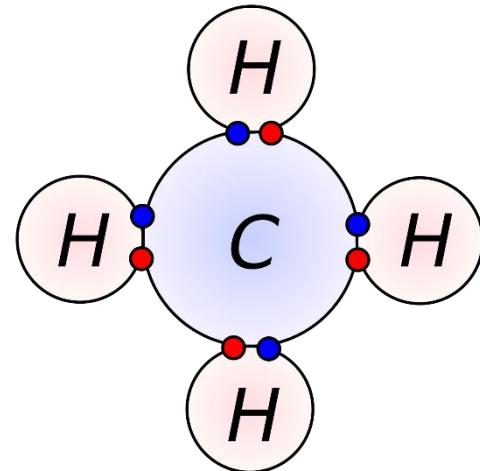
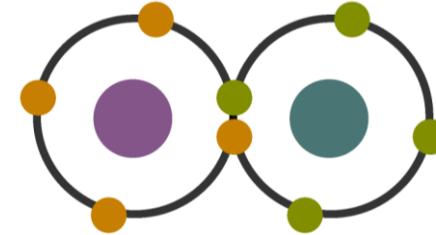
- How to read a chemical structure
- Functional groups and polarity
- Intermolecular bonding

Maximum number of bonds



Our Favorite Atoms	Max number of bonds
H	1
O	2
N	3 (4)
C	4
P	5
S	6

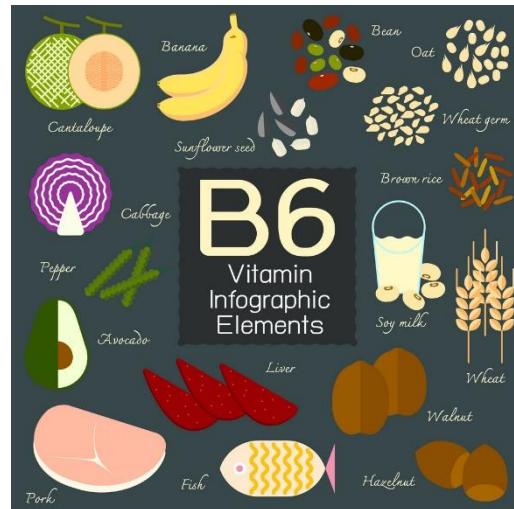
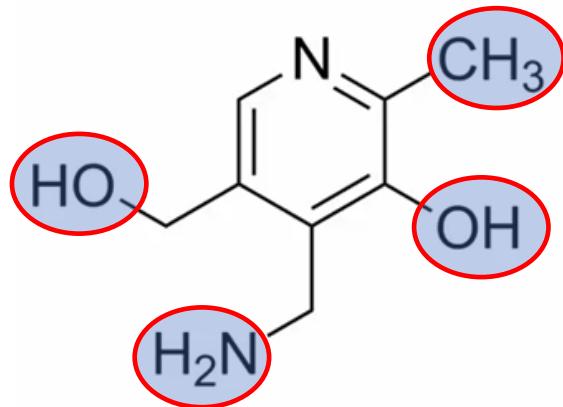
Covalent bonds



- Electron from hydrogen
- Electron from carbon

Functional groups and polar bonds

Pyridoxamine



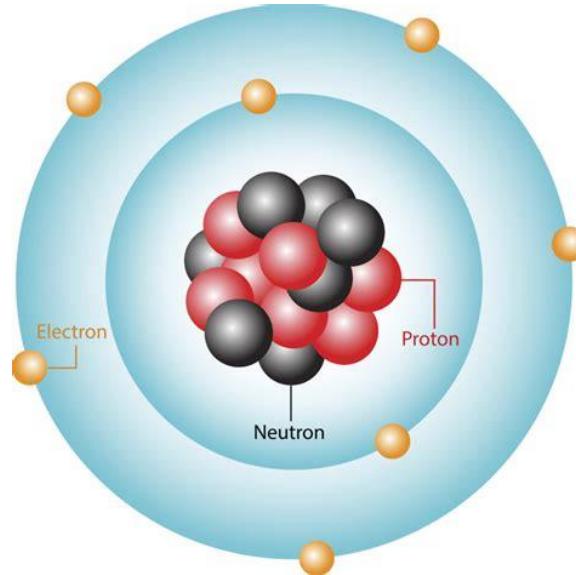
Polar or non-polar?

Molecule → Functional groups → Covalent bonds

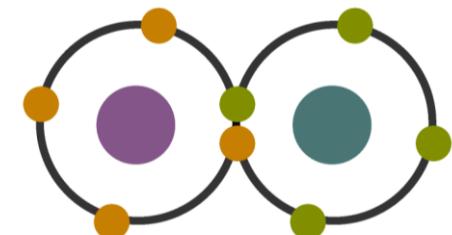
Sharing: Electronegativity and polarity

Some atoms are greedier than the others!

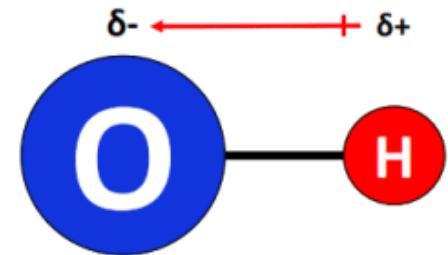
- Protons
- Distance



Non-polar



Polar



Electronegativity
O, N >> C, H

Electronegativity and polarity in terms of numbers

Greediness: Electronegativity

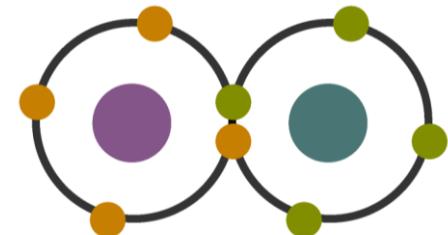
Atom's ability to attract electrons to itself when bonded to another atom

Element	Symbol	Atomic Number	Pauling Electronegativity
Phosphorus	P	17	2.19
Hydrogen	H	1	2.20
Carbon	C	12	2.55
Sulfur	S	18	2.58
Nitrogen	N	7	3.04
Oxygen	O	8	3.44

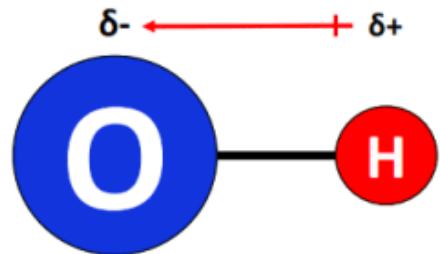


Electronegativity
 $O, N >> C, H$

Non-polar



Polar



What is the electronegativity?

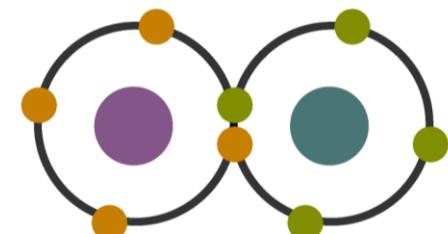
Greediness: Electronegativity

Atom's ability to attract electrons to itself when bonded to another atom

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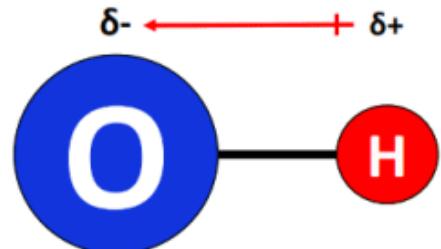
Polar

Non-polar



0

Polar



1.24

Focus on the essence, not the numbers

0 to 0.4

Nonpolar covalent

0.41 to 1.69

Polar covalent

> 1.7

Ionic

Electronegativity Difference

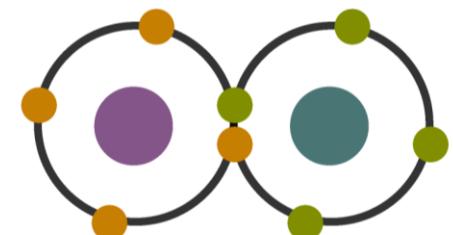
What about C-H bond? – 0.35

Greediness: Electronegativity

Atom's ability to attract electrons to itself when bonded to another atom

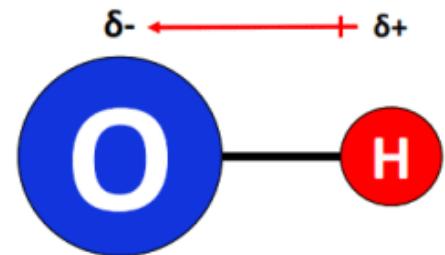


Non-polar

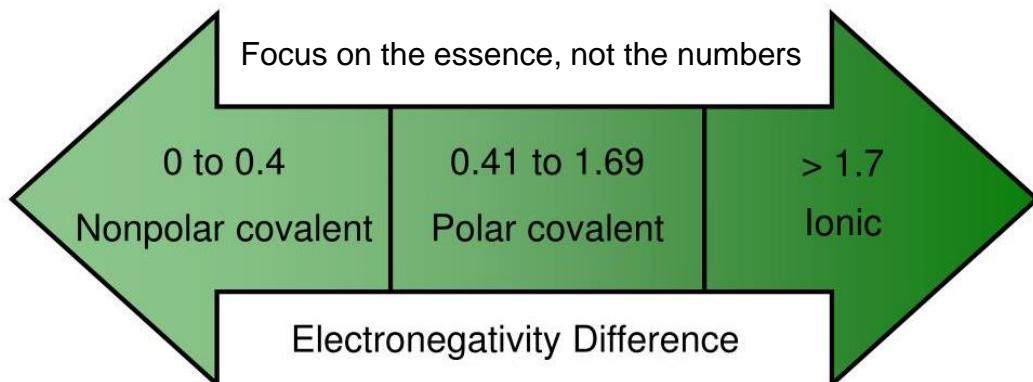


No one's winning

Polar

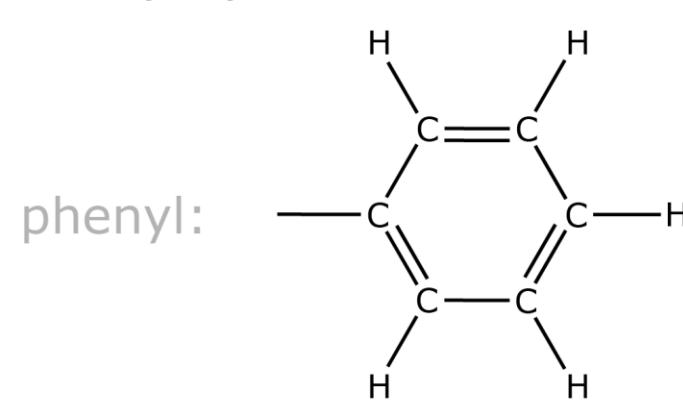
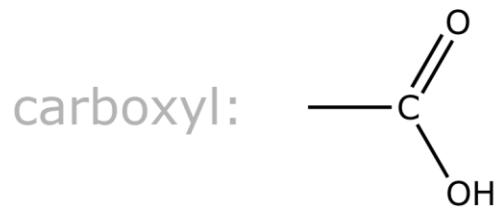
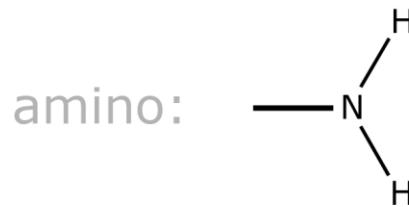
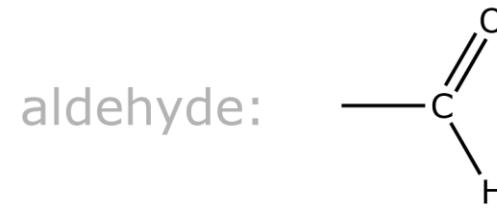
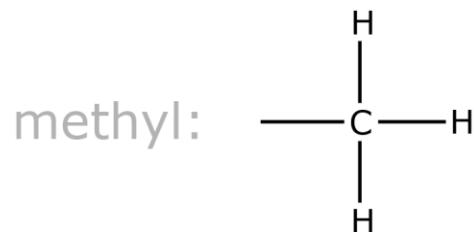


O is winning



What about C-H bond? – 0.35

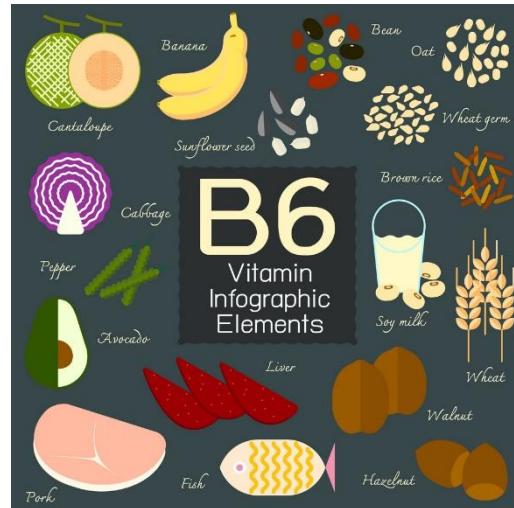
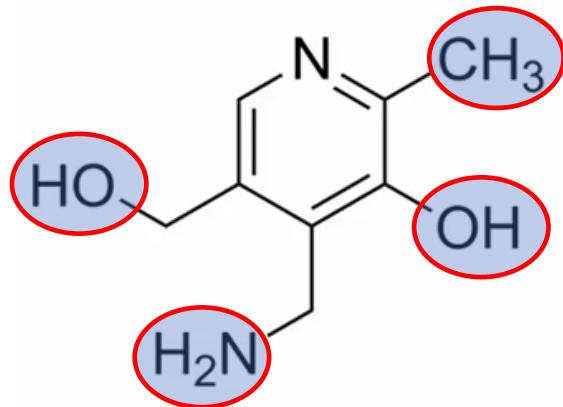
Functional groups: Polar or non-polar?



functional groups

Functional groups and polarity

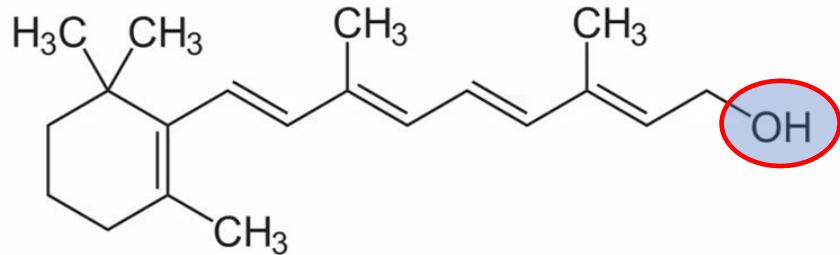
Pyridoxamine



So tell me: polar or non-polar?

Functional groups and polarity

Retinoid



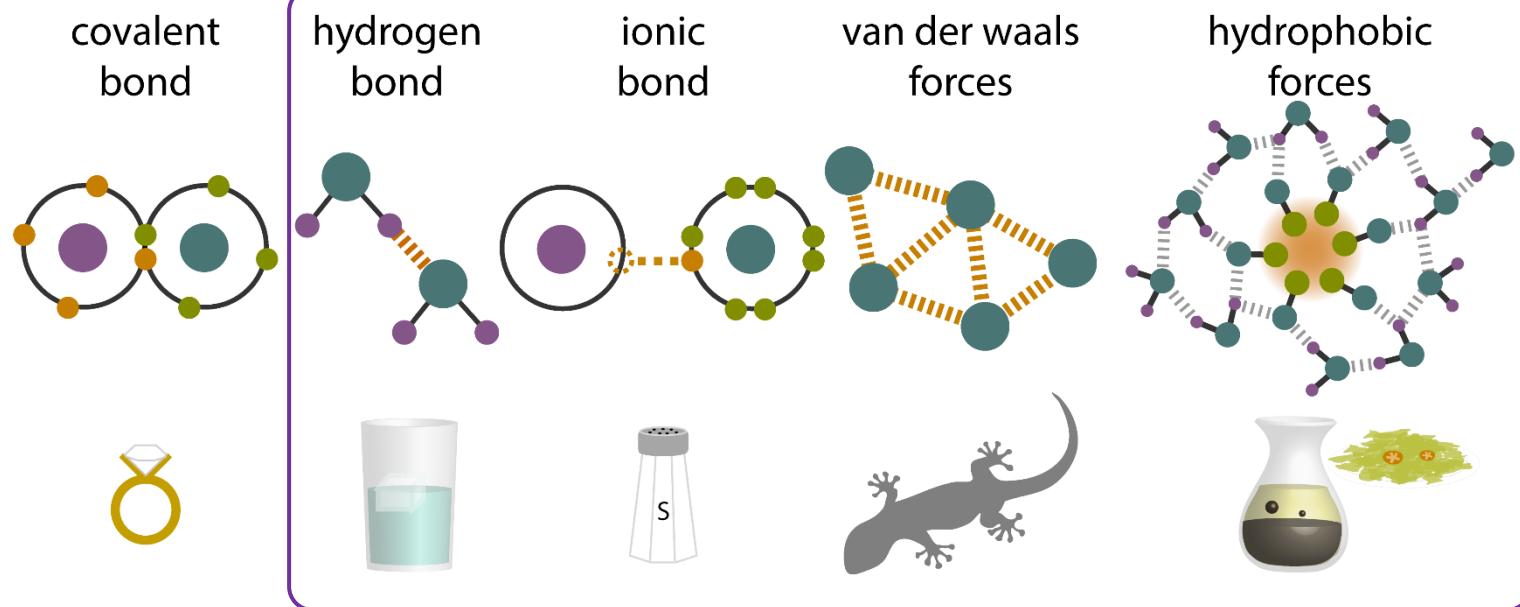
So tell me: polar or non-polar?

Some more quick basics of chemistry before we jump on to the fun stuff!

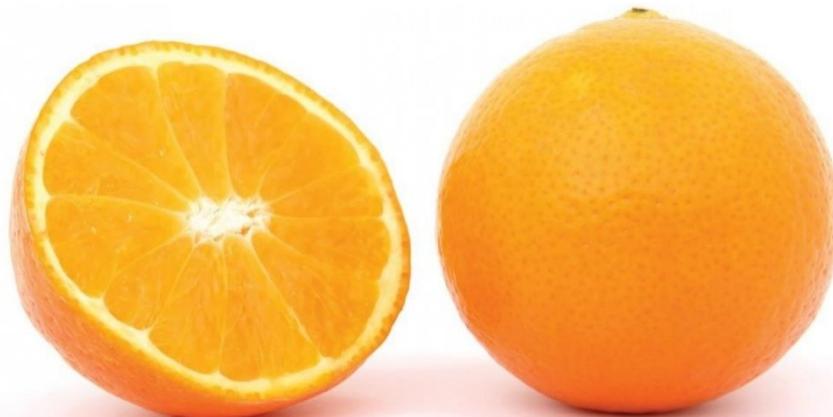
- How to read a chemical structure
- Functional groups and polarity
- **Intermolecular bonding**

Deep dive into intermolecular forces

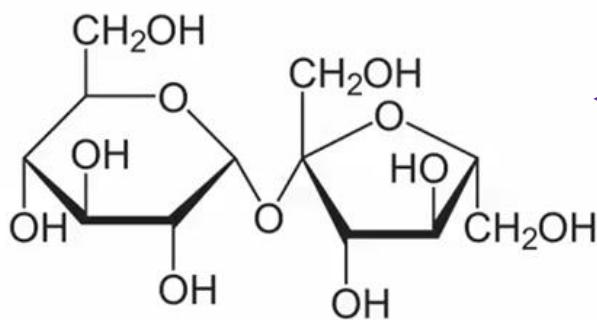
Intermolecular forces



Deep dive into intermolecular forces

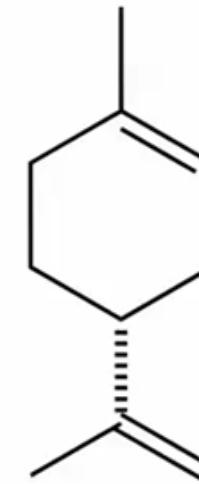


Sucrose



Polar

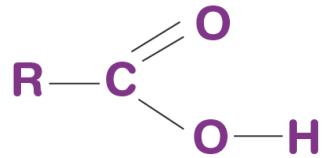
Limonene



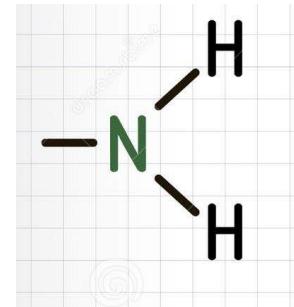
Interaction?

Non-polar

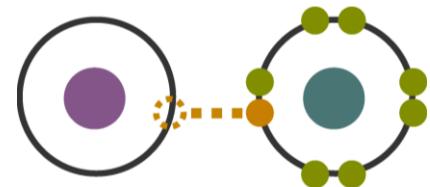
Ionic bonds



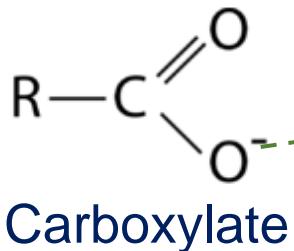
Carboxyl



Amine

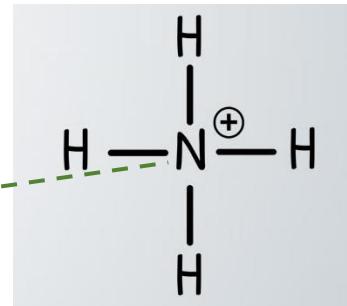


At certain pHs



Opposites attract

Carboxylate

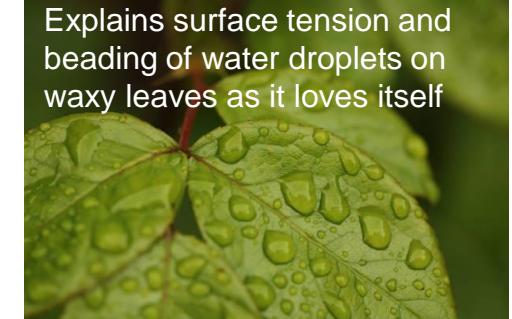
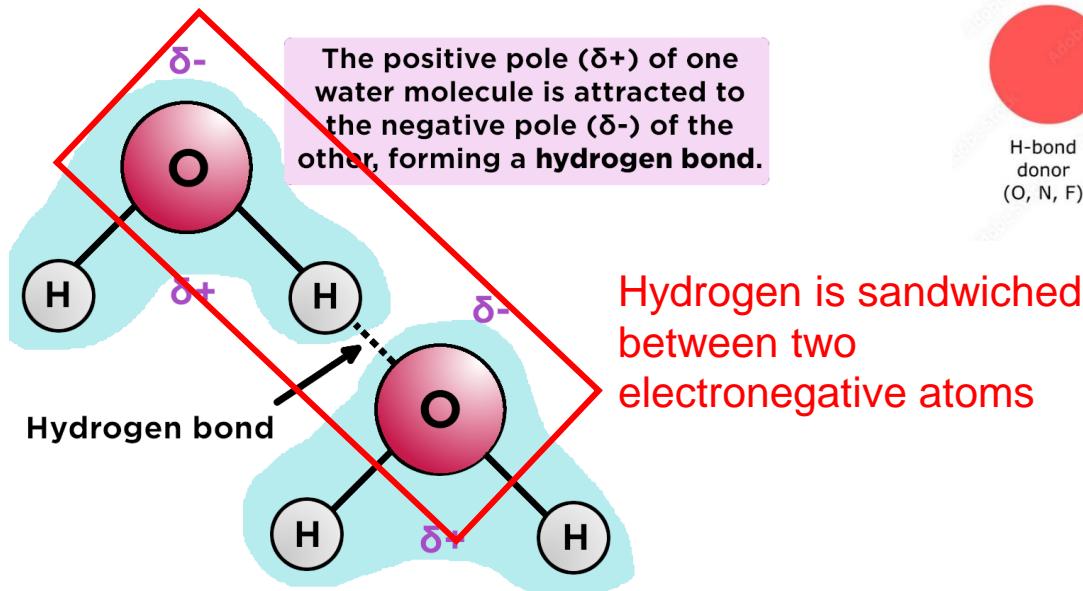


Amine ion

Ionic Bond

Hydrogen Bonds

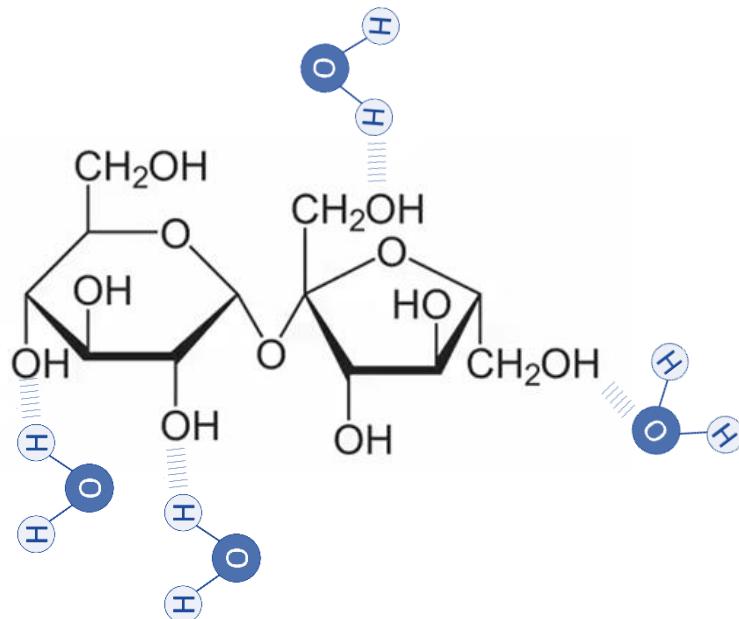
Attraction between a **polar hydrogen** in **one functional group** and an **electronegative atom** (like N or O) in another functional group



*Explains if a molecule can dissolve in water or not:
Polar molecules dissolve in water- hydrophilic*

Hydrogen bonds and water solubility

Polar sucrose



Hydrophilic: Likes to interact with water



WATER SOLUBLE

What about non-polar molecules?

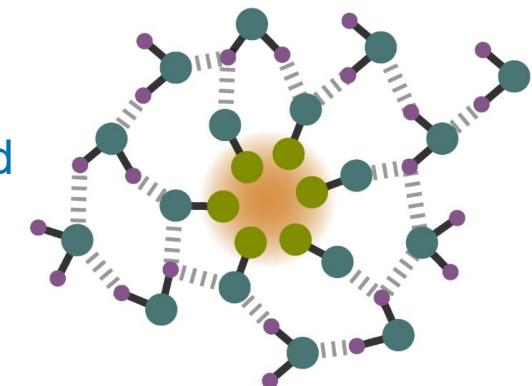
Hydrophobic forces

We know oil is insoluble in water

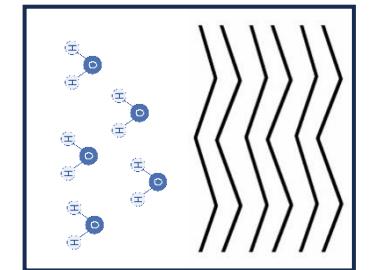
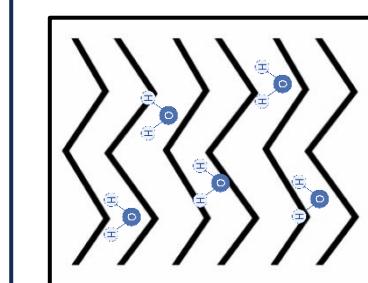


Non-polar bonds can't hydrogen bond

Oil in water



Molecular level



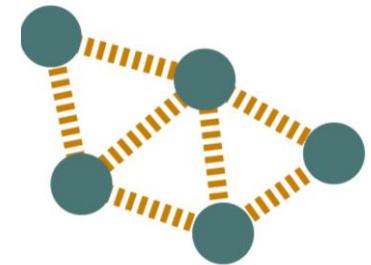
- Hydrophobic effect: Hydrophobic molecules clump together to avoid disrupting hydrogen bonds between water molecules
- This will be critical in understanding cell membranes, proteins, lipids, etc.

Van Der Waals forces

It occurs every time atoms come close to each other



Induced dipole



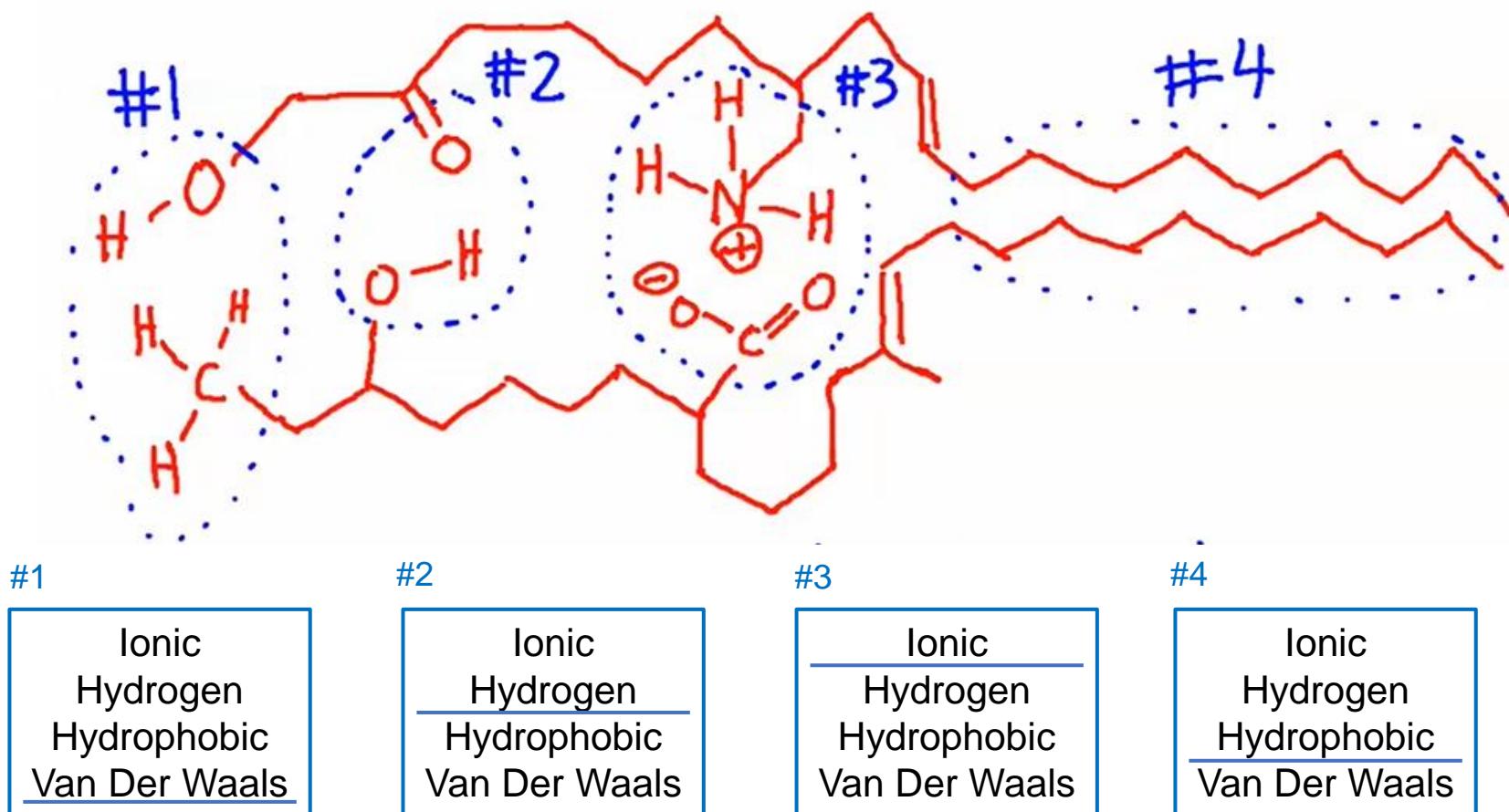
Very weak as compared to other forces

Significant in large numbers

It can be formed between all neighboring atoms;
it does not matter if any other bond exists

Example to understand the bonds in long molecules

Intermolecular bondings: long molecules in water



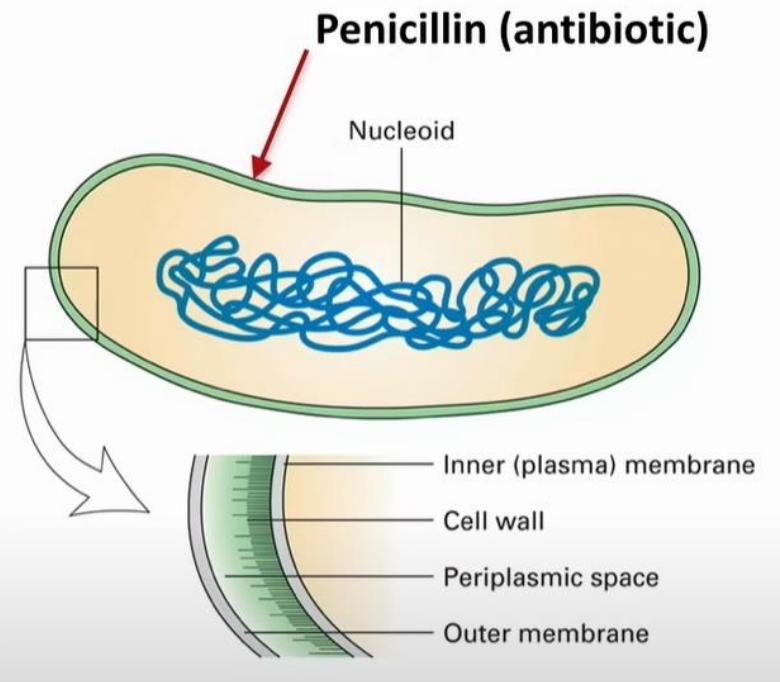
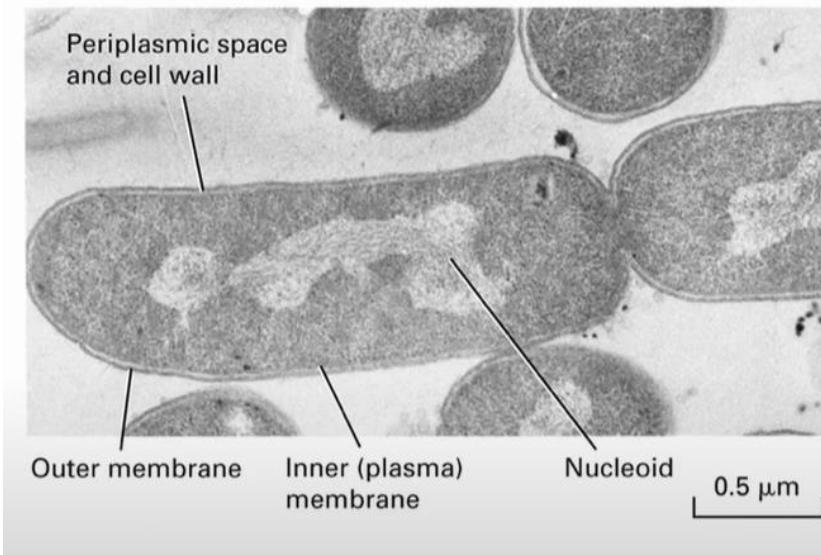
This knowledge will come in handy in understanding lipids, proteins, **membranes**

Are we ready to dive in the cool
concepts of biochemistry?

Almost there!

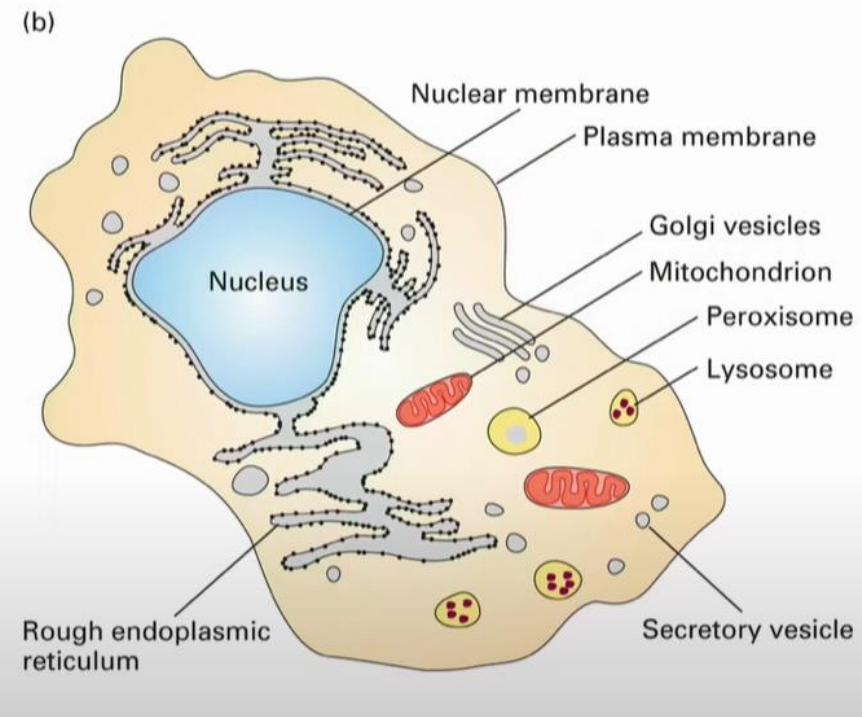
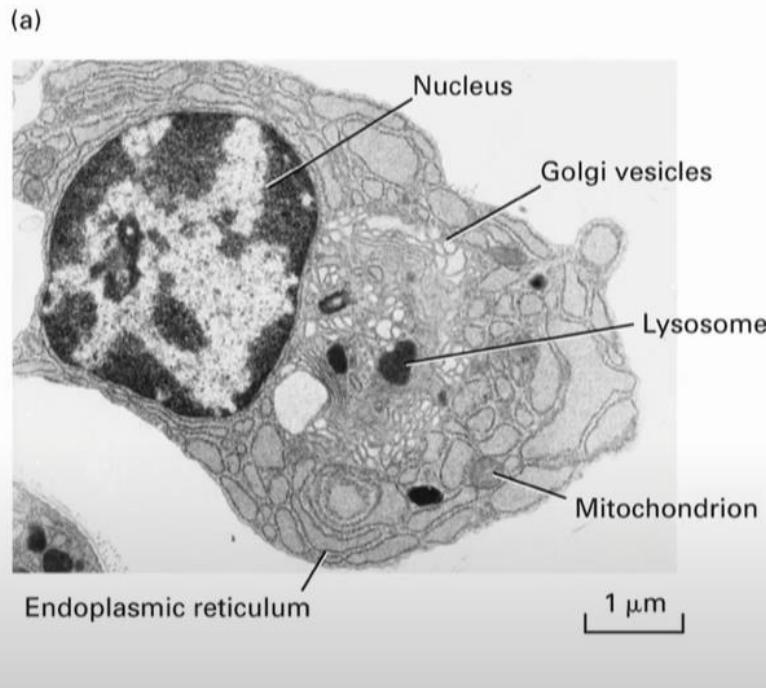
Prokaryotic cells

Prokaryotic cell have a relatively simple organization



Eukaryotic cells

Eukaryotic cells have more complex organization and membrane bound organelles



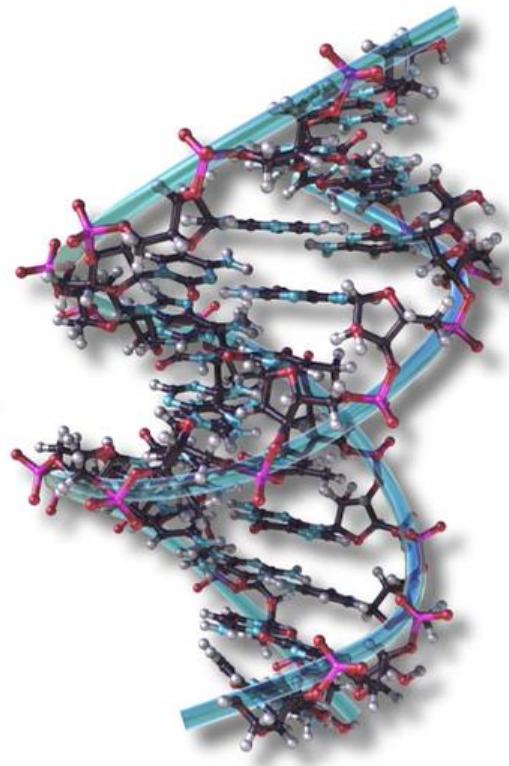
**Membranes (Lipid Bilayers) will be explained by the hydrophobic forces.
How these intermolecular forces are affecting our very core!**

Macromolecules

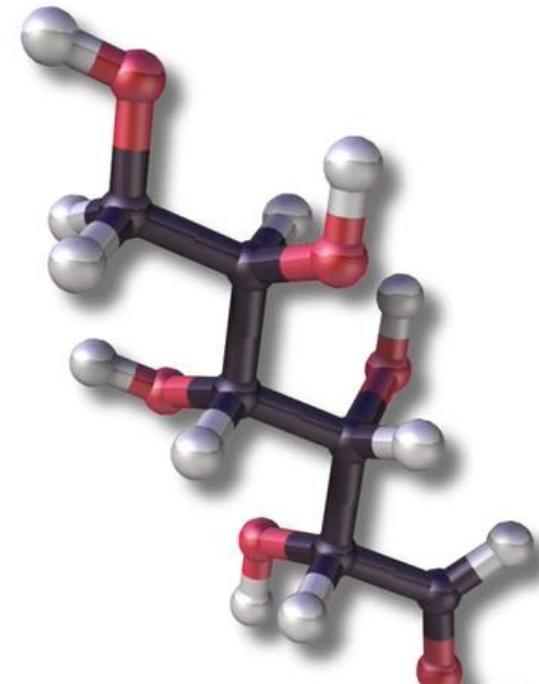
Protein



Nucleic Acid

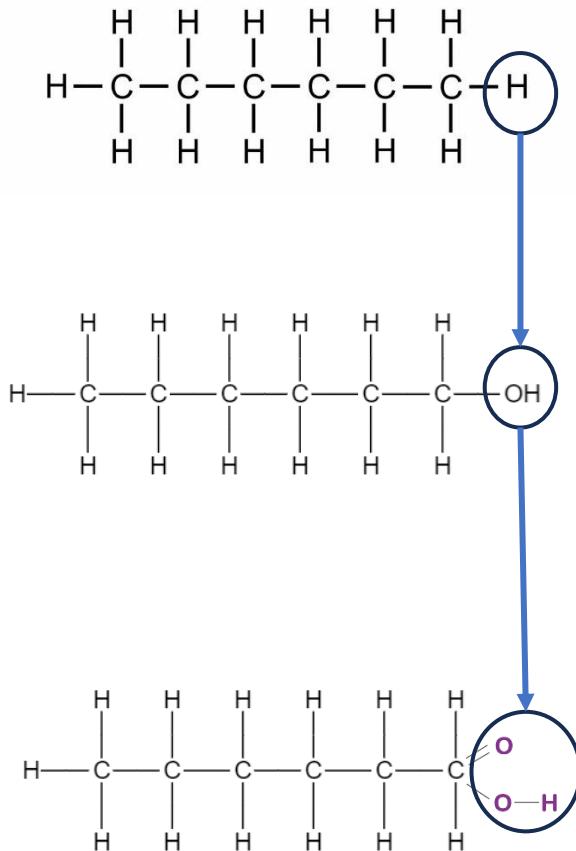


Carbohydrate



Require powerful instruments or experimental tricks
to understand their structure and functions

Let me modify it further



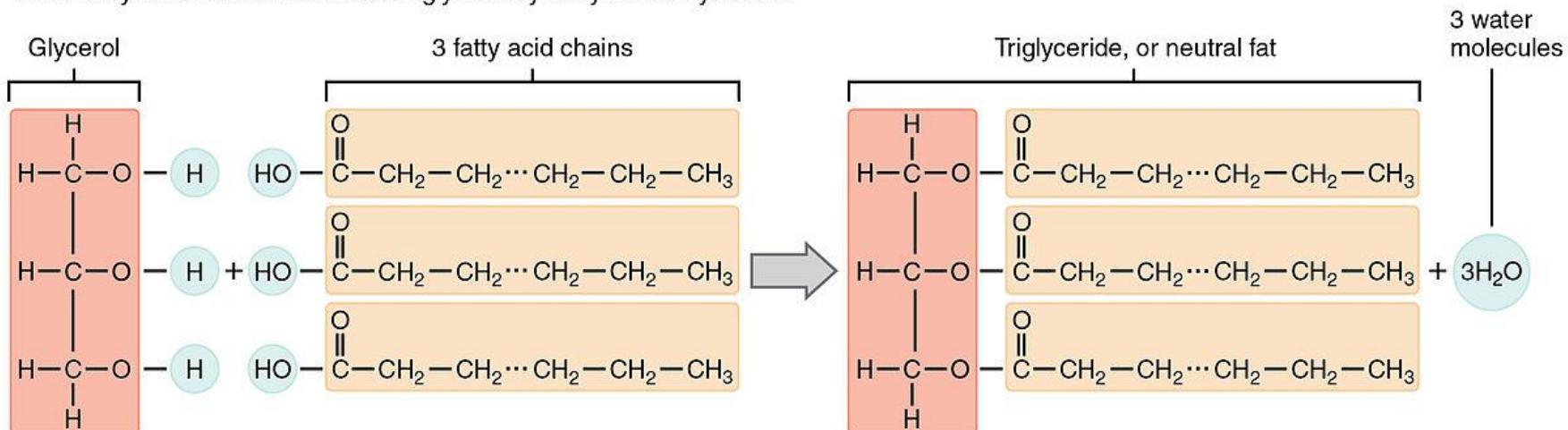
Hydrophobic / Hydrophilic?
Soluble in water or not?

Carboxyl is also hydrophilic
This is nothing but a fatty acid!
Long hydrocarbon chain with carboxyl acid!

Now let's try a chemical reaction

Let's do a reaction!

Three fatty acid chains are bound to glycerol by dehydration synthesis.



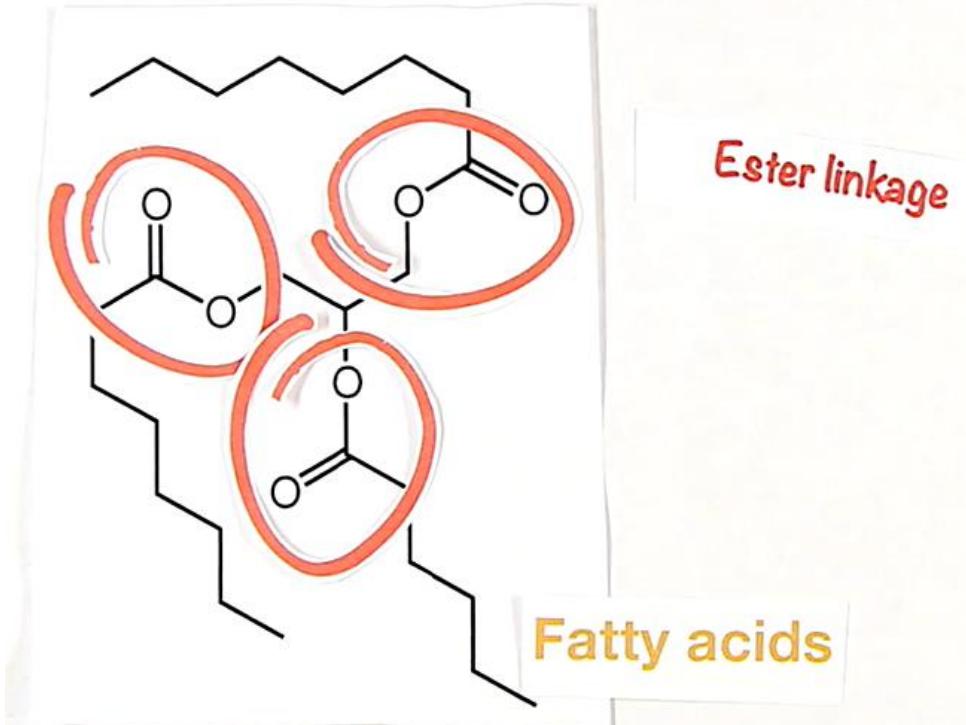
Triglyceride levels are measured by the doctors
Part of the cholesterol and fat studies

Category	Triglyceride Level
Normal	Less than 150mg/dL
Borderline high	150 to 199 mg/dL
High	200 to 499 mg/dL
Very high	500 mg/dL and above

It's a very hydrophobic molecule. What will happen if I put it in water?

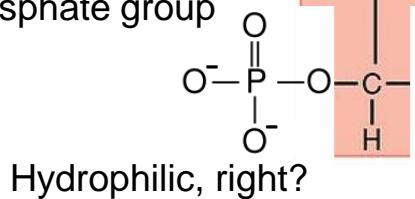
Triglycerides are lipids

Triacylglycerol



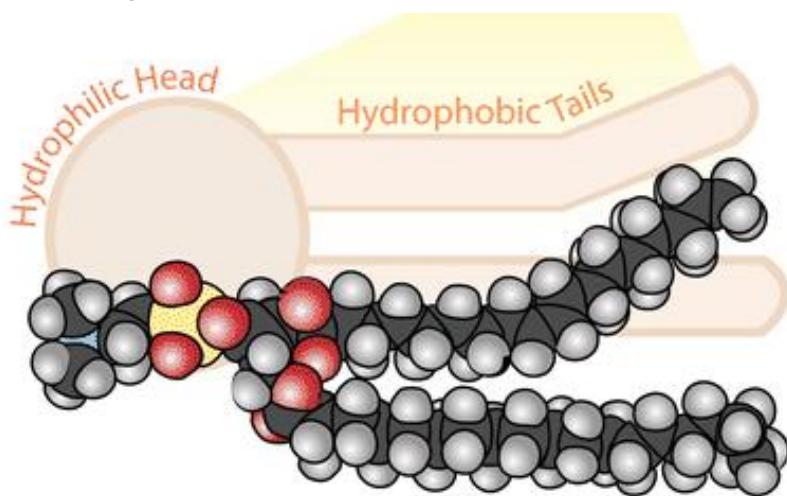
Let's take it further...

Negatively charged phosphate group



Hydrophilic, right?

But long hydrocarbon chains,
so hydrophobic – right?



Both?

Hydrophobic or Hydrophilic?

This is a confused molecule!

Amphipathic molecule!!

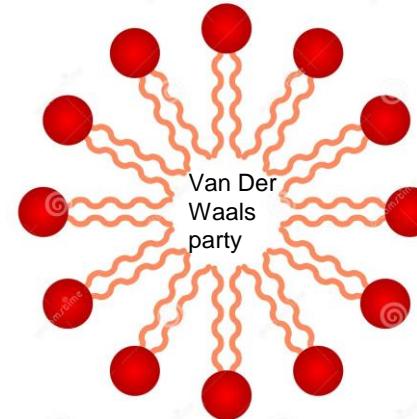
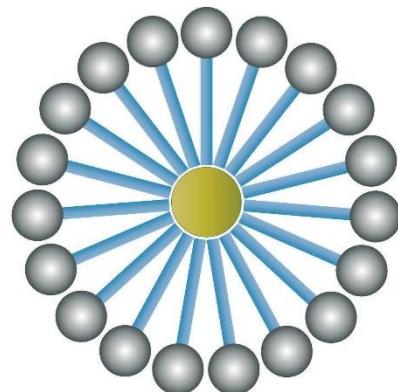
What happens if I put this confused molecule in water?

Amphipathic “confused” molecule

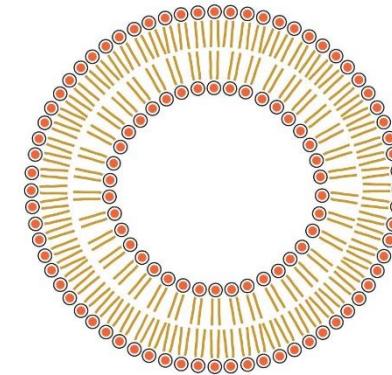
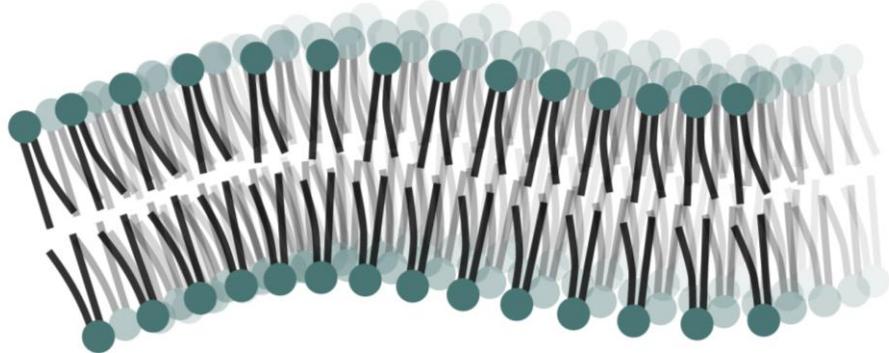
Micelle

Polar Part
Hydrophilic part

Non Polar Part
Hydrophobic Part



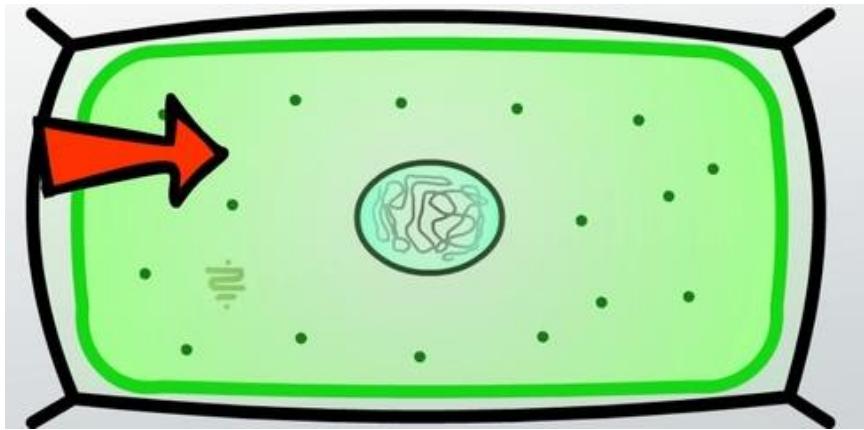
Lipid Bilayers



Supramolecular self-assembly

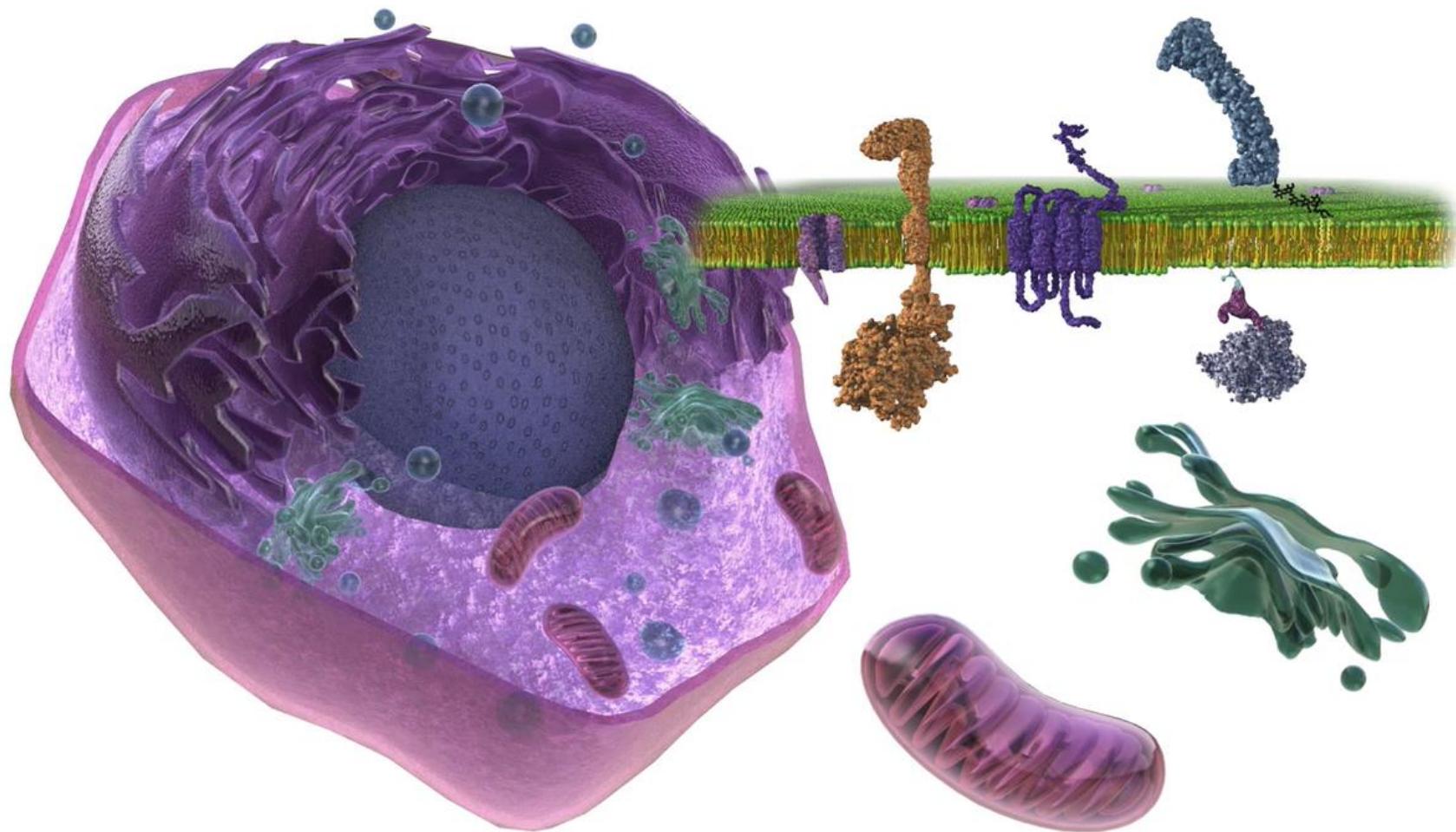
Cell “~~magical~~” membranes

- Bag or a sack that separates the inside from the outside
- Distinctive critical thing about life
- Its integrity is significant in preventing cancer



How do these magical membranes are formed?
Chemistry can explain that!

Lipid bilayers essential as membranes!



**Membranes (Lipid Bilayers) explained by the hydrophobic forces.
How these intermolecular forces are affecting our very core!**

Deep dive into lipids and how we encounter it in daily life!

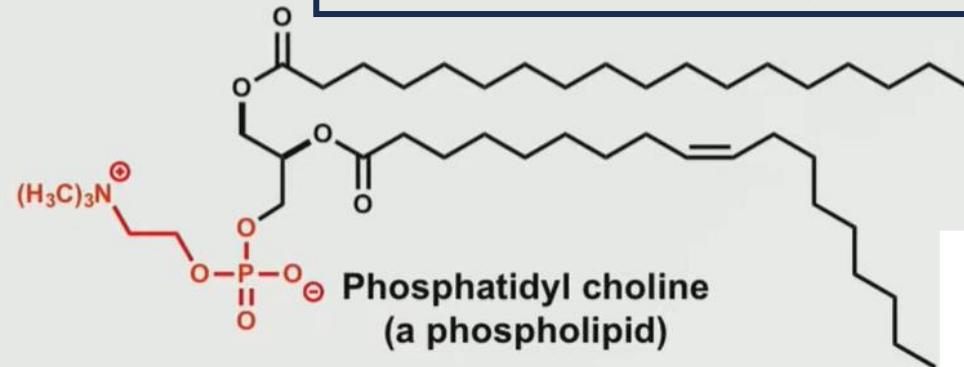
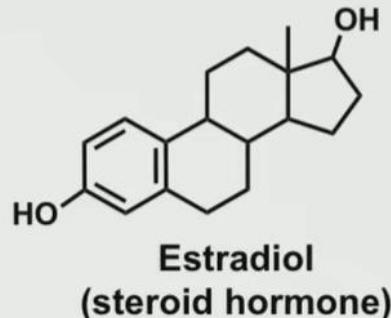
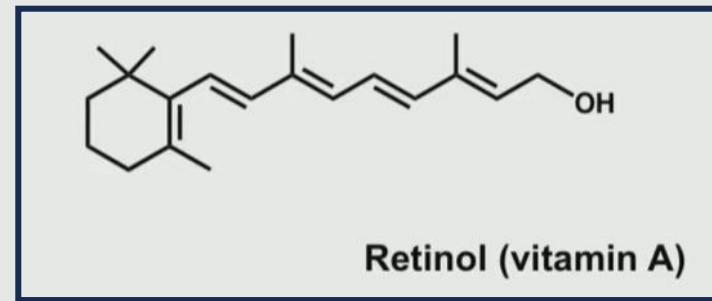
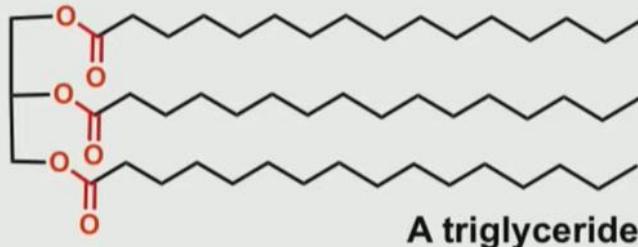
Lipids

Lipids – Diverse structures rich in C-C and C-H bonds.

Most are: “Hydrophobic”

Some are: “amphipathic” (BOTH hydrophilic and hydrophobic components)

e.g. Fats (triglycerides), steroids, some vitamins (A, D, E), phospholipids,
Involved in: Energy storage, thermal and electrical insulation, cellular signaling,
cellular membranes.

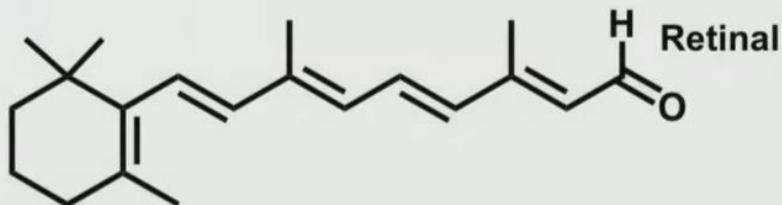


Essential lipids in vision and sight



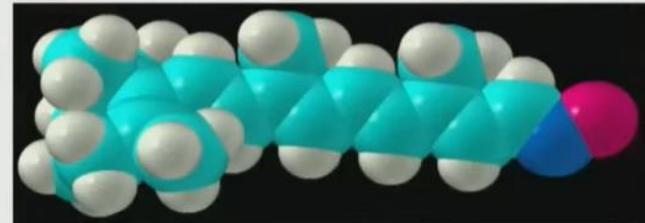
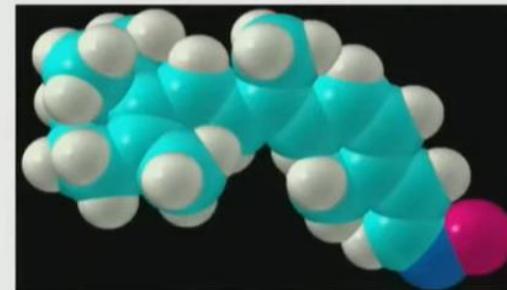
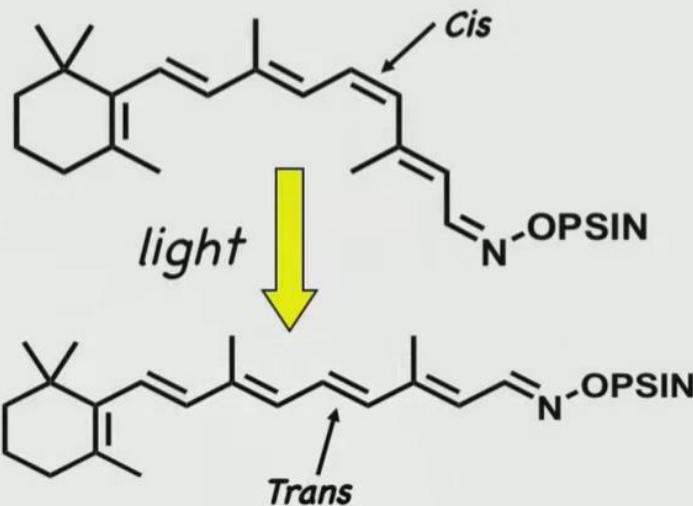
Lipids and vision

Vitamin A (RETINOL) - Precursor to the visual pigment RETINAL



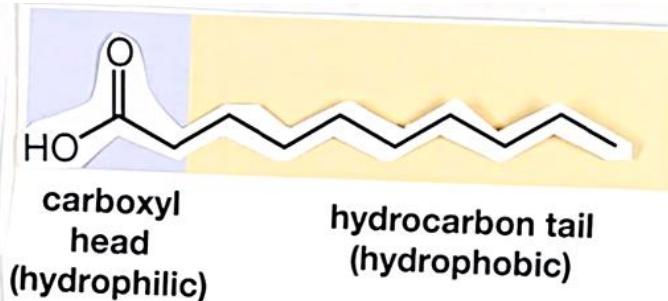
retinal (lipid) + opsin (protein) = rhodopsin

opsin



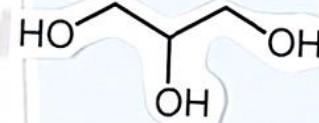
Building blocks of lipids

Fatty acid



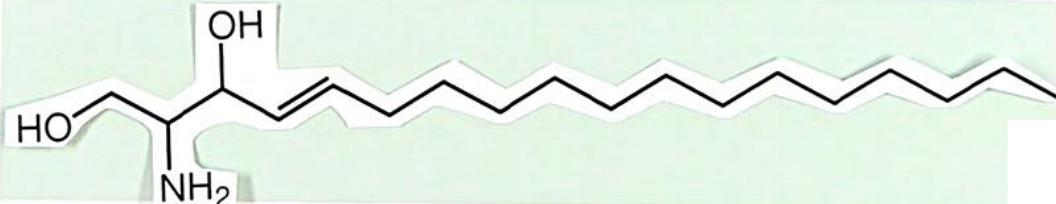
Lipid tails

Glycerol



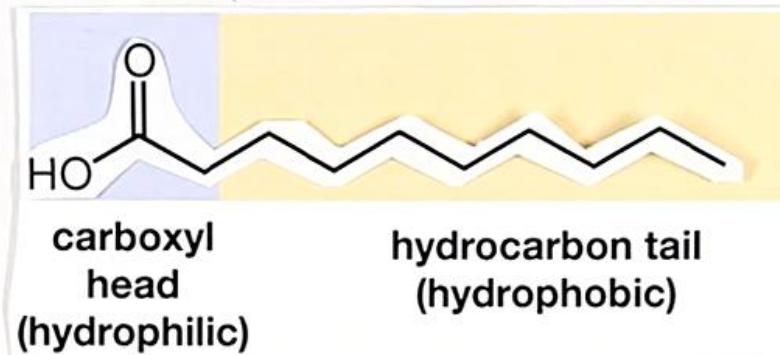
Alcohols are backbones

Sphingosine

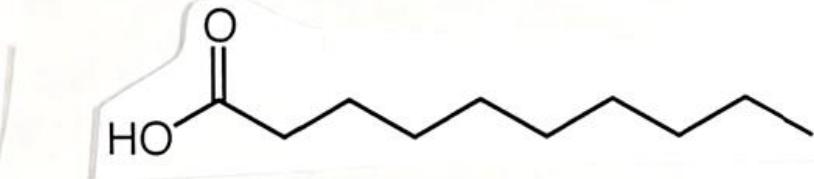
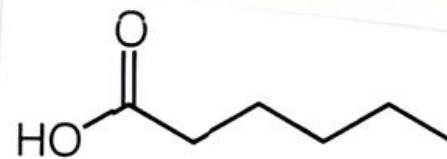


Fatty acids

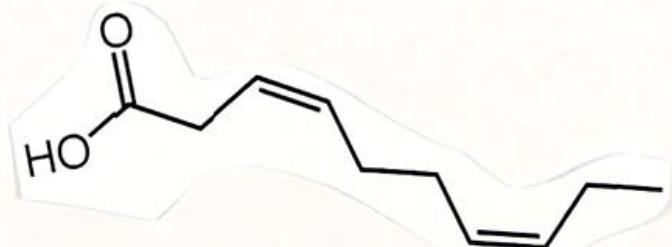
Fatty acid



saturated

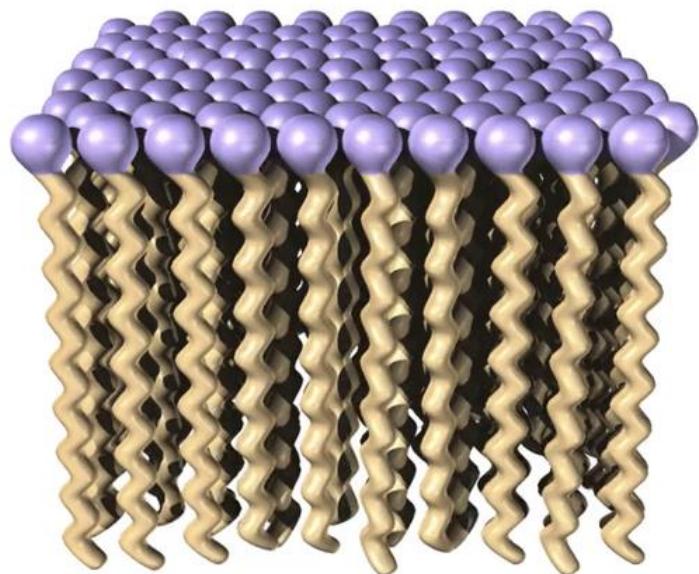


unsaturated



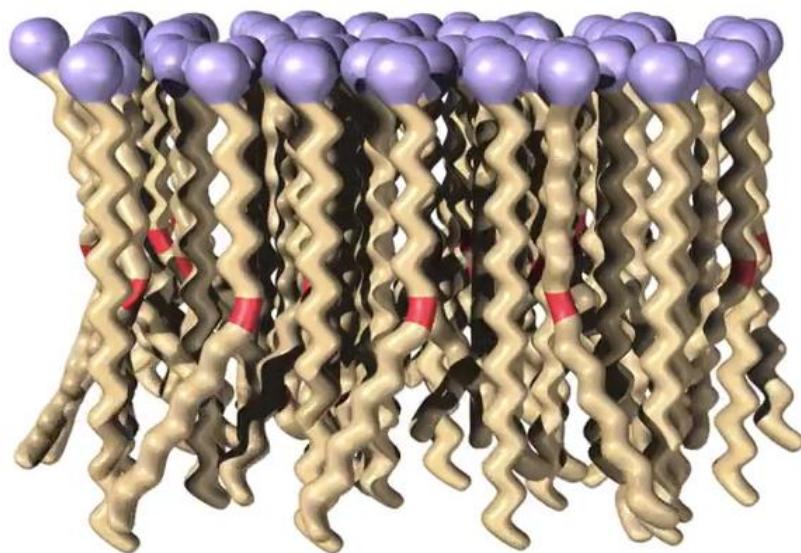
Fatty acids

Saturated fatty acids



Strong Van der Waals interactions

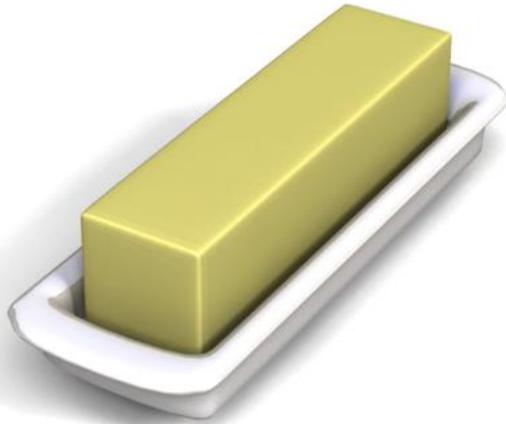
Mixture of saturated and unsaturated fatty acids



Weak Van der Waals interactions

Fatty acids

Saturated fatty acids



Increases the risk of heart disease, LDLs

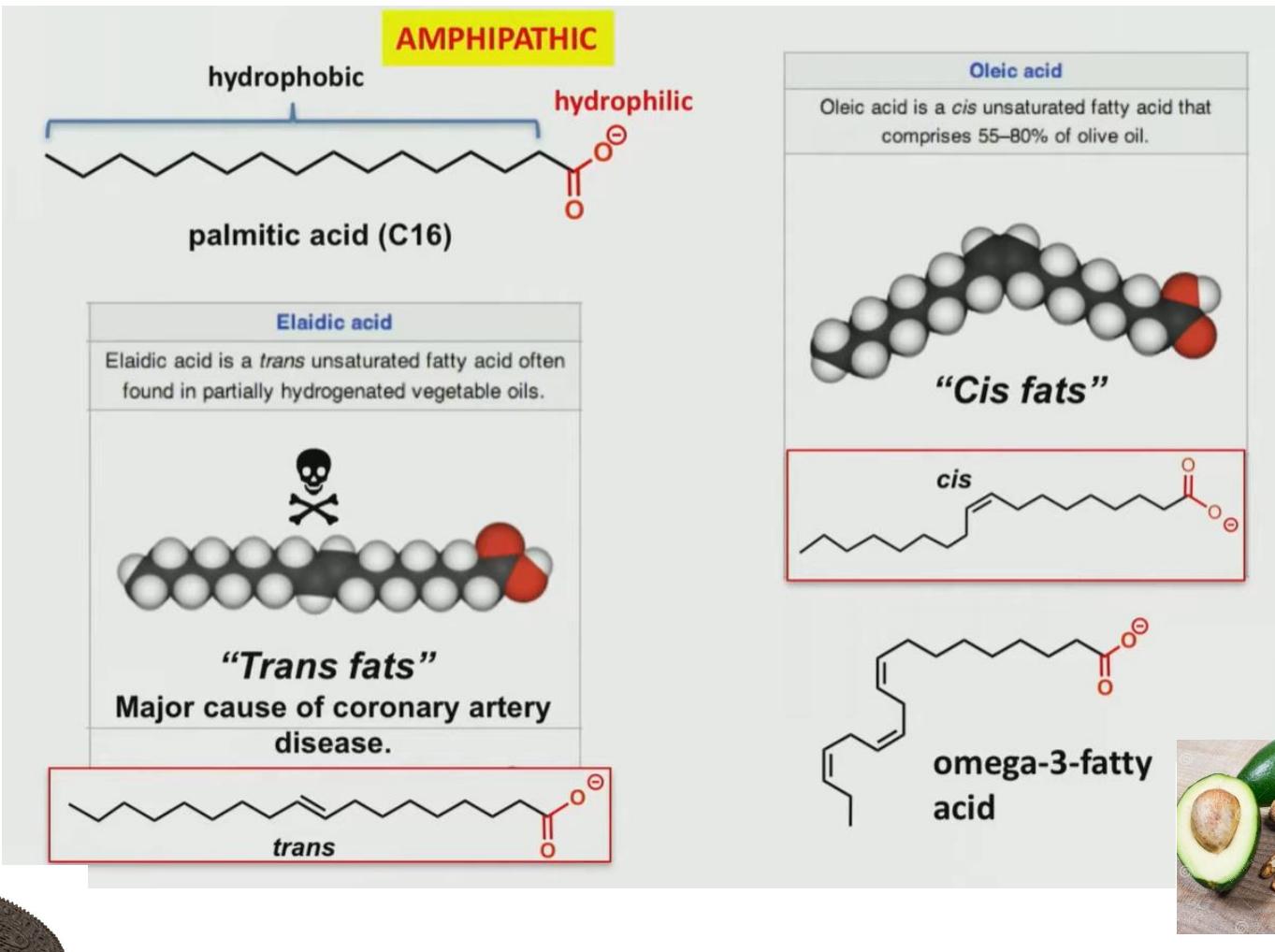
Mixture of saturated and unsaturated fatty acids



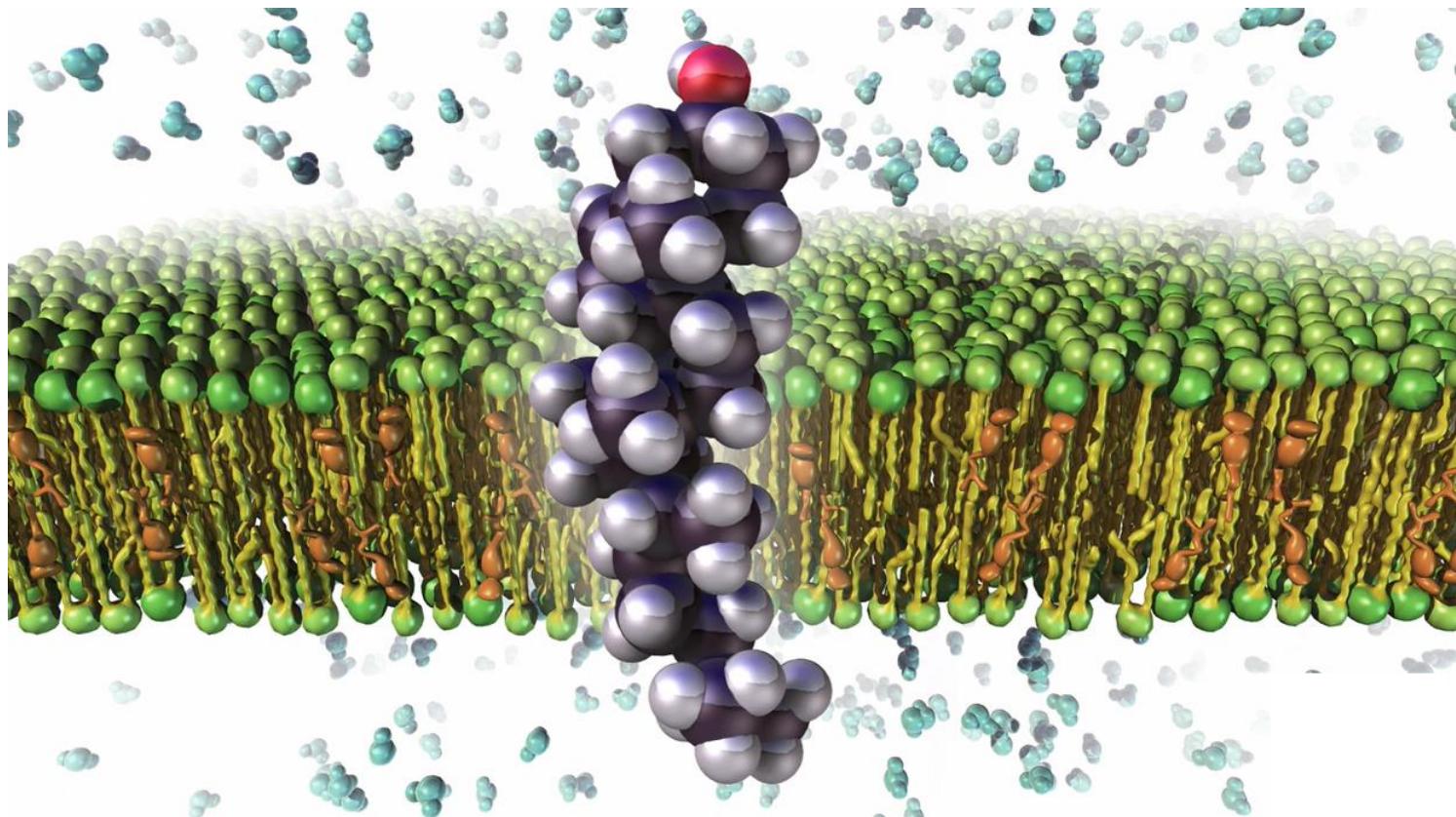
cis and trans fats



Fatty acids



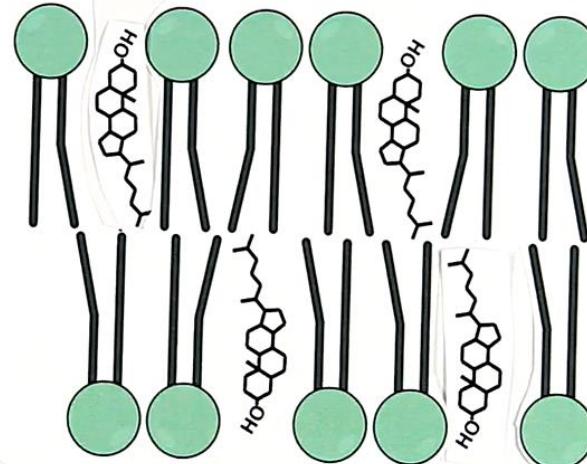
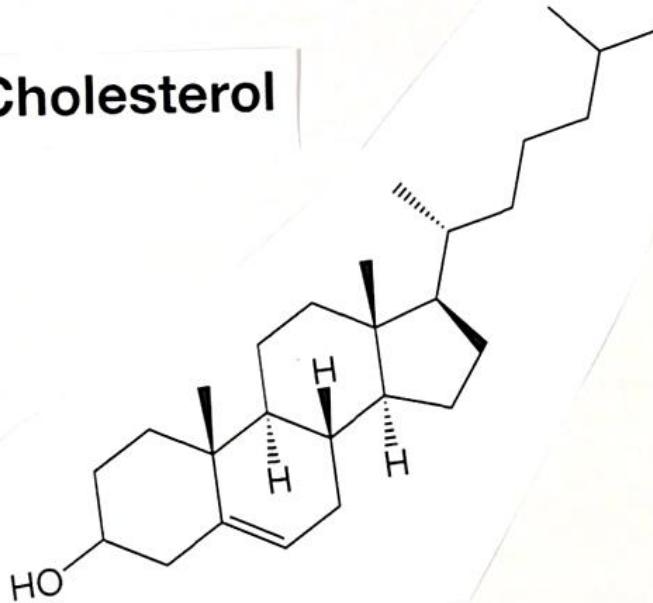
One more lipid everyone has heard of!



Cholesterol

One more lipid everyone has heard of!

Cholesterol

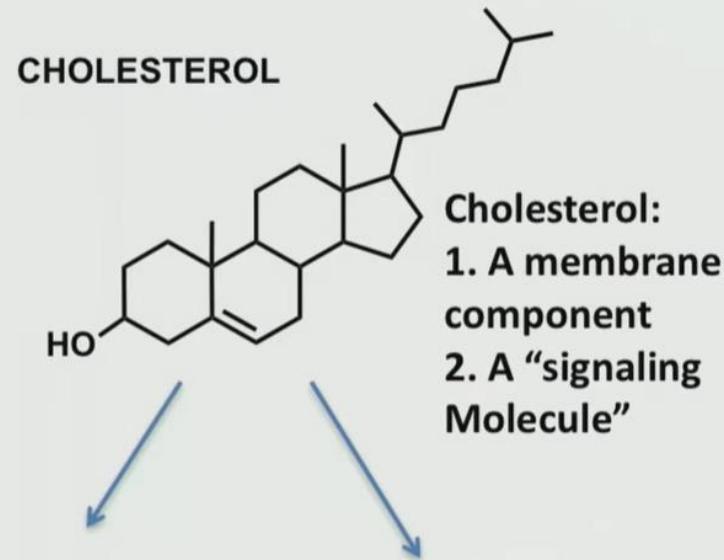
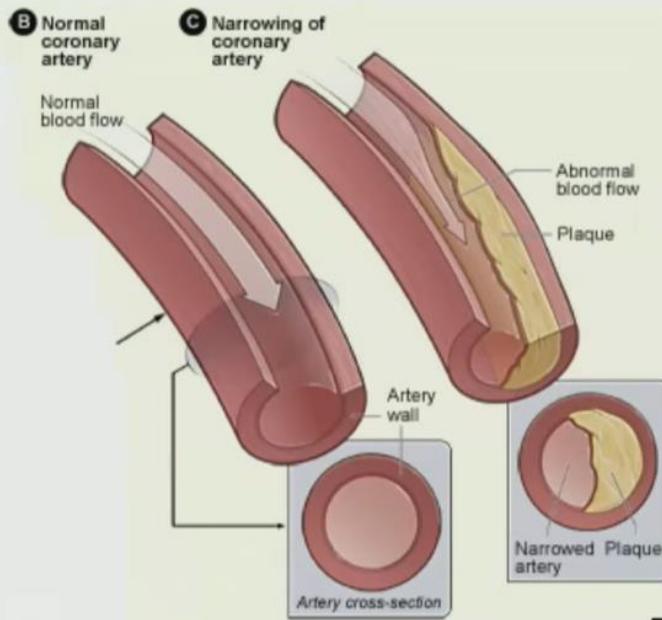


- Very hydrophobic
- Critical components in the membrane
- Needs to be moved around to different organs where it is needed, as lipoprotein
- Play vital roles in various physiological processes, including forming cell membranes and serving as a precursor for the synthesis of hormones, vitamin D, and bile acids

Cholesterol in our daily lives

Fatty acids, cholesterol levels and heart disease

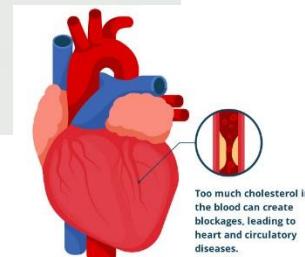
Saturated and trans fats are associated with coronary artery disease



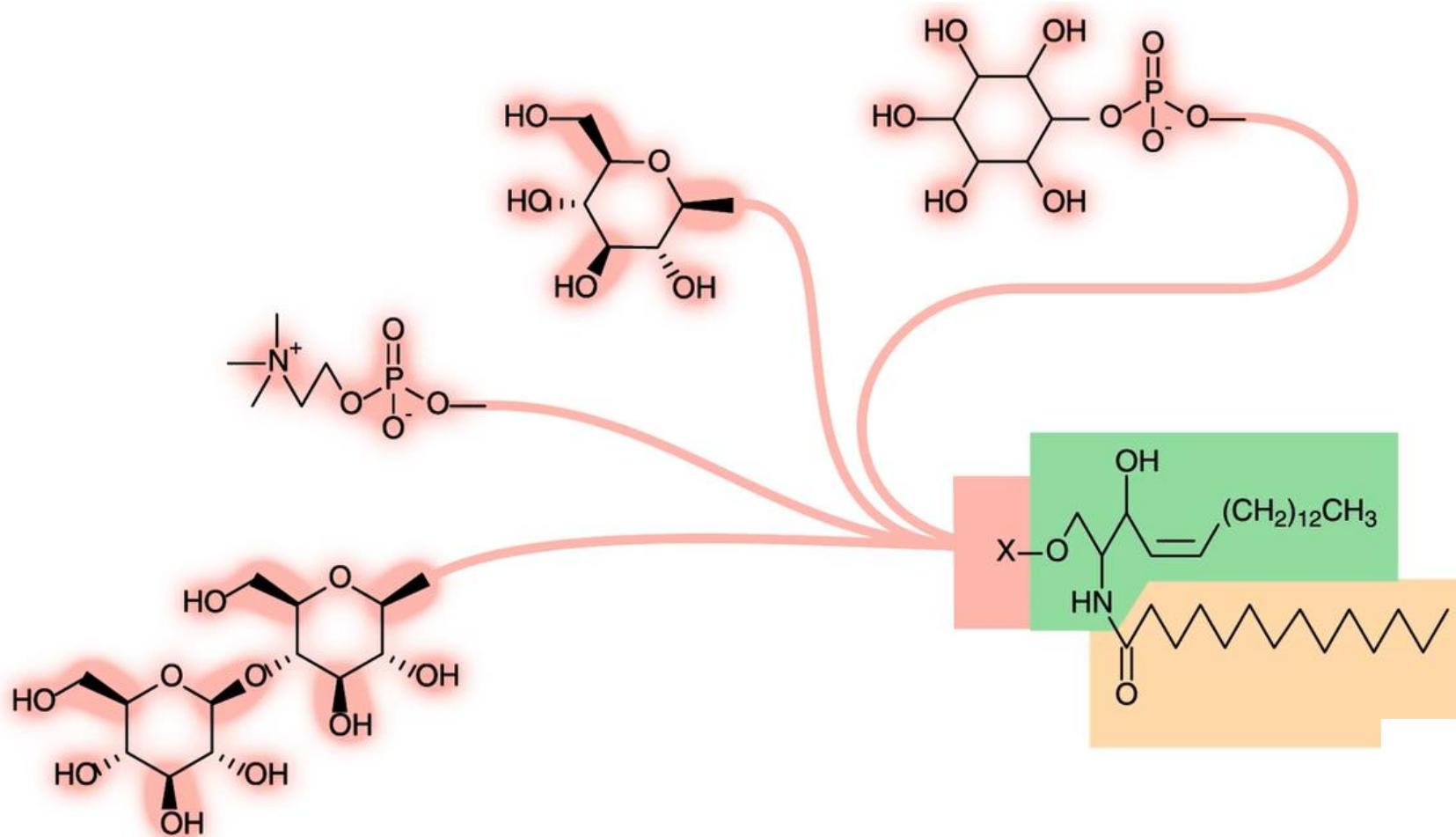
Binds low density lipoprotein (LDL):
Deposited in arteries → clogged arteries → heart attacks

Binds high density lipoprotein (HDL) and excreted by liver

Saturated and trans fats increase LDL

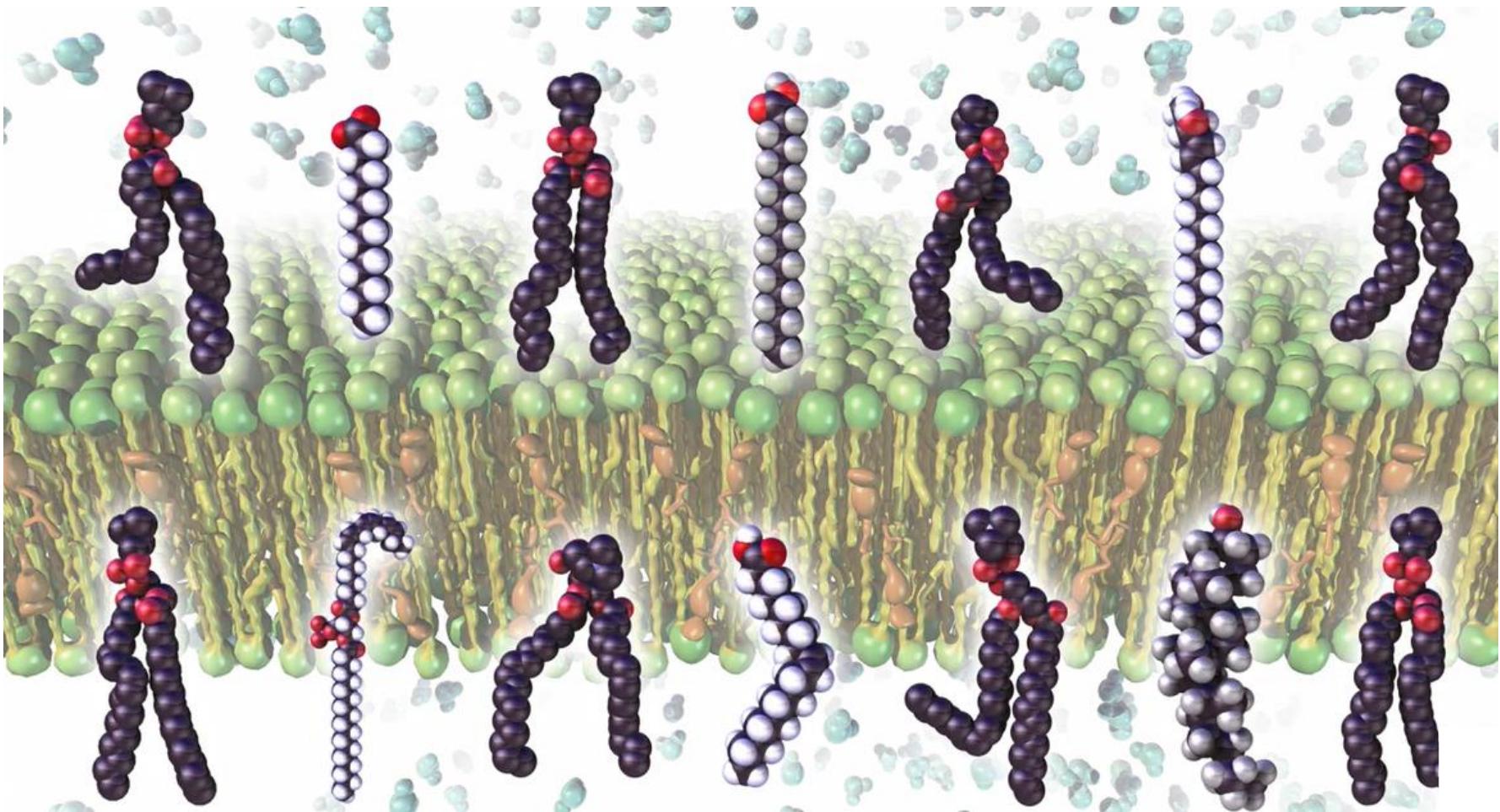


Diversity in lipids



Diversity based on the head groups, functional groups and compositions

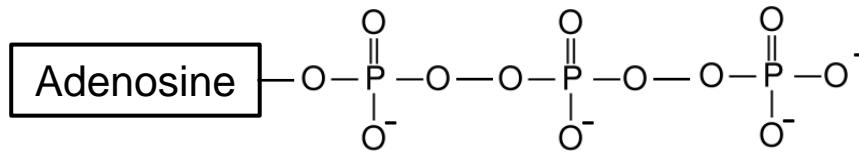
Lipids: essential part of cells



1000 different species of lipids in our cells

High energy molecules with phosphates

High energy molecules: ATP



What do negatives do?

Repel each other!

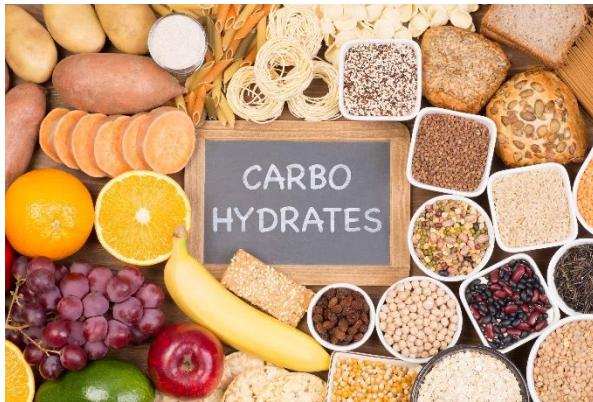
It's a great way to store energy!

If these bonds break,
it releases energy.

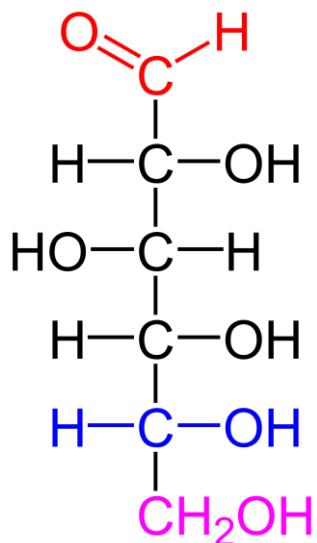


When cells need energy,
it resorts to **Adenosine Tri Phosphate (ATP)**

Carbohydrates: Glucose



Let's talk about carbohydrates



Glucose: a simple sugar

Every carbon except one carbon has –OH

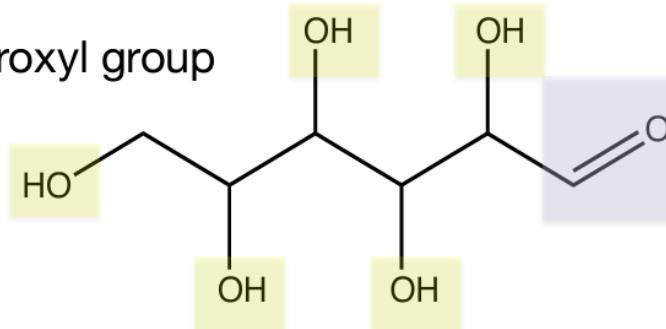
(C. H₂O)₆ or C₆H₁₂O₆

(C. H₂O) named as carbo hydrate

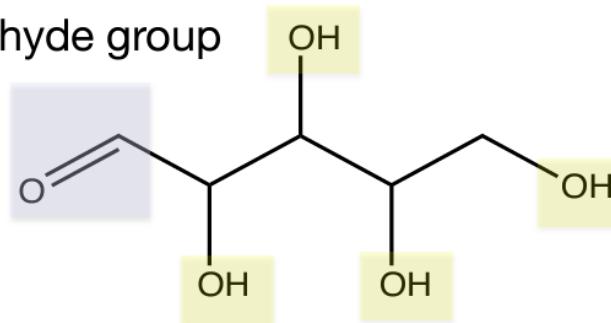
Carbohydrates- richness of functional groups

Aldoses

Hydroxyl group



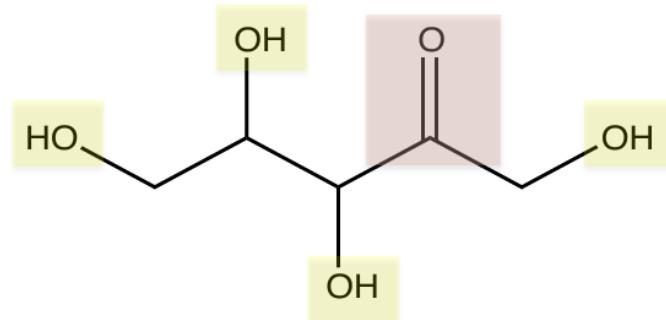
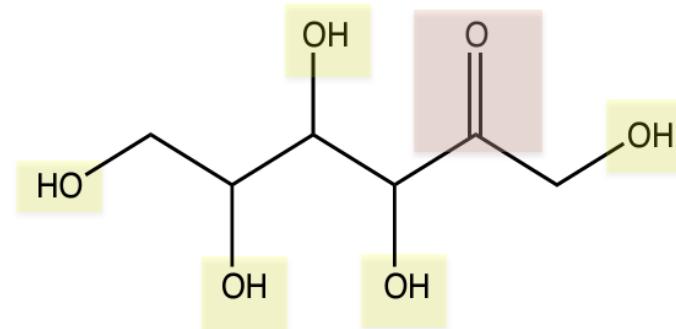
Aldehyde group



-ose

Ketoses

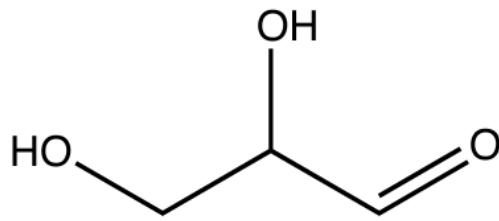
Ketone group



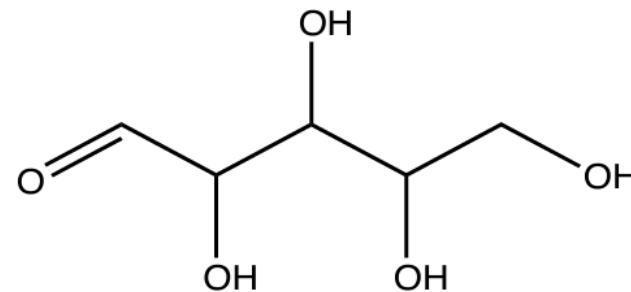
-ulose

Rich in hydroxyls: Polar or non-polar?

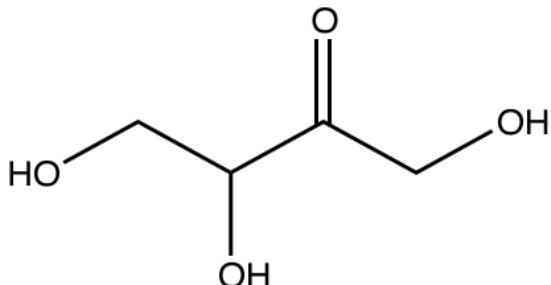
Carbohydrates



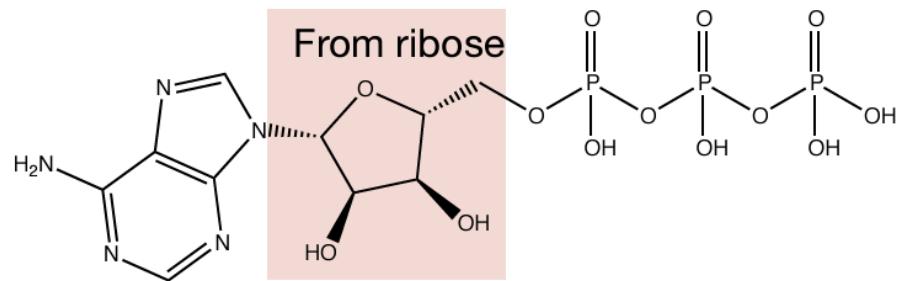
triose



pentose



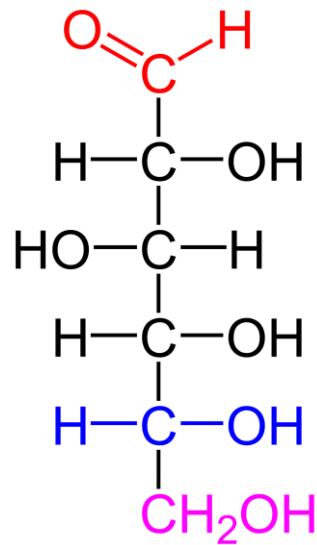
tetrose



Adenosine triphosphate (a ribonucleotide)

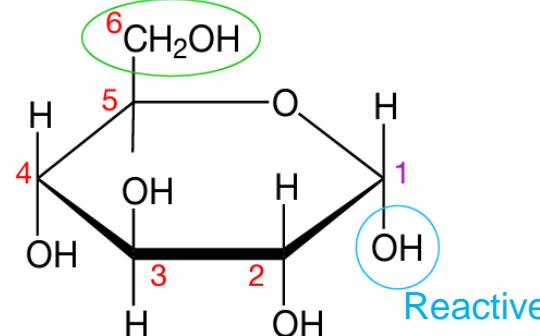
Metabolic intermediates

Monosaccharides



It spontaneously makes itself into nice little chair

Glucose is usually not found in linear chains

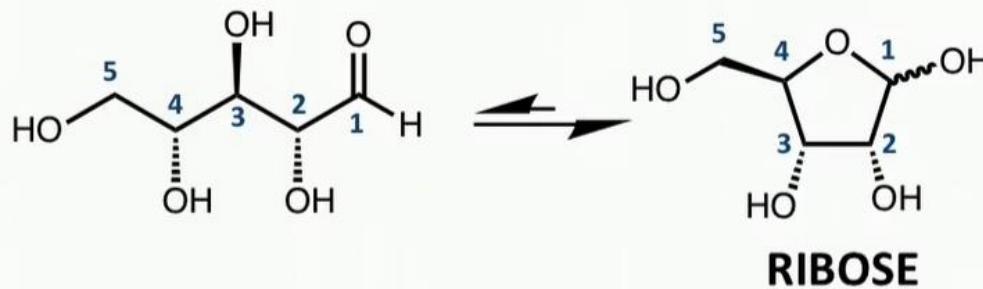
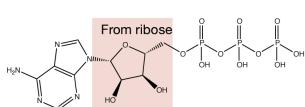


Monosaccharide

Monosaccharides

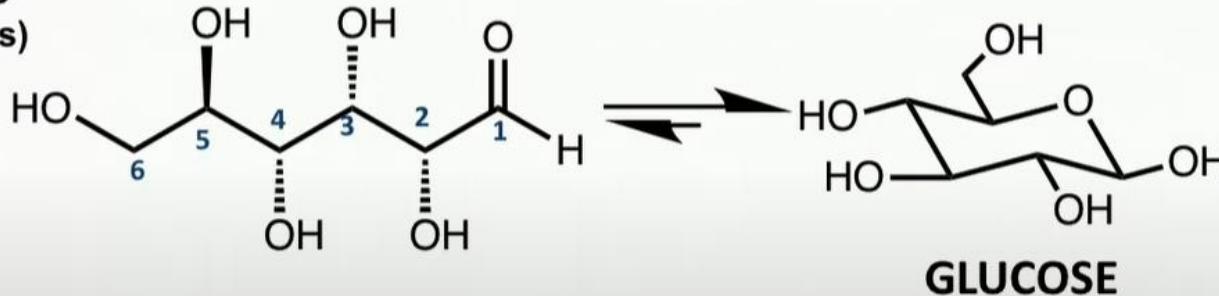
MONOSACCHARIDES

PENTOSES (5 carbons)



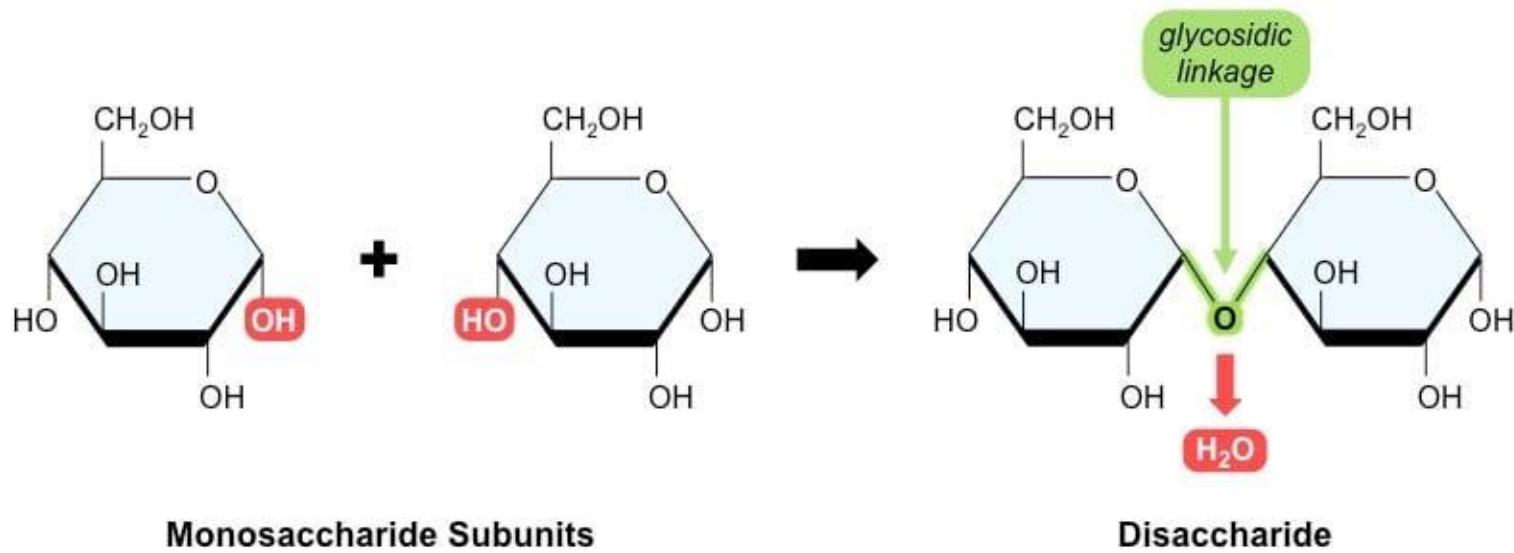
RIBOSE

HEXOSES (6 carbons)



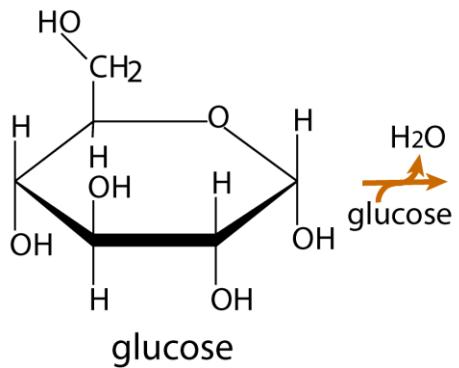
GLUCOSE

Highly reactive monosaccharides

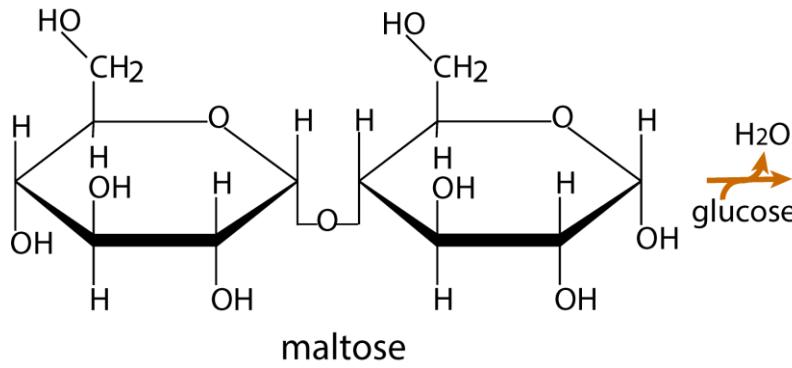


Highly reactive monosaccharides

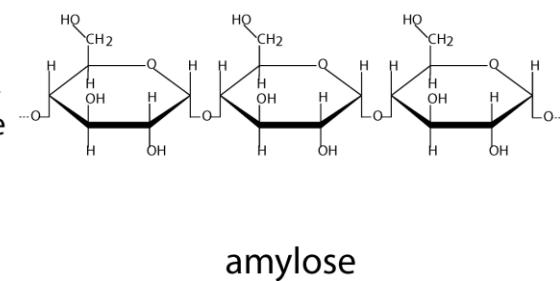
monosaccharide



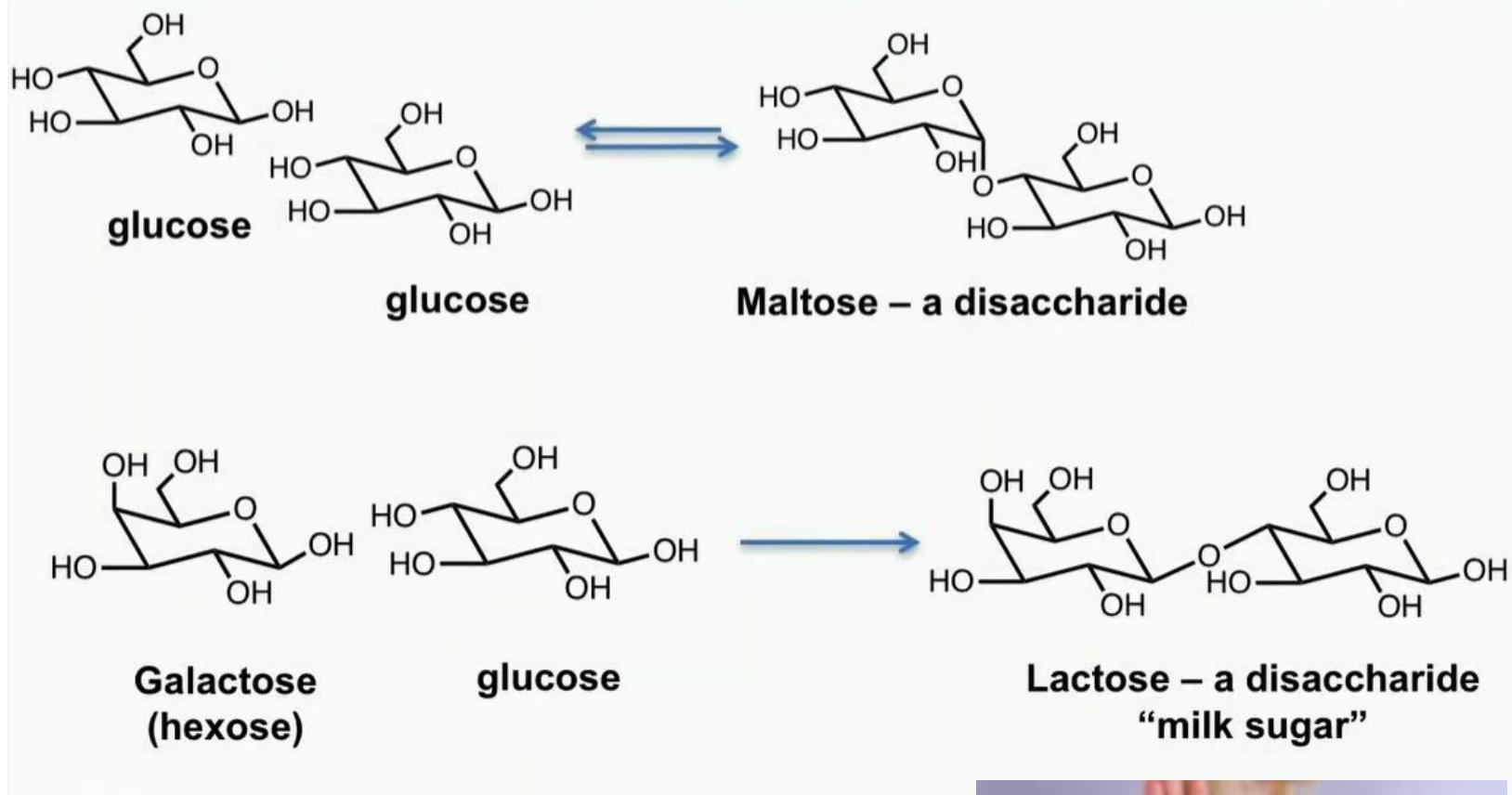
disaccharide



polysaccharide



Highly reactive monosaccharides

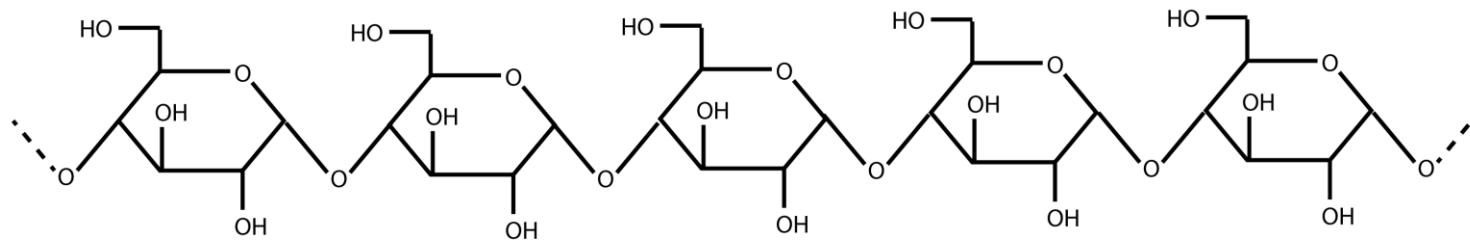


Cause for lactose intolerance
“Lactase” does not work anymore



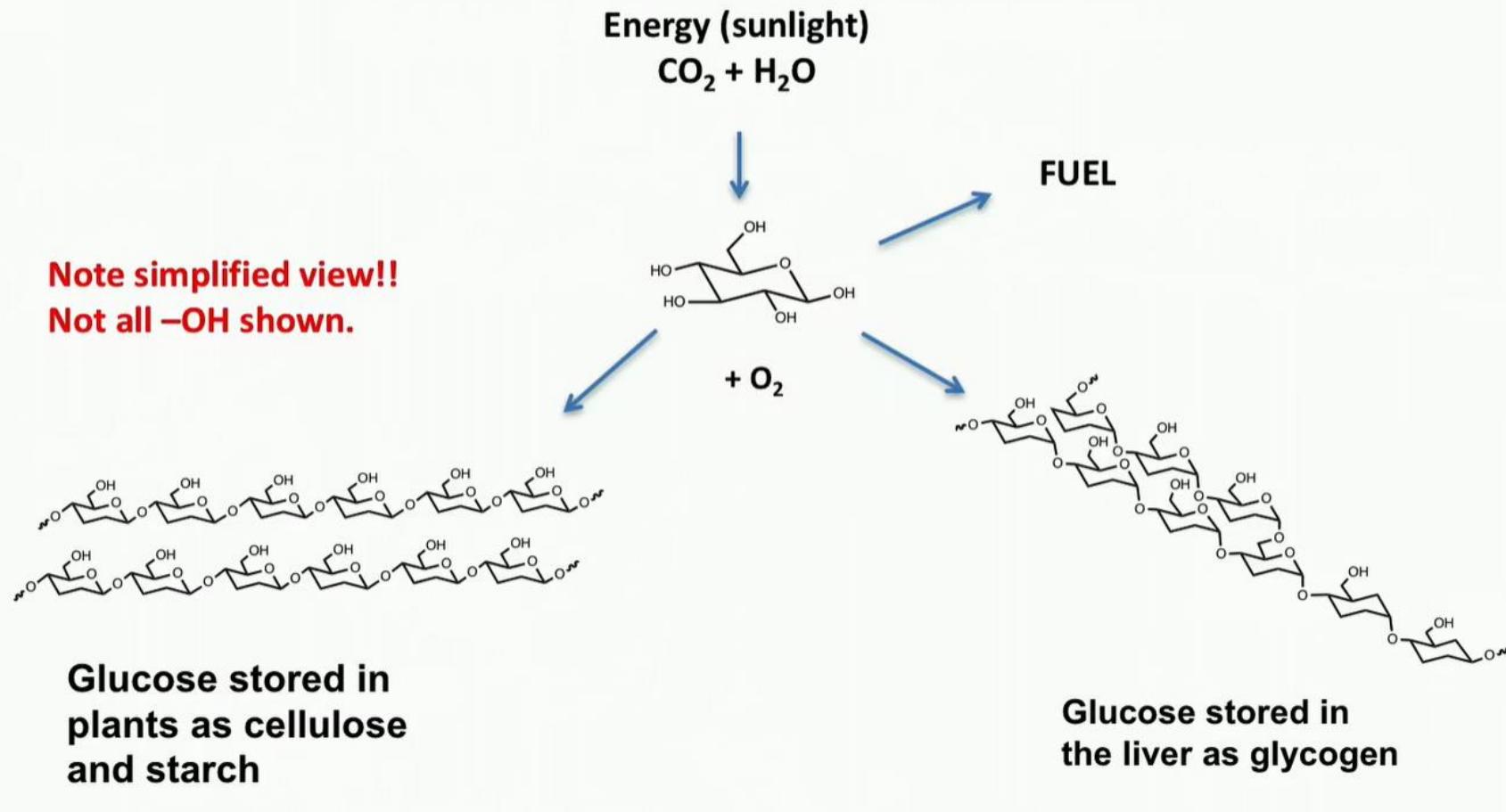
Highly reactive monosaccharides

Starch



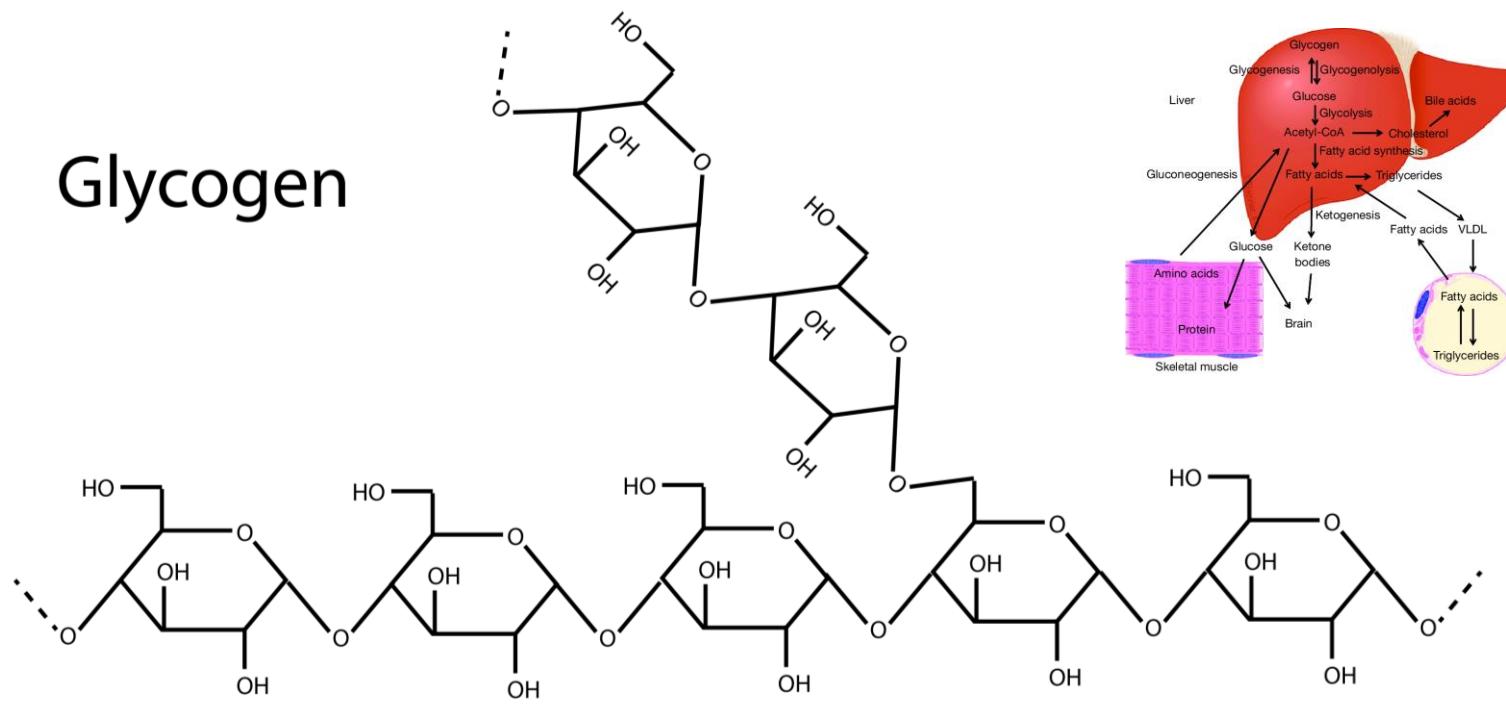
Highly reactive monosaccharides

Energy and energy storage – e.g. glucose (grape sugar, dextrose)



Glycogen: store energy in liver

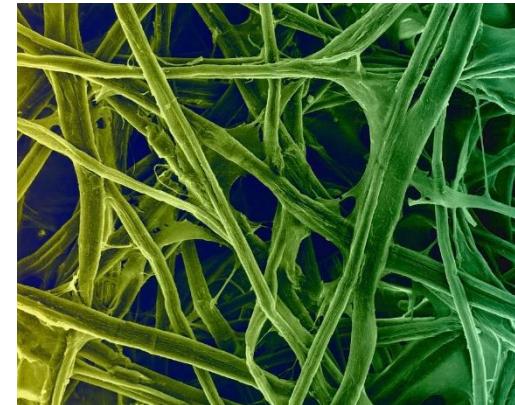
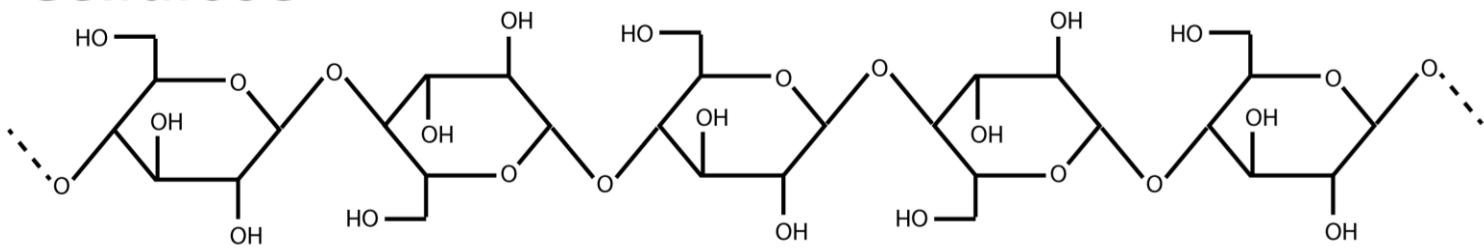
Glycogen



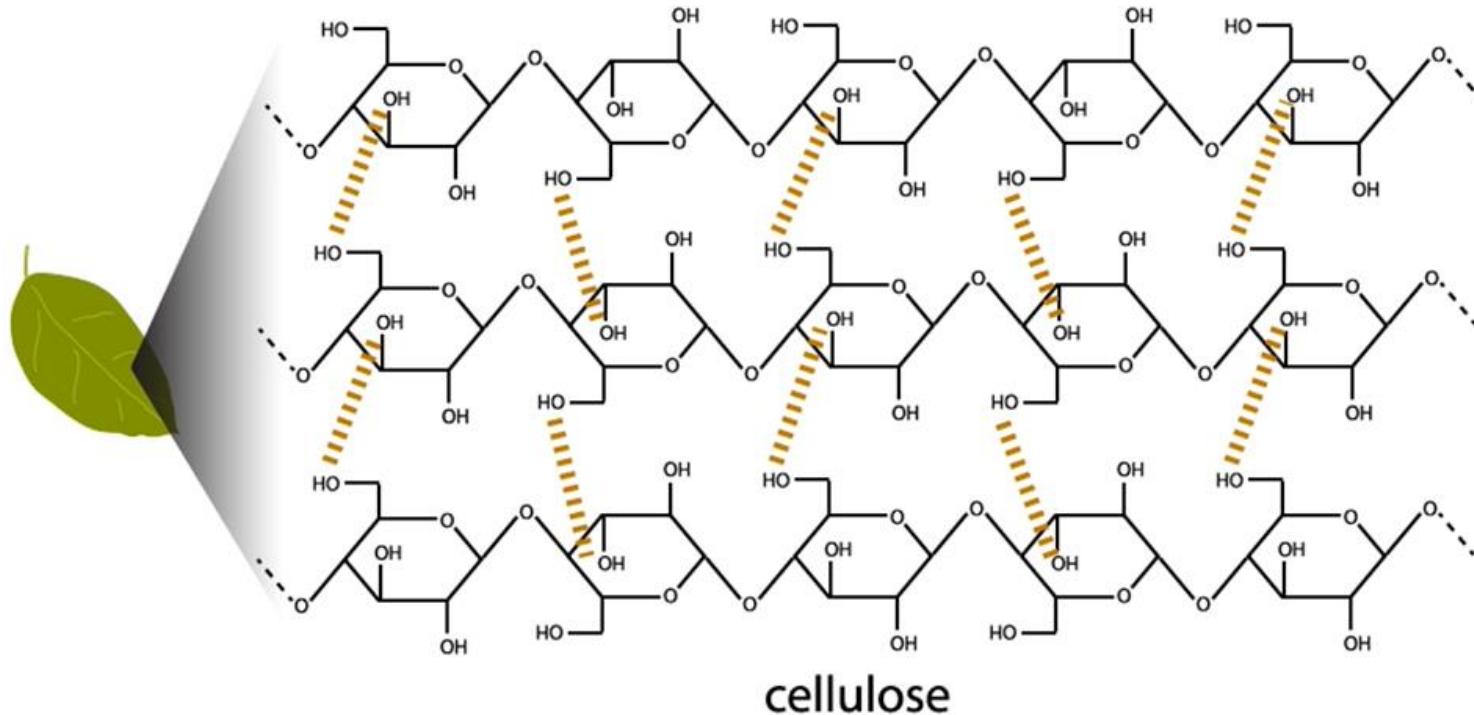
Store sugars in non-rigid ways in the liver

Cellulose

Cellulose



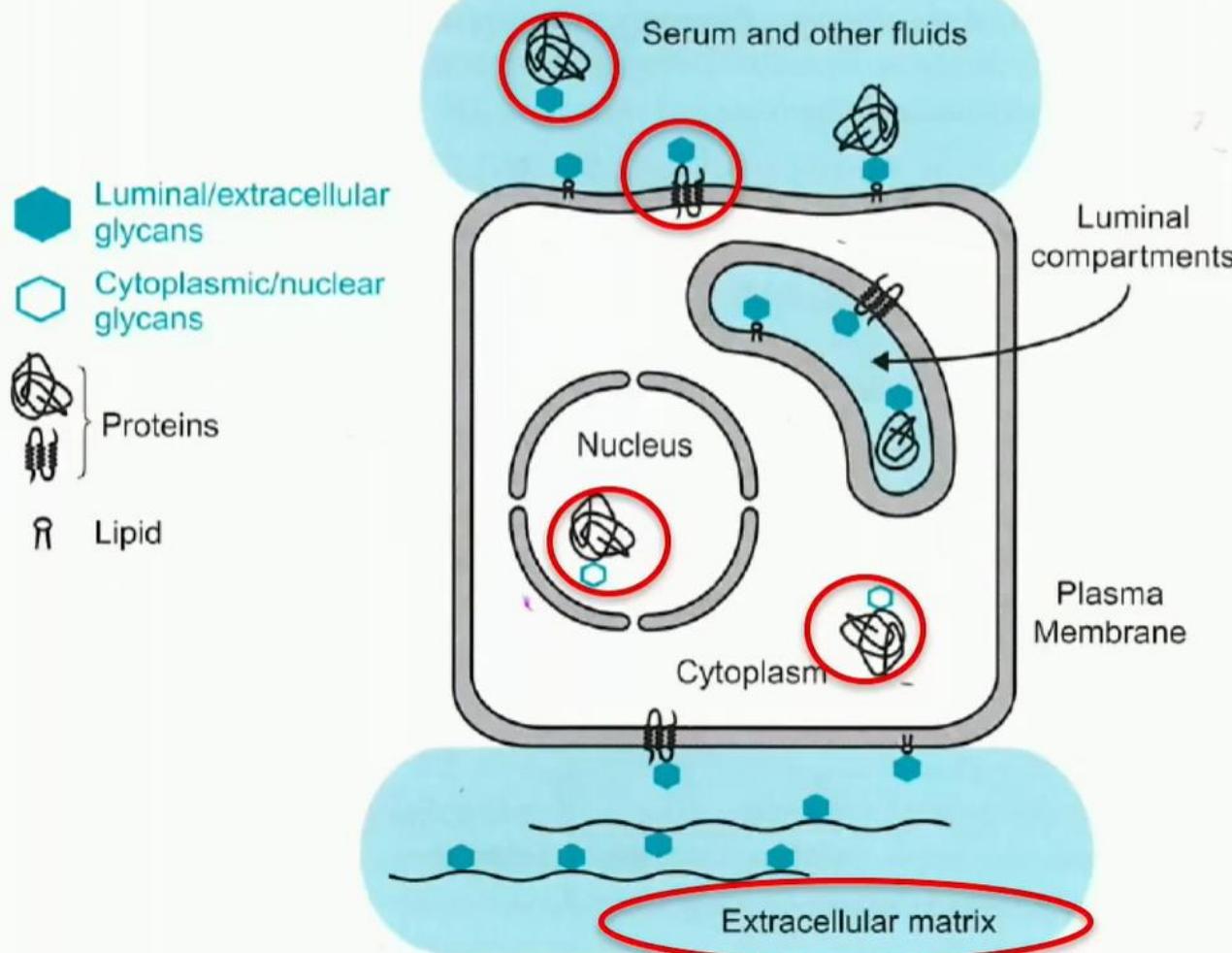
What if they line up perfectly?



Cellulose that holds up trees



Carbohydrates in a cell!



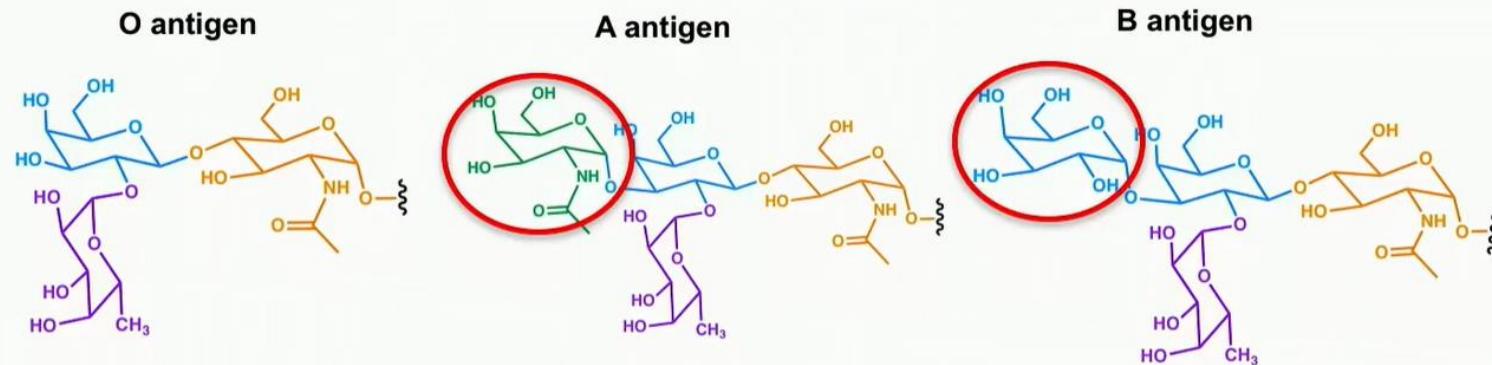
Good sugars and bad sugars



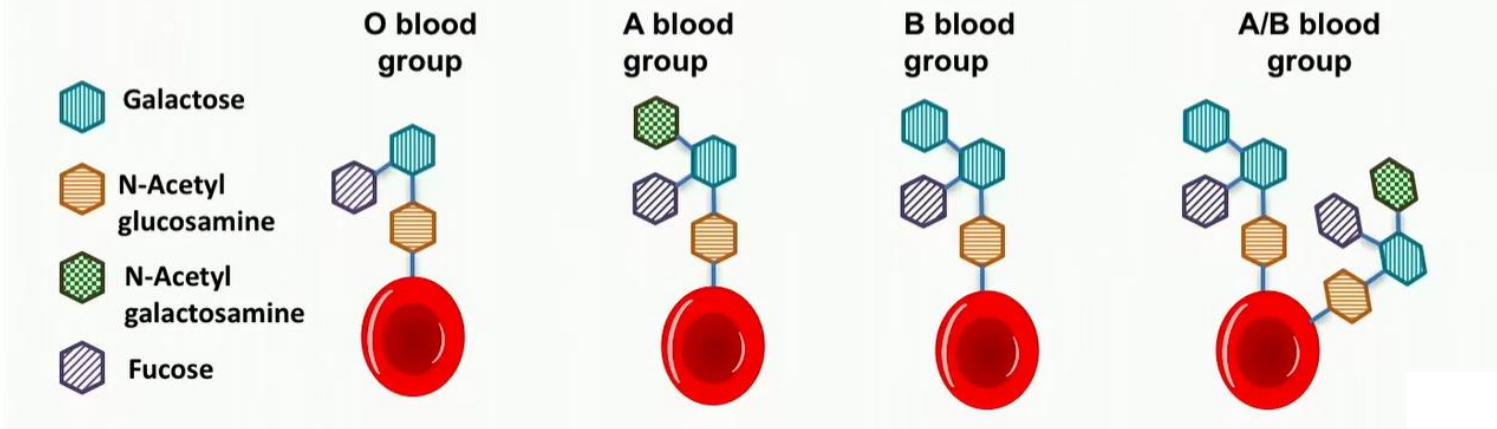
In moderation

Sugars and blood groups

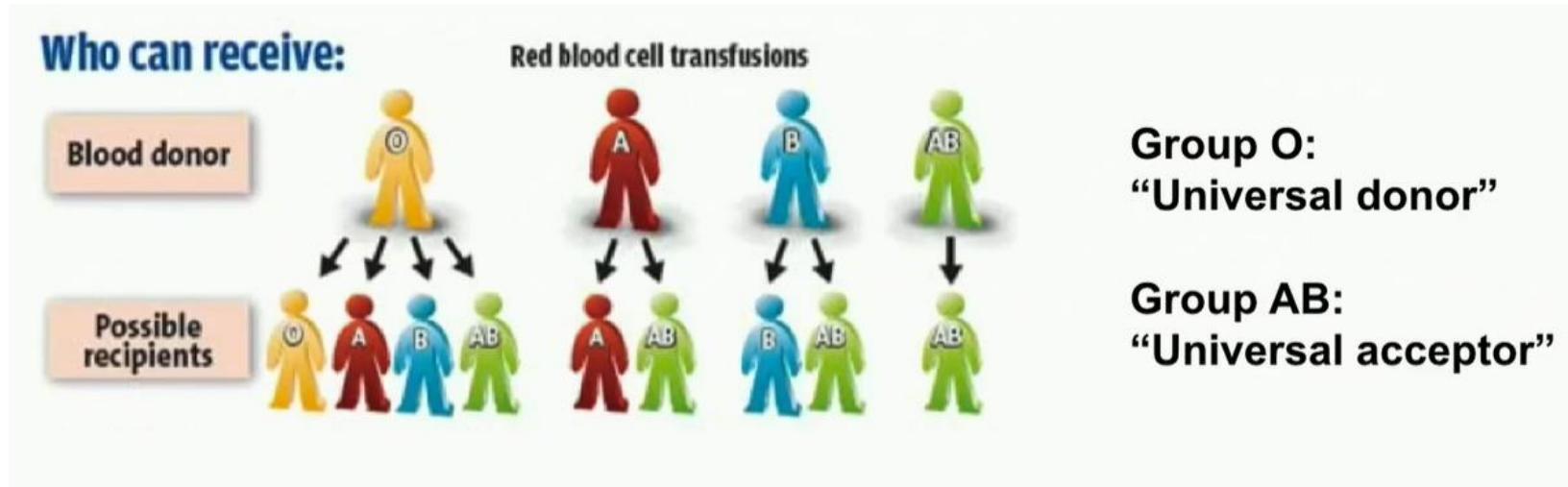
Functions of carbohydrates (saccharides, sugars)



ABO Blood group antigens are cell surface glycoconjugates (glycolipids)

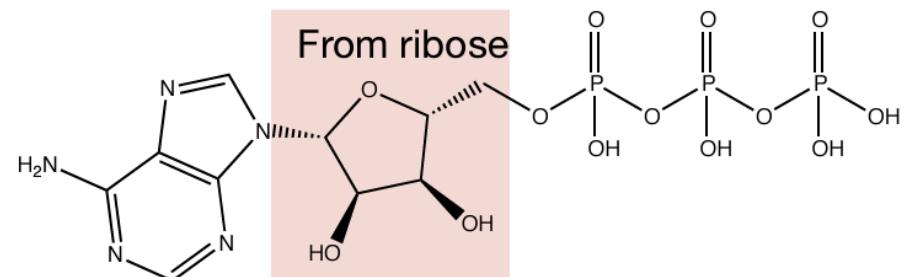
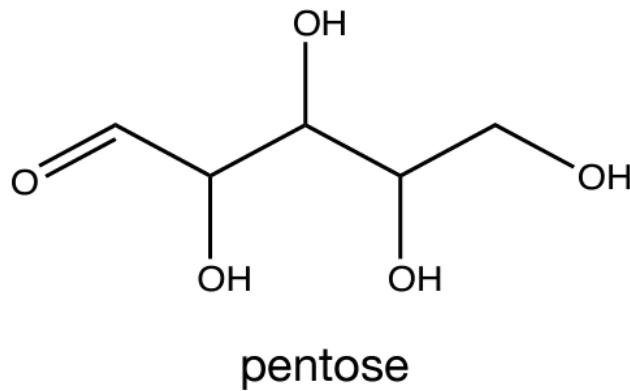


Sugars and blood groups



https://www.youtube.com/watch?v=JDM-DpAGh7U&ab_channel=AmericanChemicalSociety

Nucleotides

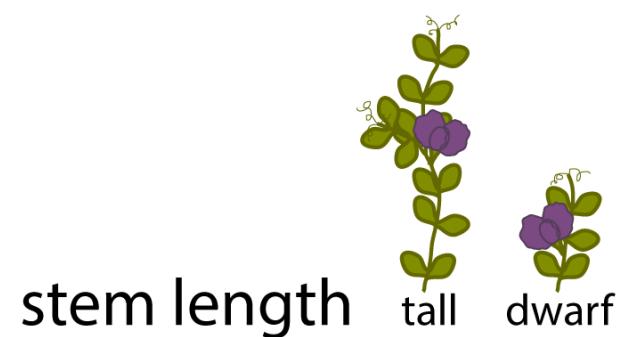
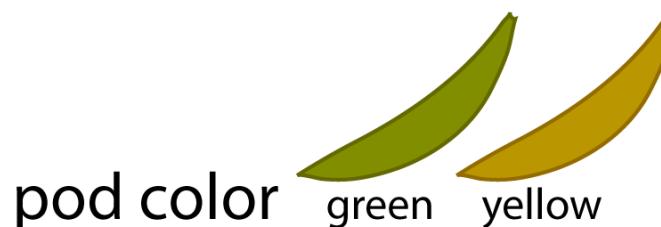
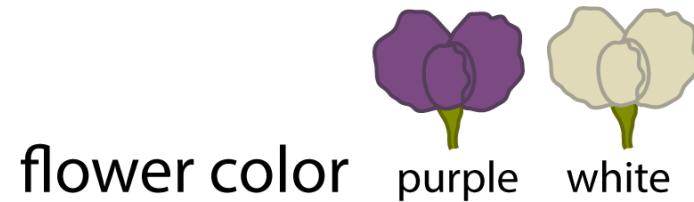


Adenosine triphosphate (a ribonucleotide)

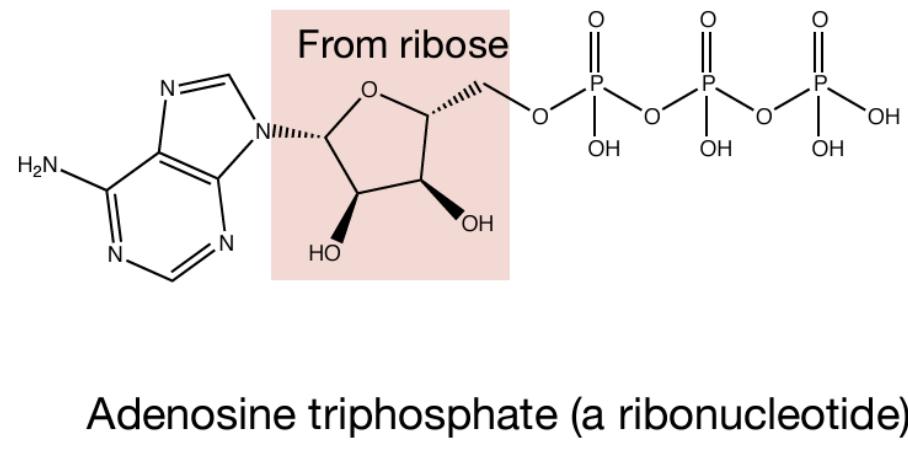
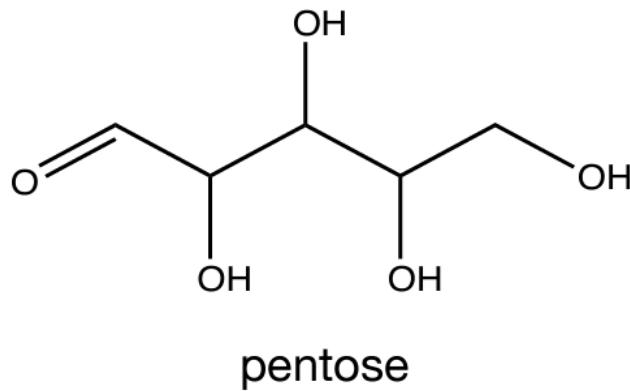
Nucleotides are building blocks for nucleic acids

Mendel and genetics

Mendel's seven pea plant traits



Nucleotides



Nucleotides are building blocks for nucleic acids

Questions that arises

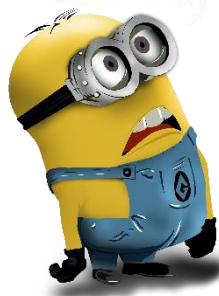
- How is biochemistry used to study genetics?
- How to purify inheritance/heredity in a test-tube?

This seems like an impossible problem!

It happened by accident!

Every new concept should have a perspective!

WHAaaa?!?!

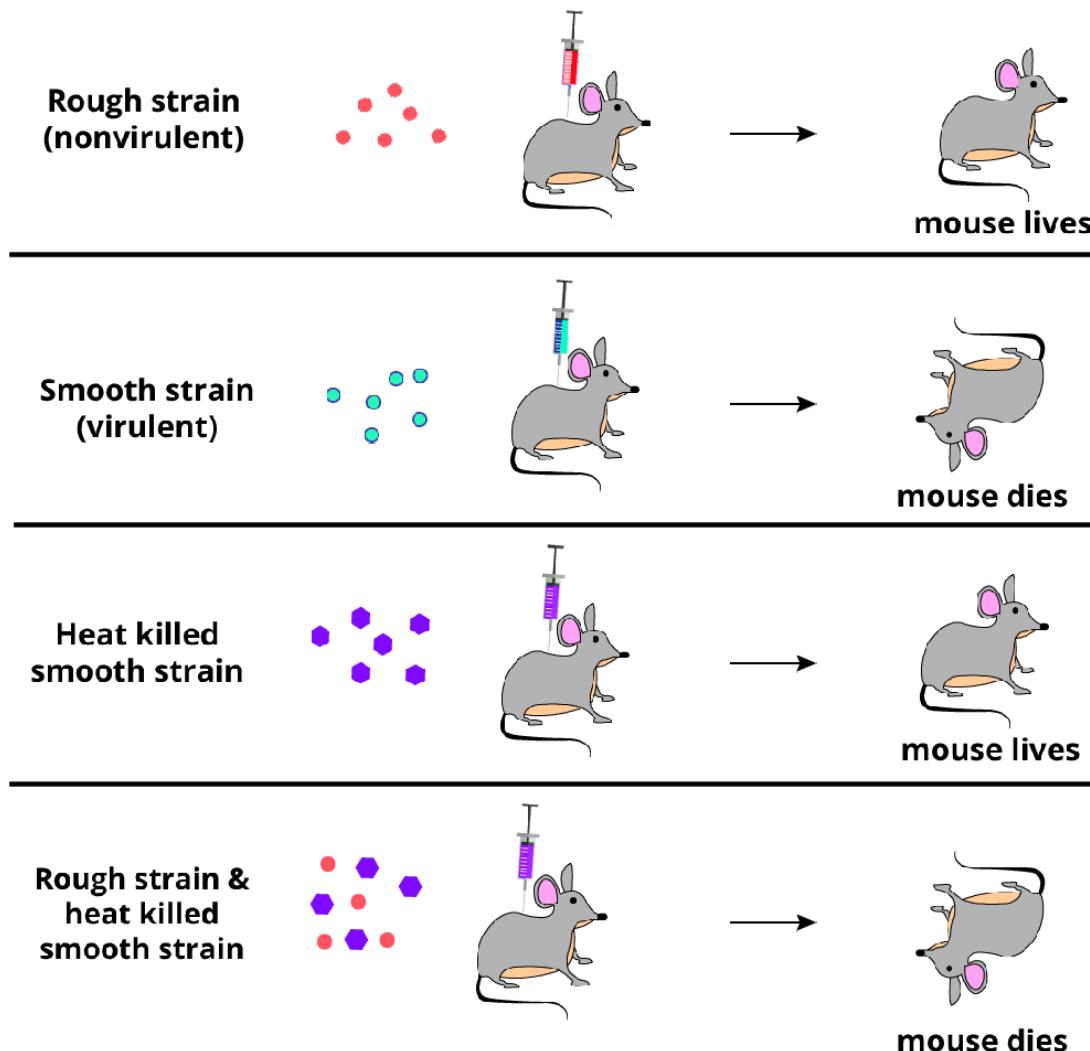


Spanish influenza, 1918-1919



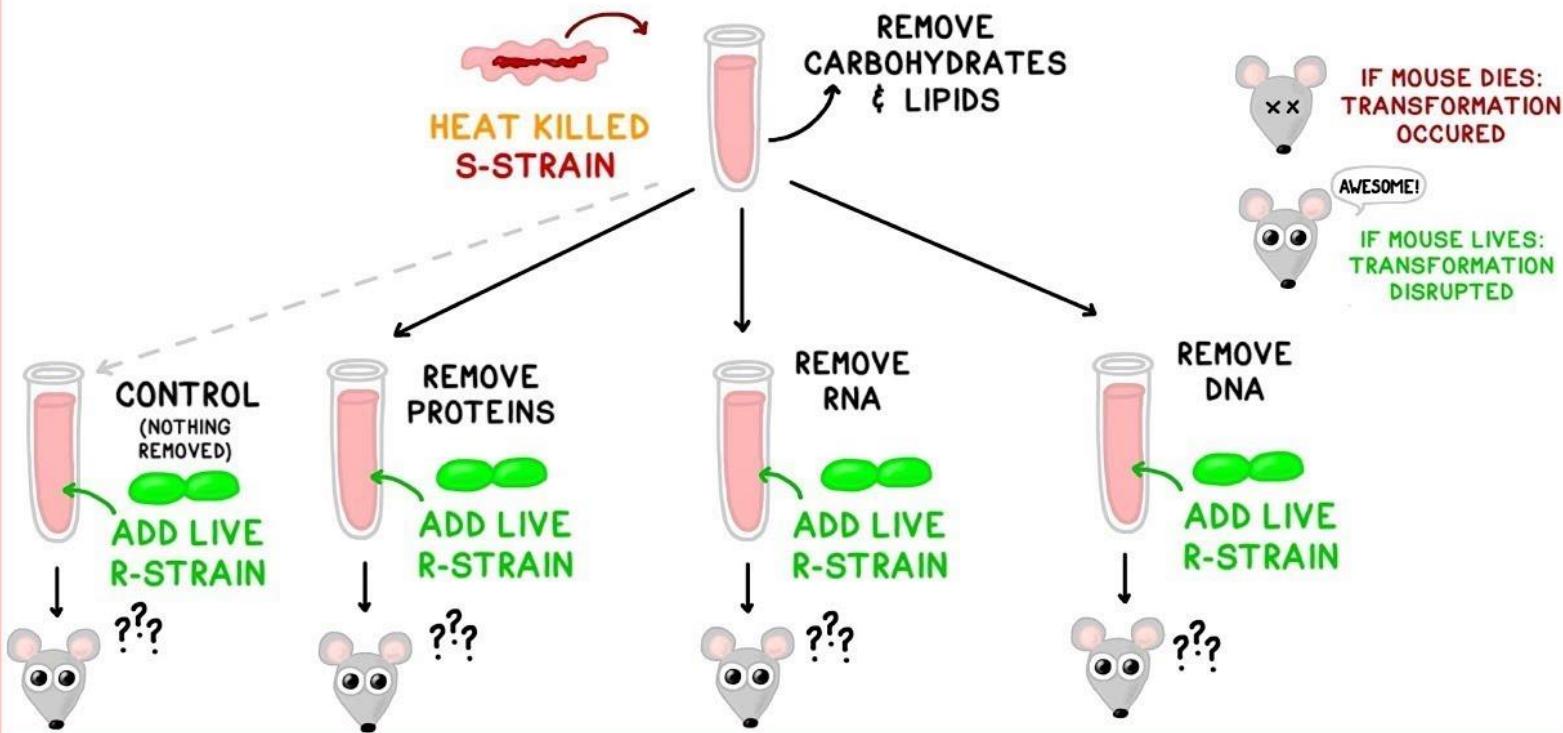
Fred Griffith's study, 1928

S-transforming principle



S-transforming principle

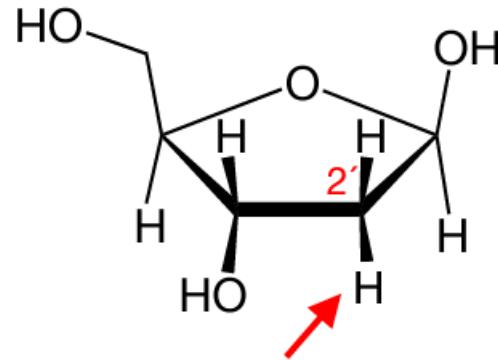
AVERY-MACLEOD-MCCARTY EXPERIMENT



DNA structure: Nucleotide component

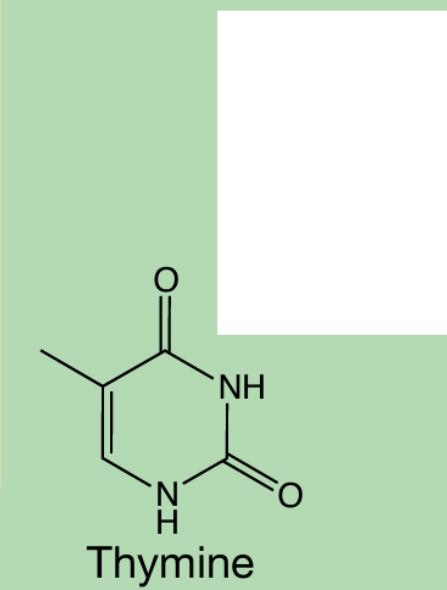
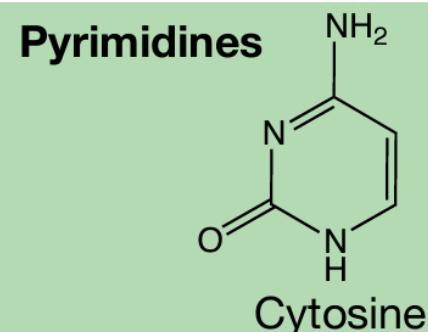
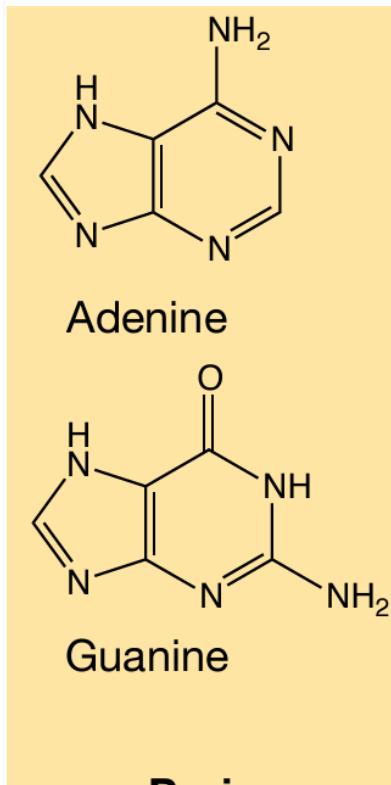
Component 1: Sugar

DNA:
Deoxyribose



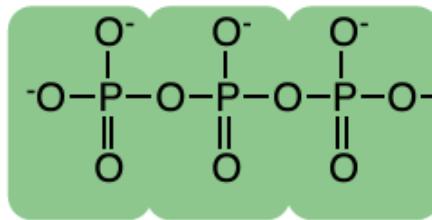
DNA structure : Nucleotide component

Component 2: Base



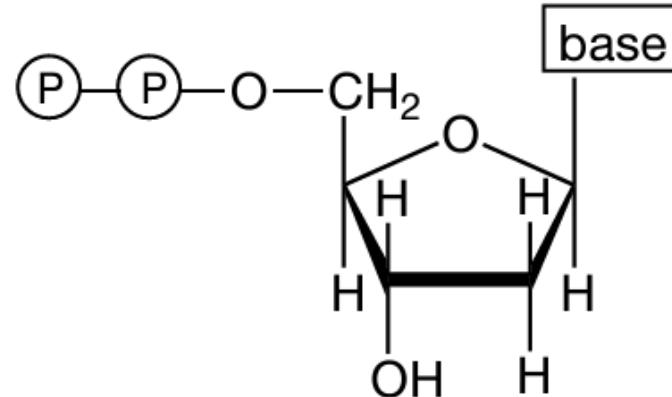
DNA structure : Nucleotide component

Component 3: Triphosphate



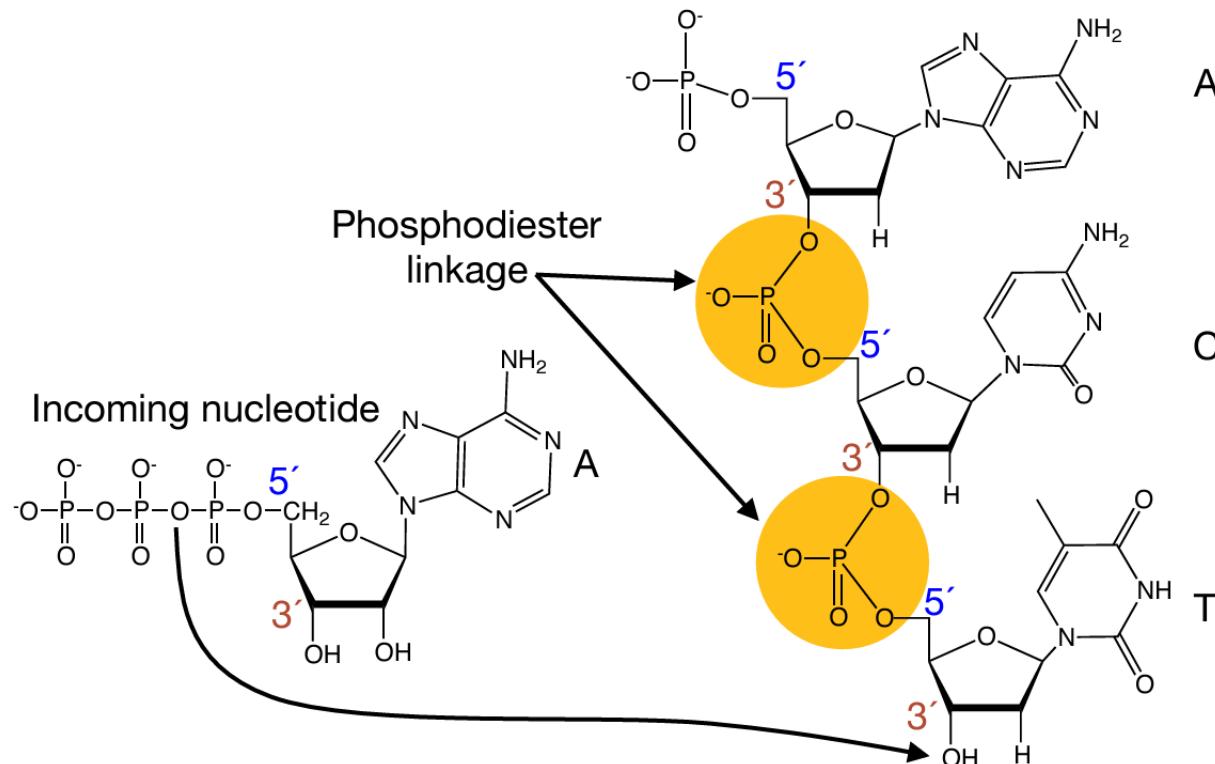
Nucleotides: Combination of the 3 components

Deoxyribonucleotide



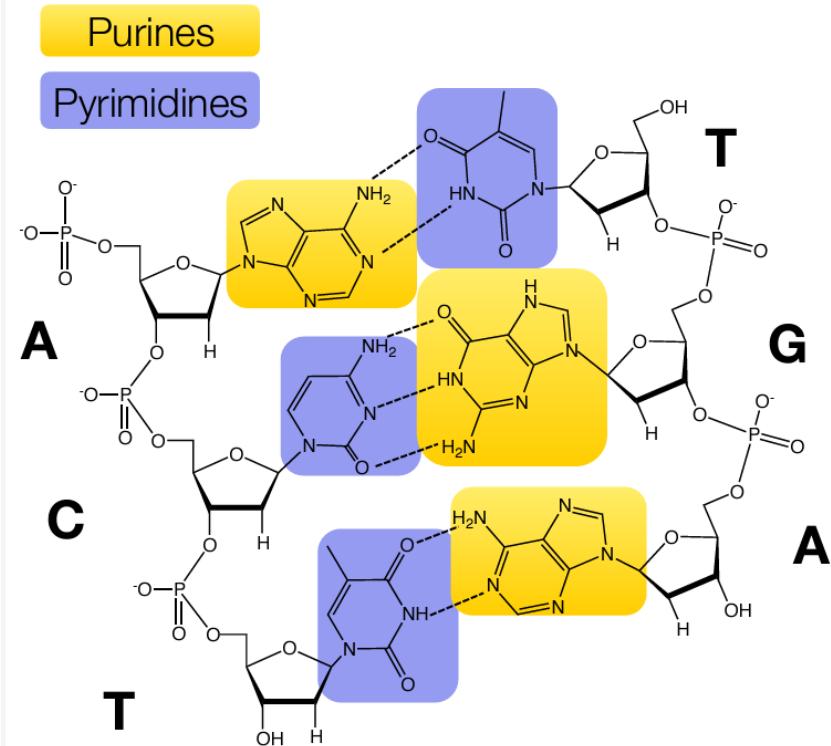
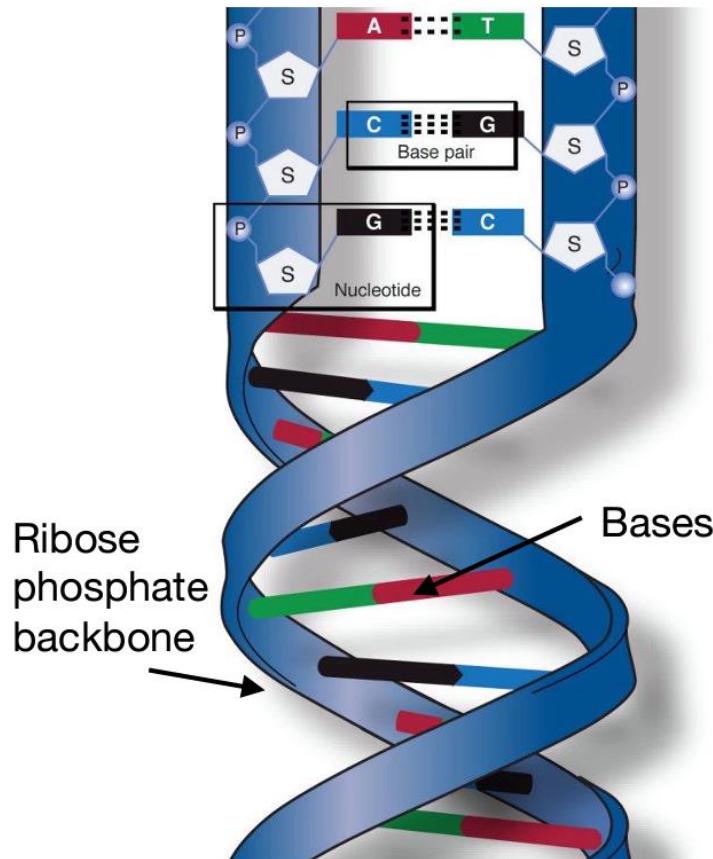
DNA structure

Polymerize by dehydration reaction



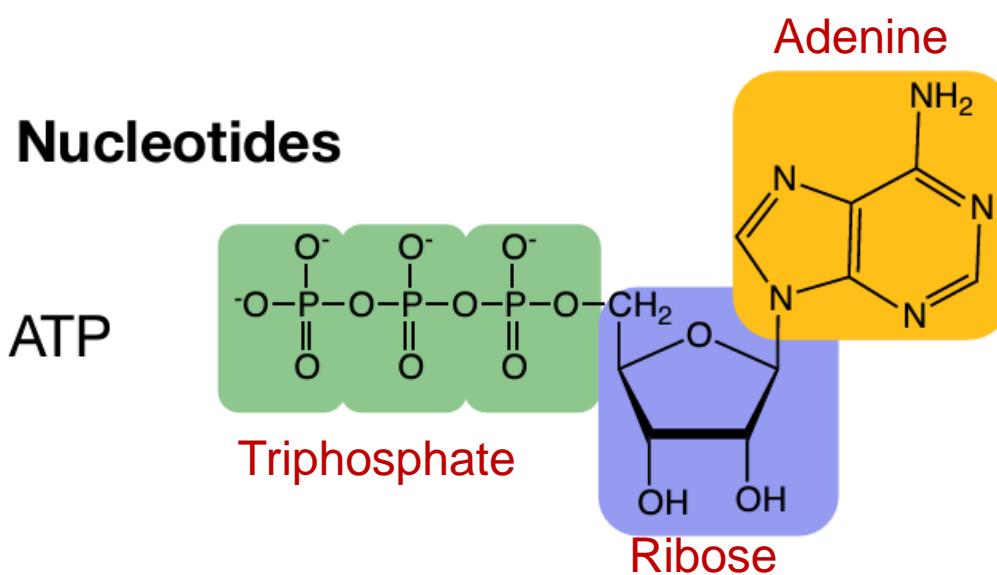
DNA structure

Phosphate backbones



DNA structure : ATP is also a nucleotide

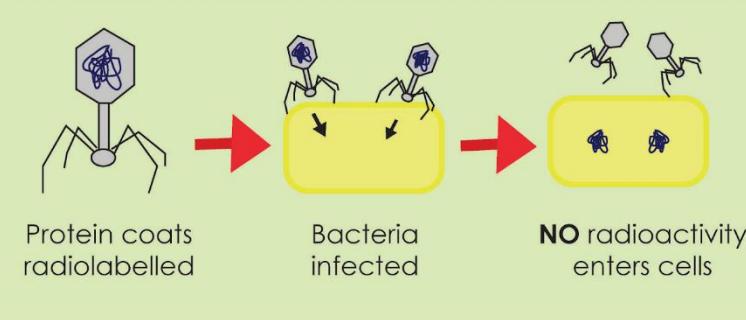
Involved in DNA synthesis



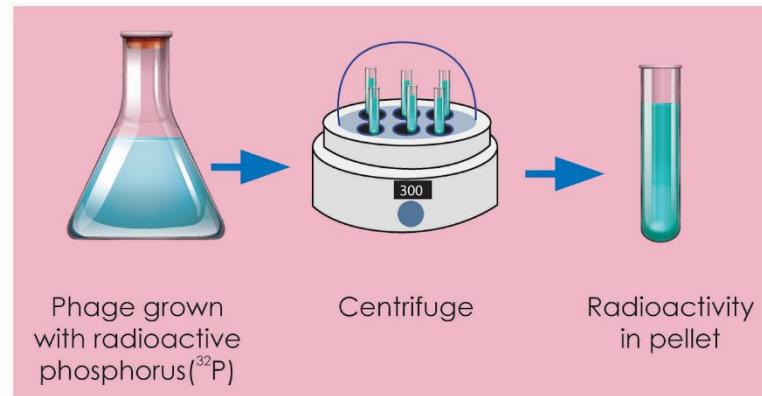
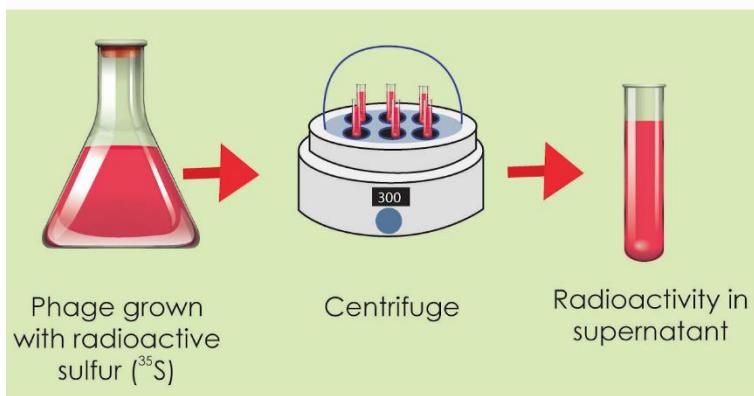
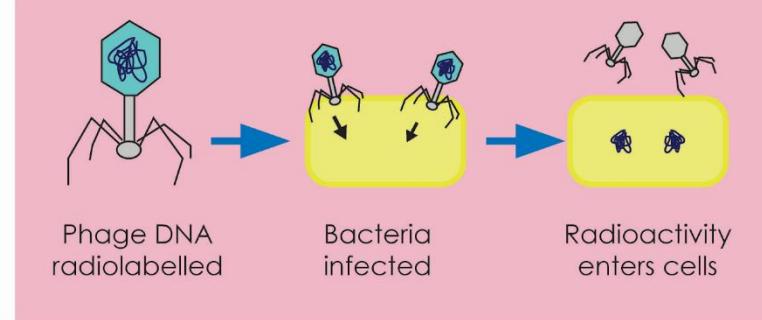
Bacterial viruses: Hershey-Chase experiment

Hershey and Chase Experiment

Experiment 1: Testing Proteins



Experiment 2 : Testing DNA



Conclusion : Proteins are not genetic material

Conclusion : DNA is the genetic material

Bacterial viruses: Hershey-Chase experiment

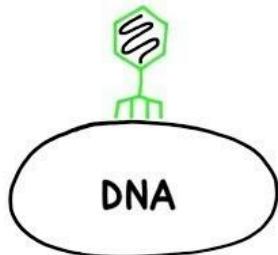
Powerful device, with some fancy technology to knock off the viruses



Bacterial viruses: Hershey-Chase experiment

HERSHEY-CHASE EXPERIMENT

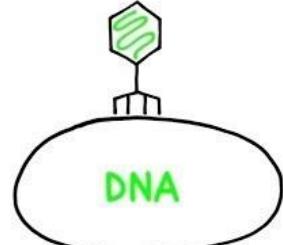
PROTEINS
RADIOACTIVE
SULFUR 35



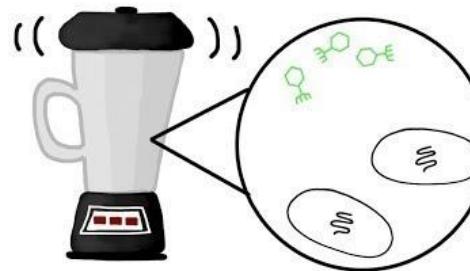
VS.

I. INFECTION

NUCLEIC
ACIDS
RADIOACTIVE
PHOSPHORUS 32



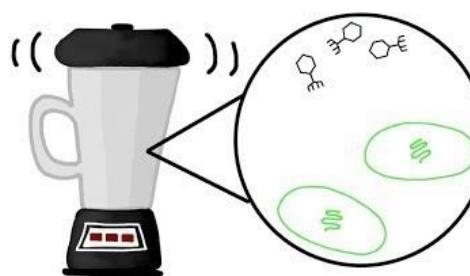
NUCLEIC ACIDS
~~PROTEINS~~ ARE THE MOLECULE OF HEREDITY



2. BLENDING



3. CENTRIFUGATION



RADIOACTIVE
PELLET



Nucleic acids

What we know so far!

- Nucleotides are the building blocks
- Genes and Mendel
- Genes are the part of DNA
- Spanish influenza: What carries heredity information-DNA or proteins?
(Avery – Mcleod - McCarty experiment)
- Introduction to DNA structure

- Proteins are complex cool macromolecules that has C, N, H, O, S

Nucleic acids

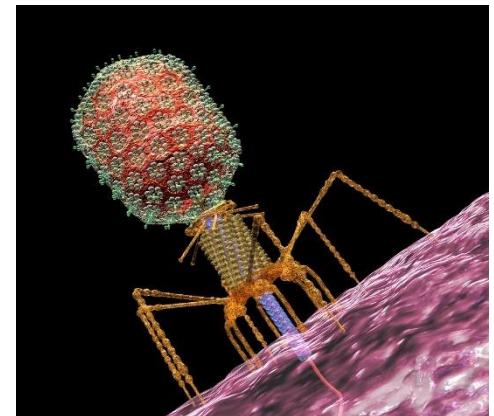
- DNA structure in detail
- What carries heredity information- DNA or proteins? - Bacterial viruses
- DNA helical structure: the race
- RNA

Bacterial viruses

What carries heredity information- DNA or proteins?

Bacterial viruses

- Bacteria get affected by viruses too
- Virus + Bacteria = Bacteria destroyed within 20-30 minutes
Bacteriophage
- In early 20th century, belief was to drink a lot of virus to cure from bacterial infection –
Hasn't worked out, has it?



Bacterial viruses and replication

- It actually got people thinking

- Didn't just kill bacteria

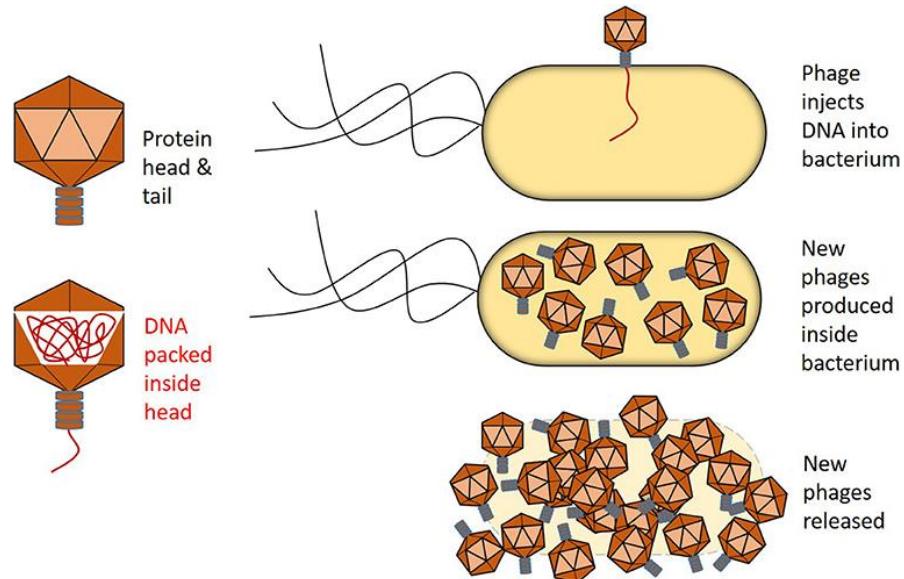
- Caused bacteria to burst open

- Lots of viruses burst out

- **Replicate**

- make thousands of copies of itself

- So, it's a living organism who replicates itself when it goes into a cell



Bacterial viruses and replication

- So 2 people **Al Hershey** and **Martha Chase** did an experiment called-
Hershey – Chase experiment

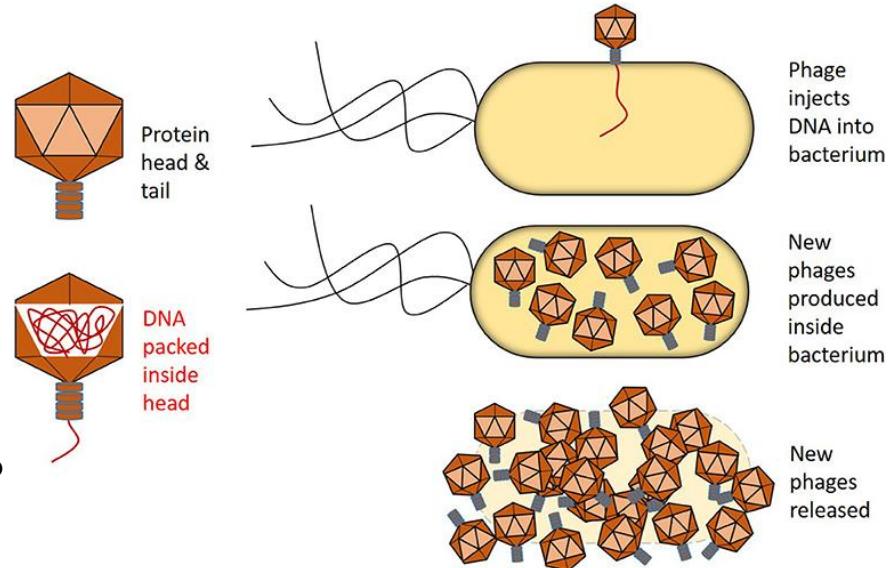
- They took bacterial viruses

- Let them attach to the bacteria

- And they asked: what went into the cell?

- Protein? DNA?

- If DNA, then DNA is carrying the instructions.



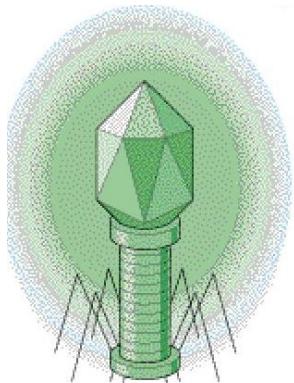
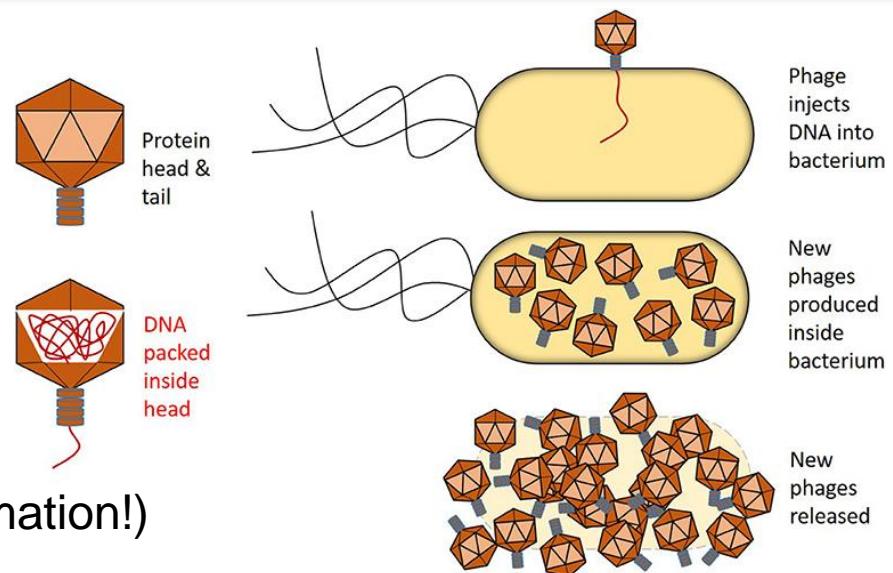
Bacterial viruses and replication

How can they tell?

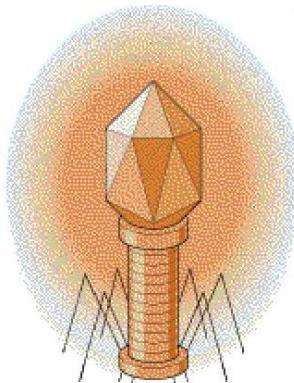
Radioactive labeling!

How? (Hint: remember the protein information!)

Which element from protein? Which one from DNA?



sulfur
label

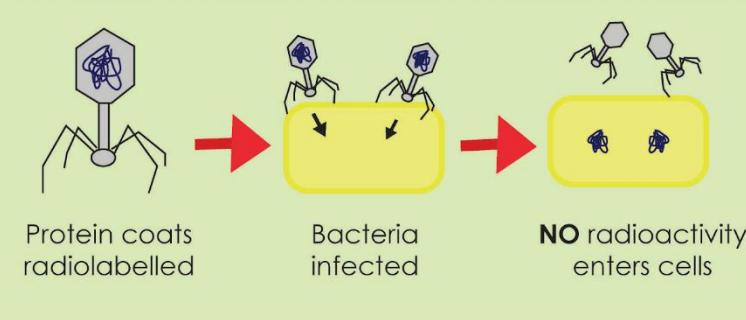


phosphorus
label

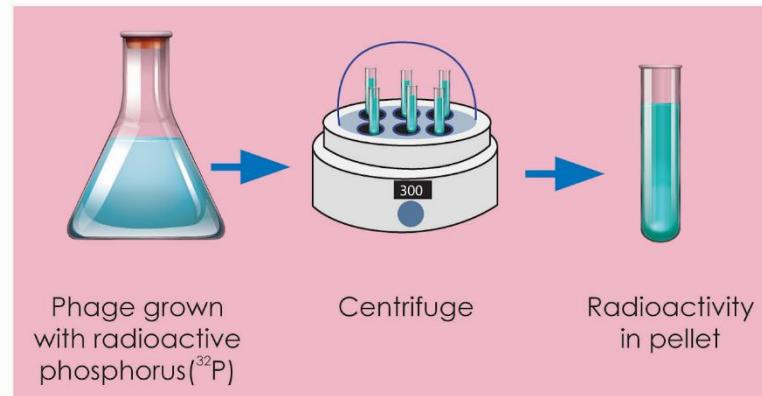
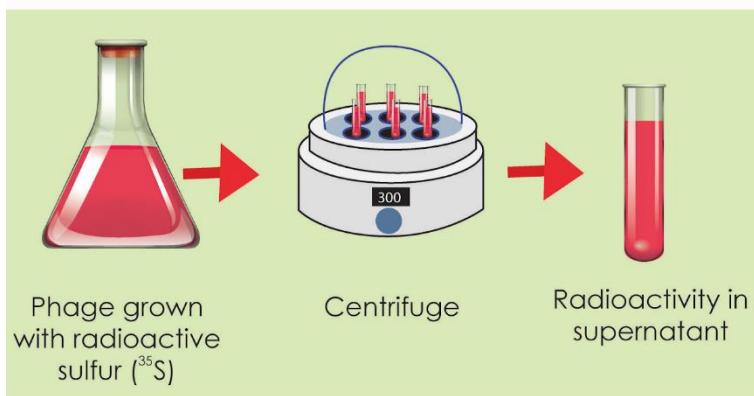
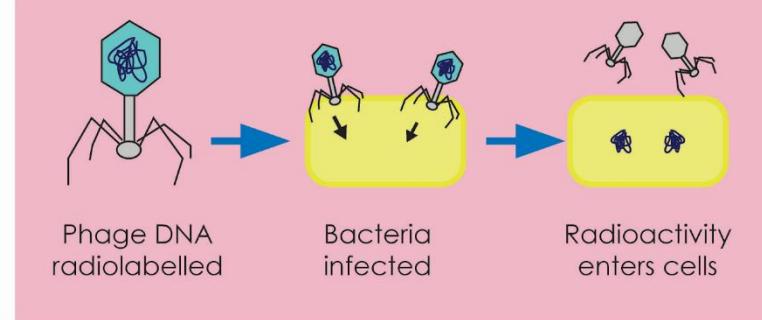
Bacterial viruses: Hershey-Chase experiment

Hershey and Chase Experiment

Experiment 1: Testing Proteins



Experiment 2 : Testing DNA



Conclusion : Proteins are not genetic material

Conclusion : DNA is the genetic material

Bacterial viruses: Hershey-Chase experiment

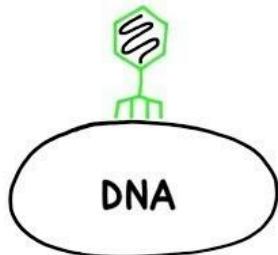
Powerful device, with some fancy technology to knock off the viruses



Bacterial viruses: Hershey-Chase experiment

HERSHEY-CHASE EXPERIMENT

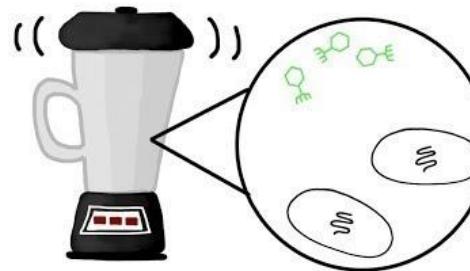
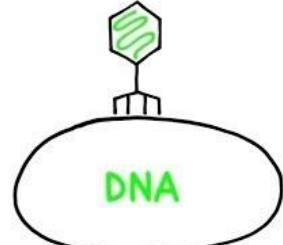
PROTEINS
RADIOACTIVE
SULFUR 35



VS.

I. INFECTION

NUCLEIC
ACIDS
RADIOACTIVE
PHOSPHORUS 32



2. BLENDING



3. CENTRIFUGATION



RADIOACTIVE
PELLET

DNA structure: the race

Why? - History behind our understanding towards the secret of life



DNA structure: the race

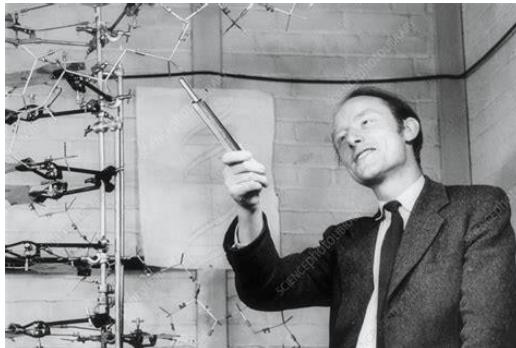
- How is it that this boring DNA molecule can carry heredity?

- Race began...



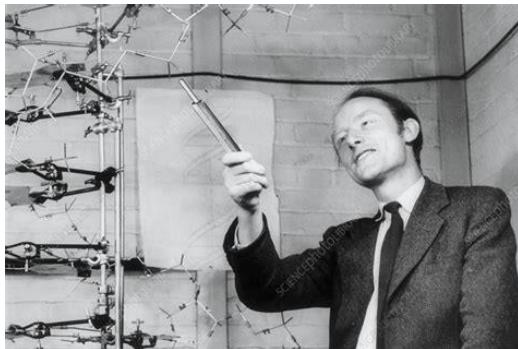
25-year-old American ornithologist: **James D Watson**

35-year-old talkative British physicist: **Francis Crick**



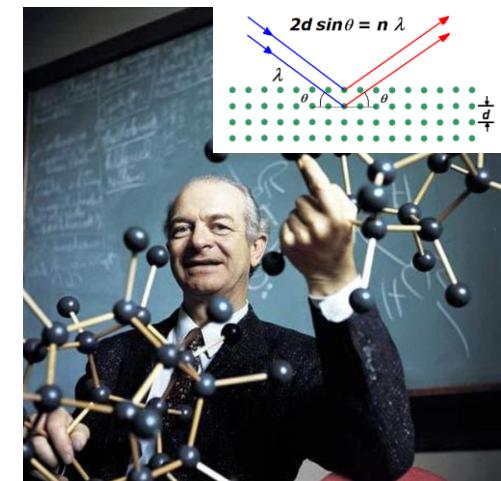
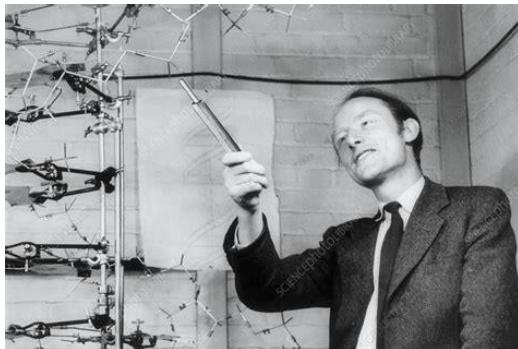
Watson comes to England

- Watson came over to on a fellowship to Medical Research Council Labs in Cambridge, England
- To work on structure of things with Crick
Crick knew a lot about crystallography and mathematics
- They were incredibly well known around the Medical Research Council – Why?
- Because they talked a lot and did nothing!



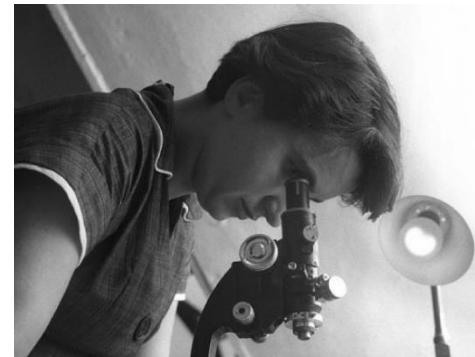
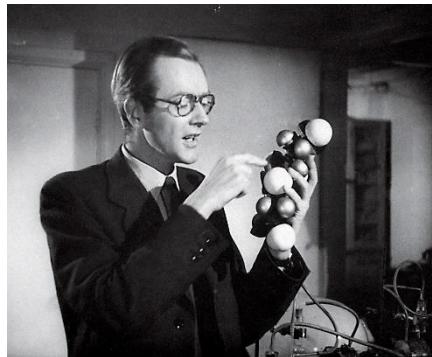
Big ideas on DNA

- They had big ideas of what they are going to do
- Including the DNA thing. They knew this was important
- They weren't supposed to be working on DNA, they were supposed to be working on something else
- The distinguished lab head, Sir Lawrence Bragg, did not want them to work on DNA
- Because another college, King's College in London, was supposed to be working on DNA stuff.



Crick and Watson went to King's College

- Kids being kids- they anyways started to work on it
- They made some models: crazy models
- Some were even embarrassing models in 1952
anybody could tell they were wrong
- They began going down to King's College, invited by **Maurice Wilkins**
- Met **Rosalind Franklin**, who was working on crystallization and DNA XRD patterns



Crick and Watson went to King's College

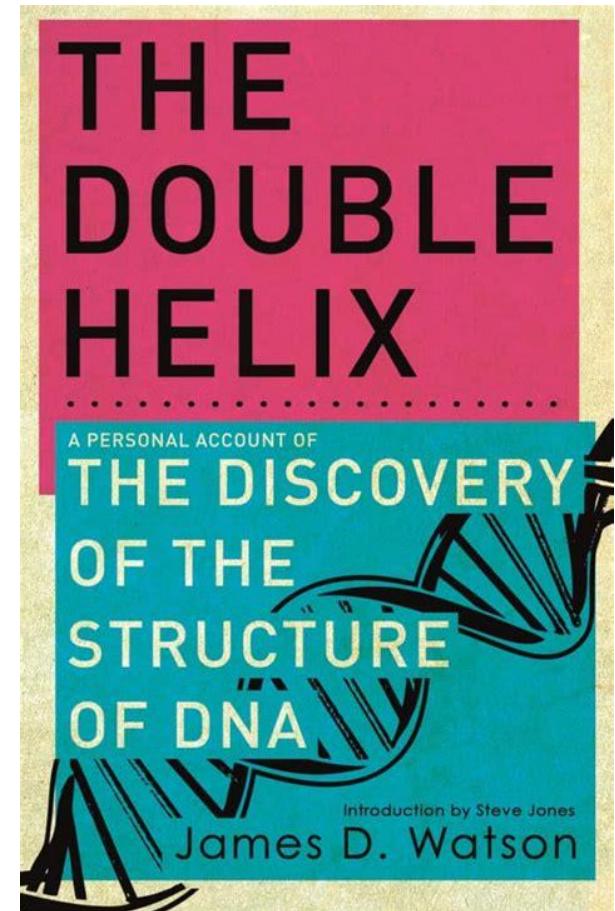
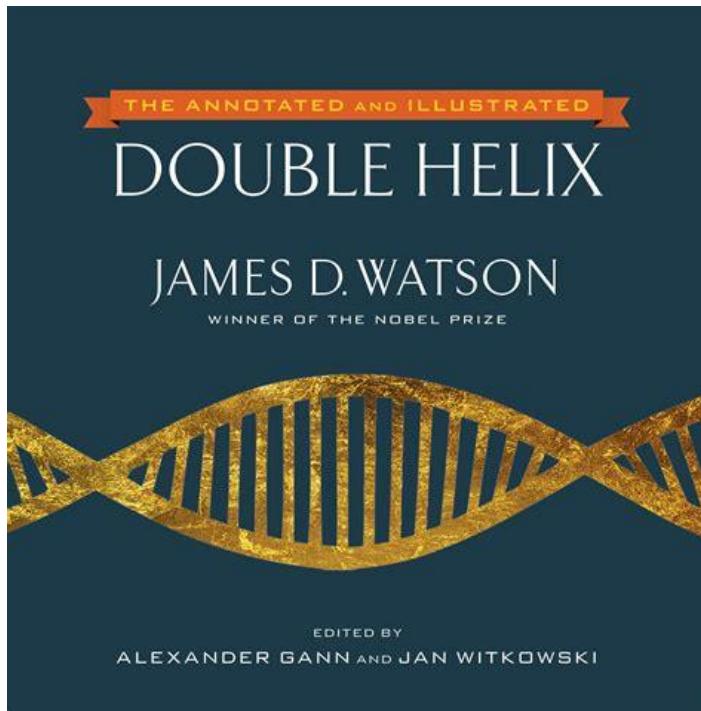
- But... Crick and Watson did not get along too well with Rosalind
- There were a bit of tensions and misunderstandings
- They came down and made a pain of themselves!
- Talked back and forth
- Rosalind hated all these abstract models
- She needed hard data
- Crick and Watson loved models
- There was this tension back and forth
- Rosalind, at one point, was certain that DNA is not a helix
She even announced the death announcement



It was comic

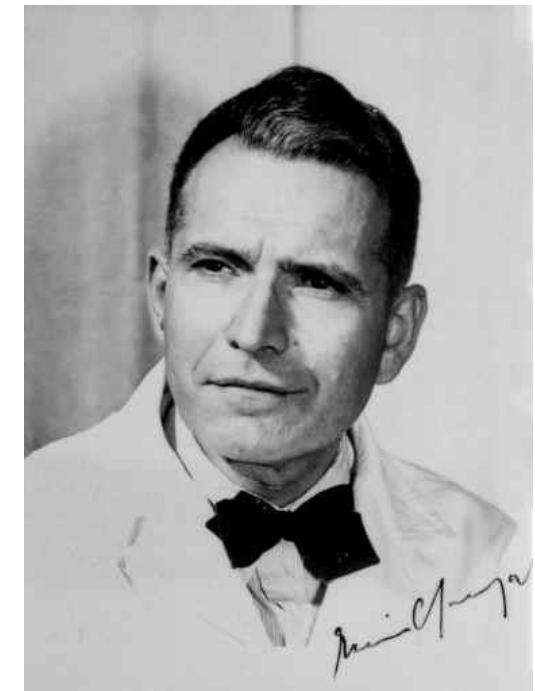
“The Double Helix”

- You got to read “The Double Helix”
- There are so many stories!



Now Chargaff comes into picture

- One smart guy called Chargaff (finally, a biochemist!) plays a vital role in the story
- He discovered that the simple established tetranucleotide story, that A T C G are stuck together in that ratio, was wrong
- Started measuring ratios of A T C G biochemically across different organisms
- They were not always equal
- He found A 28%, T 28%, G 24%, C 20%
- He did more measurements and found there were some experimental errors
- As time went by it became more clear that G and C were roughly 22%
- Checked more organisms, numbers varied
- But always: A = T and G = C



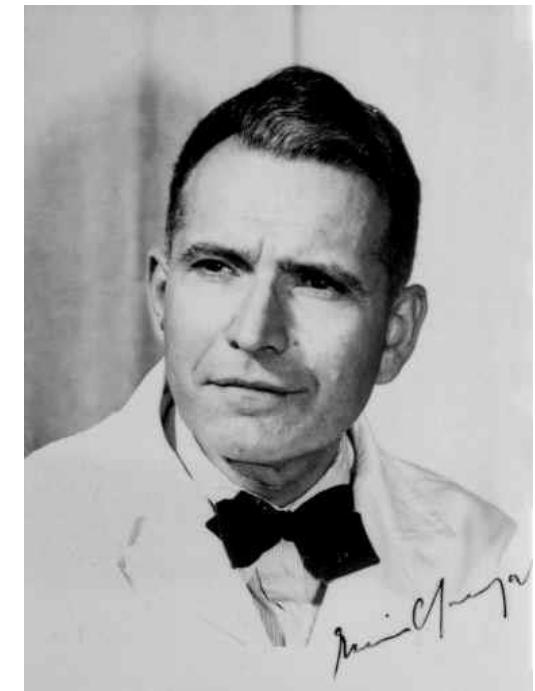
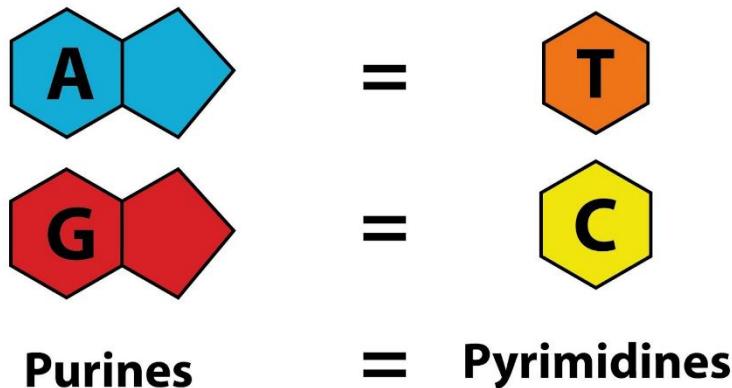
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Chargaff's DNA database composition in various species (%)

Species	A	T	G	C
<i>Homo sapiens</i>	31.0	31.5	19.1	18.4
<i>Drosophila melanogaster</i>	27.3	27.6	22.5	22.5
<i>Zea mays</i>	25.6	25.3	24.5	24.6
<i>Neurospora crassa</i>	23.0	23.3	27.1	26.6
<i>Escherichia coli</i>	24.6	24.3	25.5	25.6
<i>Bacillus subtilis</i>	28.4	29.0	21.0	21.6

c.

Now Chargaff comes into picture



- Very important observation
- DNA was not simply a boring molecule of just A, T, C, G
- They were ratios and they could differ between organisms and all that

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**Chargaff's DNA database
composition in various species (%)**

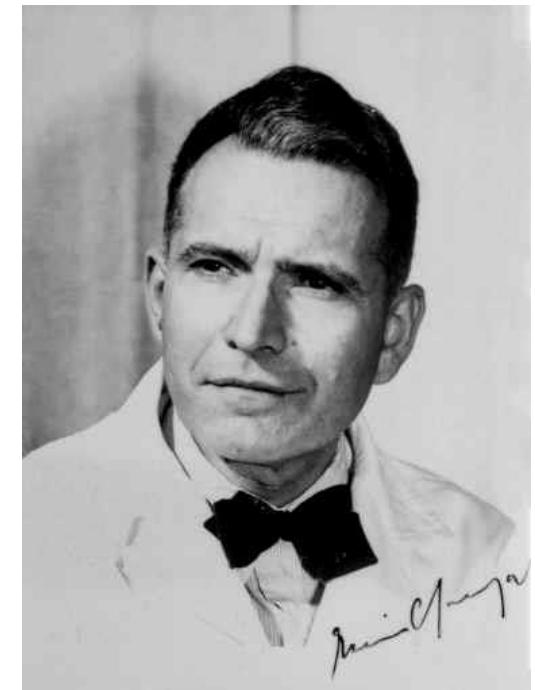
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<i>Escherichia coli</i>	24.6	24.3	25.5	25.6
<i>Bacillus subtilis</i>	28.4	29.0	21.0	21.6

c.

Chargaff meets Crick and Watson

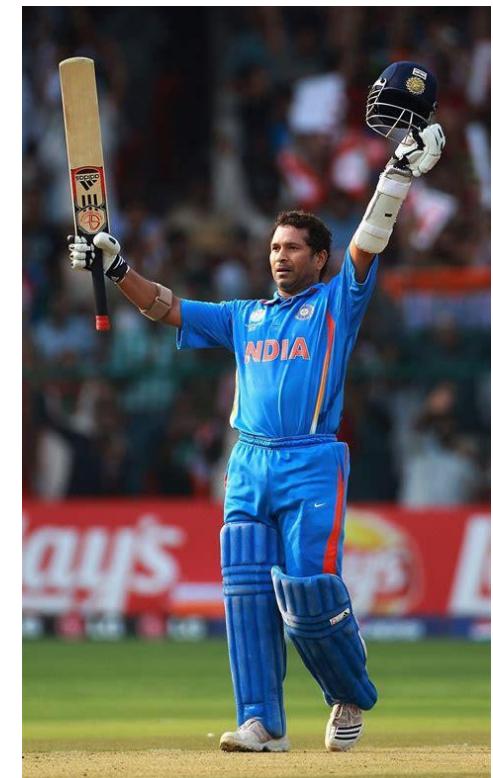
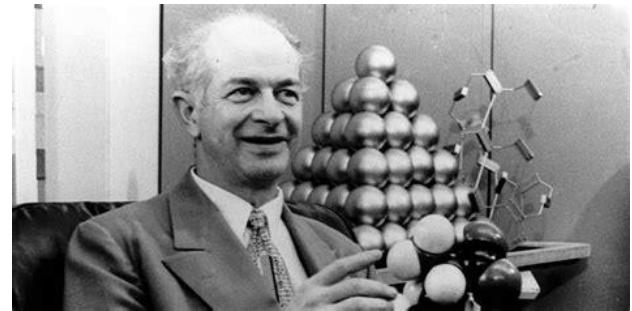
- Chargaff came to the Medical Research Council once and had lunch with Crick and Watson
- He was not impressed
- Chargaff later said:

“They impressed me by their sheer ignorance. I never met two men who knew so little and aspired to do so much.”



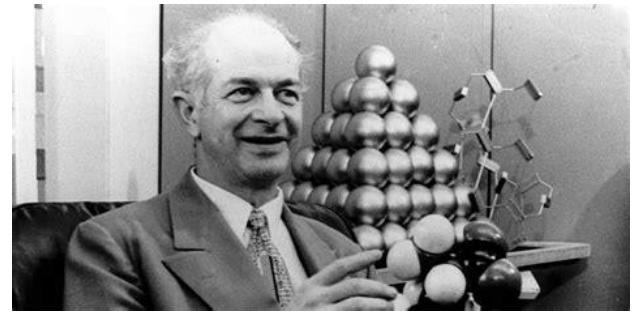
Linus Pauling comes into the picture

- Word reaches Cambridge...
- Somebody else was working on this problem
Linus Pauling
- The person who strikes fear into the hearts of anybody thinking about chemical structures
- He was Sachin Tendulkar of chemical bonds!
- The most brilliant chemist in the 20th century
- He figured out protein structures while he was sick with flu
- Now he got interested in DNA structure
- Crick and Watson are now worrying- they are facing Linus Pauling



Linus Pauling submitted his paper...

- Word reaches Cambridge...
- Pauling submitted a paper to the Proceedings of the National Academy of Sciences in the US in December 1953
- Jan 28, 1953: paper shows up in Cambridge
- One of the copy goes to his son, Peter Pauling.
- He was C and W 's good friend
- He showed them the paper and Pauling structure

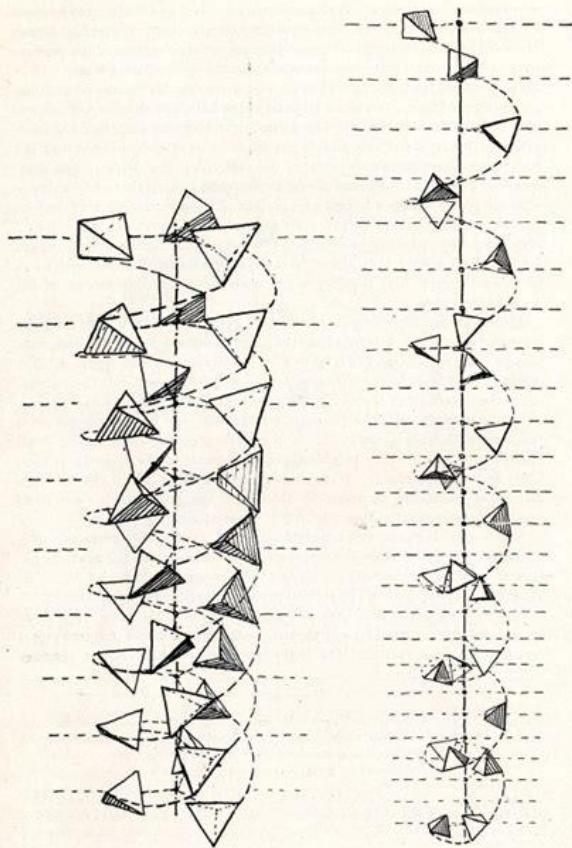


Pauling's triple helix structure

88

CHEMISTRY: PAULING AND COREY

PROC. N. A. S.



92

CHEMISTRY: PAULING AND COREY

PROC. N. A. S.

which are involved in ester linkages. This distortion of the phosphate group from the regular tetrahedral configuration is not supported by direct experimental evidence; unfortunately no precise structure determinations have been made of any phosphate di-esters. The distortion, which corresponds to a larger amount of double bond character for the inner oxygen atoms than for the oxygen atoms involved in the ester linkages, is a reason-

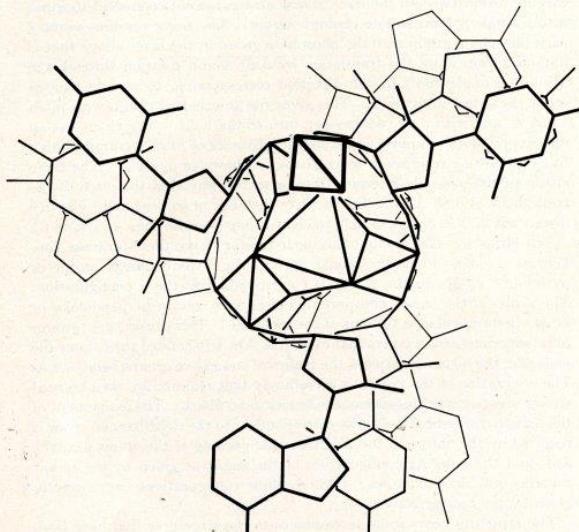


FIGURE 6

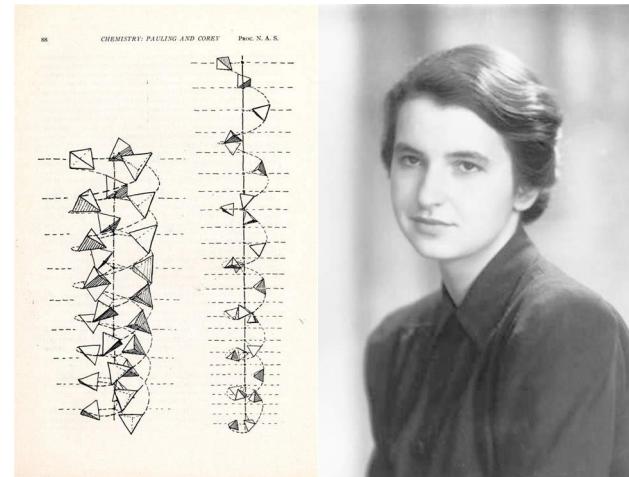
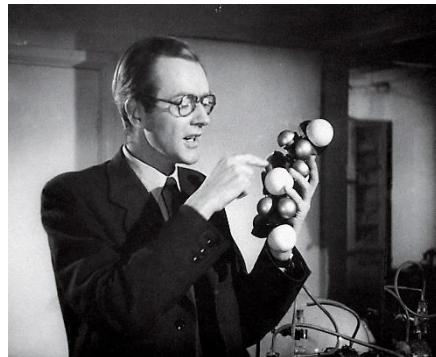
Plan of the nucleic acid structure, showing several nucleotide residues.

able one, and the assumed distances are those indicated by the observed values for somewhat similar substances, especially the ring compound S_8O_8 , in which each sulfur atom is surrounded by a tetrahedron of four oxygen atoms, two of which are shared with adjacent tetrahedra, and two unshared. The O—O distances within the phosphate tetrahedron are 2.32 Å (between the two inner oxygen atoms), 2.46 Å, 2.55 Å, and 2.60 Å. The

- Phosphate groups in the middle
- Bases sticking out in space
- Is there a problem with all the phosphate groups in the middle?

Pauling blew it!

- They showed it to Rosalind
- Rosalind realizes it too- Pauling blew it
- They immediately started arguing
- As Watson is leaving, Wilkins come over
- And they saw this iconic photo Rosalind took a while back from her crystallography



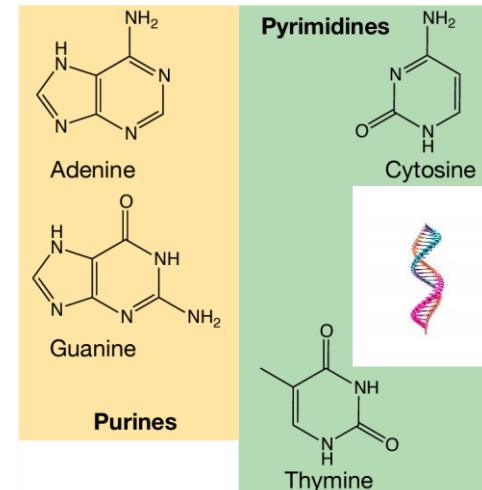
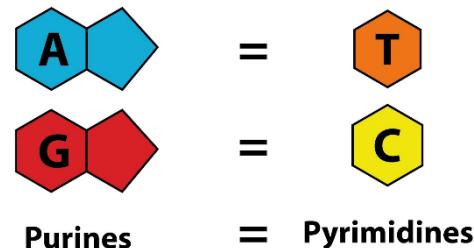
Rosalind's iconic photo

- Anybody who knows crystallography
- Can say this is a helix
- Rosalind did this amazing work
- But she was not into interpreting
- But Watson understood
- He understood it means helix
- Another piece of information:
It is same if you turn it upside down. It is symmetric
- It has axis of symmetry
- Crick and Watson takes this seriously



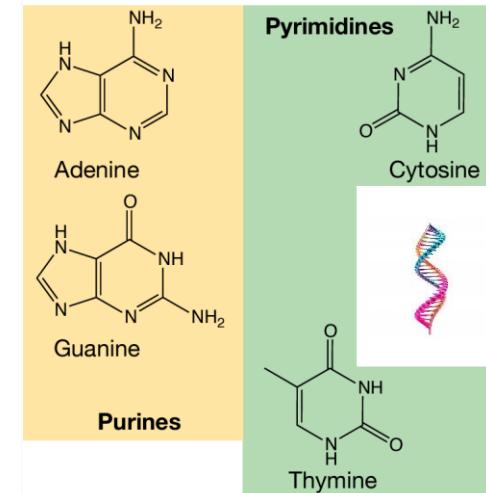
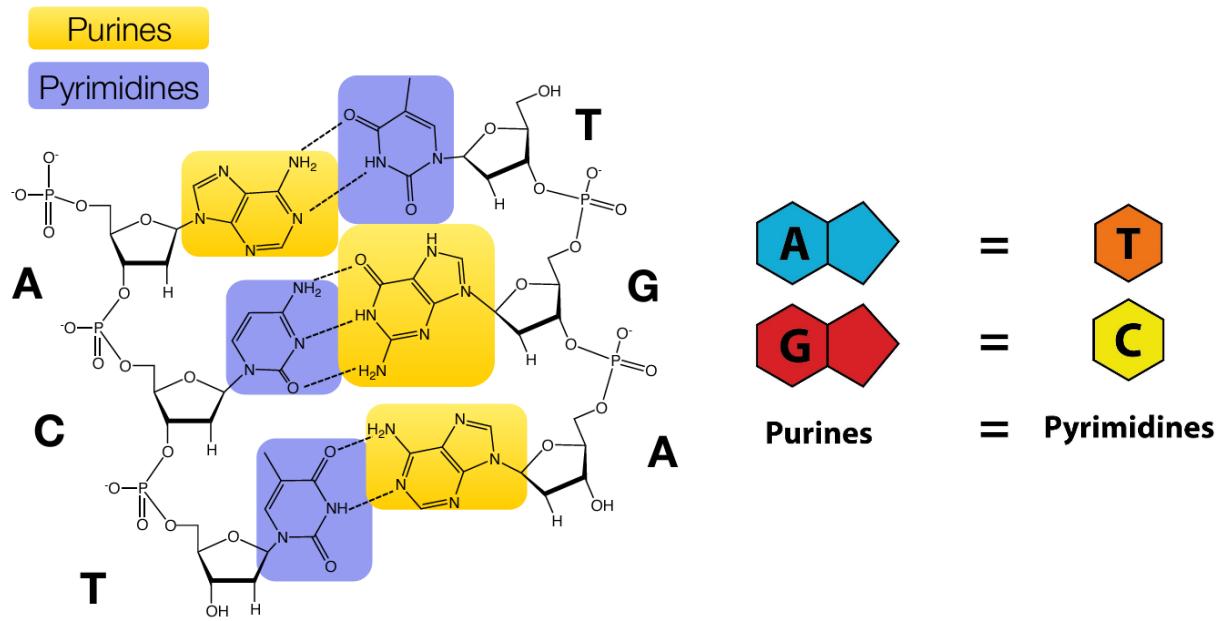
Watson starts again...

- Crick and Watson takes this seriously
- Returns to Cambridge and start working on the models
- He starts making models with sugar and phosphates outside, and bases pointing in
- Watson made model that like matches like: A matches A, T matches T etc.
- He showed to people
- People laughed at him because H-bonding is not going to work
- Crick says that it does not explain Chargaff's rules. They are needed to be explained.



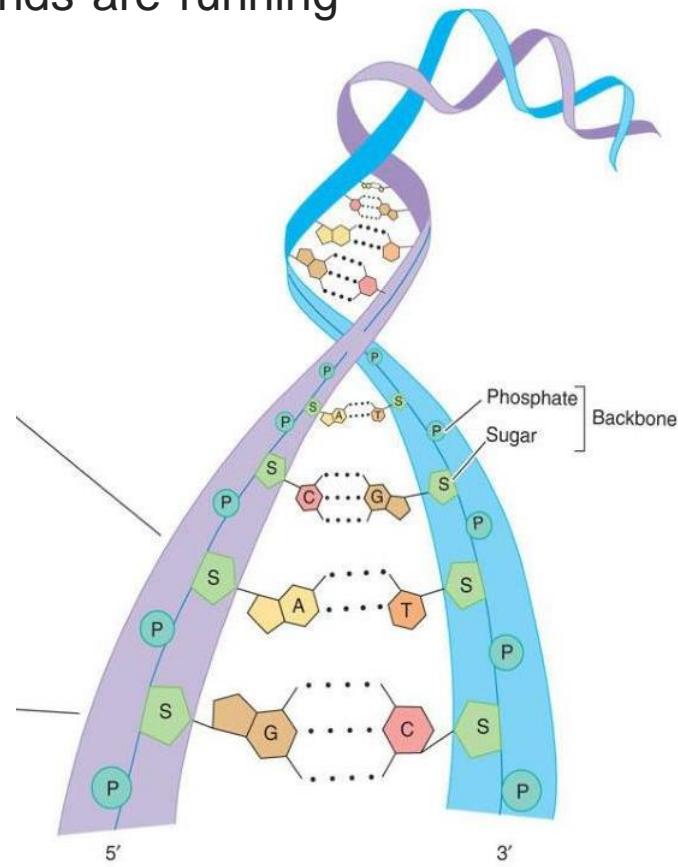
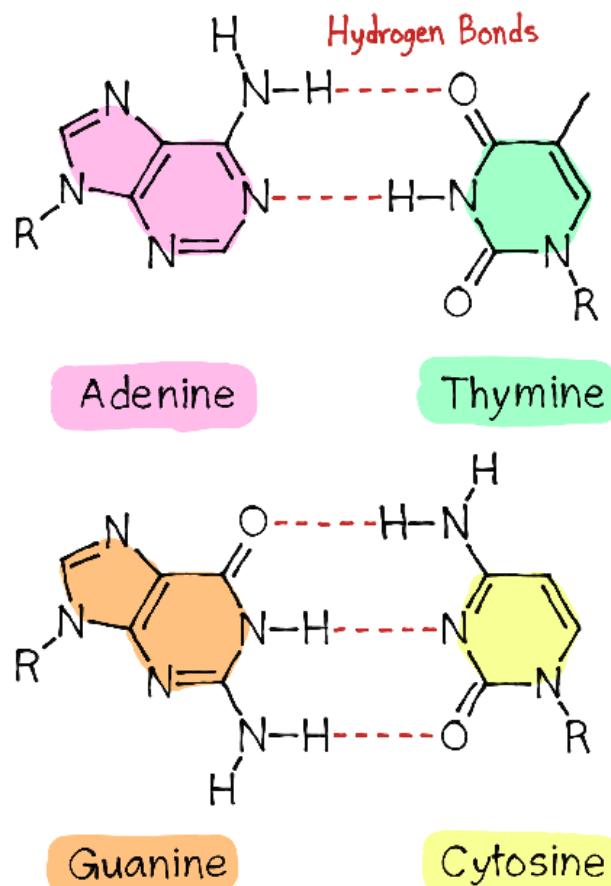
So Watson starts again...

- Give up like for like model
- Instead, starts combining different with different
- T's fit with A's and get 2 Hydrogen bonds
- C's fit with G's and get 3 Hydrogen bonds



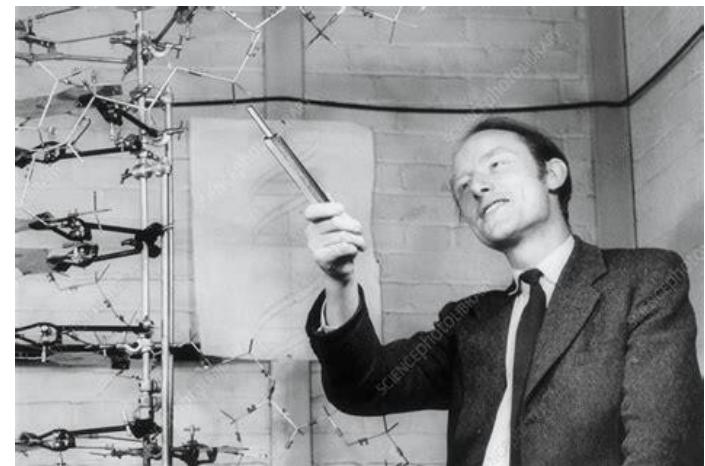
Pauling's triple helix structure

Perfectly fit in double helix if the two strands are running in the opposite directions



So Watson starts again...

- And reproduces the spacing evidence in photo 51
- Crick comes into the lab an hour later
- Watson shows this to Crick, and Crick instantly realizes this as an answer. This feels good!
- Watson is still cautious, they have blew it so many times in the past!

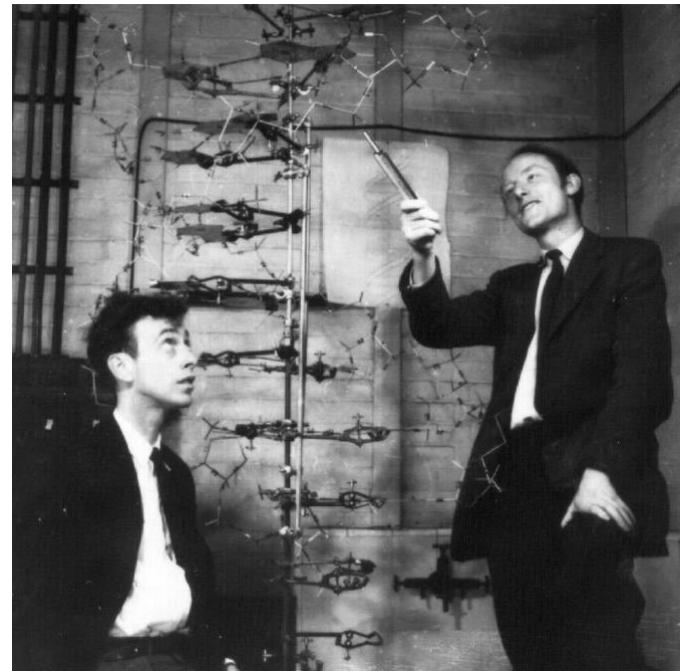


Finally they did it!

- Made it carefully redone at the machine shop to ensure the whole thing really works!
- They went to the Eagle Pub for lunch!
- Watson said to keep it quiet yet!
- Talkative Crick would say to anyone who listens

We have discovered the secret of life!

- And it turns out- they did!
- Model checks out! – Famous picture
- They write up a paper for Nature
- Rosalind and Wilkins also write up a paper for Nature describing their crystallographic experiments



Crick and Watson one-page paper!

No. 4356 April 25, 1953

NATURE

737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

¹Yates, F. B., Gerard, H., and Jeavons, W., *Phil. Mag.*, **40**, 149 (1931).

²Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Suppl.*, **5**, 285 (1949).

³Von Arx, W. S., Woods Hole Papers in Phys. Oceanogr. Meteor., **11** (3) (1960).

⁴Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

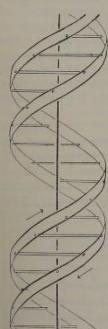
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphodiester chains of the molecule; the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

is a residue on each chain every 3.4 Å. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-coordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally^{2,3} that the ratio of the amounts of adenine to thymine, and the ratio to deoxyribose nucleic acid,

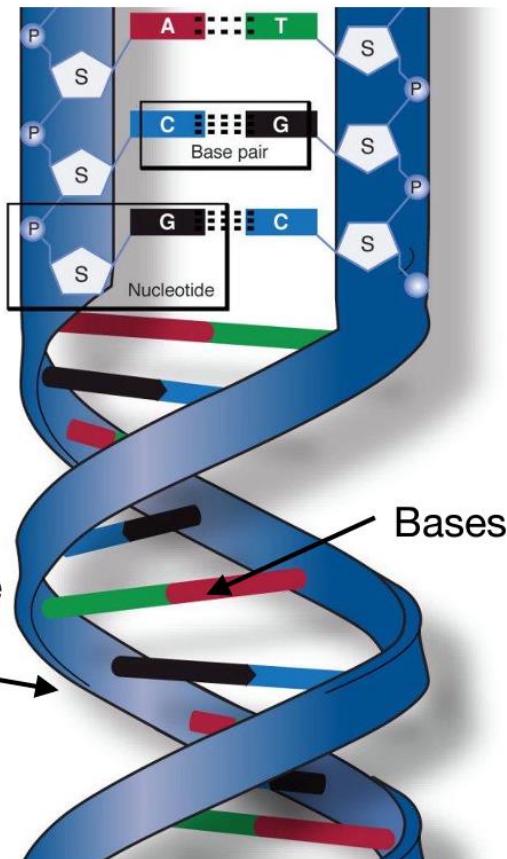
It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{5,6} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

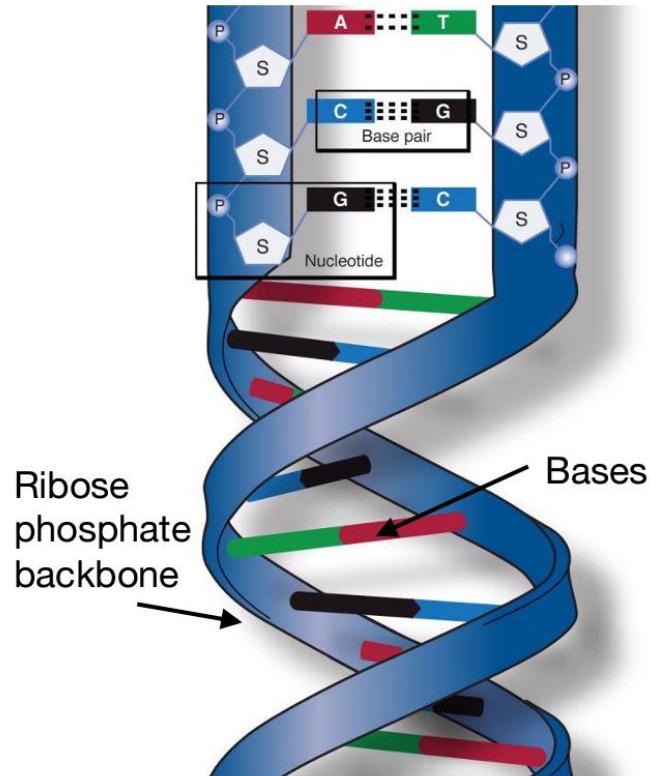
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at



Why is this model so good?

- Not just because it explained everything
Rosalind
Chargaff
- It explains something- heredity
- Because each strand alone is a complete template for the other strand
- If I give one strand: AGCTTAGG, you can fill in the other strand
- If I separate these two strands, each can be used as a template to make a copy of that information
- We talked about genes carrying information, we talked about how genes are part of these DNA and now we know we can control this information

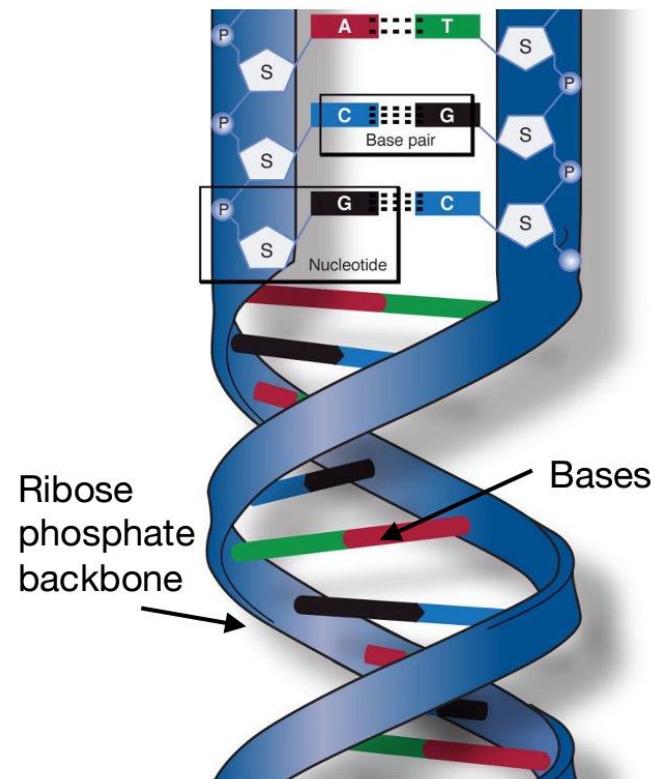


Why is this model so good?

- You can replicate information – stunning
Whole notion of replication is clear
You replicate by having double helix where information is fully specified on either of the two strands
- You can peel them apart, put them together

This is how chromosomes replicate
Transmission of genetic information
Discovered the basis of heredity
Basis of mutation: mutation is wrong letter going in

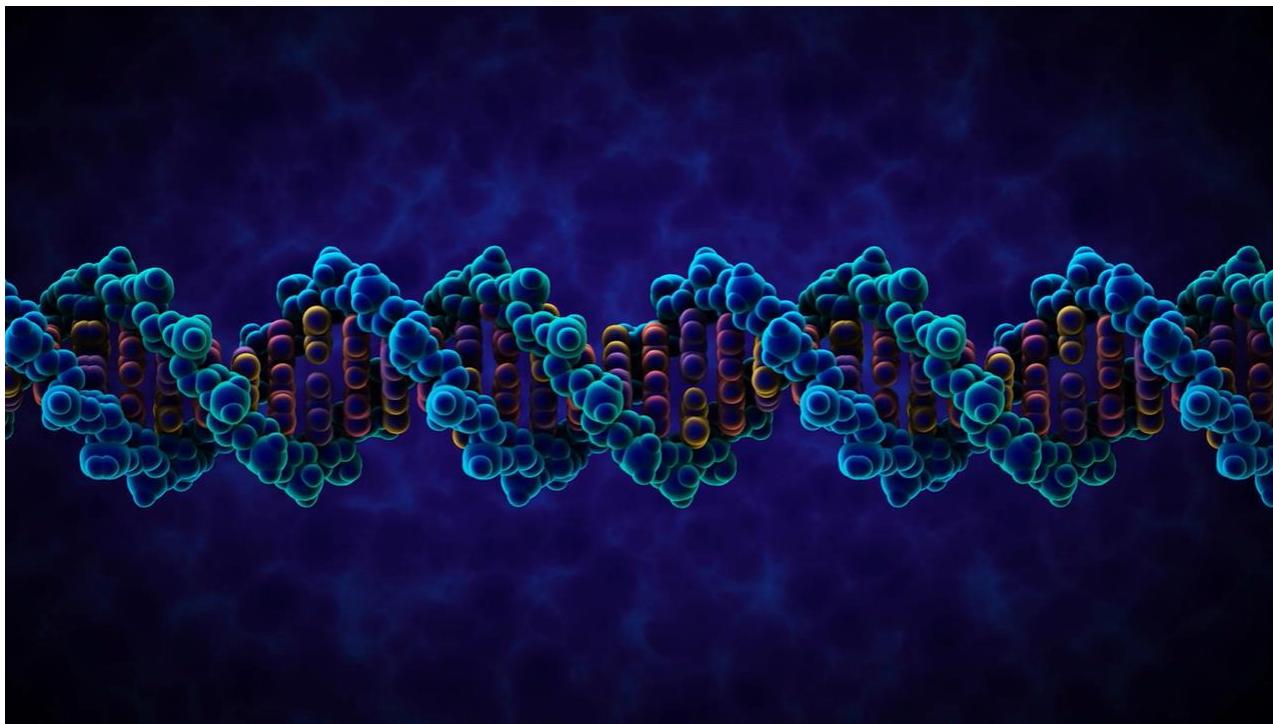
- But- how to write the paper?: They need to prove all this. Their words cannot be considered gospel of truth!
- They explained everything in the *coyest sentence in the history of molecular biology*, which is also the last sentence of the paper



It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

DNA structure

Biochemistry uses this double helix to purify and explain heredity

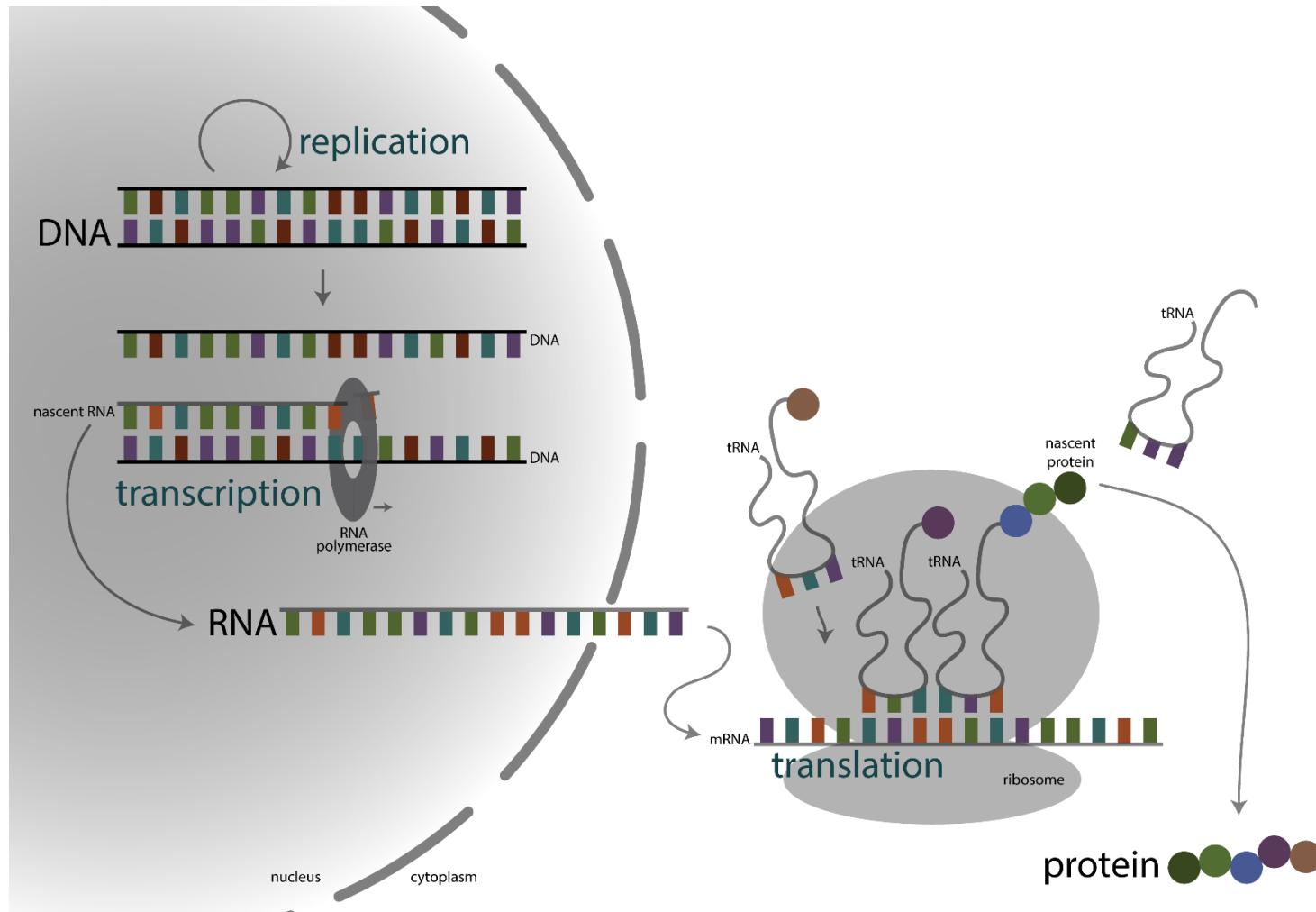


Look into DNA-based computing!

RNA

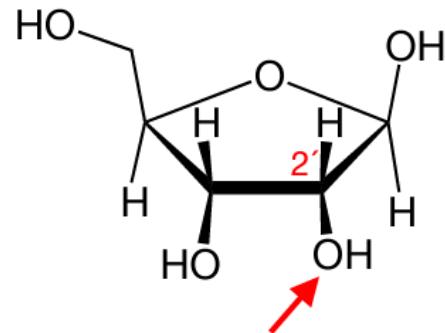
DNA (Replication) → RNA → Proteins
Transcription Translation

Central dogma of life

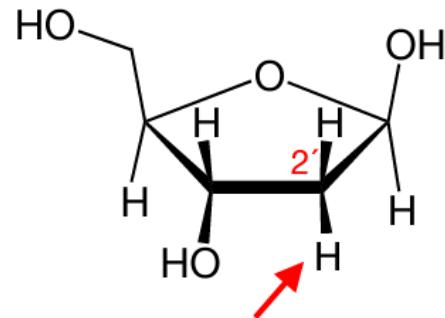


RNA

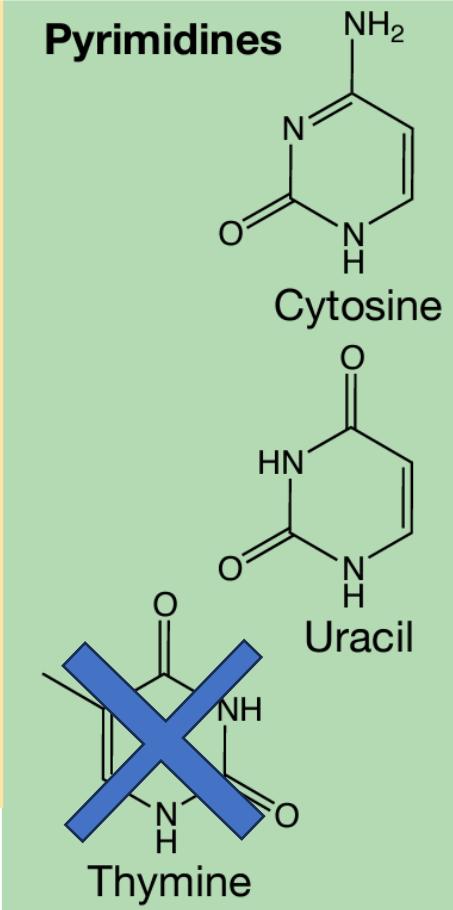
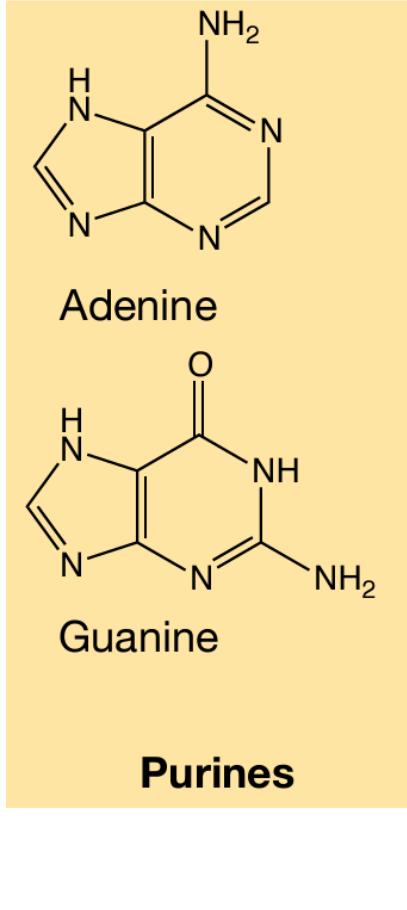
RNA:
Ribose



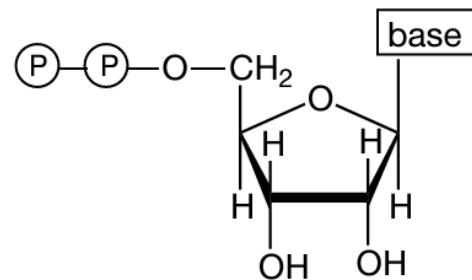
DNA:
Deoxyribose



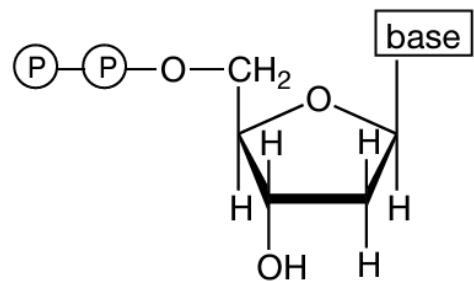
RNA



Ribonucleotide



Deoxyribonucleotide

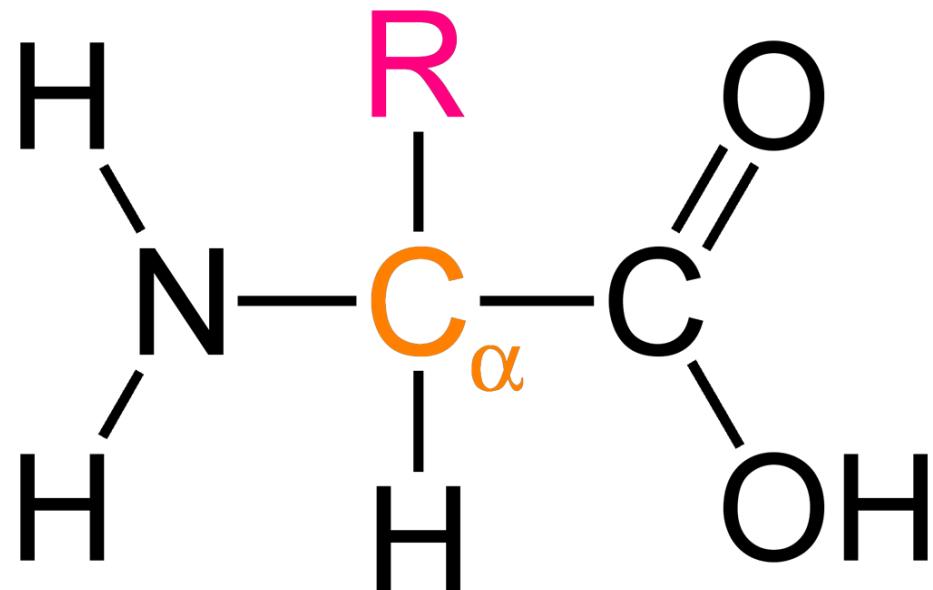


Proteins structure

Proteins: primary structure

Building blocks

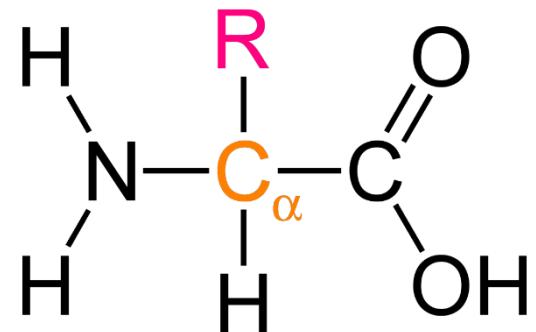
Building block: amino acids



Building blocks

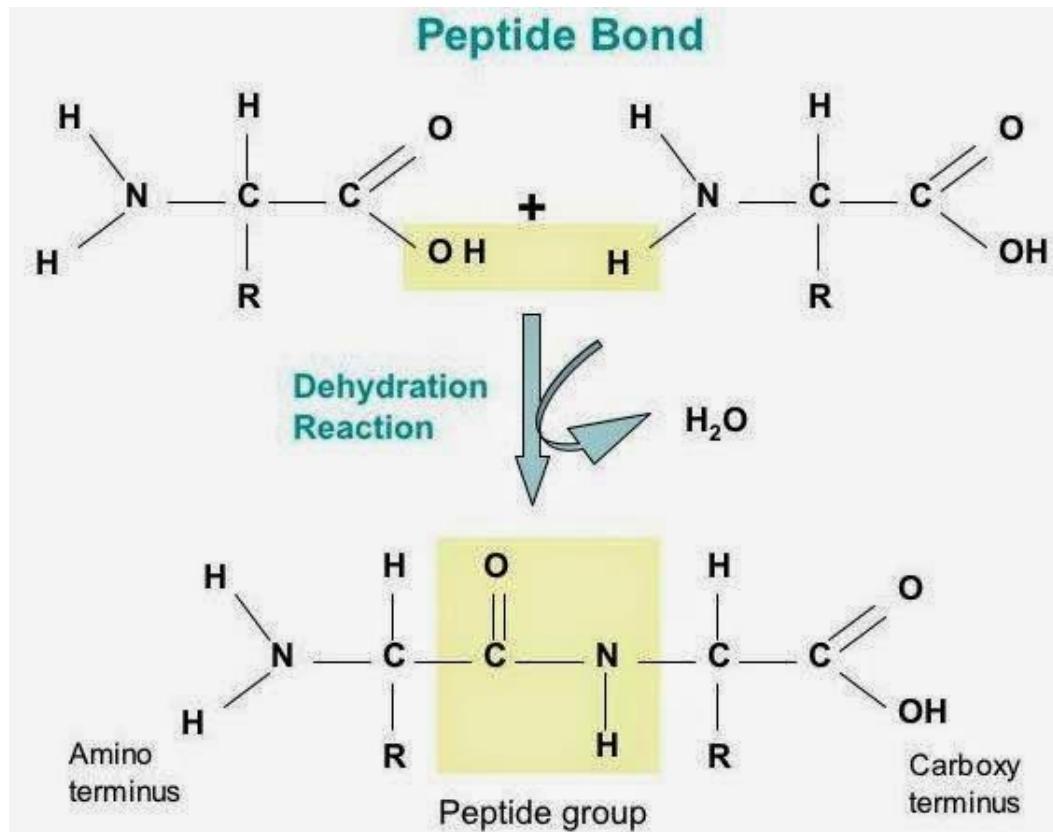
Building block: amino acids

- We have the “big-dog” carbon, or the α -carbon
- We always have a **hydrogen**
- We always have an **amino group**
- We always have a **carboxyl group**
- And some side-chain '**R**' : we will talk about it soon
- Now we are going to stick them together



Building blocks – sticking together

Building block: Stick together through peptide bonds



- Peptide bond is a very special bond: joins together the monomers
- Partial double bond character

Amino acids

hydrophobic



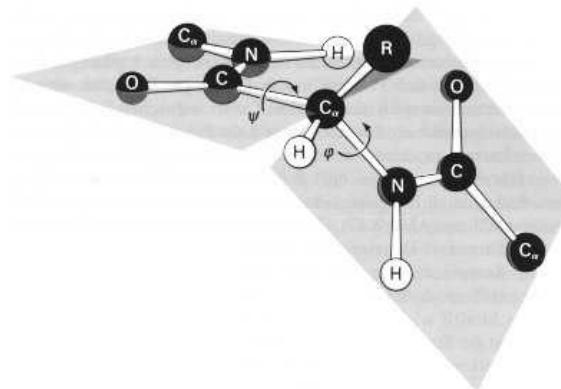
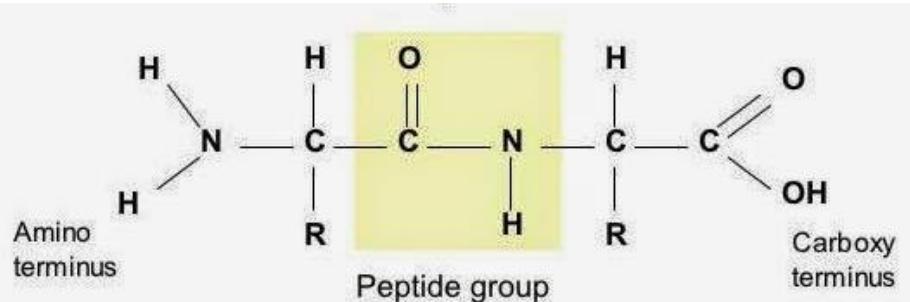
polar; uncharged



polar; charged

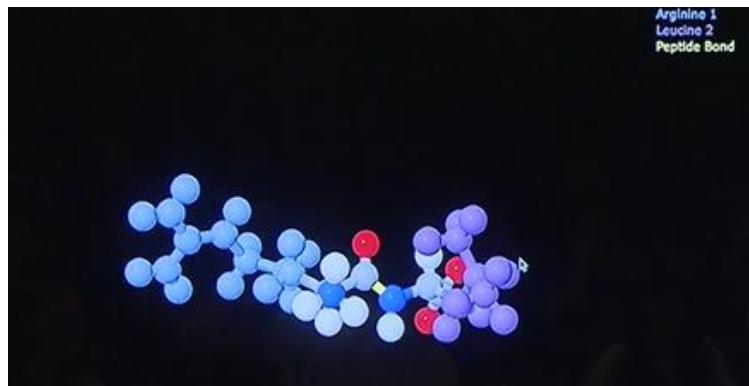


Primary structure of protein

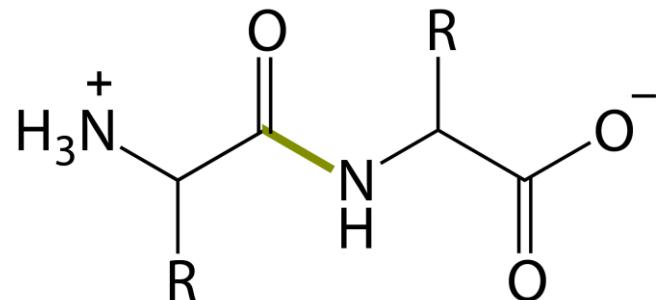


- So now I told you the protein structure was incredibly straightforward.
 - We have simply an amino acid, an amino acid, an amino acid. They get joined together.
 - All we do is we make these peptide bonds here.
 - They have some angles, and we have some different groups.
 - That makes it sound really boring.
 - Peptide means a short chain, a protein is a long chain
-
- **But**, Suppose I have a dipeptide, just two amino acids stuck together.
 - How many options do I have? How many different dipeptides exist?

Let's take a look at the dipeptide



peptide bond



- So what we've got here is the **side chain for arginine** hanging off.
- We've got the **side chain of leucine** hanging off.
hanging off the alpha carbon.
- We have carboxyls (**O**) and amines (**N**)
- What's striking is that peptide backbone we were talking about.
- It's pretty small compared to these side chains.
- The peptide backbone is the thread that's holding this all together.
- But those side chains can be pretty big, and they are very different in their chemical properties.

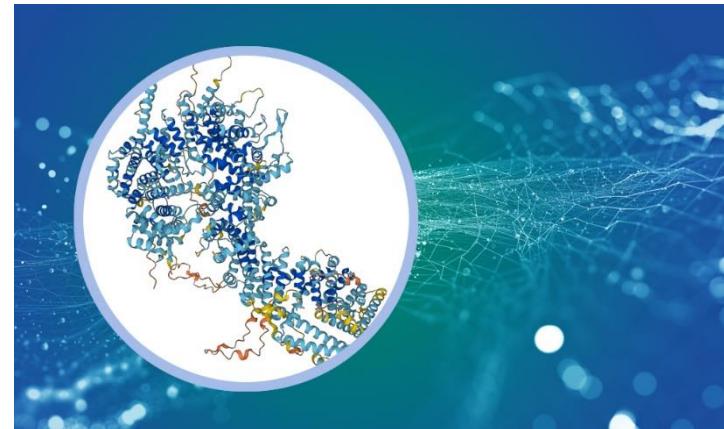
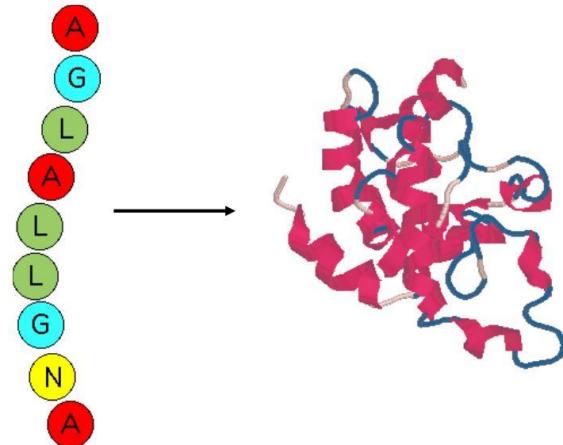
How are you going to fold the protein?



Protein folding problem!

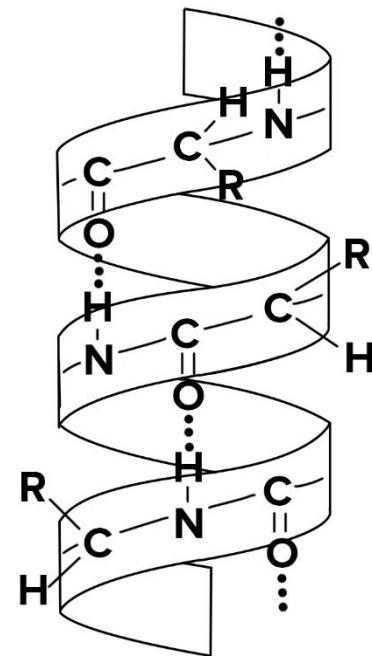
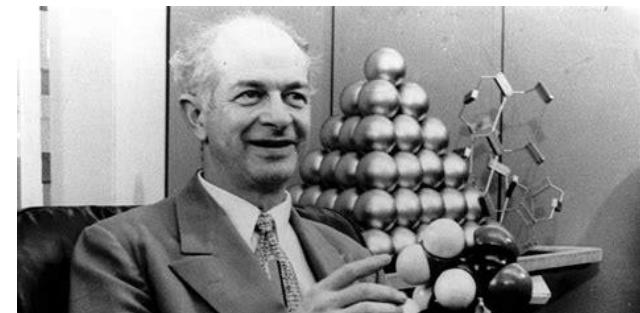
- Problem: I give you the amino acids; you give me the structure.
- What is the solution?
- The "protein folding problem" remains, to this day, unsolved.
- No one can really write a computer program that just takes the sequence of an arbitrary protein and nail it as to exactly what structure it's going to form.
- Although folks are doing better and better and better with protein folding, it's not perfect.

The Protein Folding Problem



The α -helix structure

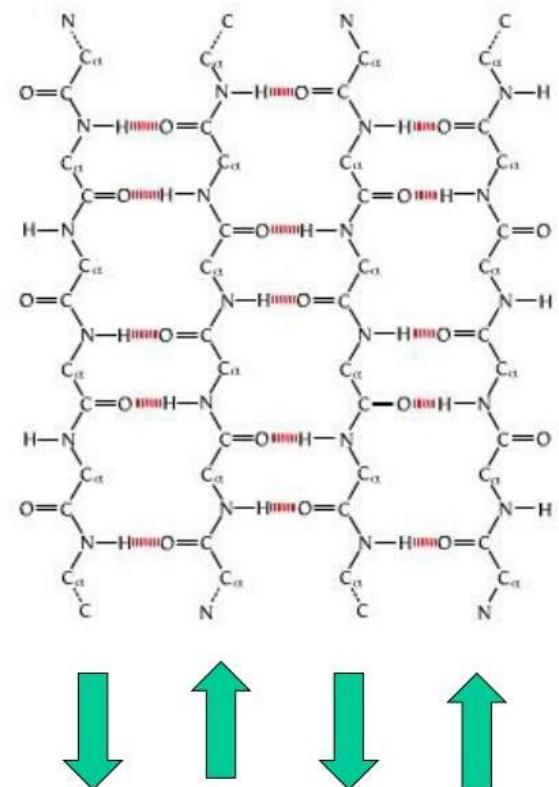
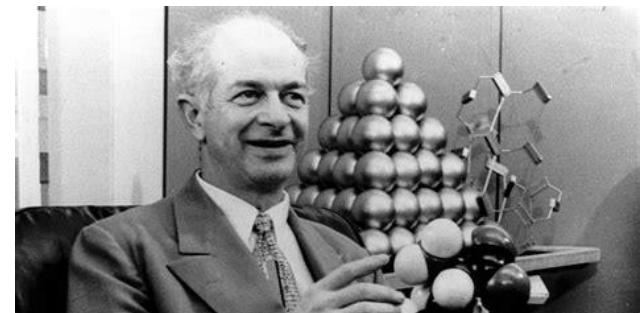
- You get a helix.
- And that helix has 3.7 amino acids per turn by just perfectly lining up the hydrogen bonds that you can have between this carboxyl and the amino.
- And then, onward and onward and onward, every one of them making this beautiful bond, and making these beautiful hydrogen bonds.
- This was the sort of thing that pissed people off.
- Because Linus Pauling, sitting in bed with the flu, is able to come up with the fundamentals of protein structure by just thinking about it.
- You know, this is amazing and did not necessarily endear him to others who didn't think of such things.



Linus calls this the alpha helix.

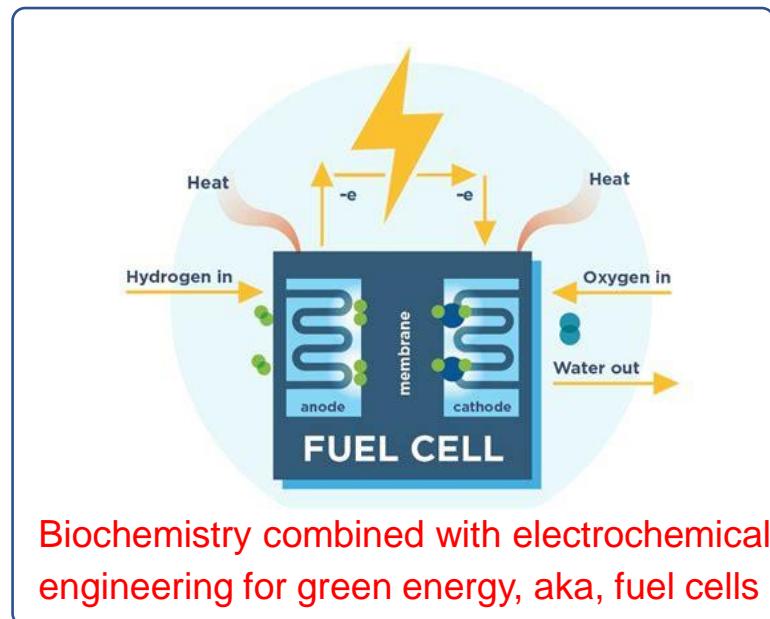
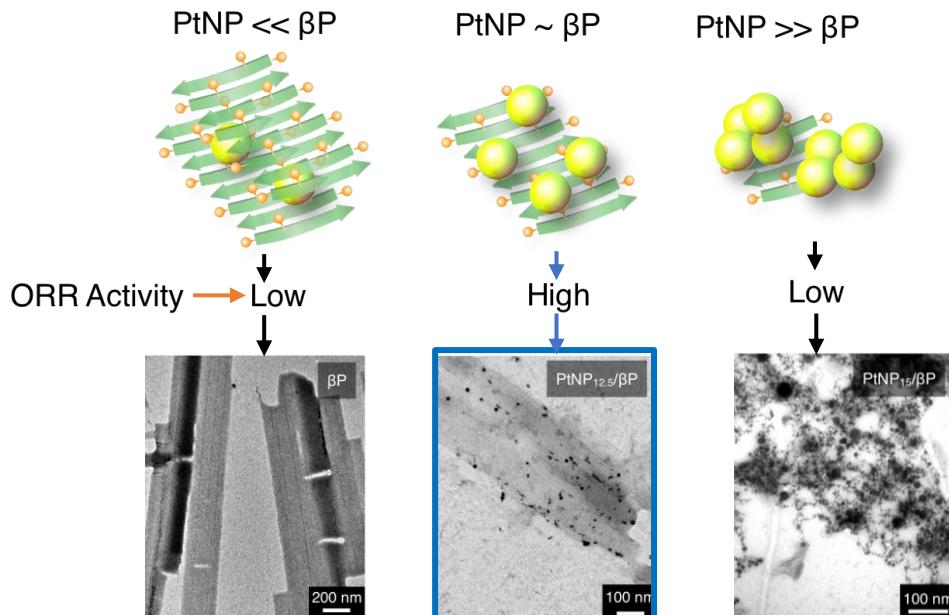
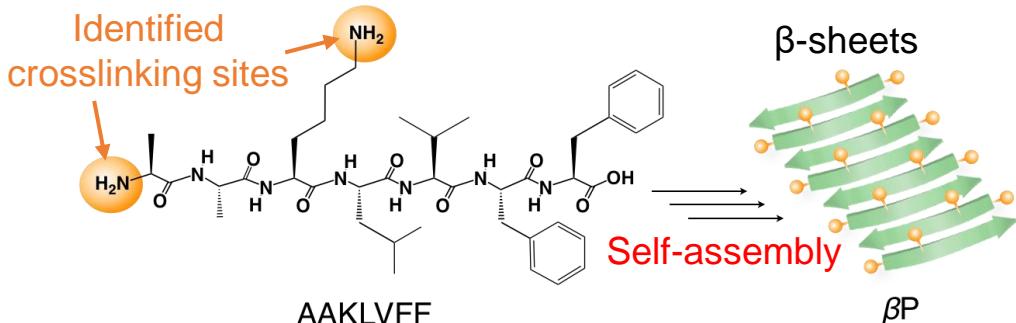
One more structure: the β -sheets!

- Not to be outdone, he also says, there's another way I could imagine using all this hydrogen bonding potential.
- Kinda suppose I have my peptide backbone here, like a chain, and make lots and lots of bonds along a long chain.
- So instead of making a spiral, a helix, I could have two strands, one strand and another strand, and I could have lots of hydrogen bonds between the strands.
- And you can get the strands going back, and again, going back.
- And what you can make are beta sheets.
- But it turns out, Linus was kind of smart here.
- He said, some amino acids are pretty fine with being in an alpha helix and some amino acids, you know, they're better for beta sheets.



Mind-blowing applications: β -sheets for fuel cells

Nanocomposites = AAKLVFF peptide + Pt nanoparticles (PtNP)



Wide range of applications due to the nature of its foldings

Granted Patent

Patent: JP7207658, granted on 2023.01.10; Complex of platinum nanoparticles and peptide carriers and their production



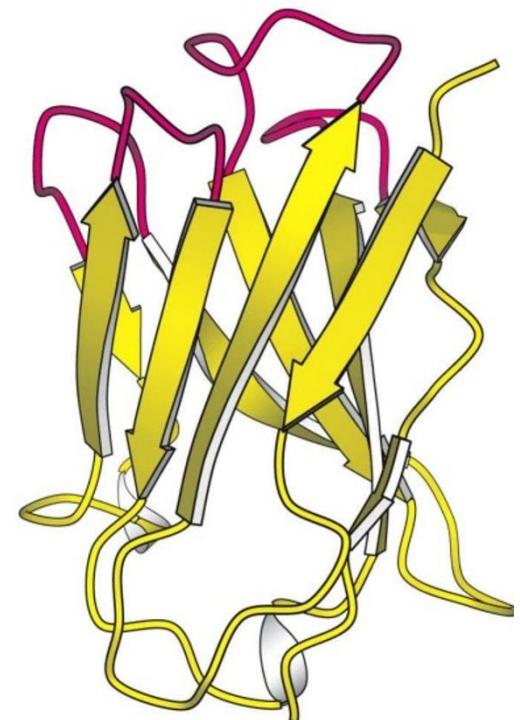
Amandeep Jindal, et. al., ACS Applied Energy Materials, 2019, 2, 6536-6542

One more structure: The Loops

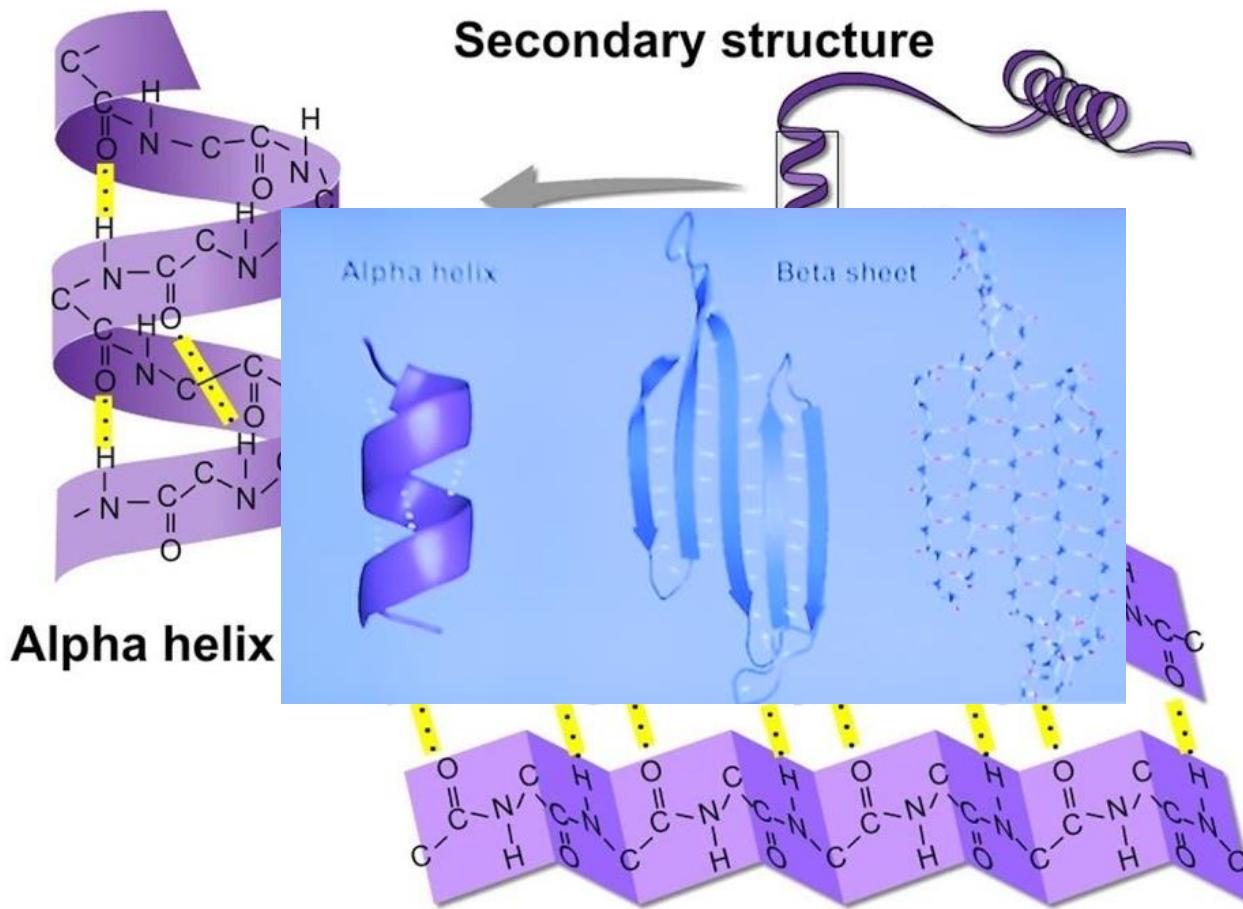
- And then there's another possibility, which is neither of the above.
- Neither of the above is called loops.
- And it means stuff, things that aren't easily classified as beautiful helices, not easily classified as sheets, but kind of more random-y loops.



We can cartoons of the proteins that has β -sheets in part of it, α -helix in part of it and random loops in part of it!



Protein secondary structures



Proteins tertiary structure

- Secondary structure means this local feature.
The local features of, is there a little bit of local alpha helix? Is there a local beta sheet?
- There is also, the next level up, **tertiary structure**.
And that refers to the whole thing.
- So that could be a protein that's got some alpha helix, loops, maybe we'll have some beta sheets there
- That is my rendering of tertiary structure: **It's the overall three-dimensional structure of the protein.**
- So primary structure: just the amino acids in order We don't really care what shape they take up in space.
- Secondary structure: local structure that they have in just little localities, little regions of the protein.
- **Overall tertiary structure, we talk about all of the shape.**

Proteins quartenary structure

- And then it turns out there is **quaternary structure**: the fourth level up--
- And what quaternary structure refers to if we have already described the entire shape of that whole protein, then the quaternary structure is actually the structure when multiple proteins come together.
- And they bond to each other.
- Which bond they might make?

They might make hydrogen bonds.

Maybe they have a nice surface that makes hydrogen bonds to each other.

- Additionally, what other cool bond might you want to make?

Covalent bonds? Maybe- can they make covalent bonds?

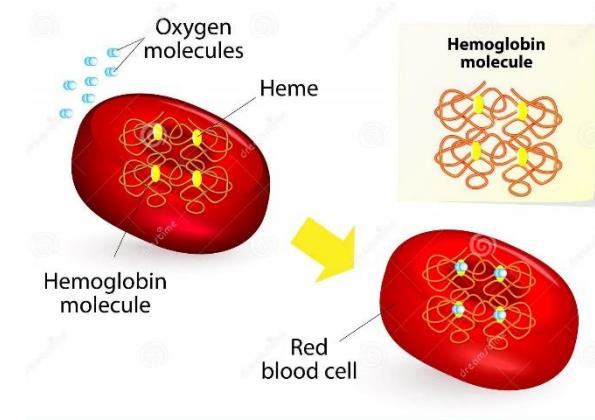
Sometimes cysteines can make covalent disulphide bonds.

Protein folding problem: incredibly hard unsolvable problem!

Some protein examples

Hemoglobin

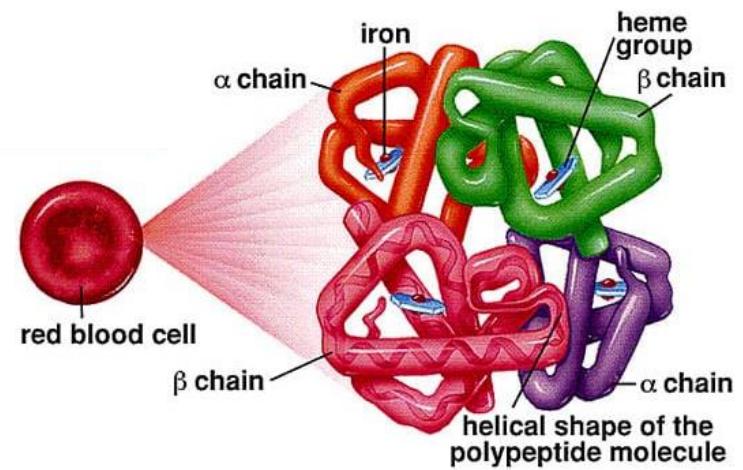
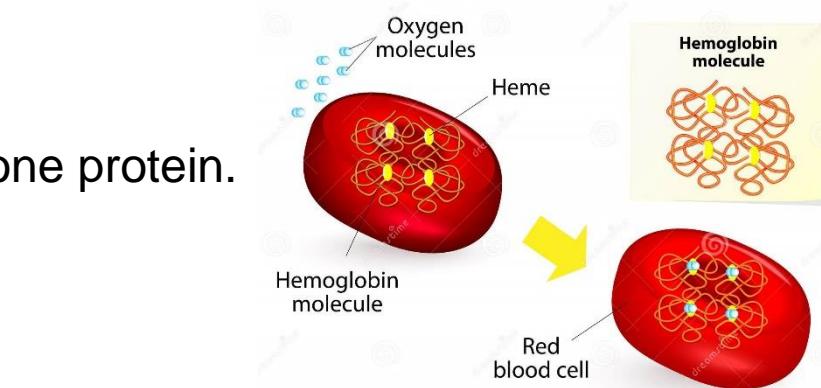
- What does hemoglobin do?
- Binds oxygen, carries it around the blood.
- Why does it bind oxygen to carry it around the blood?
- It gets oxygen to your body.
- Where does it pick up the oxygen?
- In lungs.
- Where does it deliver it?
- Every place else that needs it that isn't the lungs.
- Where does the hemoglobin reside?
- In your red blood cells.
- So your red blood cells are actually bags of almost nothing but hemoglobin,



Hemoglobin is a protein.

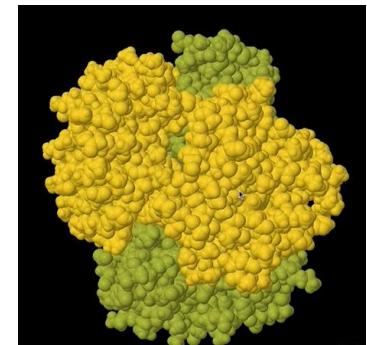
Hemoglobin

- Hemoglobin is a protein
- And in that protein, there's a group called the heme group.
- And the heme group binds an oxygen.
- Now, hemoglobin in your blood is not just one protein.
- It's actually four proteins.
- Hemoglobin has a quaternary structure.
- Hemoglobin is two proteins that are the same here that are called hemoglobin alpha and two proteins called hemoglobin beta.
- Very different from α -helix and β -sheets we were discussing

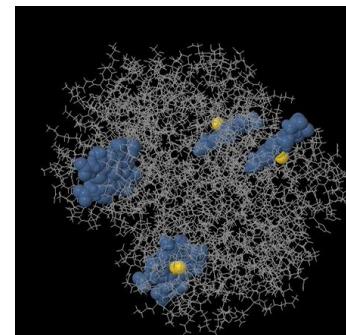


Hemoglobin

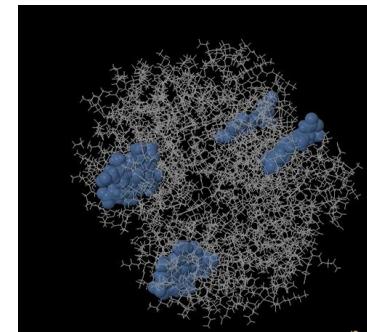
- The two alpha's here--**alpha globin**, beta globin.
- And their surfaces match very nicely, so they stick together to make this quaternary structure.



- I'm just showing the bonds.
- But I have shown those heme groups there.
- The heme groups are the groups that will bind the oxygen.
- And there are four of them here.
- And this is when it's not binding oxygen.

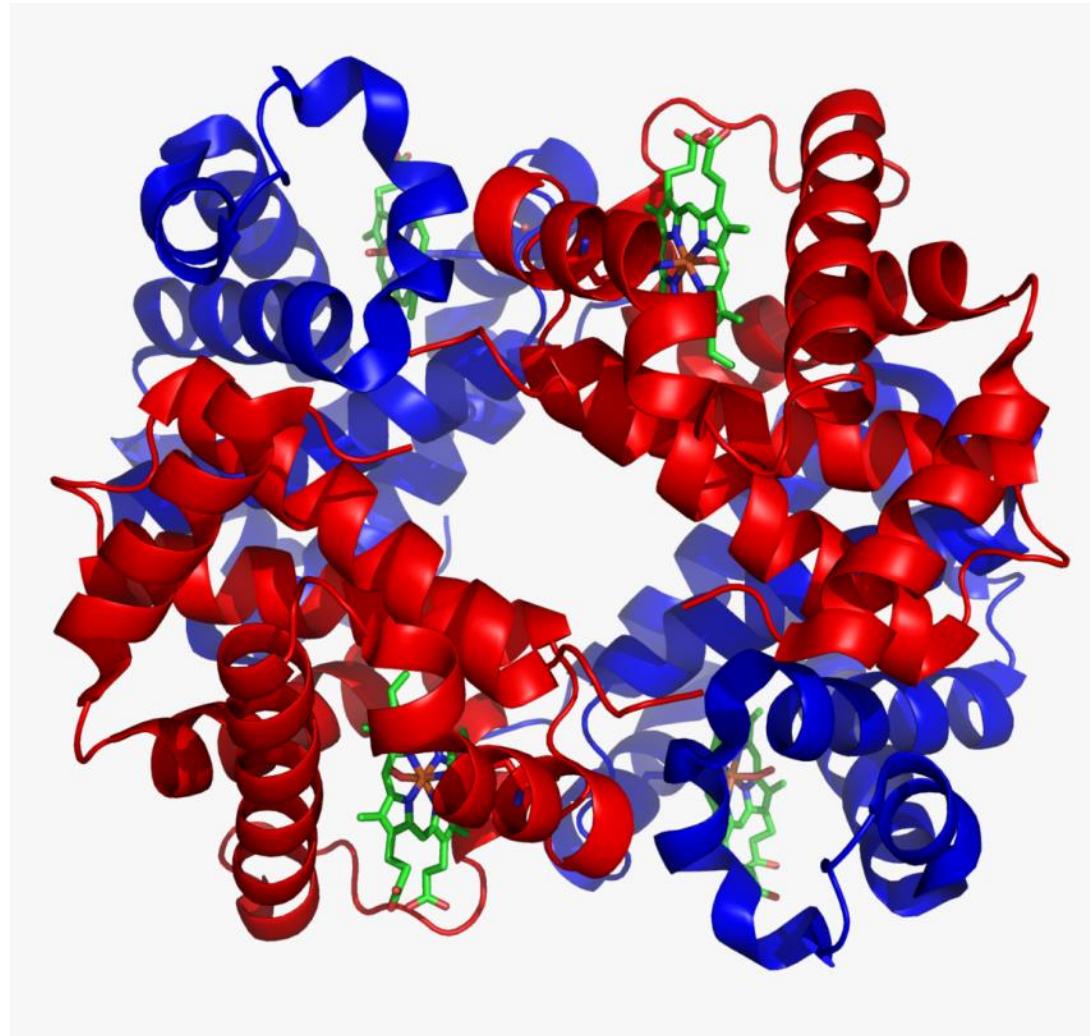


- Now, let's bind **oxygen**.
- The heme group is now binding oxygen.
- What happens to the proteins when it binds to the oxygen?
- Protein changes shape when it binds the oxygen.



Hemoglobin- ribbon structure

Lots of α -helices



Another protein

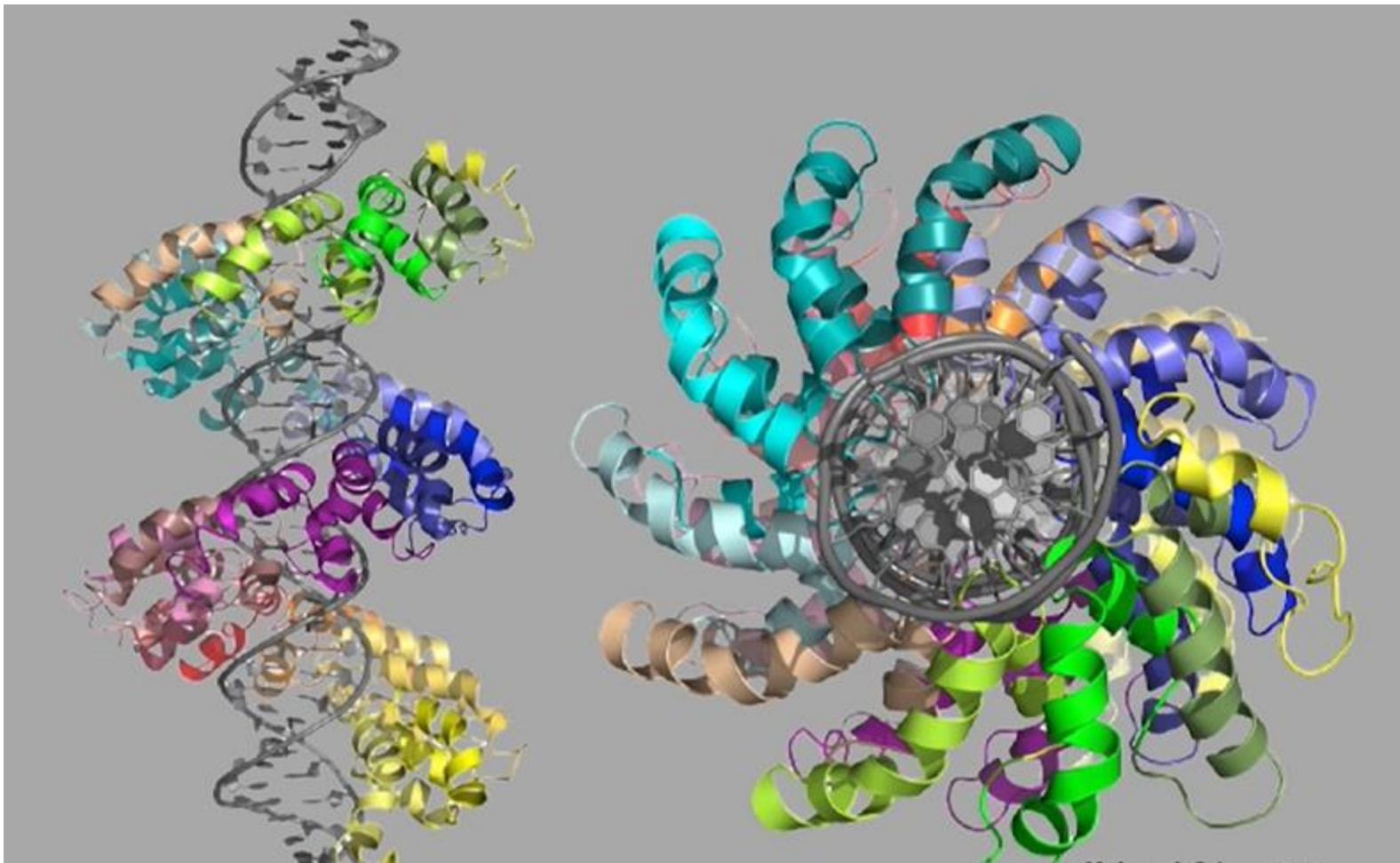
Lots of β -sheets



β -barrels



Another proteins



Enzymes

- Biochemistry
- Thermodynamics
- Kinetics
- Inhibition

Thursday onwards: Heterogenous catalysis

After mid-sems: Bioenergetics and Pathways

Enzymes

What is an enzyme?

Finally...enzymes!

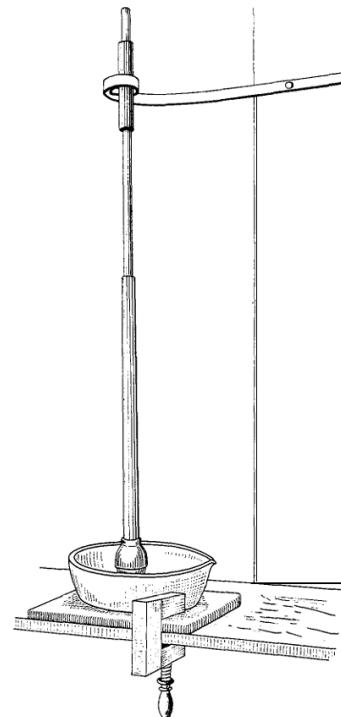
- Proteins are amazingly diverse
- Lipids are good
- Carbohydrates are ok
- Nucleic acids- definitely amazing but still came under the scanner because it looked boring!



Eduard Buchner
(1860-1917)
1897 found fermentation in
broken yeast cells
1907 Nobel Prize in Chemistry

- When we talked about Buchner and the discovery of **enzymes**

The amazing things that could carry out chemical transformations



*That's what we
are planning to
do today-
enzymes*

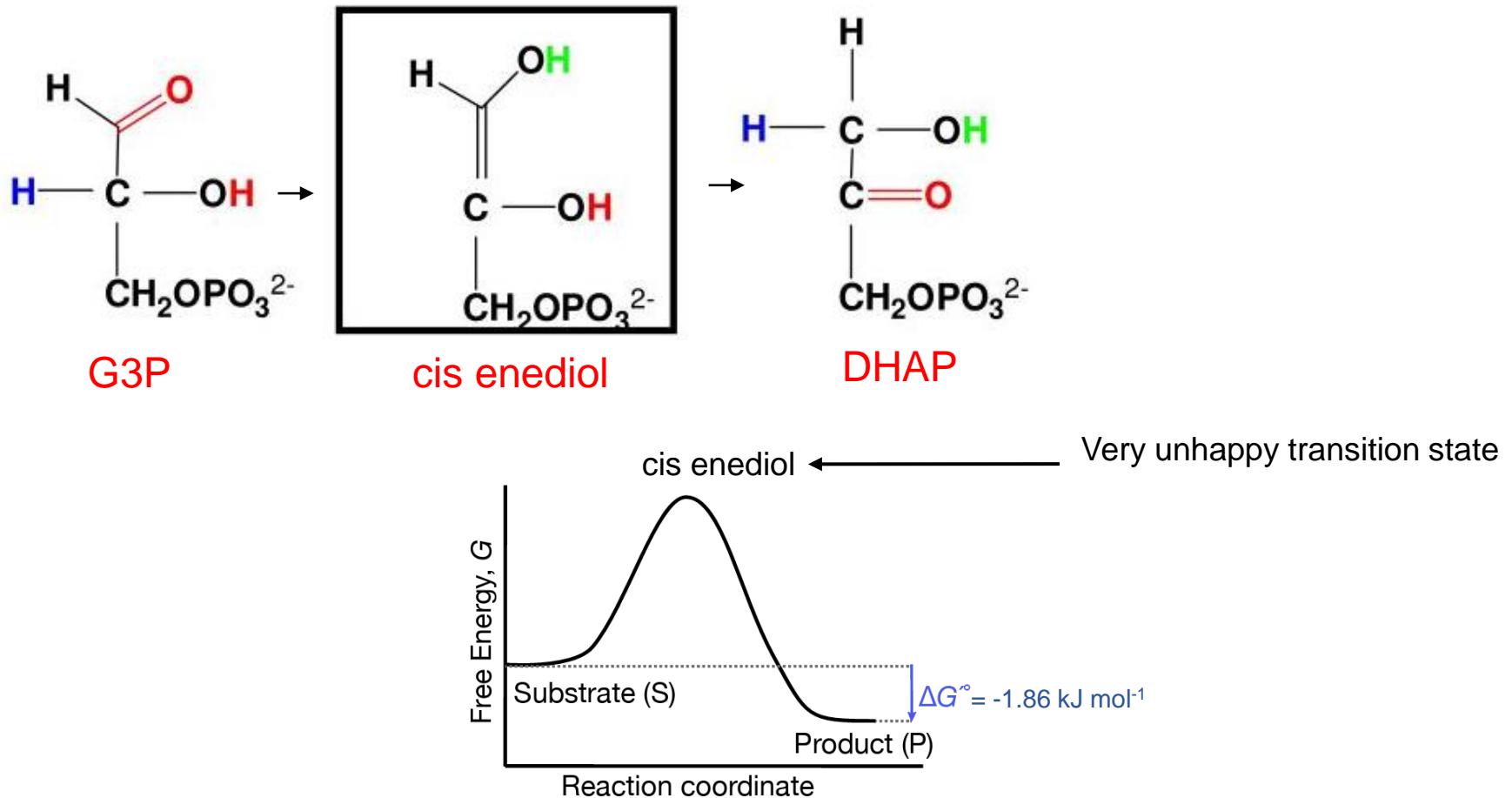
Enzymes

Enzymes are biological catalysts

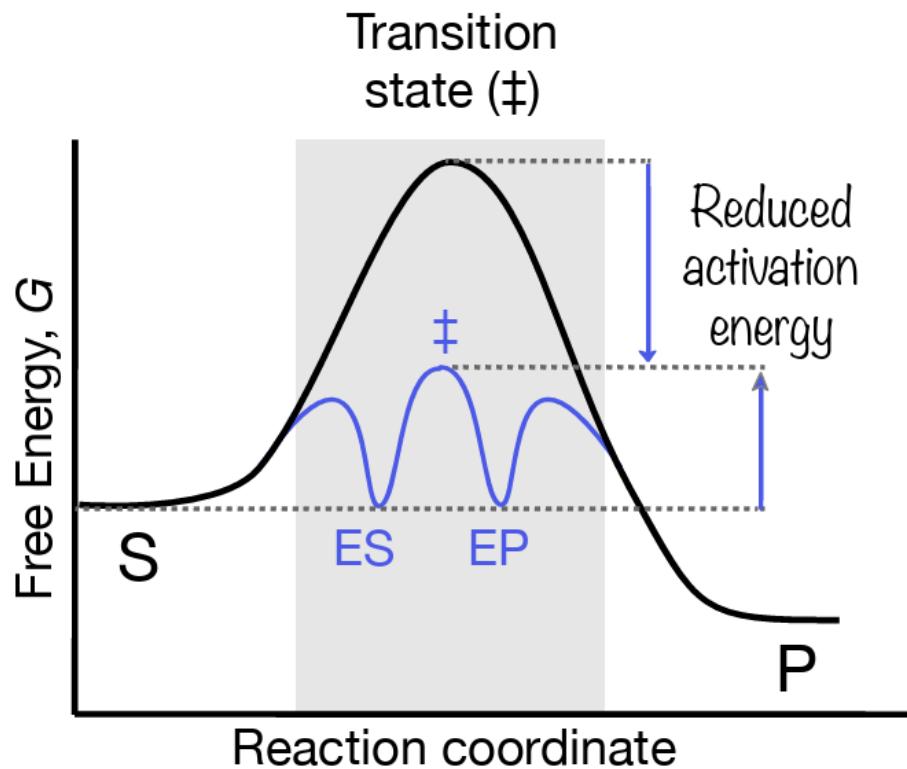
Often proteins

Some are RNA

So why does G3P does not spontaneously become DHAP?



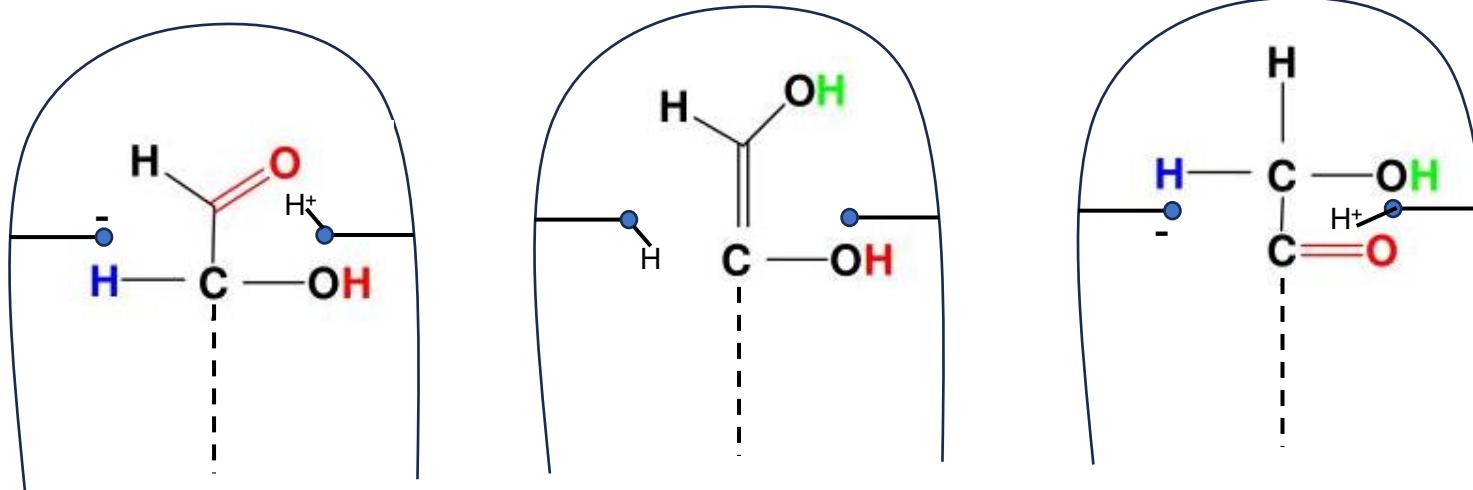
In generality!



Enzymes

How do enzymes work?

So basically this is how enzymes work...

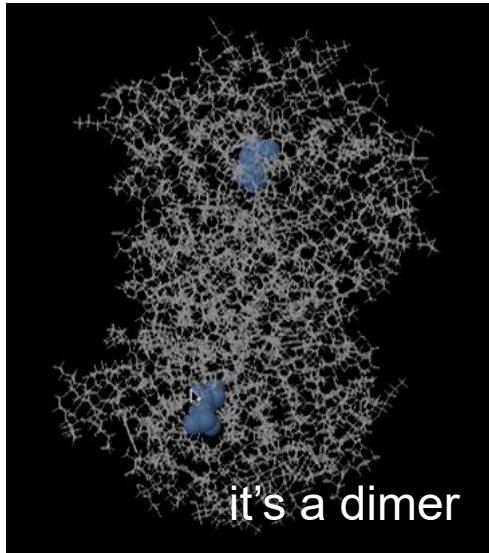
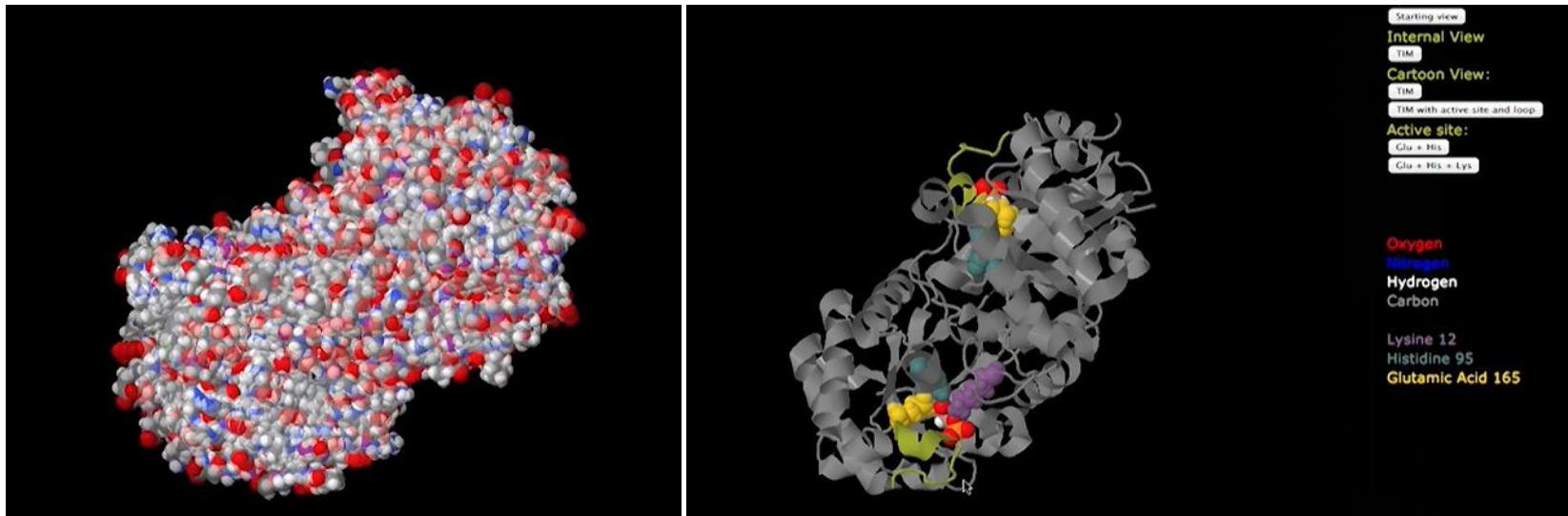


So basically this is how enzymes work:

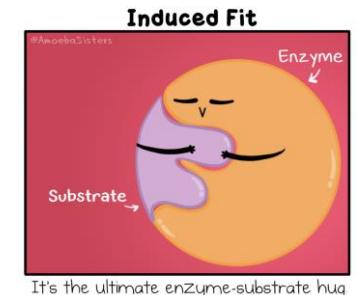
- You've got to have a pocket that binds these triose phosphates.
- And you've got to have a pocket that has amino acid sticking out at exactly the right place and exactly the right distance and exactly the right charge to move those two hydrogens.

And then we studied about the 3 tricks that stabilizes the transient state.

TIM: a better rendition



TIM is really lovingly cradling this molecule.



When I talk about one carbon shorter, it's a big deal

it's a dimer

Enzymes

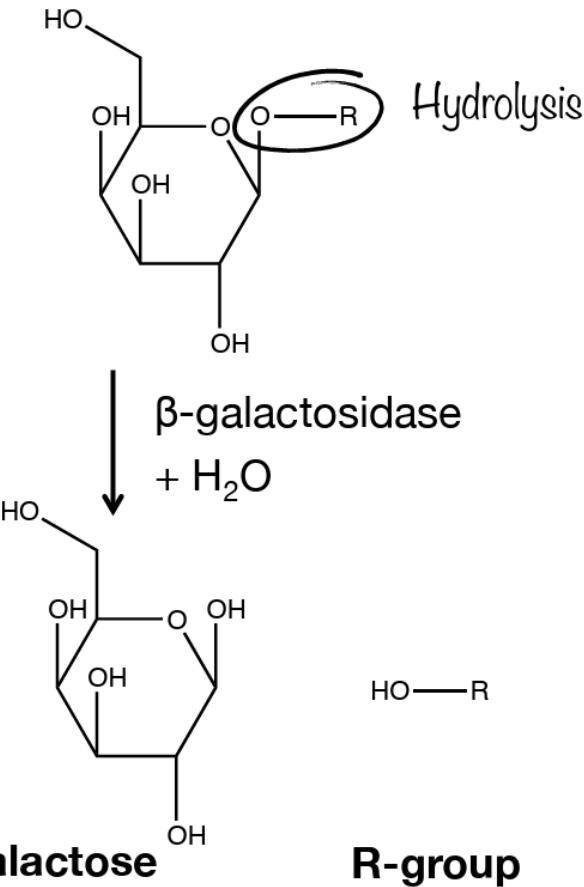
- Biochemistry
- Thermodynamics
- **Kinetics**
- Inhibition

Thursday onwards: Heterogenous catalysis

After mid-sems: Bioenergetics and Pathways

Let us consider another example!

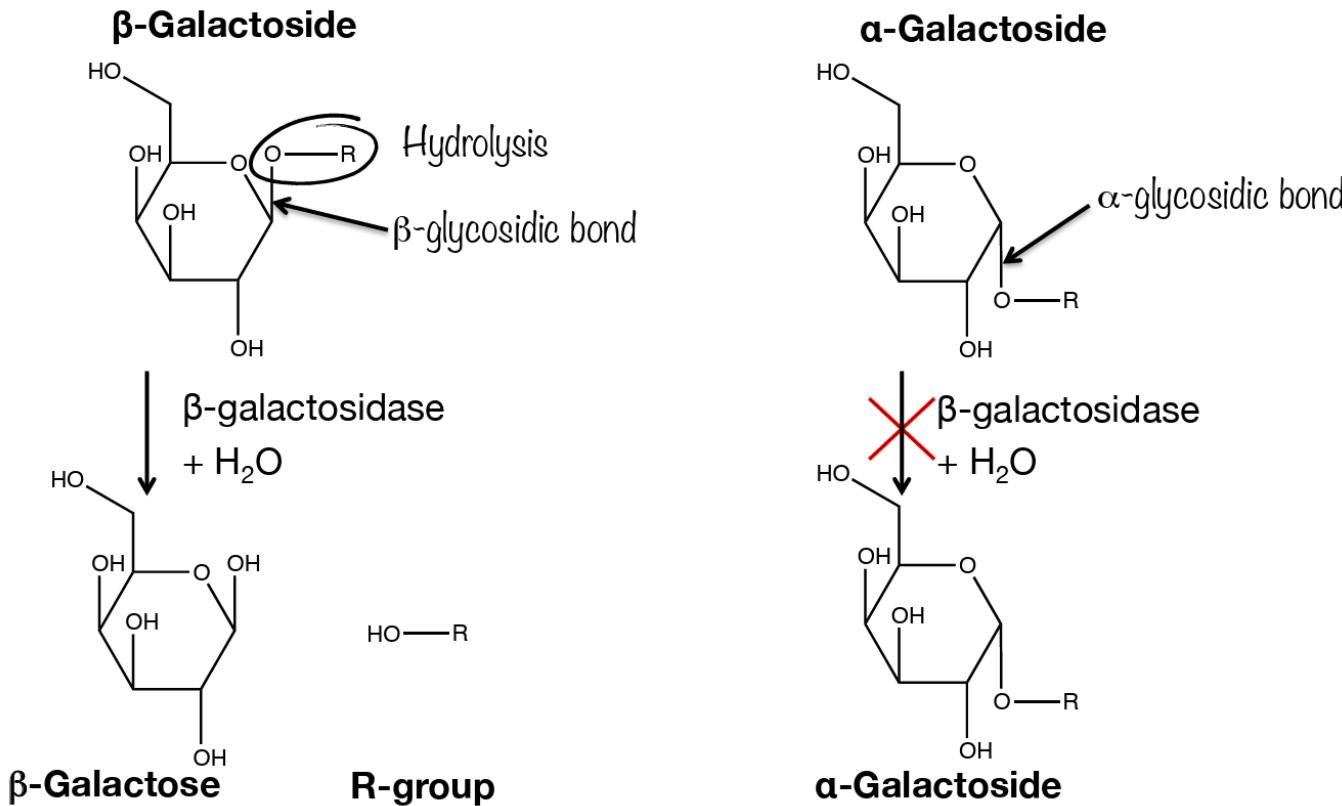
β -galactoside (Lactose)



Remember- lactose intolerance?

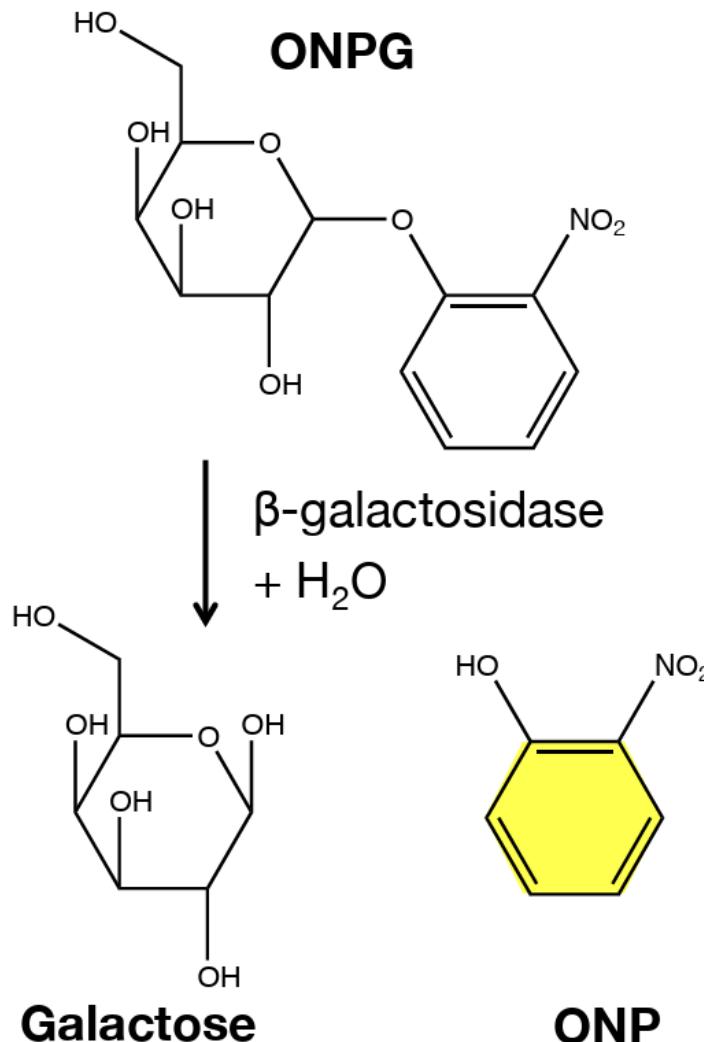


Specificity of β -Galactosidase



This small change prevents the enzyme beta-galactosidase to hydrolyze alpha-galactoside.

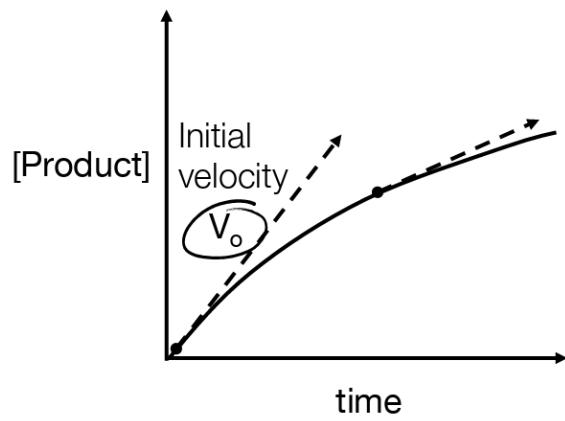
Let us consider another example!



- We are lucky with β -galactosidase as this enzymes the conversion of the structural analogous of β -galactosidase, called, ONPG (Ortho-Nitrophenyl- β -D-Galactopyranoside) into galactose and ONP (Orthonitro phenol).
- And while **ONPG is colorless**, **ONP is yellow**.

So we can have a very simple colorimetric assay that we can use to measure the velocity of the reaction.

How is the color related to the amount of ONP?

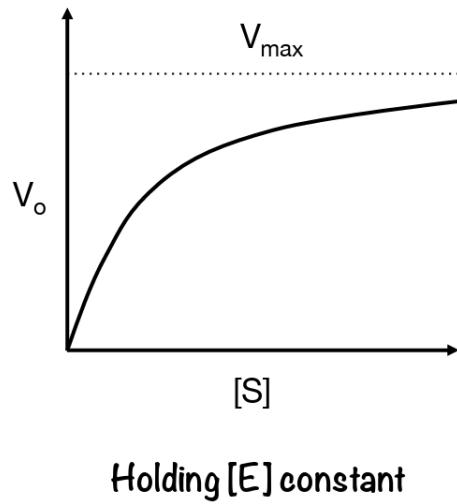


$$V = \frac{d[P]}{dt}$$

- Let's plot the concentration of product the intensity of the yellow color-- as a function of the time.
- What we get is an hyperbolic curve.
- And you remember that the velocity of the reaction is the amount of product formed over time.
- Graphically, this velocity represents in fact, the slope of the tangent at any point along the curve.

V_{max}

What we see on the graph is that

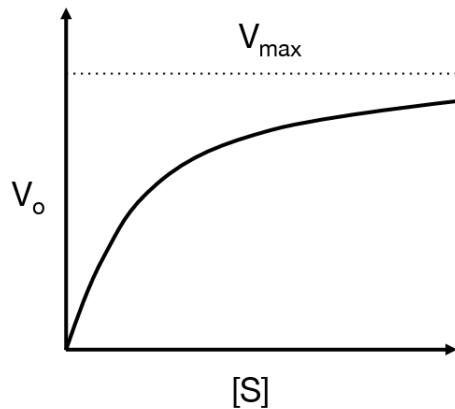


when the concentration of substrate is relatively low →

as the concentration of substrate increases, V_o increases as well.

But then after some time, we have a situation where any increase of the concentration of substrate does not affect significantly the initial velocity.

V_{max}



Holding [E] constant

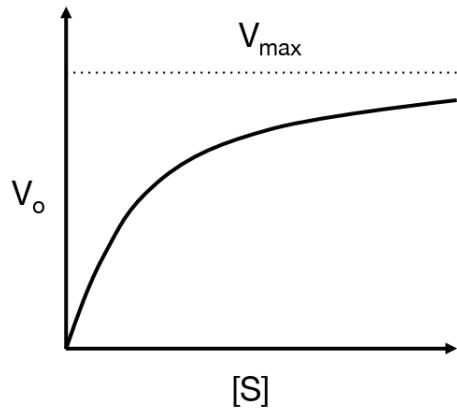
It is very easy to figure out why this initial velocity reaches a plateau.

When an enzyme reacts with a substrate, it forms an enzyme-substrate complex.

If the concentration of enzyme is maintained constant as we increase the concentration of substrate, the enzyme becomes the limiting factor.

All the enzyme is engaged into an enzyme-substrate complex.

V_{\max}



Holding [E] constant

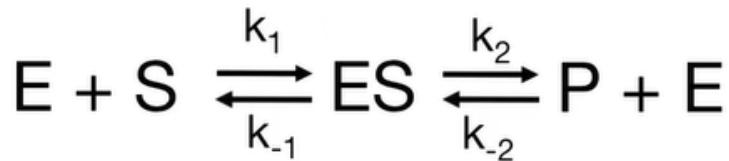
And any addition of substrate will not change the velocity of the reaction and will not change the initial velocity.

So now we can define this maximum initial velocity that we call V_{\max} .

And the V_{\max} of the reaction is a characteristic feature of our enzyme.

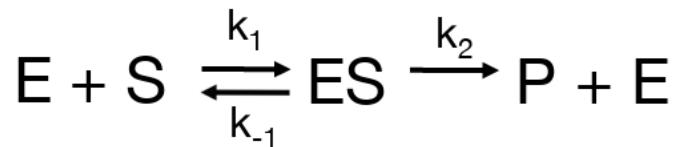
Michaelis and Menten mathematical model describing enzyme kinetics

The reaction



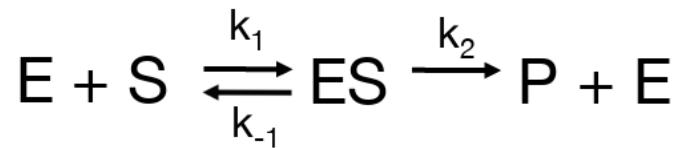
- If we look at this step of the reaction, the enzyme first reacts with a substrate to form an enzyme substrate complex.
- This reversible reaction has constant rates, which are k_1 for the forward and k_{-1} for the reverse.
- Then the substrate on the enzyme is converted into a product.
- And we have a release of the product plus the free enzyme.
- This reaction is also reversible with the rate constant k_2 and k_{-2}

Is the last step really reversible?



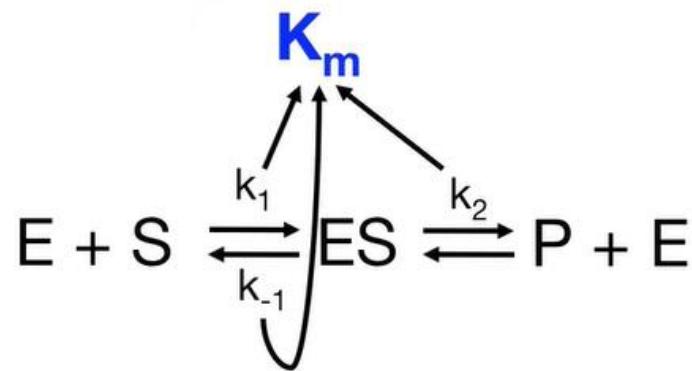
- Well, yes-- this second step of the reaction is reversible.
- However, because our study of kinetics is during the early phases of the reaction.
- At that stage there is a very, very low concentration of product.
- So we can consider that the reverse reaction-- the one with the rate constant k_{-2} -- is negligible.
- And for now on, what we will do is that we will ignore this reverse reaction.
- And consider that the second step in our reaction is irreversible.

Our first task: Combining k_1 , k_2 and k_{-1}

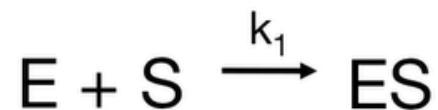


Our first task is to combine the three rate constants-- k_1 , k_2 , k_{-1} --

and create a single constant, the **Michaelis and Menten constant, or K_m**



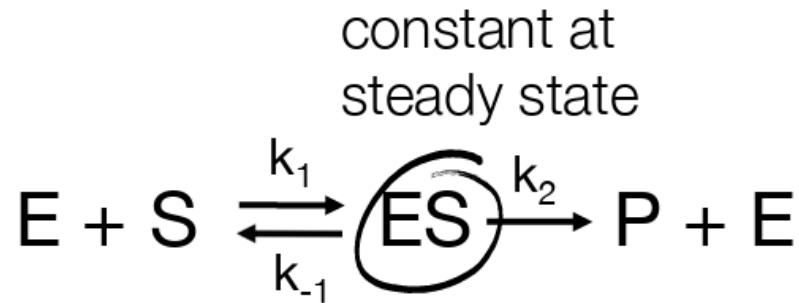
In the first few microseconds...



In the first few microseconds, the concentration of enzyme-substrate builds up

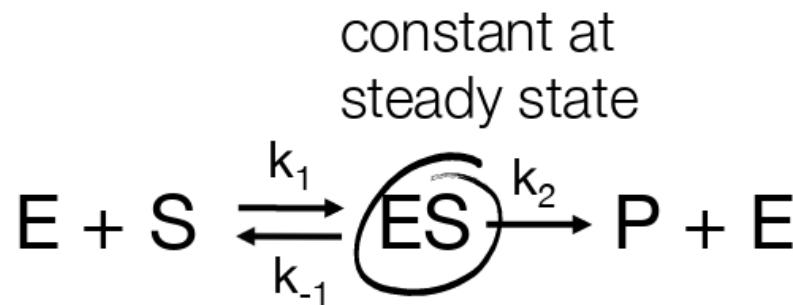
That is what we call the **presteady state**.

Then we enter the steady state....



Then we enter **steady state**, where the concentration of the enzyme-substrate complex is constant.

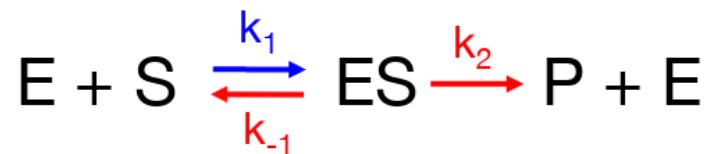
Calculate rate of formation of ES complex



formation of ES $V_1 = k_1 [E] [S]$

- When we study the kinetics of enzymes in steady state,
- the first thing that we have to do is in fact to determine the rate of formation of the enzyme-substrate complex.
- This rate is in fact a product of the rate constant-- here k_1 -- times the concentration of the starting material-- here the concentration of substrate and the concentration of enzyme.
- So what we have is V_1 -- the velocity of formation of the enzyme-substrate complex-- equals k_1 times $[E]$ times $[S]$.

Calculate rate of dissociation of ES complex



formation of ES $V_1 = k_1 [E] [S]$

degradation of ES $V_2 = k_{-1} [ES] + k_2 [ES]$
 $= [ES] (k_{-1} + k_2)$

- And this complex can dissociate in two different ways.
 1. We can have production of enzyme plus product,
 2. We can have the reverse reaction, where the enzyme dissociates from the substrate.
- So the rate of the degradation of the enzyme substrate complex is in fact the sum of k_{-1} times the concentration of the enzyme-substrate complex and k_2 times the concentration of the enzyme-substrate complex.

At steady state



formation of ES $V_1 = k_1 [E] [S]$

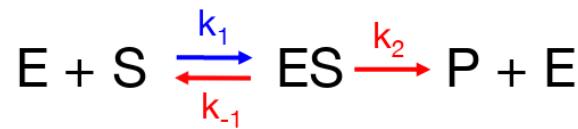
degradation of ES $V_2 = k_{-1} [ES] + k_2 [ES]$
 $= [ES] (k_{-1} + k_2)$

steady state $V_1 = V_2$

$$k_1 [E] [S] = [ES] (k_{-1} + k_2)$$

- We are at steady state.
- That means that these two rates are equal because the concentration of the enzyme-substrate complex is constant.

Rearranging to get Michaelis-Menten constant



$$k_1 [E] [S] = [ES] (k_{-1} + k_2)$$

$$\frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

K_m is Michaelis-Menten constant

What is Michaelis-Menten constant?



$$k_1 [E] [S] = [ES] (k_{-1} + k_2)$$

$$\frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

High K_m : low substrate affinity

Low K_m : high substrate affinity

What is the meaning of this value K_m ?

High $K_m \rightarrow$ means $[ES]$ is low,
which suggests that the enzyme
has a very low affinity for the
substrate.

Low $K_m \rightarrow$ means $[ES]$ is high,
means that there is very little
enzyme that is free,

which suggests that under this
condition the enzyme has a high
affinity for its substrate.

What is Michaelis-Menten constant?



$$k_1 [E] [S] = [ES] (k_{-1} + k_2)$$

$$\frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

High K_m : low substrate affinity

Low K_m : high substrate affinity

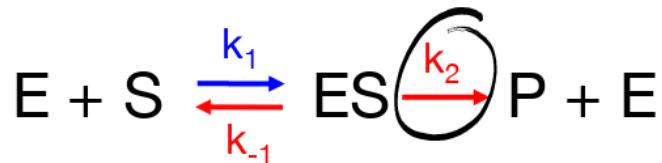
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which suggests that the enzyme
has a very low affinity for the
substrate.

Low $K_m \rightarrow$ means $[ES]$ is high,
means that there is very little
enzyme that is free,

which suggests that under this
condition the enzyme has a high
affinity for its substrate.

If the 2nd step is the rate-limiting step...



What does it mean for k_2 ?

$$k_1 [E] [S] = [ES] (k_{-1} + k_2)$$

It means k_2 is very small compared to the other rate constant.

when k_2 is
rate-limiting: $\frac{k_{-1}}{k_1} \approx K_m$

High K_m : low substrate affinity

Low K_m : high substrate affinity

Let us get serious for a moment!



$$\frac{[E][S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

- If we look at our equation right now, we have terms that are very difficult to determine.
- We know how much total enzyme we added in the reaction.
- But it's very difficult to determine the concentration of free enzyme, E, and the concentration of the enzyme-substrate complex, ES.

What we do know...



What we know is that the **total concentration of enzyme** is the sum of the concentration of free enzyme and the concentration of enzyme-substrate complex.

$$\frac{[E][S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

$$[E_T] = [E] + [ES]$$

$$[E] = [E_T] - [ES]$$

What we do know...

$$\frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

$$[E_T] = [E] + [ES]$$



$$\begin{aligned} K_m &= \frac{[E] [S]}{[ES]} \\ &= \frac{([E_T] - [ES]) [S]}{[ES]} \end{aligned}$$

$$[E] = [E_T] - [ES]$$

$$= \left(\frac{[E_T]}{[ES]} - 1 \right) [S]$$

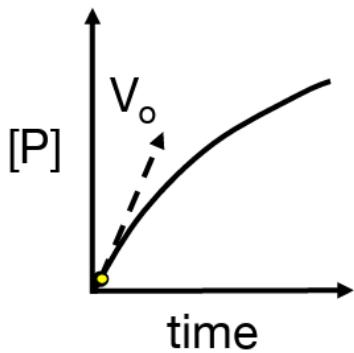
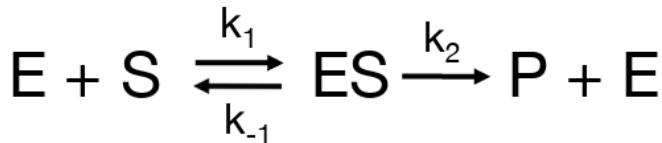
We still have an unknown term

$$K_m = \left(\frac{[E_T]}{[ES]} - 1 \right) [S]$$

We still have unknown [ES]

Let's go back...

$$K_m = \left(\frac{[E_T]}{[ES]} - 1 \right) [S]$$



$$V_o = k_2 [ES]$$
$$V_{\max} = k_2 [E_T]$$

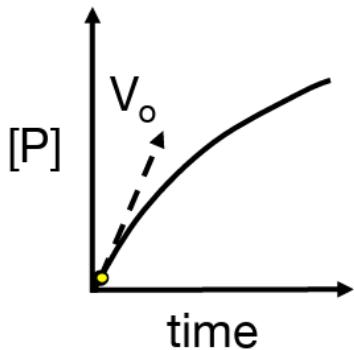
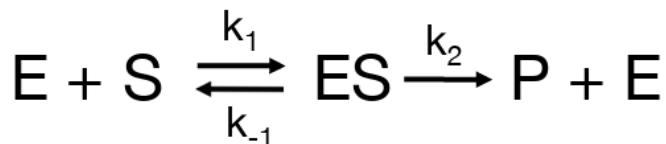
Max rate:
all enzyme is in
ES complex

At very high [S]
The reaction reached V_{\max}

All the E is associated with S
to form ES

Let's go back...

$$K_m = \left(\frac{[E_T]}{[ES]} - 1 \right) [S]$$



$$V_o = k_2 [ES]$$
$$V_{max} = k_2 [E_T]$$

Max rate:
all enzyme is in
ES complex

$$\rightarrow \frac{V_{max}}{V_o} = \frac{k_2 [E_T]}{k_2 [ES]} = \frac{[E_T]}{[ES]}$$

This ratio appears in our equation for K_m .

$$K_m = \left(\frac{[E_T]}{[ES]} - 1 \right) [S]$$

So we could replace [ES]

$$\frac{V_{max}}{V_o} = \frac{k_2 [E_T]}{k_2 [ES]} = \frac{[E_T]}{[ES]}$$

This ratio appears in our equation for K_m .

$$K_m = \left(\frac{[E_T]}{[ES]} - 1 \right) [S] \quad \Rightarrow \quad K_m = \left(\frac{V_{max}}{V_o} - 1 \right) [S]$$

So we could replace [ES]

$$K_m = \left(\frac{V_{max}}{V_o} - 1 \right) [S]$$

- Now we have two variables-- V_0 and a concentration of substrate, $[S]$.
- And we have constant terms K_m and V_{max} :
- We have eliminated all the terms that are hard to determine.
- Now if we continue to rearrange this equation,
we can express V_0 as a function of the concentration of S.

Writing V_o in terms of [S]

Finally – Michaelis Menten equation

$$K_m = \left(\frac{V_{max}}{V_o} - 1 \right) [S]$$

$$= \frac{V_{max}}{V_o} [S] - [S]$$

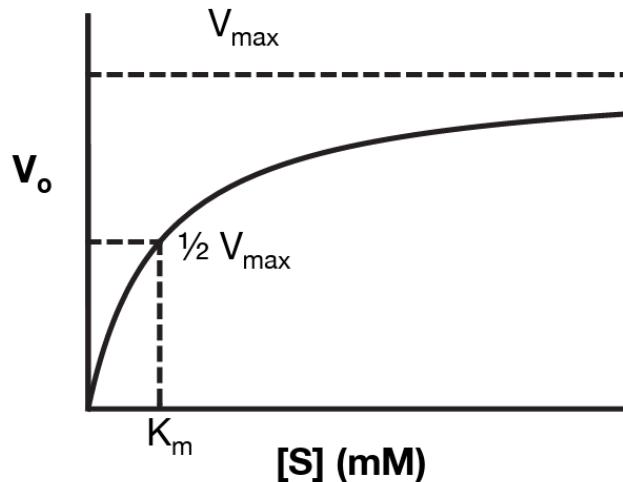
$$V_o = \frac{V_{max} [S]}{K_m + [S]}$$

Michaelis Menten equation

Michaelis Menten equation

Michaelis Menten equation

$$V_o = \frac{V_{\max} [S]}{K_m + [S]}$$



When we plot the experimental values of V_o as a function of the concentration of S , $[S]$:

we obtained a hyperbolic curve.

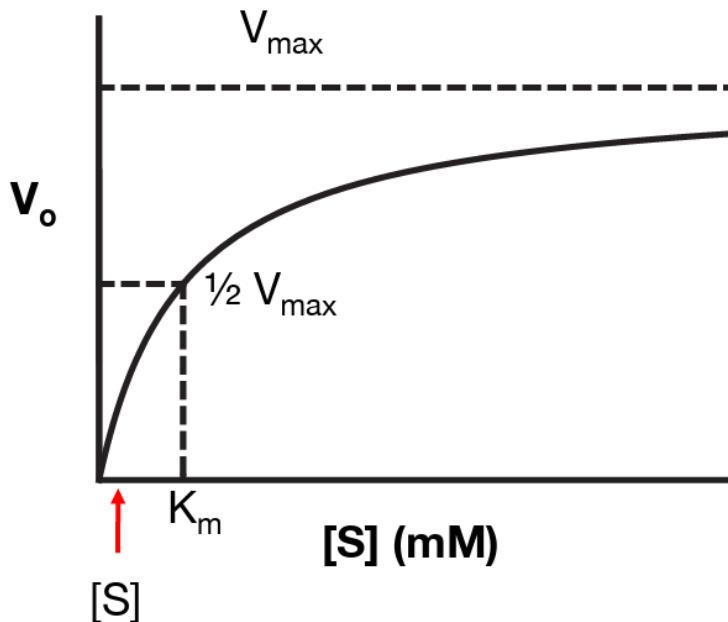
So in fact, the Michaelis and Menten equation fits the hyperbolic curve that is determined experimentally.

What can we predict from the Michaelis and Menten equation?

Consider very low [S]

Michaelis-Menten with low [S]

$$V_o = \frac{V_{max} [S]}{K_m + [S]} \quad \xrightarrow{\text{red arrow}} \quad V_o = \frac{V_{max} [S]}{K_m}$$

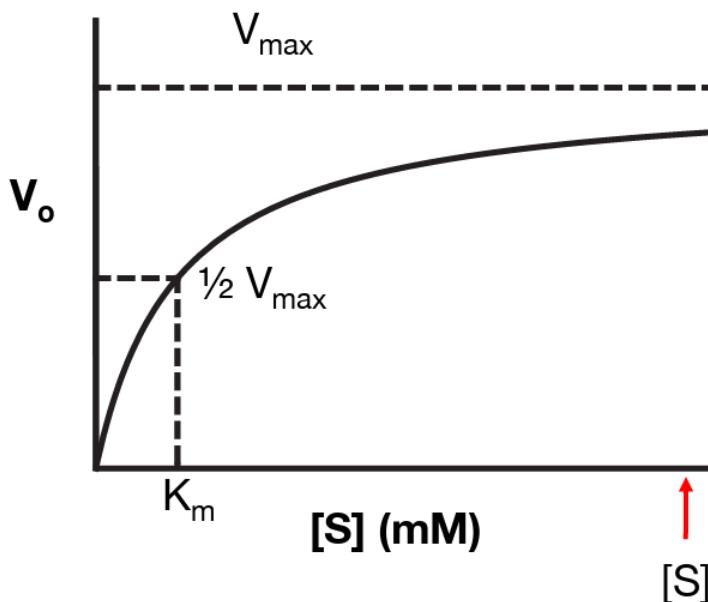


- Well, we can, for example, consider that this concentration of substrate is so low that it's negligible compared to K_m .
- In this case, the velocity of the reaction is directly proportional to the concentration of substrate.
- This is not very good for a cell where you have a very low concentration of substrate, then the reaction is very sensitive to any changes in the concentration of substrate.
- **Enzyme will never work at its full potential**

Consider very high [S]

Michaelis Menten with high [S]

$$V_o = \frac{V_{\max} [S]}{K_m + [S]} \xrightarrow{\text{red arrow}} V_o = \frac{V_{\max} [S]}{[S]} = V_{\max}$$

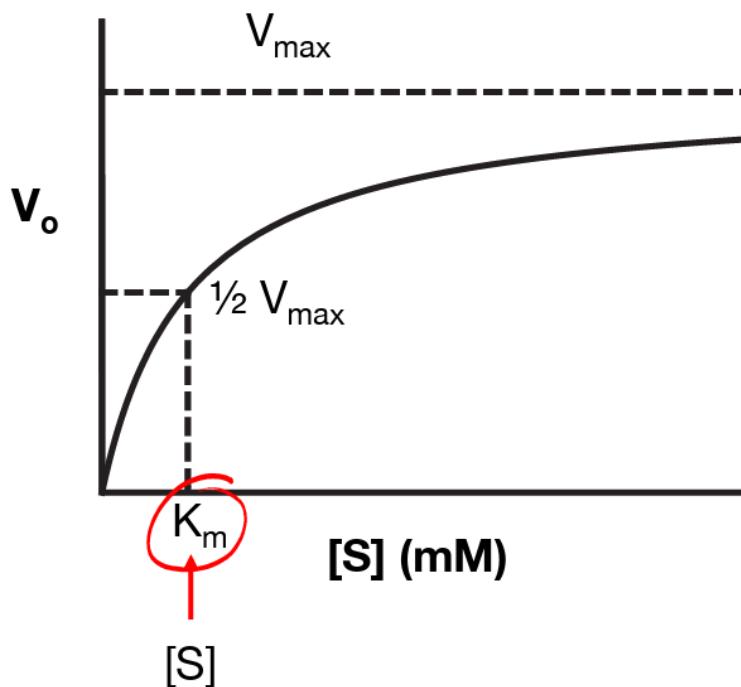


- If we take the opposite example, we have a very high concentration of substrate, much higher than K_m .
- Now V_o will be equal to V_{\max} .
- Is that better for the cell?
- Not at all, because in this condition the cell cannot fine tune the activity of the enzyme. The enzyme will always work at its maximum capacity.
- And it would be a wasteful degradation of substrate even when the product of the reaction is not needed.

Consider intermediate $[S] = K_m$

Michaelis-Menten when $[S] = K_m$

$$V_o = \frac{V_{max} [S]}{K_m + [S]} \rightarrow V_o = \frac{V_{max} [S]}{2 [S]} = \frac{V_{max}}{2}$$



- This is an optimum condition for the cell.
- Because the enzyme can both be sensitive to changes in the concentration of substrate, but can also reach its maximum velocity.
- And this also gave us a new definition for K_m .
 K_m is the concentration of substrate needed to reach half of maximum velocity.

Enzymes

- Biochemistry
- Thermodynamics
- Kinetics
- Inhibition

Thursday onwards: Heterogenous catalysis

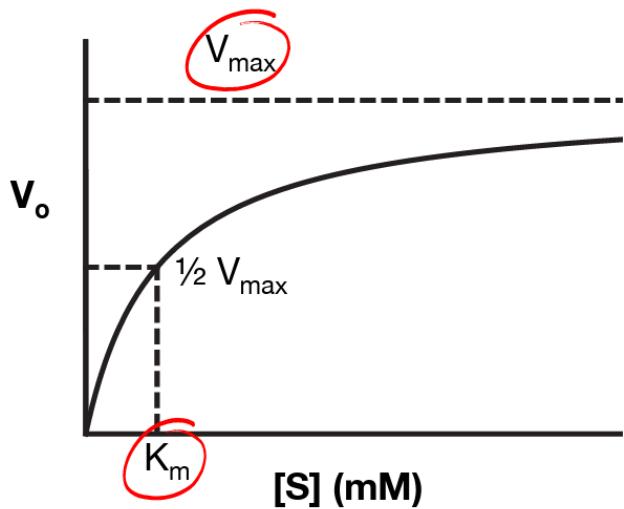
After mid-sems:

Assignment-2
Bioenergetics and Pathways

Michaelis Menten equation

Michaelis Menten equation

$$V_o = \frac{V_{\max} [S]}{K_m + [S]}$$



When we draw the hyperbolic curve that corresponds to the plot of V_o vs $[S]$,

we estimate V_{\max} and K_m

But we need a more accurate calculation

How can we improve accuracy?

It will be more accurate if we could determine K_m and V_{\max} from a straight line.

So we need to linearize the Michaelis and Menten equation?

Linearizing M M equation using Basic Maths

Michaelis
Menten $V_o = \frac{V_{max} [S]}{K_m + [S]}$

Reciprocal seems a little easy to solve!

→ $\frac{1}{V_o} = \frac{K_m + [S]}{V_{max} [S]}$

→ $\frac{1}{V_o} = \frac{K_m}{V_{max} [S]} + \frac{\cancel{[S]}}{V_{max} \cancel{[S]}}$

→ $\frac{1}{V_o} = \left(\frac{K_m}{V_{max}} \right) \left(\frac{1}{[S]} \right) + \frac{1}{V_{max}}$

Lineweaver-Burk equation

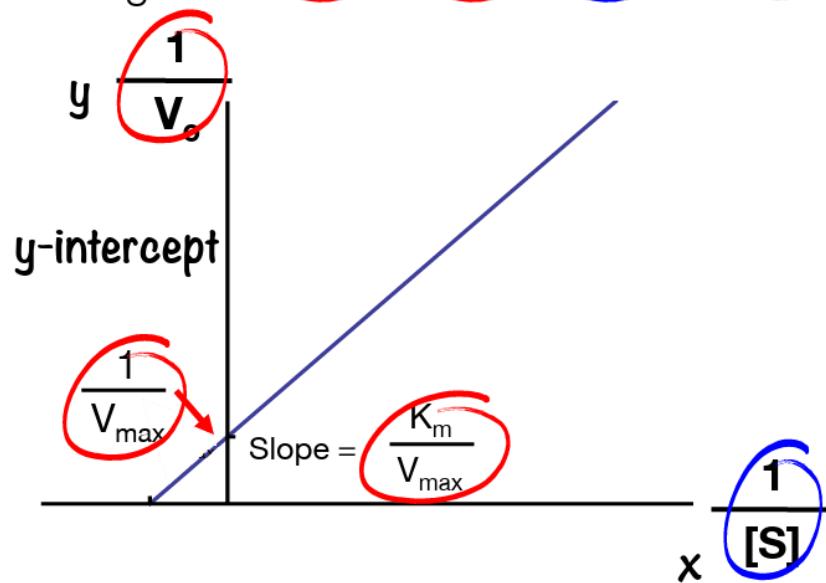
Lineweaver-Burk

Lineweaver-Burk equation

$$\frac{1}{V_o} = \left(\frac{K_m}{V_{max}} \right) \left(\frac{1}{[S]} \right) + \frac{1}{V_{max}}$$

equation of straight line

$$y = m x + b$$



Lineweaver-Burk equation

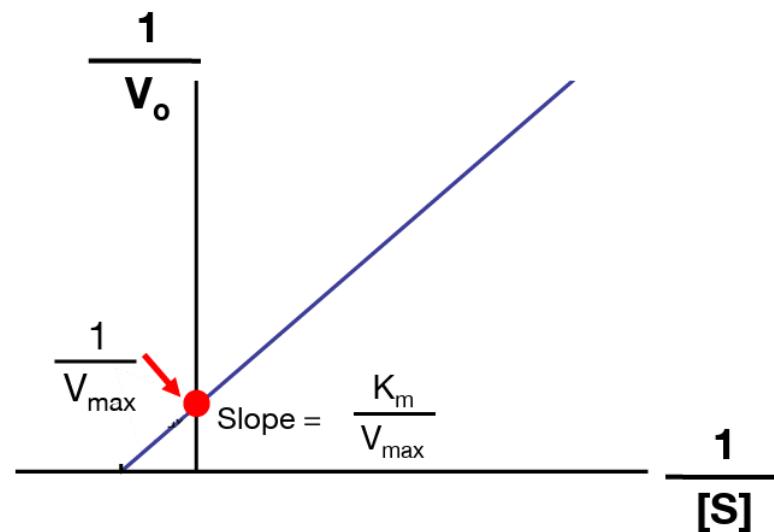
Lineweaver-Burk equation

Lineweaver Burk: y-intercept

$$\frac{1}{V_o} = \left(\frac{K_m}{V_{max}} \right) \left(\frac{1}{[S]} \right) + \frac{1}{V_{max}}$$

≈ 0

$$\rightarrow \frac{1}{V_o} = \frac{1}{V_{max}}$$



Lineweaver-Burk equation

Lineweaver Burk: x-intercept

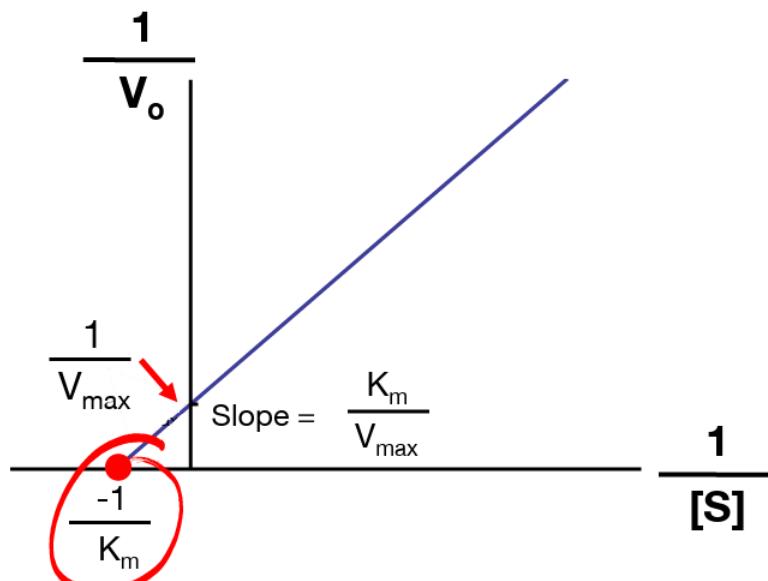
$$\frac{1}{V_o} = \left(\frac{K_m}{V_{max}} \right) \left(\frac{1}{[S]} \right) + \frac{1}{V_{max}}$$
$$\approx 0$$

$$\rightarrow \frac{K_m}{V_{max} [S]} = \frac{-1}{V_{max}}$$

$$\rightarrow \frac{K_m}{[S]} = -1$$

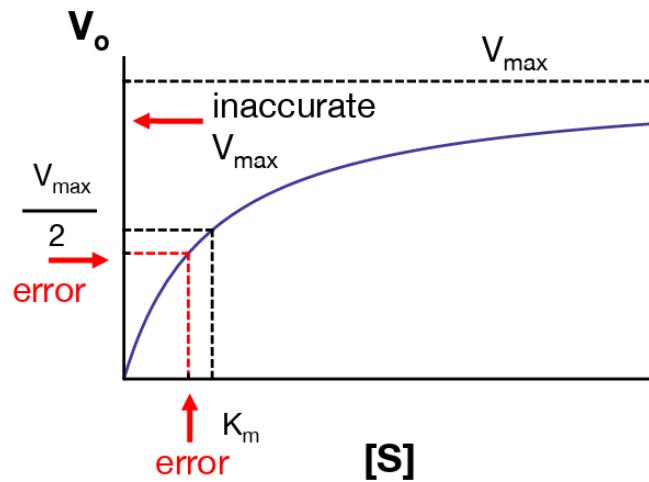
$$\rightarrow \frac{1}{[S]} = \frac{-1}{K_m}$$

Lineweaver Burk

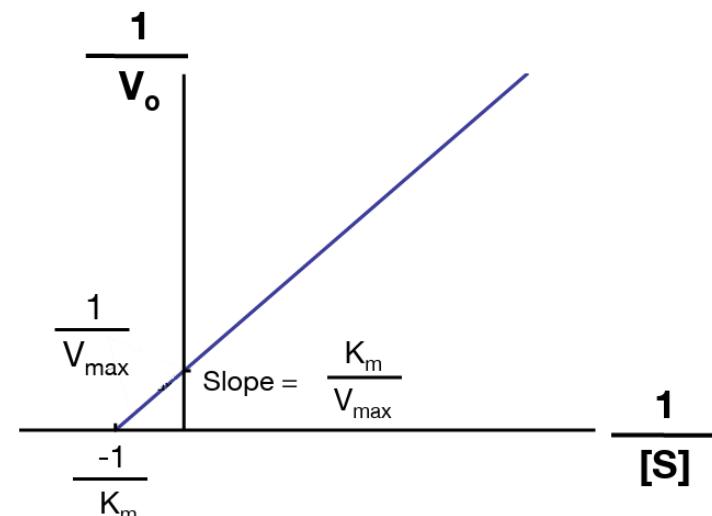


Lineweaver-Burk equation is more accurate!

Michaelis Menten



Lineweaver Burk



less error when
determining K_m

Enzymes

- Biochemistry
- Thermodynamics
- Kinetics
- Inhibition

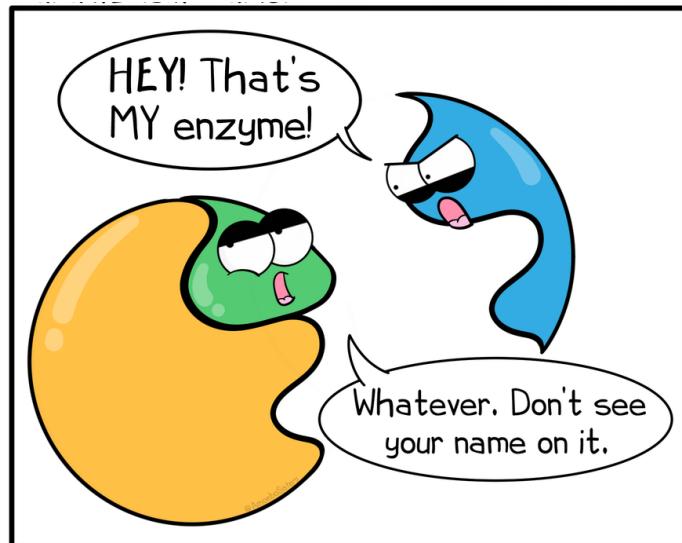
Thursday onwards: Heterogenous catalysis

After mid-sems:

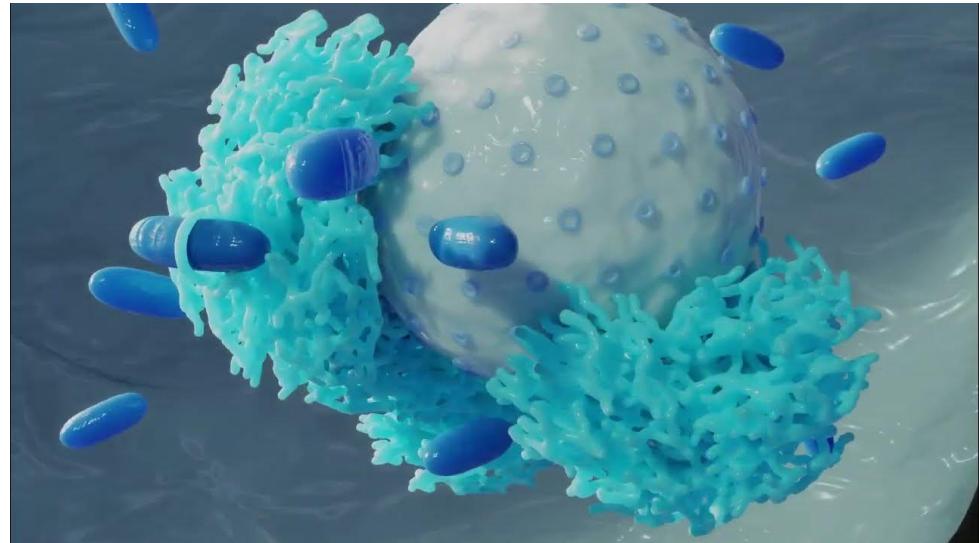
Assignment-2
Bioenergetics and Pathways

Activity of enzymes need to be regulated!

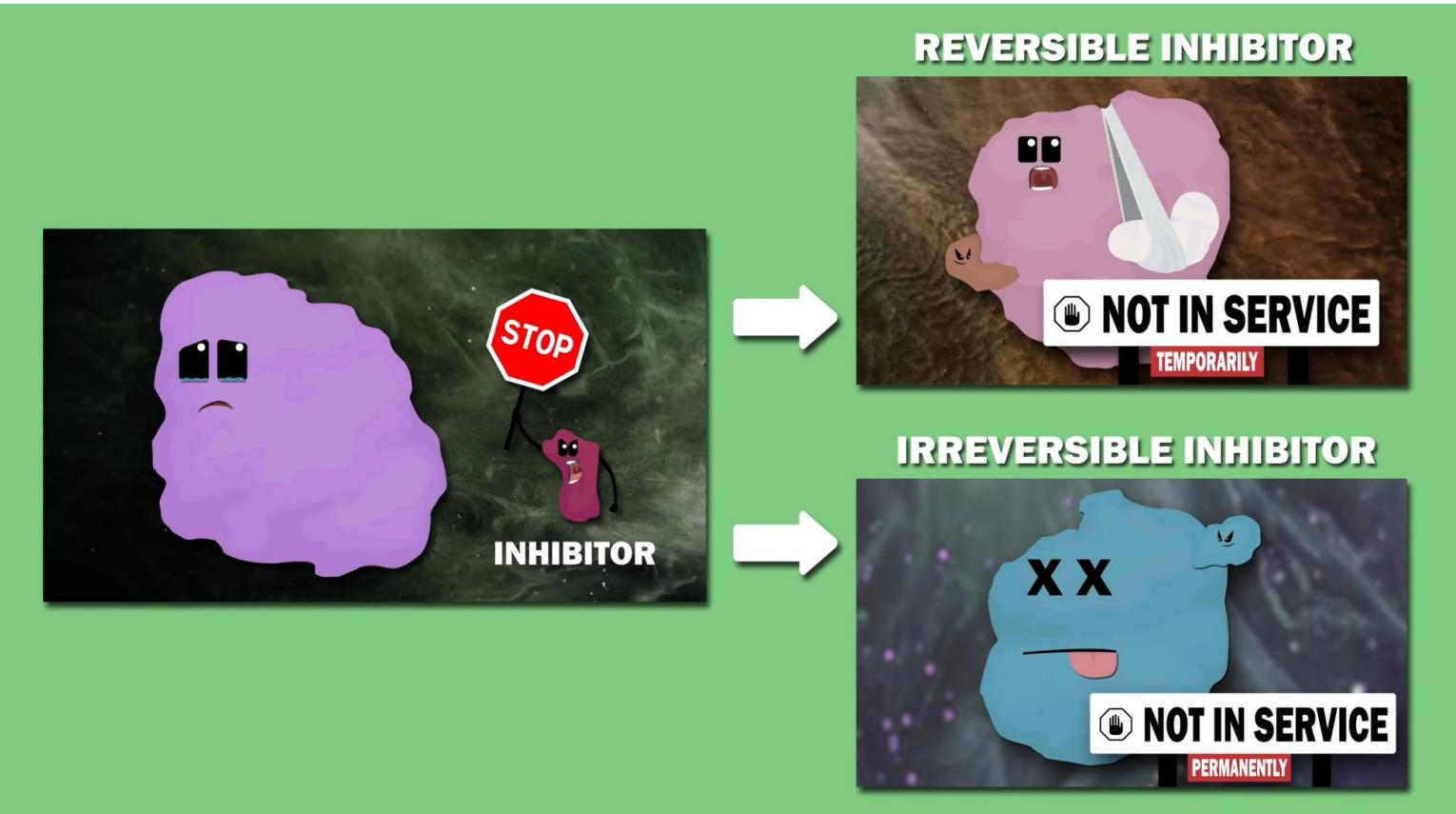
- In a cell, the activity of enzymes have to be regulated.
- A substrate cannot be constantly converted into its product.
- So the cell contains natural molecules that act as inhibitors of an enzyme.
- Inhibitors are also extremely important in medicine, because a lot of drugs used to treat disease, are in fact, inhibitors of specific enzymes.



Competitive Inhibitors: If it fits, it sits.



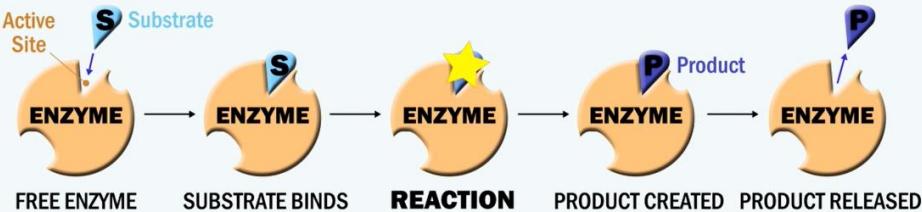
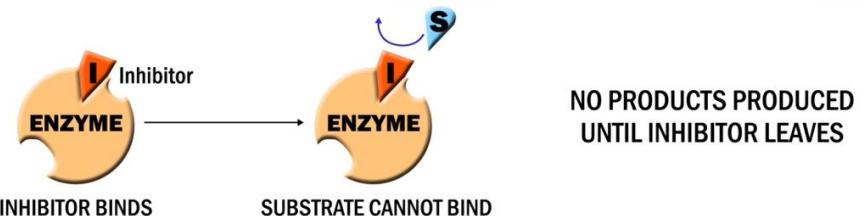
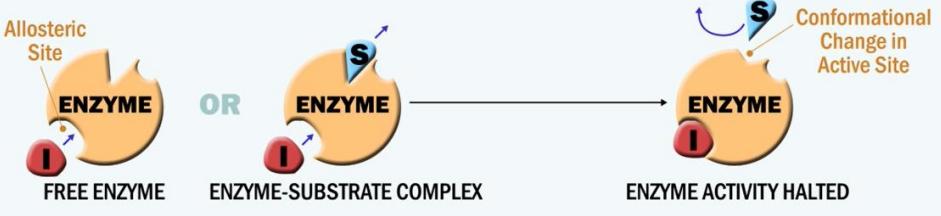
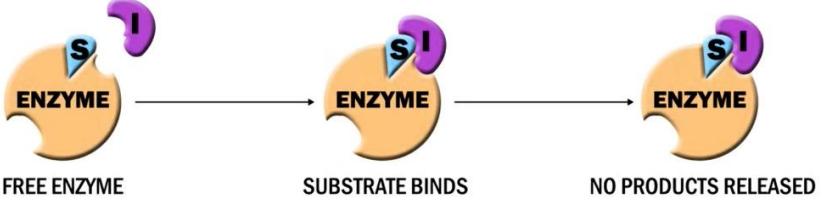
Inhibitors



3 MAJOR CATEGORIES OF REVERSIBLE INHIBITORS

**COMPETITIVE
NONCOMPETITIVE
UNCOMPETITIVE**

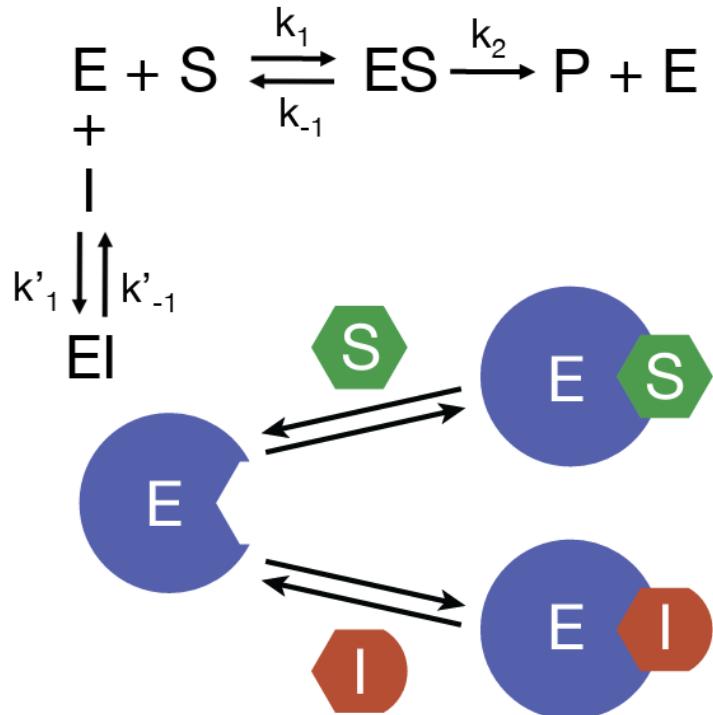
Reversible Inhibitors

NO INHIBITION	 <p>Active Site S Substrate ENZYME FREE ENZYME SUBSTRATE BINDS REACTION PRODUCT CREATED PRODUCT RELEASED</p>	SUBSTRATE BINDS ACTIVE SITE
COMPETITIVE INHIBITOR	 <p>I Inhibitor ENZYME INHIBITOR BINDS SUBSTRATE CANNOT BIND S</p> <p>NO PRODUCTS PRODUCED UNTIL INHIBITOR LEAVES</p>	COMPETITIVE INHIBITOR BINDS ACTIVE SITE
NONCOMPETITIVE INHIBITOR	 <p>Allosteric Site ENZYME FREE ENZYME OR ENZYME-SUBSTRATE COMPLEX I S Conformational Change in Active Site ENZYME ACTIVITY HALTED</p>	NONCOMPETITIVE INHIBITOR BINDS ALLOSTERIC SITE
UNCOMPETITIVE INHIBITOR	 <p>S Substrate ENZYME FREE ENZYME SUBSTRATE BOUNDS NO PRODUCTS RELEASED I SI</p>	UNCOMPETITIVE INHIBITOR BINDS ALLOSTERIC SITE

Competitive Inhibitors

Activity of enzymes need to be regulated!

Competitive Inhibitor



When inhibitor is bound,
substrate cannot bind

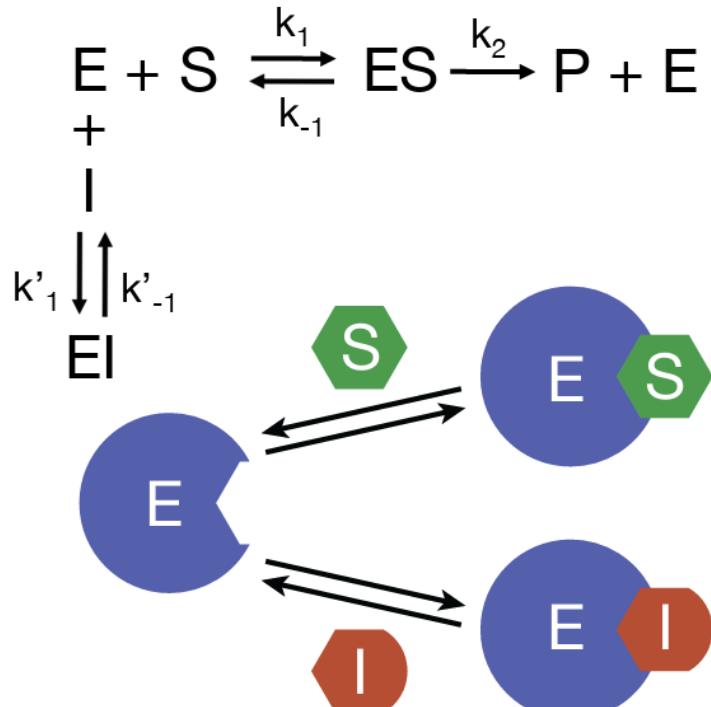
Predictions about
competitive inhibitors:

They increase K_m

They don't affect V_{max}

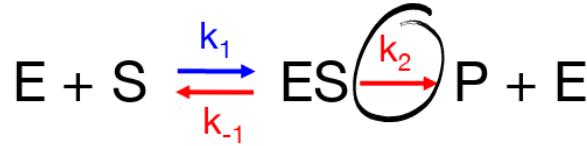
They increase K_m !

Competitive Inhibitor



When inhibitor is bound,
substrate cannot bind

We know!!!



$$k_1 [E] [S] = [ES] (k_{-1} + k_2)$$

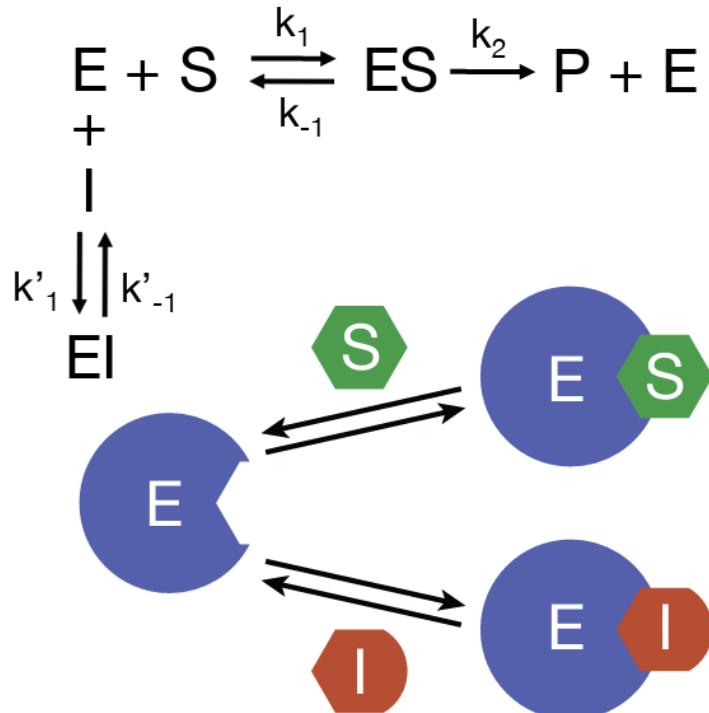
$$\frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

High K_m : low substrate affinity

Low K_m : high substrate affinity

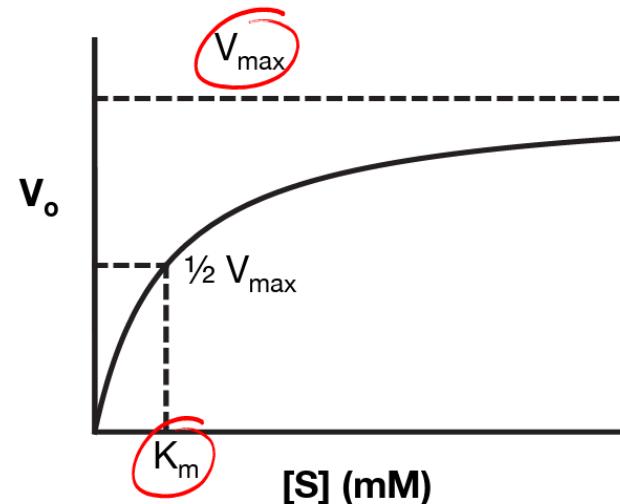
They do not affect V_{max} !

Competitive Inhibitor

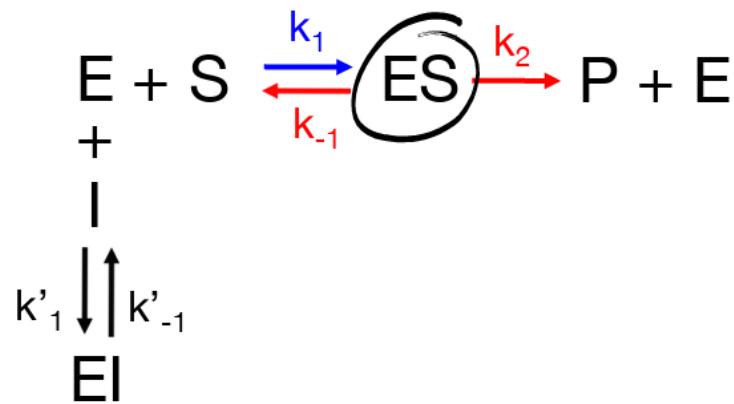


When inhibitor is bound,
substrate cannot bind

We can keep increasing [S] till
[I] becomes limiting
=> V_{max} is same



K_m

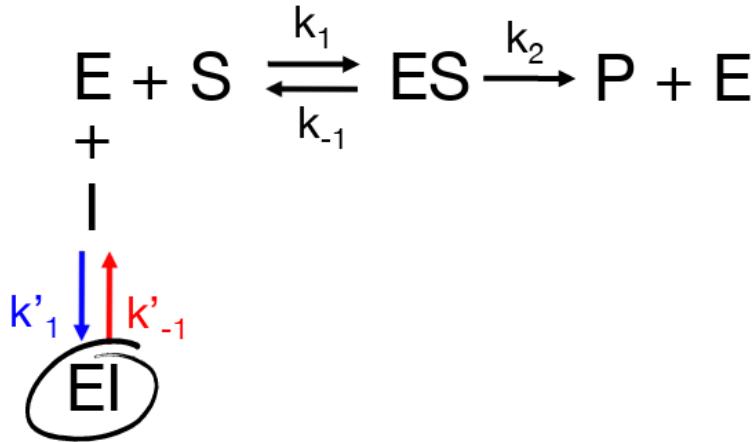


ES formation ES dissociation

$$k_1 [E] [S] = (k_{-1} + k_2) [ES]$$

$$\rightarrow \frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

$$\rightarrow \boxed{\frac{[E] [S]}{[ES]} = K_m}$$

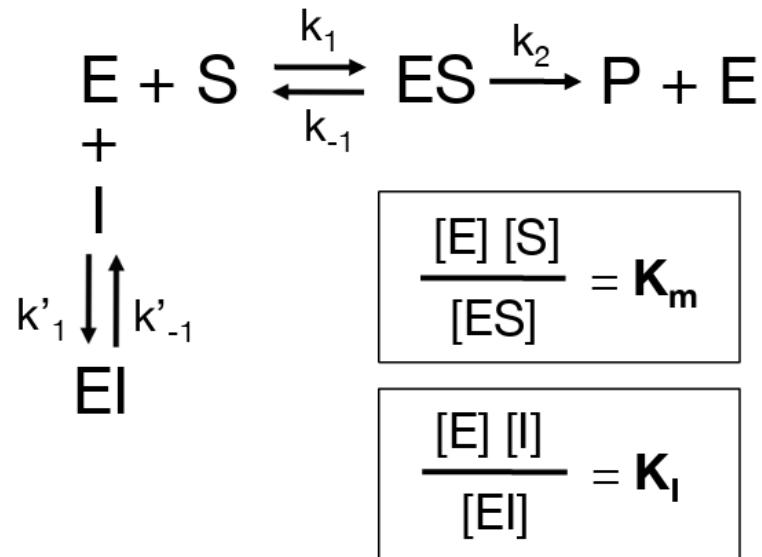


EI formation EI dissociation

$$k'_1 [E] [I] = k'_{-1} [EI]$$

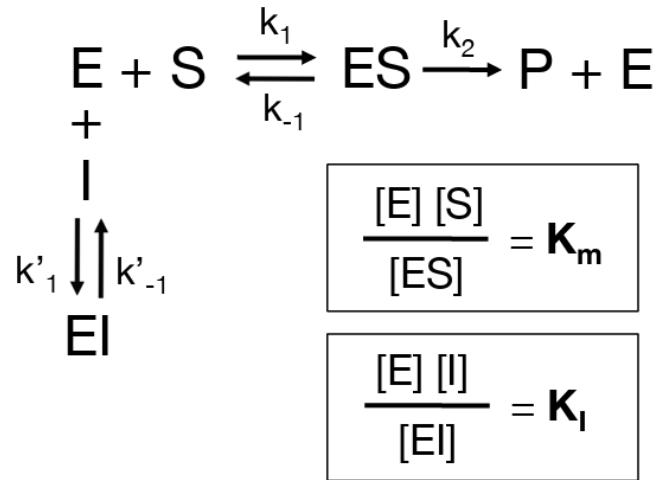
$$\rightarrow \boxed{\frac{[E] [I]}{[EI]} = \frac{k'_{-1}}{k'_1} = K_I}$$

Combining K_m and K_l



$$\frac{K_m}{K_l} = \frac{[E][S]}{[ES]} \times \frac{[EI]}{[E][I]} = \frac{\cancel{[E]} [S] [EI]}{[ES] \cancel{[E]} [I]}$$

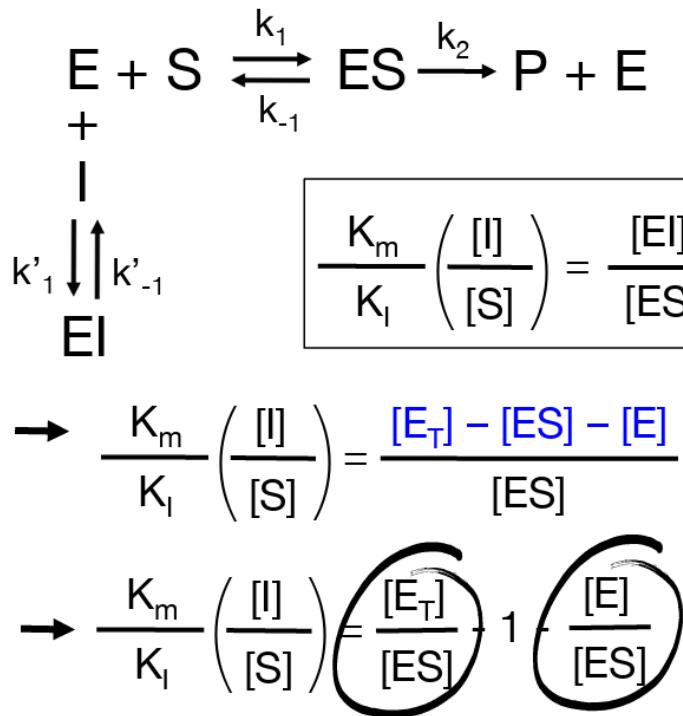
Combining K_m and K_I



$$\frac{K_m}{K_I} = \frac{[E][S]}{[ES]} \times \frac{[EI]}{[E][I]} = \frac{\cancel{[E]} [S] \cancel{[E]}}{\cancel{[ES]} \cancel{[E]} [I]}$$

$$\rightarrow \frac{K_m}{K_I} \left(\frac{[I]}{[S]} \right) = \frac{[EI]}{[ES]}$$

Rearranging



$$\frac{K_m}{K_l} \left(\frac{[I]}{[S]} \right) = \frac{[EI]}{[ES]}$$

reaction rates

$$V_o = k_2 [ES]$$

$$V_{max} = k_2 [E_T]$$

$$\frac{V_{max}}{V_o} = \frac{[E_T]}{[ES]}$$

total enzyme $[E_T] = [E] + [ES] + [EI]$

Rearranging

last slide:

$$\frac{K_m}{K_l} \left(\frac{[I]}{[S]} \right) = \frac{[E_T]}{[ES]} - 1 - \frac{[E]}{[ES]}$$

$$\rightarrow \frac{K_m}{K_l} \left(\frac{[I]}{[S]} \right) = \frac{V_{max}}{V_o} - 1 - \frac{K_m}{[S]}$$

reaction rates

$$V_o = k_2 [ES]$$

$$V_{max} = k_2 [E_T]$$

$$\frac{V_{max}}{V_o} = \frac{[E_T]}{[ES]}$$

total enzyme $[E_T] = [E] + [ES] + [EI]$

$$K_m = \frac{[E][S]}{[ES]} - \frac{K_m}{[S]} = \frac{[E]}{[ES]}$$

Trying to imitate our original Lineweaver Burk equation

last slide:

$$\frac{K_m}{K_l} \left(\frac{[I]}{[S]} \right) = \frac{V_{max}}{V_o} - 1 - \frac{K_m}{[S]}$$

$$\rightarrow \frac{V_{max}}{V_o} = 1 + \frac{K_m}{[S]} + \frac{K_m}{K_l} \left(\frac{[I]}{[S]} \right)$$

$$\rightarrow \frac{V_{max}}{V_o} = K_m \left(1 + \frac{[I]}{K_l} \right) \frac{1}{[S]} + 1$$

$$\rightarrow \frac{1}{V_o} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_l} \right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

straight line $y = m x + b$

Trying to imitate!

$$\frac{1}{V_o} = \left(\frac{K_m}{V_{max}} \right) \left(\frac{1}{[S]} \right) + \frac{1}{V_{max}}$$

equation of straight line $y = m x + b$

They do not affect V_{max}

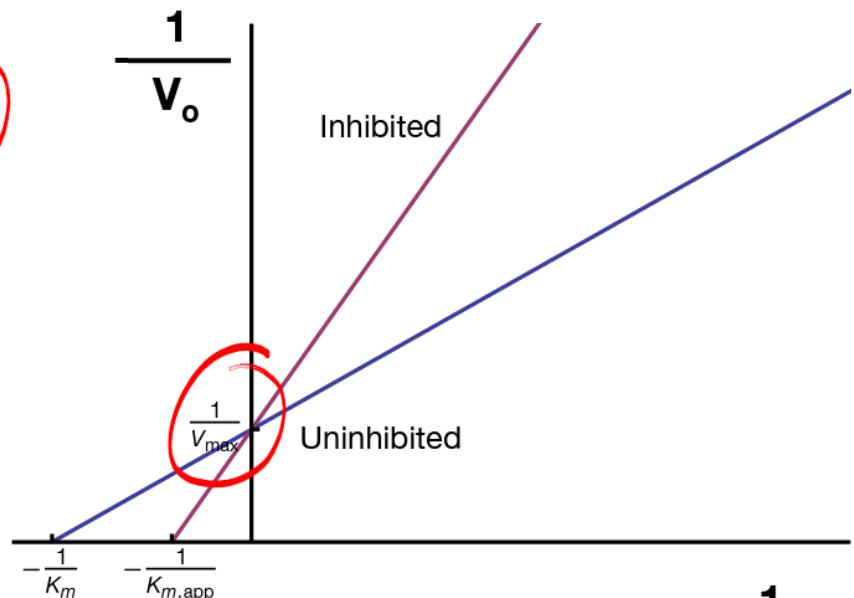
Lineweaver-Burk with competitive inhibitor

$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_I}\right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

equation of straight line $y = m x + b$

$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

Lineweaver-Burk without competitive inhibitor



Competitive inhibitors
don't affect V_{max}

$$\frac{1}{[S]}$$

They affect K_m

Lineweaver-Burk with competitive inhibitor

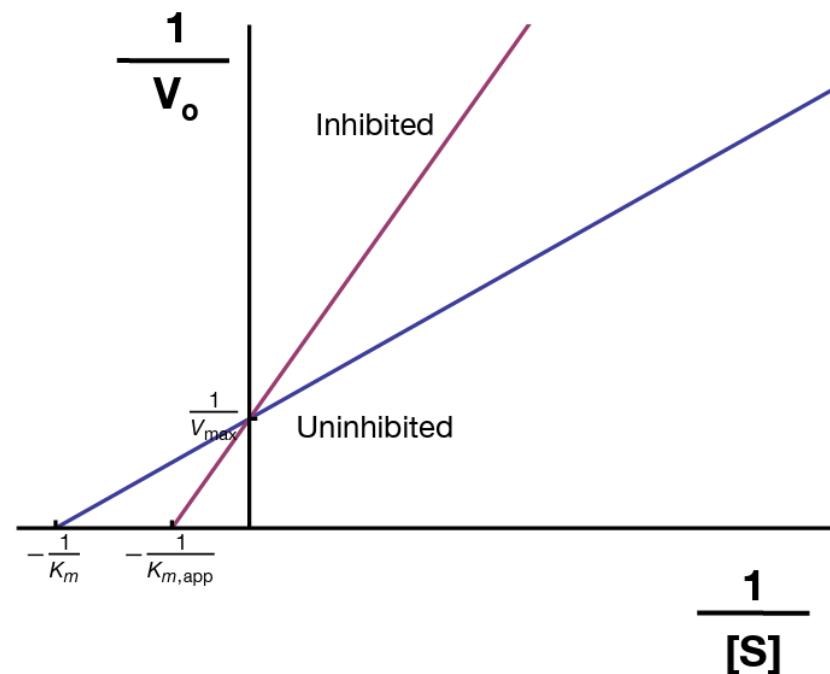
$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_I} \right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

equation of straight line

$$y = m x + b$$

$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

Lineweaver-Burk without competitive inhibitor



K_{m, app}

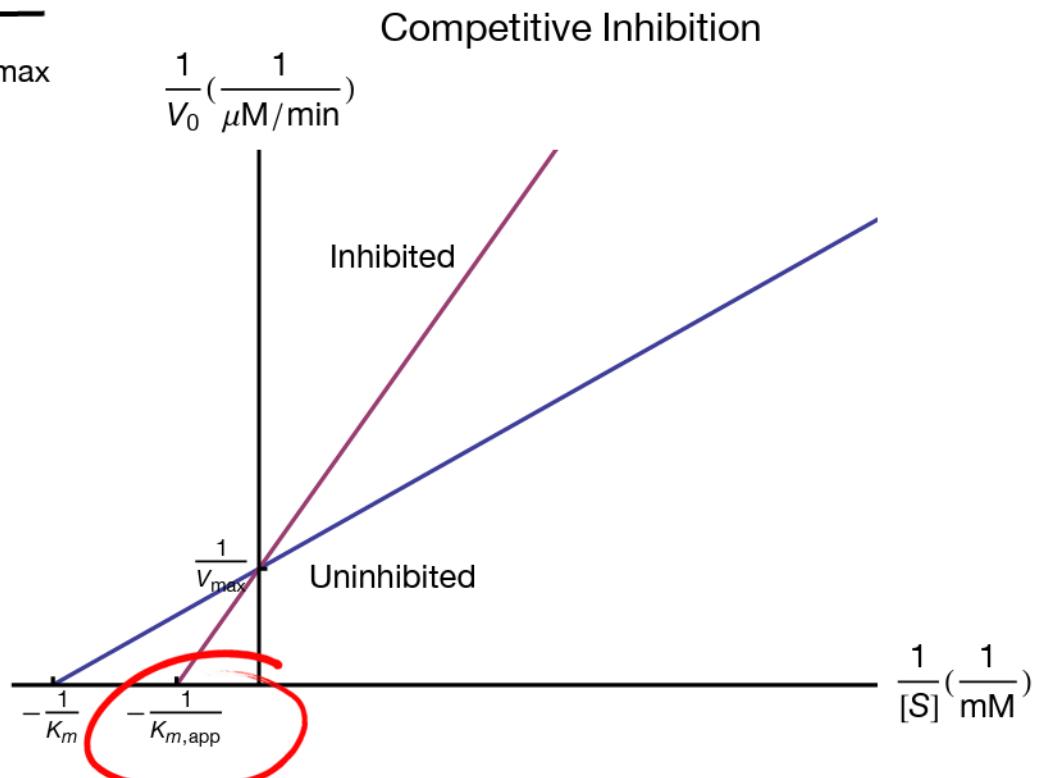
$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \left(1 + \frac{\alpha [I]}{K_I} \right) \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$\boxed{\frac{1}{V_o} = \left(\frac{\alpha K_m}{V_{max}} \right) \left(\frac{1}{[S]} \right) + \frac{1}{V_{max}}}$$

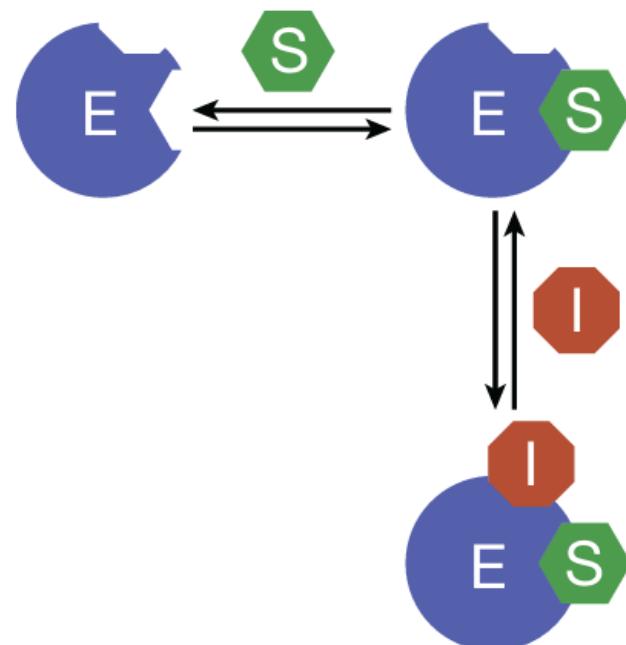
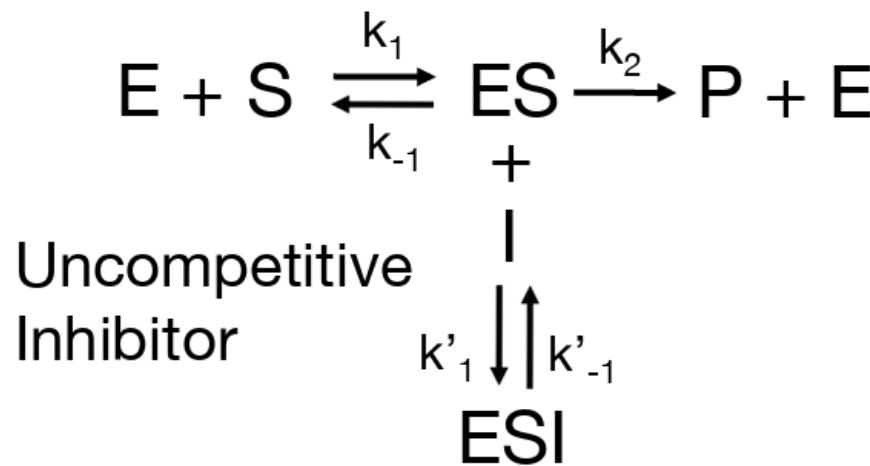
K_m with inhibitor $\alpha K_m = K_{m, app}$

$$K_{m, app} > K_m$$

Competitive inhibitors increase K_m

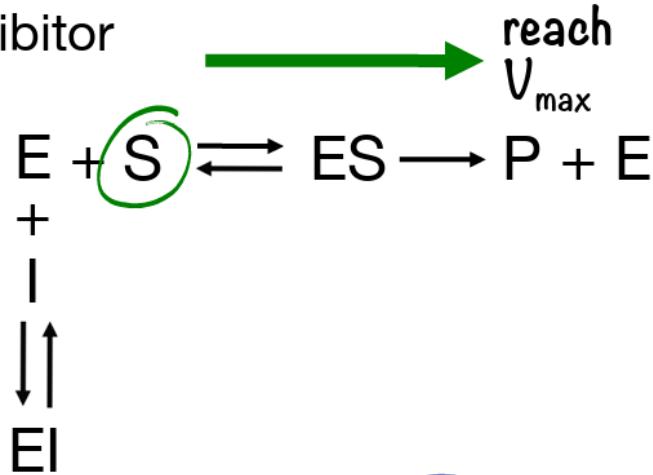


Uncompetitive inhibitors!

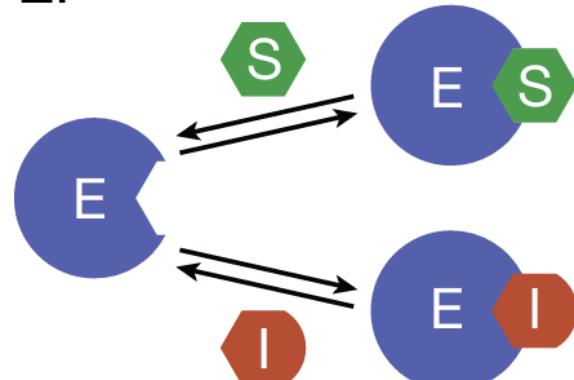
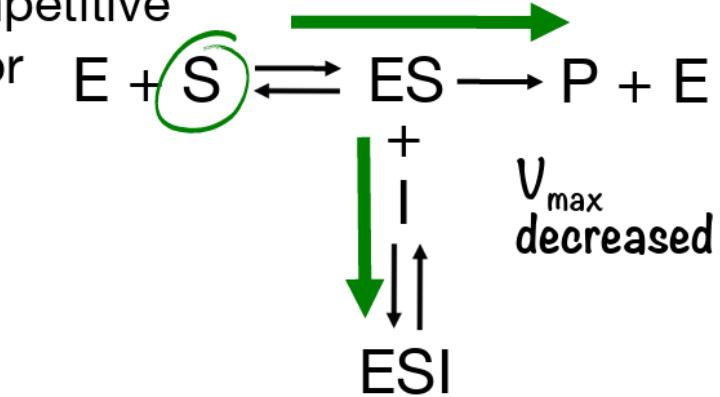


Uncompetitive inhibitors: Does it affect V_{max} !

Competitive
Inhibitor

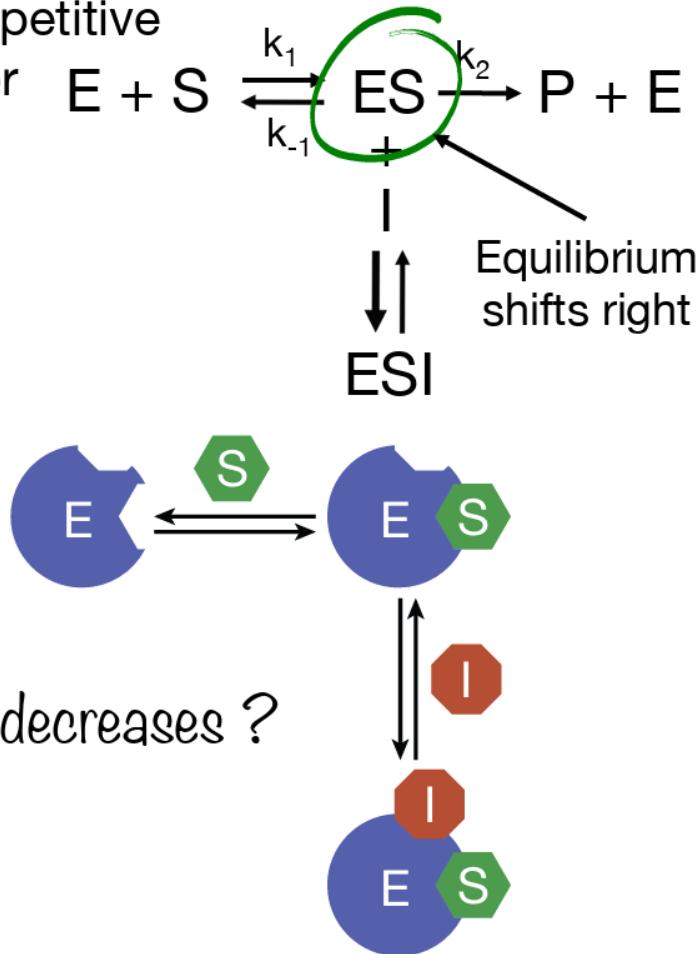


Uncompetitive
Inhibitor



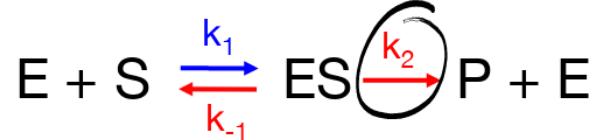
Uncompetitive inhibitors: What happens to K_m!

Uncompetitive Inhibitor



K_m decreases?

We know!!!



$$k_1 [E] [S] = [ES] (k_{-1} + k_2)$$

$$\frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_m$$

High K_m: low substrate affinity

Low K_m: high substrate affinity

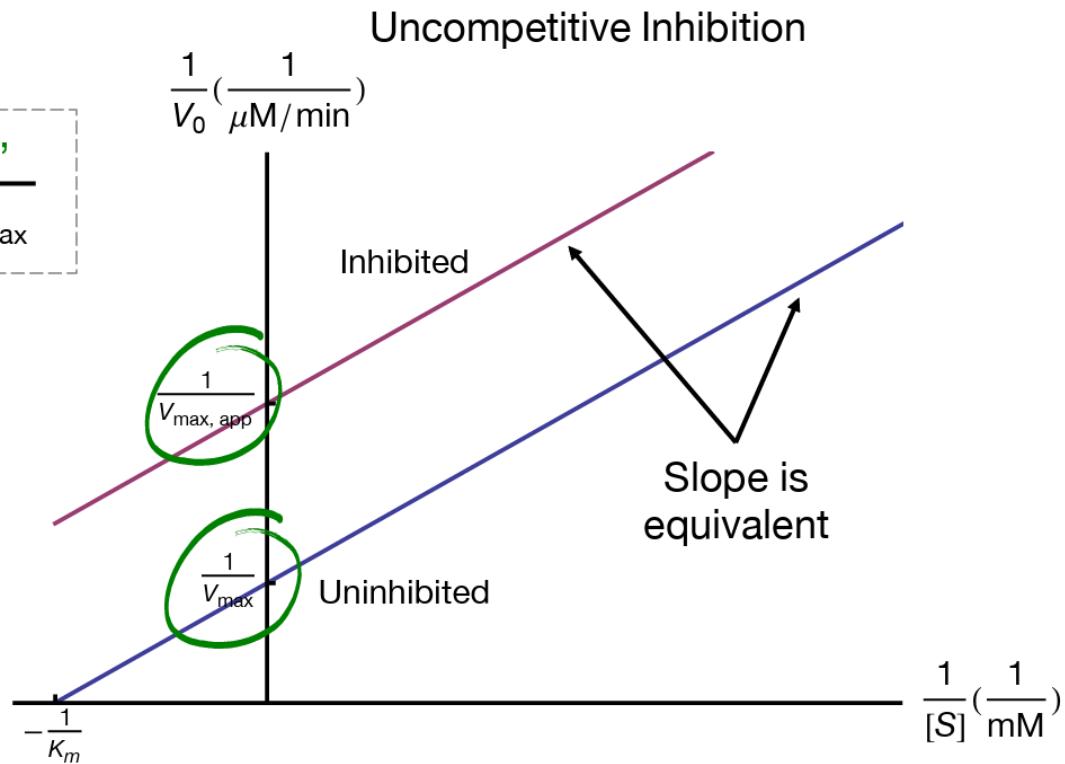
Uncompetitive inhibitors

$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

$$\alpha' = 1 + \frac{[I]}{K'_I}$$

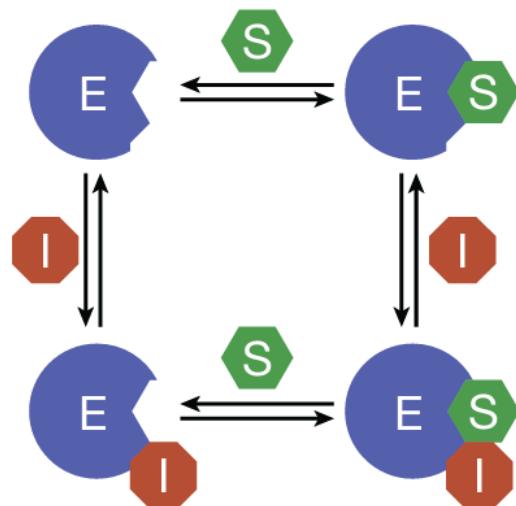
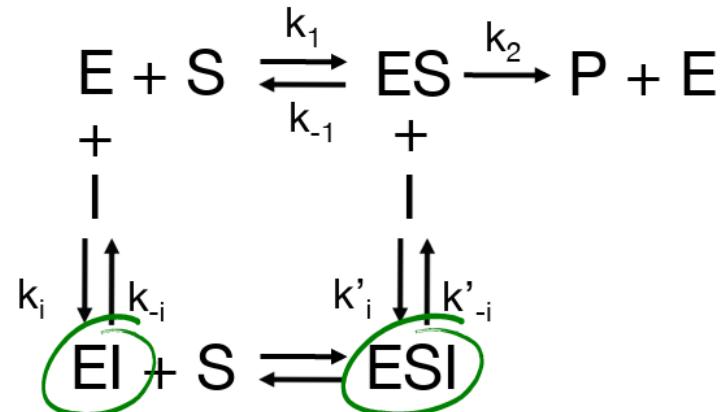
Decreased K_m

Decreased V_{max}

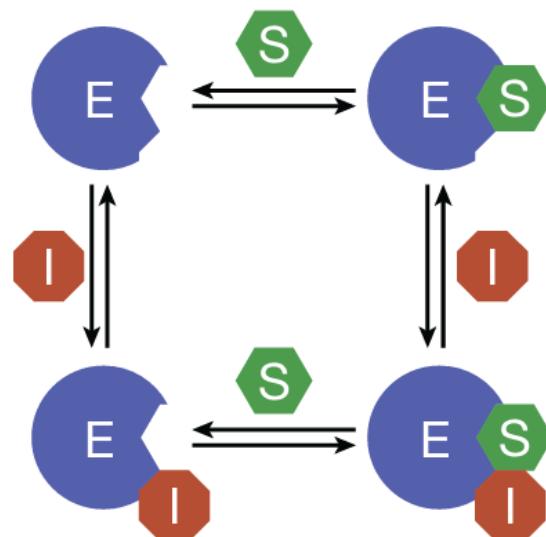
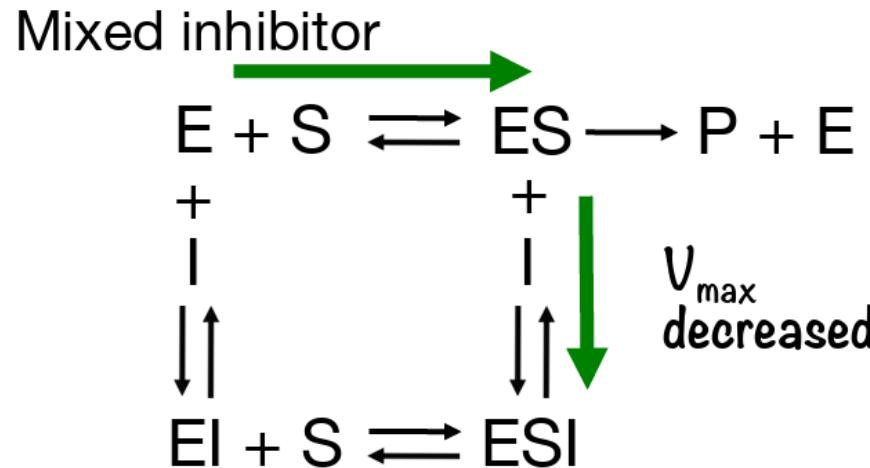


Mixed inhibitors!

Mixed inhibitor

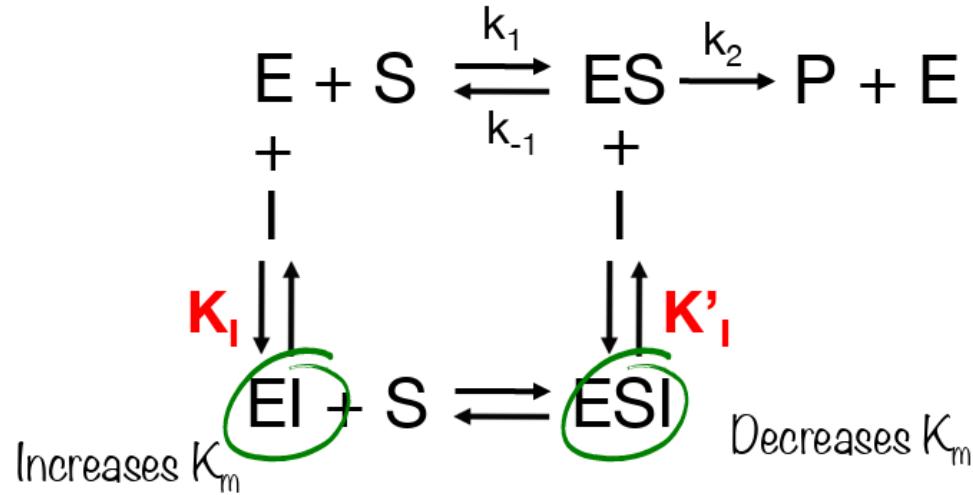


Mixed inhibitors!



Mixed inhibitors!

Mixed inhibitor



K_m can increase, decrease
or stay the same

Mixed inhibitors!

Mixed Inhibitor

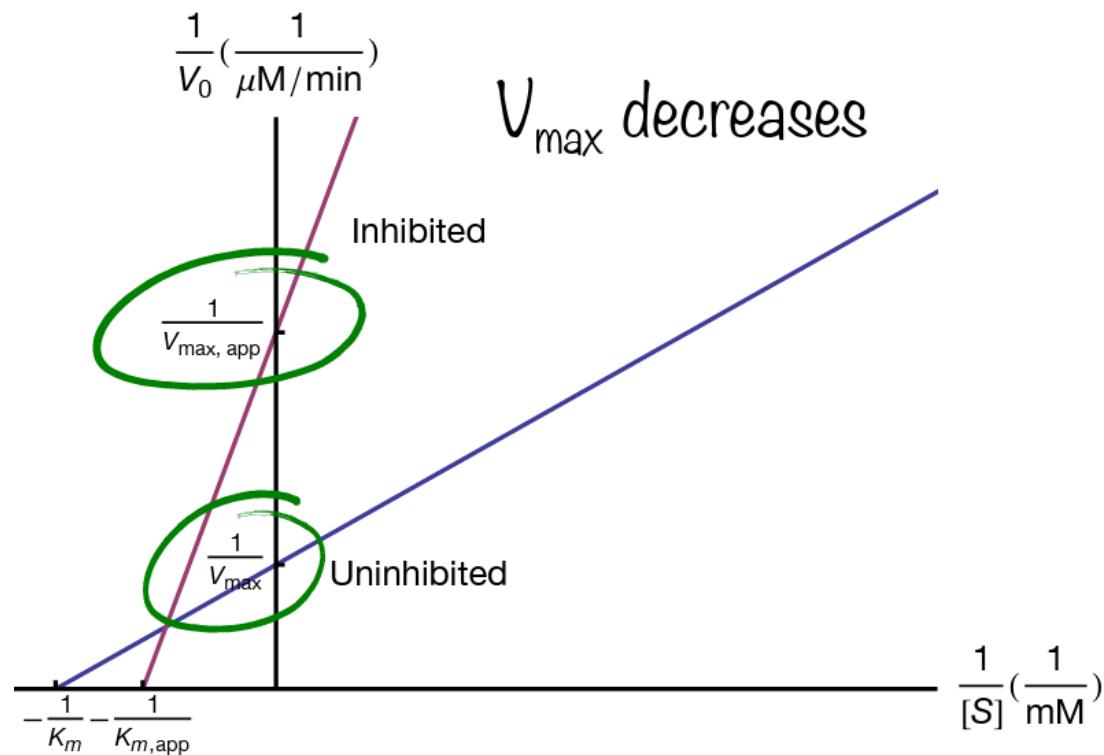
$$\frac{1}{V_o} = \frac{\alpha K_m}{V_{max}} \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

$$\alpha = 1 + \frac{[I]}{K_I} \quad \text{Effect of EI formation}$$

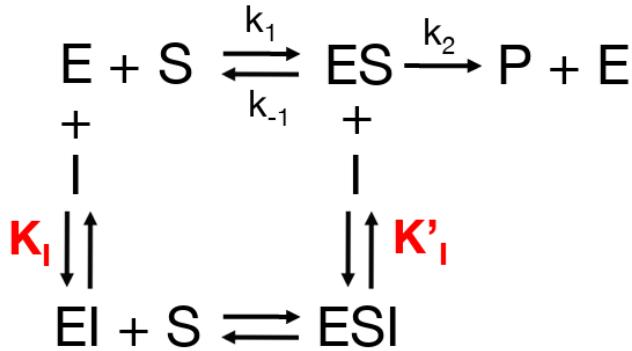
$$\alpha' = 1 + \frac{[I]}{K'_I} \quad \text{Effect of ESI formation}$$

Mixed inhibitors!

Mixed Inhibitor



Mixed inhibitors!

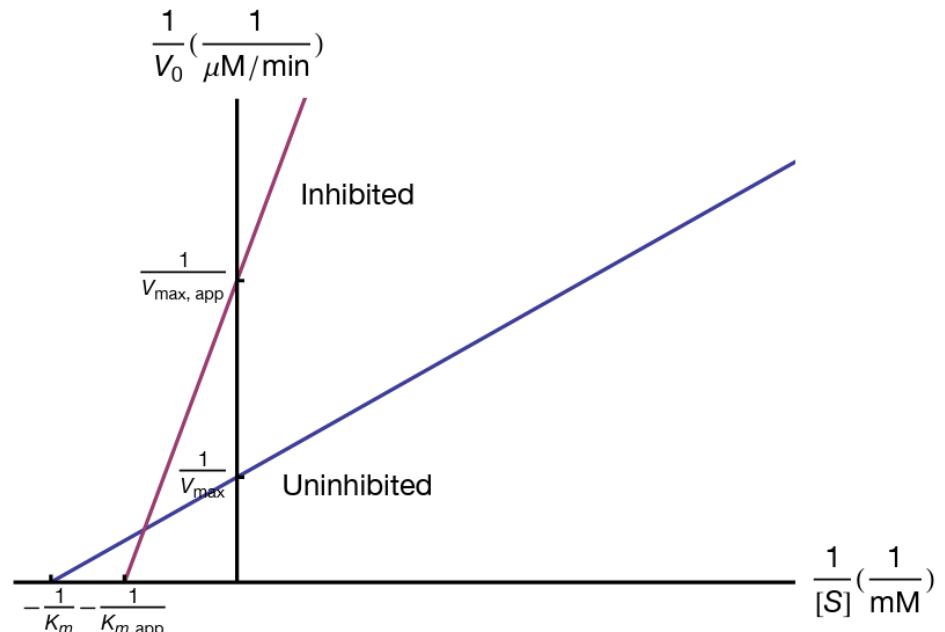


Inhibitor favors E over ES

K_I dominates over K'_I

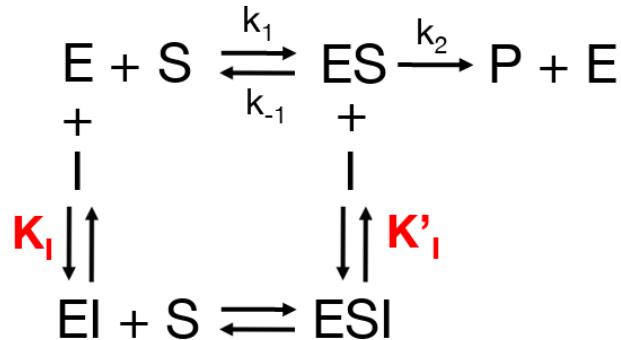
This example: K_m increases

$$K_{m,app} = \frac{\alpha K_m}{\alpha'}$$



Non-competitive inhibitors!

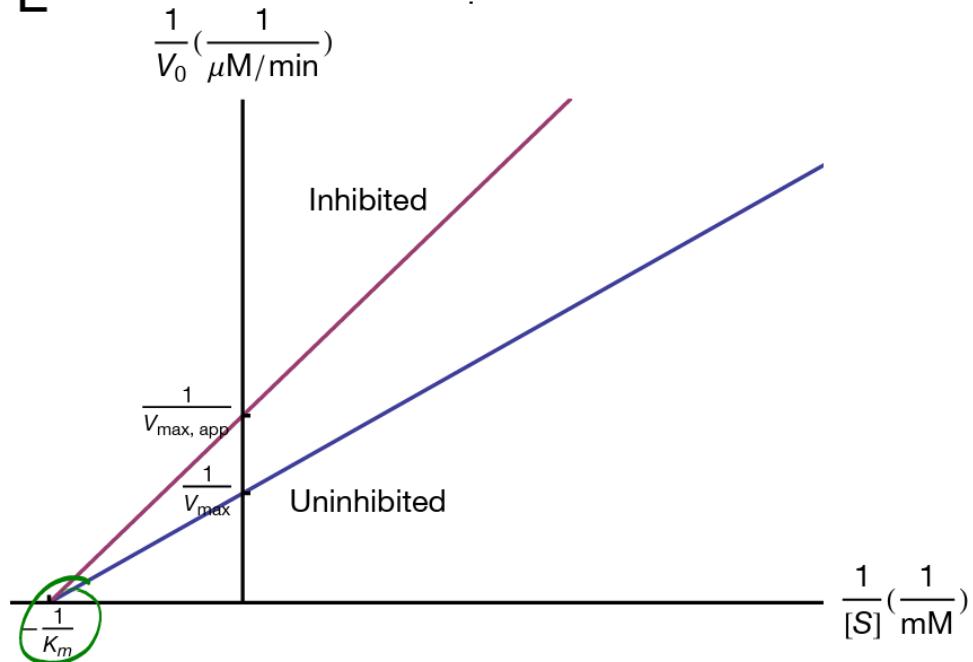
Noncompetitive Inhibitor



$$\alpha = \alpha'$$

$$K_{m,\text{app}} = \frac{\alpha K_m}{\alpha'}$$

$$K_{m,\text{app}} = K_m$$

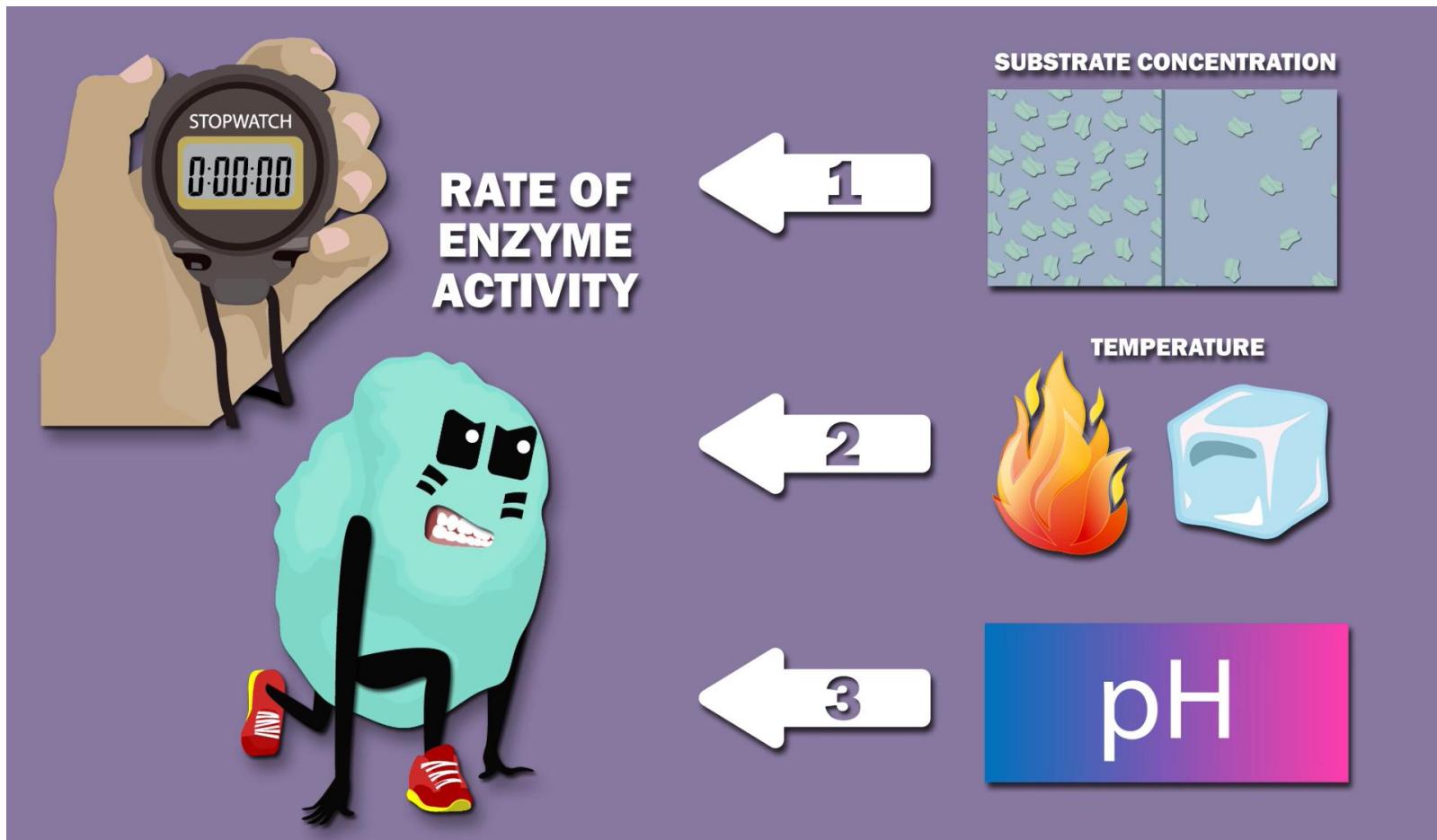


In conclusion!

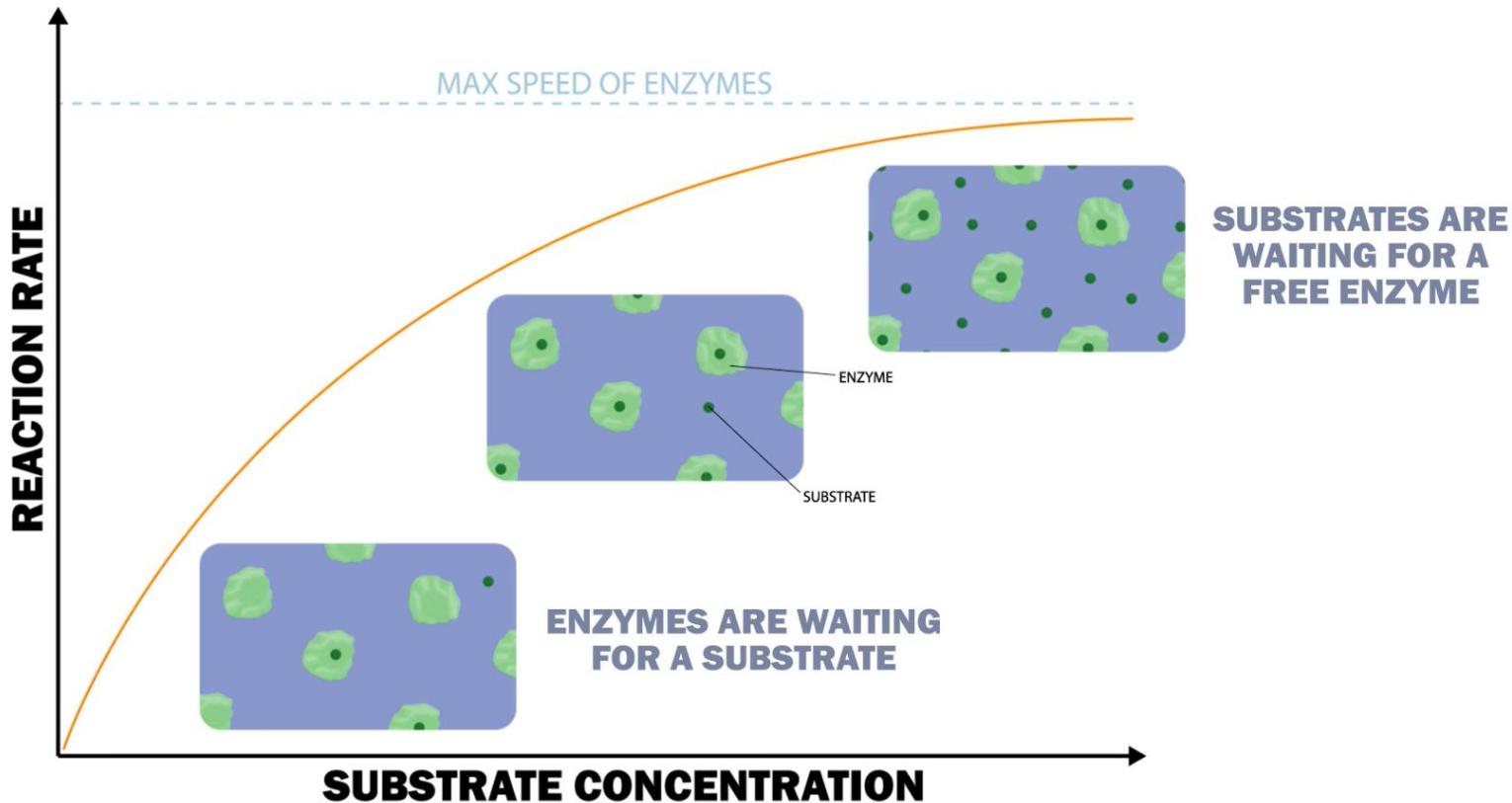
Conclusion: Effect of reversible inhibitors on $V_{max,app}$ and $K_{m, app}$

Inhibitor	$V_{max,app}$	$K_{m, app}$
Absent	V_{max}	K_m
Competitive	V_{max}	αK_m
Uncompetitive	V_{max}/α'	K_m/α'
Mixed	V_{max}/α'	$\alpha K_m/\alpha'$

Factors that affect enzyme activity

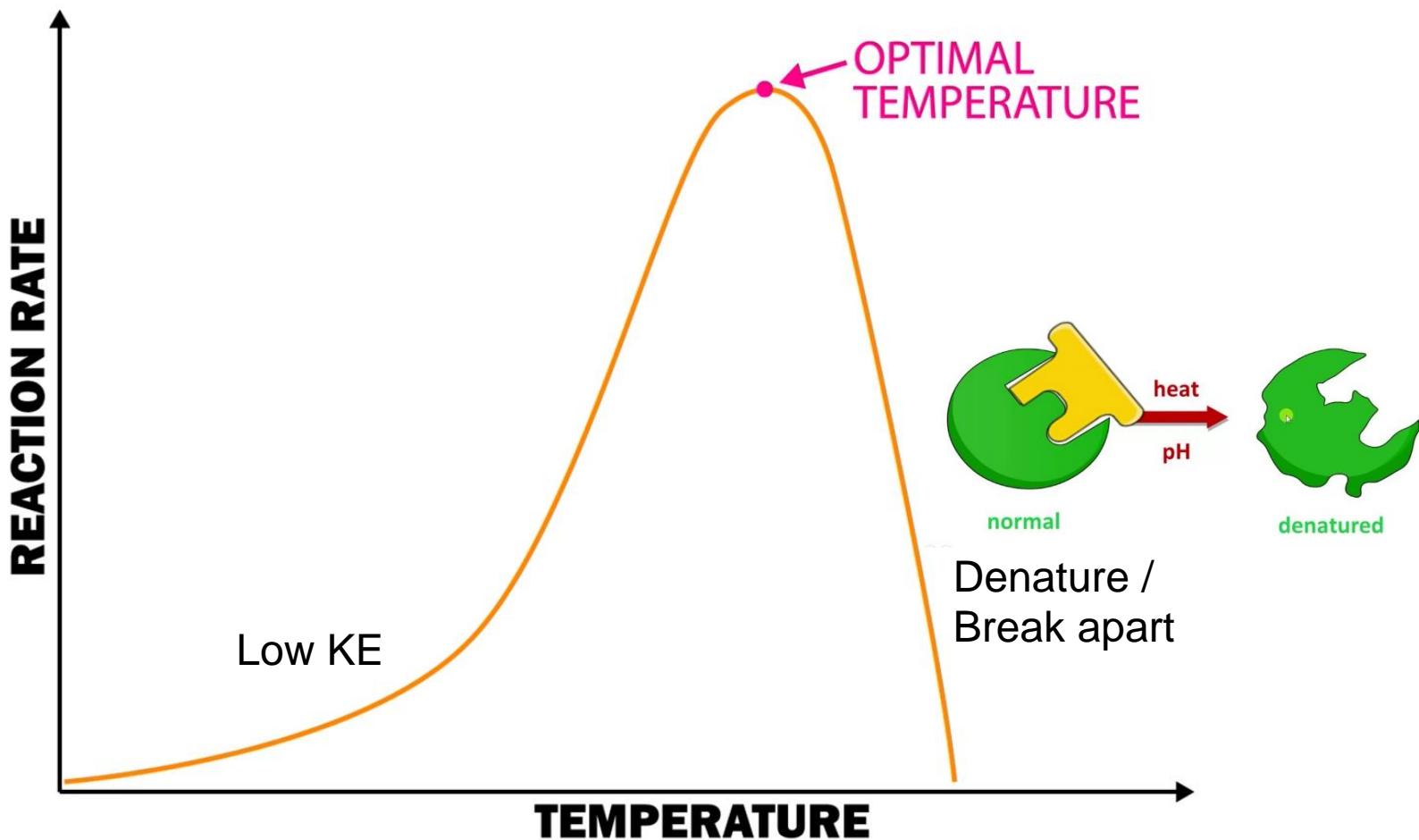


Substrate concentration



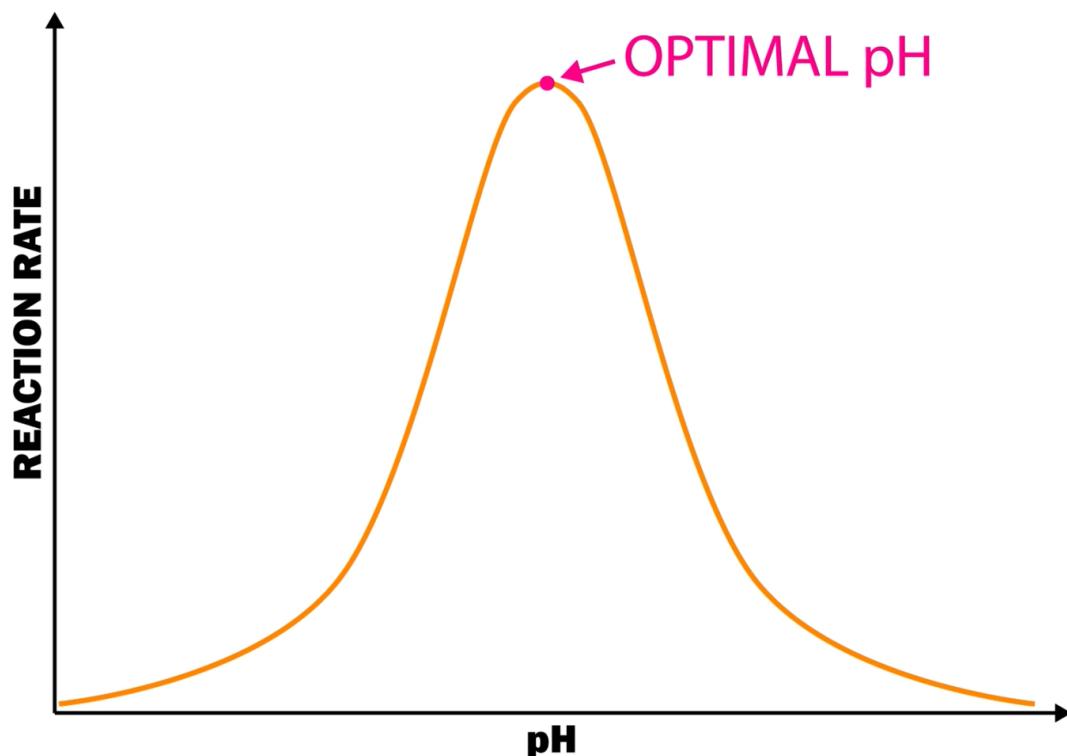
Temperature

Enzyme activity is a delicate combination of intermolecular forces

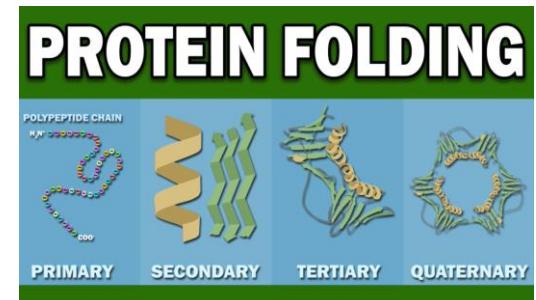
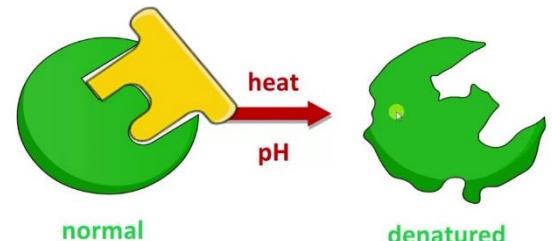


pH

Enzyme activity is a delicate combination of intermolecular forces



At non-optimal pH



Cells in different organs produce different enzymes!

