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Special Issue Preface

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Celebrating the Scientific Life of Richard A. Mathies

It is a great pleasure for me to partake in celebrating the wonderful scientific life of Richard A. Mathies. I first met Rich in 1973, when I visited Cornell to present a seminar on visual excitation. Rich was then a graduate student in Andreas Albrecht's laboratory, where he was carrying out electric field studies of the excited-state polarizabilities of benzene and other aromatic hydrocarbons.[1] What struck me right away was Rich's experimental virtuosity combined with a strong and intuitive understanding of spectroscopy. I was impressed by the ease with which Rich moved back and forth between experiment and theory and was delighted when he said he would like to join my laboratory at Yale to study the photochemistry of retinal. Rich received a Helen Hay Whitney Fellowship, a highly coveted award, and came to my laboratory soon after completing his doctoral thesis.

As a postdoctoral fellow, Rich decided to carry out resonance Raman spectroscopic studies of rhodopsin because he recognized that the method was highly sensitive and could potentially provide atomic-resolution views of the retinal chromophore both before and after photoexcitation, but it was first necessary to solve a challenging problem: the intense exciting light needed to obtain a resonance Raman spectrum of rhodopsin also isomerizes retinal. Rich's solution was to flow the sample through the light beam at a sufficiently high velocity so that the fraction of photoisomerized molecules in the illuminated volume was very low. He wanted a quantitative understanding of the process and not just an empirical solution. Rich proceeded to derive a general expression for the extent of photoalteration F of a photolabile molecule

$$F = P\epsilon\phi 3.824 \times 10^{-21}/(v\omega\pi^{1/2})$$

where v is the flow velocity, P is the laser power, ω is the equivalent radius of the beam, ϵ is the absorbance coefficient, and ϕ is the quantum yield of photoisomerization (or photodestruction).[2]

This expression for the photoalteration parameter makes it possible to control the extent of interaction between light and *any* photolabile molecule. Armed with this knowledge, Rich measured the resonance Raman spectrum of unphotolyzed rhodopsin and opened a new field of inquiry.

In his postdoctoral years at Yale, Rich also explored the excited state of retinal by electric field spectroscopy. He discovered that retinal and its Schiff bases have a highly dipolar vertically excited singlet state.[3] The change in dipole moment is 12 debye, which corresponds to a shift of 0.21 e over the length of the retinal molecule (~1.2 nm), with the ionone ring acquiring a partial positive charge. This large change in dipole moment between the ground and excited states provides a facile mechanism for tuning the absorption spectrum of retinal by electrostatic interaction of charged or dipolar groups of the protein with the excited state of retinal. The absorption maxima of the three cone visual pigments underlying color vision in humans are at 431, 531, and 561 nm. Indeed, red-shifting by stabilization of the excited state of retinal plays a key role in tuning visual pigments.[4,5] Furthermore, the large excited-state dipole moment promotes photoisomerization of retinal by preferentially reducing the bond order of the central double bonds in the polyene chain.

In 1976, Rich joined the faculty of the Department of Chemistry at UC Berkeley and initiated a creative research program centered on the excited states and conformational transitions of biologically important photoresponsive molecules. He vigorously pursued his interest in how light alters the retinal chromophore of rhodopsin to generate a trigger for vision. The challenge was two-fold: to capture the resonance Raman spectra of the transient intermediates following photoexcitation and to deduce the conformation of retinal in each by rigorously assigning the bands seen in this series of vibrational spectra. The wealth of information in these spectra was unlocked by studying a large number of isotopically substituted retinals and other analogs that were synthesized in a stimulating and effective long-term collaboration with Johan Lugtenburg of the University of Leiden. Theoretical calcu-

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lations and modeling complemented and informed the experimental studies. A detailed picture of retinal before and soon after photoexcitation began to emerge. A key finding was that the chromophore of bathorhodopsin, the first photoproduct, has a twisted all-*trans* conformation,[6] in contrast with the 11-*cis* conformation of rhodopsin. The complete assignment of the fingerprint vibrations[7] and the highly distinctive hydrogen out-of-plane wagging vibrations (HOOP modes)[8] of rhodopsin and bathorhodopsin then revealed how 33 kcal/mol of the excitation energy is stored in bathorhodopsin to generate a very reliable trigger for visual excitation.[7]

Rich also used resonance Raman spectroscopy to elucidate the conformational transitions of retinal in bacteriorhodopsin, a light-driven proton pump present in halobacteria. In 1980, he provided strong evidence that the photocycle begins with an all-*trans* to 13-*cis* isomerization.[9] Rich and coworkers then carried out an incisive series of spectroscopic and computational studies of each intermediate in the photocycle, culminating in a detailed picture of how a biological energy transduction process operates at the atomic level.[10] His laboratory has also provided insight into the interaction of light with halorhodopsin (a light-driven chloride pump),[11] phytochrome (a plant photosensor, in collaboration with J. Clark Lagarias at the University of California, Davis),[12] and bacterial photosynthetic reaction centers (in collaboration with Steve Boxer at Stanford).[13]

A deeper understanding of the photoisomerization of rhodopsin came from a series of femtosecond spectroscopic studies carried out by Rich's laboratory in collaboration with Chuck Shank's laboratory at Lawrence Berkeley Laboratory. The 11-*cis* retinal chromophore of rhodopsin was excited with a 35 fs pump pulse at 500 nm, and transient absorption changes between 450 and 580 nm were monitored with 10 fs probe pulses.[14] These elegant state-of-the-art experiments demonstrated that the 11-*cis* to all-*trans* torsional isomerization is essentially complete in less than 200 fs.[14,15] Their subsequent study revealed that the primary step in vision is a vibrationally coherent process.[16] Contrary to the traditional picture of a photochemical process, the chromophore is not relaxed on the excited-state potential energy surface. Rather, an essentially barrierless transition takes the chromophore from the excited state of the reactant to the ground state of the product. The motion can be swift because it is relatively small and not impeded by the protein environment; only the middle part of retinal needs to move to undergo isomerization. The high quantum yield of photoisomerization of rhodopsin (0.67) is a biologically advantageous consequence of the coherence of the isomerization process.[15,16]

Rich has also applied his broad knowledge of spectroscopy and instrumentation to create novel tools to advance genome sequencing, genetic polymorphism detection, proteomics, and medical diagnostics. His 1990 paper on the optimization of high-sensitivity fluorescence detection[17] stimulated him to initiate a bioanalytical chemistry program in tandem with his fundamental spectroscopic studies. His laboratory then developed capillary array electrophoresis and microfabricated lab-on-a-chip microanalysis systems that advanced DNA sequencing and genotyping.[18,19] He and his colleague Alex Glazer exploited fluorescence resonance energy transfer to generate a new class of high-performance fluorescent labeling reagents for multiplex analysis of nucleic acids.[20] These energy-transfer primers markedly improved the performance of automated DNA sequencers based on the Sanger dideoxy method and accelerated the Human Genome Project. His most recent work in the analytical arena emphasizes miniaturization, portability, and the integration of electronic and fluidic components.[21] At the same time, Rich has developed femtosecond stimulated Raman spectroscopy to map multidimensional potential energy surfaces in the quest to predict and control chemical reactivity.[22] In doing so, he has met the daunting challenge of getting key structural information in the 10 fs to 1 ps time range in which chemical reactions take place but was previously silent to the experimenter. His interests in applied and fundamental science go hand-in-hand and are mutually reinforcing. Moreover, he provides vibrant leadership as Dean of the College of Chemistry and Gilbert Newton Lewis Professor.

For 40 years, Rich has elegantly brought together physics, chemistry, and biology to illumine fundamental processes of nature that are driven by light. His many contributions in photobiology and in light-based analytical chemistry are deep and enduring. What a remarkable harvest!

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