⌘ *Author's Choice*

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 19, pp. 15164–15173, May 4, 2012

Exploring Light and Life

Published, JBC Papers in Press, March 12, 2012, DOI 10.1074/jbc.X112.361436

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Undergraduate, Medical School, and Postdoctoral Years

The interplay of light and life has fascinated me since my college years. In the introductory biology laboratory at the University of Chicago, we isolated chloroplasts and carried out the Hill reaction, in which light leads to the reduction of an indicator dye with the concomitant generation of O_2. I vividly recall my excitement on seeing this graphic demonstration of key features of photosynthesis in a test tube. Equally important were my evenings as a waiter at the Quadrangle Club, where I had the good fortune of serving James Franck, a distinguished emeritus professor of chemistry who had received the Nobel Prize in 1925 for carrying out experiments that established the validity of Bohr's model of the atom. My task was simple because he invariably ordered a lean veal sandwich for dinner, and so there was time for conversation. Franck told me about his current interest in the mechanism of photosynthesis and his early research on energy transfer. In 1923, Franck and his graduate student Günther Cario reported that excitation of mercury led to the emission of thallium in a gaseous mixture of the two atoms (1). This sensitized fluorescence revealed that electronic excitation energy can be transferred between atoms by a direct electromagnetic interaction. Later in the quarter, Franck remarked to me, "One day, you too might work on energy transfer."

That day came two years later, in 1957, when I spent a very rewarding summer as a research student at the Argonne National Laboratory under the mentorship of Douglas Smith. He introduced me to photodynamic action, a process in which light activates a photosensitizing dye in the presence of O_2 to induce cellular damage, and encouraged me to read about energy transfer because it often plays a key role in effecting the light-induced damage. I learned that energy transfer can take place between any two chromophores provided that the emission spectrum of the energy donor overlaps the absorption spectrum of the energy acceptor. I was captivated by Theodor Förster's theory for dipole-dipole energy transfer, which explicitly predicted the rate and efficiency of transfer as a function of spectroscopic and geometric variables (2). What especially grabbed me was his prediction that the transfer efficiency depends on the inverse sixth power of the distance between the donor and acceptor. During that summer and the following one, I learned how to carry out fluorescence measurements and label proteins. My first published paper dealt with energy transfer from aromatic amino acids in chymotrypsinogen to attached dansyl groups (3). At a bioenergetics meeting at Brookhaven in 1959, where I met Förster, I reviewed the literature on energy transfer in proteins and suggested that energy transfer could be used as a probe to gain insight into the conformation of biological macromolecules (4). A highlight of that year was visiting Franck to let him know that I was indeed working on energy transfer.

As a medical student at Harvard University, I carried out research under the mentorship of Elkan Blout, who was Director of Chemical Research at Polaroid by day and head of a basic research laboratory at Children's Hospital by night. At Polaroid, Elkan worked closely with Edwin Land in developing instant color photography, and at Harvard, he synthesized synthetic polypeptides as models of proteins and studied their conformations using spectroscopic techniques. I wanted to find out how dyes bound to synthetic polypeptides altered their conformation and began by monitoring the known helix-coil transitions of poly-L-glutamic acid and poly-L-lysine by

optical rotatory dispersion using a manual spectropolarimeter. I then turned to their complexes with dyes. To my surprise, the optical rotation of acriflavine bound to the helical form of poly-L-glutamic acid changed dramatically with wavelength in the vicinity of the visible absorption band of the bound dye. The helical polypeptide by itself showed only a monotonic change in optical rotation in this spectral region, and the dye alone was optically inactive. I presented these data to Elkan that evening and told him that I was puzzled by the large magnitude of the optical rotation of the dye-polypeptide complex and that it went positive and then negative in such a short-wavelength interval. Elkan's eyes sparkled as he told me, "Ah, you've just seen a Cotton effect!"; he proceeded to give me an illuminating tutorial on how optical activity arises in the absorption bands of chiral molecules and is expressed as circular dichroism or circular birefringence. We were then excited to find that acriflavine bound to the random coil form of the polypeptide exhibited no Cotton effect in the visible band. These experiments revealed that symmetric chromophores can exhibit induced optical activity when bound to helical macromolecules (5).

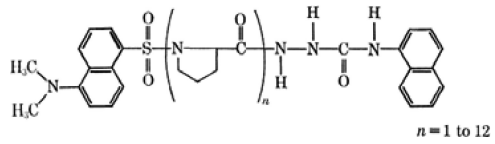
At the start of my fourth year of medical school, I decided to pursue a career in basic research. A few weeks later, Elkan had a plan for my postdoctoral years: I was to first move across the river to strengthen my background in physics, chemistry, and mathematics under the mentorship of Edward Purcell in the Department of Physics at Harvard and then join John Kendrew at the Medical Research Council (MRC) Laboratory in Cambridge, England, to learn x-ray crystallography. A fellowship from the Helen Hay Whitney Foundation made it possible for me to pursue these exceptional opportunities. Indeed, my postdoctoral years in the two Cambridges turned out to be among the best in my life. I was incredibly fortunate to have had Purcell as a very inspiring and supportive tutor. Kendrew then opened the doors of the MRC to me at an auspicious time in structural biology and molecular genetics. Together with Herman Watson, I used the difference Fourier method to see the precise mode of binding of the azide ion to the heme iron atom in azide ferrimyoglobin (6). This crystallographic study demonstrated that the mode of binding of a small molecule to a protein can readily be determined if the native and complexed crystals are isomorphous.

Fluorescent Probes

A very attractive offer of a faculty appointment at Stanford University from Arthur Kornberg and his colleagues in the biochemistry department led me to leave the MRC Laboratory earlier than planned. In the autumn of 1963, I went to Stanford to start my own research program and decided to pursue fluorescence spectroscopy as a means of gaining insight into the conformation and dynamics of protein molecules in solution. Gregorio Weber, a pioneer in fluorescence spectroscopy, had shown that some polycyclic aromatic hydrocarbons that are virtually non-fluorescent in solution become highly fluorescent on binding to proteins (7). I surmised that the markedly enhanced emission of these compounds might be due to their binding to non-polar crevices in proteins. I had in mind the non-polar niches that serve as the heme-binding sites in myoglobin and hemoglobin and proceeded to prepare the heme-free forms of these proteins. Indeed, apomyoglobin and apohemoglobin, but not their heme-bound forms, bound 1-anilino-8-naphthalene sulfonate (ANS) in a highly specific manner (8). Moreover, the fluorescence intensity of ANS increased 200-fold, and the emission spectrum shifted from green to blue by 60 nm. The fluorescence quantum yield of ANS likewise increased in going from water to less polar solvents, and there was a concomitant blue shift of the emission maximum. These experiments suggested that ANS and related chromophores would be useful in detecting changes in the tertiary and quaternary structures of proteins that alter the presence and accessibility of non-polar regions.

Energy Transfer as a Spectroscopic Ruler

During my medical school and postdoctoral years, I thought often about fluorescence energy transfer and particularly about Förster's prediction that the transfer efficiency depends on the inverse sixth power of the distance between the donor and acceptor. It seemed to me that this steep dependence on distance could be exploited to measure distances in proteins between sites bearing chromophores with appropriate spectroscopic properties, but it was essential to first test this central prediction of Förster's theory. The most direct way would be to synthesize a series of compounds in which the donor and acceptor moieties were separated by known distances. Helical synthetic polypeptides first came to mind during my medical school years, but the synthesis of a series of oligomers by conventional methods seemed very daunting and out of reach. A major breakthrough occurred in 1963 when R. Bruce Merrifield introduced solid-phase peptide synthesis, in which growing peptide chains attached to beads could be filtered and thereby washed free of reagents and byproducts at each step (9). Merrifield's solid-phase method opened the way to preparing molecular sticks of



STRUCTURE 1

different lengths to calibrate the distance dependence of energy transfer.

I chose oligomers of L-proline as spacers because it seemed likely that even short *n*-mers would form a type II *trans*-helix of the type seen in high molecular weight poly-L-proline and in the individual strands of collagen. Furthermore, the rise per residue of the prolyl *trans*-helix is 3.12 Å, compared with 1.5 Å for the α-helix, and so a wider span of distances would be obtained for a given range of *n*-mers than with an α-helix. Richard Haugland joined my laboratory as a graduate student, and we proceeded to synthesize oligomers of poly-L-proline up to the 12-mer by the solid-phase method. The energy donor was an α-naphthyl group at the carboxyl-terminal end of the peptide, and the energy acceptor was a dansyl group at the imino end. These chromophores were chosen because they fulfill the conditions for efficient transfer over distances of the order of 30 Å and have distinct absorption and emission spectra (Structure 1).

Optical rotatory dispersion measurements established that the oligomers from $n = 5$ to 12 in ethanol were in fact in the type II *trans*-helical conformation. We assumed that the helix was rigid and estimated from molecular models that the distance (r) between the centers of the chromophores ranged from 12 to 46 Å for $n = 1$–12. The transfer efficiency (E) decreased from nearly 100% for the short oligomers to 16% for the $n = 12$ oligomer. Most important, the observed dependence of the transfer efficiency on distance followed an inverse 5.9 ± 0.3 power dependence (Fig. 1) (10), in excellent agreement with the r^{-6} dependence predicted by Förster. We proposed then that energy transfer can serve as a spectroscopic ruler. Two years later, we showed that the transfer rate is proportional to J, the magnitude of the overlap between the emission spectrum of the energy donor and the absorption spectrum of the energy acceptor (11), and thereby validated another key prediction of Förster's theory.

It is very gratifying to see, forty-five years later, that fluorescence resonance energy transfer (FRET) has been used in thousands of studies to detect molecular associations, map the architecture of proteins and nucleic acids, and monitor conformational transitions. The application of recombinant DNA technology and the availability of a wide selection of fluorescent probes, exemplified by cyanine dyes and the green fluorescent proteins, have greatly

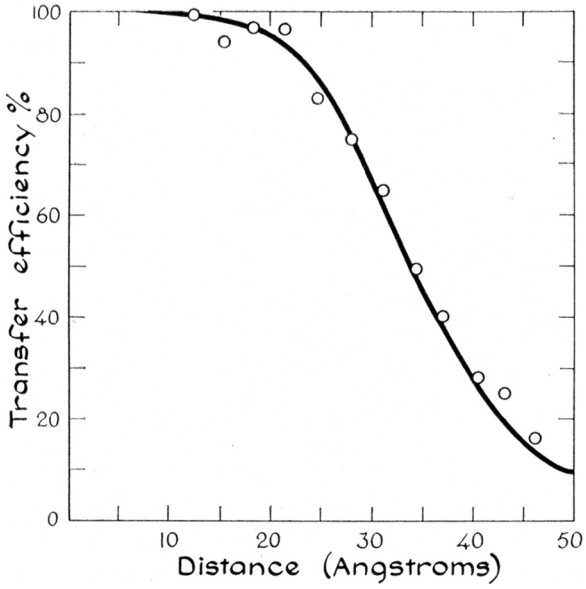


FIGURE 1. **Distance dependence of FRET.** The observed transfer efficiencies depend on the inverse 5.9 ± 0.3 power of the distance between the donor and acceptor, in excellent agreement with the r^{-6} dependence predicted by Förster. This figure has been reproduced from Ref. 10.

facilitated the specific labeling of macromolecules with donors and acceptors having appropriate spectroscopic properties. Uncertainty about the value of the dipole-dipole orientation factor has been markedly reduced by using fluorescent probes that have appreciable local rotational mobility and by using donor-acceptor pairs that have different attachment and transition moment geometries. The introduction of single-molecule FRET by Ha *et al.* (12) in 1996 was a major advance because it provides a window on discrete states and transitions between them without being blurred by ensemble averaging. The molecular ballet of life comes into view with single-molecule FRET, which has provided a wealth of information about molecular motors, signaling complexes, transcriptional and translational assemblies, membrane fusion machines, and protein folding dynamics (for an enticing sample, see Refs. 13–16). FRET has also been ingeniously employed to create highly specific sensors for cellular imaging studies (17, 18). The FRET harvest coming from many laboratories around the world has been remarkable. What began as a physical principle has been transformed into a powerful biological discovery tool.

Spectroscopic Studies of Rhodopsin

I have been intrigued by the molecular basis of visual excitation since my medical school days, when I visited George Wald's laboratory and learned that vision begins so simply, with the light-driven isomerization of 11-*cis*-retinal bound to rhodopsin to the all-*trans* form (19). I was also inspired

by Selig Hecht's earlier discovery that a retinal rod cell can be excited by a single photon (20). How remarkable that a 7-Å change in the geometry in a single rhodopsin molecule can generate a nerve signal! I conveyed my sense of awe and wonder in a lecture I gave in 1966 in Stanford's introductory biochemistry course and decided while lecturing that I should turn my research focus to the molecular basis of vision. My move from Stanford to Yale University in 1969 gave me the scope and opportunity to initiate this alluring exploration.

We began by carrying out spectroscopic studies of rhodopsin. Our FRET experiments showed that rhodopsin is an elongated molecule with retinal bound near its center (21). We also succeeded in obtaining the resonance Raman spectrum of rhodopsin, which was technically challenging, by rapidly flowing a fresh solution into the intensely illuminated volume (22). Richard Mathies brought electric field spectroscopy into the laboratory and applied it to retinal and its Schiff bases. The key finding was that retinal has a highly dipolar, vertically excited singlet state (23). The 12-debye change in dipole moment on excitation corresponds to a shift of 0.21 e^- across the retinal, with the ionone ring becoming more electropositive. One consequence of this charge shift is that the double bonds of the polyene chain acquire single-bond character, which would promote isomerization. The large increase in dipole moment on excitation also provides a facile electrostatic mechanism for tuning the absorption maximum of retinal, which is at 500 nm for rhodopsin and ranges from 431 to 561 nm for the cone visual pigments. Negatively charged groups positioned near the ionone ring would lower the energy of the excited state and thereby shift the absorption maximum of retinal to red.

Molecular Basis of Amplification in Visual Excitation

At a Gordon Conference in the summer of 1978, I was intrigued by Paul Liebman's report that a single photon can trigger the activation of hundreds of phosphodiesterase (PDE) molecules specific for cGMP in rod outer segments (ROS) provided that GTP is present (24). On my way home from this meeting, I had the good fortune of staying overnight at William Miller's home. After a delicious dinner, Miller showed me some striking electrophysiological records. He and Grant Nicol had just found that rods became depolarized when cGMP was injected in the dark into ROS (25). Furthermore, the injection of cGMP greatly increased the latency between illumination and hyperpolarization. The simplest interpretation of these data was that cGMP is the transmitter: sodium-conducting channels are opened by cGMP in the dark and close when the cGMP level drops because of hydrolysis by the light-activated PDE. Channel closure would directly hyperpolarize the rod, in agreement with Tsuneo Tomita's finding several years earlier that light hyperpolarizes vertebrate rod cells (26).

When I returned to Stanford (having moved back after seven rewarding years at Yale), I told Bernard Fung, a postdoctoral fellow in my laboratory, about these exciting results. He shared my enthusiasm for the notion that cGMP might be the transmitter, so we decided to explore the molecular mechanism by which the cGMP-specific PDE is activated by light. The requirement for GTP and Mark Bitensky's recent finding that ROS contain a light-activated GTPase (27) suggested that a G-protein might be a key participant in this process. Our first experiment was designed to detect the binding of guanyl nucleotides to ROS membranes by incubating them with [α-^{32}P]GTP and washing with a nucleotide-free buffer. We were rewarded by finding that 1) the ROS contained radioactive GDP following incubation, and 2) subsequent bright illumination led to the release of the bound GDP. Because ROS are rich in GTP, the question arose as to whether GTP influences the photorelease of tightly bound GDP. Indeed, GTP greatly enhanced the photorelease of GDP. In the presence of 1 μM GTP, release of GDP was half-maximal when fewer than 1 in 10^4 rhodopsins were excited. The next question was what happens when ROS are incubated with a hydrolysis-resistant analog of GTP such as guanosine 5′-(β,γ-imido)triphosphate (Gpp(NH)p). The striking finding was that a single photoexcited rhodopsin catalyzed the uptake of 500 molecules of this GTP analog (28). Our observation of this highly amplified GTP-GDP exchange led us to propose that the GTP form of a guanyl nucleotide-binding protein, which we subsequently called transducin, is the first amplified intermediate in visual excitation. We also proposed that the GTP form of transducin is the activator of the PDE.

The flow of information postulated by us in 1980 was R* → T-GTP → PDE*, where R* is photoexcited rhodopsin, T-GTP is transducin bearing GTP, and PDE* is the activated form of the phosphodiesterase. Our scheme predicted that 1) T-GTP can be formed in the absence of the phosphodiesterase, and 2) the phosphodiesterase can be activated in the absence of photoexcited rhodopsin. The next step was to purify transducin, a task facilitated by Hermann Kühn's report that transducin binds tightly to illuminated ROS membranes in the absence of GTP and can be released into solution by adding GTP (29). We added a final hexyl-agarose chromatography step and

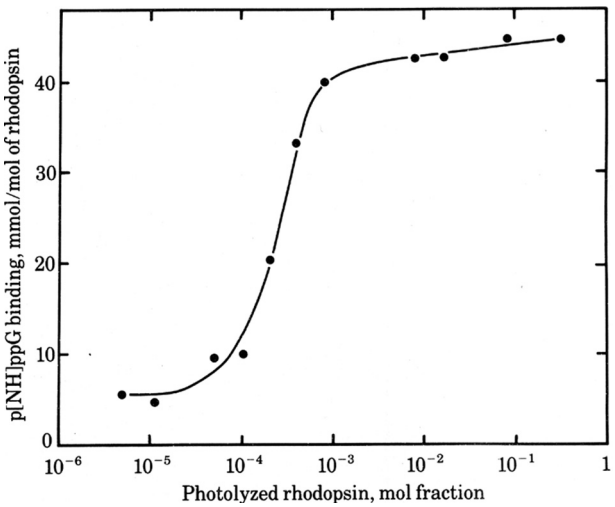


FIGURE 2. **A single photoexcited rhodopsin molecule leads to the amplified uptake of GTP by transducin.** In this reconstituted system containing only rhodopsin and transducin in phosphatidylcholine membrane vesicles, a single R* catalyzed the uptake of 71 molecules of Gpp(NH)p, a hydrolysis-resistant analog of GTP. This figure has been reproduced from Ref. 30.

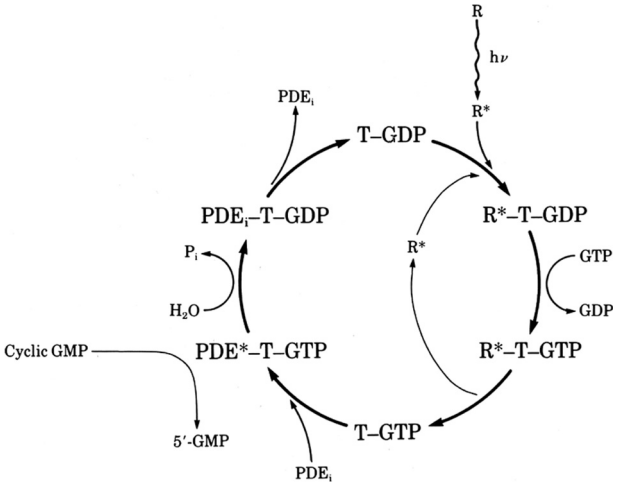


FIGURE 3. **Light-activated cGMP cascade of vision.** *R*, unexcited rhodopsin; *R**, photoexcited rhodopsin; *T*, transducin; *PDE$_i$*, inhibited phosphodiesterase; *PDE**, activated phosphodiesterase. This figure has been reproduced from Ref. 30.

observed that transducin consists of three polypeptide chains: T_α (~39 kDa), T_β (~36 kDa), and T_γ (~10 kDa). We then found that reconstituted membranes containing only rhodopsin and transducin exhibit light-activated GTPase activity and light-triggered binding of GTP or a GTP analog. A single R* led to the uptake of Gpp(NH)p by 71 molecules of transducin (Fig. 2). This reconstituted system revealed that the role of photoexcited rhodopsin is to activate transducin by catalyzing the exchange of GTP for bound GDP (30).

We were then very eager to learn whether the GTP form of transducin, by itself, activates the PDE. Fung and James Hurley, who had recently joined my laboratory, prepared transducin containing Gpp(NH)p. We encountered a pleasant surprise in purifying this complex. Transducin had dissociated into T_α and $T_{\beta\gamma}$ following the exchange of Gpp(NH)p for GDP, and it was the α-subunit that bore the GTP analog. Now we could ask a more precise question than was originally planned: do either T_α-Gpp(NH)p or $T_{\beta\gamma}$ activate the PDE? We found that T_α bearing the GTP analog was highly effective in activating PDE in the dark, whereas $T_{\beta\gamma}$ had no effect. Thus, T_α-GTP is the amplified information-carrying intermediate in the photoactivation of the PDE (30).

T_α-GTP greatly stimulates the catalytic activity of the PDE by pulling away its inhibitory subunits, which hold the PDE in check in the dark (31, 32). The subsequent hydrolysis of T_α-GTP to T_α-GDP returns the PDE to the dark state. Also crucial for the return to the dark state, as shown by Kühn, is the deactivation of R* by multiple phosphorylations of its C-terminal region, followed by the binding of arrestin, which caps these sites and prevents R* from interacting with transducin (33).

Our experiments established that transducin cycles between a quiescent T-GDP state in the dark and a transiently active T_α-GTP state following illumination (Fig. 3). The light-activated transducin cycle is powered by the hydrolysis of GTP rather than by the energy of the absorbed photon, which serves only to generate a very reliable trigger, R*. The cycle flows unidirectionally once triggered by R*, whose only role is to bind transducin to stabilize the transition state, an empty nucleotide-binding site. R* accelerates GTP-GDP exchange by a factor of $\sim 10^7$, corresponding to a lowering of the energy of the transition state by ~10 kcal/mol. The reaction partners have been designed so that R* has high affinity for transducin devoid of a bound nucleotide but not for T-GDP or T-GTP. Like other efficient catalysts, R* is specifically complementary to the transition state but not to the substrate or product. The free energy profile of the transducin cycle has been optimized for 1) the rapid formation of T_α-GTP, 2) a high degree of amplification, 3) effective operation over a wide dynamic range of illumination intensity, and 4) maintenance of a very low level of T_α-GTP in the dark (34).

Elegant electrophysiological studies by Denis Baylor and co-workers revealed that absorption of a single photon blocks the influx of millions of sodium ions into ROS by closing hundreds of channels in the plasma membrane to give the consequent hyperpolarization (35). What is the link between the light-induced lowering of the level of cGMP and channel closure? The answer proved to be unexpectedly simple. Evgeniy Fesenko and co-workers incisively demonstrated in 1985 that cGMP directly opens

cation-specific channels in excised patches of the plasma membrane (36). This key finding provided the final link in the excitation chain and gave us a mechanistic understanding of what makes a retina rod cell so exquisitely sensitive. Three stages of amplification, the generation of many T_{α}-GTP complexes by R*, the hydrolysis of many cGMPs by activated PDE, and the consequent blockage of the flow of many cations through a single cGMP-gated channel, give rise to the single-photon sensitivity of rods. Subsequent studies carried out in numerous laboratories have delineated how recovery of the dark state and adaptation over a very wide range of background light levels are achieved (for reviews, see Refs. 37 and 38). Cone cells, which mediate color vision and vision at high light levels, use a very similar transduction cascade; the cone homologs of the signaling molecules of rods are optimized for speed rather than sensitivity. What is especially satisfying is that the G-protein cascade of vision exemplifies fundamental principles of signal transduction that are ubiquitously employed in eukaryotes.

Collaborative Explorations

In 1981, Vernon Oi, a postdoctoral fellow in my laboratory with expertise in immunology, challenged me to devise better long wavelength-emitting fluorescent probes for flow cytofluorometry and fluorescence microscopy than were available at the time. He noted a particular need for orange- and red-emitting probes that would complement green-emitting fluorescein and, ideally, could be excited with a single laser line. It soon occurred to me that nature had long ago provided the solution. My good friend Alexander Glazer at the University of California, Berkeley (whom I knew since our postdoctoral days at the MRC Laboratory in Cambridge), often spoke to me about his pioneering research on phycobiliproteins, a family of highly fluorescent proteins present in the light-harvesting apparatus of cyanobacteria and red algae. Phycobiliproteins assemble into phycobilisomes, which harvest light that is not efficiently absorbed by chlorophyll, in the 470–650 nm valley between the blue and far-red absorption peaks of chlorophyll *a* and funnel it into the photosynthetic reaction center. The flow of energy is from phycoerythrin to phycocyanin to allophycocyanin and then to the reaction center. Indeed, phycobilisomes are directional light guides, nature's ultimate FRET devices (39).

Oi, Glazer, and I decided to form conjugates of phycobiliproteins with biological recognition molecules such as antibodies, protein A, and avidin and then test them in two-color analyses with T-lymphocytes. We found that phycoerythrin could readily be coupled to other proteins with full retention of its brilliant fluorescence. Because phycoerythrin contains multiple bilin chromophores and has a fluorescence quantum yield of 0.8, its conjugates are, on a molar basis, >10 times as bright as those of fluorescein. Fluorescein conjugates and phycoerythrin conjugates proved to be ideal for two-color cell sorting because they can be excited strongly with the 488-nm argon ion laser line, and their emissions at 515 and 576 nm are easy to separate (40). Three- and four-color analyses are made feasible by conjugates of phycocyanin and allophycocyanin, which absorb and emit at longer wavelengths than does phycoerythrin, and also by a tandem phycoerythrin-allophycocyanin conjugate, which has a very large Stokes shift between its absorption and emission maxima because of energy transfer (41, 42). Phycobiliprotein conjugates, built from ancient antenna proteins and molecules that confer specific binding, have proven to be choice reagents for multicolor cell analyses since our introduction of them three decades ago.

I also had the good fortune of collaborating with Denis Baylor, my colleague at Stanford, starting in 1984. As mentioned above, Baylor and co-workers were the first to detect the electrical response of a rod cell to a single absorbed photon (35). Our collaboration was directed to determining whether the light-sensitive channels of rods are controlled only by cGMP or are additionally regulated by other transmitters. The experimental strategy was to introduce hydrolysis-resistant analogs of cGMP, synthesized by Fritz Eckstein in Göttingen, through a patch electrode sealed on the inner segment membrane and concurrently monitor the membrane current of the outer segment by a suction electrode. We were joined in these experiments by two postdoctoral fellows, Anita Zimmerman and Gregory Yamanaka. The results were clear-cut: all three hydrolysis-resistant analogs (two phosphorothioate analogs and 8-Br-cGMP) markedly increased the dark current, very little of which could be shut off by light. These results strongly implied that 1) the light-sensitive channel of ROS is controlled solely by the instantaneous concentration of cGMP, and 2) the cGMP-sensitive channel of excised patches is identical to the light-sensitive channel of intact rods (43).

We pursued another collaboration two years later to measure the gating kinetics of cGMP-gated channels in excised membrane patches. Jeffrey Karpen and Zimmerman perturbed the patch current in two ways: by laser flash photolysis of a caged analog of cGMP to generate fast rises in cGMP concentration and membrane voltage jumps to alter the interaction of the channels with cGMP. Our results showed that the channel responded in times of milliseconds and that a diffusion-controlled binding of at

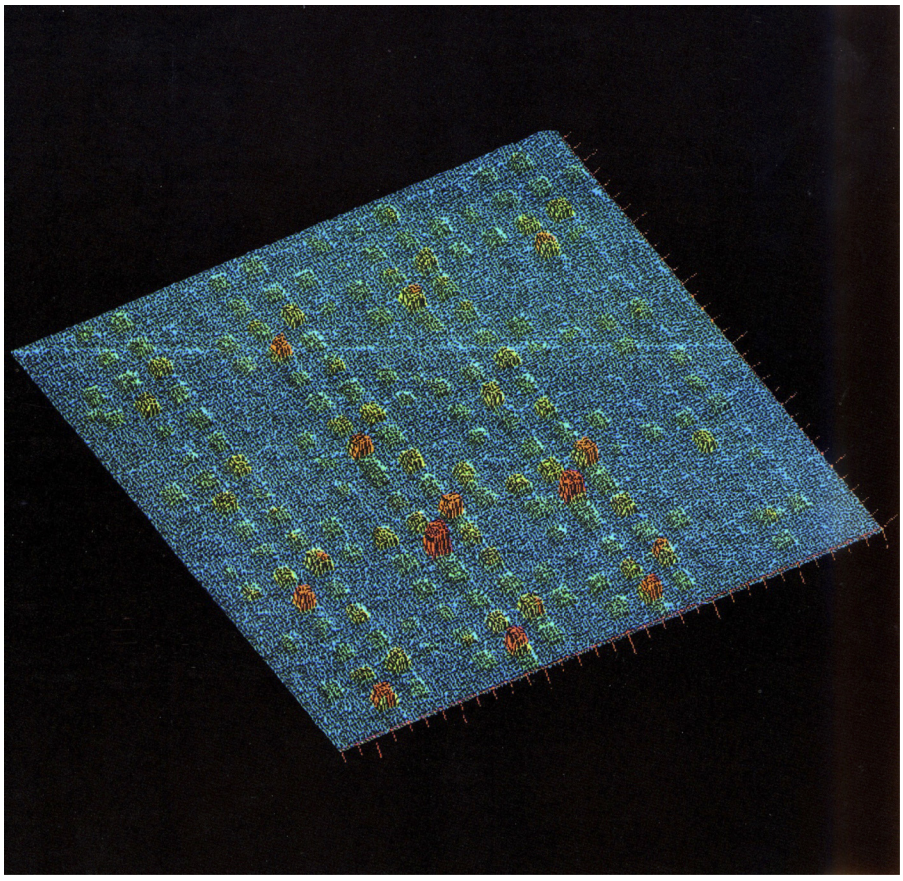


FIGURE 4. **Light-directed combinatorial synthesis of a spatially addressable array of 1024 peptides.** The array was generated by a ten-step binary synthesis. The *red peaks* identify peptides with high affinity for a specific fluorescently labeled antibody. This figure has been reproduced from Ref. 46.

least three molecules of cGMP to the closed channel was followed by a very rapid opening (44). The millisecond kinetics of the channel enables it to respond rapidly to light-induced decreases in cGMP concentration and also serves to minimize dark noise in the physiologically pertinent frequency band (45). The coming together of biochemistry, spectroscopy, and electrophysiology in these collaborations was stimulating and satisfying.

In 1989, Alejandro Zaffaroni, an innovative entrepreneur, told me that he was starting Affymax, a new company, to generate chemical diversity on a vast scale with the aim of accelerating the pace of drug discovery. He invited me to come on board as a scientific advisor. I was intrigued and, a few months later, after numerous brainstorming sessions, accepted his offer to serve as the first scientific director of Affymax and took a one-year leave of absence from Stanford. One of the ideas was to use light to direct the synthesis of many chemical compounds in a spatially addressable array on a solid support. I recruited Stephen Fodor, a postdoctoral fellow with expertise in spectroscopy, then in Mathies' laboratory in Berkeley, to direct this effort, which he did with energy and verve. In essence, synthesis occurs on a solid support, and the pattern of exposure to light determines which regions of the support are activated for chemical coupling by removal of photolabile protecting groups. After deprotection, the first of a set of building blocks (*e.g.* amino acids or nucleotides, each bearing a photolabile protecting group) is exposed to the entire surface, but coupling occurs only at sites that were deprotected in the preceding step. The pattern of masks used in a series of illuminations and the sequence of reactants define the ultimate products and their locations. In 1991, we reported the synthesis of an array of 1024 different peptides synthesized in ten chemical coupling steps by this new method of light-directed, spatially addressable parallel chemical synthesis (Fig. 4) (46). The generality of this method was also demonstrated by the light-directed synthesis of a dinucleotide. We also presented a general formalism to describe the combinatorial strategy for any desired spatially addressable synthesis. An attractive feature of combinatorial syntheses is that the number of products increases exponentially with the number of chemical steps. In a binary synthesis (in which one-half of the substrate is recursively masked), the number of products is 2^n, where n is the number of chemical steps. Moreover, in a light-directed synthesis, any desired

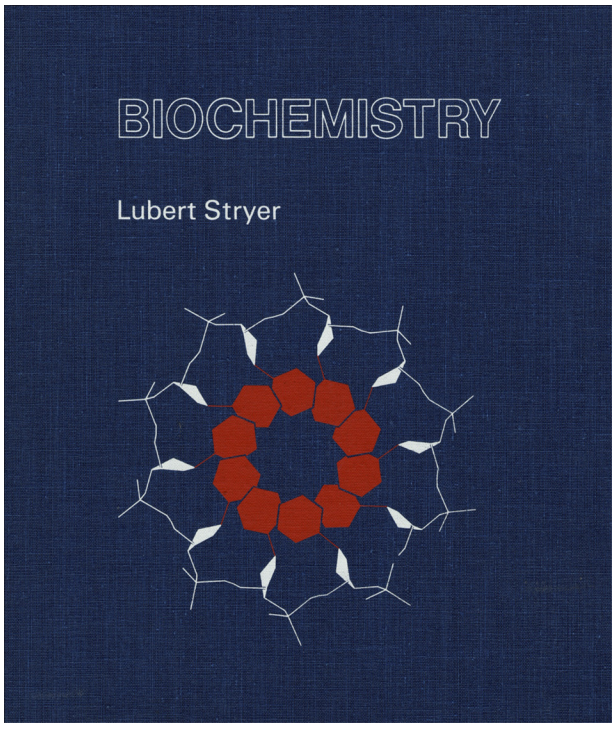


FIGURE 5. **Image of the cover of the first edition of my textbook of biochemistry, which was published in 1975.**

FIGURE 6. **Author at St. Andrews Bay, South Georgia.** Many thousands of king penguins can be seen in the background.

subset of the 2^n potential products can be made using an appropriate series of masks.

Fodor, at Affymax and then at its offspring, Affymetrix, innovatively developed light-activated synthesis of oligonucleotides and brought it to fruition to make DNA chips ultimately containing more than one million different sequences on a thumbnail-size support (47). As a scientific advisor to Affymetrix, I had the pleasure of seeing Fodor spearhead the synthesis and use of DNA chips for monitoring gene expression and detecting single-nucleotide polymorphisms. The coming together of solid-phase chemistry, photolabile protecting groups, and photolithography has greatly enhanced our capacity to read genetic information.

Color Vision and Photography

I have been captivated by visual imagery for my entire life and particularly by color, both in the natural world and in art. I used a highly visual approach in conveying fundamental concepts and illustrating chemical transformations in my textbook of biochemistry, from the first edition in 1975 to the fourth edition in 1995 (Fig. 5) (48). From the outset, multicolor graphics played a key role in enabling me to vividly depict the beautiful relations between molecular architecture and biological activity.

My retirement from Stanford eight years ago has given me the gift of time to intensively pursue two intertwined interests, photography and adventure travel (Fig. 6). My interest in photography began in childhood as a way of seeing more intensively and capturing what is so transient, and it has deepened in tandem with my research passion of many decades, the interplay of light and life. Adventure travel came into the picture soon after I married Andrea in 1958. I particularly enjoy photographing scenery and wildlife, as on our journeys to Antarctica, the Arctic, the Galapagos Islands, and Africa. Photography has heightened my awareness of color in the natural world and deepened my interest in color vision.

In 1802, Thomas Young presciently proposed the following in his Bakerian Lecture to the Royal Society (49).

> Now, as it is almost impossible to conceive each sensitive point of the retina to contain an infinite number of particles, each capable of vibrating in perfect unison with every possible undulation, it becomes necessary to suppose the number limited . . . each sensitive filament of the nerve may consist of three portions, one for each principal colour.

I recall my excitement in 1964 while reading Edward MacNichol's article "Three-Pigment Color Vision" (50), in which he presented his tour de force microspectrophotometric measurements showing that the goldfish retina has three kinds of cone cells with widely separated absorption spectra. The even more challenging studies of human ret-

inas by Paul Brown and George Wald strongly suggested that humans also have three kinds of cones (51). In 1985, I was thrilled to learn from Jeremy Nathans, an M.D.-Ph.D. student in David Hogness's laboratory, that he had cloned the genes encoding the three opsins for human color vision (52). Several years later, Baylor measured the spectral sensitivities of individual cone cells in primate retinas and found that they belong to three classes (53). These landmark studies conclusively established Young's proposal that human color vision depends on three kinds of receptors. The S, L, and M visual pigments absorb maximally at 431, 531, and 561 nm, respectively. By contrast, most mammals are dichromats. The evolution of trichromacy in primates is a complex and fascinating story that was elucidated by Gerald Jacobs and others and is lucidly recounted by Jacobs and Nathans in a recent article (54). The trichromacy of the Old World primate line, which includes humans, probably arose about thirty million years ago by a rare recombinational event. John Mollon and co-workers demonstrated, on the basis of extensive spectral studies in the field in Africa, that trichromacy enables its primate possessors to detect ripe fruits against a background of leaves (55).

I was recently privileged to see two families of gorillas in the Virunga Mountains of Rwanda. Being near a silverback male and his family of twenty and observing their social and foraging behavior as they rapidly move through the high mountain forest is awesome. Through the dense brush, I suddenly saw a gorilla savoring succulent red berries, literally enjoying the fruits of trichromacy (Fig. 7). In that instant, so many of my interests in light and life converged. The focus of my camera was on the red fruit, whose color was conferred by carotenoids, ancient molecules that arose to protect photosynthetic organisms from photodynamic death (56). They are also the principal pigments that give the distinctive colors of carrots, tomatoes, and many fruits that delight us. Moreover, β-carotene is the precursor of 11-*cis*-retinal, the chromophore in all image-forming visual systems. 11-*cis*-Retinal is ideal for vision because it is efficiently photoisomerized to all-*trans*-retinal while being very stable in the dark (35). Furthermore, as was mentioned above, 11-*cis*-retinal has a highly dipolar excited state (23), which makes its absorption spectrum highly tunable and hence well suited for color vision. Just three amino acid residues are responsible for the absorption spectrum difference between M and L visual pigments. The co-evolution of fruit color and trichromacy was so graphically displayed in that wonderful moment in the mountains of Rwanda.

FIGURE 7. **A gorilla enjoying the fruits of trichromacy in the Virunga Mountains of Rwanda.**

I have been extremely fortunate in having lived at a wonderful time in science. The DNA double helix was discovered during my scientific childhood, followed by the elucidation of the genetic code and the nerve action potential. The molecular mechanisms of channels, motors, and signal transduction cascades then came into view, followed by the recombinant DNA revolution, which profoundly enriched biology and made possible what had not even been dreamt of a few years earlier. Structural biology is now providing exquisitely detailed and dynamic pictures of molecular machines in action, and whole genome sequencing has opened the way to a deeper understanding of evolution and how genotype gives rise to phenotype. Networks consisting of many genes and proteins with novel emergent properties are now moving to center stage. How exhilarating to catch glimpses of where science is heading!

Acknowledgments—I am indebted to my students, postdoctoral fellows, and collaborators for all they did to make our research adventures so rewarding and joyful. I learned so much from them and from my colleagues at Stanford and Yale. Both institutions have provided me with extraordinarily stimulating environments for research and teaching. I am grateful to this country for opening its doors to me as an immigrant child in 1948 and providing limitless opportunities in the decades that followed and, above all, to my wife, Andrea, for her devoted support, wise counsel, and love over more than fifty years.

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REFERENCES

1. Cario, G., and Franck, J. (1923) Uber sensibilisierte fluoreszenz von gasen. *Z. Physik* **17,** 202–212
2. Förster, T. (1948) Zwischenmolekulare energiewanderung und fluoreszenz. *Ann. Physik* **437,** 55–75
3. Stryer, L. (1959) Intramolecular resonance transfer of energy in proteins. *Biochim. Biophys. Acta* **35,** 242–244
4. Stryer, L. (1960) Energy transfer in proteins and polypeptides. *Symp. Bioenerg. Radiat. Res. Suppl.* **2,** 432–451
5. Blout, E. R., and Stryer, L. (1959) Anomalous optical rotatory dispersion of dye: polypeptide complexes. *Proc. Natl. Acad. Sci. U.S.A.* **45,** 1591–1593
6. Stryer, L., Kendrew, J. C., and Watson, H. C. (1964) The mode of attachment of the azide ion to sperm whale metmyoglobin. *J. Mol. Biol.* **8,** 96–104
7. Weber, G., and Laurence, D. J. (1954) Fluorescent indicators of adsorption in aqueous solution and on the solid phase. *Biochem. J.* **56,** xxxi
8. Stryer, L. (1965) The interaction of a naphthalene dye with apomyoglobin and apohemoglobin. A fluorescent probe of non-polar binding sites. *J. Mol. Biol.* **13,** 482–495
9. Merrifield, R. B. (1963) Solid-phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Am. Chem. Soc.* **85,** 2149–2154
10. Stryer, L., and Haugland, R. P. (1967) Energy transfer: a spectroscopic ruler. *Proc. Natl. Acad. Sci. U.S.A.* **58,** 719–726
11. Haugland, R. P., Yguerabide, J., and Stryer, L. (1969) Dependence of the kinetics of singlet-singlet energy transfer on spectral overlap. *Proc. Natl. Acad. Sci. U.S.A.* **63,** 23–30
12. Ha, T., Enderle, T., Ogletree, D. F., Chemla, D. S., Selvin, P. R., and Weiss, S. (1996) Probing the interaction between two single molecules: fluorescence resonance energy transfer between a single donor and a single acceptor. *Proc. Natl. Acad. Sci. U.S.A.* **93,** 6264–6268
13. Zimmermann, B., Diez, M., Börsch, M., and Gräber, P. (2006) Subunit movements in membrane-integrated EF_oF_1 during ATP synthesis detected by single-molecule spectroscopy. *Biochim. Biophys. Acta* **1757,** 311–319
14. Komatsuzaki, T., Kawakami, M., Takahashi, S., Yang, H., Silbey, R. J., Nettels, D., and Schuler, B. (2011) Single-molecule FRET of protein folding dynamics. *Adv. Chem. Phys.* **146,** 23–48
15. Aitken, C. E., and Puglisi, J. D. (2010) Following the intersubunit conformation of the ribosome during translation in real time. *Nat. Struct. Mol. Biol.* **17,** 793–800
16. Choi, U. B., Strop, P., Vrljic, M., Chu, S., Brunger, A. T., and Weninger, K. R. (2010) Single-molecule FRET-derived of the synaptotagmin 1-SNARE fusion complex. *Nat. Struct. Mol. Biol.* **17,** 318–324
17. Tsien, R. Y. (2009) Constructing and exploiting the fluorescent protein paintbox (Nobel lecture). *Angew. Chem. Int. Ed. Engl.* **48,** 5612–5626
18. Miyawaki, A. (2011) Development of probes for cellular functions using fluorescent proteins and fluorescence resonance energy transfer. *Annu. Rev. Biochem.* **80,** 357–373
19. Hubbard, R., and Kropf, A. (1958) The action of light on rhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* **44,** 130–139
20. Hecht, S., Shlaer, S., and Pirenne, M. H. (1942) Energy, quanta, and vision. *J. Gen. Physiol.* **25,** 819–840
21. Wu, C. W., and Stryer, L. (1972) Proximity relationships in rhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* **69,** 1104–1108
22. Mathies, R., Oseroff, A. R., and Stryer, L. (1976) Rapid-flow resonance Raman spectroscopy of photolabile molecules: rhodopsin and isorhodopsin. *Proc. Natl. Acad. Sci. U.S.A.* **73,** l-5
23. Mathies, R., and Stryer, L. (1976) Retinal has a highly dipolar vertically excited singlet state: implications for vision. *Proc. Natl. Acad. Sci. U.S.A.* **73,** 2169–2173
24. Yee, R., and Liebman, P. A. (1978) Light-activated phosphodiesterase of the rod outer segment. Kinetics and parameters of activation and deactivation. *J. Biol. Chem.* **253,** 8902–8909
25. Nicol, G. D., and Miller, W. H. (1978) Cyclic GMP injected into retinal rod outer segments increases latency and amplitude of response to illumination. *Proc. Natl. Acad. Sci. U.S.A.* **75,** 5217–5220
26. Tomita, T. (1970) Electrical activity of vertebrate photoreceptors. *Q. Rev. Biophys.* **3,** 179–222
27. Wheeler, G. L., and Bitensky, M. W. (1977) A light-activated GTPase in vertebrate photoreceptors: regulation of light-activated cyclic GMP phosphodiesterase. *Proc. Natl. Acad. Sci. U.S.A.* **74,** 4238–4242
28. Fung, B. K., and Stryer, L. (1980) Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments. *Proc. Natl. Acad. Sci. U.S.A.* **77,** 2500–2504
29. Kühn, H. (1980) Light- and GTP-regulated interaction of GTPase and other proteins with bovine photoreceptor membranes. *Nature* **283,** 587–589
30. Fung, B. K., Hurley, J. B., and Stryer, L. (1981) Flow of information in the light-triggered cyclic nucleotide cascade of vision. *Proc. Natl. Acad. Sci. U.S.A.* **78,** 152–156
31. Pober, J. S., and Bitensky, M. W. (1979) Light-regulated enzymes of vertebrate retinal rods. *Adv. Cyclic Nucleotide Res.* **11,** 265–301
32. Hurley, J. B., and Stryer, L. (1982) Purification and characterization of the γ-regulatory subunit of the cyclic GMP phosphodiesterase from retinal rod outer segments. *J. Biol. Chem.* **257,** 11094–11099
33. Wilden, U., Hall, S. W., and Kühn, H. (1986) Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments. *Proc. Natl. Acad. Sci. U.S.A.* **83,** 1174–1178
34. Stryer, L. (1987) Visual transduction: design and recurring motifs. *Chem. Scripta B* **27,** 161–171
35. Baylor, D. A., Lamb, T. D., and Yau, K. W. (1979) Responses of retinal rods to single photons. *J. Physiol.* **288,** 613–634
36. Fesenko, E. E., Kolesnikov, S. S., and Lyubarsky, A. L. (1985) Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature* **313,** 310–313
37. Luo, D. G., Xue, T., and Yau, K. W. (2008) How vision begins: an odyssey. *Proc. Natl. Acad. Sci. U.S.A.* **105,** 9855–9862
38. Arshavsky, V. Y., and Burns, M. E. (2012) Photoreceptor signaling: supporting vision across a wide range of light intensities. *J. Biol. Chem.* **287,** 1620–1626
39. Glazer, A. N. (1989) Light guides. Directional energy transfer in a photosynthetic antenna. *J. Biol. Chem.* **264,** 1–4
40. Oi, V. T., Glazer, A. N., and Stryer, L. (1982) Fluorescent phycobiliprotein conjugates for analyses of cells and molecules. *J. Cell Biol.* **93,** 981–986
41. Glazer, A. N., and Stryer, L. (1983) Fluorescent tandem phycobiliprotein conjugates. Emission wavelength shifting by energy transfer. *Biophys. J.* **43,** 383–386
42. Glazer, A. N., and Stryer, L. (1984) Phycofluor probes. *Trends Biochem. Sci.* **9,** 423–427
43. Zimmerman, A. L., Yamanaka, G., Eckstein, F., Baylor, D. A., and Stryer, L. (1985) Interaction of hydrolysis-resistant analogs of cyclic GMP with the phosphodiesterase and light-sensitive channel of retinal rod outer segments. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 8813–8817
44. Karpen, J. W., Zimmerman, A. L., Stryer, L., and Baylor, D. A. (1988) Gating kinetics of the cyclic GMP-activated channel of retinal rods: flash photolysis and voltage-jump studies. *Proc. Natl. Acad. Sci. U.S.A.* **85,** 1287–1291
45. Karpen, J. W., Zimmerman, A. L., Stryer, L., and Baylor, D. A. (1988) Molecular mechanics of the cyclic GMP-activated channel of retinal rods. *Cold Spring Harbor Symp. Quant. Biol.* **53,** 325–332
46. Fodor, S. P., Read, J. L., Pirrung, M. C., Stryer, L., Lu, A. T., and Solas, D. (1991) Light-directed, spatially addressable parallel chemical synthesis. *Science* **251,** 767–773
47. Matsuzaki, H., Dong, S., Loi, H., Di, X., Liu, G., Hubbell, E., Law, J., Berntsen, T., Chadha, M., Hui, H., Yang, G., Kennedy, G. C., Webster, T. A., Cawley, S., Walsh, P. S., Jones, K. W., Fodor, S. P., Mei, R. (2004) Genotyping over 100,000 SNPs on a pair of oligonucleotide arrays. *Nat. Methods* **1,** 109–111
48. Stryer, L. (1995) *Biochemistry*, 4th Ed., W. H. Freeman and Co., New York
49. Young, T. (1802) On the theory of light and colours. *Philos. Trans. R. Soc. Lond.* **92,** 12–48
50. MacNichol, E. F., Jr. (1964) Three-pigment color vision. *Sci. Amer.* **211,** 48–56
51. Brown, P. K., and Wald, G. (1964) Visual pigments in single rods and cones of the human retina. Direct measurements reveal mechanisms of human night and color vision. *Science* **144,** 45–52
52. Nathans, J., Thomas, D., and Hogness, D. S. (1986) Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. *Science* **232,** 193–202
53. Baylor, D. A., Nunn, B. J., and Schnapf, J. L. (1987) Spectral sensitivity of cones of the monkey *Macaca fascicularis*. *J. Physiol.* **390,** 145–160
54. Jacobs, G. H., and Nathans, J. (2009) The evolution of primate color vision. *Sci. Amer.* **300,** 56–63
55. Regan, B. C., Julliot, C., Simmen, B., Viénot, F., Charles-Dominique, P., and Mollon, J. D. (2001) Fruits, foliage, and the evolution of primate color vision. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **356,** 229–283
56. Griffiths, M., Sistrom, W. R., Cohen-Bazire, G., Stanier, R. Y., and Calvin, M. (1955) Function of carotenoids in photosynthesis. *Nature* **176,** 1211–1215