BIOCHEMISTRY

Redder Than Red

Molecular engineering of a retinol binding protein defies prediction.

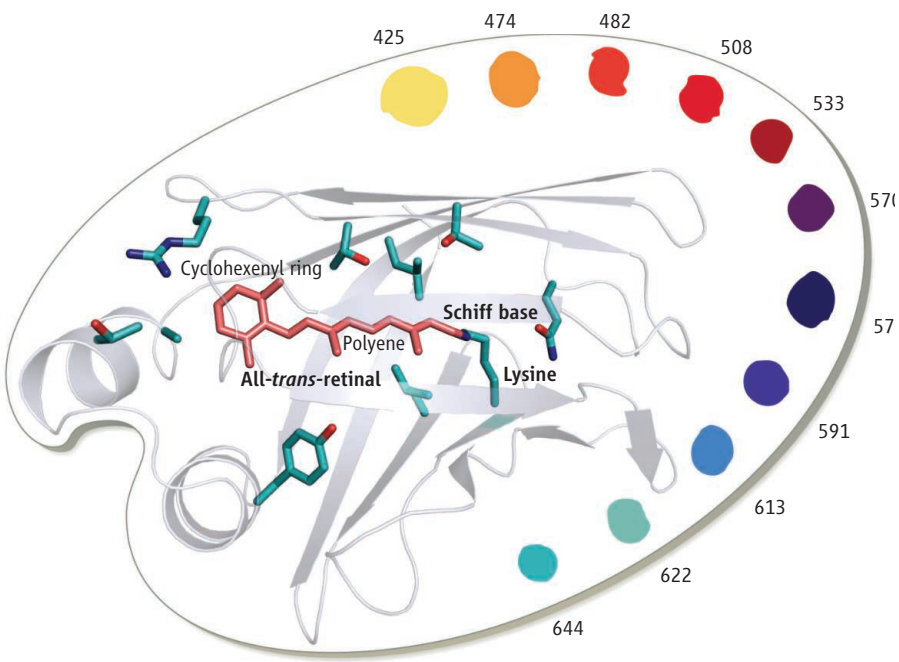
Thomas P. Sakmar

A chromophore is the part of a molecule that imparts its color. Arguably the most common chromophores in biology are carotenoids such as retinoids. Their absorption spectra can be tuned across a wide range of light energy by modulating the electronic environment within the protein to which they are attached. On page 1340 of this issue, Wang *et al*. (*1*) report a systematic study of the spectral tuning of an engineered all-*trans*-retinal pigment within a protein environment. Using protein engineering and x-ray crystallography, they create a palette of retinal-based pigments that reveal how electrostatics dominates spectral tuning (see the figure).

The physicochemical basis of retinoid chromophore spectral tuning is embodied in the term "opsin shift" (*2*). A model retinal chromophore compound absorbs maximally at 440 nm in methanol solution, but its absorption can be red-shifted to ~600 nm. The energy difference between 440 nm and the absorption of the pigment in question is the opsin shift. Factors that may affect the opsin shift include the presence of charged or polar amino acids in the chromophore-binding pocket, as well as protein-induced twisting of the normally planar polyene system. Just how a protein alters the electronic structure of the chromophore to cause dramatic spectral changes has been a matter of debate.

Knowledge of the opsin shift mechanism is important for understanding color vision (*3*). Most humans have three separate visual pigments—blue, green, and red—that absorb light maximally at ~420, ~530, and ~560 nm, respectively (*4*–*6*). In each case, the chromophore is the same: a vitamin A aldehyde linked to a lysine residue by a Schiff base (a carbon-nitrogen double bond). Spectral tuning is achieved by genetic differences in the opsin genes that encode different amino acid sequences in the binding pocket of the chromophore. Other animals are much more spectrally endowed than humans. The mantis shrimp, for example, has 12 spectral channels from ultraviolet to deep red (*7*). But in all cases, the visual chromophore is retinal (vitamin A aldehyde), or a very closely related hydroxyl or dihydro form of vitamin A.

Light-activated channelrhodopsins from the green alga *Chlamydomonas* also contain retinal and are used as tools in optogenetics (in which optics and genetics are integrated at the cellular level to control an organism's physiology and behavior). In these channelrhodopsins, desirable spectral properties such as red-shifted absorption can be engineered by site-directed mutagenesis.



Expanding the color palette of a retinal-based pigment. Wang *et al*. have engineered the CRBPII binding pocket for the all-*trans*-retinal chromophore to tune its absorption. They introduced a lysine residue to facilitate a Schiff base linkage of the chromophore to the protein, and replaced other amino acid residues by site-directed mutagenesis to create pigments with increasing red shifts. Shown along the edge are the maximal absorption wavelengths of the mutant pigments in nanometers.

As a model system to study spectral tuning and the opsin shift mechanism, Wang *et al*. used a rationally engineered version of human cellular retinol binding protein II (CRBPII), a soluble retinol transport protein found mainly in the gut. The crystal structures of the retinol-bound and retinol-free forms of this protein are virtually identical, creating a scaffold where the effects of amino acid side chain substitutions might be revealed without significant secondary effects (*8*). The key enabling mutation was to introduce a lysine residue in the proper position so that retinal could replace retinol (the usual ligand for CRBPII) and could form the covalent protonated Schiff base bond characteristic of all retinylidene pigments (*9*).

Once the stable pigment was formed, Wang *et al*. carried out systematic rational mutagenesis and solved crystal structures for several pigments to test their hypothesis that an electrostatic field completely dispersed along the polyene should allow a "super red" pigment. A key conceptual jump was to mutate the negative counterion that would normally compensate for the positive charge on the Schiff base imine.

To form a protonated Schiff base in the core of the protein without a compensatory negative counterion was counterintuitive. But in fact, the absence of a counteranion causes more charge delocalization along the polyene and facilitates red shifts. Further disruption of hydrogen bonding with the imine by replacing a glutamine with an arginine caused a massive red shift. Removal of hydroxyl-bearing amino acids along the polyene caused additional red shifts, as did the introduction of polarizable groups.

Laboratory of Chemical Biology and Signal Transduction, The Rockefeller University, New York, NY 10065, USA. E-mail: sakmar@rockefeller.edu

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Knowledge of the crystal structures of successively engineered pigments was crucial when Wang *et al*. tried to isolate the retinal binding pocket from the surrounding aqueous environment. They replaced an arginine residue at the entrance of the binding pocket by a tryptophan residue to plug the aqueous opening. Replacement of an alanine with another tryptophan further dehydrated and encapsulated the binding pocket.

The crystal structures of the most red-shifted mutants showed almost no twisting of the retinal polyene, with the cyclohexenyl ring and polyene almost coplanar. Thus, extreme red shifts were achieved with almost no apparent contribution from conformational effects. Once the binding pocket of the chromophore was dry and insulated, it was possible to distribute the electrostatic potential uniformly along the polyene, and the positive charge from the Schiff base imine became completely delocalized. The most highly engineered pigment absorbed maximally at 644 nm (see the figure), redder than any retinal-based pigment previously reported and even redder than predicted to be possible for that chromophore (*10*).

The theoreticians will have a field day with the redder-than-red structures reported by Wang *et al*. And although it is not likely that transmembrane proteins such as visual pigments and channelrhodopsins can be engineered analogously because they are more dynamic and flexible, molecular and chemical biologists will certainly try. It will be interesting to see how the principles elucidated here might be applied to other systems with the aim of expanding the color palette in photoactive biological systems.

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CHEMISTRY

Atomic Layer Electrodeposition

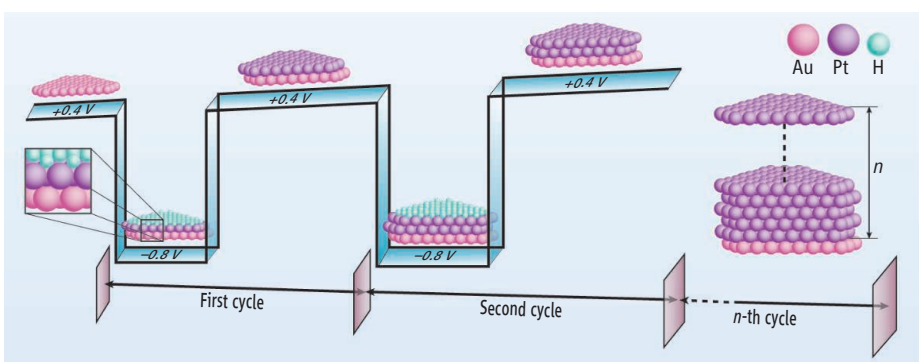
An electrochemical process provides a cheap and simple route to layer-by-layer growth of metal films.

Jay A. Switzer

The growth of ultrathin films is generally hindered by roughening and three-dimensional mound formation. Atomic layer deposition (ALD), in which atomic layer control and conformal growth are achieved through sequential, self-limiting surface reactions (*1*), can eliminate or reduce such roughening. One application of ALD is to deposit ultrathin layers of expensive metals such as Pt that are used, for example, as the catalyst in proton-exchange membrane fuel cells (*2*). Besides the economic incentives to produce ultrathin films (*3*), there are also scientific payoffs—they often have catalytic, electronic, or magnetic properties that are not found in the bulk material (*4*–*6*). Although ALD processes are usually conducted in the vapor phase, Liu *et al*. (*7*) show on page 1327 of this issue that they can sequentially electrodeposit two-dimensional Pt layer by layer by simply pulsing the applied electrochemical potential in a single plating bath. The process is inexpensive and rapid. Because each layer is produced by cycling the potential rather than by exchange of reactants, electrochemical ALD could be orders of magnitude faster than vapor-phase ALD.

Electrodeposition is a bottom-up processing method, because the solid is assembled from ionic or molecular precursors in solution. It is similar in many ways to biomineralization, because solution additives and pH can be used to control the growth. Hence, the size, shape (*8*), crystallographic orientation, and even chirality (*9*) can be tuned. Compared with deposition from ultrahigh vacuum, electrodeposition is inexpensive, and the deposition rates can be much higher. It is not a line-of-sight process, so conformal films can be grown on complex shapes—for instance, in the on-chip deposition of copper interconnects into submicrometer-sized features of semiconductor devices.

What distinguishes electrodeposition from other deposition techniques is the applied potential, a single parameter that controls the departure from equilibrium and, therefore, the rate of the reaction. The electrodeposition of metals requires only that the electrode potential be driven negative of the equilibrium potential. The difference between the applied potential and the equilibrium potential is called the over-



One step at a time. Electrochemical atomic layer deposition of ultrathin Pt films (*7*) deposited one monolayer at a time by simply pulsing the electrode potential between +0.4 and −0.8 V. A capping layer of hydrogen is produced at −0.8 V that blocks the deposition of more than one monolayer of Pt. When the potential is stepped to +0.4 V, the hydrogen layer is desorbed and the cycle can begin again. The self-limiting processing method is fast because it is performed in a single plating bath, so it is not necessary to exchange reactants. The ultrathin Pt films could lower the costs of the Pt catalyst in fuel cells and provide a platform to study how the catalytic, electronic, and magnetic properties of ultrathin films evolve with film thickness (*4*–*6*).

CREDIT: C. SMITH/*SCIENCE*

Department of Chemistry and Materials Research Center, Missouri University of Science and Technology, Rolla, MO 65409–1170, USA. E-mail: jswitzer@mst.edu

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Thomas P. Sakmar

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