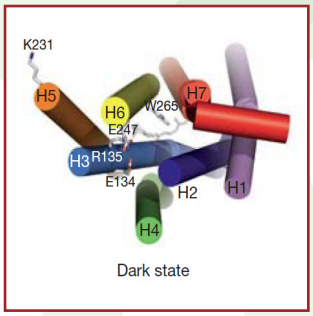
New probe technology

Lab Highlight

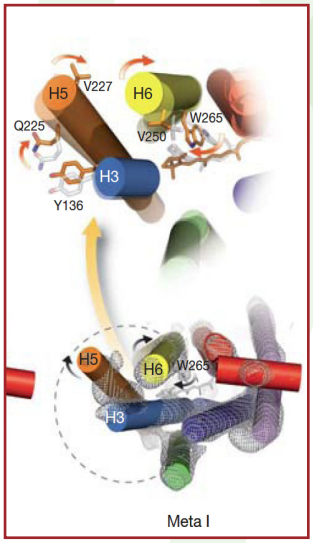
illuminates the activation of light-sensing cells

Through ingenious combinations of roughly 20 amino acids, the basic building blocks of life, genes can build the proteins that comprise everything from the simplest bacteria to the human brain. In new research published today in Nature, scientists unveil a new technique to illuminate the function of those proteins. The method of genetically targeting a non-natural amino acid to specific locations within a protein could theoretically be adapted to place a fluorescent probe at any position in any protein in a mammalian cell



Dark state

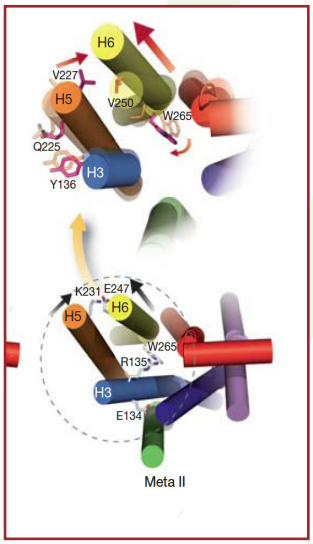
Here, in the inactive dark state, 11-cis retinal is wrapped around W265$^{6.48}$ and E247$^{6.30}$ interacts with E134$^{3.49}$/R135$^{3.50}$, stabilizing H6.



Meta I

In this model of Meta I, retinal isomerization frees W265$^{6.48}$ and weakens the H3/H5 interaction,resulting in small tilt/rotation of H5 and H6.

The top image is a close-up and shows a superimposition of Meta I (solid cylinders, orange sticks) with the inactive dark state (translucent cylinders,white sticks).



Meta II

Here, a model of the active MetaII state shows that the G-protein-binding site opens by subsequent larger movements of H5 and H6. The interaction of E247$^{6.30}$ with E134$^{3.49}$/R135$^{3.50}$ is broken, and a new H5/H6 interaction mediated by K231$^{5.66}$ and E247$^{6.30}$ is created.

The top image is again a close-up and shows the superimposition of the Meta II model (solid cylinders, red sticks) with that of Meta I.

Ultimately, Charles Darwin's "endless forms most beautiful and most wonderful" can be boiled down to a scant 20 or so amino acids, the basic building blocks of life. From this parsimonious palette, nature paints the proteins that make up the wild diversity of life on earth, from the simplest bacteria to the most complicated structure in the known universe — the human brain. Now, in work published today online by Nature, researchers from The Rockefeller University reveal a new technique for tagging proteins with non-natural amino acids to scrutinize details about how they function.

The experiments in Nature yield new findings about rhodopsin, the light sensitive cell receptor that is crucial to dim-light vision, showing that light causes changes in the structure of the protein much faster than previously believed — on the order of tens of microseconds rather than milliseconds. Thomas P. Sakmar, head of the Laboratory of Molecular Biology and Biochemistry, and postdoctoral associate Shixin Ye, worked with colleagues in Germany, England, Spain and Switzerland, to combine a variety of genetic engineering techniques to introduce an amino acid, azidoF, a relative of phenylalanine, into several points on rhodopsin. The three-nitrogen-atom azido is an especially good probe for three reasons: In contrast to other tags, azido does not exist naturally in mammals, which makes it easier to "see," or distinguish from other molecules in the cell; it is small enough to not interfere with a protein's normal functioning; and it has chemical properties that make it a good handle on which to hang other molecules, like fluorescent probes.

In fact, the method could in principle be applied to place a fluorescent probe at any point in any protein in a mammalian cell. "The long-term goal is to label receptors in live cells and do single molecule fluorescent studies," says Sakmar, who is Richard M. and Isabel P. Furlaud Professor. Such experiments could illuminate the minute functional differences that differentiate proteins the world over.

Similar approaches have been successfully used in bacteria, but last year, the researchers first showed that their method could be applied to mamma-