Bright lights: Mystery of glowing antibody solved

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A chance discovery of a uniquely luminescent monoclonal antibody nearly ten years ago has proven to be far more interesting -- and far more tenacious -- than anyone might have suspected.

Now, a group of Scripps Research scientists have shown that EP2-19G2, one of a panel of fluorescent monoclonal antibodies that were first reported in 2000, produces its distinctive bright blue glow through a rare and highly complex recombination of electrical charge.

This charge recombination involves an electron hole—the gap left by the electron as it is transferred from one molecule to the other.

The new study was published in the February 29, 2008 edition (Volume 319, Number 5867) of the journal *Science*.

“Our study describes in detail the rare and rather surprising mechanism that creates this exceptionally bright and long-lasting fluorescent antibody,” said Richard Lerner, president of The Scripps Research Institute, Lita Annenberg Hazen Professor of Immunochemistry, and member of the Skaggs Institute for Chemical Biology at Scripps Research. “These findings could have wide application in the development of novel and more broadly applicable biosensors.”

Biosensors, which relay biological reactions such as ligand binding and antibody-antigen actions into detectable signals, have a variety of uses, from signaling the presence of pathogens and toxins to monitoring blood

glucose levels for diabetic patients.

When the monoclonal antibody EP2-19G2 is combined with stilbene, a hydrocarbon commonly used in making dyes, it emits an intense blue light after exposure to ultraviolet light. While other antibody-stilbene complexes have also shown the ability to produce purple, blue-purple, and blue fluorescence after UV exposure, their fluorescent effect has been relatively weak and short lived.

“The luminescence produced by the EP2-19G2-stilbene complex lasts more than 400 times longer than that of stilbene on its own,” said Erik Debler, the first author of the new study and a former graduate student of Professor Ian Wilson’s laboratory at Scripps Research. “This is probably the longest luminescence lifetime that has ever been observed for stilbene and was totally unexpected, especially since stilbene is basically the E. coli of photochemistry, as it is extremely well characterized and understood.”

The fact that the EP2-19G2-stilbene complex emits a bright luminescence was first described in a Scripps Research study published in the October 13, 2000 edition (Volume 290, Number 5490) of the journal Science. The underlying mechanism had remained unknown until now.

A Perfect Match, A Perfect Storm

When EP2-19G2 binds stilbene, the antibody itself coordinates the joining or stacking of stilbene with a deeply buried tryptophan residue at the active site. In other fluorescent antibodies, such a stacking interaction does not exist. After exposure to UV, electron transfer between stilbene and tryptophan occurs deep within the 19G2 protein matrix.

Kim Janda, who is Ely R. Callaway, Jr. Chair in Chemistry, member of The Skaggs Institute for Chemical Biology, and director of the Worm Institute for Research and Medicine at Scripps Research, led the initial research into EP2-19G2 and was part of the new study. He described the importance of the combination this way: “This may be a perfect molecular storm. When the tryptophan residue and stilbene are matched in EP2-19G2, this perfect alignment creates a different luminescence pathway than is seen in other related antibodies.”

Unlike in other complexes, this antibody reacts with stilbene in the excited state. Antibody EP2-19G2 is deeply penetrated by the stilbene molecule and the special constellation of the stilbene-tryptophan pairing sets this antibody apart from other antibody-stilbene complexes, where binding merely enhances stilbene fluorescence by inhibiting photoisomerization, the dominant nonradiative decay pathway of stilbene in solution.

In fact, the unusual binding mode of stilbene in EP2-19GE2 is enabled by a non-canonical interface of the variable heavy and light chains of the antibody.

“This light-generating mechanism has rarely been observed in other luminescent proteins found in nature,” Debler said. “The neat thing about this system is that it acts like a molecular light switch. Neither stilbene nor the antibody themselves are notably fluorescent, but, when these two molecules combine, they yield an incredibly intense blue light.”

It is the special photophysical behavior—the extreme brightness coupled with the switch-like character—that makes the EP2-19G2 antibody attractive as a potential biosensor and as a model for the development of other equally potent types of luminescent antibody-chromophore complexes.

Source: Scripps Research Institute