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New Red Fluorescent Protein a Glowing Success

A new red fluorescent protein—derived from a brilliant red sea anemone purchased in a Moscow pet shop—can reveal body tissues more vividly than other fluorescent proteins in use today. The Russian researchers who developed the new protein said it can render cancers and other target tissues easily visible in living animals, making them glow like Christmas bulbs.

The development of the red fluorescent protein, which the researchers call Katushka, was reported by Howard Hughes Medical Institute international research scholars Andrey Zarskiy, Sergey A. Lukyanov, and their colleagues August 26, 2007, in the online version of *Nature Methods*. Principal development of Katushka was carried out by Dmitry Chudakov and colleagues in Lukyanov's laboratory at the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry in Moscow. Zarskiy and his colleagues are also at the institute.

"The palette of basic fluorescent proteins is currently almost complete, the only gap remaining in the very far-red part of the visible spectrum."

— Dmitry Chudakov

According to the researchers, Katushka solves a major problem in the field of fluorescent reporter proteins. Fluorescent proteins have become invaluable research tools for labeling specific genes and tissues. This labeling permits researchers to follow gene activity visually or to track cellular development. However, there is no tracer that glows brightly in a particular window of the far-red spectrum favorable for maximally penetrating living tissues. Thus, such proteins were not practical for optically imaging tagged genes, cells or tissues in whole animals.

The development of Katushka might never have happened had it not been for the shrewd bargaining of Lukyanov. Visiting a Moscow pet shop, Lukyanov saw a brilliant red sea anemone among the denizens of the store's aquarium. Sensing that the vivid red coloring in the anemone might provide the

blueprint for a new biological tracer, he tried to buy the anemone. He was told by the shopkeeper that it had already been sold, and the buyer was expected shortly. Unfazed, Lukyanov persistently outbid the buyer and procured the creature.

Back in Lukyanov's laboratory, Chudakov and his coworkers isolated the red protein from the anemone and then developed an enhanced version, which they named turbo red fluorescent protein (TurboRFP). The protein comprised a string of identical protein subunits, so the researchers also developed a single-unit monomeric version, which they called TagRFP.

Although TurboRFP was twice as bright as a comparable red protein, DsRed2, the researcher set out to improve its brightness in the far-red window. They created about 100,000 variants of the gene for TurboRFP, screening the resulting proteins for high brightness in the near-infrared. This screening yielded a highly bright variant protein, which they further optimized by randomly mutating the gene and selecting the brightest protein. They named this protein Katushka, a Russian diminutive for the name Ekaterina—after co-author Ekaterina Merzlyak, in recognition of her work developing TurboRFP and TagRFP. Katushka proved to be up to 10-fold brighter in the far-red, compared to the spectrally close fluorescent proteins HcRed and mPlum. mPlum was developed in 2004 by HHMI investigator Roger Y. Tsien at the University of California, San Diego, a leader in the study of fluorescent proteins for cell biology research. The researchers also generated a monomeric version of Katushka, called mKate, which is useful for molecular labeling of proteins.

Zaraisky and his colleagues tested Katushka's properties as a tracer by introducing it into living cells in culture, as well as into the muscle cells of the African clawed frog *Xenopus laevis*, widely used in research. They found the protein to be highly visible and nontoxic.

The key feature of Katushka that makes it valuable as a fluorescent marker is the unique combination of high brightness, far-red emission and fast rate of the chromophore maturation, said Zaraisky. As far as I know, none of the known fluorescent proteins demonstrates this combination of properties.

Early appearance of the Katushka protein in tissues is particularly important, said Zaraisky. It's desirable to detect transgenic expression at the earliest stage of embryonic development to be able to investigate early events of embryogenesis, he said. This means that you need to use the fastest-maturing and brightest fluorescent protein as a reporter. At the same time, since frog embryos are not transparent at early stages, your fluorescent reporter should emit as far in the infrared as possible, because the longer wavelength can more easily pass through living tissues.

As an added benefit, the researchers found that the Katushka protein persisted in labeled tissues as the frogs reached adulthood. We hoped that Katushka could be visible in adult frogs, but it was a surprise that it has appeared so bright, Zaraisky said.

Zaraisky sees broad uses for Katushka in research. We believe that Katushka has a big potential in cancer research, because it could significantly simplify some experiments that should be done only at the whole-body level. It would be possible, for example, to generate model lines of mice that would specifically express the Katushka transgene in cells of different types of cancer. Then, these lines of mice could be used to test influence of different drugs on progression of cancer metastases.

Also, Katushka is an excellent choice as a living reporter for experiments in developmental biology, especially for such semi-transparent models as frog and early mouse embryos, he said. For example, Zaraisky and his colleagues plan to use the frog embryos generated in their analysis of Katushka's properties to study muscle cell differentiation.

According to Chudakov, further improvements in Katushka are likely. The palette of basic fluorescent proteins is currently almost complete, the only gap remaining in the very far-red part of the visible spectrum, he said. We hope that further development of Katushka and mKate will finally fill the gap.