SENSORS AND PROBES

Enhancing photostability with FRET

FRET improves the photostability of fluorophores for longer single-molecule tracking.

Single-molecule tracking in cells can be technically challenging. One major obstacle is that fluorophores often have photophysical properties that limit the measurable length of a molecule's trajectory. Although several strategies to compensate for these issues have been developed, better tools are still needed.

David Klenerman, Steven Lee, Ernest Laue and their research groups at the University of Cambridge discovered a new approach to extend fluorophore longevity and showed that it can improve single-molecule tracking in mammalian cells. When they fused the fluorescent protein mEos3.2 to the HaloTag protein bound to the dye JF_{646}, they observed the expected Förster resonance energy transfer (FRET) between the two fluorophores. What they also found, however, was that the mEos3.2 molecules that underwent FRET with JF_{646} showed dramatically reduced photobleaching and emitted more total photons than mEos3.2 alone.

To optimize the increase in photostability, the researchers tested constructs with varied FRET efficiency. These experiments revealed that an increase in FRET efficiency could further improve photostability, but at the cost of signal in the green mEos3.2 channel, as fewer photons were directly emitted from mEos3.2. The team also found that the photophysical properties of another dye, JF_{549}, were similarly improved by FRET to JF_{646}.

The researchers fused mEos3.2–HaloTag–JF_{646} to the chromatin remodeler CHD4 in mouse embryonic stem cells, where they were able to obtain longer trajectories with the FRET construct than with mEos3.2 alone. They also showed that they could fuse different copies of CENP-A with either mEos3.2 or HaloTag–JF_{646} in the same cells and image extended trajectories for complexes formed between the two differently tagged versions. These results highlight the power of this tagging approach for long time-lapse single-particle tracking, and should encourage future experiments to elucidate the mechanism underlying this phenomenon.

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Research papers

Basu, S. et al. FRET-enhanced photostability allows improved single-molecule tracking of proteins and protein complexes in live mammalian cells. *Nat. Commun.* **9**, 2520 (2018).