SINGLE MOLECULE

Turning the lights on a few molecules at a time

Researchers adapt split-GFP complementation to single-molecule imaging.

How do you spot a beacon of light in a crowd? Work by Fabien Pinaud and Maxime Dahan at the Ecole Normale Supérieure in Paris provides an answer that brings ease and versatility to single-molecule imaging.

Labeling a population of molecules is akin to layering a room with light bulbs; contours become lost in the blinding glow. This is the problem with most fluorophores—the density of labeled molecules inside the cell is simply too high. But turning on just a fraction of bulbs at a time makes it possible to make out individual points of light and define objects more precisely.

Methods such as photoactivatable light microscopy overcome the resolution problem by exciting a few fluorophores at a time with laser pulses. Although effective, these techniques require a sophisticated setup.

Another approach involves sparse chemical labeling. But expressing fluorescent fusion proteins at low levels is not always feasible, and as Pinaud notes for synthetic probes, "as you dilute, molecular interactions are affected because association and dissociation rates are proportional to concentrations."

To solve the problem, Pinaud and Dahan expressed a split-GFP reporter fusion in normal amounts. The GFP was 'dark' because it lacked one of its β-sheets. By titrating synthetic versions of the 16-amino-acid complementary β-sheet, they could control the fraction of molecules that lit up. The authors dubbed the technique complementation-assisted light microscopy (CALM).

In live cells, they showed correct subcellular localization of N- and C-terminal fusions of cytosolic and extracellular split-GFP to membrane proteins by wide-field, confocal and total internal reflection fluorescence microscopy, and tracked sub-resolution movement of individual molecules.

In principle, the ease of modifying the β-sheet peptide creates limitless options. Pinaud and Dahan demonstrated the use of bright, photostable quantum dots for prolonged imaging after GFP bleaches. They also achieved robust and precise localization using fluorescence resonance energy transfer. "The power of the technique," explains Pinaud, "is that you go beyond simple imaging." As a vehicle for cellular targeting, CALM with synthetic peptides "opens the door to specific modification of biological processes with nanometer precision."

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RESEARCH PAPERS

Pinaud, F. & Dahan, M. Targeting and imaging single biomolecules in living cells by complementation-activated light microscopy with split-fluorescent proteins. *Proc. Natl. Acad. Sci. USA* advance online publication (23 May 2011).