**Advancing single-molecule fluorescence experiments**

Single-molecule experiments can yield insights into molecular behavior that cannot be discerned from ensemble measurements in which a population of molecules is studied. In this issue, two independent groups provide solutions to two different single-molecule fluorescence challenges, using microfluidics. Deniz, Groisman, Gambin and colleagues describe a rapid microfluidic mixing device in which the output flow is slowed down to be compatible with single-molecule fluorescence detection; this device allowed them to study the early folding steps of an intrinsically disordered protein, α-synuclein. In separate work, Majumdar, Weiss, Quake and colleagues used a microfluidic device to examine single-molecule behavior, including conformational changes and enzyme activity, under a variety of environmental conditions in high throughput.

**Better DNA nanostructures**

DNA has shown itself to be a versatile and powerful construction material for nanostructures, but synthesis of desired structures at high yield is challenging. By using many shorter oligonucleotides as staples to shape a long single-stranded DNA scaffold, the DNA origami technique allows more reliable synthesis of large structures than using small oligonucleotides alone. In a Perspective, Dietz and colleagues present practical advice, step-by-step instructions and a new design tool for creating nanostructures using DNA origami. Purification of the finished structures without damaging them can be one of the most problematic steps. In a Correspondence, Shih and colleagues describe modifications to agarose gel–based electroelution that aids this crucial step, particularly for large and complex structures. These reports should provide a useful starting point for researchers wishing to apply DNA origami in their own work.

**Brainbows in the fly**

The ability to label subsets of cells in a multicellular organism is key to many biological experiments. In neuroscience, visualizing multiple neurons with different colors can help resolve the intricate wiring patterns of the brain's connections. The fruit fly *Drosophila melanogaster* is an ideal organism to study both the anatomy and the function of neural networks, but labeling many individual neurons in relation to each other in the same preparation has been difficult with previous methods. In two independent papers, Simpson and colleagues, and Salecker and colleagues now report adaptations of the mouse genetic multicolor labeling technique known as Brainbow to the fly. These methods combine stochastic expression of fluorescent proteins with high control of the targeted cells via the Gal4–upstream activating sequence binary expression system.

**Fire and forget microscopy**

Automated microscopy allows high-throughput examination of a multitude of complex cellular phenotypes. It has proven to be a valuable complement to large-scale RNA interference screens and allows the detection of rare phenotypes that would otherwise go unnoticed. But detailed examination of such rare phenotypes remains challenging. The Micropilot software by Ellenberg, Pepperkok and colleagues solves this problem by identifying rare phenotypes of interest during live-cell imaging and automatically launching complex follow-up microscopy assays on these cells such as three-dimensional time-lapse imaging or fluorescence recovery after photobleaching. An investigator can fire up their experiment and forget about it until they return in the morning to analyze the data.

**Quantifying PIP₃**

As far as signaling molecules go, the lipid phosphatidylinositol(3,4,5)triphosphate (PIP₃) is as elusive as it is important. It regulates many aspects of cellular behavior, but its low abundance and amphiphilic properties make direct measurements challenging. Hawkins, Stephens and colleagues developed an integrated high-performance liquid chromatography–mass spectrometry strategy that addresses this problem. By completely methylating the phosphate groups in PIP₃, they create a species that is more easily transferred into the mass spectrometer and yields fewer ionic species, making the mass spectrometric analysis simpler and more sensitive. The researchers applied this strategy to quantify PIP₃ in stimulated human neutrophils and a breast epithelial cell line as well as in tissue samples from fat and liver.

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