Understanding living forces

For years biophysicists have been measuring the forces acting on and between single biological molecules. These infinitesimal forces are central to the actions of innumerable biological processes occurring in living cells, but to date nearly all of this work has been conducted in vitro where the system under study can be tightly controlled. In a Commentary in this issue of Nature Methods, Müller and colleagues argue that despite the extreme challenges involved, it is imperative that more of these experiments be moved into living cells where the biological molecules under investigation can operate under native conditions that are likely far different, and more biologically relevant, than those experienced in vitro. They discuss five challenges that must be overcome to make this important move.

Commentary p123

How fat is a worm?

To understand metabolic disorders such as obesity, one needs to study the genetic underpinnings of fat metabolism. Xie, Wang, Min and colleagues were not satisfied with the traditional approaches for lipid analysis in model organisms such as Caenorhabditis elegans: biochemical analysis is labor-intensive and requires a large number of worms, lipid-staining dyes are not quantitative, and coherent anti-Stokes Raman scattering has limited sensitivity. Instead, the researchers applied stimulated Raman scattering (SRS) microscopy to a screen that targeted 270 genes with potential involvement in fat metabolism. The sensitive and quantitative nature of the SRS signal allowed them to identify genes that substantially increased the fat content in worms.

Brief Communication p135, News and Views p132

Green monsters

Most genes are not islands but interact with one or more other genes to ‘create’ a phenotype. To understand their function it is thus necessary to target genes not as individuals but to knock out the entire functional group. This is where the technical challenge begins, even in a model organism as simple as yeast. Introducing sequential deletions takes time and is limited by the number of selectable markers available. Roth, Suzuki and colleagues now present a solution. They mated yeast strains, each with a separate deletion marked by a GFP reporter, and enriched the resulting strains for increased fluorescence indicative of combinations of deleted alleles. This green monster strategy allowed them to generate a strain with deletions of all 16 members of a family of drug exporters.

Article p159

A cocktail for photoprotection

Single-molecule fluorescence is being increasingly used to study molecular processes; these experiments can uncover interesting behaviors that are masked in ensembles of molecules. But because fluorescent dyes are prone to bleaching and blinking, the time resolution of single-molecule fluorescence methods has been limited to the millisecond regime. Muñoz and colleagues now describe a photoprotection ‘cocktail’ that improves the time resolution of such experiments to the microsecond regime, allowing them to resolve single-molecule sub-millisecond conformational dynamics of the α-spectrin SH3 domain and the protein BBL. The cocktail contains cysteamine, which minimizes bleaching by scavenging for oxygen radicals, as well as dissolved oxygen and Trolox, triplet quenchers that prevent blinking.

Brief Communication p143

Optogenetics in freely behaving worms

The use of light-activated ion channels to manipulate neuronal activity constitutes a powerful tool for understanding animal behavior. Ideally, in such experiments individual neurons are modulated specifically and in an unconstrained worm, but this has proved challenging. In this issue, two groups independently report illumination of specific neurons in freely moving Caenorhabditis elegans. Samuel, Fang-Yen and colleagues use machine-vision algorithms to direct the projection of a desired illumination pattern onto a moving animal with a digital micromirror device. Lu, Gottschalk and colleagues combine an off-the-shelf LCD projector with an epifluorescence microscope to apply structured illumination patterns to moving worms. Both groups achieve sufficient spatiotemporal resolution to dissect the contribution of individual neurons to sensory and motor circuits that control behavior.

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