Citations

Interference Welcome

A few years ago, backscattering interferometry (BSI) emerged as a label-free method to detect binding between molecules in solution. The equipment consists of little more than a microfluidic cell, a red helium-neon laser, a mirror, and a CCD camera for data capture. Bouncing the laser through the microfluidic chamber produces a set of light and dark regions on the detector in a pattern that relates to the refractive index of the solution. Remarkably, intermolecular binding events alter the laser's transit through the chamber enough to shift the interference fringe. By then using Fourier analysis and data processing of results at different ligand concentrations, subtle changes in refractive index can be converted to binding constants. In an article in *Nature Biotechnology*, Baksh et al. describe an adaptation of BSI for measuring membrane protein–ligand interactions. Despite its central importance to cell signaling, the binding behavior of membrane proteins has been challenging to assay. In existing measurement techniques, the readout system isn't compatible with assaying in the native membrane context, or the receptor has to be modified in some way to signal ligand binding to the readout apparatus. Although the label-free nature of BSI removes any need for protein modification, Baksh et al. needed to show that shifts in the interference fringe pattern could accurately and quantitatively capture binding events in various membrane environments. The first test used vesicle preparations from defined components and showed detection of binding between a small

molecule incorporated into a synthetic membrane and a soluble protein (the interaction between the monosialoganglioside GM1 and cholera toxin B). A more typical experimental need, however, is assaying the opposite situation, a solution-phase small molecule with an integral membrane protein. For these experiments, the authors used fatty acid amide hydrolase (FAAH) and the CXCR4 and GABA_B receptors. Sure enough, BSI picked up dose-dependent signal when FAAH was embedded in synthetic vesicles and incubated with small-molecule inhibitors. As a closer approximation of physiological conditions, Baksh et al. prepared the CXCR4 and GABA_B receptors in native membrane vesicles derived from cultured cells. Although the sample complexity did correlate with larger error bars, the dissociation constants determined from small-molecule binding to these receptors corresponded well to previously reported values. In addition to being compatible with complex membrane environments, BSI is forgiving in terms of protein requirements (~20 pmol for a binding curve) and has a dynamic range of several logs. These advantages should make BSI a go-to technique for assaying membrane proteins in basic biochemical studies or drug discovery applications.

Baksh et al. 2011. Label-free quantification of membrane–ligand interactions using backscattering interferometry. *Nat. Biotechnol.* 29:357–360.

A Place for Everything

Although electron microscopy (EM) offers a more detailed view of cellular structure than light microscopy, many protein localization studies rely on imaging via fluorescent tags. The hangup with EM lies in part with immunogold labeling, which demands low-background antibodies that can recognize crosslinked antigens, and ends up destroying some of the ultrastructural detail thanks to the fixation conditions necessary for access of the antibodies and gold particles. Another factor in fluorescence microscopy's favor is the sheer convenience of tagging proteins with a genetically encoded fluorophore. Now, one of the leading voices in the development of fluorescently tagged fusion proteins describes an engineered flavo-protein-based marker capable of creating an EM-resolvable signal. Writing in *PLoS Biology*, Roger Tsien and colleagues introduce miniSOG (mini singlet oxygen generator), a 106–amino acid protein that generates a precipitate that can be stained with the EM contrast agent osmium. MiniSOG works in combination with diaminobenzidine (DAB); specifically, blue light activates the tag to produce singlet

Correlated confocal fluorescence and electron microscopy of miniSOG-tagged α -actinin. ©PLoS 2011.

oxygen, which polymerizes DAB into a locally contained osmiophilic polymer. Because the players in this process are small molecules instead of proteins or gold particles, fixation conditions can be mild and detergent permeabilization is unnecessary. Although a biarsenical dye-tagging method can also be used to photogenerate singlet oxygen, miniSOG boasts a 20 fold–higher quantum yield and doesn't pose detectable toxicity problems. In keeping with the tag's ~15-kDa size, miniSOG protein fusions appear to localize as expected. And although miniSOG is no match for GFP as a fluorescent tag, it does produce sufficient green signal for correlating fluorescence localization with EM.

The authors show data for cells transfected with miniSOG fused with proteins localizing to actin filaments, chromatin, the mitochondrial matrix, and gap junctions. Other data demonstrate correlated fluorescence and electron microscopy in nematodes injected with a cDNA encoding mitochondrially targeted miniSOG fusion and mice electroporated in utero with DNA expressing a protein localized to cortical neuron synapses. The authors suggest that the miniSOG tag may at last unlock the potential of EM for high-resolution in situ visualization of protein localization.

Shu et al. 2011. A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. *PLoS Biol.* 9:e1001041.

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