Citations

Go For a Spin

In 1930, an article in *Science* described a combined centrifuge and microscope, a device intended for drawing inferences about the properties of intracellular structures. Eighty years later, Halvorsen and Wong reintroduce that concept in the *Biophysical Journal*, this time applying it to single-molecule observations in parallel. Halvorsen and Wong's centrifuge force microscope (CFM) is elegant in its simplicity: it comprises a rotating microscope imaging arm with a camera aimed in the direction of the centrifugal force, focusing on a coverslip that has the sample on the far side of the glass. The sample consists of a bead tethered to the coverslip by the particular molecule or molecular binding partners under study; as the arm rotates, the centrifugal force pulls the bead outward, flinging the object out of focus when the intermolecular interaction is broken. Because the force is uniform across the sample and the field of view encompasses many beads, multiple observations can be made in sync. To validate their CFM, the authors measured the dissociation of digoxigenin (DIG) and an anti-DIG antibody. For this experiment, an anti-DIG-coated coverslip was incubated with 2.8- μm beads that were conjugated to DIG by a DNA tether. After the microscope was focused to a location offset from the coverslip by the length of the DNA, the centrifuge was turned on. Beads whose tethers were not antibody-bound immediately vanished, beads that interacted nonspecifically with the coverslip surface stayed out of focus, and antibody-antigen-tethered beads snapped into focus. Over time, the antibody-antigen bonds began to rupture, evidenced by a reduction in the number of beads countable in the field of view. CFM-based measurements of intermolecular force corresponded almost

Schematic of the centrifuge force microscope. Reprinted with permission © 2010 Elsevier.

exactly to single-molecule analyses by either a micropipet-based optical trap force probe or an atomic force microscope. In addition to the ease with which multiple measurements can be made, a major advantage of the CFM is that applied force can be tuned as desired by varying bead size and density and rotation speed. The prototype machine described in the article, which the authors estimate could be constructed for ~\$5000, has a range of sub-femtoNewton to microNewton forces. In addition to being an economical alternative to currently available single-molecule analysis instruments, CFM's capacity for highly parallel analyses should enable measurement of binding phenomena not practical with current methods.

Halvorsen and Wong, 2010. Massively parallel single-molecule manipulation using centrifugal force. *Biophys. J.* 98:L53–L55.

Easy TARGET

Despite their increasing sophistication, recombinant protein expression systems aren't always adequate for producing secreted proteins of mammalian origin. Expressing such proteins in mammalian cells would guarantee authentic folding and posttranslational modifications, but the associated expense and effort are burdensome. Writing in the *Journal of Proteomics*, Tabata et al. describe a method that shortens the time required to screen for high-producer clones, and simplifies recovery of recombinant protein. Their first step in developing an improved expression system was to identify a high-affinity peptide binder to a monoclonal antibody known as P20.1, which is directed against six amino acids of human protease-activated receptor 4. The appeal of P20.1 is that elution occurs with 40% propylene glycol, conditions mild enough to preserve protein structure and function. Tabata et al. designed a 21-amino acid tag, called

TARGET (tandemly arranged recognition motif combined with gentle elution technology), with an affinity of 10 nM. As with the original, lower-affinity P20.1 epitope, the TARGET tag is compatible with elution from antibody resin using nondenaturing buffer. Such mild conditions should not only aid recovery of active protein, but also better preserve the binding capacity of the antibody column. As expected, the antibody resin held up well, with less than 1% activity loss per cycle. This resilience in the face of multiple binding and washing cycles is important because it simplifies clone screening. Typically, high-expressing clones are identified by cell sorting, but doing so requires two time-consuming culture expansion steps. With the TARGET tag, a small aliquot from a 96-well selection plate can be assayed on a surface plasmon resonance (SPR) chip bearing immobilized P20.1 antibody. Because the antibody can

be regenerated so easily, screening occurs efficiently. Using this strategy, the authors were able to purify 0.5–10 mg of recombinant protein just 30–50 days after initial transfection, depending on the protein and particular cell type used. The authors validated the TARGET tag expression/purification scheme for eight different proteins, establishing the system as an efficient, economical choice for mammalian protein expression.

Tabata et al. 2010. A rapid screening method for cell lines producing singly-tagged recombinant proteins using the "TARGET tag" system. *J. Proteomics*. [Epub ahead of print, May 20, 2010; doi: 10.1016/j.jprot.2010.05.012].

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BioTechniques 49:543 (August 2010)
doi 10.2144/000113472

Membrane Protein Overexpression: Eight is Enough

High-resolution 3-D structures of membrane proteins have been scarce, due to difficulty in the overexpression and purification of these proteins to yield sufficient quantities for crystal formation and subsequent X-ray crystallography. Existing protein overexpression systems in *Escherichia coli* have been more successful with soluble proteins than membrane proteins. But in an effort to increase the likelihood of successful overexpression of membrane proteins in *E. coli*, S. Leviatan and colleagues have devised a new combinatorial fusion protein overexpression system that they describe in the *Journal of Biological Chemistry*. The authors generated eight different expression cassettes, placing fusion domains derived from two different hydrophilic bacterial proteins at either the N- or C-terminus, or both, of the target protein. These short (~100 amino acids long) bacterial domains were chosen for their hydrophilicity and predicted α -helical, nonglobular structure to facilitate expression and purification of membrane proteins. To test the domains, 14 prokaryotic and eukaryotic transmembrane

proteins (12 transporters, 1 channel, and 1 enzyme) were each cloned into all eight expression cassettes. Prior to purification, at least two cassettes for each protein showed higher expression levels than the corresponding control construct lacking the fusion domains, and the expression for several proteins was markedly better than from the method using the membrane integrating protein Mistic. In all cases, significant amounts of protein from at least one of the overexpressing cassettes could be solubilized using mild detergents, an indication that a significant amount of the protein was inserted into the membrane, although inclusion bodies were still formed. For almost all the proteins, large-scale purification using standard methods and Fos-choline detergent solubilization yielded high amounts of pure protein that was both stable and monodispersed. And the proper folding of five different mammalian SLC17 transporters was verified using in vitro activity tests of the proteins reconstituted into liposomes.

Leviatan et al. A combinatorial method for overexpression of membrane proteins in *E. coli*. *J Biol Chem*. [Epub ahead of print, Jun 4, 2010; doi: 10.1074/jbc.M110.125492].

MapQuest

Single-particle electron microscopy has enabled the determination of protein composition within macromolecular complexes; yet identifying positions of individual subunits within such assemblies remains a challenge. Because knowledge of subunit position is essential to understanding function, multiple methods have been used to identify protein locations. While these approaches have supplied valuable data, low labeling efficiencies and flexible binding of labels has hindered more precise determination of subunit position. To work around these drawbacks, researchers often obtain very large datasets and then rely on sophisticated image-processing techniques to determine subunit locations. While studying the binding of yeast dynein light chain (Dyn2) to a dynein light chain-interacting domain (DID) in the nucleoporin complex Nup159, Flemming and colleagues from the University of Heidelberg discovered a prominent protein structural feature that could potentially be used as a tool for topological mapping of subunits within multimeric protein assemblies. As described in the journal *Nature Structural and Molecular Biology*, the recombinant Dyn2-DID complex forms a rod-like structure with five Dyn2 homodimers aligned between two extended DID strands when reconstituted in vitro. This conformation can easily be identified by negative-stain electron microscopy. To develop the Dyn2-DID complex as an electron microscopy label, the authors used two different DID strands (DID1 and DID2)

Schematic drawing of Exosome complex involved in RNA degradation. Reprinted with permission © 2007 Wikipedia Commons.

capable of binding six Dyn2 homodimers between them when arranged in opposing parallel positions. In electron micrographs, the structure is easily observable as a 25 nm-long rod-like filament with six distinct globular domains. To demonstrate the use of this label, the authors fused DID1 with two different subunits of Nup84, a well-characterized nuclear pore complex. Attachment of the 80-residue DID1 tag to the N terminus of individual subunits did not interfere with complex assembly. The structural electron microscopy marker was built upon the DID1 tag carried on a subunit within the assembled Nup84 complex by incubation with Dyn2 and DID2-Flag. Subsequent purification with anti-Flag sepharose enriched for DID-Dyn2-labeled complexes and resulted in clear micrographs showing the rod structure protruding from the expected subunit. This marker demonstrated low levels of angular flexibility and enabled precise topological

mapping of tagged N termini of subunits in the Nup84 complex. The tag may be attached to either terminus of a target protein and since it remains unfolded in its native state, is less likely to interfere with folding of the target subunit or its assembly into a complex than bulkier tags such as GFP. Another advantage of the new label is its stability over a wide range of salt concentrations, enabling use in multiple buffers preferred by differing protein complexes.

Flemming et al. 2010. Precise mapping of subunits in multiprotein complexes by a versatile electron microscopy label. *Nat Struct Mol Biol*. 17(6):775-778.

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BioTechniques 49:543-544 (August 2010)
doi 10.2144/000113472