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WHO NEEDS LABELS?
MACROMOLECULAR INTERACTION SANS LABELS

When it comes to monitoring biological interactions, whether of nucleic acids, proteins, small molecules, or cells, labels are essential for detection, right? Researchers certainly have embraced detection modalities based on tagging with radioisotopes, fluorescent dyes, and conjugated enzymes. Yet in many cases such molecular baggage is unnecessary, and possibly detrimental. But there is another way. Whether based on optical, mechanical, or electrical detection, label-free methods can probe molecular pas de deux in their native, unadulterated states. As a result, they are faster, simpler, and more physiological than their labeled counterparts.

By Jeffrey M. Perkel

The research community is taking note. Says Guenter Gauglitz, whose lab does extensive work in this area, when asked why researchers should eschew tried-and-tested label-based methods for label-free: “I would pose the question the opposite way: Why bother with labeled methods?”

Tagging methods, says Gauglitz, of the University of Tübingen, Germany, have one very obvious disadvantage: “You have to label the systems.” That, in turn, can impact the “bioactivity” of the labeled molecule, for instance through misfolding, reduced mobility, or steric hindrance. Labeling reagents are also pricey, may tag different molecules with varying efficiency, and in some cases are incompatible with live cells.

Label-free approaches, in contrast, reduce biomolecular interactions to their most essential components: molecule A, molecule B, and a detection scheme to watch them in action, often in real time.

Researchers have, to some extent, caught on, but not in droves. “To date it’s been a niche technology,” says Matthew Cooper of the University of Queensland, Australia, editor of the recently published book Label-Free Biosensors: Techniques and Applications (Cambridge University Press, 2009). “It’s been a classic early-adopter play.”

Pharmaceutical companies have been more enthusiastic, pressing label-free systems into service for fragment-based screening, lead optimization, and affinity ranking, among other applications.

At biopharmaceutical firm Amgen, as head of the company’s protein-protein interaction group, principal scientist Ching Chen uses three different label-free platforms. Those systems, from GE Healthcare, ForteBio, and Sapidyne Instruments, are used, she says, “in a complementary way,” for such applications as primary screening, epitope mapping, and affinity determination.

Label-free technologies provide “a much more straightforward answer [than labeled approaches],” she says. “They provide much better confidence when interpreting the data and give us confidence and speed in the selection of molecules.”

Three Essentials
All label-free (and for that matter, labeled) technologies, regardless of their operating principle and configuration, have at their heart three fundamental components, says Cooper: a receptor, a transducer, and an analyzer.

The receptor, of course, is what makes a biosensor, a biosensor—it specifically captures the molecule, cell, or pathogen of interest from a biological fluid or sample. The transducer, says Cooper, is the “detection modality, what converts that biological event into a photometric, electrochemical, electrical, or acoustical change.” That change, which is proportional to the extent of binding, is then quantified by the analyzer.

Observe how these pieces work together in one of the most popular label-free approaches, surface plasmon resonance (SPR). continued >
As implemented by GE Healthcare, an SPR biosensor comprises a thin gold film (about 50 nm thick) mounted on the flat face of a prism. On the side of the gold film facing away from the prism is a surface on which capture molecules (such as antibodies or DNA) are immobilized. This surface (the transducer) is exposed to a fluidics interface, such that reagents—whether capture molecules, analytes, or buffer—can flow over and interact with the biosensor surface (see figure above).

Within the instrument itself, the biosensor, light source, and detector form the vertices of a triangle, an arrangement called the Kretschmann configuration. As polarized light passes through the prism at an angle, it strikes the gold and reflects back through the prism at the “specular” angle—that is, as if the gold were a mirror. For the most part, the gold really is a mirror, and over a wide range of wavelengths (or angles), the light hitting the detector (a photodiode that measures light intensity) is 100 percent of that which left the source. But at one particular combination of wavelength and angle, reflection drops nearly to zero—a phenomenon called the “SPR dip” or “SPR resonance.”

That’s because under those precise conditions, “the light resonantly couples with the conduction electrons near the surface of the gold film to create clouds of oscillating electrons called a surface plasmon,” says Charlie Campbell, the West Professor of Chemistry at the University of Washington. “When in resonance, the momentum of this plasmon parallel to the surface matches the parallel momentum of the incoming light; since the latter is a function of the refractive index of the material under analysis in contact with the gold surface, the angle [at fixed wavelength] or wavelength [at fixed angle] of the dip can be used to monitor the refractive index of that material.”

In practice, this index of refraction varies ever so slightly as molecules bind to the sensor surface. So as intermolecular binding events unfold, the wavelength at which the SPR “dip” occurs will change, and the magnitude of that change is a function of the extent of binding.

The “refractive index change is directly proportional to the mass change,” says Stefan Lofas, principal scientist for the Biacore line at GE Healthcare Life Sciences. “So actually, you can say that we quantitatively measure the change in mass when something binds to the surface.”

And, because SPR devices are often coupled to flow cells that can deliver analytes or buffer, they can measure those events in real-time (in contrast to systems such as ELISA, which collect endpoint measurements). That makes SPR systems particularly useful for collecting kinetics data—and explains their popularity in the pharmaceutical industry as well.

Biopharmaceutical firm Xencor runs its Biacore 3000 “24/7” in its antibody development pipeline, says John Desjarlais, vice president of research; MedImmune’s SPR instruments (from Biacore and Bio-Rad) have been used for affinity kinetic characterization, epitope competition studies, and characterizing concurrent binding of multispecific antibodies, says Herren Wu, vice president of research and development.

“In the past 10 years, almost all compounds have gone through the Biacore measurement,” he says.

By all accounts, Biacore has been a dominant force in the label-free market in general and SPR in particular for nearly two decades. “Last fall, we celebrated the 10,000th publication with Biacore-related data,” Lofas says. Yet competition is growing. Bio-Rad’s ProteOn system can measure 36 interactions simultaneously via a criss-crossing array of 6x6 fluidic channels, while Fujifilm Life Science’s AP-3000 can probe six targets at once. An in-development system from Plexera, on the other hand, applies an SPR variant called imaging SPR (or SPR microscopy) to monitor interactions on arrays of up to several thousand spots by imaging light intensity using CCD cameras.

Yet all these techniques suffer from the same basic shortcomings, namely, that they are flow-based and mounted on a prism. As a result, SPR is generally incompatible with standard microtiter-plate formats, including much of the high throughput drug discovery workflow.

**Optical Alternatives**

Several companies have overcome this problem by devising SPR alternatives in standard microplate labware. Though flow-based systems have the advantage of providing kinetics data, microplate-based systems can accommodate a wider variety of sample types, from cruder samples that would gum up microfluidic channels, to live cells that are currently too large for them.

The majority of these options—like SPR—rely on evanescent waves. As described in a review by Cooper in Nature Reviews Drug Discovery (1:515, 2002), the “evanescent wave phenomenon” occurs when “total internal reflection of light at a surface–solution interface produces an electromagnetic field, or evanescent wave, that extends a short distance (~100–200 nm) into the solution.” That wave effectively probes the interface—and is in turn “tuned” by it in a dose-dependent fashion—allowing detection.

Just how that detection occurs depends on what aspect of the outbound light the detector looks at. Corning’s Epic system probes the index of refraction on its biosensor surface using a “resonant waveguide grating structure,” says Mark Krol, technology manager at Corning.

“The resonant waveguide changes the index of refraction, whose magnitude is a function of the degree of binding.”

**SRU Biosystems’ BIND performs a similar measurement using an optical device called a photonic crystal.**

“A photonic crystal is just a fancy way of saying we have a material with two different refractive indexes in it, a high refractive index and a low refractive index, that alternate from high to low to high to low in a periodic fashion,” explains company co-founder Brian Cunningham of the University of Illinois, Urbana-Champaign.

In this case, the crystal is illuminated with near-infrared light, almost all of which passes through the sensor. But one wavelength resonates with the crystal and is reflected back. “At that one wavelength, the resonant wavelength, the photonic crystal structure acts as a perfect mirror,” Cunningham explains. “So it reflects very efficiently at that one wavelength, and all other wavelengths pass through.” When a biomolecular interaction occurs on the sensor surface, that wavelength shifts, producing a measurable readout. **continued >**
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Cunningham compares the effect to a guitar string. “If you strum it, you get a certain wavelength out in terms of the sound it makes,” he says. “If you paint the string and strum it again, you will get a different resonance, a different sound. In the same way, when mass is added to this sensor, it reflects the light at a different wavelength.”

Maven Biotechnologies’ in-development Polaron instrument measures changes in light polarization, says chief scientific officer Shane Dultz. Based on a technology called LFIRE (label-free internal reflection ellipsometry), Polaron’s is an imaging approach that measures changes in the elliptical polarization of light as it interacts with the sensor surface to chronicle biomolecular interactions in miniaturized protein microarrays.

“It turns out that the phase shift, or the degree to which the light’s elliptical polarization is changing, is proportional to the number of molecules per square micron on that surface,” Dultz says.

Though still in development, Polaron will image arrays in the bottom of 96-well glass bottom plates, Dultz says, with up to 700 protein spots per well. Each spot, he adds, can be analyzed either continuously or on an endpoint basis.

Silicon Kinetics (SKI Pro system), BiOptix, and ForteBio (Octet) employ an alternative strategy called interferometry. Used, among other things, to calculate the distance to astronomical objects, interferometry may also be used to measure the thickness of the molecular layer on the biosensor surface as biochemical events occur.

In ForteBio’s case, the biosensor is a narrow tube (or fiber tip) with two parallel surfaces perpendicular to its long axis: an invariant reference surface and a sensor surface, to which capture molecules (such as antibodies) are bound. When light is directed down the barrel of the tube, it bounces off both surfaces on its way back to the detector, producing a composite signal that is a reflection of both waves.

“The two reflections interfere constructively and destructively and there is a net signal seen at the detector,” explains Sriram Kumaraswamy, product manager of ForteBio.

As molecules bind to the biosensor surface, that net signal pattern changes, producing a difference in the resulting spectrum whose magnitude depends on the size and number of molecules bound.

Electrical and Mechanical Biosensing
Though most commercial systems employ optical detection, there are other ways to detect interactions sans labels. As Cunningham puts it, “All optical biosensors take advantage of the fact that biomolecules all have a dielectric coefficient or refractive index that is higher than that of water. But what other properties does a molecule have?”

One property, of course, is mass, and several mechanical approaches exist to probe that, says Rashid Bashir, the Bliss Professor of Electrical and Computer Engineering and Bioengineering at the University of Illinois at Urbana-Champaign. One such approach is “resonance mass sensing.”

Many structures—quartz crystals, for instance—have a characteristic vibrational frequency, Bashir explains. Because that frequency depends on mass, if the device is small enough, “as I add a mass to it, I can detect that added mass by the change in the resonant frequency of the resonator.”

TTP LabTech’s RAPiD 4 system is a quartz crystal microbalance device based on this principle.

An alternative strategy, stress sensing, uses mechanical stress to follow biochemical events on the surface of, for instance, a cantilever (a kind of nanoscale diving board). “If you perform a biochemical reaction on only one surface of these cantilevers, then that change in the local surface energy will result in a change in surface stress, and the cantilever will actually bend,” Bashir explains. As with other label-free methods, the degree of that flexion depends on the extent of macromolecular interaction, and can be quantified by bouncing a laser off the cantilever’s tip.

Others have devised label-free sensing methods based on electronic fluctuations resulting from macromolecular interactions. According to Bashir, systems have been devised based on impedance or electrochemical sensing using electrodes in fluid or potentiometric sensing using field-effect transistors or silicon nanowires. Acea Biosciences and MDS Analytical Technologies have commercialized electrical impedance–based systems, which measure changes in current flow between electrodes when cells deposited between the electrodes change shape, adherence, or mass distribution as a result of, for instance, G protein-coupled receptor stimulation.

Ultimately each strategy, whether optical, mechanical, or electrical, has its pros and cons. SPR, for instance, has decades of user know-how behind it. Electrical approaches may be most amenable to miniaturization, says Bashir, as they require neither optics nor vibrational detectors, whereas electrical and mechanical strategies have the advantage of being immune to optical distortions at the biosensor surface.

Whichever molecular property you focus on, once you get over the learning curve, you might find that once you go label-free, there’s no going back.

Jeffrey M. Perkel is a freelance science writer based in Pocatello, Idaho.

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