RESEARCH PROFILES

Magnetic tweezers tease out dissociation

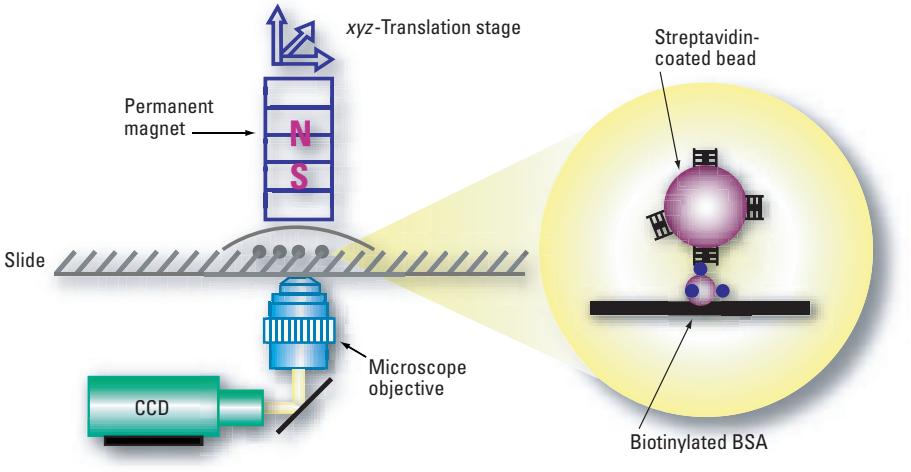
A new method that relies on magnetic tweezers is now available to measure the dissociation of receptor–ligand complexes at the single-molecule level. In the May 15 issue of *Analytical Chemistry* (pp 3023–3028), Mara Prentiss and colleagues at Harvard University use the approach to measure the dissociation of streptavidin–biotin complexes. The method is inexpensive and robust and can be carried out in parallel.

Optical tweezers, biomembrane force probes, and atomic force microscopy have been previously used to measure the forces involved as multiple short-range, noncovalent interactions are broken in receptor–ligand complexes. But it can be difficult and time-consuming to use these techniques for parallel analyses and to obtain statistically significant data. The new tool developed by Prentiss and colleagues overcomes these limitations and is capable of making 100–1000 measurements simultaneously on many single ligand–receptor complexes.

To demonstrate the approach, the researchers modified 4.5-μm superparamagnetic beads with streptavidin, which served as the receptor molecule. The corresponding ligand, biotin, was attached to bovine serum albumin (BSA) and adsorbed to a plastic slide. The beads were released over the ligands, and streptavidin–biotin complexes were allowed to form.

A permanent magnet was then brought into the vicinity of the complexes to generate a constant force. The distance between the magnet and the beads determined the force's magnitude. The force pulled the complexes apart, allowing the investigators to measure the strength of the interactions between the receptor and its ligand.

The application of force helped the investigators measure interactions in complexes that are difficult to study. "Intuitively, you can imagine, as you apply force, dissociation occurs considerably faster," says Claudia Danilowicz, a research associate in the Prentiss group. For very strong binding, such as between streptavidin and biotin, spontaneous dissociation can take a couple of days. "But if you apply force, the lifetime of the complex is significantly reduced," she says.



Schematic of the magnetic tweezers experimental setup, in which a permanent magnet moves over superparamagnetic beads modified with receptors. The receptors on the beads interact with biotinylated BSA. (Image is not drawn to scale.)

Prentiss and colleagues measured the dissociation of streptavidin–biotin complexes under a series of different forces. They extrapolated their measurements and calculated the kinetic rates and transition-state distances for streptavidin and biotin at zero force. The values they obtained were consistent with those found by other techniques.

Because the beads are commercially available and relatively inexpensive, Danilowicz says that the magnetic tweezers could be developed as part of a disposable assay platform. R. J. Colton at the U.S. Naval Research Laboratory previously proposed a similar magnetic bead assay that uses magnetoresistive detection.

But Danilowicz cautions that a caveat exists for commercially available beads. "There's a big spread in the force," she says. "We are also thinking about possible ways of purifying or doing some kind of chromatography to get a fraction of beads that are more homogeneous in terms of the force you can achieve. Otherwise, you get a spread in the force and that's not what you want. Every time you apply a nominal force, you want to have that force per bead [be] exactly the same or about the same."

Prentiss and colleagues are collaborating with George Whitesides's group at Harvard University to study the behavior of an enzyme called carbonic anhydrase. The collaborators are extending the magnetic tweezers studies to carbonic anhydrase immobilized on beads and its substrate embedded in self-assembled monolayers.

Whitesides's group has "been providing us with self-assembled monolayers where you can really tune the stoichiometry of your surface in terms of the ligand concentration very well and precisely," says Danilowicz. Because the stoichiometry can be better controlled, the investigators plan to carry out more sensitive measurements of the interactions in receptor–ligand complexes.

In addition, the investigators are looking to make the experimental procedure simpler. "We would like do these measurements in an automatic way," explains Danilowicz. "We have thought for a while [about] trying to design electromagnets where you could apply the force almost instantaneously." ■

—Rajendrani Mukhopadhyay