PROTEIN BIOCHEMISTRY

# Interaction in solution

**Using interferometry, researchers measured macromolecular interactions without labels and in free solution.**

No macromolecule is an island. Proteins interact constantly with one another, and with other entities in the cellular and extracellular environment. However, sensitive *in vitro* methods to monitor such interactions, in particular methods that are label-free and do not involve surface immobilization, have until recently been lacking. Darryl Bornhop and colleagues at Vanderbilt University now report the use of back-scattering interferometry to monitor interactions of unlabeled molecules in free solution.

"Most solutes aren't fluorescent," explains Bornhop, "and derivatization can complicate the analysis, so we've been long interested in developing universal sensing techniques. That's how we got into interferometry, which is basically a very sensitive way to monitor changes in refractive index." In previous work, the researchers showed that molecular interactions could be measured in a surface-attached format using interferometry. "Then it just dawned on me," says Bornhop, "that the majority of the signal could be coming from bulk refractive index changes and not principally from the surface. So we thought, why not try and do this in solution? And with the efforts of a number of great people in the lab, this has now become possible."

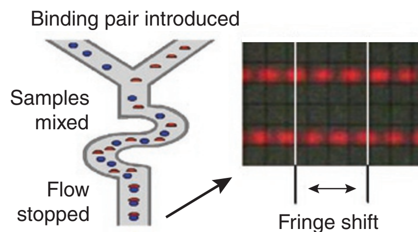


**Figure 1** | Interferometry to monitor molecular interactions. The design of the microfluidic channel, the signal and the fringe shift for a representative binding event are shown. Reprinted with permission from AAAS.

The researchers built a chip with a simple serpentine microfluidic channel followed by a constriction (**Fig. 1**), and optimized flow rates and sample mixing. Impinging coherent light onto the channel produces high-contrast interference fringes, which result from interactions between the light, the channel and the fluid. As Bornhop puts it, "the channel is the optics." Light refracts and reflects off the channel walls, making hundreds of passes and becomes, in a sense, trapped. The extent to which this occurs depends in part on the refractive index of the sample. A change in this refractive index, as is thought to occur upon molecular binding, results in a spatial shift in the interference fringes. This can be measured with high sensitivity.

Using this simple chip and a stop-flow system, the researchers monitored protein-protein, small molecule–protein, and calcium-protein interactions in solution over a dynamic range spanning six orders of magnitude. Both kinetic and end-point measurements were possible, and measured values were more or less in agreement with previous reports. The method is robust, is rapid, can measure dissociation constants

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IMAGING AND VISUALIZATION

## VARIATIONS ON ATOMIC FORCE MICROSCOPY

**The sphere of atomic force microscopy (AFM)-based applications is expanding in possibly unexpected directions.**

Since its invention in 1986, AFM applications have expanded far beyond simple topographic imaging. AFM tips can be functionalized with a wide range of molecules and used for force-distance measurements to determine binding strengths and single-molecule rupture forces. Image maps can even be generated showing the distribution of interacting molecules on biological surfaces.

Yves Dufrêne and colleagues at the Université Catholique de Louvain now report the use of a kind of AFM called chemical force microscopy (CFM) to map the hydrophobicity of live microbes (Dague *et al.*, 2007). The hydrophobic character of microbial cell surfaces is believed to be important in mediating pathogen-drug and pathogen-host interactions.

To detect hydrophobic interactions, Dufrêne and colleagues functionalized a gold AFM tip with $CH_3$ groups. They obtained nanoscale maps of the hydrophobicity of multiple live pathogens. Whereas the native mycobacteria had homogeneous surface hydrophobicity, treatment of the cells with the antimycobacterial drug isoniazid dramatically decreased the hydrophobicity.

Although AFM generally relies on interactions mediated by the passive physical characteristics of the tip and target, a variant of AFM called Kelvin probe force microscopy (KPFM) requires application of a voltage to the AFM probe. The electrified tip acts as a reference electrode that is scanned over a surface without touching it to measure changes in the surface potential.

Previous work showed that Kelvin probes could be used to detect protein or DNA arrayed on a surface. Ashe Sinensky and Angela Belcher at the Massachusetts Institute of Technology now show that KPFM combined with arrays of protein or DNA could form the basis of a new high-density and label-free class of microarrays (Sinensky & Blecher, 2007). The high spatial resolution of AFM nanoprobes potentially allows detection of spot sizes much smaller than those on existing microarrays. Producing spots small enough to exploit the resolution of KPFM, however, was a challenge.

To create such small spots Sinensky and Belcher used a lithography technique called dip-pen nanolithography (DPN). This allowed them to write sub-micrometer–sized features on a gold substrate by using an AFM tip as a pen, and 'ink' containing protein or ssDNA. Using KPFM they could detect the change in surface potential created by the deposited protein or ssDNA.

For a microarray to be usable though, a user must be able to detect the specific binding of target molecules. Sinensky and Belcher exposed their protein or ssDNA features to protein or ssDNA binding partners, respectively, and showed that KPFM was

in the picomolar range, and requires very small quantities of material. The researchers hope in the future to extend the dynamic range to span all known biological interactions.

"Being able to do this in free solution is really the big deal," says Bornhop. "People have forever complained about surface plasmon resonance being tough in terms of needing to do the surface chemistry, and also needing to have a priori information. The nice thing for us is that we don't need to know much about the proteins we're studying." Thus the method has potential exciting applications in screening for as-yet unknown binding partners of orphan receptors.

The technology may be applicable to diagnostics and therapeutic monitoring as well. "You could do this (relatively cheaply) with a diode laser, a little plastic chip, and a simple detector or bar-code scanner," says Bornhop. The researchers are developing interferometry for benchtop point-of-care analysis, which would allow patients to receive diagnostic and treatment information within minutes, eliminating the need for repeated clinic visits. In the future, given sufficient investment and effort, it is possible that the method could even be harnessed for extremely low-tech field applications.

In the meantime, Bornhop envisions that free-solution interferometry will be useful for studying intramolecular changes such as solubilization and folding as well as binding. "If the molecular dipole changes enough to produce a refractive index change, we should be able to see it," he says. And because of the universal nature of the signal, this will apply not only to proteins, but to other macromolecules as well.

**Natalie de Souza**

**RESEARCH PAPERS**

Bornhop, D.J. *et al*. Free-solution, label-free molecular interactions studied by back-scattering interferometry. *Science* **317**, 1732–1736 (2007).

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able to reliably detect the change in potential that occurred. The magnitude of the potential change for the protein-protein interaction was dependent on the ionic properties of the protein, but DNA hybridization gave a reliable twofold signal change.

Sinensky and Belcher showed that the technique is compatible with fast scanning speeds that should allow analyses similar in speed to what is achievable with fluorescent microarray systems but without the requirement for labeling. The limitation at this time is the slow speed of conventional DPN to manufacture the arrays. Once this is overcome, the high-density and label-free nature of KPFM should provide advantages over fluorescence-based arrays.

AFM is expanding quickly into new areas as the nanoscale resolution of the device finds new applications that are quite different from what AFM was developed for. Although it is unlikely that AFM-based devices will ever be as common as light-based microscopes in biology laboratories, the technology seems poised for rapid expansion.

**Daniel Evanko**

**RESEARCH PAPERS**

Dague, E. *et al.* Chemical force microscopy of single live cells. *Nano Lett.* **7**, 3026–3030 (2007).

Sinensky, A.K. & Blecher, A.M. Label-free and high-resolution protein/DNA nanoarray analysis using Kelvin probe force microscopy. *Nature Nano.* **2**, 653–659 (2007).

## NEWS IN BRIEF

CHROMATIN TECHNIQUES

### Assessing histone biology with SILAC

There is great interest in understanding how histone post-translational modifications regulate biological processes. Vermeulen *et al.* now use a combination of SILAC (stable isotope labeling by amino acids in cell culture), high accuracy mass spectrometry, and a new statistical procedure to monitor differences in transcription factor binding to methylated or nonmethylated histone H3 peptides.

Vermeulen, M. *et al. Cell* **131**, 58–69 (2007).

RNA INTERFERENCE

### MicroRNAs need accessible targets

It is still not fully understood how microRNAs recognize their mRNA targets. Sequence complementarity at key positions is an important feature, and Kertesz *et al.* now show that accessibility of the binding site on the mRNA is equally crucial. They demonstrate that the total energy balance between unwinding an mRNA and binding the microRNA is an important feature in determining the overall silencing efficacy of the microRNA.

Kertesz, M. *et al. Nat. Genet.* **39**, 1278–1284 (2007).

CHEMICAL BIOLOGY

### Promiscuous glycosyltransferases

The synthesis of glycosylated natural products would be made easier with glycosyltransferase enzymes that accept diverse sugar substrates. Williams *et al.* describe a fluorescence-based assay to screen for increased glycosyltransferase catalytic efficiency and substrate promiscuity via directed evolution. They evolved a 'universal' glycosyltransferase with broad activity.

Williams, G.J. *et al. Nat. Chem. Biol.* **3**, 657–662 (2007).

IMAGING AND VISUALIZATION

### Imaging nitrogen fixation

Using a technique called multi-isotope imaging mass spectrometry (MIMS), Lechene *et al.* monitored nitrogen fixation by symbiotic bacteria in the gills of marine shipworms. They followed the incorporation of $^{15}N$ from nitrogen gas fixed by the bacterium *Teredinibacter turnerae* by bombarding shipworm gill tissue with a primary cesium ion beam to produce secondary cyanide ions, thus mapping the distribution of $^{15}N$ and the normal isotope $^{14}N$. The technique may be useful in other microbial ecology studies.

Lechene, C.P. *et al. Science* **317**, 1563–1566 (2007).

GENE REGULATION

### Assessing genomic ultraconservation

Sequences with 100% identity between human, mouse and rat (>200 nt), so-called ultraconserved elements, are thought to represent genomic regions of great functional relevance. Ahituv *et al.* now show that deletion of four such elements in mouse had no discernible effects on the animals. Although alternative explanations are possible, the observation suggests that not all ultraconserved elements are indispensable in mammals.

Ahituv, N. *et al. PLoS Biol.* **5**, e234 (2007).