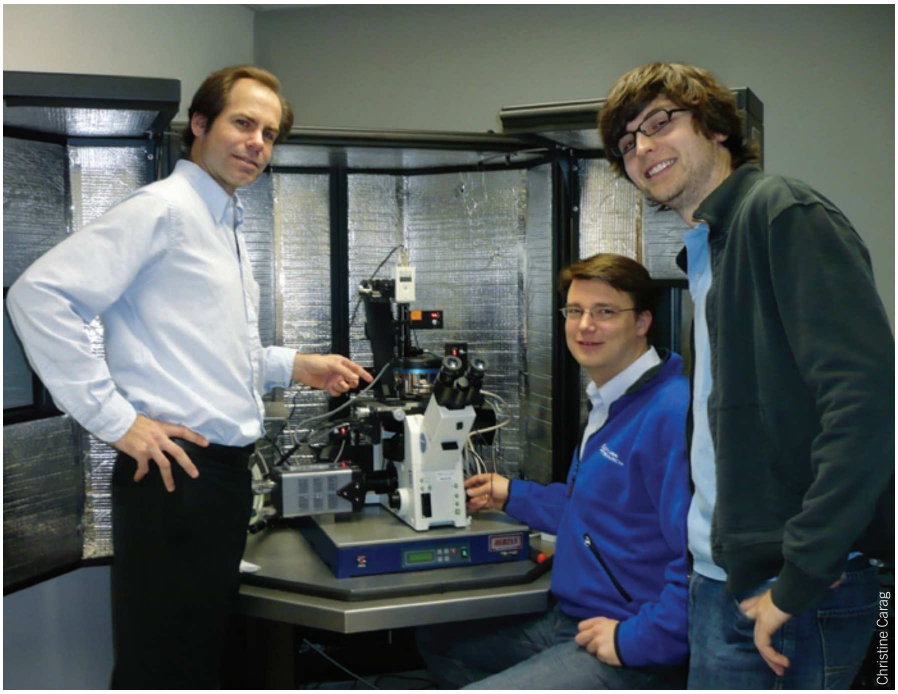
Nanotechnology in biology: big collaborations for a small world

Nathan Blow

In less than five years the Nano/Bio Interface Center at the University of Pennsylvania has gone from an idea to a nationally funded nanotechnology center. A look inside reveals how they have taken a collaborative approach to technology development.

Between bites of his lunch on a stone bench outside the Towne engineering building of the University of Pennsylvania campus in Philadelphia, Dennis Discher talks excitedly about the new directions his research on cell signaling regulated by force will take in the coming months. Discher and his team have built a system that combines polarized total internal reflection fluorescence (polTIRF) and atomic force microscopy (AFM)—no easy technical feat. But with the new ability to perform polTIRF and AFM imaging simultaneously on a sample, Discher is convinced the whole engineering effort was worthwhile.

"The development of this polTIRF-AFM has really been a collective effort," says Discher, acknowledging the instrumental role of the university's Nano/Bio Interface Center (NBIC). This center brings together research groups from ten different departments across five schools at the university—nearly 40 investigators—and promotes interactions such as those that led to Discher's microscope development.



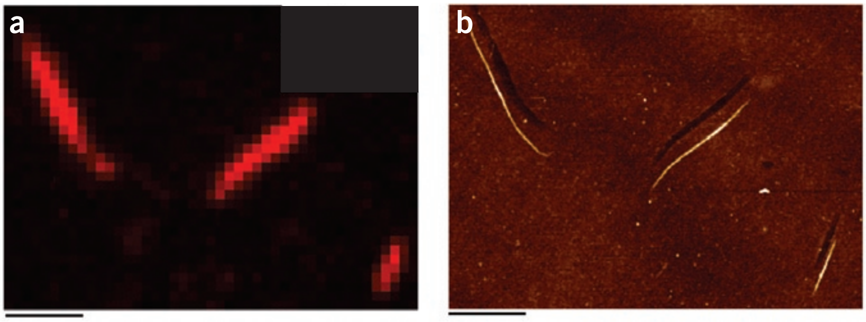
Dennis Discher, Florian Rehfeldt and Andre Brown (left to right) along with NBIC colleagues have built an integrated polTIRF-AFM microscope.

Dawn Bonnell, director of the NBIC, says interactions and diversity are critical to NBIC's theme: "molecular interactions at the interface of physical and biological systems." The center was established in 2004 with an $11.4 million grant from the US National Science Foundation and takes a somewhat 'circular' approach to nanotechnology: some members, like Discher, are applying nanotechnology to answer biological questions, whereas others are using biological materials for nanotechnology applications.

A.T. "Charlie" Johnson, for example, an NBIC physicist, is exploring the design of new proteins for electronics applications. "It brings a whole new set of thoughts into my lab," he says of the NBIC diversity. "At Penn we thought we had quite a wide range of people who could come together and form a truly distinct nanotechnology center."

Coming into the light

Tucked away in a windowless room at the back of their lab, Discher, a postdoc and a physics graduate student have toiled away for the past six months setting up their three-color polTIRF-AFM. Development of this microscope was funded through a $700,000 major research instrumentation program grant from the National Science Foundation and co-sponsored by the NBIC. And although his lab has extensive experience using AFM for force measurements and imaging, integrating polarized TIRF microscopy provided a new set of challenges that could only be met in collaboration with NBIC colleagues.



Myosin filaments examined using NBIC's polTIRF-AFM microscope. (**a**) TIRF image. (**b**) AFM image. Scale bars, 2 µm. (Courtesy of D. Discher.)

AFM measures the deflection of a cantilevered tip with a laser as the tip scans along a surface, a 'touch scanning' approach that provides a resolution exceeding that of optical microscopy. In contrast, polTIRF is an optical technique that takes advantage of evanescent waves of light, which do not penetrate samples deeply, to excite and image fluorophores very near a surface and in an orientation-dependent manner. The combination of these two imaging modalities in a single microscope allows single-molecule fluorescence measurements of both angular and conformational motion to be collected before, during or after AFM imaging, providing the opportunity to answer compositional as well as structural questions.

Why incorporation of polTIRF with AFM? "Fluorescence is king of compositional information," says Discher. But registering this 'king' with the force-sensitive structural information provided by AFM will be another challenge; right now there is limited instantaneous registration between the two. Because AFM measurements are sensitive to the environment—to the point where even the heat generated from the polTIRF set up can cause the AFM tip to drift—the group is using biological markers to register positions at different time points. They are working closely with Asylum Research (Santa Barbara, California, USA), manufacturer of the MFP-3D—the AFM component of the new microscope, and Nikon Instruments (Melville, New York, USA), manufacturer of the optical TIRF components, on the thermal drift and other registration issues.

Right now the microscope is only missing a third laser light source, which will provide a greater flexibility, but the group has started to obtain images and manipulate molecules. "You can image surface fluorescence in the [sub-micrometer] range, but true nanometer resolution on wet samples is only accessible with AFM," says Florian Rehfeldt, a postdoc in Discher's lab who is using the new microscope for ongoing studies of gels and surfaces that promote stem cell differentiation. "But you can also determine the geometry of a filament or a virus, which is not possible with fluorescence [microscopy]."

Discher and physics graduate student Andre Brown also plan to use the microscope to advance their recent work on the unfolding of coiled-coil domains in fibrinogen molecules[1]. Using AFM to uncoil sections of fibrinogen molecules, decorated with specifically placed fluorophores, they want to visualize the unfolding by following the distance between fluorophores in relation to the force applied.

There is already a demand for more applications. Discher says: "We are going to build another polTIRF-AFM soon, and in the future both will be available to NBIC members as well as external users."

BOX 1 NEXT-GENERATION PROTEIN SEQUENCING?

NBIC collaborations are starting to travel beyond the walls of the University of Pennsylvania. In November 2007, Yale Goldman, along with Barry Cooperman from the chemistry department, partnered with New Jersey, USA–based Anima Cell Metrology to develop a new technology for real-time protein analysis in living cells.

The project, funded with a $1.9 million Advanced Technology Program grant from the US Commerce Department's National Institutes of Standards and Technologies, will take advantage of instrumentation and methods developed by Goldman and Cooperman during their studies into ribosome dynamics. "This is a grant for projects that require risk," notes Goldman, adding that, although risky, the approach has the potential to advance proteomic analysis and create a new method for protein sequencing.

The group will use single-molecule fluorescence resonance energy transfer (FRET) in combination with optical waveguides and low-angle oblique microscopy to peer inside cells as protein synthesis is occurring *in vivo*. Goldman says by performing FRET with different spectral signals for each incorporated amino acid, it should be possible to 'see' protein synthesis in real time. In many ways, the idea is analogous to several 'next-next' generation DNA sequencing methods now being developed but with the added twist, and difficulty, of performing the imaging and reactions in living cells.

For the NBIC, this form of collaboration is a win-win situation: the project is going to bring in up to eight new students and postdocs in the coming months, and the instrumentation they create will be part of the NBIC to benefit other investigators.

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Fancy footsteps

A few buildings away, in his office on the seventh floor, overlooking the medical school, Yale Goldman jokes about one huge benefit to his involvement with the NBIC. "Around campus my colleagues now realize that molecular motors are fascinating."

Goldman, who is also director of the Pennsylvania Muscle Institute, has been a member of the University of Pennsylvania community most his professional life, having been away only for five years between his MD/PhD and the faculty position he got in 1980. He now focuses his research on the dynamics of molecular motors and protein synthesis at the single-molecule level. "One puzzle to me is how motors can operate so efficiently in such a chaotic nanoenvironment," says Goldman, noting that water and other molecules are constantly in motion, bombarding motor components[2].

When it comes to explaining his latest research on the stepwise movement of myosin motors along actin, Goldman rises from his chair: "To explain our myosin work, I will have to dance a little." Placing one foot in front on the other, as if starting a race, he swings his back leg forward searching for a specific spot on the floor. Why does myosin not go back to its starting position if searching for the correct landing spot on actin takes time? The current hypothesis is that moving back is prevented because as the myosin filament moves forward, the myosin head 'wiggles' back and forth to find the correct landing spot—Goldman is now wiggling his foot as he continues to stand on one leg.

The challenge of seeing the movement of myosin's 'foot' at the molecular level has been taken up by his third-year graduate student John Beausang in collaboration with NBIC investigator Phil Nelson from the physics department. They will need to be able to see the rotational movement of the myosin head at a time resolution of 5 milliseconds—tenfold faster than their current polTIRF setup allows. But advancing TIRF instrumentation is a recurring effort for Goldman; he collaborated with Discher on the polTIRF-AFM microscope and is now working with other groups on live-cell proteomics applications (**Box 1**).

Beausang and Goldman will use hardware that is more often applied to fluorescent lifetime imaging—which marks each photon with the polarization state—in combination with several different wavelengths of light to get multiple views of the myosin head as it moves forward. They hope to see the rotational movement of the head with sufficient time resolution to tell if Goldman's foot wiggle is truly the way the myosin head acts, or if he will have to learn some new steps.

Building a better machine, biology style

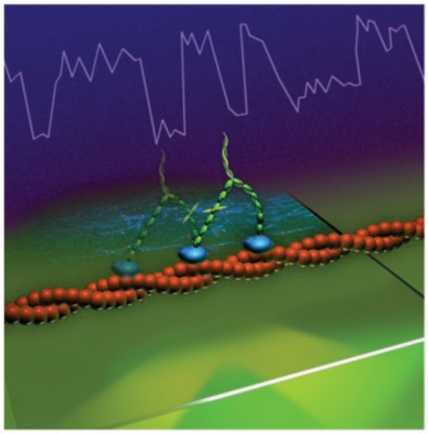
"In this world of nanotechnology, a lot has been developed by bringing together many disciplines," says Johnson. A condensed matter physicist by training, he says collaborations through the NBIC have been

Yale Goldman is exploring the dynamics of molecular motors using single-molecule approaches.

essential for his recent research into carbon nanotubes and *de novo* protein design.

"I am interested in biomolecules as amazingly sophisticated structures that I can take advantage of for novel physical applications," says Johnson. Notably, his group is working toward making molecular electronic devices using very small proteins. But Douglas Strachan, a post-doc working on the project, acknowledges such devices are still in the future: "While transistors or sensors made out of things from the body would be the ultimate goal, right now we are making small steps in that direction."

Another interest in Johnson's lab is to combine surface-modified carbon nanotubes with field effect transistors. Here the group relies on an integrated scanning tunneling microscopy (STM)-AFM system from Omicron Nanotechnology from Taunusstein and Carl Zeiss in Jena, Germany to image the surface of the nanotube with AFM while probing local electron density states as a nanotube responds to different environments or molecules using STM. One application the group is now working on is ultrasensitive gas sensing. "We are trying to mimic a dog's nose with nanotechnology," says Strachan.



Goldman's lab and others at the NBIC are examining the movement of myosin filaments along actin using optical traps and TIRF microscopy. (Courtesy of Y. Goldman.)

The NBIC is not only a boon for its own investigators: it supports 30 students and postdocs every year and provides four to five "Innovation Grants" to Penn researchers working on new topics of inquiry in the fields of bio-nanotechnology and engineering.

Strachan, who recently accepted a position as an assistant professor at the University of Kentucky after being supported by the NBIC for two years, thinks being associated with a center like the NBIC was a good career move. "This has been a great opportunity for me to experience interdisciplinary collaborations," he says. "And people always seem to perk up when you start talking about nanotechnology centers."

1. Brown, A.E., Litvinov, R.I., Discher, D.E. & Weisel, J.W. *Biophys J.* **92**, 39–41 (2007).
2. Sun Y. *et al. Mol Cell.* **28**, 954–964 (2007).

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Improvision	http://www.improvision.com
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Lightspeed Technologies	http://www.light-speed-tech.com
Mauna Kea Technologies	http://www.maunakeatech.com
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Nanoptek	http://www.nanoptek.com
Nanoscience Instruments	http://www.nanoscience.com
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Nikon Instruments	http://www.nikoninstruments.com
Novascan Technologies	http://www.novascan.com
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