ANALYTICAL CURRENTS

Nanotube and DNA ion channels

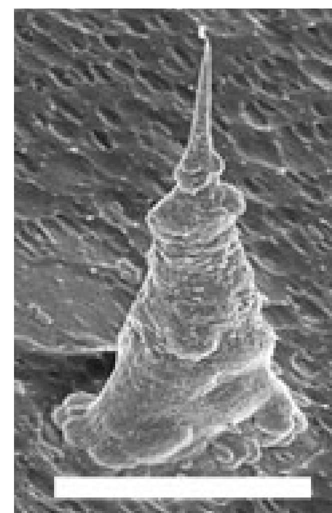
There's considerable effort to build artificial ion channels that closely mimic their biological counterparts. Charles Martin and colleagues at the University of Florida have built artificial ion channels out of nanotubes and single-stranded DNA molecules, which control the current through the ion channel in a manner similar to that observed in vivo.

The researchers created the ion channels in a series of steps. In the first step, conical pores were etched into a 12-µm-thick polycarbonate membrane. The diameter of the larger pore opening was 5 µm, and that of the smaller one was 60 nm. Gold was then deposited to coat the pores. In the third and final step, single-stranded DNA molecules with a thiol group were bound to the gold at the pore openings.

The artificial ion channels were attached to a U-tube conductivity cell to measure current–voltage curves. The investigators studied the rectification of the ion channels. Rectification, a term from electrical circuit theory, means that the ion channel changes the conductance of ions as a function of voltage, so that ions flow better in one direction than in the other.

The investigators quantified the extent of rectification using the ratio r_{max}, which is the current at –1 V divided by the current at +1 V. They found that the channels were turned on at negative potentials and turned off at positive potentials. The ratio, $r_{max,}$ increased as a function of the nanotube small-mouth diameter. Rectification by the small-mouth diameter occurred by increasingly inhibiting ion flow as the mouth diameter became smaller.

The ratio, $r_{max,}$ also increased as a function of DNA chain length. The investigators proposed that the rectification by the DNA was based on the positions of the DNA molecules. In the off state, the DNA molecules inserted themselves into the channel pore and blocked ions from passing through the channel. In the on state, the DNA molecules excluded themselves from the pore, enabling ion transport through the channel. The longer DNA chains resulted in greater rectification. The investigators suggest that rectification in these artificial ion channels could be reversed by using polycations at the nanotube mouth instead of polyanions like DNA. (*J. Am. Chem. Soc.* **2004**, *126*, 15,646–15,647)

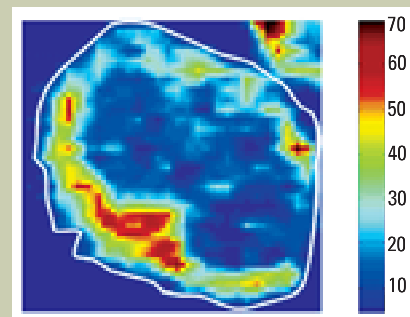


A conical artificial ion channel made out of DNA and a gold-plated nanotube. Scale bar = 5 µm.

Intracellular imaging with Raman

Henk-Jan van Manen and colleagues at the University of Twente and the University of Amsterdam (both in The Netherlands) have developed a confocal Raman microscopy method for intracellular imaging of heme-containing enzymes. The method's lack of sample preparation is a major advantage over other methods, such as fluorescence and electron microscopy, because artifacts are not introduced and the cells are not disturbed.

The researchers used the new label-free method to visualize two crucial enzymes involved in innate immunity. They excited leukocytes with a 413.1-nm laser, which enhanced the resonance Raman scattering signal of the heme-containing enzymes. They stimulated neutrophils by either phagocytosis or with the addition of phorbol 12-myristate 13-acetate (PMA) and visualized the redistribution of an enzyme involved in NADPH oxidation. In addition, they used the method to watch the enzyme eosinophil peroxidase in single eosinophils. (*J. Phys. Chem. B* **2004**, *108*, 18,762–18,771)



Resonance Raman image of a neutrophil activated with PMA for 20 min. The outline of the cell is marked in white.

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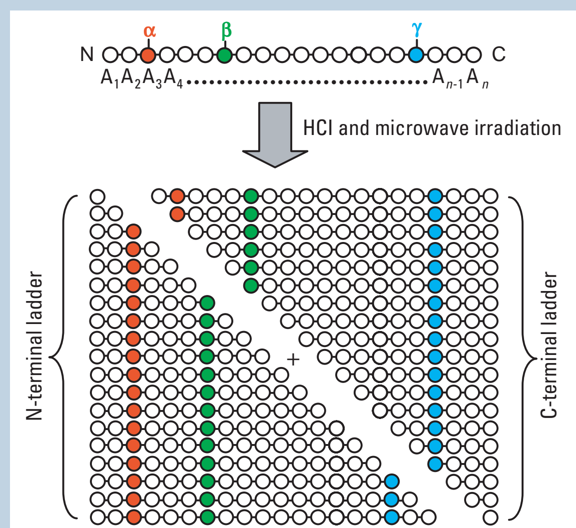
ANALYTICAL CURRENTS

Microwaving proteins

Liang Li and colleagues at the University of Alberta (Canada) have developed a microwave-based method for sequencing proteins and identifying posttranslational modifications (PTMs). Unlike traditional methods of generating polypeptide ladders, the new technique specifically produces N- and C-terminal peptides and appears to cleave many different peptides and proteins.

The researchers digest proteins and peptides by controlled acid hydrolysis. HCl is added to the sample protein or peptide, which is then microwaved for 1 min. Acid hydrolysis alone can generate peptides that are solely N- or C-terminal, but exposure of the reaction to microwave irradiation speeds up the process. Longer periods of irradiation, however, produce many internal peptides, and this can make sequencing difficult. The peptides are analyzed by MALDI-TOFMS. Peaks differ by one amino acid or by one amino acid and a PTM, so protein identification and determination of PTMs are easy and fast. Impurities of <15% of the total sample do not interfere with sequencing.

This method was used to sequence many different types of proteins, including those with internal disulfide bonds, which must be reduced prior to sequencing, and acid labile bonds. PTMs such as phosphorylations, acetylations, and heme groups were also detected. (*Nat. Biotechnol.* **2004**, *22*, 1291–1296)

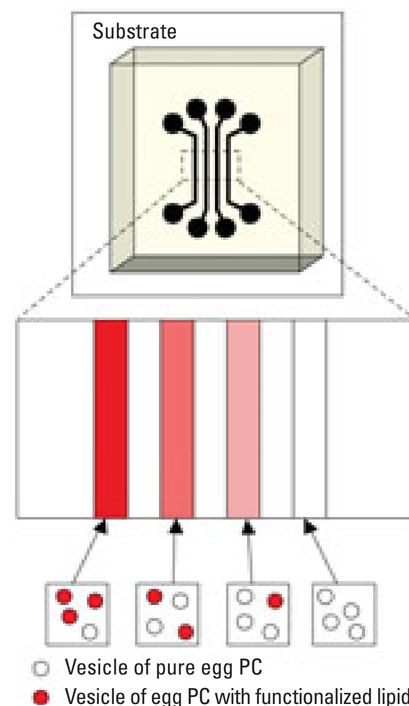


N- and C-terminal peptides generated by controlled acid hydrolysis. (Adapted with permission. Copyright 2004 Nature Publishing Group.)

Patterned lipid bilayers

Joyce Wong and colleagues at Boston University have developed a method to study different types of supported lipid bilayers in parallel under identical shear flow conditions. Supported lipid bilayers can be used as models for lipid-based drug carriers to analyze the effects of lipids on receptor–ligand interactions.

One problem in making patterned, supported lipid bilayers is that the lipids diffuse and mix together. Wong and colleagues confronted this problem by using a microfluidic device with channels. They injected mixtures of lipid vesicles into the channels and allowed them to form bilayers of various compositions. Barriers of bovine serum albumin were used to prevent mixing between adjacent lipid bilayers in the channels.



Patterned, supported lipid bilayers of various compositions can be created and studied in a microfluidic device.

The investigators used fluorescence microscopy to verify the formation of the lipid bilayers. They found that the addition of 140 mM NaCl promoted better bilayer formation than adding pure water.

Wong and colleagues incorporated biotin into some of the lipid bilayers. The biotin was attached to the bilayers via either a short or a long polymer chain. They then introduced streptavidin-coated beads into the channels and studied the interactions between streptavidin and biotin under identical shear flow conditions.

The longer the polymer chain, the more resistant the streptavidin–biotin complex was to detachment under shear flow. The researchers suggest that the longer polymer chain increased the proximity, and therefore the bonding interactions, between streptavidin and biotin.

Wong and colleagues say that their experimental approach can be applied to more than just lipid bilayers. Polymers, proteins, and even cells could be patterned into the microfluidic device. The investigators also suggest that various receptor–ligand interactions could be screened under identical flow conditions. (*Langmuir* **2004**, *20*, 10,252–10,259)

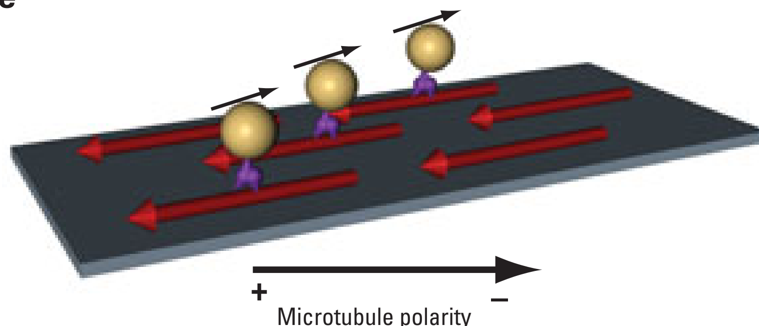
Microfluidic flow without pressure

Hiroyuki Fujita and co-workers at the University of Tokyo (Japan) have developed an alternative to pressure-driven flow in micro- and submicroscale devices. Taking a hint from nature, the researchers adapted a cellular process to move objects through tiny channels in a targeted direction.

In cells, kinesin motor molecules carry vesicles and other cargo unidirectionally along microtubules, from the minus ends to the plus ends of the fibers. To construct a one-way traffic system of microtubules in a microfluidic device, Fujita and co-workers had to ensure that all of the microtubules in a channel were aligned in the same direction and they all had the same polarity. To accomplish this, the researchers coated glass coverslip channels with kinesin molecules. They then added microtubules, which rested on the kinesin-coated surface in a direction parallel to the buffer flow.

Although the microtubules were physically aligned, they were randomly arranged with respect to polarity. To specifically retain only one population of the microtubules, the researchers added a pulse of ATP, which caused kinesin to slide the microtubules. Microtubules that slid upstream of the buffer flow fell off, leaving behind those that slid in the same direction as the buffer. The researchers immobilized the remaining microtubules by cross-linking them to the kinesin molecules.

When Fujita and co-workers added kinesin-coated beads and ATP to the immobilized microtubules, the beads moved upstream of the buffer flow, toward the plus ends of the filaments. Higher flow rates increased the orientation ratio, or the ratio of the number of beads moving upstream to the total number of moving beads, but decreased the microtubule density. An orientation ratio of ~97% was achieved with a buffer flow rate of 8 μm/s. Similar results were obtained when the process was repeated with poly(dimethyl siloxane) single- and multiple-channel devices. (*Nano Lett.* **2004**, *4*, 2265–2270)



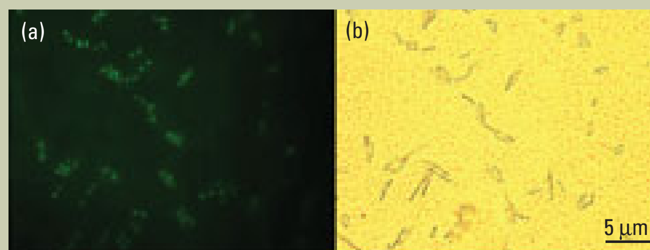
Kinesin-coated beads move to the plus ends of immobilized microtubules.

Bright carbon nanotubes

To image carbon nanotubes (CNTs) in the lab, researchers often have to resort to techniques such as electron microscopy (EM) and atomic force microscopy. Optical methods are better suited to imaging in biologically relevant aqueous environments, but current fluorescent labeling approaches either damage the CNTs or provide low signals. To overcome these difficulties, Mihrimah Ozkan and colleagues at the University of California, Riverside, used CdSe–ZnS core-shell quantum dots to visualize single-walled CNTs (SWNTs) in aqueous solutions.

The researchers prepared stable dispersions of SWNTs in 1% sodium dodecylsulfate (SDS) by shear mixing and sonication. Part of the solution was centrifuged to remove aggregates and to purify individual SWNTs and SWNT ropes. Quantum dots, stabilized by mercaptoacetic acid, were added to the original SWNT mixture and to the supernatant containing the individual SWNTs and SWNT ropes. In both cases, quantum dots attached to the SWNTs and produced strong signals that could be visualized by fluorescence microscopy.

To see even more detail, Ozkan and colleagues centrifuged the supernatant again. This second supernatant was spin-coated onto glass slides to minimize re-aggregation. By comparing light and fluorescence images, the researchers observed quantum dot labeling along the lengths of the SWNTs. Scanning EM and transmission EM images also showed that quantum dots were attached to SWNTs at multiple locations. Thus, once the quantum dots and SDS are removed by alcohol or water washes, the SWNTs can be used in devices and for other applications. (*Nano Lett.* **2004**, *4*, 2415–2419)



(a) Fluorescence and (b) light microscopy images of SWNTs labeled with quantum dots.

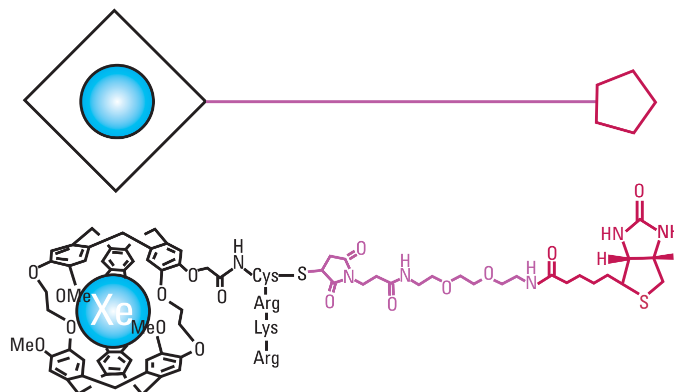
ANALYTICAL CURRENTS

Xenon as an NMR biosensor

Alexander Pines and colleagues at the University of California, Berkeley, Lawrence Berkeley National Laboratory, and the Scripps Research Institute have characterized the binding of a caged-xenon sensor to its protein target by NMR. Sensors based on caged xenon could lead to the multiplexing of analyte detection in a high-density format, such as in microarrays.

Using ^{129}Xe as an NMR sensor offers several advantages. Laser polarization of ^{129}Xe increases the S/N by several orders of magnitude over nuclear-spin measurements normally made in NMR experiments. Spectra from ^{129}Xe NMR are easier to interpret because fewer lines are present and no background signal is recorded. In addition, the chemical shift and relaxation parameters of ^{129}Xe can provide information about its local environment.

Pines and colleagues formed a biosensor by caging ^{129}Xe inside a chiral cryptophane-A molecule that was modified with biotin. The caged ^{129}Xe acted as a reporter, while the modified cryptophane-A molecule interacted with biotin's binding partner, avidin.



An NMR xenon biosensor consists of cryptophane-A with a caged ^{129}Xe (black) and a biotin tag (red), with a linker (purple) between the two.

Additional resonances appeared in the spectra when the biosensor bound to avidin. The investigators demonstrated that the signal change was due to the binding, not to the exchange rate of xenon between the free and caged forms.

Different diastereomeric forms of the cryptophane-A molecule provided distinctly different chemical shifts of ^{129}Xe when the sensor bound avidin. Pines and colleagues suggest that sets of sensors that work in parallel could be designed on the basis of diastereomeric forms of the cryptophane-A molecule. (*J. Am. Chem. Soc.* **2004**, *126*, 15,287–15,294)

Substrate for disposable microarrays

Ashish Vaidya and Michael Norton of Marshall University have prepared and characterized a surface-modified silicone elastomer for use as a substrate for DNA microarrays. The new substrate is robust and cost-effective and brings researchers one step closer to disposable microarrays.

Various materials, including gold, silicon, and glass, have been used for microarray substrates. Even plastics have been used because they are inexpensive to produce. It is difficult, however, to immobilize DNA molecules onto plastic surfaces unless the surfaces have been modified.

Vaidya and Norton used a vapor deposition process to expose a plasma-treated silicone elastomer made of poly(dimethyl siloxane) to 3-(aminopropyl)triethoxysilane (APTS) vapors under vacuum. The APTS formed a self-assembled monolayer, which was then coupled with a heterofunctional cross-linker to produce a maleimide-functionalized elastomer. The maleimide groups on the elastomer were reacted with thiol-terminated DNA sequences. The attachment of the DNA to the maleimide groups was shown to be stable, and the surface-bound DNA was capable of undergoing hybridization with fluorescently labeled complementary DNA sequences. (*Langmuir* **2004**, *20*, 11,100–11,107)

Solid-state NMR of proteins

Ann McDermott and colleagues at Columbia University and Abbott Laboratories have demonstrated that protein–ligand interactions can be studied with solid-state NMR. Although solution-phase NMR has been used to characterize protein–ligand complexes, this is the first time that NMR has been used to identify protein–ligand binding sites in solid samples. The new approach significantly increases the number of proteins that can be analyzed by NMR.

Solution-phase NMR is typically limited to highly soluble proteins with low to medium molecular weights. It excludes membrane-bound proteins as well as those that are prone to aggregation. Solid-state NMR extends the application of NMR to larger proteins and those bound to membranes.

The researchers used solid-state NMR to monitor the binding of peptides and small organic molecules to a precipitate of ^{13}C-labeled protein. The protein Bcl-xL, which belongs to a family of proteins involved in regulating cell death (apoptosis), was used as a model protein. The researchers obtained high-resolution spectra that were easily reproducible, and they detected changes in the protein that occurred upon formation of protein–ligand complexes. (*J. Am. Chem. Soc.* **2004**, *126*, 13,948–13,953)