ANALYTICAL CURRENTS

Detecting DNA using conical pores

Zuzanna Siwy and colleagues at Johnson & Johnson Pharmaceutical Research and Development, Gesellschaft für Schwerionenforschung (Germany), and Silesian University of Technology (Poland) have developed a conical polymer nanopore that permits DNA fragments of differing lengths to be distinguished with high resolution. The new sensor can detect single DNA molecules that are an order-of-magnitude smaller than those sensed by other synthetic pores.

DNA can be detected by measuring changes in current as the molecules traverse pores in an electric field. A protein pore embedded in a lipid bilayer has been used as a DNA biosensor, but this setup has limited applications because currents can leak through the unstable membrane. Synthetic pores made of various materials also have been reported, but, until now, none have performed as well as the protein pore.

Scanning electron micrographs of (a) a single pore viewed from the surface of its large opening and (b) a cross section of a single pore.

The biosensing properties of the new conical nanopore are similar to those of the protein pore, with the added benefits of mechanical robustness and stability as well as the ability to control its surface chemistry. The conical shape concentrates the resistance to the tip of the pore, which reduces its effective length. This shape also has less resistance than a cylindrical pore with the same diameter as the tip, which allows higher currents to be generated for a particular voltage.

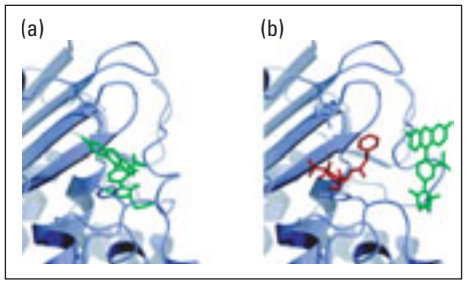
Siwy and colleagues prepared the single nanopores in polymer films by using a track-etching procedure, in which a film of polyimide was irradiated with uranium ions of 2.2 GeV energy, and the latent ion tracks were chemically etched. To demonstrate the system, they placed the nanopore-containing film between the buffer compartments of a conductivity cell and applied a constant voltage. When three DNA fragments of varying lengths were added to the system, changes in current were observed. This indicated that the DNA fragments, which were ~280, 970, and 4100 base pairs long, blocked the pore opening and translocated. The researchers could distinguish between the different-sized DNA fragments on the basis of their translocation times. In the future, they plan to study the effects of temperature and buffer conditions on DNA translocation times. (*Nano Lett.* **2004**, *4*, 497–501)

Fluorescent antibiotic biosensor

Conventional techniques for detecting β-lactam antibiotics, such as penicillin and cephalosporin, can be tedious, semiquantitative, and susceptible to interferences. But the new biosensor developed by Kwok-Yin Wong and colleagues at the Hong Kong Polytechnic University and the University of Cambridge (U.K.) is simple, quantitative, and sensitive.

Most biosensors are based on enzymes that undergo large conformational changes upon binding to ligands. These enzymes typically do not destroy the ligand, thus the interaction may be detected over a long window of time.

The new biosensor uses the enzyme β-lactamase. Although it binds well to β-lactam antibiotics, β-lactamase is a hydrolytic enzyme that undergoes only a small structural change in its Ω-loop, which is a 17-residue segment that is near the active site, after binding a ligand. Therefore, Wong and colleagues engineered a glutamic acid to a cysteine mutation in the Ω-loop of β-lactamase that reduced the enzyme's hydrolytic activity by 1800-fold compared with that of a wild-type enzyme. A thiol-specific fluorophore was placed at the cysteine residue to detect the motion of the loop. When antibiotic is not bound, the fluorophore is buried. Upon interacting with the ligand, the Ω-loop moves and the fluorophore is exposed.



Model of the β-lactamase Ω-loop (a) in the absence of antibiotic and (b) in the presence of penicillin.

The mutant had a low level of fluorescence in its buffer, but when antibiotics were added, fluorescence intensity increased in a concentration-dependent manner. Antibiotics could be detected at concentrations as low as 0.05 μM.

The sensor's activity differed according to which antibiotic was present. When penicillin was added, fluorescence increased, reached a plateau, and then decreased. When cephalosporin was added, fluorescence increased and then leveled off at a maximum, but it did not decline. This effect may occur because penicillin is a better hydrolytic substrate for β-lactamase than cephalosporin. Over time, the weakened, but still active, enzyme eventually hydrolyzes penicillin, and the fluorophore returns to its buried position. This process happens much more slowly for cephalosporin, thus β-lactamase fluorescence remains steady. (*J. Am. Chem. Soc.* **2004**, *126*, 4074–4075)