Adapting to nanoscale events

Daniel Branton and Jene Golovchenko

A hallmark of twentieth-century science has been the continual development of experimental strategies to observe individual atomic-scale ‘events’. These strategies ultimately rely on significantly amplifying the consequences of a selective microscopic interaction, for example the chemical development of a silver halide grain in a photographic emulsion, the condensation of a droplet around a single ion in a cloud chamber, or the charge amplification in electron multiplier devices.

New strategies for detecting and characterizing single-molecule events are now emerging in the biochemical sciences. The latest example comes from Gu et al. on page 686 of this issue1. This group, working in Hagan Bayley’s laboratory, show how measurement of ionic transport through a single, atomic-scale pore in an insulating membrane can be used to detect organic molecules of relative molecular mass as low as 100. Coupled with highly sensitive semiconductor electronics, the membrane–pore system can amplify these currents on timescales commensurate with the interaction times between the molecule and the pore. The system thus reveals the presence, nature and interaction of single molecules with the pore. Remarkably, a single protein channel can be adapted for simultaneous analysis of a mixture of organic molecules.

Gu et al. assemble the nanoscale chemical and mechanical building blocks of their detector from the toolbox of biochemistry; the key components are shown in Figs 1 and 2 of the paper on page 687. Seven ω-haemolysin molecules (a bacterial toxin) self-assemble to form a channel with a 15-Å diameter aqueous pore through a 50-Å-thick lipid bilayer membrane. The membrane separates two chambers filled with a conducting salt solution. Because the lipid bilayer is a nearly perfect insulator, the d.c. electrical conductance across the membrane is determined by the interaction between ions of the salt and the molecular pore in the ω-haemolysin channel. When a bias is applied across the membrane, the current flow is tiny (at the picoampere level), but it can nonetheless be measured with modern room-temperature semiconductor electronics.

The innovative step here is to sensitize the nanopore to specific organic chemical species by using β-cyclodextrin as an adapter. β-cyclodextrin, a doughnut-like molecule made of seven sugar units, diffuses into the ω-haemolysin channel, partially obstructing its water-filled pore. The authors show that the cyclodextrin molecule is exquisitely sensitive to different guest molecules. Thus, when bound to cyclodextrin, members of the adamantane family of petroleum derivatives can be distinguished, as can members of the group of tricyclic pharmaceuticals that include imipramine and promethazine. These guest molecules each bind to β-cyclodextrin for milliseconds and make their presence known by altering the electrical conductivity of the ω-haemolysin pore in which the β-cyclodextrin resides.

In neurobiology and biophysics, it is routine to measure picosecond ionic currents passing through single-channel proteins in biological membranes or planar lipid bilayers. Such measurements are usually made to determine the properties and behaviour of the channel, but they can also reveal details about the concentration and other properties of molecules that are able to pass through or into the channel. Very small alterations in the distribution of charges lining the pore can give rise to relatively large changes in the ionic flux, so a membrane channel can serve as an amplifying transducer. Such a transducer allows one to detect extremely small modulations (< 0.02 kT) in the energy barrier to ionic transport3, and does so at bandwidths that permit high-speed (< 50 µsec) measurements. Thus, membrane channels have been used to effect high-speed molecular counting and sizing5, and show promise for high-speed sequencing of polynucleotides6. Channels have also been modified to incorporate design elements that convert them into sensitive, analyte-driven switches7, or genetically engineered to create selective, divalent metal sensors7.

Bayley and his colleagues have long puzzled over how to engineer or redesign the ω-haemolysin channel as a biosensor that can discriminate between different organic molecules9. They reasoned that the sensitivity and extraordinarily rich informational content of single-channel recordings reduce the requirement for the channel to contain a highly selective binding site. By fashioning a single-channel detector, rather than one that integrates signals from numerous sensor molecules, several independent
membrane–pore systems be engineered using advanced materials science and nanolithography techniques, perhaps in conjunction with the methods of biological or chemical engineering? Can sensitive electronic circuitry and photonic sensing capabilities ultimately be integrated directly into a membrane–pore system, as they have for other chemical sensors\(1,2,3,4\)? These questions may not be answerable today. But, in the twenty-first century, exciting new nanoscale tools and molecular machines will surely emerge at this interface of biology, chemistry and physics.

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Neurobiology

Attraction is relative not absolute

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Suppose you like wine very much, and are faced with the choice of ordinary table wine or Beaujolais Nouveau—the latter is probably very attractive. But if presented with Beaujolais Nouveau and a superior wine, such as Romanée-Conti, the attraction of the Beaujolais Nouveau may subside. A similar phenomenon called ‘reinforcement contrast effect’ seems to occur in animal-learning studies, and Tremblay and Schultz now show (page 704 of this issue) that a region of the brain called the orbitofrontal cortex may be actively involved in this process.

Fifty years ago, in an experiment by Zeaman\(5\), rats were trained to run to a goal, for which they received a large reward. They were then shifted suddenly to a small reward. A second group of rats was trained the other way round; that is, they were first given a small reward and then shifted to a larger one. The animals that were shifted from a small to a larger reward immediately ran faster than would have been expected if the large reward were used alone. Conversely, those rats shifted from a large to a small reward ran more slowly than would have been expected with the small reward alone. The results indicated that the relative—but not absolute—magnitude of reward determines the attraction, as assessed by the eagerness of the rats to run to the goal.

Where in the brain is the attraction of a reward evaluated? To find out, Tremblay and Schultz\(6\) trained monkeys in a modified delayed-response task (Fig. 1). When the animal pressed a lever at the bottom of an experimental panel, a picture ‘cue’ was presented briefly on either the right or left side of the panel. Then there was a delay, after which the animal had to press the lever (right or left) above which the picture had been presented. This meant that, to obtain a reward, the monkey had to retain a memory of where the picture (although not what picture) was presented. Within a block of several tens of trials, the same two pictures were used continuously, and each picture always predicted one kind of reward.

Tremblay and Schultz found that where-as neurons in the lateral part of the prefrontal cortex showed changes in activity during the delay period, according to the position or identity of the picture, neurons in the orbitofrontal cortex were not sensitive to these factors. The orbitofrontal cortex—so called because it is located above the orbit, or eye socket—occupies the ventral surface of the prefrontal cortex (Fig. 2, overleaf). Humans and monkeys with damage to the orbitofrontal cortex often show impaired motivational and emotional behaviour\(7,8\), such as altered reward preferences\(9\). Consistent with this, the authors found that many orbitofrontal neurons were sensitive to the different rewards. They observed such responses when the picture cue was presented, during the expectation of reward, or after the monkey had received the reward.

Figure 1 shows the activity of such an orbitofrontal neuron. The neuron is more active when the monkey sees a square, which predicts a morsel of apple, than when it is presented with a triangle, which predicts a piece of lettuce (Fig. 1a). But in the next trial block, when a square predicts apple and a plus sign a piece of banana, the same neuron shows higher activity to the cue for banana, because monkeys prefer bananas to apples, and apples to lettuce (Fig. 1b). The attraction of the apple now seems to subside, and the neural activity to the square cue is reduced. In fact, it is similar to the activity for the triangle cue, which predicted the lettuce reward in the previous trial block. So, the activity of orbitofrontal neurons reflects the relative, but not absolute, attraction of the reward.

Once a monkey has had enough of the reward, the orbitofrontal neurons stop responding to the reward and to the cues that predict it\(10\). This process is specific to the nature of the reward, indicating that activity of the orbitofrontal neurons is determined by how much the monkey wants the reward\(11\). The work of Tremblay

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