both the elastic modulus and failure strength. These experiments were elegantly conceived, but the particular nanotubes used (grown by the arc-discharge method) showed rather poor strength at failure, presumably because of the defective nature of the as-grown structures. Moreover, the inability to observe the internal structure of the tubes during the testing complicated the interpretation of these results.

Now Espinosa and colleagues have performed experiments that border on the heroic. They have made use of a highly sophisticated testing methodology that they have developed over the past six years to measure the mechanical properties of the nanotubes while observing them inside a transmission electron microscope. The testing method uses a specially designed 'microelectromechanical system': this involves electrical signals being used to resistively heat a set of mechanical actuators to apply the load to the nanotube, with on-chip capacitors being used to measure the resulting displacement.

The nanotube is placed on a load frame using a nanoscale positioning robot during simultaneous observation in a scanning electron microscope, and it is then welded in place with local electron-beam-induced carbon deposition. The entire system is then transferred to a specially designed sample holder that can allow the microelectromechanical structures to be actuated inside the transmission electron microscope. This permits direct, real-time correlations to be made between the load–displacement curves and the structure of the nanotube at the atomic scale (as revealed by electron diffraction). These observations provide correlations between the nanotube chirality (which is the main indicator of structure), the number of walls, the ways in which load is transferred between the walls, and the mechanisms by which failure occurs.

The resulting experiments provide multiple new insights into the mechanical properties of carbon nanotubes. Perhaps most important is the simple confirmation that nanotubes can in fact be as strong as computational studies have predicted. Additionally, Espinosa and colleagues have shown that electron irradiation of the tubes can create mechanical linkages across the individual shells of the tubes, further increasing their capacity to carry load. This suggests that further studies of how to create these interlinks during nanotube production are merited.

Despite these results, much remains to be accomplished before carbon nanotubes are ubiquitous in load-bearing applications. The primary mode in which nanotubes are likely to be used will be as mechanical reinforcement elements in polymer-based composites, providing improvements in both stiffness and strength for little additional weight. But nanotubes are still relatively expensive, and greater insights into both growth mechanisms and improved growth methods are needed to drive their cost down. Additionally, carbon nanotubes have a strong tendency to bind together, making it difficult to disperse them uniformly in polymeric matrices[6].

Finally, decades of research have shown that in addition to the properties of the reinforcement, the properties of the interface between the matrix and the reinforcement have an important role in determining, for instance, how load is transferred between the constituents and how cracks propagate in the composite[7]. Thus, increasing our understanding of surface chemistry and interface properties remains crucial. Despite these challenges, the fact that carbon nanotubes can in fact be as stiff and strong as predicted is encouraging and indicates that we might, one day, find ourselves living in the Nanotube Age.

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PROTEIN ENGINEERING

Electrifying cell receptors

Ion channels can be attached to certain types of protein receptors in cells to make a detector–switch pair that could be used in various sensing and screening applications.

Atheir Abbas[1] and Bryan L. Roth[2]

are in [1]Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, USA; and [2]Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina 27516, USA.

e-mail: aia4@case.edu; bryan_roth@med.unc.edu

Modifying receptor proteins on the surfaces of cells so that they interact with proteins that are not their natural partners is one way of controlling signalling processes in the cell. Moreover, when isolated from their natural environment, these re-engineered proteins could be used for various sensing and drug-screening applications. On page 620 of this issue, Michel Vivaudou and co-workers[1] report that two types of proteins — G-protein coupled receptors (GPCRs) and the ion channels that control the voltage gradients across cell membranes — can be combined so that a measurable electrical signal is generated when a molecule such as a potential drug binds to the GPCR. The ability to screen potential drugs that recognize GPCRs with a generic electrical signal in this way could lead to important breakthroughs in nanobiotechnology.

GPCRs are a family of receptors that detect molecules outside the cell: they help regulate our senses, smell and mood, and are also the target for more than 50% of all modern medicinal drugs[2,3]. When ligands such as drugs, neurotransmitters, light or odorants bind to these receptors, they change their conformation and this activates G-proteins — a family of proteins that turn on downstream signalling cascades that, in turn, alter cellular function and behaviour[4]. Although this process is well-established, it is becoming clear that GPCR signalling is very complex because it can involve more than one type of G-protein[5] and can sometimes occur by other pathways that do not require G-proteins[6]. Furthermore,

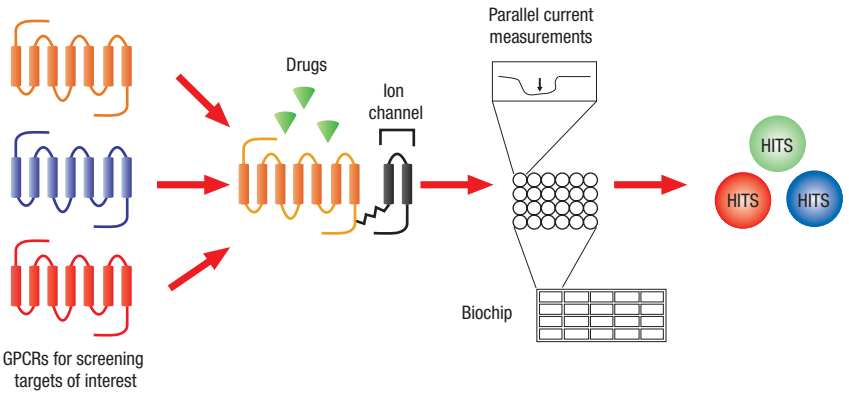

Figure 1 Advantages of ICCR-based screening. In current high-throughput screening (HTS), promising drug candidates or 'hits' could be missed because only one of many possible functional readouts from the complex GPCR signalling pathway is used to screen molecules. With the hypothetical ICCR-based HTS, one generic functional assay (known as ICCR) — formed by coupling one type of GPCR (orange, blue or red) with an ion channel — is used to screen GPCR targets of interest. The current of the ion channel changes when potential drugs (green cones) bind to the GPCRs. Using a biochip, many parallel measurements of the current can be made. This strategy increases the likelihood of detecting a wider variety of hits (red, green, blue circles) with more therapeutically optimal efficacies without relying on the complex signalling cascades of the GPCR.

different ligands can send different signals from the same receptor for one type of cell, and the same ligand can send different signals in different types of cells — a phenomenon known as 'functional selectivity'[5].

Although it is now routinely possible to screen hundreds of thousands of possible drug compounds at a single GPCR, current screening assays are still limited because the pathways that mediate the therapeutic actions for a particular GPCR are not immediately clear[7,8]. These complications imply that current large-scale screens for potential drug 'hits' are likely to be missing promising drug candidates because the 'wrong' functional readout is being screened. The main aim for this type of screening is a robust, nanoscaleable and universal high-throughput assay that can be adapted for any GPCR, and can also translate any conformational shift into a measurable generic signal.

Vivaudou and colleagues, of the Institut de Biologie Structurale and Institut de Recherches en Technologies et Sciences pour le Vivant in France, may have found a route to this assay by engineering a new type of GPCR. The membrane protein, sulphonylurea receptor, associates naturally with a potassium ion channel, modifying the behaviour of the ion channel when drugs bind to the receptor. The researchers surmised that by replacing the sulphonylurea receptor with a GPCR, it is possible to attach a GPCR directly to an ion channel to form what they call an ion-channel-coupled receptor (ICCR).

Using standard protein-engineering techniques, they mechanically coupled the well-studied M_2 muscarinic receptor with the ion channel. When the GPCR binds an agonist — a molecule that binds to a receptor and activates downstream signalling — its conformation changes and this alters the ionic current through the ion channel, which provides a measure of the binding of the agonist. The ICCR was able to record the increase and decrease in current flow when treated with agonists and antagonists (a molecule that inhibits the action of agonists), respectively. Further experiments suggested that the ICCR is modulated by the direct communication between the receptor and the channel.

To test the generalizability of this strategy, Vivaudou and co-workers created a second ICCR by coupling another well-known GPCR, the D_2 dopaminergic receptor, to the ion channel. As expected, this ICCR was sensitive to its naturally occurring agonist, dopamine. Surprisingly, however, instead of activating, dopamine inhibited the ion channel, and this was also seen with a synthetic agonist; importantly, both agonist responses were blocked by a D_2 antagonist. Despite the similarities between the M_2 and D_2 receptors, this inhibitory response suggests that the conformational shifts occurring at the M_2 receptor are qualitatively different from those of the D_2 receptor.

The possibility of engineering ICCRs from various GPCRs makes them promising candidates for the next generation of screening technologies (Fig. 1). Because the receptor and ion channel are linked directly to each other, any agonist-induced conformational shift should technically be transformed into a measurable generic electrical signal. This way, lead drug compounds can be screened without relying on the complex cascades of chemical signals typical of GPCRs.

A number of questions need to be answered, however. First, it remains to be seen whether other types of GPCRs can be used to engineer ICCRs. Second, it is a mystery why two similar GPCRs would have opposite effects on the opening of the ion channel. Third, to understand how the electrical responses are mediated by ICCRs, large numbers of agonists with widely varying efficacies should be tested. Finally, how the receptor and ion channel communicate with one another needs to be explored further.

For ICCR-based screening to become a reality, adapting the electrical measurement method with membrane protein microarray technology for parallel current recordings is necessary. The widely available dyes that measure membrane potential and fluorescent indicators for ion flux commonly used in high-throughput screening could accelerate the adoption of ICCR technology[9]. Nonetheless, these first ICCRs represent a clever feat of nanoscale protein engineering, which are likely to be quickly adopted by biotechnologists, screening centres and the greater biological community.

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