develop in a thin liquid film (less than 10 nm thick) when its two surfaces attract or repel each other through van der Waals and electrostatic forces[2]. The interplay of the capillary and disjoining pressures is critical to the stability of thin curved films, such as the wedge-like region at the edge of a wetting solution, trapped between a solid surface and the air above. If one pressure is higher than the other, liquid flows in or out of the wedge, causing the solution to wet or de-wet the surface. Integrating the capillary and disjoining pressures over the liquid volume produces Young's equation for simple liquids, which shows how to relate the angle of the wetting wedge to the surface and interfacial tensions of the three interfaces[3].

What happens when the wetting solution is not a simple liquid but is instead a concentrated dispersion of nanoparticles? Wasan and Nikolov[1] looked at the effect of using a dispersion of surfactant micelles — self-assembled particles, about 5 nm in diameter, formed of molecules with a hydrophilic and a hydrophobic end (Fig. 1). Micellar spreading is different from classical flow, as here wetting is driven by the gradient of film tension — that is, the tension acting over the depth of the wetting wedge. This phenomenon is also different from the surfactant-driven spreading of a liquid drop caused by the gradient of the surface tension on an expanding interface.

Uniform-sized micelles are known to form ordered structures[4–6] in confined spaces, such as in thin soap films, imparting stability to these structures. Wasan and Nikolov[1] have discovered that the micelles also form ordered structures near the three-phase contact line of a drop on a solid surface, which promotes wetting. The authors suggest that the micelles form ordered domains in the confined space of the wetting wedge wherever its thickness is equal to an integral multiple of the diameter of a single micelle (Fig. 1). There are two pronounced effects that result from this ordering. First, because the concentration of micelles is larger in the wedge than in the bulk suspension of the surrounding liquid, an osmotic pressure develops that attempts to separate the two interfaces, increasing the depth of the wedge between them. Second, because the film tension (the integrated value of the disjoining pressure over the width of the wedge) increases towards the vertex of the wedge, this creates an extra driving force for spreading the liquid at the wedge tip.

Wasan and Nikolov have explored these effects experimentally, observing the wetting wedge formed by an air bubble on a glass plate through an aqueous dispersion of 1-μm-diameter latex spheres (which is similar to a micellar suspension but allows visual inspection). They saw evidence that the latex particles assemble in the wedge in ordered structures: the average distances between particles followed a damped oscillatory pattern consistent with the prediction of statistical mechanics (Fig. 1b on page 156).

In a second experiment, a concentrated dispersion of 5-nm-diameter micelles was injected over an oil drop resting on a glass plate. Video microscopy showed a rapid invasion of the oil–water interface by the aqueous solution, and, as expected because of the increased disjoining pressure, efficient removal of the oil drop. But adding an electrolyte (in this case, sodium chloride) to the nanoparticle solution unexpectedly caused the oil-removal effect to disappear. Wasan and Nikolov suggest that the presence of the electrolyte caused the micellar diameter to shrink, leading to a decrease in the volume fraction of micelles in the wedge, and thereby decreasing the film tension.

The findings of Wasan and Nikolov[1] are of tremendous significance for many areas of research — from the interactions of liquid drops in complex fluids to the 'superwetting' of concentrated surfactant solutions on hydrophobic surfaces — as well as for applications such as the recovery of spilled oil. But to understand the phenomenon of wetting more fully, future studies will need to explain the roles of solvent structuring and the capillary pressure resulting from the curvature of the liquid meniscus in the stratified region of the wedge. These are important considerations, as the overall film pressure should be monotonic from the bulk solution all the way to the vertex of the wedge, even if the film pressure due to the presence of micelles oscillates. ■

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Computational biology

Biosensor design

William F. DeGrado

A series of bacterial receptor proteins have been 'redesigned' by computer so that they bind molecules that are quite different from their natural ligands. The approach might be useful for designing catalytic proteins.

On page 185 of this issue, Looger and colleagues[1] describe a powerful computational method for designing proteins that can detect small molecules. The authors have tested their approach on compounds such as trinitrotoluene (TNT) and the neurotransmitter serotonin, and their findings might see applications in, for instance, medicine and biotechnology.

Organisms use a broad repertoire of small-molecule-binding proteins — such as receptors and antibodies — to mediate cell-to-cell communication, signalling, and protection against pathogens. Binding proteins such as antibodies are also used widely to diagnose and treat diseases, and as molecular sensors. Until now, such proteins have been 'developed' *in vivo*, or by *in vitro* methods[2,3]. The *in vitro* methods require the construction of large 'libraries' of proteins containing diverse amino-acid sequences, coupled with an efficient strategy for selecting and continuously evolving those proteins that can bind the target molecule (the ligand) tightly. This approach is inevitably highly time-consuming. Looger *et al.*[1] have now accomplished the tasks of library construction and screening much more rapidly — in a computer.

The authors started with a series of periplasmic binding proteins[4] (PBPs) from the bacterium *Escherichia coli*. These Venus-flytrap-like receptor proteins have two structural domains that clamp together when they engage specific nutrient molecules. On binding their ligand they send signals into the cell, ultimately leading to the activation of appropriate genes. Looger *et al.* used a computational method to change the specificities of the PBPs entirely, so that they bind TNT, serotonin or the sugar L-lactate instead of their usual nutrient (Fig. 1).

The computational design process was initiated by placing a 'virtual' molecule of interest in the binding site of a virtual PBP receptor. The program then sequentially mutated receptor amino acids involved in binding the ligand, searching for sequences that form a surface complementary to the ligand. Typically, 12 to 18 amino acids were mutated, so this step alone created up to 10^{23} possible sequences — significantly more than can be screened *in vitro*. However, when approaching this problem computationally, not only must the amino-acid sequence of the receptor be specified, but also the orientation of the ligand, as well as the various conformations that might be adopted by the side chains of the mutated amino acids. These requirements greatly expanded the

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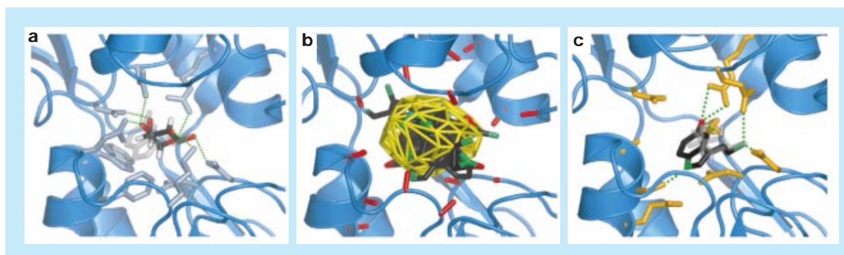

Figure 1 **Computational design of binding proteins. Looger *et al.*[1] have converted bacterial periplasmic binding proteins (PBPs) into receptors that bind serotonin, L-lactate or trinitrotoluene (TNT). Steps in the design of a serotonin receptor are shown.** a, **Close-up of a wild-type PBP that binds the sugar arabinose. The sugar is black; the complementary surface of the receptor is pale blue. A ghostly image of serotonin indicates its position in the redesigned binding pocket.** b, **An intermediate step. Amino acids in the complementary surface have been computationally truncated to alanines (red), leaving a large cavity into which sterically allowed serotonin molecules are docked. These molecules are confined to a convex polygon (yellow), restricting them to roughly the space occupied by arabinose.** c, **The serotonin receptor. The automated design algorithm has identified an orientation of serotonin that has a complementary surface (gold). This was predicted (and shown experimentally) to convert the wild-type protein into a serotonin receptor. The original arabinose is shown as a ghost image. Ligand atoms: carbons, black; nitrogens, green; oxygens, red. Dashed green lines represent hydrogen bonds. (Figure supplied by H. W. Hellinga.)**

combinatorial problem to between 10^{53} and 10^{76} permutations.

Finding productive combinations in such an astronomical sea of possibilities requires powerful computational algorithms[5]. Looger *et al.* used an enhanced version of 'dead-end elimination'[6], a tree-pruning method that considers two-body interactions to eliminate features that cannot belong to the global energy minimum. This strategy has previously been used to great advantage in the automated design of proteins, to determine amino-acid sequences that fold into well-defined three-dimensional structures[7]. Of course, the success of the computed sequence depends on how the energy (or 'fitness') of the interacting groups is calculated. Looger and colleagues' calculations were relatively simple, including terms representing hydrogen bonds, van der Waals interactions, electrostatic interactions and atomic solvation.

Seventeen of the virtually designed receptors were thereby selected for experimental testing. To carry out these experiments, the authors first mutated the real-life PBPs as specified by the computational algorithm. Then, in one set of studies, they modified the mutant PBPs with a dye whose fluorescence-emission intensity changed when the proteins bound the correct ligand. Fluorescence measurements showed that the redesigned receptors bound their new ligands specifically and with affinities ranging from relatively low values to values as high as those typically observed in antibody–ligand complexes. In further experiments, the authors inserted the mutant PBPs into *E. coli*, linking the receptors through a synthetic signalling pathway to the production of a 'reporter' enzyme. These techniques provide the possibility of experimentally enhancing the binding still further, by selection or screening.

Finally, the authors returned to the computer and calibrated the relative contribution (weight) of each term in the energy calculation against the experimental data. To do this, they used a quantitative structure–activity relationship (QSAR) that constructs a linear relationship between the observed affinity and the calculated properties of the designs. In the future, this approach should allow more accurate design of high-affinity binders.

There have been previous successful attempts to design proteins that bind specific peptide ligands. For instance, we computationally designed a protein that bound a specific region (peptide) of the calcium-activated enzyme calcineurin[8]. We did so by introducing the sequence of the peptide onto the crystal structure of a newly designed bundle of three α-helices (a common structural unit of proteins), changing the sequence of one helix, and then moulding the sequence of the remaining two-helix bundle to the shape and electrostatic profile of the peptide. Similarly, computer-aided design was used to change the peptide-binding specificity of a PDZ domain — a protein–protein-interaction motif — by first changing the sequence of the bound peptide and then selecting for mutations in the PDZ domain that would accommodate these changes[9].

So what makes Looger and colleagues' work different? First, in our earlier study[8] the backbone of the ligand was held fixed relative to that of the receptor protein. This fixed geometry led to underprediction of the number of possible binding sequences, and severely limited the method to the design of proteins that bind close analogues of the starting ligand. Second, whereas previous studies considered side chains only in their canonical ideal conformations, or in a few variations on these arrangements, Looger *et al.* used a more fine-grained sampling of dihedral angles. This approach allowed optimization of hydrogen bonds to the polar groups in the target ligand. Indeed, the highest-affinity ligands came out of the most fine-grained sampling. Finally, Looger *et al.*'s calculations required that all possible hydrogen bonds be satisfied, ensuring that the protein bound specifically to the target.

The authors suggest a variety of possible applications for their methods — for instance, following further development, the TNT receptor might be useful in detecting landmines, and the serotonin receptor in clinical diagnostics.

The approach might also have potential for the design of catalytic proteins, for applications in, for instance, the chemical industry or diagnostics. When an enzyme catalyses a particular reaction, its substrate normally passes through a transition state. Antibodies that bind analogues of the transition state can speed up the reaction, with good selectivity[10] — but the rate enhancements are generally much lower than those seen with the enzyme. A possible explanation for this is that the transition-state analogues are less than perfect mimics of the true transition state. Greater rate accelerations might be achieved by designing proteins that bind a virtual transition state that is an accurate mimic of the true transition state, and whose geometry and charge distribution can be estimated using quantum-mechanical calculations. A related approach has been used to computationally design an enzyme from an inert protein. By targeting a high-energy covalent intermediate, Bolon and Mayo[11] designed a protein that catalysed the hydrolysis of 4-nitrophenyl esters (reviewed in ref. 12). Although the rate enhancements were modest, the protein showed enzyme-like saturation kinetics, illustrating the promise of the method. The approach of Looger *et al.*, which does not require the formation of a covalent intermediate, should provide a complementary means of designing catalysts. ■

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