are not components of the dystrophin–glycoprotein complex, it was reasonable to conclude that other mechanisms could generate some of the features of muscular dystrophies.

The amino-acid sequence of dysferlin is 28% identical to that of Fer-1 protein found in the nematode worm *Caenorhabditis elegans*. In *C. elegans*, Fer-1 is proposed to mediate vesicle fusion to the plasma membrane of one cell type[5]. It is part of a growing family of 'ferlins', which include myoferlin and otoferlin, and is characterized by having six C2 domains (although only the first of them confers a calcium-dependent, phospholipid-binding ability). One LGMD2B patient has been found to have a point mutation in this C2 domain, reducing the domain's phospholipid-binding ability[6]. Myoferlin[7] is also a sarcolemma protein, and its activity is increased in mice — *mdx* mice — that lack dystrophin, while otoferlin[8] is associated with vesicle fusion in the inner ear.

Given this background, Bansal *et al.* reasoned that dysferlin could well be involved in calcium-dependent membrane repair, and they set out to test this hypothesis. To do this, they exposed isolated muscle fibres from normal — wild-type — mice to laser-induced membrane damage. Vesicles that stained positive for dysferlin accumulated beneath the sites of injury. To see if these vesicles could participate in membrane repair, Bansal *et al.* isolated muscle fibres from wild-type, *mdx* and dysferlin-null mice, exposed them to laser-induced membrane damage, and then timed how quickly their sarcolemmas resealed themselves in the presence and absence of external calcium. In both wild-type and *mdx* mice, sarcolemmas were resealed within a minute of being damaged, but only when calcium was present. The membranes of muscle fibres in dysferlin-null mice failed to reseal in any circumstances, confirming the role of dysferlin in membrane repair. So the damage seen in muscular dystrophies can indeed arise from a mechanism that is not associated with the dystrophin–glycoprotein complex.

Another group has reported[9] that vesicles containing dysferlin undergo exocytosis in 57% of muscle fibres examined from patients with defective dysferlin, suggesting that dysferlin-containing vesicles may be part of an intracellular transport pathway. If myoferlin or otoferlin (or both) are also present in these vesicles, it seems likely that other ferlin family members function in membrane repair. If so, defects in proteins other than dysferlin would be implicated in producing certain hallmarks of muscular dystrophy by preventing efficient wound-healing.

Interestingly, dysferlin has also been reported[10,11] to associate with the calcium-dependent protease calpain 3 and the sarcolemma protein caveolin-3. This is significant because defects in both of these proteins cause a type of muscular dystrophy, although neither protein belongs to the signalling routes implicated in the membrane repair process discussed here. Instead, modulation of calpain activity contributes to muscular dystrophies by disrupting cell-regulatory mechanisms, whereas caveolin-3 organizes lipid and protein constituents in the plasma membrane as part of a vesicular transport mechanism. Evidently, there may be yet more pathways to be discovered that, when disrupted in some way, ultimately lead to sarcolemma fragility. ■

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Complex fluids

Spread the word about nanofluids

Manoj K. Chaudhury

Liquid spreads and wets a surface, but wetting behaviour changes if the liquid contains nanoparticles. New experiments point to an explanation and a way to create effective detergents for cleaning oil from a surface.

The wetting of solid surfaces by liquids is usually described in terms of the surface and interfacial tensions at the 'three-phase contact line' — where solid surface, liquid and the air above meet. But this simple situation changes drastically when the liquid is a dispersion of nanoparticles. On page 156 of this issue, Darsh Wasan and Alex Nikolov[1] relate experiments and theoretical modelling that explain why this is. Ordering of the nanoparticles, they say, creates extra, osmotic pressure — an effect that could be exploited in the detergents used to clean up after oil spills.

The surface of a liquid is unique in the sense that it can bend inwards or outwards, maintaining a capillary pressure within a liquid drop that is lower or higher than the surrounding pressure. Excess pressure (known as the disjoining pressure) can also

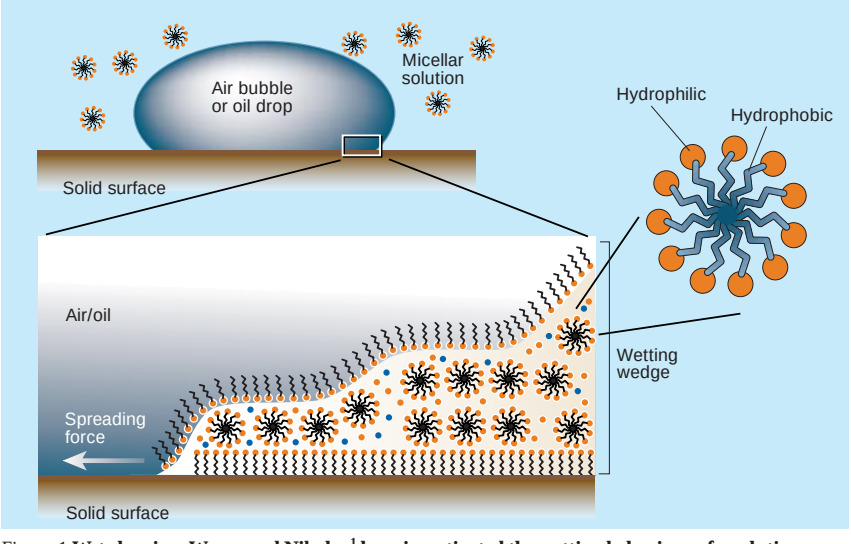

Figure 1 **Wet cleaning. Wasan and Nikolov[1] have investigated the wetting behaviour of a solution of nanoparticles — 5-nm-diameter micelles with a hydrophilic surface and hydrophobic core. By introducing air bubbles or oil drops on a glass surface in the micellar solution, they observed the 'wetting wedge' of solution in the confined space between the drop or bubble and the solid surface. It seems that the micelles organize in the wetting wedge wherever the wedge thickness matches an integral multiple of micelle diameters. This induces an osmotic component in the excess pressure directed towards the wedge from the bulk solution. The excess pressure drives further spreading of the solution and can be large enough to separate an oil drop from the solid surface.**

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 dewet 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