

## BIOCATALYSIS

# Unmasked by stretching

The biocatalytic activity of enzyme-loaded responsive layer-by-layer films can be switched on and off by simple mechanical stretching. Soft materials could thus be used to trigger biochemical reactions under mechanical action, with potential therapeutic applications.

Eugenia Kharlampieva and Vladimir V. Tsukruk

Can we create a universal responsive material with dramatically variable surface properties that can be switched by simple triggers on demand? This question has puzzled scientists ever since organized materials with molecular-level control of morphology were introduced. A variety of designs have been proposed and tested, including light-controlled Langmuir monolayers, pH-responsive polymer brushes, photoresponsive layer-by-layer films, or thermally triggered block-copolymers<sup>1</sup>. On page 731 of this issue, Voegel and co-workers<sup>2</sup> demonstrate a new approach for creating mechanically responsive materials based on layer-by-layer assembly. In contrast to conventional triggers applied to multilayer films, Voegel *et al.* show that they can catalyse an enzymatic reaction on demand by simply stretching the film. The responsive behaviour of the engineered films resembles the natural mechanism of uncovering cryptic sites in proteins that are buried inside the native folds, but which become exposed under mechanical forces. The force-regulated cryptic sites represent an effective system to convert a mechanical force into a bioactivated signalling pathway.

From the prospective of their potential responsive behaviour, layer-by-layer films assembled by an alternating deposition of oppositely charged polymers/particles onto a substrate are promising materials owing to their capability to coat substrates of virtually any shape and chemistry (even metal nanoparticles or living cells); and to precisely control the thickness and

layer composition at the nanoscale<sup>3,4</sup>. Moreover, their properties can be tuned by the incorporation of active compounds, such as biological materials or inorganic nanoparticles, nanosheets and nanowires<sup>5</sup>. Multilayer assemblies are not only attractive to academic researchers, but can also be used for devices and coatings for demanding applications such as corrosion protection, optical coatings, fuel cells, as antifogging and antireflection coatings, and biomedical implants<sup>3,6,7</sup>.

The functionality and properties of the multilayer materials can easily be manipulated by simply changing the environmental conditions. Traditional external stimuli, such as temperature, salt concentration and acidity of solutions can be further expanded into light, magnetic or electrical triggers. For example, layer-by-layer films become permeable, porous, or break apart when they encounter a particular level of acidity or a certain concentration of metal ions<sup>8</sup>. The unique properties of the film can be used to engineer microcontainers with a triggered response for drug-delivery applications, when incorporated cargo is released on demand, and as nanoreactors for growing nanoparticles<sup>9</sup>.

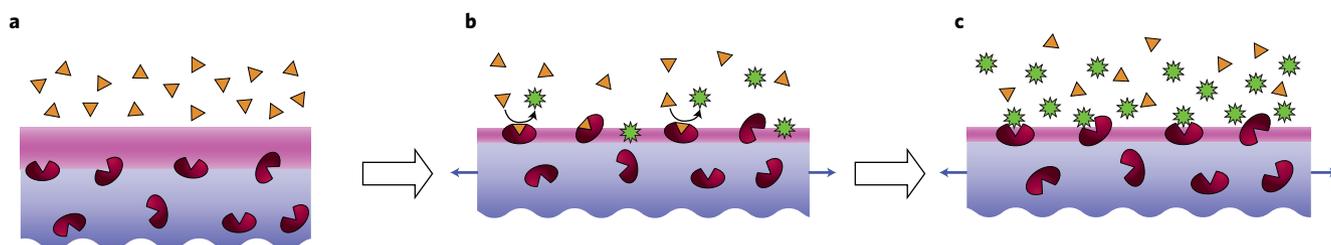
An important missing piece of the puzzle is responsive materials that readily react to mechanical stress. Very few examples have demonstrated mechanically tunable surface properties, such as one suggested for elastomeric materials<sup>10</sup>. Such mechano-responsive materials and molecules are, however, well-known in nature. The most prominent example is

of proteins, whereby during the process of mechano-transduction, mechanical deformation of the molecule reveals active sites that are normally buried within the protein, enabling mediation of chemical activity or membrane transport<sup>11</sup>.

To accomplish their goal, Voegel and colleagues combine two types of multilayer: on the bottom, a loosely bound but thick set of layers, capped with a thin, strongly bound multilayer. The weakly bound thick underlayer is used as a reservoir for enzyme molecules. The loose connections between the layers allow the enzymes that are confined in these reservoirs to diffuse through the whole structure. This bottom layer filled with enzyme is capped with a second film, strongly bound and dense, to keep the enzymes trapped and to prevent diffusion to the solution above. More importantly, this molecular barrier is designed to be elastic to allow significant stretching of the whole system.

The authors exploit the enzymatically catalysed hydrolysis of a molecule, fluorescein diphosphate (FDP), to study the reactivity of the layers. The remarkable feature of FDP is its transformation on hydrolysis: FDP is converted into fluorescein — a fluorescent molecule — and phosphate ions, allowing convenient visualization of the bioactivation process (Fig. 1).

In the non-deformed state the hydrolysis of trapped FDP is not catalysed, so the film is in an 'off' state. However, mechanical stretching above a certain level turns biocatalysis 'on' by making the barrier thinner, unmasking the enzymes



**Figure 1** | Schematic of the mechanically sensitive biocatalytic material. The designed film is a reservoir loaded with alkaline phosphatase enzymes (red shapes) and capped with a barrier. **a**, In the unstretched state, and in the presence of the FDP (yellow triangles), catalysis is switched off. **b,c**, When the film is stretched enzymes are exposed, the biocatalysis is switched on, and FDP molecules are hydrolysed into fluorescein molecules (green stars).

and making them accessible for catalysis (Fig. 1b,c). The hydrolysis reaction can be followed by the emerging fluorescence of the hydrolysed FDP.

The process of masking and unmasking the enzymes is reversible, and thus bioactivation can be turned on or off on demand by applying more or less mechanical strain. When the film is no longer stretched, the enzymes are again masked by the dense polymer barrier and the catalysis is switched off.

The researchers identified two important requirements needed for successful mechanically tunable enzymatic reactions. First, the barrier should be thick enough to prevent enzyme diffusion through the unstretched barrier, but at the same time it should be relatively thin to unmask the enzyme on stretching. Second, the

critical stretching degree required for the biocatalytic activation was found to be 70%, a significant level of deformation.

The work of Voegel and co-workers shows that the design of 'cryptic-like' surfaces capable of reversibly inducing biochemical reactions by applying a mechanical stress is feasible with layer-by-layer materials. Manipulating the construct design, for example, by varying the barrier thickness and reservoir parameters, is a way to tune the amplitude of the biocatalytic activity triggered by mechanical stimuli. The work has great potential in the design of responsive bioengineered materials aimed at triggering and modulating chemical and biochemical reactions by mechanical action, with applications ranging from smart microfluidic devices to mechanically controlled biopatches. □

Eugenia Kharlampieva and Vladimir V. Tsukruk are at the School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332, USA.  
e-mail: vladimir@mse.gatech.edu

#### References

1. Luzinov, I., Minko, S. & Tsukruk, V. V. *Prog. Polym. Sci.* **29**, 635–698 (2004).
2. Mertz, D. *et al. Nature Mater.* **8**, 731–735 (2009).
3. Decher, G. & Schlenoff, J. B. (eds) *Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials* (Wiley-VCH, 2003).
4. Jiang, C. & Tsukruk, V. V. *Adv. Mater.* **18**, 829–840 (2006).
5. Srivastava, S. & Kotov, N. *Acc. Chem. Res.* **41**, 1831–1841 (2008).
6. Hiller, J., Mendelsohn, J. D. & Rubner, M. F. *Nature Mater.* **1**, 59–63 (2002).
7. Lvov, Y. & Caruso, F. *Anal. Chem.* **73**, 4212–4217 (2001).
8. Kharlampieva, E. & Sukhishvili, S. J. *Macromol. Sci.-Polym. Rev.* **46**, 377–395 (2006).
9. Sukhorukov, G. B. & Möhwald, H. *Trends Biotechnol.* **25**, 93–98 (2007).
10. Genzer, J. & Efimenko, K. *Science* **290**, 2130–2133 (2000).
11. Betanzos, M., Chiang, C. S., Guy, H. R. & Sukharev, S. *Nature Struct. Biol.* **9**, 704–710 (2002).

## RELAXING THE VACUUM

There are few topics in physics more prone to misrepresentation than the Casimir force. In popular discourse, the term is commonly preceded by 'ghostly', as though there is something barely credible about the manifestation of an attractive interaction between two surfaces separated by a vacuum. Interpretations in terms of virtual particles or suppressed quantum fluctuations of the electromagnetic field only encourage that view. But regarded as the familiar dispersion force resulting from induced dipoles, 'slowed down' by the finite speed of photons, the Casimir force becomes altogether more prosaic.

All the same, proposals to alter its influence — to engineer it — have about them something of the marvellous, as though the inescapable exigencies of nature are somehow being cheated. This possibility, however, was already implicit in Evgeny Lifshitz's recasting of the Casimir force in 1956, when he worked out the theory for real materials with finite dielectric permittivity (that's to say, finite conductivity). It's easy to see from Lifshitz's theory that, for certain choices of plate materials and media separating them, the Casimir force can actually be made repulsive. All the same, it wasn't until earlier this year that the right combination of materials — silica, gold and an

organic liquid — was found<sup>1</sup>. (It's often overlooked that a classical analogue of this repulsive force, due to density fluctuations of a fluid at its critical point between two surfaces, was seen some time ago in superfluid helium<sup>2</sup>.)

This raises the prospect of 'quantum levitation' and of ultralow friction and contactless bearings for micro- and nanoelectromechanical systems (MEMS and NEMS, respectively). But the reality is trickier. The choice of materials, for example, is commonly dictated by other engineering considerations. Transparent dielectric surfaces such as silica will in themselves reduce the Casimir attraction relative to reflective metals, even if they don't alter its sign. But they also have a tendency to accumulate surface charges in air, which, on non-conductive media, cannot be dissipated and create a strong electrostatic attraction. A thin film of noble metal such as gold will allay that issue, but at the expense of constraining the dielectric function and leaving little scope for tuning the Casimir force.

Davide Iannuzzi and colleagues at the University of Amsterdam have now shown that it is possible to combine the best of both worlds<sup>3</sup>. Conductive transparent metal oxides such as indium tin oxide (ITO), indispensable for semiconductor display technology, offer amenable dielectric properties



PHILIP BALL

while dispersing surface charges in air. The researchers have used a customized atomic-force microscope to measure the force between a gold-coated polystyrene microbead and a flat surface coated with gold or ITO. In both cases, the Casimir force clearly dominates over any residual Coulombic force in ambient conditions for separations down to about 60 nm. But for ITO the attractive force is about a factor of two smaller. This, they say, should leave plenty of scope for tailoring the interaction in MEMS/NEMS applications. It's an intriguing example of how the right choice of materials can alter the basic physics. □

#### References

1. Munday, J. N., Capasso, F. & Parsegian, V. A. *Nature* **457**, 170–173 (2009).
2. Garcia, R. & Chan, M. H. W. *Phys. Rev. Lett.* **88**, 086101 (2002).
3. de Man, S., Heeck, K., Wijngaarden, R. J. & Iannuzzi, D. *Phys. Rev. Lett.* **103**, 040402 (2009).