The use of the nanoparticle as a delivery vehicle, demonstrated by Soike and co-workers, in effect eliminates these issues; the nanoparticle surface functionality can be manipulated independently of the molecule that it contains. As a rigid and self-contained structure it can retain its integrity, and its degradation behaviour can be manipulated by the choice of degradable polymer used to encapsulate the cargo. By removing the aforementioned constraints, one can imagine integrating a very broad range of drug systems that release at tunable but independent rates. The alternating assembly of charged microparticles using electrostatic assembly was introduced by Iler[9], and has since been extended to nanoparticles[10]. Soike *et al.* now show that by using a nanoparticle loaded with cargo, the out-diffusion of the cargo from the assembled nanoparticle film is influenced by its position within the film layers: the outermost layers release cargo at a higher flux than layers in the middle or at the bottom. This discovery might be used to tune the relative release rates of different drugs from different parts of a surface. This capability would allow greater control of an assortment of therapeutics for vascular stents (artificial tubes designed to maintain flow in blood vessels), hip or joint implants, and a wealth of other implant devices for which, for example, there is a desire to elute one drug fairly rapidly, and a second drug at a slower pace.

Another interesting finding of this work[2] is that the diffusion rates of drug molecules from the nanoparticle cores are also influenced by the drug diffusion path within the polyelectrolyte ionically crosslinked film network. As cargo is released and the nanoparticles begin to break down, several diffusion channels for molecules of various size open up, and thus co-delivery of two drugs can lead to synergistic release behaviour. In all cases, drug release rates were affected depending on the layer in which the drug nanoparticle was located, but the time point at which release begins did not change regardless of particle position or release of other drugs in the multilayer. This implies that diffusion through the LbL network is a key factor of release kinetics regardless of whether and how the principal components are encapsulated. The observation underlines the fact that the only way to acquire a staged release profile in these systems is to effectively block diffusion of molecules throughout the film, as demonstrated with the release of macromolecular model drugs in degradable multilayer films[11].

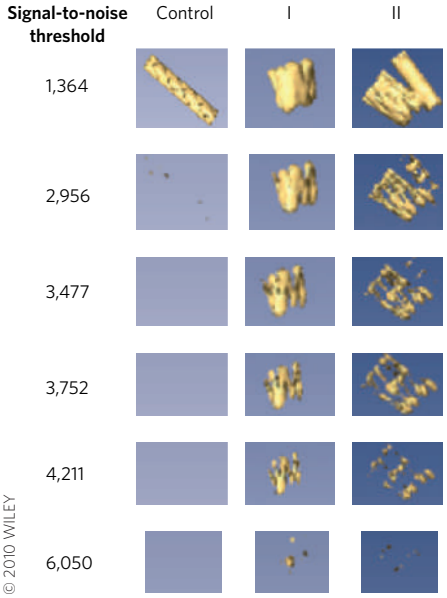


**Figure 2 |** Microcomputer-aided tomography contrast images of vascular stents coated with LbL films containing various numbers of layers of gold nanoparticles (samples I and II) compared with a control (no coating).

Finally, the team report a new and exciting advantage to using polyelectrolyte multilayer coatings for implant applications. They use the multilayer assembly approach to coat vascular stents with ultrathin films containing gold nanoparticles, barium sulphate and/or fluorescent-dye contrast agents, and demonstrate that with just a few layers, the contrast of the devices in microcomputer-aided tomography, and X-ray and fluorescence spectroscopy is significantly enhanced. The level of contrast obtained is notable — using the very thin and conformal surface coating, they were able to image the stents with signal-to-noise ratios that are challenging for typical biomedical applications. Moreover, the imaging that is achieved can be very readily tuned — they demonstrate that the imaging contrast of the stent can be varied systematically (Fig. 2), enabling a further means of identifying different implants. Dual modes of imaging can also be engaged using this approach, enabling a range of different means for visualizing implants in living tissue. The ability to combine this level of imaging with controlled drug release is an exciting development that presents a model case for the rationale of LbL assembly methods for a new generation of multifunctional responsive bioimplant device coatings. ❐

*Paula T. Hammond is a professor in the Department of Chemical Engineering and at the Institute for Soldier Nanotechnologies, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.*
*e-mail: hammond@mit.edu*

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PROTEIN CRYSTALS

# How the weak become strong

β-sheet stack structures in protein crystals are held together with some of nature's weakest links: hydrogen bonds. It turns out that the size of the crystal stack makes a difference to its strength — and smaller is better.

Christine Semmrich and Andreas R. Bausch

Proteins are the bricks of life — not only as molecular machines fulfilling many biochemical tasks and reactions but also as a class of material with an amazing diversity of properties. The requirements placed on proteins as materials in biology range from encapsulation, through simple chemical and mechanical protection by withstanding mechanical forces and cushioning of sudden mechanical impacts, to roles in more active tasks such as capturing prey or camouflage. These different applications pose often contradictory requirements to the material design. Whereas classical

man-made materials can use a wide variety of organic and inorganic substances and tailor and combine materials for special purposes, nature mainly relies on weak interactions and a few basic sets of building blocks.

A strategy often used in nature is nanostructuring at hierarchical levels to perform different functions, as beautifully seen in the most prominent examples such as bone, wood or spider silk[1]; we are just beginning to understand how this nanostructuring works. In this vein, writing in *Nature Materials*, Markus Buehler and colleagues use molecular dynamics simulations to understand the properties of protein crystals; in particular they study the way the crystals are deformed under testing as a function of their size[2]. Under shear deformation, weak hydrogen bonds, such as those found in the 'β-sheet stacks' in spider silk that they study, can surprisingly withstand the highest forces. The results therefore provide a wealth of possible new insights into materials design made by nature and pave the way for further rational design approaches for many applications, where versatility of properties and biocompatibility is needed.

The unique mechanical properties of spider silk rely on the existence of a composite structure of protein nanocrystals with the amino acids aligned in sheets — β-sheet stacks — in an amorphous matrix material. For this example and almost any biomaterial, it is still a significant challenge to unravel the link between the nanoscopic structural elements, their mechanical properties and the resulting macroscopic properties. In recent years, amazing insights into single-molecule mechanics has been achieved, yet the bridge to larger scales of ensembles of molecules is often lacking. Although for this level of complexity experimental methods such as microrehology[3] have been introduced, an understanding at the single-molecule level of complex biomaterials is rarely achieved[4]. The approach used by Buehler *et al.* harnesses the power of molecular dynamics simulations and connects them with classical engineering concepts such as continuum mechanics, which model solids as being homogeneous at all scales in contrast to approaches that include the influence of atomic structure at the nanoscale.

Molecular dynamics simulations of up to 25,000 atoms reveal that β-sheet nanocrystals confined to a few nanometres have high stiffness, strength and mechanical toughness. The β-sheet sequence that Buehler and colleagues used, which is found in *Bombyx mori* silk, is representative for many different β-sheet nanocrystals found throughout nature. The nanocrystals were studied under bending and 'pull out' geometry. Whereas the former resembles beam bending, which is familiar from daily life, in the latter the force is applied in the middle of the crystal. Both deformation modes are conducted on four differently sized β-sheet structures. Interestingly, the size dependence of the response is shown to deviate between classical beam theory and simulation results. The discrepancy can be accounted for by considering shear contributions in the deformation[5]. The smaller the crystal size, the more dominant shear contributions become, which is in contrast to systems where entropic contributions dominate the response[6].

The model here can be visualized by considering the structure of the protein stack (Fig. 1), which is held in place at top and bottom. Pulling out a strand from the middle of the stack has different effects depending on the stack size: in a large stack it tends to induce bending deformations on most of the other strands while the strand that is pulled experiences shear against its neighbours (Fig. 1, right panel); the 'stiffer' response in the short stack results in a reduced bending deformation and therefore a relative increase in the shear deformation (Fig. 1, left panel).

When the size of the crystals becomes as small as 2.5 nm, the bending deformation induces an equal shearing and stretching contribution from the hydrogen bonds. This has important consequences for the failure of the crystals, which is observed when the researchers model this pulling on individual protein strands of the crystal: at sizes smaller than 3 nm, maximum force and stiffness is required. The origin is simple: in small systems a stick–slip motion allows stepwise reformation of hydrogen bonds under shear stress as the molecules shift; the hydrogen bonds act cooperatively to dissipate the force. For bigger crystals a catastrophic failure is observed, because the increased bending deformation localizes the strain on a small number of hydrogen bonds and destroys the cooperative strength. A 'best size' of four hydrogen bonds for optimum strength using the stick–slip process is identified by the authors; this, therefore, defines the optimal-sized crystal. In recent experiments, an enhancement of the silk strength has indeed been achieved by decreasing the crystallite size to 2.1 nm (ref. 7).

The question remains how to best test such predictions and how to use them to build new materials. The simulation concentrates on single β-sheet stack geometries, for which single-molecule experiments should be feasible to test the predictions. Another main challenge will be to identify the directionality of force application to the β crystals when they are embedded in the amorphous matrix. For another protein structure based on β sheets, β barrels, it has already been shown that the directionality of force applications and the mode of deformation matters for the strength and stability of the protein structure[8,9]. The structurally much simpler β-sheet stack structures would seem from this work to still hide some surprises.

Experiments that allow all-scale observations on a well-defined model system of spider silk are still to be designed. To this end, the achievement of a bacterial expression system to produce these proteins in large quantities and their processing into fibres rich in β-sheet-stacks are extremely useful[10,11]. A combination of microfluidics, X-ray scattering techniques, biochemical analysis and molecular dynamics simulation

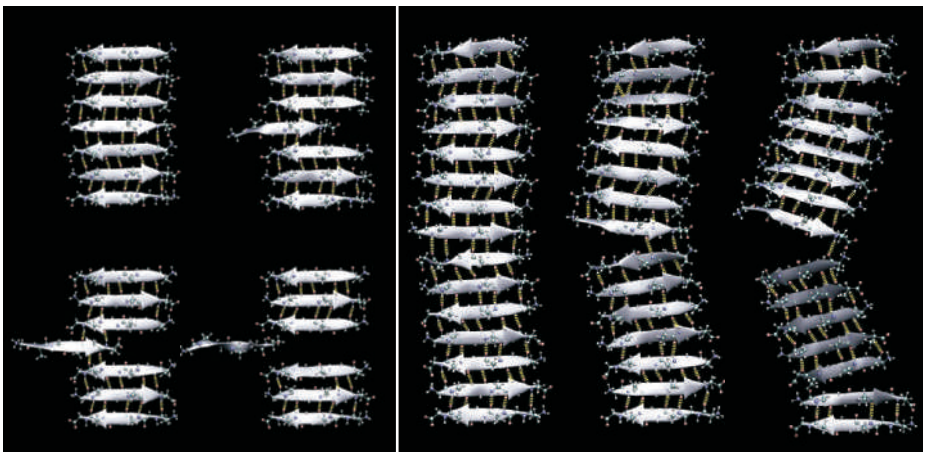

**Figure 1 |** Schematic showing how pulling a single protein strand from a short stack (left panel) induces relatively greater shear deformation than from a big stack (right panel), where bending dominates.

techniques will be a promising route to teach us the lessons needed to improve our engineering skills. ❐

*Christine Semmrich and Andreas R. Bausch are at the Lehrstuhl für Biophysik-E27, Technische Universität München, Garching, Germany.*
*e-mail: abausch@ph.tum.de*


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CRYSTALLINE MATERIALS

# Twin behaviour and size

For a Ti alloy single crystal, the stress required for deformation twinning increases dramatically as the size of the crystal decreases, until at submicrometre sizes, deformation occurs solely by dislocation motion.

Oliver Kraft

Danny de Vito would be happy to hear that for twin formation the paradigm 'smaller is stronger' still prevails. Twinning is a deformation process in crystals defined as the collective shearing of one portion of the crystal with respect to the rest. However, compared with plasticity based on dislocation glide, twinning has been scarcely studied in the deformation of metals. This is because twinning occurs (when slip by dislocation glide is suppressed) mostly for a few hexagonal-close-packed metals such as Ti, or at very low temperatures. Thus, twinning in metals has been considered of rather small technical importance. Furthermore, twinning is difficult to study as it occurs as a collective mechanism, and its origin and evolution in space and time remain puzzling. Writing in *Nature*, Yu *et al.*[1] now describe size effects on the deformation of small, micrometre- and submicrometre-sized Ti alloy crystals and, in doing so, provide new insights and questions relating to the deformation twinning process.

It has long been known that size effects have a prominent role in the deformation and strength of metals. In the 1950s, it was observed that grain size has a strong impact on the strength of pure metals and alloys. Also, it was demonstrated impressively that specimen size is important and that small whiskers, presumably dislocation-free, can have very high strength. Over the decades, such size effects have been observed for many samples and testing conditions, including metal thin films and, most recently, for small single crystals in microcompression tests[2–4]. Although not all details are understood, it is now recognized that these size effects relate to the confinement of the motion of dislocations and the availability of dislocations and dislocation sources[5]. Moreover, it has been shown that nanoscale growth twins can lead to high-strength materials because the twins confine dislocation motion[6]. This observation, however, is not to be confused with twinning as a deformation mechanism, which is discussed here.

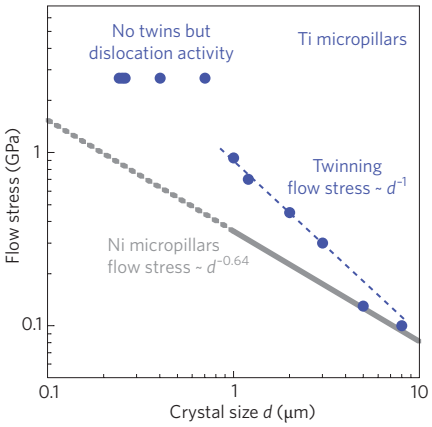


**Figure 1 |** Mechanical data as a function of the crystal size ($d$) for Ti alloy micropillars[1] compared with the typical strength values for Ni micropillars. The trend line is taken from ref. 3 in which data for the 1–10 μm region are presented. In Ti, the size effect for twinning is more pronounced with an exponent of –1 compared with –0.6 for many fcc metals[4,5].

Intuitively, one might imagine that there is no size effect for twinning of a small single crystal, which can freely deform. It could be argued that a smaller crystal twins more easily because a smaller volume needs to be sheared compared with a large crystal. However, Yu *et al.* show that the opposite is true: the smaller the crystal, the greater the stress required for deformation twinning (Fig. 1). For submicrometre-sized crystals, the flow stress is found to be constant and of the order of the theoretical strength of Ti. Using *in situ* transmission electron microscopy, it is well demonstrated that deformation is governed by dislocation motion. Compared with results from similar experiments on face-centred-cubic (fcc) metals (such as Ni, as shown in Fig. 1), it becomes clear that for twinning, the size effect is stronger than for dislocation plasticity. Therefore, the limit of theoretical strength, at which apparently homogeneous dislocation occurs, is reached for larger crystals.

The dramatic increase in flow stress with a decreasing size of single crystal is explained using a 'stimulated slip' model (Fig. 2). The model incorporates a pole, for example a screw dislocation, perpendicular to the slip plane, which acts as a promoter that is responsible for twin nucleation. The main idea is that in a large crystal, with enough dislocations, there is always a suitable promoter dislocation present; twinning occurs when the stress is high enough to drive a partial dislocation on the twinning plane that wraps around the pole, leading to a collective, stimulated-slip phenomenon. This implies that there is a certain critical crystal size, below which the initial dislocation density is not large enough to have a twinning promoter. Instead, in such a small crystal, dislocation plasticity with strain hardening occurs. The dislocation density increases until a twinning promoter is available and a twin is formed, corresponding to a large burst in the stress–strain curve. Indeed, for a critical crystal size and below, the crystal is only deformed by dislocations at a stress level near the theoretical strength of the