

A perfect marriage of materials

An absorbent gel has been integrated into the void space of a protein crystal to yield a remarkable self-healing material — it recovers its molecular order after several cycles of expansion and contraction. **SEE LETTER P.86**

FRANÇOIS BANEYX

In the best marriages, each partner brings attributes to the union that makes it both stronger and greater than the sum of its parts. On page 86, Zhang *et al.*¹ have achieved such a feat in the field of materials science. They have wed protein crystals to polymeric gels to create a self-healing hybrid material that can undergo multiple cycles of hyperexpansion and contraction without losing its ability to return to its original crystalline state. Such dynamic assemblies hold great promise for applications such as sensors, separators and actuators (devices that convert energy into movement), but have thus far been difficult to produce.

The ordered component of the wedded couple is ferritin, a protein that is ubiquitous in nature. Ferritin stores a mineralized form of iron called ferrihydrite in its hollow core, and releases iron(II) ions (Fe^{2+}) when they are needed by an organism. Each ferritin molecule is made up of 24 subunits that self-assemble to form a nearly spherical, cage-like structure.

Zhang and colleagues used a variant of ferritin, which had been engineered as previously reported², to enable amino-acid side

chains on two ferritin molecules to bind to calcium ions, forming bridges between the molecules. Each ferritin molecule connects in this way to 12 of its neighbours, enabling the protein to grow as cubic crystals that have long-range order extending to tens of micrometres.

The authors wanted to combine the crystal with a flexible component, and chose a polymeric hydrogel — a water-absorbing network of crosslinked polymers — as a suitable candidate. To bring the two components together, the authors first soaked the crystals in a solution of small hydrogel precursors; this allowed the precursors to diffuse throughout the water-filled void space of the ferritin lattice. They then polymerized the precursors by transferring the crystals to a solution of polymerization initiators that was highly saline to prevent undue crystal swelling by water infiltration. A continuous and elastic hydrogel network formed in less than two minutes in the void space, which was infiltrated soon after by the salt solution.

When the resulting composites were placed in water, they expanded isotropically (equally in all directions) to about 200% of their original size within minutes. The crystals did not lose

their faceted morphology, or release ferritin to the bulk medium, for at least 50 minutes. However, continued expansion eventually led to the loss of detectable edges of the crystals.

More strikingly, when Zhang and colleagues transferred partially expanded crystals to a concentrated solution of either sodium chloride or potassium chloride, and then incubated them in a concentrated solution of calcium chloride, the crystals contracted through dehydration, recovering both their original size and their lattice structure (Fig. 1). This expansion–contraction process could be repeated at least eight times without a noticeable change in ferritin ordering. In fact, the authors observed that X-ray structures of ferritin–hydrogel hybrids that had undergone a single expansion–contraction cycle were of higher resolution than could be achieved using conventionally prepared ferritin crystals that lacked the hydrogel. This suggests that the lattices of such hybrids are more precisely ordered than those of conventionally produced ferritin. Polymer infusion might thus be a useful approach to improve the quality of other protein structures, or to access alternative structural states of proteins.

Several additional observations are worth highlighting. First, Zhang *et al.* found that not all hydrogels are equally good at supporting the expansion–contraction process, because extensive electrostatic and hydrogen-bonding interactions are required between polymer side chains and ferritin molecules for order to be recovered. Second, the reversibility of the process is not perfect: calcium ions help the ferritin lattice to ‘snap back’ into place, both by screening undesirable electrostatic interactions and by bridging ferritin molecules, but the structure of about half of these bridges changes after an expansion–contraction cycle. Third, the polymer-infused crystals often cracked when subjected to abrupt expansion or contraction. However, these fractures were swiftly repaired because the polymeric network dynamically interacted with, and rearranged around, the ferritin molecules, even though the hydrogel was not engineered to have intrinsic self-healing properties³.

Finally, the authors showed that neither crystals loaded with ferrihydrite, nor crystals made using fluorescently labelled ferritin, were impaired in their ability to undergo isotropic expansion–contraction when infused with hydrogel. They also demonstrated that the expansion and contraction of such crystals was unaffected when a shell of ferritin was grown on them and the assembly was infused with hydrogel. By contrast, when a ferritin shell was grown on a core of fluorescently labelled ferritin crystals whose molecules had been chemically crosslinked to prevent expansion, and hydrogel was incorporated into the core–shell structure, the shell shattered on treatment of the crystals with water, owing to the generation of a lattice mismatch between the core and shell as the shell expanded.

The internal cavity of ferritin has been used

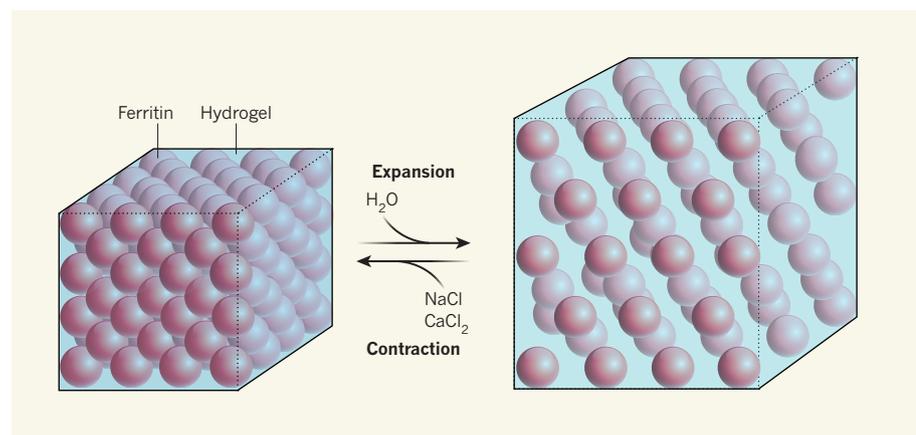


Figure 1 | Expansion and contraction of a protein–hydrogel composite. Zhang *et al.*¹ have prepared a composite material in which a hydrogel (a water-absorbing network of crosslinked polymers) fills the void spaces in the crystal lattice of the almost-spherical ferritin protein; the lattice is held together by calcium ions (not shown) that form bridges between ferritin molecules. When the crystals are soaked in water, they expand equally in all directions as the hydrogel hydrates. Most of the order in the lattice is lost, but some long-range order is retained in the diagonal direction shown (the (111) plane). When the expanded crystals are soaked in a solution of concentrated sodium chloride (which dehydrates the hydrogel) and then in a calcium chloride solution (which re-forms bridges between ferritin molecules), the crystals contract back to their original size, and lattice order is restored.

as a nanoscale reaction vessel in which to produce mineralized nanoparticles that have optical, catalytic or magnetic activity⁴, and packing nanoparticles into ordered assemblies leads to the emergence of collective behaviours that have found applications in opto-electronics, medicine and sensing⁵. Zhang and colleagues' findings might therefore provide a useful method for fine-tuning such collective phenomena by enabling controllable and reversible structural ordering in 3D nanoparticle arrays. Success will hinge on the identity and properties of the particles produced in the cavities of ferritin molecules, on the separation distances and ordering that the protein structure and crystal lattice impose on these nanoparticles, and on whether the kinetics of polymer expansion and contraction can be accelerated or otherwise precisely controlled.

Beyond ferritin, the use of other natural protein cages⁴ — and, more excitingly, of

synthetic protein cages designed from scratch^{6,7} — should provide the versatility needed to control nanoparticle separation distances and lattice systems. Furthermore, the development of creative polymer chemistry will aid efforts to modify the kinetics of expansion and contraction. ■

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NANOTECHNOLOGY

Molecular machines swap rings

A chemical system has been made in which two rings on an axle can switch places by allowing a smaller ring to slip through the cavity of a larger one. The advance opens up potential applications in molecular data storage.

STEVE GOLDUP

Many of the synthetic molecular machines¹ that have been developed in the past 40 years are based on rotaxanes: molecules in which a ring-shaped component encircles a linear axle that is terminated with large 'stoppers' to prevent the ring from slipping off. The threading of the axle through the ring limits the motion of the ring to shuttling back and forth along the axle. Such shuttling has been used in a range of molecular machines that includes switches², ratchets³, pumps⁴ and small-molecule synthesizers⁵. Rotaxanes in which more than one ring encircles the axle have also been made⁶, reminiscent of an abacus, but the rings have been unable to switch places. Writing in *Nature Chemistry*, Zhu *et al.*⁷ now report a system in which the rings can slip past one another, opening the way to new types of molecular machine.

To achieve a ring-through-ring shuttling motion, Zhu and colleagues assembled a rotaxane that contains two differently sized rings (Fig. 1). One has a circumference of 24 atoms, which is about as small as a ring can be in a rotaxane, whereas the other is almost twice as large at 42 atoms. Both rings form hydrogen bonds with nitrogen–hydrogen (N–H) units of

the axle, and this enabled the authors to probe the rings' movement using nuclear magnetic resonance (NMR) spectroscopy.

At room temperature, the authors observed two distinct N–H signals in the NMR spectrum of the rotaxane, because the signal for an N–H unit that is bonded to the small ring appears at a different frequency from that of an N–H unit bonded to the larger ring. This told Zhu and co-workers that the rings exchange places slowly at this temperature, or not at all. However, as the sample of rotaxane was heated, the signals began to broaden and then merged into a single peak. This finding confirmed that the rings change places quickly at elevated temperatures. The only way that this could have occurred is by the smaller ring passing through the larger one.

Zhu *et al.* determined that, at room temperature, the energy barrier that must be overcome for the rings to change places is about 52 kilojoules per mole of rotaxane, which corresponds to a shuttling rate of about 3,600 times per second. For comparison, in an analogous rotaxane that contains only the smaller ring, the ring hops between the N–H groups approximately 80,000 times per second, or roughly 20 times faster. On the basis of this comparison, the authors estimate that the ring-through-ring movement 'costs' about 12 kilojoules per



50 Years Ago

The British General Post Office is busy organizing a "telephone fortnight" in an attempt to silence the public criticism of its services. So far, the promotion has given everybody a chance to tell their favourite telephone stories, most of them unflattering to the GPO. The GPO's timing was inept; it is only two weeks since it announced increases in postal and telephone charges, and it might have been better to let the hubbub settle down before organizing the campaign ... In the next three years, the GPO is intending to spend £1,100 million on investment in telecommunications ... In the longer term, the GPO should be wondering how to increase the number of subscribers ... Britain still has very few telephones — 183 telephones per 1,000 of population.
From *Nature* 4 May 1968

100 Years Ago

Students of animal behaviour will find some interesting facts on the "drumming" of the ruffed grouse ... in *Forest and Stream* for April, illustrated by a series of remarkable photographs, probably the first of the kind which have ever been taken. The author, Mr. F. K. Vreeland, had the good fortune to watch at close range one of these birds while "displaying", and he is convinced that the strange drumming sound then made is produced by the use of the wings alone. This may indeed be the case, but we suspect that later investigations will show that these sounds are at least partly vocal ... The author is apparently so much of an "outdoor naturalist" that he has never read any of the voluminous literature on this theme of courtship displays. But in some respects this adds rather than detracts from the value of his observations, since his records are made without bias.
From *Nature* 2 May 1918