GOLD NANOPARTICLES

Grown in a crystal

Metal nanoparticles can be formed inside protein crystals, creating composite materials with potentially bifunctional catalytic properties.

Peter G. Vekilov

Composite materials are important in both nature and everyday life. Wood, for example, is the most common composite, bringing together the tensile strength of cellulose fibres with the compressive resistance of a lignin matrix. Alternatively, the first man-made composite was steel-reinforced concrete, which was invented in 1867 by the French gardener Joseph Monier. Writing in *Nature Nanotechnology*, Ian Robertson, Jian-Min Zuo, Yi Lu and colleagues now report on a new composite material — gold nanoparticles embedded in single crystals of the enzyme lysozyme[1].

The researchers — who are based at the University of Illinois at Urbana-Champaign, Chongqing University and Brookhaven National Laboratory — start with a solution of lysozyme and gold thiodiglycol chloride ($[AuS(CH_2CH_2OH)_2]Cl$), and add sodium chloride. After about a day, colourless, clear tetragonal lysozyme crystals form, as is typical for this protein in the presence of sodium chloride[2]. Moreover, X-ray crystallography reveals that the crystals have one gold atom (probably within a molecule of gold thiodiglycol chloride) bound to a histidine residue of the protein. The solution-filled voids between the lysozyme molecules — a common feature of all protein crystals[3] and known to occupy about 33% of the volume of the tetragonal lysozyme crystals[4] — probably contain further units of gold thiodiglycol chloride.

After a second day, the lysozyme crystals stop growing, probably as a result of the depletion of the protein concentration in the solution[5], and attain a pink colouration. The crystals remain soaked in the gold-containing solution, and as the size of the gold-containing species (~0.5 nm) is smaller than the width of the channels between protein molecules, the compound can diffuse freely in and out of the crystals.

After a fifth day, the pink colour of the crystals has evolved into a majestic imperial purple, which is then stable for up to 90 days. The pink colour is indicative of metallic gold nanoparticles, and their presence in the crystals and their crystalline nature is confirmed by optical spectroscopy, X-ray diffraction and transmission electron microscopy. Furthermore, the evolution of the size of the nanoparticles, their defect structures and their three-dimensional distribution in the lysozyme crystals are imaged with high-resolution electron microscopy and electron tomography.

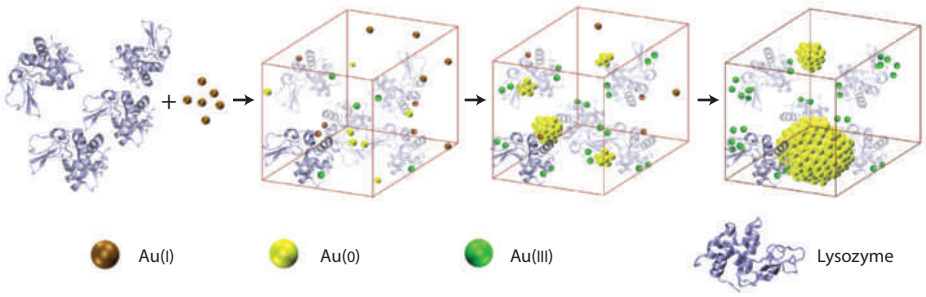

Figure 1 | Gold nanoparticles can be formed within lysozyme crystals by growing the crystals in the presence of a molecule containing Au(I). The Au(I)-containing molecules become trapped and can penetrate the intermolecular voids of the lysozyme crystal. Some of these molecules can also bind to the imidazole ring ($C_3H_4N_2$) of a histidine amino acid of the protein. Bound and free Au(I) molecules then undergo disproportionation: the gold is simultaneously oxidized and reduced, forming two species — Au(III) and Au(0). The Au(0) assembles into gold nanoparticles, while the Au(III) rebinds to other sites in the lysozyme. Over time, more Au(I) reacts to form Au(0) and the nanoparticles grow, creating particles as large as ~20 nm within the protein single crystals.

These beautiful observations of nanoparticle growth are augmented by a careful study of the underlying mechanisms (Fig. 1). A simple test using commercial gold nanoparticles demonstrates that the lysozyme crystals serve as a matrix for the growth of the nanoparticle and not vice versa: that is, the gold nanoparticles do not facilitate the heterogeneous nucleation of lysozyme crystals around themselves. Furthermore, the integrity of the protein crystals is preserved throughout the nanoparticle synthesis and this allows the process to be monitored with X-ray crystallography. The results indicate that gold from both the free thiodiglycol chloride molecules in the intermolecular voids and those attached to the histidine residues undergoes disproportionation, a reaction in which the gold is both oxidized and reduced, forming trivalent gold and metallic gold. The trivalent gold associates to and eventually saturates eight sites on the protein molecules in the crystal, while the metallic gold aggregates into gold nanoparticles. The diameter of the nanoparticles reaches about 16 nm in around 10 days and then barely increases in a further 80 days. The gold nanoparticles are relatively sparse (about 100 μm^{-3}) and randomly distributed throughout the lysozyme crystal matrix, indicating that the initial stages of their formation are controlled by nucleation. Lu and colleagues also show that the synthesis of the nanoparticles can be accelerated and slowed down by using mercury ions and an organic phosphine derivative, respectively.

Numerous questions about the mechanism remain. For example, in the absence of protein, a solution of gold thiodiglycol chloride spontaneously disproportionates within four hours. This suggests that, as well as providing a crystalline matrix for the formation of the nanoparticles, lysozyme might also inhibit the disproportionation in solution. Furthermore, each 16-nm gold particle should displace or engulf about 100 lysozyme molecules. These processes would endanger either the structure of the protein crystal or that of the gold nanoparticles,

respectively. But which of these happens to the lysozyme molecules? Also, how are the final size and number of gold nanoparticles controlled? Does their growth stop when the gold precursor molecules in the solution are depleted, or when the sites suitable for attachment of the side product of the nanoparticle synthesis (trivalent gold) are saturated? The study does, however, shed light on other issues, such as the recently discovered biomineralization of gold[6] and the molecular mechanisms of protection that some bacteria use against toxic gold and other metal ions[7].

The potential applications of the protein–gold composite are not immediately apparent. In contrast to wood and reinforced concrete, the hybrid is unlikely to find applications for its mechanical properties. However, new catalysts could perhaps be synthesized by embedding in enzyme crystals metal nanoparticles, platinum or palladium for example, with specific catalytic properties. Such a catalyst could convert part of a molecule to its enzymatically coupled product and reduce or oxidize another part of the reacting molecule to a desired functional group. The hybrid catalyst would allow both catalytic processes to occur as one kinetic step, which would offer at least two significant advantages. First, the slow transport of the reactants toward the catalyst and of the products away from the catalyst would merge into one step, and second, higher selectivity could potentially be achieved.

Of course, a number of problems would need to be solved before this catalyst could be created. In particular, metal catalysts operate at elevated temperature and pressure, whereas most proteins quickly denature at temperatures above 60 °C, even at atmospheric pressure. Another puzzle is whether crystals of other proteins could serve as matrices for the synthesis of nanoparticles of gold or other metals. Nevertheless, the work of Lu and colleagues[1] is a striking demonstration of the synthesis of an unusual class of composite material and a compelling study of its underlying mechanism. ❐

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NANOMEDICINE

Nanotubes reduce stroke damage

Pre-treating rats with single-walled carbon nanotubes can protect neurons in the brain and enhance the recovery of motor functions after injury.

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Stroke accounts for around one-tenth of the 55 million deaths worldwide every year and leaves many more patients with life-changing disability such as loss of mobility[1]. Ischaemic stroke, which accounts for most of the cases, is a form of stroke caused by disturbed blood flow to the brain owing to blockage of an artery by a blood clot, known as a thrombus. Irreversible neurological damage occurs quickly, and treatment to re-open the blocked artery with 'clot-busting' thrombolytic drugs is effective only if given within 4.5 hours of the onset of symptoms. Although this reduces subsequent disability, it is logistically difficult to deliver treatment in such a narrow time window.

Over the past two decades, many promising treatments have been evaluated, but thrombolytic therapy is the only example with encouraging results. Numerous putative 'neuroprotectant' drugs have shown apparent beneficial effects in animal models but have failed in human studies, partly because of insufficient or poor pre-clinical studies[2,3]. Recent high-profile failures of promising new drugs to treat ischaemic stroke patients[4,5] highlight the need to improve the design and interpretation of pre-clinical studies[6,7]. The limited success of pharmacotherapy means that alternative approaches to stroke treatment are also of interest. Writing in *Nature Nanotechnology*, Sung Su Kim and colleagues at Chung Ang University, Sungkyunkwan University and Harvard Medical School[8] report that rats pre-treated with amine-modified single-walled carbon nanotubes had less tissue damage and had better motor function than untreated controls after a surgically induced stroke.

Kim and colleagues prepared amine-modified nanotubes by treating commercially available carboxylated nanotubes with plasma; this resulted in the formation of many positively charged amine groups on the nanotube surface. The brains of rats were pre-treated with amine-modified nanotubes one week before surgically inducing a stroke, and the neurological damage was measured by assessing how long it took for injured animals to fall off a rotating rod. Rats pre-treated with the modified nanotubes took longer to fall off the rod than untreated controls, indicating less neurological damage and better-preserved motor function. Treated animals were seen to have a smaller volume of injured brain tissue, a key goal in the development of new strategies to treat stroke (Fig. 1).

Stroke disrupts tissue architecture. After brain injury, a series of restorative processes are triggered, including anti-apoptotic functions to prevent further cell death and angiogenic functions to regenerate new blood vessels. Mechanistic studies revealed that rats pre-treated with nanotubes had reduced levels of neuronal apoptosis markers and lowered post-ischaemic inflammation and glial responses, suggesting that amine-modified nanotubes protected the tissues from injury by limiting cell death and harmful inflammation.

Levels of N-cadherin (an adhesion protein important for neural cell adhesion and survival) remained high in treated rats compared with untreated animals, suggesting that the nanotubes