HYBRID NANOMATERIALS

Not just a pretty flower

Combining copper(II) phosphate and proteins leads to the formation of hybrid nanostructures that are shaped like flowers and have enhanced catalytic activity and stability.

Jie Zeng and Younan Xia

Roses symbolize romance and elegance and, like other flowers, they have structures that span many length scales. In recent years, this nested structure — sepals, petals and the regions inside the flower that produce pollen and seeds (Fig. 1a) — has been reproduced on the nanoscale with purely inorganic materials such as metal oxides and hydroxides[1]. Now, writing in *Nature Nanotechnology*, Jun Ge, Jiandu Lei and Richard Zare[2] of Stanford University describe the first example of a hybrid nanoflower made of both organic materials (in the form of proteins) and inorganic materials (copper(II) phosphate). Moreover, when an enzyme is used as the protein component, the nanoflowers showed enhanced catalytic activity, stability and durability.

Compared with more compact structures such as solid spheres or polyhedra, a nanoflower has a much larger surface area, and thereby a much higher surface energy, for a given volume. And like other nanostructures with branched morphologies[3], nanoflowers have to be prepared using methods based on kinetic rather than thermodynamic control. The appearance of the final product depends on both the concentration and shape of the primary nanoparticles that are formed in the nucleation step. When the concentration of particles is relatively low, the aggregation process is governed by diffusion[4] and this results in aggregates with a fractal structure characterized by random branching. When the concentration of particles is relatively high, fast aggregation occurs. In this case, because of the large surface-to-volume ratio and high collision frequency, there is a strong driving force for the particles to aggregate into more compact structures rather than branch out.

Zare and co-workers serendipitously discovered that by adding copper(II) sulphate to phosphate-buffered saline that contained a protein (such as bovine serum albumin), porous flower-like structures with nanoscale features were formed. The protein had many roles in the synthesis of the nanoflowers: by complexing with copper(II) ions via its amine groups, it served as a nucleation site for the formation of primary nanoparticles of copper(II) phosphate; the protein also acted as a capping agent that helped the particles maintain a plate-like morphology (Fig. 1b). Furthermore, the protein bound individual nanoparticles together to form petals and in the process became naturally incorporated into the nanoflowers during synthesis. In the absence of proteins, large crystals rather than nanoflowers were obtained because copper(II) phosphate would nucleate and grow through atomic addition.

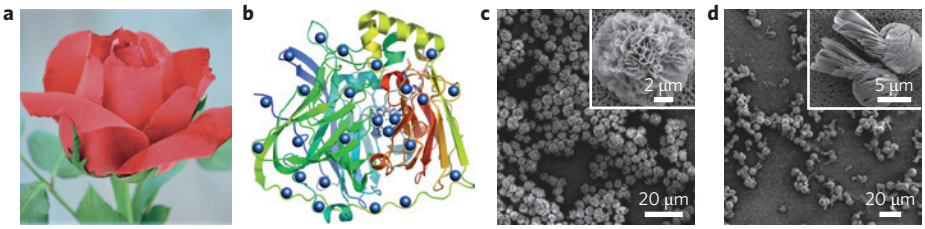

Figure 1 | Hybrid nanoflowers are formed by combining an inorganic and organic component. **a**, Photograph of a rose showing the hierarchical structure of the flower. **b**, A three-dimensional structure of laccase showing copper(II) ions (blue spheres) coordinated to amine groups of the protein (colourful strands represent the alpha helix and beta sheets of the protein). Proteins have an important role in the synthesis of hybrid nanoflowers. **c**,**d**, Scanning electron micrographs of nanoflowers made of bovine serum albumin and copper(II) phosphate[2]. Blooming flowers are formed at low concentrations of bovine serum albumin (0.02 mg ml^{-1}; **c**), whereas buds are formed at high concentrations (0.1 mg ml^{-1}; **d**). Panel **b** courtesy of Takeshi Sakurai.

For blooming nanoflowers to form, it is important to keep the protein concentration sufficiently low (for example, down to 0.02 mg ml^{-1}) to limit the number of primary particles of copper(II) phosphate that form in the nucleation step, and to ensure the particles would aggregate under diffusion control (Fig. 1c). High concentrations of proteins (for example, 0.1 mg ml^{-1}) results in high concentrations of primary particles which, in turn, reduces the influence of diffusion; this leads to the formation of nanoflowers with small buds (Fig. 1d).

By using an enzyme as the protein component, Zare and co-workers created hybrid nanoflowers with greatly enhanced catalytic performance. For example, in the case of laccase, the nanoflowers were 4.5 to 6.5 times more active at oxidizing catecholamine and syringaldazine than free laccase. Furthermore, the laccase-incorporated nanoflowers retained 95% of their initial activity after two months, whereas under the same conditions, free laccase lost 50% of its initial activity in ten days. Moreover, the nanoflowers could be repeatedly used at least five times without any drop in activity. Similar characteristics were also seen with other enzymes such as α-lactalbumin and carbonic anhydrase.

Compared with free enzymes and other immobilization systems based on nanogels[5] and silica particles[6], enzymes incorporated in the hybrid nanoflowers set a record on enzymatic activity, stability and durability. Such enhanced performance is probably a result of the large surface-to-volume ratio of the nanoflower, which helps reduce the mass-transfer limitation for a catalytic reaction. It is also possible that cooperative effects between the immobilized laccase molecules and their interactions with copper(II) ions in the nanoflower may boost the enzymatic activity.

Immobilizing enzymes on substrates is essential for biosensing and biocatalytic applications because this allows the enzymes to be easily recovered from the reaction mixture. The strategy of making hybrid nanoflowers as described by the Stanford

team can be readily extended to many other hybrid systems such as calcium phosphate and Ca^{2+}-assisted enzymes like α-amylase and calpain. The use of biocompatible inorganic materials such as hydroxyapatite will also enable the protein-incorporated nanostructures to be used for drug delivery and tissue engineering.

However, there are a number of issues that need to be addressed in future work. First, a better understanding of the interaction between the inorganic phase and protein is required. At present, it remains unclear how and at what density and orientation the protein molecules bind to the surface of an inorganic particle. Furthermore, it is difficult to determine where the immobilized protein molecules are located and whether their conformation is retained or altered during an immobilization process. Second, if the nucleation event of an inorganic phase in the presence of protein molecules at different concentrations can be described quantitatively, it is possible to develop more effective strategies for immobilizing proteins and for controlling the shape of the inorganic nanocrystals that form[7]. It is known that capping agents (such as ionic species, small molecules or macromolecules) are important in the nucleation, growth and shape evolution of nanocrystals. ❐

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NANOTOXICOLOGY

No signs of illness

Quantum dots that contain cadmium, selenium and zinc are not toxic to monkeys for periods of up to 90 days, but longer-term studies are needed to determine the ultimate fate of the heavy metals that accumulate in the organs.

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Semiconductor nanocrystals — also known as quantum dots — have significant potential to be used as fluorescent probes for medical imaging, image-guided surgery and drug delivery, but clinical applications have been delayed because some studies have shown that metals released from the degradation of quantum dots can kill cells that are grown in culture, as can reactive oxygen species generated by the transfer of energy from quantum dots to nearby oxygen molecules[1,2] (Table 1). There is, however, one rodent study that showed that cadmium selenide (CdSe) quantum dots capped with a zinc sulphide (ZnS) shell did not have any apparent toxic effects at a concentration suited for guiding tumour resection despite the breakdown of the quantum dots[3]. Although the results from the rodent study are difficult to extrapolate to humans, researchers from China, Singapore and the US now report in *Nature Nanotechnology* that quantum dots with a CdSe core, a CdS–ZnS shell and a phospholipid coating do not exhibit acute toxicity in rhesus monkeys[4], which is a primate model that closely resembles humans.

The team — led by Ling Ye of the Chinese PLA General Hospital in Beijing, Ken-Tye Yong of Nanyang Technological University in Singapore and Paras Prasad of the State University of New York in Buffalo — investigated the biodistribution and toxicity of 50-nm-diameter phospholipid-coated CdSe–CdS–ZnS quantum dots by intravenously injecting the particles into monkeys and measuring the concentrations of cadmium, selenium and zinc in the blood and various tissues of the animals at various timepoints using inductively coupled plasma mass spectroscopy. The quantum-dot clusters were cleared from the blood of the monkeys and distributed to various organs within six hours of intravenous injection. After 90 days, more than 90% of the cadmium from the injected quantum dots remained in the body with most of it accumulating in the kidneys, liver and spleen. The relative abundance of the injected elemental cadmium, zinc and selenium in each organ was different at the end of the study. These results suggest that these quantum dots slowly degrade *in vivo* and the free metal ions redistribute to various organs over time.

To evaluate acute toxicity, the team measured the number of white blood cells and the amount of different proteins in the serum. Levels of all the measured biomarkers — which are indicators of organ function and inflammation — were within normal physiological ranges, and the subjects did not display behavioural abnormality or weight loss. This suggests that these types of quantum dots were well tolerated by the animals. Histology of the tissues collected from four of the six treated animals at the end of the 90-day period did not show any major changes in the tissue structure; there were no signs of cell death or inflammatory response. All of these results suggest that the injected quantum dots did not cause any toxic damage to organs in which the particles accumulated.

This first set of non-human primate data serves to dampen some of the fears over the toxicity of quantum dots intended for applications in humans. However, much work remains to be done because current toxicological and pharmacokinetic profiles of a particular quantum dot formulation has offered little predictive power over the behaviour of another. And this has generally been the difficulty in assessing the safety of engineered nanomaterials and in interpreting published results across the literature. The term 'quantum dot' does not refer to a specific nanomaterial, but rather to a large body of semiconductor nanocrystals that vary in their geometry, composition and surface chemistry. These parameters determine the biological fate and toxicity of the engineered nanomaterial[5]. Furthermore, the *in vivo* behaviour of quantum dots also depends on their dose and routes of administration. Because there are currently no standardized metrics and experimental conditions to