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Biomimetic Crystallization of Sulfide Semiconductor Nanoparticles in Aqueous Solution

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In recent years, a new generation of nanomaterials has been revolutionizing the field of bioimaging due to their superior optical properties as compared to classical molecular probes.^[1] Among these, sulfide semiconductors such as CdS and CuS could be useful fluorescent labels due to their size-tunable emission in the visible range,^[2,3] while PbS and Ag₂S nanocrystals are promising probes for in vivo imaging and cancer therapy due to the absorption and emission in the infrared–near-infrared (IR–NIR) range.^[4,5] These cutting-edge biomedical applications require the syntheses of high-quality nanocrystals with controlled dimensions in aqueous solution. While several synthetic routes to silver sulfide semiconductor nanocrystals were reported, the use of organic capping agents was necessary for achieving high crystallinity and narrow size distribution of the resulting nanoparticles.^[6,7] For these nanoparticles, due to the poor solubility of the ligands in aqueous solutions, the reaction must be performed in the presence of organic solvents and therefore the resulting nanocrystals need to undergo tedious ligand-exchange procedures for bioimaging applications. Hence, it would be desirable to develop a green chemical approach to synthesize mono-disperse sulfide semiconductor nanocrystals in aqueous solution and in the absence of toxic ligands, for applications in living organisms. To achieve this goal, an important issue must be overcome: the reaction between S^{2−} and heavy metal ions is thermodynamically favored, as denoted by their low solubility-product constant (K_{sp}), and the fast growth kinetics of the crystals in aqueous solution with very low concentrated precursors induces the aggregation of a plethora of kinetics-controlled nuclei that leads to the least stable polymorph.^[7] As a result, shapeless particles with poor crystallinity are often obtained when the reaction is performed with water as the solvent.

Biomineralizing organisms such as mollusks may have the answer for this issue. Mollusks have evolved the capability of controlling the synthesis of calcium carbonate to render complex crystalline structures in a mild condition.^[8] For this crystallization process, the presence of both a nucleating protein sheet and soluble polyanionic proteins are crucial for controlling the orientation of crystal growth and polymorph phase.^[9] In vitro, calcium

carbonate could be biomimetically crystallized on patterned substrates, where mass transport of precursors toward the nucleation regions yielded a concentration gradient that controlled the rate of nucleation of the crystals.^[10,11] Since calcium carbonate of molluscan shell is also grown from precursors with low solubility in water, mimicking this system might enable the growth of monodisperse sulfide semiconductor nanoparticles in aqueous solution. Here, we apply these concepts learned from biological systems to the synthesis of sulfide semiconductors on an enzyme that acts as a nucleating template in mild conditions. To control the concentration gradient of the precursors around the nucleation point, we tune the hydrolysis of thiourea catalyzed by urease,^[12] an enzyme well known to have affinity for heavy metals,^[13] to produce S^{2-} ions in an adequate concentration for limiting the rate of nucleation of sulfide semiconductors. To demonstrate the proof-of-concept of this bioinspired synthetic route, we fabricate monodisperse core/shell enzyme/ Ag_2S particles in mild conditions. Silver sulfide has an ultralow K_{sp} ($7 \times 10^{-50} M^3$ at 25 °C) and therefore it is an excellent model system to examine the proposed mechanism of crystallization control of insoluble sulfide semiconductors in aqueous solution. To tune the catalytic activity of the enzyme and to control the concentration gradient of S^{2-} ions around the nucleation point, the enzyme nanoreactor is inhibited by carefully choosing the concentration of the heavy metal precursor, which is a well-known inhibitor of the enzyme.^[14] Since supersaturation of metal sulfide solutions is achieved with very low concentrations of precursors, the concentration of S^{2-} tuned by the enzyme activity of the nanoreactor is the critical factor for the crystallization of the semiconductor.

Figure 1 schematically summarizes the bioinspired crystallization of sulfide semiconductors with controlled morphology. To mimic the biological crystallization of calcium carbonate, the production of S^{2-} ions must be confined close to the surface of the nucleating protein, which works as a soft template for the reaction, and the concentration of S^{2-} ions on the enzyme surface needs to be optimized for Ag_2S crystal growth. Since the enzyme is a potent catalyst, careful inhibition is needed to spatially confine the S^{2-} ion production so that the super-saturation region created close to the surface of the nanoreactor can yield crystalline metal sulfides, as depicted in Figure 1a. In Figure 1b, when the enzyme is not inhibited, the gradient of S^{2-} ions from the surface of the enzyme to the bulk of the solution is higher, and therefore the supersaturation condition can be reached in a larger volume around the nanoreactor. While this condition should lead to the formation of larger particles, the crystallization process occurs far away from the enzyme surface and the protein cannot control the crystal growth as observed in biomineralizing organisms; hence large amorphous particles are likely produced when the enzyme is only slightly inhibited. By contrast, when the enzyme is fully inhibited it cannot generate the S^{2-} ion precursors. In this case, the enzyme cannot template Ag_2S particles and only heavily aggregated shapeless particles are expected to be generated after prolonged incubation time with the precursors (Figure 1c).

To demonstrate that the catalytic activity of urease can indeed be tuned to biomimetically crystallize metal sulfides via changing the heavy metal precursor concentration, first the enzyme was inhibited with Ag^+ solutions with various Ag^+ ion concentrations and the production of ammonia after addition of the substrate urea was monitored with a pH meter. In fact, the choice of Ag^+ ions as an inhibitor is a convenient one, since the Ag^+ ions can also be utilized as a precursor for Ag_2S growth on the enzyme. In Figure 2, the ammonia

production was slightly inhibited after addition of Ag^+ with the concentration of 10^{-13} M (squares in Figure 2), corresponding to the condition depicted in Figure 1a. A significant decrease of the activity was only observed when the concentration was increased to 10^{-7} M (open circles in Figure 2), corresponding to the condition summarized in Figure 1b. To fully inhibit the enzyme, the concentration needed to be raised to 10^{-4} M (triangles in Figure 2), corresponding to the condition shown in Figure 1c. These results demonstrate that the activity of the nanoreactor, and therefore the local concentration of ions, can be tuned by harnessing the catalysis on the enzyme via inhibition by metal ions. It should be noted that in all cases the concentration of Ag^+ is far above the solubility product of Ag_2S and therefore the supersaturation condition depends only on the concentration of S^{2-} anions on the nanoreactor.

In Figure 3, we explored the growth of crystalline Ag_2S when the enzyme nanoreactor is moderately inhibited by Ag^+ with the concentration of 10^{-7} M, as hypothesized in Figure 1a. In Figure 3a, transmission electron microscopy (TEM) images of the products revealed monodisperse nanoparticles consisting of an electrotransparent enzyme core and an electrodense Ag_2S shell. This core/shell nanostructure was further confirmed by high-resolution TEM (HR-TEM), as shown in Figure 3d. The quasi-spherical shape of the particles and the fairly narrow size distribution (Figure 3c) with a mean diameter of 35 ± 8 nm were consistent with the proposed growth model of Ag_2S templated by the enzyme. Moreover, in the TEM image, the diameter of the electro-transparent core is 13 ± 4 nm, which is comparable to the hydrodynamic radius of urease.^[15] The selected area electron diffraction (SAED, Figure 3b) pattern of these particles is consistent with the interplanar distances of the planes (211), (321), and (411) for the cubic phase of the argentite structure of Ag_2S , and HR-TEM images indicate that these particles are highly crystalline (Figure 3e). These results demonstrate that the concentration gradient of the precursors around the enzyme nanoreactors controls the nucleation via inhibition of the enzymatic activity, and this condition is crucial to obtain sulfide semiconductor nanoparticles with high crystallinity in aqueous solution.

To prove our hypothesis that the confinement of the S^{2-} ions close to the protein as a nucleation point is the critical factor for the crystallization of Ag_2S nanoparticles, the growth conditions with different ion distributions around the enzyme, as depicted in Figure 1b and c, were explored. In Figure 4a, the slightly inhibited enzyme, as determined in Figure 2, produced large and monodisperse particles with a mean diameter of 142 ± 16 nm (Figure 4a), and the absence of an electron-diffraction pattern (Figure 4a, inset) indicated that these particles were amorphous. This result is consistent with the growth scheme in Figure 1b, where the nucleation occurs uncontrollably far away from the enzyme surface, and the crystal growth is not regulated by the protein in this configuration. When the same experiment is performed with a high concentration of Ag^+ (10^{-4} M) to fully inhibit the enzyme activity (Figure 1c), as determined in Figure 2, no spherical nanoparticles were observed since urease could not produce a high-enough concentration of S^{2-} to grow the Ag_2S shell. Under this condition, only shapeless and heavily aggregated particles were obtained after prolonged reaction time, because excess Ag^+ ions dispersed in solution react with S^{2-} dissociated from thiourea without the concentration gradients (Figure 4b). This result, along with the absence of a defined electron-diffraction pattern from these particles

(Figure 4b, inset), indicates that the catalytic action of urease producing S^{2-} anions plays a central role for the synthesis of sulfide semiconductors in controlled shape and crystallinity.

In summary, we demonstrated the biomimetic crystal growth of Ag_2S nanoshells around an enzyme nanoreactor in aqueous solution. The mechanism underlying this bioinspired route is to tune the catalytic activity of the enzyme so that the ideal concentration of precursors around the nucleating protein induces the crystallization only on the enzyme surface. This procedure could be straightforwardly adapted for the synthesis of other relevant sulfide semiconductors, since the mechanism of enzyme inhibition is common for any heavy metal precursor.^[14] Moreover, this crystalline nanoparticle-growth method could be further expanded to other relevant semiconductor nanocrystals such as metal selenides and tellurides via the biomimetic route, provided that the concentration gradients around suitable nucleation points are controlled.

Experimental Section

Enzyme inhibition:

To demonstrate the mechanism of inhibition of urease by heavy metals, the enzyme (1 mg mL^{-1} in $NaNO_3$ (0.1 M)) was incubated with $AgNO_3$ to different final concentrations for 20 min. After diluting the enzyme solution (1:100 v:v) and adding the enzyme substrate urea (10 mM), the production of ammonia via the hydrolysis of urea was followed by measuring the pH with a pH meter. The enzyme activity was calculated from the linear part of the plots.

Synthesis of Ag_2S :

After the urease solution (1 mg mL^{-1} in $NaNO_3$ (0.1 M)) was incubated with thiourea (0.1 M) for 30 s, $AgNO_3$ was added to different final concentrations, as specified earlier and the mixture was incubated in the dark for 30 min. Then, a TEM grid was dipped in the solution for 30 min, washed with deionized water, and dried with the help of a filter paper. Mother solutions were filtrated with 0.2-mm-diameter polypropylene porous membranes. All glass materials used were acid cleaned. TEM images were taken with a Zeiss EM 902 TEM instrument operating at 80 kV. HR-TEM images and SAED patterns were obtained with a JEOL JEM-2100F instrument operating at 200 kV. Size-distribution plots and mean-diameter values were obtained by measuring 30 particles on the grid. Variability is expressed as the standard deviation.

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Figure 1.

Scheme of the bioinspired crystallization of sulfide semiconductors with controlled morphology. a) The moderately inhibited enzyme distributes the production of S^{2-} ions close to the surface of the bionanoreactor as a nucleation point and core/shell enzyme/nanocrystal particles are obtained. b) The less-inhibited enzyme produces an overwhelming number of S^{2-} ions to yield an amorphous material. c) The fully inhibited enzyme cannot produce S^{2-} ions, and the metal sulfide does not grow on the biocatalytic template.

Figure 2.

The degree of inhibition of the catalytic reaction of urease by Ag^+ . Enzyme activity of urease incubated with Ag^+ with the concentration of: 0 M (non-inhibited, full circles, enzyme activity, $v_0 = 0.183 \text{ s}^{-1}$); 10^{-13} M (slightly inhibited, squares, $v_0 = 0.168 \text{ s}^{-1}$); 10^{-7} M (moderately inhibited, open circles, $v_0 = 0.158 \text{ s}^{-1}$); and 10^{-4} M (fully inhibited, triangles, $v_0 = 0.000 \text{ s}^{-1}$).

Figure 3.

Biomimetic synthesis of Ag₂S nanocrystals on moderately inhibited enzymes. a) TEM image of core/shell enzyme/Ag₂S nanostructures ($[Ag^+] = 10^{-7}$ M). b) SAED pattern of Ag₂S nanoshells. c) Size distribution plot (outer diameter, D) of core/shell structures. d) HR-TEM image showing the core/shell nanostructure. e) High-magnification HR-TEM image showing a highly crystalline Ag₂S nanoshell.

Figure 4. TEM image and diffraction pattern of amorphous but spherical Ag₂S particles grown on slightly inhibited enzymes ($[Ag^+] = 10^{-13}$ M) (a) and shapeless and aggregated amorphous Ag₂S particles grown outside fully inhibited enzymes ($[Ag^+] = 10^{-4}$ M) (b).