

Center for Hierarchical Materials

Title: Supraparticles: Catalytic Assemblies for Harsh Conditions
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The development of new enzyme technologies has both immediate and future significance for the US economy. Enzymes are central for applications such as analytical techniques for virus detection, health monitoring, and forensics. Enzymes are also essential for medicaments, nutritional regiments, and portable energy generation. The persistent problem with enzyme-based technologies is their environmental instability in often harsh conditions. Both their activity loss and its unpredictability are problematic for enzyme-based device components, drugs, and reagents. We shall leverage the accumulated knowledge about protein complexes, organic-inorganic systems for enzyme stabilization, and biomimetic nanostructures to develop a new method to prevent loss and stabilize the activity of enzymes exposed to harsh operational environments. The method is expected to have general applicability for a variety of different enzymes.

The loss of enzymatic activity complicating the wide use of many industrially produced enzymes,

is primarily attributed to denaturation, proteolytic decomposition, and radical damage from reactive oxygen species (ROS). Nature has developed efficient methods to protect essential enzymes and other vital biomacromolecules against these processes. Many hierarchical biomimetic assemblies successfully replicate protective supramolecular organization of catalytic subsystems found in Nature. However, the challenge of enzyme stabilization for media and temperature conditions far away from the 'comfort zone' of enzymes remains acute. Enabling mesophilic enzymes to retain prolonged activity, under, for instance, thermophilic conditions with simultaneous free-radical attack, would be a game-changer for a wide spectrum of technologies.

Therefore, this project is focused on engineering of hierarchical catalytic assemblies for harsh environmental conditions. Achieving this goal becomes possible for bioinorganic supraparticles (SPs) - a new type of micelle-like supramolecular assemblies (**Figure 1**) that self-assemble from

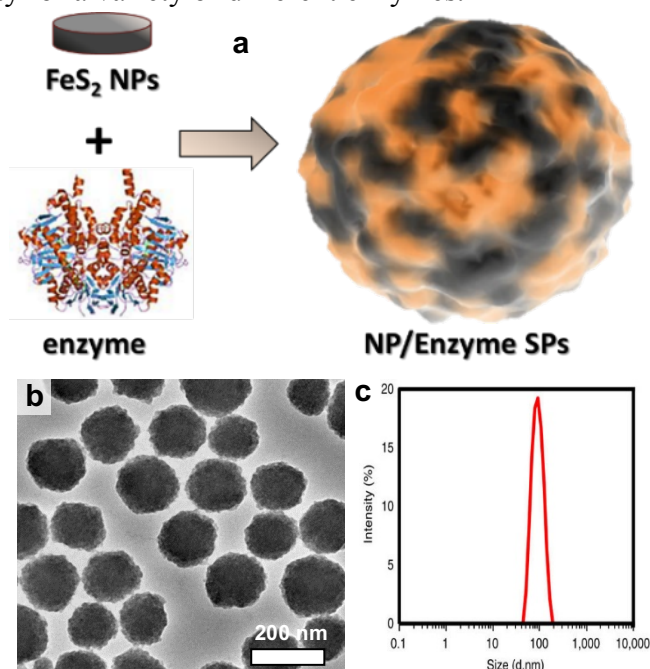


Figure 1. Catalytic supraparticles. (a) Schematics of the supraparticles from FeS₂ NPs and enzyme molecules. (b) Transmission electron microscopy (TEM) image of SPs assembled from 3.8 ± 0.4 nm CdTe NPs, stabilized by 2-(dimethylamino)ethanethiol (DMAET) and charge transfer protein Cytochrome C (CytC) (c) Ensemble size distribution of SPs from DMAET-CdTe NPs and CytC, determined by dynamic light scattering (DLS) from Ref. ¹

proteins and inorganic nanoparticles (NPs) of iron sulfide, FeS₂. The damage to enzymes will be mitigated by protective functions of FeS₂ NPs replicating ‘guardian proteins’ in biological assemblies. Their properties include robustness under thermophilic conditions, capsid-like supramolecular packing with enzymes, and efficient deactivation of ROS. These properties will be synergistically integrated with catalytic activity of enzymes.

Based on our previous studies,¹ we anticipate that the proposed SPs will have uniform size distribution (**Figure 1b,c**). The number of constituent units in SPs will be controlled by repulsive/attractive interactions between them and surface chemistry of NPs. The NPs to be used in this project will be made from the inexpensive, Earth-abundant semiconductor material iron sulfide, FeS₂,^{2,3} modified with small organic molecules and individual amino acids.

In the course of the project, we shall interact with industrial partners to collaboratively create SPs from FeS₂ NP and three enzymes--glucose oxidase (GOX), ascorbate oxidase (AOX) and serine protease (PRS). The produced SPs are expected to combine high catalytic activity and resilience against denaturation, proteolytic decomposition, and radical damage. Extensive data on structure, thermodynamics, and catalytic properties of the bioinorganic SPs will allow us to develop general methodology of enzyme stabilization. The self-repair capabilities emergent for dynamic self-organized systems will make possible to restore the activity of SPs after cumulative damage by several environmental factors. The process of self-repair will be evaluated for both NPs and enzymatic components of SPs.

The selected enzymes, GOX, AOX, and PRS, will also highlight technological relevance of the bioinorganic SPs. The network of interconnected FeS₂ NPs improves charge transport through the SPs. When combined with redox-active GOX and AOX enzymes, bioinorganic SPs can alleviate the short life time of enzymatic biofuel cells associated with loss of enzymatic activity. The thermophilic PRS-based SP will be tested for rapid proteomics analysis of tobacco mosaic virus.

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