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Single enzyme nanoparticles armored by a thin silicate network: Single enzyme caged nanoparticles

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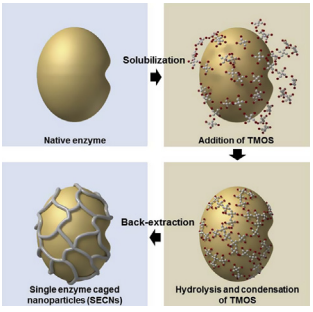
HIGHLIGHTS

- Protocol of single enzyme caged nanoparticles (SECNs) has been developed.
- SECNs have ultra-thin silicate network on the surface of individual enzyme molecule.
- SECNs minimize substrate diffusional limitation, and inhibit the enzyme denaturation.
- SECNs can be immobilized into mesoporous silica for recyclable enzymatic systems.

GRAPHICAL ABSTRACT



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ABSTRACT

For the encapsulation of biomolecules in inorganic materials, we have developed a unique enzyme-silicate conjugate material that consists of a self-assembled molecularly thin silicate layer on the surface of each individual enzyme molecule. The enzyme-silicate conjugate materials, called single enzyme caged nanoparticles (SECNs), were synthesized via the silica polymerization on the surface of enzyme molecule after solubilizing each enzyme molecule in hexane by using a tiny amount of surfactant, called "ion-pairing". SECNs possess near native enzyme activity in aqueous media with minimal substrate diffusional limitations, and are highly stable under the protection of silicate network cage. Due to their nearly molecular size, SECNs can also be adsorbed into mesoporous silica materials to yield robust and easily-recyclable enzymatic systems that can be used in a number of potential biocatalytic applications such as diagnostics, biosensors, biotransformations, biofuel production, bioremediation and CO_2 capture.

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1. Introduction

Encapsulation of biomolecules in inorganic materials has been extensively studied for potential uses in biosensors, bioconversion, imaging, and therapeutics [1–6]. The combination of reverse micellar enzyme encapsulation with silica sol-gel condensation provides a route to the preparation of silicate nanoparticles containing encapsulated enzyme molecules in a scalable fashion [3–6]. To date, however, the majority of synthetic approaches with reverse micelles do not provide tight control of silicate layer thickness and the number of enzyme molecules inside silicate layer. Moreover, the enzyme-encapsulated silicate nanoparticles has a wide range of size distribution (20 nm–100 nm), which is too big for efficient renal clearance, and therefore, can potentially restrict *in vivo* applications [7,8]. The thick silicate layer also places serious mass transfer limitations on substrate diffusion that can reduce the apparent activity of enzyme catalysis [4–6]. The large size of enzyme-encapsulated nanoparticles also limits their hierarchical incorporation into mesoporous materials, which are an ideal candidate for effective enzyme immobilization in various enzyme applications [9–13].

In the present work, we report a unique synthetic approach for the formation of ultra-thin and highly uniform silicate networks on the surface of each individual enzyme molecule, called 'single enzyme caged nanoparticles' (SECNs) (Fig. 1). The SECN synthesis starts with the solubilization of enzymes in hexane via an ion-pairing protocol [14]. Usually, the reverse micelles are prepared by using a high concentration of surfactant, which ensures the extraction of water containing enzymes into a hydrophobic solvent (Fig. S1a in Supporting Information). On the other hand, the protocol of 'ion pairing' uses a much lower concentration of surfactant that extracts single enzyme one-by-one into the hydrophobic organic solvent with some portion of its surface directly exposed to the solvent and only part of enzyme surface paired with surfactant head groups via charge interaction (Fig. S1b in Supporting Information) [14,15]. We hypothesize that the use of ion-pairing, where each enzyme molecule is associated with a much smaller number of water molecules than enzymes in reverse micelles (Fig. S1 in Supporting Information), leading to an ultra-thin and highly uniform silicate network that makes final SECNs sufficiently small for incorporation into mesoporous materials. Through the scalable and hierarchical synthesis of SECNs and their immobilization into mesoporous silicas, we achieve a biocatalytic material with excellent activity, stability and reusability for a range of *in vitro* applications.

2. Experimental sections

2.1. Materials

α-Chymotrypsin (CT) from *Bovine pancreas* (EC 3.4.21.1), lipase (LP) from *Candida rugosa* (EC 3.1.1.3), Tetramethyl orthosilicate (TMOS), Dioctyl sulfosuccinate sodium salt (AOT), bis-tris propane (BTP), sodium phosphate, sodium acetate, isopropanol, 1-octanol, calcium chloride, 4-methylumbelliferyl p-trimethylammonium ciamate chloride (MUTMAC), 4-methylumbelliferone (MU),

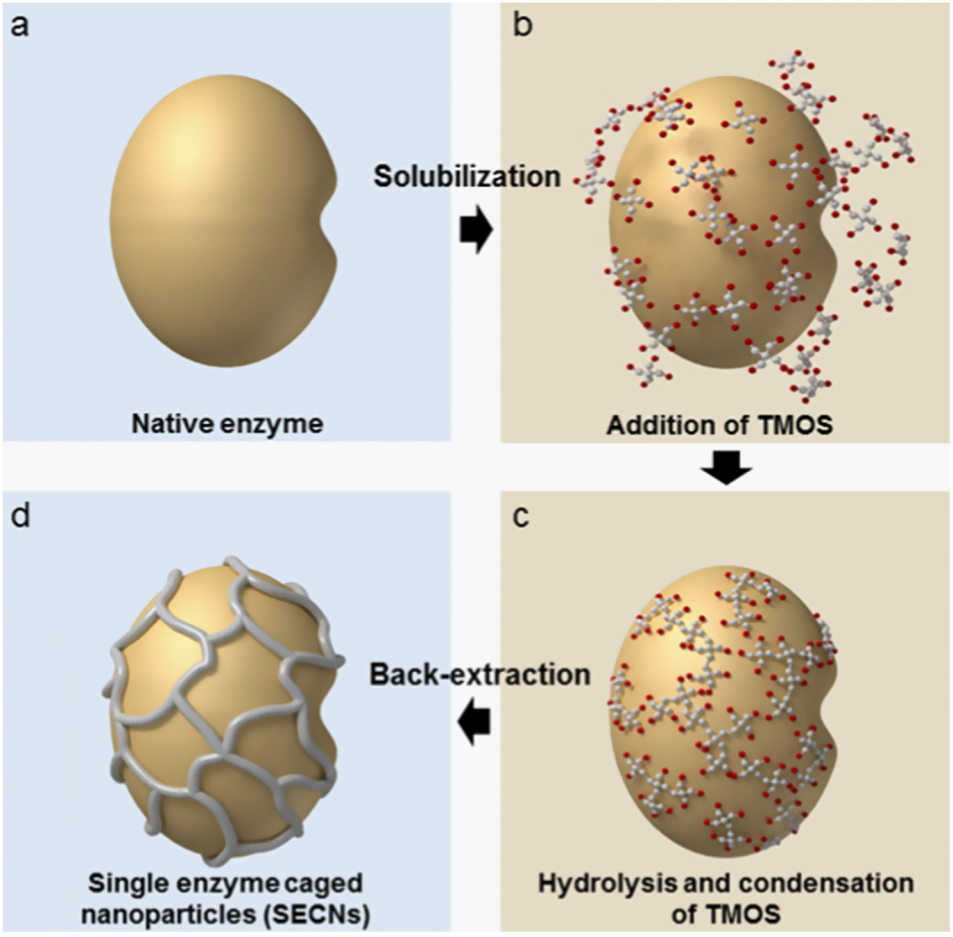

Fig. 1. Schematic representation for the synthesis of SECN-CT. (a) Native enzyme in aqueous buffer. (b) Native enzyme solubilized into hexane via ion-pairing with 2 mM AOT. Then, TMOS solution is added into hexane. (c) TMOS hydrolyzes and condenses on the surface of the enzyme molecule at 4 °C overnight. (d) Enzyme is back-extracted into aqueous buffer and stored at 4 °C for at least three days to complete the hydrolysis and condensation of TMOS on the enzyme surface.