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Biocatalytic Enzymogel Nanoparticles

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Extended Abstract

In this work we developed biocatalytic enzymogel which is designed as a core-shell nanoparticle with a superparamagnetic core and a polymer brush scaffold loaded with enzymes. The enzymogel demonstrates a novel type of phase-boundary biocatalysis with a unique combination of properties including (a) biocatalysis involving enzymes in the particle, (b) stimuli-triggered release of enzymes to the nanoparticle's surrounding and extracapsular biocatalysis, (c) biocatalytic conversion of substrates contacting the enzymogel when the enzymes act to bridge the particle and the substrate and engulf the substrate, and (d) stimuli-triggered reattachment of released enzymes for their reuse. The enzymogel nanoparticle mimics catabolism of either internal or extracapsular substrates. This versatility turns the particle into a universal biocatalytic "supercapsule" for a number of applications such as synthesis of biofuels; the storage, delivery and reuse of enzymes in biotechnology; and in multifunctional biomaterials when enzymogel nanoparticles could be remotely guided to the surface of drug-loaded containers or temporal implants (absorbable sutures) and degrade them upon contact.

The nanoparticle's design of enzymogel includes a core-shell structure with an inorganic core and a polymer brush shell. A typical example is represented by a 100±10 nm silica core with a 30±5 nm (in the dry state) poly(acrylic acid) (PAA) brush. To enable magnetic separation of the enzymogel particles, the core was made of one or few 15 nm γ-Fe_2O_3 superparamagnetic particles that were enveloped by a silica shell.

The PAA shell shrinks at pH 4.5 but still maintains a net negative charge. PAA is a weak polyelectrolyte and is negatively charged in a pH range of 4 to 8 which is optimal for the highest enzymatic activity for most enzymes. The enzymogel can be loaded with positively charged enzymes in this pH range. In our working example, we use cellulase (CEL) enzymes to cleave cellulose molecules and convert them into glucose for biofuel or biochemical production. CEL is typically isoelectric at pH 4.9 and, thus, attains a moderate positive charge at pH 4.5. CEL was loaded into the enzymogel particles at pH 4.5. The enzymogel nanoparticle provides a unique opportunity for industrial enzyme recovery. Due to the high biocatalytic activity of enzymes that reside in the enzymogel there is no need to release and extract enzymes. The enzymogel can be used for biomass conversion as integral moieties. After conversion of the cellulosic biomass, the enzymogel nanoparticles can be magnetically extracted and transferred into a freshly loaded bioreactor for reuse. The experiments demonstrated that this

methodology provides a four-fold increase in glucose per enzyme when compared with the traditional one-way use of CEL for cellulose conversion

Our work introduces novel phase boundary biocatalysis when encapsulated enzyme retains its mobility in a soft hydrophilic polymer carrier and is capable of hydrolyzing insoluble substrates using highly dynamic behavior of the enzyme and their high affinity to the polymer brush of the enzymogel and to the substrate. The enzyme remains encapsulated in the polymer brush and catalyzes the hydrolysis of the insoluble substrates attached to the enzymogel.