Single-Enzyme Nanoparticles on Nanostructured Matrices

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◆ The goal of this research is to synthesize and understand composite nanobiological materials based on enzymes. Success will have a broad impact in various DOE mission areas, including biosensing major chemical-warfare agents, enzymatic hydrogen production from biobased products and biofuel cells, bioremediation and enzymatic carbon sequestration, and fundamental understanding in the area of nanobiology. ◆

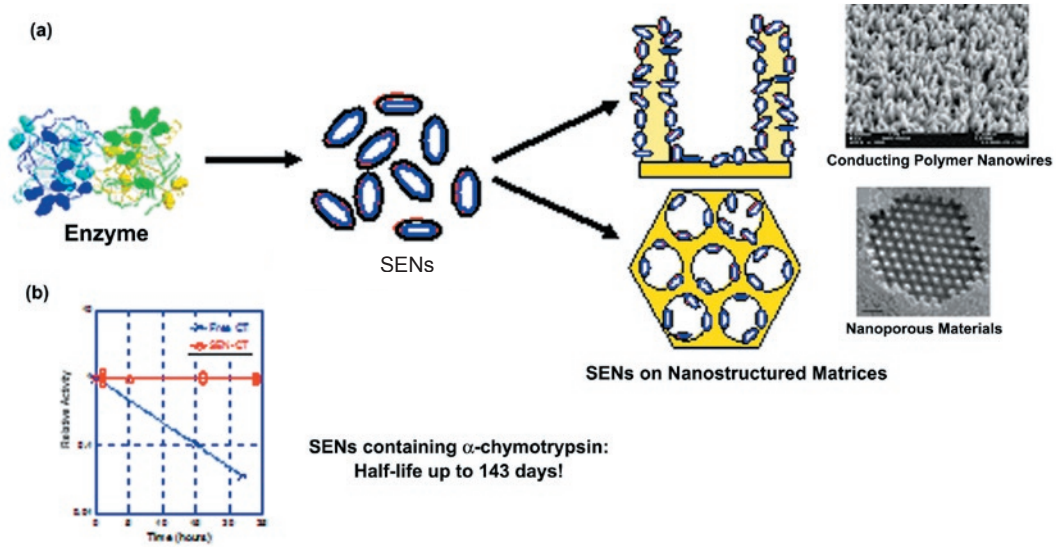
Enzymes are useful biocatalysts that regulate the chemistry of cells and organisms on the nanometer scale. The specificity of enzymes promises great improvements in various applications such as chemical conversion, biosensing, and bioremediation. However, the short catalytic lifetimes of enzymes presently limit their usefulness. Our goal is to develop new methods for the preparation of nano-bio-composites that provide high yields of enzymes stabilized in the form of single-enzyme nanoparticles (SENs). The SENs consist of enzymes surrounded and armored by silicate coating. The long-term goal is to immobilize SENs in various nanostructured matrices such as conductive polymer nanowires (for biosensing) and nanoporous silica (for bioconversion or bioremediation). These nanostructured matrices will provide a high surface area for the attachment of active and stable SENs. The figure illustrates these proposed tasks schematically. The combination of stabilized SENs and increased surface area for SENs attachment will result in an ideal enzyme system for applications in biosensors, bioremediation, and other biocatalytic processes.

To date, we demonstrated that two enzymes (α-chymotrypsin and trypsin) can be stabilized in a form of SENs (see the figure). The armor network around each enzyme molecule is sufficiently thin and porous that it does not seriously limit mass-transfer on the substrates. The synthetic reactions are carried out so that crosslinking is largely confined to individual enzyme surfaces, yielding discrete nanoparticles rather than bulk solids. The yields of enzyme activity were 38% to 73%. The storage stability of SENs in buffer solution was also impressive, and a sample solution showed negligible decrease in the enzyme activity over 5 months when stored in the refrigerator (4°C). We have further confirmed that SENs are stable in the presence of free unarmored proteases. This resistance to proteolysis will be a huge benefit when SENs are used in real applications, where proteases exist in the samples.

We have demonstrated that SENs can be immobilized in nanoporous silica, and that this provides a much more stable immobilized enzyme system than the native enzyme immobilized by either adsorption or covalent bonding in the same material. Furthermore, we found that SENs immobilized in nanoporous silica were more stable than free SENs. This hierarchical approach to immobilized enzyme architectures separates the stabilization approach from the immobilization step, so that the larger scale immobilization can then be designed with emphasis on surface area, porosity, and other factors.

Conducting polymer nanowires, which are good nanostructured matrices for biosensing purposes, were synthesized through controlled nucleation and growth using a step-wise electrochemical deposition process. In this process, molecularly aligned polyaniline molecules assembled into the desired uniform nanowires without using any template structures.

We are proceeding to study the stabilization mechanism of various SENs and the effect of controlled armor-thickness on their properties. This draws on PNNL's tools, such as atomic force microscopy, single-molecule fluorescence spectroscopy, and computational resources to perform molecular dynamic simulation of SENs.



(a) Enzyme encapsulation and further immobilization on nanostructured matrices. (b) Stabilized SENs of α-chymotrypsin.