Nanobiotechnology in Using Enzymes for Environmental Remediation

"Single Enzyme Nanoparticles"

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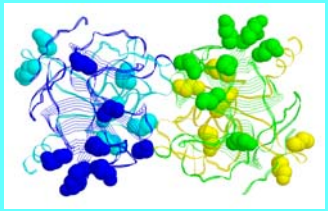
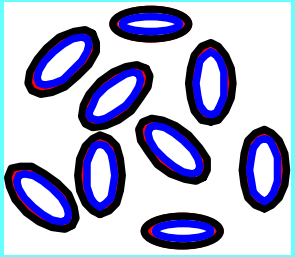
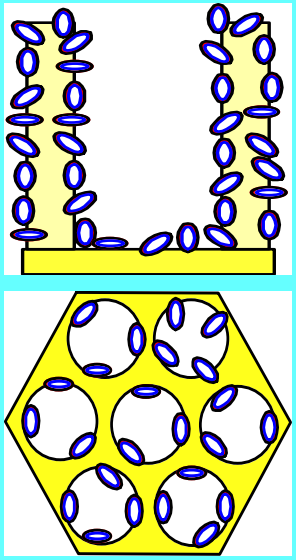


α-Chymotrypsin (CT)



Single Enzyme Nanoparticles containing CT(SEN-CT)



SENs on Nanostructured Matrices

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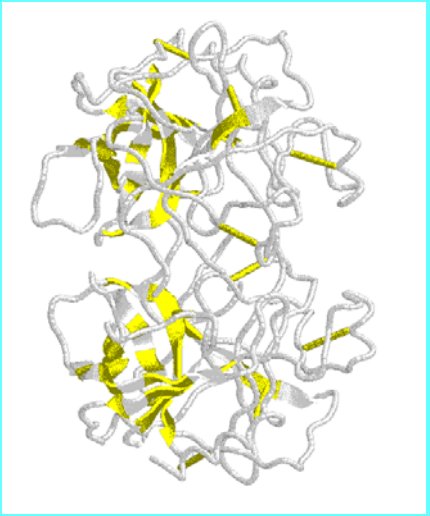
Enzymes - Biological Catalysts

- Regulate the chemistry of cells and organisms
- Natural diversity of enzyme reactions
- Enzymatic catalysis
  - $E + S \leftrightarrow ES^{\ddagger} \rightarrow E + P$
  - Binding step
  - Catalytic step
- Enzyme Technology
  - Enzyme selectivity
  - Wide range of applications
    - Biosensors
    - **Bioremediation**
    - Enzymatic synthesis in pharmaceutical and food industries
    - Detergent industry
  - Problem: Enzyme inactivation via denaturation
  - Stabilization of enzyme activity

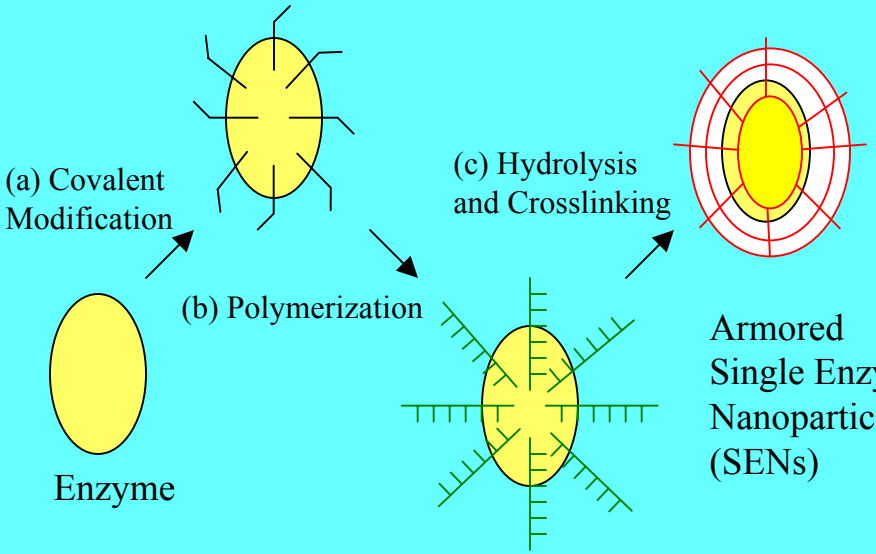


α-Chymotrypsin (CT)

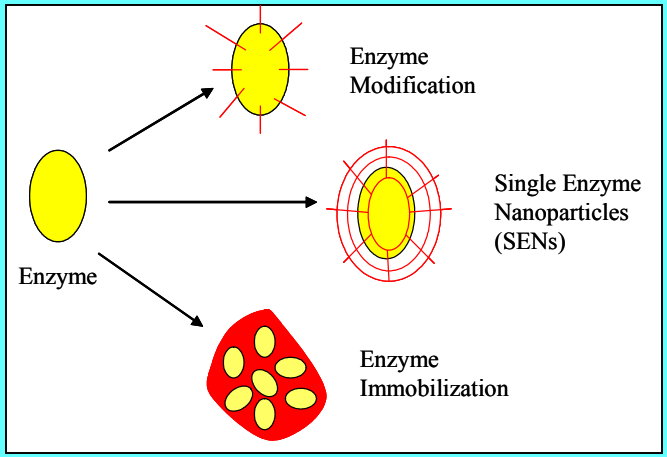
Enzymes vs. Microorganisms for Bioremediation

- Recent advances in genetic engineering, enzyme isolation and purification have lowered the cost of enzymes
- Advantages of Enzymes over Microorganisms
  - More harsh operational conditions (contaminant concentration, pH, temperature, and salinity)
  - Application to recalcitrant compounds
  - No requirement of nutrients
  - No requirement of biomass acclimation
  - No formation of metabolic by-products
  - Greatly lowered mass transfer limitation on contaminants compared to microorganisms
  - Easy-to-control process
  - Effective in small quantity
- Target organic compounds
  - Phenols
  - Chlorinated compounds
  - Polyaromatics
  - Dyes
  - Organophosphorous pesticides or nerve agents
  - TNT
- Enzymes
  - Peroxidases
  - Laccases
  - Tyrosinases
  - Organophosphorous hydrolases
  - Dehalogenases
- Problems
  - Still expensive
  - Short lifetime
- Solution
  - **Enzyme stabilization**

Single Enzyme Nanoparticles (SENs)



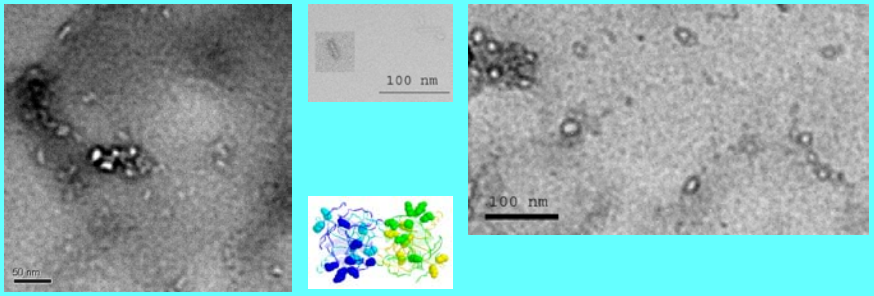
- Synthesis
  - Enzyme Modification
  - Vinyl Polymerization in Hexane
    - Solubilization of modified enzymes in hexane
    - Free radical polymerization
  - Silicate Polymerization

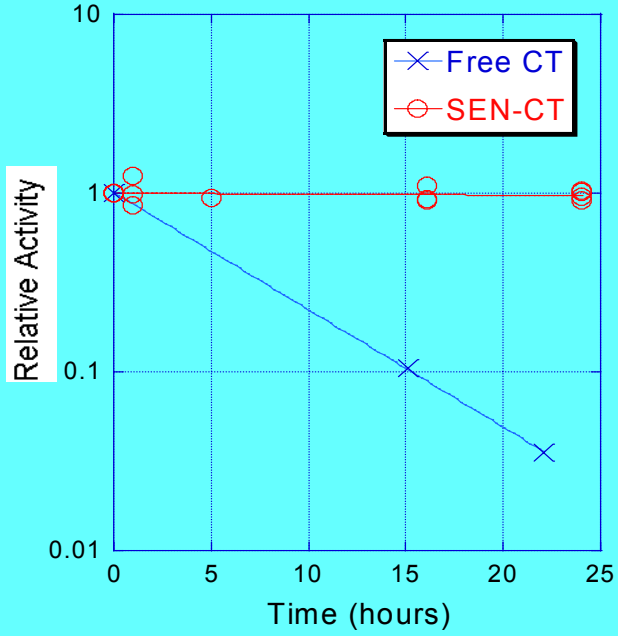


- Neither enzyme immobilization nor enzyme modification
- Nano-Bio-Composites
  - Nanometer and single-enzyme scale
  - Thin coating (a few nanometer)
- **Will SENs Stabilize Enzyme Activity?**

Success with SEN-CT

- SEN-CT: Single enzyme nanoparticles containing α-chymotrypsin (CT)
- TEM Images
  - Hollow center matches the size and shape of CT.
  - The dark image surrounding CT contains silicon (Energy Dispersive X-ray Analysis)
- Synthetic yield up to 73%
- **Vivid stabilization of CT activity**
  - Half-life at 30ºC: up to 143 days
  - No activity decrease at 4ºC for five months





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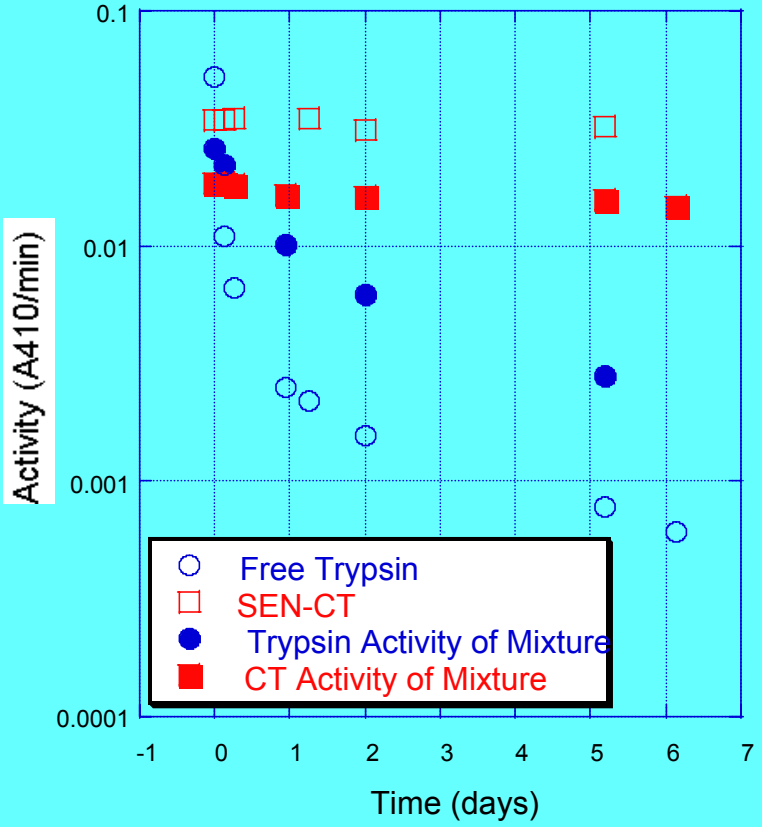
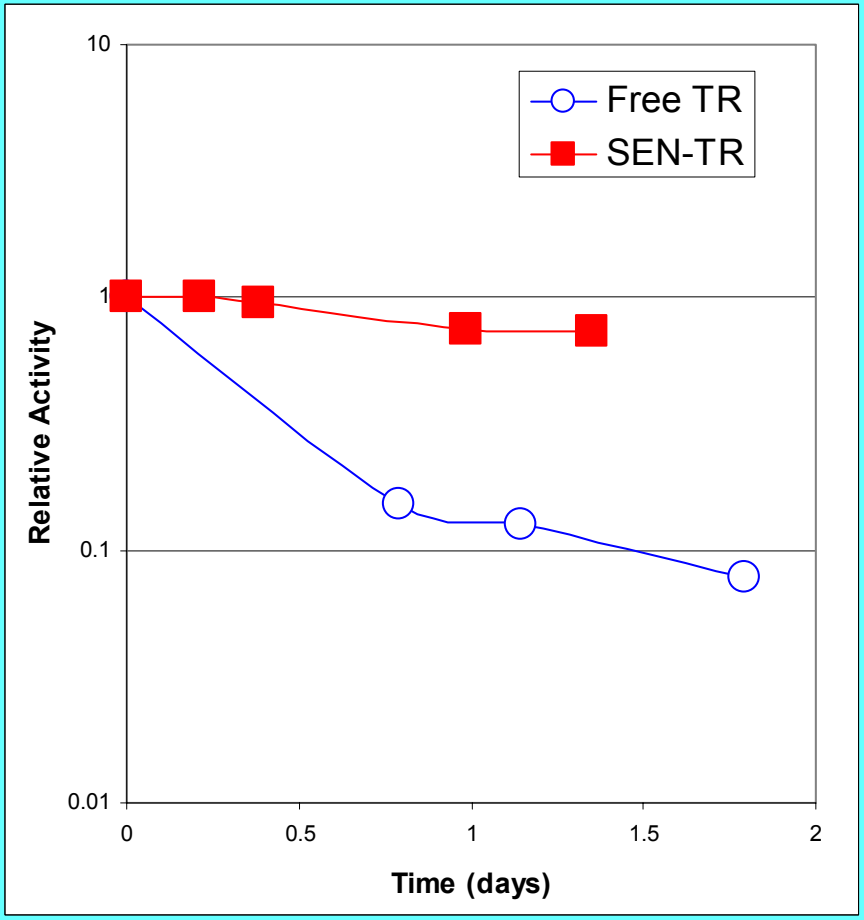
No Proteolysis of SEN-CT by Free Trypsin



- SEN-CT: Stable
- Free Trypsin: Unstable due to autolysis
- In a Mixture,
  - Orthogonal measurement of trypsin and CT activities
    - Trypsin: N-benzoyl-Arg *p*-nitroanilide
    - CT: N-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide
  - Trypsin activity: Unstable
  - CT activity: Stable
- SEN-CT was protected from the proteolysis by free trypsin.

Stabilization of Trypsin in a Form of SENs



- **SEN-TR stabilized the TR activity.**
  - **Further optimization**
- **Ongoing task: SENs of non-proteolytic enzymes such as tyrosinase**

Activity of SEN-CT

Sample	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (x 10^5 $M^{-1}s^{-1}$)
Free CT	29.9 ± 0.7	38.9 ± 2.7	7.70 ± 0.06
SEN-CT	13.8 ± 0.6	40.2 ± 4.6	3.44 ± 0.04
SEN-CT to Free CT	0.46	1.03	0.45

- Activity measurement
  - Hydrolysis of N-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide in aqueous buffer
- Catalytic efficiency
  - $k_{ca}/K_{mt}^{\text{SEN-CT}} = 0.45\ k_{ca}/K_{mt}^{\text{Free CT}}$
  - Reduced catalytic efficiency
- Catalytic step
  - $k_{cat}^{\text{SEN-CT}} = 0.46\ k_{cat}^{\text{Free CT}}$
  - Reduced flexibility of CT
- Binding step
  - $K_m^{\text{SEN-CT}} \approx K_m^{\text{Free CT}}$
  - No serious mass transfer limitation

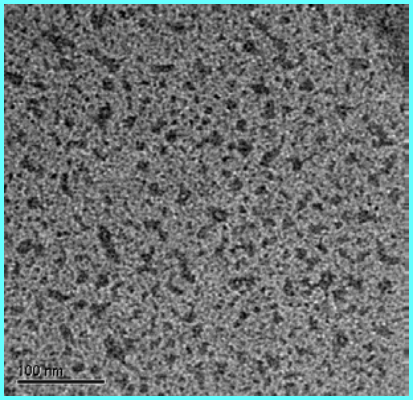
Porosity of the Armor Shell in SEN-CT

Substrate Proteins	MW of Substrate Proteins	Initial Rate w/ Free CT (μM/h)	Initial Rate w/ SEN-CT (μM/h)
Insulin	5734	31	20
Apomyoglobin	16952	4	13
Aldolase	39212	30	19
Albumin	66430	43	49

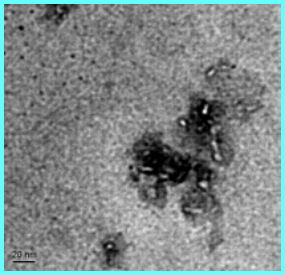
- Proteolysis of Substrate Proteins
  - Proteins of various sizes
  - Incubation with free CT or SEN-CT
  - Compare the initial rates of proteolysis
- Comparison of Initial Proteolytic Rates
  - Free CT vs. SEN-CT
  - Up's and Down's due to the complicated model system
    - Protein conformational changes
    - Stability
  - $Rate^{SEN\text{-}CT} \approx Rate^{Free\ CT}$
- No serious mass-transfer limitation through the armor shell for proteolysis of proteins

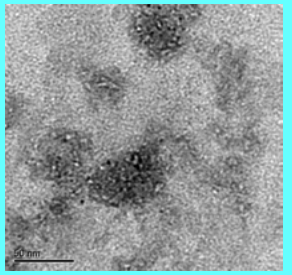
Processing of SEN-CT

Thick coating

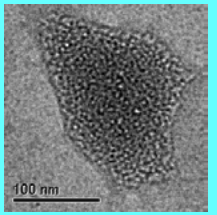


Aggregated

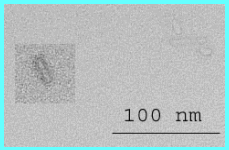




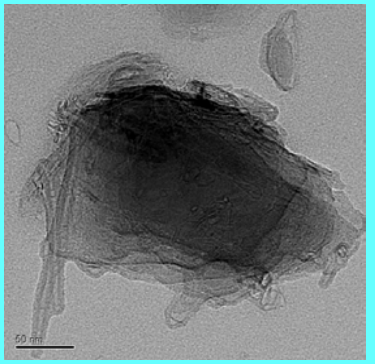
Many SENs in a particle



Thin coating



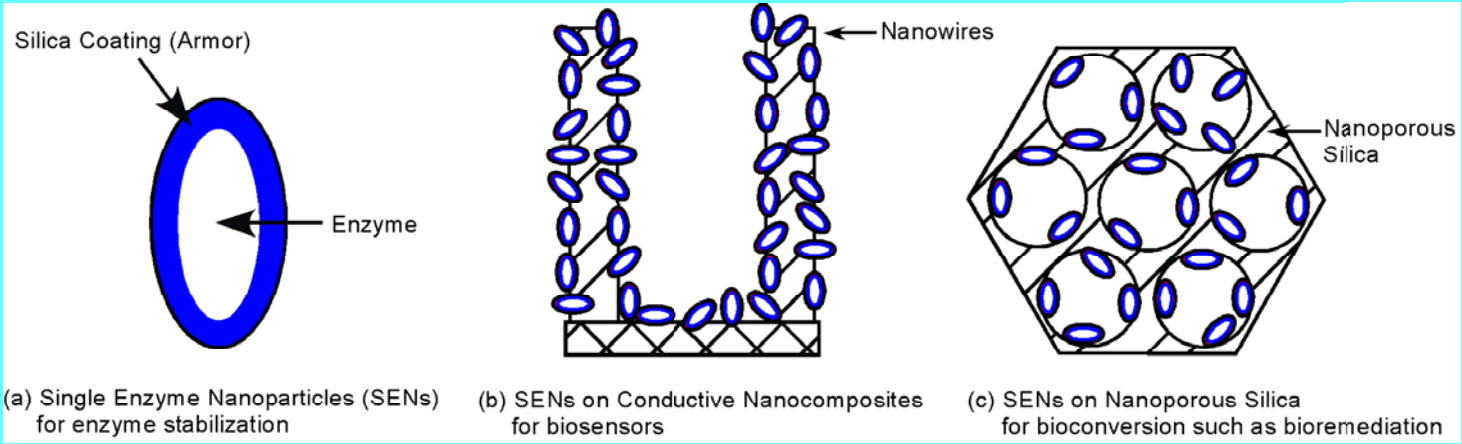
Powder form



- SEN-CT can be processed in various forms.
- Powder form (micrometer scale)
  - Biocatalytic Silicates
  - Half-life up to 350 days
- Easy attachment onto the inner surface of glass vials

SENs on Nanostructured Matrices

- SENs: Active and stable form of enzymes
- Nanostructured Matrices: Large surface area for the attachment of SENs
- Combination: Ideal enzyme system for biosensors (especially, in a miniaturized system) and bioconversion (bioremediation and for bio-based products)



(a) Single Enzyme Nanoparticles (SENs) for enzyme stabilization

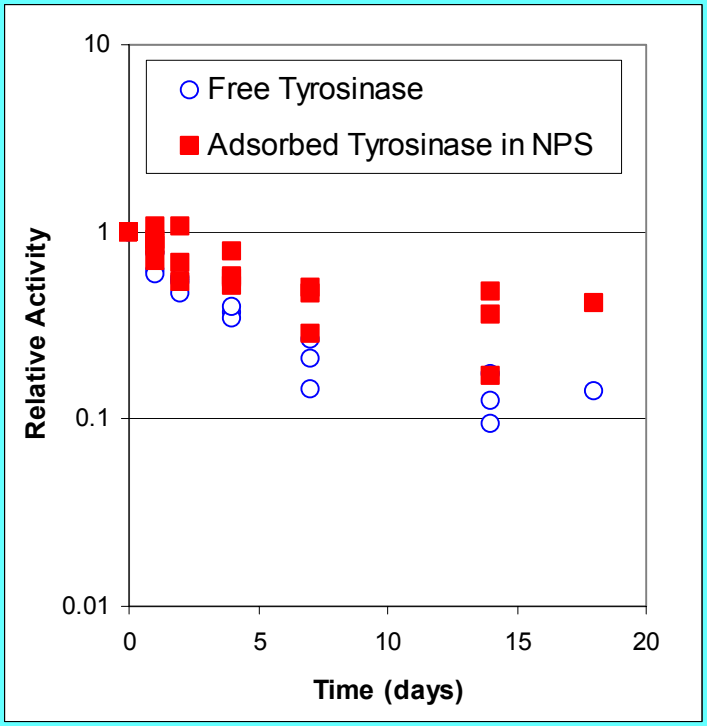
(b) SENs on Conductive Nanocomposites for biosensors

(c) SENs on Nanoporous Silica for bioconversion such as bioremediation

Tyrosinase in NPS

Target Substrates	Free Tyrosinase	Adsorbed Tyrosinase	Covalently bound Tyrosinase
Catechol	8810	14101	19483
4-Methylcatechol	1791	1743	3125
t-Butylcatechol	927	1102	1797

Activities (mol min^{-1} mg^{-1}) were measured with 1 mM substrate after seven day incubation at 30°C.



- Tyrosinase for Bioremediation and/or Soil Carbon Sequestration
- Stabilization even by adsorption
- Further stabilization by the SEN approach?

Conclusions

- We have developed a synthetic protocol for both stable and active enzyme system – “Single Enzyme Nanoparticles” (SENs).
- The armor shell did not place any serious mass transfer limitation for substrates.
- SEN-CT was not proteolysed by free trypsin and α-chymotrypsin.
- SEN-CT could be processed in various forms.
- The combination of SENs and nanostructured matrices will make a great impact in biosensors (for example, environmental monitoring) and bioconversion (for example, bioremediation).

Future Directions and Plans

- Tyrosinase
  - SENs of Tyrosinase (SEN-tyrosinase)
  - SEN-tyrosinase on Nanoporous Silica
- Dehalogenase
  - Cloning of dehalogenase
  - Cloning the haloalkane dehalogenase
  - Direct evolution of dehalogenase
  - Screening for activity toward TCE
  - Synthesis and characterization of engineered dehalogenases nanopaticles