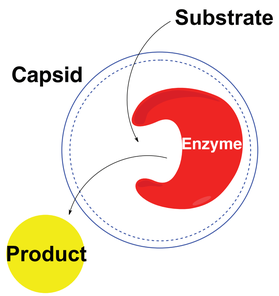
Construction of a Protein-based Nanoreactor Using Charge Complementarity

Seung-Ook Yang and Kenneth Woycechowsky*
Department of Chemistry, University of Utah
315 South 1400 East, Salt Lake City, UT 84112
*e-mail: kwoycech@chem.utah.edu

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF UTAH

1. Introduction

Figure 1. Schematic For Nanoreactor

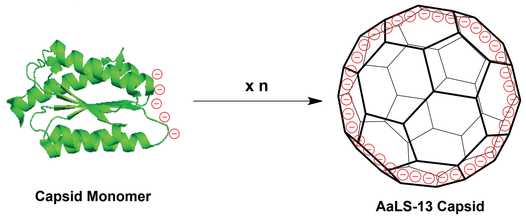


- Protein capsids can be formed by non-covalent assembly of multiple identical subunits
- In nature, encapsulation is used to regulate the activities of some enzymes
- Examples include carbon fixation, propanediol utilization and ethanolamine utilization
- We have developed a nanoreactor using charge complementarity to study how enzyme encapsulation influences catalysis

2. Engineering The Capsid (AaLS-13)

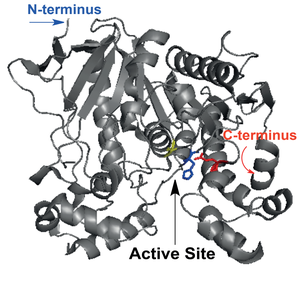
- Lumazine synthase (AaLS) is a non-viral capsid protein from Aquifex aeolicus
- An engineered variant of AaLS (AaLS-13) that possesses a negatively charged inner surface has been previously generated by rational design and directed evolution
- Previous experiments have shown that AaLS-13 can encapsulate R_{10} tagged guest proteins (GFP and HIV-protease) upon coproduction in E. coli

Figure 2. Assembly of AaLS-13



3. Guest Enzyme (Est55-R_{10})

Figure 3. Carboxylesterase (Est55)



- Est55 is a carboxylesterase from Geobacillus stearothermophilus
- Maximum activity at pH 8, which is compatible with the capsid
- Broad substrate specificity
- To promote encapsulation by AaLS-13, a deca arginine (R_{10}) tag has been fused with C-terminus of Est55 (Est55-R_{10})

4. Enzyme Activity

Figure 4. Characterization of Encapsulated Enzyme

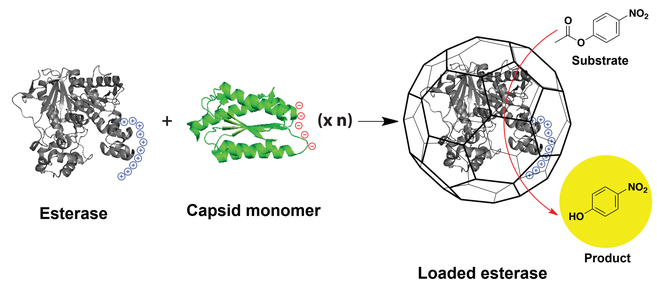
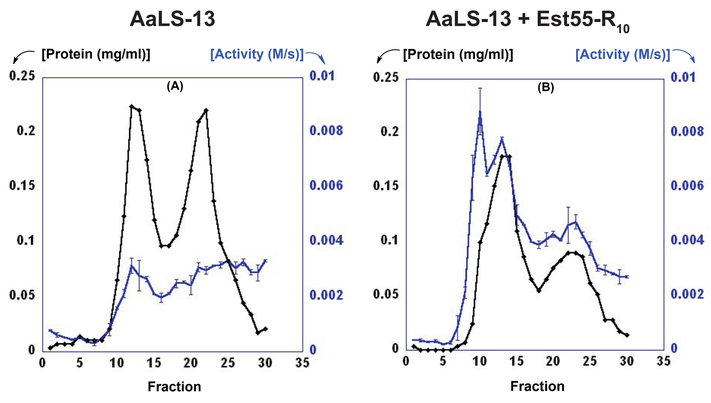


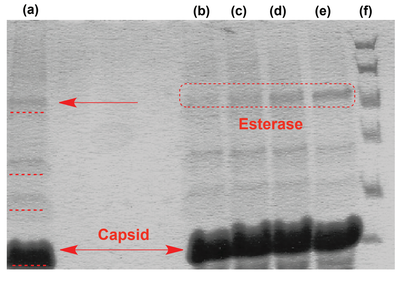
Figure 5. Specific Activity: Empty vs Loaded Capsids



	AaLS-13	AaLS-13 + Est55-R_{10}
Specific Activity (µmol of product/min/ mg of total protein)	4.0 x 10^{-3} ± 1.9 x 10^{-3}	2.6 x 10^{-1} ± 1.3 x 10^{-1}

5. Guest Enzyme Detection

Figure 6. SDS-PAGE Detection of Encapsulated Est55-R_{10}



(a): AaLS-13 co-produced with Est55-R_{10} (27 µg)
(b) ~ (e) : AaLS-13 and Est55-R_{10} were purified separately and then mixed
(b): AaLS-13 (43 µg), Est55-R_{10} (12 ng)
(c): AaLS-13 (43 µg), Est55-R_{10} (23 ng)
(d): AaLS-13 (43 µg), Est55-R_{10} (45 ng)
(e): AaLS-13 (43 µg), Est55-R_{10} (90 ng)
(f): Molecular weight standards

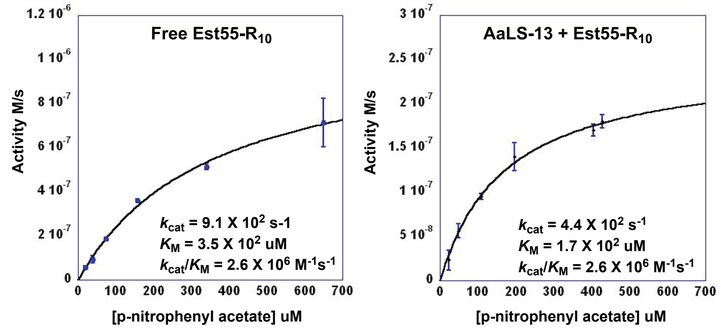
6. Enzyme Loading Efficiency

Protein Pair	AaLS-13 + Est55-R_{10}	AaLS-13 + Est55	AaLS-wt Est55-R_{10}
Loading Estimation by	Activity and SDS-PAGE	Activity	Activity
Number of Esterase per Capsid	1 / 11	1 / 76	1 / 144

- In case of AaLS-13 + Est55-R_{10}, SDS-PAGE analysis and activity gave similar loading estimation
- Charge complementarity between esterase and capsid contributes significantly enhanced loading efficiency

7. Michaelis-Menten Kinetics

Figure 7. Enzyme Kinetics: Free Enzyme vs Encapsulated Enzyme



- Upon encapsulation, k_{cat}/K_M of Est55-R_{10} is not changed, but k_{cat} and K_M both decrease by about two-fold

8. Conclusion and Future Directions

- Free Est55-R_{10} has high enzymatic activity with p-nitrophenyl acetate
- An encapsulated Est55-R_{10} is also highly active with the small model substrate and confers a 64-fold higher specific esterase activity relative to the empty capsid
- Positively supercharged Est55 variants may improve capsid loading
- Activity of different packing densities with encapsulated supercharged Est55 will be measured
- Varying molecular weights of substrates will determine porosity of the capsid