UCMERCED | CHEMISTRY & BIOCHEMISTRY

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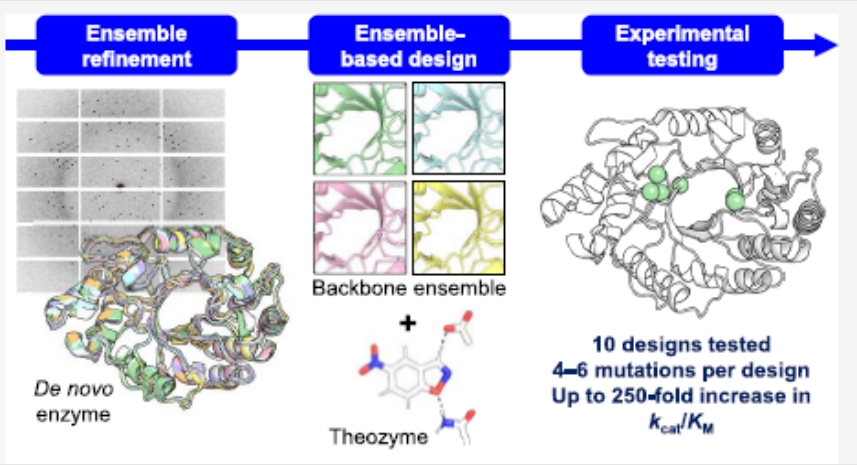
CHEMISTRY & BIOCHEMISTRY SEMINAR SERIES:
Design of Efficient Artificial Enzymes Using Crystallographically Enhanced Conformational Sampling

Speaker:

Behnoush Seifinoferest

PhD Candidate, Chemistry & Biochemistry
UC Merced



Abstract:

The ability to create efficient artificial enzymes for any chemical reaction is of great interest. Here, we describe a computational design method for increasing the catalytic efficiency of de novo enzymes by several orders of magnitude taking advantage of X-ray crystallography data and ensemble refinement. Our approach circumvents the need for labor-intensive directed evolution and high-throughput screening methods typically used to improve the activity of de novo enzymes. We used Phenix ensemble refinement (Burnley et al. 2012) to generate ensemble models from dynamics-based refinement against room temperature X-ray diffraction data collected from crystals of Kemp eliminases HG3 (kcat/KM 125 M−1 s −1) and KE70 (kcat/KM 57 M−1 s −1). Using backbone templates from these ensemble models, we designed, for each of the two enzymes, ≤10 sequences predicted to catalyze this reaction more efficiently. The most active designs display kcat/KM values improved by 100−250-fold, comparable to mutants obtained after screening thousands of variants in multiple rounds of directed evolution. Crystal structures show excellent agreement with computational models, with catalytic contacts present as designed and transition-state root-mean-square deviations of ≤0.65 Å. Our work shows how a more precise sampling of backbone dynamics and conformational sub-states through ensemble refinement can improve de novo enzyme design algorithms for producing more efficient artificial enzymes.

About the Speaker:

Behnoush is a third year PhD candidate in Michael Thompson lab working on structural analysis of engineered enzymes using advanced X-ray crystallography techniques.