Our results constitute a notable contribution to the characterization of the ssDNA-NucS interactions and more generally to the nucleotide excision repair mechanism. The method we developed furthermore constitutes a powerful way to probe DNA/protein intramolecular kinetics.

1048-Plat
Construct a Catalyst using the Beta-Peptide Bundle
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''What I cannot create, I do not understand.'' Richard Feynman, 1988.
Through the construction of an efficient catalyst using a non-natural material, this research project aims to deepen our understanding of the structural and energetic features that control catalysis. We expect our findings to inform the principles for the design and engineering of both proteinaceous enzymes and artificial catalysts.
We set out to use beta-peptides - a unique yet virtually unexplored middle ground between proteins and small molecules - to devise catalysts for the formation or hydrolysis of glycosidic bonds. Beta-peptides are smaller and thus synthetically more tractable than natural proteins; nevertheless, they can still adopt higher order structures inaccessible to small molecules. For example, the Schepartz Laboratory reported previously that the EYYK β-peptide self-assembles into an octameric bundle that displays conformational flexibility and thermodynamic properties similar to natural protein. We therefore build our first generation design around the EYYK scaffold.
Each beta-peptide bundle will be characterize by three biophysical techniques. In addition to structural information obtained by X-ray crystallography, two novel assays that evaluate the beta-peptide bundles based on their substrate binding affinity and reaction turnover rate will be carried out. Together these information provide details of the physical and chemical properties of the beta-peptides to guide the iterative design - synthesis - characterization process. The latest results of our first generation beta-peptide bundle, EYBK, will be presented. The utility of two newly developed assays for monitoring binding and turnover will also be discussed in the context of informing catalyst design.

1049-Plat
An Artificial Safranine Enzyme which Activates Chemotherapeutic Prodrugs
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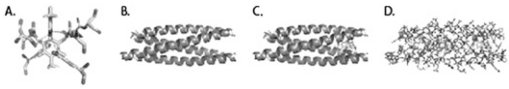
Protein design has opened new boundaries in the past decade, particularly in the area of designed enzymes capable of catalyzing reactions not yet observed in nature. We report the design and synthesis of an artificial enzyme capable of activating the class of nitroaromatic anticancer prodrugs typified by CB1954. The enzyme utilizes a synthetic flavin analogue cofactor, p-methoxysafranine, which has a reduction potential preoptimized for efficient nitroreduction. Progress toward optimizing catalytic activity and determination of the three-dimensional structure of this novel enzyme will also be reported.



1050-Plat
Redox Enzymology of Shewanella Oneidensis Cytochrome C Nitrite Reductase
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Shewanella oneidensis cytochrome c nitrite reductase (soNrfA), a dimeric enzyme that houses five c-type hemes per protomer, carries out the six-electron reduction of nitrite and the two-electron reduction of hydroxylamine. Protein film voltammetry (PFV) has previously been used to study the cytochrome c nitrite reductase from Escherichia coli (ecNrfA) adsorbed to a graphite electrode, revealing catalytic reduction of both nitrite and hydroxylamine substrates by ecNrfA that is characterized by 'boosts' and attenuations in activity depending on the applied potential. Here, we use PFV to investigate the catalytic properties of soNrfA during both nitrite and hydroxylamine turnover and compare those properties to ecNrfA. Distinct differences in both the electrochemical and kinetic characteristics of soNrfA are observed, e.g., all detected electron transfer steps are one-electron in nature, contrary to what has been observed in ecNrfA. Additionally, we find evidence of substrate inhibition during nitrite turnover and negative cooperativity during hydroxylamine turnover, neither of which have previously been observed in any cytochrome c nitrite reductase. Collectively these data provide evidence that during catalysis, potential pathways of communication exist between the individual soNrfA monomers comprising the native homodimer.

1051-Plat
Vibrational Stark Effects in the Active Site of Ketosteroid Isomerase Point to Large Electric Fields Driving Chemical Catalysis
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Enzymes are extraordinary catalysts that actuate nearly all biomolecular processes with speed and specificity. Nevertheless, the physical origins of enzymes' catalytic power remain elusive despite investigations of many enzymes' mechanisms over the last half-century. Ketosteroid isomerase (KSI) is a small and proficient enzyme - accelerating an isomerization reaction ~1 trillion-fold over its intrinsic rate in water - that has been employed as a test system to examine the catalytic strategies at Nature's disposal. Electrostatic interactions are broadly purported to play an essential role in catalysis, but this proposal has yet to be experimentally tested in a quantitative fashion. Toward this end, vibrational Stark effect spectroscopy provides a toolkit to measure electric fields in biomolecular environments. By measuring the vibrational Stark effect of an intrinsic probe that mimics the reactive species of KSI's catalytic cycle, we found that KSI's active site focuses an extremely large electric field onto the scissile bond, potentially enabling its speedy chemical conversion through electrostatic interactions. Moreover, we observed a strong correlation between active site electric field and catalytic power across several KSI mutants. These studies are building toward a highly reductionist picture of KSI's catalysis and possibly enzyme catalysis in general.

1052-Plat
Pseudosubstrate Regulation of the Type II P21-Activated Kinases (PAKs)
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The type II p21-activated kinases (PAKs) are key effectors of RHO-family GTPases involved in cell motility, survival and proliferation. There are two sub-groups of PAK kinase, denoted type I (PAK1, PAK2 and PAK3) and type II (PAK4, PAK5 and PAK6). PAK4 is the best-studied type II PAK family member, is widely expressed, and is essential for viability in mice. PAK4 is required for oncogenic or metastatic phenotypes of many cancer cell lines, can promote tumorigenesis and breast cancer cell migration, and is the only PAK that is transforming when overexpressed. Furthermore, a specific PAK4 inhibitor (PF-3758309) has shown efficacy in mouse models of cancer. Using a structure-guided approach, we discovered that type II PAKs are regulated by an N-terminal autoinhibitory pseudosubstrate motif centered on a critical proline residue, and that this regulation occurs independently of activation loop phosphorylation. We determined 6 X-ray crystal structures of either full-length PAK4 or its catalytic domain, that demonstrate the molecular basis for pseudosubstrate binding to the active state with phosphorylated activation loop. We show that full-length PAK4 is constitutively autoinhibited, but mutation of the pseudosubstrate releases this inhibition and causes increased phosphorylation of BAD and cellular morphological changes. We also find that PAK6 is regulated by the pseudosubstrate region, indicating a common type II PAK autoregulatory mechanism. Finally, we find Src SH3, but not β-PIX SH3 can activate PAK4. This clear switch-like 'on-off' regulation mechanism for the type II PAKs is distinct from regulation mechanisms previously observed for the type I PAKs. We therefore provide new understanding for type II PAK regulation.

Symposium: Regulation of Protein Synthesis

1053-Symp
Optimal Strategy for Rapid Proteome Re-Arrangements in Bacterial Populations
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When a bacterial population is subjected to environmental change, its proteome must be re-arranged for resumption of rapid growth. Upon recurrent environmental changes, rapid proteome responses are required for high average growth rate and fitness value, suggesting high selection pressure for rapid proteome re-arrangements in response to environmental changes. What, then, is the best gene expression strategy for rapid proteome change among bacteria?
Here, we demonstrate that the optimal solution to the adaptation problem is to direct all gene expression to synthesis of the proteins in the currently rate limiting set of pathways. The result is obtained by reformulating proteome dynamics in terms of flow-coupled networks in which the rate limiting protein components determine the growth rate. This approach provides a universal frame work for the description of the whole proteome and its adaptation to