530b Computational Design of Arac Protein with Novel Effector Specificity

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Computer simulations play an increasingly significant role in understanding the underlying physical principles that dictate protein folding, stability and function, and computational advances in this area have greatly improved protein design predictions. While it is not yet possible to robustly predict structure and function de novo, it is possible to assess the impact of mutations on existing, well-characterized proteins. In this study we are developing and testing a computational framework to rationally alter the effector binding specificity of the bacterial transcriptional regulatory protein AraC, belonging to the AraC/XylS family of transcriptional regulators. AraC consists of an effector binding domain and a DNA binding domain. In the absence of L-arabinose, the AraC homodimer represses transcription from promoter ParaBAD, while transcription is activated by the AraC dimer upon binding arabinose. This activation/repression is described as “the light-switch mechanism” and can be understood by examining the structure of AraC. Our goal is to engineer AraC variants which selectively bind one of a variety of very similar molecules not resembling arabinose. As an example, we are designing proteins capable of distinguishing between different oxidized forms of the bicyclic monoterpene alpha-pinene. Our interest in these and similar target molecules stems from a need to develop biocatalysts capable of converting natural resources (such as plant oils) into value-added products: regulatory proteins engineered to activate transcription upon sensing of a desired molecule enable the use of genetic selections and/or high-throughput screens to engineer enzymes and metabolic pathways which synthesize the target compound. We will describe computational and experimental research efforts toward modeling and engineering novel molecular recognition by AraC. Simulation and optimization methods have been used to accurately reflect the relative strengths with which wild-type AraC binds various compounds, and are being used to predict mutagenesis strategies resulting in altered binding selectivity. This is accomplished by identifying mutations in the protein that minimize binding scores for novel substrates (e.g., verbenol). Our procedure involves iterative backbone movements and optimal sequence redesign (near the binding pocket) based on potential energy scoring functions, which serve as a surrogate for binding affinity.