

control of the switch is known. The *Escherichia coli* biotin protein ligase, BirA, is a bifunctional protein that functions in biotin homeostasis. In its metabolic role BirA catalyzes post-translational biotin addition to the BCCP subunit of acetyl-CoA carboxylase and as a transcription repressor it homodimerizes and binds to the biotin operator sequence of the biotin biosynthetic operon. In performing its alternative functions BirA utilizes a single surface that is characterized by several loops to form two mutually exclusive protein-protein interactions. Previous results indicate the importance of these loop sequences for the energetics of homodimerization and the rates of association with BCCP. In this work DNaseI footprint titrations were employed to investigate the influence of the two dimerization interactions on the energetics of transcription repression complex assembly and the switch between the two functions. Direct footprint titrations reveal that homodimerization energetics dictate the energetics of repression complex assembly. Inhibition footprint titrations reveal a direct correlation between inhibition of repression complex assembly and the rate of heterodimer association. This correlation firmly establishes kinetics as the controlling factor in regulating the BirA functional switch.

Synthetic Biology

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Loads Bias Bistable Switches in Synthetic and Natural Systems

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A traditional view of biological modules is that they operate the same in the presence and absence of downstream targets including gene promoters and other proteins. Recent theoretical work [1] suggests that this may not be the case and the properties of biochemical networks are affected by the interactions that the output of the network has with downstream elements. Downstream components can change the dynamic and static properties of the upstream circuit without explicit feedback. Understanding the properties of network modules connected in different ways to downstream components is therefore necessary before we can reliably use these modules as parts of larger synthetic biology circuits. Bistable circuits play an important role in both natural and synthetic biology. Using computer modeling and simulations, we study a variety of bistable circuits including the synthetic genetic toggle switch under the action of a downstream binding element (a "load"). We find that the load can have profound effects on the dynamic properties of the network, significantly affecting the ability of the system to switch from one state to another. Construction of an energy landscape picture using stochastic simulations show that loads significantly bias the bistable switch towards one state. Surprisingly in the genetic toggle switch this effect is particularly strong when the load is on the other state. This is a simple but novel way of tuning bistable protein circuits in synthetic biology and may be ubiquitous in natural systems.

[1] Del Vecchio, D., A.J. Ninfa, and E.D. Sontag, *Modular cell biology: retroactivity and insulation*. *Mol Syst Biol*, 2008. 4: p. 161.

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Design and Engineering of Protein Platforms for Multiple Functions

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We have designed a simple protein, monomeric four- α -helix bundle, which can serve as a platform for diverse set of oxidoreductase related functions. We describe the progressive design steps taken from the simplest α -helical peptide toward functionalized proteins. This platform has considerable latitude for external charge patterning and internal control of stability, both globally and locally. We show how ligation of photoactive and redox cofactors can induce the stabilization and structuring of helices. We demonstrate that this platform can accomplish not one, but variety of functions, including electron transfer to natural proteins, O₂ binding sustained for seconds, CO and NO sensing, millisecond superoxide bursts and triplet-excited state-driven nanosecond charge-separation followed by micro to millisecond electron tunneling reactions. We will present our strategies for optimizing bacterial expression, assembly of natural cofactors such as hemes, flavins, iron-sulfur clusters, quinone, nicotinamide and light active cofactors (porphyrins, chlorins) and many more synthetic analogues with a control over the distance and orientation that allow tuning for specific selected function. These maquette proteins can be altered to assemble in water, membranes and on a range of surfaces including titania. Such versatility makes this protein platform suitable for further iterative designs for light energy harvesting, photochemical charge separation, oxygen transport, oxidative metabolism as well as understanding of fundamental properties of enzyme activity, stability, and folding.

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Hemoprotein Design using Minimal Sequence Information

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We present a simple method for the design of cofactor binding proteins which utilizes a low degree of sequence information. Starting with a bioinformatically derived helical porphyrin-binding consensus sequence, we generate a 'wire-frame' model of an idealized four alpha-helix bundle which contains both the target cofactor and the consensus amino acids on both ligating helices. We then use the model to determine the level of solvent exposure of each remaining unspecified side chain, using database-derived helical side-chain frequencies to randomly select these remaining side chain identities. Evaluation of this method using a ten member library demonstrated that additional sequence information, in the nonligand pair of helices, was required to create a cavity for cofactor binding. Our results allow us to estimate that there are 10^{54} sequences which should fold into a four-helix bundle and bind one or more porphyrin cofactors. These data demonstrate that, at least in the case of helical bundle proteins, functional sequence space is much too large for evolution to explore.

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Design and Characterization of a Single Chain Amphiphilic Maquette for Membrane Insertion and Electron Transfer

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The ability to design and produce artificial proteins opens up many scientific avenues for producing artificial enzymes, catalysts, novel drug therapies, bioelectronics devices, bioremediation and alternative energy sources. We are designing simplified model proteins, called maquettes, for diverse set of oxidoreductase related functions including electron transfer, catalysis, and charge separation. These maquettes can be built as four- α -helical bundles with different topologies such as homotetramer, homodimer, and single chain (monomer). They can be assembled in aqueous solution (hydrophilic maquettes) or in membranes (amphiphilic and hydrophobic maquettes). Here, we will present the design and characterization of an amphiphilic maquette that has been designed for efficient electron transfer across a lipid bilayer. This maquette contains four membrane-spanning α -helices linked into a single chain. The transmembrane electron transfer is enabled by bis-histidine ligated hemes that are positioned 7-11 Å apart (edge to edge). We have successfully developed methods for expressing this maquette in inclusion bodies using *E. coli*. Expression in inclusion bodies helps evade problems associated with insolubility and toxicity of the maquette inside the bacterial cells and therefore enables production in high yields. Since we have designed the maquette with very strong alpha-helical propensity, it refolds readily after purification. We will discuss the assembly, heme binding and redox properties of the maquette in detergent micelles and lipid vesicles.

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A Synthetic Capillary

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Tissue engineering strives to create functional tissue for organ replacement and regenerative medicine by combining living cells with a porous scaffold that promote growth and differentiation. Recent attempts to replace tissue with autologous cells on a biodegradable scaffold have been frustrated by inflammatory and healing responses. We speculate that without vascularization and hierarchical organization, gradients in effector molecules and basic nutrients can develop in engineered tissue due to the competition between convection and diffusion, cell consumption and production that compromises cell function and viability. In particular, once implanted in the patient, the cells in the engineered tissue consume the available oxygen and nutrients within a few hours, while it can take several days for the growth of new blood vessels (angiogenesis) to deliver nutrients to the implant. We have created using "live cell lithography" an in vitro model that emulates the in vivo microenvironment found in human capillaries with micron-scale precision. To create a capillary, we used a microfluidic to convey human umbilical vein endothelial cells (HUVECs) into multiple microarrays of optical tweezers, which are used to precisely position cells in a 3D hydrogel scaffold (see figure).

