Deep Mutational Learning of Protein Function for New Intracellular Biosensors

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Project Goals: Develop high-throughput computational and experimental approaches to deeply characterize natural allosteric transcription factor function so to accelerate the design and engineering of new factors that respond to molecules of special interest to the Department of Energy.

Recent progress in DNA synthesis and sequencing technologies have enabled systematic studies of protein function at a massive scale. We apply deep mutational scanning, a process whereby a library of protein variants modified at each position to every alternative amino acid are assembled and assayed in parallel, to study the sequence-structure-function relationships of allosteric transcription factors in bacteria. Such transcription factors are interesting since they intrinsically couple the binding of a small molecule to the binding of DNA and have emerged as useful tools in synthetic biology as intracellular biosensors. We constructed deep mutational scanning libraries for several allosteric transcription factors, including lacI, and screened these libraries to determine variants that can no longer bind DNA or no longer release DNA under induction from their native inducers. We also screend protein variant response to several new ligands to gain an understanding of protein-ligand binding relationships. Furthermore, we utilize advanced deep learning methodologies, integrating experimental results with large-scale measures of protein conservation and associated predictions from molecular modeling/simulation tools, to reveal essential regions of protein function and to provide useful predictions for custom biosensor design.

This project has been funded by DOE grant DE-FG02-02ER63445. Dr. Church is a founder of companies in which he has related financial interests: ReadCoor; EnEvolv; and 64-x. For a complete list of Dr. Church's financial interests, see also arep.med.harvard.edu/gmc/tech.html