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Directed Evolution of a Photochromic Protein for Long-Term Data Storage NICOLE WAGNER, Department of Molecular and Cell Biology, University of Connecticut, 91 North Eagleville Road, Storrs, Connecticut 06269-3125, USA, JORDAN GRECO, ROBERT BIRGE, Department of Chemistry, University of Connecticut, 55 North Eagleville Road, Storrs, Connecticut 06269-3060, USA — Bacteriorhodopsin has long been known as a protein with comparative advantages for photonic device applications due to its unique photochemistry, excellent thermal stability, and high quantum efficiency. Our recent work has emphasized the use of the long-lived Q state, which is a stable photoproduct with photochemical properties ideal for optical data storage and processing. The formation of the Q state is minimized in the native organism because it eliminates the biological function of the protein. Thus, mutagenesis is necessary to enhance the ability of bacteriorhodopsin to form this photoproduct. We describe here the use of directed evolution to optimize the photochemical properties of the protein, and implement an automated process to characterize microgram protein quantities. Directed evolution is a process by which proteins are optimized toward a specific characteristic via repeated iterations of genetic mutation, screening and differential selection. The mutants are generated via region specific semi-random mutagenesis and are screened with respect to Q state formation and reversion. Next, the efficient Q state mutants are selected to serve as the parent to the next generation of genetic progeny. This process is iterative and builds upon successive improvements to the protein. After six generations of optimization involving over 10,000 mutants, more than ten new proteins have been discovered with excellent Q formation and reversion efficiency, cyclicities and thermal stabilities.

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