Characterization of NADPH Binding Patterns for the Rational Design of a Photoactivatable NADPH Analog

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Time resolved X-ray crystallography produces molecular movies of proteins at femtosecond resolution. The elucidation of an enzyme's structural dynamics during its catalysis has major implications in fundamental structural biology, but this technology is limited due to the need for enzymes to be triggered in a synchronous fashion. Approximately 5.4% of proteins bind to NADPH, a ubiquitous redox active coenzyme. By engineering a coenzyme analog that becomes redox active upon light stimulation, light can be used to initiate simultaneous action of crystallized NADPH dependent enzymes. This approach can potentially lead to time resolved experiments of a larger range of protein targets including dihydrofolate reductase (DHFR). My goal was to locate the optimal site in the molecule to modify without compromising protein binding. I conducted the first large scale (data from 1654 NADPH-protein complexes) computational analysis of NADPH binding using a novel atomic score approach. The approach involves characterizing coenzyme binding by using atomic scores generated from the X-Score scoring algorithm; each atom in every NADPH-protein complex has a score which represents binding strength. Samples are then hierarchically clustered and protein families are traced within the dendrogram for the analysis of atomic score trends in different protein families. For clusters of enzymes like DHFR and nitric oxide synthase (NOS), I identified patterns of low/high scoring atoms and functional groups. Comparative analysis revealed unique patterns of low adenine and high nicotinamide scores in DHFR as compared to NOS. After score analysis and comparisons with crystallographic studies, I determined that the carbon opposite to the amide of nicotinamide is the optimal site for modification.