ABSTRACT

HIGH-RESOLUTION SEQUENCE-FUNCTION MAPPING OF PROTEIN-PROTEIN INTERACTIONS FOR CONFORMATIONAL EPITOPE MAPPING

By

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Protein-protein interactions are essential for biological signaling, including the adaptive immune system, membrane transport and cell metabolism. A protein’s sequence defines its function; however, the relationship between a protein’s sequence and its function is not a well-understood problem. Recent advances in DNA sequencing technologies have allowed the development of independent high-throughput methods to couple a protein’s sequence to its function. These methods analyze the effect of individual mutations on a protein’s fitness. However, the methods
lack standardization leading to many different experimental setups and data presentations. In this dissertation we present a validated and standardized method to determine the sequence-function relationships of protein-protein interactions. We develop a series of equations to model and optimize experimental conditions and to expand the accessibility of the technique. We further used the method to characterize the effect on binding affinity of all single-point mutations for two protein-protein interactions involved in biomass degradation. Finally, we have utilized this method to introduce a novel platform technology for rapid determination of fine conformational epitopes. This technology involves deep sequencing of yeast displayed antigen libraries and analytical equations to identify epitope positions. We show the methods effectiveness by determining critical (and previously unknown) neutralizing epitopes for pertussis toxin and a breast cancer target. We further show the implications of this method for structural-based vaccine design.