REAL-TIME AND LABEL-FREE BIOMOLECULAR INTERACTIONS ANALYSIS USING SELF-ASSEMBLED PROTEIN MICROARRAYS AND SURFACE PLASMON RESONANCE IMAGING

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Now that the human genome has largely been sequenced, one of the most important pursuits is to understand the function of proteins it encodes. Despite immense progress in molecular biology and genetics, only a small fraction of the proteome is understood at the biochemical level. Systems biology and proteomics strive to create detailed predictive models for molecular pathways based upon the quantitative behavior of proteins. Understanding these dynamic networks provides clues into the consequence of aberrant interactions and why they lead to diseases like cancer. However, collecting biochemical data about protein behavior at scale has been daunting. Historically, methods capable of collecting quantitative data on biochemical interactions could only be used for one or a few proteins at the time. Here, we show the combination of two technologies that together could lead to the ability to measure binding events in real time for many protein interactions simultaneously using a label free technology. This could revolutionize the study of protein interactions networks by enabling quantitative comparisons of binding affinities across many molecular species, as well determining the kinetics rates of binding and release.

The first technology is protein microarrays, which display thousands of proteins in high density and enable their simultaneous biochemical characterization. We use Nucleic Acid Programmable Protein Arrays (NAPPA), developed at the Harvard Institute of Proteomics (HIP), as a method for producing the microarrays, because they replace the complex process of spotting purified proteins with the simpler process of spotting plasmid DNA. The proteins can then be simultaneously transcribed/translated *in situ* at the time of the assay. The second technology is a surface plasmon resonance imaging (SPRi) device that has been adapted to multiplexed binding events from a planar surface and is compatible with the protein microarray. In addition this technique is sensitive, accurate and provides real-time date for both the equilibrium and the interaction kinetics. The project is focused at coupling NAPPA protein array technology to multiplexed real-time label-free SPRi-based detection system (which allows thousands of binding events to be monitored in real-time without any loss in sensitivity). By SPR*i* we were able to detect binary interactions using NAPPA format. The combination of both technologies allows us to generate detailed kinetic data of interactions pathways.