SELF-ASSEMBLING PROTEIN MICROARRAYS AND SURFACE PLASMON RESONANCE IMAGING TO STUDY HIGH-THROUGHPUT PROTEIN INTERACTIONS

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The overwhelming size and complexity of human proteome requires very high-throughput techniques for rapid analysis. Despite the significant advancements in molecular biology and genetic tools, this demand has not been satisfied and only a small fraction of the proteome has been understood at the biochemical level. Protein microarrays allow hundreds to thousands of proteins to be spotted onto a single array slide and analyzed simultaneously, providing an attractive option for high-throughput studies such as protein-protein interaction. In Nucleic Acid Programmable Protein Array (NAPPA), full length cDNAs corresponding to the proteins of interest are printed on the microarrays and then transcribed/translated *in situ* at the time of assay. The cDNAs are configured to append a common epitope tag to all of the proteins and along with the cDNA, a high affinity capture reagent, such as an antibody, is immobilized. For protein production, *in vitro* transcription and translation (IVTT) coupled rabbit reticulocyte lysate is used, and expressed protein is captured on the array through the high affinity reagent that recognizes the epitope tag. Protein microarrays offer a compelling method to display many proteins for testing, but label-based methods exhibit major hurdles such as synthetic challenges, multiple label issues, and interference with the binding site. Surface Plasmon Resonance (SPR) can overcome these challenges and provides direct and rapid determination of association and dissociation rates of binding process, determination of strength of the binding and specificity of interactions at large scale. We have used a novel NAPPA-SPRi technology to study protein-protein interactions of p53 (and its mutants) and MDM2 in high throughput, cKIT(and its mutants) and SCF. NAPPA-SPRi technology could revolutionize the study of protein interaction networks by enabling quantitative comparisons of binding affinities across many molecular species, as well as determining the kinetic rates of binding and release.