SCAPA (Spacially Addressable Protein Array) – a Novel Protein Array for the Differential Profiling of Ligand-receptor Induced Signalling.

PARAYIL Kumaran Ajikumar^a, Ng Jin Kiat^a, Lee Jim Yang^{a,b}, Gregory Stephanopoulos^{a,c}, Too Heng-Phon ^{a,d},

^a MEBCS, Singapore-MIT Alliance, 4 Engineering Drive 3, National University of Singapore, Singapore-117576; ^bChemical and Biomolecular Engineerin, National University of Singapore. g; ^cDepartment of Chemical Engineering, Massachusetts Institute of Technology; ^d Department of Biochemistry, Kent Ridge Crescent, National University of Singapore.

Abstract - While protein microarray technology has been successful in demonstrating its usefulness for large scale high-throughput proteome profiling, performance of antibody/antigen microarrays has been only moderately productive. Immobilization of either the capture antibodies or the protein samples on solid supports has severe drawbacks. Denaturation of the immobilized proteins as well as inconsistent orientation of antibodies/ligands on the arrays can lead to erroneous results. This has prompted a number of studies to address these by immobilizing proteins biocompatible surfaces, which has met with limited success. Our strategy relates to a multiplexed, sensitive and high-throughput method for the screening quantification of intracellular signalling proteins from a complex mixture of proteins.

Each signalling protein to be monitored has its capture moiety linked to a specific oligo 'tag'. The array involves the oligonucleotide hybridizationdirected localization and identification of different signalling proteins simultaneously, in a rapid and easy manner. Antibodies have been used as the capture moieties for specific identification of each signaling protein. The method involves covalently partnering each antibody/protein molecule with a unique DNA or DNA derivatives oligonucleotide tag that directs the antibody to a unique site on the microarray due to specific hybridization with a complementary tag-probe on the array. Particular surface modifications and optimal conditions allowed high signal to noise ratio which is essential to the success of this approach.