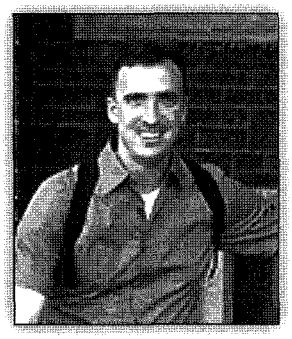
High-Throughput Interactome Mapping Using Protein Mass Spectrometry: *Moving-Forward*



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A key feature of the molecular organization of all organisms is the tendency of proteins to form larger complexes via protein-protein interactions. Understanding how all the myriad of protein components of an organism operate together dynamically to mediate the fundamental cellular processes that collectively form the basis of a functional living cell will require a complete characterization of all the interacting proteins and protein complexes present in a cell, and how this change in response to physiological signals, developmental cues and disease processes.

Using rigorous affinity tagging and purification procedures, we have elucidated a large-scale network of high-confidence interactions for ~700 gene products in *E. coli* [Butland *et al.*, Nature (2005) 433:531-7]., and for about ~3,000 gene products in budding yeast [Krogan *et al*; manuscript in preparation]. These networks include many novel interactions, and have provided insight into the putative functions of uncharacterized proteins. Since interacting proteins generally belong to the same pathway, and since genes are often conserved across evolution, companion comparative genomic studies have revealed evidence for the modular design and functional diversification of these networks across different phyla. Nevertheless, these are static maps, and we have only limited information regarding how dynamic these networks are during either normal or perturbed growth.

To address this issue, we are now developing a new analytical platform, consisting of basic liquid-chromatography-tandem mass spectrometry based shotgun profiling combined with advanced computer algorithms, statistical methods and software applications to support systematic large-scale identification, quantification and evaluation of the global patterns of soluble protein complexes across entire biological systems. The platform is designed to detect, quantify and track large numbers of protein complexes from feature-rich ion mass chromatograms, compensate for spurious fluctuations in recorded signal intensities, and reliably match related complexes across different datasets, allowing for comparative interactome studies under different experimental conditions. We hope to further refine this toolkit to enable routine genome-scale proteomic studies aimed at detecting pathological biological responses, such as biochemical signatures of disease, at the interactome level.