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**In-cell NMR technology to study protein interactions**

The etiology of many human diseases involves structural changes in multi-component protein complexes caused by aberrant posttranslational modifications (PTM). We propose to develop a novel in-cell NMR-based technology for mapping the structural changes that accompany protein-protein interactions in the cell (STINT-NMR) and use it to analyze molecular interactions of biological significance. STINT-NMR analysis greatly reduces the time required to obtain atomic resolution information on protein complexes compared to traditional *in vitro* NMR and x-ray crystallography. The resulting data define structural details of the interacting surfaces at atomic resolution. By sequentially expressing enzymatic activities that modify protein-protein interactions, STINT-NMR also allows us to perform “biochemistry inside the cell”, where we can monitor the structural consequences of post-translational modifications.

The broad objective of this proposal is **to develop a novel *in vivo* atomic resolution technique to understand on structural level how posttranslational modifications regulate protein complexes involved in important biological processes.** To fulfill this objective we have three specific aims:

1) Develop STINT-NMR methodology to structurally characterize multiprotein interactions in bacterial cells. As a biological system, we will use the interactions between Ubiquitin and unmodified or posttranslationally modified endocytic proteins Hrs, STAM2, Eps15. For this aim we will create STINT-NMR compatible plasmid constructs capable of overexpressing endocytic proteins, protein kinases, and monoubiquitination machinery in bacteria. We will perform series of STINT-NMR experiments using different combinations of unmodified and modified endocytic proteins to assess the structural changes resulting from PTM's. We will supplement our in-cell NMR experiments with *in vitro* studies of the modified protein complexes.

2) Develop STINT-NMR for eukaryotic cells to test the influence of intracellular structures on protein-protein interactions. For this aim we will optimize overexpression of Ubiquitin and STAM2 in yeast cells to perform STINT-NMR experiments.

3) Extend STINT-NMR for high-throughput screening of small molecules capable of interfering with the complexes formed between viral proteins required for budding and host endocytic proteins. We will create STINT-NMR compatible plasmid constructs capable of overexpressing viral HIV p6 domain, endocytic proteins TSG101 and Hrs, protein kinase MEK1 and ERK2, and monoubiquitination machinery in bacteria. We will perform a series of STINT-NMR experiments using different combinations of unmodified and modified proteins to establish structural changes associated with complex formation. As a positive control of STINT-NMR HTS methodology, we will screen a small library of known antagonists against TSG101-directed HIV-1 budding. Later, a library of small drug-like molecules (NCI Discover set) will be screened against the p6-TSG101-Hrs complexes using STINT-NMR to identify possible classes of the compounds capable of interfering with viral budding.