Analyzing Protein Complexes from Mammalian Cells at the Single Molecular Level

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Time: 12:00 – 1:00 p.m. CST (10:00 – 11:00 a.m. PST)
Location: 1000 MNTL at Illinois (SSM 150 at UC Merced)

Abstract:
Proteins perform most cellular functions in macromolecular complexes. Quantitative information on macromolecular complexes is scarce. The same protein often participates in different complexes to exhibit diverse functionality. Current ensemble approaches of identifying cellular protein interactions cannot reveal physiological permutations of these interactions. I will discuss a single-molecule pull-down (SiMPull) assay that combines the principles of a conventional pull-down assay with single-molecule fluorescence microscopy and enables direct visualization of individual cellular protein complexes. SiMPull can reveal how many proteins and of which kinds are present in the in vivo complex, as we show using protein kinase A. We then demonstrate a wide applicability to various signaling proteins found in the cytosol, membrane and cellular organelles, and to endogenous protein complexes from animal tissue extracts. The pulled-down proteins are functional and are used, without further processing, for single-molecule biochemical studies. SiMPull should provide a rapid, sensitive and robust platform for analyzing protein assemblies in biological pathways.

Seminar Presented by:

IGERT Integrative Graduate Education and Research Traineeship
Cellular and Molecular Mechanics and BioNanotechnology
M-CNTC NC Alliance for Nanotechnology in Cancer
Midwest Cancer Nanotechnology Training Center
CNST University of Illinois Center for Nanoscale Science and Technology