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Label-Free Single Molecule Method for Detecting Released Cellular Protein Complexes

The ability to precisely and sensitively measure the abundance and composition of intracellular proteins is vital for understanding signaling pathways and cell functions. Typically, this is achieved using gel electrophoresis and western blot. However, these require sufficient signal-to-noise ratio, which limits the detection of rare cells or low-abundant proteins. There are other techniques that have been developed to probe proteins with single cell resolutions, but they often denature protein complexes, making it difficult to interpret their native composition and function in cells.

Researchers at the Biodesign Institute of Arizona State University have developed a novel label-free single-molecule pulldown (LFSMP) technique for imaging released cellular protein and protein complexes with single-molecule sensitivity and low sample consumption. This LFSMP technique is based on plasmonic scattering microscopy (PSM), and as such can directly image the surface captured molecules without labels. By fabricating a thin flow channel, intracellular molecules from lysed adherent live cells can be directed to the bottom gold film surface, which is functionalized with antibodies, and imaged using PSM. This method was validated with the mammalian target of rapamycin (mTOR) and demonstrated specific pulldown of single molecules from lysed cells.

This method is able to effectively image and uncover the molecular mechanisms of cells, offering new insights into signaling pathways and functions with single-molecule resolution.

Potential Applications

- Imaging released cellular protein and protein complexes
  - Understanding signaling pathways and cell functions
- Analyzing molecular binding kinetics of single molecules

Benefits and Advantages

- Does not require fluorescence labelling and is not susceptible to quenching
- Non-destructive compared with other techniques
- Directly and specifically images the intact individual protein complex immediately after release from lysed cells
- Single-molecule sensitivity and low sample consumption
  - Can measure as low as 25 cells/mm2
- Provides additional information on identifying single molecules
- Can differentiate specific and nonspecific binding by analyzing the binding kinetics
- Specific pulldown of one complex from lysate should report accurate molecular weight
- Has the potential to achieve single cell resolution with single-molecule sensitivity
- Minimal perturbation to the native composition to allow signaling pathway studies and real-time binding kinetic analyses

For more information about this opportunity, please see

Ma et al - ACS Cent Sci - 2022

For more information about the inventor(s) and their research, please see

Dr. Wang's departmental webpage

Dr. Wang’s laboratory webpage