Docket #: S19-004

Quantitative, ultra-sensitive protein footprinting in living cells

Researchers at Stanford have developed a technique to quantitatively measure protein structure and interactions in living cells by protein "footprinting"—monitoring the solvent accessibility of each residue under different environmental conditions. In contrast to existing methods (HDX and hydroxyl radical footprinting), this approach combines high-throughput cysteine mutagenesis with a cell-permeable, enrichable, mass-tagged, irreversible thiol-alkylating reagent that enables highly accurate mass spectrometric quantification. With these advances, solvent accessibility can be measured at high resolution for virtually all of a protein's residues within complex environments, including living cells.

This kind of footprinting can be applied to many areas of biological study where it is useful to understand protein conformation and how that changes under different environmental stimuli, including the addition of a protein or small molecule binding partner. It is particularly valuable for proteins involved in large complexes or membrane proteins that are difficult to purify or otherwise reconstitute in vitro.

Related Technologies:
Stanford docket S19-003 – describing hydroxamate-based affinity tags with size and cost advantages for purifying biological macromolecules.

Stanford docket S19-005 – describing improved tags for separating crosslinked peptides for mass spectrometry.

Stanford docket S19-007 – describing a first-of-its-kind family of protein crosslinkers that can be cleaved and easily detected by mass spectrometry.

Stage of Development
The researchers have demonstrated that their footprinting method in live cells can map a dynamic protein-protein interface.

Applications

- Improved method for high-throughput protein footprinting
- Analysis of protein conformation, interaction and signaling (e.g., determining how different drug candidates induce conformational changes in a target protein)
- For use in vitro or in cell culture
- Could be paired with the purification of a protein of interest or used to probe cysteine solvent accessibility throughout the proteome

Advantages

- The thiol alkylating agents are irreversible, compact, cell permeable, and gas-phase cleavable.
- Provides comprehensive coverage of amino acids, quantitative accuracy and high sensitivity

Innovators

- Pehr Harbury
- Jenna Caldwell
- Bjorn-erik Wulff

Licensing Contact

Seth Rodgers

Licensing Manager, Life Sciences

Email