Docket #: S19-005

Improved extraction of crosslinked peptides for mass spectrometry

Researchers at Stanford have developed a technique for separating crosslinked peptides from unwanted linear peptides. Crosslinks, both naturally occurring and artificial, play a key role in the structure and interaction of proteins and are routinely detected by digesting the protein into shorter peptides that are examined by mass spectrometry. Therefore, it is critical to the throughput and sensitivity of mass spectrometry experiments to separate crosslinked peptides (which have two N-termini) from linear peptides (single N-terminus). The researchers took advantage of this distinction to design two novel affinity tags that bind selectively to the N-termini; only crosslinked peptides will append both tags and will be extracted for purification and analysis by mass spectrometry. The tags include a scaffold containing a hydroxamate moiety (see Related Technology below).

Previous techniques for enriching crosslinked peptides required the use of affinity tagged crosslinkers or particular digestion enzymes that minimized variation among the resulting peptides.

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

Stanford docket S19-004 – describing an ultra-sensitive, in vivo protein footprinting technique using thiol alkylating agents.

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 shown that the affinity tags specifically label N-termini without labeling any side chains and can be removed following purification. Future improvements to the technique include simplifying its application and improving

yield.

Applications

- Discovery and detection of natural and artificial crosslinks
- Analysis of protein structure and interaction

Advantages

- No limits on the crosslinkers or enzymes that can be used
- Automated purification step on commercially available equipment

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