Docket #: S19-003

Compact hydroxamate affinity tags for purifying biomolecules

Researchers at Stanford have developed hydroxamate-based affinity tags with size and cost advantages for studying macromolecules. Affinity tags are commonly used to isolate and purify proteins, lipids, polysaccharides and other molecules of interest from a background of contaminating material. The new hydroxamate tags bind selectively and reversibly to ytterbium metal ions, enabling immobilized metal affinity chromatography (IMAC) using reusable, commercially available resins.

Hydroxamate tags have several advantages relative to other affinity strategies such as biotin-streptavidin binding or alkyne functionalization. In particular, they enable reversible binding with a pH-based elution of bound material, producing pure samples for downstream analysis with high yield and specificity. In addition, hydroxamate tags are compact (60 Da), bio-orthogonal, and highly soluble in aqueous solutions. Because the hydroxamate-ytterbium interaction is distinct from other common binding modes, hydroxamate tags can be used in parallel with biotin tags, for example, in situations requiring multiple orthogonal affinity tags.

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

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
Hydroxamate affinity tags have been paired with multiple different reactive groups to label proteins at thiols or at N-termini. In one test, the new method resulted in an 86-fold improvement in fractional abundance of an affinity-tagged synthetic peptide

in a complex mixture of tryptic peptides, with a 70% yield for the peptide of interest. Subjecting enriched samples to a second enrichment step improved the fractional abundance of the affinity-tagged peptide >300-fold.

Applications

- Isolation and purification of biological macromolecules for downstream analysis (e.g., mass spectrometry)
- Can generally be used in place of or in addition to biotin affinity tags

Advantages

- Hydroxamate tags feature multiple advantages over biotin systems including:
  - Reversible ytterbium binding enables pH-based elution
  - Bio-orthogonal interaction
  - More compact (60 Da vs 244 Da)
  - Yield purer samples for downstream analysis
  - Better solubility properties in water
  - Process uses commercially available, highly reusable resins.
  - Eliminate expensive, single-use reagent (streptavidin resin)

Patents

- Published Application: WO2020154541
- Published Application: 20220054955
- Issued: 12,472,448 (USA)

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