Edman Sequencing Evolved: Precise Native Peptide *C*-Terminal Ligation

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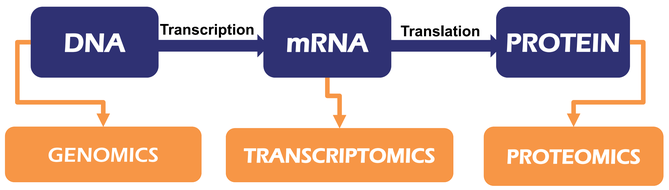
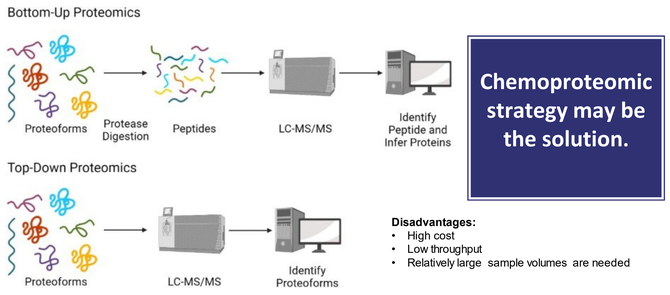
Abstract

Contemporary single-molecule protein sequencing relies on tandem mass spectrometry (LC-MS/MS), employing either top-down or bottom-up proteomics. Bottom-up approaches, which involve proteolytic fragmentation, can lose both post-translational modification data and the contextual integrity of the proteoform. While top-down methods analyze intact proteins, each approach is limited by high costs, low throughput, and large sample requirements, which hinder the analysis of low-abundance proteoforms. To address these challenges, we propose a novel Edman-based multiplexing protein sequencing strategy. This suite of technologies utilizes chemoselective probes and advanced computational techniques to enable comprehensive and quantitative analyses of proteoforms. This presentation will focus on the foundational aspect of our technology: the development of a C-terminal-specific ligation strategy, a critical step that enables subsequent Edman degradation and precise protein sequencing. Contemporary immobilization strategies utilize unnatural amino acids to facilitate the adherence of peptides to solid surfaces; otherwise, the results yield very low returns. ***Here, we report our successful ability to immobilize a native peptide onto a solid surface.***

The Central Dogma



Proteomics

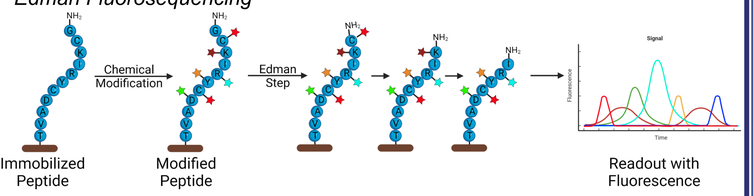


Chemoproteomic strategy may be the solution.

Disadvantages:
- High cost
- Low throughput
- Relatively large sample volumes are needed

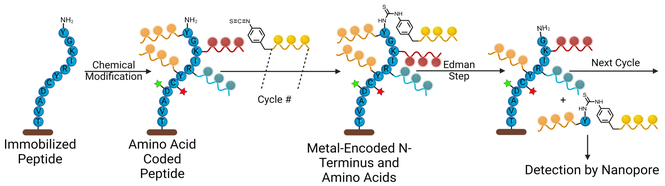
Contemporary Chemoproteomic Sequencing Strategies

Edman Fluorosequencing



- Limited total signal for single molecule detection
- Limited modifiable residues for detection
- Limited modification types only demonstrated with synthetic peptides

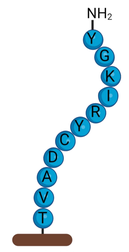
Optimization to Edman Sequencing



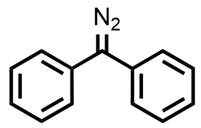
- Alteration of Edman fluorosequencing
- Allows for detection at single-cellular levels
- Improved throughput and multiplexed detection capabilities
- Increased accessibility by reducing cost

New Foundations

- *C*-terminal specific immobilization is a type of enrichment strategy necessary for Edman-based sequencing
- Current state-of-the-art uses either ***unnatural amino acids*** to facilitate the adherence of the peptide to a surface, or results are very ***low-yielding***

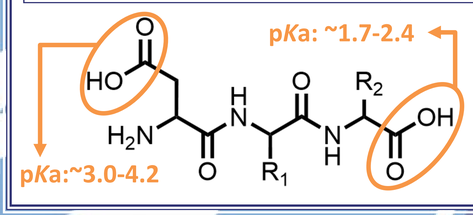


Chemoselective Probe



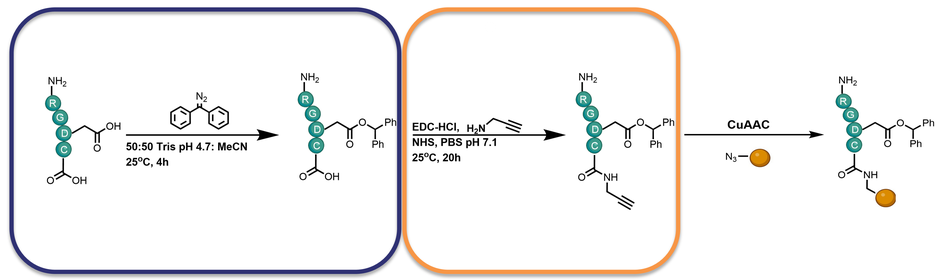
Diphenyl Diazomethane "DPDAM"

Differences in Reactivity



By strictly controlling the pH, we can selectively modify either sidechain or *C*-terminal carboxylic acid residues within a peptide.

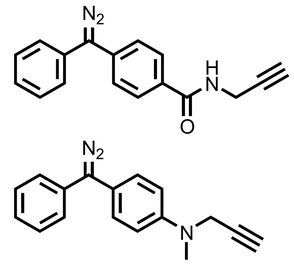
Initial Immobilization Strategy



Utilizing previously established pH control

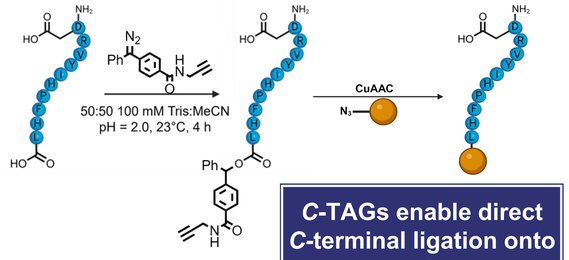
propargyl amine primes peptide for subsequent immobilization strategy

Developing C-TAGs



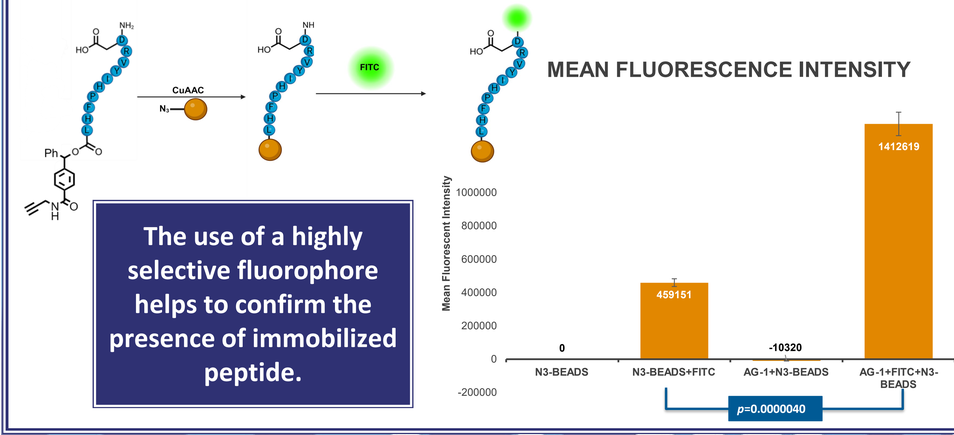
***C*-Terminal Activity Groups**

Optimized Immobilization Strategy



***C*-TAGs enable direct *C*-terminal ligation onto a surface.**

Results of Immobilization Determined via Fluorometric Analysis



The use of a highly selective fluorophore helps to confirm the presence of immobilized peptide.

References

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