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Pulse-Chase Proteomics: Adding temporal resolution to global approaches to study cell biology

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Development of new tools to study proteins in live cells

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Abstract

Cells are composed of highly interconnected networks of proteins, nucleic acids and metabolites. To better understand how proteins modulate cell function, we use mass spectrometry based proteomics to identify proteins and quantify their abundance in cells. However, traditional proteomics experiments are fundamentally limited by temporal resolution.

In collaboration with the chemistry department, we have developed a strategy to synthesize a heavy isotope molecule that can be used to add temporal resolution to proteomics.

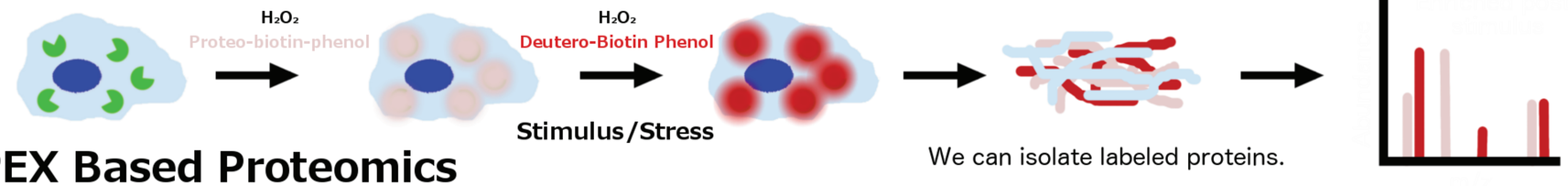
Objctive: To develop a strategy to synthesize deuterated biotin-phenol.

APEX is a promiscuous enzyme that is used to add spatial resolution to proteomics experiments.

APEX labels proteins with a molecule that we can identify.

A second round of APEX labeling can be used to label new proteins with a different molecule.

Mass Specteometry can be used to identify the proteins that were labeled by APEX at each timepoint. Therefore, we can add temporal resolution to our experiments.



APEX Based Proteomics

1. Develop Synthesis Strategy

Synthesis

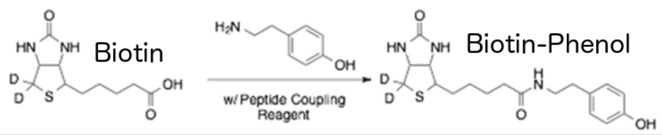
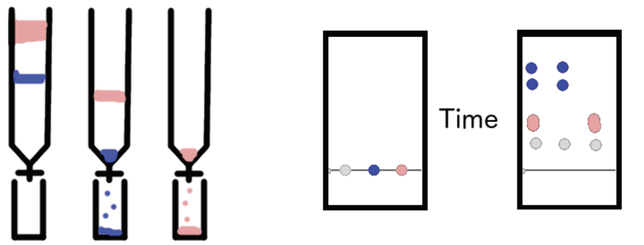


Figure 1: Organic synthesis strategy. Replace select hydrogens (H) on biotin with deuterium (D). Deuterium is a heavier isotope of hydorogen. It has the same molecular properties however is slightly heavier.

Purification



Column Chromatography Thin layer chromatography

2. Confirm Compound

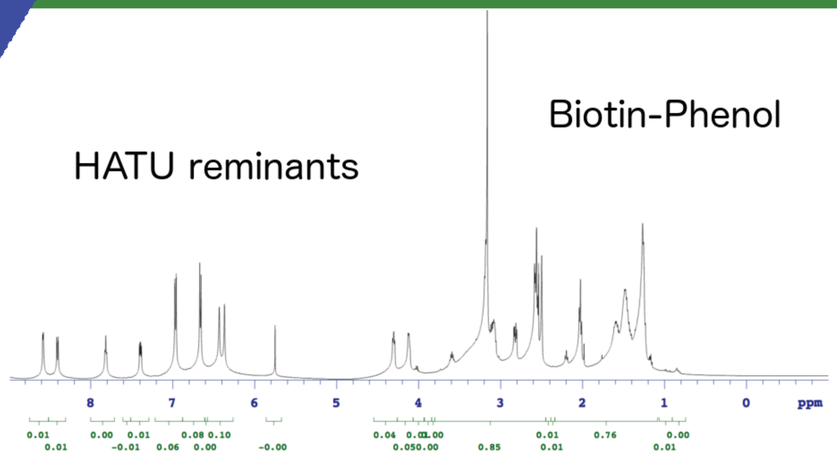


Figure 2: NMR synthesized biotin-phenol. Nuclear Magnetic Resounance (NMR) was used to determine the structure of syntehsized compound fractions. Our successfully purified coupound was 93% by mass biotin-phenol and 7% HATU.

3. Test in Live Cells

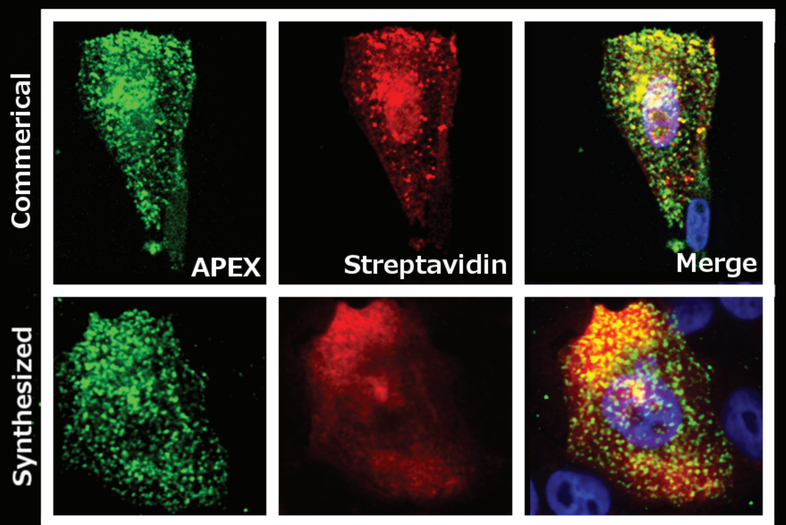
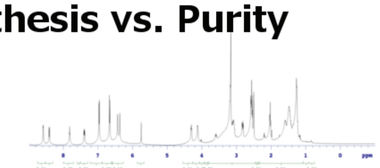


Figure 3: APEX can use both commerical and synthesized biotin-phenol to biotinylate endogenous proteins.

What We Learned

Synthesis vs. Purity



New Technologies

- Organic synthesis, colunm and thin layer chromatography
- Applications of organic synthesis to answer biological questions

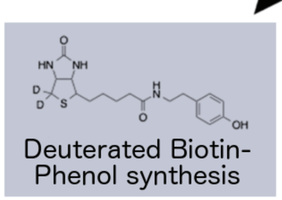
Real Life impacts

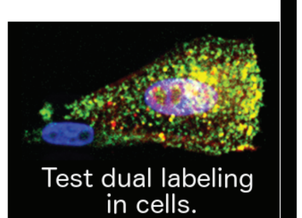
- Forge New Collaboration
- Interdisciplinary Research
- Development of a new technology that enables temporal resolution and dual labeling proteomics experiments.

Library Partnership

- Financial support
- Accelerated Collaboration
- Reference Management
- Reaxys Database Searching

Future directions


Deuterated Biotin-Phenol synthesis


Test dual labeling in cells.

Acknowledgements and References

1. Rhee, H.; Zou, R.; Udeshi, N.D.; Martell, J.D.; Mootha, V.K.; Carr, S.A.; Ting, AY. Science. 2013, 339, 6125, 1328.
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