Synthesis of MS-Labile Crosslinker to Determine Protein-Protein Interaction Networks in Various Biological Systems Using Crosslinking Mass Spectrometry

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Introduction:

- ***Crosslinkers*** are used to serve many purposes such as:
  - Determining domains of protein interactions
  - 3-D structures of proteins
- What are ***crosslinkers***?
  - ***Molecules*** that have two or more reactive ends that are particularly reactive towards specific functional groups
  - ***bind to proteins*** via these functional groups

Objective:

- We want to synthesize a crossinker that is MS-labile and that produces fragments under MS conditions that allows masses of the peptides determined by the name of ***LXR-SEB (Labile Crosslinker Reagent-Succinic Ethanolamine Biotin)***
- This crosslinker will ultimately assist in the future of healthcare by allowing scientists to not only understand more about PPIs (protein-protein interactions) but also about what occurs inside a cell when it becomes diseased

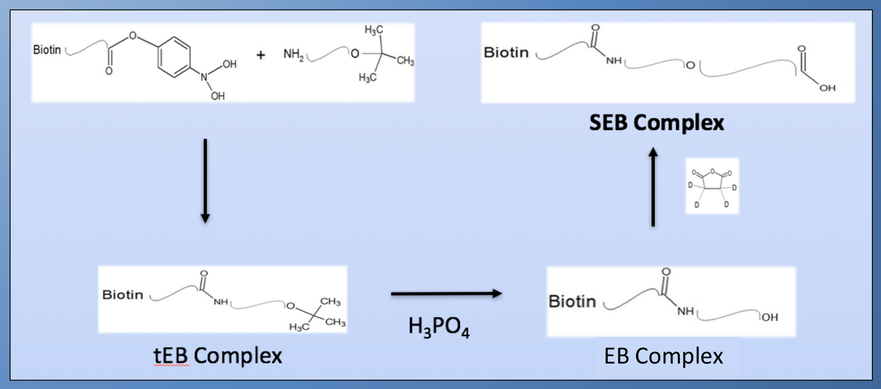
Space Needle, Seattle, WA

Mass Spectrometer, ISB

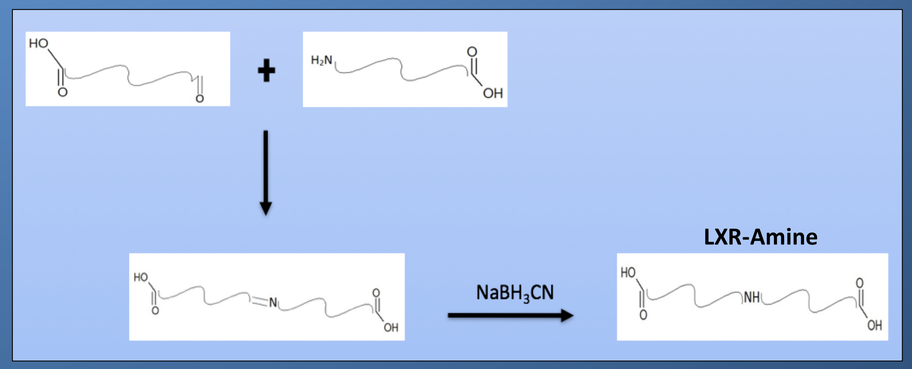
Methods:

Our hypothesis is that the crosslinker fragments, SEB and LXR-Amine, can be synthesized by using the chemical synthesis approach:

- **Synthesis of SEB**



- **Synthesis of LXR-Amine**



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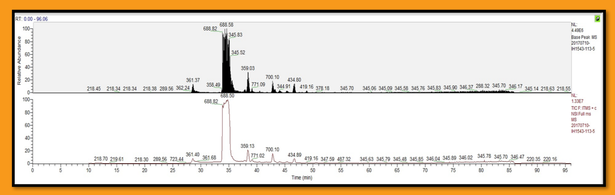
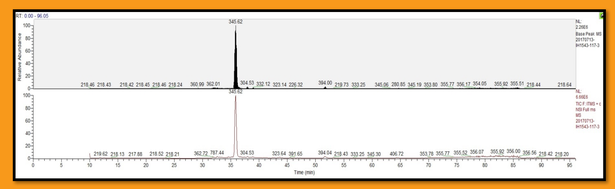
Results:

- Biotin and TBEA reacts in DMF solvent at room temperature to form tEB and side products
- Pure tEB is required for deprotection step
- **FPLC (Fast Protein Liquid Chromatography)** successfully separates tEB from the rest of the side products.
  - tEB produces MS peak at 688 m/z

Before FPLC Purification:



After FPLC Purification:



- Pure tEB can also be deprotected using strong acid
- Formation of SEB requires slightly basic medium (pH=7-9)

Conclusion and Future Studies:

- We designed a new synthetic strategy for the in-solution synthesis of LXR-SEB and the results show that the suggested route produces intermediate complexes
- Further experiments will include fine-tuning the amount of acid for deprotection
- This project will continue on to combine the eventual formation of SEB and the successfully produced LXR-Amine to form the complete crosslinker of LXR-SEB