Synthesis of NTA-Terminated PEG Thiols for CB2 Structural Characterization with Neutron Scattering Techniques

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Goal of Study

- Conventional methods of analyzing integral membrane proteins, such as X-ray crystallography, are lacking in efficacy

- Neutron scattering can be used to characterize the structure of integral membrane proteins
  - Requires that the protein is immobilized on a surface
  - Can use with G-protein coupled receptors, such as the CB2 protein

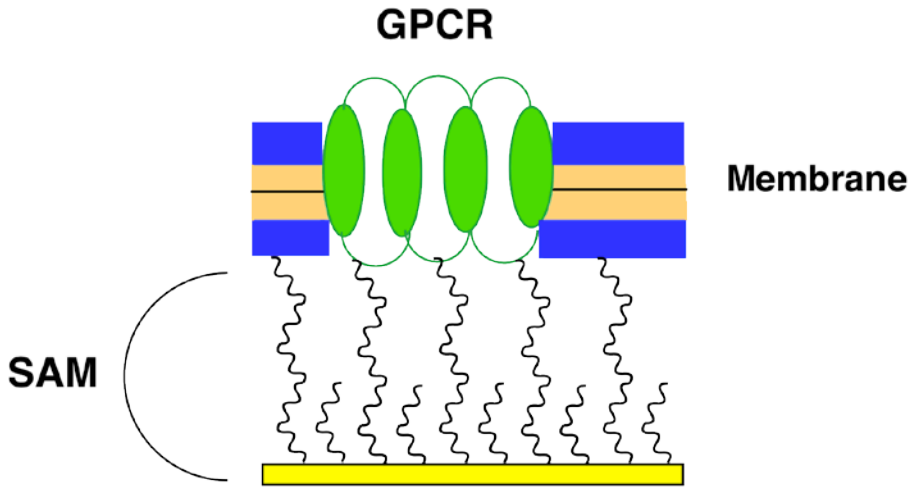
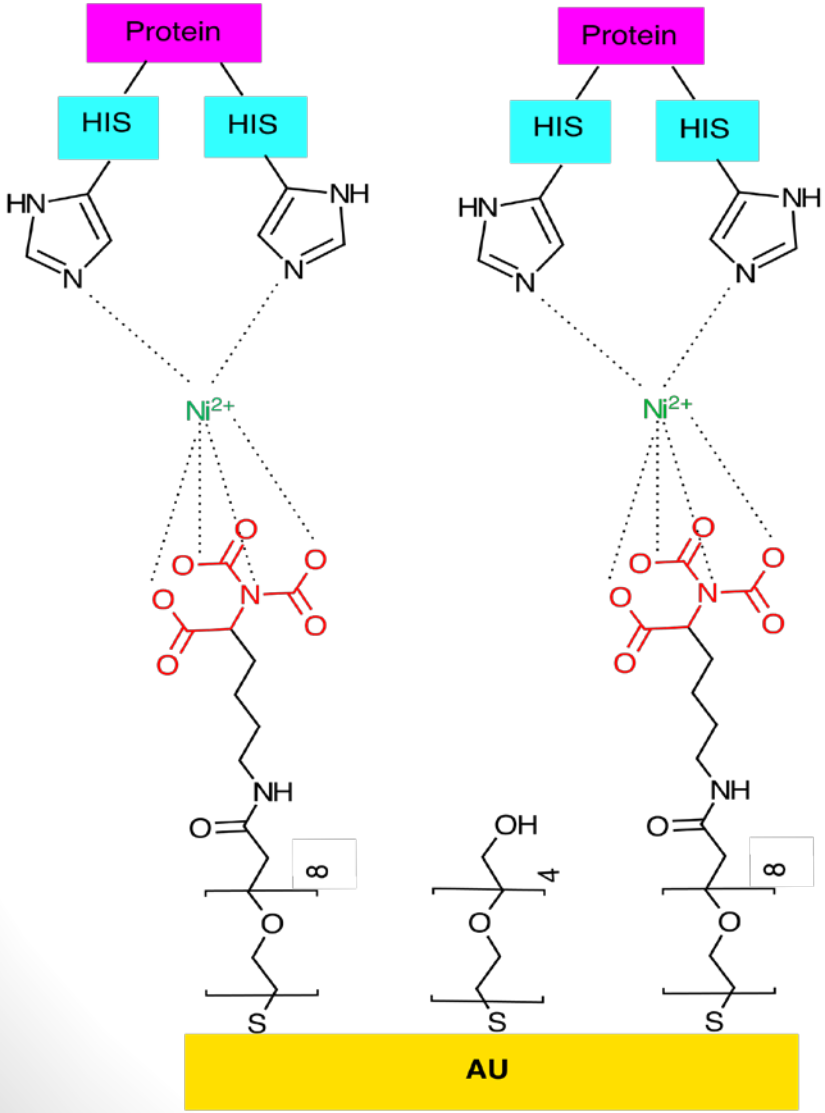


Diagram of Protein Tethering to SAM via NTA and His-Tag



- For recombinant proteins with a His-tag, nitrilotriacetic acid (NTA) groups can be used to link a protein to a SAM consisting of NTA-terminated PEG thiols and a gold surface
  - Each protein is tethered to multiple PEGs
  - Need smaller PEGs to act as spacers in between longer NTA-terminated PEGs

Objective of the Project

Synthesis

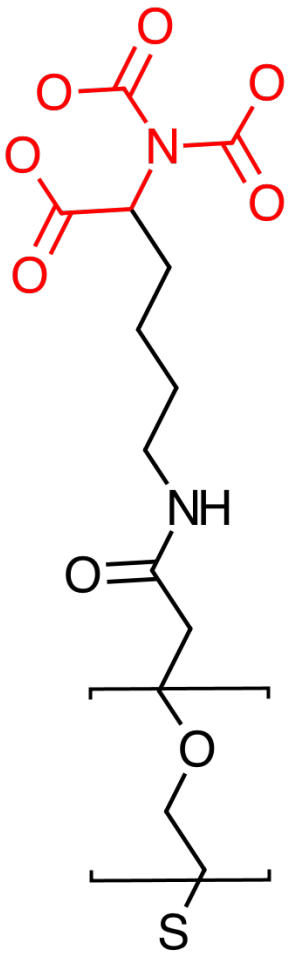
- Use organic synthetic techniques to attach Nitrilotriacetic Acid (NTA) groups to PEG compounds with 8 and 12 ethylene oxide units.
- Conduct a reaction with 4-(Bromomethyl)phenyl isothiocyanate

Purification

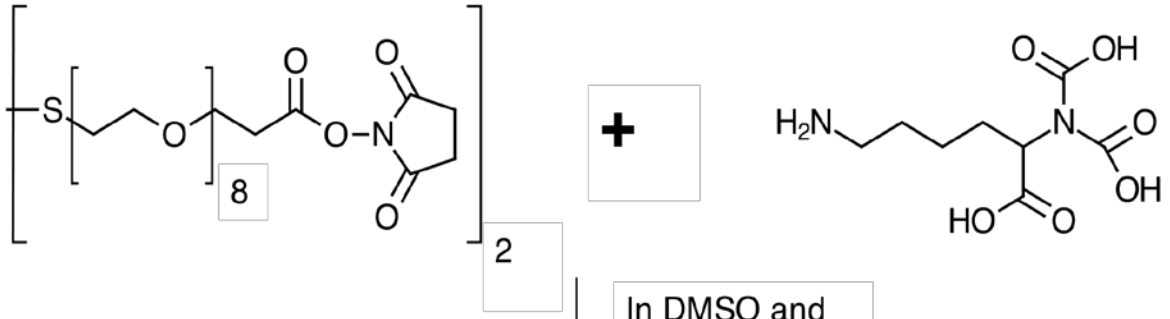
- Conduct purification with HPLC.
- Develop a procedure to scale up purification

Analysis

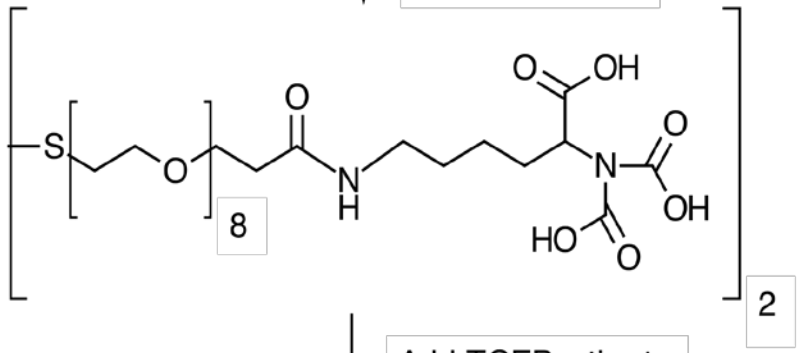
- Assess purity with MALDI-TOF Mass Spectroscopy
- Use SPR to determine if the product synthesized can adsorb proteins.



PEG(8)-NTA Synthesis (EL8)

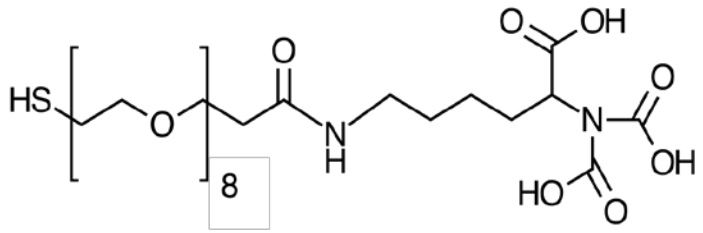


In DMSO and Triethylamine, Stir overnight at 30 C



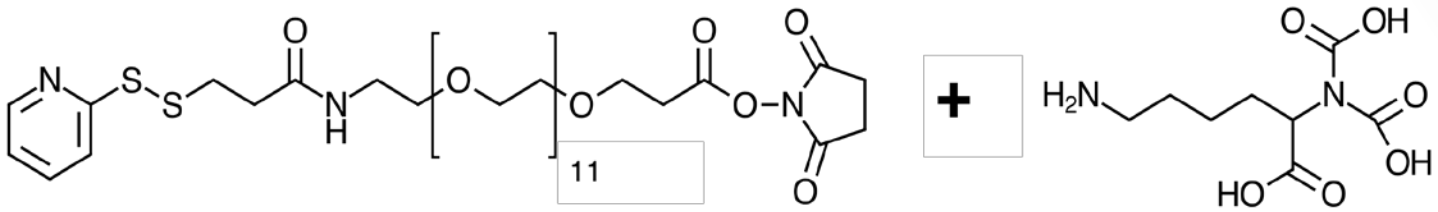
EL8-SS

Add TCEP, stir at room temperature overnight

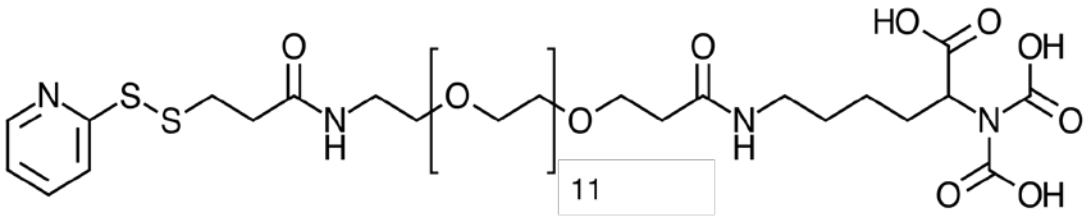


EL8

PEG(12)-NTA Synthesis (EL12)

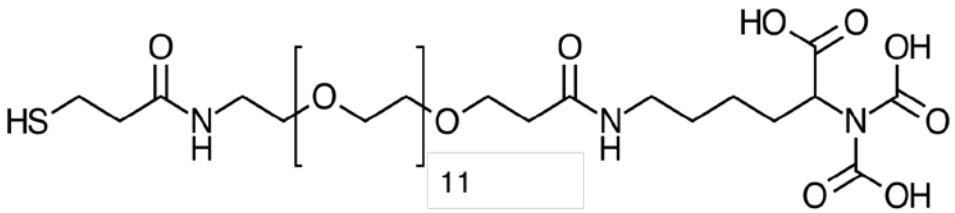


In DMSO and Triethylamine, Stir overnight at 30 C



EL12-SS

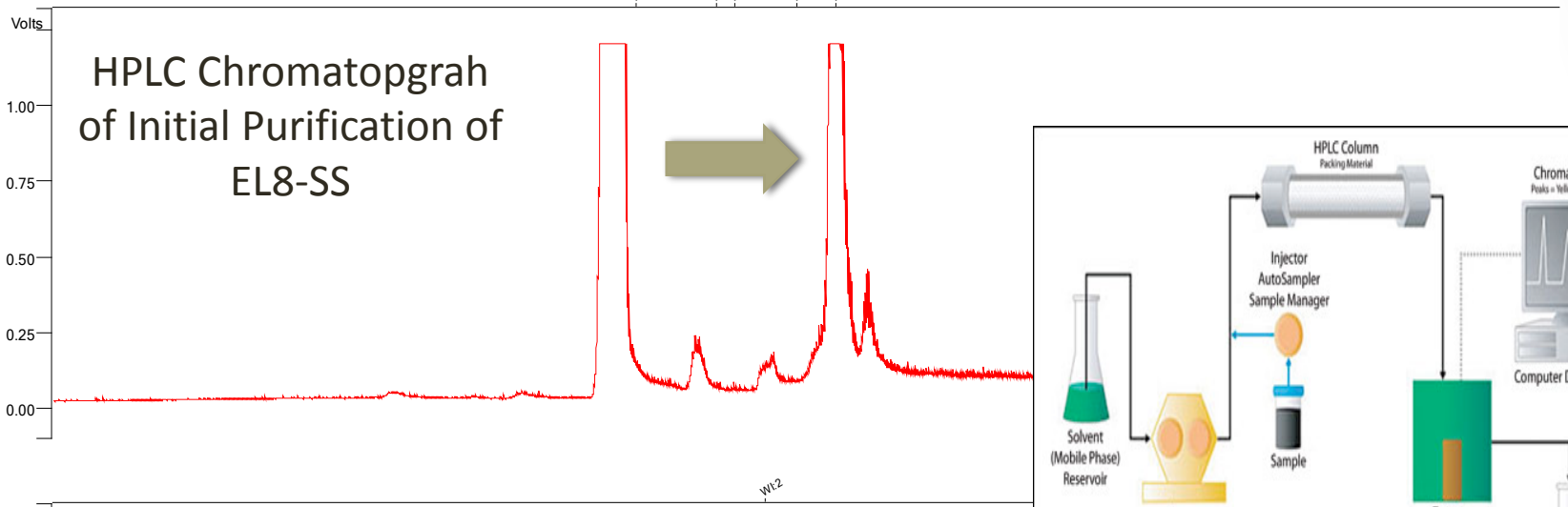
Add TCEP, stir at room temperature overnight

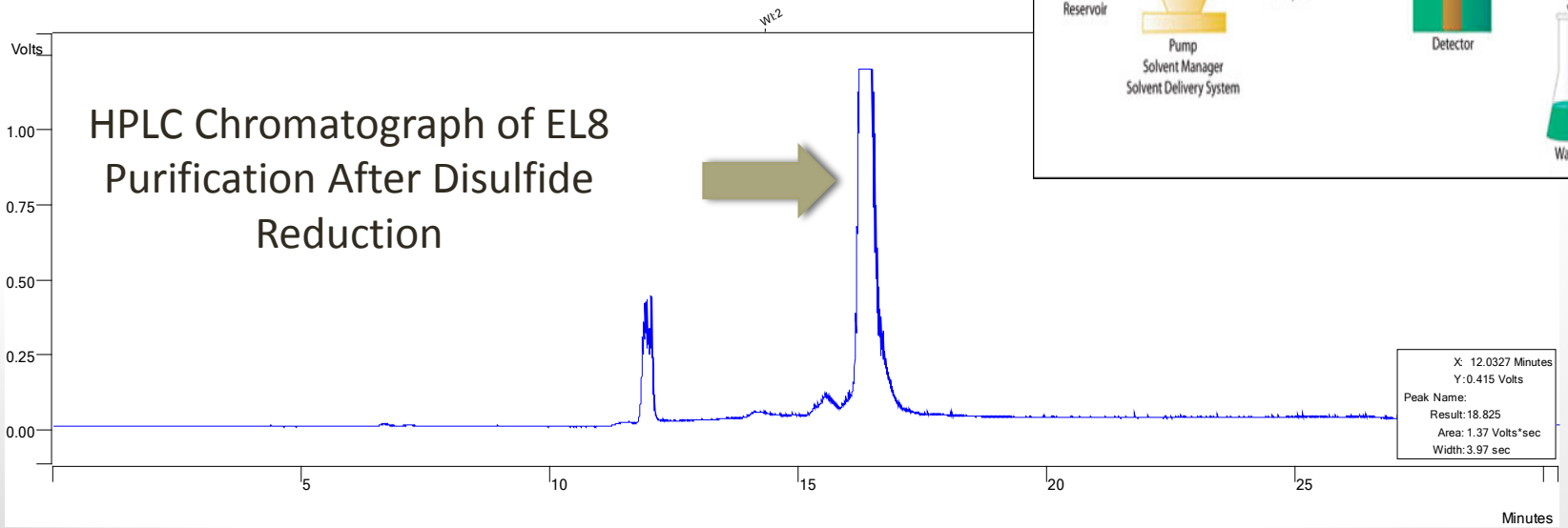


EL12

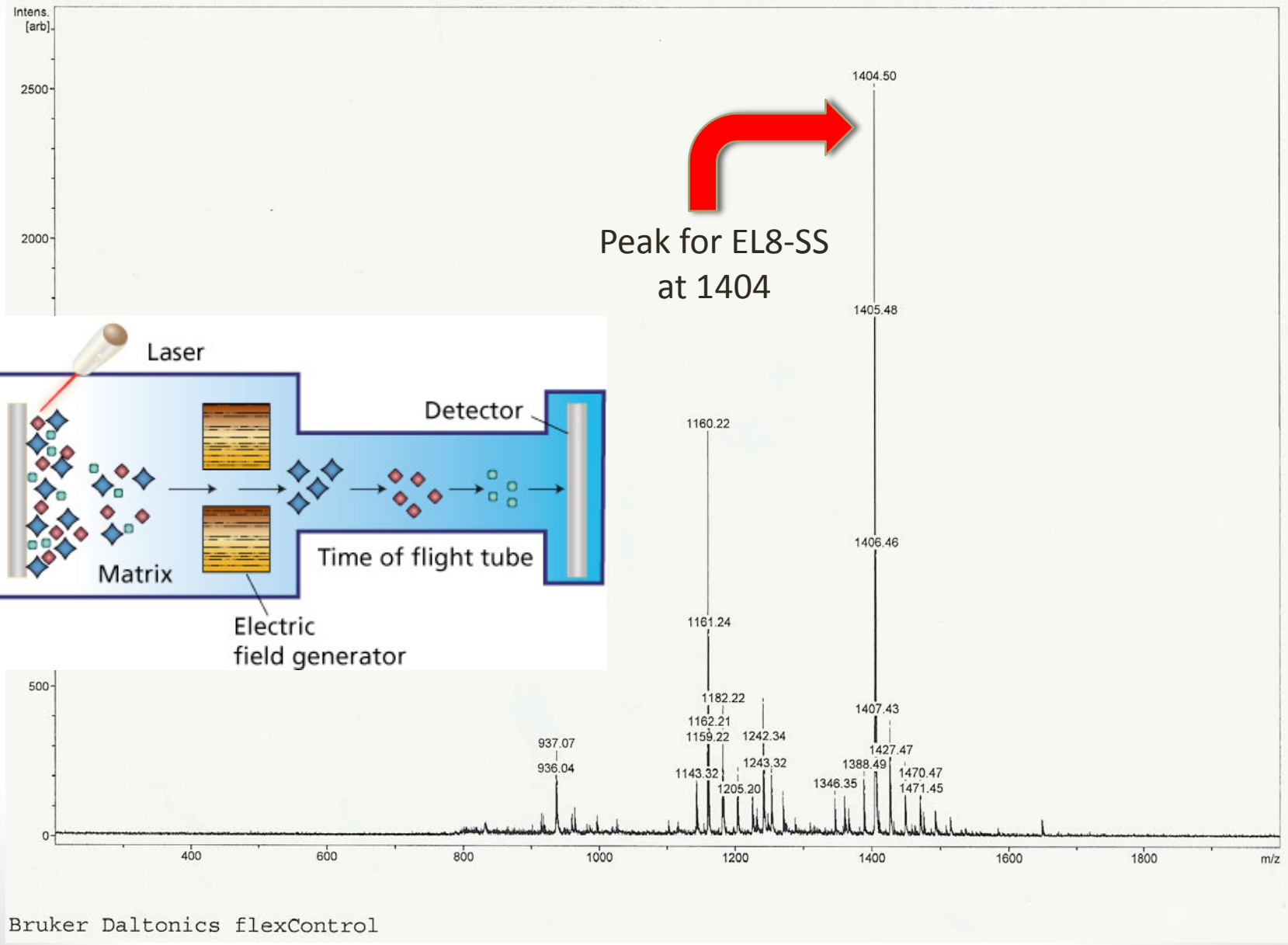
HPLC Purification of EL8

- Mobile phase was a mixture of water (pH 2) and 70% acetonitrile
- Scale up of purification involved switching from a small analytical column to a larger preparatory column, 10X scale up factor

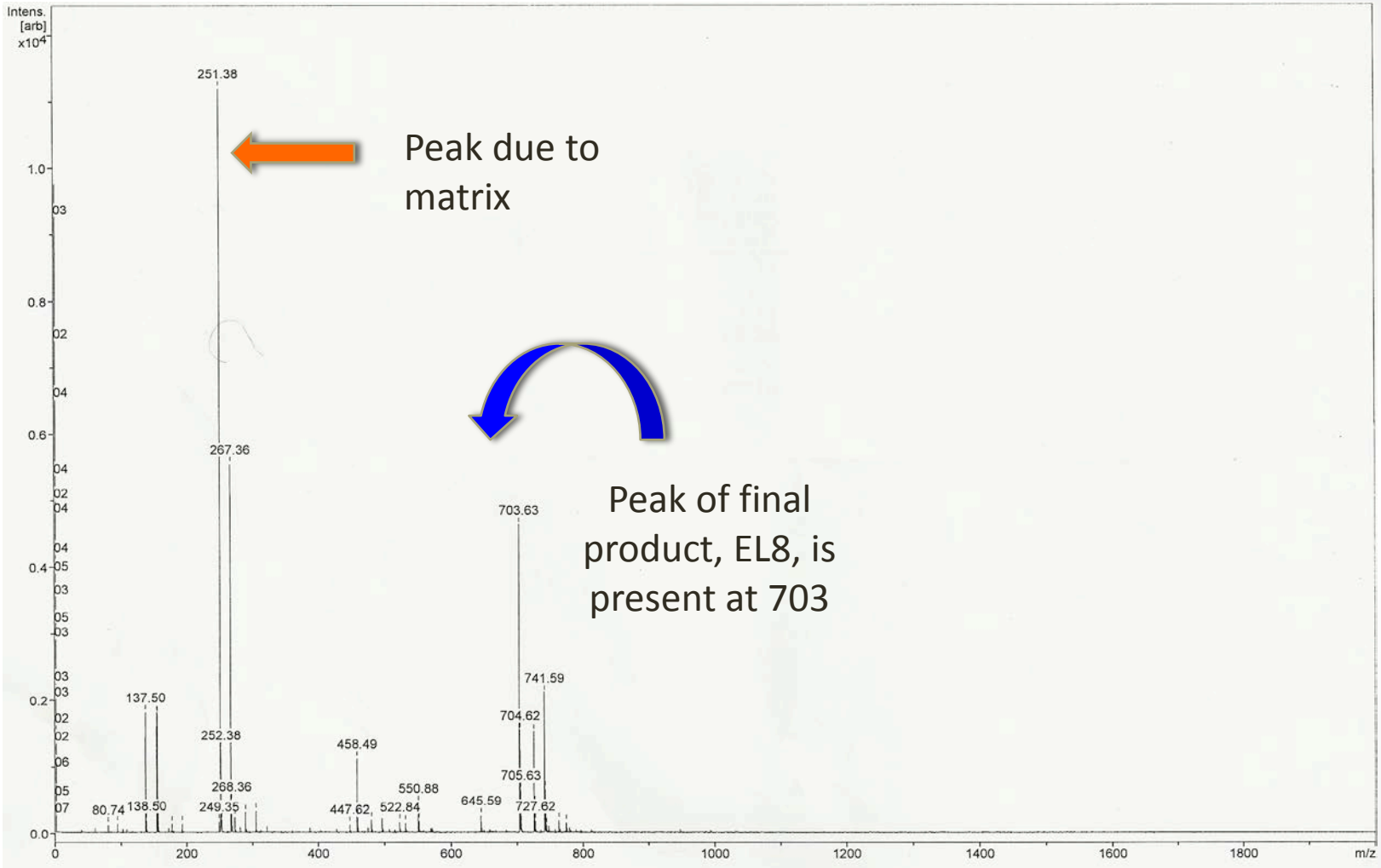




MS of EL8 After Initial Purification



MS of EL8 After Disulfide Reduction

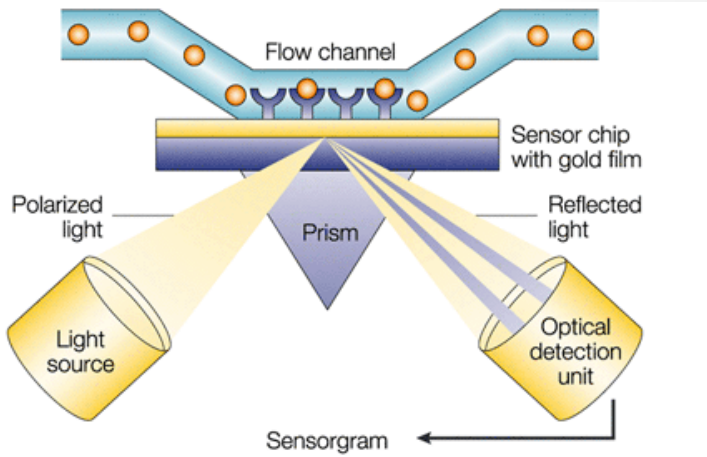


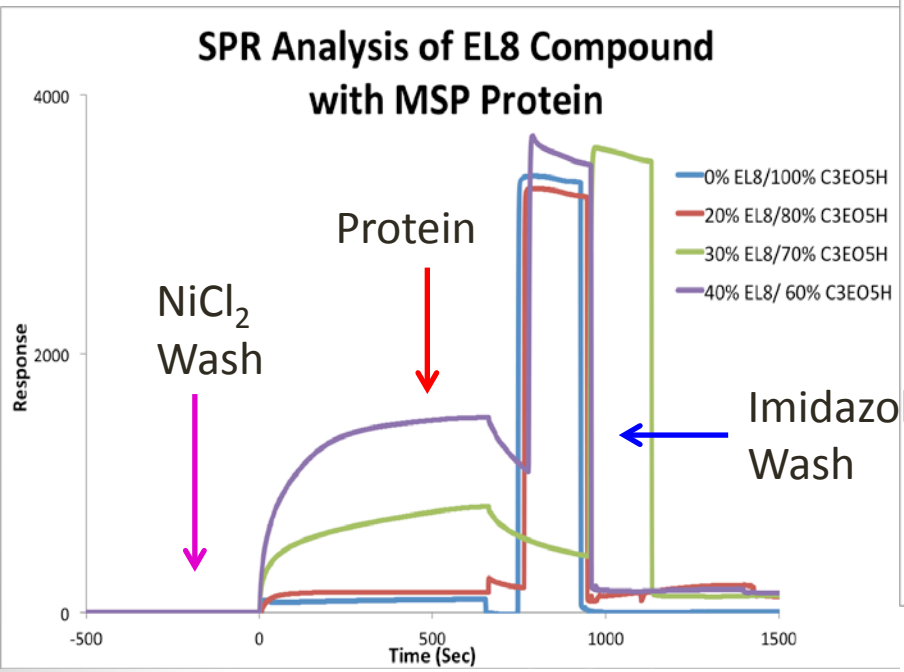
Bruker Daltonics flexControl

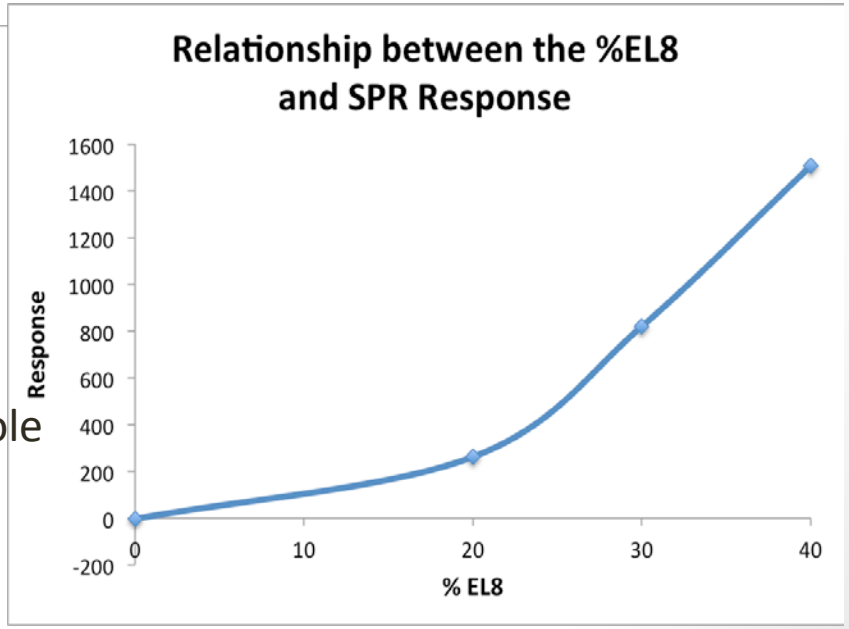
Display Screenshot - Generated On 2012-06-19 15h58m22s

SPR Analysis of EL8

- Analysis was conducted to ensure that protein could bind to the compound synthesized
- Samples for SPR were prepared by soaking the gold layer overnight in solutions of five different concentrations.
- The MSP protein was used







Conclusion

- Two NTA-terminated PEG thiols with 8 and 12 ethylene oxide units were synthesized and purified on a 100mg scale
  - EL8 reaction produced 67 mg, while the EL 12 reaction yielded 44.7 mg.
- A third reaction is currently in progress with 4-(Bromomethyl) phenyl isothiocyanate.
- SPR was successfully used to test protein adsorption onto a surface consisting of one of the compounds (EL8)

Acknowledgments

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