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)

1. In DMSO and Triethylamine, Stir overnight at 30°C

2. Add TCEP, stir at room temperature overnight

EL8-SS

EL8
PEG(12)-NTA Synthesis (EL12)

In DMSO and Triethylamine, Stir overnight at 30°C

Add TCEP, stir at room temperature overnight

EL12-SS

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

HPLC Chromatograph of Initial Purification of EL8-SS

HPLC Chromatograph of EL8 Purification After Disulfide Reduction
MS of EL8 After Initial Purification

Peak for EL8-SS at 1404
MS of EL8 After Disulfide Reduction

Peak due to matrix

Peak of final product, EL8, is present at 703

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)
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