Site Selective Conjugation for Protein Therapeutics

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Introduction

- Polymers are large macromolecules composed of repeating subunits.
- Polymers can be used for drug delivery.
- Proteins can be covalently linked via bioconjugation.
- Pharmaceutical efficacy is related to the structural stability and circulation time.

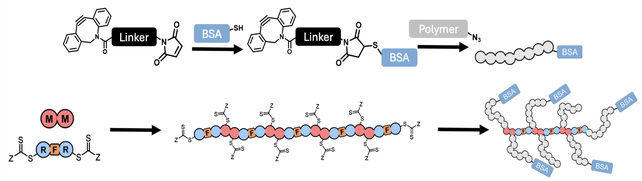


Figure 1: BSA incorporated into a linear polymer and graft copolymer

Objectives

- Conjugate linkers and polymers with model protein BSA
- Analyze conjugation products for successful reaction

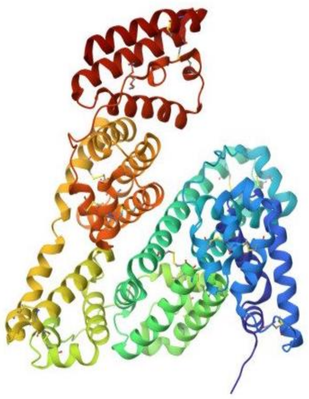
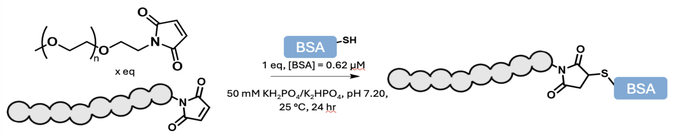
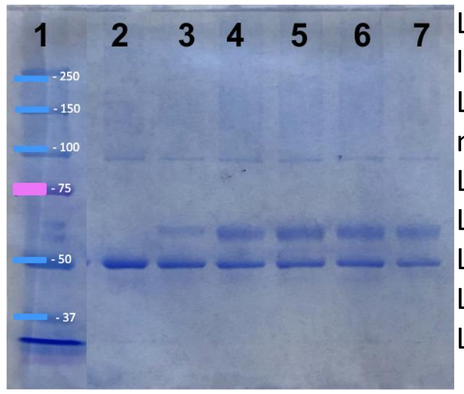


Figure 2: X-Ray Crystal Structure of BSA (PDB: 4F5S)

Data and Results

Figure 3: SDS-PAGE Utilizing Polymer

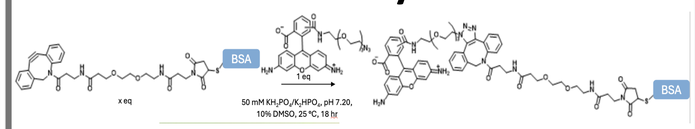


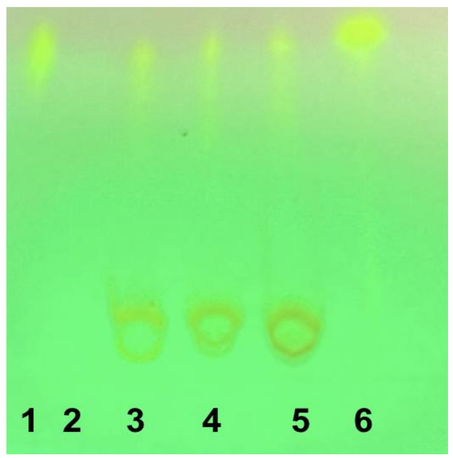


Lane 1: MW ladder
Lane 2: BSA (0.5 mg/mL)
Lane 3: x = 1 eq
Lane 4: x = 5 eq
Lane 5: x = 10 eq
Lane 6: x = 20 eq
Lane 7: x = 50 eq

SDS-PAGE conducted using 7.5% gel. Larger than 1:5 molar ratios led to constant free thiol concentrations

Figure 4: Confirming conjugation via TLC analysis

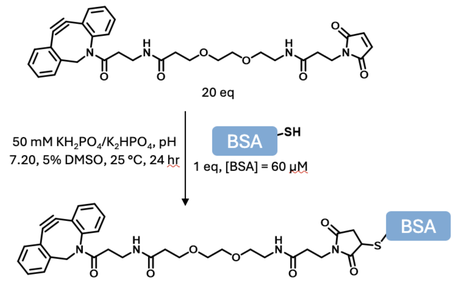




Lane 1: BSA + Fluoro-N_3
Lane 2: BSA
Lane 3: BSA-DBCO + Fluoro-N_3, x = 2
Lane 4: BSA-DBCO + Fluoro-N_3, x = 1
Lane 5: BSA-DBCO + Fluoro-N_3, x = 0.5
Lane 6: Fluoro-N_3

TLC analysis utilizing 70% ethanol as the solvent.

Figure 5: Confirming Conjugation via MALDI



MALDI of the linker with and without BSA. Results show an increase in weight.

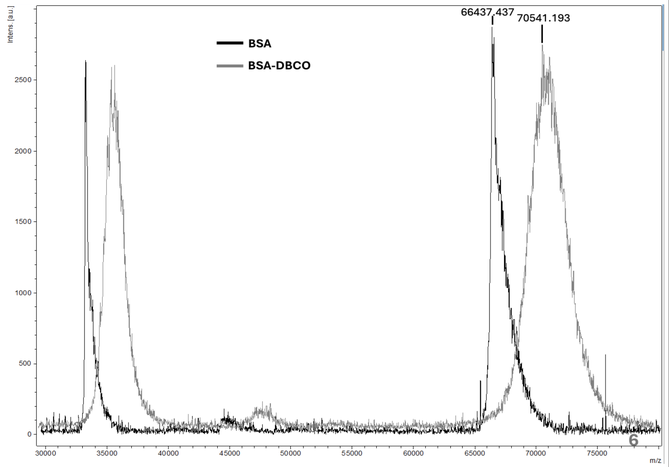
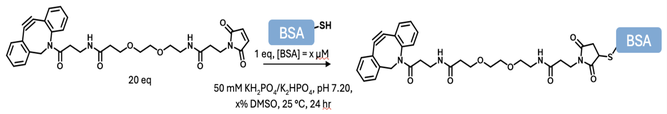
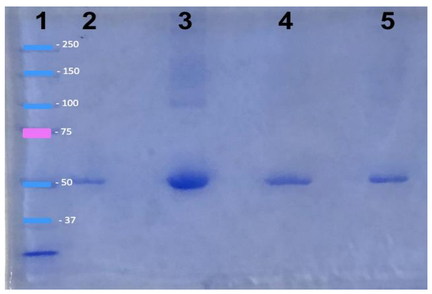


Figure 6: SDS-PAGE Utilizing Linker





Lane 1: MW ladder
Lane 2: BSA (0.5 mg/mL)
Lane 3: [BSA] = 60 μM, 5% DMSO
Lane 4: [BSA] = 6.0 μM, 0.5% DMSO
Lane 5: [BSA] = 0.6 μM, 0.05% DMSO

SDS-PAGE conducted using 7.5% gel. BSA concentrations less than 60 μM led to challenges in downstream characterization.

Conclusions

- MALDI provided uncertain results
- Free thiol concentrations reduced after reaction
- SDS-PAGE indicates bioconjugation and protein aggregation

Future Directions

- Conduct MALDI again to confirm production of products
- Conjugate other proteins to polymer such as Fab
- Utilize graft copolymers

References

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