Bioconjugation and covalent binding of native proteins using azide-alkyne cycloaddition

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INTRODUCTION

~ Bioconjugation reactions are bioorthogonal reactions in which a covalent bond is formed between two molecules, one of which is a biological molecule or its fragment. [1] Such bioorthogonal reactions are also suitable for chemical cross-linking of proteins. [2]

~ We developed a protocol for binding NHS esters, maleimides and benzotriazolides that are functionalized with either an azide group or a cyclooctyne group to proteins (lysine or cysteine residues) and an analytical method for quantifying the binding (loading) to proteins.

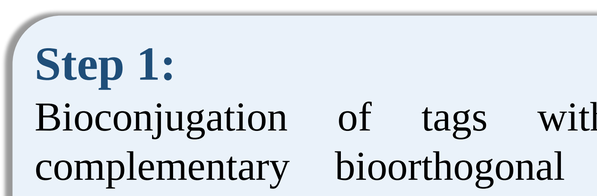
~ Labeled proteins could thus undergo dimerization via strain-promoted azide–cyclooctyne [3+2] cycloaddition reaction (SPAAC), resulting in formation of a covalent 1,2,3-triazole linker. [3]

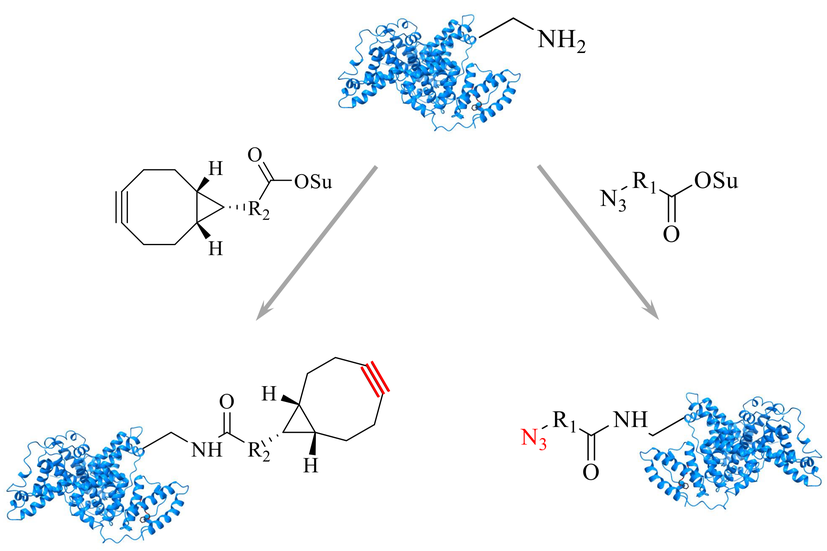
STRATEGY

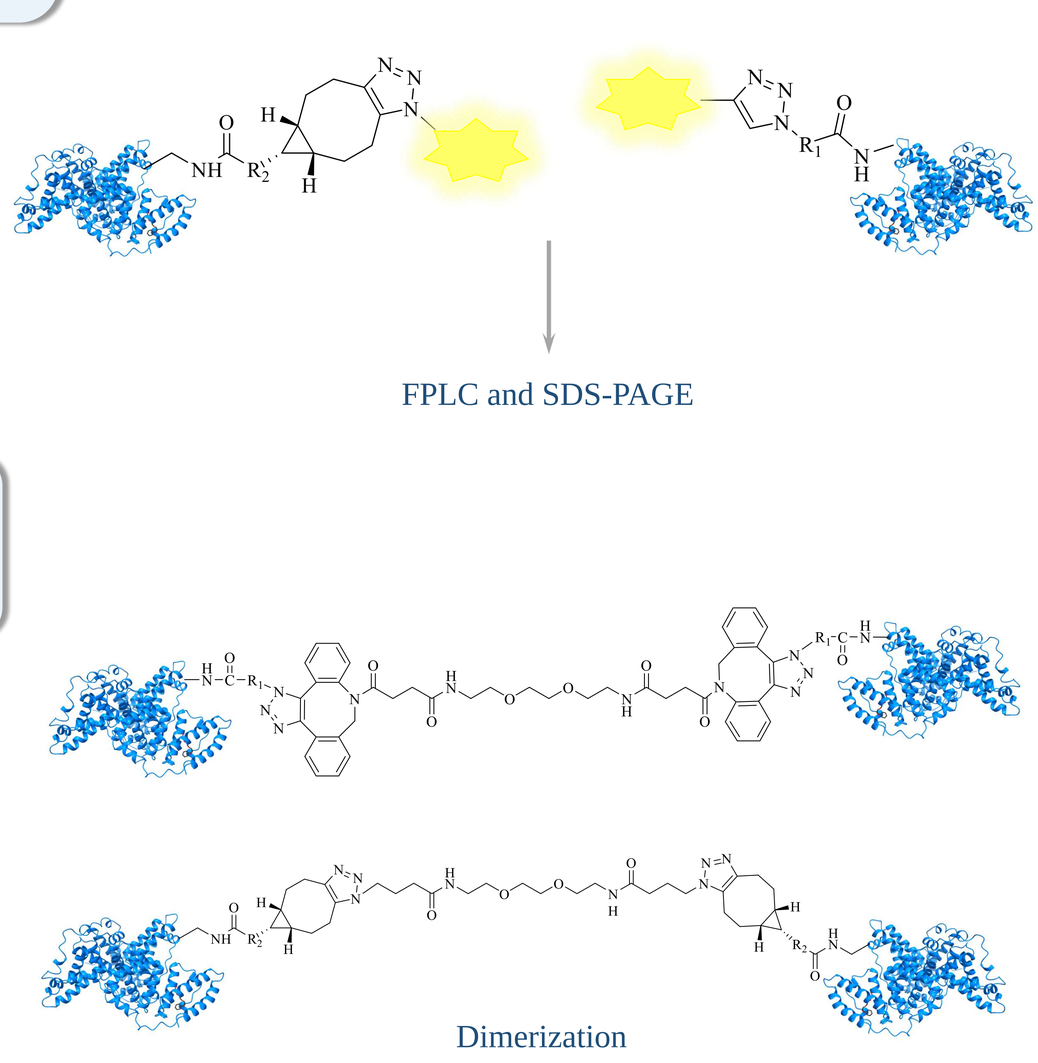
Step 1:
Bioconjugation of tags with two complementary bioorthogonal groups (azide and cyclooctyne group, separately) to the protein.

Step 2:
Spectrophotometrical determination of loading of each bioorthogonal groups with fluorescein derivatives.

Fluorescent probes:

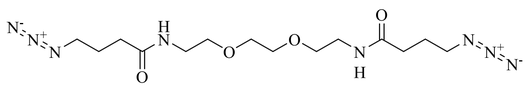




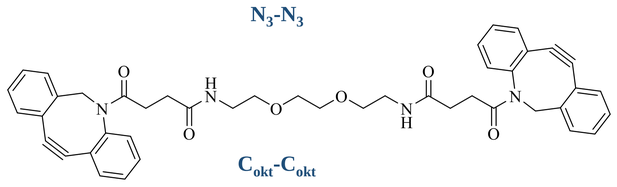


Step 3:
Determination of optimal length of a linker for desired cross-linking.

Bifunctional reagents that act as intermediate connectors:



N_3-N_3



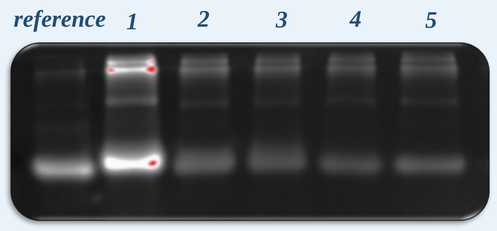
C_{okt}-C_{okt}

Dimerization

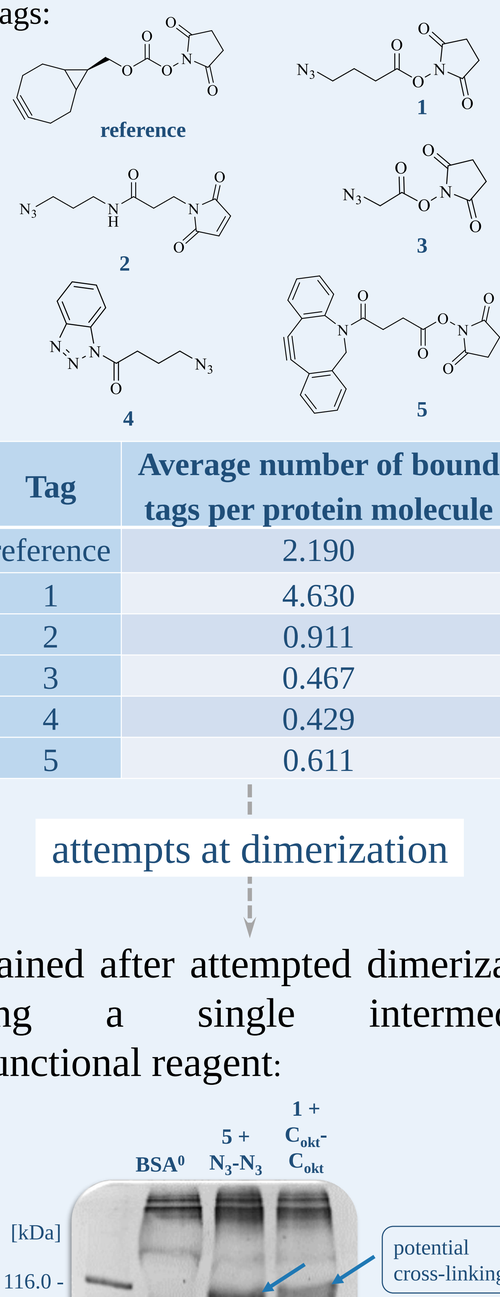
RESULTS

SDS-PAGE gels:

- obtained after reaction between BSA, labeled with tags ref, 1-5, and fluorescent probes and scanned for fluorescein fluorescence:



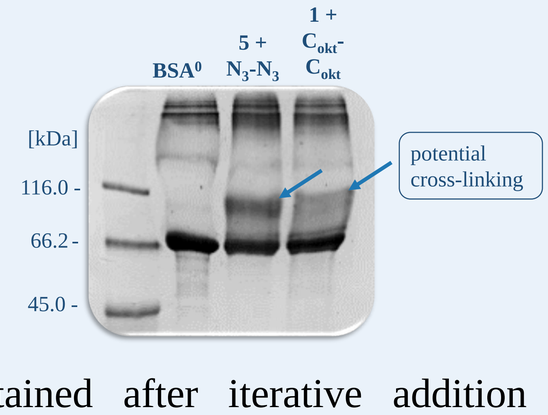
Used tags:



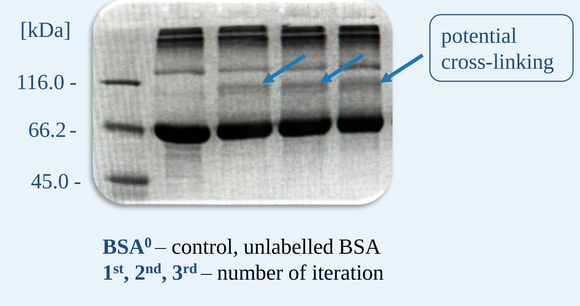
Tag	Average number of bound tags per protein molecule
reference	2.190
1	4.630
2	0.911
3	0.467
4	0.429
5	0.611

attempts at dimerization

- obtained after attempted dimerization using a single intermediate bifunctional reagent:



- obtained after iterative addition of bifunctional reagents to determine the optimal linker length for desired dimerization:



BSA[0] – control, unlabelled BSA
1st, 2nd, 3rd – number of iteration

CONCLUSION

We successfully prepared various NHS esters, benzotriazolides and maleimides that were functionalized with either an azide group or a cyclooctyne group. We developed a protocol for binding these molecules to proteins and an analytical method for quantifying the loading. With known loading of labels on the protein in hand, we were able to perform experiments on protein dimerization via azide–cyclooctyne [3+2] cycloaddition reaction. To extend the linker, we prepared bifunctional reagents that could act as intermediate connectors of the two protein molecules.

References:

[1] G. T. Hermanson: Introduction to Bioconjugation. In Bioconjugate Techniques; Elsevier, 2013; pp 1–125.
[2] N. Forte, M. Livanos, E. Miranda, M. Morais, X. Yang, V. S. Rajkumar, K. A. Chester, V. Chudasama, J. R. Baker: Tuning the Hydrolytic Stability of Next Generation Maleimide Cross-Linkers Enables Access to Albumin-Antibody Fragment Conjugates and Tri-ScFvs. Bioconjugate Chem. 2018, 29, 486–492.
[3] J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. Van Hest, D. J. Lefeber, P. Friedl, F. L. Van Delft: Readily Accessible Bicyclononynes for Bioorthogonal Labeling and Three-Dimensional Imaging of Living Cells. Angew. Chem. Int. Ed. 2010, 49, 9422–9425.

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