Fluorescent Bivalent Antibody Mimics Against Epidermal Growth Factor Receptor (EGFR)

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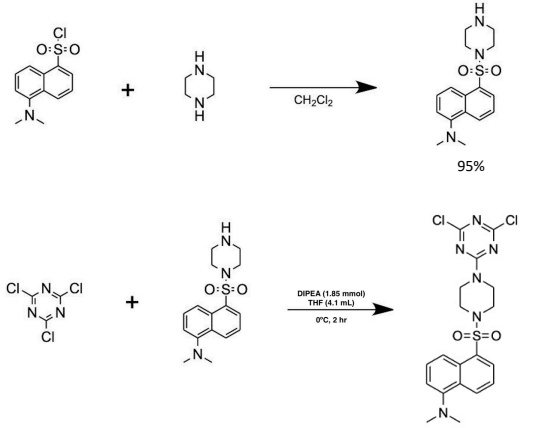
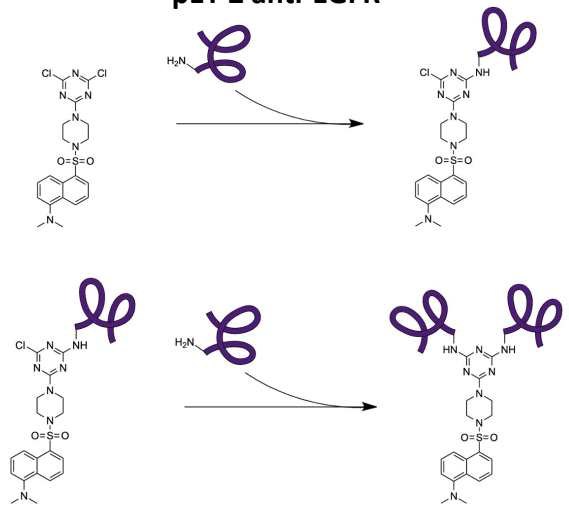
Abstract

This project is aimed to develop triazine-based fluorescent bivalent antibody mimics against the epidermal growth factor receptor (EGFR), a protein disease marker for cancer. A synthetic gene for the anti-EGFR Z-domain was constructed by overlapping extension PCR and inserted to the pET-Z plasmid to produce pET-Z anti-EGFR. The anti-EGFR Z-domain variant was expressed as a C-terminal His-tag fusion in BL21(DE3) *E. coli* cells transformed with the pET-Z anti-EGFR plasmid and purified by immobilized metal ion affinity chromatography. A dansyl fluorophore was attached to the first position of a triazine core that has three positions available for modification. To the second available position of the dansyl-triazine conjugate, an anti-EGFR Z-domain molecule was selectively attached to generate a monomeric conjugate. Another anti-EGFR Z-domain molecule will be attached to the remaining position of the triazine core to produce a dimeric conjugate. We will test the fluorescent monomeric and dimeric anti-EGFR Z-domain conjugates for binding to the EGFR by a standard ELISA method and isothermal titration calorimetry.

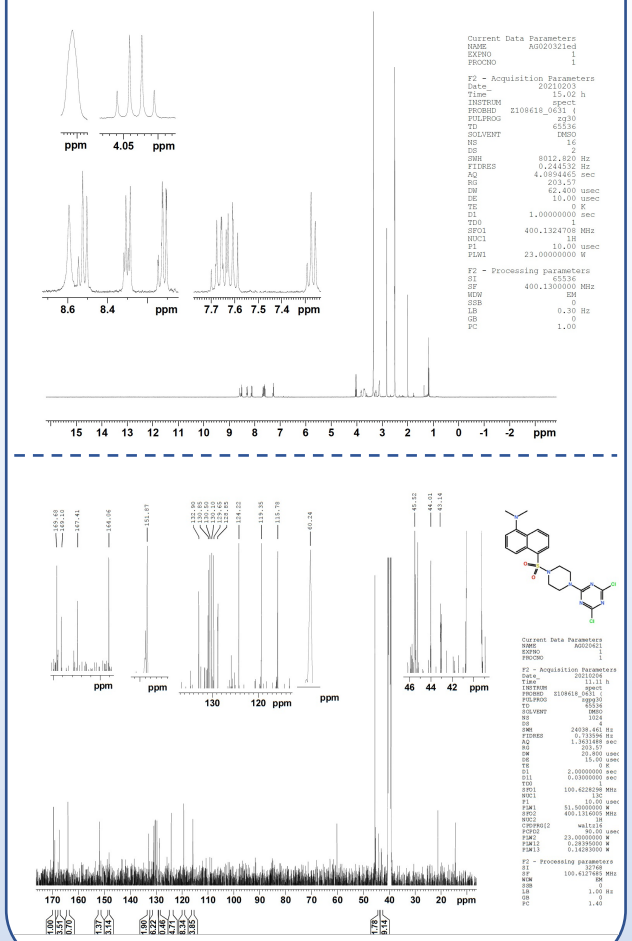
Synthesis of the Dansyl Fluorophore



Conjugation with pET-Z anti-EGFR



Proton (top) and Carbon (bottom) NMR



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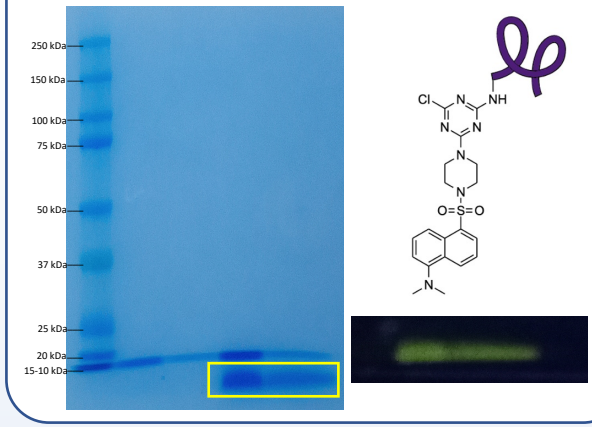
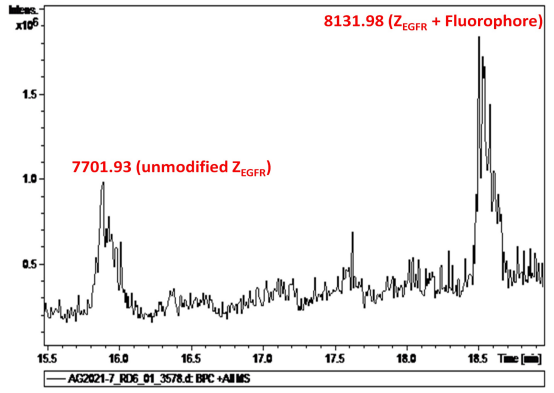


Mass Spectroscopy



Acknowledgements

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