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Application of Strain-Promoted Azide–Alkyne Cycloaddition and Tetrazine Ligation to Targeted Fc-Drug Conjugates

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Targeting
Moiety Attached

Drug Attached


 "Programmed and Armed" Fc-Sec

We have previously described an approach whereby antibody Fc fragments harboring a single C-terminal selenocysteine residue (Fc-Sec) are directed against a variety of targets by changing the peptide or small molecule to which they are conjugated. In the present work, we describe methodology for improving the efficacy of these Fc Sec conjugates by incorporating cytotoxic drugs. The Fc Sec protein is first programmed to target specific tumor cell types by attachment of a bifunctional linker that contains a “clickable” handle (e.g., cyclobutane or cyclooctyne) in addition to a tumor cell-binding peptide or small molecule. Following Fc-Sec conjugation, a cytotoxic warhead is then attached by cycloaddition reactions of tetrazine or azide-containing linker. To validate this approach, we used a model system in which folic acid (FA) is the targeting moiety and a disulfide-linked biotin moiety serves as a cytotoxic drug surrogate. We demonstrated successful targeting of Fc-Sec proteins to folate-receptor expressing tumor cells. Tetrazine ligation was found to be an efficient method for biotin “arming” of the folate-targeted Fc-Sec proteins. We also report novel bioconjugation methodologies that use [4 + 2] cycloaddition reactions between tetrazines and cyclooctynes.

Supporting Information

Materials and methods including detailed synthetic procedures, Fc-Sec programming, arming of Fc-Sec-folate and flow cytometry are included. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Targeted Fc-Drug Conjugates

Supporting Information

**Application of Strain-Promoted Azide-Alkyne Cycloaddition and Tetrazine Ligation to
Targeted Fc-Drug Conjugates**

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General Materials and Methods

All experiments involving moisture-sensitive compounds were conducted under dry conditions (positive argon pressure) using standard syringe and septa protocols. All anhydrous



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