Group : Laboratory of Organic Chemistry – Bioorganic Chemistry
Project : **Development of Masked Antibodies using Click Chemistry**
Supervisors : Maartje Peters, Bauke Albada

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Introduction

Antibody-based therapies hold great promise for current and future cancer treatment. However, their clinical effectiveness is often limited by issues such as "on-target, off-tumor" binding. This phenomenon occurs when the therapeutic antibody binds not only to cancer cells, but also to healthy tissues that express the same target antigen. As a result, less than 1% of the administered antibody typically reaches the tumor site. This poor targeting can lead to unwanted side effects and, in severe cases, even treatment discontinuation. To address these challenges, there is a strong demand for antibody-based therapies with enhanced selectivity for tumor tissue over healthy tissue. In response, we propose the development of so called "masked antibodies" – engineered antibodies that remain functionally inactive during circulation and are selectively activated in response to specific stimuli like tumor-associated enzymes.

Conceptually, we will achieve our goal by installing a masking group, such as a peptide or bulky molecule, adjacent to the antigen-binding site of the antibody that is connected via an enzyme-cleavable linker to the antibody. The enzymatic cleavage of the linker results in the removal of the mask, thereby restoring the antibody's ability to bind exclusively to its target antigen within the tumor. The masking constructs (mask + linker) can be conjugated chemically to an antibody using our established strain-promoted oxidation-controlled *ortho*-quinone (SPOCQ) click chemistry[1]. This allows us to post-translationally modify an antibody in a highly efficient and controlled manner.

Goal

The goal is to develop antibodies that are chemically modified with a masking construct in order to increase the efficiency of antibody-based therapies. The project involves the synthesis of either a linker and/or a mask and potentially the chemical installation thereof onto an antibody using SPOCQ chemistry. In this way you will obtain hands-on experience with a range of techniques focused on synthesis of the masking constructs (*e.g.* SPPS), analyzing the synthesized masking constructs (*e.g.* HPLC, MS and NMR), and evaluating the (un)masking efficiency (*e.g.* SDS-PAGE and BLI).

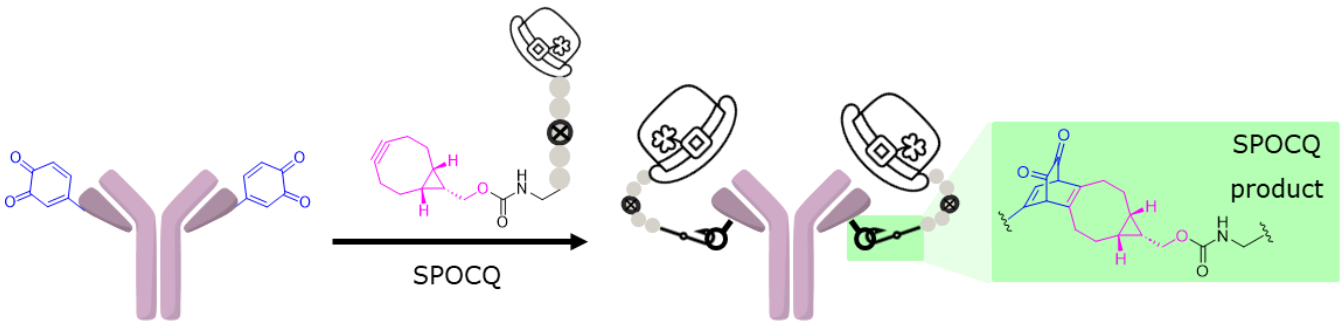

Figure 1. *Schematic representation for the modification of antibodies with masking constructs using SPOCQ.*

Techniques to be used

General organic synthesis techniques: reaction set-up, TLC, column chromatography, (preparative) HPLC, LC-HR-MS and NMR. Peptide synthesis: manual and automated SPPS. Analysis of modified antibodies: SDS-PAGE and BLI.

Information

Bauke Albada, Helix 8057, bauke.albada@wur.nl

Reference

Bruins, J. J.; Damen, J. A. M.; et al. Non-Genetic Generation of Antibody Conjugates Based on Chemoenzymatic Tyrosine Click Chemistry. *Bioconjug Chem* **2021**, 32 (10), 2167–2172.