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# Hinge region modification for the engineering of antibody function

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## COI

All authors declare no conflict of interest

## ACKNOWLEDGEMENTS

We thank Prof. Martin Bachmann (University Hospital Bern, University of Bern) and Prof. Monique Vogel (University Hospital Bern, University of Bern) for carefully reading the manuscript.

## FUNDING

This work was supported by the Swiss National Science Foundation, grant number 191782 to Cristina Hoehener and Swiss National Science Foundation, grant number 188262 to Paul Engeroff

## KEYWORDS

Anti-CD40, hinge region, IgG2, ChiLob7/4, disulfide bonds, agonistic antibody

MAIN TEXT

Monoclonal antibodies are one of the most important biotechnological tools available. They consist of a variable region that can recognize antigens of interest with high affinity, and a constant Fc region that interacts with Fc receptors expressed by immune cells. This unique combination of simultaneous antigen and immune cell receptor targeting makes monoclonal antibodies potent modular drug candidates. Most licensed therapeutic monoclonal antibodies are of the mouse, human, or chimeric IgG isotype due to its long half-life in the serum. Human IgG exists in the subclasses, IgG1, IgG2, IgG3 and IgG4 whereas the four subclasses of mouse IgG are IgG1, IgG2a, IgG2b, IgG3. While there is a strong homology between the different subclasses, their Fc regions still differ in several key aspects that have functional consequences including half-life, antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC)[1]. However, the Fc region alone cannot always explain the diversity of antibody-mediated functional effects. For example, in the case of the human IgG2 anti-CD40 antibody ChiLob7/4, hinge region conformational flexibility was proposed as a regulator of agonistic function[2]. In a recent study, Orr et al elegantly provide convincing evidence that the engineering of the hinge region may be a novel dimension by which the functional effect of monoclonal antibodies could be optimized[3].

In human IgG2, disulfide bonds are formed between cysteine residues C232, C233, C239 and C242 to connect the two heavy chains together and between heavy chain residue C127 and light chain residue C214. Orr et al. show the importance of those disulfide bonds in the hinge region for the agonistic function of ChiLob7/4. They produced several cysteine/serine (C/S) exchange variants of the antibody and analyzed the agonistic properties of those mutants. The order of increasing ability for the agonistic function between the different variants is summarized in Figure 1. Structural analysis revealed that the mutants with higher agonistic ability were able to form crossover disulfide bonds to stabilize the structure, whereas the non-agonistic variants were not able to do so. Furthermore, it was shown that the crossover disulfide bonds in the hinge region result in a more compact and less flexible structure of the monoclonal antibody. Orr et al. propose, that this may promote receptor clustering, which is known to be an important factor in CD40 activation.

Antibody flexibility has previously been shown to be an important regulator of functional activity for the Fc region, for example the elongated Fc region of IgE regulates its receptor targeting and thus shapes effector function via conformational flexibilty[4]. The here-described study was done with Fab fragments and thus demonstrates an Fc-independent effect regulating the binding to the targeted antigen. In the future, the engineering of the hinge region can be a useful tool for the optimization of antibody function including a wide array of therapeutic applications of agonistic antibodies. Anti-CD40 antibodies represent a highly relevant specific example as their ability to activate B cells is thought to be an essential feature for their utility in cancer treatment. Those modifications could also

be combined with other currently emerging engineering approaches that aim to improve the functional effects of the Fc region. For example, the engineering of post-translationally modified glycosylation sites has become increasingly relevant[5]. Furthermore, while the half-life of non-IgG isotypes is significantly lower, their functional properties could have advantages depending on the type of pathology that is being faced. For example, an interesting recent study showed that monoclonal IgA antibodies could be superior for the neutralization of SARS-CoV-2 compared to IgG[6]. In summary, a number of promising tools for the engineering of monoclonal antibodies and their functional effects are on the rise (Figure 2).

**Figure 1: Agonistic activity of hIgG2 anti-CD40 hinge region mutants described by Orr et al:** Cysteine-Serine (C/S) mutants that lead to a disulfide crossover formation present a more compact structure and a lower amount of flexible states whereas the mutants that lack the crossover are more flexible. The C/S mutants become increasingly agonistic with increasing compactness, potentially due to a close CD40 clustering effect that is facilitated by more the compact hinge structure antibodies.

**Figure 2: The major tools for the engineering of monoclonal antibodies**
Established approaches to engineer an antibody include the screening for an optimal antibody epitope (1) and the modulation of the Fc region including the choice of isotype/subclass (2) and/or glycosylations (3) to optimize antibody half-life and functional effects. Orr et al propose a novel approach to modify the structure-dependent agonistic profile of an antibody by engineering its hinge region (4)

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Figure 1

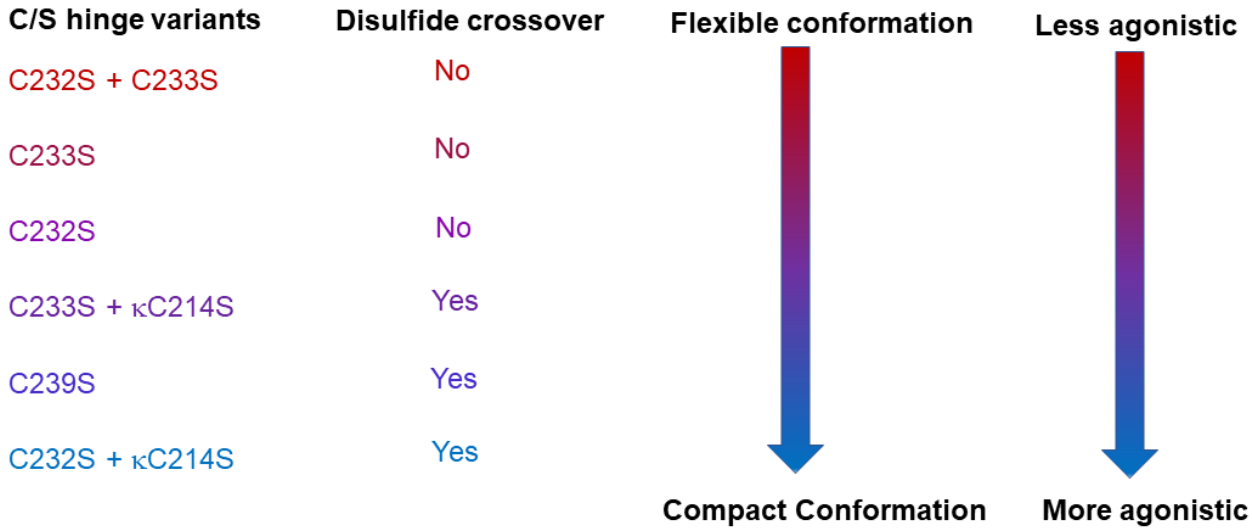

Figure 2