# From Mono- to Bicyclic: Stability and Affinity Enhancement by the Introduction of a Click-Bridge into the Cyclic Peptide 5C6

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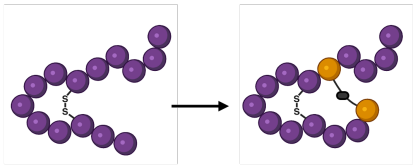
Upon exposure of non-self surfaces (*e.g.,* nanocarriers, hemodialysis filters) to the immune system, activation of the complement system can trigger thromboinflammatory responses that contribute to clinical complications and/or functional impairment.[1] Coating vulnerable surfaces with complement-regulatory modalities has thus emerged as a promising strategy to prevent these putative life-threatening immune responses.

Recruitment of the complement-regulatory protein factor H (FH) by the cyclic peptide 5C6 represents a promising peptide coating strategy to protect vulnerable surfaces.[2,3] However, limited proteolytic stability of the peptide imposes restrictions on the application spectrum. In this work, we employed copper-catalyzed azide-alkyne cycloaddition (CuAAC) to add a second structural constraint to the 5C6 peptide with the aim to enhance resistance to degradation.

1

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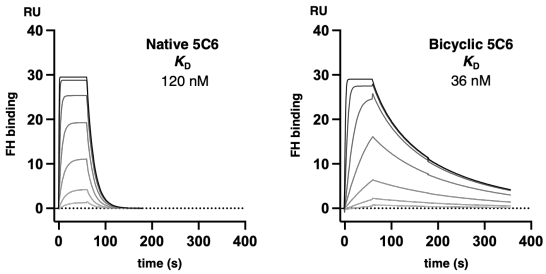


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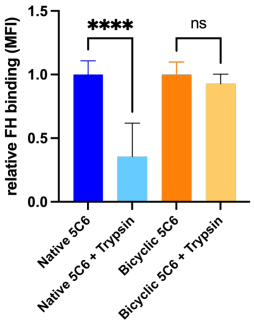


Optimization of the cyclization geometry eventually resulted in a bicyclic 5C6 analogue (**1**) that features a 3-fold enhanced target affinity for FH (**2**) and higher structural rigidity, which was revealed by molecular dynamics simulations based on solution NMR structures of the peptide. In a microparticle model, beads coated with bicyclic 5C6 retained full FH-recruiting capacity upon treatment with various proteases, *e.g.* trypsin (**3**), whereas the monocyclic 5C6-coated beads showed substantial loss of function. Therefore, the exceptional stability of the new bicyclic analogue is anticipated to broaden the application spectrum of FH-recruitment for the protection of non-self surfaces from immune recognition, such as in transplantation medicine, drug delivery, or biomedical research. Moreover, this work more broadly illustrates the facile implementation of solid-phase CuAAC to generate highly constrained bicyclic peptides starting from simple disulfide-monocyclic peptides.

[1] B. Nilsson *et al.*, *Trends Immunol*. **2010**; 31 (1), 32-38.
[2] C. Bechtler *et al.*, *Acta Biomater*. **2023**; *155*, 123-138.
[3] J. Felsch *et al.*, *Adv. Mater. Interf*. **2025**; *12*, 19.