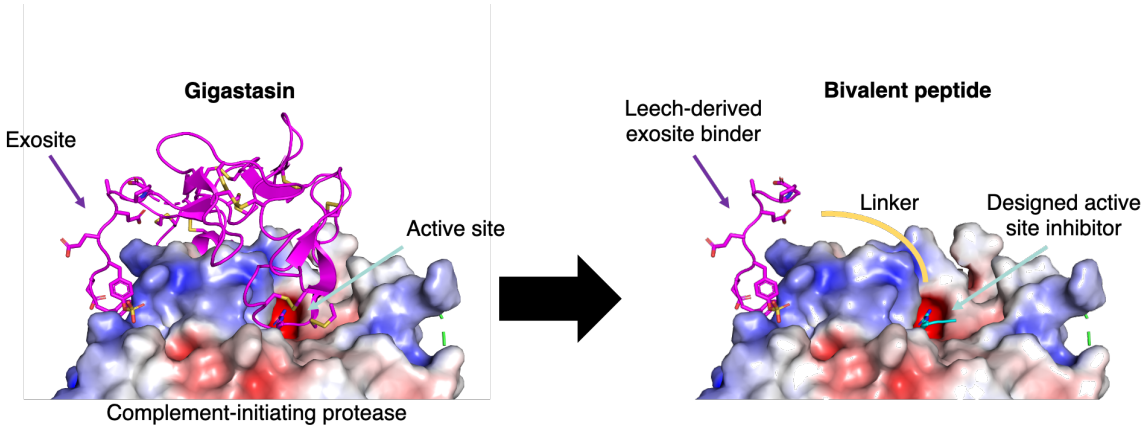
Development of a Leech-Inspired Bivalent Peptide to Inhibit Complement-Initiating Proteases

Alexander John Lander, Aleksandra Blagojevic, Peter Rüthemann, Stephanie Anita Vogt, Oliver Schwardt & Daniel Ricklin

Molecular Pharmacy Group, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056, Basel, Switzerland
landeraj@unibas.ch

The complement system, a natural host-defense mechanism for clearing damaged and microbial cells, can become dysregulated which contributes to a vast variety of inflammatory diseases. Subsequently, therapeutic modulation of complement activity has emerged as a promising clinical intervention, creating an urgent need for new therapeutic strategies.[1] Blood-feeding leeches have evolved to secrete proteins that inhibit complement activation, but their application directly as therapeutics is hindered by immunogenicity and their challenging preparation. Inspired by nature, we have utilized structural insights of the leech protein, gigastasin, in complex with a protease target as a template to design bivalent peptide analogs.[2]



Gigastasin exerts its activity by directly blocking the active site of multiple complement-initiating proteases, whilst remaining inactive against other serum circulating enzymes. Meanwhile, the sulfotyrosine-containing protein tail binds to a distal anion-binding exosite (ABE) utilized for recognition of the native protein substrates. Through rational redesign of known protease substrates, we first created short peptides capable of blocking the enzyme active-site. Conjugation of a lead inhibitor with a peptide derived from the ABE-binding tail of gigastasin, yielded a bivalent peptide with improved inhibitory activity against the complement protease C1s. Subsequently, we demonstrated that the bivalent-peptide analogs are capable of inhibiting complement-activation in human serum via both the classical (antibody-activated) and lectin (glycan-activated) pathways. The lead bivalent analogs will be subject to further structure-activity relationships, while also investigating the potential for fine-tuning the peptide towards specific target proteases within the host-defense system. Collectively, this work illustrates the potential of designing peptides inspired by naturally-evolved proteins – yielding functionality difficult to achieve using de novo discovery efforts.

[1] C. Lamers, D. Ricklin & J.D. Lambris., *Am J Hematol.*, **2023**, *98(**S4**)*, S82-S89.
[2] S. Pang, et. al., *J Immunol*, **2017**, *199(**11**)*, 3883-3891.