Structure-based design and optimization of ligands for novel antiviral strategies

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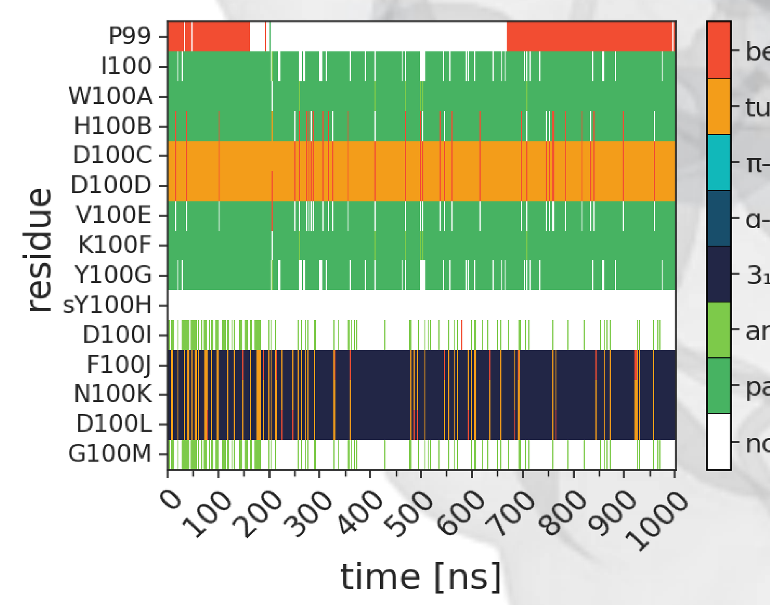
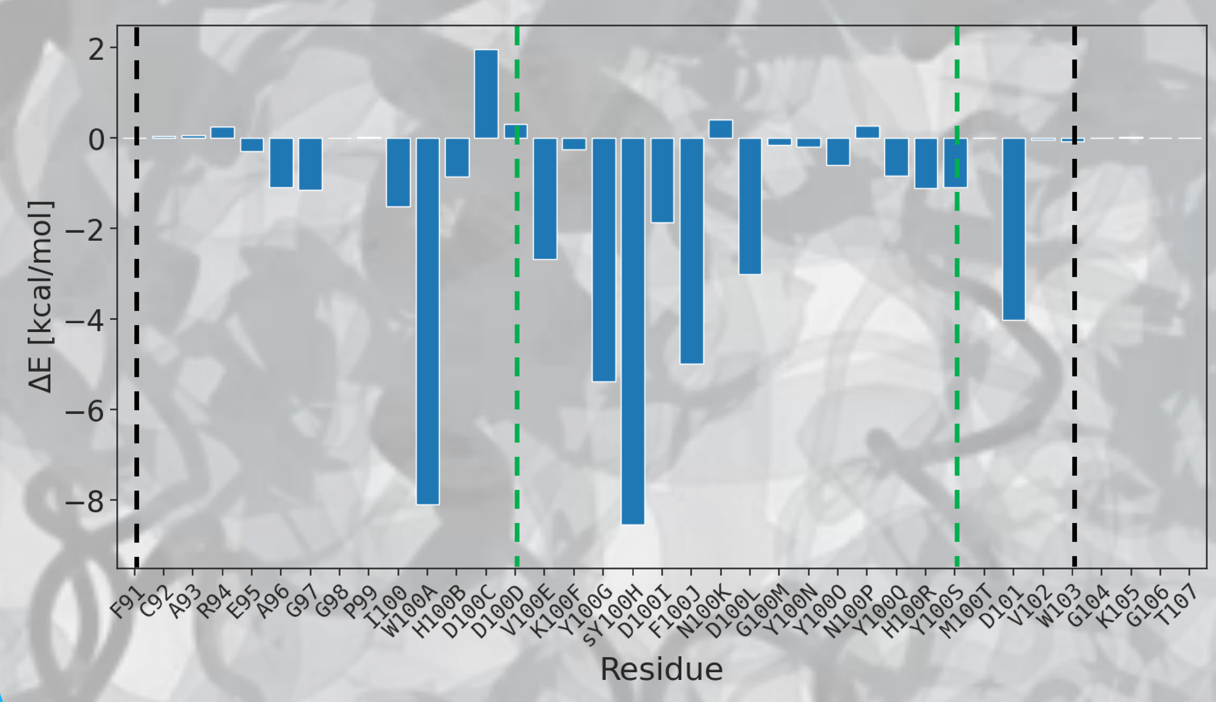
Broadly neutralizing **antibodies** bnAb that bind to viral fusion proteins represent a promising strategy for protection from viral infections. Such antibodies can be used for passive immunization and are currently tested in clinical trials, but they are expensive and difficult to produce. As an alternative, **antibody-derived peptides** may be used for this purpose. Suitable antibody sequences were identified using a newly developed computational **pipeline that identifies interfaces** in complexes of antibodies and viral fusion proteins. Application of this pipeline to 2050 interfaces of HIV-1 antibody-antigen complexes lead to several promising candidate peptides, which were investigated by molecular dynamics (MD) simulations. The first peptides investigated by this **MD-based optimization** approach are from a **sulfo-tyrosine (sY)** containing broadly neutralizing antibody PG16. Optimization of the peptide length is based on the **energetic analysis of the complex interface**, which particularly focuses on the **roles of glycans** in the interaction. In addition, the effect of peptide **cyclisation** was assessed from microsecond MD-simulations of the free peptides. This approach resulted in a high-affinity peptide ligand that was experimentally demonstrated to exhibit a nanomolar affinity for the HIV-1 gp120 protein.

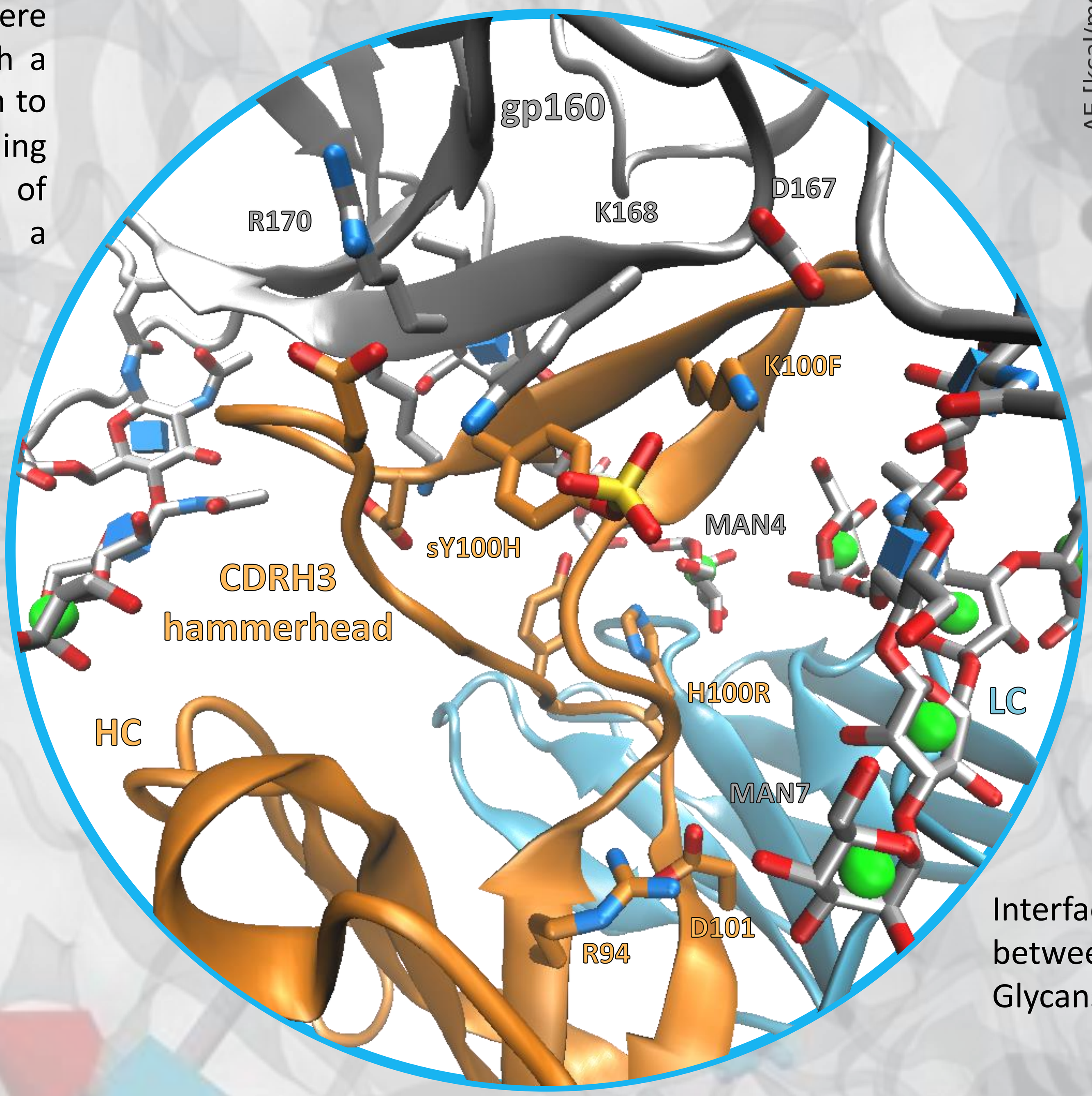
The pipeline approach yielded many top scoring antibody-antigen complex-structures containing the post-translationally modified amino acid **sulfo-tyrosine (sY).** To investigate the effects of sY in PG16's highest scoring sequence window 1 µs simulations of PG16 with sY in explicit solvent (TIP3P) where performed. The crystal structure of PG16 in complex with a fully glycosylated **gp160 trimer** (PDB: **6ULC** [1]) was chosen to further investigate the **effects of glycans** on PG16's binding ability. The complementary determining region CDR 3 of PG16's heavy chain is 26 residues long and adapts a **hammerhead-like shape.**



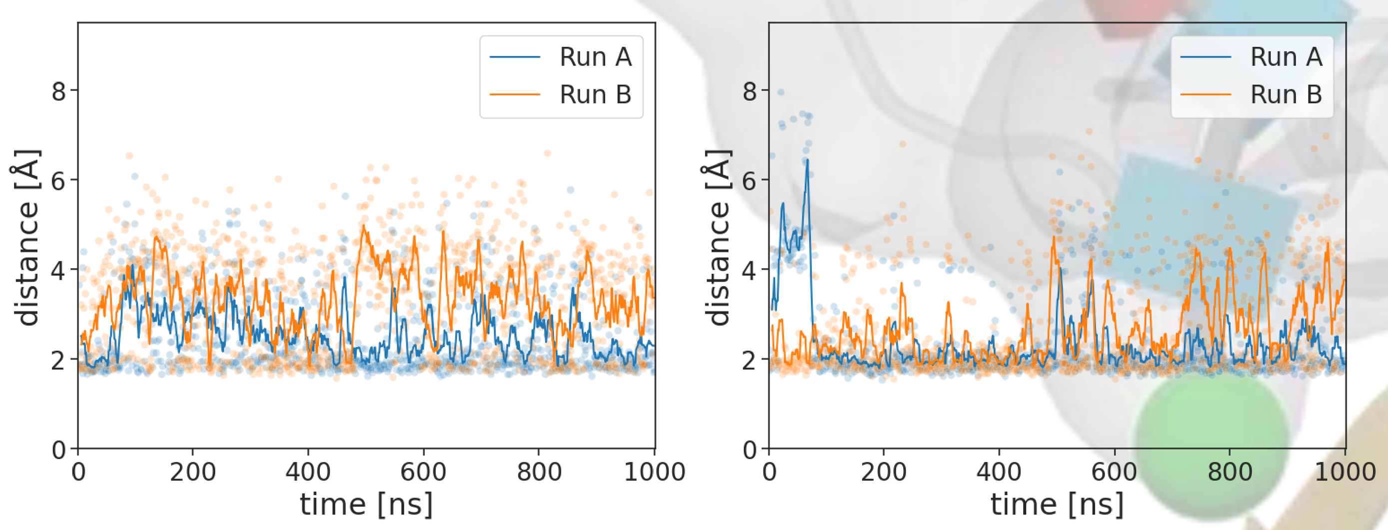
Secondary structure of PG16's hammerhead remains stable over the entire simulation time. The intricate CDR is stabilized by the antibody's framework region and intermolecular interactions



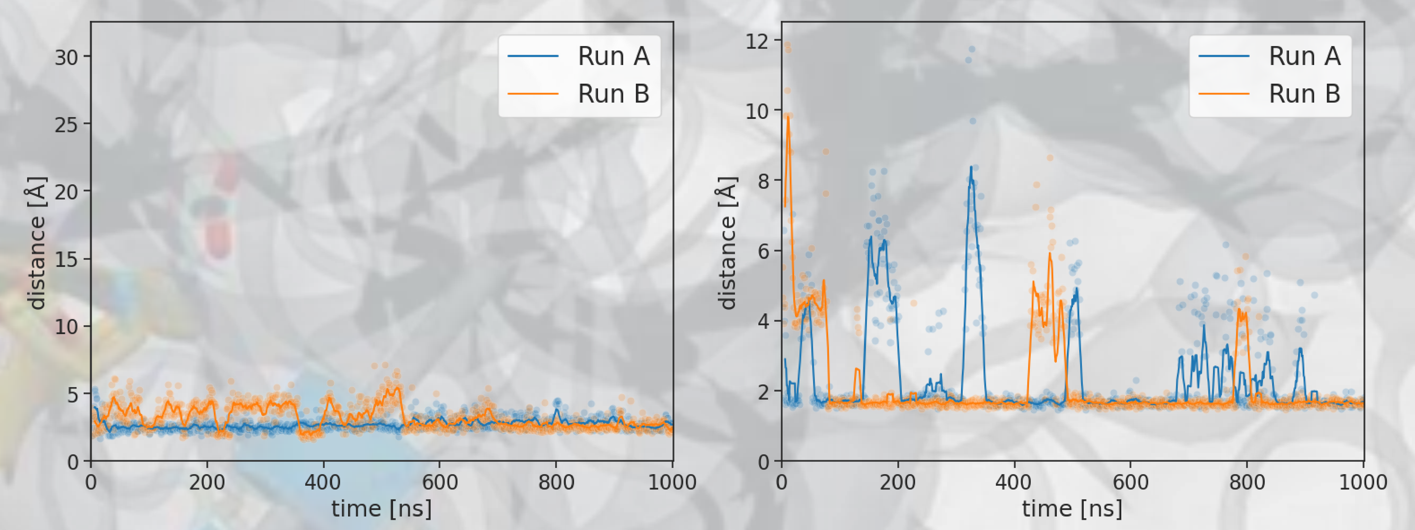
Energy contributions of individual residues in the CDRH3 of sY-PG16. The **green bars** entail the top 15 residues provided by the pipeline and the **black bars** the residues required to construct a peptide with additional major residues.



Interface of the PG16 binding site with **gp160**. Key interactions between antibody light chain and heavy chain are highlighted. Glycans are represented in SNFG nomenclature.



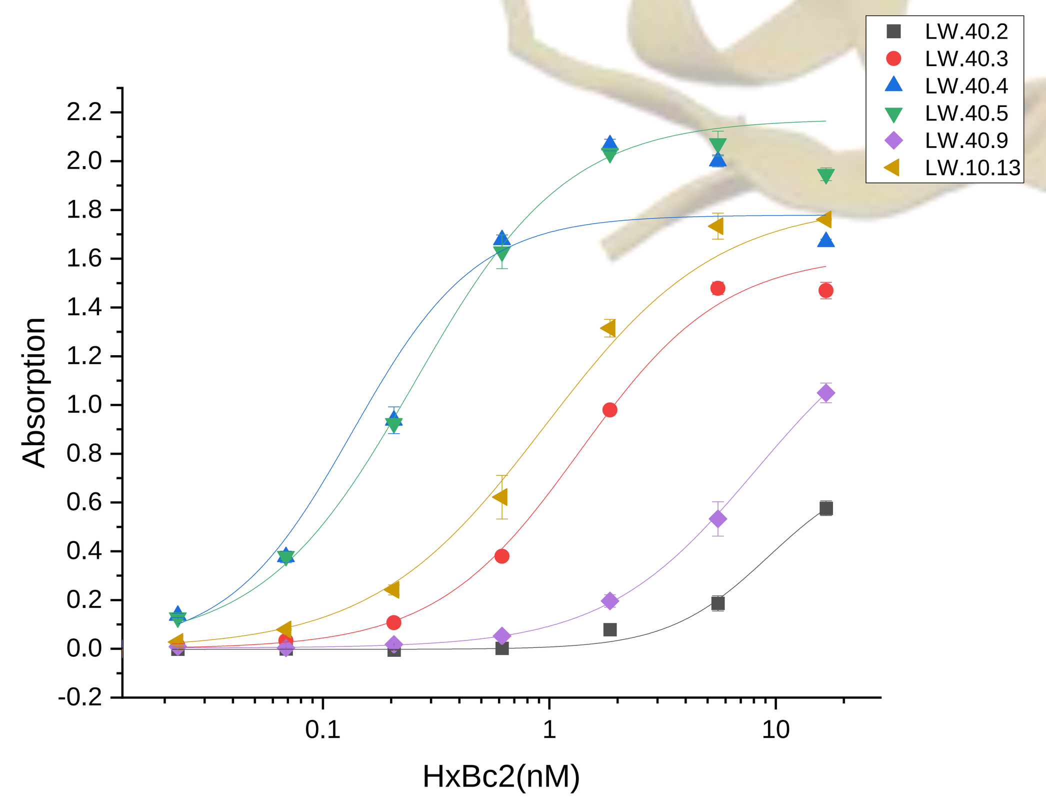
Salt bridge between sY100H of PG16 and K168 of gp160 (left) is one of the key interactions. Intermolecular salt bridge between sY100H and K100F (right) in PG16's CDRH3 is aligns key interaction partners.



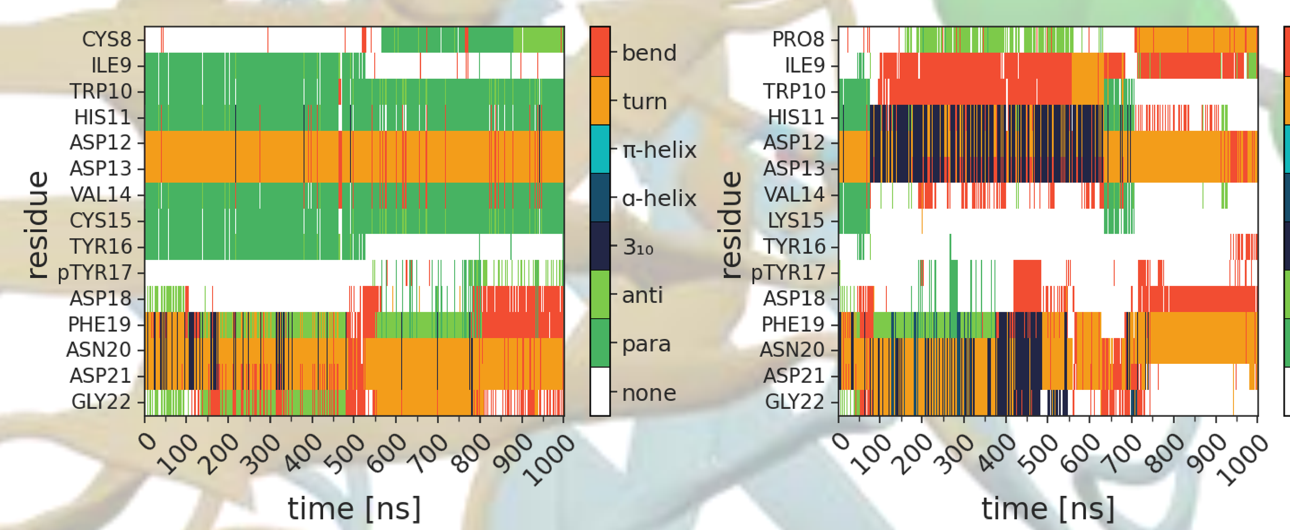
Protein-glycan interactions between H100R and Man4 (top right) and D101 and Man7 (bottom right) in sY-PG16. Glycan interactions with the base of CDRH3 are found with D101 and are more stable in sY-PG16.

Based on the characterization of the antibodies, peptides with **different disulfide bond patterns** were constructed to investigate their effects on the **pre-stabilization** of the peptides.

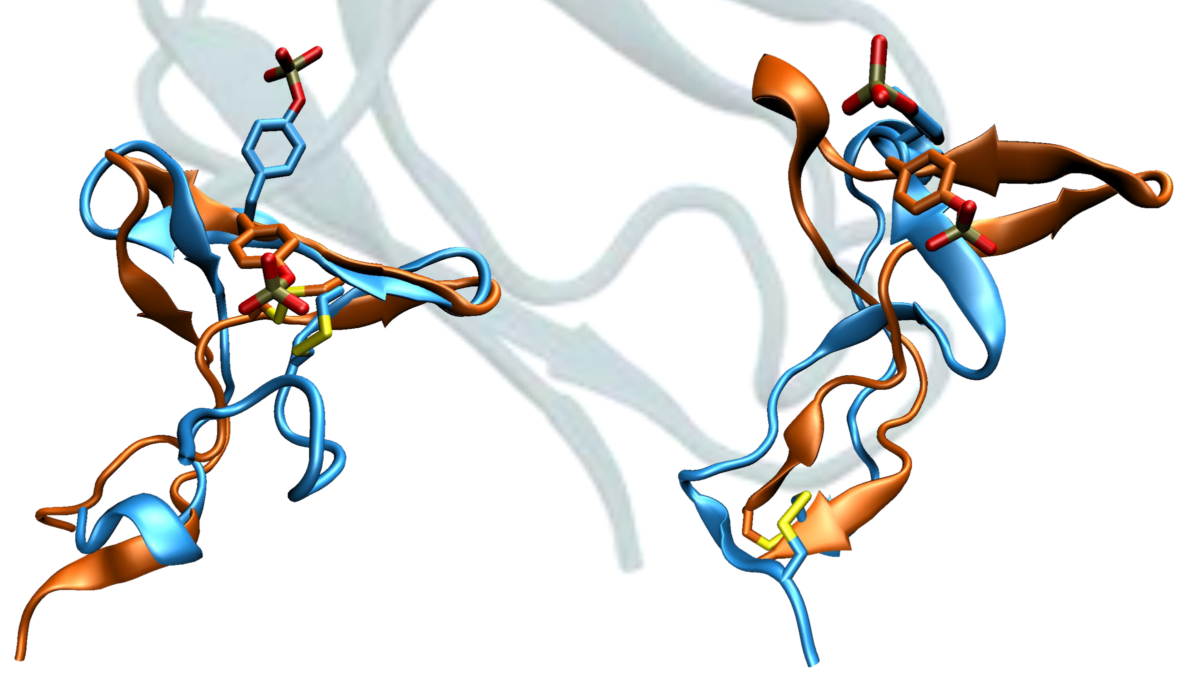
p40.04
exp-seq -SAREAGGPIWHDDVKYzDFNDGYYNYHYMDVW
p40.09



Neutralization of different PG16-based peptides in Neutravidin ELISA assay shows a one order of magnitude difference between different disulfide bonds in **p40.04** and **p40.09**.



Secondary structure analysis of p40.04 (left) and p40.09 (right) demonstrate the stabilizing effect of strategically placed disulfide bonds.



Alignment of start and end structures of the simulations of p40.04 (left) and p40.09 (right). Despite highly flexible termini of p40.04, the binding competent form of the hammerhead is retained. In p40.09 the hammerhead structure is lost.

Summary

- MD simulations revealed importance of glycan interaction in antibody binding
- Glycan interactions extend to the base of CDRH3 of PG16
- Antibody framework provides support for long CDRs
- Intermolecular interactions stabilize CDRH3 of PG16
- Hammerhead CDRH3 peptides of PG16 require stabilization for efficient binding

Outlook

- Further investigation of stabilizing disulfide bonds in Ab-derived peptides
- Application of pipeline on Sars-CoV-2 antibodies

[1] Pan, J., Peng, H., Chen, B., & Harrison, S. C. (2020). Cryo-EM structure of full-length HIV-1 Env bound with the Fab of antibody PG16. Journal of Molecular Biology, 432(4), 1158–1168. https://doi.org/10.1016/j.jmb.2020.01.012

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Novel antiviral approaches

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