GANGLIOSIDE RECEPTOR RECOGNITION BY BOTULINUM NEUROTOXIN A

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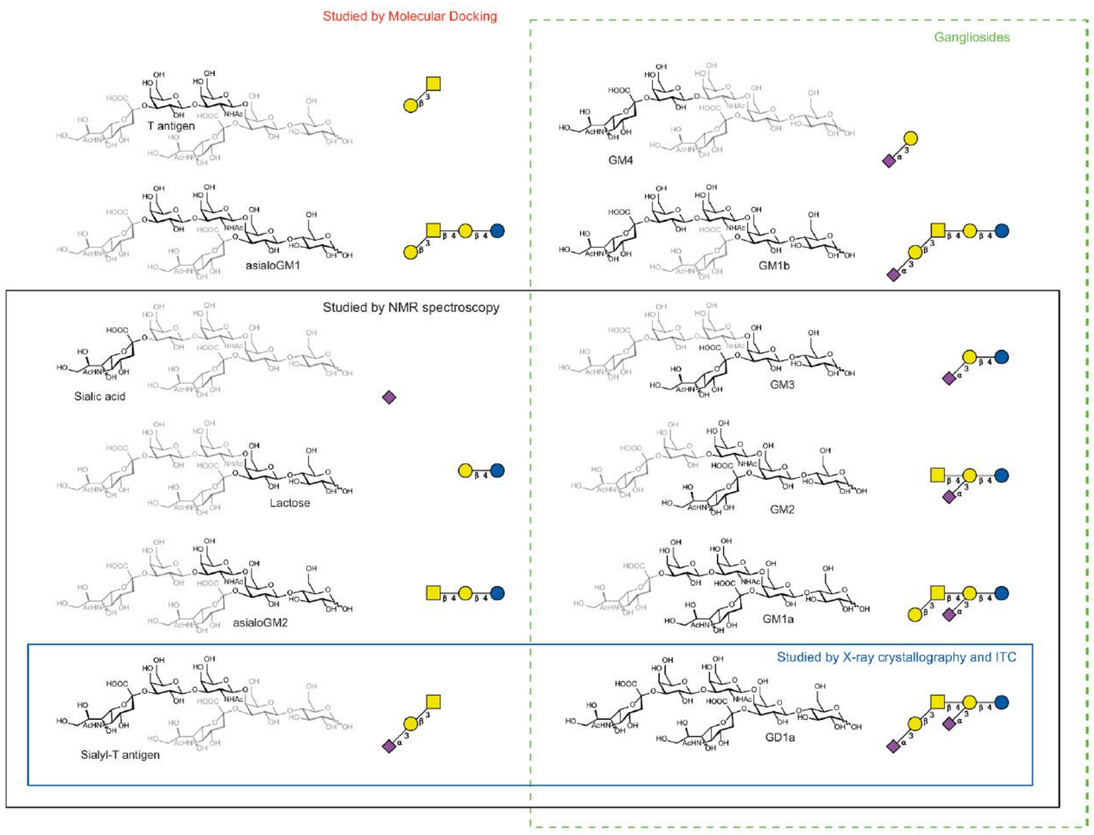
Introduction and Objectives: The anaerobic bacterium Clostridium botulinum produces the highly poisonous Botulinum neurotoxins, which associate to two receptors on neuronal cell surfaces with high affinity in the first step of host cell invasion. The initial co-receptor for the botulinum neurotoxin is the glycan moiety of a ganglioside, whereby binding of a membrane protein receptor occurs. In this study we investigate the minimal carbohydrate binding epitope of botulinum neurotoxin serotype A.

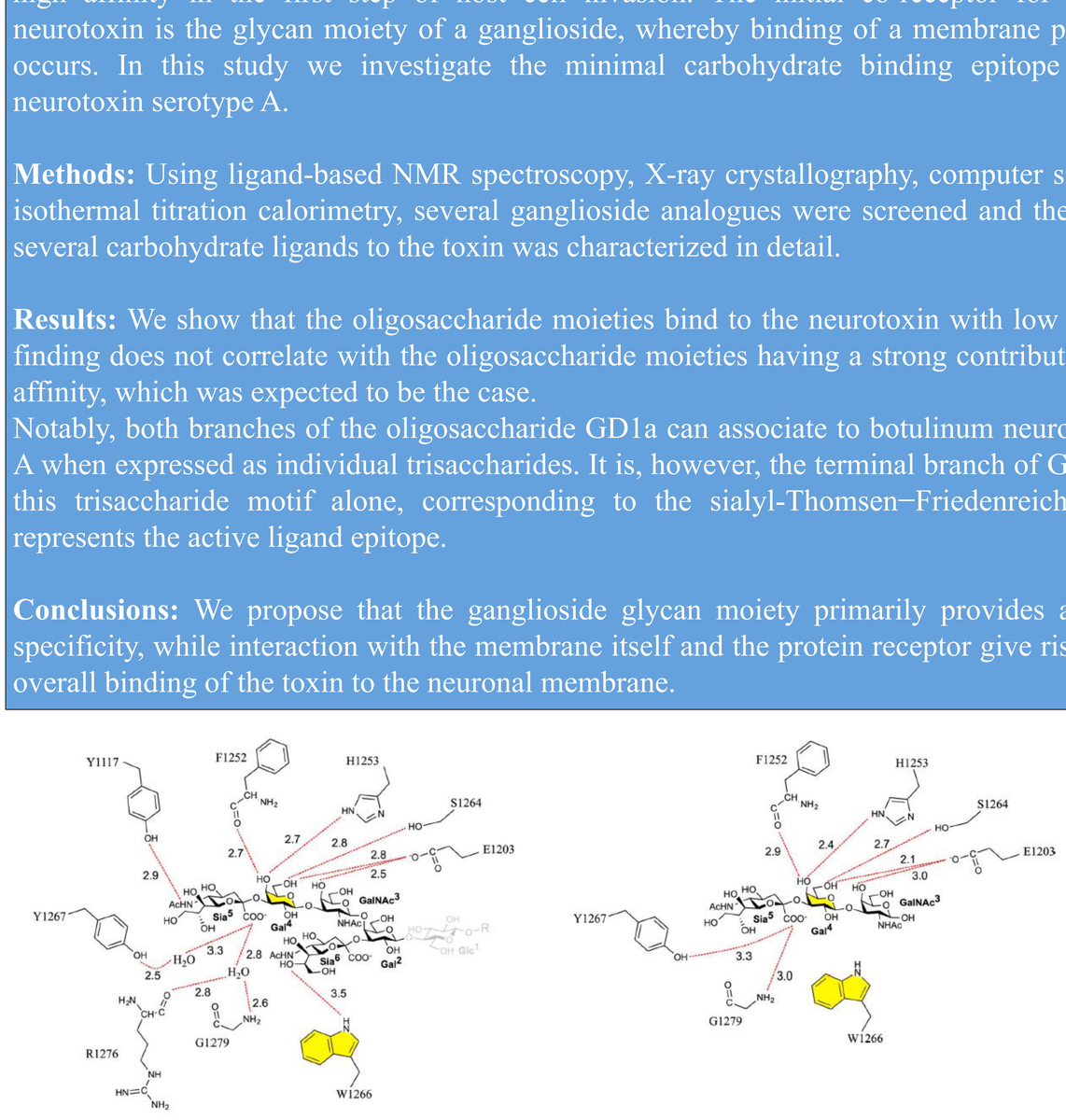
Methods: Using ligand-based NMR spectroscopy, X-ray crystallography, computer simulations and isothermal titration calorimetry, several ganglioside analogues were screened and the interaction of several carbohydrate ligands to the toxin was characterized in detail.

Results: We show that the oligosaccharide moieties bind to the neurotoxin with low affinities. This finding does not correlate with the oligosaccharide moieties having a strong contribution to the total affinity, which was expected to be the case.
Notably, both branches of the oligosaccharide GD1a can associate to botulinum neurotoxin serotype A when expressed as individual trisaccharides. It is, however, the terminal branch of GD1a as well as this trisaccharide motif alone, corresponding to the sialyl-Thomsen−Friedenreich antigen, that represents the active ligand epitope.

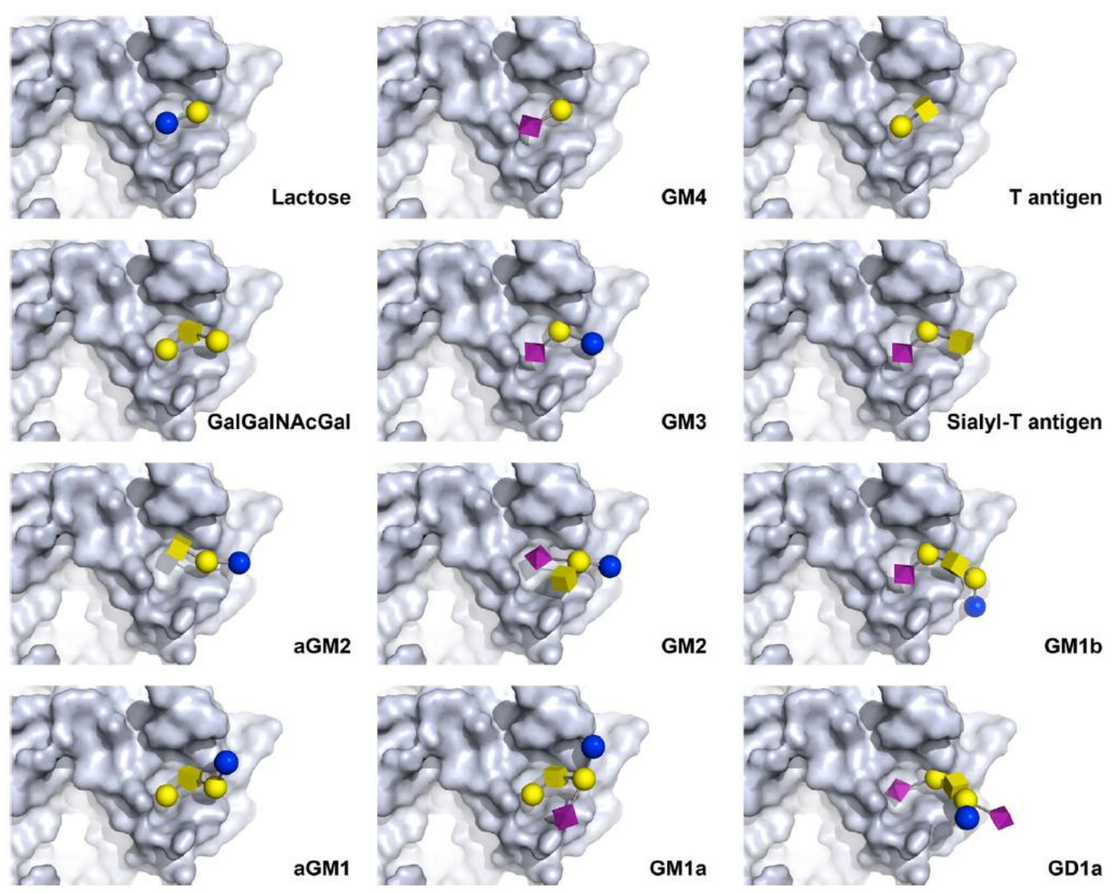
Conclusions: We propose that the ganglioside glycan moiety primarily provides abundance and specificity, while interaction with the membrane itself and the protein receptor give rise to the strong overall binding of the toxin to the neuronal membrane.



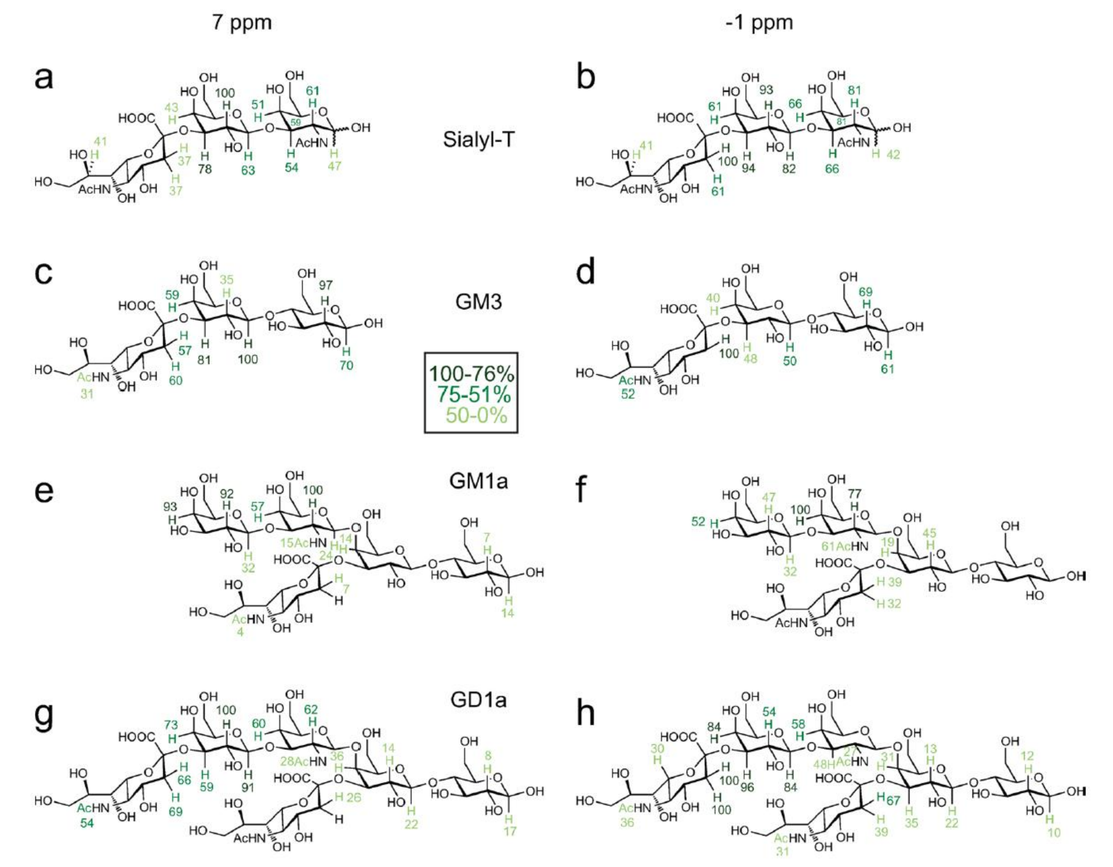
Molecular structure and CFG representation of the compounds of this study. They all represent fragments of the glycan part of GD1a, the ganglioside without its sphingolipid part. Different sets and classifications of the ligands are indicated by differently colored frames.



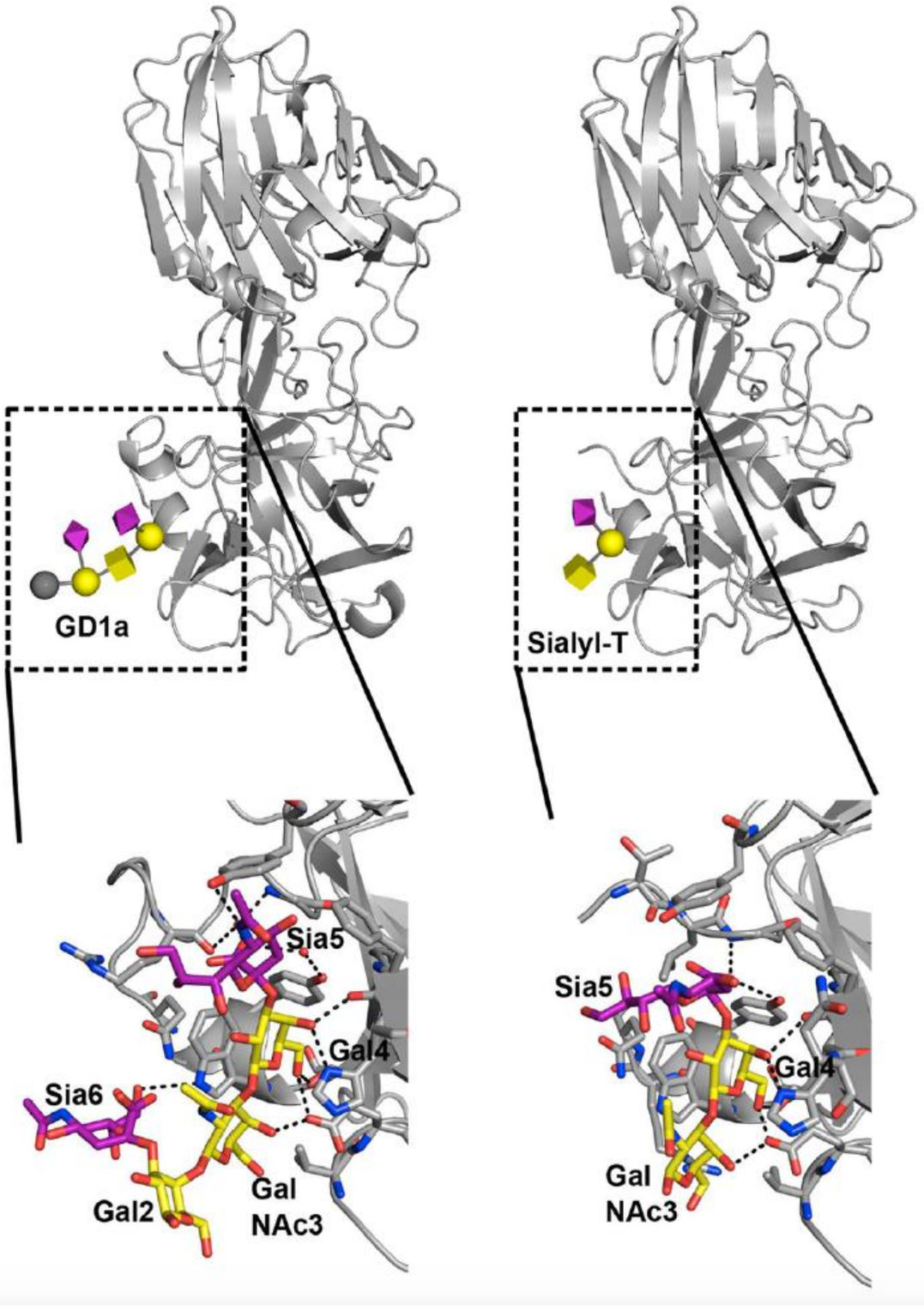
Schematic 2D plots of the glycan hydrogen bonding of GD1a (left) and sialyl-T (right) to BonT/A-H_C, as obtained by X-ray crystallography. Glc1 is disordered in the GD1a complex and thus colored gray. Possible intermolecular hydrogen bond interactions are shown as red dotted lines, with the hydrogen bond distance annotated for each bond (Å). Yellow coloring in Gal^4 and W1266 highlights the stacking interaction between these residues.



Representative output for each studied ligand of the BoNT/A-H_C•ligand models from molecular docking simulations with the Autodock VINA software. The ligands are represented as 3D-CFG symbols. The GD1a-containing complex was obtained from a redocking.



Epitope mapping presented as normalized levels of saturation (against the most intense signal arbitrarily assigned to 100%) from STD-AF_0 for sialyl-T (a, b), GM3 (c, d), GM1a (e, f), and GD1a (g, h) in complex with BonT/A-H_C with on-resonance irradiation at 7 ppm (a, c, e, g) and −1 ppm (b, d, f, h). Nonexchangeable protons explicitly presented in the figures indicate detected STD effects.



Glycan binding to BonT/A-H_C as obtained by X-ray crystallography. (left) Overall view of the BoNT/A-H_C•GD1a complex (protein in gray color and sugar in 3D-CFG representation). Glc1 is disordered in the complex and thus colored gray. The lower panel shows a zoomed-in view on the detailed interactions with the GD1a residues colored according to CFG. Possible intermolecular hydrogen bonds are shown as black dotted lines. (right) Corresponding information for the BoNT/A-H_C•sialyl-T complex.

Reference:

Hamark, C., Berntsson, R. P.-A., Masuyer, G., Henriksson, L. M., Gustafsson, R., Stenmark, P., Widmalm, G. Glycans Confer Specificity to the Recognition of Ganglioside Receptors by Botulinum Neurotoxin A. *J. Am. Chem. Soc.* Published online Dec 13 2016. DOI:10.1021/jacs.6b09534