Automating the Rational Design of Glycomimetic Drugs

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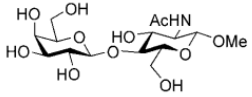
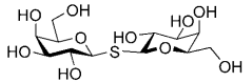
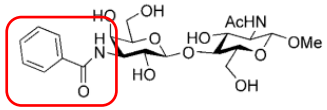
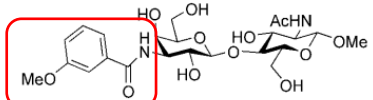
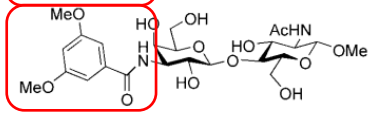
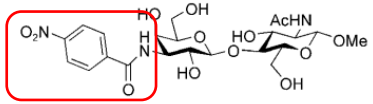
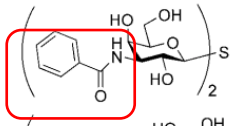
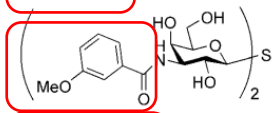
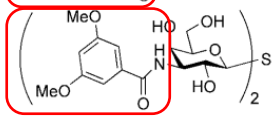
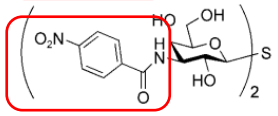
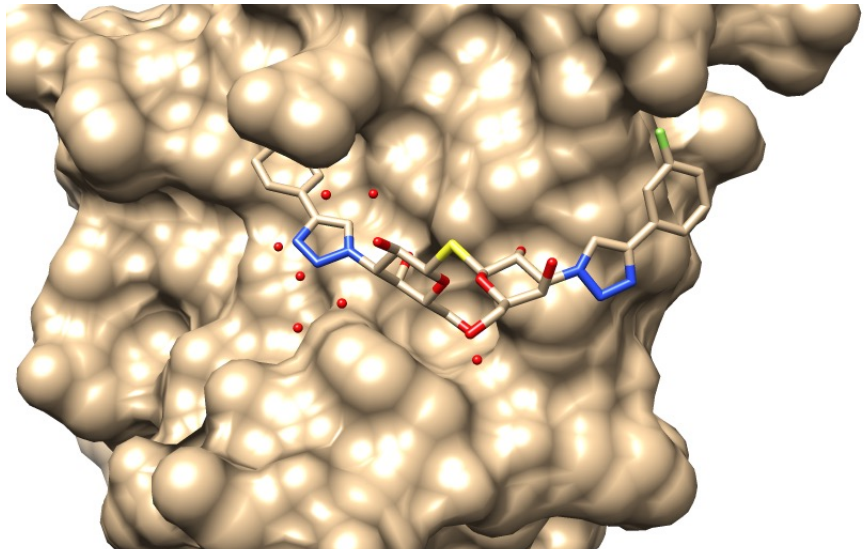
University of Georgia

GLYCAM-Web: www.glycam.org

Glycomimetics as a Therapeutic Strategy

	Compound	K_d [nM]	Relative activity[a]
9		69 000[5b]	1
3		43 000	1.6
2a		6700[5b]	10
2b		2500[5b]	28
2c		1100[5b]	63
2d		950[5b]	73
4a		3000	23
4b		61	1130
4c		50	1380
4d		33	2090

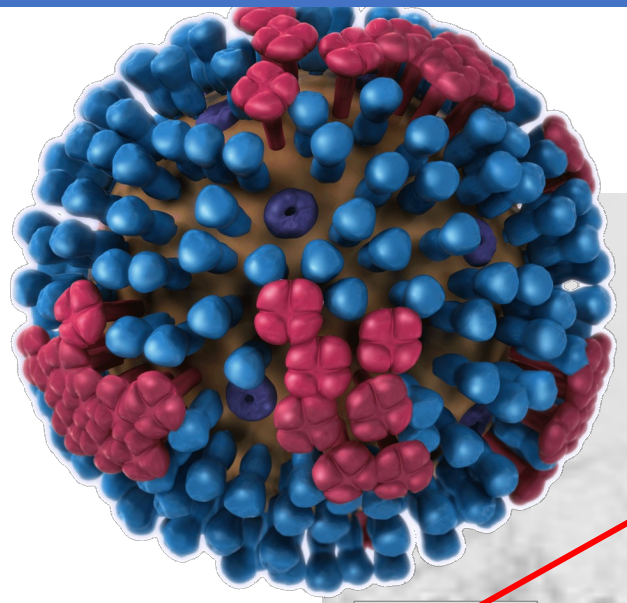
Cumstey et al. *Angew. Chem. Int. Ed.* **44**, 5110-5112



- Exploits the specificity of the endogenous carbohydrate ligand
- Employ the native carbohydrate ligand as a basis for rational design
- Examples: Relenza® and Tamiflu®
- Review: Magnani and Ernst (2009) *Discov. Med.* **8**, 247-252

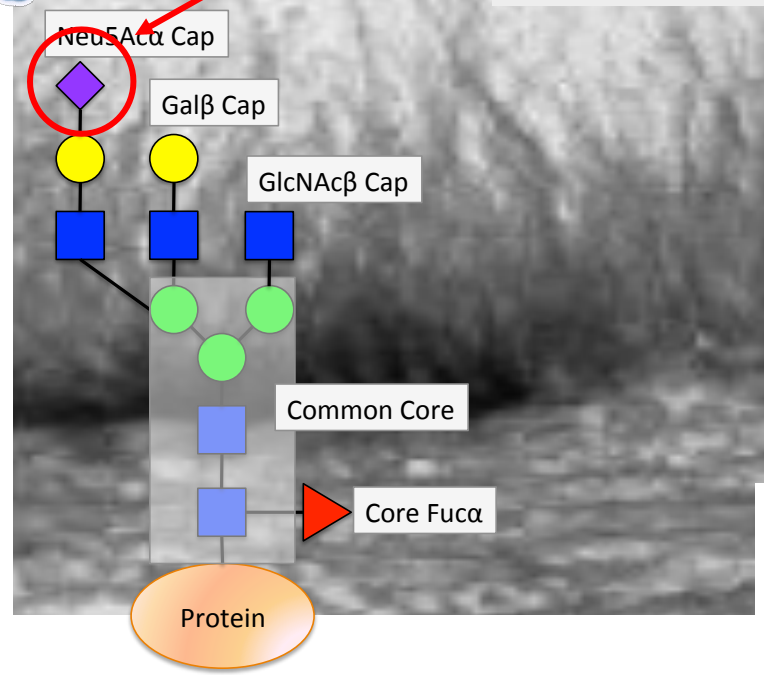
How to choose the “R” groups?

Can We Block Pathogen Adhesion with Glycomimetics?



Many pathogens exploit binding to glycans as the the first stage of infection:

Influenza, parainfluenza, mumps, corona, noro, rota, and DNA tumor viruses, chikungunya (?), zika (?), many bacteria



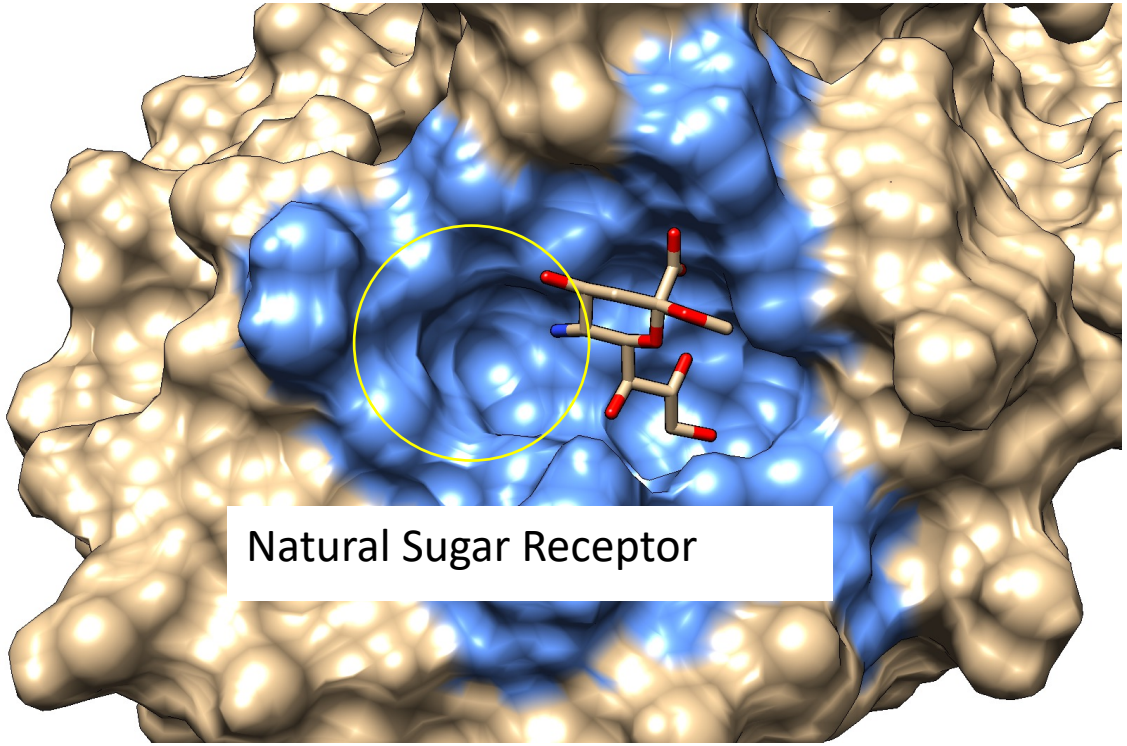
Ji et al. *Curr. Opin. Struct. Biol.* (2017) 44: 219-231
Matrosovich et al. Top. Curr. Chem. (2015) 367: 1-28
van Duijl-Richter et al. Viruses (2015) 7: 3647–3674

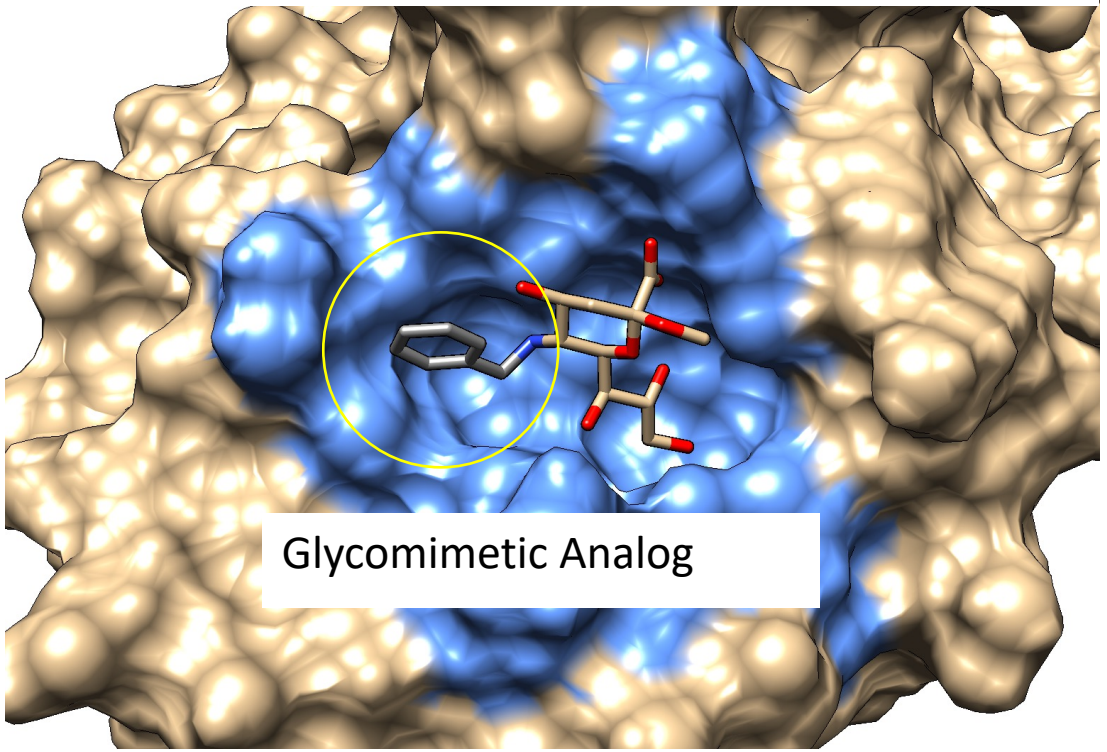
Rational Glycomimetic Design

How do we make the glycomimetic bind more strongly to the protein receptor?

- Stronger binding – smaller dose, higher efficiency, less change of side effects

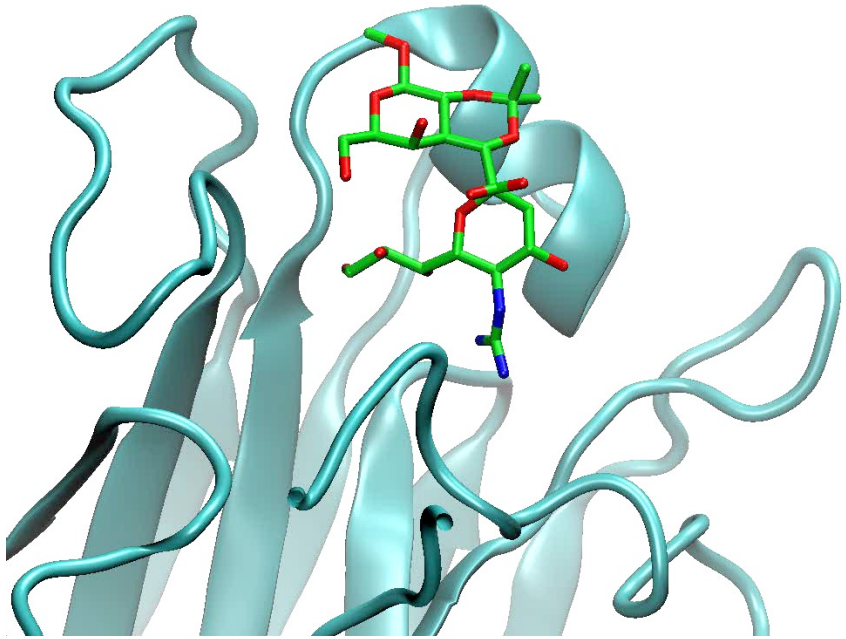
Make chemical modifications that create new contacts between the small molecule and the protein surface “rational design”



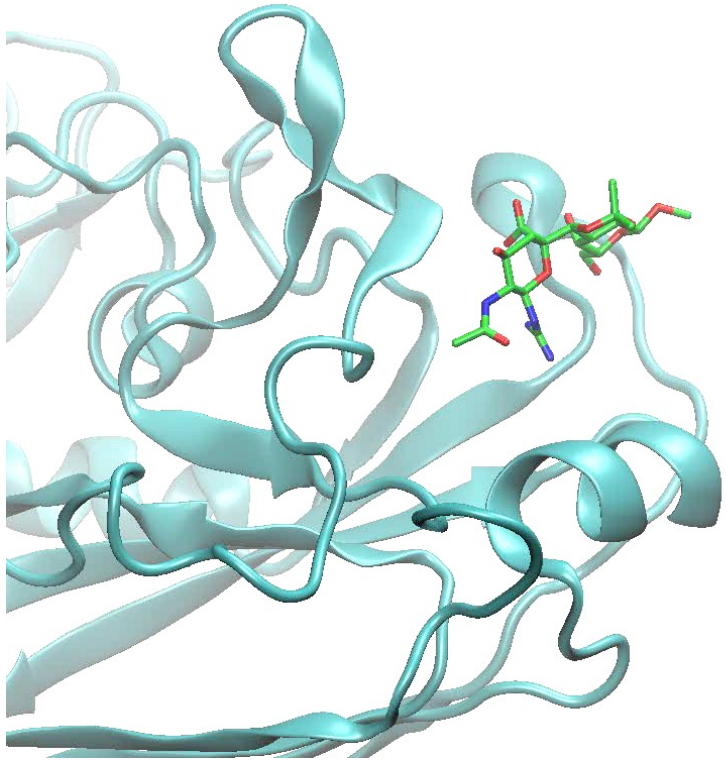


Molecular Dynamics Can Discriminate Good from Bad Binders

But – which inhibitors should we simulate?



Putative Inhibitor 1

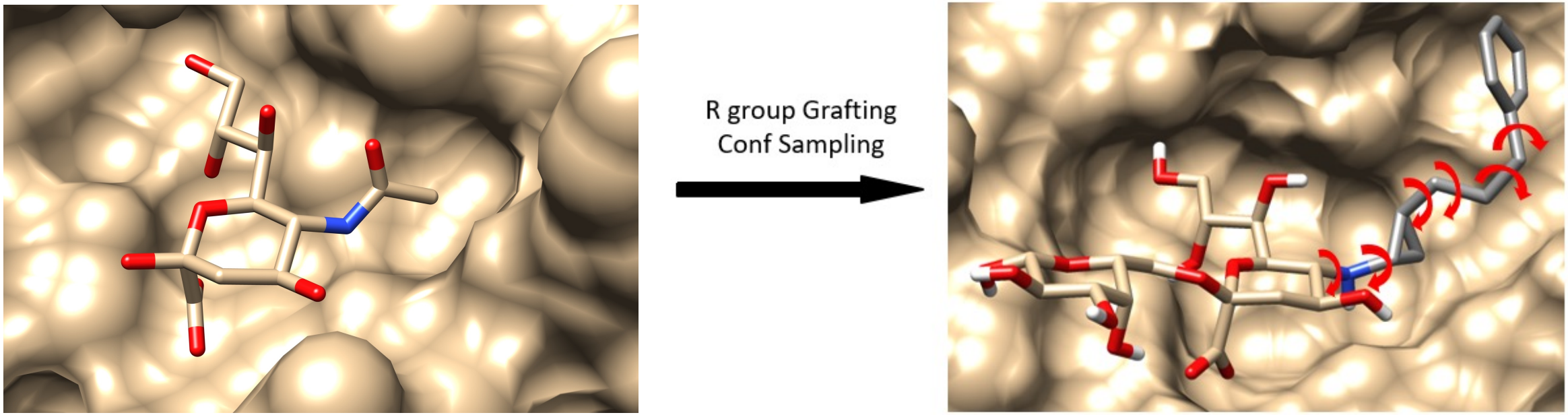


Putative Inhibitor 2

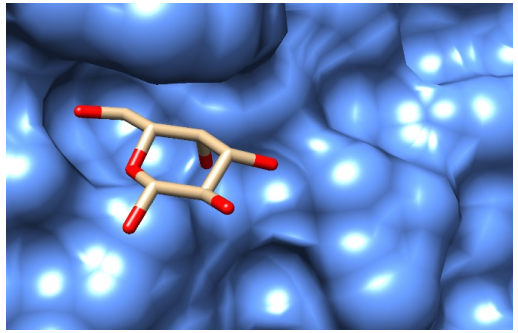
Grafting and Conformational Sampling

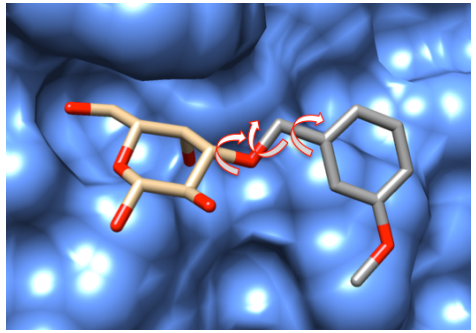
Graft R groups onto carbohydrates and conformational sampling.

- All rotatable bonds in the chemical moiety are identified and rotated
- A genetic algorithm is employed for conformational sampling
- $\Delta G_{rotamer} = \Delta G_{Vina} + \Delta G_{CH-\pi}$



Virtual Glycomimetic Screening





Upload protein-carbohydrate PDB file

Select position in ligand to modify

Select library of analogs to screen

Perform conformational search for each analog
- rotamer generation
- clash checking
- energy minimization

Pass MD snapshots at 5 ns intervals

Perform MD of receptor-carbohydrate co-complex
- explicit water
- AMBER GAFF force field
- 50 ns

Calculate GIST desolvation energy and docking scoring for all rotamers of each analog in each MD snapshot

Compute ligand conformational entropy

Return best pose and estimated binding free energy for each analog

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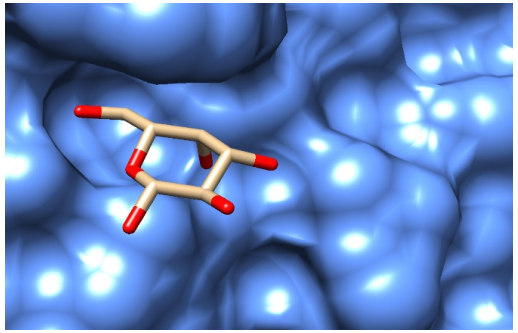

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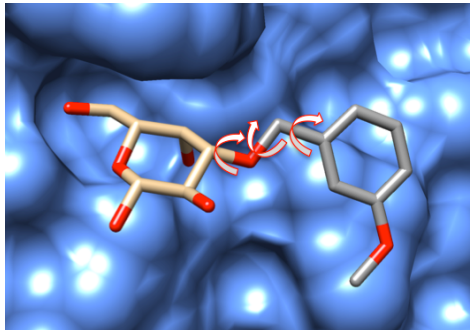
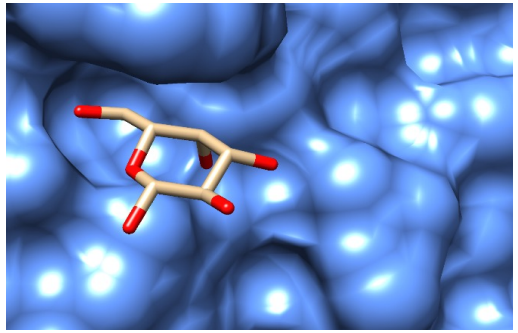


Moiety Library:
- Designed for addition to NH/OH atoms in carbohydrates
- Moieties scraped from chemical catalogs and PubChem
- Converted from SMILES to 3D
- Currently ~600 moieties

Virtual Glycomimetic Screening



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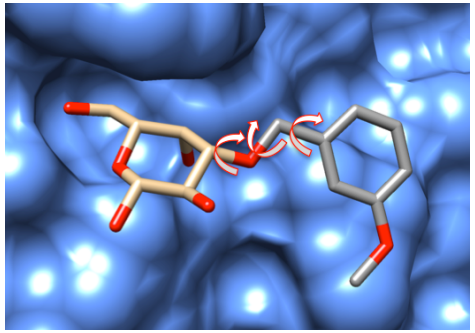

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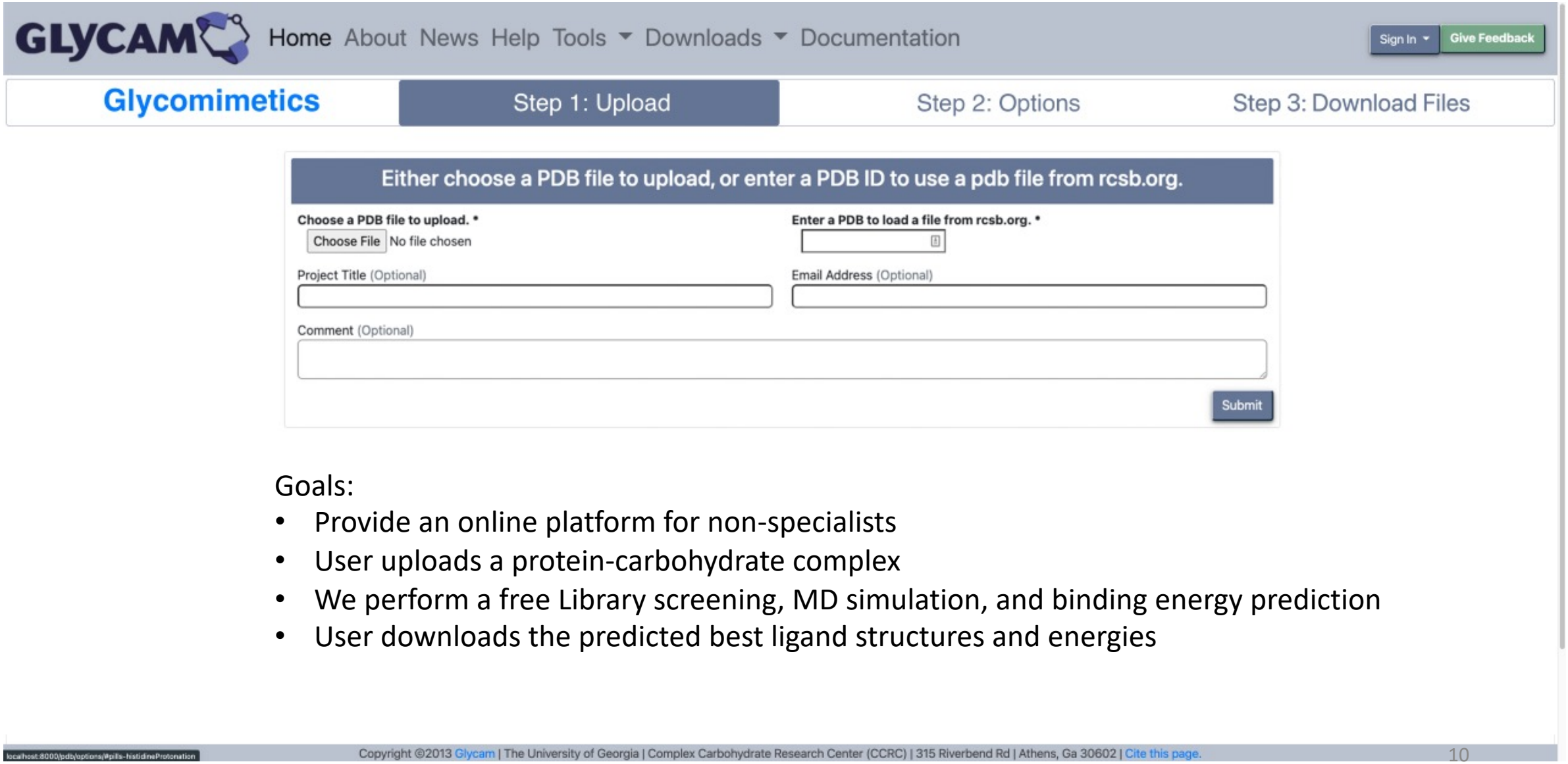
Binding Energies:
- Multiple methods implemented
  - AutoDock VINA-Carb [1]
  - AMBER/GLYCAM MM-GBSA
  - AMBER/GLYCAM MM-GIST
- New functional forms added (CH-π)

[1] Nivedha, A. K., Thieker, D. F., Makeneni, S., Hu, H., & Woods, R. J. (2016). *Journal of chemical theory and computation*, *12*(2), 892-901.

Proposed Online Webtool

Step 1: Upload/Fetch PDB



Goals:

- Provide an online platform for non-specialists
- User uploads a protein-carbohydrate complex
- We perform a free Library screening, MD simulation, and binding energy prediction
- User downloads the predicted best ligand structures and energies

Proposed Online Webtool

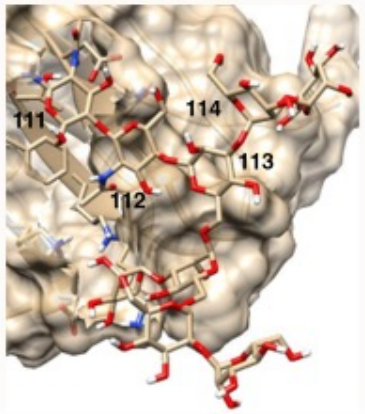
Step 2: Choose options

GLYCAM Home About News Help Tools Downloads Documentation Sign In Give Feedback

Glycomimetics | Step 1: Upload PDB File | Step 2: Options | Step 3: Download Files

Showing: Example.pdb

LiteMol Video Tutorial LiteMol User Manual



We detected the following positions available for modification:

Select Position			Select R Group Library		
Residue Index	Atom Name	Atom To Replace	Aldehydes	Sulfonyl Halides	Ketones
111	O3	HO3 ☐	☐	☐	☐
112	N2	C7 ☒	☒	☒	☐
113	O6	HO6 ☐	☐	☐	☐
114	O1	HO1 ☐	☐	☐	☐

Submit

Proposed Online Webtool

Step 3: Download Results

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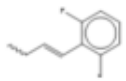
Glycomimetics | Step 1: Upload PDB File | Step 2: Options | Step 3: Download Files

Your job is complete. Please download the result: **Result.zip** Download

Select Position			Select R Group Library		
Residue Index	Atom Name	Atom To Replace	Aldehydes	Sulfonyl Halides	Ketones
111	O3	HO3 ☐	☐	☐	☐
112	N2	C7 ☒	☒	☒	☐
113	O6	HO6 ☐	☐	☐	☐
114	O1	HO1 ☐	☐	☐	☐

Top 5 Analogs

R Group	Rank	Affinity Change (kcal/mol)	RIP*
	1	-5.67	12,708
	2	-4.56	1,998
	3	-2.34	49.4
	4	-1.23	7.77
	5	-0.53	2.42

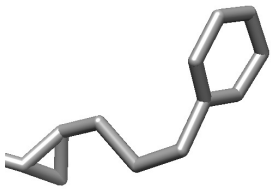
*RIP = relative inhibitory potential

Virtual Library Creation: Automated Ligand Parameterization

GAFF Forcefield

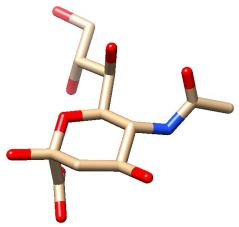
Forcefield Mixing

R group



ESP Calculation (QM)

RESP Charge Fitting



Glycan

Subgraph Isomorphism Matching

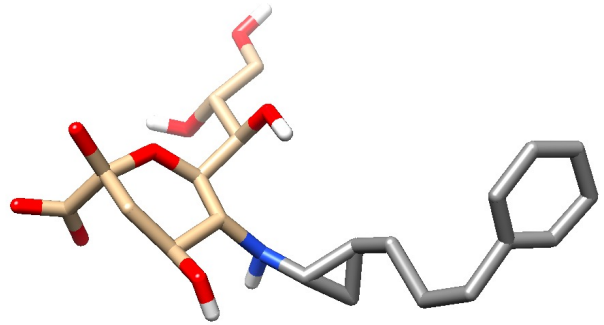
Force Field Parameter Assignment

GLYCAM Forcefield

New Bond, Angle, and Torsional Terms

Charge Adjustment

Glycomimetic Analog

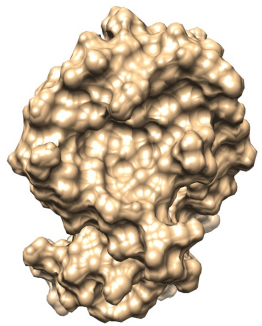


Automated MD Simulation

Preprocessing

Simulation

Protein



Remove Co-crystalization Reagents

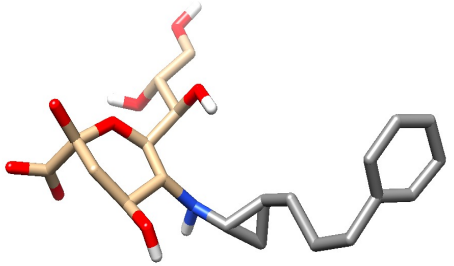
Detect Disulfide Bonds

Detect Missing Segments

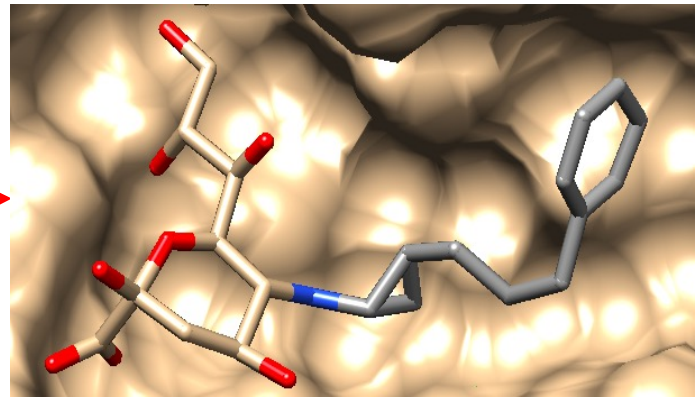
Repair (Modeler, AlphaFold, etc)

Place High Restraints

Glycomimetic Ligand

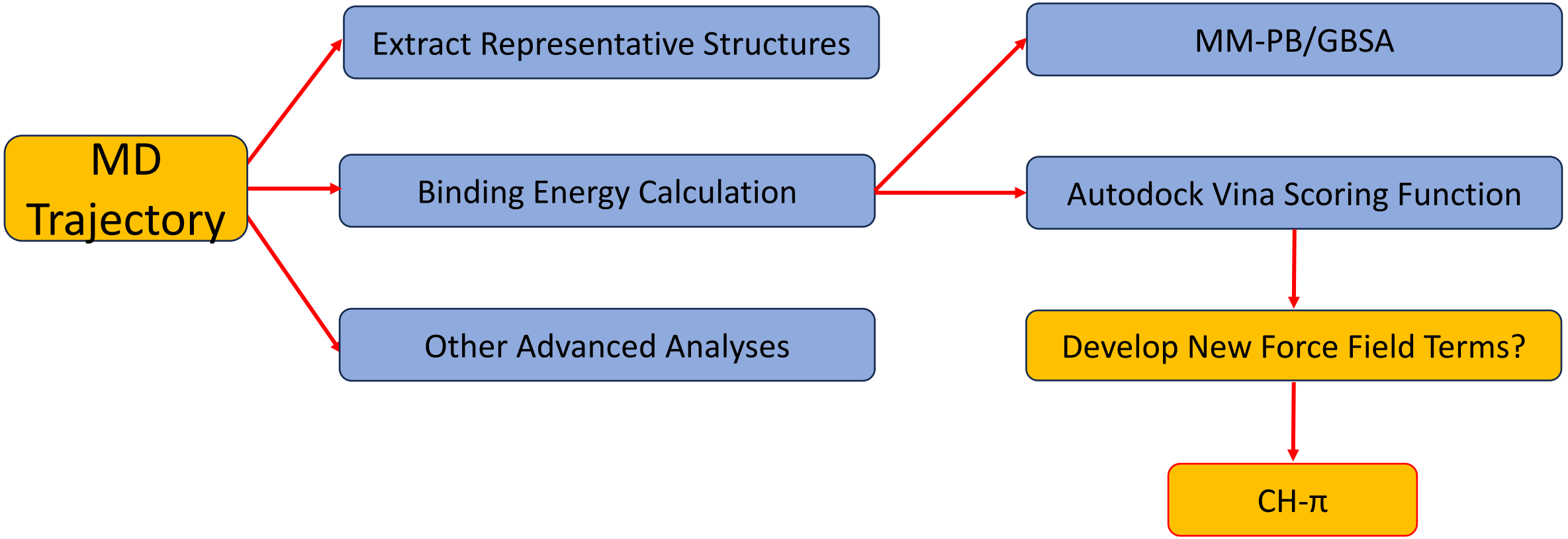


Heating, Equilibration, MD

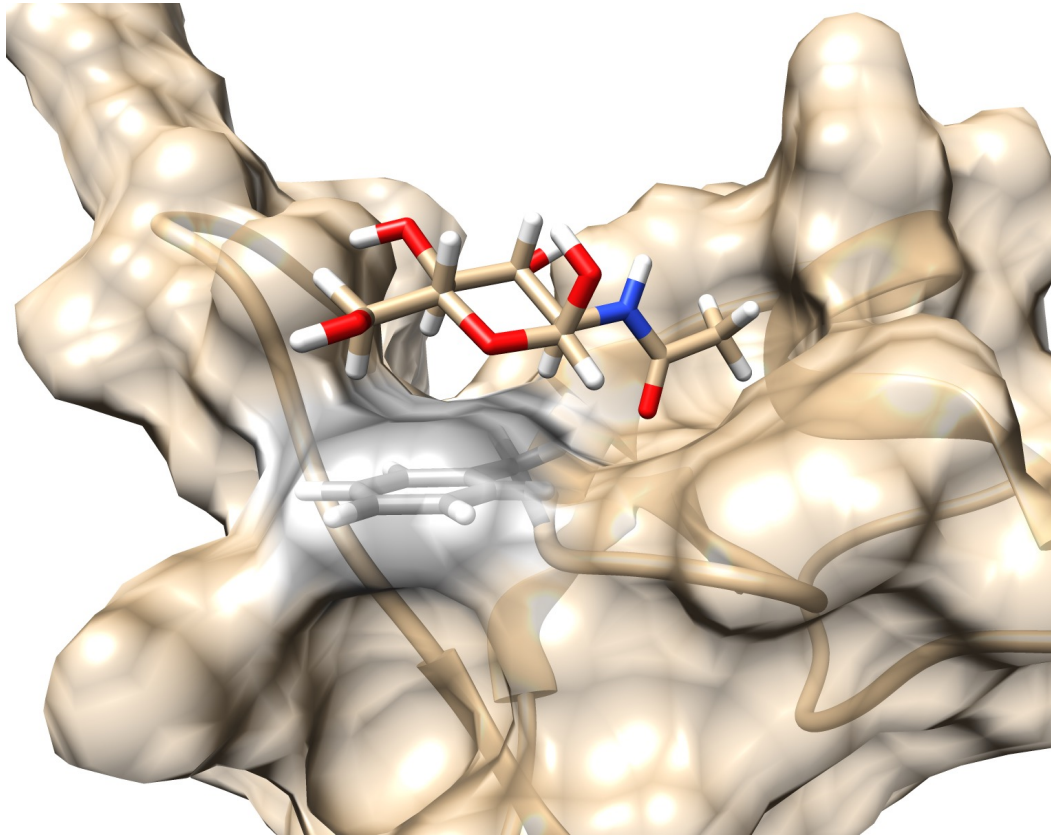


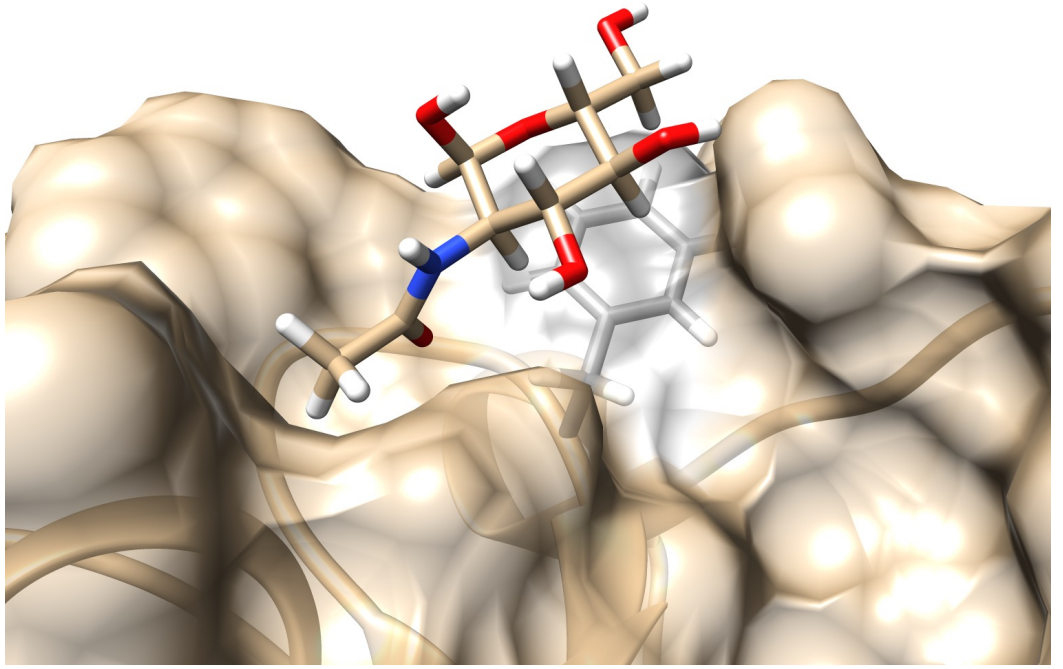
Input Files Created Automatically Without Human Intervention

MD Post-processing and Analysis



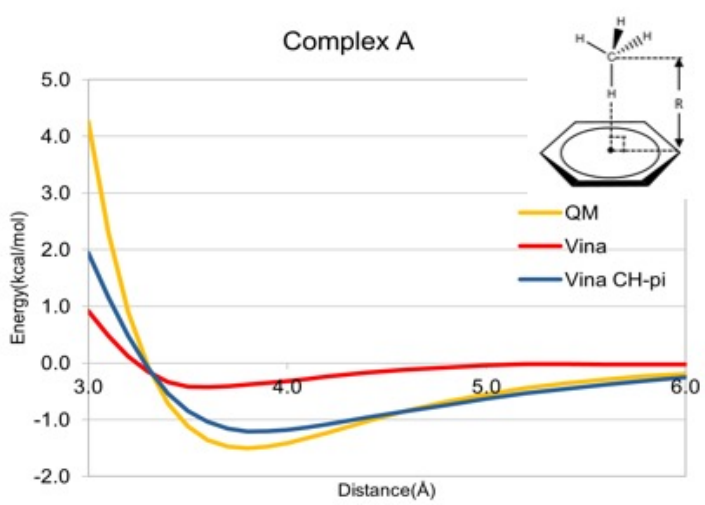
CH-π Interactions in Carbohydrate Binding

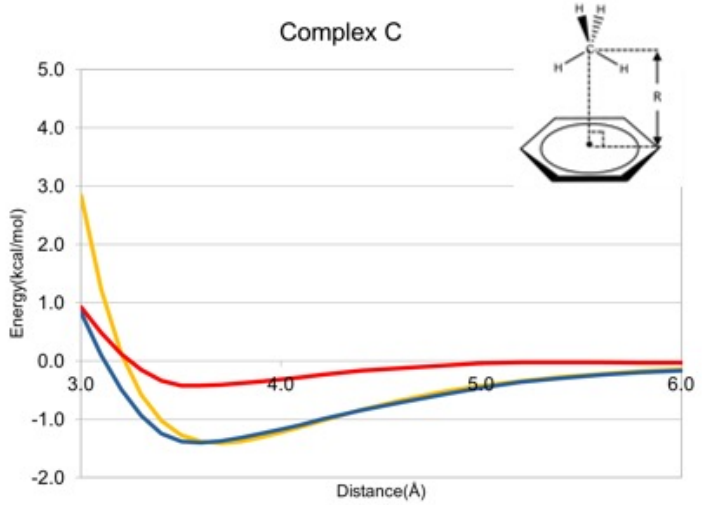


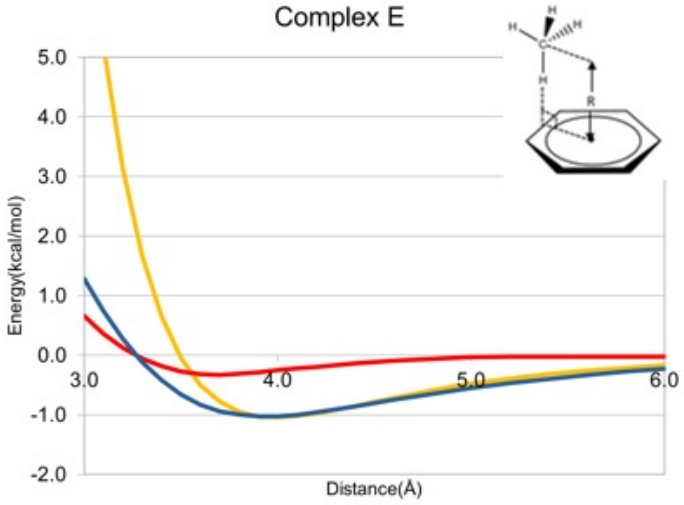


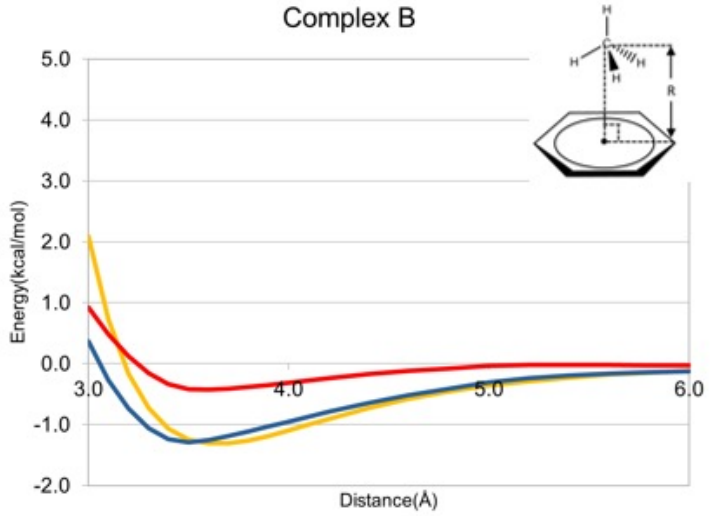
PDB 2UVO
Wheat Germ Agglutinin + GlcNAc

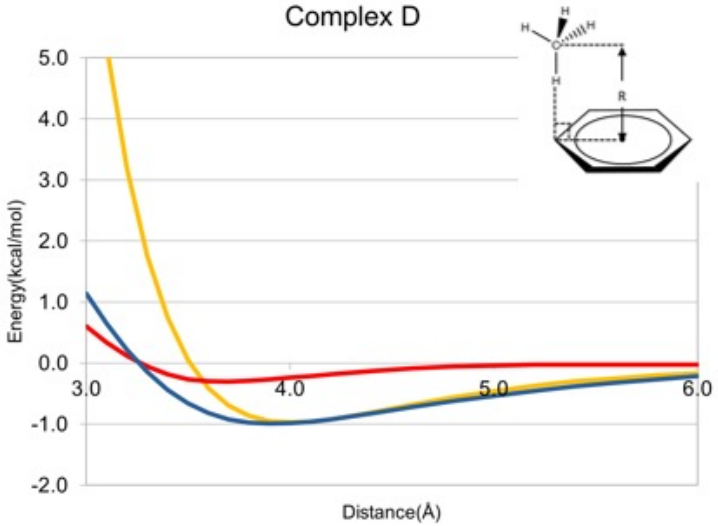
A Molecular Mechanical Model for CH-π Interaction

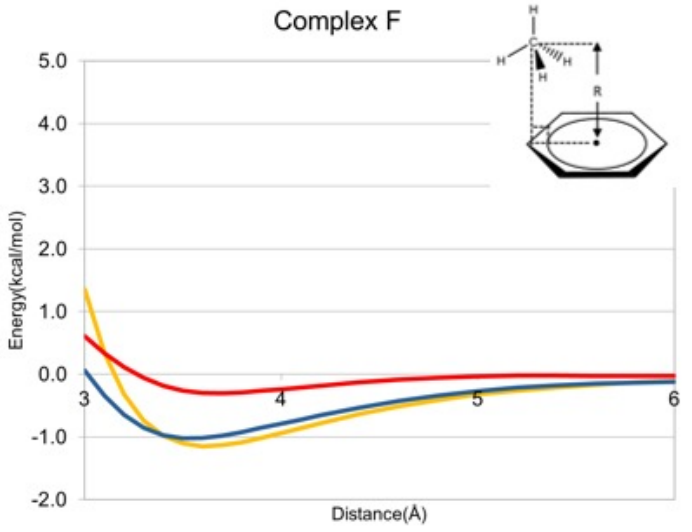






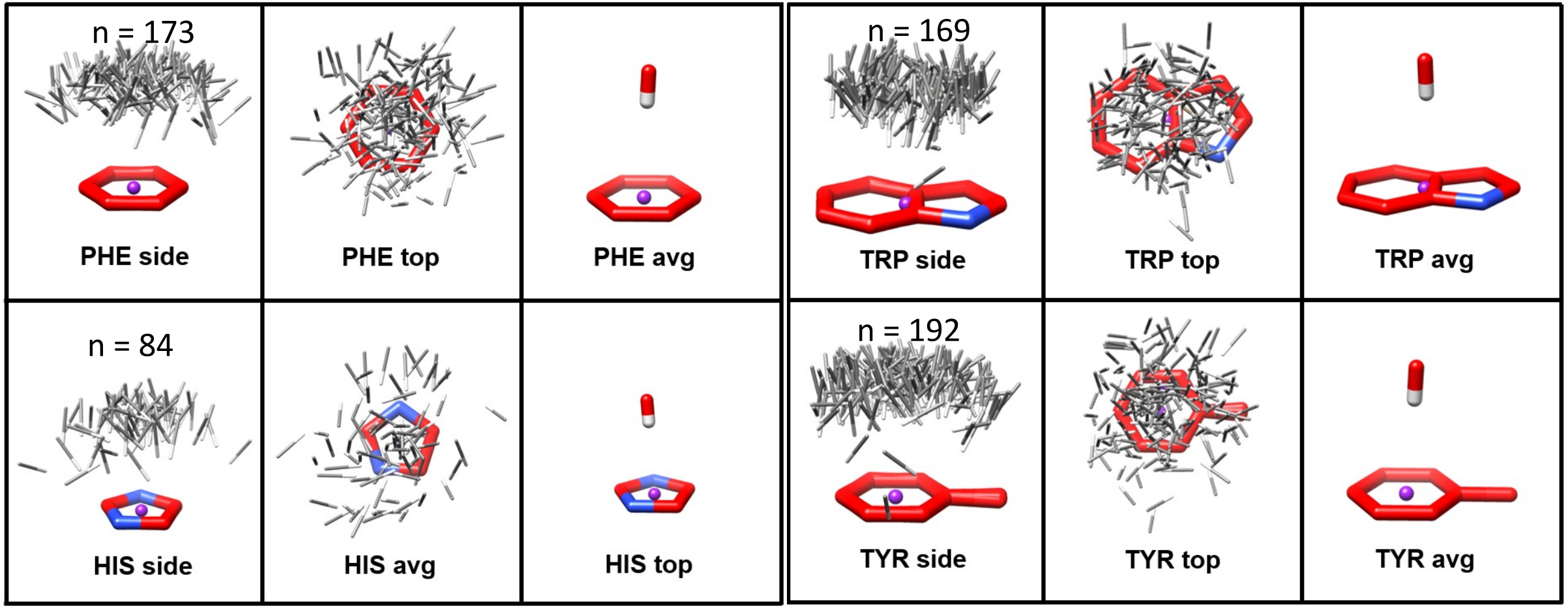






Experimental CH-π Interactions

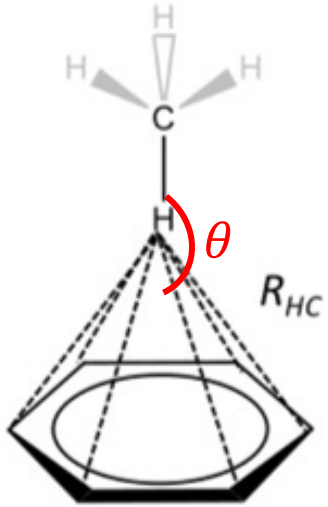
- All the CH bonds on top aromatic amino acids.
- Diffuse distribution with the average matching the canonical geometry.

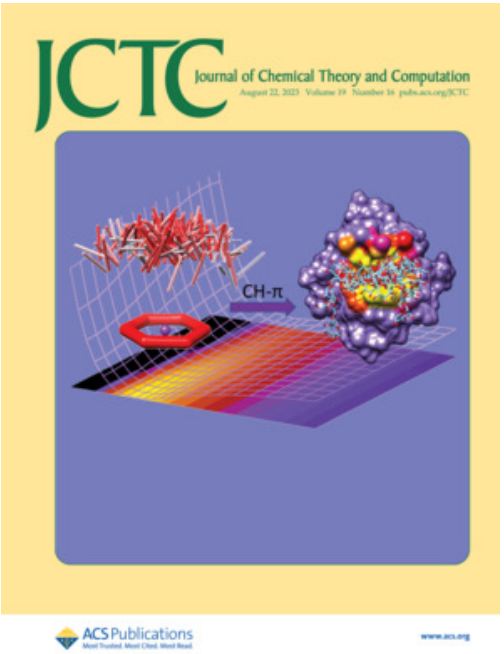


Xiao, Y., & Woods, R. J. (2023). Protein–Ligand CH− π Interactions: Structural Informatics, Energy Function Development, and Docking Implementation. *J. Chem. Theory Comput.*, *19*(16), 5503-5515.

Empirical CH-π Functional Form

- $E_{CH-\pi} = \sum \left(f(\theta) E_{HC} \cdot e^{\frac{-\left(R_{HC} - R_{HC}^{0}\right)^{2}}{2C_{HC}^{2}}} \right)$
- $f(\theta) = cos\theta (0 \leq \theta < 90°)$
- $E_{CH-Aromatic} = E_{CH-\pi} + E_{Vina}$





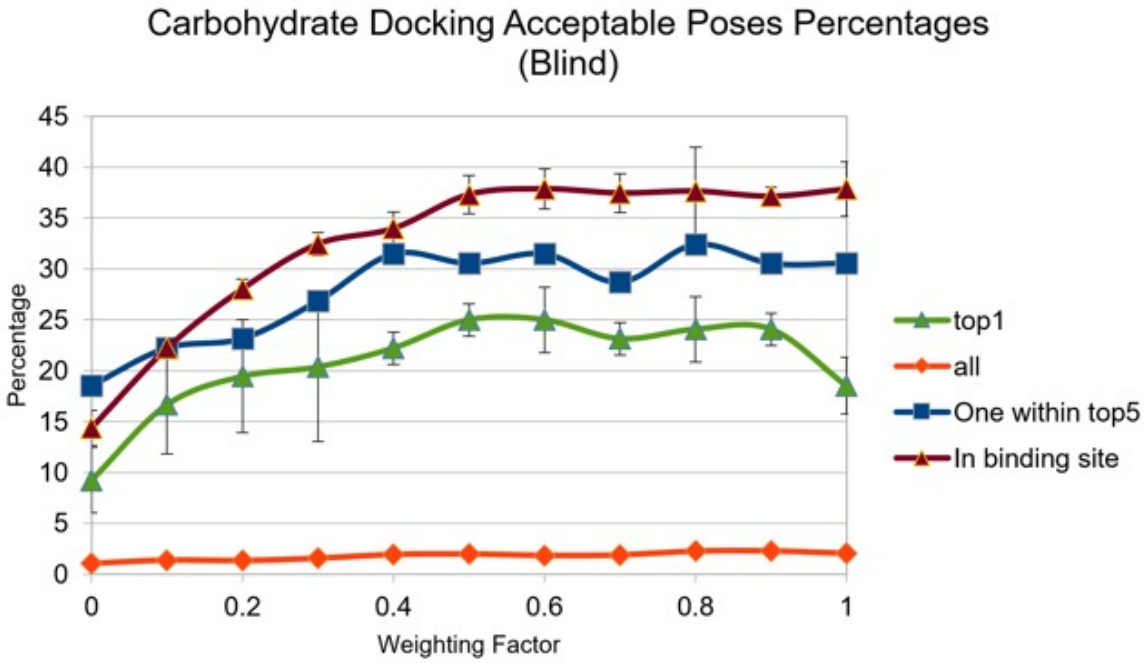
Xiao, Y., & Woods, R. J. (2023). Protein–Ligand CH− π Interactions: Structural Informatics, Energy Function Development, and Docking Implementation. *J. Chem. Theory Comput.*, *19*(16), 5503-5515.

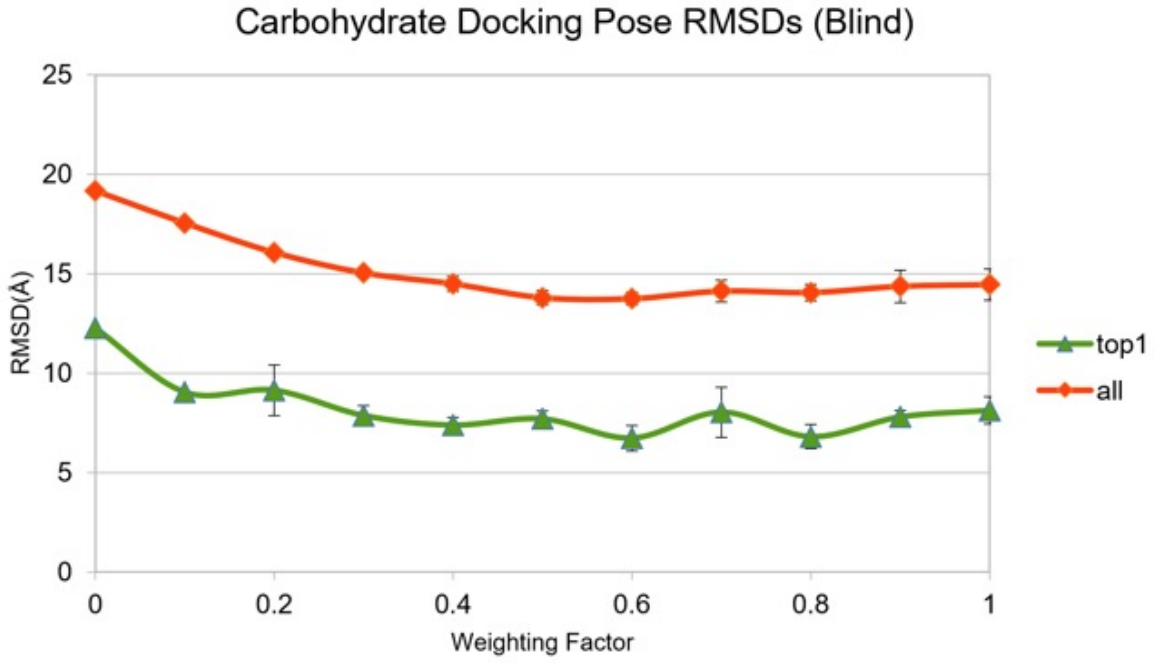
Inclusion of CH-π Interactions Improves Carbohydrate Docking

$$\Delta E_{total} = w_{CH-\pi} * \Delta E_{CH-\pi} + \Delta E_{Vina}$$

36 systems containing carbohydrate ligands (Vina-Carb[1]).

Significantly improved percentages of acceptable poses and average RMSDs.

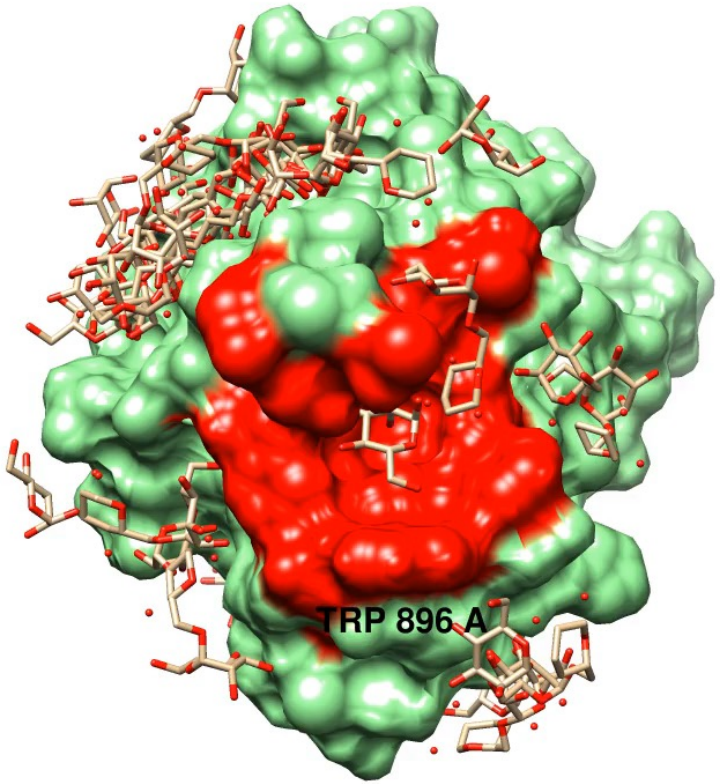




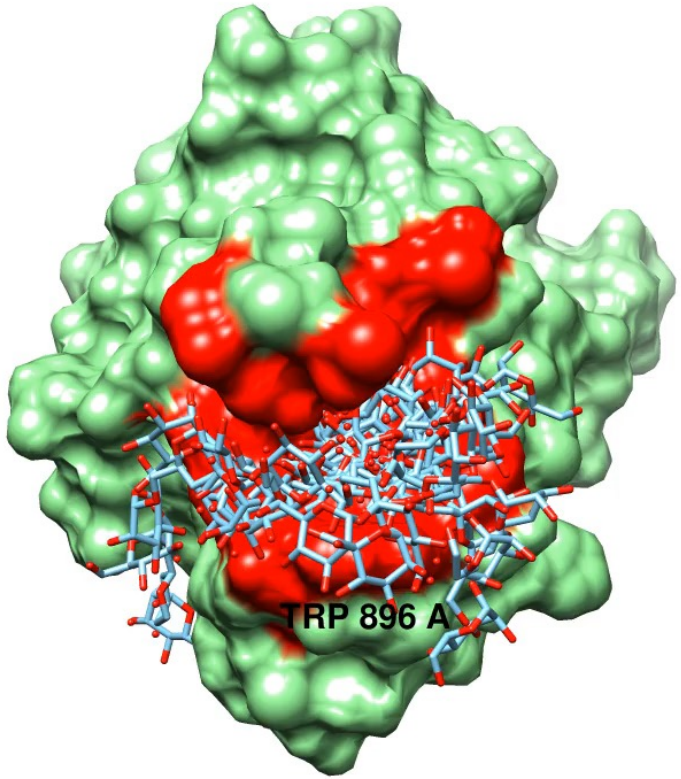
[1] Nivedha, A. K., Thieker, D. F., Makeneni, S., Hu, H., & Woods, R. J. (2016). Vina-Carb: improving glycosidic angles during carbohydrate docking. *Journal of chemical theory and computation*, *12*(2), 892-901.

Blind Docking Example (PDB 5V6F)

$w_{CH-\pi} = 0.0$



$w_{CH-\pi} = 0.8$

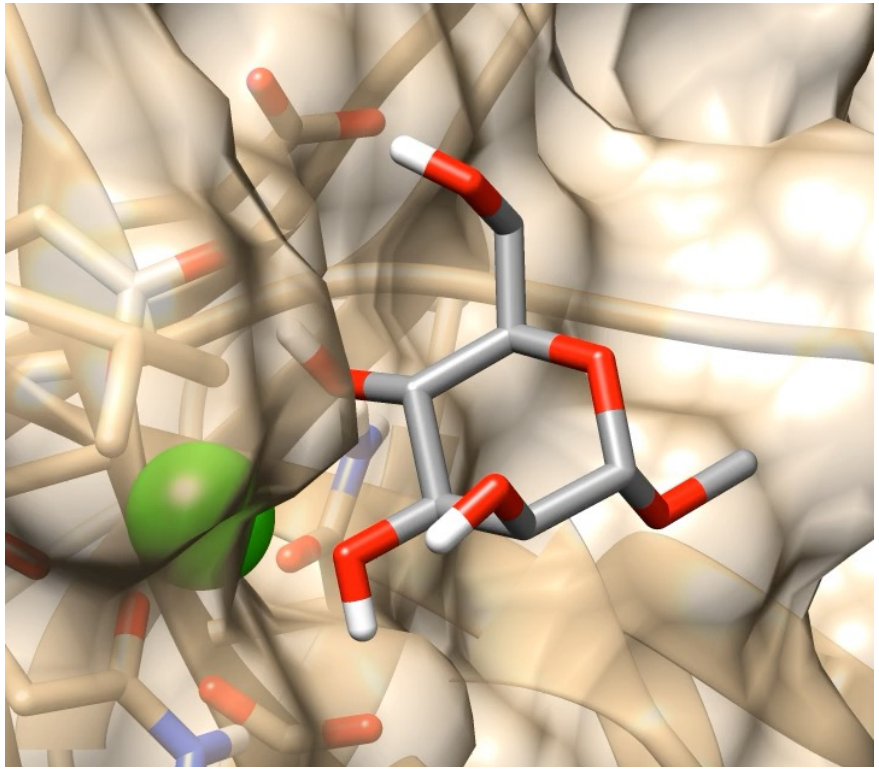


Crystal Structure of the Second beta-Prism Domain of RbmC from *V. cholerae* bound to Mannotriose

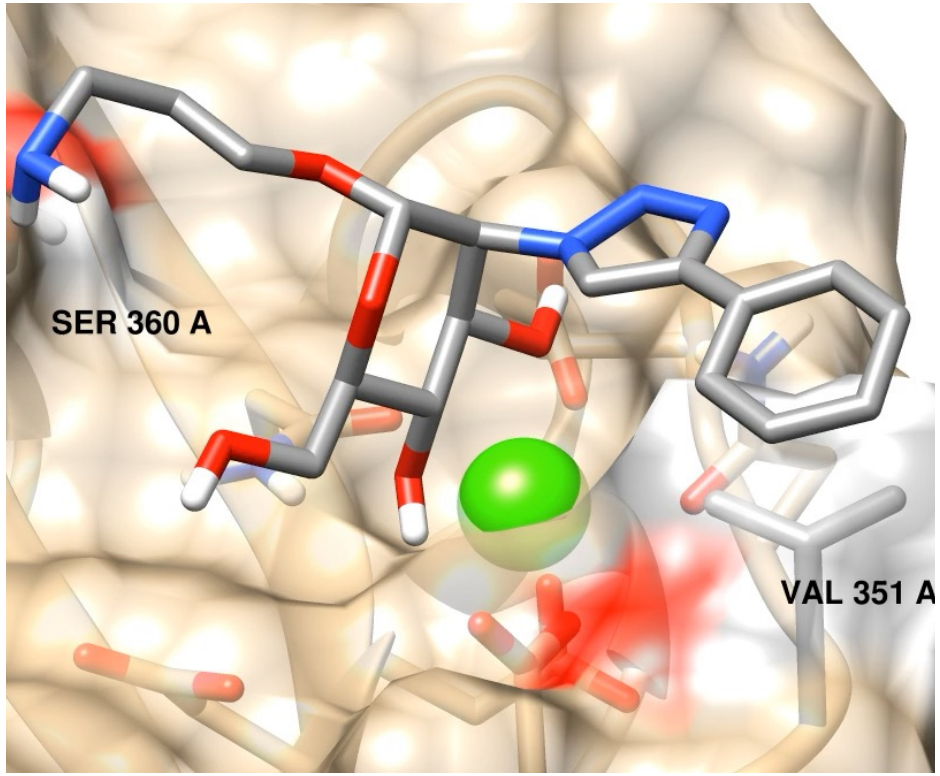
Systems Where Our Method Worked Well (DC-SIGN)

- A lectin involved in immunity. Exploited for infection by HIV[3] and COVID[4].

Mannose (control)



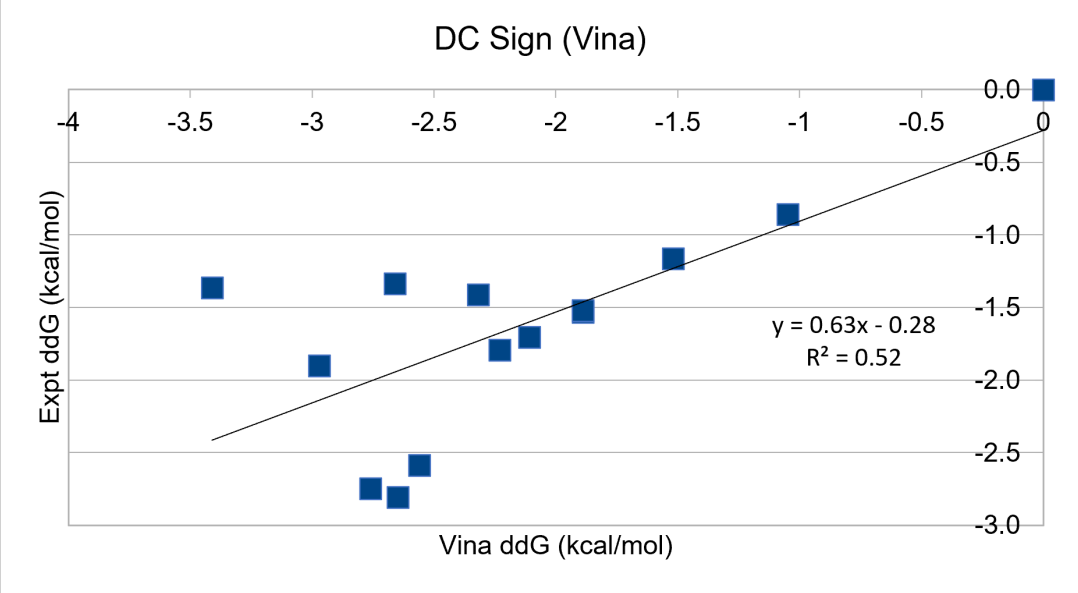
Representative Analog

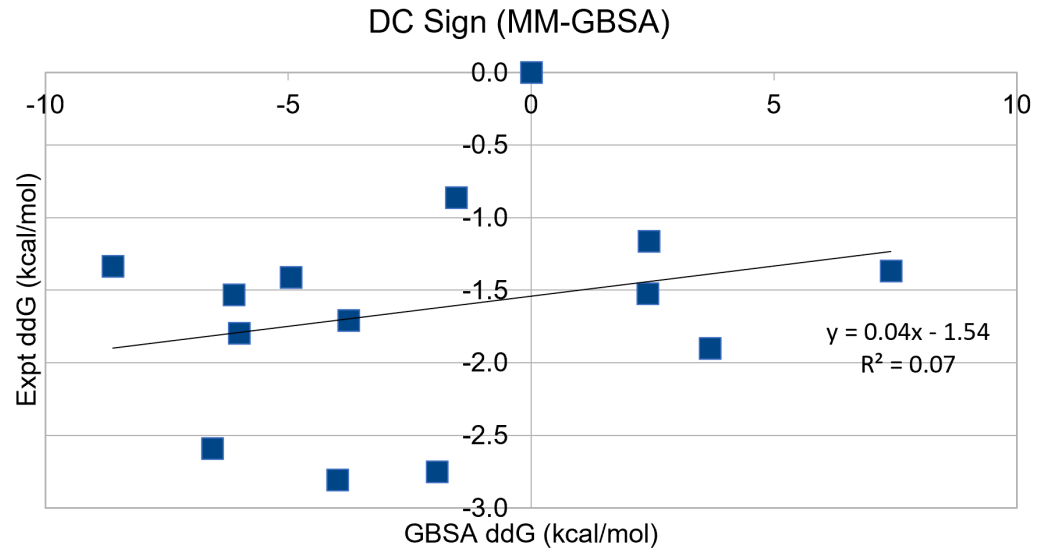


[3] Geijtenbeek, T. B. H., & Van Kooyk, Y. (2003). DC-SIGN: a novel HIV receptor on DCs that mediates HIV-1 transmission. *Dendritic Cells and Virus Infection*, 31-54.
[4] Cramer, J.; Lakkaichi, A.; Aliu, B.; Jakob, R. P.; Klein, S.; Cattaneo, I.; Jiang, X.; Rabbani, S.; Schwardt, O.; Zimmer, G., Sweet drugs for bad bugs: a glycomimetic strategy against the DC-SIGN-mediated dissemination of SARS-CoV-2. *Journal of the American Chemical Society* **2021,** *143* (42), 17465-17478

Statistical Correlation to Experimental Affinity

Vina with CH-π significantly outperformed MM-GBSA in this system





[3] Cramer, J.; Lakkaichi, A.; Aliu, B.; Jakob, R. P.; Klein, S.; Cattaneo, I.; Jiang, X.; Rabbani, S.; Schwardt, O.; Zimmer, G., Sweet drugs for bad bugs: a glycomimetic strategy against the DC-SIGN-mediated dissemination of SARS-CoV-2. *Journal of the American Chemical Society* **2021,** *143* (42), 17465-17478

Conclusions

Glycomimetic design is amenable to automation

- Expect to see it at glycam.org in 2024

Online Webtools ensure consistency and ease of use

Predicted binding energies need to be improved

- Parameterization of existing scoring functions
- Introduction of new physics (CH-π, water, entropy)
- Need to introduce CH-π into AMBER force field for MD

Need beta test users

Acknowledgements

Infrastructure	Online Modeling Tools
Lachele Foley	Dave Montgomery
Dan Wentworth	Spandana Makeneni
Yao Xiao	Amika Sood
Oliver C. Grant	

NIH National Institute of General Medical Sciences

NIH NATIONAL CANCER INSTITUTE

GlycoMIP NSF Materials Innovation Platform