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Using Computational Molecular Docking methods to further understand the structure and function of the MalA protein through binding of different sugars.



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Background

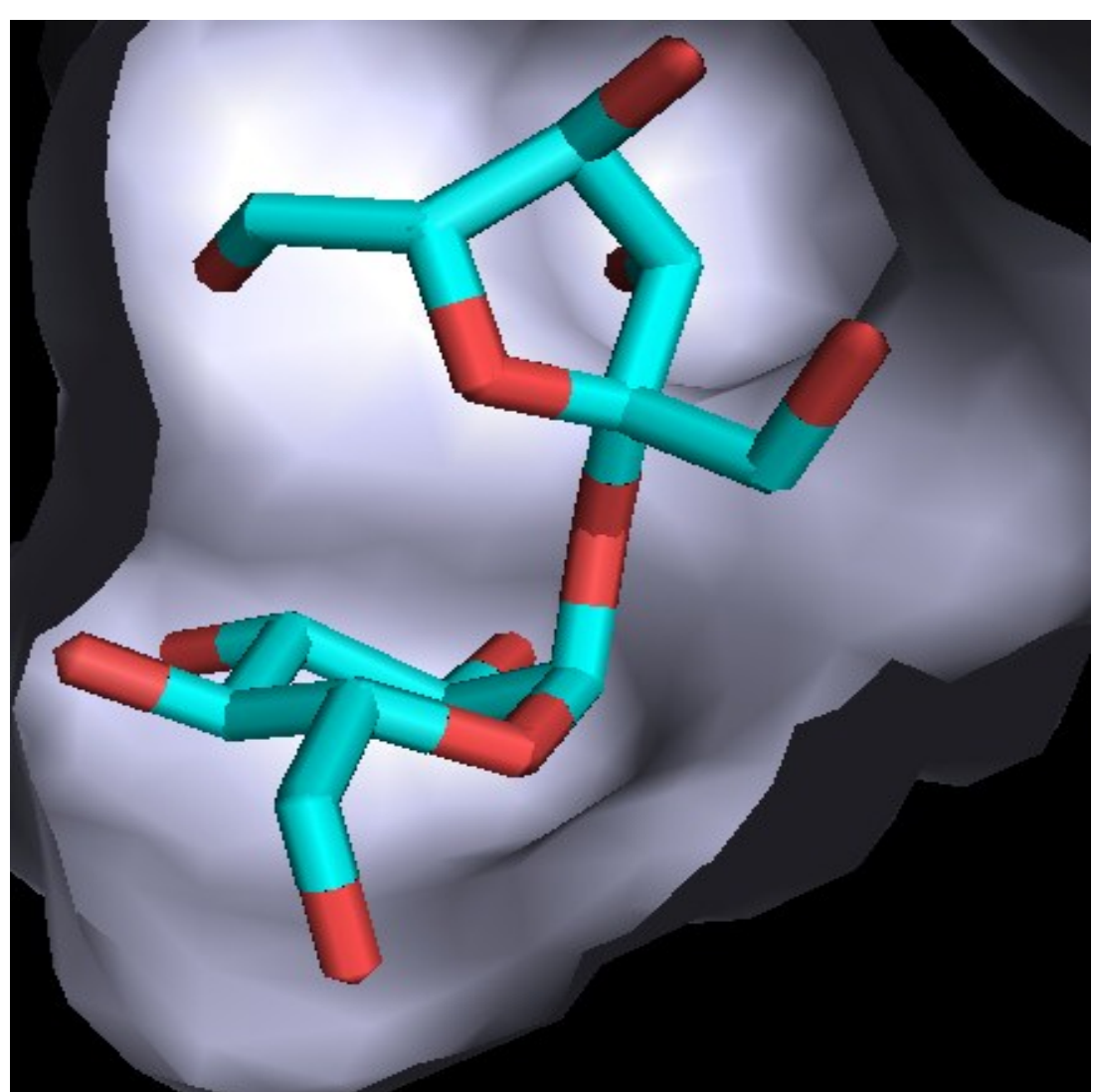
MalA is an enzyme found in the bacterium *Bdellovibrio bacteriovorus* and is a proposed member of a larger group of proteins known as the glycoside hydrolase family 13 of alpha-glucosidases (GH13). In order to understand its structure and substrate specificity, we have utilized molecular docking simulations. The active residues in the binding pocket were determined from MutB, a sucrose isomerase with a known structure and similar sequence homology (40% identity, 76% homology). The two proteins share 100% identity in the glucose-binding pocket, but the fructose-binding pocket has differences.



Experimental Questions

- Does the MalA homology model accurately describe the structure of the glucose/fructose binding pocket?
- What type of substrate molecules fit the binding pocket of the MalA model?

Docking Design



MutB-sucrose complex (PDB code 2PWE)

Residues in MutB binding pocket in contact with glucose subunit of sucrose: Asp²⁰⁰, Glu²⁵⁴, Asp³²⁷, Tyr⁶⁴, His¹⁰⁴, His³²⁸, Asp⁶¹, Phe¹⁴⁵, Phe¹⁶⁴, Gln¹⁶⁸, Arg⁴¹⁴

The distances between these residues and the protein were used as atom-specific distance restraints (to drive docking of different glucose-containing sugars into the MalA homology model).

Corresponding residues in MalA binding pocket:

Asp²⁰², Glu²⁵⁹, Asp³²⁸, Tyr⁶⁷, His¹⁰⁶, His³²⁹, Asp⁶¹, Phe¹⁴⁷, Phe¹⁶⁶, Gln¹⁷⁰, Arg⁴¹⁴

Docking Protocol

Overview:

The docking process consists of three different stages: the rigid body stage (it0), the flexible simulated annealing stage (it1), and the flexible water refinement stage. These three stages are all very important in order to first place the ligand in the active site of the protein (it0), and only then allow flexibility (it1 and water refinement).

PDB files:

Protein file: This file is the homology model of the MalA protein constructed on MutB (MalA_2pwe.pdb).

Ligand files: 3D coordinates of glucose, sucrose, maltose and turanose were cut out of pdb files 2PWE, 1YTV, and 3UER, respectfully. They were then modified in order to fit HADDOCK format.

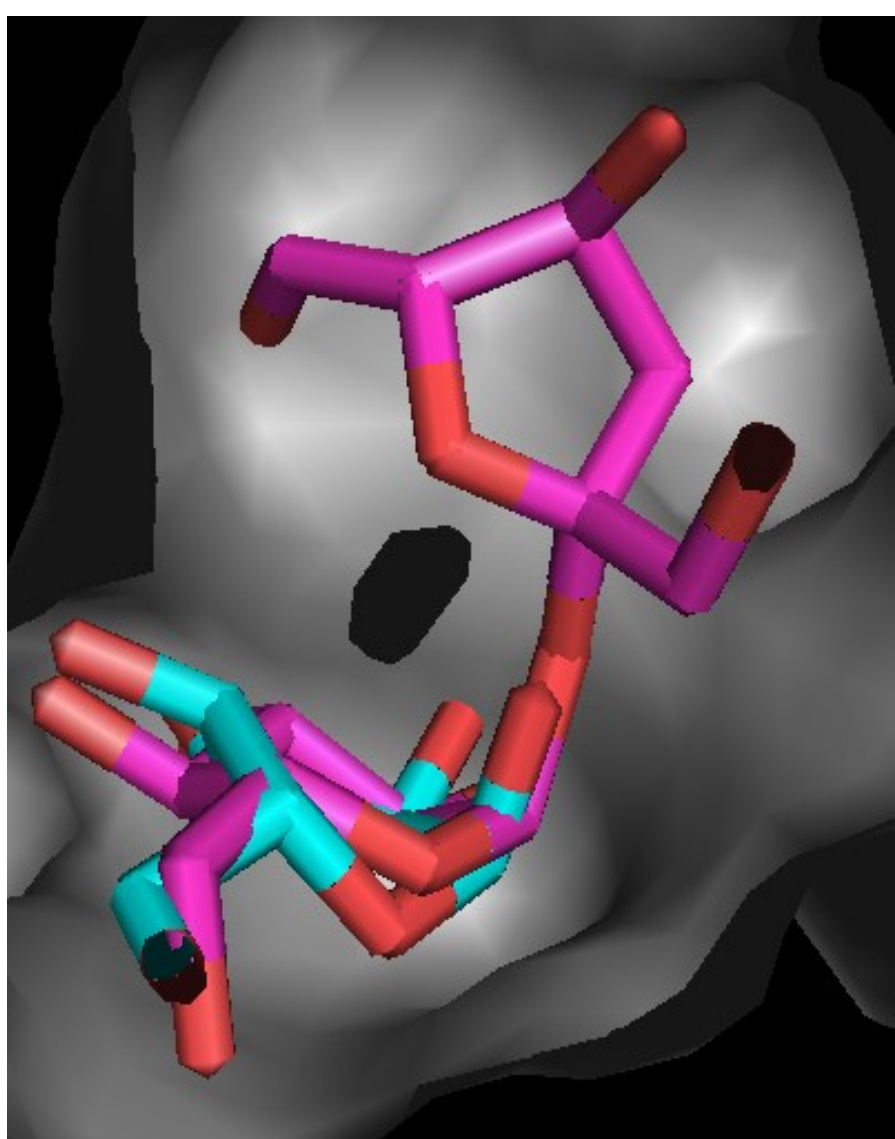
Ligand-specific settings:

- The ligand was allowed to be flexible (n_segB = -1).
- In order to ensure that the ligand would fit properly into the deeply buried active site, the intermolecular interactions for rigid body (inter_rigid) value was changed 0.01 to 0 in order for the ligand and protein atoms to not repel one another, allowing overlay. The van der waals energy scoring for the rigid-body docking stage (w_vdw_0) was changed from 0 to 0.01 in order for HADDOCK to choose the non-overlapping structures for the final water-refinement stage.

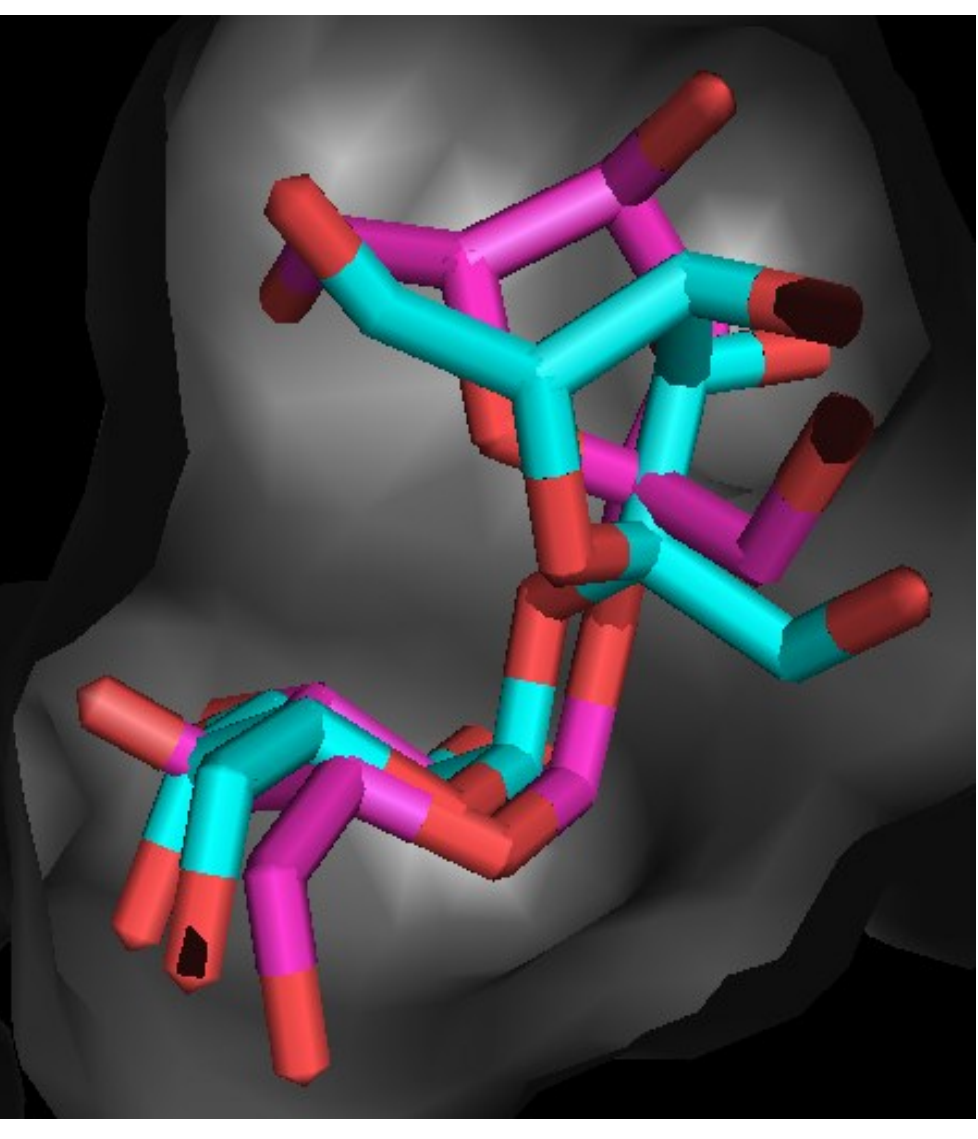
AIR files:

- The Ambiguous Interaction Restraints (AIRs) included active residues of MalA the protein, determined from a sequence alignment with MutB, a homologous protein (www.uniprot.org). The correlating active residues in MalA were determined to be Asp²⁰², Glu²⁵⁹, Asp³²⁸, Tyr⁶⁷, His¹⁰⁶, His³²⁹, Asp⁶⁴, Phe¹⁴⁷, Phe¹⁶⁶, Gln¹⁷⁰, Arg⁴¹⁴, and these active residues were inputted into the AIR files.
- Atom-specific contacts were obtained from running a contact analysis script with a 3.9 Angstrom cutoff on glucose bound to the MalA protein. The shortest distance (in Angstroms) for each specific atom on glucose to the active residues on the MalA protein were determined, and this data was inputted into the AIR files.

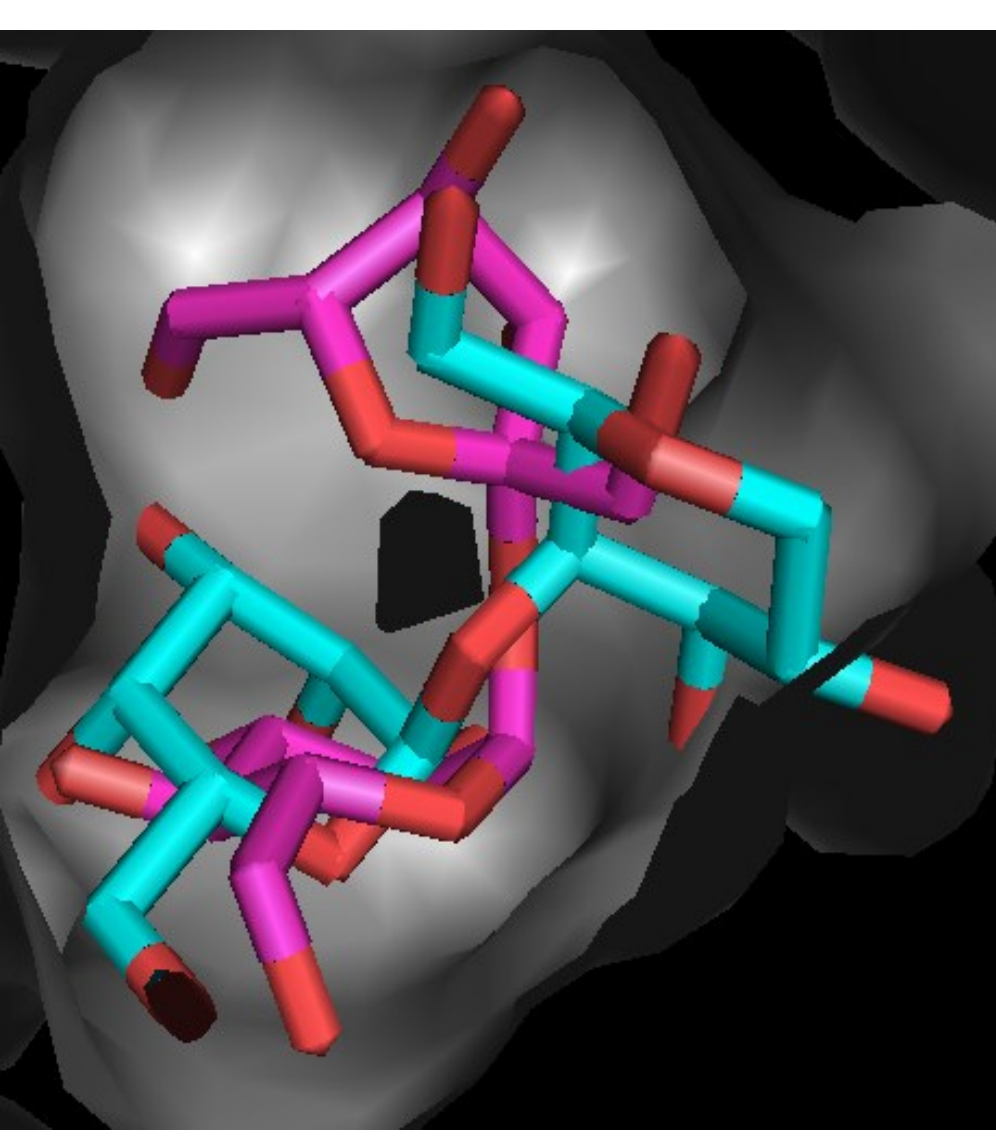
Comparison of Docked Sugars



Glucose
l-RMSD 0.773Å
HADDOCK -28.754



Sucrose
l-RMSD 0.838Å
HADDOCK -4.959



Maltose
l-RMSD 1.586Å
HADDOCK -38.043

Color Key:
Reference sucrose: purple
Docked sugar: cyan

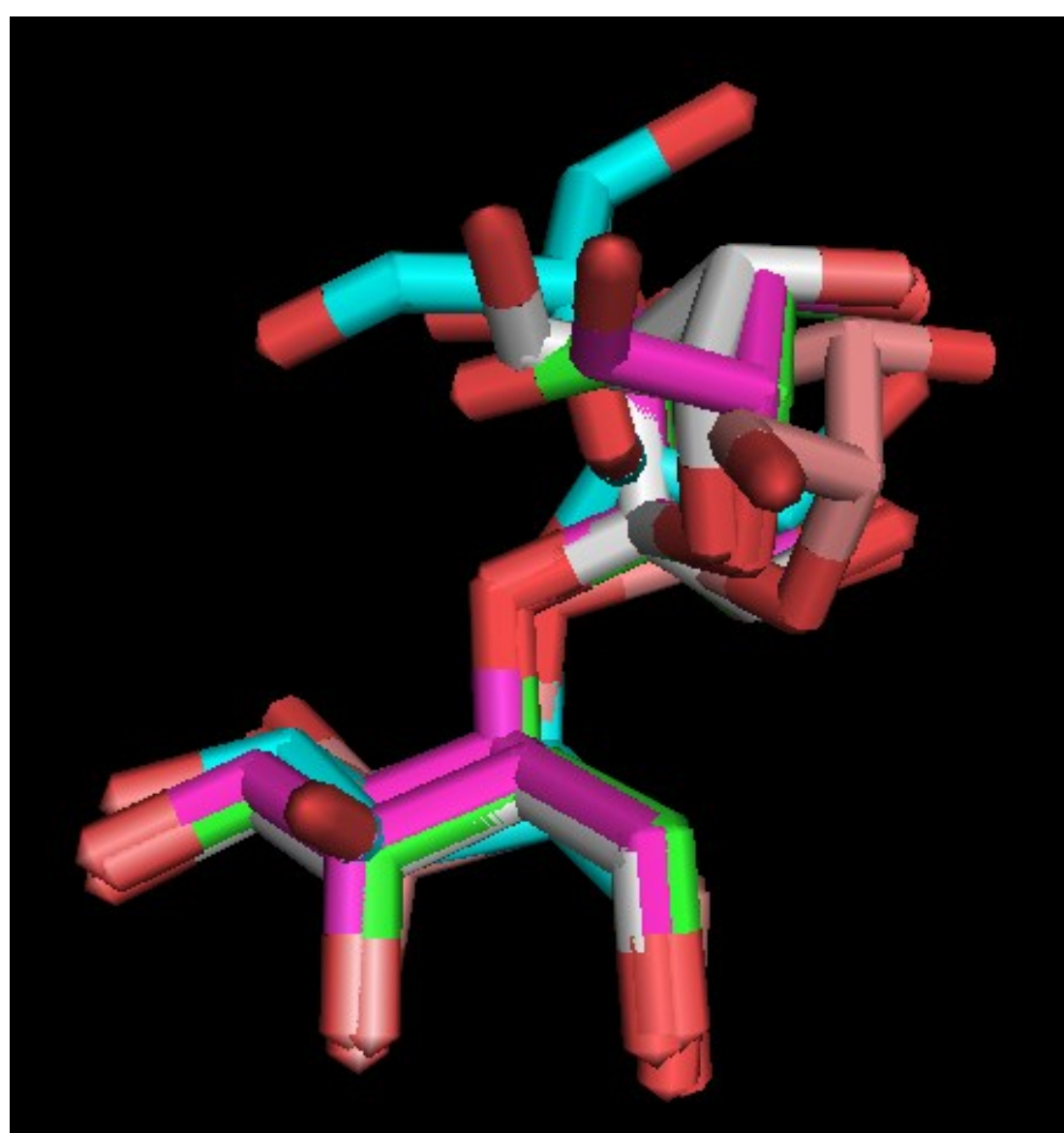
l-RMSD: fitting the backbone of protein to the reference structure, then calculating the RMSD of the common glucose atoms.

These figures show the dockings with the best HADDOCK scores, although l-RMSD values and HADDOCK scores do not have a high correlation, as seen with the lack of overlap of the glucose subunit of maltose.

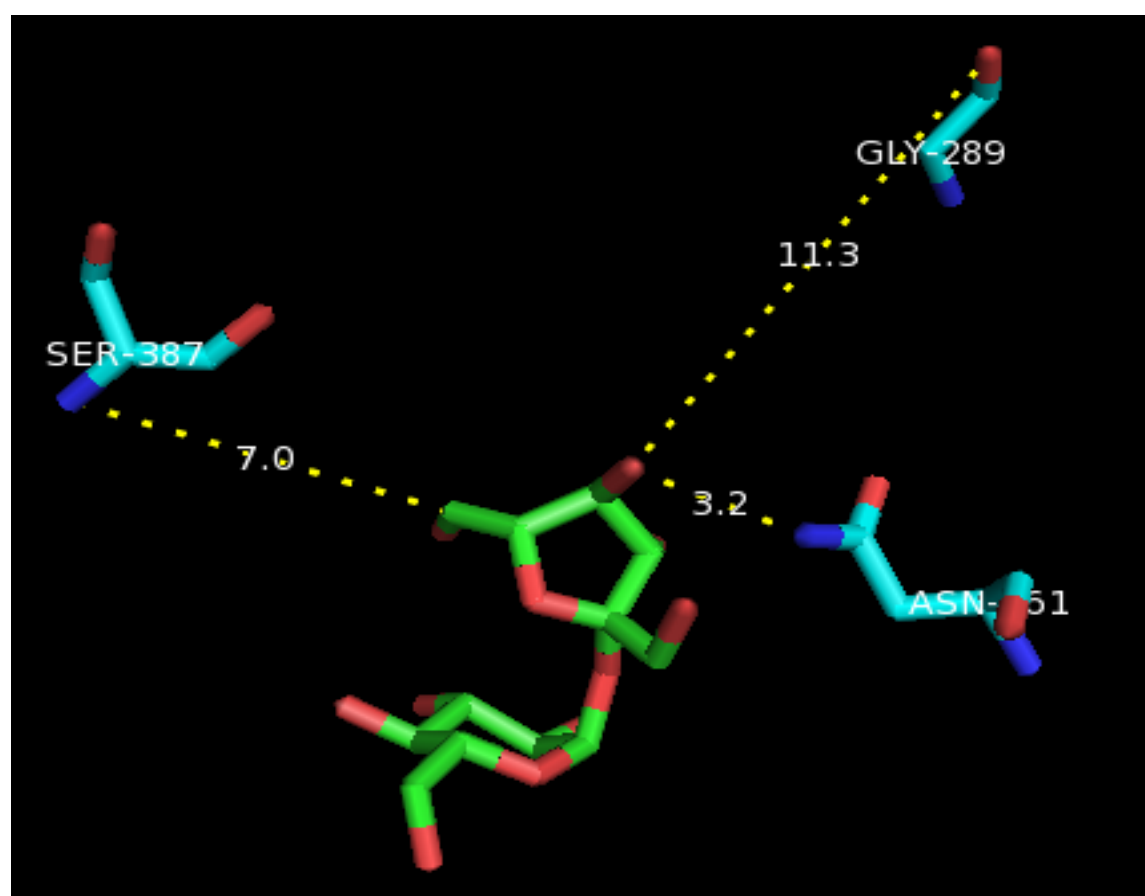
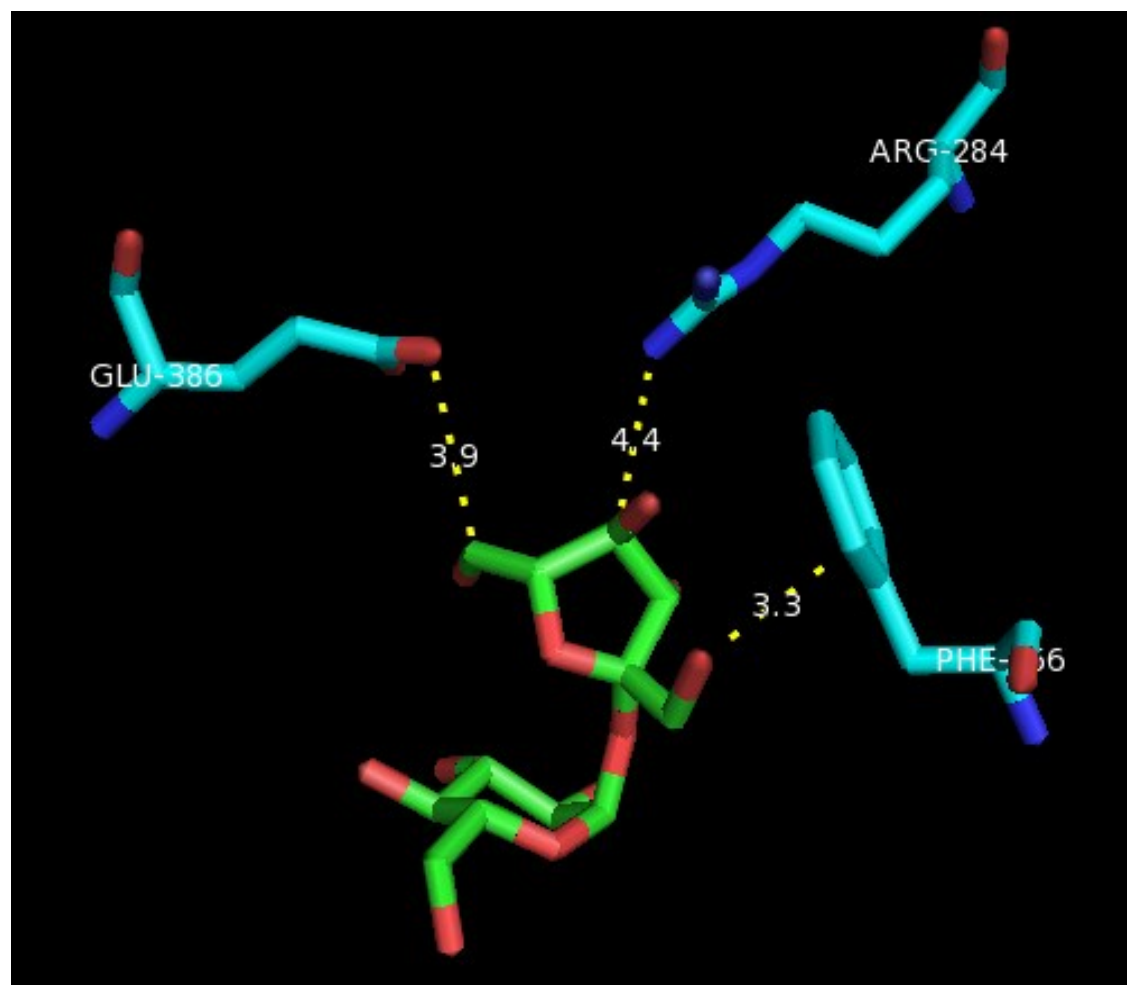
Future Directions

- Bind acarbose, a tetrasaccharide, in order to test whether longer sugars can fit in the binding pocket.
- Bind trehalose, a sugar not expected to fit well into the binding pocket, in order to determine the shape of the active site.
- Determine differences in the structure of MalA vs. MutB

Analysis of Fructose Subunit of Sucrose



Sucrose bound to MutB



Sucrose bound to MalA

MutB	MalA
Phe 256	Asn 261
Phe 164	Phe 166
Asp 327	Asp 329
Arg 414	Arg 414
Glu 254	Glu 259
Arg 284	Gly 289
Glu 386	Ser 387

- Top four scoring (by HADDOCK) structures of sucrose bound to MalA

- Glucose maintains the same position in each docking.

- Fructose rotates about the C1-O1 and O1-C1' bonds (The fructose has no distance restraints).

- The table lists residues in contact with fructose in MutB, and the corresponding residues in MalA. Four of the residues are conserved in the MalA protein.

- The residues that differ in MalA (highlighted orange) are all much smaller.

- The fructose-binding portion of the MalA active site is more open than the MutB

Discussion

- The MalA homology model structure allows binding of alpha-glucosides: glucose, sucrose, maltose
- The MalA fructose-binding region is much more open than MutB, and does not restrain the position of the fructose
- The MalA active site is larger than that of MutB, perhaps allowing longer sugars, such as acarbose (a tetrasaccharide) to fit within the binding pocket

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References

- Henrissat, B. and Davies, G. *Curr. Opin. Struct. Biol.* **2007**, 7, 637-644.
- Ravaud, S.; Xavier, R.; Watzlawick, H.; Haser, R.; Mattes, R.; Aghajari, N. Trehalulose Synthase Native and Carbohydrate Complexed Structures Provide Insights into Sucrose Isomerization *J. Biol. Chem.* **2007**, 282, 38, 28126-28136.
- Smith, P.; Hanson, J.; Martin, M. O.; Grinstead, J. Determining the Kinetic Parameters of MalA, a glucosidase from the predatory bacterium *Bdellovibrio bacteriovorus*, poster presented at summer science research symposium, **2012**
- Isabella, C.; Hanson, J.; Grinstead, J.; Martin, M. O. Exploration of the Active Site Specificity of MalA, a glucosidase from the predatory bacterium *Bdellovibrio bacteriovorus*, poster presented at *2012 ACS National meeting*, **2012**
- Ravaud, S.; Robert, X.; Watzlawick, H.; Haser, R.; Mattes, R.; Aghajari, N. Trehalulose Synthase Native and Carbohydrate Complexed Structures Provide Insight into Sucrose Isomerization *J. Biol. Chem.* **2008**, 282, 38, 28126-28136.
- Ravaud, S.; Robert, X.; Watzlawick, H.; Haser, R.; Mattes, R.; Aghajari, N. Structural determinants of product specificity of sucrose isomerases *Inst. Biol. Chim. Prot.* **2009**
- Halperin, I.; Ma, B.; Wolfson, H.; Nussinov, R. Principles of docking: An overview of search algorithms and a guide to scoring functions *Sackler Inst. Mol. Med.* **2002**, 47, 4, 409-443.
- de Vries, S. J.; van Dijk, M.; Bonvin, A. M. J. J. The HADDOCK web server for data-driven biomolecular docking *Nat. Prot.* **2010** 5, 883-897
- Dominguez, C.; Boelens, R.; Bonvin, A. M. J. J. HADDOCK: A Protein-Protein Docking Approach Based on Biochemical and Biophysical Information *J. Am. Chem. Soc.* **2003**, 125, 1731-1737.