Supplemental Information

Mannobiose binding induces changes in hydrogen bonding and protonation states of acidic residues in concanavalin A as revealed by neutron crystallography

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MATERIALS AND METHODS

General Information. Jack bean concanavalin A (lyophilized), type V, and α1-2 mannobiose used for crystallization trials were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Crystallization reagents were purchased from Hampton Research (Aliso Viejo, California, USA).

Protein crystallization. Prior to crystallization, a sample of ConA (20 mg/mL) in 1 M NaCl, 50 mM NaOAc at pH 5.0, 1 mM $CaCl_2$, 1 mM $MnCl_2$ was incubated at 42 ^{0}C for 2 hours and dialyzed against three changes of 0.4 M NaCl, 20 mM TRIS pH 7, 1 mM $CaCl_2$, 1 mM $MnCl_2$. Such treatment of the ConA sample before crystallization is set up substantially improves growth and the size of the crystals. For crystallization trials, ConA was concentrated to 14-15 mg/mL. About 1 hour prior to crystallization solution of ConA was combined with the 0.25 M stock solution of α1-2 mannobiose in water at a molar ratio of 1:10 (ConA:ligand). Crystals of the complex were grown in 600μL drops made by mixing the sample and the reservoir solution (10% PEG 6K, 0.1 M sodium citrate, pH 5 in H_2O) at a 1:1 ratio in the 9-well glass plate/sandwich box sitting drop setup. Crystals suitable for neutron diffraction measured ~1 mm^3. For the neutron diffraction data collection, a crystal was mounted in a quartz capillary containing 12 % PEG 6K, 0.1 M sodium citrate solution made with 100% D_2O. Another crystal from the same crystallization drop that provided the crystal for the neutron data collection was mounted in the same fashion for room-temperature X-ray data collection. The labile H atoms were allowed to exchange with D by D_2O vapour for 1 week for both crystals before starting data collection.

X-ray and neutron data collection. Room temperature X-ray crystallographic data set was collected on a Rigaku HighFlux HomeLab instrument equipped with a MicroMax-007 HF X-ray

generator and Osmic VariMax optics. The diffraction images were obtained using an R-Axis IV^{++} image plate detector. Diffraction data were collected, integrated, and scaled using the HKL3000 software suite.[1] The room-temperature X-ray structure of ConA-Mannobiose complex was solved by the molecular replacement method using phasing information from the structure with PDB code 1I3H.[2] The room-temperature X-ray structure was refined using SHELX-97.[3]

Quasi-Laue neutron data to 2.5 Å resolution were collected from the 1 mm^3 crystal of ConA-Mannobiose complex at room temperature on the IMAGINE beamline at the High Flux Isotope Reactor (Oak Ridge National Laboratory, Oak Ridge, TN, USA).[4] Images were collected from 3 different crystal orientations. At each orientation, the crystal was held stationary at different φ settings for each 20 hour exposure. The neutron data were processed using the Daresbury Laboratory *LAUE* suite program *LAUEGEN* modified to account for the cylindrical geometry of the detector.[5,6] The program *LSCALE*[7] was used to determine the wavelength-normalization curve using the intensities of symmetry-equivalent reflections measured at different wavelengths. No explicit absorption corrections were applied. These data were then merged in *SCALA*.[8] A summary of the experimental and refinement statistics is given in Table S1.

Joint X-ray/Neutron (XN) structure refinement. The joint XN structure of the ConA-Mannobiose complex was determined using *nCNS*[9] and manipulated in *Coot*.[10] After initial rigid-body refinement, several cycles of positional, atomic displacement parameter, and occupancy refinement followed. The structures were checked for the correctness of side-chain conformations and water molecule orientations, which were built based on the F_O-F_C difference neutron scattering length density maps. The $2F_O$-F_C and F_O-F_C neutron scattering length density maps were then examined to determine the correct orientation of hydroxyl groups, and protonation states of the enzyme residues. The protonation states of some disordered side chains could not be obtained directly, and remained ambiguous. All water molecules were refined as D_2O. Initially, water oxygen atoms were positioned according to their electron density peaks, and then were shifted slightly in accordance with the neutron scattering length density maps. The level of H/D exchange was refined for labile OD, and ND groups, but not for water molecules, through occupancy refinement of D. The occupancies of the D atoms were refined individually within the range of -0.56 to 1.00 (the scattering length of H is -0.56 times the scattering length of D). Before depositing the final structure to the PDB, a script was run that converts a record for

the coordinates of D atom into two records corresponding to an H and a D atom partially occupying the same site, both with positive partial occupancies that add up to unity. The joint XN structure of ConA-Mannobiose complex has been deposited to the Protein Data Bank with the code 5WEY.

Table S1. Room temperature crystallographic data collection and joint XN refinement statistics.

	ConA-Mannobiose, PDB ID 5WEY	
Data collection:	**Neutron**	**X-ray**
Beamline/Facility	IMAGINE/ORNL	Rigaku HighFlux HomeLab
Space group	I222	
Cell dimensions:		
a, *b*, *c* (Å)	66.56, 86.62, 91.81	
α, β, γ (°)	90, 90, 90	
Resolution (Å)	53.84-2.50 (2.63-2.50)*	50.00-1.80 (1.86-1.80)
No. reflections measured	34144 (3520)	96702
No. reflections unique	7739 (985)	23395 (2192)
R_{merge}	0.168 (0.297)	0.055 (0.539)
$I / \sigma I$	4.1 (2.3)	25.1 (2.0)
Completeness (%)	82.5 (72.1)	93.7 (89.9)
Redundancy	4.4 (3.6)	4.1 (4.0)
Joint XN Refinement:		
Resolution (neutron, Å)	40-2.50	
Resolution (X-ray, Å)	40-1.80	
Data rejection criteria	no observation & |F|=0	
Sigma cut-off	2.5	
No. reflections (neutron)	6614	
No. reflections (X-ray)	19749	
R_{work} / R_{free} (neutron)	0.247 / 0.285	
R_{work} / R_{free} (X-ray)	0.191 / 0.212	
No. atoms		
Protein including H and D	3570	
Ligand (α1-2 Mannobiose)	45	
Metal	2	
Water	417 (139 D_2Os)	
B-factors		
Protein	23.05	
Ligand	28.1	
Metal	17.5	
Water	42.0	
R.m.s. deviations		
Bond lengths (Å)	0.008	
Bond angles (°)	1.158	

* Values in parentheses are for highest-resolution shell. Data were collected from 1 crystal for each structure.

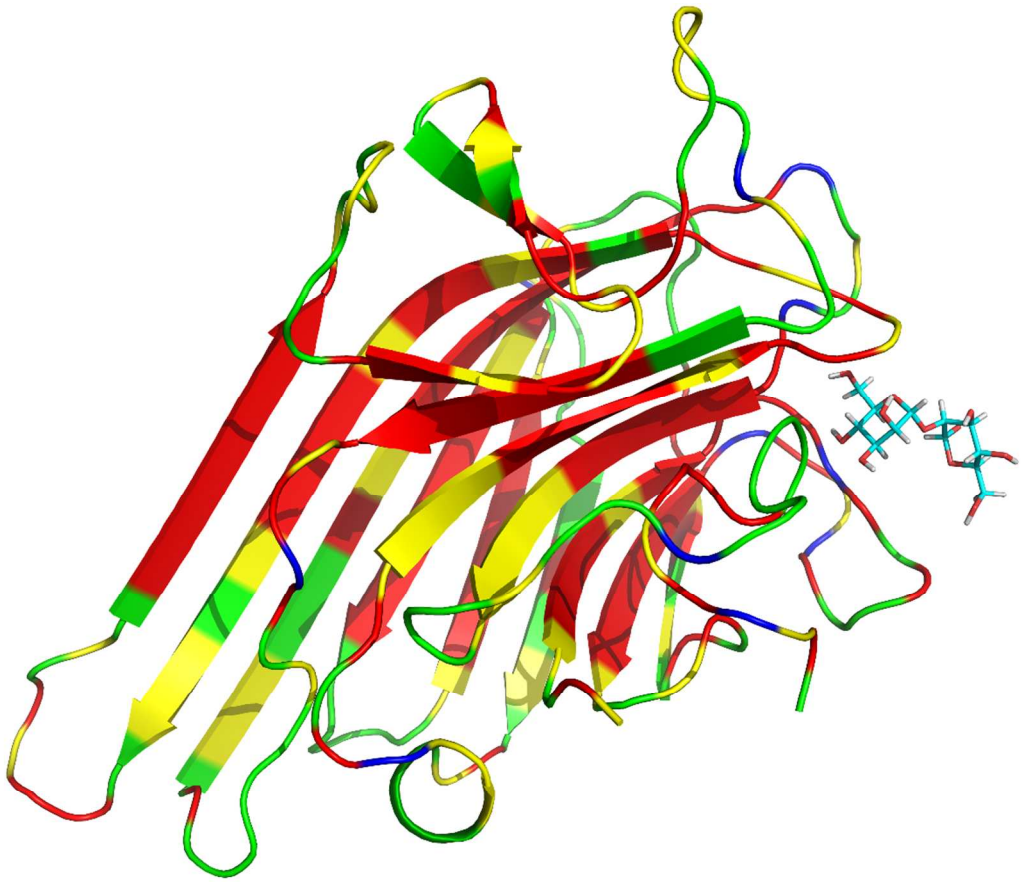

Figure S1. Backbone H/D exchange patterns for ConA-Mannobiose neutron structure depicted using cartoon representation. Green color corresponds to fully exchanged main chain amides (D occupancy 0.60-1.00), yellow for partially exchanged (D occupancy of 0.00-0.60), and red for non-exchanged NH (D occupancy of -0.56-0.00). As expected, the main chain amides involved in tight hydrogen bonds formed in β-sheets undergo less H/D exchange than at other positions. Proline residues that lack a main chain amide hydrogen are colored blue. The level of H/D exchange of the labile hydrogen atoms on the side chains is > 80%.

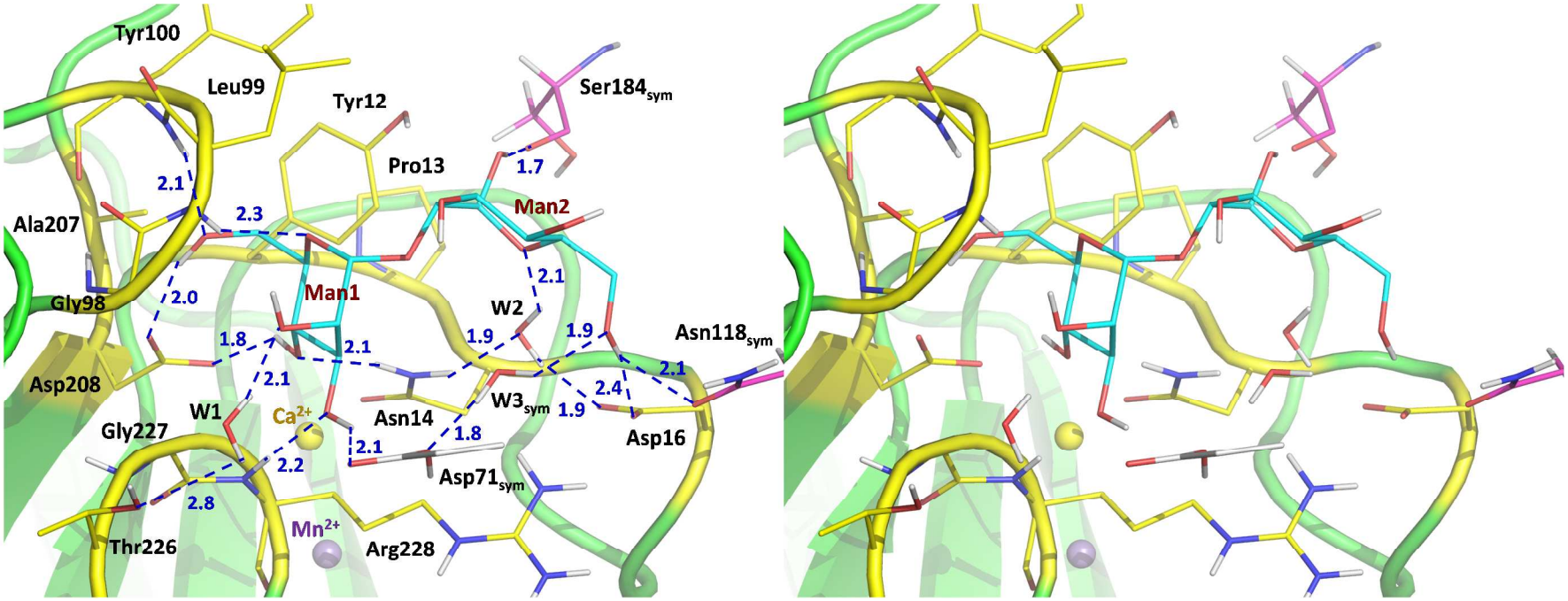

Figure S2. A cross-eye stereo-view version of Figure 1.

Hydrogen bonding and water-mediated interactions made by α1-2 D-mannobiose with ConA residues. Carbohydrate binding site residues are color-coded with carbon in yellow. The residues from the two symmetry-related ConA molecules are color coded with carbon in grey, and in magenta. D···O and D···N distances are shown by blue dashed lines, and are in Å.

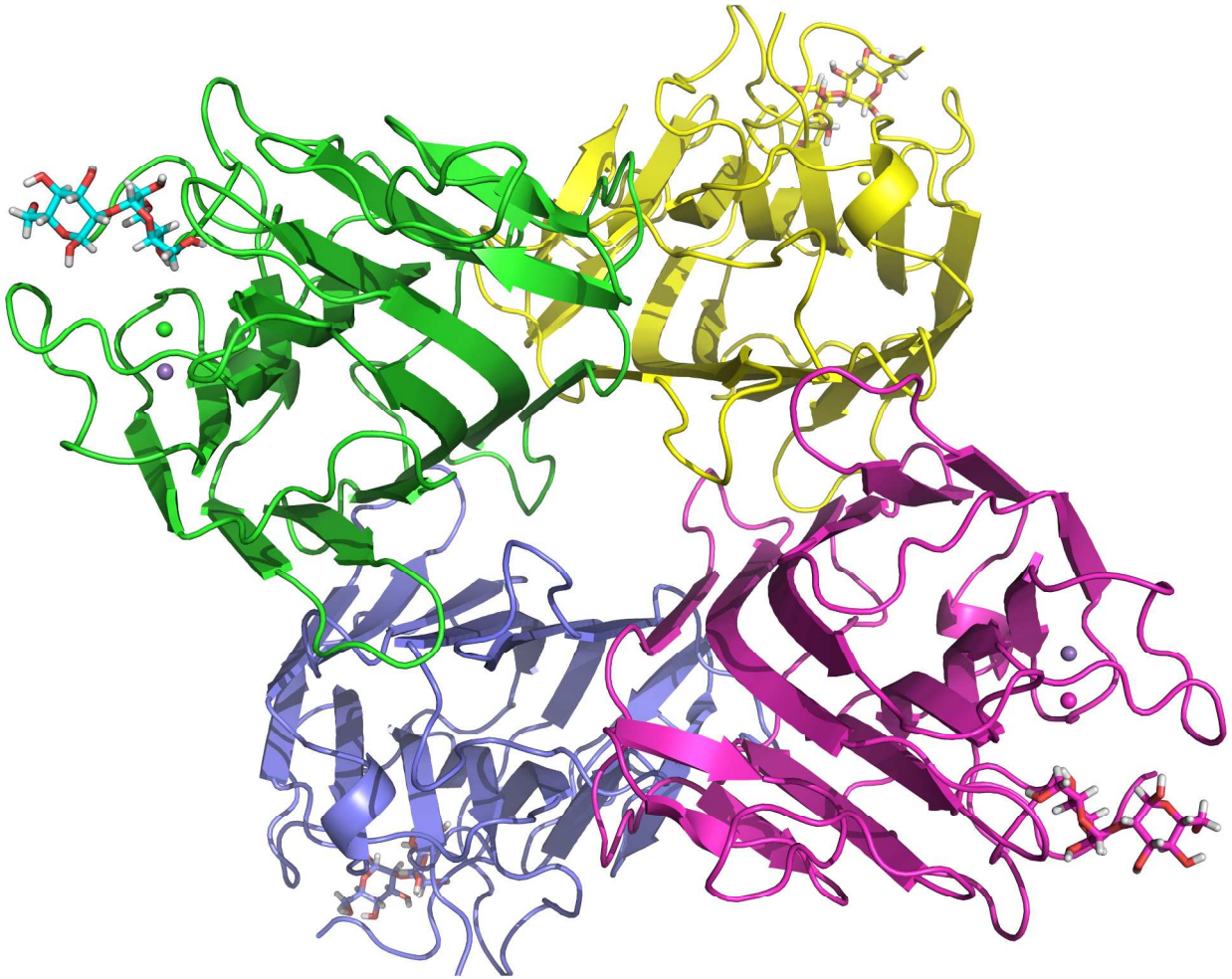

Figure S3. ConA tetramer made by crystallographic symmetry operations of the I222 space group. α1-2 Mannobiose is shown in stick representation; Ca^{2+} and Mn^{2+} metal ions are depicted as green and violet spheres, respectively, for the green-colored ConA molecule.

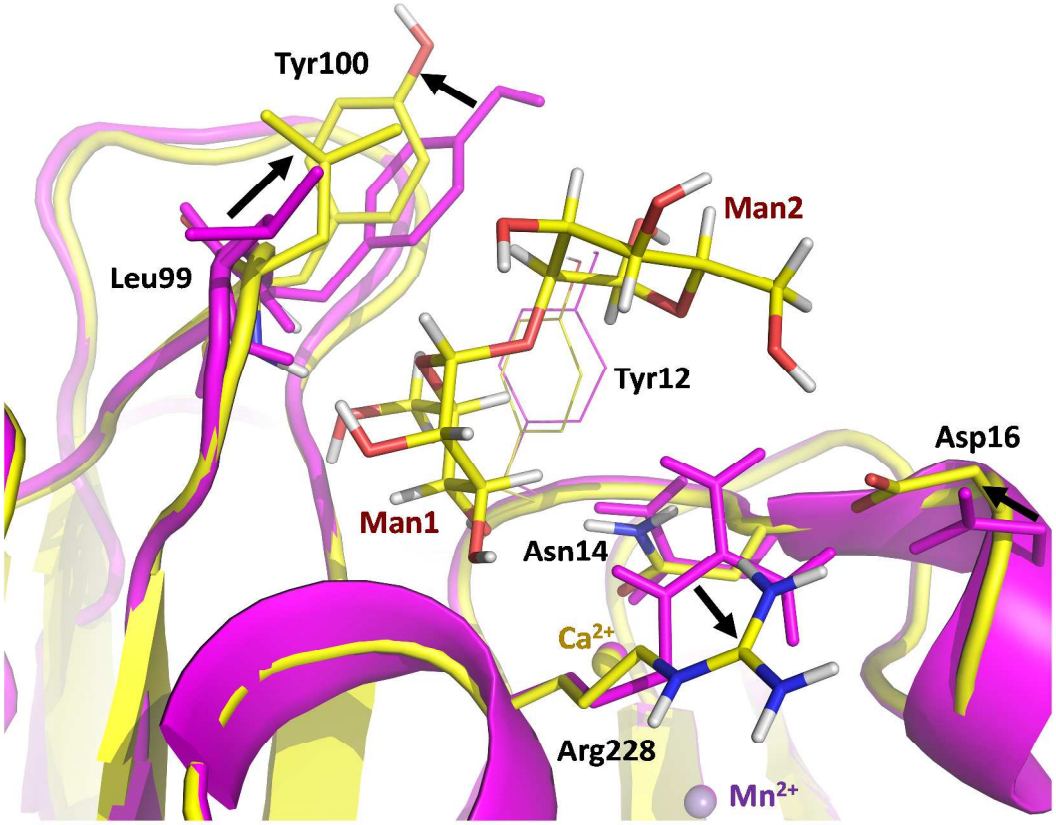

Figure S4. Superposition of the neutron structures of ConA-mannobiose complex and the apo ConA (PDB ID 2YZ4). The disaccharide complex is colored by atom type with carbon in yellow; sugar-free ConA is colored magenta. Residues that change their positions significantly to accommodate the disacchiride are shown as sticks and their movement is indicated with black arrows.

References

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