Supporting Information

In Silico Mutagenesis and Docking Study of *Ralstonia solanacearum* RSL Lectin. Performance of docking software to predict saccharide binding

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METHODS

RSL lectin production. RSL protein was prepared as previously described [1]. *E. coli* BL21 (DE3) cells harboring pET25rsl plasmid were grown in LB broth containing 100 μg ml^{-1} ampicillin at 37°C until the OD_{600} reached approximately 0.6.Then the cells were cultured for an additional three hours with addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG). After induction with IPTG, bacterial cells were harvested by centrifugation at 8,000 g, for 20 min, at 4°C and washed with equal volume of buffer containing 20mM Tris/HCl, 100 mM NaCl, (pH 7.4). The cell suspension was disintegrated by ultrasonic vibration and cell-free extract was collected by centrifugation at 20,000g for 50 min at 4°C. Recombinant RSL was purified by affinity chromatography on Mannose-agarose column. Protein extract was loaded on column equilibrated by 20mM Tris/HCl, 100 mM NaCl buffer pH 7.4 and unbound proteins were washed out with the same buffer. RSL was eluted with 0.1 M D-mannose in the same buffer. Obtained RSL was dialyzed against deionised water for 5 days at 4°C and then concentrated by lyophilization. Protein purity was assessed by SDS–PAGE and mass spectroscopy.

Surface Plasmon Resonance. SPR experiments were performed on a BIAcore 3000 instrument (GE Healthcare) at 25°C using a running buffer HEPES - Buffered Saline (HBS) (10 mM HEPES and 150 mM NaCl, pH 7.5) and a flow rate of 5 μL per minute. A research grade CM5 sensor chip was activated with an EDC/NHS solution (1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide, N-hydroxysuccinimide) for 10 minutes and 50 μl of RSL in 5 mM maleate buffer, pH 6.0, at the concentration of 100 μg/ml, was injected to a flow cell. The unreacted species on the sensor surface were blocked by 1 M ethanolamine. The blank channel was treated identically except for the lectin injection. 20 μl of carbohydrate solutions (concentrations between 0.7 μM and 10 mM, depending on the ligand) in running buffer were injected into the flow cells using the kinject mode. The equilibrium response (after subtraction from the response of the reference surface) of each experiment was used to

create curves of the carbohydrate binding, which were fitted to a 1:1 steady-state affinity model using Origin7.0 software (OriginLab Corporation, USA).

RESULTS

A surface plasmon resonance binding assay was used to determine equilibrium dissociation constants K_D for RSL binding to selected monosaccharides. Three independent experiments were performed. Figure S1 shows an example of sensorgrams obtained after injection of the low affinity binders over the lectin-covered surface. Because association and dissociation phases were rapid, binding curves were done using steady-state parts of experimental curves. K_D were investigated using Scatchard plot analysis and by fitting the data to saturation curves. Final values were obtained by curves fitting using Origin Software 7.0 using model 1:1. The calculated constants were in agreement with values obtained by linearisation methods.

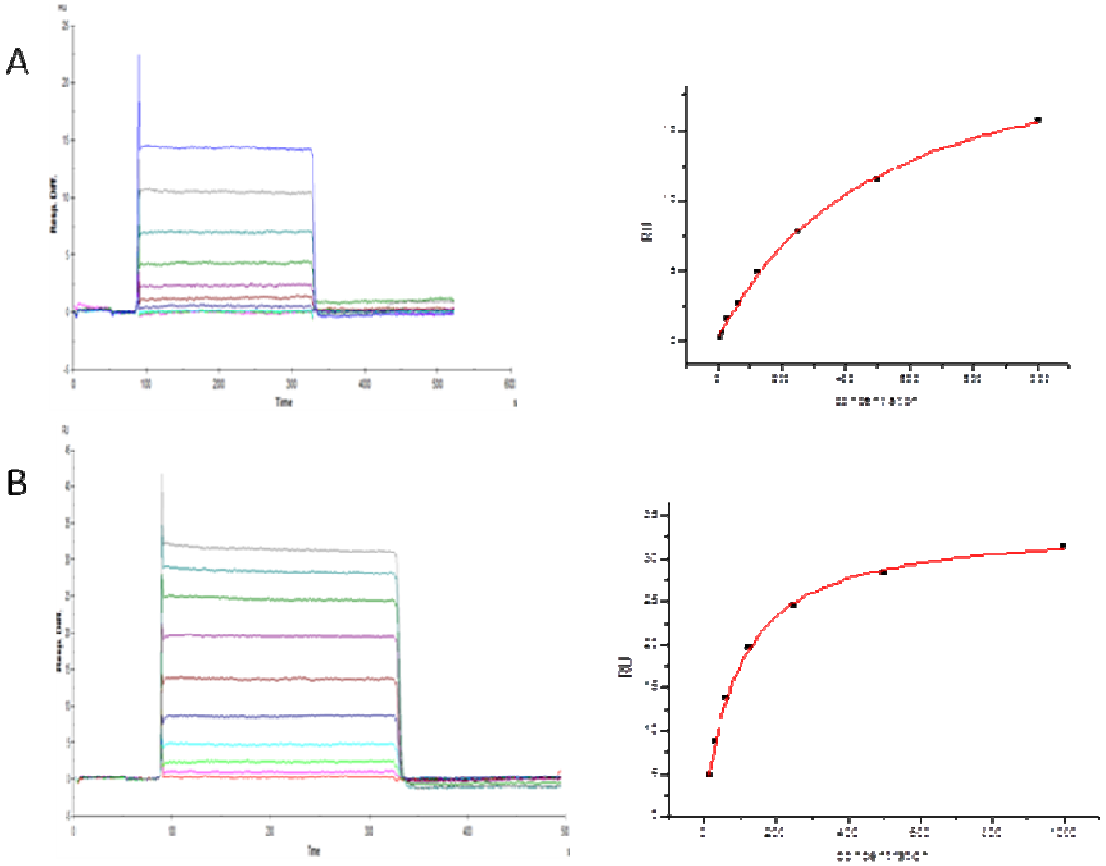

Figure S1: Example of SPR data evaluation.

Left: Equillibrium steady state curves for rhamnose (A) and mannose (B) interacting with RSL at concentrations varying from 1 to 1000 µM.

Right: Binding curves derived from steady-state equilibrium resonance units.

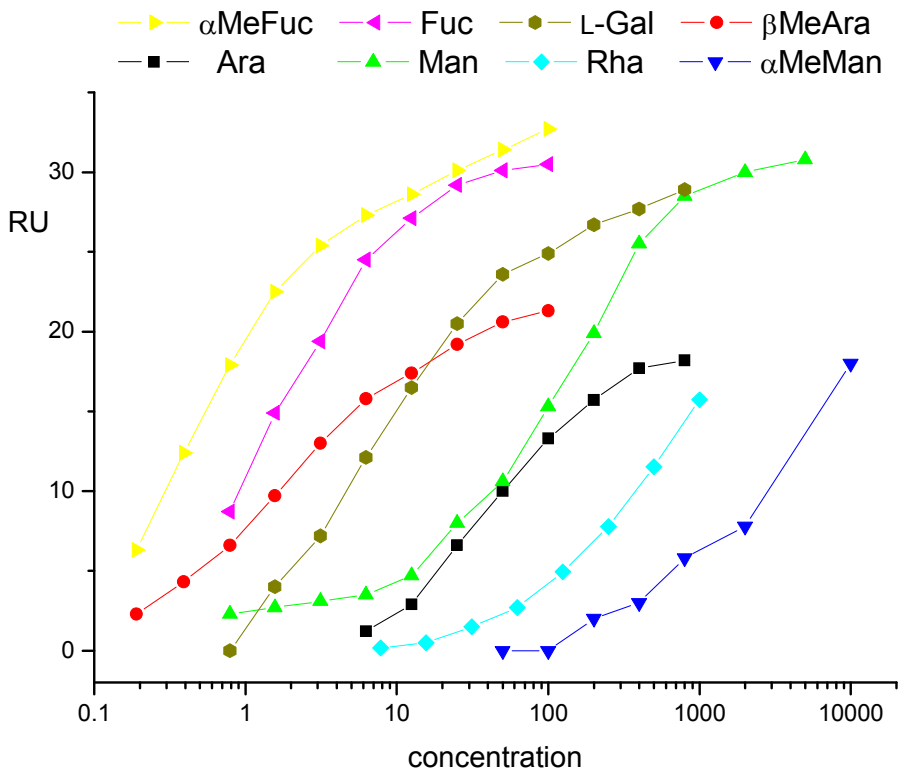

Figure S2: Equilibrium dissociation and association constants for the interaction between monosaccharides and RSL measured by SPR experiments. Binding potency is expressed in comparison with L-fucose (taken as 1.0).

Table TS1: Equilibrium dissociation and association constants for the interaction between monosaccharides and RSL measured by SPR experiments. Binding potency is expressed in comparison with L-fucose (taken as 1.0).

Sugar	K_D [μM]	K_A [mM^{-1}]	relative potency
Me-α-L-fucoside *	**0.52**	**1936.6**	**3.40**
L-fucose *	**1.75**	**570.0**	**1.00**
L-galactose *	**7.75**	**129.0**	**0.23**
Me-β-D-arabinoside	**12.45**	**80.3**	**0.14**
D-arabinose	**48.78**	**20.5**	**0.036**
D-mannose	**104.17**	**9.6**	**0.017**
D-rhamnose	**450.0**	**2.2**	**0.003**
Me-α-D-mannoside	**2588.66**	**0.4**	**0.0007**

* Taken from [1] Kostlanova at al, J Biol Chem 280, 27839–27849, 2005

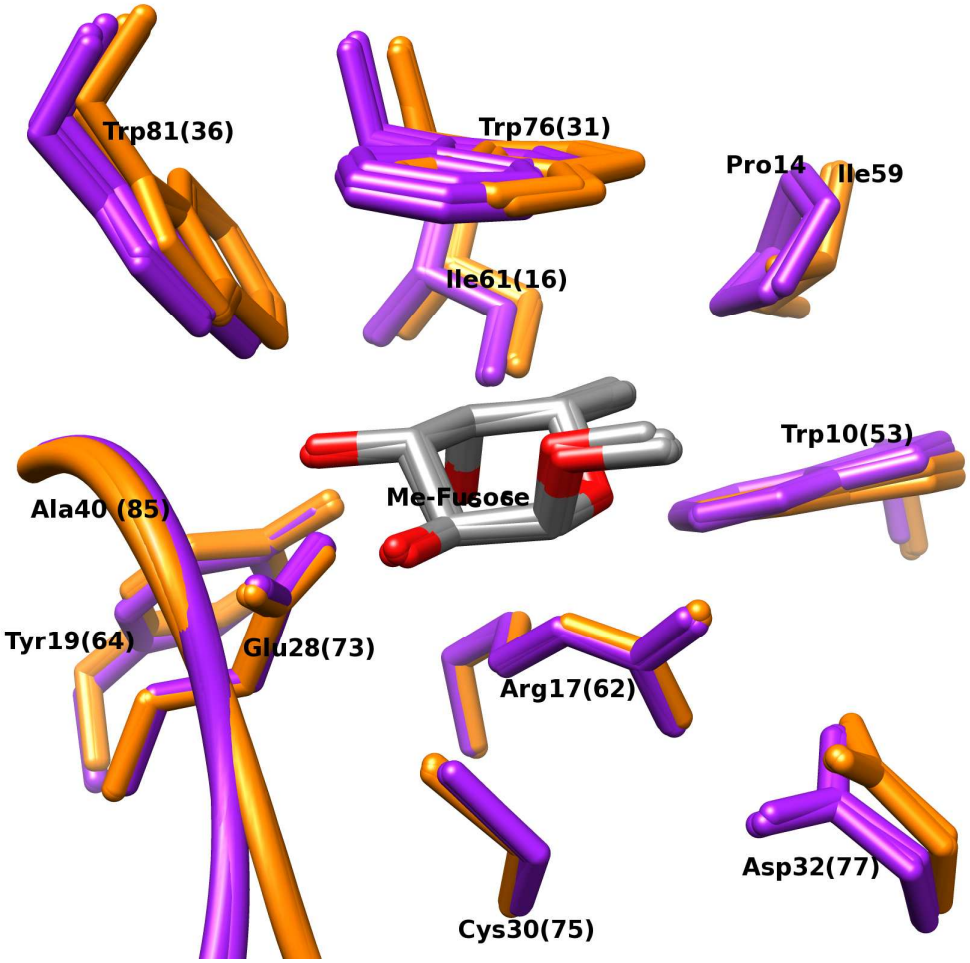

Figure S3. Superimposition of all the intra-monomeric (shown in orange) and intermonomeric (shown in purple) binding sites of RSL. It can be seen that all the sites have similar orientation of amino acid residues around the ligand except Ile59/Pro14 residue in the intra-monomeric and inter-monomeric respectively.

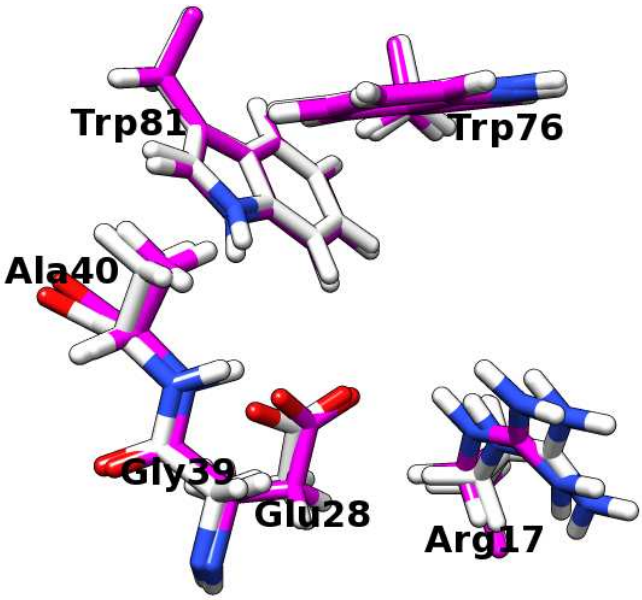

Figure S4: Superimposition of the residues of the binding site in crystal structure (gray) and energy minimized structure (magenta).

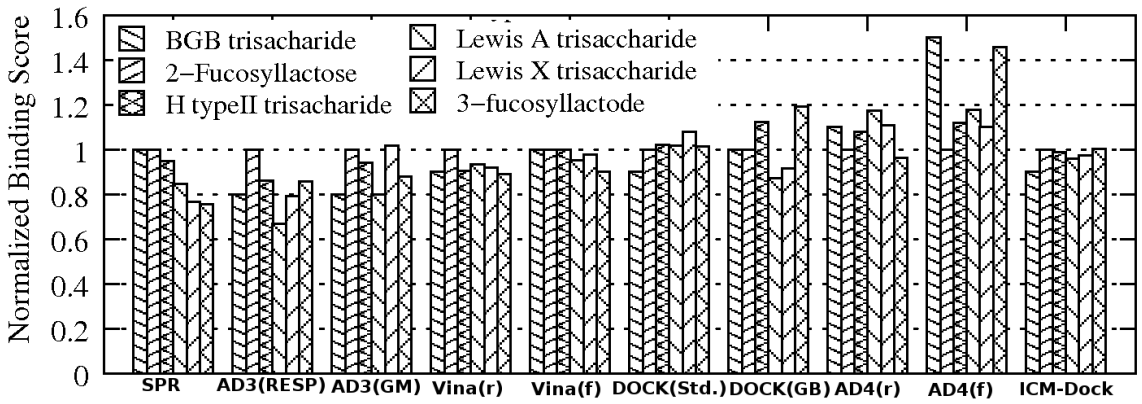

Figure S5. Normalized binding scores calculated for RSL/trisaccharide complexes from data obtained from various docking software. The X-axis represents the experiment and software used, whereas Y-axis shows corresponding scores. The 2-fucosyllactose was set as the reference (taken as 1.0).

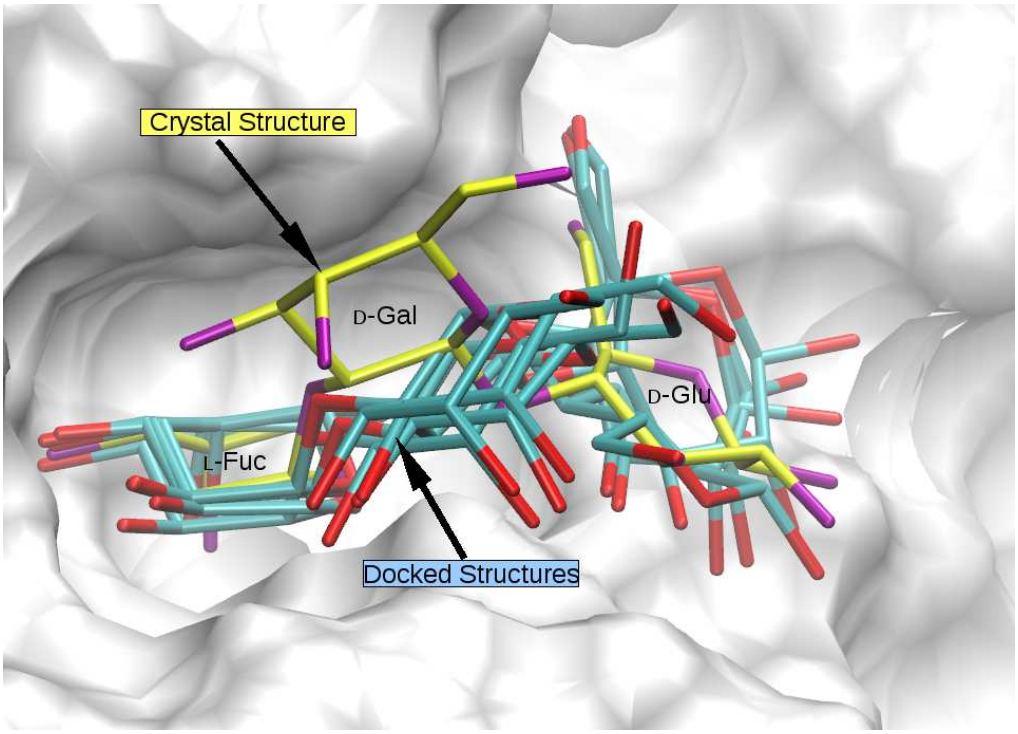

Figure S6: . Docking of 2-fucosyllactose in RSL lectin binding site by docking software. The 2-fucosyllactose from the crystal structure is shown in yellow while the docked structures from various docking software are shown in cyan.

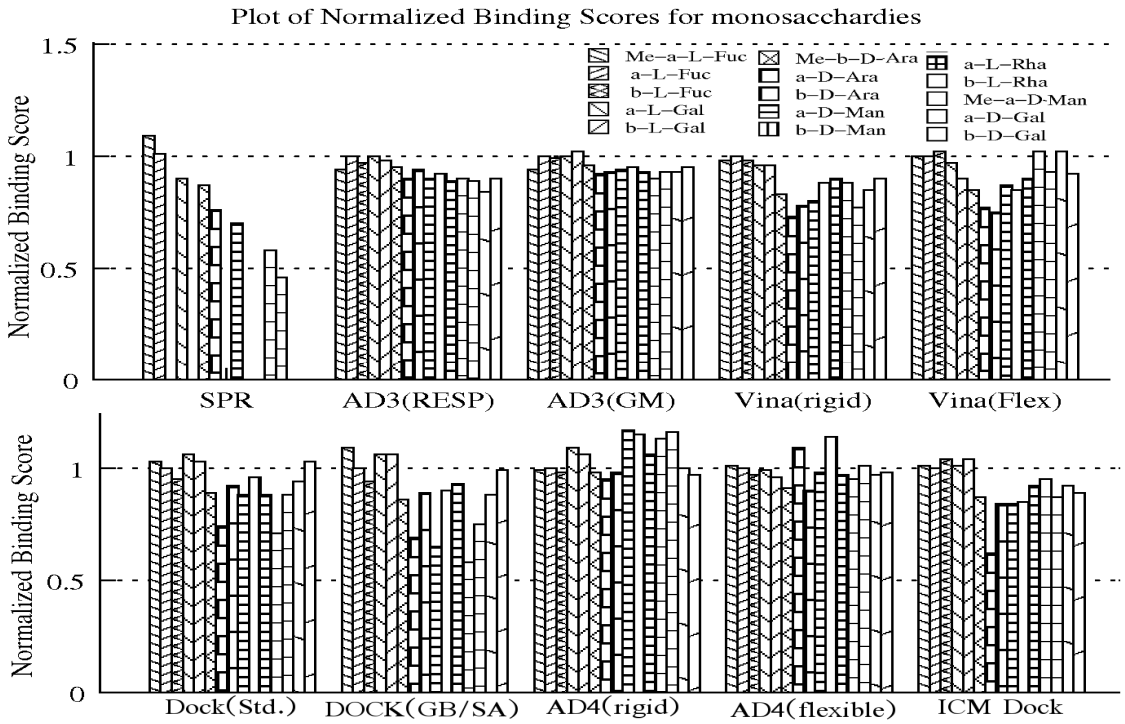

Figure S7. Normalized binding scores calculated for RSL/monosaccharide complexes from various docking software. Y-axis represents normalized binding scores relative to L-fucose (taken as 1.0). Scores of free sugar from SPR experiment are independent of its α/β anomer.

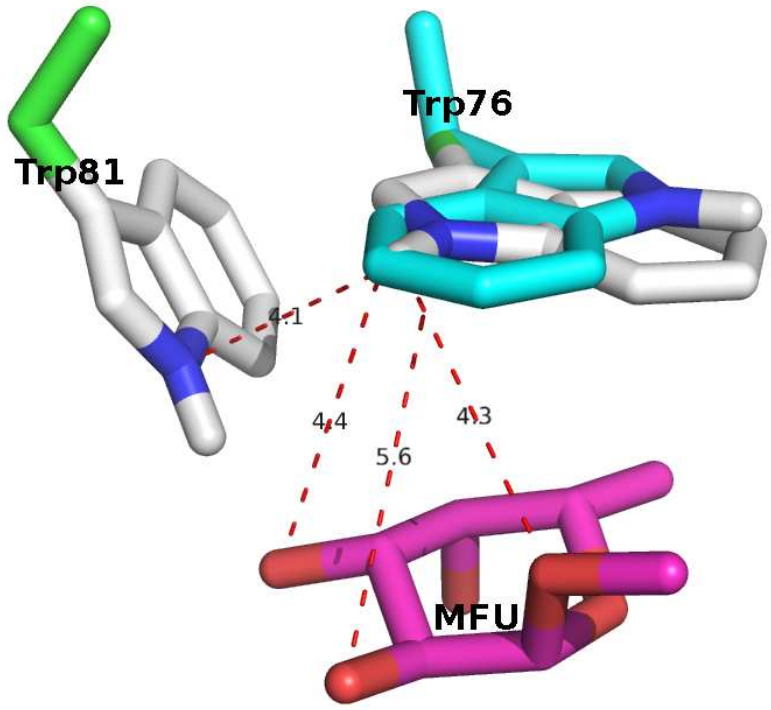

Figure S8. Orientation Trp76 (cyan) up on docking if it was allowed to be flexible, compared to its orientation in crystal structure (gray) of RSL/me-α-L-fucoside complex[1]. Possible interactions of NE1 of Trp76 established in docking are shown by red dots.

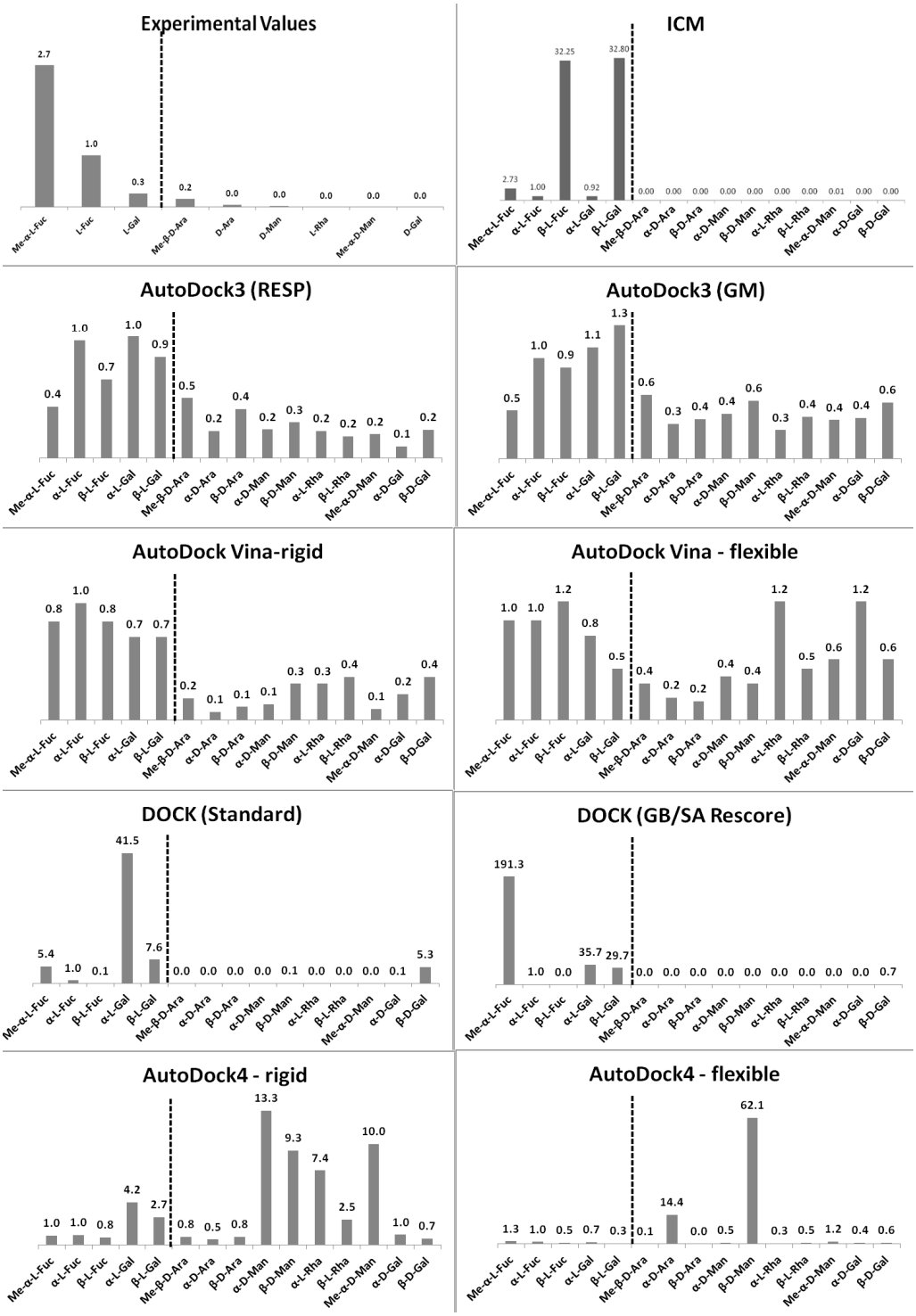

Figure S9. Graphical representation of RSL binding potency towards the studied monosaccharides. Apparent equilibrium association constants were calculated from the energy score calculated by a particular software package and the binding potency was determined. L-fucose was set as a reference (taken as 1.0). The binding potency of the free sugar from the SPR experiment was independent of its α/β anomer.

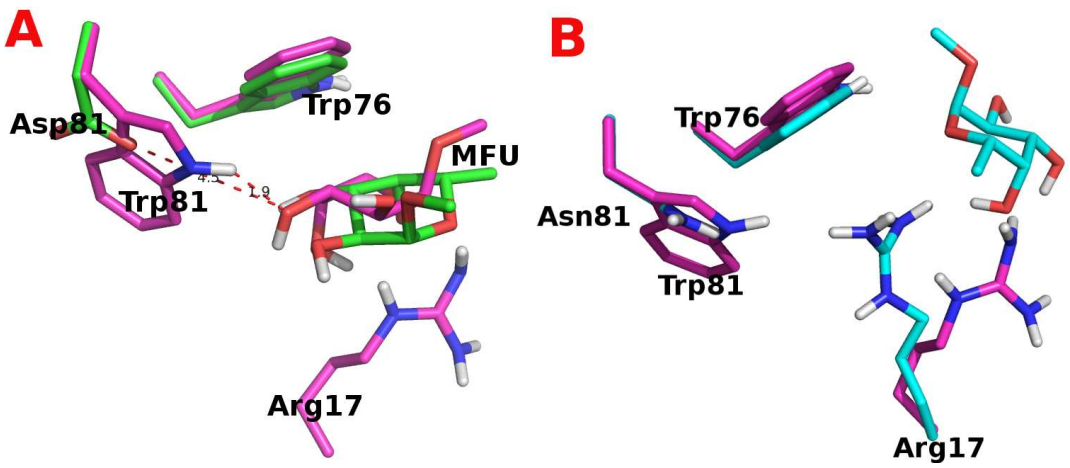

Figure S10: A) Binding mode of Me-α-Lfucoside in W81D mutant compared to wild type (Purple C). B) Binding mode of Me-α-Lfucoside in W81N mutant (Cyan "C") showing a large movement of Arg17 compared to wild type (Purple C) up on W81D mutation.

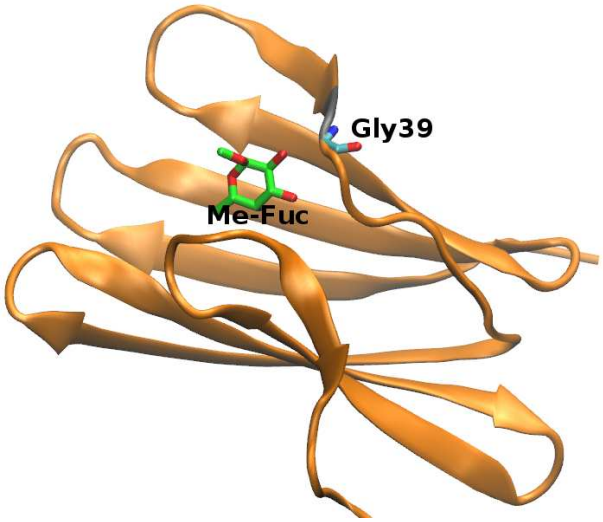

Figure S11: Secondary structure representation of RSL crystal structure[1] drawn by VMD[2]. The Gly39 is seen outside the β-sheet region here.

Table TS2: Normalized Binding Energies of Trisaccharides Calculated by Various Docking Software. Autodock Vina was tested both in the mode of rigid receptor and flexible receptor docking. The value presented here are the normalized binding score of a particular saccharide, which is the binding energy of the saccharide divided by the binding energy of 2-fucosyllactose. All values of binding energy are in kcal/mol.

	Ligand	SPR[10]	AutoDock3 (RESP)	AutoDock3 (GM)	Vina (rigid)	Vina (flexible)	DOCK (Std.)	DOCK (GB/SA)	AutoDock4 (rigid)	AutoDock4 (flex)	ICM Dock
1	**Blood group B trisaccharide** **α -Fuc(1→2)[α-Gal(1→3)]Gal**	1.05	0.87	0.88	0.92	1.03	0.95	1.04	1.17	1.52	0.95
2	**2-Fucosyllactose** **α -Fuc(1→2) β-Gal(1→4)Glc**	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3	**H type II trisaccharide** **α-Fuc(1→2)β-Gal(1→4) GlcNAc**	0.95	0.86	0.94	0.90	1.00	1.02	1.12	1.08	1.12	0.99
4	**Lewis A trisaccharide** **α -Fuc-(1→4)[β-Gal(1→3)]GlcNAc**	0.85	0.67	0.80	0.93	0.95	1.02	0.87	1.17	1.18	0.96
5	**Lewis X trisaccharide** **α -Fuc-(1→3)[β-Gal(1→4)]GlcNAc**	0.77	0.79	1.02	0.92	0.98	1.08	0.91	1.11	1.10	0.97
6	**3-Fucosyllactose** **α -Fuc(1→3)β-Gal(1→4)Glc**	0.75	0.86	0.88	0.89	0.90	1.01	1.19	0.96	1.46	0.99

Table TS3: RMS value of residues which are allowed to be flexible during the docking of trisaccharides. For Glu28 RMSD values (from AutoDock Vina) are high (about 1.28 Å) because of the flipping of the positions of the two charged side chain oxygen. However there is very minor movement seen in the rest of the atoms of Glu28.

		RMSD from AutoDock Vina (Å)			RMSD from AutoDock 4 (Å)		
	Ligand	Arg17	Glu28	Trp81	Arg17	Glu28	Trp81
1	**Blood group B trisaccharide α -Fuc(1→2)[α-Gal(1→3)]Gal**	1.01	1.28	0.114	1.65	0.35	1.38
2	**2-Fucosyllactose α -Fuc(1→2) β-Gal(1→4)Glc**	0.85	1.28	0.026	0.69	0.66	1.40
3	**H type II trisaccharide α-Fuc(1→2)β-Gal(1→4) GlcNAc**	1.00	1.28	0.077	0.69	0.63	0.14
4	**Lewis A trisaccharide α -Fuc-(1→4)[β-Gal(1→3)]GlcNAc**	1.12	0.13	0.075	0.94	0.44	0.12
5	**Lewis X trisaccharide α -Fuc-(1→3)[β-Gal(1→4)]GlcNAc**	0.89	1.28	0.101	1.52	0.48	1.37
6	**3-Fucosyllactose α -Fuc(1→3)β-Gal(1→4)Glc**	0.87	1.28	0.036	1.07	0.50	0.13

Table TS4. Normalized Binding Energy of Monosaccharides Calculated by Various Docking Software. Values shown here are the normalized binding score, which is the binding energy of the saccharide divided by the binding energy of α-L-fucose. The SPR value of the free sugars does not correspond to a particular anomer of that sugar, but rather to the α/β anomers mixture. However, in computational studies the specified anomer was docked to the RSL. All binding energy values are in kcal/mol, and the NB notation stands for non-binders that do not show significant binding during the SPR experiments, e.g. with Kd > 10 mM.

	Ligand	SPR[10]	AutoDock3 RESP	AutoDock3 GM	Vina (rigid)	Vina (flexible)	DOCK (Standard)	DOCK (GB/SA)	AutoDock4 (rigid)	AutoDock4 (flex)	ICM Dock
1	Me-α-L-fucoside	1.07	0.94	0.95	0.98	1.00	1.03	1.09	1.00	1.01	1.01
2	α-L-fucose	1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
3	β-L-fucose		0.97	0.99	0.98	1.03	0.95	0.94	0.98	0.98	1.04
4	α-L-galactose	0.89	1.00	1.01	0.97	0.98	1.07	1.06	1.10	0.99	1.01
5	β-L-galactose		0.99	1.02	0.97	0.90	1.04	1.06	1.07	0.96	1.04
6	Me-β-D-arabinoside	0.85	0.95	0.97	0.83	0.85	0.90	0.87	0.99	0.92	0.87
7	α-D-arabinose	0.75	0.90	0.92	0.73	0.78	0.74	0.69	0.96	1.09	0.62
8	β-D-arabinose		0.94	0.93	0.78	0.75	0.92	0.89	0.99	0.90	0.84
9	α-D-mannose	0.69	0.91	0.94	0.80	0.88	0.88	0.66	1.18	0.98	0.84
10	β-D-mannose		0.92	0.96	0.88	0.85	0.96	0.91	1.15	1.14	0.85
11	α-L-rhamnose	0.57	0.90	0.91	0.88	1.03	0.71	0.59	1.14	0.96	0.92
12	β-L-rhamnose		0.89	0.94	0.90	0.90	0.89	0.94	1.06	0.98	0.95
13	Me-α-D-mannoside	0.46	0.89	0.93	0.77	0.93	0.88	0.75	1.16	1.01	0.87
14	α-D-galactose	NB	0.85	0.93	0.85	1.03	0.95	0.89	1.00	0.98	0.92
15	β-D-galactose	NB	0.90	0.96	0.90	0.93	1.03	0.99	0.97	0.99	0.89

Table TS5: RMS value of residues which are allowed to be flexible during the docking of monosaccharides. For Glu28 RMSD values (from AutoDock Vina) are high (about 1.28 Å) because of the flipping of the positions of the two charged side chain oxygens. Movement of the rest atoms of the Glu28 atom's is almost negligible.

		RMSD from AutoDock Vina4 (Å)			RMSD from AutoDock4 (Å)		
	Ligand	Arg17	Glu28	Trp81	Arg17	Glu28	Trp81
1	Me-α-L-fucoside	1.924	1.286	0.027	0.77	0.17	1.40
2	α-L-fucose	1.934	1.287	0.027	1.09	0.34	1.39
	β-L- fucose	2.095	1.287	0.076	0.85	0.35	0.13
3	α-L-galactose	2.302	1.390	0.045	1.04	0.36	0.11
4	β-L-galactose	1.806	1.286	0.033	1.00	0.64	0.11
5	Me-β-D-arabinoside	1.84	0.11	0.140	0.85	0.52	0.19
7	α-D-arabinose	1.34	0.19	0.040	1.27	0.50	0.03
8	β-D-arabinose	2.11	0.15	0.050	1.00	0.45	0.12
9	α-D-mannose	1.56	0.11	0.026	0.83	0.52	0.12
10	β-D-mannose	1.33	1.29	0.05	0.89	0.60	1.40
11	Me-α-D-mannoside	1.65	0.25	0.02	1.11	0.53	0.12
12	α-L-rhamnose	1.77	1.28	0.11	1.01	0.20	0.21
13	β-L-rhamnose	2.31	1.28	0.11	0.87	0.42	0.14
14	α-D-galactose	1.941	1.28	0.027	1.03	0.62	0.12
15	β-D-galactose	1.947	1.27	0.076	0.98	0.67	0.13

References

[1] Kostlanova, N.; Mitchell, E.; Lortat-Jacob, H.; Oscarson, S.; Lahmann, M.; Gilboa-Garber, N.; Chambat, G.; Wimmerova, M.; Imberty, A. The fucose-binding lectin from Ralstonia solanacearum - A new type of beta-propeller architecture formed by oligomerization and interacting with fucoside, fucosyllactose, and plant xyloglucan. *J. Biol. Chem.* **2005**, *280*, 27839-27849.

[2] Humphrey, W.; Dalke, A.; Schulten, K. VMD: visual molecular dynamics. *J. Mol. Graph.* **1996**, *14*, 33-38.