Supporting Information

Structural Insight into Multivalent Galactoside Binding to *Pseudomonas aeruginosa* lectin LecA

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P. aeruginosa lectin LecA expression and purification

LecA was expressed and purified by affinity chromatography along an optimized protocol and in accordance to a previous report.[1] Briefly, the plasmid pET25paIL was transformed into *Escherichia coli* BL21 (DE3) cells. *E.coli* cells were grown in 10 L of LB medium (10 g tryptone; 5 g yeast extract and 5 g NaCl in 1 L of deionized water) at 30 °C. When the culture had reached an optical density of 0.5–0.6 at 600 nm, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM. Cells were harvested after overnight shaking at 220 rpm at 20 °C, washed, and resuspended in 100 ml of loading buffer (20 mM Tris–HCl and 100 μM $CaCl_2$, pH 7.5). The cells were broken by sonication. After centrifugation at 5000 rpm for 45 min the supernatant was loaded to an affinity chromatography column containing 500 ml of Sepharose 4B. LecA was eluted with 0.2 M D-galactose in buffer (20 mM Tris–HCl and 100 μM $CaCl_2$, pH 7.5). The purified protein was extensively dialyzed against distilled water containing 2 μM $CaCl_2$ for 7 days. Purified fractions of protein were lyophilized, and kept at −20 °C.

Analytical Ultracentrifugation

Mixtures of LecA with various equivalents of inhibitors were prepared within 1 h before the analysis was carried out. Samples (0.4 mL) were centrifuged in 11 mm path length 2-sector aluminum centerpiece cells with sapphire windows in a An60Ti analytical rotor running in an Optima XL-I or Optima XL-A analytical ultracentrifuge (Beckman Instruments, Inc., Palo Alto, California 94304) at 35 k rpm and at a temperature of 20 °C. Changes in solute concentration were detected by 600 absorbance scans measured at 280 nm over a period of 9-10 hours.

Analysis and fitting of the data was performed using the software SedFit.[2] A continuous c (s) distribution model was fitted to the data, taking every 3rd scan. The resolution was set at 200 over a sedimentation coefficient range of 0.0-20.0 S. Parameters were set for the partial specific volume as 0.72912, the buffer density of 1.00167 and the buffer viscosity at 0.01034, as calculated using SEDNTERP for Tris buffer 0.1 M, $CaCl_2$ 6 mM, pH 7.2. The frictional coefficient, the baseline and

the raw data noise were floated in the fitting. The meniscus and bottom of the cell path were also floated after initial estimations from the raw data. Control experiments with the inhibitors in the absence of protein failed to give an adequate absorbance at 280 nm to allow accurate data collection.

Crystallization (a)

Co-crystallization of **5u3** with LecA lectin was carried out by the sitting drop method. In brief, lyophilized protein was dissolved in water (10 $mg.ml^{-1}$) in the presence of salts (1 mM $CaCl_2$ and $MgCl_2$). The compound **5u3** was added to the protein at a 2:1 molar excess, taking in to account, that the biological unit of LecA is a homo-tetramer. Crystals were obtained within three days after mixing 2 µL of LecA ligand-complex with 2 µL of reservoir solution at 18 °C. Primary crystallization conditions were found in Crystal screens I/II, Index I/II and SaltRx I/II, respectively (Hampton Research, Laguna Niguel, CA, USA). Crystals of highest diffraction were obtained from condition A7, Index I (0.1 M Citric acid, 3.0 M sodium chloride.)

Crystallization (b)

Crystals of **3u3**.LecA and **GalAG1**.LecA were obtained by soaking. For this, LecA crystals were grown in a condition containing 1.5 M Ammonium sulfate at a pH of 4.6 which is the SaltRx II-13 condition. The crystals grew within 3-4 days. Drops of 4 µL containing the crystals were supplemented with **3u3** and with **GalAG1** at 15 binding equivalents of compound, respectively. The soaked crystals were incubated at 18°C for 3-4 days, transferred into a solution of 1.5 M Ammonium sulfate supplemented with 30 % v/v glycerol at pH 4.8 and immediately flash frozen in liquid nitrogen for storage.

LecA-galactosides crystals belong to space group P1 with the corresponding asymmetric units containing four, sixteen and four monomers for **3u3**, **5u3**, **GalAG1** respectively. Further details on data collection statistics are given in (Table S1). Crystals were cryo-cooled at 100 K after soaking

them for as short a time as possible in glycerol 30% v/v in precipitant solution. All data were collected at the SLS synchrotron (Villigen, Switzerland) at beam line PX-II/III.

Table S1. Data collection and refinement statistics.

Structural data	**3u3.LecA**	**5u3.LecA**	**GalAG1.LecA**
Beam line	PX-III	PX-III	PX-III
Wavelength(Å)	1.000030	0.91956	1.000030
Resolution(Å)	17.97 - 1.19	16.05 - 1.82	18.02 - 1.40
Cell dimension			
Space group	P1	P1	P1
Unit cell(Å)	40.77 72.32 78.71 117.23 105.00 89.94	49.57 89.31 100.01 90.04 89.83 79.61	40.87 72.98 79.02 117.52 104.99 90.00
Measured reflection/unique	724110/229498	322703/134652	380562/129508
Average multiplicity	3.2	2.4	2.9
Completeness (%)	94.0	89.0	84.9
Average I/σ(I)	6.8	6.0	7.7
R_{merge} (%)	7.7	8.9	5.8
Correlation CC (1/2) (%)	99.8	99.4	99.6
Wilson B-factor (Å^2)	9.9	10.8	11.0
Refinement			
Resolution range (Å)	17.97 - 1.19	16.05 - 1.82	18.019 - 1.40
R_{work} (%)	0.1858	0.1789	0.1861
R_{free} (%)	0.2035	0.2206	0.2058
Average Biso (Å^2)	20.3	18.7	18.1
All atoms	4620	16661	4788
Solvent atoms	744	1873	898
RMSD from ideality angles (°)	1.389	0.842	1.379
Bonds (Å)	0.011	0.006	0.009
Number of galactose	4	16	4
Calcium atoms	4	16	4
Protein Data Bank deposition code	4YWA	4YW7	4YW6

The structures were solved using the mosflm,[3] CCP4,[4] the phenix[5] program suite and the coot[6] graphical program. Pictures were done with the help of pymol[7] and PDB2PQR.[8]

Isothermal Titration Calorimetry (ITC)

Lyophilized LecA was dissolved in buffer (Tris 20 mM, NaCl 100 mM, $CaCl_2$ 100 µM, pH=7.5). Protein concentration was checked by measurement of absorbance at 280 nm using a theoretical molarity extinction coefficient of 27,600 $M^{-1}cm^{-1}$. Ligands were dissolved directly into the same buffer. ITC was performed with iTC_{200} calorimeter (MicroCal Inc.). Titration was performed on 14-91 µM LecA in the 200 µl sample cell using 2 µL injections of 10-1000 µM ligand every 150 s at 25 °C. The data were fitted with MicroCal Origin 8 software, according to standard procedures using a single-site model. Change in free energy Δ*G* was calculated from the equation: $\Delta G = \Delta H - T\Delta S$ where T is the absolute temperature, ΔH and ΔS are the change in enthalpy and entropy respectively. Two independent titrations were performed for each ligand tested.

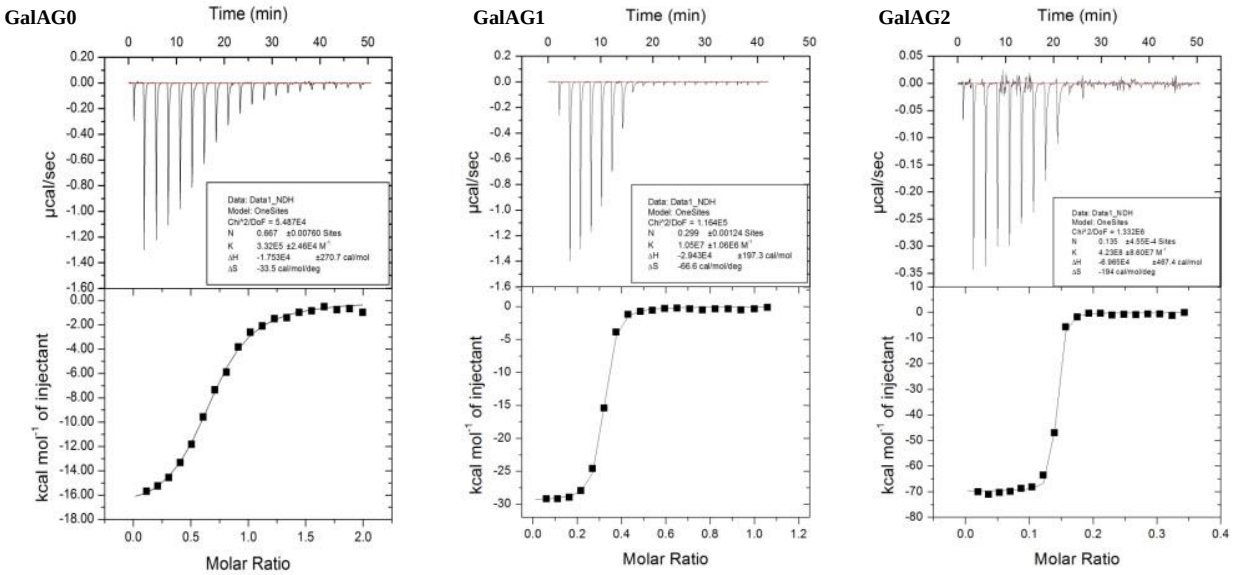

Figure S1. Isothermal Titration Calorimetric (ITC) measurements representing the raw ITC data (above) and integrated titration curves (below) for the binding of glycopeptide dendrimer ligands to LecA. Titration type and corresponding concentrations for Ligand/LecA: **GalAG0** (0.5 mM/0.0516 mM), **GalAG1** (0.25 mM/0.0486 mM), **GalAG2** (0.03 mM/0.018 mM).

Amber Modeling

Molecular Dynamics (MD) simulations with AMBER 12 package[9] were carried out for **3u3**, **5u3**, **GalAG1**, **GalAG2** and four complexes, (i) LecA in complex with **3u3**, (ii) LecA in complex with **5u3**, (iii) LecA in complex with **GalAG1** and (iv) LecA in complex with **GalAG2**. The initial structures used for modeling were taken as follows: free **3u3** and **3u3**.LecA: coordinates taken from the **3u3**.LecA crystal coordinates; free **5u3** and **5u3**.LecA: the model was constructed from the **3u3**.LecA model by Pertici *et al*.[10] by extending the CH_2 groups with two CH_2 units in both linkers using the modelling suite of Yasara, followed by energy minimization and 10 ps of molecular dynamics in which the two bound galactose units and the protein part were kept in fixed position. **GalAG1**, **GalAG2** and LecA complexes: The initial structures of dendrimers (**GalAG1** and **GalAG2**) were built using CORINA.[11] The complexes of LecA and **GalAG1**/**GalAG2** were obtained by imposing two binding units of **GalAG1**/**GalAG2** in the binding pocket of the crystal structure of LecA using protein coordinates from the **GalAG1.**LecA complex structure shown in this paper.

Electrostatic point-charges for the phenyl galactosides, triazoles, galactoses and for the branching lysines in the dendrimer structures were obtained using the standard RESP procedure.[12] The dendrimers or protein complexes were placed in a truncated octahedral water box using the TIP3P solvent model.[13] The final topologies, coordinates and parameters for refinement were then built using the Xleap module from AMBER 12 package.[9] Langevin dynamics was carried out for temperature regulation.[14] Hydrogen bonds were constrained with the SHAKE constraint algorithm,[15] and the long-range electrostatic interactions were handled by the Particle-Mesh Ewald (PME) procedure.[13] In addition, the non-bonded interactions used a cutoff of 11 Å. The trajectories from MD were analyzed using VMD.[16]

The simulation started from energy minimization on the entire system, which used the steepest descent algorithm for the first 1000 steps followed by 2500 steps of conjugate gradient

algorithm. Next approach was 200 ps MD with weak restraints on the ligands or protein complexes. During this approach, the system was heated from 0 K to 300 K at constant volume. After this procedure the whole system was energy minimized. Finally, 10 ns (ligands) or 15 ns (complexes of LecA and ligand) MD on the entire solute and solvent was performed using constant pressure periodic boundary and 300 K constant temperature.

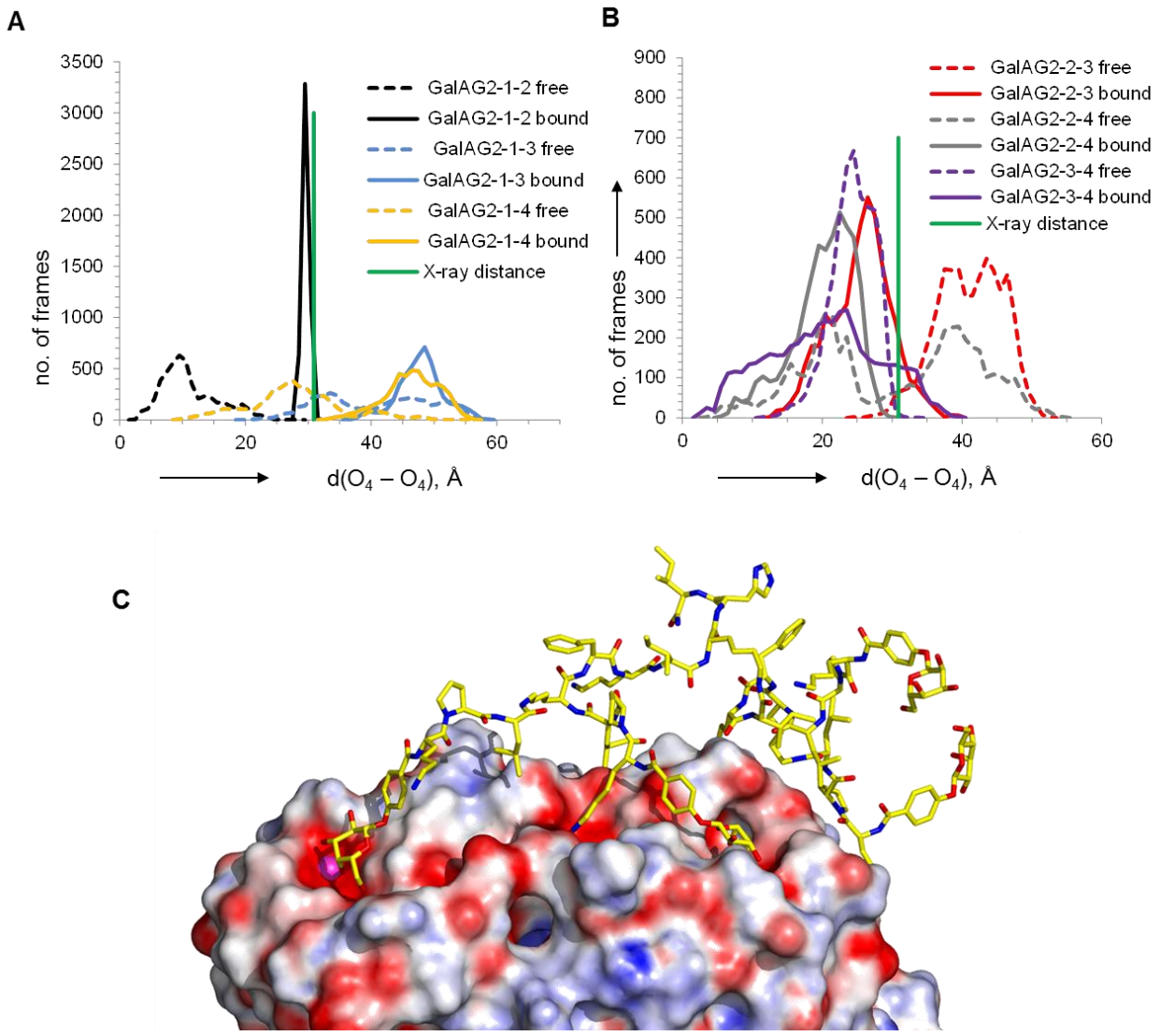

Figure S2. MD simulation of a 1:1 complex **GalAG2**.LecA complex with **GalAG2** bound via sugars 1 and 2. A-B. Frequency histograms for the distance separating the galactose C(4)-OH oxygen atoms in pairs of galactosyl groups. Numbering of galactosyl groups in **GalAG2** according to Figure 1. C. Image of the last MD frame showing the ligands in yellow stick models and the electrostatic potential map of the LecA surface (+4.8 kcal = blue, -4.8 kcal = red). **GalAG2** was simulated for 10 ns in the free state and 15 ns in the bound state using Amber12 (see methods for MD details).

Biofilm Assay on Polystyrene Microtitre Plates

A modified version of the method described by Diggle et al. was employed.[17] 96well sterile, U-bottomed polystyrene microtitre plates (TPP Switzerland) were prepared by adding 200 µl of sterile deionized water to the peripheral wells to decrease evaporation from test wells. Aliquots of 180 µl of culture medium (10% (w/v) nutrient broth No. 2, Oxoid) containing appropriate concentration of the test compound were added to the internal wells. Inoculum of *P. aeruginosa* strain PAO1 was prepared from 5 ml overnight culture grown in LB broth. Aliquots of 20 µl of overnight cultures, pre-washed in 10% (w/v) nutrient broth and normalized to an OD_{600} of 1, were inoculated into the test wells. Plates were incubated in a humid environment for 25 hours at 37°C. Wells were washed with 200 µl sterile deionized water before staining with 200 µl 10% (w/v) nutrient broth containing 0.5 mM WST-8 and 20 µM phenazine ethosulfate for 3 hours at 37°C. Afterwards, the well supernatants were transferred to a polystyrene flat bottomed 96well plate (TPP Switzerland) and the absorbance was measured at 450 nm with a plate reader (SpectraMax250 from Molecular Devices).

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