

SUPPLEMENTARY MATERIALS

NMR EXPERIMENTS

Saturation Transfer Difference (STD) NMR

All the STD experiments were acquired using AVANCE 2 600 MHz spectrometer equipped with a 5 mm QCI cryo-probe (Bruker Inc.; Billerica, MA, USA). The samples (500 μ L total in 5mm standard NMR tubes) were prepared in deuterated phosphate saline buffer (50 mM sodium phosphate, 150 mM NaCl, pH 7.4 – 2 mM of dithiothreitol-d10 (DTT-d10) was added to the samples containing Gal-1). The STD spectra acquired with different irradiations and 2 s of saturation time for ligands **2** and **3** and Gal-1 and Gal-3-CRD are reported here, together with the relative STD Amplification Factor (STD-AF) intensities and STD percentage (STD%) calculated. The STD% values obtained from the spectra with the irradiation at δ -0.5 ppm were used for the representation of the epitope mapping (Figure S1 and main test).

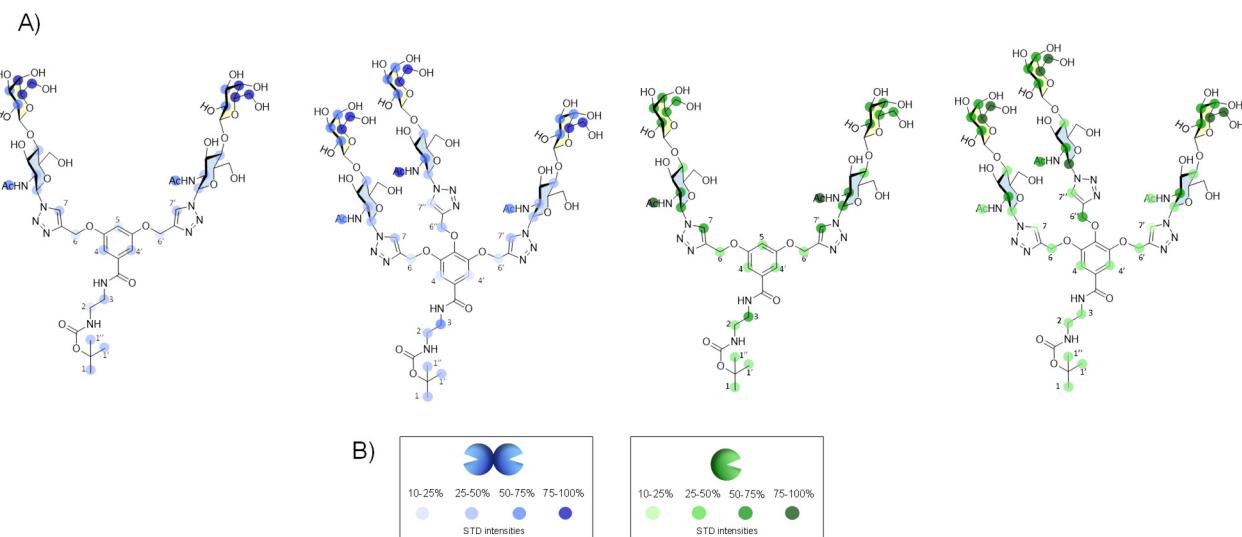
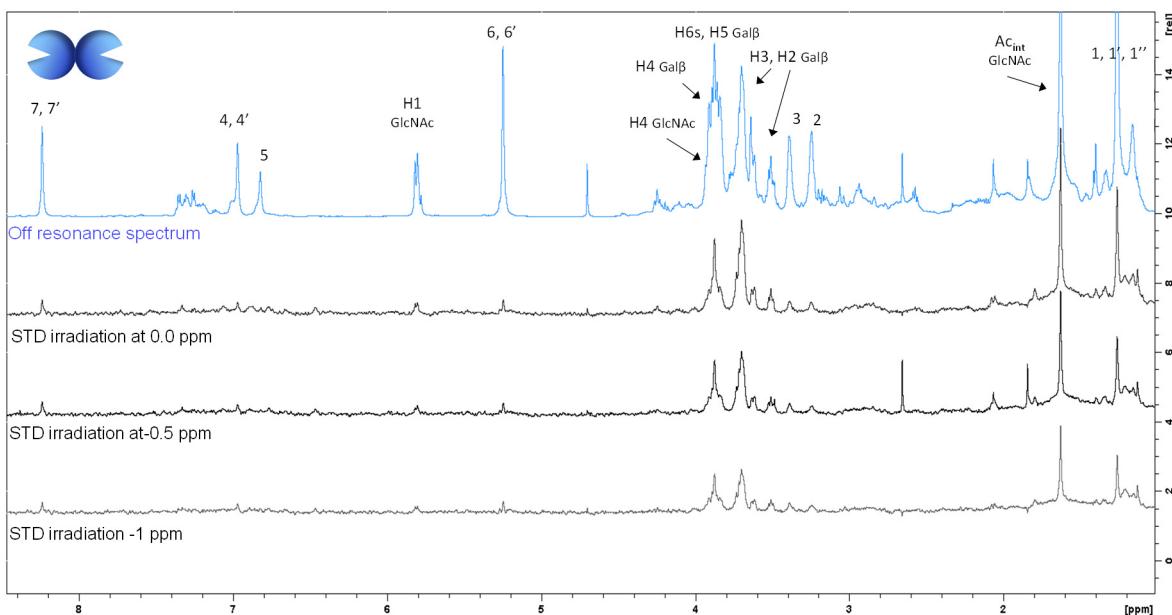


Figure S1. Binding epitopes of ligand **2** and **3** for Gal-1 and Gal-3 CRD. (A) STD binding epitopes for ligands **2** and **3** and Gal-1 (blue) and Gal-3 CRD (green) obtained from spectra acquired with irradiation at δ -0.5 ppm. (B) Color legend of the percentage of STD.

A)

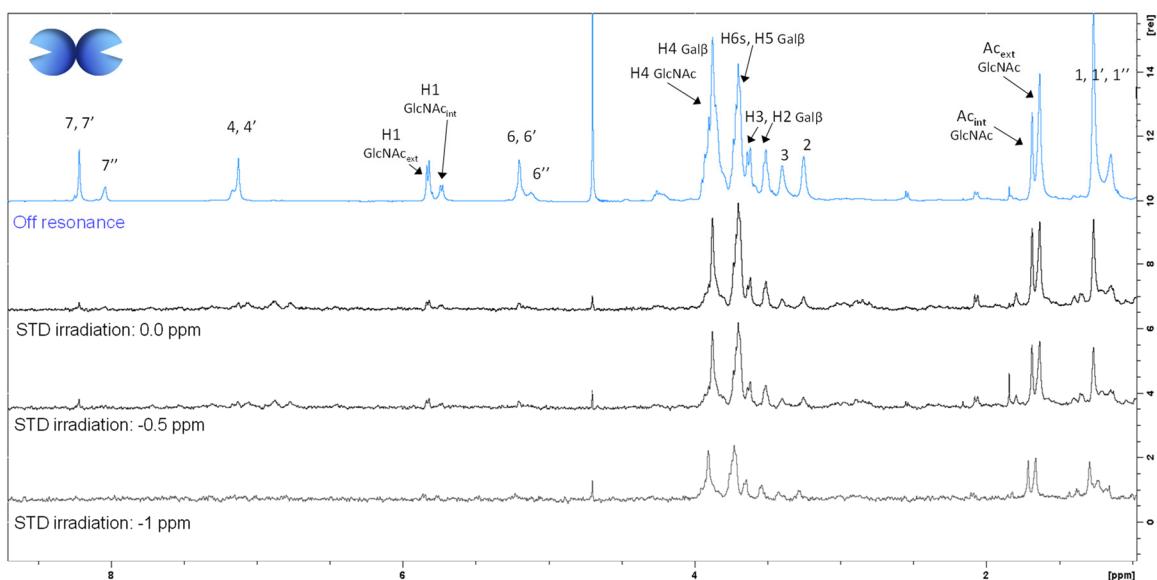


B)

0.0 ppm				-0.5 ppm				-1.0 ppm			
Residue	Atom	STD-AF	STD %	Residue	Atom	STD-AF	STD %	Residue	Atom	STD-AF	STD %
GlcNAc	H1	0.014	30%	GlcNAc	H1	0.0108	35%	GlcNAc	H1	0.0078	35%
	H2	0.0232	49%		H2	0.0156	50%		H2	0.011	50%
	H3	-	-		H3	-	-		H3	-	-
	H4	0.016	34%		H4	0.0119	38%		H4	0.009	45%
	H5	-	-		H5	-	-		H5	-	-
	H6, H6'	-	-		H6, H6'	-	-		H6, H6'	-	-
	Ac	0.034	78%		Ac	0.023	74%		Ac	0.016	72%
Galβ	H1	-	-	Galβ	H1	-	-	Galβ	H1	-	-
	H2	0.03	63%		H2	0.022	71%		H2	0.016	72%
	H3	0.03	63%		H3	0.022	71%		H3	0.016	72%
	H4	0.038	80%		H4	0.024	77%		H4	0.017	77%
	H5	0.047	100%		H5	0.031	100%		H5	0.022	100%
	H6s	0.047	100%		H6s	0.031	100%		H6s	0.022	100%
scaffold	1, 1', 1''	0.02	42%	scaffold	1, 1', 1''	0.012	38%	scaffold	1, 1', 1''	0.008	36%
	2	0.01	21%		2	0.008	25%		2	0.007	31%
	3	0.012	25%		3	0.01	32%		3	0.009	40%
	4, 4'	0.012	25%		4, 4'	0.01	32%		4, 4'	0.009	45%
	5	0.011	23%		5	0.008	25%		5	0.007	31%
	6, 6'	0.006	12%		6, 6'	0.005	16%		6, 6'	0.005	22%
	7, 7'	0.012	25%		7, 7'	0.01	32%		7, 7'	0.009	45%

Figure S2. STD Gal-1 and ligand 2. (A) Sample: Gal-1 100 μ M, 2 3 mM (ratio lectin:ligand = 1:30). Off-resonance (irradiation at 100 ppm) and STD spectra (irradiation at δ 0.0, δ -0.5 and δ -1 ppm, 50 \times). (B) Relative STD-AF intensities and STD percentage for each proton signal calculated on the basis of the relative experiment.

A)

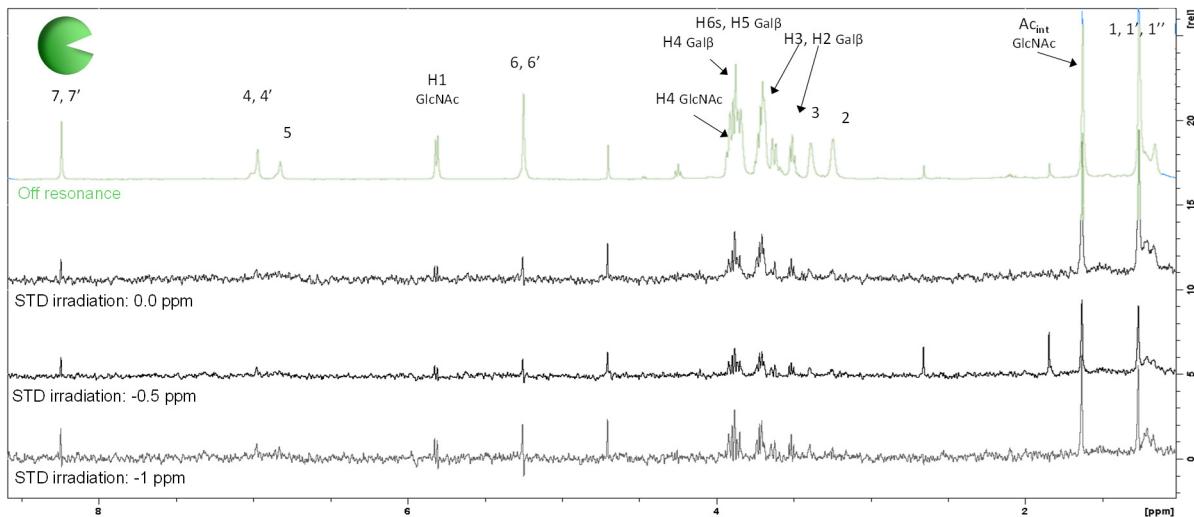


B)

0.0 ppm				-0.5 ppm				-1 ppm			
Residue	Atom	STD-AF	STD %	Residue	Atom	STD-AF	STD %	Residue	Atom	STD-AF	STD %
GlcNAc	H1 _{ext}	0.012	25%	GlcNAc	H1 _{ext}	0.01	31%	GlcNAc	H1 _{ext}	0.005	31%
	H1 _{int}	0.017	35%		H1 _{int}	0.014	45%		H1 _{int}	0.008	50%
	H2 _{ext}	0.02	42%		H2 _{ext}	0.015	48%		H2 _{ext}	0.008	50%
	H2 _{int}	0.025	52%		H2 _{int}	0.016	52%		H2 _{int}	0.009	56%
	H3	-	-		H3	-	-		H3	-	-
	H4	0.017	35%		H4	0.012	38%		H4	0.008	50%
	H5	-	-		H5	-	-		H5	-	-
	H6, H6'	-	-		H6, H6'	-	-		H6, H6'	-	-
	Ac _{ext}	0.036	75%		Ac _{ext}	0.023	74%		Ac _{ext}	0.011	68%
	Ac _{int}	0.048	100%		Ac _{int}	0.031	100%		Ac _{int}	0.016	100%
Galβ	H1	-	-	Galβ	H1	-	-	Galβ	H1	-	-
	H2	0.03	62%		H2	0.019	61%		H2	0.01	62%
	H3	0.03	62%		H3	0.019	61%		H3	0.01	62%
	H4	0.034	70%		H4	0.02	64%		H4	0.011	68%
	H5	0.042	87%		H5	0.027	87%		H5	0.015	93%
	H6s	0.042	87%		H6s	0.027	87%		H6s	0.015	93%
scaffold	1, 1', 1''	0.023	47%	scaffold	1, 1', 1''	0.013	42%	scaffold	1, 1', 1''	0.0066	41%
	2	0.015	31%		2	0.012	38%		2	0.007	43%
	3	0.016	33%		3	0.019	61%		3	0.008	50%
	4, 4'	0.0085	17%		4, 4'	0.007	22%		4, 4'	0.0045	28%
	6, 6'	0.009	18%		6, 6'	0.0075	24%		6, 6'	0.0045	28%
	6''	0.01	20%		6''	0.009	29%		6''	0.006	37%
	7, 7'	0.0073	15%		7, 7'	0.007	22%		7, 7'	0.0025	15%
	7''	0.012	25%		7''	0.009	29%		7''	0.004	25%

Figure S3. STD Gal-1 and ligand 3. (A) Sample: Gal-1 100 μ M, 3 3 mM (ratio lectin:ligand = 1:30). Off-resonance (irradiation at 100 ppm) and STD spectra (irradiation at δ 0.0, δ -0.5 and δ -1 ppm, 50 \times). (B) Relative STD-AF intensities and STD percentage for each proton signal calculated on the basis of the relative experiment.

A)



B)

0.0 ppm				-0.5 ppm				-1.0 ppm			
Residue	Atom	STD-AF	STD %	Residue	Atom	STD-AF	STD %	Residue	Atom	STD-AF	STD %
GlcNAc	H1	0.008	42%	GlcNAc	H1	0.0087	60%	GlcNAc	H1	0.009	75%
	H2	0.009	47%		H2	0.009	62%		H2	0.01	83%
	H3	-	-		H3	-	-		H3	-	-
	H4	0.007	36%		H4	0.0069	48%		H4	0.0075	62%
	H5	-	-		H5	-	-		H5	-	-
	H6, H6'	-	-		H6, H6'	-	-		H6, H6'	-	-
	Ac	0.019	100%		Ac	0.0143	100%		Ac	0.012	100%
Galβ	H1	-	-	Galβ	H1	-	-	Galβ	H1	-	-
	H2	0.01	52%		H2	0.0098	68%		H2	0.01	83%
	H3	0.009	47%		H3	0.0082	57%		H3	0.008	66%
	H4	0.0095	50%		H4	0.0077	53%		H4	0.008	66%
	H5	0.011	57%		H5	0.0085	59%		H5	0.008	66%
	H6s	0.011	57%		H6s	0.0085	59%		H6s	0.008	66%
scaffold	1, 1', 1''	0.015	78%	scaffold	1, 1', 1''	0.0105	73%	scaffold	1, 1', 1''	0.0085	70%
	2	0.006	31%		2	0.0055	38%		2	0.0050	41%
	3	0.007	36%		3	0.0078	54%		3	0.0075	62%
	4, 4'	0.008	42%		4, 4'	0.01	69%		4, 4'	0.01	83%
	5	0.01	52%		5	0.011	76%		5	0.012	100%
	6, 6'	0.006	31%		6, 6'	0.0066	46%		6, 6'	0.0077	64%
	7, 7'	0.008	42%		7, 7'	0.01	76%		7, 7'	0.01	83%

Figure S4. STD Gal-3-CRD and ligand 2. (A) Sample: Gal-3-CRD 50 μ M, 2 1.5 mM (ratio lectin:ligand = 1:30). Off-resonance (irradiation at 100 ppm) and STD spectra (irradiation at δ 0.0, δ -0.5 and δ -1 ppm, 50x). (B) Relative STD-AF intensities and STD percentage for each proton signal calculated on the basis of the relative experiment.

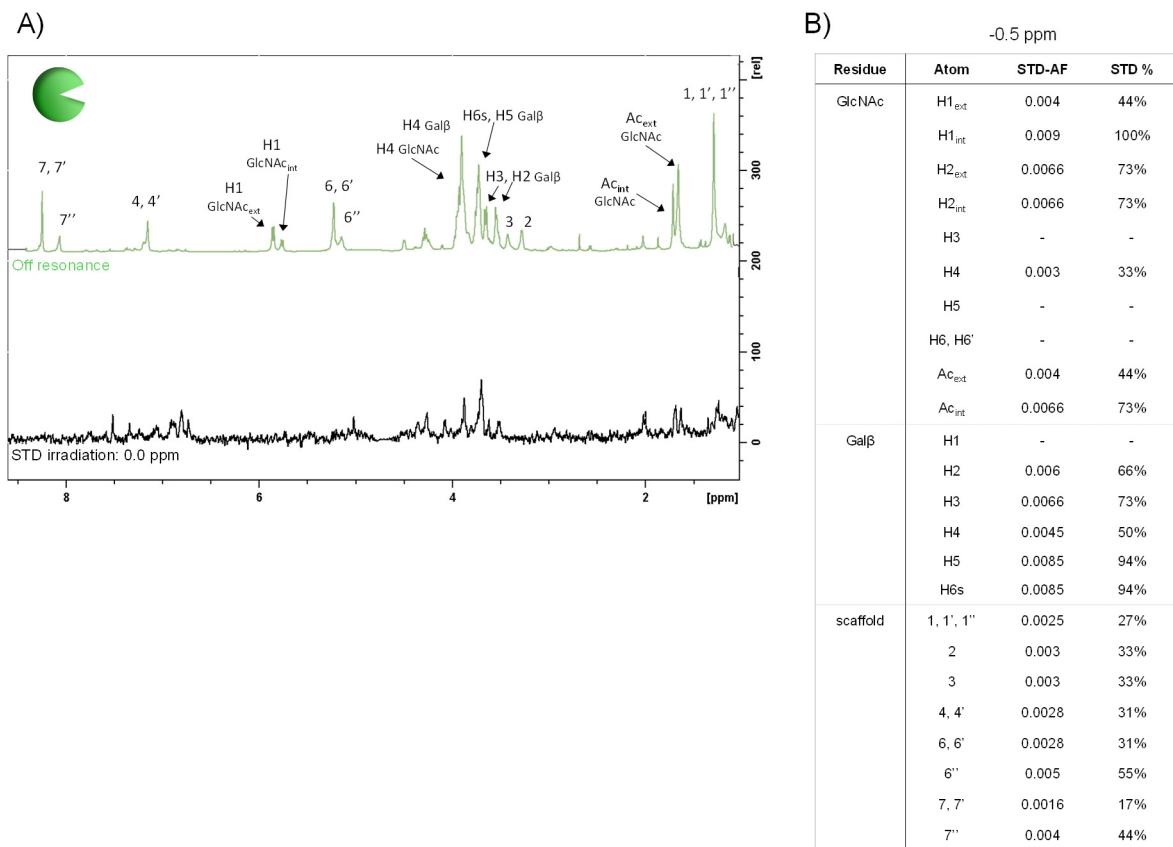


Figure S5. STD Gal-3-CRD and ligand 3. (A) Sample: Gal-3-CRD 50 μ M, 3 1.5 mM (ratio lectin:ligand = 1:30). Off-resonance (irradiation at 100 ppm) and STD spectrum (irradiation at δ -0.5, 50 \times). (B) Relative STD-AF intensities and STD percentage for each proton signal calculated on the basis of the experiment.

DYNAMIC LIGHT SCATTERING MEASUREMENTS

Gal-1	0.5 mg/ml	4.54 nm \pm 0.6
Gal-3	0.5 mg/ml	1.56 nm \pm 0.19
Ligand 4	1.5 mg/ml	7.47 nm \pm 1.8

Gal-1	0.5 mg/ml + Ligand 4	1.5 mg/mL 875.0 nm \pm 128.1
Gal-3	0.5 mg/ml + Ligand 4	1.5 mg/mL 8.43 nm \pm 1.23

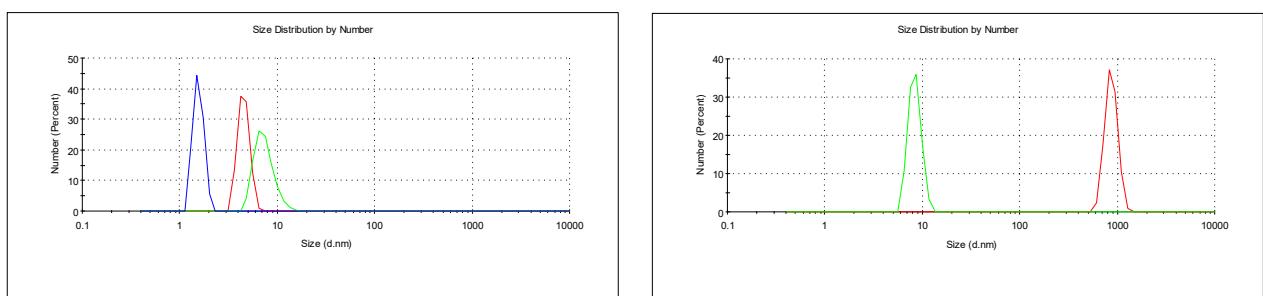


Figure S6. Hydrodynamic radius distribution by number measurements by dynamic light scattering. To the left, hydrodynamic radius distribution graphs for proteins Gal-1 and Gal-3-CRD at 0.5 mg/ml and ligand 4 at 1.5 mg/ml, indicating homogeneity by number distribution; peaks from multiple measurements indicate highly pure and homogenous preparations. To the right, hydrodynamic radius distribution graphs for ligand 4 complexed with Gal-3-CRD and Gal-1. Homogenous peaks are observed at different sizes, around 9 nm for ligand 4–Gal-3 CRD complex and around 900 nm for ligand 4 – Gal-1 CRD complex.