

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-*d*10 (DTT-*d*10) 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).

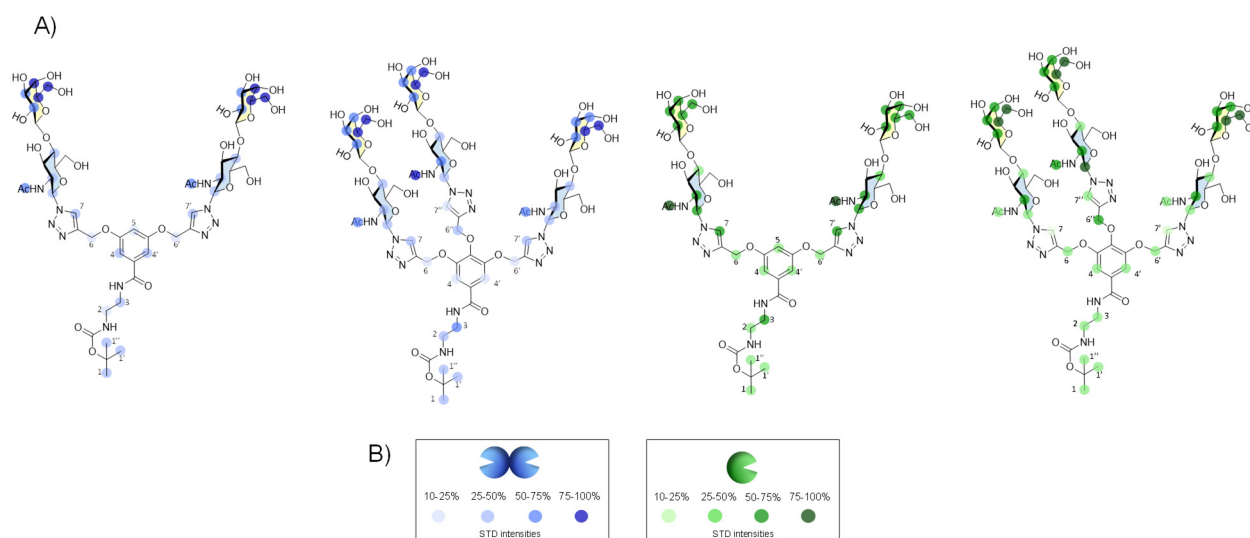
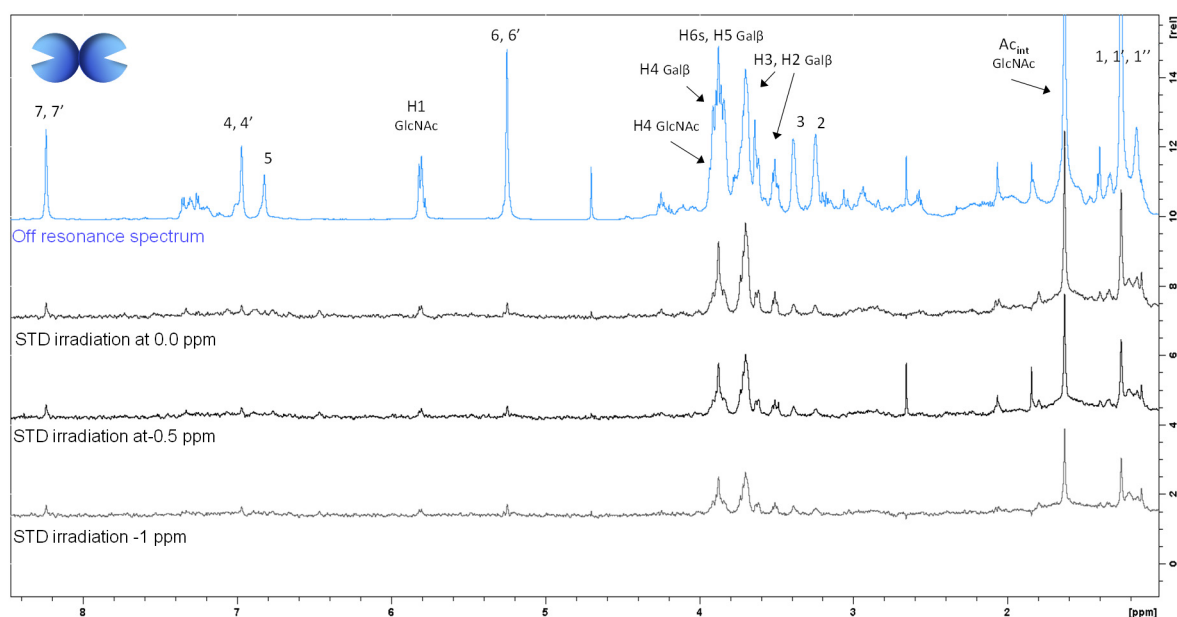


Figure S1. Binding epitopes of ligand **2** and **3** for Gal-1 and Gal-3 CRD. (A) STD binding epitopes for ligands **2** and **3** and Gal-1 (blue) and Gal-3 CRD (green) obtained from spectra acquired with irradiation at δ -0.5 ppm. (B) Color legend of the percentage of STD.

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.

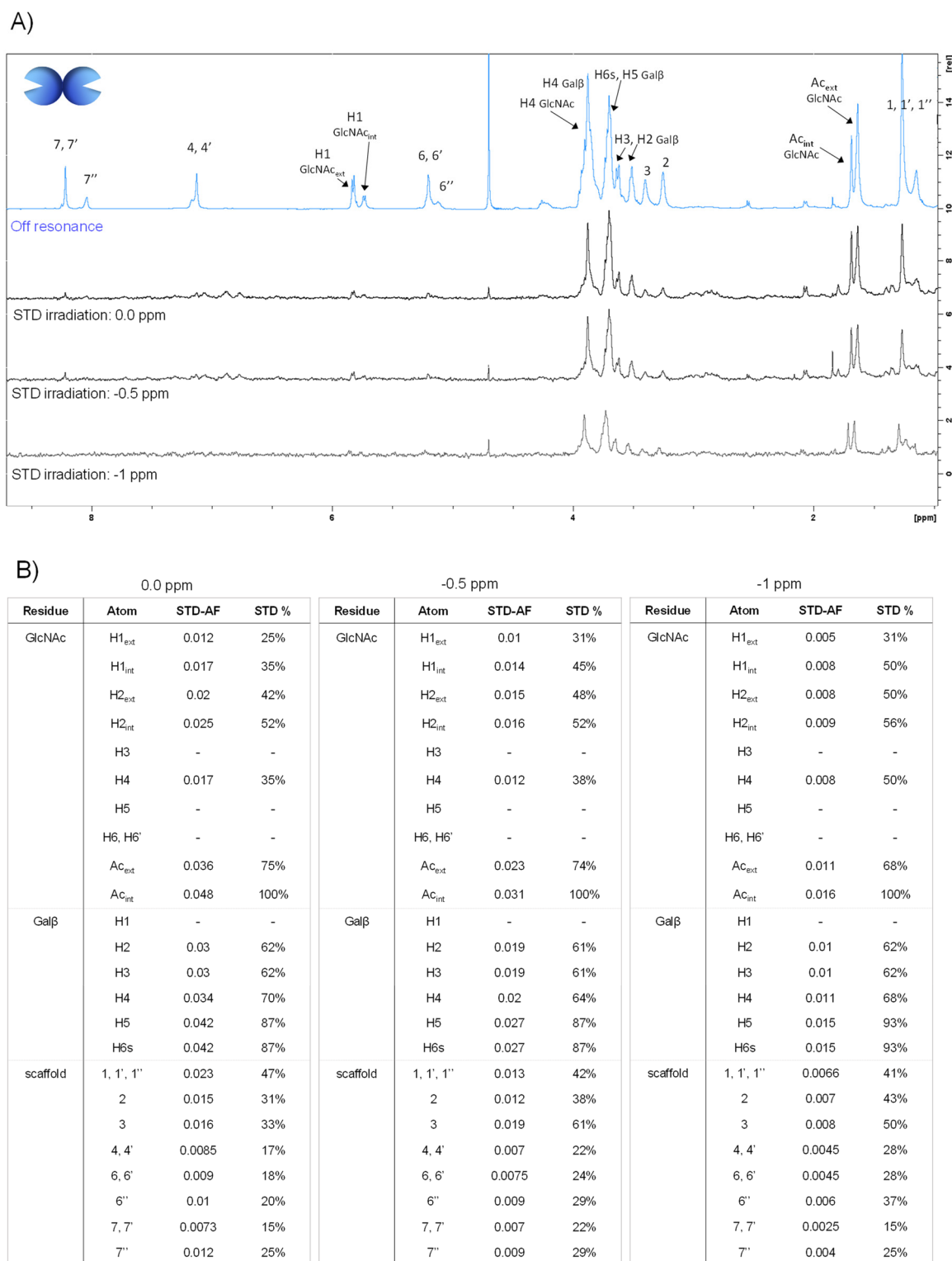
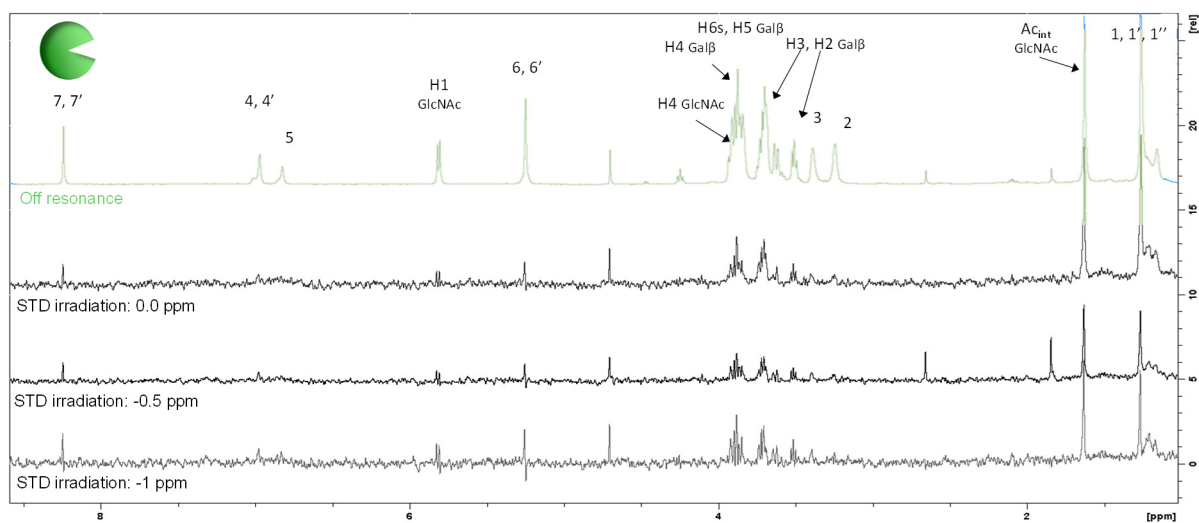


Figure S3. STD Gal-1 and ligand 3. **(A)** Sample: Gal-1 100 μ M, 3 3 mM (ratio lectin:ligand = 1:30). Off-resonance (irradiation at 100 ppm) and STD spectra (irradiation at δ 0.0, δ -0.5 and δ -1 ppm, 50 \times). **(B)** Relative STD-AF intensities and STD percentage for each proton signal calculated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

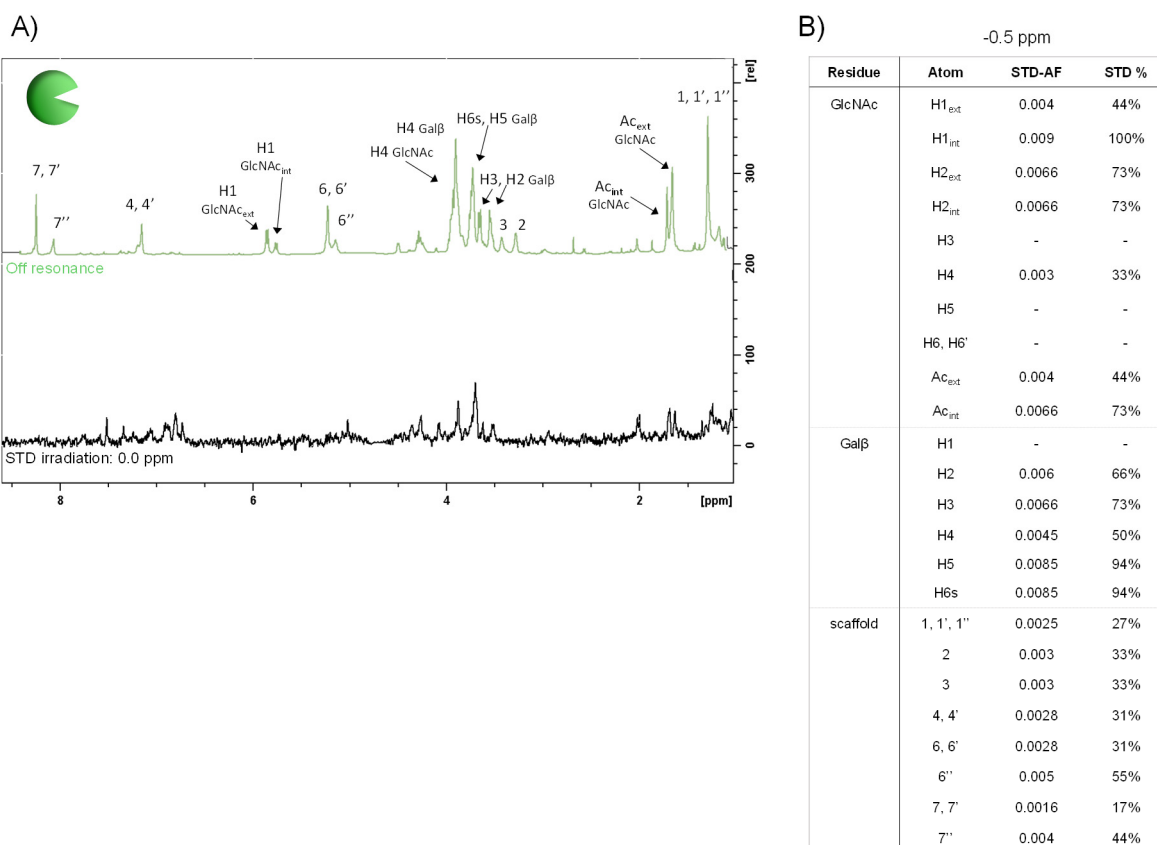


Figure S5. STD Gal-3-CRD and ligand 3. (A) Sample: Gal-3-CRD 50 μ M, 3 1.5 mM (ratio lectin:ligand = 1:30). Off-resonance (irradiation at 100 ppm) and STD spectrum (irradiation at δ -0.5, 50 \times). (B) Relative STD-AF intensities and STD percentage for each proton signal calculated on the basis of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

DYNAMIC LIGHT SCATTERING MEASUREMENTS

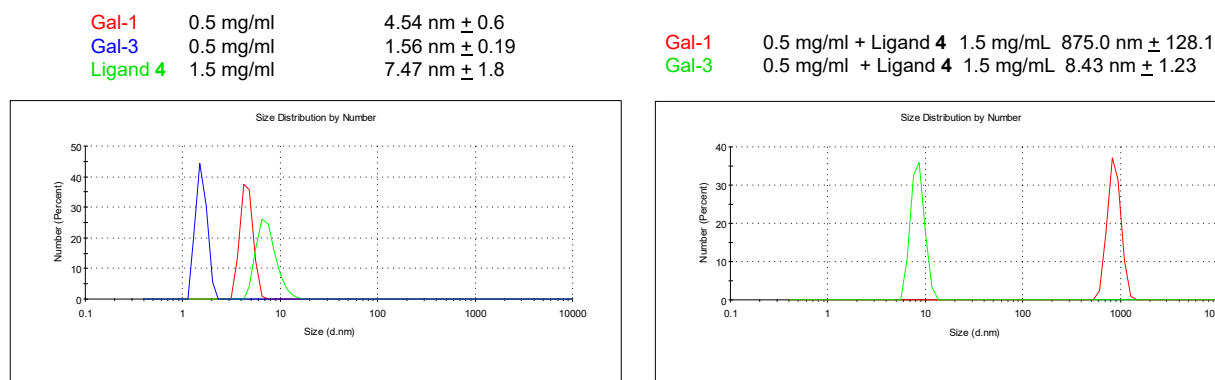


Figure S6. Hydrodynamic radius distribution by number measurements by dynamic light scattering. To the left, hydrodynamic radius distribution graphs for proteins Gal-1 and Gal-3-CRD at 0.5 mg/ml and ligand 4 at 1.5 mg/ml, indicating homogeneity by number distribution; peaks from multiple measurements 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.