Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Study of multivalent carbohydrate-protein interactions by Bio-Layer Interferometry

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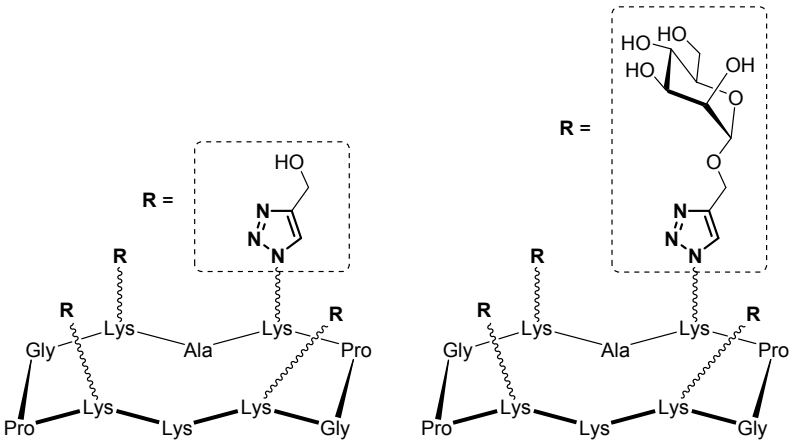
Structures of tetravalent negative controls



R4tOH **R4tMan**

Experimental procedure

Bio Layer Interferometry (BLI). BLI sensors coated with streptavidin (SA sensors) were purchased from Forte Bio (PALL). They were immerged 10 minutes in buffer solution (PBS 1x pH 7,4) before functionalization to remove the protective sucrose layer. Then, the sensors were dipped for 15 minutes in lectin solutions at 1 µM and rinsed in buffer solution for 10 minutes. The functionalized sensors were dipped in sugar solutions at different concentrations and, after each association step, rinsed in the buffer solution. The experimental parameters are reported in Table SI-1. For the strongest ligand-lectin interactions, a solution of commercial GalNAc at 1M was used to regenerate the sensor. Reference sensors not functionalized with the lectin were used as blank to subtract the non-specific adsorption to the raw data. The sensorgrams were fitted using a heterogeneous model (2:1). The reported values are obtained from the average of representative independent experiments, and the errors provided

are standard deviations from the mean. Each experiment was repeated at least three times for the multivalent ligands and two times for the references and negative controls.

Conjugate	Association time (s)	Dissociation time (s)	Concentration	Number of sensors	Sensor type
GalNAc	200	300	1, 0.75, 0.5, 0.25, 0.05, 0.01 mM	1	SA SSA
1	200	300	40, 20, 7.5, 2.5, 0.5, 0.1 µM	1	SA
3	200	300	40, 20, 7.5, 2.5, 0.5, 0.1 µM	1	SA
2	200	300	40, 20, 7.5, 2.5, 0.5, 0.1 µM	1	SA
4	200	300	40, 20, 7.5, 2.5, 0.5, 0.1 µM	1	SA
5	2000	2000	200, 150, 100, 50, 25, 10 nM	6	SA
6	2000	2000	200, 50, 25, 10, 5, 1 nM	6	SA
8	2000	2000	200, 50, 25, 10, 1, 0.5 nM	6	SA
7	2000	2000	200, 50, 25, 10, 5, 1 nM	6	SA
R4tOH	200	300	40 and 20 µM	1	SA
R4tMan	200	300	40 and 20 µM	1	SA

Table SI-1: Experimental parameters used to evaluate the lectin-ligand interaction

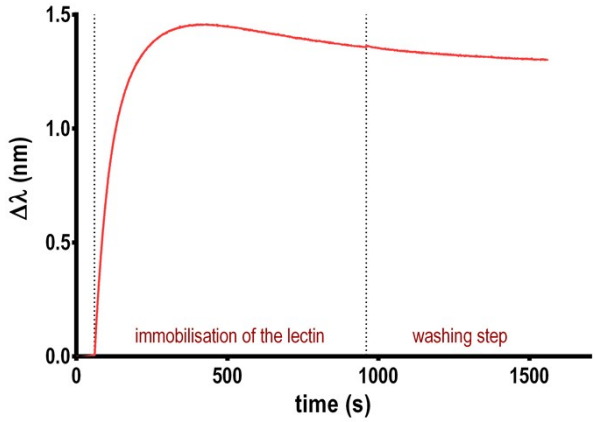


Figure SI-1: Immobilization of the HPA lectin on SA sensor by streptavidin-biotin interaction

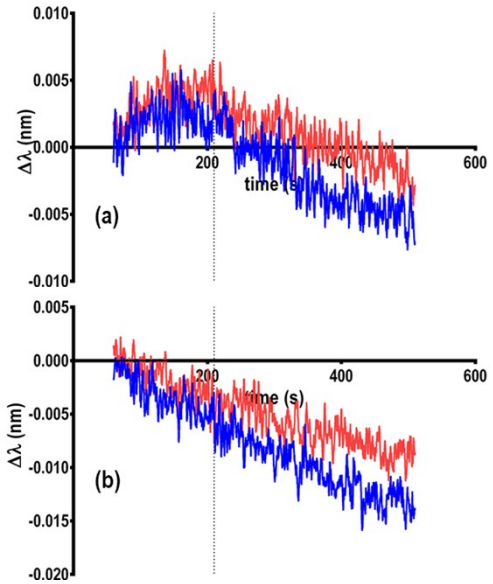


Figure SI-2: Sensorgrams obtained for : (a) R4tMan and (b) R4tOH

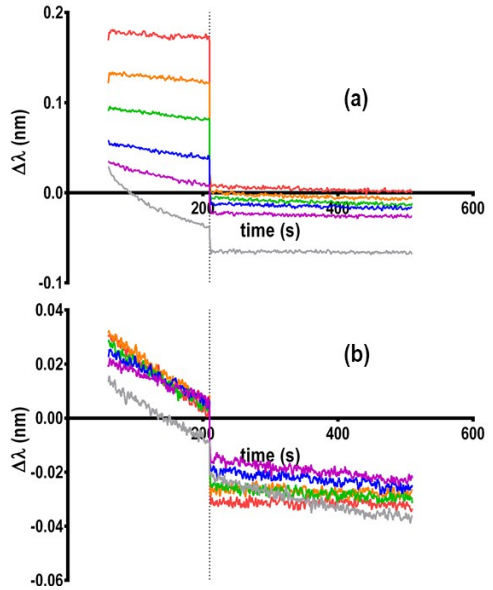


Figure SI-3: Sensorgrams obtained for commercial GalNAc by using: (a) SA and (b) SSA sensors.

Molecule	K_D (nM)	k_{on} (10^4 $M^{-1}.s^{-1}$)	k_{off} (s^{-1})
5	2.6 ± 0.2	1.1 ± 0.04	$2.8 .10^{-5} \pm 5.10^{-7}$
8	0.3 ± 0.2	39.4 ± 9.6	$9.9 .10^{-5} \pm 4.0.10^{-7}$
7	0.3 ± 0.02	25.1 ± 7.4	$7.8 .10^{-5} \pm 1.9.10^{-7}$
6	1.28 ± 0.7	19.5 ± 9.2	$1.9 .10^{-4} \pm 4.0.10^{-7}$

Table SI-2: Kinetic and thermodynamic parameters of the HPA-hexadecavalent glycoconjugate interactions.