Electronic Supplementary Material (ESI) for Molecular BioSystems

Fluorescent Labeling Agents Change Binding Profiles of Glycan-Binding Proteins

SUPPLEMENTARY INFOPMATION:

Structural drawings of 24 oligosaccharides (Figure S1). Structures of 24 oligosaccharides as listed in Table 1 are drawn in Figure S1, including that of a biotin-conjugated linker.

ECA-glycan binding profile (Figure S2). The non-labeled protein binds primarily to Galβ1-4Glcβ (OS-3), Galβ1-4GlcNAcβ (OS-5), Galβ1-4GlcNAc**6S**β (OS-6), and Galβ1-3GlcNAcβ (OS-7)[1, 2]. FITC-labeling enhances the affinity of ECA binding to Galβ1-4Glcβ (OS-3) while reducing the affinity to Galβ1-3GlcNAcβ (OS-7). In addition, FITC-ECA binds to Galβ-biotin (OS-1) while the non-labeled lectin does not.

PNA-glycan binding profile (Figure S3). The non-labeled lectin binds to 2 out of 24 glycans. The binding dissociation constants of the non-labeled lectin to Galβ1-4Glcβ (OS-1) and Galβ1-3GalNAcβ (OS-9) are 3 μM and 300 nM, respectively[3]. In comparison, FITC-labeled PNA only binds to Galβ1-3GalNAcβ (OS-9) with a higher affinity.

MAA-glycan binding profiles (Figure S4). Non-labeled lectin primarily binds to 4 α2-3-linked sialyl lactosides or α2-3-linked sialyl type II glycans (OS-11 through OS-14)[4, 5] with dissociation constants around 100 nM or lower. Additionally, it also binds to Galβ1-4Glcβ (OS-3) and Galβ1-4GlcNAc**6S**β (OS-6)[6]. Upon labeling with FITC, the affinity of MAA binding to these four α2-3-linked sialosides (OS-11 through OS-14) and Galβ1-4Glcβ decreases substantially, by as

much as 2 orders of magnitudes. In addition, FITC-MAA binds to Galβ1-4GlcNAcβ (OS-5) instead of Galβ1-4GlcNAc**6S**β (OS-6).

WFA-glycan binding profile (Figure S5). Non-labeled WFA does not bind to any sialosides tested. The lectin binds to 8 out of 9 asialoglycans (OS-1 through OS-9 except for OS-4)[7]. The overall binding affinity profile of WFA remains unchanged when the lectin is labeled with FITC. Nevertheless, the association constants change either up or down by a factor of less than 10 and a discernable pattern is not be able to be identified.

SNA-glycan binding profile (Figure S6). Non-labeled SNA binds only to α2-6-linked sialosides (OS-19 through OS-24) except for Neu5Acα2-6GalNAcα[5, 8]. The overall binding affinity profile remains unchanged upon labeling with FITC except for minor changes in association constants (within a factor of 10) with no clearly identifiable trends.

Equilibrium dissociation constants of 7 unlabeled and FITC-labeled lectins with 24 glycans are tabulated in Table-S1, Table-S2, Table-S3, Table-S4, Table-S5, Table-S6, Table-S7, and Table-S8. We only list the constants of more tightly bound lectin-glycan complexes, i.e., those corresponding to solid columns in Figure 3 through Figure 5 and Figure S2 through S6.

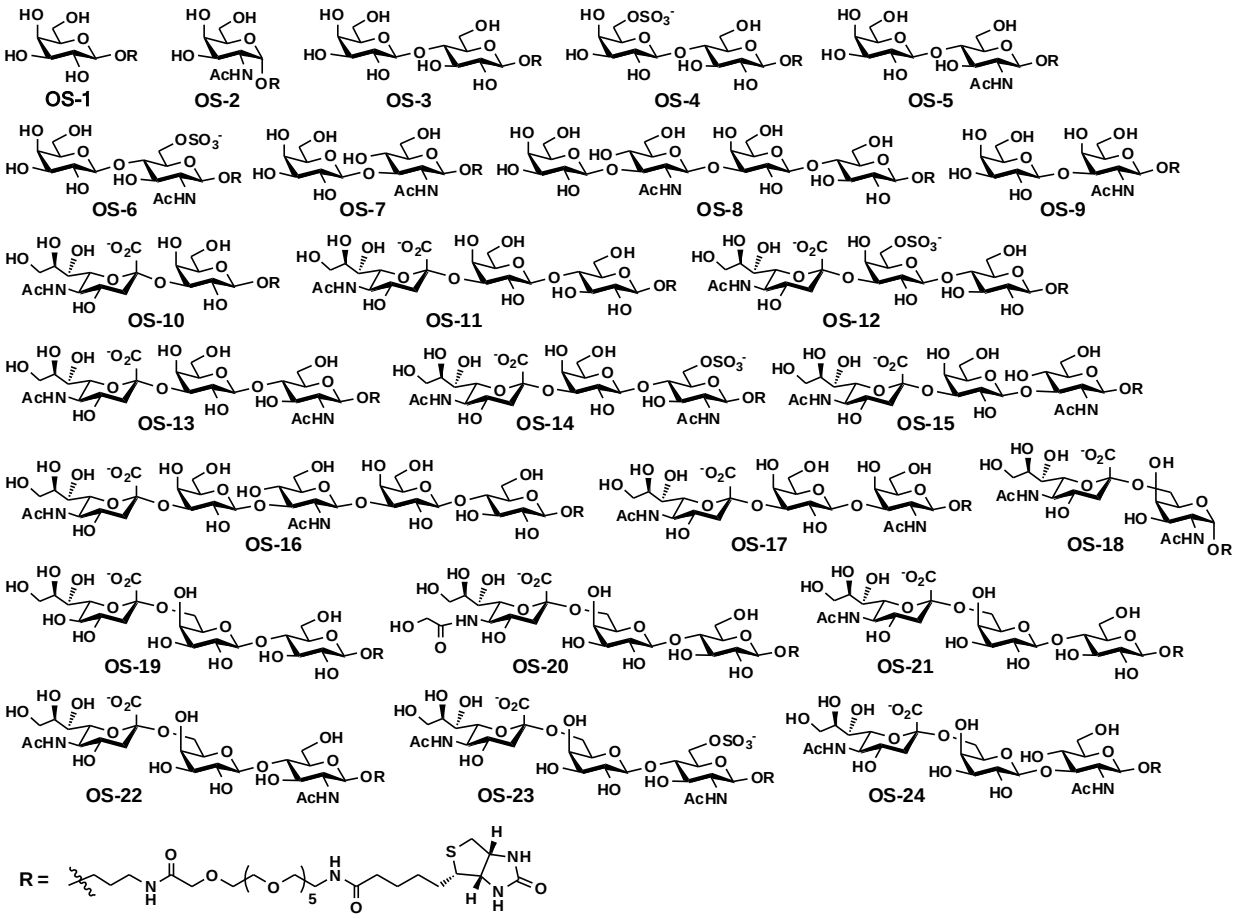

Figure S1. Structures of 24 oligosaccharides marked by the glycan ID numbers. Each oligosaccharide is conjugated to a biotin through a HEG linker so that when the biotin anchors the conjugate on a streptavidin-coated glass surface, the oligosaccharide remains functionally accessible by solution-phase glycan-binding proteins.

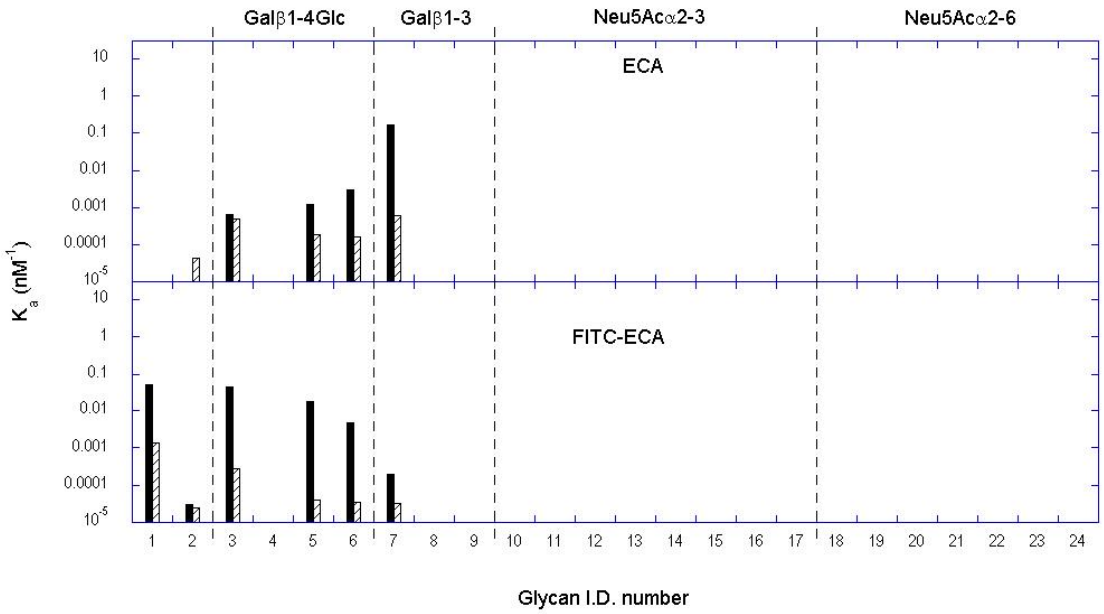

Figure S2. Equilibrium association constants of ECA (top panel) and FITC-ECA (bottom panel) binding to 24 glycans. FITC labeling mainly causes the affinity of ECA to increase except for OS-2 and OS-7.

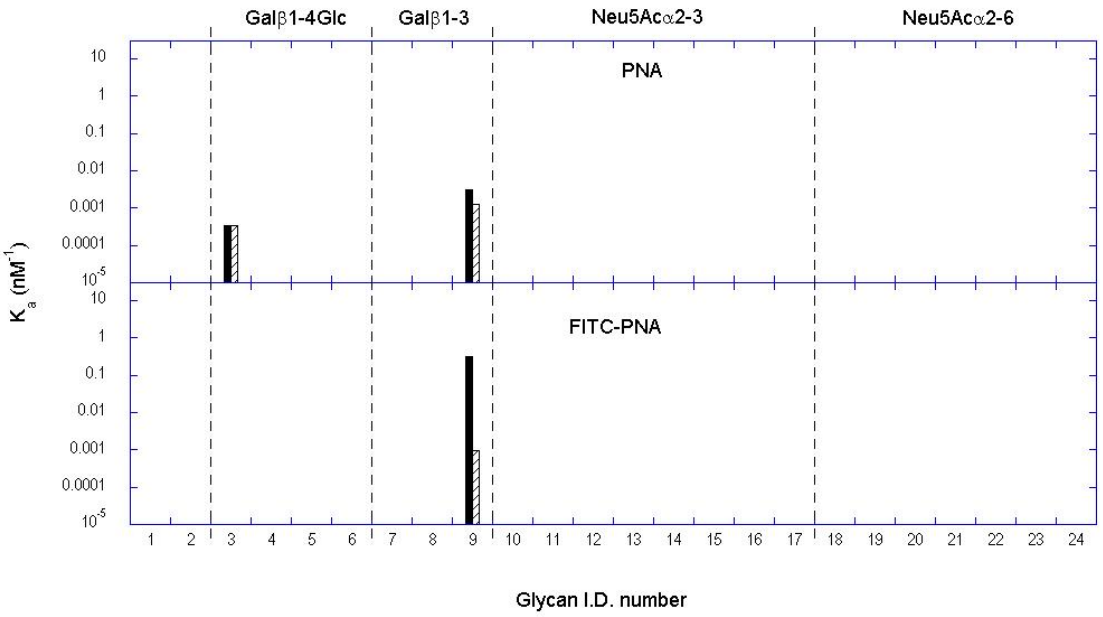

Figure S3. Equilibrium association constants of PNA (top panel) and FITC-PNA (bottom panel) binding to 24 glycans. Unlike non-labeled PNA, FITC-PNA does not bind to OS-3, yet the labeled PNA binds to OS-9 with a 100-fold increased affinity.

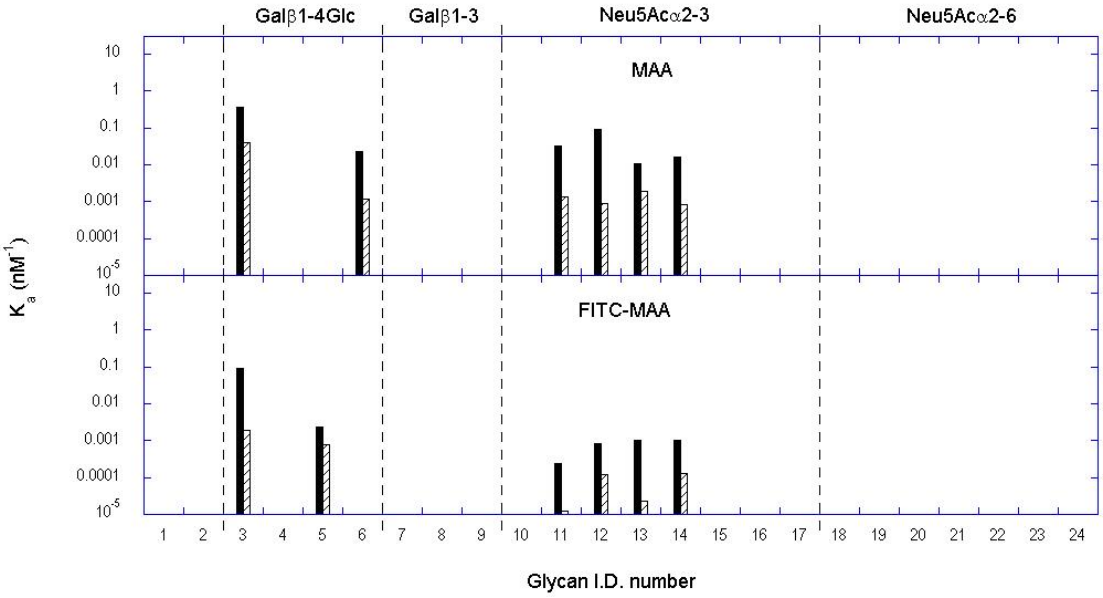

Figure S4. Equilibrium association constants of MAA (top panel) and FITC-MAA (bottom panel) binding to 24 glycans. FITC labeling mainly reduces the affinity of MAA to 4 α2-3-linked sialosides (OS-11 through OS-14) and to Galβ1-4Glcβ (OS-3). Interestingly, unlike non-labeled MAA which binds to Galβ1-4GlcNAc**6S**β (OS-6), the FITC-MAA binds to Galβ1-4GlcNAcβ (OS-5) instead.

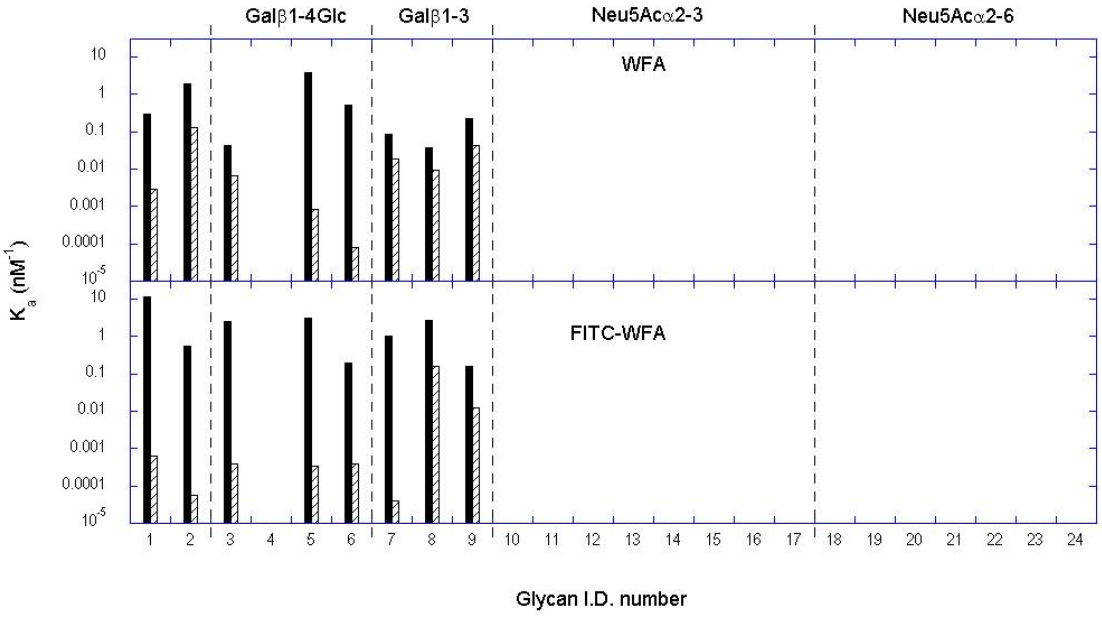

Figure S5. Equilibrium association constants of WFA (top panel) and FITC-WFA (bottom panel) binding to 24 glycans. FITC labeling only changes the magnitudes of the affinity constants (association constants) within a factor of 10, and the overall affinity profile remains intact.

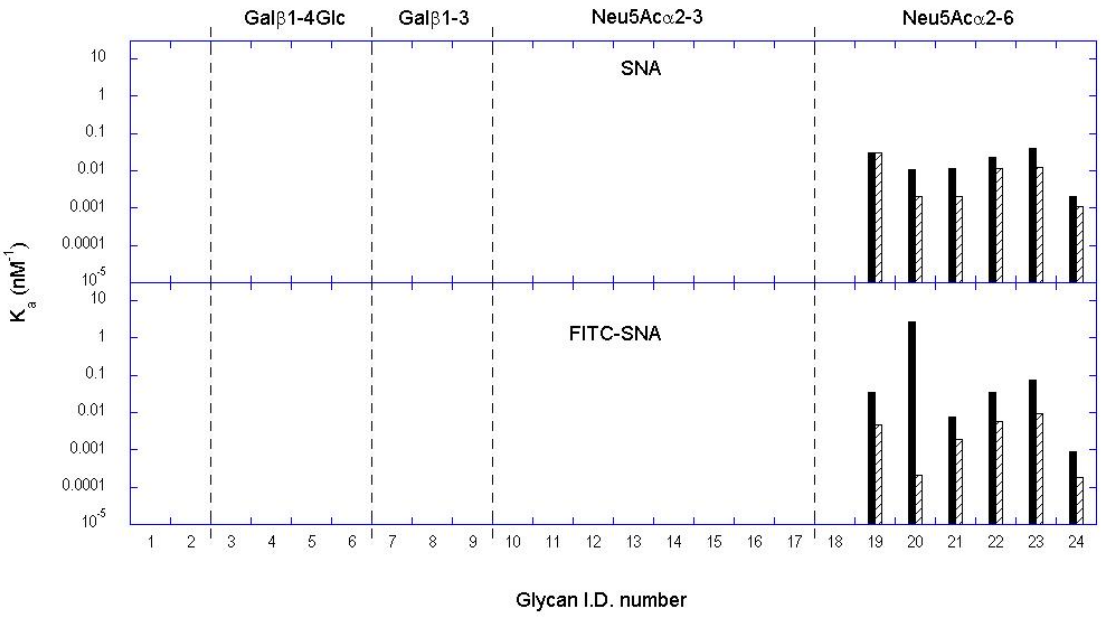

Figure S6. Equilibrium association constants of SNA (top panel) and FITC-SNA (bottom panel) binding to 24 glycans. FITC labeling does not change the overall affinity profile of SNA except for the values of association constants more or less within a factor of 10.

Table-S1: Equilibrium dissociation constants K_d of unlabeled and FITC-labeled WGA with 24 glycans. Only the constants of the more tightly bound lectin-glycan complexes are listed, corresponding to the solid columns ($K_a = 1/K_d$) in Figure 3. Symbol "∗" means $K_d > 500$ μM.

Glycan I.D.	Glycan structures	Kd (nM) (unlabeled WGA)	Kd (nM) (FITC-WGA)
OS-1	Gal β -biotin	*	*
OS-2	GalNAc α -biotin	0.87	0.92
OS-3	Gal β 1-4Glc β -	*	*
OS-4	Gal6S β 1-4Glc β -	*	*
OS-5	Gal β 1-4GlcNAc β -	0.83	20
OS-6	Gal β 1-4GlcNAc6S β -	0.09	0.52
OS-7	Gal β 1-3GlcNAc β -	*	*
OS-8	Gal β 1-3GlcNAc β 1-3Lac β -	*	*
OS-9	Gal β 1-3GalNAc β -	*	*
OS-10	Neu5Acα2-3Gal β -biotin	0.16	51
OS-11	Neu5Acα2-3Gal β 1-4Glc β -	3.15	230
OS-12	Neu5Acα2-3Gal6S β 1-4Glc β -	1.43	3.9
OS-13	Neu5Acα2-3Gal β 1-4GlcNAc β -	0.12	18
OS-14	Neu5Acα2-3Gal β 1-4GlcNAc6S β -	0.42	1460
OS-15	Neu5Acα2-3Gal β 1-3GlcNAc β -	0.79	130
OS-16	Neu5Acα2-3Gal β 1-3GlcNAc β 1, 3Lac β -	1.5	15
OS-17	Neu5Acα2-3Gal β 1-3GalNAc β -	0.05	770
OS-18	Neu5Acα2-6GalNAc α -	7.8	*
OS-19	Kdn α2-6Gal β 1-4Glc β -	*	*
OS-20	Neu5Gcα2-6Gal β 1-4Glc β -	*	*
OS-21	Neu5Acα2-6Gal β 1-4Glc β -	168	*
OS-22	Neu5Acα2-6Gal β 1-4GlcNAc β -	110	*
OS-23	Neu5Acα2-6Gal β 1-4GlcNAc6S β -	102	*
OS-24	Neu5Acα2-6Gal β 1-3GlcNAc-	470	*

Table-S2: Equilibrium dissociation constants K_d of unlabeled and FITC-labeled RCA with 24 glycans. Only the constants of the more tightly bound lectin-glycan complexes are listed, corresponding to the solid columns ($K_a = 1/K_d$) in Figure 4. Symbol "∗" means $K_d > 500$ μM.

Glycan I.D.	Glycan structures	Kd (nM) (unlabeled RCA)	Kd (nM) (FITC-RCA)
OS-1	Gal β-biotin	0.73	0.9
OS-2	GalNAc α-biotin	*	51
OS-3	Gal β1-4Glc β-	0.73	1
OS-4	Gal6S β1-4Glc β-	28	*
OS-5	Gal β1-4GlcNAc β-	2.3	0.79
OS-6	Gal β1-4GlcNAc6S β-	17	0.96
OS-7	Gal β1-3GlcNAc β-	14	1
OS-8	Gal β1-3GlcNAc β1-3Lac β-	*	115
OS-9	Gal β1-3GalNAc β-	3.6	20
OS-10	Neu5Acα2-3Gal β-biotin	*	*
OS-11	Neu5Acα2-3Gal β1-4Glc β-	*	*
OS-12	Neu5Acα2-3Gal6S β1-4Glc β-	*	*
OS-13	Neu5Acα2-3Gal β1-4GlcNAc β-	*	*
OS-14	Neu5Acα2-3Gal β1-4GlcNAc6S β-	*	*
OS-15	Neu5Acα2-3Gal β1-3GlcNAc β-	*	*
OS-16	Neu5Acα2-3Gal β1-3GlcNAc β1,3Lac β-	*	*
OS-17	Neu5Acα2-3Gal β1-3GalNAc β-	*	*
OS-18	Neu5Acα2-6GalNAc α-	110	*
OS-19	Kdn α2-6Gal β1-4Glc β-	53	69
OS-20	Neu5Gcα2-6Gal β1-4Glc β-	33	67
OS-21	Neu5Acα2-6Gal β1-4Glc β-	1.7	21
OS-22	Neu5Acα2-6Gal β1-4GlcNAc β-	58	1530
OS-23	Neu5Acα2-6Gal β1-4GlcNAc6S β-	2.9	275
OS-24	Neu5Acα2-6Gal β1-3GlcNAc-	210	*

Table-S3: Equilibrium dissociation constants K_d of unlabeled RCA and goat IgG-RCA precomplexes with 24 glycans. Only the constants of the more tightly bound lectin-glycan complexes are listed, corresponding to the solid columns (K_a = $1/K_d$) in Figure 5. Symbol "∗" means K_d > 500 μM.

Glycan I.D.	Glycan structures	Kd (nM) (unlabeled RCA)	Kd (nM) (IgG-RCA)
OS-1	Gal β -biotin	0.73	2.2
OS-2	GalNAc α -biotin	*	*
OS-3	Gal β 1-4Glc β -	0.73	0.32
OS-4	Gal6S β 1-4Glc β -	28	*
OS-5	Gal β 1-4GlcNAc β -	2.3	0.36
OS-6	Gal β 1-4GlcNAc6S β -	17	2.9
OS-7	Gal β 1-3GlcNAc β -	14	*
OS-8	Gal β 1-3GlcNAc β 1-3Lac β -	*	*
OS-9	Gal β 1-3GalNAc β -	3.6	*
OS-10	Neu5Acα2-3Gal β -biotin	*	*
OS-11	Neu5Acα2-3Gal β 1-4Glc β -	*	*
OS-12	Neu5Acα2-3Gal6S β 1-4Glc β -	*	*
OS-13	Neu5Acα2-3Gal β 1-4GlcNAc β -	*	*
OS-14	Neu5Acα2-3Gal β 1-4GlcNAc6S β -	*	*
OS-15	Neu5Acα2-3Gal β 1-3GlcNAc β -	*	*
OS-16	Neu5Acα2-3Gal β 1-3GlcNAc β 1, 3Lac β -	*	*
OS-17	Neu5Acα2-3Gal β 1-3GalNAc β -	*	*
OS-18	Neu5Acα2-6GalNAc α -	110	141
OS-19	Kdn α2-6Gal β 1-4Glc β -	53	177
OS-20	Neu5Gcα2-6Gal β 1-4Glc β -	33	82
OS-21	Neu5Acα2-6Gal β 1-4Glc β -	1.7	1.1
OS-22	Neu5Acα2-6Gal β 1-4GlcNAc β -	58	31
OS-23	Neu5Acα2-6Gal β 1-4GlcNAc6S β -	2.9	10.4
OS-24	Neu5Acα2-6Gal β 1-3GlcNAc-	210	*

Table-S4: Equilibrium dissociation constants K_d of unlabeled and FITC-labeled ECA with 24 glycans. Only the constants of the more tightly bound lectin-glycan complexes are listed, corresponding to the solid columns ($K_a = 1/K_d$) in Figure S2. Symbol "∗" means $K_d > 500$ μM.

Glycan I.D.	Glycan structures	Kd (nM) (unlabeled ECA)	Kd (nM) (FITC-ECA)
OS-1	Gal β -biotin	*	20
OS-2	GalNAc α -biotin	0.03	33700
OS-3	Gal β 1-4Glc β -	1570	21
OS-4	Gal6S β 1-4Glc β -	*	*
OS-5	Gal β 1-4GlcNAc β -	820	54
OS-6	Gal β 1-4GlcNAc6S β -	336	220
OS-7	Gal β 1-3GlcNAc β -	6.2	5100
OS-8	Gal β 1-3GlcNAc β 1-3Lac β -	*	*
OS-9	Gal β 1-3GalNAc β -	*	*
OS-10	Neu5Acα2-3Gal β -biotin	*	*
OS-11	Neu5Acα2-3Gal β 1-4Glc β -	*	*
OS-12	Neu5Acα2-3Gal6S β 1-4Glc β -	*	*
OS-13	Neu5Acα2-3Gal β 1-4GlcNAc β -	*	*
OS-14	Neu5Acα2-3Gal β 1-4GlcNAc6S β -	*	*
OS-15	Neu5Acα2-3Gal β 1-3GlcNAc β -	*	*
OS-16	Neu5Acα2-3Gal β 1-3GlcNAc β 1, 3Lac β -	*	*
OS-17	Neu5Acα2-3Gal β 1-3GalNAc β -	*	*
OS-18	Neu5Acα2-6GalNAc α -	*	*
OS-19	Kdn α2-6Gal β 1-4Glc β -	*	*
OS-20	Neu5Gcα2-6Gal β 1-4Glc β -	*	*
OS-21	Neu5Acα2-6Gal β 1-4Glc β -	*	*
OS-22	Neu5Acα2-6Gal β 1-4GlcNAc β -	*	*
OS-23	Neu5Acα2-6Gal β 1-4GlcNAc6S β -	*	*
OS-24	Neu5Acα2-6Gal β 1-3GlcNAc-	*	*

Table-S5: Equilibrium dissociation constants K_d of unlabeled and FITC-labeled PNA with 24 glycans. Only the constants of the more tightly bound lectin-glycan complexes are listed, corresponding to the solid columns ($K_a = 1/K_d$) in Figure S3. Symbol "∗" means $K_d > 500$ μM.

Glycan I.D.	Glycan structures	Kd (nM) (unlabeled PNA)	Kd (nM) (FITC-PNA)
OS-1	Gal β -biotin	*	*
OS-2	GalNAc α -biotin	*	*
OS-3	Gal β 1-4Glc β -	2900	*
OS-4	Gal6S β 1-4Glc β -	*	*
OS-5	Gal β 1-4GlcNAc β -	*	*
OS-6	Gal β 1-4GlcNAc6S β -	*	*
OS-7	Gal β 1-3GlcNAc β -	*	*
OS-8	Gal β 1-3GlcNAc β 1-3Lac β -	*	*
OS-9	Gal β 1-3GalNAc β -	317	3.2
OS-10	Neu5Acα2-3Gal β -biotin	*	*
OS-11	Neu5Acα2-3Gal β 1-4Glc β -	*	*
OS-12	Neu5Acα2-3Gal6S β 1-4Glc β -	*	*
OS-13	Neu5Acα2-3Gal β 1-4GlcNAc β -	*	*
OS-14	Neu5Acα2-3Gal β 1-4GlcNAc6S β -	*	*
OS-15	Neu5Acα2-3Gal β 1-3GlcNAc β -	*	*
OS-16	Neu5Acα2-3Gal β 1-3GlcNAc β 1, 3Lac β -	*	*
OS-17	Neu5Acα2-3Gal β 1-3GalNAc β -	*	*
OS-18	Neu5Acα2-6GalNAc α -	*	*
OS-19	Kdn α2-6Gal β 1-4Glc β -	*	*
OS-20	Neu5Gcα2-6Gal β 1-4Glc β -	*	*
OS-21	Neu5Acα2-6Gal β 1-4Glc β -	*	*
OS-22	Neu5Acα2-6Gal β 1-4GlcNAc β -	*	*
OS-23	Neu5Acα2-6Gal β 1-4GlcNAc6S β -	*	*
OS-24	Neu5Acα2-6Gal β 1-3GlcNAc-	*	*

Table-S6: Equilibrium dissociation constants K_d of unlabeled and FITC-labeled MAA with 24 glycans. Only the constants of the more tightly bound lectin-glycan complexes are listed, corresponding to the solid columns ($K_a = 1/K_d$) in Figure S4. Symbol "∗" means $K_d > 500$ μM.

Glycan I.D.	Glycan structures	Kd (nM) (unlabeled MAA)	Kd (nM) (FITC-MAA)
OS-1	Gal β-biotin	*	*
OS-2	GalNAc α-biotin	*	*
OS-3	Gal β1-4Glc β-	2.8	11
OS-4	Gal6S β1-4Glc β-	*	*
OS-5	Gal β1-4GlcNAc β-	*	430
OS-6	Gal β1-4GlcNAc6S β-	44	*
OS-7	Gal β1-3GlcNAc β-	*	*
OS-8	Gal β1-3GlcNAc β1-3Lac β-	*	*
OS-9	Gal β1-3GalNAc β-	*	*
OS-10	Neu5Acα2-3Gal β-biotin	*	*
OS-11	Neu5Acα2-3Gal β1-4Glc β-	31	4200
OS-12	Neu5Acα2-3Gal6S β1-4Glc β-	11	1220
OS-13	Neu5Acα2-3Gal β1-4GlcNAc β-	94	980
OS-14	Neu5Acα2-3Gal β1-4GlcNAc6S β-	59	990
OS-15	Neu5Acα2-3Gal β1-3GlcNAc β-	*	*
OS-16	Neu5Acα2-3Gal β1-3GlcNAc β1,3Lac β-	*	*
OS-17	Neu5Acα2-3Gal β1-3GalNAc β-	*	*
OS-18	Neu5Acα2-6GalNAc α-	*	*
OS-19	Kdn α2-6Gal β1-4Glc β-	*	*
OS-20	Neu5Gcα2-6Gal β1-4Glc β-	*	*
OS-21	Neu5Acα2-6Gal β1-4Glc β-	*	*
OS-22	Neu5Acα2-6Gal β1-4GlcNAc β-	*	*
OS-23	Neu5Acα2-6Gal β1-4GlcNAc6S β-	*	*
OS-24	Neu5Acα2-6Gal β1-3GlcNAc-	*	*

Table-S7: Equilibrium dissociation constants K_d of unlabeled and FITC-labeled WFA with 24 glycans. Only the constants of the more tightly bound lectin-glycan complexes are listed, corresponding to the solid columns ($K_a = 1/K_d$) in Figure S5. Symbol "∗" means $K_d > 500$ μM.

Glycan I.D.	Glycan structures	Kd (nM) (unlabeled MAA)	Kd (nM) (FITC-MAA)
OS-1	Gal β-biotin	*	*
OS-2	GalNAc α-biotin	*	*
OS-3	Gal β1-4Glc β-	2.8	11
OS-4	Gal6S β1-4Glc β-	*	*
OS-5	Gal β1-4GlcNAc β-	*	430
OS-6	Gal β1-4GlcNAc6S β-	44	*
OS-7	Gal β1-3GlcNAc β-	*	*
OS-8	Gal β1-3GlcNAc β1-3Lac β-	*	*
OS-9	Gal β1-3GalNAc β-	*	*
OS-10	Neu5Acα2-3Gal β-biotin	*	*
OS-11	Neu5Acα2-3Gal β1-4Glc β-	31	4200
OS-12	Neu5Acα2-3Gal6S β1-4Glc β-	11	1220
OS-13	Neu5Acα2-3Gal β1-4GlcNAc β-	94	980
OS-14	Neu5Acα2-3Gal β1-4GlcNAc6S β-	59	990
OS-15	Neu5Acα2-3Gal β1-3GlcNAc β-	*	*
OS-16	Neu5Acα2-3Gal β1-3GlcNAc β1, 3Lac β-	*	*
OS-17	Neu5Acα2-3Gal β1-3GalNAc β-	*	*
OS-18	Neu5Acα2-6GalNAc α-	*	*
OS-19	Kdn α2-6Gal β1-4Glc β-	*	*
OS-20	Neu5Gcα2-6Gal β1-4Glc β-	*	*
OS-21	Neu5Acα2-6Gal β1-4Glc β-	*	*
OS-22	Neu5Acα2-6Gal β1-4GlcNAc β-	*	*
OS-23	Neu5Acα2-6Gal β1-4GlcNAc6S β-	*	*
OS-24	Neu5Acα2-6Gal β1-3GlcNAc-	*	*

Table-S8: Equilibrium dissociation constants K_d of unlabeled and FITC-labeled SNA with 24 glycans. Only the constants of the more tightly bound lectin-glycan complexes are listed, corresponding to the solid columns ($K_a = 1/K_d$) in Figure S6. Symbol "∗" means $K_d > 500$ μM.

Glycan I.D.	Glycan structures	Kd (nM) (unlabeled MAA)	Kd (nM) (FITC-MAA)
OS-1	Gal β-biotin	*	*
OS-2	GalNAc α-biotin	*	*
OS-3	Gal β1-4Glc β-	2.8	11
OS-4	Gal6S β1-4Glc β-	*	*
OS-5	Gal β1-4GlcNAc β-	*	430
OS-6	Gal β1-4GlcNAc6S β-	44	*
OS-7	Gal β1-3GlcNAc β-	*	*
OS-8	Gal β1-3GlcNAc β1-3Lac β-	*	*
OS-9	Gal β1-3GalNAc β-	*	*
OS-10	Neu5Acα2-3Gal β-biotin	*	*
OS-11	Neu5Acα2-3Gal β1-4Glc β-	31	4200
OS-12	Neu5Acα2-3Gal6S β1-4Glc β-	11	1220
OS-13	Neu5Acα2-3Gal β1-4GlcNAc β-	94	980
OS-14	Neu5Acα2-3Gal β1-4GlcNAc6S β-	59	990
OS-15	Neu5Acα2-3Gal β1-3GlcNAc β-	*	*
OS-16	Neu5Acα2-3Gal β1-3GlcNAc β1,3Lac β-	*	*
OS-17	Neu5Acα2-3Gal β1-3GalNAc β-	*	*
OS-18	Neu5Acα2-6GalNAc α-	*	*
OS-19	Kdn α2-6Gal β1-4Glc β-	*	*
OS-20	Neu5Gcα2-6Gal β1-4Glc β-	*	*
OS-21	Neu5Acα2-6Gal β1-4Glc β-	*	*
OS-22	Neu5Acα2-6Gal β1-4GlcNAc β-	*	*
OS-23	Neu5Acα2-6Gal β1-4GlcNAc6S β-	*	*
OS-24	Neu5Acα2-6Gal β1-3GlcNAc-	*	*

References:

1. P. M. Kaladas, E. A. Kabat, J. L. Iglesias, H. Lis and N. Sharon, *Archives of biochemistry and biophysics*, 1982, **217**, 624-637.
2. H. De Boeck, F. G. Loontiens, H. Lis and N. Sharon, *Archives of biochemistry and biophysics*, 1984, **234**, 297-304.
3. M. E. A. Pereira, E. A. Karat, R. Lotan and N. Sharon, *Carbohydrate research*, 1976, **51**, 117 - 118.
4. W. C. Wang and R. D. Cummings, *The Journal of biological chemistry*, 1988, **263**, 4576-4585.
5. S. R. Haseley, P. Talaga, J. P. Kamerling and J. F. Vliegenthart, *Analytical biochemistry*, 1999, **274**, 203-210.
6. R. N. Knibbs, I. J. Goldstein, R. M. Ratcliffe and N. Shibuya, *The Journal of biological chemistry*, 1991, **266**, 83-88.
7. H. J. Allen, E. A. Johnson and K. L. Matta, *Carbohydrate research*, 1980, **86**, 123-131.
8. N. Shibuya, I. J. Goldstein, W. F. Broekaert, M. Nsimba-Lubaki, B. Peeters and W. J. Peumans, *The Journal of biological chemistry*, 1987, **262**, 1596-1601.