Supporting Information

for

Factors Contributing to Variability of Glycan Microarray Binding Profiles

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Estimation of glycan spacing on the neoglycoprotein microarray.

The surface area of BSA is about 30,000 Å². If we model a relatively tightly packed surface with a monolayer of BSA, then one could treat the surface as an extended surface of BSA. If glycans are distributed evenly on the surface, X glycans per BSA splits the total surface area into X sections. For example, 9 glycans per molecule of BSA would contain an average of 1 attachment site per 3333 Å², corresponding to an average spacing of 58 Å apart (square root of 3333). This neglects any curvature and contours, and it does not include the length of the linker. Based on this highly simplified model, a surface composed of neoglycoprotein with about 16 molecules of glycan per molecule of BSA would have an average spacing of 43 Å. A surface composed of neoglycoprotein with about 5 molecules of glycan per molecule of BSA would have an average spacing of 79 Å.

ID	Linker Structure	R	10-fold differences	50-fold differences
			(rate)	(rate)
Sp0	¹ / ₀ //NH ₂	0.93	7 (0.59%)	None
Sp8	20 NH 2	0.84	27 (1.49%)	7 (0.37%)
Sp9	↓ 0,NH ₂	0.89	None	None
Sp10	NH₂ H	0.95	None	None
Sp11	P NH₂ − NH	ND	None	None
Sp12 (Asp)	N NH₂ N N OH H O	0.93	10 (2.78%)	2 (0.56%)
Sp13 (Gly)	KO ^{NH} ₂	0.98	3 (1.67%)	None
Sp14 (Thr)	А NH ₂ О NH ₂	0.60	15 (2.98%)	5 (0.99%)
Sp15 (Ser)	О ∕О́∕́О́́́́О́́́́́́́́́́́́́́́́́́́́́́́́́́	0.83	2 (5.56%)	1 (2.78%)
Sp16	~~~~~NH2	ND	3 (2.78%)	1 (0.93%)
Sp18	$\mathcal{A}_{O} \xrightarrow{O}_{H} \xrightarrow{O}_{NH_{2}} \times \mathcal{N}_{H_{2}}$	0.98	None	None
Sp21	∕ _N o∕∕ _{NH₂}	0.94	6 (5.56%)	1 (0.93%)
Sp23	1,0	0.96	None	None
Sp24 (KVANKT)	O N H Lys-Val-Ala−N H O	0.92	8 (4.44%)	2 (1.11%)
Sp25 (VANK)	Val-Ala-N H O	0.83	3 (4.17%)	1 (1.39%)

Table S1. Pearson correlations constants (R) and large differences for linkers

Linker Pair	R	10-fold differences (rate)	50-fold differences (rate)
Sp14 and Sp8	0.60	15 (3.21%)	5 (1.07%)
Sp0 and Sp8	0.93	7 (0.63%)	none
Sp12 and Sp24	0.91	4 (3.70%)	1 (0.93%)
Sp21 and Sp24	0.90	3 (8.33%)	1 (2.78%)
Sp16 and Sp8	ND	3 (4.17%)	1 (1.39%)
Sp12 and Sp25	0.83	3 (4.17%)	1 (1.39%)
Sp15 and Sp8	0.83	2 (5.56%)	1 (2.78%)
Sp12 and Sp21	0.95	2 (5.56%)	none
Sp13 and Sp24	0.97	1 (2.78%)	none
Sp13 and Sp21	0.97	1 (2.78%)	none
Sp12 and Sp13	0.99	1 (0.93%)	none

Table S2. Pearson correlations constants (R) for linker pairs with large differences.

ND= not determined, too few values were above the floor for the given lectin/conc.