Supporting Information for: **Control of Multivalent Interactions by Binding Epitope Density**

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Characterization of Multivalent Ligands 1-7: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WP-300 or a Brucker AM-500 Fourier Transform NMR spectrometer. Polymers were synthesized using emulsion conditions with a monomer to catalyst ratio of 100:1 (M/C = 100) as previously described[1], using various ratios of mannose and galactose monomers (See: Figure 2 and Table 1 SI)[2]. The ratio of the two saccharides within the polymer was analyzed from the intensity of the anomeric protons by ^{1}H NMR: (mannose-1-H at 4.86 ppm and galactose-1-H at 4.93 ppm). As shown in Table 1 SI, the polymers obtained reflect the mannose/galactose ratios employed in their synthesis. These data indicate that there is no detectable reactivity difference between mannose- and galactose-bearing monomers.

Entry	% Man[a]	% Man (NMR)[b]	n[c]	yield/%[d]
1	100	100	143	67 (95)
2	71	71	145	78 (89)
3	45	45	115	81 (85)
4	31	33	86	77 (83)
5	18	18	102	47 (69)
6	10	10	116	70 (85)
7	2	ND	129	87 (95)

Table 1 SI: NMR Yields and characterization of polymers **1-7**. [a] Percent mannose incorporation based on ratio of mannose to galactose monomers used in the polymerization. [b] Percent mannose incorporation based on integration of mannose- and galactose-1-H by ^{1}H NMR. [c] The value n is defined as the degree of polymerization (DP) for the reaction determined from ^{1}H NMR. [d] Isolated yield. Yields estimated from NMR are in parentheses.

Emulsion polymerization for polymer 1 (DP = 143). To the solution of the mannose bearing monomer (36 mg, 0.098 mmol) and dodecyltrimethylammonium bromide (DTAB) (48 mg, 0.16 mmol, 1.6 eq) in water (310 μL), was added catalyst [$Ru{=}CHPh(Cl)_2(PCy_3)_2$] (0.8 mg, 0.00098 mmol, 0.01 eq) in CH_2Cl_2 (150 μL). The mixture was stirred vigorously for 20 hr at room

temperature. After workup and purification, polymer **1** was obtained as a colorless film (24 mg, 67%). The degree of polymerization determined from ^{1}H NMR is 143.

Quantitative Precipitation Results:

Precipitation of the tetrameric lecting concanavalin A (Con A) by polymers **1-7** was assessed by quantitative precipitation experiments as described in Experimental Methods. The raw data are shown (Figure 1 SI) fit to a sigmoid to determine relative activity. Experiments also were performed using control polymers bearing only galactose residues (Figure 2 SI) to demonstrate that polymeric galactose is unable to cause significant precipitation of Con A.

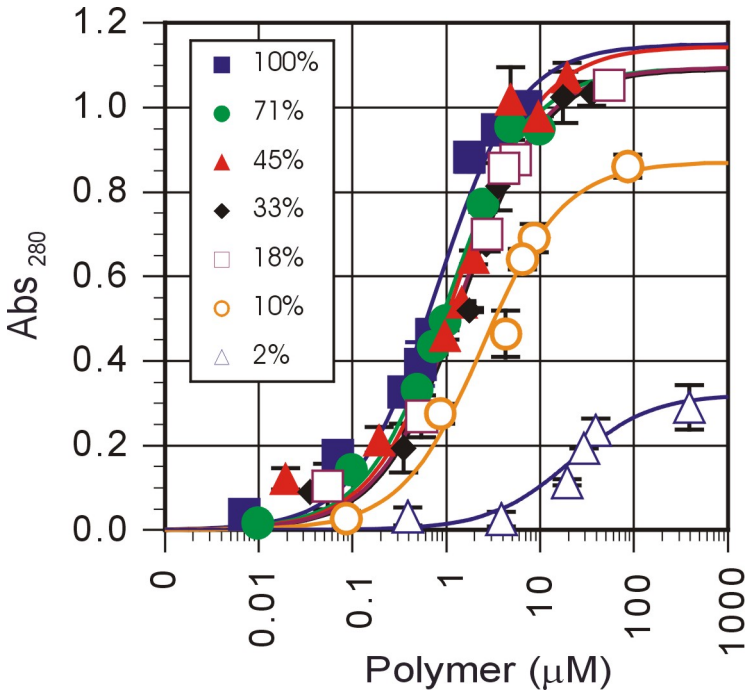

Figure 1 SI: Quantitative Precipitation Results. All series were fit to a sigmoidal curve to determine the half maximal concentration required for precipitation. The stoichiometry of complex formation was determined as previously described.[3] Measurements are the average of three independent experiments with 2 scans performed in each experiment.

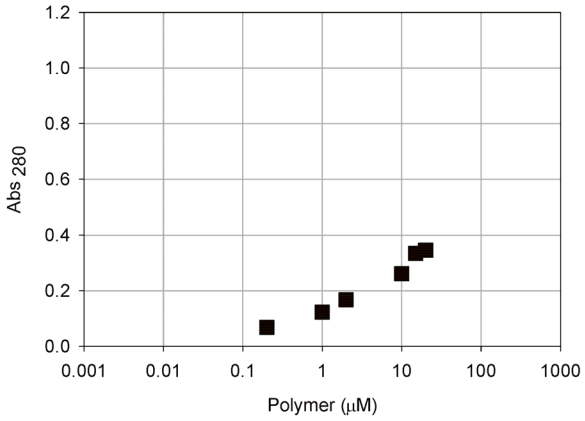

Figure 2 SI: Galactose Controls for Quantitative Precipitation Experiments. Quantitative precipitation experiments were using a ROMP-derived galactose polymer (n=50) generated from galactose monomer in Figure 2. Very little precipitation occurred in the presence of this ligand, suggesting that specific interactions with Con A are required for precipitation.

Turbidimetric Assay Results:

The rate of Con A precipitation induced by polymers **1-6** was assessed by turbidimetric assays as described in Experimental Methods. The raw data are shown below (Figure 3 SI). The relative rates of precipitation were determined by a linear fit of the steepest portion of the curve. To establish that the aggregation process was a result of specific binding of saccharide epitopes to the lectin, competition experiments were also performed. Complexes between Con A and ROMP ligands were produced as above (5 μM Con A, 50 μM saccharide). The resulting solutions were allowed to stand for 1 hr at room temperature. The turbid solution was then placed in a dry quartz micro-cuvette (100 μL volume, 1 cm pathlength) and the absorbance at 420 nm was recorded (T=0 min). A solution of methyl-α-D-mannopyranoside was then added (10 μL at 54 mM, final concentration 5 mM) and mixed for 5 seconds. The change in absorbance (at 420 nm) was monitored as a function of time. The final absorbance (T=10 min) was determined as an

average of the last 10 seconds of this run. The percent change in absorbance was determined as $(T_0-T_{10})/T_0$, the results are represented in Figure 4 SI. The resulting data indicate that the ability of monovalent methyl-α-D-mannopyranoside to disrupt the aggregatres depends on the binding epitope density of the multivalent ligand template employed.

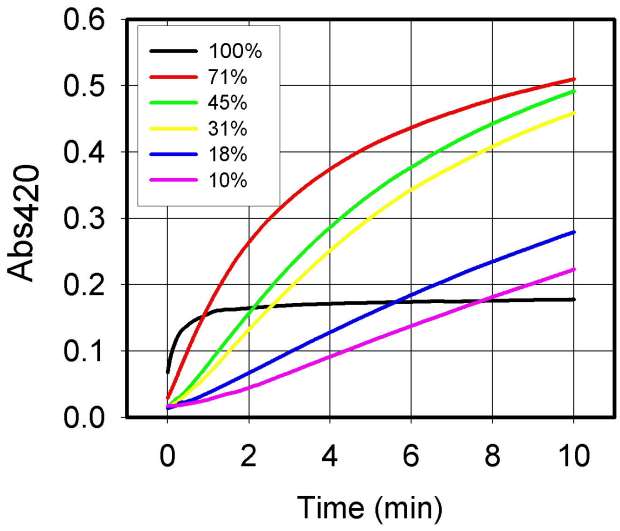

Figure 3 SI: Time course of turbidity measurements. Each curve represents the average of three independent determinations

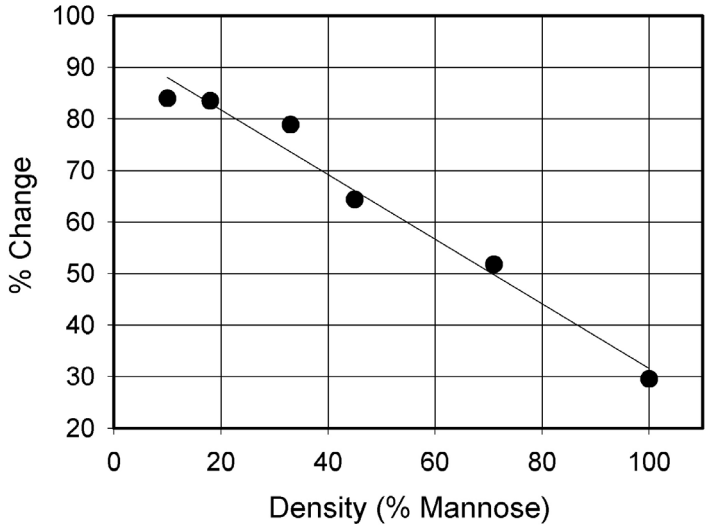

Figure 4 SI: Reversal of lectin aggregation by a competitive ligand. Each point represents the average determined using two independent runs. The ability of α-D-methyl-mannopyranoside to solubilize the complex is dependent on the binding epitope density of the multivalent ligand.

FRET Results:

FRET assays were used to determine changes in relative proximity between Con A upon treatment with polymers **1-6**. Experiments were performed as described in Experimental Methods, the raw data are shown in Figure 5 SI. The maximum observed change in fluorescence was used as a measure of the change in proximity of the receptors. The addition of polymer **6** did not result in significant changes in fluorescence. The maximum percent change in fluorescence to calculate the change in average distance between receptors is shown in Table 2 SI. It should be noted that a similar change in fluorescence could result from changes in the population of proximal receptors rather than changes in the average distance between receptors.

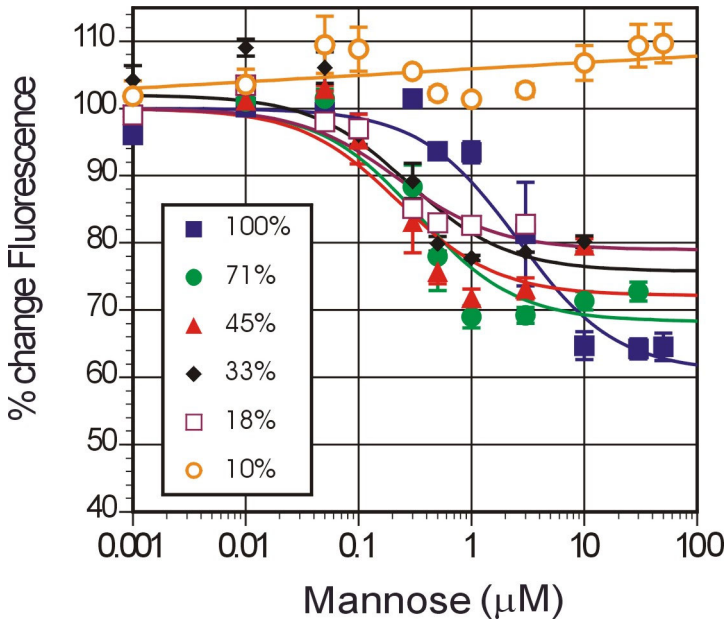

Figure 5 SI: FRET Measurements for Con A Complexes Generated from Multivalent Ligands. The data were fit to standard binding equations that result in sigmoidal curves, and the half maximal change in fluorescence was determined. Percent change was assessed by comparison to a buffer treated control.

% Mannose	*r*	*+/-*
100	60	2
71	62	1
45	63	1
33	67	1
18	70	1
10	ND	
2	ND	

Table 2 SI: Calculation of Average Inter-Receptor Distances. Average inter-Con A distances were calculated using the maximum % change in FRET as described previously.[4] The Förster distance used for calculations was 54 Å.[5]

References:

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(2) Kanai, M.; Kiessling, L. L. *in preparation*.
(3) Khan, M. I.; Mandal, D. K.; Brewer, C. F. *Carbohydr. Res.* **1991**, *213*, 69-77.
(4) Matko, J.; Edidin, M. In *Fluorescence Spectroscopy*, 1997; Vol. 278, pp 444-462.
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