Electronic Supplementary Information

Concanavalin A targeting *N*-linked glycans in spike proteins influence viral interactions

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1. Supplementary materials and methods

1.1. General methods

ConA isolated from *Canavalia ensiformis* (Jack bean) and soybean agglutinin isolated from *Glycine Max* were purchased from Sigma (St. Louis, USA, C2010 and L1395, respectively). 2-Aminobenzamide-labeled oligomannose oligosaccharide with 9-mannosyl residues (Ludger, 2AB-labeled $Man_9GlcNAc_2$, CAB-MAN9-01) and 2-aminobenzamide-labeled triantennary trigalactosylated oligosaccharide (Ludger, 2AB-labeled A_3G_3, CAB-NA3-01) were purchased for the fluorescence anisotropy experiment. 2AB-labeled $Man_9GlcNAc_2$ (Man_9-2AB, $C_{77}H_{126}N_4O_{56}$, 2,003.82 g/mol) has α-D-mannoses as its terminal carbohydrates. 2AB-labeled A_3G_3 (A_3G_3-2AB, $C_{83}H_{135}N_7O_{56}$, 2,126.97 g/mol) has β-D-galactoses as its terminal carbohydrates. ConA and SBA have molecular weights of 25,598.47 and 27,571.93 Da, respectively.

1.2. Measurements of fluorescence anisotropy between *N*-glycans-2AB and lectins

N-glycans (Man_9-2AB or A_3G_3-2AB) binding to lectins (ConA or SBA) were observed by fluorescence anisotropy (FA). Measurements were taken with FP-8300 (JASCO) with a 2/3 type cuvette (JASCO, material Q (10 X 10 mm)) with Tris (hydroxyl methyl) amino methane (Sigma) and sodium chloride (Aldrich). Full excitation and emission spectra were measured initially to determine optimal wavelengths with 2.0 nM *N*-glycans-2AB. The excitation and emission wavelengths were 337 and 433 nm in both Man_9-2AB and A_3G_3-2AB, respectively, with a 5-nm band width. The voltage of the photomultiplier tube (PMT) was fixed at 700 V, and all

experiments were performed at 25°C. Man_9-2AB or A_3G_3-2AB (2.0 nM) in 20 mM Tris-HCl and 50 mM NaCl at pH 7.4 was titrated with ConA or SBA to the saturation point. All experiments were performed at least three times to achieve averages with standard deviations (average ± s.d.). Anisotropy ($\boldsymbol{r}$) was measured by the following equation:

$$\mathbf{r} = \frac{(\boldsymbol{I}_{\parallel} - \boldsymbol{I}_{\perp})}{(\boldsymbol{I}_{\parallel} + \mathbf{2}\boldsymbol{I}_{\perp})}$$

$\boldsymbol{I}_{\parallel}$ is the fluorescence intensity from parallel mode, and $\boldsymbol{I}_{\perp}$ is the fluorescence intensity from perpendicular mode. The results of anisotropy ($\boldsymbol{r}$) were changed to fraction bound ($\boldsymbol{F_{bound}}$, fraction bound of Man_9-2AB or A_3G_3-2AB).

$$\boldsymbol{F_{bound}} = \frac{\boldsymbol{r} - \boldsymbol{r_{free}}}{\boldsymbol{r_{bound}} - \boldsymbol{r_{free}}}$$

Where $\boldsymbol{r_{free}}$ is the anisotropy of *N*-glycans-2AB that do not interact with lectins and $\boldsymbol{r_{bound}}$ represents the fluorescence anisotropy of *N*-glycans-2AB that bind lectins. In every experiment, $\boldsymbol{r_{bound}}$ was plotted against the lectin concentration and fit to the 1:1 binding model.

$$\mathbf{T} + \mathbf{C} \rightleftharpoons \mathbf{TC}$$

$$\boldsymbol{K_d} = \frac{[\boldsymbol{T}][\boldsymbol{C}]}{[\boldsymbol{TC}]}$$

$$\boldsymbol{F_{bound}} = \frac{\boldsymbol{C_{total}} + \boldsymbol{T_{total}} + \boldsymbol{K_d} - \sqrt{(\boldsymbol{C_{total}} + \boldsymbol{T_{total}} + \boldsymbol{K_d})^2 - 4\boldsymbol{C_{total}}\boldsymbol{T_{total}}}}{2\boldsymbol{T_{total}}}$$

Where C is the concentration of ConA or SBA and T is the concentration of Man_9-2AB or A_3G_3-2AB. K_d represents the dissociation constant. K_d was calcualted by Origin (2018b version) and Wolfram Mathmatica.

1.3. Preparation of apo-concanavalin A

Mn^{2+}-Ca^{2+}-ConA (250 μM, 3 mL, hereafter ConA) in 20 mM Tris-HCl (pH 7.4) and 50 mM NaCl was incubated with 2.0 M HCl (3.0 mL) for 50 min at room temperature. The mixture was transferred to a 10-kDa cut-off membrane filter (Merck) and rinsed at 3,000 rpm and 4°C three times with 2.0 M HCl in 20 mM Tris-HCl and 50 mM NaCl. This demetallization process was performed five times to form apo-ConA. The concentrations of metal ions including Mn^{2+} and Ca^{2+} were analyzed with an inductively coupled plasma-optical emission spectrometer (ICP-OES, Thermo Fisher Scientific, iCAP 7400 Duo Model) that was operated at the core facility center at Jeonbuk National University.

Supplementary Figures

Fig. S1 Three major types of *N*-linked glycans.

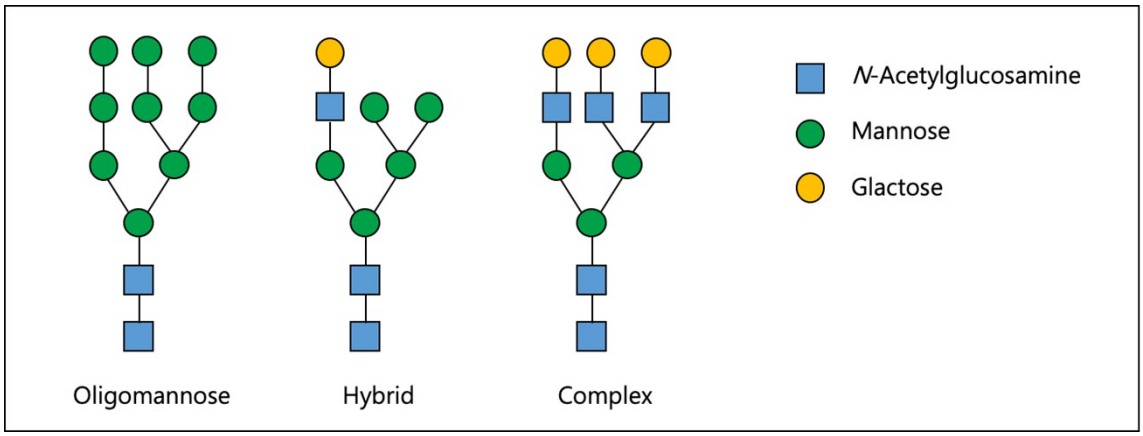

Fig. S2 Structures of (a) $Man_9GlcNAc_2$ and (b) A_3G_3.

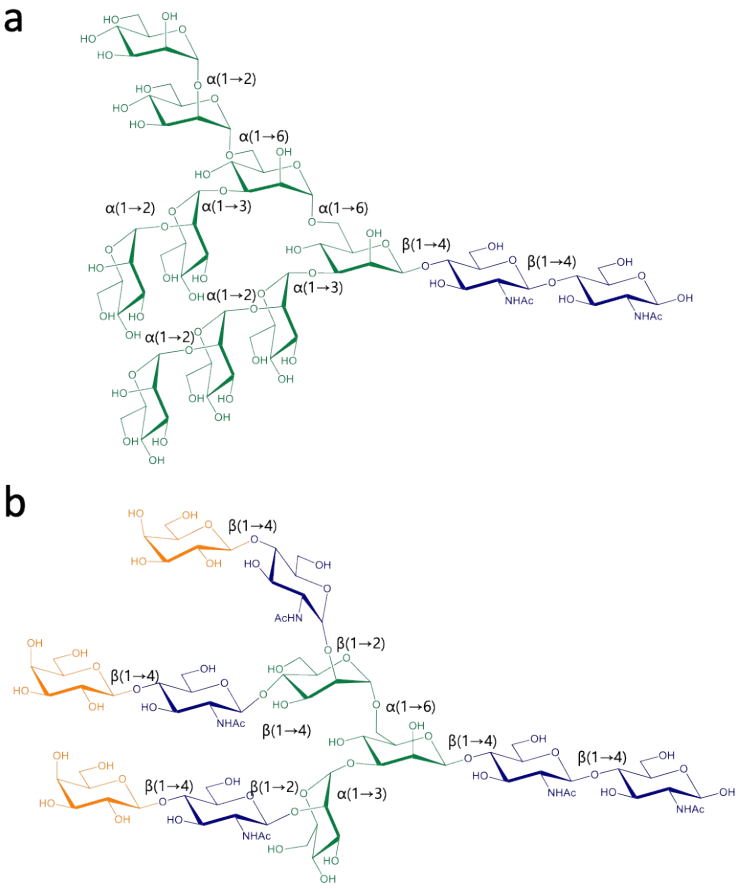

Table S1. Dissociation constants (K_ds) between lectins and *N*-glycans labeled 2-aminobenzamide (2-AB) at pH 7.4.

	$Man_9GlcNAc_2$-2AB	A_3G_3-2AB
ConA	$0.63 \pm 0.03 \times 10^{-6}$	$7.58 \pm 0.16 \times 10^{-6}$
Apo-ConA	$24.69 \pm 1.20 \times 10^{-6}$	$33.21 \pm 1.34 \times 10^{-6}$
SBA	$1.40 \pm 0.07 \times 10^{-6}$	$0.78 \pm 0.03 \times 10^{-6}$

(Unit: M)