Supporting information

A Fluorescent Lectin Array Using Supramolecular Hydrogel for Simple Detection and Pattern Profiling for Various Glyco-conjugates

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Quenching and Recovery process of F-lectins by specific Quencher.

Fluorescent titration of Fluorescein labeled 6 lectins (Con A, WGA, GSL-II, AAL, UEA-I, GSL-I) with suitable quencher and then saccharide were measured to demonstrated BFQR event. Emission of F-lectins were decreased by the addition of quenchers, and then increased by the addition of the corresponding saccharides in the presence of quenchers. These results are consistent with in aqueous solution (Fig. s-1) and in hydrogel matrix (Fig.s-2). The conditions of theses measurements were written in experimental section.

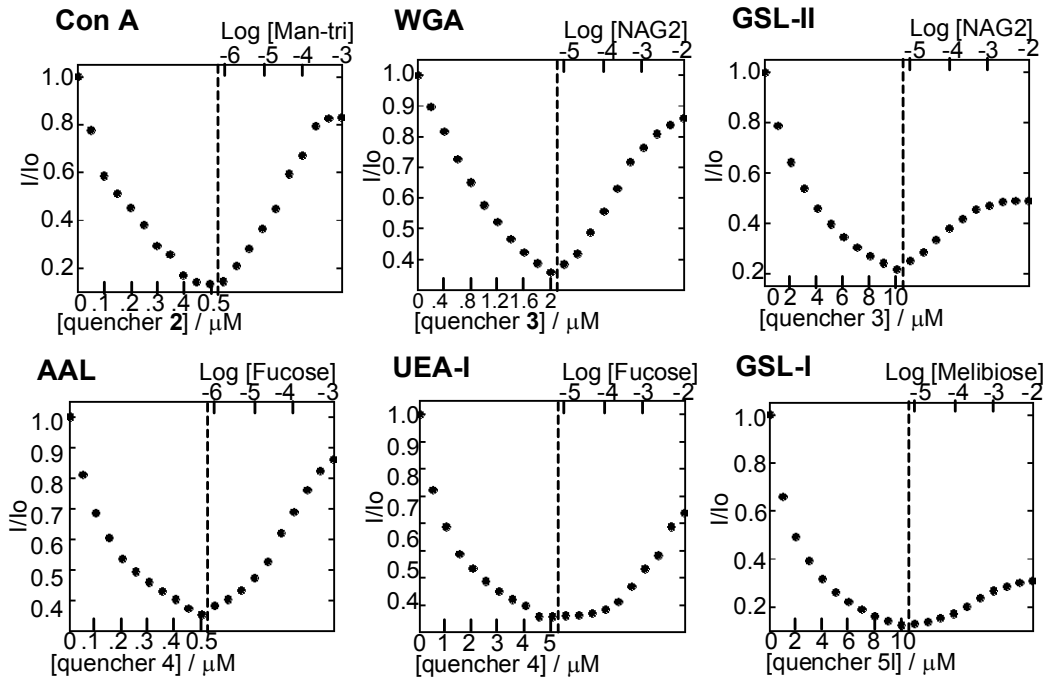

Figure s-1. Fluorescent titration curve of Fluorescein-labeled lectins with quenchers and saccharides in aqueous solution. [F-lectin] = 0.1 μM in 50 mM HEPES buffer (pH 7.5, containing 1 mM $MnCl_2$, 1 mM $CaCl_2$, 0.1 M NaCl), 20 ˚C, λ_{ex} = 488 nm.

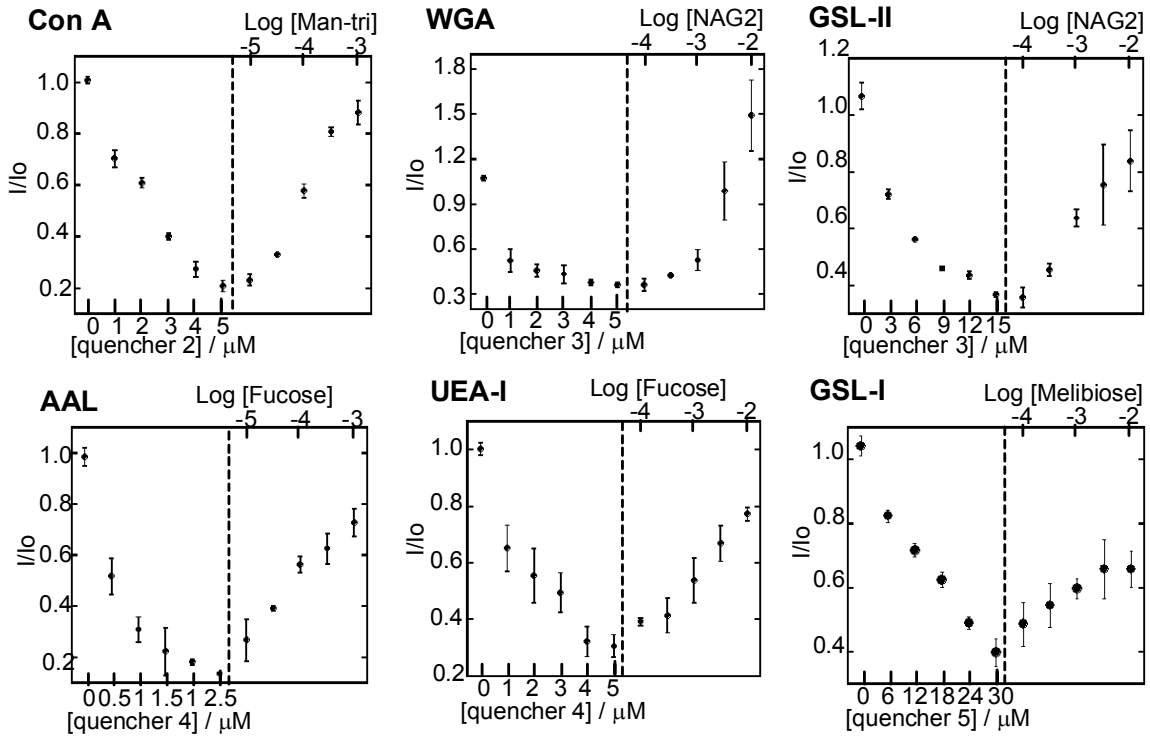

Figure s-2. Fluorescent titration curve of Fluorescein-labeled lectins with quenchers and saccharides in hydrogel matrix. [F-lectin] = 1 μM in 50 mM HEPES buffer (pH 7.5, containing 1 mM $MnCl_2$, 1 mM $CaCl_2$, 0.1 M NaCl), in 0.25 wt% of **1,** 20 ˚C, λ_{ex} = 475 nm. The error bar in graph mean standard deviation of independent 3 times measurement.

Binding constants of Lectin in hydrogelmatrix

In BFQR, the competitive binding of quencher (Q) versus saccharide (S) to lectin (L) can be formalistically represented be eq **1**.

$$\begin{array}{l} L + Q \underset{}{\overset{K_q}{\rightleftharpoons}} LQ \\ + \\ S \\ \Big\Updownarrow K_s \\ LS \end{array} \qquad (1)$$

In eq 1, K_q and K_s are the association constants of the lectin-quencher (LQ) and lectin-saccharide (LS) complexes, respectively. If the free and total concentrations of L, Q, S are represented by [L], [Q] and [S], and $[L]_t$, $[Q]_t$ and $[S]_t$, respectively, K_q and K_s can be represented as follows.

$$K_q = [LQ]/[L][Q] = [LQ]/[L]([Q]_t-[LQ]) \qquad (2)$$

$$K_s = [LS]/[L][S] = [LS]/[L]([S]_t-[LS]) \qquad (3)$$

If $[L]_t << [S]_t$, then $[S]_t$ can be taken to be the measure of [S], and by taking into account the mass balance ($[L]_t = [L]+[LS]+[LQ]$), $[L]_t$ can be represented by eq 4.

$$[L]_t =[LS]+[LS]/K_s[S]_t+K_q[LS][Q]_t/(K_s[S]_t+K_q[LS]) \qquad (4)$$

Equation 4 can be simplified to yield the dependence of [LS] on the concentrations and the binding constants of other species (eq 5).

$$[LS] = K_s[S]_t([L]_tK_q\text{-}1\text{-}K_s[S]_t\text{-}K_q[Q]_t+ ((-[L]_tK_q+1+K_s[S]_t+K_q[Q]_t)^2+4K_q[L]_t(K_s[S]_t+1))^{1/2})/2K_q(K_s[S]_t+1) \qquad (5)$$

The binding constant of the lectin-saccharide complex determined by monitoring the fluorescent recovery from the quenching state (lectin-quencher complex). Hence, the fluorescence change ($\triangle$F) as a function of the saccharide ([S]) concentration can be given by eq 6

$$\triangle F/\triangle F_{max} = [LS]/[L]_t \qquad (6)$$

where $\triangle F_{max}$ is the maximum change in the fluorescence upon complete displacement of quencher form lectin.

In Con A, K_q in the hydrogel matrix ($1.6 \times 10^5\ M^{-1}$) was determined by the titration data of Fig.2b, and K_s in hydrogel matrix was determined by the nonliner regression analysis of eq 6 from the titration data of Fig.2c using *KaleidaGraph.*

In GSL-I, K_q ($5.4 \times 10^4\ M^{-1}$) and K_s for melibiose ($1.1 \times 10^4\ M^{-1}$) in the hydorogel matrix were determined by the titration curve of Fig.s2. This K_s is almost identical with the binding constant of GSL-I for α-Gal derivatives in previous report.[s1]

Pattern detection of glycoproteins and carbohydrates in cell

Fluorescent images of Fig.s-3 were the sources of the graphs of Fig.7-b and Fig.8-b.

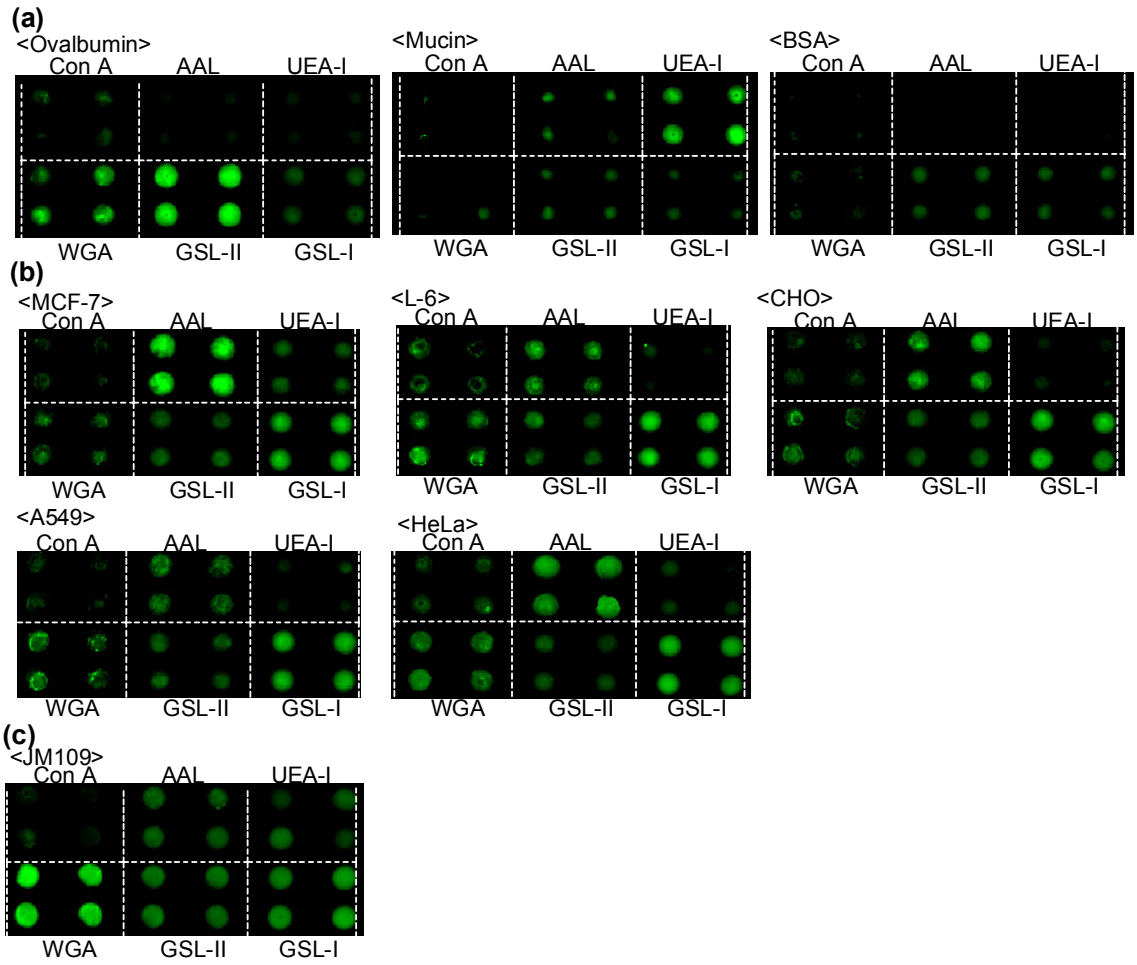

Figure s-3. Pattern detection of glycoproteins and carbohydrates in cells. (a) Fluorescent images of lectin chip about 2 glycoproteins (Ovalbmin, Mucin) and nonglycosilated protein (BSA). (b) Fluorescent images of mammalian cell lines. (c) A fluorescent image of bacteria cell.

Variability of the response pattern on the same plate or independent plate

Fig.s-4 shows the fluorescent recovery ratio of all spots of Fig.8a on the same plate. It is shown that serious variability was not observed on the same plate.

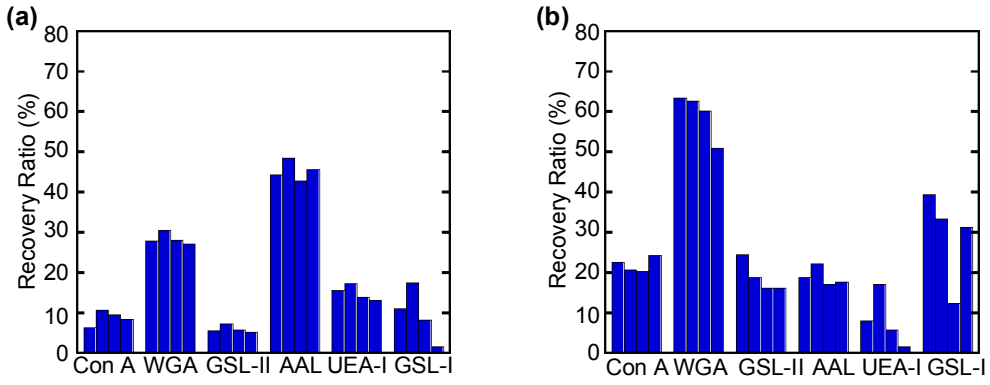

Fig.s-4 The bar graph of fluorescence recovery ratio of all spots on the same plate. (a) HepG2. (b) NM522.

Similarly, it is shown that the response patterns were practically conserved between independent arrays. This was confirmed by the comparison of pattern with a single plate (see to Fig.8-b) with the averaged pattern of 4 independent plates (see to Fig.s-6). For instance, in the MCF-7 cell lysate, the distinguished recovery at the AAL spot and the moderate response at WGA, UEA-I and GSL-I spots were conserved. In the L-6 sample, on the other hand, the major response at three lectins (WGA, AAL and GSL-I) was constantly observed. In bacteria cells, the response pattern was maintained among independent arrays, that is, not only predominant response of WGA for NM522 and JM109, but also the moderate response of Con A (in NM522) or GSL-II (in JM109).

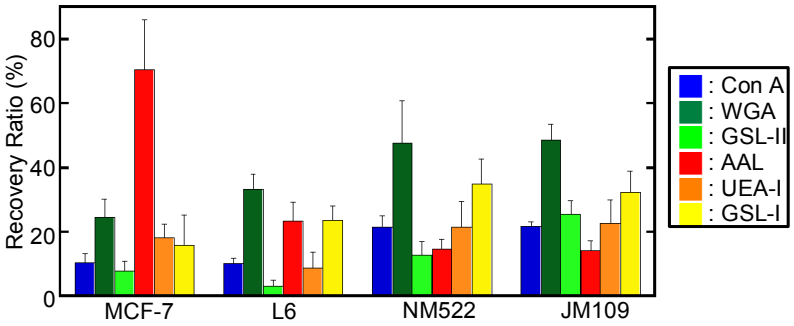

Fig.s-5 The bar graph of the averaged ratio about 4 independent plates. The bar height and the error bar in the graph display the average and standard deviation of 4 independent plates.

Detection limit of lectin chip

The detection limit of mono-saccharide, glucose, was 1 mM (see Fig.5). The detection limit is different in each glyco-conjugates because of the affinity difference. As a typical examples, the detection limit for a glycoprotein, Ribo B, was 0.5 μg/μL (30 μM) (Fig.s-6a). A typical pattern for a bacteria (NM522) lysate was detected at 0.5 μg/μL (Fig.s-6b).

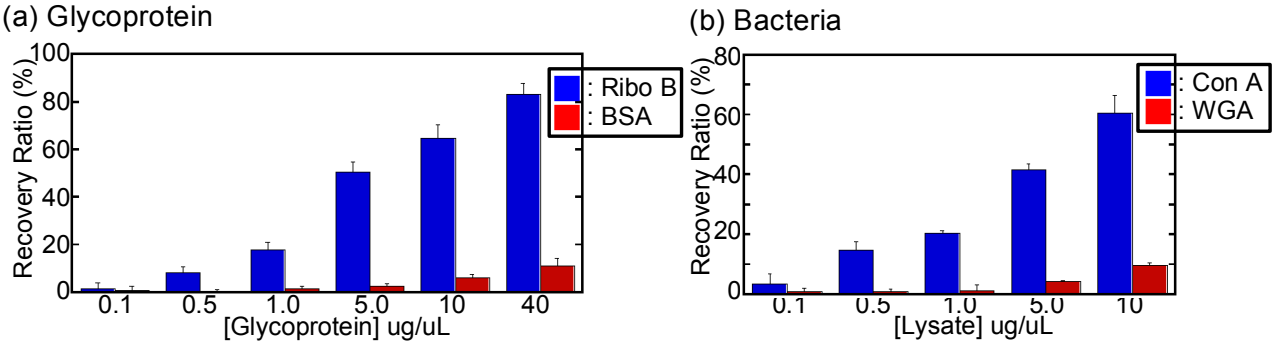

Fig.s-6 (a) The bar graph of the recovery ratio with various concentration of Ribo B or a control protein (BSA) in Con A chip. (b) The recovery ratio of Con A or WGA chip by the addition of bacteria (NM522) lysate.

Tuning the detection sensitivity

The detection sensitivity is adjustable by modulating the quencher concentration in this BFQR method. Table s-1 shows that the detection sensitivity of glucose shifted to the high concentration by the increase of quencher **2**.

Table s-1 The comparison of glucose detectable range by Con A chip to change the concentration of quencher **2**.

quencher **2**	3.0 uM	4.0 uM	5.0 uM	7.5 uM	10 uM
RC_{50} (Glc) *	5 mM	12 mM	15 mM	20 mM	24 mM

*The concentration of glucose which fluorescent ratio recovered at 50%.

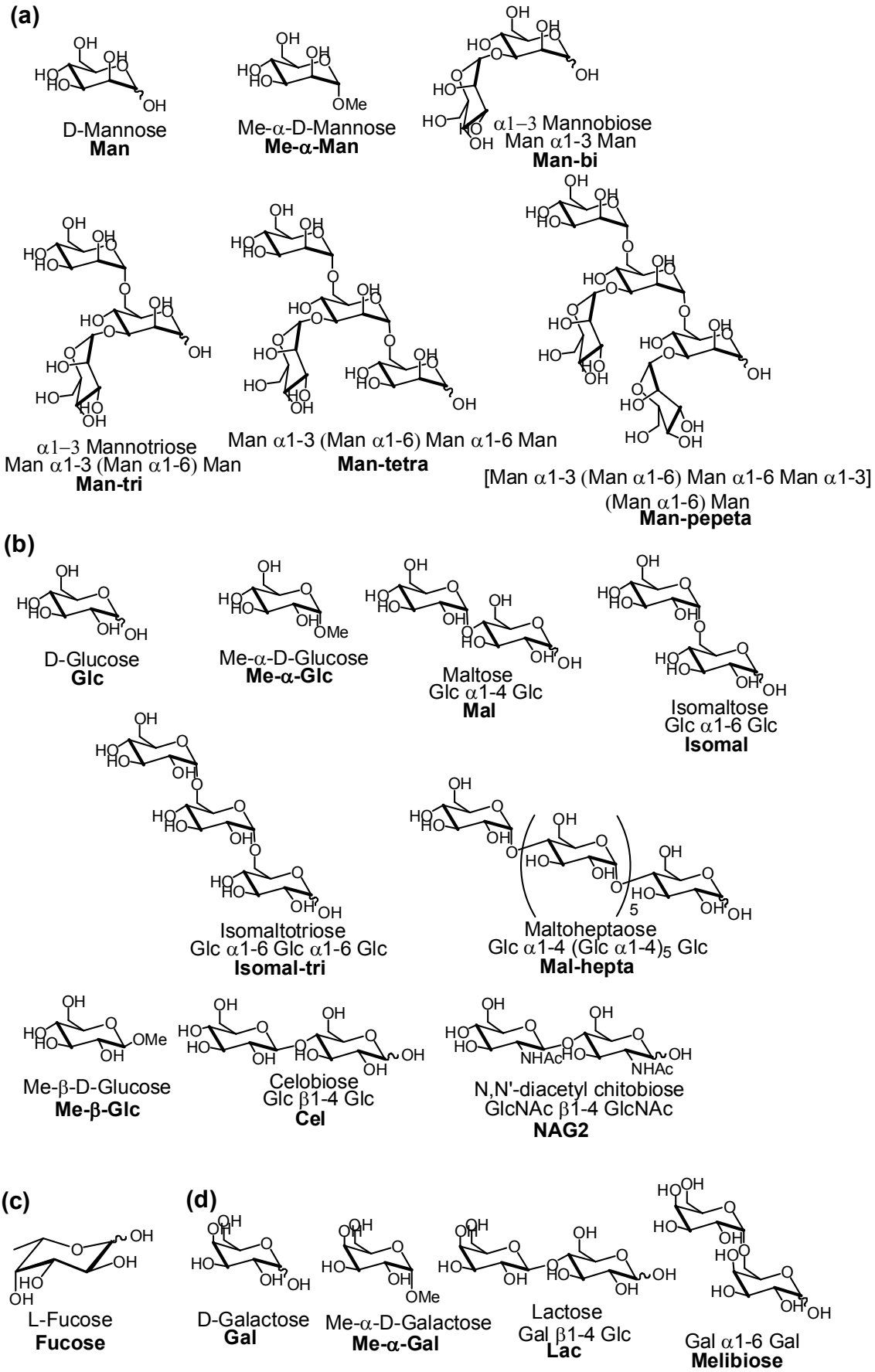

Figure s-7. Molecular structures of saccharides used in this study.

Complete Ref. 4g

(4) (g) Blixt, O.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C.-H.; Paulson, J. C. *Proc. Nat. Acad. Sci.* **2004**, *101*, 17033.

Reference

(s1) Goldstein, I. J.; Blake, D. A.; Ebisu, S.; Williams, T. J.; Murphy, L. A. *J. Bio. Chem.* **1981**, *256*, 3890.