procedures, which is not the case with a conventional confocal detection system. This specific feature enables in situ aqueous-phase detection without perturbing equilibrium conditions, which should contribute to both sensitive and reproducible analysis.

3. *Bimolecular fluorescence quenching and recovery method*

   Hamachi and coworkers manufactured a sophisticated detection principle called "bimolecular fluorescence quenching and recovery" (BFQR) [38]. They used a supramolecular hydrogel matrix, where fluorescent lectins were noncovalently fixed to act as a molecular recognition scaffold. Though the detection mechanism is rather complicated and not straightforward, it enables one to read out fluorescently a series of lectin–saccharide interactions without washing processes, in a manner similar to the evanescent-type detection principle. Moreover, the developed method BFQR does not require prior labeling of the target saccharides. The latter feature resembles that of a surface plasmon resonance detection principle used much more widely [39]. To the present, however, relatively simple, synthetic saccharide quenchers have been utilized, limiting the application of this technology to more complex samples, e.g., body fluids and tissue extracts.

3 General Procedures

A general procedure for glycan profiling experiments using the GlycoStation™ system has been described [37]. Although there might be a variety of sample forms depending on experimental purposes, e.g., sera, urine, cell lysates, and tissue extracts, the core experimental procedure is common to all of the sample forms: i.e., it comprises (1) sample preparation, (2) protein quantification, (3) labeling with an appropriate fluorescent reagent (e.g., Cy-3), (4) application of the labeled sample onto the lectin microarray plate, and (5) data analysis, essentially as described [40, 41]. General procedures are outlined in Fig. 1.

In the following section, a typical procedure using cell lysates and Cy-3 labeling is described, while variously modified procedures are also available, some of which are described later.

3.1 Sample Preparation: Cell Lysate

The following protocol assumes that a cell pellet is washed extensively with PBS, and is kept frozen at –80°C until use:

1. Melt the cell pellet gradually on ice
2. Add 1 mL of PBS-Tx (PBS containing 1% (w/v) Triton X-100) to the cell pellet and suspend the cells with a pipette

3. Disrupt the cells by sonication (1–2 min on ice)
4. Centrifuge the above sample at 14,000×*g* at 4°C for 5 min
5. Recover the supernatant with a pipette
6. Quantify protein by an appropriate method (e.g., Pierce/Micro BCA™ Protein Assay Reagent Kit, #23235)

3.2 *Fluorescent Labeling and Application to the Lectin Microarray*

1. *Cy3 labeling*
   (a) Dilute samples to 50 μg/mL by adding PBS-Tx based on the result of the above protein assay
   (b) Put 20 μL of each sample (50 μg/mL) into a tube containing 100 μg of Cy3-SE (succinimide ester), mix with a pipette, and spin down by brief centrifugation
   (c) Put the tubes into a shading bag and incubate them for 1 h at room temperature (25°C) in the dark
2. *Gel filtration to remove excess free Cy3-SE*:
   Remove excess Cy3-SE reagent by gel filtration using appropriate equipment (e.g., Pierce/Zeba™ Desalt Spin Columns, 0.5 mL, #89882)
3. *Sample application to LecChip™*
   (a) Measure each volume of Cy3-labeled samples with a pipette
   (b) Prepare a total volume of 500 μL by adding an appropriate probing buffer (e.g., GP Biosciences Probing Solution); the protein concentration becomes 2 μg/mL as 1 μg of protein is eluted
   (c) Dilute these samples to 2 μg/mL, 1 μg/mL, 500 ng/mL, 250 ng/mL, 125 ng/mL, 62.5 ng/mL, and 31.25 ng/mL by adding the same probing buffer
   (d) Put LecChip™ in a frozen state at –20°C into an incubation box to melt the keeping solution (provided by the supplier) in wells of the LecChip™
   (e) Remove the keeping solution
   (f) Apply 100 μL of these samples to each well of the LecChips™ with a pipette
   (g) Incubate the LecChips™ in an incubation box at 20°C for 16 h
4. *Scanning with GlycoStation™ Reader 1200*
   Scan the LecChips with GlycoStation™ Reader 1200. The following conditions are recommended for the first trial: gain (70–125), exposure time (133, 199 ms), cumulative count (4). In order to detect relatively weak signals while avoiding saturation of strong signals, take some other scans while adjusting the gain and the exposure time.

3.3 Data Analysis

Data analysis is a critical part of lectin microarray experiments, because the data obtained from each microarray analysis shows systematic variation in microarray quality, scanner detection stability, sample preparation reproducibility, and labeling efficiency. For this purpose, an initial step to normalize the obtained signal intensities is essential. In this section, the two fundamental procedures required for data analysis are described [42–44].

1. *Gain merging*

   Kuno et al. developed a gain-merging procedure to expand the dynamic range of signal intensities obtained by lectin microarray analysis [42]. This procedure is necessary because of the optical properties of the scanning system, but is practically useful when analyzing a series of clinical samples, which often give a wide range of binding signals. When performing an analysis, a lectin microarray slide is scanned under two different gain conditions; one is a higher gain to "rescue" weak signals below 1,000 and the other is a lower gain to "suppress" strong signals over 40,000. Here, appropriate lectins for "merging" are selected, to give moderate signal intensities (i.e., 1,000–40,000) under both higher and lower gain conditions. Then, a merging factor (F) is determined as the average of higher (Int^H)/lower (Int^L) ratios calculated for individual merging lectins by (1):

$$F = \text{Averaged}\left(\text{Int}^{\text{H}}/\text{Int}^{\text{L}}\right) \tag{1}$$

   The over-range intensities (>40,000) obtained under the higher gain condition (e.g., $Int^H{}_{(\text{lectin A})}$) are replaced with theoretical intensities ($Int^T{}_d$ lectin cT) by (2):

$$\text{Int}^{\text{T}}{}_{(\text{lectin A})} = \text{Int}^{\text{L}}{}_{(\text{lectin A})} \times F \tag{2}$$

2. *Normalization*

   Four different normalization methods are available to process lectin microarray data: "max," "mean," "particular lectin," and "median." For these normalization procedures, the signal intensity is multiplied by a normalization factor N for each array, which is calculated by $N = 1/\mu$, where μ is either the highest signal intensity of all of the lectins on the array (max), the mean of all of the lectins on the array (mean), the signal intensity of one selected lectin on the array (particular lectin), or the median of all of the lectins on the array (median). Required procedures depend significantly on experimental procedures and research purposes. For example, the author usually uses a mean normalization method for comparative purposes when dealing with a series of stem cells [45–47], because this gave the best result for glycan analysis of CHO and its mutant LEC cells [44]. On the other hand, a max-normalization procedure is widely used for differential glycan profiling targeting clinical samples for glycan-related bio-marker investigation [16, 17, 28, 48, 49].

4 Targets of the Lectin Microarray

Since its first publication in 2005, most reports of the lectin microarray have concerned its basic aspects; e.g., development of the array substrates and their fabrications. Recently, however, an increasing number of applications of lectin microarray technology have been reported, e.g., in glycan-related biomarker investigations, stem cell profiling toward regeneration medicine, microbial infections, and glycoprotein profiling in the light of functional glycomics (for representative applications, see Table 1). In the following section, relevant technologies required in various orders of hierarchy (i.e., molecular, cellular, tissue and body fluid orders) are briefly described.

4.1 Samples of Homogeneous Glycome

1. *Oligosaccharides*

   There are few reports describing the application of the lectin microarray to free oligosaccharides [10, 34], probably because a substantial merit of this microarray is its direct applicability to glycoproteins without liberation of oligosaccharides. Alternatively, most monoamine-coupling fluorescent reagents (e.g., 2-aminopyridine and 2-aminobenzamide) are of the UV-excited type, which are not compatible with the present glass substrate (UV-fluorescence positive). Uchiyama et al. used TAMRA (tetramethylrhodamine)-labeled three representative *N*-linked oligosaccharides (i.e., M6 high-mannose type, α-2-6-disialobiantennary, and asialobiantennary glycans) for analysis by an evanescent-field activated fluorescence-type scanner [34]. Notably, without a washing procedure, relatively weak bindings of these monovalent oligosaccharides could be seen toward a restricted set of lectins, while they were lost immediately after a buffer replacement procedure. Signal patterns observed for these oligosaccharides are relatively clear, as has been evident when sequential glycosidase digestion was performed for a complex-type sialobiantennary *N*-glycan [10].
2. *Purified glycoproteins*

   As described, glycoproteins are major targets for direct analysis by the lectin microarray, either in their purified (e.g., glycoprotein drugs) or crude (e.g., cell supernatant and body fluids) forms. The analysis provides glycan profiles regarding both *N*- and *O*-glycans [8–13]. Because the profiles obtained are unique to individual glycoproteins and states of the cells which produce them, the method contributes to the validation of glycoprotein drugs [12]. In combination with a specific antibody against core protein, highly sensitive monitoring of glycan profiles of target glycoproteins in the course of their production is possible (described later).

Table 1 Representative research papers reporting applications of the lectin microarray

Application fields and paper titles (year)	Reference
1. Glycan synthesis and glycoprotein production	
Pichia pastoris-produced mucin-type fusion proteins with multivalent *O*-glycan substitution as targeting molecules for mannose-specific receptors of the immune system (2011)	Gustafsson et al. [50]
Chemoenzymatic synthesis and lectin array characterization of a class of *N*-glycan clusters (2009)	Huang et al. [51]
Engineering of mucin-type human glycoproteins in yeast cells (2008)	Amano et al. [52]
A lectin array-based methodology for the analysis of protein glycosylation (2007)	Rosenfeld et al. [12]
2. Glycoprotein profiling relevant to functional glycomics	
Survey of glycoantigens in cells from α-1,3-galactosyltransferase knockout pig using a lectin microarray (2010)	Miyagawa et al. [53]
Transient expression of an IL-23R extracellular domain Fc fusion protein in CHO vs HEK cells results in improved plasma exposure (2010)	Suen et al. [54]
Glycomic analyses of glycoproteins in bile and serum during rat hepatocarcinogenesis (2010)	Nakagawa et al. [55]
Testicular angiotensin-converting enzyme with different glycan modification: characterization on glycosylphosphatidylinositol-anchored protein releasing and dipeptidase activities (2009)	Kondoh et al. [56]
Polylactosamine on glycoproteins influences basal levels of lymphocyte and macrophage activation (2007)	Togayachi et al. [57]
Functional glycosylation of human podoplanin: glycan structure of platelet aggregation-inducing factor (2007)	Kaneko et al. [58]
3. Development of disease-related glycoprotein markers	
A unique *N*-glycan on human transferrin in CSF: a possible biomarker for iNPH (2011)	Futakawa et al. [59]
Multilectin assay for detecting fibrosis-specific glyco-alteration by means of the lectin microarray (2011)	Kuno et al. [16]
Lectin microarray profiling of metastatic breast cancers (2011)	Fry et al. [60]
Identification of various types of α2-HS glycoprotein in sera of patients with pancreatic cancer: possible implication in resistance to protease treatment (2010)	Kuwamoto et al. [61]
Wisteria floribunda agglutinin-positive mucin 1 is a sensitive biliary marker for human cholangiocarcinoma (2010)	Matsuda et al. [48]
High levels of E4-PHA-reactive oligosaccharides: potential as marker for cells with characteristics of hepatic progenitor cells (2009)	Sasaki et al. [62]
4. Glycan profiling of stem cells relevant to regenerative medicine	
Possible linkages between the inner and outer cellular states of human induced pluripotent stem cells (2011)	Saito et al. [47]
Glycome diagnosis of human induced pluripotent stem cells using the lectin microarray (2011)	Tateno et al. [46]
Lectin microarray analysis of pluripotent and multipotent stem cells (2011)	Toyoda et al. [45]
5. Glycan profiling relevant to pathogen infection	
HIV-1 and microvesicles from T cells share a common glycome, arguing for a common origin (2009)	Krishnamoorthy et al. [63]
Analyzing the dynamic bacterial glycome with a lectin microarray approach (2006)	Hsu et al. [64]

3. *Eukaryotic cells*

As mentioned previously, glycan structures differ significantly between different types of cells (species and states). According to this concept, i.e., "cellular glycomics," the lectin microarray should work ideally for differentiation of different types of cells. The first demonstration was made by Ebe et al. [65] using CHO and its LEC mutant strains, and work in this context was further extended in a more systematic manner by Tao et al. [13]. These studies used detergent-solubilized cell membrane fractions as a glycoprotein source. However, significant care is necessary for the preparation of such glycoprotein fractions, which may contain interfering materials, such as non-glycosylated cytoplasmic proteins and immature glycoproteins included in the endoplasmic reticulum and Golgi apparatus.

Tateno et al. reported a convenient method to profile a cell-surface glycome using a commercial CellTracker dye (Invitrogen) [66]. This series of reagents passes freely through the cell membrane, but once inside the cell, they are transformed into cell-impermeant forms. One of the commercial products, Orange™ CMRA, can fluoresce only after it is metabolized by endogenous esterase. The procedure is simple and applicable to an extensive range of eukaryotic cells [44].

4. *Bacterial cells*

Hsu et al. [64] successfully profiled glycosylation patterns of closely related *Escherichia coli* strains, including pathogenic ones. Those authors differentiated commensal and pathogenic strains in a manner of facile fingerprinting. More recently, Yasuda et al. reported an alternative method to analyze bacterial glycomes with emphasis on differential profiling of 16 strains of *Lactobacilli casei* species [67]. They found CYTOX Orange, usually used for intracellular nuclear staining, to be the best dye to be incorporated into the bacterial cells and bound to DNA. Despite the organisms being from the same species, almost all binding patterns obtained for these 16 strains were unique. It should also be noted that the current systems of the lectin microarray, largely composed of plant lectins, can work for bacterial glycome profiling.

5. *Virus and related particles*

Krishnamoorthy et al. reported an interesting analysis of glycan profiles of the HIV virus which focused on comparison with virus-resembling microvesicles, which are secreted from host cells [63]. Lectin microarray analysis using 68 lectin probes revealed that glycome signatures of HIV and host cell microvesicles were almost identical. This observation provides important support for the "exosome" hypothesis that HIV largely relies on the biosynthetic machineries of host cells, and thus they should give totally the same glycan profiles. For this analysis, the authors utilized a sensitive ratiometric two-color detection method (described later).

4.2 Samples of Nearly Homogeneous Glycome

1. *Tissue sections*

   Matsuda et al. developed a skilful technique for differential glycan profiling targeting small areas (i.e. 1.5 mm diameter, 5 μm thickness) of paraffin-embedded and formalin-fixed tissue sections [48]. The method provides clinicians with a very useful approach to differential glycan analysis. Considering that tissue samples to be compared are of the same date and same individual, the data obtained are more reliable than those obtained from sera. In fact, the authors applied the technique to an investigation of glycoprotein markers for diagnosis of cholangiocarcinoma, a representative malignant tumor, for which no useful markers are presently available. As a result, they found that *Wisteria floribunda* agglutinin specific for GalNAc was the best probe to differentiate cholangiocarcinoma lesions from normal bile duct epithelia ($p < 0.0001$) [49]. Though not yet performed, combination with laser micro-dissection followed by high-throughput differential glycan profiling should become a powerful means for future biomarker discovery.

4.3 Samples of Highly Heterogeneous or Complex Glycomes

1. *Body fluids (bile, sera) in biomarker investigations*

   Body fluids, such as patient sera and urine, are primary targets for biomarker diagnosis. Under the concept of glycoproteomics, an increasing number of researchers of both glycomics and proteomics have been involved in the discovery phase of glycoprotein markers. For this realization, however, one should consider the fact that serum is a highly heterogeneous mixture of glycoproteins originating from many different organs. Therefore, their individual cellular glycomes are also heterogeneous. A well-organized strategy is described, which relies on the high-sensitivity technique of the lectin microarray at two different phases [6, 7]. One is the tissue-section targeted analysis described above, and the other an antibody-overlay method described below.

5 Modified Technologies of the Lectin Microarray

1. *Antibody-overlay method in biomarker qualification/verification*

   Kuno et al. reported a highly practical approach to the differential glycan profiling of an antibody-targeted glycoprotein in the course of biomarker development [28]. As the discovery phase of biomarker development proceeds, several biomarker candidates (i.e., glycoproteins) are nominated, whereas it is not yet certain that they really work as robust diagnostic markers, e.g., for hepatocellular carcinoma. For the purpose of pre-validation (i.e., qualification

or verification), it is important to carry out the analysis on several dozen clinical samples (e.g., usually body fluids such as sera). Therefore, establishment of a high-throughput procedure such as the lectin microarray is particularly important in glycoprotein biomarker development. The developed method makes maximum use of an antibody, which is raised against the protein moiety of the target glycoprotein: the antibody is used for enrichment (immunoprecipitation), semi-quantification (Western blotting), and overlay to the lectin microarray (*lower* scheme in Fig. 1). A target glycoprotein included in each clinical sample need not be fluorescence-labeled, and thus high-throughput (>100 samples) analysis is easily performed. Therefore, the lectin microarray is used in two steps in the strategy developed for glycoprotein biomarker investigation. The approach was applied to the investigation of an hepatic fibrosis marker, α-1-acid glycoprotein probed with *Maackia amurensis* lectin (MAL) and *Aspergillus oryzae* lectin (AOL) [16].

2. *Dual color measurement*

   Pilobello et al. developed a method for the rapid evaluation of glycosylation changes of heterogeneous mammalian samples using a ratiometric two-color lectin microarray approach [68]. This approach is reminiscent of a proteomic procedure called two-dimensional differential in-gel electrophoresis (2-D DIGE), which uses two differentially labeled (i.e., Cy3/Cy5) protein samples [69]. When focusing on differential analysis, this approach can be extremely useful to enhance substantial differences in the glycome. However, it should be mentioned that these two methods, i.e., proteomic 2-D DIGE and the glycomic dual color method, are essentially different in that the latter procedure includes "competition" between immobilized lectins toward a set of various glycans. In other words, quantitative comparison of lectin signals needs careful consideration.

6 Perspective

The technology of the lectin microarray described here is expanding its application fields rapidly to broad areas of life sciences, which include both basic and applied sciences. Because the method is still new, relatively few researchers appreciate its innovative features, which previous technologies have lacked, i.e., discrimination based on biological affinity. However, considering that every biological phenomenon comprises cellular communications, it is quite natural to assume that carbohydrate-protein (e.g., lectins) interactions are fundamental for carcinogenesis, embryogenesis, morphogenesis, pathogenesis, etc. Thus it should not be surprising that the lectin microarray platform works very well to analyze, differentiate, and elucidate these complex cellular processes under the basic concept of cellular glycomics/glycoproteomics. For further development of the system, however, production of a series of recombinant lectins is necessary, considering the history of restriction enzymes [18]. In this context, several groups have already shifted to

using recombinant lectins for improved sensitivity [70] and resolution [46]. If a recombinant lectin is established with a good bacterial expression system, its propagation to produce new lectins with a wider glycome coverage and a better cost effectiveness is feasible. An alternative approach is to synthesize "artificial" lectins equipped with boronic acid functionalized peptidyl ligands to carbohydrates [71]. In this regard, the lectin microarray enters its really creative phase, and is poised for further development.

References

1. Varki A (1993) Biological roles of oligosaccharides: all of the theories are correct. Glycobiology 3:97–130
2. Varki A, Lowe JB (2009) Biological roles of glycans. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME (eds) Essentials of glycobiology, 2nd edn. Cold Spring Harbor, New York
3. Laine RA (1994) A calculation of all possible oligosaccharide isomers both branched and linear yields 1.05 x 10^{12} structures for a reducing hexasaccharide: the isomer barrier to development of single-method saccharide sequencing or synthesis systems. Glycobiology 4:759–767
4. Hirabayashi J (2008) Concept, strategy and realization of lectin-based glycan profiling. J Biochem 144:139–147
5. Hirabayashi J, Kuno A, Tateno H (2011) Lectin-based structural glycomics: a practical approach to complex glycans. Electrophoresis 32:1118–1128
6. Ito H, Kuno A, Sawaki H, Sogabe M, Ozaki H, Tanaka Y, Mizokami M, Shoda JI, Angata T, Sato T, Hirabayashi J, Ikehara Y, Narimatsu H (2009) Strategy for glycoproteomics: identification of glyco-alteration using multiple glycan profiling tools. J Proteome Res 8:1358–1367
7. Narimatsu H, Narimatsu H, Sawaki H, Kuno A, Kaji H, Ito H, Ikehara Y (2010) A strategy for discovery of cancer glyco-biomarkers in serum using newly developed technologies for glycoproteomics. FEBS J 277:95–105
8. Angeloni S, Ridet JL, Kusy N, Gao H, Crevoisier F, Guinchard S, Kochhar S, Sigrist H, Sprenger N (2005) Glycoprofiling with micro-arrays of glycoconjugates and lectins. Glycobiology 15:31–41
9. Pilobello KT, Krishnamoorthy L, Slawek D, Mahal LK (2005) Development of a lectin microarray for the rapid analysis of protein glycopatterns. Chembiochem 6:985–989
10. Kuno A, Uchiyama N, Koseki-Kuno S, Ebe Y, Takashima S, Yamada M, Hirabayashi J (2005) Evanescent-field fluorescence-assisted lectin microarray: a new strategy for glycan profiling. Nat Methods 2:851–856
11. Zheng T, Peelen D, Smith LM (2005) Lectin arrays for profiling cell surface carbohydrate expression. J Am Chem Soc 127:9982–9983
12. Rosenfeld R, Bangio H, Gerwig GJ, Rosenberg R, Aloni R, Cohen Y, Amor Y, Plaschkes I, Kamerling JP, Maya RB (2007) A lectin array-based methodology for the analysis of protein glycosylation. J Biochem Biophys Methods 70:415–426
13. Tao SC, Li Y, Zhou J, Qian J, Schnaar RL, Zhang Y, Goldstein IJ, Zhu H, Schneck JP (2008) Lectin microarray: a powerful tool for glycan related biomarker discovery. Glycobiology 18:761–769
14. Hirabayashi J, Arata Y, Kasai K (2001) Glycome project: concept, strategy and preliminary application to *Caenorhabditis elegans*. Proteomics 1:295–303
15. Hirabayashi J (2004) Lectin-based structural glycomics: glycoproteomics and glycan profiling. Glycoconj J 21:35–40

16. Kuno A, Ikehara Y, Tanaka Y, Angata T, Unno S, Sogabe M, Ozaki H, Ito K, Hirabayashi J, Mizokami M, Narimatsu H (2011) Multilectin assay for detecting fibrosis-specific glycoalteration by means of lectin microarray. Clin Chem 57:48–56
17. Kuno A, Ikehara Y, Tanaka Y, Saito K, Ito K, Tsuruno C, Nagai S, Takahama Y, Mizokami M, Hirabayashi J, Narimatsu H (2011) LecT-Hepa: a triplex lectin-antibody sandwich immunoassay for estimating the progression dynamics of liver fibrosis assisted by a bedside clinical chemistry analyzer and an automated pretreatment machine. Clin Chim Acta 412:1767–1772
18. Gupta G, Surolia A, Sampathkumar SG (2010) Lectin microarrays for glycomic analysis. OMICS 14:419–436
19. Katrlík J, Svitel J, Gemeiner P, Kozár T, Tkac J (2010) Glycan and lectin microarrays for glycomics and medicinal applications. Med Res Rev 30:394–418
20. Vanderschaeghe D, Festjens N, Delanghe J, Callewaert N (2010) Glycome profiling using modern glycomics technology: technical aspects and applications. Biol Chem 391:149–161
21. Rakus JF, Mahal LK (2011) New technologies for glycomic analysis: toward a systematic understanding of the glycome. Annu Rev Anal Chem (Palo Alto Calif) 4:367–392
22. Goldstein IJ, Hughes RC, Monsigny M, Osawa T, Sharon N (1980) What should be called a lectin? Nature 285:66
23. Fukui S, Feizi T, Galustian C, Lawson AM, Chai W (2002) Nat Biotechnol 20:1011–1107
24. Blixt O, Head S, Mondala T, Scanlan C, Huflejt ME, Alvarez R, Bryan MC, Fazio F, Calarese D, Stevens J, Razi N, Stevens DJ, Skehel JJ, van Die I, Burton DR, Wilson IA, Cummings R, Bovin N, Wong CH, Paulson JC (2004) Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proc Natl Acad Sci U S A 101:17033–17038
25. Tateno H, Mori A, Uchiyama N, Yabe R, Iwaki J, Shikanai T, Angata T, Narimatsu H, Hirabayashi J (2008) Glycoconjugate microarray based on an evanescent-field fluorescence-assisted detection principle for investigation of glycan-binding proteins. Glycobiology 18:789–798
26. Angata T (2006) Molecular diversity and evolution of the Siglec family of cell-surface lectins. Mol Divers 10:555–566
27. Barondes SH, Castronovo V, Cooper DN, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, Leffler H, Liu F-T, Lotan R, Mercurio AM, Monsigny M, Pillai S, Poirer F, Raz A, Rigby PWJ, Rini JM, Wang JL (1994) Galectins: a family of animal beta-galactoside-binding lectins. Cell 76:597–598
28. Kuno A, Kato Y, Matsuda A, Kaneko MK, Ito H, Amano K, Chiba Y, Narimatsu H, Hirabayashi J (2008) Focused differential glycan analysis with the platform antibody-assisted lectin profiling for glycan-related biomarker verification. Mol Cell Proteomics 8:99–108
29. Ogata S, Muramatsu T, Kobata A (1975) Fractionation of glycopeptides by affinity column chromatography on concanavalin A-Sepharose. J Biochem 78:687–696
30. Cummings RD, Kornfeld S (1982) Characterization of the structural determinants required for the high affinity interaction of asparagine-linked oligosaccharides with immobilized Phaseolus vulgaris leukoagglutinating and erythroagglutinating lectins. J Biol Chem 257:11235–11240
31. Kasai K, Oda Y, Nishikata M, Ishii S (1986) Frontal affinity chromatography: theory for its application to studies on specific interactions of biomolecules. J Chromatogr 376:33–47
32. Nakamura-Tsuruta S, Uchiyama N, Hirabayashi J (2006) High-throughput analysis of lectin-oligosaccharide interactions by automated frontal affinity chromatography. Methods Enzymol 415:311–325
33. Tateno H, Nakamura-Tsuruta S, Hirabayashi J (2007) Frontal affinity chromatography: sugar-protein interactions. Nat Protoc 2:2529–2537
34. Uchiyama N, Kuno A, Tateno H, Kubo Y, Mizuno M, Noguchi M, Hirabayashi J (2008) Optimization of evanescent-field fluorescence-assisted lectin microarray for high-sensitivity detection of monovalent oligosaccharides and glycoproteins. Proteomics 8:3042–3050
35. Lee RT, Lee YC (2000) Affinity enhancement by multivalent lectin-carbohydrate interaction. Glycoconj J 17:543–551

36. Brewer CF, Miceli MC, Baum LG (2002) Clusters, bundles, arrays and lattices: novel mechanisms for lectin-saccharide-mediated cellular interactions. Curr Opin Struct Biol 12:616–623
37. Yamada M (2009) Lectin microarrays. In: Matson RS (ed) Microarray methods and protocols. CRC Press, Taylor & Francis Group, Boca Raton, pp 139–154
38. Koshi Y, Nakata E, Yamane H, Hamachi I (2006) A fluorescent lectin array using supramolecular hydrogel for simple detection and pattern profiling for various glycoconjugates. J Am Chem Soc 128:10413–10422
39. Abbas A, Linman MJ, Cheng Q (2011) New trends in instrumental design for surface plasmon resonance-based biosensors. Biosens Bioelectron 26:1815–1824
40. Tateno H, Kuno A, Hirabayashi J (2008) How to determine specificity: from lectin profiling to glycan profiling. In: Gabius H-J (ed) Sugar code: fundamentals of glycoscience. Willey, Weinheim, pp 247–259
41. Hirabayashi J (2009) In: Pierce M, Cummings R (eds) Handbook of glycomics. Elsevier, Amsterdam, pp 161–176
42. Kuno A, Itakura Y, Toyoda M, Takahashi Y, Yamada M, Umezawa A, Hirabayashi J (2008) Development of a data-mining system for differential profiling of cell glycoproteins based on lectin microarray. J Proteomics Bioinform 1:68–72
43. Kuno A, Matsuda A, Ikehara Y, Narimatsu H, Hirabayashi J (2010) Differential glycan profiling by lectin microarray targeting tissue specimens. Methods Enzymol 478:165–179
44. Tateno H, Kuno A, Itakura Y, Hirabayashi J (2010) A versatile technology for cellular glycomics using lectin microarray. Methods Enzymol 478:181–195
45. Toyoda M, Yamazaki-Inoue M, Itakura Y, Kuno A, Ogawa T, Yamada M, Akutsu H, Takahashi Y, Kanzaki S, Narimatsu H, Hirabayashi J, Umezawa A (2011) Lectin microarray analysis of pluripotent and multipotent stem cells. Genes Cells 16:1–11
46. Tateno H, Toyoda M, Saito S, Onuma Y, Ito Y, Hiemori K, Fukumura M, Nakasu A, Nakanishi M, Ohnuma K, Akutsu H, Umezawa A, Horimoto K, Hirabayashi J, Asashima M (2011) Glycome diagnosis of human induced pluripotent stem cells using lectin microarray. J Biol Chem 286:20345–20353
47. Saito S, Onuma Y, Ito Y, Tateno H, Toyoda M, Akutsu H, Nishino K, Chikazawa E, Fukawatase Y, Miyagawa Y, Okita H, Kiyokawa N, Shimma Y, Umezawa A, Hirabayashi J, Horimoto K, Asashima M (2011) Possible linkages between the inner and outer cellular states of human induced pluripotent stem cells. BMC Syst Biol 5(Suppl):S17
48. Matsuda A, Kuno A, Ishida H, Kawamoto T, Shoda J, Hirabayashi J (2008) Development of an all-in-one technology for glycan profiling targeting formalin-embedded tissue sections. Biochem Biophys Res Commun 370:259–263
49. Matsuda A, Kuno A, Kawamoto T, Matsuzaki H, Irimura T, Ikehara Y, Zen Y, Nakanuma Y, Yamamoto M, Ohkohchi N, Shoda J, Hirabayashi J, Narimatsu H (2010) *Wisteria floribunda* agglutinin-positive mucin 1 is a sensitive biliary marker for human cholangiocarcinoma. Hepatology 52:174–182
50. Gustafsson A, Sjöblom M, Strindelius L, Johansson T, Fleckenstein T, Chatzissavidou N, Lindberg L, Angström J, Rova U, Holgersson J (2011) *Pichia pastoris*-produced mucin-type fusion proteins with multivalent *O*-glycan substitution as targeting molecules for mannose-specific receptors of the immune system. Glycobiology 21:1071–1086
51. Huang W, Wang D, Yamada M, Wang LX (2009) Chemoenzymatic synthesis and lectin array characterization of a class of *N*-glycan clusters. J Am Chem Soc 131:17963–17971
52. Amano K, Chiba Y, Kasahara Y, Kato Y, Kaneko MK, Kuno A, Ito H, Kobayashi K, Hirabayashi J, Jigami Y, Narimatsu H (2008) Engineering of mucin-type human glycoproteins in yeast cells. Proc Natl Acad Sci U S A 105:3232–3237
53. Miyagawa S, Takeishi S, Yamamoto A, Ikeda K, Matsunari H, Yamada M, Okabe M, Miyoshi E, Fukuzawa M, Nagashima H (2010) Survey of glycoantigens in cells from α1-3galactosyltransferase knockout pig using a lectin microarray. Xenotransplantation 17:61–70

54. Suen KF, Turner MS, Gao F, Liu B, Althage A, Slavin A, Ou W, Zuo E, Eckart M, Ogawa T, Yamada M, Tuntland T, Harris JL, Trauger JW (2010) Transient expression of an IL-23R extracellular domain Fc fusion protein in CHO vs. HEK cells results in improved plasma exposure. Protein Expr Purif 71:96–102
55. Nakagawa T, Takeishi S, Kameyama A, Yagi H, Yoshioka T, Moriwaki K, Masuda T, Matsumoto H, Kato K, Narimatsu H, Taniguchi N, Miyoshi E (2010) Glycomic analyses of glycoproteins in bile and serum during rat hepatocarcinogenesis. J Proteome Res 9:4888–4896
56. Kondoh G, Watanabe H, Tashima Y, Maeda Y, Kinoshita T (2009) Testicular angiotensin-converting enzyme with different glycan modification: characterization on glycosylphosphatidylinositol-anchored protein releasing and dipeptidase activities. J Biochem 145:115–121
57. Togayachi A, Kozono Y, Ishida H, Abe S, Suzuki N, Tsunoda Y, Hagiwara K, Kuno A, Ohkura T, Sato N, Sato T, Hirabayashi J, Ikehara Y, Tachibana K, Narimatsu H (2007) Polylactosamine on glycoproteins influences basal levels of lymphocyte and macrophage activation. Proc Natl Acad Sci U S A 104:15829–15834
58. Kaneko MK, Kato Y, Kameyama A, Ito H, Kuno A, Hirabayashi J, Kubota T, Amano K, Chiba Y, Hasegawa Y, Sasagawa I, Mishima K, Narimatsu H (2007) Functional glycosylation of human podoplanin: glycan structure of platelet aggregation-inducing factor. FEBS Lett 581:331–336
59. Futakawa S, Nara K, Miyajima M, Kuno A, Ito H, Kaji H, Shirotani K, Honda T, Tohyama Y, Hoshi K, Hanzawa Y, Kitazume S, Imamaki R, Furukawa K, Tasaki K, Arai H, Yuasa T, Abe M, Arai H, Narimatsu H, Hashimoto Y (2011) A unique N-glycan on human transferrin in CSF: a possible biomarker for iNPH. Neurobiol Aging 33(8):1807–1815
60. Fry SA, Afrough B, Lomax-Browne HJ, Timms JF, Velentzis LS, Leathem AJ (2011) Lectin microarray profiling of metastatic breast cancers. Glycobiology 21:1060–1070
61. Kuwamoto K, Takeda Y, Shirai A, Nakagawa T, Takeishi S, Ihara S, Miyamoto Y, Shinzaki S, Ko JH, Miyoshi E (2010) Identification of various types of α2-HS glycoprotein in sera of patients with pancreatic cancer: possible implication in resistance to protease treatment. Mol Med Report 3:651–656
62. Sasaki N, Moriwaki K, Uozumi N, Noda K, Taniguchi N, Kameyama A, Narimatsu H, Takeishi S, Yamada M, Koyama N, Miyoshi E (2009) High levels of E4-PHA-reactive oligosaccharides: potential as marker for cells with characteristics of hepatic progenitor cells. Glycoconj J 26:1213–1223
63. Krishnamoorthy L, Bess JW Jr, Preston AB, Nagashima K, Mahal LK (2009) HIV-1 and microvesicles from T cells share a common glycome, arguing for a common origin. Nat Chem Biol 5:244–250
64. Hsu KL, Pilobello KT, Mahal LK (2006) Analyzing the dynamic bacterial glycome with a lectin microarray approach. Nat Chem Biol 2:153–157
65. Ebe Y, Kuno A, Uchiyama N, Koseki-Kuno S, Yamada M, Sato T, Narimatsu H, Hirabayashi J (2006) Application of lectin microarray to crude samples: differential glycan profiling of lec mutants. J Biochem 139:323–327
66. Tateno H, Uchiyama N, Kuno A, Togayachi A, Sato T, Narimatsu H, Hirabayashi J (2007) A novel strategy for mammalian cell surface glycome profiling using lectin microarray. Glycobiology 17:1138–1146
67. Yasuda M, Tateno H, Hirabayashi T, Iino T, Sako T (2011) Lectin microarray reveals binding profiles of *Lactobacillus casei* strains in a comprehensive analysis of bacterial cell wall polysaccharides. Appl Environ Microbiol 77:4539–4546
68. Pilobello KT, Slawek DE, Mahal LK (2007) A ratiometric lectin microarray approach to analysis of the dynamic mammalian glycome. Proc Natl Acad Sci U S A 104:11534–11539
69. Tonge R, Shaw J, Middleton B, Rowlinson R, Rayner S, Young J, Pognan F, Hawkins E, Currie I, Davison M (2001) Validation and development of fluorescence two-dimensional differential gel electrophoresis proteomics technology. Proteomics 1:377–396

70. Propheter DC, Hsu KL, Mahal LK (2011) Recombinant lectin microarrays for glycomic analysis. Methods Mol Biol 723:67–77
71. Bicker KL, Sun J, Lavigne JJ, Thompson PR (2011) Boronic acid functionalized peptidyl synthetic lectins: combinatorial library design, peptide sequencing, and selective glycoprotein recognition. ACS Comb Sci 13:232–243