Carbohydrate biomarkers for future disease detection and treatment

CHENG YunFeng[1], LI MinYong[2*], WANG ShaoRu[1], PENG HanJing[1], REID Suazette[1], NI NanTing[1], FANG Hao[2], XU WenFang[2] & WANG BingHe[1*]

[1]*Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA;*
[2]*Department of Medicinal Chemistry, School of Pharmacy, Shandong University, Jinan 250012, China*

Carbohydrates are considered as one of the most important classes of biomarkers for cell types, disease states, protein functions, and developmental states. Carbohydrate "binders" that can specifically recognize a carbohydrate biomarker can be used for developing novel types of site specific delivery methods and imaging agents. In this review, we present selected examples of important carbohydrate biomarkers and how they can be targeted for the development of therapeutic and diagnostic agents. Examples are arranged based on disease categories including (1) infectious diseases, (2) cancer, (3) inflammation and immune responses, (4) signal transduction, (5) stem cell transformation, (6) embryo development, and (7) cardiovascular diseases, though some issues cross therapeutic boundaries.

carbohydrates, biomarkers, imaging agents, boronolectins

1 Introduction

Carbohydrates, traditionally considered as bioenergy suppliers and structural components, have been found to have a wide variety of biological and physiological functions. Therefore, together with proteins and peptides, nucleic acids and oligonucleotides, and lipids, carbohydrates are considered one of the most important classes of biomacromolecules [1–4]. In addition, the relationship among these four classes of biomolecules is often intertwined. For example, protein glycosylation is very important to its conformation, transportation, function, and fate [5–16]; glycosylated lipids (glycolipids) are essential biomolecules [3, 17–22]; and nucleic acid glycosylation, whether synthetic [23] or natural [24–31], has recently been recognized as important in affecting its distribution and function. Because of all these properties, it is not surprising that carbohydrates are biomarkers for cell types, disease states, protein functions,

and developmental states [2, 32–40]. Recent years have seen a rapid increase in knowledge related to all these areas mentioned. Such advancements can largely be attributed to the development in new techniques, such as NMR and mass spectrometry, and molecular biology, and the ready availability of genomic information. As a result, recent advancements in glycobiology and glycomics have also opened new doors for the development of new therapeutics and imaging agents through carbohydrate recognition. Therefore, compounds that can specifically recognize a particular carbohydrate have very important applications [41–52]. They can be used as sensors for detection, diagnosis, and prognosis, as "blockers/inhibitors" for therapeutics development if the target carbohydrate is involved in pathogenesis, and as vectors for the targeted delivery of imaging and therapeutic agents. Critical to all these potential applications are two issues: (1) the identification of carbohydrate biomarkers and (2) the design and synthesis of "binders" that can specifically recognize the target biomarker with high affinity and specificity. In this review, we

*Corresponding author (email: wang@gsu.edu; mli@sdu.edu.cn)

present selected examples of important carbohydrate biomarkers and how they can be targeted for the development of therapeutic and diagnostic agents. It should be noted that there are many books on glycobiology and there are many review articles on how one can target carbohydrates for various applications [45, 51, 53–55] and it would be impossible to cover the entire topic in detail. Therefore, this article does not strive to be comprehensive. Instead, it is meant to be "tutorial," which we hope would help to generate more interests in carbohydrate recognition as a way for the development of new therapeutics and diagnostics. Carbohydrate recognition research impacts essentially all areas in which carbohydrates play a role. Examples include (1) infectious diseases, (2) cancer, (3) inflammation and immune responses, (4) signal transduction, (5) stem cell transformation, (6) embryo development, and (7) cardiovascular diseases. In the following sections, we divide the discussions based on disease categories though some issues cross therapeutic boundaries. In the last section, we present a few simple examples of how to target carbohydrate biomarkers for diagnostic and therapeutic applications.

2   Cancer

Among all pathologically relevant glycosylation changes, cancer is probably the most extensively studied. Even in normal cells, surface carbohydrate structures are known to be characteristic markers for different types of cells [56–61]. Transformations of normal to cancerous cells are often associated with the alteration of cell surface carbohydrates and the expression or over-expression of certain carbohydrates has been closely correlated with cancer [56–61]. Therefore, many carbohydrates are considered cancer associated antigens (CAA). Among all carbohydrate-based CAA, Globo H and the Tn antigen are probably the most common. It is known that the Tn antigen is found on the cell surface of over 90% of solid tumor [62]. The formation of the Tn antigen is because of deficiency of an enzyme named β-1,3-galactosyltransferase, which results in the incomplete conversion of the Tn antigen to the T antigen. Other important cancer-related carbohydrates include the sialylated carbohydrates. For example, sialyl Lewis X (sLe^x) has been shown to mediate lung colonization of B16 melanoma cells [63] and yet excessive sLe^x expression is shown to lead to rejection by natural killer cells [64]; serum sLe^x and cytokeratin 19 fragment are said to be predictive factors for recurrence in patients with stage I non-small cell lung cancer [65]; and sLe^x and sialyl Lewis a (sLe^a) have been shown to mediate adhesion of urothelial cancer cells to activated endothelium [66]. Changes in sLe^x and sLe^a levels in cancer have been attributed to both "neosynthesis" and "incomplete synthesis" of pathways involving sulfation or sialylation [67] and variations of enzyme levels can be directly correlated with certain changes in glycosylation [68]. With all these aberrant glycan expressions, "binders" that can recognize these carbohydrates will be very useful research tools, diagnostic agents, and possibly therapeutic agents [51].

In addition to cell surface carbohydrate biomarkers, post-/co-translational protein glycosylation often carries signatures of malignant transformations [69–93]. In terms of the biological significance of protein glycosylation, usually it is not a question of whether there is glycosylation; rather it is the glycosylation pattern that marks different pathological states including malignancy. For example, the glycosylation patterns of prostate specific antigen (PSA) from cancer cells in culture [94] and prostate cancer patients' tissue and sera [69, 93, 95] are different from that of normal prostate; human pancreatic RNase 1, a glycoprotein secreted mostly by pancreatic cells, has completely different oligosaccharide chains when produced from pancreatic tumor cells [39, 71, 96, 97]; pregnancy-related human chorionic gonadotropin (hCG) can be biomarkers for cancer, Down syndrome, and pregnancy failure depending on their glycosylation patterns [98, 99]; and specific glycosylation patterns of haptoglobin (Hp) and alpha-fetoprotein (AFP) have a much higher degree of correlation with cancer than the total Hp/AFP levels [100, 101]. In such cases, "binders" that can recognize glycosylation variations can be very useful diagnostic tools.

3   Infectious diseases

There are several ways in which carbohydrates are involved in the pathogenicity of infectious agents. In the case of viral infections, the human influenza virus is an excellent example. Flu viral infection involves sialic acid for binding to hemagglutinin and infection. After infection, the budding of mature viruses from infected cells involves the cleavage of sialic acid by neuraminidase in order for the virus to detach [102, 103]. Flu drug such as tamiflu functions by inhibiting neuraminidase and thus inhibit viral replication [104, 105].

In the case of human immunodeficiency virus (HIV), a critical protein, gp120, is glycosylated with polymannose [106, 107]. Infection of cells by HIV-1 requires the fusion of the viral membrane with cellular membrane [108]. This fusion is mediated by gp120 and gp41 along with cell surface receptors (CD4 and chemokine receptor) on the target cells [109]. Conceivably, agents that interact with gp120 may interfere with viral entry into target cells [110]. Binding to glycans on the viral envelope may also force the virus to delete a portion of its glycan shield, making the virus more susceptible to attack [111]. Along this line, several lectins have been studied for their anti-HIV activities. For example, cyanovirin-N (CV-N) is a 11kDa protein with 101 amino acids and has affinity for high-mannose glycans especially α-(1,2)-linked mannose oligomers [112, 113]. CV-N inactivates T-lymphocyte-tropic, laboratory strains of HIV type 1 and HIV type 2, as well as T-tropic, M-tropic and

dual tropic primary clinical isolates of HIV-1, presumably through inhibition of viral entry by blocking part of gp120. In CEM-SS cells, CV-N has an EC_{50} of 0.1 nM. As a control, treatment of uninfected CEM-SS cells in the presence of high concentrations (9000 nM) of CV-N did attenuate the lethal effect of the virus. Other lectins have also shown similar effect, presumably by binding to the polymannose portion of gp120. These include SVN (scytovirm), which has affinity for $\alpha(1,2)$-$\alpha(1,6)$-mannose trisaccharide units and can inhibit HIV infection in T-tropic laboratory strain HIV-1 in CEM-SS cells with an EC_{50} of 0.3 nM [114], and actinohivin, which has affinity for mannose-type glycans [115] and can inhibit T-cell and macrophage infection by HIV-1 in cell culture (IC_{50} of 60 to 700 nM). All such results indicate that the viral envelope glycans play an important role in their pathogenicity.

In bacterial pathogenicity, carbohydrates also play very important roles. One prominent example is lipopolysaccharides (LPS), also known as endotoxin, from the outer membrane of cell wall of Gram negative bacteria. One such example is *Pasteurella multocida*, an encapsulated, Gram-negative coccobacillus that causes a wide range of animal diseases including avian fowl cholera [116]. When infections by Gram-negative bacteria occur, LPS stimulate the immune system in an attempt to clear the bacteria and the infection that may result. The lipid A component of LPS is primarily responsible for this inflammatory response. As the infection proceeds, the presence of a large amount of LPS can result in an overproduction of inflammatory mediators that result in damage to tissues, septic shock, organ failure, and death [117]. LPS has also been shown to play an important role in the pathogenesis of *P. multocida*, in which modification of LPS structure negatively affects the viability of *P. multocida in vivo* [118]. In addition to functioning as toxins, carbohydrates can also be the target for bacterial recognition. For example, many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAc beta 1-4Gal found in some glycolipids [119]; *Pseudomonas aer*uginosa and *Pseudomonas cepacia* isolated from cystic fibrosis patients bind specifically to gan-gliotetraosylceramide (asialo GM1) and gangliotriaosylceramide (asialo GM2) [120]; the pili of *P. aeruginosa* strains PAK and PAO bind specifically to the carbohydrate sequence beta GalNAc(1-4)beta Gal found in glycosphingolipids asialo-GM1 and asialo-GM2 [121]; adherence of *P. aeruginosa* and *Candida albicans* to glycosphingolipid (Asialo-GM1) receptors is achieved by a conserved receptor-binding domain present on their adhesions [122]; lacto- and ganglio-series glycolipids are adhesion receptors for *Neisseria gonorr*hoeae [123]; and there are many other examples [124]. In addition to all these, serotyping of Gram-negative bacteria is primarily based on their LPS structures [125]. Because of the many ways that carbohydrates can affect bacteria (and fungi) infections, one can envision situations where artificial "binders" of the target carbohydrates can be used for detection and treatment of bacterial and fungal infections.

4 Inflammation and immune responses

Carbohydrates are involved in mediating inflammatory processes in many ways. The most widely known example is probably sLe^x-mediated white blood cell adhesion to infection/damaged sites through interactions with L-selectin. Recent studies unveiled that 6-O GlcNAc sulfate modification of the sLe^x tetrasaccharide is of importance in L-selectin activity in animal and in leukocyte invasion into different human tissues [126]. Furthermore, the *de novo* induction of endothelial sLe^x or its sulfated form through interactions with L-selectin is a common event in many organs, including thyroid gland, heart, skin and colon, thus, suggesting a crucial role for these glycans in the early-state induction of tissue inflammation [127].

The most prominent examples of carbohydrate-mediated immune responses originate from the ABO blood group antigens, which are entirely carbohydrate-based [128]. Figure 1 shows the structures of these antigens. The difference between the A and B blood types is due to their terminal galactosamine *N*-acetylation on red blood cells, while type
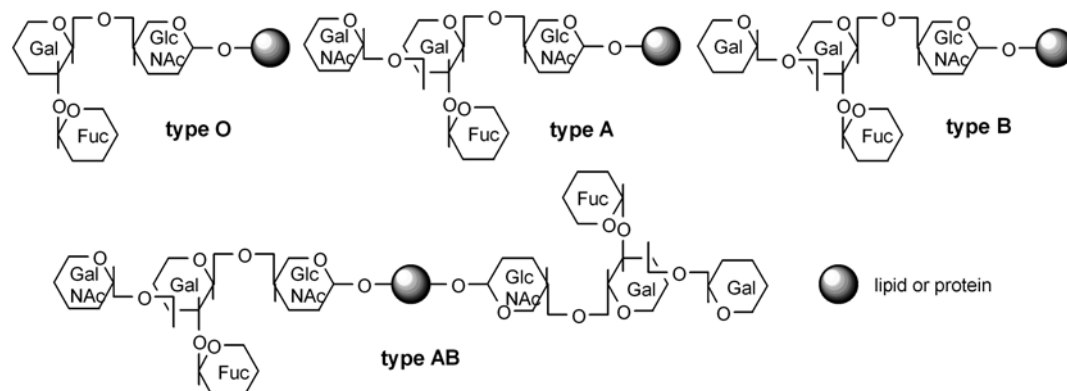
Figure 1 Diagram of the ABO blood group system.

O antigen lacks the terminal sugar. Therefore, persons of one blood type, such as A, would have natural antibodies against the other type (such as B) by targeting the difference in the terminal sugar structures. However, since O-type antigen does not have the terminal sugar and only has the common sugar structure, no antibody against the common core is produced by individuals of any blood type, which is the reason that O-type is "universal" [129].

Another type of carbohydrate-mediated immune response is the natural immunity in human against animal tissues. For example, pig cell surface has an antigen including the α-Gal-Gal moiety [130], which is absent on human cells. Therefore, humans have natural antibodies against α-Gal-Gal [131]. The acute immune response against α-Gal-Gal is a major hurdle in organ transplant using pig organs. A few years ago, genetically engineered pigs were produced [132, 133], which lack the α-Gal-Gal moiety and thus allow for organ transplant using pig organs to move one step closer to reality. In addition, targeted delivery of the α-Gal-Gal moiety has also been used to elicit immune response at a specific location or cell type [134].

5   Stem cell differentiation and embryo development

Fundamentally, stem cell differentiation and embryo development are the same. The process of transforming pluripotent stem cells into those of specialized functions is marked by different stages, which have characteristic stage-specific biomarkers. Such biomarkers are often carbohydrate-based [135–137] and can be targeted in "binder" development for various applications [138]. For example, Lewis X (Le^x) has been identified as a stage-specific embryonic antigen, which can be used for identifying and isolating specific cell types from heterogeneous populations [139, 140]. Along this line, it was reported that sorting SVZ cells on the basis of Le^x was a good strategy to enrich a restricted but highly proliferative neutral stem cell population [141]. Other examples of carbohydrate changes at various developmental stages include (1) enriched fucose incorporation into macromolecules was found on cell surface at the 8- to 16-cell stage in pre-implantation mouse embryos [142]; (2) sialic acid was found on the 12th day of incubation of metanephros while before that time only *N*-acetyl-D-glucosamine and alpha-D-mannose were found to exist ubiquitously [143]; (3) in chick embryo development, glycopeptides were found to be mainly *N*-linked on the 8-day and both O- and N-linked on the 16th day with increased sialylated small glycopeptide contents [144]; and (4) glycosylation of two identical polypeptide chains was found to be organ specific by analysis of chicken serum transferrin and ovotransferrin glycans [145]. There are also many other examples of these biomarkers, such as SSEA-1, -3, and -4 and tumor rejection antigen

(TRA)-1-60 and -1-81 [137, 139, 146]. All such results indicate that carbohydrate biomarkers play very important roles in the stem cell differentiation and embryo development. In stem cell research, one critical element is the ability to purify cells of the same differentiation stage and lineage. "Binders" that can recognize stage-specific biomarkers will be very useful for the purpose of separation and identifications. Along this line, aptamers can be very useful since their selection does not require prior knowledge of the nature and structure of the biomarkers in questions. There have been successful examples of selecting aptamers for cell-surface biomarkers using whole cells for the selection [138, 147–151]. For example, liver cancer-specific aptamers were developed by using whole live cells [152]. This study demonstrates that cell-based aptamer selection can specifically recognize cells from multiple cell lines, even for two cell lines with minor differences [152, 153]. In another study, a series of aptamers were selected for leukemia cells. These aptamers have dissociation constants (K_d) in the nano to pico-molar range. The selected aptamers could specifically recognize leukemia cells when mixed with normal human bone marrow aspirates. These aptamers were also used to identify cancer cells closely related to the target cell line in real clinical specimens [154]. Therefore, cell-based selection is a very promising method of developing specific molecular probes for cell-surface biomarker recognition. Similar approaches can be applied to stem cells for the selection of aptamers capable of recognizing stage-specific carbohydrate biomarkers. The recent development of boronic acid-modified DNA-based aptamers [155] allows for the selection of high affinity "binders" for glycoproteins with the ability to differentiate variations in glycosylation patterns and should tremendously enhance the chance of finding high affinity aptamers for such cell-surface carbohydrate biomarkers because of the intrinsic affinity of the boronic acid moiety for carbohydrates [51, 55]. Recently, the Schultz lab has developed a way of engineering boronic acid-modified protein [155, 156]. This will also be very useful in selecting artificial lectins for carbohydrate recognition.

6   Signal transduction

In the signal transduction area, the most prominent example is probably with glycosphingolipids (GSLs), which are involved in a whole host of activities. GSLs have the general structure of two hydrophobic tails (ceramide, consisting of Sph and fatty acid) and one carbohydrate chain, which are oriented perpendicularly. GSLs are held in the membrane by ceramide such that the carbohydrate chain is accessible to various ligands (antibodies, lectins, and complementary carbohydrates) (Figure 2) [157]. GSLs are an integral part of cellular membrane, which functions as antigens, receptors for microbial toxins, and mediators of cell adhesion and
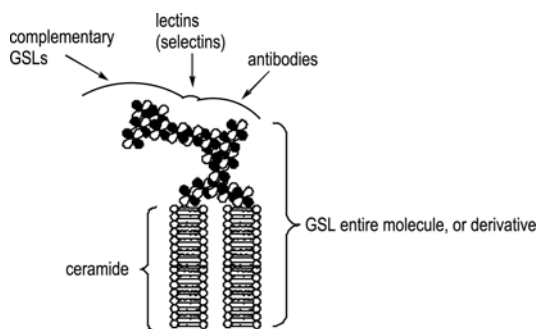
Figure 2 Structure of GSLs.

modulators of signal transduction. Many functions were attributed to the unique property of GSLs to form clusters. Glycosynapse refers to glycosphingolipids-enriched microdomain, and glycosynaptic domains, which control GSL-dependent or -modulated cell adhesion, growth, and motility, are formed by the interaction of GSL clusters and functional membrane proteins. Modulation of glycosynapse functions can lead to new strategies in cancer therapy, and elucidation of the molecular mechanism of interaction among components in glycosynaptic domain might shed lights on new possible approaches to disrupt or promote such interactions [119, 121, 122, 157–161].

Protein glycosylation is known to be involved in regulating signal transduction as well. For example, the dynamic glycosylation of serine or threonine residues on nuclear and cytosolic proteins by O-linked β-*N*-acetylglucosamine (O-GlcNAc) has been implicated in regulating protein-protein interaction(s) and/or protein function [162]; the Notch signaling pathway could be regulated by alterations of O-fucose structures [68]; O-fucose modification of Cripto is essential for Nodal-dependent signaling [68]; glycosylation of human CRLR at Asn123 is required for ligand binding and signaling [163]; elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation [164]; alternative *O*-glycosylation/*O*-phosphorylation of serine-16 regulates the activities of murine estrogen receptor beta [165]; NFkappaB activation is associated with its O-GlcNAcylation state under hyperglycemic conditions [166]; glucose deprivation stimulates O-GlcNAc modification of proteins through up-regulation of O-linked *N*-acetylglucosaminyltransferase [167, 168]; a mitotic GlcNAcylation/phosphorylation signaling complex alters the posttranslational state of the cytoskeletal protein vimentin [169]; *O*-glycosylation of Sp1 by insulin seems to enhance its nuclear recruitment and results in activation of CaM gene transcription [170]; and glycosylation of guanylyl cyclase C affects its conformation and functional ability [134]. There are many other reports of similar nature.

In 1993, a uniquely glycosylated base, β-D-glucopyranosyloxymethyluracil (also called base J), was identified in the nuclear DNA of *Trypanosoma brucei* [171]. This is a rare, if

not the only, case where DNA glycosylation is known to play a very important physiological role in signal transduction and gene silencing [24, 25, 27, 172–175]. Specifically, base J is said to be the first hypermodified base found in eukaryotic DNA in the telomeric repeats and to be present in all kinetoplastid flagellates analyzed and some unicellular flagellates closely related to trypanosomatids [176]. In one study, Sabatini and coworkers proposed a model in which chromatin remodeling by J Binding Protein (JBP2) regulates the initial sites of J synthesis within bloodstream form trypanosome DNA, with further propagation and maintenance of J by JBP1. Synthesis of J within telomeric DNA of *Trypanosoma brucei* correlates with the bloodstream-form-specific epigenetic silencing of telomeric variant surface glycoprotein genes involved in antigenic variation [177, 178]. All such results suggest that binding and modulation of glycans of various proteins, lipids, and even DNA may allow for regulation of signal transductions.

7 Cardiovascular diseases

In the cardiovascular area, the case of glycosylated hemoglobin (technically it should be glycated since the attachment of a sugar moiety is through chemical reactions) is most widely recognized. Specifically, glycated hemoglobin (GlcHb) level is a strong indicator of cardiovascular disease (CVD) risk in diabetic patients [179–184], who die of CVD at rates 2–4 times higher than those without diabetes [181]. *In vivo*, glycation, normally defined as a nonenzymatic reaction of glucose with amino groups in protein to form the Amadori product [185] (Scheme 1), is generally considered the first step in the Maillard reaction [186, 187]. Later stages of Maillard reaction lead to the formation of sugar-derived protein adducts and advanced glycation end-products (AGEs), which play an important role in the pathogenesis of chronic diseases [182]. The term GlcHb generally refers to the full spectrum of glycated hemoglobins, including those containing glycated valine (such as HbA_{1c}) and/or lysine residues. In a normoglycemic person, GlcHb accounts for ~24% of total hemoglobin at the end of the erythrocyte lifespan (~120 days), including 4% HbA_{1c} [182, 188]. However, elevated glucose concentration in diabetic patients significantly increases this glycation reaction. For example, some diabetic patients have about 2–4 fold increases in HbA_{1c} [189]. Although certain important questions regarding the prevention of CVD in diabetic patients remain unresolved, epidemiologic analyses suggested that a 1% increase in glycated hemoglobin elevates the risk for CVD by approximately 18% [181].

Another example of glycosylation's effect on cardiovascular diseases is with fibrinogen, which circulates in the blood as the precursor of fibrin, the structural component of blood clot. It plays a key role in platelet aggregation, the final step of the coagulation cascade, and is a major deter-
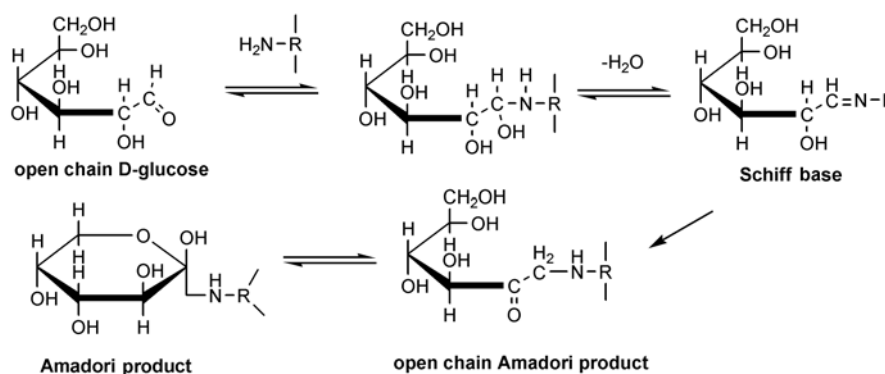
Scheme 1   Reaction between glucose and the amino group of a protein to form the Amadori product.

minant of plasma viscosity and erythrocyte aggregation [190]. Fibrinogen is a glycoprotein with a molecular weight of 340 kDa [191] and contains approximately 3% carbohydrate consisting of NeuAc, Gal, Man, and GlcNAc [192]. From amino acid sequence studies, it has been determined that carbohydrate is linked to Asn52 on the γ chain and Asn364 on the Bβ chain [193, 194]. The carbohydrate moieties play important roles in fibrinogen functions [195, 196] and are involved in the clotting process, although some contradictory results have been reported [197–199]: deglycosylated fibrinogen could accelerate polymerization and increase lateral aggregation of fibrin fiber [200]; the desialylated oligosaccharide chains of fibrinogen could potentially mediate plasma clearance via the hepatic galactose/ galactosamine binding lectin [201]; altered amounts of carbohydrate in fibrinogen are known to be directly related to certain types of dysfibrinogenemia [202]; extra negative charges (sialic acids) on fibrinogen can impair fibrin polymerization [203, 204], presumably due to the repulsive forces of the charges; and steric hindrance presented by additional glycan structures can also impair fibrin polymerization [203]. Fibrinogen-related abnormality is also implicated in other diseases such as hepatoma [202], pancreatic [33, 205] and other cancers [33, 206–213], tumor metastasis [208, 214–216], human hemopoietic cell proliferation [217], and embryogenesis and reproduction [218]. Some of these pathological changes are directly related to abnormal glycosylations [203, 204, 219–228]. In addition, there are many other situations where glycosylation affects the health of the cardiovascular system. For example, certain congenital disorder of glycosylation has been linked to intracranial hemorrhage [229].

The above examples are far from a complete list and are at best some highlights of examples where glycosylation variations play an important role in determining the biological outcome. However, even from this incomplete list, it can be seen that "binders" that can recognize a carbohydrate biomarker with high specificity and affinity would have great application potentials as sensors for concentration analysis, diagnostics, and possibly therapeutic agents. Below is a section describing the general concept of artificial "binder" design. It should be noted that there are several extensive reviews and book chapters on this subject. The following section is far from comprehensive. It can only be treated as some basic conceptual description. Readers are referred to published reviews [45, 51, 54, 55, 230, 231] and book chapters [232, 233] for more details.

8   Carbohydrate recognition

As discussed above, "binders" that can recognize certain carbohydrates with high affinity and specificity should be very useful research tools and potential diagnostic and therapeutic agents. However, achieving high affinity and high specificity carbohydrate recognition is not a trivial issue. In molecular recognition, antibody is often the gold standard. However, in the case of carbohydrates, raising antibodies is often a difficult task. More often than not, low affinity IgM antibodies are obtained. Recently, Boons [234] and colleagues have developed a remarkable synthetic approach to carbohydrate-protein conjugates, which elicits strong immune responses with very high titer. Naturally occurring protein "binders" include lectins [235, 236]. A few hundreds of lectins have been identified, about 60 of which are commercially readily available. Lectins all have certain specificity based on the overall topology and sugar compositions. For example, there are lectins that recognize polymannose structures and others that can recognize galactose connected to different structures. However, essentially all lectins have cross reactivity issues [237, 238].

Recent years have seen a great deal of interest in developing artificial lectins for various applications. These artificial lectins can generally be divided into two types: boronic acid-based and non-boronic acid based. The reason that boronic acid plays such an important role is that it can form tight complexes with diol-containing compounds. Sometimes single hydroxyl group interactions are sufficient in reinforcing highly specific macromolecular interactions. All these aspects have been discussed in detail in a recent re-

view and will not be duplicated here [51]. One thing that needs to be emphasized is the misperception that boronic acids only interact with linear diols and diols on five-membered ring. There are ample literature precedents demonstrating that boronic acid interactions with single hydroxyl groups or other nucleophiles/Lewis bases can also play a very important role in designing/selecting carbohydrate "binders" [51]. Since all boronic acid-containing carbohydrate "binders" function in a similar fashion as lectins, we have termed them as boronolectins [55]. Within the boronolectin category, there are small molecule- and macromolecule-based boronolectins including boronic acid-based carbohydrate sensors [51, 55, 239], nucleic acid-based boronolectins (NABL) [240], peptide boronolectins (PBL) [241–244], and protein boronolectins (PrBL) [155, 156]. Each of these categories is discussed briefly below.

8.1 Non-boronic acid based lectin mimics

In this approach, the design is mostly based on hydrophobic and hydrogen bond interactions for recognition. Though early efforts were mostly on recognition in organic solvent, recently there has been remarkable progress in making non-covalent carbohydrate binders which showed reasonably high affinity in water, especially those by the Davis lab [245–249]. One example comes from the successful design of such a binder (**1**, Figure 3) for all-equatorial disaccharides, such as D-cellobiose, with good affinity and selectivity in aqueous solution [248]. Receptor **1** has two building blocks: (1) a-meta-tertphenyl structure providing the "roof" and "floor" for hydrophobic interactions with carbohydrates and defining the length of the binding cavity; (2) isophthalamide units serving as pillars, with the potential to form hydrogen bonds, promote solubility, as well as prevent the cavity from collapsing. The binding constants between reporter 1 and selecting carbohydrates were determined to be
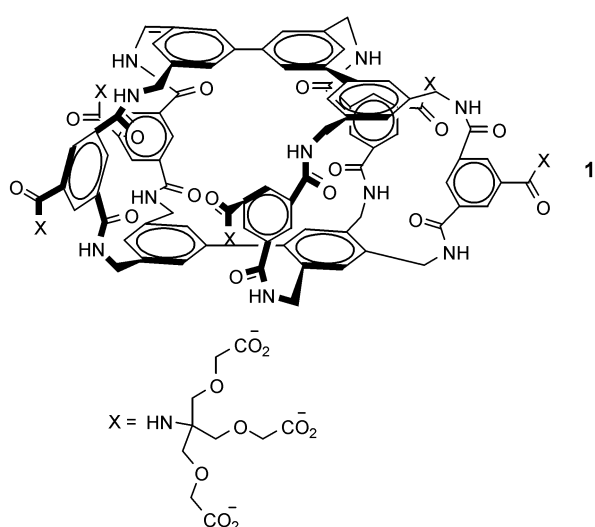
5–910 M^{-1} with good selectivity.

Recently, Boons and Davis reported another analog **2** (Figure 4) using the same design concept, with a biphenyl "roof" and "floor" as well as isophthalamide "pillars" [249]. In a previous study, receptor **2** showed weak bind for the β-glucosyl unit ($K_a = 9$ M^{-1} for D-glucose (α/β = 40/60) and 5 M^{-1} for D-glucose (α/β = 72/28); $K_a = 27$ M^{-1} for methyl-β-glucoside and 7 M^{-1} for α-anomer) [247]. In an expanded study, **2** was recently reported as a strong and selective receptor for β-GlcNAc. The apparent binding constant between GlcNac β-OMe (Figure 4) and receptor **2** was determined to be 630 M^{-1}, which competes well with one lectin (WGA, $K_a = 730$ M^{-1}). Furthermore, receptor **2** has higher selectivity for GlcNac β-OMe than for the α-anomer and other N-acetylaminosugars.

8.2 Small molecule boronolectins (SBL)

Among all the carbohydrate sensors, boronic acid emerges as the most commonly used functional group for recognition, due to its strong interactions with diols [51–55, 231, 239, 250–263], aminoalcohols [264–266], α-aminoacids [267], α-hydroxyl acids [268–271], alcohols [55, 233, 272–287] as well as cyanide [288, 289] and fluoride [290–294]. The intrinsic ability for boronic acids to interact with nucleophiles is described in Scheme 2. The boron atom has only 6 valence electrons in its trigonal neutral form, which makes boronic acid a Lewis acid and capable of strong interactions with Lewis bases/nucleophiles. As a result, the boronic acid (**3**, Scheme 2) group is able to react with a protic solvent and convert to its anionic tetrahedral form (**4**). Both **3** and **4** are able to form tight and reversible complexes with 1,2- and 1,3-substituted Lewis base donors such as hydroxyl, amino, and carboxylate groups. Several factors such as

Figure 3 Structure of all-equatorial disaccharide receptor **1**.
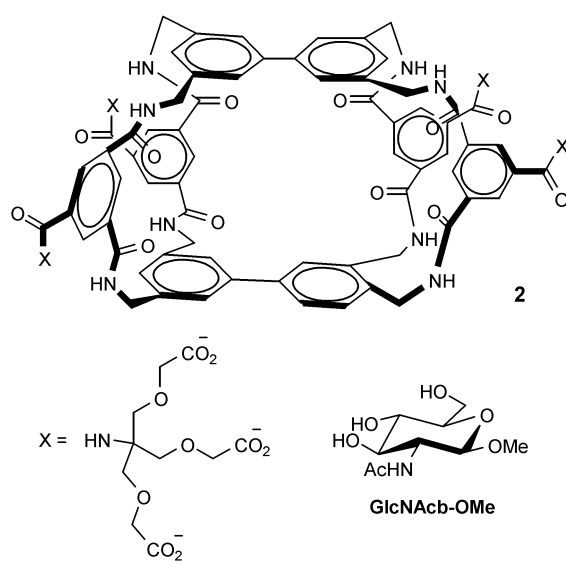

Figure 4 Structures of reporter **2** and GlcNAcβ-OMe.
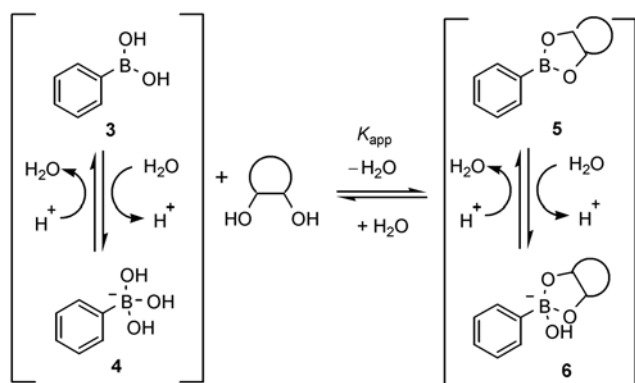
Scheme 2 Overall binding equilibria of phenylboronic acid with a diol.

Figure 5 Structures of boronic acids sensor **7**.

O-C-C-O dihedral angle, pK_a values of the diol, buffer, ionic strength, as well as solvent all affect the complexation [55, 295, 263, 296].

Small molecule boronolectins (SBLs) have drawn a great deal of attentions in carbohydrate biomarker recognition and targeting [51]. Several recent reviews and research papers comprehensively summarize the use of boronic acids in sensor designs for carbohydrates [51, 54, 55, 231, 239] fluoride [290–294], cyanides [288, 289], as well as in-depth discussions of factors [55, 263, 296] that should be considered along the line of designing such sensors. Besides, there have also been quite a few recent reviews [51, 53] and research papers [44, 156, 297–312] on boronic acids that change fluorescent properties upon binding to a nucleophilic analyte or pH changes. Readers are referred to the above-referenced papers for details. Below, several representative examples are discussed to highlight applications. One such example comes from the Wang lab, which developed an anthracene-based diboronic acid compound for sialyl Lewis X (sLe^x). The anthracene-based boronic acid was first developed by the Shinkai group [253], whose fluorescence can be quenched by nitrogen lone pair electrons and recovered if lone pair electrons are masked through protonation after binding with carbohydrates [140]. By taking advantage of this, Wang and co-workers successfully designed and synthesized a series of anthracene-based diboronic acid compounds with different linkers, rigidity, and spatial orientation for recognition of sLe^x on cell surface [254, 313]. Among all the designed compounds, sensor **7** (Figure 5) stands out as an excellent receptor, which was able to label sLe^x-expressing cells at low concentrations (0.5 μM) without cross-reactivity to Ley-expressing cells. This represents the first example of a small organic molecule used to fluorescently label cells based on the cell-surface carbohydrate structures. Further development along this line could lead to a number of small molecule boronolectins for labeling, drug delivery, and selective imaging applications.

In another example, the Hall lab in 2006 reported an ortho-hydroxymethyl phenylboronic acid (**8**, Scheme 3), which
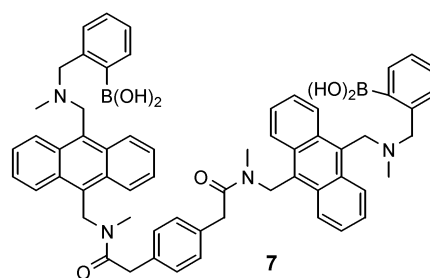
competes well with the well-established dialkylamino (Wulff-type) analogs with better binding affinity and solubility [272, 306]. The most significant finding of compound **8** was the weak but encouraging binding with model glycopyranosides. In aqueous media at physiological pH (7.4), the apparent binding constant between **8** and methyl α-D-glucopyranoside was determined to be 22 M^{-1}, which was slightly lower than that for glucose ($K_a = 36$ M^{-1}). This system has been used by the Hindsgaul lab for the detection of the terminal glycosylation of a glycoprotein with the aid of colored ortho-hydroxymethyl phenylboronic acid conjugates [314].
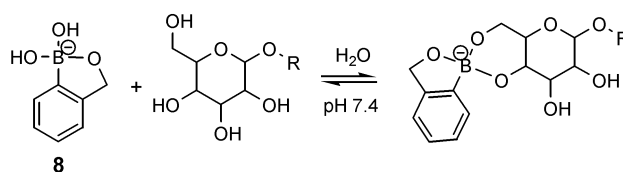
Scheme 3 Binding between ortho-hydroxymethyl phenylboronic acid **8** and glycoconjugates.

8.3 NABL

Another area developed recently in carbohydrate sensor design is nucleic acid-based boronolectins (NABL). The Wang lab is working on incorporating boronic acid-modified thymidine into DNA for aptamer selection work for glycoproteins. A boronic acid-labeled thymidine triphosphate (BTTP **9**, Figure 6) was successfully synthesized [46]. It has been demonstrated that DNA polymerase can recognize BTTP as a substrate and the boronic acid-labeled DNA as a template, which are critical issues for aptamer selection work. One challenging task of carbohydrate recognition is the differentiation of glycosylation patterns of a glycoprotein. By taking advantage of the general aptamer selection method developed about 18 years by the labs of Szostak [315], Joyce [316], and Gold [317], as well as the intrinsic affinity of boronic acids for carbohydrates, it is reasonable to believe that incorporation of a boronic acid into the DNA aptamer would allow for the selection process to gravitate toward the glycosylation site and therefore allow for differentiation of glycosylation patterns.
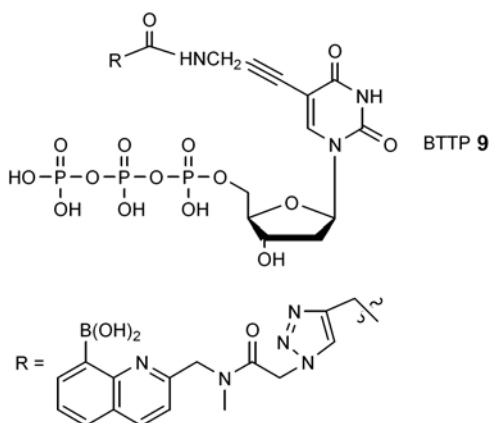
Figure 6   Structure of BTTP **9**.

Anslyn and co-workers invented a method of using aptamers to fine-tune the selectivity of boronic acid-based synthetic small molecule receptors [240]. Specifically, organic receptor **10** (Figure 7) was immobilized on glyoxal agarose beads through reductive amination to form immobilized receptor **11**, which was in complexation with tartrate as the target for the selection. Control compound **12**, incapable of binding tartrate, was used for counter selection. The progress of the selection was monitored by incorporating a radiolabel into the RNA pool. By this approach, the author successfully selected an aptamer with good selectivity for the complex between bis-boronic acid receptor **11** and tartrate (>14 for tartrate, $K_d = 2.1 \times 10^{-4}$ M and $K_d < 3 \times 10^{-3}$ M for citrate in 20% MeOH). One explanation for the selectivity is that aptamer might form a pocket more precisely to accommodate the receptor-tartrate complex, while excluding citrate via steric interactions or charge repulsion. This work can lead to the applications for improving the specificity of synthetic receptors and the development of biosensors for small organic analytes.

8.4   PBL

In addition to nucleic acid-based boronolectins (NABL), there have been efforts in making peptide boronolectins (PBL) for the same purpose [241–244]. For examples, the
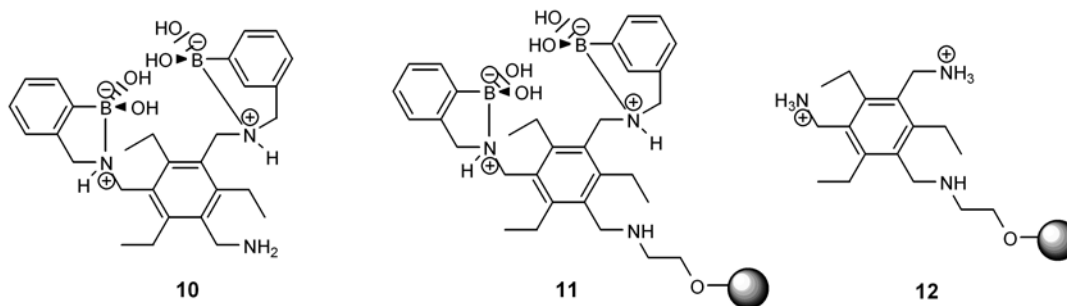
Anslyn lab group developed a chemosensor array of PBL for saccharides, saccharide derivatives, and even sucralose in a real world beverage sample with good water solubility and high sensitivity at physiological pH [241]. The Duggan lab prepared solid-supported PBL derived from 4-borono-L-phenylalanine and studied their affinity for alizarin [242]. The Hall lab developed a general solid-phase approach to the synthesis and isolation of functionalized boronic acids, which should be very useful in combinatorial library synthesis of boronic acid-based carbohydrate sensors [243]. The Lavigne lab reported their PBL sensors for glycomics recognition with the potential for cancer diagnosis [244]. Along a similar line, Hall and coworkers have also established a prototypic bead-supported split-pool library of triamine-derived triboronic acid receptors [318].

8.5   PrBL

Recently, the Schultz lab successfully demonstrated the feasibility of adding the boronate functionality to the genetic code of *E. coli* in high yield and efficiency [155, 156]. Specifically, *p*-boronophenylalanine (**13**, Figure 8) was incorporated into proteins. The intrinsic affinity of the boronic acid group allows for the selection of high affinity PrBL. In addition, this method has the potential to be used for purification of native protein sequences in a one-step scarless affinity procedure.

8.6   Carbohydrate labeling in living systems

Recently, the Bertozzi lab has developed labeling approaches to probing the functions of glycans in living system and application of these tools to studies of glycobiology such as the identification of novel glycan-based tumor biomarkers [319–334].

The general principle relies on the availability of a toolkit of "azido sugars" for metabolically labeling different classes of glycans. These azido sugars can be recognized by their respective processing/incorporation enzymes and can be used for tagging via two bio-orthogonal reactions: Staudinger ligation (Figure 9) and [2+3] cycloaddition (click reaction) (Figure 10) [335–337]. Along this line, a

Figure 7   Structures of compounds **10–12**.
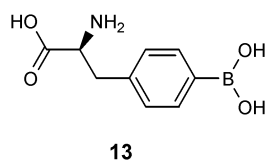
Figure 8   Structure of *p*-boronophenylalanine **13**.
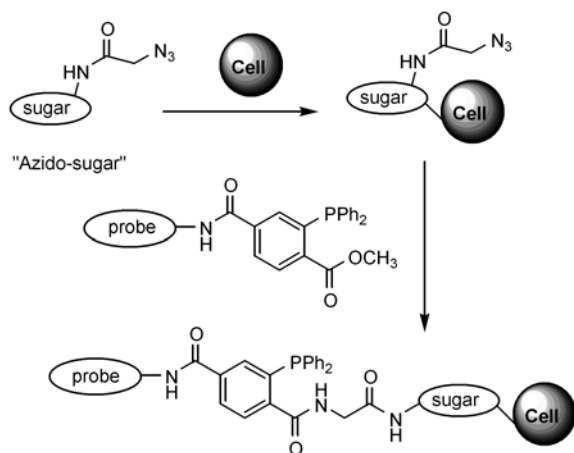
Figure 9   Incorporation of "azido-sugar" into cell and subsequent Staudinger ligation.
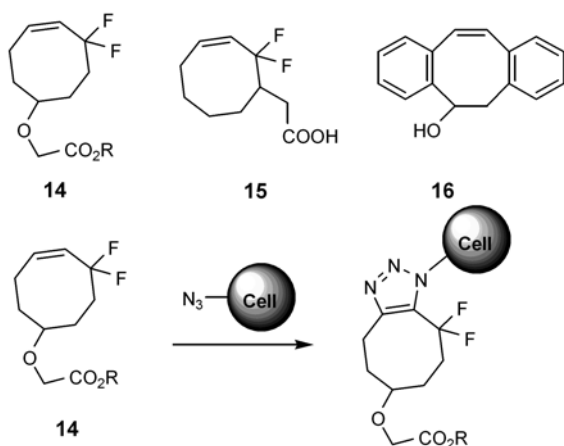
Figure 10   Cyclooctynes for strain-promoted cycloadditions with azides in living systems.

series of successful applications have been published [320, 323, 325, 330, 332, 334]. For examples, the azide-bearing ManNAz has been employed to visualize sialic acids in live cells using phosphine and cyclooctyne reagents [320, 334]; the same strategy was further developed to perform *in vivo* imaging [332]; simultaneous imaging of the expression of two different glycans was also achieved by introducing two chemical reporters (ketone and azide) into sialic acid and *N*-acetylgalactosamine (GalNAc) residues, which allowed controlled introduction of two fluorescent probes to monitor glycan expression and dynamics [323]; and a FRET-based fluorogenic phosphine for live-cell imaging with Staudinger

ligation was also exploited recently [325].

In using the Huisgen [3+2] dipolar cycloaddition with alkynes, the Bertozzi lab developed strained alkynes (**14** and **15**, Figure 10) for copper-free cycloaddition [320, 322, 324, 326, 328]. The Boons lab subsequently also reported their own strained alkynes for copper-free cycloaddition with azido compounds (**16**, Figure 10) [338]. The availability of such alkynes for copper free cycloaddition is especially important in live cell imaging because of the toxicity issues of copper in living systems. With this technique, the dynamics of glycan trafficking and a population of sialogly-coconjugates with unexpectedly rapid internalization kinetics were studied. In another example, **14** was used for the noninvasive imaging of glycans in live developing zebrafish [327]. In this experiment, zebrafish embryos were first treated with an unnatural sugar to metabolically label their cell-surface glycans with azides and then visualized by using an *in vivo* Cu-free click reaction with fluorophore-conjugated **14**. The Bertozzi group also performed a spatio-temporal analysis of glycan expression and trafficking and identified patterns by using a multicolor detection strategy [324].

In conclusion, carbohydrates serve very important biological functions in a wide variety of processes. "Binders" that can specifically recognize a carbohydrate biomarker can be used for site specific delivery of therapeutic and imaging agents. Specific recognition of carbohydrates that mediate pathological processes has the potential to be used as a way to develop novel types of therapeutic agents. Recent years have seen a tremendous amount of work in developing carbohydrate "binders," with boronolectins showing special promises. It is almost a certainty that new diagnostic and therapeutic agents will come out in the not too distant future that rely on carbohydrate recognition.

1   Timmer MSM, Stocker BL, Seeberger PH. Probing glycomics. *Curr Opin Chem Biol*, 2007, 11: 59–65
2   Varki A. Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins. *Nature*, 2007, 446, 1023–1029
3   Hecht SM. *Bioorganic Chemistry: Carbohydrates*. New York: Oxford University Press, 1999
4   Fukuda M, Hindsgaul O. *Cell Surface Carbohydrates: Cell-type Specific Expression*. New York: Oxford University Press, 2000
5   Andreotti AH, Kahne D. Effects of glycosylation on peptide backbone conformation. *J Am Chem Soc*, 1993, 115, 3352–3353
6   Bosques CJ, Tschampel SM, Woods RJ, Imperiali B. Effects of glycosylation on peptide conformation: a synergistic experimental and computational study. *J Am Chem Soc*, 2004, 126: 8421–8425
7   Brisson JR, Uhrinova S, Woods RJ, vanderZwan M, Jarrell HC, Paoletti LC, Kasper DL, Jennings HJ. NMR and molecular dynamics studies of the conformational epitope of the type III group B streptococcus capsular polysaccharide and derivatives. *Biochemistry*, 1997,

36: 3278–3292

8   Collier E, Carpentier JL, Beitz L, Caro LHP, Taylor SI, Gorden P. Specific glycosylation site mutations of the insulin-receptor alpha-subunit impair intracellular-transport. *Biochemistry*, 1993, 32: 7818–7823

9   Dennis JW, Granovsky M, Warren CE. Protein glycosylation in development and disease. *Bioessays*, 1999, 21: 412–421

10   Lis H, Sharon N. Protein glycosylation–structural and functional-aspects. *Eur J Biochem*, 1993, 218: 1–2

11   Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of igg associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nat Med*, 1995, 1: 237–243

12   Martinez-Maza R, Poyatos I, Lopez-Corcuera B, Nunez E, Gimenez C, Zafra F, Aragon C. The role of *N*-glycosylation in transport to the plasma membrane and sorting of the neuronal glycine transporter GLYT2. *J Biol Chem*, 2001, 276: 2168–2173

13   Montreuil J, Vliegenthart JFG, Schachter H. *Glycoproteins and Disease*. Amsterdam: Elsevier, 1996, Vol 30

14   Szymanski CM, Wren BW. Protein glycosylation in bacterial mucosal pathogens. *Nat Rev Microbiol*, 2005, 3: 225–237

15   Woods RJ, Dwek RA, Edge CJ, Fraserreid B. Molecular mechanical and molecular dynamical simulations of glycoproteins and oligosaccharides. 1. Glycam-93 parameter development. *J Phys Chem*, 1995, 99: 3832–3846

16   Xiao H, Cai GP, Liu MY. Fe^{2+}-catalyzed non-enzymatic glycosylation alters collagen conformation during AGE-collagen formation *in vitro*. *Arch Biochem Biophys*, 2007, 468: 183–192

17   Bucala R, Model P, Cerami A. Modification of DNA by reducing sugars: a possible mechanism for nucleic acid aging and age-related dysfunction in gene expression (nonenzymatic browning/Maillard reaction). *Proc Natl Acad Sci USA*, 1984, 81: 105–109

18   Vance DE, Vance J. *Biochemistry of Lipids, Lipoproteins and Membranes*. Amsterdam: Elsevier, 1991, Vol 20

19   Griffitts JS, Haslam SM, Yang TL, Garczynski SF, Mulloy B, Morris H, Cremer PS, Dell A, Adang MJ, Aroian RV. Glycolipids as receptors for bacillus thuringiensis crystal toxin. *Science*, 2005, 307: 922–925

20   Kochetkov NK, Smirnova GP. Glycolipids of marine invertebrates. *Adv Carbohydr Chem Biochem*, 1986, 44: 387–438

21   Lockhoff O. Glycolipids as immunomodulators: synthesis and properties. *Angew Chem Int Ed*, 1991, 30: 1611–1620

22   Wiegandt H. *Glycolipids*. Amsterdam: Elsevier, 1985

23   Lee AT. The Nonenzymatic glycosylation of dna by reducing sugars *in vivo* may contribute to dna damage associated with aging. *Age*, 1987, 10: 150–155

24   van Leeuwen F, Taylor MC, Mondragon A, Moreau H, Gibson W, Kieft R, Borst P. Beta-D-glucosyl-hydroxymethyluracil is a conserved DNA modification in kinetoplastid protozoans and is abundant in their telomeres. *Proc Natl Acad Sci USA*, 1998, 95: 2366–2371

25   Simpson L. A Base Called J. *Proc Natl Acad Sci USA*, 1998, 95, 2037–2038

26   Bright RA, Ross TM, Subbarao K, Robinson HL, Katz JM. Impact of glycosylation on the immunogenicity of a DNA-based influenza H5HA Vaccine. *Virology*, 2003, 308: 270–278

27   DiPaolo C, Kieft R, Cross M, Sabatini R. Regulation of trypanosome DNA glycosylation by a SWI2/SNF2-like protein. *Molecular Cell*, 2005, 17: 441–451

28   Mironova R, Niwa T, Handzhiyski Y, Sredovska A, Ivanov I. Evidence for non-enzymatic glycosylation of escherichia coli chromosomal DNA. *Mol Microbiol*, 2005, 55: 1801–1811

29   Reiter WD, Vanzin GF. Molecular genetics of nucleotide sugar interconversion pathways in plants. *Plant Mol Biol*, 2001, 47: 95–113

30   Rupprath C, Schumacher T, Elling L. Nucleotide deoxysugars: essential tools for the glycosylation engineering of novel bioactive compounds. *Curr Med Che*, 2005, 12: 1637–1675

31   Seifert GJ. Nucleotide Sugar interconversions and cell wall biosynthesis: how to bring the inside to the outside. *Curr Opin Plant Biol*, 2004, 7: 277–284

32   Babino A, Oppezzo P, Bianco S, Barrios E, Berois N, Navarrete H, Osinaga E. Tn antigen is a pre-cancerous biomarker in breast tissue and serum in *N*-nitrosomethylurea-induced rat mammary carcinogenesis. *Int J Cancer*, 2000, 86: 753–759

33   Bloomston M, Zhou JX, Rosemurgy AS, Frankel W, Muro-Cacho CA, Yeatman TJ. Fibrinogen Gamma overexpression in pancreatic cancer identified by large-scale proteomic analysis of serum samples. *Cancer Res*, 2006, 66: 2592–2599

34   Kyselova Z, Mechref Y, Al Bataineh MM, Dobrolecki LE, Hickey R J, Vinson J, Sweeney CJ, Novotny MV. Alterations in the serum glycome due to metastatic prostate cancer. *J Proteome Res*, 2007, 6: 1822–1832

35   Sahni A, Khorana AA, Baggs RB, Peng H, Francis CW. FGF-2 binding to fibrin(ogen) is required for augmented angiogenesis. *Blood*, 2006, 107: 126–131

36   Visser CE, Brouwersteenbergen JJE, Betjes MGH, Koomen GCM, Beelen RHJ, Krediet RT. Cancer antigen-125: a bulk marker for the mesothelial mass in stable peritoneal–dialysis patients. *Nephrology Dialysis Transplantation*, 1995, 10: 64–69

37   Xu Y, Shen ZZ, Wiper DW, Wu MZ, Morton RE, Elson P, Kennedy AW, Belinson J, Markman M, Casey G. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. *J Am Med Assoc*, 1998, 280: 719–723

38   Nimrichter L, Gargir A, Gortler M, Altstock RT, Shtevi A, Weisshaus O, Fire E, Dotan N, Schnaar RL. Intact Cell adhesion to glycan microarrays. *Glycobiol*, 2004, 14: 197–203

39   Peracaula R, Royle L, Tabares G, Mallorqui-Fernandez G, Barrabes S, Harvey DJ, Dwek RA, Rudd PM, de Llorens R. Glycosylation of human pancreatic ribonuclease: differences between normal and tumor states. *Glycobiol*, 2003, 13: 227–244

40   Tabares G, Radcliffe CM, Barrabes S, Ramirez M, Aleixandre RN, Hoesel W, Dwek RA, Rudd PM, Peracaula R, de Llorens R. Different glycan structures in prostate-specific antigen from prostate cancer sera in relation to seminal plasma PSA. *Glycobiology*, 2006, 16: 132–145

41   Boraston AB, Bolam DN, Gilbert HJ, Davies GJ. Carbohydrate-binding modules: fine-tuning polysaccharide recognition. *Biochem J*, 2004, 382: 769–781

42   Gao SH, Wang W, Wang BH. Building fluorescent sensors for carbohydrates using template-directed polymerizations. *Bioorg Chem*, 2001, 29: 308–320

43   Gao XM, Zhang YL, Wang BH. A highly fluorescent water-soluble boronic acid reporter for saccharide sensing that shows ratiometric UV changes and significant fluorescence changes. *Tetrahedron*, 2005, 61: 9111–9117

44   Gao XM, Zhang YL, Wang BH. Naphthalene-based water-soluble fluorescent boronic acid isomers suitable for ratiometric and off-on sensing of saccharides at physiological pH. *New J Chem*, 2005, 29: 579–586

45   James TD, Shinkai S. Artificial receptors as chemosensors for carbohydrates. In: Penadess, Ed. *Host-Guest Chemistry*. Berlin: Springer-Verlag, 2002, Vol 218. 159–200

46   Lin N, Yan J, Huang Z, Altier C, Li MY, Carrasco N, Suyemoto M, Johnston L, Wang SM, Wang Q, Fang H, Caton-Williams J, Wang BH. Design and synthesis of boronic-acid-labeled thymidine triphosphate for incorporation into DNA. *Nucleic Acids Res*, 2007, 35: 1222–1229

47   Lis H, Sharon N. Lectins: carbohydrate-specific proteins that mediate cellular recognition. *Chem Rev*, 1998, 98: 637–674

48   Wang JF, Jin S, Wang BH. A new boronic acid fluorescent reporter that changes emission intensities at three wavelengths upon sugar binding. *Tetrahedron Lett*, 2005, 46: 7003–7006

49   Weis WI, Drickamer K. Structural basis of lectin-carbohydrate recognition. *Annu Rev Biochem*, 1996, 65: 441–473

50   Yang WQ, Lin L, Wang BH. A new type of boronic acid fluorescent reporter compound for sugar recognition. *Tetrahedron Lett*, 2005, 46: 7981–7984

51   Jin S, Cheng YF, Reid S, Li M, Wang B. Carbohydrate recognition by boronolectins, small molecules, and lectins. *Med Res Rev*, 2009,

doi: 10.1002/med.20155

52 Striegler S. Selective carbohydrate recognition by synthetic receptors in aqueous solution. *Curr Org Chem*, 2003 7: 81–102

53 Cao HS, Heagy MD. Fluorescent chemosensors for carbohydrates: a decade's worth of bright spies for saccharides in review. *J Fluorescence*, 2004, 14: 569–584

54 Wang W, Gao X, Wang BH. Boronic acid-based sensors for carbohydrates. *Curr Org Chem*, 2002, 6: 1285–1317

55 Yan J, Fang H, Wang B. Boronolectins and fluorescent boronolectins-an examination of the detailed chemistry issues important for their design. *Med Res Rev*, 2005, 25: 490–520

56 Fukuda M. Cell surface carbohydrates in hematopoietic cell differentiation and malignancy. In: Fukuda M, Ed. *Cell Surface Carbohydrates and Cell Development*. Boca Raton: CRC Press, 1992. 127–160

57 Fukuda M. *Cell Surface Carbohydrates and Cell Development*. Boca Raton: CRC Press, 1992

58 Fukuda M. Cell surface carbohydrates: cell-type specific expression. In: Fukuda M, Hindsgaul O, Eds. *Molecular Glycobiology*. New York: Oxford University Press, 1994. 1–52

59 Fukuda M, Hindsgaul O. *Molecular Glycobiology*. New York: Oxford University Press, 1994. 1–52

60 Fukuda M. Possible roles of tumor-associated carbohydrate antigens. *Cancer Res*, 1996, 56, 2237–2244

61 Fukuda M, Hindsgaul O. *Molecular and Cellular Glycobiology*. New York: Oxford University Press, 2000

62 Springer GF. T and Tn pancarcinoma markers–autoantigenic adhesion molecules in pathogenesis, prebiopsy carcinoma-detection, and long-term breast-carcinoma immunotherapy. *Crit Rev Oncog*, 1995, 6: 57–85

63 Zhang J, Nakayama J, Ohyama C, Suzuki M, Suzuki A, Fukuda M, Fukuda MN. Sialyl Lewis X-dependent lung colonization of B16 melanoma cells through a selectin-like endothelial receptor distinct from E- or P-selectin. *Cancer Res*, 2002, 62: 4194–4198

64 Ohyama C, Tsuboi S, Fukuda M. Dual roles of sialyl Lewis X oligosaccharides in tumor metastasis and rejection by natural killer cells. *Embo J*, 1999, 18: 1516–1525

65 Mizuquchi S, Nishiyama N, Iwata T, Nishida T, Izumi N, Tsukioka T, Inoue K, Suehiro S. Serum sialyl Lewis X and cytokeratin 19 fragment as a predictive factors for recurrence in patients with stage I non-small cell lung cancer. *E J Cancer Suppl*, 2007, 6554

66 Fujii Y, Yoshida M, Chien LJ, Kihara K, Kageyama Y, Yasukochi Y, Oshima H. Significance of carbohydrate antigen sialyl-Lewis X, sialyl-Lewis A, and possible unknown ligands to adhesion of human urothelial cancer cells to activated endothelium. *Urol Int*, 2000, 64: 129–133

67 Kannagi R. Molecular mechanism for cancer-associated induction of sialyl Lewis X and sialyl Lewis A expression–the warburg effect revisited. *Glycoconjugate J*, 2003, 20: 353–364

68 Haltiwanger RS. Regulation of signal transduction pathways in development by glycosylation. *Curr Opin Struct Biol, England*, 2002, 12: 593–598

69 Tabares G, Radcliffe CM, Barrabes S, Ramirez M, Aleixandre RN, Hoesel W, Dwek RA, Rudd PM, Peracaula R, de Llorens R. Different glycan structures in prostate-specific antigen from prostate cancer sera in relation to seminal plasma PSA. *Glycobiol*, 2006, 16: 132–145

70 An HJ, Miyamoto S, Lancaster KS, Kirmiz C, Li BS, Lam KS, Leiserowitz GS, Lebrilla CB. Profiling of glycans in serum for the discovery of potential biomarkers for ovarian cancer. *J Proteome Res*, 2006, 5 1626–1635

71 Barrabes S, Pages-Pons L, Radcliffe CM, Tabares G, Fort E, Royle L, Harvey DJ, Moenner M, Dwek RA, Rudd PM, de Llorens R, Peracaula R. Glycosylation of serum ribonuclease 1 indicates a major endothelial origin and reveals an increase in core fucosylation in pancreatic cancer. *Glycobiol*, 2007, 17: 388–400

72 Beckett ML, Wright GL Jr. Characterization of a prostate carcinoma mucin-like antigen (PMA). *Int J Cancer*, 1995, 62: 703–710

73 Byrd JC, Bresalier RS. Mucins and mucin binding proteins in colorectal cancer. *Cancer Metastasis Rev*, 2004, 23: 77–99

74 Casey RC, Oegema TR, Skubitz KM, Pambuccian SE, Grindle SM, Skubitz APN. Cell membrane glycosylation mediates the adhesion, migration, and invasion of ovarian carcinoma cells. *Clin Exp Metastasis*, 2003, 20: 143–152

75 Chandrasekaran EV, Xue J, Neelamegham S, Matta KL. The pattern of glycosyl- and sulfotransferase activities in cancer cell lines: a predictor of individual cancer-associated distinct carbohydrate structures for the structural identification of signature glycans. *Carbohydrate Res*, 2006, 341: 983–994

76 Dennis JW, Granovsky M, Warren CE. Glycoprotein glycosylation and cancer progression. *Biochim Biophys Acta Gen Sub*, 1999, 1473: 21–34

77 Dimitroff CJ, Pera P, Dall'Olio F, Matta KL, Chandrasekaran EV, Lau JTY, Bernacki RJ. Cell surface *N*-acetylneuraminic acid alpha 2,3-galactoside-dependent intercellular adhesion of human colon cancer cells. *Biochem Biophy Res Comm*, 1999, 256: 631–636

78 Dwek MV, Brooks SA. Harnessing changes in cellular glycosylation in new cancer treatment strategies. *Current Cancer Targets*, 2004, 4: 425–442

79 Hallouin F, Goupille C, Bureau V, Meflah K, Le Pendu J. Increased tumorigenicity of rat colon carcinoma cells after alpha 1,2-fucosyltransferase FTA *anti*-sense cdna transfection. *Int J Cancer*, 1999, 80: 606–611

80 Hanisch FG, Hanski C, Hasegawa A. Sialyl Lewis(x) antigen as defined by monoclonal antibody AM-3. Is a marker of dysplasia in the colonic adenoma-carcinoma sequence? *Cancer Res*, 1992, 52: 3138–3144

81 Hanisch FG, Stadie TRE, Deutzmann F, PeterKatalinic J. MUC1 glycoforms in breast cancer–cell line T47D as a model for carcinoma-associated alterations of *O*-glycosylation. *Eur J Biochem*, 1996, 236: 318–327

82 Hernandez JD, Baum LG. Ah, sweet mystery of death! Galectins and control of cell fate. *Glycobiol*, 2002, 12: 127R–136R

83 Kirmiz C, Li B, An HJ, Clowers BH, Chew HK, Lam KS, Ferrige A, Alecio R, Borowsky AD, Sulaimon S, Lebrilla CB, Miyamoto S. A serum glycomics approach to breast cancer biomarkers. *Mol Cell Proteomics*, 2007, 6: 43–55

84 Meichenin M, Rocher J, Galanina O, Bovin N, Nifant'ev N, Sherman A, Cassagnau E, Heymann MF, Bara J, Fraser RH, Le Pendu J. Tk a new colon tumor-associated antigen resulting from altered *O*-glycosylation. *Cancer Res*, 2000, 60: 5499–5507

85 Miyamoto S. Clinical applications of glycomic approaches for the detection of cancer and other diseases. *Curr Opin Mol Ther*, 2006, 8: 507–513

86 Pousset D, Piller V, Bureaud N, Monsigny M, Piller F. Increased alpha 2,6 sialylation of *N*-glycans in a transgenic mouse model of hepatocellular carcinoma *Cancer Res*, 1997, 57: 4249–4256

87 Weston BW, Hiller KM, Mayben JP, Manousos GA, Bendt KM, Liu R, Cusack JC. Expression of human alpha(1,3)fucosyltransferase antisense sequences inhibits selectin-mediated adhesion and liver metastasis of colon carcinoma cells. *Cancer Res*, 1999, 59: 2127–2135

88 Wong NK, Easton RL, Panico M, Sutton-Smith M, Morrison JC, Lattanzio FA, Morris HR, Clark GF, Dell A, Patankar MS. Characterization of the oligosaccharides associated with the human ovarian tumor marker CA125. *J Biol Chem*, 2003, 278: 28619–28634

89 Zhao J, Qiu W, Simeone DM, Lubman DM. N-linked glycosylation profiling of pancreatic cancer serum using capillary liquid phase separation coupled with mass spectrometric analysis. *J Proteome Res*, 2007, doi: 10.1021/pr0604458

90 Zhao J, Simeone DM, Heidt D, Anderson MA, Lubman DM. Comparative serum glycoproteomics using lectin selected sialic acid glycoproteins with mass spectrometric analysis: application to pancreatic cancer serum. *J Proteome Res*, 2006, 5: 1792–1802

91 Dargan E, Thompson S, Cantwell BMJ, Wilson RG, Turner GA. Changes in the fucose content of haptoglobin in breast and ovarian cancer: association with disease progression. *Glycoconj J*, 1984, 1: 37–43

92 Ohyama C, Hosono M, Nitta K, Oh-eda M, Yoshikawa K, Habuchi T, Arai Y, Fukuda M. Carbohydrate structure and differential binding of

prostate specific antigen to maackia amurensis lectin between prostate cancer and benign prostate hypertrophy. *Glycobiol*, 2004, 14: 671–679

93 Kyselova Z, Mechref Y, Al Bataineh MM, Dobrolecki LE, Hickey RJ, Vinson J, Sweeney CJ, Novotny MV. Alterations in the serum glycome due to metastatic prostate cancer. *J Proteome Res*, 2007, 6: 1822–1832

94 Peracaula R, Tabares G, Royle L, Harvey DJ, Dwek RA, Rudd PM, de Llorens R. Altered glycosylation pattern allows the distinction between prostate-specific antigen (PSA) from normal and tumor origins. *Glycobiol*, 2003, 13: 457–470

95 Tabares G, Jung K, Reiche J, Stephan C, Lein M, Peracaula R, de Llorens R, Hoesel W. Free PSA forms in prostatic tissue and sera of prostate cancer patients: analysis by 2-DE and western blotting of immunopurified samples. *Clin Biochem*, 2007, 40: 343–350

96 Peracaula R, Tabares G, Lopez-Ferrer A, Brossmer R, de Bolos C, de Llorens R. Role of sialyltransferases involved in the biosynthesis of Lewis antigens in human pancreatic tumour cells. *Glycoconj J*, 2005, 22, 135–144

97 Fernandez-Salas E, Peracaula R, Frazier ML, de Llorens R. Ribonucleases expressed by human pancreatic adenocarcinoma cell lines. *Eur J Biochem*, 2000, 267: 1484–1494

98 Birken S. Specific measurement of O-linked core 2 sugar-containing isoforms of hyperglycosylated human chorionic gonadotropin by antibody b152. *Tumour Biol*, 2005, 26: 131–141

99 Cole LA. Hyperglycosylated hCG. *Placenta*, 2007, 28: 977–986

100 Ang IL, Poon TC, Lai PB, Chan AT, Ngai SM, Hui AY, Johnson PJ, Sung JJ. Study of serum haptoglobin and its glycoforms in the diagnosis of hepatocellular carcinoma: a glycoproteomic approach. *J Proteome Res*, 2006, 5: 2691–2700

101 Johnson PJ, Poon TC, Hjelm NM, Ho CS, Blake C, Ho SK. Structures of disease-specific serum alpha-fetoprotein isoforms. *Br J Cancer*, 2000, 83: 1330–1337

102 Colman PM. In the influenza viruses: influenza virus neuraminidase, enzyme and antigen. In: Krug RM, Ed. *The influenza viruses*. New York: Plenum Press, 1993. 175–218

103 Liu C, Air GM. Selection and characterization of a neuraminidase-minus mutant of influenza virus and its rescue by cloned neuraminidase genes. *Virology*, 1993, 194: 403–407

104 Crusat M, de Jong MD. Neuraminidase inhibitors and their role in avian and pandemic influenza. *Antivir Ther*, 2007, 12: 593–602

105 Lowen AC, Palese P. Influenza virus transmission: basic science and implications for the use of antiviral drugs during a pandemic. *Infect Disord Drug Targ*, 2007, 7: 318–328

106 Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature*, 1998, 393: 648–659

107 Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science*, 1998, 280: 1884–1888

108 Chan DC, Kim PS. HIV entry and its inhibition. *Cell*, 1998, 93: 681–684

109 Damodaran D, Jeyakani J, Chauhan A, Kumar N, Chandra NR, Surolia A. CancerLectinDB: a database of lectins relevant to cancer. *Glycoconj J*, 2008, 25: 191–198

110 Dacheux L, Moreau A, Ataman-Onal Y, Biron F, Verrier B, Barin F. Evolutionary dynamics of the glycan shield of the human immunodeficiency virus envelope during natural infection and implications for exposure of the 2G12 epitope. *J Virol*, 2004, 78: 12625–12637

111 Balzarini J. Targeting the glycans of gp120: a novel approach aimed at the achilles heel of HIV. *Lancet Infect Dis*, 2005, 5: 726–731

112 Bewley CA. Solution structure of a cyanovirin-N:Man alpha 1-2Man alpha complex: structural basis for high-affinity carbohydrate-mediated binding to gp120. *Structure*, 2001, 9: 931–940

113 Bolmstedt AJ, O'Keefe BR, Shenoy SR, McMahon JB, Boyd MR. HIV-inhibitory natural products part 71–cyanovirin-N defines a new class of antiviral agent targeting N-linked, high-mannose glycans in an oligosaccharide-specific manner. *Mol Pharmacol*, 2001, 59: 949–954

114 Bokesch HR, O'Keefe BR, McKee TC, Pannell LK, Patterson GM, Gardella RS, Sowder RC 2nd, Turpin J, Watson K, Buckheit RW Jr, Boyd MR. A potent novel anti-HIV protein from the cultured cyanobacterium scytonema varium. *Biochemistry*, 2003, 42: 2578–2584

115 Muller WE, Renneisen K, Kreuter MH, Schroder HC, Winkler I. The D-mannose-specific lectin from gerardia savaglia blocks binding of human immunodeficiency virus type I to H9 cells and human lymphocytes *in vitro*. *J Acquir Immune Defic Syndr*, 1988, 1: 453–458

116 Boyce JD, Adler B. The capsule is a virulence determinant in the pathogenesis of pasteurella multocida M1404 (B:2). *Infect Immun*, 2000, 68: 3463–3468

117 Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. *Annu Rev Biochem*, 2002, 71: 635–700

118 Harper M, Boyce JD, Wilkie IW, Adler B. Signature-tagged mutagenesis of pasteurella multocida identifies mutants displaying differential virulence characteristics in mice and chickens. *Infect Immun*, 2003, 71: 5440–5446

119 Krivan HC, Roberts DD, Ginsburg V. Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAc beta 1-4Gal found in some glycolipids. *Proc Natl Acad Sci USA*, 1988, 85: 6157–6161

120 Kirvan HC, Ginsburg V, Roberts DD. Pseudomonas aeruginosa and pseudomonas cepacia isolated from cystic fibrosis patients bind specifically to gangliotetraosylceramide (asialo GM1) and gangliotriaosylceramide (asialo GM2). *Arch Biochem Biophys*, 1988, 260: 493–496

121 Sheth HB, Lee kk, Wong WY, Srivastava G, Hindsqaul O, Hodges RS, Paranchych W, Irvin RT. The pili of pseudomonas aeruginosa strains PAK and PAO bind specifically to the carbohydrate sequence beta GalNAc(1-4)beta Gal found in glycosphingolipids asialo-GM1 and asialo-GM2. *Mol Microbiol*, 1994, 11: 715–723

122 Yu L, Lee KK, Hodges RS, Paranchych W, Irvin RT. Adherence of pseudomonas aeruginosa and candida albicans to glycosphingolipid (asialo-GM1) receptors is achieved by a conserved receptor-binding domain present on their adhesins. *Infect Immun*, 1994, 62: 5213–5219

123 Deal CD, Kirvan HC. Lacto- and ganglio-series glycolipids are adhesion receptor for neisseria gonorrhoeae. *J Biol Chem*, 1990, 265: 12774–12777

124 Scharfman A, VanBrussel E, Houdret N, Lamblin G, Roussel P. Interactions between glycoconjugates from human respiratory airways and pseudomonas aeruginosa. *Ame J Resp Crt Care Med*, 1996, S163–S169

125 Popoff MY, Bockemuhl J, Brenner FW, Gheesling LL. Supplement 2000 (no. 44) to the Kauffmann-White scheme. *Res Microbiol*, 2001, 152: 907–909

126 Lowe JB. Glycosylation in the control of selectin counter-receptor structure and function. *Immunol Rev*, 2002, 186: 19–36

127 Renkonen J, Tynninen O, Hayry P, Paavonen T, Renkonen R. Glycosylation might provide endothelial zip codes for organ-specific leukocyte traffic into inflammatory sites. *Am J Pathol*, 2002, 161: 543–50

128 Milland J, Sandrin MS. ABO blood group and related antigens, natural antibodies and transplantation. *Tissue Antigens*, 2006, 68: 459–466

129 Yamamoto F. ABO blood group system: ABH oligosaccharide antigens, anti-A and anti-B, A and B glycosyltransferases, and ABO genes. *Immunohematology*, 2004, 20: 3–22

130 Oriol R, Ye Y, Koren E, Cooper DK. Carbohydrate antigens of pig tissues reacting with human natural antibodies as potential targets for hyperacute vascular rejection in pig-to-man organ xenotransplantation. *Transplantation*, 1993, 56: 1433–1442

131 Sandrin MS, McKenzie IF. Gal alpha (1,3)Gal, the major xenoantigen(s) recognised in pigs by human natural antibodies. *Immunol Rev*, 1994, 141: 169–190

132 Sandrin MS. Gal Knockout pigs: any more carbohydrates? *Transplantation*, 2007, 84, 8–9

133 Zhong R. Gal knockout and beyond. *Am J Transplant*, 2007, 7: 5–11

134 Galili U. The alpha-gal epitope and the anti-gal antibody in xenotransplantation and in cancer immunotherapy. *Immunol Cell Biol*, 2005, 83: 674–686

135 Lapidot T, Petit I. Current understanding of stem cell mobilization: the

roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Exp Hematol*, 2002, 30: 973–981

136 Yu J, Vodyanik MA, He P, Slukvin II, Thomson JA. Human embryonic stem cells reprogram myeloid precursors following cell-cell fusion. *Stem Cells*, 2006, 24: 168–176

137 Muller T, Eildermann K, Dhir R, Schlatt S, Behr R. Glycan stem-cell markers are specifically expressed by spermatogonia in the adult non-human primate testis. *Hum Reprod*, 2008, 23: 2292–2298

138 Guo KT, Schafer R, Paul A, Ziemer G, Wendel HP. Aptamer-based strategies for stem cell research. *Mini Rev Med Chem*, 2007, 7: 701–705

139 Lanctot PM, Gage FH, Varki AP. The glycans of stem cells. *Curr Opin Chem Biol*, 2007, 11: 373–380

140 Muramatsu T, Muramatsu H. Carbohydrate antigens expressed on stem cells and early embryonic cells. *Glycoconj J*, 2004, 21: 41–45

141 Capela A, Temple S. LeX/ssea-1 is expressed by adult mouse CNS stem cells, identifying them as nonependymal. *Neuron*, 2002, 35: 865–875

142 Kimber SJ, Bagley PR. Cell-surface enrichment of fucosylated glycoconjugates in the 8- to 16-cell mouse embryo. *Dev Gene Evol*, 1987, 196: 492–498

143 Gheri G, Bryk SG, Sgambati E, Russo G. Chick Embryo metanephros: the glycosylation pattern as revealed with lectin conjugates. *Acta Histochem*, 1993, 94: 113–124

144 Codogno P, Bernard B, Font J, Aubery M. Changes in protein glycosylation during chick embryo development. *Biochim Biophys Acta*, 1983, 763: 265–275

145 Jacquinot P-M, Lèger D, Wieruszeski JM, Coddeville B, Montreuil J, Spik G. Change in glycosylation of chicken transferrin glycans biosynthesized during embryogenesis and primary culture of embryo hepatocytes. *Glycobiol*, 1994, 4: 617–624

146 Nagano K, Yoshida Y, Isobe T. Cell surface biomarkers of embryonic stem cells. In: *Proteomics*, Germany, 2008. Vol 8, 4025–4035

147 Ulrich H, Magdesian MH, Alves MJM, Colli W. *In vitro* selection of rna aptamers that bind to cell adhesion receptors of trypanosoma cruzi and inhibit cell invasion. *J Biol Chem*, 2002, 277: 20756–20762

148 Zhen B, Song YJ, Guo ZB, Wang J, Zhang ML, Yu SY, Yang RF. *In vitro* selection and affinity function of the aptamers to bacillus anthracis spores by SELEX. *Acta Biochem Biophys Sinica*, 2002, 34: 635–642

149 Daniels DA, Chen H, Hicke BJ, Swiderek KM, Gold LA Tenascin-C aptamer identified by tumor cell selex: systematic evolution of ligands by exponential enrichment. *Proc Natl Acad Sci USA*, 2003, 100: 15416–15421

150 Wang CL, Zhang M, Yang GA, Zhang DJ, Ding HM, Wang HX, Fan M, Shen BF, Shao NS. Single-stranded dna aptamers that bind differentiated but not parental cells: subtractive systematic evolution of ligands by exponential enrichment. *J Biotech*, 2003, 102: 15–22

151 Cerchia L, Duconge F, Pestourie C, Boulay J, Aissouni Y, Gombert K, Tavitian B, de Franciscis V, Libri D. Neutralizing aptamers from whole-cell SELEX inhibit the RET receptor tyrosine kinase. *Plos Biology*, 2005, 3: 697–704

152 Shangguan DH, Meng L, Cao ZHC, Xiao ZY, Fang XH, Li Y, Cardona D, Witek RP, Liu C, Tan WH. Identification of liver cancer-specific aptamers using whole live cells. *Anal Chem*, 2008, 80: 721–728

153 Kim YM, Liu C, Tan WH. Aptamers generated by cell Selex for biomarker discovery. *Biomark Med*, 2009, 3: 193–202

154 Shangguan D, Li Y, Tang ZW, Cao ZHC, Chen HW, Mallikaratchy P, Sefah K, Yang CYJ, Tan WH. Aptamers evolved from live cells as effective molecular probes for cancer study. *Proc Natl Acad Sci USA*, 2006, 103: 11838–11843

155 Liu CC, Mack AV, Tsao M-L, Mills JH, Lee HS, Choe H, Farzan M, Schultz P, Smidere VV. Protein evolution with an expanded genetic code. *Proc Natl Acad Sci USA*, 2008, 105: 17688–17693

156 Brustad E, Bushey ML, Lee JW, Groff D, Liu W, Schultz PG. A genetically encoded boronate-containing amino acid. *Angew Chem Int Ed*, 2008, 47: 8220–8223

157 Hakomori SI, Yamamura S, Handa K. Signal Transduction through glyco(sphingo)lipids – introduction and recent studies on glyco (sphingo) lipid-enriched microdomains. In: Ledeen RW, Hakomori S, Yates AJ, Schneider JS, Yu RK, Eds. *Sphingolipids as Signaling Modulatorsi in the Nervous System.* New York: New York Acad Sciences, 1998; Vol. 845 pp 1–10

158 Todeschini AR, Hakomori SI. Functional role of glycosphingolipids and gangliosides in control of cell adhesion, motility, and growth, through glycosynaptic microdomains. *BBA-GS*, 2008, 421–433

159 Hakomori S. Glycosynapses: microdomains controlling carbohydrate-dependent cell adhesion and signaling. *An Acad Bras Cienc*, 2004, 76: 553–572

160 Iwabuchi K, Yamamura S, Prinetti A, Handa K, Hakomori S. GM3-enriched microdomain involved in cell adhesion and signal transduction through carbohydrate-carbohydrate interaction in mouse melanoma B16 cells. *J Biol Chem*, 1998, 273: 9130–9138

161 Iwabuchi K, Zhang YM, Handa K, Withers DA, Sinay P, Hakomori S. Reconstitution of membranes simulating "glycosignaling domain" and their susceptibility to lyso-GM3. *J Biol Chem*, 2000, 275, 15174–15181

162 kamemura K, Hart GW. Dynamic Interplay between *O*-glycosylation and *O*-phosphorylation of nucleocytoplasmic proteins: a new paradigm for metabolic control of signal transduction and transcription. *Prog Nucleic Acid Res Mol Biol*, 2003, 73: 107–136

163 Kamitani S, Sakata T. Glycosylation of human CRLR at Asn123 is required for ligand binding and signaling. *Biochim Biophys Acta-Mol Cell Res*, 2001, 1539: 131–139

164 Vosseller K, Wells L, Lane MD, Hart GW. Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. *Proc Natl Acad Sci USA*, 2002, 99: 5313–5318

165 Cheng XG, Hart GW. Alternative *O*-glycosylation/*O*-phosphorylation of serine-16 in murine estrogen receptor beta- post-translational regulation of turnover and transactivation Activity. *J Biol Chem*, 2001, 276: 10570–10575

166 Yang WH, Park SY, Nam HW, Kim do H, Kang JG, Kim YS, Lee HC, Kim KS, Cho JW. NFkappaB Activation is associated with its *O*-GlcNAcylation state under hyperglycemic conditions. *Proc Natl Acad Sci USA*, 2008, 105: 17345–17350

167 Taylor RP, Parker GJ, Hazel MW, Soesanto Y, Fuller W, Yazzie MJ, McClain DA. Glucose deprivation stimulates O-GlcNAc modification of proteins through up-regulation of O-linked *N*-acetylglucosaminyltransferase. *J Biol Chem*, 2008, 283: 6050–6057

168 Golks A, Guerini D. The O-linked *N*-acetylglucosamine modification in cellular signalling and the immune system. Protein modifications: beyond the usual suspects' review series. *Eu Mol Bio Org*, 2008, 9: 748–753

169 Slawson C, Lakshmanan T, Knapp S, Hart GW. A mitotic GlcNAcylation/phosphorylation signaling complex alters the posttranslational state of the cytoskeletal protein vimentin. *Mol Biol Cell*, 2008, 19, 4130–4140

170 Majumdar G, Harmon A, Candelaria R, Martinez-Hernandez A, Raghow R, Solomon SS. *O*-glycosylation of Sp1 and transcriptional regulation of the calmodulin gene by insulin and glucagon. *Am J Physiol -Endocrinol Metab*, 2003, 285: E584–E591

171 Gommers-Ampt J, Van Leeuwen F, de Beer A, Vliegenthart J, Dizdaroglu M, Kowalak J, Crain P, Borst P. Beta-D-glucosyl-hydroxymethyluracil: a novel modified base present in the DNA of the parasitic protozoan T. *Brucei Cell*, 1993, 75: 1129–1136

172 Sabatini R, Meeuwenoord N, van Boom JH, Borst P. Site-specific interactions of JBP with base and sugar moieties in duplex J-DNA. Evidence for both major and minor groove contacts. *J Biol Chem*, 2002, 277: 28150–28156

173 Sabatini R, Meeuwenoord N, van Boom JH, Borst P. Recognition of base J in duplex DNA by J-binding protein. *J Biol Chem*, 2002, 277: 958–966

174 Borst P, van Leeuwen F. Beta-D-glucosyl-hydroxymethyluracil, a novel base in african trypanosomes and other kinetoplastida. *Mol Biochem Parasitol*, 1997, 90: 1–8

175 Cross M, Kieft R, Sabatini R, Wilm M, de Kort M, van der Marel GA, van Boom JH, van Leeuwen F, Borst P. The modified base J is the target for a novel dna-binding protein in kinetoplastid protozoans. *Embo J*, 1999, 18: 6573–6581

176 Borst P, Sabatini R. Base J. Discovery, biosynthesis, and possible functions. *Annu Rev Microbiol*, 2008, 62: 235–251

177 DiPaolo C, Kieft R, Cross M, Sabatini R. Regulation of trypanosome DNA glycosylation by a SWI2/SNF2-like protein. *Mol Cell*, 2005, 17: 441–451

178 Kieft R, Brand V, Ekanayake DK, Sweeney K, DiPaolo C, Reznikoff WS, Sabatini R. JBP2, a SWI2/SNF2-like protein, regulates de novo telomeric dna glycosylation in bloodstream form trypanosoma brucei. *Mol Biochem Parasitol*, 2007, 156: 24–31

179 Khaw KT, Wareham N. Glycated hemoglobin as a marker of cardiovascular risk. *Curr Opin Lipidology*, 2006, 17: 637–643

180 Dailey G. Assessing glycemic control with self-monitoring of blood glucose and hemoglobin-A(1c) measurements. *Mayo Clin Proc*, 2007, 82, 229–236

181 Goff DC, Gerstein HC, Ginsberg HN, Cushman WC, Margolis KL, Byington RP, Buse JB, Genuth S, Probstfield JL, Simons-Morton DG. Prevention of cardiovascular disease in persons with type 2 diabetes mellitus: current knowledge and rationale for the action to control cardiovascular risk in diabetes (ACCORD) Trial. *Am J Cardiol*, 2007, 99: 4I–20I

182 Zhang QB, Ames JM, Smith RD, Baynes JW, Metz TOA. Perspective on the Maillard reaction and the analysis of protein glycation by mass spectrometry: probing the pathogenesis of chronic disease. *J Proteome Res*, 2009, 8: 754–769

183 Roman G, Hancu N. Early insulin treatment to prevent cardiovascular disease in prediabetes and overt diabetes. *Horm Metab Res*, 2009, 41: 116–122

184 Gerstein HC. Is it possible to reduce cardiovascular risk with glucose-lowering approaches? *Nat Rev Endocrinol*, 2009, 5: 270–275

185 Amadori M. Products of condensation between glucsoe and *p*-phenetidine. *Atti Accad Naz Lincei*, 1925, 2: 337–342

186 Hadden JW. Review Article The immunology and immunotherapy of breast cancer: an update. *Int J Immunopharmacol*, 1999, 21: 79–101

187 Hodge JE. Chemistry of browning reactions in model systems. *J Agric Food Chem*, 1953, 1, 928–943

188 Zucolotto V, Ferreira M, Cordeiro MR, Constantino CJL, Moreira WC, Oliveira ON. Nanoscale processing of polyaniline and phthalocyanines for sensing applications. *Sens Actuators B*, 2006, 113: 809–815

189 Bunn HF, Shapiro R, McManus M, Garrick L, McDonald MJ, Gallop PM, Gabbay KH. Structural heterogeneity of human hemoglobin A due to nonenzymatic glycosylation. *J Biol Chem*, 1979, 254: 3892–3898

190 Pulanic D, Rudan I. The past decade: fibrinogen. *Coll Antropol*, 2005, 29: 341–349

191 Mosesson MW. Fibrinogen and fibrin structure and functions. *J Thromb Haemost*, 2005, 3: 1894–1904

192 Townsend RR, Hilliker E, Li YT, Laine RA, Bell WR, Lee YC. Carbohydrate structure of human fibrinogen. use of 300-MHz 1H-NMR to characterize glycosidase-treated glycopeptides. *J Biol Chem*, 1982, 257, 9704–9710

193 Watt KW, Takagi T, Doolittle RF. Amino acid sequence of the beta chain of human fibrinogen. *Biochemistry*, 1979, 18: 68–76

194 Wolfenstein-Todel C, Mosesson MW. Carboxy-terminal amino acid sequence of a human fibrinogen gamma-chain variant (gamma'). *Biochemistry*, 1981, 20: 6146–6149

195 Gilman PB, Keane P, Martinez J. The role of the carbohydrate moiety in the biologic properties of fibrinogen. *J Biol Chem*, 1984, 259: 3248–3253

196 Cote HCF, Lord ST, Pratt KP. γ-Chain dysfibrinogenemias: molecular structure-function relationships of naturally occurring mutations in the g-chain of human fibrinogen. *Blood*, 1998, 92: 2195–2212

197 Raisys V, Molnar J, Winzler RJ. Study of carbohydrate release during the clotting of fibrinogen. *Arch Biochem Biophys*, 1966, 113: 457–460

198 Nishibe H, Takahashi N. The release of carbohydrate moieties from human fibrinogen by almond glycopeptidase without alteration in fibrinogen clottability. *Biochim Biophys Acta*, 1981, 661: 274–279

199 Hamulyak K, Nieuwenhuizen W, Devilee PP, Hemker HC. Reevaluation of some properties of fibrinogen, purified from cord blood of normal newborns. *Thromb Res*, 1983, 32: 301–310

200 Langer BG, Weisel JW, Dinauer PA, Nagaswami C, Bell WR. Deglycosylation of fibrinogen accelerates polymerization and increases lateral aggregation of fibrin fibers. *J Biol Chem*, 1988, 263: 15056–15063

201 Grewal PK, Uchiyama S, Ditto D, Varki N, Le DT, Nizet V, Marth JD. The ashwell receptor mitigates the lethal coagulopathy of sepsis. *Nat Med*, 2008, 14: 648–655

202 Gralnick HR, Givelber H, Abrams E. Dysfibrinogenemia associated with hepatoma. Increased carbohydrate content of the fibrinogen molecule. *N Engl J Med*, 1978, 299: 221–226

203 Maekawa H, Yamazumi K, Muramatsu S, Kaneko M, Hirata H, Takahashi N, Arocha-Pinango CL, Rodriguez S, Nagy H, Perez-Requejo JL. Fibrinogen lima: a homozygous dysfibrinogen with an alpha-arginine-141 to serine substitution associated with extra *N*-glycosylation at a alpha-asparagine-139. impaired fibrin Gel formation but normal fibrin-facilitated plasminogen activation catalyzed by tissue-type plasminogen activator. *J Clin Invest*, 1992, 90: 67–76

204 Ridgway HJ, Brennan SO, Loreth RM, George PM. Fibrinogen kaiserslautern (gamma 380 Lys to Asn): a new glycosylated fibrinogen variant with delayed polymerization. *Br J Haematol*, 1997, 99: 562–569

205 Radhi JM, Lukie BE. Pancreatic cancer and fibrinogen storage disease. *J Clin Pathol*, 1998 51, 865–867

206 Yamaguchi T, Yamamoto Y, Yokota S, Nakagawa M, Ito M, Ogura T. Involvement of interleukin-6 in the elevation of plasma fibrinogen levels in lung cancer patients. *Jpn J Clin Oncol*, 1998, 28: 740–744

207 Vejda S, Posovszky C, Zelzer S, Peter B, Bayer E, Gelbmann D, Schulte-Hermann R, Gerner C. Plasma from cancer patients featuring a characteristic protein composition mediates protection against apoptosis. *Mol Cell Proteomics*, 2002, 1: 387–393

208 Gerner C, Steinkellner W, Holzmann K, Gsur A, Grimm R, Ensinger C, Obrist P, Sauermann G. Elevated plasma levels of crosslinked fibrinogen gamma-chain dimer indicate cancer-related fibrin deposition and fibrinolysis. *Thromb Haemost*, 2001, 85: 494–501

209 Kuhns DB, Nelson EL, Alvord WG, Gallin JI. Fibrinogen induces IL-8 synthesis in human neutrophils stimulated with formyl-methionyl-leucyl-phenylalanine or leukotriene B(4). *J Immunol*, 2001, 167: 2869–2878

210 Lee JH, Ryu KW, Kim S, Bae JM. Preoperative plasma fibrinogen levels in gastric cancer patients correlate with extent of tumor. *Hepatogastroenterology*, 2004, 51: 1860–1863

211 Nagy JA, Meyers MS, Masse EM, Herzberg KT, Dvorak HF. Pathogenesis of ascites tumor growth: fibrinogen influx and fibrin accumulation in tissues lining the peritoneal cavity. *Cancer Res*, 1995, 55, 369–375

212 Rybarczyk BJ, Simpson-Haidaris PJ. Fibrinogen assembly, secretion, and deposition into extracellular matrix by mcf-7 human breast carcinoma cells. *Cancer Res*, 2000, 60: 2033–2039

213 Simpson-Haidaris PJ, Rybarczyk B. Tumors and fibrinogen. the role of fibrinogen as an extracellular matrix protein. *Ann NY Acad Sci*, 2001, 936: 406–425

214 Palumbo JS, Degen JL. Fibrinogen and tumor cell metastasis. *Haemostasis*, 2001 31 Suppl 1: 11–15

215 Palumbo JS, Potter JM, Kaplan LS, Talmage K, Jackson DG, Degen JL. Spontaneous hematogenous and lymphatic metastasis, but not primary tumor growth or angiogenesis, is diminished in fibrinogen-deficient mice. *Cancer Res*, 2002, 62: 6966–6972

216 Palumbo JS, Talmage KE, Massari JV, La Jeunesse CM, Flick MJ, Kombrinck KW, Jirouskova M, Degen JL. Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. *Blood*, 2005, 105: 178–185

217 Hatzfeld JA, Hatzfeld A, Maigne J. Fibrinogen and its fragment D

stimulate proliferation of human hemopoietic cells *in vitro. Proc Natl Acad Sci USA*, 1982, 79: 6280–6284

218 Palumbo JS, Zogg M, Talmage KE, Degen JL, Weiler H, Isermann BH. Role of fibrinogen- and platelet-mediated hemostasis in mouse embryogenesis and reproduction. *J Thromb Haemost*, 2004, 2: 1368–1379

219 Hamano A, Mimuro J, Aoshima M, Itoh T, Kitamura N, Nishinarita S, Takano K, Ishiwata A, Kashiwakura Y, Niwa K, Ono T, Madoiwa S, Sugo T, Matsuda M, Sakata Y. Thrombophilic dysfibrinogen Tokyo V with the amino acid substitution of GammaAla327Thr: formation of fragile but fibrinolysis-resistant fibrin clots and its relevance to arterial thromboembolism. *Blood*, 2004, 103: 3045–3050

220 Keller MA, Martinez J, Baradet TC, Nagaswami C, Chernysh IN, Borowski MK, Surrey S, Weisel JW. Fibrinogen philadelphia, a hypodysfibrinogenemia characterized by abnormal polymerization and fibrinogen hypercatabolism due to Gamma S378P mutation. *Blood*, 2005, 105: 3162–3168

221 Lacombe M, Soria J, Soria C, D'Angelo G, Lavallee R, Bonny Y. Fibrinogen montreal. A new case of congenital dysfibrinogenemia with defective aggregation of monomers. *Thromb Diath Haemorrh*, 1973, 29: 536–546

222 Lounes KC, Lefkowitz JB, Coates AI, Hantgan RR, Henschen-Edman A, Lord ST. Fibrinogen Longmont. A heterozygous abnormal fibrinogen with B beta Arg-166 to Cys substitution associated with defective fibrin polymerization. *Ann NY Acad Sci*, 2001, 936: 129–132

223 Matsuda M, Baba M, Morimoto K, Nakamikawa C. "Fibrinogen Tokyo II". An abnormal fibrinogen with an impaired polymerization site on the aligned DD domain of fibrin molecules. *J Clin Invest*, 1983, 72: 1034–1041

224 Sherman LA, Gaston LW, Kaplan ME, Spivack AR. Fibrinogen ST. Louis: a new inherited fibrinogen variant, coincidentally associated with hemophilia A. *J Clin Invest*, 1972, 51: 590–597

225 Soria J, Soria C, Samama M, Poirot E, Kling C. Fibrinogen troyes: fibrinogen metz. two new cases of congenital dysfibrinogenemia. *Thromb Diath Haemorrh*, 1972, 27: 619–633

226 Sugo T, Nakamikawa C, Takano H, Mimuro J, Yamaguchi S, Mosesson MW, Meh DA, DiOrio JP, Takahashi N, Takahashi H, Nagai K, Matsuda M. Fibrinogen niigata with impaired fibrin assembly: an inherited dysfibrinogen with a Bbeta Asn-160 to Ser substitution associated with extra glycosylation at Bbeta Asn-158. *Blood*, 1999, 94: 3806–3813

227 Sugo T, Sakata Y, Matsuda M. Structural alterations in hereditary dysfibrinogens. *Curr Protein Pept Sci*, 2002, 3: 239–247

228 Okumura N, Terasawa F, Hirota-Kawadobora M, Yamauchi K, Nakanishi K, Shiga S, Ichiyama S, Saito M, Kawai M, Nakahata T. A novel variant fibrinogen, deletion of Bbeta111Ser in coiled-coil region, affecting fibrin lateral aggregation. *Clin Chim Acta*, 2006, 365: 160–167

229 Cohn RD, Eklund E, Bergner AL, Casella JF, Woods SL, Althaus J, Blakemore KJ, Fox HE, Hoover-Fong JE, Hamosh A, Braverman NE, Freeze HH, Boyadjiev SA. Intracranial hemorrhage as the initial manifestation of a congenital disorder of glycosylation. *Pediatrics*, 2006, 118: e514–521

230 Striegler S, Tewes E. Investigation of sugar-binding sites in ternary ligand-copper(II)-carbohydrate complexes. *Eur J Inorg Chem*, 2002, 487–495

231 Yang W, Gao X, Wang B. Boronic acid compounds as potential pharmaceutical agents. *Med Res Rev*, 2003, 23: 346–368

232 Yang X, Shan J, Cheng Y, Wang B. Boronic acid-based chemosensors. In: Mirsky VM, Yatsimirsky AK, Eds. *Artificial Receptors for Chemical Sensors*. New York: Wiley-VCH, 2010

233 Yang W, Gao S, Wang B. Biologically active boronic acid compounds. In: Hall D, Ed. *Organoboronic Acids*. New York: John Wiley and Sons, 2005. 481–512

234 Ingale S, Wolfert MA, Gaekwad J, Buskas T, Boons GJ. Robust immune responses elicited by a fully synthetic three-component vaccine. *Nature Chem Biol*, 2007, 3: 663–667

235 Gabius H-J, Gabius S. *Lectins and Glycobiology*. New York: Springer-Verlag, 1993

236 Brooks S. *Lectin Histochemistry (Microscopy Handbooks)*. 1 ed. Oxford UK: Garland Science, 1996. 177

237 Kilpatrick DC. *Handbook of Animal Lectins: Properties and Biomedical Applications*. Chichester: John Wiley & Sons, 2000

238 Van Damme EJM, Peumans WJ, Pustzai A, Bardocz S. *Handbook of Plant Lectins: Properties and Biological applicaions*. New York: John Wiley and Sons, 1998

239 James TD, Shinkai S. Artificial receptors as chemosensors for carbohydrates. *Top Curr Chem*, 2002, 218: 159–200

240 Manimala JC, Wiskur SL, Ellington AD, Anslyn EV. Tuning the specificity of a synthetic receptor using a selected nucleic acid receptor. *J Am Chem Soc*, 2004, 126: 16515–16519

241 Edwards NY, Sager TW, McDevitt JT, Anslyn EV. Boronic acid based peptidic receptors for pattern-based saccharide sensing in neutral aqueous media, an application in real-life samples. *J Am Chem Soc*, 2007, 129: 13575–13583

242 Duggan PJ, Offermann DA. Remarkably selective saccharide recognition by solid-supported peptide boronic acids. *Tetrahedron*, 2009, 65: 109–114

243 Stones D, Manku S, Xiaosong L, Hall D. Modular solid-phase synthetic approach to optimize structural and electronic properties of oligoboronic acid receptors and sensor for the aqueous recognition of oligosaccharides. *Chem Eur J*, 2004, 10: 92–100

244 Zou Y, Broughton DL, Bicker KL, Thompson PR, Lavigne JJ. Peptide borono lectins (PBLs): a new tool for glycomics and cancer diagnostics. *Chembiochem*, 2007, 8: 2048–2051

245 Davis AP, Wareham RS. A tricyclic polyamide receptor for carbohydrates in organic media. *Angew Chem Int Ed*, 1998, 37: 2270–2273

246 Lecollinet G, Dominey AP, Velasco T, Davis AP. Highly selective disaccharide recognition by a tricyclic octaamide cage. *Angew Chem Int Ed*, 2002, 41: 4093–4096

247 Klein E, Crump MP, Davis AP. Carbohydrate recognition in water by a tricyclic polyamide receptor. *Angew Chem Int Ed*, 2005, 44: 298–302

248 Ferrand Y, Crump MP, Davis AP. A synthetic lectin analog for biomimetic disaccharide recognition. *Science*, 2007, 318: 619–622

249 Ferrand Y, Klein E, Barwell NP, Crump MP, Jimenez-Barbero J, Vicent C, Boons GJ, Ingale S, Davis AP. A synthetic lectin for O-linked beta-*N*-acetylglucosamine. *Angew Chem Int Ed*, 2009, 48: 1775–1779

250 Asher SA, Alexeev VL, Goponenko AV, Sharma AC, Lednev IK, Wilcox CS, Finegold DN. Photonic crystal carbohydrate sensors: low ionic strength sugar sensing. *J Am Chem Soc*, 2003, 125: 3322–3329

251 Bielecki M, Eggert H, Norrild JC. A fluorescent glucose sensor binding covalently to all five hydroxy groups of α-d-glucose. A reinvestigation. *J Chem Soc, Perkin Trans 2*, 1999, 449–455

252 Eggert H, Frederiksen J, Morin C, Norrild JC. A new glucose-selective fluorescent bisboronic acid. first report of strong α-furanose complexation in aqueous solution at physiological pH. *J Org Chem*, 1999, 64: 3846–3852

253 James TD, Sandanayake KRAS, Iguchi R, Shinkai S. Novel saccharide-photoinduced electron transfer sensors based on the interaction of boronic acid and amine. *J Am Chem Soc*, 1995, 117: 8982–8987

254 Yang W, Fan H, Gao S, Gao X, Ni W, Karnati V, Hooks WB, Carson J, Weston B, Wang B. The first fluorescent diboronic acid sensor specific for hepatocellular carcinoma cells expressing sialyl Lewis X. *Chem Biol*, 2004, 11: 439–448

255 Yoon J, Czarnik AW. Fluorescent chemosensors of carbohydrates. a means of chemically communicating the binding of polyols in water based on chelation-enhanced quenching. *J Am Chem Soc*, 1992, 114: 5874–5875

256 Badugu R, Lakowicz JR, Geddes CD. Anion sensing using quinolinium based boronic acid probes. *Curr Anal Chem*, 2005, 1: 157–170

257 Lim SH, Musto CJ, Park E, Zhong W, Suslick KS. A colorim etric sensor array for detection and identification of sugar. *Org Letl*, 2008, 10: 4404–4408

258 Kim SK, Kim HN, Xiaoru Z, Lee HN, Lee HN, Soh JH, Swamyand KMK, Yoon J. Recent development of anion selective fluorescent

chemosensors. *Supramol Chem*, 2007, 19: 221–227

259 Mader HS, Wolfbeis OS. Boronic acid based probes for microdetermination of saccharides and glycosylated biomolecules. *Microchimica Acta*, 2008, 162: 1–34

260 Pickup JC, Hussain F, Evans ND, Rolinski OJ, Birch DJS. Fluorescence-based glucose sensors. *Biosens Bioelectr*, 2005, 20: 2555–2565

261 Norrild JC, Eggert H. Evidence for mono- and bisdentate boronate complexes of glucose in the furanose form. application of $^1J_{C-C}$ coupling constants as a structural probe. *J Am Chem Soc*, 1995, 117: 1479–1484

262 Springsteen G, Wang B. Alizarin red as a general fluorescent reporter for studying the binding of boronic acids and carbohydrates. *Chem Commun*, 2001, 1608–1609

263 Yan J, Springsteen G, Deeter S, Wang B. The relationship among pK_a, pH, and binding constants in the interactions between boronic acids and diols-it is not as simple as it appears. *Tetrahedron*, 2004, 60: 11205–11209

264 Aharoni R, Bronstheyn M, Jabbour A, Zaks B, Srebnik M, Steinberg D. Oxazaborolidine derivatives inducing autoinducer-2 signal transduction *in vibrio* harveyi. *Bioorg Med Chem*, doi:10.1016/j.bmc.2007.11.032

265 Cho BT. Oxazaborolidines as asymmetric inducers for the reduction of ketones and ketimines. In: Hall DG, Ed. *Boronic Acids*. Weinheim: Wiley-VCH, 2005. 411–439

266 Jabbour A, Steinberg D, Dembitsky VM, Moussaieff A, Zaks B, Srebnik M. Synthesis and evaluation of oxazaborolidines for antibacterial activity against streptococcus mutans. *J Med Chem*, 2004, 47: 2409–2410

267 Mohler LK, Czarnik AW. α-Amino acid chelative complexation by an arylboronic acid. *J Am Chem Soc,* 1994, 116: 2233

268 Gamsey S, Miller A, Olmstead MM, Beavers CM, Hirayama LC, Pradhan S, Wessling RA, Singaram B. Boronic acid-based bipyridinium salts as tunable receptors for monosaccharides and alpha-hydroxycarboxylates. *J Am Chem Soc,* 2007, 129: 1278–1286

269 Gray CW Jr, Houston TA. Boronic acid receptors for X-hydroxycarboxlates: high affinity of shinkai's glucose receptor for tartrate. *J Org Chem*, 2002, 67: 5426–5428

270 Wang Z, Zhang DQ, Zhu DB. A new saccharide sensor based on a tetrathiafulvalene-anthracene dyad with a boronic acid group. *J Org Chem*, 2005, 70, 5729–5732

271 Wiskur SL, Lavigne JL, Ait-Haddou H, Lynch V, Chiu YH, Canary JW, Anslyn EV. pK_a Valuaes and geometries of secondary and tertiary amines complexed to boronic acids-implications for sensor design. *Org Lett*, 2001, 3: 1311–1314

272 Dowlut M, Hall DG. An improved class of sugar-binding boronic acids, soluble and capable of complexing glycosides in neutral water. *J Am Chem Soc*, 2006, 128: 4226–4227

273 Hiratake J, Oda J. Aminophosphonic and aminoboronic acids as key elements of a transition state analogue inhibitor of enzymes. *Biosci Biotech Biochem*, 1997, 61, 211–218

274 Gallardo-Williams MT, Maronpot RR, Wine RN, Brunssen SH, Chapin RE. Inhibition of the enzymatic activity of prostate-specific antigen by boric acid and 3-nitrophenyl boronic acid. *Prostate*, 2003, 54: 44–49

275 Adams J, Kauffman M. Development of the proteasome inhihitor veleade((TM)) (Bortezomib). *Can Invest*, 2004, 22: 304–311

276 Bacha U, Barrila J, Velazquez-Campoy A, Leavitt SA, Freire E. Identification of novel inhibitors of the SARS coronavirus main protease 3CLpro. *Biochemistry*, 2004, 43: 4906–4912

277 Holyoak T, Wilson MA, Fenn TD, Kettner CA, Petsko GA, Fuller RS, Ringe D. 2.4 Angstrom resolution crystal structure of the prototypical hormone-processing protease Kex2 in complex with an Ala-Lys-Arg boronic acid inhibitor. *Biochemistry*, 2003, 42: 6709–6718

278 Snow RJ, Bachovchin WW, Barton RW, Campbell SJ, Coutts SJ, Freeman DM, Gutheil WG, Kelly TA, Kennedy CA, Krolikowski DA, Leonard SF, Pargellis CA, Tong I, Adams J. Studies on proline boronic acid dipeptide inhibitors of dipeptidyl peptidase – IV – identifycation of a cyclic species containing a B–N bond. *J Am Chem Soc*, 1994, 116: 10860–10869

279 Geele G, Garrett E, Hageman HJ. Effect of benzene boronic acids on sporulation and on production of enzymes in bacillus subtilis cells. Spores VI. *Am Soc Micro*, 1975, 391–396

280 Farr-Jones S, Smith SO, Kettner CA, Griffin RG, Bachovchin WW. Crystal versus solution structure of enzymes: NMR spectroscopy of a peptide boronic acid-serine protease complex in the crystalline state. *Proc Natl Acad Sci USA*, 1989, 86: 6922–6924

281 Groll M, Berkers CR, Ploegh HL, Ovaa H. Crystal structure of the boronic acid-based proteasome inhibitor bortezomib in complex with the yeast 20s proteasome. *Structure*, 2006, 14: 451–456

282 Matthews DA, Alden RA, Birktoft JJ, Freer ST, Kraut J. X-ray crystallographic study of boronic acid adducts with subtilisin BPN' (novo). a model for the catalytic transition state. *J Biol Chem*, 1975, 250: 7120–7126

283 Powers RA, Blazquez J, Weston GS, Morosini MI, Baquero F, Shoichet BK. The complexed structure and antimicrobial activity of a non-beta-lactam inhibitor of AmpC beta-lactamase. *Protein Sci*, 1999, 8, 2330–2337

284 Powers RA, Shoichet BK. Structure-based approach for binding site identification on AmpC beta-lactamase. *J Med Chem*, 2002, 45: 3222–3234

285 Stoll VS, Eger BT, Hynes RC, Martichonok V, Jones JB, Pai EF. Differences in binding modes of enantiomers of 1-acetamido boronic acid based protease inhibitors: crystal structures of gamma-chymotrypsin and subtilisin carlsberg complexes. *Biochemistry*, 1998, 37: 451–462

286 Zhong S, Jordan F, Kettner CA, Polgar L. Observation of tightly bound boron-11 nuclear magnetic resonance signals on serine proteases. direct solution evidence for tetrahedral geometry around the boron in the putative transition-state analogs. *J Am Chem Soc*, 1991, 113: 9429–9435

287 Bone R, Frank D, Kettner CA, Agard DA. Structural analysis of specificity: a-Lytic protease complexes with analogues of reaction intermediates. *Biochemistry* 1989, 28: 7600–7609

288 Badugu R, Lakowicz JR, Geddes CD. Excitation and emission wavelength ratiometric cyanide-sensitive probes for physiological sensing. *Anal Chem*, 2004, 327: 82–90

289 Badugu R, Lakowicz JR, Geddes CD. Cyanide-sensitive fluorescent probes. *Dyes Pigments*, 2005, 64, 49–55

290 Badugu R, Lakowicz JR, Geddes CD. A wavelength-ratiometric fluoride-sensitive probe based on the quinolinium nucleus and boronic acid moiety. *Sensors Actuators B-Chem*, 2005, 104: 103–110

291 DiCesare N, Lakowicz JR. New sensitive and selective fluorescent probes for fluoride using boronic acids. *Anal Biochem*, 2002, 301: 111–116

292 Oehlke A, Auer AA, Jahre I, Walfort B, Ruffer T, Zoufala P, Lang H, Spange S. Nitro-substituted stilbeneboronate pinacol esters and their fluoro-adducts. fluoride ion induced polarity enhancement of arylboronate esters. *J Org Chem*, 2007, 72: 4328–4339

293 Swamy KMK, Lee YJ, Lee HN, Chun J, Kim Y, Kim SJ, Yoon J. A new fluorescein derivative bearing a boronic acid group as a fluorescent chemosensor for fluoride Ion. *J Org Chem*, 2006, 71: 8626–8628

294 Cooper CR, Spencer N, James TD. Selective fluorescence detection of fluoride using boronic acids. *Chem Commun*, 1998, 1365–1366

295 Jin S, Cheng YF, Reid S, Li M, Wang B. Carbohydrate recognition by boronolectins, small molecules, and lectins. *Med Res Rev*, 2009, doi: 10.1002/med.20155

296 Springsteen G, Wang B. A detailed examination of boronic acid-diol complexation. *Tetrahedron*, 2002, 58: 5291–5300

297 Yang W, Lin L, Wang BA. Novel type of fluorescent boronic acid for carbohydrate recognition. *Tetrahedron Lett*, 2005, 46: 7981–7984

298 Zhang Y, Ballard CE, Zheng S, Gao X, Ko KC, Yang H, Brandt G, Lou X, Tai PC, Lu C-D, Wang B. Design, synthesis and evaluation of efflux substrate-metal chelator conjugates as potential anti-microbial agents. *Bioorg Med Chem Lett*, 2007, 17: 707–711

299 Zhang Y, Li M, Chandrasekaran S, Gao X, Fang X, Lee H-W, Hardcastle K, Yang J, Wang B. A unique quinolineboronic acid-based supramolecular structure that relies on double intermolecular B–N bonds for self-assembly in solid state and in solution. *Tetrahedron*, 2007, 63: 3287–3292

300 Zheng SL, Lin N, Reid S, Wang BH. Effect of extended conjugation with a phenylethynyl group on the fluorescence properties of water-soluble arylboronic acids. *Tetrahedron*, 2007, 63: 5427–5436

301 Jin S, Wang JF, Li MY, Wang BH. Synthesis, evaluation, and computational studies of naphthalimide-based long-wavelength fluorescent boronic acid reporters. *Chem Eur J*, 2008, 14: 2795–2804

302 Jin S, Zhu CY, Li MY, Wang BH. Identification of the first fluorescent alpha-amidoboronic acids that change fluorescent properties upon sugar binding. *Bioorg Med Chem Lett*, 2009, 19: 1596–1599

303 Han F, Chi LN, Liang XF, Ji SM, Liu SS, Zhou FK, Wu YB, Han KL, Zhao JZ, James TD. 3,6-Disubstituted carbazole-based bisboronic acids with unusual fluorescence transduction as enantioselective fluorescent chemosensors for tartaric acid. *J Org Chem*, 2009, 74: 1333–1336

304 Swamy KMK, Ko SK, Kwon SK, Lee HN, Mao C, Kim JM, Lee KH, Kim J, Shin I, Yoon J. Boronic acid-linked fluorescent and colorimetric probes for copper ions. *Chem Commun*, 2008, 5915–5917

305 Luvino D, Gasparutto D, Reynaud S, Smietana M, Vasseur JJ. Boronic acid-based fluorescent receptors for selective recognition of thymine glycol. *Tetrahedron Lett*, 2008, 49: 6075–6078

306 Berube M, Dowlut M, Hall DG. Benzoboroxoles as efficient glycopyranoside-binding agents in physiological conditions: structure and selectivity of complex formation. *J Org Chem*, 2008, 73: 6471–6479

307 Xu WZ, Huang ZT, Zheng QY. Highly efficient fluorescent sensing for alpha-hydroxy acids with c-3-symmetric boronic acid-based receptors. *Tetrahedron Lett*, 2008, 49: 4918–4921

308 Chi L, Zhao JZ, James TD. Chiral mono boronic acid as fluorescent enantioselective sensor for mono alpha-hydroxyl carboxylic acids. *J Org Chem*, 2008, 73: 4684–4687

309 Liang XF, James TD, Zhao JZ. 6,6'-Bis-substituted BINOL boronic acids as enantio selective and chemoselective fluorescent chemosensors for D-sorbitol. *Tetrahedron*, 2008, 64: 1309–1315

310 Akay S, Yang WQ, Wang JF, Lin L, Wang BH. Synthesis and evaluation of dual wavelength fluorescent benzo[b]thiophene boronic acid derivatives for sugar sensing. *Chem Biol Drug Des*, 2007, 70: 279–289

311 Schiller A, Wessling RA, Singaram B. A fluorescent sensor array for saccharides based on boronic acid appended bipyridinium salts. *Angew Chem Int Ed*, 2007, 46: 6457–6459

312 Cao Z, Nandhikonda P, Heagy MD. Highly water-soluble monoboronic acid probes that show optical sensitivity to glucose based on 4-sulfo-1,8-naphthalic anhydride. *J Org Chem*, 2009, 74: 3544–3546

313 Yang W, Gao S, Gao X, Karnati VR, Ni W, Wang B, Hooks WB, Carson J, Weston B. Diboronic acids as fluorescent probes for cells expressing sialyl Lewis X. *Bioorg Med Chem Lett*, 2002, 12: 2175–2177

314 Sorensen MD, Martins R, Hindsgaul O. Assessing the terminal glycosylation of a glycoprotein by the naked eye. *Angew Chem Int Ed, Engl*, 2007, 46: 2403–2407

315 Ellington AD, Szostak JW. *In vitro* selection of rna molecules that bind specific ligands. *Nature*, 1990, 346 : 818–822

316 Robertson DL, Joyce GF. Selection *in vitro* of an RNA enzyme that specifically cleaves single-stranded DNA. *Nature*, 1990, 344: 467–468

317 Gold L, Tuerk C. Systematic Evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*, 1990, 249: 505–510

318 Manku S, Hall DG. Synthesis, decoding, and preliminary screening of a bead-supported split-pool library of triboronic acid receptors for complex oligosaccharides. *Aust J Chem*, 2007, 60: 824–828

319 Agard NJ, Baskin JM, Prescher JA, Lo A, Bertozzi CR. A comparative study of bioorthogonal reactions with azides. *ACS Chem Biol*, 2006, 1: 644–648

320 Agard NJ, Prescher JA, Bertozzi CR. A strain-promoted [3+2] azide-alkyne cycloaddition for covalent modification of biomolecules in living systems. *J Am Chem Soc*, 2005, 127: 11196–11196

321 Baskin JM, Bertozzi CR. Bioorthogonal click chemistry: covalent labeling in living systems. *QSAR Comb Sci*, 2007, 26: 1211–1219

322 Baskin JM, Prescher JA, Laughlin ST, Agard NJ, Chang PV, Miller IA, Lo A, Codelli JA, Bertozzit CR. Copper-free click chemistry for dynamic *in vivo* imaging. *Proc Natl Acad Sci USA*, 2007, 104: 16793–16797

323 Chang PV, Prescher JA, Hangauer MJ, Bertozzi CR. Imaging cell surface glycans with bioorthogonal chemical reporters. *J Am Chem Soc*, 2007, 129: 8400

324 Codelli JA, Baskin JM, Agard NJ, Berozzi CR. Second-generation difluorinated cyclooctynes for copper-free click chemistry. *J Am Chem Soc*, 2008, 130: 11486–11493

325 Hangauer MJ, Bertozzi CR. A FRET-based fluorogenic phosphine for live-cell imaging with the staudinger ligation. *Angew Chem Int Ed*, 2008, 47: 2394–2397

326 Laughlin ST, Agard NJ, Baskin JM, Carrico IS, Chang PV, Ganguli AS, Hangauer MJ, Lo A, Prescher JA, Bertozzi CR. Metabolic labeling of glycans with azido sugars for visualization and glycoproteomics. In: *Glycobiology*. San Diego: Elsevier Academic Press Inc, 2006, Vol 415. 230–250

327 Laughlin ST, Baskin J M, Amacher S L, Bertozzi C R. *In vivo* imaging of membrane-associated glycans in developing zebrafish. *Science*, 2008, 320: 664–667

328 Laughlin ST, Bertozzi C. R. Metabolic labeling of glycans with azido sugars and subsequent glycan-profiling and visualization via staudinger ligation. *Nat Protoc*, 2007, 2: 2930–2944

329 Laughlin ST, Bertozzi CR. Imaging the glycome. *Proc Natl Acad Sci USA*, 2009, 106, 12–17

330 Prescher JA, Bertozzi CR. Chemistry in living systems. *Nat Chem Biol*, 2005, 1: 13–21

331 Prescher JA, Bertozzi CR. Chemical technologies for probing glycans. *Cell*, 2006, 126: 851–854

332 Prescher JA, Dube D. H, Bertozzi CR. Chemical remodelling of cell surfaces in living animals. *Nature*, 2004, 430: 873–877

333 Rabuka D, Hubbard SC, Laughlin ST, Argade SP, Bertozzi CR. A chemical reporter strategy to probe glycoprotein fucosylation. *J Am Chem Soc*, 2006, 128: 12078–12079

334 Saxon E, Bertozzi C. R. Cell surface engineering by a modified staudinger reaction. *Science*, 2000, 287: 2007–2010

335 Lewis WG, Green LG, Grynszpan F, Radic Z, Carlier PR, Taylor P, Finn MG, Sharpless KB. Click chemistry *in situ*: acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. *Angew Chem Int Edit*, 2002, 41: 1053–1057

336 Meldal M, Tornoe CW. Cu-catalyzed azide-alkyne cycloaddition. *Chem Rev*, 2008, 108, 2952–3015

337 Huisgen R. 1.3-dipolar cycloadditions. Past and future. *Angew Chem Int Ed*, 1963, 2: 565–598

338 Ning XH, Guo J, Wolfert MA, Boons GJ. Visualizing metabolically labeled glycoconjugates of living cells by copper-free and fast huisgen cycloadditions. *Angew Chem Int Ed*, 2008, 47: 2253–2255