Sugared biomaterial binding lectins: achievements and perspectives†

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Lectins, a distinct group of glycan-binding proteins, play a prominent role in the immune system ranging from pathogen recognition and tuning of inflammation to cell adhesion or cellular signalling. The possibilities of their detailed study expanded along with the rapid development of biomaterials in the last decade. The immense knowledge of all aspects of glycan–lectin interactions both in vitro and in vivo may be efficiently used in bioimaging, targeted drug delivery, diagnostic and analytic biological methods. Practically applicable examples comprise photoluminescence and optical biosensors, ingenious three-dimensional carbohydrate microarrays for high-throughput screening, matrices for magnetic resonance imaging, targeted hyperthermal treatment of cancer tissues, selective inhibitors of bacterial toxins and pathogen-recognising lectin receptors, and many others. This review aims to present an up-to-date systematic overview of glycan-decorated biomaterials promising for interactions with lectins, especially those applicable in biology, biotechnology or medicine. The lectins of interest include galectin-1, -3 and -7 participating in tumour progression, bacterial lectins from *Pseudomonas aeruginosa* (PA-IL), *E. coli* (Fim-H) and *Clostridium botulinum* (HA33) or DC-SIGN, receptors of macrophages and dendritic cells. The spectrum of lectin-binding biomaterials covered herein ranges from glycosylated organic structures, calixarenes and fullerenes cores over glycopeptides and glycoproteins, functionalised carbohydrate scaffolds of cyclodextrin or chitin to self-assembling glycopolymer clusters, gels, micelles and liposomes. Glyconanoparticles, glycan arrays, and other biomaterials with a solid core are described in detail, including inorganic matrices like hydroxyapatite or stainless steel for bioimplants.

†This work is dedicated to the memory of our dear friend and tutor Dr. Gerben Visser, Utrecht University, The Netherlands, who passed away in April 2015.

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1. Introduction

The complexity of the carbohydrate structure conceals a bulk of biological information and its decoding belongs to the major challenges in current interdisciplinary science. The sugar message imprinted on most cellular surfaces in living organisms is translated by a range of specific molecules, among which lectins occupy a privileged position. Understanding of the principle of how the sugar code is cracked by lectins gives us the power to encompass the fundamentals of life. Therefore, a thorough study of lectin–carbohydrate interactions represents a yet unexplored route for applications like drug design, in vivo imaging, targeted drug delivery, diagnostic, and analytic methods.

The subject of glycan-decorated biomaterials has been reviewed in current years. However, most authors presented just isolated aspects of this topic and, to the best of our knowledge, no recent work has given a comprehensive overview of biomaterial binding lectins and applications resulting thereof. This review aims to cover all types of glycan-decorated biomaterials including polymers, saccharide and non-saccharide scaffolds as well as solid carriers like glycoarrays, which have shown promising potential in lectin-mediated interactions. The hallmark of glycan-coated materials is multivalency, provided by simultaneous presentation of multiple sugar epitopes in a particular pick and arrangement. Since the monovalent lectin–glycan interaction is often relatively weak, i.e., with the association constant \(K_a\) in a micro- to millimolar range, the biological response in vivo is amplified by the cluster glycoside effect. Through the multivalent display of sugar ligands, the sugar–lectin interaction is enhanced by several orders of magnitude, resulting in \(K_a\) values of up to \(10^9\) M\(^{-1}\). Thus, glycomaterials successfully mimic the natural design, which makes them utmost effective and precise tools in biology and medicine; for example, in analysis using glyco-biosensors and microarrays, in magnetic resonance imaging as well as in targeted treatment of tumour tissue.

2. Lectin ligands of sugar-coated biomaterials – biological ABC

The roots of the term “lectin” can be traced back to the 1950s, when William C. Boyd, an American immunochimist, recognised the need to distinguish a special group of proteins. They were not produced in response to antigens like antibodies but still selectively interacted with specific sugar structures without changing their biological nature. The word “lectin” comes from Latin lectus, (legere, lat. read, pick; perfect passive). Lectins encompass carbohydrate-binding proteins besides antibodies, transport proteins, and enzymes, which are either secreted or localised on the cell surface and recognise specific glycan motifs presented typically on protein or lipid backbones. In the literature, they are often considered within a larger group of GBPs (glycan-binding proteins). The diversity of the lectin group reflects on their ubiquity in all parts of the living universe, from bacteria and viruses to plants, animals and humans. Merely the animal/human group includes fourteen 3D folds. The lectin family as such has been extensively reviewed elsewhere. Here we focus on three lectin groups that are most intensively studied from the viewpoint of interacting with glycomaterials: galectins, C-type lectins, and siglecs. They represent the majority of ca. 70 known mammalian GBPs and they all have irreplaceable roles in the immune system. As such, they are well documented in terms of their glycan specificity and cell-specific presentation.

2.1. Galectins

Galectins are widely spread animal lectins typical of high evolutionary conservation of a carbohydrate-recognition domain (CRD) and sequence similarity. They specifically recognise \(\beta\)-galactose-containing glycans and participate in basic cellular processes such as cell growth, development and apoptosis, inter-cell adhesion, trafficking and signalling (Fig. 1). The involvement of galectins in defence and pathophysiological processes like infection, atherosclerosis and cancerogenesis has been thoroughly documented in recent years. So far, fifteen members of the galectin family have been described (Gal-1–15). They all feature a globular fold comprising two antiparallel \(\beta\)-sheets (5–6 strands each) and one or two concave-shaped CRD domains; sometimes also one non-lectin domain. Since the CRD domain is highly conserved, the differences in binding affinity are usually given by variations in \(\beta\)-sheets and the surrounding loop regions. The galectin function is based on supramolecular assembly into oligomeric structures, leading to cross-linking into “lattices” on the cell surface, which activates various signalling pathways. For example, the bivalent galectin-1 can induce specific pro-apoptotic glycoprotein receptors through crosslinking and thus affect the homeostasis and survival of T-cells. It is the major regulator of immune responses involving T-cell disorders, inflammation, allergies, and host–pathogen interactions; it participates in tumour metastasis, immunoregulation as well as in neurodegenerative pathologies. The roles of galectin-1 and -3 in tumour progression and proliferation, angiogenesis, resistance to drugs and masking against recognition by the immune system have recently been reviewed. Besides the role as a cancer marker, galectin-3 is implicated in a range of metabolic disorders, such as complications of diabetes; its expression accompanies processes connected to heart disease and stroke, fibrosis, and tissue repair. Upon binding to multivalent ligands, galectin-3 assembles into pentamers. More specialised than the previously mentioned counterparts, galectin-9 is involved in the regulation of the glucose level in blood and in related metabolic disorders like diabetes. Human galectin-7 is involved in the metabolism of keratocytes and related epithelia formation, development and regeneration at the level of cornea and epidermis; it also contributes to the regulation of apoptosis.
2.2. C-type lectins and Siglecs

The C-type (calcium-dependent) lectin receptors (CLRs) are the biggest and most varied family of animal lectins. Their carbohydrate recognition domain is typical of binding sugar ligands by ligation to Ca\(^{2+}\) ions.\(^{34}\) The diverse group of C-type lectins comprises endocytic receptors, selectins, collectins, and proteoglycans, both of secreted and transmembrane types. The degree of conservation varies throughout the family – receptors for adhesion and endocytosis of endogenous mammalian glycans are often conserved whereas pathogen-binding receptors on immune cells show more variability.\(^{35}\) Besides their function in cell adhesion, and glycoprotein metabolism, C-type lectins also strongly participate in immune response\(^{36}\) and pathogen recognition\(^{37}\) (Fig. 1). It was shown that pathogens and tumour antigens abuse CLRs in order to escape recognition by the host system leading to degradation.\(^{38}\) The communication of CLRs and Toll-like receptors of dendritic cells results either in the onset of inflammatory response or in maintaining tolerance by the defense system.\(^{39}\) Thus, CLRs are able to modify signalling pathways activated by Toll-like receptors. This behaviour is typically observed in DC-SIGN (dendritic cell specific intercellular adhesion molecule-3-grabbing nonintegrin)\(^{40}\) and the macrophage galactose receptor (MGL)\(^{41}\) and it shows a new pathway to antiviral and anticancer therapeutics as also described in section 7.2.

Siglecs (sialic acid-binding immunoglobulin-like lectins) are a group of membrane proteins of type 1 that selectively bind glycans containing sialic acid.\(^{42}\) They are ranked under I-type lectins since they contain a homologous immunoglobulin-like domain. They form two distinct groups: (1) an evolutionary conserved group consisting of sialoadhesin/Siglec-1, CD22/Siglec-2, and myelin-associated glycoprotein/MAG/Siglec-4, and (2) CD33-related siglecs (CD33/Siglec-3 and Siglec-5 to -13). To date, thirteen siglec family representatives have been found in humans, particularly on immune cells like B-cells, monocytes, and dendritic cells.\(^{15}\) Siglecs are involved in cell signalling and adhesion, and they are supposed to participate in pathogen recognition and endocytosis\(^{13}\) (Fig. 1).

3. Glycomaterials – mode d’emploi

The expansive development of glycomaterials would not have been possible, were it not for novel synthetic methods like automated solid-phase synthesis,\(^{44}\) programmable one-pot synthesis,\(^{45}\) and ingenious multi-enzyme synthetic methods,\(^{46}\) which amplified the pool of glycans required by the high-throughput approach (Fig. 2). The use of synthetic glycans is particularly valuable when, for example, the binding nuances around a known cancer glyco-motif are examined. Anyway, the major challenge still remains to assemble a sufficient bulk of diverse carbohydrate motifs suitable for display. Especially needed are structures containing naturally occurring glycans, which are recognised as ligands by biologically or medically interesting GBPs. Such glycans may be isolated directly from natural sources but there arises the problem of sufficient purification and of reliable and easy structural verification. Despite these bottlenecks, natural glycans represent the most signifi-
cant stock of carbohydrate structures for biomedical research to date. Commonly used natural glycans comprise milk oligosaccharides, proteoglycans, glycans, and their fragments released from glycolipids and glycoproteins by means of chemical or enzymatic degradation as well as bacterial and plant polysaccharides. The diversity of glycan libraries may further be increased by means of genetic engineering (Fig. 2 and Table 1), such as by mass production of glycophages containing sugar epitopes of interest, using recombinant bacteria. Advantageously, these glycan-displaying phages are readily isolated from bacterial supernatants, and are highly suitable for high-throughput screening methodologies.

Successful determination of the specificity of a particular GBP consists of presenting an exhaustive choice of glycans and comparing the strength of binding of individual structural features. Ideally, the complete glycome of the target tissue or cell should be displayed. The cellular glycome is estimated to comprise ca. 100 to 500 thousand glycans but the crucial structural information is contained within a limited number of structural variations in strategic positions of the glycan chain. Therefore, the glycan libraries of ca. 500–600 items existing nowadays (Table 1) substantially cover the major informational potential of the examined glycome.

With the still expanding stock of glycan motives available for screening, correct analysis and interpretation of the mined data may become difficult and time-consuming. In order to facilitate combing through the screening data, several software programmes have been developed, such as Outlier Motif Analysis, GlycoSearch software, Quantitative Structural Activity Relationship (QSAR), and GlycanMotifMiner. In principle, they are based on quantitative evaluation of how individual structural motives correspond to the GBP binding affinity, usually in the form of numerical scores of statistical calculations.

Importantly, the very structure of the glycan motif is just one parameter to consider. Other factors matter like the orientation and density of the glycans displayed, depending on the nature, valency and geometry of the scaffold, and even the type of glycan immobilisation. Therefore, in order to obtain reliable data, glycan probes must be optimised including the structure, geometry and density of the linker, carrier and labels.

![Fig. 2 Generation of glycans for carbohydrate libraries. Glycans prepared by synthetic or enzymatic methods, by genetic engineering or from natural sources. LG, leaving group; PG, protecting group.](image-url)

### Table 1 Examples of glycan libraries for the screening of lectin affinities

<table>
<thead>
<tr>
<th>Research group</th>
<th>Glycans</th>
<th>Characteristics</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Feizi</td>
<td>~600</td>
<td>Amino-linked neoglycolipids</td>
<td>Liu et al.50</td>
</tr>
<tr>
<td>Cummings</td>
<td>~200</td>
<td>Natural fluorescently tagged glycans</td>
<td>Song et al.51</td>
</tr>
<tr>
<td>Derda</td>
<td>86</td>
<td>Glycopeptides with Man-WYD motif</td>
<td>Ng et al.52</td>
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<tr>
<td>Lepenies</td>
<td>52</td>
<td>Glycans binding CLRs</td>
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</tr>
<tr>
<td>Percec</td>
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<td>Dendrimers with Man/Gal/Lac</td>
<td>Percec et al.54</td>
</tr>
<tr>
<td>Winsinger</td>
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<td>Glycans tagged to peptide-nucleic acids</td>
<td>Huang et al.55</td>
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<td>Paulson</td>
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<td>9-Acyl substituted sialosides</td>
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<tr>
<td>Smith</td>
<td>26</td>
<td>High-Man phosphorylated N-glycans</td>
<td>Blixt et al.57</td>
</tr>
<tr>
<td>Paulson</td>
<td>26</td>
<td>Glycans with Neu5Ac0,2,6-Gal</td>
<td>Song et al.58</td>
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<tr>
<td>Wong</td>
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<td>Sialosides binding influenza HA</td>
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<td>Asymmetrically branched N-glycans</td>
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<td>DeLisa</td>
<td>8</td>
<td>Fluorescent engineered glycophages</td>
<td>Çelik et al.61</td>
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</table>
4. Glycans on solid carriers – glycoarrays, nanoparticles, and quantum dots

The biomaterials containing a solid core of various types and shapes – planar, cluster-type or tubular – are covered in glycan ligands displayed on the surface in a 3D mode.

4.1. Glyconanoparticles

Besides the scaffold function, the carrier solid brings in other practical features. Metal oxides such as Fe₂O₃ are excellent contrast agents in non-invasive magnetic resonance imaging (MRI) of human soft tissues due to their superparamagnetic properties. In the last two decades, many of these magnetic materials have appeared on the commercial market, such as Super-Paramagnetic Iron Oxide (SPION) nanoparticles, Very Small Iron Oxide Particles (VSOP), Feraheme, Primovist, and others. Moreover, iron oxide glyconanoparticles exhibit exothermic behaviour in alternating current magnetic field, directly applicable in, e.g., hyperthermia therapy of tumours. Their nontoxic and biodegradable nature are significant advantages in biomedical applications, compared to heavy-metal-containing quantum dots; for example in drug delivery, detection of altered (cancer) cells, magnetic resonance imaging, and in vivo thermotherapy (see section 7 for details).

Gold nanoparticles exhibit an excellent response in colorimetric bioassays; the reason is the extremely high extinction coefficient of gold (10⁶–10⁷ M⁻¹ cm⁻¹ depending on the size and shape of the nanoparticle and its ligands) and also good self-assembling potential typical of gold in colloidal form. The colour change from red to violet, induced by aggregation upon binding of glycopeptide gold nanoparticles to the lectin ligand (e.g., ConA), is easy to observe even with the naked eye. In another set-up, a gold nanocluster with glycoproteins from chicken egg-white was prepared that showed significant red photoluminescence properties, which were attributed to the presence of Cys and Tyr amino acids in the glycoprotein.

Tethered with specific glycans, gold glyconanoparticles are ideal for efficient binding of lectins of choice, such as in the case of PA-IL (LecA) adhesive lectin of Pseudomonas aeruginosa as shown by Reynolds et al. The multivalent presentation of Gal ligands in the nanoparticle arrangement caused an immense 3000-fold increase in lectin affinity (Kₐ per Gal ligand = 50 nM) over the monovalent counterpart, which corresponds to the strongest PA-IL inhibitor found to date. This model is promising for designing anti-adhesives that could prevent pathogen invasion in vivo. An example of a potent anticancer therapeutic platform was reported by Biswas et al. Gold nanoparticles coated with Thomsen–Friedenreich antigen, a disaccharide specific for many carcinoma cells, efficiently bound galectin-3, and, as a result, inhibited tumour cell growth (Fig. 3).

4.2. Quantum dots

The term “quantum dots”, coined by Prof. Mark A. Reed, denotes semiconductor nanocrystals, typically containing binary or ternary compounds of heavy metals such as Cd or Pb with characteristic fluorescent properties. Similar to classical nanoparticles, quantum dots are abundantly used especially as inexpensive and efficient analytical and diagnostic tools. With suitable glycan coating, they can label selected lectins even in complex mixtures. Again, the binding potency is immensely multiplied thanks to the multivalent glycan presentation, as shown in selective labelling of FimH lectin of E. coli flagella or of galectin-3 tumour marker. Quantum dots and nanoparticles may even be used together in one bioprobe, as shown by Hu et al. Thus, beneficial features of both systems, namely super-paramagnetic and fluorescent properties, are elegantly combined, enabling a time-saving simultaneous detection of multiple components in one pot. This approach promises to show new possibilities in high-throughput assay and screening techniques. A major drawback of quantum dots, their considerable toxicity, is conveniently decreased in the presence of a glycopolymer envelope, thanks to covalent conjugation. Pei et al. prepared quantum dots coated with a star-shaped glycopolymer hull and showed their use as targeted fluorescent probes. The hybrid quantum dots were shown to bind ConA in vitro and enter human carcinoma cells by endocytosis. Upon internalisation by Hep G2 cells, green fluorescence was emitted.

4.3. Glycoarrays

Glycoarrays were invented in 2002 as followers of DNA and protein arrays, and filled the achingly perceived gap in high-throughput methodologies. Since then, the number of publications regarding the design and biological applications of glycoarrays have grown exponentially and the topic has been reviewed. The main advantages of the glycoarray set-up are simultaneous monitoring of numerous samples in the high-throughput mode, and minute (pg amounts) consumption of analytes.

There are numerous methods of immobilisation of glycan ligands on the array surface. Besides noncovalent strategies based on adhesion, the most demanded way is site-specific covalent immobilisation of glycans on the array surface, preferably without changing their natural orientation and conformation. To accomplish this, the glycans in question must be equipped with a suitable functional group (thiol, amine, etc.) to react with the solid support. The immobilisation of underivatised reducing oligosaccharides still remains the major challenge. Beckmann et al. immobilised a range of unprotected reducing sugars on functionalised glass slides by means of Diels–Alder ligation with inverse electron demand. Binding assays were performed with fluorescently labelled lectins. Another gentle immobilisation method that fully preserves the original glycan structure including conformation comprises cheap cyanuric chloride as a linker. The intact structure of fifteen model saccharide ligands was confirmed by LC-MS and NMR and binding was tested with standard lectins. A novel bifunctional spacer, 2-amino-N-(2-aminoethyl)benzamide, by Song et al. was specially designed for immobilisation and fluorescent detection of underivatised natural
glycans. It is directly conjugated to the sugars via its arylamino
group by reductive amination. Thus, over 200 glycans from
various sources were immobilised on functionalised glass
slides and tested for binding to galectin-1 and -3. This
approach should facilitate the preparation of natural glycan
microarrays, consisting of naturally occurring glycans directly
isolated in bulk from target cells/tissues. Immobilisation
becomes more complicated if the array contains sensitive
glycan derivatives such as glycosphingolipids, present in all
eukaryotic membranes. In this case, special attention must be
paid to the intactness of both the hydrophilic glycans and the
hydrophobic ceramide moiety; if only the glycan part is ana-
lysed, the assay may give incomplete or misleading infor-
mation. Arigi et al.91 solved this problem by cleaving the fatty-
N-acyl moiety of the ceramide aglycone with sphingolipid
N-deacylase and derivatising the free amide with a fluorescent
tag. In contrast, Song et al.92 performed ozonolysis of the
sphingosine moiety and derivatisation of the originated alde-
hyde. Tagged fluorescent glycosphingolipids were chromato-
graphically separated, quantified and covalently coupled to
glass slides. The microarrays were then assayed with biological
samples of patients with Lyme disease in order to identify rele-
vant glycosphingolipids, prospective for further structural
identification. Thus, time-consuming structural analysis was
to be performed solely with pre-selected target ligands and not
with the whole glycome; this approach was termed “shotgun
glycomics”. Analogously, this approach was shown with O- and
N-glycans released from glycoproteins.51,93

Elling and coworkers94–96 presented an elegant green one-
pot preparation of a library of poly(N-acetyllactosamine) poly-
mers of varying lengths as ligands for fungal CGL2 galectin
from Coprinus cinereus. The defined mix of poly(LacNAc) units
was prepared by a combined action of human β1,4-galactosyl-
transferase-1 and Helicobacter pylori β1,3-N-acetylglucosaminyl-
transferase in one reaction step and the glycans were
covalently attached to functionalised microtiter plates. The
glycan-lectin interaction was measured by ELLA assay.

The density of glycan coating on the array surface is critical
for an efficient multivalent interaction with analysed lectins.
In a two-dimensional arrangement, the ligand density reaches
saturation at some point. Therefore, efforts were made to
further increase the ligand density by fabricating the glycan
coating in a 3D mode (Fig. 4). This may be accomplished by,
e.g., conjugating the array to a polymer scaffold decorated with
pendant glycans97 or by constructing arrays coated in multi-
valent dendrimers instead of monovalent glycan units.98 The
response increase due to the multivalency effect largely
depends on how the distances within the polymer/dendrimer
“brush” fit the lectin ligand morphology. Another extension of
classical arrays that largely increases the array multivalency are

![Fig. 3](image-url) Gold nanoparticles tethered with Thomsen–Friedenreich antigens. The epitope is O-linked via a threonine alkane/PEG linker. The binding to Gal-3 positive breast cancer cells (orange) efficiently induced apoptosis.78
microarrays based on glyconanoparticles or quantum dots printed on a polymer matrix (Fig. 4). Thus, the threshold of saturation of ligand density is much shifted to higher levels. The presence of a flexible polymer base film on the wafers is imperative in order to ensure the adaptation to conformational requirements of nanoparticle solids. Tong et al. 99 prepared such a hybrid microarray using gold glyconanoparticles and poly(allyl amine) perfluorophenyl azide by means of photocoupling chemistry. Notably, the hybrid array unproportionally amplified the response to high-affinity ligands compared to the low-affinity ones, in contrast to the standard array printed with free sugar ligands.

Functionalisation of inert solid materials with carbohydrates helps to increase their biocompatibility, in vivo tolerability and functionality, and to minimise undesirable side effects like formation of blood clots on the implant material when in the body, its potential carcinogenic and/or allergenic effects. For example, stainless steel suitable for all sorts of bioimplants may be conjugated with a nanomolar layer of passivation silica coating and functionalised with N-acetyl-D-glucosamine or D-galactose by means of alkoxysilane chemistry.100 Inorganic material like hydroxyapatite is suitable as a matrix for bone tissue regeneration; however, interaction and communication with the extracellular matrix must be ensured by adding biological cues. Russo et al. 101 presented innovative nanostructured hydroxyapatite decorated with α-glucosides via Huisgen cycloaddition and its binding to ConA. Nanoporous gold, useful as a matrix for the formation of self-assembled monolayers, for separation techniques or for immobilisation of biomolecules, was derivatised with α-mannoside and tested for lectin interaction.102

5. Glycodendrimers and glycoclusters

Biomaterials containing organic or biological dendrimer scaffolds decorated with glycan chains may be based on, e.g., resorcinarene, calixarene, fullerene, aromatic cores, or neo-glycopeptides, as well as on saccharide scaffolds such as cyclodextrins, branched oligo- and polysaccharides (Fig. 5).103–109 There are some recent reviews on this topic.110,111

5.1. Glycodendrimersomes

In analogy to polymersomes described in section 7.2, Zhang et al. 103 constructed eighteen amphiphilic glycodendrimers of a novel design with three different glycosylation patterns — so-called glycodendrimersomes — that self-assemble into stable
vesicle-like structures (Fig. 5A). The most efficient binding of lectins from various sources was shown in glycodendrimersomes with heterogenic display of glycans, compared to those with ligands of only one type. The supramolecular multivalency of glycodendrimersomes mimics natural biomembranes and it is universally applicable in many areas of nanomedicine. Glycodendrimersomes were used as diagnostic tools in a study with artificial lectin ligands, in order to reveal diverse aspects of protein–glycan multivalent interactions. Importantly, the surface pattern of displayed ligands was pro-

Fig. 5 Examples of glycodendrimer structures used for lectin binding. (A) glycodendrimerosome; (B) lactosyl calix[4]arene; (C) α-mannosylated tetranuclear [2 × 2] grid with Zn²⁺ cations (blue); (D) α-mannosyl β-lactosyl β-cyclodextrin glyocluster; (E) tetravalent thiogalactoside glycomimetic, and (F) neoglycoconjugate with human serum albumin.
grammable in terms of topology and local density. The programming of surface glycans was realised through self-assembly of selected monomeric building blocks of Janus dendrimers. In another study, Zhang et al.\textsuperscript{113} used glycodendrimersomes in a structure–activity relationship study with native forms of galectin-8.

In general, the idea of ligand heterogeneity is much closer to the real situation than the presentation of just one type of glycans. This is because the naturally occurring cells usually tune the composition of the surface glycan envelope to modify their affinity and selectivity. Ponader et al.\textsuperscript{114} showed another implementation of the heterogeneous multivalent concept. In this case, the ligands were displayed at a defined sequence and positions along the oligomer backbone, which originated through solid-phase synthesis from defined functionalised building blocks.

5.2. Glycocalixarenes

Glycocalixarene dendrimers are among the most popular scaffolds in glycodendrimer chemistry.\textsuperscript{115} Calixarenes (or resorcinols) are cyclic oligomers originated from condensation of phenols (or resorcinols) and aldehydes. They are especially valuable for their ability to accommodate guest molecules and transport them to particular destinations. The calixarene macrocycles may vary in size; the even-numbered conjugates \((n = 4, 6, 8)\) are cheap and readily chemically and commercially available, contrary to their odd-numbered counterparts.

Calixarenes tethered with carbohydrates at the upper and/or lower rim were reported as strong ligands of a variety of lectins, some of them are of pathogenic nature. For instance, calix[4]arene\textsuperscript{116} and calix[5]arene\textsuperscript{117} derivatives acted as good inhibitors of cholera toxin (the lowest IC\textsubscript{50} fell into the picomolar range). Adhesive lectins of the \textit{Pseudomonas aeruginosa} opportunistic bacterium were inhibited by galactosyl calixarenes of diverse conformations\textsuperscript{118} as well as by resorcin[4]arene, tetragalactosylated at the lower rim.\textsuperscript{119} The former substance reported by Cecioni et al.\textsuperscript{120} was patented as a base for a pharmaceutical composition.

For potential clinical applications, the core conformers of lactosyl calixarenes with a thioureido linker were shown to efficiently bind tandem-repeat type galectin-4\textsuperscript{121} whereas its counterparts containing a triazole linker prepared by click chemistry showed high affinity to galectin-3 (Fig. 5B).\textsuperscript{104}

5.3. Glycosylated aromatic scaffolds

Aromatic cores are a basic structural element of organic dendrimer scaffolds. They often dispose of additional favourable properties such as luminescence in the case of tetraphenylethylene.\textsuperscript{122} Faint luminophores \textit{per se}, they enhance the emitted photoluminescence intensity by up to three orders of magnitude upon aggregation of the glycodendrimer with target lectins – exhibiting the so-called aggregation-induced emission (AIE). The reversible character of ligand–dendrimer aggregation ensures the turn-on/turn-off character of biosensors constructed on this principle. An example is 6'-sialyl-lactosyl tetraphenylethylene fluorescent probe, used by Kato \textit{et al.}\textsuperscript{123} for detecting influenza virus.

Aromatic scaffolds based on pyridine or pyrimidine aldehydes by Chmielewski et al.\textsuperscript{105} self-assemble into supramolecular grid-shaped tetranuclear complexes of the \([2 \times 2]\) type, bearing a coordinated zinc cation and eight glycan residues (Fig. 5C). The programmed formation is reversibly dependent on pH and dilution. The complexes strongly interact with tetra-valent ConA upon assembly into polymeric networks leading to almost quantitative precipitation of aggregates from the solution. Another supramolecular structure based on \([2]\)protaxane aims at LeC and LeC bacterial lectins.\textsuperscript{124} André \textit{et al.}\textsuperscript{125} presented a symmetrical tetravalent aromatic dendri-inhibitor that efficiently blocked binding of the human macrophage galactose-binding C-type lectin (MGL) to cells and the matrix at the nM concentration.

5.4. Glycocyclodextrins

Heterogeneous display of ligands was demonstrated using a cyclodextrin core.\textsuperscript{106} The described synthetic procedure based on a modular convergent strategy enables the preparation of conjugates with defined density and orientation of ligands; in this case, \(\alpha\)-mannosyl and \(\beta\)-lactosyl moieties (Fig. 5D). The heterogeneous display of ligands results in the so-called “heterocluster effect” – the binding affinity of a glycan ligand is synergically increased in the presence of another sugar non-ligand.

Hydrophobic self-assembly of cyclodextrin-containing building blocks reported by Grünstein \textit{et al.}\textsuperscript{126} yielded heptamannosylated cyclodextrin scaffolds with a fluorescent ruthenium(ii) core. The resulting homogeneously glycosylated multivalent cyclodextrin sensors exhibited strong binding to Man-specific receptors of \textit{E. coli}. Self-assembling functionailised cyclodextrin dendrimers yielded a promising platform for preparing bilayer vesicles and membrane mimics.\textsuperscript{127,128} In this case, the hydrophobic cyclodextrin cavity accommodates a guest molecule of a suitable size and shape, such as adamantane (\(K_d = 4.10^4\) M\textsuperscript{-1}), conjugated with selected sugar(s). They can interact with certain lectin ligands to simulate behaviour in an artificial glycoalyx (Fig. 6). It seems that in order to reach maximum agglutination with lectin ligands, the density of glycans on the cyclodextrin surface must fall into certain ranges that e

5.5. Oligo- and polysaccharidic glycodendrimers

Analogous to cyclodextrins, uniform cyclic glycosyl scaffolds may be prepared in various sizes and with various types of sugar moieties, such as di-, tri-, and tetra-glucosamine cycles with glycosyl linker arms conjugated through the sugar amino groups.\textsuperscript{123} They were tested as inhibitors of \textit{Pseudomonas aeruginosa} LeC lectin; the best results were reached with a tetra-valent structure containing aromatic linkers (\(K_d = 79\) nM).
A tetrasaccharide glycomic invented by Magnani et al.\textsuperscript{130} served for diagnosis and therapeutic intervention against \textit{Pseudomonas} bacteria. Our group prepared bivalent glycomimetics with linear spacers of different lengths for studying the binding to various lectins, such as galectin-1 and -3.\textsuperscript{131, 132} François-Heude \textit{et al.}\textsuperscript{133} presented veracious mimics of high-mannose oligosaccharides, in which some mannose moieties were substituted by triazole rings. Thus, with the help of click chemistry, substantial reduction of synthetic cost was accomplished. In bioaffinity tests with ConA and human macrophage mannose receptor, these mimics copied structural preferences of natural high mannose oligosaccharides, which shows how close their design and properties are to the original structures. Paulson \textit{et al.}\textsuperscript{134} patented an original oligosaccharide motif containing sialic acid that binds Siglecs. This motif may be attached to a variety of carriers including nanoparticles, chromatographic matrices, polymers, dendrimers and even therapeutic agents. Besides analysis and separation, it serves for targeted intracellular delivery of agents into Siglec-overexpressing cancer cells. Carbohydrate polymers such as chitin may also be used as matrices for the construction of glycodendrimers. For instance, Zhou \textit{et al.}\textsuperscript{135} tagged chitin nanocrystals with a fluorescent dye and conjugated them with miscellaneous glycan ligands. The fluorescent label facilitates the optical monitoring of glycan–lectin interaction. Functionalised chitin nanocrystals were used, \textit{e.g.}, for imaging of \textit{E. coli}. Oligosaccharide clusters may also be exploited as inert scaffolds for target glycosylation. To ensure their inertness against enzyme-induced hydrolysis, they contain unnatural bonds such as in thio-\textsuperscript{107} (Fig. 5E) or selenoglycosides.\textsuperscript{136}

5.6. Other glycodendrimers

A variety of other dendrimer scaffolds have been proved to be useful in promoting lectin–carbohydrate interactions, for example glycosyl fullerenes,\textsuperscript{137} described also in section 7.2. If branched peptides are used as a matrix, the results are impressive “apple trees” of several dozens of amino acids with various topologies, of molecular weights approaching even 5 kDa. They show interesting biological activities, such as inhibition of biofilm formation, anticancerogenic and antimicrobial activities.\textsuperscript{138} They may also exhibit self-assembling properties leading to micelle/cluster formation as described in section 6. Cyclopeptides provide defined peptide scaffolds that may be used for the construction of a range of dendrimers, from low-molecular structures to second- and third-generation conjugates with up to 64 glycans.\textsuperscript{139} Glycoprotein-based dendrimer ligands employed by Wang \textit{et al.}\textsuperscript{119} (Fig. 5F) efficiently blocked binding of galectin-3 to prostate and lung cancer cells, with potential application as antimetastatic agents. The presented elegant conjugation of a functionalised BSA matrix and egg-yolk N-glycans by click chemistry designs a pathway to a new type of neoglycoproteins. Functionalised organic dendrons were used, \textit{e.g.}, for obtaining high resolution crystal structural data in the complex with lectins, such as in the case of human galectin-7 with three- and hexaglycosylated branched long-armed dendrimers prepared by means of click-chemistry.\textsuperscript{140} These studies help to reveal the details of interaction and crosslinking between lectins and multivalent glycoligands. Lactose-decorated (polyamidoamine; PAMAM) dendrimers were able to modulate Gal-3-mediated aggregation in three different cancer lines. The PAMAM dendrimer with fifteen lactose units was found to be the most efficient inhibitor of Gal-3-induced cell aggregation.\textsuperscript{141}

6. Glycosylated polymer scaffolds

6.1. Glycopolymer synthesis

Various synthetic approaches are of choice for the preparation of synthetic glycopolymeres with one or more types of glycan units;\textsuperscript{142} for example, ionic polymerisation, ring-opening metathesis, click chemistry or radical polymerisation. The latter approach involves the techniques of Nitroxide-Mediated Radical Polymisation (NMP),\textsuperscript{143} Reversible Addition Fragmentation Transfer (RAFT) polymerisation,\textsuperscript{144} and Atom Transfer Radical Polymisation (ATRP).\textsuperscript{145}

Great interest is currently laid on the control of the sequence of incorporated monomers in the glycopolymer chain, ideally including the influence on folding and formation of tertiary structures.\textsuperscript{146} To this aim, Ponader \textit{et al.}\textsuperscript{147} employed solid-phase synthesis for the preparation of defined glycopolymer segments, which were clicked on the poly(amidoamine) backbone. The main drawbacks of this approach are low isolated yields and numerous reaction steps. More promising results have been reached with the method of “single electron transfer living radical polymerisation” (SET-LRP) that enables to build multiblock glycopolymeres from small sugar monomers.\textsuperscript{148}

A high degree of control over the chain and linker length as well as over the glycan density may be exercised using a tandem post-polymerisation modification strategy\textsuperscript{149} (Fig. 7).
These parameters are impossible to control in glycopolymers prepared by conventional approaches, such as chain-growth or step-growth polymerisation. In this case, binding preferences of the B subunit of cholera toxin were studied with a series of galactose glycopolymers of varying defined linker lengths. A clear preference for longer linkers was revealed, probably due to the deep binding pocket in the toxpin, in contrast to peanut agglutinin control. Thus, the structure–activity relationship of the lectin binding process could be studied.

Preparation of sequence-defined glycopolymers according to Lutz and coworkers consists of placing reactive mal- 
emides with various N-substitutions at defined locations in a bioinert polystyrene chain. This is accomplished in a particular kinetic regime when donor and acceptor co-monomers are successively added into the reaction mixture under precise time-control. Subsequent selective deprotection, derivatisation and substitution with selected hexoses afforded a single-chain glycopolymer with exact positioning of hexoses, useful in therapeutic or biomedicinal applications, such as trapping of bacteria and viruses.

6.2. Three-dimensional organisation of glycopolymers

A much valued property of glycopolymers is their ability to form three-dimensional nano-sized clusters in the shape of micelles, vesicles or rods. The most used protocol is based on a non-covalent self-assembly of amphiphilic block copolymers. Its main disadvantage, however, is the dynamic nature, which may result in insufficient stability. Particularly spherical micelles are perfectly suitable as substance carriers, targeted through the display of apt glycans on the particle surface. The glycans are recognised by lectins on target structures, such as tumour tissue. The transported substance is then taken up and accumulated at the tumour site, penetrating through the leaky surface with the typical pores of ca. 200 nm; this phenomenon is known as enhanced permeability and retention effect (EPR). Ideally, a controlled nanocluster size (hydrodynamic diameter larger than ca. 5 nm) should be sufficient in order to ensure a prolonged blood circulation time and delayed renal clearance. The targeted transporting function of glycopolymer nanoclusters ("polymersomes") is described in section 7.2.

6.2.1. Glycopolymer micelles, vesicles and nanoparticles.

Depending on the type of the copolymer used for the self-assembly, the size, shape and surfacial glycan distribution of the clusters may be varied. The starting polymer blocks are as different as glycopolyacrylate with adjustable branching, glycopolyamide dendrons or tree-like glycopolypeptides. Glycopolypeptides are especially attractive thanks to their structural similarity to natural glycoproteins. Understandably, the organisation of glycan units on the micelle surface is a critical factor for binding affinity to lectin ligands, as shown in the comparison of micelles formed from two amphiphilic polymers of comparable sizes, compositions and glycosylation degrees – one consisting of a solely linear glycopolymer, the other containing a dendritic end functionality. The latter showed much faster clustering rate when tested with ConA. Dal Bò et al. presented an efficient preparation of amphiphilic glycopolymers using click chemistry on azido-terminated PEG-tetra(p-phenylene) precursors. Advantageously, the carbohydrate structure is attached without protecting group manipulation. Shorter PEG900 building blocks (MW 600) gave access to vesicles whereas regular spherical micelles originated from longer PEG900 conjugates (MW 900). The nano-structures were tested with peanut agglutinin and wheat germ agglutinin. If PEG900 esters of various fatty acids were employed for conjugation, the resulting micelles proved to be of a similar size (ca. 11 nm) irrespective of the lipid nature, which offers the possibility of using crude fatty acid mixtures from, e.g., vegetable oil feedstock.

The cluster size is also tunable depending on the method of preparation from the same starting components: for instance, nanoprecipitation from solution or formation through an aerosol flow reactor of either pre-glycosylated or post-glycosylated particles resulted in quite different particle diameters (97, 357 or 197 nm, respectively).

Solid polymer nanoparticles represent an alternative that combines the advantages of a solid inorganic particle and the synthetic variability of polymers. Hybrid fluorescent poly- (styrene)-poly(amiido acid) copolymers reported by Jacobs et al. showed strong binding to chinese hamster ovary cells and outlined a promising route to bioimaging agents. The fluorescent dye – Nile Red – was encapsulated in the particle core.
Polystyrene particles grafted with S-linked glycans by Kohri et al.\textsuperscript{163} were resistant to chemical and enzymatic hydrolysis.

6.2.2. Linear glycopolymers. Glycopolymer biofilms and membranes. The self-assembling properties of glycopolymers described in section 6.2.1. may also be exploited with glycopolymer systems in a linear arrangement. Geng et al.\textsuperscript{164} prepared a linear water-soluble supramolecular glycopolymer with a backbone of a covalent methacrylate polymer prepared by radical polymerisation. The backbone was decorated with non-covalently assembled conjugates of glycosylated cucurbit[8]uril attached on the principle of "supramolecular handcuffs" on 2-naphthol "pegs" (Fig. 8). The supramolecular self-assembly is reversible and adapts to the system's morphological requirements, which is close to the ligand-receptor binding mechanisms in vivo.

Elegant glycosylation of polymer structures may be well utilised in enhancing the biocompatibility and adhesive parameters of non-glycosylated polymers for use in tissue engineering, wound healing, cell growth or cartilage repair. Silk fibroin protein, a product of Bombyx mori silk worm, is a matrix of eminent interest due to its excellent mechanical properties. Its click conjugation with synthetic glycopeptides yielded a hybrid water-soluble brush-like polymer with outstanding affinity towards ConA, in a water-soluble vine-format or as an insoluble biofilm.\textsuperscript{165} Russo et al.\textsuperscript{166} performed multiple glycosylation of collagen without affecting its morphology. Lactosylation was realised through reductive amination at lysine side chains.

An example of a glycopolymer-coated membrane was constructed by Yang and Ulbricht.\textsuperscript{167} The poly(ethylene terephthalate) membrane surface was grafted with either linear or comb-like galactose glycopolymers. Efficient specific binding of peanut agglutinin was observed especially in the comb-like set-up during convective flow through the membranes. The glycopolymer functionalised membranes are promising particularly for protein separation, capture of viruses or bacteria.

7. Top applications of biomaterials

The most important applications of lectin-binding biomaterials comprise especially biosensors and bioprobes for analytic/diagnostic uses, targeted delivery agents for medication or therapy\textsuperscript{168} as well as specific inhibition and bioimaging agents for magnetic resonance techniques.

7.1. Biosensors and imaging methods

Biosensors rapidly and efficiently release a detectable signal upon interaction with the target substance. In general, they serve for measuring the kinetics of glycan–lectin interactions, for determining the specificity of binding in an array design or as diagnostic tools for detecting and quantifying tiny concentrations of lectin analytes in complex mixtures. For example, bio-functionalised nanostructures based on polydiacetylene make excellent membrane mimics and, at the same time, exhibit rapid colorimetric transition (blue-red) when they come into contact with the biological target. An example of such biosensors is glycoliposomes\textsuperscript{7} constructed by photopolymerisation and subsequent click glycosylation of resulting polydiacetylene vesicles. Tethered with relevant carbohydrates, they may aim at any sort of lectin, e.g., \textit{C. botulinum} or \textit{E. coli} toxins. In the past, their versatility was shown in the search of new antimicrobial peptides\textsuperscript{169} or in the detection of bacteria.\textsuperscript{170}

A low-cost, robust, fast and sensitive biosensor system is based on optical detection of a noncovalent complex of boronic acid tagged with a fluorescent dye, and glyco-gold nanoparticles. When binding to the target lectins occurs, the complex is released and fluorescence is turned on. This system is able to detect as little as the nM concentration of ConA.\textsuperscript{171}

Biosensors based on magnetic nanoparticles are predestined for \textit{in vivo} imaging through magnetic resonance (MRI) since their super-paramagnetic properties save the need for further derivatisation. In a nanoparticle array set up pre-

Fig. 8  Self-assembling linear glycopolymers based on the strategy of non-covalent "supramolecular handcuffs."\textsuperscript{164} The circular molecule of cucurbit[8]uril accommodates the 2-naphthol "pegs" of the methacrylate polymer backbone as well as α-mannosyl viologen, resulting in a "brush-like" supramolecular ternary glycopolymer complex that binds to ConA. The polymer scaffold is tagged with fluorescent rhodamine B. (B), structures of the glycan/concurbit[8]uril/java polymer complex, depending on the conditions in the system (addition of Na2S2O4 or O2).
sented by El-Boubbou et al.\textsuperscript{172} it was possible to clearly differentiate between normal, malignant and potentially metastatic cells on the basis of their different MRI responses. Thus, complete information could be gathered on the glycan specificity of tumour cells, which is directly applicable, e.g., in the development of anti-adhesive agents. A gold biosensor with immobilised neoglycopeptides was able to detect pM amounts of \textit{Ricinus communis} agglutinin and Gal-1 using surface plasmon resonance,\textsuperscript{173} which approaches the concentrations of Gal-1 as a marker in the sera of cancerogenic patients. A biosensor chip with attached glycopolymer brushes prepared through RAFT and click chemistry dramatically lowered the sensor detection limit by enhancing the lectin binding affinity.\textsuperscript{174}

7.2. Biomedical applications

Besides their super-paramagnetic nature that predestines them to be quality contrast agents (see section 7.1), glyconanoparticles with an iron oxide core (Fe$_3$O$_4$) have optimum heat dissipation properties. As a result, they are used in clinical practice for targeted hyperthermia treatment of cancerogenic tissues. Lartigue \textit{et al.}\textsuperscript{172} evaluated the magnetic, relaxometric and heat transfer properties of glyconanoparticles of varying sizes to find the optimum inorganic core size to be 16–18 nm. In order to enhance the biodegradability, water dispersibility and tolerability of glyconanoparticles \textit{in vivo}, the solid metal core may be conveniently enveloped with glycopolymer coating. These glycopolymer-topped hybrid nanoparticles represent new-generation nanoparticles with optimised properties for \textit{in vivo} usage. The presence of glycans on the particle surface ensures good dispersion and controlled size of aggregates in aqueous medium and enables endocytosis into cells. Muñoz-Bonilla \textit{et al.}\textsuperscript{179} constructed hybrid glycopolymer nanoparticles with covalently attached glycopolymer coating originated through radical polymerisation at the particle interface. They exhibited good heat dissipation properties suitable for hyperthermia treatment. Fluorescent hybrid nanoparticles by Pfaff \textit{et al.}\textsuperscript{9} contained an additional thin silica shell between magnetic particles and the glycopolymer coating. They were shown to enter lung cancer cells and be targeted to their nuclei, presumably by means of interaction with galectins. It was proved that decoration of nanoparticles with glycopolymer chains of varying lengths dramatically increases the efficiency of multivalent binding due to improved ligand mobility.\textsuperscript{175}

Vesicles formed from self-assembled amphiphilic glycopolymer (so-called polymersomes) have recently attracted increased attention as directed drug delivery agents.\textsuperscript{176} In the hollow spherical cavity, they can accommodate any compounds of a suitable size and shape and transport them to the destination marked by respective lectin ligands. Eissa \textit{et al.}\textsuperscript{177} constructed large polymersomes of 25–50 μm diameter coated with various sugars. They could internalise a fluorescent dye and show binding to ConA (Fig. 9A). Recently it was found\textsuperscript{178} that gold nanoparticles functionalised with amphiphilic glycopolymer self-assembled into spherical aggregates or vesicles of a tunable size with hydrophilic glycan coating that could also be potentially used as targeted transport agents while maintaining the beneficial properties of both nanoparticles.

![Fig. 9](image-url) Lectin-targeted delivery systems. (A) $\beta$-D-Glucosylated click polyethylene–polyethylene glycol) polymersomes carrying a hydrophobic fluorescent Nile red dye (red) conjugated with tetrameric ConA.\textsuperscript{177} (B) gold nanoparticles tethered with a lactose epitope and $\beta$-cyclodextrin carrier (red) bound Gal-3 and was able to carry the methotrexate drug load (blue).\textsuperscript{179}
and glycopolymers. Besides the transport in a polymer envelope, drug delivery may also be accomplished by other methods, as shown by Aykaç et al.\textsuperscript{179} Gold nanoparticles decorated with lactose ligands and β-cyclodextrin vesicles on flexible linkers were designed to serve as efficient delivery agents of anticancer drugs like methotrexate to cancer tissue, marked by the presence of galectin-3 (Fig. 9B). Advantageously, the transported drug was unmodified and noncovalently loaded in the cyclodextrin pocket.

A high degree of polyvalent glycosylation on carriers that faithfully mimic natural systems in size and shape may efficiently block binding of pathogens \textit{in vivo}. For example, DC-SIGN (dendritic cell specific intercellular adhesion molecule-3-grabbing nonintegrin)\textsuperscript{180} is an important pathogen-recognising surface receptor of the C-type lectin family, found on dendritic cells as well as macrophages. Through binding to this receptor, some pathogens are able to evade the normal degradation processes involving antigen-presenting cells. Thus, blocking of binding of pathogens to this receptor is a promising strategy for new antiviral agents. A crucial parameter is the optimum ligand structure that should fit the 20 nm distance of carbohydrate-recognition domains of DC-SIGN.\textsuperscript{180} Using this strategy, Ribeiro-Viana et al.\textsuperscript{181} constructed a unique glyco-dendri-protein-nanoparticle with the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{glyco-dendri-protein-nanoparticle.png}
\caption{Glyco-dendri-protein nanoparticles featuring “nested” 1620 glycans.\textsuperscript{181} The azido-functionalised multivalent glyco-dendron was clicked to the L-homopropargylglycine tag on the protein cluster to yield the glyco-dendri-protein nanoparticle. The particles bind to DC-SIGN receptors (red) on T-lymphocytes (blue), same as pseudotyped Ebola virus (black).}
\end{figure}
highest degree of glycosylation constructed to date (1620 glycans in a “nested” design), which efficiently inhibited pseudotyped Ebola viral infection of mammalian cells in the nM-pM range (Fig. 10).

Pseudotyped Ebola viral particles were also used by Luczko-wiak et al.\textsuperscript{182} with C\textsubscript{60} glycodendrofullerene inhibitors. Tethering long spacers with twelve Man units brought the inhibitory potency of pseudo-Ebola virus in a sub-nanomolar range.\textsuperscript{183} Another popular target virus, which may prove the inhibitory potency of pseudo-Ebola virus in a sub-nanomolar range, is HIV, as in the case of a tetravalent mannoside dendrimer\textsuperscript{184} or mannose-containing glycopolymer.\textsuperscript{185} Other dendrimer-based inhibitors of medically important lectin targets were recently described,\textsuperscript{186} including Shiga-like toxin, enterotoxin, cholera toxin, and LecA, the virulence factor of \textit{P. aeruginosa}.

8. Conclusions

A deep understanding of lectin–carbohydrate interactions opens immense and yet unexplored possibilities in many areas of biology and medicine. Biomaterials with tailored lectin affinities are applicable in targeted delivery of bioimaging agents and therapeutics \textit{in vivo}, inhibition of pathogen adhesion and breakage into cells, construction of organism-friendly bioimplants and tissue substitues, specific and exact analysis and separation of complex biological samples, efficient screening for specific GBPs in a high-throughput setup, production of artificial biomembranes and many other uses. Intensive research is currently devoted to fine-tuning of biomaterial properties, such as in sequence-controlled polymers or in hybrid polymer-layered nanoparticles, with the involvement of modern technologies like genetic engineering. The current trend envisages perfectly biotolerable materials with properties specified for individual applications, decorated with tailored carbohydrate structures in a controlled pattern and density. Such materials have good potential to step out of the proof-of-principle routine and enter everyday practice.

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