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Multivalent Sialosides: A Tool to Explore the Role of Sialic Acids in Biological Processes

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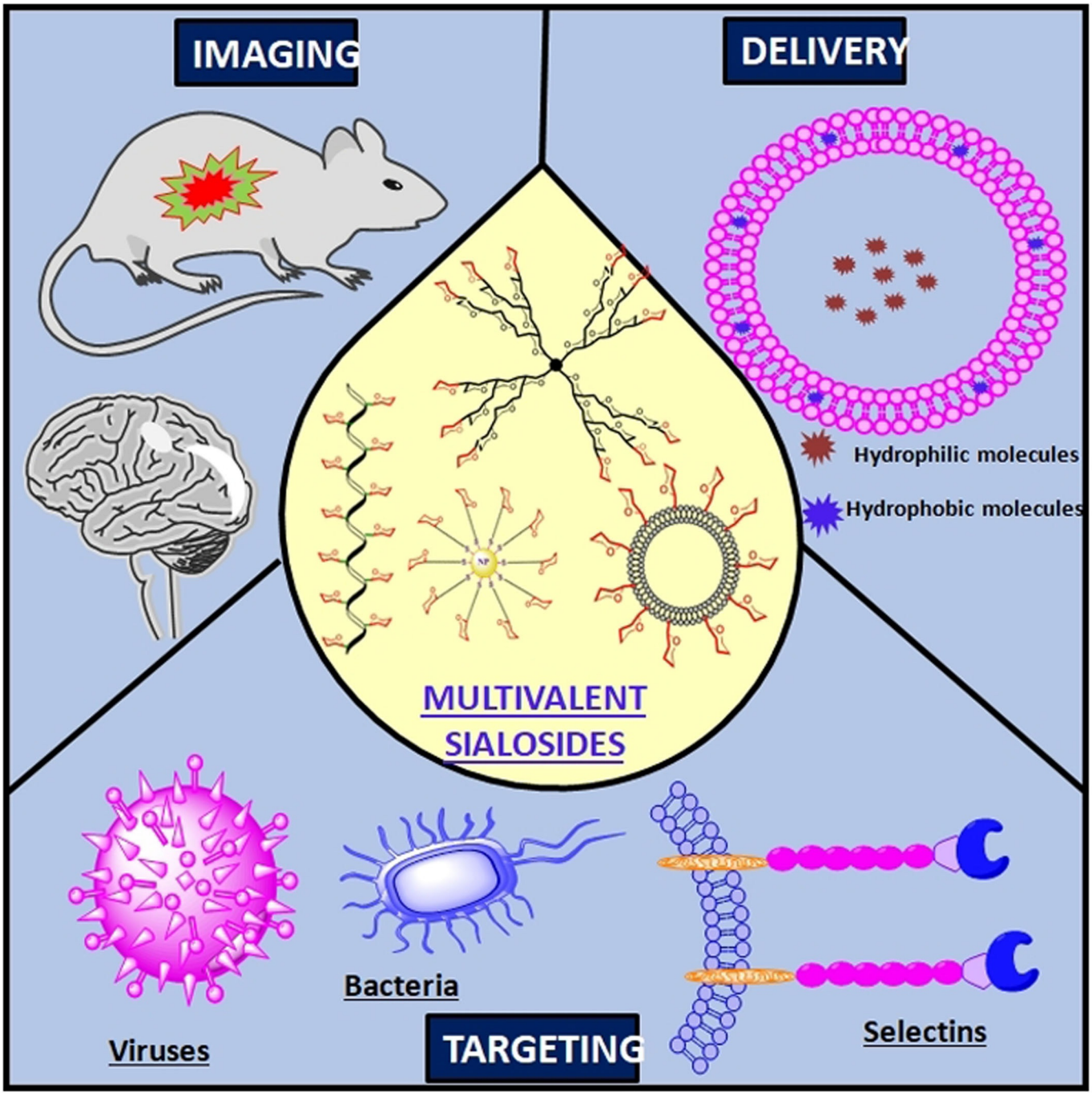

Abstract: Sialic acids (Sias) are fascinating nine-carbon monosaccharides that are primarily found on the terminus of the oligosaccharide chains of glycoproteins and glycolipids on cell surfaces. These Sias undergo a variety of structural modifications at their hydroxy and amine positions, thereby resulting in structural diversity and, hence, coordinating a variety of biological processes. However, deciphering the structural functions of such interactions is highly challenging, because the monovalent binding of Sias is extremely weak. Over the last decade, several multivalent Sia ligands have been synthesized to modulate their binding affinity with proteins/lectins. In this Minireview, we highlight recent developments in the synthesis of multivalent Sia probes and their potential applications. We will discuss four key multivalent families, that is, polymers, dendrimers, liposomes, and nanoparticles, and will emphasize the major parameters that are essential for the specific interactions of these molecules with proteins in biological systems.

1. Introduction

Sialic acids (Sias) are the major constituents of a wide variety of glycoconjugates, including N- and O-linked glycoproteins and glycolipids. Sialic acids share a characteristic nine-carbon backbone, with carboxylic acid residues attached to the C2 carbon atom, and they occupy the distal end of glycan chains, a location that makes them suitable for interactions with other cells and with environmental agents. The negative charge on the Sia unit has many modulatory roles. In mucins, sialoglycan increases the viscosity of the proteins and protects epithelial cells from bacterial infection.[1] Whereas in cancer cells, sialoglycans are overexpressed to safeguard malignant cells from the immunoevasion of natural killer (NK) cells.[2] In addition to their function of physically masking cellular biomolecules, Sias participate in major biological events through specific carbohydrate–protein interactions (CPIs). There are more than 50 different types of naturally occurring Sias, which differ in the type of substituents on the hydroxy and amine groups.[3] *N*-Glycolyl, *N*-hydroxy, *N*-acetimidoyl, *N*-acetyl, and *N*-lactyl substituents are found on the 5-amino group of Sia, whilst *O*-acetyl, *O*-lactyl, *O*-phosphate, and *O*-sulfate are examples of substituents that are found on the hydroxy groups. Furthermore, Sias are also linked to penultimate carbohydrate residues, either through α(2–6), α(2–3), α(2–8) or by α(2–9) glycosidic linkages.[4] The structural diversity and variety of glycosidic linkages articulate a wide range of cellular events through specific CPIs.

Sia-binding proteins are ubiquitous. These proteins utilize specific sialoglycan structures on cell surfaces to regulate pathogenicity and a multitude of physiological events. For example, human H3N2 influenza viruses utilize the Sia α(2–6) linkage to induce virulence, whereas avian H5N1 viruses recognize the Sia α(2–3) linkage on cell surfaces.[5] Furthermore, Influenza C and coronaviruses preferential bind to *N*-glycolylneuraminic acid (Neu5Gc) and 9-*O*-acetylated Sia to infect mammalian cells,[6,7] whilst the simian virus (SV40) recognizes GM1 ganglioside (GM = ganglioside monosialic) as a preferred ligand.[8] A large number of bacteria, such as *E. coli*, *Streptococcus*, *Helicobacter,* and parasites, such as *Plasmodium falciparum*, attack vertebrate cells through Sia-mediated interactions. *B. streptococcus* displayed a specific Sia α(2–3)Gal linkage, which binds to a Sia-binding protein on neutrophils, weakens the immune responses, and invades the human immune system.[9] Toxins from *Vibrio* and *Clostridium* species also bind to sialoganglioside.[10,11] Surprisingly, the AB5 toxin secreted by Shiga toxigenic *E. coli* binds to Neu5Gc glycans and induces gastrointestinal disease in humans.[12]

On mammalian cell surfaces, Siglecs (sialic-acid-binding immunoglobulin-type lectins) and selectins represent two distinct classes of lectin that recognize sialoglycan. Siglecs belong to a major subfamily of type-I lectins, which are widely expressed on immune cells and provide an array of diverse functions based on specific sialoglycan-mediated interactions.[13] Among the family of Siglecs, Siglec-2 and Siglec-7 are attractive targets for cancer therapy.[14,15] Siglec-2 (CD22) is overexpressed on B-cell lymphomas and leukemia; as such, human anti-CD22 has been used to treat B-cell non-Hodgkin's lymphoma.[15] Siglec-7 is expressed on natural killer (NK) cells and monocytes cells and it selectively recognizes α(2–8) di-Sia ligands.[16] It has been shown that up-regulating cell-surface Sia on cancer cells helps them to escape Siglec-7-dependent attacks of NK cells; therefore, compounds that exhibit an inhibitory effect towards Siglec-7 can activate NK cells and be used for cancer therapy.[17] Another set of Sia-binding lectins are found on endothelial cells, leukocytes, and platelets, which are classified as E-selectins, L-selectins, and P-selectins, respectively.[18] P-selectin is present on activated platelets and endothelial cells, whereas E-selectin is exposed on the endothelium after activation by cytokines, and leukocytes express L-selectin. Targeting P- and E-selectins can be used to monitor endothelial cells in the early phases of inflammation. Binding to L-selectin enables the visualization of local concentrations of leukocytes or can even modulate their function.[19] These lectins bind explicitly to sialyl Lewis^x (SL^x) and sialyl Lewis^a (SL^a) glycans, respectively. Interest-

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ingly, tumor cells also express the SL^x residue, which binds to selectins. Such selectins have a role in tumor-cell migration and metastasis.[20] Finally, the discovery that β-amyloid binds to Sia has had a significant impact on the field of Alzheimer's disease targeting.[21]

All of these aforementioned biological events and many more demonstrate the importance of sialoglycans in biological processes and highlight the potential of these molecules. However, investigating biological processes by using Sia monosaccharide in the solution phase is a huge challenge, owing to its very weak binding interactions with lectins/proteins, which are within the range mM–μM.[22] To overcome this difficulty, multivalent systems have been developed in which clusters that contain many Sia molecules with different topologies have been developed, thus amplifying the effect of the CPIs.[23] Several reviews have been published on the use of multivalent probes in targeting a wide range of carbohydrate ligands.[24–28] This Minireview primarily focuses on multivalent Sia probes to investigate CPIs and biological potency. We will mainly highlight the detailed synthesis of four important multivalent scaffolds, that is, sialo-polymers, sialo-dendrimers, sialo-nanoparticles, and sialo-liposomes, which have been used to decipher specific CPIs (Figure 1). We shall also describe the potential applications of multivalent Sia probes in drug delivery, imaging, and biosensors.

2. Sialo-polymers

The primary recognition between lectins and sialoside conjugates relies on interactions between the non-reducing end of the carbohydrate residue and the hydrophilic part of the lectin, thus indicating that the inner skeleton of a glycoprotein

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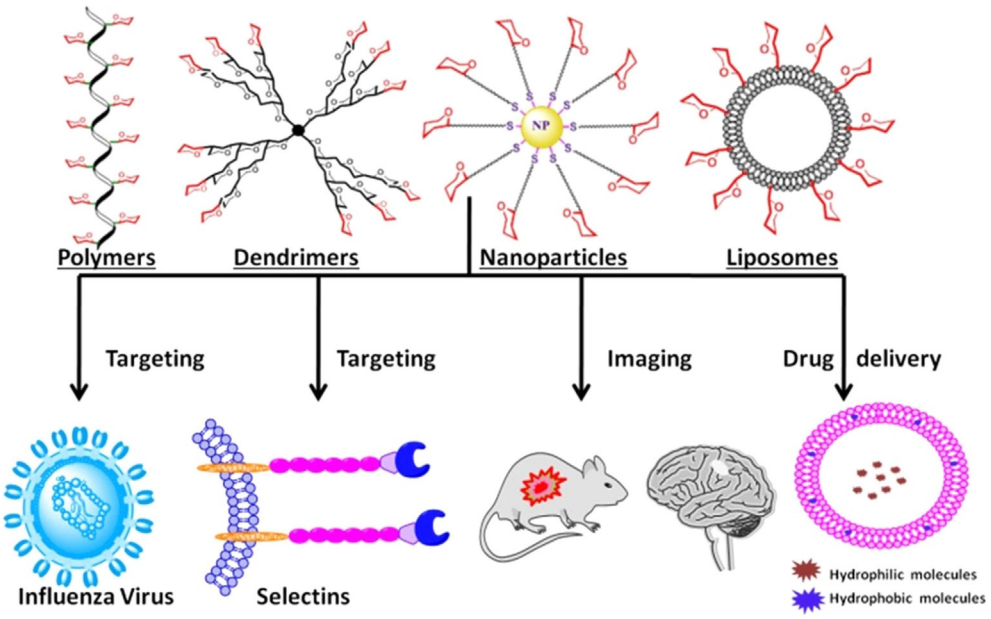

Figure 1. Multivalent sialosides and their potential biological applications.

or glycolipid could be modified without changing the CPIs. The use of a polymeric network is one of the earliest known examples of multivalent Sia probes.[29] The main synthetic approaches that have been developed to obtain glycopolymers are: 1) the assembly of monomeric sialoside ligands by using an acrylate free-radical polymerization method (convergent strategy); and 2) the coupling of sialoside ligands on commercially available well-defined polymer scaffolds (divergent strategy). In 1995, Whiteside's group reported a convergent free-radical-based polymerization method to synthesize acrylamide-based Sia copolymers.[30] They tested various adjuvants, such as ionic, hydrophobic/hydrophilic, and bulky substituents, with Sia ligands to modulate influenza-virus-mediated hemagglutinin activity and found that the presence of hydrophobic and charged species in the sialo-polymers enhanced the virus–protein binding affinity. Furthermore, the number of pendant Sia residues on the polymer backbone had a direct influence on the agglutination and illustrated the importance of multivalency in interfering with the viral binding to cell surfaces.

The synthesis of glycopolymers by using a convergent strategy is typically suitable for monosaccharide ligands, because they are easy to functionalize with polymerizable groups (e.g., acrylate derivatives). However, such functionalization of higher oligosaccharides is laborious and requires multistep syntheses. To get around this problem, Kimura and co-workers combined the copper(I)-mediated click reaction with an acrylate-polymerization strategy and synthesized sialo-oligosaccharide copolymers (Figure 2a).[31] These sialo-polymers (Neu5Acα(2–3)galβ(1–4)gal, sialylated *N*-glycan) were functionalized on a quartz crystal microbalance (QCM) to study their binding affinity with influenza viruses. The sialylated *N*-glycan polymer was found to exhibit stronger binding with human and avian influenza A viruses compared to the Neu5Ac-polymer, which was attributed to multivalency and synergistic effects of the higher-oligosaccharide to afford sensitive virus–protein recognition.

Free-radical-based polymerization methods lead to a high rate of ligand conjugation, polydispersity, and wide molecular mass distribution, which limit their potential biological applications. To overcome these drawbacks, the Kiessling group employed ring-opening metathesis polymerization (ROMP) to synthesize sialo-copolymers.[32] They constructed a copolymer that was composed of a B-cell receptor (BCR)-binding epitope and the Siglec-2 (CD22) specific ligand to investigate the *trans* interactions between Siglec-2 and Sia glycans, which were regulated by the activation of BCR. They prepared the copolymers by using BCR and CD22 antigens, such as 2,4-dinitrophenylnorbornene and Siaα(2–6) LacNAc–norbornene monomers (LacNAc = *N*-acetyl-D-lactosamine), in the presence of a ruthenium–carbene initiator. These copolymers enabled the simultaneous targeting of BCR and CD22 (Siglec-2) receptors, induced *trans* interactions in the CD22 signaling, and activated several pathways (Figure 2b). Working under the same notion, Olsen and co-workers synthesized a brush-like sialo-polymer by using a ROMP synthetic strategy.[33] Based on the structural similarities between the native mucins and synthetic sialo-polymers, it was possible to use them as model systems to analyze antiviral activities. Furthermore, their results also suggested that the brush topology also contributed to the viscosity of the polymer and the process of infection suppression.

Notably, free-radical and ROMP methods for direct polymerization were found to be efficient techniques for nonfunctional carbohydrate ligands. However, most cell-surface glycans are negatively charged sialoside ligands and it has been shown that functional groups, such as sulfate or acid groups, lower the efficiency of free-radical polymerization. Hence, an effective method for the synthesis of structurally well-defined glycopolymers is still required. Recently, Tanaka and co-workers reported

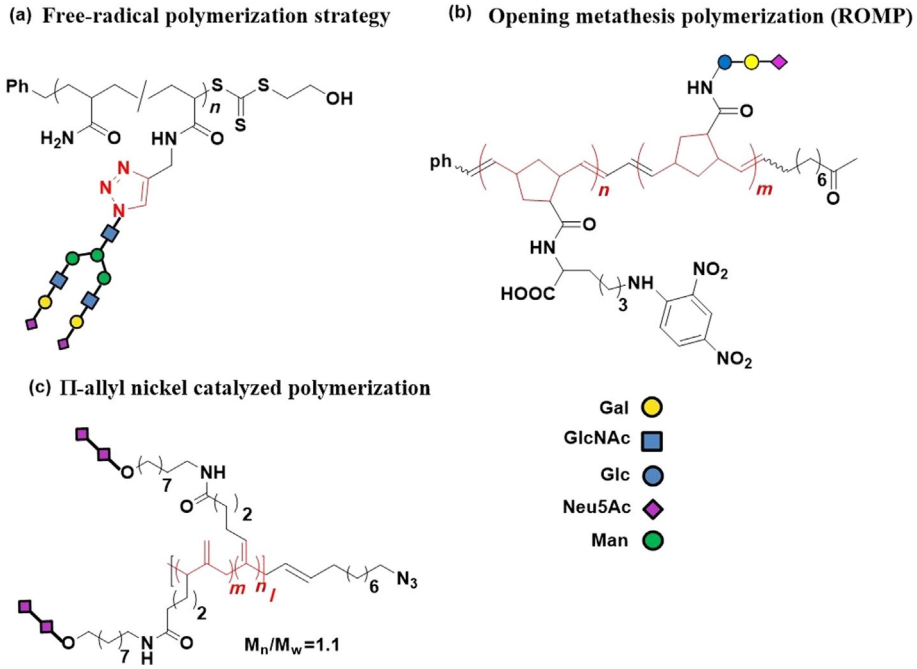

Figure 2. Structures of sialo-polymers that were synthesized by using a convergent strategy. Gal = galactose, GlcNac = *N*-acetylglucosamine, Glc = glucosamine, Neu5Ac = *N*-acetylneuraminic acid, Man = mannose.

the π-allyl–nickel-catalyzed coordination polymerization of allene monomers as an alternative method for the synthesis of sialo-polymers that generated an excellent dispersity index.[34] By using this technique, they prepared sialo-polymers that could target Siglec-7 on NK-cells (Figure 2c). Overall, in a convergent strategy, several challenges must be met to synthesize defined sialo-polymers. Alternatively, the functionalization of sialoside ligands on polymer scaffolds, commonly termed a "divergent strategy", typically allows the large-scale synthesis of glycopolymers. Savasta and co-workers explored a divergent strategy for the synthesis of GM3-ganglioside-based polymers to alter cell-signaling, thereby modulating cell-proliferation.[35] The galactose/lactose ceramide ligands were coupled with an acrylic acid polymer scaffold in different stoichiometric ratios to generate lactose–ceramide polymers. These polymers were enzymatically reacted with a Sia precursor to synthesize GM3 polymers. The cell-proliferation assay and cell-signaling assay in the presence of these polymers revealed that the number of GM3 units per polymer and hydrophobic residue of GM3 directly influenced the inhibition of cell proliferation.

A divergent strategy was also applied to the synthesis of sialo-polypeptides, dextran-based sialo-polymers, and conductive sialo-polymers. Hidari and co-workers synthesized Neu5Gc-carrying glycopeptides by using γ-polyglutamic acid and LacNAc/lactose precursors (LacNAc = *N*-acetyl-D-lactosamine; Figure 3a).[36] The lactose-conjugated polypeptide was enzymatically reacted with Neu5Gc to synthesize Neu5Gcα(2–3)/α(2–6)-polypeptides (Figure 3a). These polypeptides were subjected to a hemagglutination inhibition (HI) assay with influenza viruses, which revealed that the Ne5Gcα(2–3)-polypeptides selectively inhibited the H3N8-EIV-strain-mediated hemagglutinations (EIV = equine influenza virus). Similarly, Usui and co-workers also synthesized a variety of sialo-polypeptides by using a divergent strategy to test their selective inhibition of influenza virus strains (Figure 3b).[37] They found that Neu5Acα(2–6)-polypeptides selectively inhibited human virus H3N2 and the compositions of the inner glycans in the sialo-polypeptides had the least influence on influenza recognition. These studies illustrated the crucial role of terminal Sia compositions in the selective recognition of influenza virus strains. Finally, a divergent strategy was employed to synthesize a dextran-based disialic acid-polymer (Figure 3c).[38] These polymers showed high binding affinity toward Siglec-7, thereby effectively inhibiting Siglec 7-GD3 interactions.

Conductive polymers, such as poly(3,4-ethylene dioxythiophene) (PEDOT), have found tremendous application in biosensors in bioelectronic devices. As such, conductive sialo-polymers would constitute ideal scaffolds for the detection of the influenza virus in blood and the development of point-of-care devices. Miyahara and co-workers employed a divergent strategy to synthesize PEDOT-based conductive sialo-polymers (Figure 3d).[39] QCM (quartz crystal microbalance) and potentiometric analysis of α(2–6)-linked sialyllactose–PEDOT polymers showed strong selectivity for the human influenza A virus (H1N1), thus illustrating the potential of these polymers in point-of-care devices.

Despite this broad range of methods that have been developed for the synthesis of sialo-polymers, these convergent and divergent strategies failed to produce well-controlled sialo-polymers with end-group functionalization, which is essential for cell-surface engineering. Godula and co-workers tackled this problem by using reversible addition–fragmentation chain-

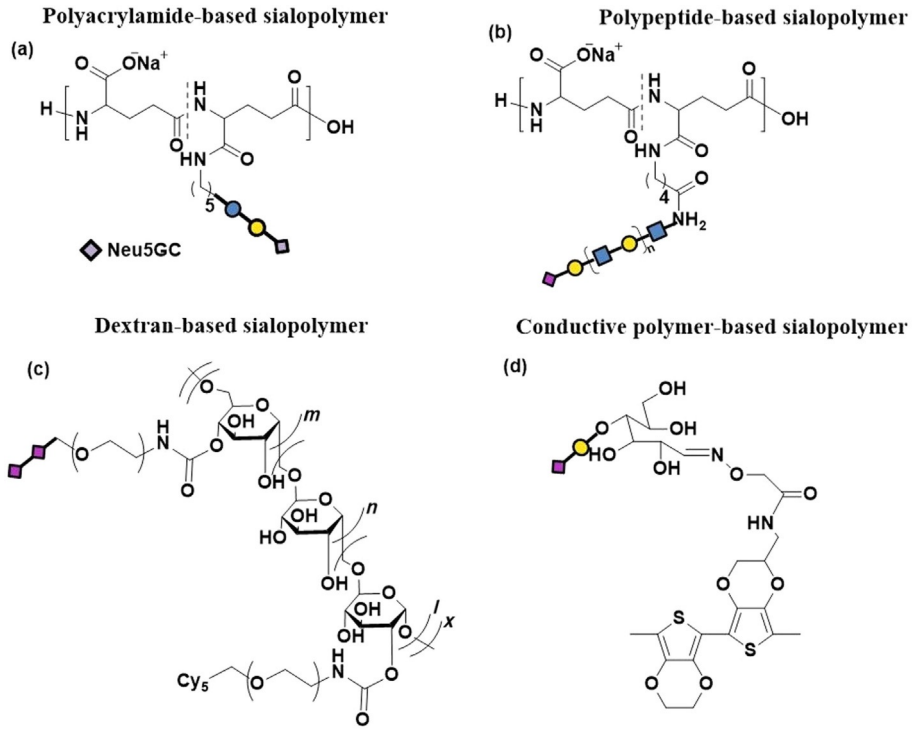

Figure 3. Structures of sialo-polymers that were synthesized by using a divergent strategy. Neu5Gc = *N*-glycolylneuraminic acid, Cy = cysteine.

transfer (RAFT) polymerization to synthesize low-polydispersity multivalent glycopolymers with simultaneous end-group functionalization with a lipid chain and a fluorescent tag (Figure 4a).[40–44] The sialo-polymers were prepared from the condensation of Sia glycans with polymethyl vinyl ketone scaffolds through the formation of an oxime, which enabled the generation of a panel of sialo-polymers, which were used in cell-surface bioengineering, modulating neural development and stem-cell differentiation. Working under the same notion, Bertozzi and co-workers reported the synthesis of sialo-RAFT copolymers and decorated them onto cancer cells to modulate the activity of NK-cells through Sia–Siglec-7 interactions (Figure 4b).[45]

3. Sialo-dendrimers

Dendrimers are important multivalent scaffolds that are used to decorate ligands of interest in a distinct homogeneous symmetrical form. In comparison to polymers, dendrimers offer more-controllable monodispersity and persistent shapes. Moreover, the 3D geometry of sialo-dendrimers closely mimics the decoration of Sia groups over cell surfaces and it allows for the fine-tuning of the microenvironment, which is essential for amplifying specific CPIs.

Sialo-dendrimers are typically prepared by using a divergent synthesis from commercial or synthetic dendrimers. Good and co-workers used poly(amidoamine) (PAMAM), a commercially

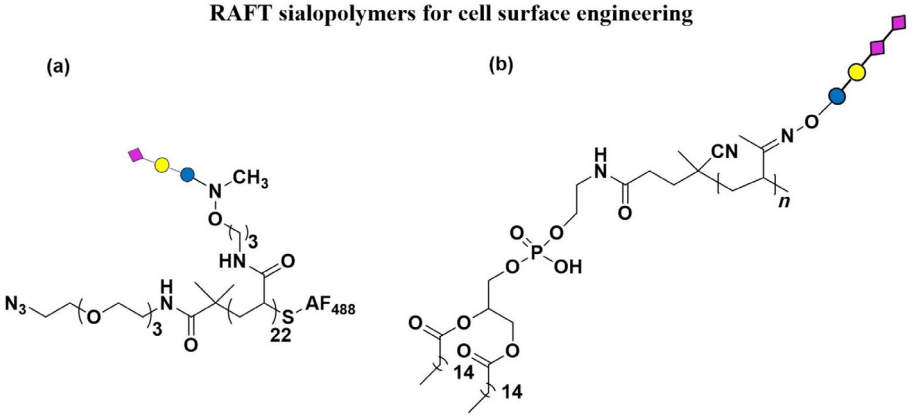

Figure 4. Schematic representation of RAFT polymers. AF_{488} = Alexa Fluor 488.

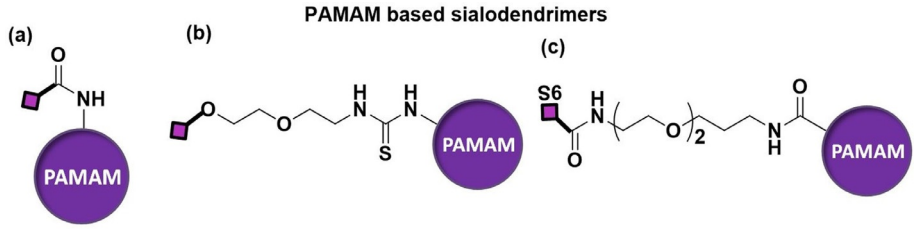

Figure 5. Structures of PAMAM sialo-dendrimers that were synthesized by using a divergent strategy.

available scaffold, to decorate Sia ligands.[46,47] They used two different strategies to conjugate the Sia ligands on PAMAM: 1) a direct amide-coupling reaction between the Sia ligand and PAMAM (Figure 5a); and 2) a thiourea-based cross-coupling reaction between Sia–amine-linker and the PAMAM scaffold (Figure 5b). These sialo-dendrimers were used to target amyloid β (Aβ)-induced neurotoxicity. Both sialo-dendrimers showed similar binding affinities with Aβ-protein, but the thiourea-based sialo-PAMAM displayed stronger inhibition of Aβ-mediated neurotoxicity. These results clearly illustrated the significance of having native Sia ligands on the PAMAM to control its biological activities. In another development, McReynolds and co-workers synthesized sulfated sialo-PAMAM dendrimers by using a divergent strategy to assess their anti-HIV activity (Figure 5c).[48] They found that sulfated-Sia ligands on PAMAM dendrimers showed strong binding to the gp120 of HIV protein and displayed micromolar-range inhibition of four host-cell infections with HIV-1 strains.

Matsuoka and co-workers constructed a library of carbosilane-based synthetic dendrimer scaffolds to decorate Sia ligands.[49–52] They employed different types of linker between the dendron scaffold and the Sia moiety to study the structure–activity relationships (Figure 6). A sialidase-inhibition assay showed that the ether- and amide-linkers were more active than an aliphatic linker. Moreover, the sialyl α(2–3) lactose moiety displayed strong antiviral activity.[51] Overall, the authors concluded that the ether linker was the most suitable for the biological study of Sia ligands. Working under the same notion, Haag and co-workers prepared a series of polyglycerol-based Sia dendrimers with diameters within the range 1–100 nm by using a divergent strategy.[53] The antiviral activities of these dendrimers revealed that large particle sizes improved their activity compared to smaller-sized clusters. Overall, the antiviral activity of sialo-dendrimers relies on the number of sulfated groups on the Sia moiety and on the nature of the linker between the Sia group and the dendrimer scaffold.

Even though dendrimers are nanostructural macromolecules that have been successfully used to study carbohydrate–protein interactions, drug delivery, and targeting, their toxicity issues limit their use in biological systems.

4. Sialo-liposomes

Apart from covalently conjugated glycodendrimers, multivalent glycoclusters can also be synthesized through the self-assembly of supramolecular architectures. In this way, liposomes and micelles have been synthesized to target specific Sia-mediated CPIs and carbohydrate–carbohydrate interactions. Furthermore, liposomes and micelles can also be used to encapsulate drugs, proteins, genes, and fluorescent dye molecules for applications in imaging and controlled drug release. Sialo-liposomes can be prepared from sialosidic phospholipids and amphiphilic precur-

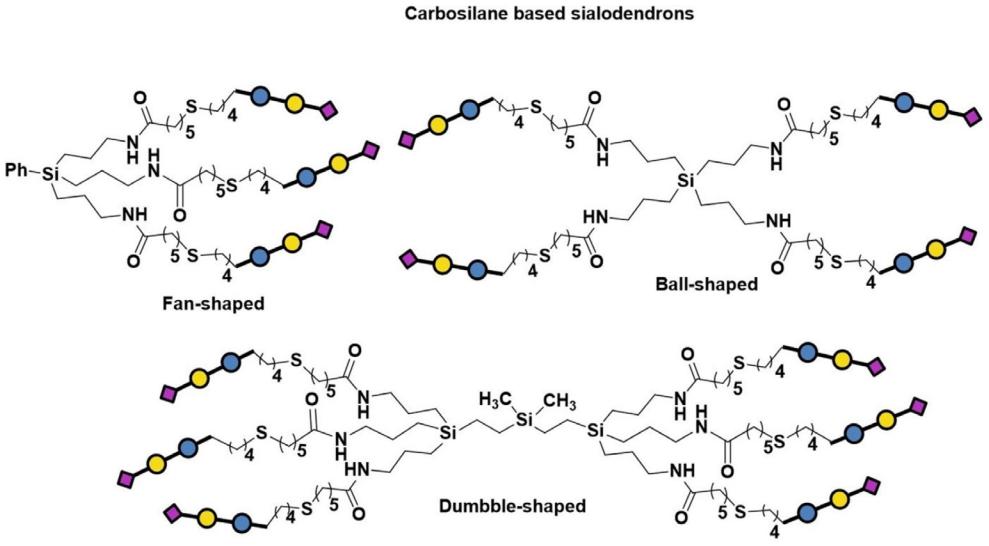

Figure 6. Different shapes of carbosilane sialo-dendrimers.

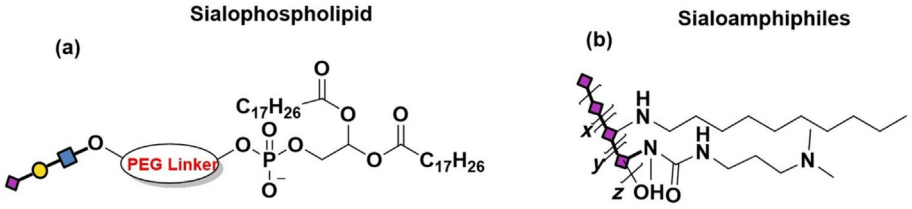

Figure 7. Structures of sialo-liposome precursors. PEG = poly(ethylene glycol).

sors. These precursors were subjected to mechanical, ultrasonic, cosolvation, dehydration–rehydration, and freeze–thaw–extrusion methods to generate specific sizes of liposomes. The nature of the liposomes also relied on their physicochemical characteristics and on the concentrations of the precursors. Paulson and co-workers synthesized liposomes that were labeled with Alexa Fluor 647 by using sialo-phospholipids of Siglec-2 (Sia-binding immunoglobulin-like lectin-2) and sialoadhesin (Sn)-specific Sia ligands to target B-cell- and Sn-positive macrophages in vitro and in vivo (Figure 7a).[54,55]

Finally, the same authors reported a selective sialo-liposome-mediated delivery of antigens and therapeutic agents into macrophages and B-cells. Similarly, Yamazaki and co-workers decorated liposomes with SL^x-phospholipids and loaded them with the drug *cis*-diammine(dinitrato)platinum(II) (CDDP).[56] They used these liposomes to target tumor cells, driven by selectin-mediated interactions. In another study, GM3[57] and GD3-appended liposomes[58] (GD = disialoganglioside) were prepared and loaded with the anticancer drugs paclitaxel and docetaxel to generate systems that simultaneously released two different potent drugs at the same time and site.[59] Zhang and co-workers prepared polySia (PSA)-based amphiphiles that self-assembled in aqueous solution to form spherical micelles (Figure 7b).[60] These micelles were used to encapsulate hydrophobic molecules and served as drug carriers that possessed acceptable properties for the targeting of diseased regions. The authors demonstrated their idea by using Cyclosporine A, an immunosuppressant medication that is used to treat autoimmune diseases. Similarly, PSA–octadecylamine-decorated liposomes were successfully used for the neutrophil-mediated delivery of pixantrone.[61] In our lab, we synthesized sialo-amphiphiles by conjugating aliphatic linkers at the C2 and C9 positions to determine the importance of the orientation of Sia in CD22-mediated CPIs.[62]

Overall, liposomes offer prolonged circulation in the blood and the slow release of drug molecules. However, the high cost of production and stability issues have hampered further clinical applications of this multivalent system.

5. Sialo-nanoparticles

Nanoparticles are well-organized, robust dendrimers that exhibit large surface areas and have inherent optical, electrochemical, and magnetic properties that facilitate the sensing and imaging of specific interactions. Several types of sialo-nanoparticles have been reported in the literature, the most prevalent of which are gold, silver, silica, iron, cadmium, selenium, and virus-like nanoparticles. Owing to their unique physical properties, these nanoparticles are attractive biomaterials for Sia research. Gold nanoparticles (AuNPs) have been widely used in sialo-nanotechnology, because of their large surface area, electronic conductivity, nontoxicity, facile synthesis, and functionalizability, which characterize the sialo-conjugates.[63] AuNPs were synthesized by reducing gold(III) chloride in the presence of a suitable surfactant and sodium borohydride. Finally, these nanoparticles were functionalized by using suitable sialoside ligands. Sialo-AuNPs can be formed by: 1) direct ligand exchange between sialo-functionalized thiol linkers and AuNPs; or 2) coupling reactions between Sia derivatives and functionalized AuNPs. Process (1) offers several advantages, including control over the number of sialoside ligands per nanoparticle and the resultant sialo-AuNPs do not contain additional functional groups, which may induce nonspecific interactions during CPIs. Ijiro and co-workers synthesized sialo-AuNPs by using directed ligand exchange between sialylated oligosaccharides that contained a thiol linker and AuNPs (5, 10, and 15 nm).[64] These nanoparticles were used to detect the John Cunningham (JC) virus, which displayed 360 copies of Sia-binding virus protein 1 (VP1). The specific carbohydrate–protein interactions between VP1 and sialo-AuNPs resulted in clustering of the AuNPs on the surface of the viruses, and led to a significant plasmonic shift in UV/Vis absorption spectrum of the AuNPs, which ultimately allowed the sensitive detection of the virus system. Similarly, Fairbanks and co-workers reported the synthesis of bi-sialo bi-antennary *N*-glycan-AuNPs to detect the influenza virus at nanomolar concentrations (Figure 8a).[65] First, they isolated *N*-glycan from egg yolk and conjugated it to NHS-(*S*)-acetylthioacetic acid active ester (NHS = *N*-hydroxysuccinimide). Mild deacetylation of the glycan afforded a good amount of the *N*-glycan with the thiol linker for direct conjugation onto the AuNPs. The resultant sialo-AuNPs were subjected to colorimetric detection of the influenza virus and the results from these studies confirmed the development of an inexpensive, sensitive platform for the detection of the influenza virus. A similar goal has also driven Haag and co-workers to synthesize Sia-conjugated AuNPs (2 and 14 nm) for a sialo-ligand-dependent hemaglutination assay with the influenza virus (Figure 8b).[66] In yet another development, Russell and co-workers synthesized sialo-AuNPs by using direct ligand exchange to detect mSiglec-E which expressed on CHO cell lines by using a colorimetric method (Figure 8c).[67] Overall, direct ligand exchange afforded quick access to the sialo-AuNPs, which was an efficient platform for the detection of the influenza virus. However, the major limitation of this strategy was the requirement

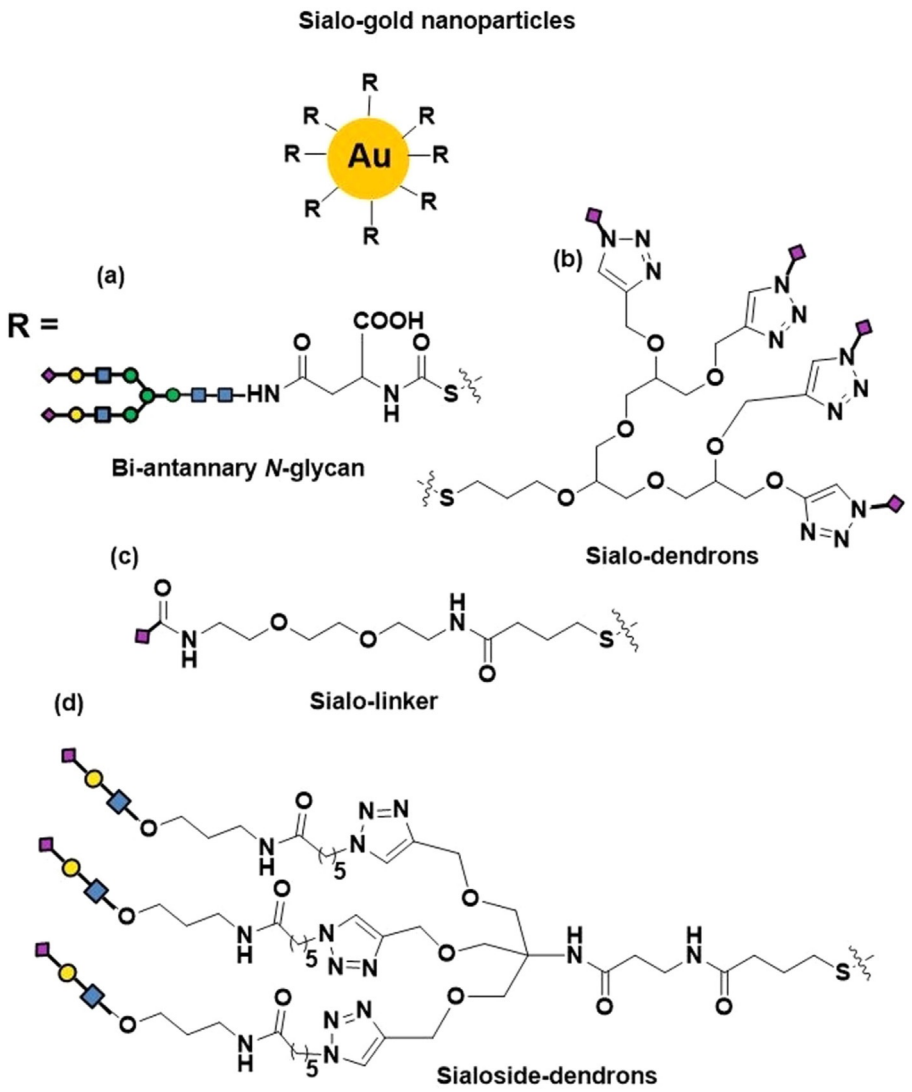

Figure 8. Sialo-gold nanoparticles that were synthesized by using a direct-ligand-exchange strategy.

for a large quantity of sialoside ligands. In such a scenario, process (2) is ideal. However, the critical requirement of process (2) is to identify mild, efficient coupling reactions that result in neutral epitopes. Tamiya and co-workers employed a click chemistry reaction between a sialoside azide linker and acetylene-functionalized AuNPs to synthesize sialo-AuNPs, which were coated onto a carbon electrode to fabricate an electrochemical Aβ-peptides biosensor.[68] Similarly, Russell and co-workers synthesized sialo-AuNPs by using click chemistry to develop a biosensor for the detection of the influenza A virus (Figure 8d).[69] Nishimura and co-workers employed an oxide-coupling reaction between aminooxy-functionalized AuNPs and a sialoside ketone linker to synthesize sialo-AuNPs, which were used to detect the influenza A virus.[70]

Gold and silver nanoparticles have similar optical properties, large surface areas, and good conductance. In addition, silver nanoparticles (AgNPs) display distinct antimicrobial activities. As such, sialo-AgNPs are useful probes for the treatment of health-threatening bacteria. Park and co-workers synthesized sialo-AgNPs through the direct autoclaving or microwave irradiation of Sia ligands and silver(I)nitrate solution.[71] These nanoparticles showed both antibacterial (*pseudomonas aeruginosa*, *Escherichia coli*, and *salmonella typhimurium*) and antiviral (influenza virus) activities.

Iron NPs exhibit a unique superparamagnetic nature and, hence, enhanced contrast with background water molecules to detect specific interactions by using magnetic resonance imaging (MRI) techniques. Huang and co-workers synthesized sialo-iron NPs or sialo-magnetic nanoparticles (Sialo-MGNPs) by using an amide-coupling reaction between the Sia ligand and amine-functionalized MGNPs.[72] These sialo-MGNPs displayed low toxicity and bound to most of the cancer and normal cell lines, thus indicating the presence of Sia-active receptors on these cell lines. In another work, the same group utilized sialo-MGNPs to decrease Aβ-mediated cytotoxicity in neuroblastomal cell lines and in vivo imaging of Aβ-aggregation (Figure 9a).[73] MRI analysis of sialo-MGNPs was well-documented by the Seeberger[74] (Figure 9b) and Davis groups[75] (Figure 9c) by synthesizing sialyl-Lewis^x- and Lewis^x-conjugated MGNPs through amide-coupling reactions between sugar ligands and

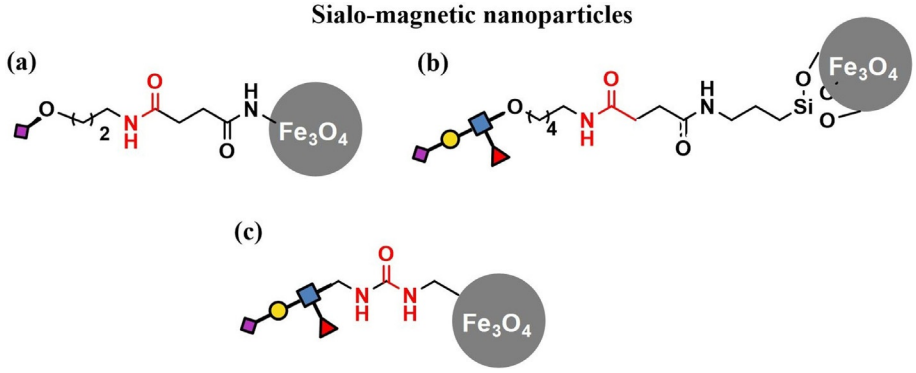

Figure 9. Sialo–magnetic-nanoparticles that were synthesized by using a divergent strategy.

iron nanoparticles. These MGNPs exhibited binding to E-selectin protein in cerebral inflammation in mice models, thus giving an opportunity for the live imaging of brain damage during multiple sclerosis and ischemic strokes.

Sialo-quantum dots (sialo-QDs) are powerful multivalent scaffolds that are widely used as imaging agents and sensor platforms. Nishimura and co-workers synthesized sialo-QDs by coupling aminooxy-functionalized QDs with a sialyl-Lewis^x–ketone linker.[76] Intravenous injection of these nanoparticles led to prolonged lifetimes and wide biodistributions, thus illustrating that it is a versatile platform for bioimaging (Figure 10a). Varki and co-workers used nanometal surface energy transfer (NSET) signals between lectin-conjugated quantum dots and carbohydrate-conjugated AuNPs to quantitatively measure Sia levels in serum.[77] Similarly, Ju and co-workers reported a method for the highly sensitive and selective in situ evaluation of Sia groups on cell surfaces through the multiplex sandwich binding of boric acid groups that were attached to quantum dots to Sia groups on living cells.[78] Recently, we reported the synthesis of sialo-QDs that contained α(2–6) and α(2–6) sialosides by using a divergent strategy (Figure 10b).[79] The in vivo pharmacokinetics and biodistribution of these two sialo-QDs in mice and zebrafish models illustrated the applicability of the simple zebrafish model in Sia research.

Beside gold, silver, and magnetic sialo-nanoparticles, Sia ligands have also been conjugated to fullerene, virus-like nanoparticles, and selenium- and silica-based nanoparticles to develop biosensors and imaging probes. Herczegh and co-workers synthesized a sialo-fullerene by using a click reaction between fullerene–pyrrolidine and sialoside–azide-linker (Figure 10c).[80] These sialo-fullerenes inhibited neuraminidase at micromolar concentrations. Finn and co-workers prepared bifunctional sialo-virus-like particles (sialo-VLPs) by using a click chemistry reaction between alkyne-derivatized VLPs and sialoside–azide/porphyrin (photodynamic therapeutic agent) azide linkers.[81] These particles were able to bind Siglect-2 and overexpressed on CHO cell lines. Photoirradiation of these particles resulted in an increase in the local concentration of singlet-oxygen damage and killed the cells. Behrens and co-workers prepared polySia-conjugated nonporous silica nanoparticles by using click and amide coupling reactions between functionalized nanoparticles and polySia precursors.[82] An in vitro assay showed that the click-chemistry-based nanoparticles decreased cell viability and toxicity at high concentrations, thus indicating that copper ions may be trapped in the porous silica and may induce toxicity. Hence, amide coupling reactions are ideal for nanoparticle-based sialoside functionalization and further biological studies. Chen and co-workers prepared sialo-selenium nanoparticles and utilized the anticancer activity of selenium to enhance the uptake and apoptosis of cancer cells.[83] In an another development, Liu and co-workers synthesized sialo-selenium nanoparticles that contained B6 peptides to cross the blood/brain barrier and target amyloid-β-peptide aggregation in Alzheimer's disease models.[84]

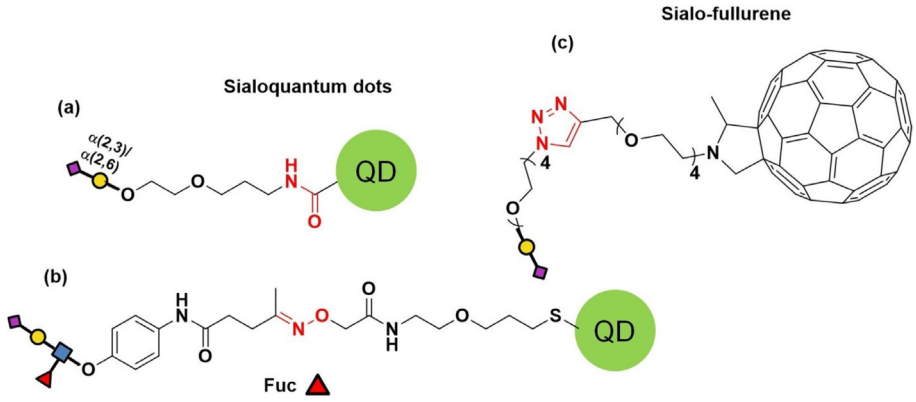

Figure 10. Structures of sialo-nanoparticles. Fuc = Fucose, QD = quantum dot.

6. Conclusions

Over the past few decades, our knowledge of the functions of sialic acid has improved substantially, thanks to multivalent sialosides. Many multivalent tools, such as polymers, dendrimers, liposomes, and nanoparticles, have been functionalized with sialoglycans, either through convergent or divergent strategies and these probes have provided an in-depth knowledge of the Sia-mediated influenza virus, Siglecs, selectin, and β-amyloid interactions. However, despite the remarkable advances that have been made in their synthesis, there are still tremendous challenges in expanding the use of multivalent systems in clinical research, primarily owing to their toxicity and synthetic challenges. Thus, it is imperative to establish more-efficient strategies for their synthesis. Suitable chemical and chemoenzymatic synthetic strategies are expected to procure sialoglycans on the gram scale to understand biological recognition and have a positive influence on clinical studies. Moreover, the solubility problems and aggregation of sialo-nanoparticles does not allow in vivo applications. Therefore, we need appropriate linker systems between the Sia ligand and the nanoparticles to enhance solubility and improve the selective targeting. With the sialo multivalent probes, there are still no defined rules for the design of universal multivalent probes that can meet the needs of a wide range of biological problems. Each time, we need to synthesize a library of multivalent probes to establish the efficacy of multivalency, ligand composition, and concentration for a specific biological function. To alleviate this problem, we need a standard sialo multivalent probe to compare the relative activities.

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Conflict of interest

The authors declare no conflict of interest.

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[1] M. A. McGuckin, S. K. Linden, P. Sutton, T. H. Florin, *Nat. Rev. Microbiol.* **2011**, *9*, 265 – 278.
[2] O. M. Pearce, H. Läubli, *Glycobiology* **2016**, *26*, 111 – 128.
[3] T. Angata, A. Varki, *Chem. Rev.* **2002**, *102*, 439 – 469.
[4] A. Varki, R. D. Cummings, J. D. Esko, H. H. Freeze, P. Stanley, C. R. Bertozzi, G. W. Hart, M. E. Etzler, *Essentials of Glycobiology*, 2nd ed., Cold Spring Harbor Press, New York, **2009**, pp. 199 – 218.
[5] Y. Suzuki, T. Ito, T. Suzuki, R. E. Holland, Jr., T. M. Chambers, M. Kiso, H. Ishida, Y. Kawaoka, *J. Virol.* **2000**, *74*, 11825 – 11831.
[6] C. Schwegmann-Wessels, G. Herrler, *Glycoconjugate J.* **2006**, *23*, 51 – 58.
[7] G. Herrler, S. Szepanski, B. Schultze, *Behring Inst. Mitt.* **1991**, *89*, 177 – 184.
[8] N. Ursula, K. Woellner, G. Gauglitz, T. Stehle, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5219 – 5224.
[9] A. F. Carlin, S. Uchiyama, Y. C. Chang, A. L. Lewis, V. Nizet, A. Varki, *Blood* **2009**, *113*, 3333 – 3336.
[10] C. S. Kim, J. H. Seo, H. J. Cha, *Anal. Chem.* **2012**, *84*, 6884 – 6890.
[11] M. Oda, Y. Terao, J. Sakurai, M. Nagahama, *Toxins* **2015**, *7*, 5268 – 5275.
[12] J. C. Löfling, A. W. Paton, N. M. Varki, J. C. Paton, A. Varki, *Kidney Int.* **2009**, *76*, 140 – 144.
[13] O. J. Adams, M. A. Stanczak, S. von Gunten, H. Läubli, *Glycobiology* **2017**, *28*, 640 – 647.
[14] H. Cao, P. R. Crocker, *Immunology* **2011**, *132*, 18 – 26.
[15] M. K. O'Reilly, J. C. Paulson, *Trends Pharmacol. Sci.* **2009**, *30*, 240 – 248.
[16] M. S. Alphey, H. Attrill, P. R. Crocker, D. M. van Aalten, *J. Biol. Chem.* **2003**, *278*, 3372 – 3377.
[17] G. Nicoll, T. Avril, K. Lock, K. Furukawa, N. Bovin, P. R. Crocker, *Eur. J. Immunol.* **2003**, *33*, 1642 – 1648.
[18] T. F. Tedder, D. A. Steeber, A. Chen, P. Engel, *FASEB J.* **1995**, *9*, 866 – 873.
[19] S. R. Barthel, J. D. Gavino, L. Descheny, C. J. Dimitroff, *Expert Opin. Ther. Targets* **2007**, *11*, 1473 – 1491.
[20] A. Natoni, M. S. Macauley, M. E. O'Dwyer, *Front. Oncol.* **2016**, *6*, 93.
[21] S. Schedin-Weiss, B. Winblad, L. O. Tjernberg, *FEBS J.* **2014**, *281*, 46 – 62.
[22] T. K. Dam, R. Roy, D. Page, C. F. Brewer, *Biochemistry* **2002**, *41*, 1359 – 1363.
[23] M. Delbianco, P. Bharate, S. Varela-Aramburu, P. H. Seeberger, *Chem. Rev.* **2016**, *116*, 1693 – 1752.
[24] A. K. Adak, B. Y. Li, C. C. Lin, *Carbohydr. Res.* **2015**, *405*, 2 – 12.
[25] M. Marradi, F. Chiodo, I. García, S. Penadés, *Chem. Soc. Rev.* **2013**, *42*, 4728 – 4745.
[26] R. Roy, M. G. Baek, *J. Biotechnol.* **2002**, *90*, 291 – 309.
[27] Y. Miura, Y. Hoshino, H. Seto, *Chem. Rev.* **2016**, *116*, 1673 – 1692.
[28] P. M. Chaudhary, R. V. Murthy, R. Kikkeri, *J. Mater. Nanosci.* **2014**, *1*, 7 – 11.
[29] M. N. Matrosovich, L. V. Mochalova, V. P. Marinia, N. E. Byramova, N. V. Bovin, *FEBS Lett.* **1990**, *272*, 209 – 212.
[30] M. Mammen, G. Dahmann, G. M. Whitesides, *J. Med. Chem.* **1995**, *38*, 4179 – 4190.
[31] T. Tanaka, H. Ishitani, Y. Miura, K. Oishi, T. Takahashi, T. Suzuki, S.-I. Shoda, Y. Kimura, *ACS Macro Lett.* **2014**, *3*, 1074 – 1078.
[32] A. H. Courtney, E. B. Puffer, J. K. Pontrello, Z.-Q. Yang, L. L. Kiessling, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 2500 – 2505.
[33] S. Tang, W. B. Puryear, B. M. Seifried, X. Dong, J. A. Runstadler, K. Ribbeck, B. D. Olsen, *ACS Macro Lett.* **2016**, *5*, 413 – 418.
[34] S. Ohira, Y. Yasuda, I. Tomita, K. Kitajima, T. Takahashi, C. Satob, H. Tanaka, *Chem. Commun.* **2017**, *53*, 553 – 556.
[35] S. Woerly, S. Fort, I. Pignot-Paintrand, C. Cottet, C. Carcenac, M. Savasta, *Biomacromolecules* **2008**, *9*, 2329 – 2337.
[36] M. Ogata, A. Koizumi, T. Otsubo, K. Ikeda, M. Sakamoto, R. Aita, T. Kato, E. Y. Park, T. Yamanaka, K. I. P. J. Hidari, *Biosci. Biotechnol. Biochem.* **2017**, *81*, 1520 – 1528.
[37] M. Ogata, K. I. Hidari, W. Kozaki, T. Murata, J. Hiratake, E. Y. Park, T. Suzuki, T. Usui, *Biomacromolecules* **2009**, *10*, 1894 – 1903.
[38] S. Yamaguchi, A. Yoshimura, Y. Yasuda, A. Mori, H. Tanaka, T. Takahashi, K. Kitajima, C. Sato, *ChemBioChem* **2017**, *18*, 1194 – 1203.
[39] W. Hai, T. Goda, H. Takeuchi, S. Yamaoka, Y. Horiguchi, A. Matsumoto, Y. Miyahara, *ACS Appl. Mater. Interfaces* **2017**, *9*, 14162 – 14170.
[40] M. L. Huang, M. Cohen, C. J. Fisher, R. T. Schooley, P. Gagneux, K. Godula, *Chem. Commun.* **2015**, *51*, 5326 – 5329.
[41] M. L. Huang, S. C. Purcell, S. Verespy, Y. Wang, K. Godula, *Biomater. Sci.* **2017**, *5*, 1537 – 1540.
[42] M. Cohen, H. P. Senaati, C. J. Fisher, M. L. Huang, P. Gagneux, K. Godula, *ACS Cent. Sci.* **2016**, *2*, 710 – 714.
[43] L. Huang, K. Godula, *ACS Chem. Neurosci.* **2014**, *5*, 873 – 875.
[44] M. L. Huang, R. A. Smith, G. W. Trieger, K. Godula, *J. Am. Chem. Soc.* **2014**, *136*, 10565 – 10568.
[45] J. E. Hudak, S. M. Canham, C. R. Bertozzi, *Nat. Chem. Biol.* **2014**, *10*, 69 – 75.
[46] D. Patel, J. Henry, T. Good, *Biochim. Biophys. Acta Gen. Subj.* **2006**, *1760*, 1802 – 1809.
[47] D. A. Patel, J. E. Henry, T. A. Good, *Brain Res.* **2007**, *1161*, 95 – 105.
[48] R. Clayton, J. O. Hardman, C. C. LaBranche, K. D. McReynolds, *Bioconjugate Chem.* **2011**, *22*, 2186 – 2197.

[49] J.-I. Sakamoto, T. Koyama, D. Miyamoto, S. Yingsakmongkon, K. I. P. J. Hidari, W. Jampangern, T. Suzuki, Y. Suzuki, Y. Esumi, K. Hatano, D. Terunuma, K. Matsuoka, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 717 – 721.
[50] J.-I. Sakamoto, T. Koyama, D. Miyamoto, S. Yingsakmongkon, K. I. P. J. Hidari, W. Jampangern, T. Suzuki, Y. Suzuki, Y. Esumi, T. Nakamura, K. Hatano, D. Terunuma, K. Matsuoka, *Bioorg. Med. Chem.* **2009**, *17*, 5451 – 5464.
[51] M. M. Rahman, S. Kitao, D. Tsuji, K. Suzuki, J.-I. Sakamoto, K. Matsuoka, F. Matsuzawa, S.-I. Aikawa, K. Itoh, *Glycobiology* **2013**, *23*, 495 – 504.
[52] H. Oka, T. Onaga, T. Koyama, C.-T. Guo, Y. Suzuki, Y. Esumi, K. Hatano, D. Terunuma, K. Matsuoka, *Bioorg. Med. Chem.* **2009**, *17*, 5465 – 5475.
[53] I. Papp, C. Sieben, A. L. Sisson, J. Kostka, C. Bçttcher, K. Ludwig, A. Herrmann, R. Haag, *ChemBioChem* **2011**, *12*, 887 – 895.
[54] S. Has, B. E. Collins, P. Bengtson, J. C. Paulson, *Nat. Chem. Biol.* **2005**, *1*, 93 – 97.
[55] W. C. Chen, G. C. Completo, D. S. Sigal, P. R. Crocker, A. Saven, J. C. Paulson, *Blood* **2010**, *115*, 4778 – 4786.
[56] M. Hirai, H. Minematsu, N. Kondo, K. Oie, K. Igarashi, N. Yamazaki, *Biochem. Biophys. Res. Commun.* **2007**, *353*, 553 – 558.
[57] F. Xua, A. Bandara, H. Akiyama, B. Eshaghia, D. Stelter, T. Keyesa, J. E. Strauba, S. Gummuluru, B. M. Reinhard, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E9041 – E9050.
[58] G. N. Shenoy, J. Loyall, C. S. Berenson, R. J. Kelleher, Jr., V. Iyer, S. V. Balu-Iyer, K. Odunsi, R. B. Bankert, *J. Immunol.* **2018**, *201*, 3750 – 3758.
[59] V. Leonhard, R. V. Alasino, I. D. Bianco, A. G. Garro, V. Heredia, D. M. Beltramo, *J. Controlled Release* **2012**, *162*, 619 – 627.
[60] R. A. Bader, A. L. Silvers, N. Zhang, *Biomacromolecules* **2011**, *12*, 314 – 320.
[61] X. Luo, L. Hu, H. Zheng, M. Liu, X. Liu, C. Li, Q. Qiu, Z. Zhao, X. Cheng, C. Lai, Y. Su, Y. Deng, Y. Song, *Drug Delivery* **2018**, *25*, 1200 – 1212.
[62] Y. R. Kikkeri, *Chem. Commun.* **2012**, *48*, 7265 – 7267.
[63] J. M. de la Fuente, S. Penadés, *Biochim. Biophys. Acta.* **2006**, *1760*, 636 – 651.
[64] K. Niikura, K. Nagakawa, N. Ohtake, T. Suzuki, Y. Matsuo, H. Sawa, K. Ijiro, *Bioconjugate Chem.* **2009**, *20*, 1848 – 1852.
[65] V. Poonthiyil, P. T. Nagesh, M. Husain, V. B. Golovko, A. J. Fairbanks, *ChemistryOpen* **2015**, *4*, 708 – 716.
[66] I. Papp, C. Sieben, K. Ludwig, M. Roskamp, C. Böttcher, S. Schlecht, A. Herrmann, R. Haag, *Small* **2010**, *6*, 2900 – 2906.
[67] C. L. Schofield, M. J. Marín, M. Rejzek, P. R. Crocker, R. A. Field, D. A. Russell, *Analyst* **2016**, *141*, 5799 – 5809.
[68] M. Chikae, T. Fukuda, K. Kerman, K. Idegami, Y. Miura, E. Tamiya, *Bioelectrochemistry* **2008**, *74*, 118 – 123.
[69] M. J. Marín, A. Rashid, M. Rejzek, S. A. Fairhurst, S. A. Wharton, S. R. Martin, J. W. McCauley, T. Wileman, R. A. Field, D. A. Russell, *Org. Biomol. Chem.* **2013**, *11*, 7101 – 7107.
[70] F. Feng, Y. Sakoda, T. Ohyanagi, N. Nagahori, H. Shibuya, M. Okamastu, N. Miura, H. Kida, S.-I. Nishimura, *Antiviral Chem. Chemother.* **2013**, *23*, 59 – 65.
[71] H. J. Noh, A.-R. Im, H.-S. Kim, J. K. Sohng, C.-K. Kim, Y. S. Kim, S. Cho, Y. Park, *J. Nanosci. Nanotechnol.* **2012**, *12*, 3884 – 3895.
[72] K. El-Boubbou, D. C. Zhu, C. Vasileiou, B. Borhan, D. Prosperi, W. Li, X. Huang, *J. Am. Chem. Soc.* **2010**, *132*, 4490 – 4499.
[73] H. Kouyoumdjian, D. C. Zhu, M. H. El-Dakdouki, K. Lorenz, J. Chen, W. Li, X. Huang, *ACS Chem. Neurosci.* **2013**, *4*, 575 – 584.
[74] T. D. Farr, C.-H. Lai, D. Grünstein, G. Orts-Gil, C.-C. Wang, P. Boehm-Sturm, P. H. Seeberger, C. Harms, *Nano Lett.* **2014**, *14*, 2130 – 2134.
[75] S. I. van Kasteren, S. J. Campbell, S. Serres, D. C. Anthony, N. R. Sibson, B. G. Davis, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 18 – 23.
[76] T. Ohyanagi, N. Nagahori, K. Shimawaki, H. Hinou, T. Yamashita, A. Sasaki, T. Jin, T. Iwanaga, M. Kinjo, S.-I. Nishimura, *J. Am. Chem. Soc.* **2011**, *133*, 12507 – 12517.
[77] R. Kikkeri, V. Padler-Karavani, S. Diaz, A. Verhagen, H. Yu, H. Cao, M. A. Langereis, R. J. De Groot, X. Chen, A. Varki, *Anal. Chem.* **2013**, *85*, 3864 – 3870.
[78] E. Han, L. Ding, H. Ju, *Anal. Chem.* **2011**, *83*, 7006 – 7012.
[79] R. Yadav, P. M. Chaudhary, B. Subramani, S. Toraskar, H. Bavireddi, R. V. Murthy, S. Sangabathuni, R. Kikkeri, *ACS Appl. Mater. Interfaces* **2018**, *10*, 28322 – 28330.
[80] S. Tollas, I. Bereczki, A. Borbás, G. Batta, E. Vanderlinden, L. Naesens, P. Herczegh, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2420 – 2423.
[81] J.-K. Rhee, M. Baksh, C. Nycholat, J. C. Paulson, H. Kitagishi, M. G. Finn, *Biomacromolecules* **2012**, *13*, 2333 – 2338.
[82] S. Williams, A. Neumann, I. Bremer, Y. Su, G. Dräger, C. Kasper, P. Behrens, *J. Mater. Sci.: Mater. Med.* **2015**, *26*, 125.
[83] J.-S. Zhenga, S.-Y. Zheng, Y.-B. Zhang, B. Yu, W. Zheng, F. Yang, T. Chen, *Colloids Surf. B* **2011**, *83*, 183 – 187.
[84] T. Yin, L. Yang, Y. Liu, X. Zhou, J. Sun, J. Liu, *Acta Biomater.* **2015**, *25*, 172 – 183.

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