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Perspective

Polymers Inspired by Heparin and Heparan Sulfate for Viral Targeting

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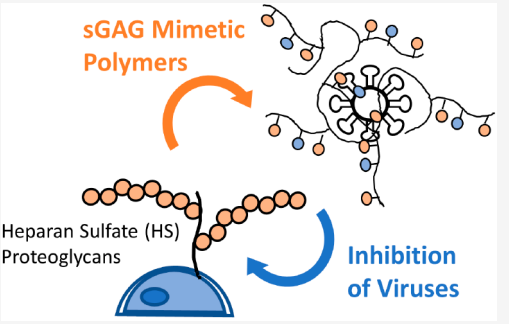
ABSTRACT: Heparin (HP) and heparan sulfate (HS) are linear, anionically charged polysaccharides well-known for their diverse biological activities. While HP is generally localized in mast cells and in connective tissues, HS is part of the glycocalyx and involved in the attachment of viruses to host cells, constituting the first step of an infection. HP and HS also exhibit antiviral activity by blocking viral receptors, thereby inhibiting viruses from engaging with host cells. Inspired by their structural features, such as their high molecular weight and polyanionic character, various synthetic polymers mimicking HP/HS have been developed and used as model systems to study bioactivity, as well as for therapeutic applications. This Perspective provides an overview of the roles of HP/HS in viral engagement, and examines historical and recent approaches toward oligo-/polysaccharide, glycopolymer, and anionic polymer HP/HS mimetics. An overview of current applications and future prospects of these molecules is provided, demonstrating their potential in addressing current and future epidemics and pandemics.



INTRODUCTION

Heparin (HP) and heparan sulfate (HS) are linear, structurally diverse polysaccharides that belong to a class of sugars known as glycosaminoglycans (GAGs), a diverse class of linear, negatively charged polysaccharides. With the exception of hyaluronic acid (HA), all GAGs are attached to protein cores to form proteoglycans (PG). HP is most frequently found in mast cells and is known for its anticoagulant properties, while HS can be found within the glycocalyx, a complex matrix of glycoproteins and glycolipids that surround the cell membrane.[1−5] Notably, HS is one of the most abundant GAGs making up 50−90% of the total amount of the GAG pool.[6] Together, HP and HS have been shown to be involved in several critical physiological and pathological processes from embryonic development to inflammation, angiogenesis, neurodegeneration, cardiovascular disorders, cancers, and infection.[7−9] HP is also used commercially as an anticoagulant, resulting in billions of dollars in sales annually.[4,5]

HP/HS are biosynthesized beginning with the assembly of a linkage region connecting the protein core with the polysaccharide. This region is composed of GlcA-β-1,3-Gal-β-1,3-Gal-β-1,4-Xyl-Ser. The reducing xylose is first phosphorylated and glycosylated to a serine residue of the protein. After completion of the core, the main polysaccharide is assembled by using two monosaccharides: a uronic acid, D-glucuronic acid (GlcA), and a hexosamine, *N*-acetyl-D-glucosamine (GlcNAc). These monosaccharides, which are introduced in an alternating fashion, are then further diversified by *N*-deacetylation, *N*- and *O*-sulfation, and C5-epimerization of D-glucuronic acid to L-iduronic acid (IdoA). Variations in saccharide composition, glycosidic linkage, and sulfation patterns introduced during biosynthesis result in the extraordinary heterogeneity and structural complexity of these biopolymers (see Figure 1).[1,10] For example, up to 48 unique uronic acid/hexosamine disaccharide combinations can be used in the assembly of HS.[11,12] While HP carries the highest number of sulfate groups in the form of uniform overall sulfation patterns, which are also found in related sulfated GAGs (sGAGs) such as chondroitin sulfate (CS), HS shows distinct sequences, or patches of higher sulfation, interspersed by less or nonsulfated sequences resulting in characteristic sequenced sulfation patterns (see Figure 1).[1,10,13]

HP/HS fragments derived from natural and/or synthetic sources continue to play an important role in elucidating the importance of sulfation patterning, the so-called sulfation code,[14] on the HS interactome.[15−18] One important example comes from seminal work on the role of HS in binding fibroblast growth factor (FGF). Studies revealed a complex series of interactions governed by salt bridges and hydrogen-bonding interactions between the sulfate groups of HS and the

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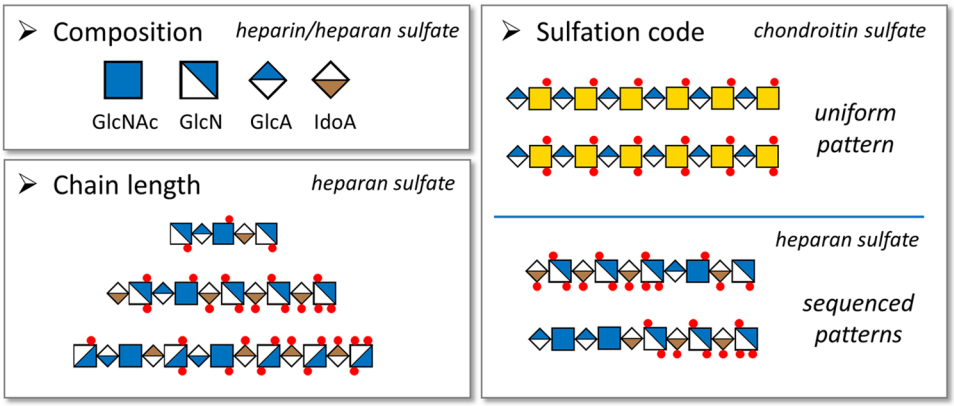

Figure 1. Structural parameters of sGAGs including heparin and heparan sulfate (shown here: CS, yellow square = *N*-acetyl-D-galactosamine) affecting their biological function (red dots = sulfate groups).

polar amino acid residues of FGF.[19] Site-selective modification of shorter HS oligosaccharides introducing uniform sulfation patterns (Figure 1) revealed even more distinct preferences between HS and FGF; FGF2 selectively bound to HS fragments with 2-*O*-sulfation but not 6-*O*-sulfation, while FGF10 is selectively bound by HS fragments with 6-*O*-sulfation but not 2-*O*-sulfation.[20] Studies have also revealed that the amount and positioning of the sulfate groups can also impact the stiffness of the overall HSPG, creating yet another dimension of variation that has shown to be important for biological activity.[21−24] For example, the interaction between HS and FGF is also governed by van der Waals contacts and HS conformational dynamics, both of which are influenced by sulfation.[25] Other biological processes where the sulfation code has been shown to influence HS-receptor interactions include neuronal development,[26,27] Alzheimer's disease,[28] cancer,[29] and viral pathogenesis.[30−33] Deciphering the sulfation code and, in general, targeting HS−protein interactions require access to well-defined structures. HP is often used as an abundant and readily available analogue of HS for *in vitro* identification of sGAG-binding proteins and in studies examining its pharmaceutical potential. In contrast, *in vivo* interactions with proteins mainly involve HSPGs.[21,34] Notably, isolation of HP and HS from natural sources has its limitations due to their inherent heterogeneity, difficulty in purification, and risk of contamination, especially when sourced from animals.[35−37] Chemical and chemoenzymatic synthesis, which results in the production of well-defined structures, presents an important alternative, and the examples that have been reported in the literature have contributed greatly to ongoing research on the specificity of sGAG−protein interactions.[14,18,38,39] However, chemoenzymatic processes remain challenging and complex and often lead to the production of only small quantities of material for evaluation. Therefore, because of their structural variety and the remaining limitations of sourcing HP/HS with controlled structural variations, the map of their structure−property correlations is still incomplete, and many questions remain unanswered.

Role of HP and HS in Viral Engagement. As we write this Perspective, it has been two years since the beginning of the COVID-19 pandemic, and so it is no surprise that there has been a resurgence in the interest of the role of HSPGs in viral infections and the potential of soluble HP and HS fragments and other sGAGs as antivirals. Almost 50 years prior to some of the first studies on the role of HSPG's on herpes simplex virus (HSV) infection, polysaccharides were observed to exhibit antiviral activity related to their polyanionic character.[40,41] Today, we understand that these polysaccharides act as inhibitors, blocking viral HSPG binding sites required for attachment of the virus to the host cell, one of the first steps of an infection.[42−46] One factor that remains under debate is whether virus−HSPG binding is the result of the natural history of the virus, or rather an evolutionary adaptation due to multiple passages in human tissue culture or through laboratory directed mutations.[45,46] Regardless of the mechanism, these studies, and many others that have followed, have established that HSPGs are involved in viral engagement at the extracellular level; negatively charged polysaccharides interact with positively charged amino acid residues on exposed viral capsid proteins to initiate direct viral entry or to localize the virus to engage receptors required for viral entry. Intracellular effects that occur upon extracellular binding have also been shown to be important for virulence and include transcriptional modulation and cellular signaling.[46−57]

Several models for how viruses engage native HSPGs or therapeutic sGAGs have been proposed. As previously mentioned, and as shown in Figure 2A, viruses may engage HSPGs as a primary receptor required for infection. HSV-1 and HSV-2 are currently the only viruses known to require direct engagement of specific HS motifs to infect cells.[42,58] More commonly, as shown in Figure 2B, HSPGs serve as a coreceptor providing localization of the virus at the cell surface, leading to increased viral concentration while bringing the virus closer to other, more specific receptors which are then required for entry. Here, HSPGs mediate virus−cell attachment. Several viruses, including several human papilloma viruses (HPV), human immunodeficiency virus (HIV), dengue viruses (DENV), hepatitis B viruses (HBV), hepatitis C virus (HCV), hepatitis E virus, (HEV), Merkel cell polyomavirus (MCPyV), rabies virus (RABV), respiratory syncytial virus (RSV), coronaviruses such as SARS-CoV-2 and HCoV-NL63, and human cytomegalovirus (HCMV), have been shown to use this mechanism in laboratory studies.[31,33,56,59−64] On the other hand, HSPGs can also serve to protect a cell from viral engagement by shielding key receptors, as shown in Figure 2C. For example, studies with Venezuelan equine encephalitis virus (VEEV) revealed that HS binding variants had lower fitness *in vivo* in contrast to non-HS binding variants.[45] Although the mechanism for this is not well understood, these effects are

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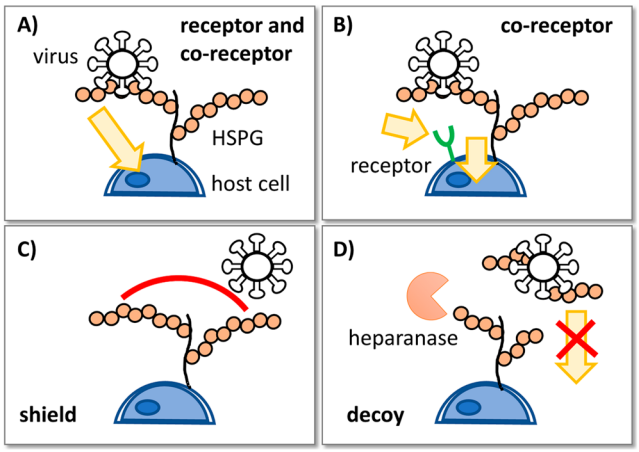

Figure 2. Schematic representation of the role of native HSPGs and therapeutic sGAGs in virus attachment and infection: (A) HSPG acts as a receptor enabling cell infection; (B) virus binding to HSPG as co-receptor facilitating subsequent binding to more specific receptors; (C) HSPGs shield cell surface from virus; (D) soluble sGAGs act as decoy blocking viral binding sites.

presumed to be due to attenuated virulence, which results in accelerated clearance. Infection by members of the Flaviviridae family, including DENV, Japanese encephalitis virus (JEV), and West Nile virus (WNV), has also been shown to be attenuated through this mechanism.[52] Finally, as shown in Figure 2D, soluble sGAGs can serve as decoys by occupying the binding sites of viral proteins, thus attenuating/blocking cell surface binding and subsequent infection.[65−69] The regulation of cellular heparanases upon viral engagement suggests that this mechanism may also play an important role in inhibiting viral attachment and infection.[55] Notably, there are still several viruses that engage HSPGs through mechanisms that are yet to be determined, demonstrating that there is still much to be discovered in this area.[46,70]

The structural heterogeneity and high abundance of HSPGs on cell surfaces make them ideal targets for pathogen attachment. Indeed, other pathogens, including bacteria, parasites, and fungi, use GAGs, and specifically HSPGs, as receptors, often for first attachment to the host cell or tissue.[70−72] The important role of HSPGs in the virus life cycle has prompted numerous investigations into the use of HS, HP, and structural analogues thereof as potential active compounds for the prophylactic and therapeutic treatment of viral infections. For example, patients treated for SARS-CoV-2 infections receiving HP to reduce the risk of thrombosis, likely benefited from an additional antiviral effect; HP treatment resulted in a less severe course of infection and reduced mortality, potentially due to its antiviral activity as an inhibitor of cell attachment and subsequent infection.[73]

HS MIMETICS AS VIRAL INHIBITORS

General Features and Classification of HS Mimetics.

The therapeutic potential of soluble HP/HS as viral inhibitors has inspired a variety of sGAG mimetics over the past 40 years. It is important to note that HP/HS protein engagement is generally governed by specific interactions between the differentially sulfated regions of the polysaccharide and the protein. Most of the mimetics covered in this Perspective aim to emulate these key interactions, albeit generally through nonspecific, fully sulfated structures that generally resemble sGAGs. Therefore, in the following section, we will generalize the HP/HS mimetics described as sGAG mimetics while noting, where possible, when specific HP/HS mimicry was targeted.

For this Perspective, we differentiate sGAG mimetics first into low-molecular-weight compounds, particle-based compounds, including dendritic structures, and polymers (see

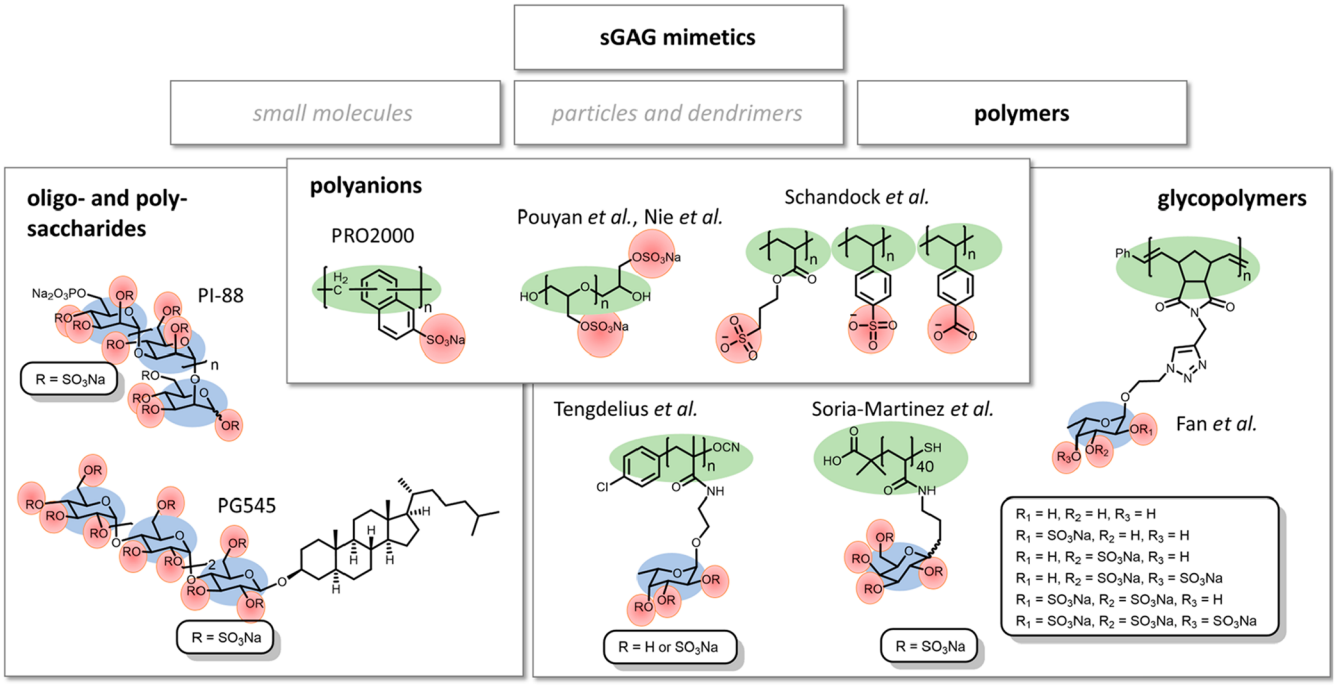

Figure 3. sGAG mimetics with focus on polymeric sGAG mimetics classified as oligo- and polysaccharides, polyanions, or glycopolymers (color code: red = anionic charge; blue = glycan content; green = synthetic polymer scaffold). Exemplary structures are shown.

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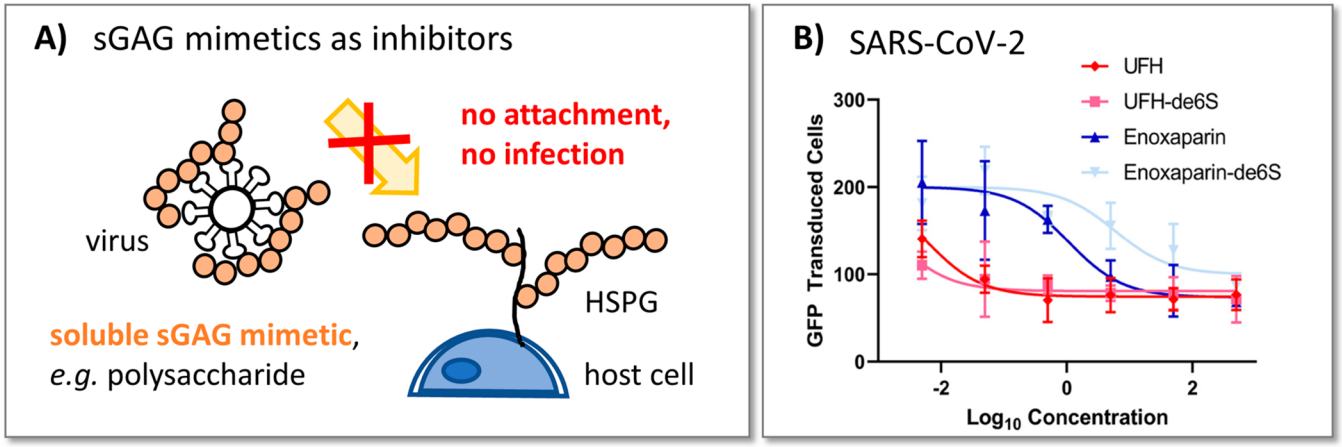

Figure 4. (A) Schematic presentation of the inhibitory activity of sGAG mimetics by blocking binding sites of the virus usually engaged in HSPG-mediated cell attachment. (B) Inhibition of SARS-CoV-2 by unfractionated (high-molecular-weight) heparin (UFH), UFH-de6S (UFH selectively desulfated at the 6-*O*-position), enoxaparin (low-molecular-weight heparin), and enoxaparin-de6S (enoxaparin selectively desulfated at the 6-*O*-position). Reproduced with permission from ref 163. Copyright 2021 American Society for Microbiology.

Figure 3). We then exclusively focus on polymer-based mimetics and refer the reader to recent reviews highlighting work on the other classes of sGAG mimetics.[74−83] Within the class of polymeric sGAG mimetics, we focus on oligo-/polysaccharides, glycopolymers consisting of a synthetic backbone and glycan side chains, and anionic polymers with no glycan component.

Oligo- and polysaccharide-based sGAG mimetics consist of a series of fully or selectively sulfated monosaccharide units linked together in a linear fashion through glycosidic linkages. Historically, they are derived from natural, sulfated carbohydrate polymers,[84,85] by chemical sulfation of naturally occurring poly-/oligosaccharides,[86] and by enzymatic degradation of naturally occurring polysaccharides.[87,88] In recent years, synthetic[89−102] and chemoenzymatic methods[103−108] have also been used to generate a diverse array of structures adding to a growing body of work in this area.

In contrast, the other two classes of polymeric sGAG mimetics generally consist of a synthetic backbone with pendant functional groups and can be loosely organized into those presenting globally or selectively sulfated glycosides (glycopolymers), and those containing no sugar but other anionic residues (polyanions).[74,75,109−114] Polymerization techniques used to obtain such mimetics include cyanoxyl-mediated free radical copolymerization,[115−120] step growth copolymerization,[121−123] ring-opening metathesis polymerization,[124−128] free radical,[129−137] and reversible addition−fragmentation chain transfer (co)polymerization.[138−146] Notably, controlled polymerization techniques have been used to synthesize sGAG mimetics with low dispersity and high structural control while tolerating numerous functional groups and reaction conditions.

A key challenge in the synthesis of both types of polymeric sGAG mimetics is the introduction of site-selective sulfation. Glycopolymer sulfation can be introduced at different stages. One possibility is to apply global sulfation postpolymerization.[144] If desired, the degree of sulfation can be adjusted by varying the equivalents of sulfating reagent.[123,129] For site-selective sulfation the sequence is reversed, and sulfation is conducted prepolymerization.[124,125,127,133−136,139] Copolymerization of different monomers also enables decoupling of carbohydrate and sulfate/sulfonate motifs.[143] Finally, for non-carbohydrate-based polymers, charges can be introduced through the different functional groups.[121,122,130,132,137,138,140−142,147−155] The flexibility of the synthesized compounds can be tuned by variation of the backbone composition which depends, among others, on the choice of monomer and the respective polymerization technique.[111,123,125]

Combined, these approaches have yielded numerous sGAG mimetics with varying properties and in some cases tunability.[74,109−114] In the past 40 years, a number of these approaches have been applied toward the synthesis of sGAG mimetics for viral targeting, and they have been studied as model systems to learn more about the roles of native HSPGs and therapeutic HP and HS in viral engagement and/or with the goal of developing pharmacologically relevant molecules to prevent and treat viral infections.

Polymeric sGAG Mimetics Targeting Viruses. As previously mentioned, most sGAG mimetics act as soluble inhibitors of viral entry, though a few have been shown to have virucidal properties. In natural systems, HSPG−virus engagement is mediated by multiple electrostatic interactions between the negatively charged sulfate groups of the HS polysaccharide and clusters of basic amino acids including arginine, lysine, and histidine arranged along exposed three-dimensional channels on capsid proteins.[54] The domain organizations of the HS chains have also been shown to play an important role in viral engagement; *N*-sulfated domains of approximately 12−20 residues alternate with *N*-acetyl-rich domains which are generally longer in length, resulting in conformational variations that affect polysaccharide rigidity and the multivalent presentation of the sulfates in three-dimensional space.[54] Natural oligo-/polysaccharides, glycopolymers, and anionic polymers have been designed to mimic these properties and are the primary focus of this Perspective. While here the focus is exclusively on sGAG mimetic polymeric compounds, there are also other classes of polymers with antiviral activity, and we direct the readers to recent reviews on the topic.[156,157]

Oligo-/Polysaccharides as sGAG Mimetics for Viral Targeting. The antiviral potential of anionic oligo-/polysaccharides was serendipitously discovered over four decades before the formal identification of HSPGs as receptors for HSV.[40,41] Today, many anionic polysaccharides with antiviral

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properties are known and have been recently reviewed.[84,85,158,159] Examples include HP, CS, dermatan sulfate, fucoidan, galactan sulfates, galactan hybrids, members of the carrageenan family, rhamnan sulfate, ulvan, and alginic acid. Combined, these polysaccharides have shown inhibition against several classes of viruses including HSV-1 and HSV-2, HCMV, HIV, DENV, HIV, HPV, RSV, human metapneumovirus (HPMV), Ebola virus (EBOV), Marburg virus (MARV), VSV, enterovirus A71 (EV-A71), and SARS-CoV-2.[56,84,85,156,158−163] Studies with natural, anionic polysaccharides have provided valuable information about numerous viral interactions resulting in several clinical trials. However, it is important to note that their use has been somewhat limited by the ability to access pure structures; most polysaccharides are produced as heterogeneous mixtures.[38,39]

HP was one of the first polysaccharides used to inhibit viral entry of HSV.[164] In this case, HP was found to act as a competitive inhibitor preventing engagement of the viral spike (S) protein in binding cell−surface HSPGs, a mechanism that has more recently been shown for SARS-CoV-2 (see Figure 4).[60,163,165−167] Results from studies with HP ultimately inspired the exploration of the oligosaccharide-based heparanase inhibitors Muparfostat (PI-88) and Pixatimod (PG545) (see Figure 3) as antiviral HS mimetics.[75,168] The oligosaccharide mixture Muparfostat (the chemical structure of the major component is shown in Figure 3) was shown to inhibit cell-to-cell spread of HSV.[169] Pixatimod, an advanced derivative of Muparfostat with improved features including a fully sulfated, isomerically pure oligosaccharide core and a hydrophobic cholestanol aglycone component, was shown to exhibit virucidal activity against HSV and protection against a variety of other viruses including SARS-CoV-2.[75,170−175] Similarly, another heparanase inhibitor Roneparstat (SST0001), a 15−25 kDa *N*-acetylated and glycol split version of heparin,[176,177] was shown to exhibit antiviral activity against SARS-CoV-2 as well as human T-lymphotropic virus 1 (HTLV-1) and HIV.[178] Notably, both Pixatimod and Roneparstat are in clinical trials and are being investigated for their immunomodulatory properties.[176,179]

A host of natural marine glycans have shown broad-spectrum inhibition of several viruses.[85] One of the earliest examples were the carrageenans which have shown potent broad-spectrum activity against HSV-1 and HSV-2,[180] DENV2,[181] HIV,[180,182] HPV,[183,184] HMPV,[185] EBOV and MARV.[186] For this reason they have been explored in clinical trials for the prevention and treatment of both HIV[180] and HPV.[184]

Natural and chemoenzymatically modified fucoidans have recently gained attention for their broad-spectrum activity against several viruses including HSV-1, HSV-2, and HIV.[187] For example, a vaginitis challenge model revealed that natural and chemoenzymatically synthesized fucoidan analogues from *F. evanescens* could inhibit HSV-2 infection *in vivo* with the native glycan having a higher selectivity index (SI) of >40 compared to >20 for the chemoenzymatically modified fucoidan when applied before or in the early stages of the HSV-2 infection. In comparison, *in vitro* studies showed little difference in HSV-2 viral inhibition for either compound, requiring 10 mg/kg to prevent lethal infection by 44−56%. Another recent study revealed that a branched fucoidan could serve as an effective antiviral against SARS-CoV-2 with an EC_{50} of 8.3 ± 4.6 μg/mL.[188]

Chemoenzymatic methods for the preparation of defined sGAG mimetics for targeting viruses have gained attention in recent years. Copeland and co-workers incubated an octasaccharide produced from heparin through enzymatic degradation with HS 3-*O*-sulfotransferase isoform 3 to produce the corresponding 3-*O*-sulfated oligosaccharide.[189] Their goal was to mimic the active HS domain of the HSV-1 entry receptor which is known to engage 3-*O*-sulfated HS. The corresponding 3-*O*-sulfated octasaccharide was shown to completely block HSV-1 infection at 60 μM; the corresponding 3-OH octasaccharide showed only 50% viral blocking at the same concentration. In addition to producing a strong, selective inhibitor of HSV-1, this work highlighted the importance of specific sulfation patterns in viral infectivity. Notably, similar compounds were later synthesized and evaluated by Hu and colleagues that complemented the results by Liu et al.[190]

Chemical approaches to the synthesis of sGAG mimetics have also played an important role in their development. One early example was the synthesis of cellulose sulfate as a topical contraceptive antimicrobial agent known as Ushercell. Ushercell was shown to serve as a broad-spectrum antiviral against HSV-1, HSV-2, and HIV with IC_{50} values of 59 ng/mL, 24 ng/mL, and 3−78 μg/mL, respectively.[191] Ushercell was also shown to inhibit HPV with an IC_{50} of 10−100 μg/mL.[192] Another recent example is the use of poly carboxymethylglucose sulfate, also known as Cacicol, as an inhibitor of both HSV-1 and varicella zoster virus (VZV).[193]

A more recent example of the chemical synthesis of an sGAG mimetic was reported by Möller and co-workers, who produced fully sulfated HA and CS analogues for biological evaluation.[194] Inhibition studies with SARS-CoV-2 infections showed greater inhibition potential of the fully sulfated HA analogue with an SI = 232.68 compared to the fully sulfated CS analogue (SI = 82.39) despite having the same degree of sulfation. The authors speculated that the substantially high molecular weight of the fully sulfated HA derivative (nearly 3 times that of the CS analogue) might be the reason for this observation.

An interesting application for using oligo-/polysaccharides as antivirals is illustrated by the work by Kim and co-workers, who used a cell-surface-inspired approach to develop GlycoGrip as a GAG-based lateral flow assay for detecting SARS-CoV-2.[195] Sulfated oligosaccharides, including HS, 6-*O*-sulfo HS (HS6S), CS, and sulfated dextran, were used to capture and detect different variants of SARS-CoV-2 with no cross-reactivity to other viruses and thus high specificity. At the same time, their setup could be easily adapted and applied for the detection of other viruses known to engage in GAG mediated cell attachment. Although it is important to recognize that the glycans prepared in this study were not used to directly inhibit SARS-CoV-2 entry, today we are aware of the importance of also detecting and identifying viral infections in fighting against ongoing and potential future pandemics.

Glycopolymers as sGAG Mimetics and as Viral Inhibitors. Non-polysaccharide sGAG mimetics targeting viruses benefit from straightforward syntheses and tunable properties by taking advantage of the full arsenal of tools that synthetic polymer chemistry has to offer. In this way, systematic alterations of the polymer structure can be used to examine structure−property correlations and to potentially increase their selectivity and potency for biomedical applications. In this

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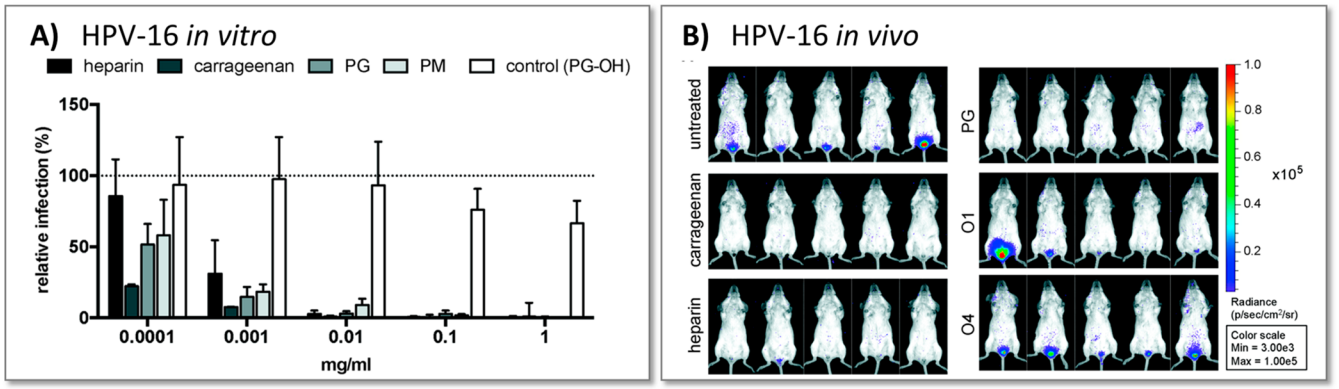

Figure 5. (A) Inhibition of HPV-16 by polysaccharides and glycopolymers in an infection cell assay. (B) Inhibition of HPV-16 in vaginal mouse study (glucose side chain = PG, mannose side chain = PM, unsulfated control = PG-OH, oligomers of different valency = O1 and O4).[144]

section, we highlight selected examples of sGAG mimetic glycopolymers used as viral inhibitors.

Tengdelius et al. used a cyanoxyl-mediated free radical polymerization technique to prepare two sulfated polyfucosylated glycopolymers from fucose monomers bearing a polymerizable methacrylamido handle (see Figure 3) in an effort to mimic sulfated fucoidan from *Laminaria saccharin* as a novel GAG mimetic.[120] These compounds were shown to have the ability to inhibit HSV-1 infection in a manner similar to fucoidan at 100 μg/mL.[120] The same team later improved their methodology by using a RAFT (reversible addition−fragmentation chain-transfer) polymerization approach to generate sGAG mimetics with lower polydispersity, bearing terminating octadecyl, dioctadecyl, and cholesterol groups.[145] The corresponding glycopolymers were shown to stop HSV-1 spread but were not shown to have virucidal properties.

Fan and co-workers reported on a series of selectively sulfated glycopolymers mimicking fucoidan as inhibitors against influenza A virus (IAV) (see Figure 3).[128] Their applied synthetic approach required the synthesis of differentially protected azidoethyl-α-fucopyranosides, followed by their conjugation to an alkynylated exo-norbornene via CuAAC (copper(I)-catalyzed azide−alkyne cycloaddition) and subsequent polymerization using microwave-assisted ROMP (ring-opening metathesis polymerization, see Figure 5). When desired, introduction of site-selective sulfation was performed either before or after CuAAC, and in one case only after polymerization. Results from inhibition assays revealed an influence of sulfation patterns on the inhibition of IAV, H1N1, and H3N2 multiplication *in vitro* demonstrating the potential of structures with sulfation at the 2-*O*-position to serve as powerful inhibitors.[128] This work demonstrates the importance of the development of tools for generating designed structures with controlled variation of structural parameters to be used in antiviral studies and to learn more about structure−property correlations.

In the development of polymeric sGAG mimetics for potential pharmacological applications, one strategy is to generate molecules that not only target one virus but rather several virus families to achieve broad-spectrum antiviral activity. In general, broad-spectrum antivirals can serve as promising therapeutics, as they have the potential to overcome the challenges posed by emerging and re-emerging viruses. However, detailed insights into the features enabling broad-spectrum activity are needed. One general challenge is to better understand the balance between broad-spectrum applicability and sufficient specificity while avoiding off-target effects, for example, the anticoagulant activity of HP and HS, which can cause serious side effects.[74,111]

While work in this area for natural HP/HS as well as polysaccharide-based sGAG mimetics is actively ongoing, the rapid and modular synthesis of polymeric sGAG mimetics is already providing researchers with tools that can be used to obtain mechanistic insights into viral entry as well as the factors governing HS specificity on antiviral inhibitory potential, both of which are crucial for applying sGAG mimetics as pharmaceuticals.[114] For example, prior to the start of the pandemic, we reported on the systematic preparation of a series of sulfated synthetic glycomimetics varying in both composition and chain length.[144] This work initially focused on targeting human papillomavirus 16 (HPV16) as an effort to address the role of this virus in the development of invasive cancers such as cervical cancer. First experiments revealed that two of the higher molecular-weight sGAG mimetics, a sulfated galactose-based and a sulfated mannose-based glycopolymer (see Figure 3 for the chemical structure of the galactose polymer by Soria-Martinez et al.[144]) prepared through RAFT polymerization, reduced relative viral infection in HeLa cells comparable to HP at concentrations between 0.0001 and 1 mg/mL, and were comparable to carrageenan at concentrations above 0.001 mg/mL. The galactose-based polymer was also effective in an intravaginal inoculation study using mice, demonstrating the translational potential of this work (see Figure 5). We then explored the generalizability of this approach. Additional studies with HSV, IAV, and MCPyV revealed that our sGAG polymer mimetics could serve as broad-spectrum inhibitors of viral infection. Parallel studies with a series of short, linear sulfated glycooligomers revealed that while not as effective as the corresponding glycopolymers, they were able to prevent viral infection via a different mechanism and with different antiviral efficiencies[144] which are currently under exploration. Recently, we have adapted our synthetic platform to now also provide access to controlled variations of the sulfation patterns as well as rigidity.[196] Studies on the effects of these structural parameters in viral engagement as well as the development of additional broad-spectrum antivirals are currently ongoing.

Non-Glycan Polyanionic sGAG Mimetics and Their Antiviral Activity. Like polysaccharide and glycopolymer-based sGAG mimetics, non-glycan polyanionic mimetics of highly sulfated polysaccharides, namely HP, have been shown to inhibit viral entry and in some cases provide virucidal

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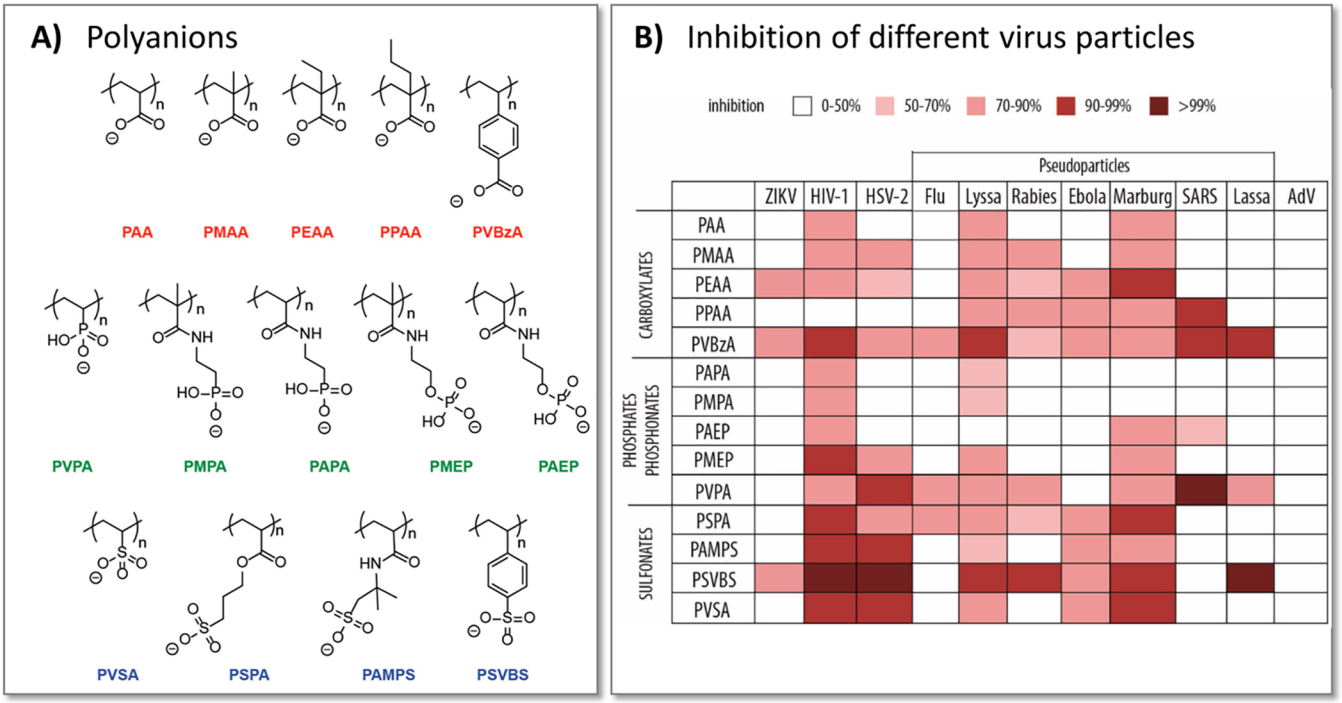

Figure 6. (A) Chemical structures of polyanions. (B) Inhibition of different pseudoparticles carrying viral glycoproteins by different types of polyanions. Reproduced with permission from ref 146. Copyright 2017 John Wiley and Sons.

activity. Early work on HP provided inspiration for the preparation of a variety of polyanionic structures which were shown to exhibit broad antiviral activity, with HIV and HSV being the primary targets of these investigations.[131,156,197−210] Polymers active against both HIV and HSV include poly(vinyl alcohol) sulfate (PVAS) and the respective copolymer with biphenyl disulfonic acid urea (PAVAS),[197] aurintricarboxylic acid (ATA) polymers,[202] poly(4-styrenesulfonic acid) (PSS),[204] polyhydroxcarboxylates,[205] a mandelic acid condensation polymer (SAMMA),[206] and poly(4-styrenesulfonic acid-*co*-maleic acid) (PSM).[207]

One early example is the compound MDL 101028, a biphenyl disulfonic acid urea copolymer with an average number of six repeating units. The synthesis of MDL 101028 was inspired by molecular modeling studies on the V3 loop immunodominant region of gp120 of HIV. MDL 101028 was shown to exhibit antiviral activity against several laboratory adapted strains of HIV and SIV that were grown in different T-cell lines, as well as against HSV-1 and HSV-2 cultivated in human embryonic diploid fibroblasts (MRC-5) and monkey kidney cells (Vero), while exhibiting negligible anticoagulant activity.[200,201]

An example of a polymeric drug candidate resulting from extensive studies on HIV is PRO2000 (see Figure 3). PRO2000, a naphthalenesulfonate polymer obtained by aldehyde−naphthalenesulfonic acid condensation polymerization, was formulated as a gel and entered into clinical trials as a potential microbicide against HIV infections in 1997.[211−214] After successful assessment in phase I, II, and IIb studies, which showed safety and inhibitory activity, PRO2000 failed to show efficacy in phase III clinical trials.[213,214] Although no definitive explanations for the failure of PRO2000 were provided, follow-up studies revealed that different factors such as local pH, the presence of seminal plasma, concentration and retention of the compound, and selectivity likely influenced the clinical trial results and rendered the application as a microbicide especially demanding. In general, the lack of virucidal activity for tested microbicides represents a significant hurdle, and HIV has remained challenging due to its high mutation rates.[214]

In the past years, Haag and co-workers developed a variety of polysulfated materials and polymers as HP mimetics with great antiviral potential.[153−155,215,216] Polyglycerol sulfate (PGS), originally developed as an HP analogue with anticoagulant and anticomplement activity, was rapidly identified as a potential antiviral agent (see Figure 3). Polyglycerol-based systems as antivirals are especially interesting due to their inherent biocompatibility and lower anticoagulant activity compared to HP. In a comparative study, PGS of linear (LPGS), dendritic (DPGS), and hyperbranched (HPGS) architecture, synthesized via monomer-activated ring-opening polymerization, were evaluated against HSV-1. In the pre- and postinfection setups of a plaque reduction assay, the significance of scaffold flexibility was revealed; highly flexible LPGS was the most active compound (IC_{50} in preinfection assay = 0.03 nM), in contrast to the rigid HPGS with the lowest activity (IC_{50} in preinfection assay = 374.17 nM). Notably, LPGS was shown to exhibit inhibitory activity comparable to acyclovir and higher than HP.[153] Recently, synthetic modifications of DPGS and HPGS accomplished by introducing hydrophobic alkyl motifs and sulfation have provided for a new class of antivirals with both inhibitory and virucidal properties.[215−217]

Polyanionic polymer-based broad-spectrum antivirals were recently investigated by Schandock and co-workers.[146] Fourteen polymers with pendant carboxylates, phosphate/phosphonates, or sulfonates were synthesized with distinct structural modifications of the backbone and charged residues (see Figure 6). The inhibitory potential of the polymers against virus entry was tested for several zoonotic viruses including the

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Macromolecules XXXX, XXX, XXX−XXX

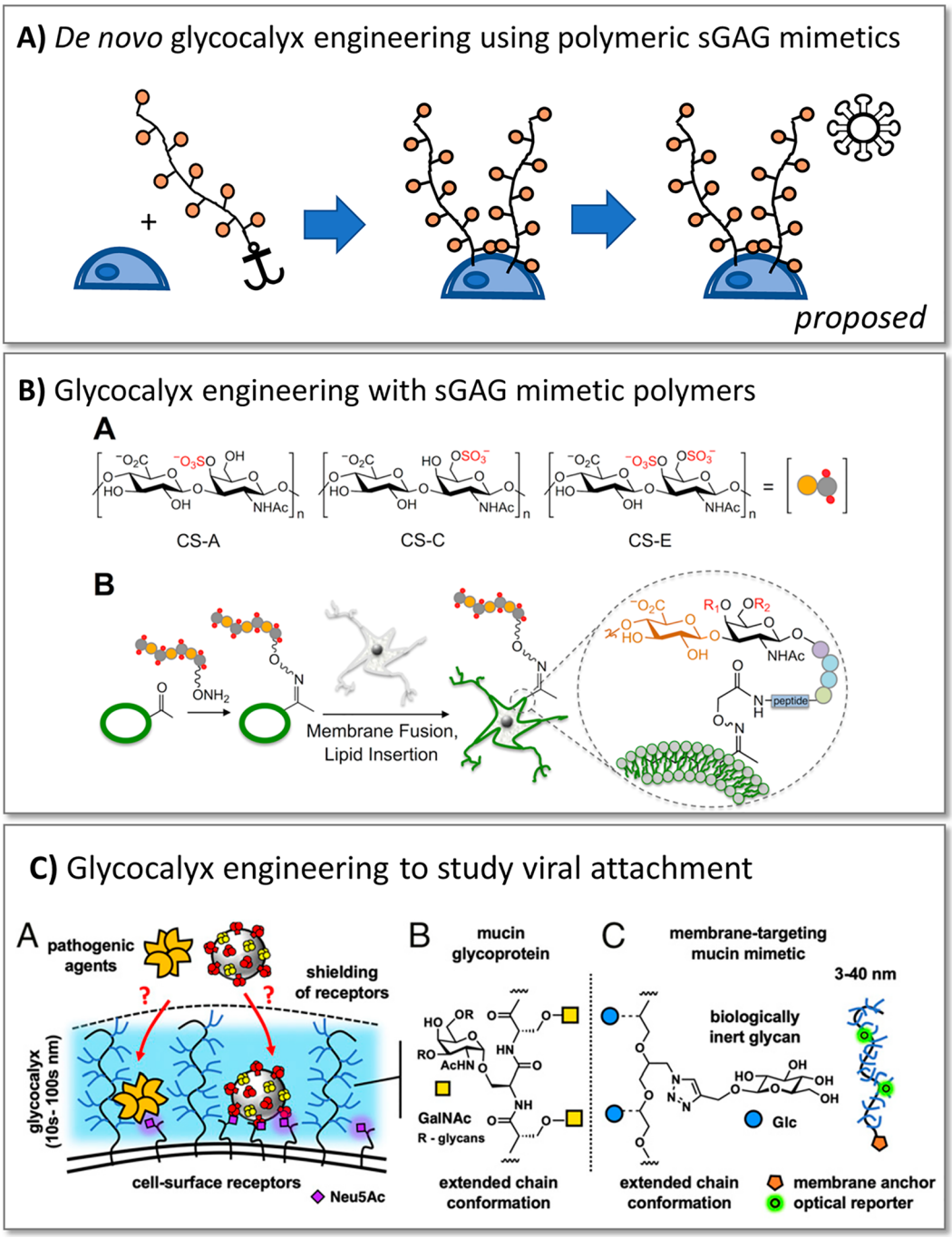

Figure 7. (A) Proposed use of *de novo* glycocalyx engineering to introduce sGAG mimetics and study effects of viral engagement. (B) An example for glycocalyx engineering with CS polysaccharides as GAG mimetics and introduction into neuronal cells.[225] (C) An example for glycocalyx engineering with mucin mimetic glycopolymers to study effects of nonbinding, high-molecular-weight compounds in viral attachment. Reproduced with permission from ref 230. Copyright 2021 The Proceedings of the National Academy of Sciences.

Zika virus (ZIKV), EBOV, Lassa virus (LASV), Lyssa virus (ABLV), RABV, and MARV as well as influenza virus, HSV, and HIV. A poly(vinylbenzoic acid) (PVBzA) analogue was identified as a lead candidate with broad-spectrum antiviral activity against all tested viruses. Further examination revealed that glycoprotein density on the viral envelope increases susceptibility of viruses to polymeric inhibitors. With a view to the polymer, more hydrophobic polymer backbones enhanced antiviral activity. It is surprising that the polysulfonates, which are strong polyanions and typical candidates in other studies on viral inhibition, lacked noteworthy broad-spectrum antiviral potential. In addition, with the exception of poly((2-methacrylamidoethyl)phosphonic acid), the poly(phosphates/phosphonates) were the least active group of polyanions. Overall, the results showed that polymeric inhibition of viral infection can be considered a universal principle and that distinct variations of structural features in polyanions can lead to valuable insights into structure−activity relationships.[146]

Shortly after this study, Yadavalli and co-workers demonstrated that three previously FDA-approved polycarboxylates used for coating drugs in oral formulations,—PVAP (poly-

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Macromolecules XXXX, XXX, XXX−XXX

(vinyl acetate phthalate)), HPMCP-55S (hydroxypropylmethylcellulose phthalate), and Eudragit S100 (methacrylic acid methyl methacrylate copolymer, ratio 1:2)—exhibited antiviral activity against HSV infections.[218] Whereas HPMCP-55S was active against both HSV-1 and HSV-2, PVAP only showed neutralizing activity against HSV-1 infections, while Eudragit S100 only showed significant antiviral efficacy against HSV-2, indicating a potential selectivity of the different polyanions against different viruses.[218]

To the best of our knowledge, the only examples of non-saccharide-based polymeric HP mimetics targeting SARS-CoV-2 were published by Nie and co-workers.[154] Here, in a similar fashion to their work on HSV, the potential to inhibit SARS-CoV-2 infection for LPGS and HPGS was evaluated with plaque reduction assays and compared to HP and pentosan sulfate. Once again, the more flexible LPGS with a high degree of sulfation and molecular weight showed the best inhibitory activity.[154]

Beyond sGAG Mimetics as Soluble Inhibitors. sGAG mimetics successfully serve as model compounds in viral engagement studies and have demonstrated great potential for the development of new bioactive, pharmacologically relevant compounds. However, in both cases, the focus so far has been exclusively on soluble compounds. In the natural setting, such as the glycocalyx, multiple HS chains are attached to a protein core and located within the cell membrane. This presentation and localization thus offer several additional levels of structural control and spatial organization that need to be considered when trying to understand or manipulate phenomena, such as HSPG-mediated viral attachment. Indeed, glycocalyx engineering now enables researchers to tune selected components of the glycocalyx, for example, by enzymatic treatment or glycan labeling, and study the effects, not at the level of the single molecule but on the larger ensemble.[219,220] Recently, the so-called *de novo* glycocalyx engineering has gained increasing attention.[221,222] Here, synthetic glycocalyx building blocks equipped with a membrane anchor, for example, a cholesterol unit, are inserted into a live cell surface, thereby artificially altering or reconstituting the cell's glycocalyx (Figure 7A).[223] Through this method, polymeric sGAG mimetics have successfully been used to derive new insights into the functional role of such carbohydrates within the complex environment of the cell surface.[224−230] For example, the Hsieh-Wilson lab used CS to engineer the cell surface of neurons and could show increased activation of neurotrophin-mediated signaling pathways and enhanced axonal growth in dependence of the sulfation pattern installed through the choice of polysaccharide (Figure 7B).[225] In another example, the Godula lab used short HS fragments displayed in a multivalent fashion on a polymeric scaffold to derive HS mimetic glycopolymers that were installed into embryonic stem cells deficient in natural HS biosynthesis. With these systems in hand, they could demonstrate that this approach led to regained functions associated with HSPG's presence, specifically the HS-mediated interaction with FGF2.[224,230]

Surprisingly, there are no studies so far making use of cell-surface bound sGAG mimetics to study their role in pathogen attachment. However, the applicability and potential impact of GAG mimetic glycocalyx engineering were demonstrated by a recent study from the Godula lab using a glucose-based glycopolymer as noncharged, biologically inert mimetic of mucins, another class of high-molecular-weight carbohydrate−protein conjugates within the glycocalyx (Figure 7C).[231] In this example, they studied how influenza virus (H1N1) cell attachment and entry mediated through endogenous sialic acid receptors on red blood cell (RBC) surfaces is altered by introduction of glycopolymers. As expected, virus attachment was reduced due to steric shielding of the receptor sites by the high-molecular-weight glycopolymers. Notably, the use of higher concentrations of glycopolymers increased viral retention time on the cell surface, leading to cluster formation, an effect that can be expected to promote the overall infection process. We predict that by bringing together new and advanced methods in deriving polymeric sGAG mimetics, for example, now with control also over sequenced patterns of sulfation,[196] and the ability to use them in *de novo* glycocalyx engineering will enable new insights into the mechanisms of sGAG-mediated pathogen interactions and open up opportunities to improve the use of polymeric sGAG mimetics against viral infections (Figure 7A).

CONCLUSION

State of the Art. Native HSPGs and therapeutic HP/HS are well established for their role in viral engagement and hold great promise to detect, prevent, and treat viral infections. sGAG mimetics have proven to be important tools as model compounds in gaining new insights into the mechanisms of HS in viral engagement. Notably, they can also be applied as drugs against viral infections and in the development of methods for viral detection and identification. Despite great promise and encouraging results in the last years, there remain limitations and challenges. A major challenge is that HP/HS as well as their mimetics must often simultaneously fulfill multiple criteria including high structural control, synthetic reproducibility, high binding strength, and specificity. Addressing these factors is crucial for generating sGAG mimetics suitable for therapeutic applications.

In the past, the interplay of polymer chemistry and glycobiology has demonstrated how interdisciplinary research between these two fields can greatly impact and inspire new insights into the biological function and medical applications of glycans and glycan mimetic polymers and materials, including but not limited to sGAG mimetics. In the following section, we will highlight where we envision future contributions and possibilities for polymer chemistry to further promote the development of sGAG mimetics for the fight against viral infections.

Future Perspectives. *Precision Polymeric sGAG Mimetics for Fine-Tuning Macroscopic Properties.* While the structure of shorter GAG and sGAG fragments and their interaction with proteins has been extensively studied, it is now clear that the "sulfation code" and structural properties of the polymer, such as the chain stiffness and overall length, critically affect selectivity, binding strength, and therefore biological activity. For example, most studies so far focus on an all-or-nothing approach where the structures are either fully sulfated (test structures) or non-sulfated (controls). Considering the important role of charge distribution and patterning on protein engagement, efforts that can produce more well-defined structures with precise sulfation patterns are of utmost importance. On the basis of their synthetic versatility, polymeric sGAG mimetics offer important tools to control and tune such properties. Recent progress in creating polymers with sequence control and monodispersity, so-called precision polymers, needs to be applied to derive sGAG mimetics of the next generation.[232−236]

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Macromolecules XXXX, XXX, XXX−XXX

Polymeric sGAG Mimetics in De Novo Glycocalyx Engineering. The biological activity of HS and HSPGs is determined not only by their structural parameters but also by their localization and dynamic organization within the glycocalyx. *De novo* glycocalyx engineering has been demonstrated as a powerful tool to tune and study effects of HSPGs as part of the larger ensemble of the cell surface, and to gain new insights into various biological functions such as their effect in stem cell differentiation or neuronal growth.[219,220] However, until today, *de novo* glycocalyx engineering has not been applied to study the role of HS, HSPGs, or sGAG mimetics in viral engagement. It is an open question which of the many different sGAG molecules and conjugates on the cell surface engage in virus binding and how their localization affects their role in mediating the different types of HS and other GAG-dependent cell contacts. Glycocalyx engineering approaches, especially in combination with precision polymeric sGAG mimetics, can further unravel the mechanisms and functional roles of HS and its conjugates within the ensemble of the cell surface and, in the long run, could provide new opportunities to develop improved or new therapeutics against viral infections based on this knowledge.

Polymeric sGAG Mimetics Targeting Different Pathogens. Other pathogens such as bacteria, parasites, and fungi are known to engage in HS and HSPG binding to promote infection.[70,71] So far, most studies with both natural and sGAG mimetic structures focus only on one class of pathogens. Comparing different pathogens, for example, in their interactions with selected polymeric sGAG mimetics, could further unravel commonalities in pathogens subverting GAGs in their infection processes and how, in turn, we could use this knowledge to derive broad-spectrum treatments against pathogens.

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Notes

The authors declare no competing financial interest.

Biography

Miriam Hoffmann (photo left) received her M.Sc. in Chemistry in 2018 from the Heinrich-Heine-University of Düsseldorf, Germany, while working under the supervision of Prof. Dr. Laura Hartmann in the Department of Organic Chemistry and Macromolecular Chemistry. Her master's thesis focused on the synthesis of liposomes functionalized with sequence-defined glycomacromolecules for galectin targeting. Currently, she is pursuing her Ph.D. in the group of Prof. Dr. Laura Hartmann. Her current research efforts are focused on the design of precision glycomacromolecules as sGAG mimetics. *Nicole L. Snyder* (photo right) received her B.S. degrees in Chemistry and Biology from Westminster College in New Wilmington, PA, in 2000. She obtained her Ph.D. in 2005 working under the direction of Prof. Mark W. Peczuh in the Department of Chemistry at the University of Connecticut in Storrs, CT, on the synthesis of a class of seven-membered ring sugars known as septanoses. She is currently a Professor of Chemistry and the interim Associate Dean of Research and Creative Works at Davidson College in Davidson, NC. Her current research program focuses on the synthesis and evaluation of carbohydrate-based construct for exploring carbohydrate−protein interactions. *Laura Hartmann* (photo middle) received her diploma in Chemistry in 2004 from the Albert-Ludwigs-University Freiburg, Germany. She obtained her Ph.D. in 2007 working with Prof. H.G. Börner and Prof. M. Anonietti at the Max-Planck-Institute of Colloids and Interfaces (MPI CI), Potsdam, Germany. She worked as a postdoctoral student at Stanford University in the group of Prof. C. W. Frank and returned to Germany in 2009, starting her independent research career as an Emmy Noether fellow in the Department of Biomolecular Systems, MPI CI, headed by Prof. P. Seeberger. Since 2014 she holds the chair for Macromolecular Chemistry at the Heinrich-Heine-University Duesseldorf, Germany. Her current research program focuses on the synthesis of polymeric biomimetics, including glycan mimetics, with control of the monomer sequence and their use in biomedical applications.

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