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RESEARCH

# Inhibition of sulfated glycans on the binding of dengue virus envelope protein to heparin

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**Abstract**
Dengue viruses (DENV) are transmitted to humans through mosquito bites and infect millions globally. DENV uses heparan sulfate (HS) for attachment and cell entry by binding the envelope protein to highly sulfated HS on target cells. Therefore, inhibiting the binding between DENV and HS could be a promising strategy for preventing DENV infection. In the current study, the interactions between DENV envelope protein (from Type 2 DENV) and heparin (a surrogate for HS) were analyzed using competition solution SPR. Results demonstrate that heparin binds to DENV envelope protein with high affinity ($K_D$ = 8.83 nM). Competitive Solution SPR assays using surface-immobilized heparin and a series of naturally-sourced and semi-synthetic sulfated glycans demonstrated significant inhibitory activity against the binding of DENV envelope proteins to heparin. This study of molecular interactions could provide insights into the development of therapeutics for DENV infection.

**Keywords** Heparin · Sulfated glycans · Marine Sourced glycans · Dengue virus · Surface plasmon resonance

## Introduction

Dengue is a mosquito-borne zoonotic disease [1]. The disease symptoms of the disease can vary from asymptomatic to severe cases of hemorrhagic fever and even death. The severity is influenced by various factors, such as viral strain, the patient's overall health, autoimmune disorders, genetic predisposition, and lifestyle. In 2023, the CDC reported that more than 4.6 million cases were recorded in the Americas [2]. In the U.S., dengue is a nationally notifiable disease, and six U.S. territories have been designated areas of risk, including American Samoa, the Federated States of Micronesia, Puerto Rico, the Republic of Marshall Islands, the Republic of Palau, and the U.S. Virgin Islands [2]. More recently, sporadic cases were reported in Florida, Hawaii, Texas, Arizona and California [2]. WHO reported that dengue is endemic in > 100 countries in the WHO Regions [3]. However, currently, there are no antiviral-approved medications for dengue [2].

The causative agent of dengue fever is Dengue virus (DENV), a single-stranded positive-sense RNA virus [1]. DENV comprises four serotypes, DEN-1, DEN-2, DEN-3, and DEN-4 [1]. DENV (type 1 - type 4) is transmitted through infected mosquito vectors (e.g., *Aedes aegypti* and *Aedes albopictus*). DENV uses the host cell surface heparan sulfates (HS) to attach to cells [4]. Heparan sulfates are highly negatively charged molecules that assist the virus in binding to cells [5]. It has been suggested that DENV may bind to diverse cell-surface putative receptors and attachment factors, and the binding may result in the

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virus's internalization in host cells [5, 6]. Once inside the host cell, the virus particles release the viral RNA in the cytoplasm. The viral RNA (genome) translocates into the ER (endoplasmic reticulum) [7]. The virus then hijacks the host's translational and post-translational machinery to produce mature viral particles [7]. The mature virus progeny are exocytosed into the extracellular matrix, ready to infect other cells [7].

Glycans are an emerging group of polysaccharide-based antiviral therapeutics. In recent years, marine organisms (e.g., *Monostroma sps* and *Undaria pinnatifida)* have emerged as a rich untapped natural resource for sulfated glycans, including fucoidans, carrageenans, and rhamnan sulfates [8–11]. Marine sulfated polysaccharides have been shown to exhibit antiviral activity against Herpes simplex virus type 1 and type 2, human immunodeficiency virus, human cytomegalovirus, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), and influenza A virus [12–14]. Recent advances in biotechnology and analytical tools have enabled designing synthetic, semi-synthetic, and bioengineered glycans. HS and heparin are naturally occurring linear glycosaminoglycans. HS and heparin share similar biosynthetic pathways. A wide range of proteins (e.g., growth factors and chemokines) bind to HS/heparin, and the interaction leads to cell function, including cell-cell communications and cell homeostasis [15]. Recently, it was shown that heparin inhibits the binding of SARS-CoV-2 spike protein to HS, suggesting that heparin can disrupt the SARS-CoV-2 pathogenesis, partly by disrupting the entry of the virus into the host cells [16–21]. The biosynthesis of HS/heparin is well understood, and advanced tools for critically examining the structure and function are available. Heparin and chemically modified heparin have been shown to exhibit antiviral activity [16–21]. Our previous study demonstrated that the interaction between the DENV envelope protein and heparin is influenced by sulfation patterns, highlighting the potential of semisynthetic and chemically modified glycans as inhibitors of DENV infection [22].

The current study analyzes the interactions between the DENV envelope protein and heparin and assesses how sulfated glycans (Fig. 1) inhibit these interactions using surface plasmon resonance (SPR). This technique allows for label-free, real-time analysis of biomolecular interactions. Understanding how the pathogenic virus interacts with naturally occurring and engineered glycans could reveal potential antiviral therapeutics, highlighting the significance and relevance of this research. This work investigates a broader spectrum of glycans, including semisynthetic derivatives and glycans derived from diverse marine organisms, providing deeper insights into the structure-function relationships of DENV proteins and glycan interactions, and proposing new potential competitive inhibitors of DENV infection.

## Materials and methods

### Materials

Porcine intestinal heparin (molecular weight, Mw ~ 15 kDa) was purchased from Celsus Laboratories (Cincinnati, OH, USA). The 6-*O*-desulfated heparin (6-DeS, MW 13 kDa) was kindly provided by Dr. Lianchun Wang (University of South Florida, USA). The 2-*O*-desulfated IdoA heparin (2-DeS, MW 13 kDa) and *N*-desulfated heparin (N-DeS, Mw 14 kDa) were prepared based on a previously described method [12]. Pentosan polysulfate (PPS, Mw 6.5 kDa) was obtained from Bene Pharma (Munich, Germany). Mucopolysaccharide polysulfate (MPS, Mw 14.5 kDa) was obtained from Luitpold Pharma (Munich, Germany). Marine-sourced sulfated glycans IbSF (Mw ≥ 100 kDa), IbFucCS (Mw ~ 75 kDa), PpFucCS (Mw 10–60 kDa), LvSF (Mw ≥ 100 kDa), HfSF (Mw ≥ 100 kDa), and HfFucCS (Mw ~ 50 kDa) were purified in Dr. Pomin's Lab (University of Mississippi, USA). The algal-sources sulfated glycan (RPI-27, ~ 100 kDa) was kindly provided by Dr. Weihua Jin (Zhejiang University of Technology, P.R. China). Using a previously described method, rhamnan sulfate (RS, ~ 290 kDa) was purified from *Monostroma nitidum* [12, 13]. The Dengue virus envelope protein (DENV Type 2, strain New Guinea C/PUO-218 hybrid E, Accession # AAC59274.1, Molecular Weight 45.7 kDa) was purchased from Sino Biologicals (Catalog Number #40471-V08B6). SPR analyses were performed using the Biacore T200 SPR System (Cytiva, Uppsala, Sweden). The HBS-EP + buffer as running buffer and Streptavidin (SA) sensor chips were purchased from Cytiva.

### Kinetic measurement of interaction between heparin and DENV envelope proteins using heparin biochip

Kinetic measurements of the interaction between heparin and DENV envelope protein were performed using a heparin biochip. The heparin biochip was prepared using a previously described method, where biotinylated heparin was immobilized onto an SA sensor chip [12].

To measure the binding kinetics, different concentrations of protein were prepared in HBS-EP + buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA and 0.05% v/v Surfactant P20, pH 7.4) and injected onto a heparin biochip at a flow rate of 30 µL/min for 180 s, followed by flowing the HBS-EP + buffer on the chip for 180 s (to monitor the disassociation rate). After each cycle, the heparin biochip was regenerated by injection of 2 M NaCl for 60 s. SPR analysis was performed at 25 °C. Binding kinetics and affinity were

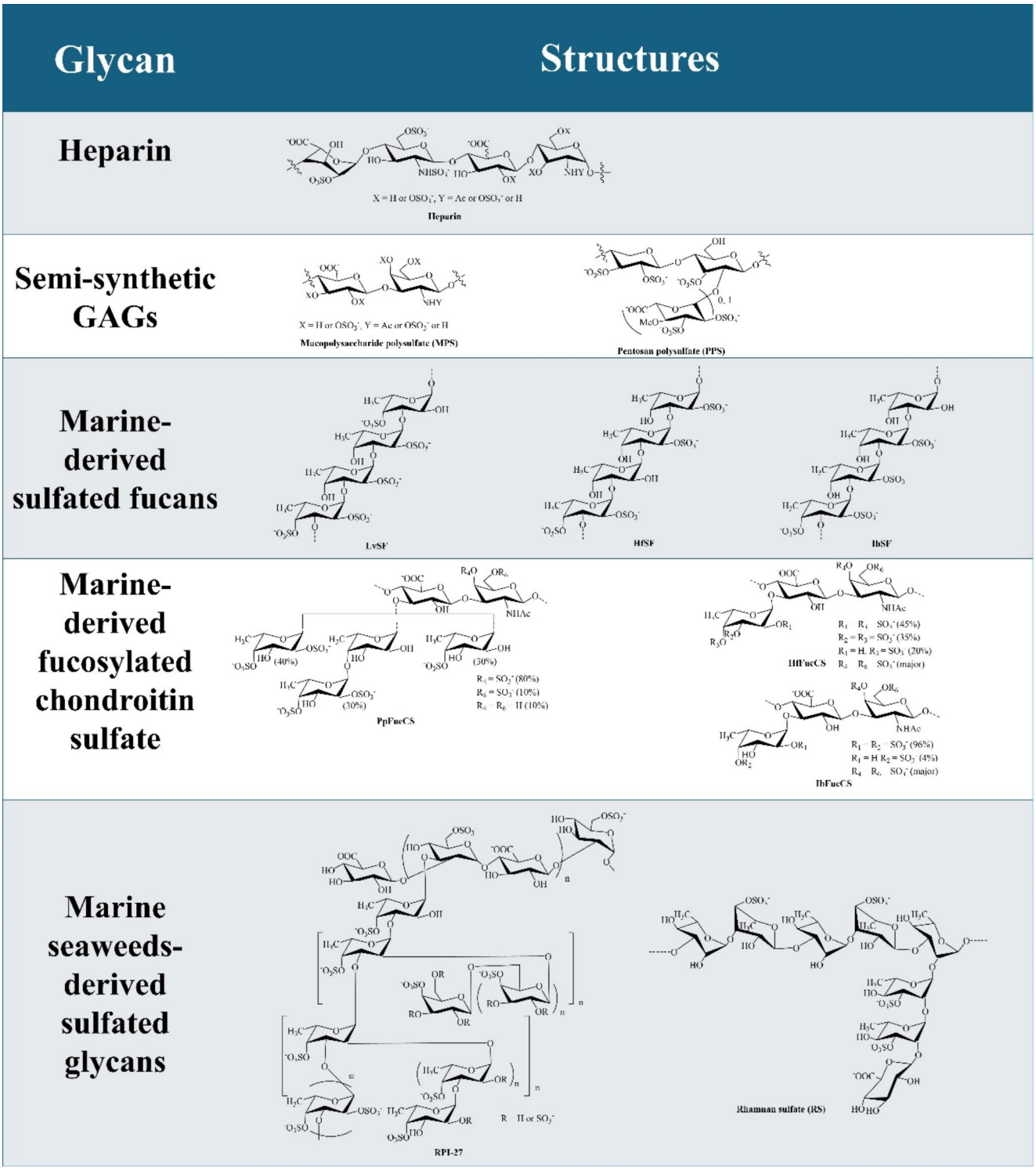


**Fig. 1** Structures of sulfated glycans

calculated using Biacore T200 Evaluation software version 3.2 (Cytiva, Uppsala, Sweden).

### Solution competition SPR to study inhibition activity of heparin, desulfated heparin, heparin-analogs, and marine sulfated glycans on heparin-DENV envelope protein interactions

To evaluate the inhibition of the interaction between DENV envelope proteins and heparin, DENV type 2 envelope proteins (50 nM) were premixed with various sulfated glycans in HBS-EP + buffer. The mixtures were injected over the heparin biochip at a flow rate of 30 µL/min. Following each cycle, the sensor surface was regenerated with a 30 µL injection of 2 M NaCl. Control experiments using only DENV envelope protein confirmed the complete regeneration of the surface. A decrease in the binding signal (Response Unit, RU) indicated that the glycan occupied the binding sites of the DENV envelope protein, thereby inhibiting their binding to the heparin-immobilized chip surface. This method was also used to measure the $IC_{50}$ values of sulfated glycans inhibiting the heparin-DENV envelope protein interactions.

### Statistical analysis

Statistical analyses were performed using GraphPad Prism (Version 9). Treatments were compared using one-way ANOVA and a *post-hoc* Tukey test. Statistical significance is defined as non-significant (ns, $wherep > 0.05$) and significant (*/#, $wherep < 0.05$; **/##, $wherep < 0.01$; ***/###, $wherep < 0.001$; and ****/#### $wherep < 0.0001$).

## Results and discussion

### Binding kinetics and affinity analysis of DENV envelop protein with heparin

The heparin biochip has been successfully utilized to study the binding kinetics of viral envelope protein with naturally occurring and engineered HS/heparin mimetics [12, 13, 23]. Here, the heparin-SPR chip was used to evaluate the binding affinity of dengue envelope protein with heparin. SPR sensorgrams of the interaction of heparin with DENV envelope protein are shown in Fig. 2. The SPR sensorgrams were used to quantify the binding kinetics and affinity by global fitting the sensorgrams (complete association and dissociation phases) with a 1:1 Langmuir binding model.

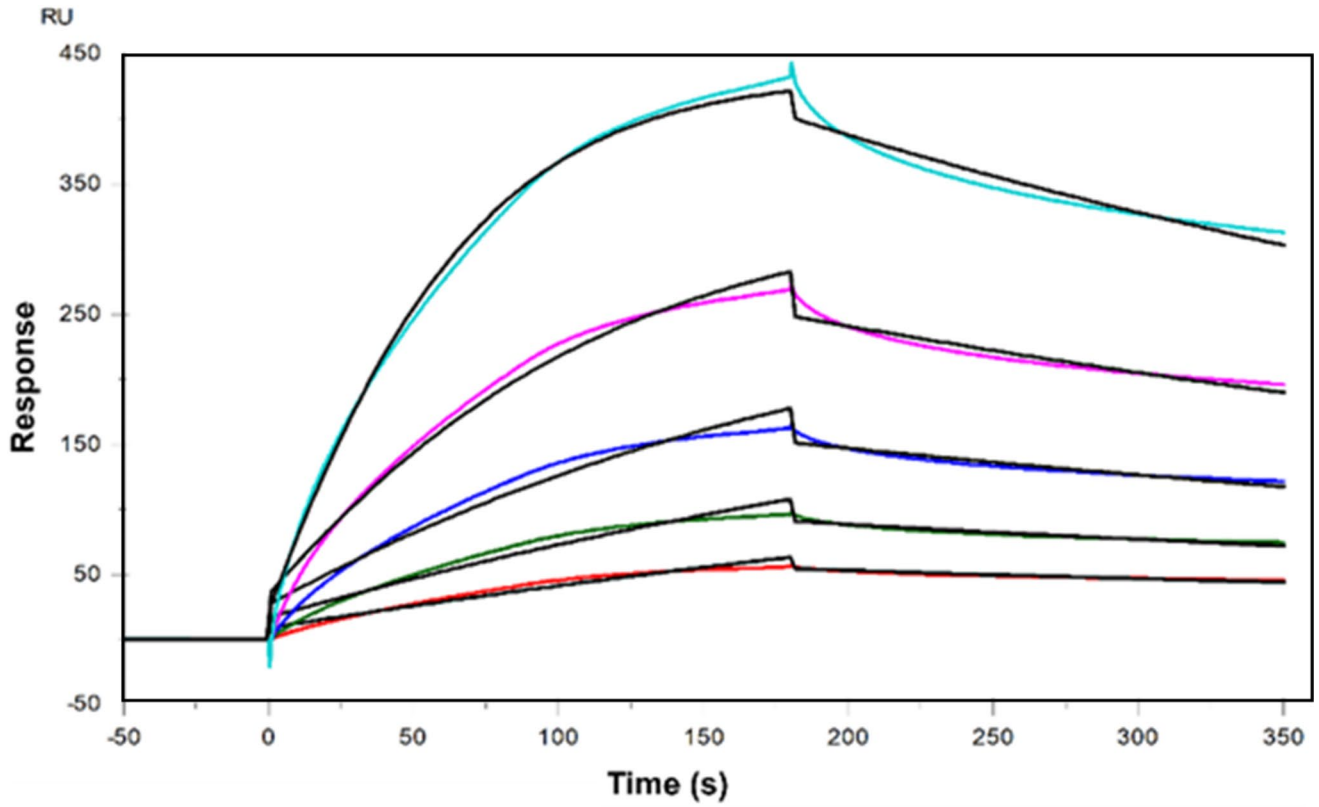


**Fig. 2** SPR sensorgrams of DENV Type 2 protein binding with heparin. Concentrations of DENV protein were 100 nM, 50 nM, 25 nM, 12.5 nM, and 6.25 nM (from top to bottom, respectively). Black curves are the fitting data using BIAcore T200 Evaluation 3.2

**Table 1** Kinetic data of DENV envelope protein binding with heparin: association rate constant (ka), dissociation rate constant (kd), equilibrium dissociation constant ($K_D$, $K_D$ = kd/ka)

| Protein | ka (1/MS) | kd (1/S) | $K_D$ (M) |
|---|---|---|---|
| DENV (Type 2) E / Envelope Protein | $2.19\times10^{5}$ ($\pm2.50\times10^{3}$)* | $1.87\times10^{-3}$ ($\pm8.40\times10^{-6}$) * | $8.83\times10^{-9}$ ($\pm9.94\times10^{-10}$)** |

*The data with (±) in parentheses represent the standard deviation (SD) obtained from the global fitting of five injections. ** SD based on triplicate measurements

The kinetics and affinity of the interaction of DENV with heparin are tabulated in Table 1. The envelope protein from DENV Type 2 showed a strong binding affinity to heparin ($K_D = 8.83\times10^{-9}$ M), which further confirmed our previous finding: dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate [4].

## Solution competition SPR analysis on the interaction of DENV protein with immobilized heparin and desulfated heparins

The solution competition SPR was conducted to evaluate the effect of sulfation of heparan sulfate (HS)/heparin on the binding interactions of the DENV envelope protein with various desulfated heparins (2-DeS, 6-DeS, and N-DeS), all of which have comparable chain lengths. DENV envelope proteins (50 nM) were premixed with heparin (as a positive control) or different desulfated heparins and then injected over the heparin biochip. The sensorgrams (Fig. 3A) indicate that all three desulfated heparins exhibited reduced inhibition of the DENV envelope protein binding to surface-immobilized heparin compared to the heparin control. However, the differences among the three desulfated samples did not reach statistical significance (Fig. 3B), suggesting that the binding of the DENV envelope protein may not be solely dependent on the sulfation pattern of heparin.

## Inhibition of interaction of DENV envelope proteins with immobilized heparin in the presence of naturally occurring algal-sourced sulfated glycans

Next, we evaluated two algal (seaweed) sourced sulfated glycans, RPI-27 and rhamnan sulfate (RS). RPI-27 is fucoidan with a complex branched high-molecular-weight sulfated glycan isolated from brown seaweed *Saccharina japonica* and has been shown to possess antiviral properties [13]. RS is a rhamnose-rich, high-molecular-weight, highly sulfated glycan isolated from green seaweed (e.g., *Monostroma latissimum* and *Monostroma nitidum*) [8, 9]. This array of test marine-sourced glycans has shown to possess antiviral properties via directly interacting with the viral envelope proteins [8–11, 23–25].

Solution competition SPR was used to evaluate the interaction of DENV envelope protein (50 nM) with immobilized heparin in the presence and absence of heparin and the two sulfated glycans (5 µg/mL). Interaction of DENV envelope protein (50 nM) with immobilized heparin was used as a control. The sulfated glycans bind to DENV envelope protein and thereby significantly inhibit the binding of DENV envelope protein to surface-immobilized heparin (Fig. 4A). The normalized % binding of DENV envelope protein with immobilized heparin was performed in the presence of controls and the test sulfated glycans. DENV envelope protein

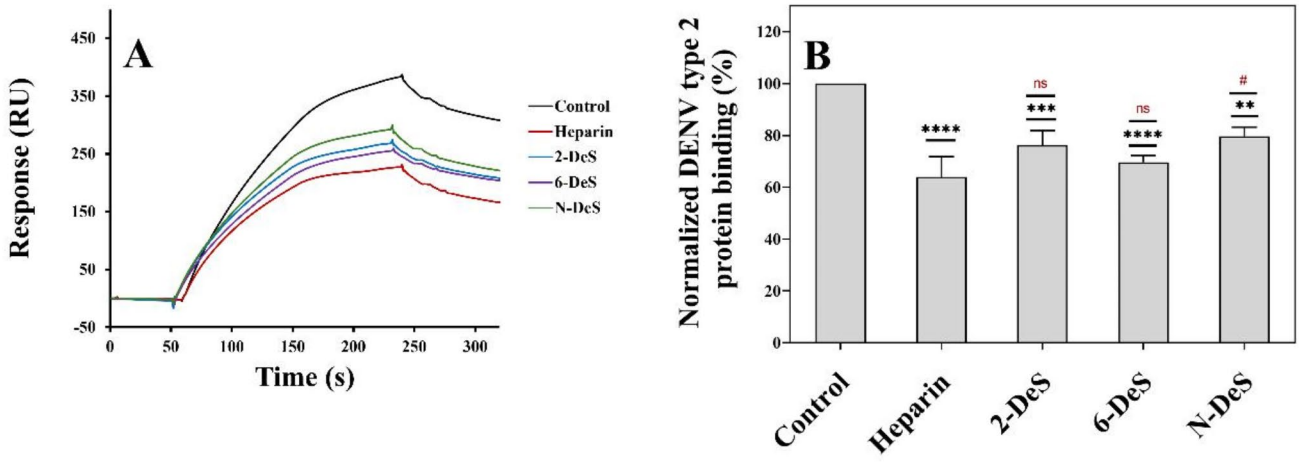


**Fig. 3** DENV protein-heparin interaction inhibited by desulfated heparins using solution competition. (**A**) SPR sensorgrams of DENV protein-heparin interaction competing with different desulfated heparins. (**B**) Bar graphs of normalized DENV protein binding preference to surface heparin by competing with different desulfated heparins. Data are shown as mean ± SD, *wheren* = 3 (triplicate measurements were performed in one experiment) and are analyzed using a one-way ANOVA/Tukey tests (* in black indicates comparison to the control, # in red indicates comparison to the heparin)

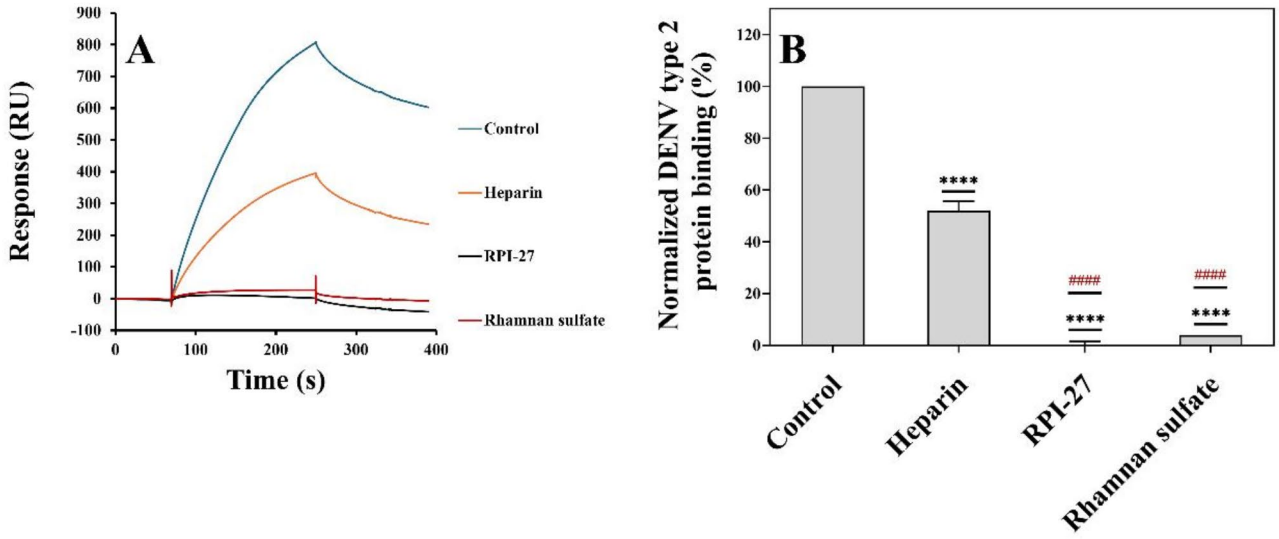


**Fig. 4** Solution competition between heparin and alga sourced sulfated glycans. (**A**) SPR sensorgrams of DENV protein-heparin interaction competing with different alga sourced sulfated glycans. (**B**) Bar graphs of normalized DENV protein binding preference to surface heparin by competing with different sulfated glycans. Data are shown as mean ± SD, *wheren* = 3 (triplicate measurements were performed in one experiment) and are analyzed using a one-way ANOVA/Tukey tests (* in black indicates comparison to the control, # in red indicates comparison to the heparin)

strongly binds to RS and RPI-27 with a > 80% inhibition (Fig. 4B).

## Inhibition of interaction of DENV envelope proteins with immobilized heparin in presence of semi-synthetic sulfated glycans

Semi-synthetic glycans are a group of naturally occurring glycans that are chemically modified. The study integrated an assessment of the anti-DENV potential of two semi-synthetic sulfated glycans: mucopolysaccharide polysulfate (MPS) and pentosan polysulfate (PPS). MPS is a linear sulfated glycan composed of repeating units of monomeric amino sugars and uronic acid moieties, potential sulfation sites of MPS include the N, C4, and C6 positions of amino sugars and the C2 and C3 positions of uronic acids [26]. MPS is obtained from mammalian sources (e.g., cartilage), followed by chemical sulfation. The degree of sulfation of the MPS plays a critical role in determining its bioactivity, including anti-inflammatory, antithrombotic, antiviral, and fibrinolytic properties [27, 28]. PPS is a sulfated glycan composed of repeating units of β-D-xylopyranose (Xyl), forming a poly-Xyl backbone, which is interweaved with a 4-*O*-methyl-glucuronic acid [29, 30]. PPS is obtained from plants (e.g., German beechwood), followed by chemical sulfation of the 4-methyl glucuronoxylans. Both MPS and PPS have shown potential antiviral properties against SARS-CoV-2 and MERS-CoV [12, 19].

Solution competition SPR was also used to evaluate the interaction of DENV envelope protein (50 nM) with immobilized heparin in the presence and absence of heparin and the two semi-synthetic sulfated glycans (5 µg/mL). DENV envelope protein strongly binds to RS and RPI-27 with a > 60% inhibition (Fig. 5). The results suggest that both MPS and PPS have antiviral potential against the DENV Type 2 strain.

## Inhibition of interaction of DENV envelope proteins with immobilized heparin in presence of marine-sourced sulfated glycans

Marine organisms are a rich source of sulfated glycans. For the current study, fucosylated chondroitin sulfate (FucCS) and sulfated fucans (SF) extracted from sea cucumbers and sea urchins were explored. Sea cucumbers and sea urchins are marine invertebrates belonging to the phylum *Echinodermata sps*. Chondroitin sulfates (CS) naturally occur in highly sulfated glycosaminoglycans and are composed of repeating GlcA and *N*-acetylgalactosamine sugar molecules that are highly modified (e.g., sulfated). Marine organisms have been shown to have diverse chondroitin sulfate chains that are modified further *via* sulfated fucose residues. For the current study, FucCS was sourced from three sea cucumber species, namely, *I. badionotus*, *H. floridana*, and *P. pygmaea* [31–34]. SFs are naturally occurring sulfated polysaccharides. For the current study, SFs were sourced

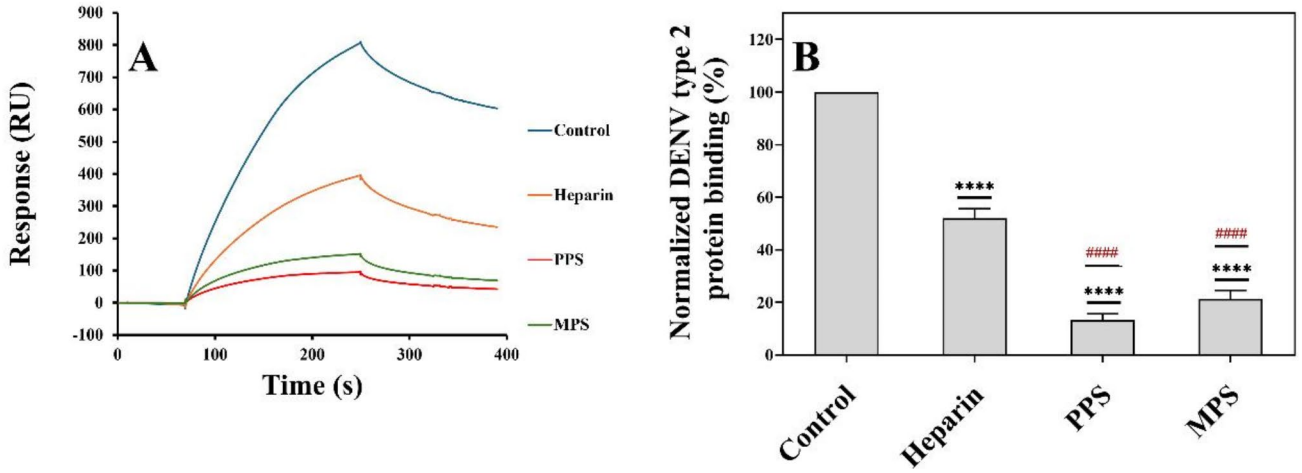


**Fig. 5** Solution competition between heparin and MPS or PPS. (**A**) SPR sensorgrams of DENV protein-heparin interaction competing with MPS or PPS. (**B**) Bar graphs (based on triplicate experiments with standard deviation) of normalized DENVprotein binding preference to surface heparin by competing with MPS or PPS. Data are shown as mean ± SD, where $n = 3$ (triplicate measurements were performed in one experiment) and are analyzed using a one-way ANOVA/Tukey tests (* in black indicates comparison to the control, # in red indicates comparison to the heparin)

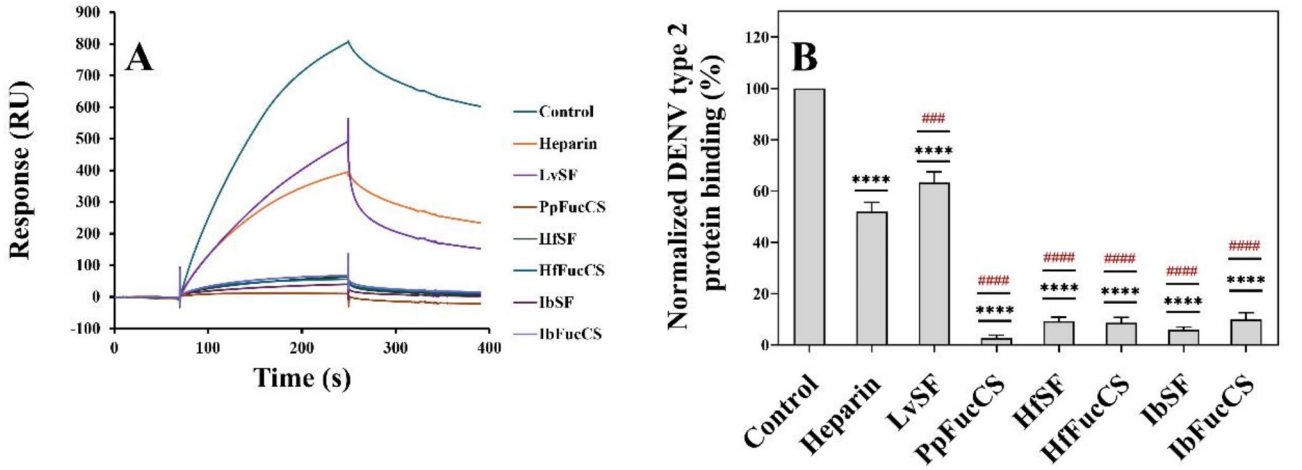


**Fig. 6** Solution competition between heparin and marine-soured sulfated glycans. (**A**) SPR sensorgrams of DENV protein-heparin interaction competing with different marine-soured sulfated glycans. (**B**) Bar graphs (based on triplicate experiments with standard deviation) of normalized DENV protein binding preference to surface heparin by competing with different marine-soured sulfated glycans. Data are shown as mean ± SD, where $n = 3$ (triplicate measurements were performed in one experiment) and are analyzed using a one-way ANOVA/Tukey tests (* in black indicates comparison to the control, # in red indicates comparison to the heparin)

from two sea cucumber species, namely, *I. badionotus* and *H. floridana*, and a sea urchin species, *L. variegatus* [34].

Again, we used solution competition SPR to assess the interaction of DENV envelope protein (50 nM) with immobilized heparin in the presence and absence of heparin and six marine-sourced sulfated glycans (5 µg/mL). The interaction of DENV envelope protein (50 nM) with immobilized heparin served as a control. The sulfated glycans bind to DENV envelope protein, significantly inhibiting its binding to surface-immobilized heparin (Fig. 6A). The normalized percentage of DENV envelope protein binding to immobilized heparin was assessed in the presence of controls and the six marine-sourced sulfated glycans. DENV envelope protein exhibited strong binding to PpFucCS, HfSF, HfFucCS, IbSF, and IbFucCS, with over 80% inhibition (Fig. 6B), suggesting the antiviral potential of these marine-sourced sulfated glycans.

### Concentration-dependant inhibitory effects of sulfated glycans

To further evaluate the competitive binding kinetics, subsequent experiments focused on the effect of increased concentration of test glycans on the binding kinetics of DENV envelope protein. DENV envelope proteins were premixed with a series of concentrations of heparin and selected marine-sourced glycans (rhamnan sulfate, IbSF, and PpFucCS). Each concentration was individually mixed with 50 nM of DENV envelope protein. Results show all the tested biomolecules displayed a concentration-dependent inhibition of DENV envelope protein binding to immobilized heparin (Fig. 7). The $IC_{50}$ results indicate that rhamnan sulfate, IbSF, and PpFucCS exhibited inhibitory activity on the interaction between immobilized heparin and DENV envelope protein, with estimated $IC_{50}$ values ranging from 43 to 172 ng/mL, compared to heparin at 5495 ng/mL These sulfated glycans could serve as lead compounds for further investigation into their structure-activity relationships and for *in vitro/in vivo* analyses as anti-DENV prophylaxis and therapeutics.

## Conclusions

The present study focuses on characterizing the binding affinity of DENV envelope protein (from Type 2 strain) with heparin and the inhibition of this interaction by sulfated glycans, namely, naturally-sourced and semi-synthetic glycans. Competitive SPR assays using surface-immobilized heparin and glycans (namely, RPI-27, RS, PpFucCS, HfSF, HfFucCS, IbSF, and IbFucCS) demonstrated a > 80% inhibition of binding of DENV envelope proteins to the heparin. Taking together the SPR-based inhibitory results regarding the series of SF (linear 3-linked α-fucose-based polymers sulfated at C2 and/or C4 positions and Mw ≥ 100 kDa) the series of FucCS (chondroitin sulfate backbones composed of GalNAc units sulfated at either or both C4 and C6 positions, decorated with α-fucose branches 3-linked to the GlcA units and Mw varying from 10 to 60 for PpFucCS, ~ 50 for HfFucCS, and ~ 75 for IbFucCS), it was interesting to note that almost all SF and FucCS molecules exhibited great activity with similar intensities. The only exception was the most sulfated SF LvSF, structurally composed of a pentasulfated tetrasaccharide-repeating unit, which showed weaker activity as compared to the the other two SFs (HfSF,

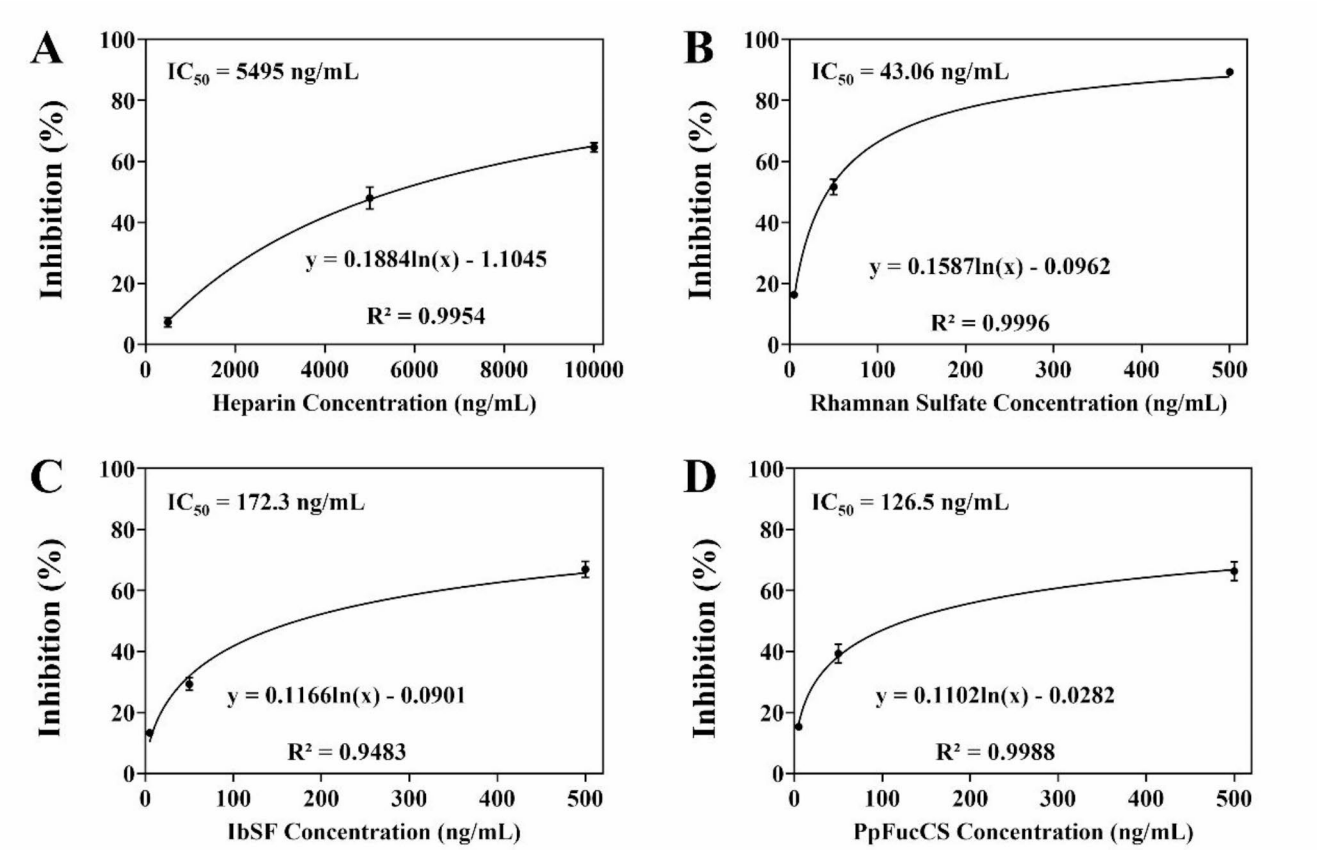


**Fig. 7** $IC_{50}$ measurement of the inhibition of DENV protein binding to heparin using solution competition SPR by sulfated glycans. (**A**): heparin; (**B**): rhamnan sulfate; (**C**): IbSF; (**D**): PpFucCS. $IC_{50}$ values were calculated using dose-response-inhibition equations in GraphPad Prism 9. Data are shown as mean ± SD, *wheren* = 3 (triplicate measurements were performed in one experiment)

and IbSF) and the three FucCSs (PpFucCS, HfFucCS, and IbFucCS), but similar activity as compared to heparin which is mostly composed of trisulfated disaccharide-repeating units. This set of observations indicates that (i) fucose branches in FucCS vs. linear fucose chains, (ii) difference in high MWs, and (iii) sulfation content is not ultimately dictating the inhibitory activity, but (i) sulfation pattern and (ii) monosaccharide composition can markedly play a role in the inhibition. Future studies will focus on improving the structure-activity relationships raised in this work and conducting functional cell-based assays to further explore the anti-DENV activity of these sulfated glycans.

**Author contributions** F.Z. and R.J.L provided the conceptualization; J. Y. and P.D. performed the analysis and drafted the manuscript; V.H.P. provided marine glycans and revision; C.W., F.Z., K.X. Q. M. and J.S.D did the acquisition of funding. All authors reviewed the manuscript.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Competing interests** The authors declare no competing interests.

## References

1. Wong, J.M., Adams, L.E., Durbin, A.P., Muñoz-Jordán, J.L., Poehling, K.A., Sánchez-González, L.M., Volkman, H.R., Paz-Bailey, G.: Dengue: A growing problem with new interventions. Pediatrics. **149** (2022). https://doi.org/10.1542/PEDS.2021-055522
2. Centers for Disease Control and Prevention (CDC): Increased risk of Dengue virus infections in the United States., Accessed September 09, 2024. https://emergency.cdc.gov/han/2024/han00511.asp
3. World Health Organization (WHO). Dengue and severe dengue. Accessed September 09: (2024). https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
4. Chen, Y., Maguire, T., Hileman, R.E., Fromm, J.R., Esko, J.D., Linhardt, R.J., Marks, R.M.: Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. Nat. Med. **3**(8), 866–871 (1997). https://doi.org/10.1038/nm0897-866
5. Cruz-Oliveira, C., Freire, J.M., Conceição, T.M., Higa, L.M., Castanho, M.A.R.B., Da Poian, A.T.: Receptors and routes of dengue virus entry into the host cells. FEMS Microbiol. Rev. **39**, 155–170 (2015). https://doi.org/10.1093/FEMSRE/FUU004
6. Liu, P., Ridilla, M., Patel, P., Betts, L., Gallichotte, E., Shahidi, L., Thompson, N.L., Jacobson, K.: Beyond attachment: Roles of DC-SIGN in Dengue virus infection. Traffic. **18**, 218 (2017). https://doi.org/10.1111/TRA.12469
7. Sinha, S., Singh, K., Ravi Kumar, Y.S., Roy, R., Phadnis, S., Meena, V., Bhattacharyya, S., Verma, B.: Dengue virus pathogenesis and host molecular machineries. J. Biomed. Sci. **31**, 43 (2024). https://doi.org/10.1186/S12929-024-01030-9
8. Song, Y., He, P., Rodrigues, A.L., Datta, P., Tandon, R., Bates, J.T., Bierdeman, M.A., Chen, C., Dordick, J., Zhang, F., Linhardt, R.J.: Anti-Sars-CoV-2 activity of rhamnan sulfate from *Monostroma Nitidum*. Mar. Drugs. **19**, 685 (2021). https://doi.org/10.3390/MD19120685/S1
9. Wang, S., Wang, W., Hao, C., Yunjia, Y., Qin, L., He, M., Mao, W.: Antiviral activity against enterovirus 71 of sulfated rhamnan isolated from the green alga *Monostroma latissimum*. Carbohydr. Polym. **200**, 43–53 (2018). https://doi.org/10.1016/J.CARBPOL.2018.07.067
10. Hayashi, K., Lee, J.B., Nakano, T., Hayashi, T.: Anti-influenza a virus characteristics of a fucoidan from sporophyll of *Undaria pinnatifida* in mice with normal and compromised immunity. Microbes Infect. **15**, 302–309 (2013). https://doi.org/10.1016/J.MICINF.2012.12.004
11. Terasawa, M., Hayashi, K., Lee, J.B., Nishiura, K., Matsuda, K., Hayashi, T., Kawahara, T.: Anti-influenza a virus activity of rhamnan sulfate from green algae *Monostroma Nitidum* in mice with normal and compromised immunity. Mar. Drugs. **18**, 254 (2020). https://doi.org/10.3390/MD18050254
12. Yang, J., Song, Y., Jin, W., Xia, K., Burnett, G.C., Qiao, W., Bates, J.T., Pomin, V.H., Wang, C., Qiao, M., Linhardt, R.J., Dordick, J.S., Zhang, F.: Sulfated glycans inhibit the interaction of MERS-CoV receptor binding domain with heparin. Viruses. **16** (2024). https://doi.org/10.3390/V16020237
13. Song, Y., Singh, A., Feroz, M.M., Xu, S., Zhang, F., Jin, W., Kumar, A., Azadi, P., Metzger, D.W., Linhardt, R.J., Dordick, J.S.: Seaweed-derived fucoidans and rhamnan sulfates serve as potent anti-SARS-CoV-2 agents with potential for prophylaxis. Carbohydr. Polym. **337** (2024). https://doi.org/10.1016/J.CARBPOL.2024.122156
14. Liyanage, N.M., Nagahawatta, D.P., Jayawardena, T.U., Sanjeewa, K.K.A., Jayawrdhana, H.H.A.C.K., Kim, J., Il, Jeon, Y.J.: Sulfated polysaccharides from seaweeds: A promising strategy for combatting viral diseases - a review. Mar. Drugs. **21** (2023). https://doi.org/10.3390/MD21090461
15. Gómez Toledo, A., Sorrentino, J.T., Sandoval, D.R., Malmström, J., Lewis, N.E., Esko, J.D.: A systems view of the heparan sulfate interactome. J. Histochem. Cytochem. **69**, 105–119 (2021). https://doi.org/10.1369/0022155420988661
16. Paiardi, G., Richter, S., Oreste, P., Urbinati, C., Rusnati, M., Wade, R.C.: The binding of heparin to spike glycoprotein inhibits SARS-CoV-2 infection by three mechanisms. J. Biol. Chem. **298** (2022). https://doi.org/10.1016/J.JBC.2021.101507
17. Chau, T., Joseph, M., Solomon, D.M., Lee, B., Igneri, L.A.: Heparin resistance in SARS-CoV-2 infected patients with venous thromboembolism. Hosp. Pharm. **57**, 737–743 (2022). https://doi.org/10.1177/00185787221111743
18. Gelbach, A.L., Zhang, F., Kwon, S.J., Bates, J.T., Farmer, A.P., Dordick, J.S., Wang, C., Linhardt, R.J.: Interactions between heparin and SARS-CoV-2 spike glycoprotein RBD from omicron and other variants. Front. Mol. Biosci. **9** (2022). https://doi.org/10.3389/FMOLB.2022.912887
19. Shi, D., Bu, C., He, P., Song, Y., Dordick, J.S., Linhardt, R.J., Chi, L., Zhang, F.: Structural characteristics of heparin binding to SARS-CoV-2 spike protein RBD of Omicron sub-lineages BA.2.12.1, BA.4 and BA.5. Viruses. **14** (2022). https://doi.org/10.3390/V14122696
20. Tandon, R., Sharp, J.S., Zhang, F., Pomin, V.H., Ashpole, N.M., Mitra, D., McCandless, M.G., Jin, W., Liu, H., Sharma, P., Linhardt, R.J.: Effective inhibition of SARS-CoV-2 entry by heparin

and enoxaparin derivatives. J. Virol. **95** (2021). https://doi.org/10.1128/JVI.01987-20
21. Kim, S.Y., Jin, W., Sood, A., Montgomery, D.W., Grant, O.C., Fuster, M.M., Fu, L., Dordick, J.S., Woods, R.J., Zhang, F., Linhardt, R.J.: Characterization of heparin and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) spike glycoprotein binding interactions. Antiviral Res. **181** (2020). https://doi.org/10.1016/J.ANTIVIRAL.2020.104873
22. Marks, R.M., Lu, H., Sundaresan, R., Toida, T., Suzuki, A., Imanari, T., Hernáiz, M.J., Linhardt, R.J.: Probing the interaction of dengue virus envelope protein with heparin: Assessment of glycosaminoglycan-derived inhibitors. J. Med. Chem. **44**(13), 2178–2187 (2001). https://doi.org/10.1021/jm000412i
23. He, P., Shi, D., Li, Y., Xia, K., Kim, S.B., Dwivedi, R., Farrag, M., Pomin, V.H., Linhardt, R.J., Dordick, J.S., Zhang, F.: SPR sensor-based analysis of the inhibition of marine sulfated glycans on interactions between Monkeypox virus proteins and glycosaminoglycans. Mar. Drugs 2023. **21**(21), 264 (2023). Page 264https://doi.org/10.3390/MD21050264
24. Suzuki, K., Terasawa, M.: Biological activities of rhamnan sulfate extract from the green algae *Monostroma Nitidum* (Hitoegusa). Mar. Drugs. **18**, 228 (2020). https://doi.org/10.3390/MD18040228
25. He, P., Shi, D., Li, Y., Xia, K., Kim, S.B., Dwivedi, R., Farrag, M., Pomin, V.H., Linhardt, R.J., Dordick, J.S., Zhang, F.: SPR sensor-based analysis of the inhibition of marine sulfated glycans on interactions between Monkeypox virus proteins and glycosaminoglycans. Mar. Drugs. **21**, 264 (2023). https://doi.org/10.3390/MD21050264
26. Samanta, P., Mishra, S.K., Pomin, V.H., Doerksen, R.J.: Docking and molecular dynamics simulations clarify binding sites for interactions of novel marine sulfated glycans with SARS-CoV-2 spike glycoprotein. Molecules. **28** (2023). https://doi.org/10.3390/MOLECULES28176413
27. Zoepfl, M., Dwivedi, R., Kim, S.B., McVoy, M.A., Pomin, V.H.: Antiviral activity of marine sulfated glycans against pathogenic human coronaviruses. Sci. Rep. **13** (2023). https://doi.org/10.1038/S41598-023-31722-5
28. Ergin Kizilçay, G., Ertürk Toker, S., Matur, D.: Analytical method development and validation of mucopolysaccharide polysulfate in topical formulations by size exclusion chromatography. Anal. Methods. **13**, 4114–4119 (2021). https://doi.org/10.1039/D1AY00673H
29. Hoppensteadt, D.A., Neville, B., Schultz, C., Jeske, W., Raake, W., Fareed, J.: Comparative studies on the topical administration of mucopolysaccharide and heparin ointments in nonhuman primates. Clin. Appl. Thromb. Hemost. **16**, 13–20 (2010). https://doi.org/10.1177/1076029609345688
30. Alekseeva, A., Raman, R., Eisele, G., Clark, T., Fisher, A., Lee, S., Larry), Jiang, X., Torri, G., Sasisekharan, R., Bertini, S.: In-depth structural characterization of pentosan polysulfate sodium complex drug using orthogonal analytical tools. Carbohydr. Polym. **234**, 115913 (2020). https://doi.org/10.1016/J.CARBPOL.2020.115913
31. Chen, S., Xue, C., Yin, L., Tang, Q., Yu, G., Chai, W.: Comparison of structures and anticoagulant activities of fucosylated chondroitin sulfates from different sea cucumbers. Carbohydr. Polym. **83**, 688–696 (2011). https://doi.org/10.1016/J.CARBPOL.2010.08.040
32. Shi, D., Qi, J., Zhang, H., Yang, H., Yang, Y., Zhao, X.: Comparison of hydrothermal depolymerization and oligosaccharide profile of fucoidan and fucosylated chondroitin sulfate from *Holothuria floridana*. Int. J. Biol. Macromol. **132**, 738–747 (2019). https://doi.org/10.1016/J.IJBIOMAC.2019.03.127
33. Chen, S., Hu, Y., Ye, X., Li, G., Yu, G., Xue, C., Chai, W.: Sequence determination and anticoagulant and antithrombotic activities of a novel sulfated fucan isolated from the sea cucumber Isostichopus badionotus. Biochim. et Biophys. Acta (BBA) - Gen. Subj. **1820**, 989–1000 (2012). https://doi.org/10.1016/J.BBAGEN.2012.03.002
34. Pomin, V.H., Pereira, M.S., Valente, A.P., Tollefsen, D.M., Pavão, M.S.G., Mourão, P.A.S.: Selective cleavage and anticoagulant activity of a sulfated fucan: Stereospecific removal of a 2-sulfate ester from the polysaccharide by mild acid hydrolysis, preparation of oligosaccharides, and heparin cofactor II–dependent anticoagulant activity. Glycobiology. **15**, 369–381 (2005). https://doi.org/10.1093/GLYCOB/CWI021