

Understanding Endothelial Glycocalyx Function Under Flow Shear Stress from a Molecular Perspective

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Understand EG function from molecular dynamics

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18 **Abstract**

19 **BACKGROUND:**

20 The endothelial glycocalyx plays a pivotal role in regulating blood flow, filtering blood components,
21 sensing and transducing mechanical signals. These functions are intimately related to its dynamics at the
22 molecular level.

23 **OBJECTIVE:**

24 The objective of this research is to establish the relationship between the functions of the endothelial
25 glycocalyx and its dynamics at the molecular level.

26 **METHODS:**

27 To establish such a relationship, large-scale molecular dynamics simulations were undertaken to
28 mimic the dynamics of the glycocalyx and its components in the presence of flow shear stresses.

29 **RESULTS:**

30 First, motions of the glycocalyx core protein and the pertinent subdomains were scrutinised. Three-
31 directional movements of the glycocalyx core protein were observed, although the flow was imposed
32 only in the x direction. Such an observation contributes to understanding the glycocalyx redistribution
33 as reported in experiments. Unsynchronised motion of the core protein subdomains was also spotted,
34 which provides an alternative explanation of macroscopic phenomena. Moreover, the dynamics, root-
35 mean-square-deviations and conformational changes of the sugar chains were investigated. Based on the
36 findings, an alternative force transmission pathway, the role of sugar chains, and potential influence on
37 signalling transduction pathway were proposed and discussed.

38 **CONCLUSIONS:**

39 This study relates the functions of the glycocalyx with its microscopic dynamics, which fills a
40 knowledge gap about the links between different scales.

41 **1. Introduction**

42 The glycocalyx, which is an integral part of the vascular barrier, covers all healthy vascular
43 endothelium. It has been confirmed that in patients with cardiovascular risk factors, such as diabetes and
44 hypertension, endothelial glycocalyx damage drives progression of kidney disease and cardiovascular
45 disease (1). The glycocalyx is a complex layer of membrane-bound proteoglycans (e.g. syndecans or
46 glypicans), glycoproteins and glycolipids. A Proteoglycan element consists of a core protein that carries
47 one or more covalently attached glycosaminoglycan (GAG) chains. The main GAGs found on
48 proteoglycans in the endothelial surface layer are heparan sulfate (HS), chondroitin sulfate and dermatan
49 sulfate (2) featuring the highly negative charges (3). It is widely believed that the negatively charged
50 GAGs in the endothelial glycocalyx capture circulating plasma protein and form an interconnected gel-
51 like structure in an aqueous environment (4). The gel-like structure lines the luminal endothelial surface
52 and acts as a barrier against albumin filtration, which is crucial in maintaining the normal function of
53 the glycocalyx (5).

54 The endothelial glycocalyx is exposed to the mechanical forces of blood flow. Thus, another function
55 of the glycocalyx is a medium for mechanotransduction. Mechanotransduction means the glycocalyx
56 senses the shear stress of flowing blood and transmits the mechanical signal into the cytoplasm (6, 7).
57 The primary evidence that supports a major role for the endothelial glycocalyx layer in
58 mechanotransduction comes from the enzyme degradation experiments. By removing specific
59 components of the glycocalyx, any loss of the glycocalyx functional can be recognised, and the role of
60 the removed part is assessed (2, 8). However, as reviewed in Ref. (9), the drawback of the enzyme
61 degradation method is that it may cause biased estimation of the contribution from the component.
62 Meanwhile, the highly dynamic and fragile sugar chains aggravate the difficulty in *ex vivo* experiments
63 (10).

64 The functions of the endothelial glycocalyx reported in experiments are intimately related to its
65 dynamics at the molecular scale. However, due to a lack of studies focusing on the dynamics of the
66 glycocalyx, how to establish such a relationship is still unclear. To fill the knowledge gap, we use large-
67 scale molecular dynamics (MD) simulations to mimic the dynamics of the glycocalyx and its
68 components in the presence of flow shear stresses. By discussing the results in the context of
69 experimental studies, functions of the glycocalyx are explained from the perspective of the molecular
70 motions.

71 **2. Methods**

72 *2.1 System construction*

73 The up-to-date structure of glycocalyx with the finest resolution (11) has been used to construct the
74 flow/glycocalyx system. As the majority (50% ~ 90% (12)) of GAG chains added to the core proteins
75 of syndecans are of the HS type (13) and for the sake of simplification, only HS sugar chains are
76 considered in the construction of the system. In the proposed system, one glycocalyx element is
77 modelled by the combination of Syndecan-4 (Syn-4) proteoglycan and HS sugar chains. The glycocalyx
78 element can be separated into three parts in accordance with the positions to the lipid membrane: Syn-4
79 ectodomain connected with sugar chains; Syn-4 transmembrane dimer with a diameter of about 45 Å
80 implanted into a lipid bilayer; and cytoplasmic part of the Syn-4 dimer. The HS length is assumed to be
81 100 sugar residues (with 4 sugar residues as a linker to Syn-4). Apart from the 4 linker sugar residues,
82 there are 48 disaccharide units for each chain. The end-to-end distance of the HS sugar chains varies
83 from 23 to 41 nm. The HS sugar chains are covalently attached to three serine residues in the Syn-4
84 ectodomain. The disaccharide sequences of HS sugar chains are introduced in detail in Ref. (11). As
85 reported in Ref. (11), the mean value for bending stiffness of HS sugar chains is $68 \text{ pN}\cdot\text{nm}^2$, which is
86 approximately an order-of-magnitude lower than the value commonly used in continuum glycocalyx

87 models (490 pN·nm²). As a real endothelial surface environment is far more complicated and
88 heterogenous than the system we proposed in this research, the high value for glycocalyx cannot be
89 attributed to the HS chains alone. All the other macromolecules (e.g. plasma proteins) which are not
90 considered in this research can contribute to the high values for bending stiffness of the glycocalyx.
91 Most of the HS sugar chains in the proposed system are set perpendicular (or nearly perpendicular) to
92 the endothelial cell surface, which corresponds to the newly published observation from Stochastic
93 Optical Reconstruction Microscopy (14). The Syn-4 intracellular domain is not linked to the
94 cytoskeleton.

95 Figure 1a illustrates an overview of the flow/glycocalyx system with its initial configuration. The
96 lipid bilayer separates the simulation domain into two regions. Ectodomain, where blood flows in the
97 lumen, is over the lipid bilayer. The ectodomain is filled with the ectodomain part of the Syn-4 core
98 protein, HS sugar chains connected to the protein, ions and water molecules. The cytoplasm, namely the
99 inner domain of the cell, is below the lipid bilayer. The cytoplasmic part of the Syn-4 protein, ions and
100 water molecules are the main components of this region. The glycocalyx features its negative charge
101 and the charge distribution depends on the geometry distributions of the sugar chains. A NaCl aqueous
102 solution with a concentration of 0.1 M was used to neutralize and solvate the biomolecules. According
103 to our previous study (15), these charges (the negative sugar chains, Na⁺ and Cl⁻) are not uniformly
104 distributed along the space in the ectodomain. The spatial distributions of the charges can be referred to
105 Ref. (15). For the three directions (i.e. *x*-, *y*- and *z*-directions), periodic boundary conditions were applied.
106 Details about the set-up for the boundary conditions can be found in previous publications (16-18).

107 In the flow/glycocalyx model, three glycocalyx elements are constructed and implanted on the lipid
108 bilayer. Each glycocalyx element (i.e. proteoglycan) bears six HS chains glued to the ectodomain of the
109 Syn-4 core protein dimer. The blood flow is simulated by driving the ectodomain water molecules via

110 external forces on water oxygens. The dimension of the domain simulated is a hexagonal area of 820
111 nm² by 72 nm in height. About 5,800,000 atoms are contained in the entire flow/glycocalyx system (18).

112 ***2.2 Protocol details***

113 The TIP3P water model (19) was adopted to simulate water molecules. A CHARMM biomolecular
114 force field (20) was applied on the proteins and the lipid bilayer.

115 An equilibrium simulation was first conducted at 1 atm and 310K (NPT ensemble), using a Langevin
116 thermostat and a Nosé-Hoover Langevin piston for 2 ns, followed by another simulation using a
117 Langevin thermostat to maintain temperature at 310K for 0.5 ns (NVT ensemble). The last frame of the
118 NVT simulation was then used as the initial configuration (as shown in Figure 1a) of the follow-up
119 “production” flow simulations. In the flow simulations, the Lowe-Andersen thermostat was selected to
120 maintain the temperature at 310K.

121 The velocity Verlet integration method (21) was used to advance the positions and velocities of the
122 atoms in time. A 2-fs timestep, and particle mesh Ewald (22) electrostatics with a grid density of 1/Å³
123 are used. The SETTLE algorithm (23) was used to enable the rigid bonds connected to all hydrogen
124 atoms. The van der Waals interactions were calculated using a cutoff of 12 Å with a switching function
125 starting at 10 Å.

126 All MD simulations were performed using the software NAMD 2.9 (24). The visualisation of the
127 molecular structures was performed by the VMD (25) package. Post-processing of the MD results was
128 accomplished using PYTHON (Python Software Foundation, Wilmington, De) scripts. All parallel
129 simulations and non-visualised post-processing were conducted on ARCHER, UK’s national
130 supercomputing service. To obtain a simulation result with a physical period of 1 ns, 9,000 compute
131 cores have been simultaneously employed for about 2 hours.

132 ***2.3 Flow simulation and case set-up***

133 In this research, NaCl solution was used as a simplification of the blood flow. To mimic flow, external
134 forces in the x direction were imposed on oxygen atoms of water molecules in the ectodomain, and the
135 tactic was successfully practiced in previous studies (16, 18, 26). In one of our previous studies (18), we
136 reported that an external force of 0.003 fN would generate a laminar flow with a physiological bulk flow
137 velocity (The order of magnitude of the bulk flow velocity is assumed 0.1~1 cm/s, which corresponds
138 to that expected in the human microcirculation.); the presence of the glycocalyx disturbs the flow profiles,
139 resulting in an oscillating velocity distribution in space. To study the dynamics of the endothelial
140 glycocalyx in various situations, four scenarios were established as listed in Table 1. Case I is for
141 mimicking a physiological flow under the intact EG situations with 18 sugar chains. In Case II, the
142 external force is set to 0 to simulate a stationary state. Cases III and IV are two scenarios with shedding
143 of sugar chains by removing of 3 and 9 sugar chains, respectively. The strategy for removal of sugar
144 chains is illustrated in Figures 1b to 1d. Detailed information about the removal strategy was introduced
145 in Ref. (17).

146 **3. Results**

147 ***3.1 Dynamics of core protein***

148 The trajectory of the core protein of the central glycocalyx element (Figure 2a) is tracked in the case
149 with intact glycocalyx configuration and physiological flow (i.e. Case I). Figure 2b shows the trajectory
150 of the core protein. The curve in Figure 2b represents the trace of the protein in the XOY plane. The
151 circles represent the relative z positions of the core protein compared to that at the start point; the area
152 of a circle means the deviation of core protein from its original position in the z direction, with the blue
153 colour being higher than the position at the start point and red lower than the start point. As illustrated
154 in the Figure 2b, the core protein travels in the same direction as the flow, as expected. Meanwhile, it
155 also moves in the Y and Z directions.

156 **3.2 Dynamics of core protein subdomains**

157 The motions of the central glycolyx core protein subdomains are further examined for Case I. The
158 core protein is a Syn-4 dimer, comprising the ectodomain and transmembrane parts with the secondary
159 structures illustrated in Figure 3a. Secondary structures (Figure 3a) show that flexible linkages between
160 subdomains of ectodomain Syn-4 (EA1 and EA2 from Chain A, and EB1 and EB2 from Chain B) as
161 well as Syn-4 ectodomain and transmembrane parts (TA1 and EA1 for Chain A, and TB1 and EB1 for
162 Chain B) encourage the glycolyx to perform like a soft matter. To study the dynamics of the soft matter,
163 Four distances (d_{A1} , d_{A2} , d_{B1} and d_{B2}) are employed to record positions of the ectodomain and
164 transmembrane Syn-4. As shown in Figure 3b, the first 10 ns witnesses different trends of the four
165 distances. After 10 ns, the average distances with standard errors for the four distances are calculated.
166 The differences in the distance variations further validate the model of the glycolyx as a soft matter.

167 **3.3 Dynamics of sugar chains**

168 The dynamic and fragile feature of the glycolyx sugar chains increases the difficulty in glycolyx-
169 related experiments (9, 10). To gain additional insight into the dynamics of the sugar chain, four
170 segments from two typical sugar chains, labelled P1 to P4 in Figure 4a, are selected for motion inspection.
171 Segments P1 and P3, individually composed of five residues, are located at 40% of the total length of
172 chains 1 and 2, respectively. Segment P2 and segment P4 each comprise the five ending residues of
173 sugar chain tails. Their motions in three directions are recorded as shown in Figures 4b and 4c. In Figure
174 4b, the z positions of these segments indicate that the four segments swing up and down as flow passes
175 by. Figure 4c records the trajectories of the four segments in the XOY plane, and white lines represent
176 their routes during the 30-ns simulation. As illustrated in Figure 4c, these segments also swirl freely in
177 space (18).

178 **3.4 Root-mean-square deviation (RMSD) of sugar chains**

179 Figure 2a suggests that the dynamic sugar chains may interfere with each other. To study how they
180 impede each other, the RMSDs of three sugar chains, as an alternative measure of sugar chain
181 movements, are investigated under situations with varying numbers of sugar chains (i.e. Cases I, III and
182 IV in Table 1). The values of RMSDs are calculated by a build-in plugin of the VMD package (25).
183 Figure 5a highlights the three mutual sugar chains. Their RMSDs under situations with changing sugar
184 chain numbers are summarised in Figure 5b. As the statistics suggest, the RMSD values increase as the
185 number of sugar chain decreases, which implies their interference with each other.

186

187 ***3.5 Conformations of sugar chains***

188 The sugar chains are flexible and dynamic even in the stationary situations. In the stationary case
189 (Case II in Table 1), to describe the dynamics of sugar chains the conformations of two sugar chains, i.e.
190 sugar chains AS and BS in Figure 6a, are investigated. The conformation of a sugar chain is measured
191 via a centre-to-centre vector connecting the two centres of mass of a bisected sugar chain, which is
192 reported effective in describing polymer rotational dynamics (27). The distributions (counted by the
193 occurrence in the recorded frames) for magnitudes, r , of centre-to-centre vectors for the two sugar chains
194 are summarised in Figure 6b. Due to the corner structure, sugar chain BS has a shorter centre-to-centre
195 distance than its stretching counterpart, sugar chains AS.

196 As illustrated in Figure 6a, the major initial conformational difference of the two sugar chains resides
197 in segments with corner shapes. The sugar chains attract Na^+ ions due to their highly negative charges.
198 To reveal how the corner shape affects the interaction between Na^+ and sugar chains, two segments with
199 identical residue sequence but one featuring a corner shape and the other with a stretching shape are
200 selected. The numbers of Na^+ around both segments (highlighted purple in Figure 6a) throughout the
201 no-flow simulation are recorded. To further explore how the corner conformation influences their

202 interaction with Na⁺ ions, the residence rates of initial Na⁺ ions around the corner and stretching
203 conformations are calculated. The residence rate is calculated as

$$\text{residence rate} = \frac{n_{Na,j}}{n_{Na,0}} \quad (1)$$

204
205 In Eq. (1), $n_{Na,j}$ is the number of Na⁺ ions retained from the initial frame of the simulation at the instant
206 j , and $n_{Na,0}$ is the number of Na⁺ ions at the initial frame of the simulation. The distributions (counted by
207 the occurrence in the recorded frames) for the residence rates of both sugar chains are summarised, as
208 shown in Figure 6c., The higher residence rate of Na⁺ of sugar chain BS indicates that more ions stay
209 around the corner sugar chain. In other words, the corner conformation accumulates Na⁺ by confining
210 the ions within its “realm”. By contrast, the stretching structure of sugar chain AS facilitates the motion
211 of ions.

212 **4. Discussion**

213 In this section, the biological significance of the dynamics of the glycocalyx is discussed.

214 **4.1 Glycocalyx redistribution**

215 When exposed to the flow shear stress, the glycocalyx is redistributed as witnessed in experiments
216 (28, 29). For example, the percentage area of the cell membrane coated by the glycocalyx increases after
217 the cells being exposed to the flow shear stress for a certain period (29). It is noteworthy that the
218 simulation time scale is far smaller than its experiment counterpart. However, it is still worthwhile to
219 relate the MD findings with experimental observations, as MD results can capture fine and basic
220 movements of atoms and molecules, which could complement experimental observations. According to
221 our results (Figure 2b), the glycocalyx core protein not only travels along the flow direction but also
222 moves actively in the other two directions. As sugar chains are anchored to the core protein, the flexible
223 movement of the core protein consequently results in varying overlap areas between the sugar chains,

224 which explains the changes in the percentage area of the cell membrane covered by the glycocalyx as
225 reported in Ref. (29). Although the cytoskeleton is not included in the present simulation, a disturbance
226 in the well-structured cytoskeleton can still be inferred from the dynamic movement of the core protein.
227 The structure disturbance could then cause the reorganisation of the cytoskeleton, thereby resulting in
228 the cell migration along the direction of flow, as reported in previous studies (30, 31).

229 ***4.2 Deformation of core protein from an atomic perspective***

230 Molecular dynamics methods provide a unique perspective to understand some macroscopic matrix
231 in continuum studies. For example, in a previous continuum study (32), the authors discussed the
232 bending rigidity of the core proteins that enables them to resist the deformation by fluid shear stresses.
233 If an object is a rigid body, any two points of the body will move simultaneously. Yet, according to our
234 results in Figure 3b, the subdomains of the core protein move unsynchronised. The presumed
235 deformation is indeed the unsynchronisation of the subdomains, with the bending rigidity being a
236 measure of the pertinent synchronization (18).

237 ***4.3 Mechanotransduction pathways***

238 Based on the dynamics of the subdomain of the core protein (mentioned in 3.2) and the sugar chains
239 (mentioned in 3.3), the potential mechanotransduction pathway of the glycocalyx can be proposed. One
240 topic about the mechanotransduction of the glycocalyx is to sort out the route via which the flow shear
241 stress is transmitted into the cytoplasm. Previous studies (32, 33) favour that the flow shear stress is first
242 transmitted to the sugar chains and then transmitted to the cytoplasm via the core protein. In our research,
243 the results in Sections 3.2 and 3.3 suggest that the movements of the core protein and the sugar chains
244 are not strongly correlated (with the Pearson correlation coefficients between the z-direction movements
245 of the selected sugar chain segment P1 and Syn-4 ectodomain being -0.35, P2 and Syn-4 ectodomain
246 0.35, P3 and Syn-4 ectodomain 0.55, and P4 and Syn-4 ectodomain 0.53), which implies that the force

247 may be transmitted to the cytoplasm via the core protein without the transduction of the sugar chains.
248 These two routes are both potential force transmission pathways via the glycocalyx.

249 ***4.4 Function of sugar chains***

250 Figure 5 suggests that the sugar chains interfere with each other, as the RMSD values increase with
251 the reducing number of the sugar chains. Given that the sugar chains are connected to the core protein,
252 albeit weakly correlated, the fierce movement in the sugar chain reduced situations suggests a dynamic
253 movement of the core protein, thereby influencing the mechanotransduction. Therefore, one function of
254 the sugar chains is to prevent the severe movement of the core protein and to maintain the normal
255 function of glycocalyx.

256 ***4.5 Implications for initiating signal transduction pathways***

257 As discussed in Ref. (34), blood flow can uncoil the glycocalyx in the direction of flow and the
258 corresponding conformational change can increase Na^+ ion binding sites that initiate signal transduction
259 pathways. In our research, the number of Na^+ ion binding sites was not determined; however, Figure 6
260 can still demonstrate the importance of the glycocalyx in activating signalling transduction pathways.
261 The conformational difference leads to the variations in the residence rate of Na^+ ions. Combined with
262 the results of sugar chain dynamics (Section 3.3), it is rational to expect an activation of signal channels
263 sensed by the glycocalyx when blood flow velocity changes.

264 **5. Conclusions**

265 In this research, large-scale molecular dynamics simulations were conducted to investigate the
266 dynamics of the glycocalyx on a small patch of the lipid membrane. The motions of the glycocalyx core
267 protein and the pertinent subdomains were scrutinised. Movements of the core protein were observed in
268 all three directions, although the flow was imposed only in the x direction. Such a finding contributes to
269 understanding the glycocalyx redistribution as reported in experiments. Unsynchronised motion of the

270 core protein subdomains provides an alternative explanation of deformation from the molecular
271 perspective. Moreover, the dynamics, RMSDs and conformational changes of the sugar chains were
272 investigated. Based on these findings, an alternative force transmission pathway, the role of sugar chains,
273 and potential influence on signalling transduction pathway have been proposed and discussed. The force
274 from the flow shear stresses can be probably transmitted from the blood flow via the core protein and
275 then to the cytoplasm without going to the sugar chains. One function of the sugar chains is to prevent
276 the severe movement of the core protein and to maintain the normal function of glycocalyx. The changes
277 in blood flow velocities may activate the signalling channels via the pertinent conformational changes
278 of sugar chains.

279 This study relates the macroscopic behaviour and functions of the glycocalyx with its molecular
280 dynamics, which contributes to filling the knowledge gaps about the links between different scales.
281 Future molecular dynamics studies can focus on the functionality of the glycocalyx serving as a
282 molecular sieve and on the response of the glycocalyx to changes in physiological conditions.

283

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Table 1 Summary of simulation cases in this study

Case	external force / fN	Number of sugar chains, N	Physical time / ns
I	0.003	18	30
II	0	18	8
III	0.003	15	15
IV	0.003	9	15

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382 **Figure legends**

383 **Figure 1 Initial configuration of the all-atom glycoalyx-flow system and the strategy of removing**
384 **sugar chains.** a. Initial configuration. The system includes 3 Syn-4 dimers as proteoglycans, 18 sugar
385 chains attached on the apexes of Syn-4 dimers and a lipid bilayer. External forces are imposed in the x
386 direction on the water molecules in the ectodomain. Water molecules and ions are not shown. b. Top
387 view of sugar chain layout in the complete flow/glycoalyx system for Cases I and II in Table 1. c. Top
388 view of a reduced flow/glycoalyx system (Case III in Table 1) with three sugar chains (highlighted red
389 in Panel b) removed from the central glycoalyx element. d. Top view of a reduced flow/glycoalyx
390 system (Case IV in Table 1) with half of sugar chains removed (highlighted sugar chains in Panels b and
391 c). In Panels b, c and d, only sugar chain layouts are illustrated. Details can be found in Ref. (13).

392 **Figure 2 Motion of the central glycoalyx core protein.** a. Top view of the glycoalyx elements with
393 the lipid membrane (Only the glycoalyx elements and the lipid membrane are shown). b. The 30-ns
394 trajectory of the core protein of the central glycoalyx element from case I. The core protein moves in
395 three directions.

396 **Figure 3 Motion of subdomain of central glycoalyx core protein.** a. Central glycoalyx core
397 protein with its secondary structure. b. Four distances used to depict the motions of the subdomains of
398 the central glycoalyx core protein.

399 **Figure 4 Dynamics of four segments from two sugar chains.** a. Four segments from two sugar
400 chains are selected to investigate the dynamics of sugar chains. b. The z -direction positions of these four
401 sugar chains as flow passes by. c. The four segments swirl freely in the XOY plane.

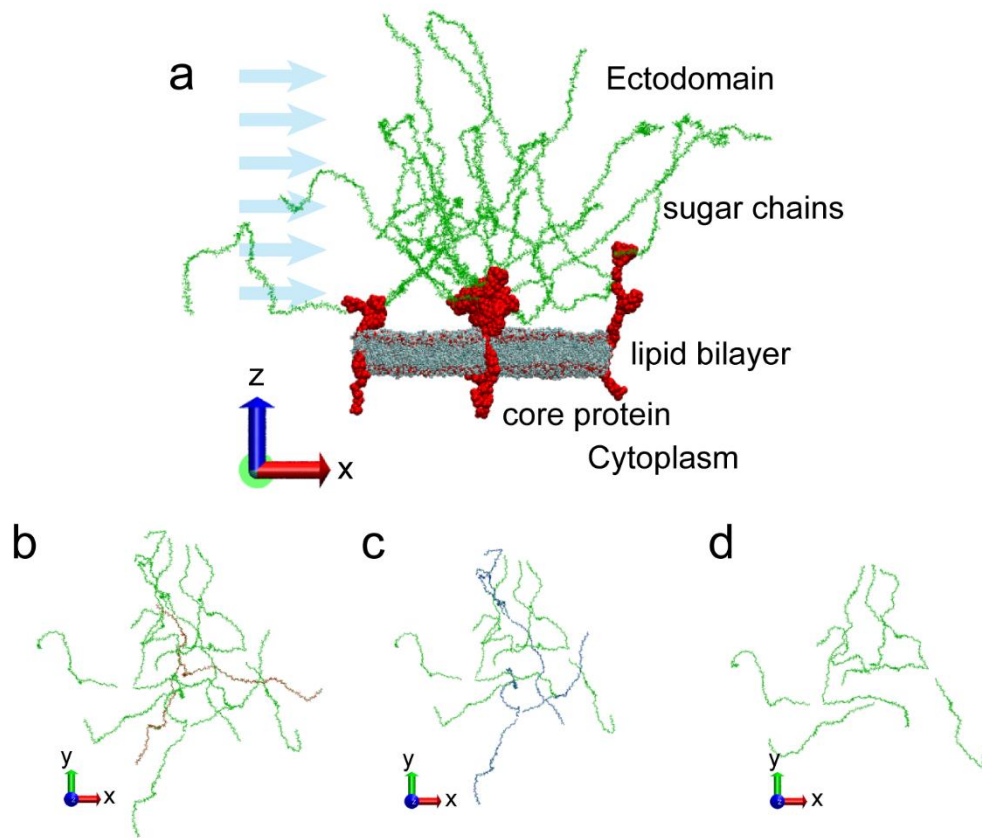
402 **Figure 5 The root-mean-square-deviations (RMSDs) of the intact sugar chains of the central**

403 **glycocalyx element.** a. The intact sugar chains are highlighted in magenta. b. The RMSDs of the three
404 sugar chains in three sugar-chain shedding scenarios. N=18 (Case I in Table 1) means no shedding sugar
405 chains, N=15 (case III in Table 1) refers to the scenario with the removal of the three sugar chains of the
406 central glycocalyx element, and N=9 (case IV in Table 1) represents the scenario with the removal of
407 half number of the sugar chains. Generally, the surrounding sugar chains impede the movement of the
408 central sugar chains, according to the small variations in the N=18 case. ($*p < 0.05$; $**p < 0.01$; $***p <$
409 0.001 by F-test)

410 **Figure 6 Conformations of two sugar chains and residence rate distributions of Na⁺ around**
411 **individual sugar chains.** a. Two sugar chains with identical residue sequence but different initial
412 conformations. b. Distributions for centre-to-centre lengths, r , of both sugar chains. c. The residence rate
413 distributions of Na⁺ ions around the corner segment and its stretching counterpart.

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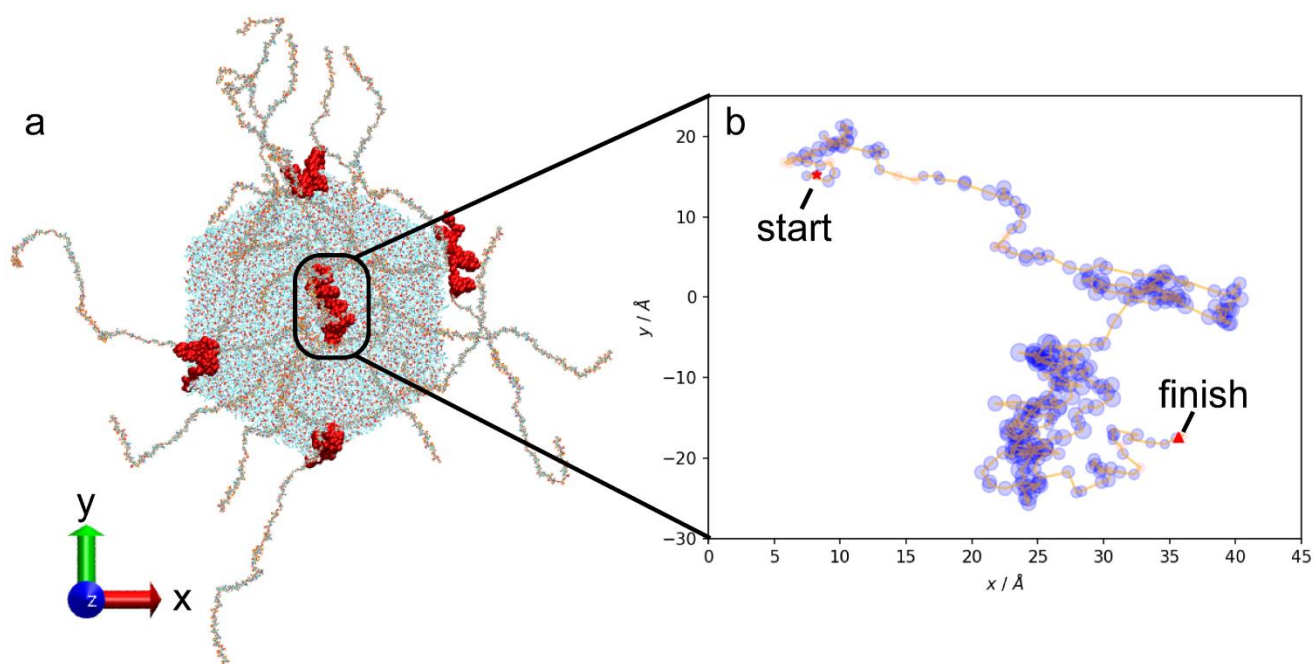
416 **Figure 1**



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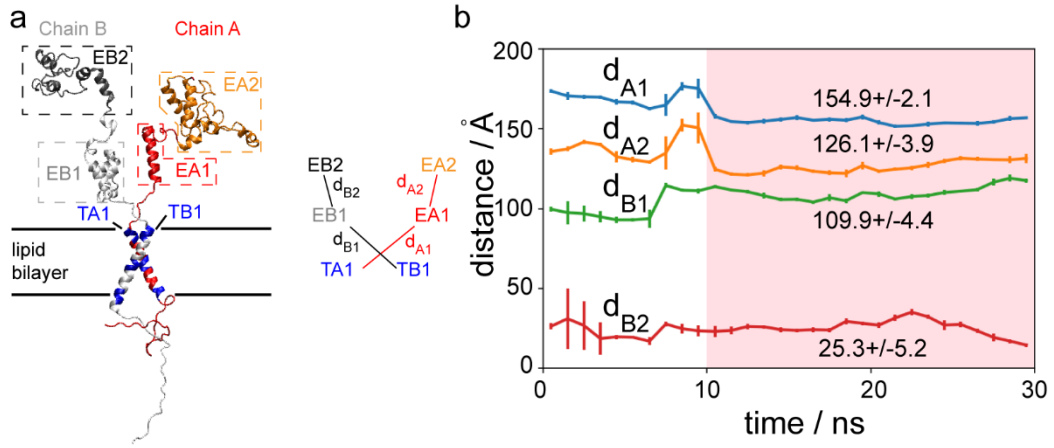
419 **Figure 2**



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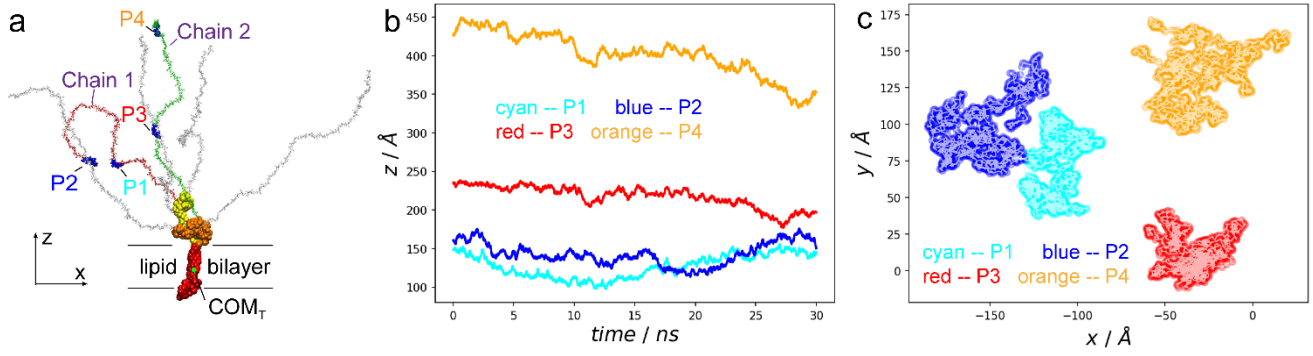
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422 **Figure 3**



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425 **Figure 4**

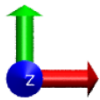
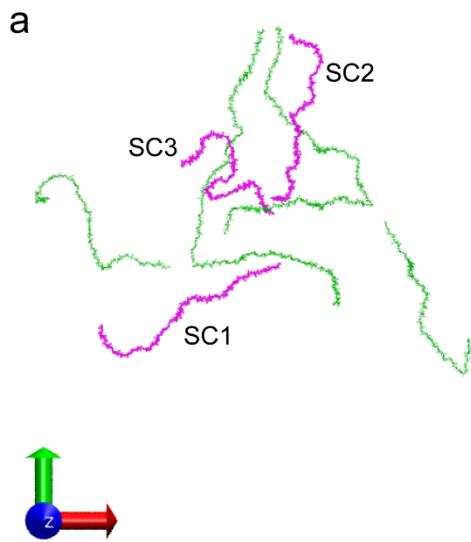


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428 **Figure 5**

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