Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

On the interaction of hyaluronic acid with synovial fluid lipid membranes: Supplementary Information

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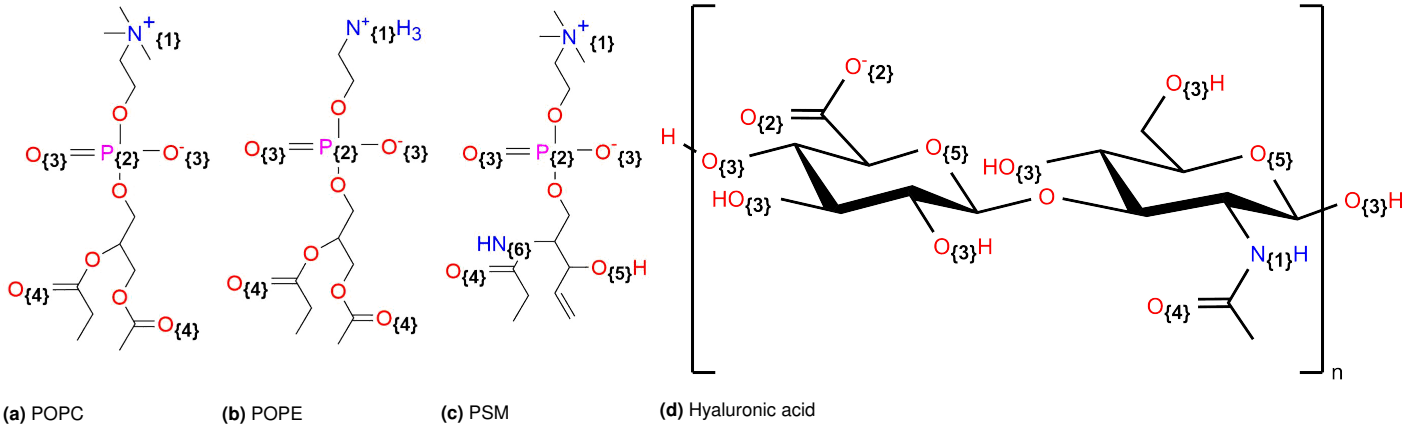
Labels of atoms in hyaluronic acid and the lipid headgroups

To make the description of interactions between specific moeities simpler, Fig 1 and Table 1 provide a list of atoms and labels of these atoms for easy reference.



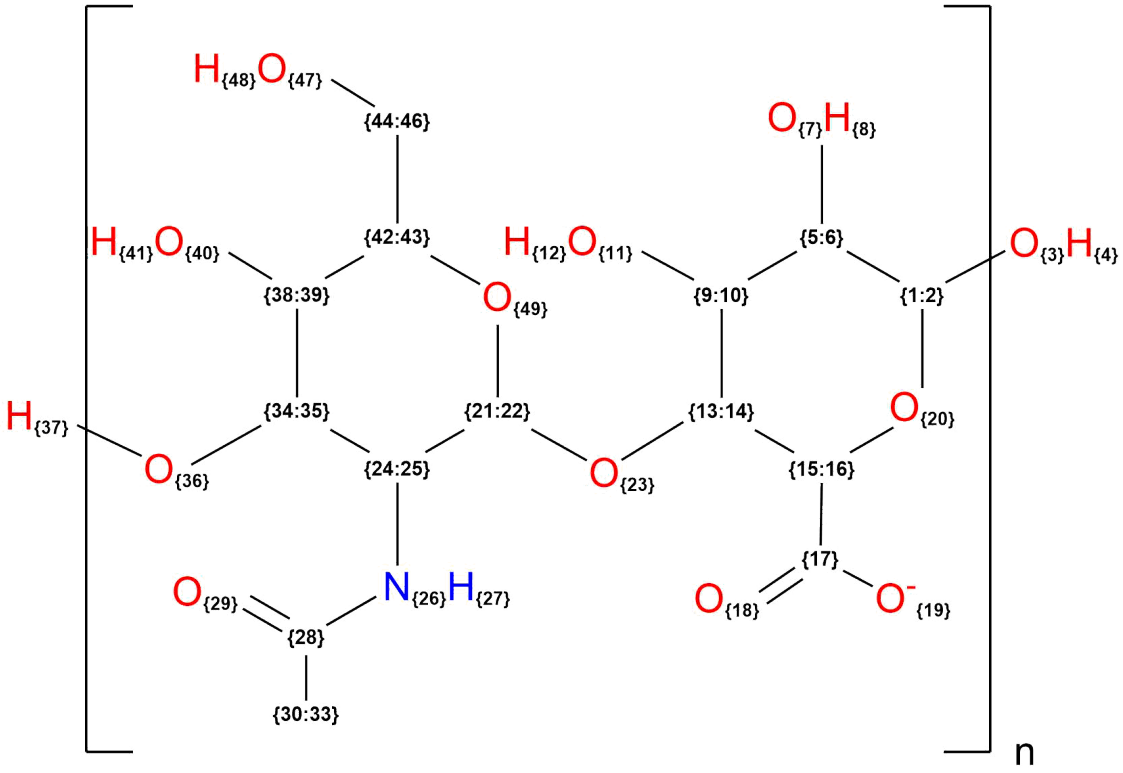
SI Fig. 1 The chemical structure of the **(a)** POPC, **(b)** POPE and **(c)** PSM headgroups and **(d)** hyaluronic acid. Atoms of functional groups that are important in the interaction between hyaluronic acid and the lipid membrane are numbered. To allow for easy reference to these atoms, Table 1 provides a list of labels for the numbered atoms.

	POPC		POPE		PSM		Hyaluronic acid	
Id	Element	Label	Element	Label	Element	Label	Element	Label
1	N	N_{PC}	N	N_{PE}	N	N_{SM}	N	NH_{HA}
2	P	P_{PC}	P	P_{PE}	P	P_{SM}	O	OC_{HA}
3	O	$O2_{PC}$	O	$O2_{PE}$	O	$O2_{SM}$	O	OH_{HA}
4	O	O_{PC}	O	O_{PE}	O	O_{SM}	O	O_{HA}
5	-	-	-	-	O	OH_{SM}	O	OO_{HA}
6	-	-	-	-	N	NH_{SM}	-	-

SI Table 1 Table of labels for the atoms that are important in the interaction between hyaluronic acid and the lipid membrane. In the main text, N_L is used as an abbreviation of N_{PC}, N_{PE} and N_{SM}, and likewise for the other lipid headgroup atoms in this table.

Parameters for hyaluronic acid

The chemical structure of hyaluronic acid is shown in Fig 2. All atoms are given numeric ids, and Table 2 provides the corresponding partial charges and CHARMM atom names. Due to having subtly different chemical environments, the partial charges may differ depending to whether an atom is part of a single disaccharide, a repeat unit in a polysaccharide or a terminal disaccharide in a polysaccharide.

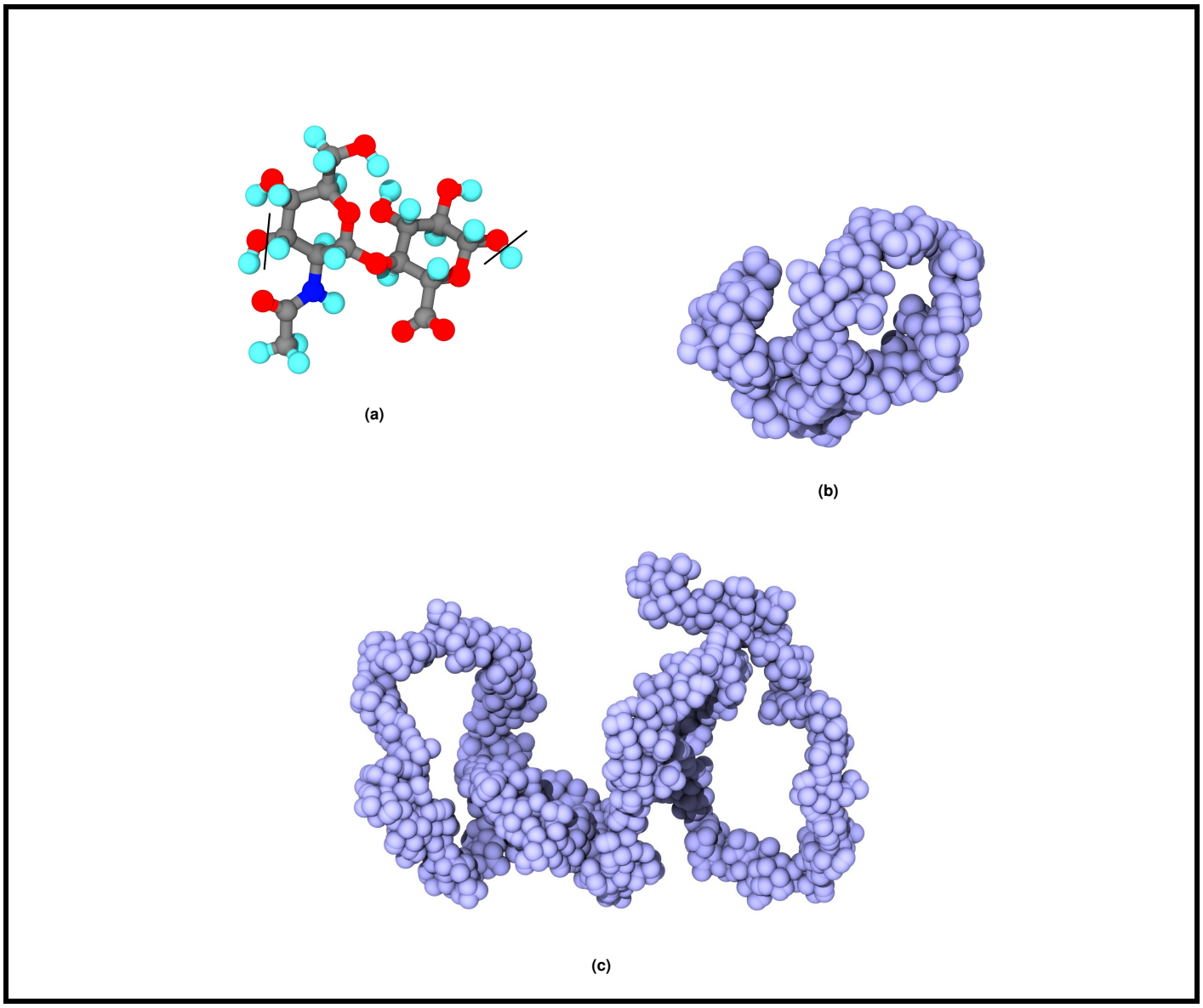


SI Fig. 2 The chemical structure of hyaluronic acid. Atoms are given numeric ids, shown here in curly braces.

	Disaccharide		Polysaccharide					
			First unit		Repeat unit		Last unit	
Id	Name	Charge	Name	Charge	Name	Charge	Name	Charge
1	CG311	0.346	CG311	0.276	CG311	0.276	CG311	0.346
2	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
3	OG311	-0.653	-	-	-	-	OG311	-0.653
4	HGP1	0.413	-	-	-	-	HGP1	0.413
5	CG311	0.151	CG311	0.153	CG311	0.153	CG311	0.151
6	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
7	OG311	-0.658	OG311	-0.657	OG311	-0.657	OG311	-0.658
8	HGP1	0.419	HGP1	0.419	HGP1	0.419	HGP1	0.419
9	CG311	0.139	CG311	0.140	CG311	0.140	CG311	0.139
10	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
11	OG311	-0.656	OG311	-0.656	OG311	-0.656	OG311	-0.656
12	HGP1	0.419	HGP1	0.419	HGP1	0.419	HGP1	0.419
13	CG311	0.105	CG311	0.105	CG311	0.105	CG311	0.105
14	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
15	CG311	-0.018	CG311	-0.018	CG311	-0.018	CG311	-0.018
16	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090

	Disaccharide		Polysaccharide					
			First unit		Repeat unit		Last unit	
Id	Name	Charge	Name	Charge	Name	Charge	Name	Charge
17	CG2O3	0.624	CG2O3	0.624	CG2O3	0.624	CG2O3	0.624
18	OG2D2	-0.760	OG2D2	-0.760	OG2D2	-0.760	OG2D2	-0.760
19	OG2D2	-0.760	OG2D2	-0.760	OG2D2	-0.760	OG2D2	-0.760
20	OG3C61	-0.389	OG3C61	-0.386	OG3C61	-0.386	OG3C61	-0.389
21	CG311	0.270	CG311	0.270	CG311	0.270	CG311	0.270
22	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
23	OG301	-0.345	OG301	-0.345	OG301	-0.345	OG301	-0.345
24	CG311	0.052	CG311	0.052	CG311	0.052	CG311	0.052
25	HGA	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
26	NG2S1	-0.452	NG2S1	-0.452	NG2S1	-0.452	NG2S1	-0.452
27	HGP1	0.304	HGP1	0.304	HGP1	0.304	HGP1	0.304
28	CG2O1	0.517	CG2O1	0.517	CG2O1	0.517	CG2O1	0.517
29	OG2D1	-0.510	OG2D1	-0.510	OG2D1	-0.510	OG2D1	-0.510
30	CG331	-0.268	CG331	-0.268	CG331	-0.268	CG331	-0.268
31	HGA3	0.090	HGA3	0.090	HGA3	0.090	HGA3	0.090
32	HGA3	0.090	HGA3	0.090	HGA3	0.090	HGA3	0.090
33	HGA3	0.090	HGA3	0.090	HGA3	0.090	HGA3	0.090
34	CG311	0.146	CG311	0.146	CG311	0.076	CG311	0.076
35	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
36	OG311	-0.654	OG311	-0.654	OG301	-0.346	OG301	-0.346
37	HGP1	0.419	HGP1	0.419	-	-	-	-
38	CG311	0.148	CG311	0.148	CG311	0.150	CG311	0.150
39	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
40	OG311	-0.656	OG311	-0.656	OG311	-0.654	OG311	-0.654
41	HGP1	0.419	HGP1	0.419	HGP1	0.419	HGP1	0.419
42	CG311	0.104	CG311	0.104	CG311	0.105	CG311	0.105
43	HGA1	0.090	HGA1	0.090	HGA1	0.090	HGA1	0.090
44	CG321	0.049	CG321	0.049	CG321	0.049	CG321	0.049
45	HGA2	0.090	HGA2	0.090	HGA2	0.090	HGA2	0.090
46	HGA2	0.090	HGA2	0.090	HGA2	0.090	HGA2	0.090
47	OG311	-0.649	OG311	-0.649	OG311	-0.649	OG311	-0.649
48	HGP1	0.420	HGP1	0.420	HGP1	0.420	HGP1	0.42
49	OG3C61	-0.386	OG3C61	-0.386	OG3C61	-0.387	OG3C61	-0.387
Total charge		-1.000		-0.823		-1.000		-1.177

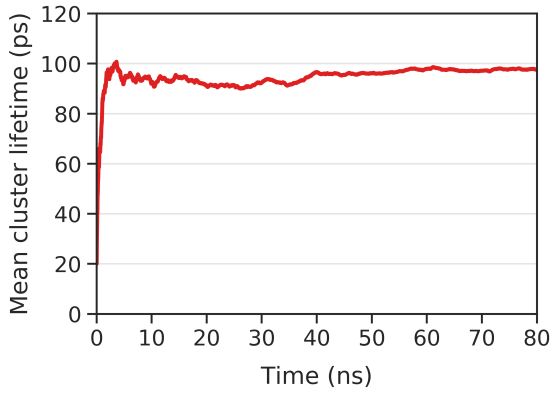
SI Table 2 Table of the partial charges and CHARMM atom names for all atoms in the hyaluronic disaccharides and polysaccharides. A single disaccharide has an overall charge of -1, localised around the two oxygen atoms of the carboxylate group. The charge on the terminal units of a polysaccharide sum to -2, and the repeating units have a net charge of -1, as expected.



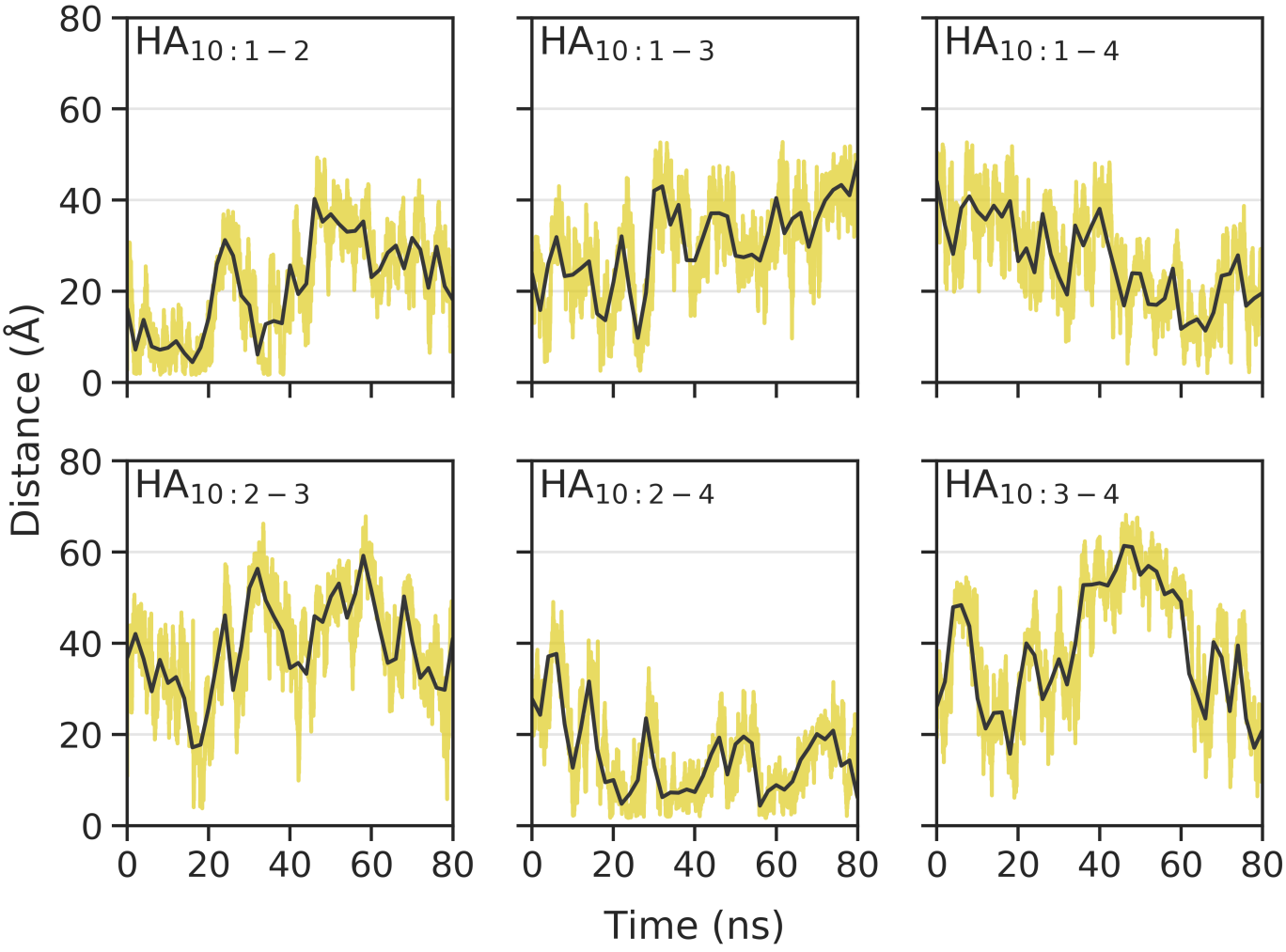
SI Fig. 3 Images of the HA_1, HA_{10} and HA_{40} that were inserted into the pre-equilibrated membrane system. **(a)** A monomer of hyaluronan, produced using Avogadro and its geometry optimisation tool. The negative charge is localised around the two oxygen atoms seen here on the right of the molecule. To make a hyaluron dimer, a 1,4-glycosidic bond is formed between two monomers, with the bond connecting atom 1 the first monomer to atom 36 of the second monomer. Through a continuation of this procedure, a hyaluronan decamer and tetracontamer were made. The hyaluronan **(b)** decamer and **(c)** tetracontamer were equilibrated using the NPT ensemble[1,2] and the CHARMM EEF1 implicit solvent for 20 ps and 40 ps respectively.[3] In **(a)**, carbon atoms are rendered in gray, oxygen in red, nitrogen in blue and hydrogen in cyan.

Aggregation of HA

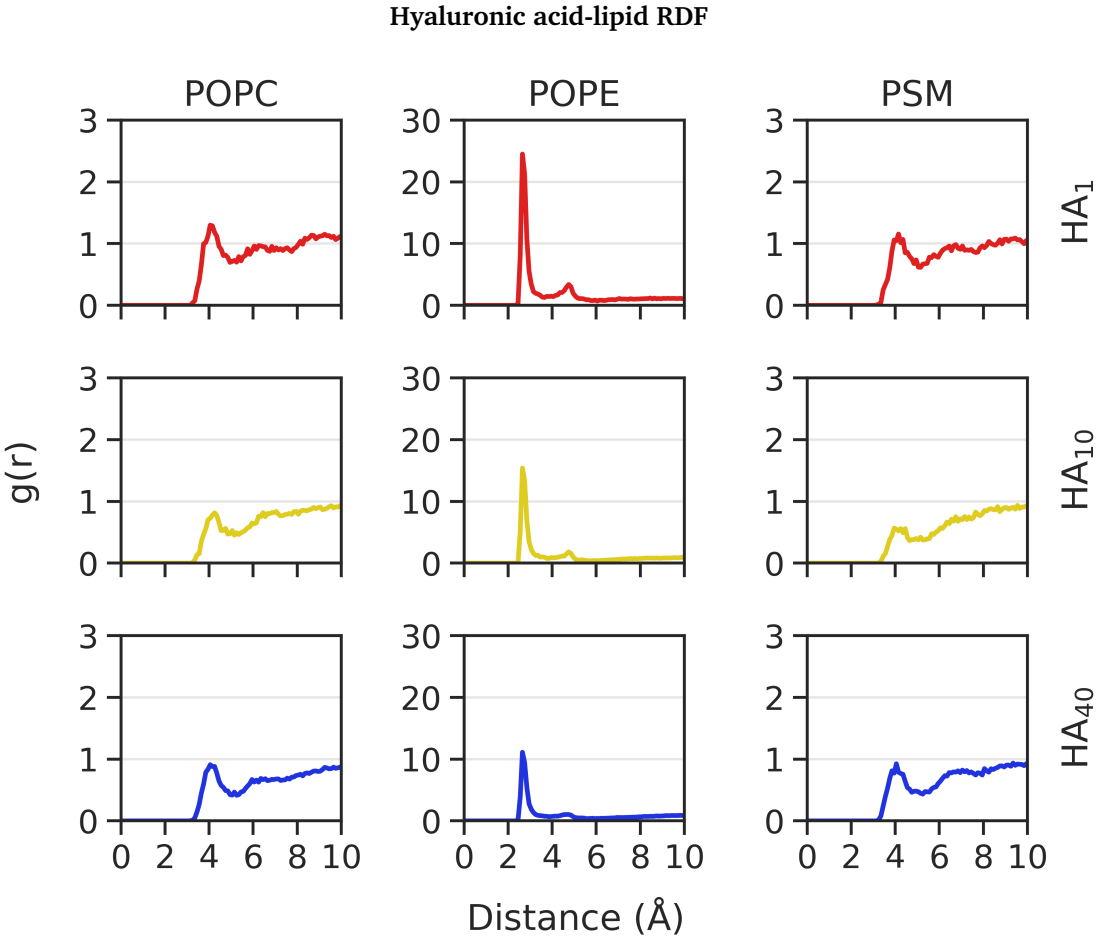
Before any cluster analysis can be performed, it is first necessary to define the intermolecular distance at which two monomers can be considered to form a dimer. Rather than using a general cluster cut-off distance, pair-specific cut-off distances are defined based on the geometric mixing of their atoms' values of σ used in calculating the non-bonded potential (LJ 6-12). The pair-specific cluster cutoff distances are determined by first calculating the relevant $\sigma_{\text{mixed}} = (\sigma_{\text{a}} + \sigma_{\text{b}})/2$ and then multiplying by 1.4. This multiplication factor was determined through a consideration of the RDFs of the polar atoms of HA. In each case, the distance of the first minimum in the RDF was multiplied by 1.15 to give a pair-specific cutoff distance that would allow for thermal fluctuations briefly disrupting clustering. These cut-off distances are all equal to the corresponding σ_{mixed} value multiplied by 1.4 ($\pm$ 0.2). This pair-specific criterion is then used for all atoms of HA, including the non-polar atoms for which no RDFs were calculated. For example, for an intermolecular hydrogen-hydrogen interaction with $\sigma_{\text{H}} = 2.39$ Å the clustering cut-off distance is 3.3 Å.



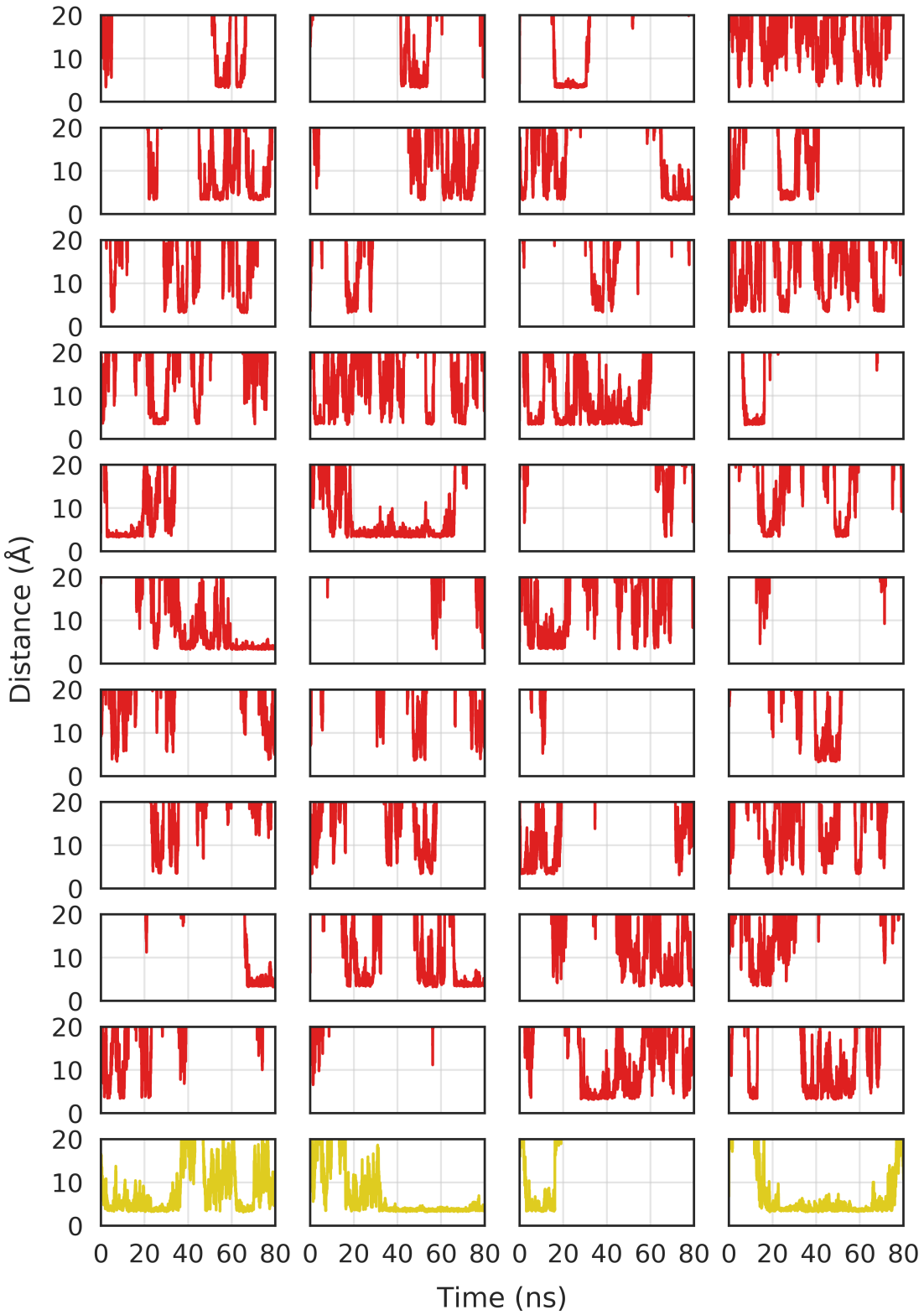
SI Fig. 4 The running average lifetime (ps) of HA clusters in the HA_1 system.



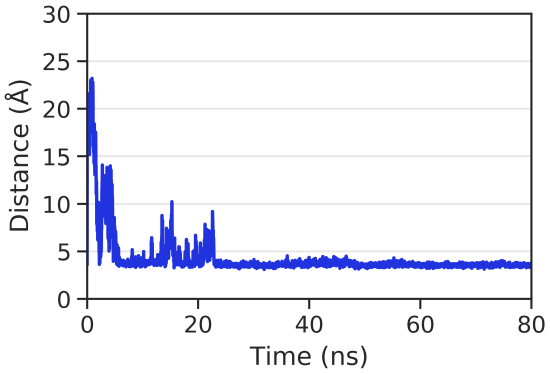
SI Fig. 5 Minimum distance between each of the four HA_{10} at each configuration (yellow) and for 5 ns block averages (black). The pot label $HA_{10:i\text{-}j}$ denotes decamers *i* and *j* are being considered.



SI Fig. 6 RDF of the nitrogen atoms (N_L) in each of the lipid headgroups around the negatively charged oxygen atoms (OC_{HA}) of hyaluronic acid.



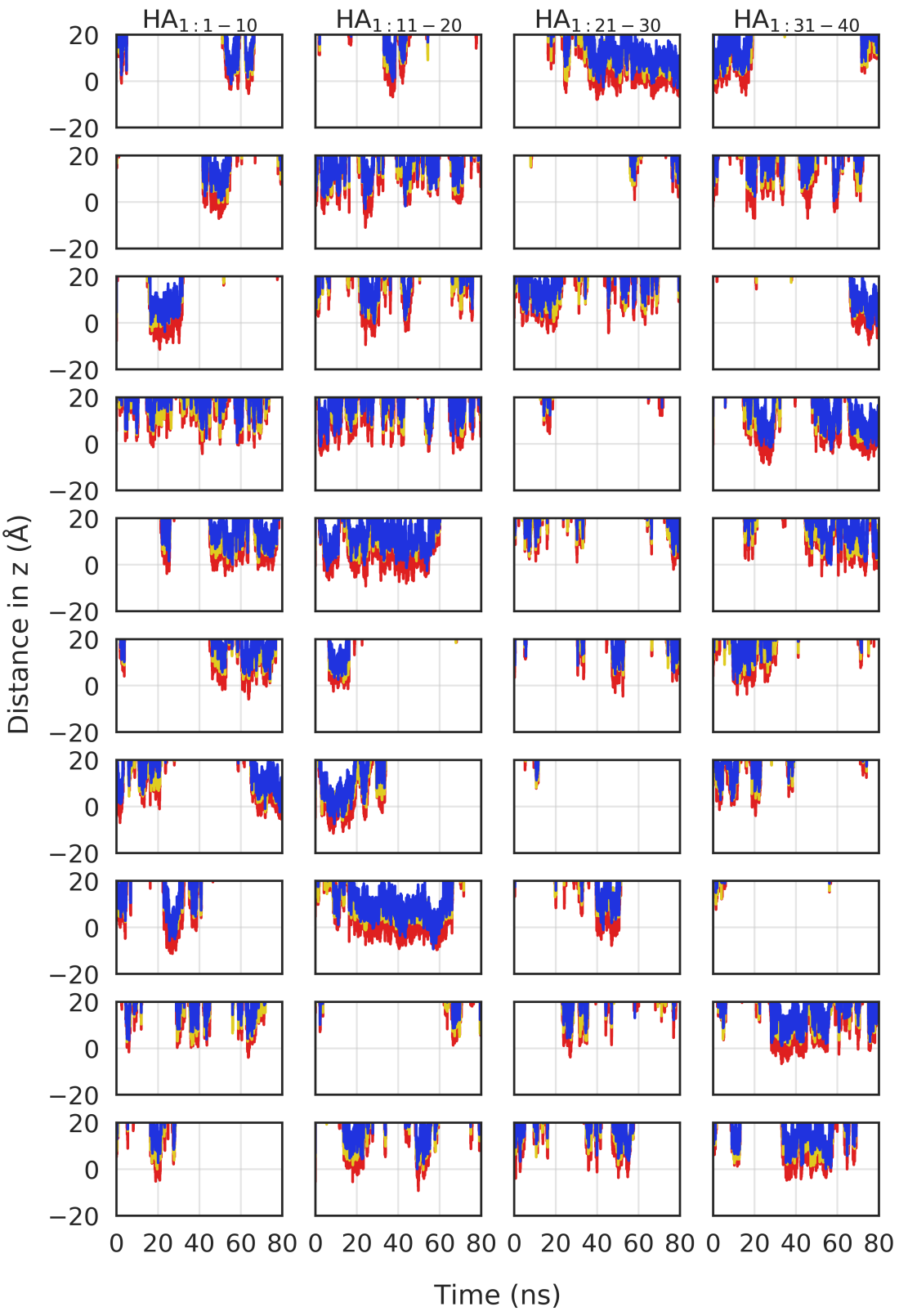
(a) Approach curves of the forty HA_1 molecules (red) and the and four HA_{10} molecules (yellow).



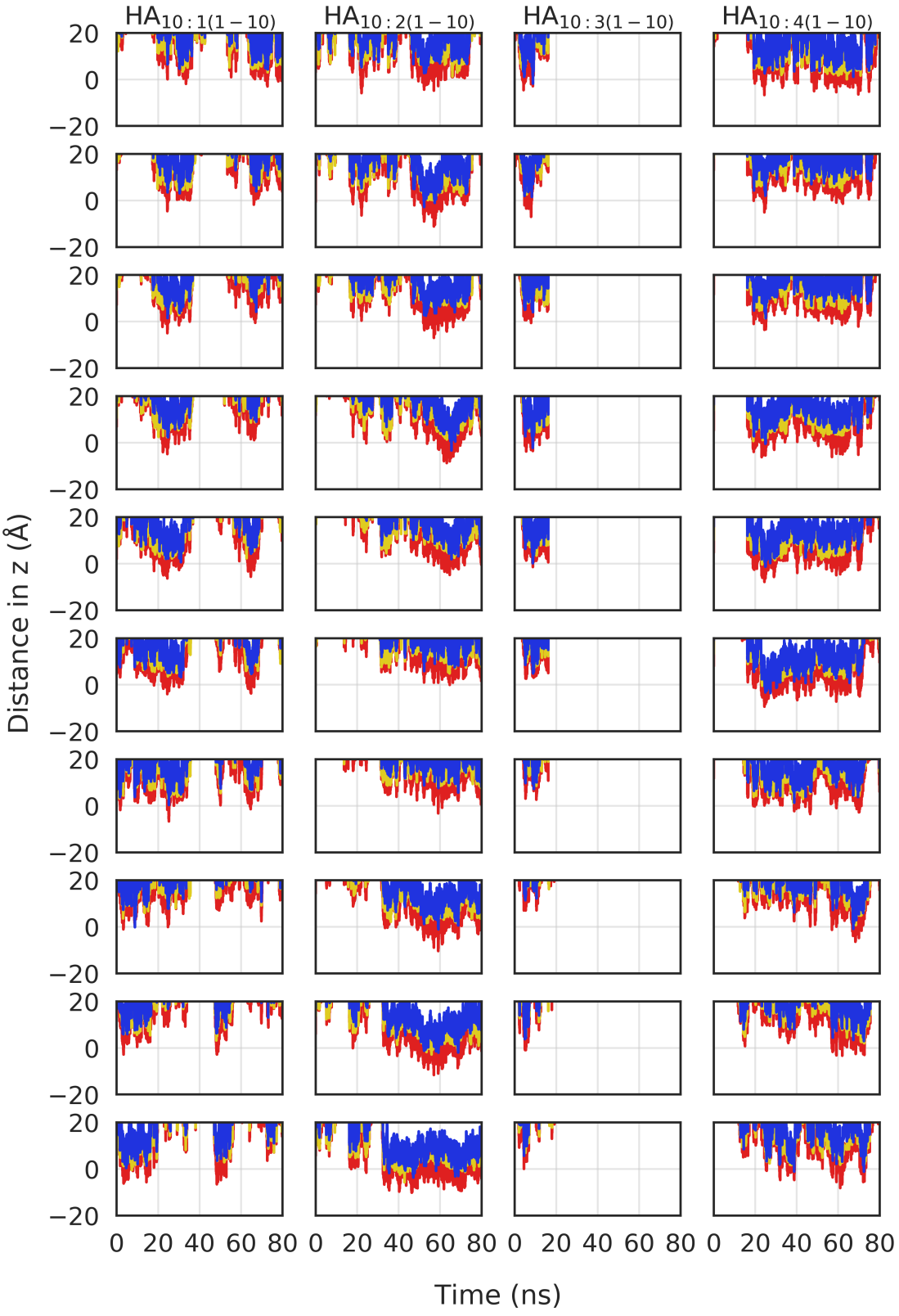
(b) Approach curve of the HA_{40} molecule.

SI Fig. 7 Approach curve for each individual hyaluronic acid molecule in **(a)** the HA_1 and HA_{10} systems and **(b)** the HA_{40} system. The approach curves show the minimum distance, at each timestep, between any P_L atom in the upper leaflet and any OC_{HA} atom of each HA molecule.

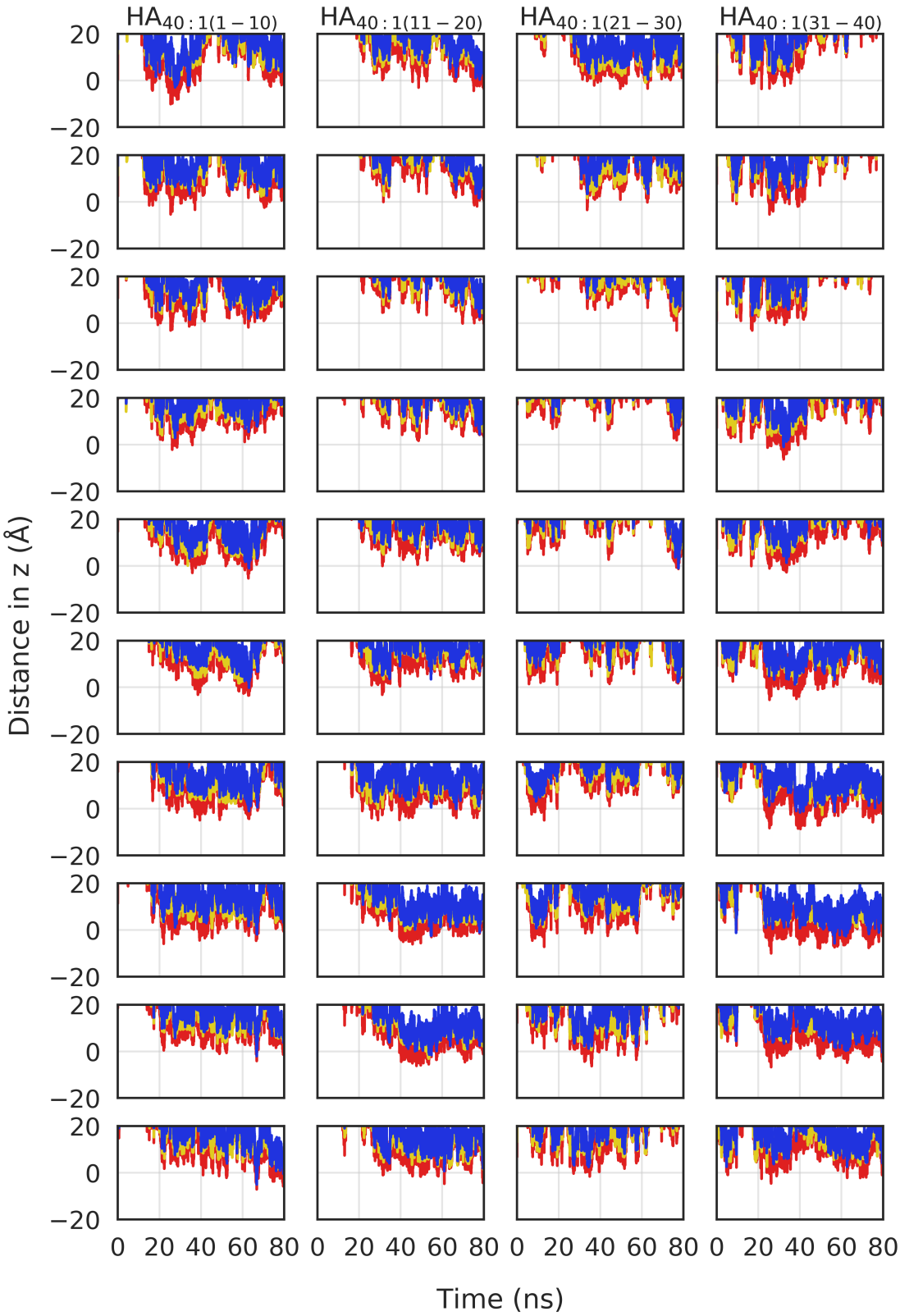
Depth of insertion.



(a) HA_1

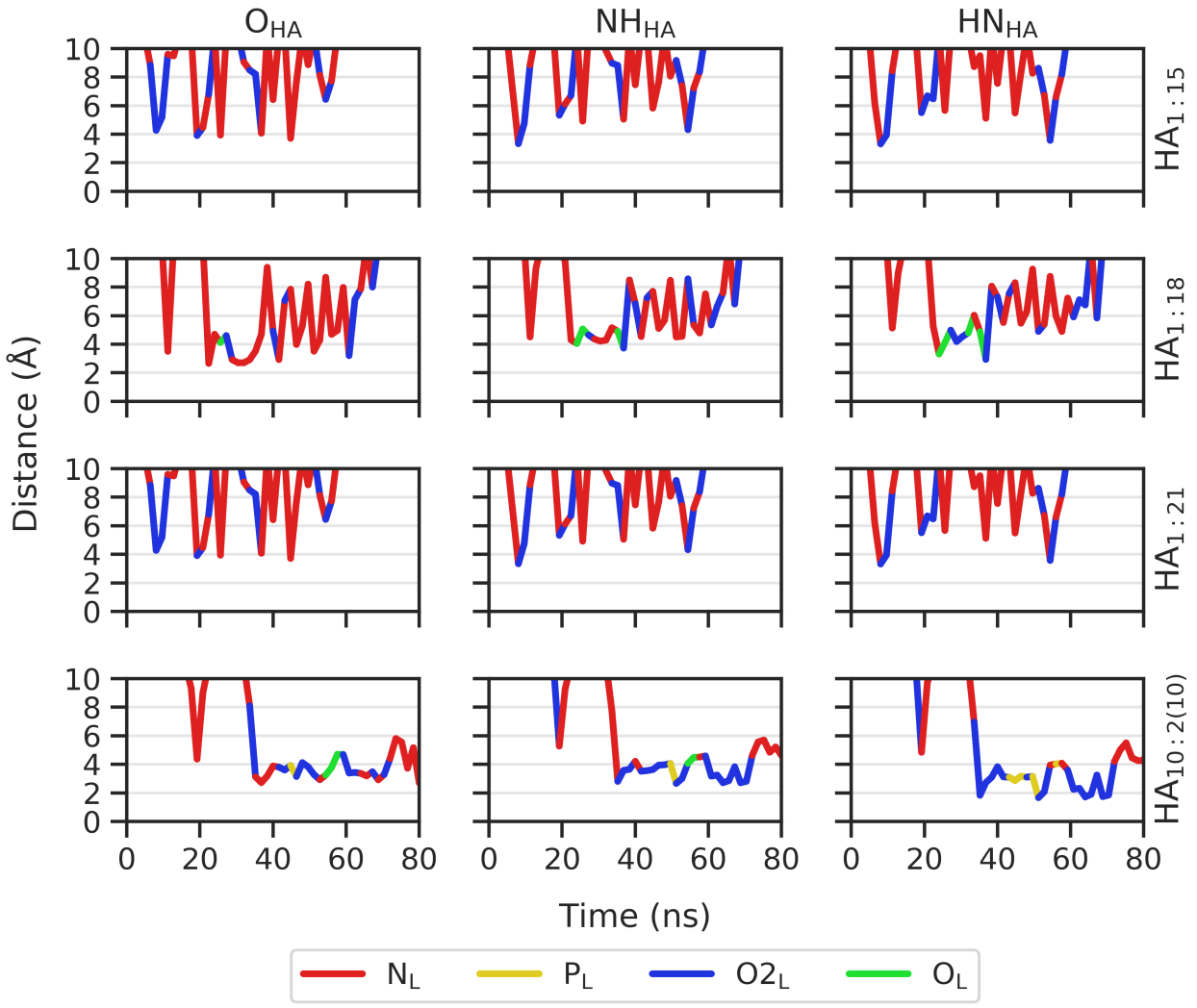

HA10 : 1(1 − 10)
HA10 : 2(1 − 10)
HA10 : 3(1 − 10)
HA10 : 4(1 − 10)
Distance in z (Å)
20
0
−20
0 20 40 60 80
Time (ns)

(b) HA_{10}

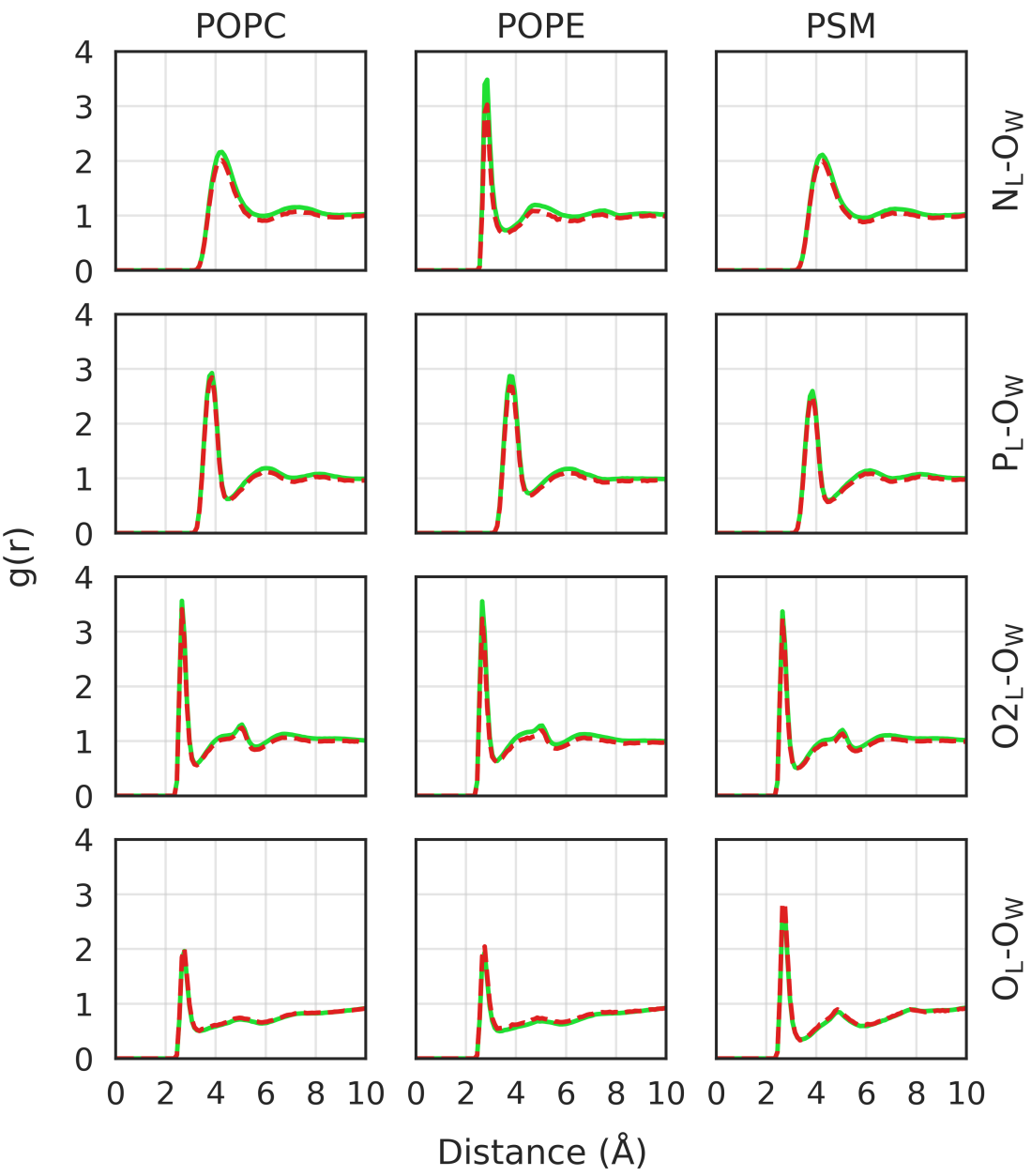


(c) HA_{40}

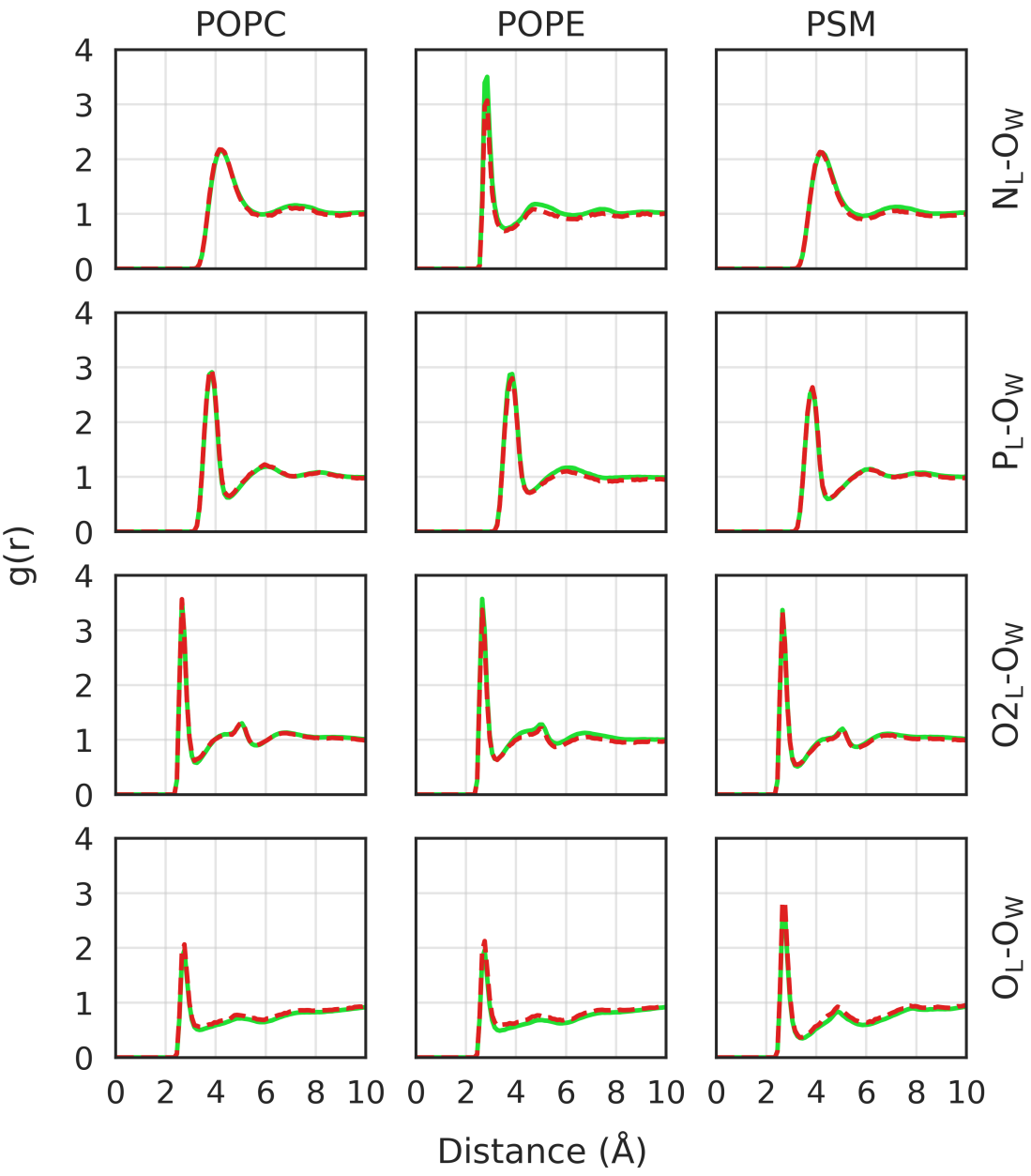
SI Fig. 8 Depth of insertion of individual HA monomeric units into the membrane surface for the **(a)** HA_1 **(b)** HA_{10} and **(c)** HA_{40} systems. Three separate intrinsic surfaces are defined using the N_L (red), P_L (yellow) and O_L (blue) atoms in the upper leaflet, and the depth of penetration into each surface is calculated as the distance in z from each surface to each monomeric unit. For the lower leaflet, no contact is made in the cases of HA_{10} and HA_{40}, whilst for HA_1 the depths of insertion seen in **(a)** are representative of those seen in the lower membrane. For the column headers, $HA_{a:b\text{-}c(d\text{-}e)}$ indicates that this column shows the depth of insertion of units *d-e* of molecules *b-c* that have a total of *a* repeat units each.



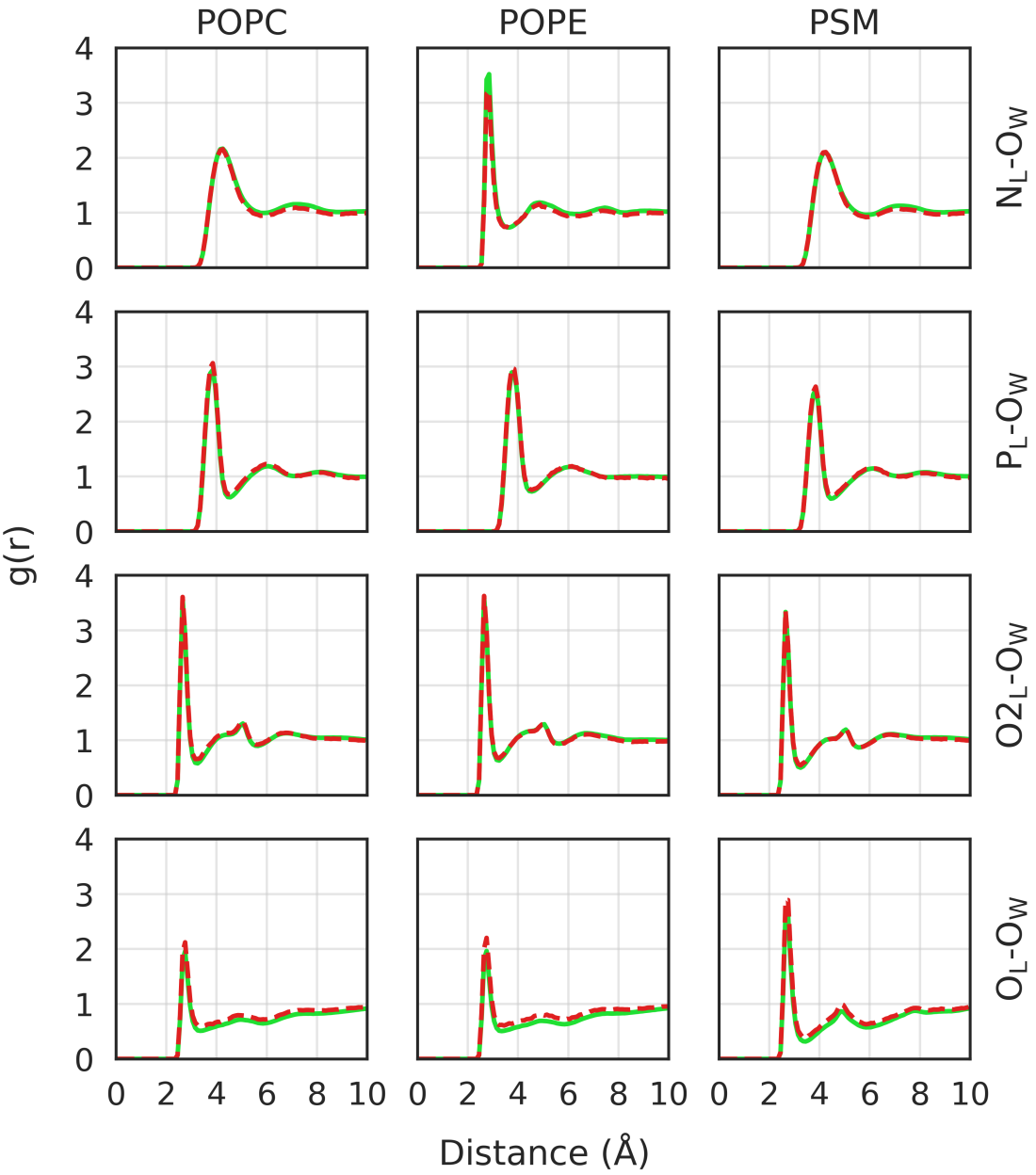
SI Fig. 9 Minimum distance from O_{HA}, NH_{HA} and amide hydrogen (HN_{HA}) to any polar atom in the lipid headgroups. Shown here are the four HA monomeric units that penetrate most deeply into the lipid membrane: the fifteenth, eighteenth and twenty-first monomers from the HA_1 system ($HA_{1:15}$, $HA_{1:18}$ and $HA_{1:21}$), and the terminal monomeric unit of the second decamer from the HA_{10} system ($HA_{10:2(10)}$) system. The colour of a curve signifies the type of lipid headgroup atom closest to a given HA atom: N_L (red), P_L (yellow), $O2_L$ (blue) and O_L (green). Electrostatic interactions between OC_{HA} and N_L atoms, and hydrogen bonding between OH_{HA} and $O2_L$ are observed during periods of close contact (< 3.0 Å) between HA and the lipids head groups.



(a) RDFs of O_W atoms around the polar atoms of the POPC, POPE and PSM lipid headrgoups for free (solid green) and bound (dashed red) lipids in the HA_1 system.

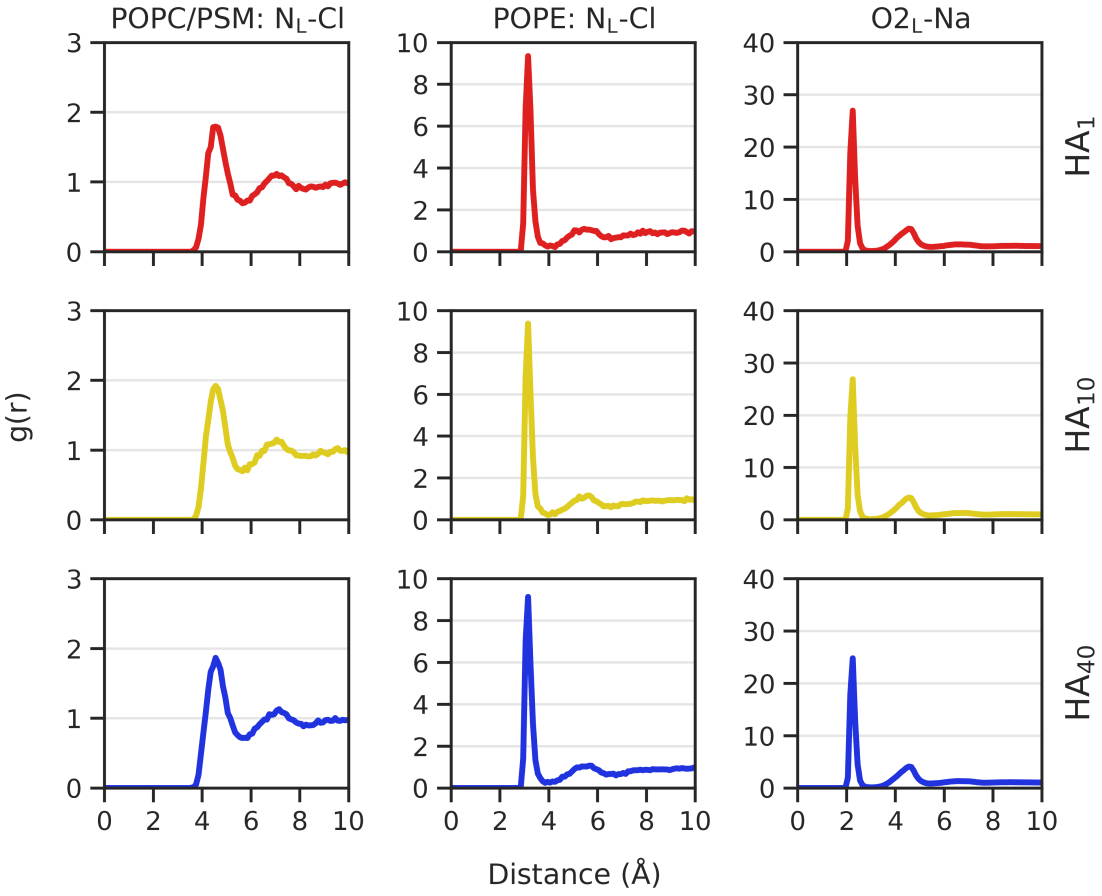


(b) RDFs of O_W atoms around the polar atoms of the POPC, POPE and PSM lipid headrgoups for free (solid green) and bound (dashed red) lipids in the HA_{10} system.



(c) RDFs of O_W atoms around the polar atoms of the POPC, POPE and PSM lipid headrgoups for free (solid green) and bound (dashed red) lipids in the HA_{40} system.

SI Fig. 10 RDF of O_W atoms around the polar atoms of the POPC, POPE and PSM lipid headrgoups for free (solid green) and bound (dashed red) lipids in the **(a)** HA_1 **(b)** HA_{10} and **(c)** HA_{40} systems. The adsorption of hyaluronic acid to the membrane surface has no significant effect on the hydration profile of the lipid membrane.



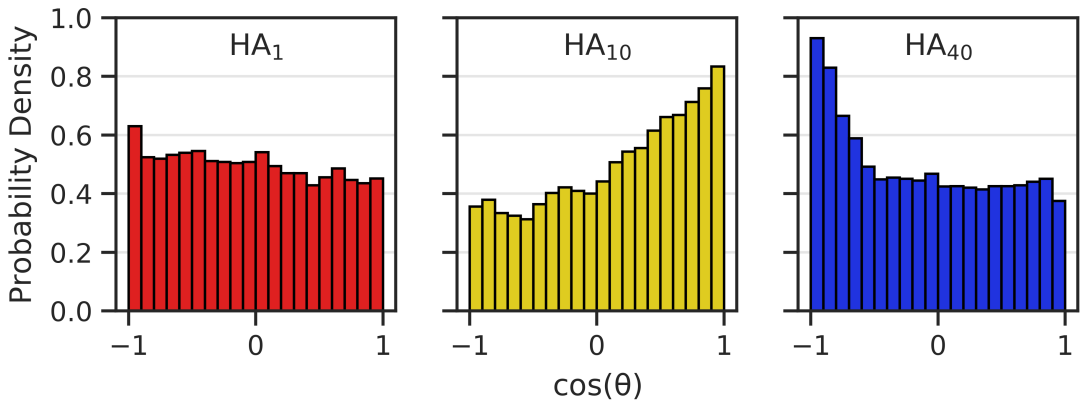
SI Fig. 11 RDF of Na^+ ions around nitrogen (N_L) and phosphate oxygen ($O2_L$) atoms of the lipid headgroups for each of the three systems. POPC and PSM share the same phosphocholine choline headgroup moiety and a single RDF of O_W around the nitrogen atom was calculated for these two lipids, whilst the smaller ethanolamine headgroup was considered separately. A single RDF was calculated for O_L.

		HA_1		HA_{10}		HA_{40}	
Pair	r_{min} (Å)	Free	Adsorbed	Free	Adsorbed	Free	Adsorbed
OC_{HA} — O_W	3.45	3.64	3.41	3.48	3.19	3.56	3.35
OH_{HA} — O_W	3.65	3.40	3.00	3.26	2.97	3.13	2.87
O_{HA} — O_W	3.55	2.83	2.46	2.78	2.53	2.67	2.46
N_{HA} — O_W	5.15	9.44	8.25	8.68	8.03	8.57	7.90

SI Table 3 Table of the first coordination number of O_W around the polar atoms of HA for free and adsorbed monomeric units, for each of the three systems.

Angle that adsorbed HA makes with the xy-plane

The angle (θ) that the vector from atom 1 to atom 38 (see SI Fig 2 for atom ids) makes with the xy-plane was calculated for each adsorbed HA unit, for each of the three systems. The distributions of $\cos(\theta)$ is plotted below.



SI Fig. 12 Distribution of $\cos(\theta)$, where θ is the angle that adsorbed HA makes with the xy-plane. The HA plane is defined at the vector from atom 1 to atom 38 (see SI Fig 2 for atom ids)

Intramolecular HA hydrogen bonds

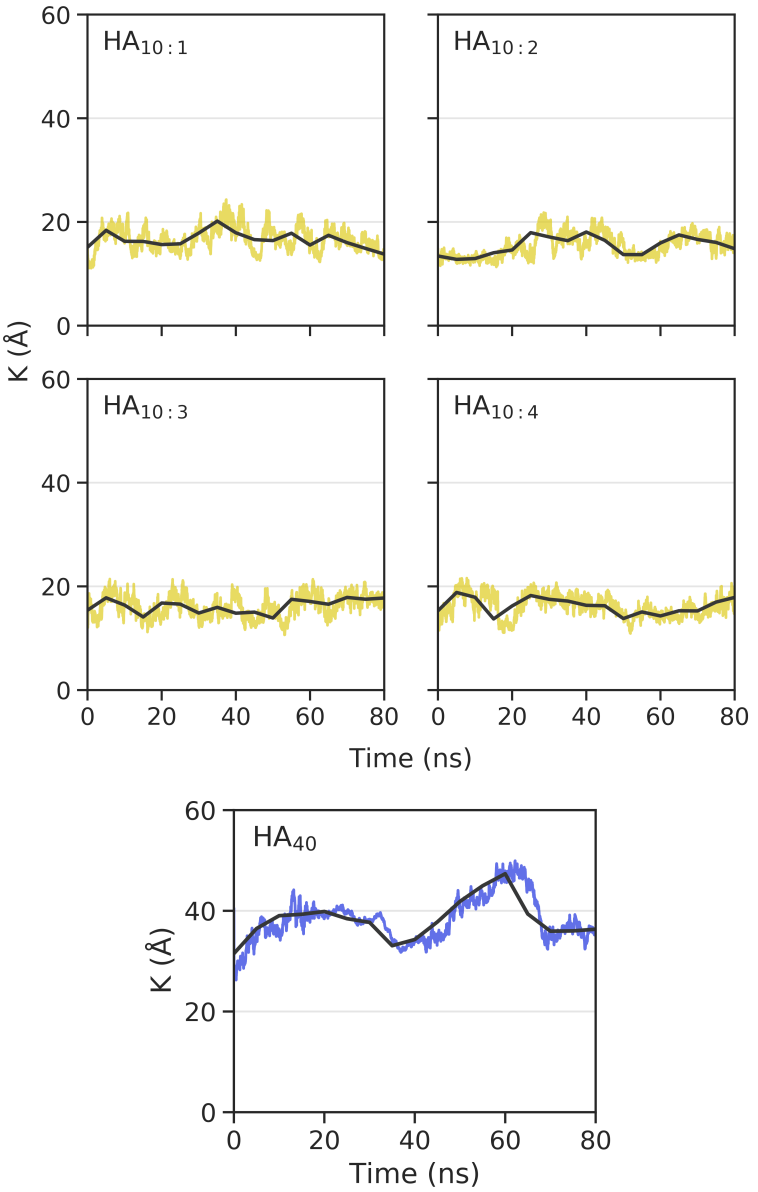
Four intramolecular hydrogen bonds have previously been identified as being important to the structure of HA in solution.[4] These are defined in SI Table 4. The total number of intramolecular hydrogen bonds was calculated for each HA_{10} and the HA_{40}, and SI Table 5 and gives the ratio of occurance of each type of hydrogen bond.

		Atom type		Atom ID	
Bond Type	Linkage	Donor	Acceptor	Donor	Acceptor
A	β-1,4	OH	OO	11	49
B	β-1,4	N	OC	26	18, 19
C	β-1,3	OH	O	7	29
D	β-1,3	OH	OO	40	20

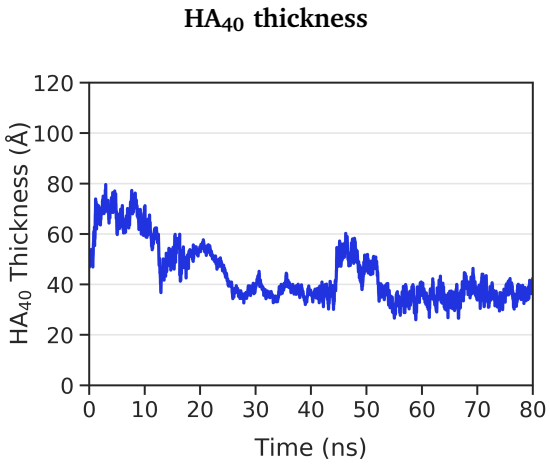
SI Table 4 The donor and acceptor atoms, and the linkage over which they are bonded, for the four intramolecular hydrogen bonds that have previously been found to be important to the structure of HA in solution.

	H-bond type			
Molecule	A	B	C	D
$HA_{10:1}$	0.45	0.33	0.10	0.12
$HA_{10:2}$	0.53	0.32	0.02	0.15
$HA_{10:3}$	0.51	0.08	0.22	0.19
$HA_{10:4}$	0.27	0.46	0.01	0.26
HA_{40}	0.30	0.54	0.02	0.14

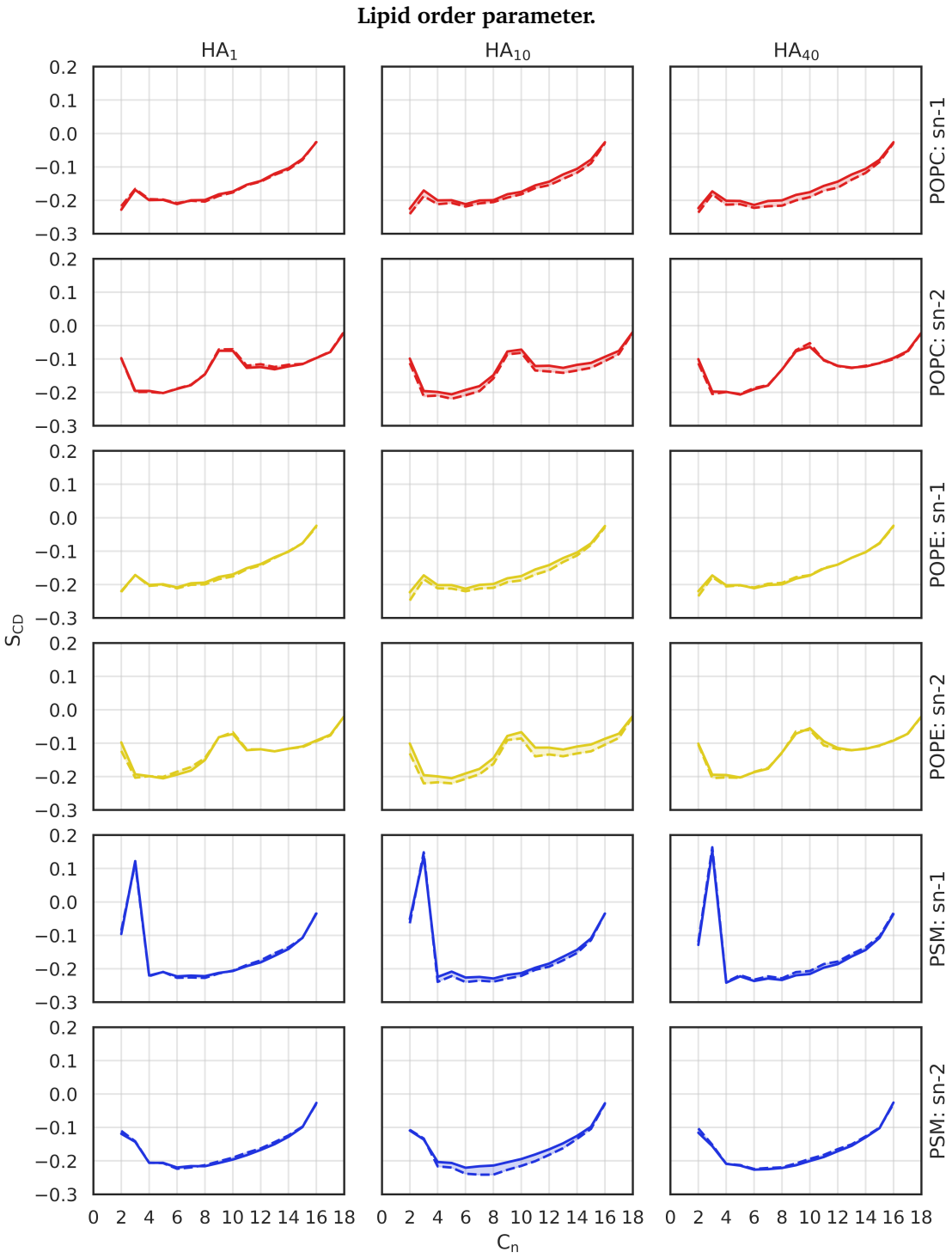
SI Table 5 Count of occurance of each type of hydrogen bond, normalised by the total number of hydrogen bonds. Ratio of occurance of each type of intramolecular hydrogen bond, for each of the four HA_{10} molecules and HA_{40}.



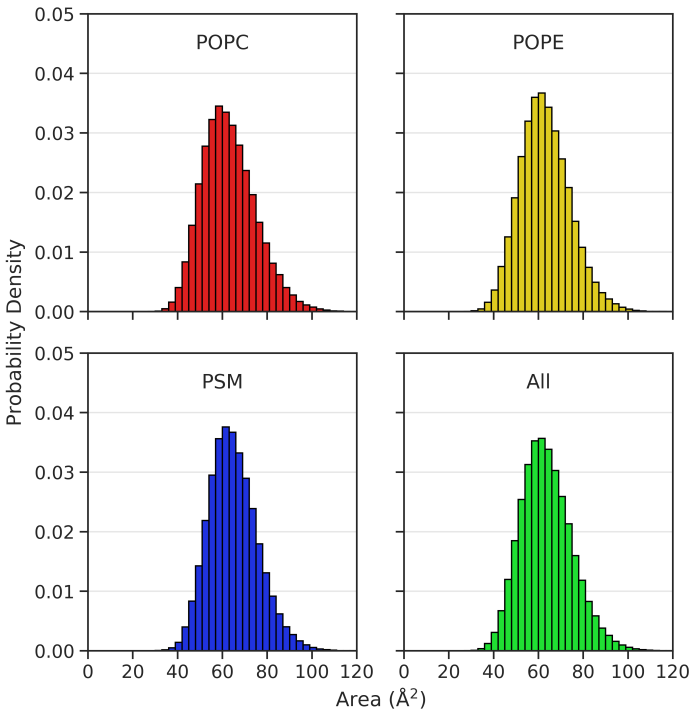
SI Fig. 13 Radius of gyration (K) as a function of time for each of the four HA_{10} molecules and the HA_{40} molecule.



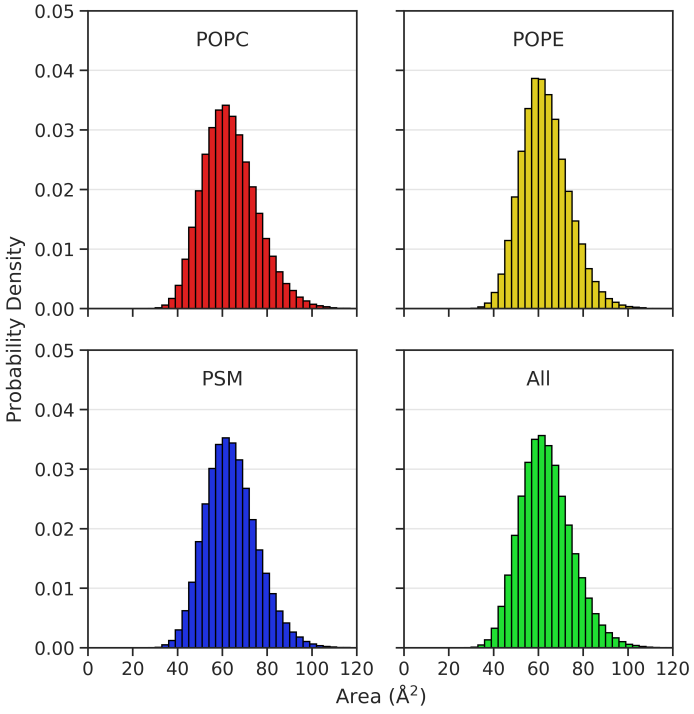
SI Fig. 14 The thickness of HA_{40} over time, as defined by the maximum distance in z between any two heavy (non-hydrogen) atoms of HA_{40}. After around $55\,\mathrm{ns}$ the thickness of HA_{40} converges to approximately $40\,\text{Å}$.



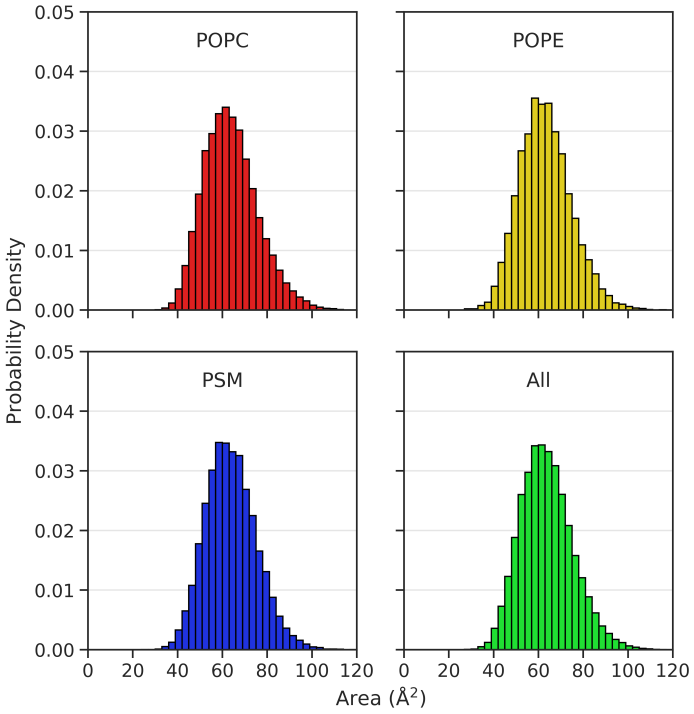
SI Fig. 15 Comparison of the lipid order parameter ($S_{C\text{-}D}$) of free lipids (solid lines) and those adsorbed to HA (dashed lines), for each tail of POPC (red), POPE (yellow) and PSM (blue). The shaded regions highlight any differences in the ($S_{C\text{-}D}$ of free and adsorbed lipids.


Area per lipid
POPC
POPE
PSM
All
Probability Density
Area (Å^2)
0.00
0.01
0.02
0.03
0.04
0.05
0
20
40
60
80
100
120

(a) HA_1


POPC
POPE
PSM
All
Probability Density
Area (Å^2)
0.00
0.01
0.02
0.03
0.04
0.05
0
20
40
60
80
100
120

(b) HA_{10}



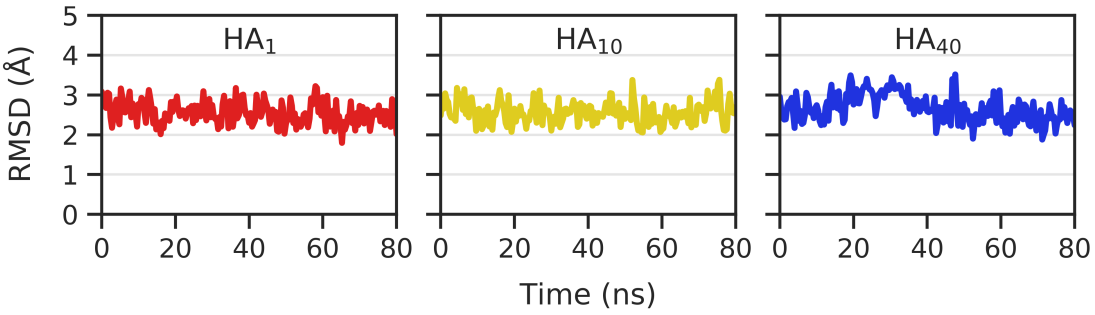
(c) HA_{40}

SI Fig. 16 Area per lipid distributions for POPC (red), POPE (yellow), PSM (blue) and all lipids (green). The python package PyVoro[5] was used to perform a Voronoi tesselation of lipids in the upper leaflet, from which the areas were then calculated.

Lipid	Reference Atoms
POPC	C2 C21 C31
POPE	C2 C21 C31
PSM	C2S C1F C4S

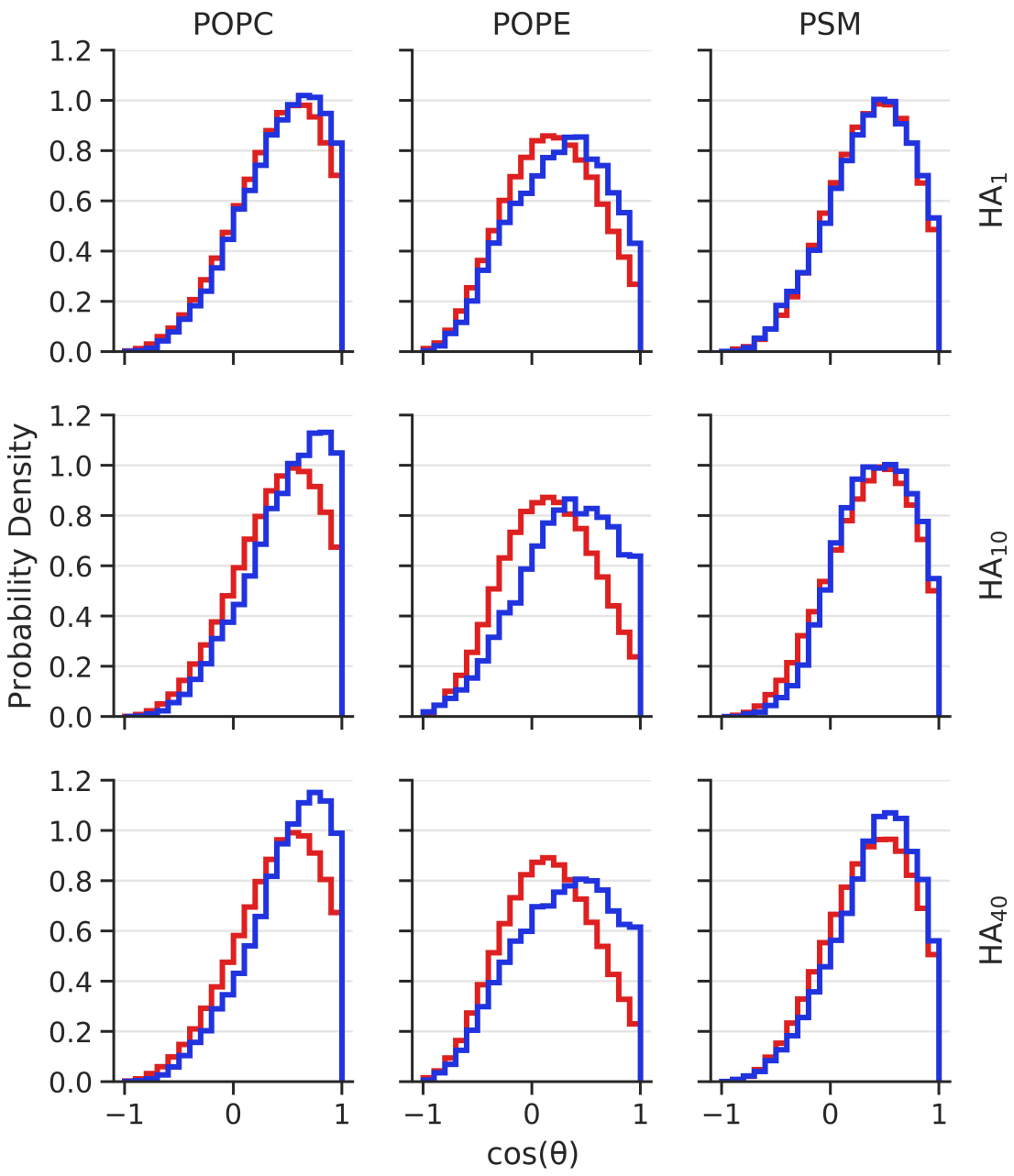
SI Table 6 Table of the CHARMM atom names of the atoms used as seeds in the Voronoi tesselation of the upper leaflet.

RMSD of the lipid membrane surface.

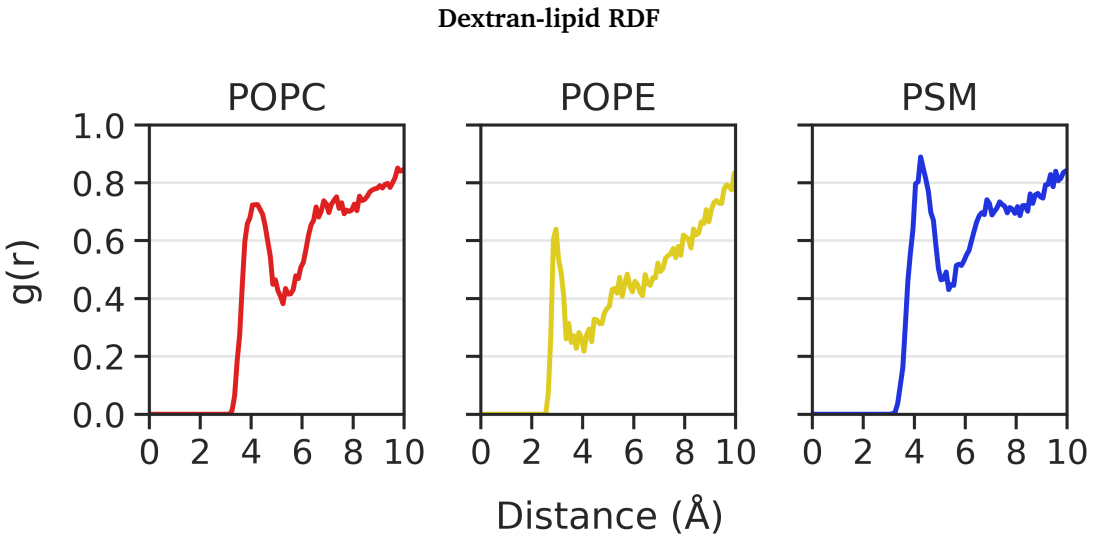


SI Fig. 17 The roughness of the membrane headgroup region as defined by the RMSD of atomic positions of P_L atoms in the upper leaflet.

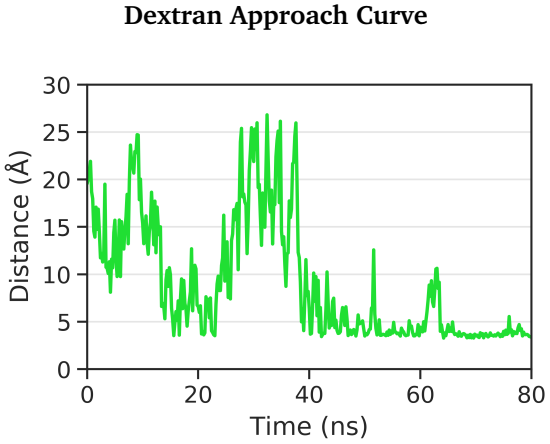
Affect of HA adsorption on lipid headgroup orientation.



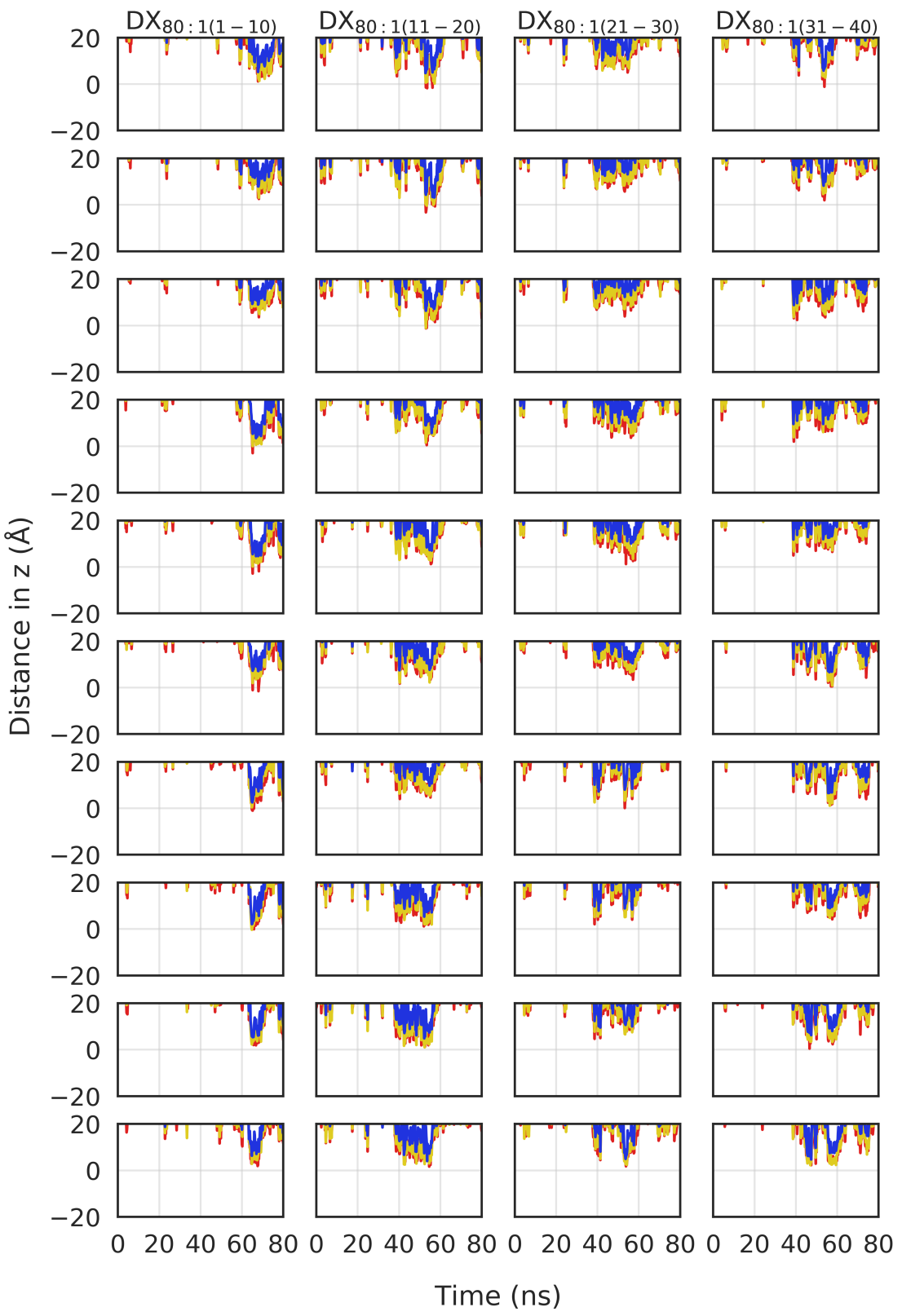
SI Fig. 18 Distribution of $\cos(\theta)$ for each lipid headgroup for free (red) and adsorbed (blue) lipids, where θ is the angle that the lipid headgroup makes with the z-plane. The lipid headgroup orientation is defined as the vector from P_L to N_L.



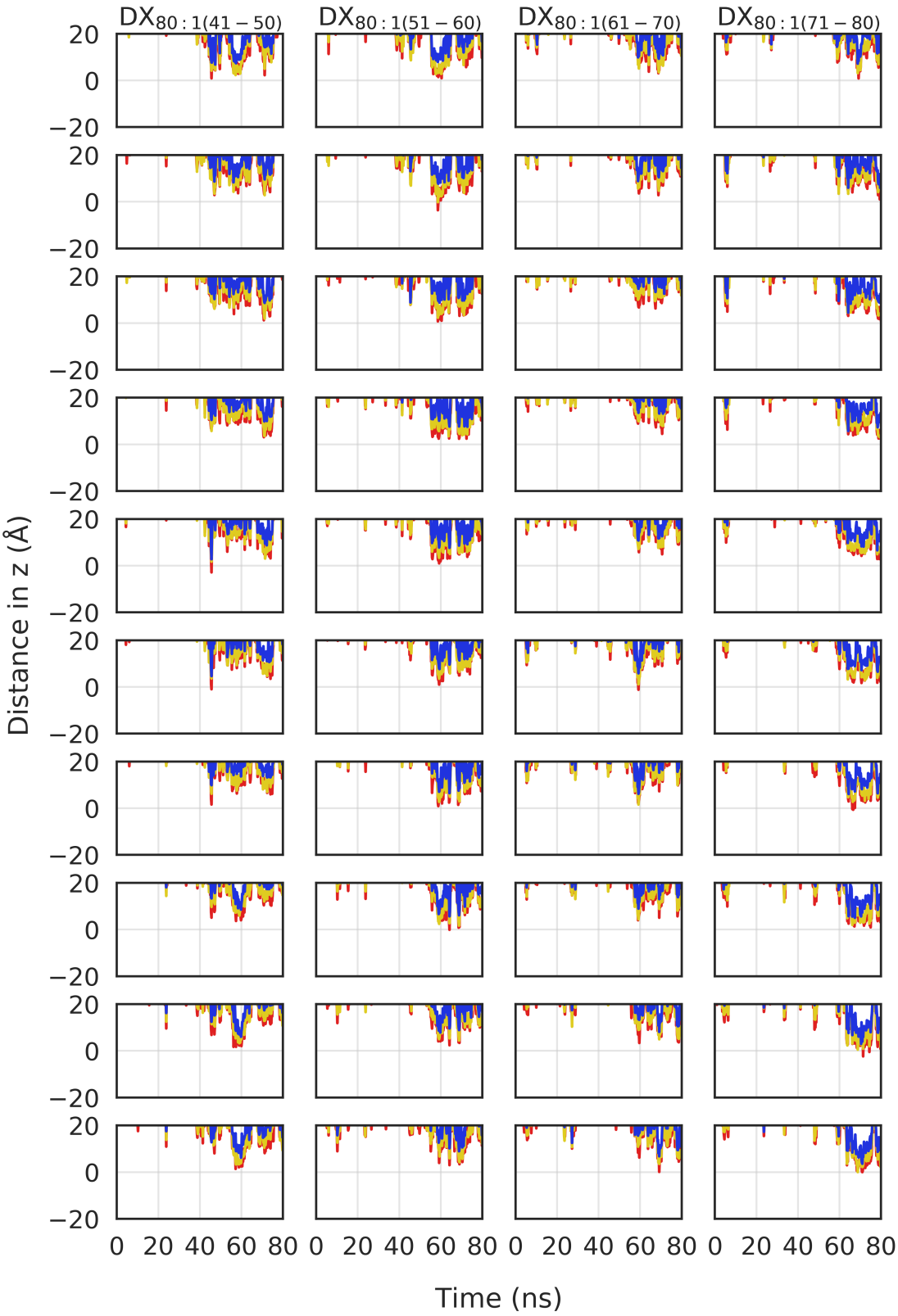
SI Fig. 19 RDF of the nitrogen atoms (N_L) in each of the lipid headgroups around the hydroxyl oxygen atoms (OH_{DX}) of dextran.



SI Fig. 20 Approach curve of DX_{80}, showing the minimum distance at each timestep between any P_L atom any OH_{DX}. DX_{80} interacts with both the upper and lower leaflets during the simulations (although never both simultaneousy), and so the distances shown here are the minimum distances to either the upper or lower leaflet.

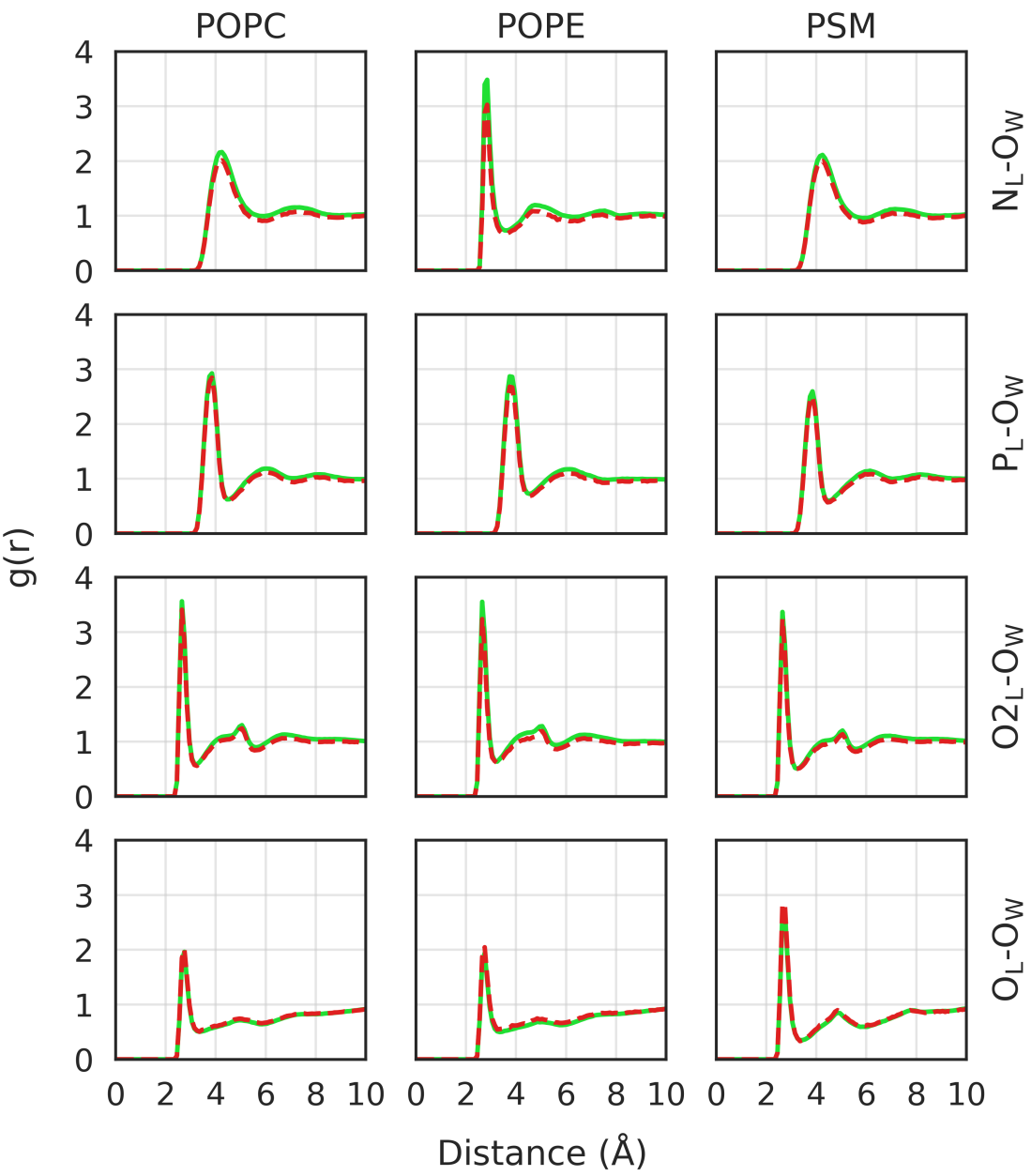

Dextran depth of insertion
DX80 : 1(1 – 10)
DX80 : 1(11 – 20)
DX80 : 1(21 – 30)
DX80 : 1(31 – 40)
Distance in z (Å)
20
0
−20
0 20 40 60 80
Time (ns)

(a) $DX_{80:1-40}$



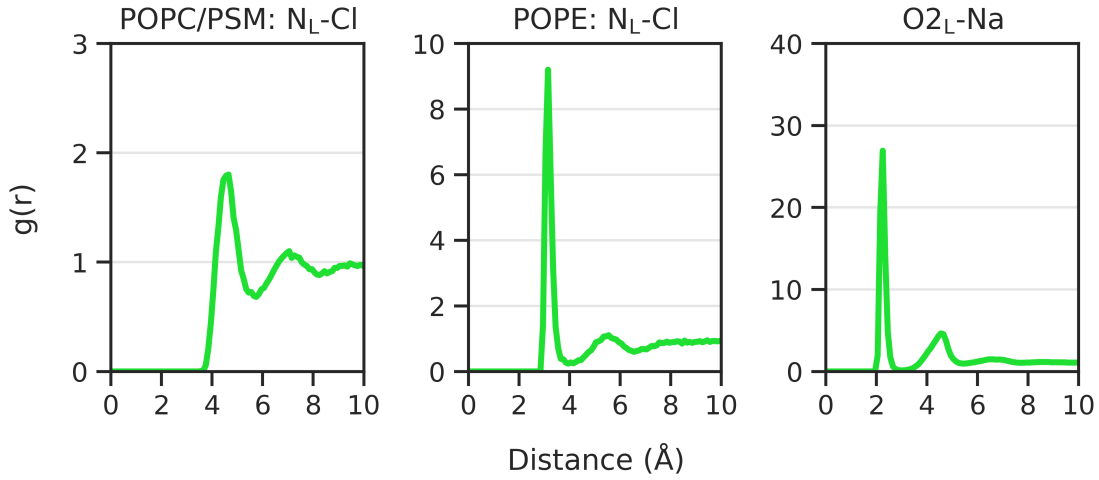
(b) $DX_{80:41-80}$

SI Fig. 21 Depth of insertion of individual DX monomeric units into the membrane surface. Six separate intrinsic surfaces are defined using the N_L (red), P_L (yellow) and O_L (blue) atoms in the upper leaflet and lower leaflets (three surfaces per leaflet). The depth of penetration into either the upper or lower leaflet is calculated as the absolute distance in z that from each monomeric unit to a given surface type (N_L, P_L or $O2_L$). For the column headers, $DX_{80:a-b}$ indicates that this column shows the depth of insertion of units *a-b* of the DX_{80} molecule.



SI Fig. 22 RDFs of O_W atoms around the polar atoms of the POPC, POPE and PSM lipid headrgoups.

Ions at the lipid-water interface in the dextran systems



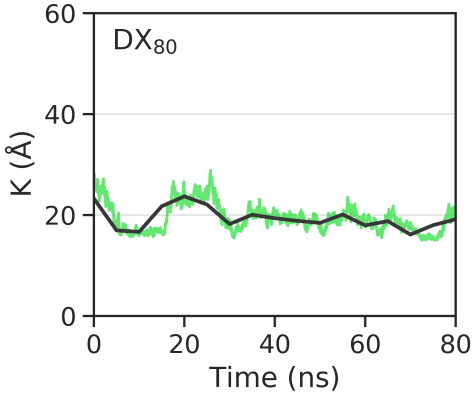
SI Fig. 23 RDF of Na^+ ions around nitrogen (N_L) and phosphate oxygen ($O2_L$) atoms of the lipid headgroups. POPC and PSM share the same phosphocholine choline headgroup moiety and a single RDF of O_W around the nitrogen atom was calculated for these two lipids, whilst the smaller ethanolamine headgroup was considered separately. A single RDF was calculated for O_L.

Hydration of dextran

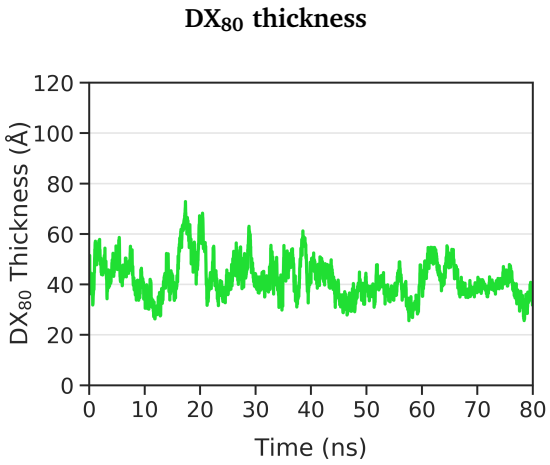
		DX_{80}	
Pair	r_{min} (Å)	Free	Adsorbed
OH_{DX} — O_W	3.65	2.87	2.80

SI Table 7 Table of the first coordination number of O_W around the polar atoms of DX for free and adsorbed monomeric units.

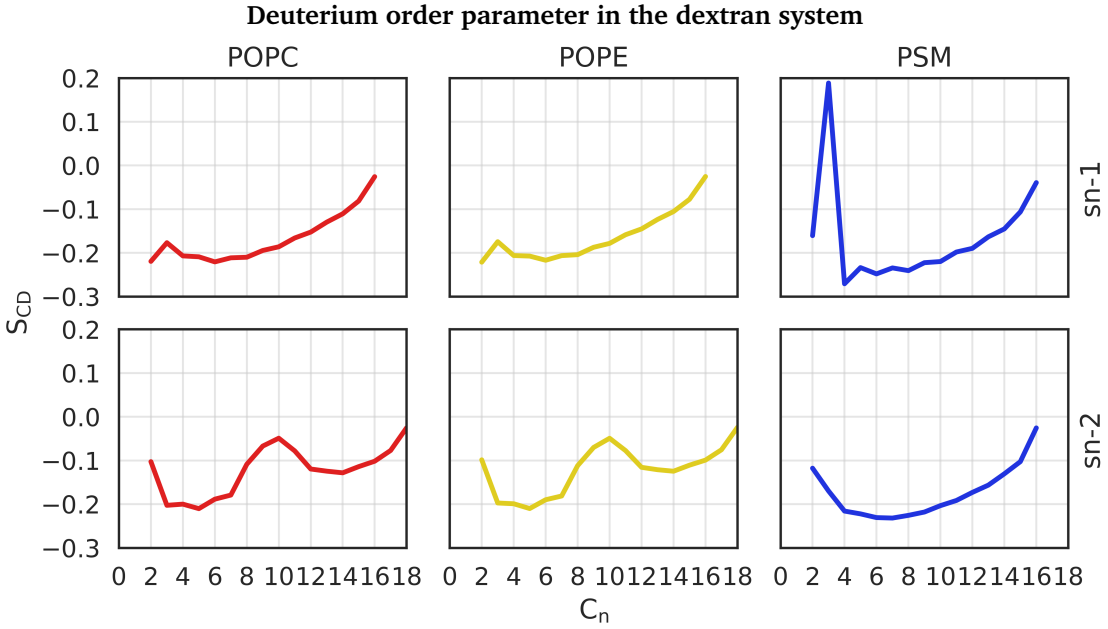
Dextran radius of gyration



SI Fig. 24 Radius of gyration (K) of DX_{80} as a function of time.

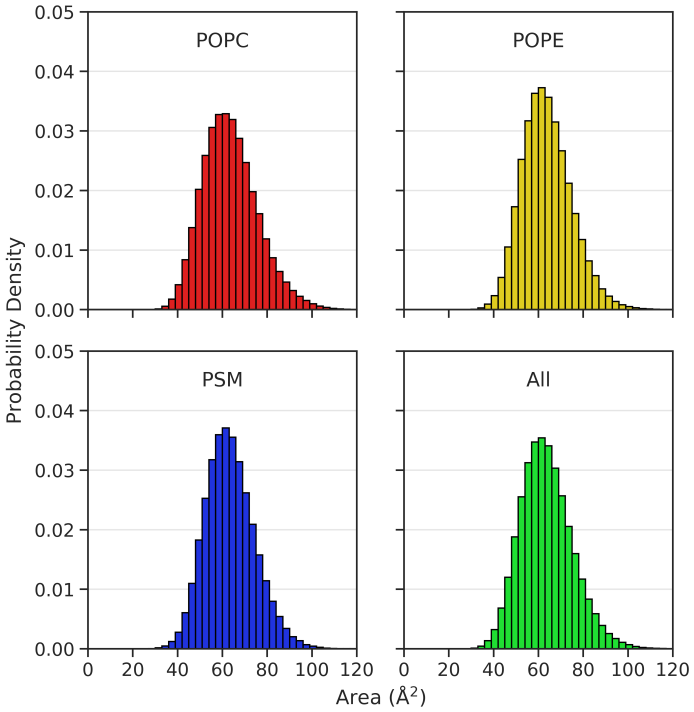


SI Fig. 25 The thickness of DX_{80} over time, as defined by the maximum distance in z between any two heavy (non-hydrogen) atoms of DX_{80}.



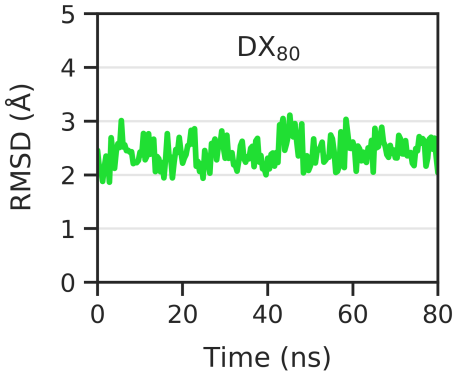
SI Fig. 26 Deuterium order parameter ($S_{C\text{-}D}$) of each tail of POPC (red), POPE (yellow) and PSM (blue).

Area per lipid in the dextran system



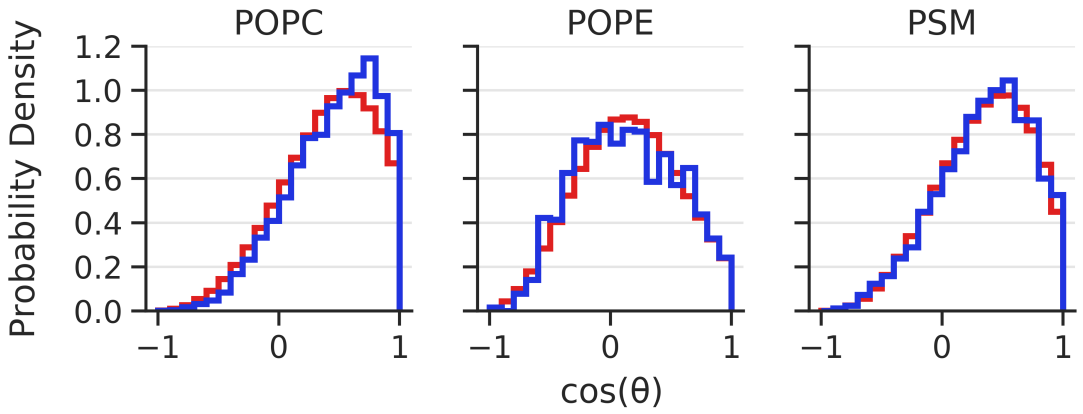
SI Fig. 27 Area per lipid distributions for POPC (red), POPE (yellow), PSM (blue) and all lipids (green). The python package PyVoro[5] was used to perform a Voronoi tesselations of lipids in the upper lower leaflets, from which the areas were then calculated.

RMSD of the lipid membrane surface.



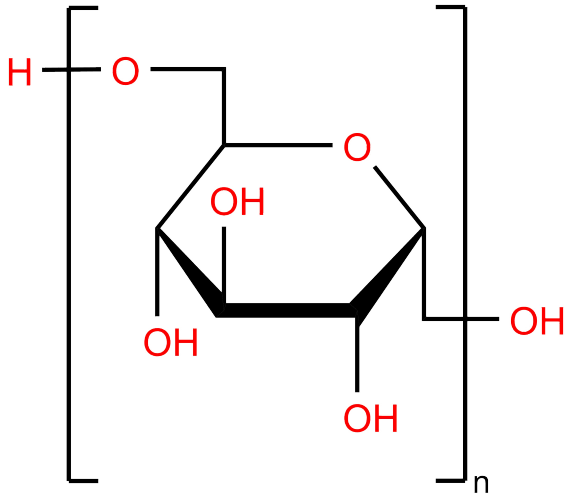
SI Fig. 28 The roughness of the membrane headgroup region as defined by the RMSD of atomic positions of P_L atoms in the upper leaflet.

Effect of dextran adsorption on lipid headgroup orientation.



SI Fig. 29 Distribution of $\cos(\theta)$ for each lipid headgroup for free (red) and adsorbed (blue) lipids, where θ is the angle that the lipid headgroup makes with the z-plane. The lipid headgroup orientation is defined as the vector from P_L to N_L.

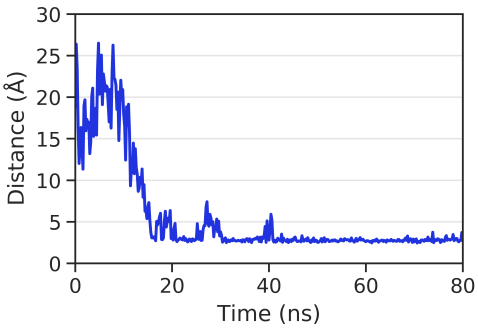
Chemical structure of dextran



SI Fig. 30 Dextran is a high molecular weight polysacharide consisting of repeating units of D-glucose linked primarily by α-1,6 glycosidic bonds, with α-1,3 linked branches of D-glucose. We have modelled dextran as a linear chain of D-glucose as short chains of dextran, such as the DX_{80} studied here, do not contain branching points.[6]

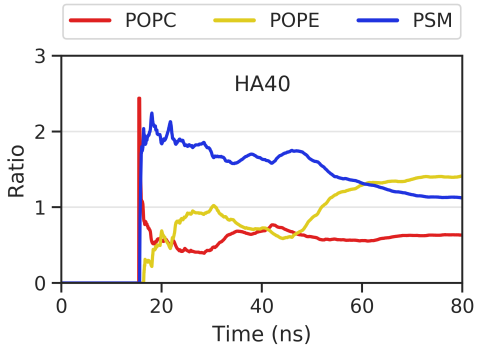
HA_{40} Replica 2

Approach curve

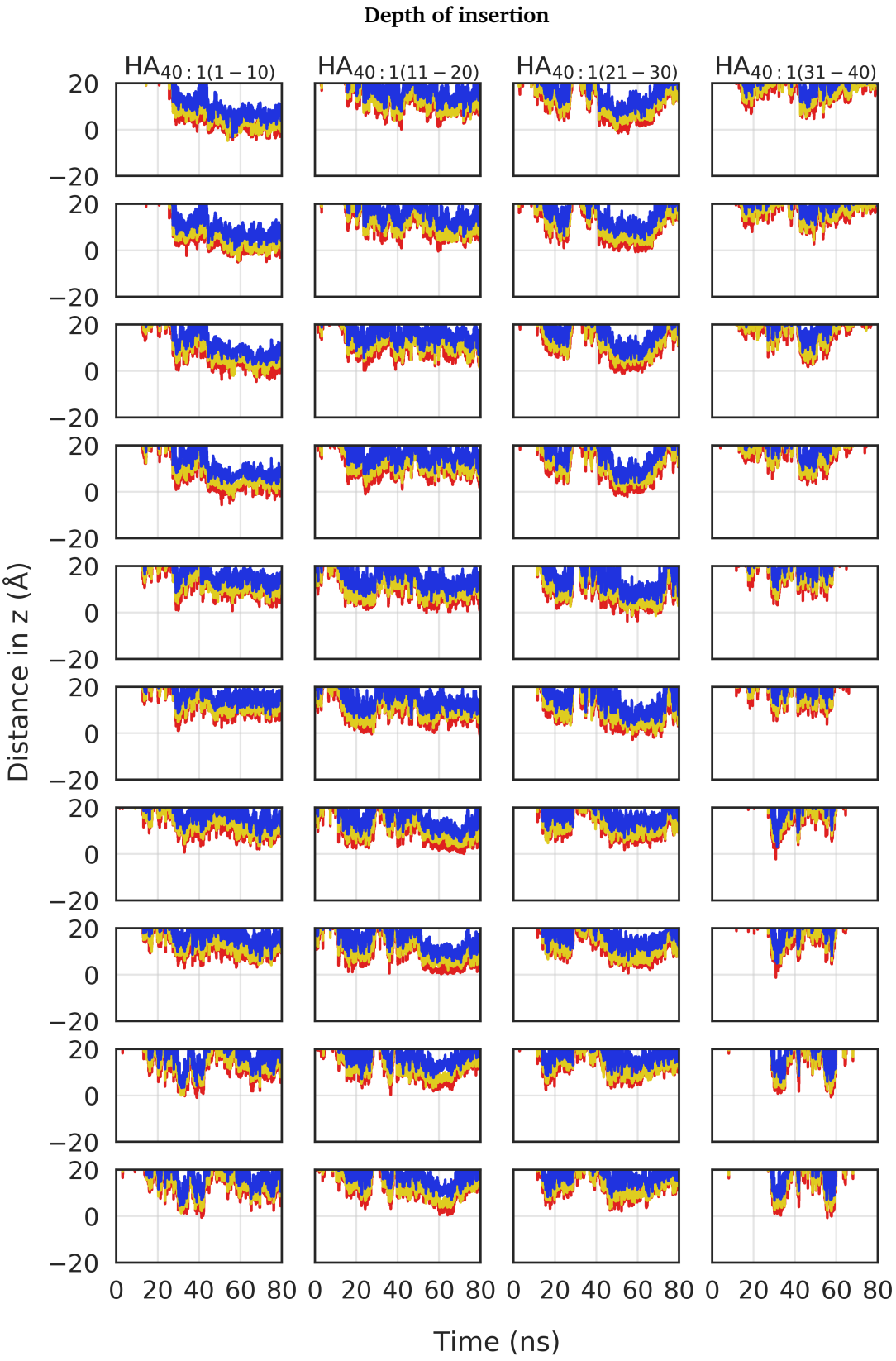


HA_{40} R2 Fig. 1 Approach curve of HA_{40}, showing the minimum distance at each timestep between any P_L atom in the upper leaflet and any OC_{HA} atom of HA.

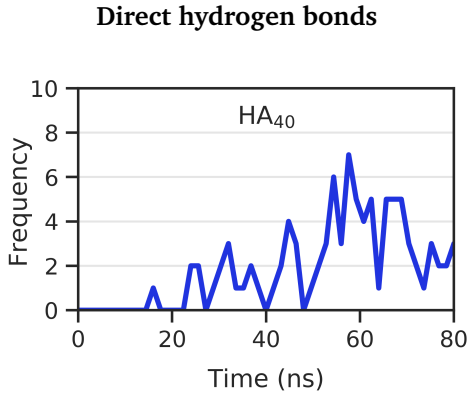
Ratio of adsorbed lipids



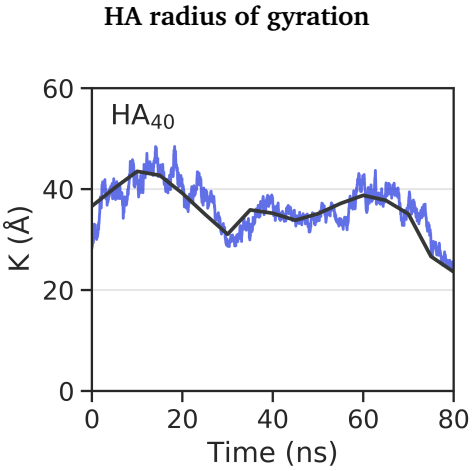
HA_{40} R2 Fig. 2 Ratio of POPC, POPE and PSM adsorbed to HA, normalised by the composition of the membrane.



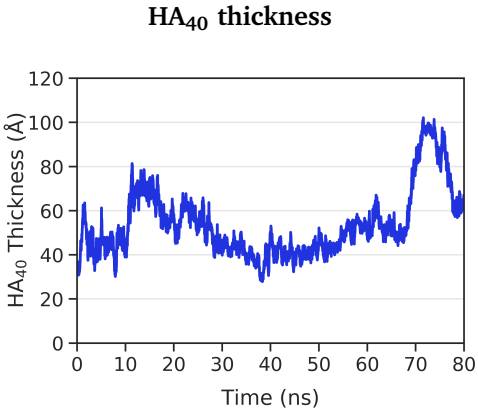
HA_{40} R2 Fig. 3 Depth of insertion of individual HA monomeric units into the membrane surface. Three separate intrinsic surfaces are defined using the N_L (red), P_L (yellow) and O_L (blue) atoms in the upper leaflet. The depth of penetration into either the upper or lower leaflet is calculated as the distance in z between a monomeric unit and a given surface type (N_L, P_L or $O2_L$). For the column headers, $HA_{40:a\text{-}b}$ indicates that this column shows the depth of insertion of units *a-b* of the HA_{40} molecule.



HA_{40} R2 Fig. 4 The number of direct hydrogen bonds between HA and the lipid membrane as a function of time. Note, for clarity, the number of direct hydrogen bonds is shown for every 20_{th} configuration; these values are representative of those seen across all snapshots.

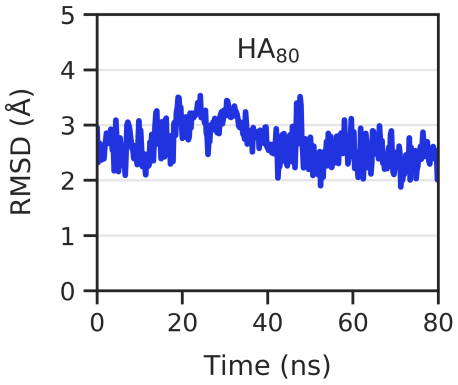


HA_{40} R2 Fig. 5 Radius of gyration (K) as a function of time for the HA_{40} molecule.

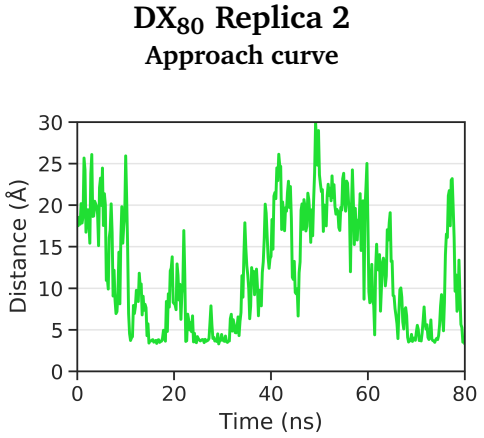


HA_{40} R2 Fig. 6 The thickness of HA_{40} over time, as defined by the maximum distance in z between any two heavy (non-hydrogen) atoms of HA_{40}.

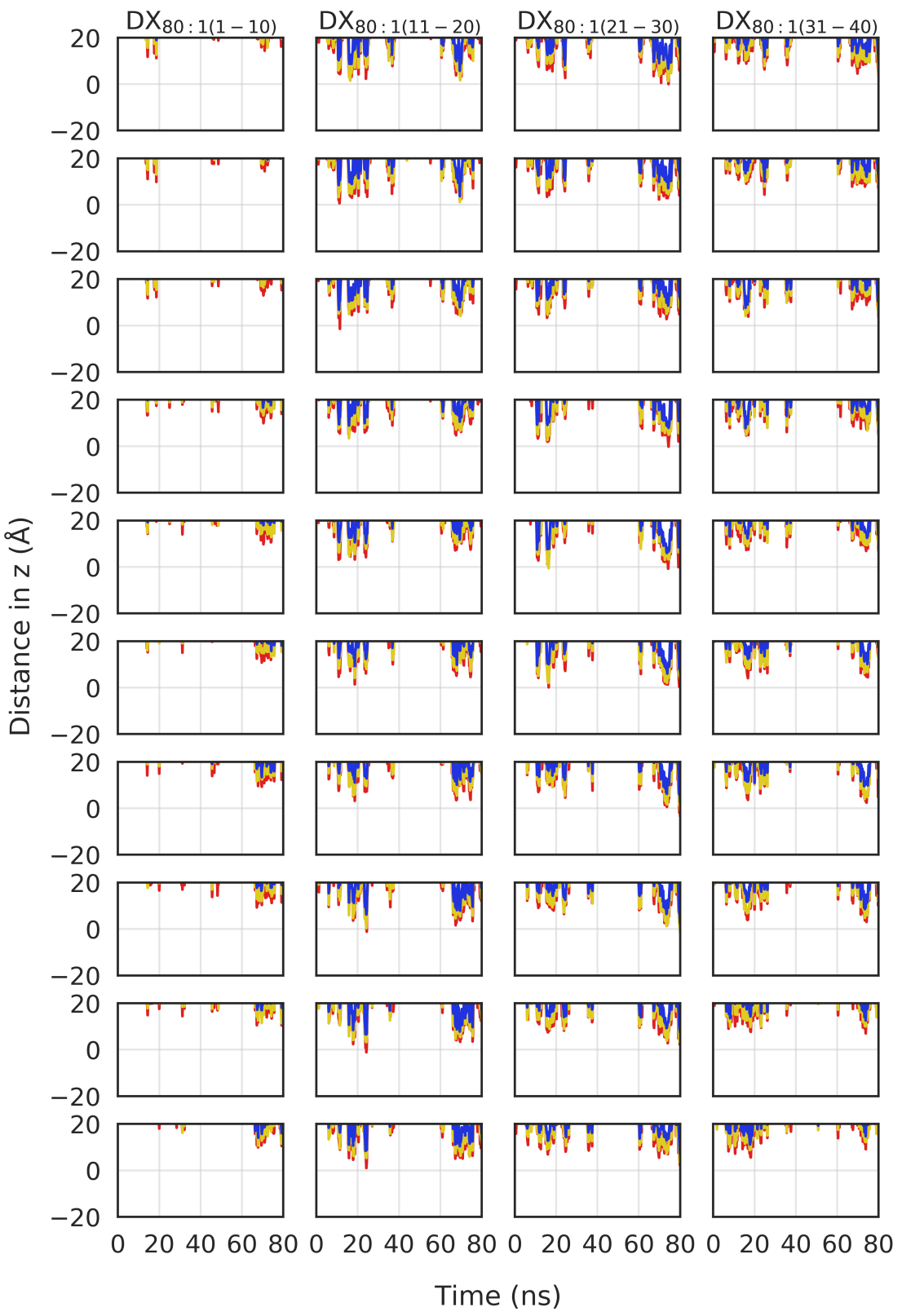
RMSD of the lipid membrane surface.



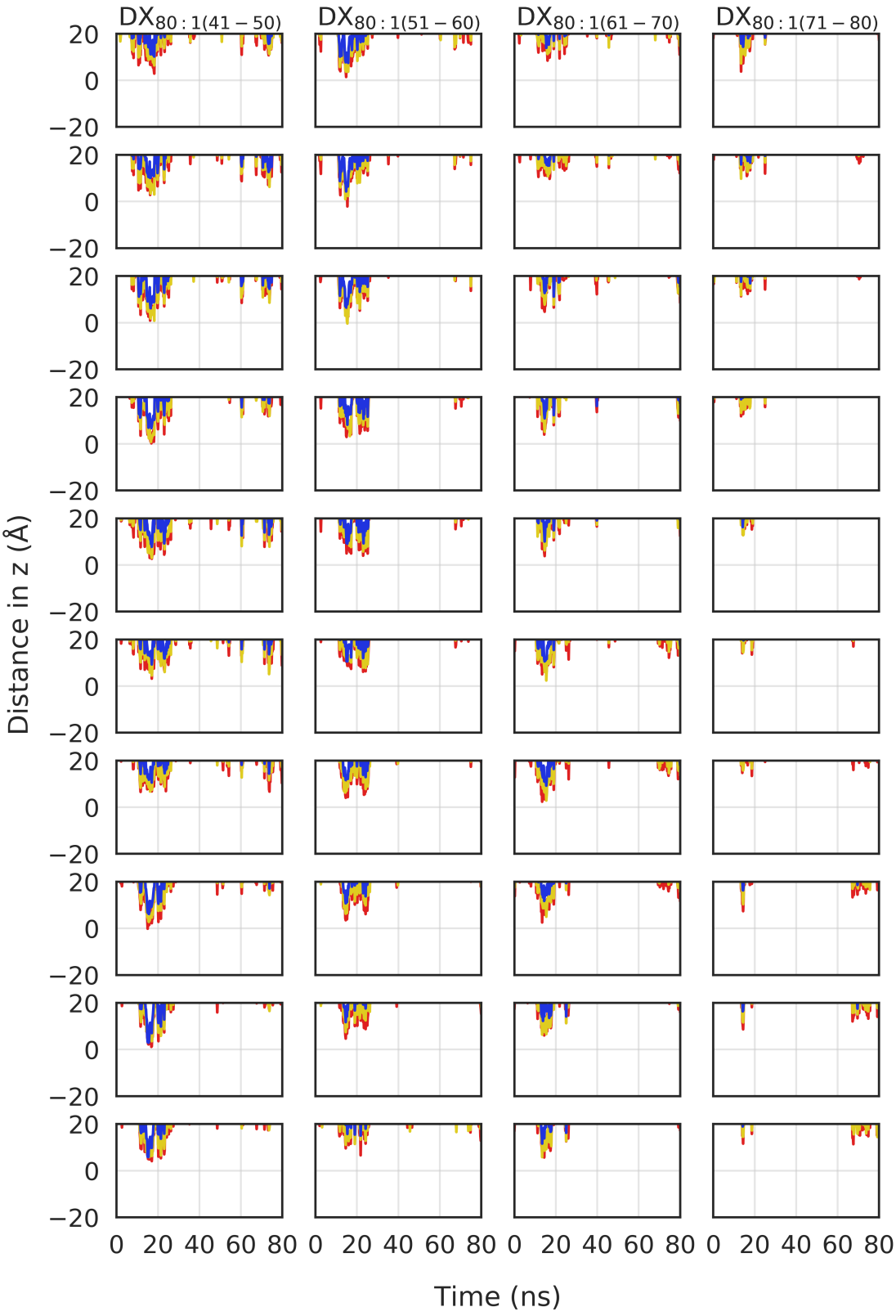
HA_{40} R2 Fig. 7 The roughness of the membrane headgroup region as defined by the RMSD of atomic positions of P_L atoms in the upper leaflet.



DX_{80} R2 Fig. 1 Approach curve of DX_{80}, showing the minimum distance at each timestep between any P_L atom any OH_{DX}. DX_{80} interacts with both the upper and lower leaflets during the simulations (although never both simultaneousy), and so the distances shown here are the minimum distances to either the upper or lower leaflet.


Depth of insertion
DX80 : 1(1 – 10)
DX80 : 1(11 – 20)
DX80 : 1(21 – 30)
DX80 : 1(31 – 40)
Distance in z (Å)
20
0
−20
0 20 40 60 80
Time (ns)

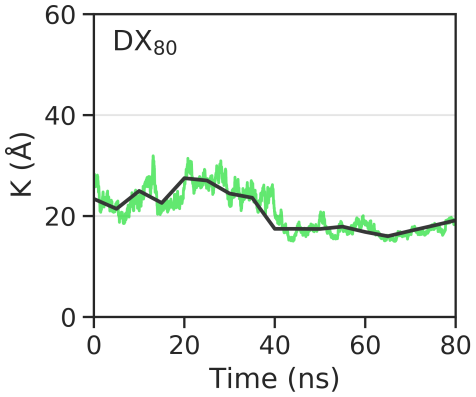
(a) $DX_{80:1-40}$



(b) $DX_{80:41-80}$

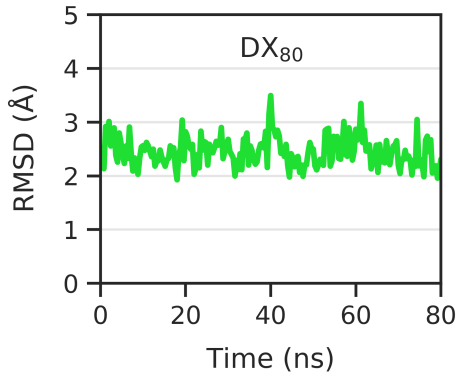
DX_{80} R2 Fig. 2 Depth of insertion of individual DX monomeric units into the membrane surface. Six separate intrinsic surfaces are defined using the N_L (red), P_L (yellow) and O_L (blue) atoms in the upper leaflet and lower leaflets (three surfaces per leaflet). The depth of penetration into either the upper or lower leaflet is calculated as the absolute distance in z that from each monomeric unit to a given surface type (N_L, P_L or $O2_L$). For the column headers, $DX_{80:a\text{-}b}$ indicates that this column shows the depth of insertion of units *a-b* of the DX_{80} molecule.

Dextran radius of gyration



DX_{80} R2 Fig. 3 Radius of gyration (K) as a function of time for the DX_{80} molecule.

RMSD of the lipid membrane surface.



DX_{80} R2 Fig. 4 The roughness of the membrane headgroup region as defined by the RMSD of atomic positions of P_L atoms in the upper leaflet.

References

1 W. Shinoda, M. Shiga and M. Mikami, *Phys. Rev. B.*, 2004, **69**, 134103.
2 M. E. Tuckerman, J. Alejandre, R. López-Rendón, A. L. Jochim and G. J. Martyna, *J. Phys. A.*, 2006, **39**, 5629–5651.
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5 C. H. Rycroft, *Chaos*, 2009, **19**, 041111.
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