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Supplementary Information

Table S1 List of the MD (setups 1-3) and SMD (setups 4-11) simulations performed in this study without (setups 1-2) or with (setups 3-10) harmonic-constraints. t_0 indicates the time after equilibration at which the pulling of Gal started. The last two columns list the RMSD values (Å) ± standard deviations calculated for each setups.

Table S2 (A) Coordination distances (Å) and (B) interaction energies (kcal/mol, non-bonded energies) of the Na^+ ions in each monomer are listed as average values ± standard deviations for setup 1. Interaction energies were calculated for the side-chains of residues Asp189, Glu192, Ser364 and 365 or the backbone of residues Ala62, Ala63, Ile65 and Ala361.

Fig. S1 Coordination and mobility of sodium in and beyond the binding site. Distances (Å) (*top*) and interaction energies (*bottom*) between the Na^+ ion and the indicated residues are shown for monomer B in setup 1.

Fig. S2 Interaction energy of sodium with water and/or transporter as a function of time, calculated for each monomer in setup 1.

Fig. S3 Selected distances (Å) of some of the possible interactions between the non-H atoms of the transporter and Gal are shown for monomer A in setup 1.

Table S3 (A) Comparison of the average distances (Å) ± standard deviation for each pair of non-H atoms in Gal and the transporter for both monomers in setup 1 with those measured in the crystal structure. Interactions where a H-bond absent in the crystal was observed in at least one of the monomers or one where an H-bond distance was reported in the crystal but a H-bond was mostly absent in the simulation in setup 1 are underlined. Interactions within H-bond distance reported in the crystal structure are shown in **bold**. (B) Average energy (kcal/mol) ± standard deviation of each Gal with the transporter groups calculated for setup 1.

Fig. S4 Trajectory of the non-bonded interaction energy of Gal with water, the transporter or the Na^+ ion calculated for each monomer in setup 1.

Fig. S5 Force-displacement profiles where Gal(A) was pulled at an angle of 60° to the –z axis. Results are shown for two different pulling velocities, 2 and 4 Å/ns, corresponding to setups 9 and 10, respectively, and are plotted as force (pN) (*top*) or z coordinate (Å) (*bottom*) vs time (ns).

Table S4 (A) Properties and dimensions of the system used in this study. (B) RMSD values (Å) ± standard deviation in the backbone coordinates compared with the energy-minimized structure.

Table S1 Setups of simulations performed in this study

Setup	time (ns)	Gal+Na^+	Constraints	pulled group	velocity (Å/ns)	t_0 (ns)	pulling angle	RMSD mon. A	RMSD mon. B
1	30	+	-	-	-	-	-	1.61 ± 0.13	1.92 ± 0.26
2	10	-	-	-	-	-	-	1.61 ± 0.16	1.51 ± 0.15
3	10	+	+	-	-	-	-	1.72 ± 0.20	1.69 ± 0.15
4	5	+	+	Gal(A)	2	0.75	0	1.24 ± 0.14	1.53 ± 0.30
5	2.5	+	+	Gal(A)	4	0.75	0	1.15 ± 0.18	1.17 ± 0.21
6	6	+	+	Gal(A)	2	0	0	1.60 ± 0.17	1.63 ± 0.20
7	5	+	+	Gal(A)	4	0	0	2.06 ± 0.21	1.87 ± 0.20
8	7	+	+	Gal(B)	2	0	0	1.51 ± 0.10	1.64 ± 0.21
9	5	+	+	Gal(A)	2	0	60	2.13 ± 0.26	1.87 ± 0.14
10	5	+	+	Gal(A)	4	0	60	1.51 ± 0.12	1.59 ± 0.15
11	5	+	+	Gal(B)	4	0	60	1.57 ± 0.16	1.52 ± 0.20

Table S2.A **Coordination distances of the Na^+ ion (Å)**

	simulation		crystal	
	monomer A	monomer B	monomer A	monomer B
Ala62(O)	3.95 ± 1.52	3.94 ± 1.95	3.64	3.72
Ala63(O)	5.59 ± 1.48	5.20 ± 1.98	5.27	5.17
Ile65(O)	5.28 ± 2.27	4.58 ± 2.56	3.32	3.19
Asp189(Oδ1)	4.38 ± 1.62	4.41 ± 1.36	4.43	4.63
Asp189(Oδ2)	4.95 ± 1.50	4.64 ± 1.77	6.14	6.32
Gln192(Oε)	4.61 ± 1.55	6.36 ± 1.40	5.65	5.75
Ala361(O)	5.19 ± 1.76	6.78 ± 1.63	3.23	3.11
Ser364(Oγ)	3.94 ± 1.63	5.76 ± 1.45	3.13	3.11
Ser365(Oγ)	5.60 ± 1.85	6.61 ± 1.97	3.68	3.50

Table S2.B Energy of interaction with sodium (kcal/mol)

	monomer A	monomer B
monomer	-107.89 ± 23.71	-110.29 ± 17.59
water	-66.35 ± 25.31	-74.24 ± 22.64
total	-174.25 ± 16.45	-184.53 ± 15.64
Ala62(O)	-26.53 ± 11.24	-29.46 ± 13.47
Ala63(O)	-23.27 ± 6.19	-24.84 ± 8.55
Ile65(O)	-25.75 ± 13.42	-33.10 ± 17.14
Asp189(Oδ1+2)	-74.49 ± 31.08	-76.34 ± 35.85
Gln192(Oε)	-9.24 ± 8.35	-3.05 ± 4.28
Ala361(O)	-22.54 ± 9.36	-15.56 ± 7.04
Ser364(Oγ)	-10.82 ± 9.17	-1.16 ± 5.51
Ser365(Oγ)	-4.25 ± 8.05	-3.24 ± 5.49

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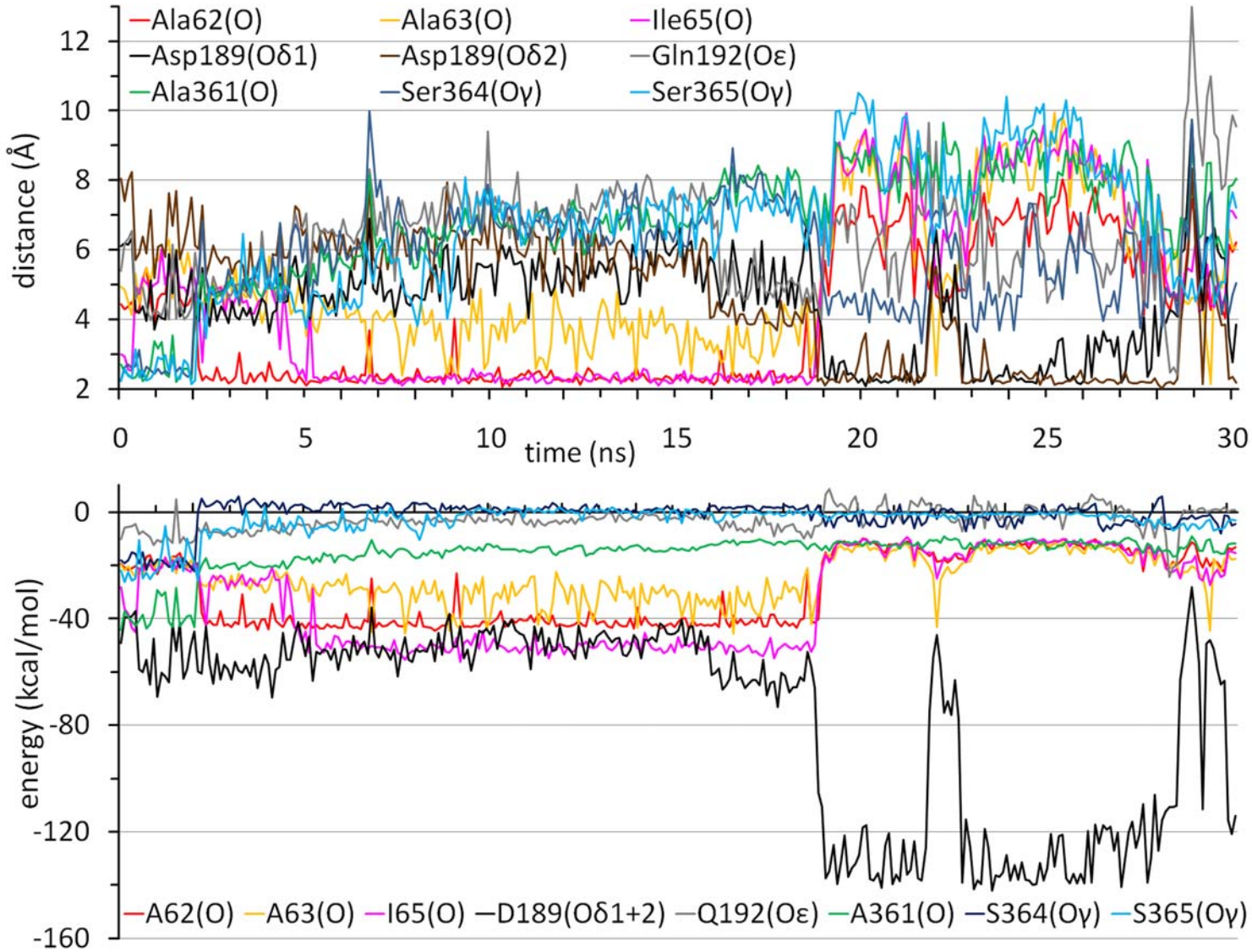

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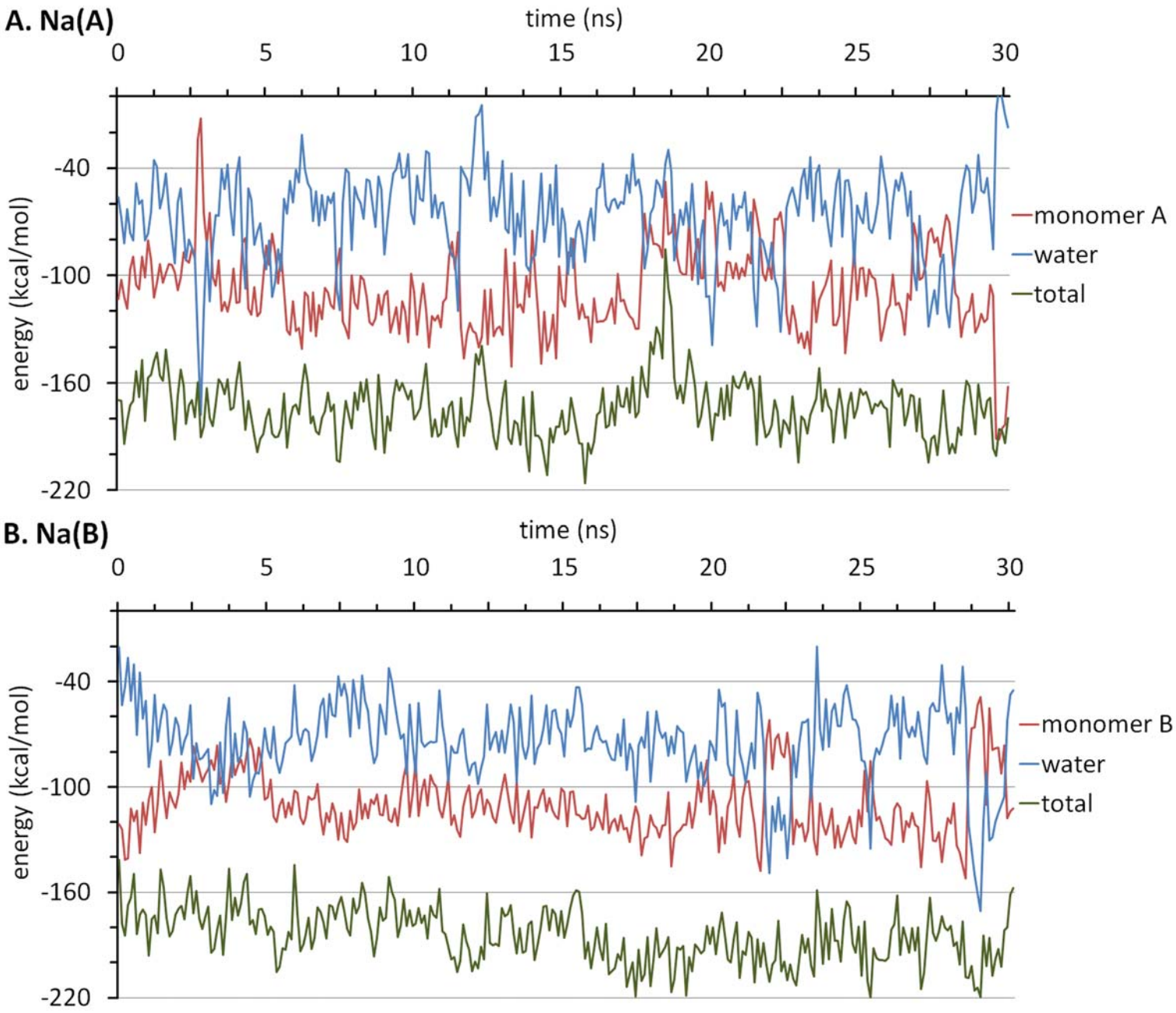

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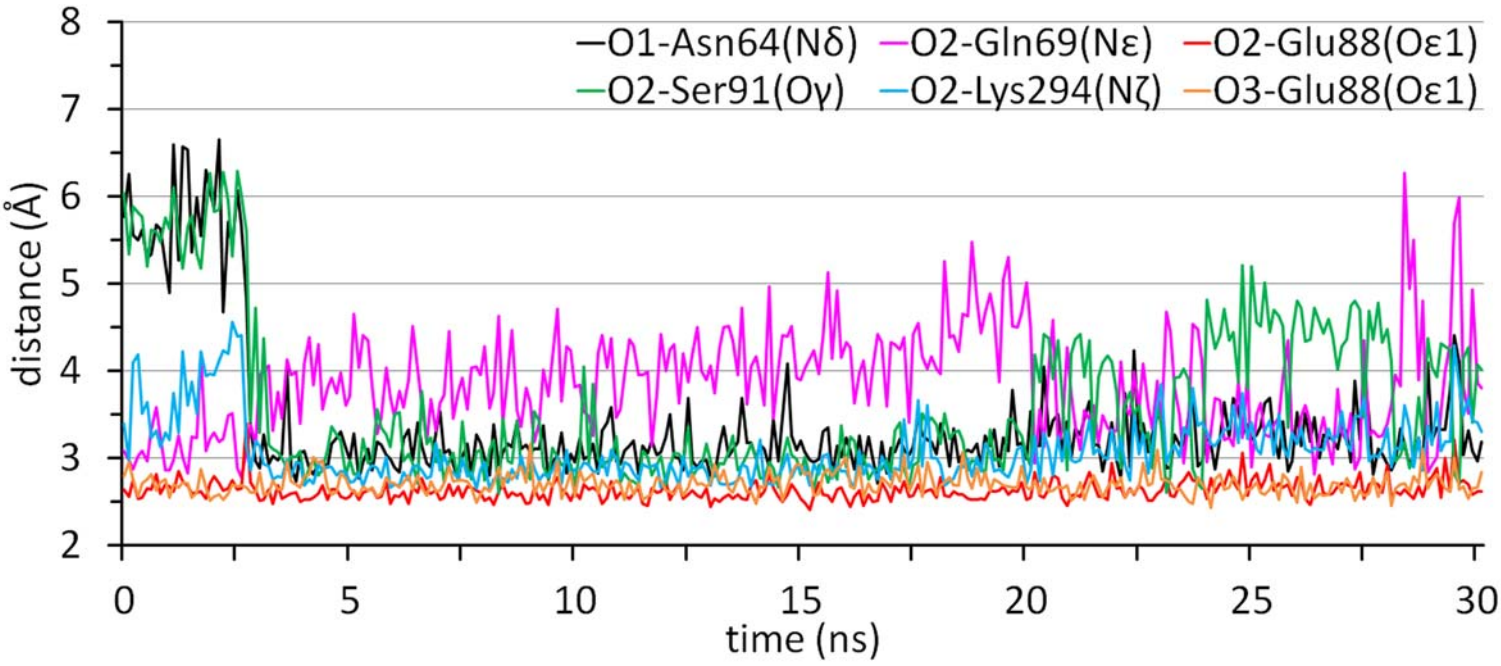

Table S3.A Coordination distances of galactose

		simulation		crystal	
		monomer A	monomer B	monomer A	monomer B
1	Gal(O1)-Asn64(Nδ)	3.41 ± 0.80	4.70 ± 1.24	5.19	5.19
2	Gal(O1)-Glu68(Oε1)	8.04 ± 1.58	9.17 ± 2.16	4.47	4.51
3	**Gal(O1)-Asn69(Nε)**	**4.80 ± 0.84**	**5.34 ± 1.04**	**3.20**	**3.27**
4	Gal(O1)-Lys294(Nζ)	4.76 ± 1.11	6.44 ± 1.05	5.57	5.57
5	**Gal(O2)-Asn64(Nδ)**	**4.33 ± 0.78**	**3.55 ± 0.73**	**3.22**	**3.24**
6	Gal(O2)-Gln69(Nε)	3.84 ± 0.59	4.65 ± 1.29	3.62	3.61
7	**Gal(O2)-Gln69(Oε)**	**4.79 ± 0.71**	**5.10 ± 1.15**	**2.81**	**2.79**
8	Gal(O2)-Glu88(Oε1)	3.80 ± 0.37	4.25 ± 1.55	3.69	3.66
9	**Gal(O2)-Glu88(Oε2)**	**2.63 ± 0.11**	**4.52 ± 0.58**	**2.98**	**2.94**
10	Gal(O2)-Ser91(Oγ)	3.61 ± 0.91	4.74 ± 1.33	5.44	5.43
11	**Gal(O2)-Lys294(Nζ)**	**3.07 ± 0.36**	**4.14 ± 0.92**	**2.76**	**2.75**
12	**Gal(O3)-Glu88(Oε2)**	**2.70 ± 0.12**	**3.57 ± 0.94**	**3.09**	**3.09**
13	**Gal(O3)-Ser91(Oγ)**	**4.97 ± 0.71**	**4.29 ± 0.91**	**2.56**	**2.57**
14	Gal(O3)-Trp264(Nε)	7.25 ± 0.67	6.25 ± 1.29	3.49	3.47
15	**Gal(O3)-Lys294(Nζ)**	**4.95 ± 0.59**	**4.01 ± 1.26**	**2.85**	**2.83**
16	Gal(O4)-Glu88(Oε2)	5.07 ± 0.22	4.63 ± 1.90	4.58	4.48
17	**Gal(O4)-Asn260(Nδ)**	**5.17 ± 0.62**	**5.88 ± 0.79**	**2.73**	**2.80**
18	Gal(O4)-Asn260(Oδ)	6.23 ± 1.11	4.92 ± 0.61	4.59	4.66
19	Gal(O4)-Gln428(Nε)	4.85 ± 1.40	4.87 ± 0.85	5.31	5.17
20	**Gal(O5)-Glu428(Nε)**	**6.29 ± 1.72**	**4.96 ± 1.70**	**3.04**	**2.97**
21	Gal(O6)-Ala259(O)	4.31 ± 0.90	4.83 ± 0.77	3.93	3.98
22	Gal(O6)-Gln428(Nε)	5.01 ± 1.63	4.41 ± 1.02	3.33	3.23
23	**Gal(O6)-Gln428(Oε)**	**4.64 ± 1.68**	**4.64 ± 1.72**	**2.69**	**2.68**

Table S3.B	**Galactose interaction energies (kcal/mol)**		
		monomer A	monomer B
1	full monomer	-63.07 ± 10.21	-60.75 ± 9.53
2	H2	-4.07 ± 5.29	-4.44 ± 3.14
3	H3	-32.70 ± 4.26	-31.41 ± 6.52
4	H7	-11.66 ± 2.49	-11.29 ± 2.45
5	H8	-7.44 ± 9.82	-4.90 ± 8.17
6	H11	-5.81 ± 3.59	-7.35 ± 4.14
7	Asn64 (H2)	-3.59 ± 1.96	-3.89 ± 1.82
8	Glu68 (H2)	2.17 ± 3.23	1.90 ± 1.42
9	Gln69 (H2)	-1.27 ± 3.26	-2.08 ± 2.31
10	Glu88 (H3)	-28.29 ± 3.87	-27.19 ± 6.36
11	Ser91 (H3)	-1.88 ± 3.06	-1.25 ± 3.05
12	Ala259(O) (H7)	-0.94 ± 1.31	-1.06 ± 0.98
13	Asn260 (H7)	-4.69 ± 1.53	-5.00 ± 1.81
14	Trp264 (H7)	-1.70 ± 1.43	-0.86 ± 0.78
15	Lys294 (H8)	-7.50 ± 9.84	-5.84 ± 9.00
16	Gln428 (H11)	-3.67 ± 3.29	-4.91 ± 3.96
17	water	-18.06 ± 6.31	-19.73 ± 7.72
18	sodium	-1.32 ± 0.72	-1.34 ± 0.70

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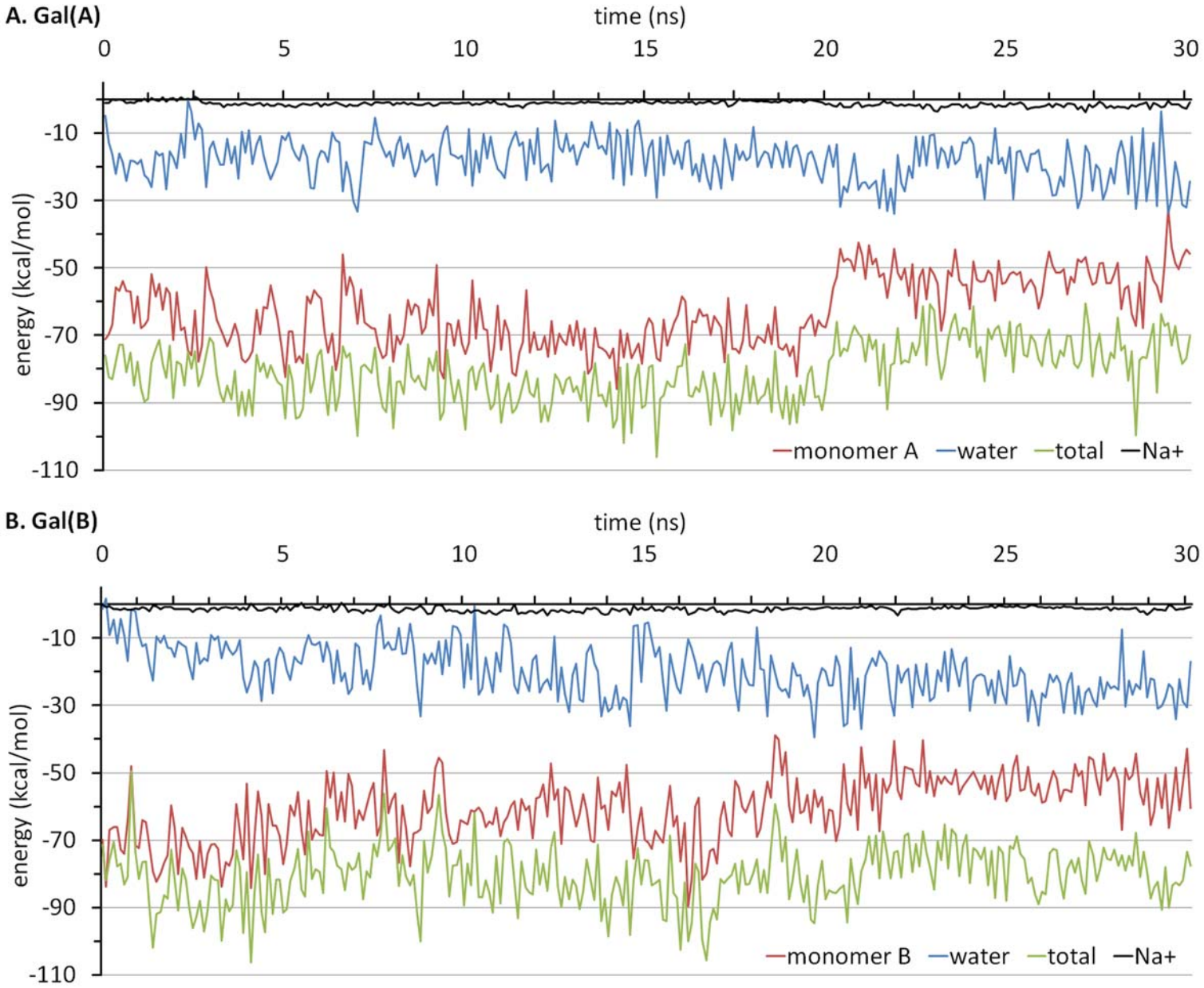

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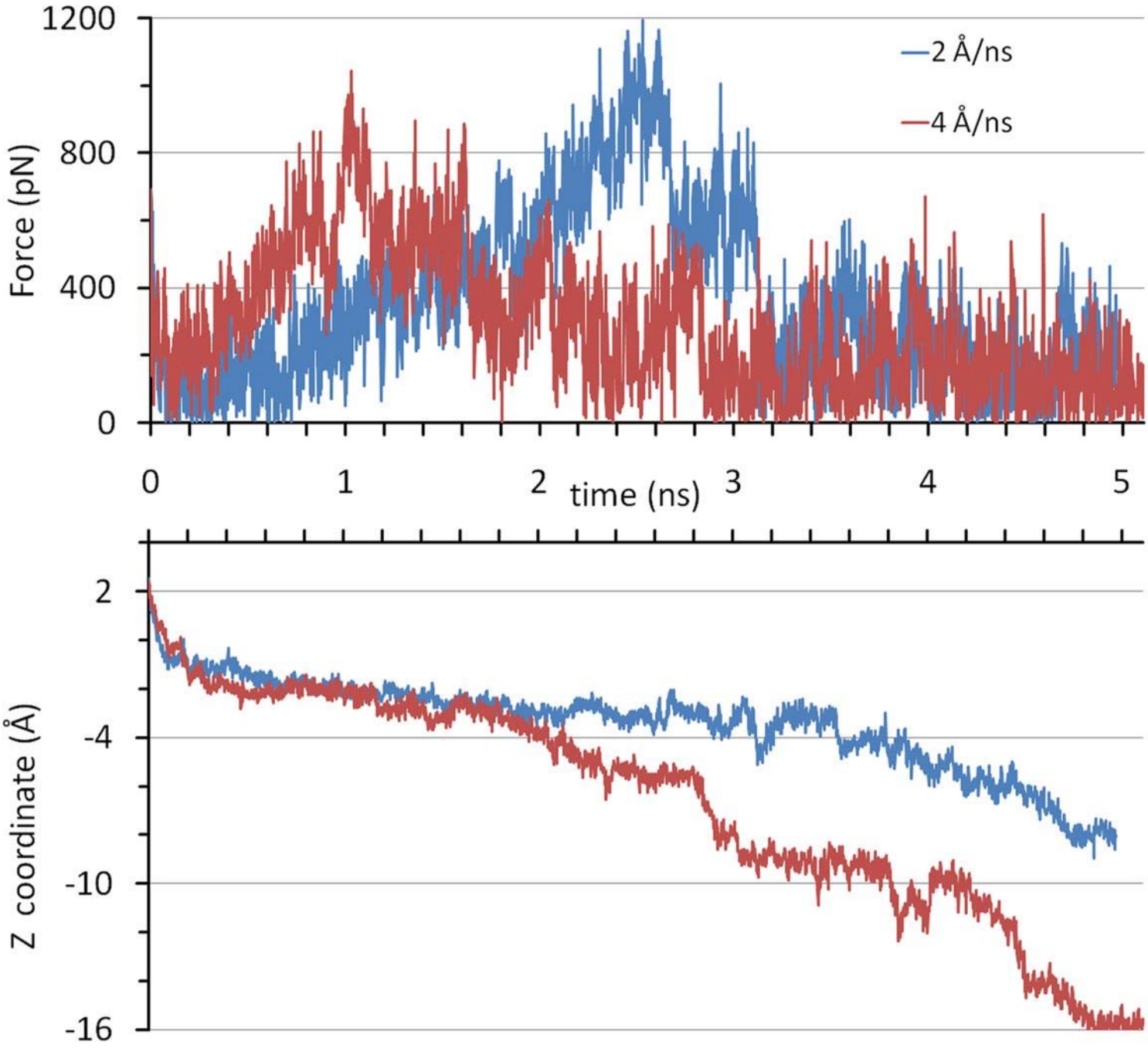

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Table S4 System properties and dimensions

total number of atoms	113’510
residues in dimer	1086
lipid molecules	321
water molecules	19’269
water layers (Å)	13 intra-, 25 extracellular
x (all) (Å)	140
y (all) (Å)	118
z (all) (Å)	92
x (protein) (Å)	~ 100
y (protein) (Å)	~ 60
z (protein) (Å)	~ 74
x (lipid padding) (Å)	at least 15
y (lipid padding) (Å)	at least 15
z (water padding) (Å)	9 intra-, 9 extracellular