Supplementary Information

Insights into the Polyhexamethylene Biguanide (PHMB) Mechanism of Action on Bacterial Membrane and DNA: A Molecular Dynamics Study

Shahin Sowlati-Hashjin[1,2], Paola Carbone[3], Mikko Karttunen[1,2,4,*]

[1]Department of Chemistry, the University of Western Ontario, London, Ontario, Canada. [2]The Centre of Advanced Materials and Biomaterials Research, the University of Western Ontario, London, Ontario, Canada. [3]Department of Chemical Engineering and Analytical Science, The University of Manchester, Oxford Road, Manchester, UK. [4]Department of Applied Mathematics, the University of Western Ontario, London, Ontario, Canada

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Table S1. Average number of hydrogen bonds (d(H···O) ≤ 0.2 nm and α (∠(Hydrogen–Donor–Acceptor) ≤ 30°) for PHMB chains (with solvent, bilayer, and self interaction) in the different systems. Standard deviations are provided in parentheses.

	Solvent	Per Bgd$^+$	Bilayer	Per Bgd$^+$	PHMB	Per Bgd$^+$	Total
1 PHMB-solvent	39.9	3.3	–	–	1.1	0.1	3.4 (0.3)
4 PHMBs-solvent							
Chain 1	39.6	3.3	–	–	1.1	0.1	3.4 (0.3)
Chain 2	38.0	3.2	–	–	1.1	0.1	3.3 (0.3)
Chain 3	38.6	3.2	–	–	1.1	0.1	3.3 (0.3)
Chain 4	39.3	3.3	–	–	1.1	0.1	3.4 (0.3)
4 PHMBs-bilayer							
Chain 1	18.6	1.6	21.5	1.8	0.5	0.0	3.4 (0.4)
Chain 2	17.7	1.5	23.5	2.0	0.7	0.1	3.6 (0.3)
Chain 3	18.9	1.6	22.3	1.9	0.5	0.0	3.5 (0.3)
Chain 4	18.4	1.5	23.5	2.0	0.5	0.0	3.5 (0.3)
4 PHMB dimers-solvent							
Chain 1	6.8	3.4	–	–	0.2	0.1	3.5 (0.8)
Chain 2	6.9	3.5	–	–	0.2	0.1	3.6 (0.8)
Chain 3	6.9	3.5	–	–	0.2	0.1	3.6 (0.8)
Chain 4	6.9	3.5	–	–	0.2	0.1	3.6 (0.8)
4 PHMB dimers-bilayer							
Chain 1	2.4	1.2	4.8	2.4	0.1	0.1	3.7 (0.8)
Chain 2	2.3	1.2	4.5	2.3	0.1	0.0	3.5 (0.8)
Chain 3	2.2	1.1	4.3	2.2	0.2	0.1	3.4 (0.8)
Chain 4	2.6	1.3	4.1	2.0	0.1	0.1	3.4 (0.8)

Table S2. Average number of phospholipid-solvent and solvent-solvent hydrogen bonds (d(H···O) ≤ 0.2 nm and α (∠(Hydrogen–Donor–Acceptor) ≤ 30°). Standard deviations are provided in parentheses.

	POPE···solvent	POPG···solvent	solvent···solvent
1 PHMB-solvent	–	–	3.3 (0.1)
4 PHMBs-solvent	–	–	3.3 (0.1)
4 PHMBs-bilayer	5.8 (0.1)	5.8 (0.2)	3.3 (0.1)
4 PHMB dimers-solvent	–	–	3.3 (0.1)
4 PHMB dimers-bilayer	5.8 (0.2)	5.9 (0.3)	3.1 (0.1)
Pure bilayer	6.0 (0.2)	6.2 (0.3)	3.1 (0.1)
Pure bilayer*	5.8	5.1	–

*Data from reference 2

Table S3. Average number of hydrogen bonds (d(H···O) ≤ 0.2 nm and α (∠(Hydrogen–Donor–Acceptor) ≤ 30°) in the PHMB-DNA system. Standard deviations are provided in parentheses.

	PHMB···solvent	Per Bgd$^+$	Total	DNA···DNA
1 PHMB-DNA	23.3	1.9	3.1 (0.3)	42.9 (2.4)
	PHMB···DNA	**Per Bgd$^+$**		
	13.0	1.1		
	PHMB···PHMB	**Per Bgd$^+$**		
	1.0	0.1		

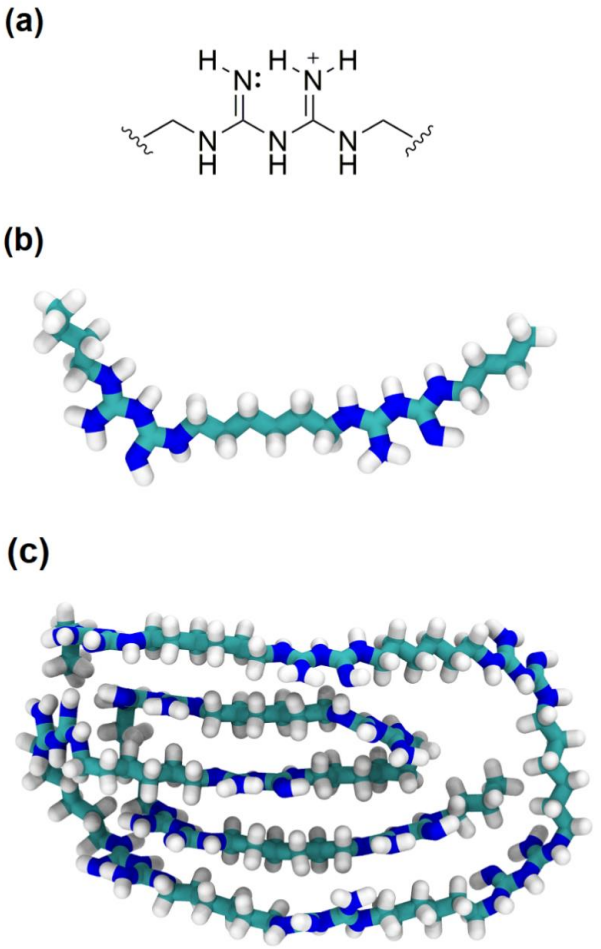

Figure S1. (a) 2D representation of the biguanide functional group. Structures of biguanide (b) dimer and (c) dodecamer. Carbon, nitrogen and hydrogen atoms are shown in cyan, blue and white, respectively.

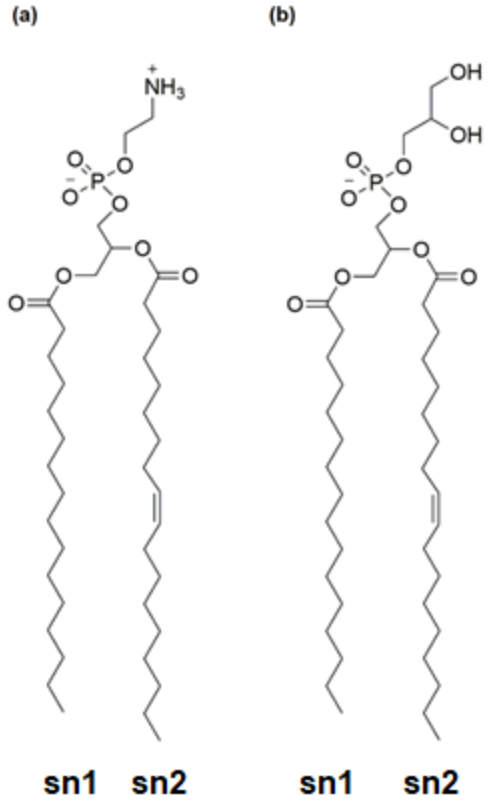

Figure S2. 2D representations of (a) phosphatidylethanolamine (POPE) and (b) phosphatidylglycerol (POPG) lipids.

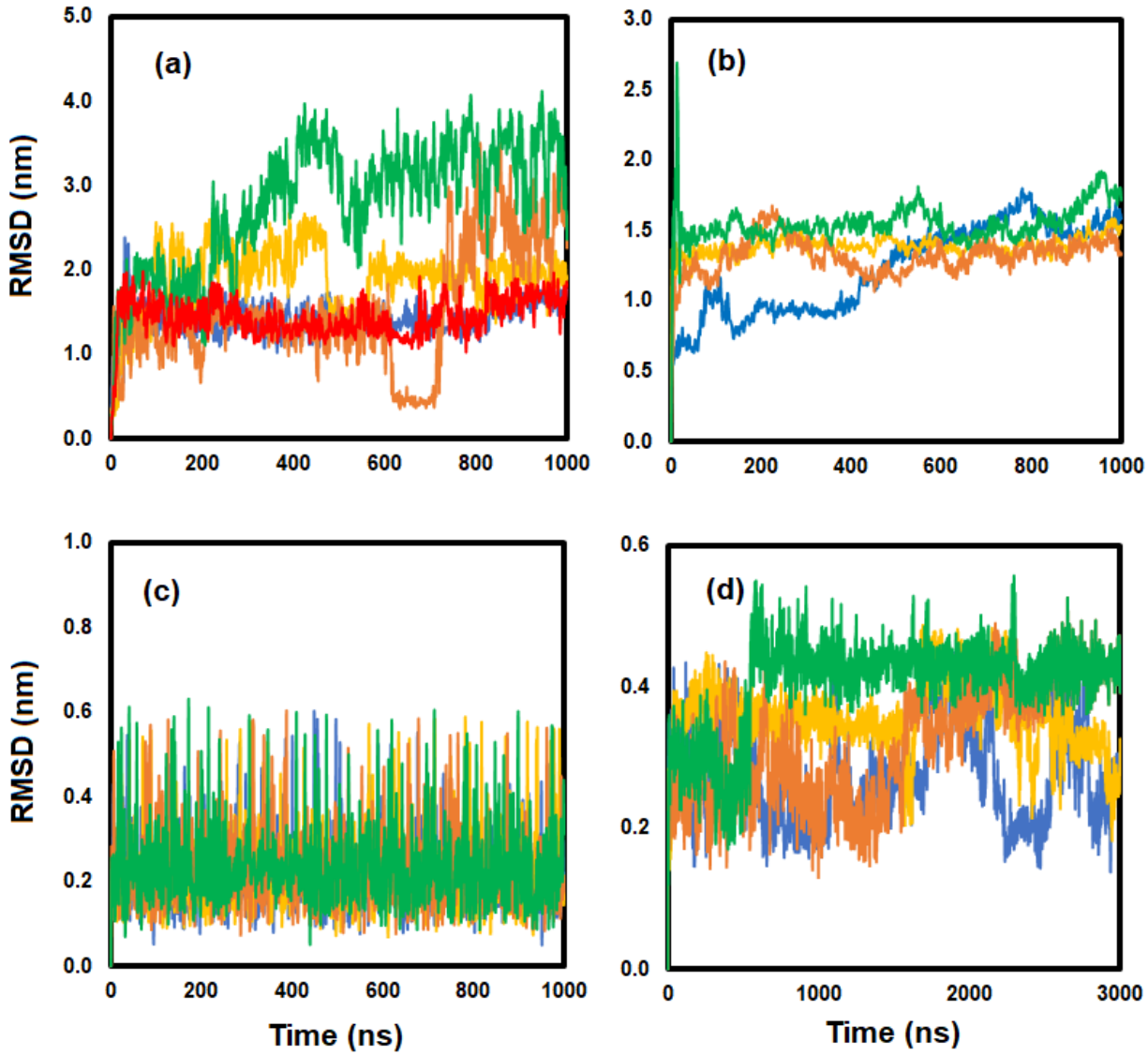

Figure S3. RMSD of (a) 1 PHMB polymer (red) and 4 PHMB polymers in solvent (individual chains are represented in different colors: green, blue, orange, and brown). (b) RMSD of 4 PHMB polymers on the bilayer. (c) RMSD of 4 PHMB dimers in solvent and (d) of 4 PHMB dimers on the bilayer. As is seen, in comparison with the solvent system, both the PHMB polymer and dimer chains show lower RMSDs on the bilayer, indicating a reduced degree of mobility when involved in interactions with the phospholipids.

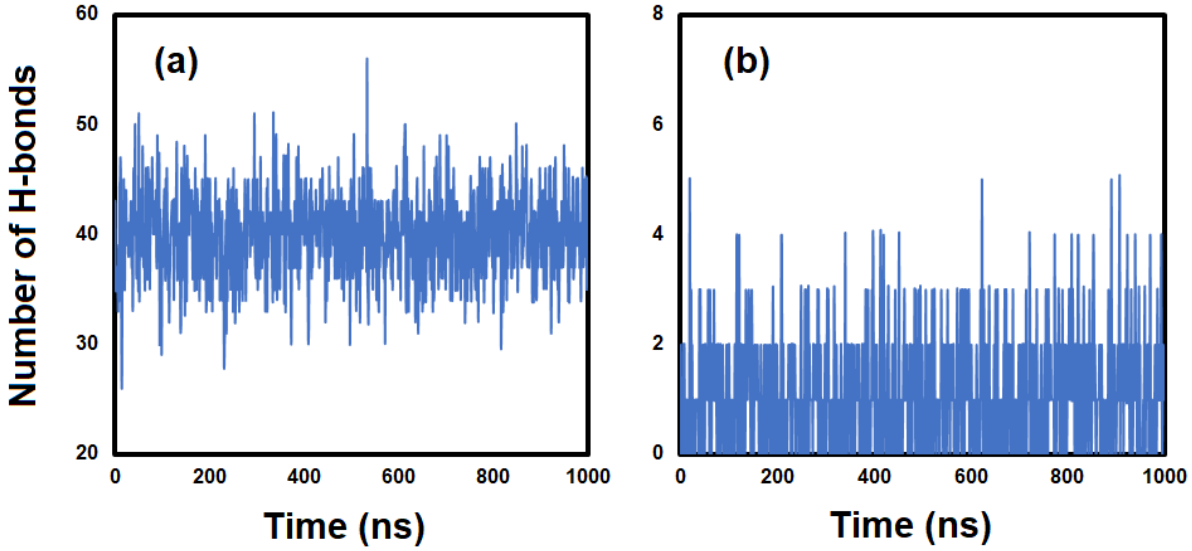

Figure S4. (a) Hydrogen bonds between PHMB and solvent and (b) PHMB intra-molecular hydrogen bonds in 1 PHMB polymer-solvent system.

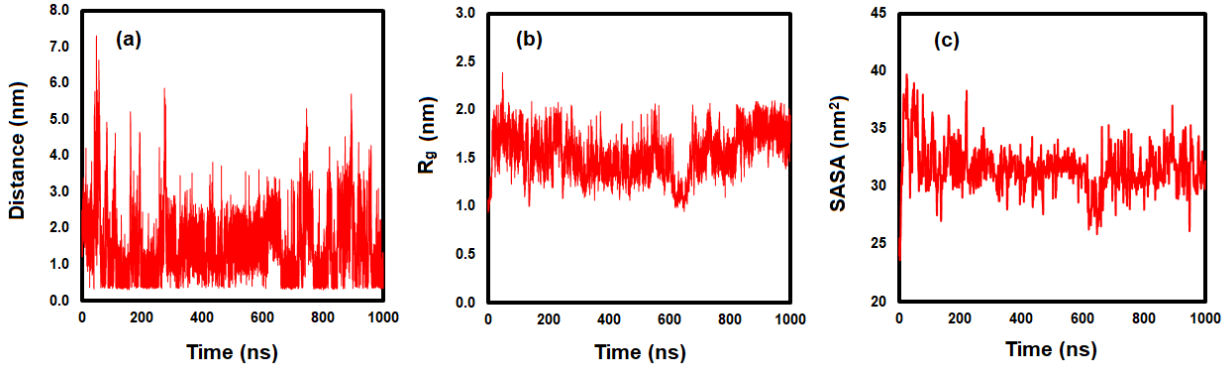

Figure S5. (a) End-to-end distance, (b) radius of gyration and (c) solvent accessible surface area (SASA) of PHMB polymer in solvent. SASA was calculated based on the numerical Double Cubic Lattice Method (DCLM)[1] as implemented in the GROMACS package using 1 ns intervals.

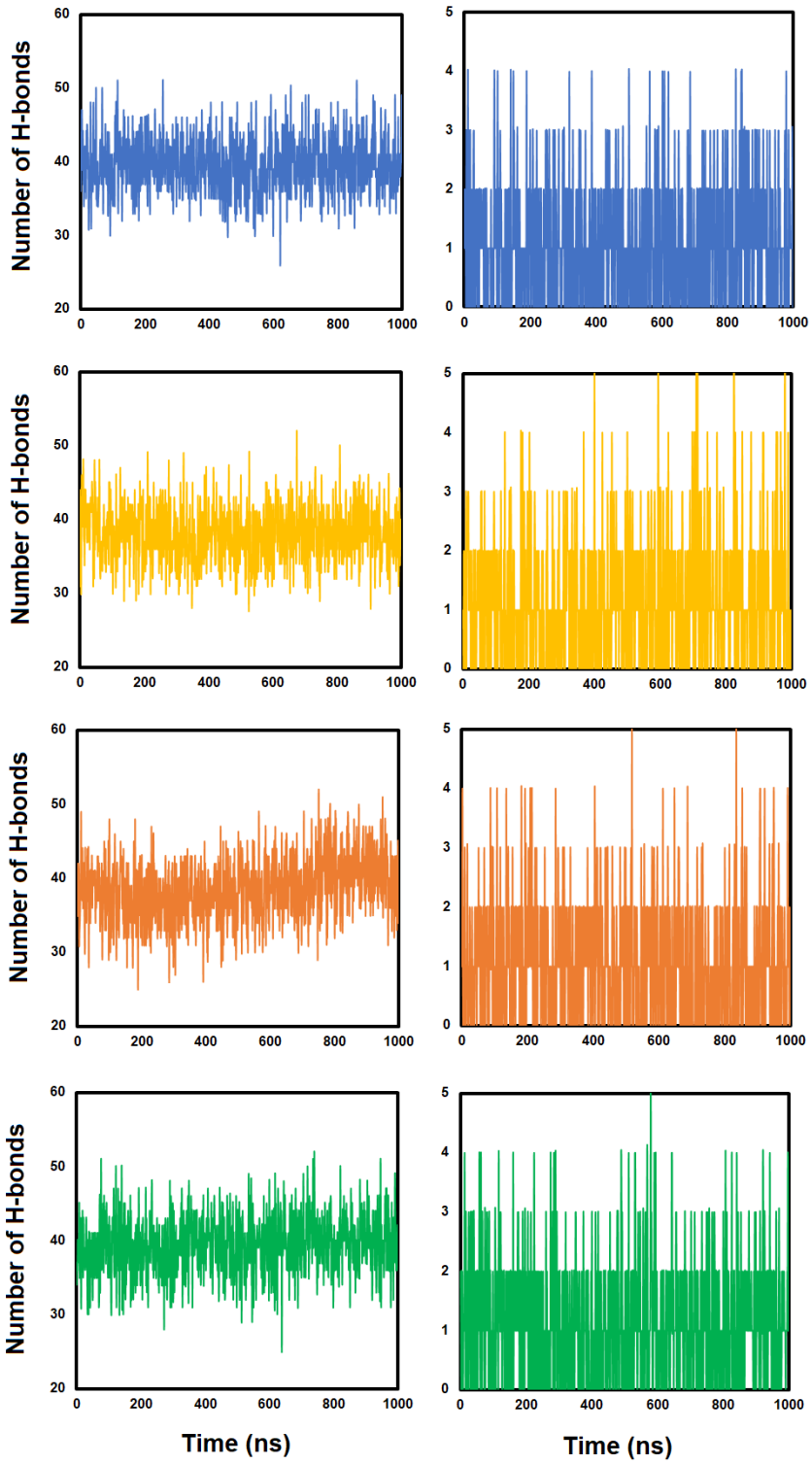

Figure S6. Left panel: Hydrogen bonds between dodecamers and solvent, and right panel: PHMB intra-molecular hydrogen bonds in four dodecamer-solvent system. Each row (shown in different colors) represents one of the dodecamer chains.

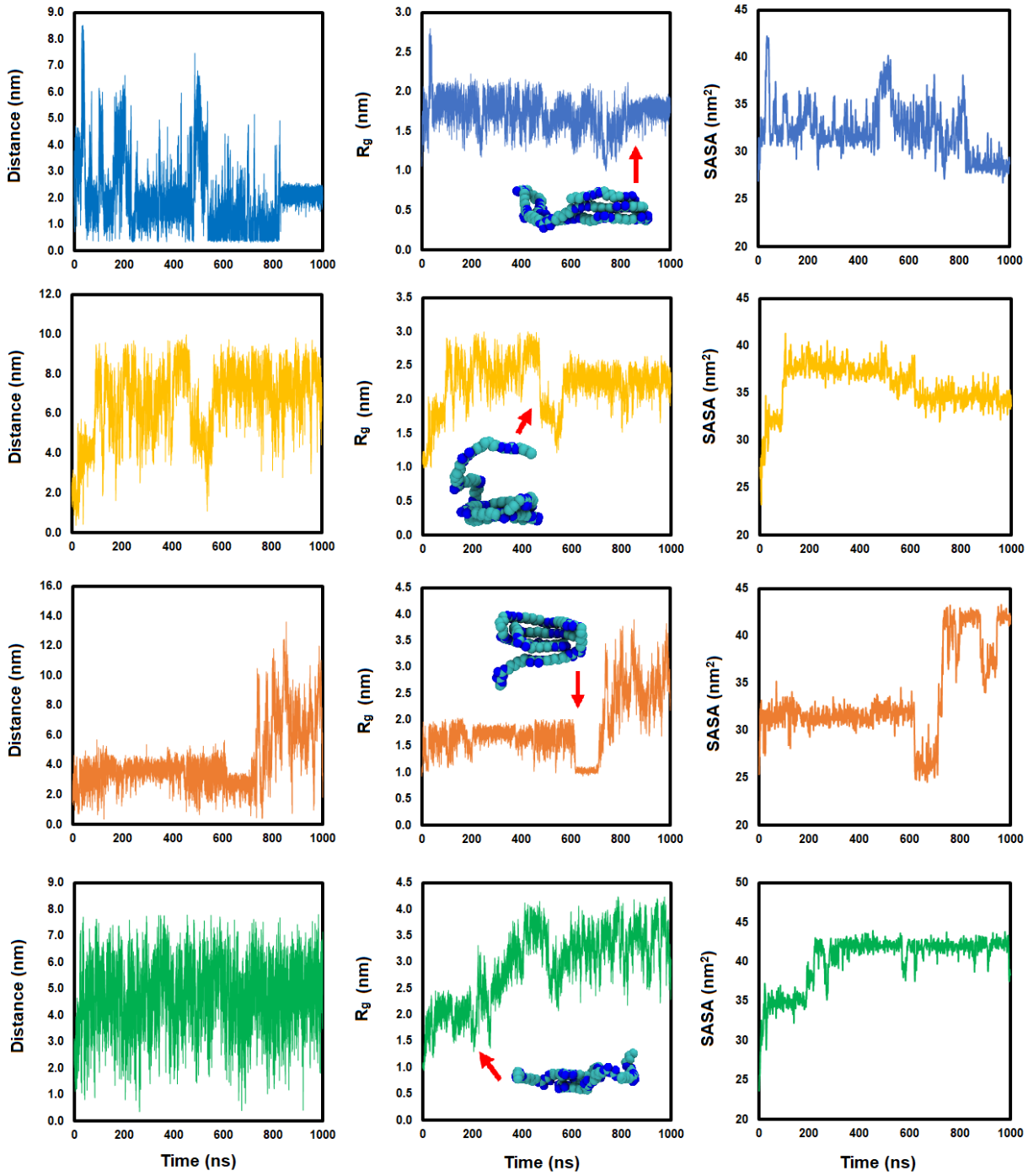

Figure S7. Left panel: End-to-end distance, middle panel: radius of gyration and right panel: solvent accessible surface area (SASA) of each individual PHMB dodecamer molecule in the four dodecamer-solvent system. Each row (shown in different colors) represents one of the dodecamer chains. SASA was calculated based on the Double Cubic Lattice Method (DCLM)[1] as implemented in the GROMACS package using 1 ns intervals.

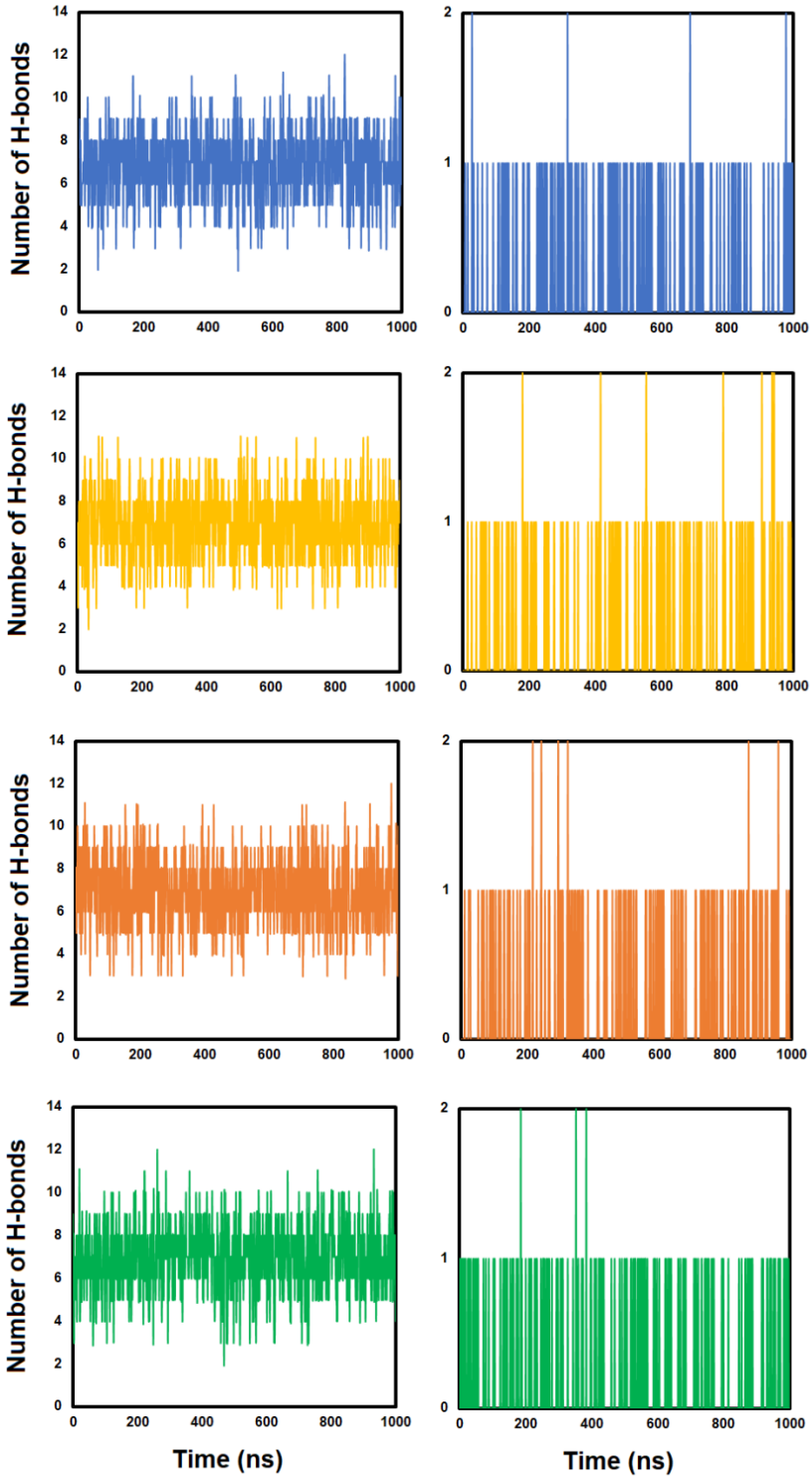

Figure S8. Left panel: Hydrogen bonds between PHMB dimers and solvent. Right panel: PHMB intra-molecular hydrogen bonds in four PHMB dimers-solvent system. Each row (shown in different colors) represents one of the dimer chains.

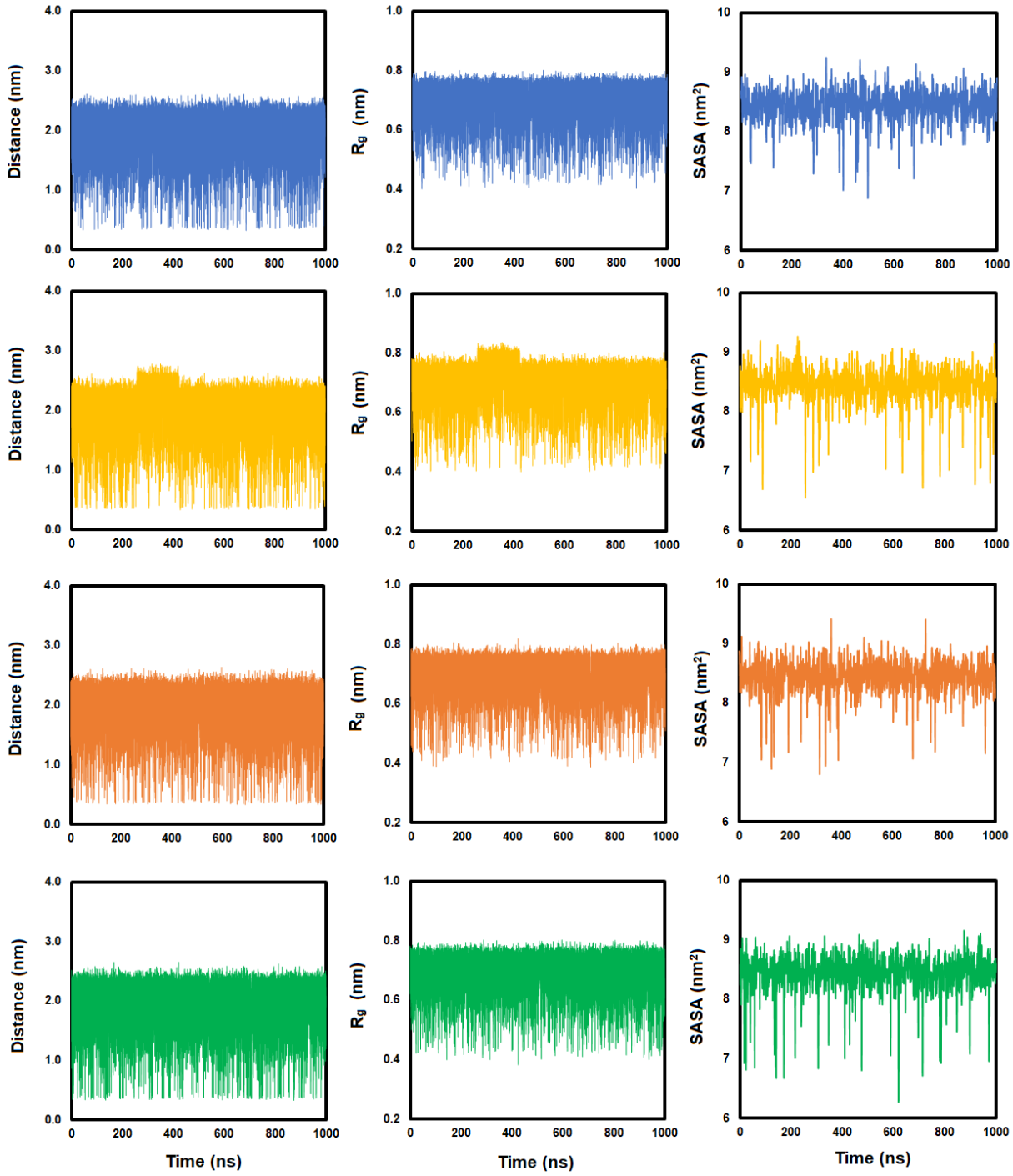

Figure S9. Left panel: End-to-end distance, middle panel: radius of gyration, and right panel: solvent accessible surface area (SASA) of each individual PHMB dimer in the four dimer-solvent system. Each row (shown in different colors) represents one of the dimer chains. SASA was calculated based on the Double Cubic Lattice Method (DCLM)[1] as implemented in the GROMACS package using 1 ns intervals.

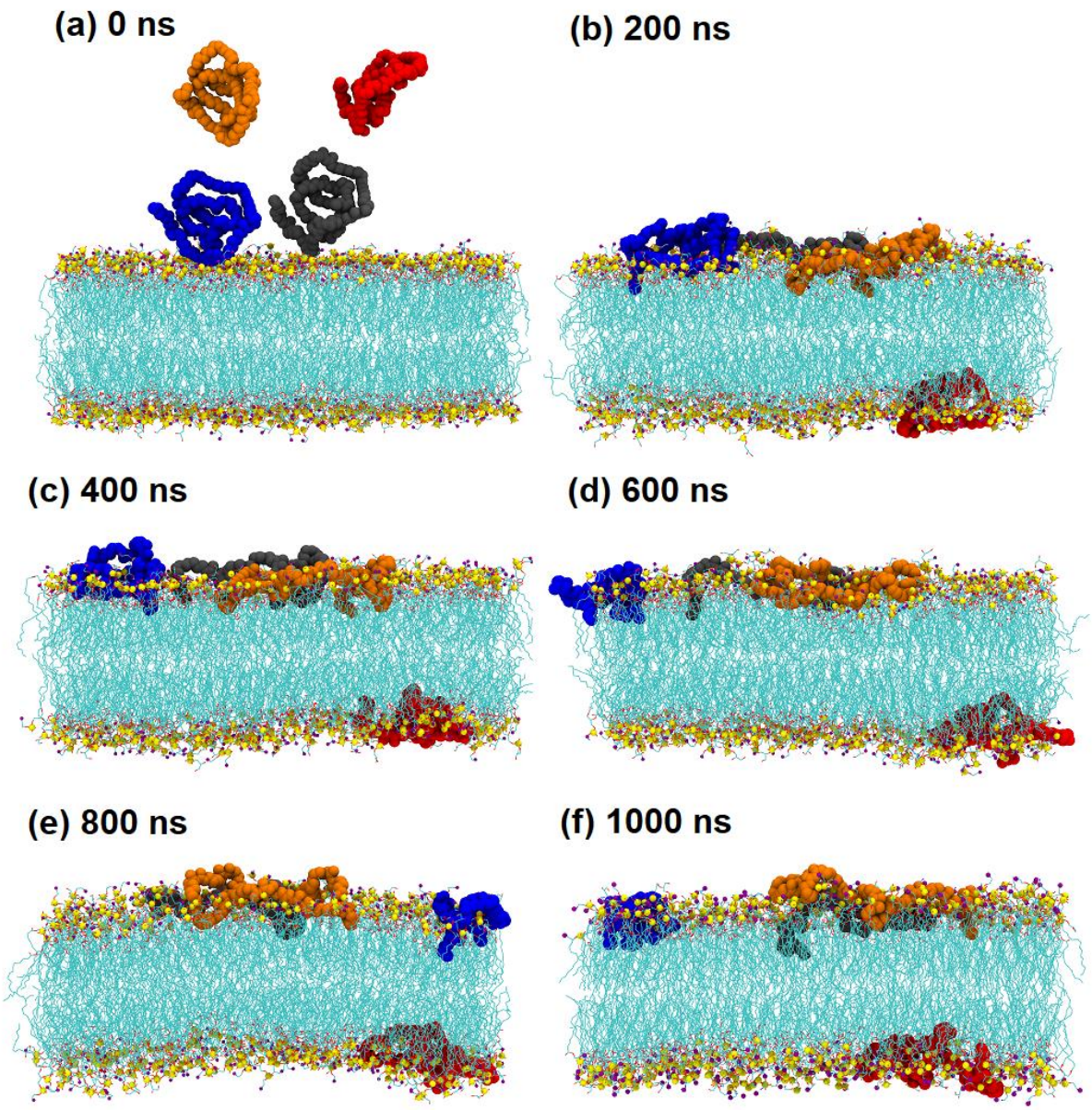

Figure S10. Snapshots of the four PHMB polymers at different times. Nitrogen atoms are shown in purple, phosphorus in yellow and oxygen in red. Note that although all the polymers were placed closer to one of the membrane surfaces, one of the polymers (shown in red) still migrated to the other side. This is a stochastic event that occurred through the water phase, that is, there was no translocation through the membrane.

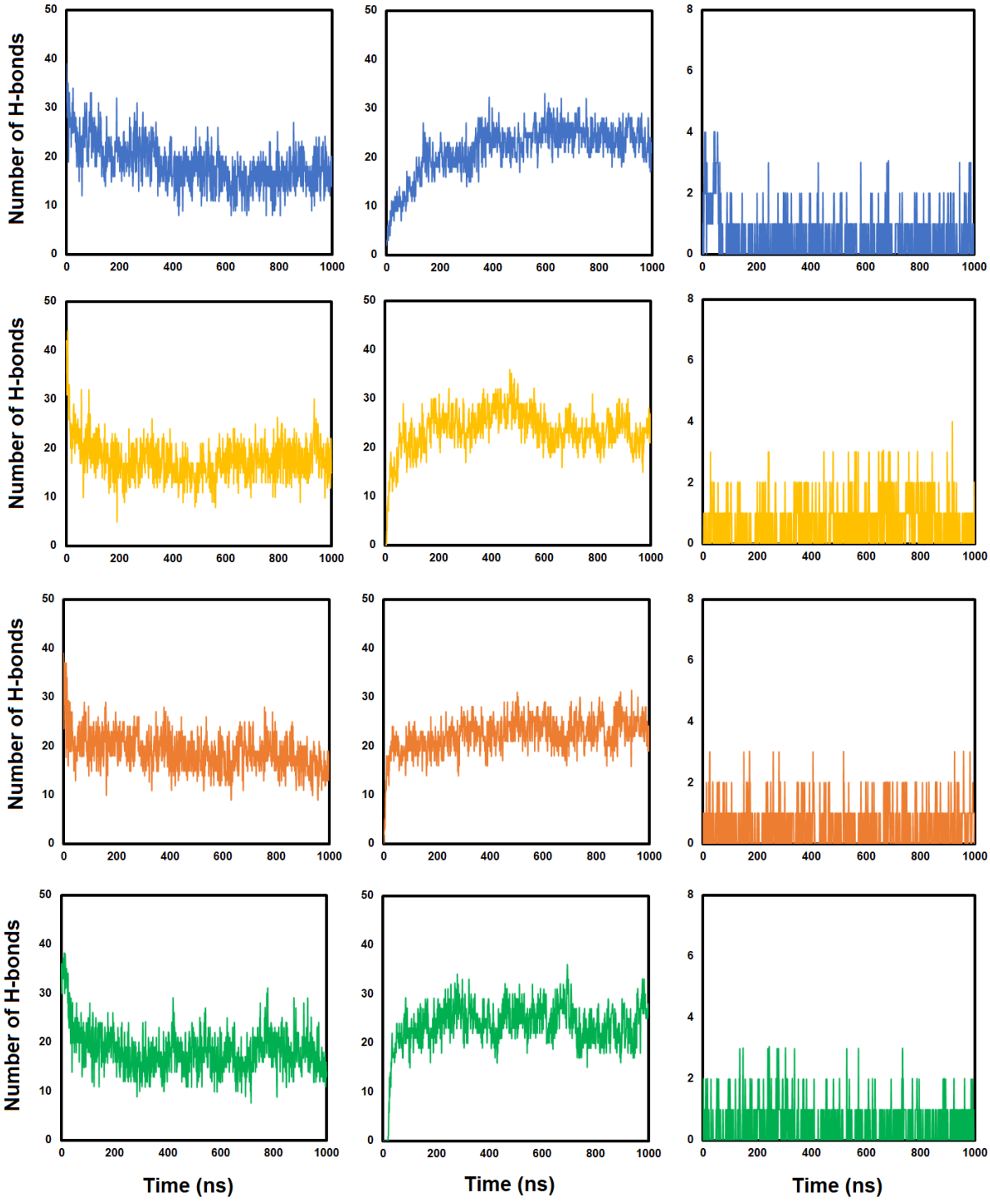

Figure S11. Left panel: Hydrogen bonds between PHMB dodecamers and solvent. Middle panel: PHMB dodecamer-bilayer hydrogen bonds, and right panel: PHMB intra-molecular hydrogen bonds in four PHMB dodecamer-solvent system. Each row (shown in different colors) represents one of the dodecamer chains.

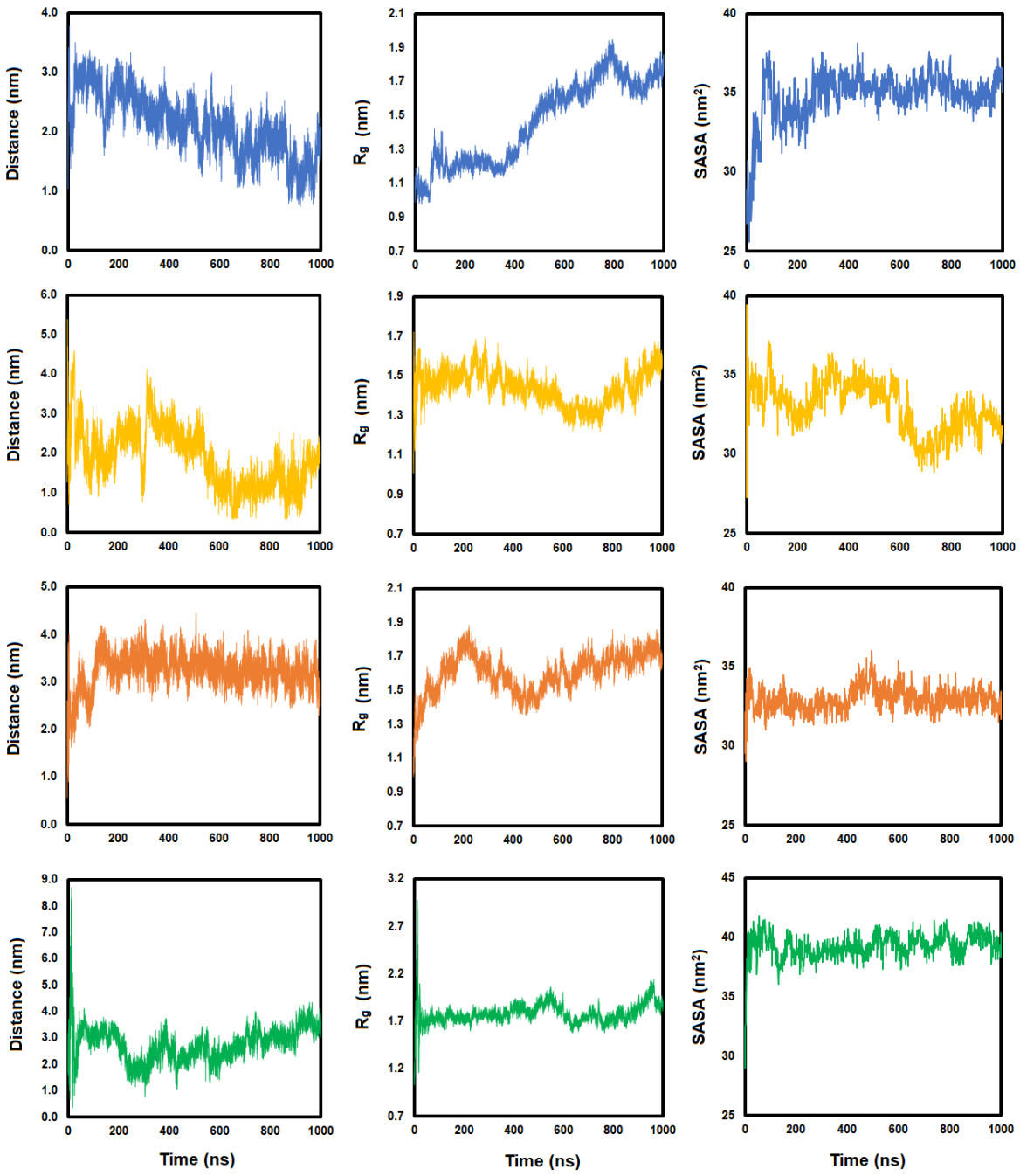

Figure S12. Left panel: End-to-end distance, middle panel: radius of gyration and right panel: solvent accessible surface area (SASA) of each individual PHMB dodecamer in the four dodecamer-bilayer system. Each row represents one of the dodecamer chains. SASA was calculated based on the Double Cubic Lattice Method (DCLM)[1] as implemented in the GROMACS package using 1 ns intervals.

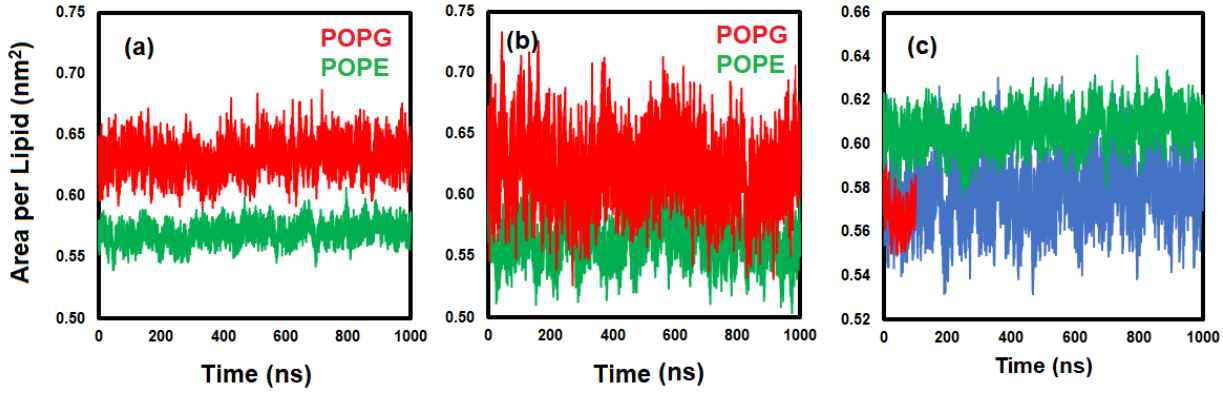

Figure S13. Area per lipid (red: POPG, green: POPE) over time for (a) four PHMB polymers-bilayer and (b) four PHMB dimers-bilayer systems. (c) Overall area per lipid for (red) pure bilayer, (green) four PHMB dodecamers-bilayer, and (blue) four PHMB dimers-bilayer.

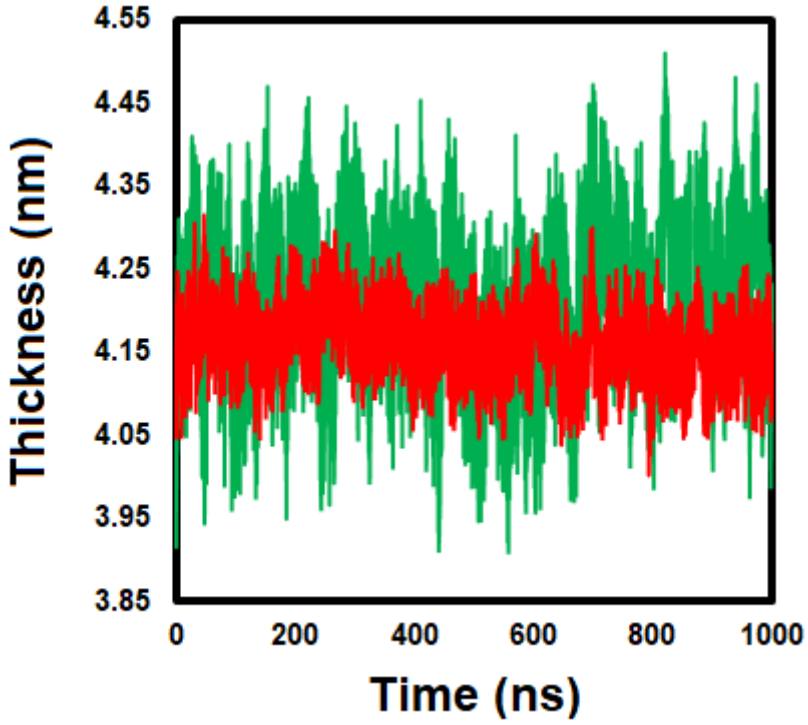

Figure S14. Bilayer thickness over time for (red) four PHMB dodecamers-bilayer and (green) four PHMB dimers-bilayer systems based on the average position of the PO_4 groups (see Figure S2 for the structure).

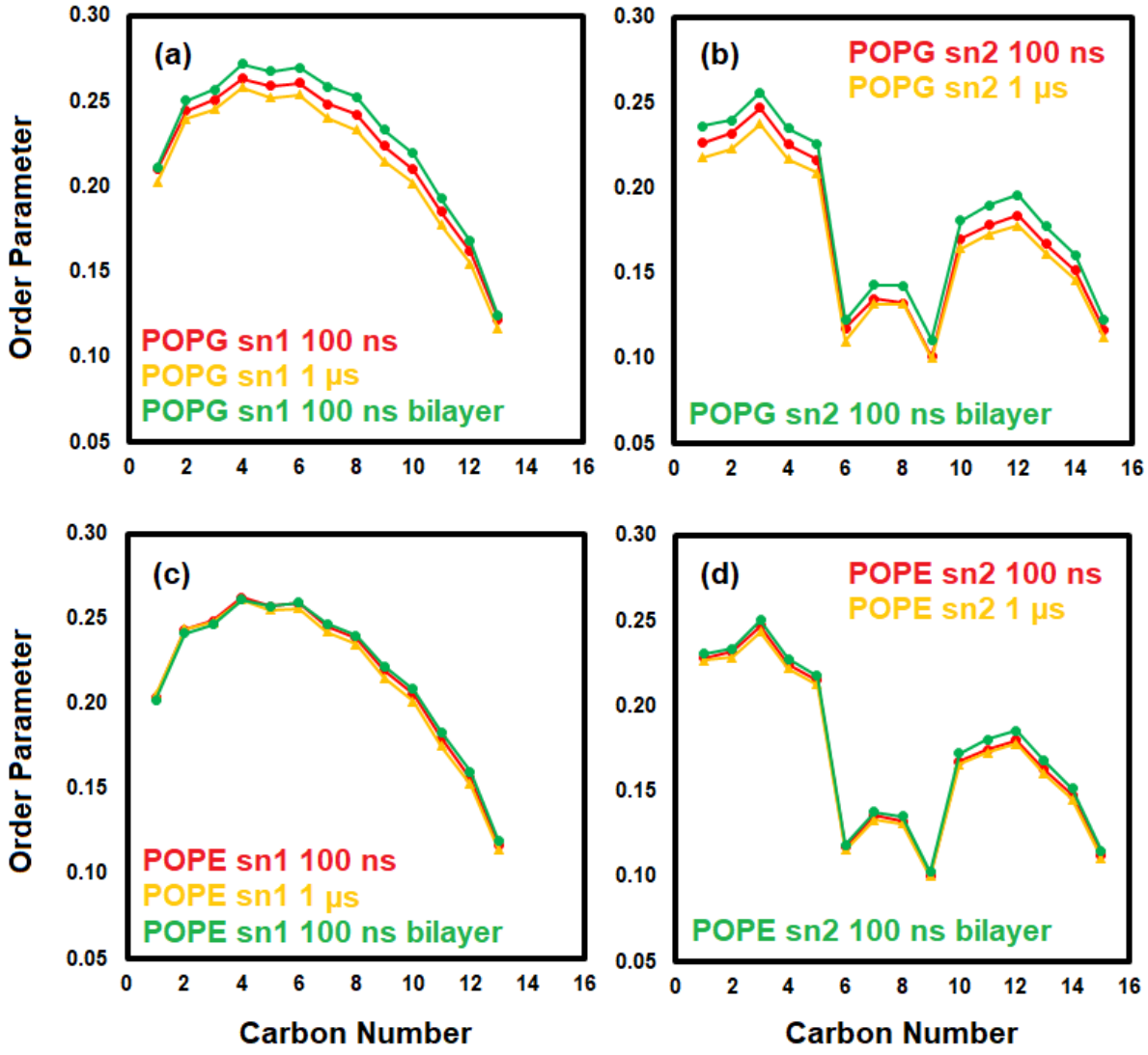

Figure S15. Order parameter of (a) POPG sn-1, (b) POPG sn-2, (c) POPE sn-1, and (d) POPE sn-2 chains (0 – 100 ns) (green for the bilayer in the absence of the polymer) and (900 – 1000 ns) for the 4 PHMB dodecamers-bilayer system.

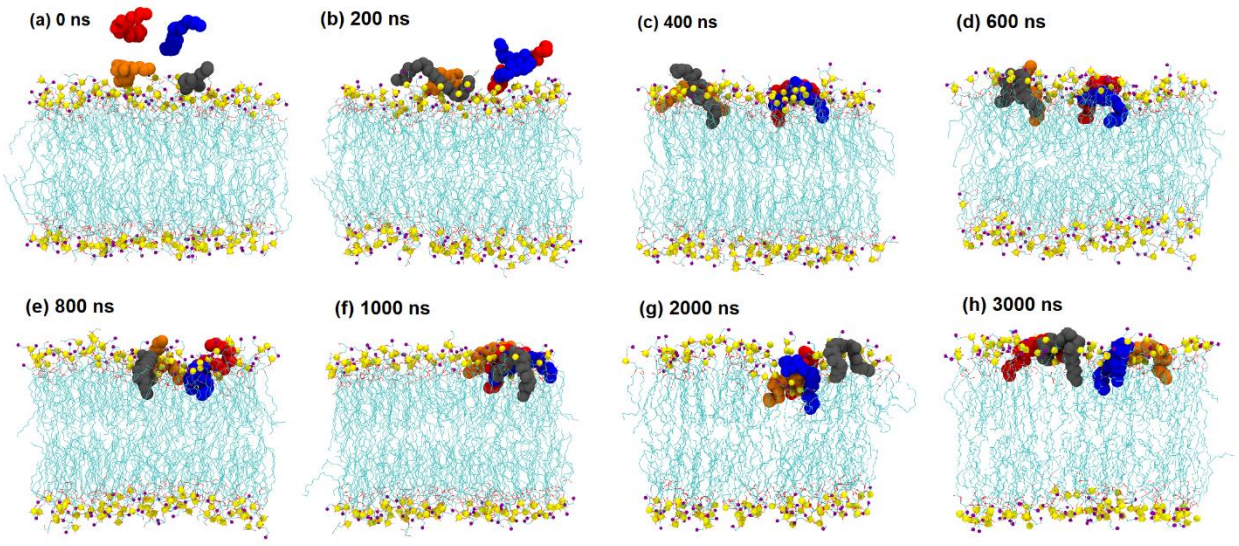

Figure S16. Snapshots of the four at different times. Nitrogen is shown in purple, phosphorus in yellow, and oxygen in red.

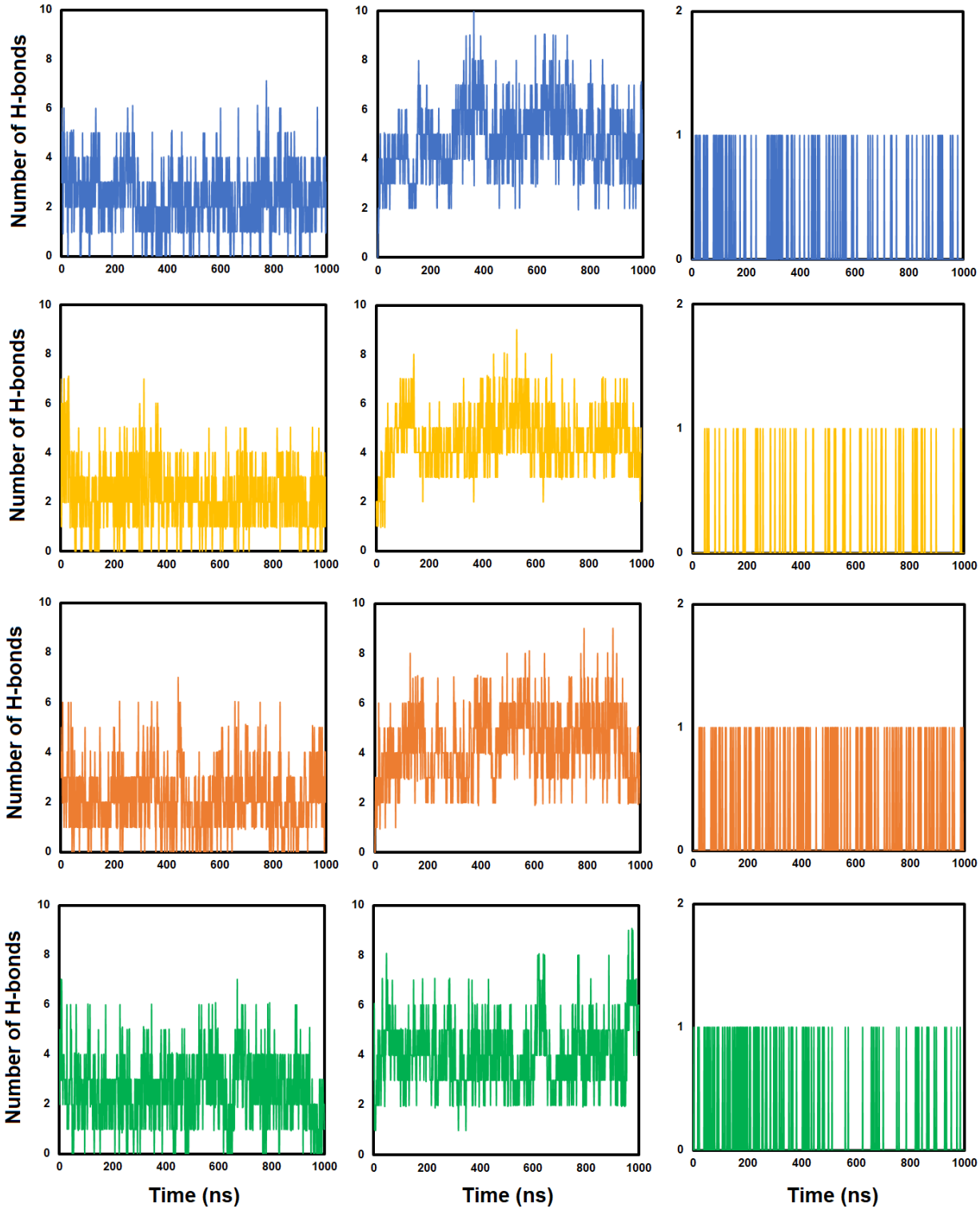

Figure S17. Left panel: Hydrogen bonds between dimers and solvent, middle panel: PHMB dimer-bilayer hydrogen bonds, and right panel: PHMB intra-molecular hydrogen bonds in four dimer-solvent system. Each row (shown in different colors) represents one of the dimer chains.

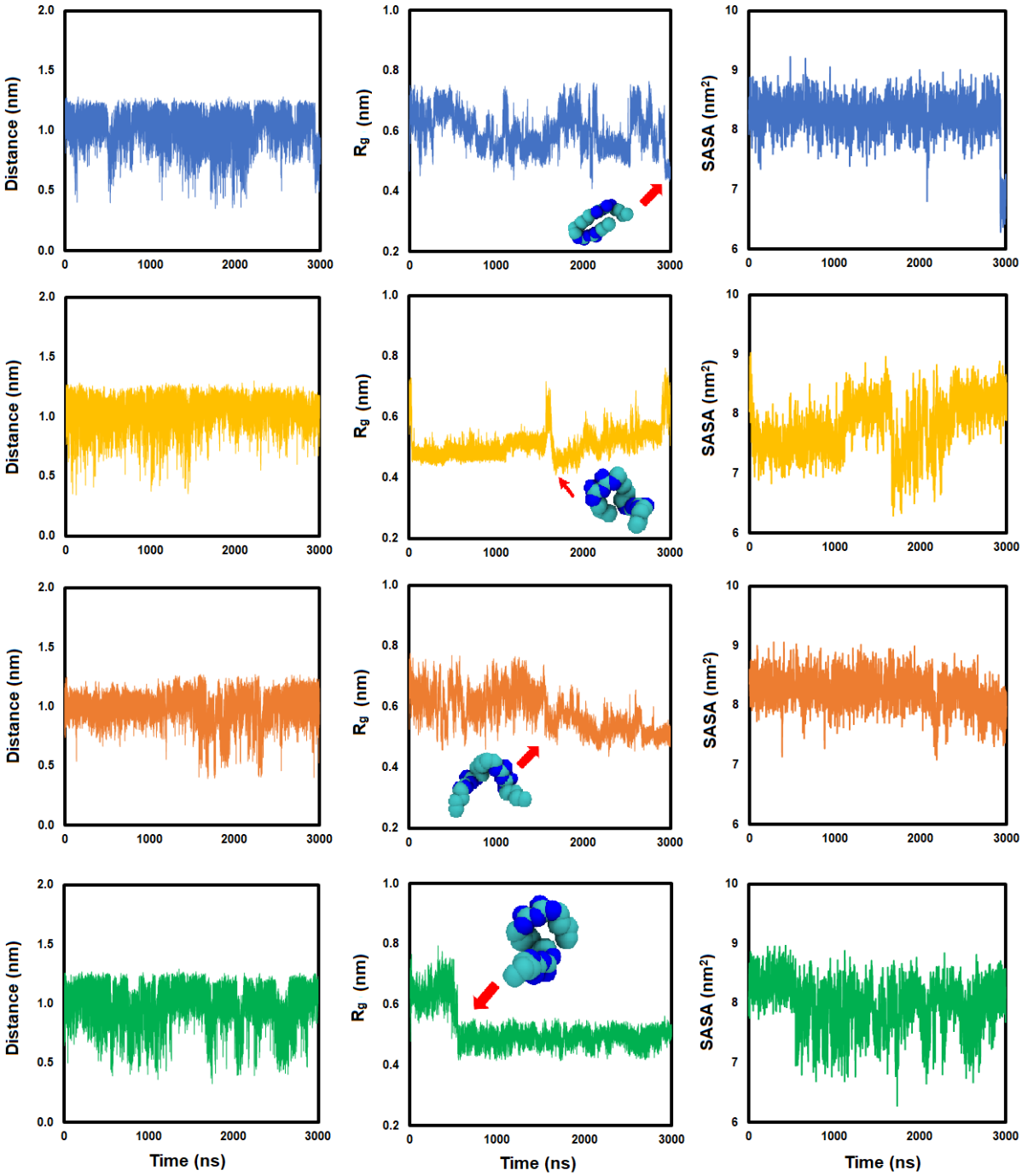

Figure S18. Left panel: End-to-end distance, middle panel: radius of gyration, and right panel: solvent accessible surface area (SASA) of each individual PHMB dimer in the four dimer-bilayer system. Each row (shown in different colors) represents one of the dimer chains. SASA was calculated based on the Double Cubic Lattice Method (DCLM) as implemented in the GROMACS package using 1 ns intervals.

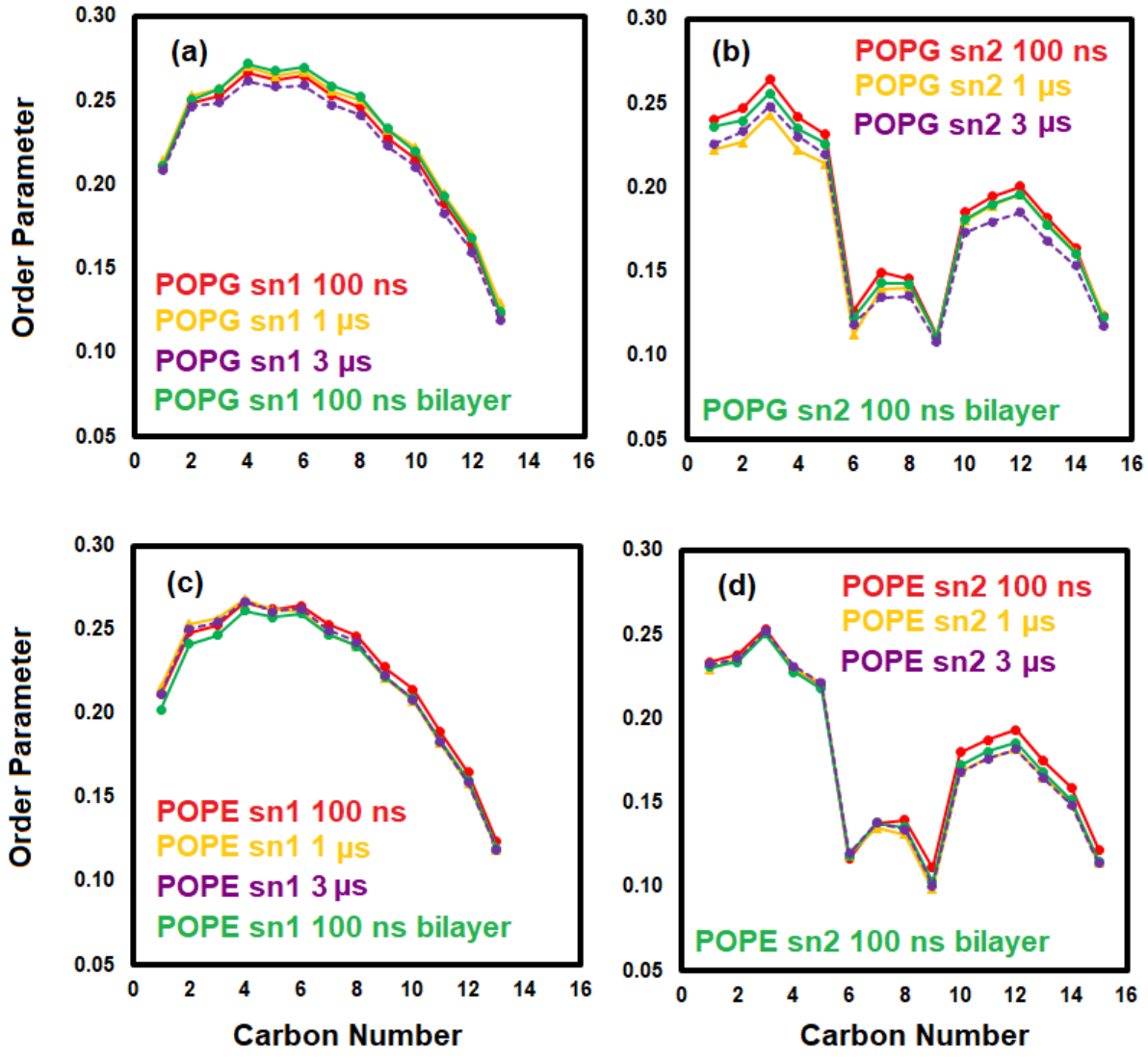

Figure S19. Order parameter of (a) POPG sn-1, (b) POPG sn-2, (c) POPE sn-1, and (d) POPE sn-2 chains (0 – 100 ns) (green for bilayer in the absence of the PHMB chains) and (900 – 1000 ns) (orange) and (2900 – 3000 ns dashed purple) for the four PHMB dimers-bilayer system.

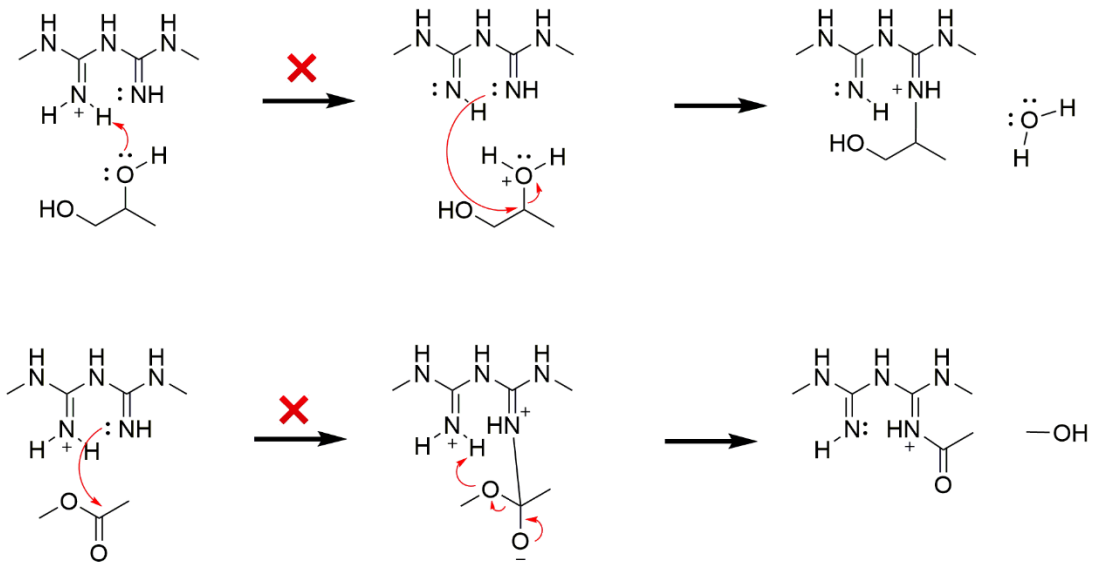

Figure S20. Potential reactions between biguanide and phospholipids that could not be characterized. Transition structures for these reactions were not found due to the unlikeliness of the reactions.

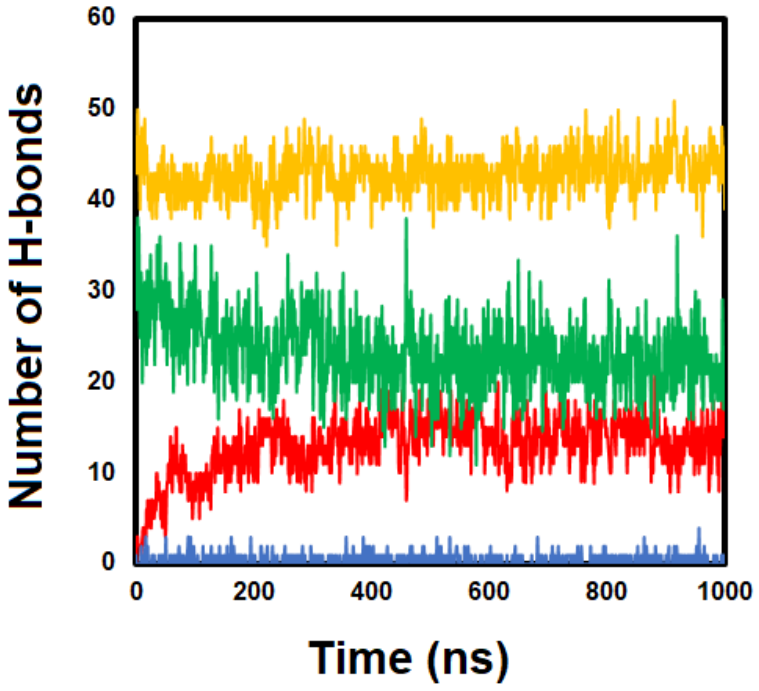

Figure S21. Average number of base pair hydrogen bonds in DNA (orange), PHMB–DNA (red), PHMB–solvent (green), and PHMB-PHMB (blue) hydrogen bonds.

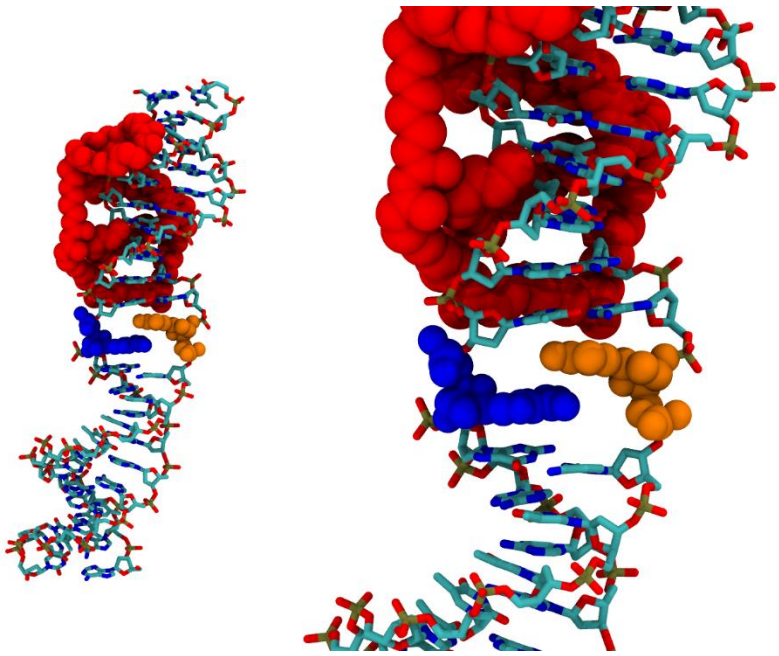

Figure S22. Base-pair dislocation in the DNA oligomer and its relative position to PHMB polymer.

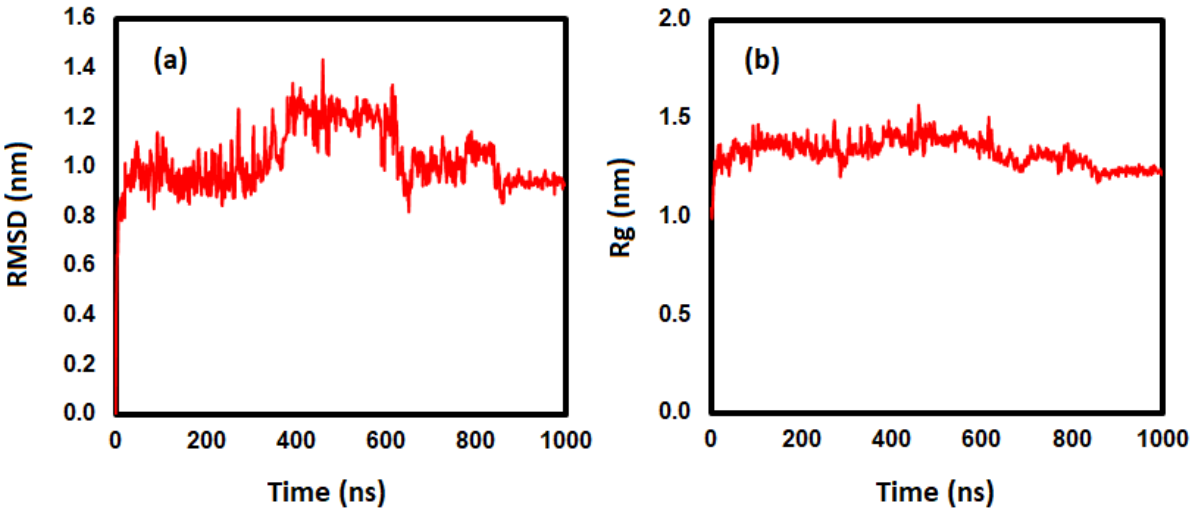

Figure S23. (a) RMSD and (b) radius of gyration of PHMB in the polymer–DNA system.

References

1. Eisenhaber, F.; Lijnzaad, P.; Argos, P.; Sander, C.; Scharf, M., The double cubic lattice method: Efficient approaches to numerical integration of surface area and volume and to dot surface contouring of molecular assemblies. *J. Comput. Chem.* **1995,** *16*, 273-284.

2. Murzyn, K.; Róg, T.; Pasenkiewicz-Gierula, M., Phosphatidylethanolamine-Phosphatidylglycerol Bilayer as a Model of the Inner Bacterial Membrane. Biophys. J. **2005**, *88*, 1091-1103.