SUPPLEMENTARY MATERIAL

CHARMM-GUI Martini Maker for Modeling and Simulation of Complex Bacterial Membranes with Lipopolysaccharides

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Table S1. The composition of the bilayer systems.

Systems	Protein	Lipid types (# Lipids)		Ion types (# Ions)			# Water	System size (xyz; Å)	Simulation time (μs)
		Outer	Inner	Na^+	Cl^-	Ca^{2+}			
OM-only	–	56 RaLPS	144 POPE 8 POPG 8 CDL2	395	35	112	4390	100,100,100	9
OM-OmpA	OmpA	56 RaLPS	144 POPE 8 POPG 8 CDL2	390	29	112	4585	100,100,100	5
OM-OmpF	OmpF	102 Ra LPS	270 POPE 15 POPG 15 CDL2	753	60	204	9455	150,150,100	5.5

Table S2. The composition of the nanodisc systems.

Systems	Protein	Lipid types (# Lipids)	Ion types (# Ions)			# Water	System size (xyz; Å)	Simulation time (μs)
		Outer / Innter	Na^+	Cl^-	Ca^{2+}			
Re LPS + MSP	MSP1D1	37 / 37 Re LPS	227	227	227	17321	140, 140, 140	1
	MSP1D1	37 / 37 Re LPS	681	227	0	17321	140, 140, 140	
Ra LPS + MSP	MSP1D1	33 / 33 Ra LPS	297	297	335	22466	150, 150, 150	
	MSP1D1	33 / 33 Ra LPS	967	297	0	22466	150, 150, 150	

Table S3. The composition of the vesicle systems.

Systems	Protein	Lipid types (# Lipids)		Ion types (# Ions)			# Water	System size (xyz; Å)	Simulation time (μs)
		Outer	Inner	Na^+	Cl^-	Ca^{2+}			
150-Å Re OMV-only	–	2076 Re LPS	3006 POPE 167 POPG 167 CDL2	11689	7036	4152	627652	444, 444, 444	9
150-Å Re OMV-OmpF	OmpF	2076 Re LPS	3006 POPE 167 POPG 167 CDL2	5436	9051	8304	623277	444, 444, 444	5
150-Å Ra OMV-only	–	1844 Ra LPS	3006 POPE 167 POPG 167 CDL2	21382	9817	3688	831366	487, 487, 487	5.5
100-Å Ra OMV-OmpF	OmpF	919 Ra LPS	1273 POPE 70 POPG 70 CDL2	2492	7760	7352	421440	387, 387, 387	10

Table S4. The composition of micelle and random systems using standard (W) or polarizable (PW) water models.

Systems	Protein	# Lipids	Ion types (# Ions)			# Water	System size (Å)	Simulation time (μs)
			Na^+	Cl^-	Ca^{2+}			
Micelle + Ra LPS	–	5	198	168	10	15305 W	124	10
	–	10	304	244	20	22378 W	141	10
	–	15	357	267	30	24477 W	145	10
	–	20	395	275	40	25157 W	147	10
	–	20	475	275	–	25117 W	147	10
	–	20	421	301	40	27383 PW	149	10
	OmpF	40	835	583	80	54062 W	191	10
Micelle + Re LPS	–	5	84	74	10	6692 W	95	10
	–	10	124	104	20	9512 W	106	10
	–	15	135	105	30	9573 W	107	10
	–	20	162	122	40	11156 W	113	10
Random + Ra LPS	–	5	278	248	10	22309 W	140	10
	–	10	308	248	20	21951 W	140	10
	–	15	338	248	30	21500 W	140	10
	–	20	368	248	40	21106 W	140	10
	–	20	448	248	–	21066 W	140	10
	–	20	368	248	40	20993 PW	140	10
	OmpF	40	773	521	80	44135 W	180	10
Random + Re LPS	–	5	131	121	10	10753 W	110	10
	–	10	141	121	20	10471 W	110	10
	–	15	151	121	30	10248 W	110	10
	–	20	161	121	40	9869 W	110	10

Table S5. The average and standard error of mean (SEM) of the APL and bilayer thickness over the last microsecond of the 5-μs *Bilayer Builder* production runs. Even though the SEM is pretty low for all systems and lipids, the insertion of a protein causes a systematic error in the Voronoi procedure at the protein lipid interphase.

OM-only	**Parameter**	**RAMP (Å^2)**	**POPE (Å^2)**	**POPG (Å^2)**	**CDL2 (Å^2)**	**Bilayer Thickness (Å)**
	Mean	173.33	58.27	60.69	103.87	37.61
	SEM	0.22	0.31	0.11	0.53	0.15
OM-OmpA	**Parameter**	**RAMP (Å^2)**	**POPE (Å^2)**	**POPG (Å^2)**	**CDL2 (Å^2)**	**Bilayer Thickness (Å)**
	Mean	170.61	57.72	59.95	103.18	37.25
	SEM	1.31	0.14	0.72	0.44	0.20
OM-OmpF	**Parameter**	**RAMP (Å^2)**	**POPE (Å^2)**	**POPG (Å^2)**	**CDL2 (Å^2)**	**Bilayer Thickness (Å)**
	Mean	176.93	59.50	62.59	104.82	37.18
	SEM	0.17	0.34	0.64	2.0	0.12

Table S6. The average and the standard error of mean (SEM) of OMV area per lipid over the last 500 ns of total simulation time.

150-Å Re OMV-only	**Parameter**	**REMP (Å^2)**	**POPE (Å^2)**	**POPG (Å^2)**	**CDL2 (Å^2)**
	Mean	179.2	61.7	63.4	66.9
	SEM	0.014	0.01	0.012	0.01
150-Å Re OMV-OmpF	**Parameter**	**REMP (Å^2)**	**POPE (Å^2)**	**POPG (Å^2)**	**CDL2 (Å^2)**
	Mean	179.4	62.1	64.3	65.6
	SEM	0.015	0.002	0.021	0.011
150-Å Ra OMV-only	**Parameter**	**RAMP (Å^2)**	**POPE (Å^2)**	**POPG (Å^2)**	**CDL2 (Å^2)**
	Mean	200.5	60.7	62.3	68.7
	SEM	0.018	0.001	0.012	0.010
100-Å Ra OMV-OmpF	**Parameter**	**RAMP (Å^2)**	**POPE (Å^2)**	**POPG (Å^2)**	**CDL2 (Å^2)**
	Mean	200.8	61.1	59.7	66.1
	SEM	0.029	0.004	0.017	0.016

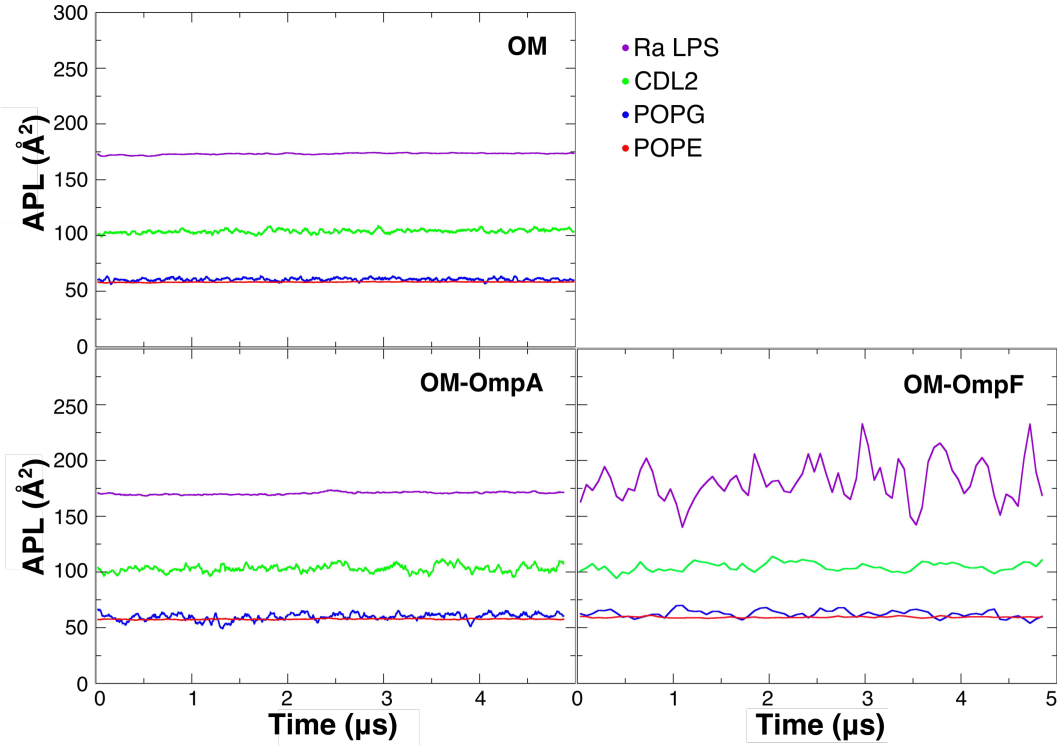

Figure S1. Time-series of area per lipid (APL) of the OM systems. A larger deviation is observed for OM-OmpF due to instability in the analysis program with regard to Voronoi tessellation on frames with multiple beads on the similar x,y coordinates (z is dumped). A skip of 10 was used to reduce the chance of hitting such a frame. Even in this case the average could be defined with a good certainty.

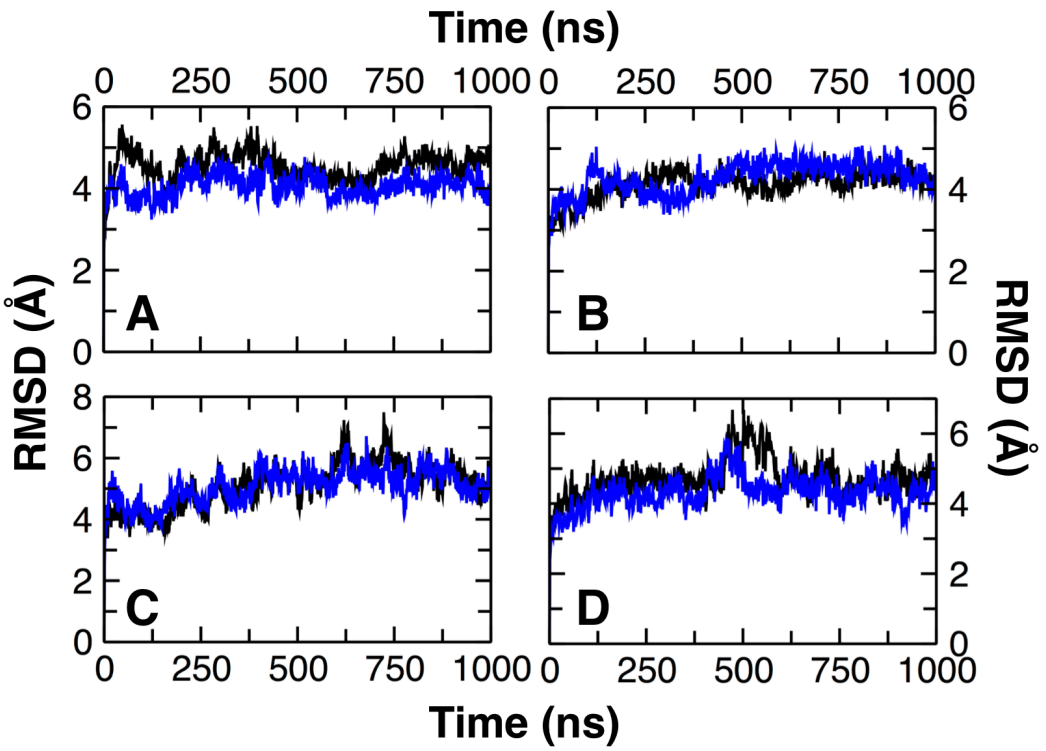

Figure S2. Time-series of the root-mean-square deviation (RMSD) of MSP1D1 proteins in the Ra LPS nanodiscs neutralized with (A) Na^+ or (B) Ca^{2+} ions and Re LPS nanodiscs neutralized with (C) Na^+ or (D) Ca^{2+} ions.

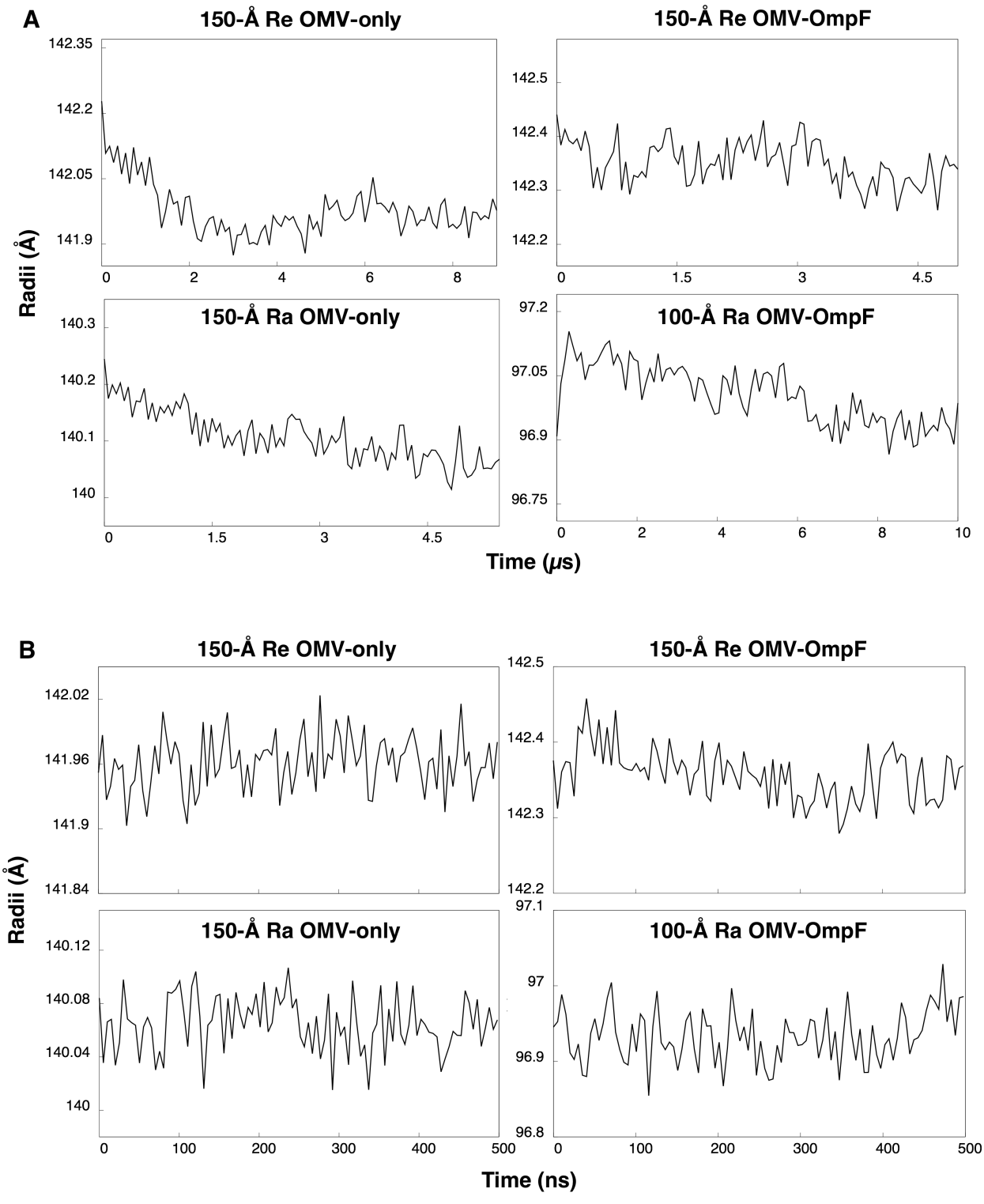

Figure S3. Time-series of the OMV radii calculated as the radial distance between the OMV center of and the center of mass of the encapsulating membranes for (A) the total simulation time and (B) the last 500 ns simulation time.

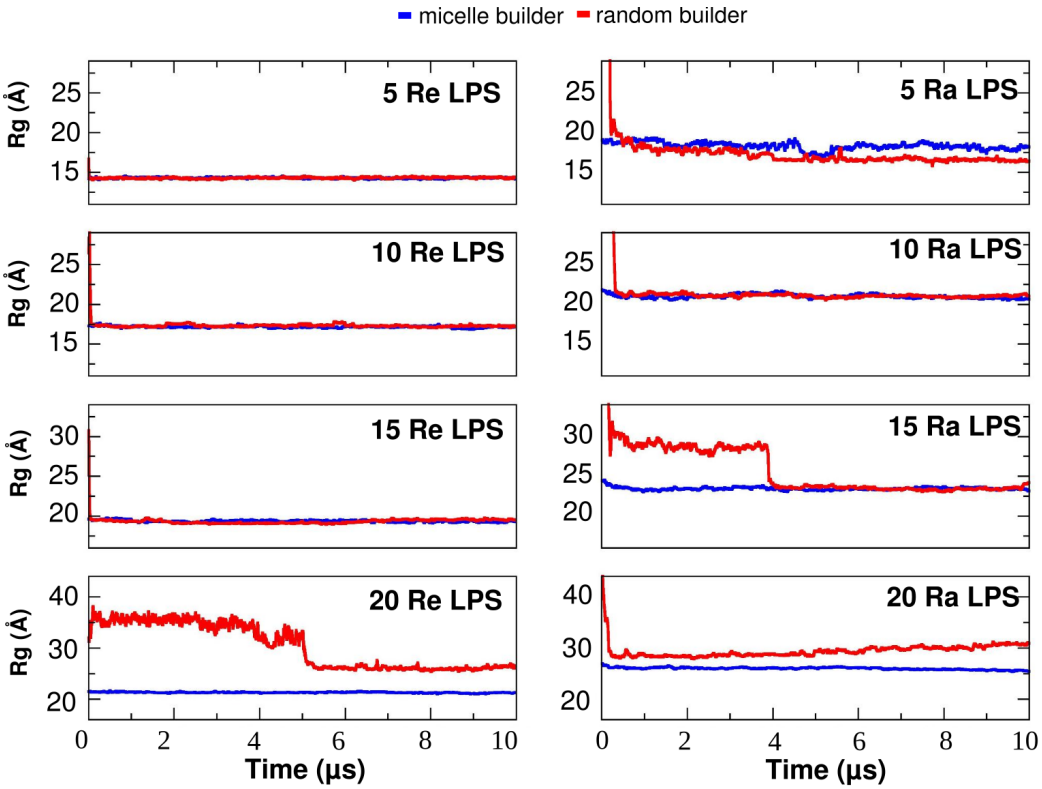

Figure S4. Time-series of the radius of gyration of the LPS molecules in various micelle and random systems.

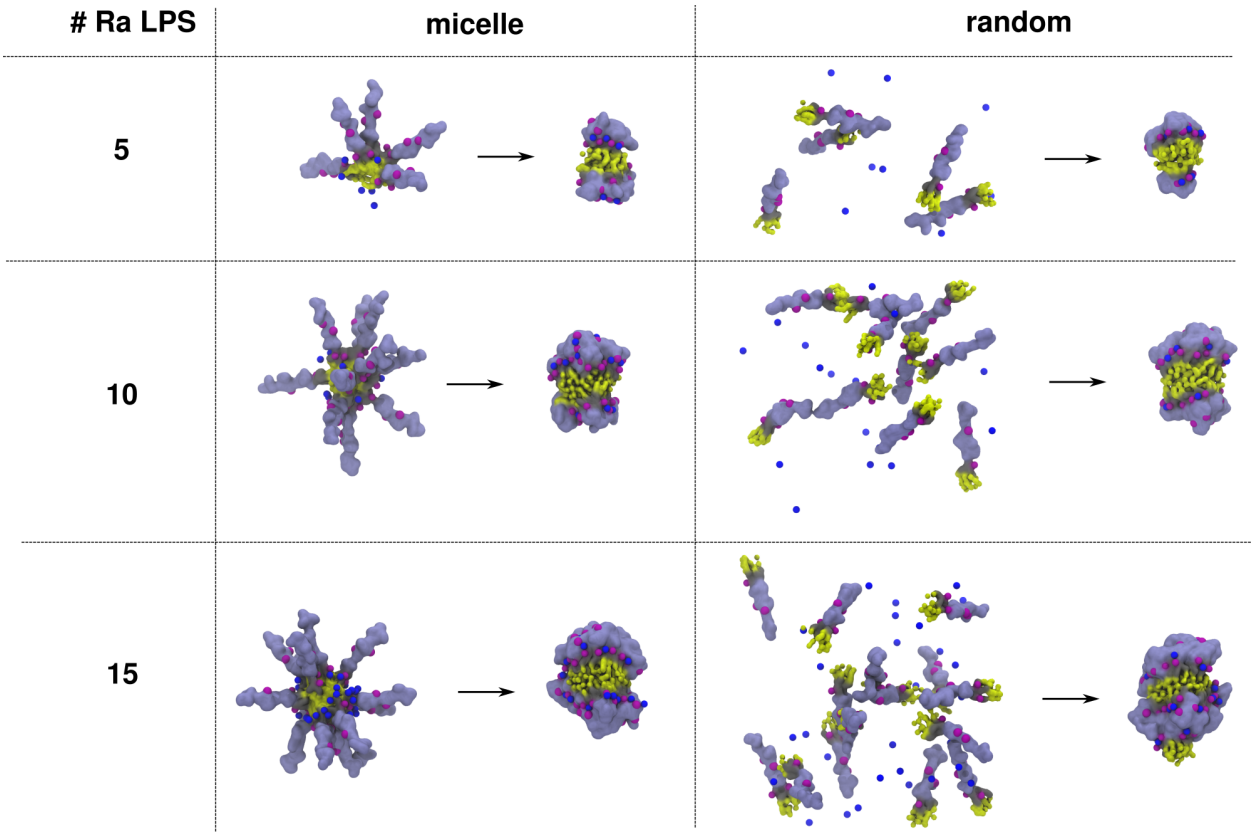

Figure S5. Initial structure and the last snapshot of MD simulations performed with Ra LPS micelle and random systems (5, 10, and 15 LPS molecules).

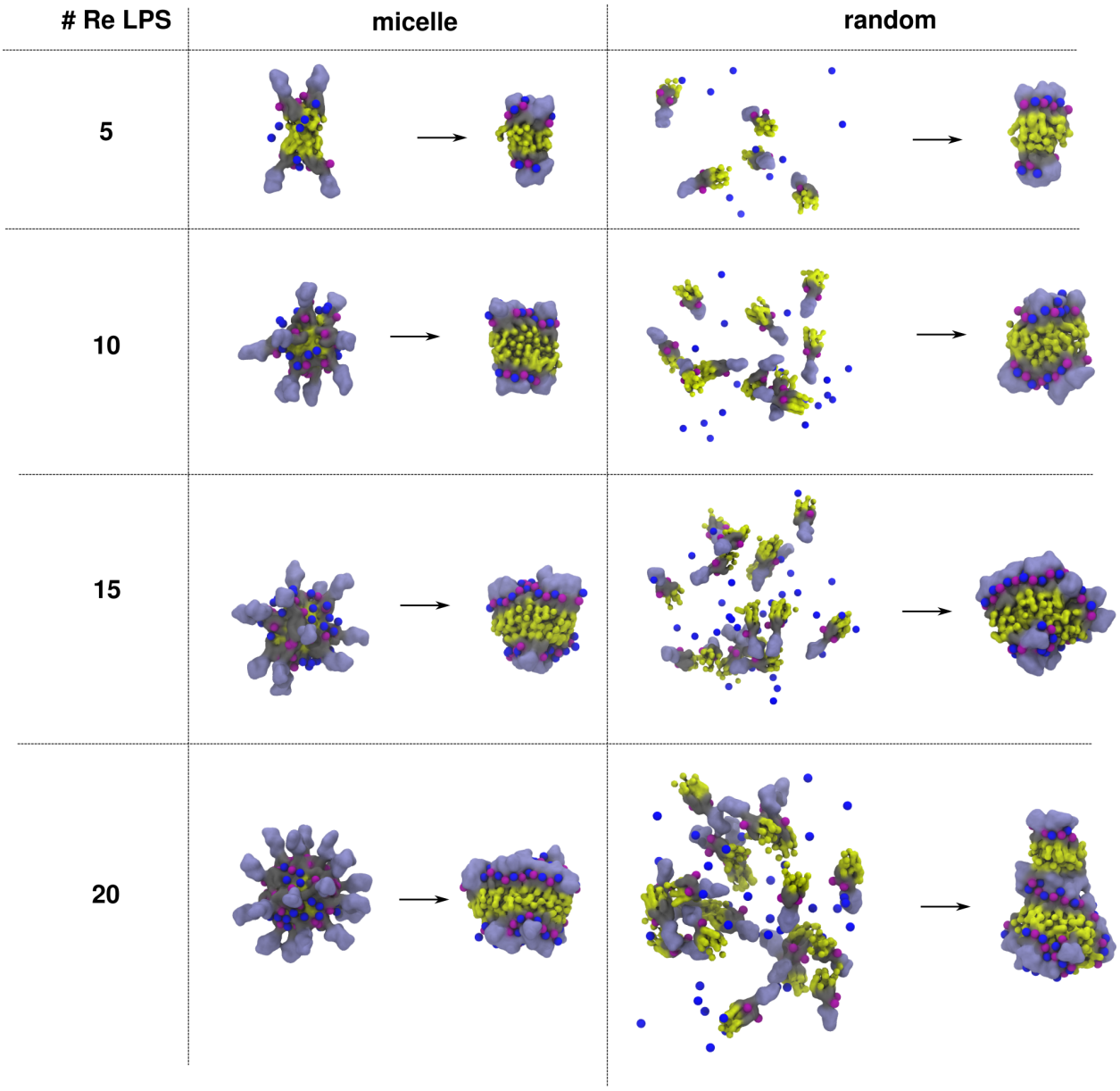

Figure S6. Initial structure and the last snapshot of MD simulations performed with Re LPS micelle and random systems (5, 10, 15, and 20 LPS molecules).