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

Supplementary information for

Fluorescence of nitrobenzoxadiazole (NBD) labeled lipids in model membranes is connected not to lipid mobility, but to probe location

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1. *Supplementary tables*

Table S1. Atomic charges used for NBD-PS. Identically to the host lipid, acyl-chain CH_n united atoms (unnumbered in Fig. 1C) were modeled with zero charge.

Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
N1	-0.28	O8	-0.56	C15	0.16	C22	0.31	C44	0.92
C2	0.02	N9	-0.34	C16	0.24	C23	0.32	O45	-0.61
C3	-0.08	C10	0.33	O17	-0.51	O24	-0.68	C65	0.70
C4	-0.02	C11	0.45	P18	1.21	C25	0.82	O66	-0.73
C5	-0.22	O12	-0.04	O19	-0.78	O26	-0.68	O67	-0.73
N6	0.81	N13	-0.25	O20	-0.78	C42	0.25		
O7	-0.60	H14	0.32	O21	-0.49	O43	-0.48		

Table S2. Atomic charges used for NBD-PSH. Identically to the host lipid, acyl-chain CH_n united atoms (unnumbered in Fig. 1C) were modeled with zero charge.

Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
N1	0.20	O8	-0.48	C15	-0.14	C22	0.31	C46	0.92
C2	-0.09	N9	-0.34	C16	0.36	C23	0.32	O47	-0.61
C3	-0.10	C10	0.43	O17	-0.50	O24	-0.68	C65	0.63
C4	0.10	C11	0.36	P18	1.16	C25	0.82	O66	-0.50
C5	-0.29	O12	0.04	O19	-0.71	O26	-0.68	O67	-0.52
N6	0.80	N13	-0.31	O20	-0.71	C44	0.25	H68	0.30
O7	-0.51	H14	0.07	O21	-0.42	O45	-0.48		

Table S3. Calculated absorption data of NBD-C_2 in different solvents and main orbitals involved in the transition. Experimental data (*Exp*) is taken from references for (NBD-C_3) [1] and (NBD-C_1) [2].

		PCM-PBE0/6-31+G(d)		PCM-PBE0/6-311+G(d,p)		SMD-M06/6-31+G(d)		SMD-M06/6-311+G(d,p)			
Solvent	State	λ (nm)	*f*	λ (nm)	*f*	λ (nm)	*f*	λ (nm)	*f*	*Exp*	Major MO → MO transitions
Cyclohexane	S_1	407	0.37	411	0.36	413	0.35	415	0.34	425[a] 425[b]	HOMO->LUMO (98%) HOMO->L+1 (2%)
	S_3	306	0.22	308	0.22	308	0.21	309	0.21	305[a]	HOMO->L+1 (97%) HOMO->LUMO (2%)
Tetrahydrofuran	S_1	416	0.39	420	0.38	422	0.35	425	0.35	457[a] 454[b]	HOMO->LUMO (97%) HOMO->L+1 (3%)
	S_3	316	0.25	318	0.25	317	0.25	319	0.25	325[a]	HOMO->L+1 (96%) HOMO->LUMO (3%)
Acetone	S_1	417	0.38	421	0.37	424	0.34	427	0.34	461[a] 456[b]	HOMO->LUMO (96%) HOMO->L+1 (3%)
	S_3	318	0.26	320	0.26	320	0.26	322	0.26	332[a]	HOMO->L+1 (96%) HOMO->LUMO (3%)
Dimethylsulphoxide	S_1	420	0.41	424	0.40	425	0.35	429	0.35	478[a] 474[b]	HOMO->LUMO (96%) HOMO->L+1 (3%)
	S_2	320	0.26	323	0.27	321	0.26	323	0.26	343[a]	HOMO->L+1 (96%) HOMO->LUMO (3%)
Ethanol	S_1	417	0.38	421	0.38	425	0.39	428	0.39	464[a] 459[b]	HOMO->LUMO (96%) HOMO->L+1 (4%)
	S_2	319	0.26	321	0.27	326	0.26	328	0.26	331[a]	HOMO->L+1 (95%) HOMO->LUMO (4%)
Water	S_1	417	0.38	421	0.37	425	0.39	429	0.39	482[a] 478[b]	HOMO->LUMO (95%) HOMO->L+1 (4%)
	S_3	319	0.26	321	0.27	328	0.26	330	0.26	348[a]	HOMO->L+1 (95%) HOMO->LUMO (4%)

f: oscillator strength

(1) Fery-Forgues, S.; Fayet, J.-P.; Lopez, A. *J. Photochem. Photobiol. A Chem.* **1993**, *70*, 229.

(2) Uchiyama, S.; Santa, T.; Imai, K. *J. Chem. Soc. Perkin Trans. 2* **1999**, 2525.

Table S4. Calculated emission data of NBD-C_2 in several solvents. Experimental data is taken from references for (NBD-C_3) [1] and (NBD-C_1) [2].

	PCM-PBE0/6-31+G(d)	PCM-PBE0/6-311+G(d,p)	SMD-M06/6-31+G(d)	SMD-M06/6-311+G(d,p)	Experimental
Solvent	λ (nm)	λ (nm)	λ (nm)	λ (nm)	λ (nm)
Cyclohexane	466	471	473	477	495[a] 499[b]
Tetrahydrofuran	484	489	495	499	525[a] 516[b]
Acetone	489	495	501	505	529[a] 521[b]
Dimethylsulphoxide	491	496	504	508	545[a] 532[b]
Ethanol	490	495	500	505	537[a] 524[b]
Water	491	497	503	508	566[a] 541[b]

(1) Fery-Forgues, S.; Fayet, J.-P.; Lopez, A. *J. Photochem. Photobiol. A Chem.* **1993**, *70*, 229.
(2) Uchiyama, S.; Santa, T.; Imai, K. *J. Chem. Soc. Perkin Trans. 2* **1999**, 2525.

Table S5. Dipole moments (μ /D) of NBD-C_2 in different solvents and in the gas phase, with different solvent models and different basis sets.

Solvent	PCM-B3LYP/6-31+G(d)			SMD-B3LYP/6-31+G(d)			PCM-PBE0/6-31+G(d)			PCM-PBE0/6-311+G(d,p)		
	μ_g	μ_e	$\Delta\mu$	μ_g	μ_e	$\Delta\mu$	μ_g	μ_e	$\Delta\mu$	μ_g	μ_e	$\Delta\mu$
Cyclohexane	12.24	14.38	2.14	12.11	14.33	2.22	12.16	14.30	2.14	12.20	14.34	2.13
Tetrahydrofuran	14.47	16.40	1.93	14.06	16.29	2.23	14.34	16.28	1.94	14.40	16.33	1.94
Acetone	15.16	16.96	1.80	14.76	16.89	2.12	15.02	16.83	1.81	15.08	16.89	1.81
Dimethylsulphoxide	15.40	17.15	1.75	14.87	17.03	2.16	15.25	17.01	1.76	15.32	17.08	1.76
Ethanol	15.22	17.01	1.79	15.91	17.54	1.62	15.08	16.88	1.80	15.14	16.94	1.80
Water	15.47	17.21	1.74	16.34	17.81	1.47	15.33	17.07	1.75	15.39	17.14	1.75
Gas phase	B3LYP/6-31+G(d)						PBE0/6-31+G(d)			PBE0/6-311+G(d,p)		
	10.27	12.33	2.05				10.23	12.28	2.05	10.26	12.30	2.04

(μ_g) dipole moment of the ground state
(μ_e) dipole moment of the excited state
$\Delta\mu = \mu_g - \mu_e$

Table S6. Average molecular area (*a*) and bilayer thickness values for all simulated systems.

System	*a*(phospholipid) (nm^2)	*a*(Chol) (nm^2)	Bilayer thickness (nm)
DOPC	0.684 ± 0.015	———	3.70 ± 0.10
DOPC/NBD-PS	0.673 ± 0.015	———	3.74 ± 0.10
DOPC/NBD-PSH	0.675 ± 0.018	———	3.74 ± 0.19
DOPC/Chol	0.602 ± 0.015	0.288 ± 0.008	4.08 ± 0.10
DOPC/Chol/NBD-PS	0.583 ± 0.015	0.285 ± 0.007	4.17 ± 0.12
DOPC/Chol/NBD-PSH	0.590 ± 0.015	0.288 ± 0.008	4.17 ± 0.12

2. *Supplementary figures*

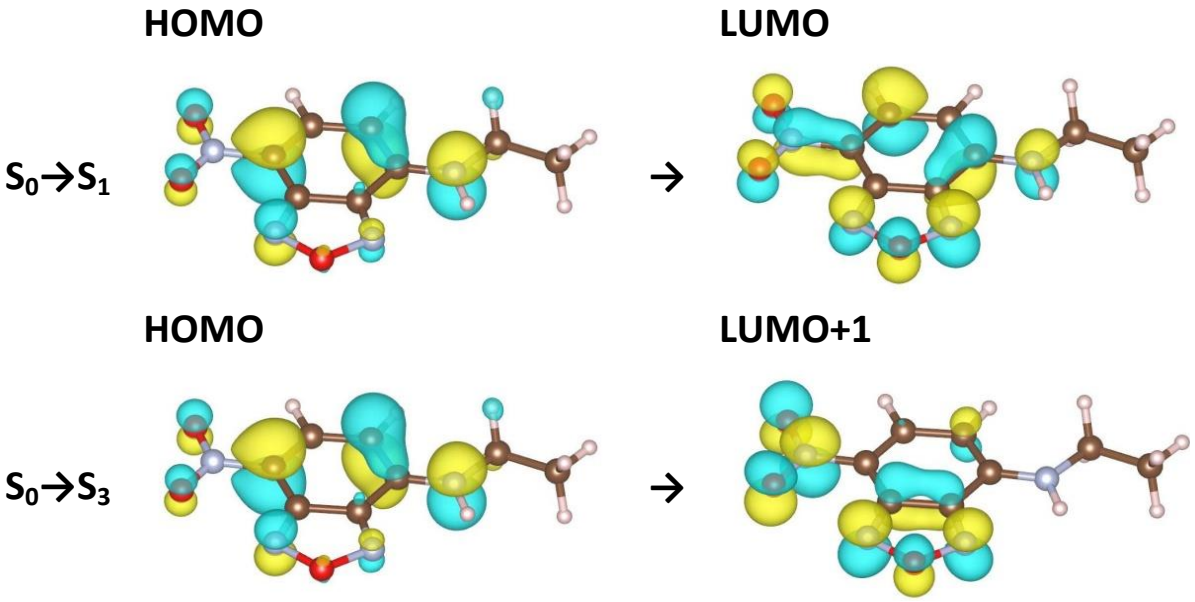

Figure S1. Plot of the orbitals mainly involved in the two lowest energy transitions of NBD-C_2 in solution.

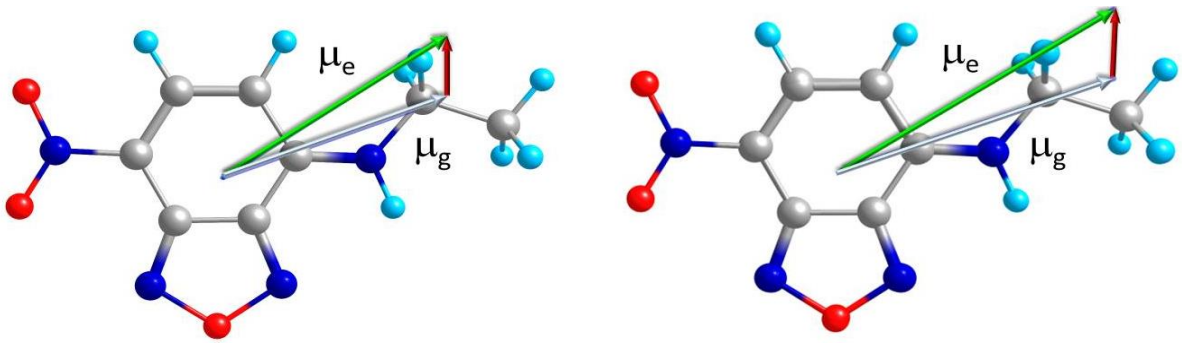

Figure S2. Representation of the calculated ground and excited state dipole moment vectors (labeled as μ_g and μ_e), as well as its vector difference (red arrow), for NBD-C2 in cyclohexane (left) and water (right).

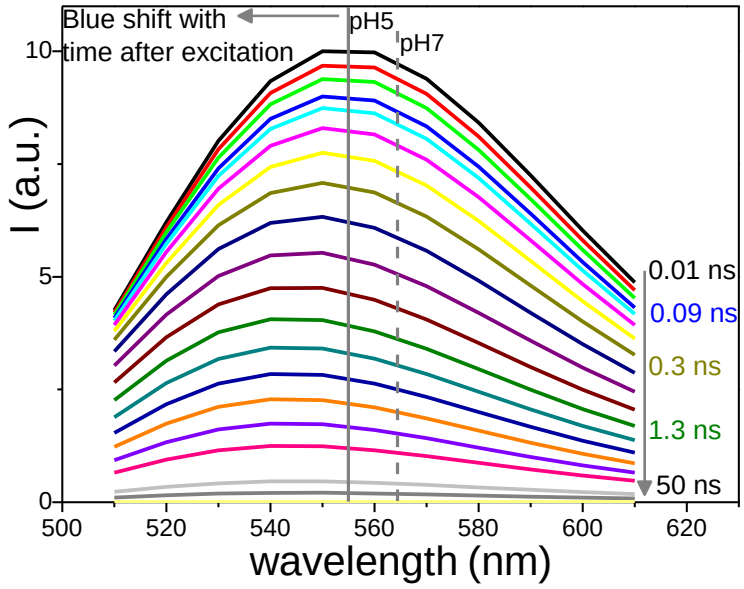

Figure S3. Selected time-resolved emission spectra (TRES) of NBD-PS in DOPC LUVs at pH 5, 20ºC. TRES were generated from 0.01ns to 50ns in a logarithmic time-scale of 100 steps. The grey vertical line is positioned at the maxima of the first generated spectra and is meant to be a guide for the eye. The dashed vertical line is the equivalent for NBD-PS in DOPC at pH 7, 20ºC (shown as Figure 7 in the main text). The dashed line is displayed to illustrate the how, at pH 5, the first generated spectrum is already shifted to shorter wavelengths compared to pH 7.

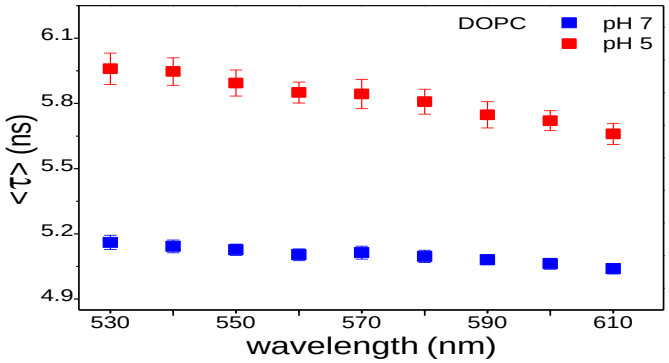

Figure S4. Amplitude-averaged lifetime values calculated from the 3-exponential fits of the lifetime decays of NBD-PS (T = 20ºC) in DOPC and DOPC/Chol, at both pH 5 and pH 7. The average lifetimes were calculated as $\langle\tau\rangle = \sum_{i=1}^{n} f_i \tau_i$, where $f_i = \frac{\alpha_i \tau_i}{\sum_{i=1}^{n} \alpha_i \tau_i}$.

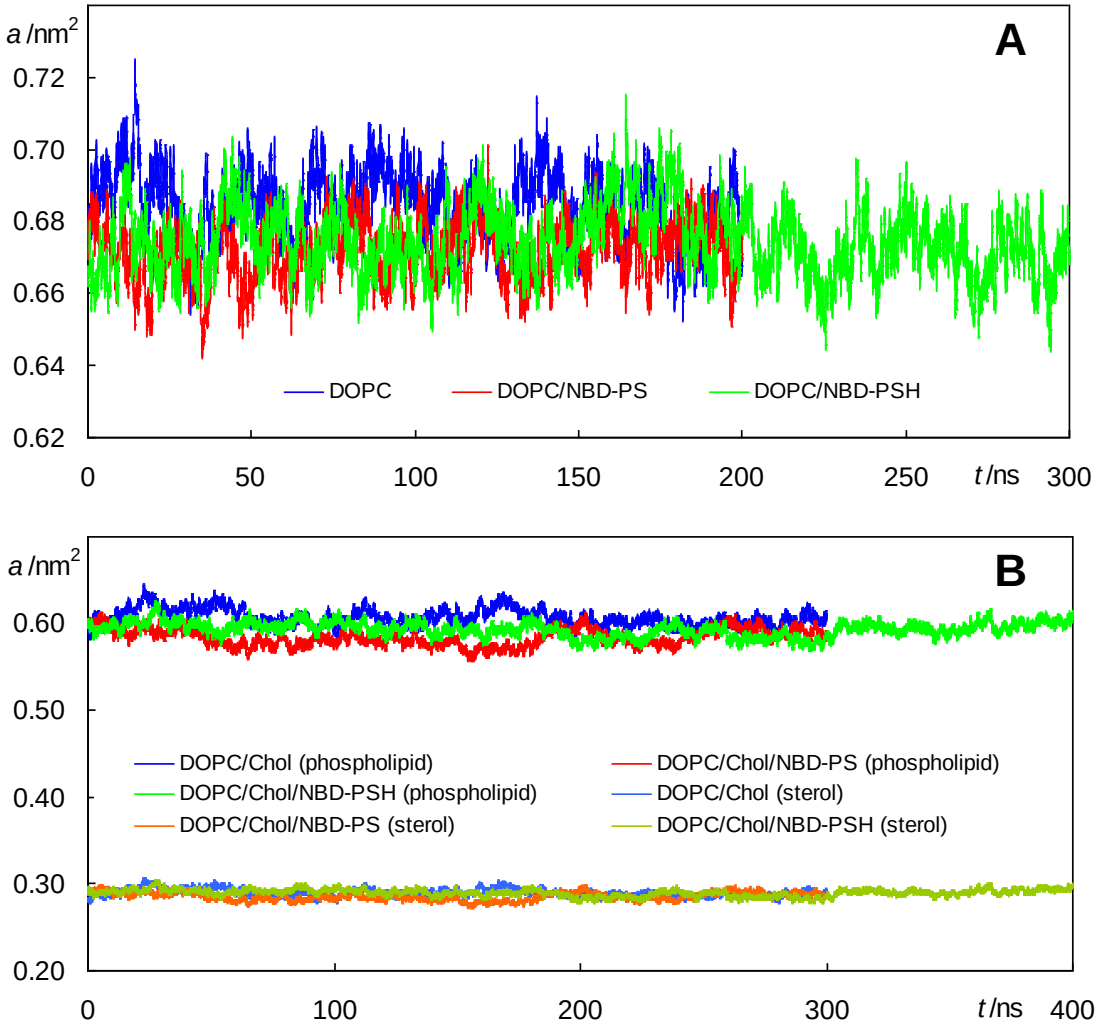

Figure S5. Time evolution of the molecular areas (*a*) for the systems without Chol (A) and with 20 mol% Chol (B).

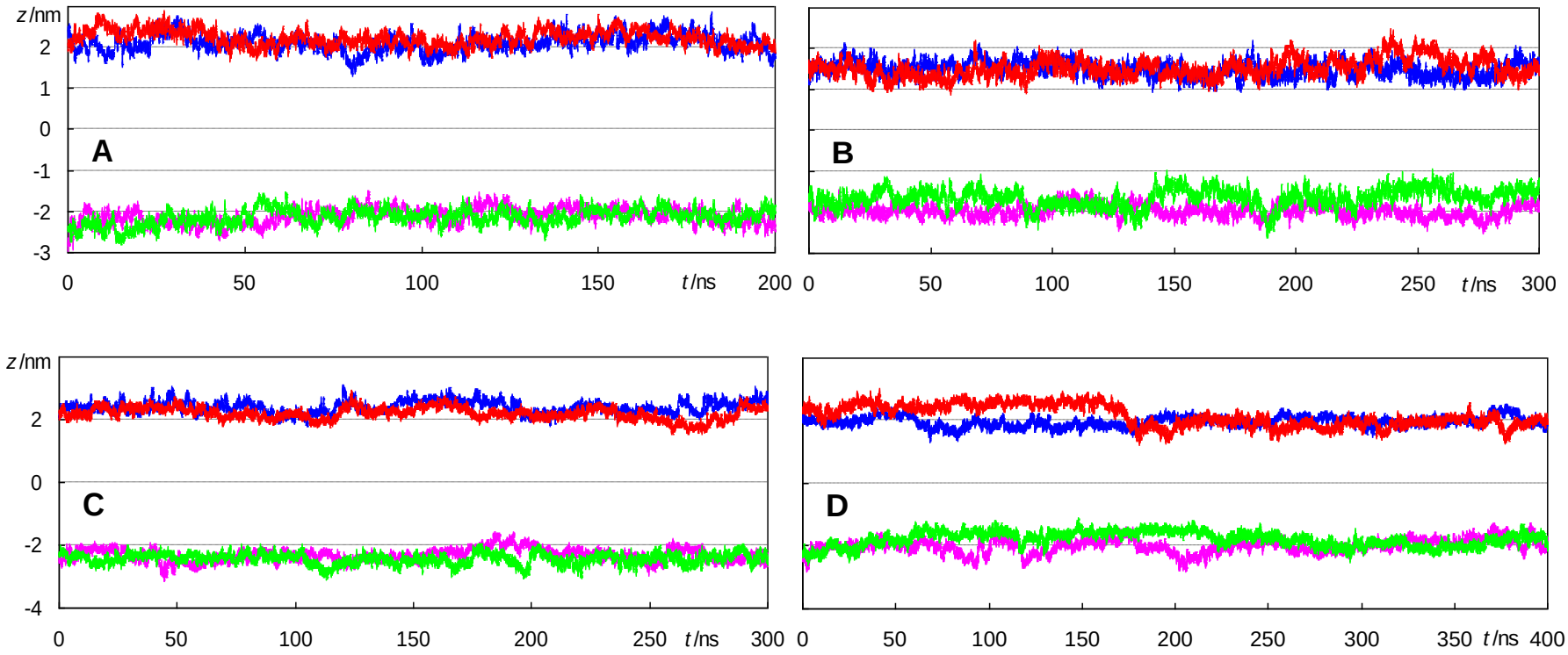

Figure S6: Time evolutions of the transverse position *z* of the fluorophore center of mass, for the 4 individual NBD-PS or NBD-PSH probes in each system (from A to D: DOPC/NBD-PS, DOPC/NBD-PSH, DOPC/Chol/NBD-PS, DOPC/Chol/NBD-PSH).

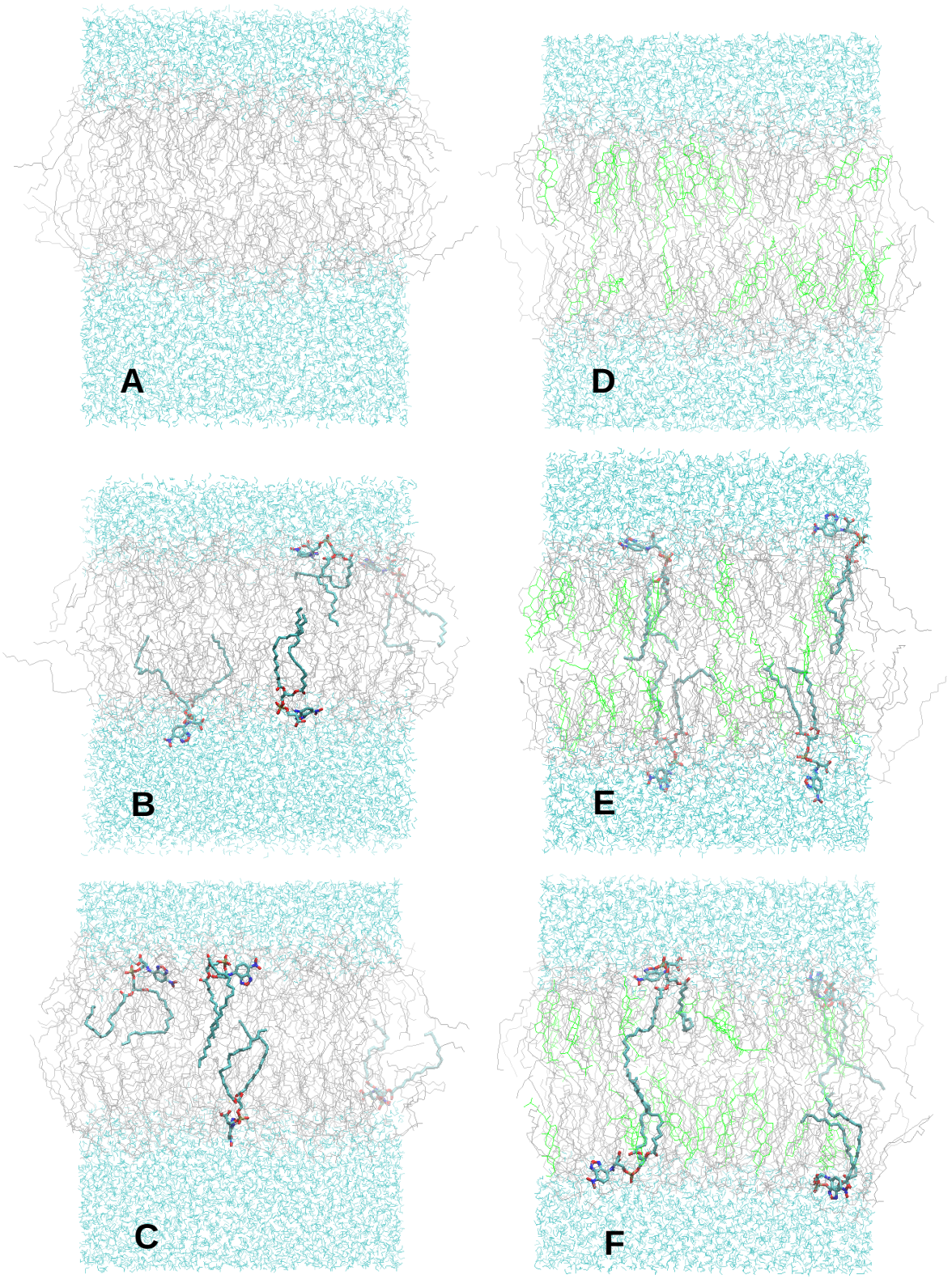

Figure S7. Final structures of all simulated systems. From A to F: DOPC, DOPC/NBD-PS, DOPC/NBD-PSH, DOPC/Chol, DOPC/Chol/NBD-PS, DOPC/Chol/NBD-PSH.

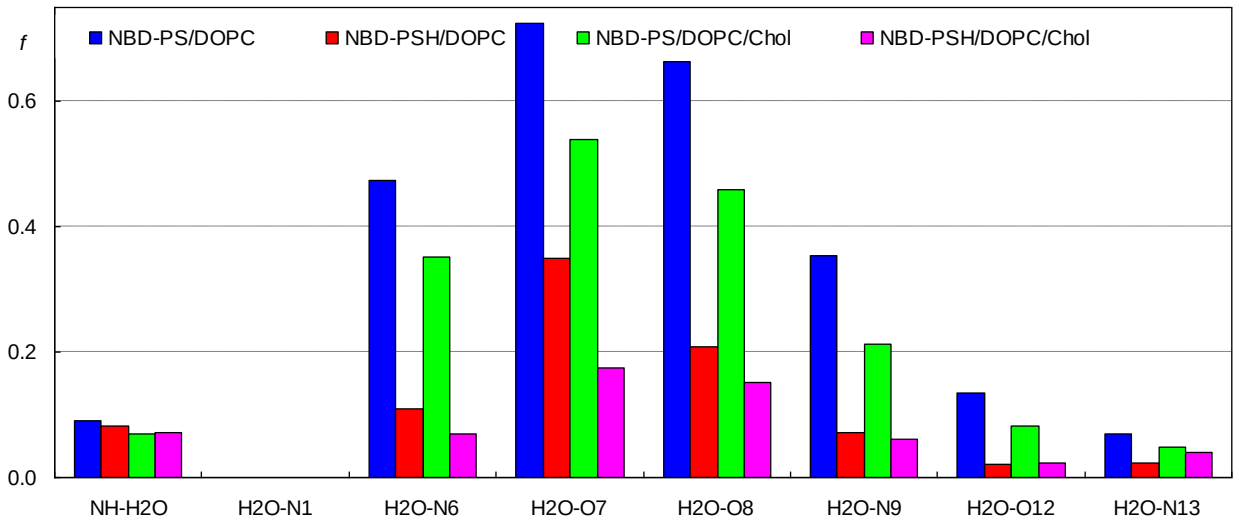

Figure S8. H-bonding between the NBD NH (donors) and H_2O O (acceptors) atoms (far left), and between H_2O (donors) and all NBD fluorophore nitrogen and oxygen atoms. For NBD atom numbering, see Fig. 1D. *f* denotes the fraction of configurations for which H-bonds are formed (relative to the maximal theoretical number).

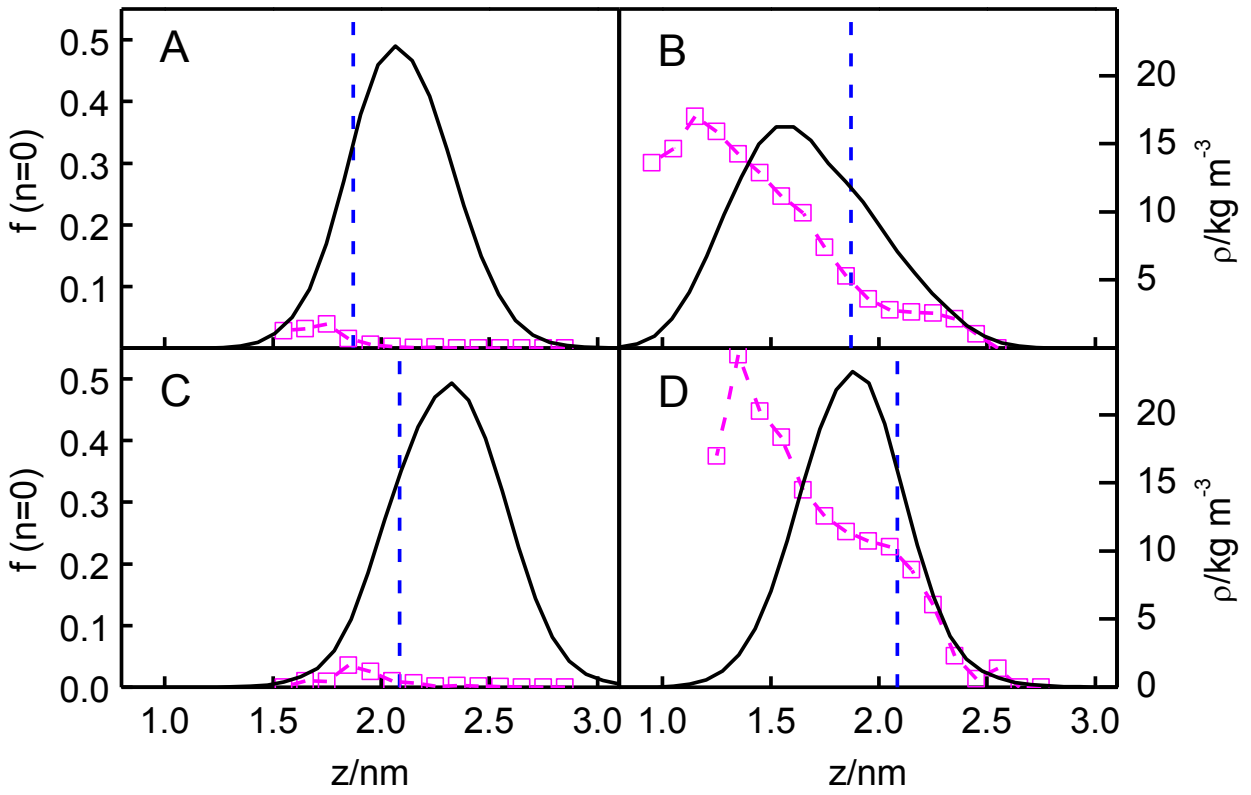

Figure S9. Fraction *f*(n = 0) of conformations for which no H-bonding is observed (magenta line), as a function of the transverse location of the fluorophore centre of mass relative to the bilayer midplane (*z*), for the (A) DOPC/NBD-PS, (B) DOPC/NBD-PSH, (C) DOPC/Chol/NBD-PS, and (D) DOPC/Chol/NBD-PSH systems. The dotted blue line represents the average DOPC phosphorus atom location in each system, while the black curve is the NBD mass distribution profile ρ(*z*).

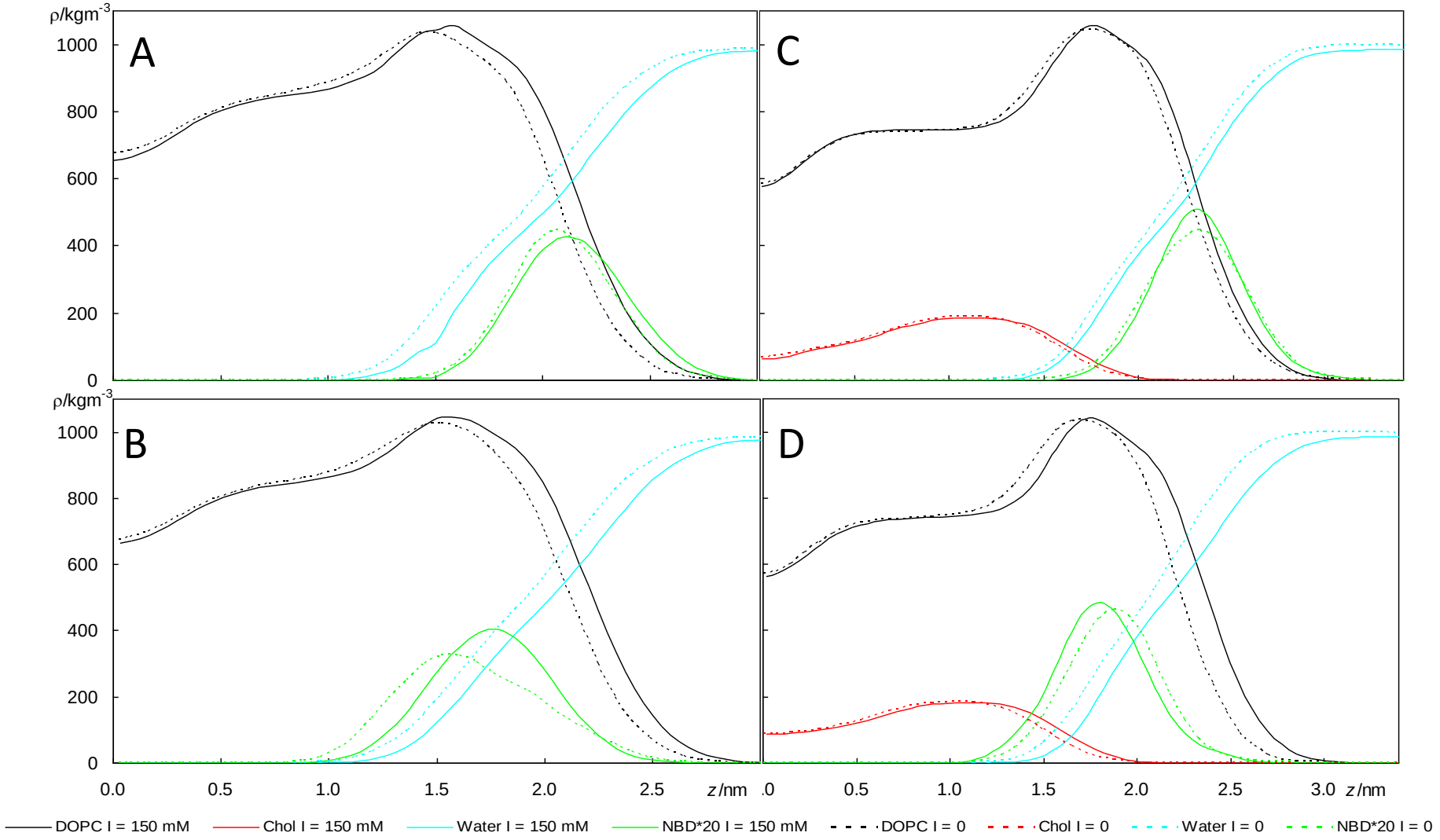

Figure S10. Mass density profiles of host lipid, water and NBD fluorophore, simulated in the absence (I = 0) and presence (I = 150 mM) of added NaCl, for the (A) DOPC/NBD-PS, (B) DOPC/NBD-PSH, (C) DOPC/Chol/NBD-PS, and (D) DOPC/Chol/NBD-PSH systems.