Electronic Supporting Information (ESI)
for
How Does Aggregation of Doxorubicin Molecules Affect Its Solvation and Membrane Penetration?

Sadaf Shirazi-Fard,[a] Amin Reza Zolghadr*,[a], Axel Klein*,[a,b]

[a] *Department of Chemistry, Shiraz University, Shiraz, 71946-84795, Iran*
[b] *University of Cologne, Faculty of Mathematics and Natural Sciences, Department of Chemistry, Greinstrasse 6, D-50939 Cologne, Germany*
**E-mail: arzolghadr@shirazu.ac.ir; Tel: +98 71 3613 7157; ORCID: 0000-0002-6289-3794*
**E-mail: axel.klein@uni-koeln.de; Tel.: +49-221-470-4006; ORCID: 0000-0003-0093-9619*
ORCID: 0000-0002-3276-7906 (SSF)

Contents:

Supporting Figures

Supporting Tables

References

Supporting Figures

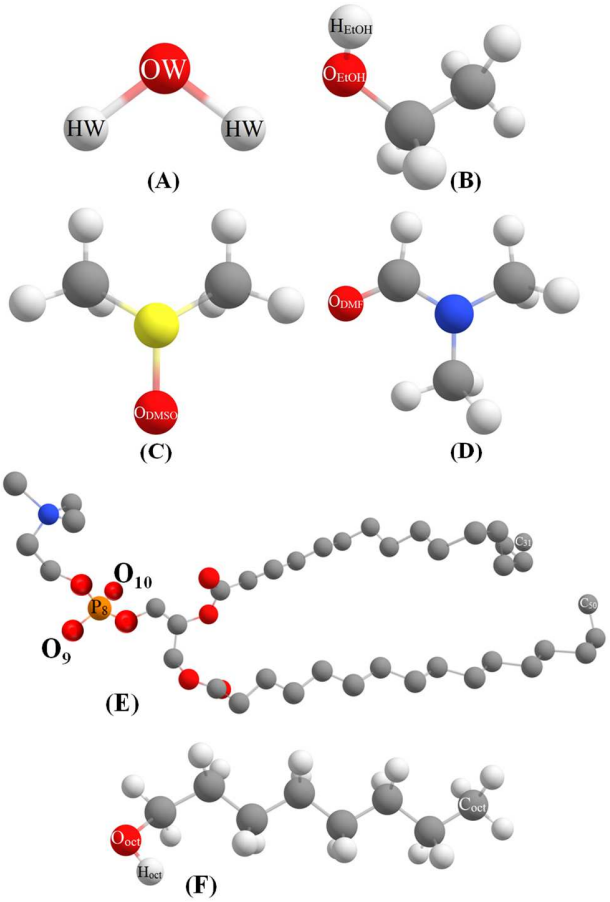

Fig. S1 DFT-calculated optimised structures of (A) water, (B) EtOH, (C) DMSO, (D) DMF, (E) DPPC, and (F) octanol molecules with atom labelling.

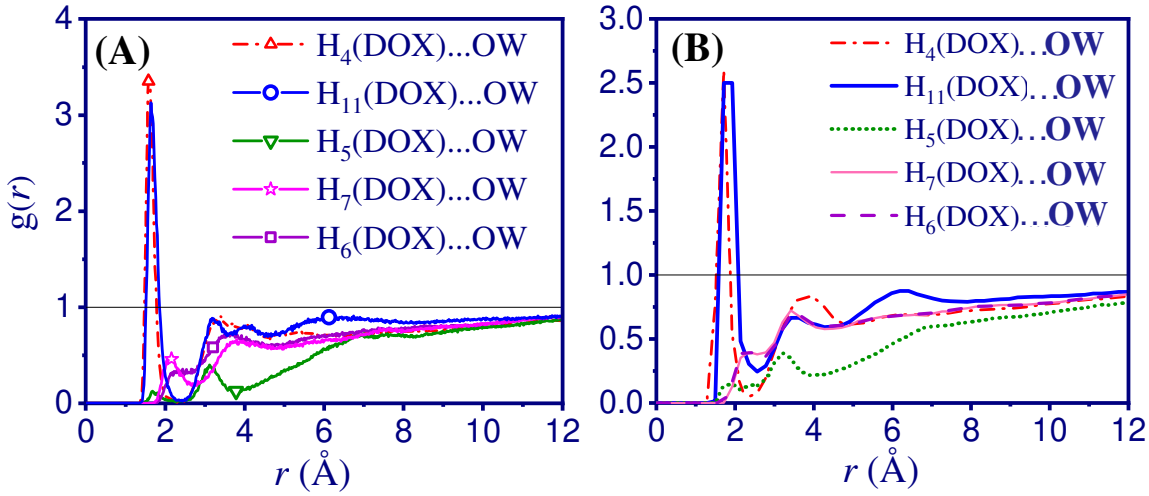

Fig. S2 Radial distribution functions (*RDF*s) of DOX···water interactions using calculated charges, (A) from the NBO method, and (B) from the CHELPG method.

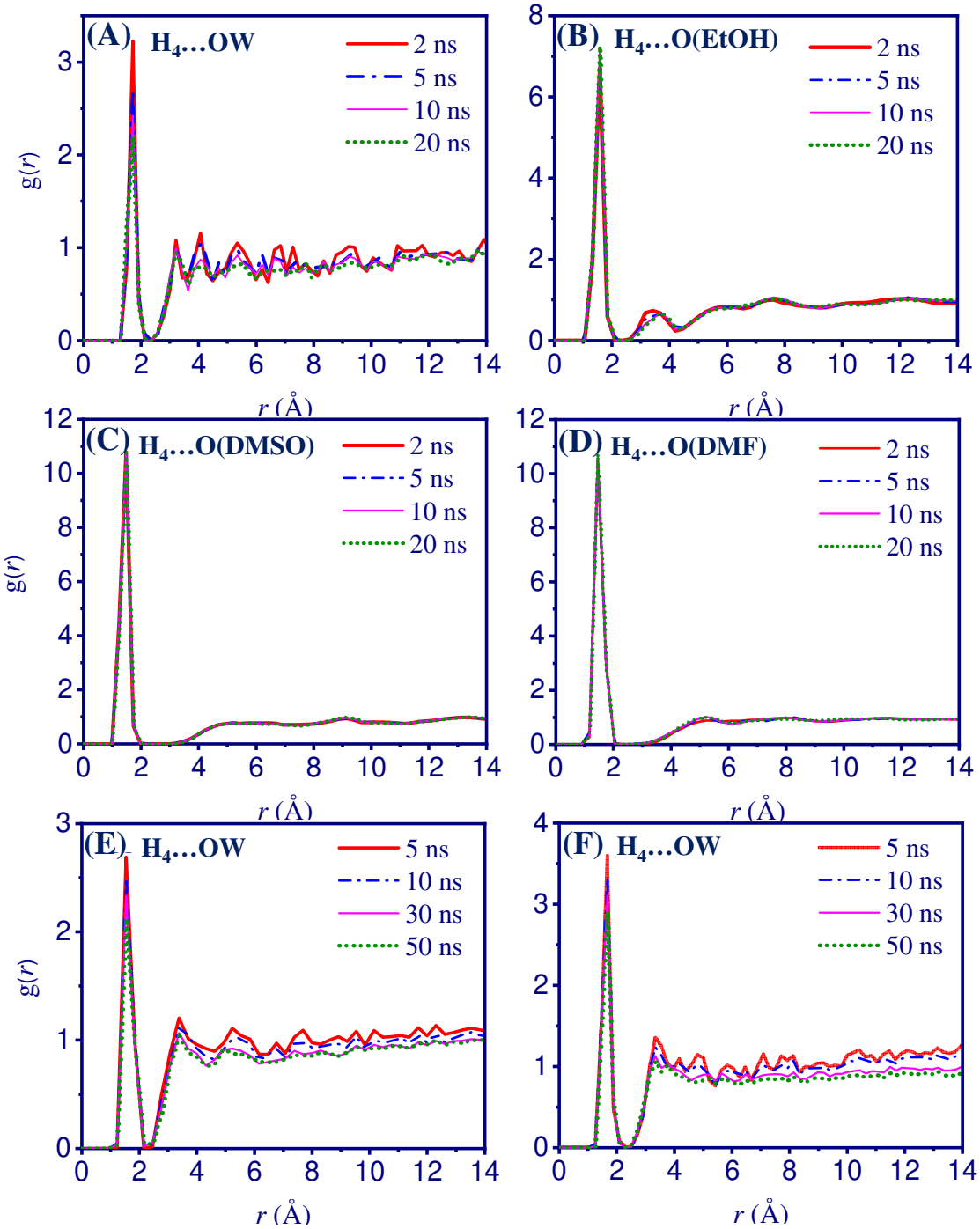

Fig. S3 Calculated radial distribution functions (*RDFs*) of DOX in (A) water, (B) EtOH, (C) DMSO, (D) DMF, (E) at the water/DPPC interface, and (F) at the water/octanol interface at different simulation times.

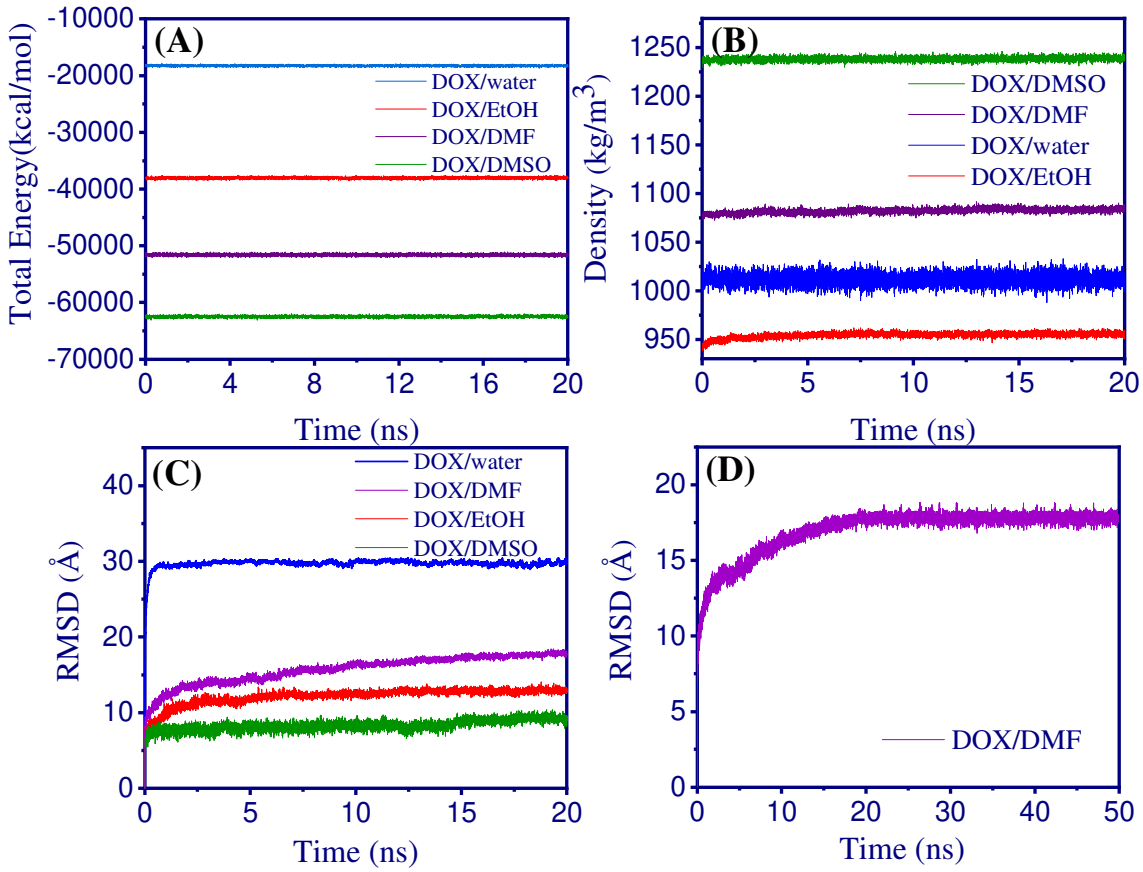

Fig. S4 (A) Total energy, (B) Density, and (C) *RMSD*s of the DOX/water, DOX/EtOH, DOX/DMSO and DOX/DMF systems, and (D) *RMSD*s of DOX/DMF, showing the equilibration of the systems during the simulation time.

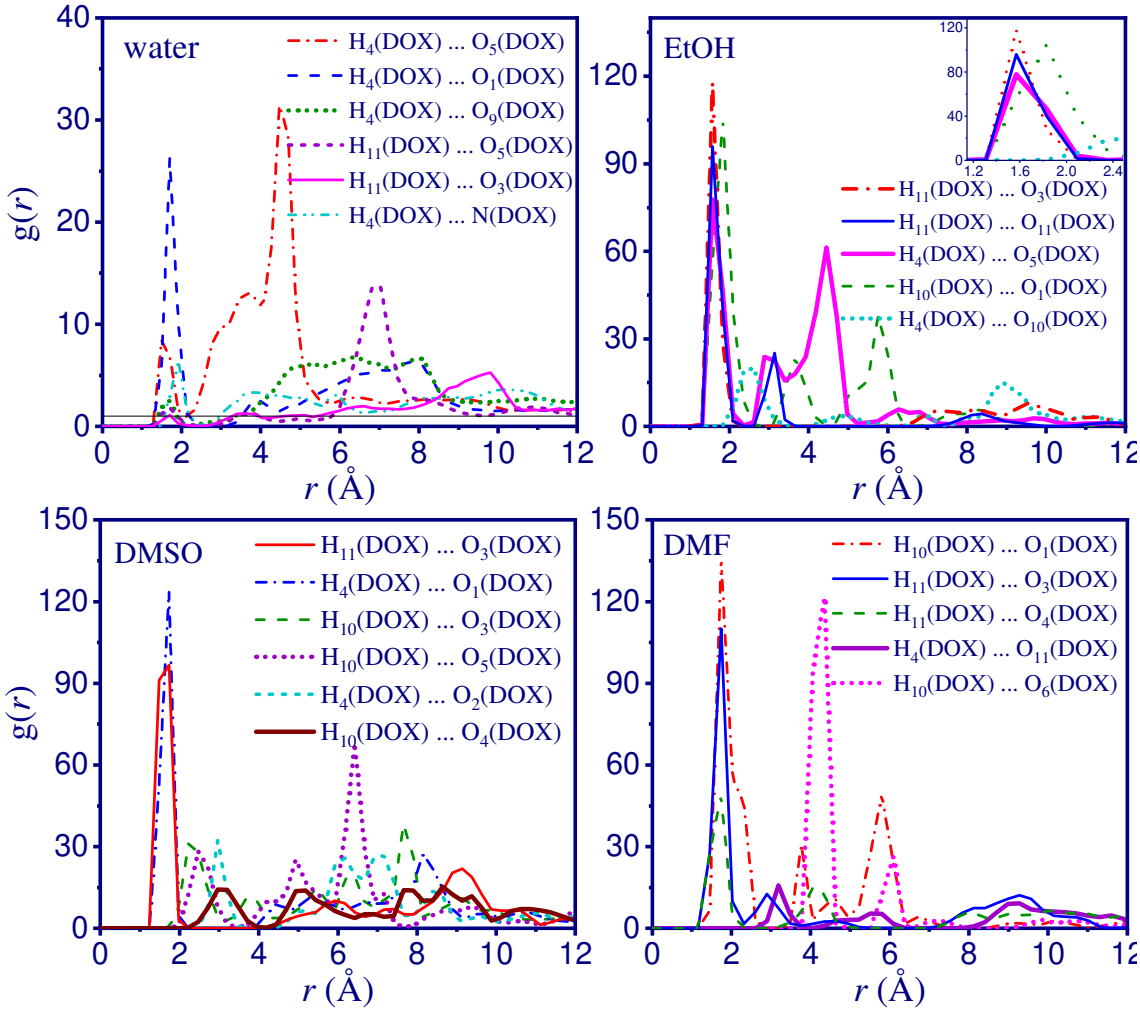

Fig. S5 Most probable *RDF*s between selected DOX H atoms and DOX O or N atoms in different solvents.

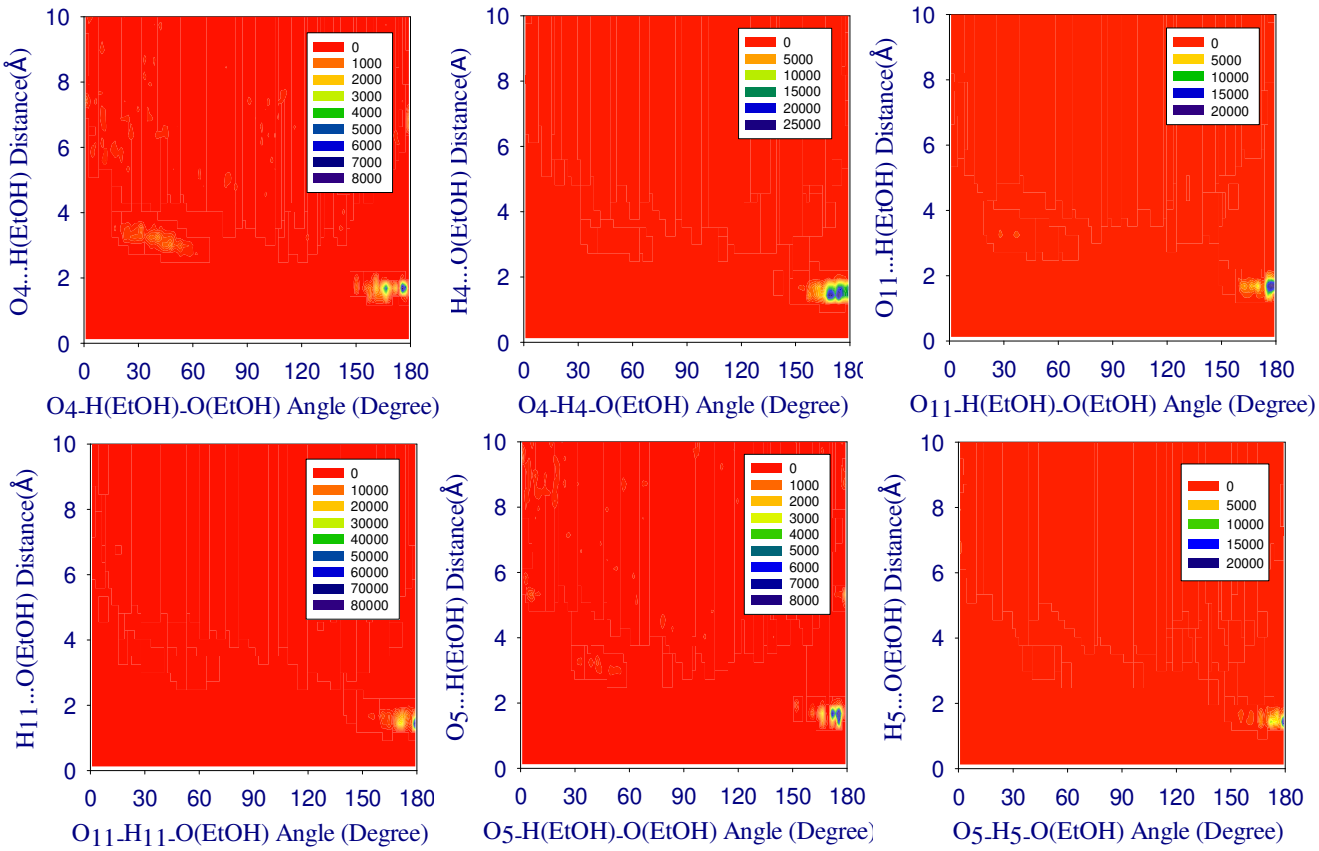

Fig. S6 Combined distribution functions (*CDF*s) of the angular distribution functions (*ADF*s) (x-axis) and radial distribution functions (*RDF*s) (y-axis) for the hydrogen bonds of DOX molecules in EtOH. The probability values were not normalised such that the highest probability value is not equal in each case and the colour scheme ranges from red (low probability) to dark blue (high probability).

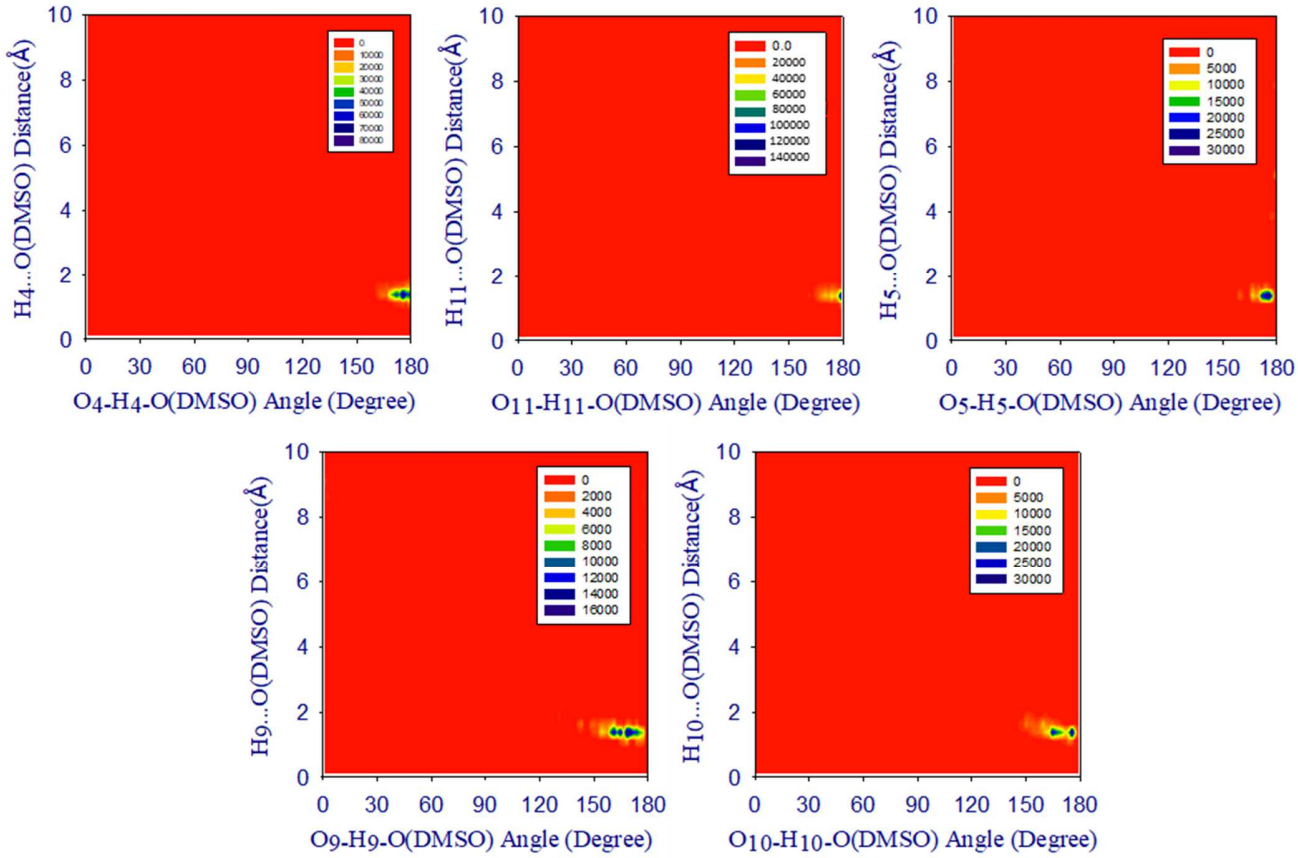

Fig. S7 *CDF*s of the *ADF*s (x-axis) and *RDF*s (y-axis) for the hydrogen bonds of DOX molecules in DMSO. The probability values were not normalised such that the highest probability value is not equal in each case and the colour scheme ranges from red (low probability) to dark blue (high probability).

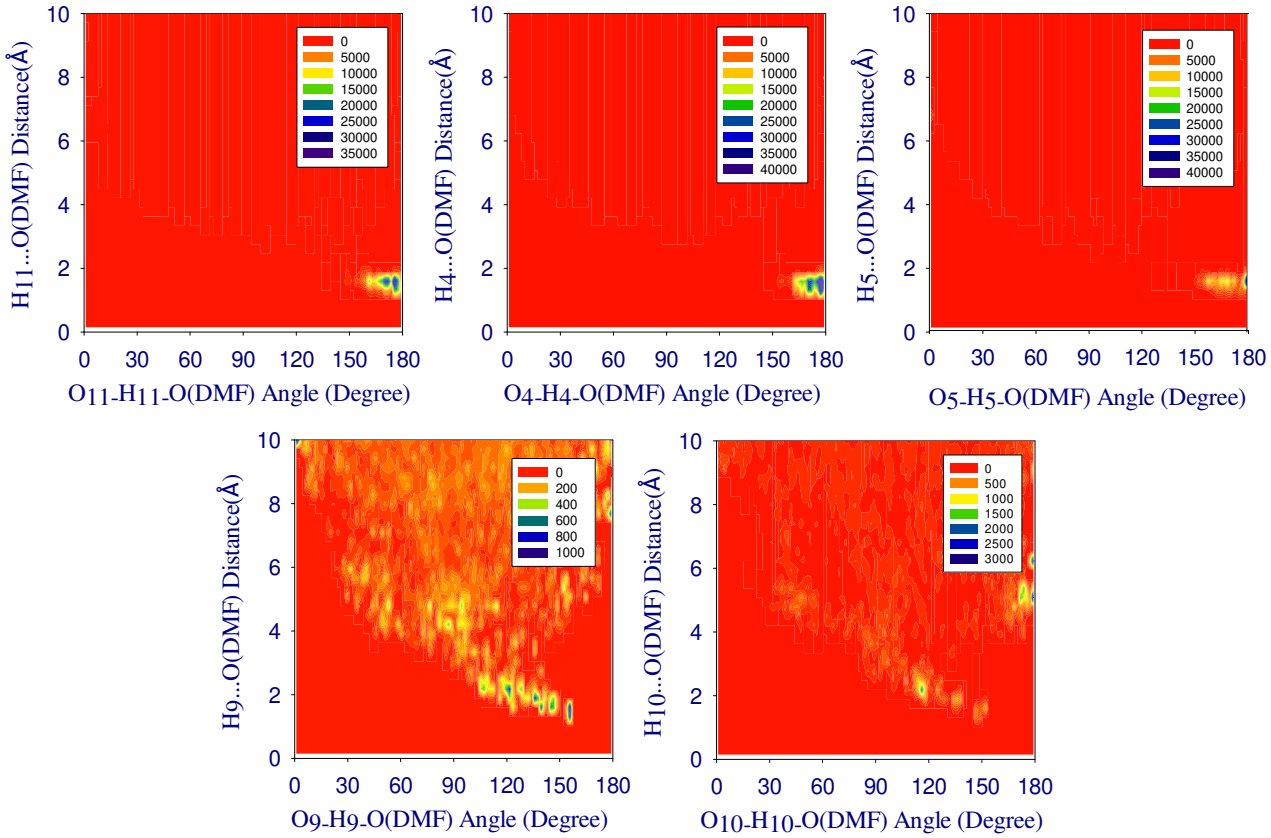

Fig. S8 *CDF*s of the *ADF*s (x-axis) and *RDF*s (y-axis) for the hydrogen bonds of DOX molecules in DMF. The probability values were not normalised such that the highest probability value is not equal in each case and the colour scheme ranges from red (low probability) to dark blue (high probability).

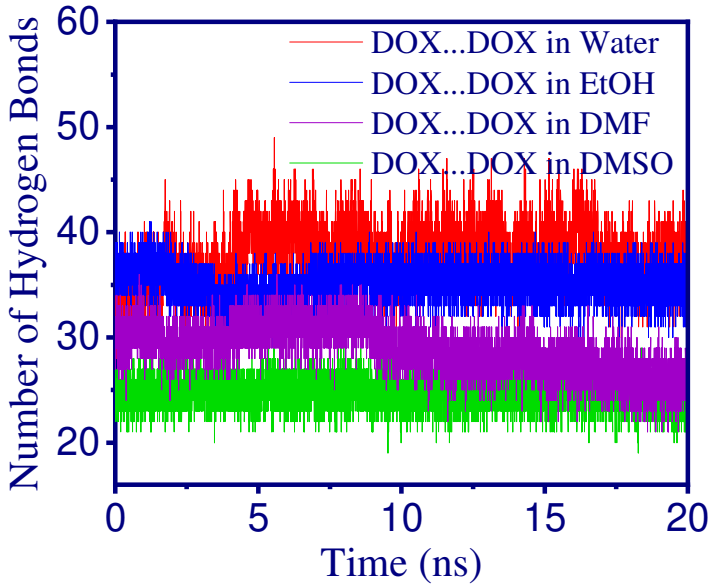

Fig. S9 Number of hydrogen bonds between DOX···DOX in different solvents.

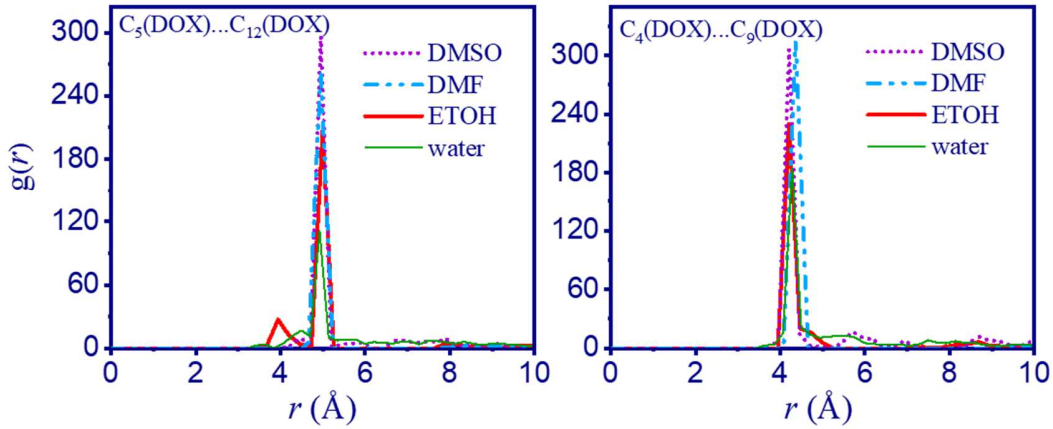

Fig. S10 DOX···DOX *RDF*s between DOX C atoms in different solvents.

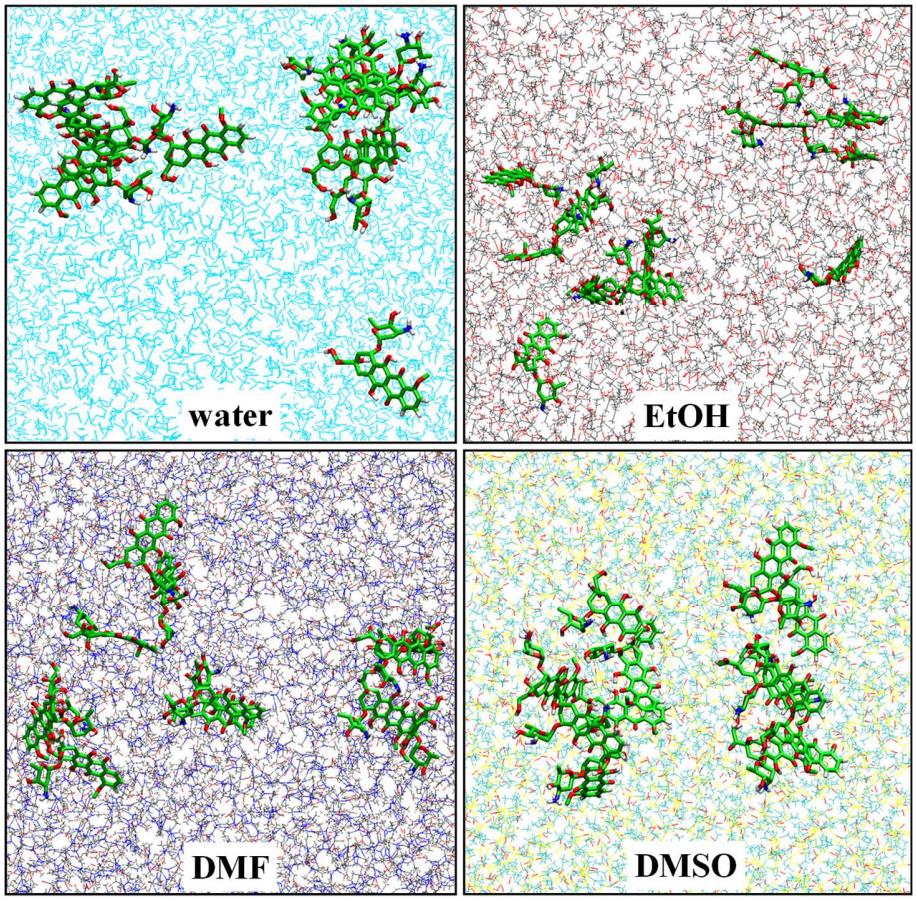

Fig. S11 Representative simulation snapshots of DOX in different solvents after 30 ns.

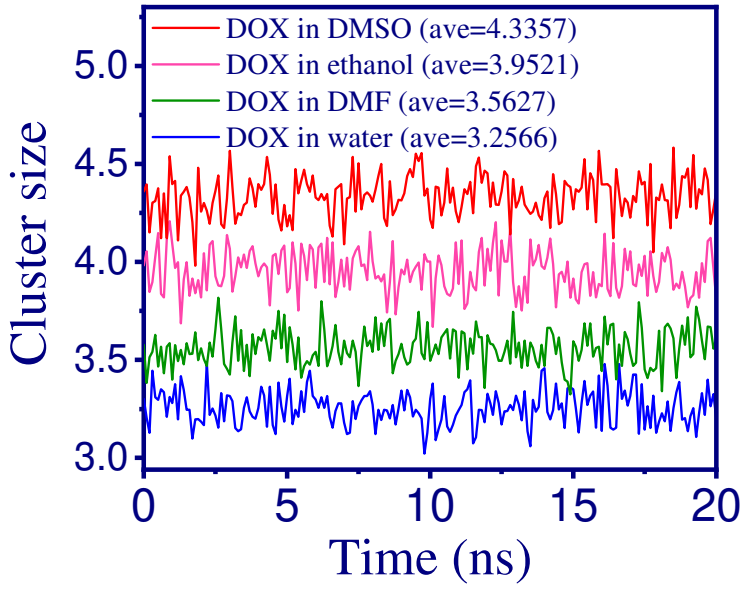

Fig. S12 Average cluster sizes of DOX in different solvents.

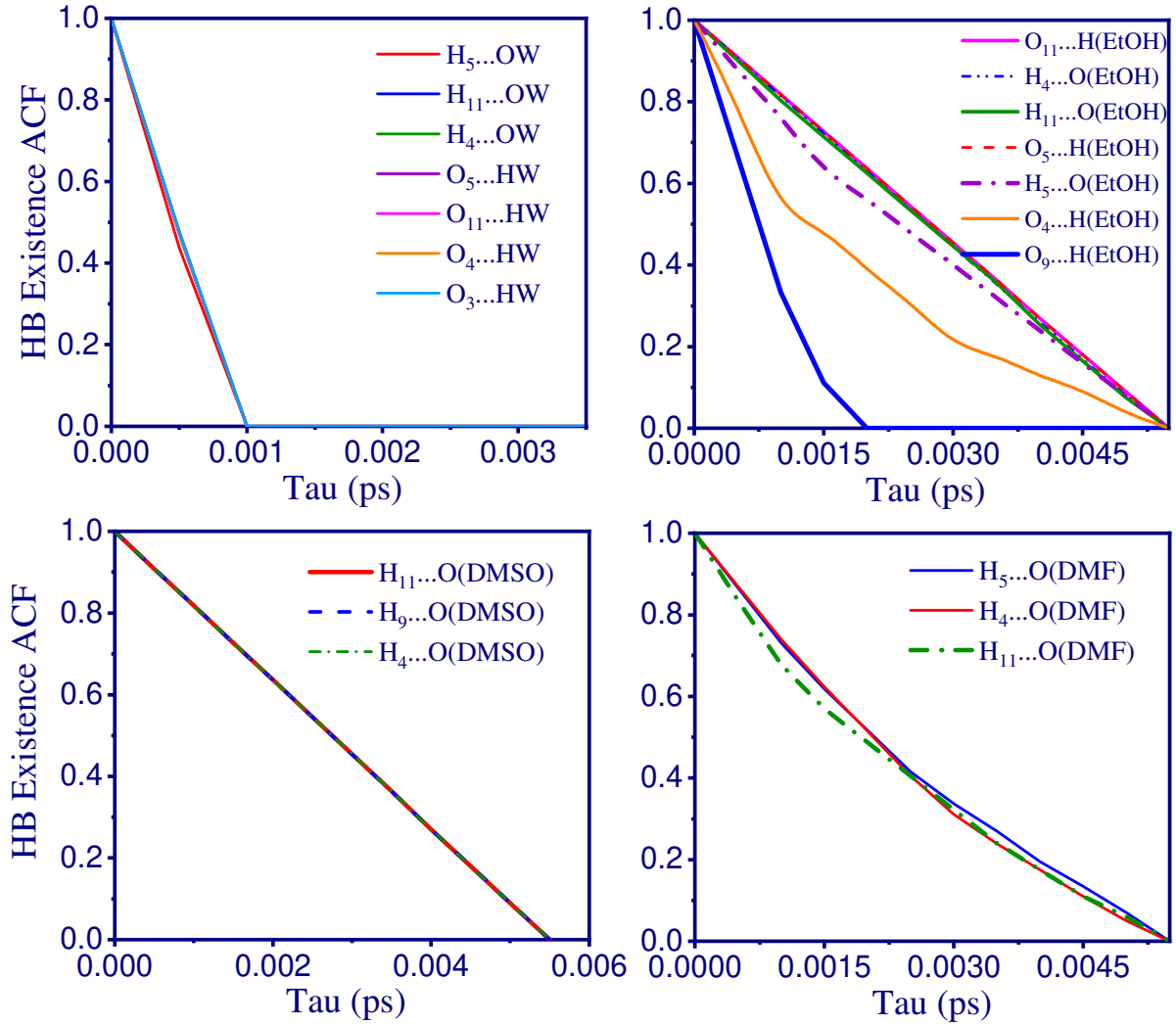

Fig. S13 Average HB Existence autocorrelation functions (*ACF*s) of DOX in (A) water, (B) EtOH, (C) DMSO, and (D) DMF.

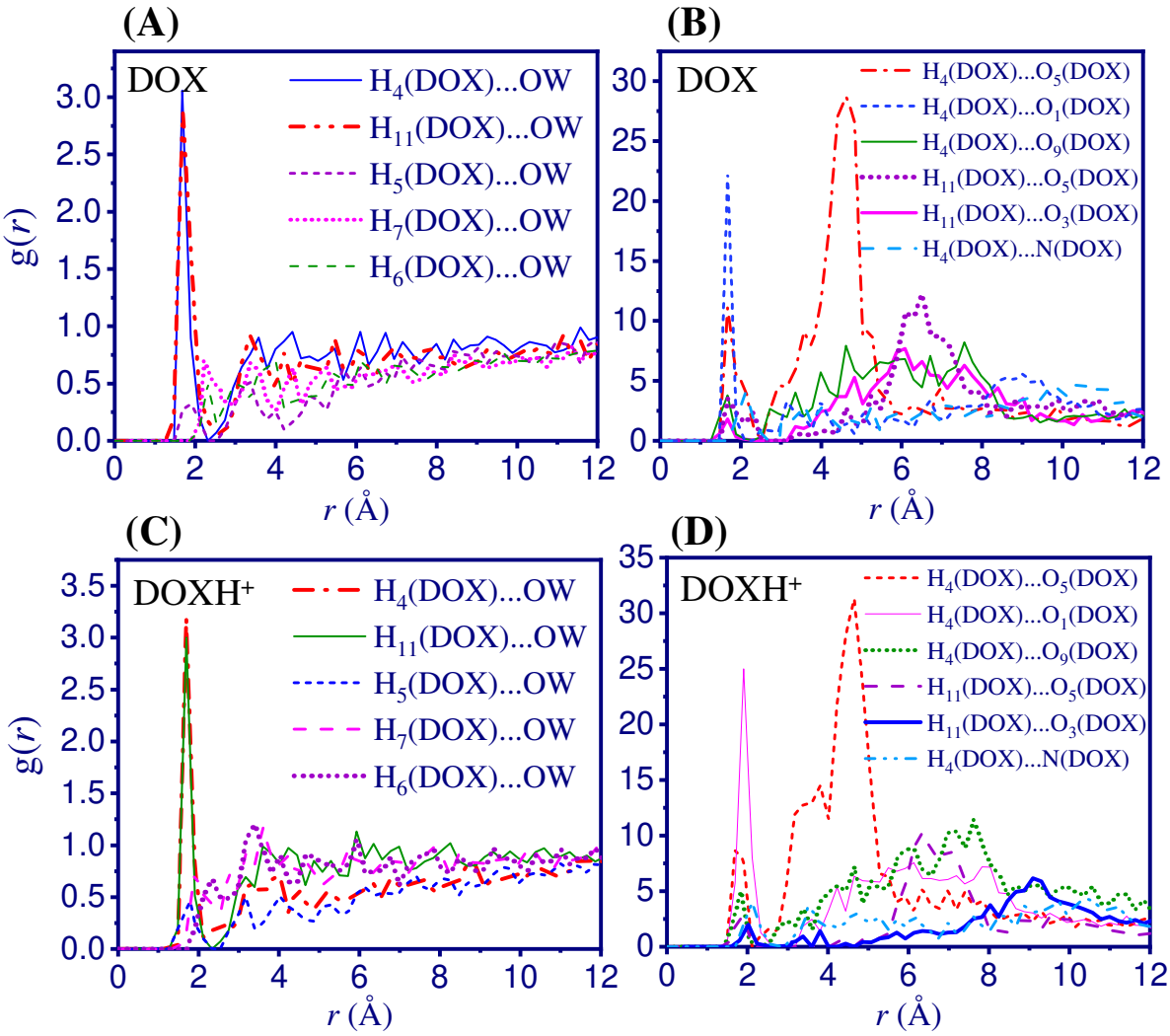

Fig. S14 *RDF*s of (A) neutral DOX atoms with water, (B) selected neutral DOX H atoms and DOX O or N atoms, (C) protonated DOX ($DOXH^+$) atoms with water, and (D) selected protonated DOX ($DOXH^+$) atoms and DOX O or N atoms using Amber force field.

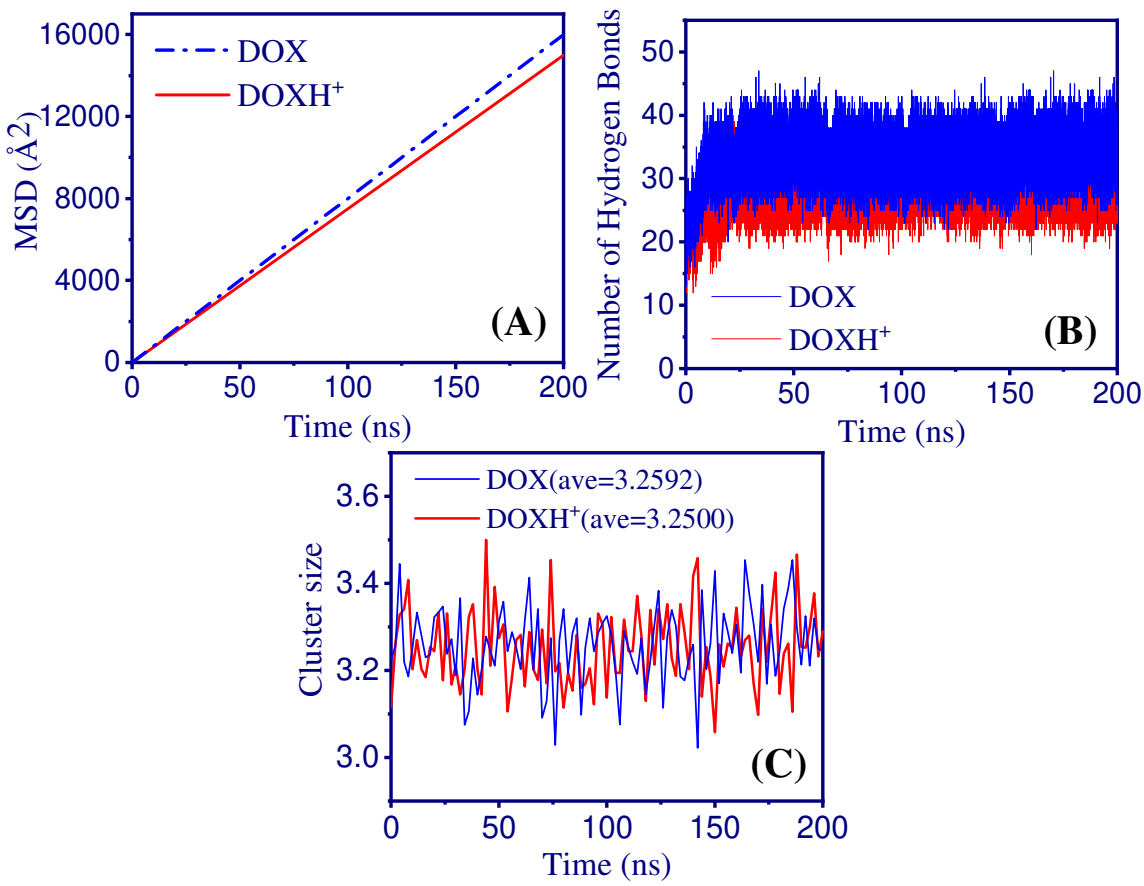

Fig. S15 (A) Mean-square displacement (*MSD*) of the geometrical centre of DOX, (B) Number of hydrogen bonds between DOX···DOX, and (C) Average cluster sizes of neutral DOX (DOX) and protonated DOX ($DOXH^+$) in water using Amber force field.

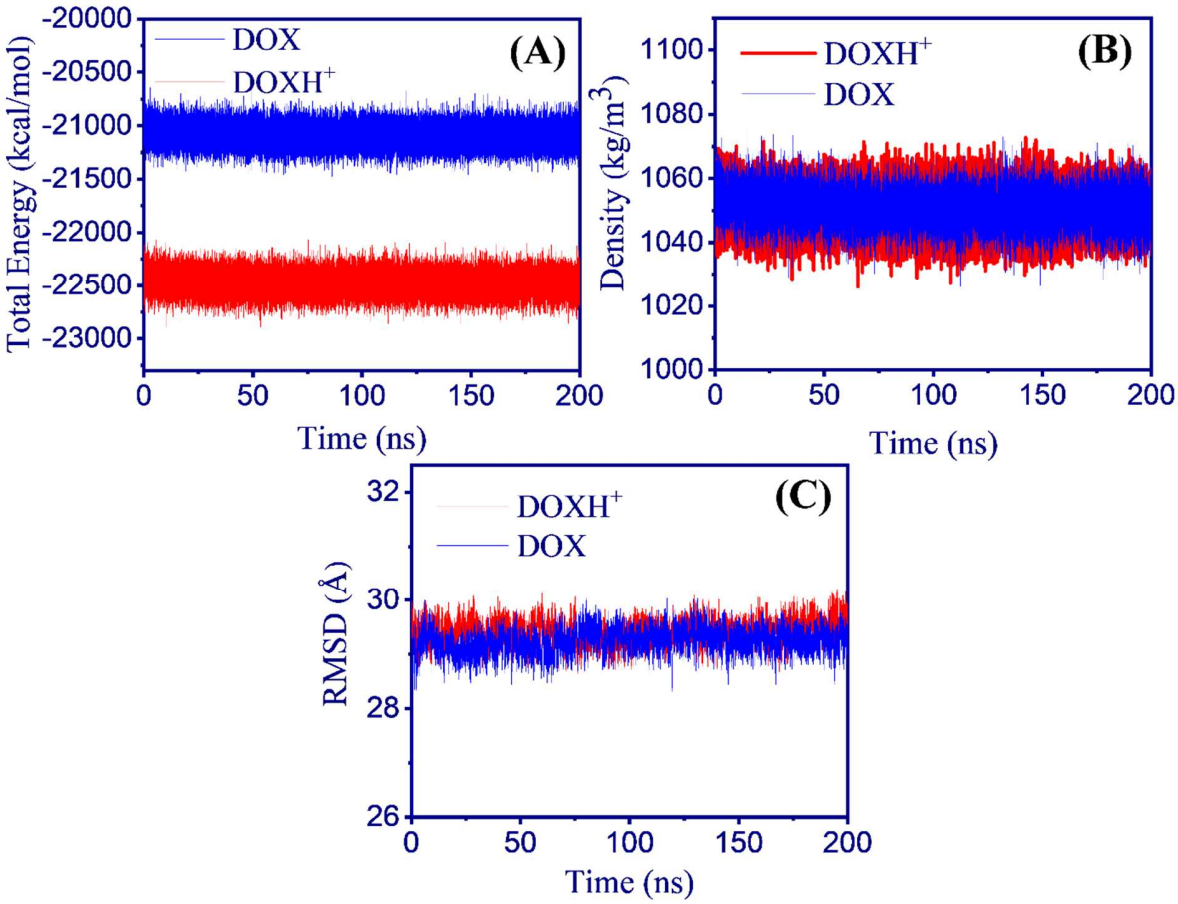

Fig. S16 (A) Total energy, (B) Density, and (C) *RMSD*s of DOX/water system for neutral DOX (DOX) and protonated DOX ($DOXH^+$) using Amber force field, showing the equilibration of the systems during the simulation time.

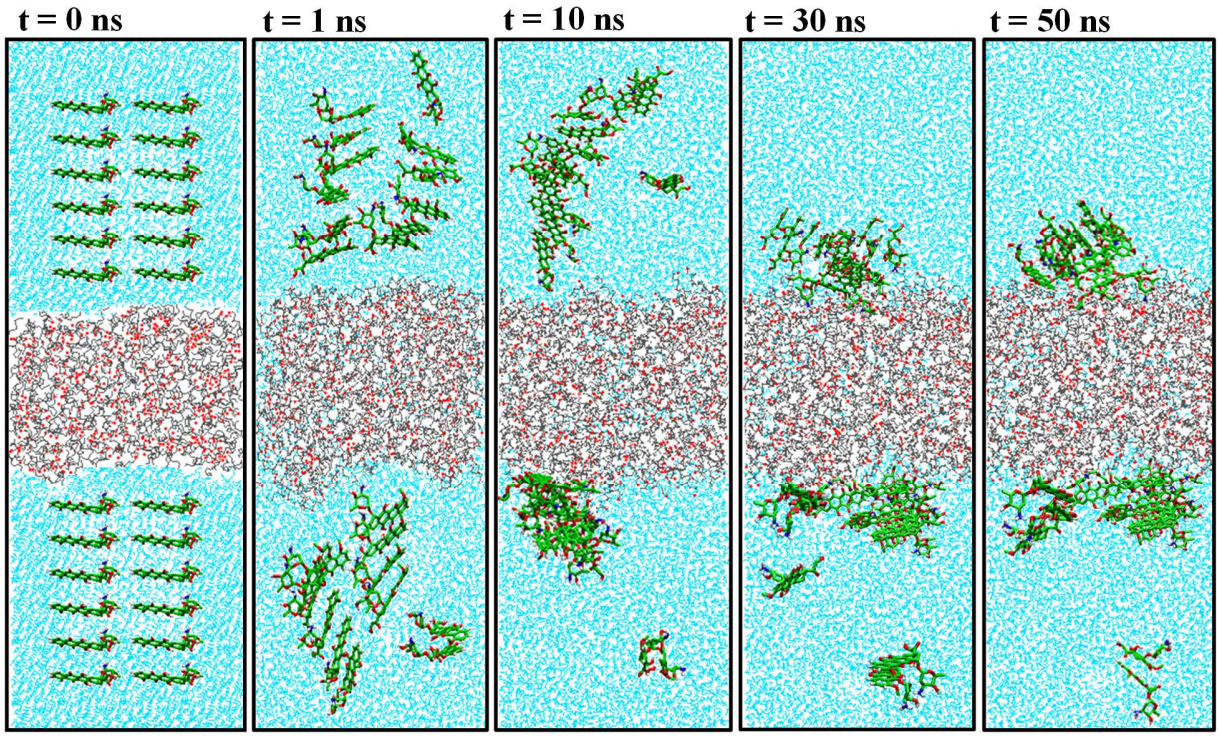

Fig. S17 Representative simulation snapshots of DOX at octanol/water interfaces at different simulation times. The DOX atoms are shown as green (C), red (O), and blue (N) balls. The hydrocarbon chains of octanol are shown in grey, while the hydroxy groups are depicted in red. The water phase is in cyan.

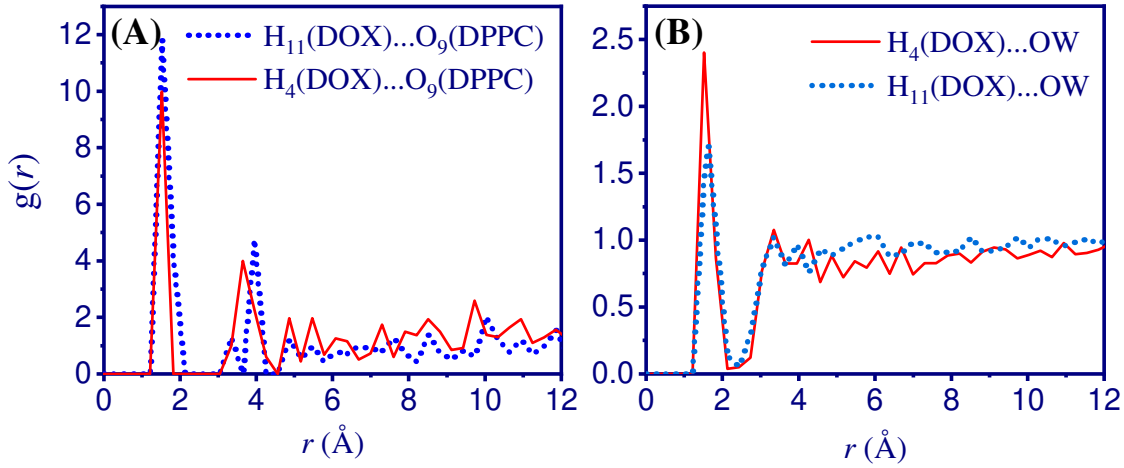

Fig. S18 *RDF*s of (A) DOX H atoms with O_9 of DPPC, and (B) DOX H atoms with the water O atom in the system of DOX/water/DPPC after 300 ns.

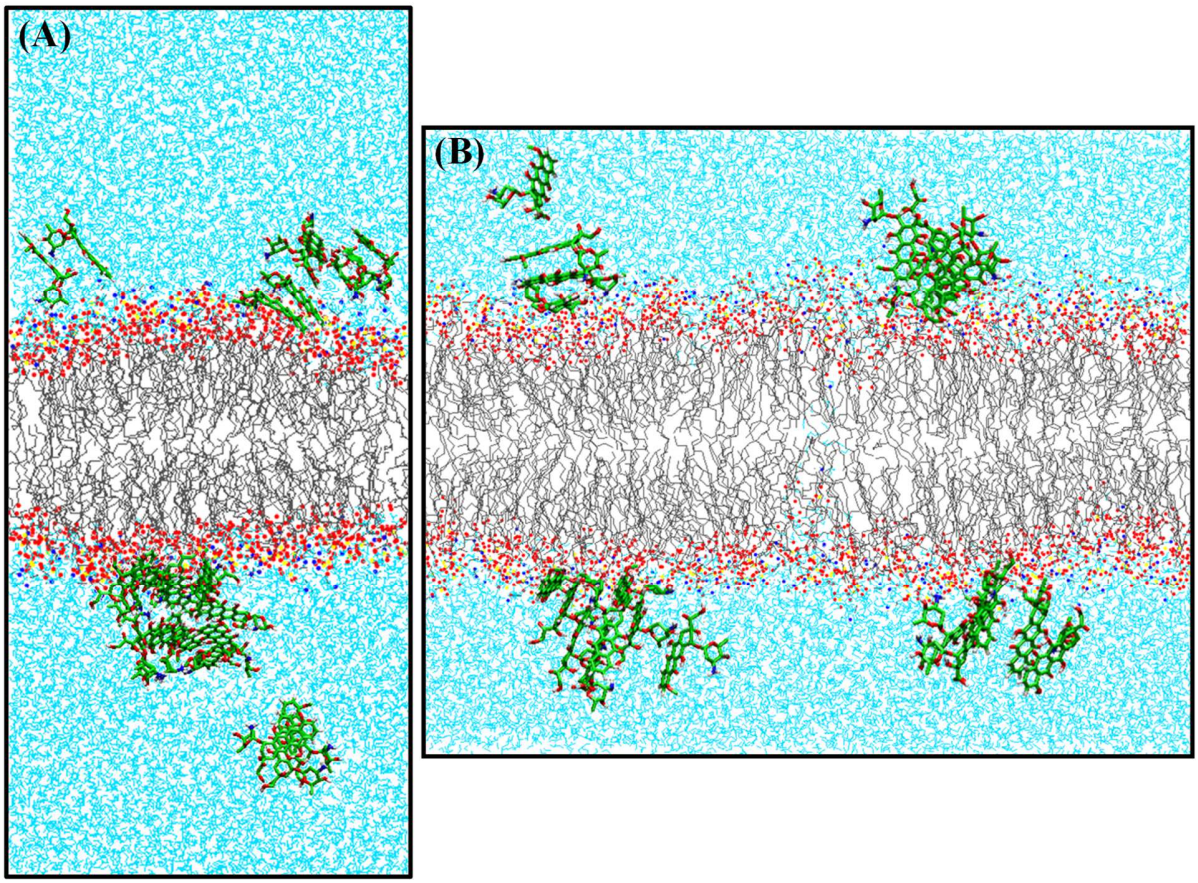

Fig. S19 Representative simulation snapshots of DOX at DPPC/water interfaces (A) with 128, and (B) with 256 lipid molecules after 300 ns. The DOX atoms are shown as green (C), red (O), and blue (N) balls. The hydrocarbon chains of octanol are shown in grey, while the hydroxy groups are depicted in red. The water phase is in cyan.

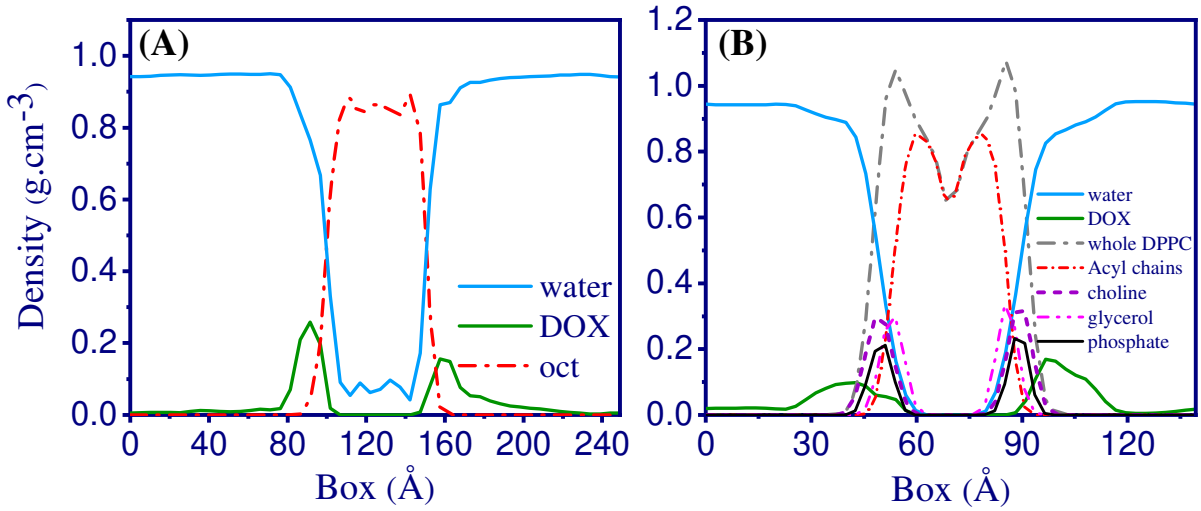

Fig. S20 Total density profiles of the (A) octanol/DOX/water, and (B) DPPC/DOX/water systems.

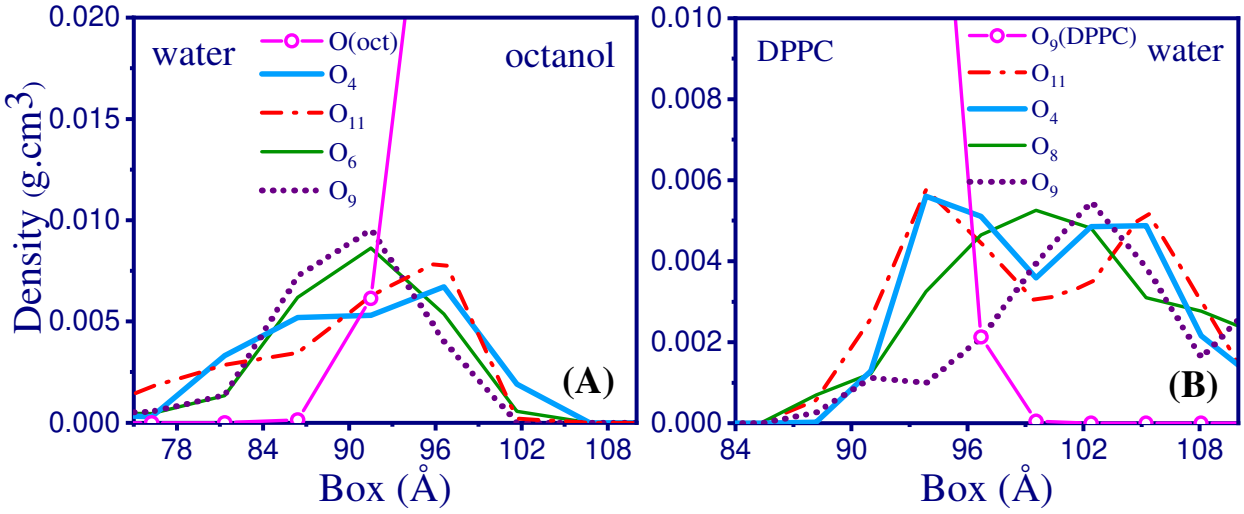

Fig. S21 Atomic density profiles of the (A) octanol/DOX/water, and (B) DPPC/DOX/water systems.

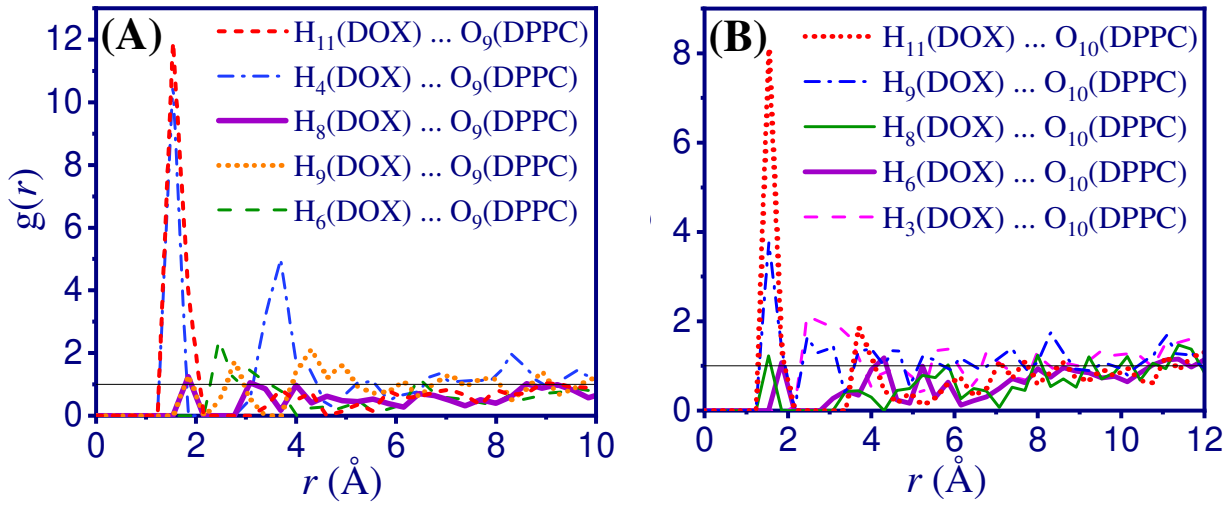

Fig. S22 DOX···DPPC *RDF*s DOX H atoms with (A) O_9, and (B) O_{10} of DPPC.

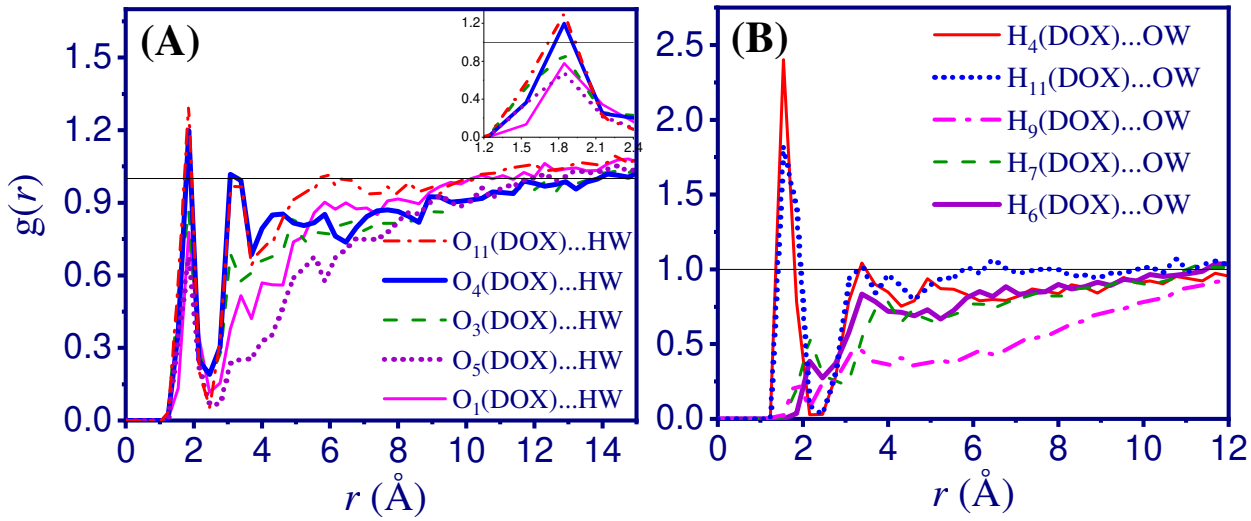

Fig. S23 DOX···water *RDF*s in the DPPC/DOX/water system. (A) DOX O atoms with water H atoms, and (B) DOX H atoms with the water O atom.

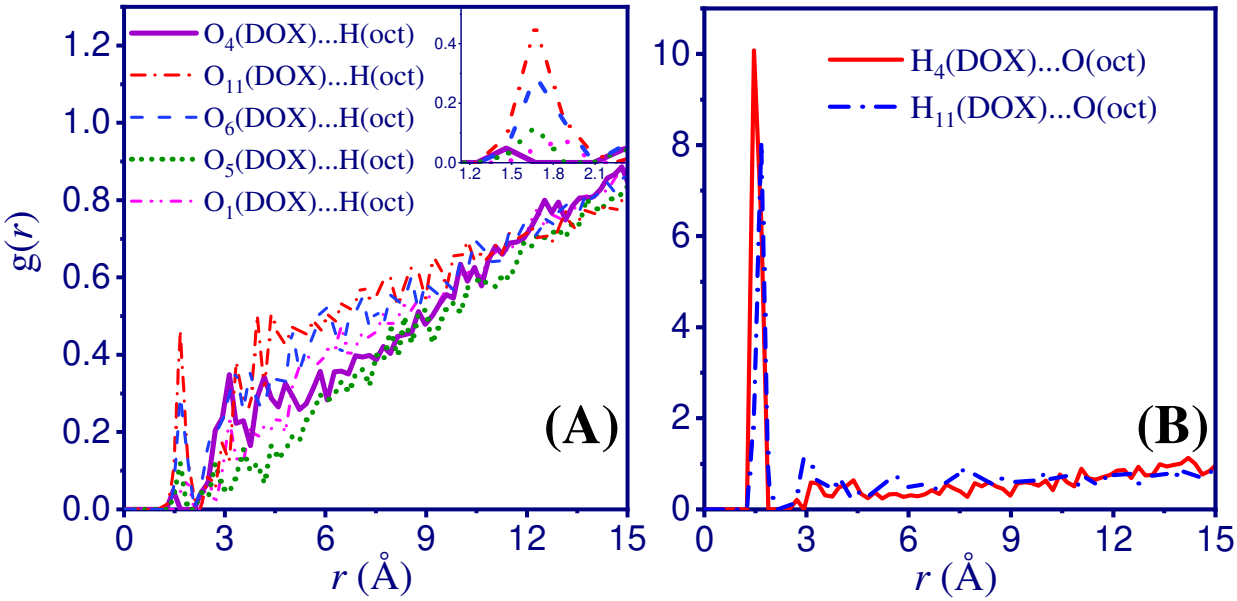

Fig. S24 DOX···octanol *RDF*s in the octanol/DOX/water system, (A) DOX O atoms with octanol H atoms, and (B) DOX H atoms with the octanol O atom.

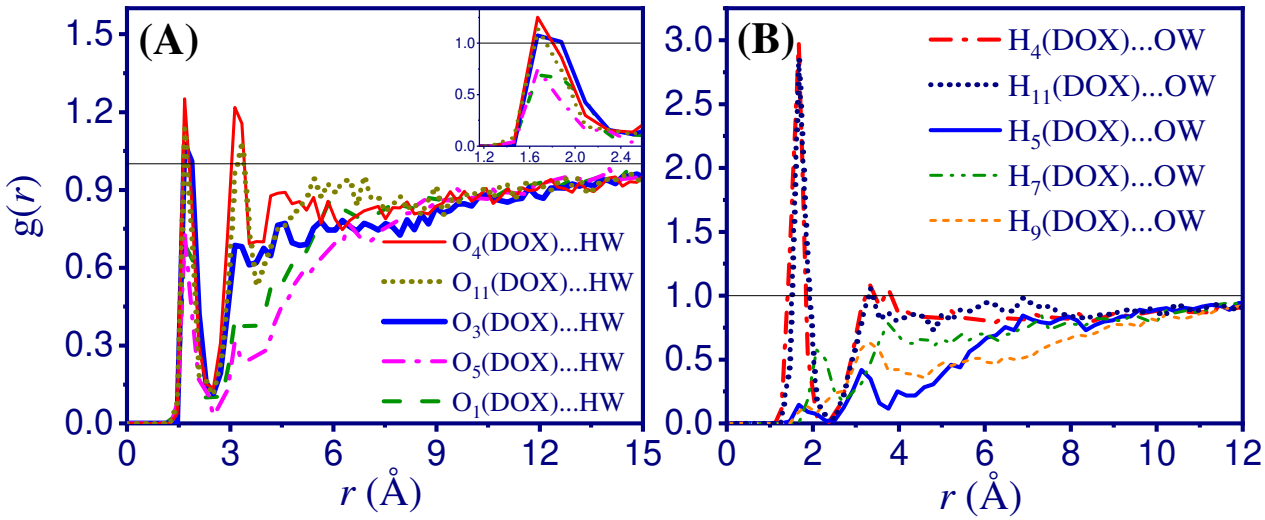

Fig. S25 DOX···water *RDF*s in the octanol/DOX/water system. (A) DOX O atoms with water H atoms, and (B) DOX H atoms of with the water O atom.

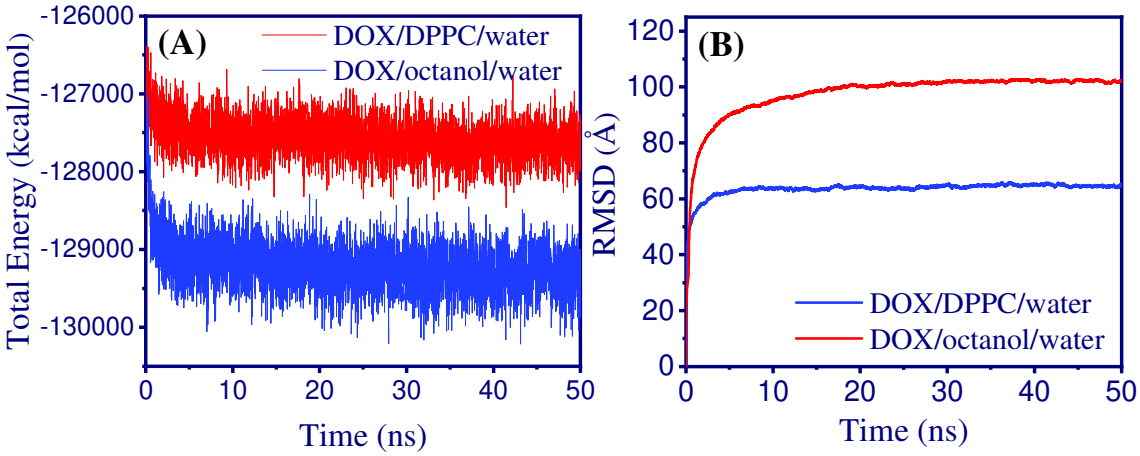

Fig. S26 (A) Total energy, and (B) *RMSD*s of the DOX/octanol/water and DOX/DPPC/water systems, indicating the system's equilibration during the simulation time.

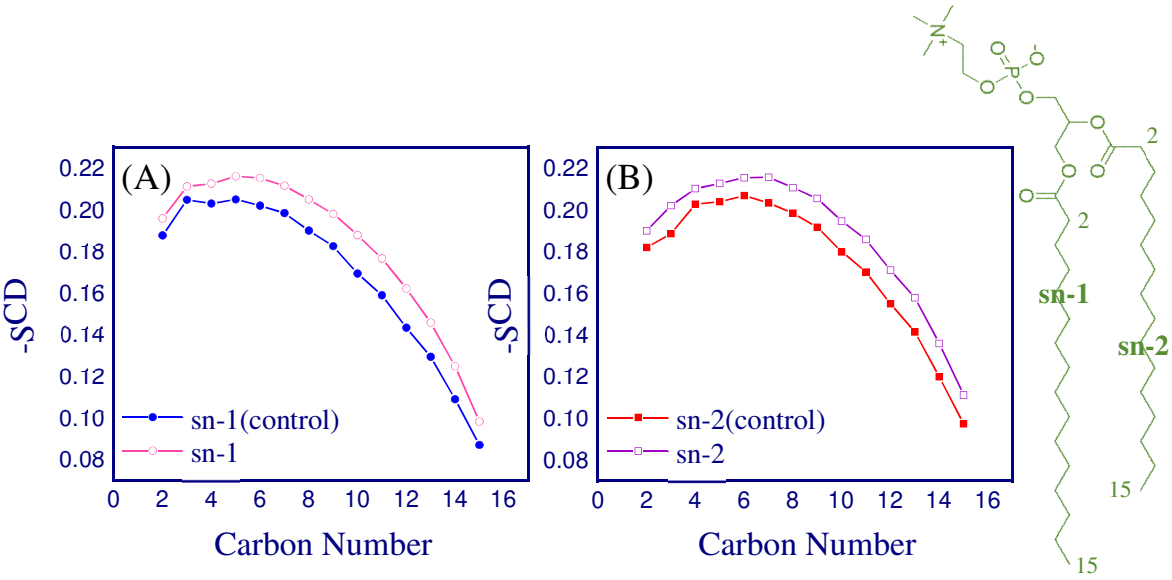

Fig. S27 Average deuterium order parameters obtained for the sn-1 and sn-2 chains of DPPC. The chemical formula of DPPC and the numbering of the C atoms in the chain is shown to the right.

Supporting Tables

Table S1 DFT-calculated partial atomic charges of DOX using the NBO method.

Atom	q/e B3LYP NBO	Atom	q/e B3LYP NBO	Atom	q/e B3LYP NBO	Atom	q/e B3LYP NBO
C_1	–0.258	C_{14}	–0.087	C_{27}	0.339	H_1	0.210
C_2	–0.218	C_{15}	0.032	O_1	–0.642	H_2	0.215
C_3	–0.201	C_{16}	0.182	O_2	–0.590	H_3	0.268
C_4	–0.112	C_{17}	0.041	O_3	–0.585	H_4	0.512
C_5	–0.149	C_{18}	0.296	O_4	–0.763	H_5	0.519
C_6	0.363	C_{19}	0.555	O_5	–0.766	H_6	0.380
C_7	0.551	C_{20}	0.297	O_6	–0.570	H_7	0.393
C_8	0.547	C_{21}	0.607	O_7	–0.520	H_8	0.109
C_9	–0.152	C_{22}	0.017	O_8	–0.526	H_9	0.505
C_{10}	0.391	C_{23}	0.135	O_9	–0.669	H_{10}	0.528
C_{11}	–0.107	C_{24}	0.28	O_{10}	–0.689	H_{11}	0.503
C_{12}	–0.137	C_{25}	0.299	O_{11}	–0.779		
C_{13}	0.352	C_{26}	0.031	N	–0.937		

Table S2 DFT-calculated partial atomic charges of EtOH.

Atom	q/e B3LYP NBO	Atom	q/e B3LYP NBO
C	–0.125	H	0.198
C	–0.72	H	0.241
O_{EtOH}	–0.776	H	0.243
H_{EtOH}	0.487	H	0.227
H	0.225		

Table S3 DFT-calculated partial atomic charges of DMSO.

Atom	q/e B3LYP NBO	Atom	q/e B3LYP NBO
C	–0.896	H	0.257
C	–0.896	H	0.251
O_{DMSO}	–0.967	H	0.269
S	1.205	H	0.251
H	0.269	H	0.257

Table S4 DFT-calculated partial atomic charges of DMF.

Atom	q/e B3LYP NBO	Atom	q/e B3LYP NBO
C	0.518	H	0.231
C	–0.467	H	0.239
C	–0.482	H	0.231
O_{DMF}	–0.635	H	0.228
N	–0.51	H	0.271
H	0.148	H	0.228

Table S5 DFT-calculated partial atomic charges of octanol.

Atom	q/e B3LYP NBO	Atom	q/e B3LYP NBO	Atom	q/e B3LYP NBO
C_{oct}	–0.684	H_{oct}	0.489	H	0.230
C	–0.472	H	0.238	H	0.233
C	–0.462	H	0.231	H	0.231
C	–0.461	H	0.231	H	0.230
C	–0.460	H	0.231	H	0.224
C	–0.468	H	0.231	H	0.242
C	–0.496	H	0.230	H	0.238
C	–0.114	H	0.230	H	0.228
O_{oct}	–0.776	H	0.230	H	0.196

Table S6 DFT-calculated partial atomic charges of DOX using the CHELPG method.

Atom	CHELPG	Atom	CHELPG	Atom	CHELPG	Atom	CHELPG
C_1	–0.149	C_{14}	0.610	C_{27}	0.006	H_1	0.139
C_2	–0.095	C_{15}	–0.610	O_1	–0.074	H_2	0.100
C_3	–0.005	C_{16}	0.490	O_2	–0.068	H_3	0.094
C_4	–0.108	C_{17}	–0.285	O_3	–0.650	H_4	0.421
C_5	–0.143	C_{18}	0.223	O_4	–0.368	H_5	0.321
C_6	0.275	C_{19}	1.105	O_5	–0.657	H_6	0.299
C_7	0.496	C_{20}	–0.355	O_6	–0.105	H_7	0.278
C_8	0.591	C_{21}	0.006	O_7	–0.386	H_8	0.250
C_9	–0.205	C_{22}	–0.174	O_8	–0.515	H_9	0.244
C_{10}	0.380	C_{23}	–0.200	O_9	–0.291	H_{10}	0.319
C_{11}	–0.375	C_{24}	0.066	O_{10}	–0.510	H_{11}	0.349
C_{12}	–0.111	C_{25}	0.224	O_{11}	–0.476		
C_{13}	0.014	C_{26}	–0.174	N	–0.212		

Table S7 DFT-calculated partial atomic charges of $DOXH^+$ using the RESP method.[1]

Atom	RESP	Atom	RESP	Atom	RESP	Atom	RESP
C_1	-0.155	C_{14}	-0.062	C_{27}	0.207	H_1	0.135
C_2	-0.144	C_{15}	0.157	O_1	-0.347	H_2	0.178
C_3	-0.105	C_{16}	0.251	O_2	-0.297	H_3	0.154
C_4	-0.187	C_{17}	0.042	O_3	-0.509	H_4	0.429
C_5	-0.163	C_{18}	0.026	O_4	-0.679	H_5	0.485
C_6	0.274	C_{19}	0.416	O_5	-0.755	H_6	0.488
C_7	0.624	C_{20}	0.352	O_6	-0.261	H_7	0.488
C_8	0.704	C_{21}	0.274	O_7	-0.573	H_8	0.126
C_9	-0.178	C_{22}	0.056	O_8	-0.480	H_9	0.411
C_{10}	0.127	C_{23}	0.143	O_9	-0.525	H_{10}	0.388
C_{11}	-0.059	C_{24}	0.201	O_{10}	-0.432	H_{11}	0.484
C_{12}	-0.196	C_{25}	0.155	O_{11}	-0.643		
C_{13}	0.236	C_{26}	0.085	N	-0.346		

Table S8 DFT-calculated partial atomic charges of DOX using the RESP method.[1]

Atom	RESP	Atom	RESP	Atom	RESP	Atom	RESP
C_1	-0.127	C_{14}	-0.035	C_{27}	0.211	H_1	0.151
C_2	-0.166	C_{15}	0.162	O_1	-0.356	H_2	0.160
C_3	-0.076	C_{16}	0.218	O_2	-0.286	H_3	0.144
C_4	-0.243	C_{17}	0.043	O_3	-0.514	H_4	0.423
C_5	-0.039	C_{18}	0.034	O_4	-0.679	H_5	0.415
C_6	0.203	C_{19}	0.430	O_5	-0.715	H_6	0.398
C_7	0.569	C_{20}	0.330	O_6	-0.355	H_7	0.398
C_8	0.724	C_{21}	0.195	O_7	-0.463	H_8	0.138
C_9	-0.246	C_{22}	0.084	O_8	-0.505	H_9	0.396
C_{10}	0.246	C_{23}	0.248	O_9	-0.522	H_{10}	0.386
C_{11}	-0.069	C_{24}	0.179	O_{10}	-0.495	H_{11}	0.419

C_{12}	-0.192	C_{25}	0.177	O_{11}	-0.580		
C_{13}	0.197	C_{26}	0.006	N	-1.021		

Table S9 DFT-calculated partial atomic charges of DOX in the gas phase and different solvents.

Atom	Gas Phase	water	DMSO	DMF	EtOH
O_4	–0.763	–0.787	–0.787	–0.786	–0.786
O_9	–0.669	–0.687	–0.687	–0.686	–0.686
O_{10}	–0.689	–0.711	–0.711	–0.711	–0.710
O_{11}	–0.779	–0.797	–0.796	–0.796	–0.796
N	–0.937	–0.947	–0.947	–0.947	–0.947

Table S10 MD-calculated first peak position of *RDF*s between DOX and solvent atoms.

DOX/water	r (Å)	DOX/EtOH	r (Å)	DOX/DMSO	r (Å)	DOX/DMF	r (Å)
H_4…OW	1.58	H_4…O(EtOH)	1.48	H_4…O(DMSO)	1.38	H_4…O(DMF)	1.48
H_{11}…OW	1.64	H_{11}…O(EtOH)	1.56	H_{11}…O(DMSO)	1.44	H_{11}…O(DMF)	1.54
H_5…OW	1.68	H_5…O(EtOH)	1.56	H_9…O(DMSO)	1.44	H_5…O(DMF)	1.54
H_7…OW	2.04	H_9…O(EtOH)	1.56	H_{10}…O(DMSO)	1.44	H_{10}…O(DMF)	1.64
H_6…OW	2.14	H_{10}…O(EtOH)	1.58	H_5…O(DMSO)	1.44	H_8…O(DMF)	1.90

Table S11 Average of hydrogen bond lifetimes ($\tau(HB)$) between DOX and solvent atoms.

System	Donor…Acceptor	$\tau \times 10^{-3}$(ps)
Water	OW…H_5	0.938
	OW…H_{11}	0.975
	OW…H_4	0.976
	OW…H_5	0.975
	O_{11}…HW	0.970
	O_4…HW	0.974
	O_3…HW	0.977
DMSO	O(DMSO)…H_{11}	5.495
	O(DMSO)…H_9	5.495
	O(DMSO)…H_4	5.495
EtOH	O(EtOH)…H_5	5.020
	O(EtOH)…H_{11}	5.379
	O(EtOH)…H_4	5.422
	O_5…H(EtOH)	5.495
	O_9…H(EtOH)	1.611
	O_{11}…H(EtOH)	5.495
	O_4…H(EtOH)	3.673
DMF	O(DMF)…H_5	4.653
	O(DMF)…H_{11}	4.382
	O(DMF)…H_4	4.538

Table S12 Calculated first peaks of *RDF*s between different pairs of atoms in DPPC/DOX/water and octanol/DOX/water systems.

DPPC/DOX/water				octanol/DOX/water			
DOX/DPPC	***r* (Å)**	**DOX/water**	***r* (Å)**	**DOX/octanol**	***r* (Å)**	**DOX/water**	***r* (Å)**
$H_{11}…O_9$(DPPC)	1.53	$O_{11}…HW$	1.84	$O_4…H$(oct)	1.46	$O_4…HW$	1.67
$H_4…O_9$(DPPC)	1.53	$O_4…HW$	1.84	$O_{11}…H$(oct)	1.67	$O_{11}…HW$	1.67
$H_8…O_9$(DPPC)	1.84	$O_3…HW$	1.84	$O_6…H$(oct)	1.67	$O_3…HW$	1.67
$H_9…O_9$(DPPC)	1.84	$O_5…HW$	1.84	$O_5…H$(oct)	1.67	$O_5…HW$	1.67
$H_6…O_9$(DPPC)	2.46	$O_1…HW$	1.84	$O_1…H$(oct)	1.87	$O_1…HW$	1.67
$H_{11}…O_{10}$(DPPC)	1.53	$H_4…OW$	1.53	$H_4…O$(oct)	1.46	$H_4…OW$	1.67
$H_9…O_{10}$(DPPC)	1.53	$H_{11}…OW$	1.53	$H_{11}…O$(oct)	1.67	$H_{11}…OW$	1.67
$H_8…O_{10}$(DPPC)	1.53	$H_9…OW$	1.84	-------	-------	$H_5…OW$	1.67
$H_6…O_{10}$(DPPC)	1.84	$H_7…OW$	2.15	-------	-------	$H_7…OW$	2.08
$H_3…O_{10}$(DPPC)	2.46	$H_6…OW$	2.15	-------	-------	$H_9…OW$	2.50

References

1 A. Jagusiak and T. Pańczyk, Interaction of Congo Red, Evans Blue and Titan Yellow with doxorubicin in aqueous solutions. A molecular dynamics study, *J. Mol. Liq.*, 2019, **279**, 640–648.