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

Supporting Information

Aggregation patterns of curcumin and piperine mixtures in different polar media

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Simulation box and atomic charges

Table S1: Composition and box side of each system employed in the MD simulations using pure ethanol and water as solvents.

# CEK molecules	# H_2O molecules	# Ethanol molecules	box side (nm)
1	9560	2886	6.60385
2	18995	5784	8.32034
4	38264	-	10.48297
# CKK molecules			
1	9563	2887	6.60385
2	19000	5778	8.32034
4	38267	-	10.48297
# PIP molecules			
1	9564	2885	6.60385
2	19000	5786	8.32034
4	38282	-	10.48297
# CEK:PIP molecules			
1:1	18997	5778	8.32034
2:2	38273	11743	10.48297
# CKK:PIP molecules			
1:1	18995	5784	8.32034
2:2	38277	11740	10.48297

Table S2: Composition and box side of each system employed in the MD simulations using ethanol:water (30:70) mixture as solvent.

# CEK molecules	# H_2O molecules	# Ethanol molecules	box side (nm)
1	6273	1030	6.60385
2	12561	2068	8.32034
4	25622	4127	10.48297
# CKK molecules			
1	6273	1029	6.60385
2	12557	2066	8.32034
4	25626	4127	10.48297
# PIP molecules			
1	6274	1029	6.60385
2	12569	2065	8.32034
# CEK:PIP molecules			
1:1	12557	2066	8.32034
2:2	25615	4134	10.48297
# CKK:PIP molecules			
1:1	12563	2067	8.32034
2:2	25622	4131	10.48297

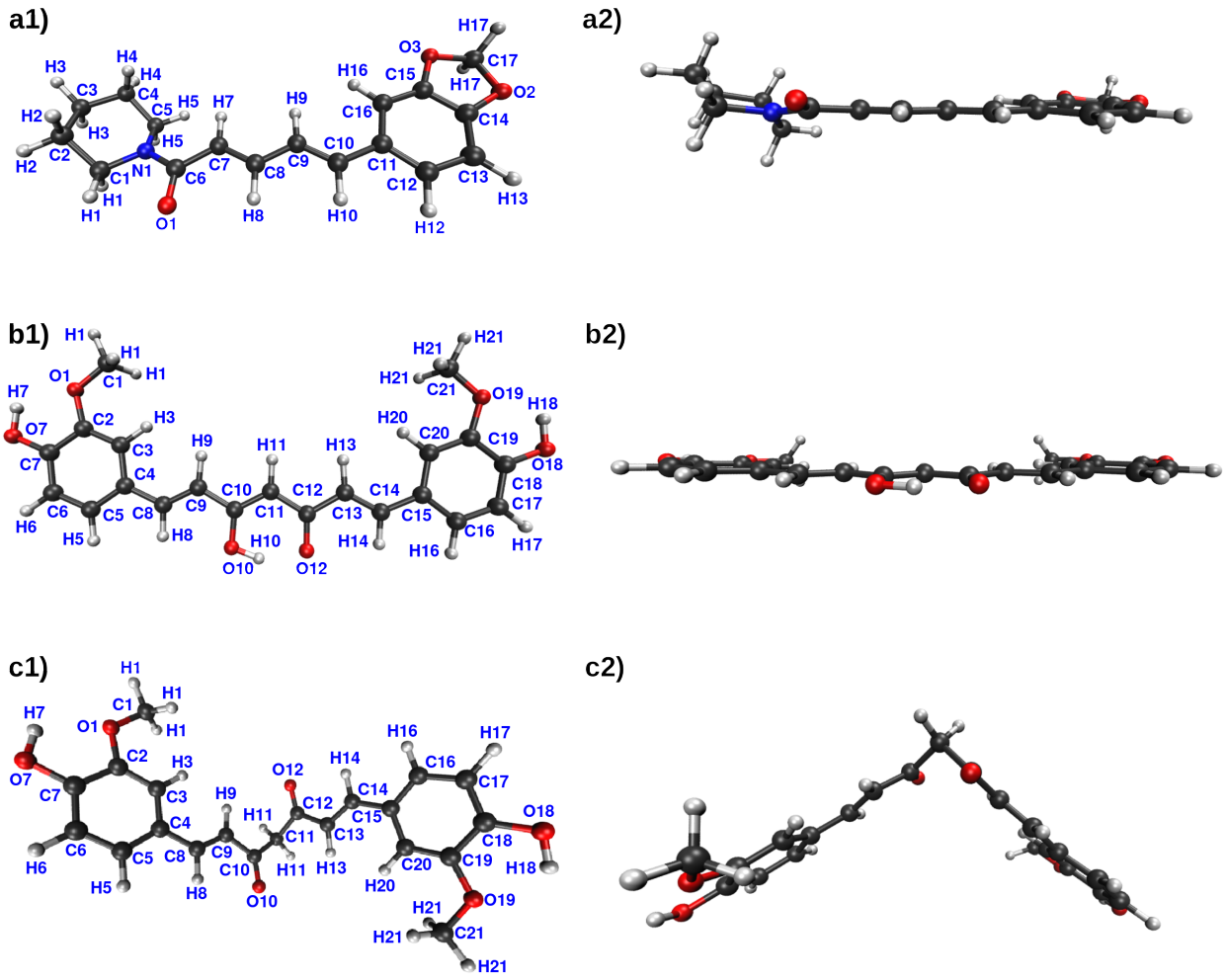

Figure S1: Molecular structures of the solute molecules from two different views: a1) PIP (front view); a2) PIP (side view); b1) CEK (front view); b2) CEK (side view); c1) CKK (front view); c2) CKK (side view). In the left views, the corresponding force-field label is shown for each atom of the molecule. Equivalent atoms have the same label.

Table S3: Atomic charges of piperine (PIP) used in the MD simulations. Labels for the atoms are shown in Figure S1.

PIP					
Atom	Charge	Atom	Charge	Atom	Charge
C1	0.0185	C13	-0.3372	H4	0.0141
C2	-0.0130	C14	0.3375	H5	0.0383
C3	-0.0443	C15	0.2193	H7	0.1320
C4	0.0085	C16	-0.2373	H8	0.1318
C5	-0.0336	C17	0.2377	H9	0.1317
C6	0.3689	O1	-0.4761	H10	0.0981
C7	-0.2563	O2	-0.3499	H12	0.1396
C8	-0.0443	O3	-0.3313	H13	0.1851
C9	-0.1601	N1	-0.0828	H16	0.1567
C10	-0.0547	H1	0.0371	H17	0.0757
C11	-0.0295	H2	0.0216	-	-
C12	-0.1172	H3	0.0143	-	-

Table S4: Atomic charges of CEK and CKK used in the MD simulations. Labels for the atoms are shown in Figure S1. Only non-equivalent atoms are indicated.

CEK				CKK			
Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
C1	-0.1112	O7	-0.4965	C1	-0.1030	O1	-0.2231
C2	0.1220	O10	-0.5384	C2	0.1084	O7	-0.5018
C3	-0.1467	O12	-0.5475	C3	-0.1599	O10	-0.4663
C4	-0.0505	O18	-0.4981	C4	-0.0139	O12	-0.4662
C5	-0.1847	O19	-0.2158	C5	-0.1835	O18	-0.4918
C6	-0.2079	H1	0.0949	C6	-0.2149	O19	-0.2288
C7	0.2223	H3	0.1257	C7	0.2469	H1	0.0949
C8	0.0013	H5	0.1577	C8	-0.0417	H3	0.1492
C9	-0.3515	H6	0.1747	C9	-0.2665	H5	0.1530
C10	0.4862	H7	0.3874	C10	0.4101	H6	0.1724
C11	-0.5785	H8	0.1315	C11	-0.0696	H7	0.3883
C12	0.5870	H9	0.1756	C12	0.4095	H8	0.1335
C13	-0.3793	H10	0.4434	C13	-0.2861	H9	0.1245
C14	0.0133	H11	0.1609	C14	-0.0450	H11	0.0391
C15	-0.0446	H13	0.1643	C15	-0.0094	H13	0.1430
C16	-0.1861	H14	0.1183	C16	-0.1690	H14	0.1368
C17	-0.2243	H16	0.1608	C17	-0.2237	H16	0.1476
C18	0.2424	H17	0.1773	C18	0.2282	H17	0.1768
C19	0.1067	H18	0.3829	C19	0.1307	H18	0.3849
C20	-0.1442	H20	0.1304	C20	-0.1643	H20	0.1358
C21	-0.1006	H21	0.0908	C21	-0.0897	H21	0.0919
O1	-0.2228	-	-	-	-	-	-

Mixed solvent box

To build a simulation box containing ethanol and water 30 % (m/m) at 25°C, we adopted the following procedure (this was also used previously to build acetonitrile and water boxes in Ref. 1 by two of the authors, but it was not described there). The density of the mixture was obtained from the Engineering Toolbox website[2] ($\rho = 0.951\,\mathrm{g\,cm^{-3}}$) and we chose to simulate a cubic box of 100 Å side. In this box we placed 3734 molecules of ethanol and 22271 molecules of TIP4P-2005 water using packmol.[3] The number of molecules was calculated to replicate the density and the concentration of the solution. The ethanol force field parameters were obtained from Refs. 4 and 5 where the authors created a 20 % (m/m) ethanol/water box for studies of solvent mapping of proteins, and were generated and converted from AMBER format to a format suitable to be used in GROMACS with acpype. After generating the initial configuration we used the conventional MD procedure: a minimization step, and two equilibration steps (an NVT ensemble followed by an NPT ensemble, each of duration of 500 ps) which allowed the system to reach the temperature of 298.15 K and pressure of 1 bar. The production stage of the MD simulation was run for 100 ns, and used an NVT ensemble. We used the same parameters as the ones used in the simulations of the main system described in the text. The last frame of the simulation was used as the solvation box used in subsequent simulations. The density of the production run is $0.950\,\mathrm{g\,cm^{-3}}$ with a root mean square deviation of $1.66 \times 10^{-3}\,\mathrm{g\,cm^{-3}}$ in accord to the experimental value.
This box, the system coordinates and topology files can be downloaded from:
https://github.com/peabreu/Mixed-Solvents/tree/main/ETA+WAT30

Atom-atom RDFs

Only the most relevant RDF plots are represented here; all the remaining ones are available in the "additionalmaterials" file at:

`https://github.com/comput-chem-uc/Curcumin_Piperine.git`

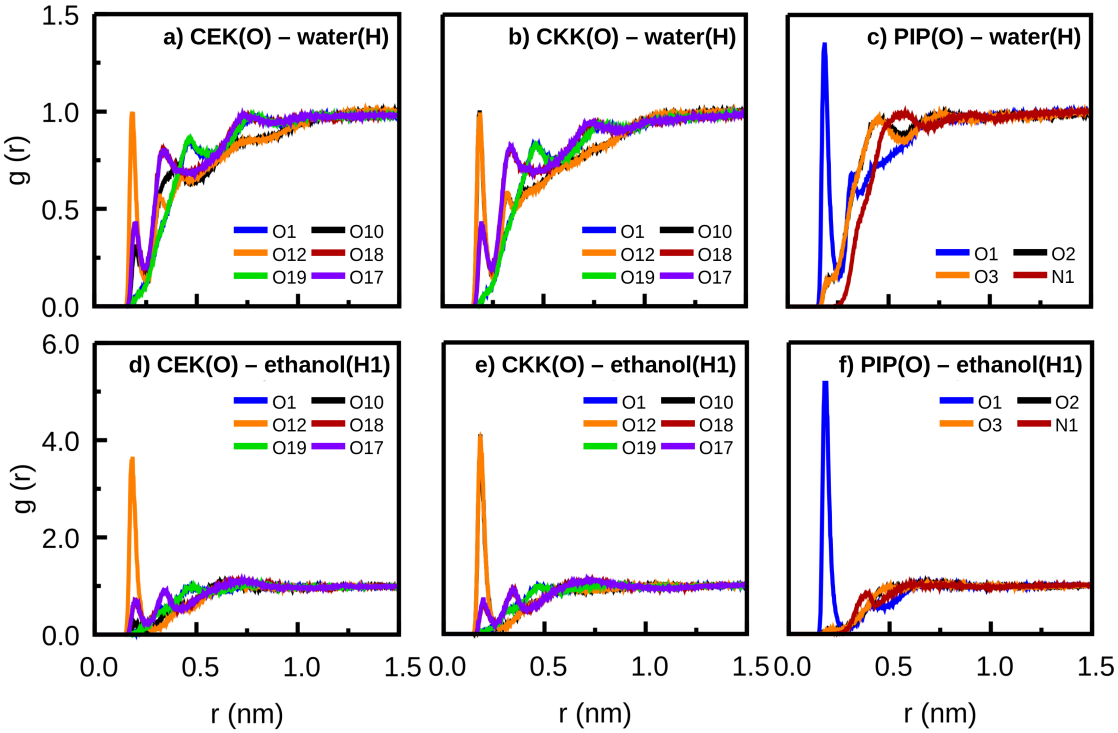

Figure S2: Solute-solvent atom-atom RDFs between one of the oxygens of the solute molecules and the hydrogen of water (a-c) or ethanol (d-f) for three simulations of distinct pairs of solute molecules in water (a-c) or ethanol (d-f): a) CEK-CEK; b) CKK-CKK; e) PIP-PIP. The represented oxygens are identified in Figure S1, the H1 of ethanol stands for the hydrogen of the -OH group of ethanol. Note that, in panels b and e, O10 line is not visible due to the overlap with the O12 line.

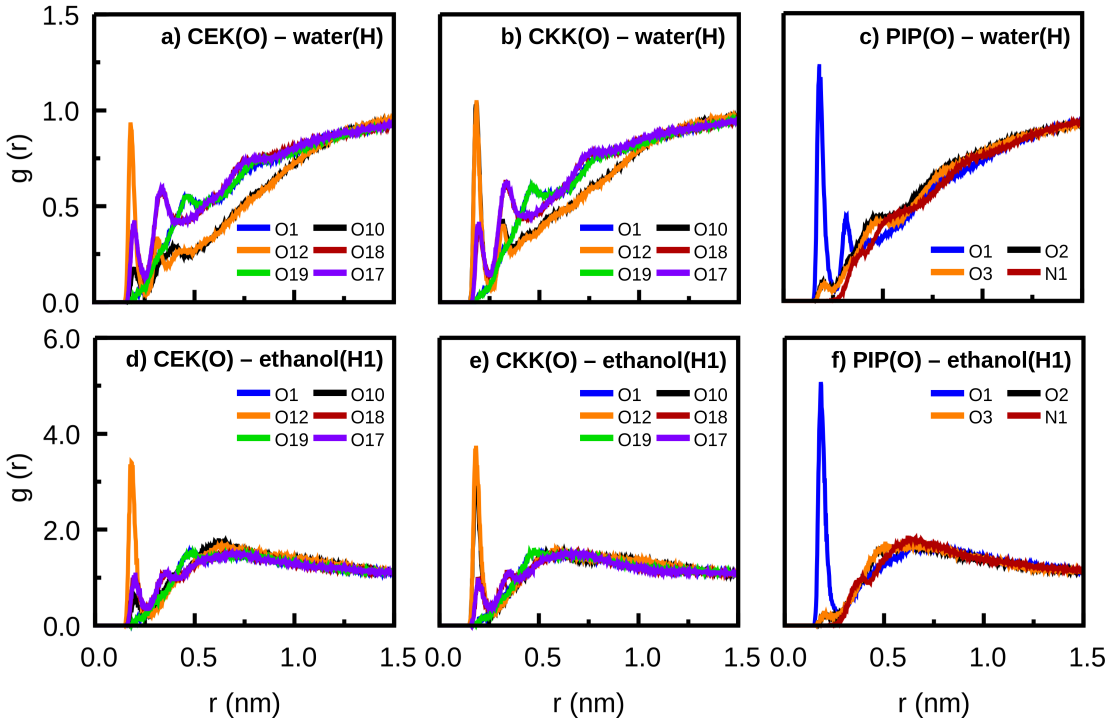

Figure S3: Solute-solvent atom-atom RDFs between one of the oxygens of the solute molecules and the hydrogen of water (a-c) or ethanol (d-f) for three simulations of distinct pairs of solute molecules in (30:70) solvent mixture: a) CEK-CEK; b) CKK-CKK; e) PIP-PIP. The represented oxygens are identified in Figure S1, the H1 of ethanol stands for the hydrogen of the -OH group of ethanol. Note that, in panels b and e, O10 line is not visible due to the overlap with the O12 line.

Movies of CEK-CEK and CKK-CKK dimers in ethanol

Movies showing how both CEK-CEK and CKK-CKK dimers can be separated apart by the action of the ethanol; for clarity, the solvent molecules are not displayed in the movies. The corresponding trajectories begin with the dimers, which dissociate into the monomers after a few nanoseconds.

Simulations with the (30:70) solvent mixture

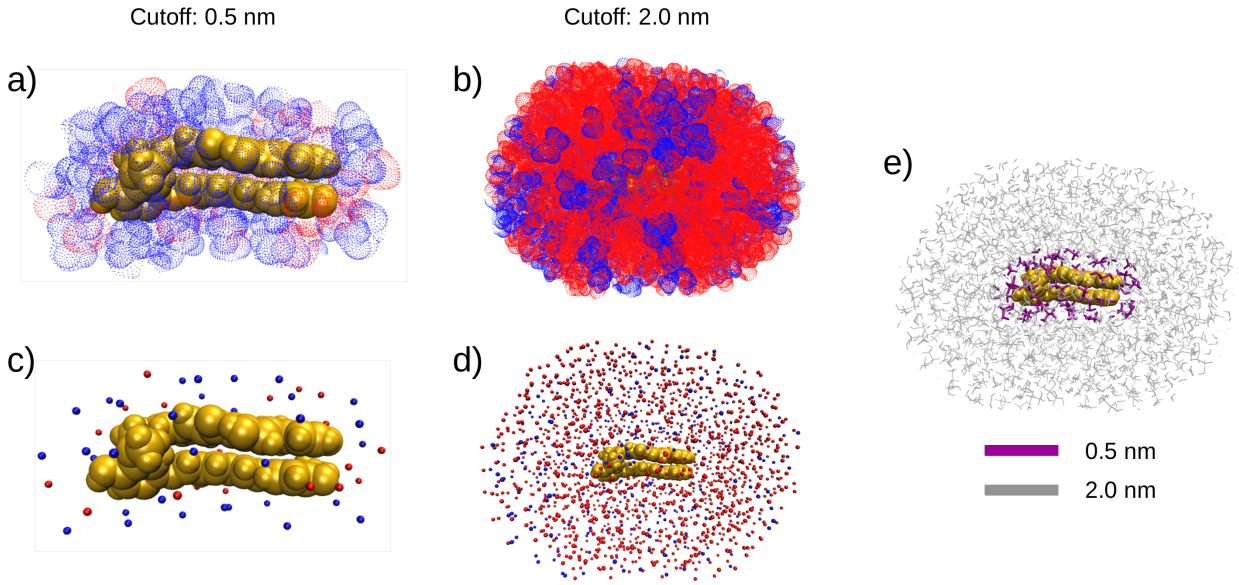

Figure S4: Typical trajectory-frame representing the solvation around the CEK dimer (yellow) in the solvent mixture of ethanol+water. A cutoff of 0.5 nm (a and c) and 2 nm (b and d) to the CEK surface for the solvent that is represented. Key for colors of a-d: blue for ethanol and red for water. Panel e) illustrates schematically how the two cutoffs employed in panels a and c (0.5 nm, with solvent molecules assigned in purple) and panels b and d (2 nm, with solvent molecules assigned in grey) are defined.

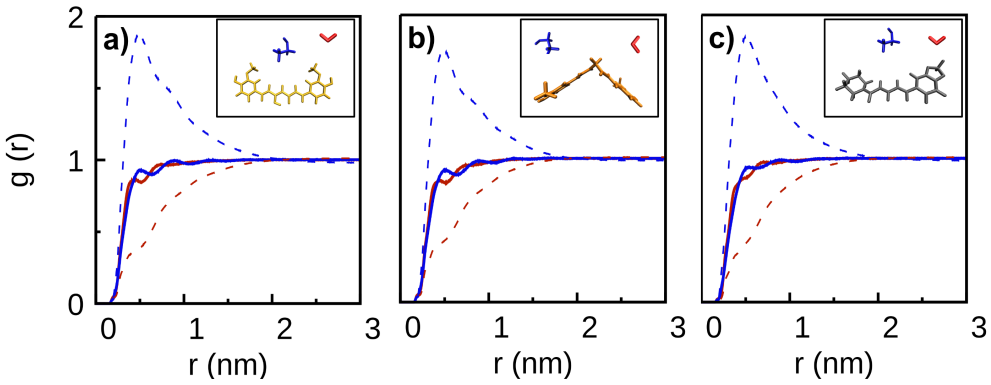

Figure S5: Solute-solvent RDFs for simulations with a single CEK (a), CKK (b) and PIP (c) molecules in a (30:70) solvent mixture. Center-of-mass RDFs related to water (ethanol) are represented by red (blue) lines; solid lines refer to simulations in pure solvents (water or ethanol), while dashed curves are for the (30:70) solvent mixture. The inserts represent the corresponding monomers, as well as one molecule of water (in red) and one molecule of ethanol (in blue).

Energetic analysis

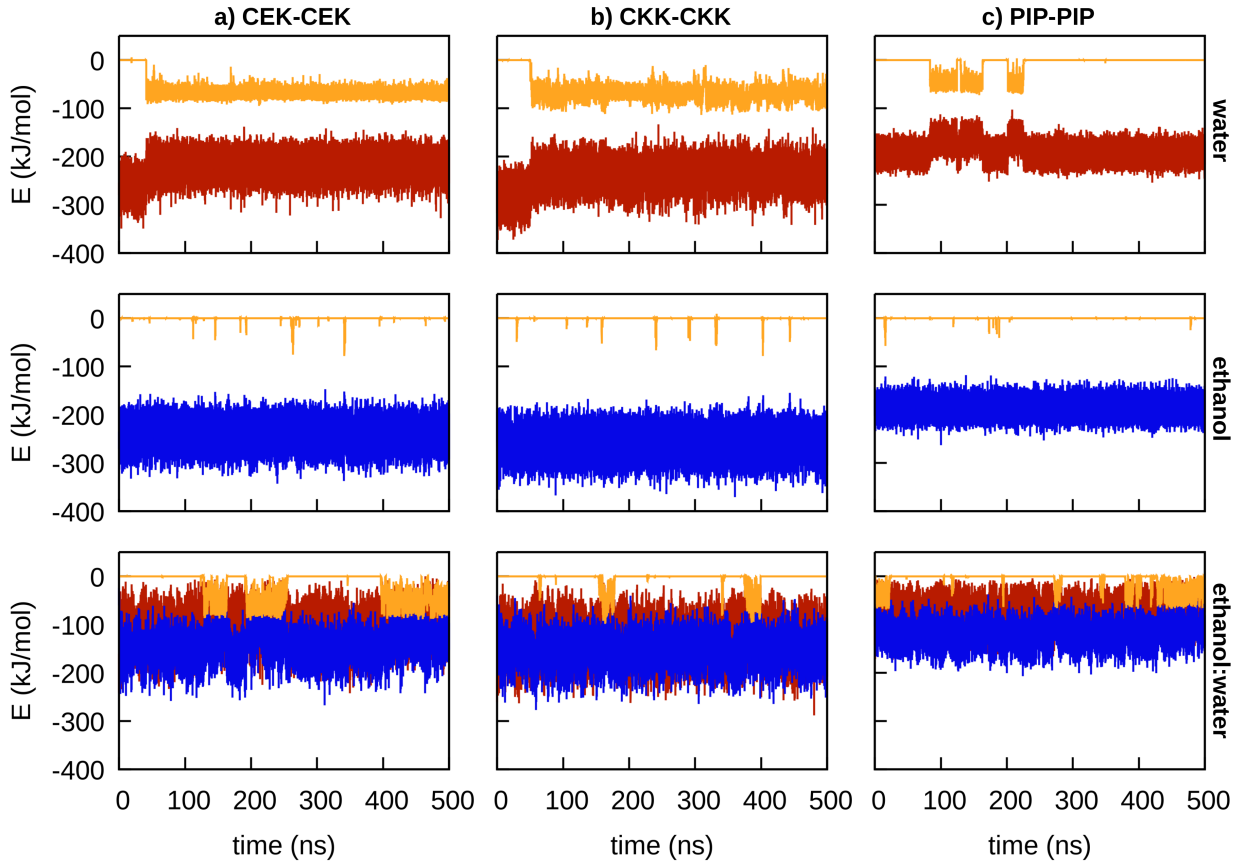

Figure S6: Solute-solute (yellow), solute-water (red), and solute-ethanol (blue) interaction energies as a function of time for simulations of distinct pairs of solute molecules in water (upper), ethanol (middle), and solvent mixture (bottom): a) CEK-CEK; b) CKK-CKK; c) PIP-PIP. Regarding the solute-solvent energy, for clearness, only the component corresponding to the interaction of one molecule of solute with the solvent is represented, since the component involving the other solute molecule with the solvent originates a similar plot.

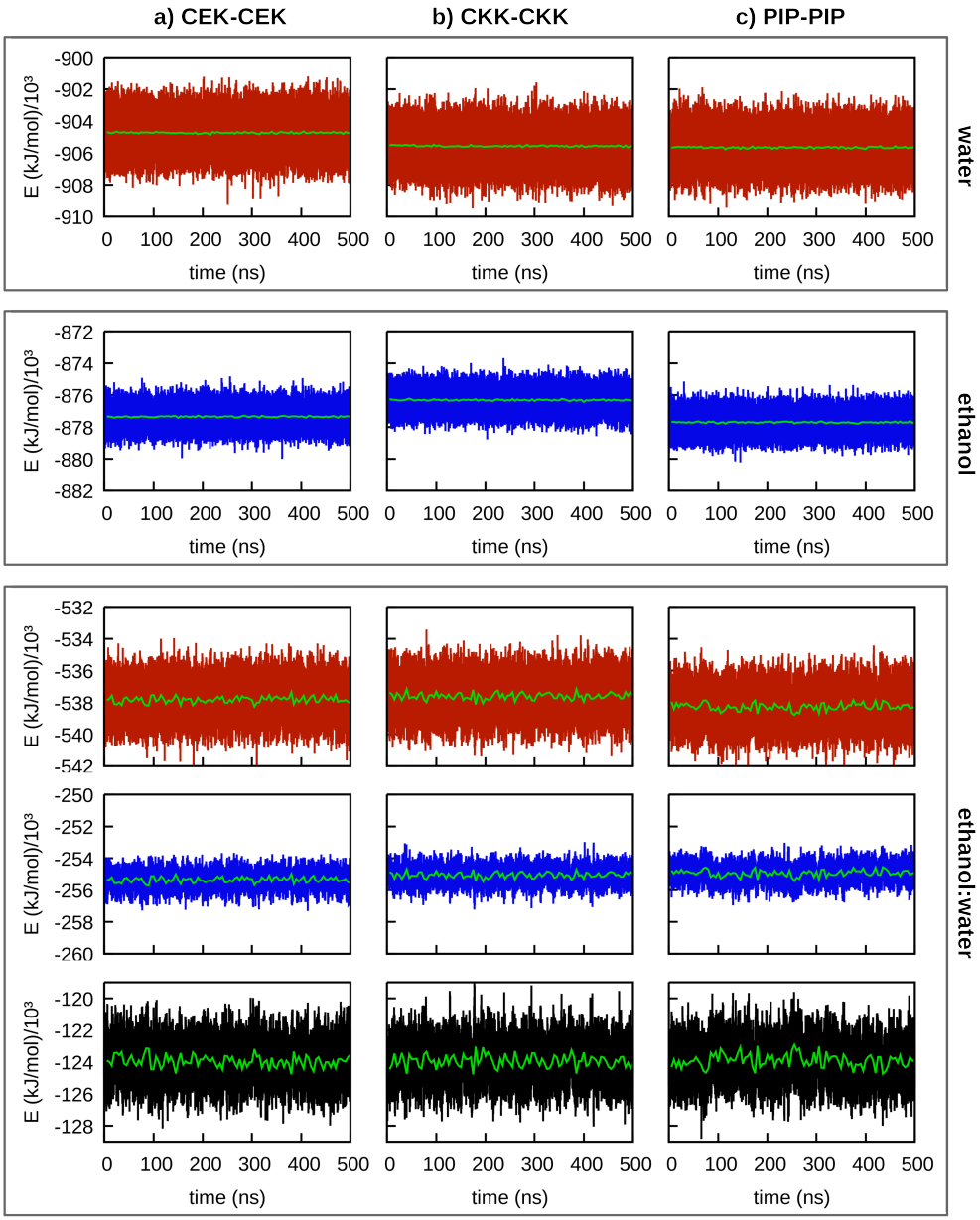

Figure S7: Water-water (red), ethanol-ethanol (blue), and water-ethanol (black) interaction energies as a function of time for simulations of distinct pairs of solute molecules in water (upper), ethanol (middle), and solvent mixture (bottom): a) CEK-CEK; b) CKK-CKK; c) PIP-PIP. The green lines represent an average energy value calculated for each 5 ns of time interval.

Table S5: Average values of interaction energies obtained from the plots of Fig. S7.

	CEK-CEK	CKK-CKK	PIP-PIP
Pure Solvents Simulations			
$E_{water-water}/10^3\,\mathrm{kJ\,mol^{-1}}$	-904.74 ± 0.95	-905.57 ± 0.95	-905.66 ± 0.94
$E_{ethanol-ethanol}/10^3\,\mathrm{kJ\,mol^{-1}}$	-877.35 ± 0.58	-876.31 ± 0.59	-877.71 ± 0.58
Solvent Mixture (30:70) Simulations			
$E_{water-water}/10^3\,\mathrm{kJ\,mol^{-1}}$	-537.82 ± 0.96	-537.60 ± 0.97	-538.27 ± 0.97
$E_{ethanol-ethanol}/10^3\,\mathrm{kJ\,mol^{-1}}$	-255.35 ± 0.48	-255.05 ± 0.47	-254.94 ± 0.48
$E_{ethanol-water}/10^3\,\mathrm{kJ\,mol^{-1}}$	-123.95 ± 1.08	-123.94 ± 1.06	-123.91 ± 1.08

Solute-solute and solute-solvent hydrogen bonds

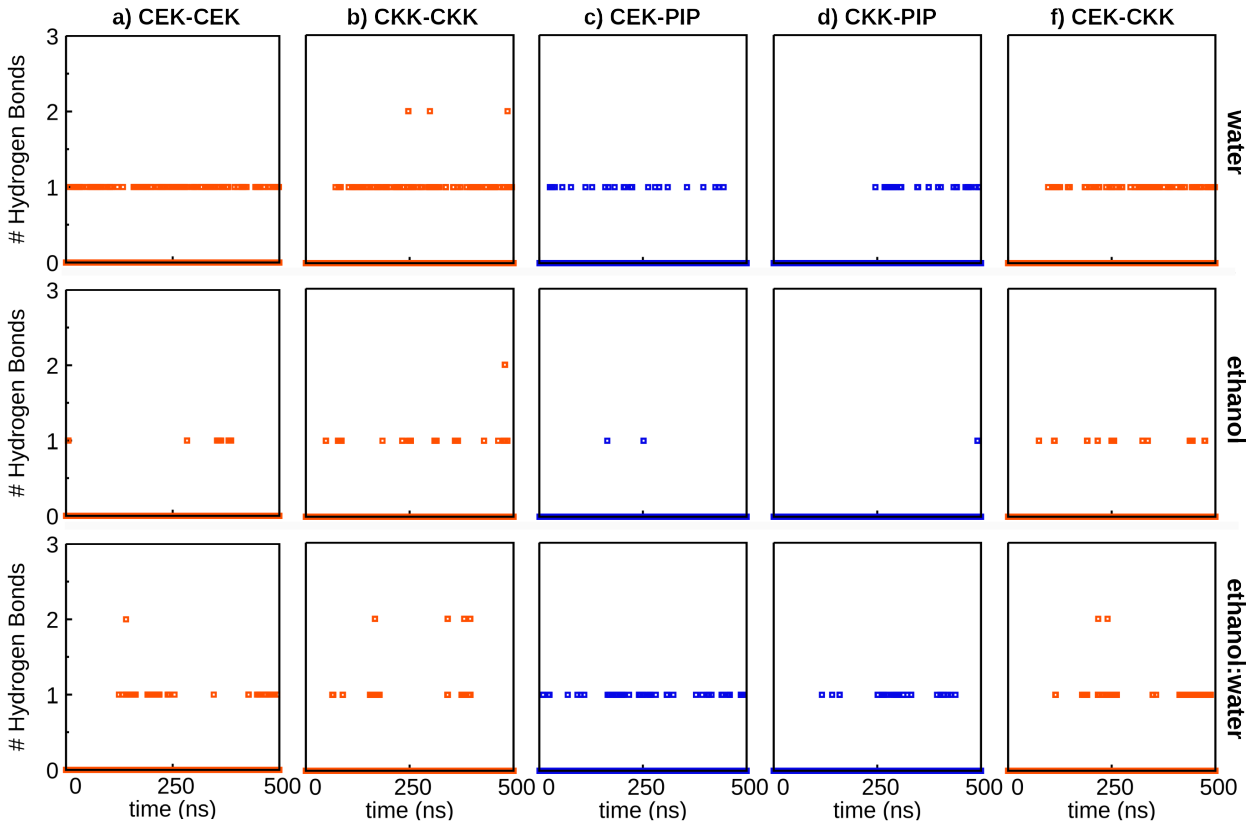

Figure S8: Solute-solute number of hydrogen bonds of six simulations of distinct pairs of solute molecules in water, (30:70) solvent mixture, and ethanol: a) CEK-CEK; b) CKK-CKK; c) CEK-PIP; d) CKK-PIP; f) CEK-CKK. For defining a hydrogen bond, we have employed the default angle and distance criterion implemented in GROMACS.

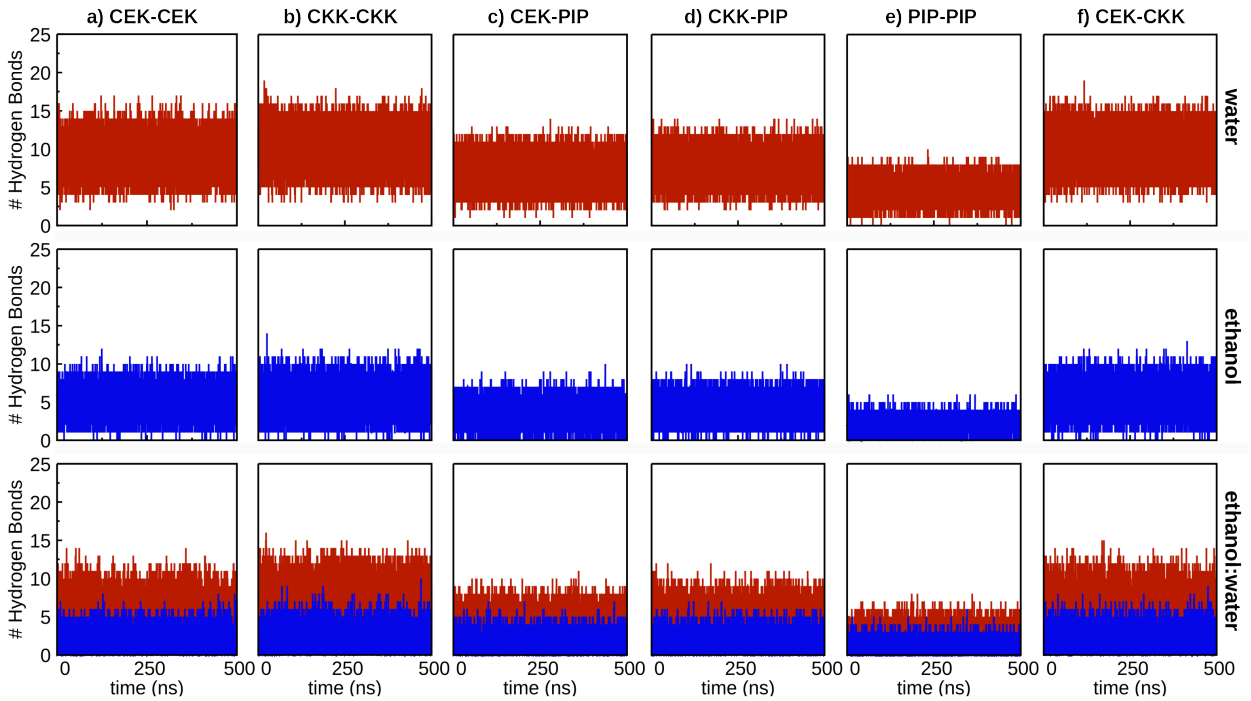

Figure S9: Solute-solvent number of hydrogen bonds of six simulations of distinct pairs of solute molecules in water, eta:water or ethanol solution: a) CEK-CEK; b) CKK-CKK; c) CEK-PIP; d) CKK-PIP; e) PIP-PIP; f) CEK-CKK.; The number of hydrogen bonds is calculated between each solute and water (red) or ethanol (blue lines). For defining a hydrogen bond, we have employed the default angle and distance criterion implemented in GROMACS.

Simulation of 2CEK+2CKK in ethanol

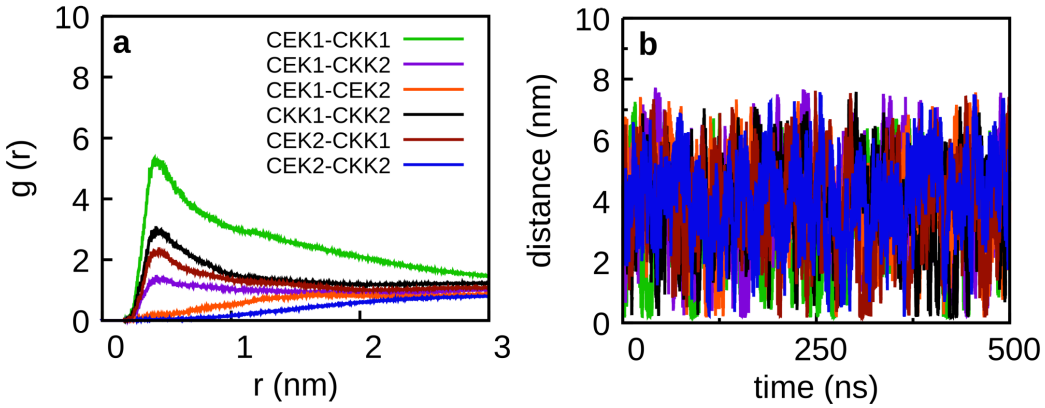

Figure S10: Simulation of 2CEK + 2CKK molecules in ethanol: a) RDFs for pairs of solute molecules; b) distance between pairs of solute molecules. Such molecules are labelled as CEK1, CEK2, CKK1 and CKK2. Key for colors inserted in panel a also applies for panel b.

Additional plots from curcumin-piperine simulations

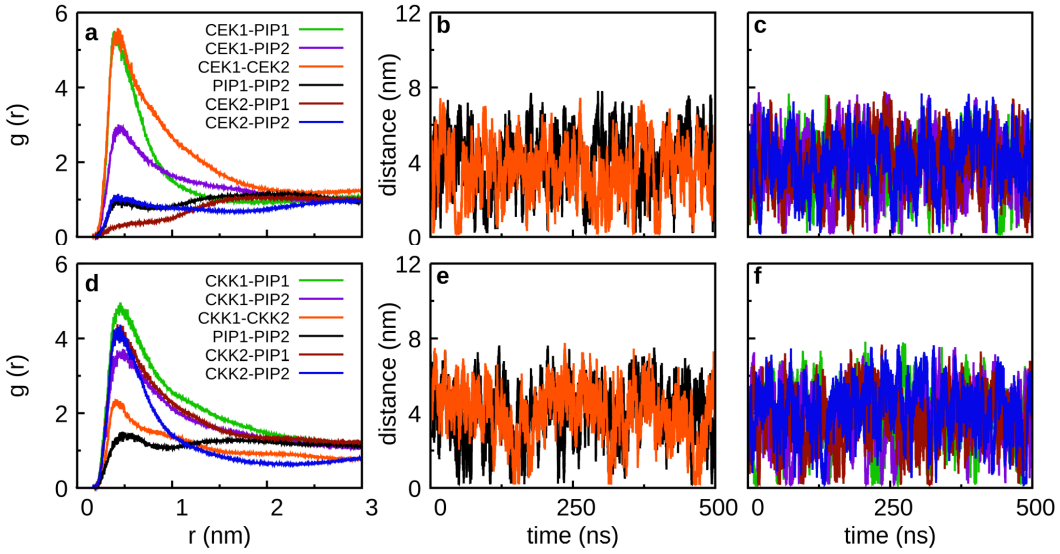

Figure S11: Simulation of 2CEK + 2PIP (panels a-c) and 2CKK + 2PIP (panels d-f) in ethanol: RDFs for pairs of solute molecules (panels a and d) and the corresponding inter-monomer distances (panels a, c, e, and f). Monomers are labelled as CEK1, CEK2, CKK1, CKK2, PIP1, and PIP2. Key for colors inserted in panel a (or d) also applies for panels b and c (or e and f).

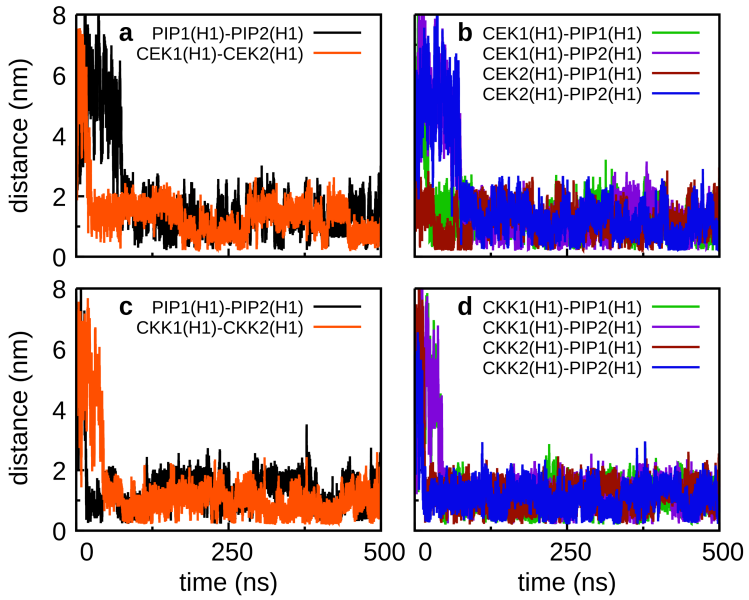

Figure S12: Simulations of 2CEK+2PIP (top panels) and 2CKK+2PIP (bottom panels) in water. Panels a and c (panels b and d) represent distances between H1 atoms of alike (distinct) monomers.

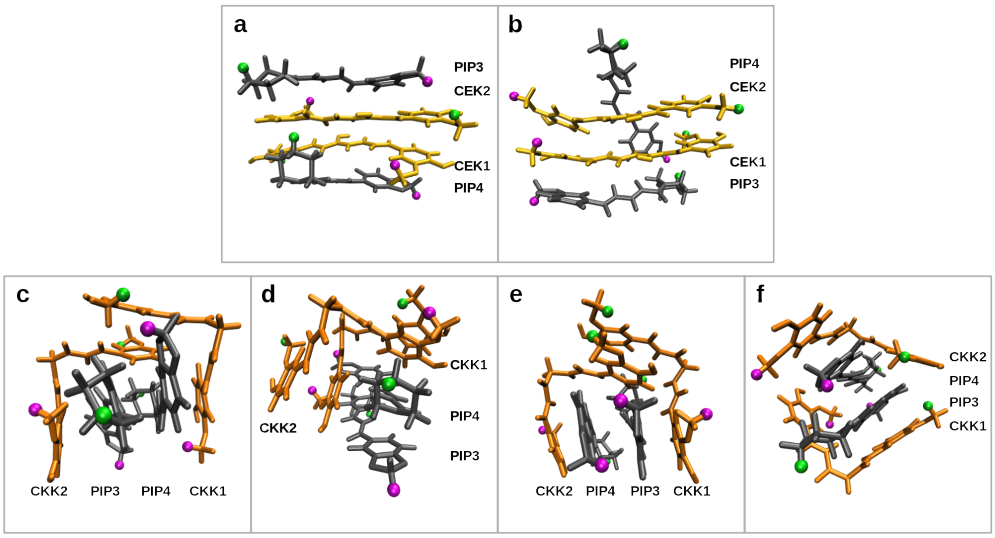

Figure S13: Most representative structures of 2CEK+2PIP (panels a and b) and 2CKK+2PIP (panels c-f) obtained by cluster analysis of the corresponding MD trajectories performed in water. The frequencies of appearance of the represented structures are: 19% (a), 10% (b), 34% (c), 7% (d), 7% (e), and 6% (f). RMSD cutoffs are (in nm): 0.25 (panels a and b), 0.20 (panels c-f). Key for colors: CEK (yellow), CKK (orange) and PIP (dark grey); H1 atoms are displayed in green, while the H15 (H18) ones of curcumin (piperine) are in magenta.

References

(1) Cunha, C.; Peixoto, M. S.; Santos, J. R.; Abreu, P. E.; Paixão, J. A.; Pineiro, M.; Seixas de Melo, J. S., Practical Design of 3,6-Di-tert-butyldiphenyldibenzofulvene Derivatives with Enhanced Aggregation-Induced Emission. *ACS Applied Optical Materials*, in press. DOI: 10.1021/acsaom.2c00067.

(2) `https://www.engineeringtoolbox.com/ethanol-water-mixture-density-d_2162.html`.

(3) Martínez, L.; Andrade, R.; Birgin, E. G.; Martínez, J. M. PACKMOL: A package for building initial configurations for molecular dynamics simulations. *Journal of Computational Chemistry* **2009**, *30*, 2157–2164, _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/jcc.21224.

(4) Alvarez-Garcia, D.; Barril, X. Molecular Simulations with Solvent Competition Quantify Water Displaceability and Provide Accurate Interaction Maps of Protein Binding Sites. *Journal of Medicinal Chemistry* **2014**, *57*, 8530–8539, Publisher: American Chemical Society.

(5) `http://www.ub.edu/cbdd/?q=content/organic-solvent-boxes`.