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Chemistry & Drug Discovery

Supporting Information

Structured Water Molecules in the Binding Site of Bromodomains Can Be Displaced by Cosolvent

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cmdc_201300156_sm_miscellaneous_information.pdf

Gromacs input parameters used in the simulations

integrator = md

dt = 0.002

nsteps = 500000000

nstcomm = 1

nstcalcenergy = 1

nstxout = 5000

nstvout = 5000

nstfout = 5000

nstlog = 5000

nstenergy = 5000

nstlist = 100

ns_type = grid

pbc = xyz

rlist = 1.0

coulombtype = PME

rcoulomb = 1.0

fourierspacing = 0.1

fourier_nx = 0

fourier_ny = 0

fourier_nz = 0

pme_order = 4

ewald_rtol = 1e-5

optimize_fft = yes

vdwtype = cutoff

rvdw = 1

Tcoupl = v-rescale

ld_seed = -1

tau_t = 0.1 0.1

tc-grps = Protein non-protein

ref_t = 310 310

; Pressure coupling is on

Pcoupl = berendsen

pcoupltype = isotropic

tau_p = 2

compressibility = 4.5e-5

ref_p = 1

gen_vel = no

gen_temp = 310

constraints = all-bonds

constraint_algorithm= lincs

Water positions	CREBBP	BAZ2B	ATOM	Distance (nm)
	Ala1164	Cys1940	N	0.45
1	Asn1163	Asn1939	O	0.45
	Tyr1125	Tyr1901	OH	0.35
	Tyr1125	Tyr1901	OH	0.5
2	Met1160	Val1936	O	0.35
	Ala1164	Cys1940	N	0.4
	Met1133	Met1909	O	0.35
3	Asp1134	Asp1910	CA	0.45
	Met1133	Met1909	N	0.6
	Pro1110	Pro1888	O	0.35
4	Gln1113	Gln1891	O	0.35
	Val1115	Val1893	N	0.45
	Pro1110	Pro1888	O	0.35
5	Pro1110	Pro1888	CB	0.45
	Val1115	Val1893	CA	0.6
	Pro1110	Pro1888	O	0.4
6	Gln1113	Gln1891	O	0.4
	Pro1114	Pro1892	O	0.4
	Met1133	Met1909	N	0.35
7	Tyr1125	Tyr1901	OH	0.35
	Met1133	Met1909	O	0.35

Table S1. Triplets of protein atoms used for calculation of occupancy. The distances listed in the column on the right are threshold values. The threshold values are decided based on the crystal structure of CREBBP (PDB code 3P1C). Protein atoms close to the corresponding crystal waters are selected to define their relative positions. Preference is given to non-hydrogen atoms which locate at protein backbone as they have lower flexibility than the side chain atoms. In case a selected atom can form a hydrogen bond with the corresponding water a threshold value 0.35 nm is applied, otherwise a larger threshold based on the original distance in the crystal structure is used. A position is considered occupied if all three distances are below the threshold values.

		Occupancy (%)		
Protein	Water position	Methanol	Ethanol	DMSO
BAZ2B	1	3.0	10.3	3.3
	2	0.2	0.3	0.0
	3	0.8	0.5	0.0
	4	0.1	0.1	0.0
	5	3.6	4.3	0.5
	6	0.2	0.3	0.0
	7	0.0	0.0	0.0
CREBBP	1	0.5	5.1	2.1
	2	0.2	0.2	0.0
	3	1.3	0.5	0.0
	4	0.0	0.0	0.0
	5	0.8	0.9	0.2
	6	0.3	0.9	0.0
	7	0.0	0.0	0.5

Table S2: Occupancies of the cosolvents at the seven water positions during 1 μs simulation. The same triplets of protein atoms and distance thresholds as for the water occupancy (Table S1) were used.

binding;unbinding thresholds (nm)	methanol τ_{on} (ns)	methanol τ_{off} (ns)	methanol k_{off}/k_{on} (mM)	ethanol τ_{on} (ns)	ethanol τ_{off} (ns)	ethanol k_{off}/k_{on} (mM)	DMSO τ_{on} (ns)	DMSO τ_{off} (ns)	DMSO k_{off}/k_{on} (mM)
BAZ2B									
0.3;0.7	1.2	1.3	431	1.2	3.5	148	2.7	6.7	179
0.4;0.7	1.0	0.8	603	1.1	2.6	184	2.5	5.1	212
0.5;0.7	0.8	0.7	560	0.7	2.0	158	2.2	3.8	250
0.3;0.8	1.2	1.8	301	1.2	5.3	102	2.6	9.5	118
0.4;0.8	**1.1**	**0.9**	**543**	**0.7**	**2.4**	**124**	**2.6**	**8.9**	**128**
0.5;0.8	0.9	0.8	475	0.8	2.7	129	2.3	6.9	148
0.3;0.9	1.2	2.7	194	1.2	6.7	79	2.4	10.9	95
0.4;0.9	1.0	1.2	397	1.1	5.5	85	2.7	10.5	111
0.5;0.9	0.9	1.0	421	0.8	3.7	97	2.3	9.6	104
CREBBP									
0.3;0.7	1.3	1.3	439	1.1	4.3	108	1.9	25.2	32
0.4;0.7	1.2	1.1	482	0.9	3.9	106	2.1	23.6	39
0.5;0.7	1.0	0.8	551	0.9	3.2	118	2.1	21.7	42
0.3;0.8	1.4	1.3	463	1.1	4.8	98	1.8	28.2	28
0.4;0.8	**0.5**	**1.3**	**176**	**1.0**	**4.5**	**94**	**2.1**	**26.0**	**36**
0.5;0.8	1.1	1.1	436	0.9	3.9	97	2.1	25.0	36
0.3;0.9	1.4	1.5	413	1.1	6.7	70	1.7	30.2	24
0.4;0.9	1.3	1.5	393	0.9	5.8	71	2.0	30.4	28
0.5;0.9	1.1	1.3	384	0.9	4.8	77	1.8	27.8	28

Table S3: **Robustness analysis.** Dissociation constant from fitting of cumulative distributions of unbinding and binding times using different thresholds. Values in boldface are reported in Table 1 of the main text. The characteristic time of the slow phase of the double-exponential fitting is used to calculate the binding rate $k_{on} = 1/(\tau_{on}[\text{cosolvent}])$ and unbinding rate $k_{off} = 1/\tau_{off}$. The concentration of the cosolvent in the simulation box is 440 mM.

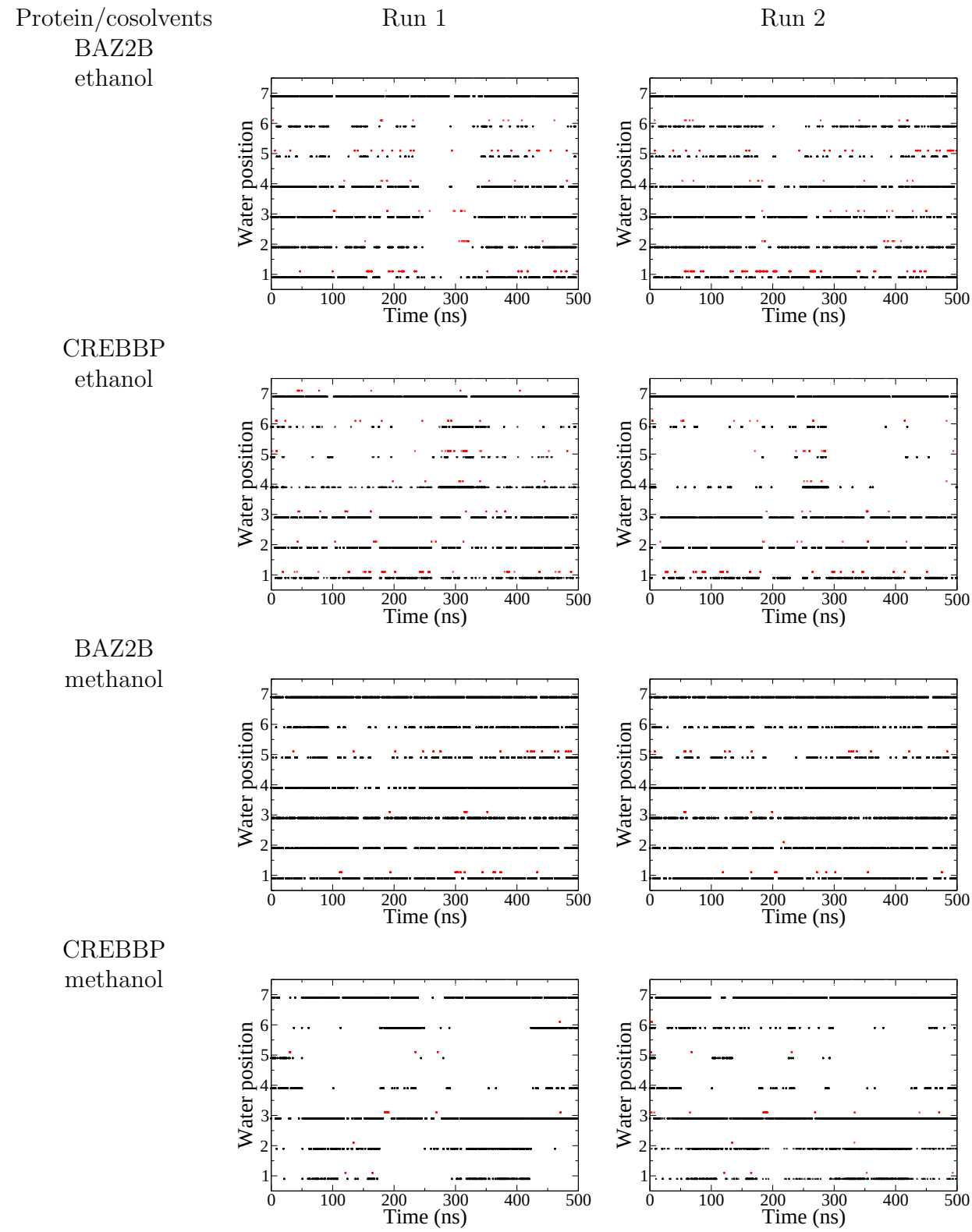
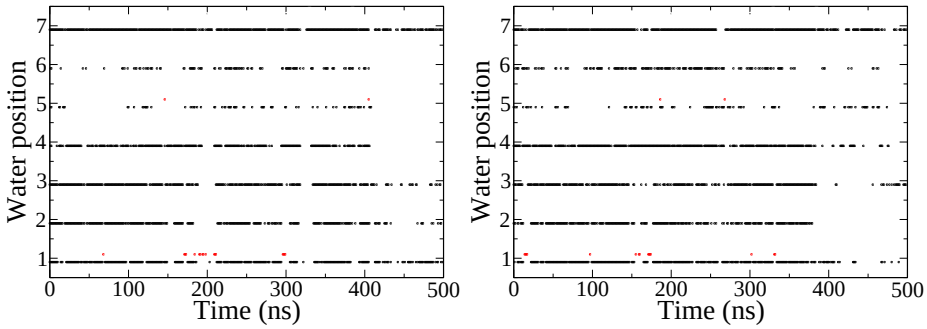


Figure S1: Caption on next side.

BAZ2B
DMSO



CREBBP
DMSO

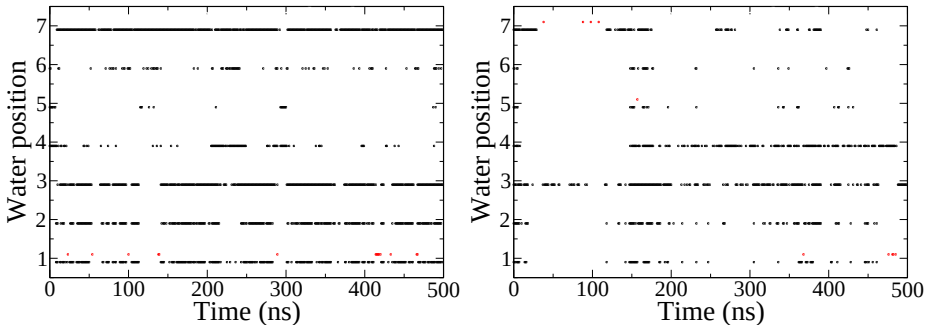


Figure S1. Cosolvent occupancy at the positions of the structured water molecules. The time series show the presence of water (black dot) or cosolvent (red dot) along time intervals of 1 ns.

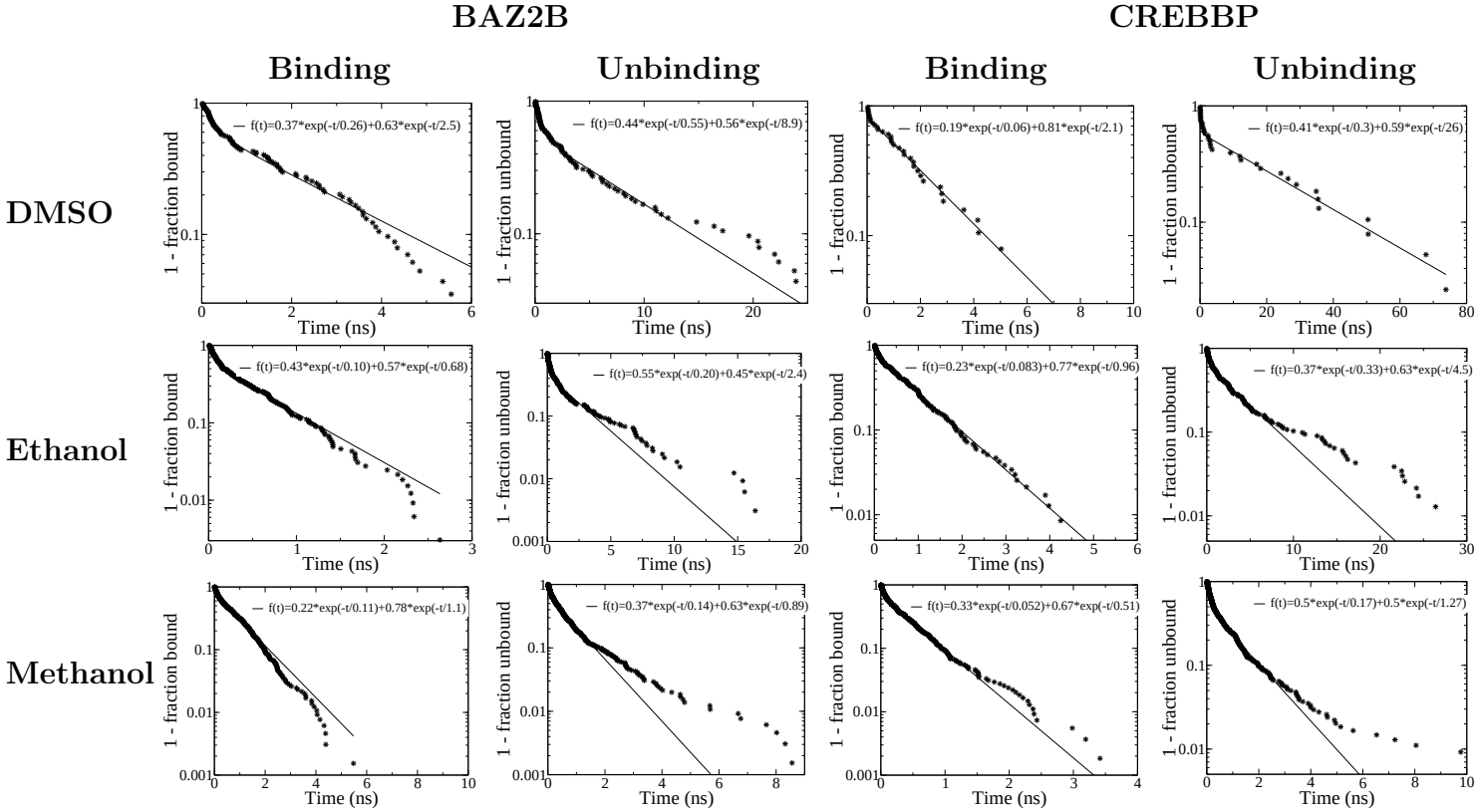


Figure S2: Cumulative distribution of the binding time and unbinding time f(t) $= \int_t^\infty p(\tau)d\tau$, where p is the probability distribution of the binding and unbinding time, respectively. Binding and unbinding events are defined by a separation between the centers of mass of the acetyl-lysine binding site and cosolvents smaller than 0.4 nm and larger than 0.8 nm, respectively. These threshold values were chosen upon visual analysis of the time series. The stars represent the binding and unbinding events observed in the two MD runs. The solid lines are double-exponential fits.

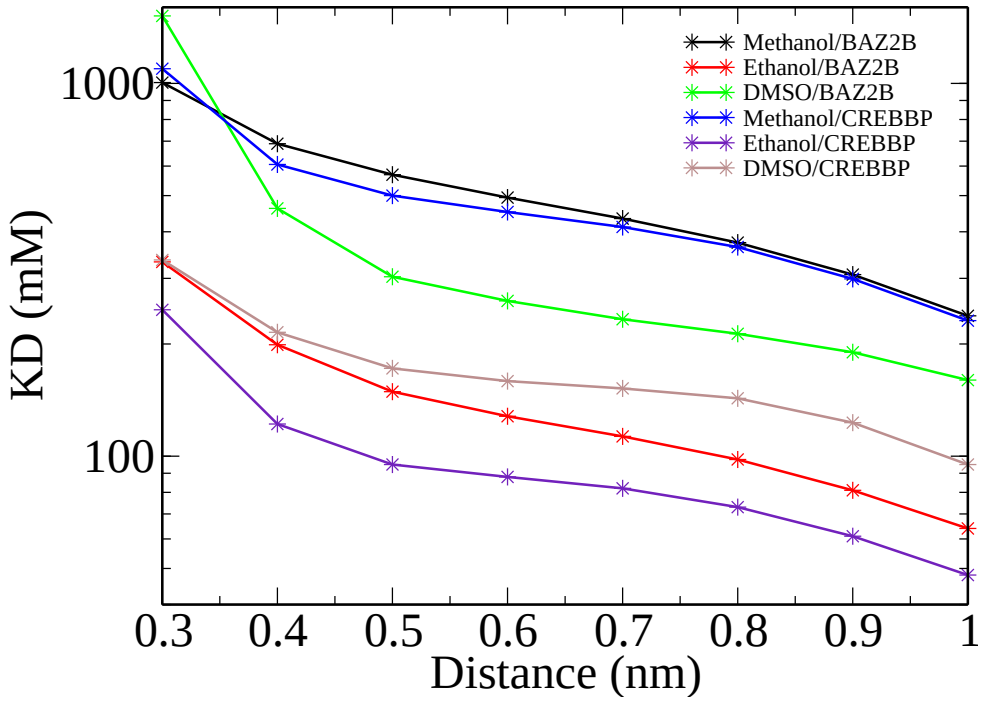


Figure S3: **Robustness of dissociation constant upon changes of occupancy threshold.** Values of the threshold ranging from 0.3 to 1.0 nm are used to calculated the occupancy of the acetyl-lysine binding site. The dissociation constant is calculated from the occupancy using K_D = [cosolvent] [unbound protein] / [bound protein] = [cosolvent] (100 - occupancy) / occupancy.