Potential mean force from umbrella sampling simulations: what can we learn and what is missed?

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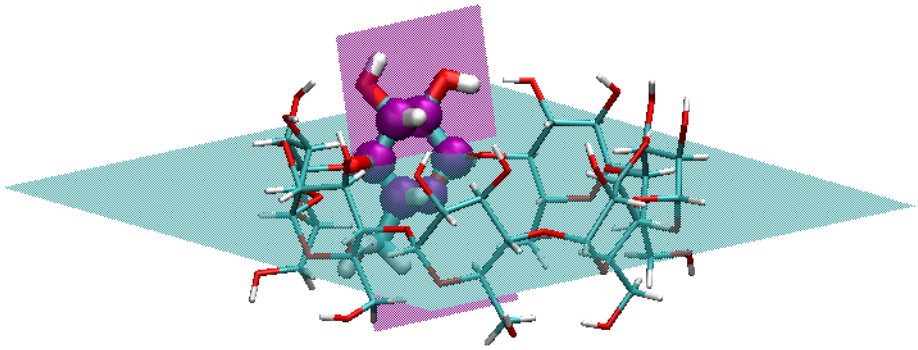
Artifact of Restraints

Due to the limitation of distance restraint algorithm used in AMBER, when COM of ligand was close to 0 Å on X axis, where COM of β-CD was located, ligand could jump back and forth between path A and B. The harmonic potential added to restrain the distance between β-CD and ligand was irrelevant from directions and therefore causing an issue shown in Figure S3. When the distance between COMs of β-CD and ligand was larger than 1 Å, the problem disappeared because the gap had become too large for ligand to jump through. We ran multiple runs for RC smaller than 1 Å to get a rough closer PMF. The future version of restraint setting in AMBER may consider restraining vector instead distance, so this issue can be revisited and solved. It's also noted that for the 0 Å on X axis, where the COMs of β-CD and ligand were overlapped, the peak split into two located on both sides. It's an artifact of restraint setting in AMBER, which caused the unusual high energy barrier at 0 Å position in Figure 5.

Figures

(A)



(B)

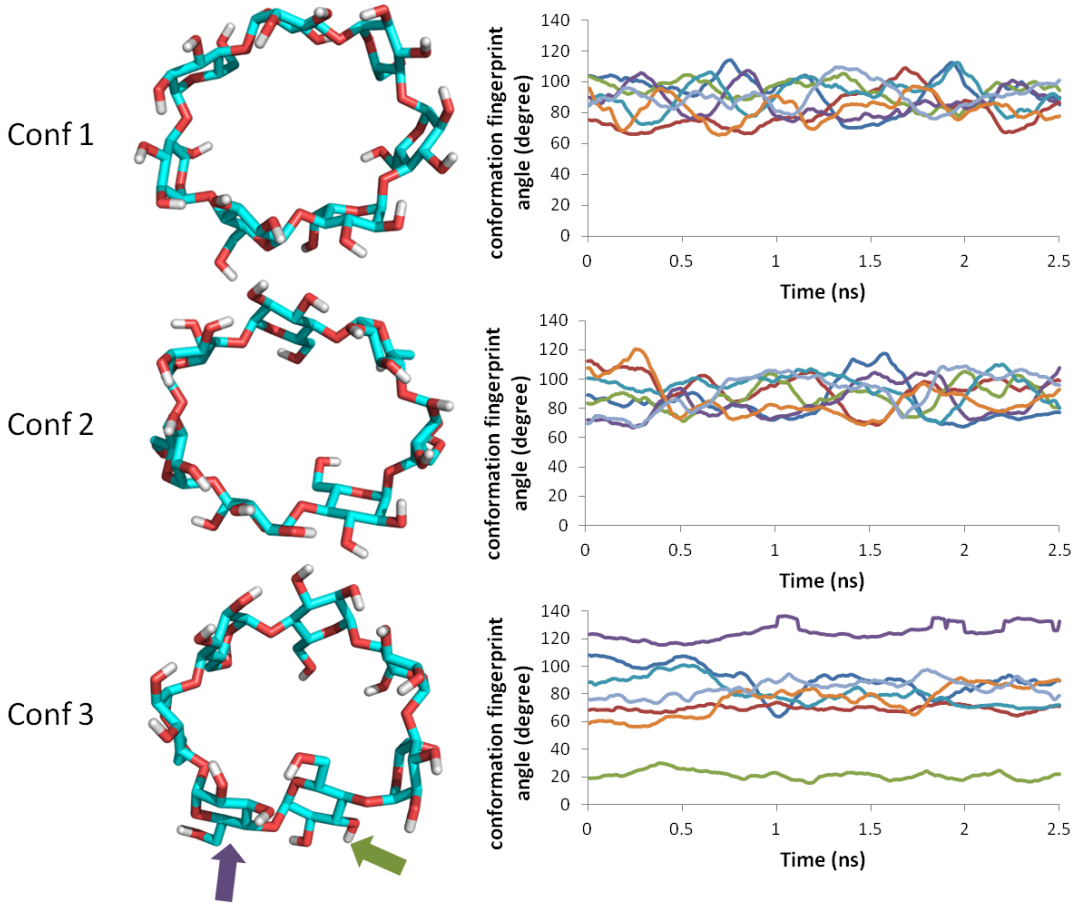


Figure S1. (A) Representation of one fingerprint angle in β-CD. The regression plane of entire molecule is in shown in cyan color and the regression plane of the six atoms highlighted by purple balls in one glucose unit is shown in purple color. The fingerprint angle of one glucose unit is defined by the dihedral angle between the two regression planes. (B)Three different conformations of β-CD. The plots of their conformation fingerprint angles (defined in A) along trajectories are on the right. The fingerprint dihedral plots of β-CD are from biased MD of one US window where

distance between center of mass (COM) of β-CD and aspirin is restrained to 10 Å. Before measurement of fingerprint angles, the trajectories were smoothed by averaging 100 forward and 100 backward frames on the concurrent frame throughout the whole trajectory to remove the noise.

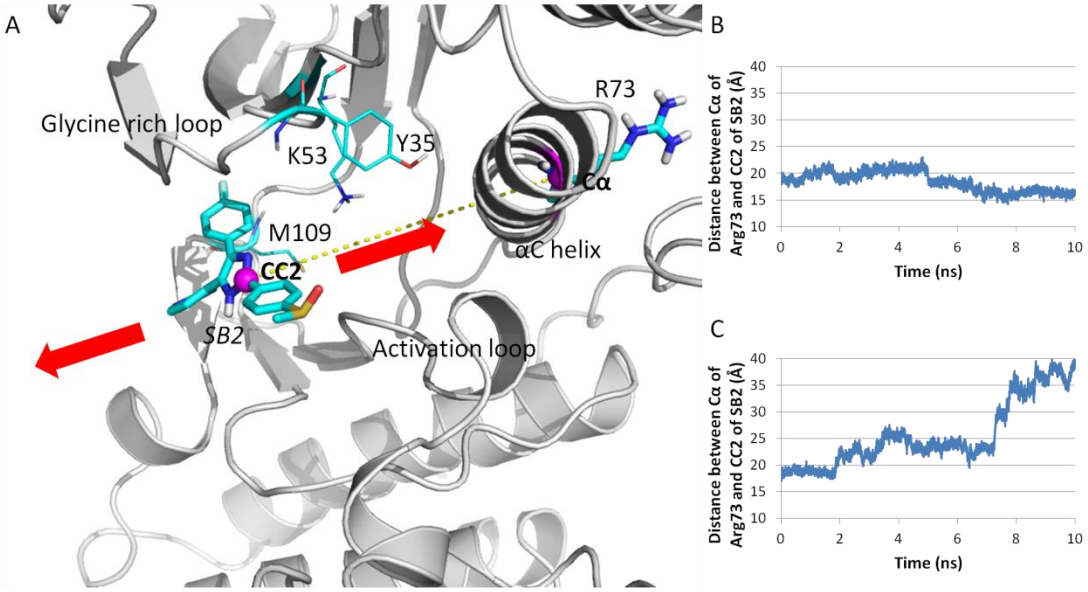


Figure S2. Reconstruction of dissociation path from AMD. Path 1 is built from AMD path that conformational relaxed by two 10 ns conventional MD. (A) SB2 in one of the two 10 ns conventional MD moves towards inside the cavity, while SB2 in the other conventional MD moves towards outside. Arg73 and SB2 are shown in bold licorice structure, Cα of Arg73 and CC2 of SB2 are indicated by purple ball structure, other interacting residues are shown in thin licorice structure. (B) SB2 moving inside the cavity indicated by the decreasing distance between SB2 and Arg73. (C) SB2 moving outside the cavity indicated by the increasing distance between SB2 and Arg73.

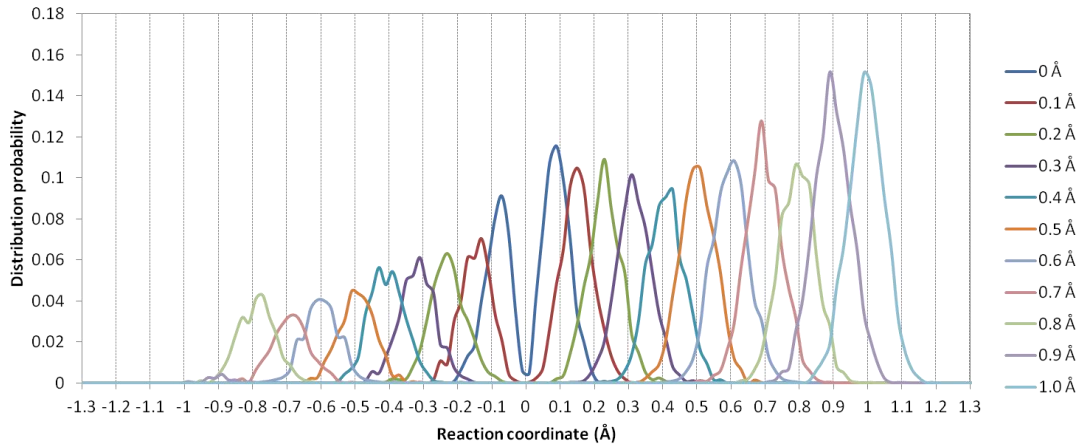


Figure S3. Selected distribution probability of ligand in US along path A. When distance of COMs of aspirin and β-CD is smaller than 1 Å, ligand jumps back and forth between path A and B during dissociation process of aspirin from β-CD in Conf 1 along path A, ligand stops jumping when RC distance reaches 1 Å.

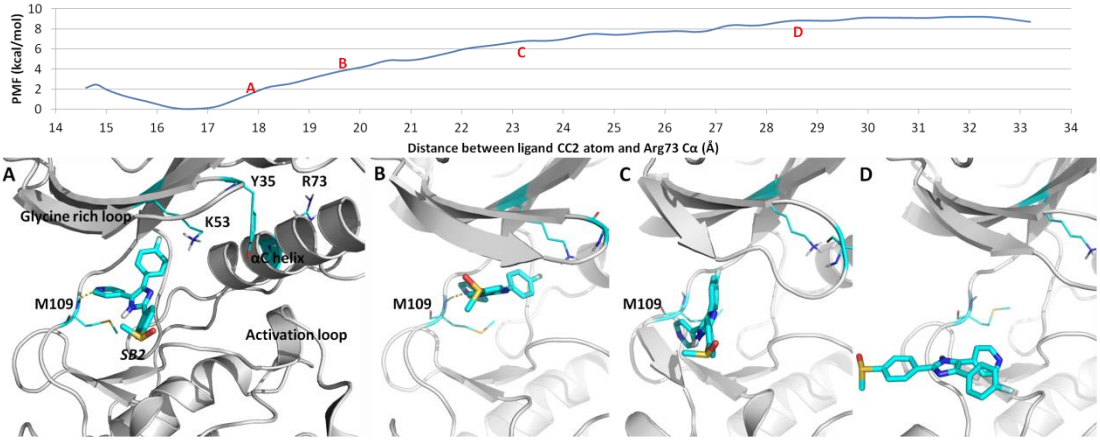


Figure S4. PMF of path 1 from US and the selected snapshots during dissociation. Hydrogen bonds between SB2 and p38α are shown in dash line. (A) SB2 breaks hydrogen bond with Lys53 side-chain and stacking interaction with Tyr35. (B) 4-methylsulfinylphenyl group of SB2 starts diffusing towards outside the cavity (C) fluorophenyl ring of SB2 moves out of the hydrophobic pocket and hydrogen bond between pyridine nitrogen and Met109 breaks. (D) SB2 is outside the edge of binding cavity.

Table S1. MM/PBSA calculations for the conformation energy of the free cyclodextrin, ligand and bound complex and the interactions energy between the ligand and three initial β-CD conformations Conf 1-3. Unit: kcal/mol

1-butanol and β-CD

Free cyclodextrin

cyclodextrin	E_bonded	E_PB	E_np	E_VdW	E_EEL	E_total
Conf 1	203.0	-101.4	-6.4	8.9	-5.3	98.9
Conf 2	203.1	-101.4	-6.5	9.1	-5.7	98.7
Conf 3	194.0	-106.7	-5.2	6.0	9.3	97.4

Free 1-butanol

cyclodextrin	E_bonded	E_PB	E_np	E_VdW	E_EEL	E_total
Conf 1	9.8	-6.9	1.0	1.2	-16.9	-11.8
Conf 2	10.3	-6.8	0.9	1.3	-16.9	-11.2
Conf 3	10.1	-7.0	1.0	1.3	-16.9	-11.6

CD-butanol complex

cyclodextrin	E_bonded	E_PB	E_np	E_VdW	E_EEL	E_total
Conf 1	212.8	-99.5	1.2	-2.7	-23.2	88.6
Conf 2	213.4	-99.2	1.0	-2.7	-24.0	88.5
Conf 3	204.1	-102.5	1.1	-5.6	-13.2	83.9

Interaction energy

cyclodextrin	ΔE_bonded	ΔE_PB	ΔE_np	ΔE_VdW	ΔE_EEL	ΔE_total
Conf 1	0.0	8.8	6.7	-12.9	-1.1	1.5
Conf 2	0.0	9.0	6.5	-13.0	-1.4	1.1
Conf 3	0.0	11.2	5.3	-12.9	-5.5	-1.8

Aspirin and β-CD

Free cyclodextrin

cyclodextrin	E_bonded	E_PB	E_np	E_VdW	E_EEL	E_total
Conf 1	205.5	-101.3	-6.4	9.0	-6.6	100.2
Conf 2	205.2	-98.4	-6.1	9.7	-10.9	99.5
Conf 3	198.7	-105.2	-5.4	7.8	2.5	98.5

Free aspirin

cyclodextrin	E_bonded	E_PB	E_np	E_VdW	E_EEL	E_total
Conf 1	23.9	-15.6	0.0	5.2	-112.9	-99.5
Conf 2	21.2	-13.4	-0.2	7.7	-116.6	-101.2
Conf 3	20.4	-12.9	-0.5	9.2	-118.0	-101.8

CD-aspirin complex

cyclodextrin	E_bonded	E_PB	E_np	E_VdW	E_EEL	E_total
Conf 1	229.4	-100.8	3.8	-9.7	-121.9	0.8
Conf 2	226.4	-93.4	4.0	-7.0	-132.4	-2.4
Conf 3	219.1	-100.6	3.9	-8.1	-120.3	-6.1

Interaction energy

cyclodextrin	ΔE_bonded	ΔE_PB	ΔE_np	ΔE_VdW	ΔE_EEL	ΔE_total
Conf 1	0.0	16.2	10.2	-23.8	-2.4	0.1

Conf 2	0.0	18.5	10.2	-24.4	-4.9	-0.6
Conf 3	0.0	17.5	9.7	-25.2	-4.8	-2.7