Supplementary Information for
Kinetics of Barrier Crossing Events from Temperature Accelerated Sliced Sampling Simulations

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S1. SCHEMATIC OF THE CONSTRUCTION OF THE BIAS POTENTIAL $V_0^{\mathrm{b}}(\mathbf{s})$

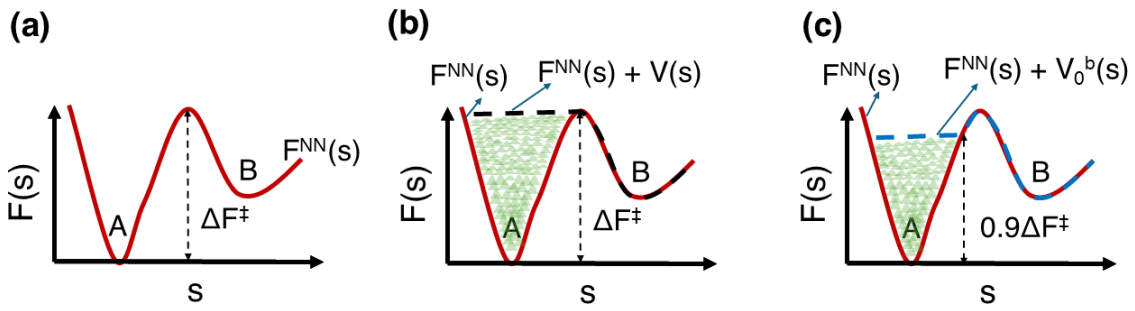

FIG. S1. Schematic of $V_0^{\mathrm{b}}(\mathbf{s})$ construction from the ANN FES; (a) ANN FES $F^{\mathrm{NN}}(\mathbf{s})$; (b) Bias potential $V(\mathbf{s})$, which when added to $F^{\mathrm{NN}}(\mathbf{s})$ yields nearly a flattened profile $F^{\mathrm{NN}}(\mathbf{s})+V(\mathbf{s})$ in basin A, corresponding to filling the barrier $\Delta F^{\ddagger}$; (c) reduced bias $V_0^{\mathrm{b}}(\mathbf{s})$, so that the effective profile $F^{\mathrm{NN}}(\mathbf{s})+V_0^{\mathrm{b}}(\mathbf{s})$ fills $0.9\Delta F^{\ddagger}$.

S2. ALANINE DIPEPTIDE IN *VACUO*

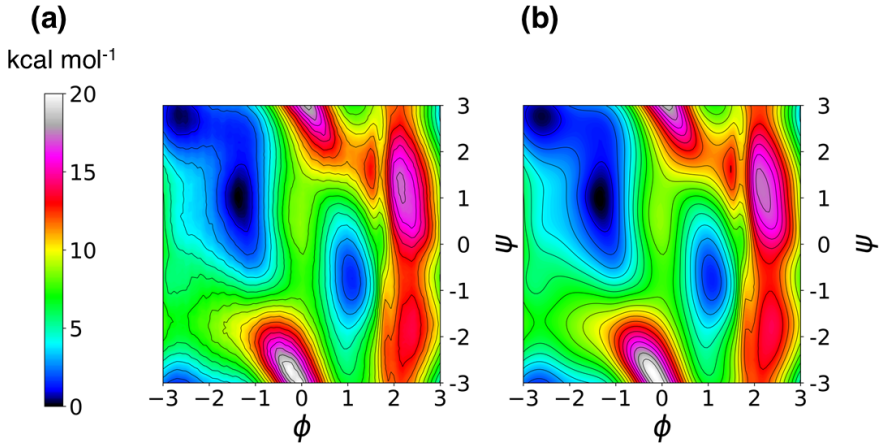

FIG. S2. Free energy surfaces, $F(\phi,\psi)$, for alanine dipeptide *in vacuo* (a) computed using TASS and (b) computed from the trained ANN.

A. L^2 Error

The L^2 error is computed using the following formula:

$$L^2 \text{ error} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left[F(\mathbf{s}_i) - F_{\text{ref}}(\mathbf{s}_i)\right]^2} \tag{1}$$

Here, F_{ref} denotes the reference free energy surface computed at the end of a long simulation, F is the free energy surface at a specified simulation time t, and n is the number of grid points included within the chosen free energy cutoff.

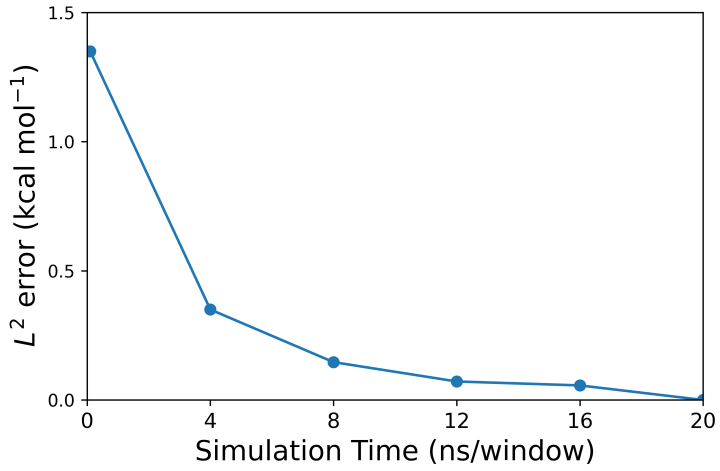

FIG. S3. Internal convergence of $F(\phi, \psi)$ for alanine dipeptide in *vacuo* is monitored by computing the L^2 error by taking the 20 ns data as the reference.

S3. BENZAMIDINE UNBINDING FROM TRYPSIN

A. Chemical Structure of Benzamidine

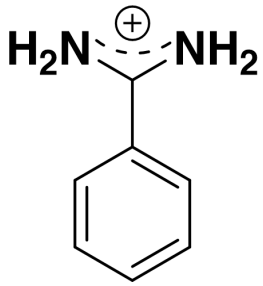

FIG. S4. Chemical structure of benzamidine

B. Collective Variables

1. **Dis**: Distance (**Dis**) between C_7 (carbon having the diamine group) of benzamidine and C_δ of Asp189 in trypsin (Figure S5).

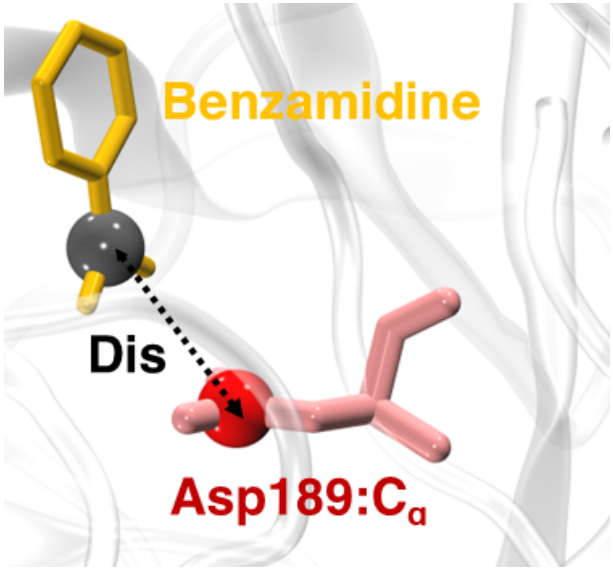

FIG. S5. **Dis** CV is defined as the distance between C_δ (sphere, red) carbon of Asp189 and C_7 (carbon having the diammine group, shown as grey sphere) of benzamidine (stick representation, yellow).

2. **Hbonds**: Coordination number of the trypsin active site residues (Asp189:OD1, Asp189:OD2, Val227:O, Val213:O, Tyr228:OH, Gly196:O, Ser190:O_γ) to a selected set of atoms of benzamidine (N9, N10) (Figure S6). For atoms i and j, the coordination number (CN) is defined as

$$\mathrm{CN}_{ij} = \frac{1-(\frac{r_{ij}}{r_0})^n}{1-(\frac{r_{ij}}{r_0})^m} \tag{2}$$

where r_{ij} is the distance between the atoms i and j. Here, r_0 is the distance cutoff. We used r_0 =3.5 Å, $n = 6$, and $m = 12$.

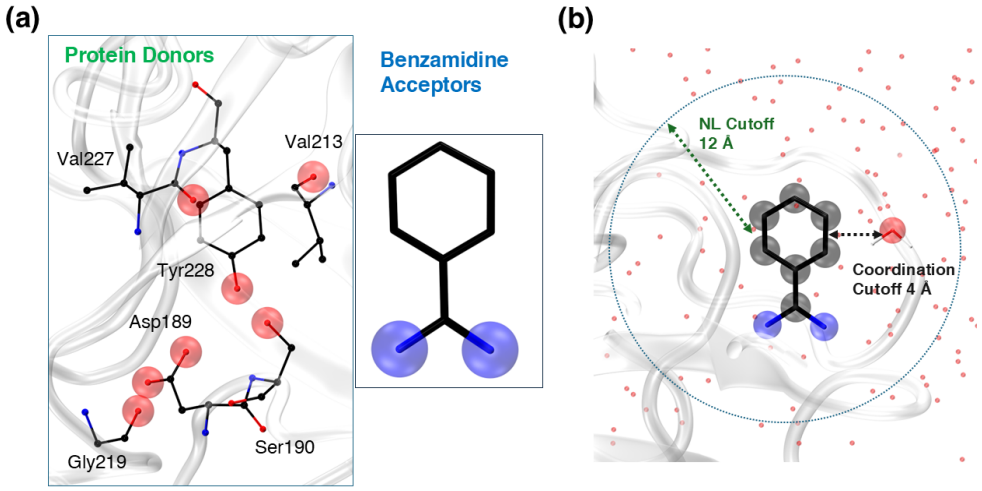

FIG. S6. (a) All the hydrogen-bond donor atoms (highlighted as spheres) of the trypsin active site residues (shown in ball and stick representations) and the corresponding hydrogen bond acceptor atoms (highlighted as spheres) of benzamidine (shown in ball and stick representations). Atom color codes: C (black), O (red), and N (blue). (b) Atoms of the benzamidine chosen for the **LigSolv** CV definition are shown (transparent spheres).

3. **LigSolv**: Coordination number between benzamidine heavy atoms and water oxygen atoms; See Figure S6. We chose $r_0 = 4$ Å, and $n = 6$, and $m = 12$ in the definition of the CN function. A neighbor list (NL) cutoff of 12 Å was chosen, and the list updated at every 50 ps while computing the CN to speed-up the calculation. We used $r_0 = 4$ Å; A Neighbor list (NL) cutoff distance of 12 Å taken.

C. Free Energy Surface

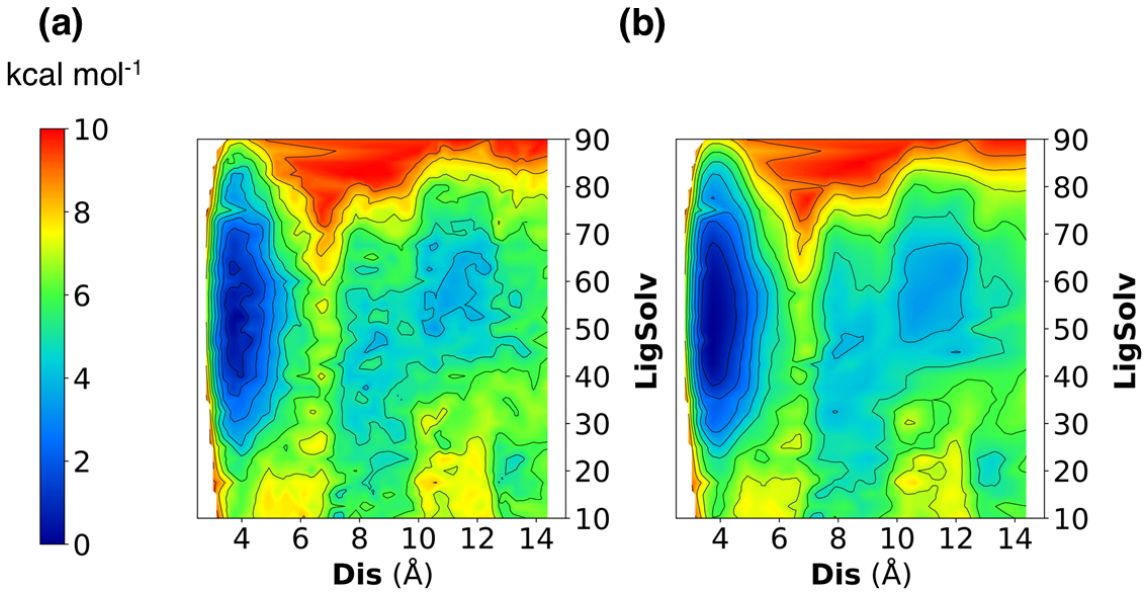

FIG. S7. Trypsin-benzamidine system: Free energy surface $F(\mathbf{Dis}, \mathbf{LigSolv})$ as (a) computed using TASS and (b) from a trained ANN.

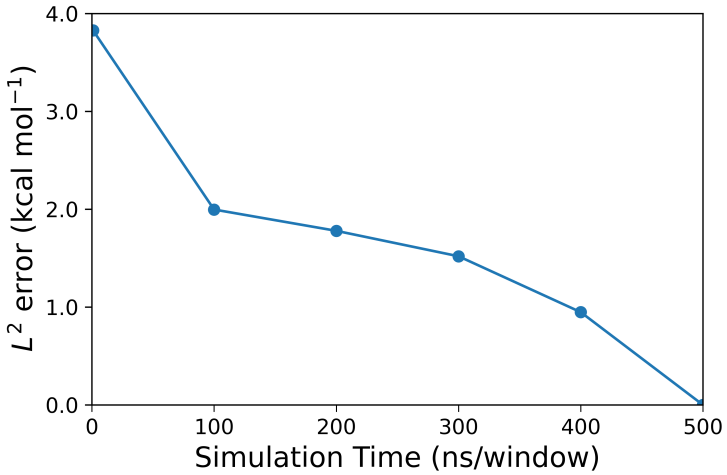

FIG. S8. Trypsin-benzamidine system: Internal convergence of $F(\mathbf{Dis}, \mathbf{Hbonds}, \mathbf{LigSolv})$ monitored through the L^2 error.

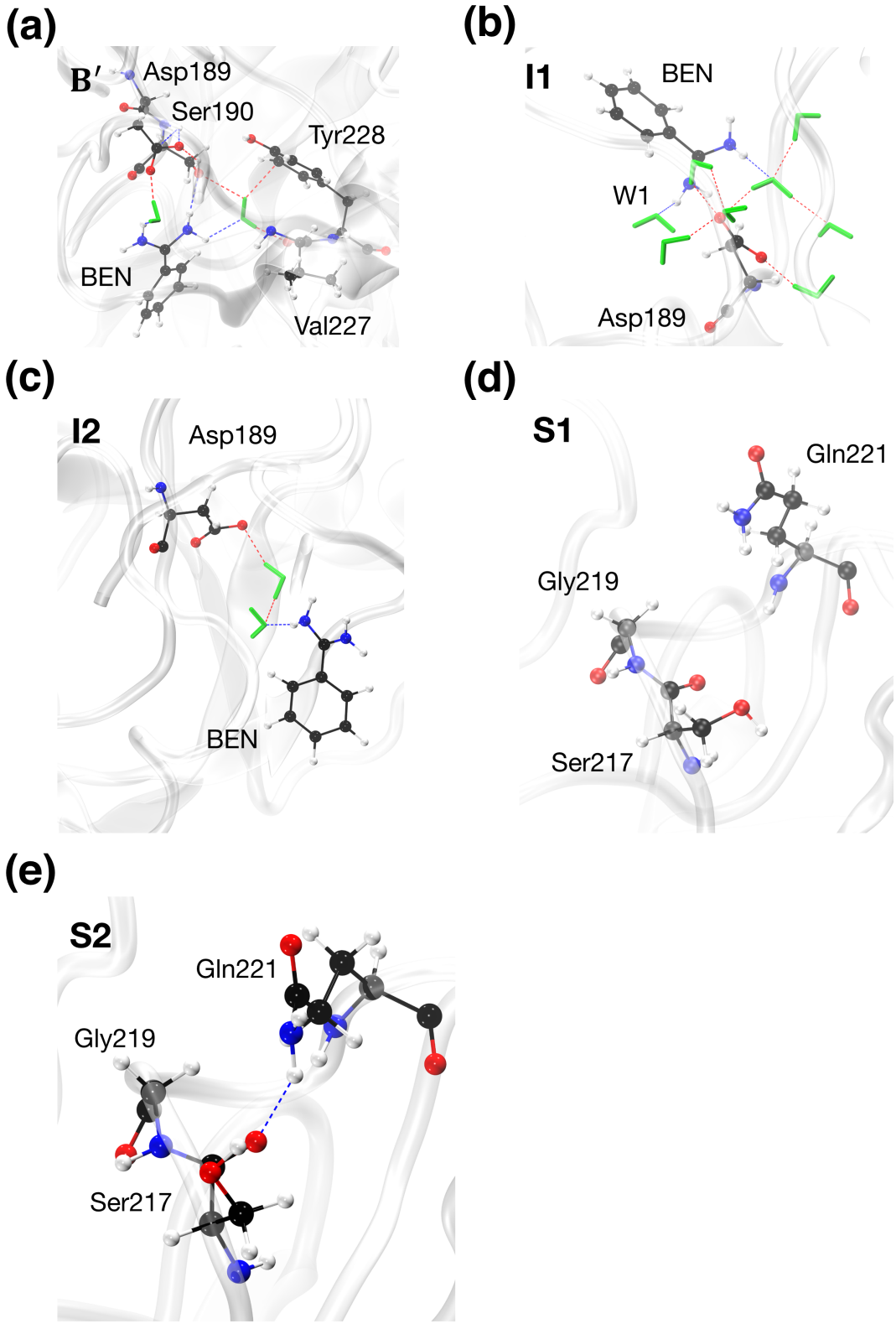

FIG. S9. Various conformational states observed in the TASS simulation of benzamidine dissociation from trypsin are shown here: (a) **B′** ; (b) **I1**; (c) **I2**; (d) **S1**; (e) **S2**. Color code: water molecules (green).

S4. ASPIRIN UNBINDING FROM β-CYCLODEXTRIN

A. Collective Variables

1. **Dis**: Distance between the center of mass of the host and the guest (Figure S10).

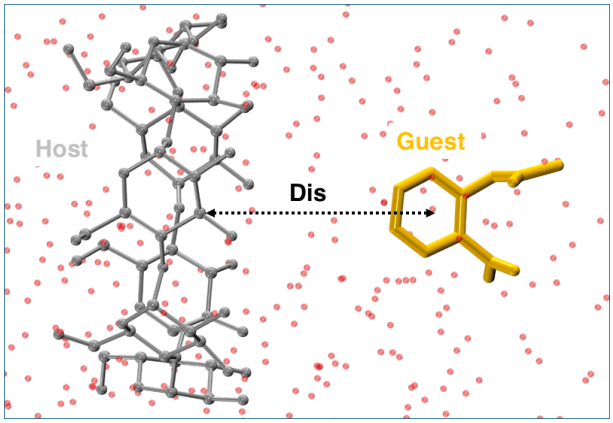

FIG. S10. **Dis** CV showing the distance between the center of mass of β-CD (silver) and aspirin (yellow).

2. **NContacts**: CN between β-CD and aspirin (Figure S11). We used $r_0 = 6$ Å and $n = 6$ and $m = 12$ in the definition of the CN function.

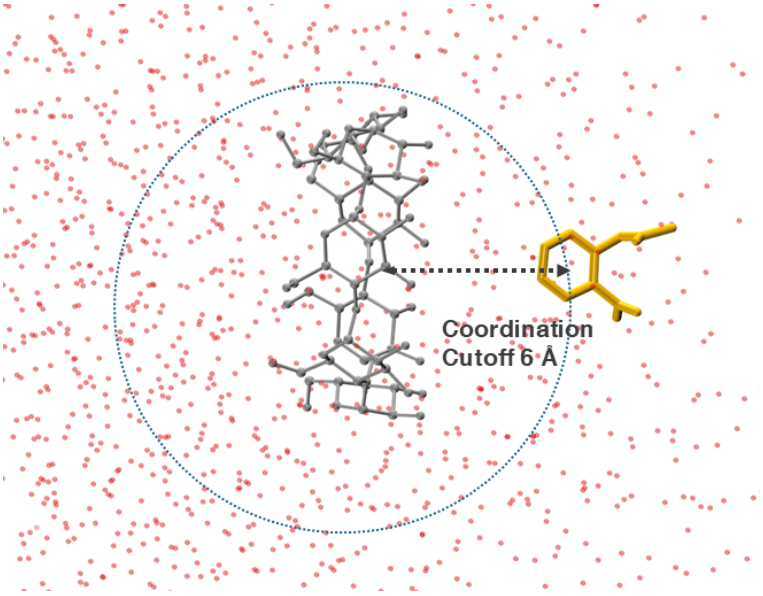

FIG. S11. Coordination of β-CD (silver) and aspirin (yellow) in the definition of the **NContacts** CV.

3. **LigSolv**: Coordination number between center of mass of the guest and water oxygen atoms (Figure S12). Here we used $r_0 = 2.5$ Å, $n = 6$ and $m = 12$ to define the CV. Atom color codes: C (black) and O (red).

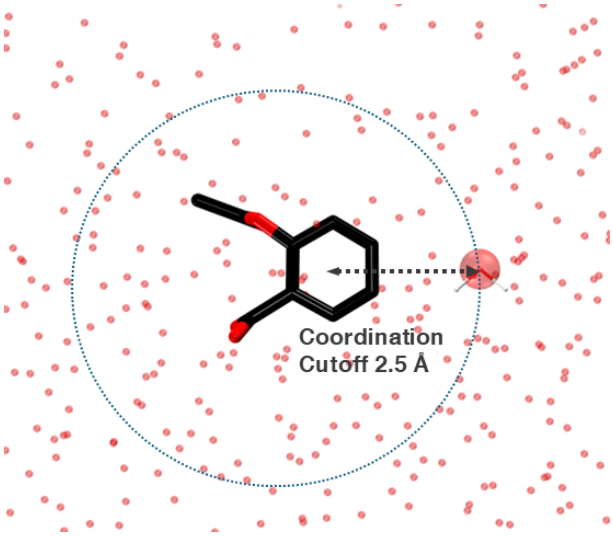

FIG. S12. Coordination between the center of mass of β-CD and oxygen atoms of water molecules is defined as the **LigSolv** CV.

B. Free Energy Surface

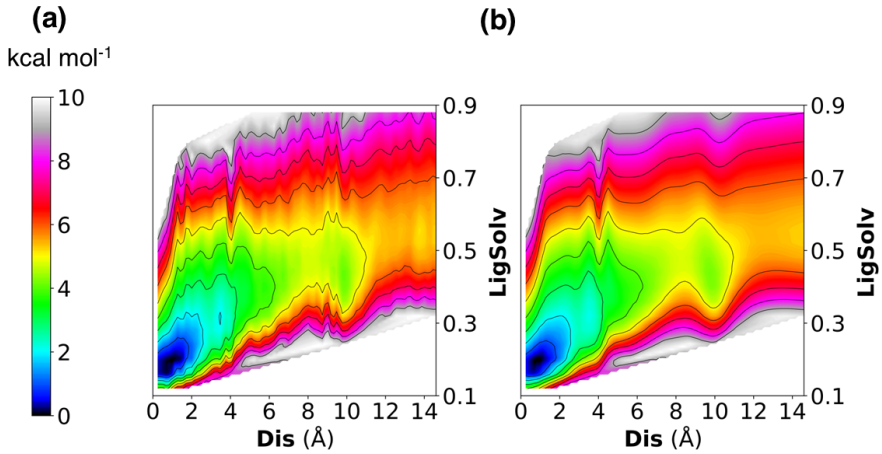

FIG. S13. β-CD-aspirin system: Free energy surface F(**Dis**,**LigSolv**) (a) computed from TASS and (b) computed from a trained ANN.

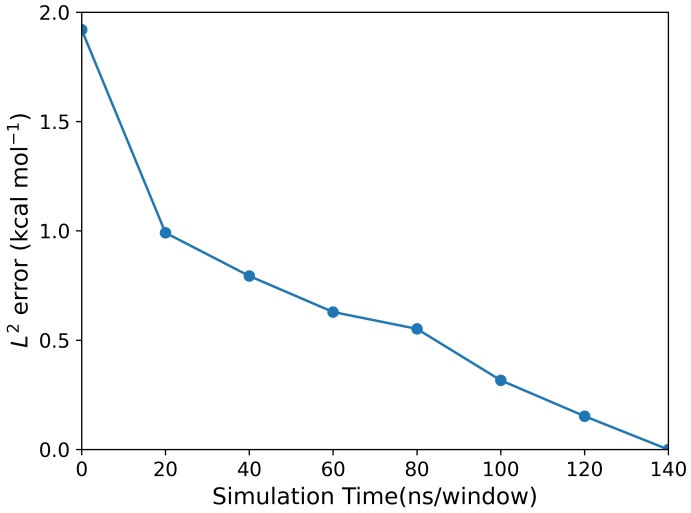

FIG. S14. β-CD-aspirin system: Internal convergence of F(**Dis**, **NContacts**, **LigSolv**) monitored through L^2 error by taking the free energy surface at 140 ns as the reference.

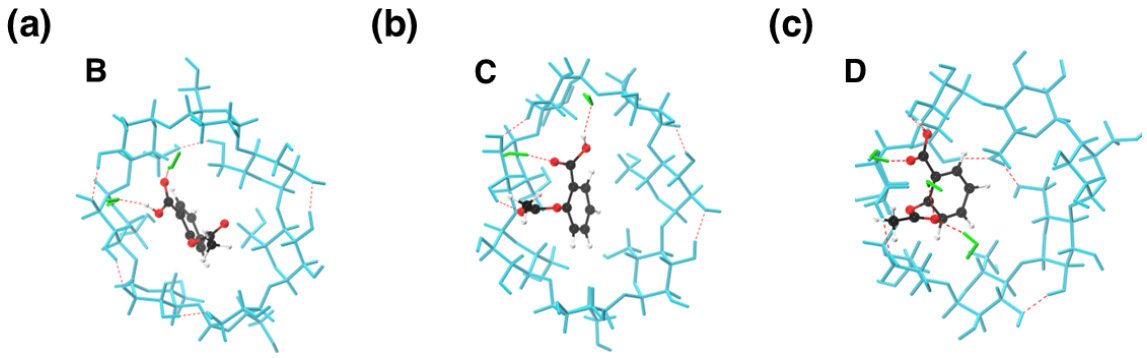

FIG. S15. Various conformational states observed in the simulation of dissociation of aspirin from β-CD are shown here: (a) **B**; (b) **C**; (c) **D**. Color code: β-CD (cyan), water molecules (green), aspirin atoms are with C (black), O (red), and H (white).