Supporting Information: The role of water in host-guest interaction

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S-1 Simulation Details

We follow the plead from [1] where the authors advocated the use of standard setups with a prescribed set of and simulation parameters for benchmarking methods within identical conditions. We have taken the input files from the public repository at https://github.com/michellab/Sire-SAMPL5. This simulation setup was used by a number of research groups [2, 3, 4] to investigate ligand binding with different methodologies.

We perform the simulations with GROMACS 2019.4 [5] in combination with the PLUMED plugin 2.5.4 [6] and the Pytorch library 1.4 [7]. We use the GAFF force field [8] with RESP charges [9] and the TIP3P water model [10]. Our timestep is 2 fs and the temperature is set at 300 K via a velocity rescale thermostat [11] with time constant 0.1 ps. The simulation box is cubic with a side of about 40 Å and it contains 2100 water molecules in solution together with the host OAMe and the chosen guest molecule. Sodium ions are included to counterbalance excess charges. At every simulation step, the coordinates are aligned so that the z axis of the box coincides with the binding axis and the simulation box is centred on the virtual atom V_1.

In the following sections, we analyse the most relevant aspects of the strategy proposed in the paper.

S-1.1 The descriptor set

Our descriptor set $\mathbf{d}$ specialises in measuring the water presence around chosen points and includes two species. The first species is centred on a set of ligand atoms L and the second one on virtual atoms V along the binding axis. The L descriptors specialise in measuring the local water solvation in the vicinity of the ligand, while the V descriptors control the host's solvation.

We choose 4 points L for every guest molecule and 8 points V, starting from the centre of the lower phenyl rings of the host and upward, with a spacing of 2.5 Å, as indicated in Fig. 1 in the main text. The water coordination number (CN) of every point i is calculated with

$$\mathrm{d}_i^0 = \sum_j^{r_{ij}<r_{\mathrm{NL}}} \frac{1-\left(\frac{r_{ij}}{r_0}\right)^n}{1-\left(\frac{r_{ik}}{r_0}\right)^m} \tag{S-1}$$

where r_{ij} measures the distance between point i and the water oxygen atom j within a sphere of radius $r_{\mathrm{NL}} = 10\,\text{Å}$ and $r_0 = 2.5\,\text{Å}$.

The parameters n and m are crucial for tuning the descriptor's effectiveness in enhanced sampling applications. Every descriptor has to be sufficiently short range to focus on the important water molecules in the vicinity of i, but also sufficiently long range to make its derivative smooth with respect to the more distant water molecules. For $d_i \in \mathrm{L}$ we choose $n = 6$ and $m = 10$ that provide a good compromise between short range focus and long range outreach. For $d_i \in \mathrm{V}$, there is no electrostatic repulsion that prevents the water molecules from reaching $r_{ij} \approx 0$, so we choose $n = 2$ and $m = 6$ that produces a switching function with an analogous long range behaviour, but a softer core at short distances. The CNs are then normalised so that their values d_i lie between -1 and 1. For $d_i \in \mathrm{L}$, we normalise the descriptor by $d_i = d_i^0/2.5 - 1$, while for $d_i \in \mathrm{V}$ we normalise it by $d_i = d_i^0/2.8 - 1$.

S-1.2 Neural network architecture and training

The Deep-LDA strategy that we use in this paper is completely akin to the one presented in [12], so we refer the interested reader to that paper for more details. A tutorial of Deep-LDA is present here https://github.com/luigibonati/data-driven-CVs. Our neural network (NN) architecture is sketched in Fig. 2 in the main text and it consists of a sequence of layers with $12, 10, 8, 6, 4$ nodes, with the rectified linear unit as activation function. Therefore $N_{\mathrm{d}} = 12$ and $N_{\mathrm{h}} = 4$.

The first layer takes as input short trajectories of the normalised descriptors $\mathbf{d}$ calculated in state B and U. Given the presence of the funnel restraint in the subsequent enhanced sampling simulations, in the state U simulations used for training we trap the ligand in a cylindrical volume above the host (see Sec. S-1.3). For state B, we take as a starting configuration the binding pose provided by the standard setup with randomised velocities. Typically, for each state we collect 5 trajectories of 4 ns where the descriptors are printed every 0.25 ps. Out of the resulting 80000 configurations, we randomly select 25600, put them in small batches of 512 elements and feed them to the NN for training.

A regularisation is applied to the within class scatter matrix $\mathbf{S}_w$ defined in the main text so that it becomes $\mathbf{S}'_w = \mathbf{S}_w + \lambda \mathbf{I}$ with $\lambda = 0.05$. Maximising Fisher's ratio in Eq. 1 is equivalent to solving the generalised eigenvalue problem

$$\boldsymbol{S}_{\mathrm{b}} \boldsymbol{w}_i = v_i \boldsymbol{S}'_{\mathrm{w}} \boldsymbol{w}_i \quad \forall i = 1, 2, \tag{S-2}$$

where $\boldsymbol{S}_{\mathrm{w}}$ and $\boldsymbol{S}_{\mathrm{b}}$ are $N_{\mathrm{h}} \times N_{\mathrm{h}}$-dimensional matrices calculated on the last hidden layer $\mathbf{h}$. In this 2-class problem, the largest eigenvalue $v = v_1$ measures the separation between states B and U along direction $\boldsymbol{w} = \boldsymbol{w}_1$.

The loss function that we use for training the NN is

$$\mathcal{L} = -v \; - \; \alpha \, \frac{1}{1 + (s^2 - 1)^2} \; + \; \gamma \sum_i |\theta_i|^2 \tag{S-3}$$

where the first term is the LDA eigenvalue, the second term prevents the NN output s from becoming too narrow over the training data, and the third one is an L2 regularization over the weights θ_i of the network, with $\gamma = 10^{-5}$. We set $\alpha = 2/\lambda$.

We optimise the model with ADAM [13] using a learning rate of $2.5 \cdot 10^{-5}$. We stop the training when the model reaches convergence, that we define through the condition $v > 1.28/\lambda$. The converged LDA eigenvector $\boldsymbol{w}$ generates $s = \boldsymbol{w}^T \boldsymbol{h}$. Throughout the paper, we transform the NN output by $s_{\mathrm{w}} = s + s^3$. This transformation improves the behaviour of the Deep-LDA CV s_{w} in enhanced sampling, as it increases its width in the important input states B and U.

S-1.3 The Funnel restraint

In the enhanced sampling simulations, we use a funnel restraint [14] equivalent to the one previously employed by [4, 15] on the same system. The funnel limits the space available to the ligand in state U by confining it to a cylindrical volume above the binding site, as sketched in Fig. 1 in the main text. As the

ligand approaches the binding site, the funnel restraint becomes wider so that its presence does not affect the binding process itself.

We define s_z as the projection on the binding axis z of the center of the carbon atoms of each ligand and r its radial component. When $s_z > 10\,\text{Å}$, the funnel surface is a cylinder with radius $R_{\text{cyl}} = 2\,\text{Å}$ with its axis along the z direction. When $s_z < 10\,\text{Å}$, the funnel opens into an umbrella-like shape with a 45 degree angle whose surface is defined by $r = 12 - s_z$.

The force that pushes the ligand for displacements x away from the funnel's surface is harmonic $-k_{\text{F}}x$ with $k_{\text{F}} = 20\,\text{kJ/mol}\,\text{Å}^{-2}$. A further harmonic restraint is applied on s_z to prevent the ligand from getting too far from the host reaching the upper boundary of the simulation box. The corresponding force is $-k_{\text{U}}(s_z - 18)$ for $s_z > 18$Å and $k_{\text{F}} = 40\,\text{kJ/mol}\,\text{Å}^{-2}$.

During training, we set boundaries to state U so that the training configurations match the ones that occur in the subsequent enhanced sampling simulations. We activate the funnel described above and two additional restraints $-k_{\text{U}}(s_z - 18)$ for $s_z > 18\,\text{Å}$ and $-k_{\text{U}}(s_z - 14)$ for $s_z < 18\,\text{Å}$, with $k_{\text{U}} = 20\,\text{kJ/mol}\,\text{Å}^{-2}$.

Because of the funnel presence, the free energy difference between the bound and the true unbound state that we extract from enhanced sampling simulations needs a correction. It can be calculated from

$$\Delta G = -\frac{1}{\beta}\log\left(C^0\pi R_{\text{cyl}}^2\int_{\text{B}}\text{d}z\,\exp\left(-\beta(W(z) - W_{\text{U}})\right)\right) \tag{S-4}$$

where $\beta = 1/k_{\text{B}}T$, $C^0 = 1/1660\text{Å}^{-3}$ is the standard concentration, z is the coordinate along the funnel's axis, $W(z)$ is the free energy along the funnel axis and W_{U} its reference value in state U. More precisely, we define W_{U} as the average free energy value in the interval $1.5\,\text{Å} < z < 1.8\,\text{Å}$. The integral is performed over the state B region that we define as $0.3\,\text{Å} < z < 0.8\,\text{Å}$.

S-1.4 Enhanced sampling simulations

We perform enhanced sampling with OPES [16], a recently developed evolution of Metadynamics that helps achieving a fast and robust convergence. For every guest molecule, we independently train 3 different Deep-LDA CVs: s_{w}^a, s_{w}^b and s_{w}^c. With each one of them, we perform an OPES simulation where both the s_z and the Deep-LDA CV s_{w} are biased. We use a deposition rate of 1 ps and a barrier estimate of 50 kJ/mol for ligands G1, G2, of 60 kJ/mol for G3 and of 40 kJ/mol for G4, G5 and G6. Each simulation includes 4 replicas running in parallel, sharing and building together the same bias potential through PLUMED's Multiple Walkers feature. Each replica runs for 140 ns, for a total simulation time of 560 ns.

All the simulation inputs can be found on the PLUMED-NEST repository XXX. We include below here an example of a PLUMED input for the G4 case.

```
# --- (1) ATOMS DEFINITIONS and ALIGNMENT ---

HOST: GROUP ATOMS=29-224      #host atoms
LIGC: GROUP ATOMS=1-11,10-12  #carbon atoms in the ligand
l1: GROUP ATOMS=2             #ligand selected atoms
l2: GROUP ATOMS=3
l3: GROUP ATOMS=11
l4: GROUP ATOMS=14
WO: GROUP ATOMS=234-6533:3    #water oxygen atoms

WHOLEMOLECULES ENTITY0=HOST
FIT_TO_TEMPLATE STRIDE=1 REFERENCE=conf_template.pdb TYPE=OPTIMAL #coordinates alignment
lig: CENTER ATOMS=LIGC

cyl: DISTANCE ATOMS=v1,lig COMPONENTS
radius: MATHEVAL ARG=cyl.x,cyl.y FUNC=sqrt(x*x+y*y) PERIODIC=NO
```

```
v1: FIXEDATOM AT=2.0136,2.0136,2.0   #virtual atoms
v2: FIXEDATOM AT=2.0136,2.0136,2.25
v3: FIXEDATOM AT=2.0136,2.0136,2.5
v4: FIXEDATOM AT=2.0136,2.0136,2.75
v5: FIXEDATOM AT=2.0136,2.0136,3.0
v6: FIXEDATOM AT=2.0136,2.0136,3.25
v7: FIXEDATOM AT=2.0136,2.0136,3.5
v8: FIXEDATOM AT=2.0136,2.0136,3.75

# --- (2) DESCRIPTORS ---

L1: COORDINATION GROUPA=l1 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=6 MM=10}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
L2: COORDINATION GROUPA=l2 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=6 MM=10}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
L3: COORDINATION GROUPA=l3 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=6 MM=10}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
L4: COORDINATION GROUPA=l4 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=6 MM=10}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
V1: COORDINATION GROUPA=v1 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=2 MM=6}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
V2: COORDINATION GROUPA=v2 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=2 MM=6}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
V3: COORDINATION GROUPA=v3 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=2 MM=6}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
V4: COORDINATION GROUPA=v4 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=2 MM=6}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
V5: COORDINATION GROUPA=v5 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=2 MM=6}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
V6: COORDINATION GROUPA=v6 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=2 MM=6}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
V7: COORDINATION GROUPA=v7 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=2 MM=6}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5
V8: COORDINATION GROUPA=v8 GROUPB=WO SWITCH={RATIONAL D_0=0.0 R_0=0.25 NN=2 MM=6}
NLIST NL_CUTOFF=1.0 NL_STRIDE=5

d1: MATHEVAL ARG=L1 FUNC=(x/2.5)-1.0 PERIODIC=NO  #normalized descriptors
d2: MATHEVAL ARG=L2 FUNC=(x/2.5)-1.0 PERIODIC=NO
d3: MATHEVAL ARG=L3 FUNC=(x/2.5)-1.0 PERIODIC=NO
d4: MATHEVAL ARG=L4 FUNC=(x/2.5)-1.0 PERIODIC=NO
d5: MATHEVAL ARG=V1 FUNC=(x/2.8)-1.0 PERIODIC=NO
d6: MATHEVAL ARG=V2 FUNC=(x/2.8)-1.0 PERIODIC=NO
d7: MATHEVAL ARG=V3 FUNC=(x/2.8)-1.0 PERIODIC=NO
d8: MATHEVAL ARG=V4 FUNC=(x/2.8)-1.0 PERIODIC=NO
d9: MATHEVAL ARG=V5 FUNC=(x/2.8)-1.0 PERIODIC=NO
d10: MATHEVAL ARG=V6 FUNC=(x/2.8)-1.0 PERIODIC=NO
d11: MATHEVAL ARG=V7 FUNC=(x/2.8)-1.0 PERIODIC=NO
d12: MATHEVAL ARG=V8 FUNC=(x/2.8)-1.0 PERIODIC=NO

# --- (3) DEEP-LDA CV and other quantities ---
```

```
s: PYTORCH_MODEL MODEL=modelG4_a.pt ARG=d1,d2,d3,d4,d5,d6,d7,d8,d9,d10,d11,d12  #NN output
sw: MATHEVAL ARG=s.node-0 FUNC=x+x^3 PERIODIC=NO   #Deep-LDA CV

funnel: MATHEVAL ARG=radius,cyl.z VAR=r,z FUNC=(r+1.0*(-1.2+z))*step(-z+1.)+(r-0.2)*step(z-1.)
PERIODIC=NO
UPPER_WALLS AT=0 ARG=funnel KAPPA=2000.0 LABEL=funnelwall  #funnel restraint
UPPER_WALLS AT=1.8 ARG=cyl.z KAPPA=4000.0 EXP=2 LABEL=sz_wall  #upper limit of s_z

ang: ANGLE ATOMS=v3,v5,6,11   #angle of a ligand's axis with z
cosang: MATHEVAL ARG=ang FUNC=cos(x) PERIODIC=NO

# --- (4) OPES  ---

OPES_WT ...
   LABEL=opes
   ARG=cyl.z,sw
   FILE=Kernels.data
   PACE=500
   BARRIER=40
   WALKERS_MPI
... OPES_WT

PRINT ARG=* STRIDE=250 FILE=COLVAR FMT=%8.4f

ENDPLUMED
```

S-1.5 Calculating average properties

For calculating average properties, such as binding free energies and estimating their statistical error, we apply the following strategy. Given a guest and a Deep-LDA CV, for each simulation we invert the trajectories of each of the 4 replicas and merge them into one longer trajectory. This way, the last configurations of each replica at the last timestesp 140 ns become the first 4 configurations in the new trajectory and so on. This step helps when calculating free energies, as it simplifies the discarding of the initial non-equilibrated part of the simulations.

Then, for numerical stability, we filter out the outlier configurations where the funnel restraint has a bias energy larger than 10 kJ/mol. We split the resulting trajectory into 5 blocks of 100 ns each, where block 1 corresponds to the last part of the simulations and block 5 to the initial one. The very beginning of the simulations is not taken into account in a natural way. In each block b, we independently evaluate the FES and the binding free energy ΔG_b through Eq. S-4. Every block has a different statistical weight given by $w_\mathrm{b} = \sum_j e^{\beta V(j)}$ where index j runs over the configurations in the block and V is the bias from OPES.

The mean ΔG is then simply the weighted average over the blocks

$$\Delta G = \frac{\sum_{b=1}^{5} w_b \, \Delta G_\mathrm{b}}{\sum_{b=1}^{5} w_b} \tag{S-5}$$

and its error the weighted standard deviation

$$\sigma(\Delta G) = \sqrt{\frac{1}{N_\mathrm{eff} - 1} \, \frac{\sum_{b=1}^{5} w_b \, (\Delta G_\mathrm{b} - \Delta G)^2}{\sum_{b=1}^{5} w_b}}. \tag{S-6}$$

The effective sample size

$$N_{\text{eff}} = \frac{\left(\sum_{b=1}^{5} w_b\right)^2}{\sum_{b=1}^{5} w_b^2} \tag{S-7}$$

measures the level of correlations between the blocks. In the ideal case of low correlation, $N_{\text{eff}} \to 5$, i.e. the number of blocks. Our calculations present a reasonably low level of correlations between blocks as the lowest value that we observed is $N_{\text{eff}} \approx 4.5$.

In the results breakdown below, for each guest molecule and Deep-LDA CV we show in tables ΔG_b, w_b and the average ΔG with its error. In the main text, we also present in Tab. 1 the average binding energy for every ligand where we applied the weighted averaging procedure to all the blocks of all the CVs.

S-2 Results

S-2.1 Water behaviour in the ligand-free state

We first present a study of the interaction of the host with the water in absence of a guest molecule. The simulations are plain molecular dynamics of about 20 ns.

In order to calculate the number of water molecules inside the host $P(n)$ in Fig 6 (b) in the main text, we create an alpha-shape using the backbone carbon atoms coordinate of the host molecule (see Fig. S-1). The volume enclosed in the alpha-shape makes it a convenient to define whether a point (here, every water oxygen atom) lies inside or outside of the host binding pocket.

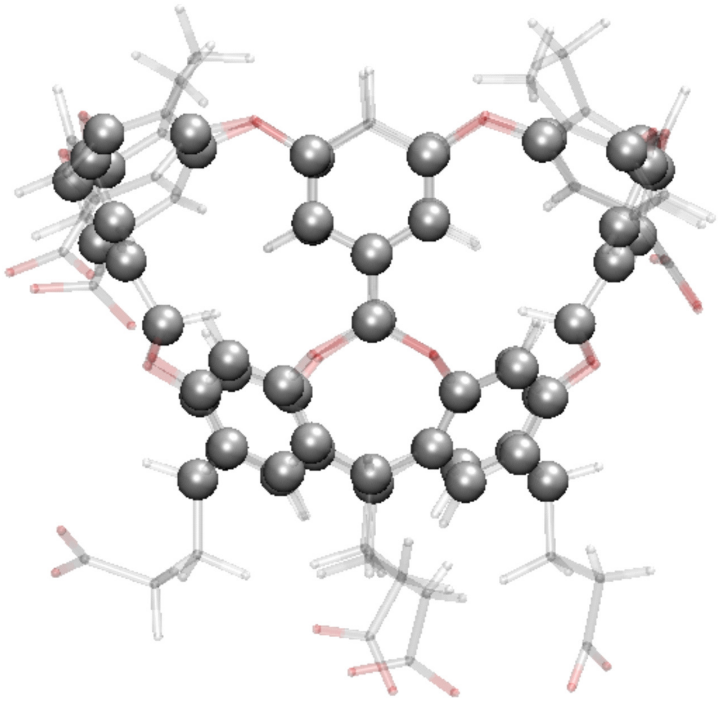

Figure S-1: Gray spheres indicate the Carbon atoms of the host molecule that we use to construct an alpha-shape.

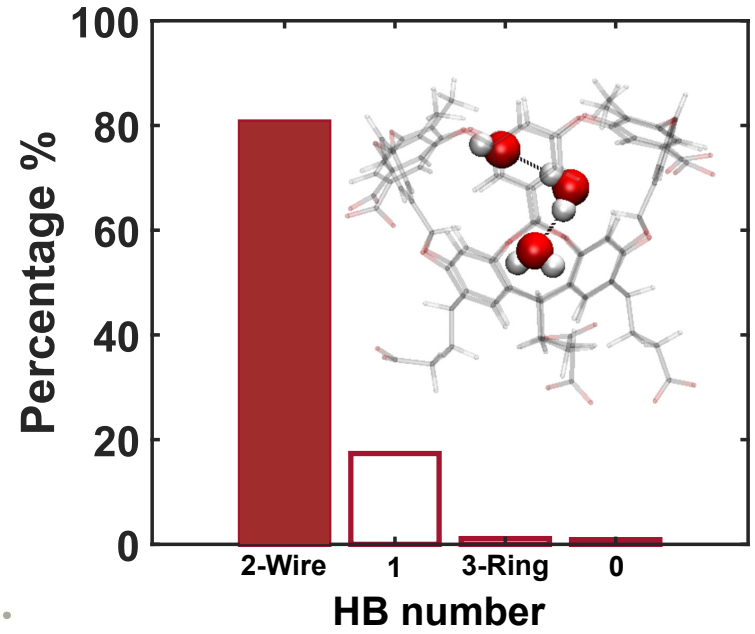

Figure S-2: Probability distribution of the number of hydrogen bonds between water molecules in the case where 3 water molecules are inside the pocket. In the inset, we show one representative snapshot of the most probable case where cavity presents three water molecules in a wire configuration.

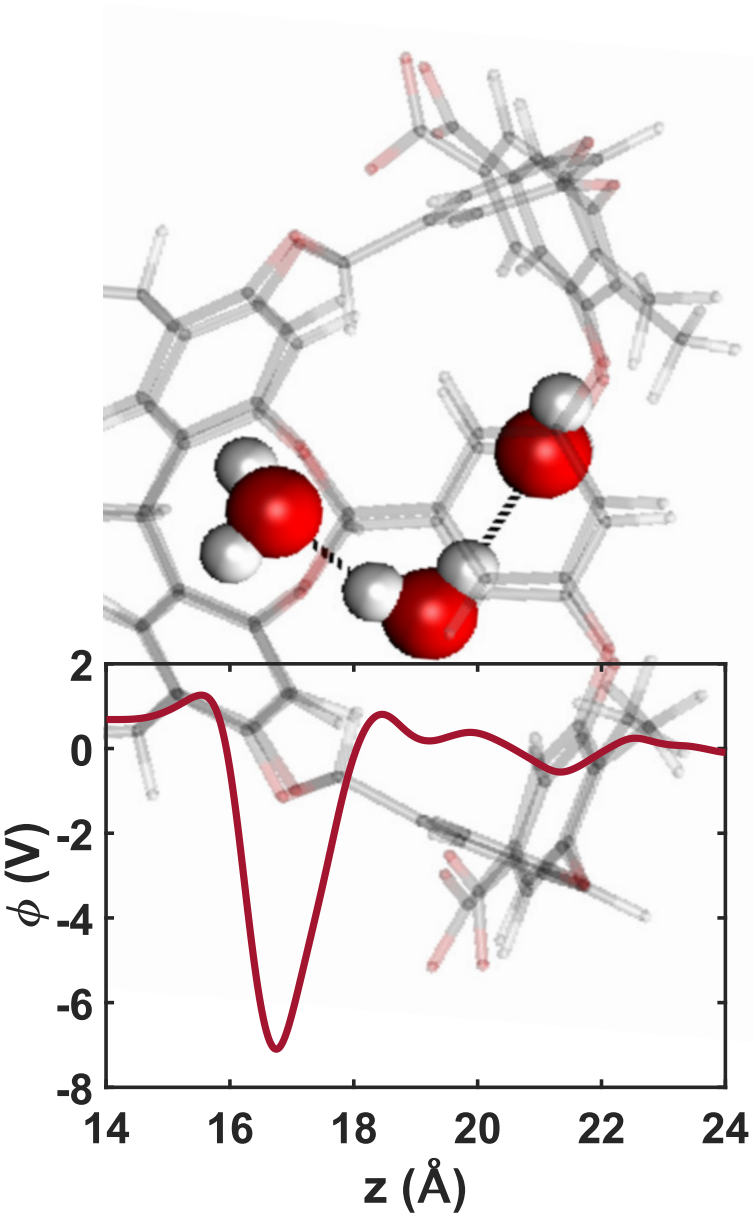

Figure S-3: Electrostatic potential along the binding axis with a corresponding snapshot of a typical configuration with 3 water molecule aligned in the pocket.

S-2.2 Ligand Binding

For each ligand we present a number of figures and tables. At first, we show a trajectory comparison. We perform identical simulations with respect to the Deep-LDA ones, with the only difference that instead of biasing a Deep-LDA CV we bias another commonly used physical variable, $\cos(\theta)$, where θ is the angle between the z axis and a ligand axis. We show the dynamics of the 4 replicas in this case and compare with the corresponding Deep-LDA simulations. Visual inspection of the trajectories indicates that in most cases the use of the Deep-LDA CV brings about a faster transition rate that leads to an improved phase space exploration.

The trajectories are coloured with the instantaneous value of the Deep-LDA CV and we notice a clear difference between the two. The simulations with CV $\cos(\theta)$ stay for long stretches of time in a constant colour area, which corresponds to the system staying in the same state, not performing any transition. On the other hand, the Deep-LDA simulations tend to have a dynamics that rapidly covers the whole range of colours which indicates a thorough phase space exploration.

Then, we present Figures analogous to Fig. 5 in the main text, where we plot a 2-dimensional FES over s_z and s_w and analyse the water position in different states. At last, we present a 2-dimensional FES over s_z and the coordination number around virtual atom V_2 and highlight the different states.

G1

Table 1: Binding energy ΔG (kcal/mol) of ligand G1 and its corresponding statistical weight w (a.u.) in every simulation block of calculations using Deep-LDA CVs from three different training.

Block	CV s_w^a		CV s_w^b		CV s_w^c	
	ΔG	w	ΔG	w	ΔG	w
1	6.54	409	6.48	331	6.47	452
2	6.30	592	6.64	583	6.40	529
3	5.76	421	6.45	598	6.16	407
4	6.19	459	6.01	263	6.33	449
5	6.00	360	6.19	370	6.41	434
all	$\mathbf{6.17} \pm 0.13$		$\mathbf{6.41} \pm 0.11$		$\mathbf{6.36} \pm 0.05$	

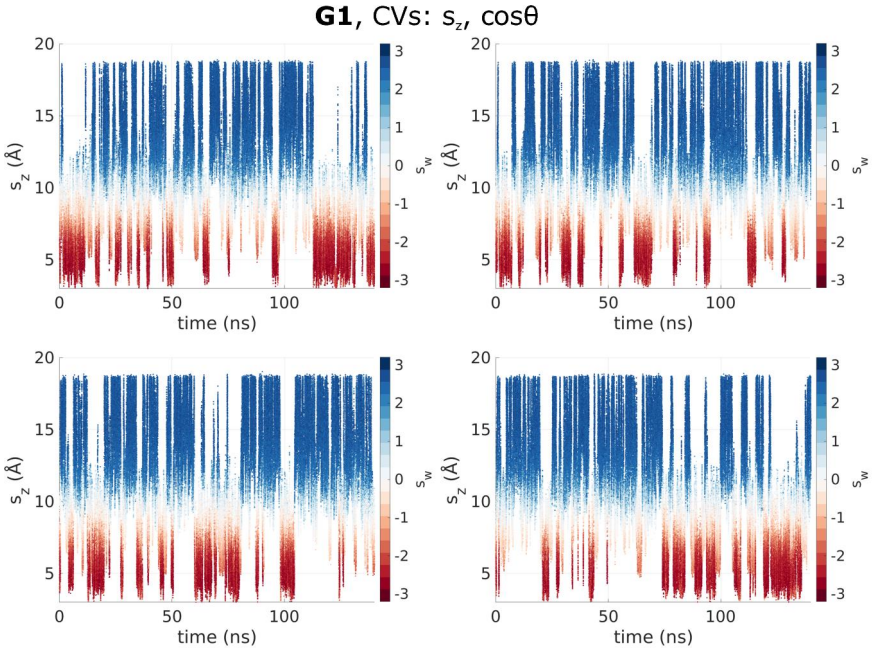

Figure S-4: Dynamics of s_z in an OPES simulation where s_z and $\cos\theta$ are biased. The plot is coloured with the instantaneous value of s_w.

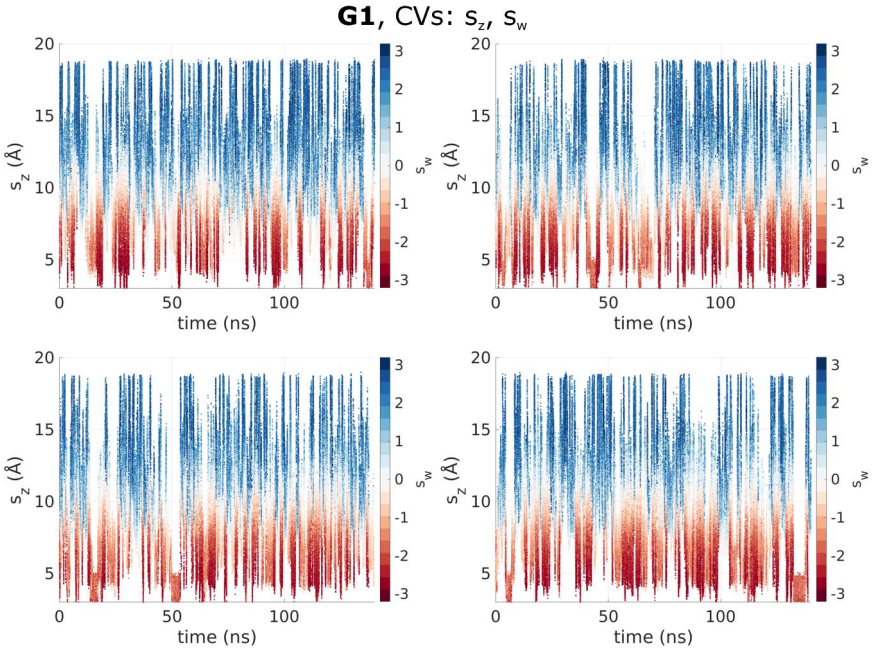

Figure S-5: Dynamics of s_z in an OPES simulation where s_z and s_w are biased. The plot is coloured with the instantaneous value of s_w.

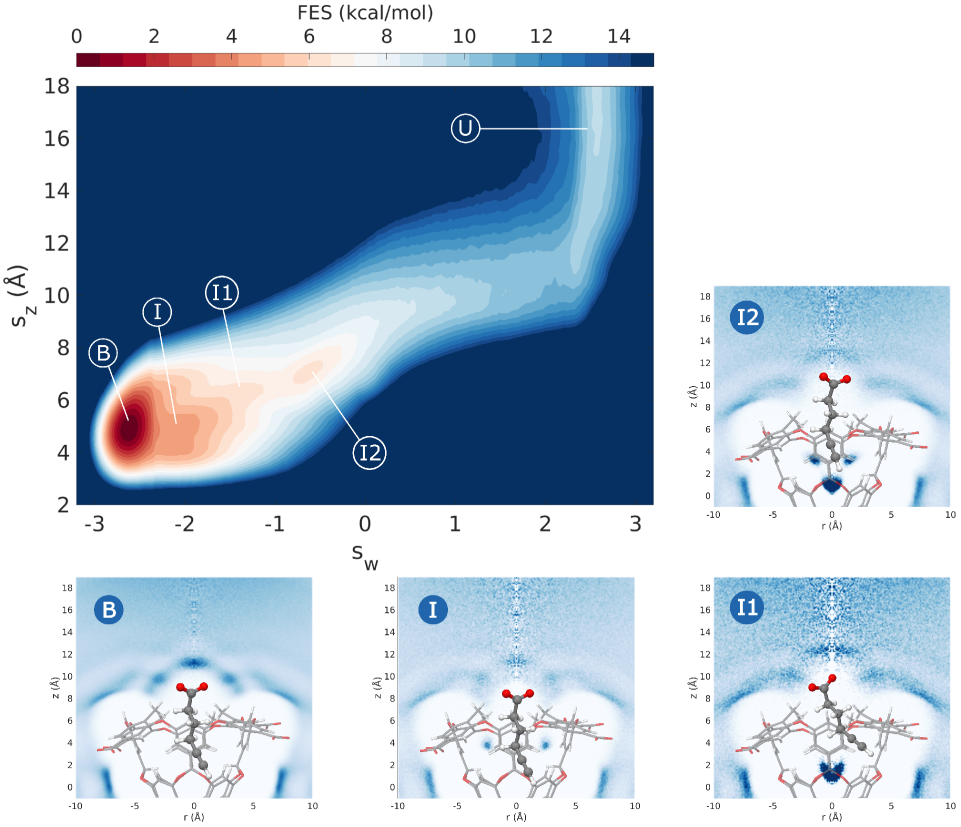

Figure S-6: FES with respect to s_z and s_w. We select some relevant states and perform unbiased simulations to measure the presence of water. We show histograms of the water oxygen atoms density in cylindrical coordinates z, r in these states with an illustrative sketch of the guest and the host. Darker colours correspond to a higher water density.

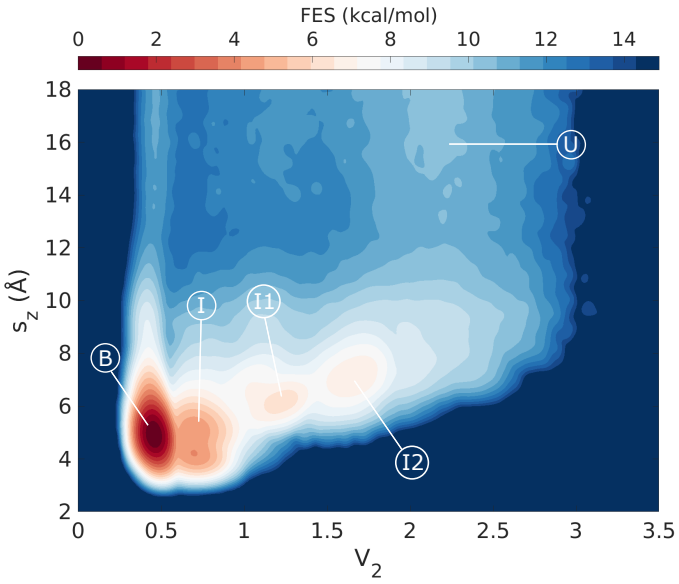

Figure S-7: FES with respect to s_z and the non-normalized coordination number V_2 that measures the water presence in the cavity.

G2

Table 2: Binding energy ΔG (kcal/mol) of ligand G2 and its corresponding statistical weight w (a.u.) in every simulation block of calculations using Deep-LDA CVs from three different training.

Block	CV s_w^a		CV s_w^b		CV s_w^c	
	ΔG	w	ΔG	w	ΔG	w
1	6.50	585	5.88	376	6.58	781
2	5.89	373	6.08	446	5.94	463
3	6.25	493	6.06	415	5.74	307
4	6.02	406	6.46	425	6.50	591
5	6.01	391	6.49	491	5.56	356
all	**6.17** $\pm$ 0.12		**6.21** $\pm$ 0.12		**6.19** $\pm$ 0.22	

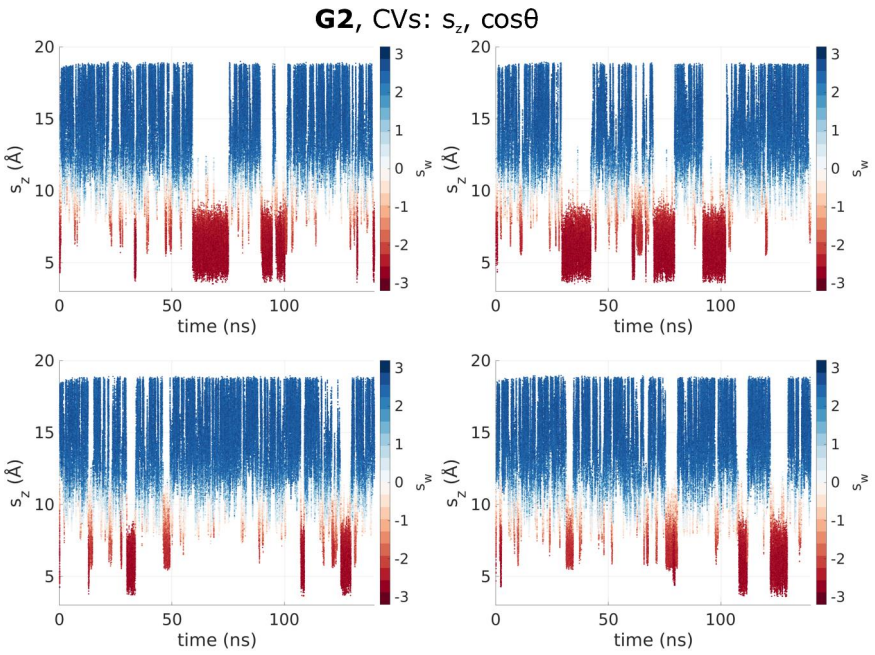

Figure S-8: Same as plot of Fig. S-4 for ligand G2.

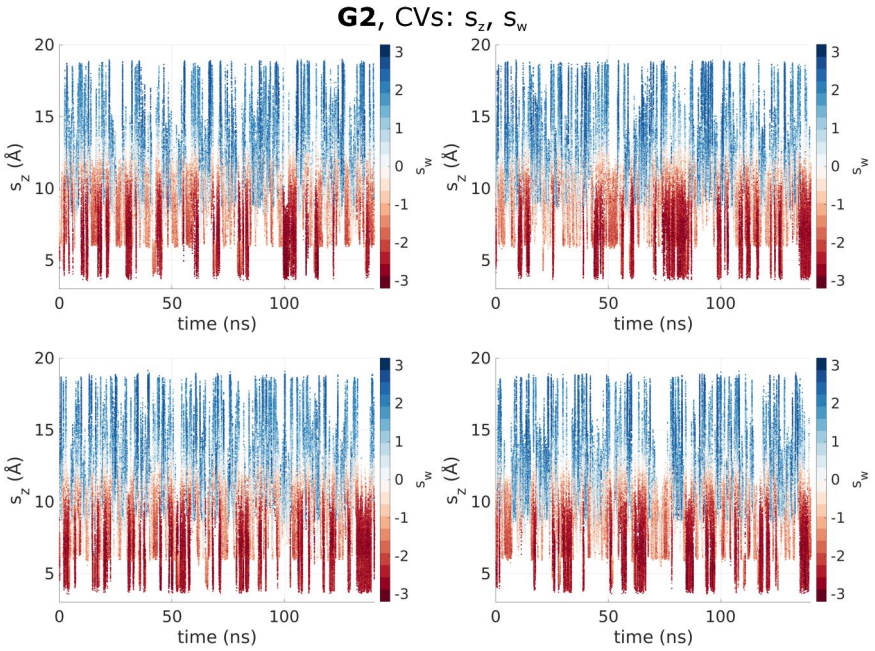

Figure S-9: Same as plot of Fig. S-5 for ligand G2.

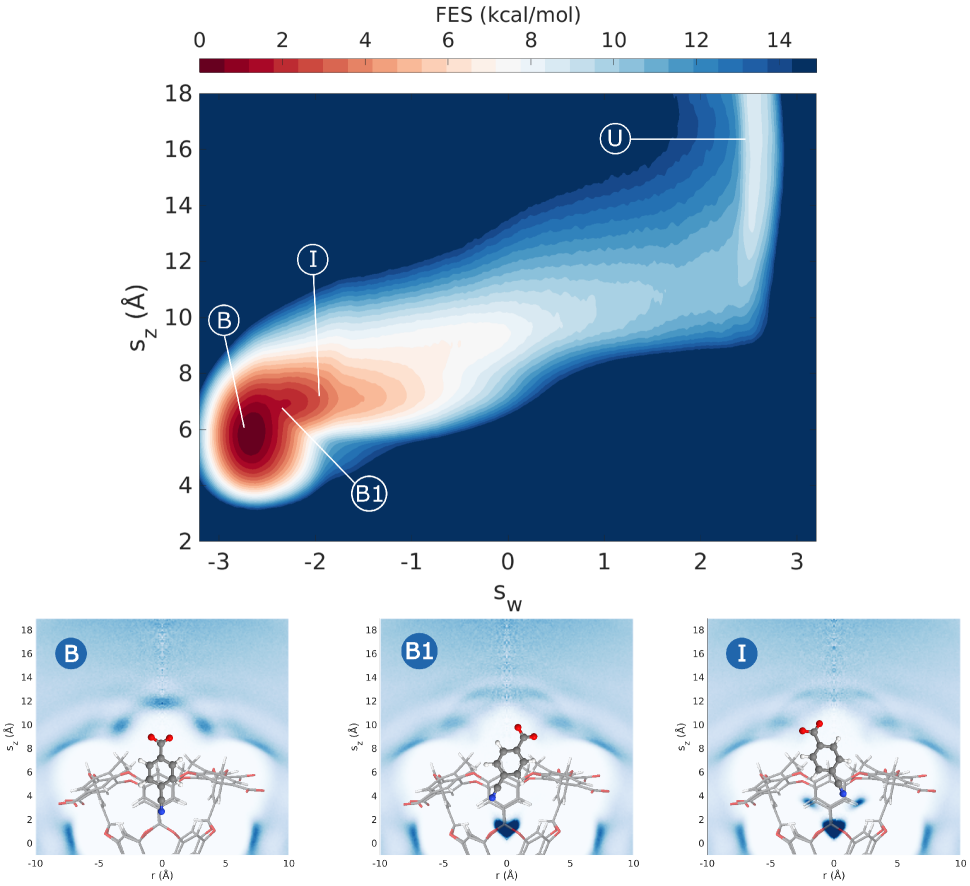

Figure S-10: Same as plot of Fig. S-6 for ligand G2.

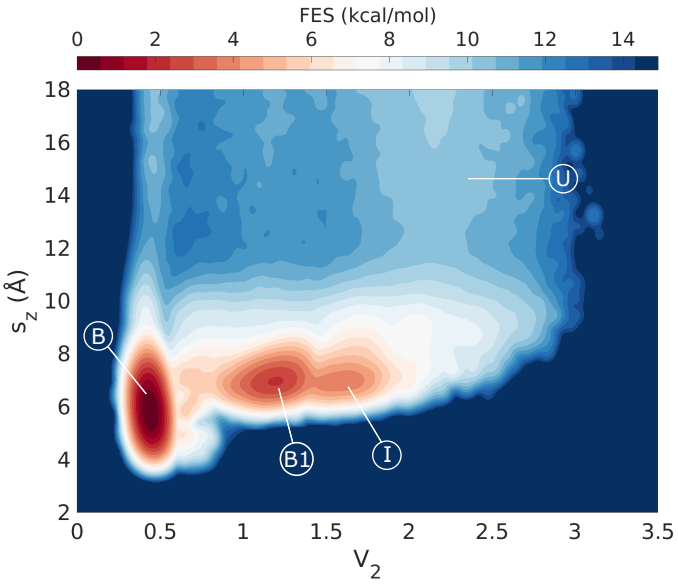

Figure S-11: Same as plot of Fig. S-7 for ligand G2.

G3

Table 3: Binding energy ΔG (kcal/mol) of ligand G3 and its corresponding statistical weight w (a.u.) in every simulation block of calculations using Deep-LDA CVs from three different training.

Block	CV s_w^a		CV s_w^b		CV s_w^c	
	ΔG	w	ΔG	w	ΔG	w
1	6.31	526	6.58	368	5.43	305
2	6.14	380	6.07	283	6.01	381
3	6.24	569	6.30	440	6.48	456
4	6.53	407	6.41	350	6.19	447
5	6.46	596	5.97	242	6.39	459
all	$\mathbf{6.34} \pm 0.07$		$\mathbf{6.30} \pm 0.11$		$\mathbf{6.15} \pm 0.17$	

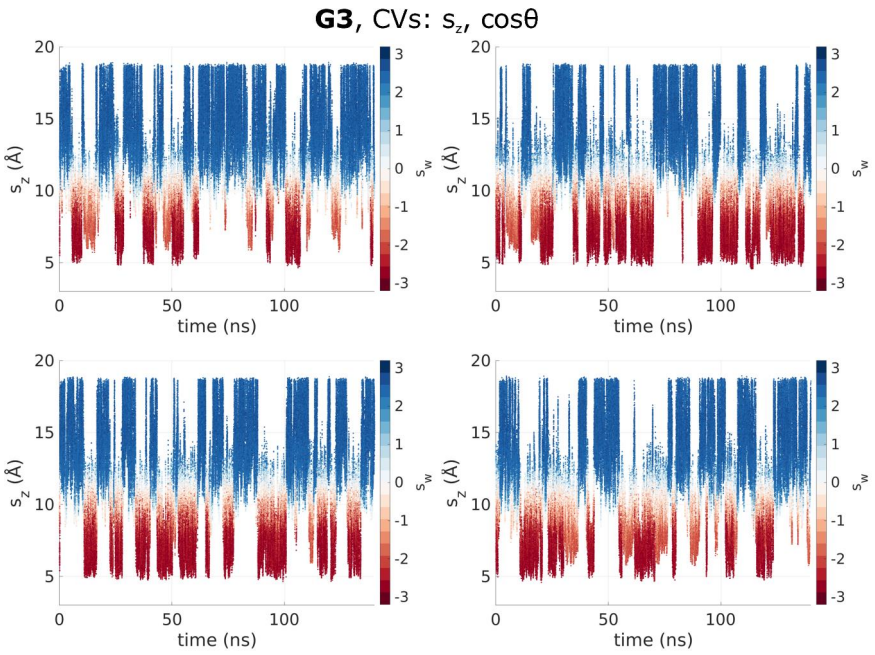

Figure S-12: Same as plot of Fig. S-4 for ligand G3.

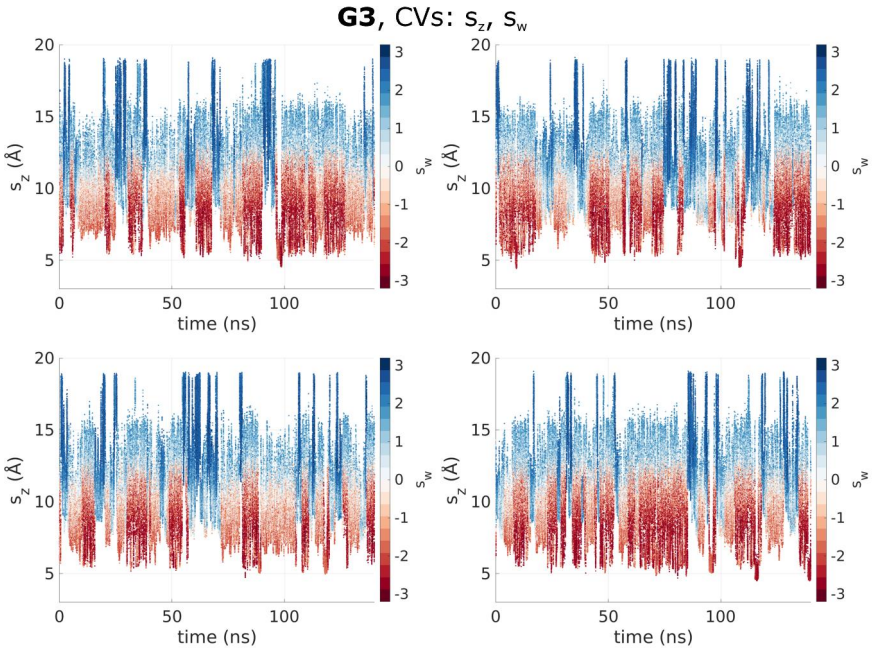

Figure S-13: Same as plot of Fig. S-5 for ligand G3.

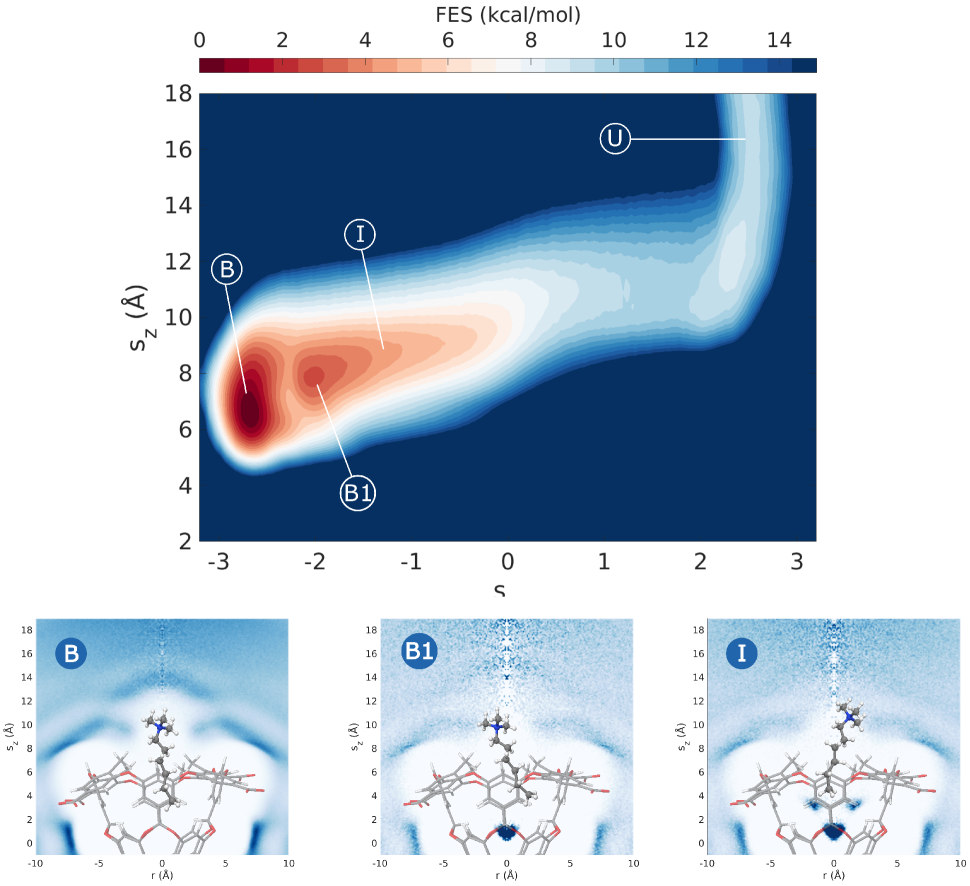

Figure S-14: Same as plot of Fig. S-6 for ligand G3.

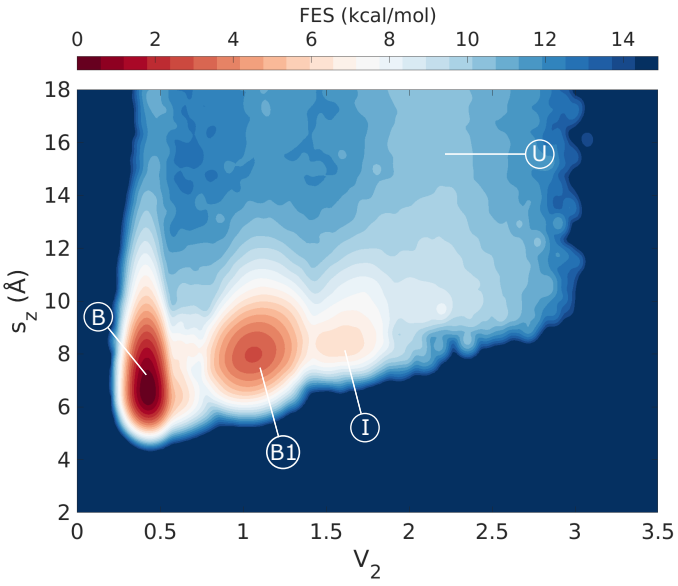

Figure S-15: Same as plot of Fig. S-7 for ligand G3.

G4

Table 4: Binding energy ΔG (kcal/mol) of ligand G4 and its corresponding statistical weight w (a.u.) in every simulation block of calculations using Deep-LDA CVs from three different training.

Block	CV s_w^a		CV s_w^b		CV s_w^c	
	ΔG	w	ΔG	w	ΔG	w
1	2.53	356	2.94	476	2.41	468
2	2.40	377	1.95	231	2.12	278
3	2.39	296	2.58	481	2.38	299
4	2.80	305	2.55	349	2.17	205
5	2.45	251	2.63	396	3.07	197
all	**2.51** $\pm$ 0.08		**2.60** $\pm$ 0.15		**2.40** $\pm$ 0.15	

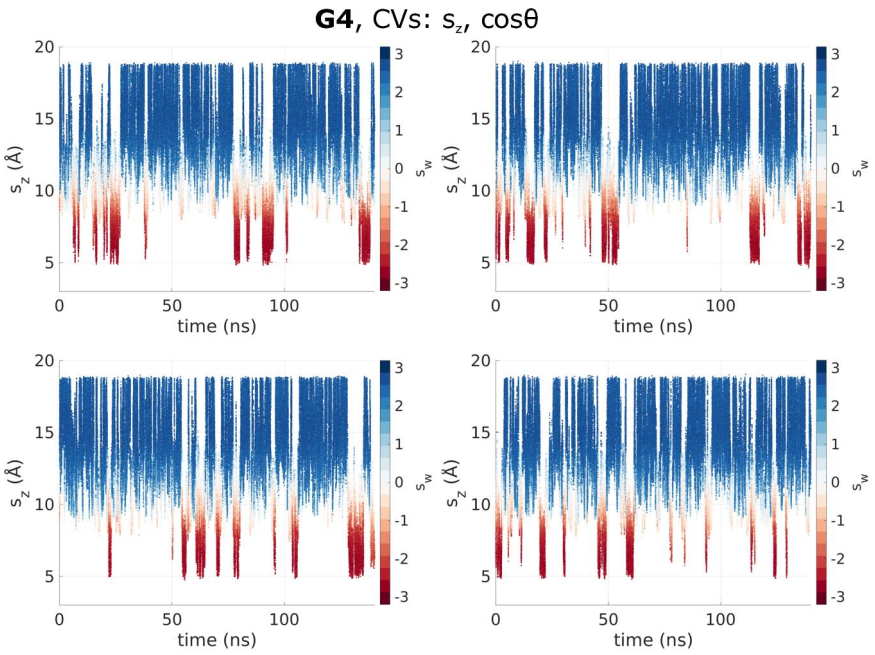

Figure S-16: Same as plot of Fig. S-4 for ligand G4.

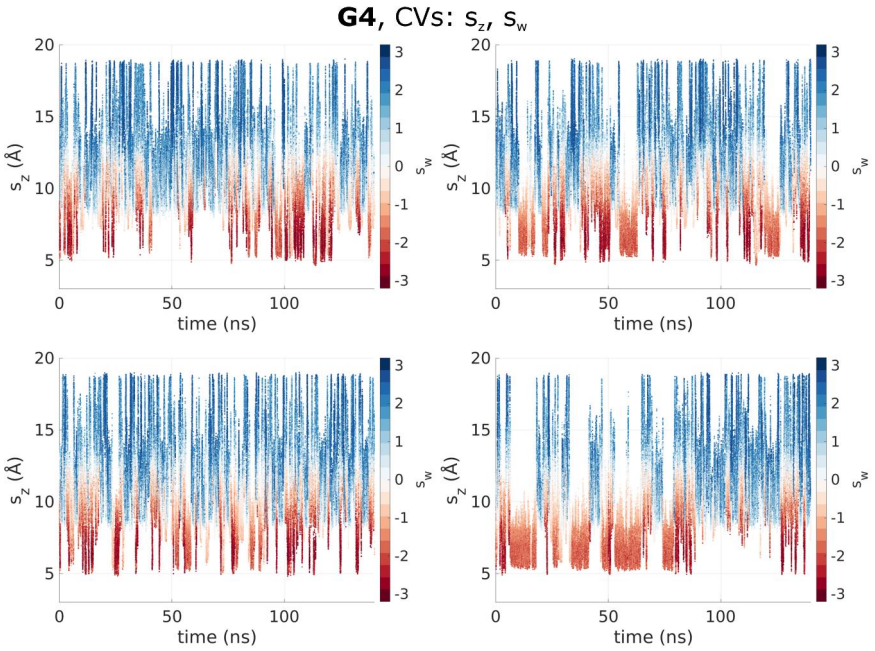

Figure S-17: Same as plot of Fig. S-5 for ligand G4.

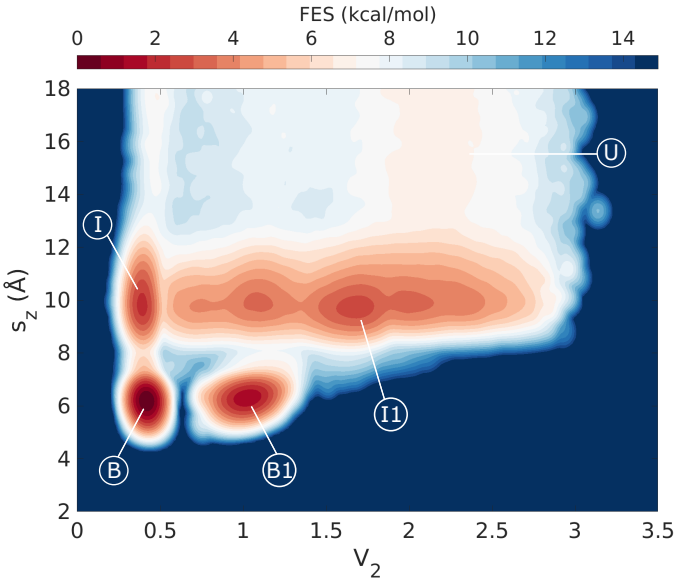

Figure S-18: Same as plot of Fig. S-7 for ligand G4.

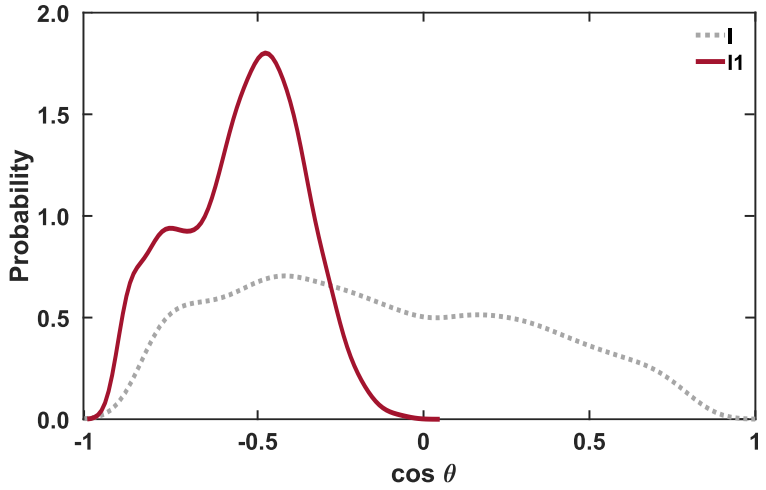

Figure S-19: Probability distribution of $\cos\theta$ for the G4 molecule in the intermediate states I and I1, where θ is the angle between the binding axis and the -COO group.

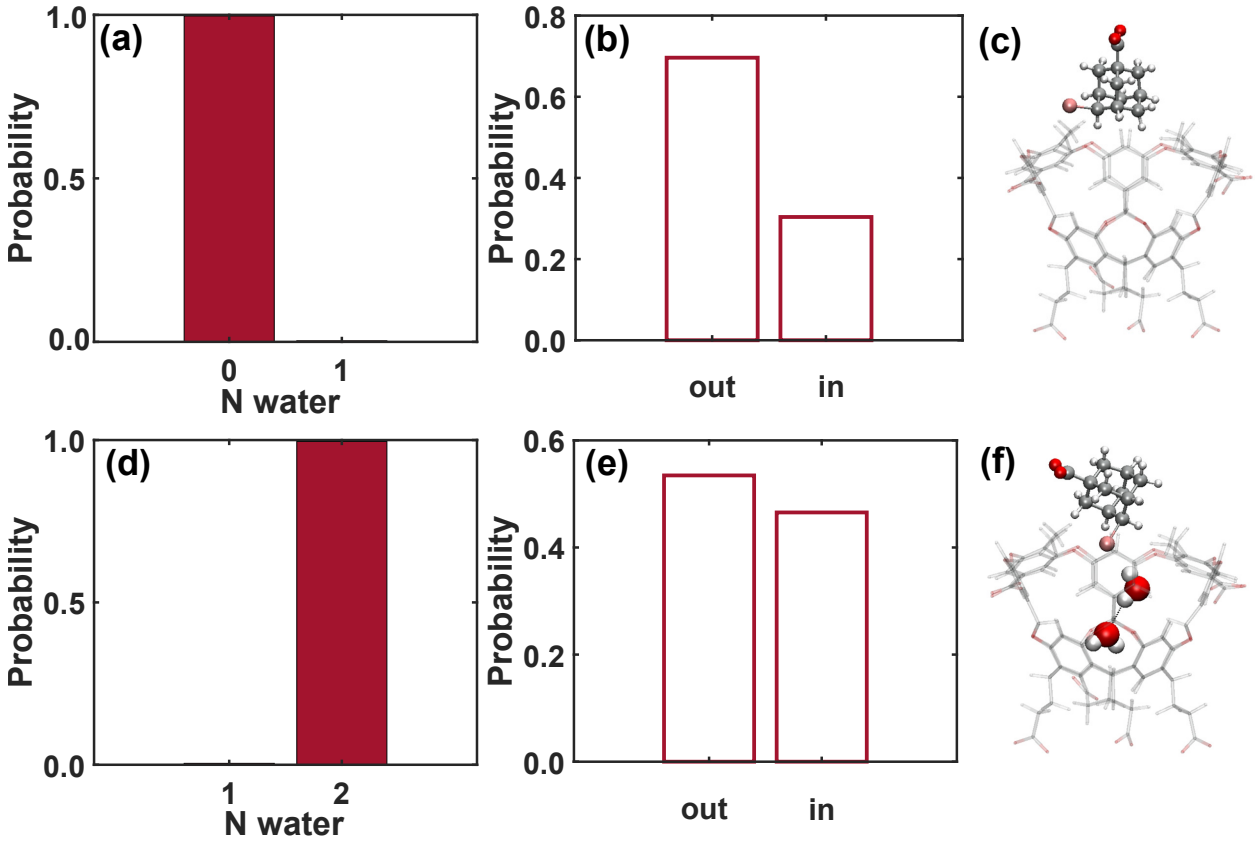

Figure S-20: In (a) and (d), probability distributions of the number of water molecules within the pocket for respectively intermediate states I and I1. In (c) and (d), probability distribution of the Br atom orientation with respect to the pocket for respectively intermediate states I and I1. In (c), a snapshot of the dry pocket with the "out" configuration for the Br atom in state I. In (f), a snapshot of the wet pocket with 2 water molecules with the "in" configuration for the Br atom in state I1.

G5

Table 5: Binding energy ΔG (kcal/mol) of ligand G5 and its corresponding statistical weight w (a.u.) in every simulation block of calculations using Deep-LDA CVs from three different training.

Block	CV s_w^a		CV s_w^b		CV s_w^c	
	ΔG	w	ΔG	w	ΔG	w
1	3.69	269	3.54	334	3.71	326
2	3.97	396	3.83	412	3.96	441
3	4.10	378	3.06	265	4.18	479
4	4.11	398	3.43	326	4.15	525
5	4.45	374	4.13	395	3.68	426
all	**4.09** $\pm$ 0.12		**3.65** $\pm$ 0.18		**3.96** $\pm$ 0.11	

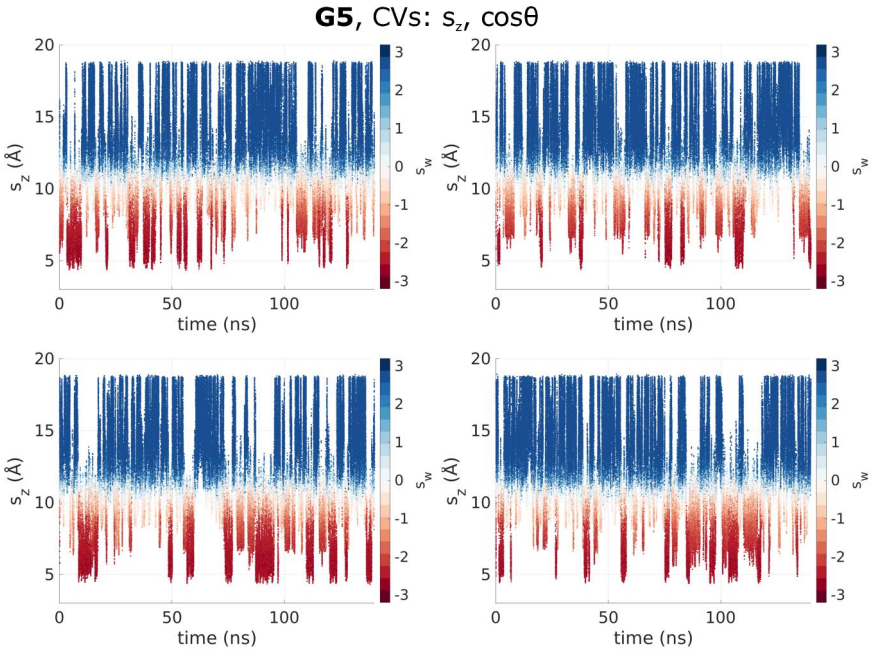

Figure S-21: Same as plot of Fig. S-4 for ligand G5.

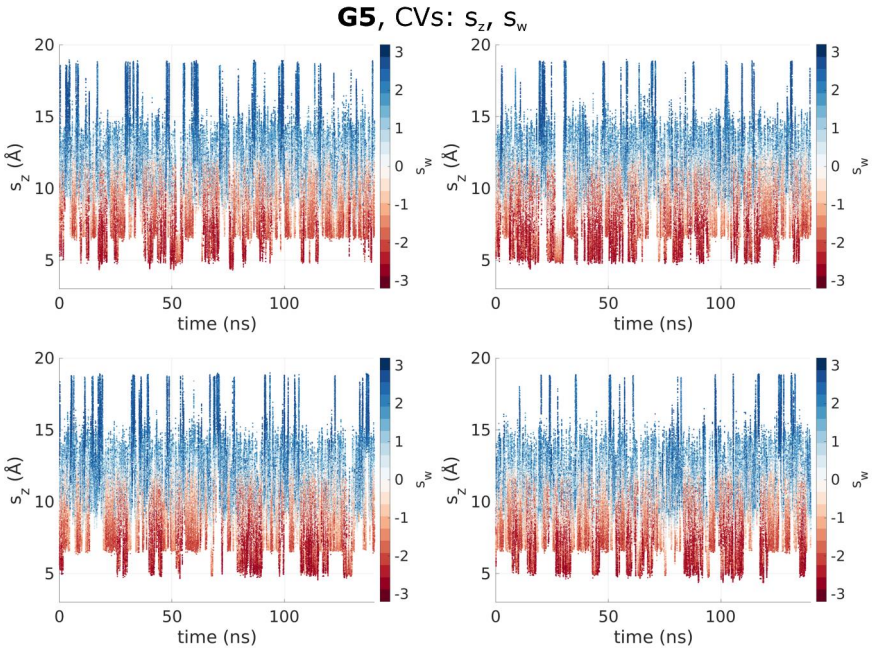

Figure S-22: Same as plot of Fig. S-5 for ligand G5.

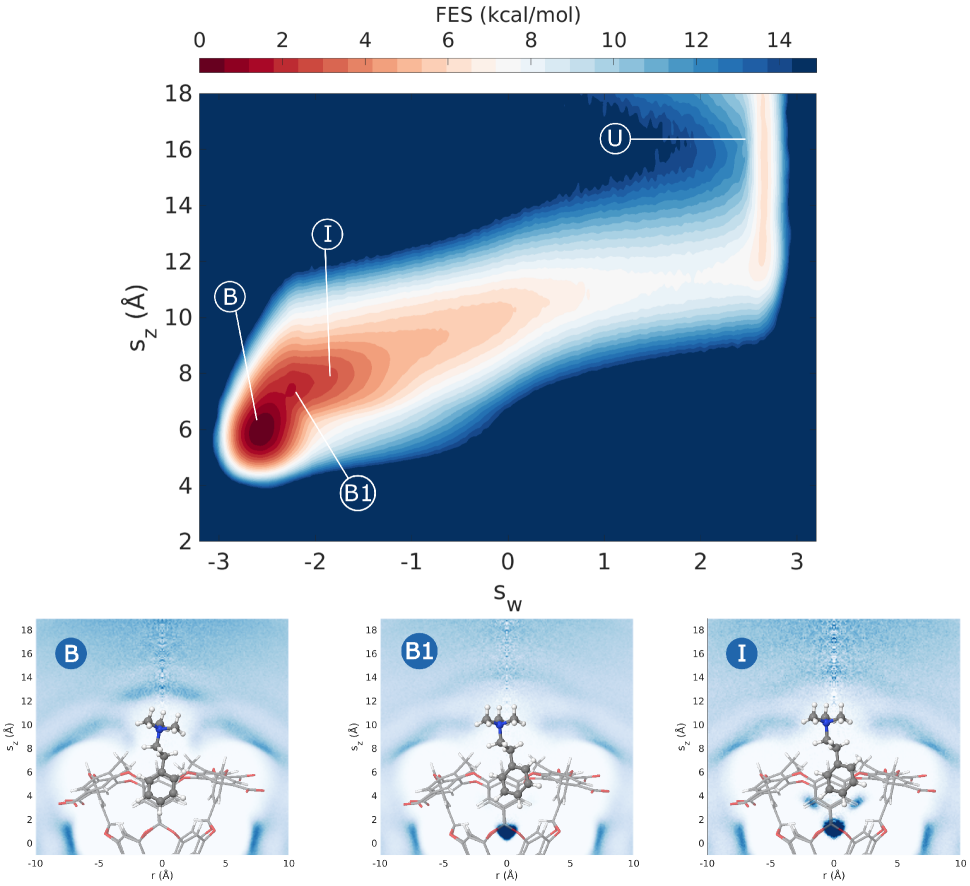

Figure S-23: Same as plot of Fig. S-6 for ligand G5.

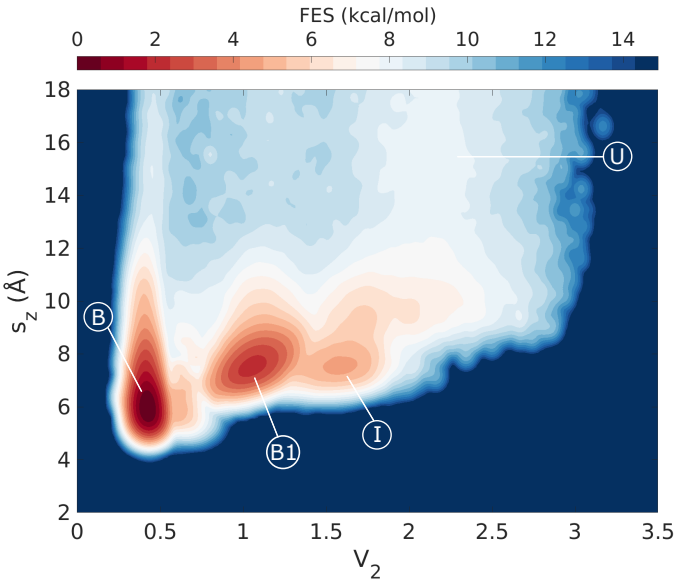

Figure S-24: Same as plot of Fig. S-7 for ligand G5.

G6

Table 6: Binding energy ΔG (kcal/mol) of ligand G6 and its corresponding statistical weight w (a.u.) in every simulation block of calculations using Deep-LDA CVs from three different training.

Block	CV s_w^a		CV s_w^b		CV s_w^c	
	ΔG	w	ΔG	w	ΔG	w
1	4.84	438	5.05	469	4.78	213
2	5.08	525	5.37	701	5.06	487
3	4.92	405	4.93	412	5.01	420
4	4.79	443	4.53	395	5.32	586
5	5.01	531	4.25	256	4.70	370
all blocks	**4.94** $\pm$ 0.05		**4.94** $\pm$ 0.20		**5.03** $\pm$ 0.12	

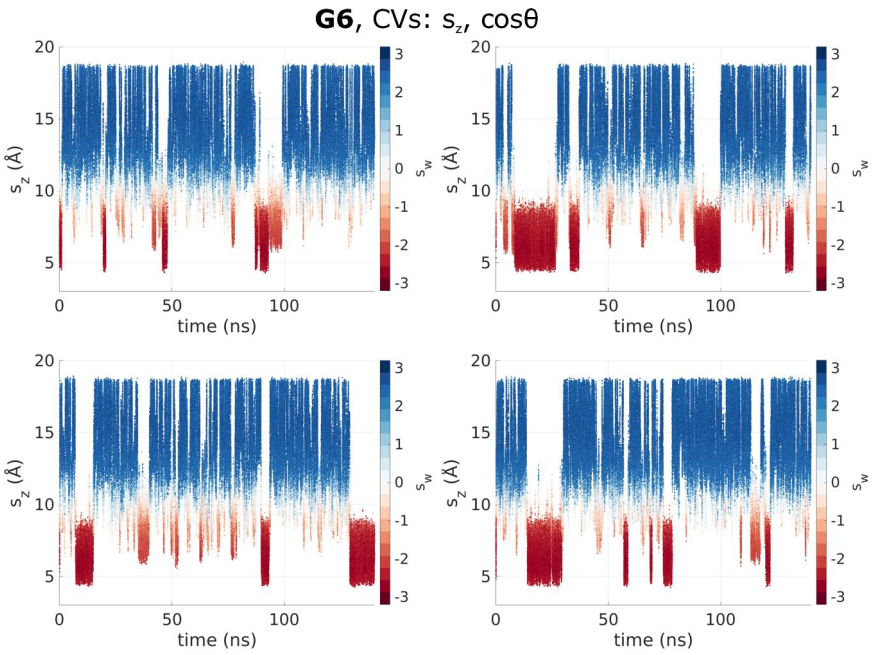

Figure S-25: Same as plot of Fig. S-4 for ligand G6.

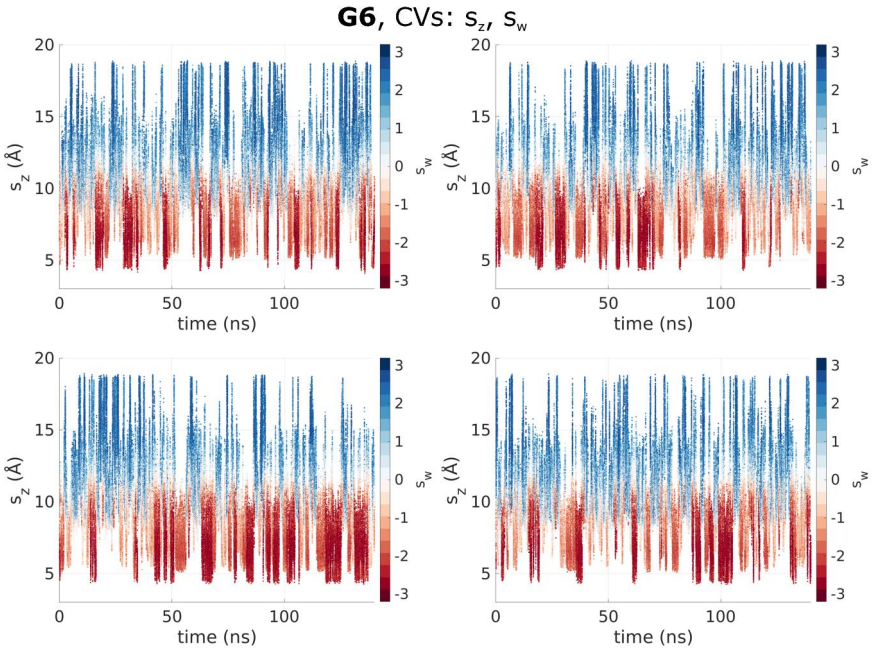

Figure S-26: Same as plot of Fig. S-5 for ligand G6.

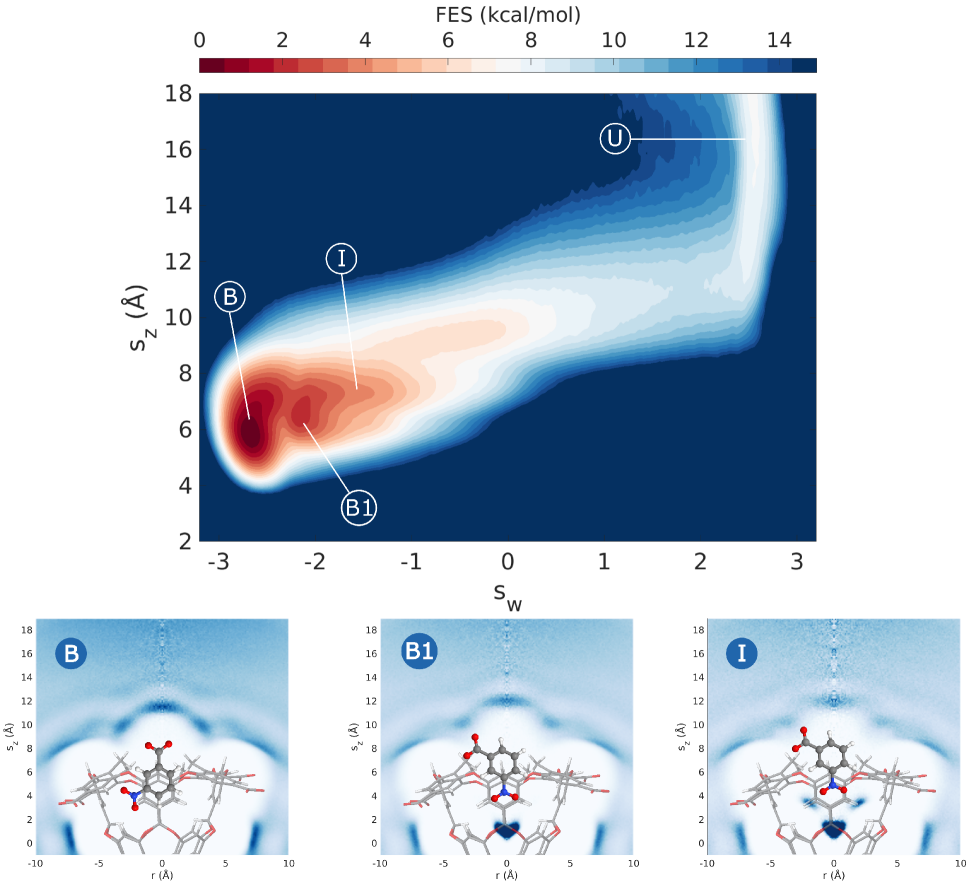

Figure S-27: Same as plot of Fig. S-6 for ligand G6.

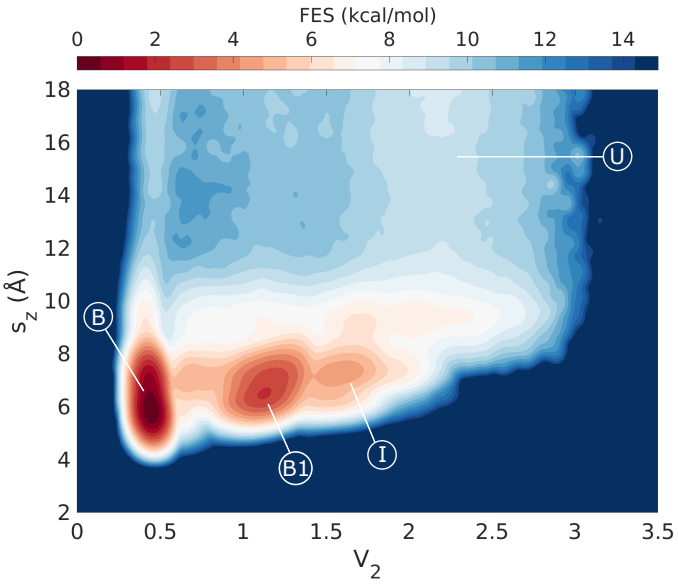

Figure S-28: Same as plot of Fig. S-7 for ligand G6.

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