Supporting Information:

Ion-Specific Induced Fluctuations and Free Energetics of Aqueous Protein Hydrophobic Interfaces: Towards Connecting to Specific-Ion Behaviors at Aqueous Liquid-Vapor Interfaces

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S1. **LJ PARAMETERS FOR IONS**

To model the properties of ions in solution properly, care must be taken to choose the parameters for ions. These concerns include combining rules, water model and treatment of the electrostatics\(^1\). In this study, TIP3P water model was applied. Correspondingly, only ion models parametrized to work with TIP3P could be taken into account. In CHARMM 22 force field, the only available anion parameters in TIP3P water are for Cl\(^-\), which are taken from Beglov and Roux parameters;\(^2,3\). Given this, we use the parameters from Cheatham’s work\(^4\) that is originally desired for TIP3P water model in AMBER force field, which is shown in Table 1, Supporting Information. To verify if these parameters are suitable to TIP3P water model in CHARMM force field, we did some tests to compare the single water-ion binding distances and the single water-ion binding energies of the ions for the two cases. In these tests, one single ion and one water molecule were placed in a large enough simulation box and the non-bonded cutoff distances were also set as large as possible. Since our production simulations were performed with NAMD simulation package using CHARMM force field, ideally, we would like to also perform the test in the same way. Unfortunately, because the default minimization algorithm in NAMD has a conflict with SHAKE algorithm which is used to constrain bond lengths involving hydrogen atoms, it is impossible to accurately minimize the structures with fixed water bond distances. Therefore, the energy minimization was performed with CHARMM package using CHARMM force field. With a stable structure, the binding energies can be recalculated using NAMD. The test results are shown in Table 1, Supporting Information. For the cations (K\(^+\), Na\(^+\), Cs\(^+\)), test results match well with the original; while for the anions (Cl\(^-\), I\(^-\)), we observe very small deviations for both single water-ion binding distances and single water-ion binding energies. We note that this is due to the fact that the TIP3P water model implemented in the CHARMM force field is slightly different from that of AMBER. In the CHARMM version of TIP3P, Lennard-Jones parameters on hydrogen is nonzero, whereas in the original version, Lennard-Jones interaction contributions from water hydrogen atoms not included. We verify this with a further test where we applied non-bonded fixed (NBFIX) strategy in CHARMM to ensure that there are no Lennard-Jones interactions involving hydrogen atoms from water; we obtained an exact matching result of binding distances and binding energies compared with original report. Despite this minor issue, we still transfer the ion parameters in TIP3P AMBER to the CHARMM force field in this study. We consider this as a valid combination because this empirical model could reproduce the most important characteristics that we would like to address between the two distinct anions: for Cl\(^-\), it is small, fully hydrated with rigid hydration shell; while for I\(^-\), it is large, soft, partial hydrated with malleable hydration shell. These characters can be proved by the ion-water RDF in Figure 1, Supporting Information. We note that currently no ion parameters could be considered...
as absolutely correct in conjunction with proteins during the simulation, because essentially no ion are parameterized
based on the interactions with proteins. Therefore, in discussion about ions’ effects related to the proteins, as long as
the model could reproduce currently accepted experiment observables, it could be considered as validation.

S2. POTENTIAL OF MEAN FORCE

Potential of Mean Force is associated with the reversible work necessary to bring two particles, from an infinite-
separation to a close contact.\(^5\) The connection between the PMF and free energy can be found in early work by
Kirkwood\(^6\) as expressed in Equation 1. Recently, a refined expression has been discussed by Wong et al.:\(^7\)

\[
\frac{dA(\xi_0)}{d\xi_0} = -\langle F_{\xi_0} \rangle_{\xi_0}
\]

\[
\frac{dA(\xi_0)}{d\xi_0} = \left\langle \left( \frac{\partial V(x)}{\partial q_{\xi}} \right)_{\{q_{m \neq \xi}\}^{N-1}} \right\rangle_{\xi_0} - \left\langle \frac{1}{\beta} \left( \frac{\partial \ln |J|}{\partial \xi_0} \right)_{\{q_{m \neq \xi}\}^{N-1}} \right\rangle_{\xi_0}
\]

\[
- \frac{1}{\beta} \sum_{m \neq \xi} \left[ \delta(q_m - l_{U_m}) \frac{dU_m}{dq_{\xi}} \delta(q_m - l_{L_m}) \frac{dL_m}{dq_{\xi}} \right]_{\xi_0}
\]

where \(V\) is the potential energy and \(q_{\xi}\) is the generalized coordinate so the first term represents the negative value
of mean force exerted on the reaction coordinate of interest and integration of the mean force along the domain of
the reaction coordinate produce the PMF. The second term is due to the volume scaling of the transformation from
Cartesian to generalized coordinates. The last term is considered as Leibnitzian contribution, which accounts the
interchange of integral and differential operators during Jacobian transformation. In our current study, since the
Cartesian representation of reaction coordinate is retained, the Jacobian and Leibniz terms vanish. For this case,
Equation 2 returns to Equation 1, so the thermodynamic free energy equals to the PMF. Thus we discuss the PMF
in terms of the free energy for the remainder of the paper.

S3. FIXED PROTEIN AND FIXED ION SIMULATION

For a comparison, we performed another set of simulation with both anion and protein fixed during the simulation.
Initial structure and position of HFBII protein remain identical as restrained protein case with most hydrophobic

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interface perpendicular to the z direction of the box. The K$^+$ was fixed at position (0, 0, -15) and the Cl$^-$/I$^-$ was frozen at position (0, 0, 16.0); (0, 0, 16.1); (0, 0, 16.2) ...... (0, 0, 24.8); (0, 0, 24.9); (0, 0, 25.0) respectively for each window. Therefore, 91 continuous windows were constructed along the reaction coordinate. Correspondingly, in Figure 3A, Supporting Information, it depicts the PMF for single Cl$^-$/I$^-$ approaching the hydrophobic protein-solvent interface and in Figure 3B, it is the fluctuation profile at (x = 0, y = 0) as a function of anion Z-position for Cl$^-$ and I$^-$ in this case. For both PMF profiles and fluctuation profiles, they actually match well with that from restrained anion simulation, which is a strong verification of the restrained simulation protocol.

S4. VERIFICATION OF FLUCTUATION ALGORITHM

In this section, we further verify the algorithm of interfacial fluctuation. First, we want to probe the ion size dependence issue. We consider single Cl$^-$/I$^-$ fixed at the position of z = 19 Å, with the frozen of the water molecules around the ion’s first hydration shell. We select this separation because judging from Figure 5B in the main test, the first hydration shells of both Cl$^-$ and I$^-$ keep intact. This includes 8 water molecules around Cl$^-$ and 9 water molecules around I$^-$. In this way, we could consider that there should be no differences of malleability of the hydration shell around Cl$^-$ and I$^-$ since they are both fixed. Therefore, the fluctuation difference will come from the size effect. In Table 4, Supporting Information, it presents the fluctuation values of various replicates with different starting structures of fixed hydration water around single Cl$^-$/I$^-$. For the same type of anion with different arrangements of water molecules in the initial structures, the fluctuation values are quite different. For a statistical meaningful result, the fluctuations should be computed with the consideration of as many as possible configurations of water molecules in the first hydration shell. Here we consider 20 replicates over 6ns simulation for both Cl$^-$ and I$^-$. The interfacial fluctuation value for Cl$^-$ is 0.13Å$^2$ ± 0.035 and for I$^-$ is 0.21Å$^2$ ± 0.07. The suppression of fluctuations with fixed hydration shell for both Cl$^-$ and I$^-$ implies the importance of hydration shell malleability for the induced fluctuations. Without consideration of shell malleability, the difference in the magnitude of fluctuation is small between Cl$^-$ and I$^-$. To further validate our algorithm, we consider the criteria to define the interface. Previously, points are defined to belong to the interface if $\rho(r_0, \theta, \phi) = 0.6\rho_{bulk}$. We test another way to define the interface with $\rho(r_0, \theta, \phi) = 0.5\rho_{bulk}$. The fluctuation profile is shown in Figure 6A, Supporting Information. Although the absolute fluctuation values are different in this case compared with that using 0.6 as criteria (Figure 6B, Supporting Information), the general trends are similar in which surface stable I$^-$ could actually induce a larger interface fluctuation compared with
nonsurface stable Cl\(^{-}\). Finally, we computed the entropic contributions \(-T\Delta S\) as a function of \(z\) position as single anion approaching the hydrophobic protein interface. For range of small temperature change, we could consider enthalpic contribution \((\Delta H)\) and entropic contribution \((-T\Delta S)\) as constant values. As a result, we could get entropy by using free energy data at 300K and 310K in the following equation:

\[
\Delta S(z) = -\frac{\Delta G_{310}(z) - \Delta G_{300}(z)}{T_{310} - T_{300}}
\]

(3)

Based on this, entropic component \((-T_{300}\Delta S)\) contribution profiles at 300K could be further obtained and shown in Figure 7, Supporting Information. As single anion moves closer to the interface, the entropic components are positive and entropies are negative in both cases, which is consistent with the results of negative adsorption entropy of ion around L-V interface from Otten et al and Vaikuntanathan et al. We further claim that according to Figure 7 in the main text, single anion would induce different magnitudes of interface fluctuation at \((x = 0, y = 0)\) as anion resides at distinctive position along \(z\)-axis. Our statement that surface stable iodide induces significantly larger interface fluctuations is actually based on the largest fluctuation value along the profile, in which case \(z\) is around 18 \(\AA\). Due to the less charge-dense of I\(^{-}\) compared with Cl\(^{-}\), causing a 'looser' or 'more malleable' hydration layer around the I\(^{-}\), this hydration layer then able to communicate with further water around protein interface, inducing a larger fluctuation. We see this correlation of larger surface stability with higher largest induced fluctuation before PMF minimum around both L-V interface and aqueous hydrophobic protein interface. Furthermore, as single anion moves closer to the interface, the induced fluctuation decreases dramatically. Around PMF minimum, \(z = 16.5\ \AA\), the fluctuation is suppressed relative to the bulk, which is also consistent with the negative entropy around protein interface we obtain here.
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| Non-Bonded Parameters | \( \sigma \) (Å) | \( \varepsilon \) (kcal/mol) |
|-----------------------|-----------------|-----------------|
| Cl\(^-\)              | 5.026           | -0.0355910      |
| I\(^-\)               | 5.720           | -0.0536816      |
| K\(^+\)               | 3.410           | -0.1936829      |

| Single Water-Ion Binding Distances | Cheatham (Å) | this research (Å) |
|-----------------------------------|--------------|-------------------|
| K\(^+\)                           | 2.66         | 2.66              |
| Na\(^+\)                          | 2.29         | 2.29              |
| Cs\(^+\)                          | 3.00         | 2.98              |
| Cl\(^-\)                          | 3.09         | 3.13              |
| I\(^-\)                           | 3.48         | 3.50              |

| Single Water-Ion Binding Energies | Cheatham (kcal/mol) | this research (kcal/mol) |
|-----------------------------------|---------------------|---------------------------|
| K\(^+\)                           | -18.51              | -18.52                    |
| Na\(^+\)                          | -24.29              | -24.30                    |
| Cs\(^+\)                          | -15.08              | -15.10                    |
| Cl\(^-\)                          | -14.26              | -14.15                    |
| I\(^-\)                           | -11.34              | -11.37                    |

**TABLE S1**: LJ parameters for ions applied in this work and verification. Note: for the case of single water-anion binding case, there are two geometries for the binding structure, one is \( C_s \), another is \( C_{2v} \); the binding distances and binding energies for Cl\(^-\) and I\(^-\) shown here are from \( C_s \) geometry.
| Parameter                              | Value 1               | Value 2               | Value 3               |
|----------------------------------------|-----------------------|-----------------------|-----------------------|
| exclude                                | scaled1-4             | langevinPistonPeriod  | 50                    |
| 1-4scaling                             | 1                     | langevinPistonDecay   | 25                    |
| COMmotion                              | no                    | langevinPistonTemp    | 300                   |
| zeroMomentum                           | no                    | useFlexibleCell       | no                    |
| dielectric                             | 1.0                   | useGroupPressure      | yes                   |
| switching                              | on                    | cellBasisVector1      | 60.00 0.00 0.00       |
| switchdist                             | 10                    | cellBasisVector2      | 0.00 60.00 0.00       |
| cutoff                                 | 12                    | cellBasisVector3      | 0.00 0.00 60.00       |
| pairlistdist                           | 14                    | cellOrigin            | 0.00 0.00 0.00        |
| timestep                               | 1.0                   | wrapAll               | on                    |
| stepspercycle                          | 20                    | PME                   | yes                   |
| nonbondedFreq                          | 1                     | PMEGridSizeX          | 60                    |
| fullElectFrequency                     | 2                     | PMEGridSizeY          | 60                    |
| rigidBonds                             | all                   | PMEGridSizeZ          | 60                    |
| langevin                               | on                    | constraints           | on                    |
| langevinDamping                        | 5                     | selectConstraints     | on                    |
| langevinTemp                           | 300                   | selectconstrX         | on                    |
| langevinHydrogen                       | off                   | selectconstrY         | on                    |
| langevinPiston                         | on                    | colvars               | on                    |
| langevinPistonTarget                  | 1.01325               |                       |                       |

**TABLE S2: NAMD input parameters for the simulations**
| Residue Name | Atom Type | Angle(°) |
|--------------|-----------|----------|
| L21          | N         | 113      |
| L21          | CA        | 110      |
| L21          | CB        | 91       |
| L21          | CG        | 86       |
| L21          | CD1       | 72       |
| L21          | CD2       | 87       |
| L22          | CD        | 75       |
| L63          | CD1       | 76       |
| L63          | CD2       | 90       |

TABLE S3: Angle between positive z vector and the line connecting central position of the patch (0,0,12) with each of the heavy atom position on the patch
| Replicate Number | Fluctuation Value (Å²) | Replicate Number | Fluctuation Value (Å²) |
|------------------|------------------------|------------------|------------------------|
| Cl-1             | 0.095                  | I – 1            | 0.159                  |
| Cl-2             | 0.101                  | I – 2            | 0.083                  |
| Cl-3             | 0.182                  | I – 3            | 0.129                  |
| Cl-4             | 0.098                  | I – 4            | 0.357                  |
| Cl-5             | 0.096                  | I – 5            | 0.223                  |
| Cl-6             | 0.064                  | I – 6            | 0.226                  |
| Cl-7             | 0.166                  | I – 7            | 0.102                  |
| Cl-8             | 0.154                  | I – 8            | 0.189                  |
| Cl-9             | 0.161                  | I – 9            | 0.091                  |
| Cl-10            | 0.106                  | I – 10           | 0.132                  |
| Cl-11            | 0.122                  | I – 11           | 0.236                  |
| Cl-12            | 0.117                  | I – 12           | 0.121                  |
| Cl-13            | 0.103                  | I – 13           | 0.133                  |
| Cl-14            | 0.106                  | I – 14           | 0.154                  |
| Cl-15            | 0.115                  | I – 15           | 0.115                  |
| Cl-16            | 0.153                  | I – 16           | 0.149                  |
| Cl-17            | 0.102                  | I – 17           | 0.235                  |
| Cl-18            | 0.190                  | I – 18           | 0.079                  |
| Cl-19            | 0.171                  | I – 19           | 0.126                  |
| Cl-20            | 0.102                  | I – 20           | 0.082                  |

TABLE S4: Aqueous protein interface fluctuation value at x = 0 and y = 0 with fix ion and fix water molecules in the first hydration shell. Each replicate is based on 6ns simulation and considering all the data in each replicate, the fluctuation value induced by Cl\(^-\) is 0.174Å² and by I\(^-\) is 0.212Å².
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