Electronic Supplementary Material for PCCP

Electronic Supplementary Information: Classical free energy calculation methods

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This document contains additional information regarding the details of the three methods for free energy calculations employed in the study. All three methods are based on molecular dynamics. For methods I and II the binding free energy is obtained by numerical integration as:

$$\Delta F_{\text{bind}} = F_{\text{bound}} - F_{\text{free}} = \int_0^1 \left\langle \frac{\mathrm{d}\mathcal{H}}{\mathrm{d}\lambda} \right\rangle_{NVT;\,\lambda} \mathrm{d}\lambda \qquad . \tag{1}$$

The ensemble average is calculated as a time average from a molecular dynamics simulations in the canonical (NVT) ensemble. The coupling parameter, λ, is chosen such that the Hamiltonian is changed from describing the bound state to describing the free state of the Zn ion. If the transformation process is reversible, the free energy change is independent of the reaction path and only depends on the initial and final states. Therefore we are free to choose the path of integration in Eq. (1) as long as the reversibility condition is met.

Determining differences between two large numbers, as is the case in Eq. (1), can be problematic since the statistical errors involved are large as well. If λ is constructed wisely, large energy differences and their errors can be made to cancel in the final result. In general

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it is non-trivial to find ways which allow for such cancellations. In our case we are comparing two states where the long-range electrostatics are very similar and therefore minimises the otherwise serious issues linked to changing charges in free energy calculations.

I. GROWING/DELETING THE ZN ION IN THE PEPTIDE AND IN THE BULK WATER

We consider the free energy states in the reversible thermodynamic cycle for the binding/unbinding process of the Zn ion to the peptide, which we can sketch in the following manner:

$$\begin{array}{ccc} \text{Peptide(sol)} & \xrightarrow{\Delta F_1} & \text{Peptide–Zn(sol)} \\ {\scriptstyle \Delta F_4=0}\uparrow & & \uparrow{\scriptstyle \Delta F_3=\Delta F_{\text{bind}}} \\ \text{Peptide(sol)} & \xrightarrow[\Delta F_2]{} & \text{Peptide(sol)} + \text{Zn(sol)} \end{array} \tag{2}$$

Peptide(sol) refers to the dissolved peptide stripped from the Zn ion and Peptide–Zn(sol) denotes the same dissolved peptide but with the Zn ion bound. Completing the cycle results in no change of free energy, i.e. the sum of the free energy changes is zero so that we can write

$$\Delta F_1 - \Delta F_2 - \Delta F_3 + \Delta F_4 = 0 \quad . \tag{3}$$

But from diagram (2) we can see that $\Delta F_4 = 0$ because it is the free energy difference between two identical states. Hence the binding free energy is

$$\Delta F_{\text{bind}} \equiv \Delta F_1 - \Delta F_2 \quad . \tag{4}$$

Thus, to calculate ΔF_{bind} we have to perform two separate simulations to obtain ΔF_1 and ΔF_2, respectively. In the first simulation, the Zn ion is created in the binding site, by which the free energy of the system changes with ΔF_1. In the second simulation, the Zn ion is grown in the solvent and the free energy changes with ΔF_2. Since the peptide is included on both sides of the reaction but remains unchanged it can be left out of the second simulation. It gives no contribution to the free energy change. In both simulations the net charge is changed by $+2e$. This is compensated by a continuous smeared out net charge of $-2e$. The contribution from this will cancel when $\Delta F_{\text{bind}} = \Delta F_1 - \Delta F_2$ is calculated. ΔF_1 and ΔF_2 are large negative numbers. ΔF_1 is, however more negative resulting in a negative ΔF_{bind},

favouring the Zn ion bound to the peptide. In principle the two reactions can be performed in opposite directions in the same simulation, but this may also be done in separate simulations to facilitate convergence of the calculations.

The numerical integrations of Eq. (1) were performed for the peptide with both four and three cysteines, as well as a Zn ion in bulk water, using 11 evenly spaced values for the parameter $\lambda = 0, 0.1, 0.2, \ldots, 1$. Further, the charge of the Zn ion and its Lennard-Jones parameters (C6 and C12) were taken proportional to λ, and soft-core potentials were employed to avoid numerical problems near $\lambda = 0$ and $\lambda = 1$. For each value of λ, the systems were equilibrated for 1 ns and then sampled during 1 ns. An average was determined over the last ns, including an estimate of the statistical error from the fluctuations and their time correlations [1].

II. PULLING THE ZN ION OUT OF THE BINDING SITE

The former method is a bit unphysical since it involves the creation and annihilation of the Zn ion at different places in the system. This does, however, not affect the free energy difference between the two final states. There are many choices for the path of integration in Eq. (1) and an alternative is to pull the ion out of the binding site [2]. We choose to introduce a reaction coordinate that is the length of the vector connecting the binding site and the actual position of the Zn ion:

$$\xi = |\mathbf{r}_{\mathrm{Zn}} - \mathbf{r}_{\mathrm{Zn\text{–}Site}}|. \tag{5}$$

We then apply a harmonic biasing potential,

$$U(\xi) = \frac{k}{2}(\xi - \xi')^2 \qquad , \tag{6}$$

that restrains the reaction coordinate close to a specified value ξ'. By slowly varying ξ' from zero to a value ξ_1 that is large enough to bring the Zn ion clear of the peptide out in the bulk water, we can perform the unbinding reaction. This does not force the ion to move along a certain path. The path may be unphysical and the barriers obtained from this are certainly not trustworthy. If the process is performed slow enough to maintain equilibrium, it is possible to calculate the free energy difference between the two end states from the

(reversible) work performed on the system by the biasing potential:

$$\Delta F_{\mathrm{bind}} = F\left(0\right) - F\left(\xi_1\right) = -\int_0^{\xi_1} \left\langle \frac{\mathrm{d}U}{\mathrm{d}\xi'} \right\rangle \mathrm{d}\xi' = k \int_0^{\xi_1} \left\langle \xi - \xi' \right\rangle \mathrm{d}\xi' \quad . \tag{7}$$

The force constant k is in principle arbitrary, but the choice affects the statistics and efficiency of the sampling [2]. It has to be chosen sufficiently large to restrain the position of the ion with respect to the binding site well enough to avoid large fluctuations that would make the force evaluation statistically uncertain. If the force constant is chosen too large, one will need simulations at a very large number of discrete ξ' when discretising the integral. It is necessary to have some overlap between the ionic distributions for the different ξ' values. $k = 10^4$ kJ/ (mol $\cdot$ nm^2) was found to be a value of suitable compromise to ensure this overlap (Fig. 1). The pulling process was extended for 10 ns of simulation time.

III. LINEAR RESPONSE AND THE LINEAR INTERACTION ENERGY (LIE) METHODS

Free energy calculations by the coupling parameter approach are computationally expensive, especially when employing window techniques and separate simulations have to be carried out for each value of the coupling parameter. To a first order a simpler and more approximate method can prove useful. Such a method is the linear response method which is exact for growing a charge in a linear continuum solvent [3]. When the solvent no longer is continuum or linear or the system contains a peptide that may undergo conformational transitions the approximations may or may not be useful. The linear response binding free energy is calculated from

$$\Delta F_{\mathrm{bind}} = \frac{1}{2} \left[\left\langle E^{\mathrm{el}}_{\mathrm{Zn-env}} \right\rangle_{\mathrm{bound}} - \left\langle E^{\mathrm{el}}_{\mathrm{Zn-env}} \right\rangle_{\mathrm{free}} \right] \quad . \tag{8}$$

An extension is the linear interaction energy (LIE) method [4, 5], in which a Lennard-Jones term is added but this is negligible in a case with strong electrostatics as the present one. Here, $\langle \ldots \rangle$ denotes ensemble averages derived as time averages in a molecular dynamics simulation. The energy terms refer to the interactions of the Zn ion with its surrounding environment (peptide, solvent) when it is bound in the site and free in the solvent. Since only two free energy states are considered with this method, the computational cost compared to an ordinary free energy integration using about 10 separate windows is cut by almost an

order of magnitude. The same MD trajectories as generated for method I were used for the linear response analysis.

IV. REFERENCES

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Figures

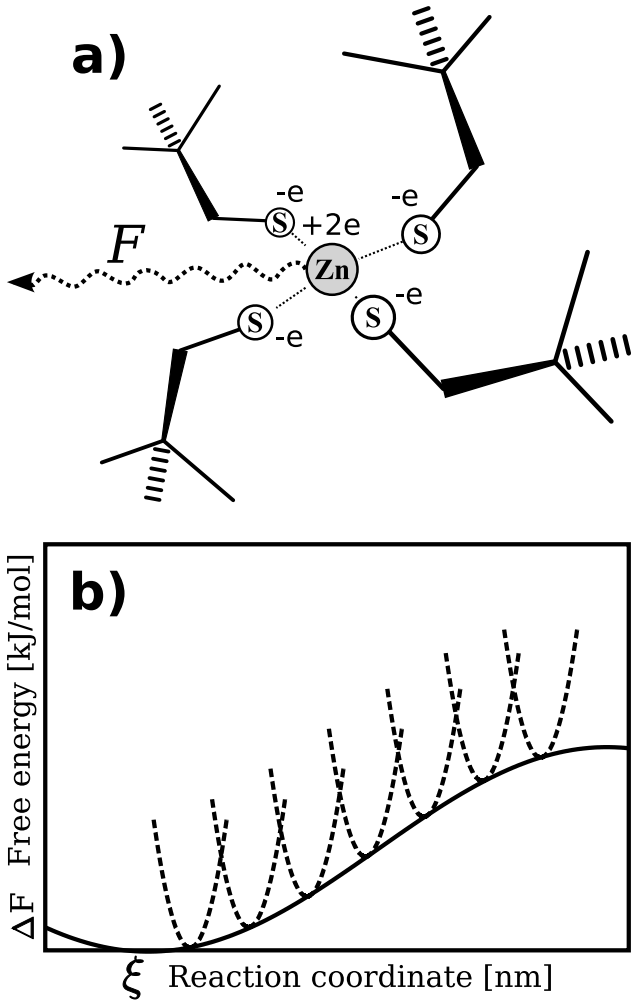

Figure 1: a) The Zn ion is pulled (with an external force F) along the reaction coordinate from the binding site in Peptide(4Cys) into the bulk water. b) The Hamiltonian of the system is biased by the external force from overlapping harmonic potentials so that the Zn ion moves along the reaction coordinate.