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The effect of intercalated water on potassium ion transport through Kv1.2 channels studied via on-the-fly free-energy parameterization

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Abstract

We introduce a two-dimensional version of the method called on-the-fly free energy parameterization (OTFP), to reconstruct free-energy surfaces using Molecular Dynamics simulations, which we name OTFP-2D. We first test the new method by reconstructing the well-known dihedral angles free energy surface of solvated alanine dipeptide. Then, we use it to investigate the process of K^+ ions translocation inside the Kv1.2 channel. By comparing a series of two-dimensional free energy surfaces for ion movement calculated with different conditions on the intercalated water molecules, we first recapitulate the widely accepted *knock-on* mechanism for ion translocation, and then confirm that permeation occurs with water molecules alternated among the

ions, in accordance with the latest experimental findings. From a methodological standpoint, our new OTFP-2D algorithm demonstrates the excellent sampling acceleration of temperature-accelerated molecular dynamics, and the ability to efficiently compute 2D free-energy surfaces, and will therefore be useful in a large variety complex biomacromolecular simulations.

1 Introduction

Free-energy surfaces (FES) are widely used to investigate the physical and chemical processes of complex systems like proteins and macromolecules, since they allow direct identification of important features of reaction mechanisms such as metastable and intermediate states, or the barriers that separate them.¹ The calculation of FES requires defining a set of collective variables (CV) that reduce the system description from an intractable atomically resolved level to a low-dimensional, albeit non-trivial, system. FES can be obtained via integration over the CV domain of mean forces computed at discrete points using restrained² or constrained³ molecular dynamics (MD) simulations. In practice, however, this operation is extremely resource-intensive, and the search for efficient algorithms for computing FES's is an active and prolific field.

On-the-fly free-energy parameterization (OTFP) is a recent CV-biasing approach to accelerate FES reconstruction.^{4,5} OTFP differs from other methods like metadynamics⁶ or adaptive bias force (ABF)⁷ in the way the CV-space sampling acceleration is achieved and/or the FE is generated. OTFP uses temperature-accelerated molecular dynamics (TAMD),^{8,9} which has the main advantage of relying on a history-*independent* bias potential, in contrast for example to metadynamics or ABF. This makes TAMD more efficient in principle, since the computational burden of a history-dependent simulation increases rapidly with time, particularly when many collective variables are used, since in that case one has to fill a multi-dimensional volume. Indeed, OTFP was recently shown to be highly efficient in overcoming the severe problem of hidden barriers in FES calculations, thus possessing improved

convergence behavior relative to other commonly used approaches.⁵ Although the presence of hidden barriers is not a consequence of the method, but rather of the definition of an incomplete set of CVs, dealing with new and complex systems increases the probability of encountering them.

The acceleration of sampling in CV-space and the actual reconstruction of the associated FES can be viewed as two separate problems. While TAMD can be used to efficiently accelerate a large number of CVs,⁹ the reconstruction of high-dimensional FES is still a numerical challenge,^{2,10,11} and, even when possible, interpreting the results can be cumbersome. For this reason, low dimensional FES are still preferred. However, one has to keep in mind that the smaller the number of CVs is, the higher the probability of hidden barriers, highlighting the importance of a robust FES reconstruction method. This scenario makes non-adaptive methods as OTFP or the replica-exchange OTFP extension (RE-OTFP) preferable.^{5,12}

In the first OTFP papers,^{4,5,12} 1D FE profiles are reconstructed by using a representation of the FE in terms of 1D *chapeau* basis functions. Although other functions can be used for the purpose, the *chapeau* functions are easy to manipulate and yield accurate results. Here, we extend the method to two-dimensional problems, by using as basis functions a set of 2D *chapeau* functions. As far as we know, this is the first use of OTFP to reconstruct 2D FESs. For this reason, we first test the performance of OTFP-2D by calculating the backbone dihedral angles FES of alanine-dipeptide (AD) in water, the most popular system for algorithm testing and 2D FES reconstruction (see for example^{7,13–16}). After having verified the reconstruction properties of OTFP-2D on the simple AD system, we employ it to study a more significant biophysical problem, the mechanism of ion translocation through the pore of Kv1.2 K⁺-channel.

Potassium channels are mainly involved in establishing the resting membrane potential of excitable cells. Malfunctions of these proteins can result in several types of muscular, neurological or autoimmune “channelopathies”. For instance, in muscle cells, channelopathies are characterized by hyperexcitability or nonexcitability (periodic paralysis). Drugs target-

ing K^+ -channels are effective in periodic paralysis.¹⁷ This highlights the importance of full comprehension of the functional mechanism and the structural characteristics of this system. Several computational studies have been dedicated to this task.^{18–26}

Kv1.2 is a member of the voltage-gated K^+ -channels family. These channels exhibit a high selectivity for K^+ ions, with a permeation rate that can approach the diffusion limit. The ion permeation process through the selectivity filter is nowadays believed to proceed mostly via the so-called *knock-on* mechanism,^{25,27–32} where a single-file of three K^+ ions alternated by water molecules moves through five ion-binding sites, called S0 to S4, along the filter. Although other mechanisms have been proposed, particularly regarding co-translocation of water molecules,^{33–36} the knock-on hypothesis was recently confirmed by ultrafast time resolution of 2D IR spectroscopy, which revealed spontaneously occurring structures of ions in a single file separated by water molecules.³⁷ In this work, we address this problem using OTFP-2D, in order to elucidate the most favorable mechanism in terms of free energy barriers.

2 Method

The free-energy surface (FES) of an N -atom system, with potential energy $U(\mathbf{x})$ and at temperature T , can be defined as (up to an additive constant):

$$G(\mathbf{z}) = -k_B T \ln \int \delta(\boldsymbol{\theta}(\mathbf{x}) - \mathbf{z}) e^{-U(\mathbf{x})/k_B T} d\mathbf{x} \quad (1)$$

This integral runs over system configurations $\mathbf{x} \in \mathbb{R}^{3N}$, and $\mathbf{z} \in \mathbb{R}^M$ represents a point in the lower-dimensional space of CVs obtained via the transformation $\boldsymbol{\theta} : \mathbb{R}^{3N} \mapsto \mathbb{R}^M$. The reconstruction of a FES using the above equation and conventional molecular dynamics (MD) can be extremely resource-intensive, even for simple systems, because the presence of high energy and entropy barriers confine the system in limited regions of the CVs space. Temperature-accelerated molecular dynamics (TAMD) introduces a set of M auxiliary variables $\mathbf{z}$ that are

1
2
3 tied by a spring to the system CVs and evolve according to their own equation of motions:^{8,9}
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5

$$\begin{cases} \gamma \dot{x}_i = -\frac{\partial V(\mathbf{x})}{\partial x_i} + \kappa \sum_j^M (z_j - \theta_j(\mathbf{x})) \frac{\partial \theta_j(\mathbf{x})}{\partial x_i} + \text{bath at } T \\ \bar{\gamma} \dot{z}_j = -\kappa (z_j - \theta_j(\mathbf{x})) + \text{bath at } \bar{T} \end{cases} \quad (2)$$

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12 where γ and $\bar{\gamma}$ are the friction coefficient of the real and auxiliary variables respectively and
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14 κ is the spring constant. Note that we use overdamped motion for simplicity, but any other
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16 dynamics can be employed. It has been demonstrated analytically^{8,38} that for the limit of
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18 large $\bar{\gamma}$ and large κ the auxiliary variables follow a dynamics driven by the mean force, i.e.:
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20

$$\kappa (z_j - \theta_j(\mathbf{x})) \approx \frac{\partial G(\mathbf{z})}{\partial z_j} \quad (3)$$

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26 This result is true regardless the auxiliary temperature used. Therefore, when a high auxil-
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28 iary temperature is used, the $\mathbf{z}$ variables are able to drag the system smoothly through the
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30 CVs space, even crossing high free-energy barriers, and thus improving sampling.

31
32 The on-the-fly free-energy parameterization (OTFP) method^{4,5} allows one to efficiently
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34 recover the FES sampled by a TAMD simulation. It begins with the approximation of the
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36 free-energy using a set of basis functions $\phi_m(\mathbf{z})$:
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38

$$G(\mathbf{z}) \approx \sum_m^L \lambda_m \phi_m(\mathbf{z}). \quad (4)$$

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43 Using this decomposition and equation 3, the following error function is constructed:
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$$E(\boldsymbol{\lambda}) = \left\langle \sum_j \left[\kappa (z_j - \theta_j(\mathbf{x})) - \frac{\partial}{\partial z_j} \left(\sum_m^L \lambda_m \phi_m(\mathbf{z}) \right) \right]^2 \right\rangle. \quad (5)$$

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51 where $\boldsymbol{\lambda}$ is the $\mathbb{R}^L$ -vector of the λ_m coefficients and $\langle \rangle$ indicates the average computed during
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53 a TAMD simulation. By minimizing $E(\boldsymbol{\lambda})$, it is possible to recover the expansion coeffi-
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55 cients and $G(\mathbf{z})$ via equation 4. This minimization translates into solving a linear system of
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equations:

$$\mathbf{A}\boldsymbol{\lambda} = \mathbf{b}, \quad (6)$$

$$A_{mn} = \left\langle \sum_j \frac{\partial \phi_m(\mathbf{z})}{\partial z_j} \frac{\partial \phi_n(\mathbf{z})}{\partial z_j} \right\rangle, \quad (7)$$

$$b_n = \left\langle \sum_j \kappa(z_j - \theta_j(\mathbf{x})) \frac{\partial \phi_n(\mathbf{z})}{\partial z_j} \right\rangle \quad (8)$$

Note that $\mathbf{A}$ is symmetric, and for the case of localized basis functions it will be band-diagonal.

In our previous publications using OTFP^{4,5,12} we found that the definition of the basis set using simple *chapeau* functions was sufficient to recover the FES in different scenarios:

$$\phi_m(z) = \begin{cases} 1 - m + z/h & \text{if } m - 1 < z/h < m \\ 1 + m - z/h & \text{if } m < z/h < m + 1 \\ 0 & \text{otherwise,} \end{cases} \quad (9)$$

where h is the grid spacing in the discretization of the CV space. The main reason of this success is based in the negligible overhead that the number of basis has in the computational effort. In other words, it is possible to compensate such a simple basis definition by increasing the number of basis functions in the set. This increment does not have a great impact on computational performance. For instance, the extra memory required to accumulate $\mathbf{A}$ and $\mathbf{b}$ is negligible considering that A_{mn} is symmetric and tridiagonal for the *chapeau* basis set. On the other hand, when solely reconstruction of the FES is sought, the increment in the number of basis functions does not imply additional computational effort, since the resolution of equation 6 need be made only at the end of the simulation. An improvement in the basis quality could be desirable in the case that the free-energy estimate is used internally, as in the RE-OTFP approach,¹² but we defer this subject to future research.

Using the above arguments as motivation, we extend the *chapeau* basis set for the 2D

case. Consider the 2D-*chapeau* function defined by:

$$\psi(x, y) = \begin{cases} 1 + x + y & \text{if } y < 0 \wedge x < 0 \wedge y > -x - 1 \\ 1 + y & \text{if } y < 0 \wedge x > 0 \wedge y < -x \\ 1 - x & \text{if } y < 0 \wedge x > 0 \wedge y > -x \\ 1 - x - y & \text{if } y > 0 \wedge x > 0 \wedge y < x + 1 \\ 1 - y & \text{if } y < 1 \wedge x < 0 \wedge y > -x \\ 1 + x & \text{if } y > 0 \wedge x > -1 \wedge y < -x \\ 0 & \text{otherwise.} \end{cases} \quad (10)$$

Here, $\psi(x, y)$ is a piecewise function with a hexagonal pyramidal shape and constant piecewise partial derivatives. Using this function, a basis set for a discretized 2D space can be expressed as:

$$\phi_m(z_1, z_2) = 2\psi\left(\frac{z_1 - z_{1m}}{h_1} + (m - 1) \bmod N_1, \frac{z_2 - z_{2m}}{h_2} - \left\lfloor \frac{m - 1}{N_1} \right\rfloor\right) \quad (11)$$

where z_{1m} and z_{2m} are the lower CV values in the grid and h_1 and h_2 the corresponding step lengths (see Fig. 1). The approximated free-energy value for the position (z_1, z_2) in the m -th cell of this grid will depend only of the nearest three bases $\phi_m, \phi_{m+1}, \phi_{m+n_x}$ or $\phi_{m+1+n_x}, \phi_{m+1}, \phi_{m+n_x}$, depending on whether (z_1, z_2) belongs to the upper or lower triangle of the cell (see figure 1). Thus, the computation of the thermodynamical averages for $\mathbf{A}$ and $\mathbf{b}$ requires the updating of only a few elements (e.g. $\mathbf{A}_{m,m}, \mathbf{A}_{m,m+1}$ and $\mathbf{A}_{m+1,m+1}$ for an upper triangle) per time step. Furthermore, if h_y is chosen to be a multiple of h_x , the redefinitions $\mathbf{A} := h_x^2 \mathbf{A}$, $\mathbf{b} := h_x^{-1} \mathbf{b}$ and $\mathbf{x} := h_x^{-1} \mathbf{x}$ render Eq. 6 dimensionless and the elements of $\mathbf{A}$ integers.

A comment must be added with respect to the numerical solution of Eq. 6. Large differences in the probability density of the auxiliary variables $\mathbf{z}$ will eventually appear

Figure 1: The discretization of CV space and the 2-D chapeau basis functions (Eq. 10).

between high and low free-energy states in long simulations. These differences can introduce numerical instabilities during the inversion of the $\mathbf{A}$ matrix. If the auxiliary temperature $\bar{T}$ is chosen high enough to frequently visit the CV values of interest, those extremely high free-energy states that produce the instability are irrelevant and can be safely dropped from the FES reconstruction. This can be done by reducing the matrix $\mathbf{A}$; that is, removing the columns/rows that are associated with these locations in CV space. The problematic columns/rows can be easily detected during the factorization step of the matrix inversion or using a singular value decomposition algorithm.

MD simulations were performed using NAMD 2.11³⁹ and the CHARMM36 force field⁴⁰ with CMAP corrections⁴¹ and NBFIX for KCl.⁴² Electrostatic interactions were treated using the particle-mesh Ewald method using a grid spacing of near 1 Å (91 grid points along each direction). NAMD is also used to integrate the atomic equations of motion in the OTFP simulations, while the auxiliary particles' movement and the FES reconstruction is handled by a home made C-code via NAMD's Tclforces module.⁴³ For alanine dipeptide, covalent bonds involving hydrogen atoms were kept fixed in length using the RATTLE algorithm and the time step used was 2 fs. Constant pressure at 1 bar is maintained using a Langevin piston with a constant decay of 50 ps⁻¹ and an oscillation time of 100 fs. Constant temperature

at 298 K is controlled through a Langevin thermostat with a damping constant of 50 ps⁻¹. The FESs for this system began to be refined after 5 ns of an initial equilibration period.

The initial configuration of the K⁺ channel corresponds to the pore domain of the Kv1.2 crystal structure (residues 312–421, PDB ID 2A79⁴⁴) immersed in POPE lipid bilayer solvated in ~83 mM KCl. The ionization state of the protein residues correspond to the neutral pH and all the simulations were performed in the zero-voltage regime. The backbone dihedral angles of residues 374–378 in the selectivity filter (SF) were constrained to the values in the crystallographic structure as in reference³² to maintain the filter open, and the Cα atoms of the linker helices connecting the pore to the voltage sensor domains (not included in the model) are fixed to hold the pore at the center of the box. The system was equilibrated during 21 ns of NPT molecular dynamics at 310 K and 1 atm. Constant pressure was maintained along the z-axis (perpendicular to the membrane) using a Langevin piston with a constant decay of 100 ps⁻¹ and an oscillation time of 200 fs. Covalent bonds to hydrogen atoms were constrained using the SHAKE algorithm, and a time step of 1 fs was used. At the end of the equilibration the simulation box sidelength along the z-axis oscillates around 85.00 ± 0.17 Å. We note that there are efforts devoted to achieving reliable description of electrostatic effects by including in the underlying simulation model effects of electrolytes and transmembrane potential, as for example in coarse-grained models.^{45,46} We resorted here to the widely used full-atom, fixed charges approach, in order to be able to compare our results with available literature showing FESs for ion translocation.²¹ We stress that the OTFP-2D method does not depend on the representation of the underlying system, and it can be applied to atomistic or coarse-grained simulations.

Figure 2: FES for alanine dipeptide in water at 300K obtained via OTFP-2D using an auxiliary temperature of 5000 K (a-c) and direct MD (d).

3 Results and Discussion

3.1 Validating OTFP-2D with alanine dipeptide

We use OTFP-2D to reconstruct the FES of alanine dipeptide in water using ψ and ϕ angles as CVs. Since this is one of the most popular and well-known systems for algorithm testing, the results presented here may be helpful in further comparison with other methodologies.

In Figures 2(a-c) we show the FESs obtained after 20, 40 and 60 ns of OTFP-2D simulation. The auxiliary temperature was 5000 K and the friction $\bar{\gamma} = 1500$ (kcal/mol)ps/ $\text{\AA}^2$ for the two auxiliary particles. For comparison, Fig. 2d shows the FES obtained after histogramming both ϕ and ψ from 150 ns of a standard direct MD simulation. Note that, in spite of this long simulation time, the surface for the ϕ angle is still incomplete. Conversely, the FES is completely recovered by OTFP at around 30 ns of simulation time. This demonstrates the improvement in both accuracy and efficiency when using OTFP rather than standard MD for this simple system.

Figure 3: Positions of the relevant groups in the selectivity filter (SF) along the z -axis (perpendicular to the membrane) and their temporal evolution during a standard MD simulation. The origin of the z -coordinate is aligned with the center of mass of the protein. The K^+ ions inside the SF are shown in red and the K^+ ions outside are blue. Water molecules and membrane lipids are shown in gray and yellow respectively. The temporal evolution of the carbonyl-ring centers formed by the TVGYG sequence (residues 374–378) are shown in black. Positions S0–S4 are defined by the carbonyl rings of the main chain of this sequence. Position S5 is defined by the carbonyl ring formed by the side chain of residue T374.

Figure 4: K^+ ions trajectories in the SF during OTFP-2D simulation. The CVs used are the position of the lower ion (K_1^+) and the center of mass of the other two ($CM_{2,3}$, shown as yellow). The color code is analogous to that used in Fig. 3.

3.2 Pore domain of Kv1.2

The Kv1.2 channel is a tetramer consisting of six membrane-spanning domains (S1–S6). In the center of the tetramer, the linker between the S5 and S6 helices forms the selectivity filter (SF) and contains the highly conserved sequence TVGYG, which is the signature of K^+ channels.⁴⁷ Fig. 3 shows the trajectory of the relevant groups in the SF during a standard MD simulation. Also shown in Fig. 3 are the definitions of the S0–S6 sites (not to be confused with the S1–S6 protein domains). Note that the lower ion K_1^+ exhibit a high mobility, since it is in the wider, intracellular part of the channel, while the upper K_2^+ and K_3^+ ions maintain their initial S2 and S4 positions in the filter.

We have performed OTFP-2D simulations on this system using the following two CVs: (1) the z -coordinate of the lower ion and (2) the z -coordinate of the geometric center between the K_2^+ and K_3^+ ions ($CM_{2,3}$). These were defined in previous work by others,²¹ and we use them here to facilitate comparison. For both CVs, the auxiliary temperature was 3000 K, the auxiliary friction 800 kcal/mol ps/Å² and the spring constant 1500 kcal/mol Å⁻². Half-harmonic constraints were used to create soft walls that prevent K^+ ions from fully leaving the SF. These soft walls activate below $z = -3$ and above $z = 18$ using a spring constant of 300 kcal/mol Å⁻². In Fig. 4 it is possible to observe the enhanced sampling obtained by one of these simulations. Note for instance the transition between the (S0,S2,S4) and (S2,S4,S6) states after the first 8 ns.

We have reconstructed the FESs for 3 independent OTFP-2D simulations, each one having a duration of 50 ns. The reference value of these surfaces is set to their respective free-energy minima. Then, for each point in CV-space where the 3 FESs are defined, we take the average of their free-energy values. Fig. 5 shows the resulting averaged FES and the associated standard deviations at each point in its domain. The A, B and D minima shown in Fig. 5 are in excellent agreement with the identically labeled states observed at low voltages (<200 mV) by Jensen *et al.*²¹ However, the transition state C presents here as a saddle point and not as the smooth minimum shown in that work. We can rationalize

Figure 5: (a) Averaged FES for the K^+ ions in the Kv1.2 selectivity filter. The minimum free-energy path is shown in green. Labels A–D correspond to the ions in the positions (S6,S4,S2), (S5,S4,S2), (S5,S3,S1) and (S4,S2,S0) respectively. (b) Standard deviation of the average FES.

this difference when considering the fact that the C minimum becomes smoother when the voltage decreases²¹ and our simulations correspond to the zero-voltage regime.

The FES obtained in this work supports the “knock-on” permeation mechanism.^{26,30,33,48} According to this mechanism, initially there are two K^+ ions in the selectivity filter with intervening water molecules (Fig. 5a). When the third K_1^+ ion enters into the SF from the intracellular side (A→B), the single-file K_2^+ -water advances to the extracellular side (B→C→D). Note that this mechanism results in the translocation of one K^+ ion and one water molecule. However, from observation of the FES shown in Fig. 5 alone, it is not

possible to determine whether the water is co-translocated with the ions or not.

The co-translocation of water molecules and the relevance of those states that have empty site between neighbor K^+ ions have been debated for years.^{34–36,49} In order to explore this issue, we performed two different kinds of simulations. First, we ran OTFP-2D while keeping the intercalated water molecules tied to their neighbor K^+ ions by a half-harmonic boundary potential. This potential activates for distances greater than 4 Å (spring constant 300 kcal/mol Å⁻²) and is used to confine the two water molecules between the $K_1^+ - K_2^+$ and $K_2^+ - K_3^+$ ions. The resulting force is only applied to the water molecules to avoid a direct perturbation of the ions motion. On the other hand, we run OTFP-2D while keeping the water molecules outside the SF. This was achieved by adding the potential term

$$U_{nw}(\mathbf{x}) = k_{nw} \sum_i^w \left(1 - \cos \left(2\pi \frac{z_i - c_1}{c_2 - c_1} \right) \right) \quad (12)$$

where the summation runs over the water molecules inside the SF; i.e., inside the rectangle delimited by the coordinates $(-5, -5, 4)$ and $(5, 5, 15)$. Using $c_1 = 4$, $c_2 = 15$ and $k_{nw} = 600$ kcal/mol, this potential introduces a prohibitive barrier for the water molecules to enter the SF. Note that U_{nw} is only smooth in the z -direction, since no water molecule is expected to enter the SF from the sides.

In Fig. 6a,b we show the FESs obtained from the simulations with (FES_{sw}) and without water (FES_{nw}, respectively). As we did previously, each of this FESs correspond to the average of the FESs obtained from 3 independent OTFP-2D simulations, each of them having a duration of 50 ns. From now on, we use the term “free-water” to refer to the calculation that did not include any kind of constraint on the filter water molecules. Note that FES_{sw} in Fig. 6a resembles the shape of the free-water FES shown in Fig. 5a. The A, B and D minima are still distinguishable, retaining their positions and shapes. The C state continues to appear as a saddle point. Although the absolute free-energy values of these states seem to differ, the relative free-energy differences between A, B and C do not change significantly, as

Figure 6: (a) Averaged FES with intercalated water between the K^+ ions in the SF. (b) Averaged FES with water molecules forced to be outside the SF. Minimum free energy paths are shown in green. The standard deviation of these FESs can be found in the supporting information.

we will show in detail below. On the other hand, consider now the FESnw shown in Fig. 6b, corresponding to the system without intercalated water. It is possible to see that this surface has significant differences with the free-water FES. The domain area associated with the A, B and C states is now associated with one extended and rough free-energy basin. Near the coordinates where we observe the C saddle point in the free-water, it is possible to see a deep minimum in FESnw. Another minimum appears in FESnw between states C and D which is labeled as C' in Fig. 6b.

Using the string method^{50,51} we have computed the minimum free energy path of FESww,

FESnw and the free-water FES. The resulting paths have been included with a green trace over their corresponding surfaces, as can be seen in Figs. 6 and 5a. Fig. 7 shows the resulting free-energy profiles along each path. A, B, C, D and the new C' states are labeled accordingly. In the figure it is possible to observe that there is no significant difference in the relative free-energy difference among the A, B and C states of the free-water FES and FESww. For example, the activation barrier for both surfaces, which is the free-energy difference between state A and C states, is ≈ 4.5 kcal/mol. This results seems to support the idea that the translocation occurs with intercalated water molecules between the K^+ ions. An interesting difference occurs in free-energy of state D, which is ≈ 2.8 kcal/mol below state A in the free-water and ≈ 0.6 kcal/mol above A in the FESww. This indicates that the presence of intercalated water tends to destabilize the D state. A reasonable explanation for this behavior can be found by analyzing the configuration associated with the D state. In this configuration, K^+ ions are in positions (S4,S2,S0) and water molecules in positions S3 and S1. Note that sites S0 and S1 are highly exposed to the extracellular space (see Fig. 3). Thus, it is reasonable to expect that the S1 water molecule might leave the site or exchange with other water molecules, events that are not considered in the free-energy of the D state of FESww.

The minimum-free energy path for the FESnw shown in Fig. 7 reflects the significant differences between this system and the free-water one. Interestingly, this profile does not exhibit a free-energy barrier between the A and D states. Moreover, the profile is relatively flat, where the deeper basin at state C is only separated from state D by a small barrier of ≈ 2 kcal/mol. This indicates that in a system without intercalated water between the K^+ ions the translocation process might occur following two different ways: (1) diffusively without any activation energy associated, or (2) actively, where the system is thermalized in state C and a small ≈ 2 kcal/mol barrier has to be overcome. The diffusive or almost diffusive translocation process found in FESnw gives a renewed impetus to the question of whether or not there is any contribution of configurations without intercalated waters or configurations

with ions occupying adjacent sites. Although the similarities obtained here between FES_{ww} and the free-water FES indicate that the process occurs with intercalated water molecules, it is important to note that the presented results correspond to the zero-voltage case. The contribution of the FES_{nw} at higher voltages should be addressed in future work.

Figure 7: Minimum free-energy paths obtained for the simulation with and without water in the SF and for the simulation free of water constraints.

4 Conclusion

We have used a set of 2D *chapeau* basis function to reconstruct 2D free-energy surfaces (FESs) via OTFP. Tests with alanine dipeptide’s dihedral-angle space validated the approach and illustrate that the key feature of OTFP’s enhanced sampling via Temperature-Accelerated MD avoids sampling limitations normally encountered in reconstructing multidimensional FESs. The acceleration achieved by this method allowed us to study the process of K^+ ion translocation through the selectivity filter of the Kv1.2 protein channel. FESs were obtained separately for the cases when water molecules are intercalated between the ions and when they are not. The comparison of these FESs allowed us to determine that our system favors a “knock-on” mechanism with intercalated water molecules. The present approach can be

used in further works to determine the translocation mechanism and the role of intercalated water in different environmental conditions, and/or in other channels, such as for example the voltage gated sodium channels, which, thanks to the recently determined atomic structures, are becoming increasingly subject of studies on permeation.⁵²

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Supporting Information Available

Standard deviation of the average FESs for water constrained simulations (S1). This material is available free of charge via the Internet at <http://pubs.acs.org/>.

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