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Translocation process of structured polypeptides across nanopores

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Abstract. The progress of molecular manipulation technology has made it possible to conduct controlled experiments on translocation of polynucleotide and polypeptide chains across alpha-Hemolysin channels and solid-state nanopores. To study the translocation process we combined Molecular Dynamics at coarse-grained level and appropriate drift-diffusion Smoluchowski equations as an integrated statistical physics approach. In particular, we performed simulations of the passage across a cylindrical nanopore of Ubiquitin described by a coarse-grained native-centric model to investigate the influence of protein structural properties on translocation mechanism. The kinetic characterization of the process is achieved by studying the statistics of blockage times, the mobility and translocation probability as a function of the pulling force F acting in the pore. We find that the transport dynamics displays a threshold F_c depending on a free-energy barrier that Ubiquitin overcomes to translocate. Our simulations show this barrier to be the result from competition of the unfolding energy and the entropy associated to the confinement effects of the pore.

Keywords: Proteins, translocation, mobility, free-energy, MD simulations

1. Introduction

The transport of proteins (translocation) across cellular membranes [8,16] has been fascinating researchers for almost forty years also due to its relationship with biosynthesis stage. The interest has recently increased also thanks to the possibility to integrate artificial α-Hemolysin pores into lipid bilayers to build nanopore systems, allowing accurate voltage-driven translocation experiments of polynucleotides [7], peptides [13] and proteins [11]. As α-Hemolysin channels, solid-state nanopores and carbon nanotubes [18] are capable of characterizing and discriminating polynucleotide molecules [7], under appropriate conditions, they can work as spectroscopy and sequencing devices.

The computational approach at atomic resolution remains a challenge due to the enormous number of degrees of freedom required to describe the full system: i.e., the translocating polymers, the membrane pores and the solvent [1]. Even when the reduction of simulation times operated by current parallel architectures allows "brute-force" MD simulations to generate a few complete translocation trajectories, the statistics remains still insufficient for a robust estimation of the relevant observables. As an alternative, steered MD simulations [14,15] employ forces at least one order of magnitude larger than physiological ones to generate several translocation events compatible with available CPU-times, however, the resulting statistics may be not representative of the real biological process. This difficulty can be overcome

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by employing methods aimed at sampling directly the relevant statistical properties of the system and discarding the degrees of freedom not involved in translocation kinetics (coarse-graining) [6,10]. In particular free-energy calculations allow an efficient coarse-graining while retaining the relevant aspects of the problem. In this context, we compute the free energy of Ubiquitin translocation as a function of its center of mass (collective coordinate) and this information is then used to develop a one dimensional phenomenological model in this reaction coordinate which explains and reproduces the behavior of the observables during the translocation. The present approach simplifies the Ubiquitin to its C_α-carbon backbone. The Ubiquitin structural properties and network of internal interactions are approximated by promoting the formation of those native interactions stabilizing the PDB structure (1UBI), according to the Gō-like force-field by Clementi et al. [4]. The pore is modeled by a potential with cylindrical symmetry around the x-axis (translocation direction) $V_p(x,y,z) = V_0\psi(y,z)[1-\tanh(\alpha(x-L)x)]$, where $\psi(y,z) = [(y^2+z^2)/R_p^2]^q$; V_0, L and R_p define the pore properties. The parameter q tunes the potential stiffness and α characterizes the step-like profile in the x-direction. A homogeneous force, F, collinear to the cylinder and acting only at the interior of the pore mimics the average importation mechanism. Besides the pulling force, the pore-protein interaction amounts to a simple confinement in a channel of section πR_p^2 and length L. Since R_p is smaller than the native Ubiquitin gyration radius, the pore allows translocation to occur only in a unfolded conformation.

In this phenomenological approach, the translocation process is regarded as the passage of a protein-like polymer through a pore with a simple cylindrical shape (see also [6,10]).

Specifically, the issue we address concerns the interplay between translocation and unfolding: the role of energy/entropic barriers associated both with the unfolding and translocation of proteins across narrow pores. Proteins, indeed, tend to resist to the unfolding and to the sudden confinement experienced when passing a narrow path. This determines a great conformational entropy reduction leading to the appearance of free-energy barriers opposing translocation.

The umbrella sampling method [12] has been applied to extract from simulations the free-energy barriers that the Ubiquitin feels across the pore during its translocation. We employed an harmonic umbrella potential $V_U(X) = K_U/2(X_{\rm CM}-X)^2$ which restrains the coordinate, $X_{\rm CM} = \sum_{i=1}^{N} x_i/N$, to fluctuate around the value X inside the channel.

2. Methods and results

The preliminary thermal-unfolding MD simulations of the 1UBI structure (by Langevin thermostat) identified a unfolding temperature $T^* = 0.77$ (units R/ε) corresponding to the experimental denaturation temperature $T = 338$ K [17].[1] The kinetics of the translocation process was simulated at temperatures $T_{\rm ref} = 198$ K (reference) and $T_{\rm ph} = 308$ K (physiological) by performing different runs in which the protein was imported into the channel by the force applied to the N-terminus. The simulation was run until all the protein exited the right end of the channel, and partial refolding occurred.

The transport is characterized by measuring the average *mobility* $\mu = V/F$ as a function of the importing force F, where V is computed as $L/\mathcal{M}\sum_{k=1,\mathcal{M}} 1/t_k = L\langle t^{-1}\rangle$, t_k being the translocation time measured in the kth run. Averages are performed over $\mathcal{M}$ independent successful runs, i.e. excluding those in which Ubiquitin does not succeed in crossing the channel from one edge to the other. The probability of translocation $P_{\rm Tr}$ can be estimated as number of translocation successes over number of total runs. Figure 1a reports the plots of $P_{\rm Tr}$ versus F at temperatures $T_{\rm ph}$, $T_{\rm ref}$. At both temperatures,

[1] This sets the model energy scale to the value $\varepsilon \simeq 0.88$ Kcal mol^{-1}.

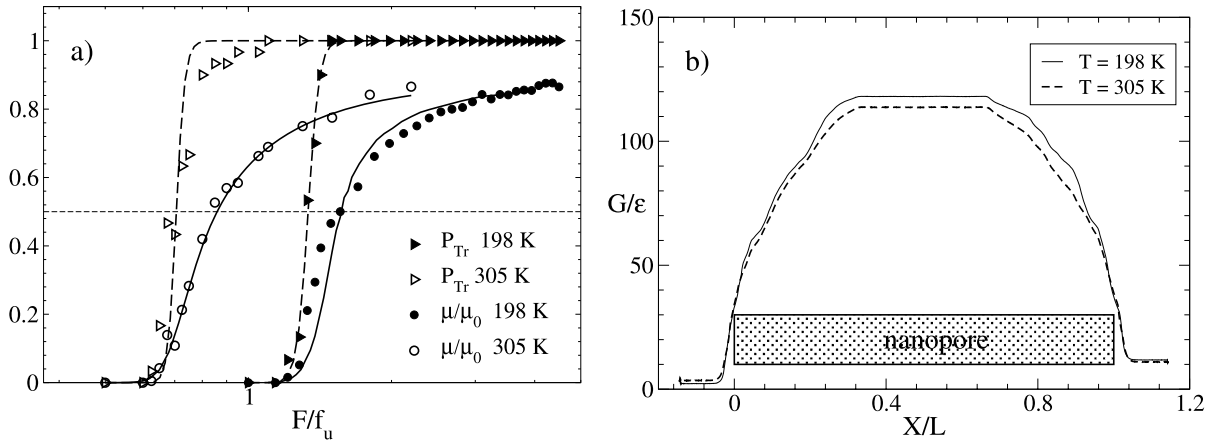

Fig. 1. (a) Translocation probability $P_{\rm Tr}$ and dimensionless mobility μ/μ_0 (μ_0 mobility without pore hindrance) as a function of F, from MD simulations and their fitting (curves) via formulas (6) (see below). The plots correspond to a pore of radius $R_p = 4$ and length $L = 300$, temperatures $T_{\rm ref} = 198$ K and $T_{\rm ph} = 305$ K. (b): Umbrella-sampling free energy profiles, obtained by an umbrella potential of constant $K_U = 0.5$, at temperatures 198 and 305 K.

translocation becomes probable ($P_{\rm Tr} > 0.5$) when the force exceeds a critical value $F_c(T_{\rm ph}) \simeq 0.6$, $F_c(T_{\rm ph}) \simeq 1.22$. Whereas, below such thresholds the probability goes rapidly to zero. The mobility $\mu = V/F$ as a function of the force F (Fig. 1a) shows a non linear characteristic indicating the existence of a free-energy barrier which the molecule has to overcome to activate its translocation.

Typical free-energy profiles $G(X)$ in the absence of force, experienced by Ubiquitin inside the pore are reported in Fig. 1b as a function of the "natural" reaction-coordinate: the molecule center of mass position X. The major variation of $G(X)$ occurs near the boundaries of the channel $[0, L]$, as the protein tends to be spontaneously expelled. The plateau in the middle indicates that once the Ubiquitin has placed enough residues inside the pore (i.e., $X \simeq 100$ in Fig. 1b), it can slide without further free-energy cost. The shape $G(X)$ can be fit by a one-dimensional potential $G_{\rm fit}(X) = \sum_{s=1}^{3} G_s g\{\mu_s[(X - L/2)^2 - (\ell_s/2)^2]\}$ which is a combination of step-like functions $g(u) = [1 - \tanh(u)]$ depending on the tuning parameters $\{G_s\}$, $\{\mu_s\}$ and $\{\ell_s\}$.

The results of Fig. 1 suggest a description based on drift-diffusion Smoluchowski equation [9] for $P(X, t)$ probability density of the reaction coordinate X

$$\partial_t P = D_0 \partial_X \{ \mathrm{e}^{-\beta U(X)} \partial_X \mathrm{e}^{\beta U(X)} P \}, \tag{1}$$

$$J(0, t) = -R_0 P(0, t), \qquad J(L, t) = R_L P(L, t), \tag{2}$$

where $U(X) = G(X) - FX$, $\beta = (RT)^{-1}$, D_0 is the effective diffusion constant of the protein and F the pulling force, the *radiation boundary conditions* (RBC) (2) for the current

$$J(X, t) = -D_0 \mathrm{e}^{-\beta U(X)} \partial_x [\mathrm{e}^{\beta U(X)} P(X, t)] \tag{3}$$

are "natural" in translocation problems [3] as they account for the possibility for the protein to exit the channel spontaneously at rates R_0 and R_L from the left and right, respectively.

Since we are interested on the events for which the molecule occupies the channel for a given time (blockage time), we consider the probability that Ubiquitin has not yet escaped the channel (*survival probability* $S(t)$) and *the distribution of blockage times* $\psi(t)$:

$$S(t) = \int_0^L \mathrm{d}X\, P(X,t), \qquad \psi(t) = -\frac{\mathrm{d}S(t)}{\mathrm{d}t}. \tag{4}$$

The solution $P(X,t)$ of Eq. (1) with RBC and the initial condition $P(X,0) = \delta(X - X_0)$ [2] provides the translocation probability P_{Tr} and the average time $\tau(F)$ spent by the molecule in the channel (blockage time in experiments):

$$P_{\mathrm{Tr}} = R_L \int_0^\infty \mathrm{d}t\, P(L,t), \qquad \tau(F) = \int_0^\infty \mathrm{d}t \int_0^L \mathrm{d}X\, P(X,t). \tag{5}$$

After some algebraic manipulations and approximations [2], we get to the explicit formulas:

$$P_{\mathrm{Tr}}(F) = \frac{D_0 K_L \mathrm{e}^{\beta F L}}{D_0(K_0 + K_L \mathrm{e}^{\beta F L}) + K_0 K_L \mathrm{e}^{\beta F L} M_+(F)}, \qquad \mu(F) = \frac{L D_0}{F M_0(F)}, \tag{6}$$

where, we have introduced: $K_0 = R_0 \exp\{-\beta G(0)\}$, $K_L = R_L \exp\{-\beta G(L)\}$, and

$$M_\pm(F) = \int_0^L \mathrm{d}x\, \mathrm{e}^{\pm\beta[G(x) - Fx]}, \qquad M_0(F) = \int_0^L \mathrm{d}x\, \mathrm{e}^{-\beta[G(x)-Fx]} \int_x^L \mathrm{d}y\, \mathrm{e}^{\beta[G(y)-Fy]}$$

to simplify the notation. For each value F, the integration is carried out numerically replacing $G(x)$ by its fitting function $G_{\mathrm{fit}}(x)$. Formula (6) allow one to explain the simulated translocation phenomenology at different forces and to fit the data, once parameters D_0, v_0, R_0, R_L have been properly adjusted. The agreement between numerical data and theory can be appreciated in Fig. 1a where the behavior of simulated translocation probability and mobility of Ubiquitin as function of F are well approximated. The estimated value of $D_0 \simeq 10^{-4}$ cm^2/s. Additional information on the translocation process can be gained by studying the statistics of translocation times, accessible to voltage-driven experiments. In such experiments [7], ion current drops correspond to the blockage of the channel by the passing molecule. In our simulations translocation times can be measured as first arrival times τ at the channel end $x = L$ of the protein center of mass. Figure 2 shows the histograms of translocation times collected in about 10^3 MD runs of Ubiquitin translocation, on regimes of small and large forces. Such distributions are skewed, with the skewness depending on the force in agreement with experiments [7].

3. Conclusions

We proposed a statistical model of translocation where a small globular protein, the Ubiquitin, is imported by a uniform pulling force across a channel of finite length. The simulations have shown that translocation of protein-like chains is different from the translocation of small peptides and unstructured polymers. For proteins, indeed, the process occurs via the stages, *unfolding* → *translocation* → *refolding*. This introduces two time-scales that combine to generate the total blockade time τ_B of the pore. One is associated to delay due to resistance to unfolding processes occurring at the entrance of the channels,

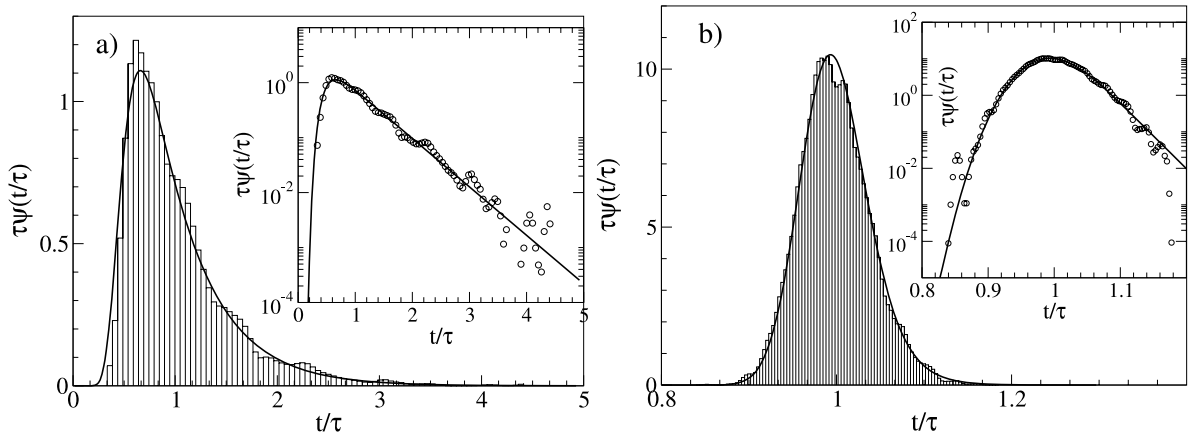

Fig. 2. Distributions of translocation times across a channel of length $L = 300$, radius $R_p = 4$, and temperature $T = 198$ K from MD simulations. The rescaling is by average time τ. (a) Corresponds to field $F = 1.38$ near the critical force region (b) to field $F = 3.0$, far from the critical region. Solid line indicates the semi-analytical result obtained after solving Eq. (1) in the approximation $G(x) \simeq G_0 - Fx$ and RBC (see [2]). The inset shows the same data in semilog scale.

and the other one corresponds to transport. Implementation of Umbrella sampling simulations have allowed to compute the free-energy profile associate to Ubiquitin translocation. This knowledge has been used to develop a one-dimensional drift-diffusion model for the probability $P(x,t)$ that Ubiquitin has its center of mass X at the position x at time t. The model is amenable to analytic treatment, and upon tuning the free parameters plus the free-energy barrier at the entrance of the channel, it explains qualitatively and quantitatively the behavior of the observables characterizing the translocation as a function of the force. It is interesting to note that both mobility and probability feature a clear non-linear behavior typical of an activated transport phenomena observed in other physical systems (e.g., granular gases) where transport requires cooperative behavior [5].

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