Protein Translocation through Nanopores: Insights from Computational and Theoretical approaches

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Abstract

Voltage driven translocation in nanopores is a promising experimental technique capable of inferring the physical chemical properties of the passing molecule by the variation of the resistance of the nanopore based devices. The tecnique needs some improvements especially when applied to complex bioploymers like proteins. In this work we discuss some computational and theoretical approaches that allow to characterizing and clarifying some elementary mechanisms, such as molecule capture and transport rates, that constitute the key elements of the working principle of the whole technique. The results we present can suggest some improvement and optimization of the efficiency of nanopore devices for protein sensing.

Keywords: Nanopore Sensing; Co-translocational unfolding; Nanopore tweezer; Simulations.

1. Introduction

During the past two decades, nanopores have been exploited for the development of innovative approaches for DNA sequencing and several research groups have focused their activities on the interpretation of current signals associated with nucleic acid translocation (Mikheyev et al, 2014, Schneider et al., 2010). Much less effort has been devoted to protein and polypeptide sensing and, only in recent years, protein analysis via nanopores has become a massive subject of research with the purpose of suggesting possible applications to sequence and structure analysis (Cressiot et al, 2014, Rodriguez-Larrea and Bayley, 2013, Boynton and Di Ventra, 2015). A typical experimental set-up, Fig 1A, employs the α-Hemolysin (αHL) nanopore, a toxin from Staphylococcus aureus (Song et al. 1996). The sensing site allows the passage of only one monomer, while the vestibule has enough room for accommodating secondary structure elements and partially folded chains that can give raise to a multilevel current signal, Fig 1B.

The uniform charge of polynucleotides (nucleic acid) makes their electrophoretic translocation in nanopore relatively easy and regular, on the contrary, polypeptides (proteins), being not uniformly charged polymers, translocate in complex pathways. In addition, the not uniform charge cannot allow a fine control of the translocation rates by tuning the electric field intensity. This issue affects directly the sensitivity of a nanopore device; a good sensitivity requires that just one residue at a time has to occupy the pore sensing site and that the residence time has to be long enough to record a stable current signal. Finally, proteins, unlike DNA, spontaneously assume compact native conformations and their passage in nanopores is possible only when accompanied to unfolding. All these issues pose a great challenge in developing reliable sensing nanopore devices for proteins and peptides.

In the present proceeding, we briefly discuss our recent theoretical and computational approaches aimed at controlling the translocation rate and unravel the complex pathway associated to protein translocation.

2. Characterization of co-translocational unfolding

As mentioned in the introduction, a crucial issue in nanopore protein sensing is the achievement of protein capture and the control of translocation rates. Oukhaled et al., 2007, studies the translocation of the Maltose Binding Protein, that at physiological pH has a net charge such that electrophoretic transport is possible. The same happens also for certain peptides studied by Mereuta et al., 2014. However, in both cases, the chains need to be completely unfolded before the translocation starts. This results in a very high translocation rate that gives rise to flat current signals that do not resolve nature of the translocating residues. A crucial improvement in this respect was achieved in 2013, when two different groups proposed a novel strategy to control protein translocation by adding a charged linker to one of the two termini of the molecule to be analyzed, Nivala et al., 2013 and Rodriguez-Larrea and Bayley, 2013. In particular, this

second set-up allowed, for the first time, to measure a multistep current signal associated with the transport of a protein inside a nanopore.

In the above cited experiments, the only accessible information on the nanopore-macromolecule interaction is the current signal. Hence, the interpretation of the actual translocation pathway relies on both the indirect evidences extracted from the current behaviour and on a wide number of tests that involves designed mutants (for instance deleting portion of the peptide or destabilizing specific regions). Simulations have the possibility to provide a molecular level description of the process.

On atomic scale simulations are extremely informative about the protein microscopic dynamics, as they take into account the finer details of structures and interactions. Although the high complexity of the systems prevents atomic-scale simulations from cumulating a necessary number of events for a meaningful statistical mechanical description, the current computational resources allow to reproduce quite accurately the experimental systems. Concerning the translocation of thioredoxin (Trx) we analyzed the process both in αHL pore and in a graphene pore the diameter of which is similar to the αHL sensing site, see Bonome et al., 2015 and Di Marino et al., 2015.

In the simulation protocols, we induce the protein translocation applying a constant force to its C-terminal, see Bonome et al 2015. The resulting pathway shows a not uniform translocation where the proteins "stall" at specific and quite reproducible conformations. Right panels of Fig 2 report the time evolution of the number of untranslocated amino acids Np (108 indicates the initial condition where the whole protein is untranslocated, while 0 means that the whole protein has translocated) for two replicas of the pulling protocol. The main stalls, appearing as plateaus in the Np curves, are associated with the pore "clogged" by more than one amino acid, i.e. not only by the translocating amino acid but also by the portion of the untranslocated region of the chain. The Trx translocation is hence characterized by two main stages. In the early stage the protein gradually unfolds and only one residue occupies the pore. The passage of the central β-strand of the Trx β-sheet (β3 in the left panels of fig. 2) destroys the native fold and also drags with itself part of the untranslocated structure at the pore mouth. This pathway results in several rearrangements of the Trx generating various stalls (plateaus in Np curves). All of them correspond to conformations where amino acids originally belonging to β2 occupy the pore. Interestingly, the different stalls are associated to different ionic currents and, in particular, the pore-clogging conformations result in greater current reductions, allowing to speculate on the possible causes of the multistep signal observed in experiments. A similar co-translocational unfolding pathway was also observed for Trx translocation through αHL pore (Di Marino et al., 2015).

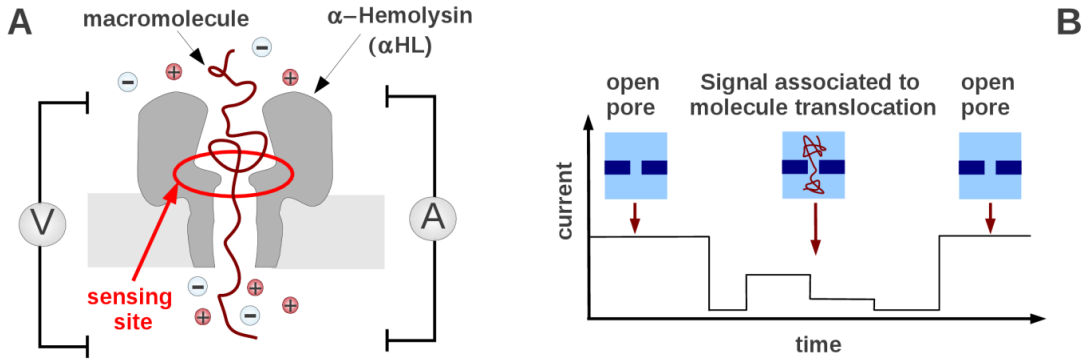

Figure 1: Nanopore sensing principle. Panel A) A nanopore is embedded in a membrane. When a voltage is applied between the two chambers (V in panel A) a ionic flux sets in. This ionic flux can be easily measured using an amperometer (A in panel A). The presence of a molecule inside the pore results in an alteration of the current. The reduction in the conductance is due to the conformation of the macromolecules inside the pore however, the main contribution comes from the narrowest section (sensing site). In the case of α-Hemolysin (αHL), the constriction is 1.4 nm in diameter and it allows the passage of only a single residue (panel A). Panel B reports a sketch of a possible multistep current signal associated to the passage of a protein that unfolds during the translocation process (co-translocational unfolding).

3. Peptide trapping

A second crucial issue in nanopore protein and peptide sensing is the control of the translocation rate. In this respect is was recently show that the addition of a positive and a negative tail to a neutral peptide, increases the residence time of the molecule inside the nanopore, Asandei et al., 2015. We shortly discuss the fundamental mechanism behind the trapping of peptides by the pore. The applied potential ΔV induces a non-homogeneous electrical field that is more intense inside the nanopore. The artificial polarity of the molecule favors its capture since the dipole tends to orient along the field lines that converge into the pore axis, Fig 3a. Indeed, as long as the molecule enters the pore, it experiences an increasing importing force due to the larger electrical field, Fig 3b. When also the other charged tail

engages the pore, an opposite couple of forces generates a sort of "tug-of-war" for which the analyte becomes stably trapped in the pore center, Fig 3c and 3d. Since the intensity of the electrical field can be controlled by changing the voltage ΔV, both capture and escape rates can be easily tuned. In particular, the capture rate increases with voltage while the escape rate decreases. In a recent work (Chinappi et al, 2015), we recasted this phenomenological interpretation in a more rigorous approach based on the estimation of the free-energy barriers associated to the translocations. Sketches of the model system and of the free-energy profile are reported in figure 3e and 3f. The particle has to overcome a capture barrier to enter the pore (due to entropic cost of the confinement), once it is inside the pore it gets trapped in the minimum of the free-energy and it has to pay a further free-energy cost to escape. The escape and the capture barrier depend on the applied potential ΔV. In particular, escape barrier increases with ΔV, realizing the trapping mechanism. Remarkably, this approach, dubbed nanopore tweezer, is not limited to macromolecules but, in principle, can work also at larger scales, since the only ingredients are the nonhomogeneous electrical field induced by the nanopore in presence of an applied voltage ΔV and a particle with high dipole moment.

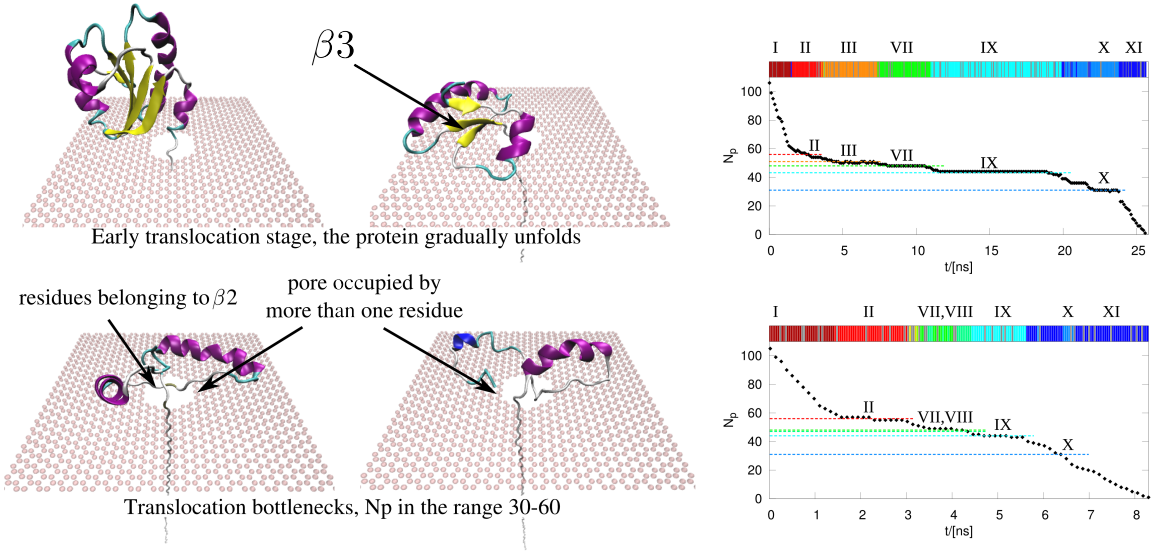

Fig 2. Multistep translocation pathway of Thioredoxin through a graphene nanopore. Left panels show the main phases of the translocation pathway. In the early stage the protein gradually unfolds and only one residue occupies the pore. The passage of the strand β3 of the thioredoxin β-sheet destroys the native fold and drags part of the untranslocated structure at the pore mouth. This results in several rearrangements of the thioredoxin that give raise to various stalls (plateaus in Np curves indicated via roman digit in the right panels). Right panels were obtained using VMD, (Humphrey et al 1996), while the simulations where performed using NAMD, Phillips et al 2015.

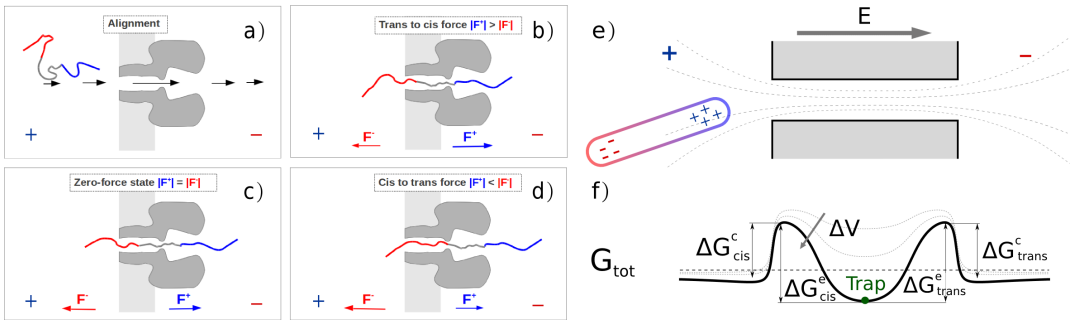

Figure 3. Nanopore tweezer. a) The applied voltage results in an inhomogeneous electrical field that is larger inside the nanopore. The field aligns the peptide with the positive tail towards the pore mouth. b) The resulting electrostatic force drives the polypeptide into the pore. As the positive residues exit from the pore side and negative residues enters, |F+| decreases while |F-| increases until the two forces approximately balance (zero net force

stage, panel c). Further movements of the polypeptide towards the positive (b) or the negative electrode (d) result in an electrical force that drives the peptide back to the zero force state, Asandei et al, 2015. Panel e and f report the interpretation of the tweezer principle in terms of free-energy barriers, Chinappi et al 2015.

4. Future developments

Both the results briefly presented here are associated to experimental set-ups where specific modifications of the peptide chain termini are needed. In this respect, recent experimental developments in manipulating the peptide structure, such as the "click" reaction proposed by Biswas et al, 2015, can potential pave the way to a more systematic employment of the discussed approaches. On the computational side, it is nowadays relatively easy to completely reproduce the nanopore experimental systems in full details (Belkin et al, 2015), although ad hoc protocols have to be employed to induce the translocation on accessible time scales. This allows to numerically test theoretical ideas and to have an immediate interpretation of the experimental results in terms of specific peptide conformations occupying the pore sensing regions.

Acknowledgements

This research used the computational resource from CINECA (pr. GRAPUNA) and TGCC (PRACE pr. 2014112673).

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