Protein translocation through graphene nanopores

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Nanopore sensing is an emerging technique to analyze macromolecules at single-molecule level. In nanopore sensing a voltage is applied between the two sides of the membrane and, consequently, a ionic current flows through the nanopore [1]. When a molecule translocates across the nanopore, a change in the current is measured. The current signal, in principle, allows to obtain information on the translocating molecule. A number of different nanopores (both biological and solid state) have been employed for different applications, such as DNA sequencing, protein-DNA interaction and polypeptide folding [2].

We focus our attention on proteins and polypeptides. Protein translocation presents two crucial differences with respect to DNA translocation. Indeed, on the one hand proteins are not uniformly charged and hence, they are not easily imported into the pore by the applied electrical field, on the other hand, in typical conditions, proteins need to partially or completely unfold in order to cross the pore. A crucial advance in controlling the protein translocation was achieved by Rodriguez-Larrea and Bayley [3] that, adding a DNA tail to the protein C-term, were able to control the protein translocation. The authors show that a multilevel current signal is associated to the thioredoxin transport, suggesting that the co-translocational unfolding of thioredoxin occurs as a multistep process where the protein undergoes to specific and reproducible structural arrangements.

In this study we present a computational analysis of thioredoxin translocation through a graphene nanopore (fig 1A). We employed a computational protocol based on several non equilibrium molecular dynamics simulations steps. As a first step we induced the protein translocation by applying a constant force to its C-terminus (fig 1C). Different translocation intermediates (stalls) were observed, these intermediate were found robust among simulation replicas (fig 1D). Then we applied a structural cluster analysis to identify representative conformations associated to the stalls. Interestingly, in presence of the stalls the pore is partially clogged not only by the translating residue (fig 1E) but by an hairpin-like structure on the entrance of the pore (fig 1F). The current levels associated to the representative conformations were then calculated by means of dedicated non equilibrium runs, showing that different current levels are associated to specific translocation intermediates (fig 2). The current is anti-correlated with the number of thioredoxin atoms that clog the pore (fig 3), i.e. the steric hindrance is the dominant contribution to the current determination. Our study shows the potentiality of graphene pores as sensitive tools to determine the volume of the molecule portion engaging the pore. This suggests possible applications to the analysis of protein sequences as well as to the single-residue detection of post translational modifications characterized by alteration of the residue volume.

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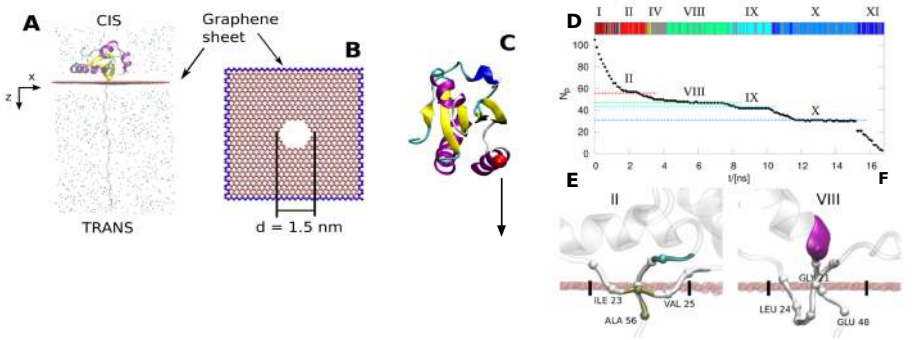

Figure 1: A) Translocation of Thioredoxin (Trx) from Cis to Trans compartment through the graphene nanopore. B) The graphene sheet lies on the Oxy plane (d = diameter 1.5 nm). C) The translocation of Trx in the pore is achieved by Constant force steered Molecular Dynamics (cfSMD). In this simulation a constant force is applied to C terminus of the protein and this is pulled in the pore. D) The cfSMD results. Representation of thioredoxin residues that are located on the CIS side of the graphene at the time t. Different stalls are observed and in presence f the stalls the pore is partially clogged by the translating residue (E) and by an hairpin-like structure on the entrance of the pore (F).

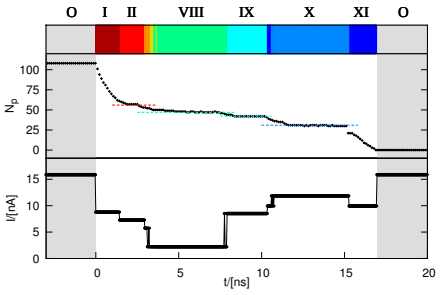

Figure 2: Current levels associated to specific translocation intermediates. The top panel shows the cfSMD as fig 1D. The bottom panel reports the current values I measured in voltage applied simulations on a representative conformations of the protein during the translocation. Different current levels are associated to specific translocation intermediates.

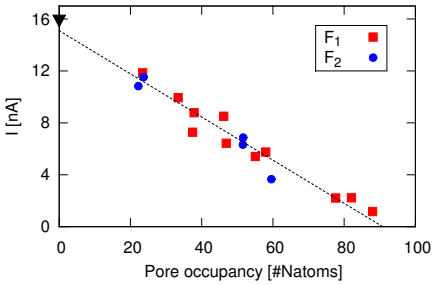

Figure 3: Correlation between current and pore occupancy. The average current I is reported as function of the number of thioredoxin atoms that occupy the pore. The trend shows a anti-correlation between current value and the number of atoms.