Jamming signatures observed in peptide translocation

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Upon peptide passage through membrane pores, peptides undergo conformational transitions and sample intermediates that block the transmembrane current [1–8] that would otherwise flow in an open pore under a potential drop. These intermediate states can be considered jammed states. On the other hand, the phenomenology of jamming has been widely studied, separately in the context of dynamical arrest of macroscopic granular matter and in that of macroscopic glasses [9–14]. Jamming can be identified by signature characteristics in the force distributions, $p(f)$ - the force profile of a jammed system will exhibit a peak in $p(f)$, that deviates from the exponential decay in the forces of an unjammed state [15–20]. In previous work, we have applied such jamming analyses to study protein folding in aqueous solution [21]. Herein we investigate the force distributions associated with an unstructured peptide, CAMA [22, 23] both in free state and within the constriction of a pore lumen.

Translocation of an unstructured peptide, **C**ecropin **A** - **MA**gainin(CAMA) with a sequence of 20 amino acids ($KWKLFKKIGIGKFLQSAKKF - NH_2$), through biological nano-pore α-HL [24], was studied using single molecule electrophysiology, inserted from the trans-end [25, 26]. Molecular dynamics simulations of the above process at complete atomistic resolution [27–32], was successful in capturing the sub-states, which induce partial or complete blockage of the pore, reflected as spikes in the current blockage signature. Three states of CAMA are chosen. First is the free peptide, which assumes a heterogeneous kinked shape due the presence of two alternate glycines(G) at positions 9 and 11 separated by one Isoleucine (I) at position 10, most reminiscent of an un-jammed peptide. Second, in which the peptide is unfolded within the pore constriction and translocated through the beta-barrel of α-HL in a linear fashion (less jammed), and lastly where the CAMA traverses the pore in a wound, condensed state, a configuration most akin to a jammed peptide. Representative snapshots of the conformational ensemble assumed at each state are depicted with their respective non-bonded force profiles in Figure 1. All three states exhibit jamming signatures, as marked by the pronounced peak above the mean force, though this peak is shifted more to the right the more unjammed the peptide, *e.g.* the free CAMA, which is shifted farthest. Thus we may surmise that the position of the peaks depends upon the relative degree of jamming between the configurations. Note that the simulation for the free CAMA was carried out at room temperature where jamming is shown, from our previous study, to be extant in peptides. A simulation at a much higher temperature will most likely wash out the jamming traits in the force profile, which can be investigated in a future study.

Translocation trajectories of the peptide traversing the pore in condensed and linear state as well as the dynamics of the free peptide were generated using the CHARMM-27 force field in ACE implicit solvent model (continuum with dielectric constant of 80) using Langevin dynamics (friction coefficient 10 ps^{-1}) at 300K. Translocation is effected by using an accelerated electric field applied to the peptide only, calculated from the applied trans-membrane voltage along the z-axis, given by, $V = -EL_z$. The non-bonded or van der Waals interactions were computed using switching function with a cutoff of 12$\mathring{A}$. The SHAKE algorithm was used to constrain the hydrogen bonds permitting a time step of 2 fs. Unfolding of the peptides within the pore constriction was effected by adding a half quadratic perturbation term to the Hamiltonian, dependent on both time and reaction coordinate, forcing the peptide to move away from its initial configuration using a half harmonic force constant through the use of the biased MD method [33, 34] in CHARMM simulation package.

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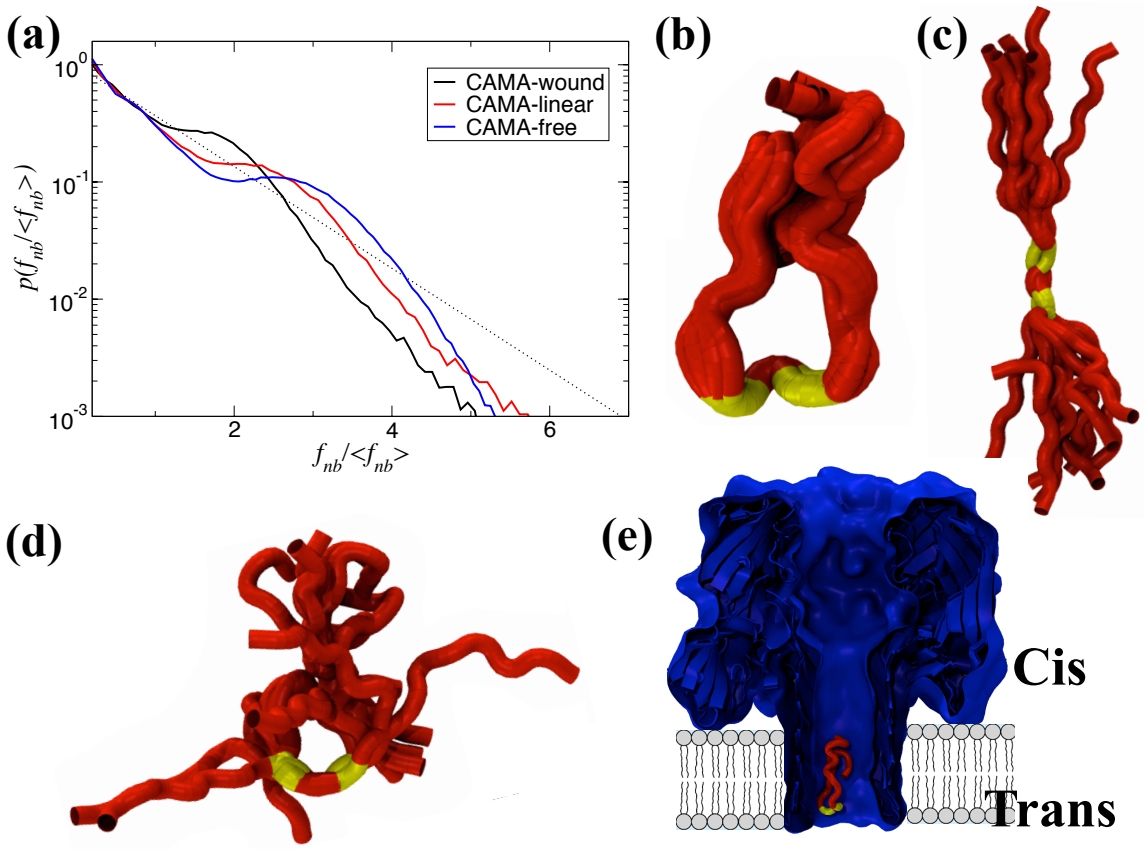

FIG. 1: (a) The non-bonded, normalized force distributions for three different states of CAMA, ranging from jammed (wound) to marginally jammed to relatively un-jammed (free). All three exhibit jamming signatures, namely a pronounced peak about the exponential line (dashed), though this is shifted to higher values the more apparently unjammed the peptide. Additionally, the tails of those that are less jammed are closer to the exponential line. Conformational ensembles of CAMA P6 in (b) wound state (c) Linear and (d) free state. (e) Translocation of peptide CAMA - P6 through lumen of α-hemolysin

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