Electronic Supplementary Material (ESI) for Chemical Science.

New Insights into the Folding-Unfolding Mechanism and Conformations of Cytochrome C

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Electronic Supplementary Information

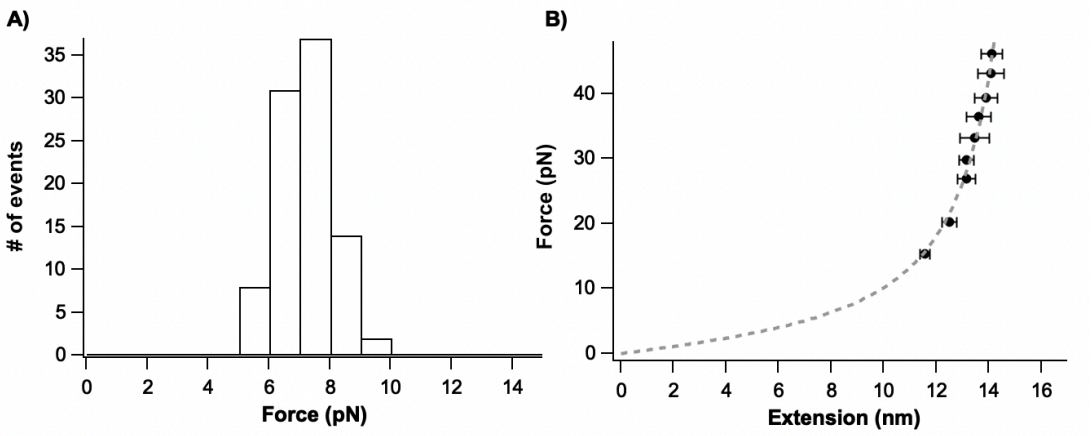

Figure S1. Unfolding-folding signature of NuG2. A) Refolding force histogram of NuG2 in Cys-cytc-NuG2-Cys. B) Force-extension relationship of the unfolding/folding of NuG2. WLC model of polymer elasticity fit to the data shows a persistence length of 0.7 nm and a ΔLc of 17. 4 nm.

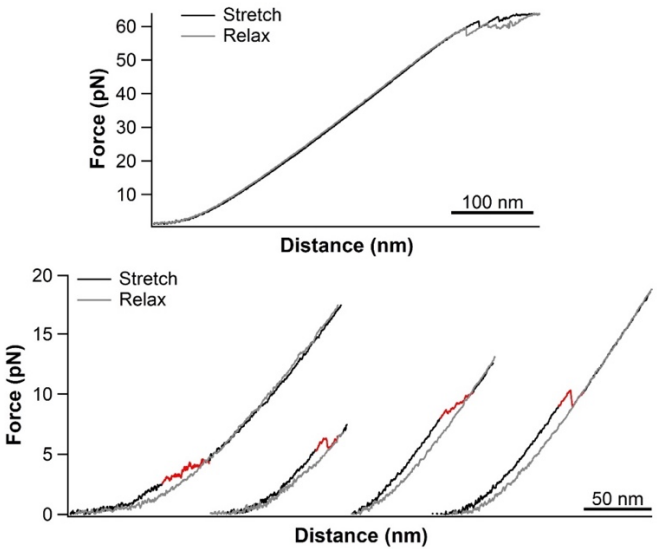

Figure S2. Typical stretching-relaxation cycles of DNA-apo-cytc-DNA. Upper panel shows typical force-distance curves in which no unfolding/refolding events of apo-cytc were observed. Lower panel shows representative force-distance curves that exhibit low-force unfolding-like events of apo-cytc.

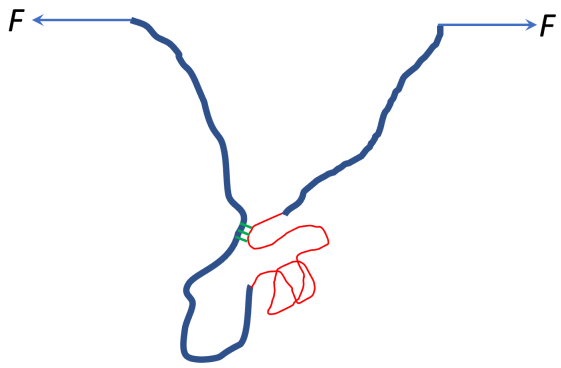

Figure S3. Rupturing non-specific interactions between apo-cytc (colored in red) and dsDNA (colored in blue) could lead to unfolding-like events with ΔLc greater than the contour length of apo-cytc (37 nm). The figure shows a highly schematic drawing of such hypothetical interactions, where one end of apo-cytc interacted with a dsDNA handle. The fact that we never observed any ΔLc greater than 35 nm in apo-cytc strongly argues against that the unfolding-like events observed in apo-cytc are due to the non-specific interactions between apo-cytc and dsDNA handles.

The low-force unfolding-like events of apo-cytc are not due to the interactions of apo-cytc and dsDNA hands

The low-force unfolding-like events of apo-cytc are due to the intrachain interactions of apo-cytc and reflect the residual structures present in apo-cytc. Although one may argue that non-specific interactions between apo-cytc and dsDNA handles could give rise to the short rips, a few evidences argue against this possibility. 1) The persistence length of dsDNA handles is significantly higher than that of unfolded polypeptide chain (50 nm vs. 0.7 nm). Hence, in the pulling experiments, the dsDNA extends much more than the unfolded polypeptide chain, effectively minimizing the probability of the polypeptide chain interacting with the dsDNA handles. 2) The ΔLc we observed for the apo-cytc spreads a broad range of values (from a few nm to 35 nm), but always smaller or equal to the contour length of cytc (37nm). The spreading of ΔLc suggests that the interactions are not specific, regardless of the nature of the interactions. If such interactions were due to the interactions between cytc polypeptide chain and dsDNA handles, such non-specific interactions should readily generate rips with ΔLc bigger than 37 nm, as schematically shown in Fig. S3, in which case the obtained ΔLc includes the length of the polypeptide chain and the length of the segment of dsDNA handle. However, we never observed an ΔLc bigger than 37 nm. Taken together, these evidences strongly argue against the possibility that these interactions are originating from protein-DNA interactions.