Supplemental Information

Nanopore detachment kinetics of Poly(A) Binding Proteins from RNA molecules reveals critical role of C-terminus interactions

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1. The Poly(A)/PABP complex and alpha-Hemolysin structures

Though currently the structure of full length PABP and poly(A) complex is not available, Deo et al. has succeeded to determine the cocrystal structure of the N-terminal RRM 1/2 of PABP and poly(A) down to the resolution of 2.6 Å (1). In Fig. S1, we overlaid this cocrystal structure on top of the crystal structure of alpha-Hemolysin (α-HL) (2). It is clear that this complex is too large to enter the α-HL's vestibule. Considering the proposed full length PABP binding configuration with its four RRMs arranged in a linear manner along the poly(A) molecule, it is reasonable to assume that the full length PABP with poly(A) will be held entirely outside the vestibule of α-HL.

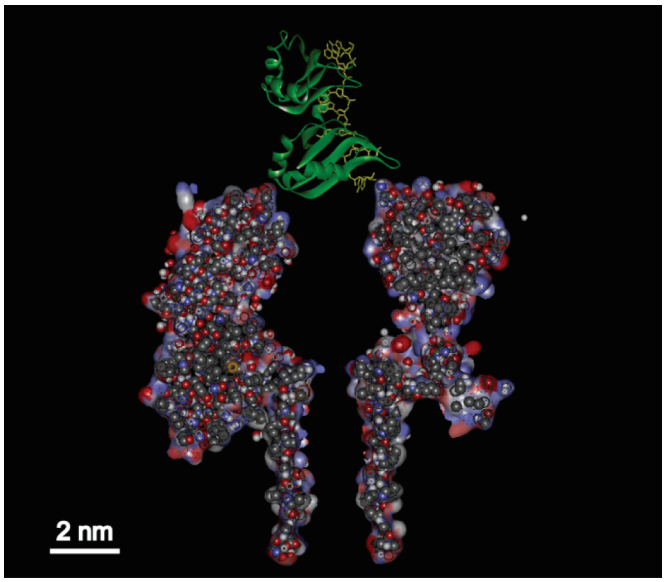

Figure S1. The co-crystal structure of the N-terminal RRM 1/2 of PABP and poly(A) on top of the crystal structure of α-HL, to scale.

2. Voltage-dependent detachment of $C_{50}A_{25}$/PABP complex

In order to rule out the possibility that the RNA/PABP complexes retract from the pore against the electrical force direction, we preformed a series of detachment experiment of the $C_{50}A_{25}$/PABP complex at various voltages. In these experiments the voltage was incrementally increased from 100 mV to 200 mV in 20 mV steps and at least 1,000 detachment events were

acquired for each voltage level. The semi-log plots of the dwell-time histogram for all voltages are shown in Fig. S2.

Mono-exponential fits to the distributions indicate that the process can be characterized by a first order kinetics. These fits are used to extract the characteristic time-scale τ at the different voltages. As can be seen from Fig. S2, increasing the applied voltage results in a sharp decrease in τ (226 ms to 47 ms for 100 mV to 200 ms, respectively). These results confirm that the events in this population indeed represent detachment of the protein from the RNA, rather than escape (retraction) of the complex back to the *cis* chamber against the voltage gradient. If the later mechanism has prevailed, an *increase* of the average dwell time with growing voltage, rather than *decrease*, would have been observed.

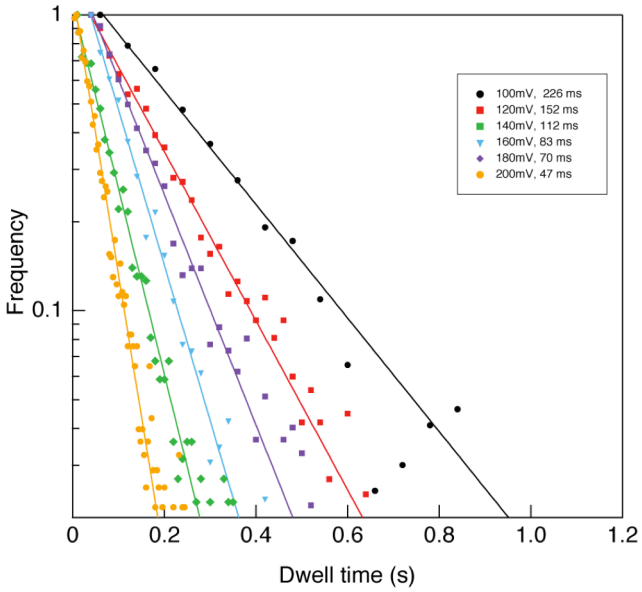

Figure S2. Voltage-dependent detachment of $C_{50}A_{25}$/PABP complex at 6 different voltages (100 mV to 200 mV) and 23 °C. The tail of each histogram is fitted with single exponential function to yield characteristic dissociation time.

References

1. Deo, R. C., J. B. Bonanno, N. Sonenberg, and S. K. Burley. 1999. Recognition of polyadenylate RNA by the poly(A)-binding protein. Cell 98:835-845.
2. Song, L. Z., M. R. Hobaugh, C. Shustak, S. Cheley, H. Bayley, and J. E. Gouaux. 1996. Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274:1859-1866.