

**Supporting Information for**  
**“Molecular Dynamics Study of MspA Arginine-Mutants Predicts**  
**Slow DNA Translocations and Ion Current Blockades Indicative of**  
**DNA Sequence ”**

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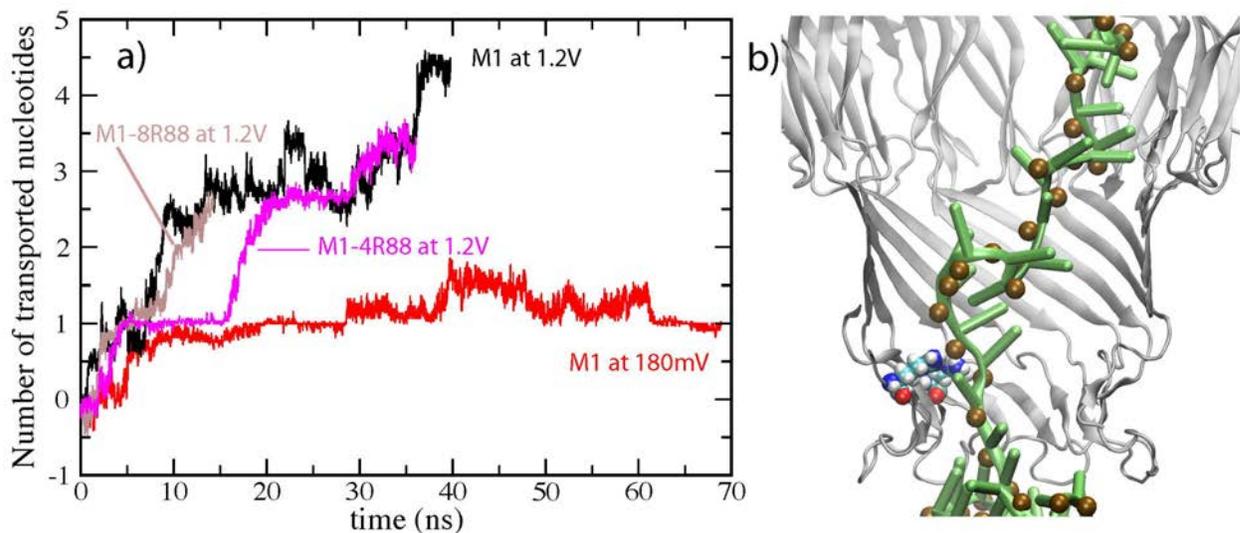
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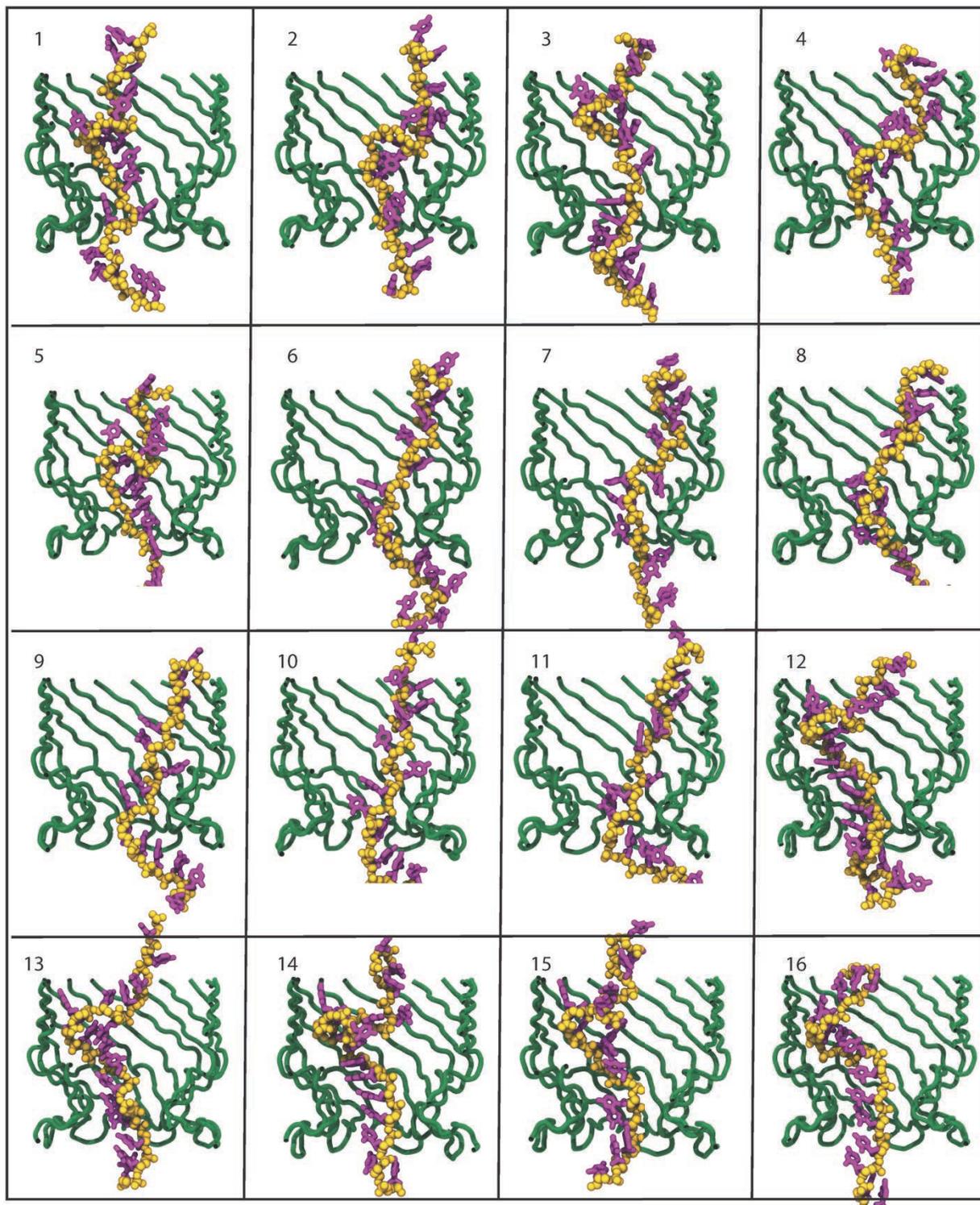
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**MD Simulations of Full-length MspA.** We used full-length MspA systems (see Methods and Figure 1-a) to determine the feasibility of observing the effect of arginine mutations on the transport rate of ssDNA through MspA. Each simulation system contained one copy of the protein, a 58-nucleotide DNA strand, lipid bilayer membrane and 1M KCl solution were simulated under a 1.2-V transmembrane bias.<sup>1</sup> In the initial conformation, ssDNA has already been threaded through the MspA pore using the phantom pore method.<sup>2</sup> Simulations with DNA in the same conformations were simulated in M1-NNN, and pores with the additional substitution L88R (denoted L88R-NNN), where all leucine residues at position 88 were replaced by arginines and L88R-4-NNN, where four adjacent Leu88 were replaced by arginines.

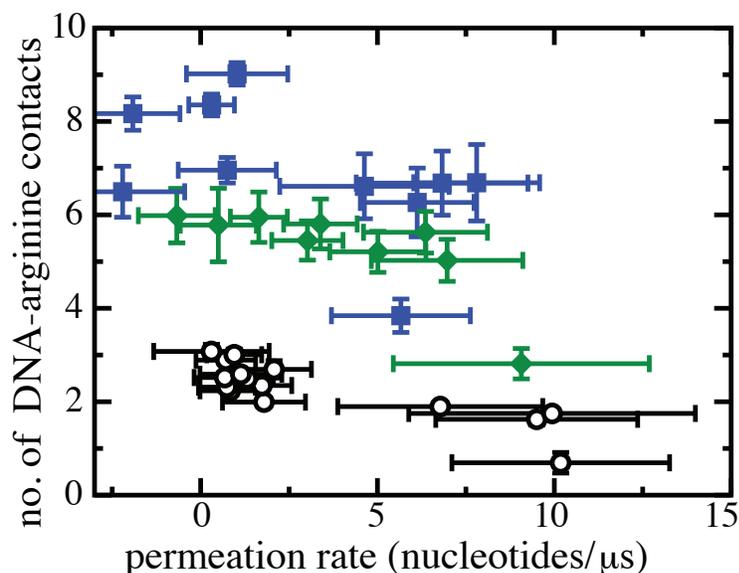
The simulated rate of nucleotide translocation for different variants of the MspA pore is shown in **Figure S1**. In the L88R-NNN and L88R-4-NNN systems, the DNA backbone was observed to develop ionic salt-bridge contacts with one (L88R-NNN) or two (L88R-4-NNN) Arg88 within less than a nanosecond of the respective simulation. As shown in **Figure S1**, the L88R mutation had no noticeable effect on DNA transport in the M1-8R88 system, whereas in the M1-4R88 system the mutation led to transient arrests of the transport (plateau at 4-17 and 20-30 ns). Visual analysis of the simulated trajectories revealed that the transient arrests of the translocation were associated with multiple Arg88–DNA backbone interactions; **Figure S1(b)** illustrates one such conformation. We note that at a 1.2V bias, a complete halt of the nucleotide transport due to the L88R mutation is unlikely as the energy gain from transporting one nucleotide through the constriction is about  $e \cdot 1.2V = 46 k_B T$  and a typical energy of an ionic salt bridge in water is about  $5 k_B T$ . With a 180 mV bias, the energy gain would be  $\sim 7 k_B T$  for suggesting that at the reduced voltage, the effect of L88R mutation could be quite prominent. A test simulation of ssDNA permeation through full-length M1 pore revealed very slow translocation kinetics ( $\sim 1$  nucleotide/100 ns, Figure 1-a), consistent with experimental estimates.



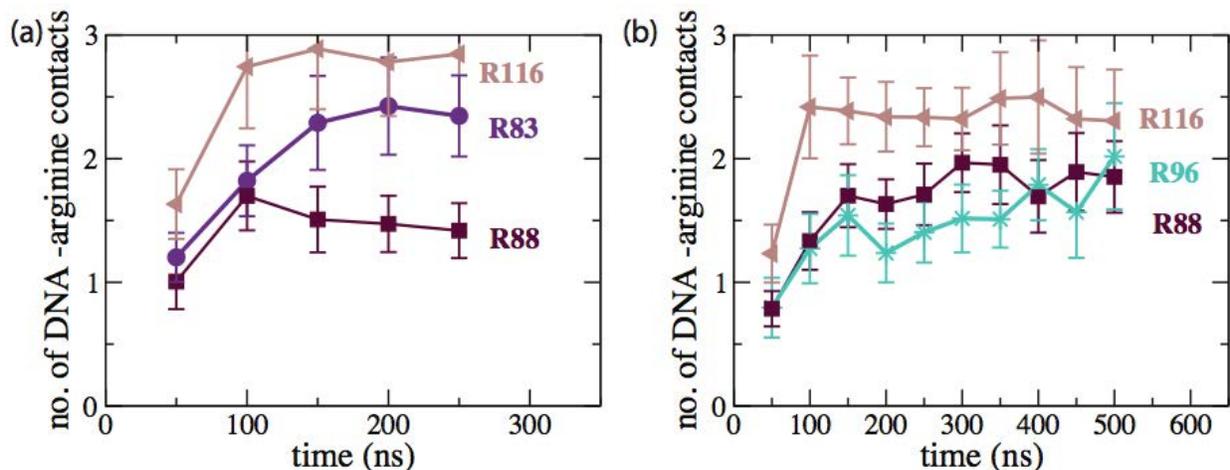
**Figure S1.** MD simulation of DNA transport through full-length MspA. (a) Number of nucleotides transported through MspA constriction (residues 90-91) versus simulation time. (b) The conformation of DNA in L88R-4-NNN mutant corresponding to the first plateau in the nucleotide transport trace (4-17s). Two Arg88 residues forming ionic salt-bridges with DNA backbone are shown in vdW representation.



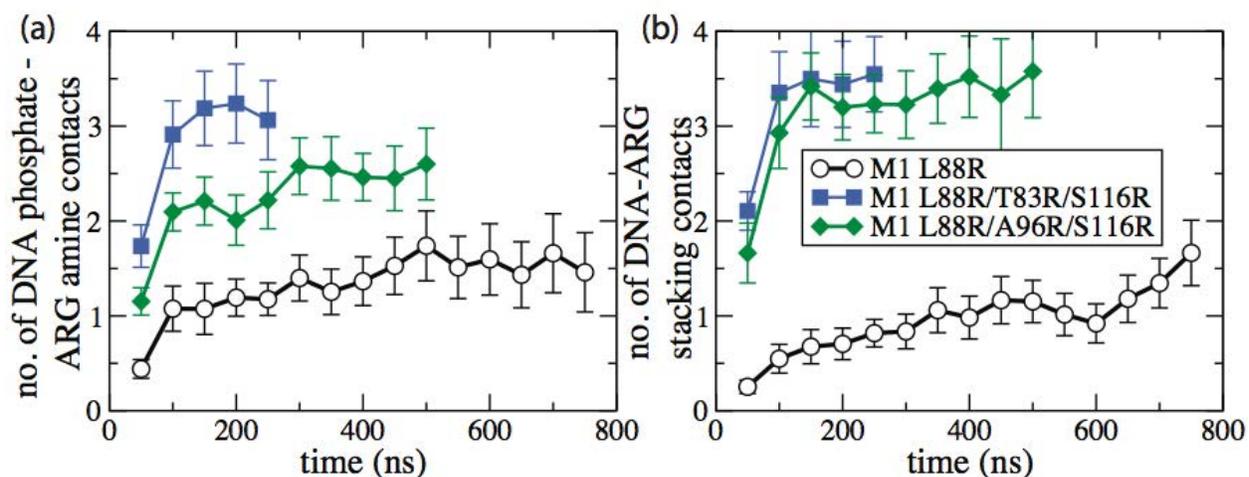
**Figure S2.** Starting conformations of sixteen reduced-MspA systems. (1-16) Cut-away views of the 16 reduced-length MspA systems. The protein backbone is shown as green tubes, the backbone and nucleobases of the DNA strand are shown as yellow and magenta vdW spheres. The lipid, water and ions are not shown.



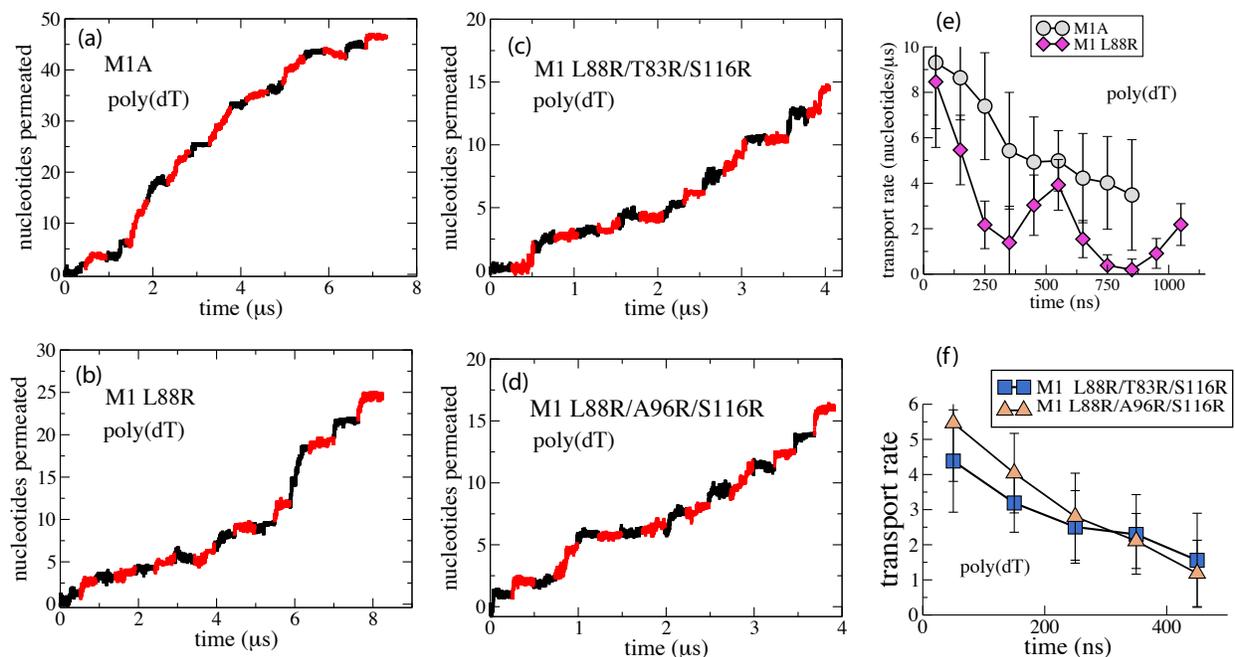
**Figure S3.** Scatter diagram showing the total number of persistent arginine-DNA contacts vs. the permeation rates of the nucleotides. The data for the L88R-NNN pore are shown as black circles, for L88R/T83R/S116R-NNN as blue squares and for L88R/A96R/S116R-NNN as green diamonds.



**Figure S4.** The total number of persistent contacts between DNA and specific arginine residues in the MD simulations of triple mutants M1/T83R/S116R (a) and M1/L88R/A96R/S116R (b). The data represent ensemble average over 16 systems; the error bars indicate the standard deviation of the average. Our definition of a persistent contact is given in the caption to Figure 3 in the main text.



**Figure S5.** Salt-bridge and stacking contacts in triple mutants. The number of persistent contacts between arginines and (a) phosphate oxygen atoms of the DNA backbone or (b) non-hydrogen atoms of the cytosine bases are plotted versus simulation time. The data represent ensemble average over 16 systems; the error bar indicates the standard deviation of the average. Our reduced-length model of M1 MspA did not contain arginine residues. Our definition of a persistent contact is given in the caption to Figure 3 in the main text.



**Figure S6.** Ensemble simulation of ssDNA transport through mutant variants of MspA. The number of nucleotides translocated through the constriction of MspA at a 180 mV bias is plotted as a function of time for 16 independent systems in the case of (a) M1, (b) M1 L88R, (c) M1 L88R/A96R/S116R and (d) M1 L88R/T83R/S116R variants of the MspA pore and thymine homopolymers. Each trace is shown in two colors, red and black, indicating multiple trajectories (differing in their initial DNA conformation) that were added consecutively to produce the total permeation trace. (e,f) Averaged over 16 independent trajectories, the transport rate of thymine homopolymers is plotted versus the simulation time for M1 and M1 L88R (e) and triple mutants M1 L88R/T83R/S116R and M1 L88R/A96R/S116R (f) pores. The error bars represent the standard deviation of the mean.

**Movie 1.** (700ns.mpg) Simulated trajectory of a homocytosine strand permeating through a truncated M1-NNN pore at a 180 mV transmembrane bias. The current trace and the number of nucleotides permeated in the trajectory are plotted in Figure 1 (panels d and e respectively). About 30 nucleotides are seen to permeate in < 800 ns.

**Movie 2.** (Mutant.mpg): A 4 μs trajectory of a poly(dC) strand permeating through a truncated L88R/T83R/S116R mutant pore at a 180 mV transmembrane bias. The permeation of the strand appears to be halted owing to the formation of contacts with the arginine residues on the wall of the pore (shown in blue).

## **References**

1. Aksimentiev, A.; Schulten, K., Imaging alpha-hemolysin with molecular dynamics: Ionic Conductance, Osmotic Permeability, and the Electrostatic Potential Map. *Biophys. J.* 2005, 88, 3745-3761.
2. Mathe, J.; Aksimentiev, A.; Nelson, D. R.; Schulten, K.; Meller, A., Orientation Discrimination of Single-Stranded DNA inside the Alpha-Hemolysin Membrane Channel. *Proc. Natl. Acad. Sci. U. S. A.* 2005, 102, 12377-12382.