Supplementary information for the manuscript by D. B. Wells, V. Abramkina and A. Aksimentiev "Exploring transmembrane transport through alpha-hemolysin with Grid-Steered Molecular Dynamics"

Sequence	Effective bias (V)	Translocation velocity (nuc./ns)	Simulation time (ns)	Translocated nucleotides
(*cis*) 3'-dA_{58}-5' (*trans*)	1.2	0.02[a]	27.8	0.8
	2.4	0.10[a]	19.6	2.5
	4.8	1.10	9.3	9.5
	9.6	6.94	3.6	24.3
		5.75	3.6	21.2
		6.46	3.3	21.0
		6.21	3.5	21.9
	13.2	9.07	1.2	11.2
		8.96	1.2	10.9
		9.13	1.2	11.1
		7.56	1.1	9.0
(*cis*) 5'-dA_{58}-3' (*trans*)	1.2	0.07[a]	29.9	2.0
	2.4	0.09[a]	18.8	2.3
	4.8	1.25	8.9	10.7
	9.6	6.84	3.4	22.9
		7.66	3.0	22.2
		8.07	2.7	21.6
		7.70	2.8	20.9
	13.2	13.5	1.1	14.1
		11.6	1.2	13.0
		9.05	1.2	11.2
		11.5	1.4	14.7
(*cis*) 3'-dC_{58}-5' (*trans*)	4.8	1.32	9.8	11.8
		0.89[a]	6.6	5.9
	9.6	6.48	4.0	25.4
		6.38	4.0	25.9
		8.89	3.8	30.1
		7.26	4.0	27.9
(*cis*) 5'-dC_{58}-3' (*trans*)	4.8	1.73	10.8	18.4
	9.6	13.5	1.5	19.3
		12.2	1.9	20.1
		12.1	1.6	18.8
		10.6	1.6	17.3
(*cis*) 5'-$(dAdC)_{29}$-3' (*trans*)	9.6	10.3	2.2	22.2
		9.25	2.2	20.4
		8.68	2.3	20.1
		8.38	2.2	19.0
DNA hairpin	15	n/a	4.2	n/a
Peptide	10	n/a	17.7	n/a
	20	n/a	3.8	n/a

TABLE I: G-SMD simulations of DNA tranlocation through α-hemolysin. The effective bias is computed as $(N+1) \times 1.2\,\mathrm{V}$, where N is the scaling factor applied to the steering potential. The DNA translocation velocities were computed by applying a linear regression fit to the cumulative DNA currents.

[a]These data yielded correlation coefficients from a linear regression fit of less then 0.95.

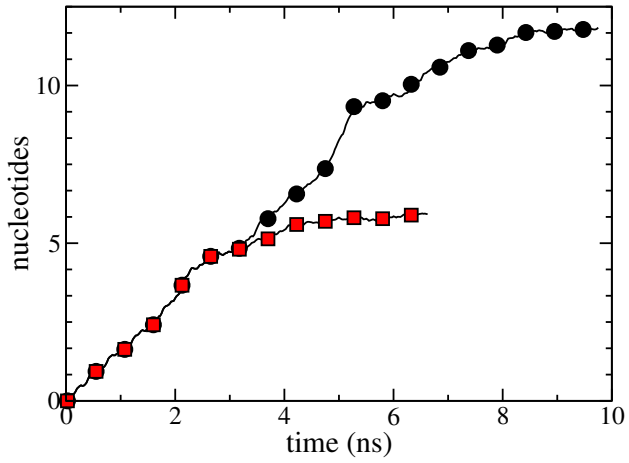


FIG. 11: We carried out two simulations of C5' translocation. In the first simulation (red squares), after ∼3 ns the DNA strand collided with the cap of α-hemolysin, which halted the DNA translocation. Fig. 12 below illustrates the conformation of the C5' strand at the end of this simulation. In the second simulation (black circles) we, for a short period of time (200 ps), applied a force to the upper portion of the DNA strand parallel to the lipid bilayer membrane in order to shift the DNA away from the cap, after which the simulation was continued normally.

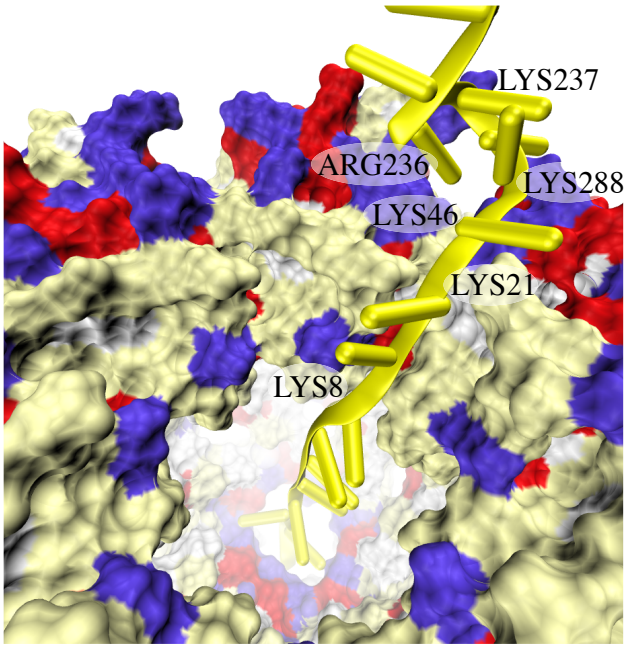
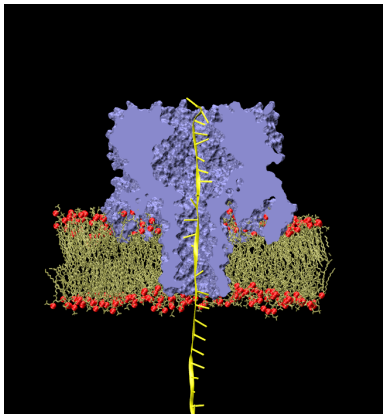
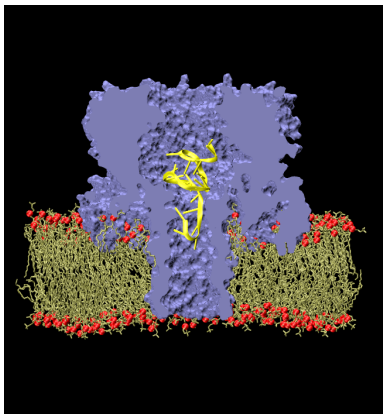


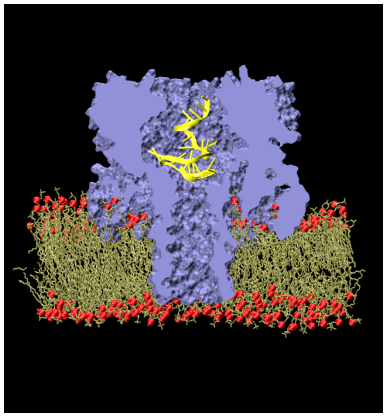
FIG. 12: Protein-DNA interaction halts translocation. The image illustrates the final state of a 6.5 ns MD simulation carried out with G-SMD under a 4.8 V effective bias. The translocation of a poly$(dC)_{58}$ strand (yellow) halted after encountering positively charged residues at the cap of α-hemolysin: Lys8, Lys21, Lys46, Arg236, Lys 237, and Lys288. The fragment of the DNA strand in the vestibule extends to its full contour length but the bonds between the DNA and the protein persist. The surface of α-hemolysin is colored according to the type of the exposed residues: red, blue, green and white correspond to negatively charged, positively charged, polar and nonpolar side chains, respectively.



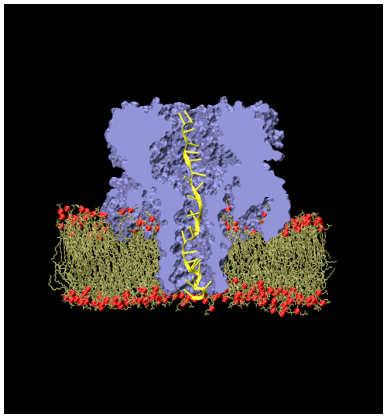
MOVIE 1: `smd_1st.mpg` — SMD force applied to the phosphorous atom of the first nucleotide of the DNA strand. The DNA stretches as it traverses through the pore constriction. This 3 ns simulations was done using an SMD pulling velocity of 43 Å/ns, and a spring constant of 500 kcal/mol·Å^2.



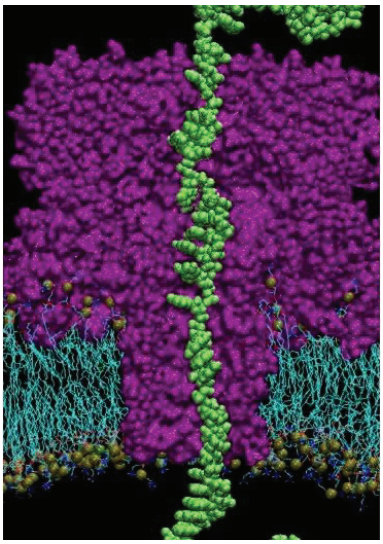
MOVIE 2: `smd_com.mpg` — SMD force applied to the center of mass of the DNA strand. The DNA is first compressed at the constriction, after which it transits the pore. This 2.8 ns simulation was done with a pulling velocity of 43 Å/ns, and a spring constant of 500 kcal/mol·Å^2.



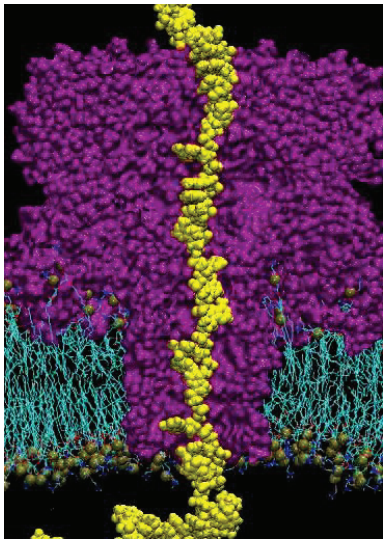
MOVIE 3: `efield.mpg` — DNA permeation driven by a uniform electric field. This simulation was performed by applying constant forces to individual DNA atoms. The magnitude of each force was computed as the product of the atomic charge and the electric field equivalent to a 12 V transmembrane bias. The total simulation time is 4 ns.



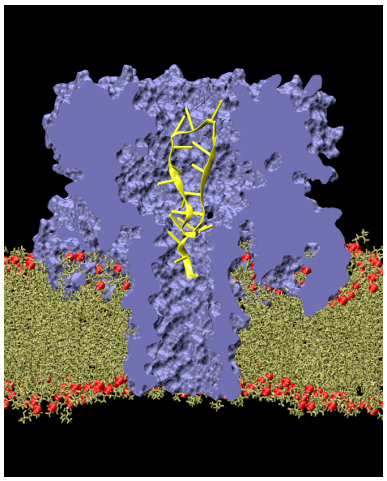
MOVIE 4: `grid.mpg` — G-SMD simulation of DNA translocation. The transmembrane bias was set to 1.2 V with the scaling factor of $N = 10$, for an effective bias of 13.2 V. The DNA is observed to permeate the pore in a conformation similar to that of DNA under an electrostatic bias only. The total simulation time is 5 ns.



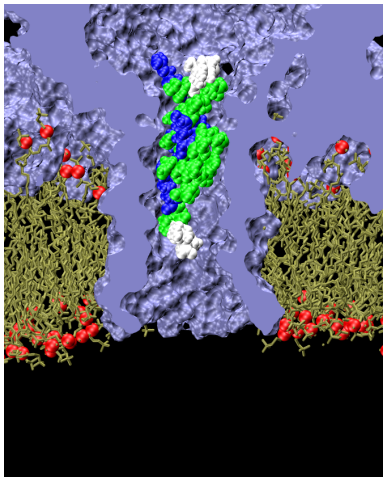
MOVIE 5: `grid_dna_A5down.mpg` — Translocation of a poly$(dA)_{58}$ DNA strand through the α-hemolysin pore in the (*cis*) 3'-dA_{58}-5' (*trans*) orientation. The DNA is drawn as green spheres, the channel is drawn as purple spheres. The DPPC lipid bilayer is shown in blue with phosphorous atoms shown as tan spheres. Water and ions are not shown. The system comprises 356,065 atoms. The applied effective bias is 4.8 V. The movie covers 9.3 ns of G-SMD simulation.



MOVIE 6: `grid_dna_A3down.mpg` — Translocation of a poly(dA)$_{58}$ DNA strand through α-hemolysin pore in the (*cis*) 5'-dA$_{58}$-3' (*trans*) direction. The DNA is drawn as yellow spheres. The pore is drawn as purple spheres. The lipid bilayer is shown in blue with phosphorous atoms shown as tan spheres. Water and ions are not shown. The system comprises 356,065 atoms. This simulation was carried out at 4.8 V effective bias. The movie covers 8.9 ns of G-SMD simulation.



MOVIE 7: `grid_hairpin.mpg` — Translocation of a DNA hairpin through the α-hemolysin pore. The DPPC lipids are shown in tan with the phosphorous atoms shown as red spheres. The pore is shown as a purple surface, cut by a plane perpendicular to the lipid bilayer. Water and ions are not shown. The model comprises 288,618 atoms. An effective bias of 19.2 V was applied in this simulation. The duration of the movie is 4.2 ns.



MOVIE 8: `grid_peptide.mpg` — Translocation of a peptide through the α-hemolysin pore. The peptide is colored according to residue type: positively charged (blue), uncharged polar (green) and non-polar (white). An effective bias of 13.2 V was applied for the first 17.7 ns, while an effective bias of 25.2 V was applied for the last 3.8 ns. The duration of the movie is 21.5 ns.