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Supporting Information

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Single DNA Rotaxanes of a Transmembrane Pore Protein**

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DNA-PEG Hybrid Strands Synthesis. Synthesis was accomplished on an Applied Biosystems 391 PCR-MATE synthesizer (Applied Biosystems, Foster City, CA) using standard cyanoethylphosphoramidite chemistry. Nucleobase, hexaethylene glycol and biotin β-cyanoethylphosphoramidites derivatives, solvents and reagents were used as provided (Glen Research, Sterling, VA). DNA-PEG hybrid strands were purified by preparative denaturing polyacrylamide gel electrophoresis, isolated by the crush and soak method, and desalted using C18 Sep-Pak cartridges (Waters, Milford, MA). Quantification was determined from the absorbance at 260 nm, and stored at –20 °C.

Planar Lipid Bilayer Experiments

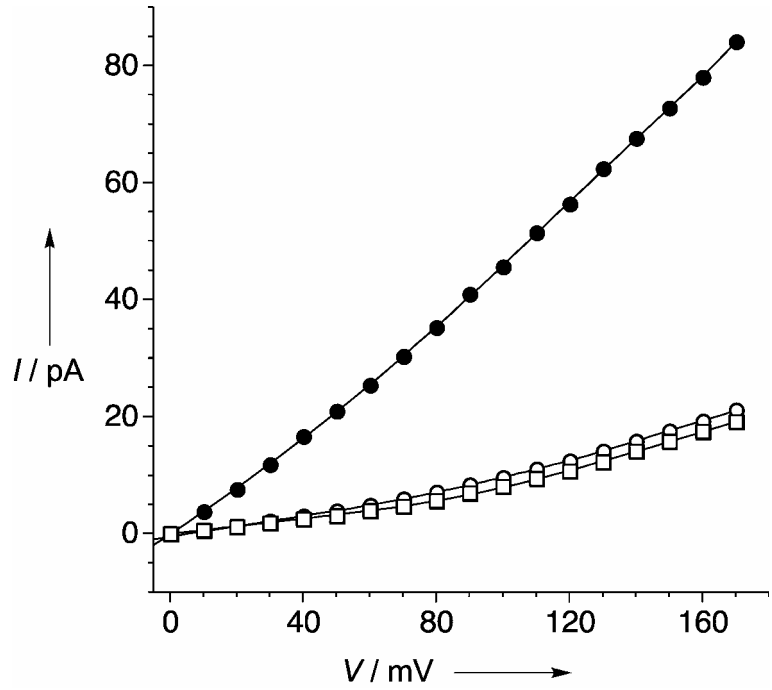
Lipid bilayers of 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids, Alabaster, AL) were formed on a 80-100 μm orifice in a 25 μm thick Teflon film (Goodfellow Corporation, Malvern, PA) separating the two chambers of a Teflon lipid bilayer apparatus following the procedure described by Montal and Mueller. Chambers were filled with 0.5 M KCl solutions containing 5 mM MOPS (3-(N-morpholino)propanesulfonic acid) and titrated to pH 7.5 with KOH. Chambers were named *cis* and *trans*, *cis* chamber was at virtual ground, hence a positive potential indicates a higher potential in the *trans* side and a positive current is one in which cations flow from the *trans* to the *cis* chamber. Monomeric α-hemolysin (α-HL) was added to a final concentration of approximately 50 nM in the *cis* chamber leading to single channel appearance in a matter of minutes, chamber was then flushed with fresh buffer solution to avoid further incorporation of additional α-HL channels. Thread molecules were added to final concentrations of 0.5 – 1.0 μM, short complementary DNA strand and streptavidin were employed at 5 – 10 μM final concentration. Transmembrane currents were recorded with an Axopatch-1D amplifier (Axon

Instruments, Foster City, CA). The electrodes were 1 mm diameter Ag/AgCl pellets connected to a silver wire surrounded by a wax-sealed Teflon tube (Axon Instruments). For analysis, currents were further low-pass filtered with an eight-pole Bessel filter (model 902, Frequency Devices, Haverhill, PA) with the corner frequency set at 5 or 2 kHZ (-3dB), and sampled by computer at 6-200 μs (Digidata A/D converter and pClamp 8 software, Axon Instruments).

Charge Selectivity Measurements. Experiments were performed in independent experiments under non-symmetrical conditions with planar bilayers separating solutions of 300 and 500mM KCl in *cis* and *trans* sides, respectively. Reverse potentials or potentials at zero current were determined from both graphical representations of current versus potential and readings from the amplifier output. Permeability ratios were calculated by substituting reverse potentials values and ionic activities for the solutions employed into the Goldman-Hodgkin-Katz equation:

$$\exp\left(\frac{VF}{RT}\right) = \frac{P_{K^+}\left[K^+\right]_{trans} + P_{Cl^-}\left[Cl^-\right]_{cis}}{P_{K^+}\left[K^+\right]_{cis} + P_{Cl^-}\left[Cl^-\right]_{trans}}$$

An additional experiment was performed to prove the assignment of the DNA blocking level. A DNA single strand of $d(A)_{40}$ was prepared containing a biotin group at the 5′ terminus. When this strand was added to the *cis* side in the presence of streptavidin, pseudorotaxane formation was observed by the decrease in conductance of the αHL pore to a similar level to the one observed when hybrid **2** was employed. This was an indication that neither the nature of the stopper nor the orientation of the strand (3′ to 5′ and 5′ to 3′) shows any remarkable effect in the blocking current in the system studied.



Current versus voltage representations for α-HL (●), thread molecule **2** added to the *cis* chamber (□) and $d(A)_{40}$-biotin +

streptavidin to the *cis* side(O). 500mM KCl, 5mM MOPS, pH 7.5.