Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information for

Facilitated Alkyl Detection by a DNA Conjugate with an α-Hemolysin Nanopore

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Experimental Section

Reagents and Chemicals. α-hemolysin (α-HL), decane (anhydrous, ≥ 99%) and potassium chloride (KCl, ≥ 99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,2-Diphytanoyl-sn-glycero-3-phosphocholine (chloroform, ≥ 99%) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Ethylensdiaminetetraacetic acid (EDTA, 99.995%) and Tris (hydroxymethyl) aminomethane (Tris, ≥ 99.9%) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). The DNA conjugate, B20-$(CH_2)_3$-B20 (5'-GTCACGATGGCCCAGTAGTT-CH_2-CH_2-CH_2-TTGATGACCCGGTAGCACTG-3') was synthesized and HPLC-purified by Sangon Biotech Co., Ltd (Shanghai, China). Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. The ultrapure water (reaching a resistivity of 18.2 MΩ cm at 25°C) was obtained using a Milli-Q System (EMD Millipore, Billerica, MA, USA).

Electrical Recording. We conducted the nanopore detection method according to our previous studies[1, 2]. The lipid bilayer membrane was formed spanning a 50 μm orifice in the center of a 1-mL Delrin bilayer cell (Warner Instruments, Hamden, CT, USA). The cell consisted of two chambers named as *cis* and *trans*, and the *cis* side is defined as virtual ground. We added 1mL solution into the two chambers, which contains 1.0 M KCl, 1 mM EDTA and 10 mM Tris (pH = 8.00). One could estimate the stability of the bilayer by measuring its resistance and capacitance. We added the α-HL near to the aperture in the *cis* chamber to form a nanopore. An easily recognized increase in ionic current would produce when the protein pore was inserted into the bilayer from the *cis* side of the cell. Also, we added the analytes from the *cis* side. Because we gave the voltage from the *trans* solution and the *cis* solution was grounded, the negatively charged DNA could be driven to translocate through the pore from *cis* side to *trans* side. A ChemClamp (Dagan Corporation, Minneapolis, MN, USA) instrument was utilized to amplify and record the nanopore currents with a low-pass Bessel filter of 3 KHz. Then, a DigiData 1440 converter and a PC running PClamp 10.3 (Axon Instruments, Forest City, CA, USA) were used to gain the data at a sampling rate of 100 KHz. The Origin 8.0 (OriginLab Corporation, Northampton, MA, USA) and a home-designed software http://people.bath.ac.uk/yl505/nanoporeanalysis.html were applied to analyze the data[3]. Nanopore measurements were conducted at 24 ± 2 °C.

Ultraviolet melting curve

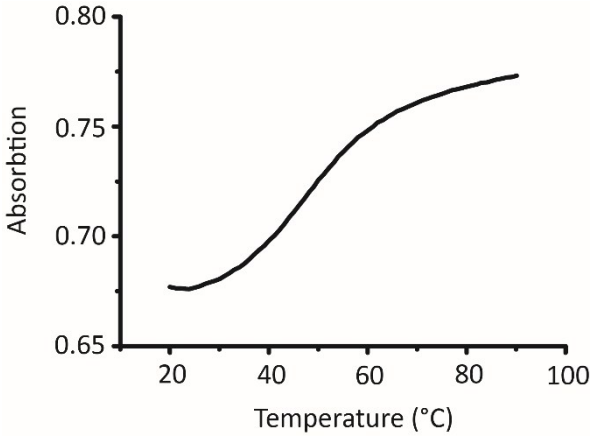

Figure S1. The melting curve of B20-$(CH_2)_3$-B20. The melting temperature (T_m) of B20-$(CH_2)_3$-B20 was 47.02 °C. The ultraviolet melting experiment was measured to investigate the stability of the DNA hairpin structure by monitoring the absorbance at 260 nm using Cary 100 Ultraviolet-Visible spectrometer in 1.0 M KCl buffered with 10 mM Tris and 1 mM EDTA (pH = 8.00). The concentration of B20-$(CH_2)_3$-B20 was fixed in 1 μM.

Analysis of Type I

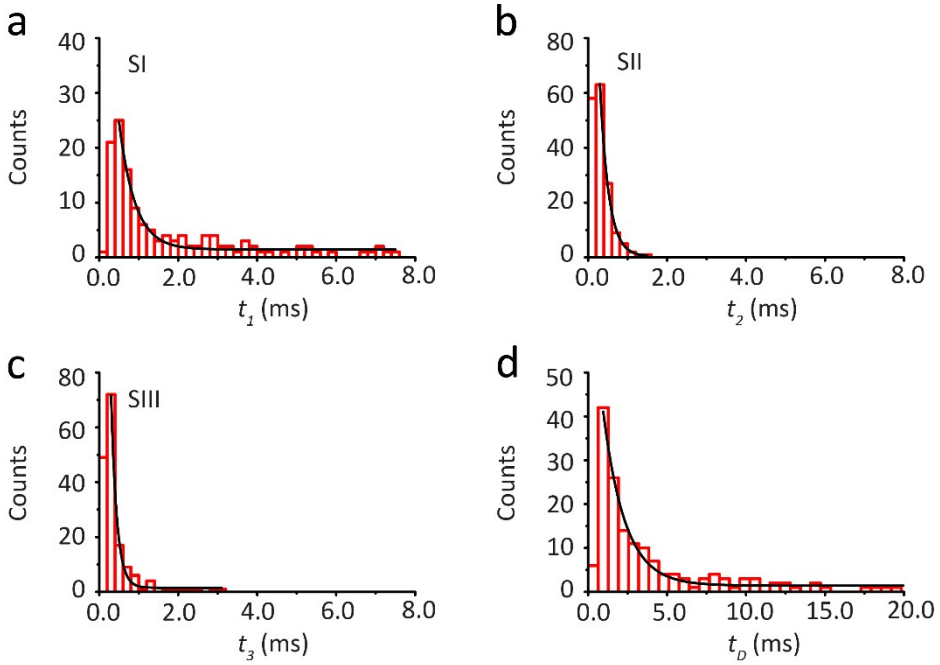

Figure S2. (**a**) The duration histograms of SI for Type I; (**b**) The duration histograms of SII for Type I; (**c**) The duration histograms of SIII for Type I; (**d**) The entire duration time of Type I for B20-$(CH_2)_3$-B20. All of the duration histograms were fitted by an Exponential function. The experiments were measured in 1.0 M KCl buffered with 10 mM Tris and 1 mM EDTA (pH = 8.00) at an applied potential of + 150 mV.

Analysis of Type II

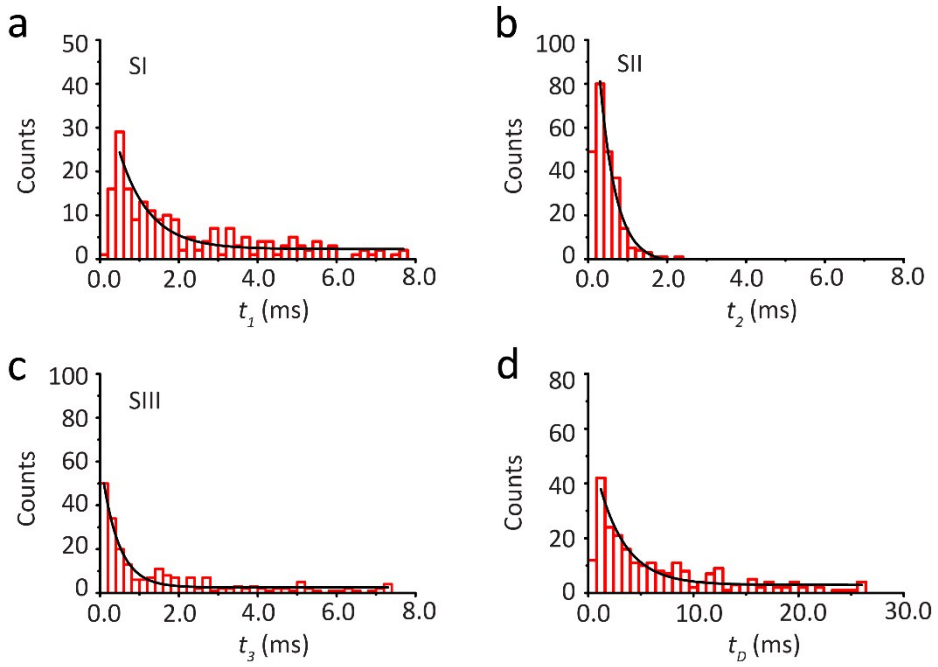

Figure S3. (**a**) The duration histograms of SI for Type II; (**b**) The duration histograms of SII for Type II; (**c**) The duration histograms of SIII for Type II; (**d**) The entire duration time of Type II for B20-$(CH_2)_3$-B20. All of the duration histograms were fitted by an Exponential function. The experiments were measured in 1.0 M KCl buffered with 10 mM Tris and 1 mM EDTA (pH = 8.00) at an applied potential of + 150 mV.

Analysis of Type III

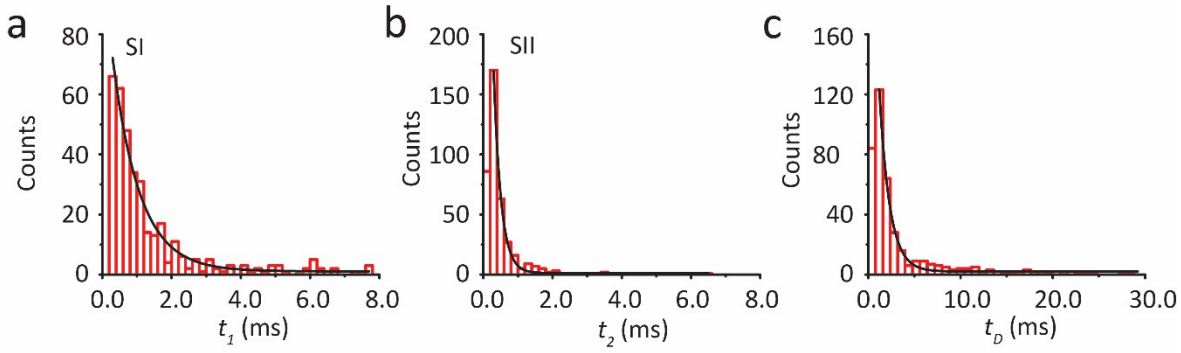

Figure S4. (**a**) The duration histograms of SI for Type III; (**b**) The duration histograms of SII for Type III; (**c**) The entire duration time of Type III for B20-$(CH_2)_3$-B20. All of the duration histograms were fitted by an Exponential function. The experiments were measured in 1.0 M KCl buffered with 10 mM Tris and 1 mM EDTA (pH = 8.00) at an applied potential of + 150 mV.

Original data of current traces

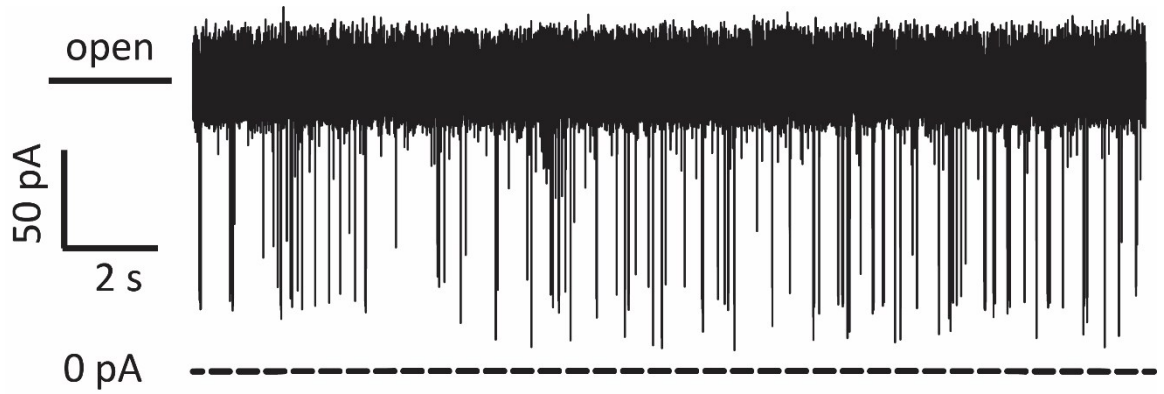

Figure S5. The raw data of current traces for the translocation of B20-$(CH_2)_3$-B20 molecules through the *α*-HL pore in the presence of analytes in the *cis* compartment. The experiments were measured in 1.0 M KCl buffered with 10 mM Tris and 1 mM EDTA (pH = 8.00) at an applied potential of + 150 mV.

References

1. Y. L. Ying, C. Cao, Z. Gu, and Y. T. Long, *Anal. Chem.*, 2014, **86**, 11946-11950.
2. D. W. Li, Y. L. Ying, Y. Liu, S. K. Dey, H. B. Kraatz and Y. T. Long, *Chem. Commun.*, 2012, **48**, 8784–8786.
3. Y. L. Ying, Z. Gu, C. Cao, P. G. He, and Y. T. Long, *Anal. Chem.*, 2015, **87**, 907-913.