Supporting information

Target-Controlled Liposome Amplification for Versatile Nanopore Analysis

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Experimental Section

Chemicals and Materials

1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine(DPhPc), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(biotinyl) (Biotin-DPPE) were purchased from Avanti Polar Lipids (USA). Avidin (≥98.0%), Avidin-FITC and inositol hexaphosphate (IP_6) (≥98.0%) were obtained from Sigma-Aldrich. The thickness of Teflon film (Goodfellow) was 25 μm. Biotinylated short polypeptide hormone thyrotropin-releasing factor (TRF) was purchased from SciLight Biotechnology, Beijing, China.

Preparation of Small Unilamellar Phospholipid Vesicles.

The SUVs were prepared by classical film dispersion method.[1,2] Briefly, 5 mL chloroform solution of DPhPc/cholesterol (40mg/10mg) or DPhPc/Biotin-PE/cholesterol (40mg/4mg/10mg) mixture were added into a clean glass round bottom flask. Then the chloroform was removed by rotary evaporation under reduced pressure at room temperature leaving a thin film of lipid on the bottom of flask. The dried lipid film were then hydrated with 2mL 10mM Tris buffer (pH 7.4) for 2h at 65^{o}C, followed by continuing rotated 2h under room temperature. Sonication under ice bath was conducted on a probe sonicator with a 10s on 30s off procedure for 1h. Final lipid concentration of the as-prepared vesicles is ~20 mg/mL. All suspensions were used immediately after the preparation.

The capsulation of IP_6 is achieved through simply substitute 2mL 10mM Tris buffer (pH 7.4) in hydration for 0.1M IP_6 in same buffer solution. Five cycles of freeze-thawing of prepared SUVs were performed in liquid nitrogen or room temperature to enhance the capsulation ratio of IP_6. Free IP_6 was removed through dialysis against 10mM Tris buffer (pH 7.4).

WT-αHL Monomers and Homoheptamer Pores.

The α-HL M113R RL2 monomers were expressed in Escherichia coli BL-21 (DE3) pLysS and purified by SDS-polyacrylamide gel electrophoresis. The assembly and purification of heptametrical protein pores were carried out as reported previously.[3]

Single-Channel Current Recording.

A bilayer of 2-diphytanoylphosphatidylcholine was formed over a 130~150 μm aperture in a Teflon septum that divided a planar bilayer chamber into cis and trans compartments. The formation of the bilayer was achieved using the Montal−Mueller method.[4] Each solution contained 1 M KCl and was buffered with 10 mM Tris (pH 7.4). α-HL(M113R)$_7$ was added to the cis compartment which was connected to ground. The trans compartment was connected to the head-stage of the amplifier. Then, liposome was mixed and vortexed with certain amount of α-HL(M113R)$_7$ for 15 minutes. Multiple αHL nanochannels would be formed on the lipid bilayer of liposome and induced IP6 release. Then the mixture solution was added into the cis or trans compartment according to the scheme. The final concentration of the α-HL proteins used for the single-channel insertion was 0.05−0.3 ng/mL.

In competitive immunoassay of biotinylated TRF, biotin-TRF was firstly mixed with certain amount of avidin, and kept vortex for 5minutes. Biotin-SUVs aqueous solution was then added in the mixture dropwise and kept vortex for 15minutes before addition in the cis chamber for further single channel current recording. The final concentration of avidin and biotin-SUVS is 1.5 pM and 0.25 μg/mL.

Currents were recorded with a patch clamp amplifier (Axopatch 200B, Molecular Devices, Sunnyvale, CA) at ±30 mV, filtered with a built-in four-pole Bessel filter at 5 kHz, sampled at 100 kHz by a computer equipped with a Digidata 1440 A/D converter (Molecular Devices), and acquired with Clampex 10.3 software (Molecular Devices). Single-channel event amplitude and duration were processed and analyzed by Clampfit 10.5 (Molecular Devices) and origin 8.0 software (Microcal, Northampton, MA). τon represents the statistical value of time between neighbor translocation events which is the inverse of the frequency of events. The mean values of ΔI (ΔI is the current different between the current of partially blocked by IP_6 and that of the unoccupied current) were obtained from ΔI histograms by fitting the distributions to Gaussian functions.

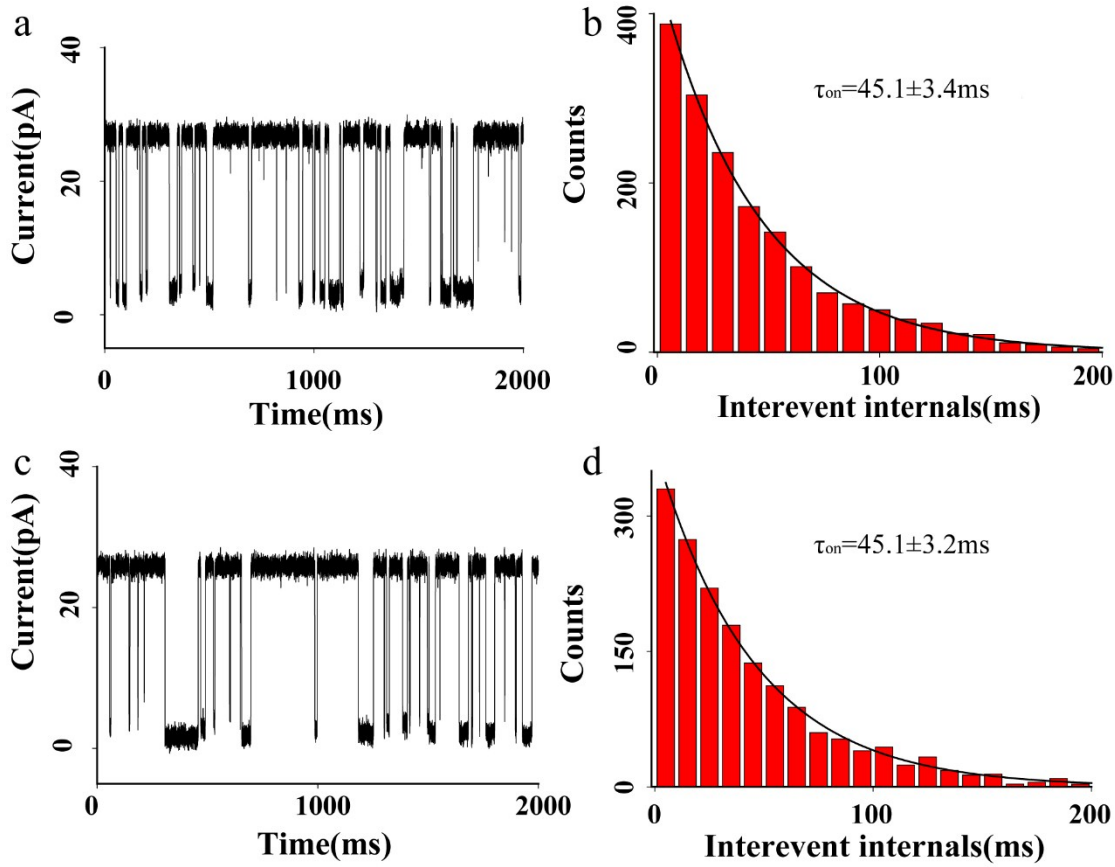

Fig. S1 Biotin modification has less effect on IP_6 release from SUVs. Representative single-channel current traces and histogram of interevent internal (τ_{on}) of IP_6 released from SUVs (DPhPc 40mg / cholesterol 10mg, a and b) and biotin-SUVs (DPhPc 40mg / Biotin-DPPE 4mg / cholesterol 10mg, c and d) passing through a single α-HL pore. The inserted black line in (b) and (d) were exponential decay fitting. The experiments were performed in solution containing 1M KCl buffered with 10 mM Tris-HCl (pH=7.4). The transmembrane potential is +30 mV relative to the cis side of the bilayer. The final concentration of liposome and biotin-liposome is ~0.25 μg/mL for all these expriments. The concentration of α-HL in cis chamber is 0.5 ng/mL.

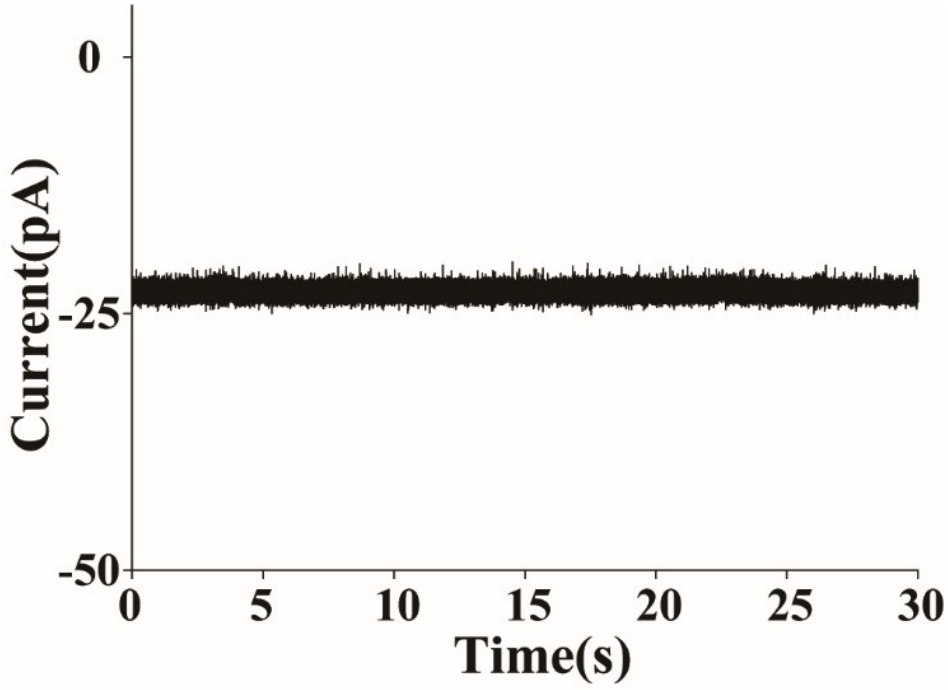

Fig. S2 Representative current trace recorded when IP_6 capsulated SUVs were added in the trans-side of the chamber, and bias potential was -30 mV. No transfer event was detected. The trace was recorded with 1.25 ug/mL SUVs, 1M KCl, and 10mM Tris (pH=7.4).

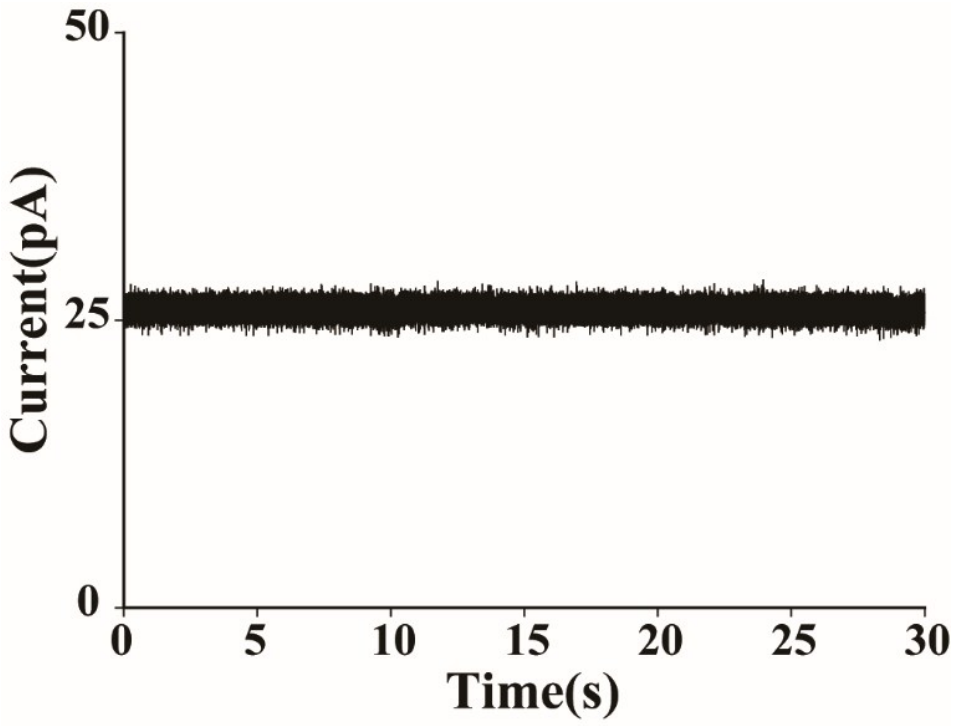

Fig. S3 Representative current trace recorded when 60 μL aqueous solution out of dialysis bag after the last dialysis was added in cis side chamber. The bias potential was +30 mV. No transfer event was detected. The trace was recorded with 1M KCl, and 10mM Tris (pH=7.4).

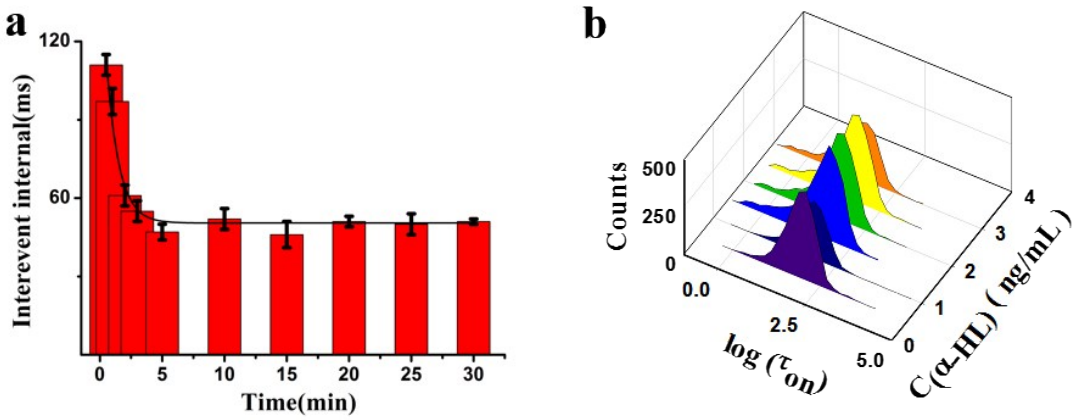

Fig. S4 The release dynamic of IP_6 from biotin-SUVs was represented by plot of τ_{on} change versus time in (a). The effect of concentration of α-HL on τ_{on} is represented in (b). The experiments were performed in solution containing 1M KCl buffered with 10 mM Tris-HCl (pH=7.4). The transmembrane potential is +30 mV relative to the cis side of the bilayer. The final concentration of liposome and biotin-liposome is ~0.25 μg/mL for all these experiments.

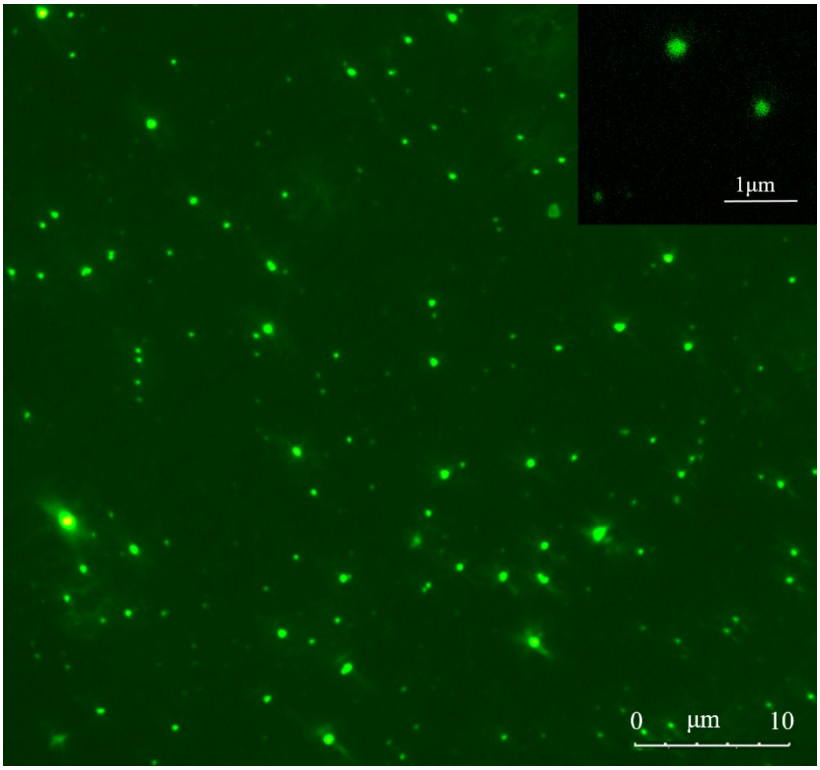

Fig. S5 Representative of fluorescent microscopy images of FITC-avidin tagged biotin-SUVs. Inset is confocal fluorescent image for clarity. Scale bars were labeled on the images.

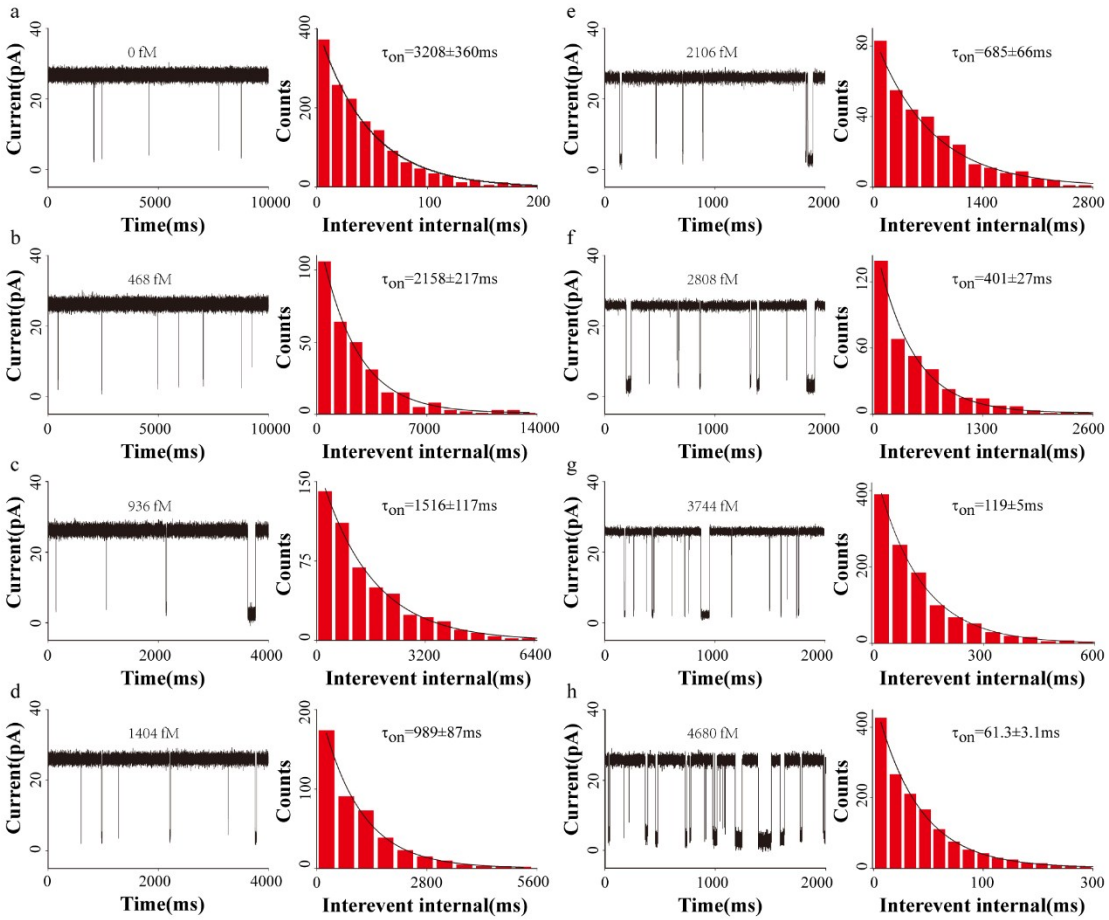

Fig. S6 Representative single-channel current traces (left) and histogram of interevent internal (τ_{on}, right) of IP_6 through a α-HL(M113R)$_7$ nanopore recorded with different biotin-TRF concentration. The experiments were performed at +30 mV in 10 mM Tris solutions containing 1 M KCl (pH 7.4). The concentration of biotin-TRF increased from (a) 0 fM, (b) 468 fM, (c) 936 fM, (d) 1404 fM, (e) 2106 fM, (f) 2808 fM, (g) 3744 fM, (h) 4680 fM.

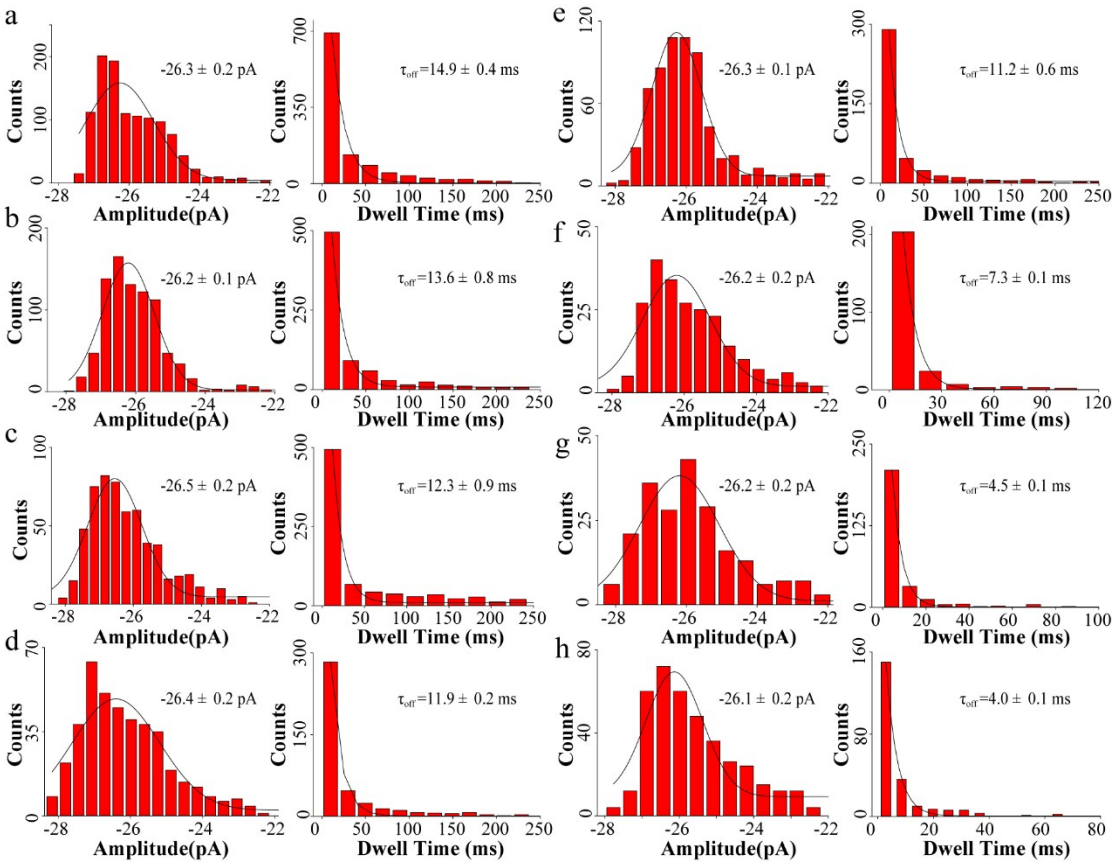

Fig. S7 Representative histogram of current (left) and dwell time (τ_{off}, right) of IP6 through α-HL $(M113R)_7$ nanopore recorded with different avidin concentration. The concentration of avidin increased from (a) 0 fM, (b) 250 fM, (c) 500 fM, (d) 750 fM, (e) 925 f M, (f) 1125 fM, (g) 1375 fM to (h) 1625 fM. The reason for the slight increase of τ_{off} is unknown. It should be related to the increase of the IP_6 concentration. However the change of τ_{off} will not affect the determination of avidin and biotin-TRF (Fig. S8).

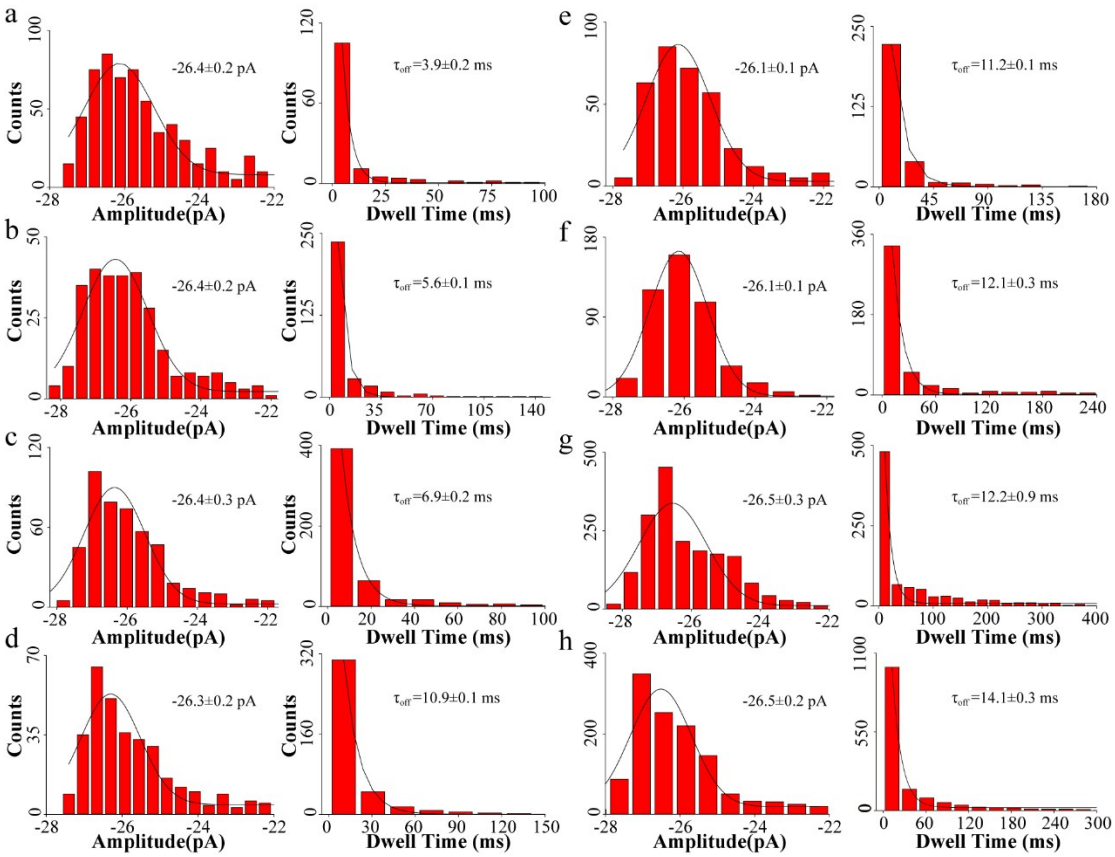

Fig. S8 Representative histogram of current (left) and dwell time (τ_{off}, right) of IP_6 through a α-HL(M113R)$_7$ nanopore recorded with different biotin-TRF concentration. The experiments were performed at +30 mV in 10 mM Tris solutions containing 1 M KCl (pH 7.4). The concentration of biotin-TRF increased from (a) 0 fM, (b) 468 fM, (c) 936 fM, (d) 1404 fM, (e) 2106 fM, (f) 2808 fM, (g) 3744 fM, (h) 4680 fM.

References

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