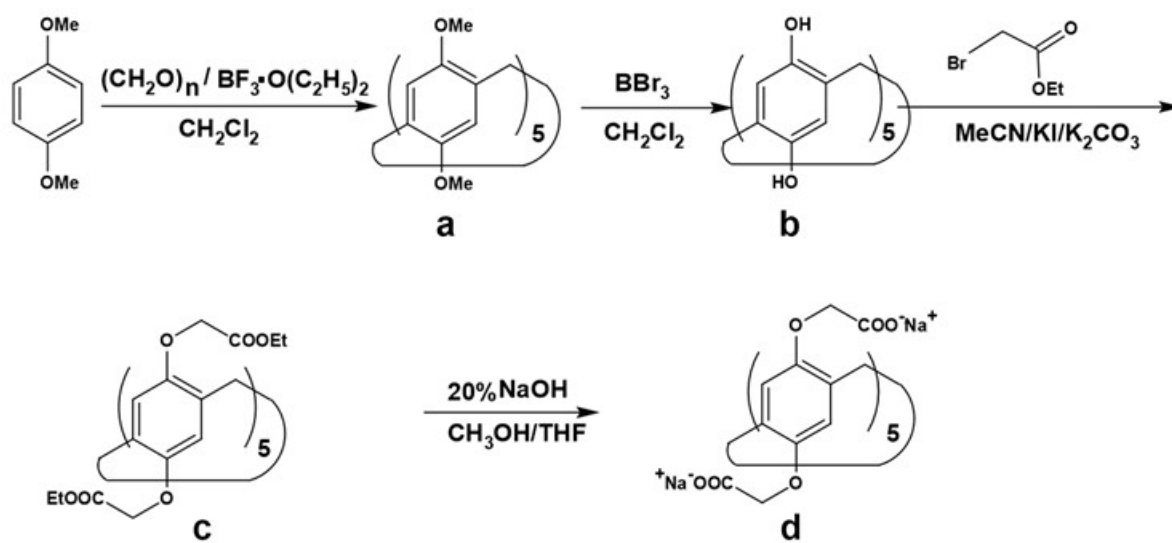


Supporting Information

Highly Sensitive Detection of Paraquat with Pillar[5]arene as an aptamer in α -Hemolysin Nanopore

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Scheme S1: The synthetic route of Carboxylatopillar[5]arene (CP[5]A)



$^1\text{H-NMR}$ spectrum of CP[5]A

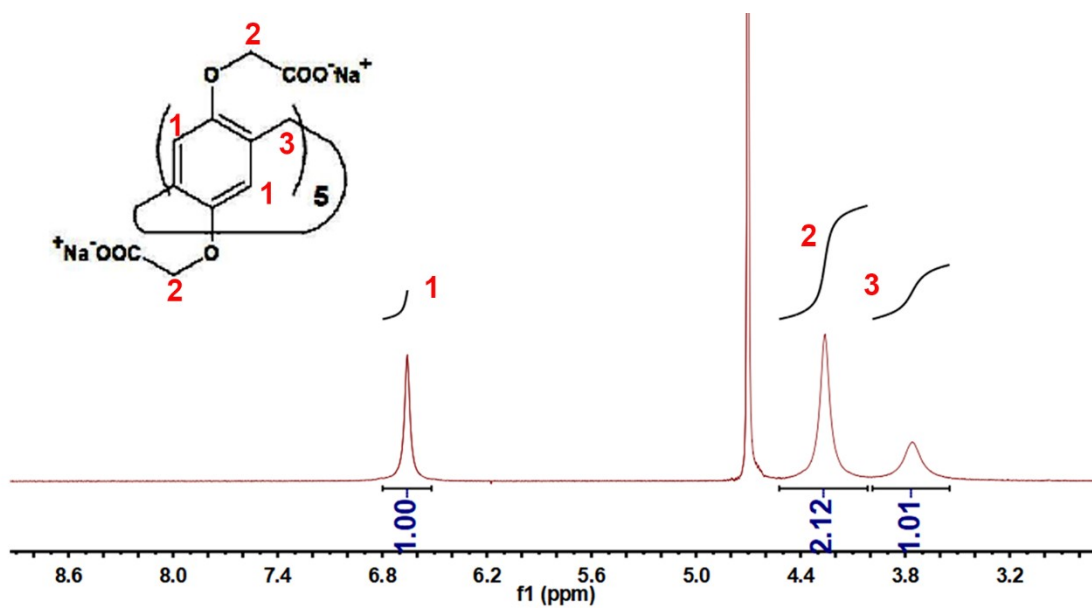


Fig S1. $^1\text{H-NMR}$ spectrum of CP[5]A. (500 MHz, D_2O , δ 6.71 (s, 10H), 4.22 (m, 20H), 3.86 (s, 10H).

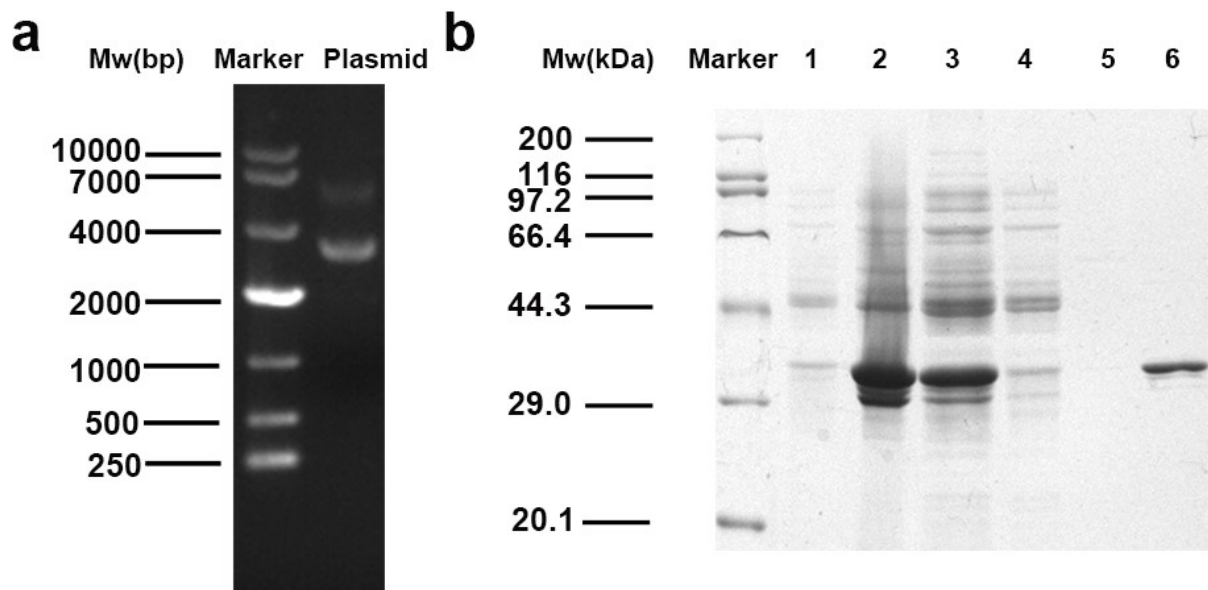


Fig S2. a) Agarose gel electrophoresis of PCR product of (E111R/K147R)₇ αHL (left lane: DNA marker; right lane: the plasmid of (E111R/K147R)₇ αHL; b) the SDS-PAGE analysis of (E111R/K147R)₇ αHL protein monomer, the lanes indicated protein marker, induced without IPTG, induced with IPTG, precipitation, supernatant, eluted solution in 20 mM imidazole and purified (E111R/K147R)₇ αHL protein monomer, respectively.

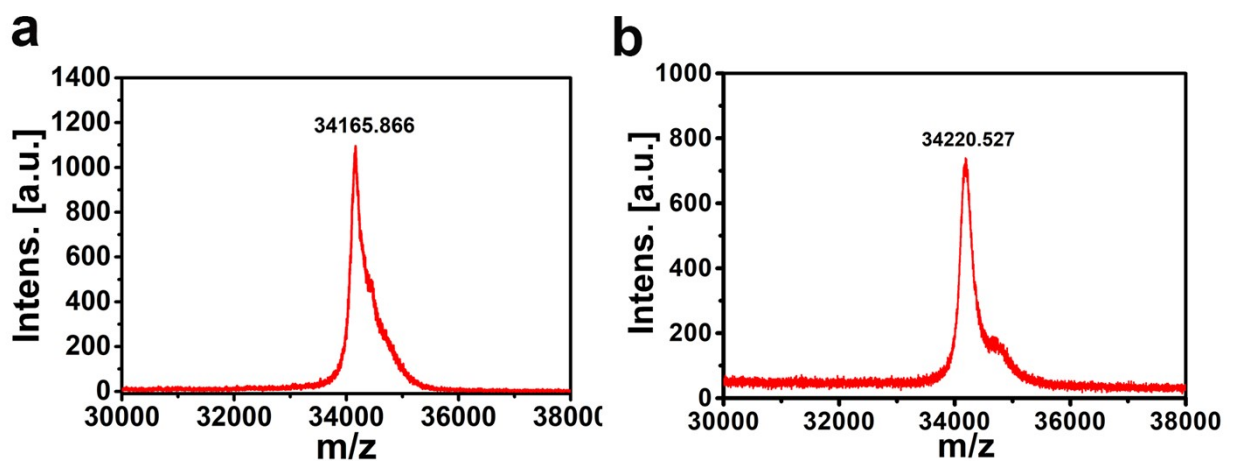


Fig S3. MALDI-TOF-MS spectra of wild-type αHL (a) and mutant (E111R/K147R)₇ αHL protein (b).

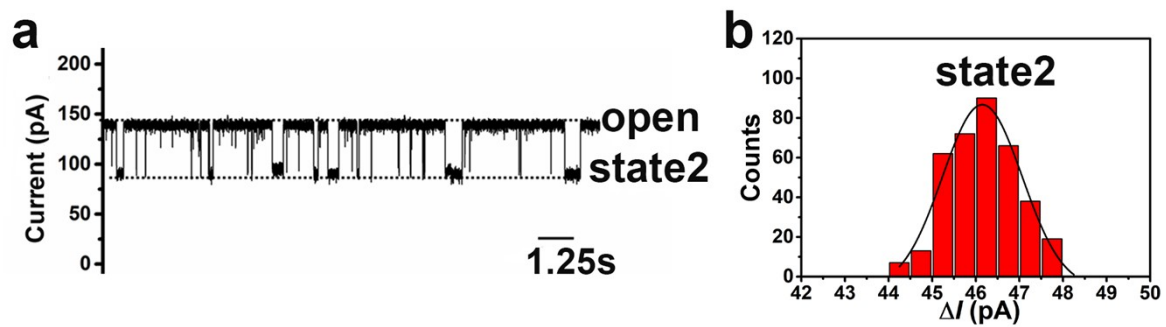


Fig S4. a) The characteristic current of state2 of CP[5]A . b) Event histogram of the current reduction of state2 in 30min single-channel current recordings. Gaussian fitting is performed for binding events of state2.

Concentration (μM)	$1/t_{\text{on}}$ (s^{-1})	k_{on} ($\text{M}^{-1}\text{s}^{-1}$)	k_{off} (s^{-1})	K_{d} (M)
2.5	4.89 ± 0.01	$(1.96 \pm 3.11) \times 10^6$	8.87 ± 0.03	4.53×10^{-5}
5	5.41 ± 0.02	$(1.08 \pm 2.02) \times 10^6$	8.19 ± 0.04	7.58×10^{-5}
10	11.57 ± 0.01	$(1.57 \pm 2.03) \times 10^6$	8.53 ± 0.01	5.43×10^{-5}

Table S1. The constant for CP[5]A with the mutant (E111R/K147R)₇ αHL nanopore.

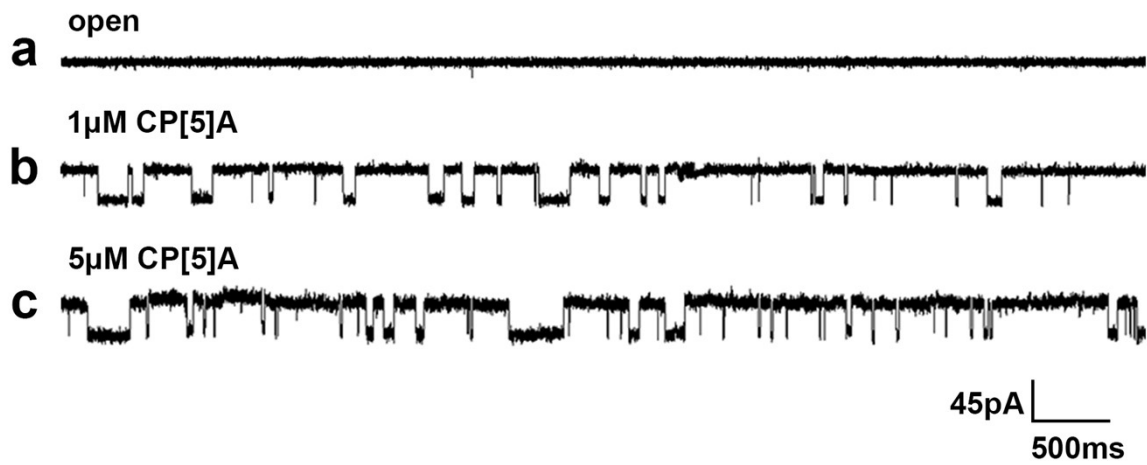


Fig S5. The characteristic current of CP[5]A lodged into wild-type α HL nanopore. a) The open current of wild- type α HL nanopore without CP[5]A. b, c) The current reduction amplitude of CP[5]A under different concentrations (1 μ M and 5 μ M) respectively. Conditions: wild-type α HL nanopore and CP[5]A were added in cis side, +100 mV.

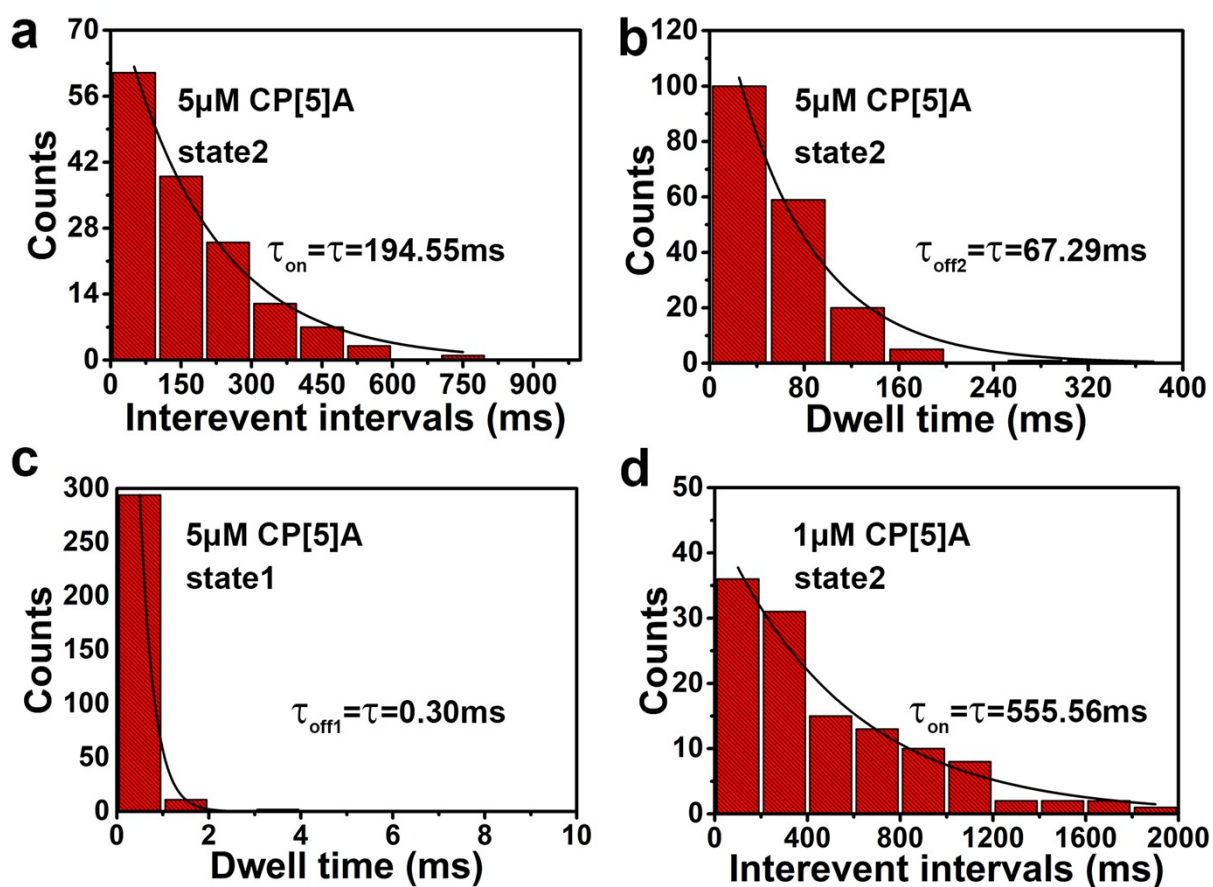


Fig S6. a, b and c) Represented the τ_{on} and the τ_{off} of 5 μ M CP[5]A binding events lodged into wild-type α HL nanopore. d) Represented the τ_{on} of 1 μ M CP[5]A binding events lodged into wild-type α HL nanopore. The statistical data were from a continuous 30min recording. τ_{on} , τ_{off} are from three independent experiments ($n = 3$) with 20 min recording for each condition. Conditions: wild-type α HL nanopore and CP[5]A were added in cis side, +100 mV.

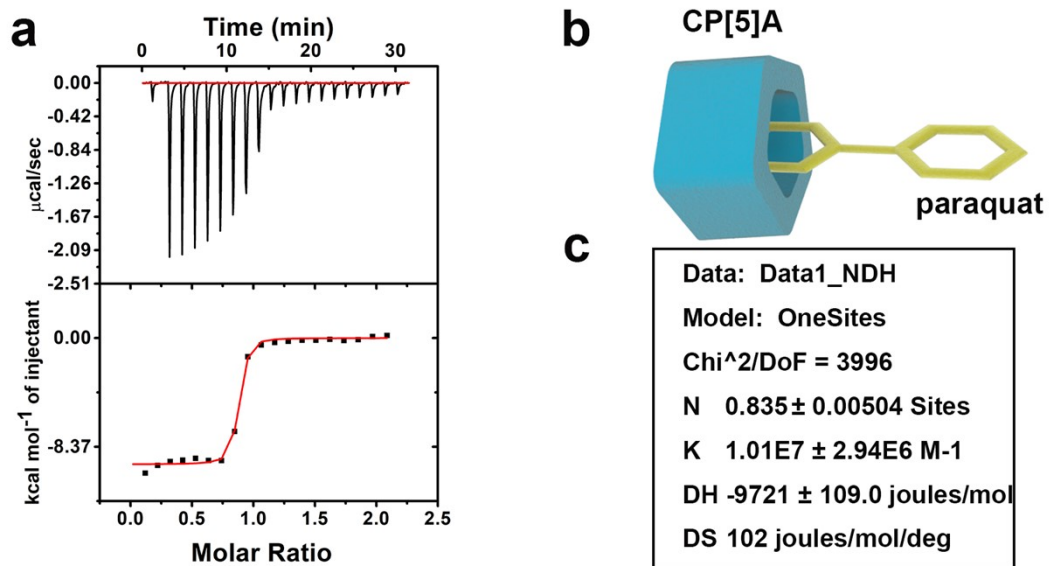


Fig S7. The ITC analysis of CP[5]A and PQ. a) The titration curve of CP[5]A and PQ. b) The representation of CP[5]A and PQ. c) The thermodynamic parameters between CP[5]A and PQ.

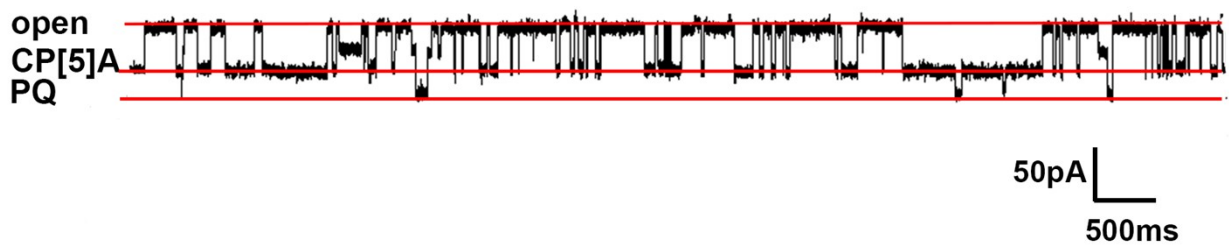


Fig S8. a) The characteristic current of PQ when 2 nM PQ was added in trans and 5 μ M CP[5]A added in cis. Conditions: 1.5 M KCl, +100 mV.