Designing a Polycationic Probe for Simultaneous Enrichment and Detection of MicroRNAs in a Nanopore

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Supplementary Materials

Table S1. Sequences of miRNAs and probe used in this study

Target and probe	Sequence
P_{7b}	N′-YGRKKRRQRRR-AACCACACAA-C′
Let-7b	5′-ugagguaguagguuguugugguu-3′
Let-7c	5′-ugagguaguagguuguaugguu-3′
miR-155	5′-uuaaugcuaaucgugauaggggu-3′
miR-21	5′-uagcuuaucagacugauguuga-3′

Table S2. Properties of the Let-7b•P_{7b} signatures in the absence and in the presence of background RNAs miR-155 and miR-21

Properties	Without background	With background	*t*-test (*p*)
I_R (pA)	74.0±1.5	73.2±1.4	0.51
τ_{off} (ms)	28±4	24±5	0.58
f (s^{-1})	8.0±0.9	7.1±3.7	0.42

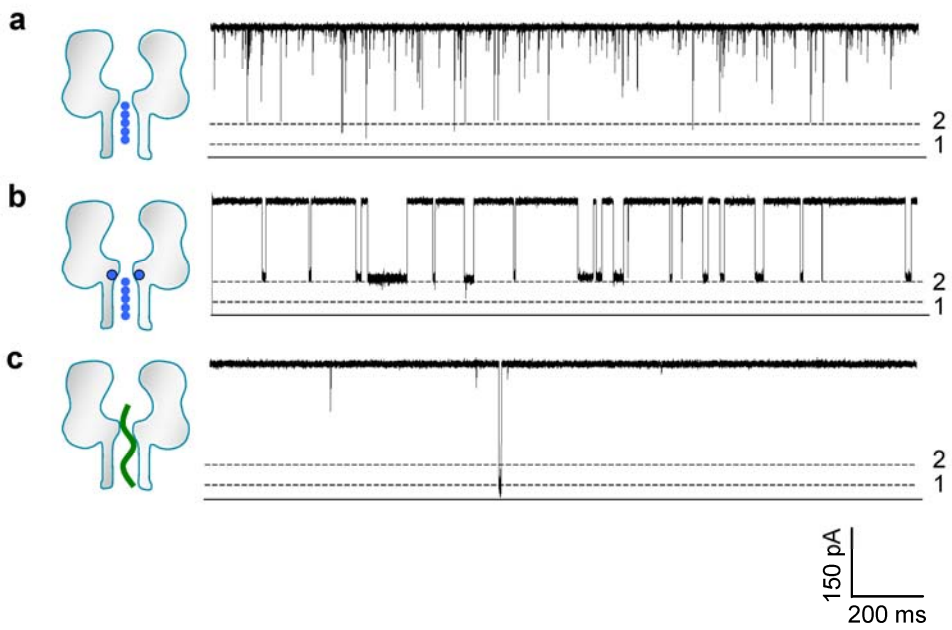

Figure S1. Current traces showing translocation or binding of various polymers in the nanopores. The currents were recorded at +180 mV in 1 M KCl solution buffered with 10 mM Tris (pH7.2). All polymers were added in trans solution. **a,** Translocation of the HIV-TAT peptide in the K131D pore; **b,** Encapsulation of the HIV-TAT peptide in the M113R pore. Blue dots marked in the nanopore model represents the arginine ring (R113) constructed in the constrictive region lining the pore lumen; and **c,** Translocation of PNA alone in the K131D pore. PNA rarely translocate or binds the pore from trans opening.

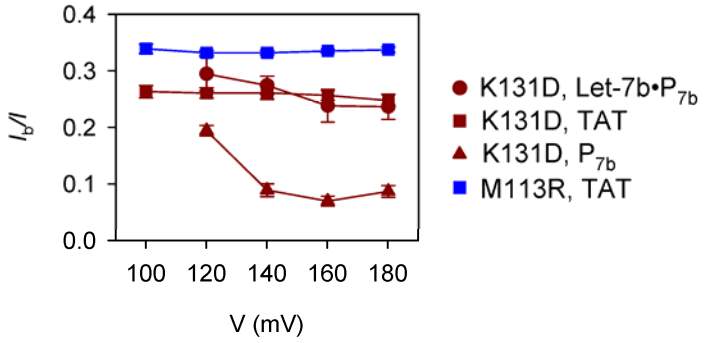

Figure S2. Voltage-dependent relative block currents for various polymers in the nnopores. The currents were recorded at +180 mV in 1 M KCl solution buffered with 10 mM Tris (pH7.2). All polymers were added in trans solution. Red circle: Let-7b•P_{7b} complex in the K131D pore; Red square: TAT peptide in the K131D pore; Red triangle, P_{7b} probe in the M113R pore; and blue square: TAT peptide in the M113R pore.

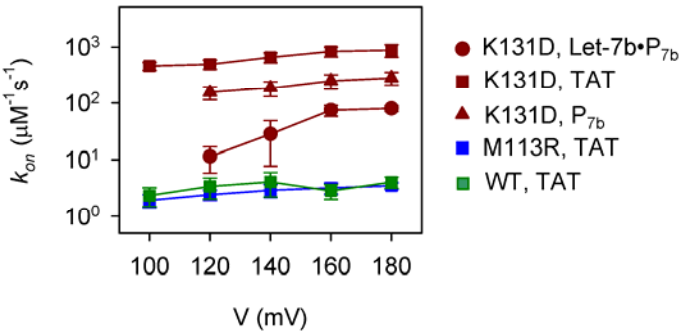

Figure S3. Voltage-dependent capture rates for trapping various polymers in the nanopores. The currents were recorded at +180 mV in 1 M KCl solution buffered with 10 mM Tris (pH7.2). All polymers were added in trans solution. Red circle: Let-7b•P_{7b} complex in the K131D pore; Red square: TAT peptide in the K131D pore; Red triangle, P_{7b} probe in the M113R pore; and blue square: TAT peptide in the M113R pore.

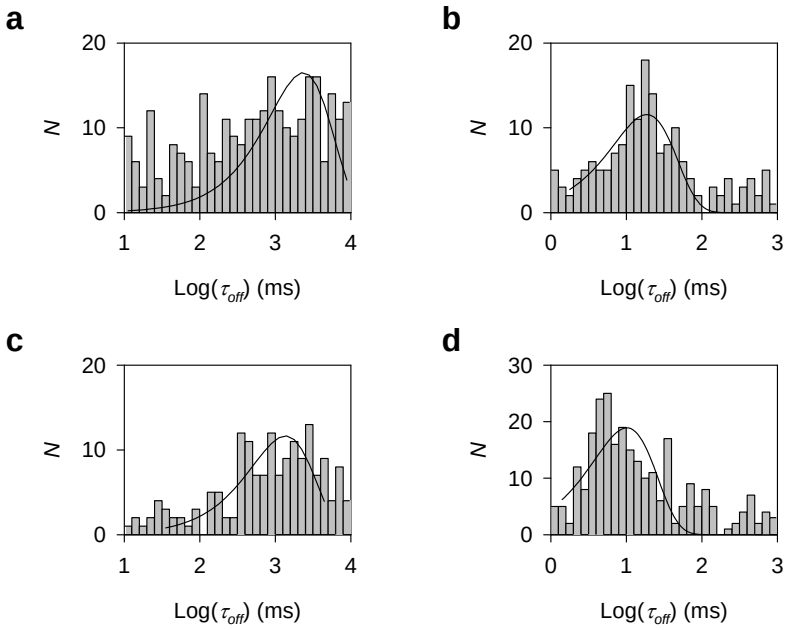

Figure S4. Duration histograms of signature blocks for fully-matched Let-7b•P_{7b} and one-mismatched Let-7c•P_{7b} at different voltages. **a** and **b.** were for (a) Let-7b•P_{7b}, τ_{off}=2.3±0.5 s, and (b) Let-7c•P_{7b}, τ_{off}=19±0.7 ms at +140 mV; **c** and **d.** were for (c) Let-7b•P_{7b}, τ_{off}=1.7±0.6 s, and (d) Let-7c•P_{7b}, τ_{off}=11±3 ms at +180 mV. Solutions contained 3 M/0.5 M cis/trans KCl and 10 mM Tris (pH7.2).

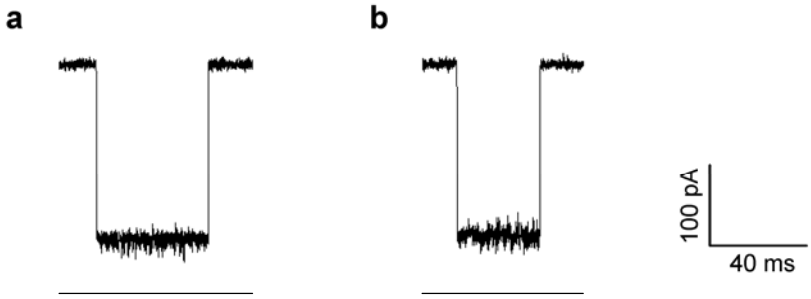

Figure S5. Typical current blocks produced by the Let-7b•P_{7b} complex trapped in the K131D pore (**a**) and by the spontaneous gating of the K131D pore (**b**). The two types of blocks are distinguishable in the current pattern. The Let-7b•P_{7b} signature is characterized by fast downward flicking, while the pore gating block does not feature it.

S1. Computational structure model of the microRNA•probe complex

The sequences of miRNA Let-7b and its probe P_{7b} are shown in Table S1. The Let-7b•P_{7b} complex consists of three domains: the 11-amino acid peptide domain including 8 cationic residues, the 10-basepair miRNA•PNA duplex, and the 12-nucleotide single-stranded miRNA (ss-miRNA) domain. The conformation for each domain was built separately. The three domains were finally assembled into a Let-7b•P_{7b} structure. The conformation of the miRNA•PNA duplex was created using an experimentally determined structure (PDB id: 176D) as the template. For the peptide and ss-miRNA domains, both oligomers are flexible and can adopt a large number of conformations. We used the CABS and PULCHRA models[1-3] to generate peptide conformations, and the Vfold model[4] to construct ss-miRNA conformations. The CABS model gives a reduced representation for protein conformations[1,2] and the PULCHRA model provides a computationally efficient method for constructing the all-atom protein structure from the backbone Cα atoms[3]. Vfold is a free energy-based model for RNA 3D structure prediction[4]. The model has been successful in predicting a broad range of RNA structures and functions such as miRNA-target interactions[5]. The structure of the Let-7b•P_{7b} complex was constructed as the assembly of the conformations of the three domains. Specifically, the Cα atoms between the peptide C-terminal and the PNA N-terminal were joined, and the 5'-end phosphate of the miRNA•PNA duplex was linked with the 3'-end C4' atom of ss-miRNA. Using these models, we have randomly generated 20 peptide conformations and 100 ss-miRNA conformations, then constructed an ensemble of a total of 2000 conformations for the probe and ss-miRNA complex. By calculating the electrostatic free energies for different conformations, we computed the Let-7b•P_{7b} complex structure that has the lowest electrostatic free energy (see Fig. 3a).

Reference List

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