SUPPORTING INFORMATION

Nucleobase Recognition By Truncated α-Hemolysin Pores

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Table S1. Sequences of the oligonucleotides used in this study. B represents the 3' biotin-TEG tag and linker (**Figure S1**).

Oligonucleotide name	Oligonucleotide sequence (5'→3')
poly(dC)$_{40}$	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC-B
poly(dC)^{A7}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCC-B
poly(dC)^{A8}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCC-B
poly(dC)^{A9}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCC-B
poly(dC)^{A10}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCC-B
poly(dC)^{A11}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A12}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A13}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A14}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A15}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A16}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A17}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A18}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A19}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{A20}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACCCCCCCC-B
poly(dC)^{T14}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCTCCCCCCCC-B
poly(dC)^{G14}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCGCCCCCCCC-B
poly(dC)^{T16}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCTCCCCCCCC-B
poly(dC)^{G16}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCGCCCCCCCC-B
poly(dC)^{T18}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCTCCCCCCCC-B
poly(dC)^{G18}	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCGCCCCCCCC-B

Table S2. Residual currents ($I_{RES\%}$) for poly(dC) and oligonucleotides that contain a single adenine nucleobase for the αHL TBMΔ2 and TBMΔ4 proteins. The position of a single adenine (A_X) nucleobase (nucleobase position between 7-20) is numbered relative to the 3' biotin tag. The $I_{RES\%}$ values are the mean values (± sd) from n ≥ 3 experiments. $\Delta I_{RES\%}$ is defined as the difference in residual current between an A_x oligonucleotide and poly(dC) ($I_{RES\%}^{Ax\ oligo} - I_{RES\%}^{pC}$). The mean of the $\Delta I_{RES\%}$ values from individual experiments and the associated error (± sd) are given. The data were collected by using the voltage protocol described in Experimental Methods. Briefly, +160 mV was applied for 900 ms to drive the negatively charged DNA into the pore, followed by -140 mV for 50 ms, to eject the immobilized DNA and a final step to 0 mV for 50 ms.

Position of adenine	TBMΔ2			Position of adenine	TBMΔ4		
	I_{RES}^{Ax} (%)	I_{RES}^{pC} (%)	ΔI_{RES} (%)		I_{RES}^{Ax} (%)	I_{RES}^{pC} (%)	ΔI_{RES} (%)
7	35.2 ± 0.2	35.2 ± 0.2	0.0 ± 0.0	7	31.3 ± 0.1	31.3 ± 0.1	0.0 ± 0.0
8	35.4 ± 0.4	35.2 ± 0.3	+0.2 ± 0.1	8	30.9 ± 1.5	30.8 ± 1.4	+0.1 ± 0.1
9	34.9 ± 0.6	35.3 ± 0.6	−0.5 ± 0.0	9	30.2 ± 1.0	30.8 ± 1.0	−0.6 ± 0.0
10	34.2 ± 0.4	35.0 ± 0.5	−0.8 ± 0.1	10	29.3 ± 2.7	29.8 ± 2.9	−0.4 ± 0.2
11	34.9 ± 0.4	35.2 ± 0.4	−0.3 ± 0.1	11	29.3 ± 2.2	29.1 ± 2.2	+0.2 ± 0.1
12	35.1 ± 0.5	35.1 ± 0.5	0.0 ± 0.0	12	30.5 ± 3.0	30.0 ± 2.9	+0.5 ± 0.1
13	35.2 ± 1.1	34.6 ± 1.2	+0.5 ± 0.1	13	31.5 ± 2.3	30.8 ± 2.3	+0.7 ± 0.0
14	35.9 ± 0.4	35.3 ± 0.4	+0.6 ± 0.0	14	30.7 ± 1.6	30.7 ± 1.7	−0.1 ± 0.1
15	35.5 ± 0.7	35.5 ± 0.7	+0.0 ± 0.0	15	29.2 ± 1.6	30.5 ± 1.6	−1.4 ± 0.0
16	33.5 ± 0.3	34.7 ± 0.4	−1.3 ± 0.1	16	29.2 ± 1.3	30.5 ± 1.3	−1.3 ± 0.0
17	33.9 ± 0.3	35.2 ± 0.3	−1.4 ± 0.0	17	30.7 ± 1.7	31.3 ± 1.8	−0.6 ± 0.1
18	34.0 ± 0.4	34.5 ± 0.4	−0.5 ± 0.1	18	31.3 ± 1.5	31.3 ± 1.5	0.0 ± 0.0
19	34.1 ± 0.9	34.1 ± 0.9	0.0 ± 0.0	19	31.9 ± 1.7	31.9 ± 1.7	0.0 ± 0.0
20	34.4 ± 1.5	34.4 ± 1.5	0.0 ± 0.0	20	31.4 ± 0.1	31.4 ± 0.1	−0.1 ± 0.1

Table S3. Residual currents ($I_{RES\%}$) for poly(dC) and oligonucleotides that contain a single adenine nucleobase for the TBMΔ6 and TBMΔ6/M113G pores. The position of a single adenine (A_X) nucleobase (nucleobase position between 7-20) is numbered relative to the 3' biotin tag. The $I_{RES\%}$ values are the mean values (± sd) from n ≥ 3 experiments. $\Delta I_{RES\%}$ is defined as the difference in residual current between an A_x oligonucleotide and poly(dC) ($I_{RES\%}{}^{Ax\ oligo}$ - $I_{RES\%}{}^{pC}$). The mean of the $\Delta I_{RES\%}$ values from individual experiments and the associated error (± sd) are given. The data were collected by using the voltage protocol described in Experimental Methods. Briefly, +160 mV was applied for 900 ms to drive the negatively charged DNA into the pore, followed by -140 mV for 50 ms, to eject the immobilized DNA and a final step to 0 mV for 50 ms.

Position of adenine	TBMΔ6			Position of adenine	TBMΔ6/M113G		
	$I_{RES}{}^{Ax}$ (%)	$I_{RES}{}^{pC}$ (%)	ΔI_{RES} (%)		$I_{RES}{}^{Ax}$ (%)	$I_{RES}{}^{pC}$ (%)	ΔI_{RES} (%)
7	21.3 ± 1.4	21.3 ± 1.4	0.0 ± 0.0	7	23.5 ± 0.5	23.5 ± 0.5	0.0 ± 0.0
8	21.3 ± 1.8	21.3 ± 1.8	0.0 ± 0.0	8	25.5 ± 2.0	25.5 ± 2.0	0.0 ± 0.0
9	22.2 ± 1.0	22.6 ± 1.1	−0.4 ± 0.1	9	24.6 ± 1.3	24.6 ± 1.3	0.0 ± 0.0
10	21.6 ± 0.3	21.6 ± 0.3	0.0 ± 0.0	10	24.1 ± 0.2	23.7 ± 0.3	+0.4 ± 0.1
11	22.8 ± 1.3	23.7 ± 1.2	+0.9 ± 0.1	11	26.0 ± 1.4	24.6 ± 1.3	+1.4 ± 0.1
12	23.0 ± 0.5	21.9 ± 0.6	+1.1 ± 0.0	12	25.0 ± 0.2	23.7 ± 0.2	+1.3 ± 0.1
13	22.1 ± 0.6	22.1 ± 0.6	0.0 ± 0.0	13	25.9 ± 1.8	25.9 ± 1.8	0.0 ± 0.0
14	20.8 ± 0.2	22.1 ± 0.2	−1.3 ± 0.0	14	23.1 ± 1.0	24.4 ± 1.0	−1.3 ± 0.0
15	21.0 ± 0.1	21.8 ± 0.1	−0.9 ± 0.1	15	22.8 ± 0.2	23.7 ± 0.2	−0.9 ± 0.1
16	21.5 ± 0.1	21.5 ± 0.1	0.0 ± 0.0	16	25.5 ± 1.9	25.8 ± 1.9	−0.3 ± 0.1
17	23.0 ± 1.4	23.0 ± 1.4	0.0 ± 0.0	17	25.2 ± 1.8	25.2 ± 1.8	0.0 ± 0.0
18	22.2 ± 0.1	22.2 ± 0.1	0.0 ± 0.0	18	23.5 ± 0.4	23.5 ± 0.4	0.0 ± 0.0

Table S4. Residual currents ($I_{RES\%}$) for poly(dC) and oligonucleotides that contain a single substituted base at position 18 (αHL NN), 16 (TBMΔ2 and TBMΔ4) or 13 (TBMΔ6 and TBMΔ6/M113G) blocking pores. $I_{RES\%}$, $\Delta I_{RES\%}^{OVERALL}$ and δ values are shown for n ≥ 3 experiments. $\Delta I_{RES\%}^{OVERALL}$ is defined as the difference in residual current between the two most widely dispersed current peaks. The δ value is the product of the successive differences between the peaks. If any two peaks overlap, then the δ value is equal to zero. Mean values (± s.d.) are given.

	Residual Current (%)				Residual Current Difference (%)	
NN	C_{18}	T_{18}	A_{18}	G_{18}	$\Delta I_{RES}^{OVERALL}$ (%)	δ
	36.9 ± 0.5	36.4 ± 0.5	36.0 ± 0.5	35.7 ± 0.5	1.2 ± 0.0	0.07 ± 0.00
Δ2	C_{16}	T_{16}	A_{16}	G_{16}	$\Delta I_{RES}^{OVERALL}$ (%)	δ
	35.7 ± 0.1	34.2 ± 0.1	34.2 ± 0.1	35.0 ± 0.1	1.5 ± 0.1	0.00 ± 0.00
Δ4	C_{16}	T_{16}	A_{16}	G_{16}	$\Delta I_{RES}^{OVERALL}$ (%)	δ
	31.4 ± 2.8	30.0 ± 2.7	30.0 ± 2.7	30.0 ± 2.7	1.4 ± 0.1	0.00 ± 0.00
Δ6	C_{13}	T_{13}	A_{13}	G_{13}	$\Delta I_{RES}^{OVERALL}$ (%)	δ
	22.9 ± 1.4	20.9 ± 1.3	21.6 ± 1.3	22.2 ± 1.3	2.0 ± 0.2	0.29 ± 0.06
Δ6/M113G	C_{13}	T_{13}	A_{13}	G_{13}	$\Delta I_{RES}^{OVERALL}$ (%)	δ
	24.2 ± 0.3	22.4 ± 0.3	22.8 ± 0.3	23.7 ± 0.3	1.8 ± 0.1	0.19 ± 0.03

Table S5. Kinetic parameters for the interaction of $am_7\beta CD$ with the homoheptameric TBMΔ6 and TBMΔ6/M113N mutant pores. $k_{on} = 1/\tau_{on}[am_7\beta CD]$, where τ_{on} is the mean inter-event interval. $k_{off} = 1/\tau_{off}$, where τ_{off} is the mean dwell time of $am_7\beta CD$ in the pore. $K_D = k_{off}/k_{on}$. Values are shown for n ≥ 3 experiments. Mean values (± s.d.) are given.

		at +120 mV, 40 µM $am_7\beta CD$ (added to *trans*)	
	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (mM)
TBMΔ6	33000 ±1000	4600 ±300	143 ±8
TBMΔ6/M113N	21200 ±1000	2200 ±200	104 ±4

Table S6. Kinetic parameters for dNMP and rNMP identification. Comparison of the kinetic parameters of mononucleotide binding to $am_7\beta CD$ within the TBMΔ6, TBMΔ6/M113F and TBMΔ6/M113N αHL pores, at pH 6.0 (+120 mV). Mean τ_{off}, k_{off}, $I_{RES\%}$, $\Delta I_{RES\%}{}^{OVERALL}$ and δ values are shown for n ≥ 3 experiments. Values of k_{off} were determined by using $k_{off} = 1/\tau_{off}$, where τ_{off} is the mean dwell time of each NMP in the pore. I_O and $I_{RES\%}$ values are mean values (±s.d.) taken from Gaussian fits to event histograms. $I_{RES\%} = (I_{RES}/I_O) \times 100$. $\Delta I_{RES\%}{}^{OVERALL}$ is defined as the difference in residual current between the two most widely dispersed current peaks. The δ value is the product of the successive differences between the peaks. If any two peaks overlap, then the δ value is equal to zero. Mean values (± s.d.) are given.

	40 μM $am_7\beta CD$ (*trans*) at +120 mV (n = ≥3)								
	TBMΔ6			TBMΔ6/M113F			TBMΔ6/M113N		
Mononucleotide	τ_{off} (ms)	k_{off} (s^{-1})	I_{RES} (%)	τ_{off} (ms)	k_{off} (s^{-1})	I_{RES} (%)	τ_{off} (ms)	k_{off} (s^{-1})	I_{RES} (%)
dGMP	15.0 ±1.0	69.0 ±1.0	44.2 ±0.3	18.0 ±1.0	54.0 ±2.0	19.9 ±2.0	8.0 ±1.0	121.0 ±1.0	22.8 ±0.4
dAMP	14.0 ±1.0	73.0 ±2.0	45.3 ±0.3	17.0 ±1.0	60.0 ±2.0	22.5 ±1.8	6.0 ±2.0	164.0 ±2.0	25.2 ±0.8
dCMP	10.0 ± 2.0	98.0 ±1.0	46.8 ±0.3	15.0 ±1.0	65.0 ±1.0	25.9 ±1.8	14.0 ±1.0	72.0 ±2.0	25.6 ±0.5
dTMP	11.0 ±1.0	90.0 ±2.0	46.3 ±0.4	21.0 ±1.0	47.0 ±1.0	21.5 ±2.4	17.0 ±2.0	58.0 ±2.0	23.1 ±0.4
$\Delta I_{RES}{}^{OVERALL}$(%)	2.6 ±0.4			6.0 ±1.4			2.8 ±0.6		
δ	0.5 ±0.1			5.4 ±0.4			0.3 ±0.1		
rGMP	16.0 ±1.0	61.0 ±1.0	41.6 ±0.6	7.0 ±2.0	152.0 ±1.0	14.9 ±1.2	15.0 ±3.0	68.0 ±2.0	30.4 ±0.3
rAMP	14.0 ±1.0	70.0 ±2.0	43.2 ±0.4	14.0 ±1.0	70.0 ±2.0	17.1 ±1.5	9.0 ±2.0	108.7 ±2.0	29.5 ±0.3
rCMP	19.0 ±1.0	52.0 ±2.0	43.9 ±0.7	9.0 ±1.0	111.0 ±1.0	17.9 ±1.2	18.0 ±1.0	55.2 ±2.0	28.2 ±0.4
rUMP	12.0 ±1.0	85.0 ±1.0	44.1 ±0.6	6.0 ±1.0	167.0 ±2.0	20.4 ±1.4	10.0 ±2.0	96.2 ±3.0	27.9 ±0.6
$\Delta I_{RES}{}^{OVERALL}$(%)	2.3 ±0.6			5.5 ±1.2			2.5 ±0.3		
δ	0.2 ±0.1			4.4 ±0.6			0.4 ±0.1		

Table S7. Voltage dependences of the residual currents ($I_{RES\%}$), dwell times (τ_{off}) and dissociation rate constants ($k_{off} = 1/\tau_{off}$) for dNMP bound to the TBMΔ6/M113F•am$_7$βCD αHL pore. Mean τ_{off}, k_{off}, $I_{RES\%}$ are shown for n ≥ 3 experiments. Values of k_{off} were determined by using $k_{off} = 1/\tau_{off}$, where τ_{off} is the mean dwell time of each NMP in the pore. I_O and $I_{RES\%}$ values are mean values (±s.d.) taken from Gaussian fits to event histograms. $I_{RES\%} = (I_{RES}/I_O) \times 100$.

Nucleotide	Potential	τ_{off}	$k_{off} = 1/\tau_{off}$	I_{RES}	Nucleotide	Potential	τ_{off}	$k_{off} = 1/\tau_{off}$	I_{RES}
	mV	ms	s^{-1}	(%)		mV	ms	s^{-1}	(%)
dGMP	100	16.0 ±1.0	63.0 ±1.0	15.3 ±1.2	dCMP	100	13.0 ±1.0	77.0 ±2.0	23.2 ±1.3
	120	18.0 ± 1.0	54.0 ±2.0	19.9 ±2.0		120	15.0 ±2.0	65.0 ±1.0	25.9 ±1.8
	140	23.0 ±2.0	43.0 ±1.0	21.6 ±1.6		140	17.0 ±1.0	59.0 ±1.0	27.4 ±1.4
	160	27.0 ±2.0	37.0 ±1.0	24.6 ±1.6		160	18.0 ±1.0	56.0 ±1.0	29.1 ±1.6
	180	32.0 ±2.0	31.0 ±1.0	27.5 ±2.2		180	21.0 ±2.0	48.0 ±1.0	31.3 ±1.5
	200	30.0 ±2.0	33.0 ±1.0	31.1 ±1.4		200	24.0 ±2.0	42.0 ±2.0	33.1 ±1.4
dAMP	100	16.0 ±1.0	63.0 ±1.0	20.4 ±0.8	dTMP	100	19.0 ±1.0	53.0 ±1.0	19.8 ±1.6
	120	17.0 ±1.0	60.0 ±2.0	22.5 ±1.8		120	21.0 ±1.0	47.0 ±1.0	21.5 ±2.4
	140	21.0 ±2.0	48.0 ±1.0	29.7 ±1.6		140	27.0 ±1.0	37.0 ±2.0	24.6 ±1.6
	160	29.0 ±2.0	34.0 ±2.0	32.4 ±1.6		160	31.0 ±1.0	32.0 ±1.0	27.3 ±1.3
	180	28.0 ±2.0	36.0 ±2.0	34.1 ±0.8		180	29.0 ±1.0	34.0 ±1.0	29.8 ±1.4
	200	33.0 ±1.0	30.0 ±1.0	35.8 ±1.9		200	32.0 ±1.0	31.0 ±2.0	32.3 ±1.8

Table S8. Voltage dependences of the residual currents ($I_{RES\%}$), dwell times (τ_{off}) and dissociation rate constants ($k_{off} = 1/\tau_{off}$) for rNMP bound to the TBMΔ6/M113F•$am_7\beta$CD pore. Mean τ_{off}, k_{off}, $I_{RES\%}$ are shown for n ≥ 3 experiments. Values of k_{off} were determined by using $k_{off} = 1/\tau_{off}$, where τ_{off} is the mean dwell time of each NMP in the pore. I_O and $I_{RES\%}$ values are mean values (±s.d.) taken from Gaussian fits to event histograms. $I_{RES\%} = (I_{RES}/I_O) \times 100$.

Nucleotide	Potential	τ_{off}	$k_{off}= 1/\tau_{off}$	I_{RES}	Nucleotide	Potential	τ_{off}	$k_{off}= 1/\tau_{off}$	I_{RES}
	mV	ms	s^{-1}	(%)		mV	ms	s^{-1}	(%)
rGMP	100	6.0 ±1.0	167.0 ±2.0	9.3 ±1.2	rCMP	100	8.0 ±1.0	125.0 ±1.0	15.4 ±1.2
	120	7.0 ±2.0	152.0 ±1.0	14.9 ±1.2		120	9.0 ±1.0	111.0 ±1.0	17.9 ±1.2
	140	12.0 ±1.0	83.0 ±1.0	19.3 ±2.0		140	13.0 ±1.0	77.0 ±2.0	23.3 ±1.4
	160	13.0 ±2.0	77.0 ±1.0	22.7 ±2.0		160	17.0 ±2.0	59.0 ±1.0	27.6 ±1.6
	180	16.0 ±2.0	63.0 ±2.0	29.2 ±2.0		180	21.0 ±1.0	48.0 ±1.0	31.1 ±1.2
	200	17.0 ±2.0	59.0 ±1.0	31.1 ±1.4		200	20.0 ±1.0	50.0 ±1.0	34.8 ±2.0
rAMP	100	13.0 ±1.0	77.0 ±2.0	14.6 ±1.2	rUMP	100	5.0 ±1.0	200.0 ±1.0	19.2 ±2.0
	120	14.0 ±1.0	70.0 ±2.0	17.1 ±1.5		120	6.0 ±1.0	167.0 ±2.0	20.4 ±1.4
	140	16.0 ±2.0	62.0 ±1.0	19.6 ±1.4		140	10.0 ±1.0	100.0 ±1.0	24.4 ±2.0
	160	17.0 ±1.0	59.0 ±1.0	23.5 ±1.2		160	13.0 ±2.0	77.0 ±2.0	29.6 ±1.8
	180	20.0 ±1.0	50.0 ±1.0	28.6 ±2.0		180	17.0 ±1.0	59.0 ±2.0	32.3 ±2.0
	200	22.0 ±2.0	45.0 ±2.0	32.3 ±1.6		200	21.0 ±2.0	48.0 ±1.0	35.8 ±1.6

Figure S1. The structure of the biotin-TEG linker used to biotinylate the 3' end of the DNA oligonucleotides. The structure was produced with ChemBioDraw Ultra 12.0. The length of the linker, from the biotin to the 3'-phosphate of the first DNA base is ~3 nm, as measured by using Pymol.

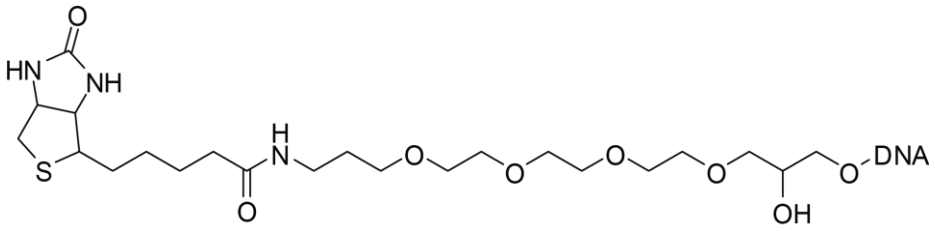

Figure S2. Current-voltage (IV) curves for αHL NN, TBMΔ6, TBMΔ6/M113G, TBMΔ6/M113F and TBMΔ6/M113N. The mean current levels for individual pores (± s.d.) were determined (n ≥ 3) in 1 M KCl, 25 mM Tris.HCl, pH 8.0, containing 0.1 mM EDTA, over a range of applied potentials: TBMΔ6 (navy) and (A) αHL NN (yellow); (B) TBMΔ6/M113G (purple); (C) TBMΔ6/M113F (red); (D) TBMΔ6/M113N (green).

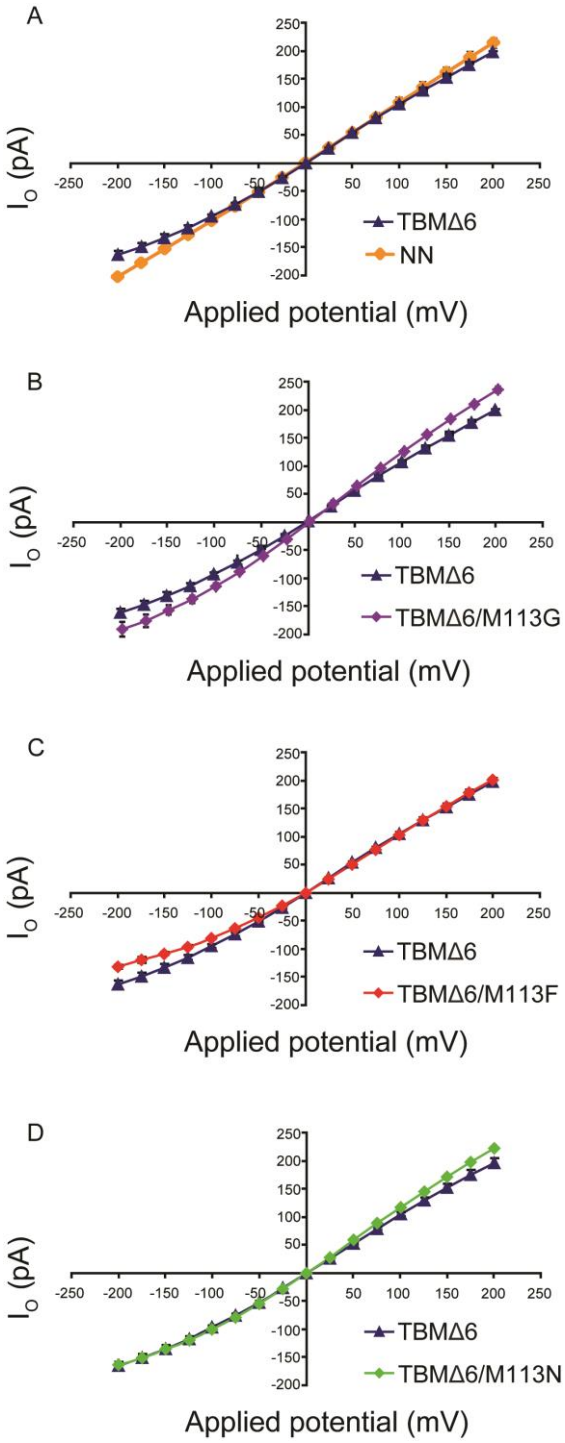

Figure S3. Representative single-channel recordings with the αHL TBMΔ6, Δ6/M113N and Δ6/M113F pores, showing CD adapter binding (40 μM $am_7\beta CD$, *trans*). In the case of TBM Δ6/M113F, the $am_7\beta CD$ remains bound for >1.5 h. The traces were recorded in 1 M KCl, 25 mM Tris.HCl, at pH 6.0.

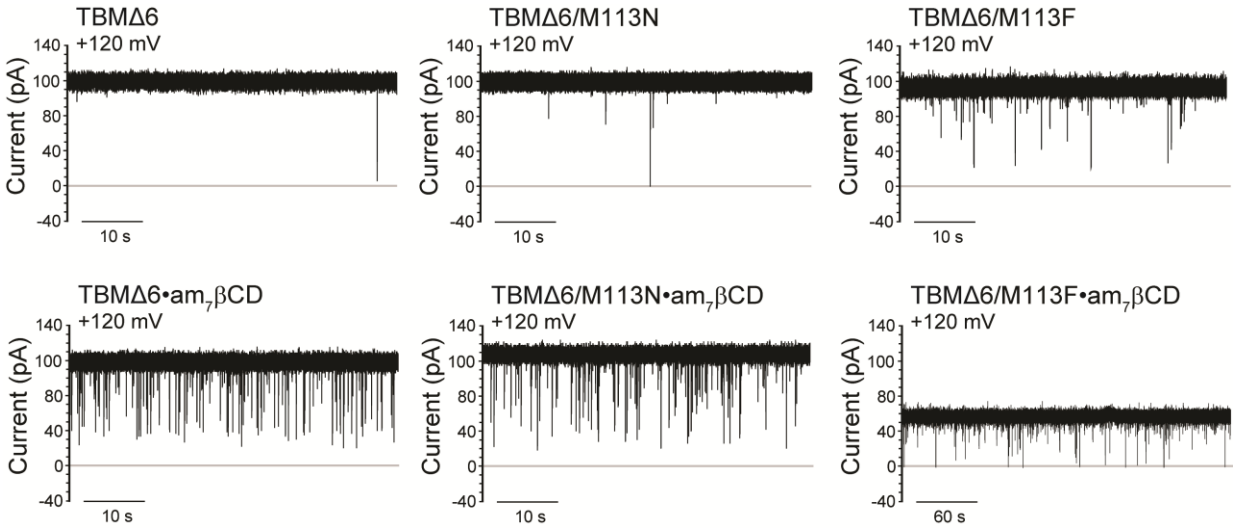

Figure S5. (A) Variation of residual currents ($I_{RES\%}$) with applied potential for each dNMP detected with the TBMΔ6/M113F•am$_7$βCD pore. (B) Variation of k_{off} with applied potential for each dNMP detected with the TBMΔ6/M113F•am$_7$βCD pore. Number of experiments: n ≥ 3. The data are tabulated in **Table S7.**

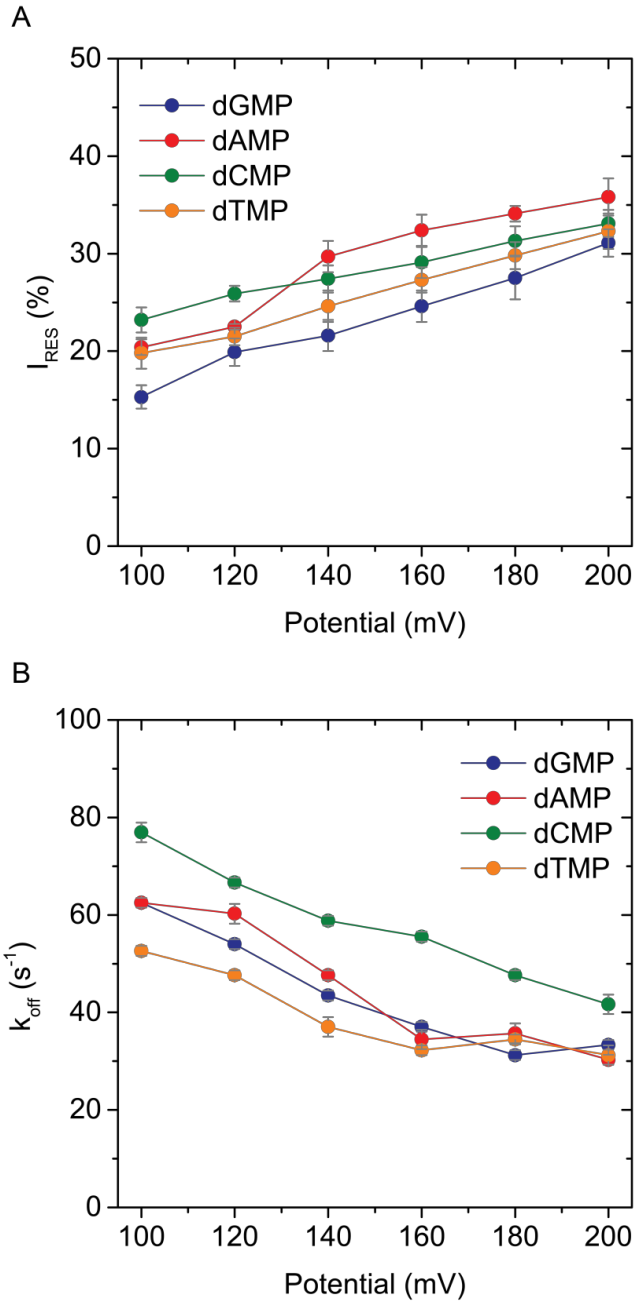

Figure S6. (A) Variation of residual currents ($I_{RES\%}$) with applied potential for each rNMP detected with the TBMΔ6/M113F•am$_7$βCD pore. (B) Variation of k_{off} with applied potential for each rNMP detected with the TBMΔ6/M113F•am$_7$βCD pore. Number of experiments: n ≥ 3. The data are tabulated in **Table S8.**

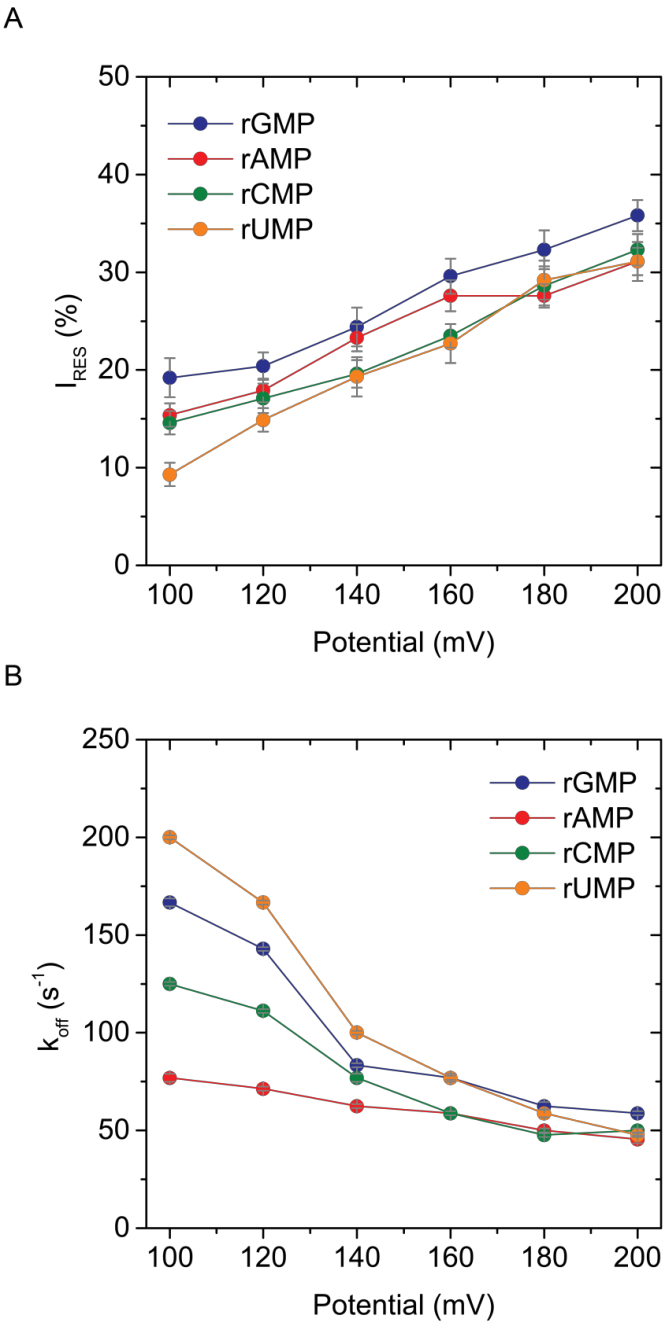

Figure S7. Typical current traces for the TBMΔ6 pore when blocked with immobilized poly(dC)$_{40}$ or poly(dC)^{A15} oligonucleotides.