

Supplementary information to

**Short-range DNA translocation initiates long-range 1-dimensional
DNA diffusion by the Type III restriction enzyme EcoP15I**

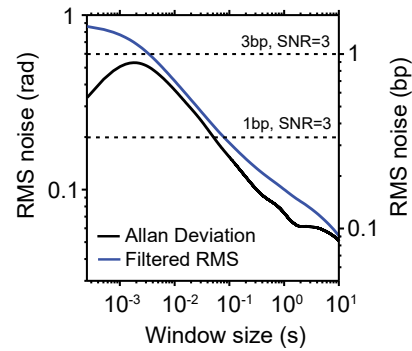
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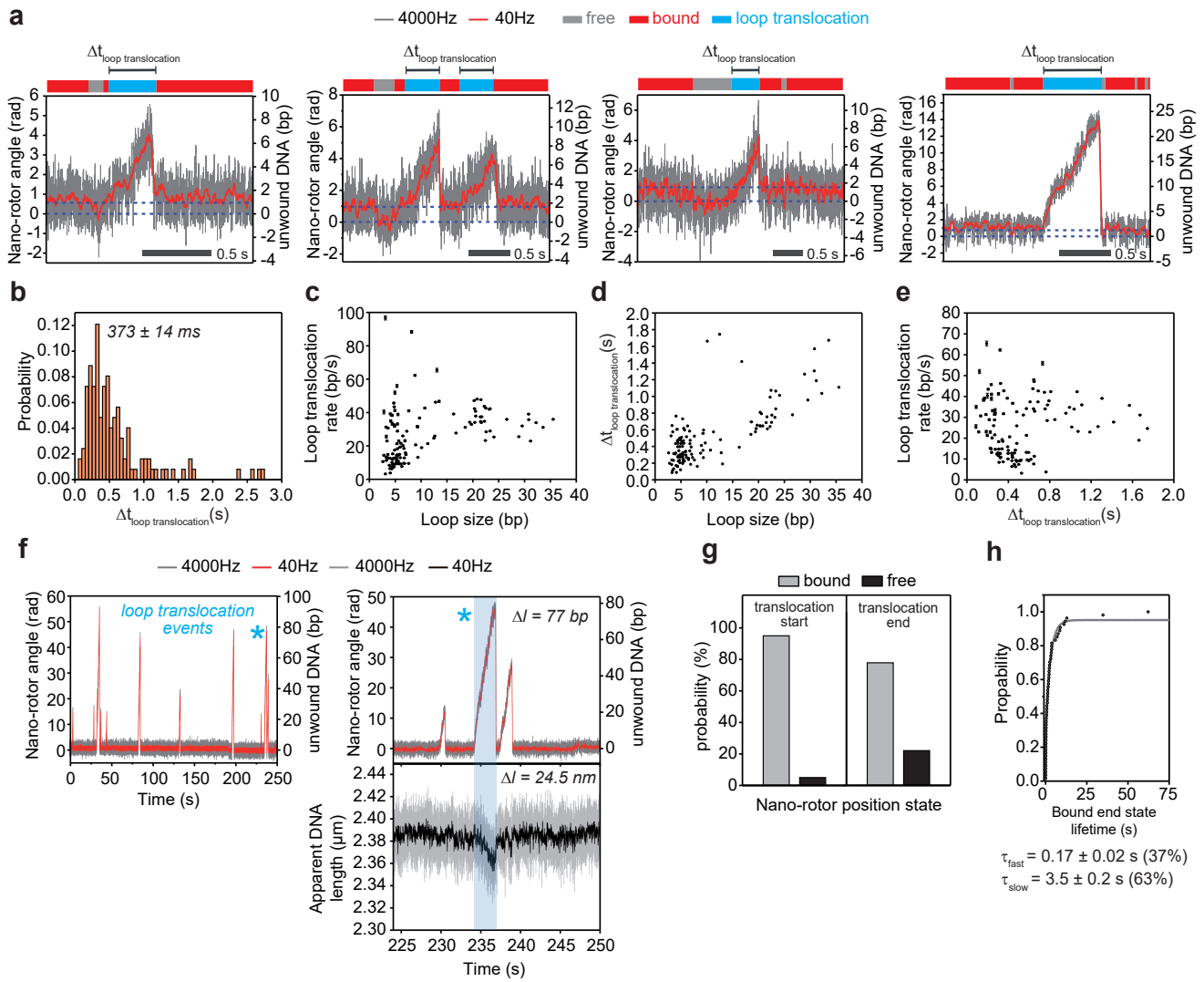
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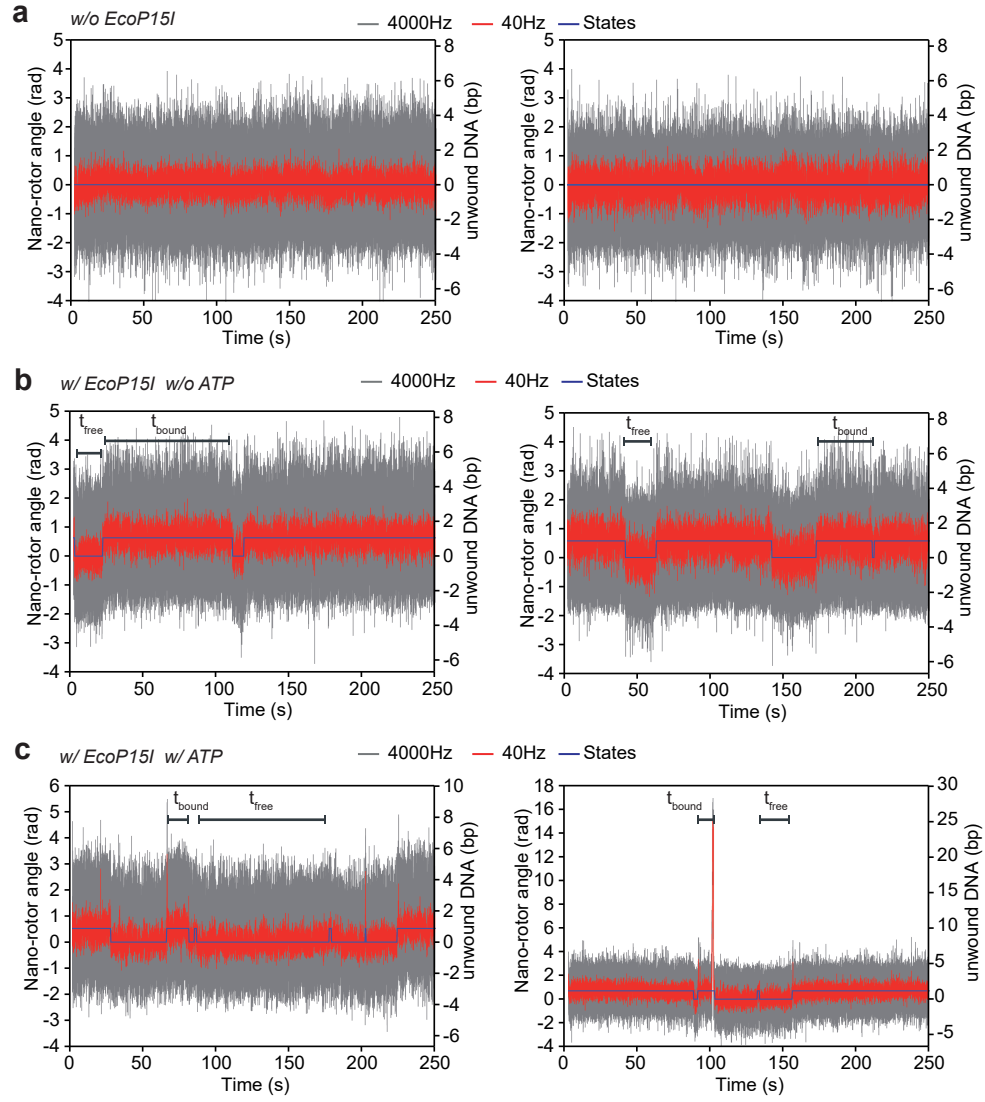
[#]co-communicating authors



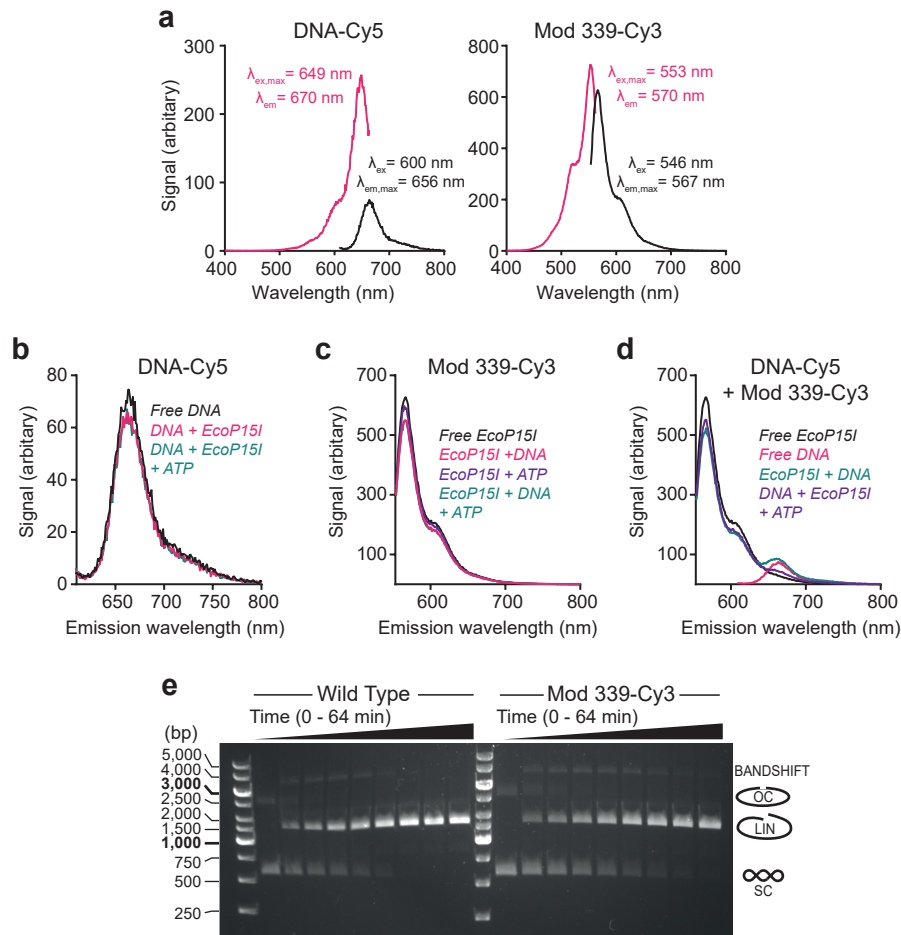
Supplementary Fig. 1: Spatio-temporal resolution of the nano-rotor. Allan Deviation (black) and root mean square (RMS) noise after filtering with a sliding average (blue). Horizontal dashed lines show the noise level at which twist changes of 3 bp or 1 bp could be resolved while assuming a signal to noise ratio (SNR) of 3. The intersections of these lines with the RMS provide information about the temporal resolution to observe these twist changes. This yields a temporal resolution of ~ 4 ms for 3 bp and ~ 80 ms for 1 bp.



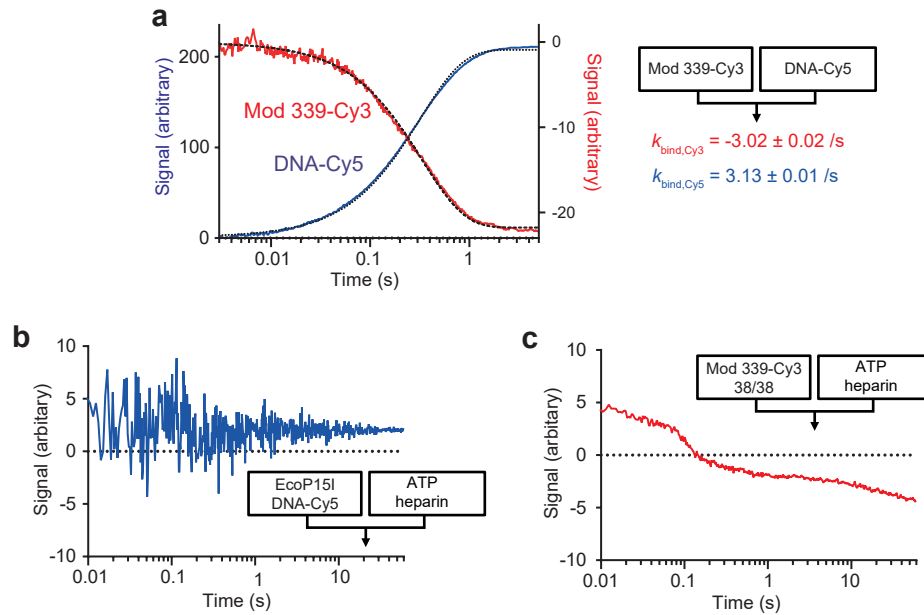
Supplementary Fig. 2: DNA loop translocation properties. **a**, Further examples of translocation events measured with the nano-rotor assay (grey, at 4000 Hz; red, after 100-point sliding average ± 40 Hz) after the addition of 4.66 nM EcoP15I. **b**, Distribution of the loop translocation time ($\Delta t_{\text{loop translocation}}$) with a mean time of $373.7 \pm 14.1 \text{ ms}$. **c-e**, Plots of the loop translocation rate vs. loop collapse size (panel c), $\Delta t_{\text{loop translocation}}$ vs loop collapse size (panel d) and the loop translocation rate $\Delta t_{\text{loop translocation}}$ (panel e) plotted for individual events. The loop translocation time increased with the loop collapse size, consistent with a constant stepwise translocation rate, whereas the other parameters did not follow a trend. **f**, (*left panel*) Time trajectory of the angular position of the nano-rotor including long loop translocation events with loop collapse sizes of $>40 \text{ bp}$. Asterisk marks the event shown in the right panel. (*right panel*) Trajectory of the angular position of the nano-rotor for the long loop translocation event shown together with the length of the DNA construct revealing the DNA length reduction upon loop translocation. The loop collapse size of $\sim 77 \text{ bp}$ matches with the apparent DNA length reduction of 24.5 nm ($\sim 72 \text{ bp}$) assuming helix translocation with $1 \text{ bp} \approx 0.34 \text{ nm}$. **g**, Probabilities that loop translocation events started and ended either in the free or bound state ($N = 113$). Only 5% of loop translocation events started in the free state and 95% in the bound state. 77% of loop translocation events terminated in a bound state and 23% in the free state. **h**, The bound state after loop translocation exhibited a bi-exponential lifetime distribution with an average lifetime of $\sim 2.3 \text{ s}$ (37%: $0.17 \pm 0.02 \text{ s}$ and 63%: $3.5 \pm 0.2 \text{ s}$; $N = 54$).



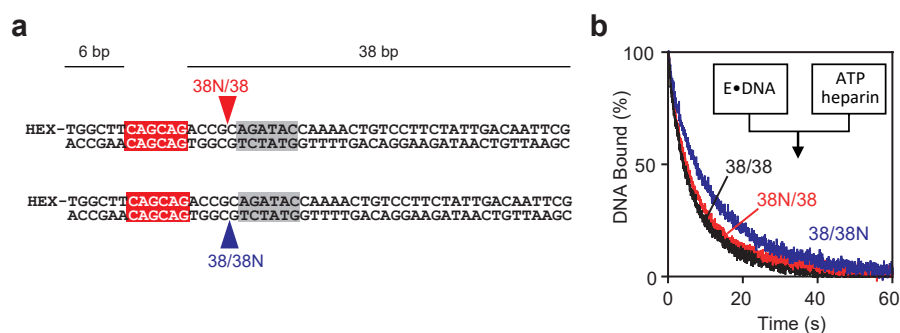
Supplementary Fig. 3: Analysis of the lifetimes of free and bound states of the EcoP15I-DNA interaction. Representative time trajectories of the angular position of the nano-rotor (grey, at 4000 Hz; red, after 100-point sliding average ± 40 Hz). A two-state approximation of the trajectories was determined using vbFRET (vbFRET_June10)⁶¹ (blue). The analysis was done for: **a**, control measurement without EcoP15I; **b**, measurements with EcoP15I but without ATP; and **c**, measurements with both EcoP15I and ATP. Indicated are examples for the times spent in the free state (t_{free}) and the “bound” state (t_{bound}) used to determine lifetime distributions displayed in Fig. 2f.



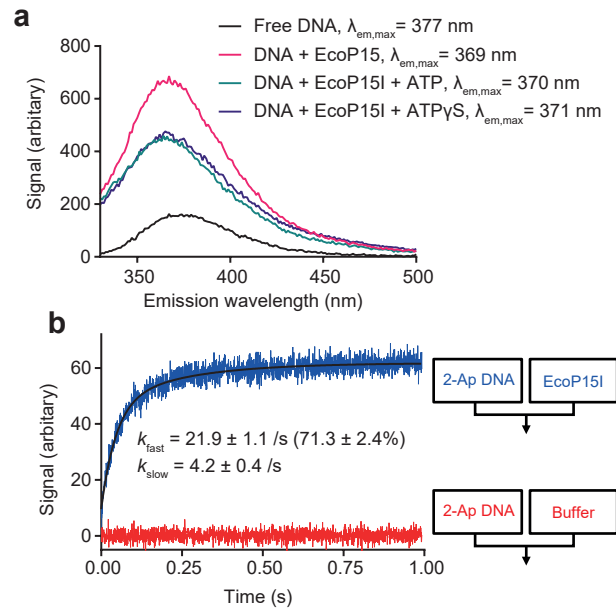
Supplementary Fig. 4: Characterisation of cyanine dye-labelled EcoP15I and DNA. **a**, The DNA-Cy5 substrate (Fig. 3b) was made by annealing 2AP_38_Fwd and 38_Rev (Supplementary Table 5). Steady-state fluorescence excitation (magenta) and emission (black) spectra for the Cy5 label in 25 nM DNA-Cy5 (*left*) and the Cy3 label in 75 nM EcoP15I 339-Cy3 (*right*). For excitation spectra, the wavelength was set to 570 nm for Cy3 and 670 nm for Cy5. For emission spectra, the excitation wavelength was set to 546 nm for Cy3 and 600 nm for Cy5. **b**, Steady-state fluorescence Cy5 emission spectra for 25 nM DNA-Cy5 and where indicated 75 nM unlabelled EcoP15I and 4 mM ATP. Excitation wavelength was set to 600 nm. **c**, Steady-state fluorescence Cy3 emission spectra for 75 nM EcoP15I 339-Cy3 and, where indicated, 25 nM unlabelled 38/38 oligoduplex and 4 mM ATP. Excitation wavelength was set to 546 nm. **d**, Steady-state fluorescence emission spectra for 75 nM EcoP15I 339-Cy3 and DNA-Cy5 mixed with 4 mM ATP, as indicated. Excitation wavelength was set to 546 nm except for the free DNA trace which was set to 600 nm. **e**, Cleavage activity assays for 50 nM Wild Type EcoP15I (*left*) or EcoP15I 339-Cy3 (*right*) and 2 nM pKA16. Time points were separated on a 1% (w/v) agarose gel. Time points were 1, 0.5, 1, 2, 4, 8, 16, 32 and 64 min. As well as the supercoiled (SC) substrate, nicked open circle (OC) intermediate and linear (LIN) product, a band corresponding to a DNA-protein bandshift was also observed.



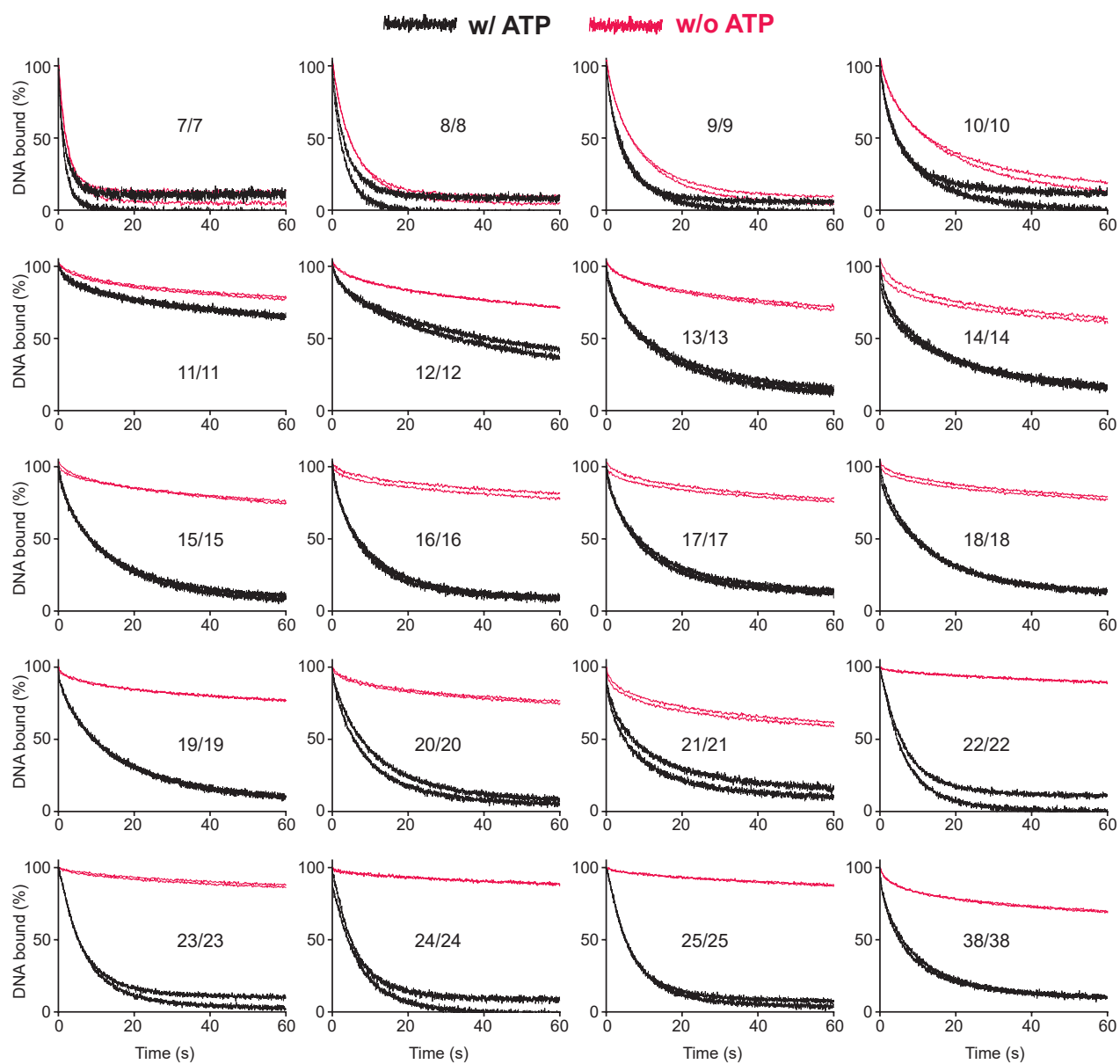
Supplementary Fig. 5: Fluorescence changes associated with DNA binding and ATP-dependent dissociation of cyanine dye-labelled EcoP15I and DNA. **a**, Stopped flow fluorescence measurement of binding of 75 nM EcoP15I 339-Cy3 and 25 nM DNA-Cy5. Excitation wavelength was set to 546 nm and the emission of Cy3 (red, left y-axis) and Cy5 (blue, right y-axis) measured simultaneously. The dashed and dotted lines are single exponential fits to give the rate constants shown (errors S.E.M.). **b**, Stopped flow fluorescence measurement of the ATP-dependent dissociation of 75 nM unlabelled EcoP15I from 25 nM DNA-Cy5, with final concentrations of 4 mM ATP and 2.5 μ M heparin trap. Excitation wavelength was set to 600 nm and the emission of Cy5 (blue) was measured. **c**, Stopped flow fluorescence measurement of the ATP-dependent dissociation of 75 nM EcoP15I 339-Cy3 from 25 nM unlabelled 38/38 oligoduplex, with final concentrations of 4 mM ATP and 2.5 μ M heparin trap. Excitation wavelength was set to 546 nm and the emission of Cy3 (red) measured.



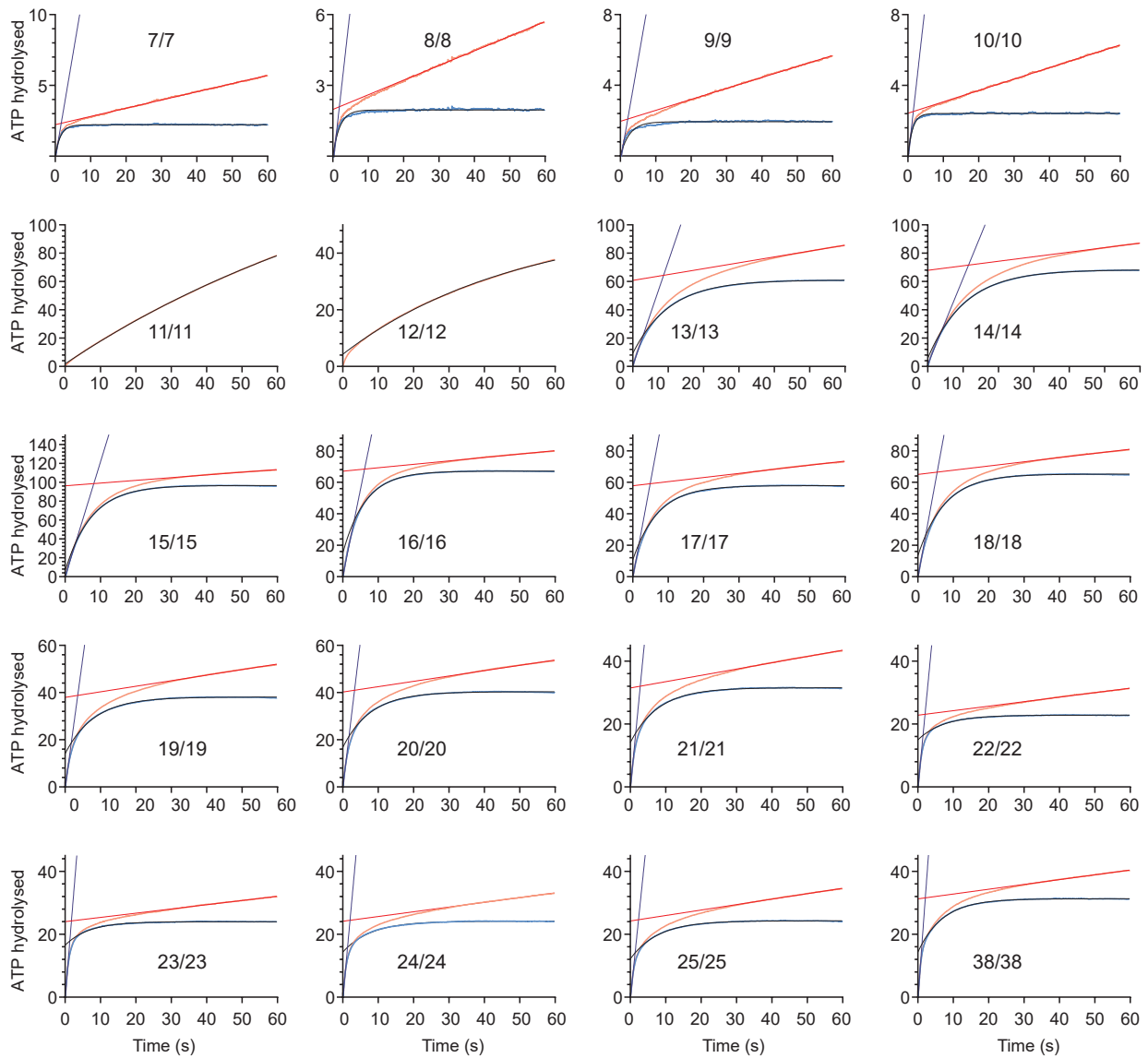
Supplementary Fig. 6: A nick in either strand of the translocated loop does not significantly affect ATP-dependent DNA dissociation. **a**, Nicked oligoduplex substrates. 38N/38 (*upper*), nicked on the MS 4 bp downstream of the recognition site, was made by annealing the DNA oligonucleotides Nick3_Fwd_Hex, N35_Fwd_Phos and 38_Rev (Supplementary Tables 6 & 7). 38/38N (*lower*), nicked on the TS 4 bp downstream of the recognition site, was made by annealing 38_Fwd_Hex, N23_Rev and N15_Rev_Phos (Supplementary Tables 6 & 7). **b**, Release of prebound enzyme from its target site with ATP and heparin measured using stopped flow fluorescence anisotropy. Data is the average of two independent repeats.



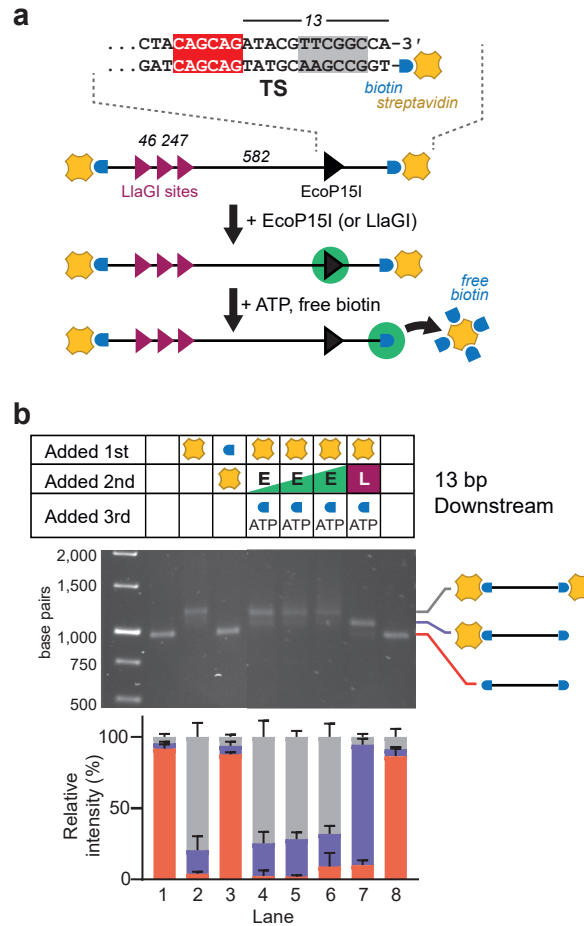
Supplementary Fig. 7: Fluorescence changes associated with EcoP15I association with a 2-aminopurine labelled oligoduplex. **a**, The 2-Ap oligoduplex was made by annealing 20/50_P15F_11Cy5 and 20/50_P15R (Supplementary Table 5) Steady-state 2-aminopurine fluorescence emission spectra for 500 nM 2-Ap oligoduplex (Fig. 3d) and, where indicated, 600 nM EcoP15I, 4 mM ATP and 4 mM ATPyS. Excitation wavelength was set to 311 nm. **b**, Stopped flow fluorescence measurement at 311 ± 3 nm and using a 360 nm long-pass filter of 125 nM 2-Ap oligoduplex mixed with either 150 nM EcoP15I or a buffer control. The association curve was fitted with a double exponential (solid black line) to give the parameters shown (errors S.E.M.).



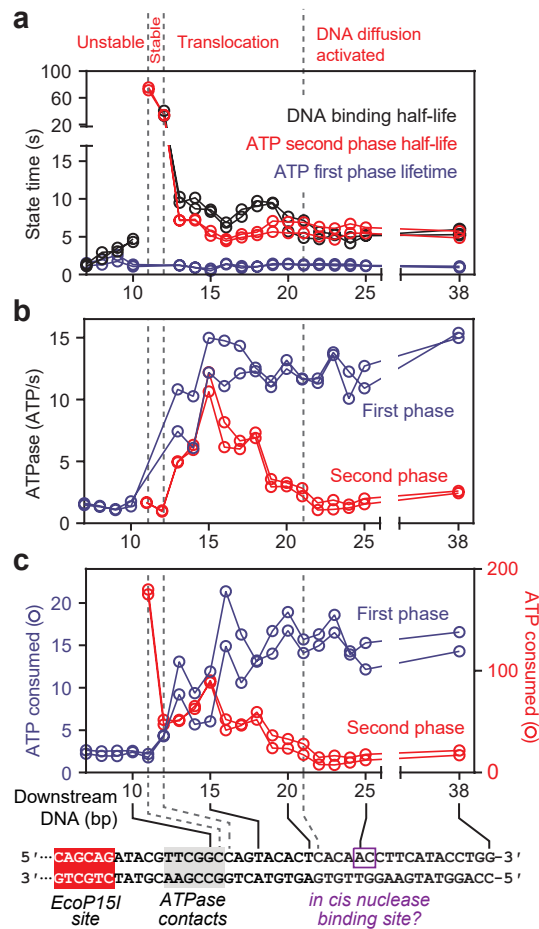
Supplementary Fig. 8: ATP-dependent DNA dissociation of EcoP15I as a function of downstream DNA length measured using stopped flow anisotropy. Release of prebound enzyme from its target site following mixing with heparin and with (black) or without (magenta) ATP, measured using stopped flow fluorescence anisotropy. Data from two independent repeats are shown.



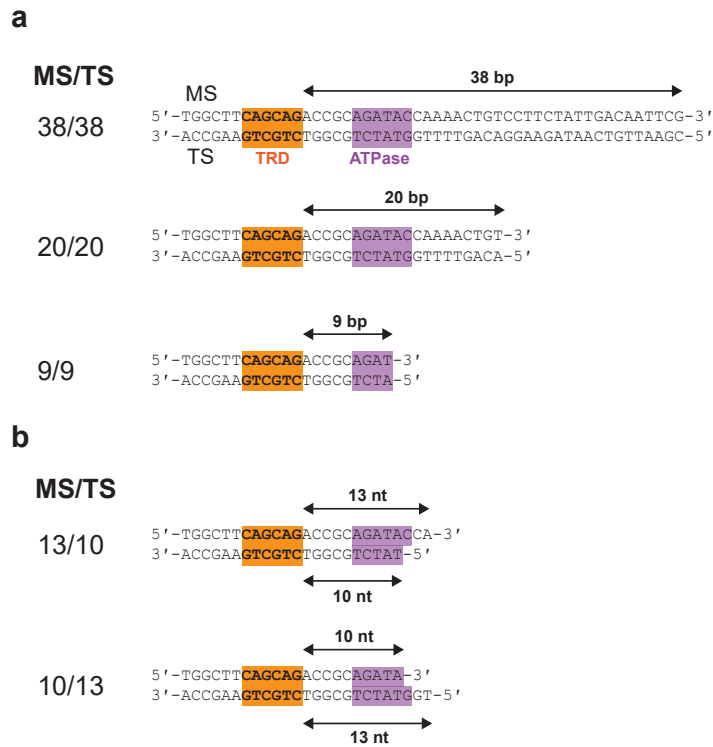
Supplementary Fig. 9: ATP hydrolysis by EcoP15I as a function of downstream DNA length measured using stopped flow fluorescence. Pre-bound EcoP15I and oligoduplexes were mixed with 4 mM ATP and the phosphate release measured using phosphate binding protein (Supplementary Fig. 17). Data was separated into different phases as in Toth et al (ref 31). The background steady-state rate of ATP hydrolysis due to heparin binding to free EcoP15I was estimated by linear regression (red line) to the raw data (light red) for $t > 45.0$ s. The raw data was corrected for the background ATPase to produce the DNA-dependent data (light blue) which was fitted for $t > 3.5$ s to $y = (A_2 \cdot (1 - \exp(-k_2 \cdot t))) + A_1$ (black line), to estimate the ATP amplitudes for the first (A_1) and second (A_2) ATPase bursts. k_2 was used as a measure of the lifetime of the second burst and $A_2 \cdot k_2$ as a measure of the ATPase rate of the second burst. The corrected data was fitted for $t < 0.35$ s by linear regression to estimate the ATPase rate of the first burst. The lifetime of the first burst was estimated by dividing A_1 by the ATPase rate. 11/11 and 12/12 use the data uncorrected for heparin as their off rate is low and so heparin binding effect is negligible and produce a first burst that was too small to be reliably fitted. 7/7 to 10/10 only give a first burst phase. Fitted values are shown in Supplementary Fig. 11. One representative dataset shown from two independent repeats.



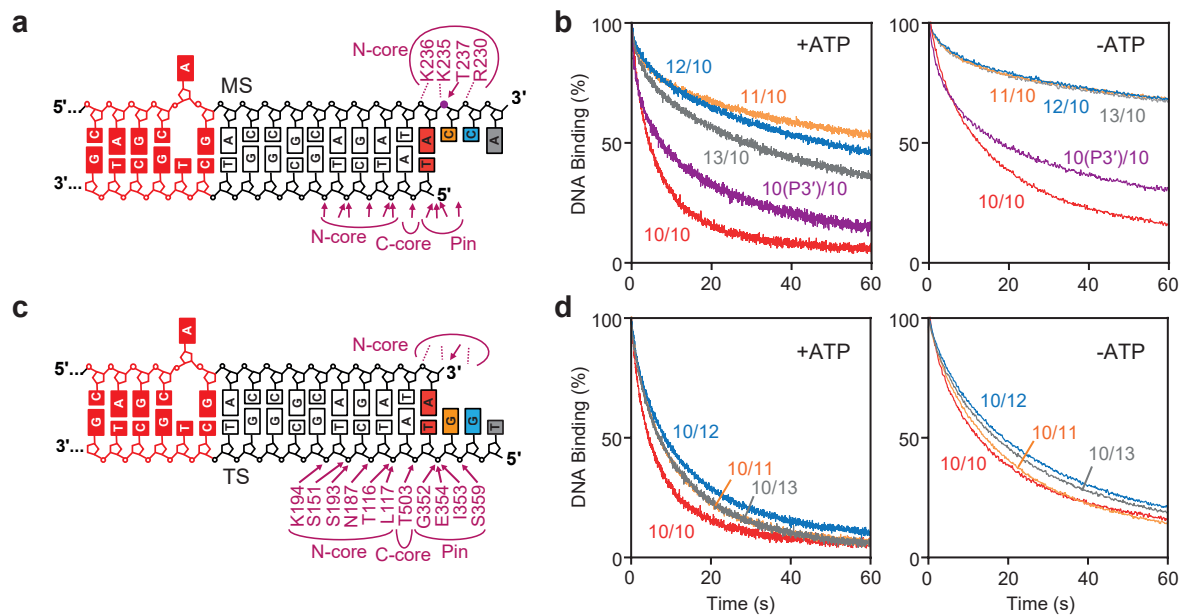
Supplementary Fig. 10: EcoP15I cannot displace streptavidin from biotin-labelled DNA despite its translocase activity. **a**, The DNA substrate from Fig. 3c, with a single EcoP15I site 13 bp from the DNA end and with three sites for the Type ISP restriction enzyme LlaGI (5'-CTnGAYG-3') in direct repeat (purple) where the arrowheads indicate the direction of ATP-dependent LlaGI translocation. The 13 bp spacing was chosen to be within the average loop translocation distance of EcoP15I (Fig. 2). The grey box indicates the helicase binding site. EcoP15I or LlaGI were pre-incubated with the DNA and then the reaction started with ATP and free biotin. If motor activity displaced the streptavidin from the biotin-labelled DNA end, the released streptavidin would be bound by the free biotin and unable to rebind the DNA. **b**, Example agarose gel of reactions with 8 nM DNA and the order of addition shown for 855 nM streptavidin (yellow), 17.1 μ M free biotin (blue), EcoP15I (from left to right, 264, 528 or 792 nM), nuclease mutant LlaGI D78A (50 nM) and/or ATP and biotin mix (4 mM and 17.1 μ M, respectively). DNA with none, one or two streptavidin molecules can be separated. The relative intensities of the three species were quantified from 3 repeat reactions (errors S.D.). Free biotin was sufficient to prevent streptavidin binding to the DNA ends (lane 3). Increasing concentrations of EcoP15I did not displace the streptavidin (lanes 4-6). Contrastingly, LlaGI can displace >80% of the streptavidin from one DNA end (lane 7) consistent with the expected directional translocation. Lane 2 shows incomplete biotin labelling of both DNA ends. Lanes 1 and 8 are the same control marker.



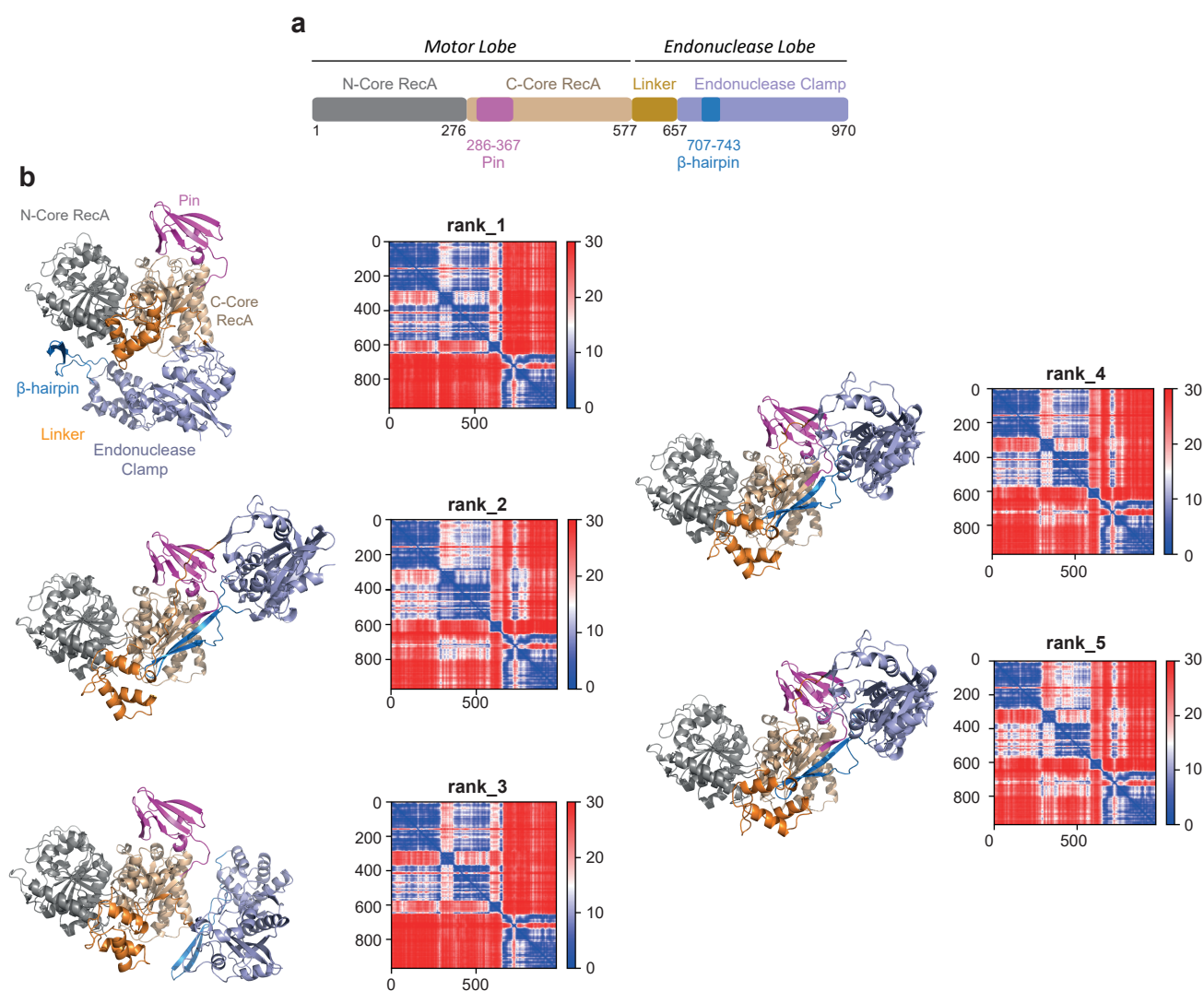
Supplementary Fig. 11: Downstream length-dependence of DNA dissociation and ATPase kinetics. Circles show fitted parameters from two repeat experiments (Supplementary Figs. 8-9). Vertical dotted lines align to the downstream DNA sequence map below panel c. **a**, Lifetimes of the first (blue) and second (red) ATPase phases and of DNA dissociation with ATP (black). Second phases were not observed for 7/7 – 9/9. Only an uncoupled second phase was observed for 11/11 and 12/12. **b**, Rates of ATP hydrolysis of the first (blue) and second (red) phases. **c**, Number of ATPs consumed in the first (blue, lefthand y-axis) and second (blue, righthand y-axis) ATPase phases.



Supplementary Fig. 12: Examples of DNA substrate nomenclature. The DNA recognition site is highlighted in orange and the region contacted by the helicase domain is highlighted in pink. Each substrate contains six fixed residues of DNA upstream of the DNA recognition site and a variable number of base pairs downstream. The naming system is “X/Y”, where “X” and “Y” represent the lengths in nucleotides downstream of the target site of the MS and TS, respectively. **a**, Sequence of the 50 bp “full length” DNA substrate 38/38 on which EcoP15I is known to have normal activity and examples of substrates with truncated downstream dsDNA. **b**, Examples of substrates with 5’ or 3’ overhangs downstream of the DNA recognition site used to investigate the effect of strand polarity on EcoP15I activity.

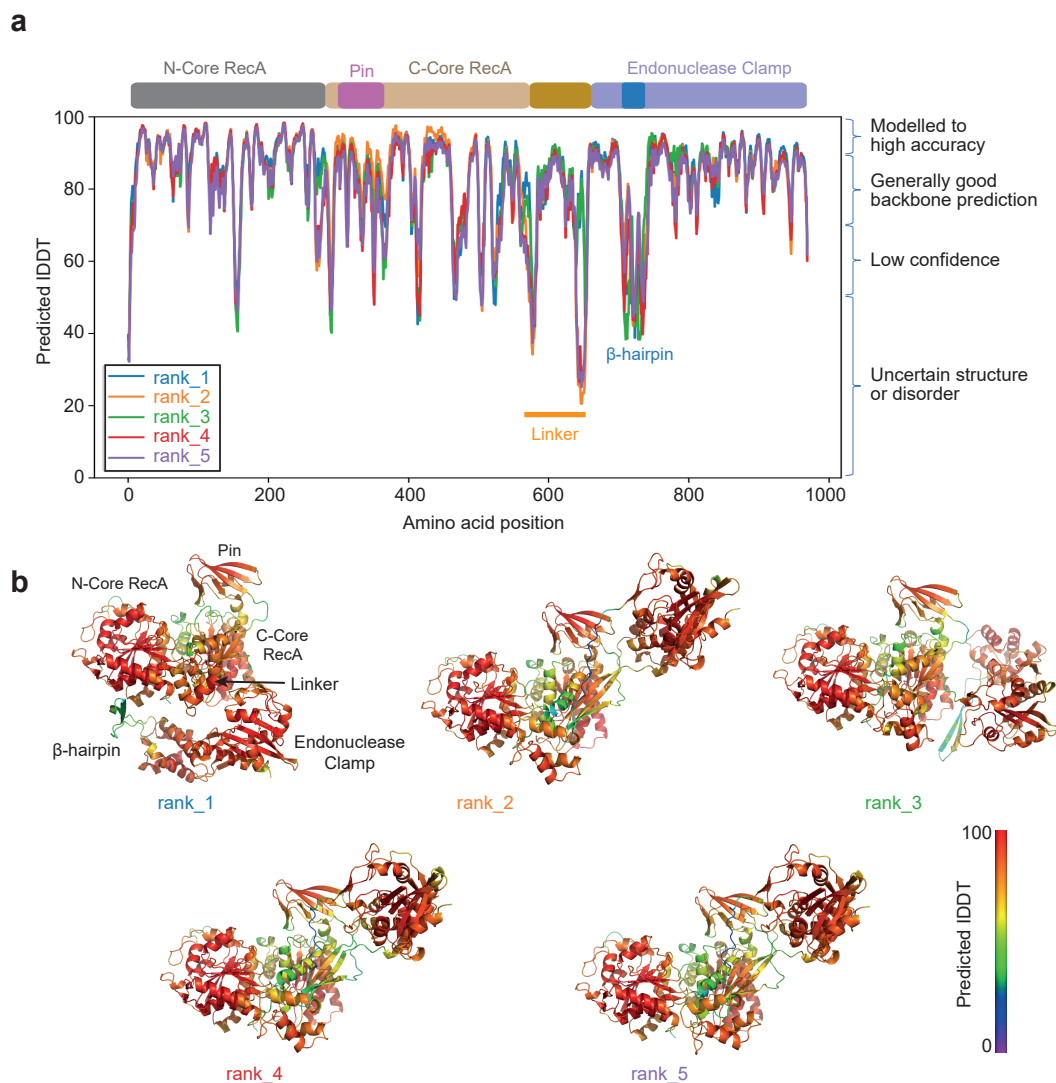


Supplementary Fig. 13: N-core RecA contacts to the methylating strand backbone are necessary for stable DNA association. **a**, Cartoon of the sequences of the DNA substrates with an extended MS (upstream DNA sequence not shown). 10(P3')/10 had an MS with 10 nt downstream and a 3' phosphate. Curved lines show regions of Res-DNA contact from PDB:4ZCF¹⁵ with arrows indicating side chain contacts and dotted lines indicating main chain contacts. **b**, Release of prebound enzyme from its target site with heparin trap and with (left) or without (right) ATP measured using HEX anisotropy. The weakly-bound 10/10 DNA was extended on the MS strand. An additional 3' phosphate produces a small increase in stability but the additional base at position 11 is also important, possibly to help orient the phosphate-T237 interaction. Further extension of the MS has only a moderate effect. **c**, Cartoon of DNA substrates with an extended TS (upstream DNA sequence not shown). **d**, Release of prebound enzyme from its target site with heparin trap and with (left) or without (right) ATP measured using HEX anisotropy. Extension of the TS strand had little effect compared to the weakly-bound 10/10 DNA. One representative dataset shown from two independent repeats.

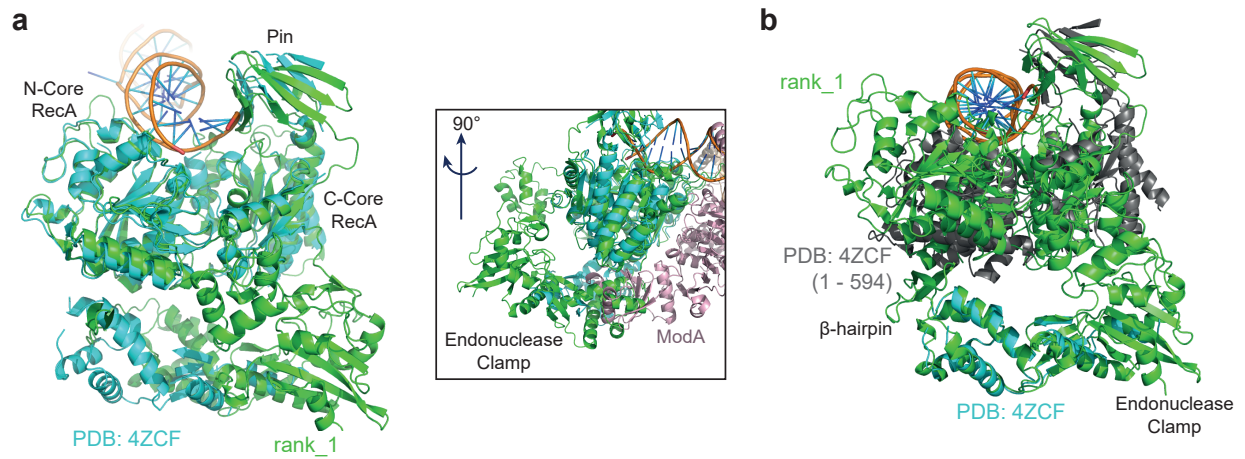


Supplementary Fig. 14: Predicted Aligned Error (PAE) of ranked AlphaFold 2 structures of EcoP15I

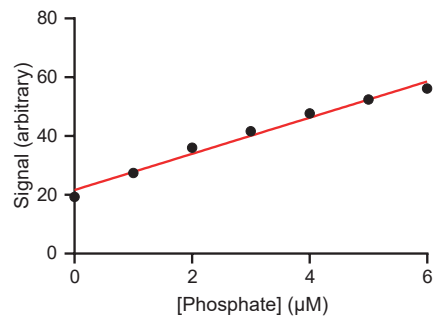
Res. a, Domain arrangement of Res determined from crystal structure¹⁰ and AlphaFold 2 structural predictions. **b**, AlphaFold 2 structural predictions and PAEs. In the structures, domains are coloured as indicated top left and panel a. In all structures, the PAEs indicate that AlphaFold 2 predicts well-defined relative positions and orientations for residues of the helicase subdomains, the Pin, the α -helical bundle of the linker and the core Endonuclease Clamp⁴⁸. The relative orientations of the N-core and C-core RecA folds are also well defined, particularly for rank_1, but the Pin has a less well-defined relative position that might reflect necessity for motion during translocation. The nuclease lobe is independent of the helicase lobe, suggesting relative flexibility that might explain the absence of this lobe in the crystal structure¹⁵ and might be necessary to allow binding to the DNA cleavage site. The PAE for the Linker and β -hairpin indicate flexibility relative to the nuclease core. In rank_1 and rank_3, there are interactions between the Linker and both RecA cores. In rank_2, rank_4 and rank_5, the β -hairpin forms a pseudo-continuous β -sheet with the C-core RecA fold.



Supplementary Fig. 15: Predicted local distance difference test (pLDDT) of ranked AlphaFold 2 structures of EcoP15I Res. a, Plot of pLDDT (corresponding to each model's predicted score on the IDDT-C α metric) against residue number for each ranked structure. The domains are indicated in the cartoon and labelled on the plot. The reliability interpretations of the pLDDT scores are indicated on the right. A pLDDT < 50 is a reasonably strong predictor of disorder⁴⁸ and corresponds to loops within the RecA and Pin folds, to the boundaries of the Linker and to the β -hairpin. **b,** Cartoon views of the ranked structures coloured according to the predicted pLDDT score (key, right). The domains are labelled for the rank_1 structure.



Supplementary Fig. 16: Alignment of the rank_1 AlphaFold 2 structure to the EcoP15I crystal structure. **a**, Alignment of rank_1 (green) to PDB:4ZCF¹⁵ (blue) via the helicase lobe (residues 1 – 594). (*inset*) Rotated alignment to show that the Endonuclease Lobe of rank_1 clashes with ModA of PDB:4ZCF (pink). Clashes are also seen for other predicted structures. **b**, Alignment of rank_1 (green) to the partial nuclease regions of PDB:4ZCF¹⁵; (discontinuous residues 620 – 633, 667 – 705, 744 – 774, 804 – 810) in blue with the helicase lobe (residues 1 – 594) in grey.



Supplementary Fig. 17: Conversion of raw fluorescence signal into phosphate released. A calibration plot of the raw fluorescence signal in the stopped flow at fixed concentrations of inorganic phosphate using a final concentration of 8 μM PBP. The slope of the linear regression was used to convert the observed signal to released phosphate and hence ATPs hydrolysed.

Name	5'-3' sequence
EcoP15I target I	5'-TCAGCCAGCAGACCGCAGATACCAAACTGTCCTTCTATTGACAATTCGG-3'
EcoP15I target II	5'-GGCCCCGAATTGTCAATAGAAGGACAGTTTTGGTATCTGCGGTCTGCTGGCTGAGGGACACT-3'
DNA spacer primer I	5'- GCAGCCACTAGTTGCCATAGATTATAGCTAAGG-3'
DNA spacer primer II	5'-CTCCCTCAGCAGGAGAACGAGGATATT-3'

Supplementary Table 1: Oligodeoxyribonucleotide sequences used for EcoP15I target sequence and PCR for dsDNA spacer shown in Fig. 2a. Oligodeoxyribonucleotides synthesised by Sigma Aldrich.

Name		Downstream Length	5'-3' Sequence
SlideF10	Variable primers	10 bp	CCGAACGTATCTGCTGTAGT
SlideF11		11 bp	GCCGAACGTATCTGCTGTAG
SlideF12		12 bp	GGCCGAACGTATCTGCT
SlideF13		13 bp	TGGCCGAACGTATCTGCT
SlideF14		14 bp	CTGGCCGAACGTATCTGCT
SlideF15		15 bp	ACTGGCCGAACGTATCTGC
SlideF16		16 bp	TACTGGCCGAACGTATCTGC
SlideF17		17 bp	GTACTGGCCGAACGTATCTG
SlideF18		18 bp	TGTACTGGCCGAACGTATCT
SlideF19		19 bp	GTGTACTGGCCGAACGTATCT
SlideF20		20 bp	AGTGTACTGGCCGAACGTATC
SlideF21		21 bp	GAGTGTACTGGCCGAACGTAT
SlideF22		22 bp	TGAGTGTACTGGCCGAACG
SlideF23		23 bp	GTGAGTGTACTGGCCGAACG
SlideF24		24 bp	TGTGAGTGTACTGGCCGAAC
SlideF25		25 bp	TTGTGAGTGTACTGGCCGAAC
SlideF26		26 bp	GTTGTGAGTGTACTGGCCGAA
slideF27		27 bp	GGTTGTGAGTGTACTGGCCGA
SlideF28		28 bp	AGGTTGTGAGTGTACTGGCCGA
SlideF29		29 bp	AAGGTTGTGAGTGTACTGGCCGA
SlideF30		30 bp	GAAGGTTGTGAGTGTACTGGCCGA
SlideF31		31 bp	TGAAGGTTGTGAGTGTACTGG
SlideF32		32 bp	ATGAAGGTTGTGAGTGTACTGG
slideF33		33 bp	TATGAAGGTTGTGAGTGTACTGG
slideF34		34 bp	GTATGAAGGTTGTGAGTGTACTGG
SlideF35		35 bp	GGTATGAAGGTTGTGAGTGTACTG
SlideF36		36 bp	AGGTATGAAGGTTGTGAGTGTACTG
SlideF37		37 bp	CAGGTATGAAGGTTGTGAGTGT
SlideF38		38 bp	CCAGGTATGAAGGTTGTGAG
SlideRbio	constant primer	N/A	TCTAGATCACACCTCTTCCTC

Supplementary Table 2: Primer sequences used for PCR to make substrates in Fig. 3c-e. Oligodeoxyribonucleotides synthesised by Integrated DNA Technologies.

Tail-to-tail EcoP15I & EcoPI DNA	TCTAGATCACACCTCTTCCTTCAACAATTTCTT CTTGATG TGCTCCGCCAAGATACGGCTGAAA AGGGGCGGAACAGCATTACCCA CTTGATG TCCTTGTTGGCGACGGTTGCCCTTAAAGATCAT CCAATCAGGGAATGTCTGAATGCGGGCGGCCTCGCGAACTGTAAGAGAGCGGTTTAAGATC GGGTGGATAGGGAAAGCATCGTTACCGGGAACCATGGTTAATGCGGTACAGTTCTGGGGGA AGATCGTTTTCGGGTAAACGCCCGCCTTCTGGAATCAGCTTATAGCGTTCGATGATCTTTTCC GTGTGCTTAAGGGGGACGTGATTGAAGTCCTTATCCTCCTTAATTTCAACTAAGTC CTGGATG GCATACCAACAGTTCTCCCAAGAAAATGTCCCATCTTCTCCGTTTTCTGAATGGGTTGGCAAT GGGAAATTGACTTCATGCCCCAGACGGTTTCCAATGATAAAGACACGTTTCGCGCAACTGAGG CACACCATAATCAGCCATATTCAGAAGCTTTAATTTGTAGTCATAGCCTGTTTTATCAAGTTCC TTATAAACACCTTTGATAAAATCACCTTTATCCATAGTCAGAAGCCCCTTGACATTCTCAATCA CGAAGTATTTTCGGCTTCAACTGATTCAAAATGCGGATATATTCTAAGATTAACCTATTGCGTTT ATCTTCTGCGGTGCGACGTTCAAGTTTCGACGATACAC GGTCT CCGATAGTTGAGAACCCCT GACAGGGAGGTCCTCCGATTAACACGTCGATCTCTTCTCCTTCGATTAAATTAATAATTCAA AACCTGTCAACTGATTAATGTCGTCGCTAAAGAAAGGGATCTCGGGGAAATTGAATTCGTGT GTTTTTCGCGACAATAGGATTCGAGTCTACAGCCAGTTTAATTTGAAGCCGGCTTCTTGGAAG CCCAGGTGCATACTA CAGCAG ATACGTTTCGGCCAGTACACTACAACCTTCATACCTGG
Head-to-head EcoP15I DNA	TCTAGATCACACCTCTTCCTTCAACAATTTCTTCTTGATGTGCTCCGCCAAGATACGGCTGAAA AGGGGCGGAACAGCATTACCCACTTGATGTCCTTGTTGGCGACGGTTGCCCTTAAAGATCAT CCAATCAGGGAATGTCTGAATGCGGGCGGCCTCGCGAACTGTAAGAGAGCGGTTTAAGATC GGGTGGATAGGGAAAGCATCGTTACCGGGAACCATGGTTAATGCGGTACAGTTCTGGGGGA AGATCGTTTTCGGGTAAACGCCCGCCTTCTGGAATCAGCTTATAGCGTTCGATGATCTTTTCC GTGTGCTTAAGGGGGACGTGATTGAAGTCCTTATCCTCCTTAATTTCAACTAAGTCCTGGATG GCATACCAACAGTTCTCCCAAGAAAATGTCCCATCTTCTCCGTTTTCTGAATGGGTTGGCAAT GGGAAATTGACTTCATGCCCCAGACGGTTTCCAATGATAAAGACACGTTTCGCGCAACTGAGG CACACCATAATCAGCCATATTCAGAAGCTTTAATTTGTAGTCATAGCCTGTTTTATCAAGTTCC TTATAAACACCTTTGATAAAATCACCTTTATCCATAGTCAGAAGCCCCTTGACATTCTCAATCA CGAAGTATTTTCGGCTTCAACTGATTCAAAATGCGGATATATTCTA CAGCAG AACCTATTGCGT TCATCTTCTGCGGTGCGACGTTCAAGTTTCGACGATACACGGTGCCGATAGTTGAGAACCC CTGACAGGGAGGTCCTCCGATTAACACGTCGATCTCTTCTCCTTCGATTAAATTAATAATTCA AAACCTGTCAACTGATTAATGTCGTCGCTAAAGAAAGGGATCTCGGGGAAATTGAATTCGTG TGTTTTTCGCGACAATAGGATTCGAGTCTACAGCCAGTTTAATTTGAAGCCGGCTTCTTGGA GCCAGCCAGGTATGAAGGTTGTGAGTGTACTGGCCGAACGTAT CTGCTG TAGTATGCAC

Supplementary Table 3: Sequences of dsDNA fragments used as PCR templates to make substrates in Figure 2. Sites for EcoP15I (CAGCAG), EcoPI (AGACC) and LlaGI (CTNGAYG) are underlined and in bold font. DNA fragments synthesised by Integrated DNA Technologies.

Name		Upstream Length	5'-3' Sequence
15I_0F	Variable primers	0 bp	CAGCAGATACGTTTCGGCCAGT
P15I_1F		1 bp	ACAGCAGATACGTTTCGGCCAGT
P15I_2F		2 bp	TACAGCAGATACGTTTCGGCCAGT
P15I_3F		3 bp	CTACAGCAGATACGTTTCGGCCAGT
P15I_4F		4 bp	ACTACAGCAGATACGTTTCGGCCAGT
P15I_5F		5 bp	TACTACAGCAGATACGTTTCGGCCAG
P15I_6F		6 bp	ATACTACAGCAGATACGTTTCGGCCAG
P15I_7F		7 bp	CATACTACAGCAGATACGTTTCGGCCA
P15I_8F		8 bp	GCATACTACAGCAGATACGTTTCG
P15I_9F		9 bp	TGCATACTACAGCAGATACGTTTCG
P15I_10F		10 bp	GTGCATACTACAGCAGATACGTTTCG
SlideRbio	Constant primer	N/A	TCTAGATCACACCTCTTCCTTC

Supplementary Table 4: Primer sequences used for PCR to make substrates in Fig. 3f,g.
Oligodeoxyribonucleotides synthesised by Integrated DNA Technologies.

Name	X = fluorophore	5'-3' Sequence
2AP_38_Fwd	X = 2- aminopurine	TGGCTTCAGC X GACCGCAGATACCAAACTGTCCTTCTATTGACAATTCG
38_Rev		CGAATTGTCAATAGAAGGACAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
20/50_P15F_11Cy5	X = Cy5	GCCAGTGAAX T AACTGGCTTCAGCAGACCGCAGATACCAAACTGTCCTT
20/50_P15R		AAGGACAGTTTTGGTATCTGCGGTCTGCTGAAGCCAGTTAATTCAGTGGC

Supplementary Table 5: Internally fluorescent labelled oligodeoxyribonucleotide sequences and complementary strands annealed to make substrates for stopped-flow spectroscopy. Oligodeoxyribonucleotides synthesised by Integrated DNA Technologies (2-aminopurine) or Eurofins (Cyanine 5).

Name	5'-3' sequence
38_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACTGTCCTTCTATTGACAATTCG
25_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACTGTCCTTC
24_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACTGTCCTT
23_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACTGTCCT
22_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACTGTCC
21_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACTGTC
20_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACTGT
19_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACTG
18_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAACT
17_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAAC
16_Fwd	TGGCTTCAGCAGACCGCAGATACCAAAA
15_Fwd	TGGCTTCAGCAGACCGCAGATACCAAA
14_Fwd	TGGCTTCAGCAGACCGCAGATACCAA
13_Fwd	TGGCTTCAGCAGACCGCAGATACCA
12_Fwd	TGGCTTCAGCAGACCGCAGATACC
11_Fwd	TGGCTTCAGCAGACCGCAGATAC
10_Fwd	TGGCTTCAGCAGACCGCAGATA
9_Fwd	TGGCTTCAGCAGACCGCAGAT
8_Fwd	TGGCTTCAGCAGACCGCAGA
7_Fwd	TGGCTTCAGCAGACCGCAG
38_Rev	CGAATTGTCAATAGAAGGACAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
25_Rev	GAAGGACAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
24_Rev	AAGGACAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
23_Rev	AGGACAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
22_Rev	GGACAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
21_Rev	GACAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
20_Rev	ACAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
19_Rev	CAGTTTTGGTATCTGCGGTCTGCTGAAGCCA
18_Rev	AGTTTTGGTATCTGCGGTCTGCTGAAGCCA
17_Rev	GTTTTGGTATCTGCGGTCTGCTGAAGCCA
16_Rev (12+4T_Rev)	TTTTGGTATCTGCGGTCTGCTGAAGCCA
15_Rev (12+3T_Rev)	TTTGGTATCTGCGGTCTGCTGAAGCCA
14_Rev (12+2T_Rev)	TTGGTATCTGCGGTCTGCTGAAGCCA
13_Rev (12+1T_Rev)	TGGTATCTGCGGTCTGCTGAAGCCA
12_Rev	GGTATCTGCGGTCTGCTGAAGCCA
11_Rev	GTATCTGCGGTCTGCTGAAGCCA
10_Rev	TATCTGCGGTCTGCTGAAGCCA
9_Rev	ATCTGCGGTCTGCTGAAGCCA
8_Rev	TCTGCGGTCTGCTGAAGCCA
7_Rev	CTGCGGTCTGCTGAAGCCA
12+5T_Rev	TTTTTGGTATCTGCGGTCTGCTGAAGCCA
N35_Fwd_Phos	PO ₃ ⁻ -GCAGATACCAAAACTGTCCTTCTATTGACAATTCG
N23_Rev	CGAATTGTCAATAGAAGGACAGTTTTGGTATCTGC
N15_Rev_Phos	PO ₃ ⁻ -GGTCTGCTGAAGCCA

Supplementary Table 6: Oligodeoxyribonucleotide sequences annealed to make substrates for stopped-flow spectroscopy. Oligodeoxyribonucleotides synthesised by Integrated DNA Technologies.

Name	5'-3' sequence
38_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACTGTCCTTCTATTGACAATTCG-3'
25_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACTGTCCTTC-3'
24_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACTGTCCTT-3'
23_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACTGTCCT-3'
22_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACTGTCC-3'
21_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACTGTC-3'
20_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACTGT-3'
19_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACTG-3'
18_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAACT-3'
17_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAAC-3'
16_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAAA-3'
15_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAAA-3'
14_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCAA-3'
13_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCA-3'
12_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACC-3'
11_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATAC-3'
10_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATA-3'
10_Phos_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATA-3'-PO ₃ ⁻
9_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGAT-3'
8_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGA-3'
7_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAG-3'
12+1T_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCT-3'
12+2T_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCTT-3'
12+3T_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCTTT-3'
12+4T_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCTTTT-3'
12+5T_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACCGCAGATACCTTTTT-3'
Nick3_Fwd_Hex	HEX-5'-TGGCTTCAGCAGACC-3'

Supplementary Table 7: Hexachlorofluoroscein-labelled oligodeoxyribonucleotide sequences annealed to make substrates for stopped-flow spectroscopy. Oligodeoxyribonucleotides synthesised by Integrated DNA Technologies.