Supplementary Information

Nano Positioning System reveals the course of upstream and nontemplate DNA within the RNA polymerase II elongation complex

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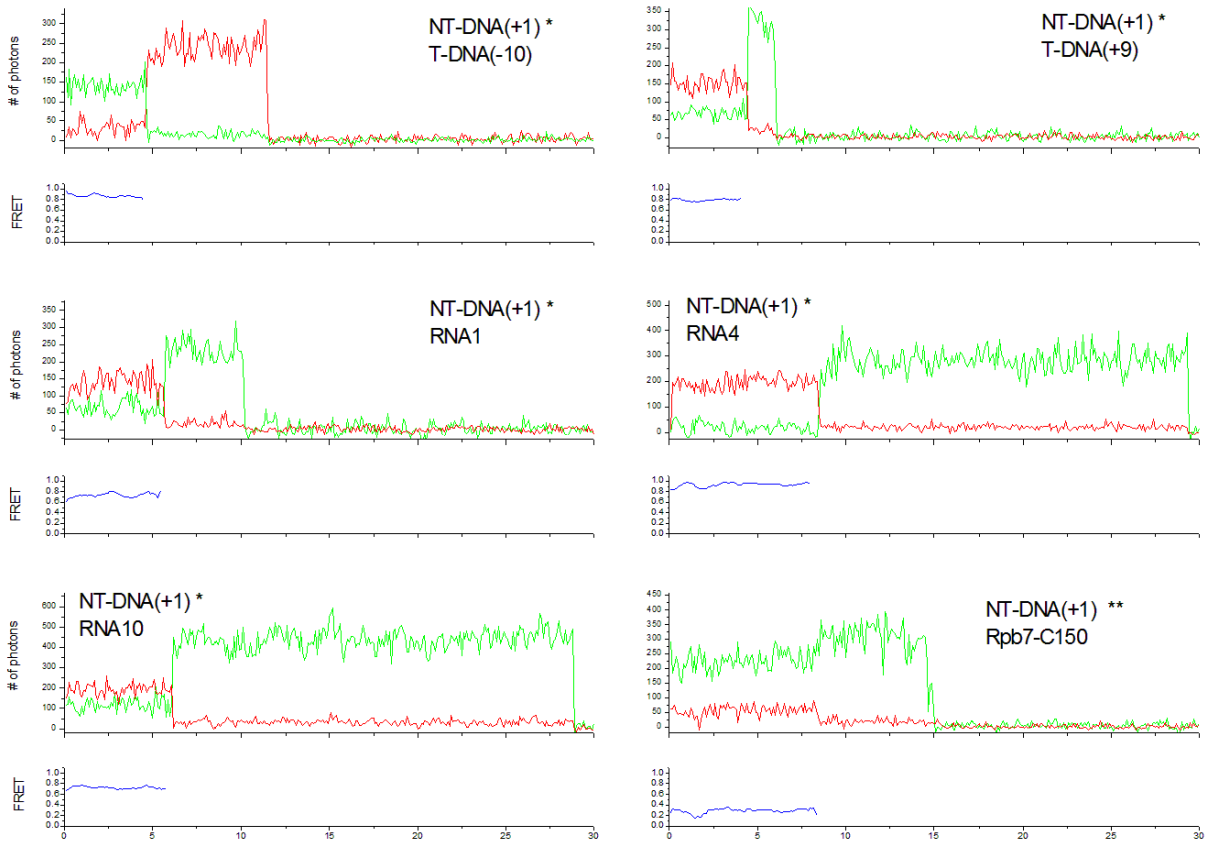
Antenna (Donor)	Satellite (Acceptor)	Anisotropy Donor	Anisotropy Acceptor	Ro (Å)	#	FRET efficiency	SE
NT-DNA(+1) *	T-DNA(-10)	0.29	0.30	54	255	86	0.00136
NT-DNA(+1) *	T-DNA(+9)	0.29	0.31	54	194	80	0.00211
NT-DNA(+1) *	RNA1	0.29	0.15	54	108	80	0.00476
NT-DNA(+1) *	RNA4	0.29	0.15	54	204	96	0.00141
NT-DNA(+1) *	RNA10	0.29	0.20	53	247	73	0.00188
NT-DNA(+1) **	Rpb7/C150	0.19	0.23	64	110	32	0.00223
NT-DNA(-2) *	T-DNA(-10)	0.31	0.30	50	465	84	0.00208
NT-DNA(-2) *	T-DNA(+3)	0.31	0.32	50	442	91	0.00202
NT-DNA(-2) *	T-DNA(+9)	0.31	0.31	50	244	75	0.00421
NT-DNA(-2) *	RNA1	0.31	0.15	50	183	64	0.00166
NT-DNA(-2) *	RNA4	0.31	0.15	50	393	90	0.0014
NT-DNA(-2) *	RNA10	0.31	0.20	49	330	76	0.00163
NT-DNA(-2) **	Rpb7/C150	0.19	0.23	62	94	35	0.0034
NT-DNA(-4) *	T-DNA(-10)	0.30	0.30	49	330	78	0.00135
NT-DNA(-4) *	T-DNA(+3)	0.30	0.32	49	281	88	0.00224
NT-DNA(-4) *	T-DNA(+9)	0.30	0.31	49	818	61	0.00179
NT-DNA(-4) *	RNA1	0.30	0.15	49	237	46	0.00166
NT-DNA(-4) *	RNA4	0.30	0.15	49	342	81	0.00117
NT-DNA(-4) *	RNA10	0.30	0.20	48	626	57	0.00034
NT-DNA(-4) **	Rpb7/C150	0.21	0.23	64	104	36	0.00508
NT-DNA(-7) *	T-DNA(-10)	0.29	0.30	46	356	80	0.00116
NT-DNA(-7) *	T-DNA(+3)	0.29	0.32	46	522	83	0.00439
NT-DNA(-7) *	T-DNA(+9)	0.29	0.31	46	463	57	0.00305
NT-DNA(-7) *	RNA1	0.29	0.15	46	199	40	0.00214
NT-DNA(-7) *	RNA4	0.29	0.15	46	477	78	0.00159
NT-DNA(-7) *	RNA10	0.29	0.20	45	461	60	0.00275
NT-DNA(-7) **	Rpb7/C150	0.18	0.23	64	173	54	0.00277
NT-DNA(-12) *	T-DNA(+3)	0.27	0.32	49	89	53	0.00207
NT-DNA(-12) **	T-DNA(+3)	0.18	0.32	59	592	90	0.00456
NT-DNA(-12) *	T-DNA(+9)	0.27	0.31	49	257	46	0.00432
NT-DNA(-12) *	RNA1	0.27	0.15	49	142	45	0.00419
NT-DNA(-12) *	RNA4	0.27	0.15	49	295	86	0.0089
NT-DNA(-12) **	Rpb4/C73	0.18	0.27	62	132	32	0.00442
NT-DNA(-12) **	Rpb7/C150	0.18	0.23	64	139	57	0.00247
NT-DNA(-15) *	T-DNA(-10)	0.31	0.30	49	175	89	0.00094
NT-DNA(-15) *	T-DNA(+3)	0.31	0.32	49	108	38	0.00612
NT-DNA(-15) **	T-DNA(+3)	0.16	0.32	60	107	68	0.00487
NT-DNA(-15) *	T-DNA(+9)	0.31	0.31	49	234	30	0.0045
NT-DNA(-15) *	RNA1	0.31	0.15	49	290	33	0.00499
NT-DNA(-15) *	RNA4	0.31	0.15	49	412	63	0.00458
NT-DNA(-15) **	Rpb4/C73	0.16	0.27	63	73	29	0.00613
NT-DNA(-15) **	Rpb7/C150	0.16	0.23	64	129	48	0.00202
NT-DNA(-18) *	T-DNA(-10)	0.27	0.30	48	1315	68/35[§]	0.005/0.011[§]
NT-DNA(-18) **	T-DNA(+3)	0.17	0.32	58	378	40/60[§]	0.004/0.007[§]
NT-DNA(-18) **	T-DNA(+9)	0.17	0.31	58	471	37/71[§]	0.004/0.014[§]
NT-DNA(-18) *	RNA1	0.27	0.15	48	325	27/56[§]	0.004/0.004[§]
NT-DNA(-18) *	RNA4	0.27	0.15	48	99	51/35[§]	0.007/0.016[§]
NT-DNA(-18) *	RNA10	0.27	0.20	48	555	63/31[§]	0.004/0.012[§]
NT-DNA(-18) **	Rpb7/C150	0.17	0.23	62	94	31/39[§]	0.027/0.013[§]

* alexa 555 used as donor
** TMR used as donor
§ second peak

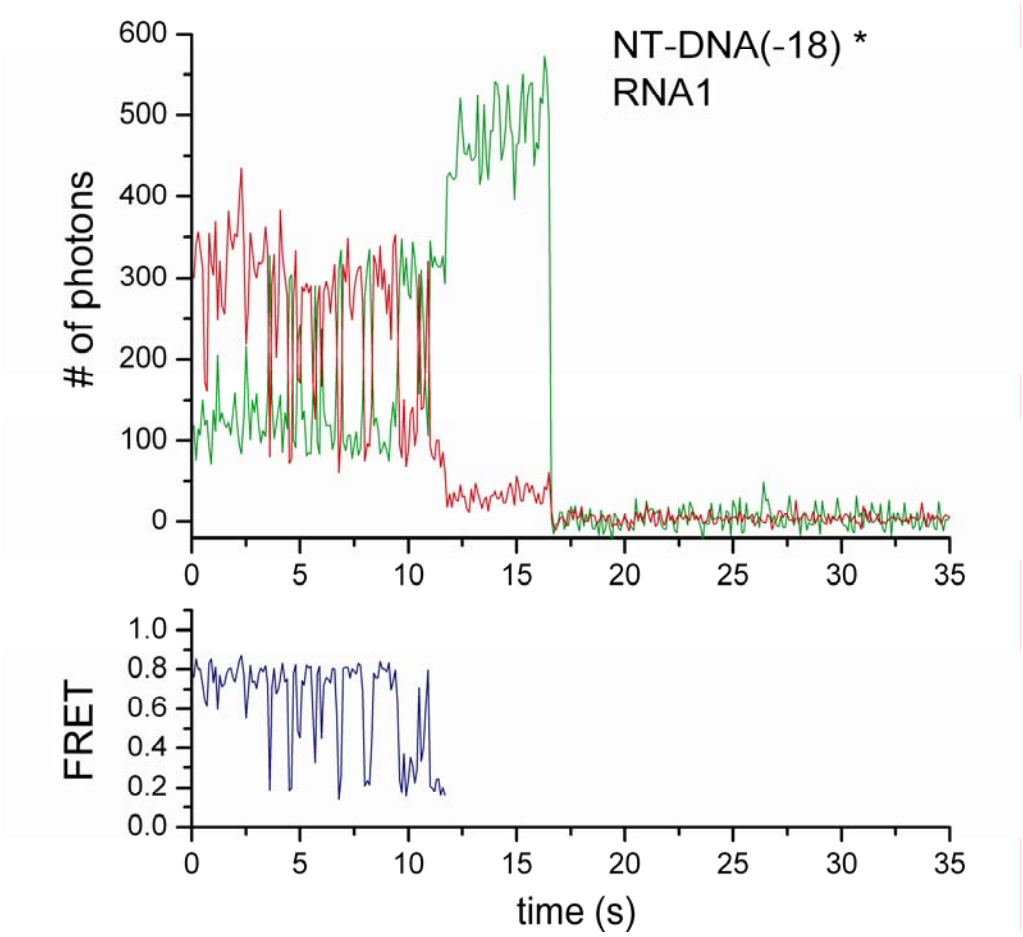
Supplementary Table 1: Overview of experimental data



Supplementary Figure 1: Exemplary single-molecule data.

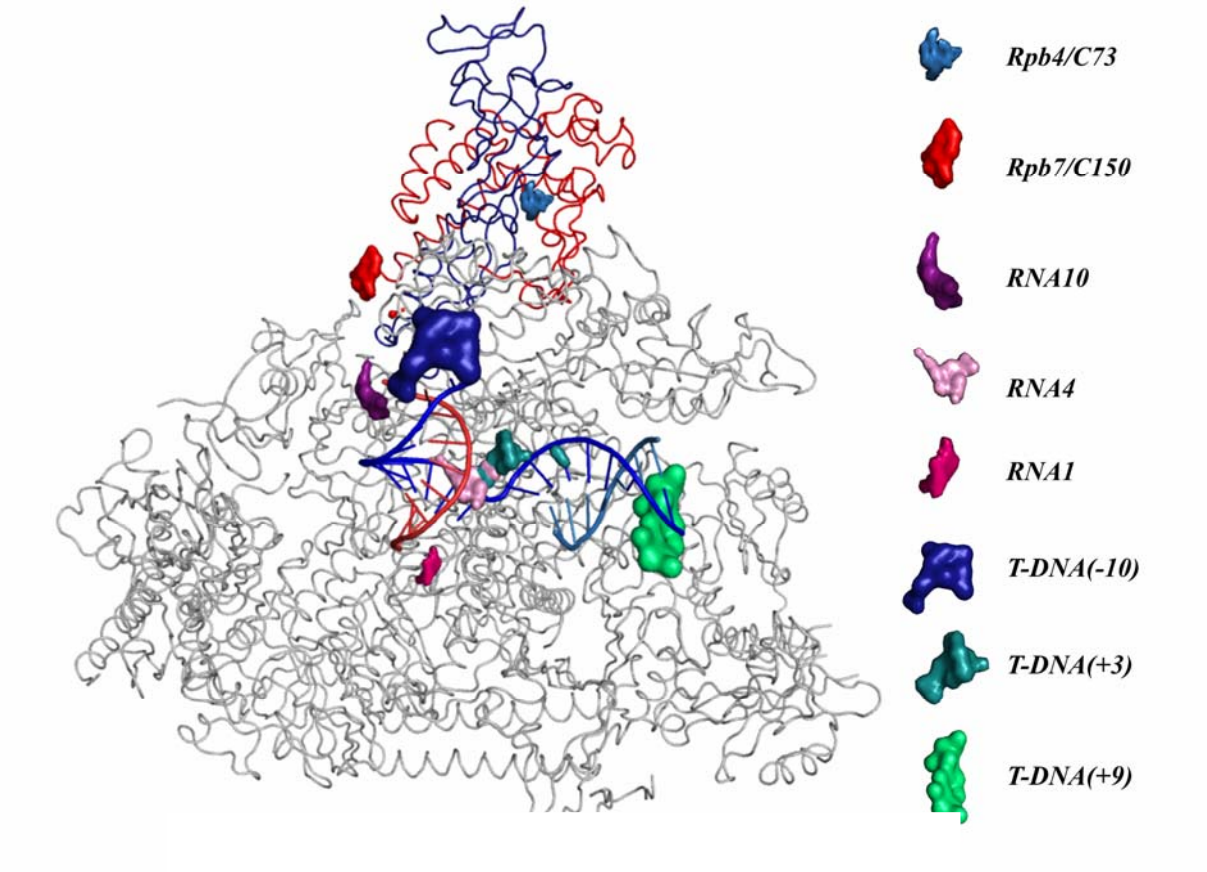
Donor fluorescence (green), acceptor fluorescence (red) and calculated FRET efficiency are shown for different donor-acceptor positions (as indicated). Integration time for all traces was 100ms. For calculation of FRET efficiency a 10 point boxcar averaging was applied.

* alexa 555 used as donor, ** TMR used as donor.



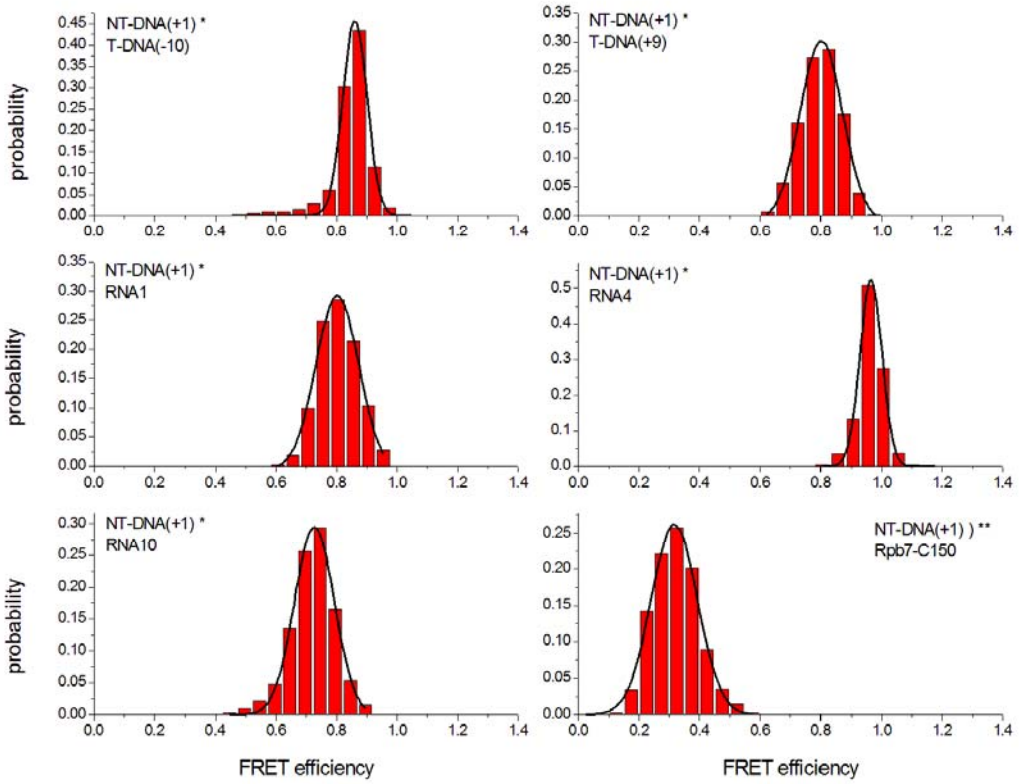
Supplementary Figure 2: Dynamic switching.

The upstream DNA becomes increasingly flexible. For the position -18 dynamic switching was observed. The figure shows an exemplary trace recorded with 100ms integration time. Fluorescence intensity of donor (green) and acceptor (red) are shown as a function of time. Here, in order to capture the dynamics in the analysis, no filtering of the data was applied.

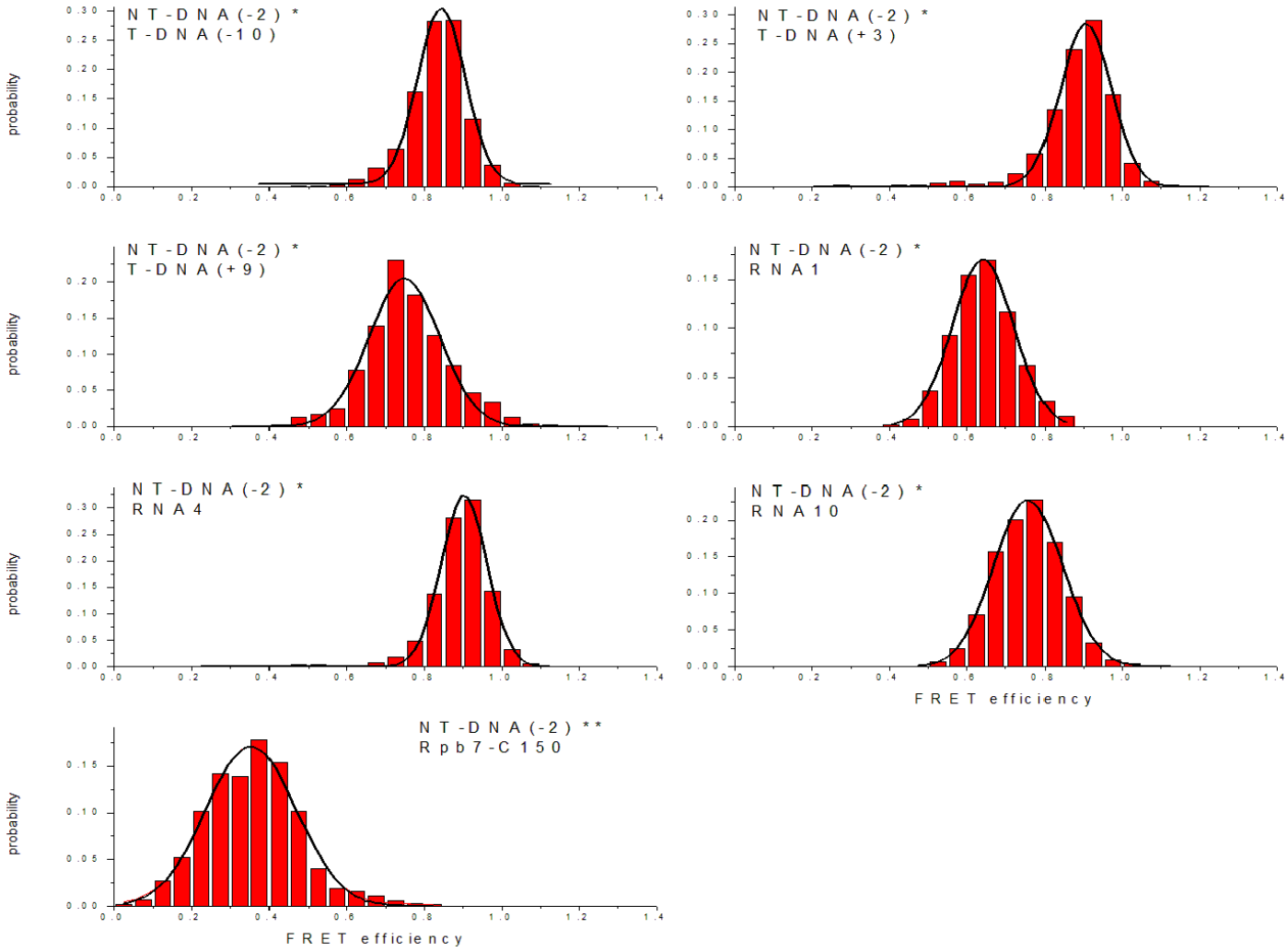


Supplementary Figure 3: Locations of the 'satellite dye molecule' positions.

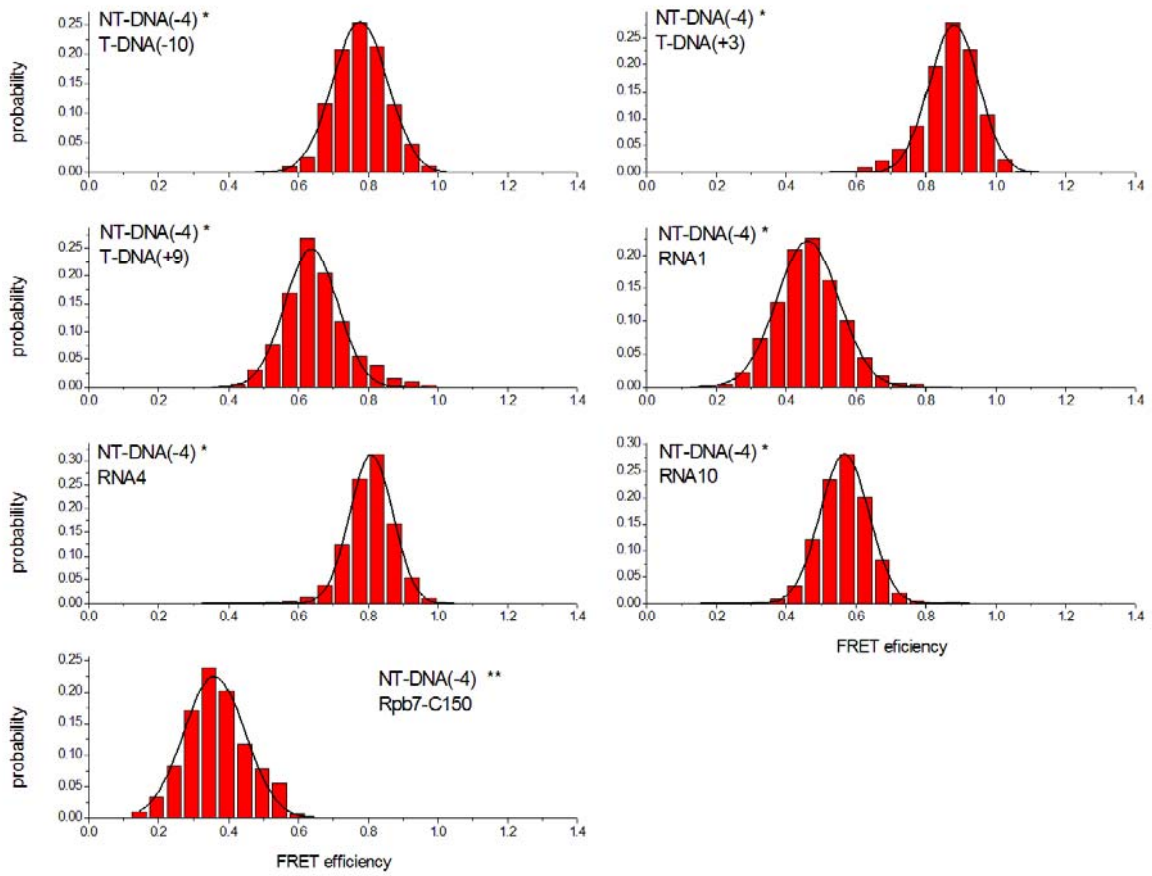
Top view of the elongation complex with Pol II core (grey), Rpb4 (blue), Rpb7 (red), template DNA strand (blue), non-template DNA strand (cyan) and RNA product (red). The location of the satellite dye molecule positions is indicated by the small icons.



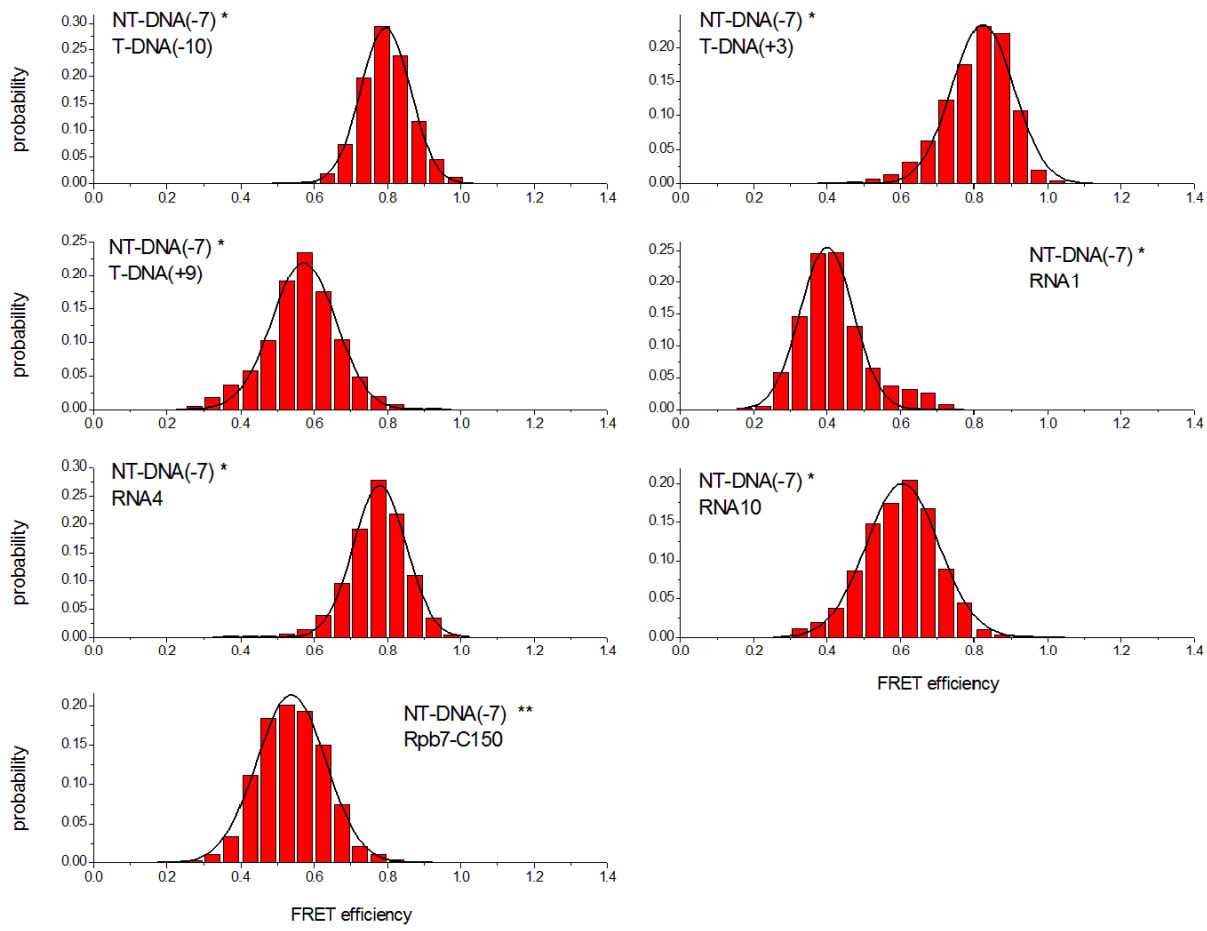
Supplementary Figure 4: Histograms of FRET efficiencies for ADM at position +1. Histograms for the SDM attached to different known positions on the EC (as indicated) are shown. All histograms were fitted with single Gaussians. The extracted data are summarised in Supplementary Table 1. * alexa 555 used as donor, **TMR used as donor.



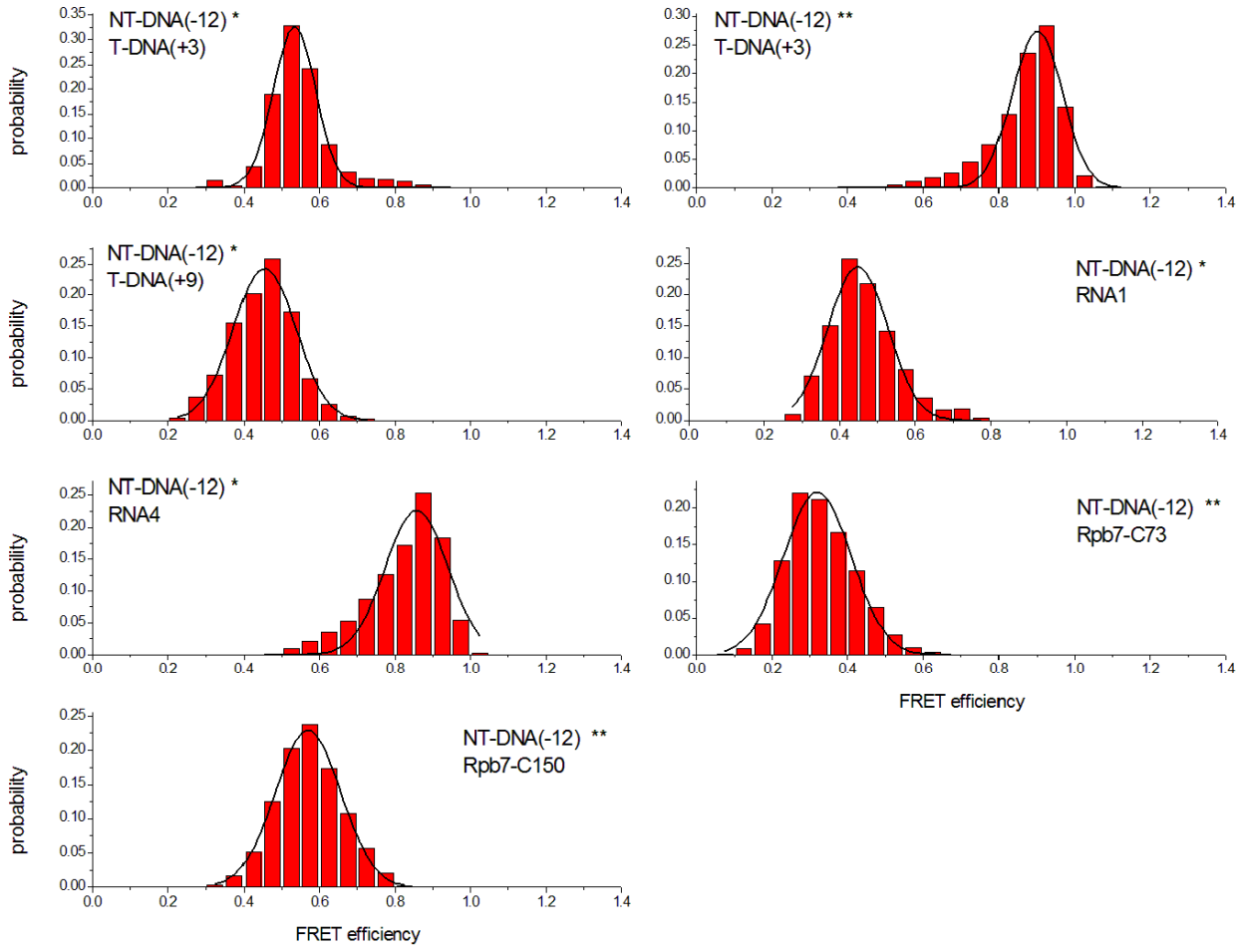
Supplementary Figure 5: Histograms of FRET efficiencies for ADM at position -2. Histograms for the SDM attached to different known positions on the EC (as indicated) are shown. All histograms were fitted with single Gaussians. The extracted data are summarised in Supplementary Table 1. * alexa 555 used as donor, ** TMR used as donor.



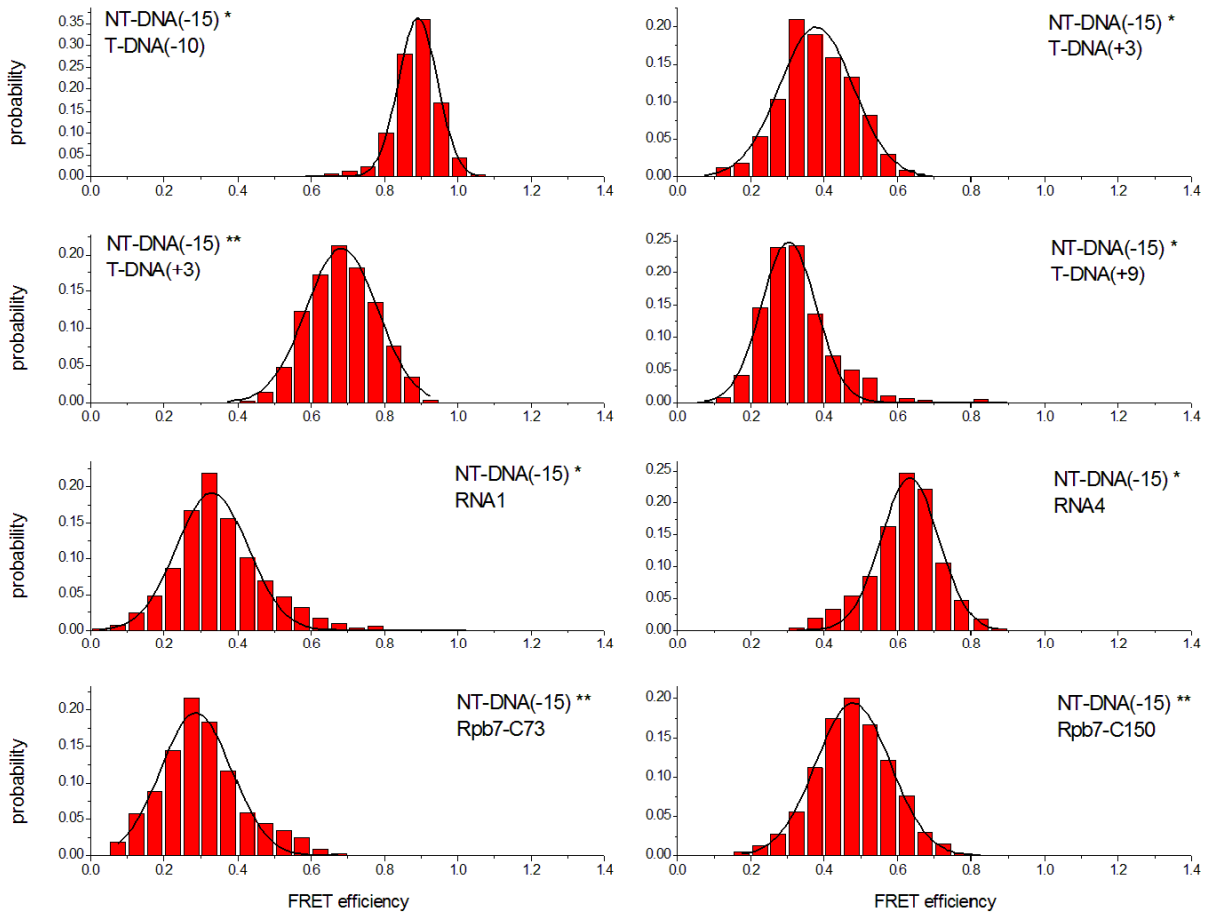
Supplementary Figure 6: Histograms of FRET efficiencies for ADM at position -4.
Histograms for the SDM attached to different known positions on the EC (as indicated) are shown. All histograms were fitted with single Gaussians. The extracted data are summarised in Supplementary Table 1. * alexa 555 used as donor, ** TMR used as donor.



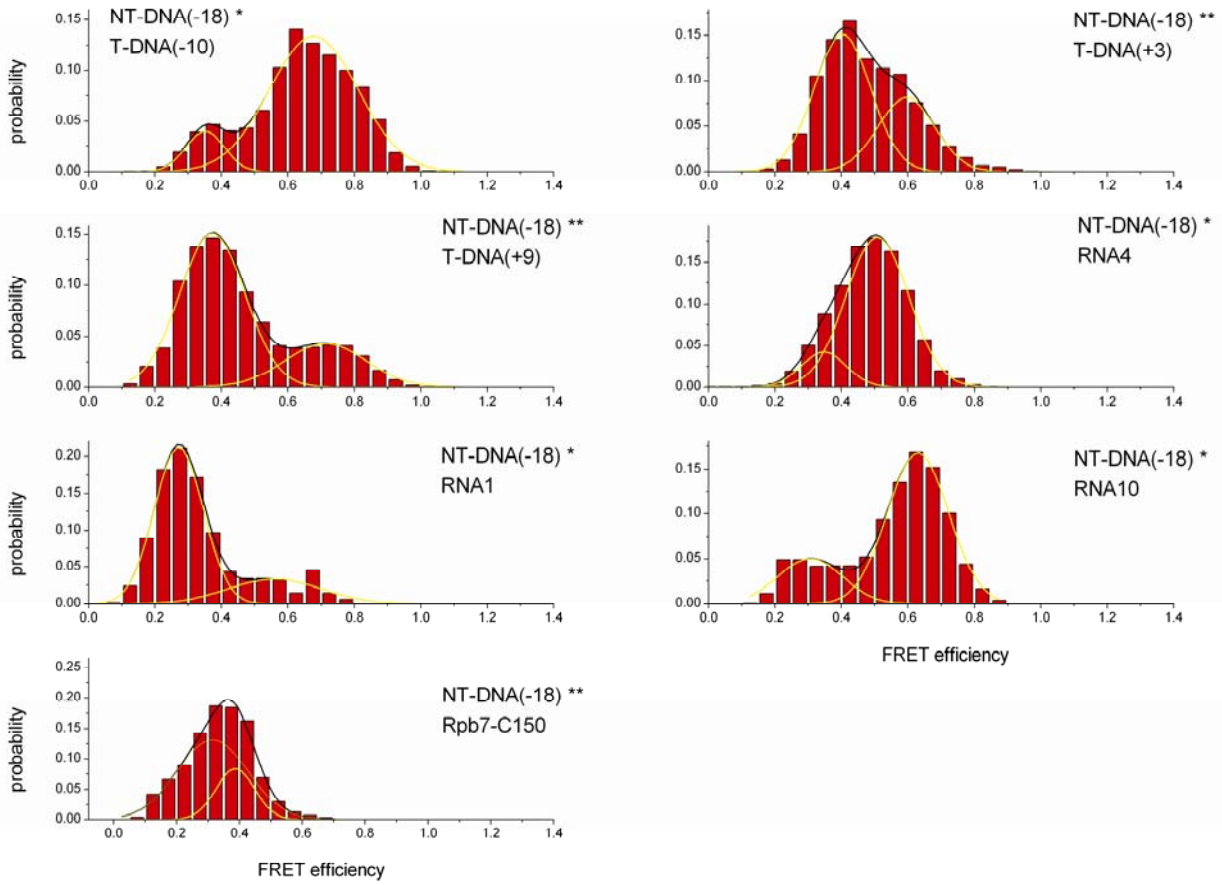
Supplementary Figure 7: Histograms of FRET efficiencies for ADM at position -7. Histograms for the SDM attached to different known positions on the EC (as indicated) are shown. All histograms were fitted with single Gaussians. The extracted data are summarised in Supplementary Table 1. * alexa 555 used as donor, **TMR used as donor.



Supplementary Figure 8: Histograms of FRET efficiencies for ADM at position -12. Histograms for the SDM attached to different known positions on the EC (as indicated) are shown. All histograms were fitted with single Gaussians. The extracted data are summarised in Supplementary Table 1. * alexa 555 used as donor, ** TMR used as donor.



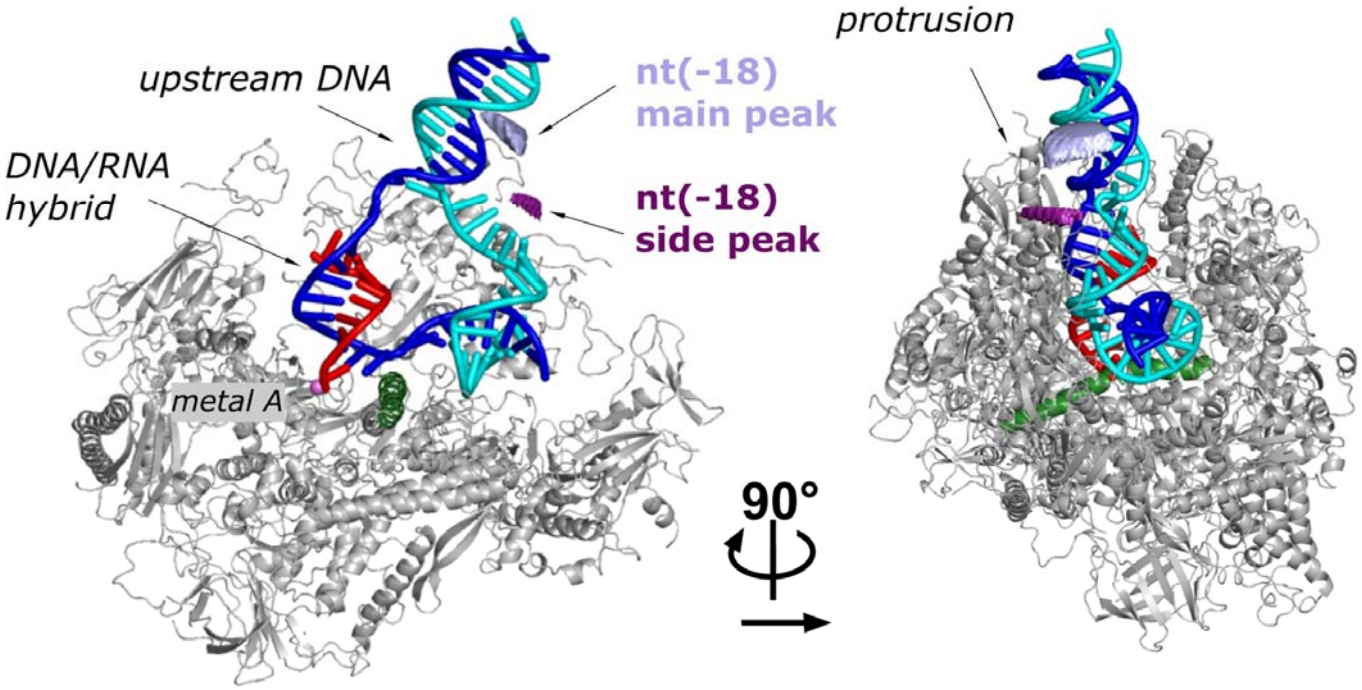
Supplementary Figure 9: Histograms of FRET efficiencies for ADM at position -15. Histograms for the SDM attached to different known positions on the EC (as indicated) are shown. All histograms were fitted with single Gaussians. The extracted data are summarised in Supplementary Table 1. * alexa 555 used as donor, ** TMR used as donor.



Supplementary Figure 10: Histograms of FRET efficiencies for ADM at position -18

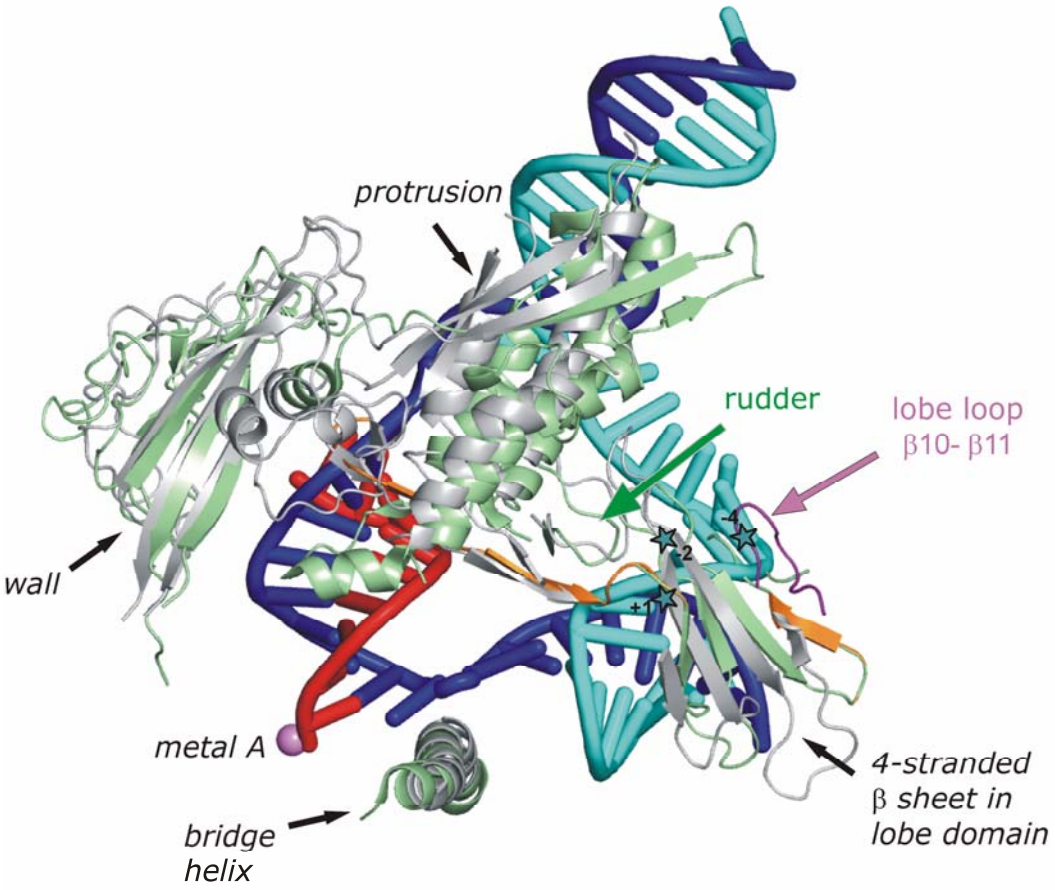
Histograms for the SDM attached to different known positions on the EC (as indicated) are shown. The single-molecule time-traces showed dynamic switching between two states (Supplementary Figure 2), therefore, all histograms were fitted with single Gaussians. The extracted data are summarised in Supplementary Table 1.

* alexa 555 used as donor, ** TMR used as donor.



Supplementary Figure 11: Two observed positions of upstream DNA.

For the most upstream ADM attached to nontemplated DNA at postion -18 dynamic switching between two states was observed (Supplementary Figure 2), resulting in double peaked histograms (Supplementary Figure 10). Thus, we used NPS to model both positions. The most likely position which accounts for about 70% of the data was used for building the model of the upstream DNA. The position of the sidepeak in the histograms (~30% probability) is shifted by about 10Å towards the protrusion as displayed in this figure. The presentation uses the same orientations as in Figures 2 and 4 of the main paper. Displayed are 38% credibility volumes for both positions together with the model of the nontemplate and upstream DNA.



Supplementary Figure 12: Comparison to crosslinking in the bacterial polymerase

Overlay of the elongation complex structure of Pol II from yeast (grey, 1Y1W) and the Thermus thermophilus enzyme (green, 1IW7) using side view. Chemical crosslinking between bases at positions +1, -2 and -4 (indicated by stars) were observed to residues 130-183 of the β subunit (orange). The crosslinking regions agree perfectly with our model for the course of the nontemplate DNA.