Supplementary Information

Wrapping of single-stranded DNA by Replication Protein A and modulation through phosphorylation

Rahul Chadda[1,#], Vikas Kaushik[1,#], Iram Munir Ahmad[2], Jaigeeth Deveryshetty[1], Alex Holehouse[3], Snorri Th.d Sigurdsson[2], Brian Bothner[4], Reza Dastvan[1], Sofia Origanti[5], and Edwin Antony[1,*]

Supplementary Tables 1-5

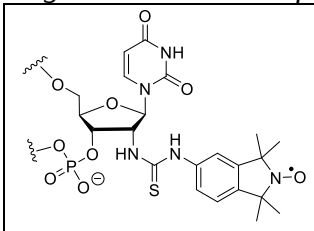
Supplementary Figures 1-7

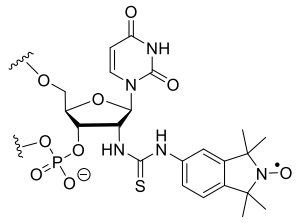
Supplementary Table 1. Oligonucleotides used in this study

Oligonucleotides used for the smFRET experiments

5′ Cy5-(dT)$_{15}$-Cy3 3′	Cy5-TTT TTT TTT TTT TTT-Cy3
5′ Cy5-(dT)$_{30}$-Cy3 3′	Cy5-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT -Cy3
5′ Cy5-(dT)$_{45}$-Cy3 3′	Cy5-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT -Cy3
5′ Cy5-(dT)$_{60}$-Cy3 3′	Cy5-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT -Cy3
5′ Cy5-(dT)$_{80}$-Cy3 3′	Cy5-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TT -Cy3
5′ Cy5-(dT)$_{97}$-Cy3 3′	Cy5-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T -Cy3

Oligonucleotides used for the DEER experiments

 **(M)**	**(dT)$_{22}$** **DEER**	5'-d(**M** TT TTT TTT TTT TTT TTT TTT TT **M** T)-3'

 **(M)**	**(dT)$_{50}$** **DEER**	5'-d (TTT **M**TT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT **M**T) 3'

Supplementary Table 2. Composition of media and reagents used for Phosphoserine incorporation media.

A) ZY-non inducing media (ZY-NIM)

Components	For 50 mL
ZY media	47.25 mL
25x M-salts	0.1 mL
1 M $MgSO_4$	2 mL
40 % (w/v) α-D-glucose	0.625 mL
Trace metals solution (5000x)	0.01 mL

B) ZY-auto inducing media (ZY-AIM)

Components	For 1 L
ZY media	940 mL
1 M $MgSO_4$	2 mL
25x M-salts	40 mL
50x 5052 solution	20 mL
Trace metals solution (5000x)	0.2 mL

C) Composition of 25X M-salts

Components	For 1 L
Sodium phosphate dibasic	88.73 g
Potassium phosphate dibasic	85.05 g
Ammonium chloride	66.86 g
Sodium sulfate anhydrous	17.75 g

D) 50x 5052 solution

Components	For 1 L
α-D-glucose	2.5 g
Lactose	50 g
Glycerol (v/v)	125 mL

Supplementary Table 3. Comparison of FRET efficiencies measured using the EI-FLEX and the Picoquant MT200

	FRET efficiency		Nbursts	
	EI-FLEX	**MT200**	**EI-FLEX**	**MT200**
$(dT)_{15}$	0.83 (±0.064)	0.89 (±0.11)	765	10260
$(dT)_{30}$	0.37 (±0.086)	0.426 (±0.097)	268	9056
$(dT)_{45}$	0.15 (±0.060)	0.17 (±0.088)	366	11788
$(dT)_{60}$	0.074 (±0.049)	0.066 (±0.078)	511	18453
$(dT)_{80}$	0.021 (±0.05)	0.022 (±0.075)	426	40425
$(dT)_{97}$	0.027 (±0.37)	0.032 (±0.088)	1257	636

Supplementary Table 4. FRET efficiencies measurements (smFRET)

$(dT)_{25}$ and hRPA	**FRET efficiency**		
	Free	**Bound**	**N bursts**
$(dT)_{25}$	0.49 (±0.093)	-	1072
$(dT)_{25}$ + 0.02 nM hRPA	0.49 (±0.095)	0.07 (±0.032)	1086
$(dT)_{25}$ + 0.1 nM hRPA	0.49 (±0.11)	0.075 (±0.047)	852
$(dT)_{25}$ + 1 nM hRPA	-	0.07 (±0.2)	912

$(dT)_{25}$ and yRPA	**FRET efficiency**		
	Free	**Bound**	**N bursts**
$(dT)_{25}$	0.49 (±0.093)	-	1072
$(dT)_{25}$ + 0.02 nM ScRPA	0.48 (±0.098)	0.09 (±0.031)	773
$(dT)_{25}$ + 0.1 nM ScRPA	0.47 (±0.11)	0.089 (±0.085)	783
$(dT)_{25}$ + 1 nM ScRPA	-	0.091 (±0.12)	1172

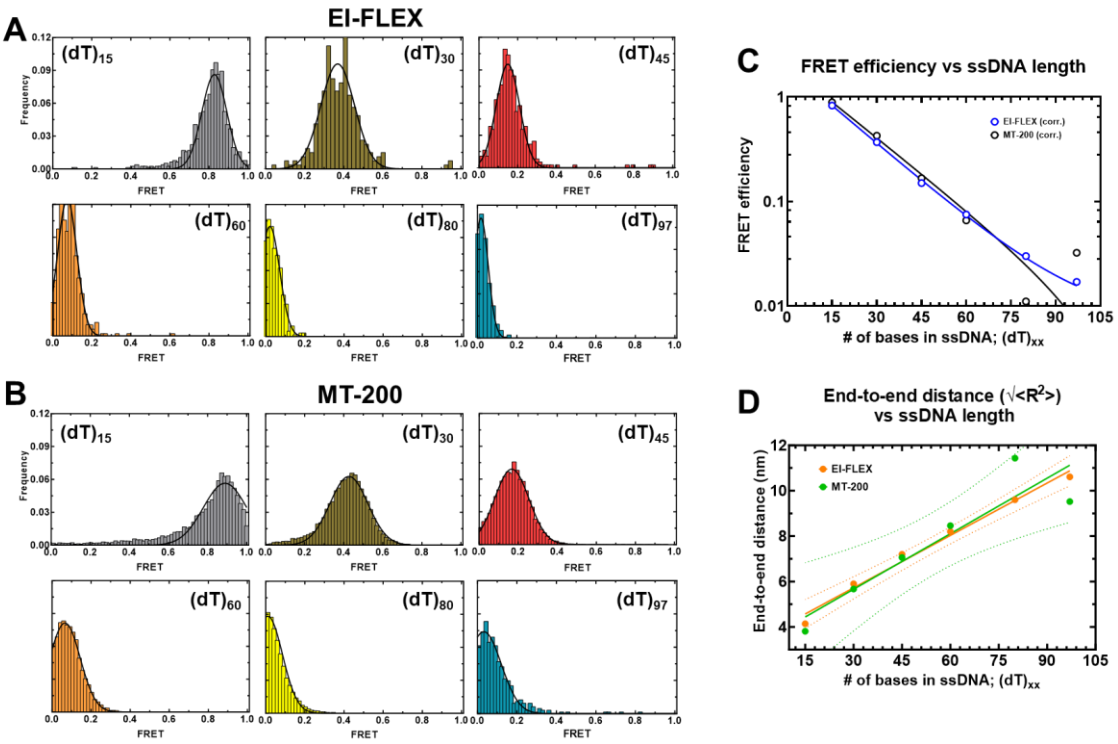
Supplementary Table 5. FRET efficiencies measurements (smFRET)

$(dT)_{30}$ and hRPA	**FRET efficiency**		
	Free	**Bound**	**N bursts**
$(dT)_{30}$	0.37 (±0.098)	-	585
$(dT)_{30}$ + 0.03 nM hRPA	0.38 (±0.095)	0.04 (±0.055)	654
$(dT)_{30}$ + 0.1 nM hRPA	0.37 (±0.092)	0.025 (±0.068)	568
$(dT)_{30}$ + 1 nM hRPA	-	0.055 (±0.047)	569

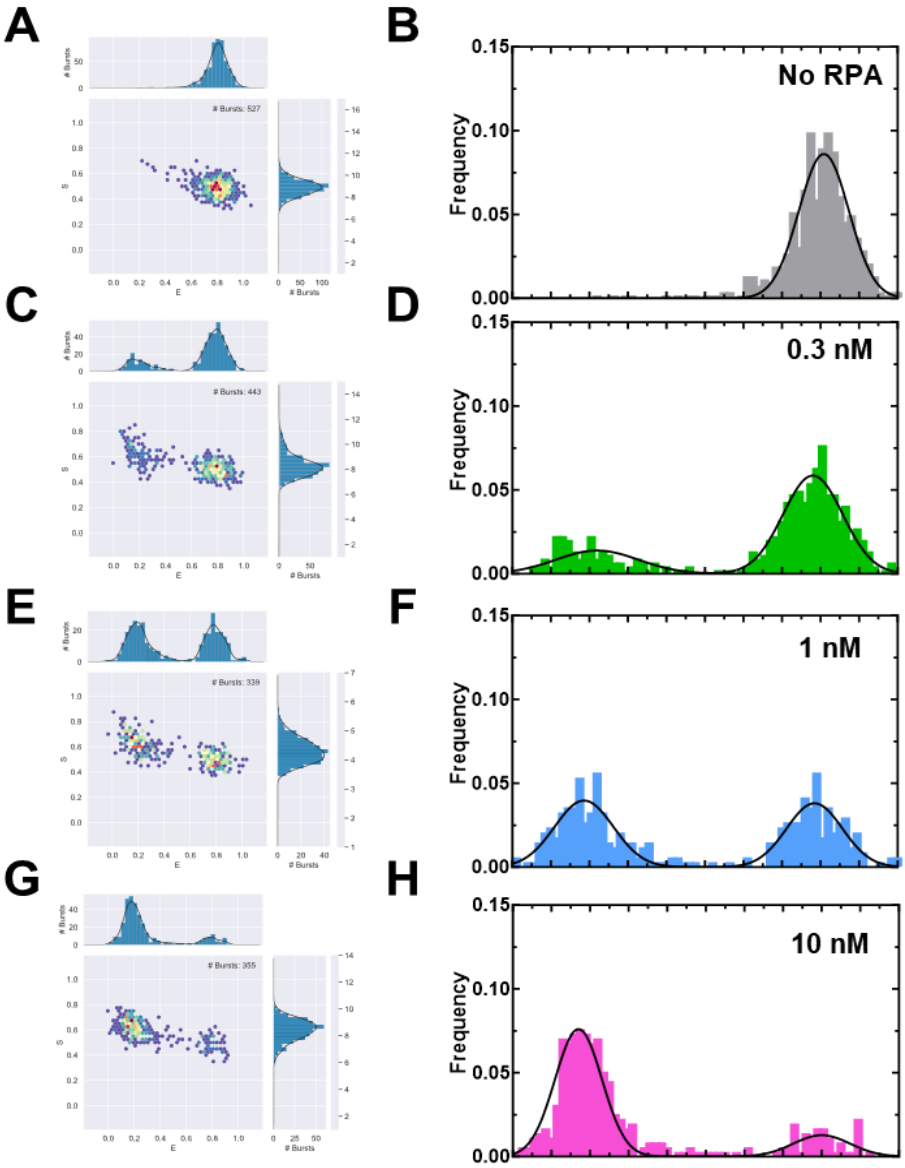
$(dT)_{45}$ and hRPA	**FRET efficiency**		
	Free	**Bound**	**N bursts**
$(dT)_{45}$	0.17 (±0.075)	-	1108
$(dT)_{45}$ + 0.03 nM hRPA	0.17 (±0.081)	0.02 (±0.044)	773
$(dT)_{45}$ + 0.1 nM hRPA	0.17 (±0.059)	0.021 (±0.033)	779
$(dT)_{45}$ + 1 nM hRPA	-	0.02 (±0.035)	677

Supplementary Table 6. DEER measurements

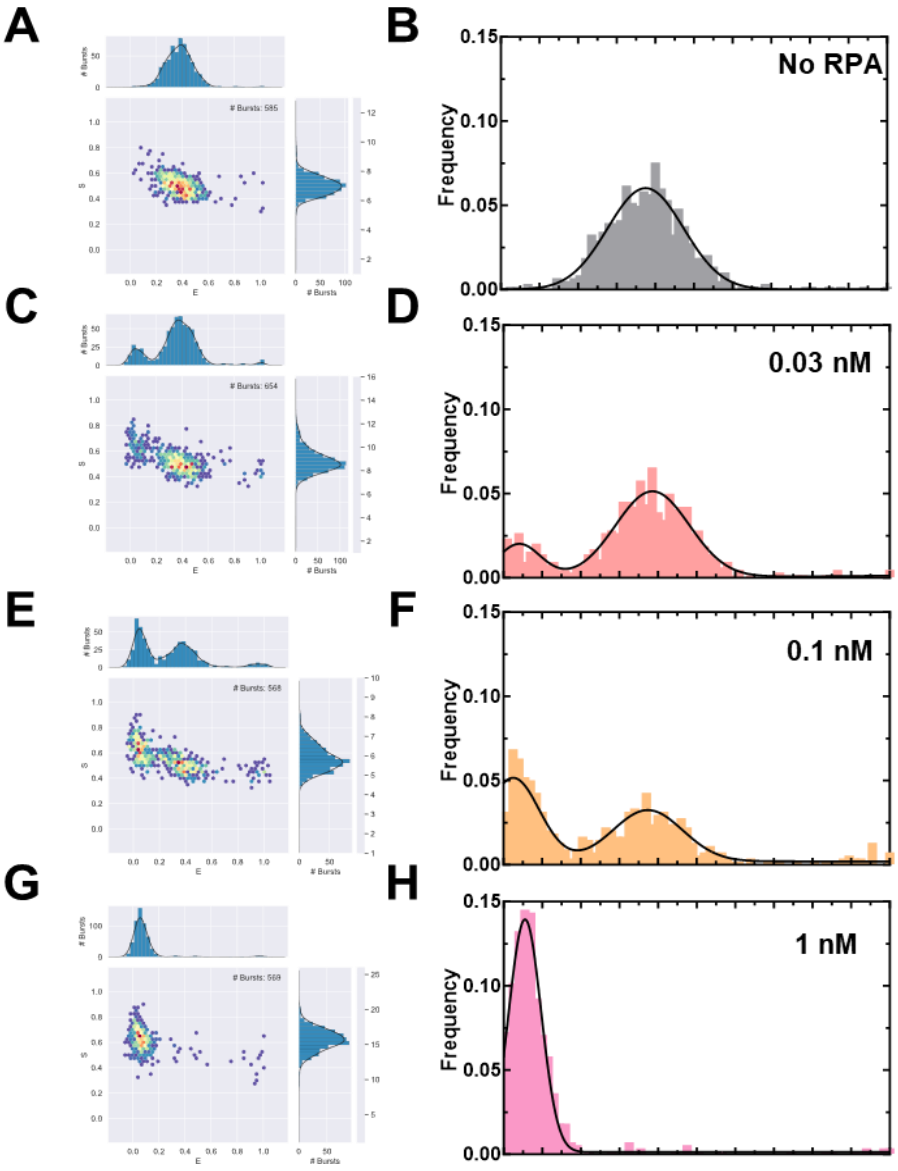
	<*R*> [nm]	σ [nm]	*f* (%)
$(dT)_{22}$	3.1±1.0	0.7±0.6	36
	5.1±2.0	1.1±3.1	64
$(dT)_{22}$+hRPA	4.6±0.4	1.0±0.7	5
	6.8±0.8	0.4±1.2	95
$(dT)_{22}$+hRPA^{pSer384}	4.6±0.4	1.0±0.7	30
	6.8±0.8	0.4±1.2	70
$(dT)_{22}$+yRPA	6.9±0.3	1.0±0.3	100



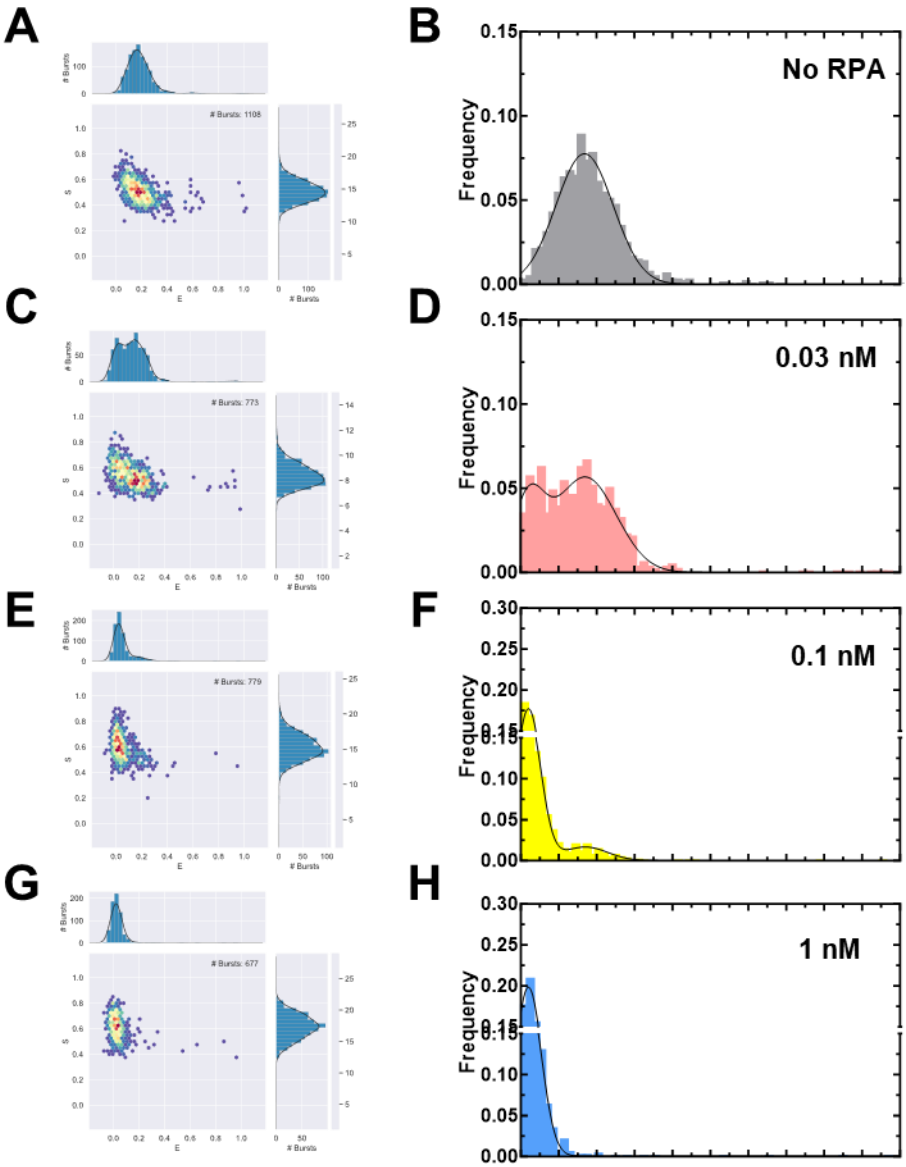
Supplementary Figure 1. Comparison of smFRET data collected using the EI-FLEX and MT-200 microscopes. smFRET data collected for various lengths of ssDNA using the **A)** EI-FLEX and **B)** Picoquant MT-200 microscopes. **C)** Plot of the FRET efficiencies versus ssDNA shows excellent agreement between measurements made using both instruments. **D)** Similarly, end-to-end distance measurements calculated from the smFRET data show excellent agreement for both instruments.



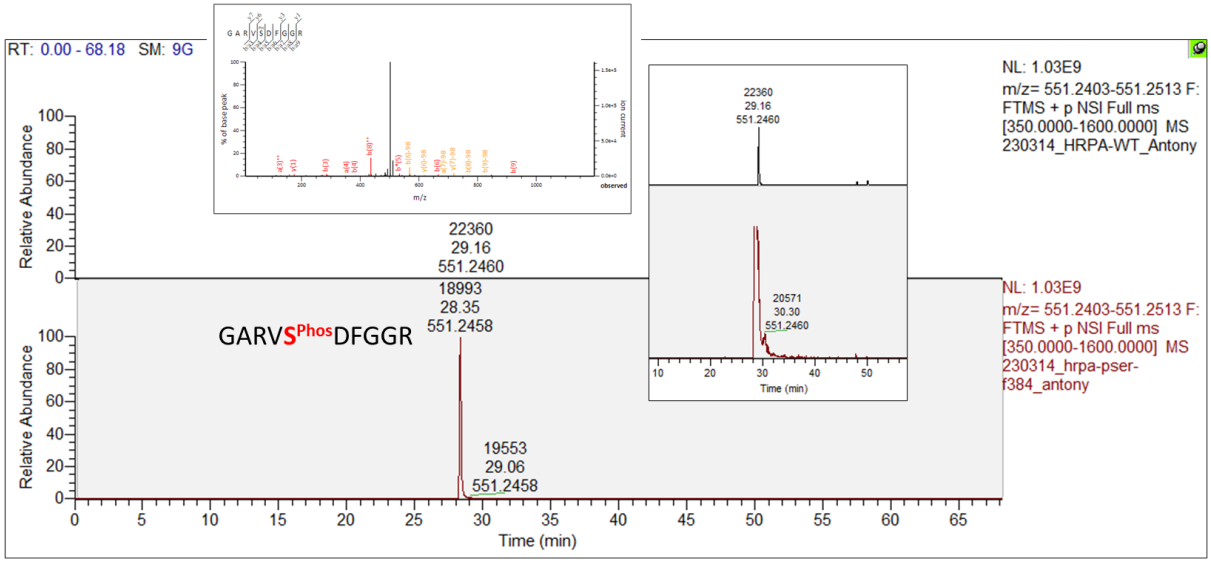
Supplementary Figure 2. smFRET data for RPA-(dT)$_{15}$ complexes. smFRET data collected for the (dT)$_{15}$ substrate in the absence of RPA **(A & B)** and increasing concentrations of RPA **(C-H)**.



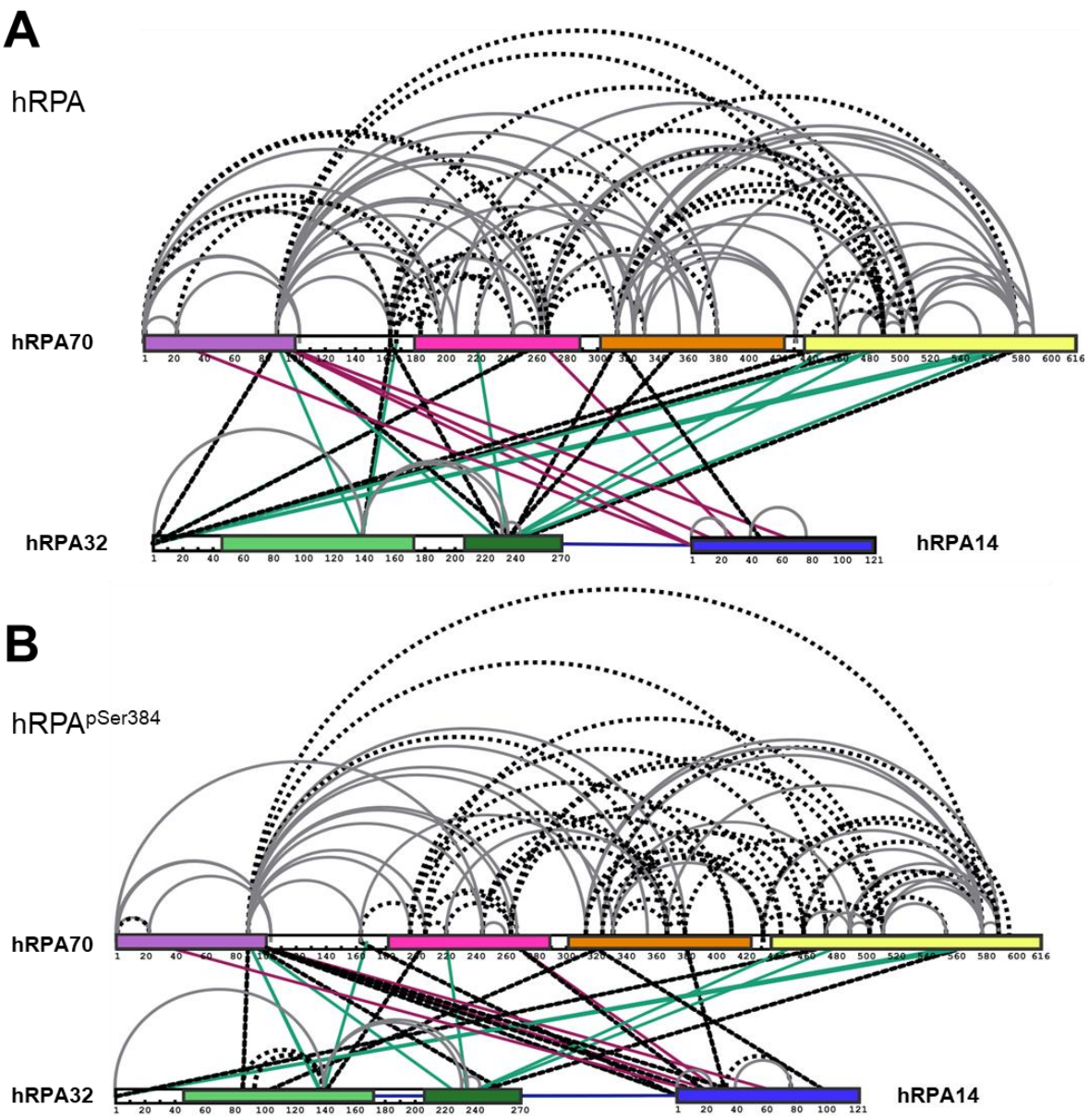
Supplementary Figure 3. smFRET and mass photometry data for RPA-$(dT)_{30}$ complexes. smFRET data collected for the $(dT)_{30}$ substrate in the absence of RPA **(A & B)** and increasing concentrations of RPA **(C-H)**.



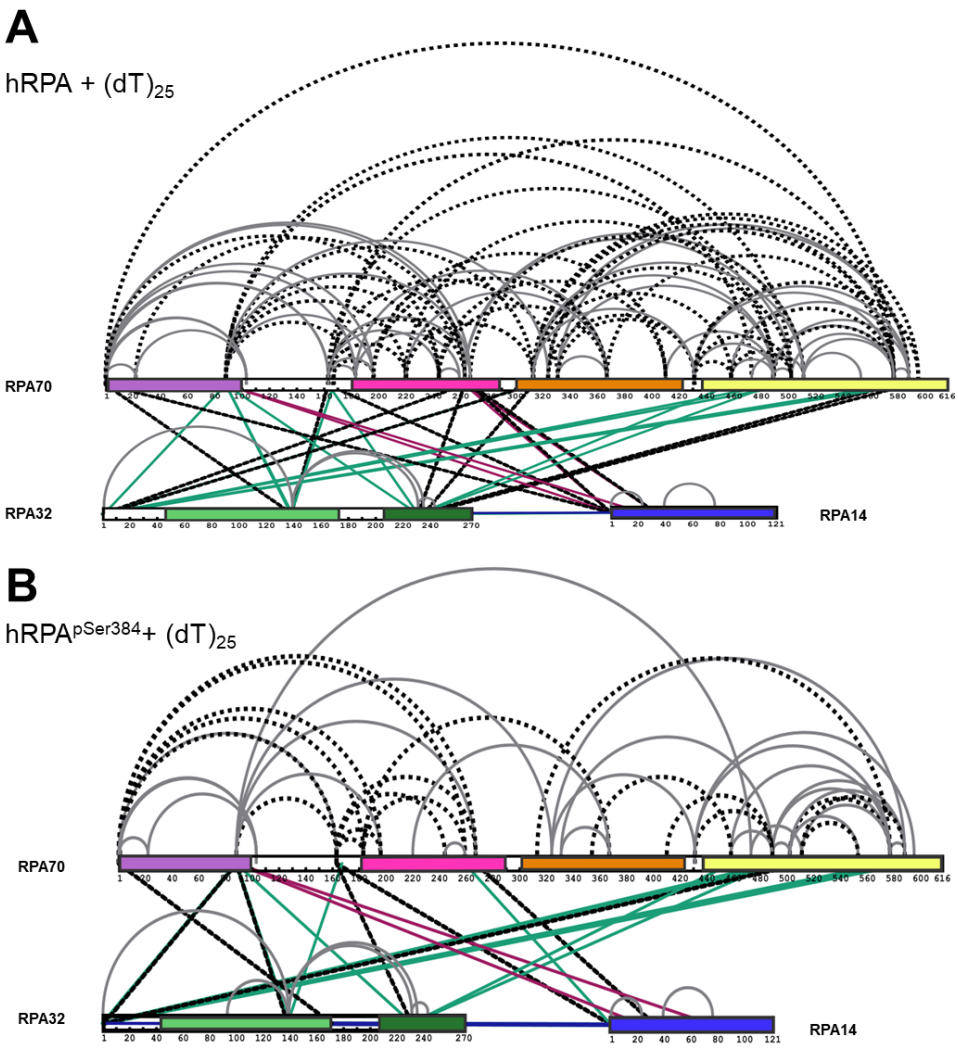
Supplementary Figure 4. smFRET and mass photometry data for RPA-(dT)$_{45}$ complexes. smFRET data collected for the (dT)$_{45}$ substrate in the absence of RPA **(A & B)** and increasing concentrations of RPA **(C-H)**.



Supplementary Figure 5. Mass spectrometry analysis of pSer incorporation in hRPA. Mass spectrometry analysis of RPA-pSer^{384} shows site-specific phosphoserine incorporation.



Supplementary Figure 6. Unique XLs in the RPA introduced by phosphorylation at Ser-384 in RPA70. Crosslinking mass spectrometry (XL-MS) analysis of **A)** RPA and **B)** RPA-pSer^{384} are shown. The two datasets are compared relative to each other and the crosslinks unique to each sample are shown in dotted black lines. Crosslinks in grey are common to both datasets and depict intra-subunit crosslinks within RPA70-, RPA32 and RPA14. Inter-subunit crosslinks between RPA70, RPA32, and RPA14 are shown in green and red, respectively.



Supplementary Figure 7. Unique XLs in the RPA-ssDNA complex introduced by phosphorylation at Ser-384 in RPA70. Crosslinking mass spectrometry (XL-MS) analysis of **A)** RPA-ssDNA and **B)** RPA-pSer^{384}-ssDNA complexes are shown. The two datasets are compared relative to each other and the crosslinks unique to each sample are shown in dotted black lines. Crosslinks in grey are common to both datasets and depict intra-subunit crosslinks within RPA70-, RPA32 and RPA14. Inter-subunit crosslinks between RPA70, RPA32, and RPA14 are shown in green and red, respectively.