FIGURE LEGENDS

Fig. S1. Kinetics of PCNA opening. Opening of PCNA-WC^{AEDANS} was measured as described in Fig. 2A, except at higher RFC concentration (final concentrations: 2 μM RFC, 0.25 μM PCNA and 0.5 mM ATP). A 2-exponential fit of the data yields $k_{open(1)} = 2.5\ s^{-1}$ and $k_{open(2)} = 0.6\ s^{-1}$.

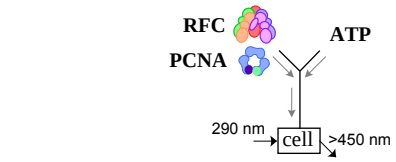
Fig. S2. DNA binding to RFC measured under various conditions. (A) $ptDNA_{TAMRA}$ (5 nM) and $ssDNA_{TAMRA}$ (5 nM, 5'-labeled 55-nt ssDNA) were titrated with RFC (0 – 50 nM) in the presence of ATPγS (0.1 mM) and PCNA (200 nM) in buffer A. The fluorescence signal (normalized to free DNA) was plotted *versus* RFC concentration, and the data fit to a quadratic function yielded $K_D \leq 1$ nM for both DNA substrates. (B) Kinetics of $ptDNA_{TAMRA}$ binding/release measured as in Fig. 3A, at Δt: 0.02 and 3s, except with higher protein concentrations (final concentrations: 0.1 or 1 μM RFC, 3 μM PCNA, 0.04 μM $ptDNA_{TAMRA}$ and 0.5 mM ATP). The exponential binding rate is 17 s^{-1} at 1 μM RFC, Δt: 3s. (C) Kinetics of $ptDNA_{TAMRA}$ binding measured as in Fig. 3A, at Δt: 10s, except with ATPγS instead of ATP in the reaction (final concentrations: 0.1 RFC, 0.4 μM PCNA, 0.04 μM $ptDNA_{TAMRA}$ and 0.1 mM ATPγS). The relative change in $ptDNA_{TAMRA}$ fluorescence intensity on binding RFC•ATPγS•PCNA is 1:2.1 (from A, C).

Fig. S3. Data from Fig. 5 shown on a log scale for time. (A) Overlay of data from PCNA opening/closing, DNA binding/release, ATP hydrolysis and Pi release experiments provides a visual of rapid ptDNA binding to RFC•ATP•PCNA complex (formed during pre-incubation, Δt:

3 s) triggering ATP hydrolysis, PCNA closure, Pi release, PCNA•ptDNA dissociation and catalytic turnover. Data for (B) PCNA opening (Δt: 0.1, 7 s), (C) PCNA opening/closing, (D) ptDNA binding/release, (E) ATP hydrolysis (Δt: 2 s), and (F) Pi release (only Δt: 0.02, 0.2, 2 s shown in C, D, F for clarity), were all fit simultaneously to the model shown in Scheme I. The black lines are simulations generated by the model based on parameters listed in Table I.

Fig. S4. Confidence contour analysis. Confidence contours for the global fit to rate constants derived by simultaneously fitting all the data to the model in Scheme I. All 6 pair wise combinations of the 4 unknown rate constants: k_2 (linked with k_{-2}, k_3, k_{-3}, k_4), k_8, k_{11}, k_{12} (linked with k_{-12}) are shown. The central red zone shows the area of good fit. The yellow band between the red and green zones delineates a 10% increase in χ^2 value, which was used to estimate the upper and lower confidence limits on each kinetic parameter listed in Table I.

Fig. S5. DNA dependence of RFC-catalyzed ATP hydrolysis and Pi release. RFC-catalyzed Pi release is measured by change in fluorescence intensity of MDCC-PBP. Pre-incubation of RFC, PCNA with ATP (Δt: 2 s) followed by addition of ptDNA results in a burst of Pi release followed by a linear steady state phase. In contrast, no burst phase is detectable with ssDNA, only a linear rate of 1.1 μM s^{-1}.



(a) RFC-catalyzed PCNA opening

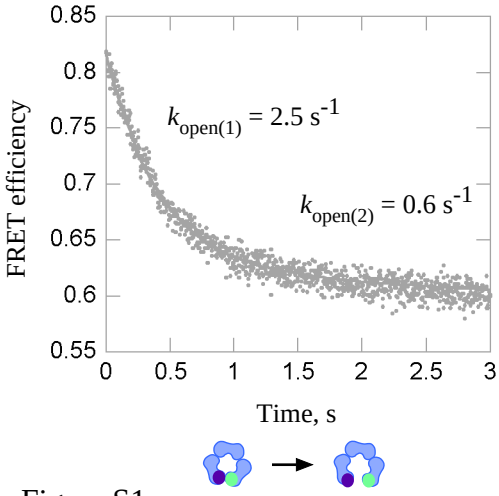


Figure S1

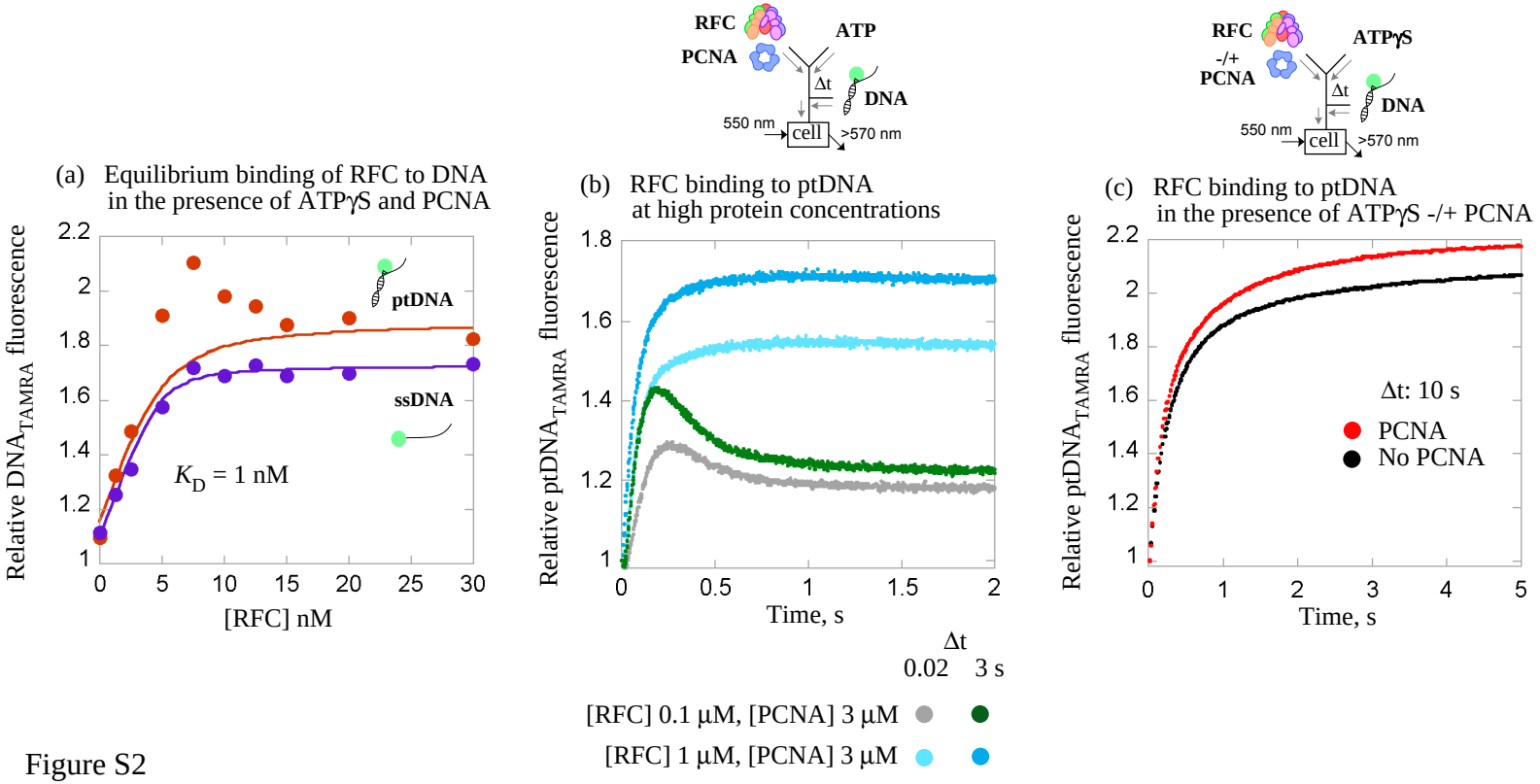

RFC
PCNA
ATP
Δt
DNA
550 nm
cell
>570 nm
(a) Equilibrium binding of RFC to DNA
in the presence of ATPγS and PCNA
Relative DNA$_{TAMRA}$ fluorescence
ptDNA
ssDNA
K_D = 1 nM
[RFC] nM
(b) RFC binding to ptDNA
at high protein concentrations
Relative ptDNA$_{TAMRA}$ fluorescence
Time, s
Δt
0.02
3 s
[RFC] 0.1 μM, [PCNA] 3 μM
[RFC] 1 μM, [PCNA] 3 μM
RFC
-/+
PCNA
ATPγS
Δt
DNA
550 nm
cell
>570 nm
(c) RFC binding to ptDNA
in the presence of ATPγS -/+ PCNA
Relative ptDNA$_{TAMRA}$ fluorescence
Δt: 10 s
PCNA
No PCNA
Time, s

Figure S2

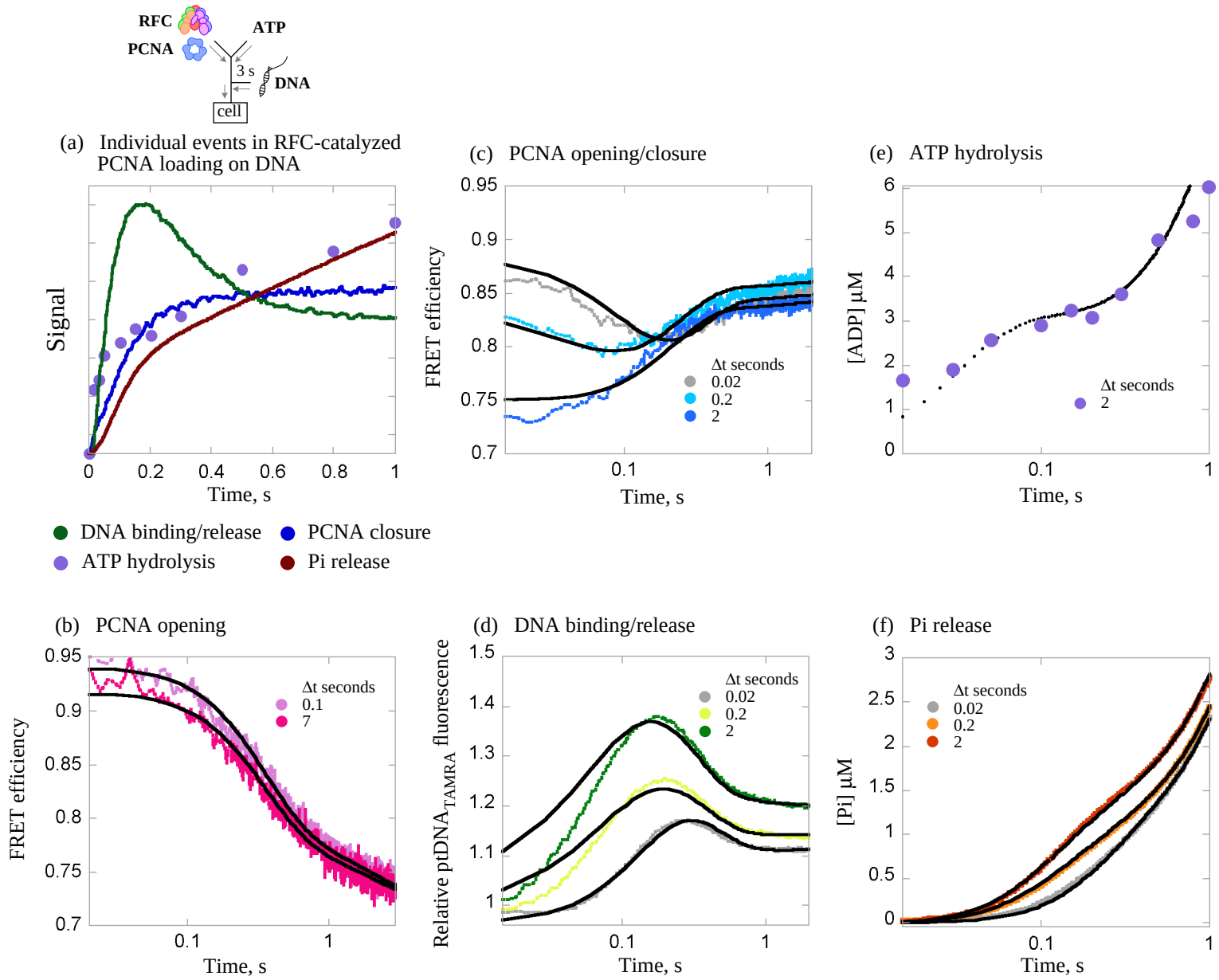

RFC
PCNA
ATP
3 s
DNA
cell
(a) Individual events in RFC-catalyzed PCNA loading on DNA
Signal
Time, s
DNA binding/release
PCNA closure
ATP hydrolysis
Pi release
(c) PCNA opening/closure
FRET efficiency
Δt seconds
0.02
0.2
2
Time, s
(e) ATP hydrolysis
[ADP] μM
Δt seconds
2
Time, s
(b) PCNA opening
FRET efficiency
Δt seconds
0.1
7
Time, s
(d) DNA binding/release
Relative ptDNA$_{TAMRA}$ fluorescence
Δt seconds
0.02
0.2
2
Time, s
(f) Pi release
[Pi] μM
Δt seconds
0.02
0.2
2
Time, s

Figure S3

Confidence contours for unknown rate constants derived from global fitting of kinetic data to a minimal model (Scheme I)

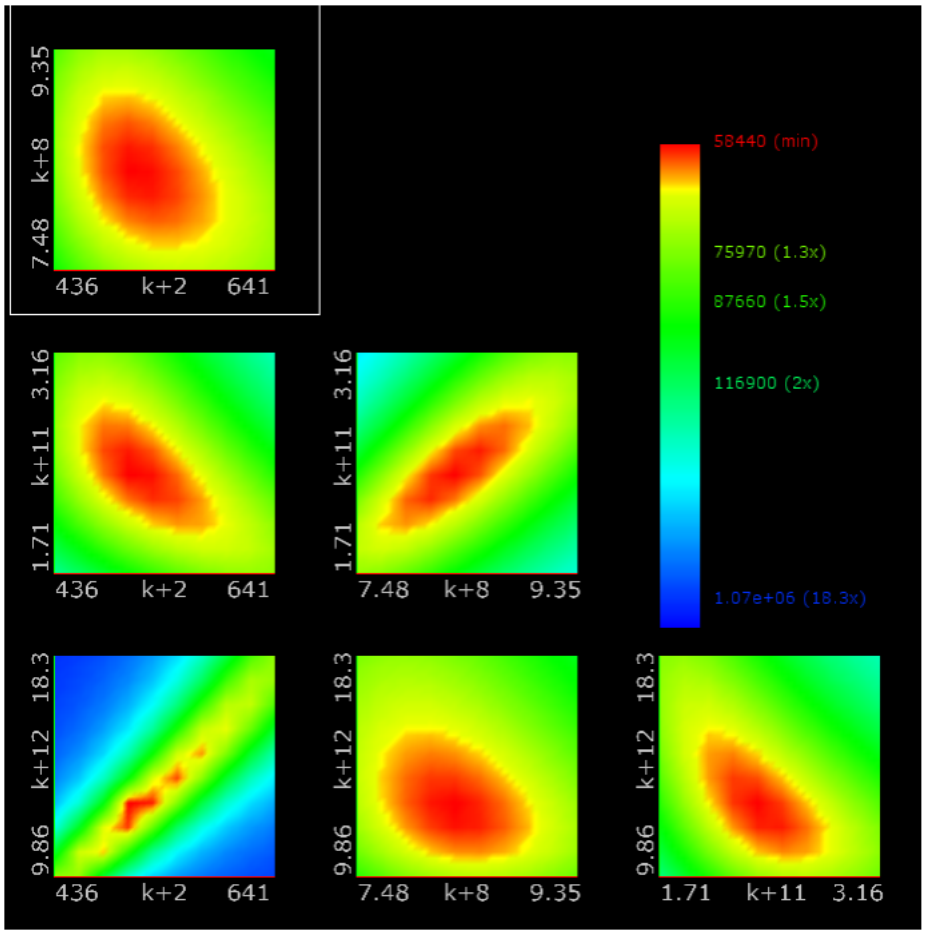
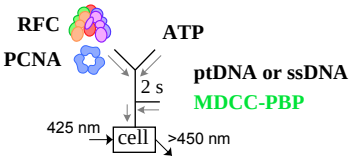


Figure S4


RFC
PCNA
ATP
ptDNA or ssDNA
MDCC-PBP
2 s
425 nm
cell
>450 nm

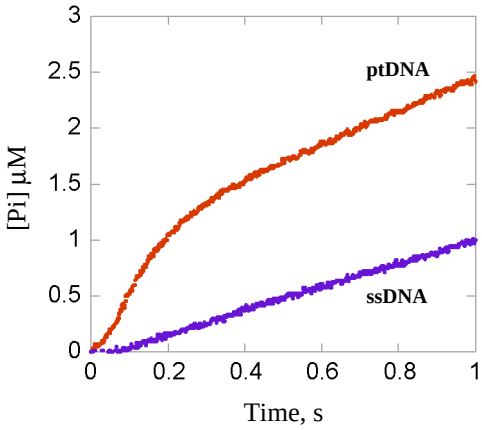

RFC-catalyzed ATP hydrolysis and phosphate (Pi) release
[Pi] µM
Time, s
ptDNA
ssDNA
0
0.5
1
1.5
2
2.5
3
0.2
0.4
0.6
0.8

Figure S5