# Supplementary materials.

**Table S1. ATPase rates ($min^{-1}$) of RecF in the presence of different DNA substrates and RecR in the conditions with a crowding agent (5% PEG10K). 33 μM of the DNA substrate was added to 10 μM of RecF and a sample of the reaction mixture was removed at different time points, quenched and Pi release was measured with Malachite Green assay. 20 μM of RecR was included where indicated. "ss" and "ds" represent ssDNA and dsDNA, respectively, with the length of oligonucleotides in parenthesis. "ds/ss" is a junction DNA with 15-mer ssDNA and 15-mer dsDNA with 5' ssDNA overhang, and "ss/ds" with 3' ssDNA overhang. The error of measurements calculated from 3 independent measurements was ~ 0.02 $min^{-1}$.**

| DNA substrate | -R | +R |
|---|---|---|
| No | 0.03 | 0.03 |
| ss(15) | 0.08 | 0.17 |
| ss(30) | 0.08 | 0.13 |
| ds(30) | 0.20 | 0.11 |
| ds/ss | 0.13 | 0.15 |
| ss/ds | 0.15 | 0.11 |

# Supplementary figures

**Fig. S1:** ***The theoretical model of RecF dimer bound to dsDNA*****.** The RecF dimer is shown in ribbon representation with one monomer in yellow and the second with β-strands in green and α-helixes in red. The DNA is shown by cyan sticks. The alanine 355, which was substituted with a cysteine for labeling, is shown by cyan sticks and transparent CPK representation.

**Fig. S2:** ***Time dependence of RecF dimerization in the presence of ATP and DNA***. The slow ATPase activity in the presence of DNA reduces the dimerization at extended time and low ATP concentration, and this effect in negligible at high ATP concentrations within 10 min. The dimerization of a mixture of 0.5 μM RecF-FAM and 0.5 μM RecF-Cy3 was measured upon titration with ATP in the presence of 5 μM ss- or dsDNA. 8 μM RecR was present where indicated. Reactions were carried out as described in Experimental Procedures and in Fig. 1. Each line of different color represents the FRET dependence on ATP concentration at different time point, as indicated in the figure.

**Fig S3:** ***The DNA binding fluorescence polarization assay performed in the presence of a crowding agent***. Interaction between RecF and DNA was measured as described in Figs. 2 and 3, with 5% of PEG10K included in the reaction mixture. Under this condition, the binding saturation was achieved within available concentration range of RecF in an assay similar to those employed in Fig. 2. Panels A, C, E represent the binding in the absence of RecR to 30-mer ssDNA, dsDNA and ds/ssDNA junction, respectively; and panels B, D, F represent similar measurements done in the presence of RecR.

**Fig S4:** ***Fluorescence polarization measurements of the RecF interactions with different DNA substrates in the presence of 0.2M KCl***. Interaction between RecF and DNA was measured under conditions similar to those in Fig.3, except the KCl concentration was changed to 0.2 M instead of 0.05 M. Solid lines with open symbols and dashed lines with black symbols represent interaction of RecF with DNA in the absence or in the presence of RecR, respectively; circles, triangles and squares represent ss-, ds, and ds/ss DNA substrates, respectively.

**Fig S5:** ***The FRET measurements of RecF dimerization in the presence of ATPγS, dsDNA and RecR.*** Reactions were carried out as described in Experimental Procedures and Fig. 1. FRET signal was measured for a 4 μM RecF-FAM and RecF-Cy3 mixture in the presence of 2mM ATPγS, without RecR (red line) or with 8 μM RecR (green line). Change in arbitrary units of the FRET signal was calculated as described in Fig. 1 and plotted against corresponding concentrations of the D1 DNA substrate.

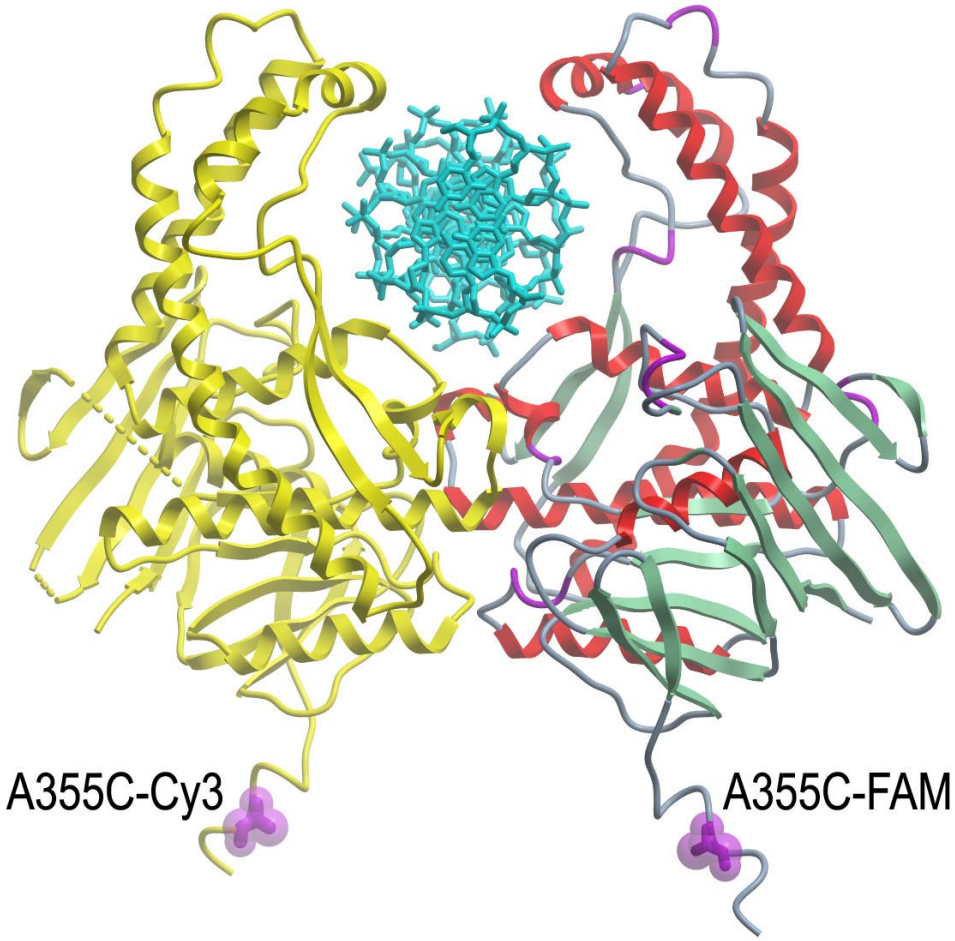


**Fig. S1**

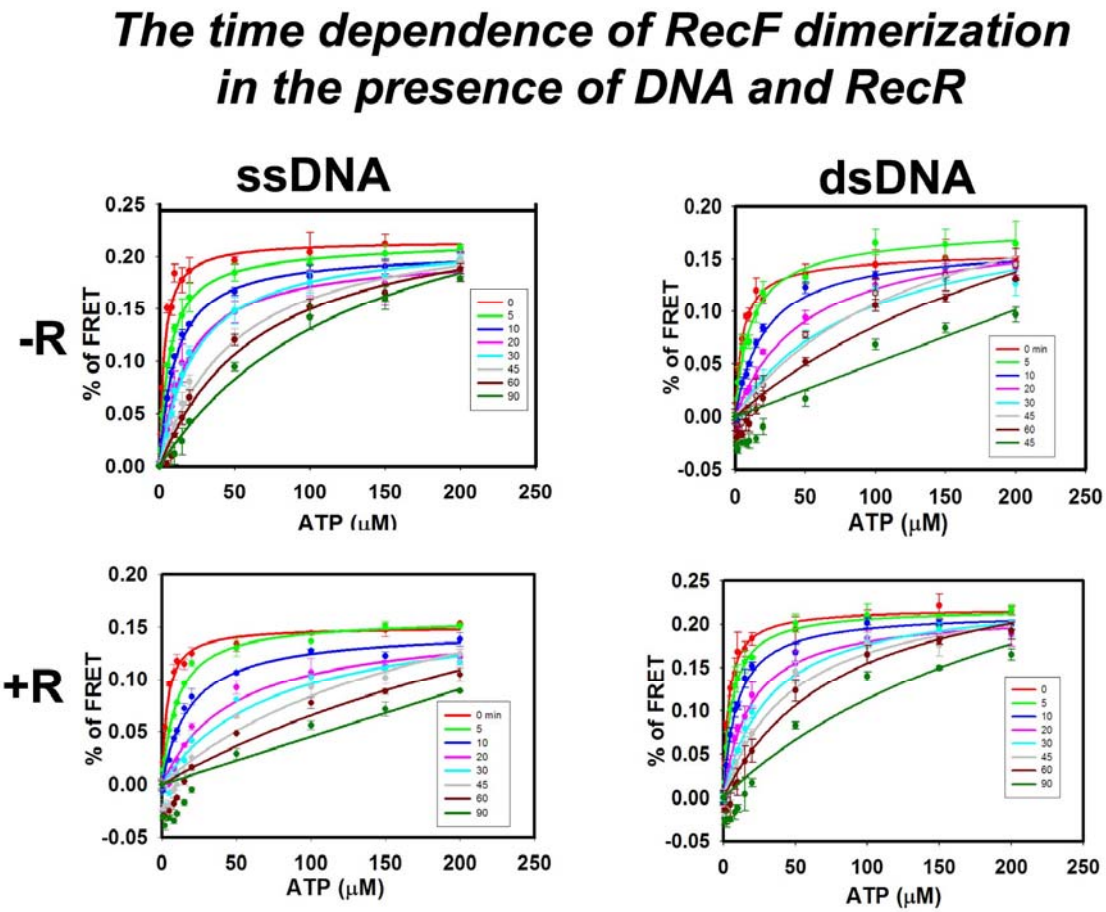
The time dependence of RecF dimerization in the presence of DNA and RecR
ssDNA
dsDNA
-R
+R
% of FRET
ATP (μM)
0
5
10
20
30
45
60
90
0 min

**Fig. S2**

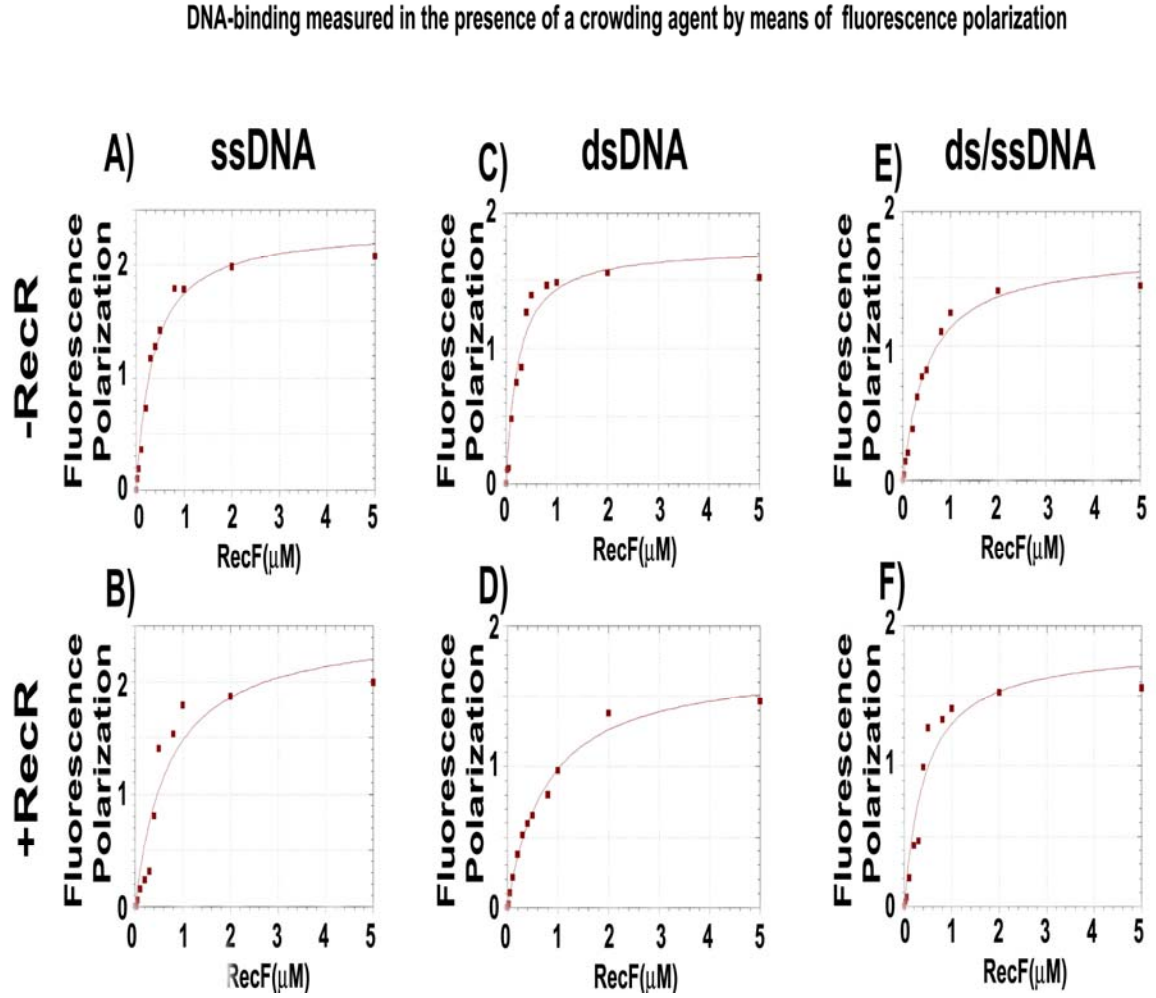
DNA-binding measured in the presence of a crowding agent by means of fluorescence polarization
A) ssDNA
C) dsDNA
E) ds/ssDNA
B)
D)
F)
-RecR
+RecR
Fluorescence Polarization
RecF(μM)

**Fig. S3**

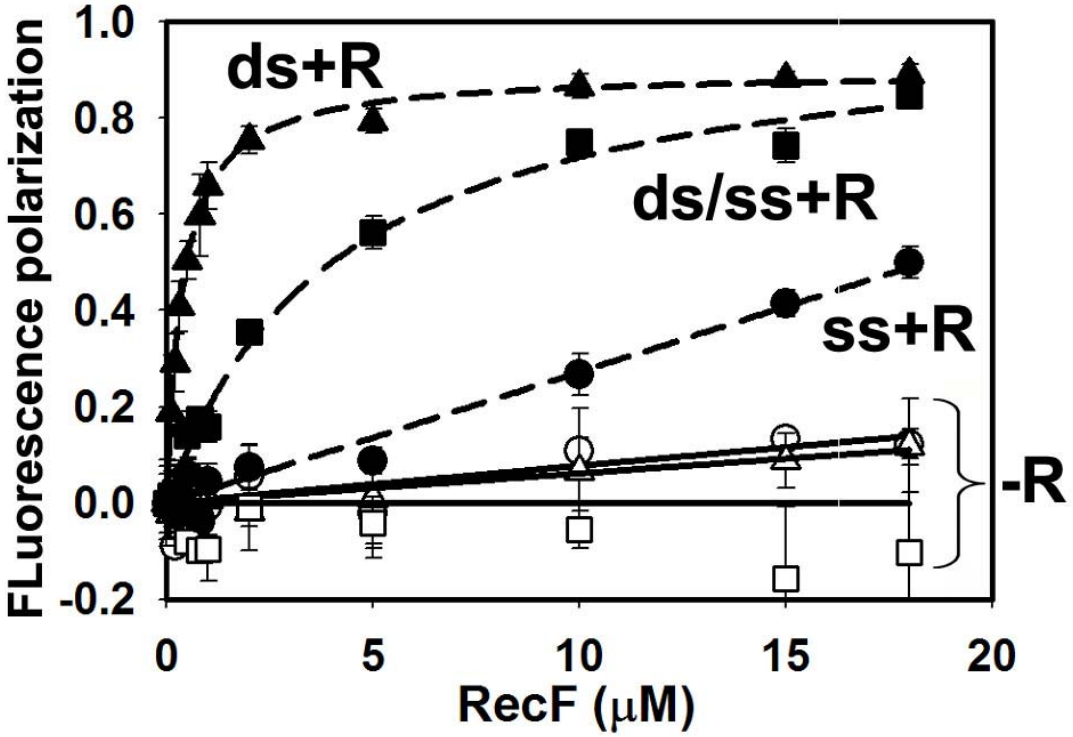
FLuorescence polarization
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
ds+R
ds/ss+R
ss+R
-R
0
5
10
15
20
RecF (μM)

**Fig. S4**

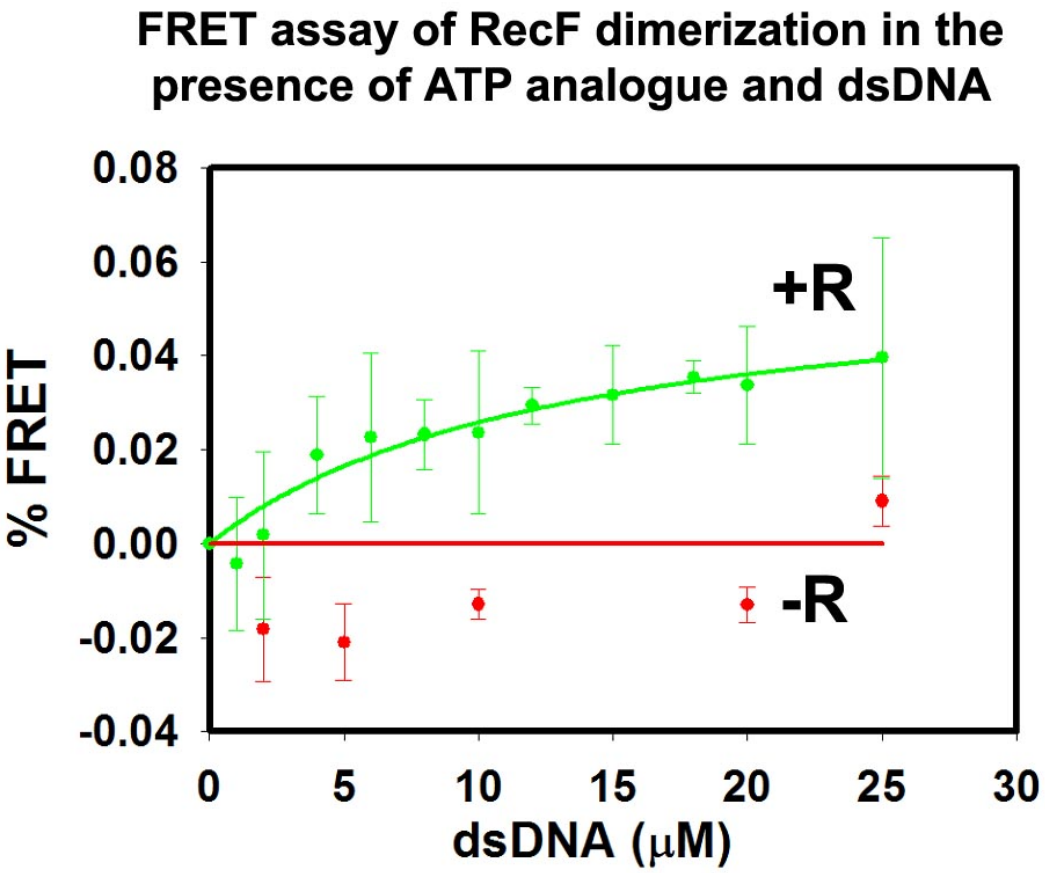
FRET assay of RecF dimerization in the presence of ATP analogue and dsDNA
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
% FRET
+R
-R
0
5
10
15
20
25
30
dsDNA (μM)

**Fig. S5**

# Equations employed in calculations using program Scientist.

**RecF dimerization FRET assay:**

**Method #1:** Datasets obtained at different RecF concentrations were fitted into a single-site binding equation to obtain maximal FRET values (Bmax). These values were plotted against the concentration of RecF to derive the dimerization constant ($L_d$). Then the FRET data were analyzed as a one-step reaction model as described in the scheme [1] using numerical nonlinear regression in the program SCIENTIST (Micromath Inc).

$$\mathbf{2\ RecF_{ATP} \overset{L_d}{\leftrightarrow} (RecF_{ATP})_2} \qquad \text{[scheme 1]}$$

where $RecF_{ATP}$ is the RecF-ATP complex, and $(RecF_{ATP})_2$ is a dimer of the RecF-ATP complex. $L_d$ ($L_a$) is the dissociation (association) constant.

The calculations in the Scientist software were done using the following equations:

Independent Variable: $RecF_{total}$
Dependent variable: FRET
Parameters to fit: $L_a$ (dimerization association constant) and Bmax

Equations
Note: for simplicity RecF(A355C)-FAM is denoted as FAM, and RecF(A355C)-Cy3 - as Cy3:

$$[FAM_{free}]=0.5\ [F_{total}]/(1+2\ L_a\ [Cy3_{free}]) \qquad \text{(equation 2a)}$$

$$[Cy3_{free}]=0.5\ [F_{total}]/(1+2\ L_a\ [FAM_{free}]) \qquad \text{(equation 2b)}$$

$$FRET = L_a\ [FAM_{free}]\ [Cy3_{free}]\ Bmax/[F_{total}] \qquad \text{(equation 2c)}$$

The data were fitted into the equilibrium equation [2]:

$$FRET = L_a\ [FAM_{free}]\ [Cy3_{free}]\ Bmax/[F_{total}], \qquad \text{[eq 2]}$$

where $[FAM_{free}]$ is the concentration of free RecF-FAM, $[F_{total}]$ is the concentration of total RecF, $[Cy3_{free}]$ is the concentration of free RecF-Cy3, and Bmax is the maximal FRET signal observed. Concentrations of free RecF-FAM and RecF-Cy3 species were calculated numerically for each point according to the corresponding mass conservation equations for the used model.

**Method #2:** All data sets corresponding to six RecF concentrations were analyzed as a two-step reaction model described by the scheme [2] to estimate nucleotide binding and dimerization constants using numerical nonlinear regression with SCIENTIST program (Micromath Inc).

$$\mathbf{2\ RecF + 2\ ATP \overset{K_d^{ATP}}{\leftrightarrow} 2\ RecF_{ATP} \overset{L_d}{\leftrightarrow} (RecF_{ATP})_2} \qquad \text{[scheme 2]}$$

The calculations in the Scientist software were done using the following equations:

Independent Variable: $ATP_{total}$
Dependent variable: FRET
Parameters to fit: $K_a^{(ATP)}$ and $L_a$,
Concentrations for proteins: 0.1 μM, 0.2 μM, 0.4 μM, 1 μM, 2 μM, 4 μM.

Equations:

$$[ATP_{free}]=[ATP_{total}]/(1+K_a^{(ATP)}[FAM_{free}]+K_a^{(ATP)}[Cy3_{free}]+2K_a^{(ATP)}K_d^{(ATP)}L_a[FAM_{free}][FAM_{free}][ATP_{free}]+2K_a^{(ATP)}K_a^{(ATP)}[FAM_{free}][Cy3_{free}][ATP_{free}]+2K_a^{(ATP)}K_a^{(ATP)}L_a[Cy3_{free}][Cy3_{free}][ATP_{free}])$$ (equation 3a)

$$[FAM_{free}]=[FAM_{total}]/(1+K_a^{(ATP)}[ATP_{free}]+L_aK_a^{(ATP)}K_a^{(ATP)}[Cy3_{free}][ATP_{free}][ATP_{free}]+2L_aK_a^{(ATP)}K_a^{(ATP)}[FAM_{free}][ATP_{free}][ATP_{free}])$$ (equation 3b)

$$[Cy3_{free}]=[Cy3_{total}]/(1+K_a^{(ATP)}[ATP_{free}]+L_aK_a^{(ATP)}K_a^{(ATP)}[FAM_{free}][ATP_{free}][ATP_{free}]+2L_aK_a^{(ATP)}K_a^{(ATP)}[Cy3_{free}][ATP_{free}][ATP_{free}])$$ (equation 3c)

$$[FAM_{ATP}]=K_a^{(ATP)}[FAM_{free}][ATP_{free}]$$ (equation 3d)

$$[Cy3_{ATP}]=K_a^{(ATP)}[Cy3_{free}][ATP_{free}]$$ (equation 3e)

$$[FAM_{ATP}/Cy3_{ATP}]=L_a[FAM_{ATP}][Cy3_{ATP}]$$ (equation 3f)

$$\text{FRET signal} = [FAM_{ATP}/Cy3_{ATP}]/F_{total}$$ (equation 3g)

where $[ATP_{free}]$ is the concentration of free ATP, $[ATP_{total}]$ is the concentration of total ATP added, $K_a^{ATP}$ is the association constant of the RecF-ATP complex, $[FAM_{ATP}]$ and $[Cy3_{ATP}]$ are the corresponding concentrations of the RecF-$FAM_{ATP}$ and RecF-$Cy3_{ATP}$ complexes, and $F_{total}$ is the total RecF concentration ($[RecF\text{-}FAM_{total}] + [RecF\text{-}Cy3_{total}]$)

**ATPase assay:** Experimental data were fitted into a one site saturation curve (Scientist, MicroMath Scientific Software; and Sigmaplot8.0, SPSS Inc.) and two values obtained: $K_d$ that represents an apparent dissociation constant of the RecF-DNA complex; and Bmax that is a maximum ATPase activity in the presence of a given DNA substrate.

The calculations in the Scientist software were done using the following equations:

Independent Variable: $DNA_{total}$
Dependent variable: ΔOD
Parameters to fit: $K_a$ and Bmax
Concentration of the RecF protein (as a dimer): 5 μM.

Equations:

$$[RecF_{free}]=[RecF_{total}]/(1+K_a[DNA_{free}])$$ (equation 5a)

$$[DNA_{free}]=[DNA_{total}]/(1+K_a[RecF_{free}])$$ (equation 5b)

$$\Delta OD = K_a[DNA_{free}][RecF_{free}]Bmax/[RecF_{total}]$$ (equation 5c)

where ΔOD is change of the optical density of the stain in comparison to 0 minute time point; $K_a$ – an association constant of the corresponding RecF-DNA complex; $[DNA_{free}]$ and $[RecF_{free}]$ – concentrations of the free DNA and RecF components.

ΔOD was converted to the amount of hydrolyzed ATP using the following formula:

ATP (μM) = ΔOD * "dilution factor" (41) * "μM to ΔOD conversion coefficient" (19.78 μM/OD)