**Supplemental Information**

**for**

**Srs2 prevents Rad51filament formation by repetitive scrunching of DNA**

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includes:

Supplemental Figure Legend

Extended Experimental Procedures

Supplemental References

**Supplemental Information**

**Supplemental Figure Legends**

**Figure S1. Rad51 binds primarily as monomer and dimer** (A) Schematic of FRET-DNA construct, same as Figure 1A. (B) Single molecule FRET traces that exhibit Rad51 monomer mixed with higher oligomer binding to T15. (C) TDP built out of all the binding events found in Rad51 binding toT15 DNA. Each cluster in TDP can be classified into monomer, dimer, trimer, tetramer and pentamer binding as shown. (D) The majority of (>75%) of Rad51 bind as monomer or dimer whereas less than 25% bind as a higher oligomer.

**Figure S2. 5’ to 3’ directionality is not due to Rad51 formation on DNA duplex** (A) Schematic diagram of DNA used for Rad51 formation on duplex DNA. Cy3 and Cy5 are located across the duplex DNA such that Rad51 formation results in FRET decrease. (B) FRET histogram of DNA only (gray) vs. Rad51 formed (orange) shows a transition from mid to low FRET induced by Rad51 formation on duplex DNA. (C) Single molecule trace showing a slow FRET decrease due to Rad51 formation on duplex DNA. (D, E) Rad51 filament formation rate deduced from fitting FRET traces collected from over fifty traces. Errors in fit results are in SEM. (The rate in S2D is higher than that shown in Fig 2E (3’Cy3) likely due to the different distance sensitivity exhibited by FRET (3-8 nm) and PIFE (0-4nm) i.e PIFE is not sensitive to initial binding of Rad51 monomers.)

**Figure S3. Srs2 repetitive movement is ATP dependent** (A) In the absence of ATP, Srs2 shows only a static binding to DNA, but no fluctuation. (B) FRET peak to peak dwell times collected in varying ATP concentrations were built as histograms and fit with a Gaussian distribution fit. (C) Single molecule traces collected at low ATP concentrations show markedly slower FRET fluctuations.

**Figure S4. Srs2 anchors near duplex junction while scrunching a finite length of ssDNA** (A) Schematic diagram of FRET-DNA construct. Three DNA substrates had tail length of 30, 40, 50 nucleotides and the same fixed Cy3-Cy5 distance of 15 nucleotide as shown. (B) Single molecule traces taken from Srs2 on three DNAs are highly analogous to one another. (C) FRET histograms obtained from the three DNA experiments are identical. (D) Dwell time collected form the three experiments are also the same. Error bars denote SEM. (E) Single molecule surface of Cy3 and Cy5 channel show single molecules of Cy3 labeled DNA and Atto 647N labeled Srs2 side by side. The presence of the single dye is confirmed by single step photobleaching as well as intensity corresponding to one fluorophore. (F) Single molecule intensity and corresponding FRET traces. As indicated by arrows, binding of Srs2 shows up as an abrupt appearance of FRET, followed by FRET fluctuations resulting from the repetitive scrunching activity of Srs2. Red signal disappears when the protein dissociates from DNA. (G) DNA constructs (3’-Cy3 labeled partial duplex with T20-40 nucleotide ssDNA) were used to probe scrunching activity of fluorescently labeled Srs2 (NTA-Atto 647N). (H) Single molecule FRET traces from pdT20, 25, 30 and 40. (I) FRET histograms collected from 30-70 molecules that exhibit FRET fluctuations. (J) DNA construct (same partial duplex DNA with Cy3 at duplex junction) were used to visualize scrunching of labeled Srs2. (K) Single molecule FRET traces from pdT20 and pdT40. (L) FRET histograms collected from 50 traces that show FRET arising from Srs2 binding and scrunching. (M) Proposed model for Srs2 translocation and repetitive scrunching of a short segment of ssDNA while anchoring near the duplex junction.

**Figure S5. Srs2 removal strength and specificity** (A) Single molecule FRET traces obtained from an experiment where Srs2CΔ276 was added to preformed Rad51. (B) Dwell time of Srs2CΔ276 scrunching duration (marked in red arrow in (A)) which represents the period Srs2 prevents Rad51 reformation. (C) Single molecule FRET traces from an experiment where Srs2 was preloaded to DNA then Rad51 was added. (D) Histogram and a Gaussian distribution fit of dwell time of Srs2CΔ276 scrunching duration which represents the period Srs2 prevents Rad51 initiation. Error in fit result is in SEM. (E) Single molecule trace of Srs2 subject to RecA removal shows that Srs2 cannot remove RecA filament.

**Figure S6. Srs2 monomer cannot unwind DNA** (A) FRET-DNA applied to surface tethered Srs2. (B) Single molecule traces show steady high FRET, indicating no unwinding by Srs2 monomer.

**Extended Experimental Procedures**

**DNA Sequence**

pdT(10-70): 5’-GCCTCGCTGCCGTCGCCA-3’ + 5’-TGGCGACGGCAGCGAGGC-(dT)10 or (dT)13 or (dT)15 or (dT)18 or (dT)20 or (dT)25 or (dT)30 or (dT)40 or (dT)70 -3’;

Internal amino modifier is represented as (C6 dT), this can be used to label DNA with an internal Cy3 or Cy5 dye.

pdT3+17: 5’-GCCTCGCTGCCGTCGCCA-3’ + 5’-TGGCGACGGCAGCGAGGC-(dT)3-(C6 dT)-(dT)17-3’;

pdT15+(15-35): 5’-GCCTCGCTGCCGTCGCCA-3’ + 5’-TGGCGACGGCAGCGAGGC-(dT)15-(C6 dT)-(dT)15 or (dT)25 or (dT)35 -3’.

**DNA Substrate Preparation**

Partial duplex DNA substrates for immobilized-DNA experiments were prepared by mixing the appropriate biotinylated and non-biotinylated oligonucleotides in a 1:2 molar ratio at 10μM in T50 buffer (50mM NaCl , 10mM Tris-HCl (pH 8.0)). Oligonucleotide mixtures were incubated at 95°C for 2 minutes followed by slow cooling to room temperature to complete the annealing reaction. Partial duplex DNA substrates for tethered-protein experiments were prepared using non-biotinylated strands of oligonucleotides with the same sequences as the biotinylated oligos.

**Single-Molecule Fluorescence Assay**

smFRET and smPIFE measurements were done using a wide-field total internal reflection fluorescence microscope ([Joo and Ha, 2008](#_ENREF_3)). Cy3 (donor) on DNA was excited by an Nd:YAG laser (532nM, 75mW, Coherent CUBE) via total internal reflection. The fluorescence signals from Cy3 and Cy5 were collected through an objective (Olympus Uplan S-Apo; X100 numerical aperture; 1.4 oil immersion) and detected at 100ms time resolution using an EMCCD (electron multiplying charge-coupled device) camera (iXon DU-897ECS0-#BV; Andor Technology). The camera was controlled using home-made C++ program. Single-molecule traces were extracted from the recorded video file by IDL software.

**Srs2 Translocation on ssDNA**

*Saccharomyces cerevisiae* Srs2 was mixed at 5nM with Rad51 reaction buffer and ATP and added to a flow chamber that had 100pM DNA specifically immobilized on a PEG-coated quartz surface through biotin-neutravidin linkage ([Ha et al., 2002](#_ENREF_2)). ATP concentration varies from 0 to 1mM for data fitted to Michaelis-Menten equation (Figure 3C).

**Srs2 Unwinding DNA**

Srs2 was mixed at 200nM with Rad51 reaction buffer and ATP and added to immobilized DNA as described previously. For counting unwound DNA molecules (loss of Cy3 signals), short movies (5-10 seconds) were taken for over 40 minutes.

**Rad51 Filament Formation**

Yeast Rad51 was mixed at 1 μM with Rad51 reaction buffer and ATP and added to immobilized DNA as described previously.

**Removal of Rad51 Filament by Srs2**

Srs2 (CΔ276) and Rad51 were mixed at 200nM and 1μM respectively with Rad51 reaction buffer and ATP and added to the flow chamber with the preformed Rad51 filament (described previously).

**RecA Filament Formation**

Bacterial RecA was mixed at 1 μM with RecA reaction buffer and ATP and added to a flow chamber that had 100pM specifically immobilized DNA.

**Removal of RecA Filament by Srs2**

Srs2 and RecA were mixed at 200nM and 1μM respectively with RecA reaction buffer and ATP and added to the flow chamber with the preformed RecA filament (described previously).

**Tethered Srs2 Protein**

The truncated Srs2 protein, Srs2CΔ276 has 9x histadine tags which are tethered to the PEG-coated quartz surface through neutravidin-biotin-tris-NTA linkage. Biotin-tris-NTA was a generous gift from Prof. Paul J. Hergenrother, Department of Chemistry at University of Illinois Urbana Champaign ([Lata et al., 2006](#_ENREF_4)).

For Srs2 translocation experiments, biotin-tris-NTA (20nM) was mixed NiCl2 (50nM) in T50 buffer (50mM Tris pH 7.5, 50mM NaCl) and incubated on ice for 15 minutes. The mixture is then added to a flow chamber that already had neutravidin immobilized to the PEG-coated surface, then allowed to incubate for 10 minutes at room temperature. 0.5-1nM of Srs2 in T50 buffer were then added to the flow chamber and incubated for 5 minutes at room temperature. Finally, non-biotinylated partial duplex DNA substrate in Rad51 reaction buffer and ATP was added to the flow chamber to initiate the reaction.

For Srs2 monomer removal of Rad51 filaments, 10nM of Srs2 was tethered to the quartz surface as previously described. Non-biotinylated partial duplex DNA substrates were first incubated with 1μM of Rad51 in Rad51 reaction buffer and ATP for 15 minutes at room temperature before added to the flow chamber to complete the reaction.

**Srs2 Protein Labeling**

NTA-Atto-647 dye was kindly provided by Prof. Robert Tampé, Institute of Biochemistry at Goethe-University Frankfurt ([Grunwald et al., 2011](#_ENREF_1)). Srs2 and dye were mixed at a ratio of 1:1.5 (5nM and 7.5 nM, respectively) with Rad51 reaction buffer, ATP, 2mM DTT, incubated at room temperature for 5 minutes, and added to a flow chamber that had 100pM Cy3-only DNA substrate immobilized.

**Data Analysis**

For various dwell time analyses: Peak-to-peak dwell time analysis to obtain δt was measured manually from individual FRET traces (Figure 4D) within Matlab, and the resulting histograms and fittings were generated using Origin (OriginLab Corporation, Northampton, MA). Binning sizes vary based on the type and range of data collected. Rad51 removal dwell time (Figure 6G) is measured as the time that it took from the appearance of low FRET signal to change to high FRET (Figure 6F). Srs2 loading duration (Figure S6B and D) is measured as the time that DNA is occupied by Srs2 before being covered by Rad51 (Figure S6A and C). DNA unwinding time by Srs2 (Figure 7K and L) is measured as the time it took from high FRET fluctuations (Srs2 occupying DNA) to go to low FRET before signal disappearance due to DNA unwinding (Figure 7I and J).

Overall $\frac{δt-R}{δt-S}$ ratio measures the time that the DNA spent coated with Rad51 versus free of Rad51. It is calculated by averaging the sum of the individual ratio of each pair of δt-R (DNA covered with Rad51 filament) and δt-S (DNA cleared of Rad51 filament) from individual single molecule traces. δt is measured as shown in Figure 6C.

**Supplemental References**

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