SUPPLEMENTAL INFORMATION

Molecular mechanism of resolving trinucleotide repeat hairpin by helicases

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FIGURE S1

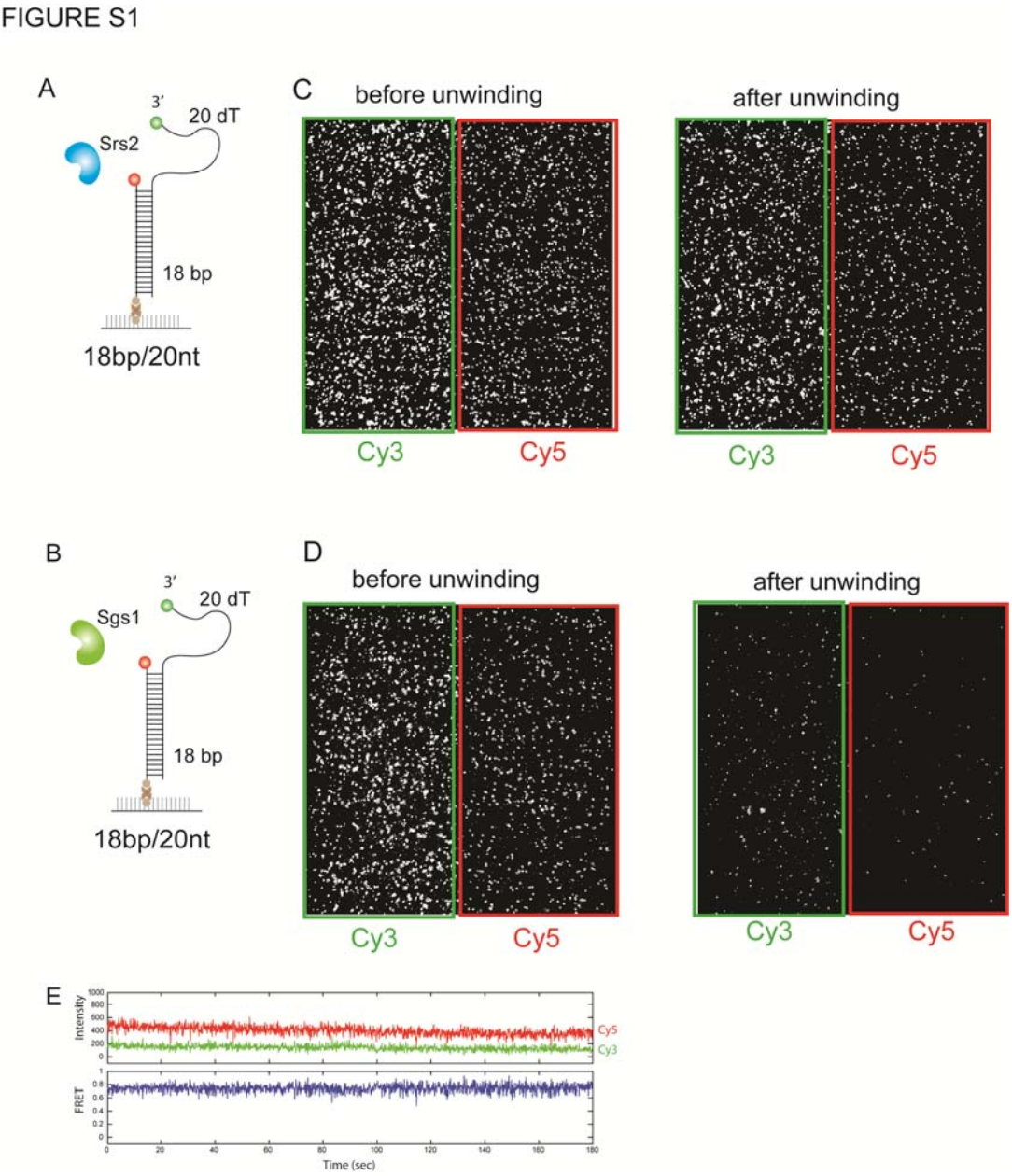

Figure S1 Related to Figure 1 (A, B) Schematic of DNA identical to Figure 1A. (C, D) Screen capture of Cy3 (donor) and Cy5 (red) signals from single molecules of FRET DNA. At 10 nM concentration, Srs2 cannot unwind DNA duplex (Cy3 and Cy5 signals remain after unwinding reaction), but Sgs1 unwinds, leading to a rapid disappearance of Cy3 and FRET signal. (E) Example single molecule trace of DNA prior to unwinding.

FIGURE S2

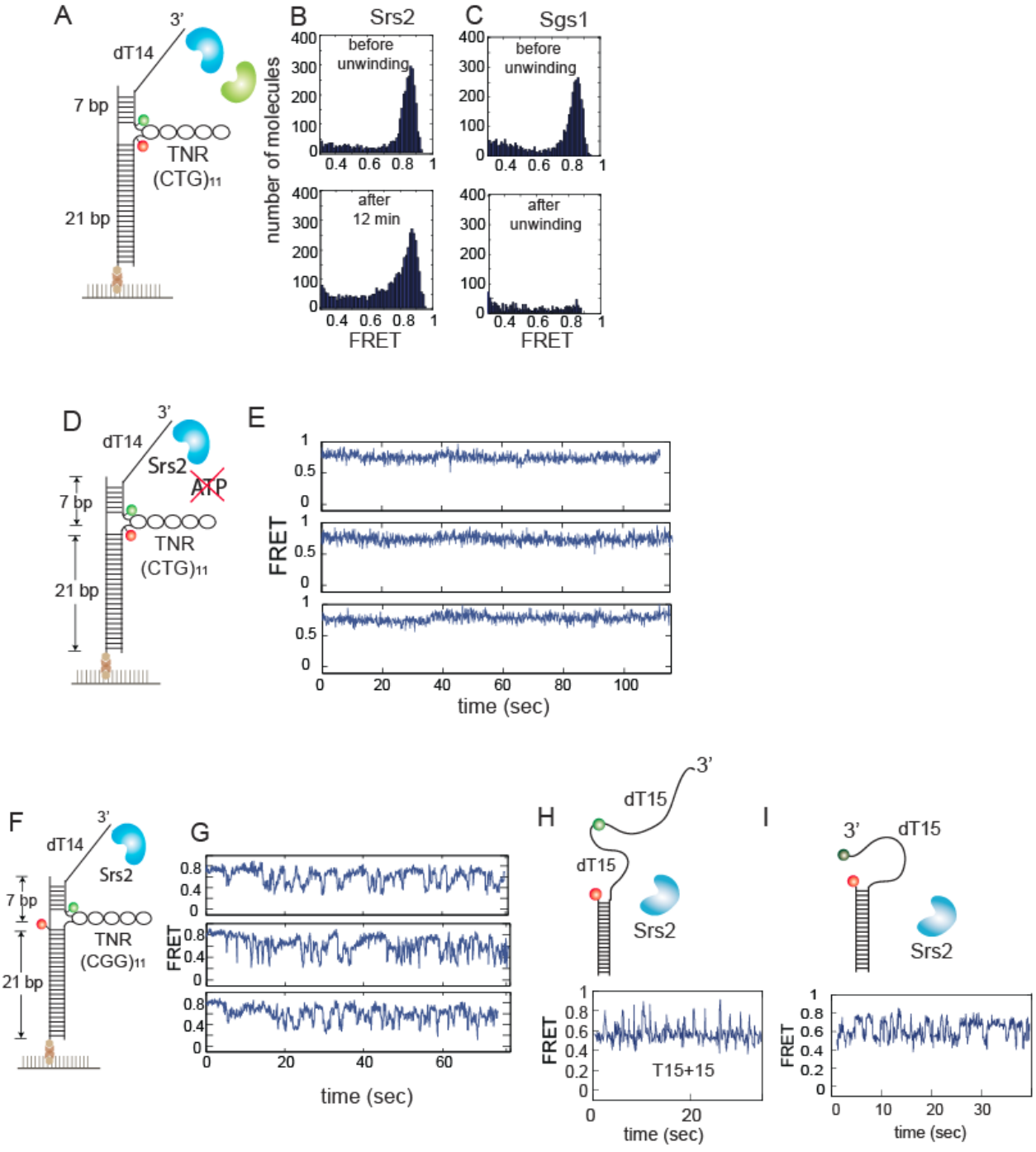
A
3'
dT14
7 bp
TNR
(CTG)11
21 bp
B
Srs2
before unwinding
after 12 min
number of molecules
FRET
C
Sgs1
before unwinding
after unwinding
FRET
D
3'
dT14
Srs2
ATP
7 bp
TNR
(CTG)11
21 bp
E
FRET
time (sec)
F
3'
dT14
Srs2
7 bp
TNR
(CGG)11
21 bp
G
FRET
time (sec)
H
3'
dT15
dT15
Srs2
FRET
T15+15
time (sec)
I
3'
dT15
Srs2
FRET
time (sec)

Figure S2 Related to Figure 2 (A) Schematic of TNR DNA with FRET dyes to which Srs2 is added without ATP. (B) smFRET traces display constant high FRET, suggesting that the FRET fluctuation depends on Srs2 activity in the presence of ATP. (C) Schematic of TNR DNA with FRET dye pairs. FRET histogram of before and after unwinding by Srs2 (D) and Sgs1 (E). (F) Alternate FRET DNA where Cy5 (red dye) is moved away from the hairpin junction to avoid potential perturbation of the dye in Srs2's activity. (G) smFRET traces that show the similar FRET fluctuation seen in other DNA constructs, suggesting that the dye at the hairpin opening did not cause disruption. (H, I) From previous study, we have shown that the dye location did not cause any difference in the repetitive translocation of Srs2 (1). Both the internally positioned and end-labeled dyes yielded same activity of Srs2.

FIGURE S3

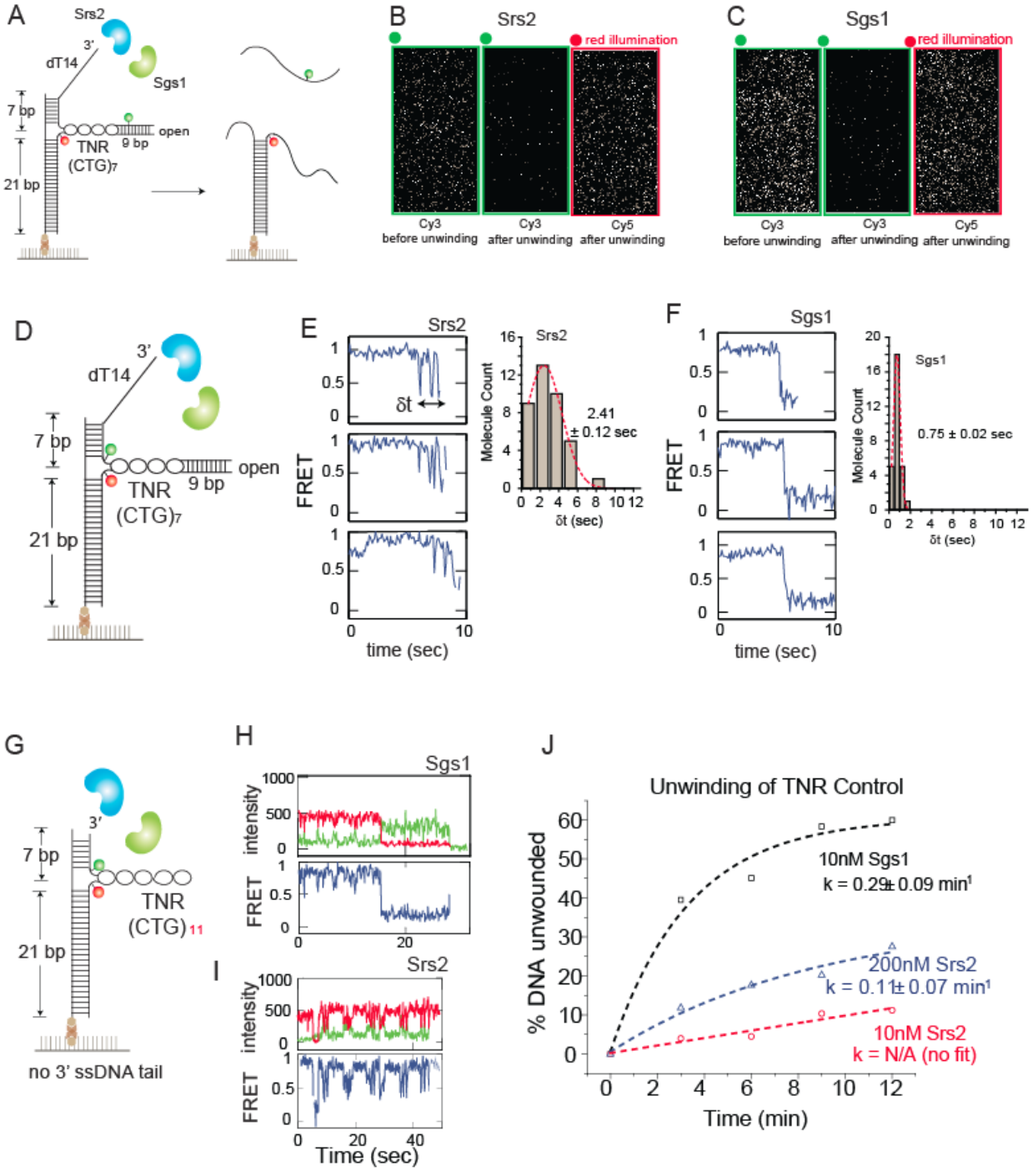

Figure S3 Related to Figure 3 (A) Schematic of open-ended TNR DNA. (B, C) Both Srs2 and Sgs1 lead to complete unfolding of open-ended TNR hairpin. The green illumination shows disappearance of Cy3

(green) signal, yet red illumination displays remaining signals of red labeled DNA that remains intact after the TNR unwinding. (D) Schematic of an alternate open-ended hairpin DNA where two FRET dyes are positioned at the entry of hairpin junction. (E, F) Both smFRET traces of Srs2 and Sgs1 show the same pattern i.e Srs2 exhibits FRET fluctuation followed by unwinding whereas Sgs1 displays an immediate unwinding without FRET fluctuation. The dwell time distribution is also similar to the case shown in Figure 3E, F. This experiment shows that the dye position did not make a difference in the activity of both proteins. (G) TNR DNA without 3' ssDNA tail. (H) Sgs1 leads to rapid unwinding, signified by the fast FRET decrease, followed by disappearance of green dye, which results from dissociation of the entire strand. (I) Srs2 displays repetitive unfolding of TNR as seen before. (J) Unwinding by Sgs1 and two different concentrations of Srs2 shows that Sgs1 even at 10nM unwinds the TNR containing DNA proficiently whereas Srs2 is shows great deficiency in unwinding capability even at 200nM concentration. Data are represented as mean ± SEM.

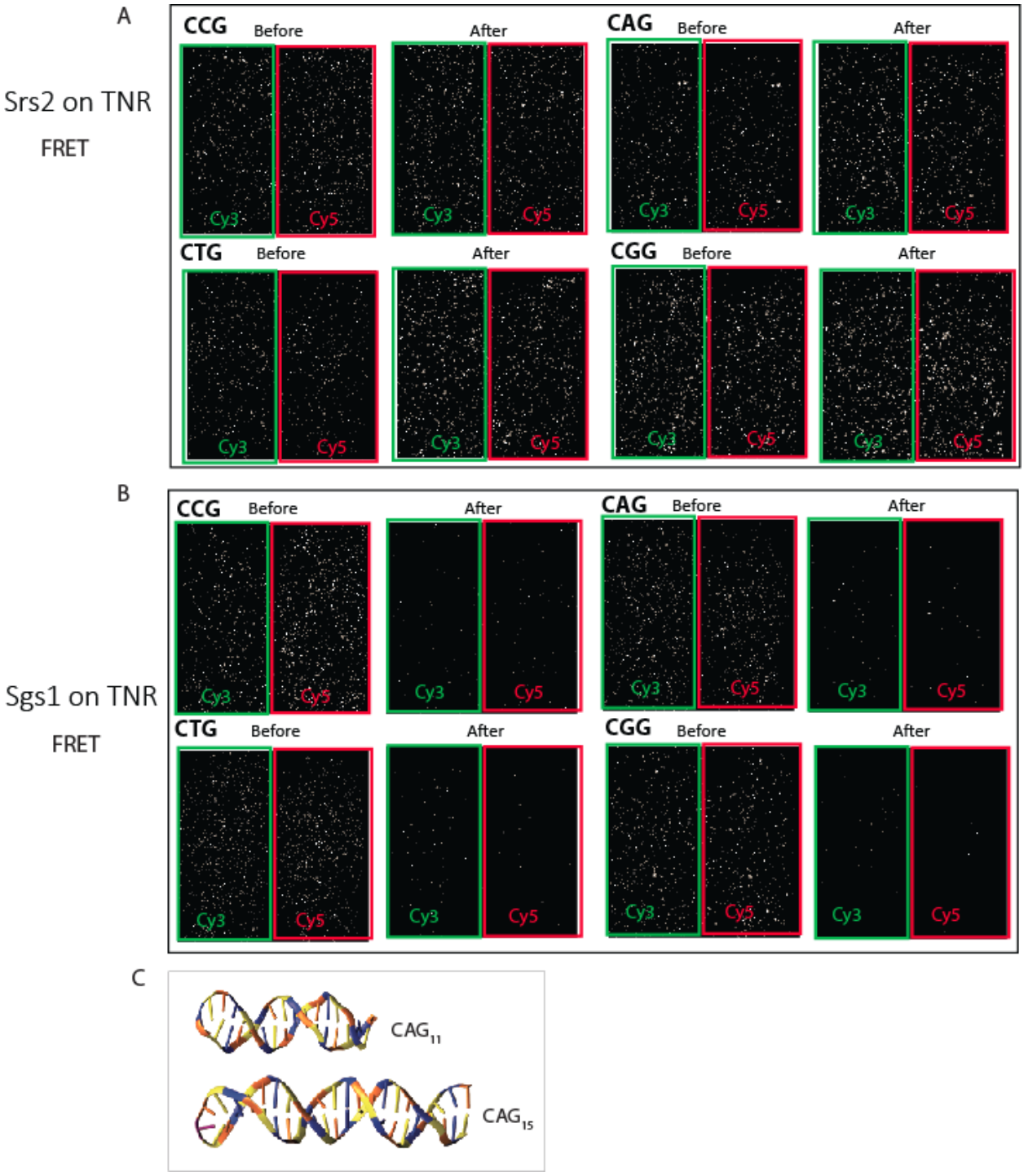

Figure S4 Related to Figure 4 Screen capture of Cy3 and Cy5 channels taken before and after the unwinding of TNR DNAs by Srs2 (upper panel, A) and Sgs1 (lower panel, B). The four TNR DNAs include 11 repeats of CCG, CAG, CTG and CGG. Srs2 exhibits repetitive unfolding of all DNAs. The lack of

unwinding by Srs2 is evident from the density of Cy3 and Cy5 signals that remain the same before and after the unwinding reaction. Sgs1 leads to rapid unwinding of all DNAs as shown by the disappearance of both Cy3 and FRET signals (red channel). (C) MD simulation of $(CAG)_{11}$ and $(CAG)_{15}$ shows that the TNR hairpin resembles that of double helical structure of DNA.

1. Qiu, Y., Antony, E., Doganay, S., Koh, H.R., Lohman, T.M. and Myong, S. (2013) Srs2 prevents Rad51 filament formation by repetitive motion on DNA. *Nature communications*, **4**, 2281.