

The recombinase protein is a torque sensitive molecular switch.

Equilibrium

Mechanical Energy:

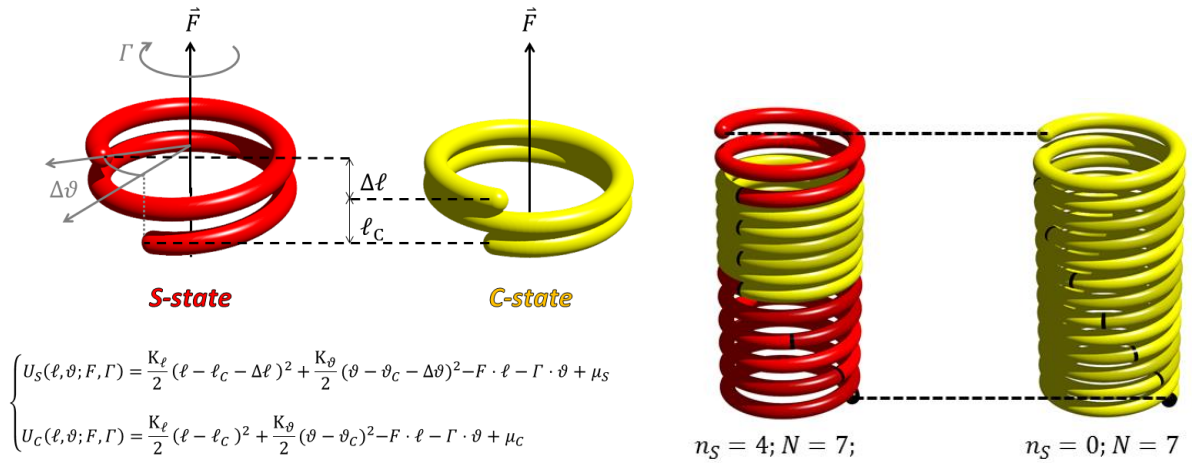


Figure S11: (left) Mechanical energy of a single hRad51 protein in the magnetic tweezers, both in the S and C-state. (right) Two configurations of the Nucleoprotein Filament of same length, but with a different number of hRad51 in the S-state. If the filament on the left is mechanically relaxed, with 4 hRad51 proteins in the S-state, the one on the right is under mechanical stress as the proteins in the C-state are partially stretched. The two filaments have same length, but different internal energies.

The Free Energy :

$$\begin{aligned} G(L, \theta, n_S; F, \Gamma, N) &= \frac{K_\ell}{2N} [L - (N\ell_C + n_S\Delta\ell)]^2 + \frac{K_\vartheta}{2N} [\theta - (N\vartheta_C + n_S\Delta\vartheta)]^2 - F \cdot L - \Gamma \cdot \theta \\ &+ Nk_B T \left[\frac{n_S}{N} \ln\left(\frac{n_S}{N}\right) + \left(1 - \frac{n_S}{N}\right) \ln\left(1 - \frac{n_S}{N}\right) \right] + n_S \cdot \Delta\mu \end{aligned}$$

is minimal when

$$\begin{cases} \frac{\partial G}{\partial n} = \frac{K_\ell}{N} \Delta\ell (L - N\ell_c - n_S \Delta\ell) + \frac{K_\vartheta}{N} \Delta\vartheta (\Theta - N\vartheta_c - n_S \Delta\vartheta) - \Delta\mu - k_B T \left[\ln\left(\frac{n_S}{N}\right) - \ln\left(1 - \frac{n_S}{N}\right) \right] = 0 \\ \frac{\partial G}{\partial L} = \frac{K_\ell}{N} (L - N\ell_c - n_S \Delta\ell) - F = 0 \\ \frac{\partial G}{\partial \Theta} = \frac{K_\vartheta}{N} (\Theta - N\vartheta_c - n_S \Delta\vartheta) - \Gamma = 0. \end{cases}$$

Equation 1

This implies:

$$\begin{cases} \frac{n_S}{N} = \frac{1}{1 + e^{-(F \cdot \Delta\ell + \Gamma \cdot \Delta\vartheta - \Delta\mu)/k_B T}} \\ \frac{L}{N} = \frac{F}{K_\ell} + \ell_c + \frac{n_S}{N} \cdot \Delta\ell \\ \frac{\Theta}{N} = \frac{\Gamma}{K_\vartheta} + \vartheta_c + \frac{n_S}{N} \cdot \Delta\vartheta. \end{cases}$$

Equation 2

Torsion locked to $\sigma = 0$ ($\Theta = N \cdot \vartheta_c$)

If the nucleoprotein filament is kept locked to the initial topology $\sigma = 0$, the equations become:

$$\begin{cases} \Gamma = \frac{k_B T \left[\ln\left(\frac{n_S}{N}\right) - \ln\left(1 - \frac{n_S}{N}\right) \right] - F \cdot \Delta\ell + \Delta\mu}{\Delta\vartheta} \\ \frac{n_S}{N} = \frac{\frac{L}{N} - \ell_c - \frac{F}{K_\ell}}{\Delta\ell} \\ K_\vartheta = -\frac{\Gamma}{\frac{n_S}{N} \cdot \Delta\vartheta} \end{cases}$$

Equation 3

$$\begin{cases} \Gamma = -30 \pm 4 \text{ pN} \cdot \text{nm} \\ \frac{n_S}{N} = 0.095 \pm 0.05 \\ K_\vartheta = 400 \pm 200 \text{ pN} \cdot \text{nm} \end{cases}$$

Equation 4

Nucleoprotein filament in ADP

After hydrolysis of ATP, the *C-state* becomes more favorable than the *S-state*, indicating a change in the sign of the chemical potential $\Delta\mu$. In order to evaluate the new chemical potential $\Delta\mu_{ADP}$, we rewrite the Equation 1 in the limit of $\Gamma = 0$:

$$\begin{cases} \Delta\mu_{ADP} = F \cdot \Delta\ell - k_B T \left[\ln\left(\frac{n_S}{N}\right) - \ln\left(1 - \frac{n_S}{N}\right) \right] \\ \frac{n_S}{N} = \frac{\frac{L_{ADP}}{N} - \ell_C - \frac{F}{K_\ell}}{\Delta\ell} \\ \theta/N = \vartheta_C + p\Delta\vartheta. \end{cases}$$

Equation 5

For a normalized length of 1.14 ± 0.02 (standard deviation $\sigma = 0.04$, measurement on 4 different molecules) and a stretching force $F = 8$ pN, we obtain:

$$\begin{cases} \Delta\mu_{ADP} = 8 \cdot 0.5 - 4[\ln(0.26) - \ln(1 - 0.26)] = 8 \pm 1 \text{ pN} \cdot \text{nm} \\ \frac{n_S}{N} = \frac{0.14 - 8/800}{0.5} = 0.26 \pm 0.04 \\ \theta/N = 1.68 \pm 0.03 \text{ Rad} \quad (\sigma = -0.110 \pm 0.002) \end{cases}$$

Magnetic Tweezers

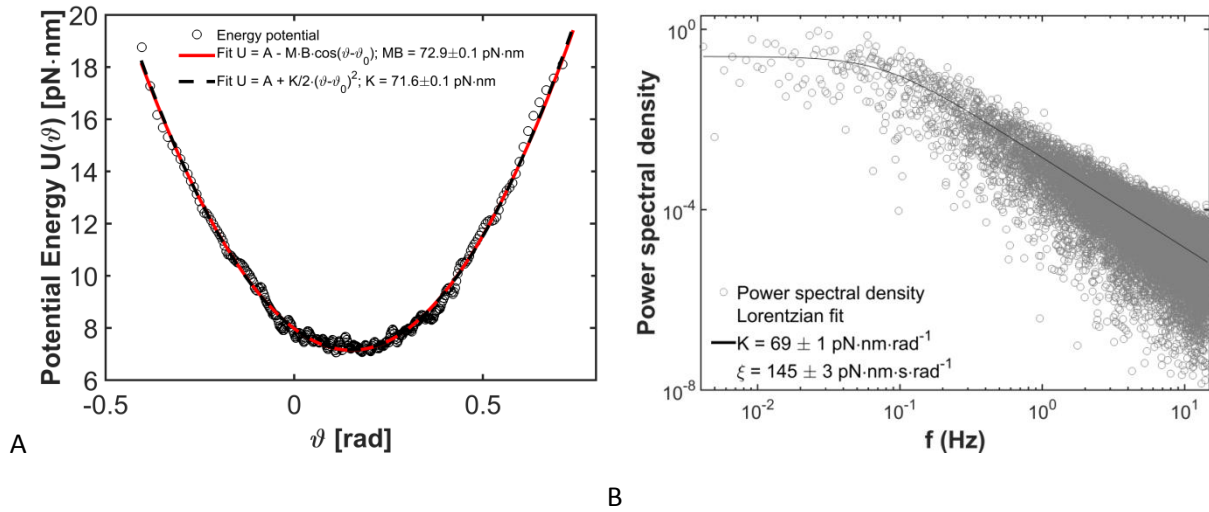


Figure S12: (A) The potential energy $U(\theta)$ in the magnetic tweezers, deduced from the thermal fluctuations of the magnetic bead. (B) Power spectrum density of the angular fluctuations of the bead. Fitting the spectrum by a Lorentzian distribution (harmonic approximation) provides both the stiffness of the trap K_θ and the viscous drag of the bead ξ .

Equilibrium state of the ADP-bound nucleoprotein filament

By not imposing the supercoiling degree, one may release the system and observe the spontaneous evolution of the hRad51-dsDNA nucleoprotein filaments. We use this spontaneous evolution to determine the equilibrium conformation of the nucleoprotein filament in the ADP state. Releasing torsion is achieved by using dsDNA molecules, on which single strand nicks have been induced. Any excess torsion within the filament is released through rotation around the monovalent phosphodiester bonds at the nick sites with no energy cost.

The formation of hRad51 filaments on nicked dsDNA yielded filaments in stretched form in both ATP-Ca²⁺ and ATP-Mg²⁺ suggesting that the unwound stretched form is the most energetically favorable regardless of the cation present when ATP is present.

On the contrary, the ADP-bound filaments are in a semi-condensed state with an elongation of approximately 1.2 times the length of B-DNA, as shown in Figure S13. The final length of the ADP-filament slightly fluctuates during the experiment and a simple change of buffer changes it from 1.26 ± 0.02 to 1.14 ± 0.02 times the crystallographic length of B-DNA (standard deviation $\sigma = 0.04$, values obtained from 4 different molecules). Nevertheless, after injection of ATP-rich buffer (without ADP) the nucleoprotein filament reaches the nominal extension of the *S-State*. This indicates that the DNA molecule was fully covered by hRad51 even in the semi-condensed state. In the following, we will refer to this semi-condensed state as *M-State*.

Chemical potential of the *M-State* in ADP.

The results shown in Figure S13 indicate that the ADP-bound nucleoprotein filament (*M-state*) has an equilibrium length in the range of 1.14-1.26 times the crystallographic length of B-DNA. Obtained when the hRad51 protein is absent from the buffer, the value of 1.14 ± 0.04 (standard deviation $\sigma = 0.04$, $N = 4$ different molecules) is in good agreement with the observations by Yu *et al*¹, who reported an intermediately condensed extension with an helical pitch of 76\AA on ssDNA. Converted into a normalized extension, this pitch corresponds to a normalized extension of 1.16. More recent studies also revealed that hRad51 filaments formed on linear dsDNA molecules can reversibly transit several times between *S-state* and *M-state* depending on whether ATP was added or removed from the reaction bufferⁱ.

After ATP hydrolysis the *C-state* becomes more favorable than in ATP, indicating a change in potential $\Delta\mu_{S \rightarrow C}$. In order to evaluate this new chemical potential $\Delta\mu_{ADP}$, we use the equilibrium equations imposing $\Gamma = 0$ (see Equation 5 in SI). With a normalized length of 1.14 and a stretching force $F = 8$ pN, we obtain $\Delta\mu_{ADP} 8 \pm 1$ pN · nm .

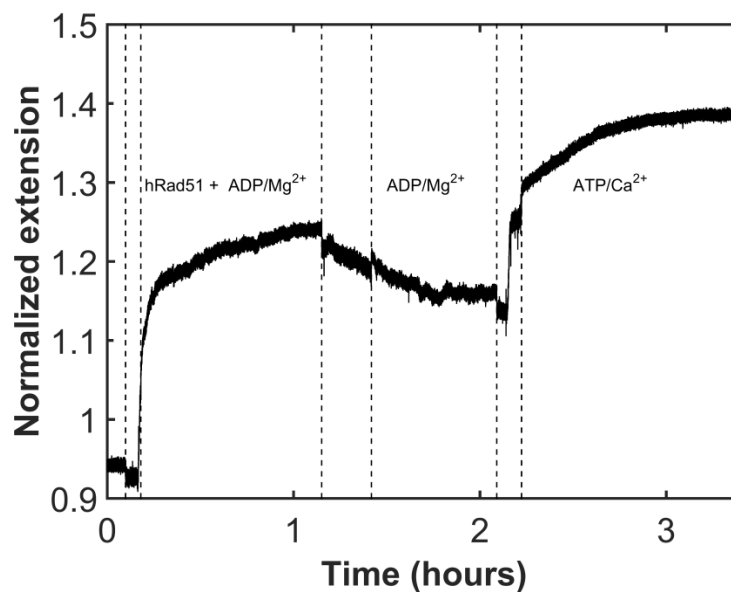


Figure S13 : Filament formed on a nicked dsDNA molecule in the presence of ADP-Mg²⁺. A partially stretched filament was formed with a stretching of 20 or 32%, depending on the presence of hRad51 in solution ($L = 1.14$ or 1.26 times the crystallographic length of the DNA molecule).

When a buffer containing ATP-Ca²⁺ was injected, the molecule's extension reaches ~ 1.5 times its initial length, i.e. the *S-State*. During the experiment, a pulling force of $F=8$ pN was maintained.

ⁱ R. B. Robertson, D. N. Moses, Y. H. Kwon, P. Chan, P. Chi, H. Klein, P. Sung, and E. C. Greene, "Structural transitions within human Rad51 nucleoprotein filaments.," Proc. Natl. Acad. Sci. U. S. A., 106 (2009) 12688–93