

**Supplemental Figure S1**

**(a) Length dependence of DrRecA nucleation times.** Assembly processes took place using 186 bp (left) and 382 bp (right) dsDNA with Buffer B (pH 6.16) and 2 mM ATP. The fitted time is  $90.62 \pm 2.30$  s, N=91 (left) and  $43.84 \pm 1.60$  s, N=118 (right). **(b) Estimation of the nucleation times using different methods.** Here showed the results of fitting with exponential formula:  $y = y_0 + A \cdot \exp(-t/\tau)$ ,  $y = A \cdot \exp(-t/\tau)$  and the maximum likelihood estimation (MLE) method. All three approaches returned with a similar nucleation rate within experimental error.

**Supplemental Figure S2**

**Estimation of the extension rates using different methods.** (a) The extension rate distribution determined by linear fitting to the same traces. (b) The rate determined by dividing the total number of RecA subunits that could be bound at saturation (equivalent to one third of the available base pairs of DNA) by the duration time of a continuous Brownian motion change. Both approaches returned with very similar distributions under our experimental resolution; we thus defined the slope of the continuous BM increase region as the extension rate.

**Supplemental Figure S3**

**Conversion factor relating BM to the number of RecA subunits added at various DNA lengths.** Factors were derived from dividing the number of RecA protein binding sites (one third of the number of DNA base pairs) by the BM value difference (in nm) observed between the maximum RecA-dsDNA value and that observed with naked dsDNA (the value shown in Figure 1b) at each DNA length. Error bars indicate the standard deviation.

**Supplemental Figure S4**

**The extension rate is independent of pH for EcRecA.** The rate distribution of EcRecA with 382 bp dsDNA was determined using Buffer B at the indicated pH (5.90, 6.06, 6.16 from top to bottom).

**Supplemental Figure S5**

**The extension rate is independent of pH for DrRecA.** The rate distribution for DrRecA binding to 186bp dsDNA was determined using the same buffer as Figure S5 at the indicated pH (5.90, 6.06, 6.16 from top to bottom).

## References:

- 1 Yu, X. & Egelman, E. H. (1997) The RecA hexamer is a structural homologue of ring helicases. *Nat. Struct. Biol.*, **4**, 101-104.
- 2 Baumann, P., Benson, F. E., Hajibagheri, N. & West, S. C. (1997) Purification of human Rad51 protein by selective spermidine precipitation. *Mutat. Res. DNA Repair*, **384**, 65-72.
- 3 Passy, S. I. *et al.* (1999) Human Dmc1 protein binds DNA as an octameric ring. *Proc. Natl. Acad. Sci. USA*, **96**, 10684-10688.
- 4 Yang, S. X., Yu, X., Seitz, E. M., Kowalczykowski, S. C. & Egelman, E. H. (2001) Archaeal RadA protein binds DNA as both helical filaments and octameric rings. *J. Mol. Biol.*, **314**, 1077-1085.

Figure S1(a)

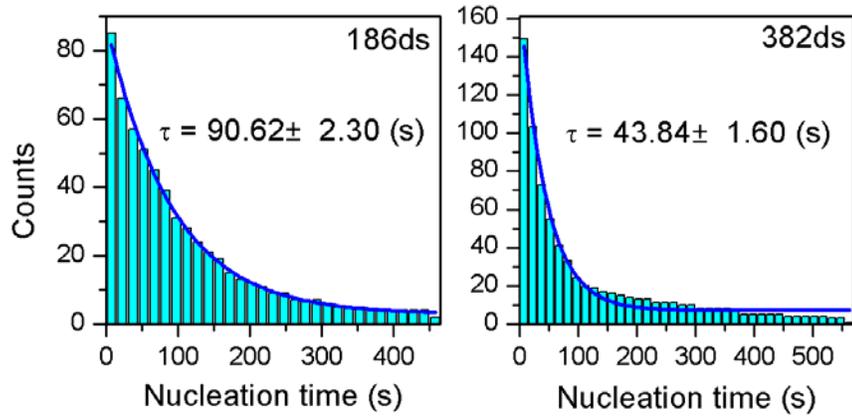
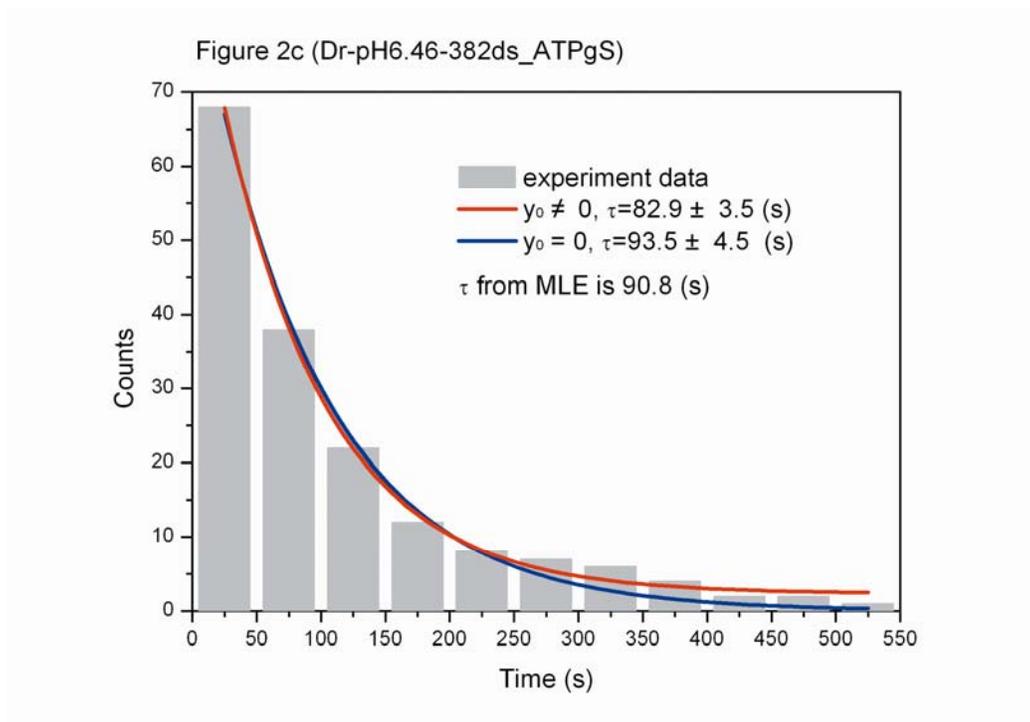


Figure S1(b)



	nucleation time $\tau$ (s)			nucleation rate ( $\text{bp}^{-1} \text{min}^{-1}$ )		
	$y = y_0 + A \cdot \exp(-t/\tau)$		MLE	$y = y_0 + A \cdot \exp(-t/\tau)$		MLE
	$y_0 \neq 0$	$y_0 = 0$		$y_0 \neq 0$	$y_0 = 0$	
Figure 2a	74.8 ( $R^2=0.99$ )	95.6 ( $R^2=0.98$ )	82.4	$2.1 \times 10^{-3}$	$1.6 \times 10^{-3}$	$1.9 \times 10^{-3}$
Figure 2b	55.5 ( $R^2=0.99$ )	64.9 ( $R^2=0.98$ )	61.4	$2.1 \times 10^{-3}$	$1.7 \times 10^{-3}$	$1.8 \times 10^{-3}$
Figure 2c EcATP	182.9 ( $R^2=0.99$ )	150.7 ( $R^2=0.99$ )	143.0	$8.6 \times 10^{-4}$	$1.0 \times 10^{-3}$	$1.1 \times 10^{-3}$
Figure 2c EcATP $\gamma$ S	81.4 ( $R^2=0.99$ )	71.7 ( $R^2=0.99$ )	80.9	$1.9 \times 10^{-3}$	$2.2 \times 10^{-3}$	$1.9 \times 10^{-3}$
Figure 2c DrATP	137.6 ( $R^2=0.99$ )	143.4 ( $R^2=0.99$ )	141.3	$1.1 \times 10^{-3}$	$1.1 \times 10^{-3}$	$1.1 \times 10^{-3}$
Figure 2c DrATP $\gamma$ S	82.9 ( $R^2=0.99$ )	93.5 ( $R^2=0.99$ )	90.8	$1.9 \times 10^{-3}$	$1.7 \times 10^{-3}$	$1.7 \times 10^{-3}$

Figure S2

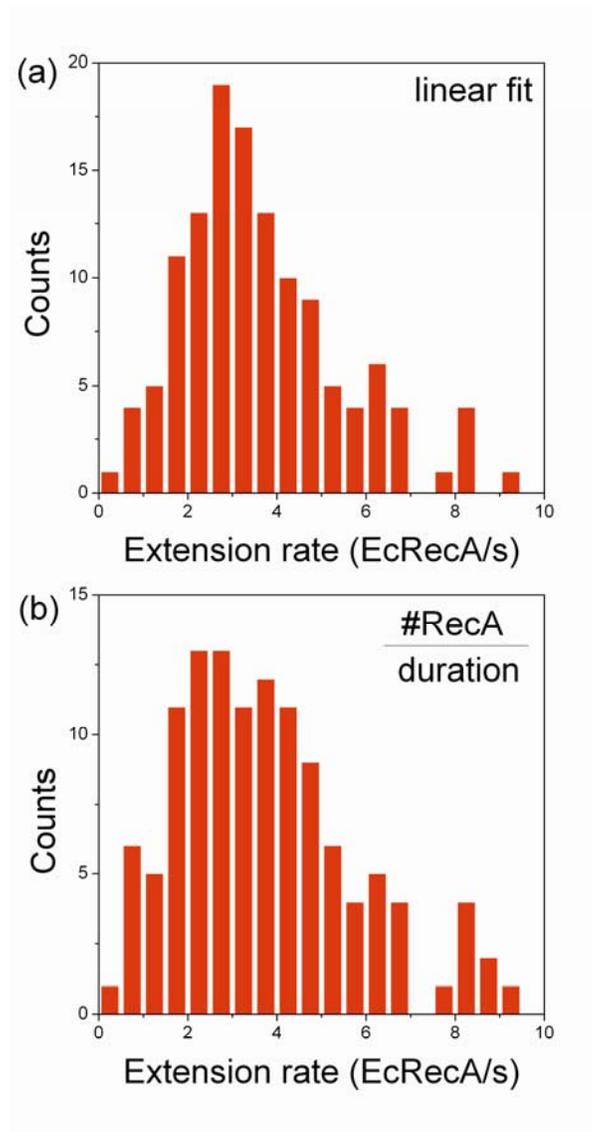


Figure S3

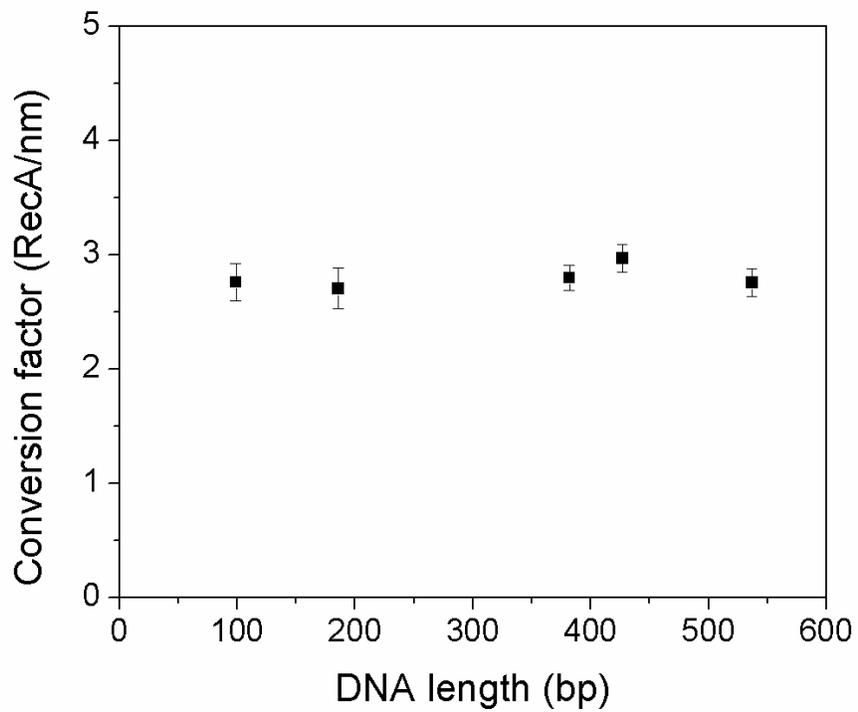


Figure S4

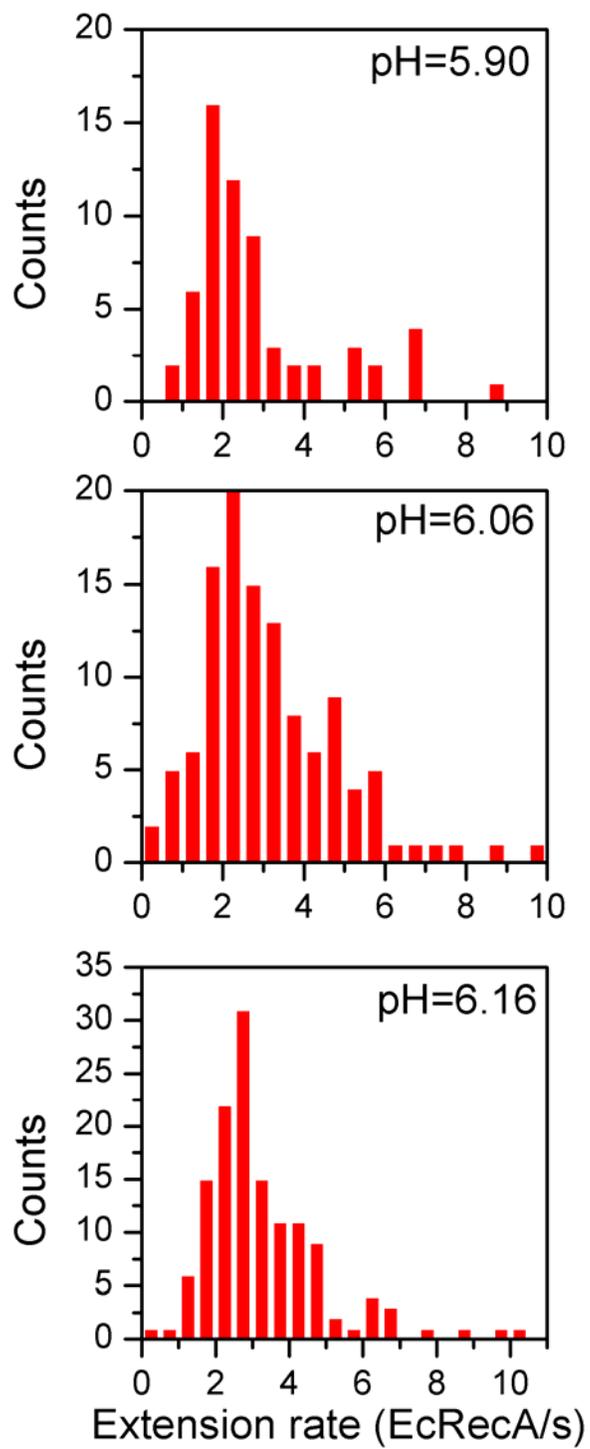


Figure S5

