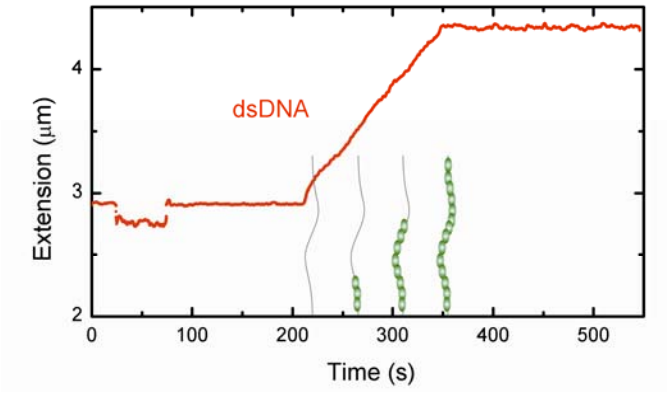
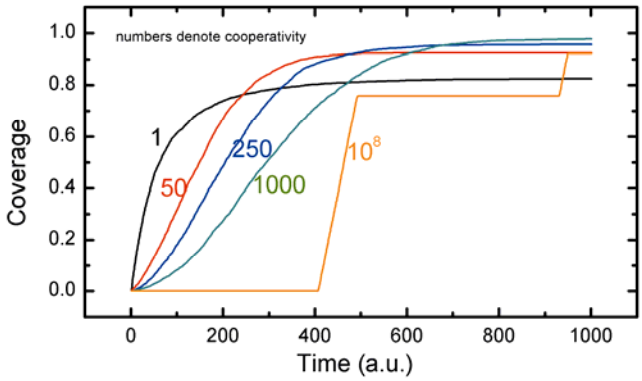
Supplementary Information

Figure S1



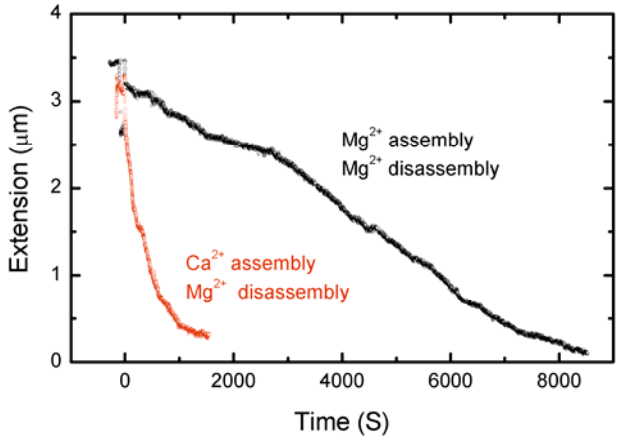
RecA polymerization on dsDNA. After flushing in 1 μM RecA, a pause is observed after which a filament nucleates and extends with a linear growth profile corresponding to the extension of a single RecA-dsDNA filament.

Figure S2



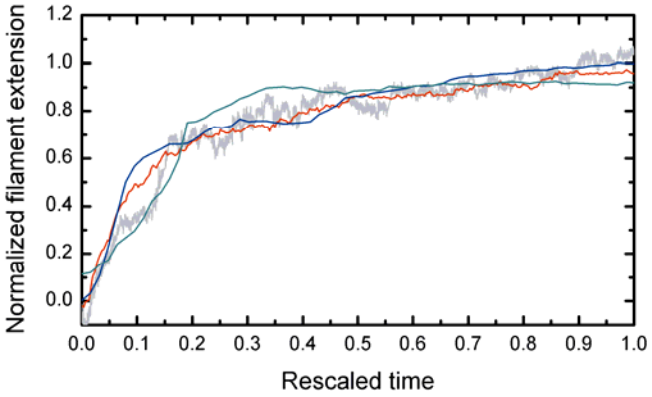
Shape of the binding profile changes from exponential for a cooperativity of 1 (black line) to sigmoidal for higher cooperativity (red, blue, green). Very large cooperativity leads to linear growth profiles (orange), the stepwise behavior in this curve is due to the unidirectional growth used in these simulations.

Figure S3



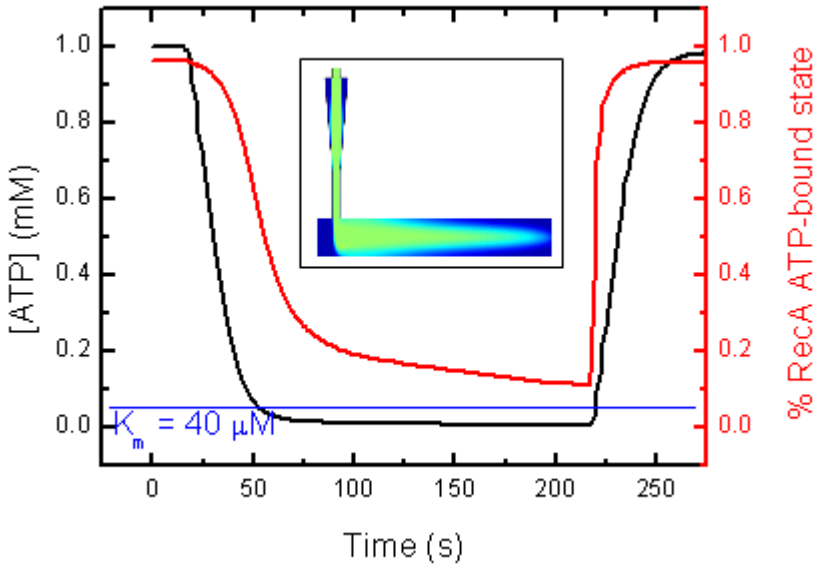
RecA disassembly after assembly at a high stretchin force (20 pN) preventing the formation of secondary structure in ssDNA. An exponential disassembly profile is observed for filaments assembled in Ca^{2+} buffer indicating the presence of gaps (red curve). A filament formed in the presence of Mg^{2+} at a stretching force of 20 pN shows a linear dissociation profile indicative of dissociation from a single continuous RecA-ssDNA filament.

Figure S4



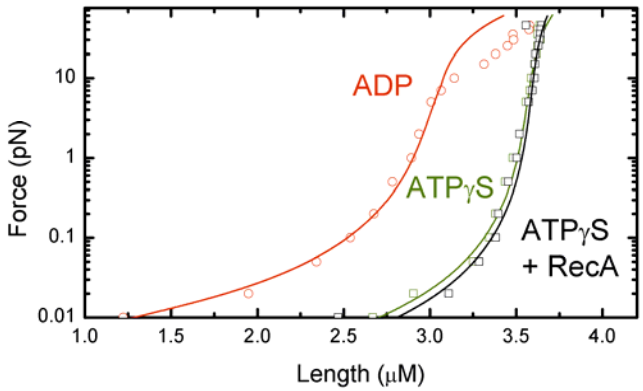
Binding profiles at different RecA concentrations: [RecA]=0.1 μM (gray), 0.4 μM (red), 0.7 μM (green), and 1.0 μM (blue). The time axis for all four traces were rescaled by the inverse of the nucleation rate determined from Monte Carlo simulation fits. The shape of the binding profile at different RecA concentrations is conserved confirming that filament nucleation and extension occur with the same binding unit.

Figure S5



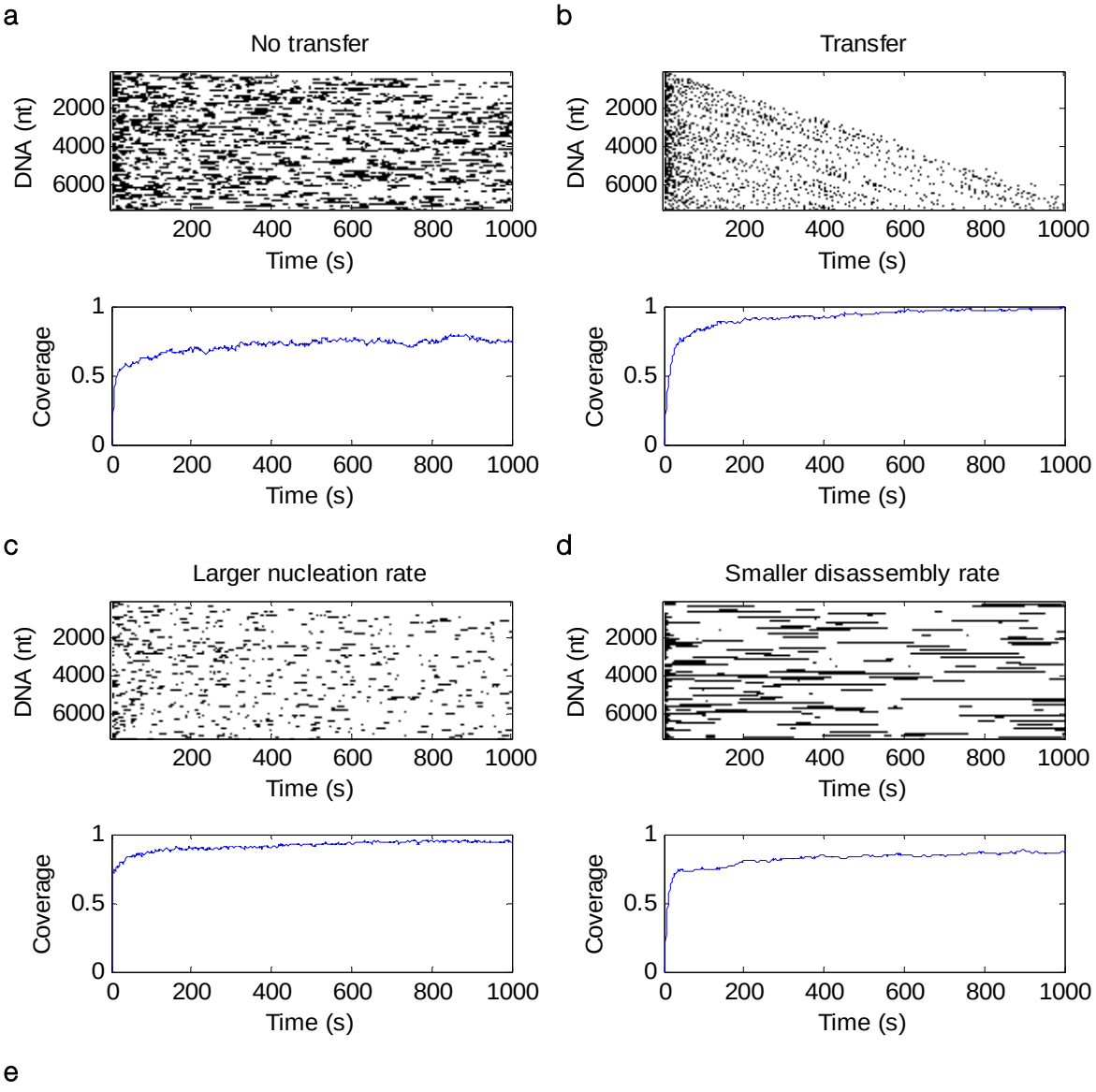
Time dependent ATP concentrations and ATP binding by RecA. Time dependent ATP concentrations were calculated using a commercial computer program Comsol multiphysics (black line). Inset shows the flow cell geometry (not to scale) at t = 15 s. The fraction of RecA in the ATP-bound state, Θ was calculated as $\Theta = [ATP] / (K_m + [ATP])$, where K_m was taken as 40 μM.

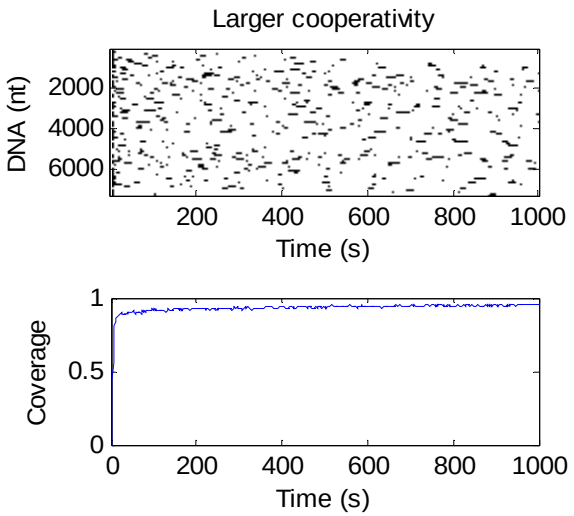
Figure S6



A force-extension curve for a RecA-ssDNA filament in the ADP-bound state showed a marked shift when ATPγS was added (red to green curve). Subsequently changing the buffer for one containing ATPγS and RecA did not noticeably change the force extension curve. This proves that no appreciable disassembly of RecA from the ADP filament takes place which would have resulted in a length increase when RecA was added (black).

Figure S7


a
No transfer
DNA (nt)
2000
4000
6000
200
400
600
800
1000
Time (s)
Coverage
1
0.5
0
0
200
400
600
800
1000
Time (s)
b
Transfer
DNA (nt)
2000
4000
6000
200
400
600
800
1000
Time (s)
Coverage
1
0.5
0
0
200
400
600
800
1000
Time (s)
c
Larger nucleation rate
DNA (nt)
2000
4000
6000
200
400
600
800
1000
Time (s)
Coverage
1
0.5
0
0
200
400
600
800
1000
Time (s)
d
Smaller disassembly rate
DNA (nt)
2000
4000
6000
200
400
600
800
1000
Time (s)
Coverage
1
0.5
0
0
200
400
600
800
1000
Time (s)
e



Monte Carlo simulations of RecA filament formation. Upper panels show kymographs of the simulated RecA-ssDNA filament. The evolution of a 7.3 kb ssDNA molecule, depicted on the *y*-axis, is shown with time. Gray areas show bound protein; at $t = 0$ no RecA is bound to the ssDNA, as time progresses RecA rapidly nucleates and a filament forms. The lower panels show the coverage of the molecule. Simulation parameters were obtained from experimental data as described in the main text. The table below list the simulation parameters. The modified parameters are shown in bold. (a) Monte Carlo simulation of RecA-ssDNA filament formation excluding transfer. Many gaps still remain after 1000s when transfer of monomers is not taken into account. Including transfer (b) yields a continuous filament. Monte Carlo simulation of RecA-ssDNA filament formation excluding transfer but with a 10 fold increased nucleation rate (c). Many gaps still remain after 1000s when transfer of monomers is not taken into account. Monte Carlo simulation of RecA-ssDNA filament formation excluding transfer but with a 10 fold reduced disassembly rate (d). Many gaps still remain after 1000s when transfer of monomers is not taken into account. Monte Carlo simulation of RecA-ssDNA filament formation excluding transfer but with a 10 fold increased cooperativity (e). Many gaps still remain after 1000s when transfer of monomers is not taken into account

Table 1: Simulation Parameters for Figure S7

	Fig 5a	Fig 5b	Fig 5c	Fig 5d	Fig 5e
Nucleation unit (monomers)	6	6	6	6	6
Extension unit (monomers)	6	6	6	6	6
Binding size (nt)	3	3	3	3	3
Nucleation rate ($s^{-1}nt^{-1}$)	0.006	0.006	**0.06**	0.006	0.006
Cooperativity	15	15	15	15	**150**
Disassembly (mon/s/filament)	0.3	0.3	0.3	**0.03**	0.03
Transfer (mon/s/filament)	0	**2**	0	0	0