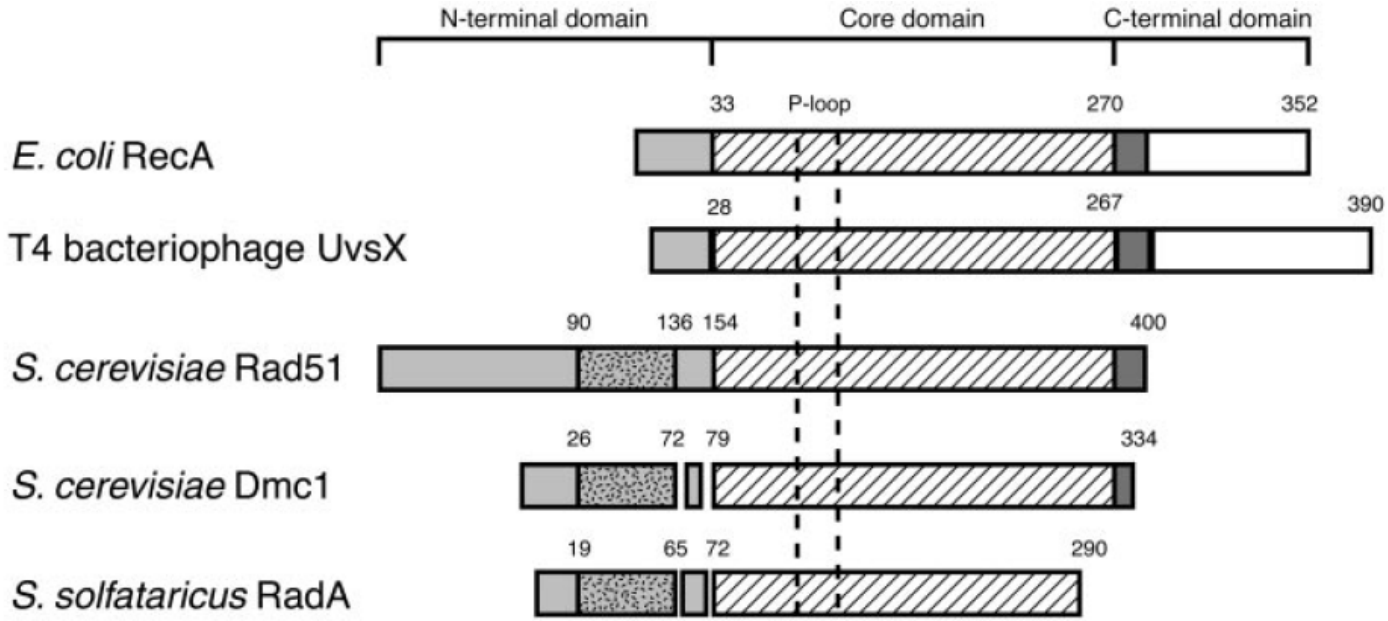
Real-time assembly and disassembly of human RAD51 filaments on individual DNA molecules

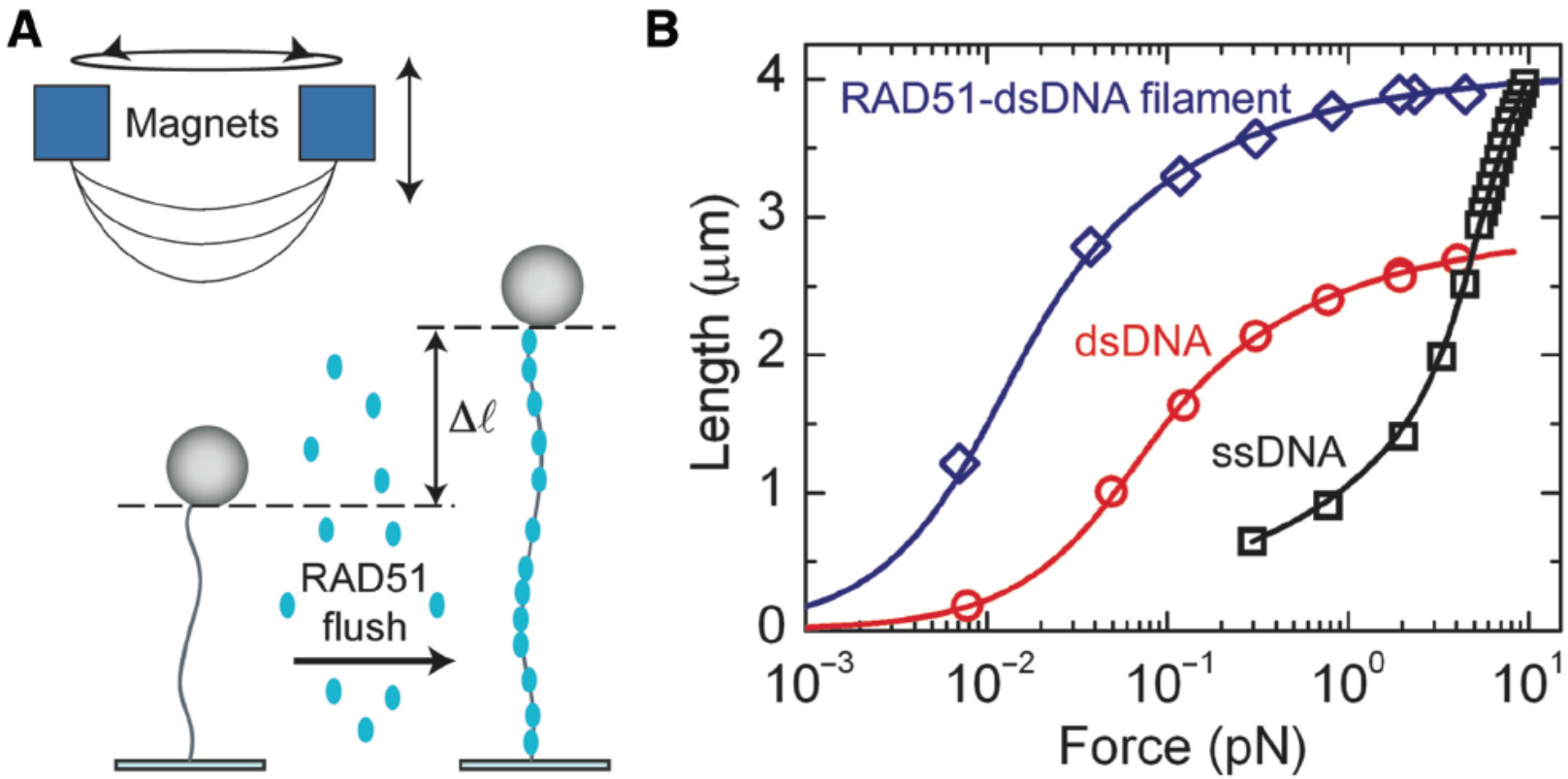
Thijn van der Heijden[1], Ralf Seidel[1], Mauro Modesti[2], Roland Kanaar[2,3], Claire Wyman[2,3,*] and Cees Dekker[1,*]

[1]Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, [2]Department of Cell Biology and Genetics and [3]Department of Radiation Oncology, Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands

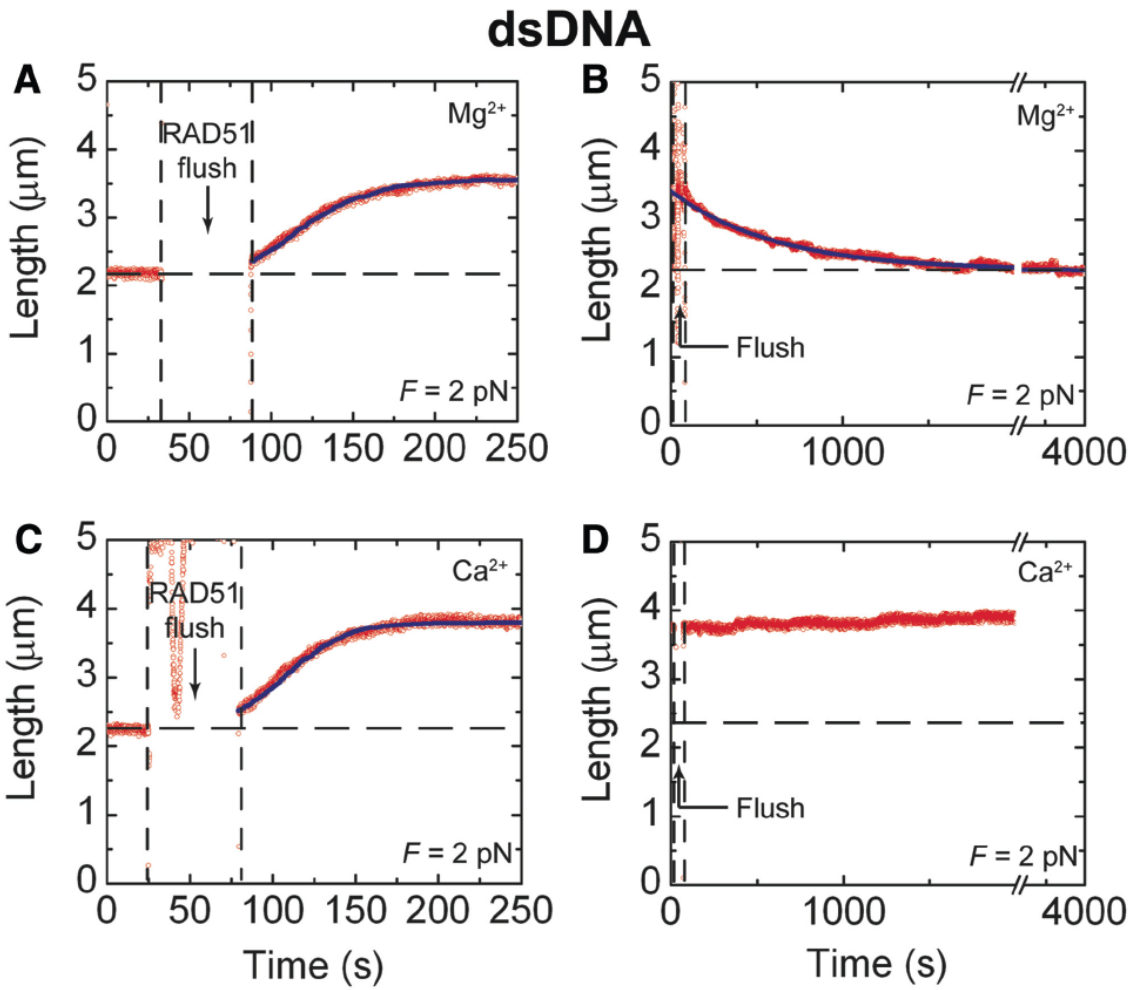
RecA family



Experimental Realization

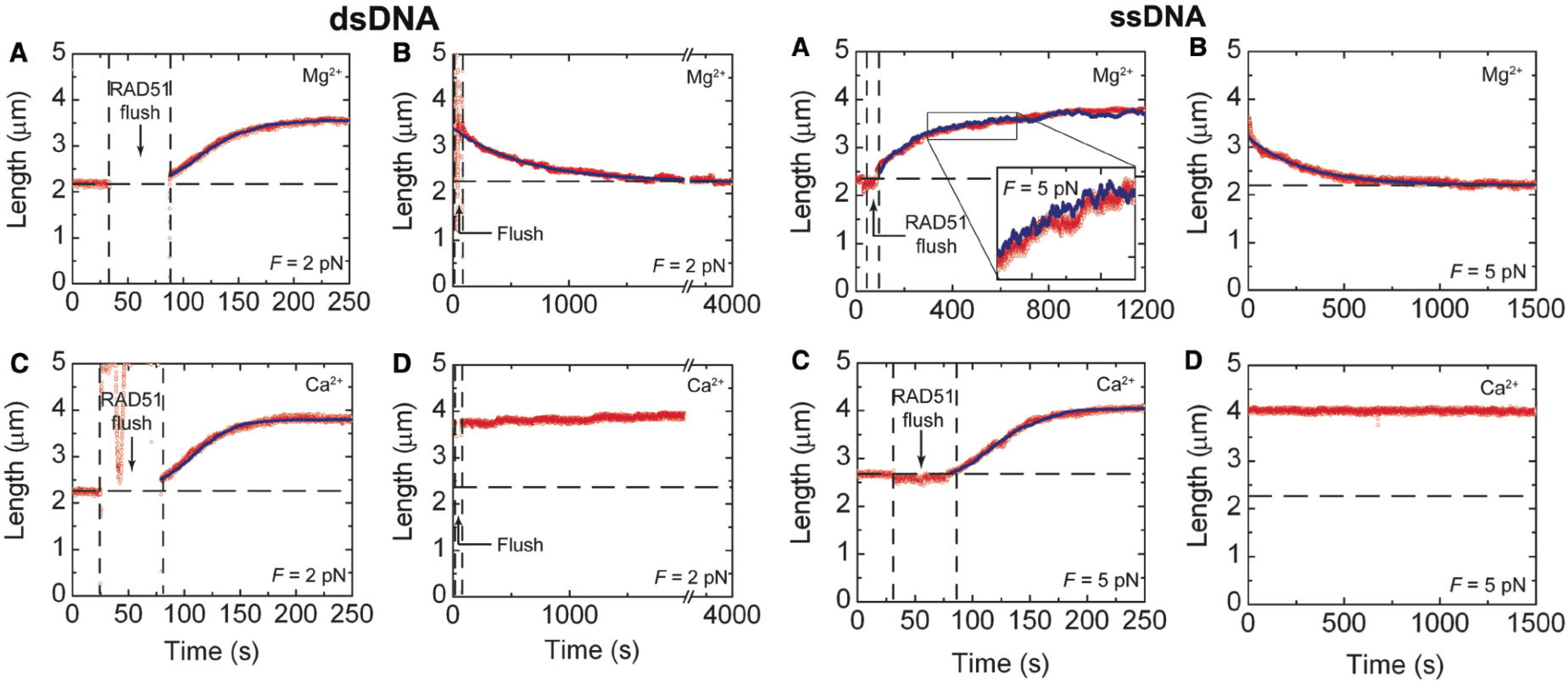


Rad51 + dsDNA



- ATP-Ca2+ is none hydrolysable causing no dissociation

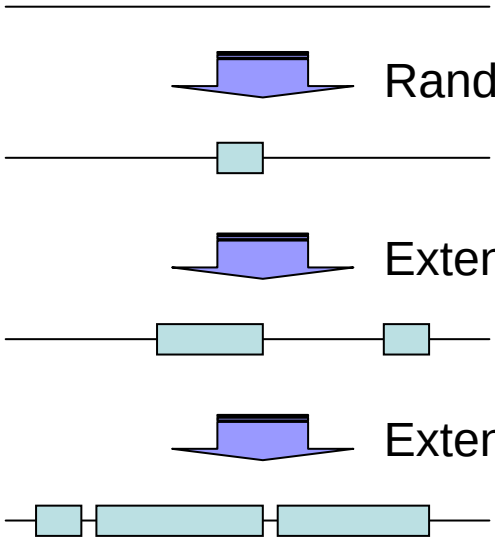
dsDNA vs. ssDNA

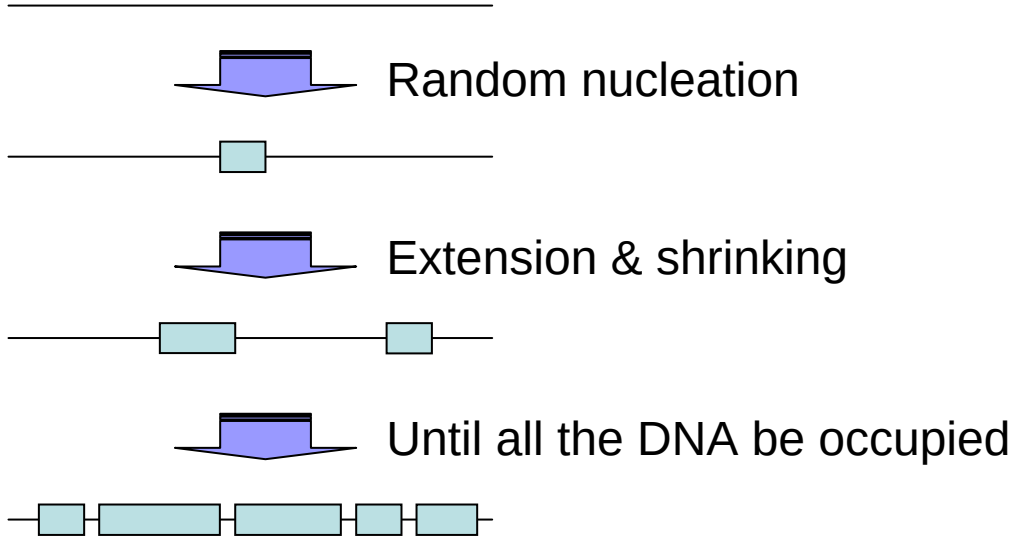


- Assembly on ssDNA is slower then that on dsDNA (for Mg2+)
- Assembly on ssDNA is similar to that on dsDNA (for Ca2+)

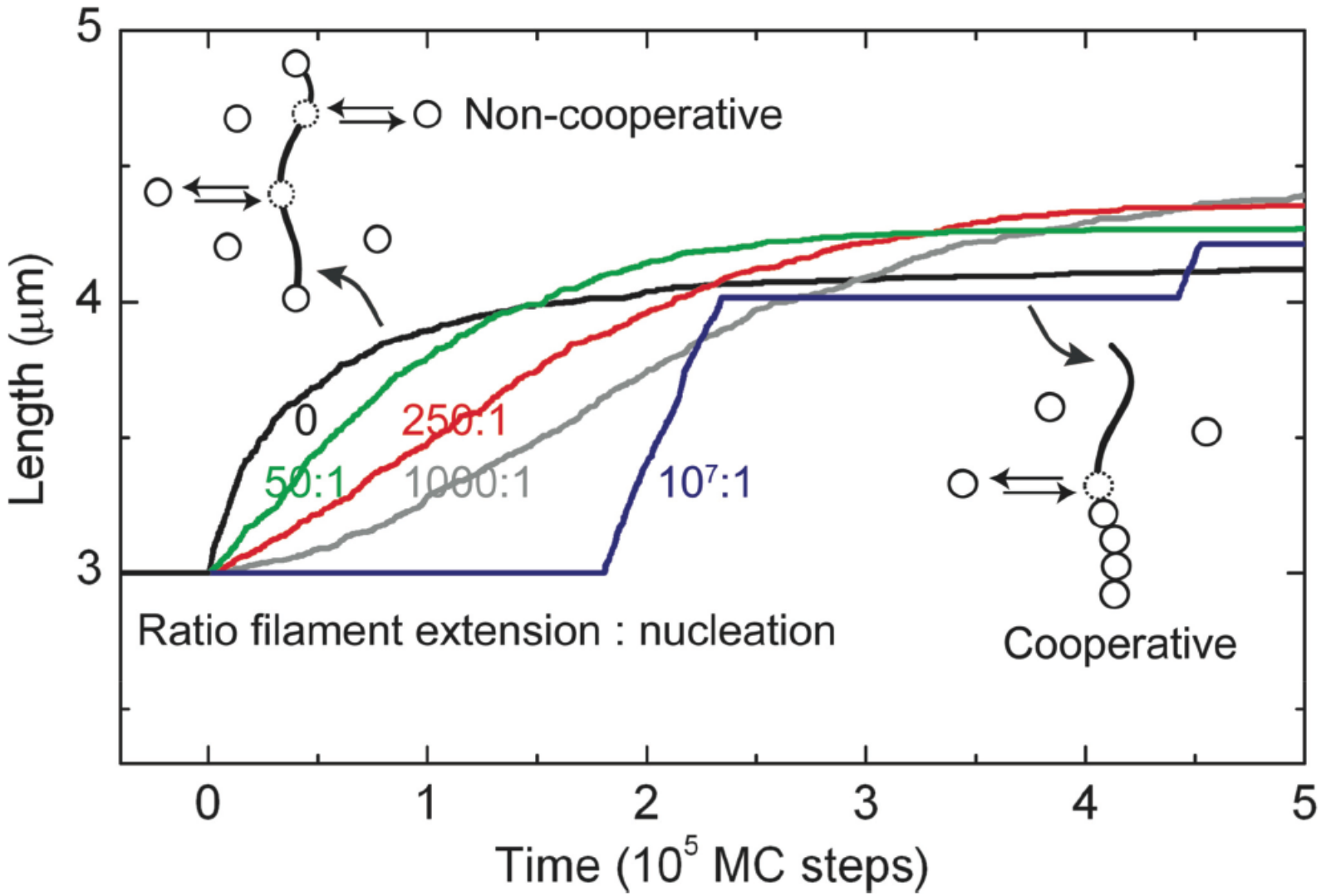
- Disassembly curve was fit w. 2-exp.
- Disassembly from ssDNA is faster then that from dsDNA (for Mg2+)

Monte Carlo simulation



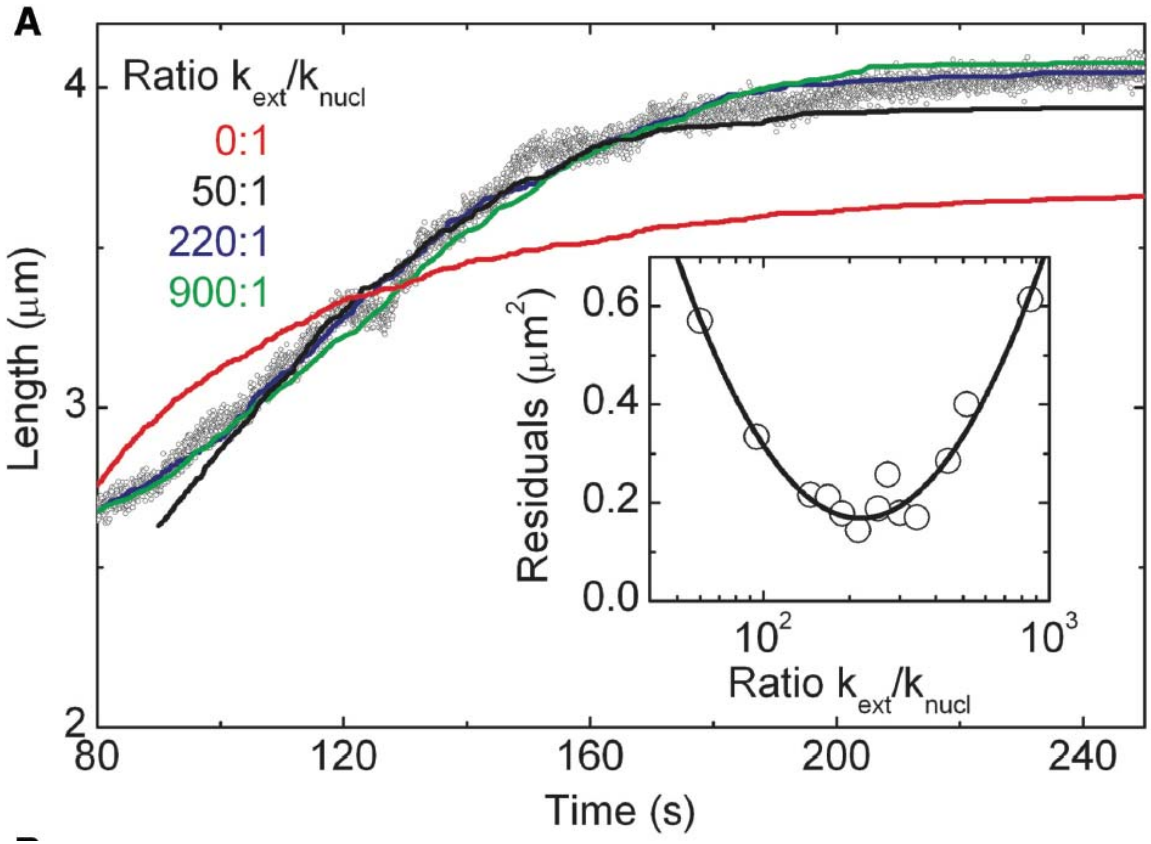


Monte Carlo simulation

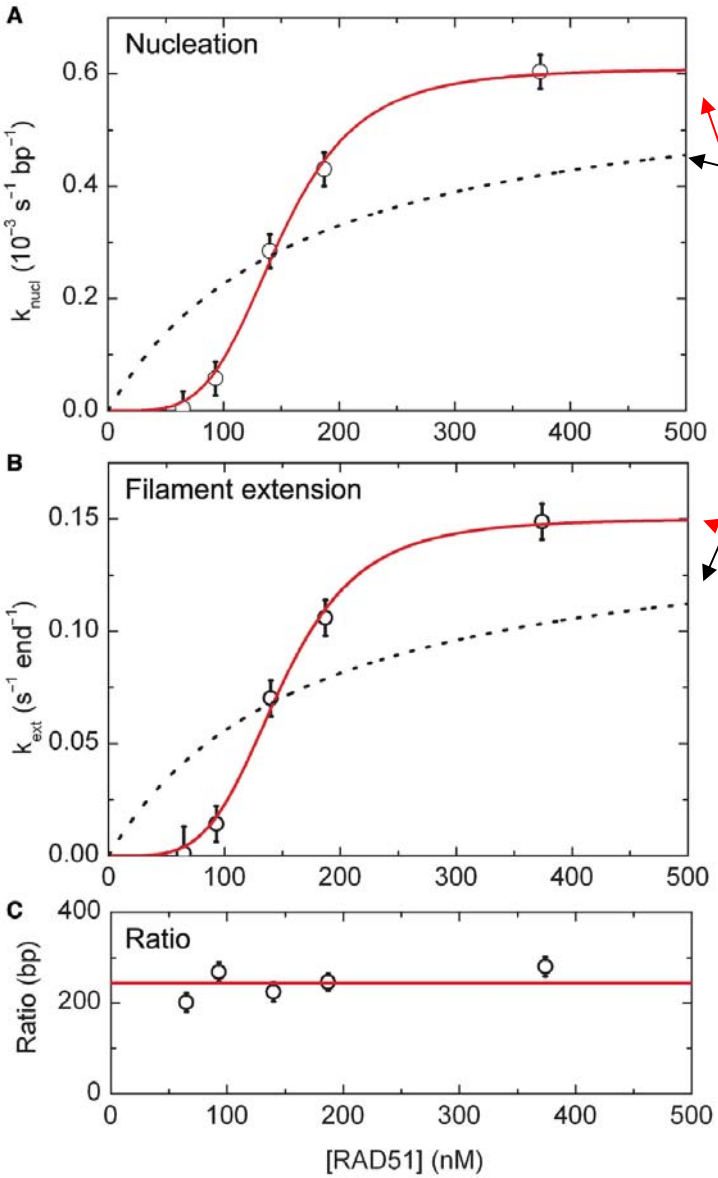


Fitting w. Monte Carlo simulation

ssDNA w. Ca2+



Assembly rate vs. [Rad51]



Michaelis-Menton eq.

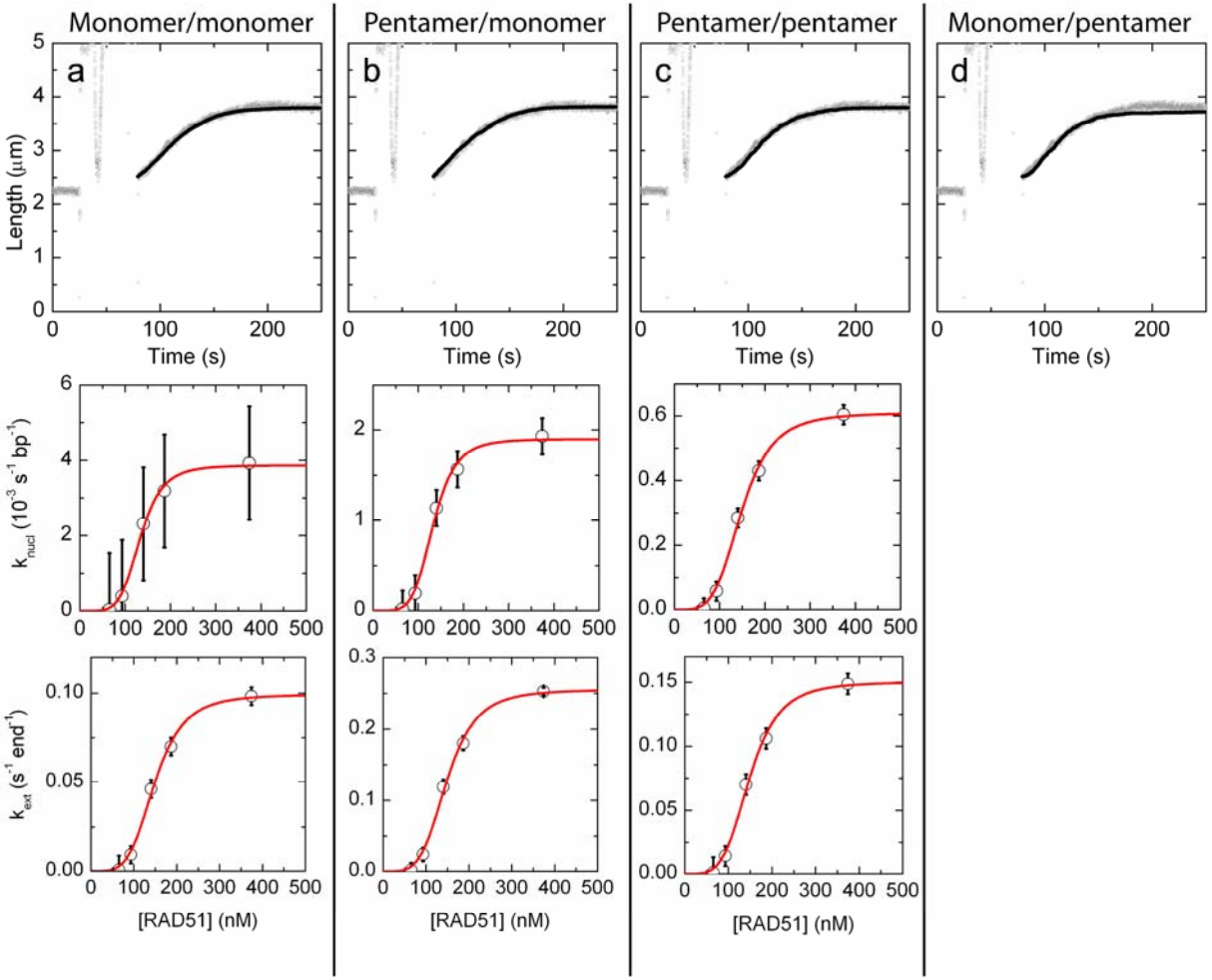
$$k_i = \frac{k_{i,\max}\left[RAD51\right]}{s_{0.5} + \left[RAD51\right]}$$

Hill relation

$$k_i = \frac{k_{i,\max}\left[RAD51\right]^n}{s_{0.5}^n + \left[RAD51\right]^n}$$

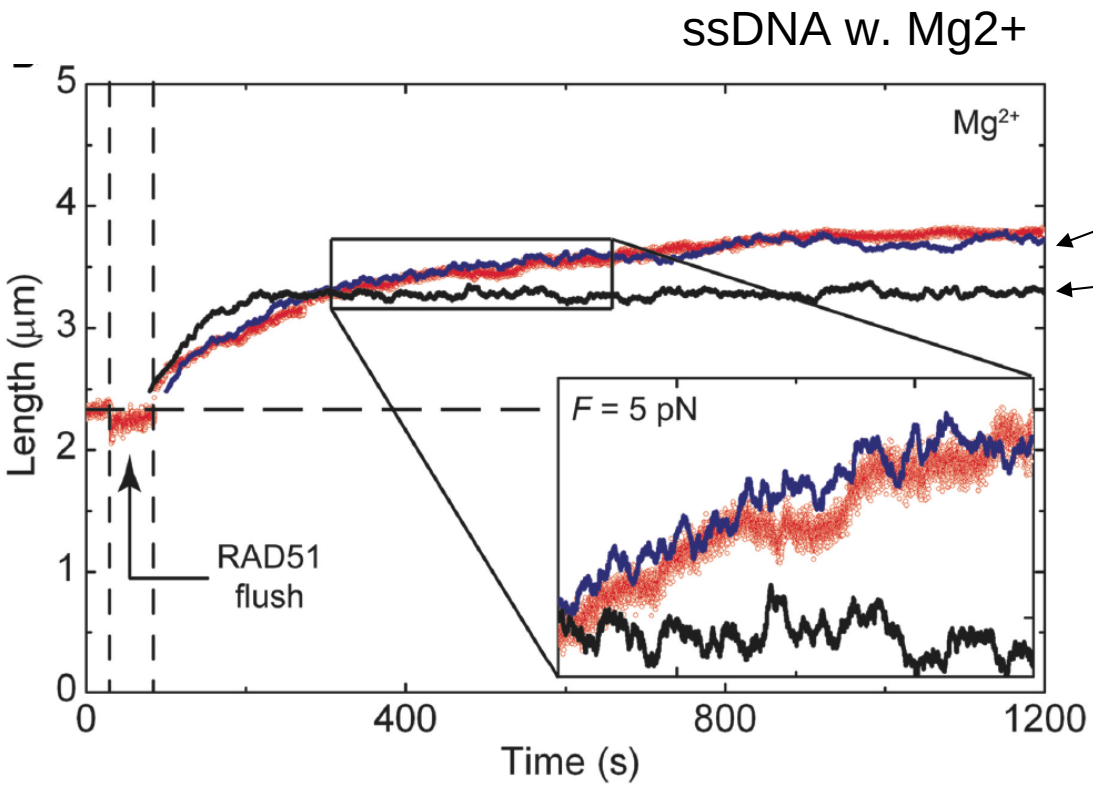
n=4.3±0.5

Nucleation/filament extension binding size



	monomer monomer	pentamer monomer	pentamer pentamer	monomer pentamer
Nucleation	5.4 ± 0.8	5.4 ± 0.8	4.3 ± 0.6	N.D.
Filament extension	4.3 ± 0.6	4.3 ± 0.6	4.3 ± 0.5	N.D.

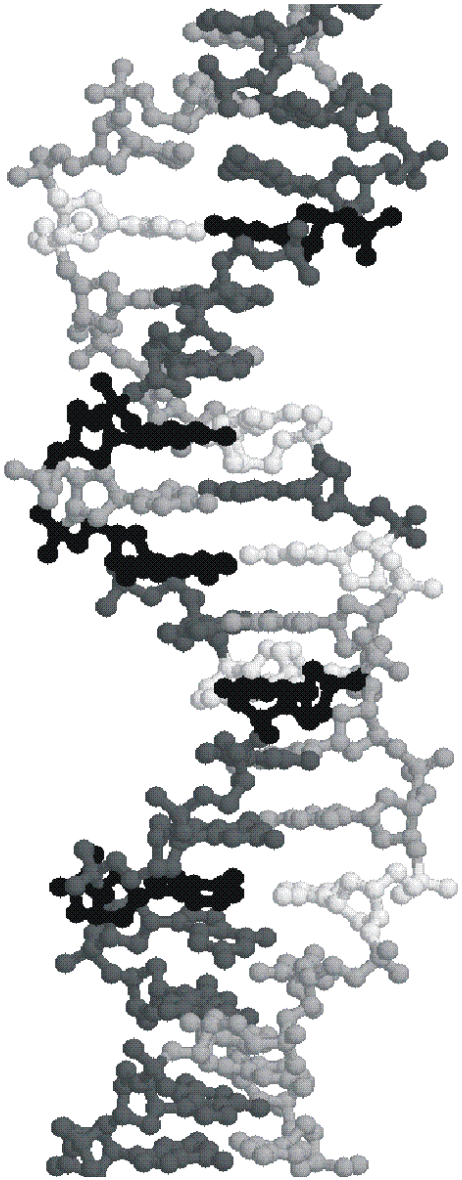
Location of disassembly

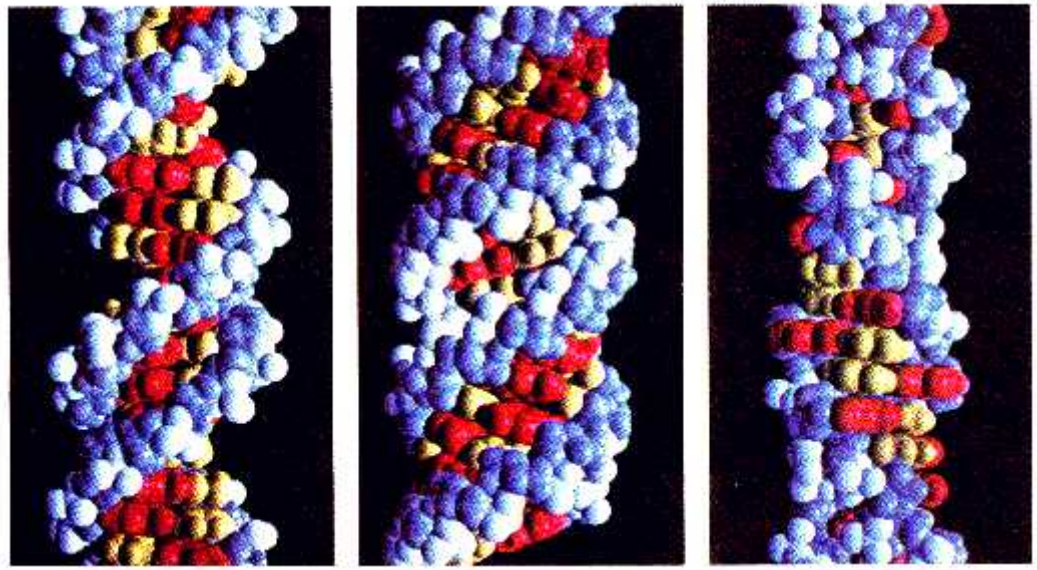


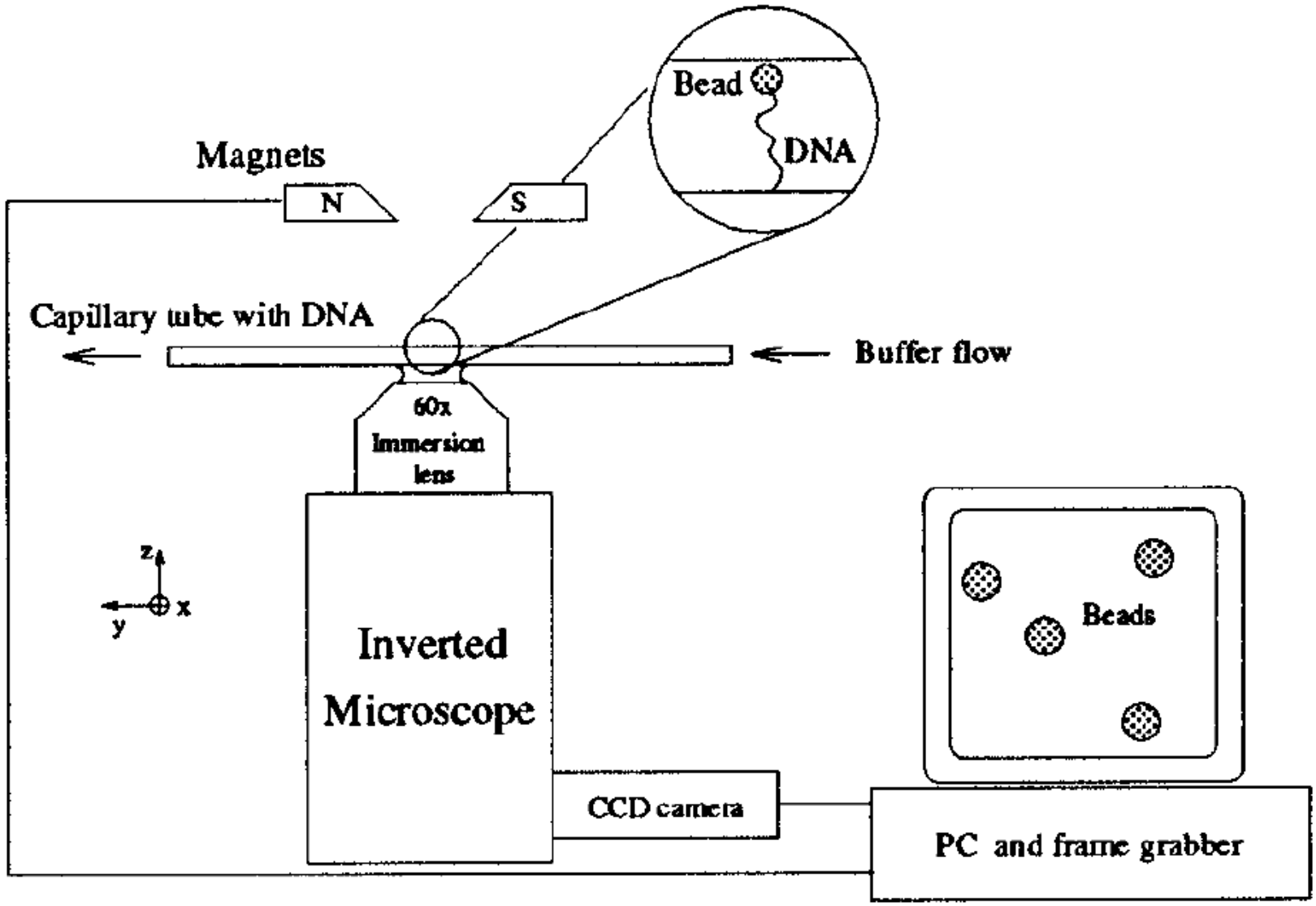
Disassembly at the end

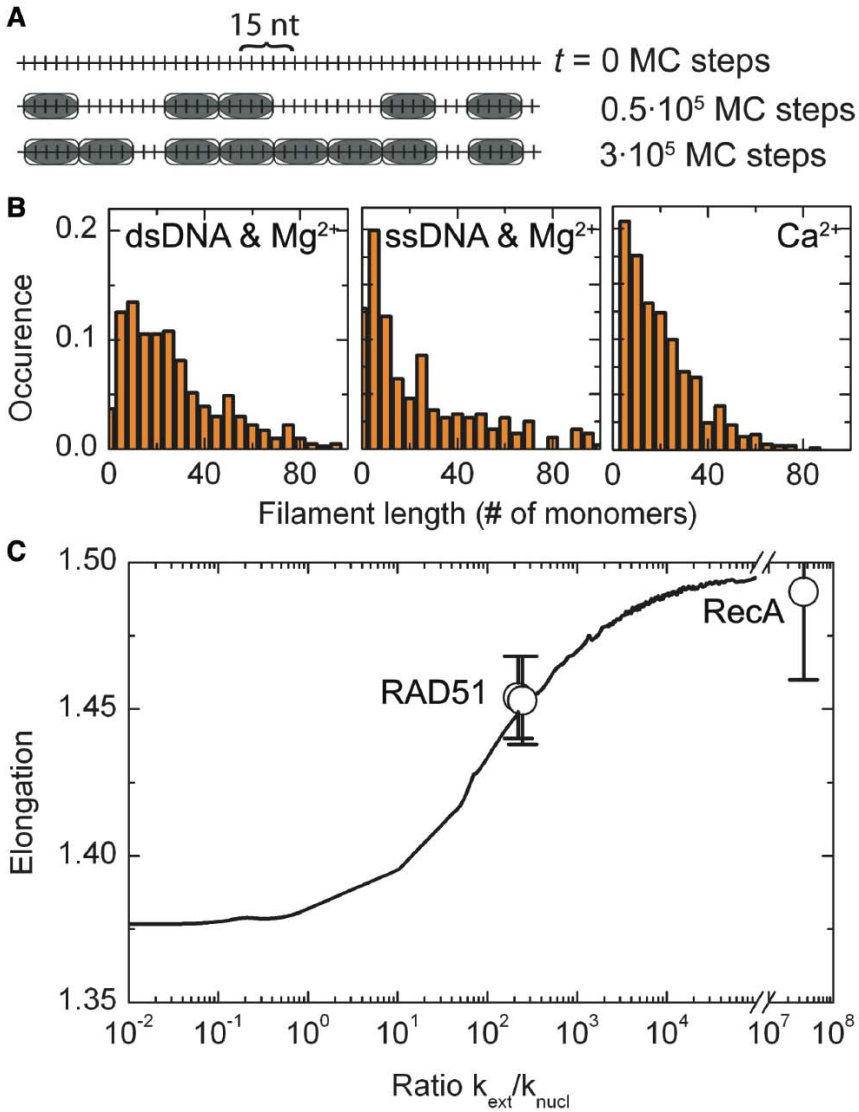
Random disassembly

Supplement Pages






Bead
DNA
Magnets
N
S
Capillary tube with DNA
Buffer flow
60x
Immersion
lens
z
x
y
Inverted
Microscope
Beads
CCD camera
PC and frame grabber


A
15 nt
t = 0 MC steps
0.5·10^5 MC steps
3·10^5 MC steps
B
dsDNA & Mg2+
ssDNA & Mg2+
Ca2+
Occurence
0.2
0.1
0.0
0
40
80
Filament length (# of monomers)
C
Elongation
1.50
1.45
1.40
1.35
RAD51
RecA
10^-2
10^-1
10^0
10^1
10^2
10^3
10^4
10^7
10^8
Ratio k_ext/k_nucl