Rad51

Single molecule
Real-time disassembly
and Structural transitions

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Structural transitions within human Rad51 nucleoprotein filaments

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Rad51 is a core component of the eukaryotic homologous recombination machinery and is responsible for key mechanistic steps during strand invasion. Higher order oligomers of Rad51 display a remarkable degree of structural variation, forming rings, compressed filaments, and elongated filaments. It is unclear whether Rad51 can transition directly between these different oligomeric structures without disassembling first into monomers. We have used single-molecule microscopy to investigate the behavior of human Rad51 assembled on double-stranded DNA. Our results show that human Rad51 can form elongated nucleoprotein filaments on DNA, but ATP hydrolysis causes a decrease in their length without concomitant dissociation of protein. Compressed Rad51 filaments can re-elongate when presented with either ATP or the non-hydrolyzable analog AMP-PNP, and these cycles of elongation and compression are reversible. A Rad51 mutant deficient in ATP hydrolysis is locked into an extended conformation that is incapable of transitioning to a compressed filament. Similarly, wild-type Rad51 bound to DNA in the presence of AMP-PNP was trapped in the elongated state. Proteins incapable of transitioning to the compressed state were also highly resistant to dissociation from the DNA. Taken together, our results indicate that nucleotide hydrolysis by human Rad51 triggers a reversible structural transition leading to filaments with reduced helical pitch.

DNA curtain | homologous recombination | single molecule imaging

Double-stranded DNA breaks (DSBs) are 1 of the most deleterious forms of DNA damage and can lead to cell death or oncogenic transformation. Homologous recombination (HR) is an evolutionarily conserved pathway used to repair DSBs, and is essential for maintaining genomic stability (1, 2). When a DSB occurs, the 5′ ends of the DNA are resected, yielding long 3′ single-stranded DNA (ssDNA) overhangs, which are the loading site for a DNA recombinase. The recombinase aligns the ssDNA with a homologous double stranded DNA (dsDNA) and then invades the duplex to form a D-loop. The invading end can then serve as a primer for the replication machinery, which uses the homologous duplex as a template, and the resulting products are resolved to restore the continuity of the chromosomes.

The DNA transactions that take place during HR are mediated by members of the *RAD52* epistasis group of proteins (1–3). This includes the recombinase Rad51, which plays a central role in HR and assembles into a nucleoprotein filament on the ssDNA overhangs generated at the DSB (4, 5). This filament is responsible for catalyzing the pairing, alignment, and strand invasion steps during recombination (6). Rad51 is sufficient to catalyze these reactions in vitro (7), however, numerous accessory factors are required in vivo and their functions range from facilitating Rad51 loading at the outset of the reaction to promoting the disassembly of Rad51 upon completion of strand invasion (1, 3, 8, 9).

Human Rad51 has a flexible N-terminal domain and a central ATP-binding core closely related to bacterial RecA (10). Rad51 forms right-handed helical filaments that extend the bound DNA by approximately 50% relative to B-form DNA (11, 12). The structural parameters defining the geometry of the filaments are variable, and can even vary within the same filament. In general, filaments active for DNA strand exchange have a steeper helical pitch (~90–130 Å) compared to inactive forms, which are more compressed (~65–85 Å). All evidence suggests that the elongated filaments are in the ATP bound state, whereas compressed filaments represent the ADP bound state. Ring-like structures have also been observed, and these are comprised of 6–8 subunits with an internal pore large enough to allow passage of DNA (13–16). These ring-like forms have no known function, but may represent an inactive storage form of the protein. The relationship between the different recombinase structures remains unknown, and it is not clear whether 1 structural form can directly transition to another without first disassembling into monomeric units.

To evaluate the properties of Rad51 we have established a single-molecule assay that allows us to probe individual nucleoprotein filaments in real time. Here we use this assay to examine the behavior of human Rad51 assembled onto dsDNA. Our results demonstrate that human Rad51 can transition between an elongated nucleoprotein filament and a more compressed structure. These transitions are triggered by ATP hydrolysis, and the Rad51 filaments could reversibly interconvert between the elongated and compressed forms when nucleotide cofactor was replenished. We suggest that the transitions between these structural forms may be a target for regulation during homologous recombination.

Results

Visualizing Rad51 Nucleoprotein Filaments. We have previously used total internal reflection fluorescence microscopy (TIRFM) to monitor the assembly of human Rad51 filaments on YOYO1-stained DNA curtains (17). However, Rad51 displaces YOYO1 from DNA, making it difficult to detect the fluorescence signal at high protein concentrations (17). To avoid this problem, we developed an assay that uses DNA substrates tagged at 1 end with a fluorescent quantum dot (QD; Fig. 1). Fig. 1 illustrates the procedure for visualizing Rad51 filaments with TIRFM. After locating a DNA curtain (17, 18), filament assembly was initiated by injecting 1 μM Rad51 in buffer containing 40 mM Tris (pH 7.8), 0.2 mg/mL BSA, 1 mM $MgCl_2$, 1 mM DTT, and 1 mM ATP, unless otherwise stated, these conditions were used for all experiments reported below. Under these conditions, Rad51

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The authors declare no conflict of interest.

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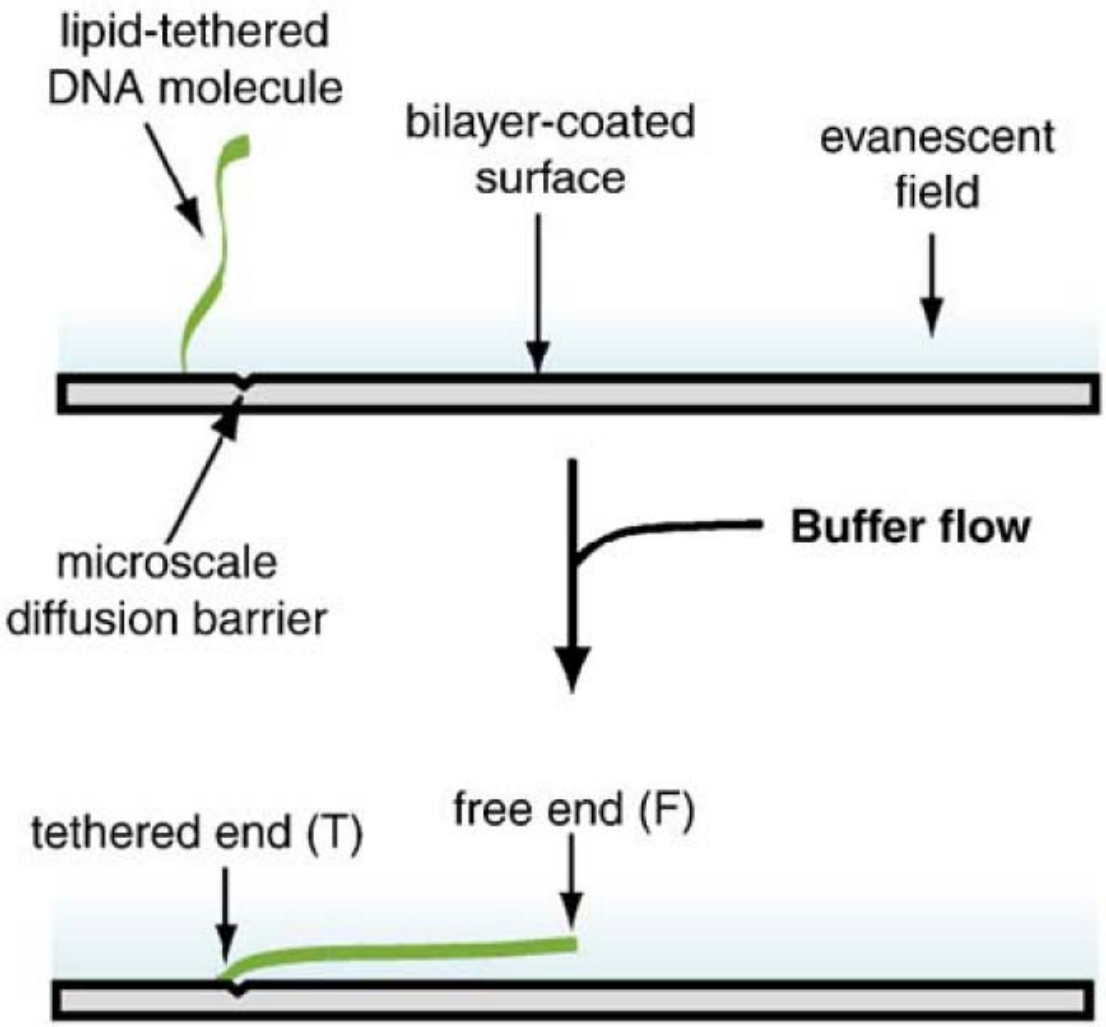
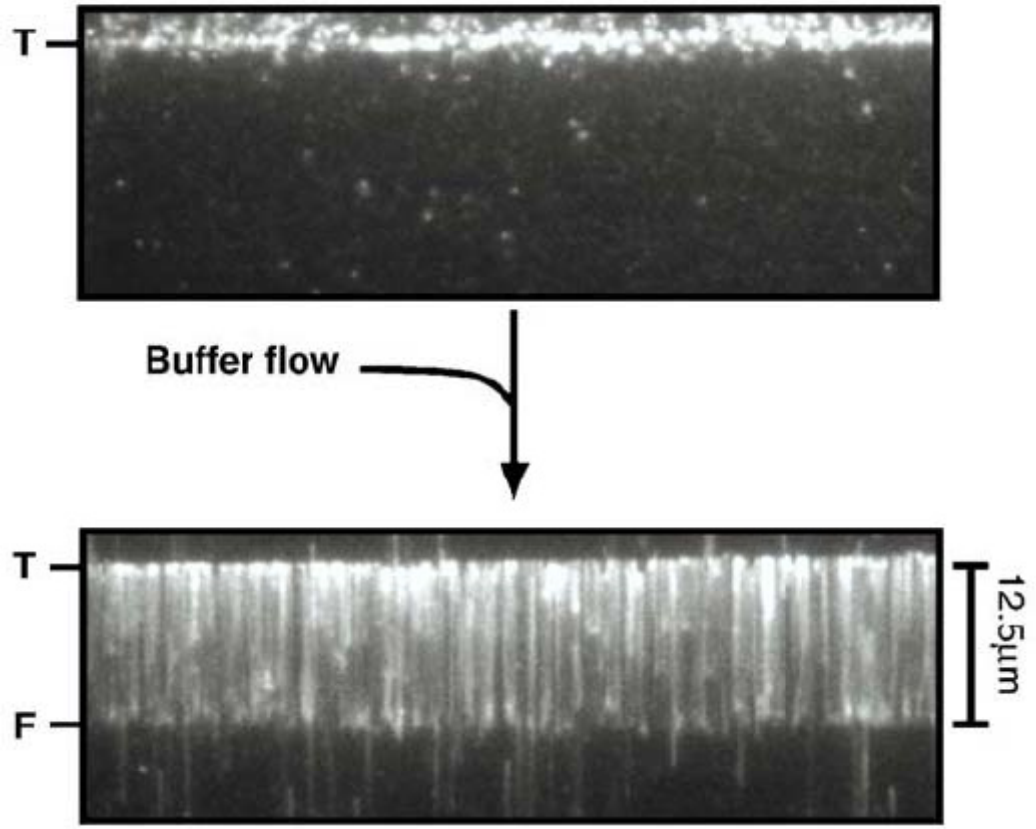
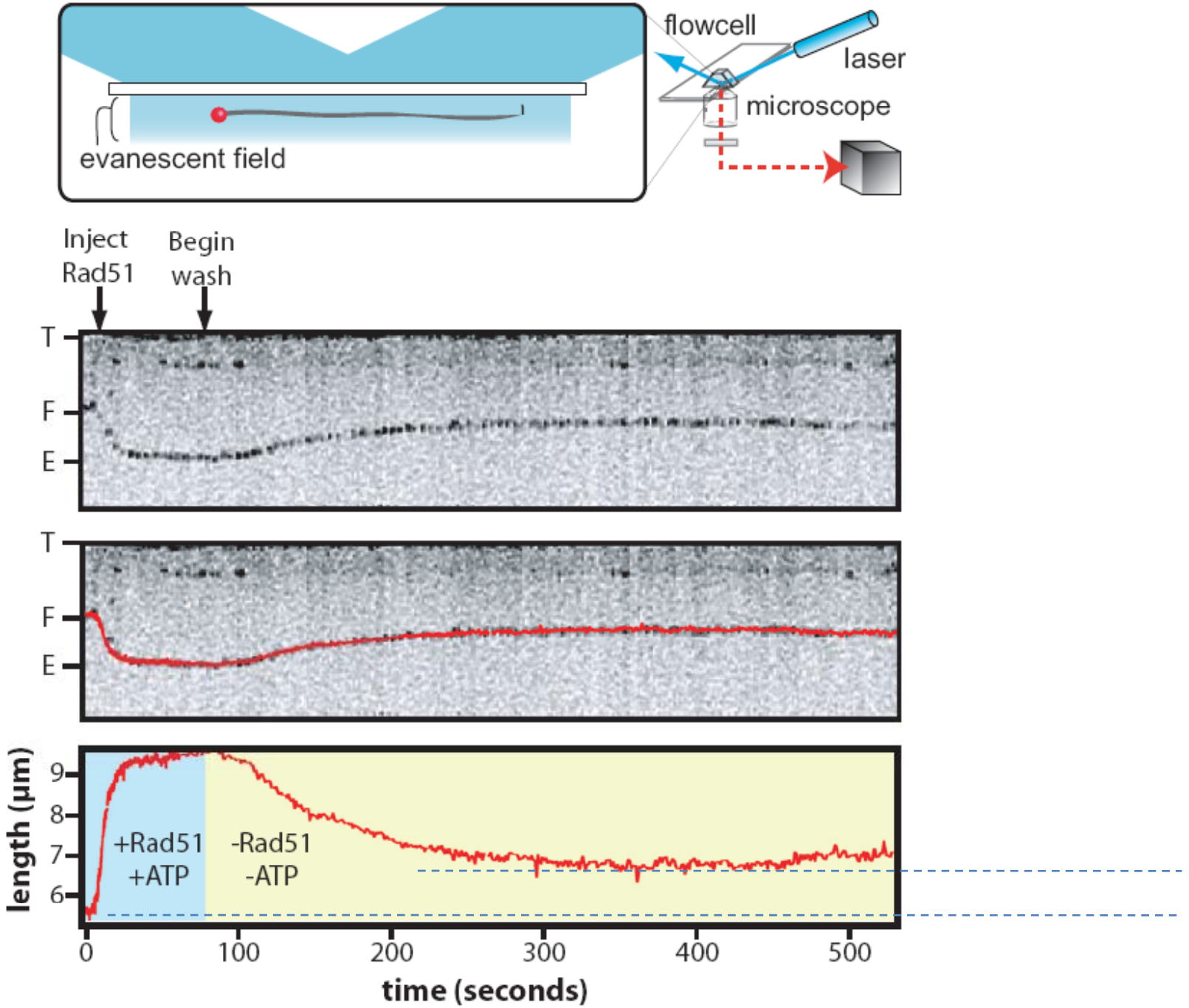
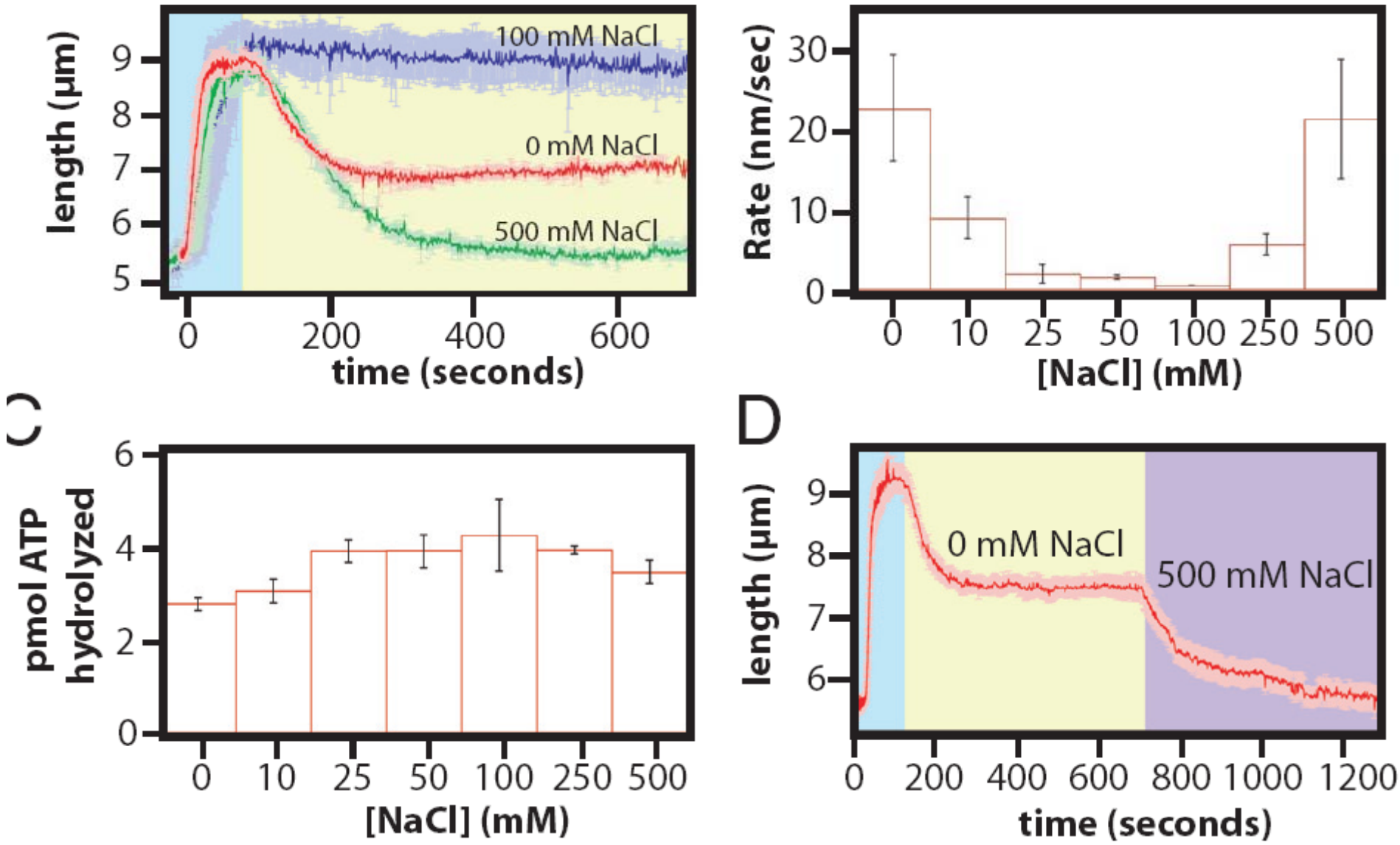
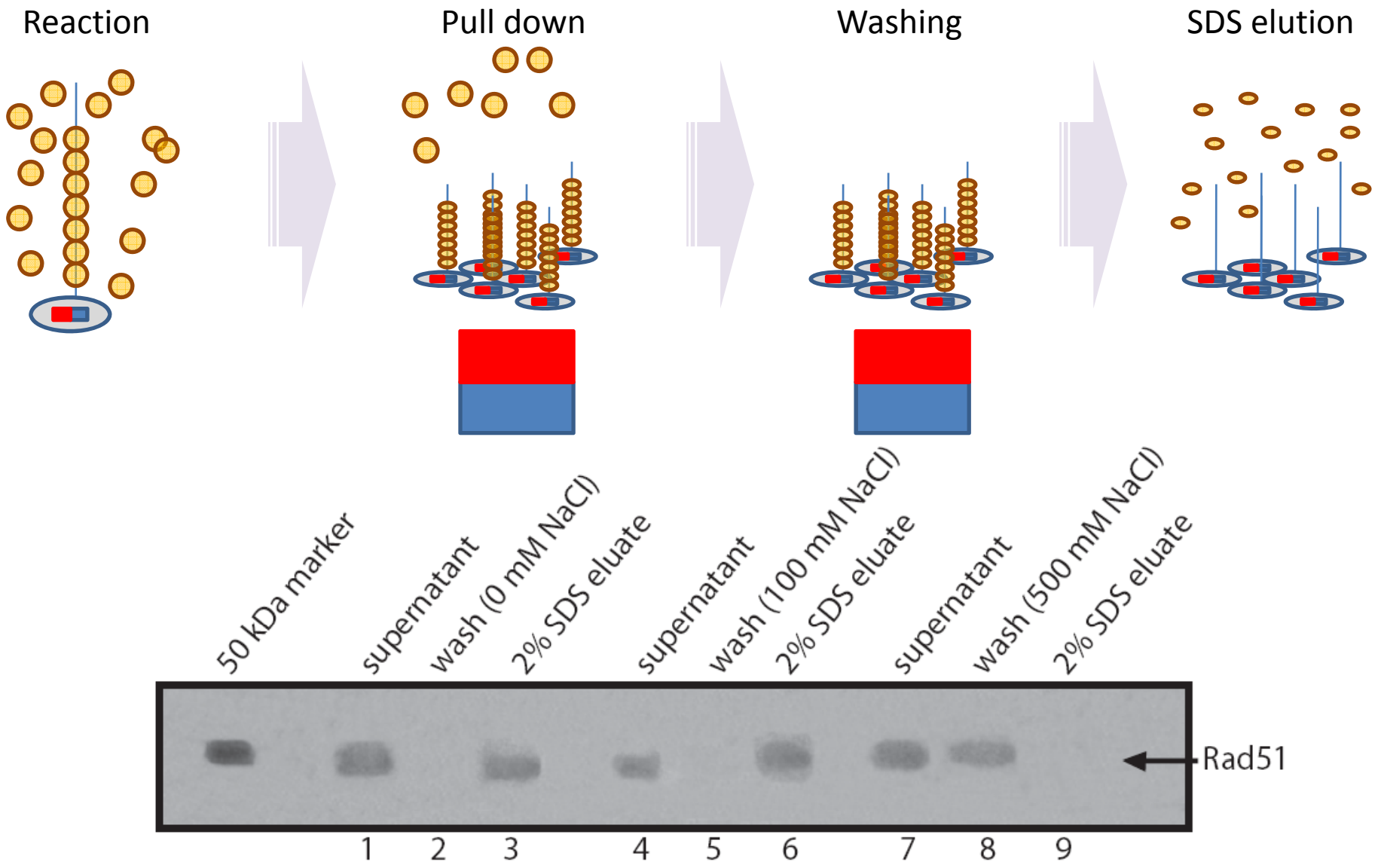
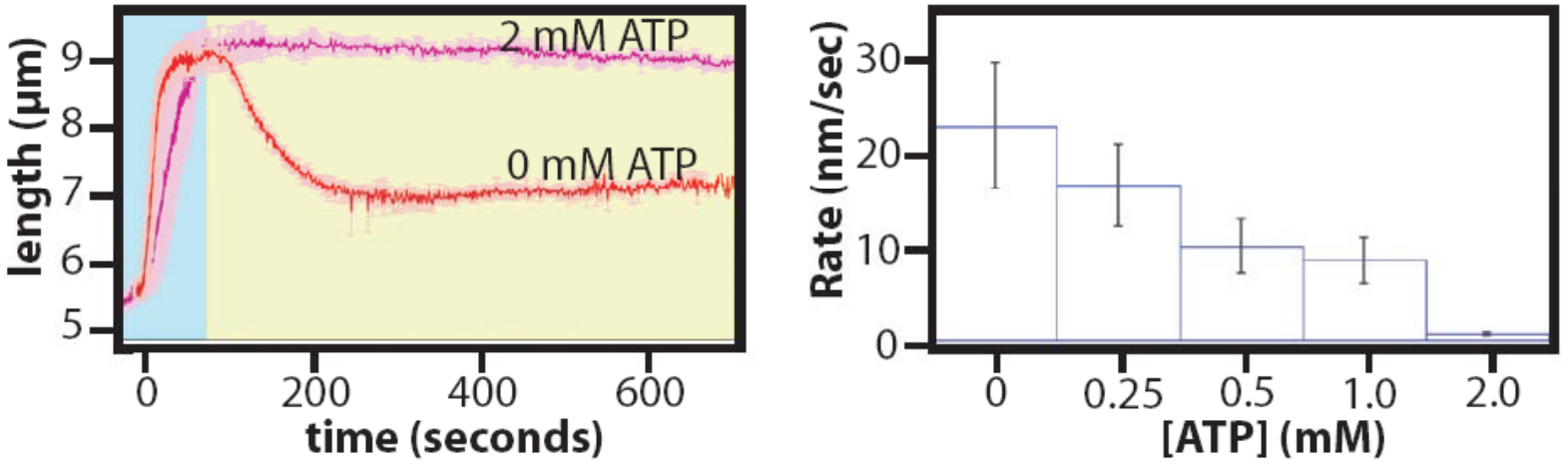

lipid-tethered
DNA molecule
bilayer-coated
surface
evanescent
field
microscale
diffusion barrier
Buffer flow
tethered end (T)
free end (F)


T
Buffer flow
T
F
12.5μm

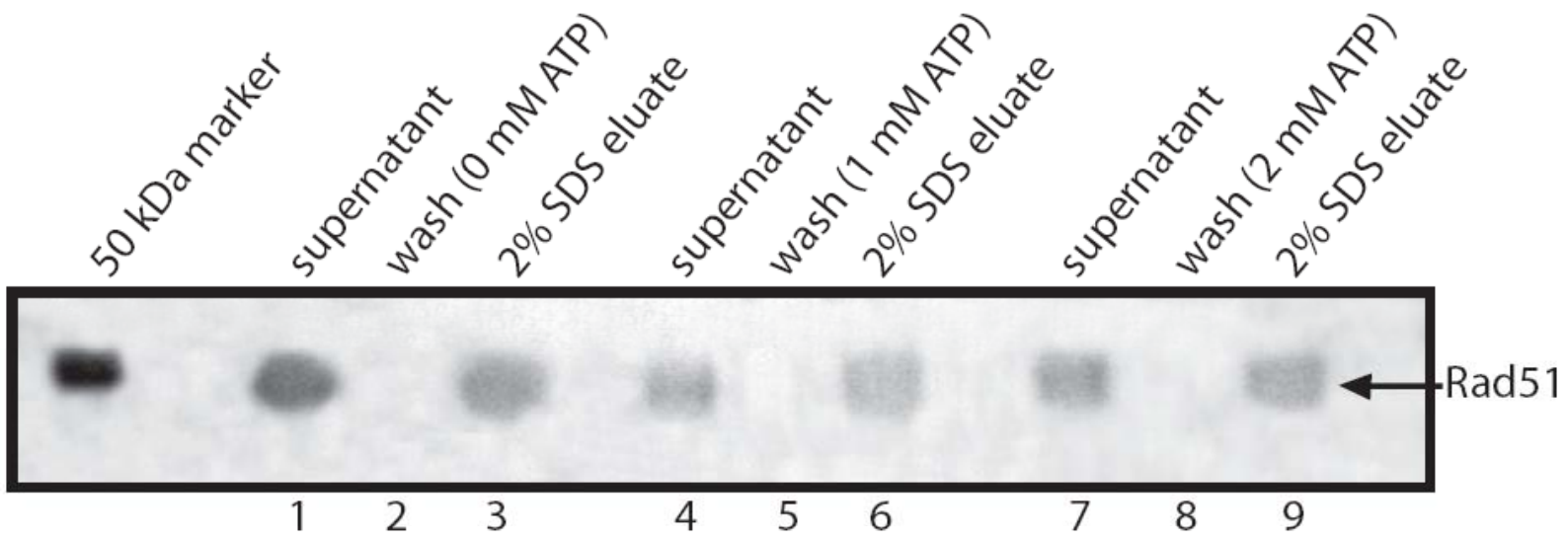

flowcell
laser
microscope
evanescent field
Inject Rad51
Begin wash
T
F
E
T
F
E
length (µm)
9
8
7
6
+Rad51 +ATP
-Rad51 -ATP
0
100
200
300
400
500
time (seconds)


length (µm)
100 mM NaCl
0 mM NaCl
500 mM NaCl
time (seconds)
Rate (nm/sec)
[NaCl] (mM)
pmol ATP hydrolyzed
[NaCl] (mM)
D
length (µm)
0 mM NaCl
500 mM NaCl
time (seconds)

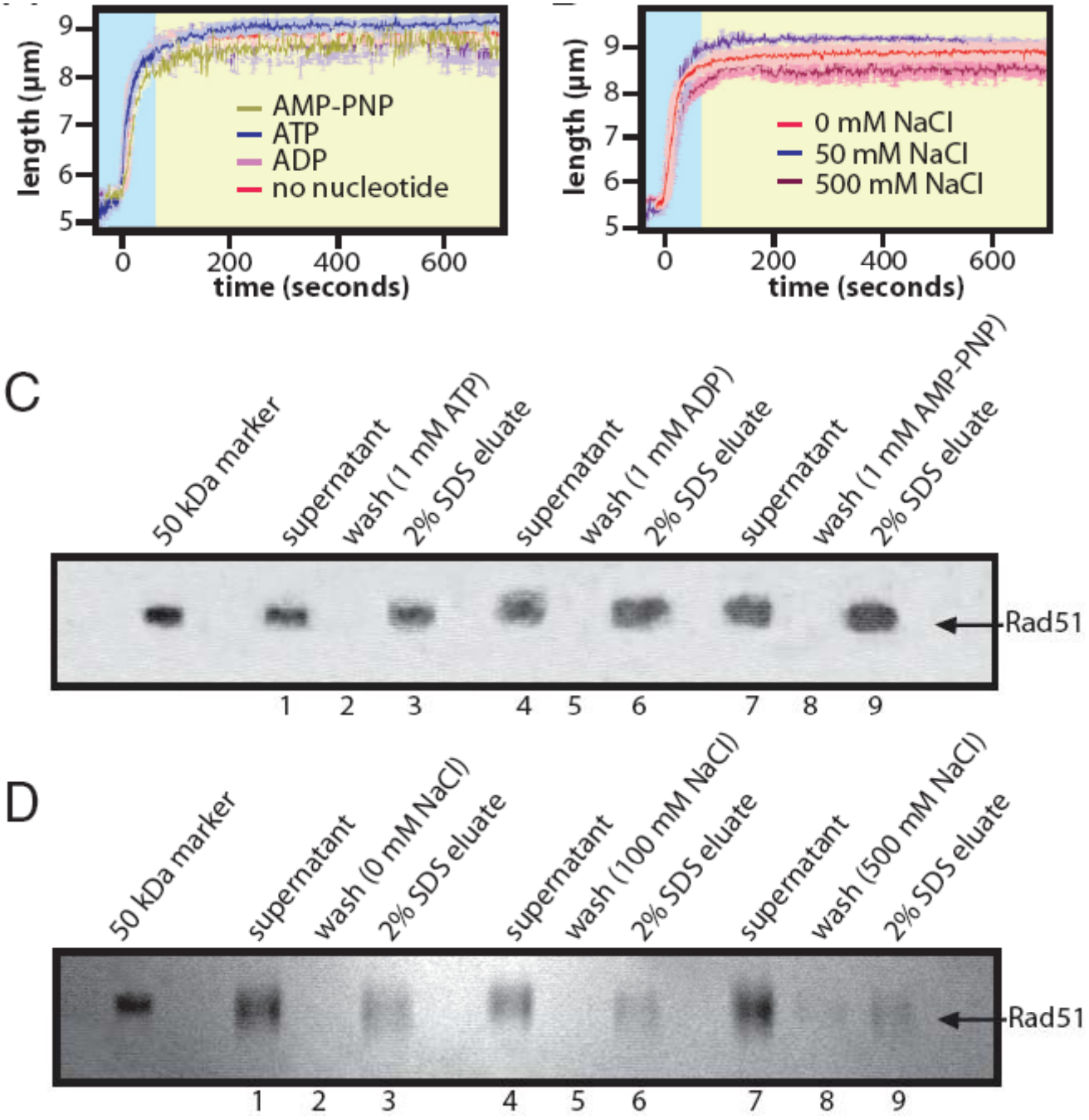

Reaction
Pull down
Washing
SDS elution
50 kDa marker
supernatant
wash (0 mM NaCl)
2% SDS eluate
supernatant
wash (100 mM NaCl)
2% SDS eluate
supernatant
wash (500 mM NaCl)
2% SDS eluate
Rad51
1
2
3
4
5
6
7
8
9


2 mM ATP
0 mM ATP
length (µm)
9
8
7
6
5
0
200
400
600
time (seconds)
Rate (nm/sec)
30
20
10
0
0
0.25
0.5
1.0
2.0
[ATP] (mM)

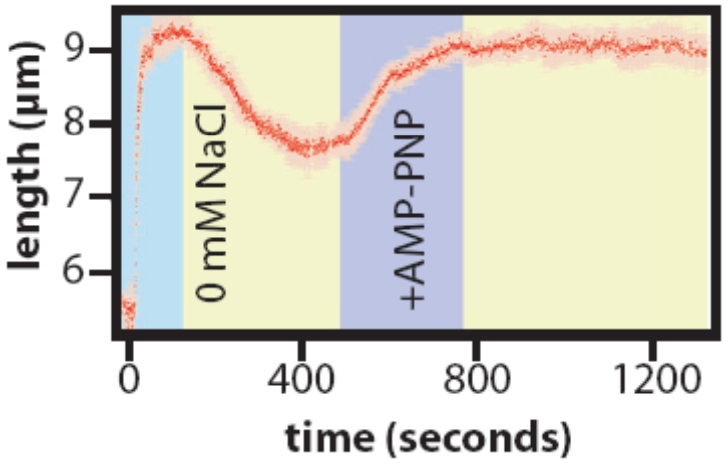
C

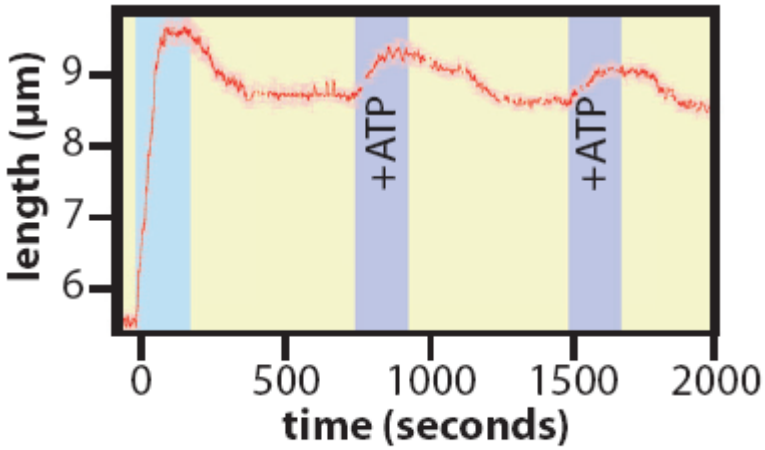

50 kDa marker
supernatant
wash (0 mM ATP)
2% SDS eluate
supernatant
wash (1 mM ATP)
2% SDS eluate
supernatant
wash (2 mM ATP)
2% SDS eluate
Rad51
1
2
3
4
5
6
7
8
9

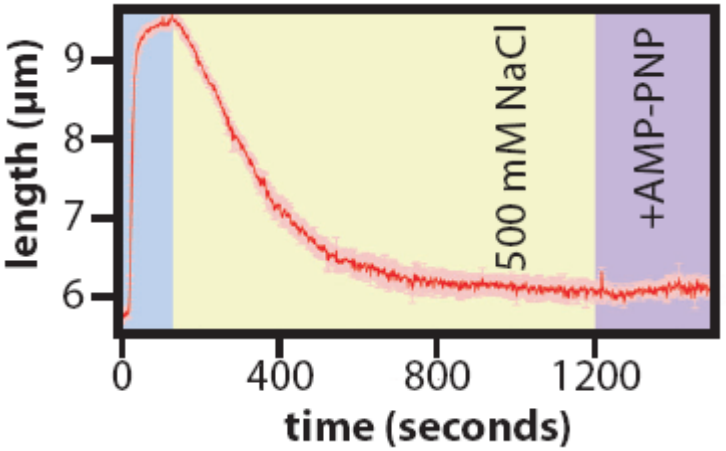
ATP hydrolysis



Reversible!!!

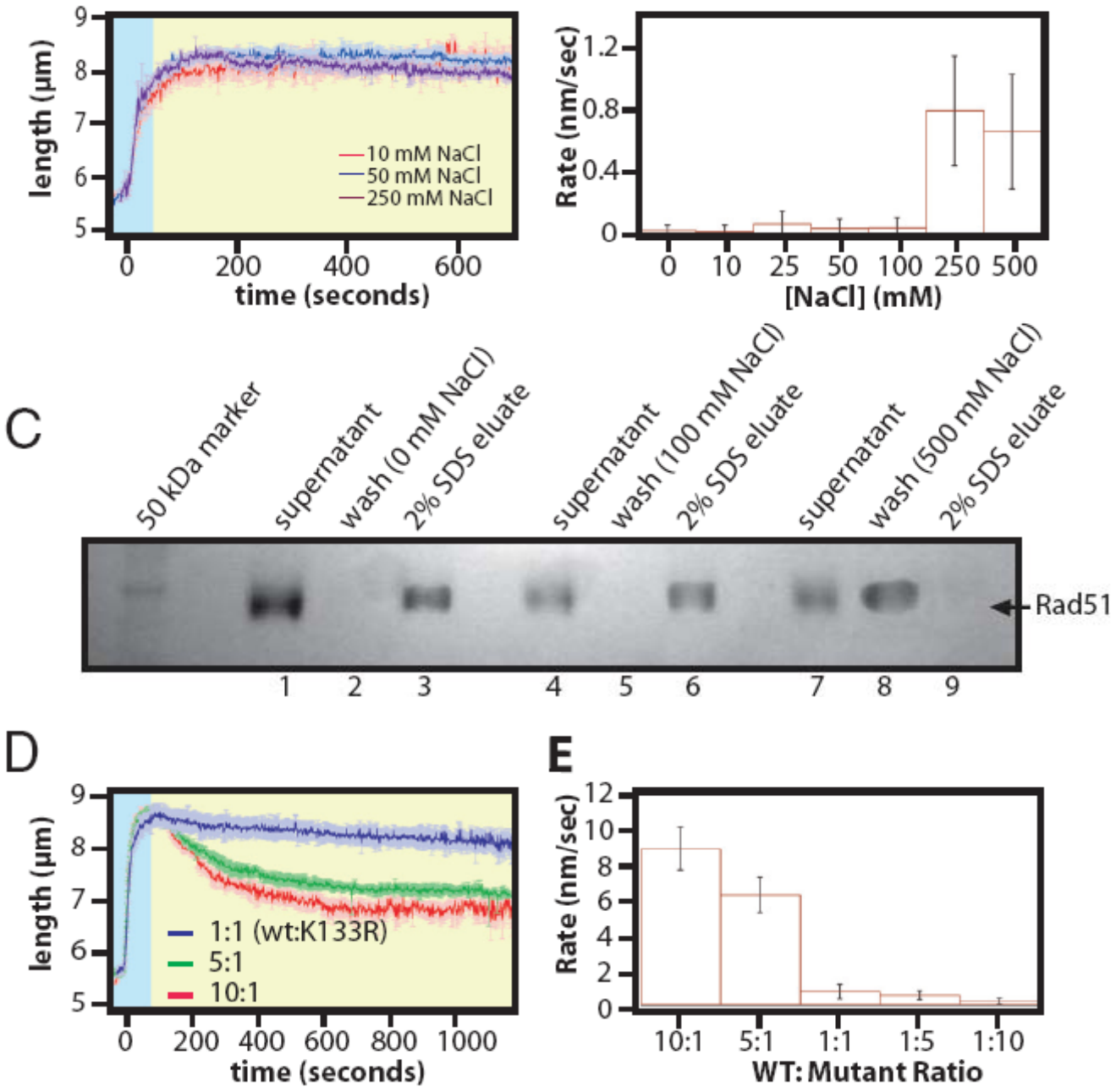

length (µm)
9
8
7
6
0 mM NaCl
+AMP-PNP
0
400
800
1200
time (seconds)


length (µm)
9
8
7
6
+ATP
+ATP
0
500
1000
1500
2000
time (seconds)


length (µm)
9
8
7
6
500 mM NaCl
+AMP-PNP
0
400
800
1200
time (seconds)

For Summary,

Just have a look again.!!!!


length (µm)
time (seconds)
10 mM NaCl
50 mM NaCl
250 mM NaCl
Rate (nm/sec)
[NaCl] (mM)
C
50 kDa marker
supernatant
wash (0 mM NaCl)
2% SDS eluate
supernatant
wash (100 mM NaCl)
2% SDS eluate
supernatant
wash (500 mM NaCl)
2% SDS eluate
Rad51
D
1:1 (wt:K133R)
5:1
10:1
E
WT: Mutant Ratio