An accidental biophysicist

Physics degrees and training
(Seoul ⇒ UC Berkeley ⇒ Stanford)
Physics professor
Howard Hughes Medical Institute

Cancer & infectious disease

"My purpose is to set forth
a very new science
dealing with
a very ancient subject.
There is, in nature, perhaps nothing older than motion.

Galileo Galilei

Two types of motion in biology

- ***Diffusion***

-thermally driven.

-rapid on short length scales: 1 μs for 10 nm.

-slow on long length scales: 3 hours for 1 mm.

- ***Directed motion***

-driven by ***molecular motors*** that consume free energy, e.g. ATP hydrolysis.

-slow on short length scales: 10 ms for 10 nm.

-rapid on long length scales: 20 minutes for 1 mm.

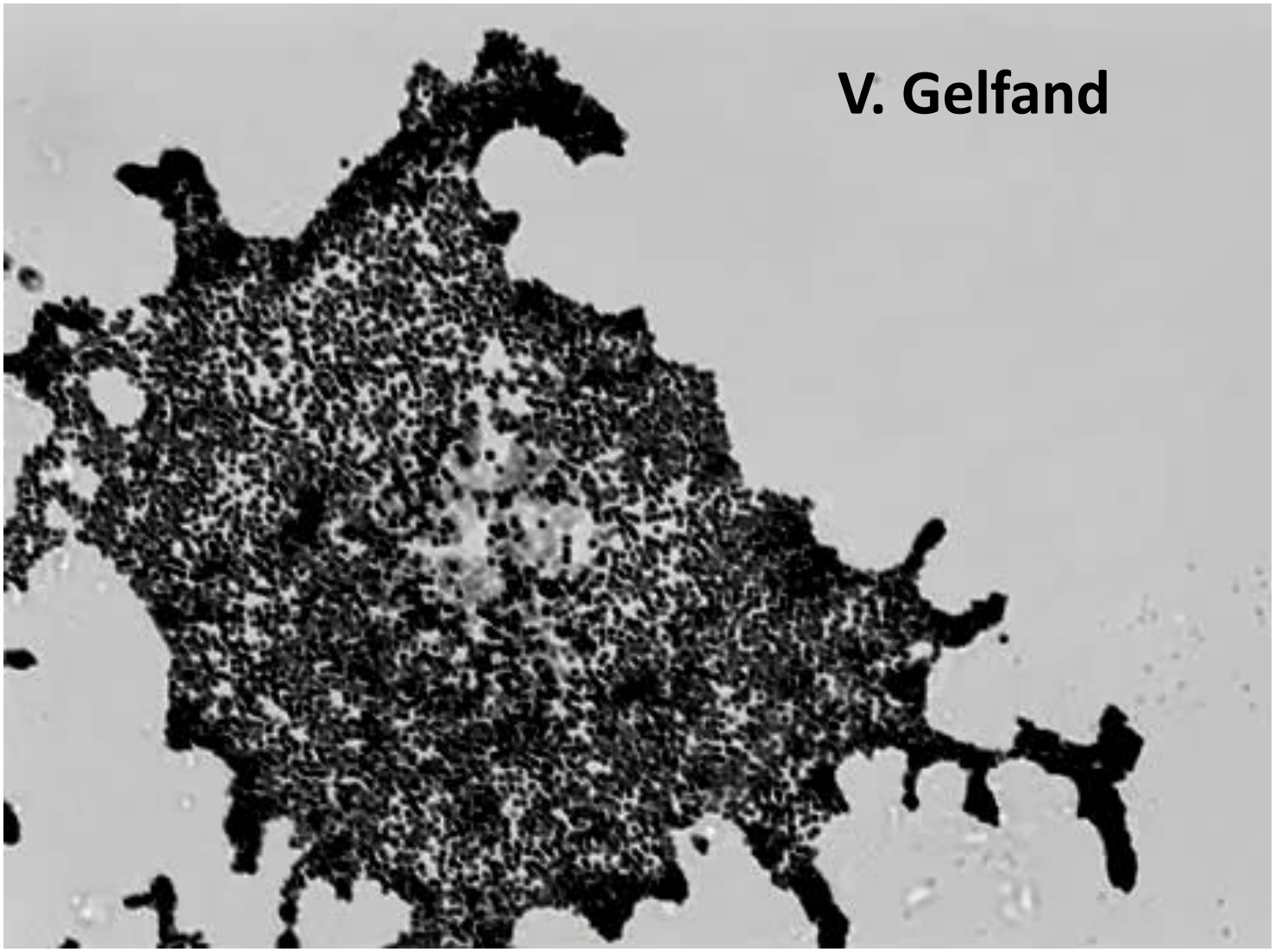
kinesin carries cargo

Motor	Track	Fuel
Car	highway	gasoline
Kinesin	microtubule	ATP

Molecular motors:

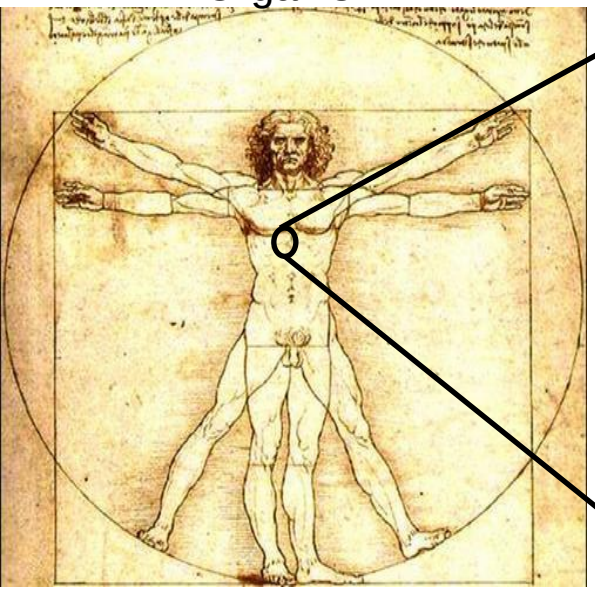
proteins that move on cellular highways directionally

Zebrafish cell

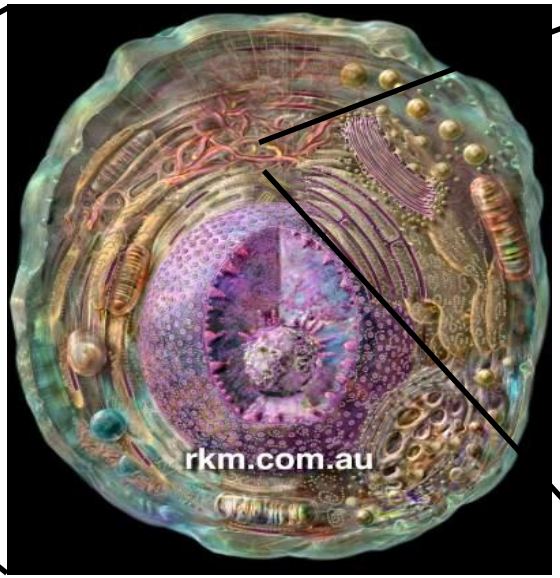


How small is a protein?

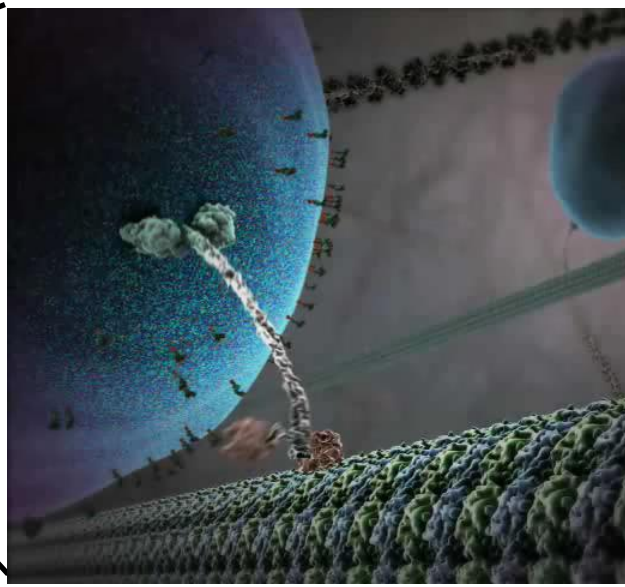
Organism



Cell



Molecule



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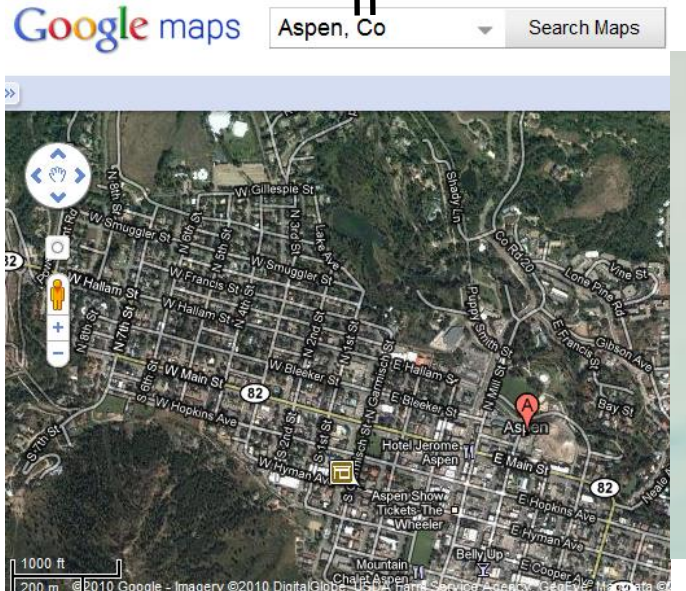

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Kinesin carries cargo

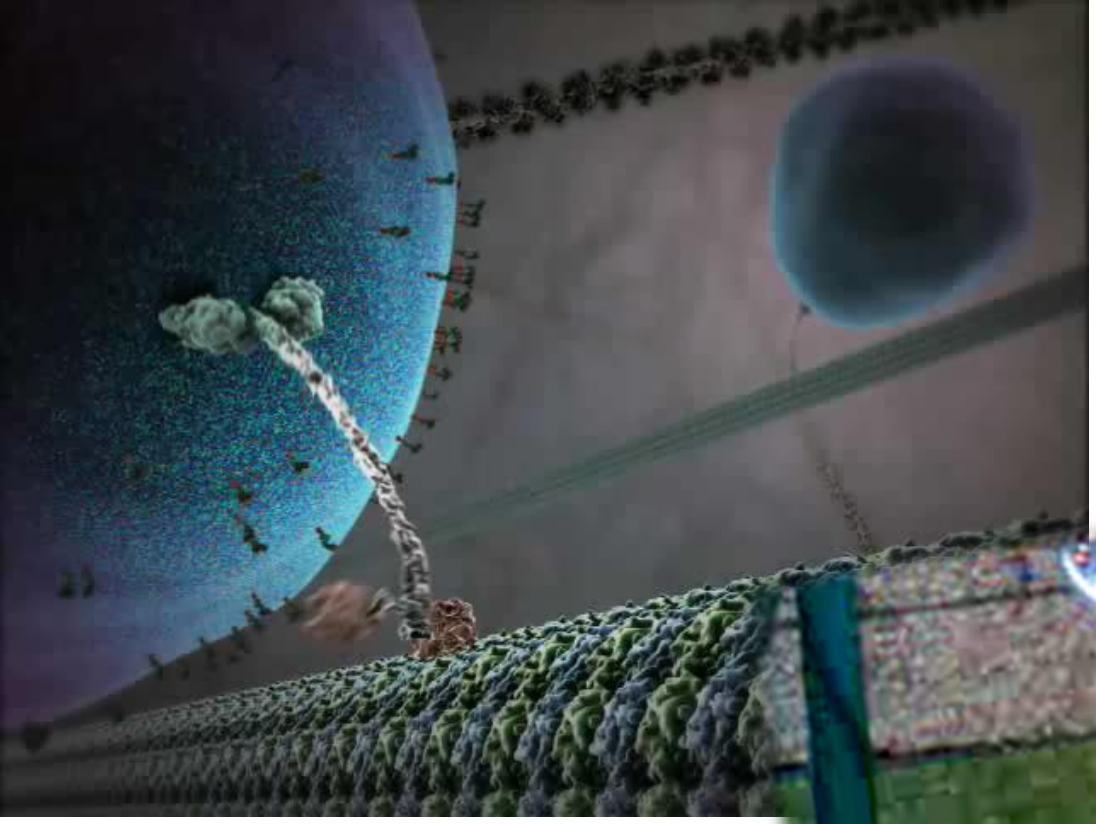
Motor	Track	Fuel
Kinesin	microtubule	ATP

Hand-over-hand mechanism

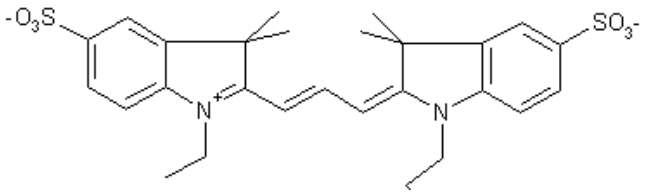
Two heads alternate

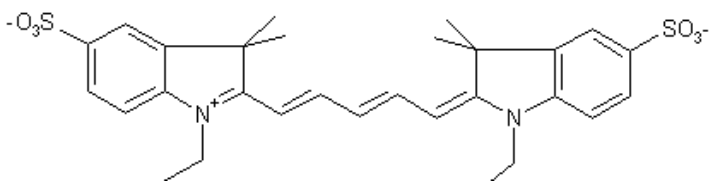
Hand-over-hand mechanism

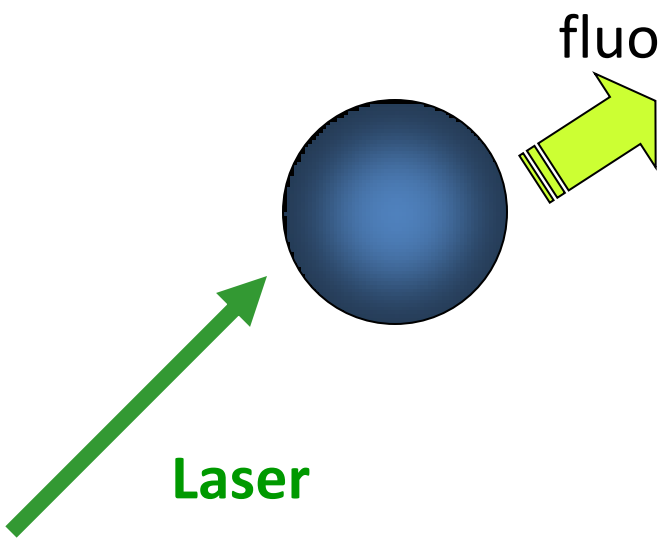
Highly processive!

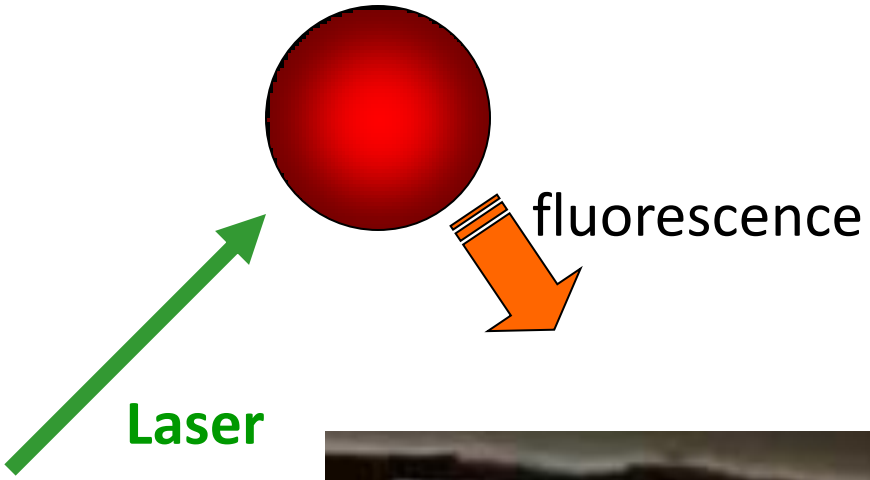


Fluorescence

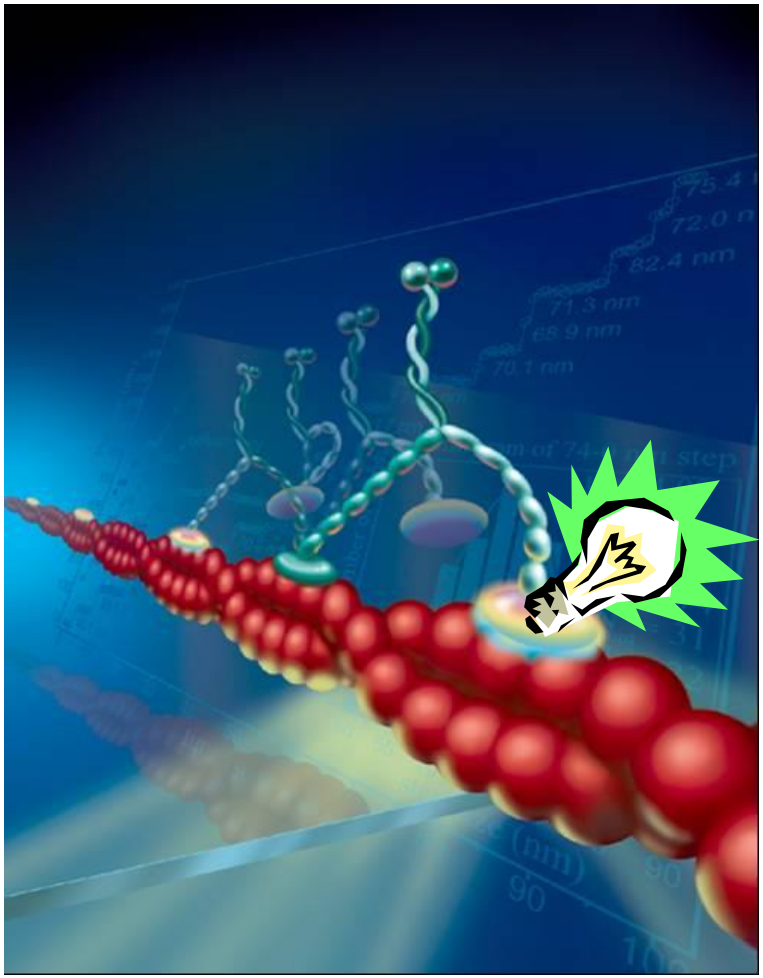






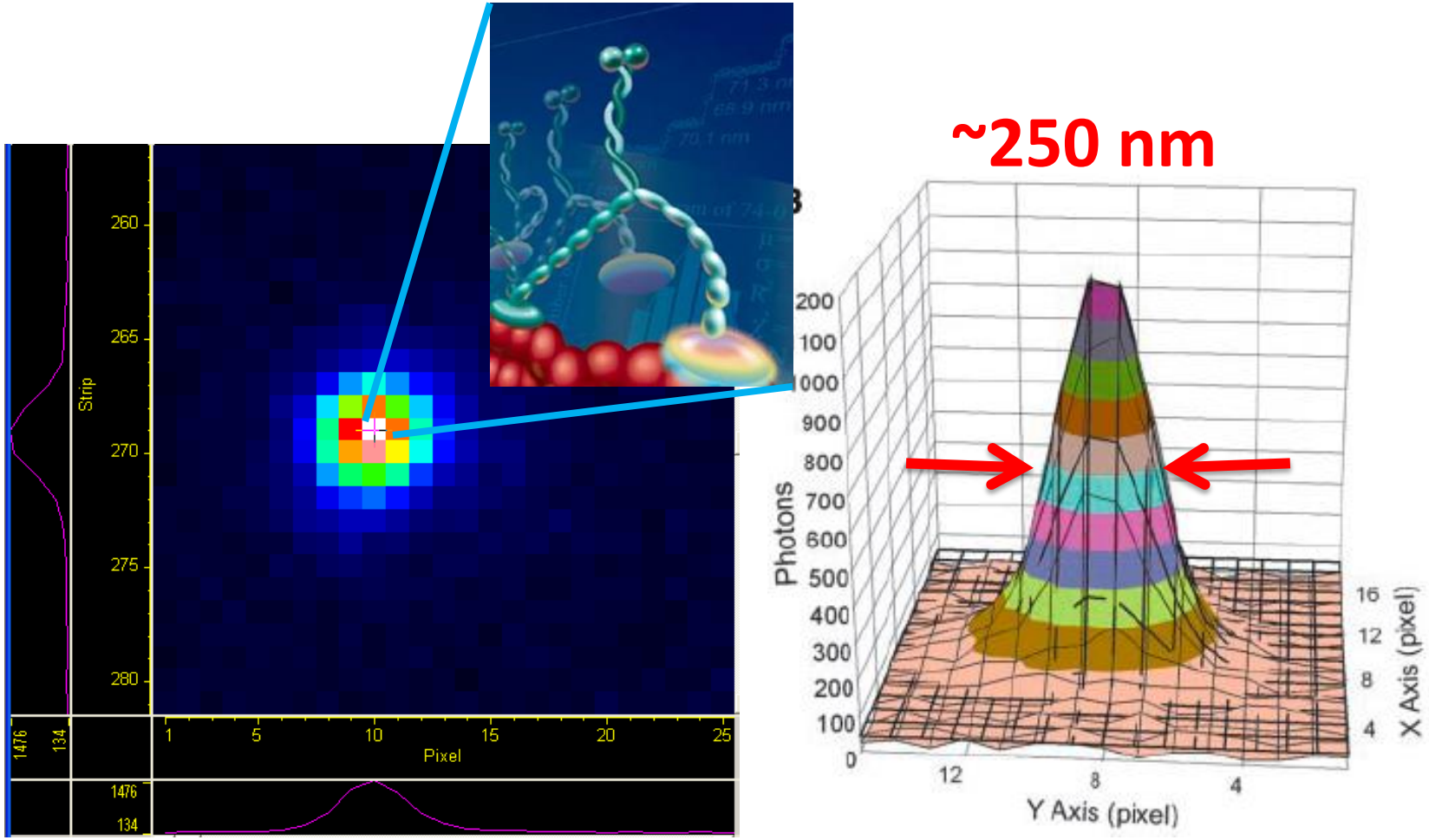


Imaging Single Molecules via *Fluorescence*



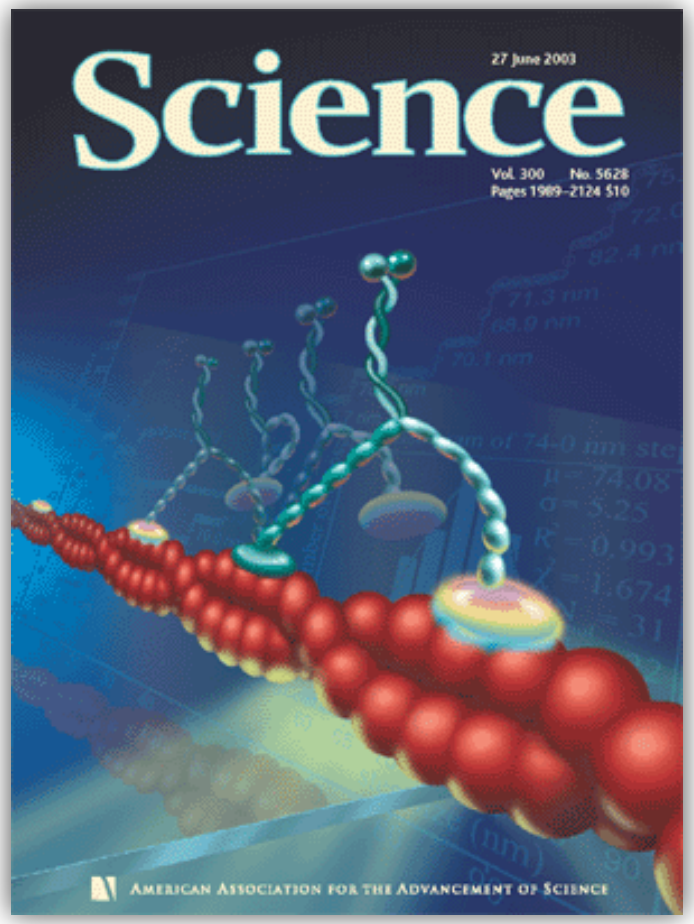
Myosin V on actin
Center-of-mass step size = 37 nm

Heisenberg's Uncertainty Principle



Center can be determined to ~ 1.5 nm!

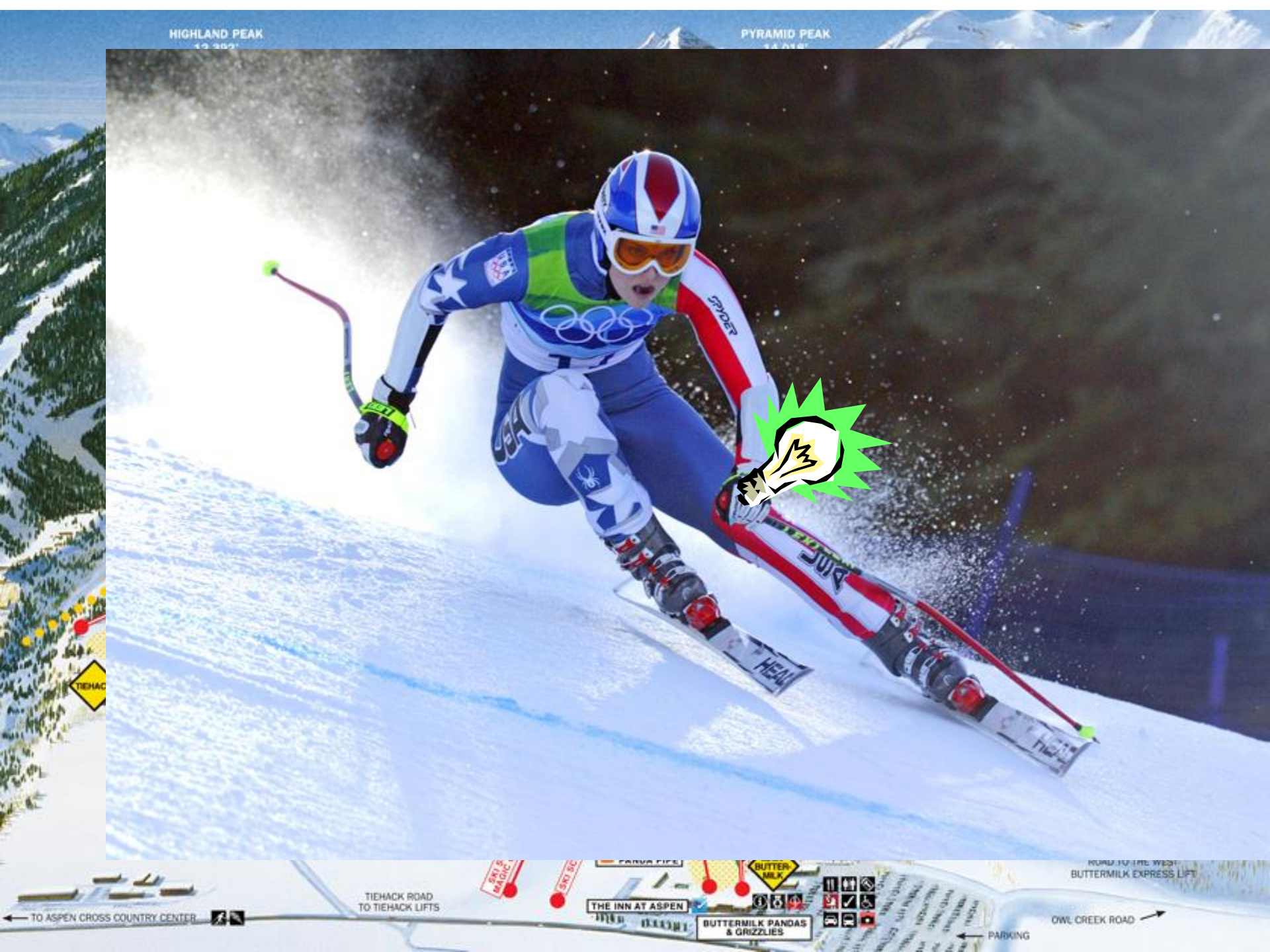
It walks!



Yildiz, Forkey, McKinney, Ha, Goldman and Selvin, Science (2003)

74 nm stepping of a head

HIGHLAND PEAK
12,392'
PYRAMID PEAK
14,018'
WEST SUMMIT
9,900'
SKI & SNOWBOARD SCHOOLS PARK (S3)
SKI AREA BOUNDARY
THE CLIFFHOUSE
9,690'
EAST SUMMIT
9,720'
TOM'S THUMB
RED'S ROVER
LARKSPUR
HOMESTEAD ROAD
WESTWARD HO
BIG FACE HOLLOW
WEST BUTTERMILK EXPRESS
BLUE GROUSE
CAMP BIRD
TEASER
LITTLE TEASER
LOWER LARKSPUR
MIDWAY LOADING STATION
LIMITED DAY PARKING
UNCLE CHUCK'S GLADES
LOVER'S LANE
TRAIL
MIDWAY AVENUE
LOWER HOMESTEAD ROAD
BEAR
SAVIO
JACOB'S LADDER
BUTTERMILK PARK
BABY DOE
HOMESTEAD ROAD
GOVERNMENT
SPRUCE
SPRUCE FACE
SUPERPIPE
COLUMBINE
SUMMIT EXPRESS
PANDA PEAK
PANDA HILL
PANDA PIPE
SKI SCHOOL MAGIC CARPET
SKI SCHOOL LIFT
MAIN BUTTER-MILK
BUMPS AT BUTTERMILK MOUNTAIN LODGE
7,880'
THE INN AT ASPEN
BUTTERMILK PANDAS & GRIZZLIES
ROAD TO THE WEST BUTTERMILK EXPRESS LIFT
TIEHACK ROAD TO TIEHACK LIFTS
TO ASPEN CROSS COUNTRY CENTER
OWL CREEK ROAD
PARKING
TIEHACK
LIMITED DAY PARKING
TIEHACK PARKWAY
TIEHACK TRAIL
TIEHACK EXPRESS
TIMBER
DOODLE GLADE
PTARMIGAN GLADE
PTARMIGAN
BUCKSKIN
STEERNER
RACER'S EDGE
JAVELIN
STERNER GULCH
EAGLE HILL
RABBIT RUN
MAGIC CARPET
STERNER CATWALK
OREGON TRAIL (TO TIEHACK)
SKI AREA BOUNDARY
ELEVATION TOP: 9,900 ft/3,016 m
VERTICAL RISE: 2,030 ft/618 m
TERRAIN: 470 acres/190 hectares
NUMBER OF TRAILS: 44
LONGEST RUN: 3 miles/4.83 km
LIFTS: 3 high-speed quads, 1 double, 4 ski/snowboard school lifts
LIFT CAPACITY: 6,000 riders per hour
AVERAGE ANNUAL SNOWFALL: 200 inches/508 cm
SNOWMAKING CAPABILITIES: 108 acres/44 hectares (27% of area)
EASIEST TERRAIN: 35%
MORE DIFFICULT TERRAIN: 21%
MOST DIFFICULT TERRAIN: 26%
EXPERT TERRAIN: 5%
SEASON DATES: December 15, 2012 - April 7, 2013


HIGHLAND PEAK
PYRAMID PEAK
SPYDER
HEAD
TIEHACK ROAD
TO TIEHACK LIFTS
TO ASPEN CROSS COUNTRY CENTER
THE INN AT ASPEN
BUTTER-
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PARKING

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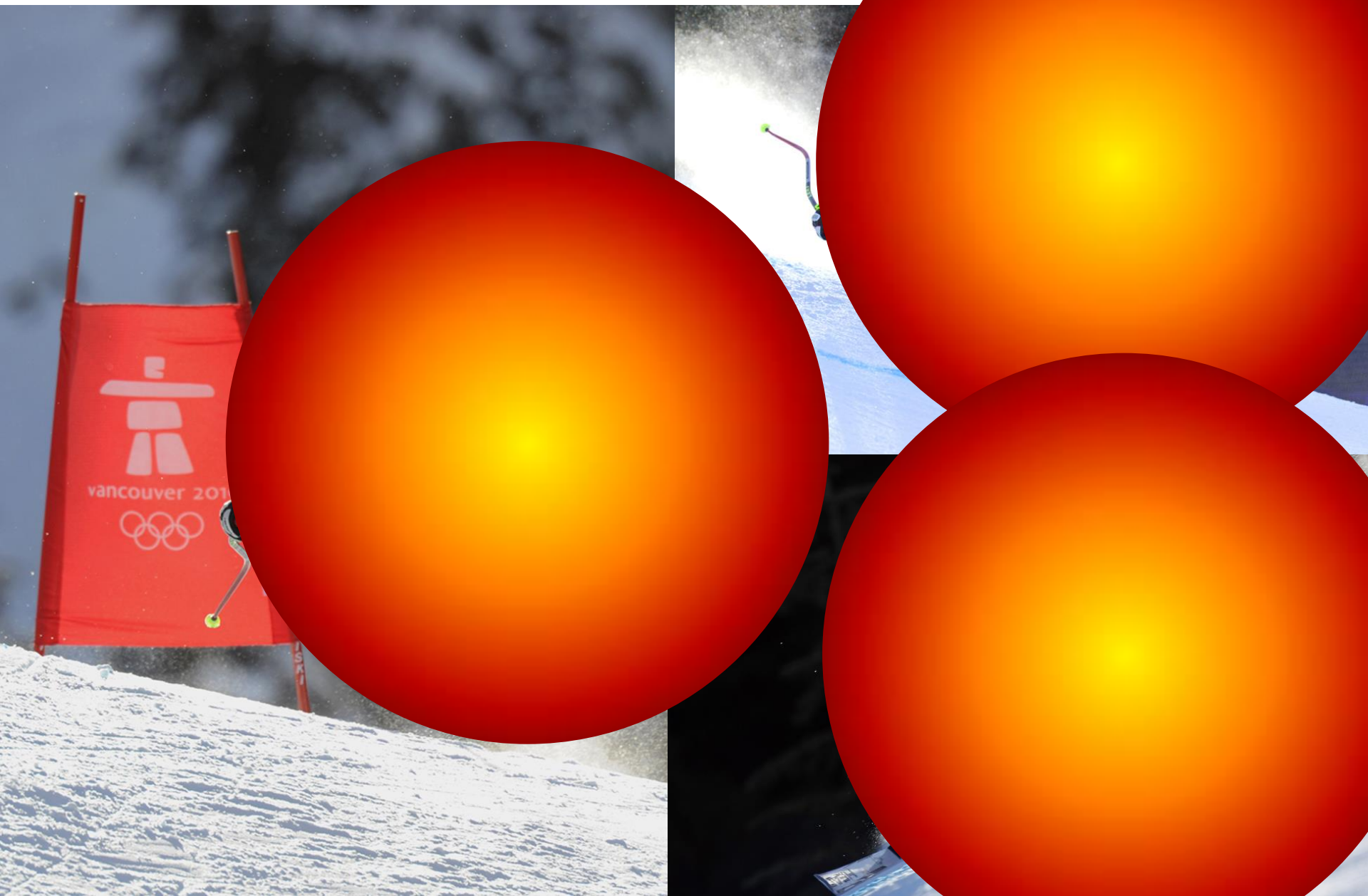
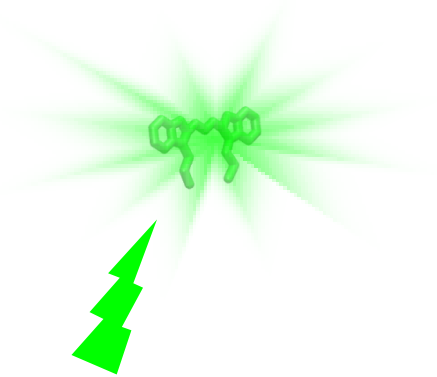
Multiple Conformati

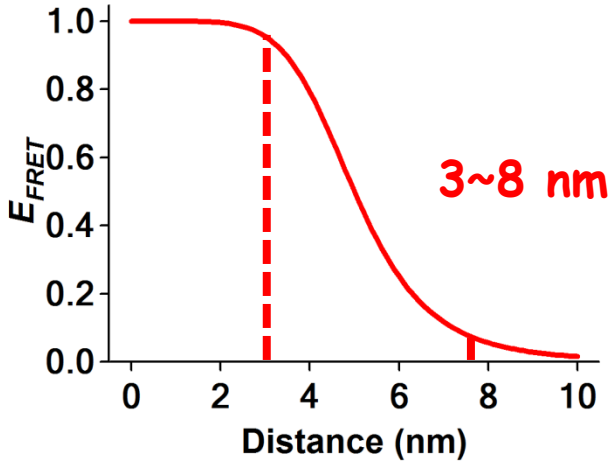


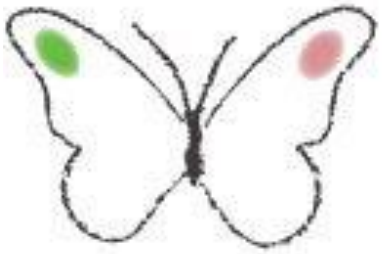
FRET

Donor: Cy3



excitation

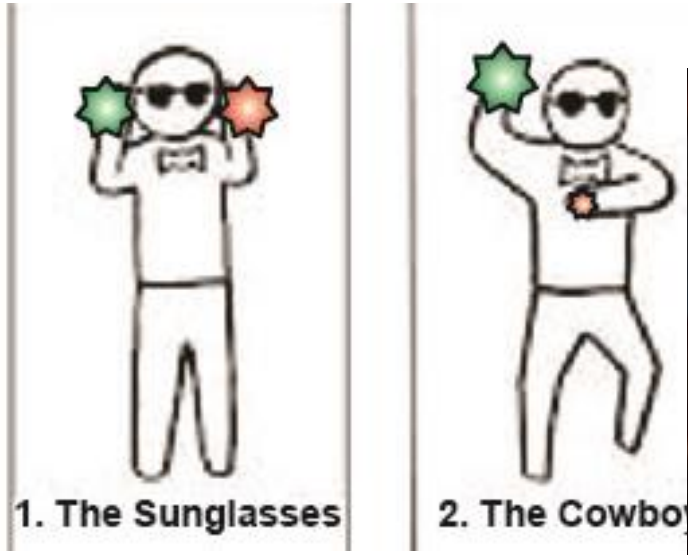


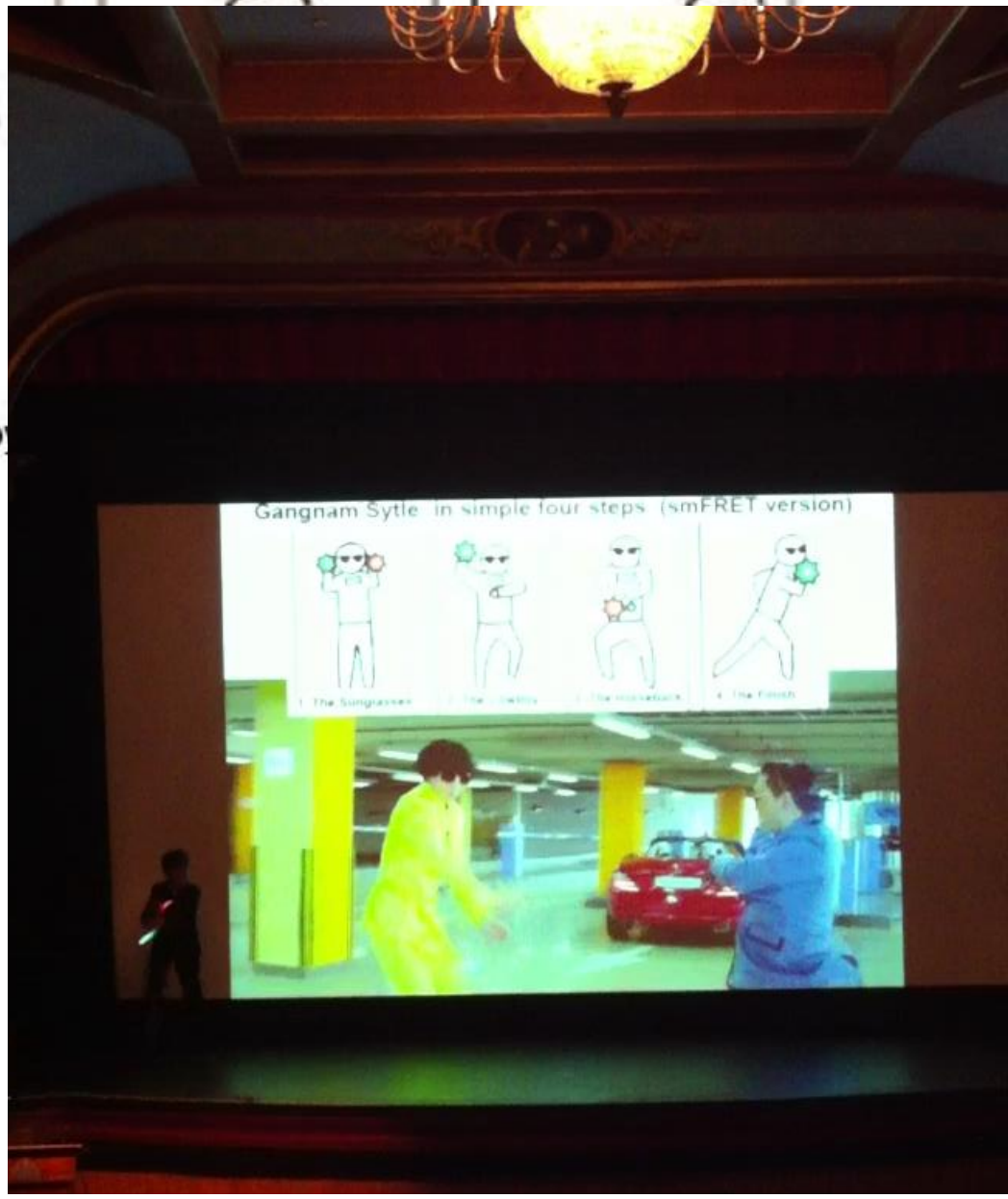


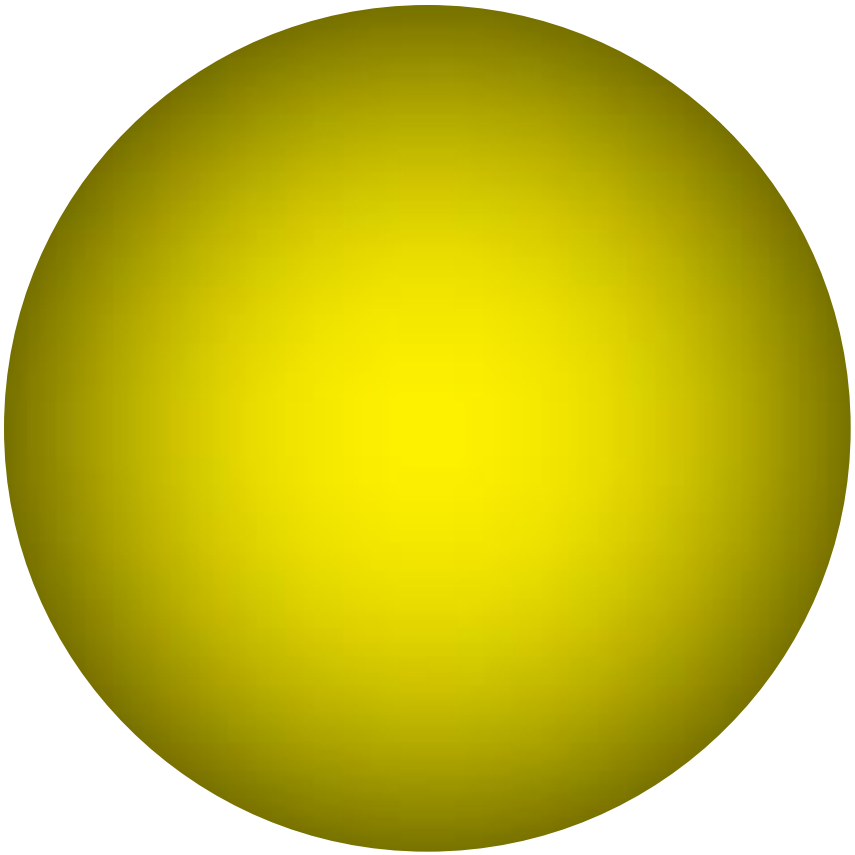
Green signal
from low FRET

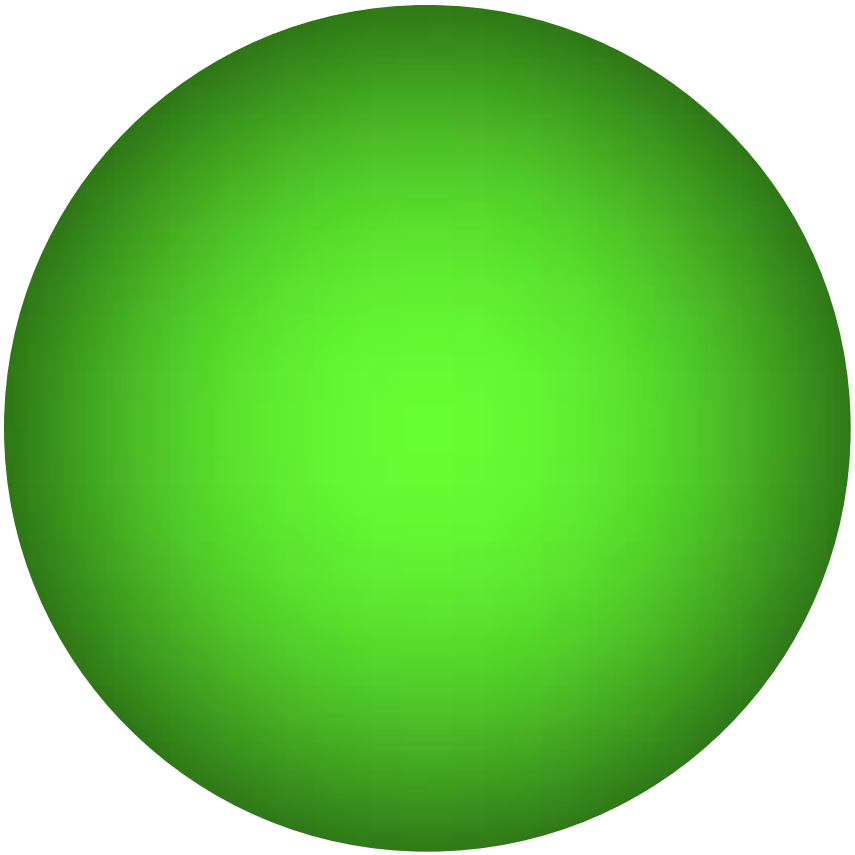
Red signal
from high FRET

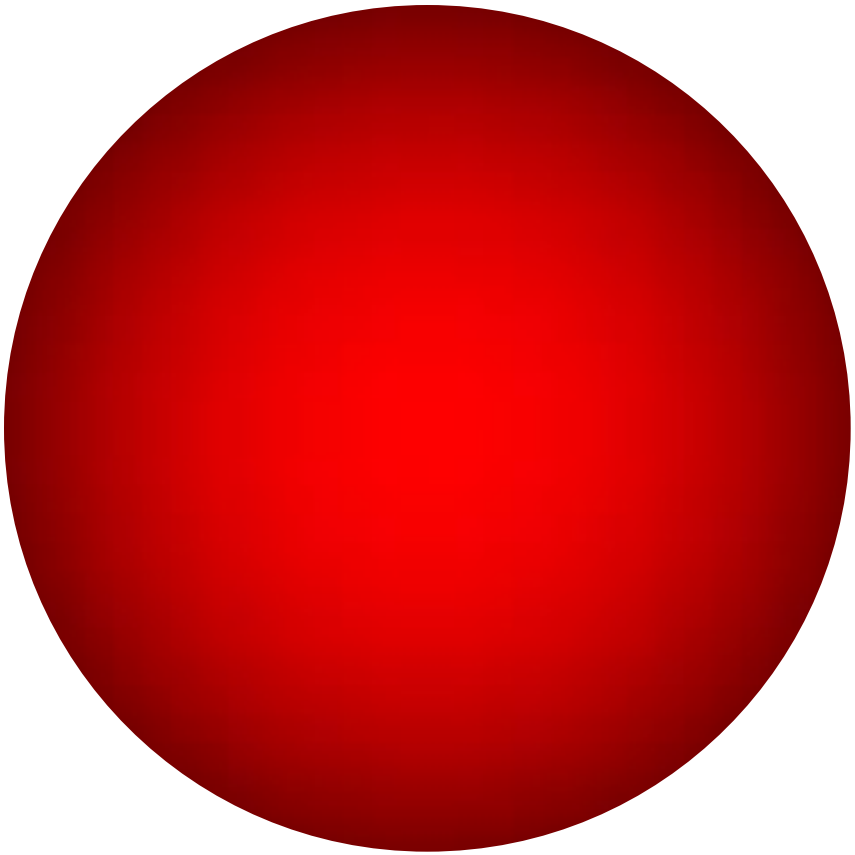
Gangnam Style in simple four steps (smFRET version)

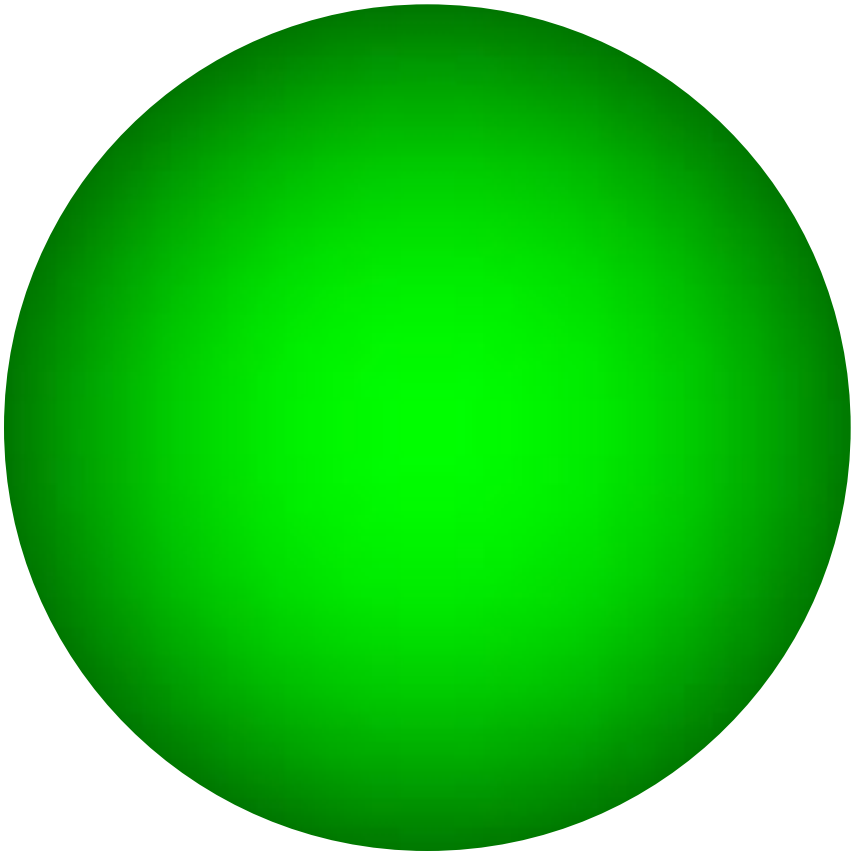




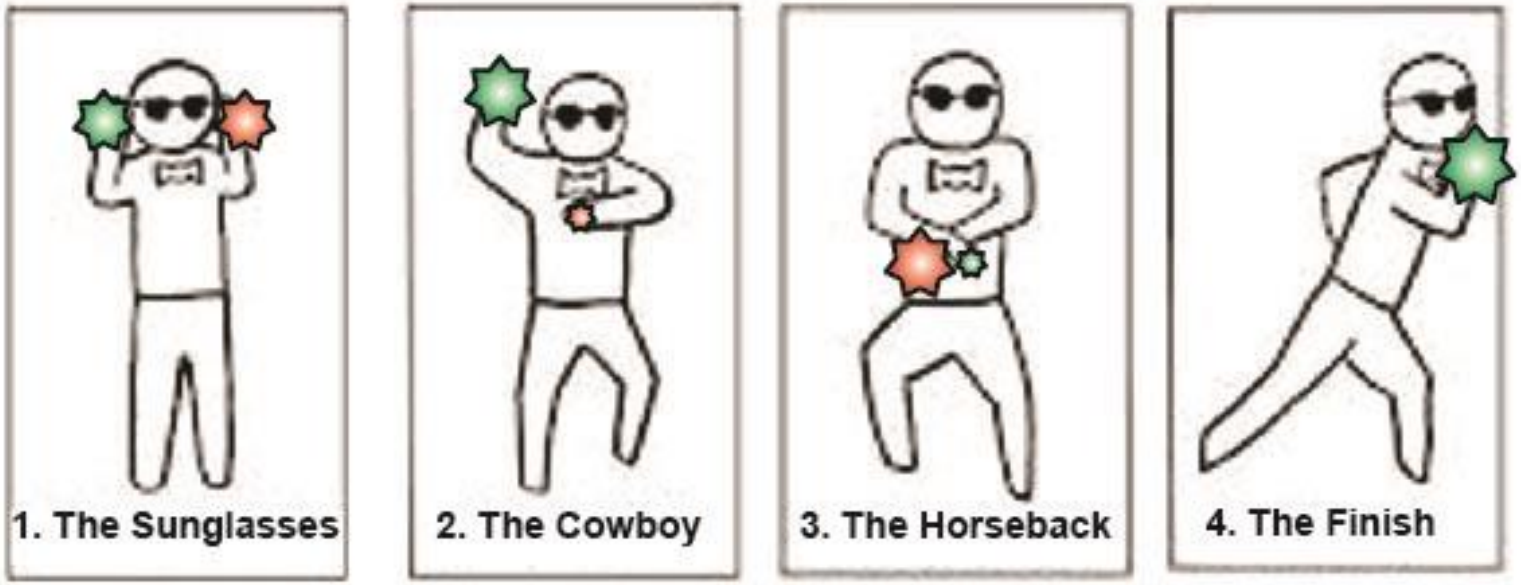


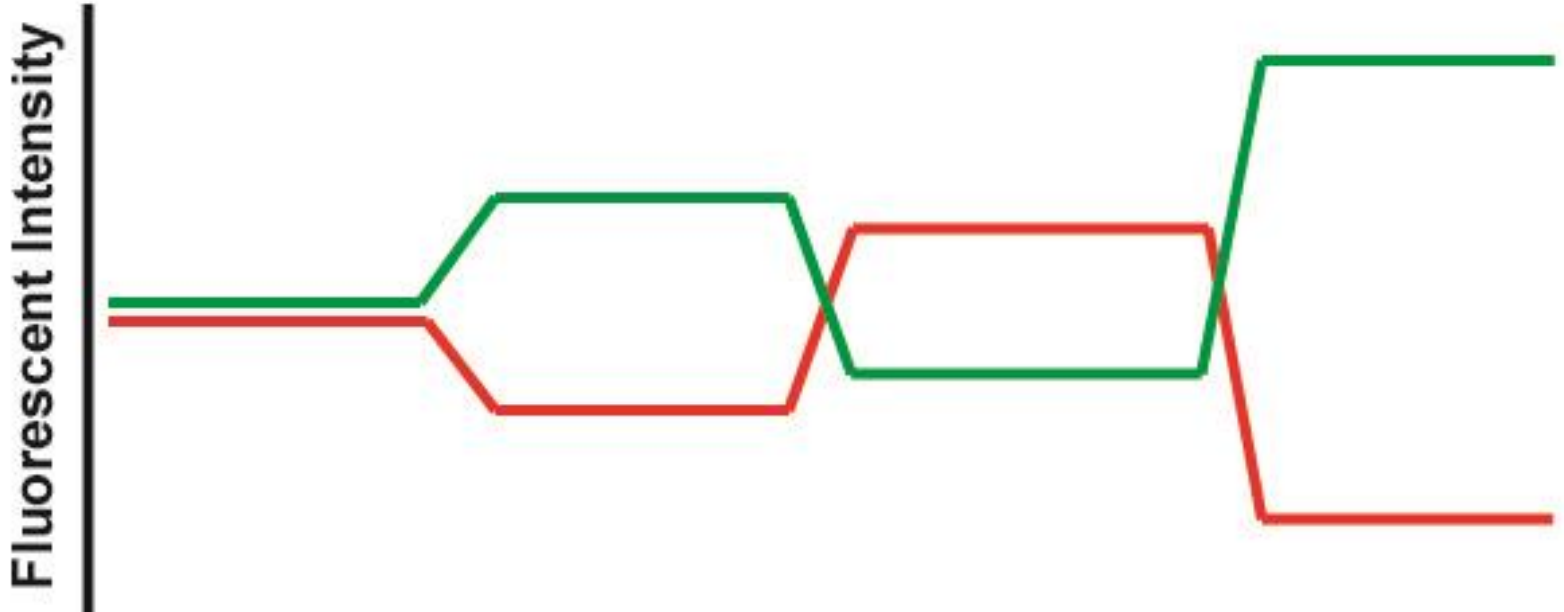




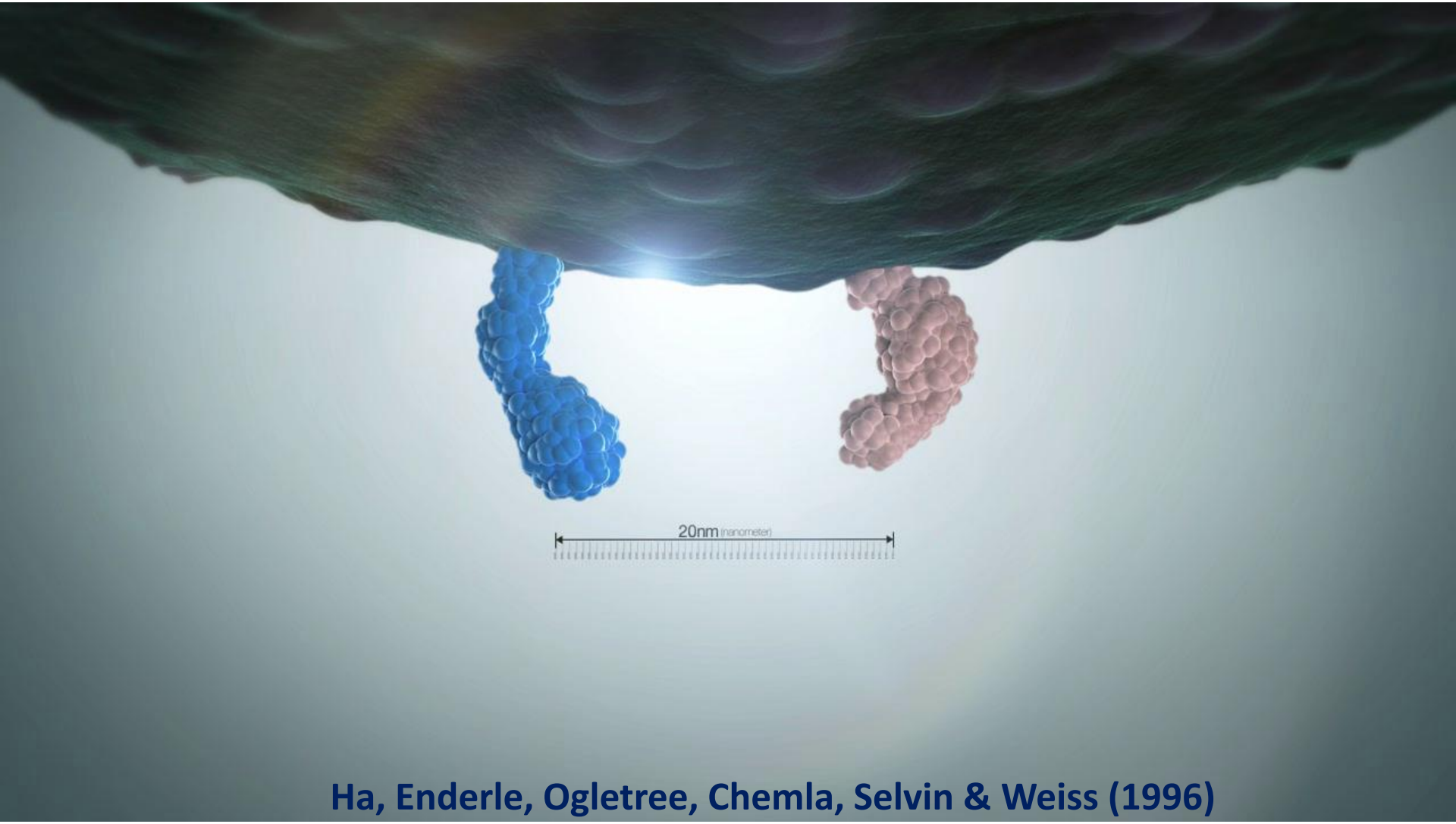


Gangnam Sytle: in simple four steps (smFRET version)

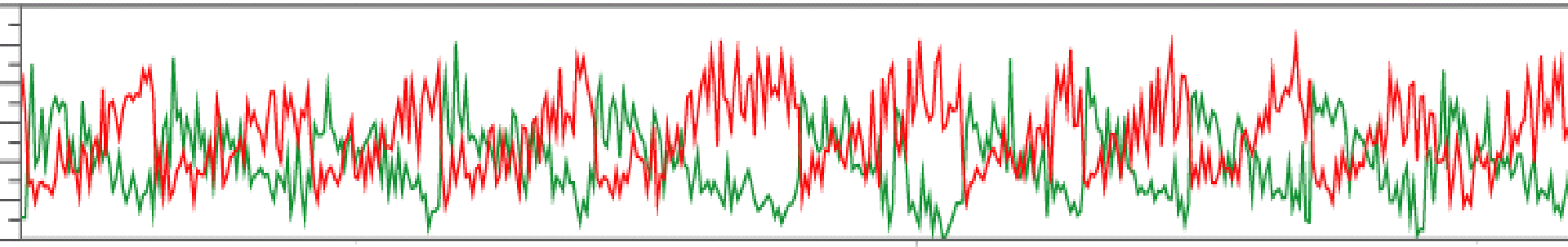


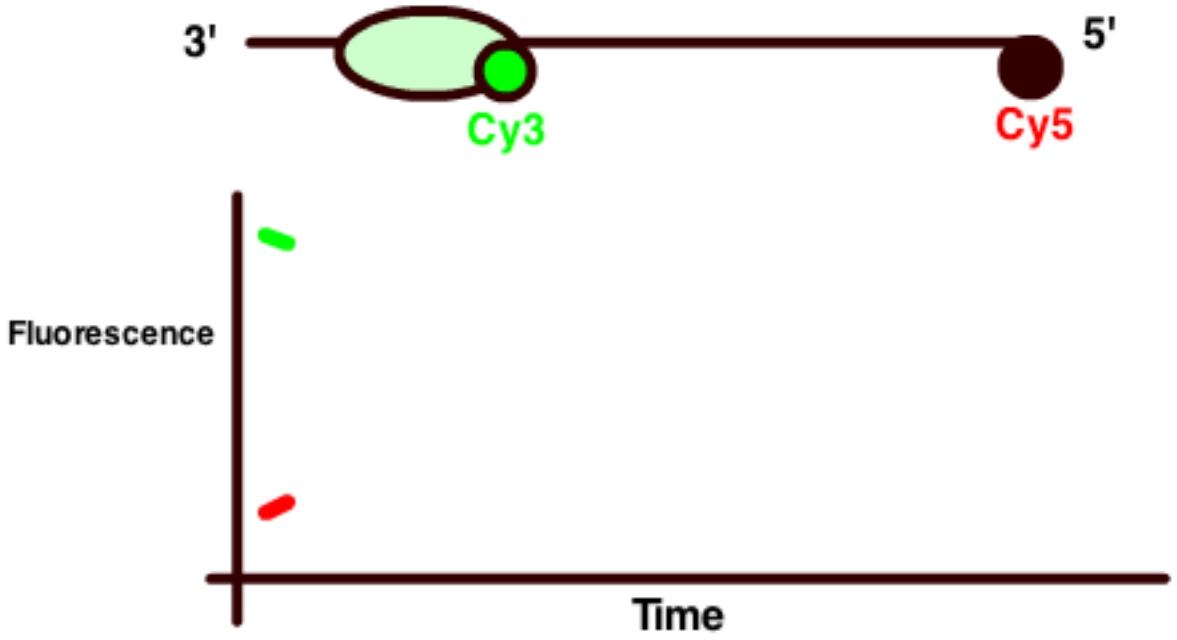


Single molecule FRET

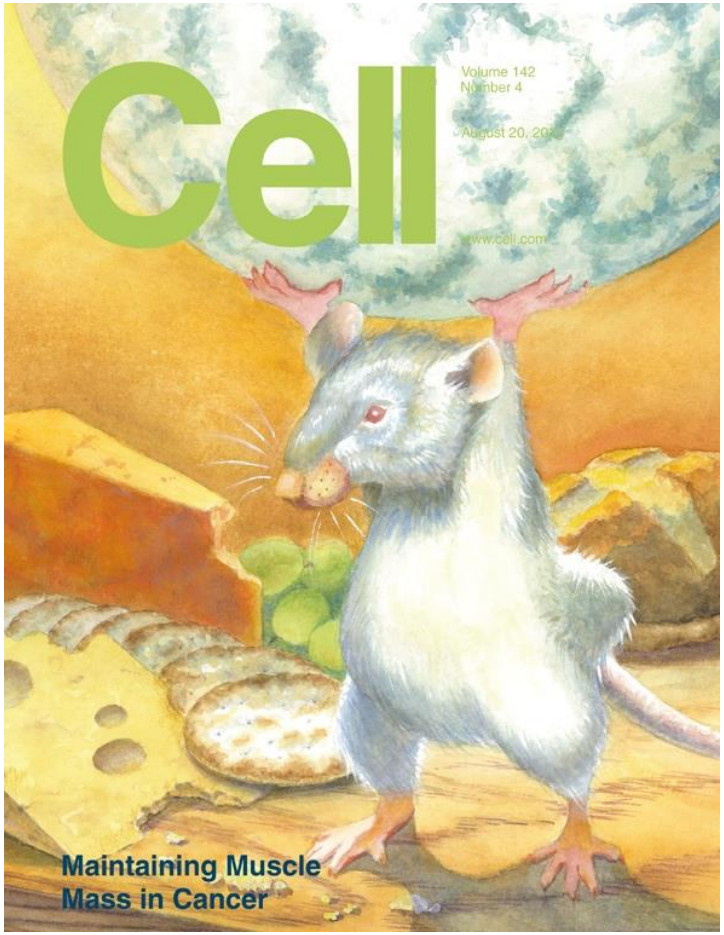


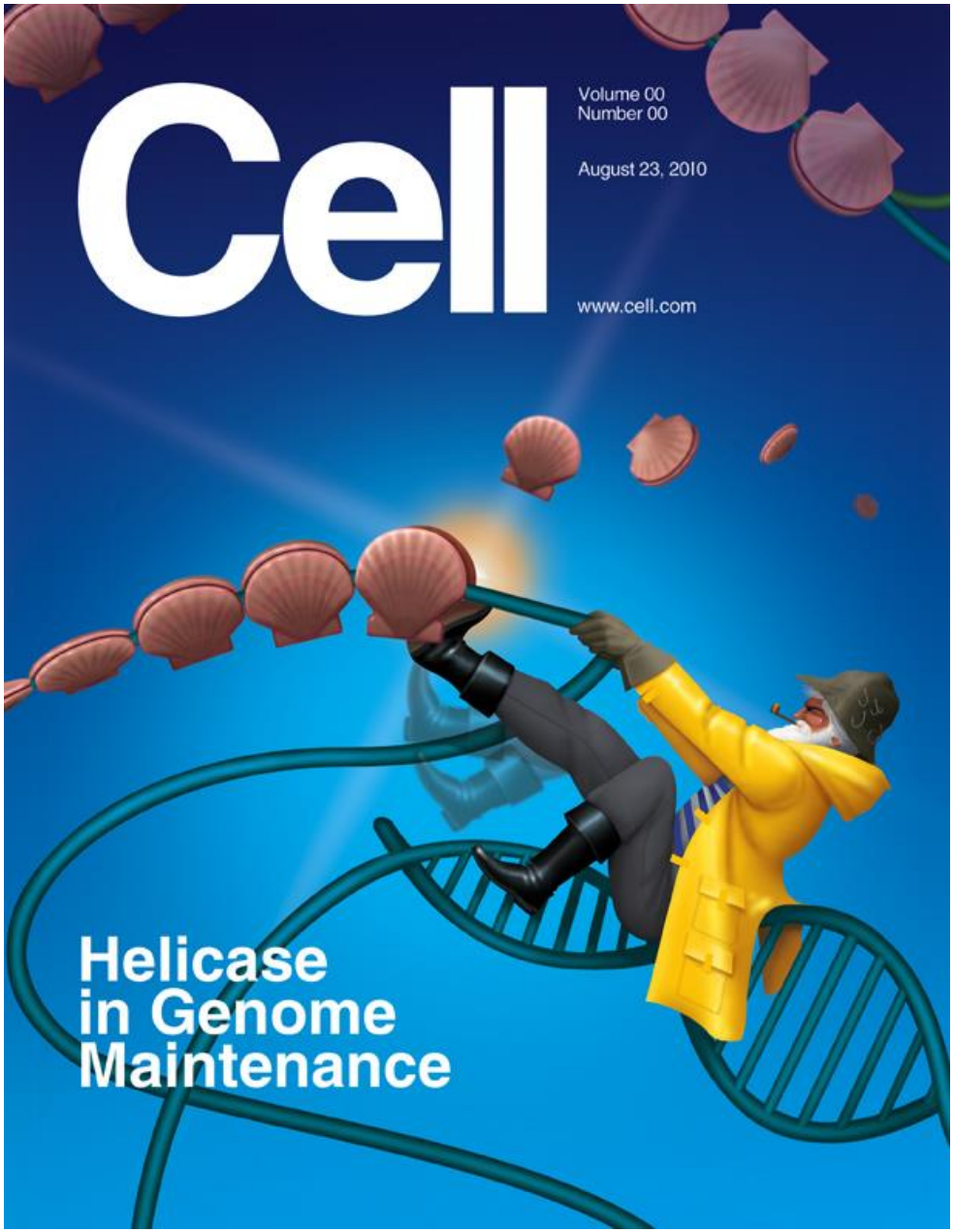
Ha, Enderle, Ogletree, Chemla, Selvin & Weiss (1996)




3'
5'
Cy3
Cy5
Fluorescence
Time

Park et al. "PcrA helicase dismantles RecA filaments by reeling in DNA in uniform steps", *Cell* 142, 544-555 (2010)





Jeehae Park

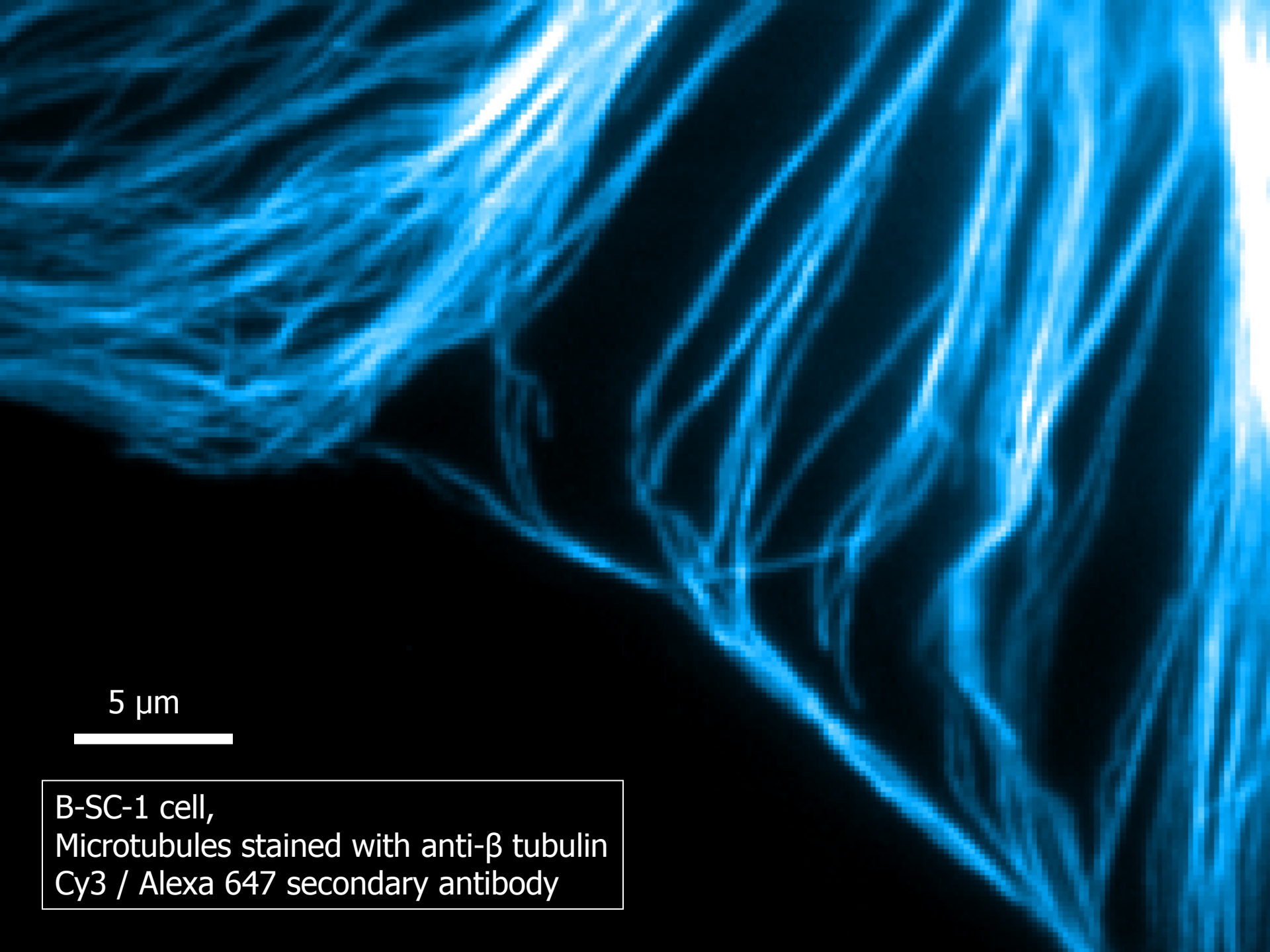
Abbe's diffraction limit

A factor of ten

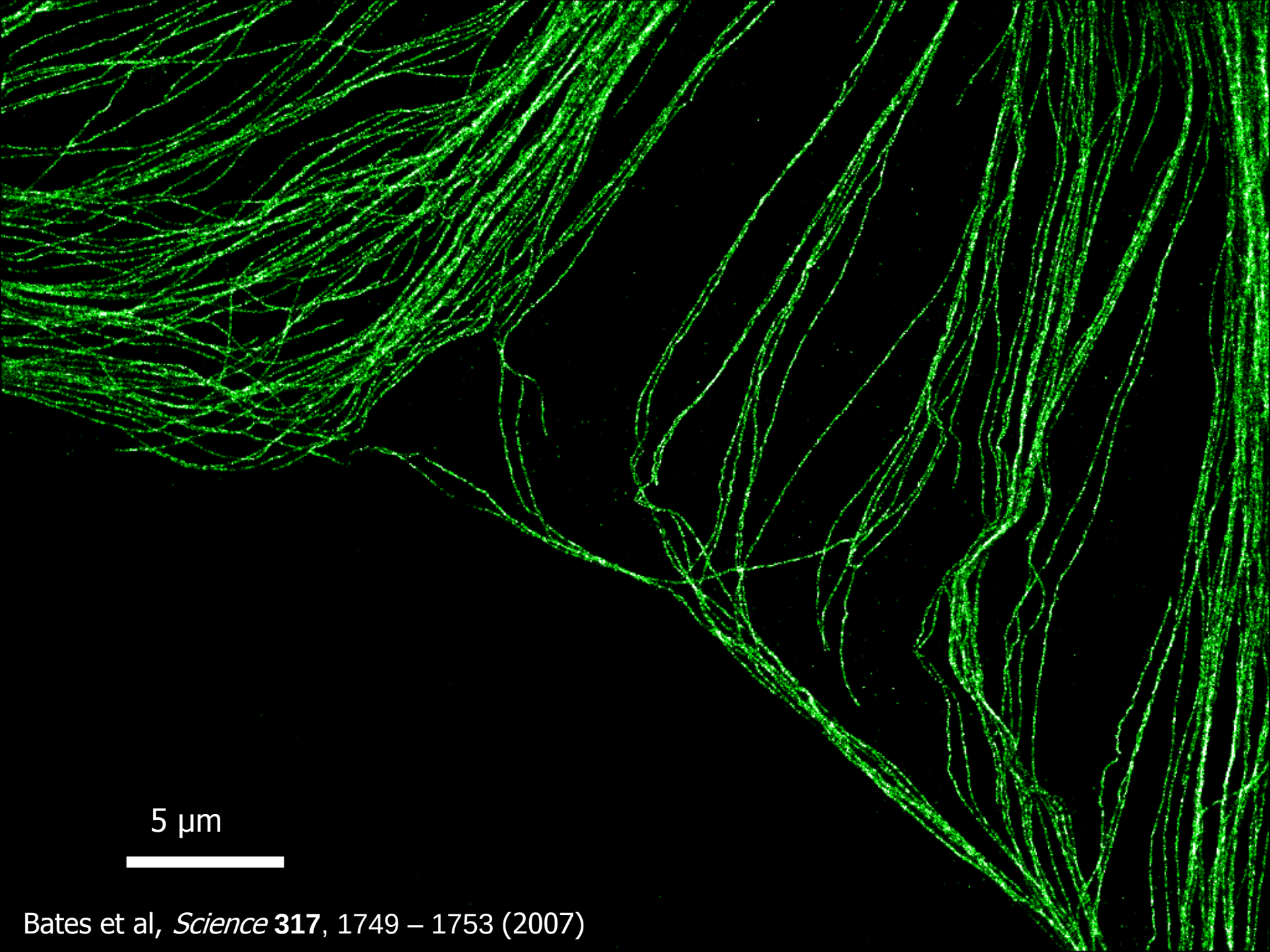
- CD -> DVD
- Conventional microscopy

->PALM/STORM

Single Molecule Based Technology

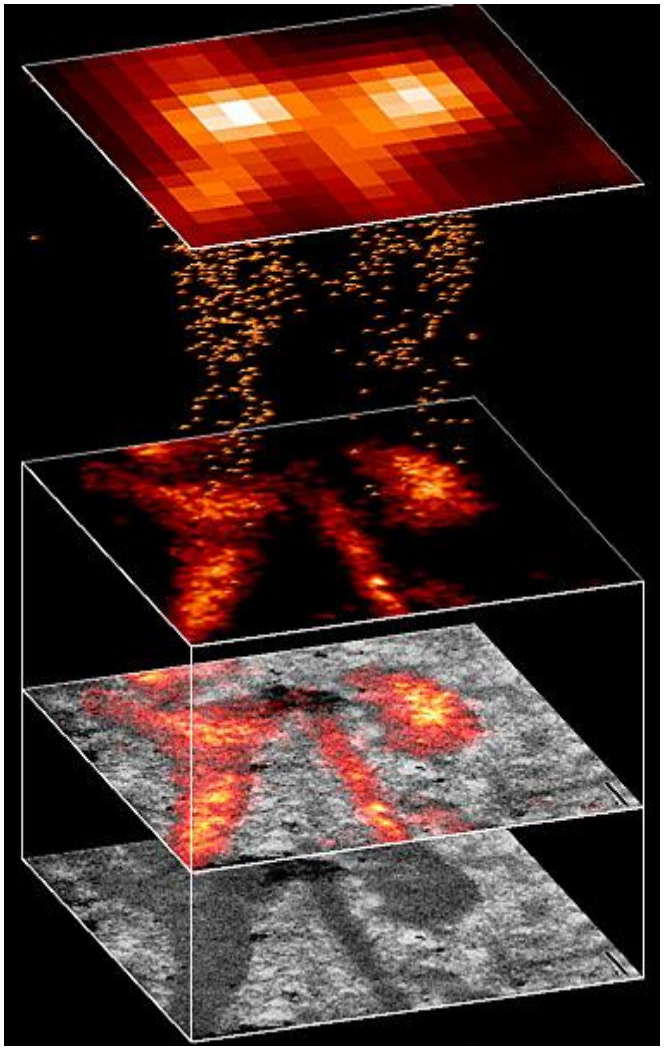


B-SC-1 cell,
Microtubules stained with anti-β tubulin
Cy3 / Alexa 647 secondary antibody



Bates et al, *Science* **317**, 1749 – 1753 (2007)

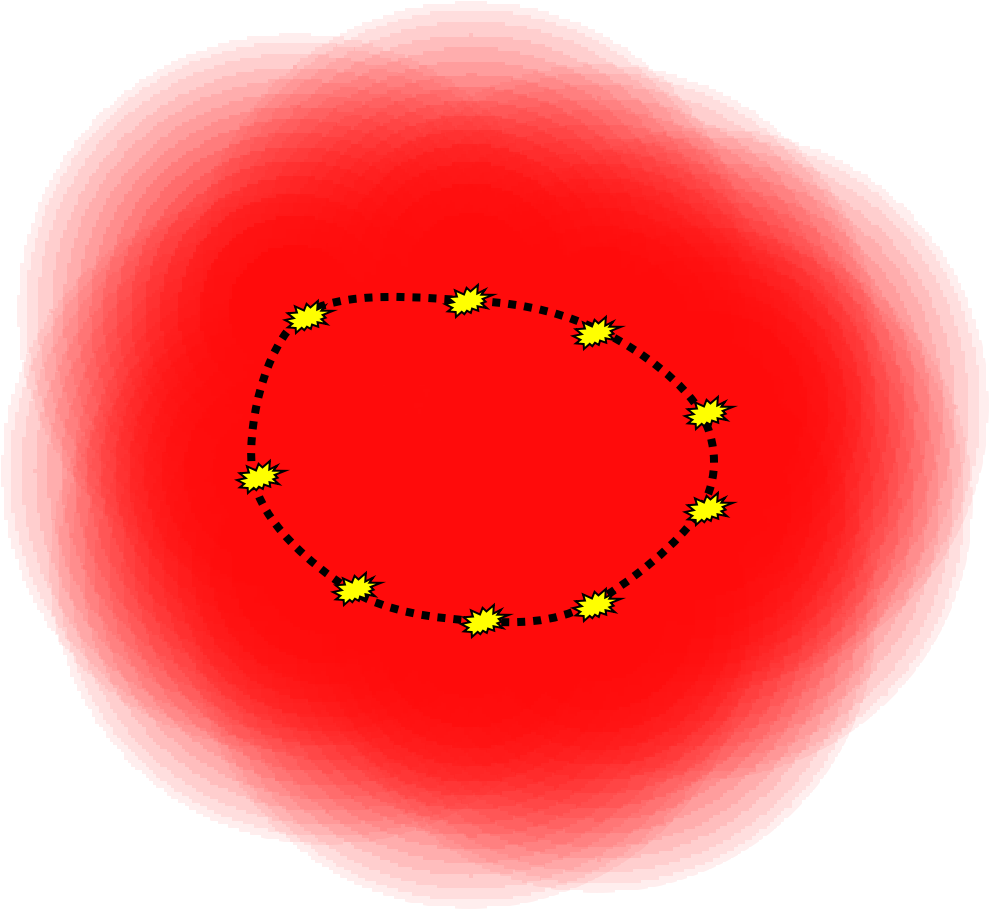
PALM (PhotoActivation Localization Microscopy) principle



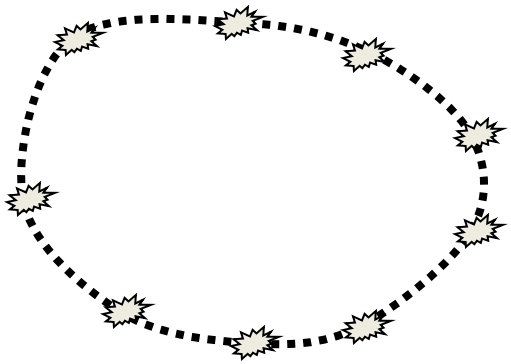
STORM
(Stochastic Reconstruction Microscopy)

Betzig et al, Science (2006)

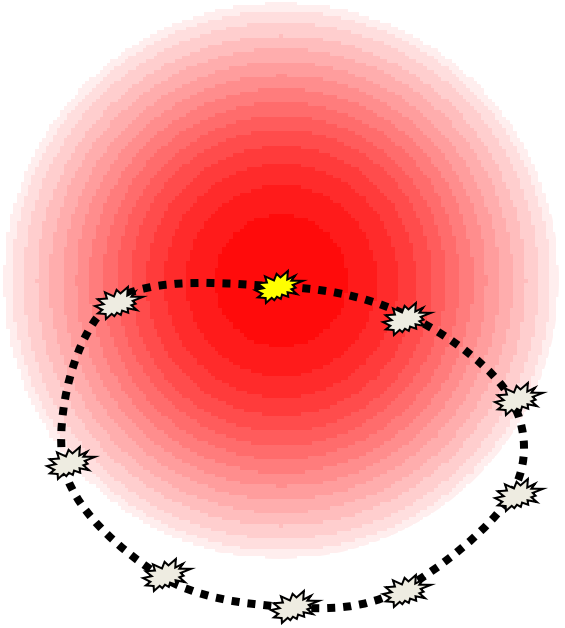
Many Molecules

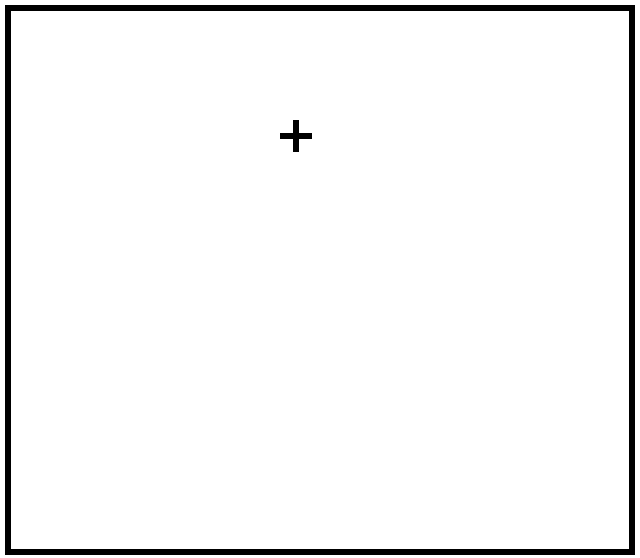


The principle of STORM

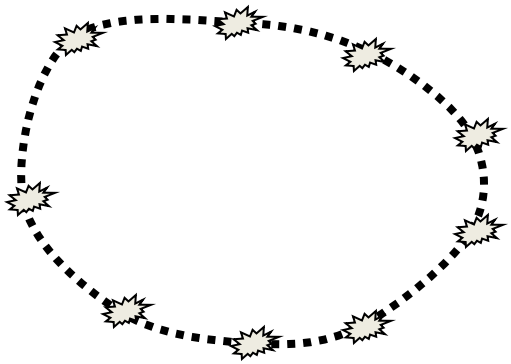


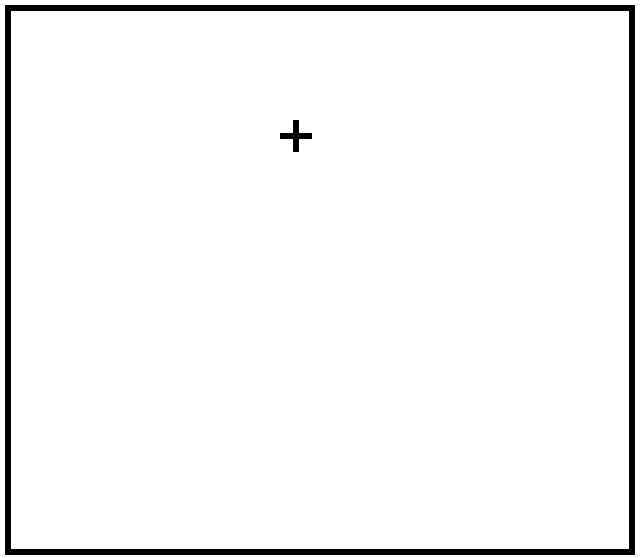
The principle of STORM



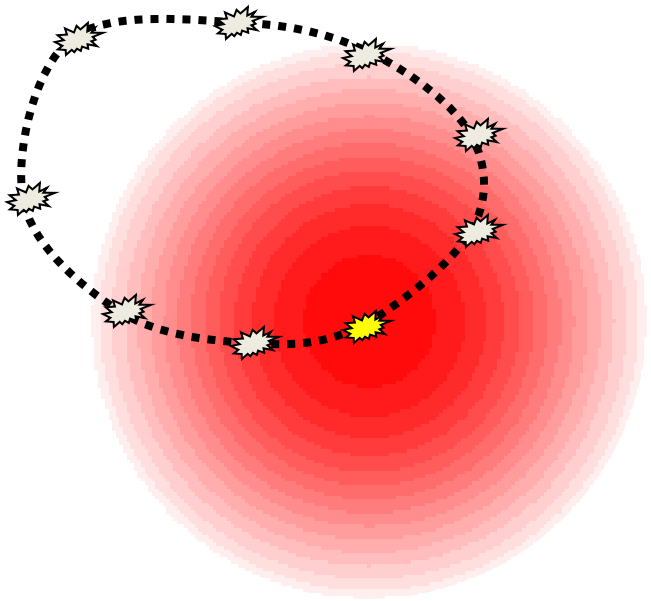


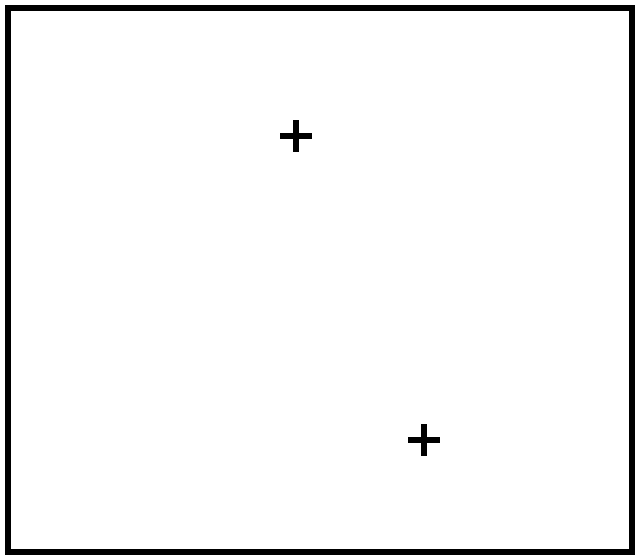
The principle of STORM



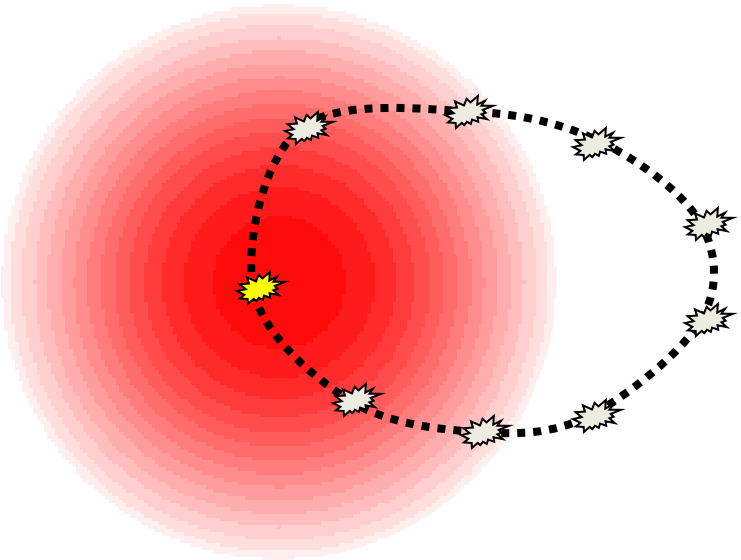


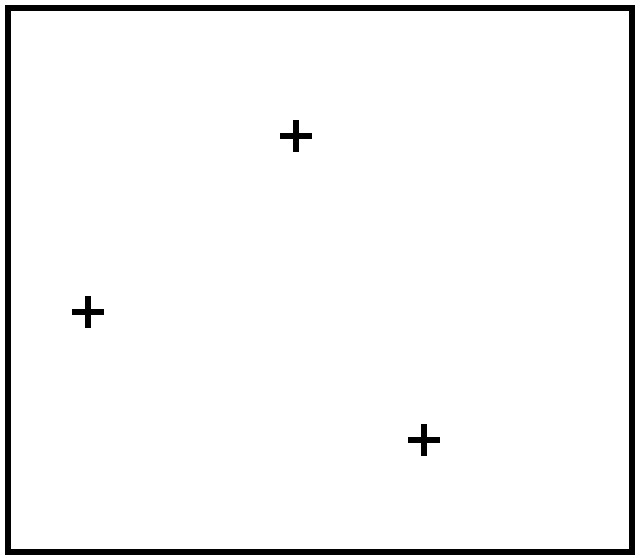
The principle of STORM



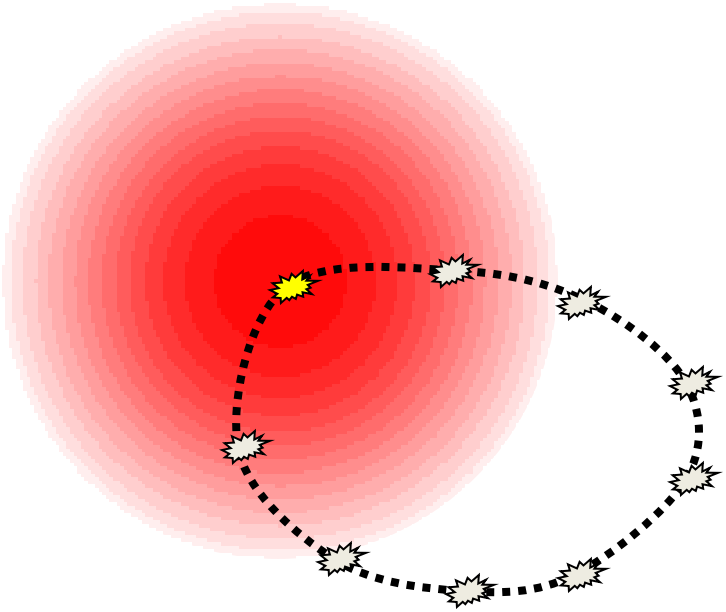


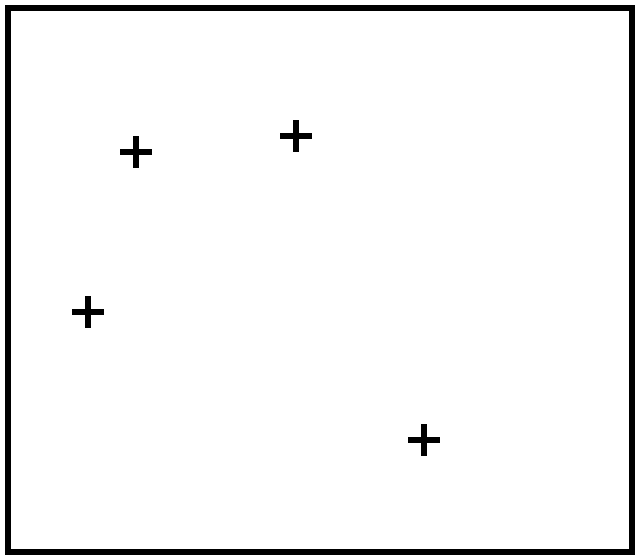
The principle of STORM



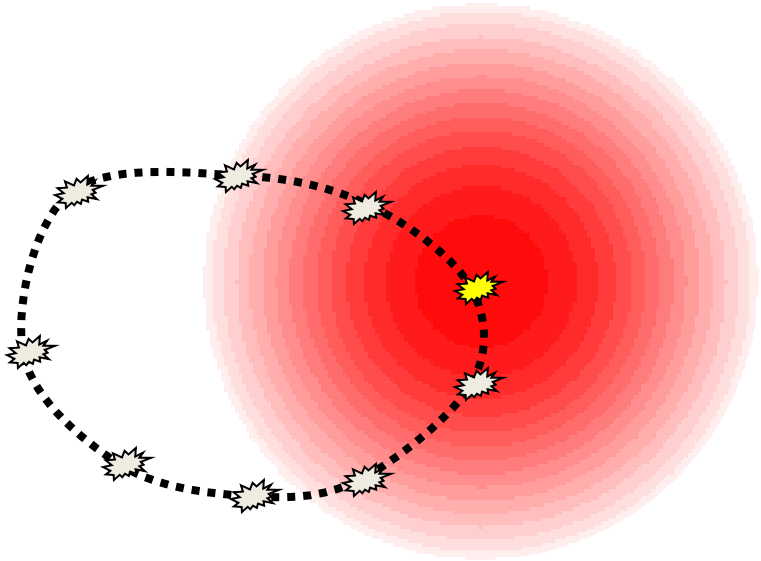


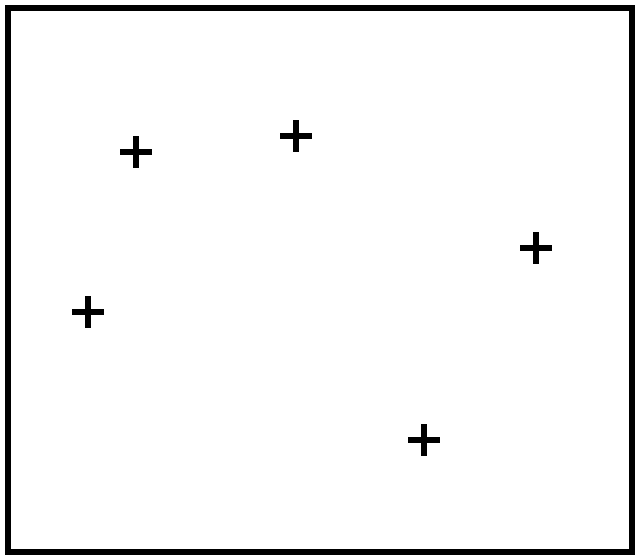
The principle of STORM



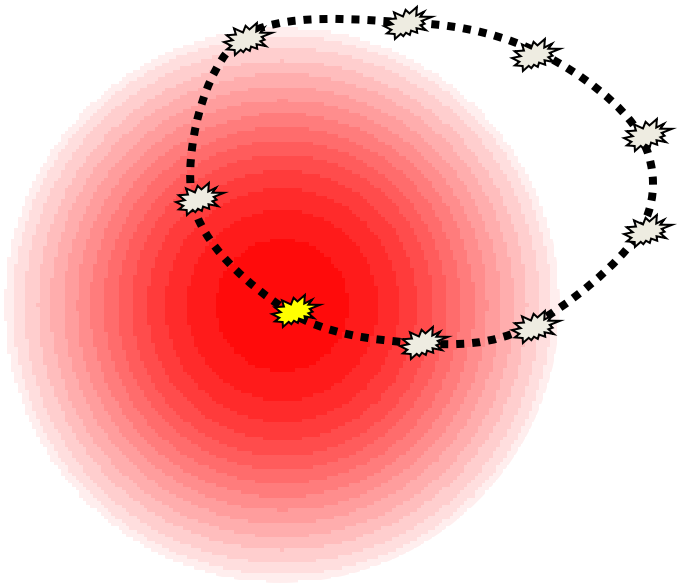


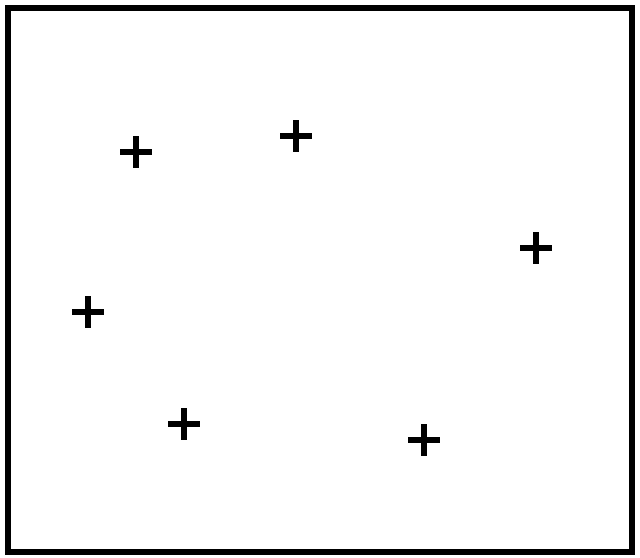
The principle of STORM



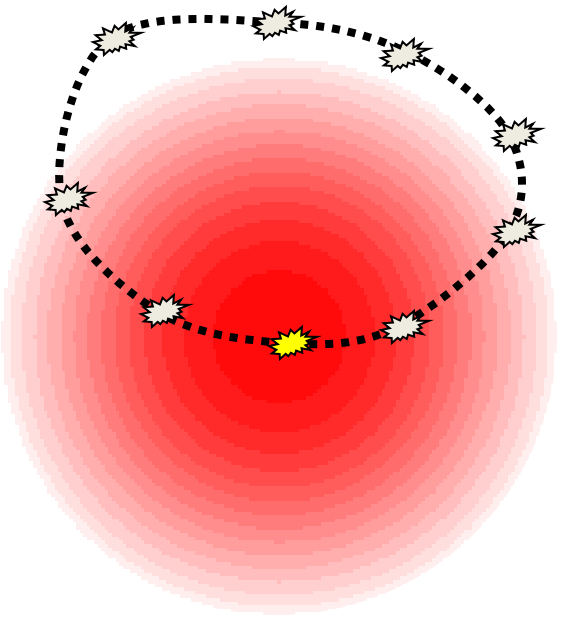


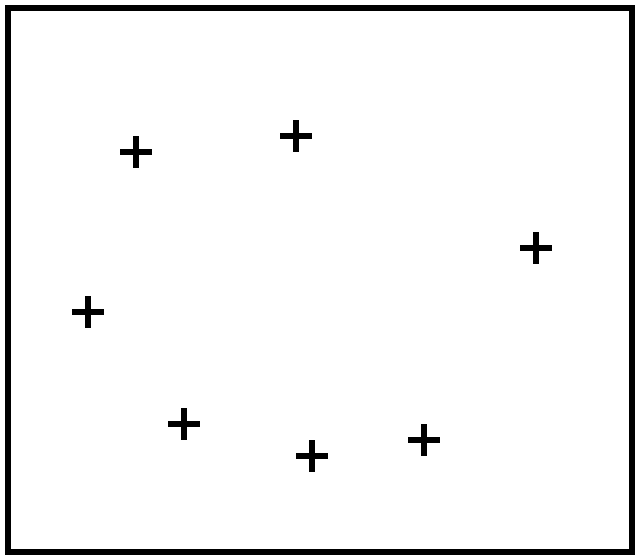
The principle of STORM



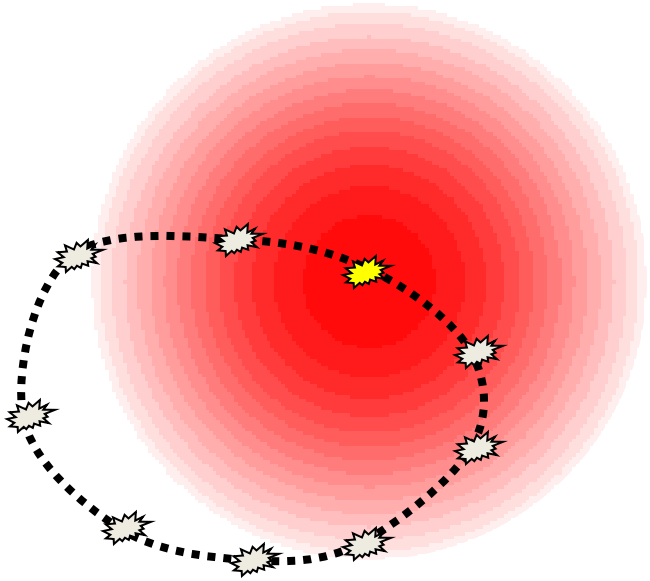


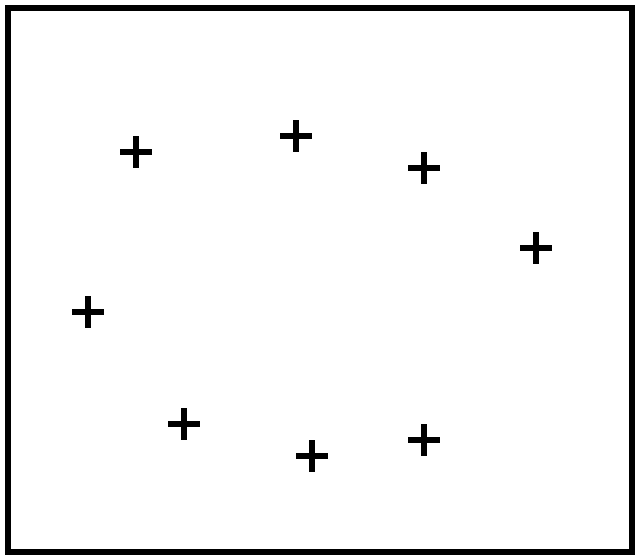
The principle of STORM



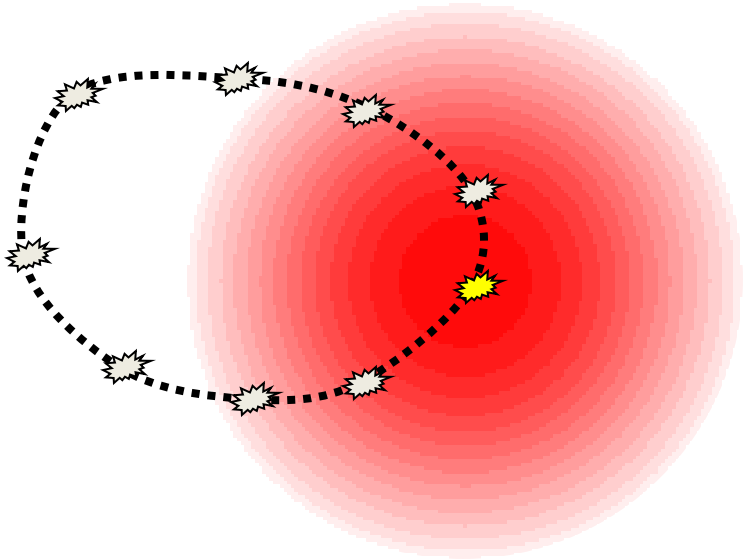


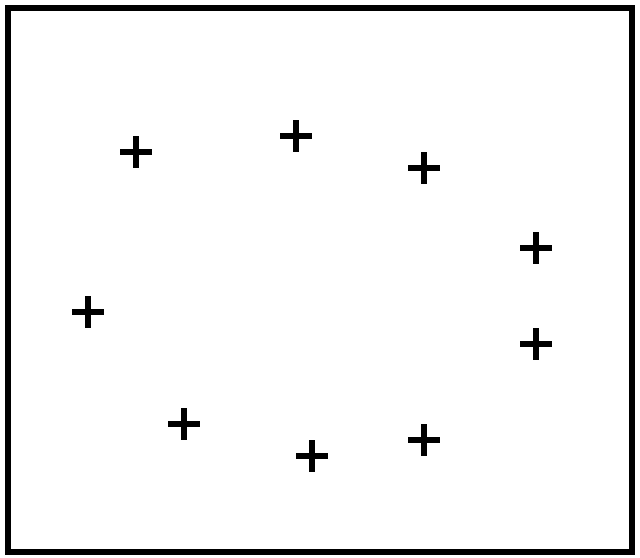
The principle of STORM



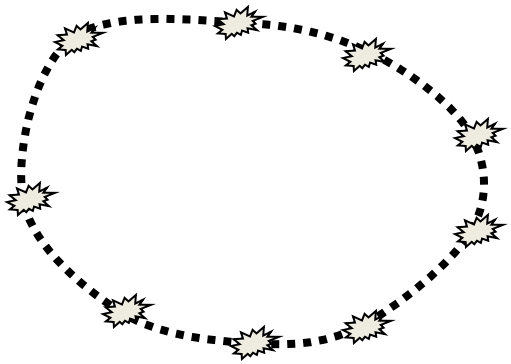


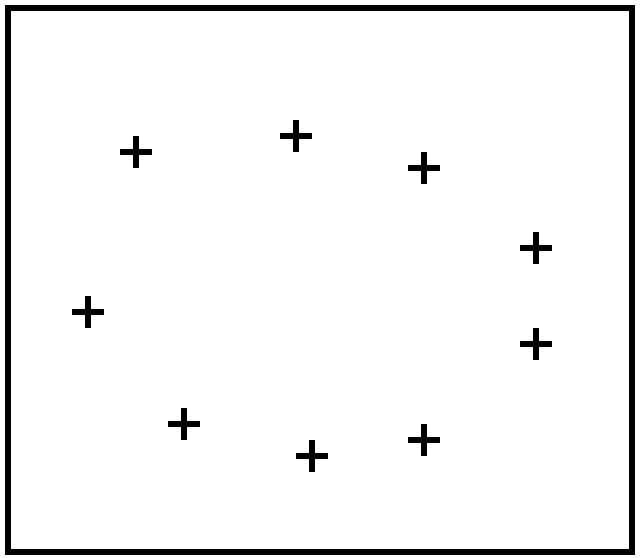
The principle of STORM

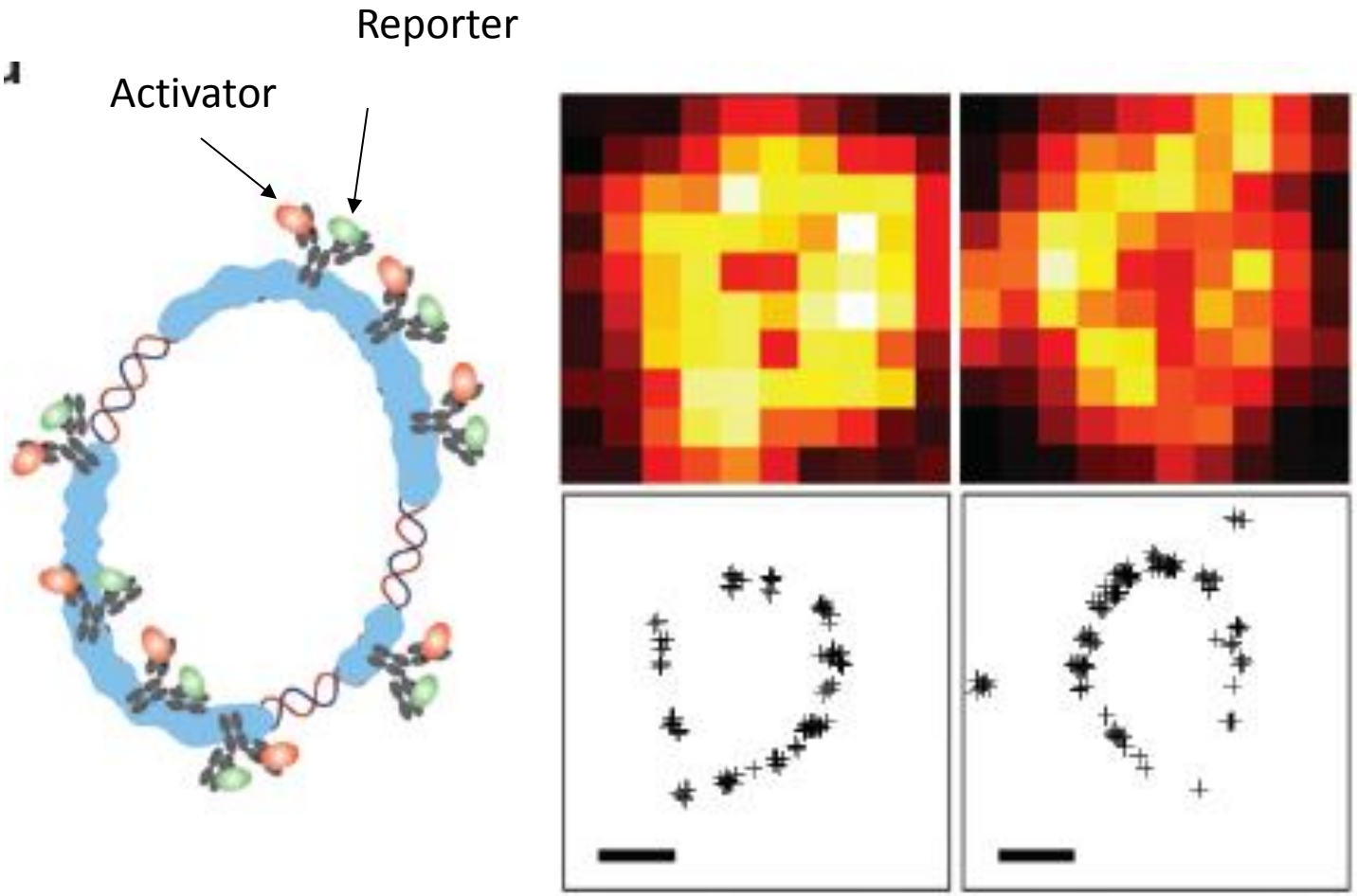


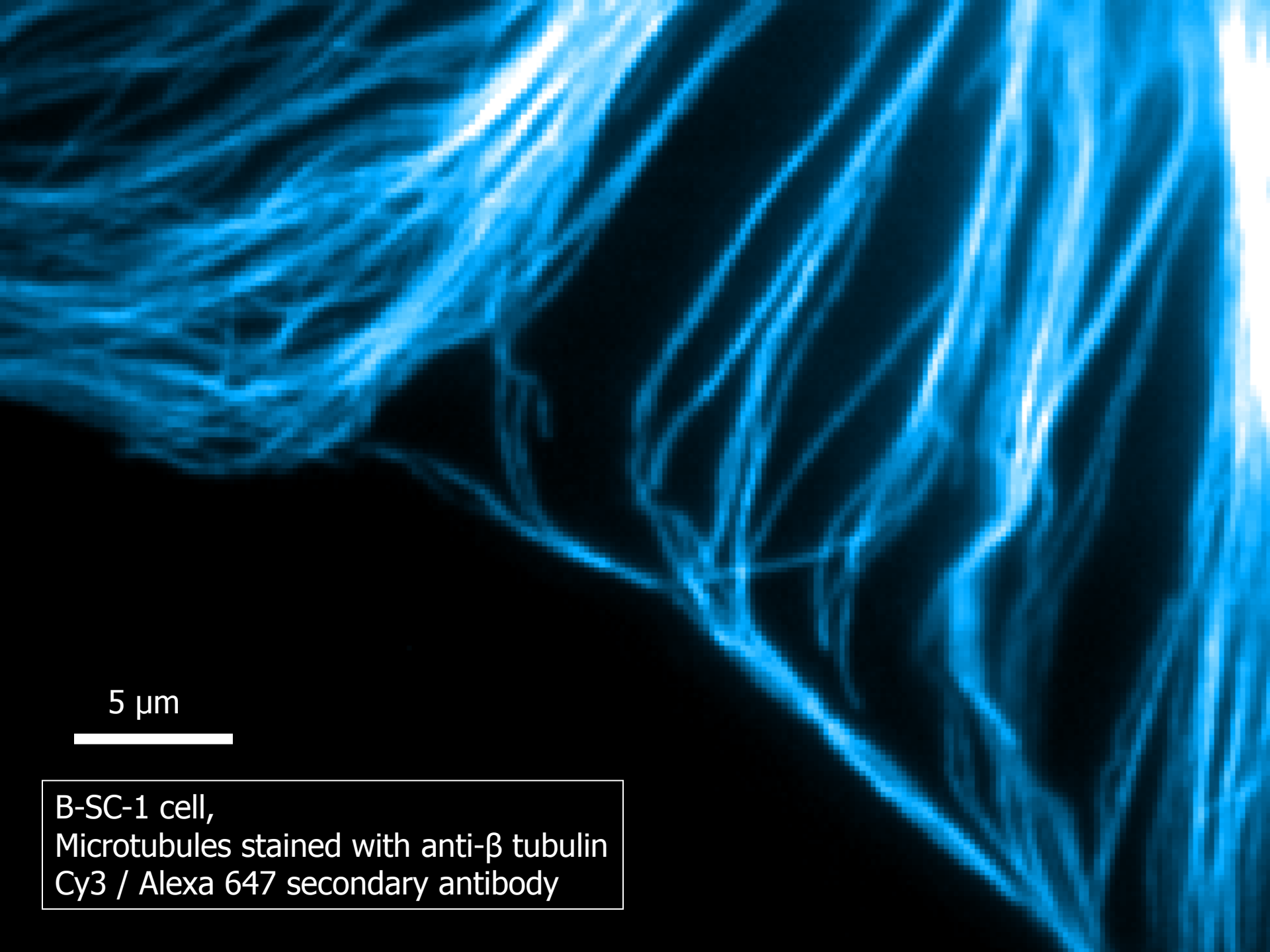


The principle of STORM

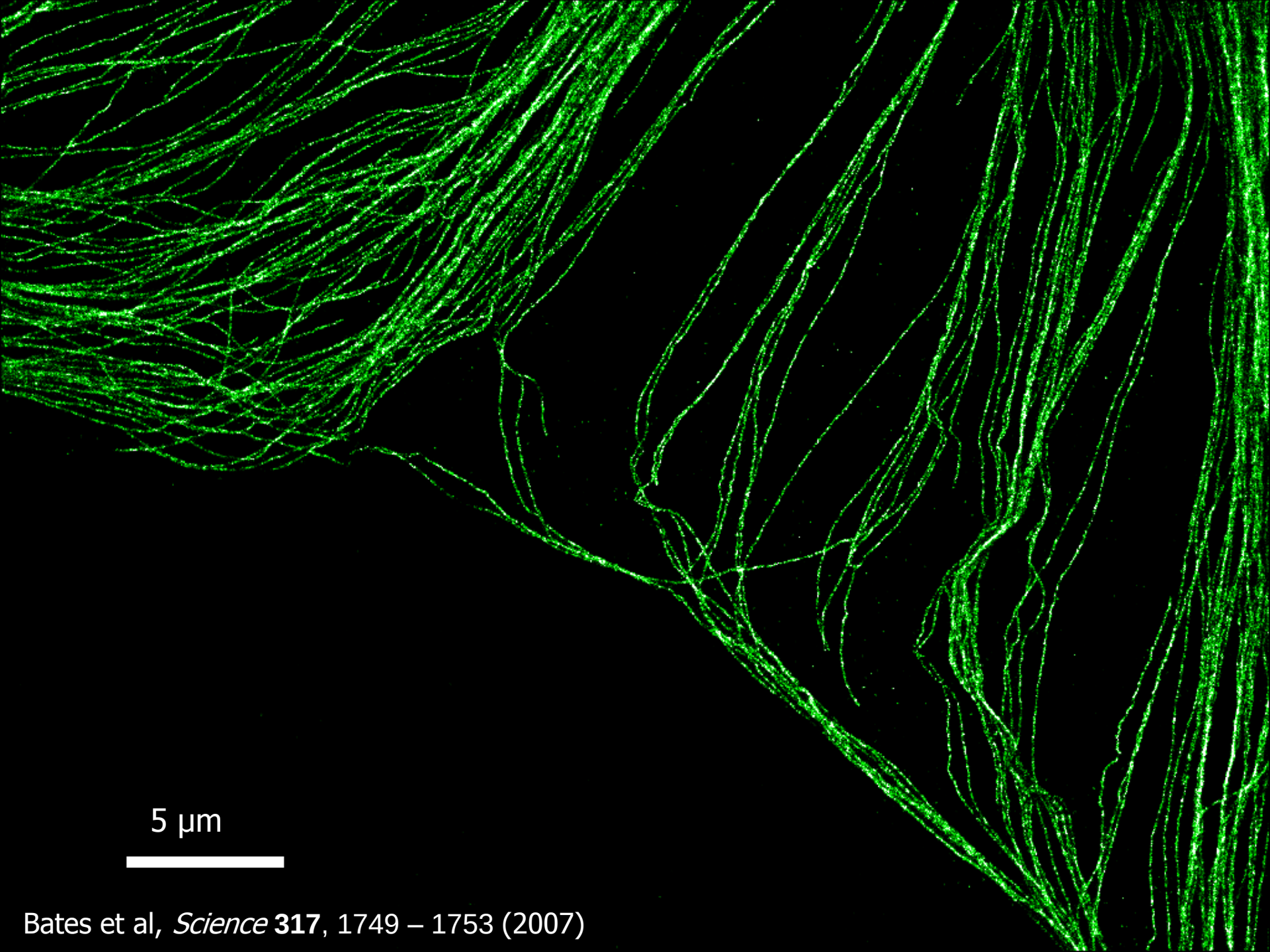




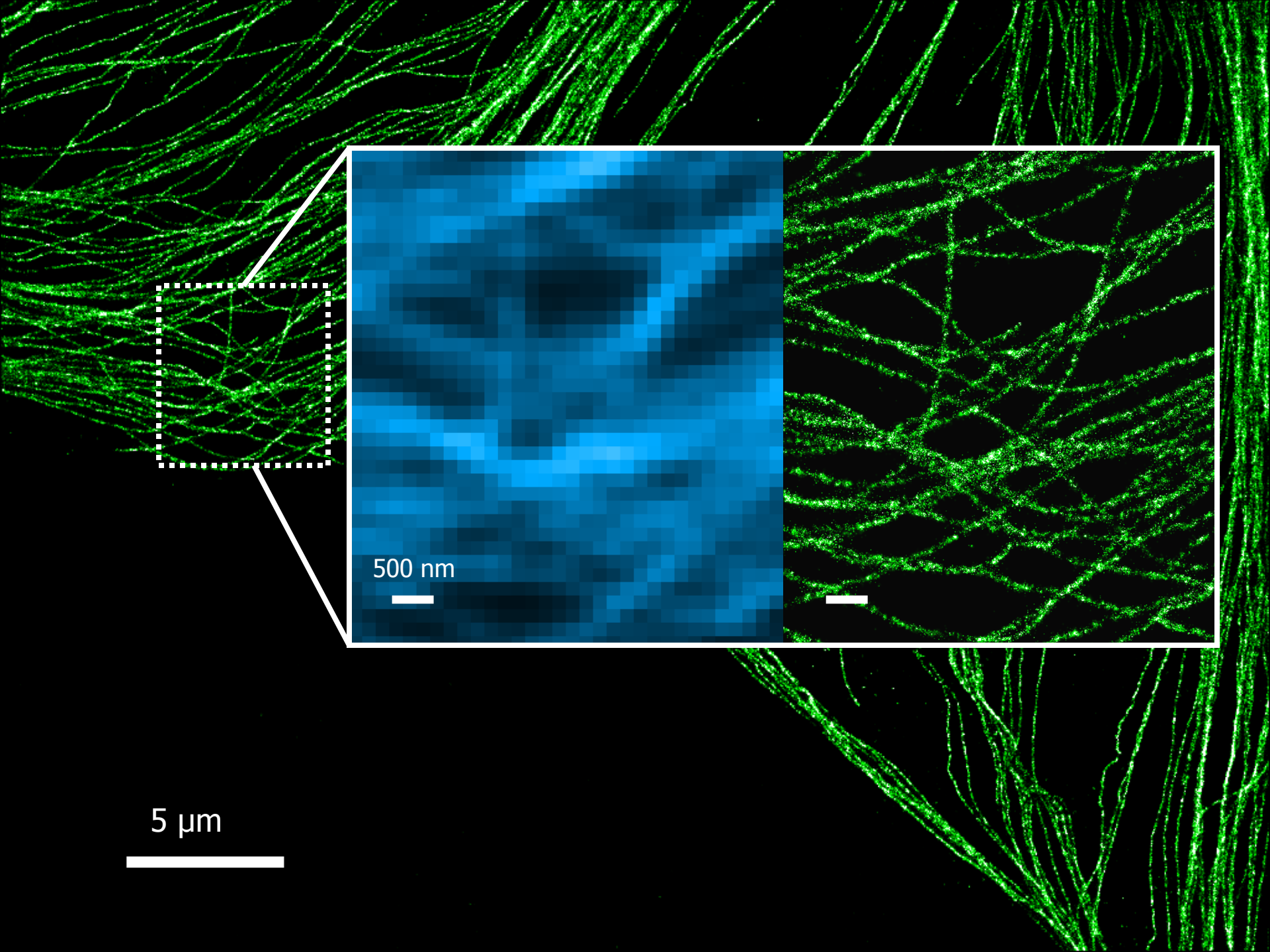

Reporter
Activator

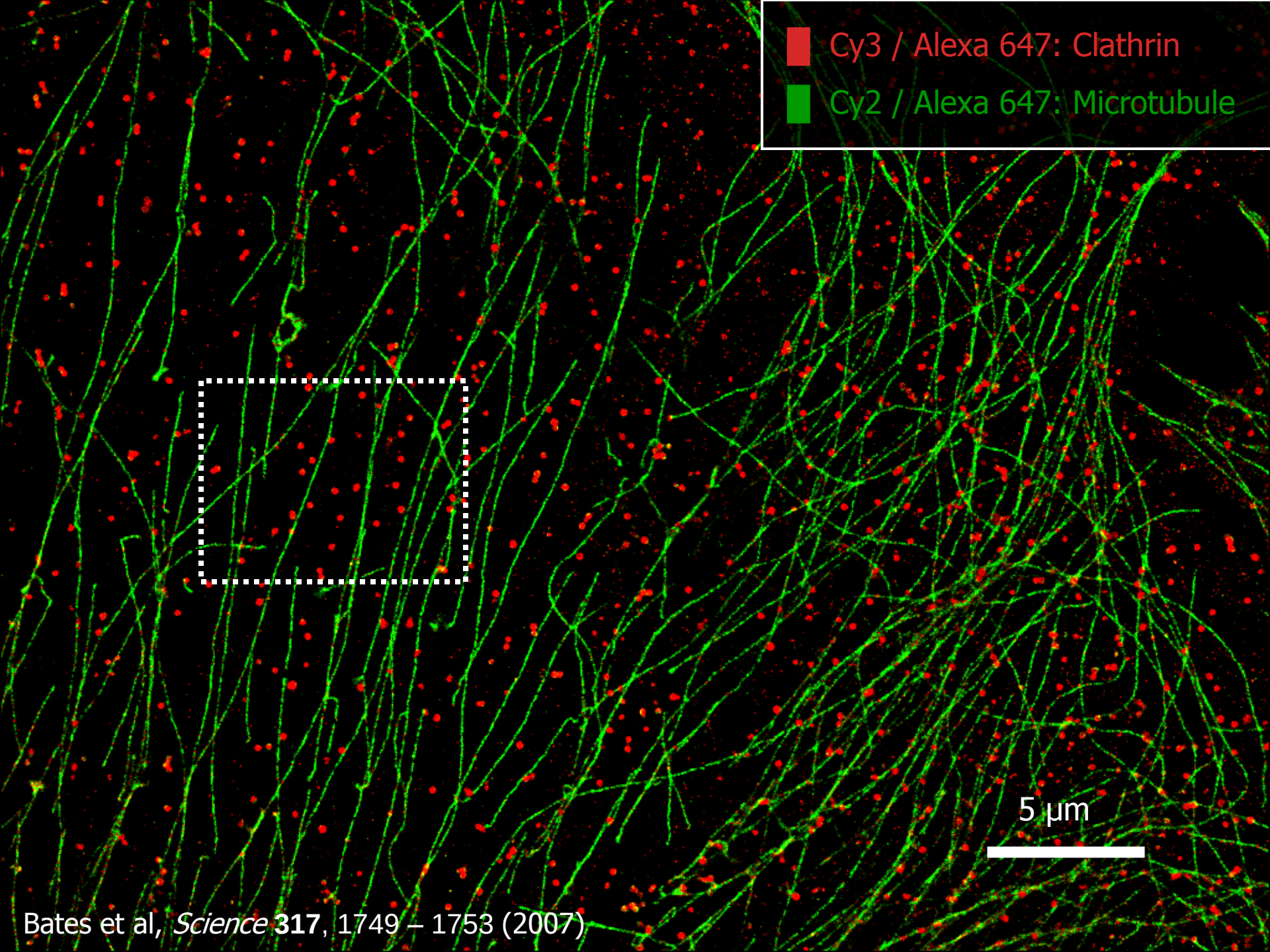


B-SC-1 cell,
Microtubules stained with anti-β tubulin
Cy3 / Alexa 647 secondary antibody

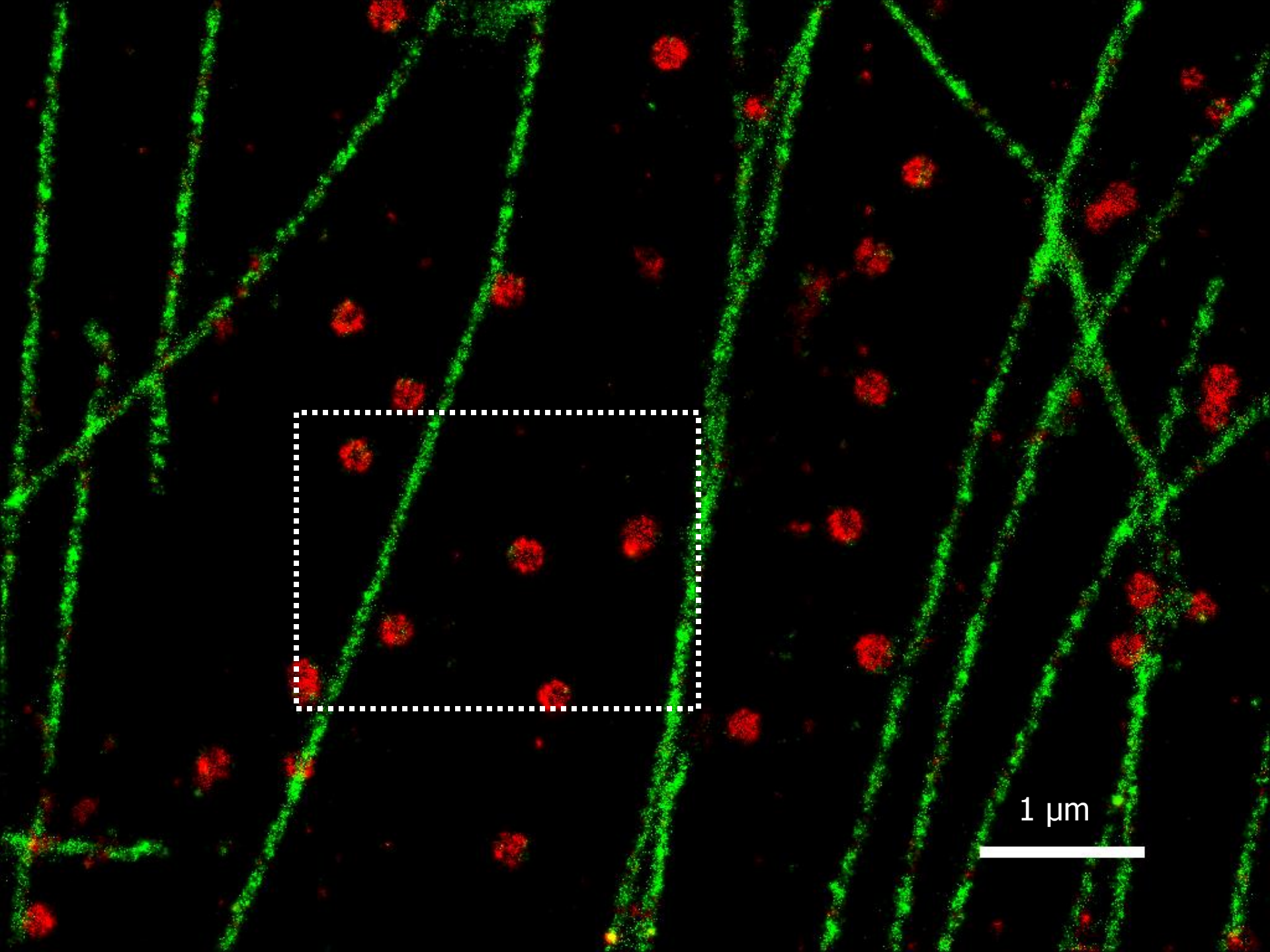


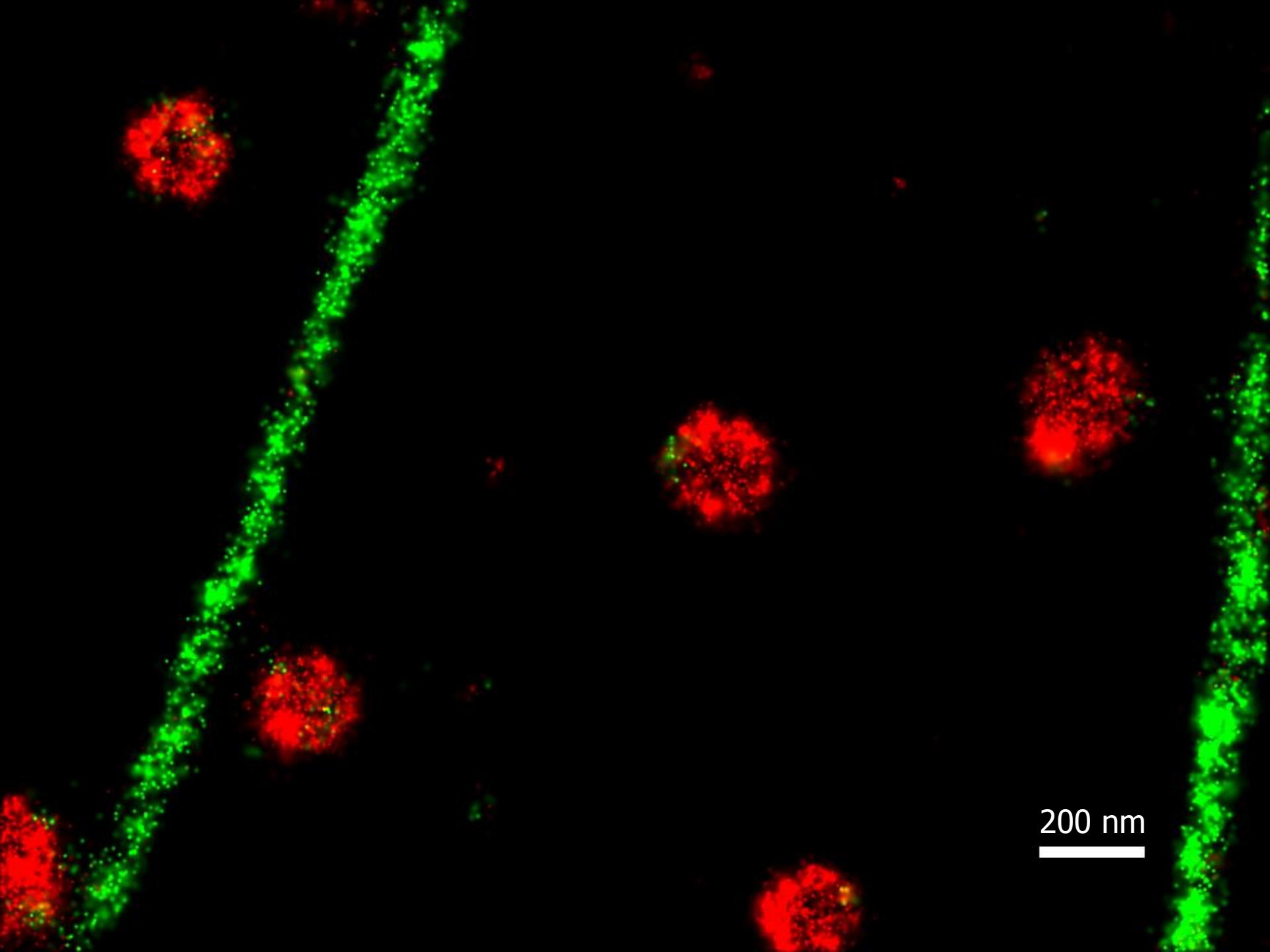
Bates et al, *Science* **317**, 1749 – 1753 (2007)


500 nm
5 µm

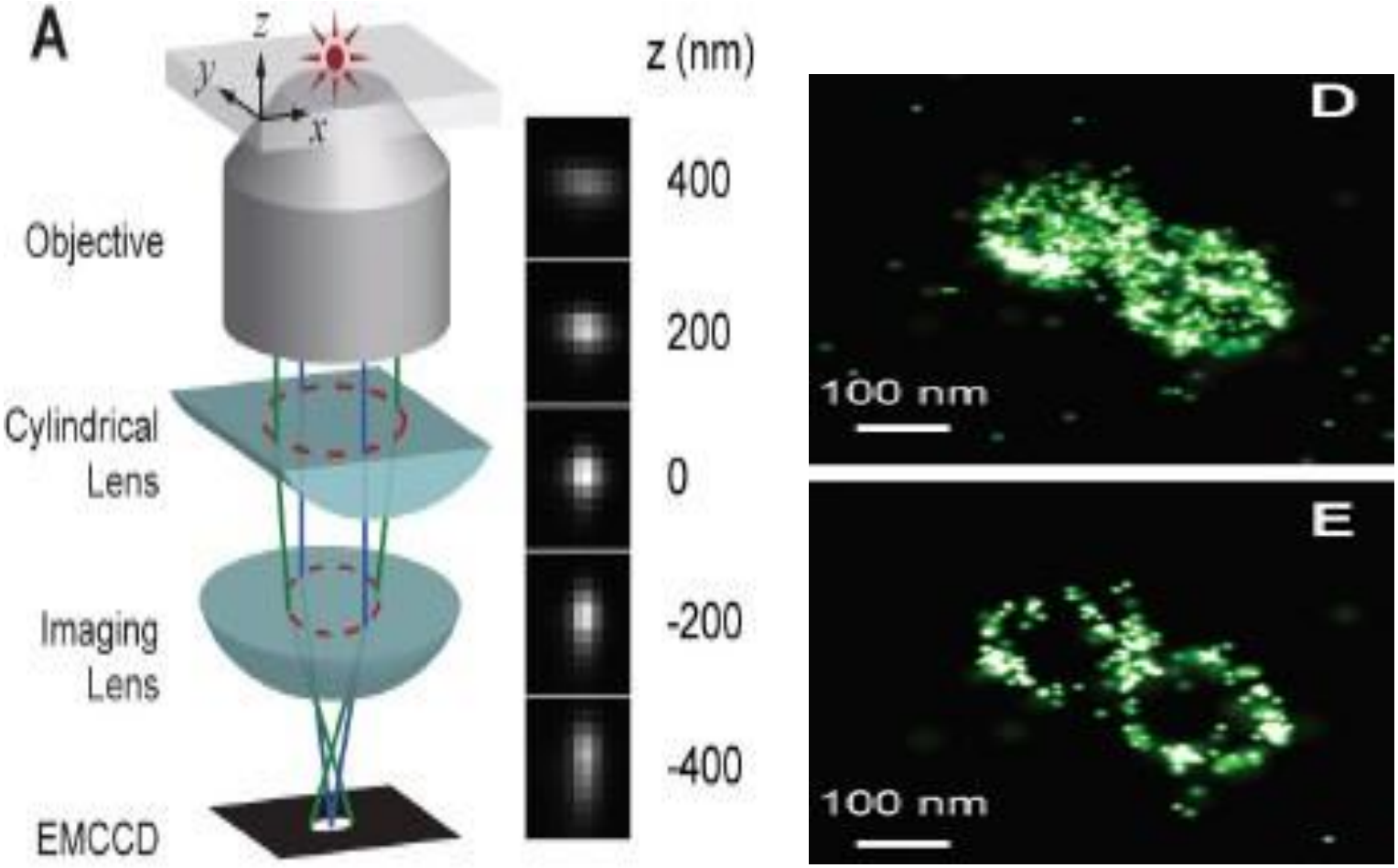


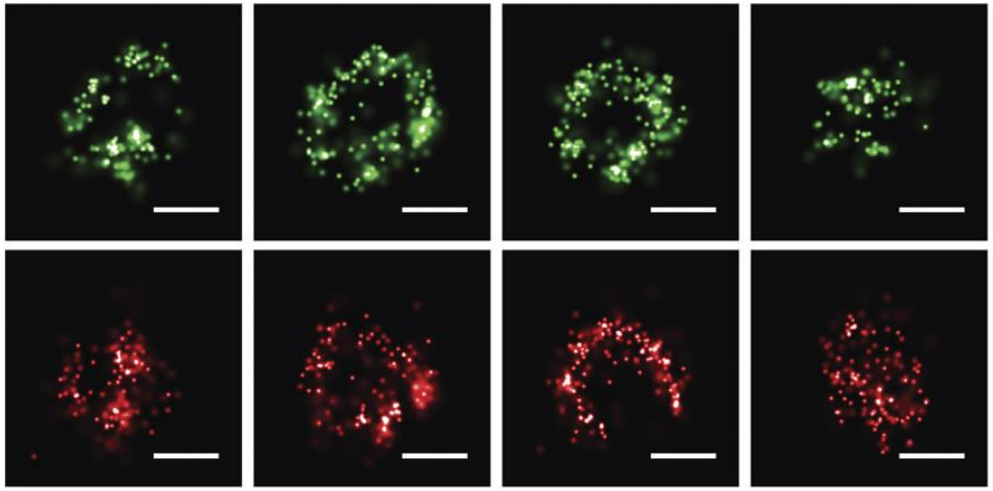
Bates et al, *Science* **317**, 1749 – 1753 (2007)

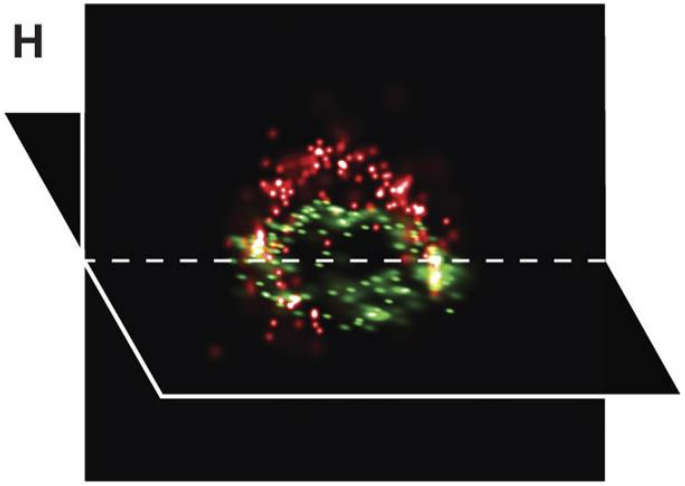

1 µm


200 nm

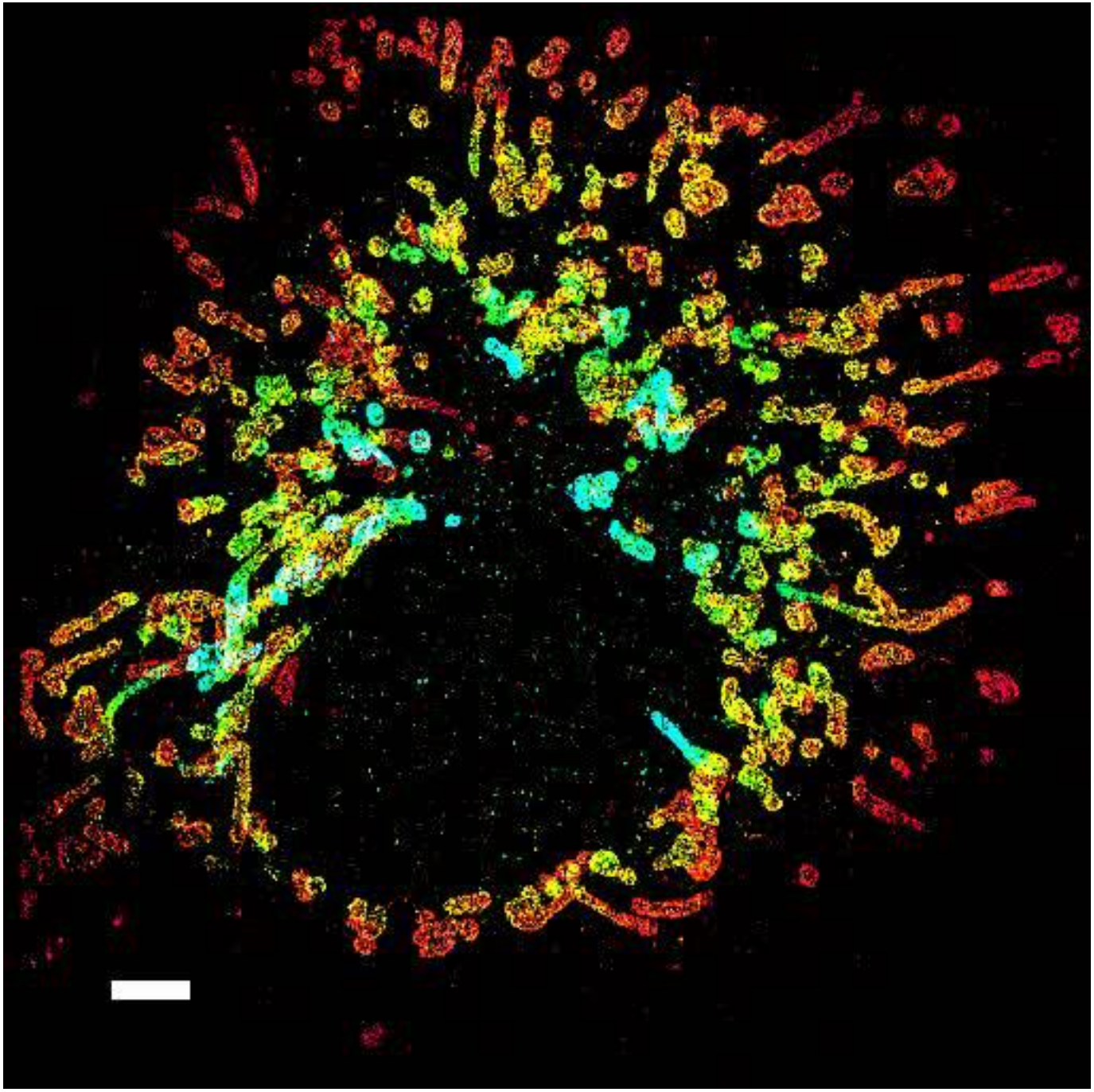
3D STORM


A
z
y
x
Objective
Cylindrical Lens
Imaging Lens
EMCCD
z (nm)
400
200
0
-200
-400
D
100 nm
E
100 nm




H

Zhuang lab, 2008
Mitochondria
Whole Cell
3D STORM



3 micron scal e bar

Optogenetics *Optomechanics* *Deep Imaging*

New Optical Techniques and Their Impact on the Life Sciences:

Making what's invisible today, visible tomorrow.

Center for the Physics of Living Cells

- ***Use light to excite neurons -> Revolutionizing brain science!***

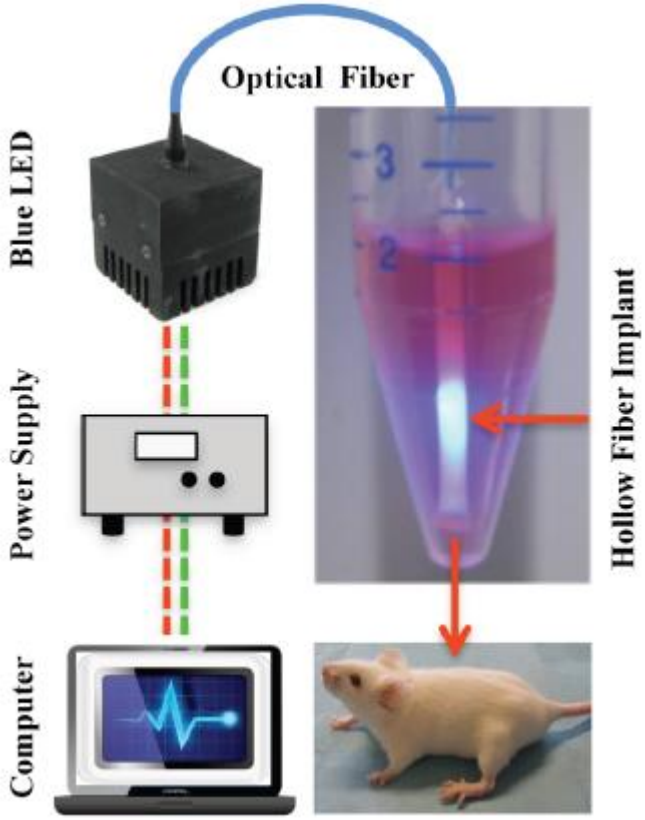
Boyden, …, Deisseroth
Nature Neurosci. (2005)

Incepting False Memories into Mice

Ramirez, … ,Tonegawa
Science (2013)

- *Use light to excite neurons -> Revolutionizing brain science!*
- ***Use light to control gene expression -> All of biology!***

Light-controlled insulin secretion in a diabetic mouse



Ye, … ,Fussenegger, Science (2011)

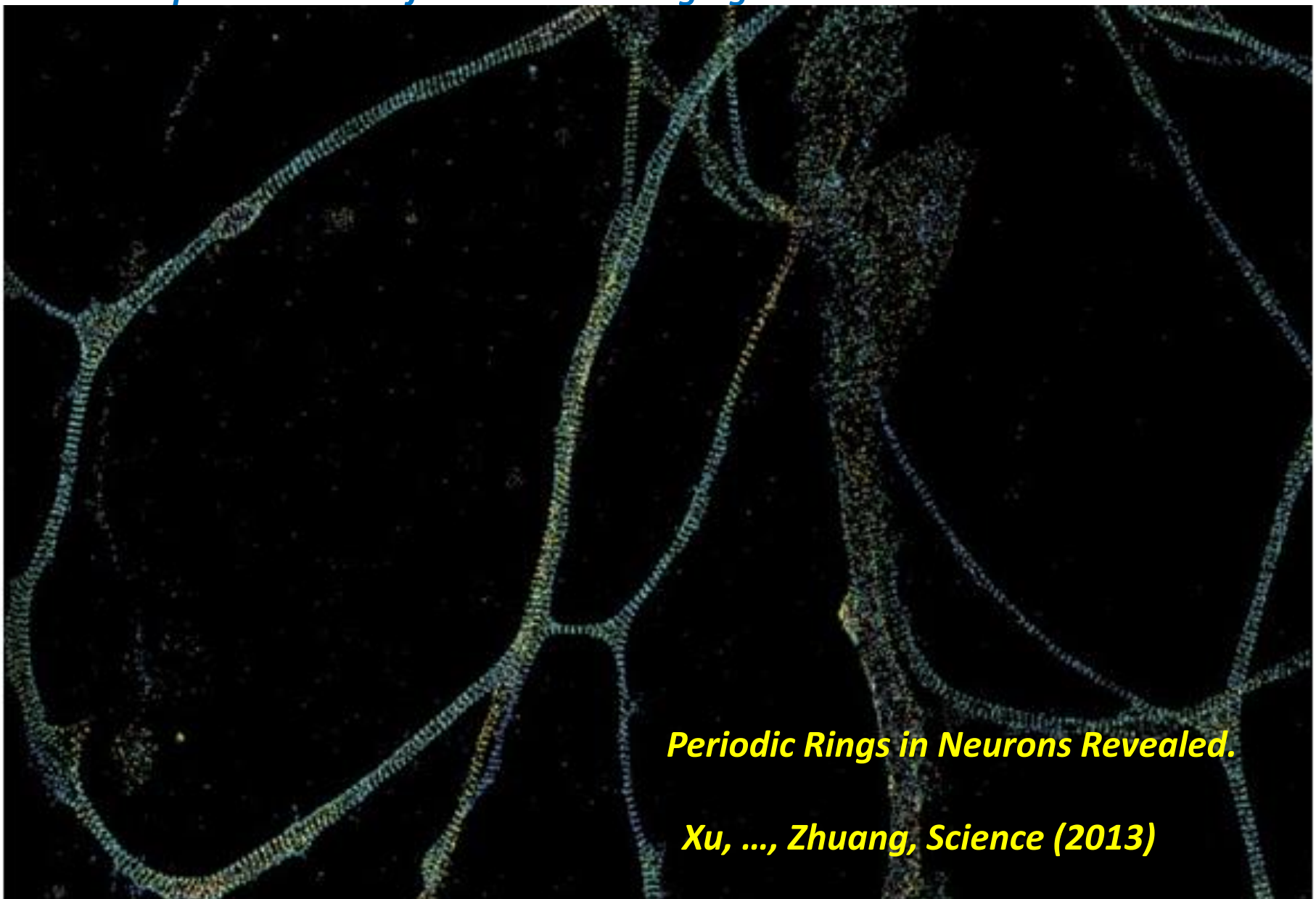
- *Use light to excite neurons -> Revolutionizing brain science!*
- *Use light to control gene expression -> All of biology!*

Gaps

- ***At best, diffraction limited resolution***
- ***Scattering and background fluorescence from thick samples***
- ***Multiplexing is still lacking***
- ***Photo-toxicity***

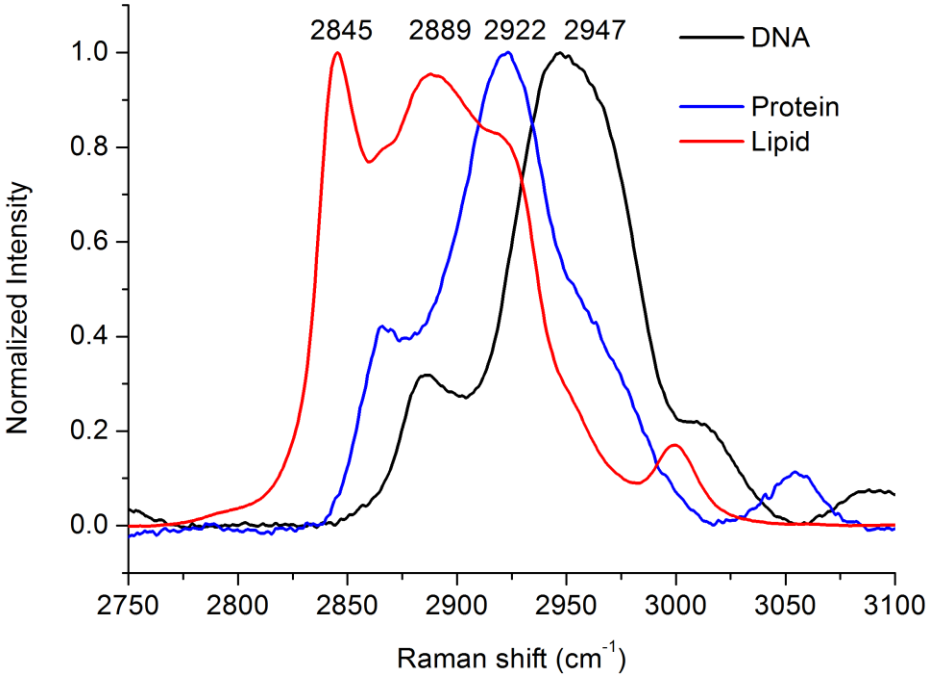
Opportunities

- Super-resolution fluorescence imaging

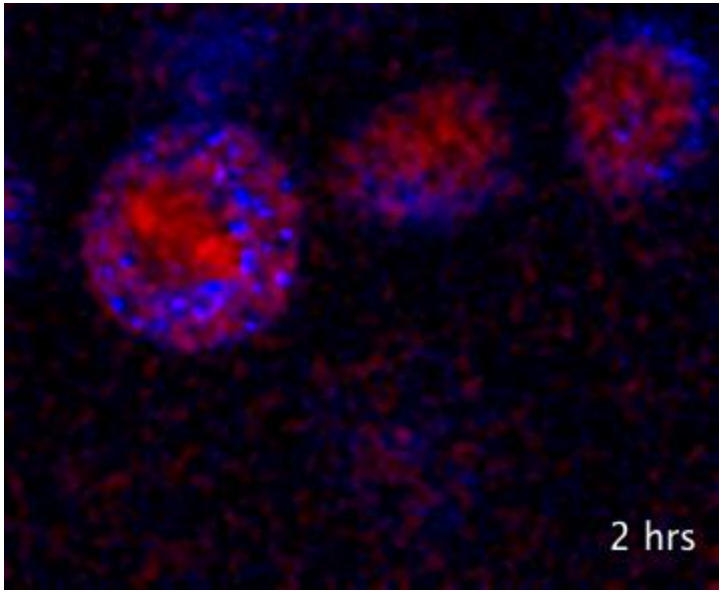


Opportunities

- *Label-free microscopy with vibrational contrast*



Label-free imaging of live cell division



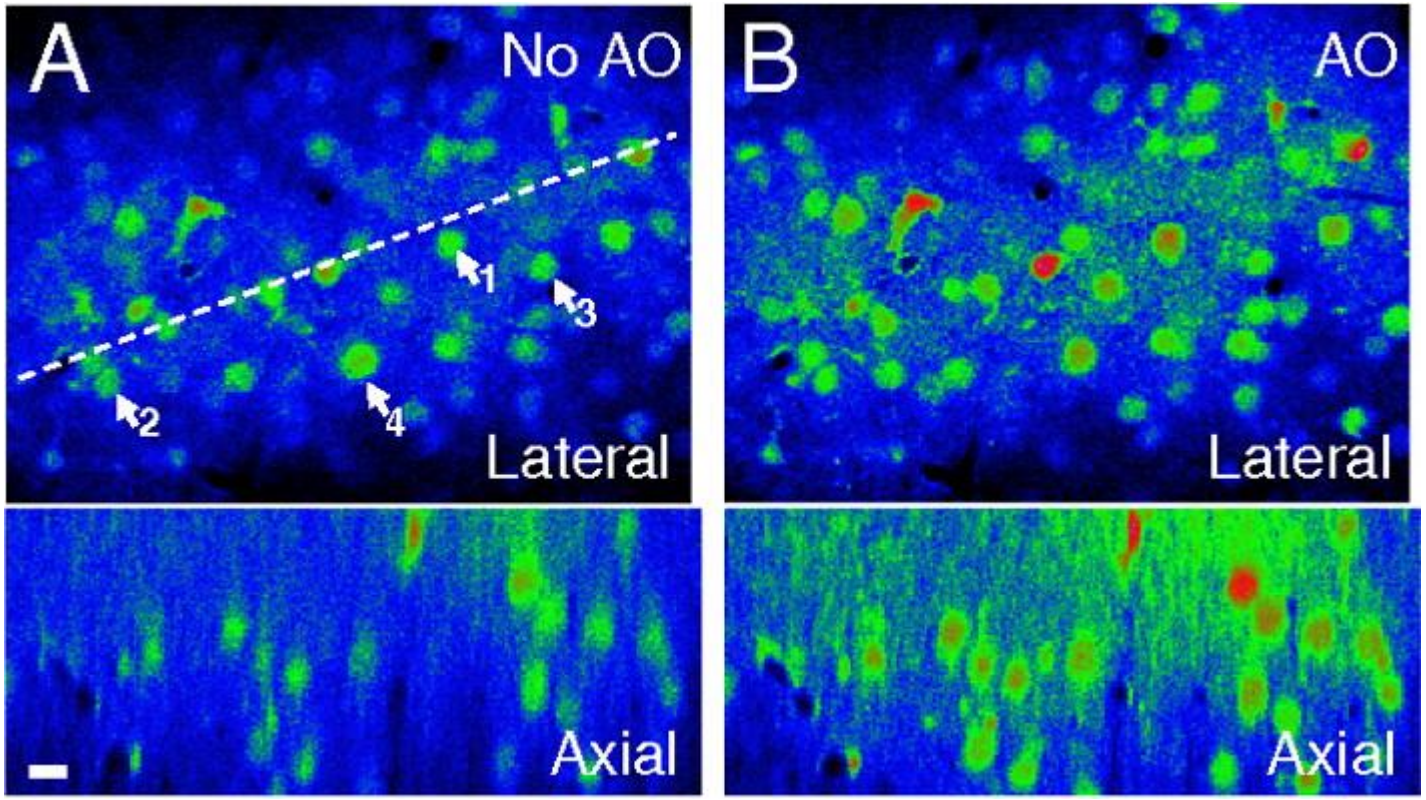
Red: DNA Blue: Lipids

Stimulated Raman Scattering Microscopy
Saar,…,Xie, Science (2010)

Opportunities

- Adaptive optics (borrowed from astronomy!)

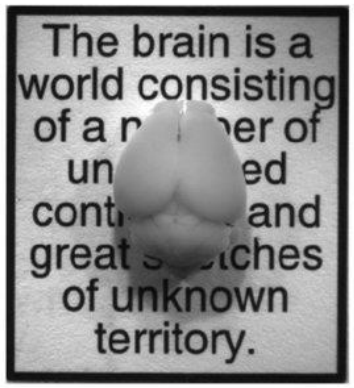
Adaptive optics correction improves calcium imaging in living mouse brain

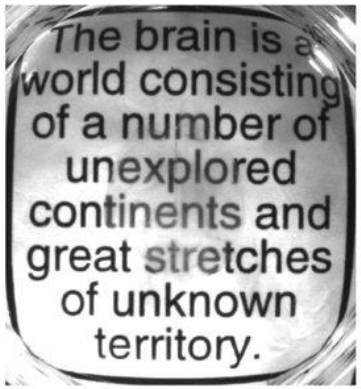


Ji,…, Betzig, PNAS (2012)

Opportunities

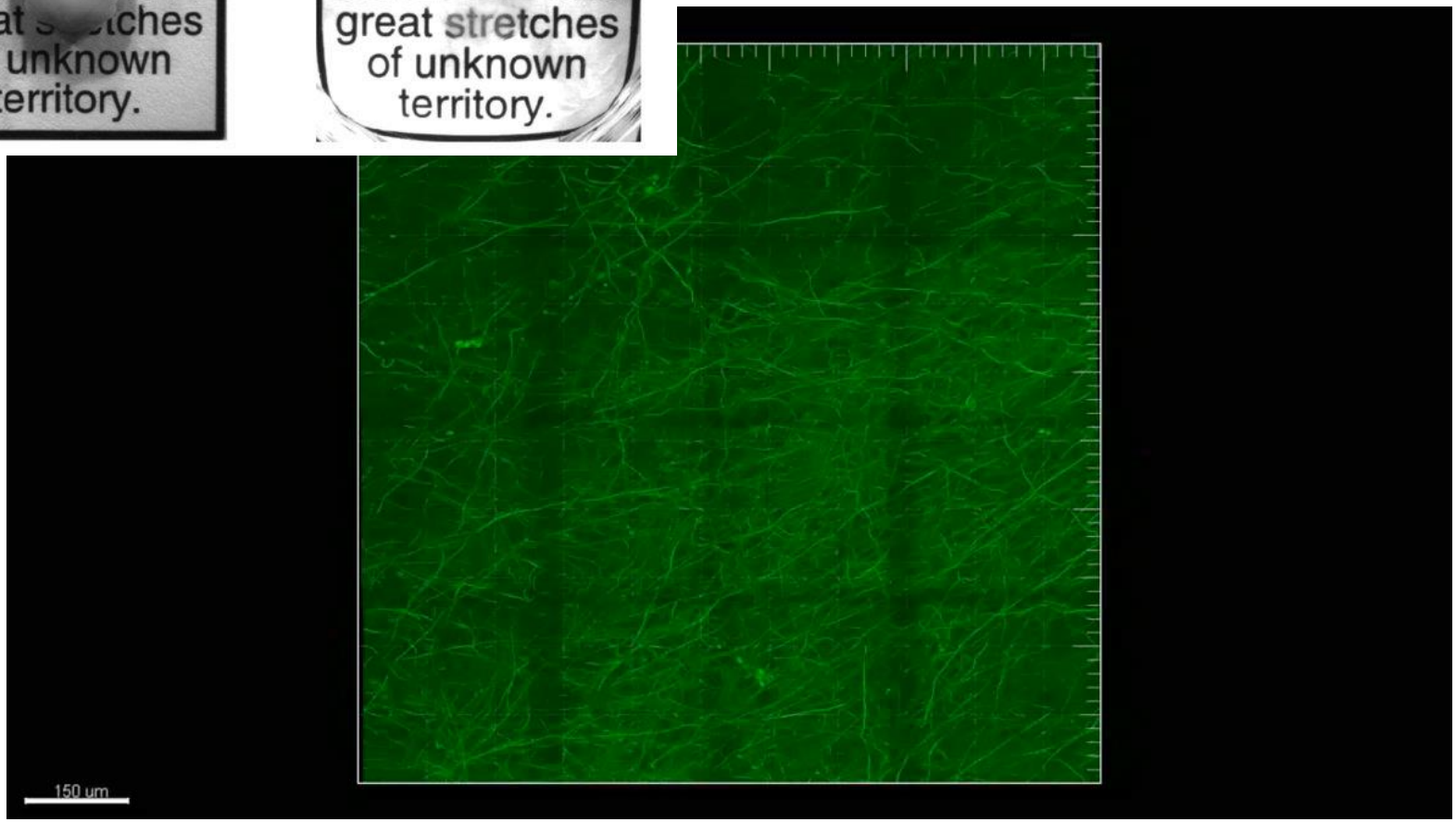
- *CLARITY -> Can image deep in organs without scattering from lipids!*





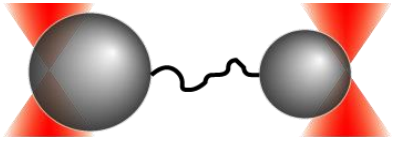
Brain as clear as a Jell-O

Chung, …, Deisseroth, Nature (2013)



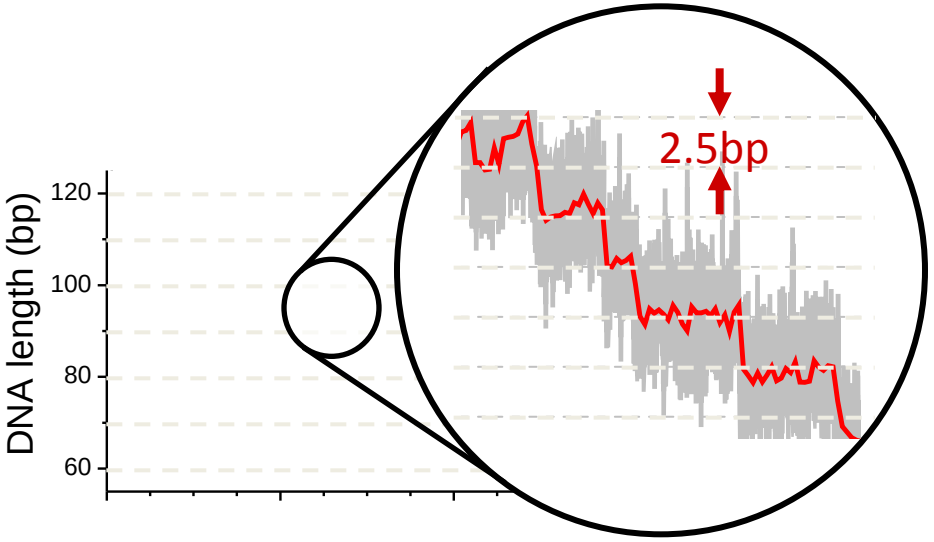
- ***Ultrahigh resolution optical trap***

1 basepair (bp) of DNA = 3.4Å



Abbondanzieri, …, Block, Nature (2005)

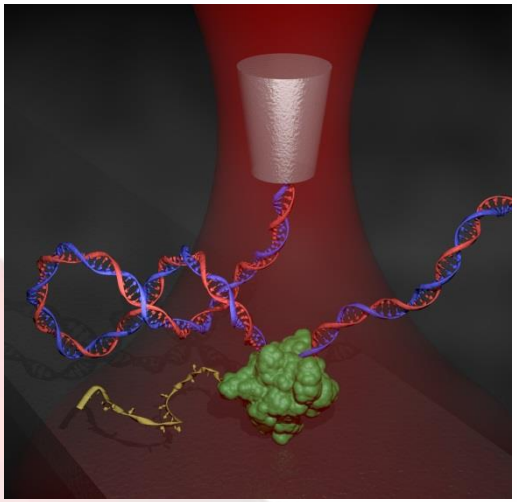
Life at the Ångström scale
Viral DNA packaging



Moffitt, Chemla, …, Bustamante, *Nature* (2009)

Optogenetics **Optomechanics** *Deep Imaging*

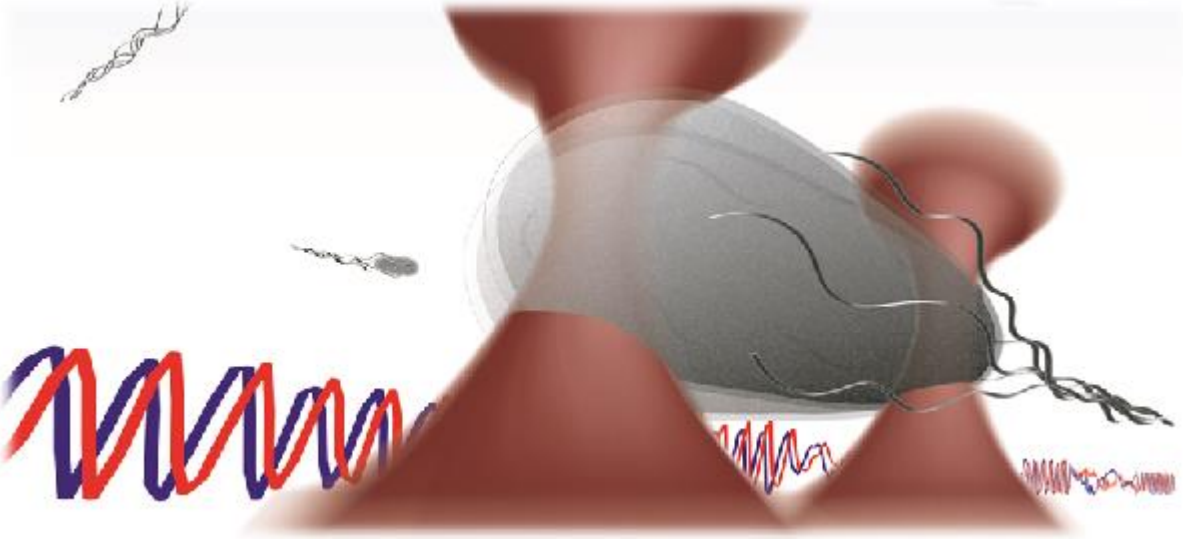
- *Ultrahigh resolution optical trap*
- ***Angular optical trap (torque)***



Transcription under torsion
Ma,... Wang, Science, 2013

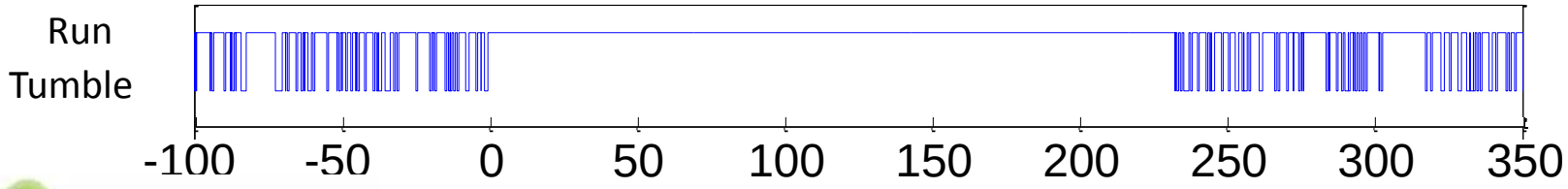
LaPorta, Wang, Phys. Rev. Lett., 2004
Deufel... Wang, Nature Methods, 2007

- *Ultrahigh resolution optical trap*
- *Angular optical trap (torque)*
- ***Bacterial treadmill***



"the bacterial treadmill"

How bacteria adapt to good news



Center for the Physics of Living Cells

- **Min, …, Chemla, *Nature Methods* (2009)**
- **Min, …, Chemla, *PNAS* (2012)**

- *Ultrahigh resolution optical trap*
- *Angular optical trap (torque)*
- *Bacterial treadmill*

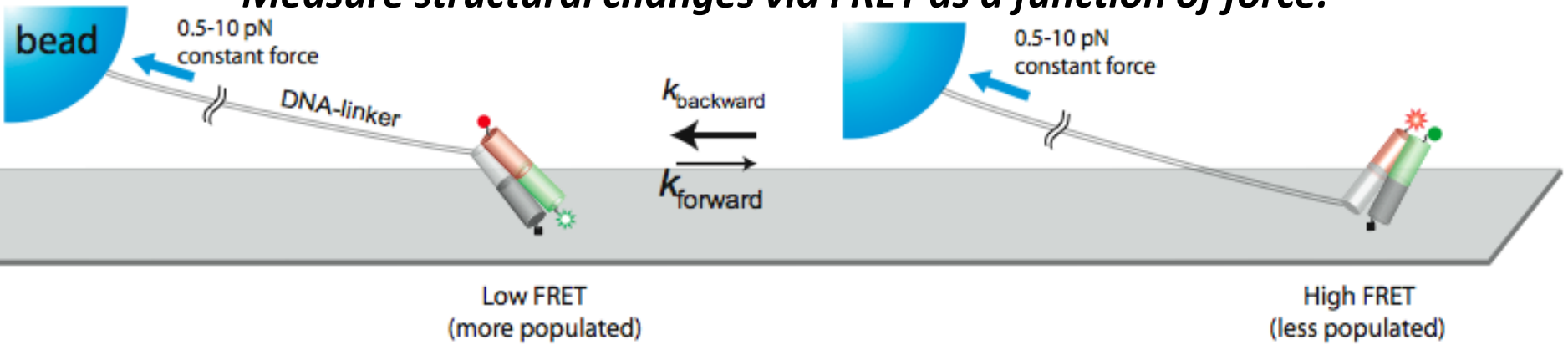
Gaps

- ***Blind to other properties of the molecule or cell***
- ***Very low throughput***

Opportunities

- Fluorescence + Force

Measure structural changes via FRET as a function of force.



- **Hohng, …, Ha, *Science* (2007)**
- **Grashoff, Hoffman, …, Ha, Schwartz, *Nature* (2010)**
- **Zhou, …, Ha, *Cell* (2011)**
- **Wang & Ha, *Science* (2013)**

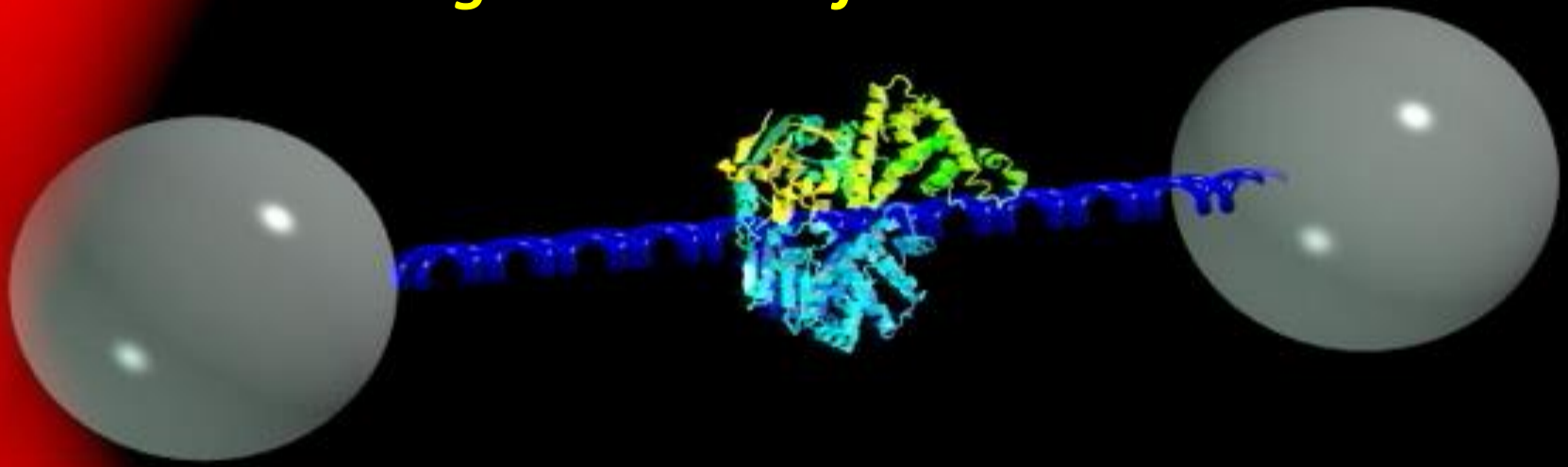
-> single molecular force sensing in vivo

Center for the Physics of Living Cells

Opportunities

- Fluorescence + Force

Ultrahigh resolution optical trap with single molecule fluorescence

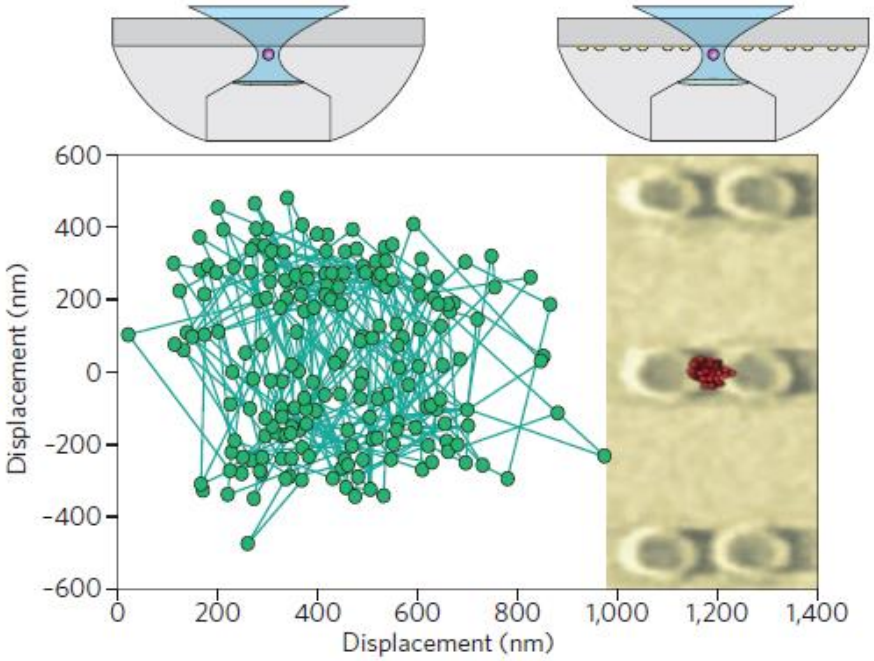


Comstock, Ha, Chemla, Nature Methods (2011)

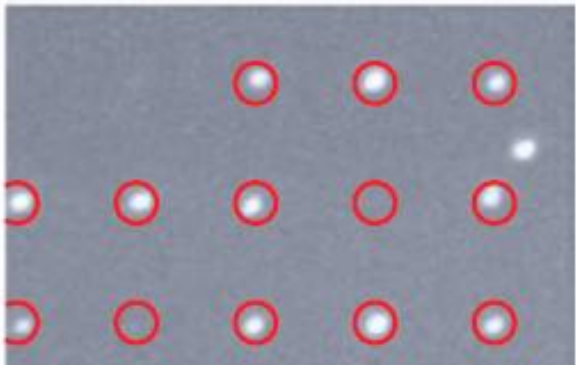
Center for the Physics of Living Cells

Opportunities

- Fluorescence + Force
- Plasmonic traps and holographic traps



Antenna trapping



Grigorenko, … , Zhang, *Nature Photonics* (2008)

Deep Imaging (with apologies to deep sequencing!)

-High information content and high throughput.

-Hypothesis-generating, not hypothesis-driven.

-Detect rare species in a heterogeneous population with confidence.

-Big data: automated acquisition and analysis.

Gaps

"…progress in unraveling basic sub-cellular interactions relies on inferences… from laborious, capital-intensive experiments, … Further developments in the visualization of single molecules will …advance the field of genomics and … personalized .. medicine."

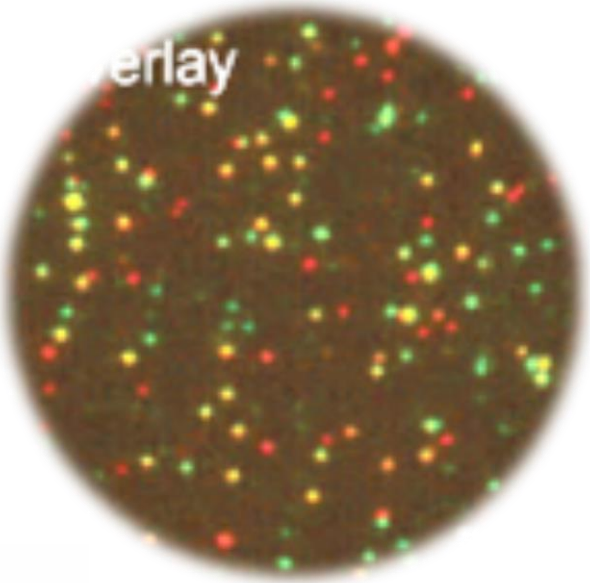
-from the NSF internal report

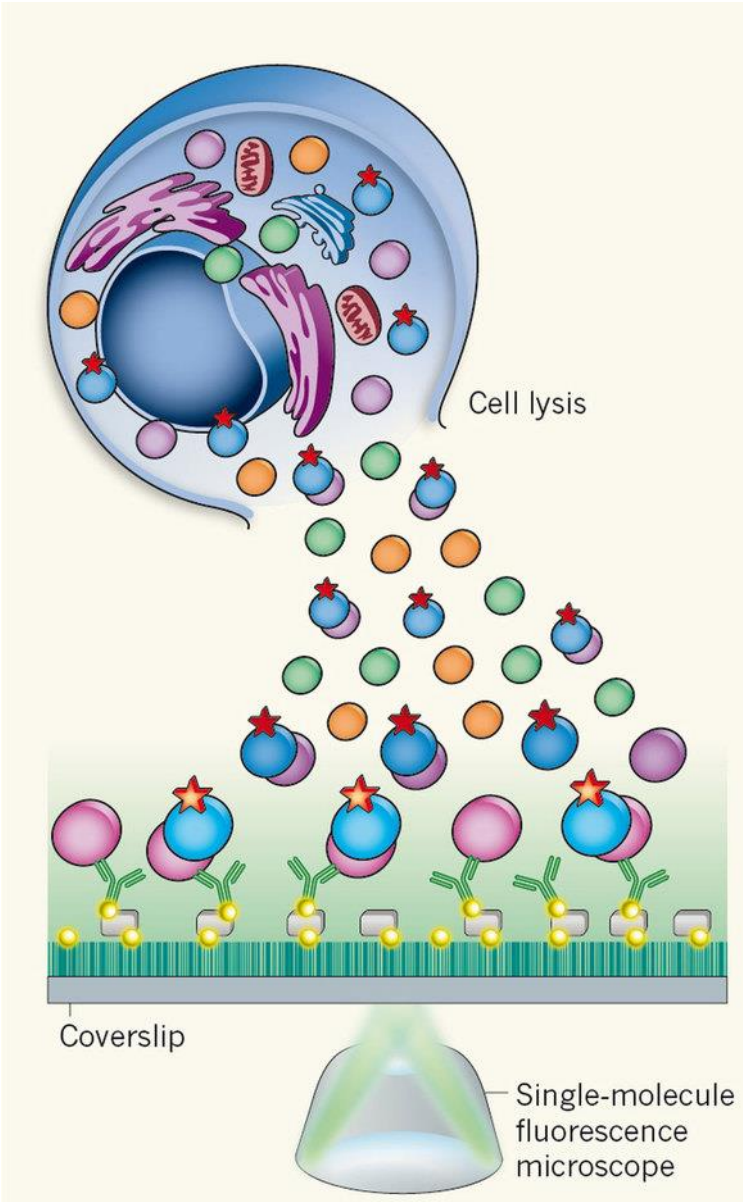
Opportunities

- *Single molecule/cell interactomics via SiMPull*

Probing Cellular Protein Complexes using Single-Molecule Pull-Down

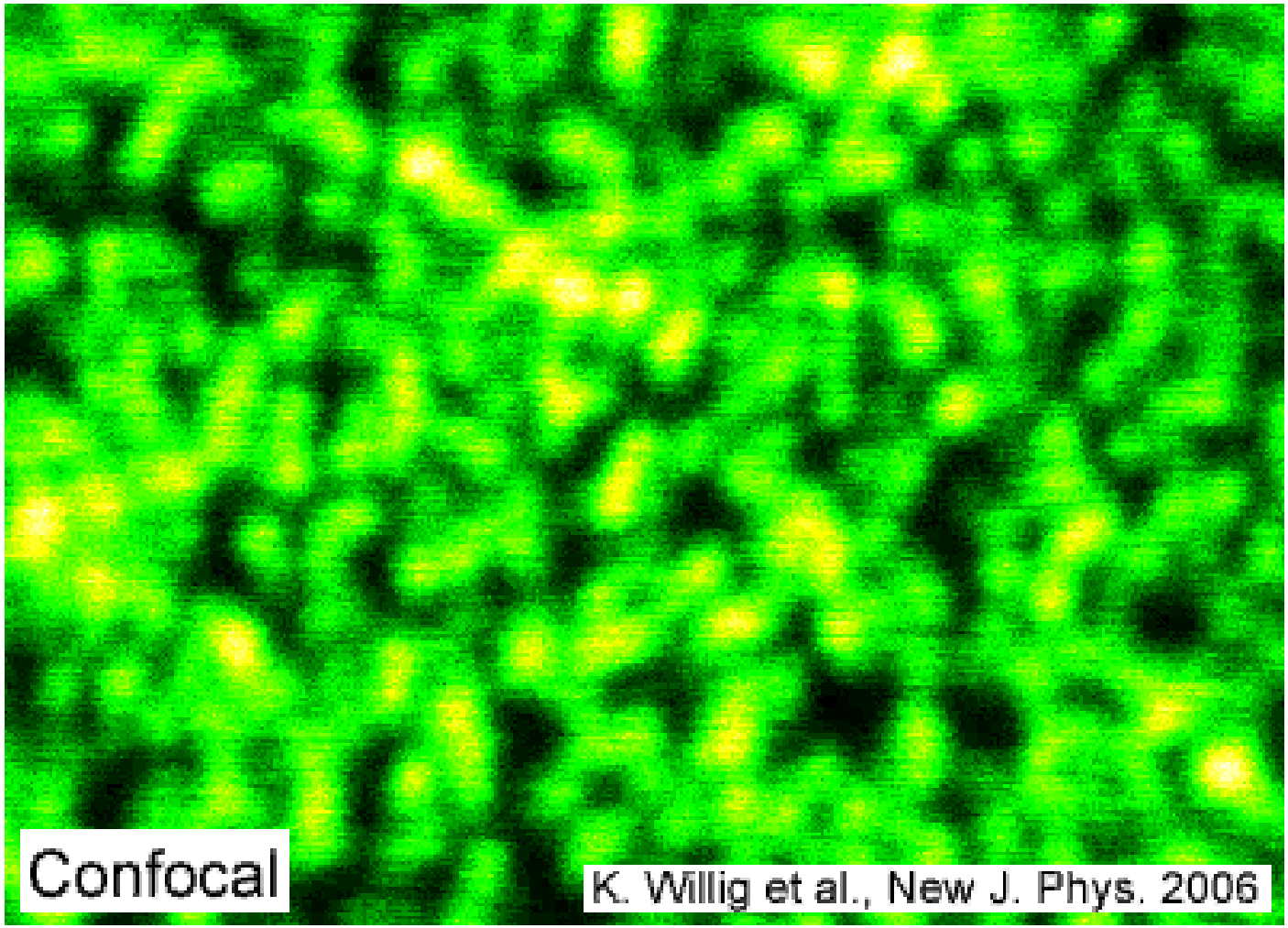
Jain, …, Ha, *Nature* (2011)



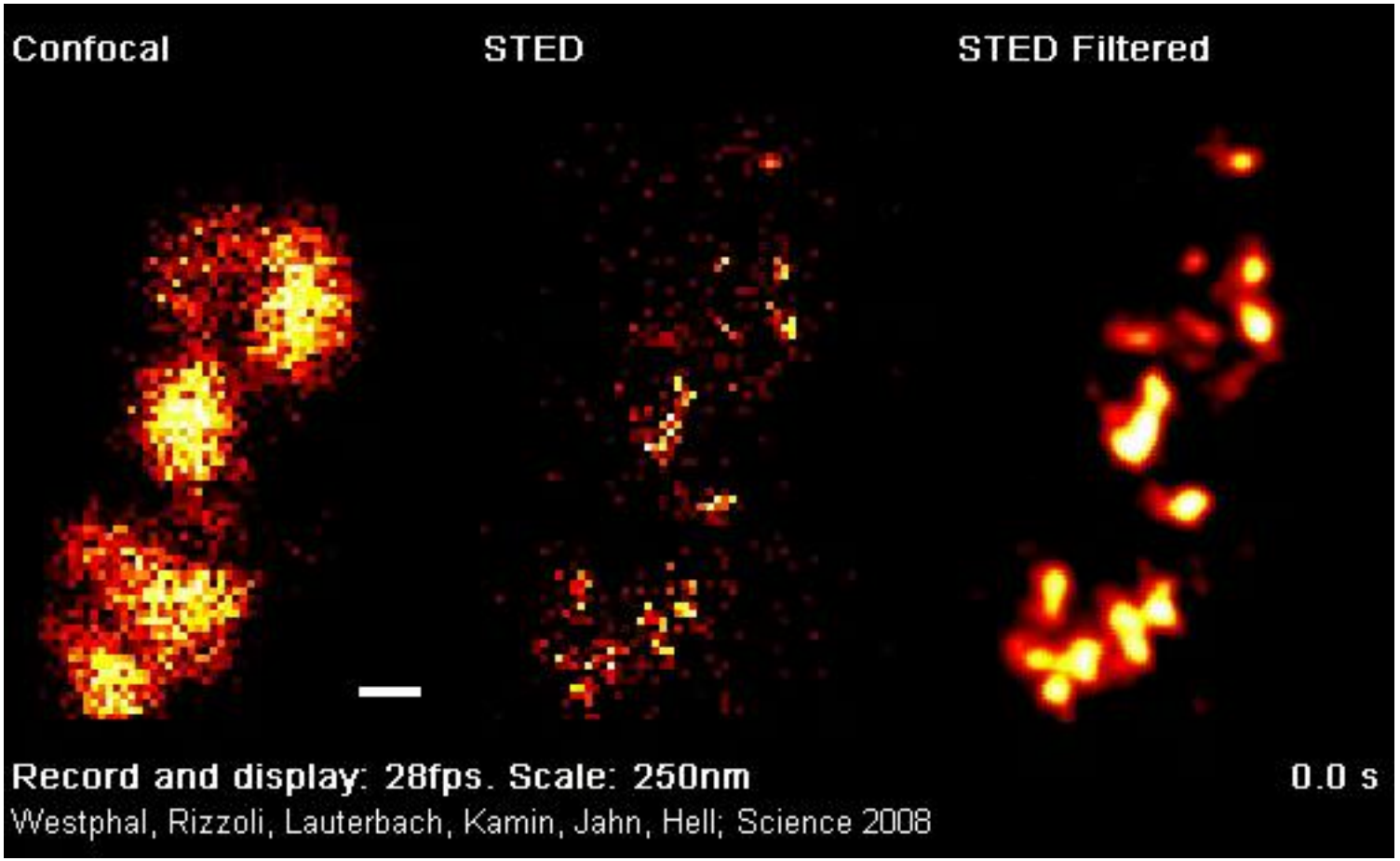


Center for the Physics of Living Cells

STED Example

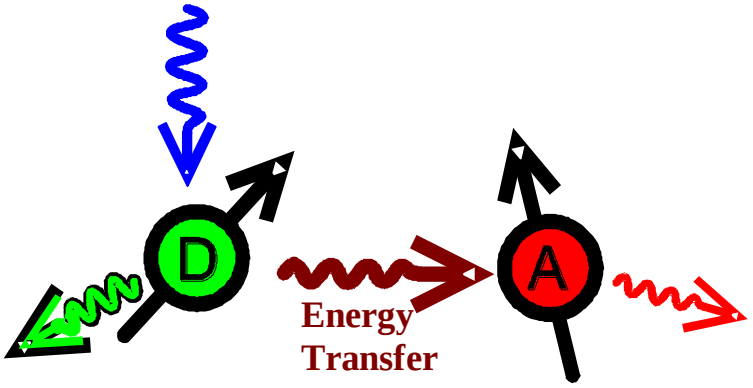


Video Rate STED Imaging

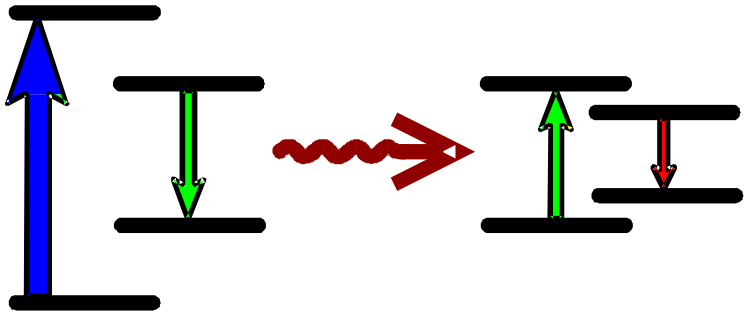


S. Hell group, Science (2008)

Fluorescence Resonance Energy Transfer (FRET)



DONOR **ACCEPTOR**

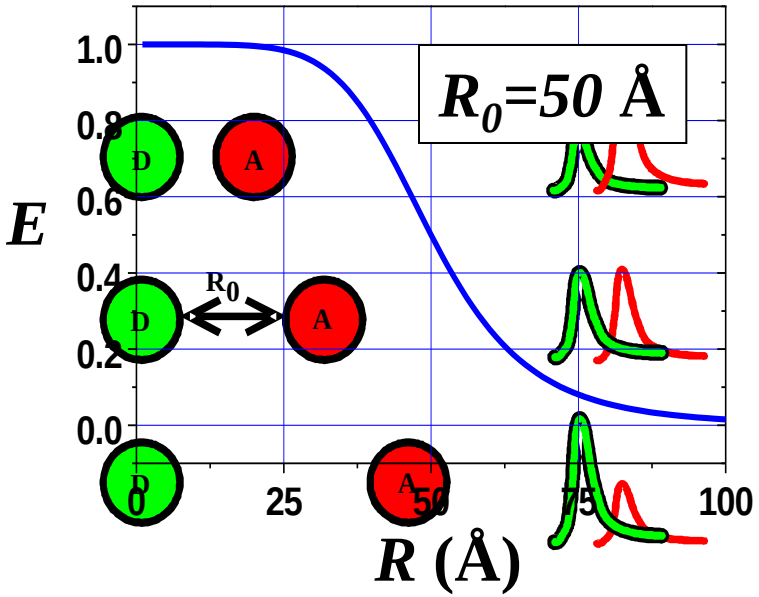


Energy transfer efficiency

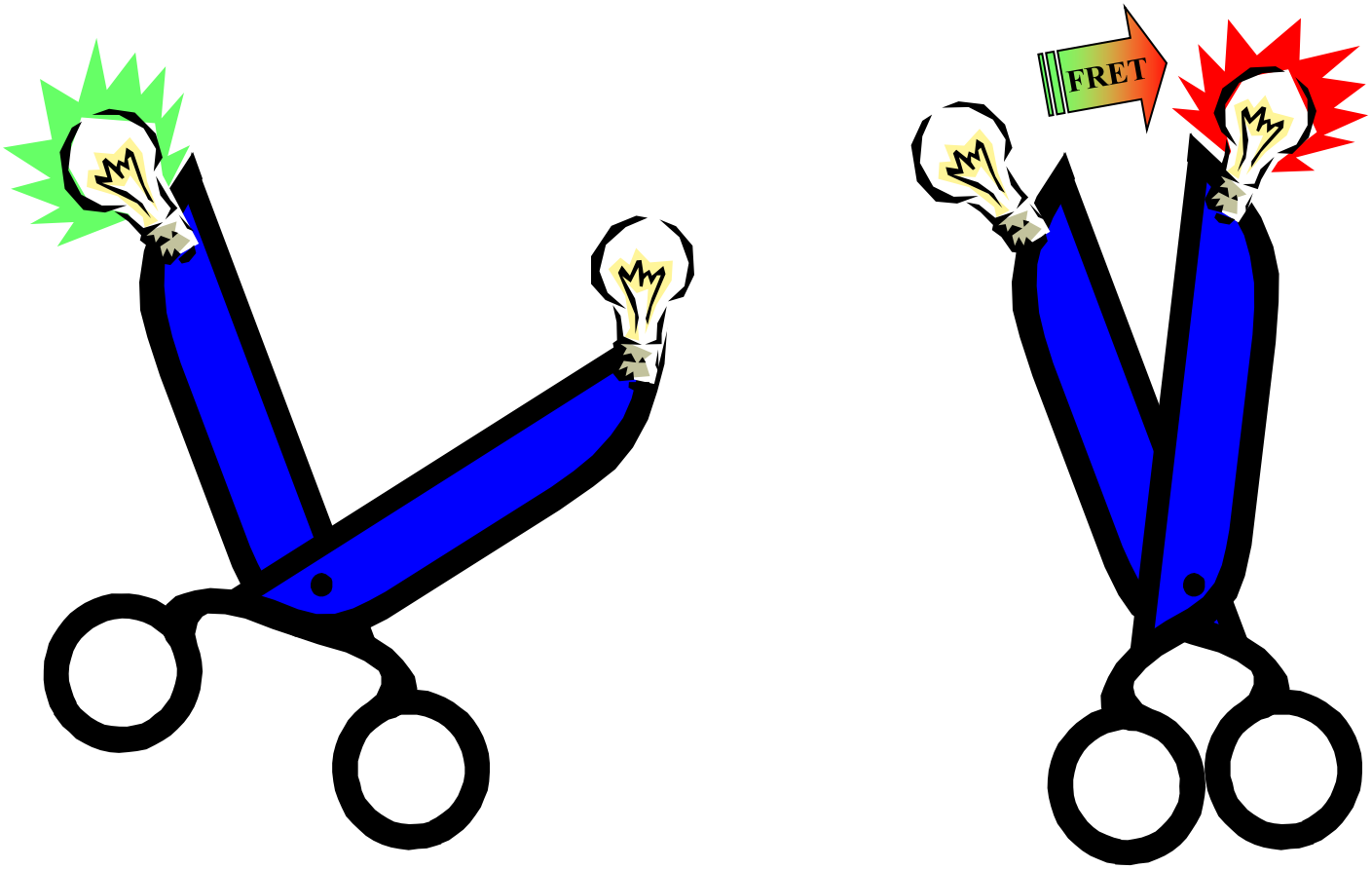
$$E = \frac{1}{1+(R/R_0)^6}$$

R0=50% transfer efficiency distance
3nm~7nm

☞ **"Spectroscopic Ruler"**



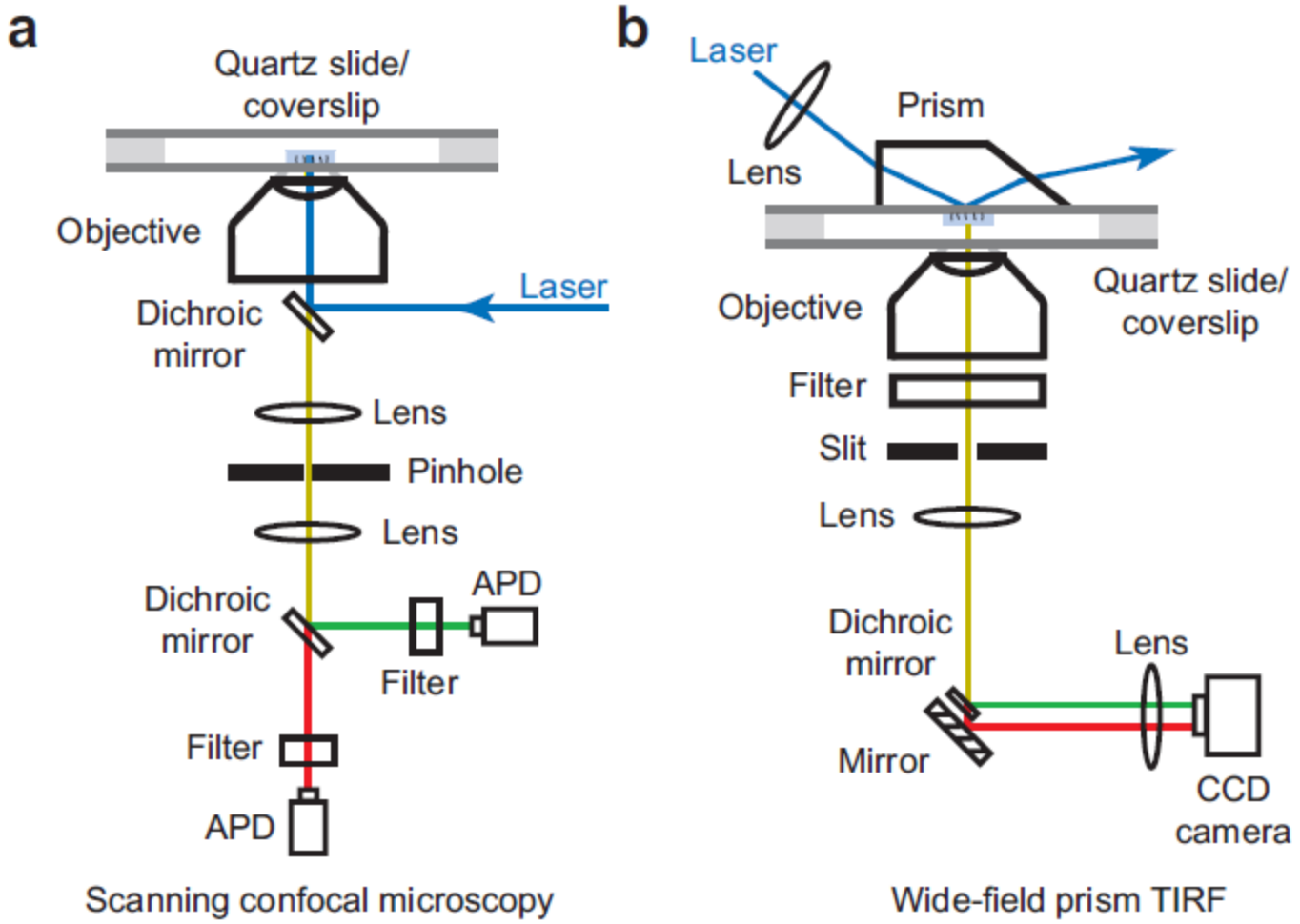
Fluorescence Resonance Energy Transfer (FRET): conformational changes of single biomolecules



Distance dependent interactions between green and red light bulbs can be used to deduce the shape of the scissors during the function.

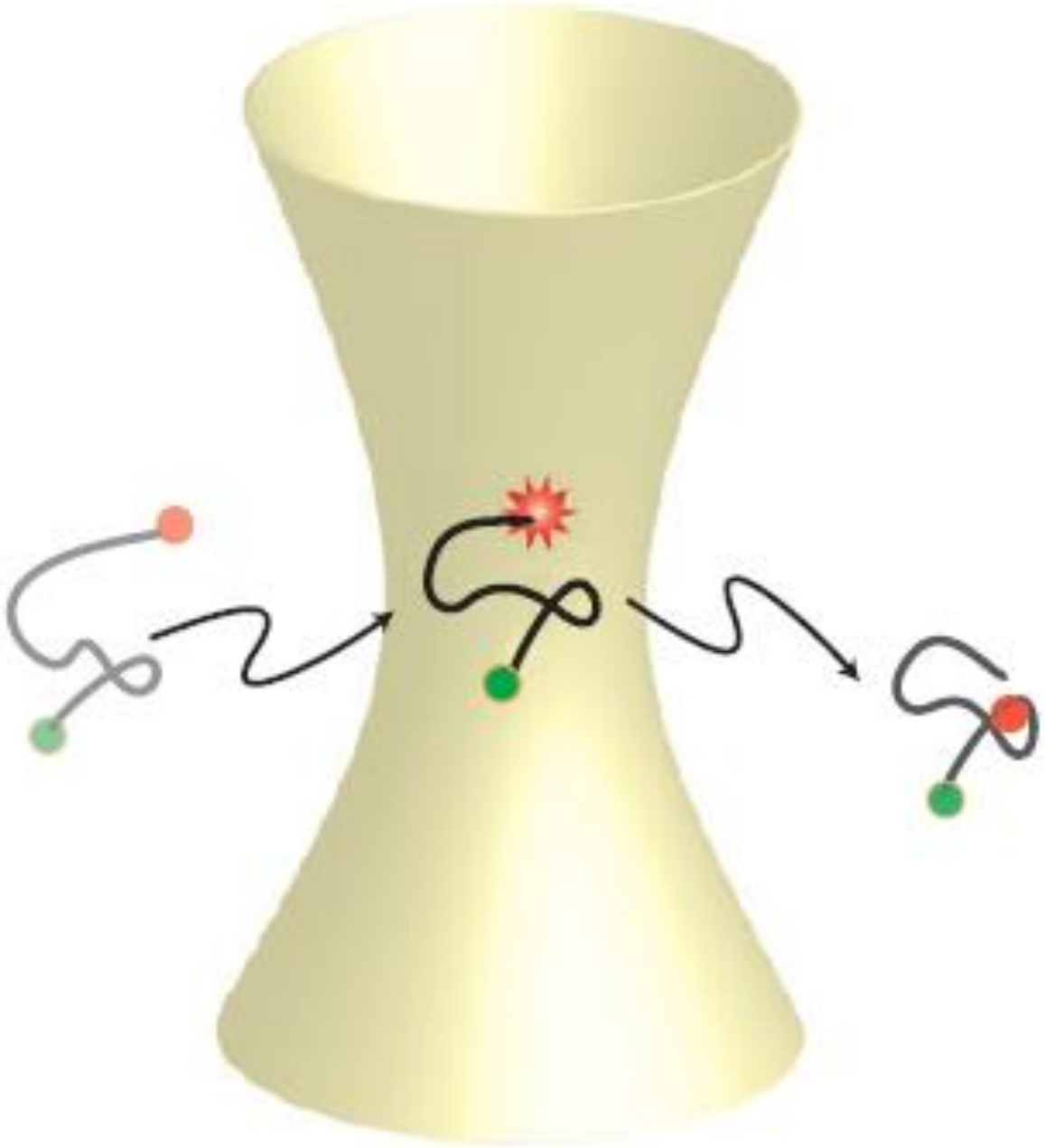
	sm-tracking	FRET
distance precision	> 1.5 nm	< 0.5 nm
photon budget	10,000 photons	100 photons
maximum distance	unlimited	< 10 nm
requirements for low drift, low vibration equipments	very high	low

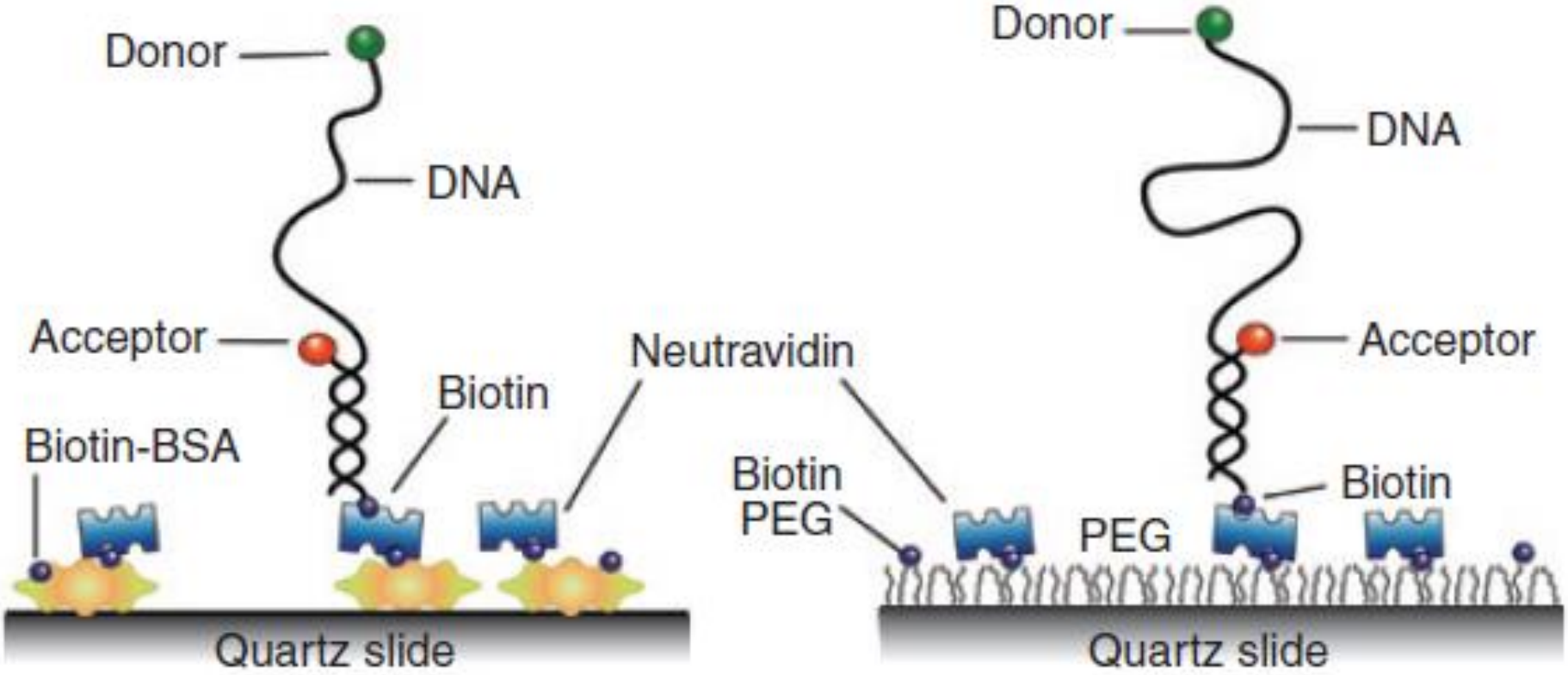
Two microscopic tools for smFRET



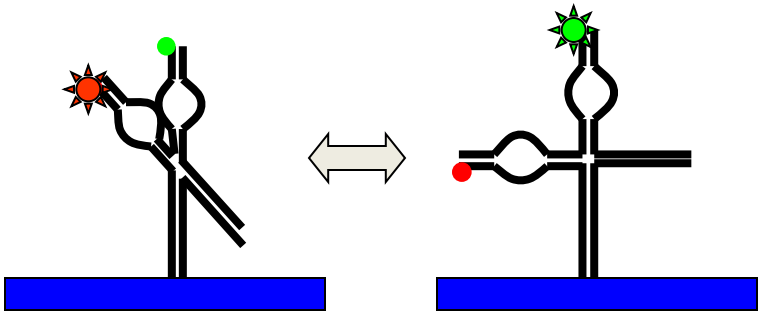
One molecule at a time
Higher time resolution (~100 us)
Complete commercial system exists.

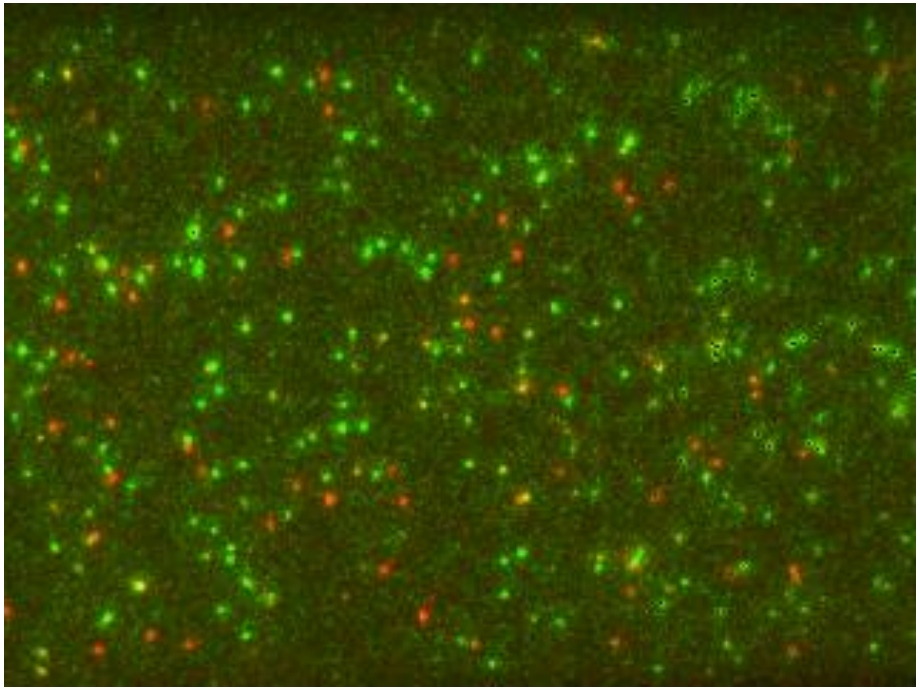
Hundreds of molecules in parallel
Lower time resolution (~10 ms)
Build your own for $80K

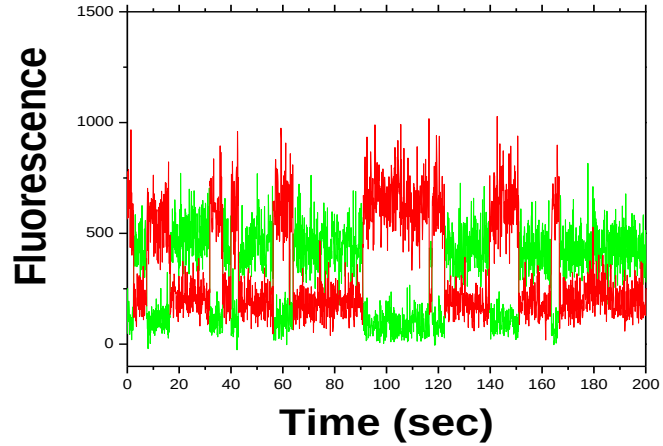


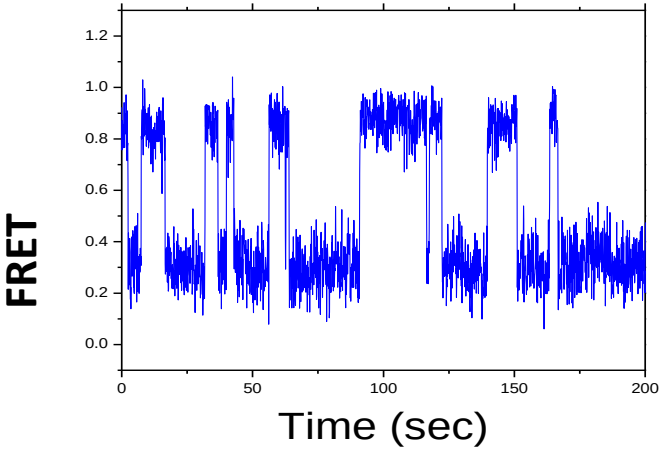

Donor
DNA
Acceptor
Biotin
Biotin-BSA
Neutravidin
Quartz slide
Donor
DNA
Acceptor
Biotin
Biotin
PEG
PEG
Quartz slide

FRET Movie (RNA folding)
10 frames per second



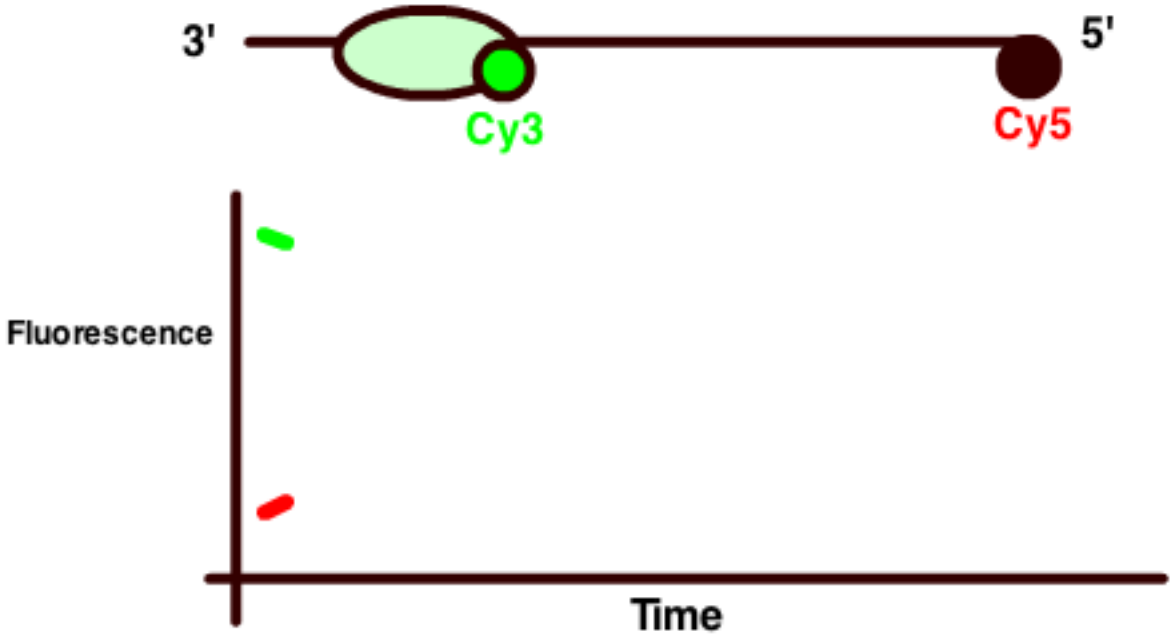


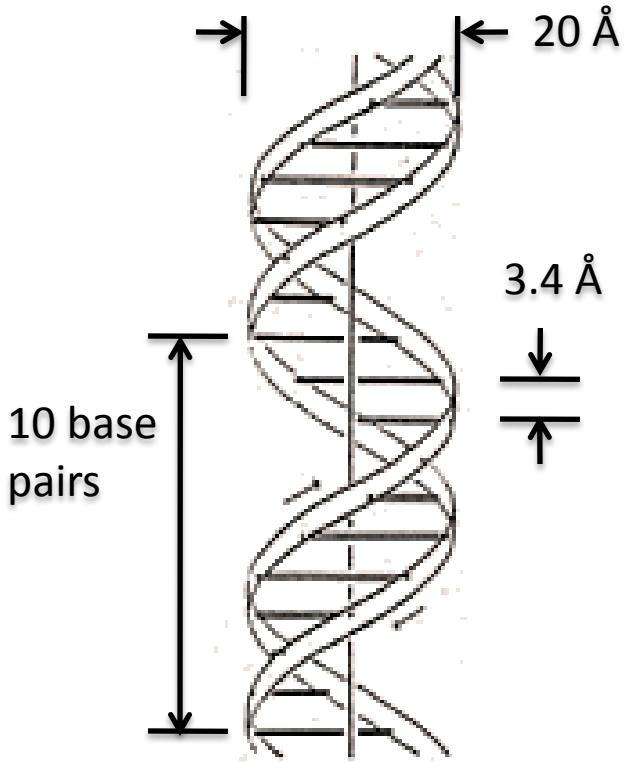




How the kitten uses her paws to pop the balloon

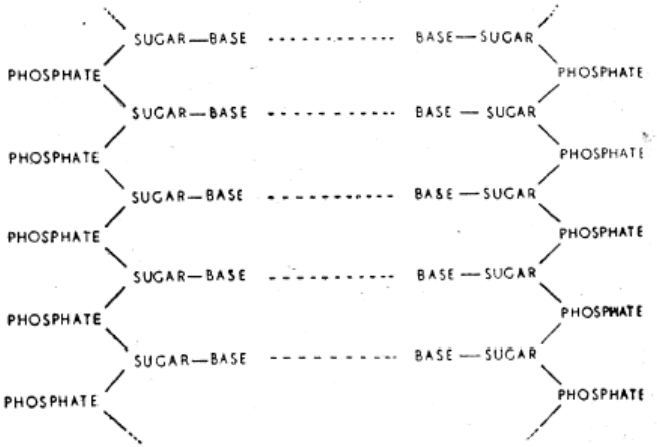
Visualizing ssDNA translocation of a helicase via FRET





"It has not escaped our notice that the specific pairing … suggests a possible copying mechanism for the genetic material."

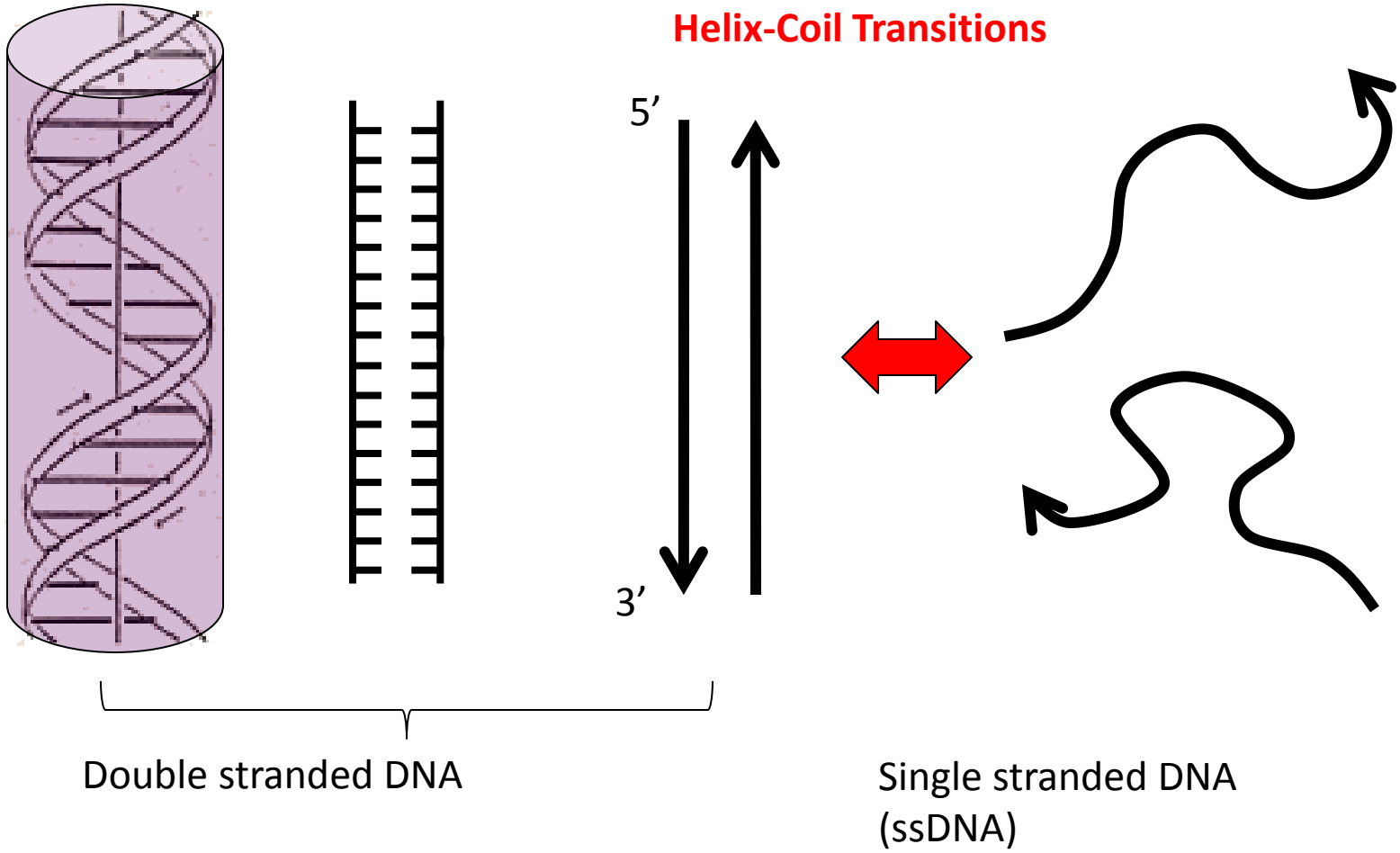
-Watson & Crick (Nature, April 25th, 1953)



"Even if it is correct, … much remains to be discovered before the picture of gene duplication can be described in detail. … What makes the pair of chains unwind and separate? …

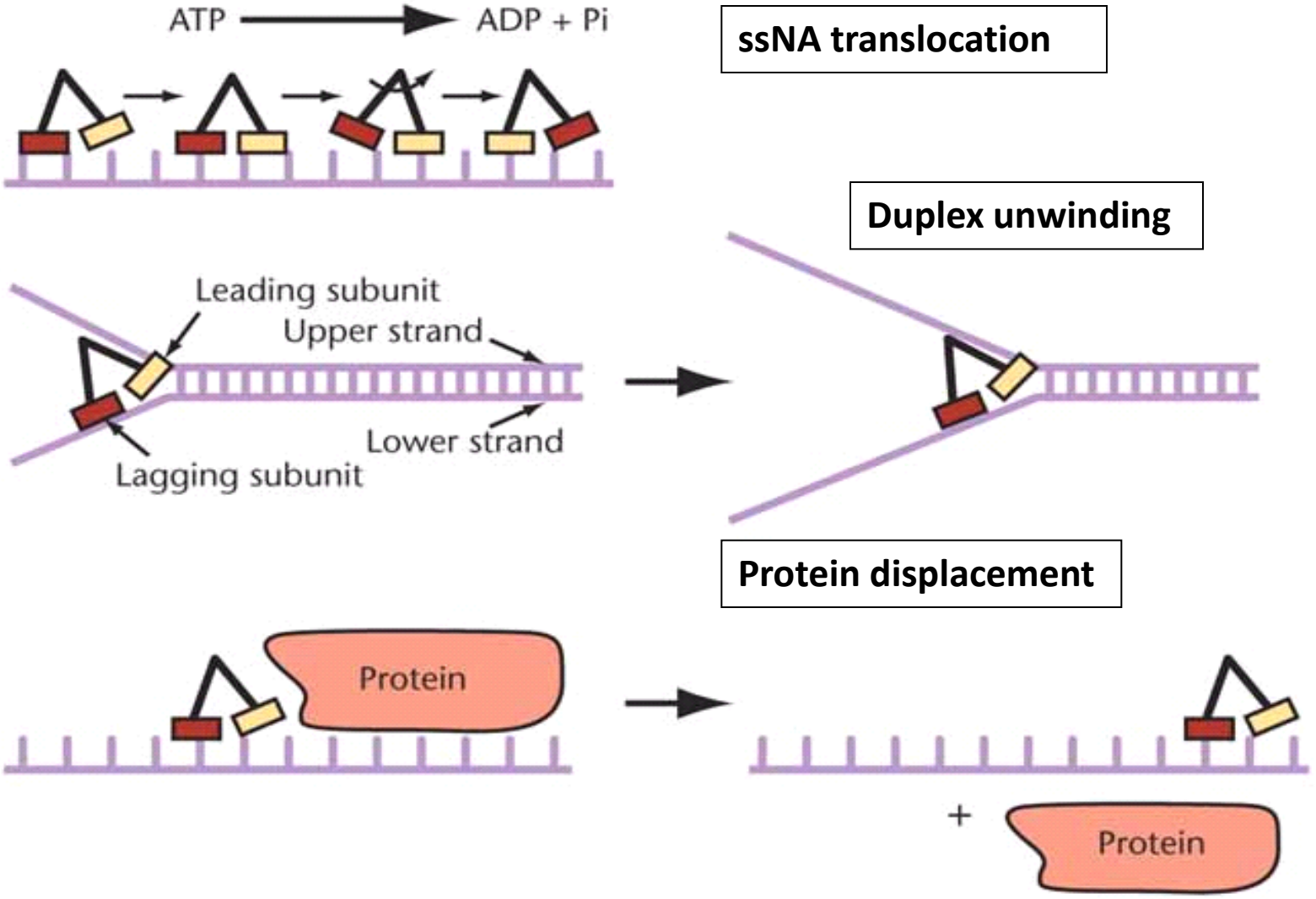
-Watson & Crick (Nature, May 30th, 1953)

Helicase!



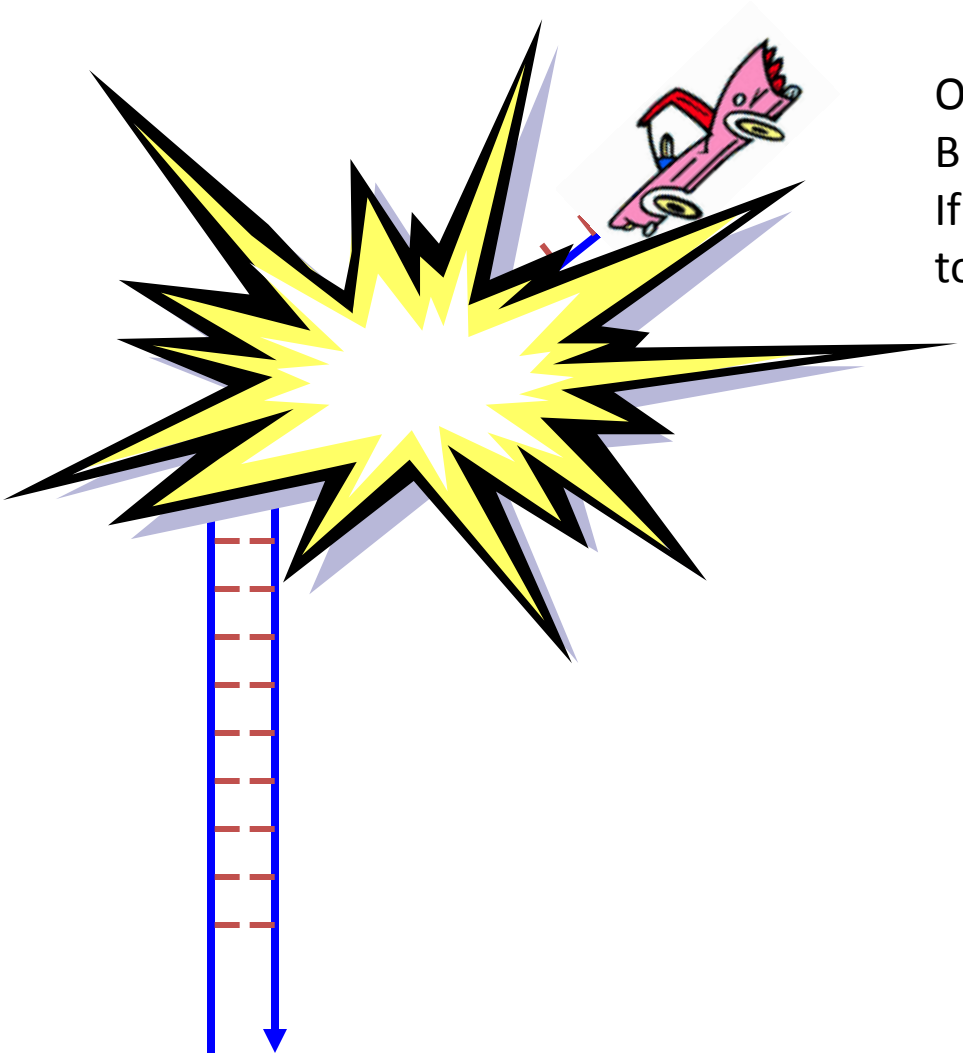
DNA in abstraction

Many faces of helicase



Von Hippel, Nature Struct. & Molec. Biol. (2004)

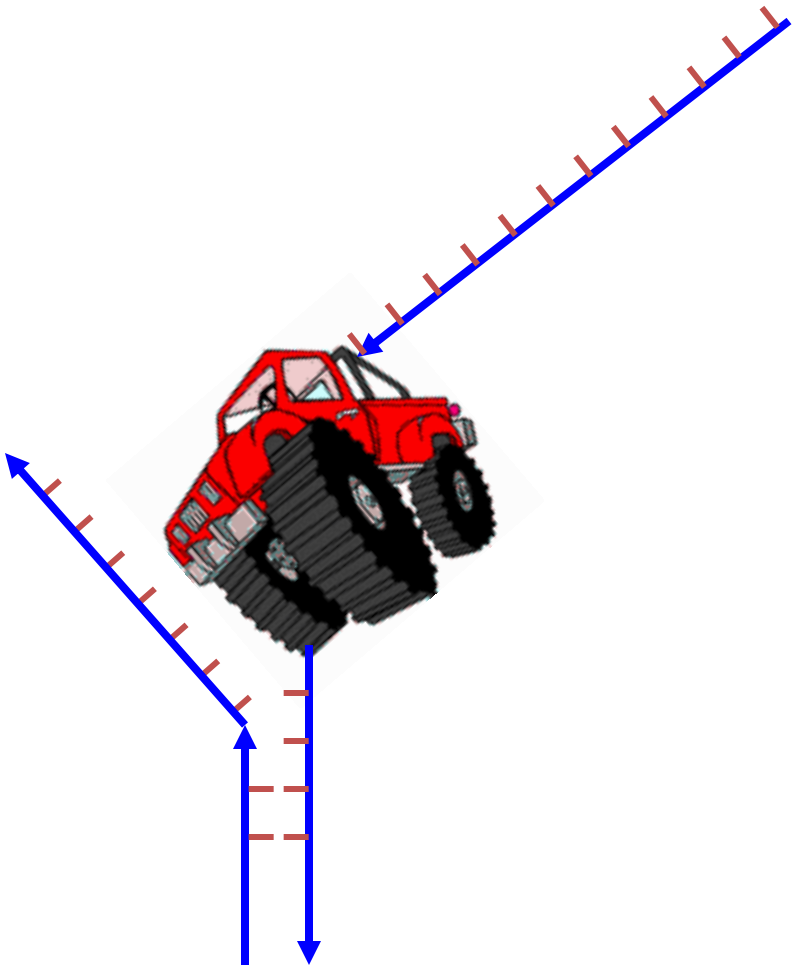
Previous findings (*E. coli* Rep helicase)



One protein can move on DNA
But cannot unwind DNA
If two can combine, they can unwind DNA together.

[Cheng et al, JMB, 2001]
[Ha et al, Nature, 2002]

Previous findings (*E. coli* Rep helicase)



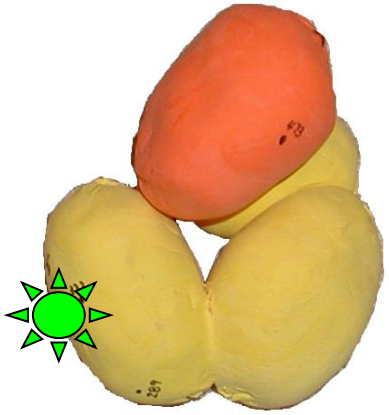
One protein can move on DNA
But cannot unwind DNA
If two can combine, they can unwind DNA together.

[Cheng et al, JMB, 2001]
[Ha et al, Nature, 2002]

Monster Truck Mechanism

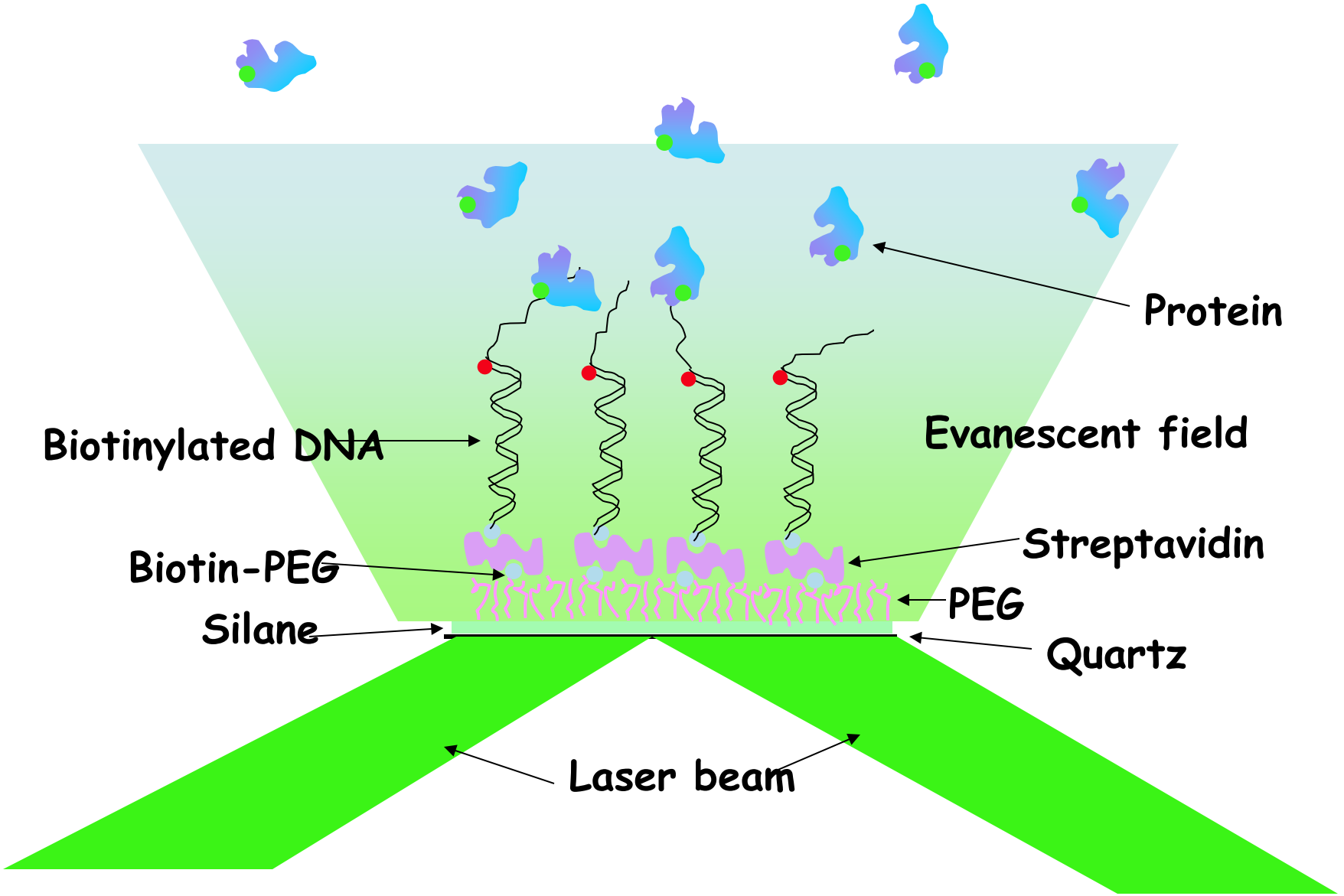
Cys-light Rep helicase

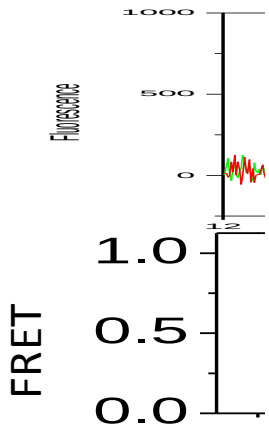
- 5 native cysteines replaced
- Unique cysteines introduced and labeled with Cy3-maleimide (>90% efficiency)
- Retains *in vivo* and *in vitro* activities

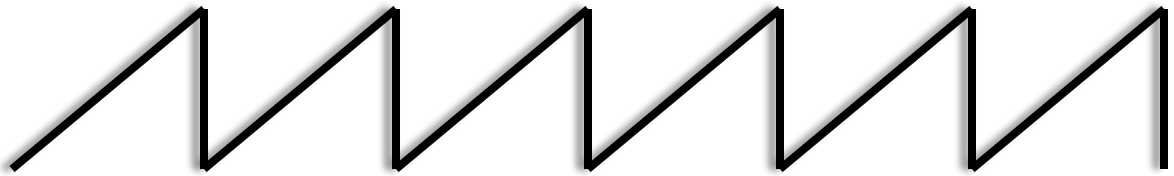


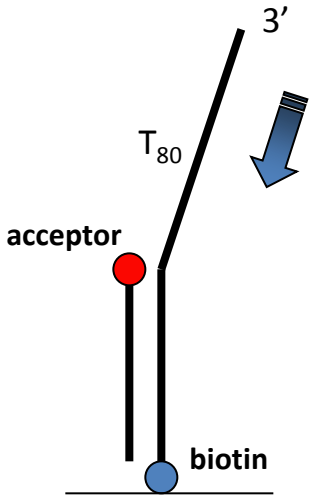
[Rasnik et al, JMB, 2004]

On the slide


Protein
Biotinylated DNA
Evanescent field
Streptavidin
Biotin-PEG
PEG
Silane
Quartz
Laser beam



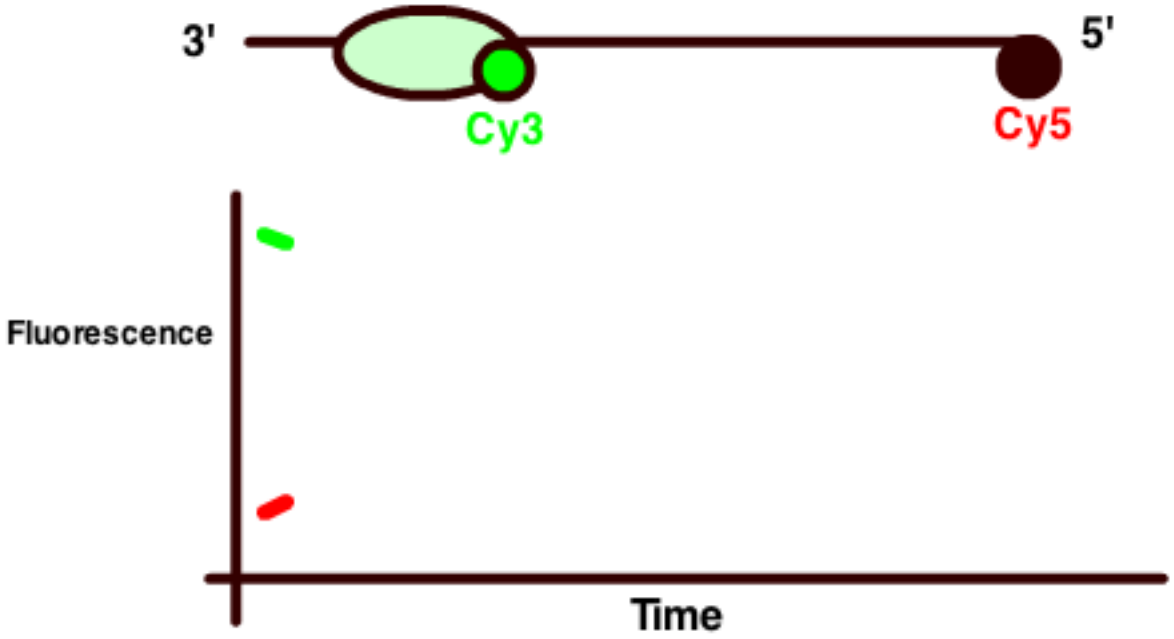




Sawtooth!

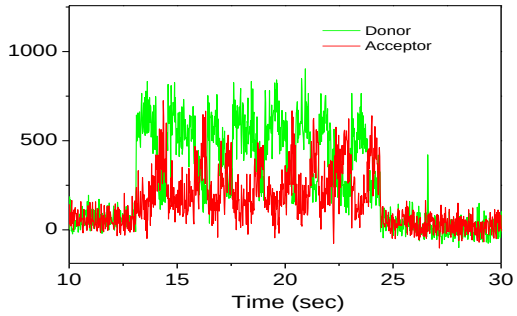
Myong, Rasnik, Joo, Lohman & Ha, Nature 2005

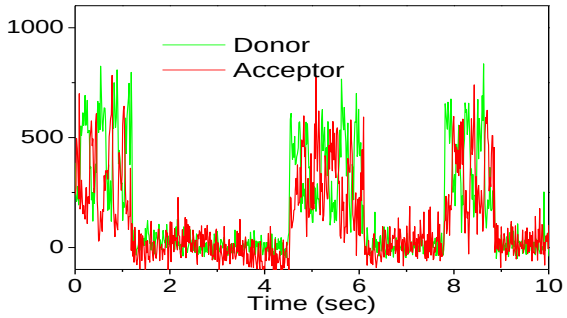
Visualizing ssDNA translocation of a helicase via FRET



Sawtooth pattern is due to a single Rep monomer

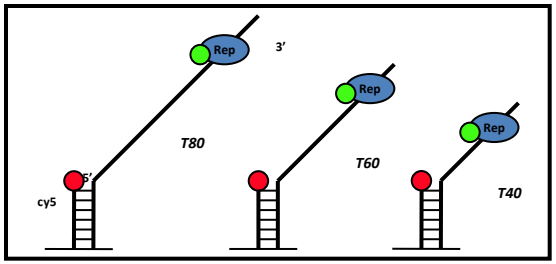
- Fluorescent labeling yield ~ 90%
- Isolated events at ~100 pM protein

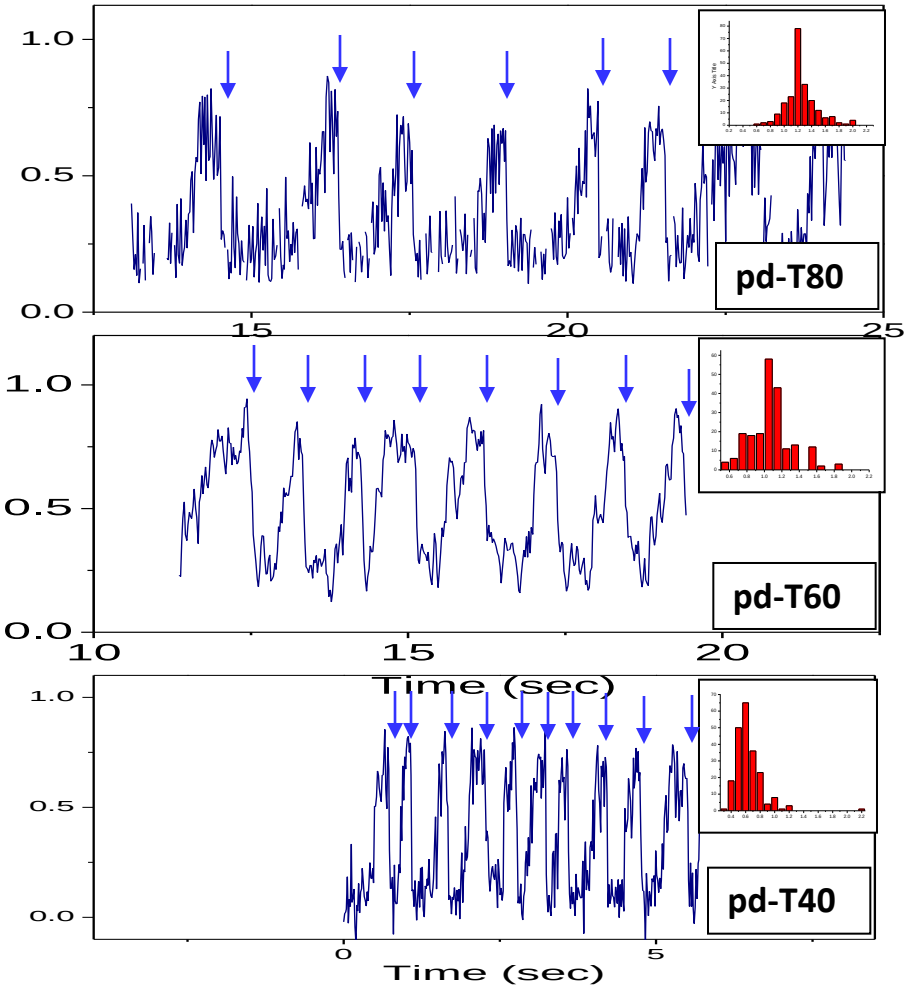




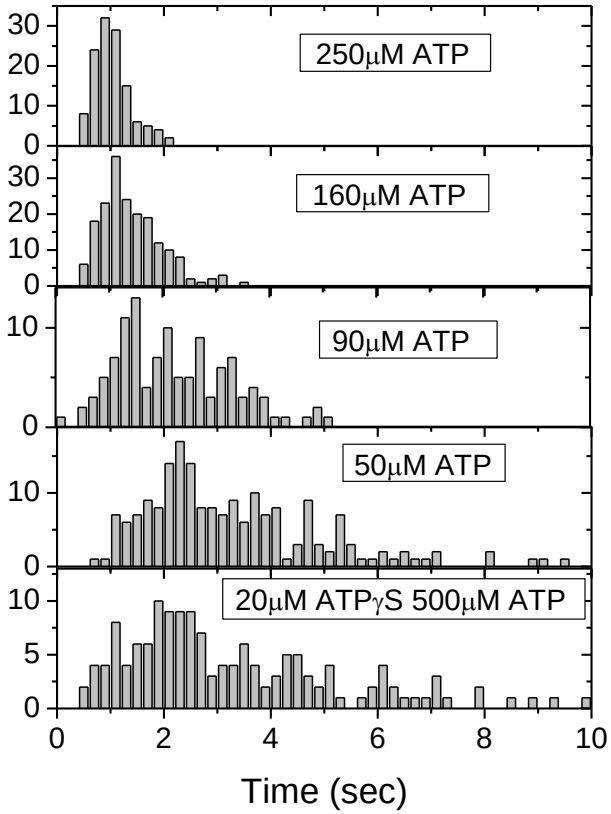
- Sawtooth pattern persists even after flow removal of free proteins in solution

Tail length dependence



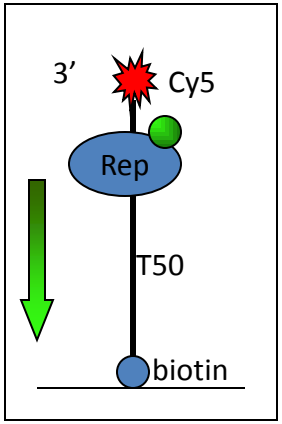


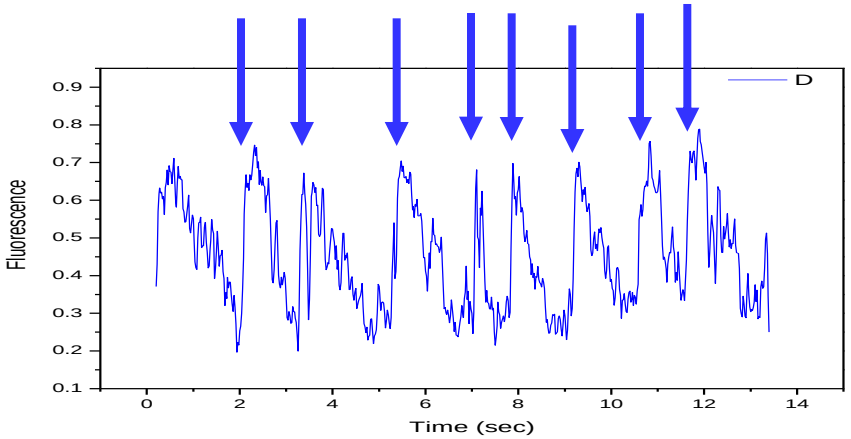
Effect of ATP concentration and ATPγS

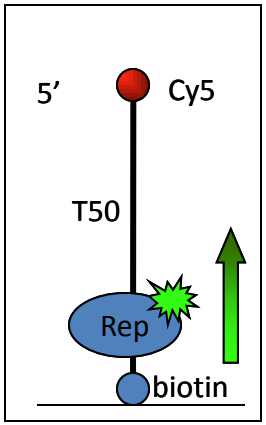


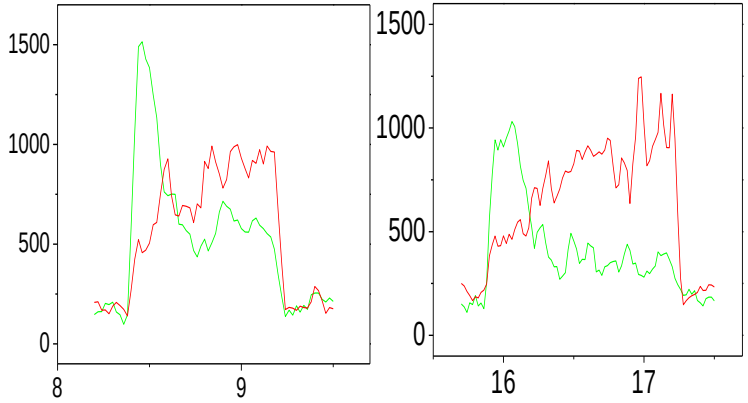
Slow phase is due to ATP powered ssDNA translocation!

Observation of sawtooth pattern requires a physical blockade







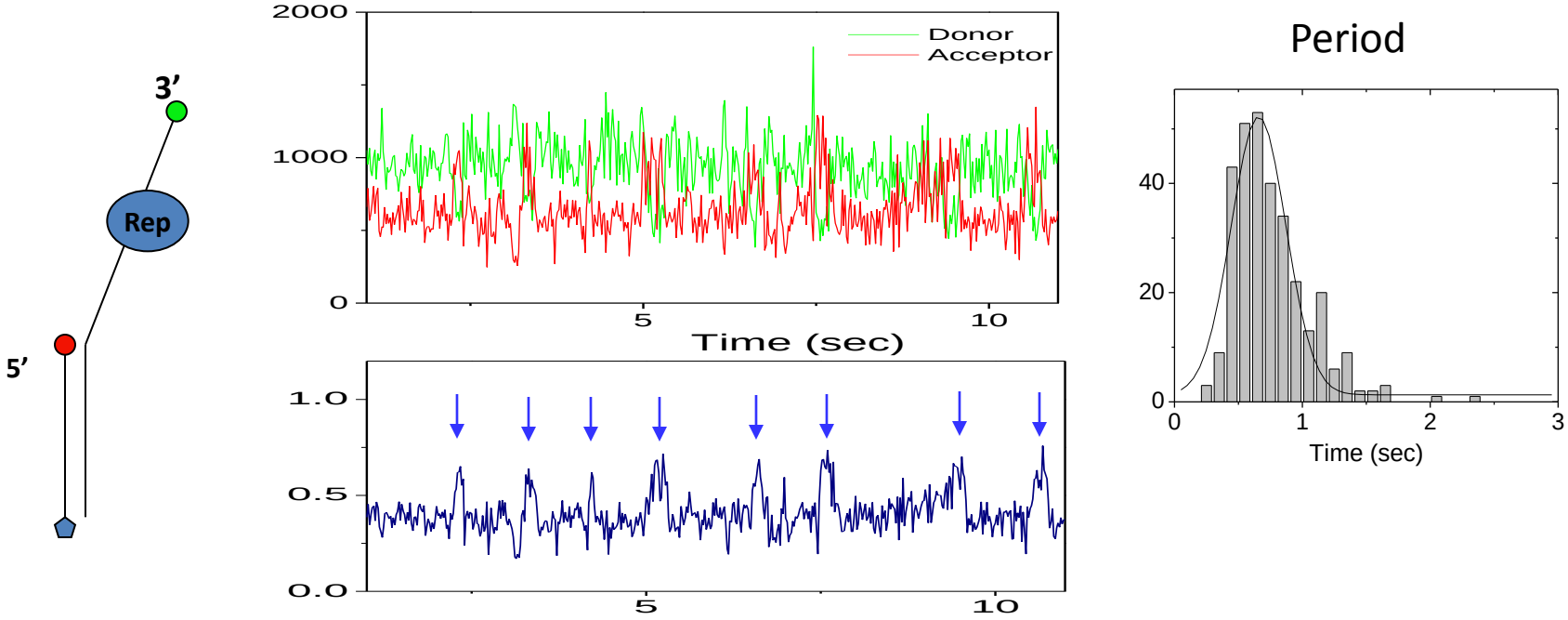


Physical Mechanism of sawtooth

- Dissociation & rebinding?
  - Unlikely. k_{on} to ssDNA 20 times slower than diffusion limited reaction [Cheng et al, 2001]
- There has to be another DNA binding site
  - Case I. Maintain a contact with 3' end of ssDNA during translocation? Unlikely.

Case II. Blockade triggers contact with 3' end?

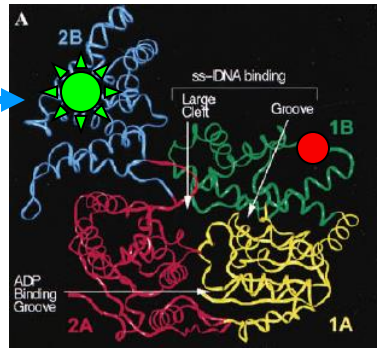
Blockade-induced contact of 3’ end



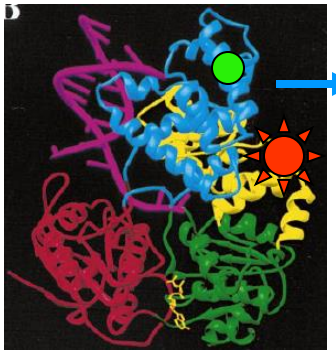
How does the protein sense the blockade?

2B domain closes near junction

2B domain



open

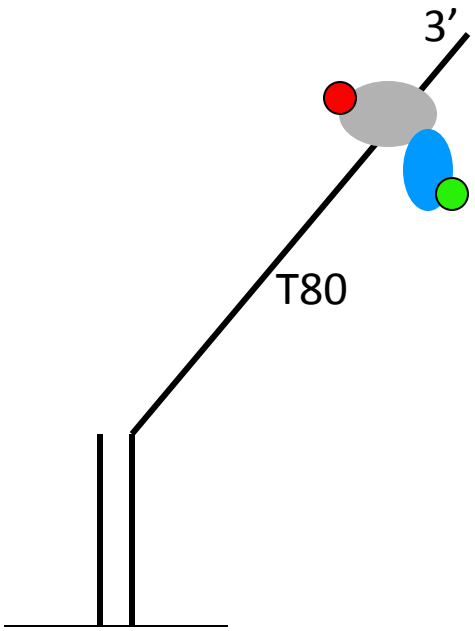


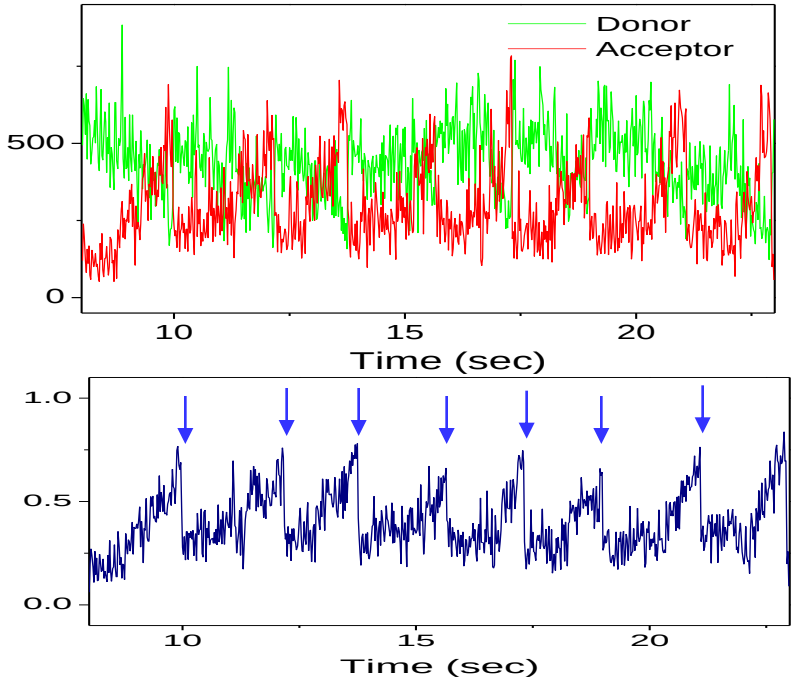
closed

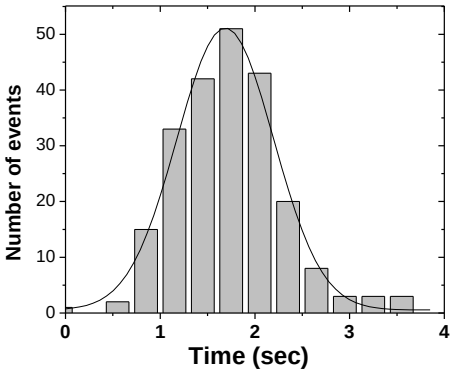
2B domain

Rep [Korolev et al, 1997]

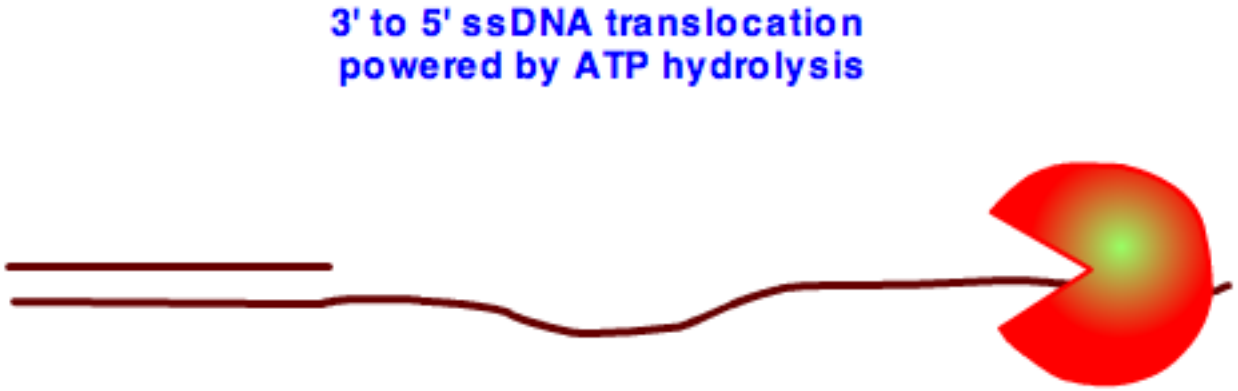
PcrA [Velankar et al, 1999]

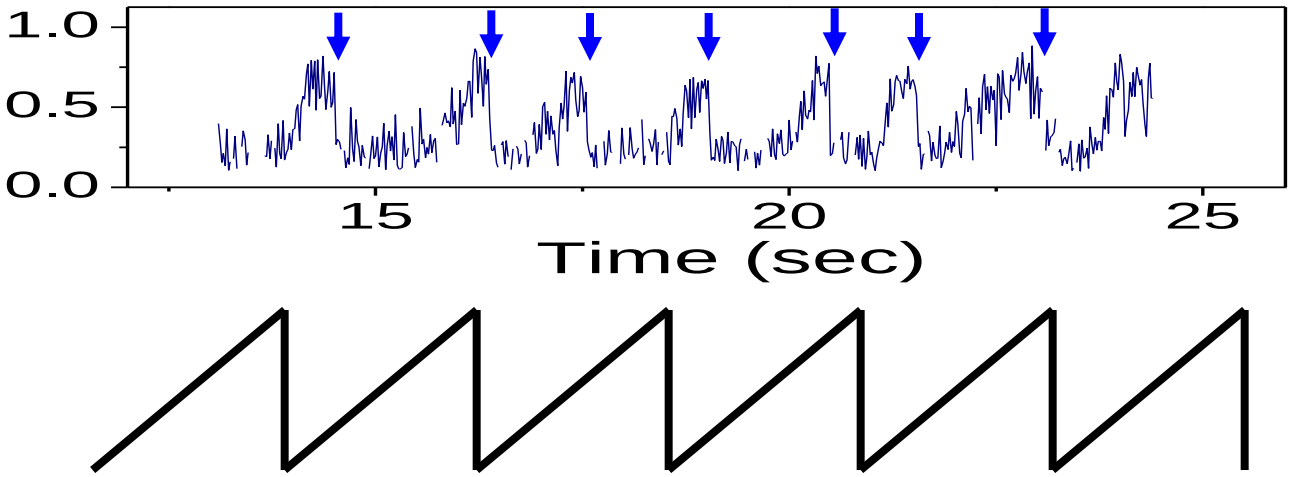






Proposed mechanism



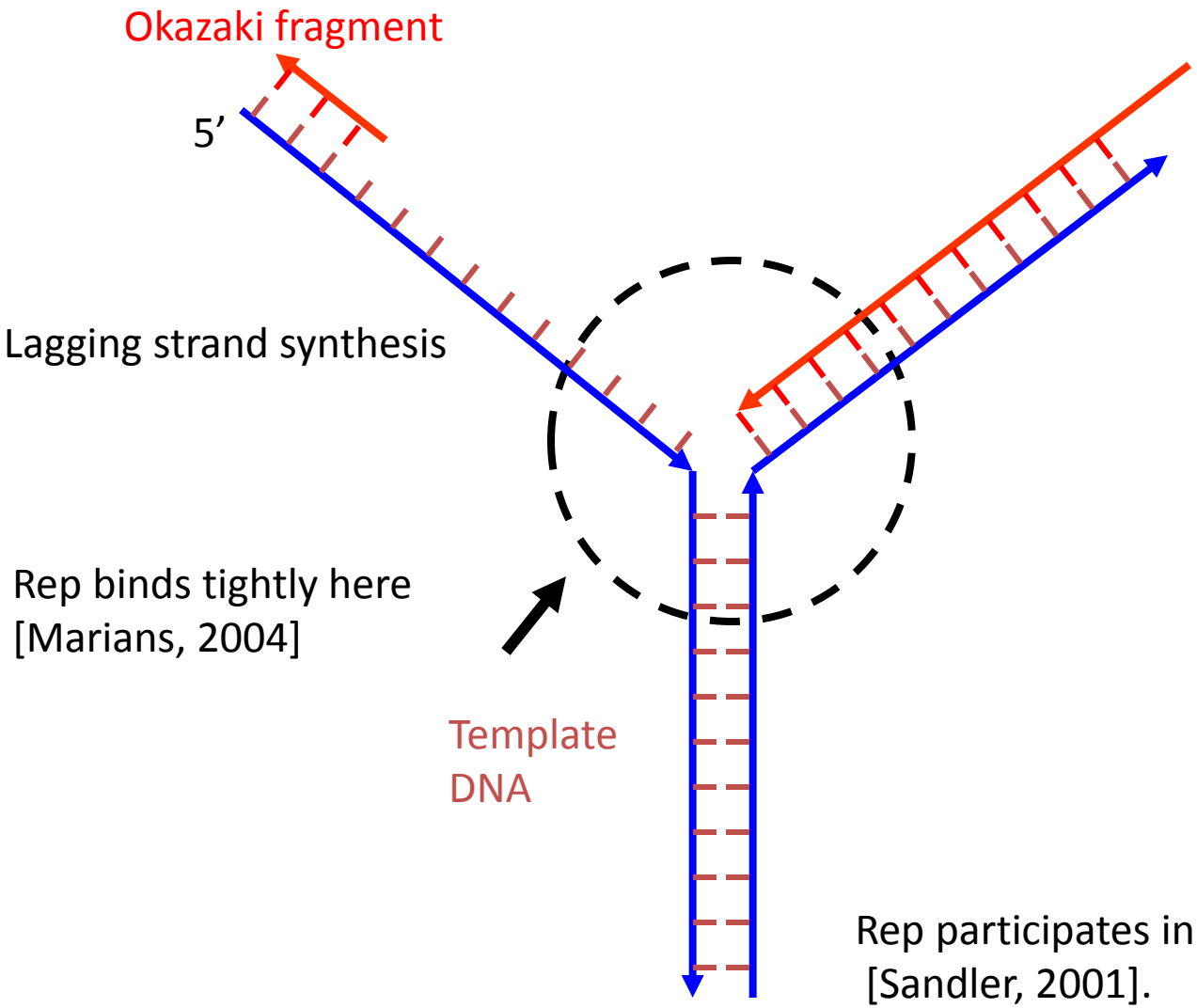


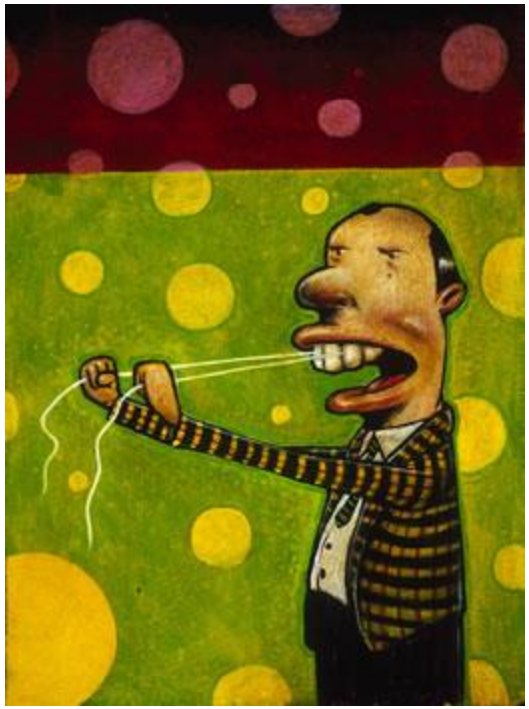
Molecular Sisyphus!

Sisyphus metaphor is resoundingly unsatisfying.

Is there any meaning in this suffering?

Stalled replication fork

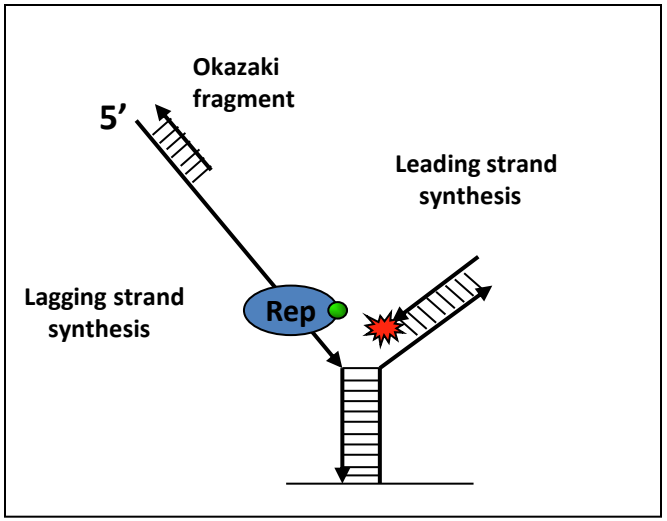


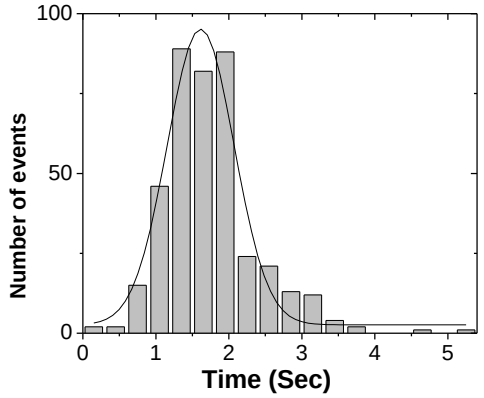


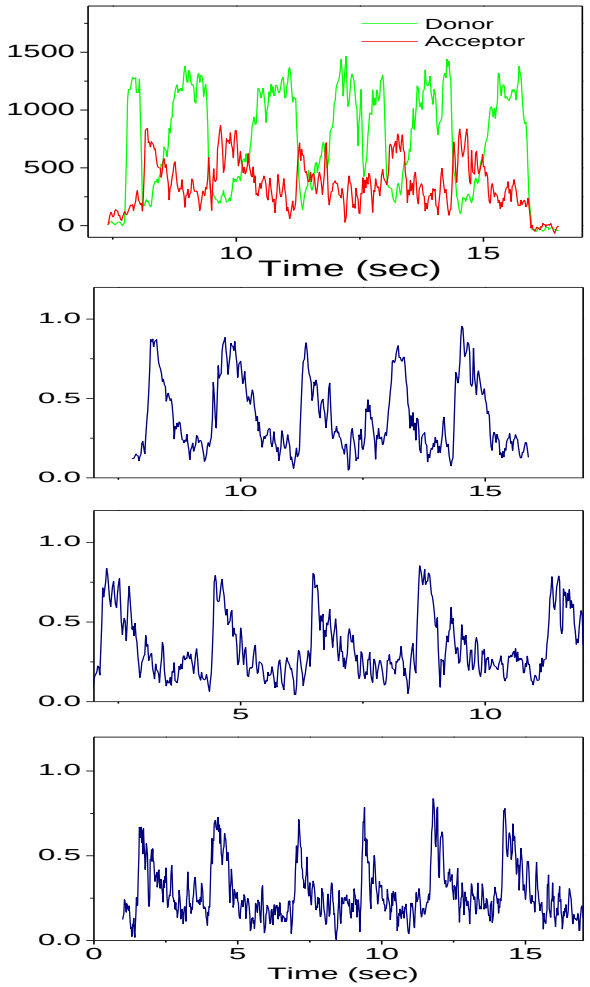
Molecular floss

Rep participates in replication restart
[Sandler, 2001].

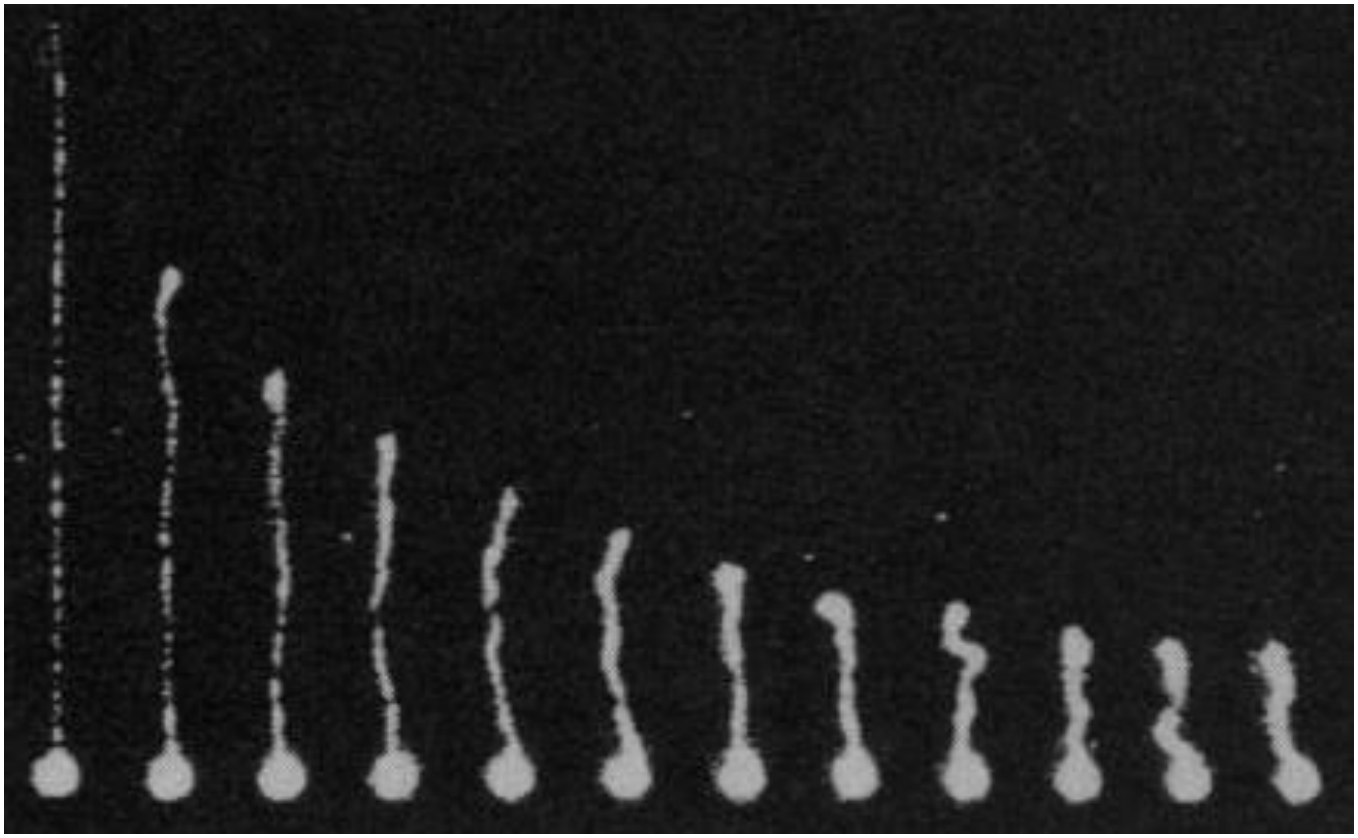
Rep monomer moves repeatedly in a stall replication fork







Part I. Double stranded DNA



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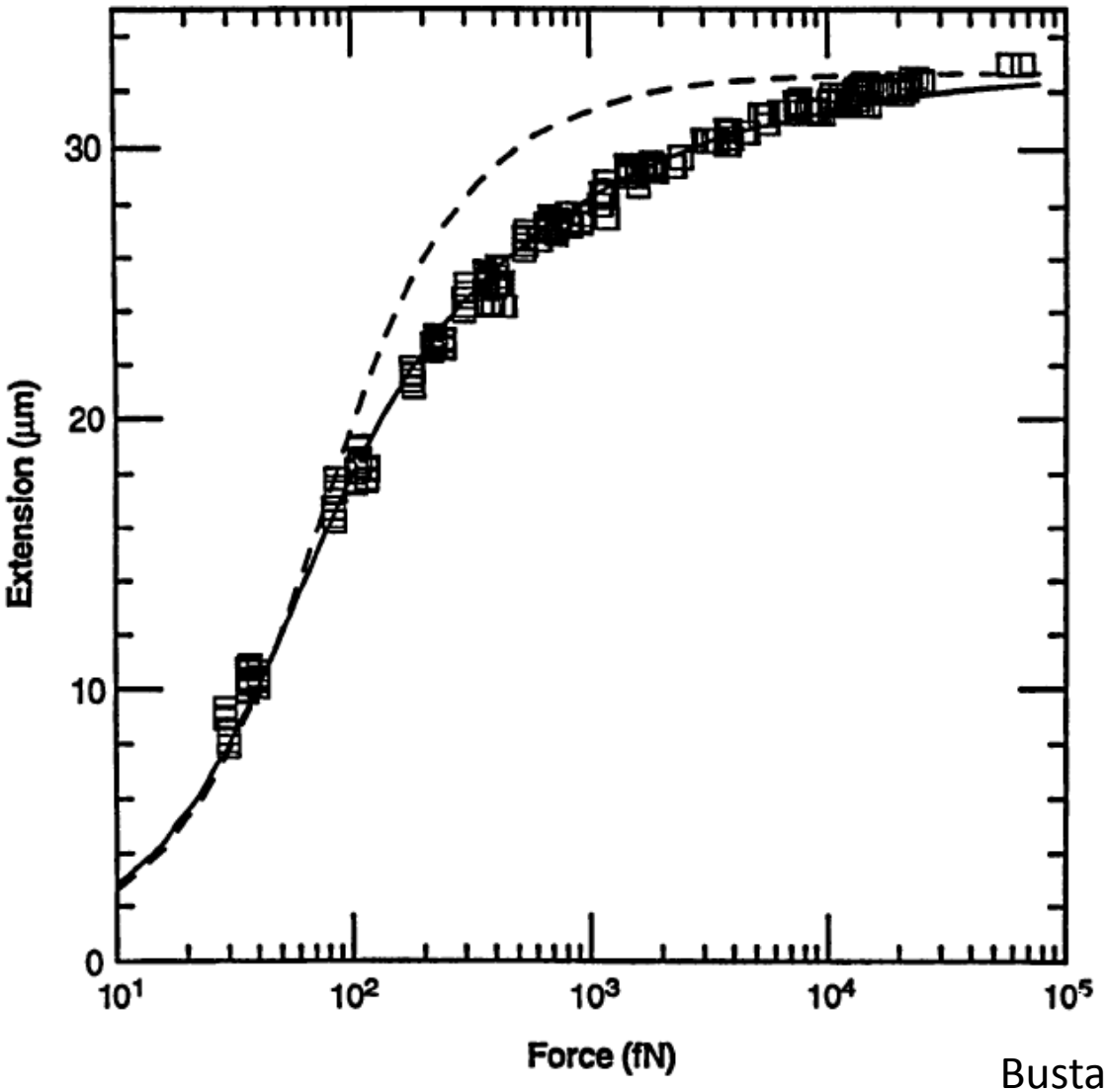
DNA as a Worm-Like Chain



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