In vitro and *in vivo* molecular imaging

Gulliver multi-scale Imaging Workshop
18 May, 2007

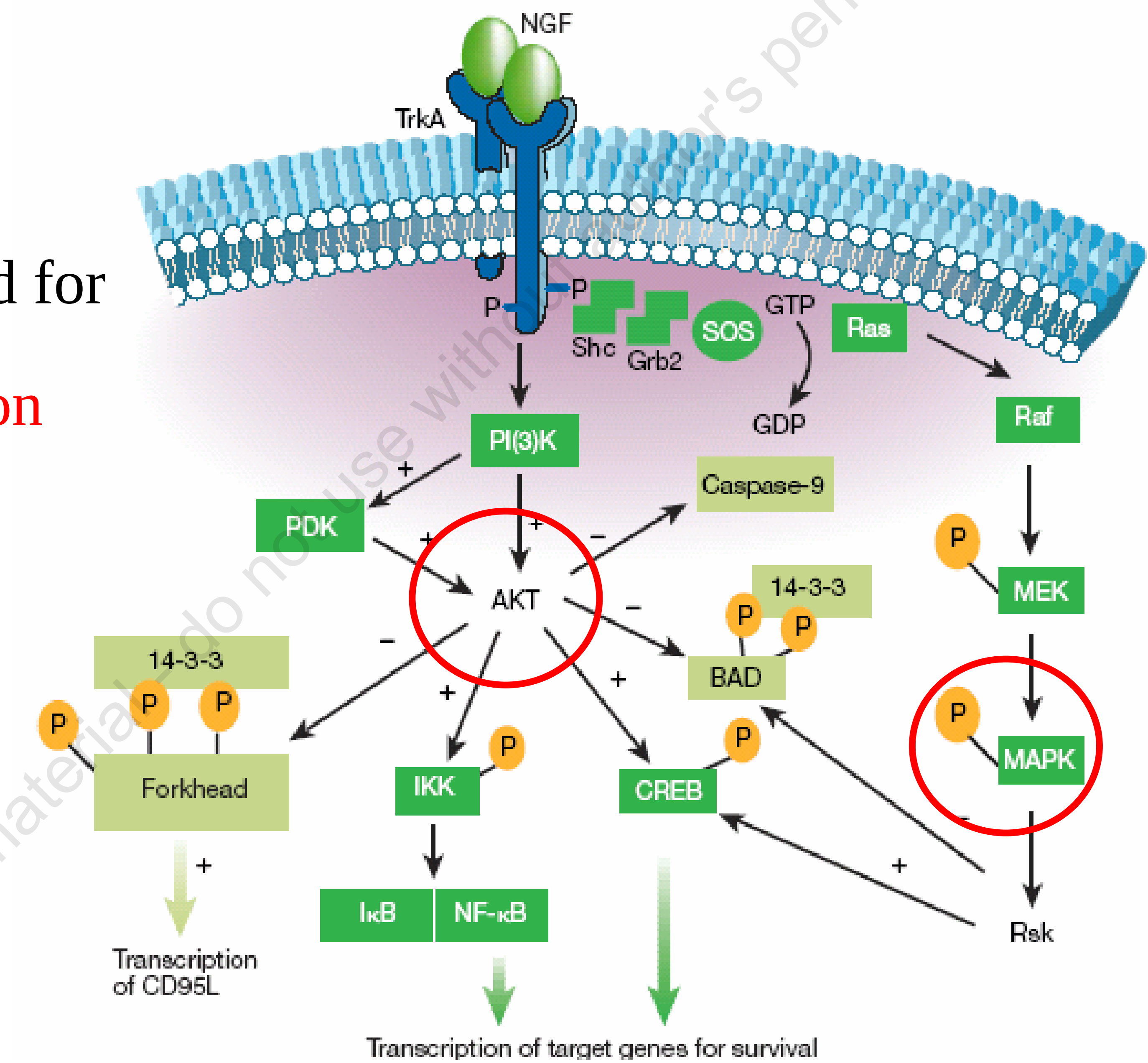
in vivo imaging of NGF retrograde transport in neurons

Bianxiao Cui, Chengbiao Wu, Liang Chen,
Alfredo Ramirez, Bill Mobley, Steve Chu

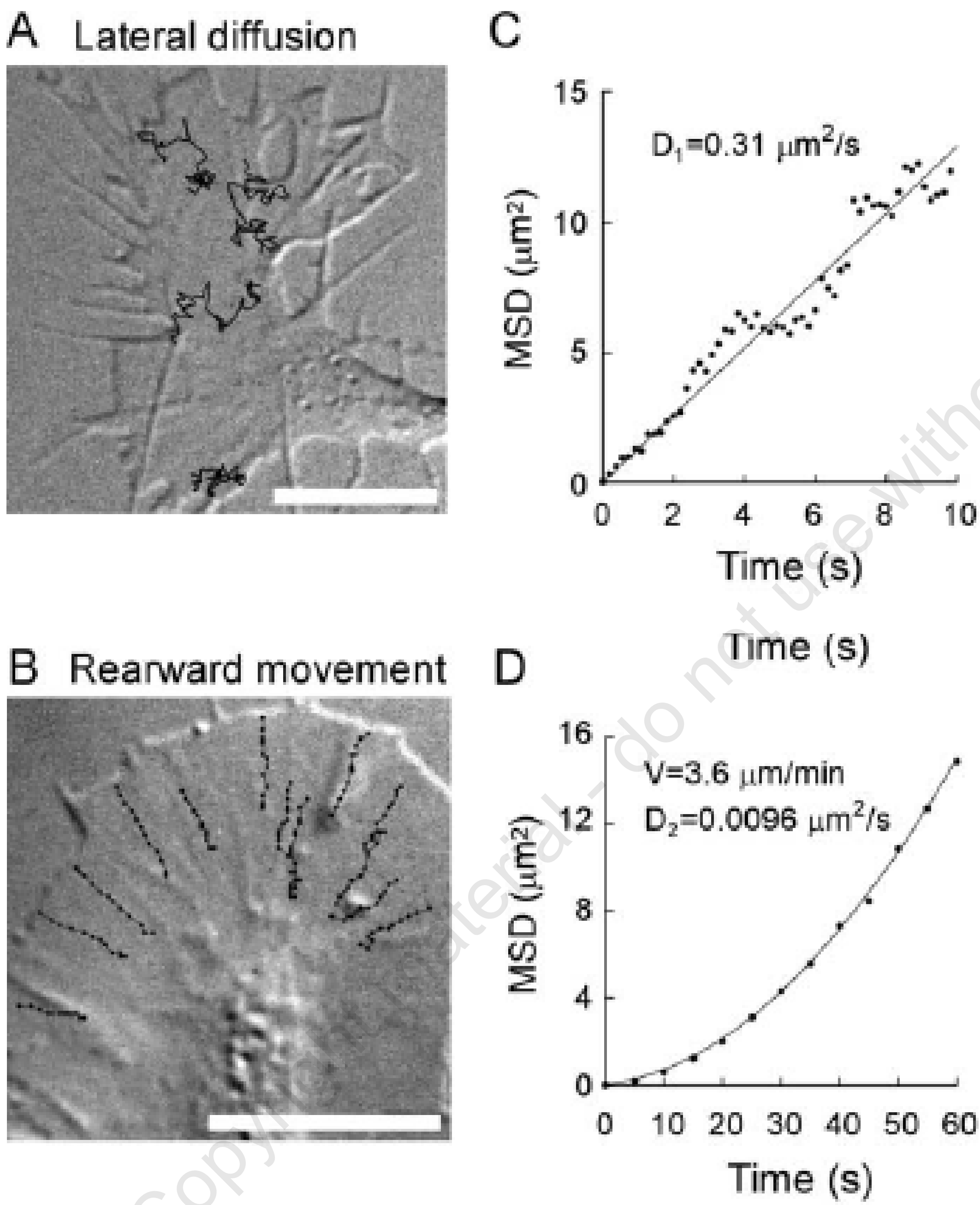
Neural Growth Factor signaling pathways

NGF is required for

- Differentiation
- Survival
- Maintenance
- Repair



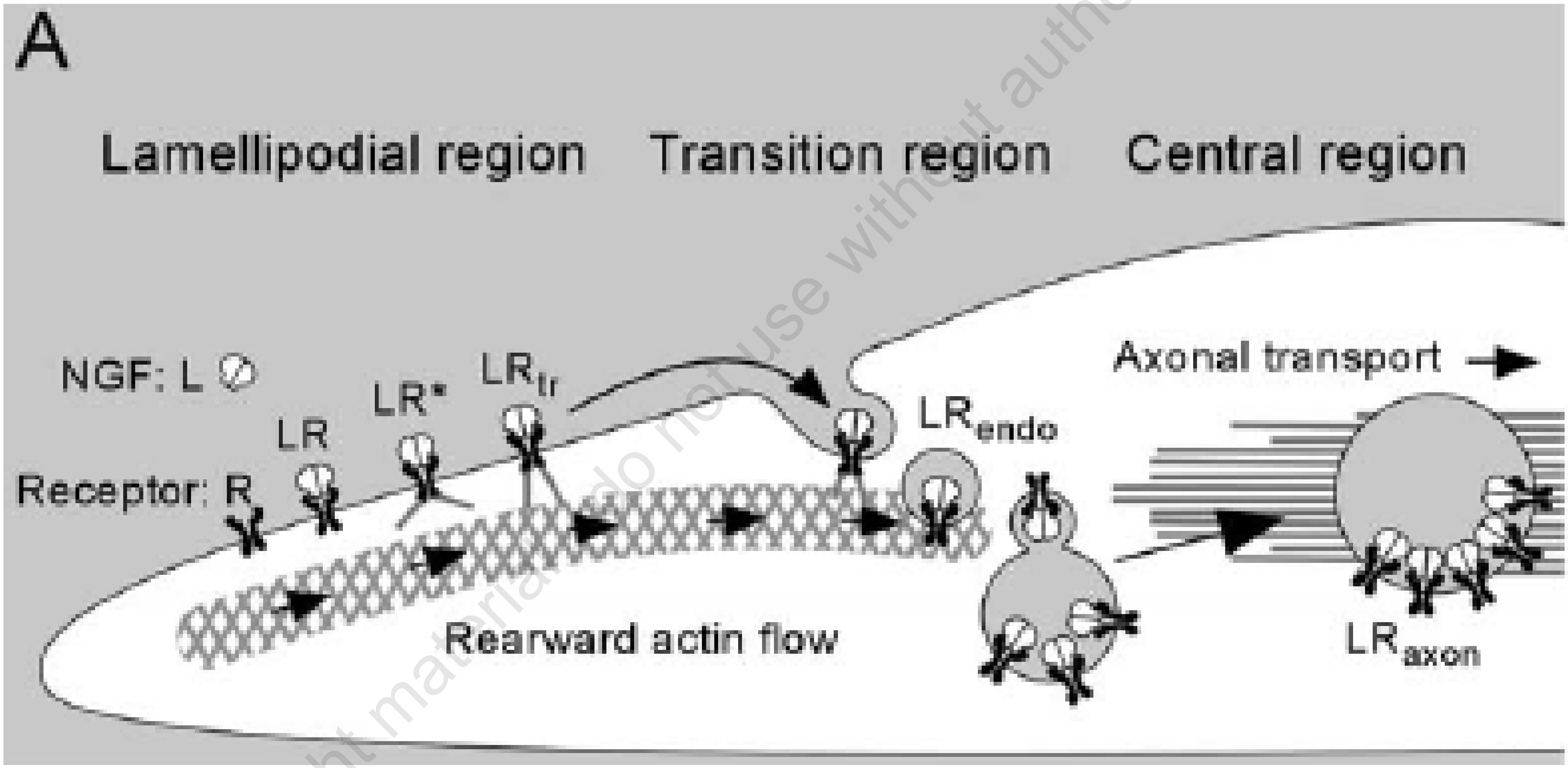
T. Tani, et al. J. Neuroscience **25**, 2181–2191 (2005)



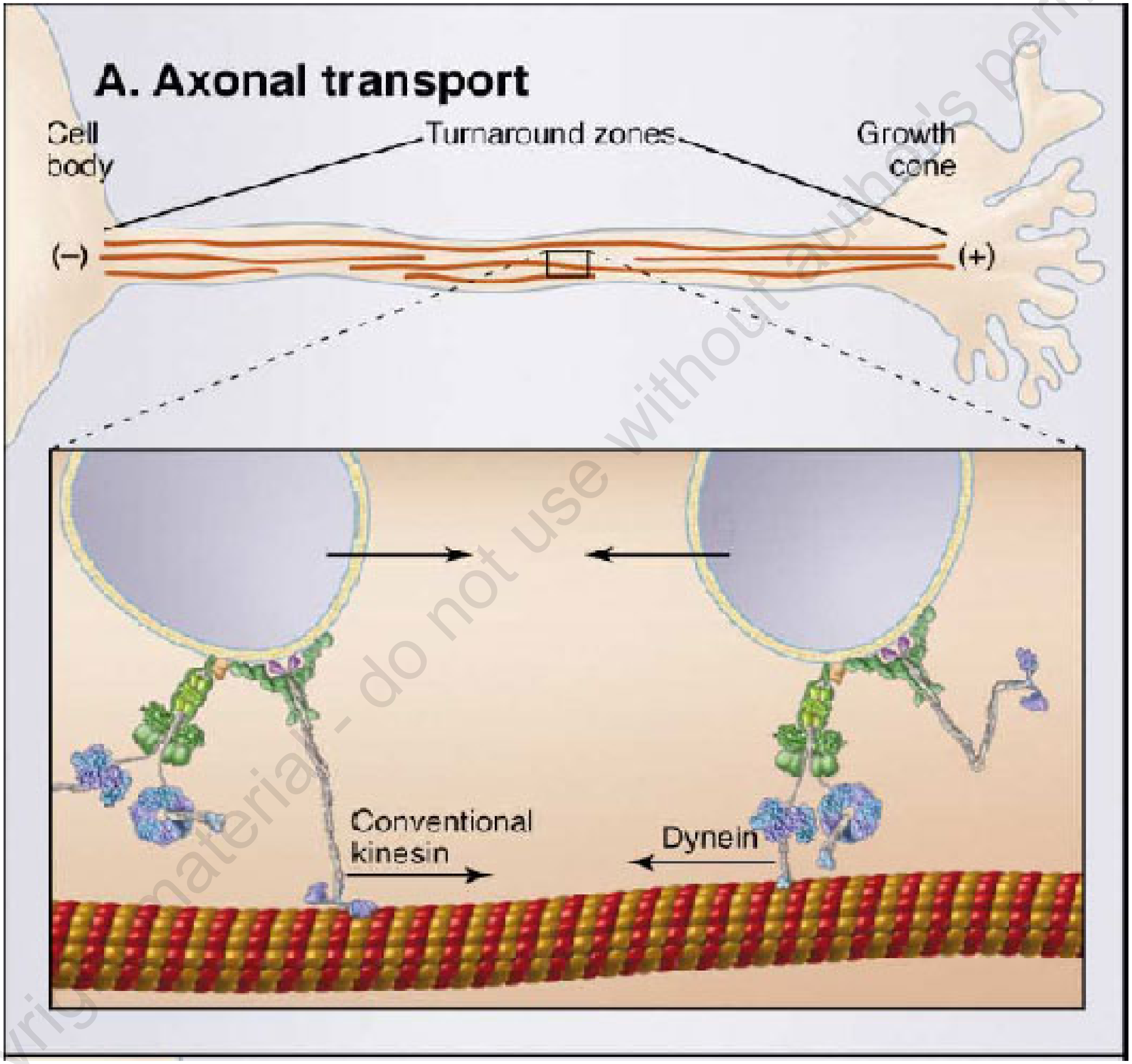
After binding to the receptor, Cy3-NGF diffuses on the membrane of the growth cones

Motion then becomes one-directional toward center of growth cone. Movement could be stopped by adding latrunculin B, a potent inhibitor of actin polymerization.

Model of NGF binding, trafficking, and uptake at the dorsal root ganglion growth cone.

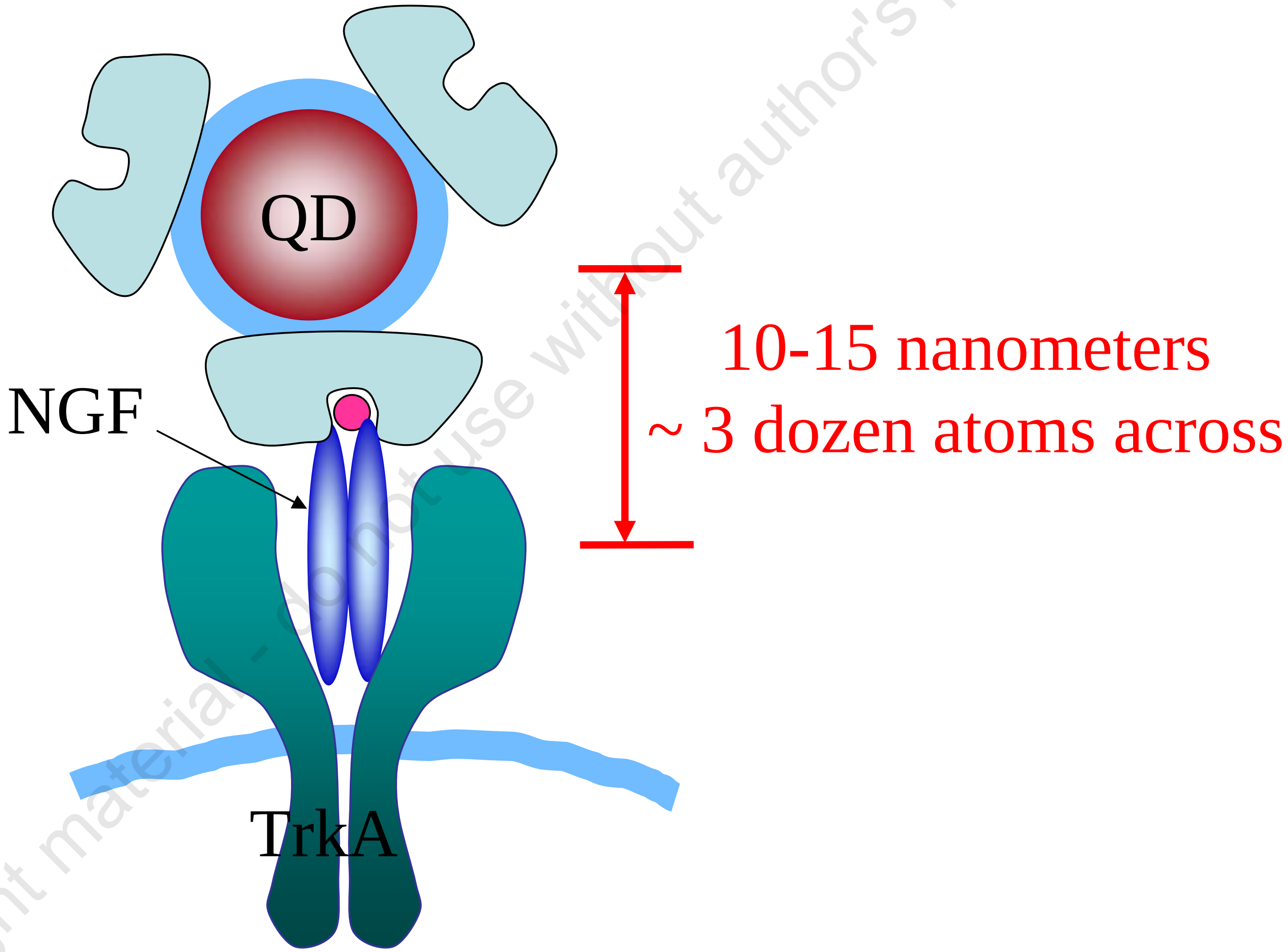


Axonal transport

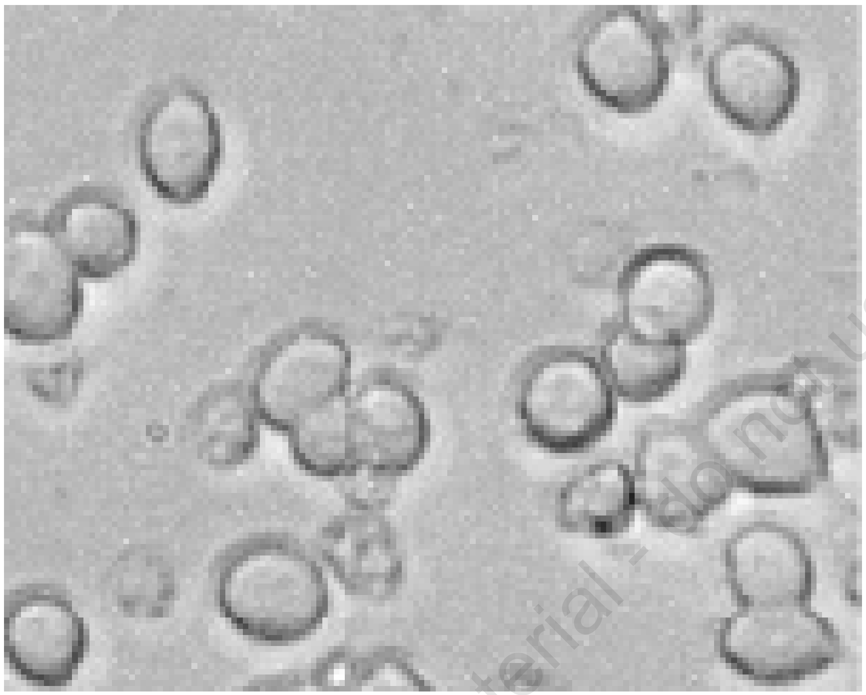


http://courses.biology.utah.edu/bastiani/3230/Neuro%206040/Bastiani/Lecture%202%20MTs

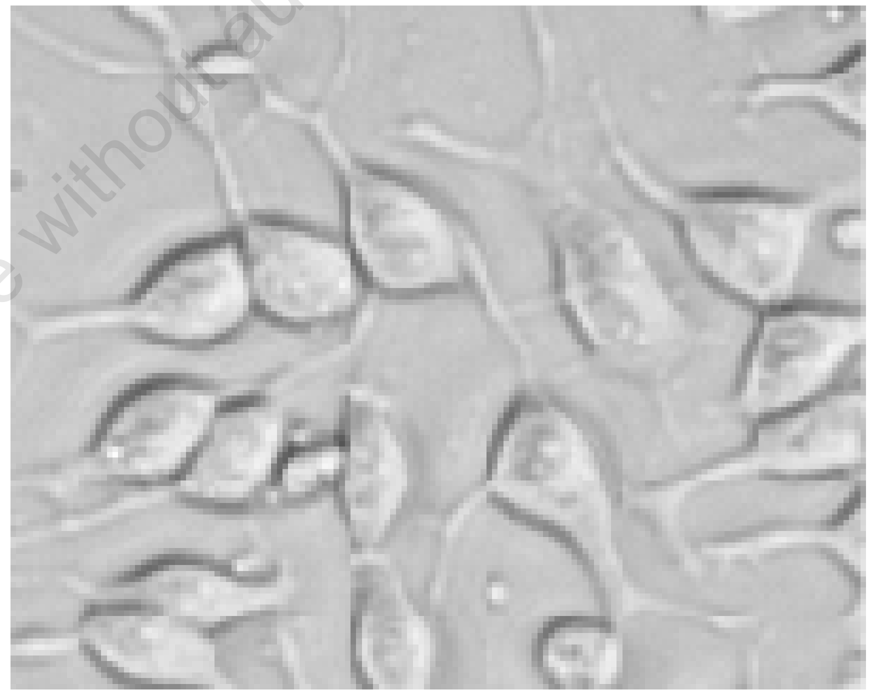
Quantum dot - Neuro-growth Factor



Qdot-NGF stimulates neurite outgrowth in PC12 cells

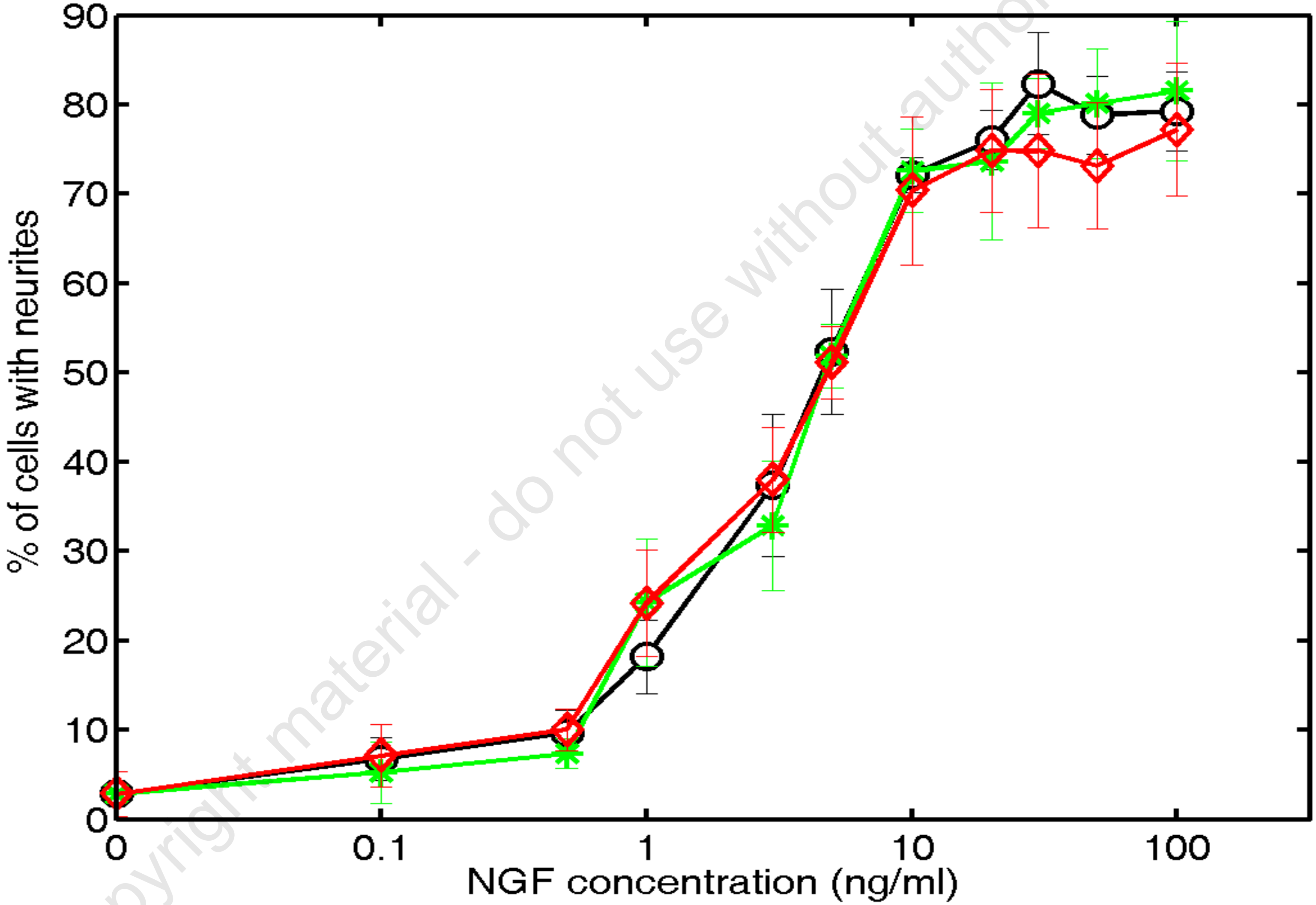


No NGF treatment

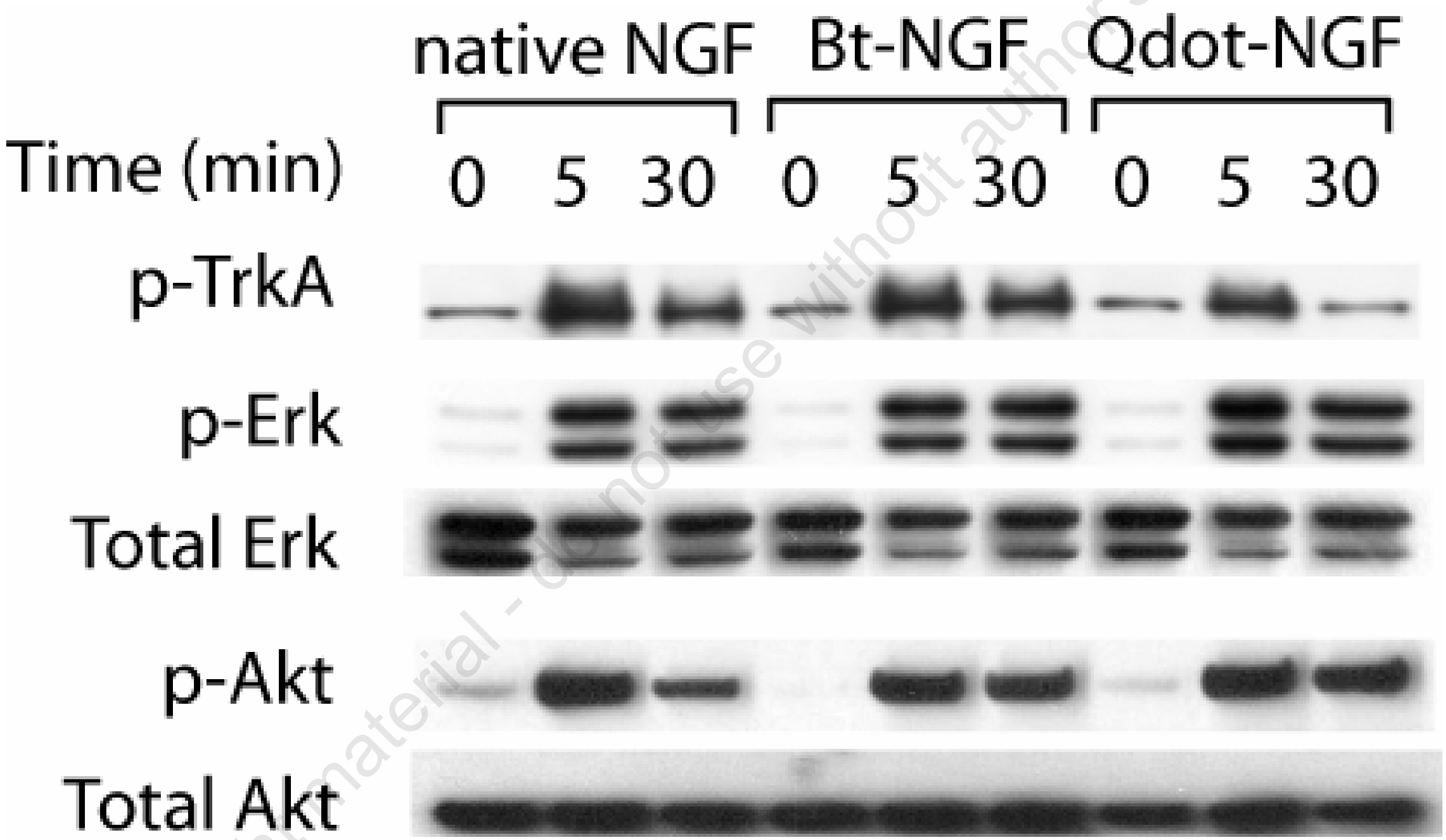


Treated with 20ng/ml Qdot-NGF for 48hrs

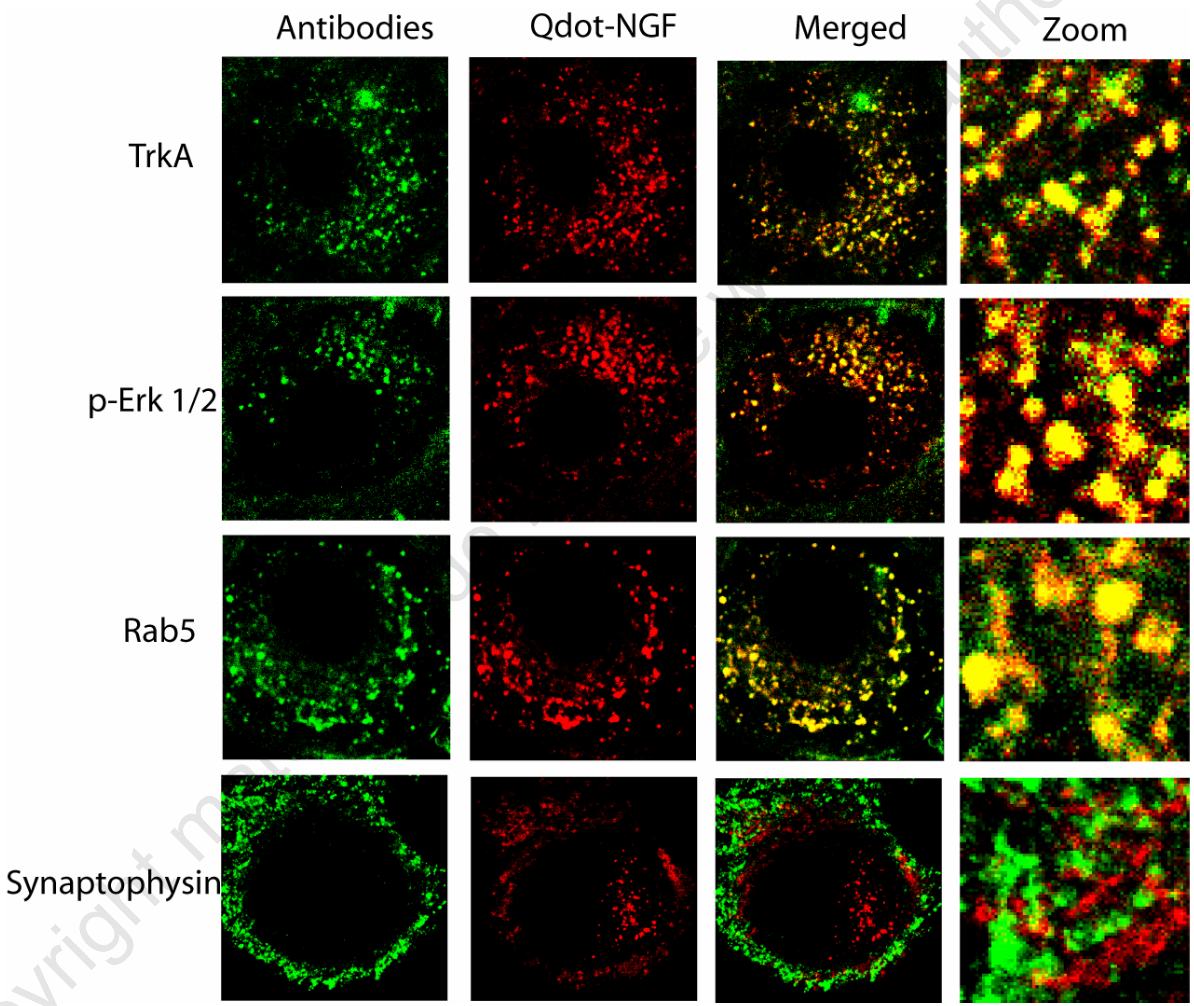
Dose response of Qdot-NGF, Bt-NGF and native NGF



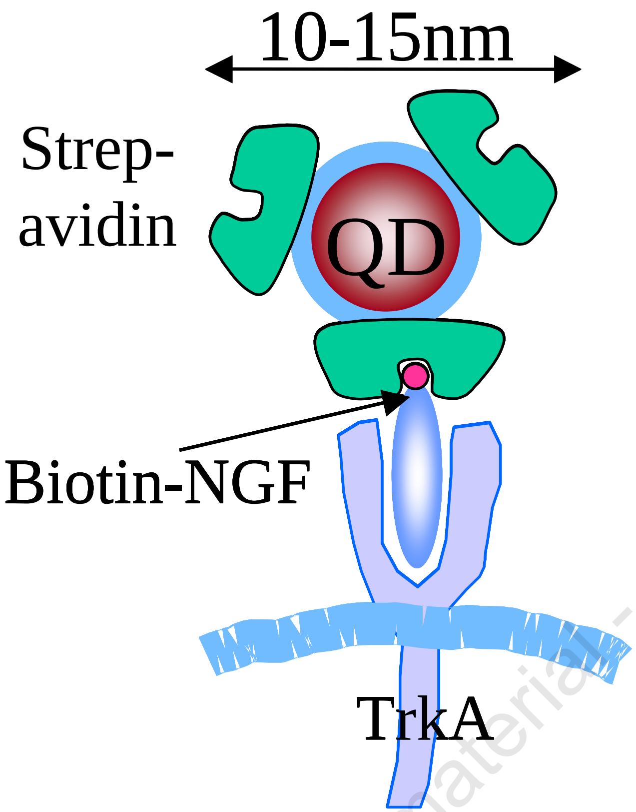
Qdot-NGF activates signaling proteins

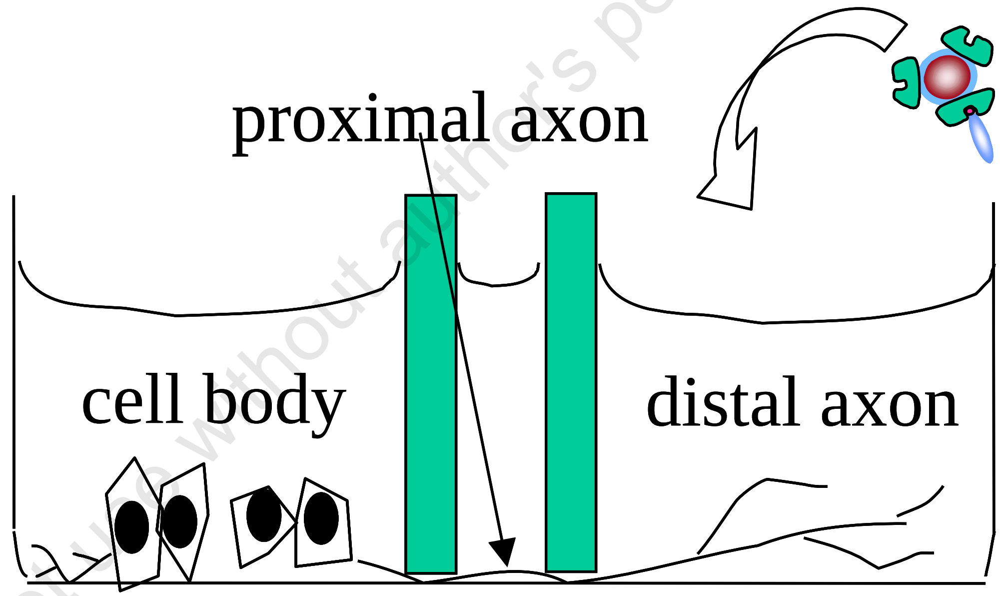


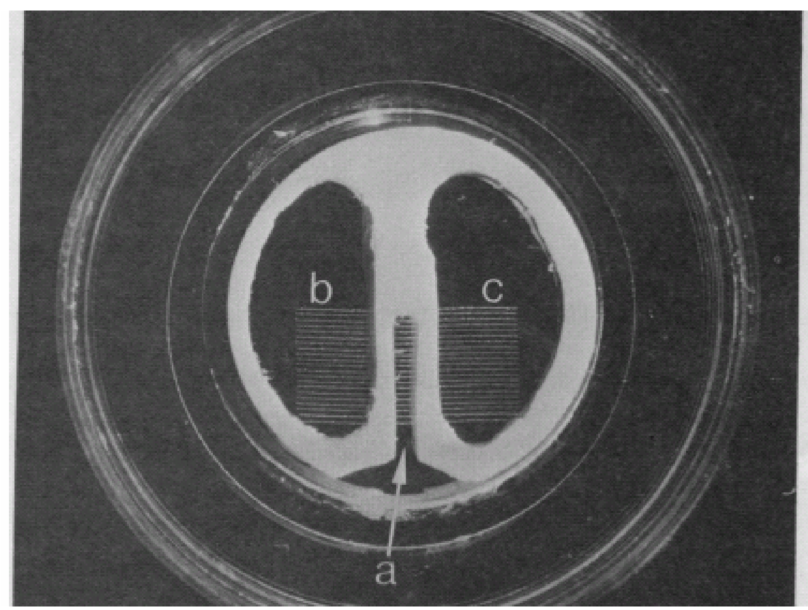
Co-localization of Qdot-NGF with signaling proteins



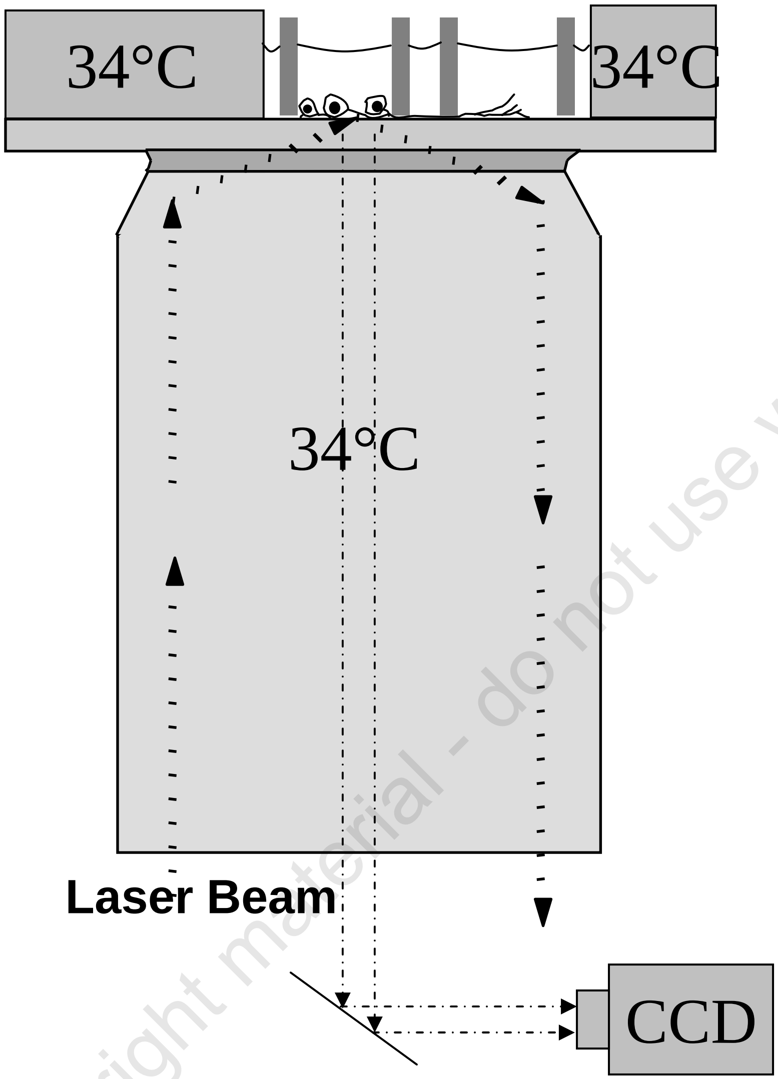
Q-dot tracking of NGF motion





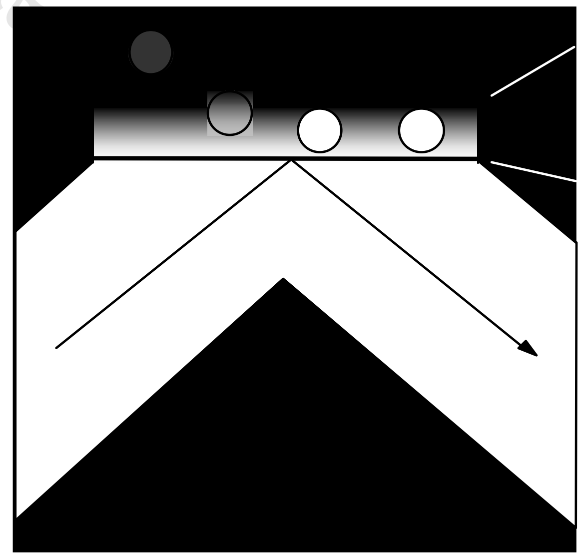


Live imaging experiment setup

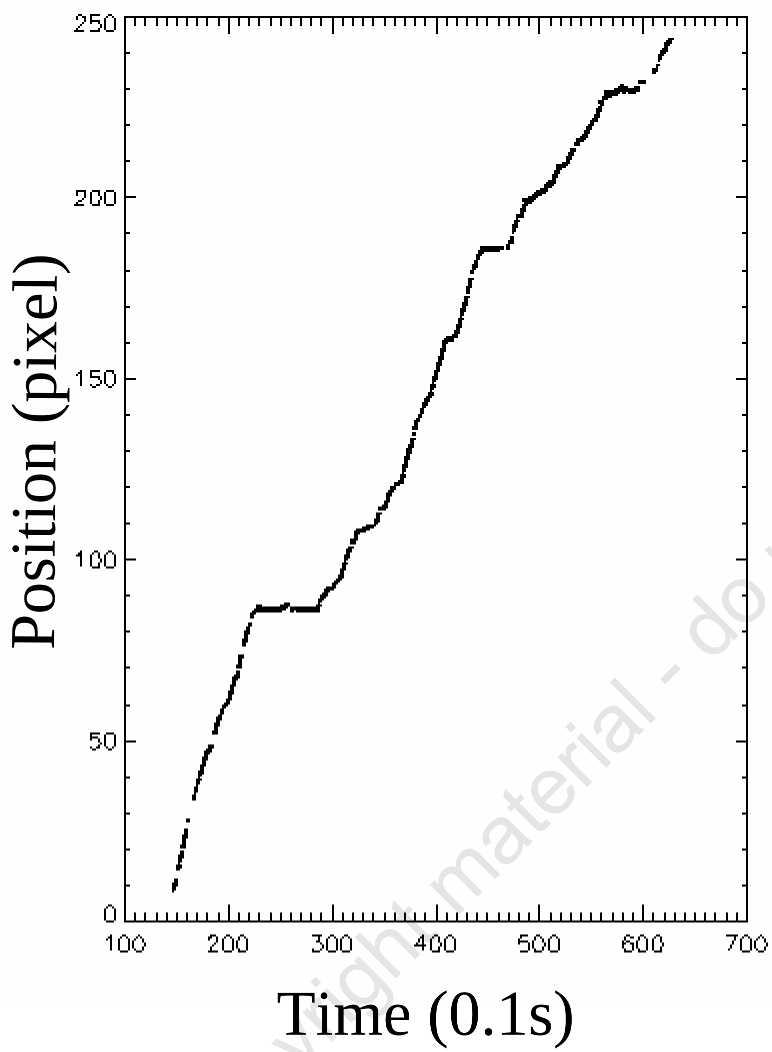


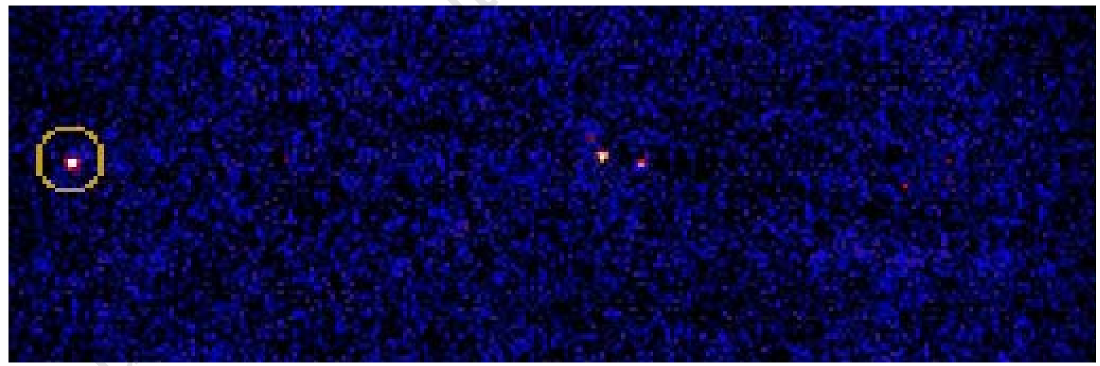
Total Internal Reflection Microscope

Evanescent Wave



Tracking an individual endosome

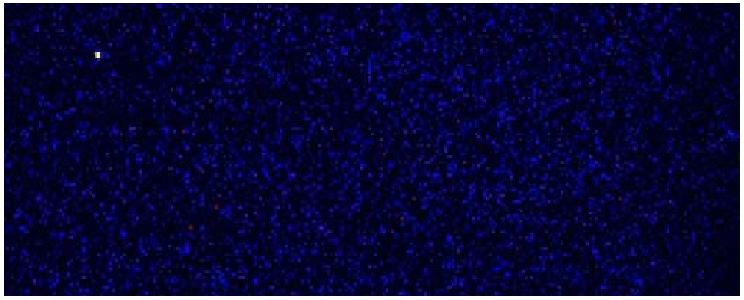




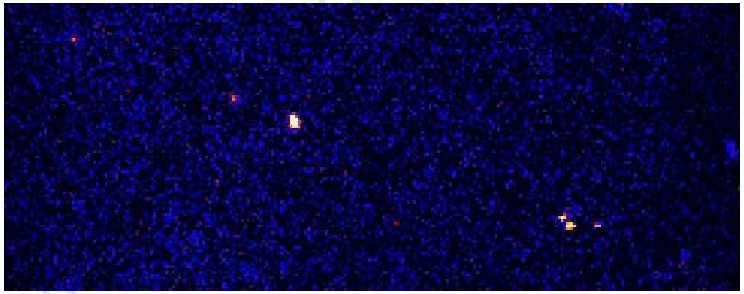
Time corresponds to 30
seconds of real time
(~5x faster)

Live imaging of NGF transport

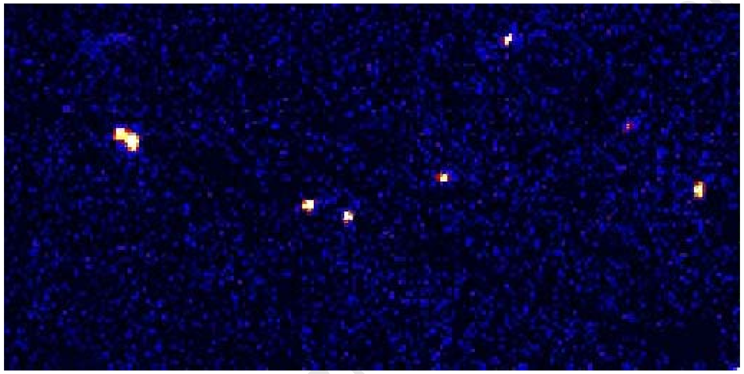
Cui, Wu, Chen, Ramirez, Mobley, Chu



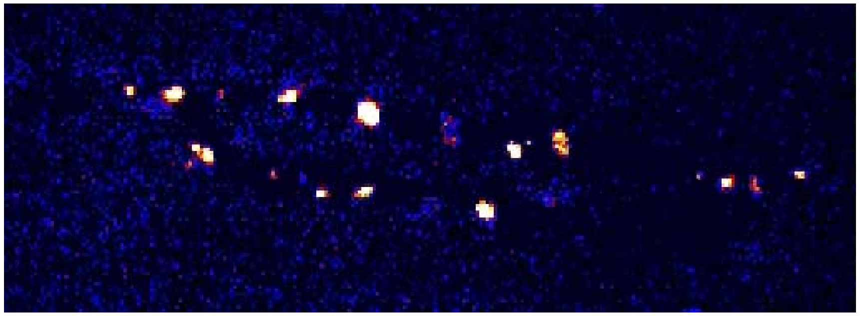
5 ng/ml NGF



25 ng/ml NGF



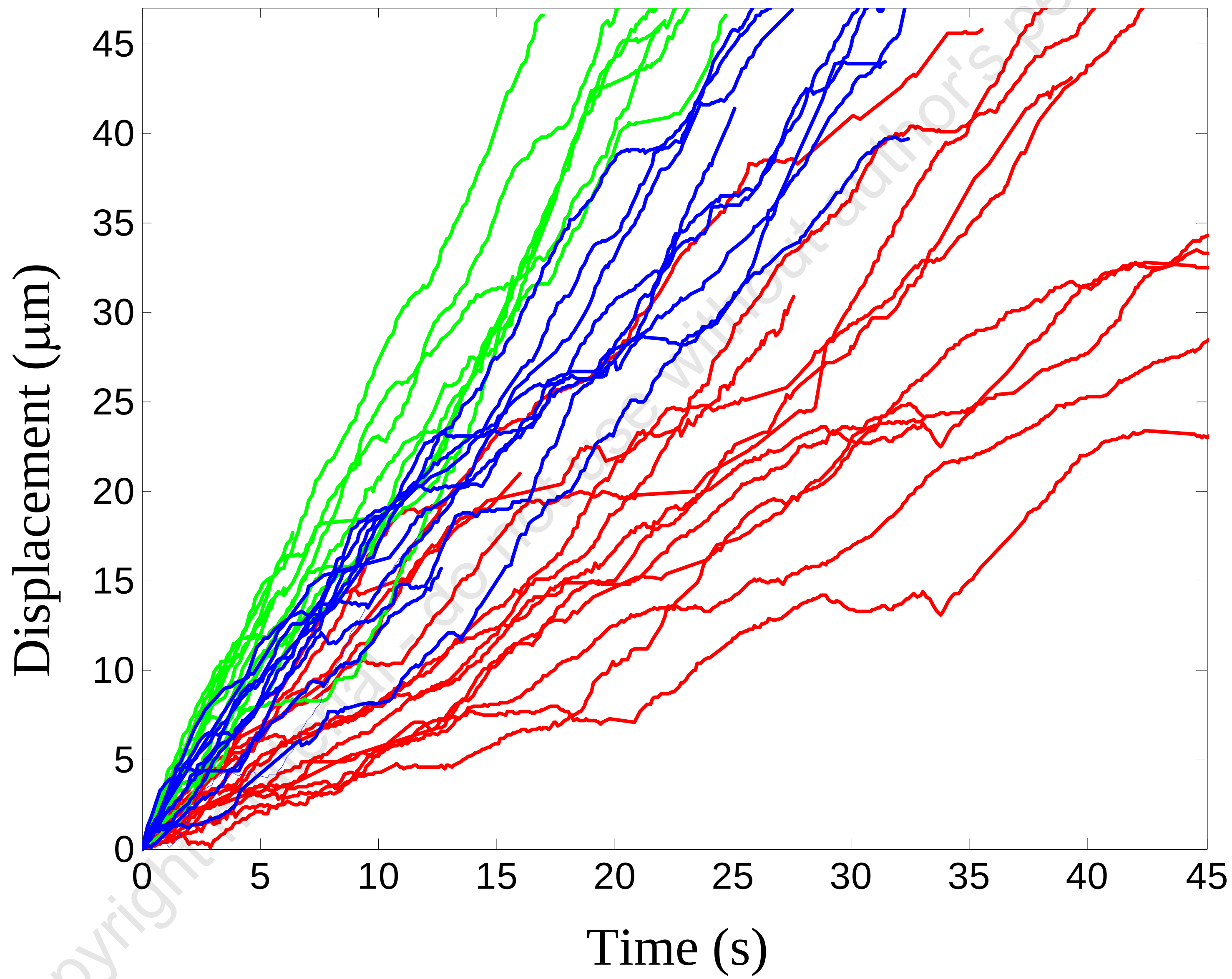
50 ng/ml NGF



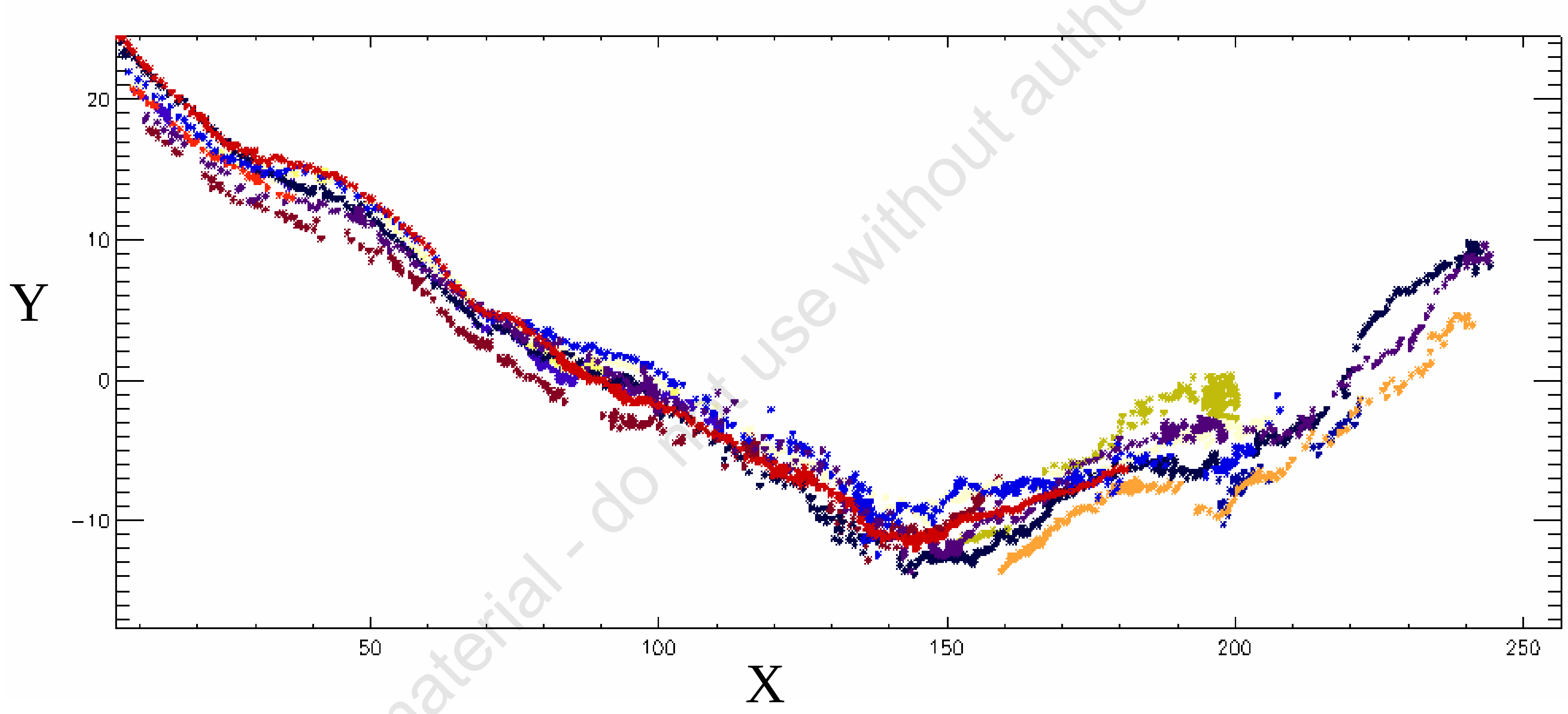
500 ng/ml NGF

Movies correspond to 30s real time

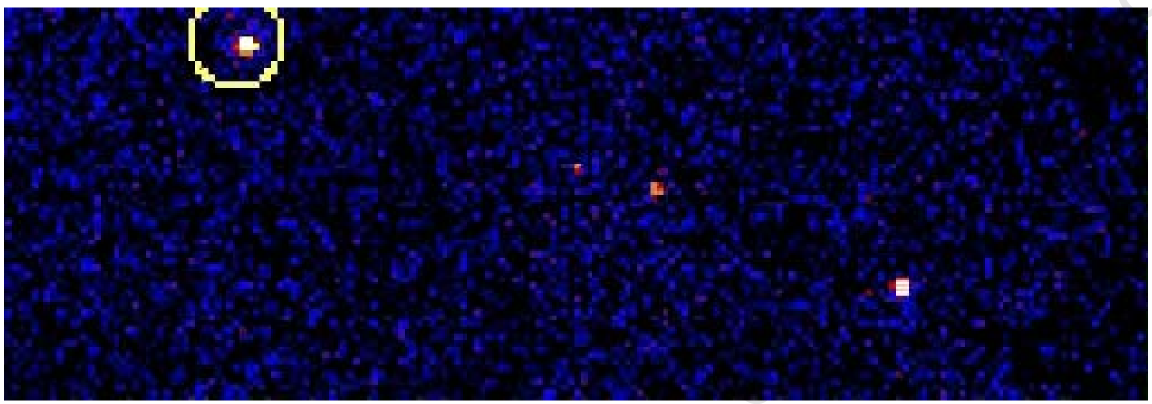
Endosomes moving in different axons

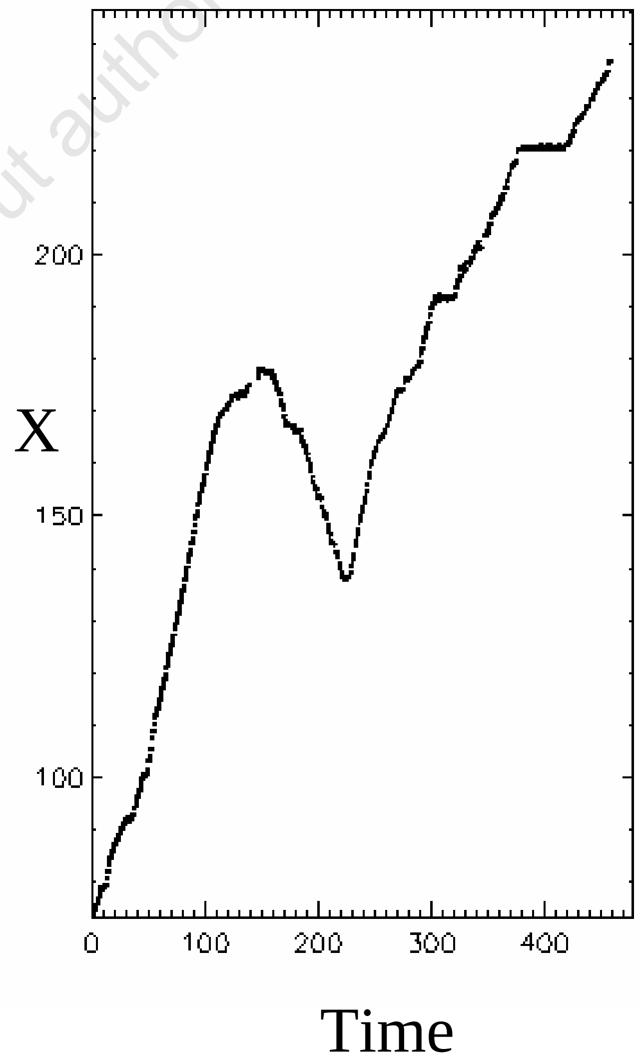


Each axon is a multi-lane highway

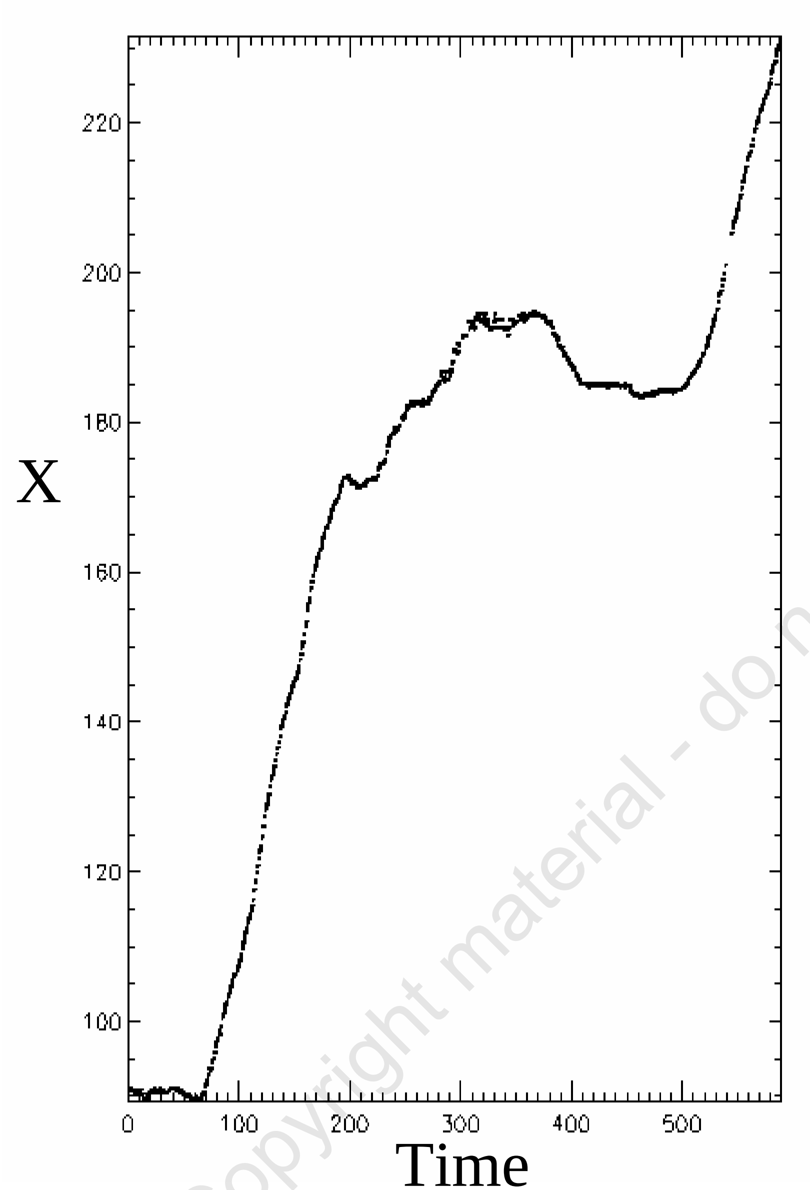


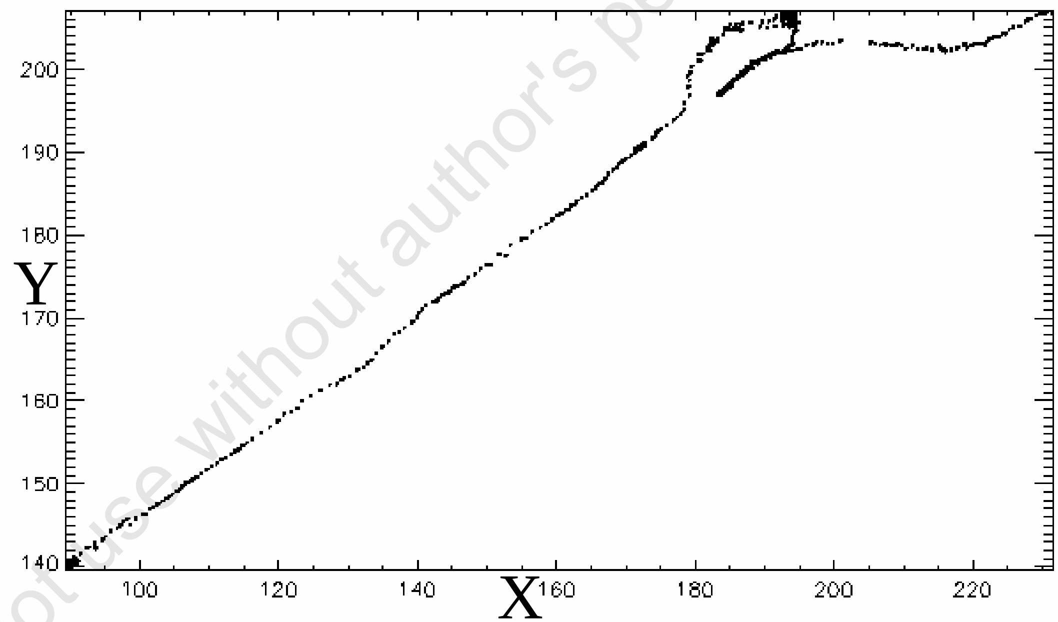
Anterograde motion

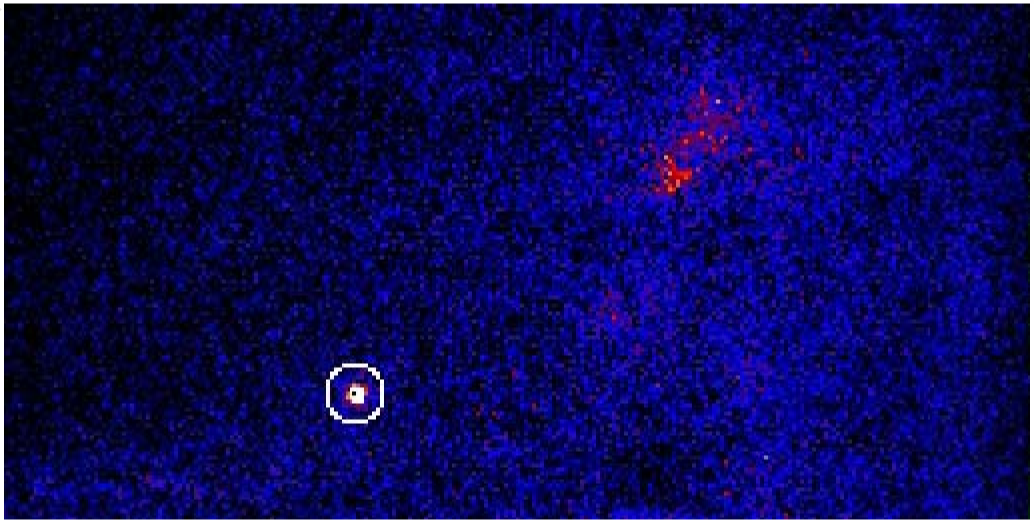




Endosome changing microtubules?

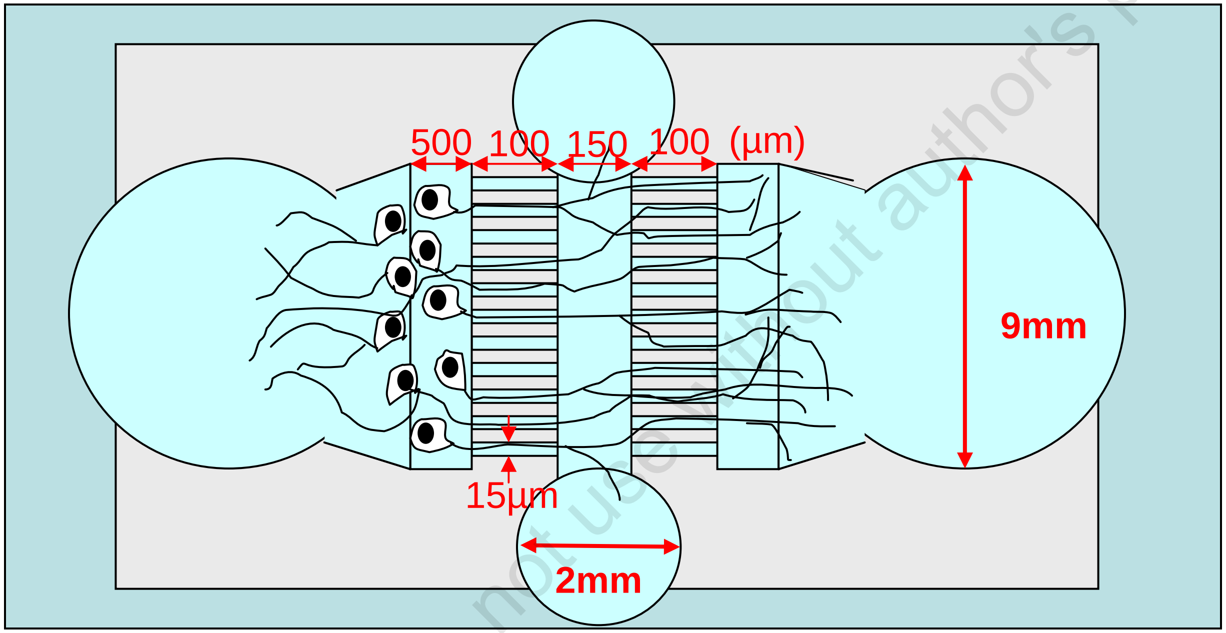




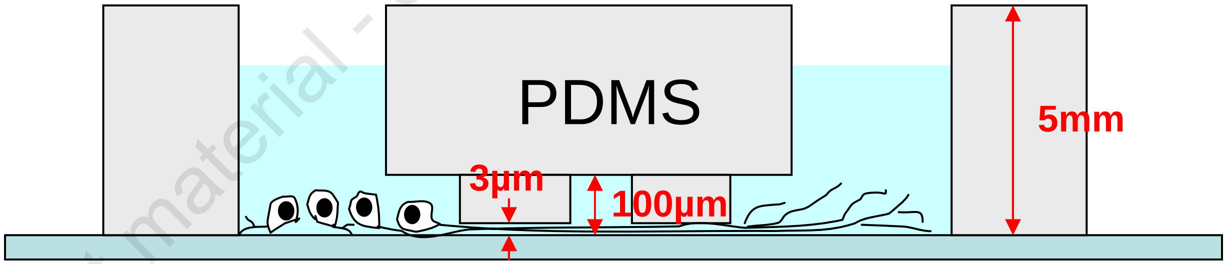


Microfluidic device diagram

Top view

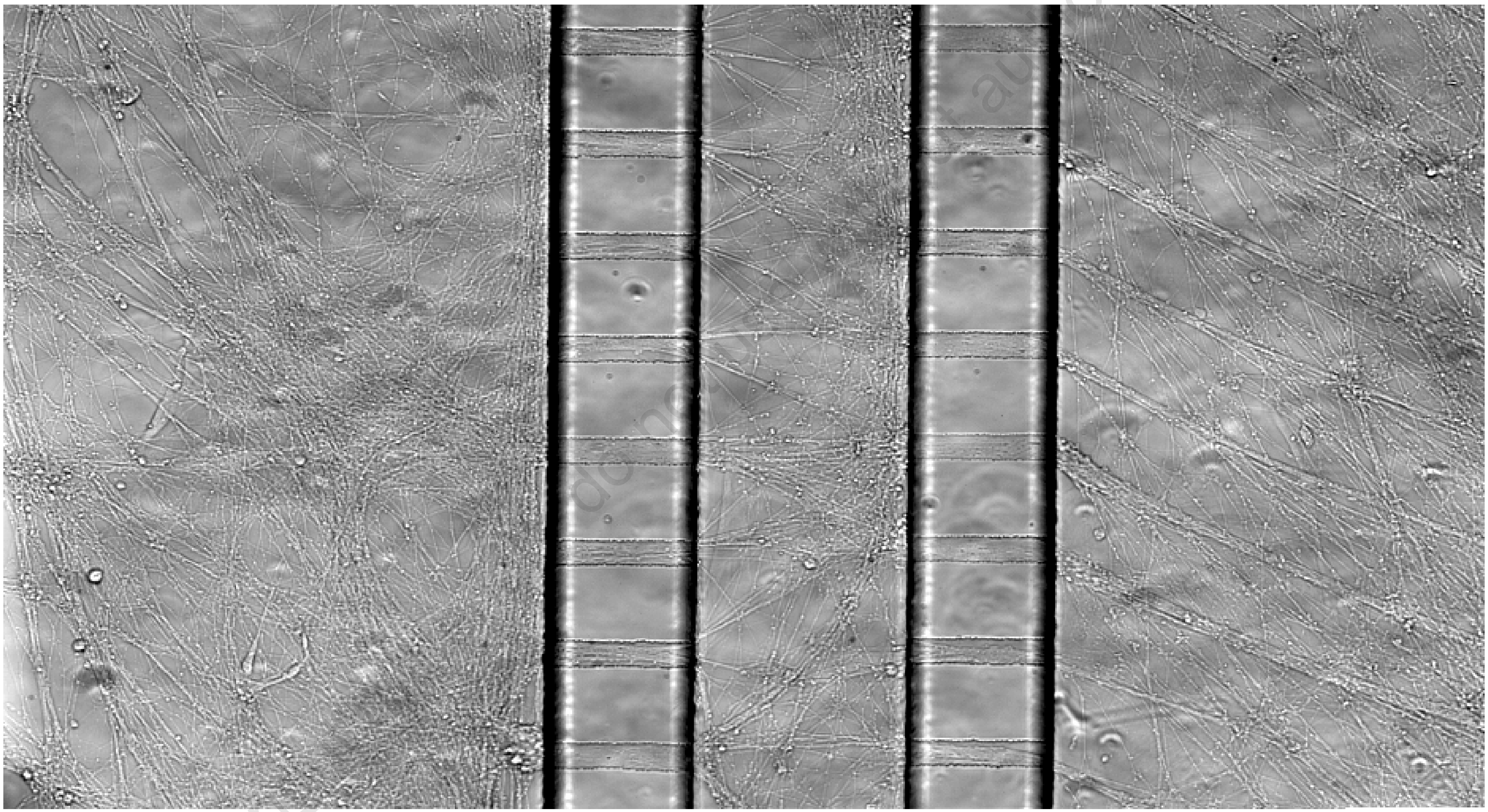


Side view



Taylor, AM, *et. al. Langmuir*, **19,** 1551-1556, 2003; *Nature Methods*, **2,** 599, 2005.

Three-chamber – day 10



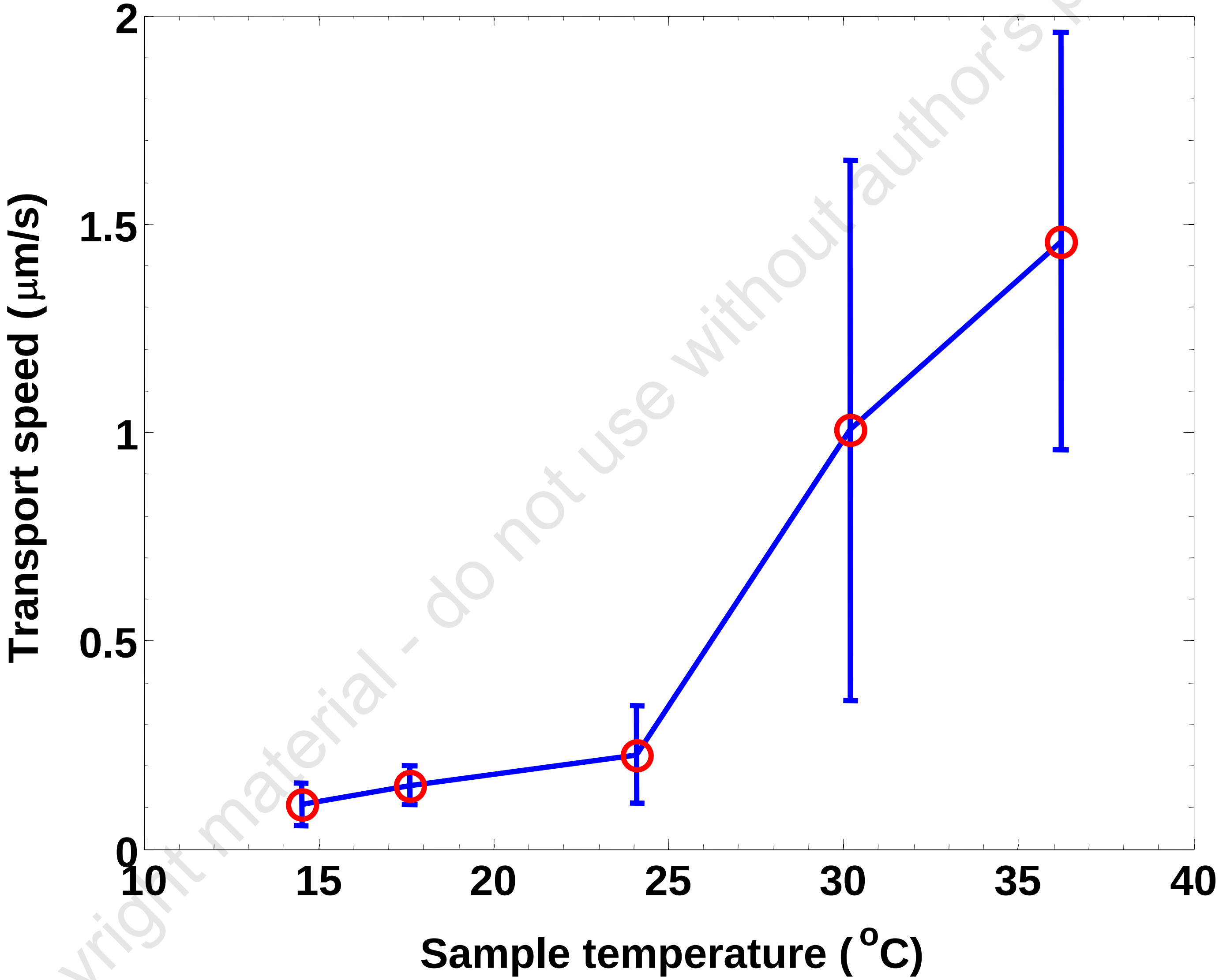
Distal axon chamber

Cell body chamber

Micro-fluidic advantages

- Axons tend to grow along the flow direction
- No leaking of fluorescent Qdots was detected, even after overnight incubation of 100ng/ml Qdot-NGF in the distal axon well.
- Rat embryonic DRG, cortical and hippocampus neurons, and mouse P0 DRG neurons, have been cultured in microfluidic devices and extend axons to the distal axon well.

Transport Speed vs. Temperature



Summary

1. NGF-containing endosomes move retrogradely along axons to cell bodies.
2. Most endosomes exhibit "stop-and-go", unidirectional motion at an average **moving speed** of up to 2.1 um/s.
3. Motion reversal is seen.
4. A single NGF dimer is sufficient to induce retrograde transport of signaling event.
5. The speed of transport varies by more than an order of magnitude between 14° and 37° C.

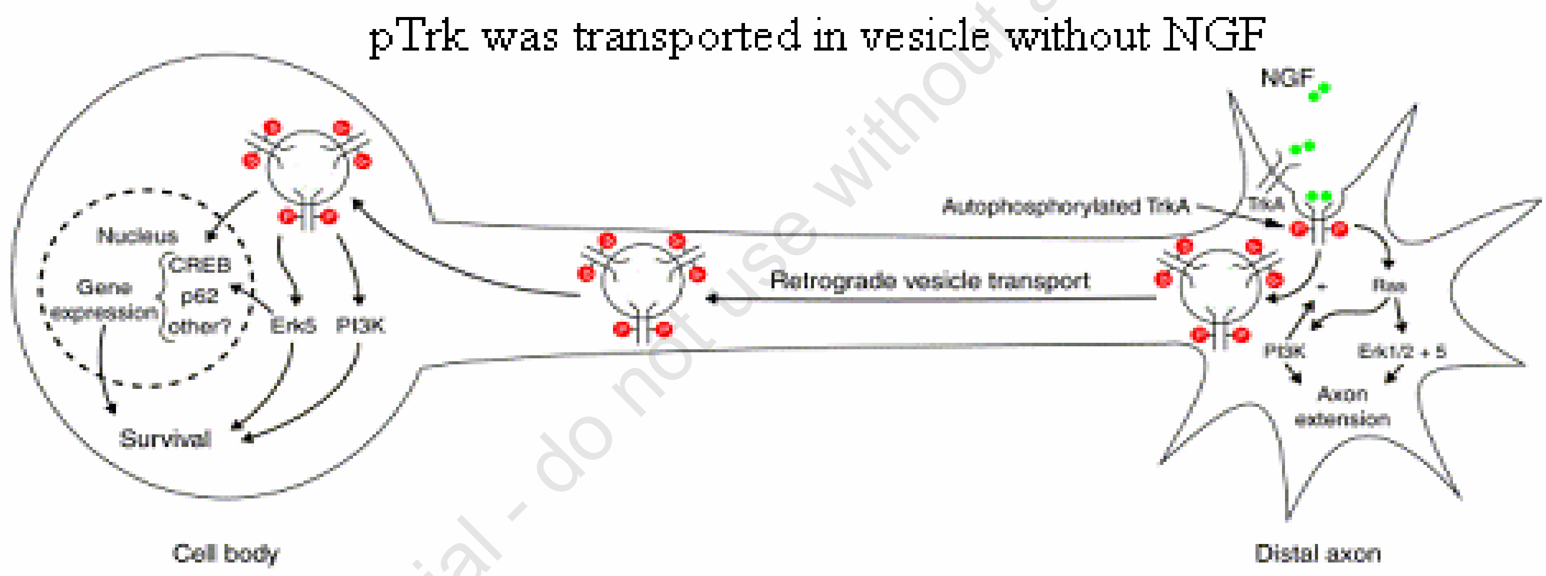
in vivo imaging and retrograde transport of Trk_A in neurons

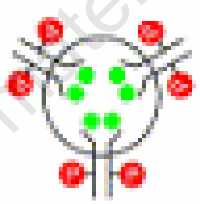
Liang Chen, Bianxiao Cui, Steve Chu

Chengbiao Wu, Alfredo Ramirez, William C. Mobley

Is pTrk-A, activated by NGF transported to the cell body?

Senger and Campenot, *JCB* 1997
MacInnis and Campenot, *Science*. 2002

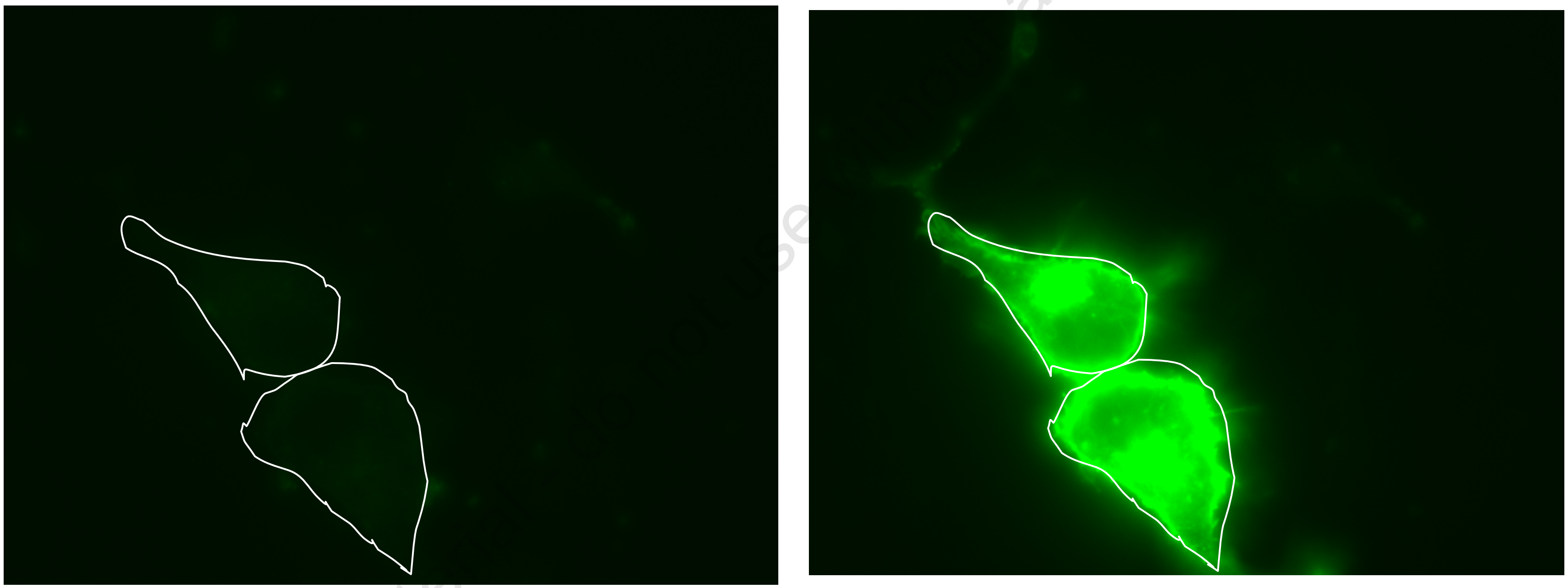




and/or

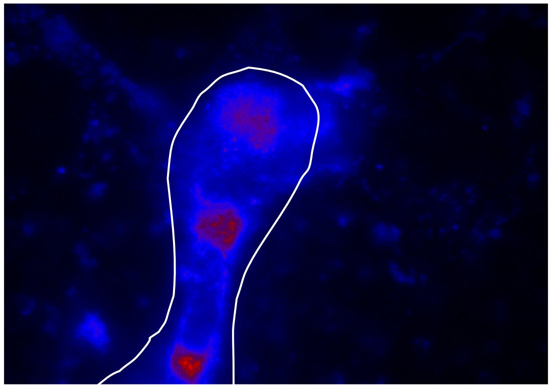
?

TrkA_ PAGFP

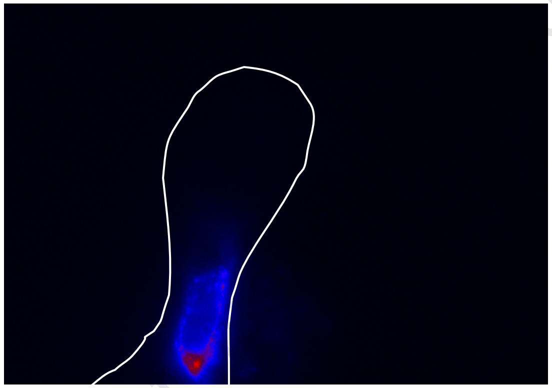


1s after UV excitation

PAGFP can be partially excited



1



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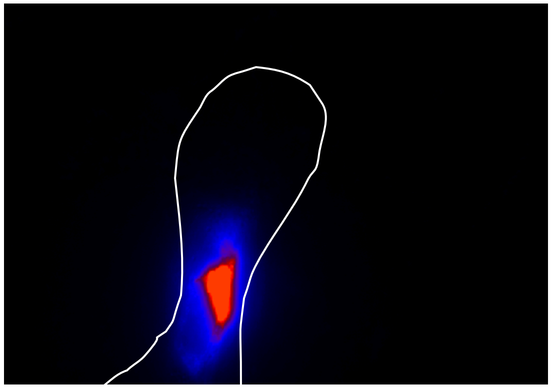

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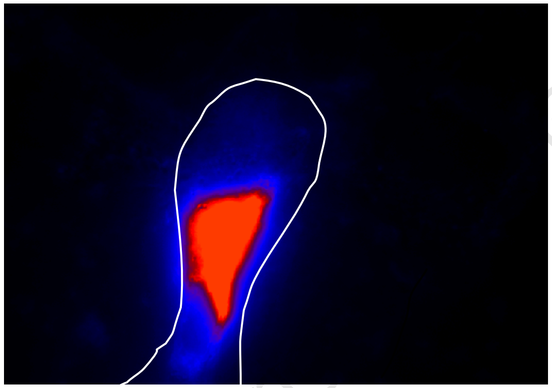
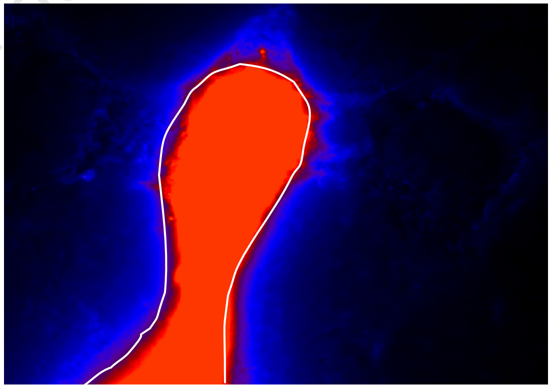
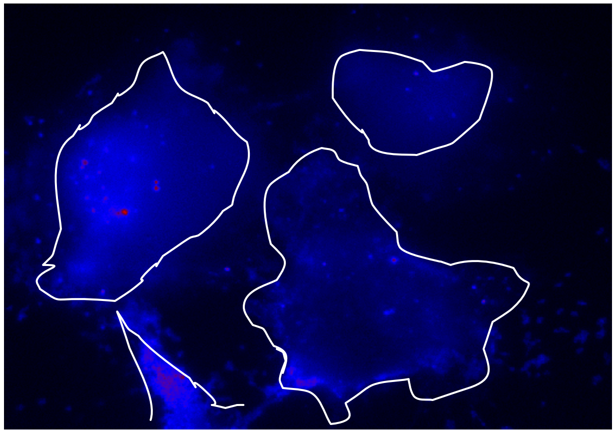


4

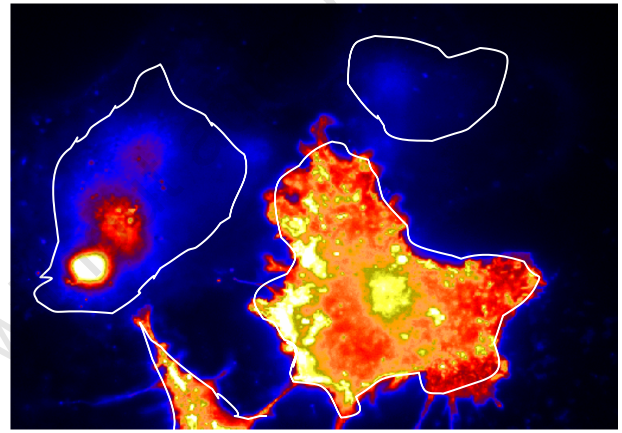


5

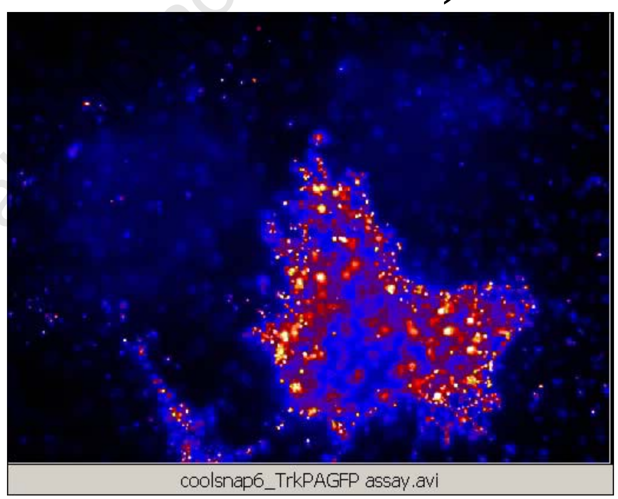
NGF binds to TrkA_PAGFP specifically on surface of Cos7 cells



a) PAGFP before UV excitation

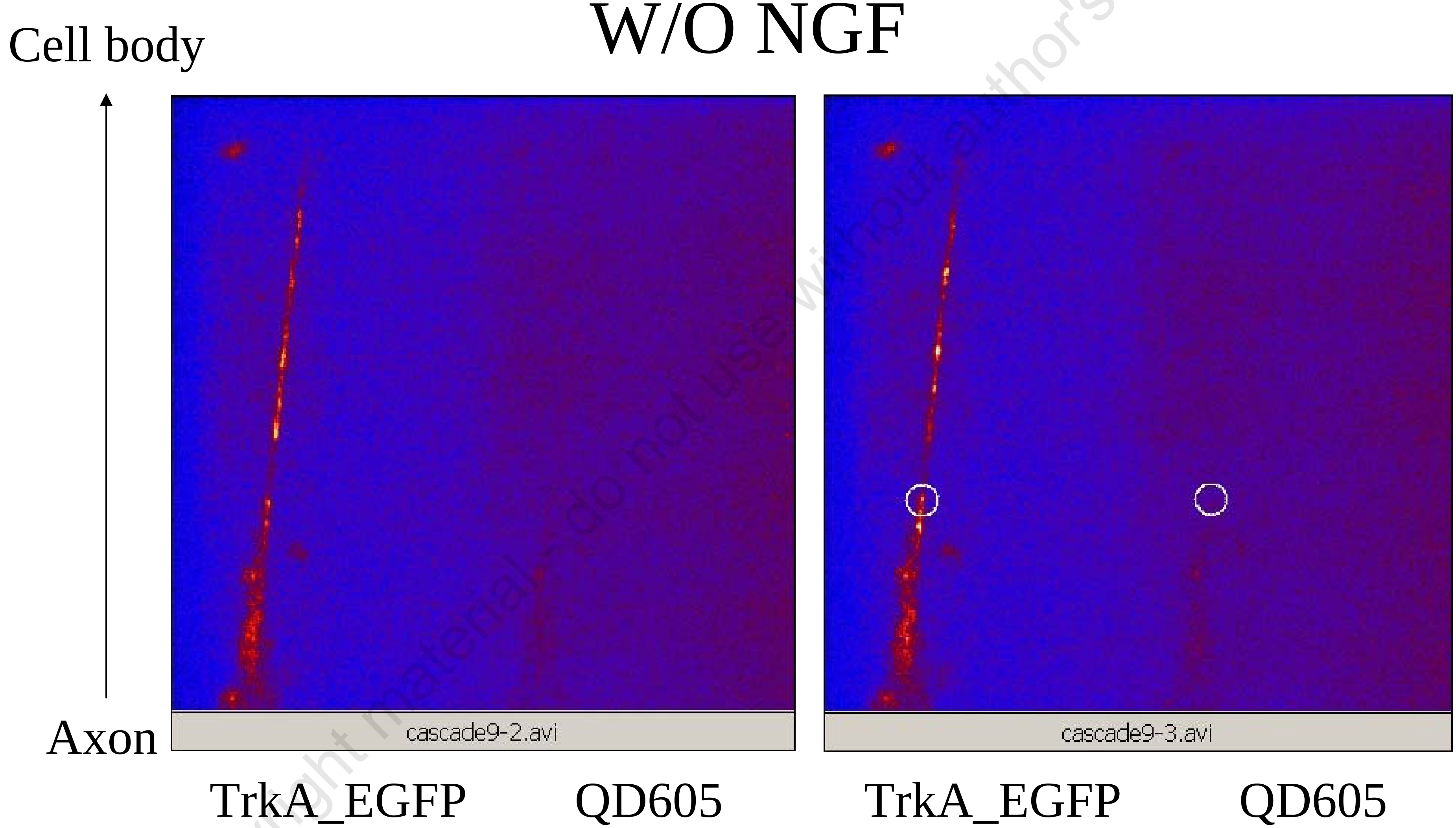


b) PAGFP after UV excitation



c) QD 605 filter

Bi-directional transport of TrkAEGFP W/O NGF

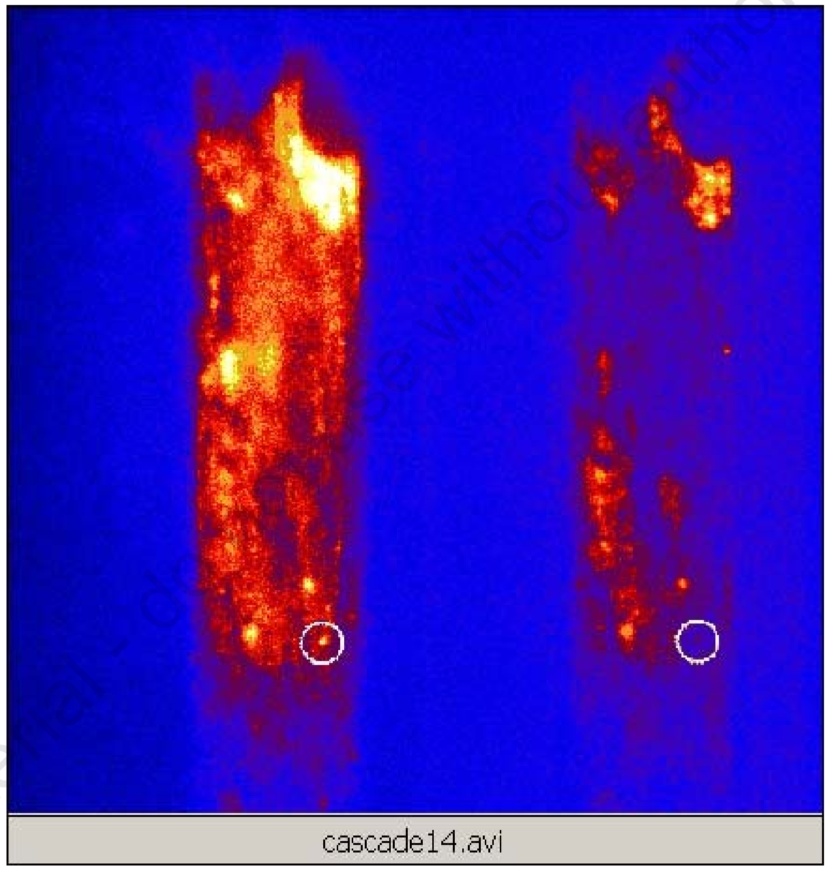


Condition: DIV 8 Over night starvation with GAM, No NGF or Qdot NGF

Colocalization of TrkAEGFP and Qdot

Cell body

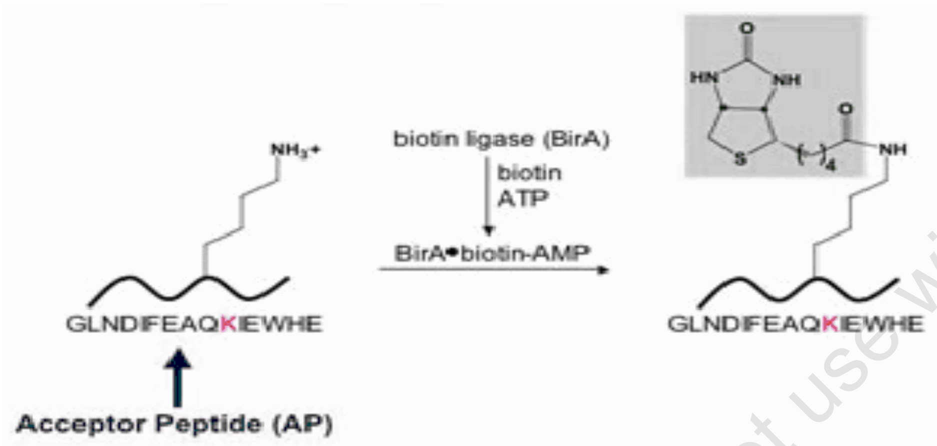
Axon

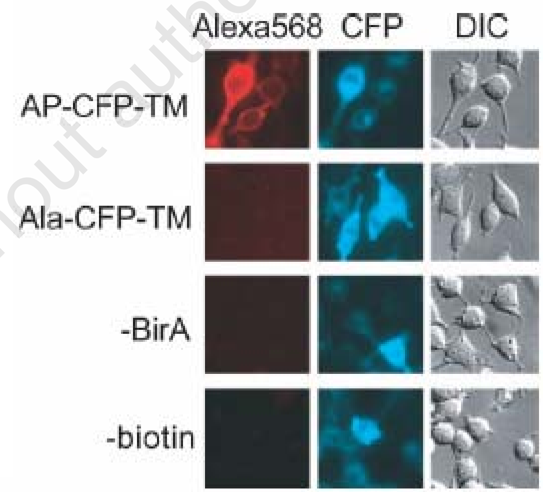


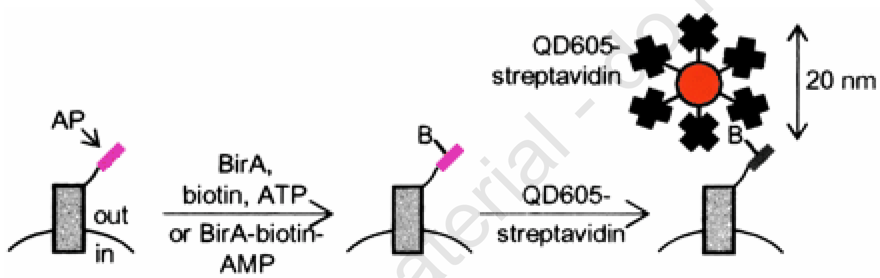
TrkA_EGFP QD605

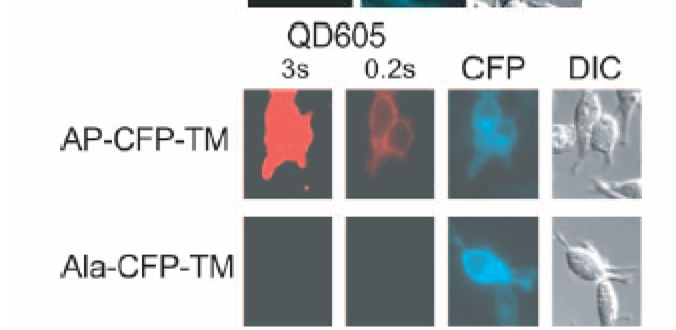
Condition: DIV 8 Over night starvation, add 120ng/ml QdotNGF

Targeting quantum dots to surface proteins in living cells with biotin ligase



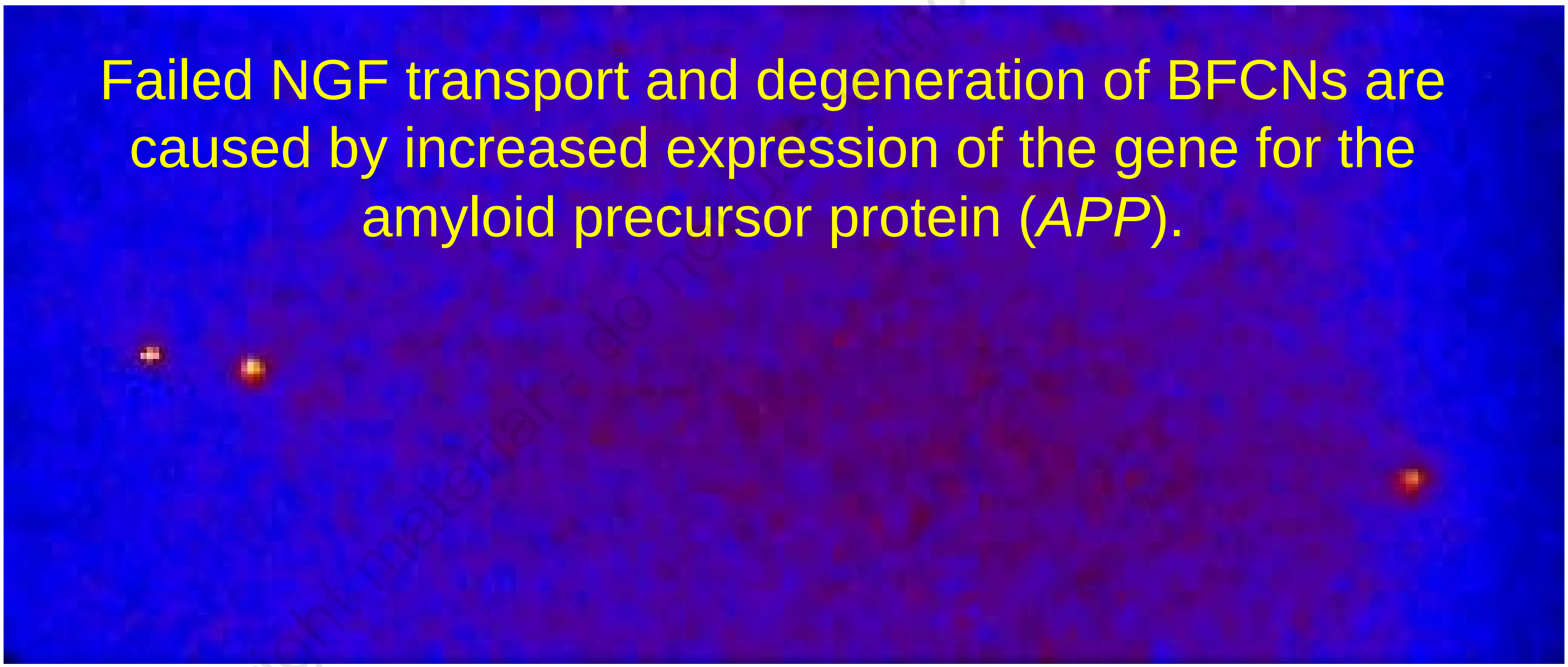




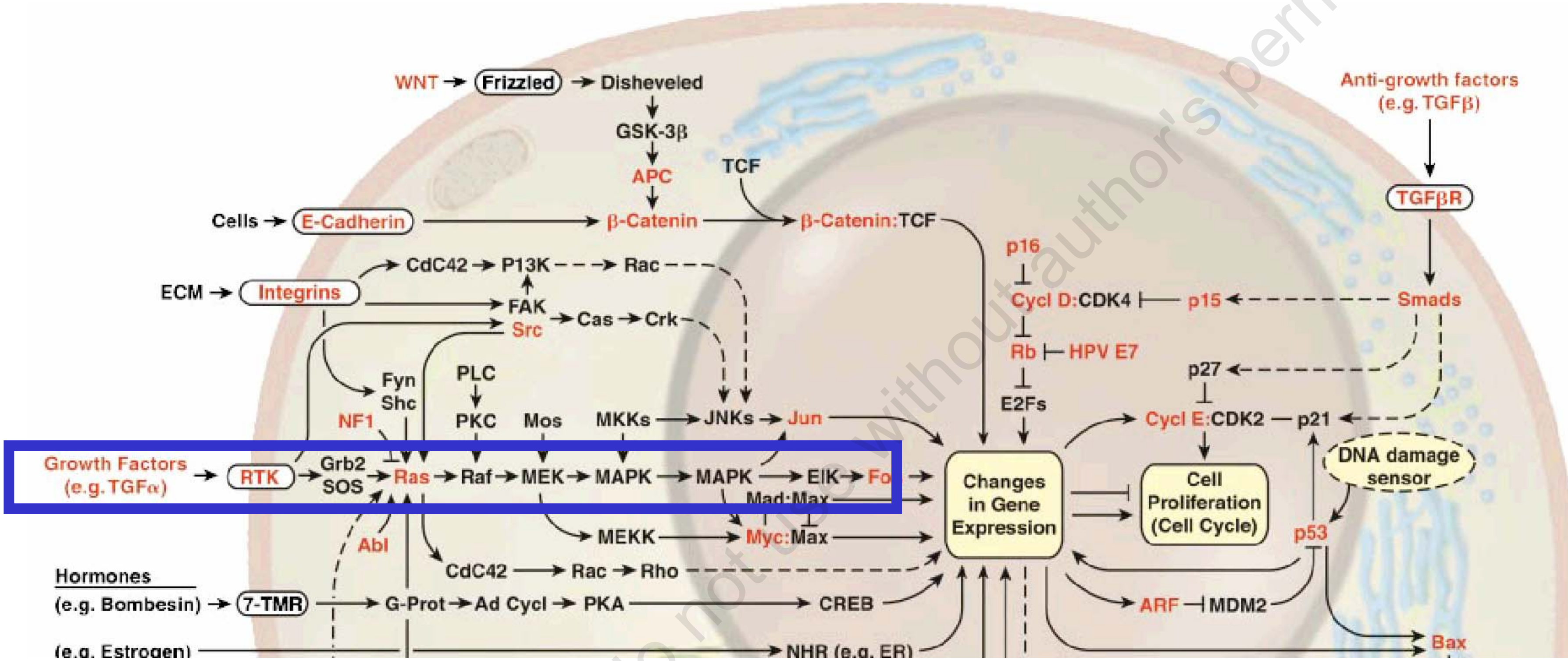


Mark Howarth, Alice Y. Ting etc. *PNAS* May 24, 2005

Alzheimer's disease: Degeneration of neurons, including basal forebrain cholinergic neurons (BFCNs). BFCN atrophy contributes to loss of attention and memory. Loss of BFCNs is linked to failed retrograde axonal transport of nerve growth factor (NGF).



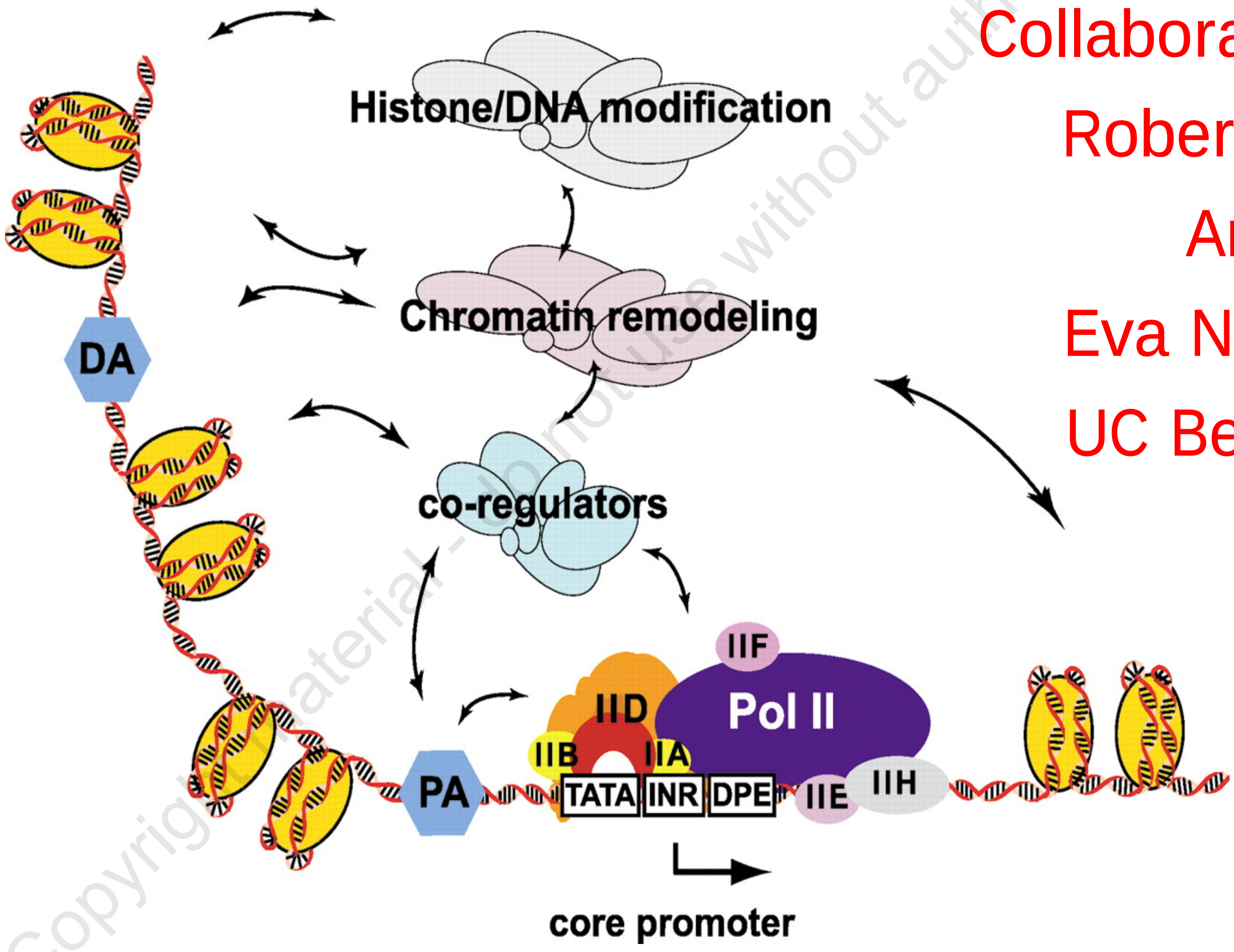
Cellular signaling networks



“Deregulation of the EGFR-MEK-ERK signaling networks contributes to the initiation or progression of many, if not most, human carcinomas.”

Joe Gray, Assoc. Berkeley Lab Director, Life Sciences, Professor, UCSF

Control of transcription is a complex process: proximal and distal promoters, co-regulators, chromatin and histone modifications



Collaboration with

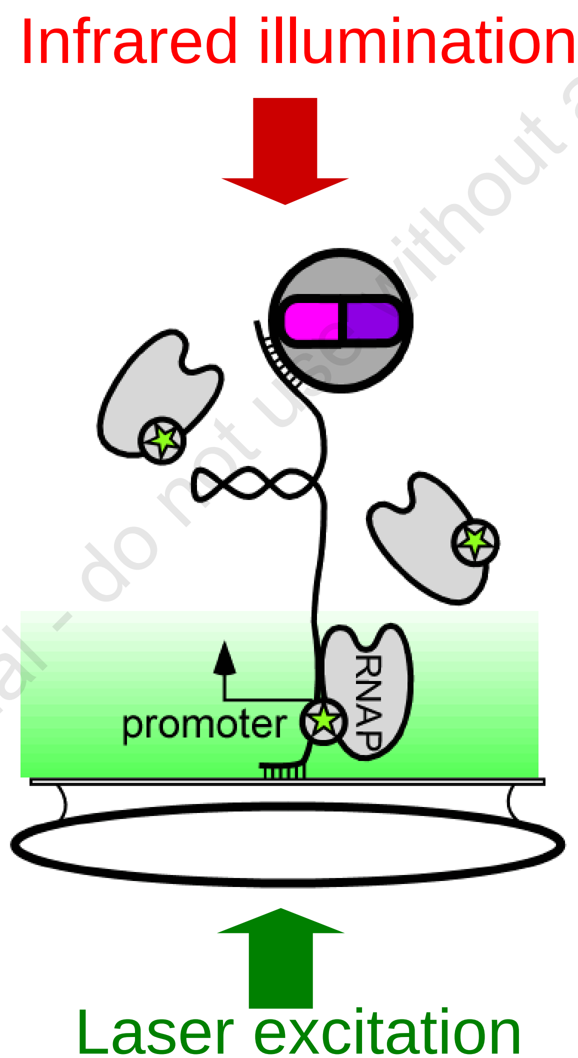
Robert Tjian

And

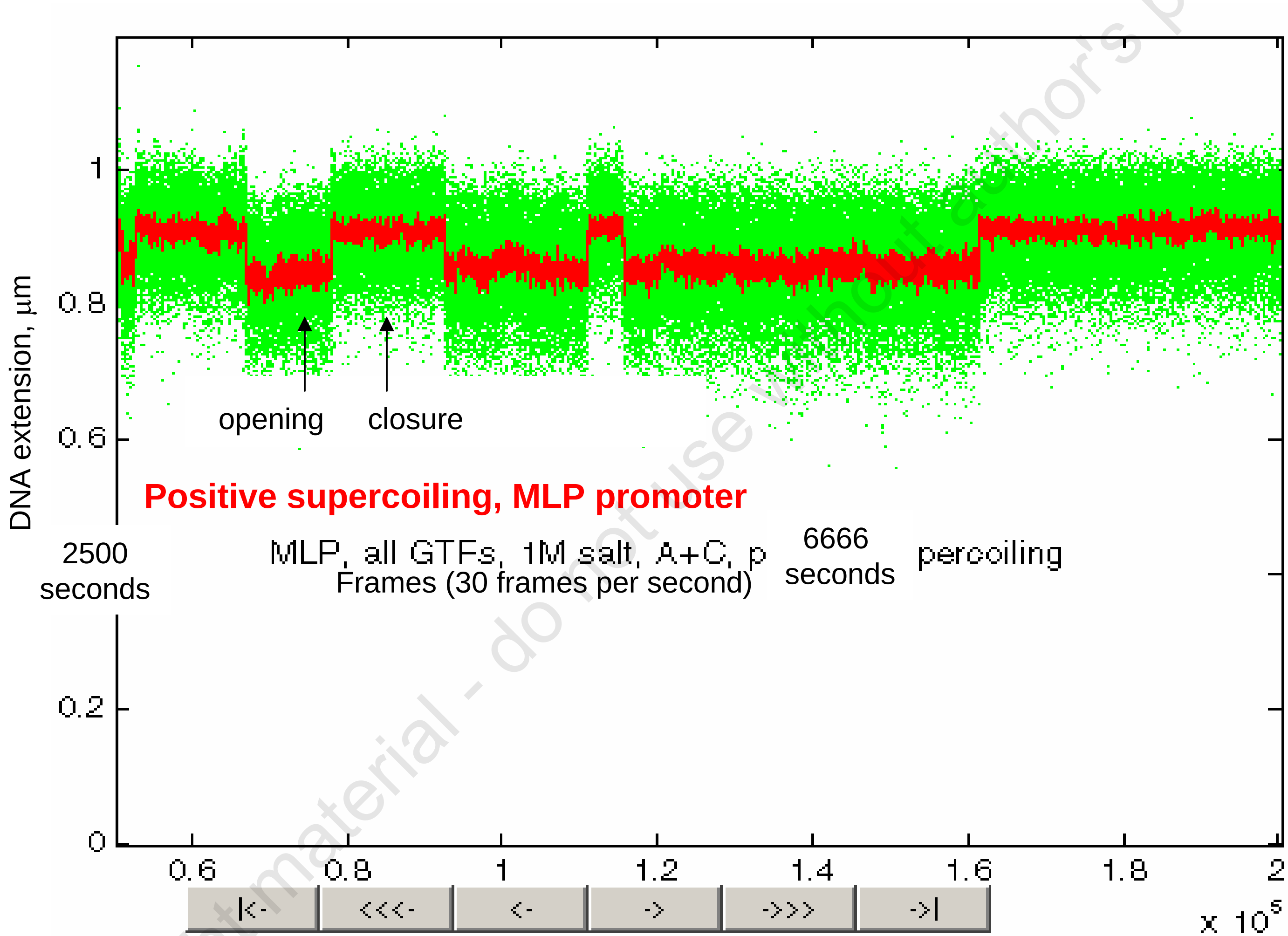
Eva Nogales,

UC Berkeley

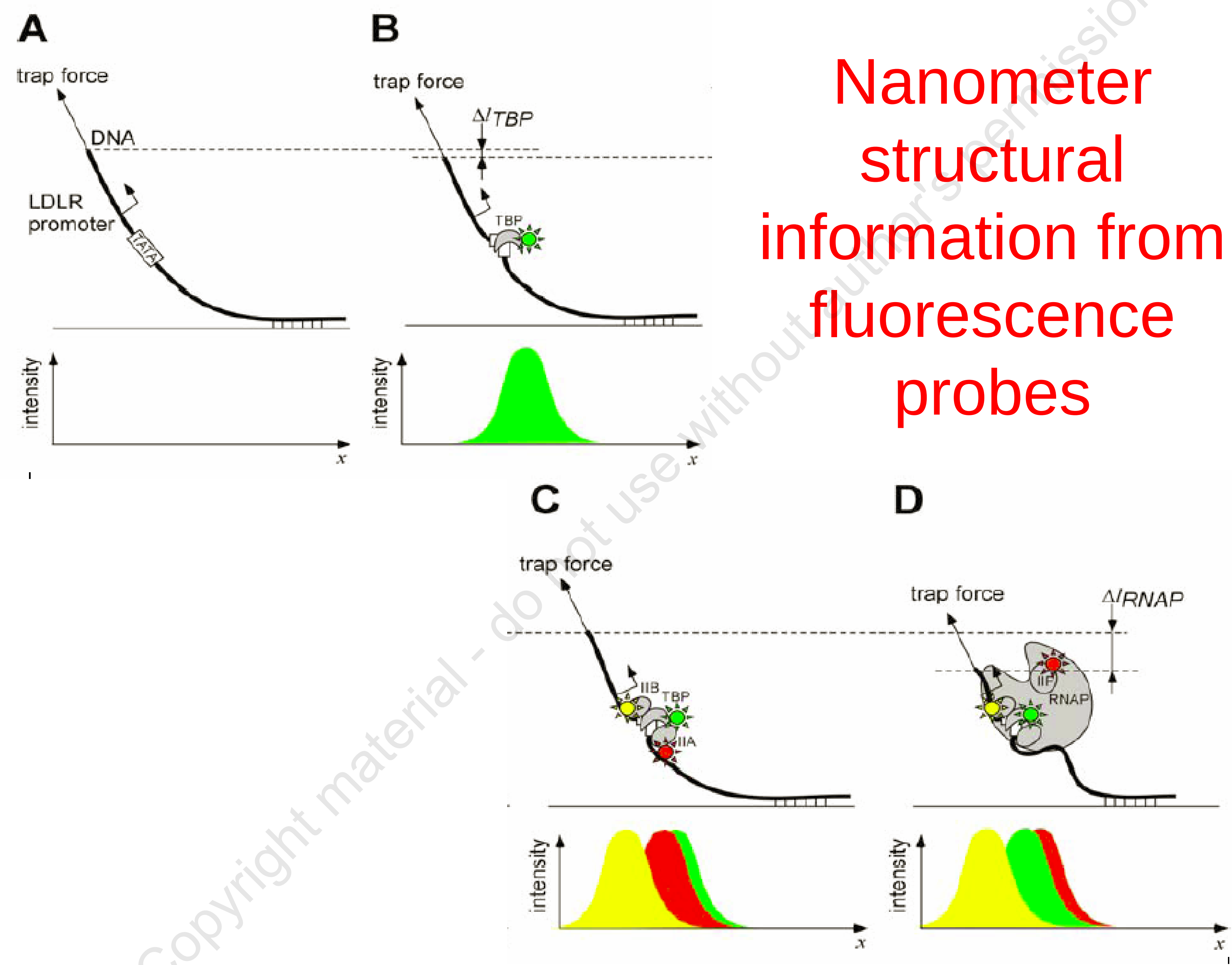
Promoter melting and promoter binding by fluorescent *E coli* RNAP

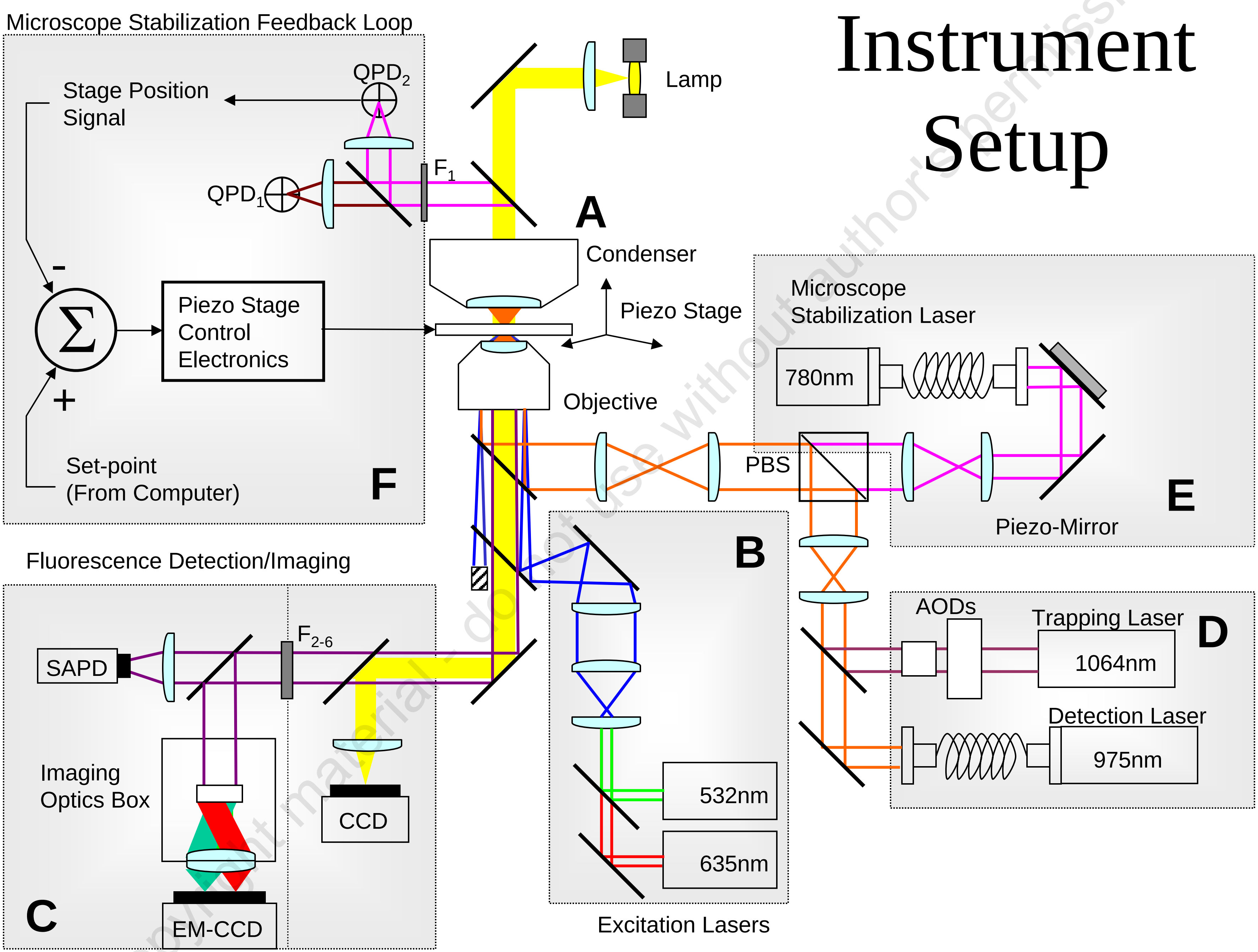


Single-molecule detection of pol II activity



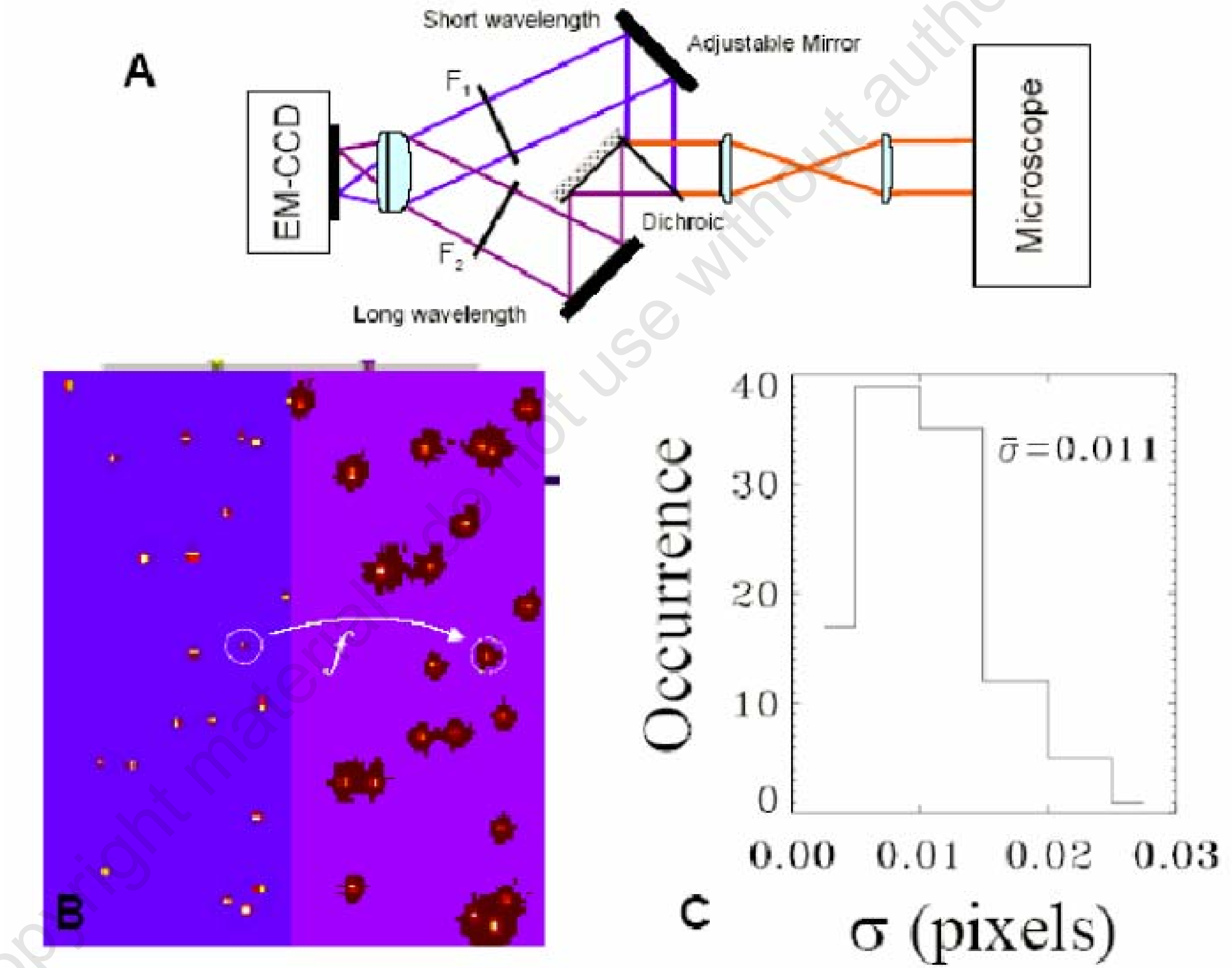
Nanometer structural information from fluorescence probes



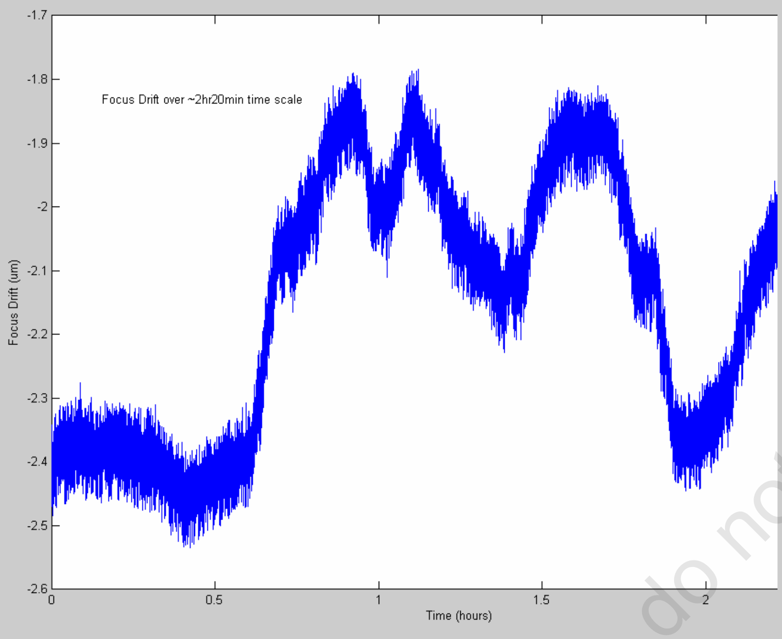


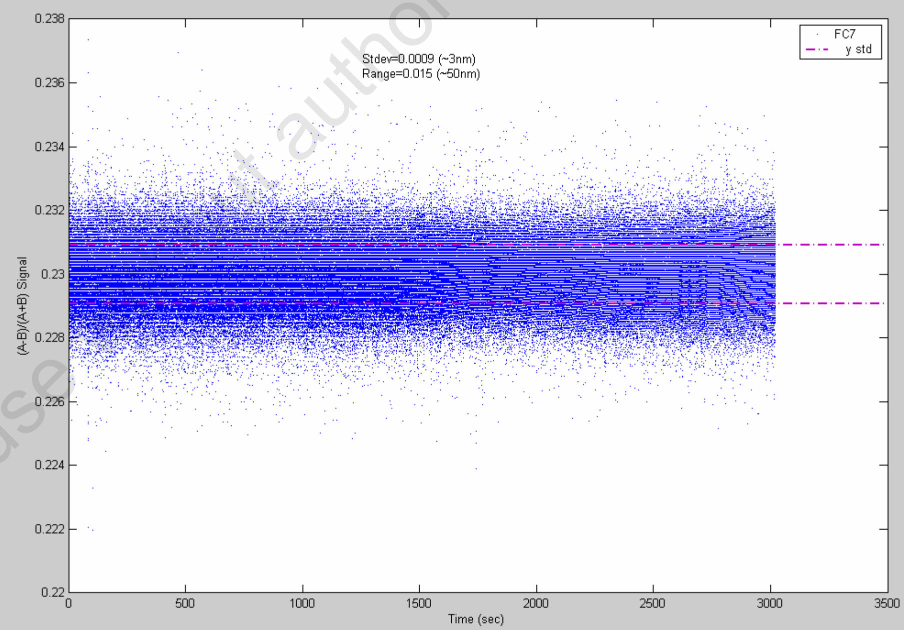
Instrument Setup
Microscope Stabilization Feedback Loop
Stage Position Signal
QPD2
QPD1
F1
Lamp
A
Condenser
Piezo Stage
-
+
Σ
Piezo Stage Control Electronics
Set-point (From Computer)
F
Objective
Microscope Stabilization Laser
780nm
PBS
E
Piezo-Mirror
B
Fluorescence Detection/Imaging
F2-6
SAPD
Imaging Optics Box
CCD
EM-CCD
C
AODs
Trapping Laser
D
1064nm
Detection Laser
975nm
532nm
635nm
Excitation Lasers

Mapping of bead positions between green and red images
Current progress: ~ 0.8 nm resolution



Microscope Stage Stability





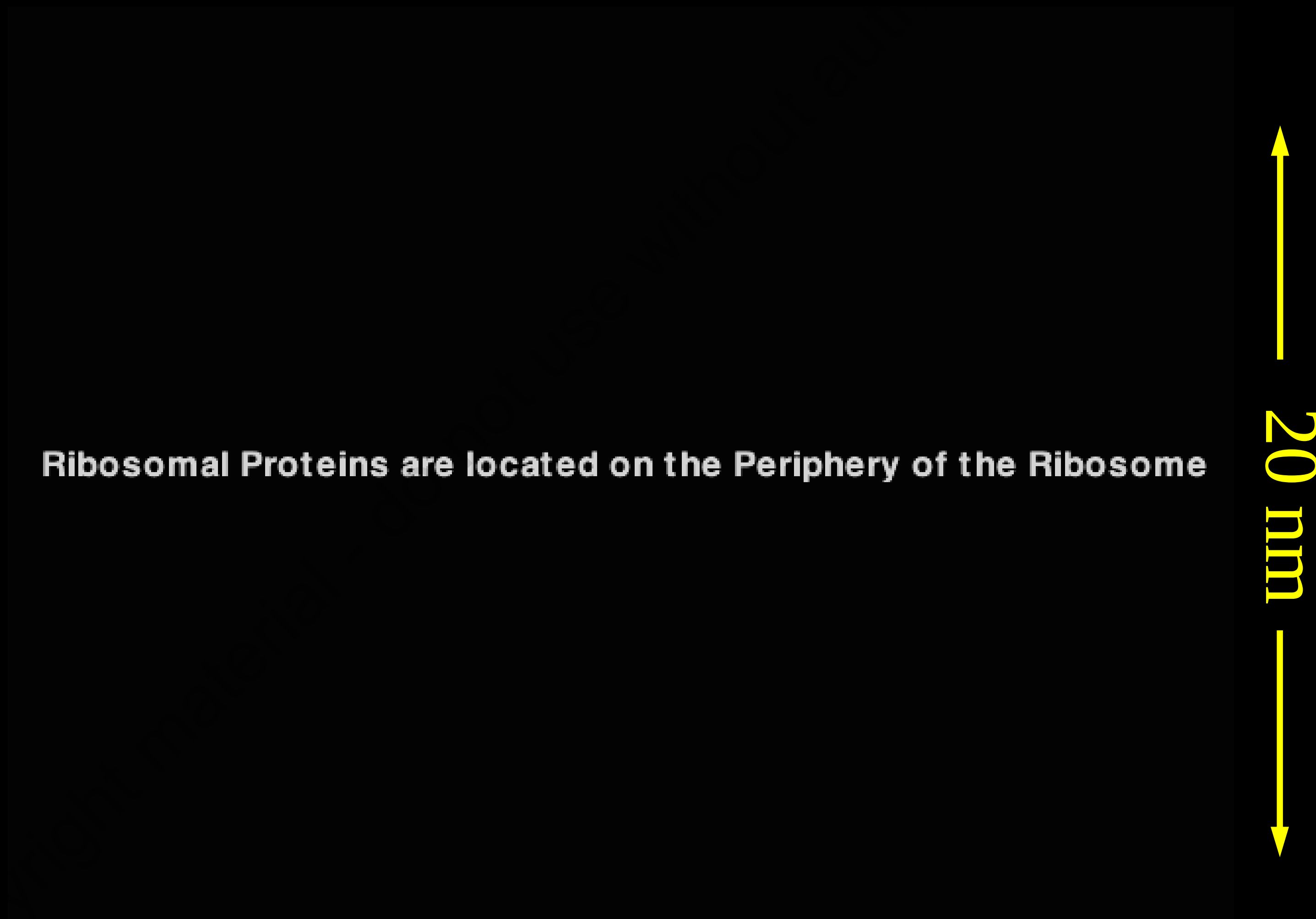
Correction signal

Error signal

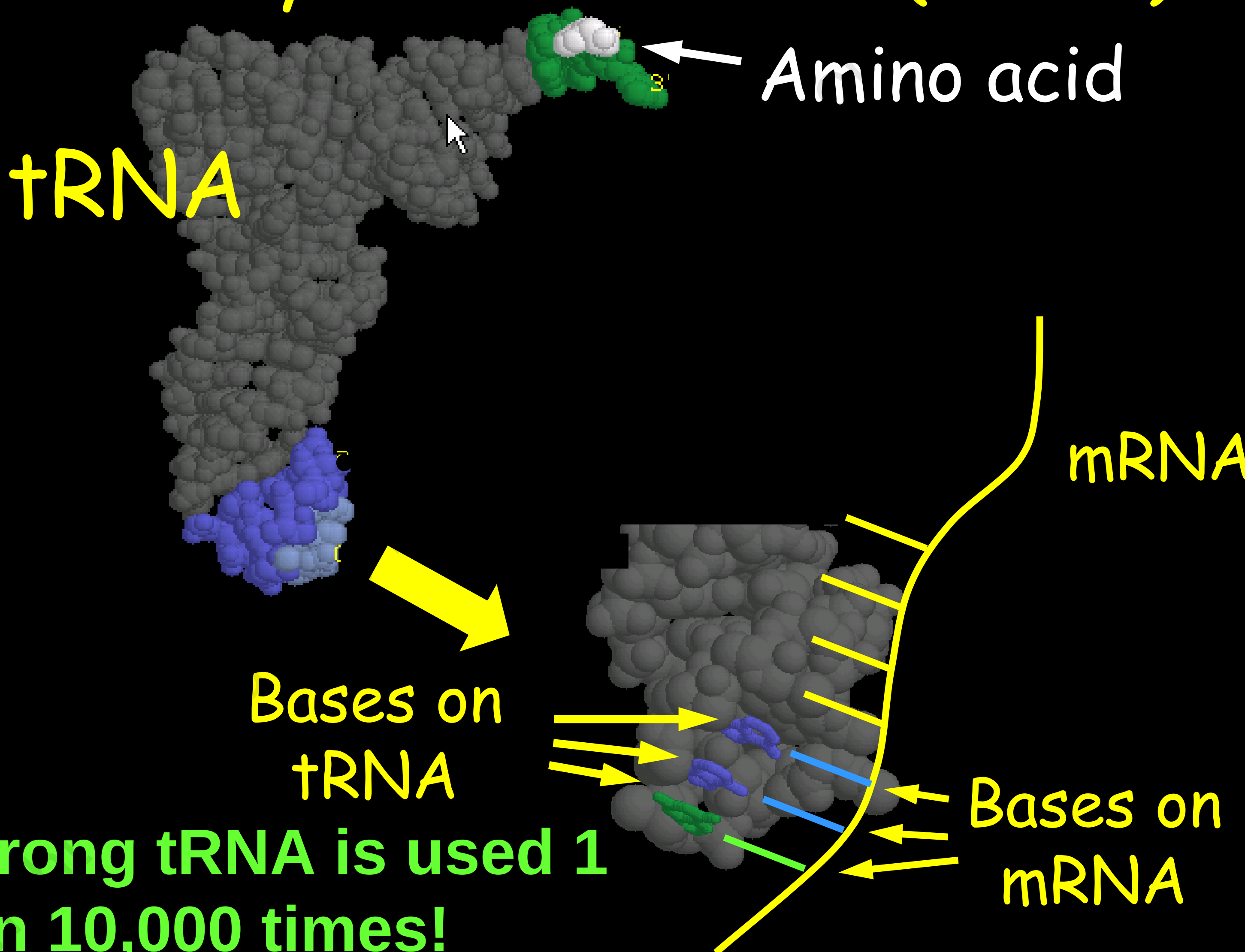
~1nm stability in X/Y, 3nm in Z.

The ribosome is an RNA enzyme !

From Harry F. Noller, Marat M. Yusupov, Gulnara Z. Yusupova, Albion Baucom and J.H.D. Cate; *FEBS Letters* (2002)



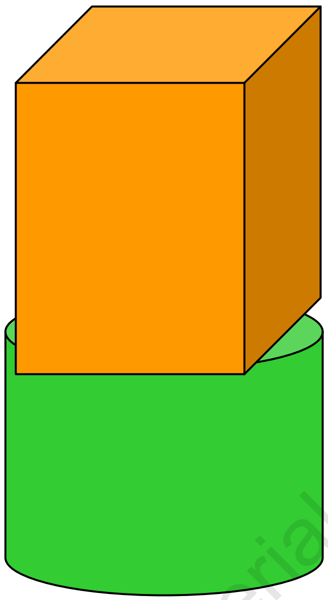
Each amino acid is brought into the ribosome by transfer RNA (tRNA)

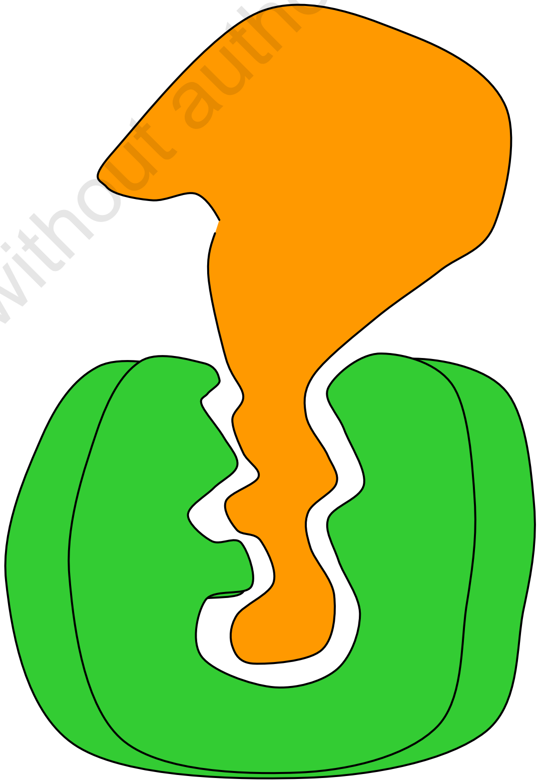


The wrong tRNA is used 1 in 10,000 times!

Selection based on shape discrimination

J. Davies, W. Gilbert, L. Gorini (1964)

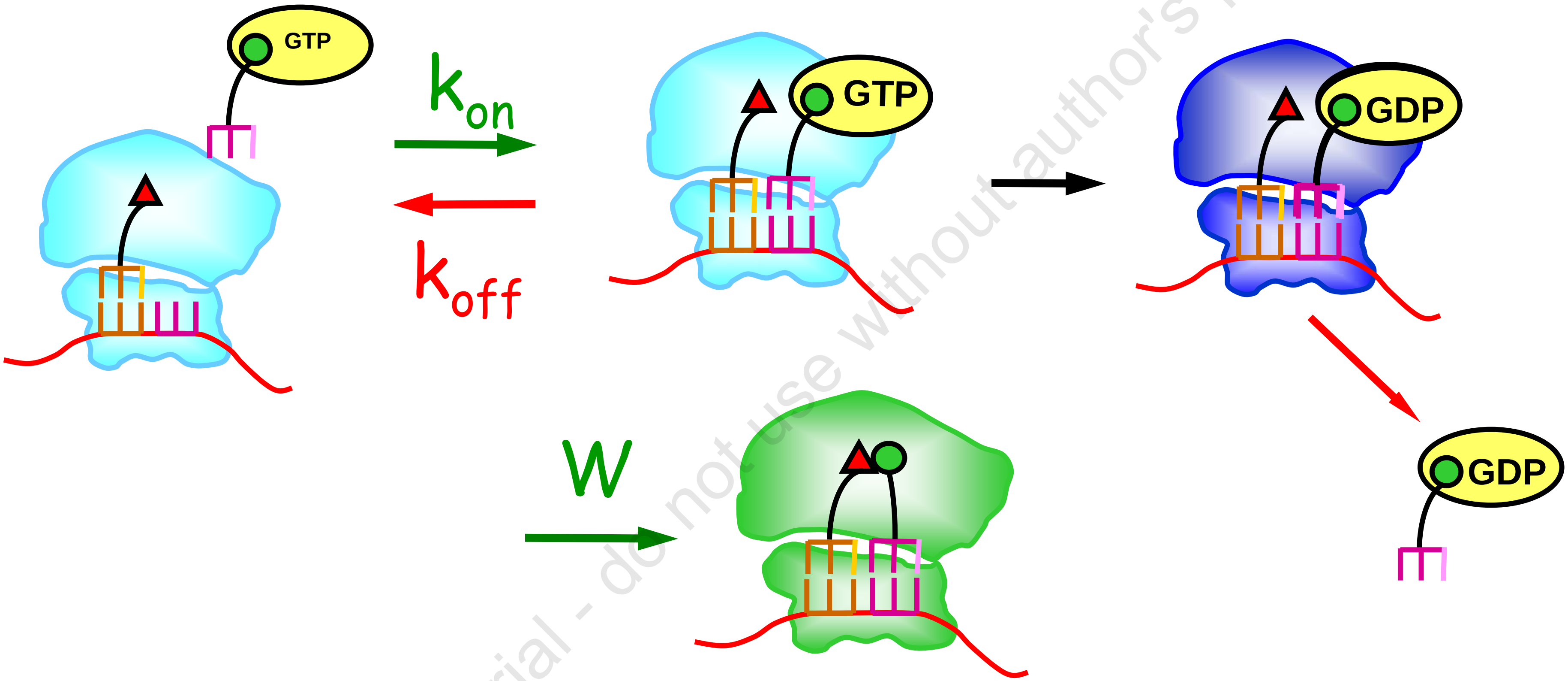




Shape selection can also be due to an "induced fit".

"Kinetic Proofreading" model

J. Hopfield (1974)



If the probability of making a mistake in the selection and proof-reading steps is 1%, then the overall probability is 0.01 x 0.01 = 0.0001%.

Previously published work

- tRNA dynamics on the ribosome
  S. Blanchard, H. Kim, R. Gonzalez, J. Puglisi, S. Chu
  PNAS 101, 12893–12898 (2004).

- tRNA selection and kinetic proofreading in translation
  S. Blanchard, R. Gonzalez, H. Kim, S. Chu, J. Puglisi
  Nature Structural Biology **11**, 1008 - 1014 (2004).

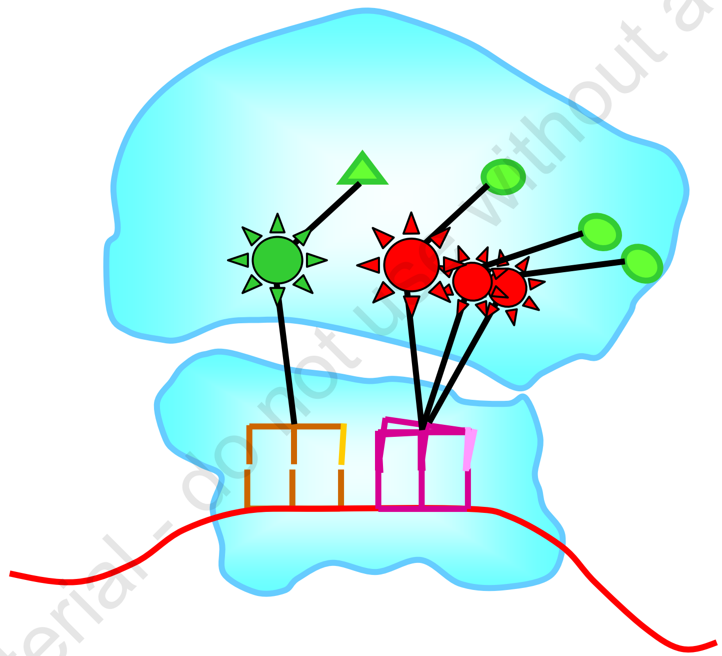
- The role of fluctuations in initial selection by the ribosome: *submitted 2007*, T-H. Lee, S. Blanchard, H. Kim, J. Puglisi, S. Chu

- Direct observation of allostery during aminoacyl-tRNA selection by the ribosome: submitted 2007 R. Gonzalez, S. Chu, J. Puglisi

- tRNA fluctuations between classical and hybrid states submitted, 2007, H. Kim, J. Puglisi, S. Chu

- Force measurements on the ribosome and a new allostery: *Nature, 2007*, U. Sotaro, M. Dorywalska, T-H. Lee, J. Puglisi, S. Chu

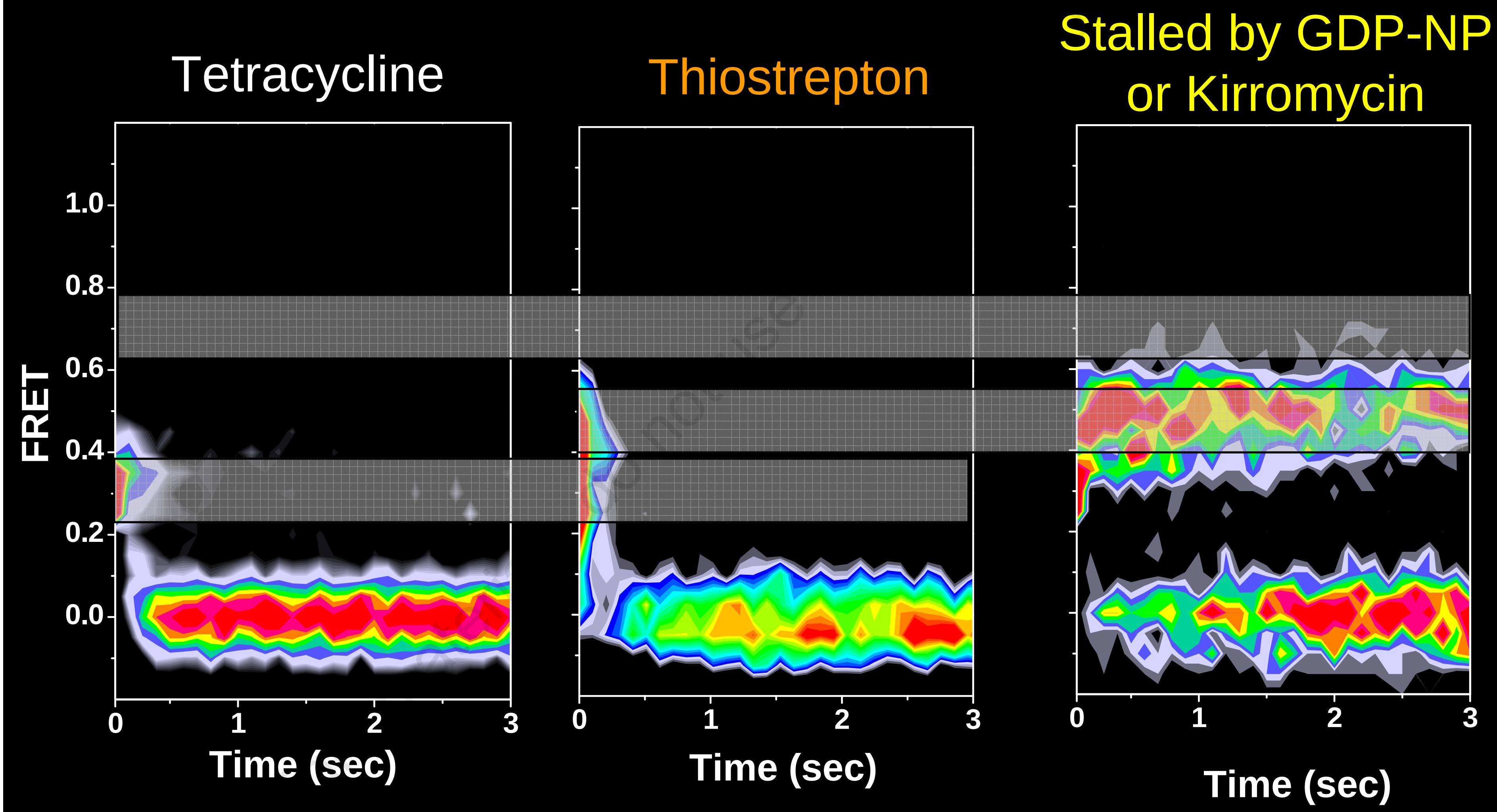
Watching t-RNA enter the ribosome using **single molecule** Fluorescence Resonant Energy Transfer (FRET)



S. Blanchard, H. Kim, R. Gonzales, T-H. Lee, J. Puglisi, S. Chu

PNAS, 2005; *Nat. Struct. Bio*, 2004

Separate steps in accommodation are seen by stalling the ribosome



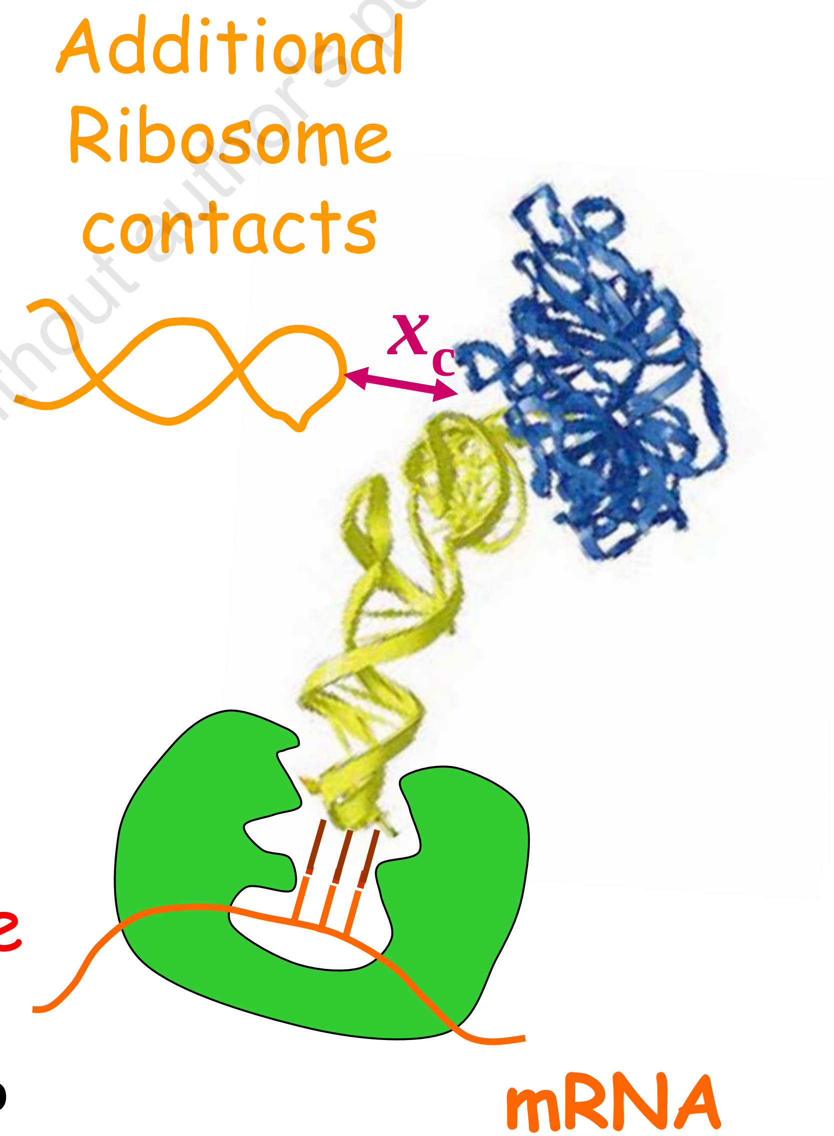
Our proposed selection mechanism

1) Proper base-paring causes the ribosome to wrap around the base of the tRNA

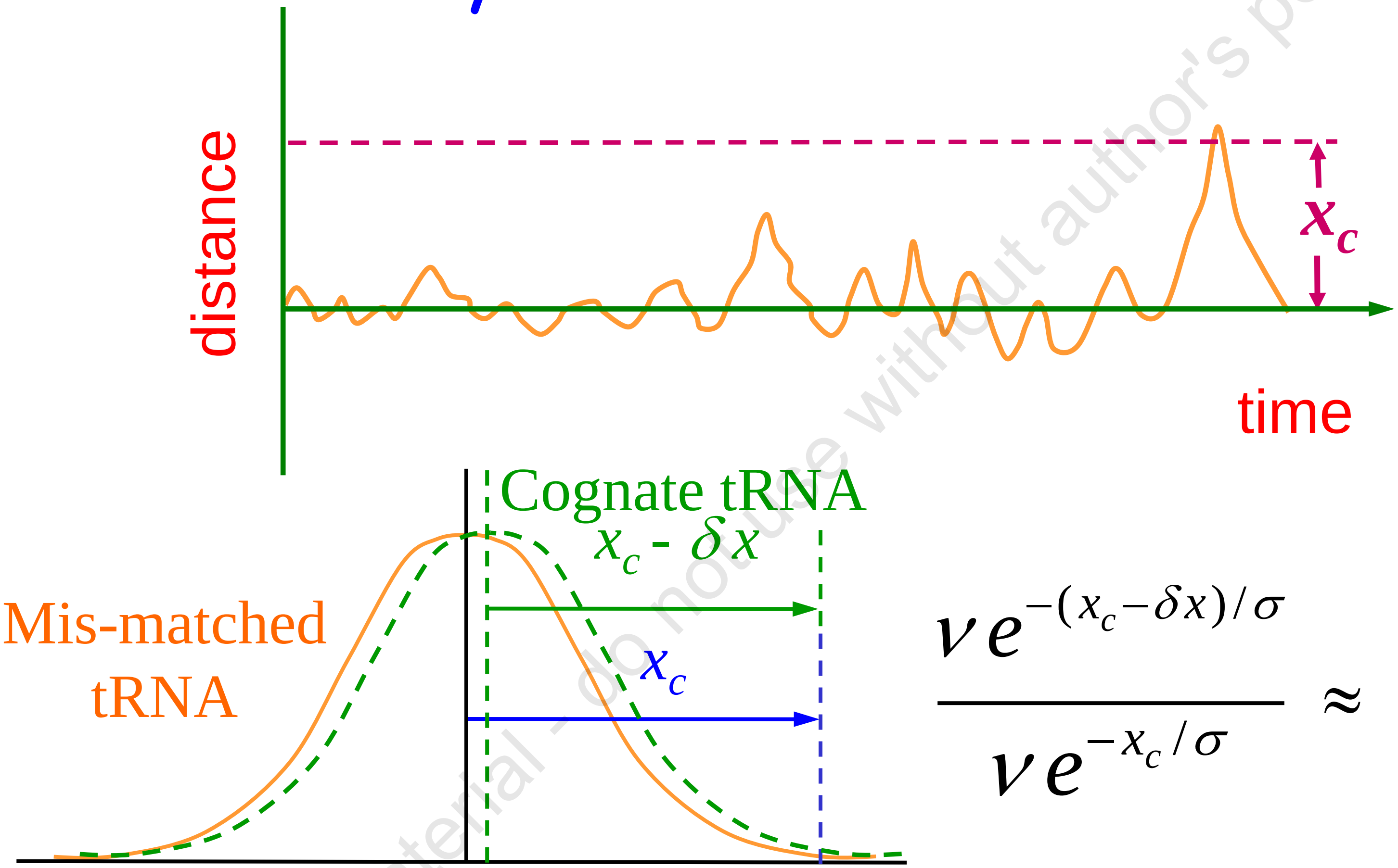
V. Ramakrishnan, *et al.* 2001

2) The wrapping of the ribosome causes the tRNA to move into a position so it is more likely to make stabilizing contacts with the Ribosome.

Tae-Hee Lee, et. al. submitted to PNAS, 2007



If you were an Intelligent Designer, how would you make the ribosome?

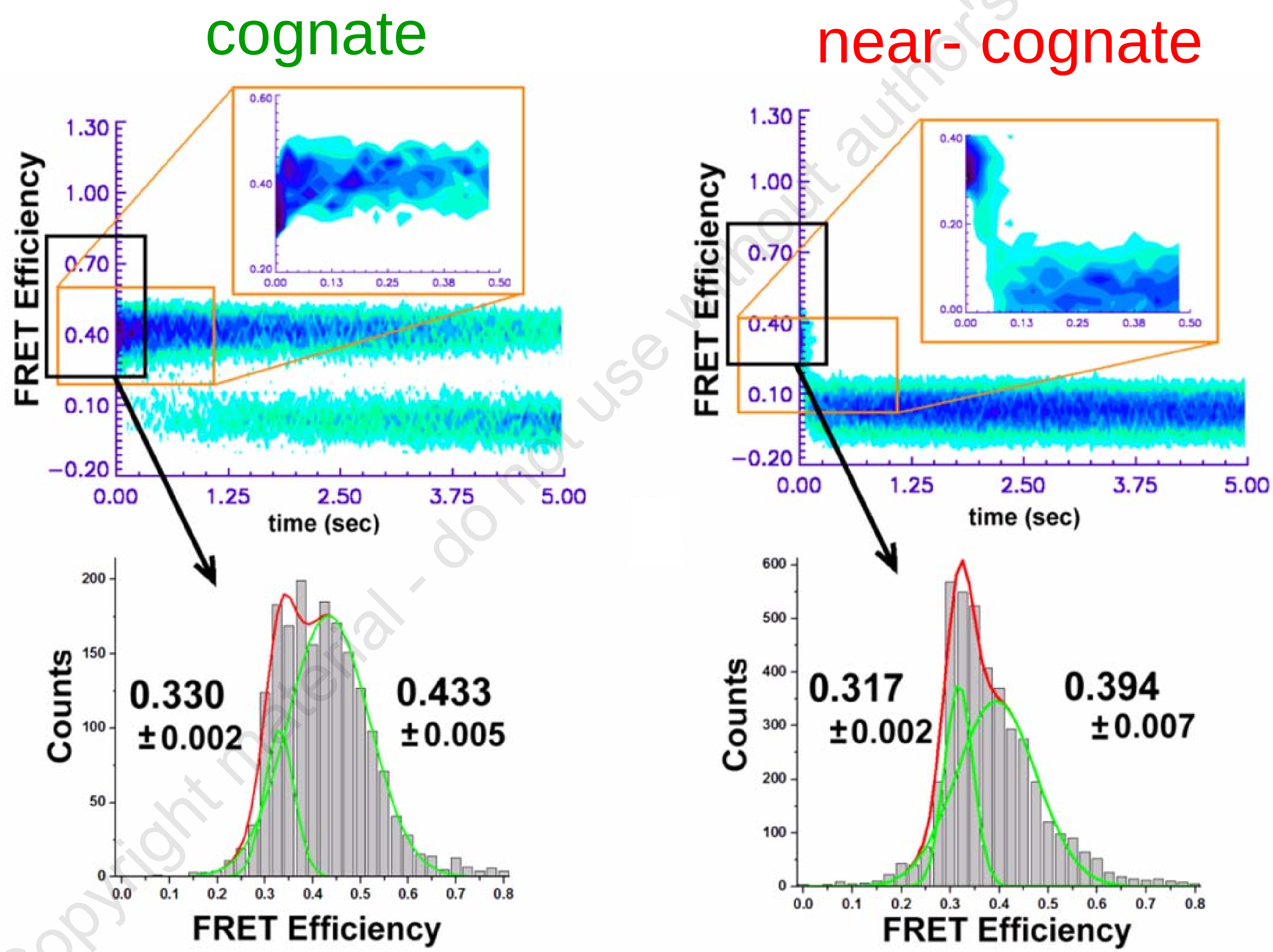


$$\frac{\nu e^{-(x_c - \delta x)/\sigma}}{\nu e^{-x_c/\sigma}} \approx e^{+\delta x/\sigma}$$

Prediction: the initial 0.3 FRET states of the correct tRNA and incorrect tRNA are different.

Post-synchronized FRET > 0.27

Tae-Hee Lee, at al. unpublished (2006)

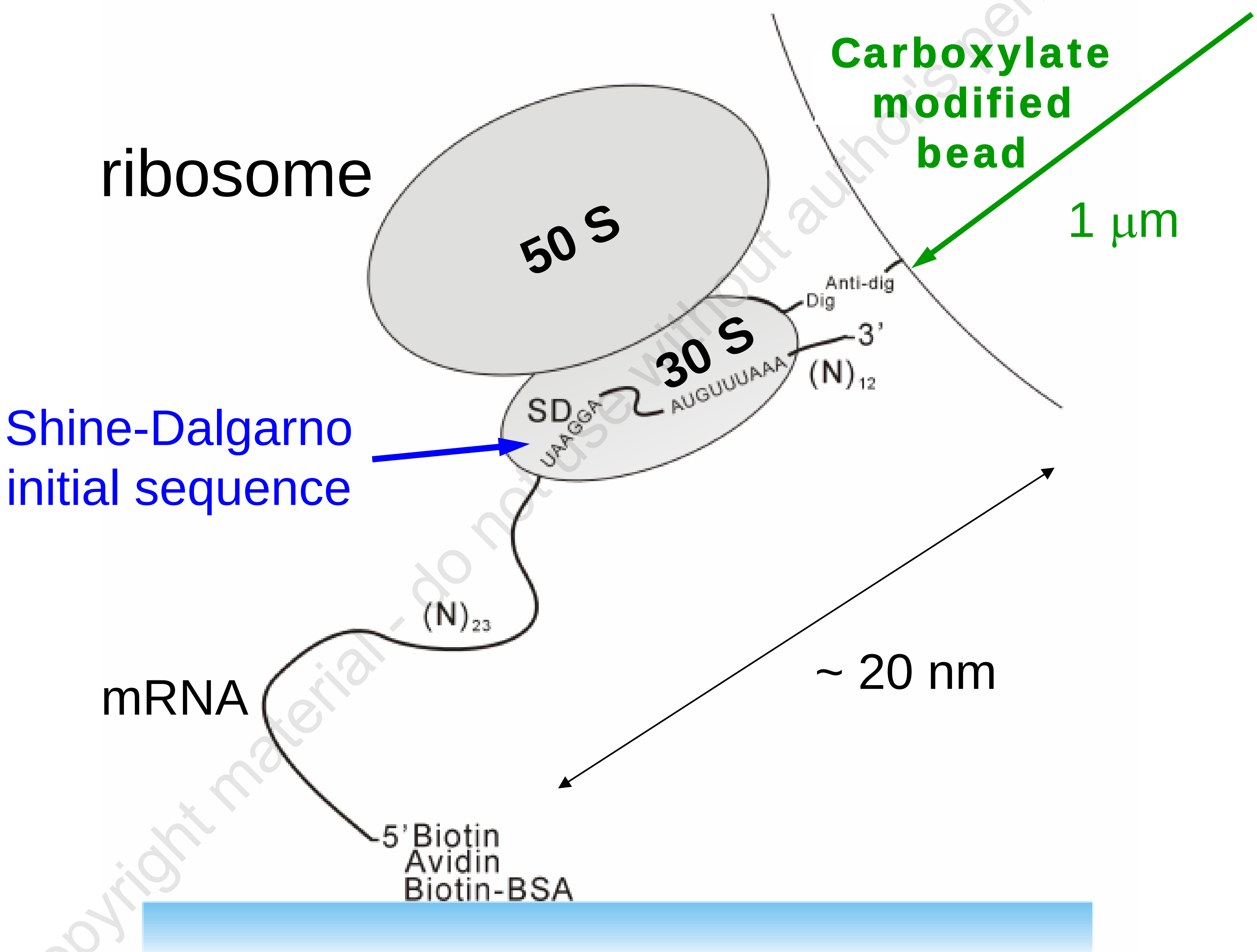


Direct force measurement between 30S in ribosome and mRNA with optical tweezers

Nature, 2007

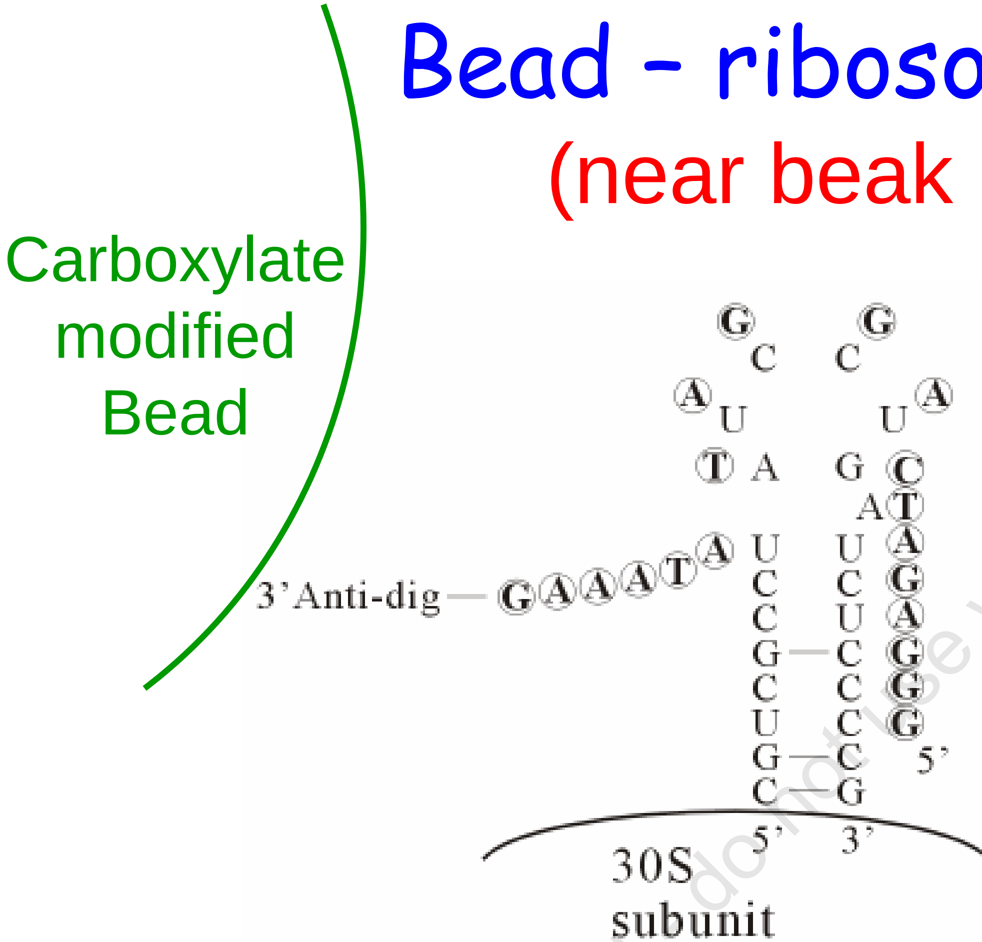
Sotaro Uemura, Magdalena Dorywalska, Tae-Hee Lee, Harold Kim, Jody Puglisi and Steve Chu

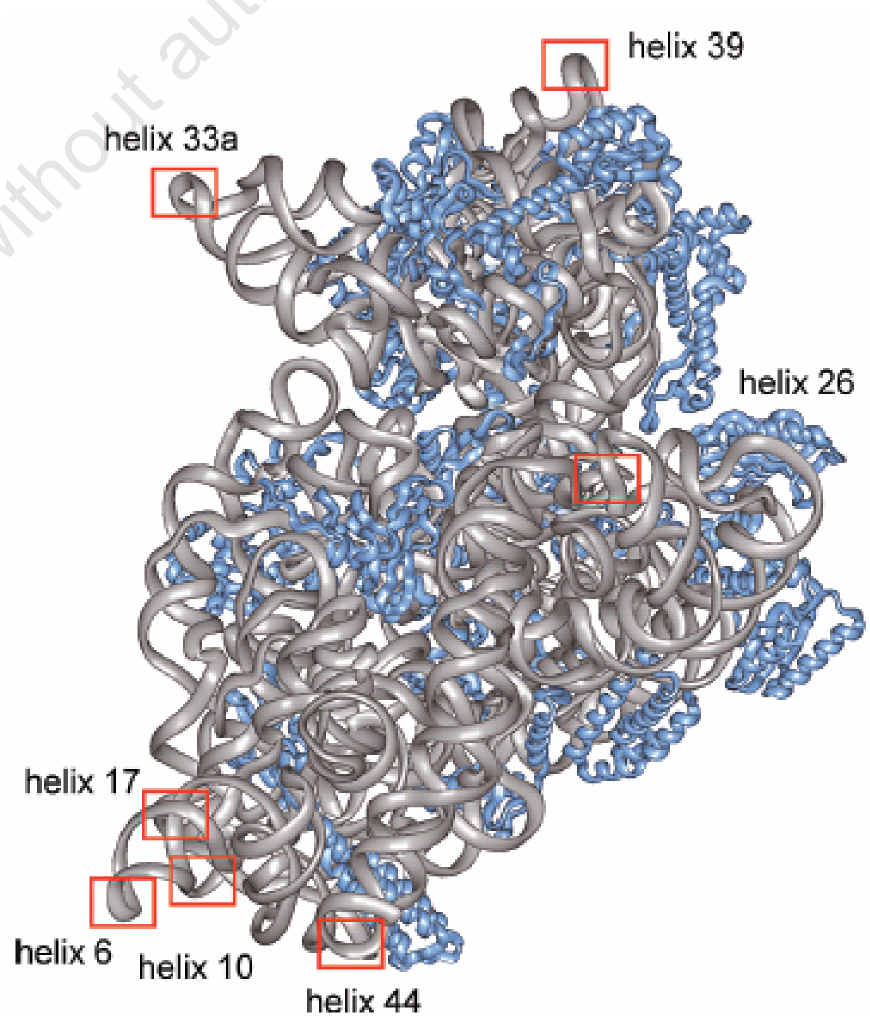
Pulling on the 30 S subunit of the Ribosome



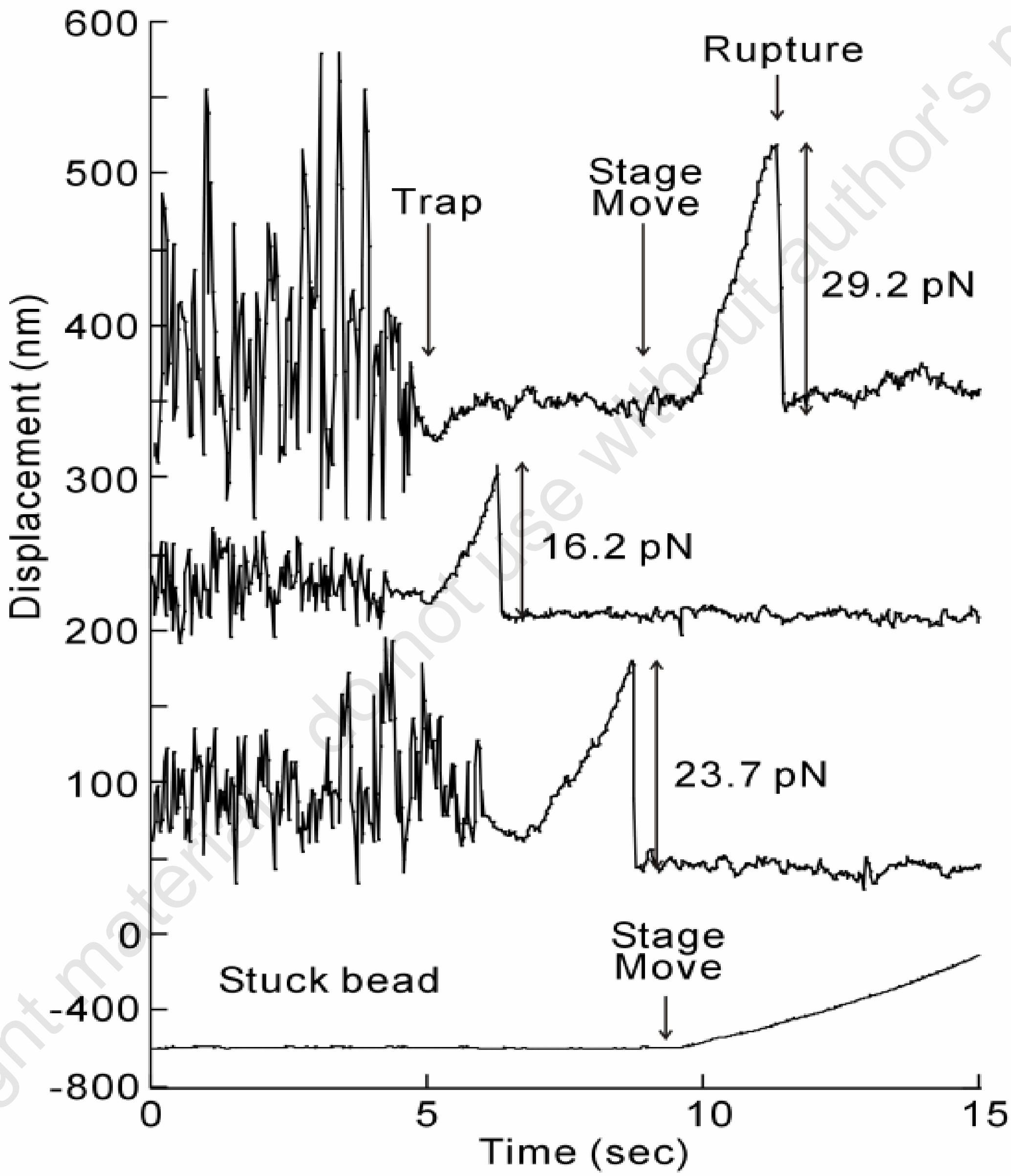
Bead - ribosome attachment

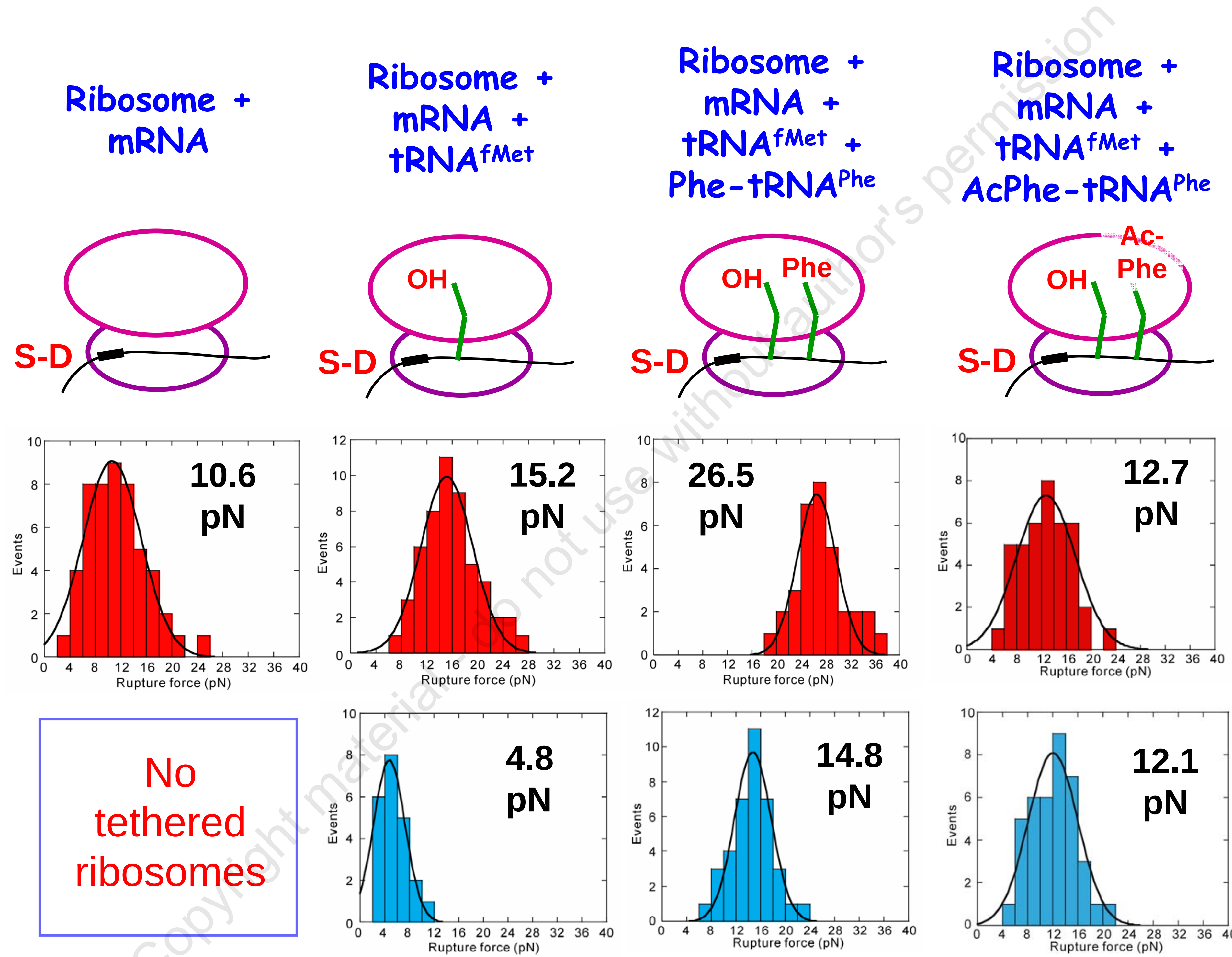
(near beak region of 30S)



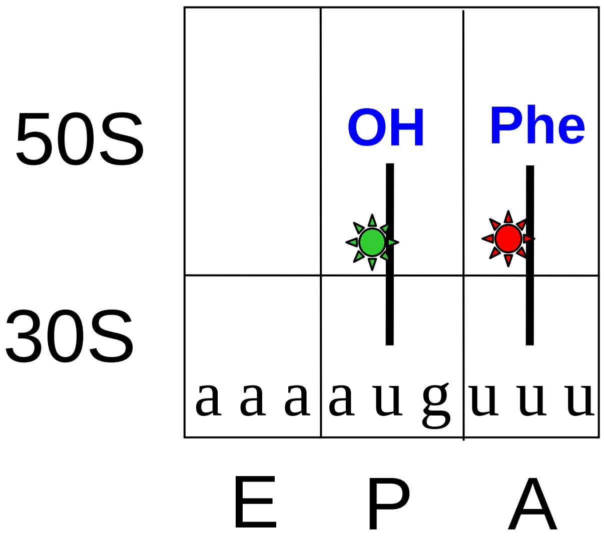


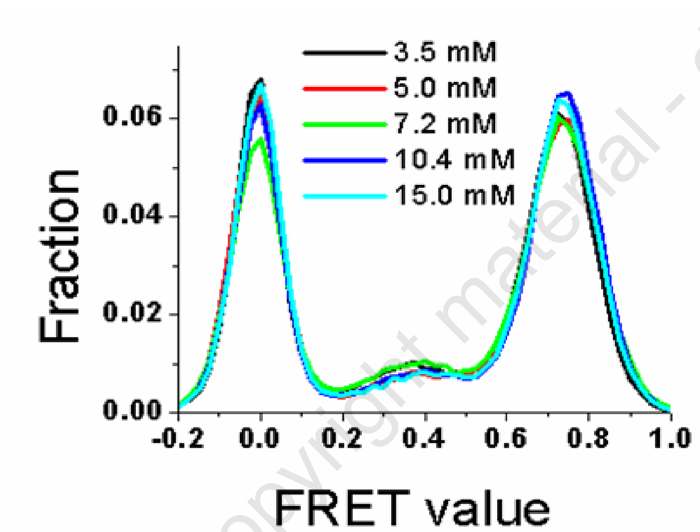
Sample force measurements



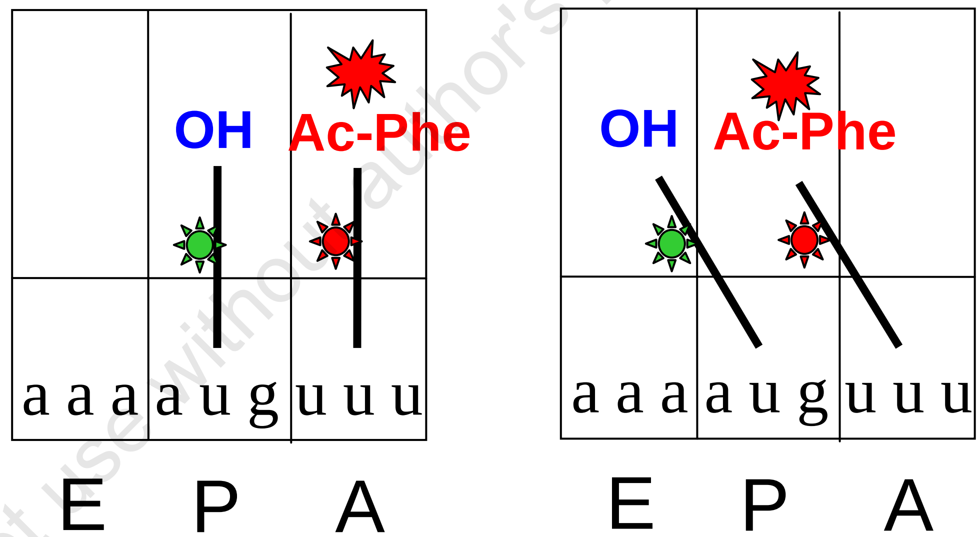

Ribosome + mRNA
Ribosome + mRNA + tRNAfMet
Ribosome + mRNA + tRNAfMet + Phe-tRNAPhe
Ribosome + mRNA + tRNAfMet + AcPhe-tRNAPhe
S-D
OH
Phe
Ac-Phe
10.6 pN
15.2 pN
26.5 pN
12.7 pN
No tethered ribosomes
4.8 pN
14.8 pN
12.1 pN
Events
Rupture force (pN)

Phe on A-site tRNA





aminoacylated Phe on tRNA



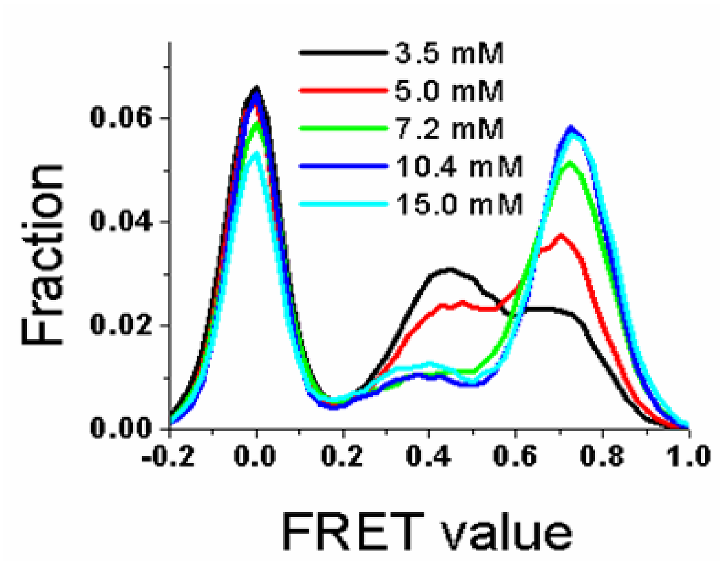
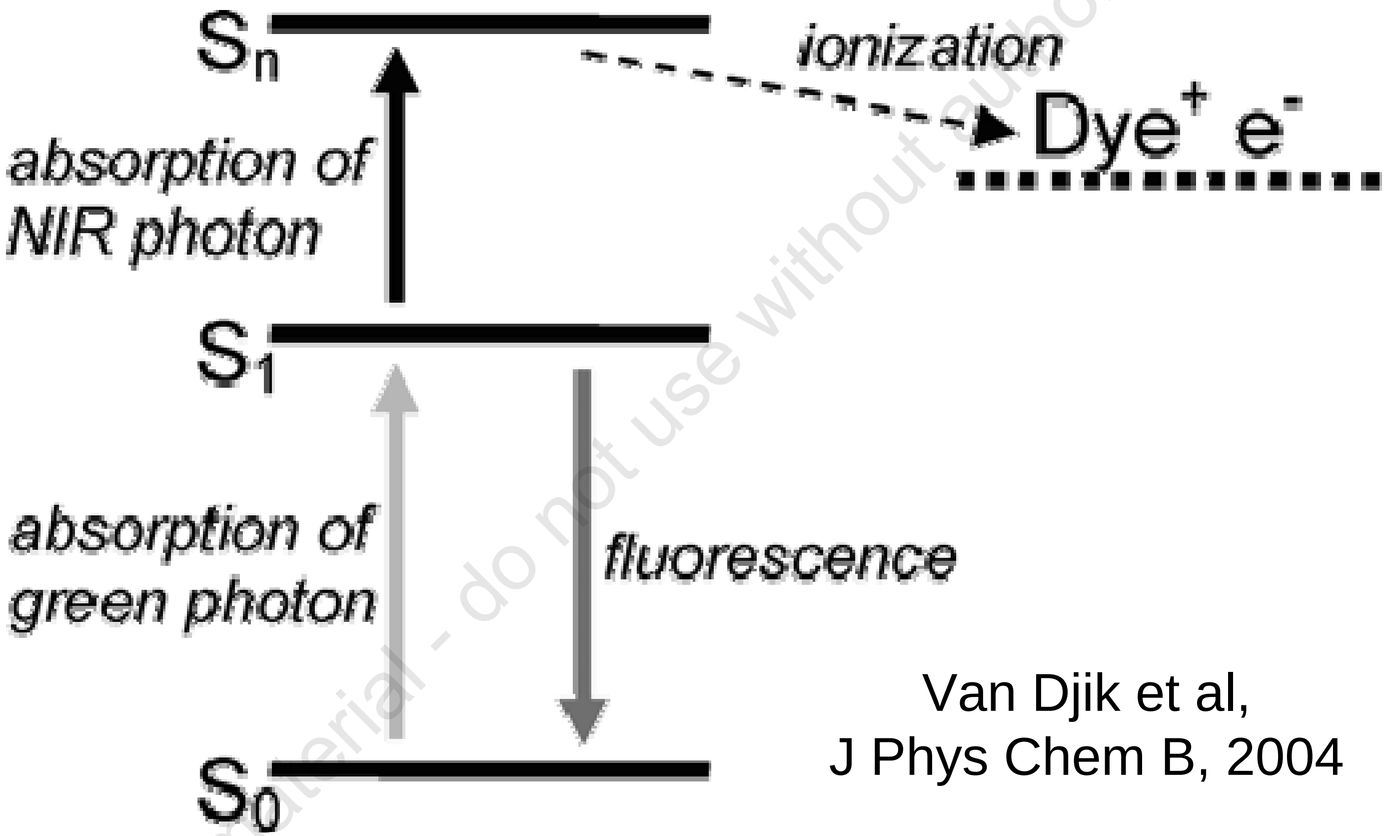


Photo-destruction Pathway



Van Djik et al,
J Phys Chem B, 2004

Enhanced Photo-bleaching from Excited State

Time-Scale Separation

- Excited-state lifetime: ~10nsec
- Viscous Relaxation Time: 100μsec-1msec
- Single molecule biology: msec-sec

Take advantage of window at ~1msec

RF Electronics Layout