Individual and population approaches to cell surface receptor motion

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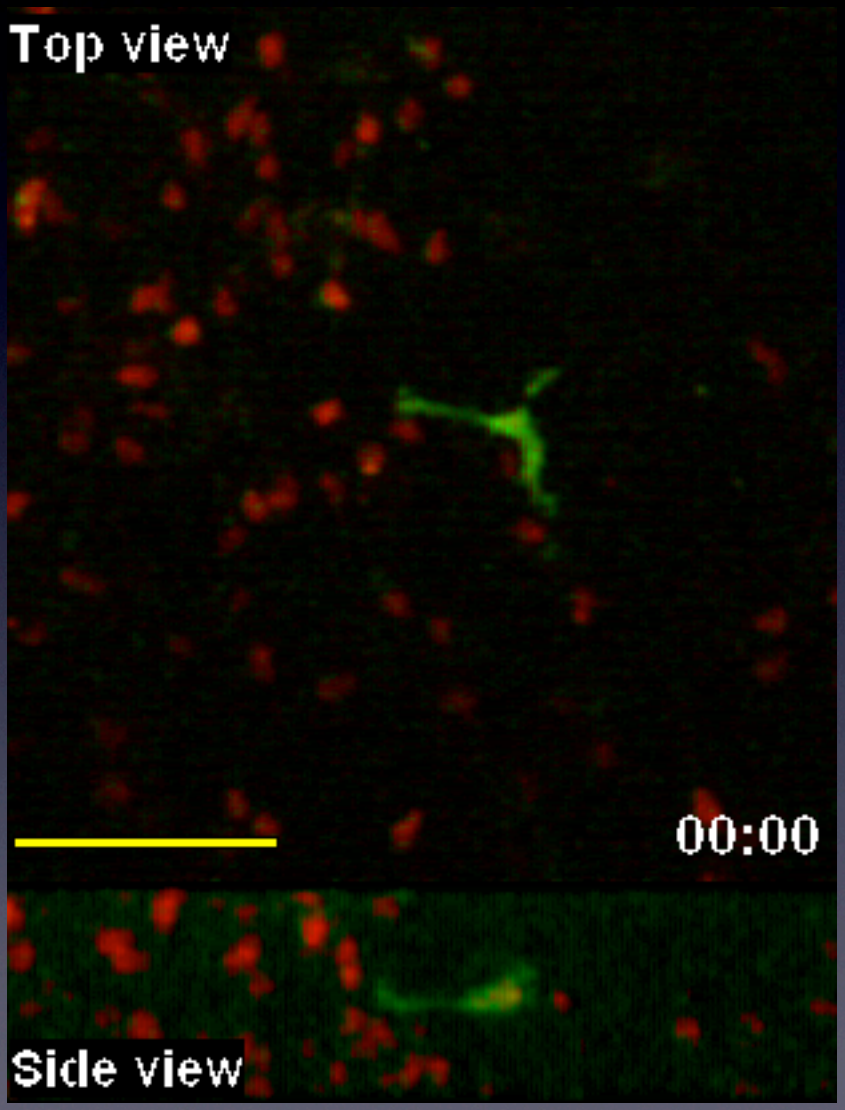
IISER 2014

- Cells sense and interact with their environment using an array of membrane-bound surface receptors
- e.g. immune cell response to foreign stimuli

- engineering effector cell responses is huge
  - intelligent vaccine design
  - immunotherapy for autoimmunity and tumours
    - monoclonals and cell therapies

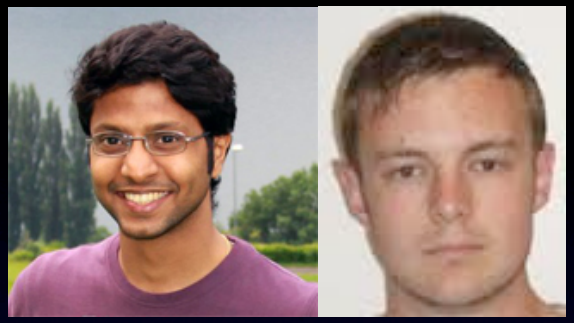
Natural Context: Immune cell patrol

- Molecular signatures of infection are presented on the surface of dendritic cells.
- T cells of the immune system patrol continuously.
- 2-photon imaging of mouse lymph node.



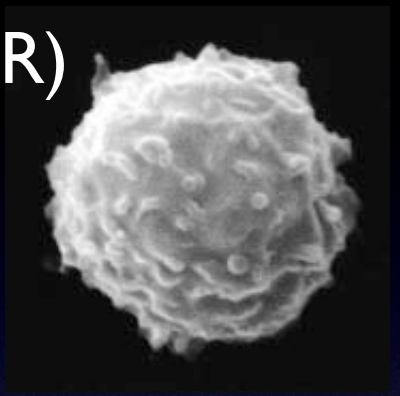
Video: Miller/Cahalan Nature 2004

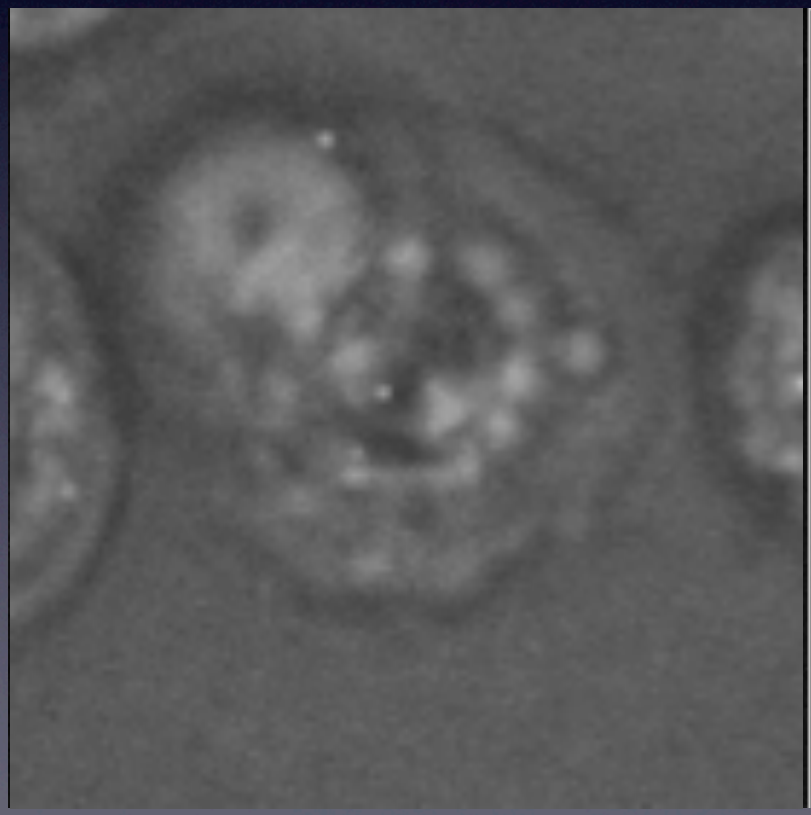
Visualizing receptor mobility

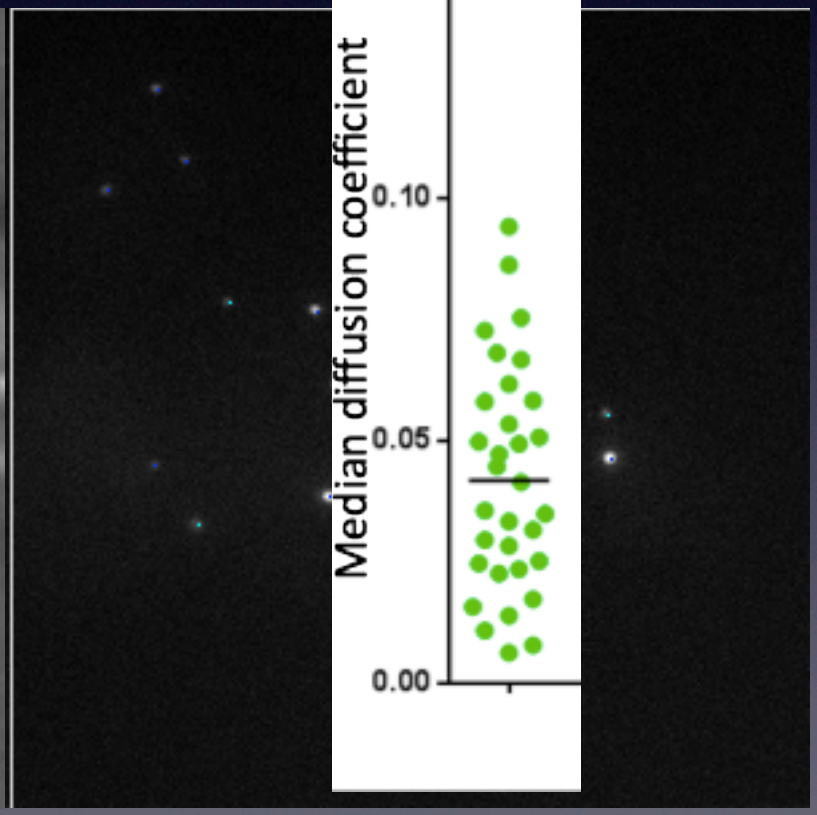


Libin Abraham Josh Scurll

Single B cell receptors (BCR) labelled on
B cells stimulated with LPS

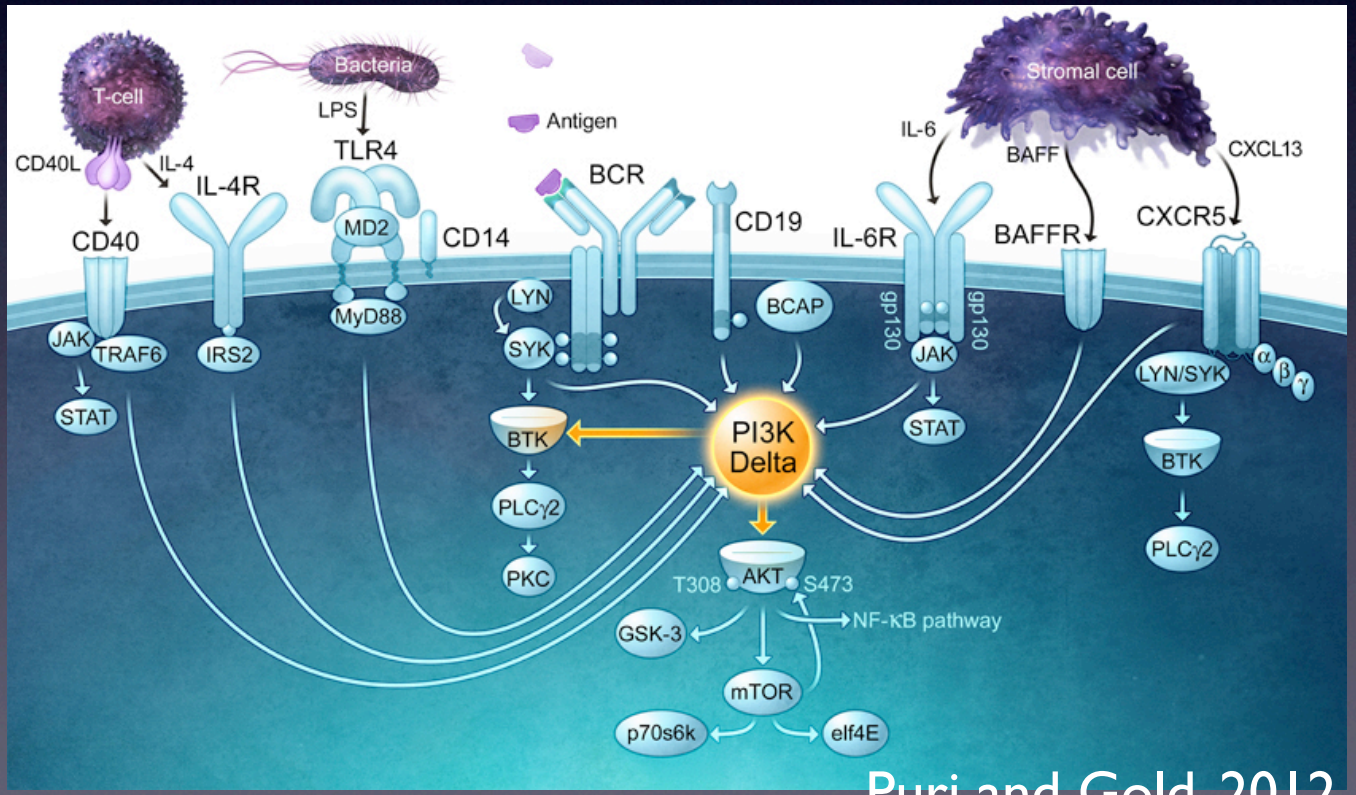






Biological questions:

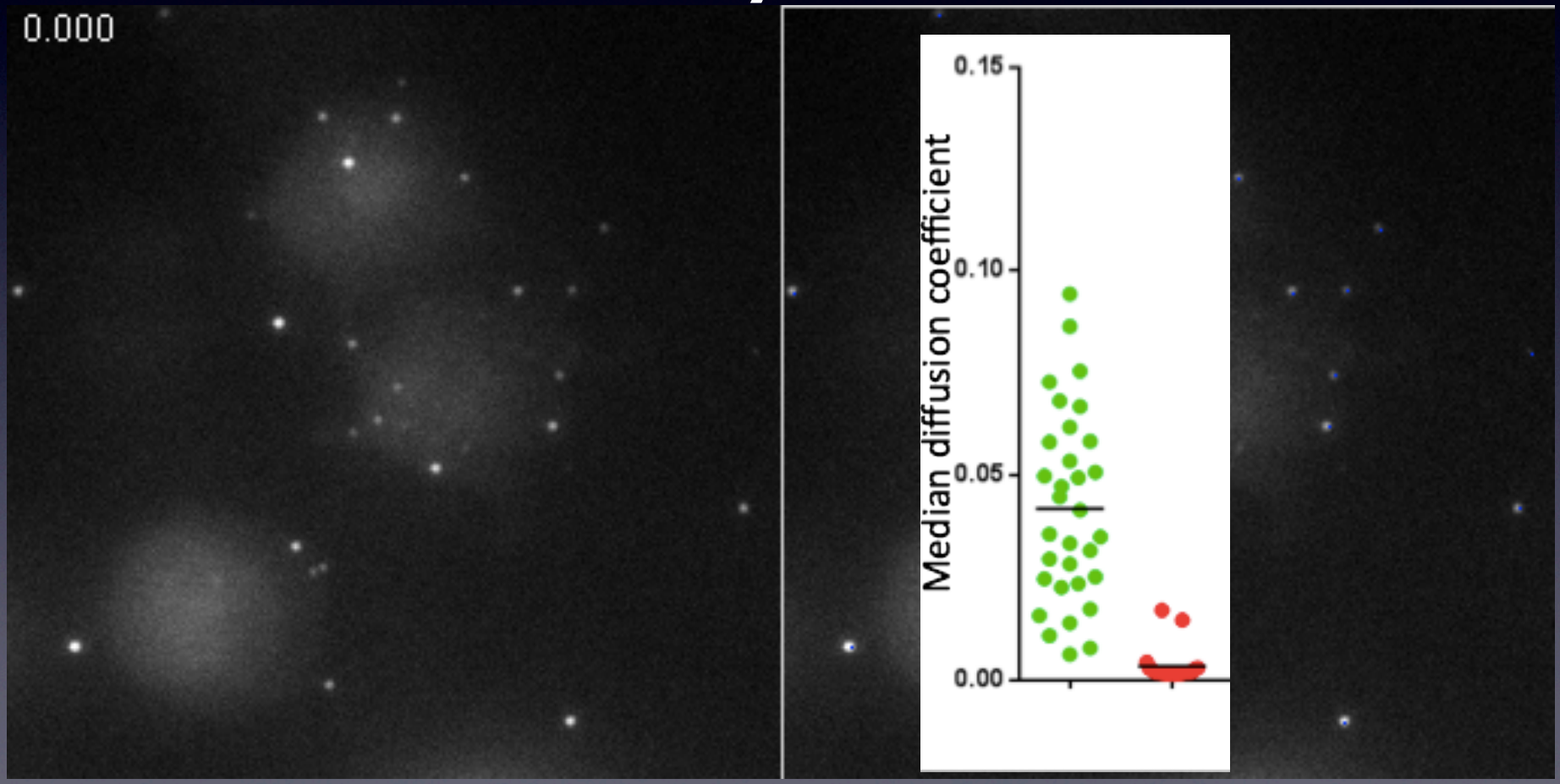
- B cell receptor (BCR) mobility dramatically decreases after BCR signaling (the BCR "controls its own mobility")
  - BCR mobility control is dependent on Syk kinase
- What happens in LPS-activated cells when we inhibit Syk?



Puri and Gold, 2012

Visualizing receptor mobility

BCR labelled on
B cells stimulated with LPS
with Syk inhibitor



- Biological questions:
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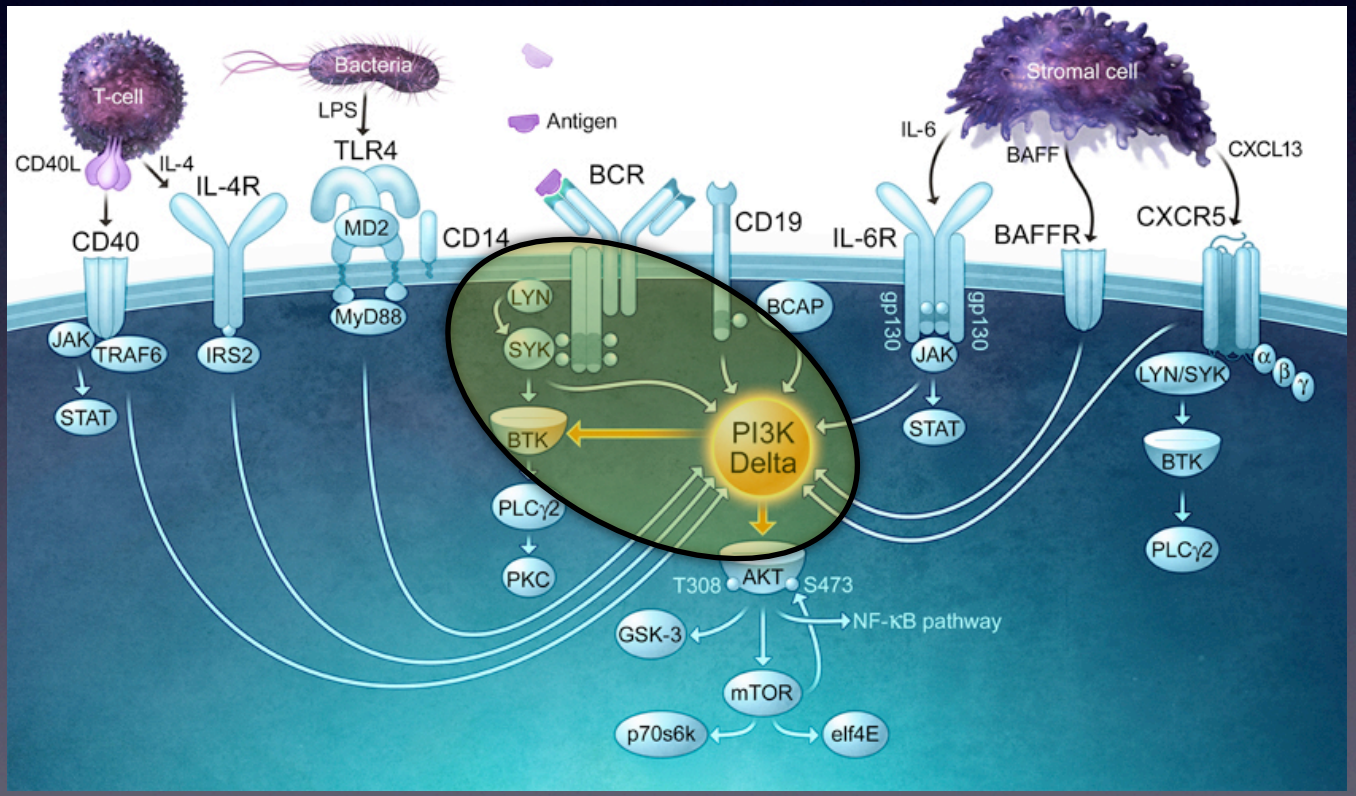
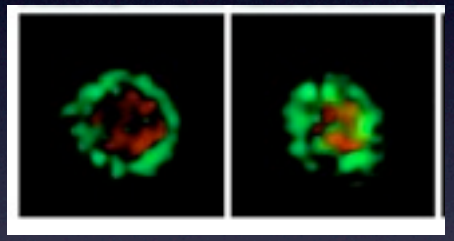


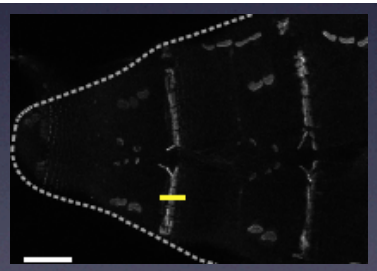
Puri and Gold, 2012

Receptor mobility is tightly integrated with detection, signaling and response of (immune) cells

Making experiments quantitative: measuring and classifying cell receptor motion

- Three examples from work at UBC:

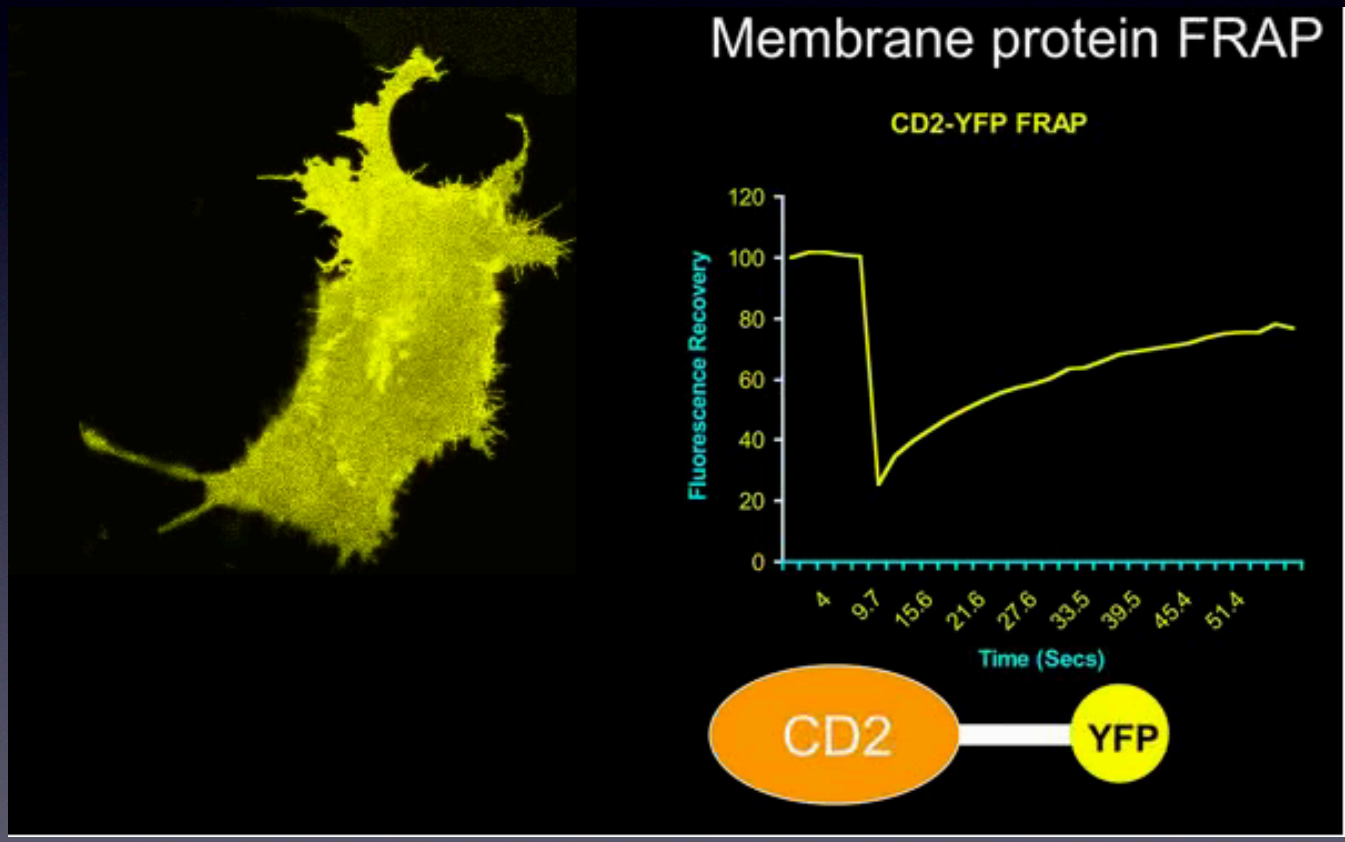




1. Improving protocols for Fluorescence Recovery experiments
2. FRAP and adhesion receptor trafficking
3. Classifying single particle mobility

Fluorescence Recovery after Photobleaching (FRAP)

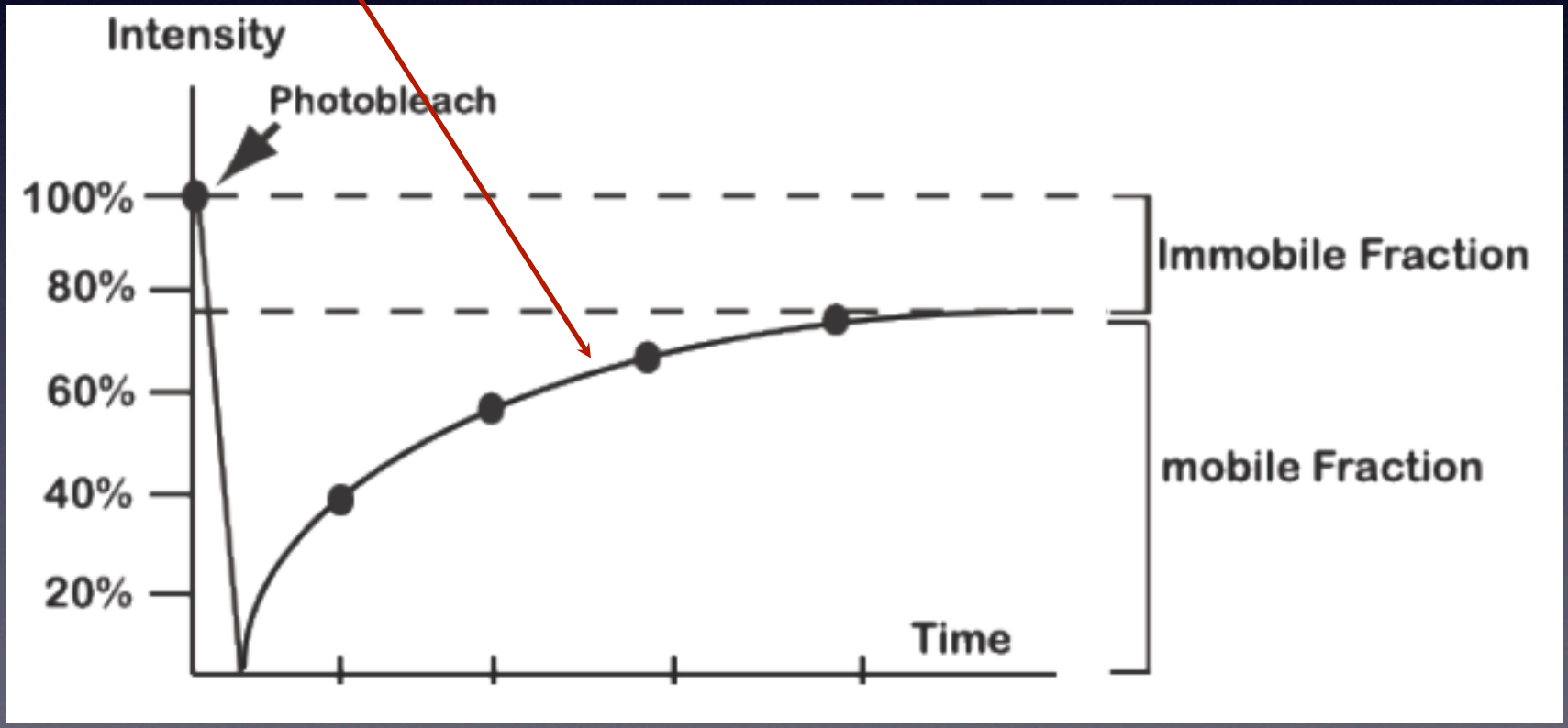
- Protein of interest is fluorescently tagged
- High-intensity laser destroys fluorescence in a defined region
- Recovery is followed
- Software-driven in many confocal microscopes



Qualitative version: assess recovery rate and immobile fraction

$$M\left(1 - e^{-t/\tau}\right)$$

τ is context-dependent



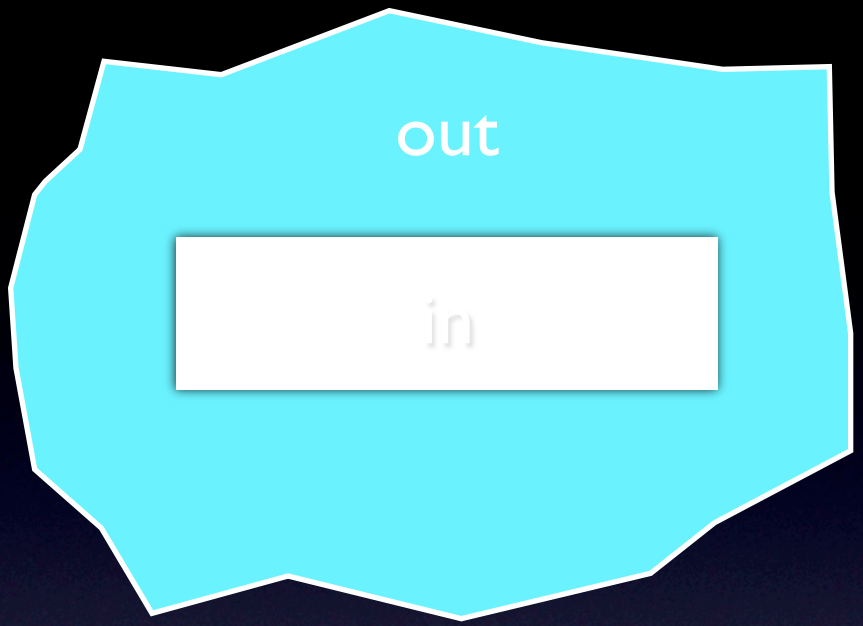
“Quantitative” FRAP analysis:

- Effectively averaging over many thousands of molecules : central limit theorem : diffusion approximation
- So we seek to find physical diffusion constant D and mobile fraction M
- Solve diffusion equation with appropriate BCs and ICs, and the possibility of an immobile fraction.

Omer Dushek

$$\frac{\partial g}{\partial t} = D\nabla^2 g$$

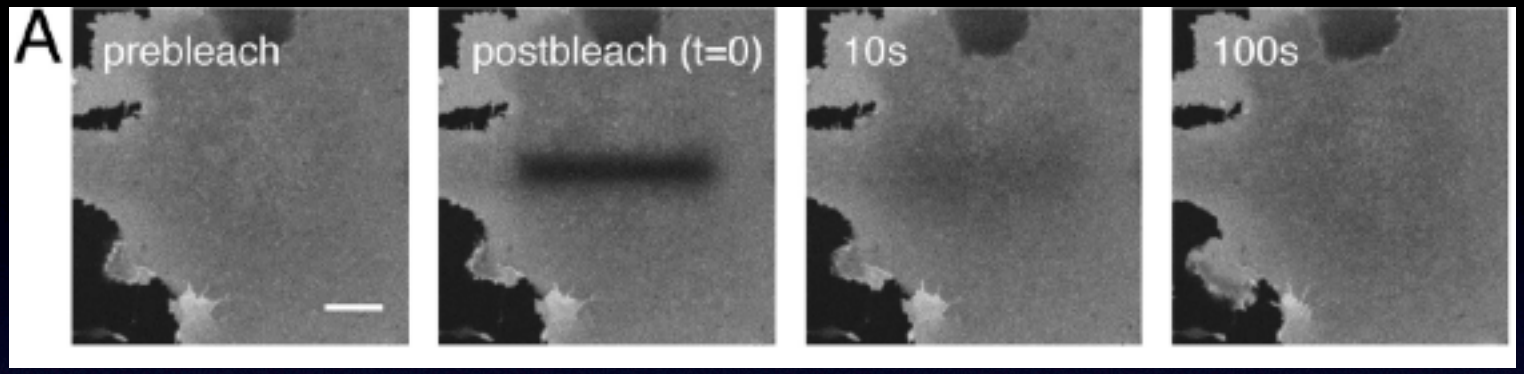
$$g(x,0) = \begin{cases} 0 & \text{in} \\ 1 & \text{out} \end{cases}$$

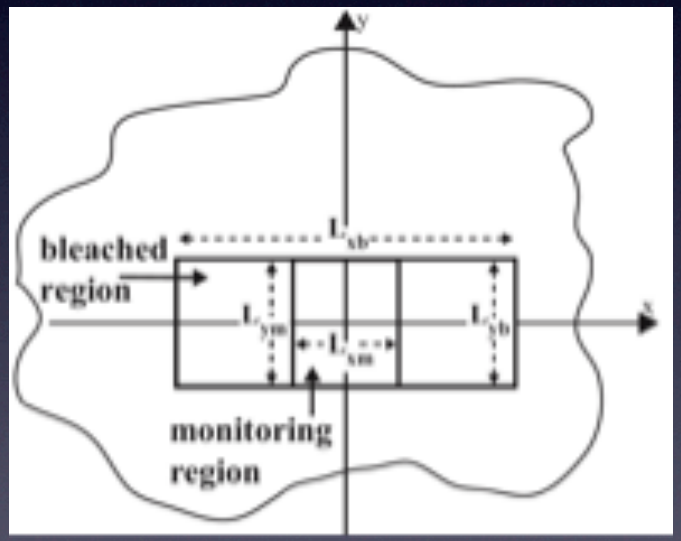


$$\frac{F(t)}{F_{\text{prior}}} = \frac{F_0}{F_{\text{prior}}} + M\left(1 - \frac{F_0}{F_{\text{prior}}}\right)\int_{\text{in}} g(x,t)\,dA$$

- Choice of geometry: simplicity vs accuracy
  - infinite plane, 1D vs 2D
  - spherical geometry

Typical geometries: flat cells

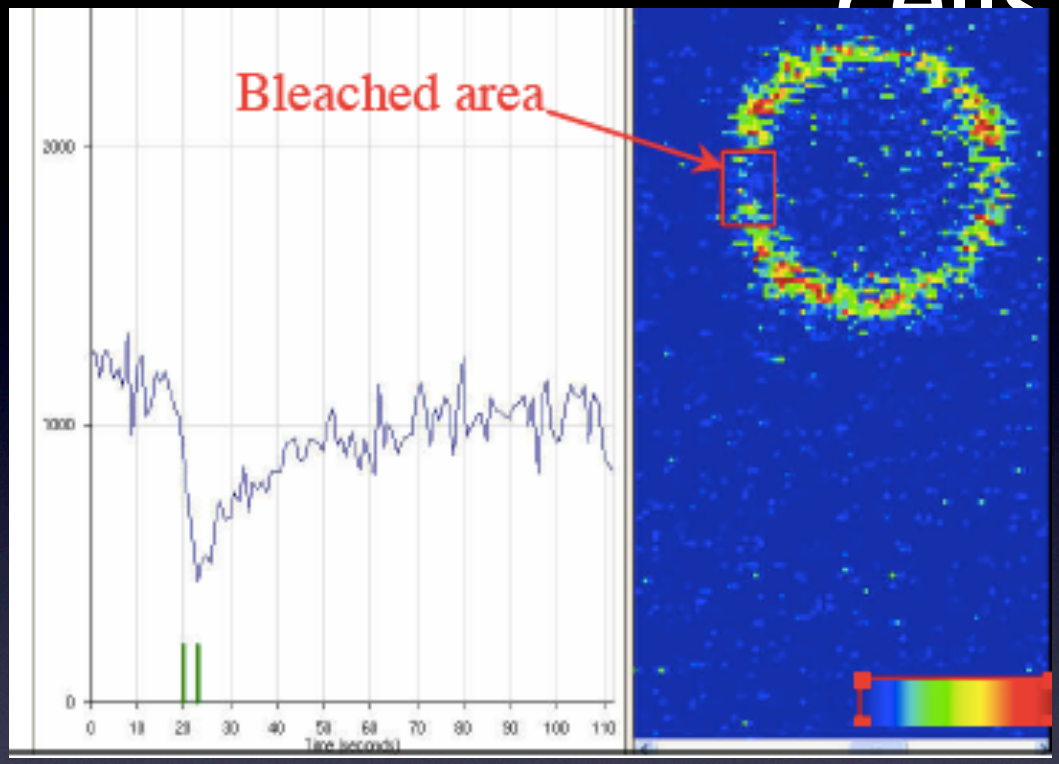


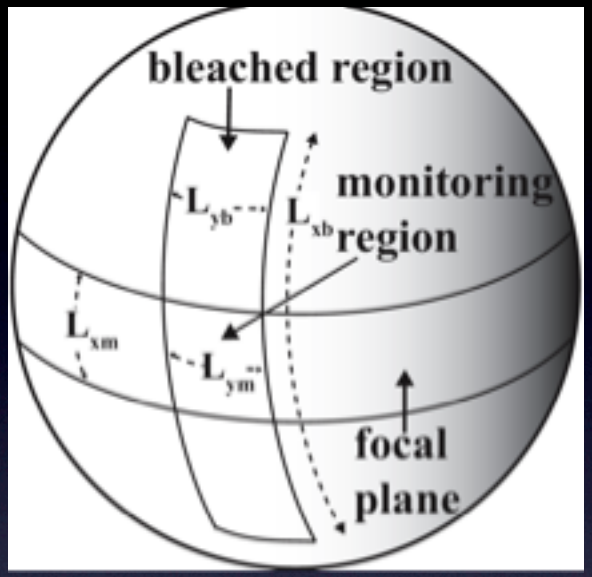


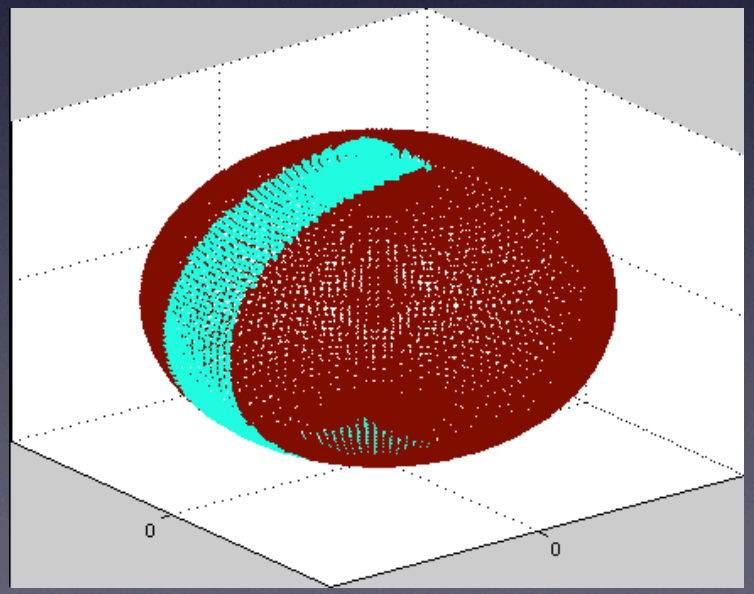
- *One-dimensional* empirical approximation in common use (approximates 1-D Fourier series well).
- Is that a good idea?

$$\frac{F(t)}{F_p} = \left(1 - \frac{F_o}{F_p}\right) M_f \left(1 - \left(\frac{4\pi D t}{(L_{yb})^2} + 1\right)^{-\frac{1}{2}}\right) + \frac{F_o}{F_p}$$

Typical geometries: round cells







- Small, round cells:
- a *vertical* section is bleached
- Can we use the 1D formula?

Potential sources of error in fit for D and M:

1. Using 1D or 2D approximation instead of solution to diffusion problem on sphere (or relevant geometry)
2. Time to bleach should be short compared to characteristic recovery time - violated for large regions
3. Bleach region must be small compared to total cell surface
    - effect of finite molecule number is reduced
    - so distant fluorescence is “constant at infinity” (like approximate model)

- Diffusion on sphere solution using special functions converges slowly and is slow to compute.
- Finite difference solution too slow for fitting
- 2-D infinite plane solution (Fourier Transform):

$$
\begin{aligned}
G(t,D) = 1 - \frac{1}{8\pi L_{xm} L_{ym}} \Bigg\{ & \sqrt{\pi} L_{xm} \left[\operatorname{erf}\left(\frac{L_{xb}-L_{xm}}{4\sqrt{Dt}}\right) + \operatorname{erf}\left(\frac{L_{xb}+L_{xm}}{4\sqrt{Dt}}\right) \right] \\
& + \sqrt{\pi} L_{xb} \left[\operatorname{erf}\left(\frac{L_{xb}+L_{xm}}{4\sqrt{Dt}}\right) - \operatorname{erf}\left(\frac{L_{xb}-L_{xm}}{4\sqrt{Dt}}\right) \right] \\
& + 4\sqrt{Dt} \left[\exp\left(-\frac{(L_{xm}+L_{xb})^2}{16Dt}\right) - \exp\left(-\frac{(-L_{xm}+L_{xb})^2}{16Dt}\right) \right] \Bigg\} \\
& \times \Bigg\{ \sqrt{\pi} L_{ym} \left[\operatorname{erf}\left(\frac{L_{yb}-L_{ym}}{4\sqrt{Dt}}\right) + \operatorname{erf}\left(\frac{L_{yb}+L_{ym}}{\sqrt{16Dt}}\right) \right] \\
& + \sqrt{\pi} L_{yb} \left[\operatorname{erf}\left(\frac{L_{yb}+L_{ym}}{4\sqrt{Dt}}\right) - \operatorname{erf}\left(\frac{L_{yb}-L_{ym}}{4\sqrt{Dt}}\right) \right] \\
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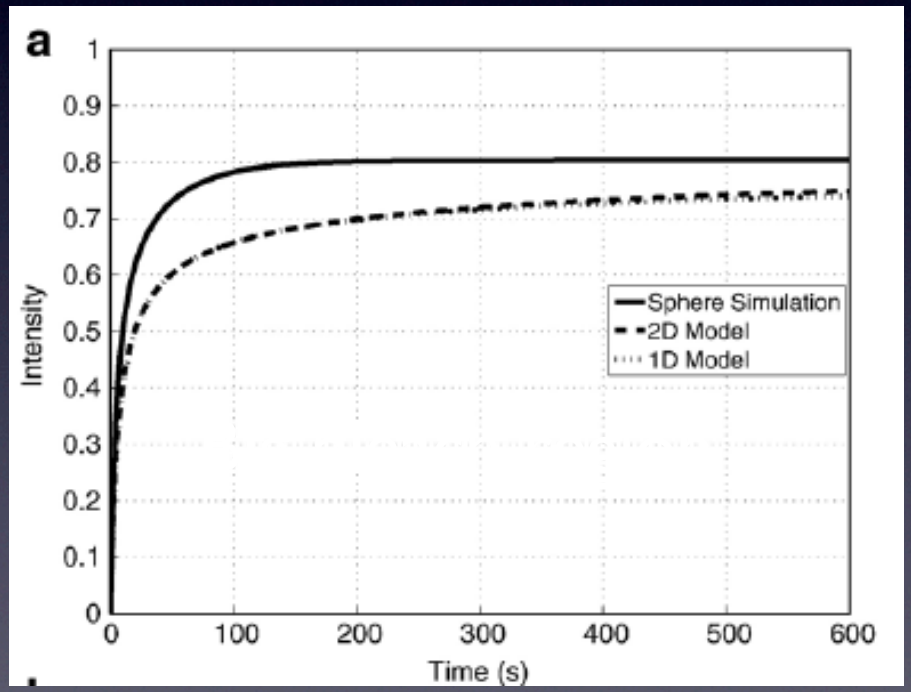
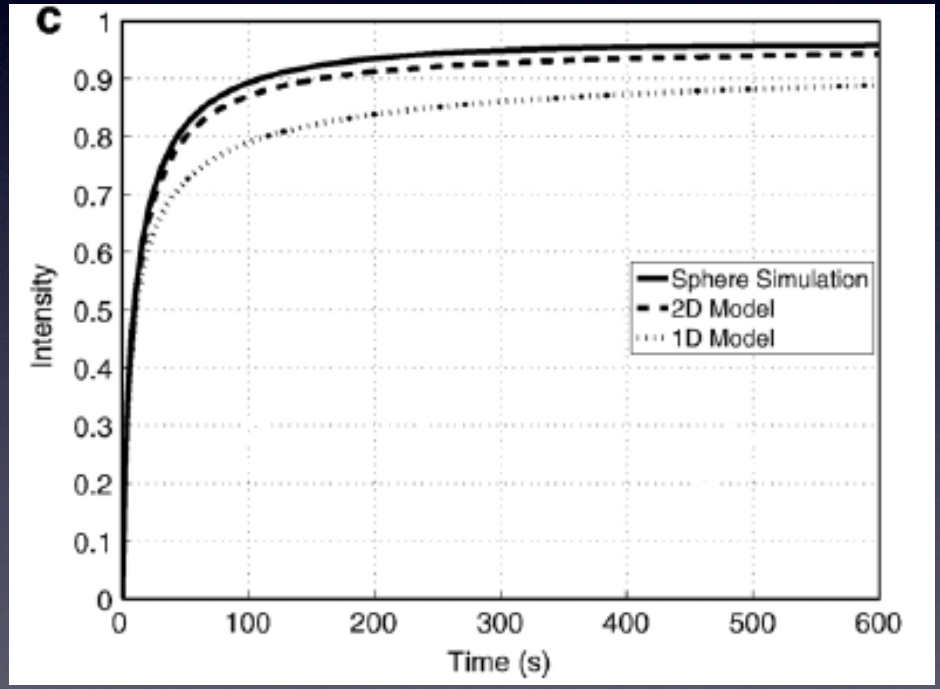
\end{aligned}
$$

Theoretical testing for small cells:

- Fix parameters D and M
- Compare output of 1-D and 2-D models with full numerics (round cell spherical geometry)





Theoretical testing for small cells:

- *Simulate* FRAP on a sphere using a high-resolution finite difference scheme
- *Fit* approximate models (1-D and 2-D)
- Compare fit parameters to actuals

Fit values:

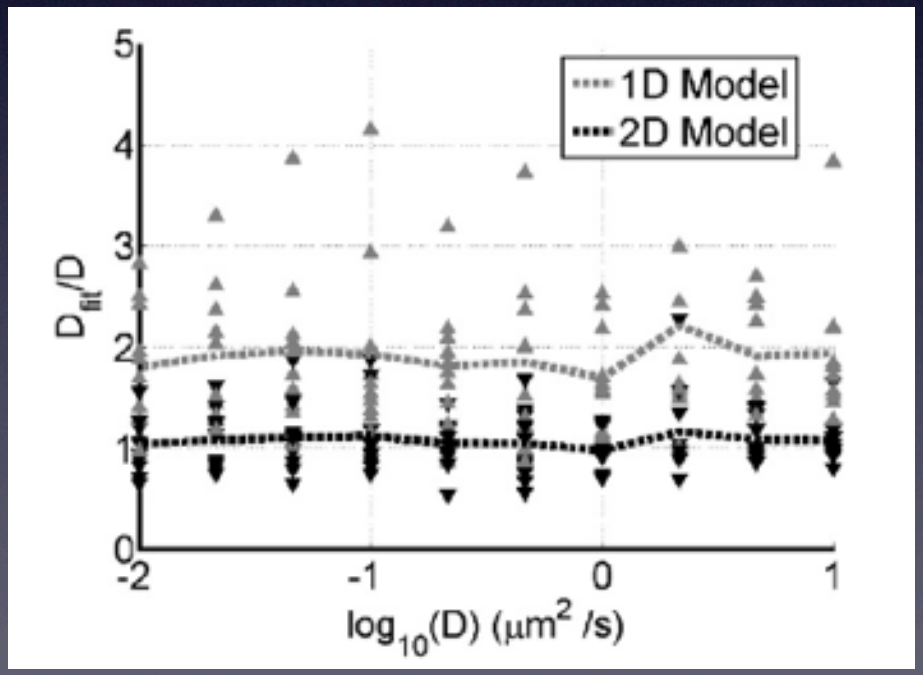
1D: $D_{fit}=2.7D_{true}$ $M_{fit}=M_{true}$ 1D: $D_{fit}=1.5D_{true}$ $M_{fit}=1.1M_{true}$

2D: $D_{fit}=3.6D_{true}$ $M_{fit}=M_{true}$ 2D: $D_{fit}=D_{true}$ $M_{fit}=1.1M_{true}$

Conclude: use a small bleach region! But what about noise?

Testing noise effect:

- *Simulate* FRAP (small bleach region)
- *Add 15% Gaussian noise*
- *Fit* approximate models (1-D and 2-D)
- Compare fit parameters to actuals

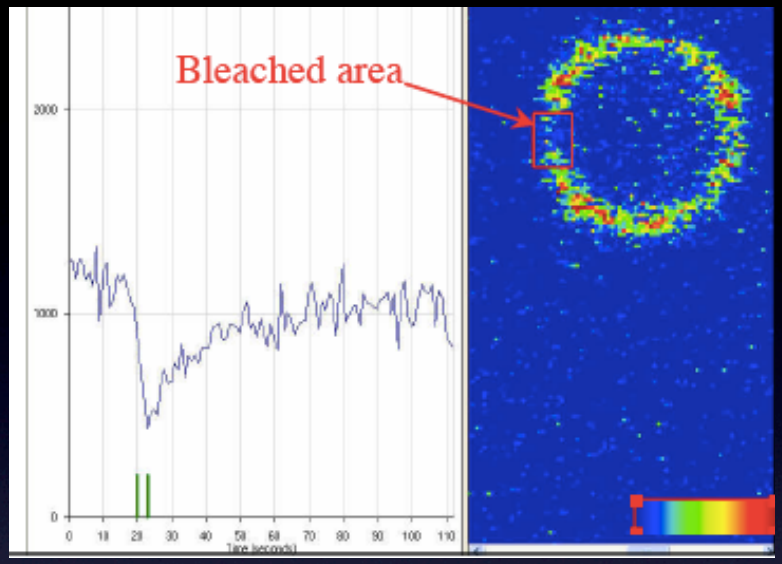


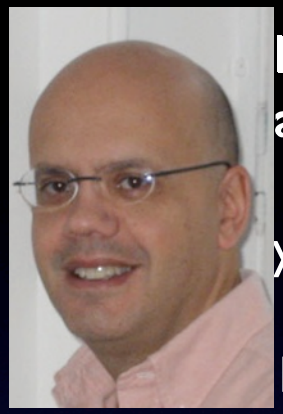
- 1-D model is highly sensitive to noise

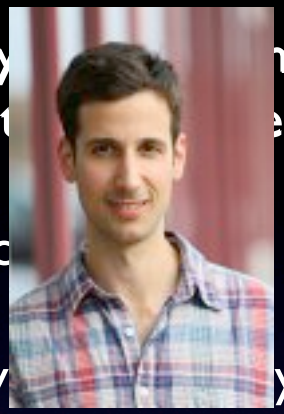
Recommendations:

- *Keep bleach spot small*
- *Maintain SNR<15%*
- *Fit best geometry model*

Application: Signaling control of T cell receptor mobility







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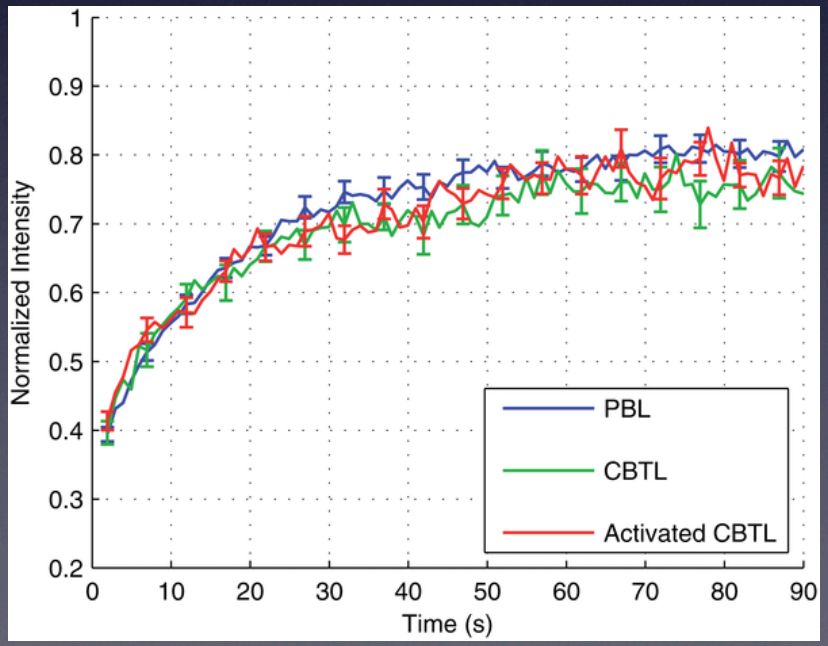
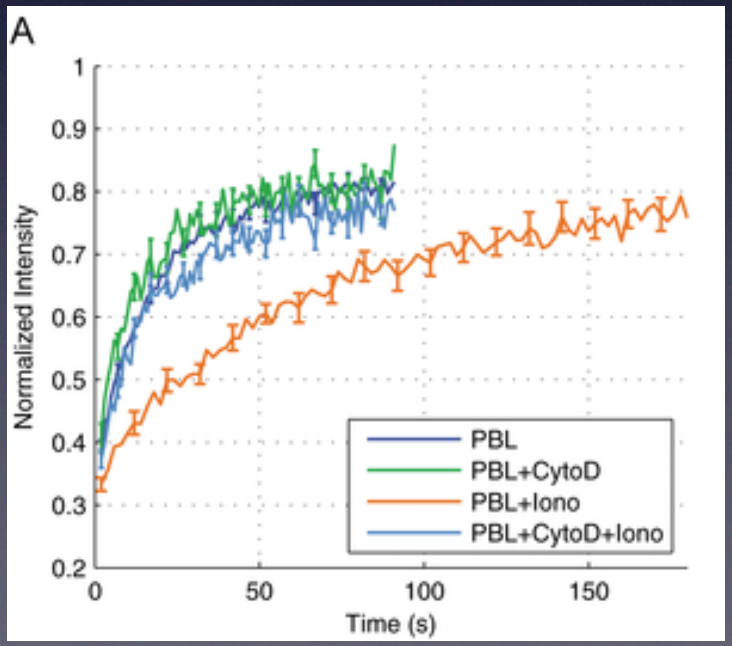
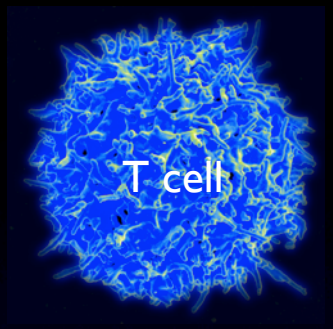
Salvatore Valitutti Omer Dushek

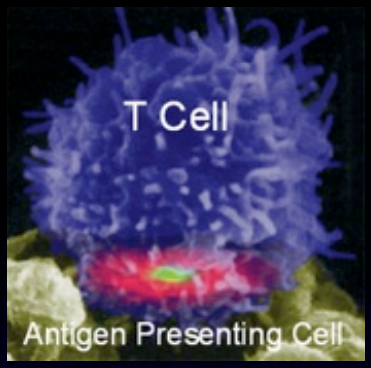
- cytoskeleton-TCR interaction can be quantified by careful fitting

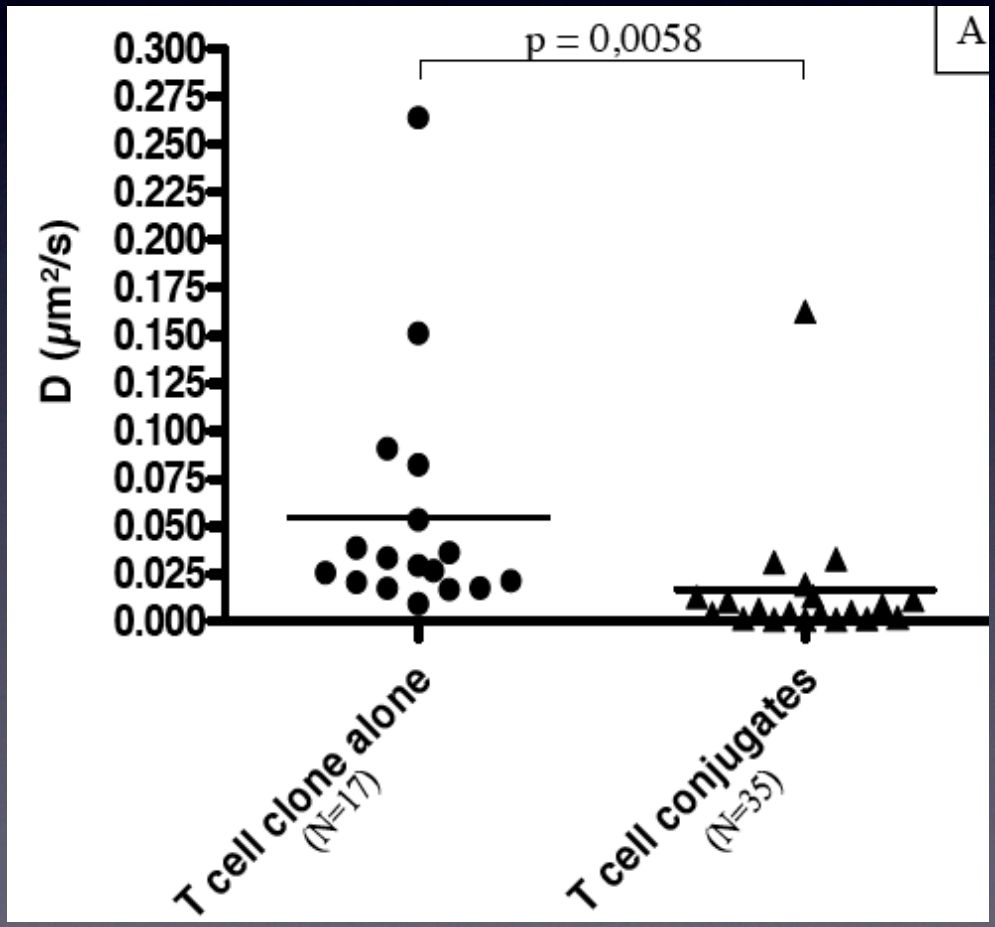




Signaling control of T Cell Receptor mobility





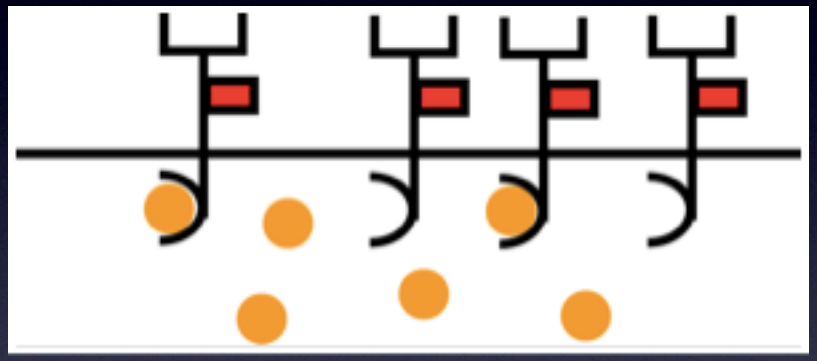


- TCR mobility is reduced by synapse formation and signaling

- Sustained calcium signaling following TCR binding may be the signal for global, actin-dependent TCR mobility reduction.

Subsequent and ongoing work:

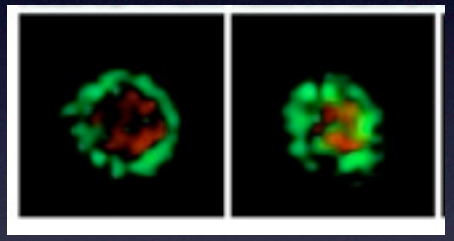
- Making confocal FRAP a quantitative tool…..
- Can FRAP measure kinetics for particles that bind and slow down, then unbind and speed up?

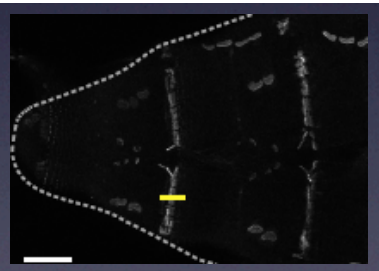


- Fluorescent tags are photo-unstable and there is a *background bleaching* effect.
  - Typically handled by fitting exponential decay parameter.
  - Optimize just a few FRAP acquisition times to estimate parameters?

Making experiments quantitative: measuring and classifying cell receptor motion

- Three examples from work at UBC:





1. Improving protocols for Fluorescence Recovery experiments
2. FRAP and adhesion receptor trafficking
3. Classifying single particle mobility

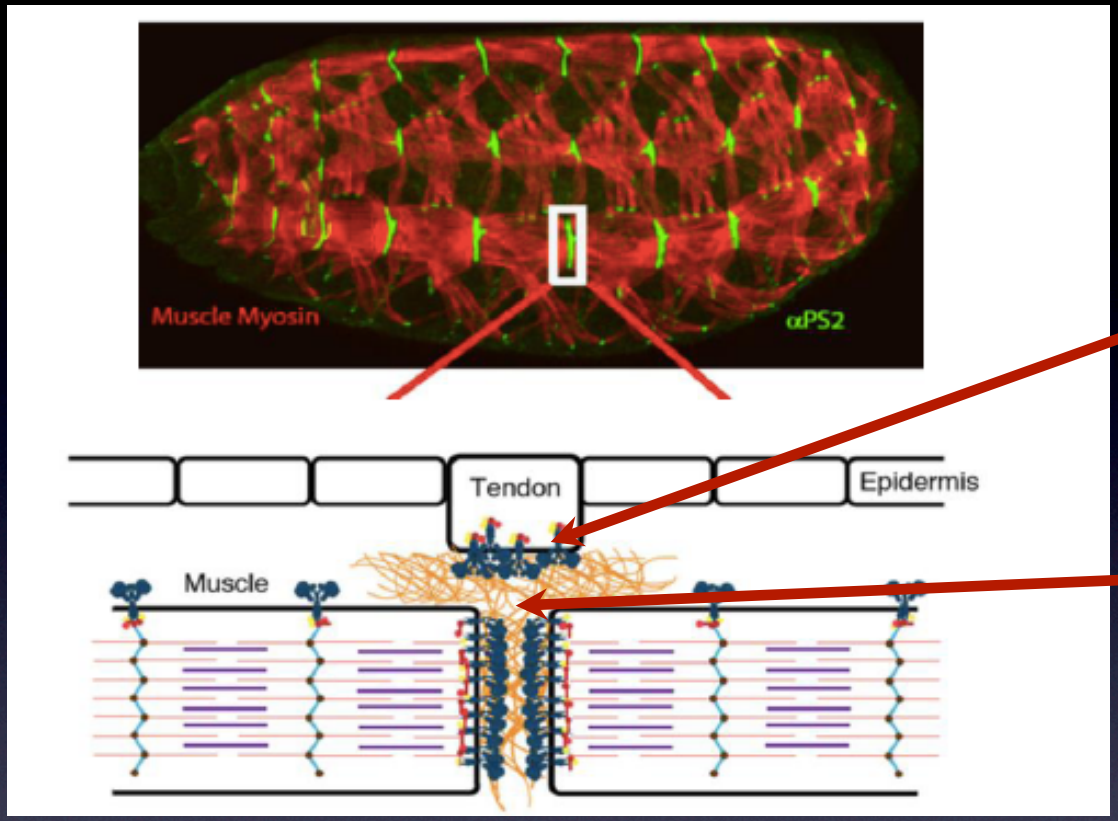
Recovery of adhesion receptors at muscle-tendon junction in fruit fly embryos

Guy Tanentzapf

Mary Pines

Dodo Das

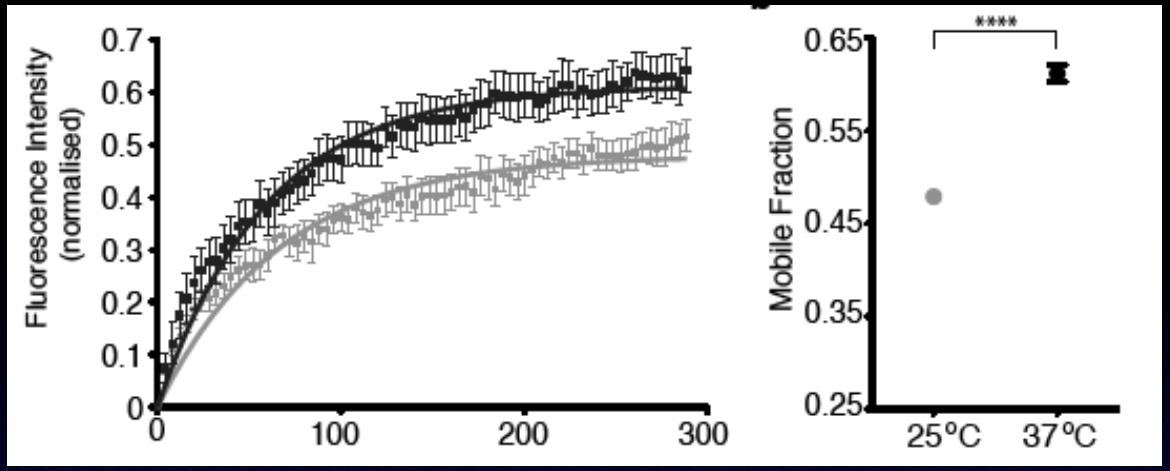
Muscle-tendon junction in *drosophila*



Adhesion molecules (β -integrins)

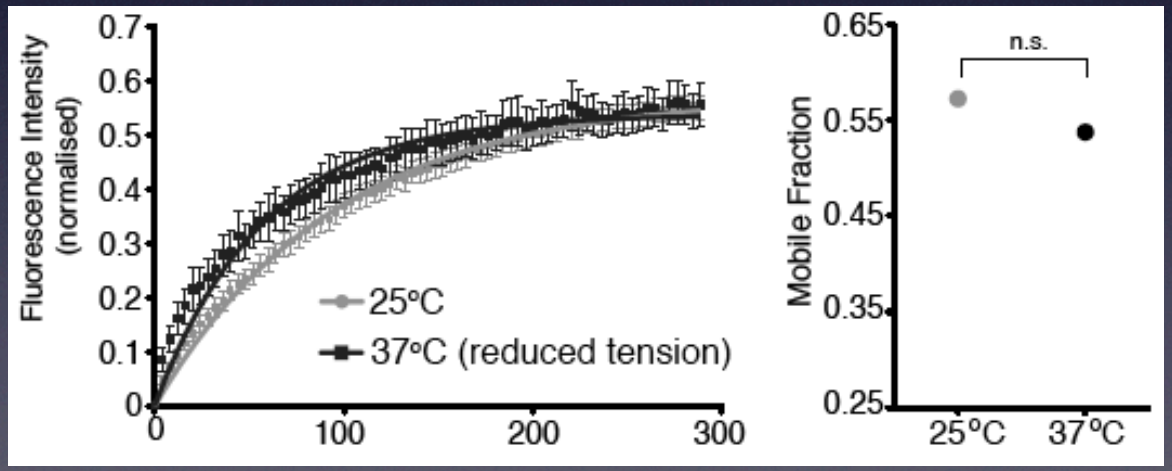
Extracellular matrix

- Breakdance (BRK) temperature-sensitive mutant
  - high force on junctions at 37C
- Para temperature-sensitive mutant
  - low force on junctions at 37C
- Concurrent integrin mutants



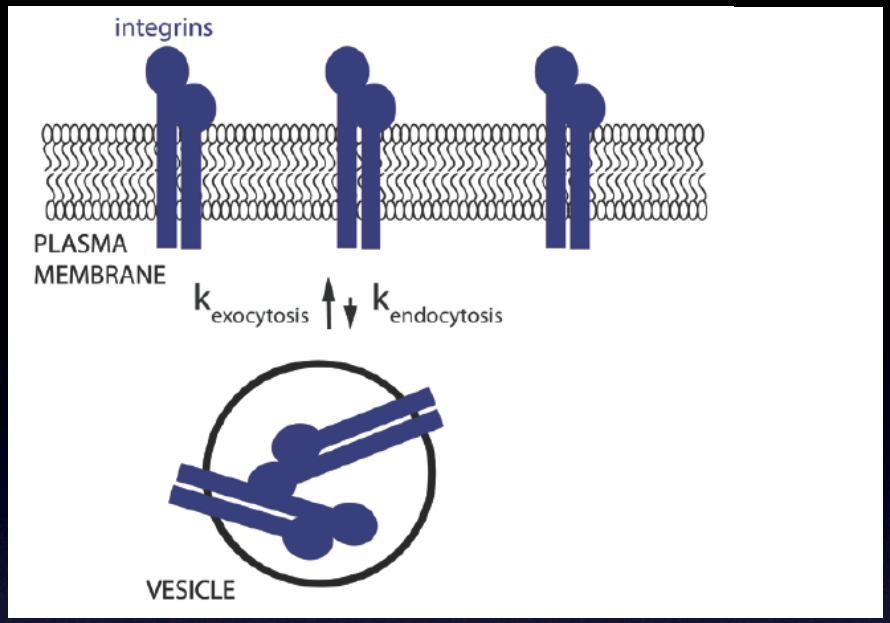
Control
BRK Increased Force

This data had a clear message



Para (decreased force)
Control

This data was problematic...



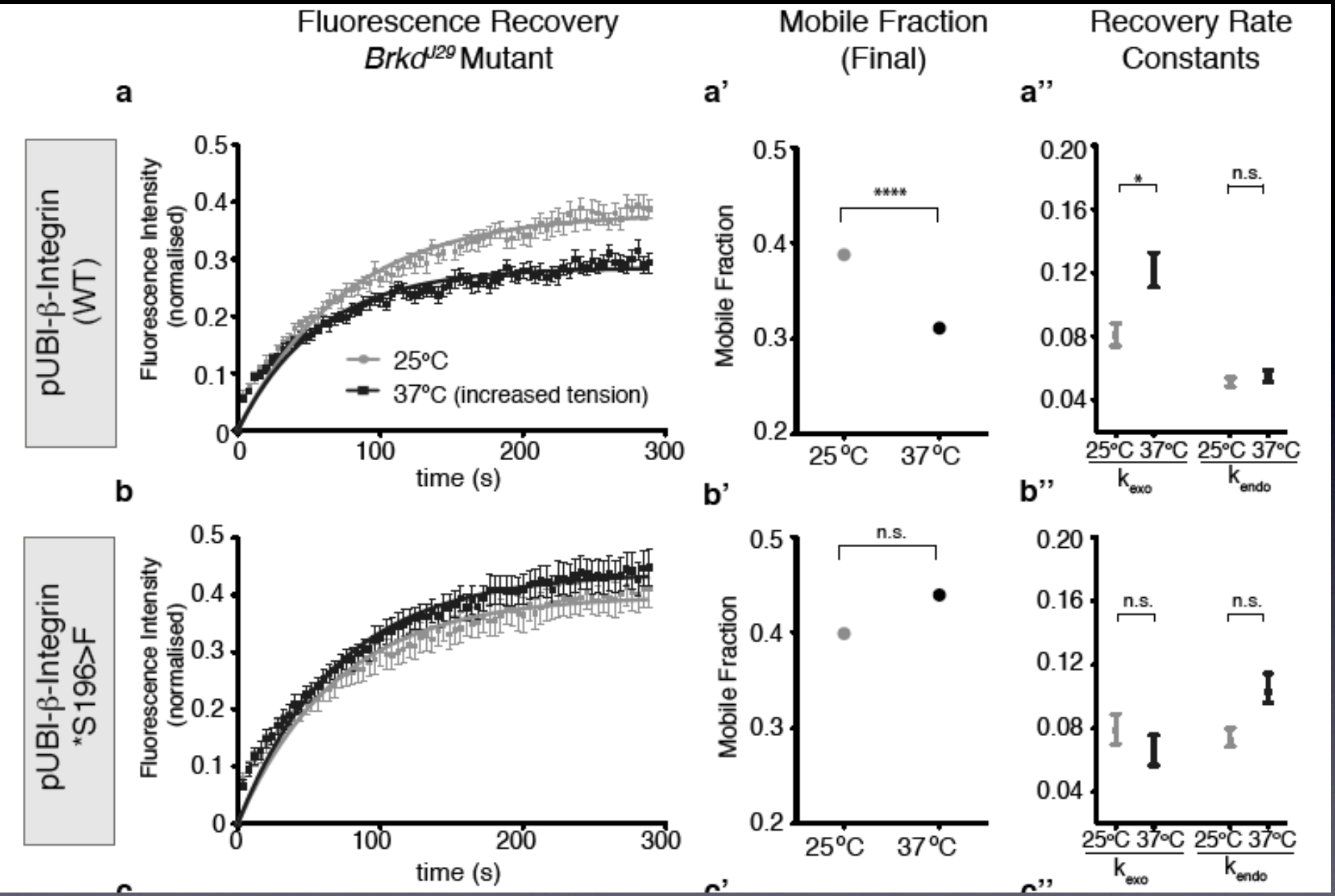
- Biological hypothesis about receptor recycling
- simple mathematical model
- fit for k_{endo} and k_{exo}

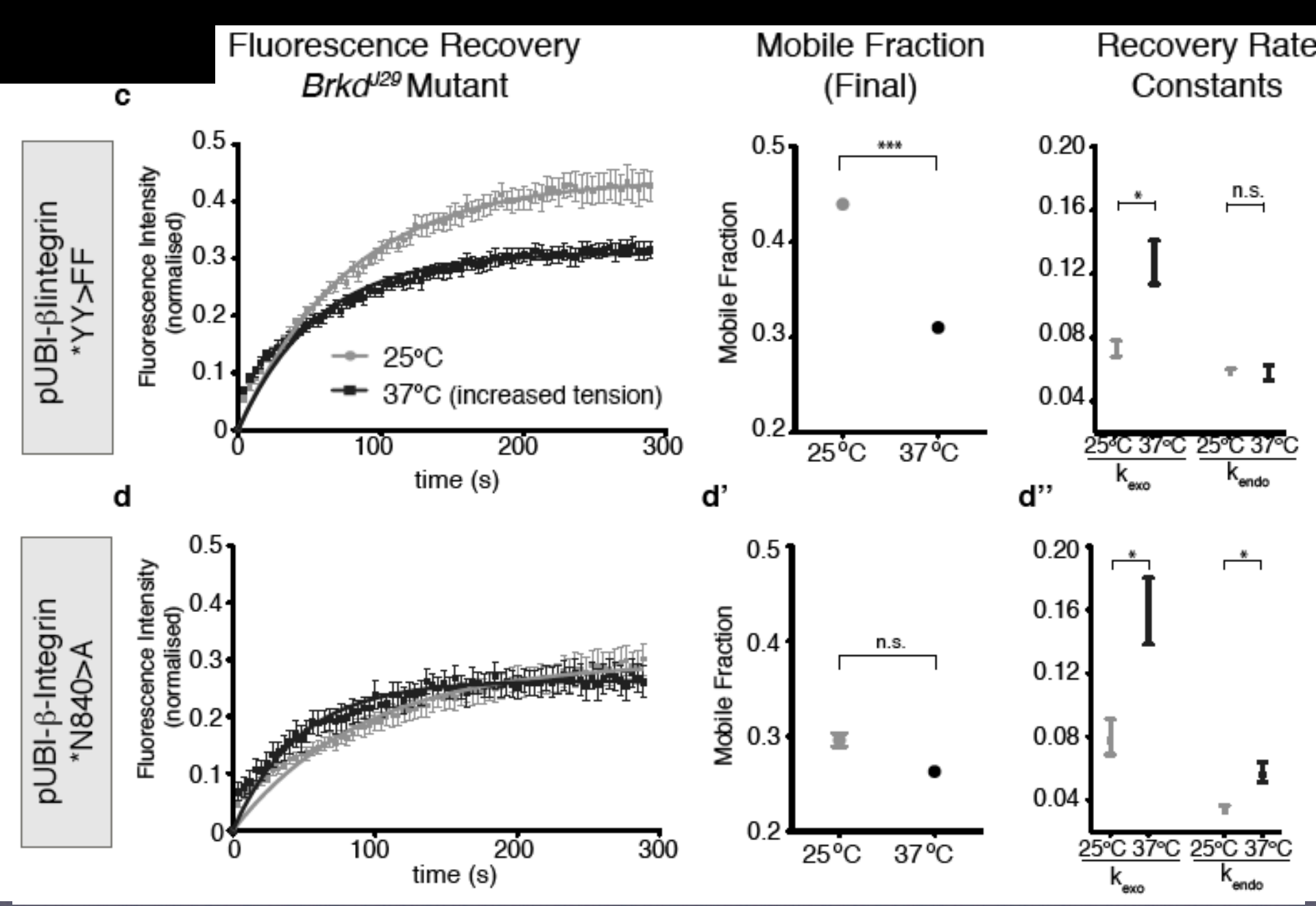
$$\frac{dP}{dt} = -k_{\text{endo}}P + k_{\text{exo}}V$$

$$P + V = \text{constant}$$

$$\cdots\cdots$$

$$\text{fluorescence} \sim \frac{k_{\text{endo}}}{k_{\text{endo}} + k_{\text{exo}}}\left[1 - e^{-(k_{\text{endo}}+k_{\text{exo}})t}\right]$$


Fluorescence Recovery
Brkd^J29 Mutant
Mobile Fraction
(Final)
Recovery Rate
Constants
a
a'
a''
pUBI-β-Integrin
(WT)
Fluorescence Intensity
(normalised)
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
time (s)
25°C
37°C (increased tension)
Mobile Fraction
****
25°C
37°C
0.20
0.16
0.12
0.08
0.04
*
n.s.
25°C 37°C
25°C 37°C
k_exo
k_endo
b
b'
b''
pUBI-β-Integrin
*S196>F
n.s.
n.s.
n.s.


Fluorescence Recovery
Brkd^J29 Mutant
Mobile Fraction
(Final)
Recovery Rate
Constants
c
pUBI-βIintegrin
*YY>FF
Fluorescence Intensity
(normalised)
25°C
37°C (increased tension)
time (s)
Mobile Fraction
***
25°C
37°C
*
n.s.
25°C 37°C
k exo
25°C 37°C
k endo
d
d'
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pUBI-β-Integrin
*N840>A
n.s.
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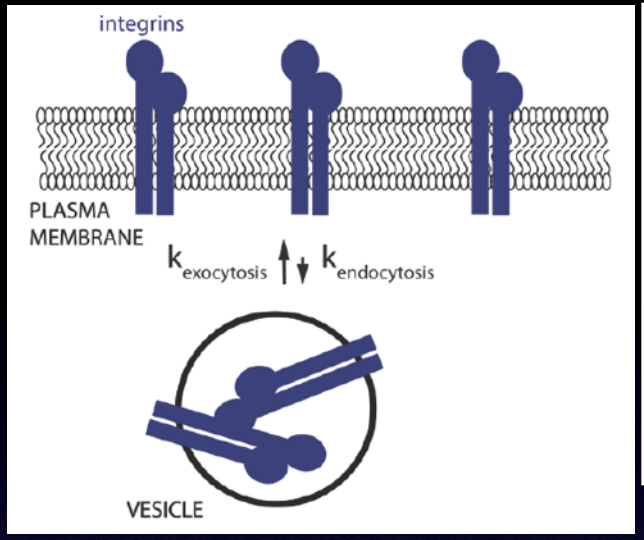


$$\frac{dP}{dt} = -k_{\text{endo}}P + k_{\text{exo}}V$$

$$P + V = \text{constant}$$

$$\cdots\cdots$$

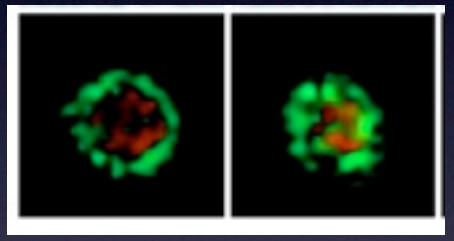
$$\text{fluorescence} \sim \frac{k_{\text{endo}}}{k_{\text{endo}} + k_{\text{exo}}}\left[1 - e^{-(k_{\text{endo}}+k_{\text{exo}})t}\right]$$

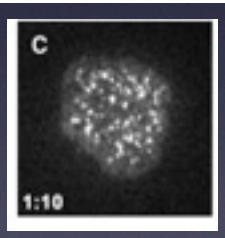
- Biological hypothesis about receptor recycling
- Simple mathematical model fit for k_{endo} and k_{exo}
- New hypotheses:
  - detailed description of endo/exo rates for integrin mutants under high/low force conditions
  - propose integrin residues that control endo/exo

- Ongoing work: FRAP studies of intracellular integrin binding partners to elucidate these ideas.

Making experiments quantitative: measuring and classifying cell receptor motion

- Three examples from work at UBC:

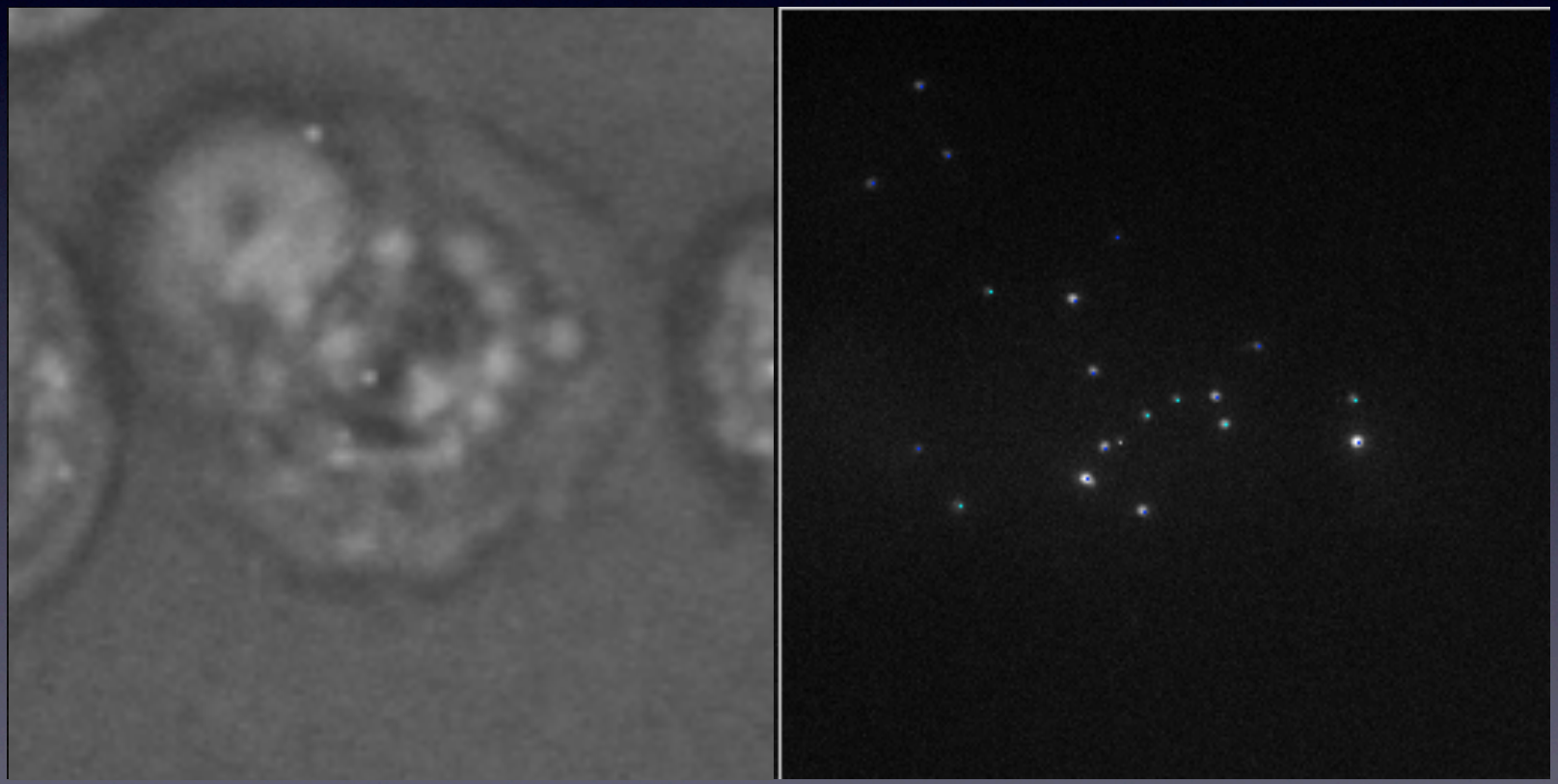




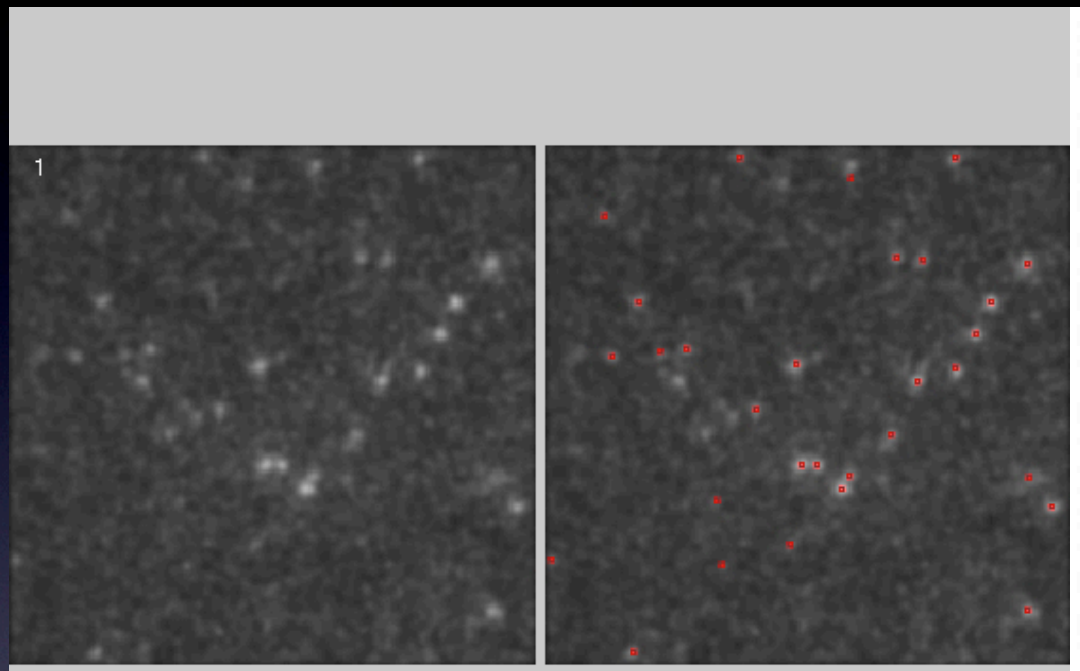
1. Improving protocols for Fluorescence Recovery experiments
2. FRAP and adhesion receptor trafficking
3. Classifying single particle mobility

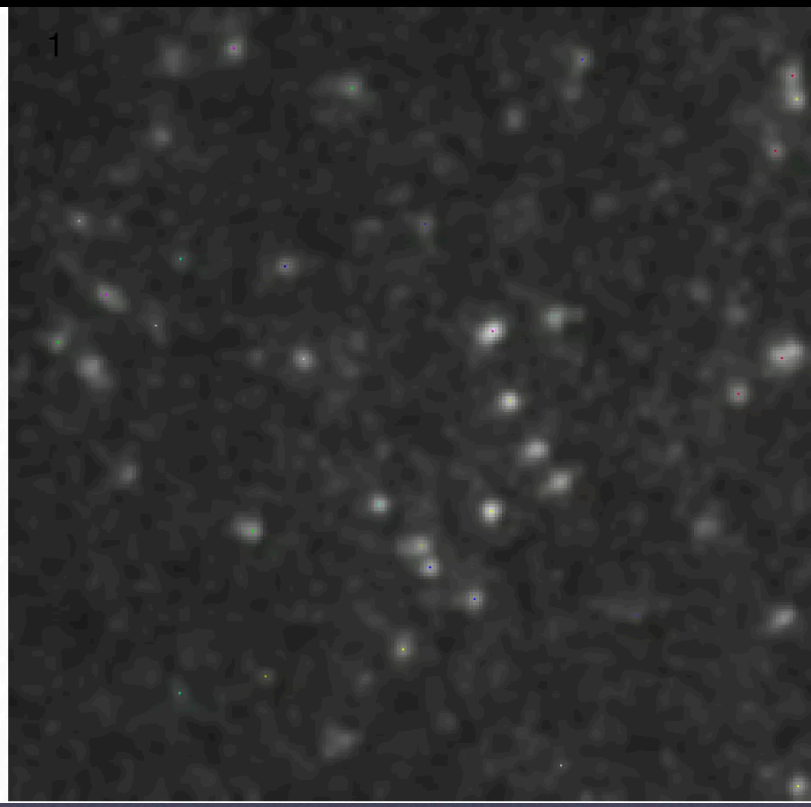
Single-Particle Tracking

- Directly observe mobility of individual tagged biomolecules with high resolution.



Single particle tracking (SPT)



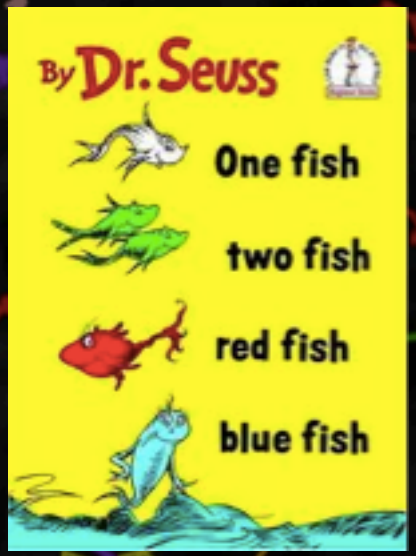


- Step 1: Identify “particles”.
- Step 2: Connect particles from frame to frame.

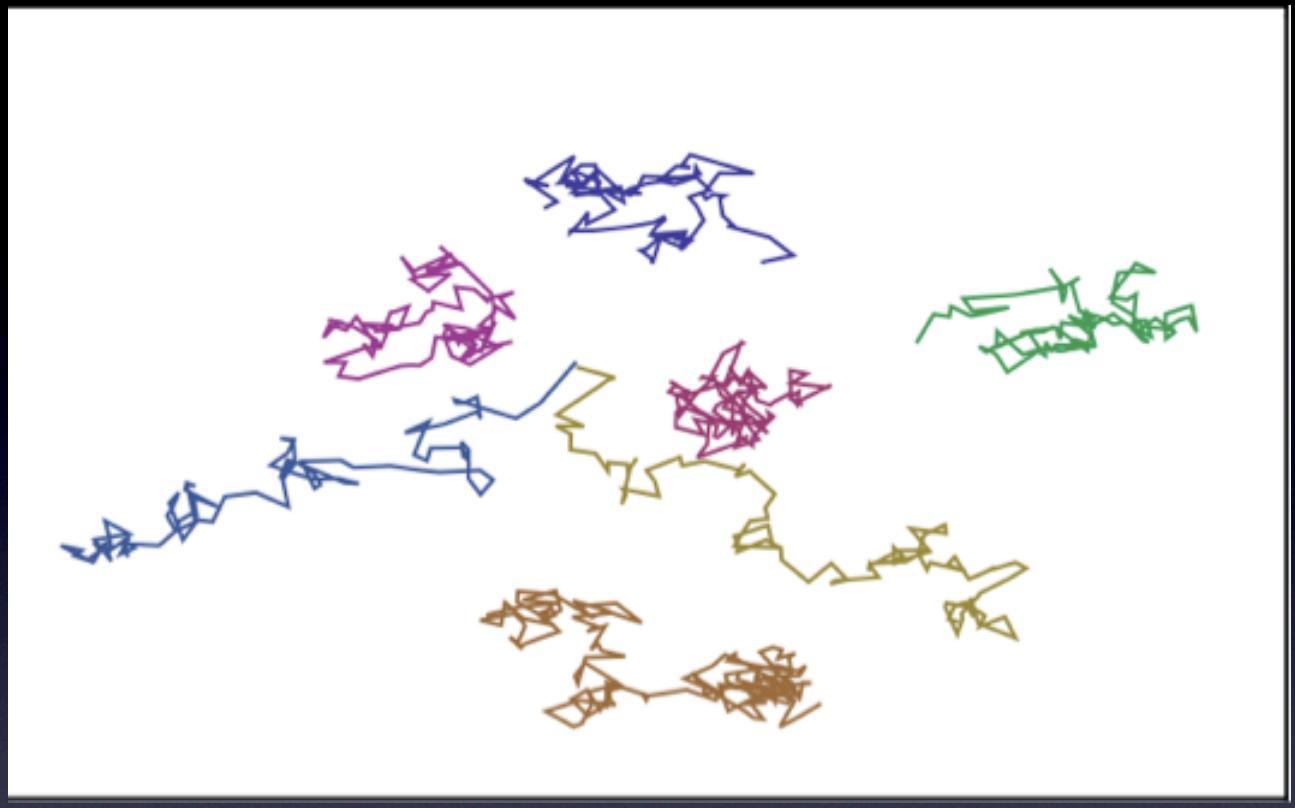
What can we say about the motion?

Some are fast
And some are slow
Some are high
And some are low

Not one of them is like another.
Don't ask us why.
Go ask your mother.

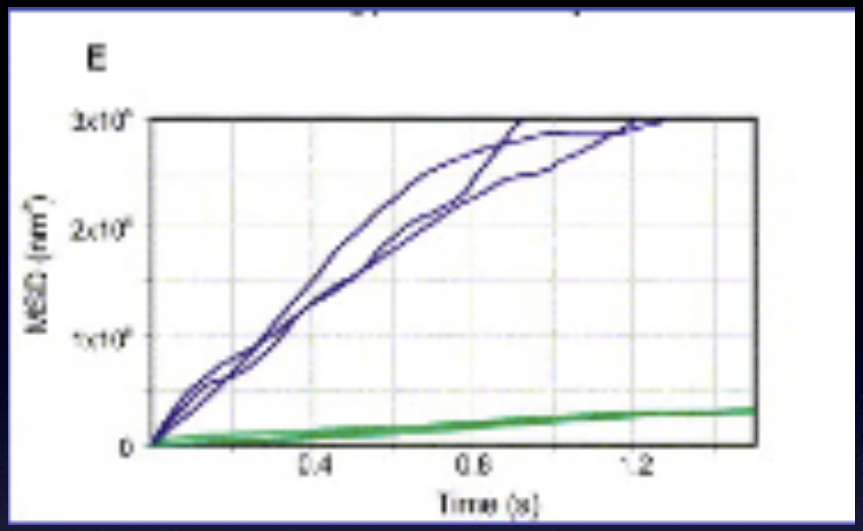


Quiz:



- Goal: given well-defined models for particle behaviour, compute the relative likelihood of each model and correlate with biological control variables
- Ideally, combine motion model with tracking algorithm

Mean-square-displacement (MSD) analysis

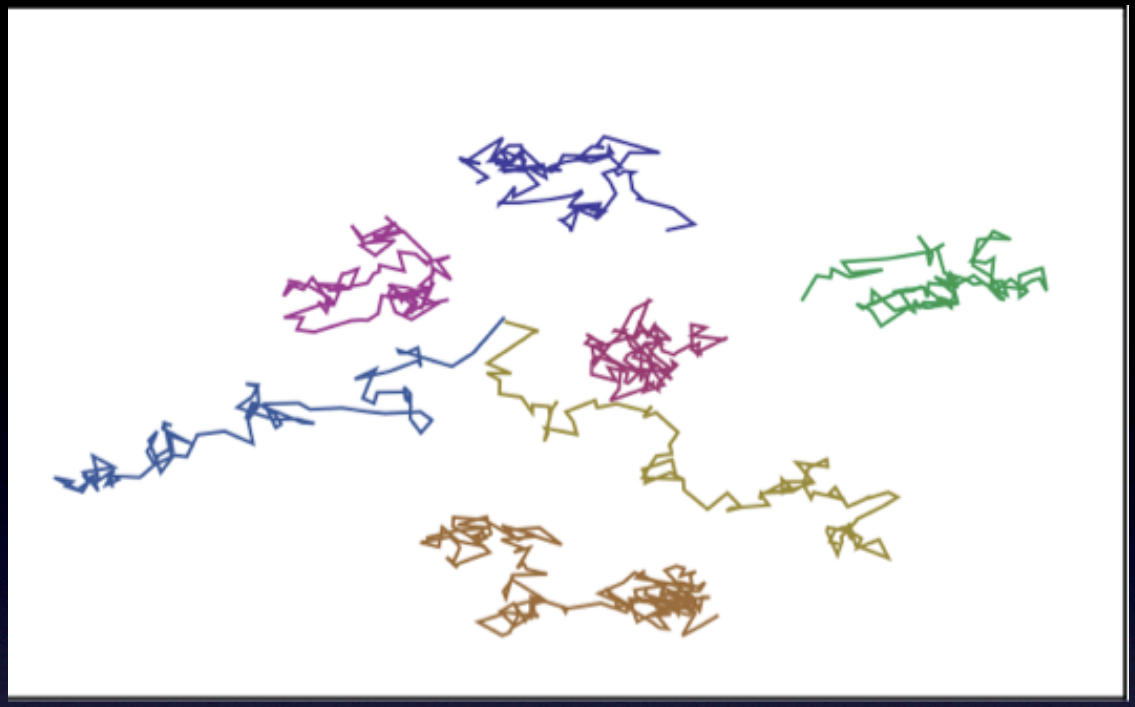


- Plot the average square displacement against time (sliding window)
- Linear is indicative of Brownian diffusion
  - sublinear: confined
  - superlinear: directed

Pure diffusion in maximum likelihood framework:

$$L(D|O) \sim \frac{1}{4\pi D\tau} e^{-r_1^2/4D\tau} \cdot \frac{1}{4\pi D\tau} e^{-r_2^2/4D\tau} \cdots \frac{1}{4\pi D\tau} e^{-r_N^2/4D\tau}$$
$$= \frac{1}{(4\pi D\tau)^N} \exp\left[-\sum_{i=1}^{N} r_i^2/(4D\tau)\right]$$

$$D_{\mathrm{ML}} = \frac{1}{4\tau N}\sum_{i=1}^{N} r_i^2 = \frac{1}{4\tau}\langle r_i^2 \rangle$$

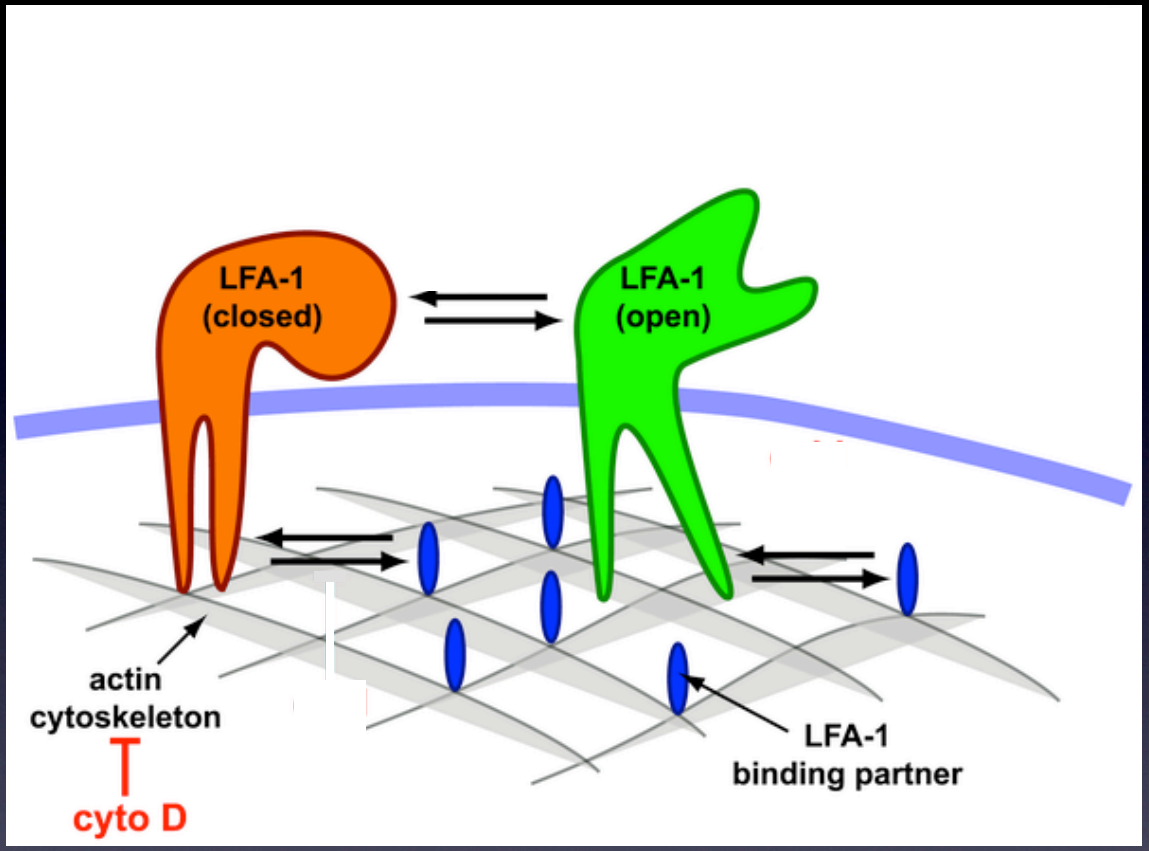


- For a single track, MSD analysis does not give good confidence.
  - average over all your data
- But what if particle behaviour changes within one track?
  - very interesting insight into particle behaviour
  - can we probe protein interactions using SPT?

SPT study of LFA-1 on T cells

Chris Cairo, Alberta

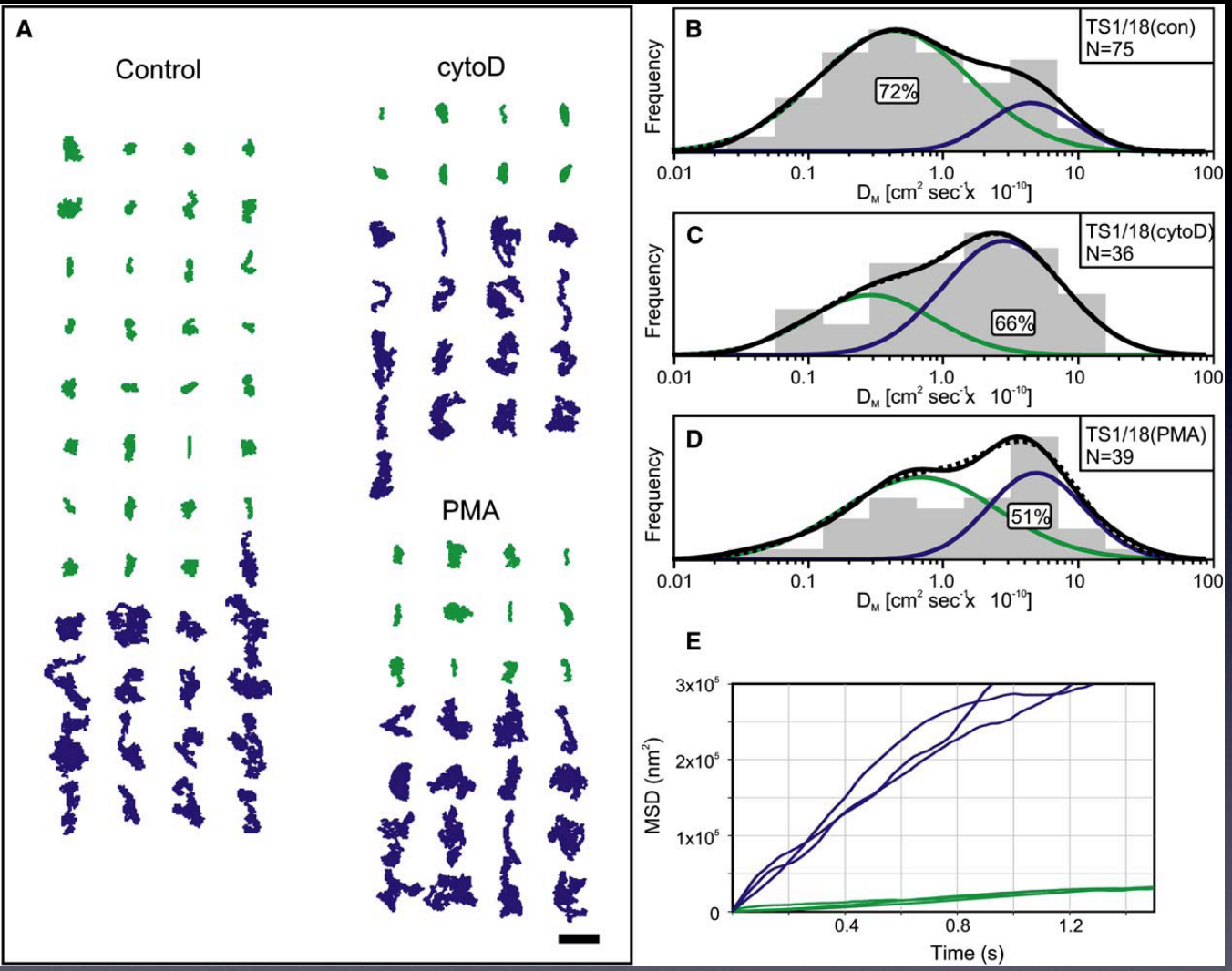
Dodo Das



- Conformational changes modulate interaction with the actin cytoskeleton

- Controls T lymphocyte adhesion and migration

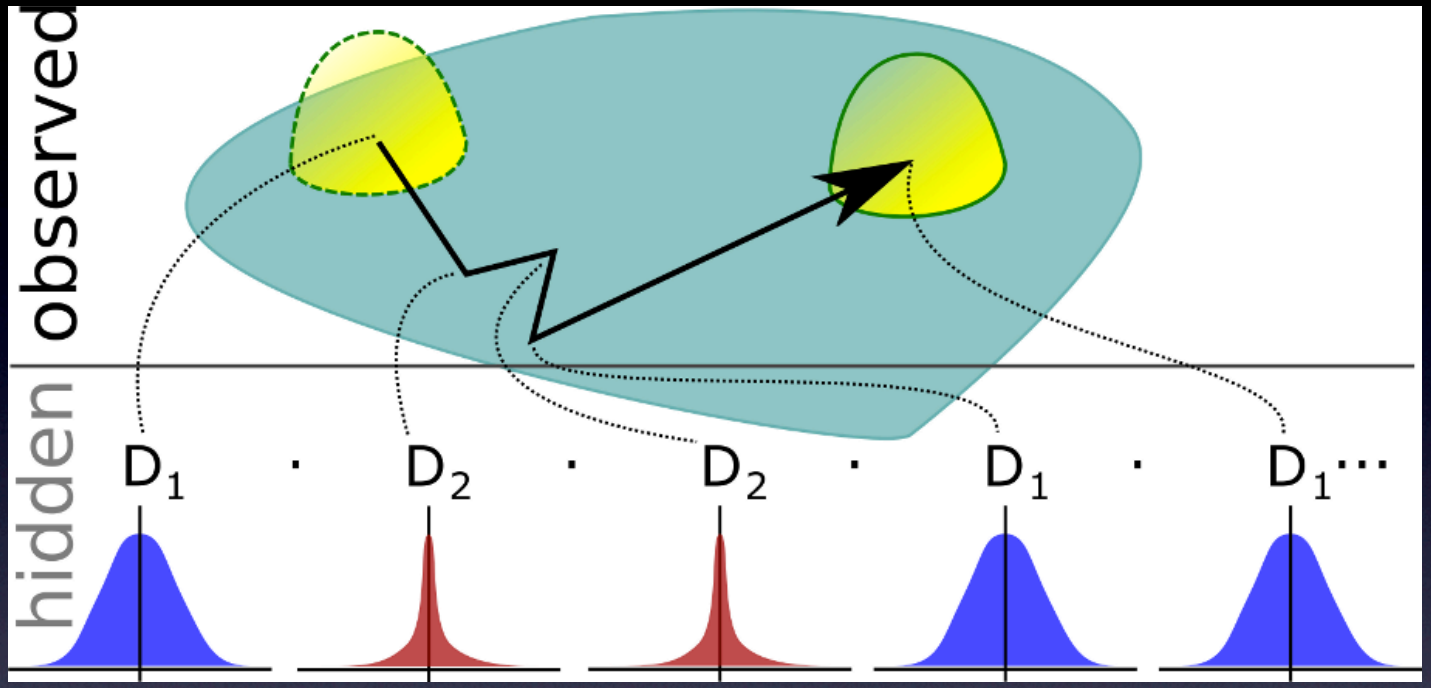
SPT study of LFA-1 on T cells



- Classify individual tracks as slow or fast based on MSD
- Distribution of diffusion coefficients changes with cell treatment
- Consistent with known interactions between LFA-1 and cytoskeleton

- this analysis captures an equilibrium distribution
- do particles undergo transitions from fast to slow?

Dynamic two-state analysis



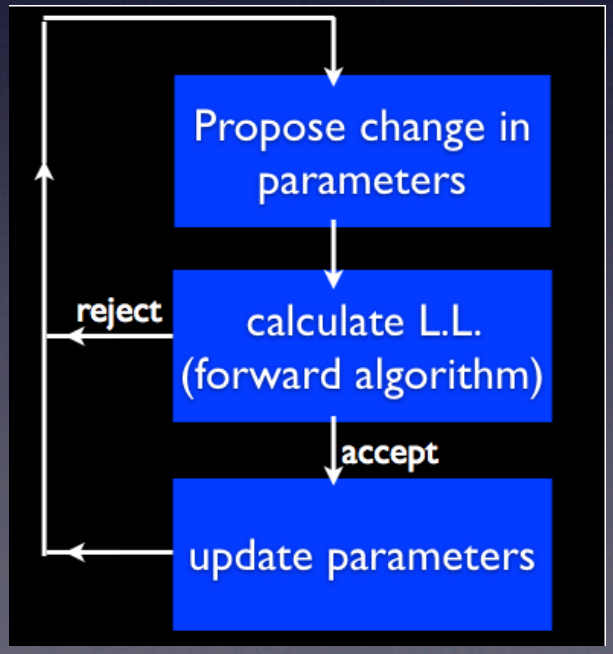
$$D_1 \underset{p_{21}}{\overset{p_{12}}{\rightleftharpoons}} D_2$$

- Suppose: LFA-1 binds and unbinds from the cytoskeleton,
- Forms a Hidden Markov model
- require transition rates slower than imaging frame-rate
  - but fast enough to find transitions in dataset.

We evaluate the likelihood of observing $O = O_1, O_2, \ldots, O_M$

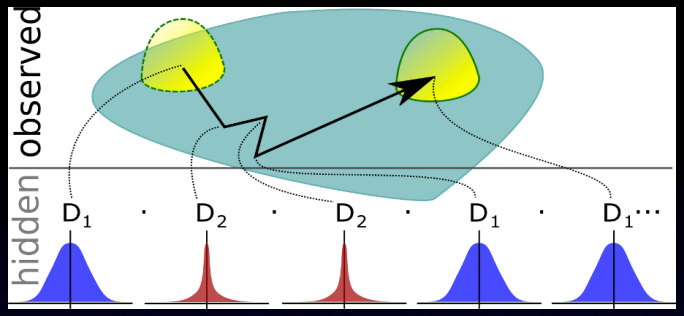
$$L(O|\{D_1, D_2, p_{21}, p_{12}\}) = \sum_{q_1, q_2, \ldots q_M} \pi_{q_1} b_{q_1}(O_1) \times p_{q_1 q_2} b_{q_2}(O_2) \times \ldots \cdots \times p_{q_{M-1} q_M} b_{q_M}(O_M)$$

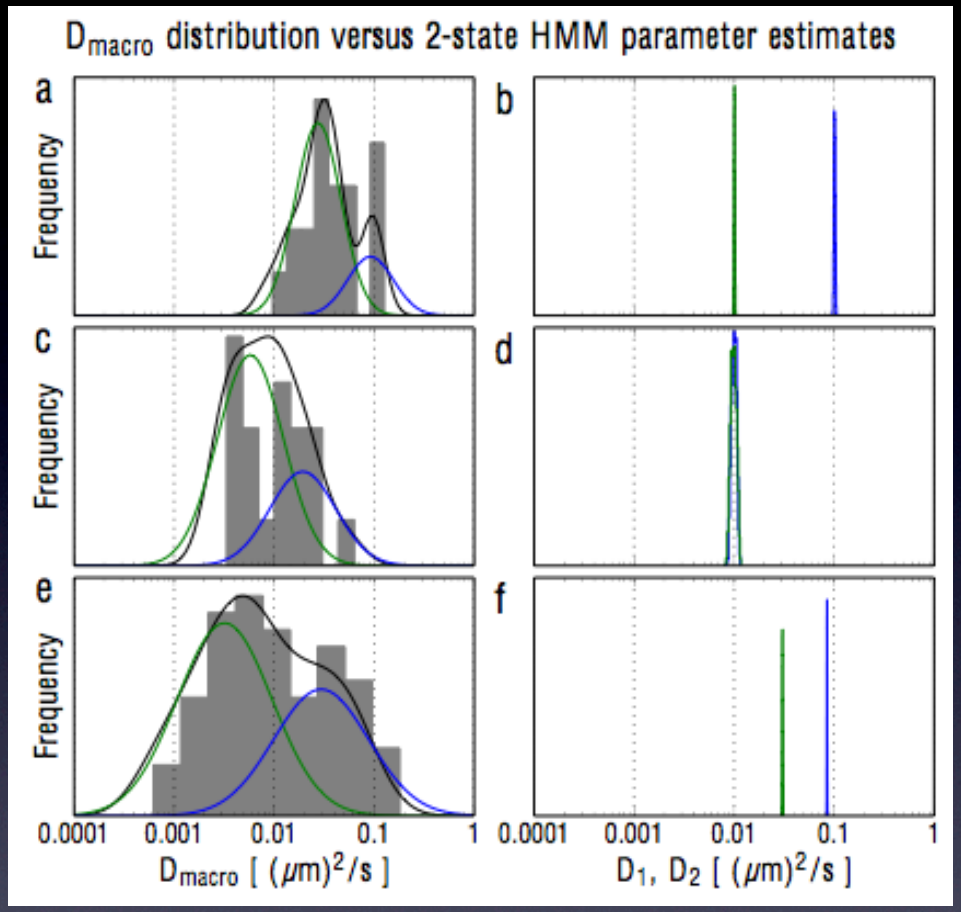
$$b_j(O_i) = \frac{1}{\sqrt{4\pi D_j \tau}} e^{-r_i^2/4D_j\tau} \qquad j = 1, 2$$



- Use the forward-backward scheme to evaluate L, input to a standard MCMC optimizer.
- Viterbi algorithm to get most likely state sequence

[simulated data]





- Precise estimates of the diffusion coefficients (D_1 , D_2)
- Generally worse estimates of transition probabilities (p_{12} , p_{21})
- If $D_1 \sim D_2$, cleanly reduces to 1-state model with undetermined transition probs.
- Statistical test correctly selects 1-state vs 2-state model.

Cytoskeleton and cell activation alter LFA-1 mobility

$$D_1 \underset{p_{21}}{\overset{p_{12}}{\rightleftarrows}} D_2$$

Treatment	D_1	D_2	p_{12}	p_{21}	D_{eff}
Control	0.081	0.015	3.9	9.1	0.062
Cyto-D	0.088	0.019	2.5	3.9	0.076
PMA	0.057	0.008	23	3.4	0.035

- Labeled with ICAM-1 on 1-micron beads
- Cyto-D inhibits cytoskeleton
- PMA "activates" cells
- Diffusivities in micron^2/s
- Transition rates in Hertz

Disrupting actin cytoskeleton shifts the equilibrium toward the free state.

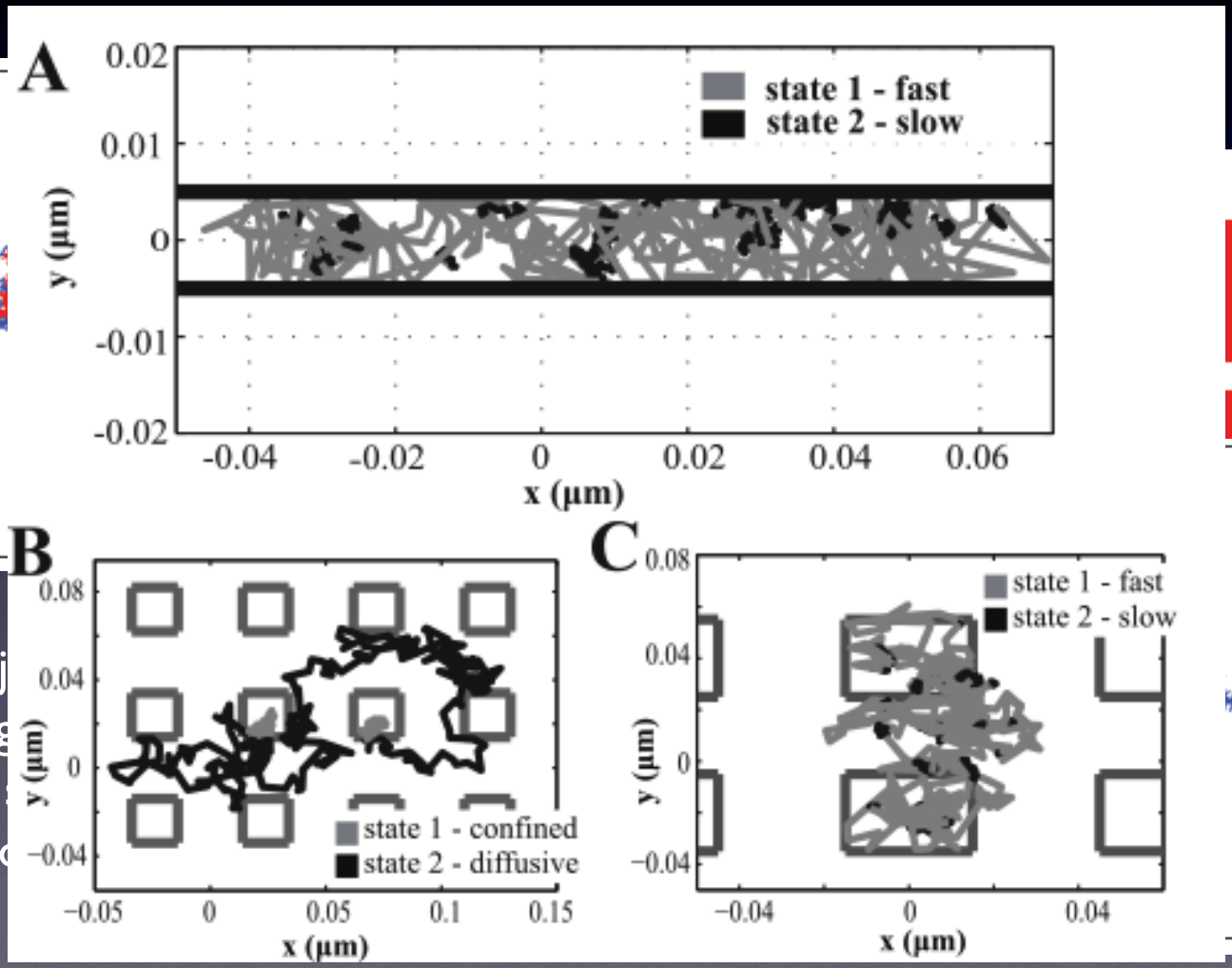
Multiple effects of PMA-induced activation:

1. Possible changes in binding partner(s).
2. Dynamic remodelling of the actin cytoskeleton

[In all cases, 2-state model is statistically preferred to 1-state diffusion model]

Detecting spatial variability

- Break tracks into fast/slow segments
- Find possible transient confinement zones



- LFA-1 traj
of switching
a role for
and/

SPT Two-State Analysis

- Likelihood-based method to detect transient changes in mobility within single trajectories.
- suggesting and quantifying biological models

- Parameter measurement: interactions of LFA-1 with actin cytoskeleton

- Segmentation of tracks into component states - inference of spatial heterogeneity.

- Extensions to more complex modes of mobility

- Problem of understanding the underlying modes of motion is generic!
- e.g. 2-photon cell tracking data (e.g. T and B cells in lymph nodes)
- animal tracking (wolves, tuna, etc) in nature

Challenges for SPT analysts:

- Optimizing and automating particle detection and tracking
- Designing algorithms to infer defined physical models of motion
  - free diffusion
  - multistate diffusion
  - confined motion (de Vries)
  - drift
- Comparing the likelihood of different models in a consistent framework
- Interpretation with other imaging methods (FRAP, super-resolution on fixed cells)

Summary

- Modern microscopic imaging opens a window on the protein-level functioning of healthy and diseased cells.
- Modeling and parameter estimation essential for quantitative, reproducible work
- Modeling as a tool for experimental design
- Generation of quantitative predictive models

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