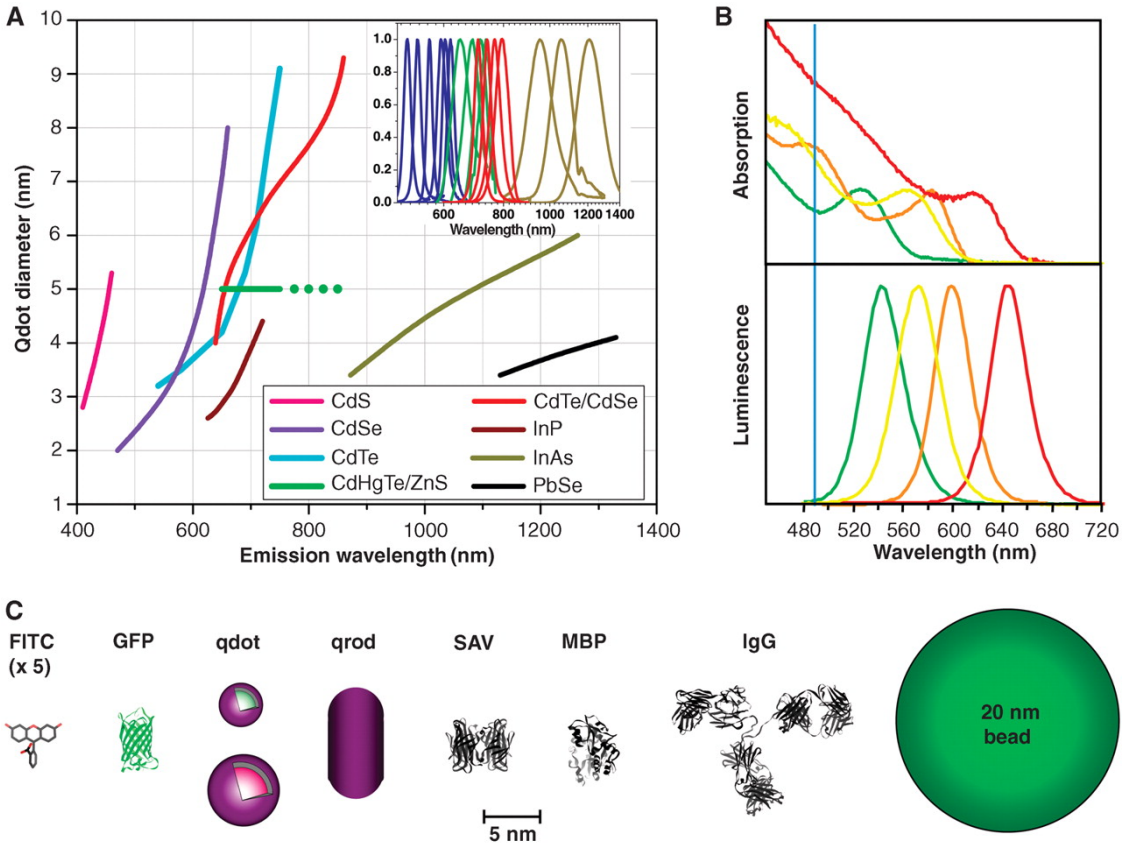
Three dimensional tracking of individual quantum dots

(plus some other stuff, time permitting)

Jim Werner
Center for Integrated Nanotechnologies
Los Alamos National Laboratories

Quantum dots as fluorescent labels for tagging biomolecules



"Quantum dots for live cells, in vivo imaging and diagnostics,"
Michalet, Piinaud, Bentolila, Tsay,Doose, Sendaresan, Wu, Gambhir, and Weiss

28 JANUARY 2005 VOL 307 SCIENCE www.sciencemag.org

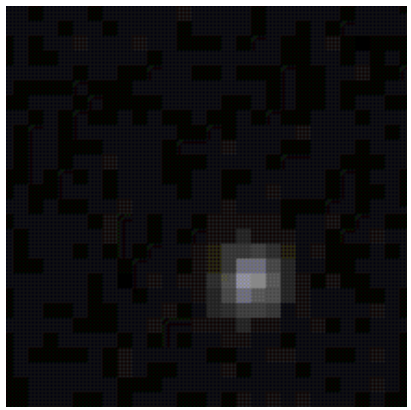
Why track a single, small particle? examples from one and two dimensions

Los Alamos NATIONAL LABORATORY EST. 1943

Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5-nm Localization

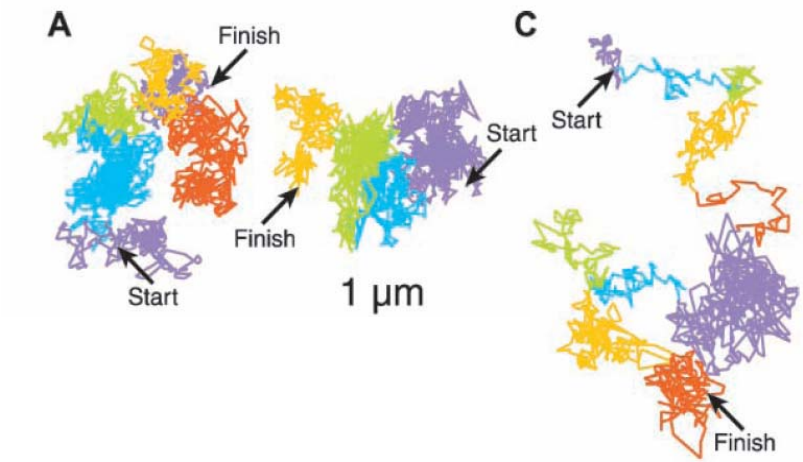
Ahmet Yildiz,[1] Joseph N. Forkey,[3] Sean A. McKinney,[1,2] Taekjip Ha,[1,2] Yale E. Goldman,[3] Paul R. Selvin[1,2]*

SCIENCE VOL 300 27 JUNE 2003



Potential for ~nm spatial precision

Can follow dynamic, stochastic processes



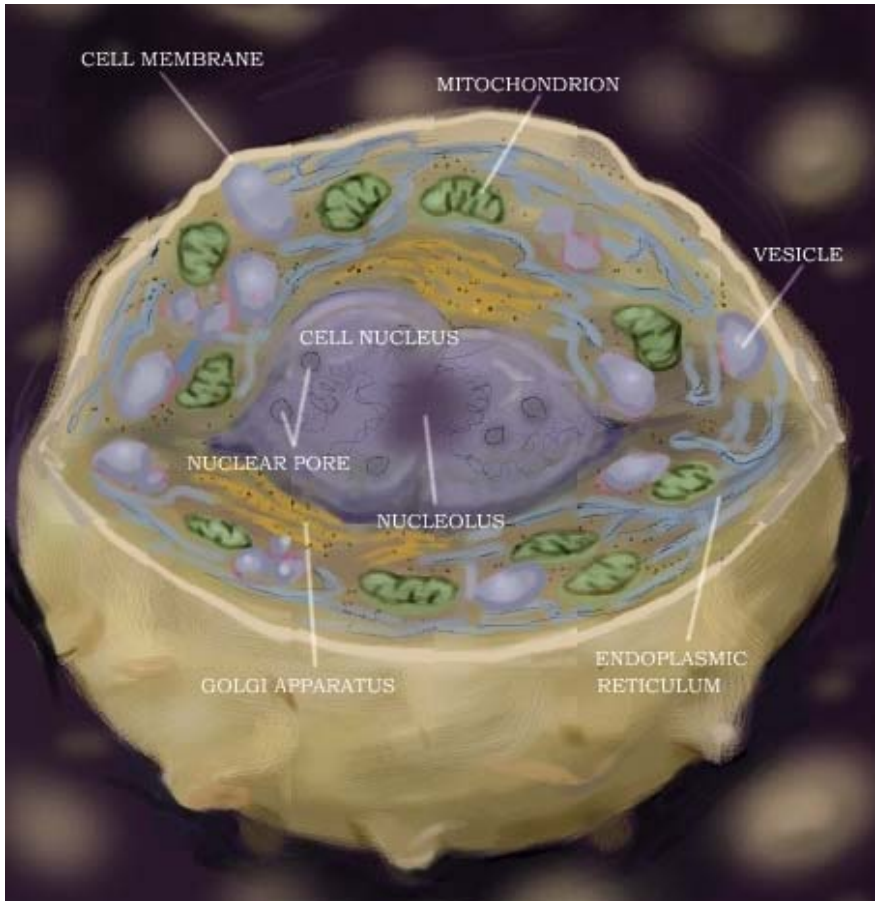
Phospholipids undergo hop diffusion in compartmentalized cell membrane

Fujiwara, Ritchie, Murakoshi, Jacobson, Kusumi

The Journal of Cell Biology

Volume 157, Number 6, 2002

We're not living in flatland:



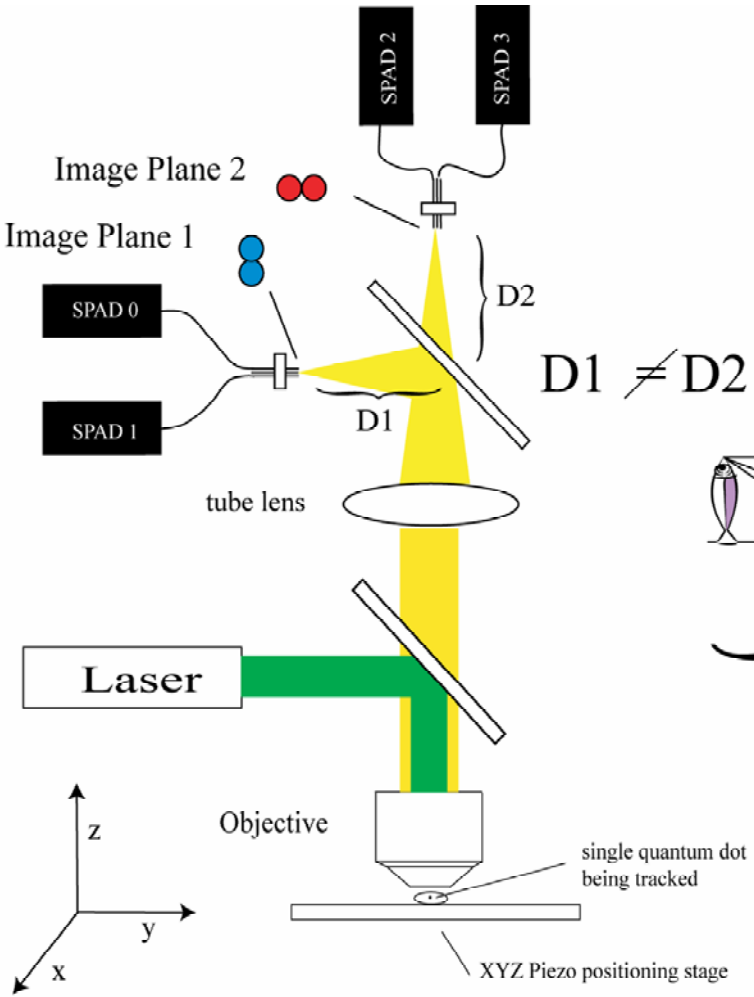
Diffusive Motion of a protein

cytoplasm ~ 4 $\mu m^2/s$ to 20 $\mu m^2/s$
membrane ~0.01 to 1$\mu m^2/s$

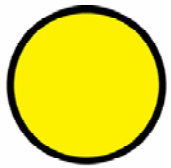
Directed Motion

kinesins ~ 0.02-2 $\mu m/s$
myosins ~ 0.2 to 60 $\mu m/s$

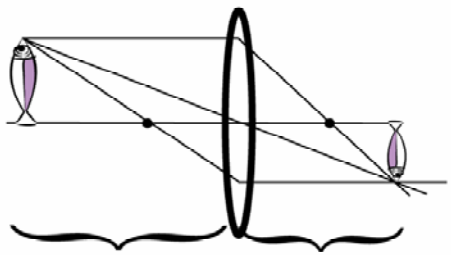
For 3D tracking, start with confocal microscopy as a base:



Fibers are 50 μm core
2.5 μm cladding

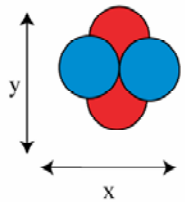


$D1 \neq D2$

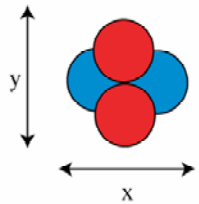


$$\frac{1}{Si} + \frac{1}{So} = \frac{1}{f}$$

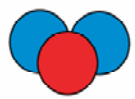
Top View of Probe Volume



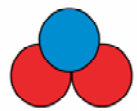
Bottom View of Probe Volume



View looking down Y axis (Z=0)



View looking down X axis (Z=0)



Simulating our microscope: Using a few things we might know

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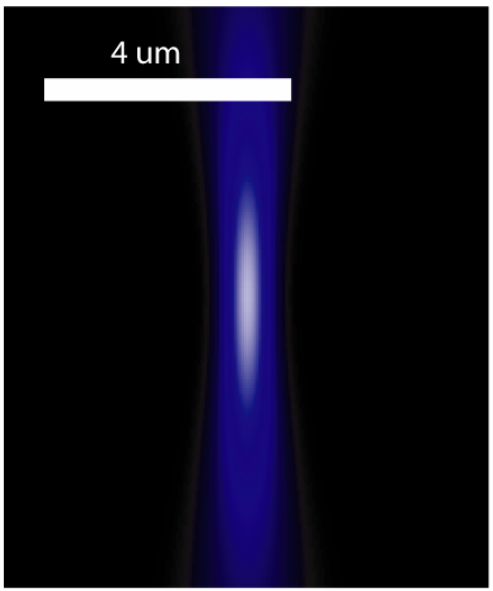
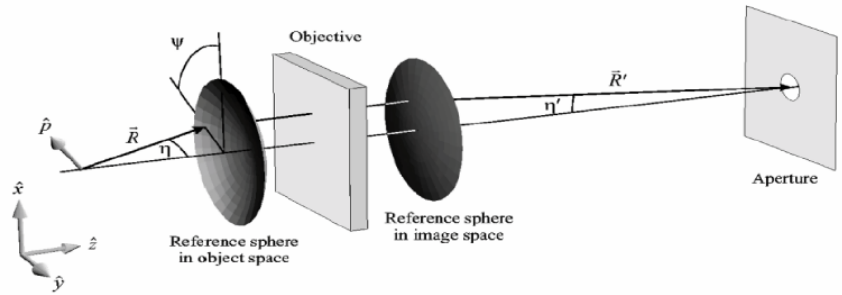
laser excitation profile



Yields # of photons absorbed per second,
Yields # of photons emitted per second

Collection Efficiency Function for a high NA objective



Yields fraction of light for a dipole at X, Y, Z that makes it through an aperture in the image plane

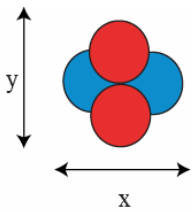
APPLIED OPTICS (1991) Hong Qian and Elliot Elson
OPTICS LETTERS (2000) Jorg Enderlein

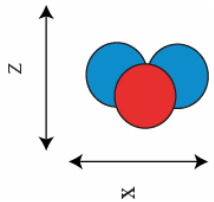
How to simulate diffusion via a random walk

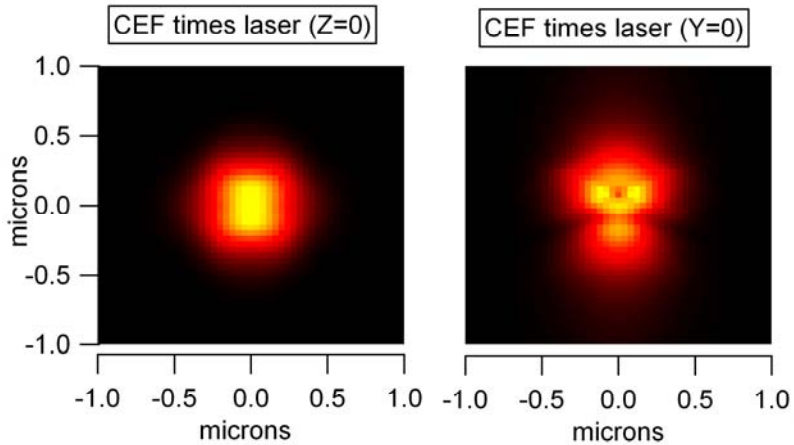
← ? →

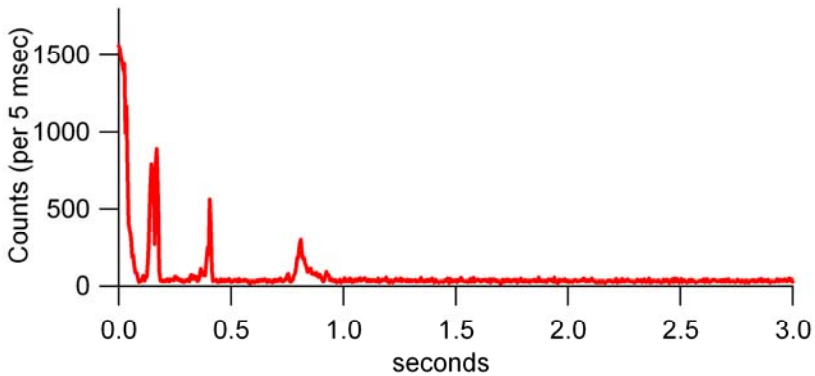
"Three dimensional tracking of fluorescent particles" Lessard, Goodwin, Werner SPIE Vol. 6092 (2006) 609205-1 to 609205-8.

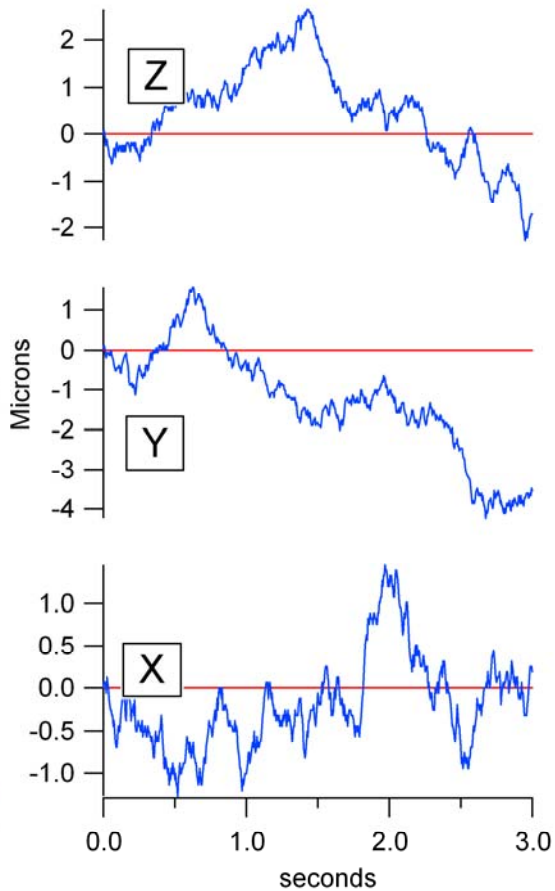
Simulation, quantum dot
D=1.0 um^2/s; NO TRACKING



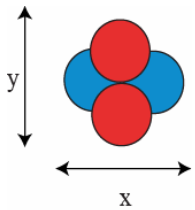


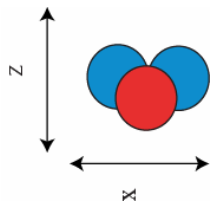


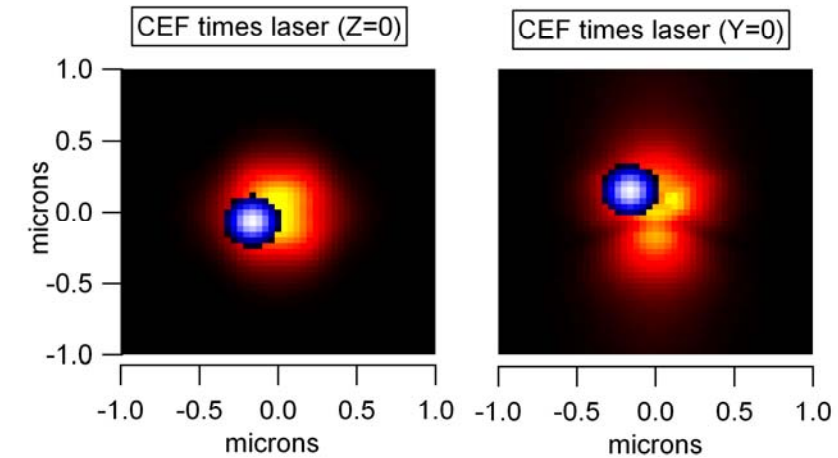


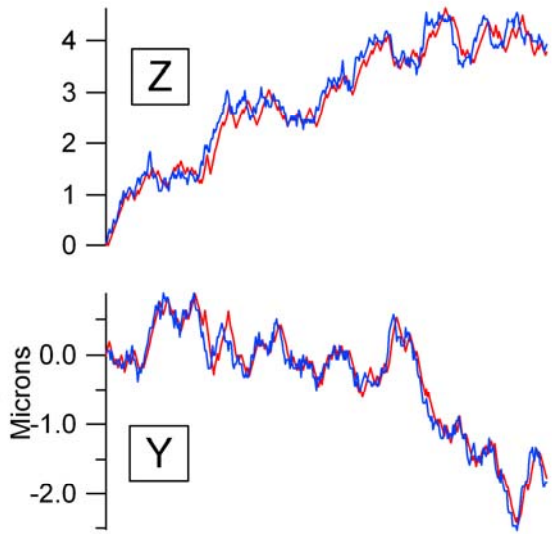


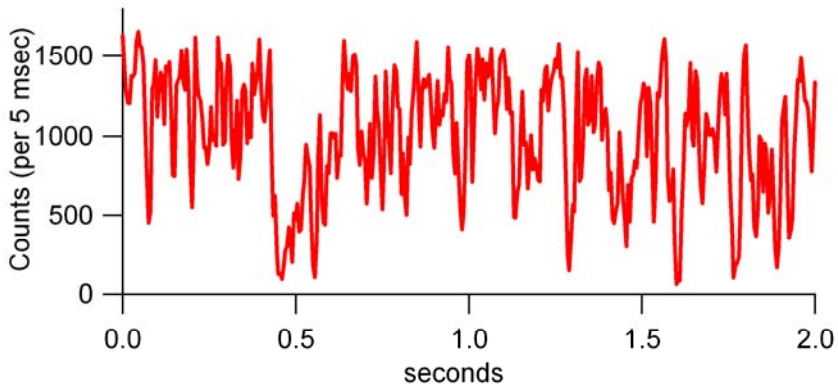
Tracking simulation, quantum dot D=1.0 um^2/s



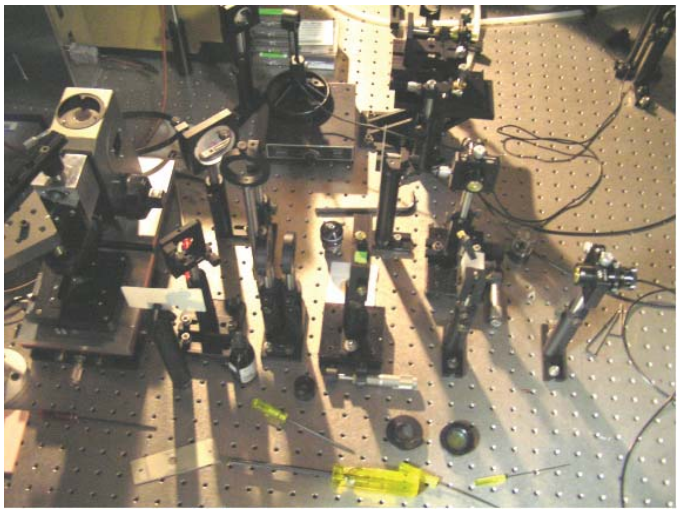


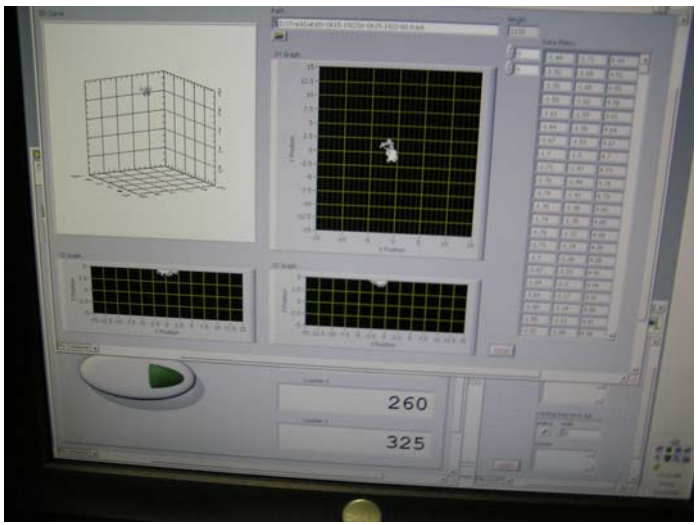






The tracking apparatus (Hardware):

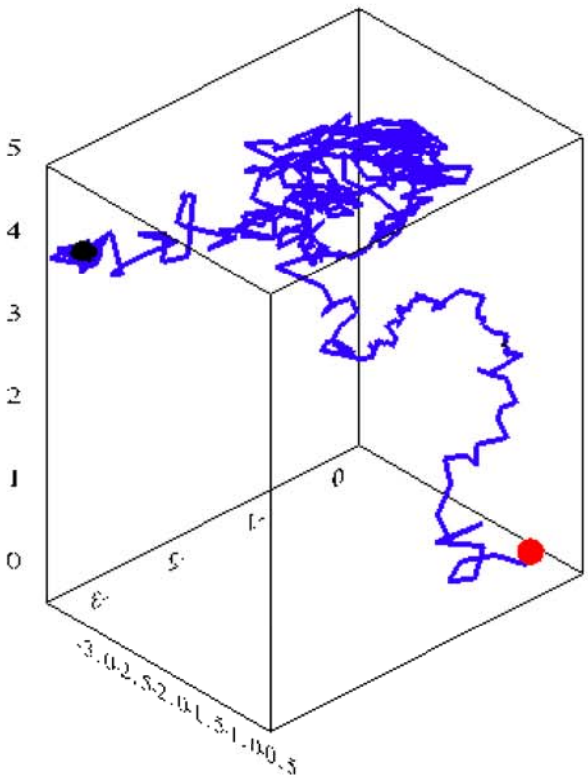


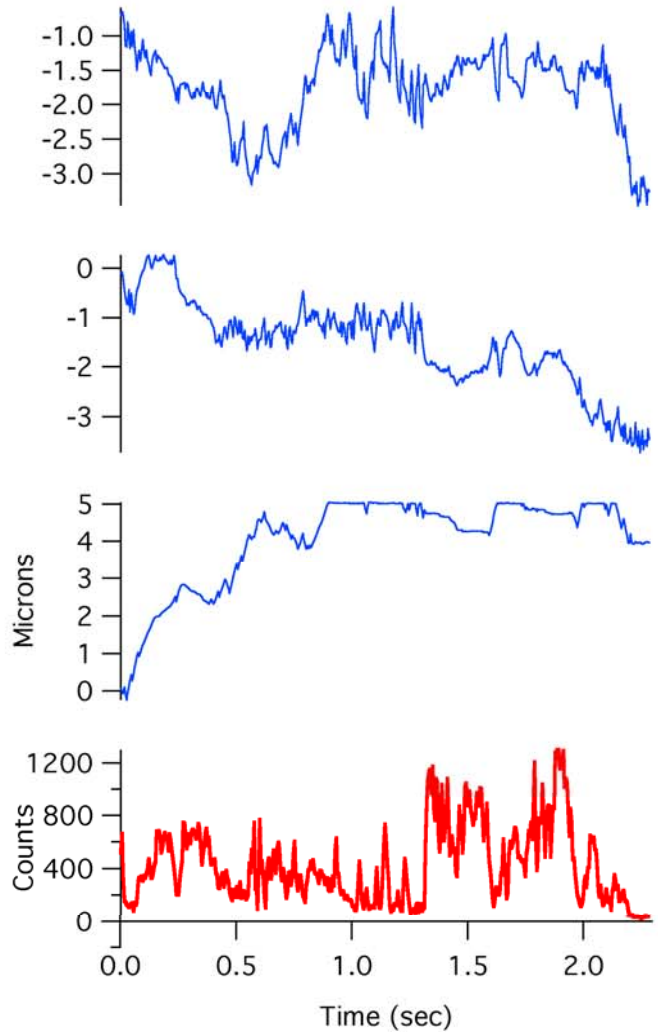


Equipment:

A Fast closed loop XYZ Piezo stage (PI-733-3DD)
SPC 630 (not used for tracking)
Four SPADs
Pulsed semiconductor diode laser
60x, 1.2 NA water immersion objective
LabView REALTIME

Experimental Data:
Glycerol/water mixture, D~ 1 um^2/s





Randomly selected 3-D trajectories

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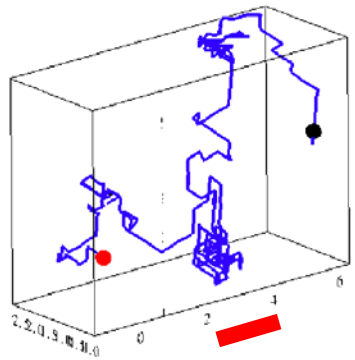
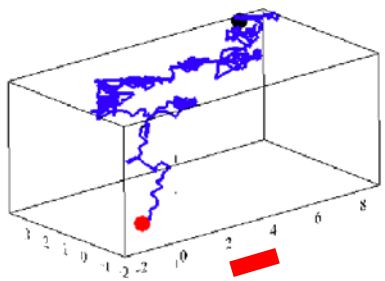
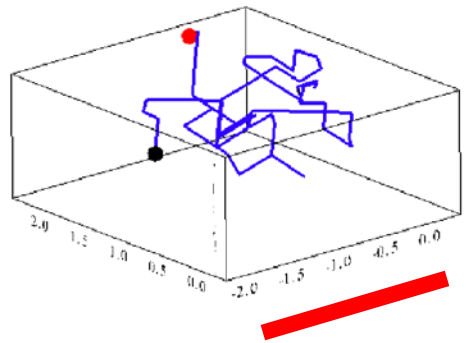
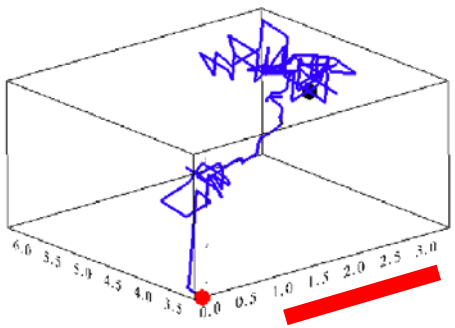
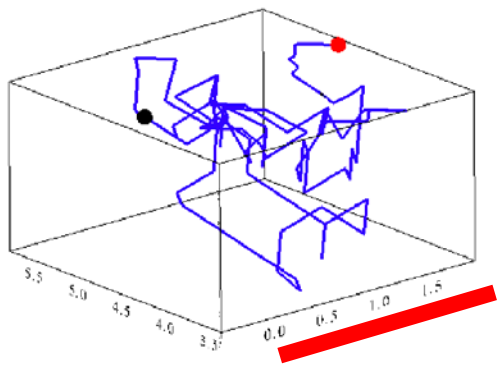
2 μm scale bar

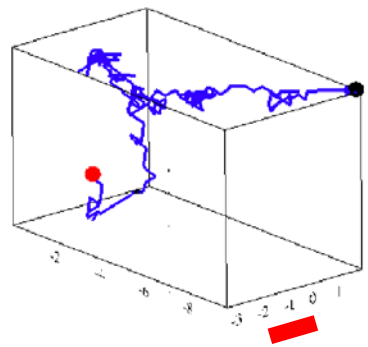




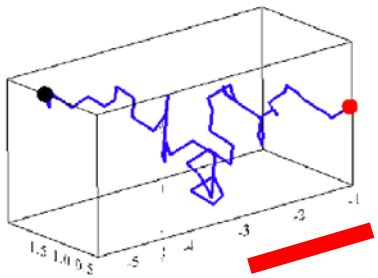


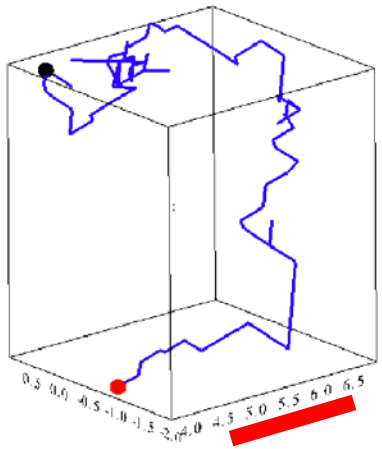


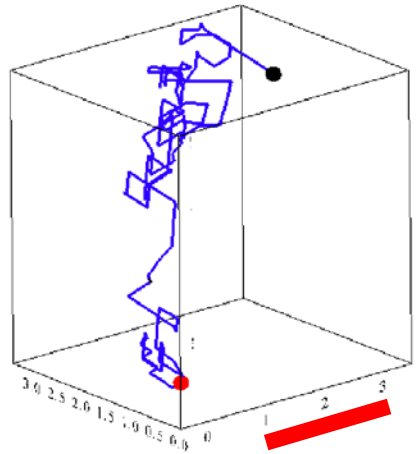


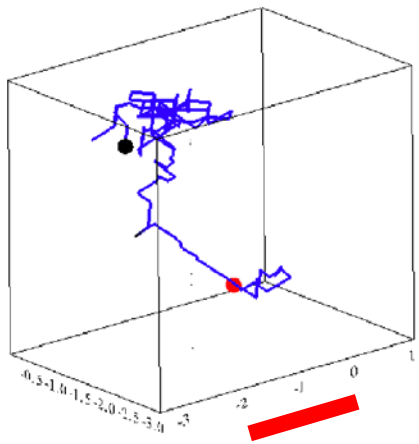


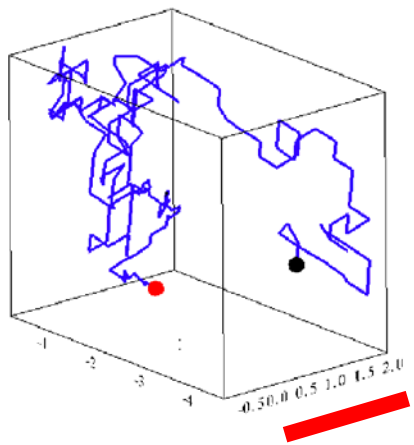
More Randomly selected 3-D trajectories

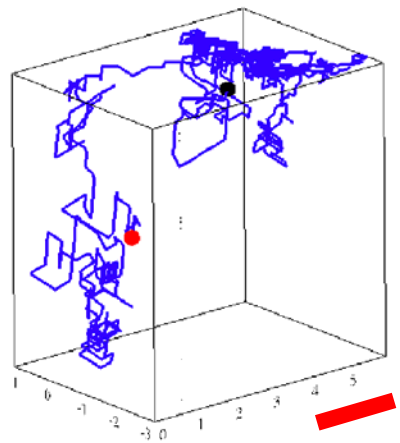








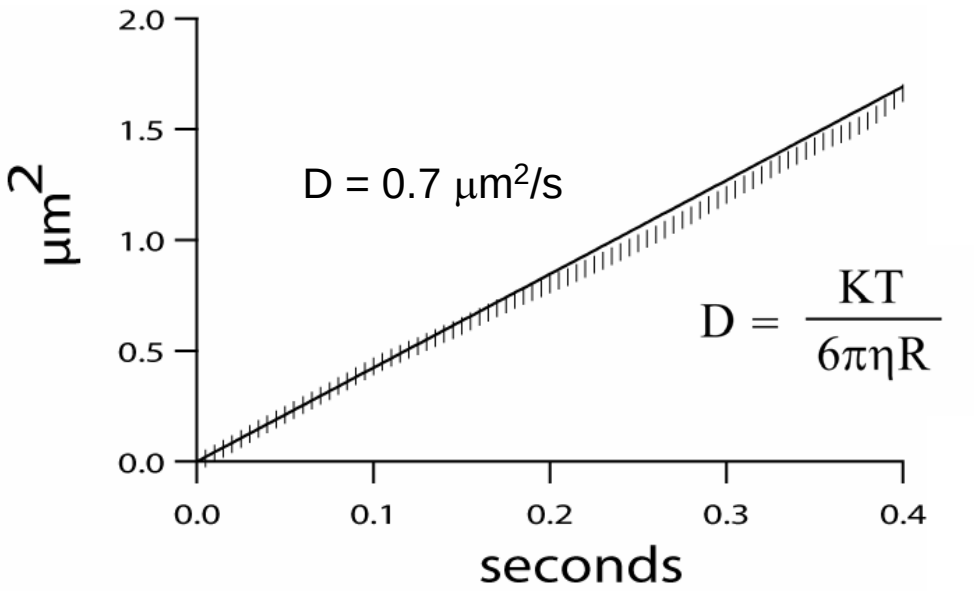




How do you know you're tracking a single qdot?

1. **Count rate is what you'd expect from a single quantum dot.**

2. **The mean squared displacement of the measured trajectories reflects particle size:**



From 3D trajectories:

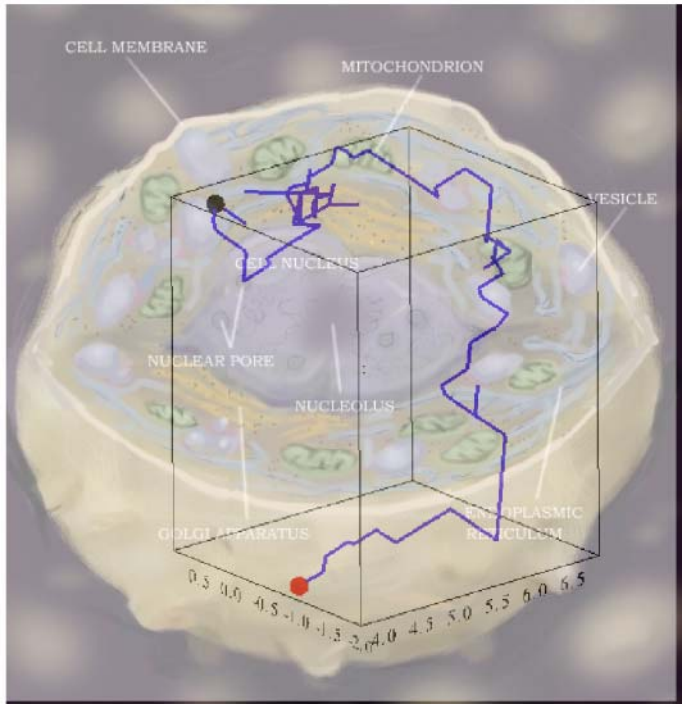
R_H = 16 nm

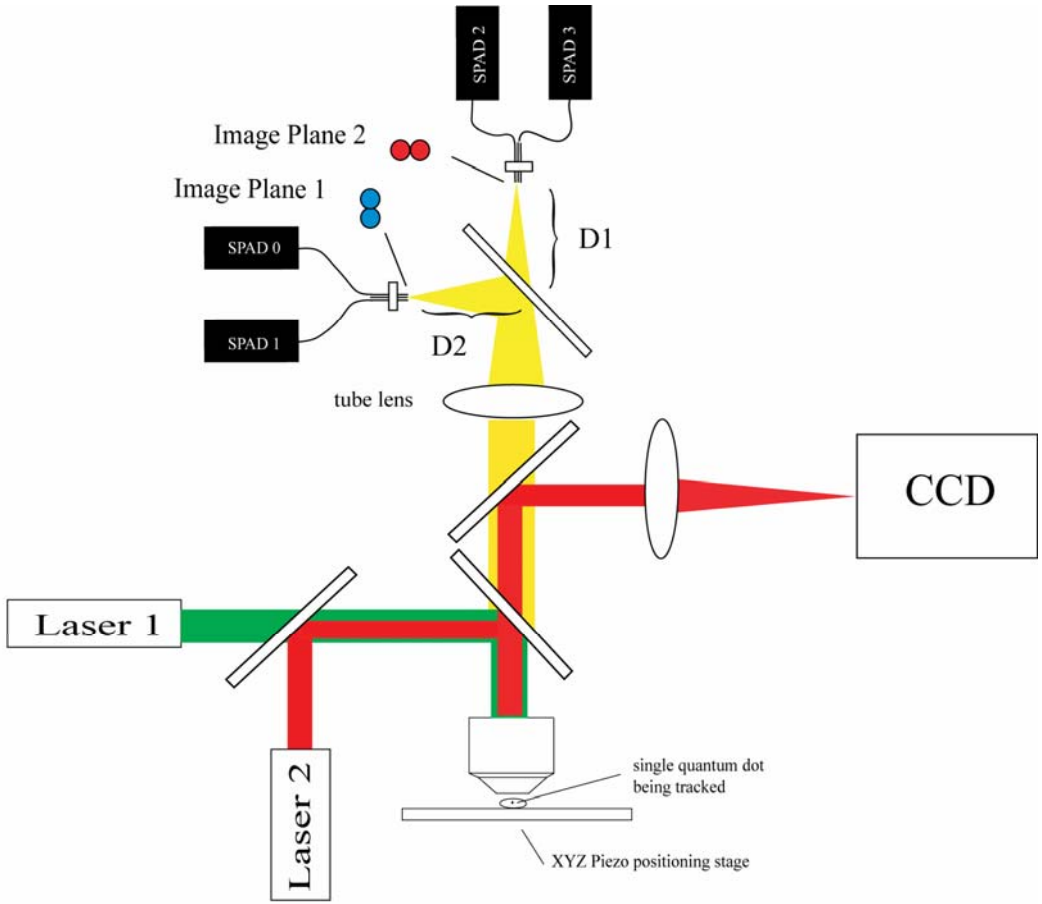
From FCS:

R_H = 15 nm

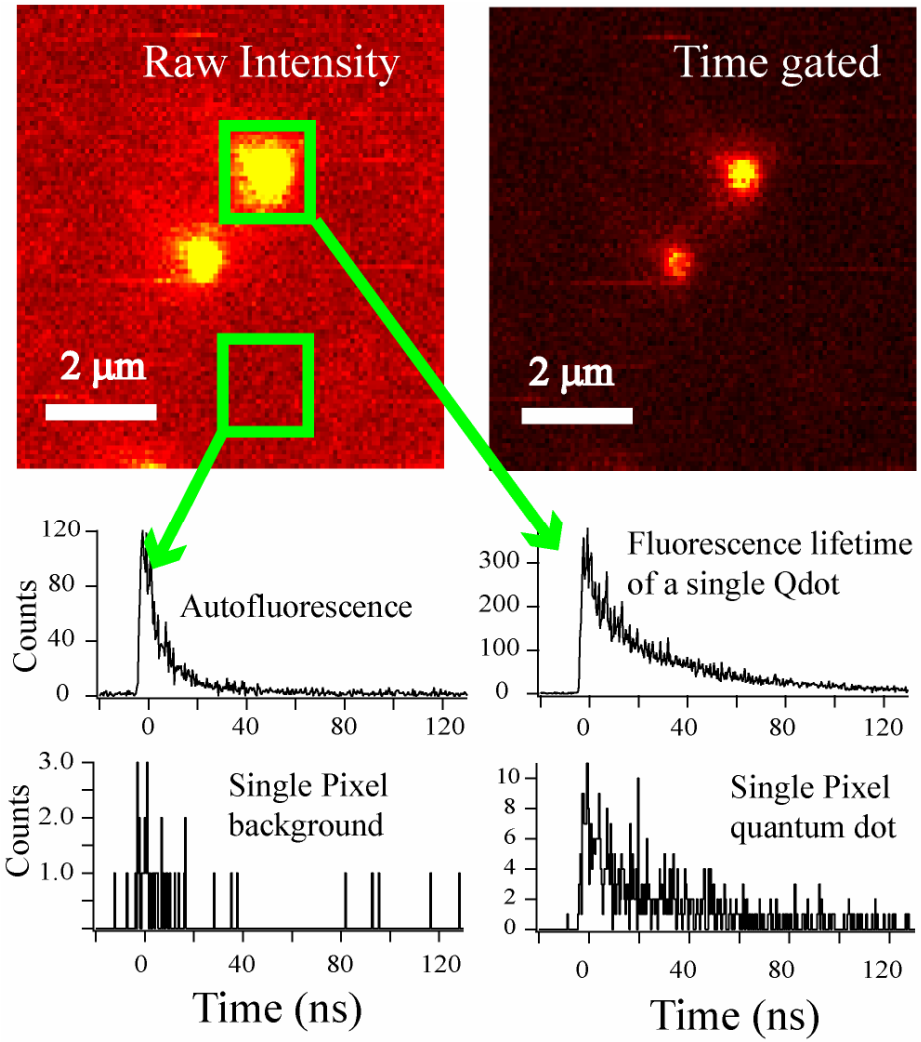
"Three dimensional tracking of individual quantum dots"
Lessard, Goodwin, Werner
(submitted)

Future directions: 3D trajectories in cells, over-lapped with structure





Time-resolved spectroscopy while tracking



Raw Photons:
ANY analysis method

Fluorescence lifetime measurements:
Proximity to a FRET partner
Conformation of molecule

Not limited by camera "frame rate"

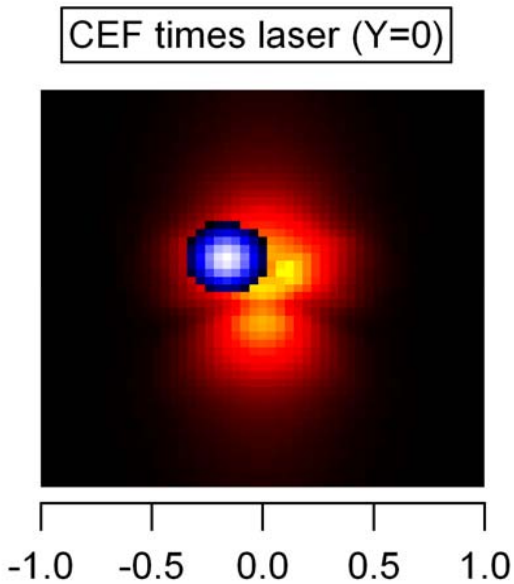
Window on cellular process spanning 100 ps to 10 seconds!

Conclusions: 3D tracking

We can track single quantum dots in 3D at rates faster than many intracellular transport processes

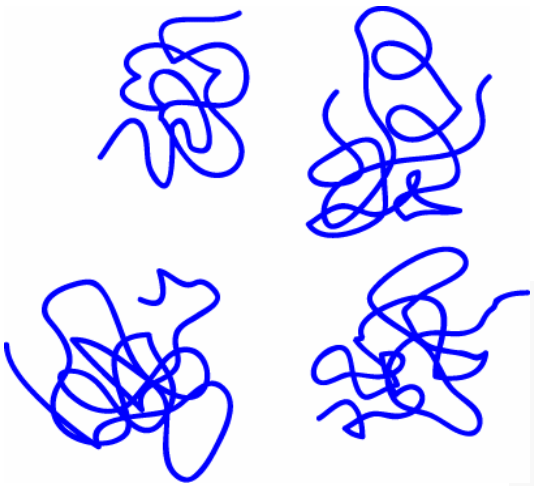
Next Steps

Into the cell
Rates of motion
Spatial accuracy
Measure CEF

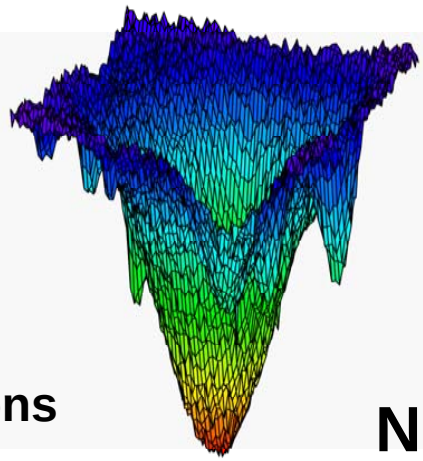


Protein folding

Unfolded states



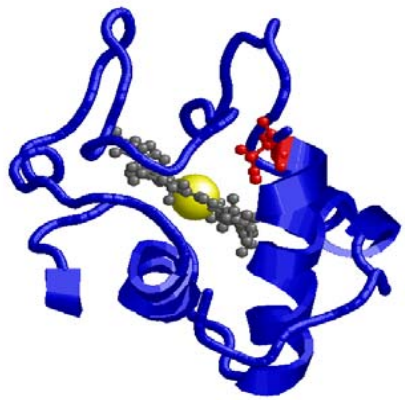
$$U \underset{k_u}{\overset{k_f}{\rightleftharpoons}} N$$



U

N

Native state



Astronomical number
of different conformations

$\sim 10^{30}$

Very similar
conformations

Yeast cytochrome c labeled with TRITC

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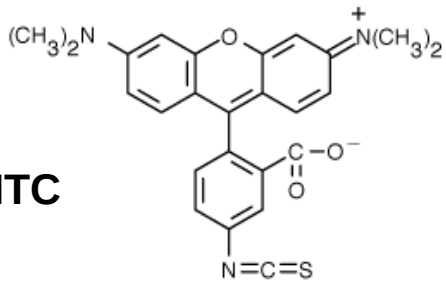
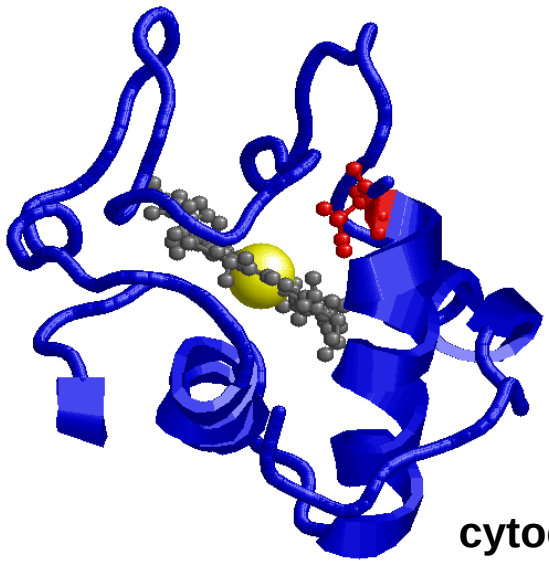
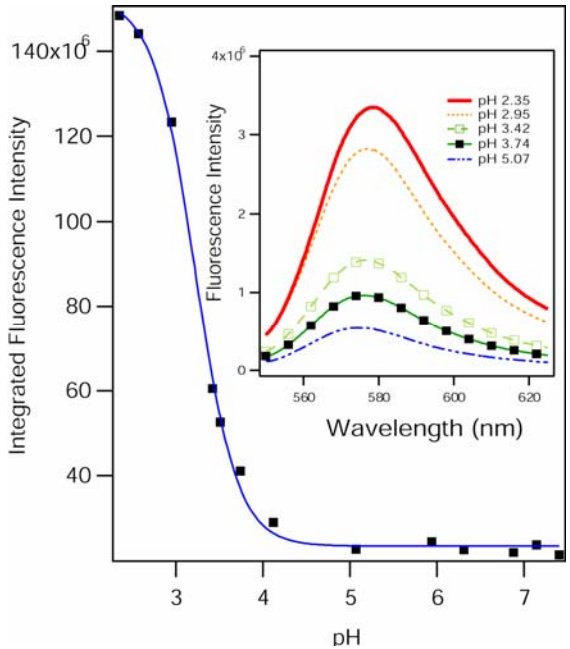


TRITC



cytochrome c



J|A|C|S ARTICLES

Published on Web 04/20/2002

Mapping the Cytochrome *c* Folding Landscape

Julia G. Lyubovitsky, Harry B. Gray,* and Jay R. Winkler*

Contribution from the Beckman Institute, California Institute of Technology, Pasadena, California 91125

Received October 26, 2001

Dansyl label (lifetime only)

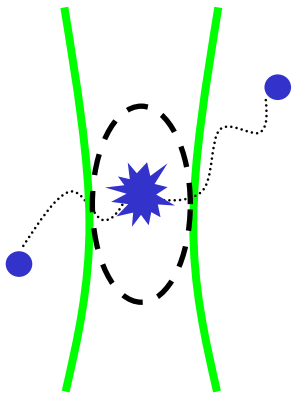
Fluorescence Correlation Spectroscopy (FCS)

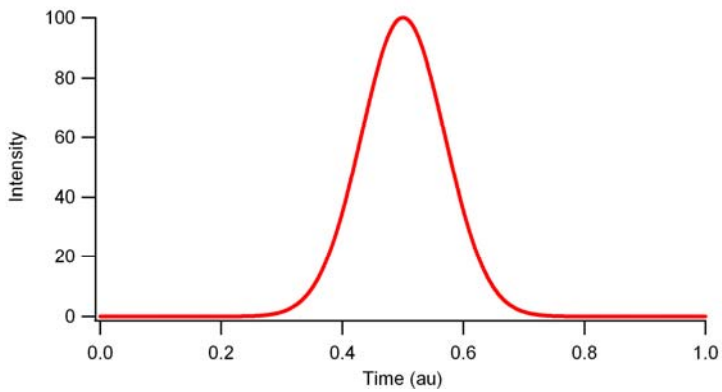
to measure gross conformation and fluctuations in fluorescence intensity

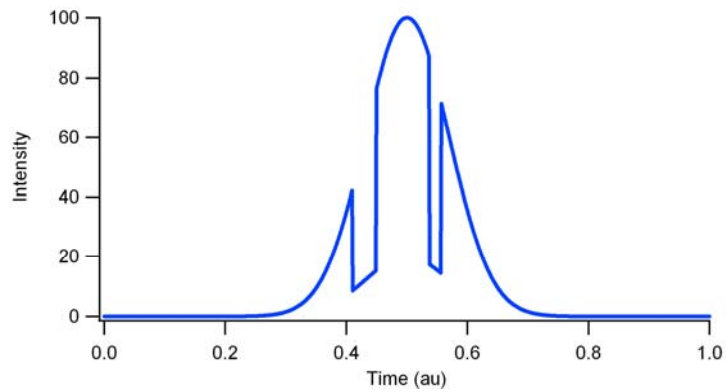
Los Alamos NATIONAL LABORATORY EST.1943

Normal emission

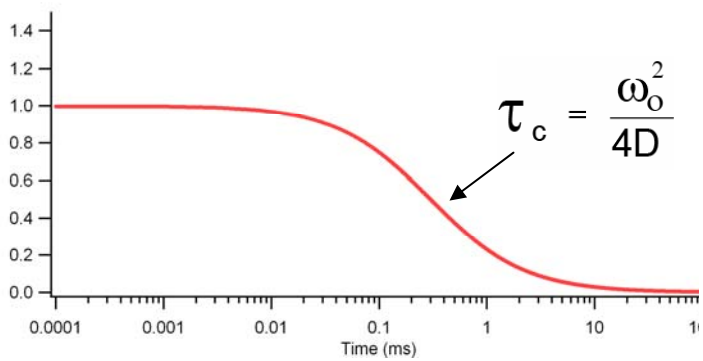
"Flickering"

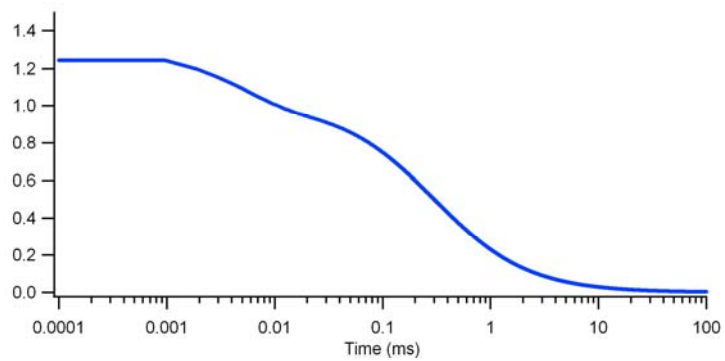




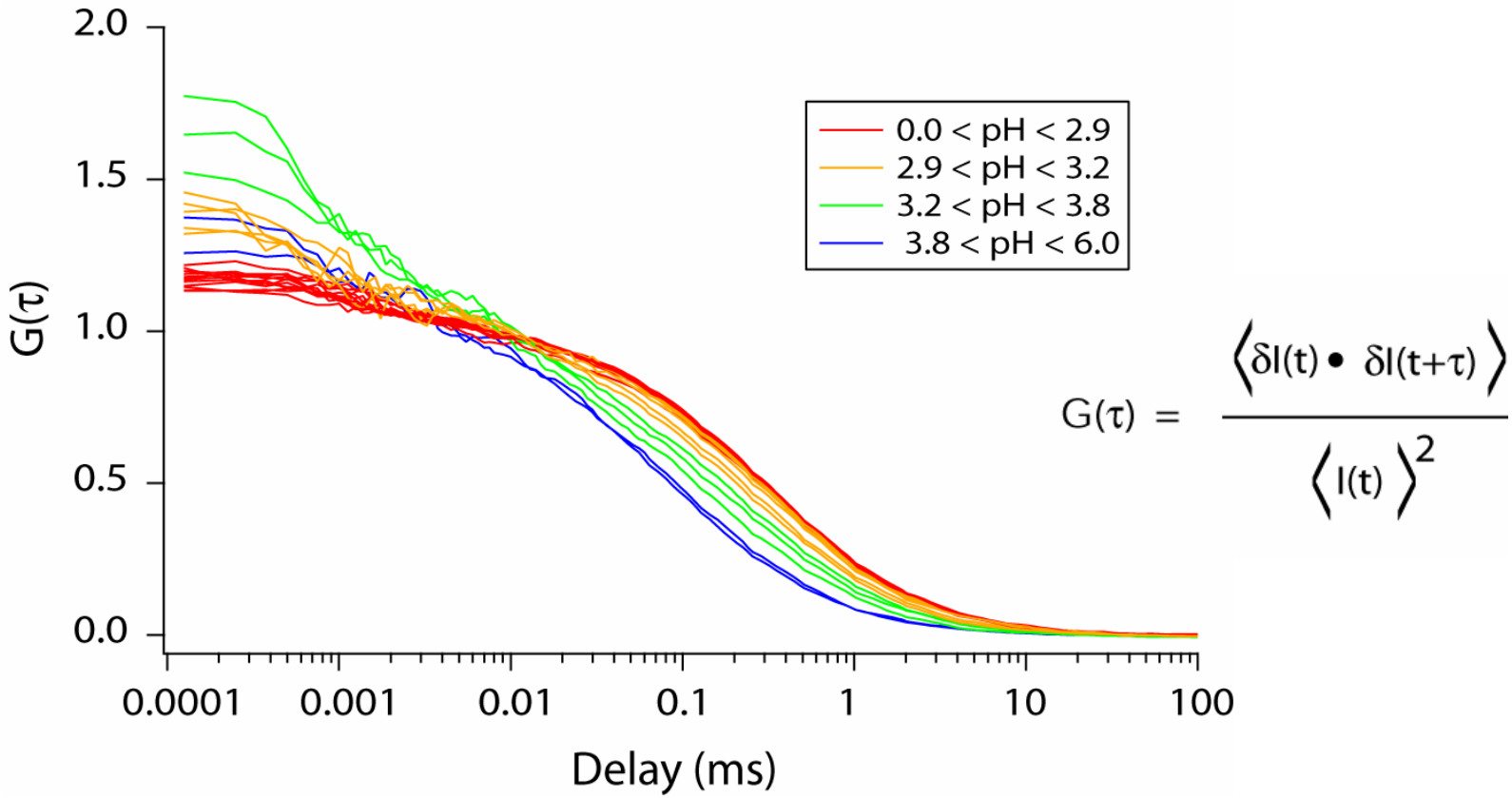


$$G(\tau) = \frac{\langle \delta I(t) \bullet \delta I(t+\tau) \rangle}{\langle I(t) \rangle^2}$$



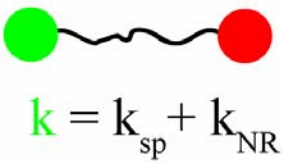


Fluorescence correlation spectroscopy of cyt c-TMR



Werner, Joggerst, Dyer, and Goodwin *"A two dimensional view of the folding energy landscape of cytochrome c,"* **Proc. Natl. Acac. Sci, *103*, 11130-11135 (2006).**

Site-specific distance distributions measured by FRET via TCSPC

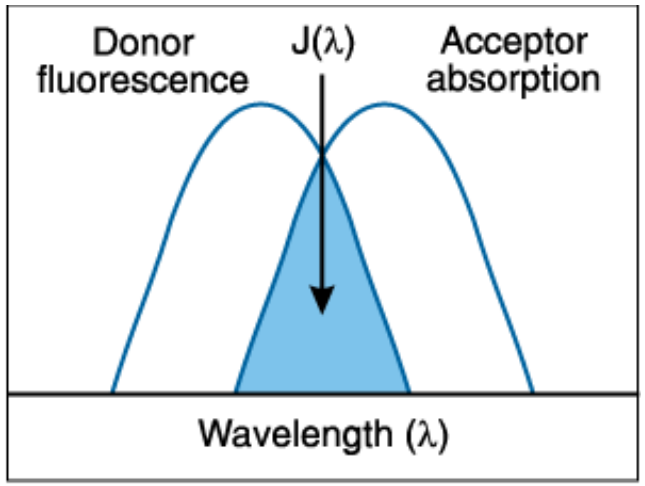


$$k = k_{sp} + k_{NR}$$

$$k_{TR} \sim |\langle d^*a| M |da^*\rangle|^2 = \frac{1}{\tau}\frac{R_o^6}{R^6}$$

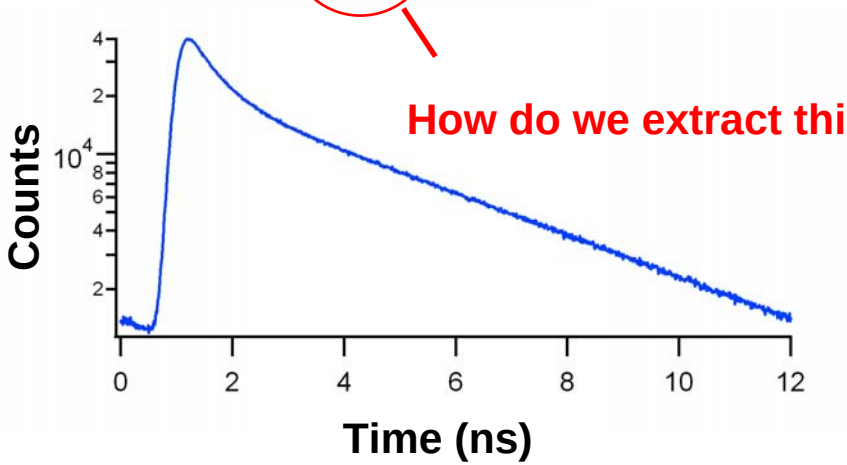
$$k = k_{sp} + k_{NR} + k_{TR}$$

$$R_o \sim 40\ \text{Å}$$



$$I(t) = \int p(k) e^{-kt}\, dk$$

How do we extract this?



Maximum Entropy Methods

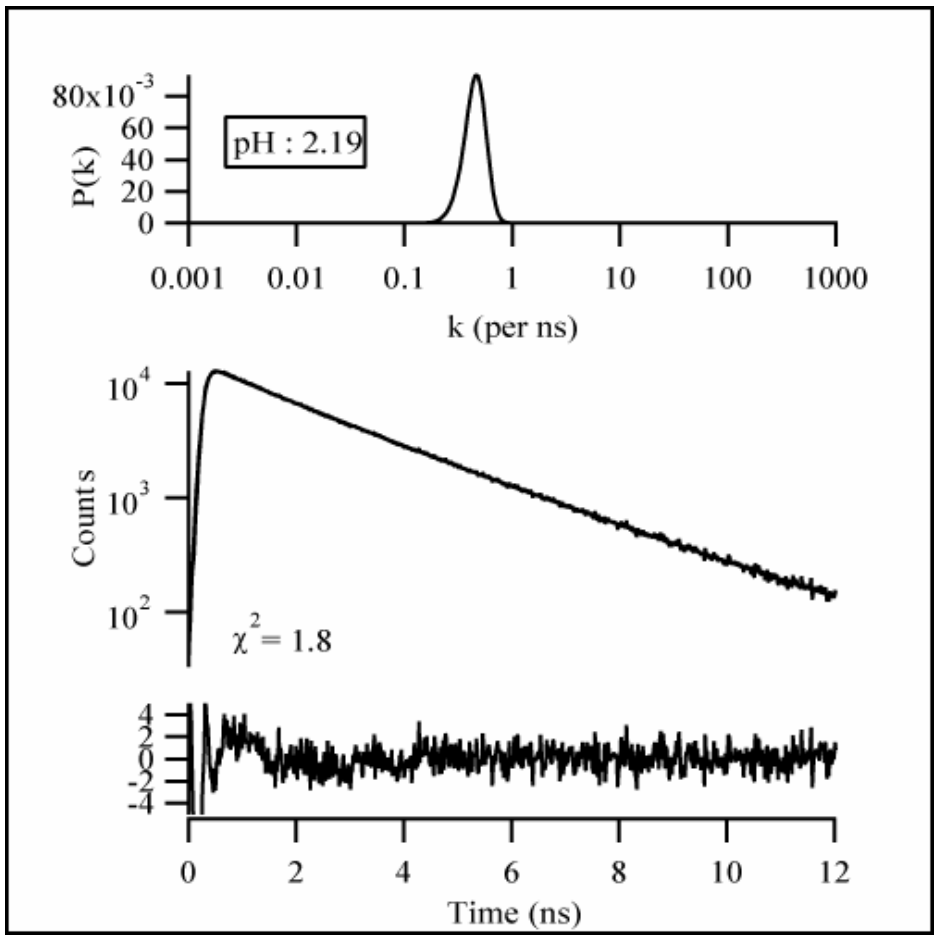
Minimize

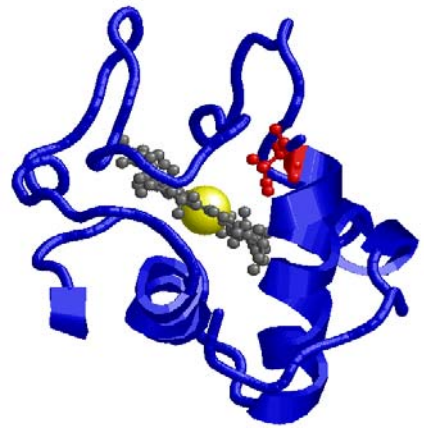
$$\chi^2$$

Maximize

$$S = \sum_i p_i \bullet \log(p_i)$$

P(k) distribution from TCSPC MEM





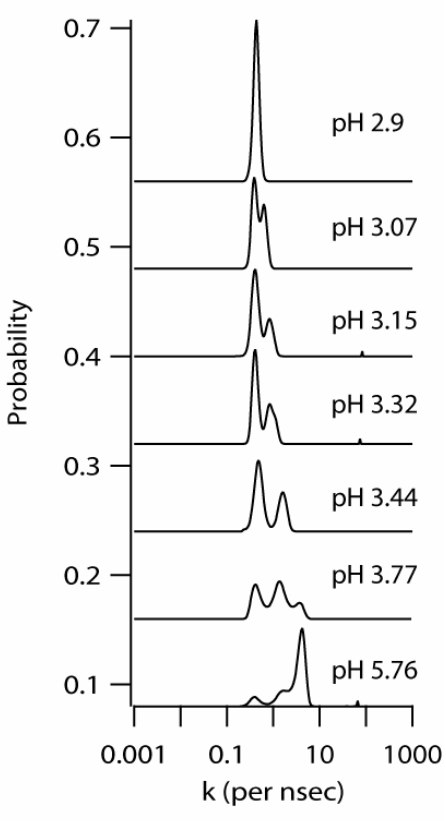
Structural characterization of folding intermediates in cytochrome *c* by H-exchange labelling and proton NMR

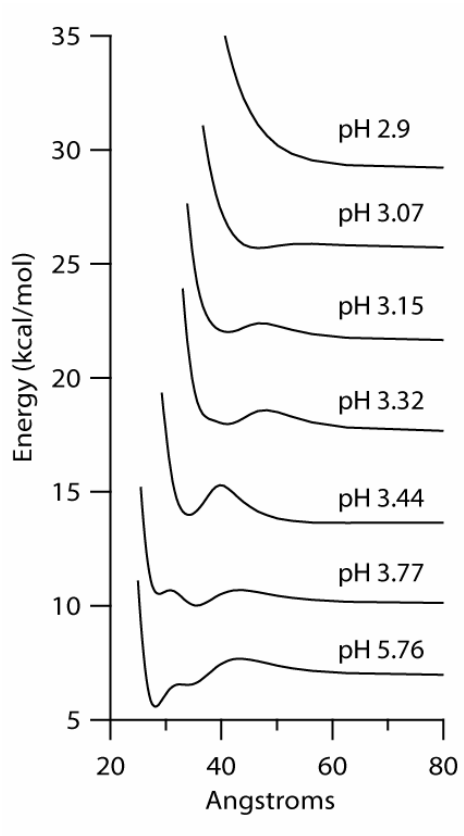
Heinrich Roder, Gülnur A. Elöve & S. Walter Englander

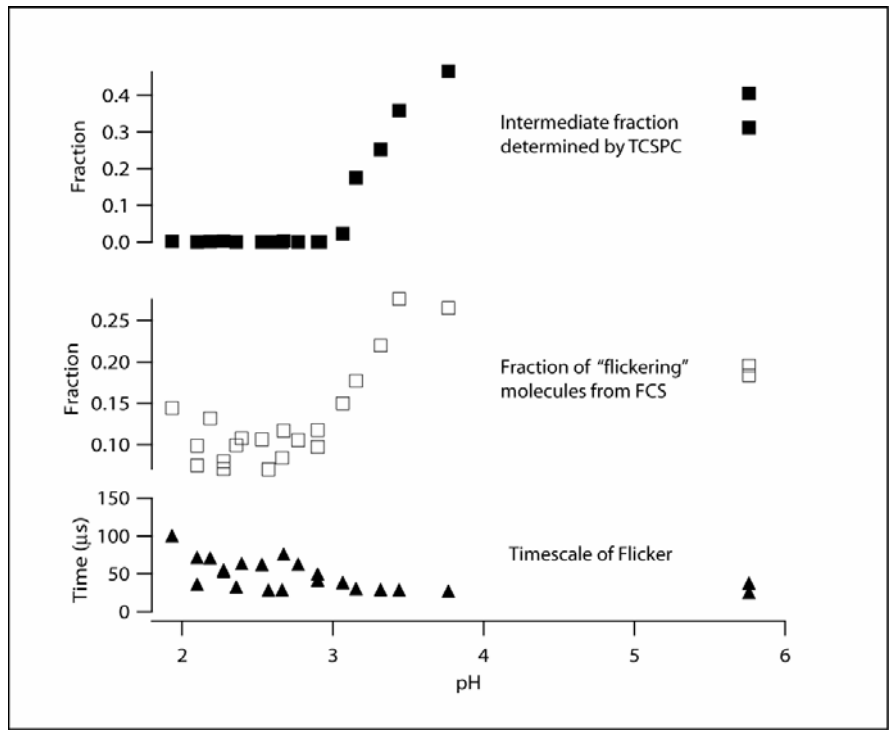
Nature **335, 700 - 704 (20 October 1988)**

U ⇄ I ⇄ N

Correlation between FCS and TCSPC







Werner, Joggerst, Dyer, and Goodwin *"A two dimensional view of the folding energy landscape of cytochrome c,"* **Proc. Natl. Acac. Sci, *103*, 11130-11135 (2006).**

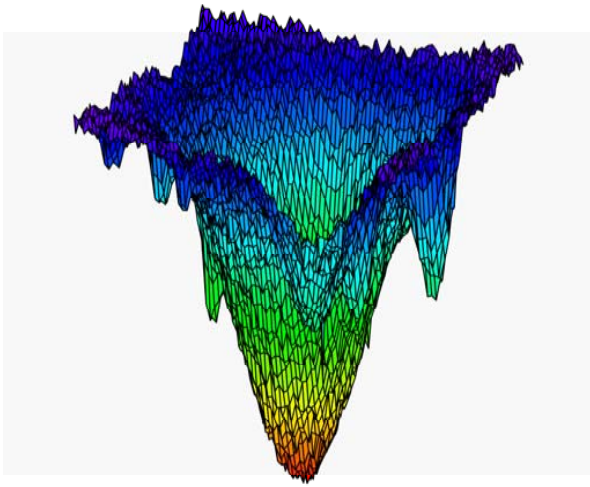
cyt c folding conclusions

Los Alamos NATIONAL LABORATORY EST.1943

Combination of methods reveals details that can't be easily discerned by either independently

Not only "2D" static view of landscape

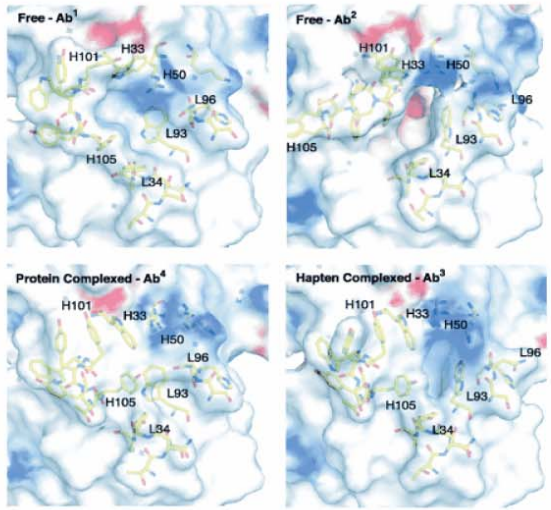
Possible use of 3D tracking.



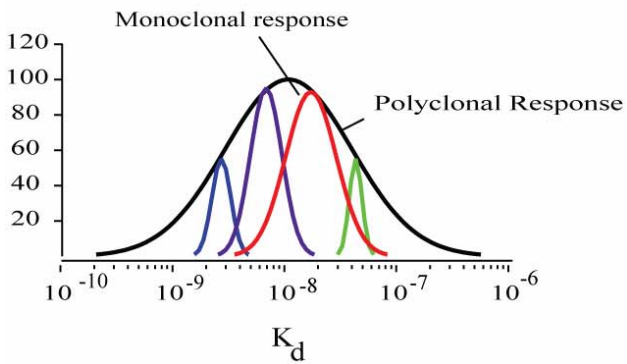
Single Molecule Studies of Antigen-Antibody Binding: Why

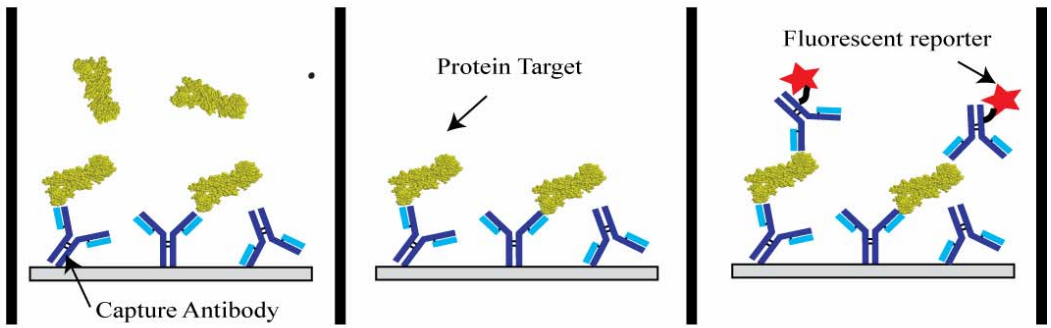
Scientific Goals:

1. Explore "conformational memory" effects
2. Correlate average affinity with deviation from average
3. Examine reasons for loss of affinity due to surface immobilization
4. Observe single molecule dynamics for decades of timescales

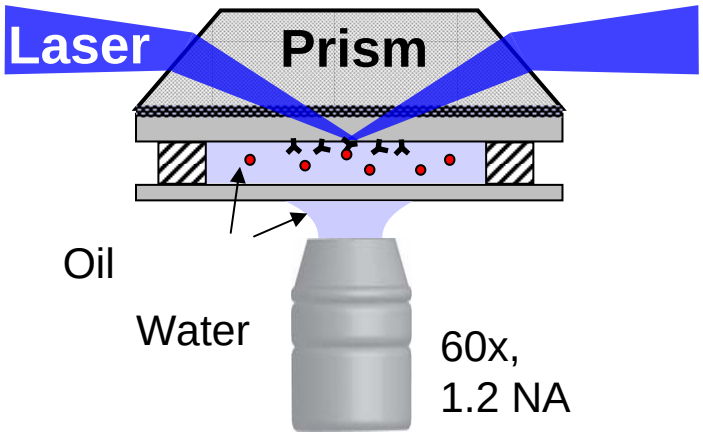


"Antibody Multispecificity Mediated by Conformational Diversity"
L. C. James, P. Roversi, and D. S. Tawfik
Science 299 1362-1367 (2003)

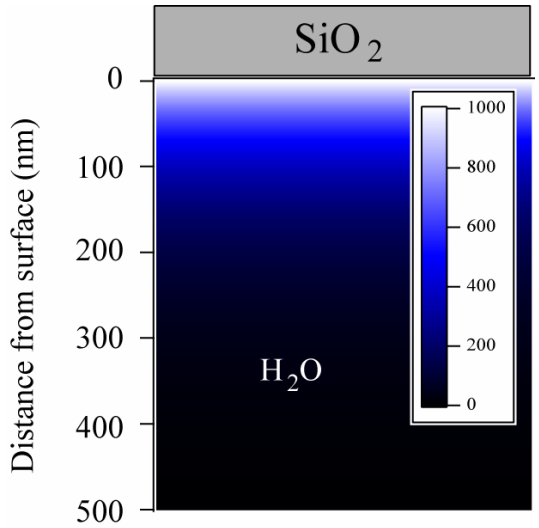




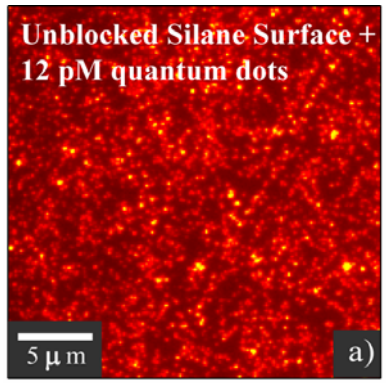
Wide-field imaging by total internal reflection microscopy

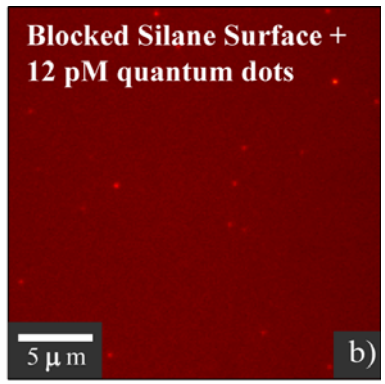


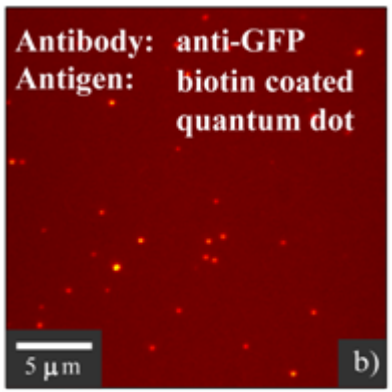
- Single fluorescent molecule or quantum dot

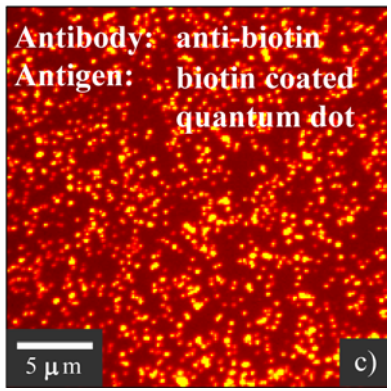


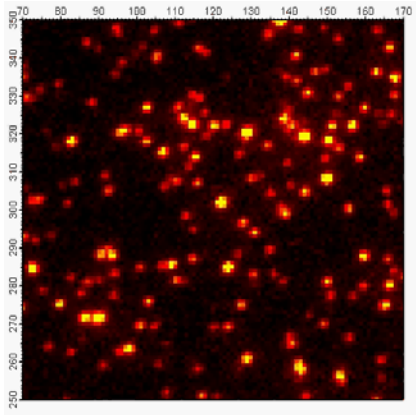
The importance of surface blocking











"Surface-immobilized antibody-antigen binding affinity studies by single molecule fluorescence imaging"
Temirov, J, Bradbury, A., Werner, JH. Proceedings SPIE Vol. 6092 (2006)

Antigen-Antibody Conclusions

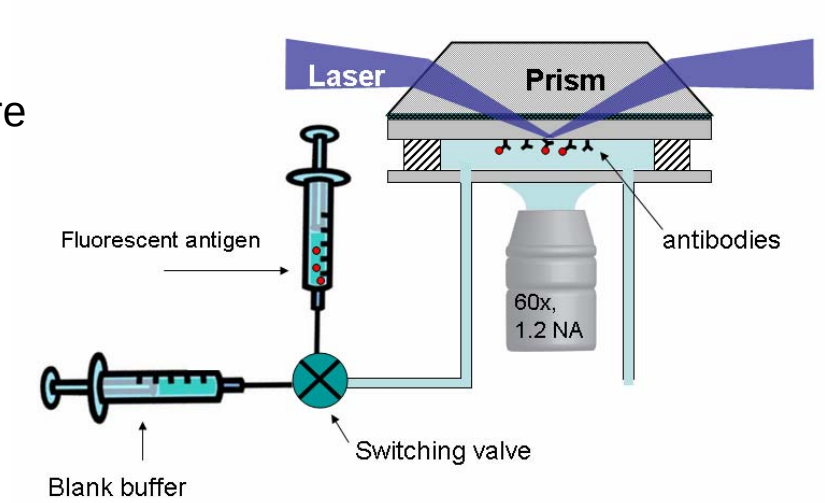
Progress thus far:

Surface Chemistry:

- "Clean" enough for single molecule detection
- Prevents non-specific binding to levels needed for single molecule studies
- Preserves antibody activity

Data Acquisition:

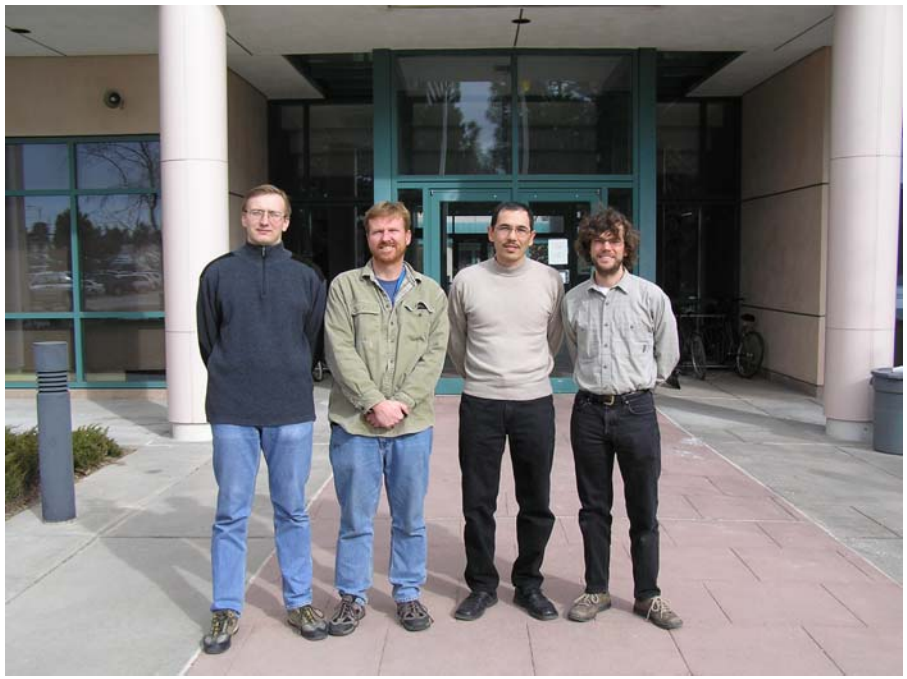
- Image and data analysis software written



Future:

Need to distinguish binding from blinking
Learn how to account for it in the data
Switch to a different fluorescent reporter

Acknowledgements



3D Tracking

Guillaume Lessard
Peter Goodwin
Jim Werner
Funding: LDRD-ER, Tech Mat, NNEDC

Protein Folding

Jim Werner
Raymond Joggerst
Peter Goodwin
Brian Dyer
Dick Keller (PI, LDRD-DR)

Single Molecule Sorting

Anton Malko
Mike Ward
Jim Werner

Antigen-Antibody Binding

Jamshid Temirov
Andrew Bradbury
Jim Werner