The Role of New Physical Tools in Advancing Biology

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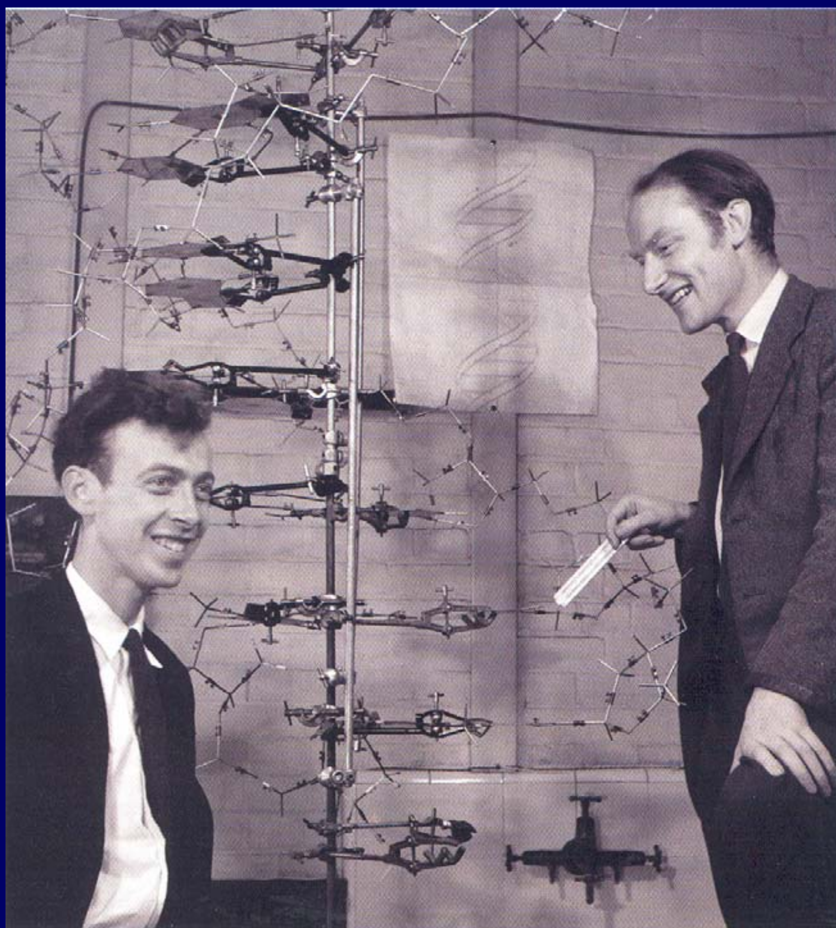
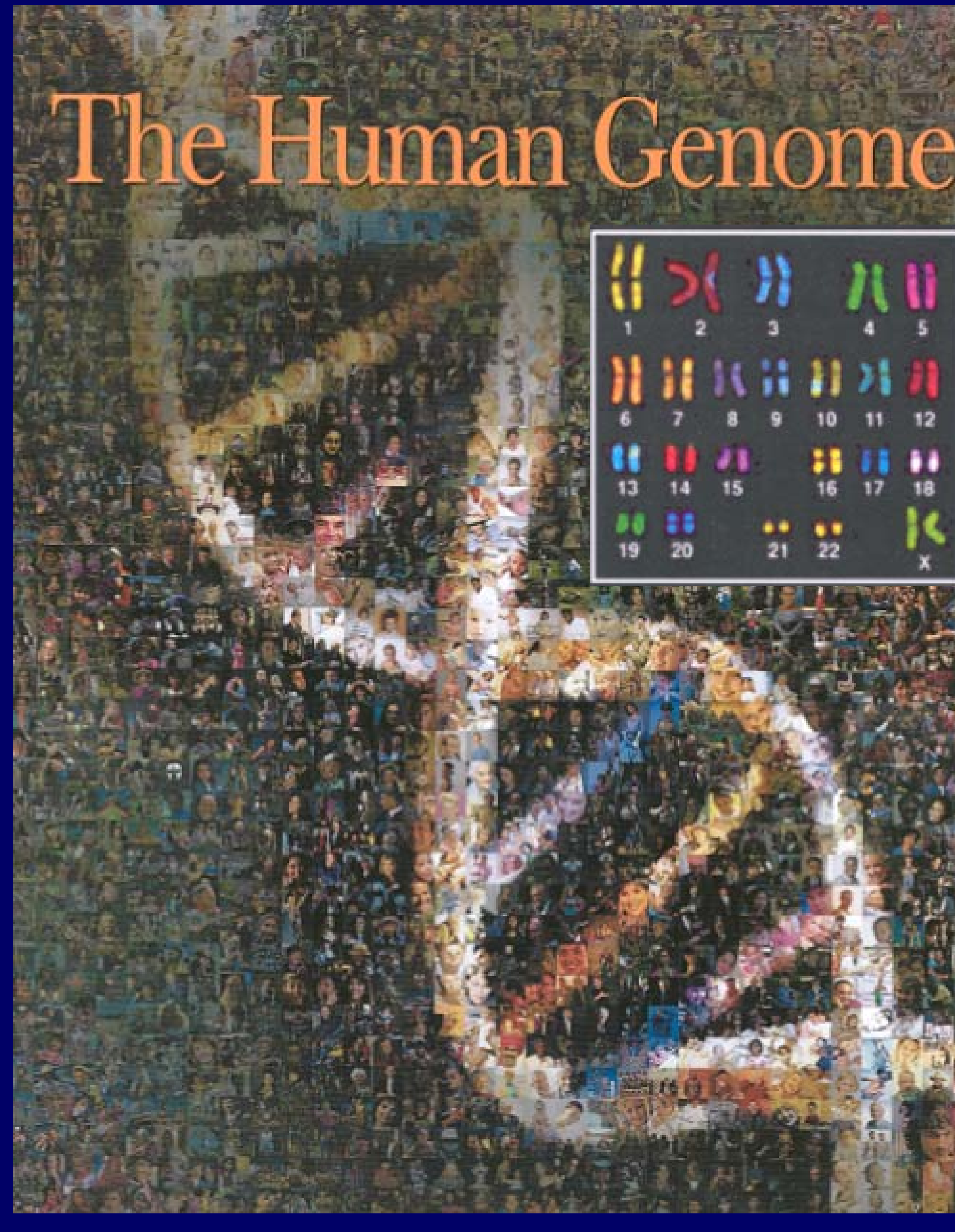
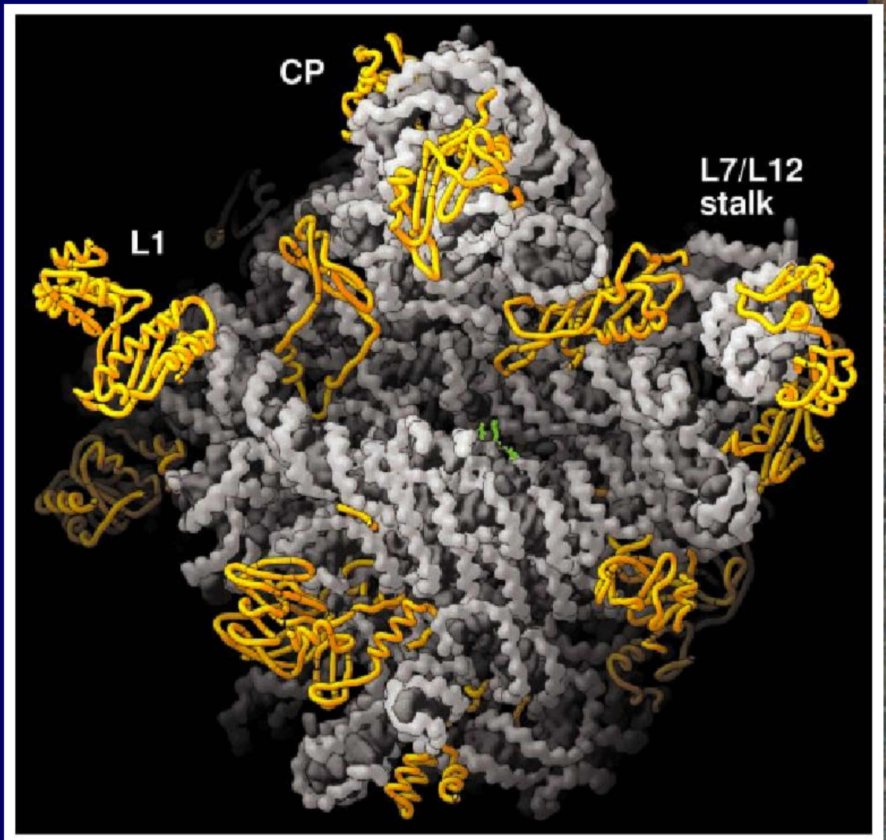
Department of Chemistry and Chemical Biology

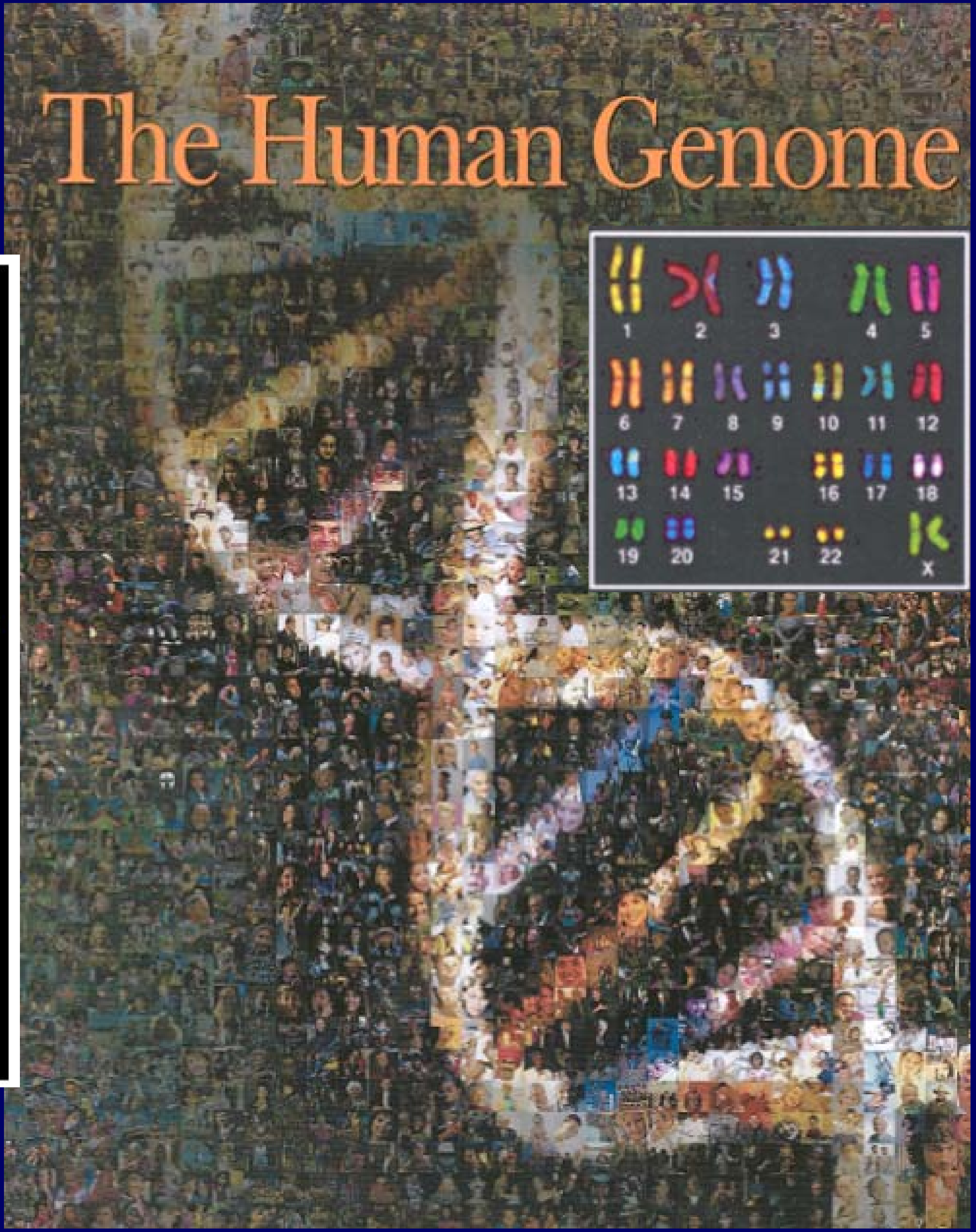



The Human Genome
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 X

Crystal Structure of Ribosome



Ban, et al., *Science*, **289**, 905 (2000)



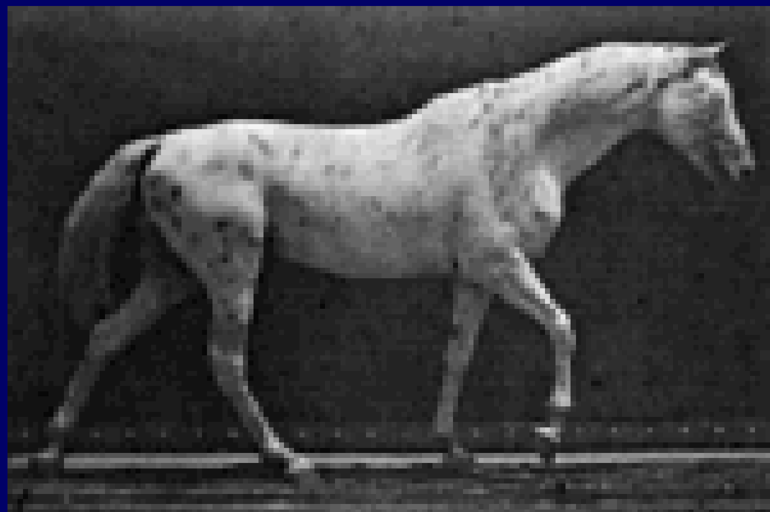
- Biology can be understood on a molecular basis.
- Biology is becoming a quantitative and data rich science.
- Experimental observations are crucial in biology.
- Biology advances are facilitated by physical tools.

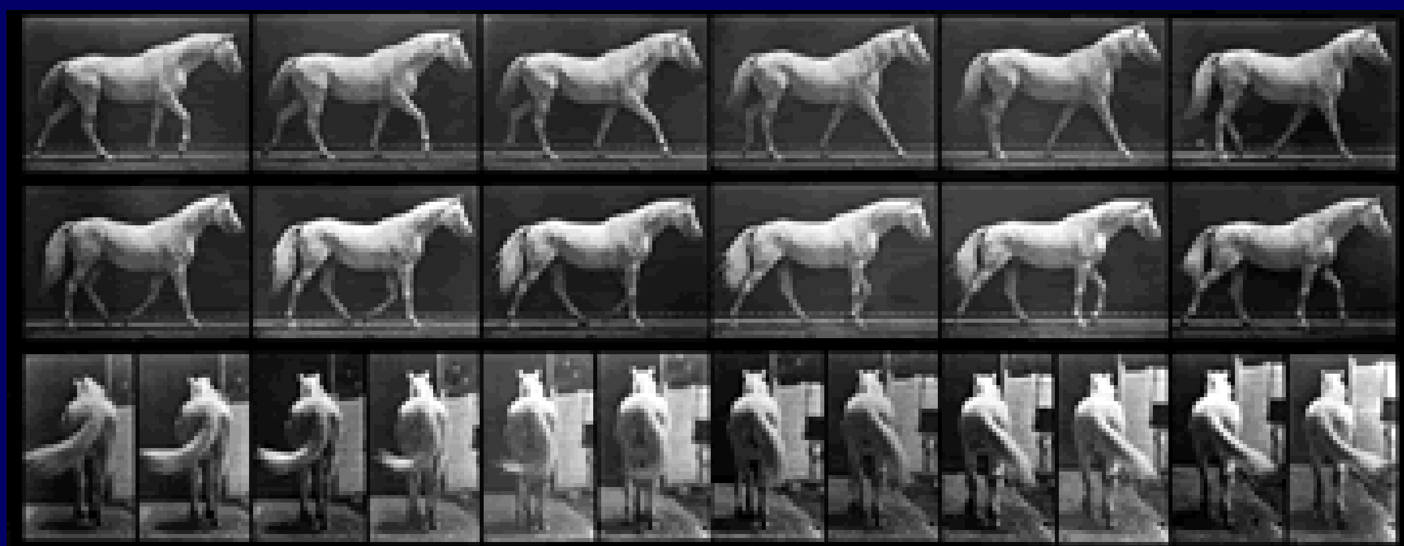
Why Study Single Molecules?

- **Measure Distributions of Molecular Properties**

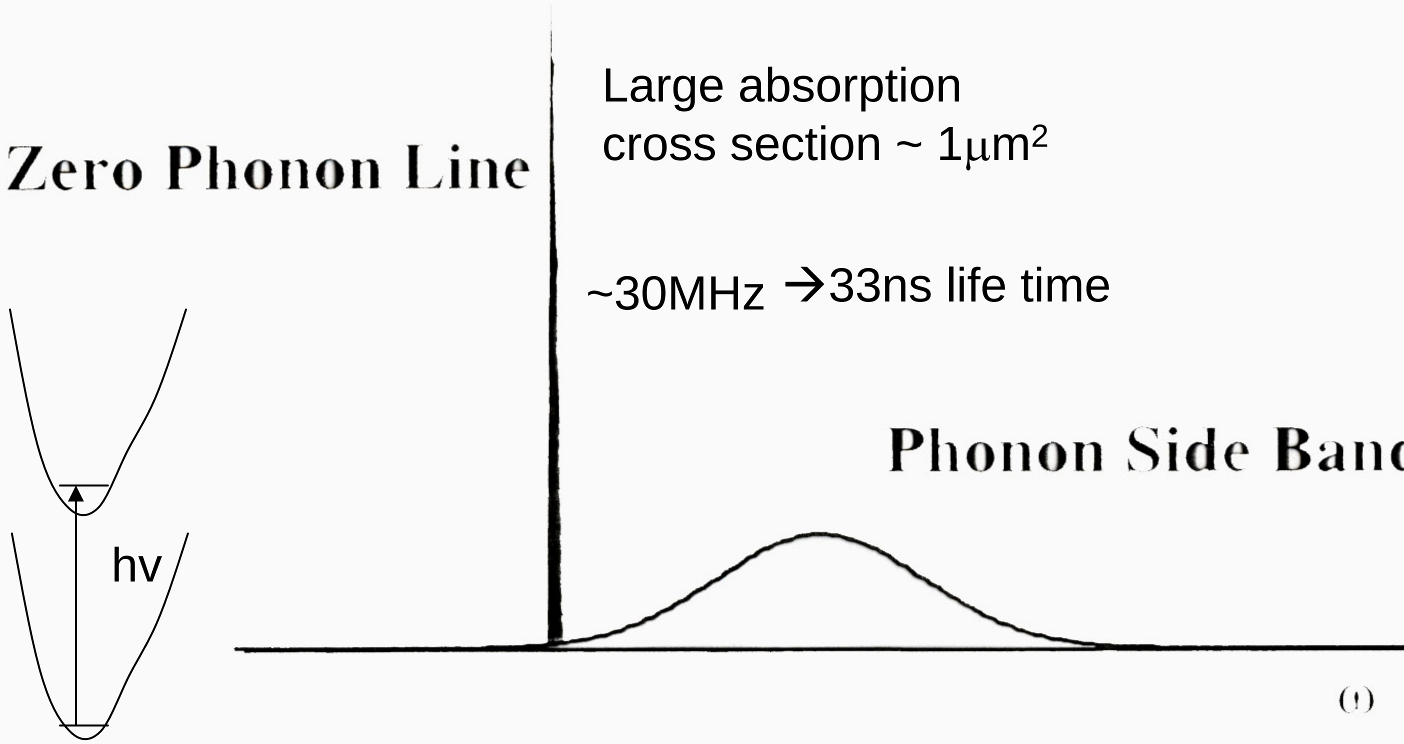
- **Take movies of molecular motions and chemical reactions**

Eadweard Muybridge, *Animal Locomotion*, 1887





Single-Molecule Absorption Spectrum at 1K



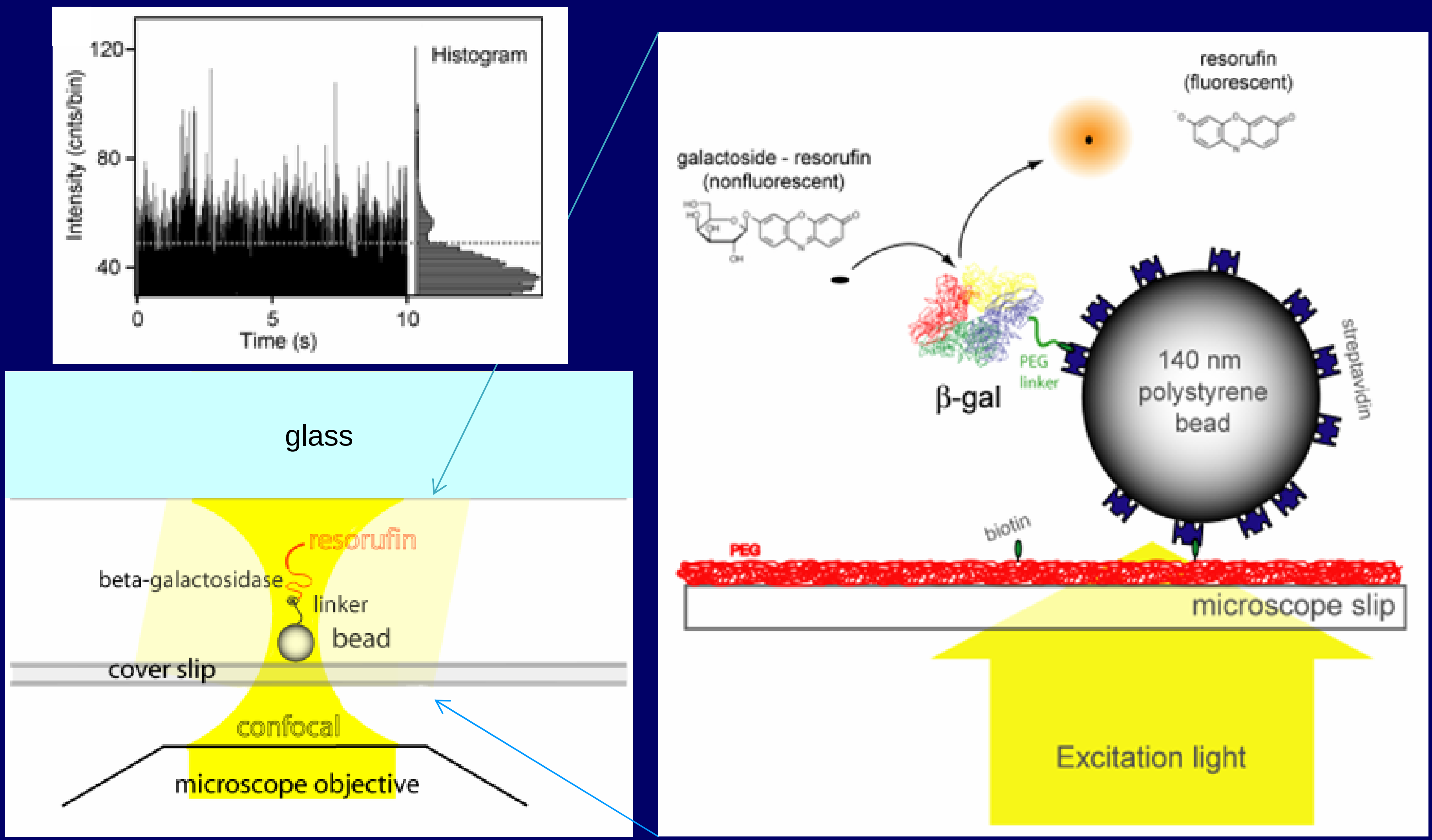
Pentacene

W.E. Moerner et al 1989, *PRL*, 62:2535

M. Orrit et al 1990, *PRL*, 65:2716

Single Molecule Turnover Experiment of ß-galactosidase

Each enzymatic turnover creates a fluorescent burst



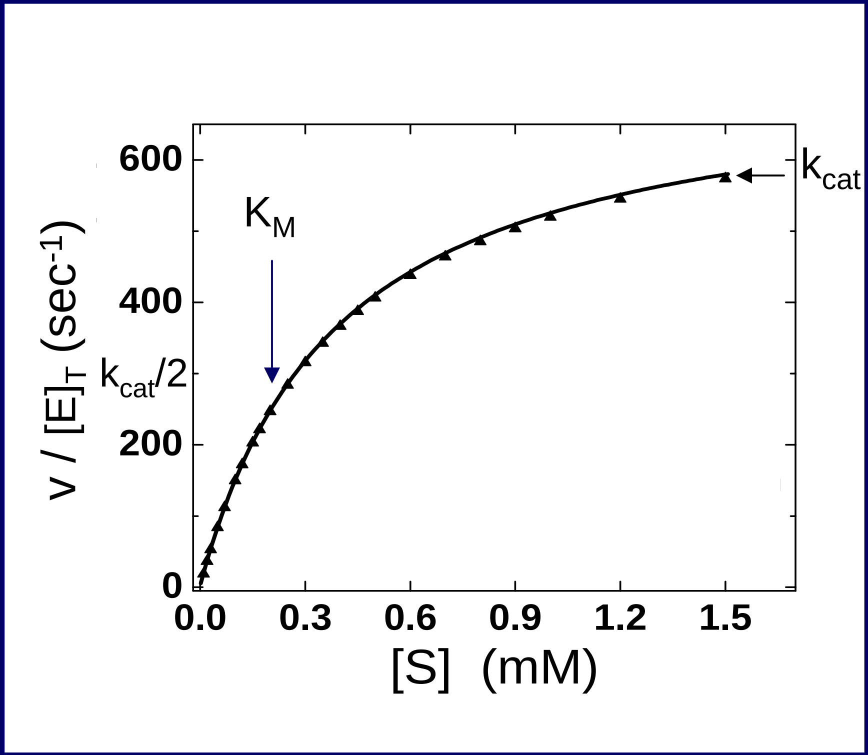
Michaelis-Menten Equation

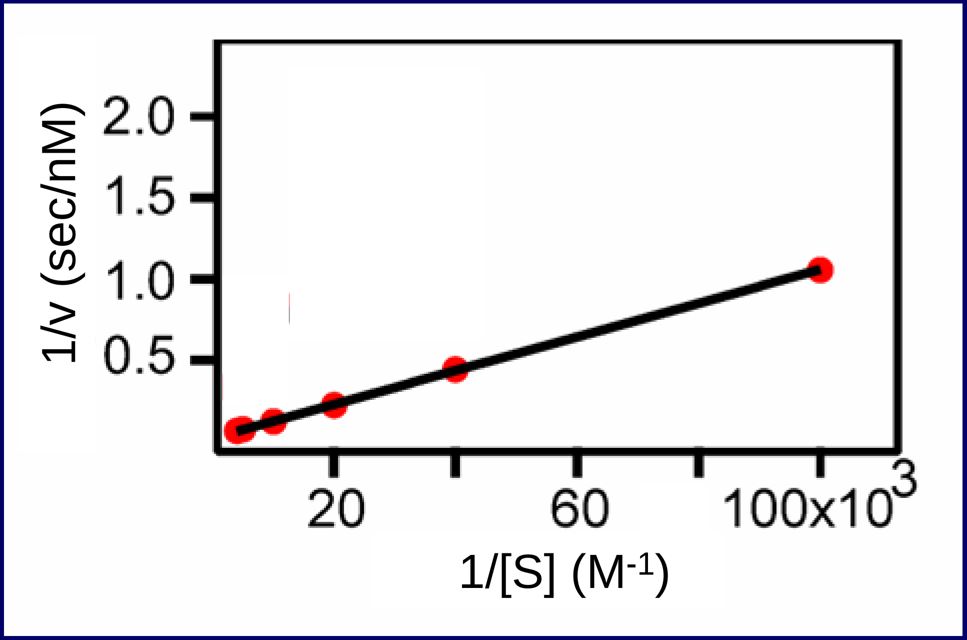
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \bullet S \xrightarrow{k_{cat}} E + P$$

$$v \propto \frac{k_{cat}[S]}{[S] + K_M} \qquad K_M = \frac{k_{cat} + k_{-1}}{k_1}$$

Leonor Michaelis
1875-1949

Maud Menten
1879-1960

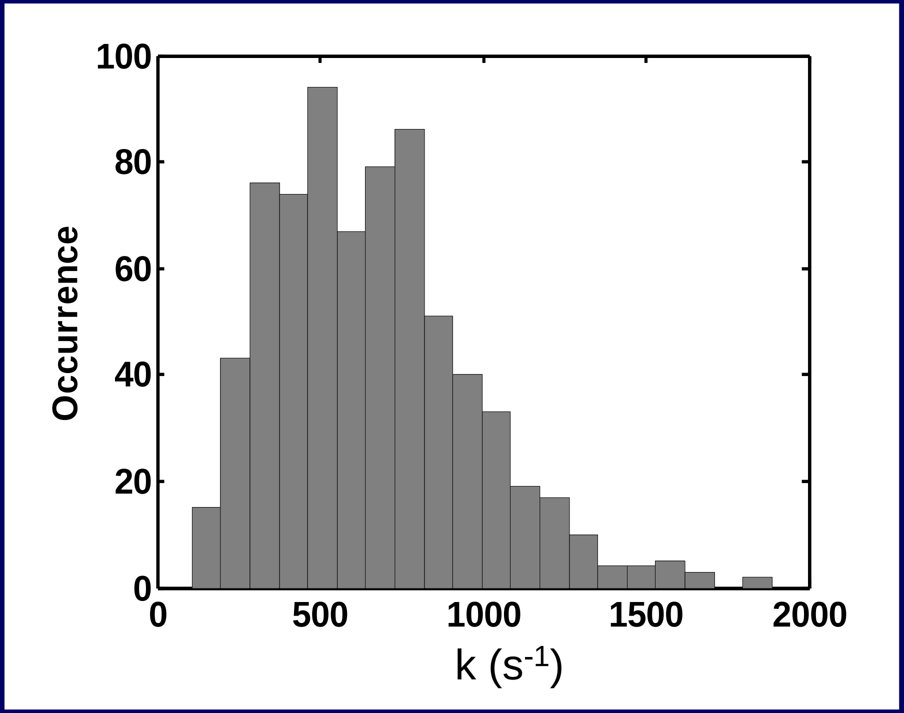




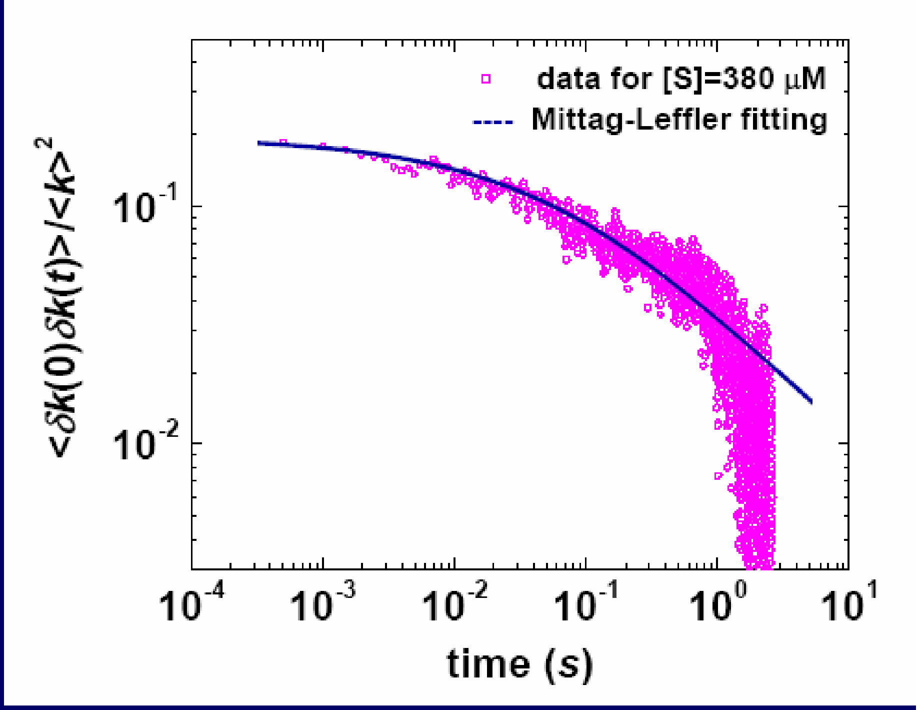
Lineweaver-Burke Plot

Fluctuation of Turnover Rate of a Single Enzyme

Histogram Turnover Rate , k, at 380 μM



Autocorrealtion of Turnover Rates



Not a Small Effect !

Ever Fluctuating Enzyme!

Single Molecule Michaelis-Menten Equation

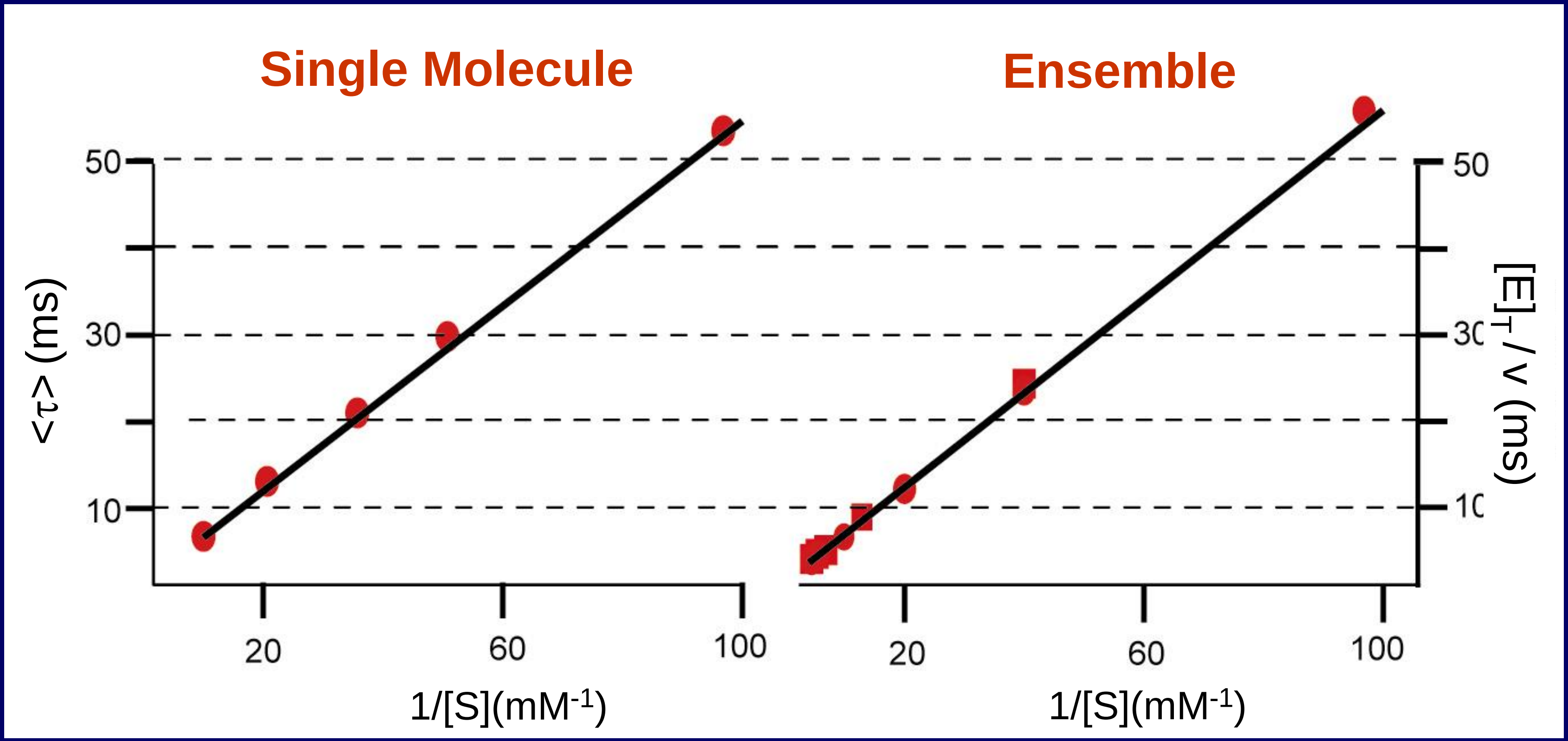
$$\frac{1}{\langle \tau \rangle} = \frac{\gamma_2 [S]}{[S] + C_M}$$

Kou et al. *J. Phys. Chem.* 2005

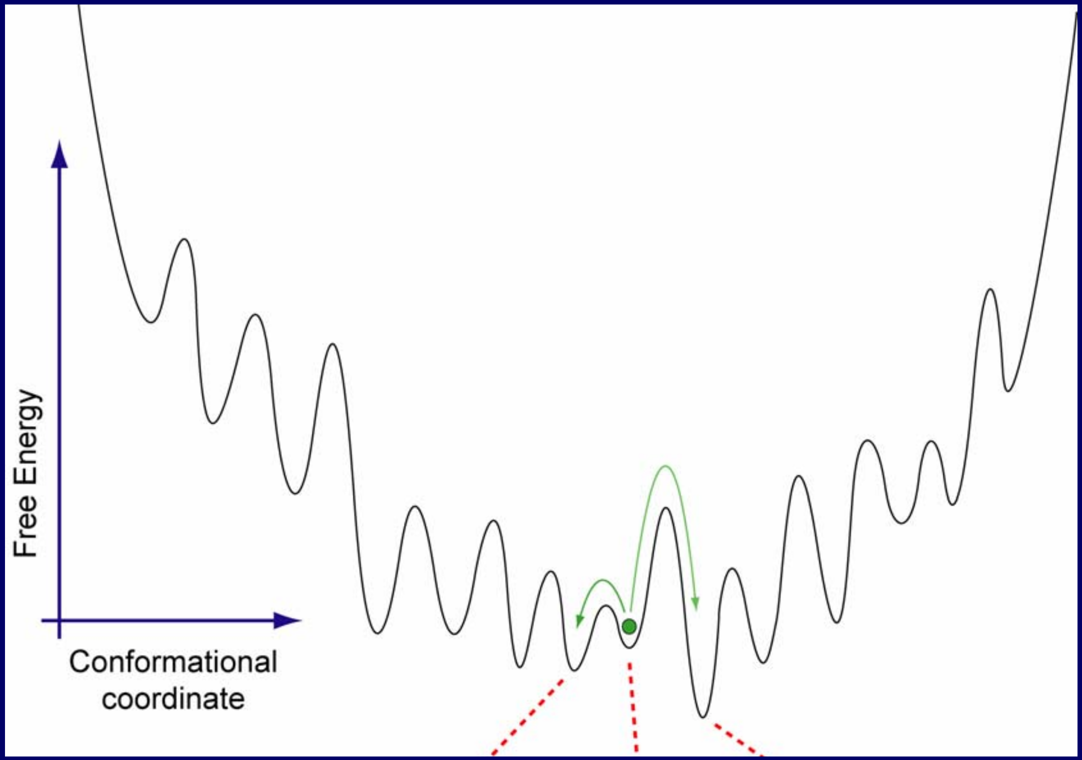
English et al. *Nature Chem. Bio.* 2006

$$\gamma_2 = \left[\int_0^\infty \frac{p(k_{cat})}{k_{cat}} dk_{cat} \right]^{-1} \qquad C_M = \frac{\gamma_2 + k_{-1}}{k_1}$$

$$\frac{v}{[E]_T} = \frac{k_{cat}[S]}{[S] + K_M}$$



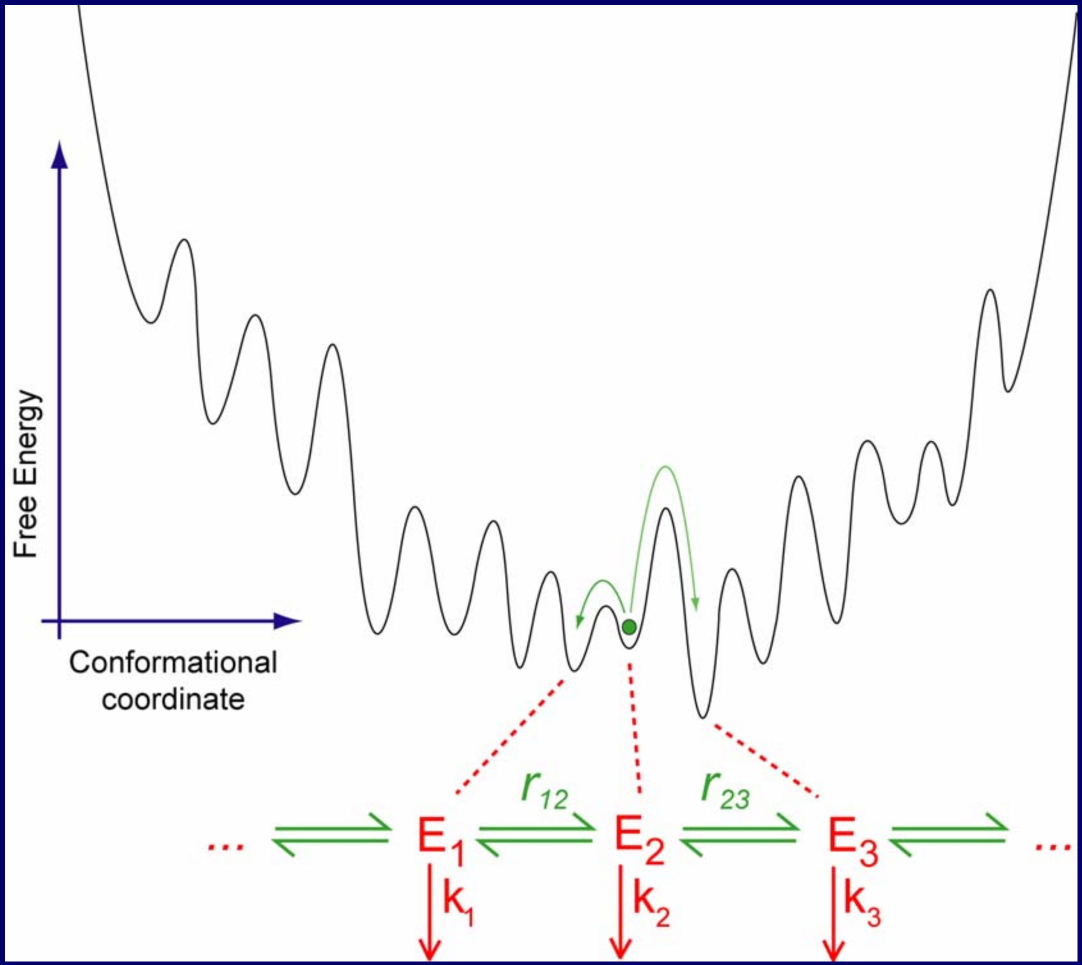
Rugged Energy Landscape



Austin, et al., *Biochemistry* **14**, 5355 (1975).

Frauenfelder, Sligar, Wolynes, *Science* **254**, 1598 (1991)

Rugged Energy Landscape



Austin, et al., *Biochemistry* **14**, 5355 (1975).

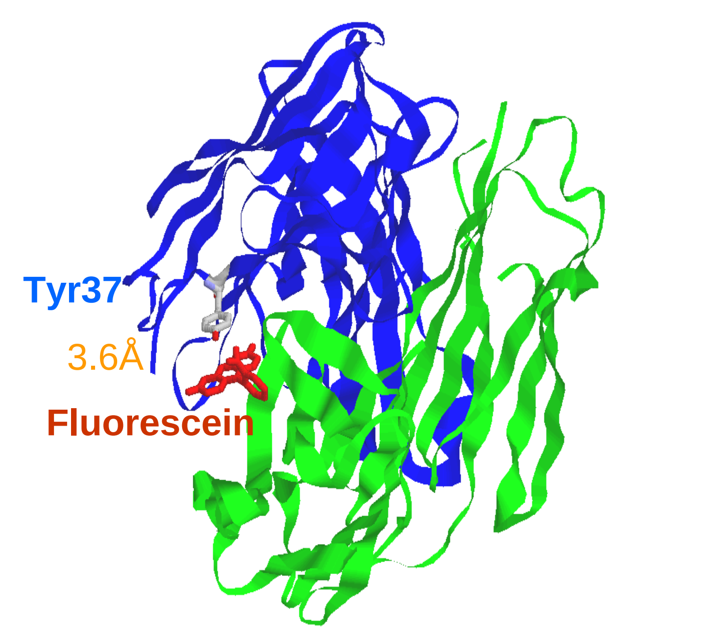
Frauenfelder, Sligar, Wolynes, *Science* **254**, 1598 (1991)

Lu, Xun, Xie *Science* **282**, 1877 (1998).

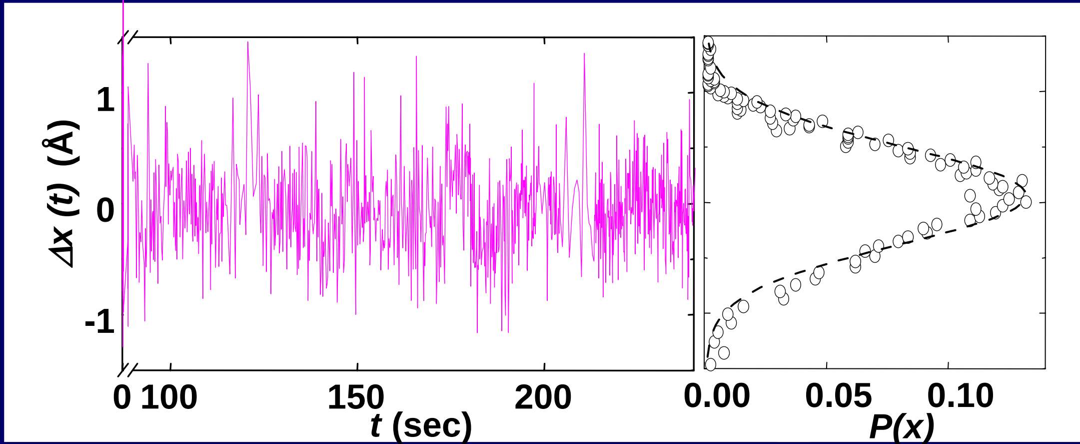
Yang, et al. *Science* **302**, 262 (2003).

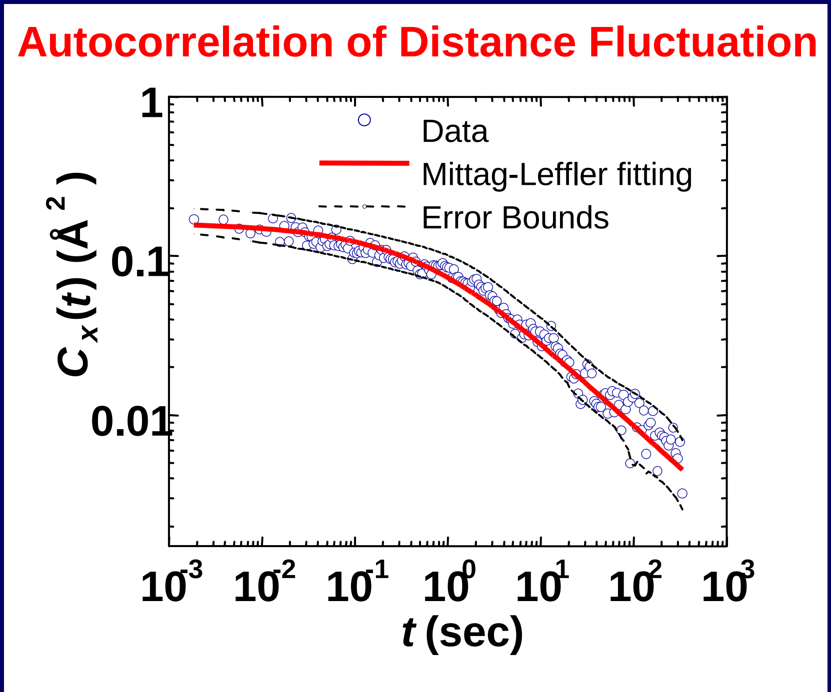
Conformational Dynamics within a Single Protein Molecule

Fluctuation of the distance between Tyr and fluorescein



M. Whitlow, *et al.*, *Protein Eng.* **8**, 749 (1995).





Wei et al, *Phys. Rev. Lett.* **94**, 198302 (2005).

The rate of electron transfer (k_{ET}) is a distance dependent probe for conformational fluctuation!

$$k_{ET} = k_o \exp(-\beta x)$$

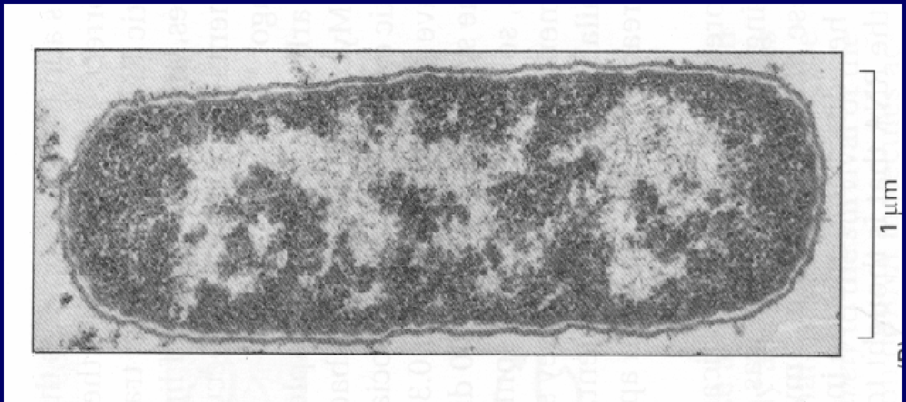
β = 1.4 A^{-1} for proteins

"Living cells are the test tubes in the 21st century."

- Jonathan Widom

- Nonequilibrium steady state
- Complex reaction network
- Biomolecules (DNA, mRNA) in low copy numbers

Gene Expression Is A Single-Molecule Problem!



EM picture of *E. coli*

E. Coli has 4,288 genes.

Kristin Xie

Kara Xie

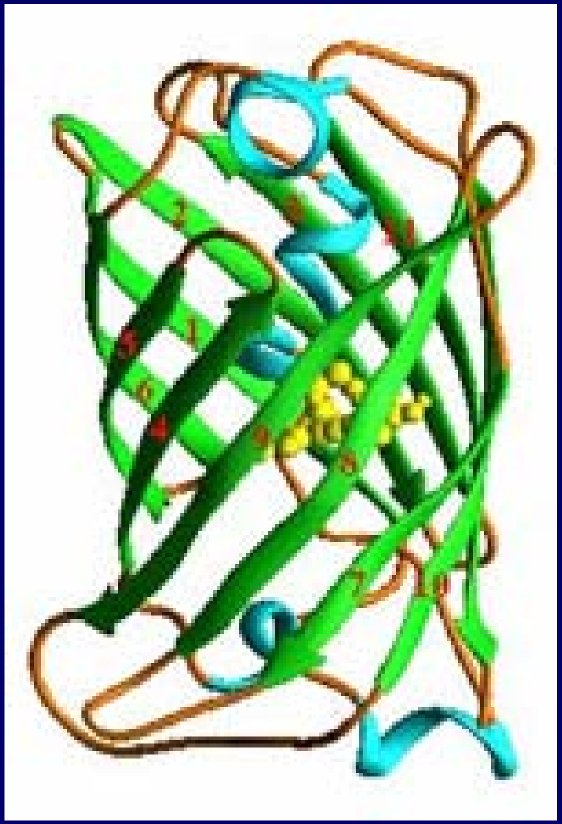
Central Dogma of Molecular Biology

RNA polymerase — Ribosome

DNA → mRNA → Protein

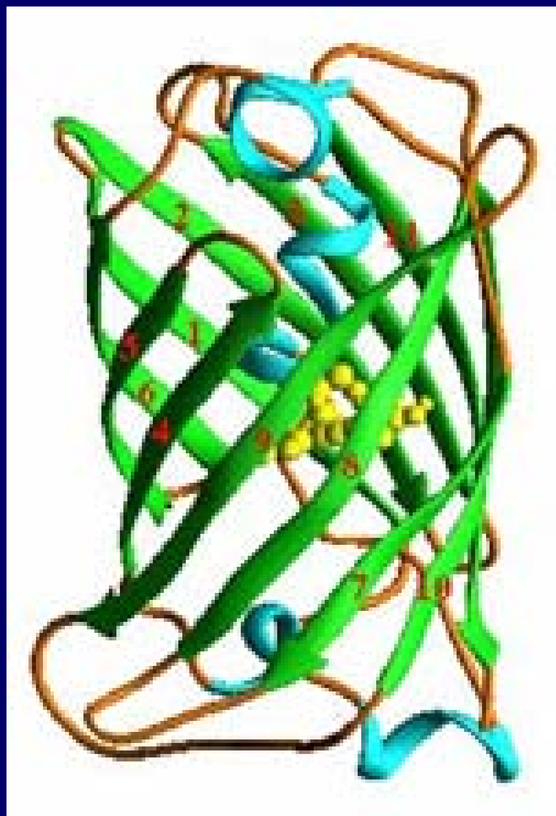
Transcription — *Translation*

Green Fluorescent Protein (GFP)



Naturally fluorescent protein in jellyfish

Green Fluorescent Protein (GFP)

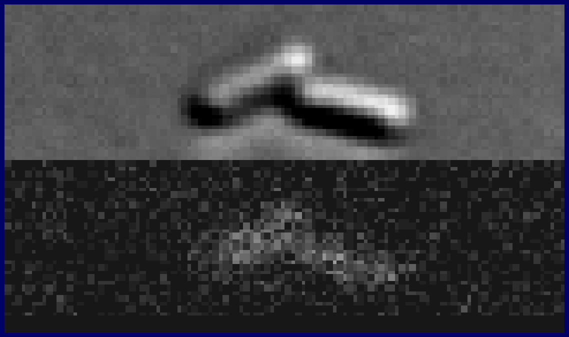


Immobilizing GFP for Single Molecule Sensitivity

A GFP molecule in cytoplasm undergoes fast diffusion. Its signal is overwhelmed by the strong autofluorescence background.

DIC Image

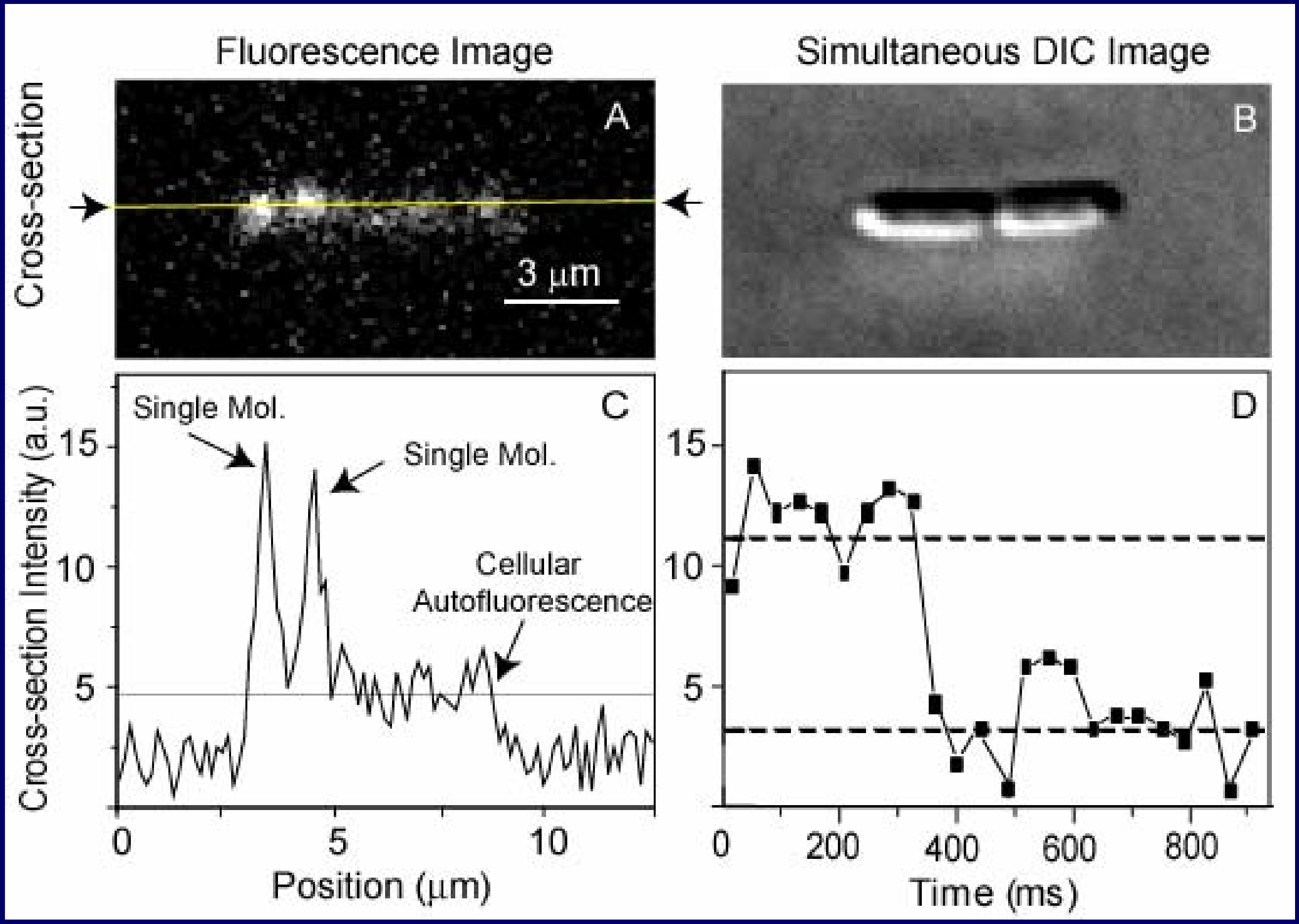
Fluorescence Image



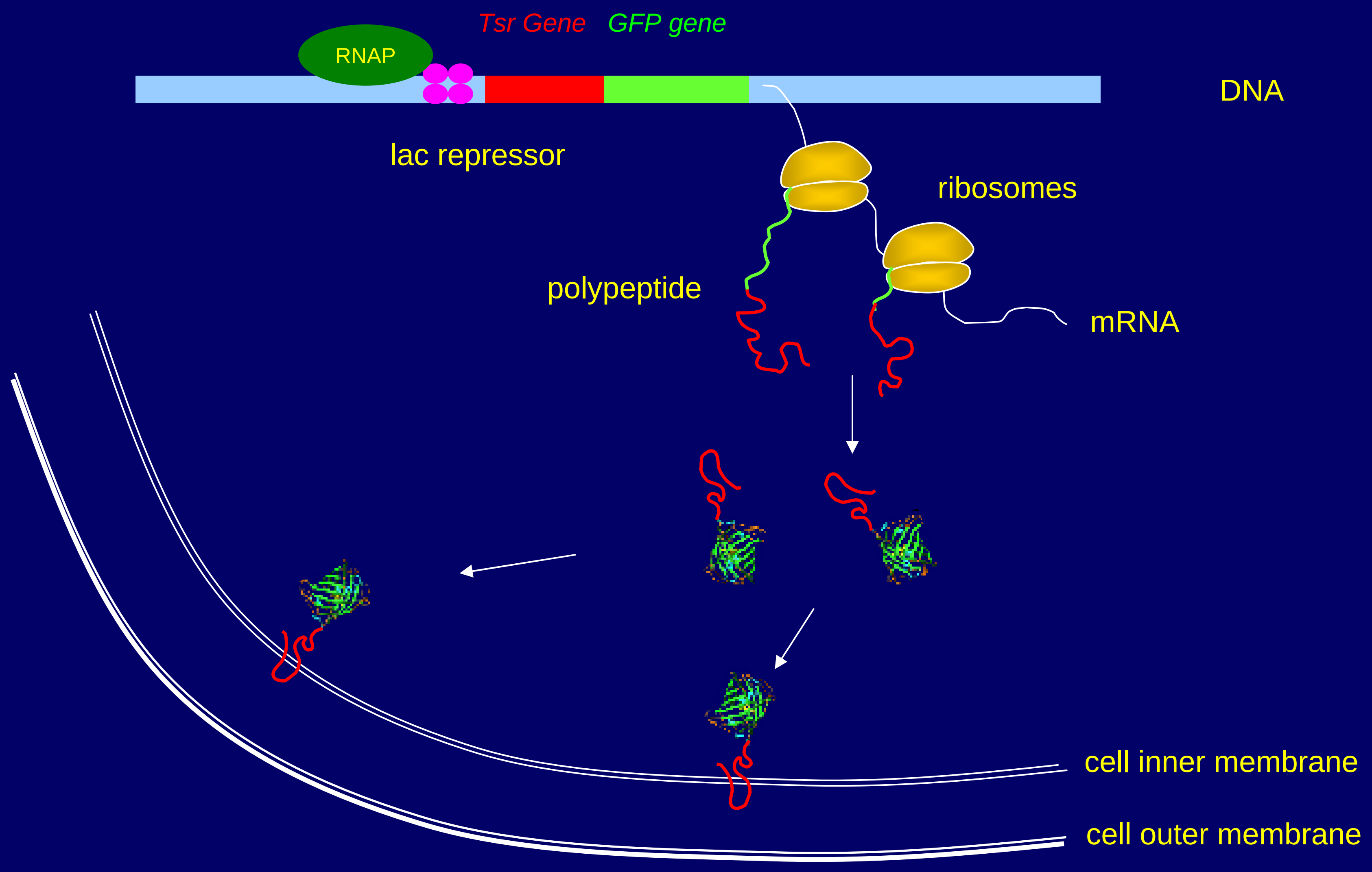
A few diffusing GFP molecule

Immobilizing GFP for Single Molecule Sensitivity

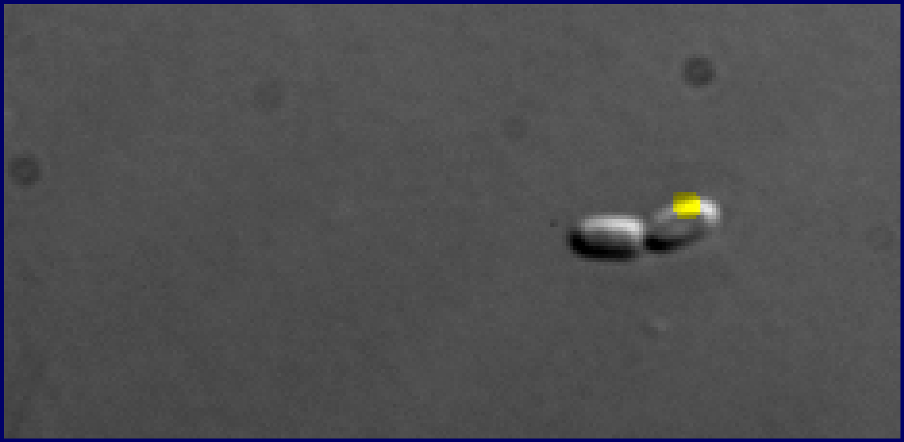
A GFP molecule in cytoplasm undergoes fast diffusion. Its signal is overwhelmed by the strong autofluorescence background.



Imaging Gene Expression in a Live *E. coli* Cell

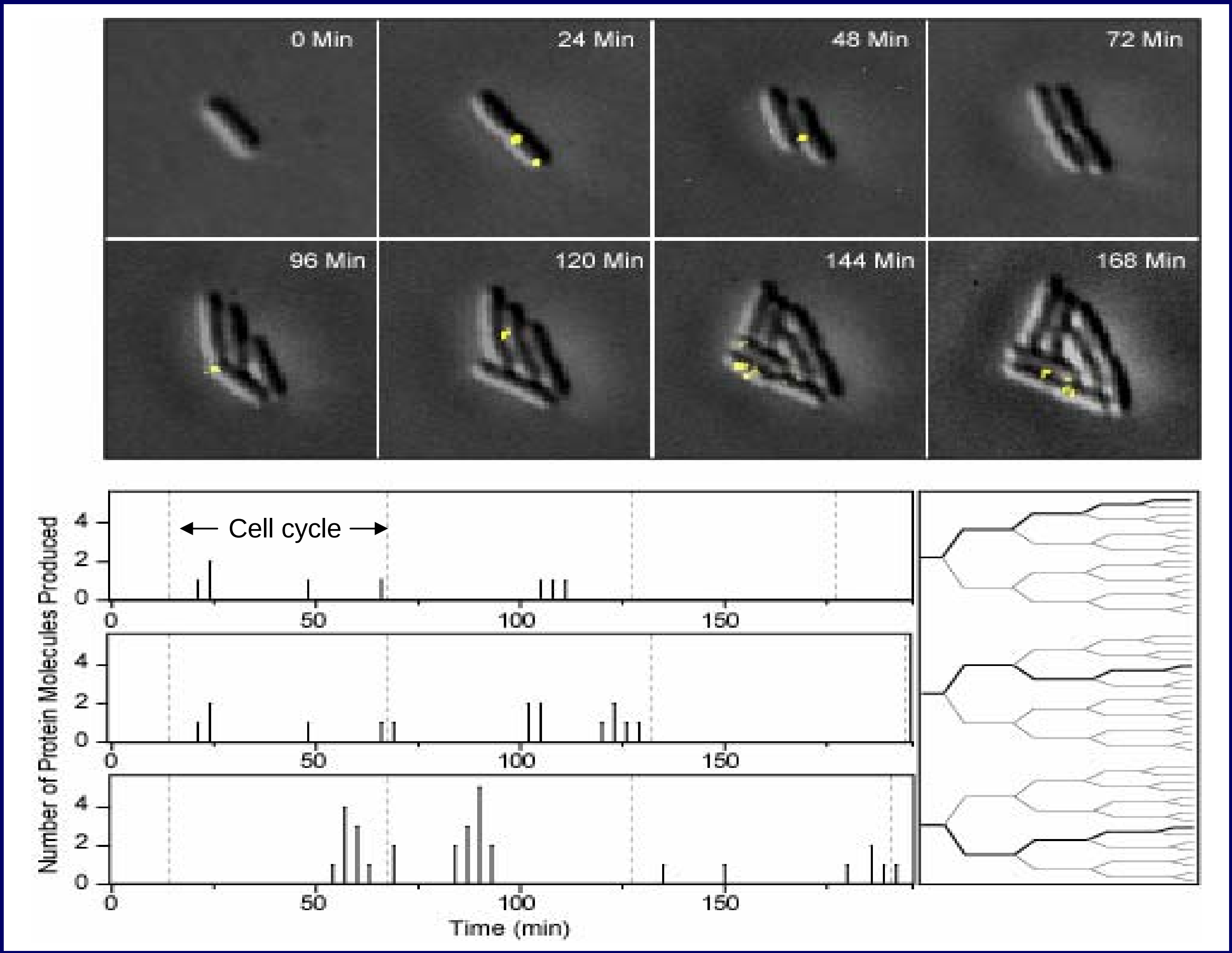


Single Molecules of Membrane Immobilized GFP: Spontaneously Expressed from Chromosome

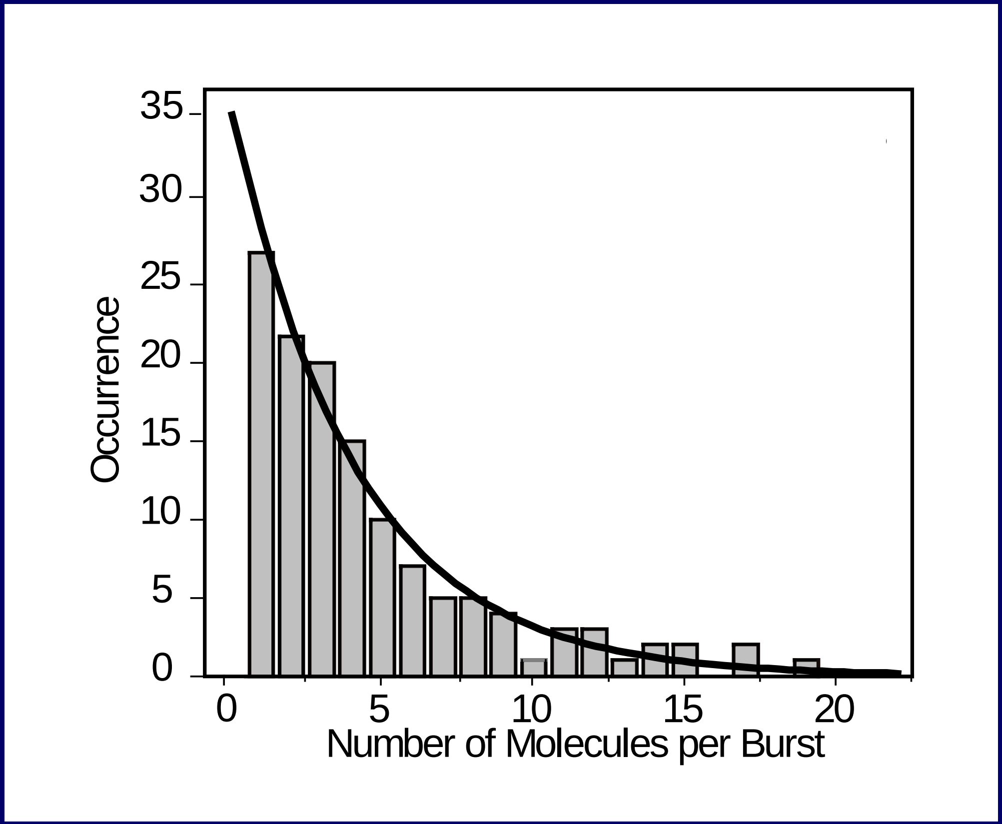


Cell division cycle: 40 min

Stochastic Gene Expression Bursts of Cell Lineages

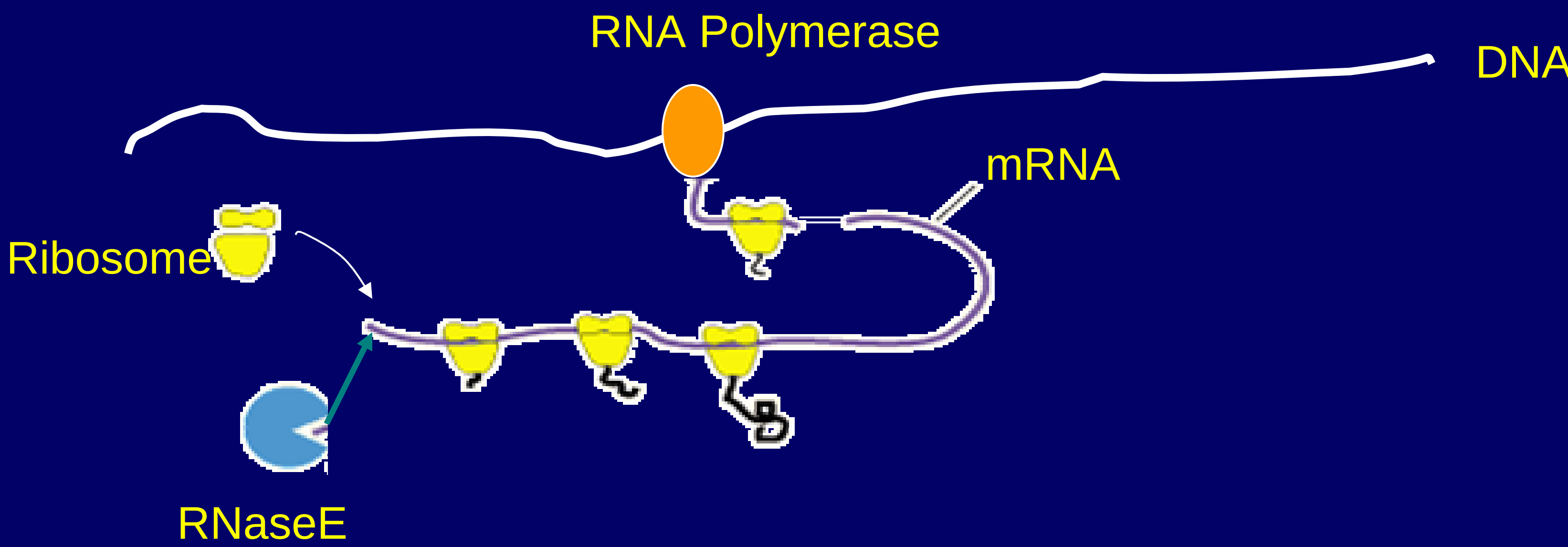


Distribution of GFP Molecules per Burst

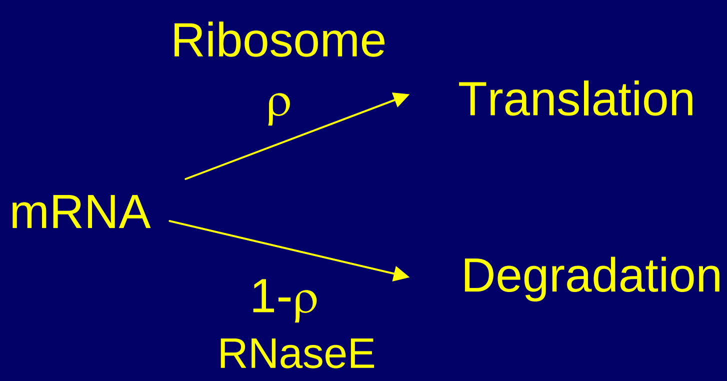


An exponential distribution with an average of b = 4.2 mol.

mRNA Degradation Determines the Burst Size



Probability of binding



Number of protein per mRNA, N, follows an exponential distribution:

$$p(N) = \rho^N(1-\rho)$$

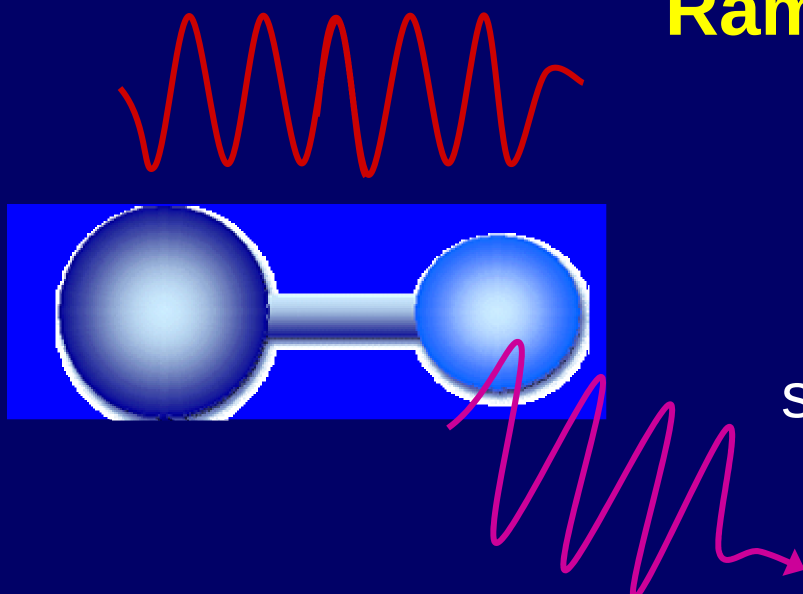
What Have We Learned?

- Cai et al., *Nature,* in press
- Yu et al., *Science,* in press

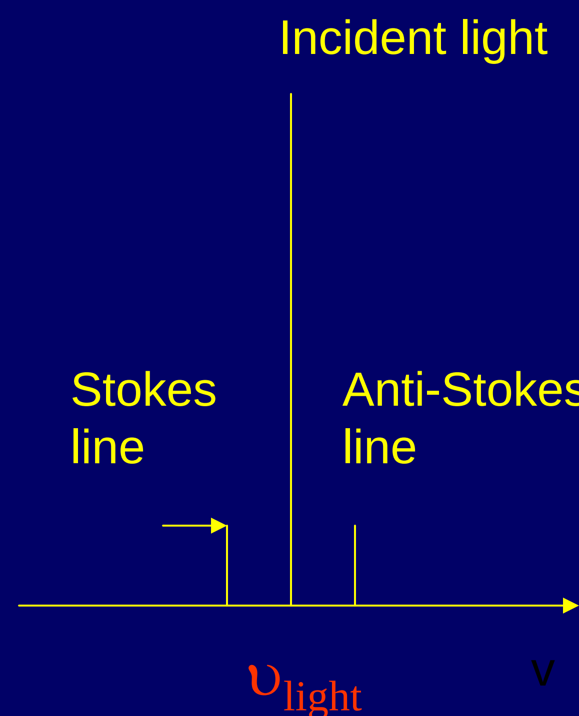
- Transcription is a Random (Poisson) process in *E. coli* under the repressed condition.
- Under the repressed condition, protein but not m-RNA expression occurs in bursts, with one mRNA generating a few protein molecules.
- The copy number of protein molecules in each burst follows an exponential distribution.

Sir Chandrasekhara Venkata Raman (1888-1970)

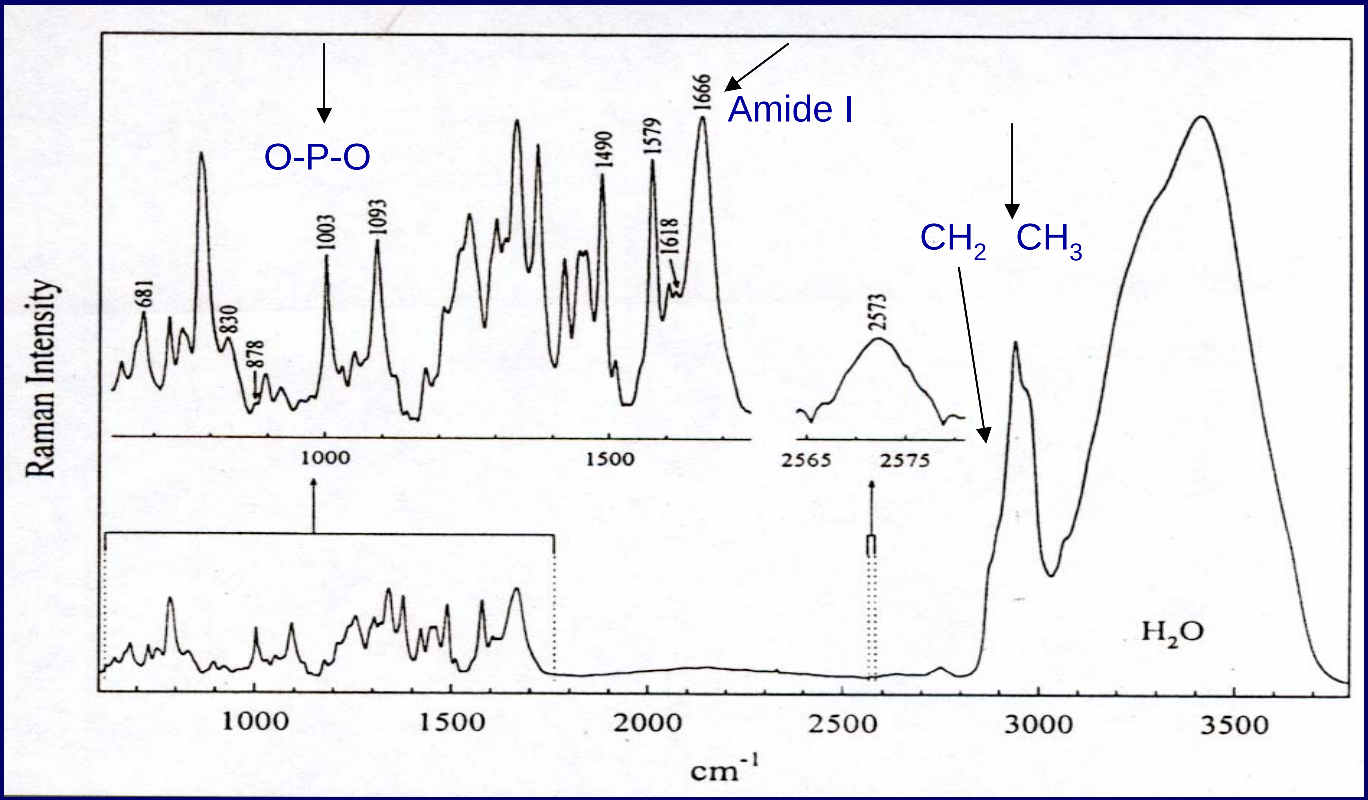
Raman Effect



inelastic
scattering



Spontaneous Spectrum of Bacteriophage P22
(DNA and capsid proteins)



Thomas, Annu. Rev. Biophys. Struct. 28, 1 (1999)

The First CARS

PHYSICAL REVIEW VOLUME 137, NUMBER 3A 1 FEBRUARY 1965

Study of Optical Effects Due to an Induced Polarization Third Order in the Electric Field Strength

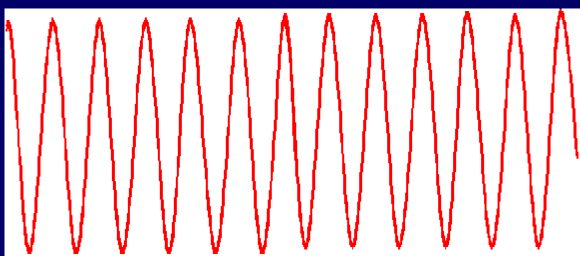
P. D. Maker and R. W. Terhune
Scientific Laboratory, Ford Motor Company, Dearborn, Michigan

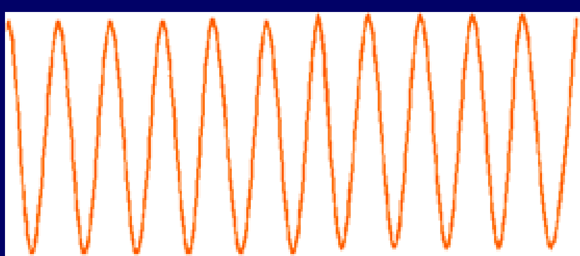
(Received 19 August 1964)

This paper presents the results of a series of experiments in which a giant pulsed ruby laser is used to study several different nonlinear optical effects arising from an induced optical polarization third order in the electric field strength. The various phenomena studied are special cases of either frequency mixing or intensity-dependent changes in the complex refractive index, including Raman laser action at a focus. A wide range of crystalline and isotropic materials was studied. The theory for these effects is extended to cover resonant interactions. The experimental results are interpreted in terms of simplified models, and quantitative values for the nonlinear polarizability coefficients are given. The rather large experimental uncertainties in these coefficients are discussed.

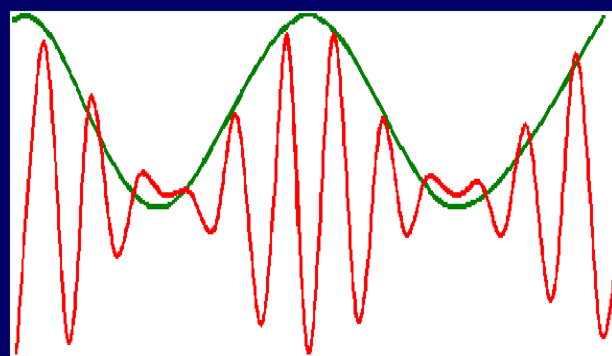
Coherent Anti-Stokes Raman Scattering (CARS)

ω_{pump}





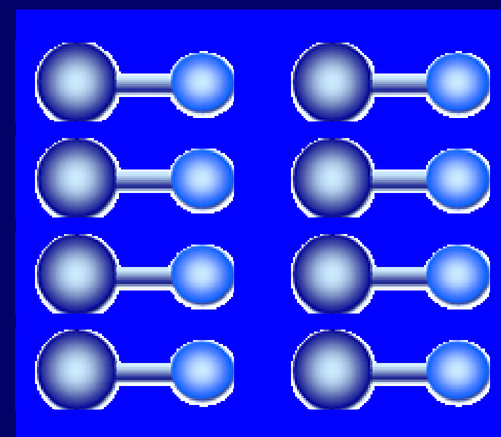
ω_{Stokes}



Beating at

$\omega_{pump} - \omega_{Stokes}$

$\omega_{pump} - \omega_{Stokes} = \omega_{vib}$

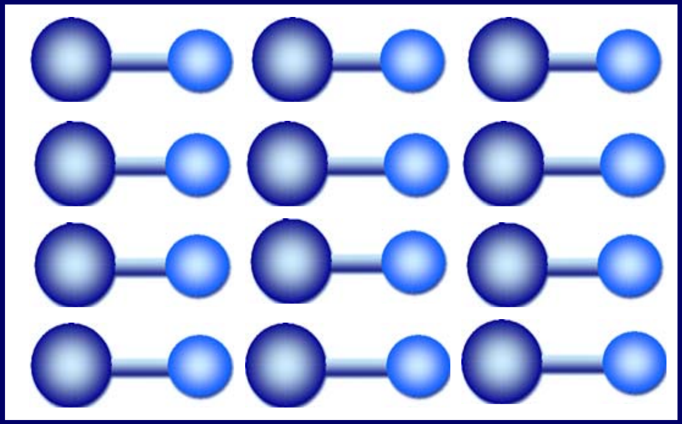


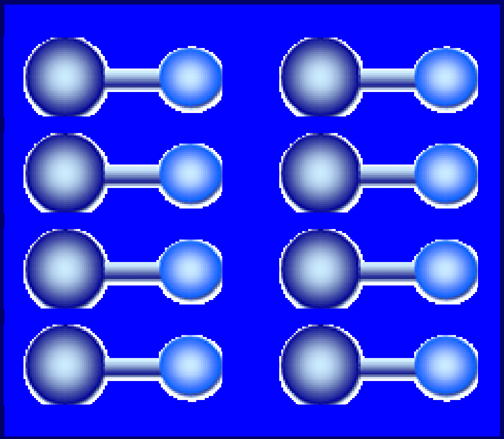
Stimulated excitation of coherent molecular vibration

Spontaneous Raman

CARS

$\omega_{pump} - \omega_{Stokes} = \omega_{vib}$





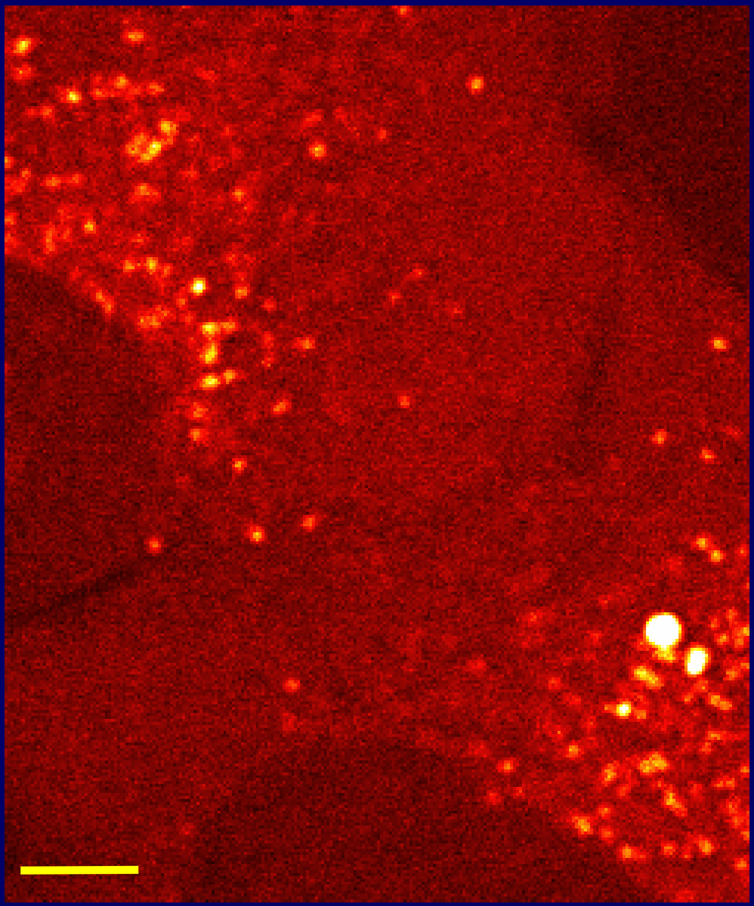
Incoherent excitation of molecular vibration

Stimulated excitation of coherent molecular vibration

Why CARS microscopy?

- No staining, no photobleaching
- Vibration contrast, chemical selectivity
- 3D sectioning
- Highly sensitive

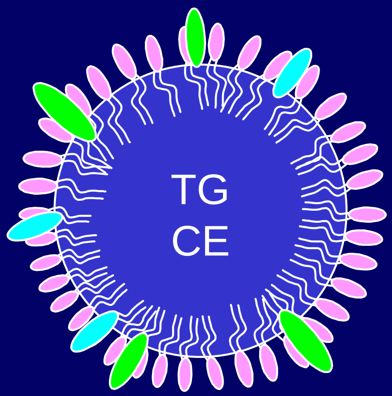
CARS Imaging of Live Cells



5 μm

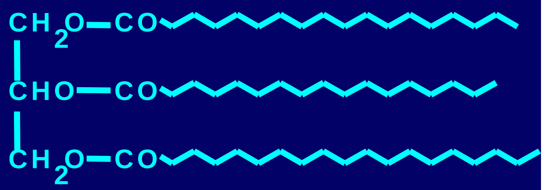
Y-1 mouse adrenal cortex cells
Movie Sped up 15 times
Pump: 1 mW Stokes: 0.5 mW
Tuned into C-H streching

Lipid Droplet

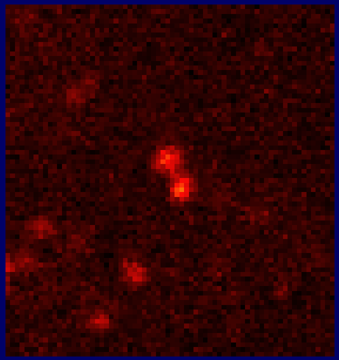


phospholipid monolayer

TG: Triglyceride



CE: Cholesterol Ester

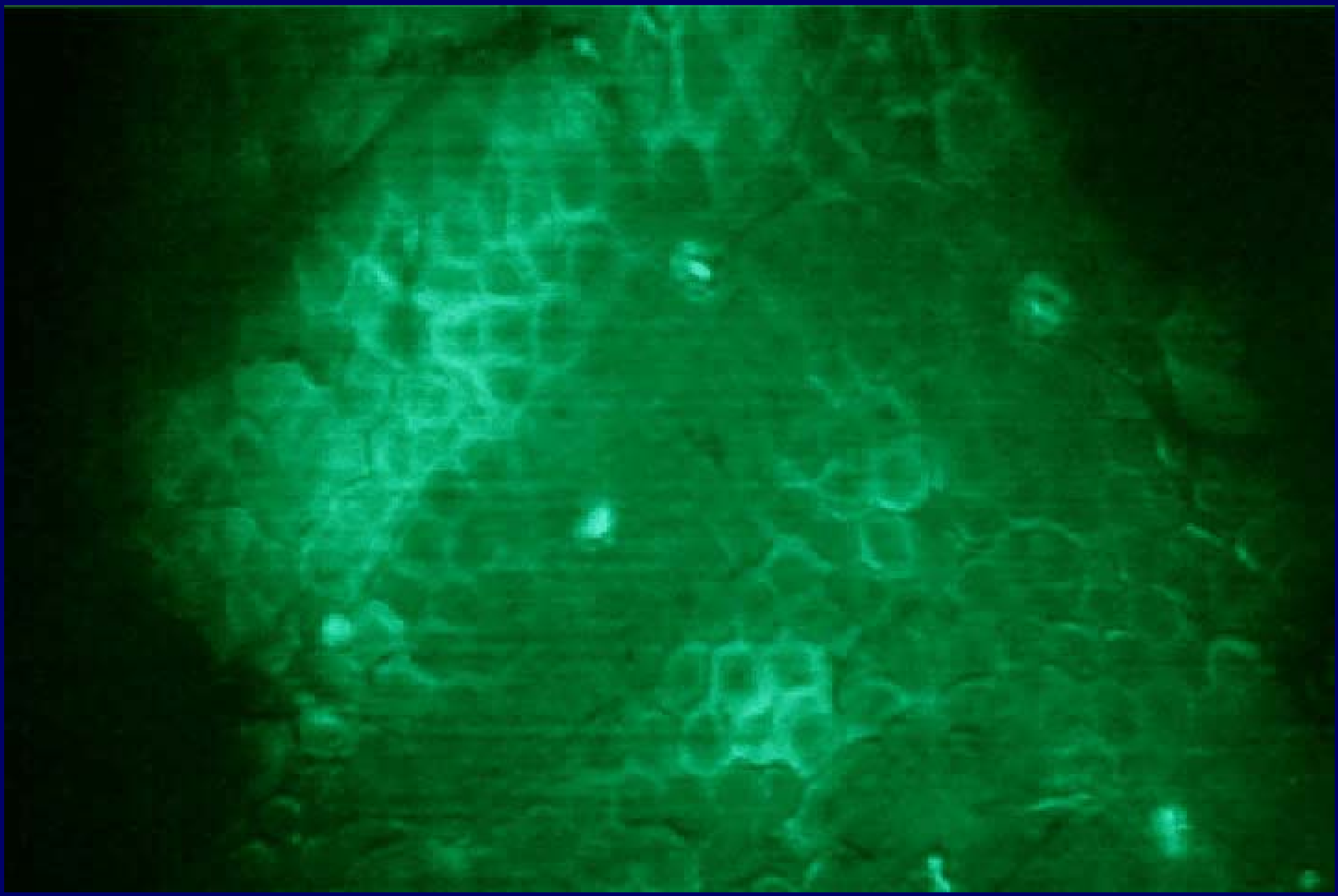


Fusion of two LDs

Not Brownian Diffusion but Active Transport Mediated by Molecular Motors

CARS Moving into Hospitals

Video Rate CARS Imaging Skin Tissue of a Mouse Ear

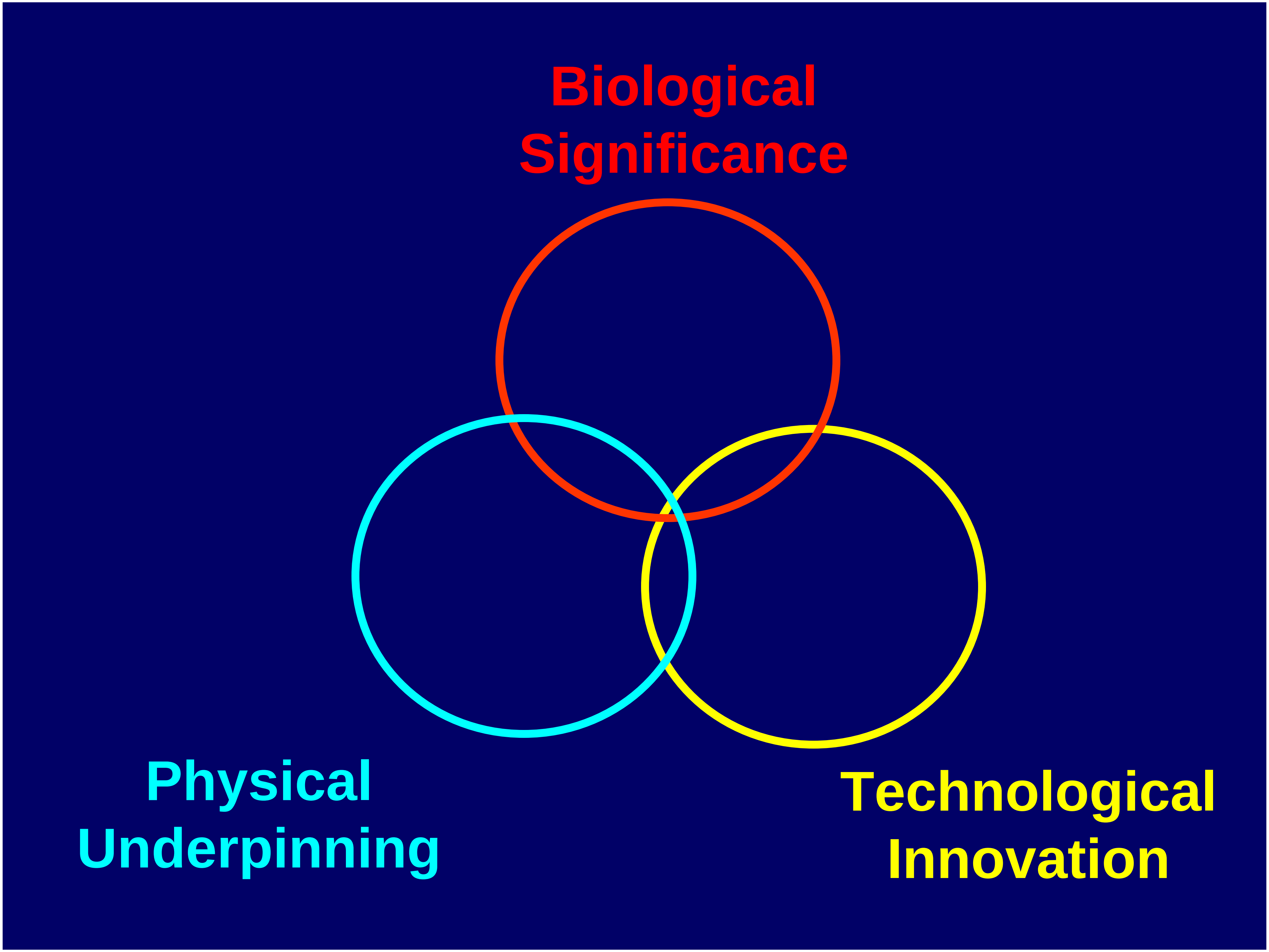


800 μm x 600 μm

Evans, et al., PNAS, 2005

20 frame/sec, Penetration depth: 200 μm,
50 mW total power for pump and Stokes beams

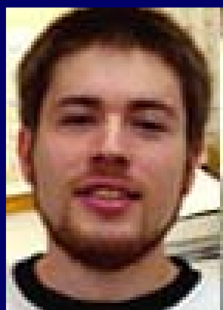
In collaboration with Charles Lin's group at MGH


Biological
Significance
Physical
Underpinning
Technological
Innovation

Acknowledgements

Enzyme Dynamics

Brian English   Dr. Antoine van Oijen → HMS



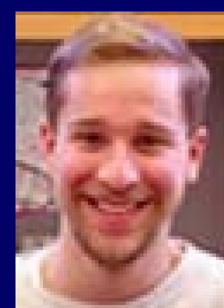
Wei Min

Prof. Sam Kou
– Harvard Statistics
Prof. Binny Cherayil
– India Institute Technology

Gene Expression

Dr. Jie Xiao   Dr. Ji Yu

Dr. Eric Potma → UV Irvine   Conor Evans



Dr. Wei Yang

Funding: NIH – NIH Director's Pioneer Award, NIGMS, NIGMS
DOE – Office of Science, Genomics:GtL