The Role of New Physical Tools in Advancing Biology

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Crystal Structure of Ribosome

• 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
Large absorption cross section $\sim 1 \mu m^2$

$\sim 30 MHz \rightarrow 33 ns$ life time

Pentacene


Single Molecule Turnover Experiment of β-galactosidase

Each enzymatic turnover creates a fluorescent burst
Michaelis-Menten Equation

\[ E + S \xrightarrow{k_1} E \cdot S \xrightarrow{k_{cat}} E + P \xleftarrow{k_{-1}} E + S \]

\[ v \propto \frac{k_{cat}[S]}{[S] + K_M} \]

\[ K_M = \frac{k_{cat} + k_{-1}}{k_1} \]
Fluctuation of Turnover Rate of a Single Enzyme

Histogram Turnover Rate, k, at 380 µM

Autocorrelation of Turnover Rates

Not a Small Effect!

Ever Fluctuating Enzyme!
Single Molecule Michaelis-Menten Equation

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

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

\[
C_M = \frac{\gamma_2 + k_{-1}}{k_1}
\]

Kou et al. J. Phys. Chem. 2005


\[
\frac{v}{[E]_T} = \frac{k_{cat}[S]}{[S] + K_M}
\]
Rugged Energy Landscape

Rugged Energy Landscape


Conformational Dynamics within a Single Protein Molecule

Fluctuation of the distance between Tyr and fluorescein


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

$$k_{ET} = k_0 \exp (-\beta x)$$

$\beta = 1.4 \text{Å}^{-1}$ 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.
Central Dogma of Molecular Biology

DNA → mRNA → Protein

Transcription

Ribosome

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
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 $b = 4.2 \text{ mol.}$
mRNA Degradation Determines the Burst Size

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.
Raman Effect

Sir Chandrasekhara Venkata Raman (1888-1970)

Inelastic scattering

Incident light

Stokes line

Anti-Stokes line

$\nu_{\text{light}}$
Spontaneous Spectrum of Bacteriophage P22 (DNA and capsid proteins)

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)

\[ \omega_{\text{pump}} \quad \omega_{\text{Stokes}} \]

Beating at
\[ \omega_{\text{pump}} - \omega_{\text{Stokes}} = \omega_{\text{vib}} \]

Stimulated excitation of coherent molecular vibration
Spontaneous Raman

Incoherent excitation of molecular vibration

CARS

\[ \omega_{\text{pump}} - \omega_{\text{Stokes}} = \omega_{\text{vib}} \]

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

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

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

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

Evans, et al., PNAS, 2005

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

Gene Expression

Dr. Jie Xiao
Dr. Ji Yu

Dr. Eric Potma → UV Irvine
Conor Evans

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

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

Dr. Wei Yang