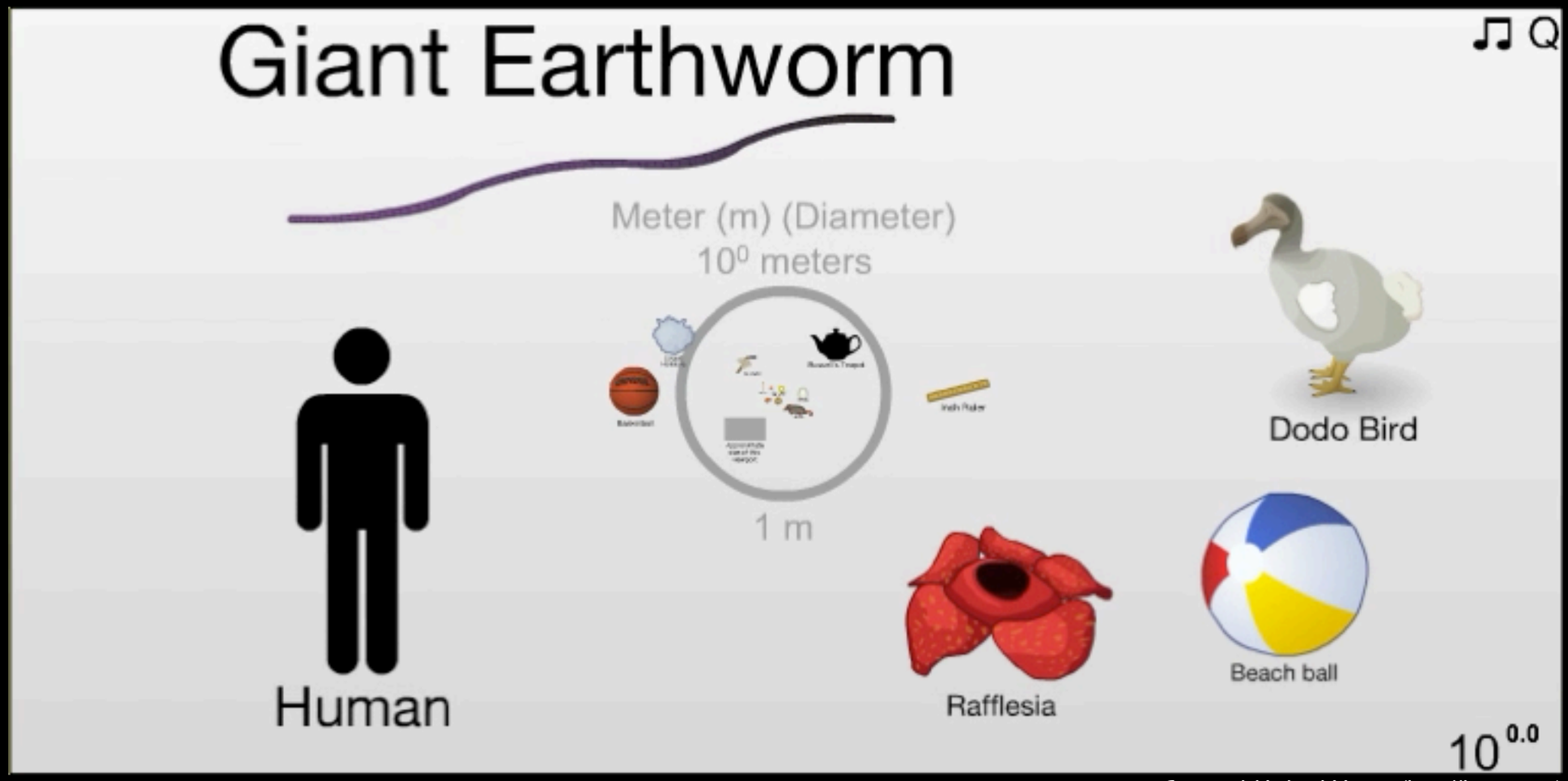
Structure and dynamics of Biomolecular systems

X-ray diffraction, mass spectrometry, IR spectroscopy, single-molecule biophysics

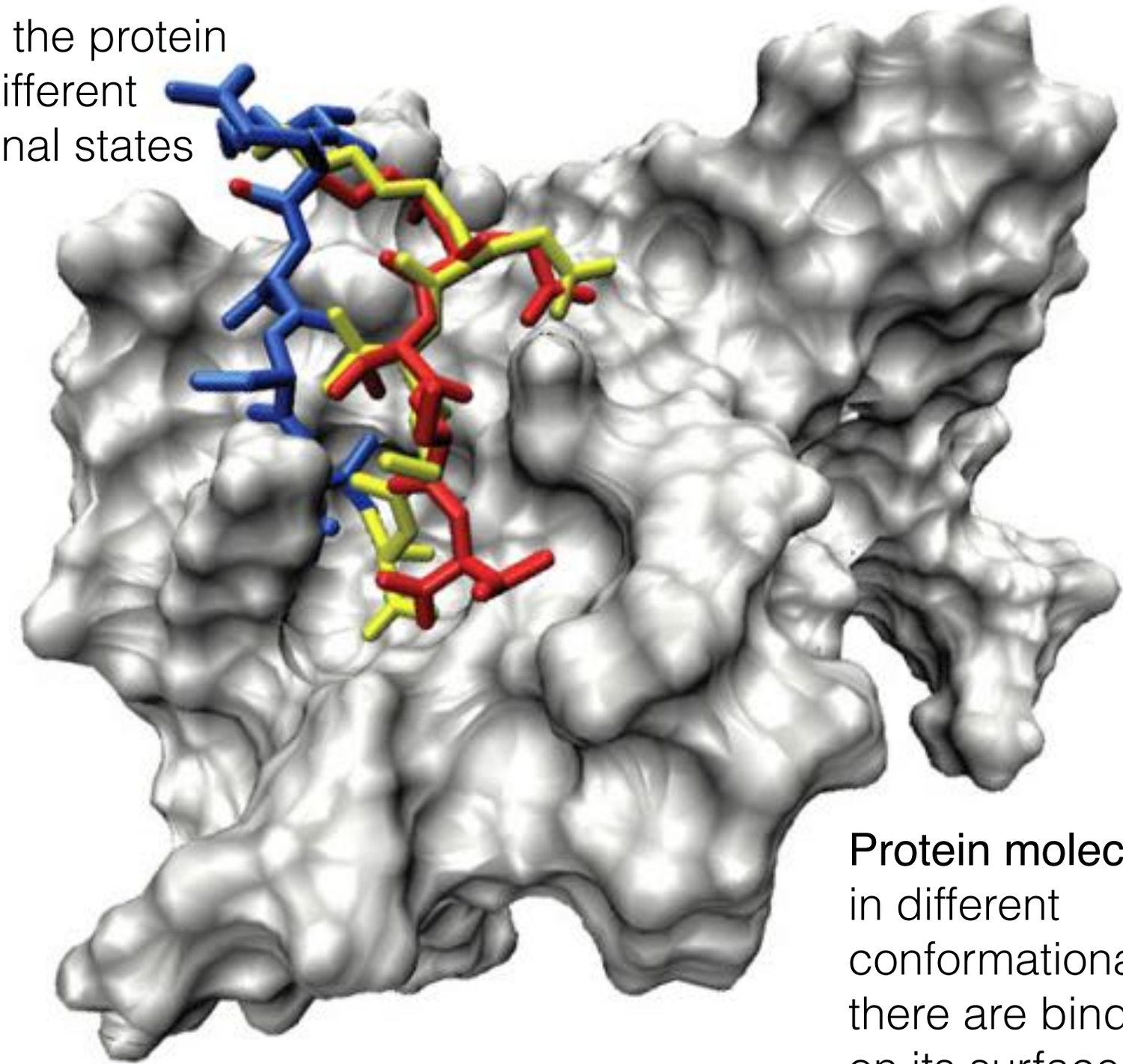
Miklós Kellermayer



Cary and Michael Huang (http://htwins.net)

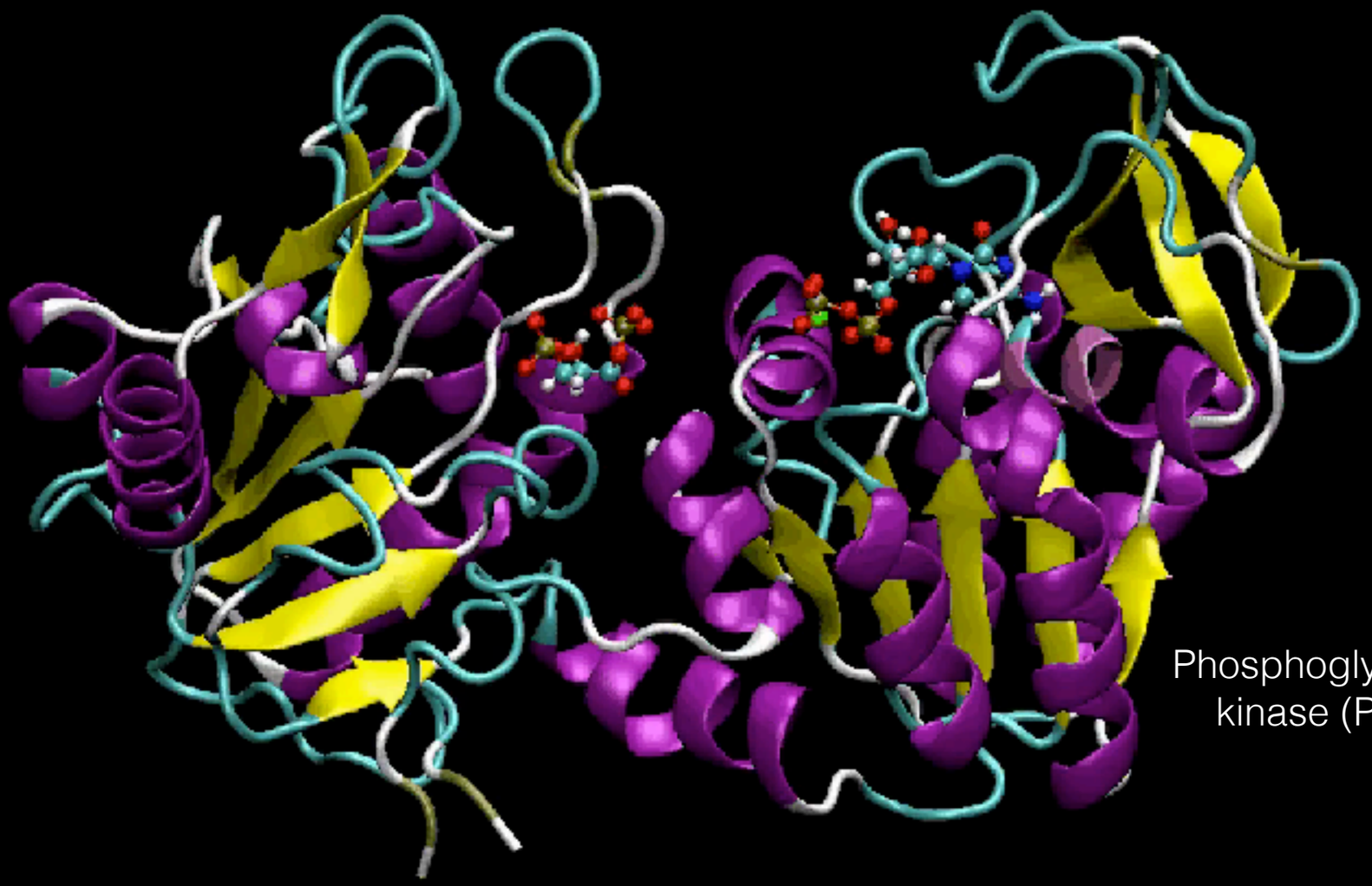
Structure

Small molecule (ligand):
may bind to the protein surface in different conformational states



Protein molecule:
in different conformational states; there are binding sites on its surface

Dynamics



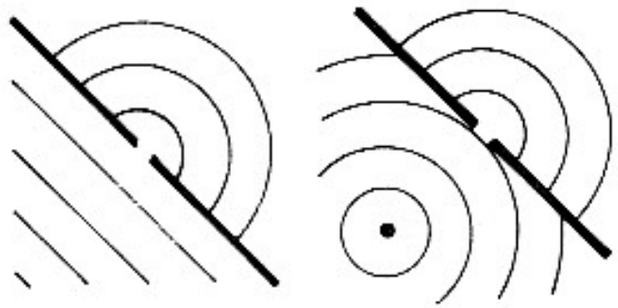
Phosphoglycerate kinase (PGK)

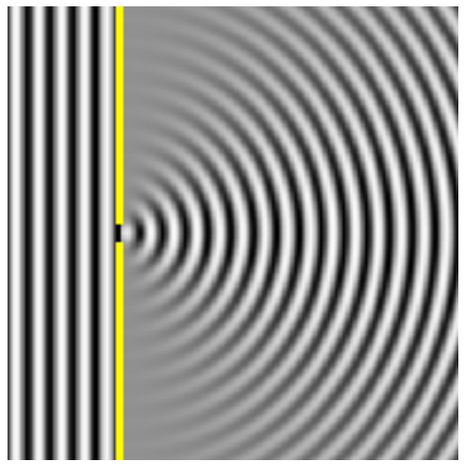
Molecules are in constant rapid motion. In complex molecules (e.g., proteins) the hierarchy of different dynamic modes (e.g., vibration, rotation) results in extremely complex motions. Certain global motions are related to function of the molecule (e.g., domain rotation in a motor protein).

X-ray diffraction, crystallography

Foundations: wave diffraction and interference

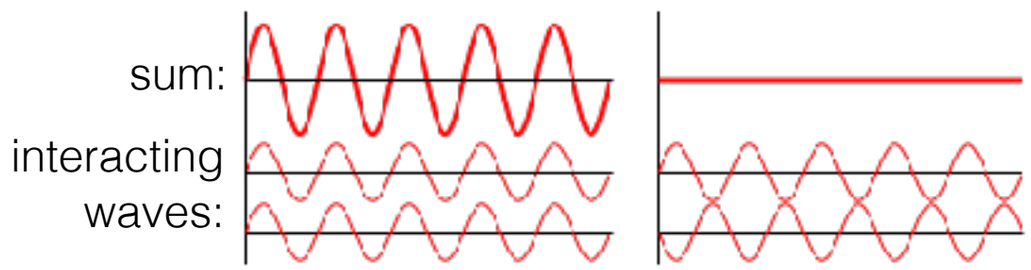
slit smaller than wavelength



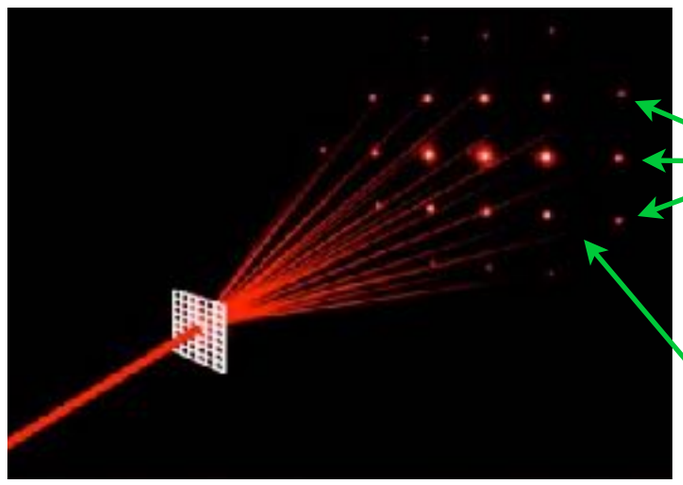


Waves in phase (φ=0): amplification

Ha φ=π : destruction

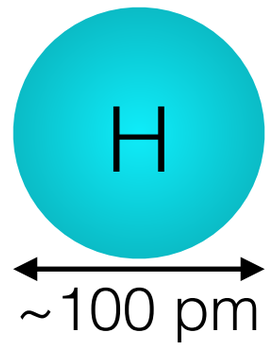


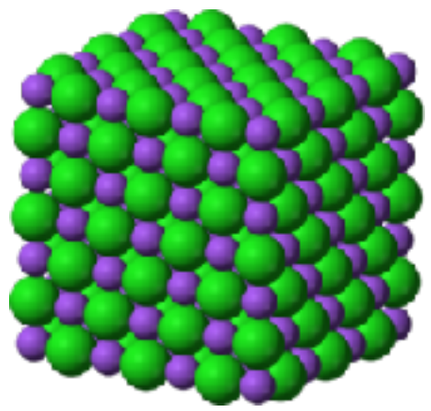
Diffraction pattern of a 2D optical grating



Condition: lattice spacing (d) and wavelength (λ) are comparable: $d \gtrsim \lambda$

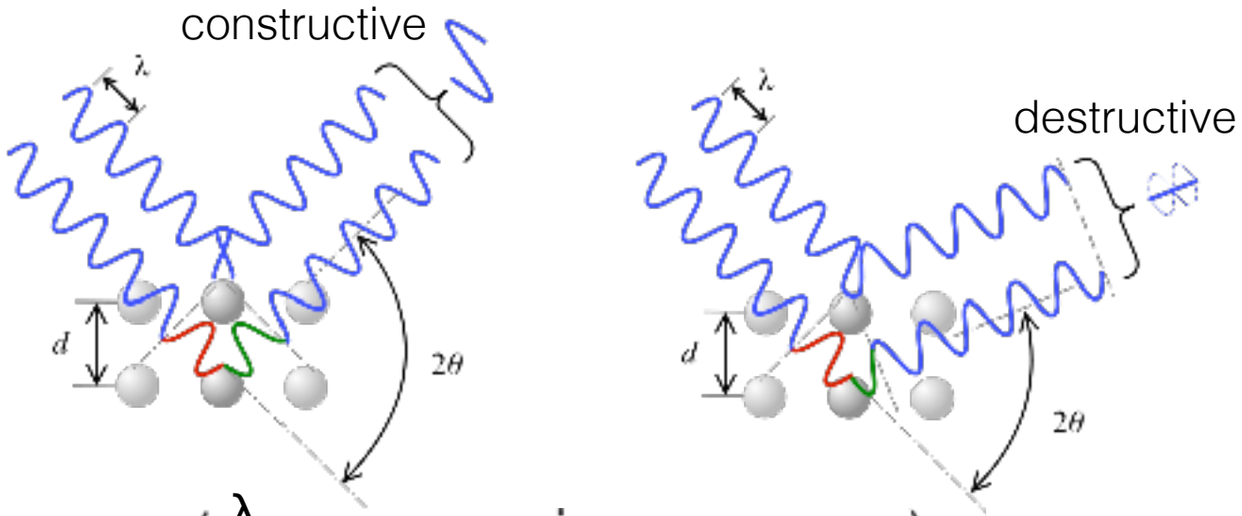
What grating is comparable with x-ray?
$\lambda_{X\text{-ray}}$: 10-200 pm

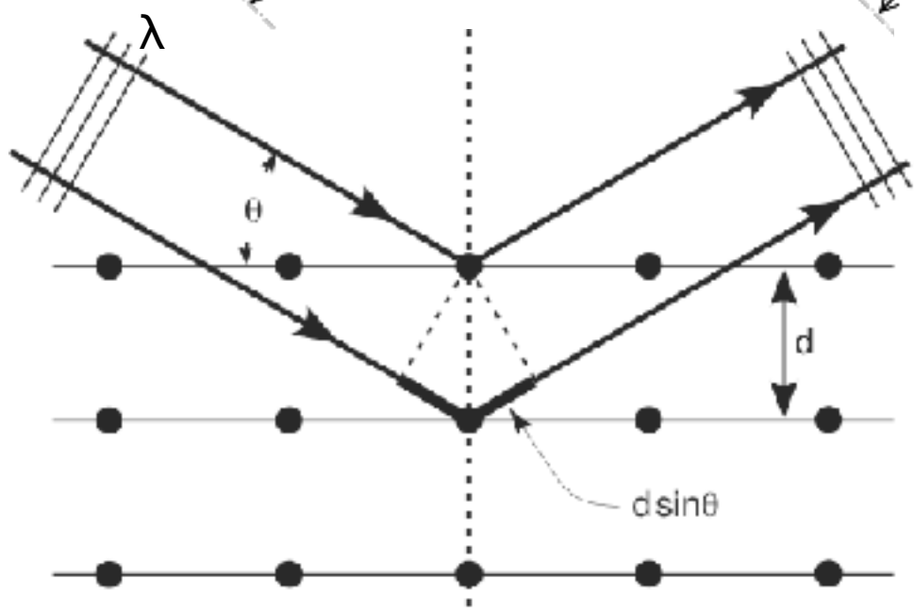




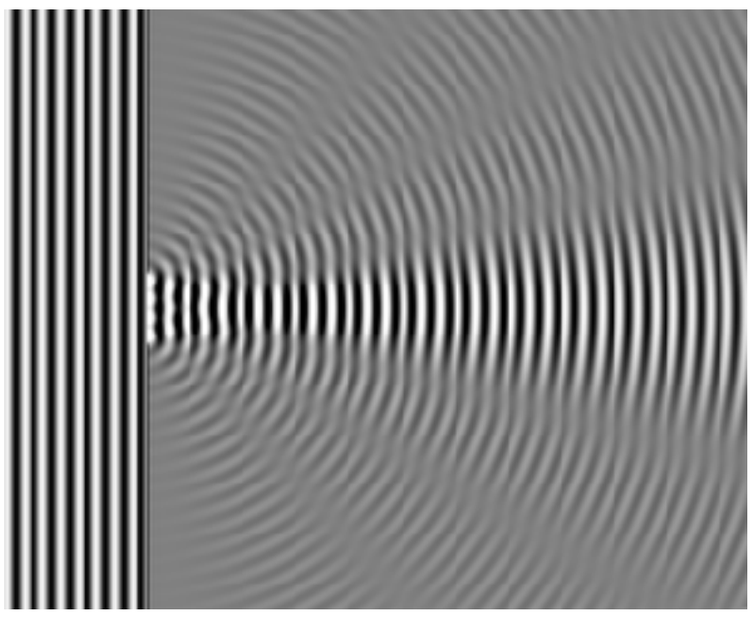
d_{NaCl}: 564 pm

Information obtained with x-ray diffraction: spatial coordinates of atoms
→ spatial structure of the molecule

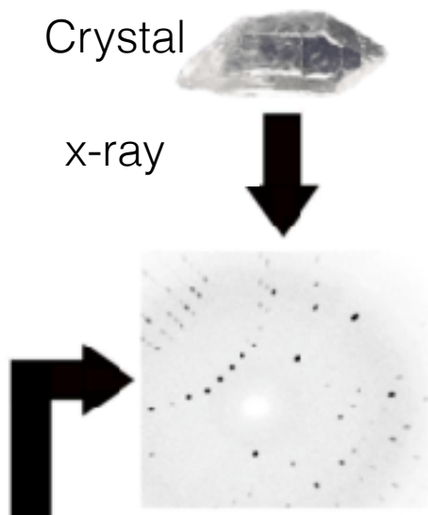


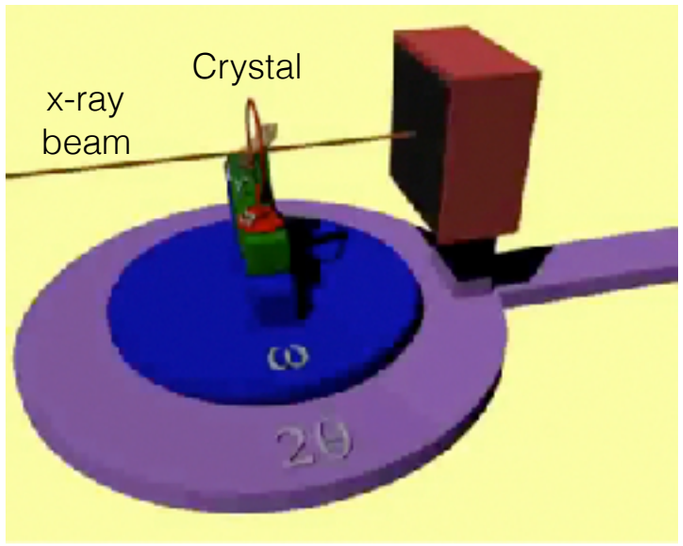


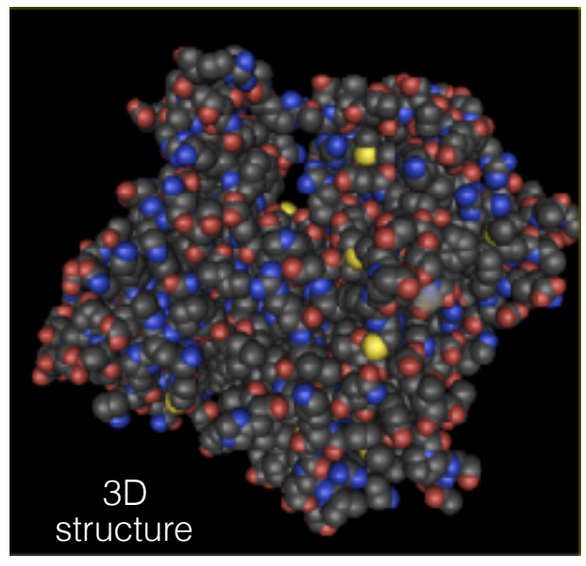
Condition of constructive interference: $2d\sin\theta = n\lambda$



x-ray—crystallography

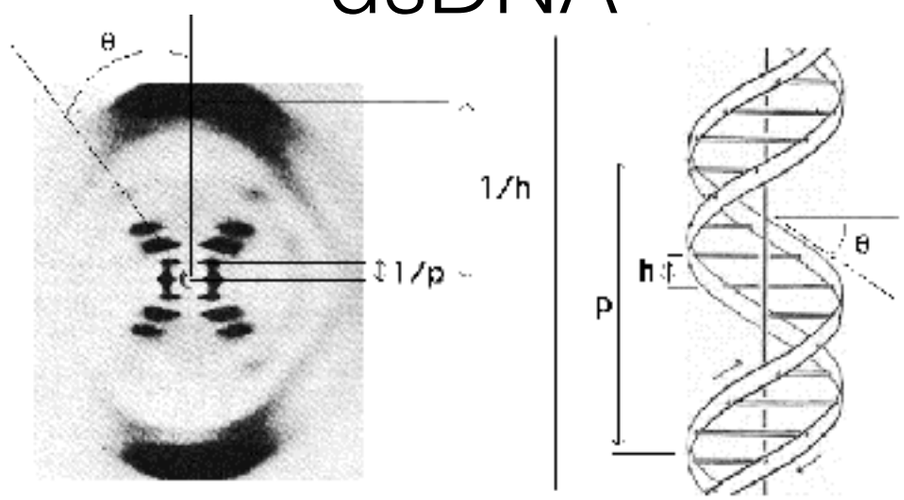






Solving molecular structure with x-ray crystallography

dsDNA



θ tilt of helix

h – 3.4 Å distance between bases

p – 34 Å repeat unit of helix (one pitch)

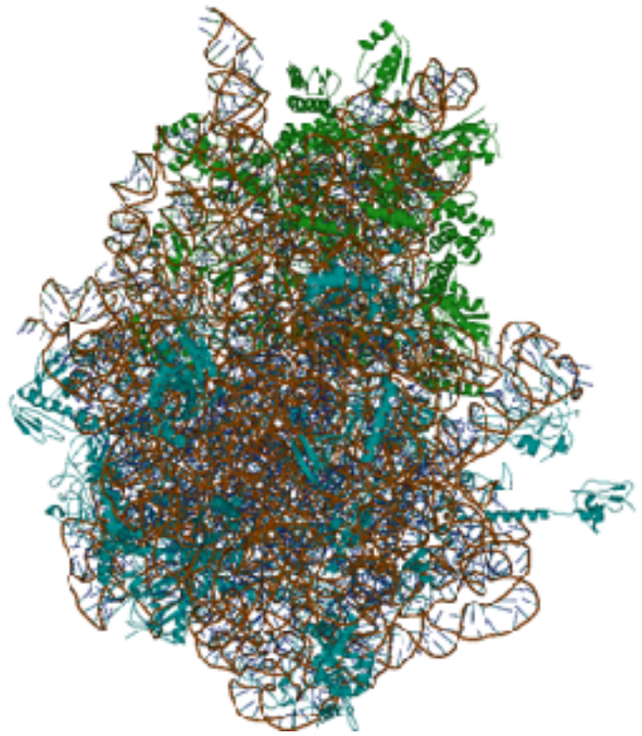
J.D. Watson and F. Crick, 1953
Nobel-prize1962

Globular protein: myoglobin

~1200 atoms

M. F. Perutz, J. C. Kendrew
Nobel-prize1962

Molecular complex: ribosome



30S subunit: ~35000 atoms,
50S subunit:~64000 atoms

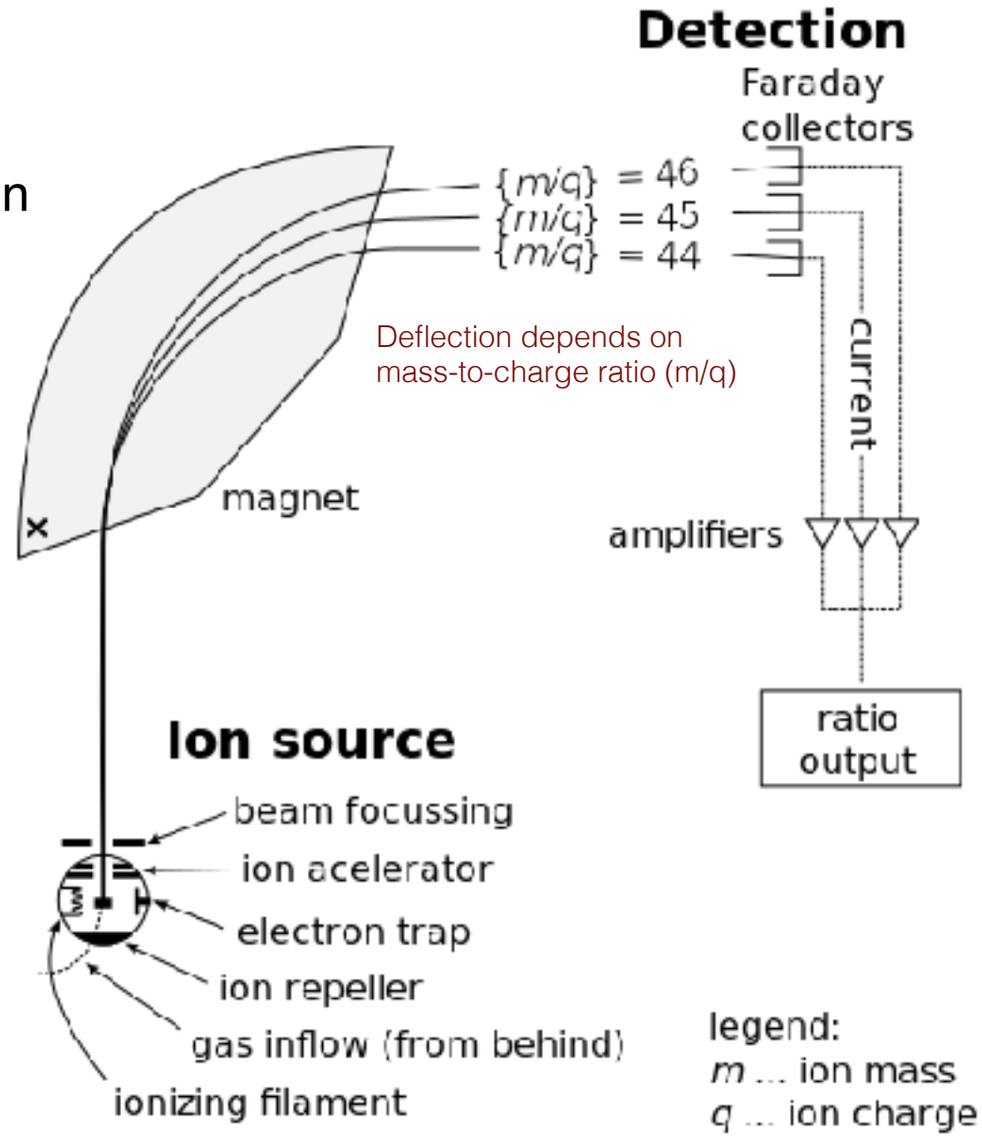
V. Ramakrishnan, T. A. Steitz, A. E. Yonath
Nobel-prize 2009

Mass spectrometry

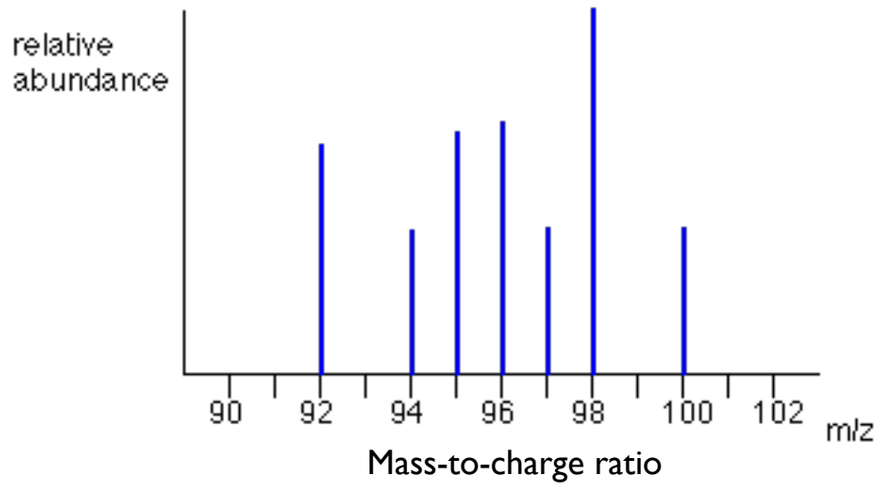
Mass spectrometry (MS): analytical technique producing spectra of the masses of the atoms or molecules in a sample. The spectra are used to determine the elemental or isotopic signature, thereby elucidating the chemical structures of molecules.

Steps:

1. Ionization
2. Acceleration
3. Deflection
4. Detection



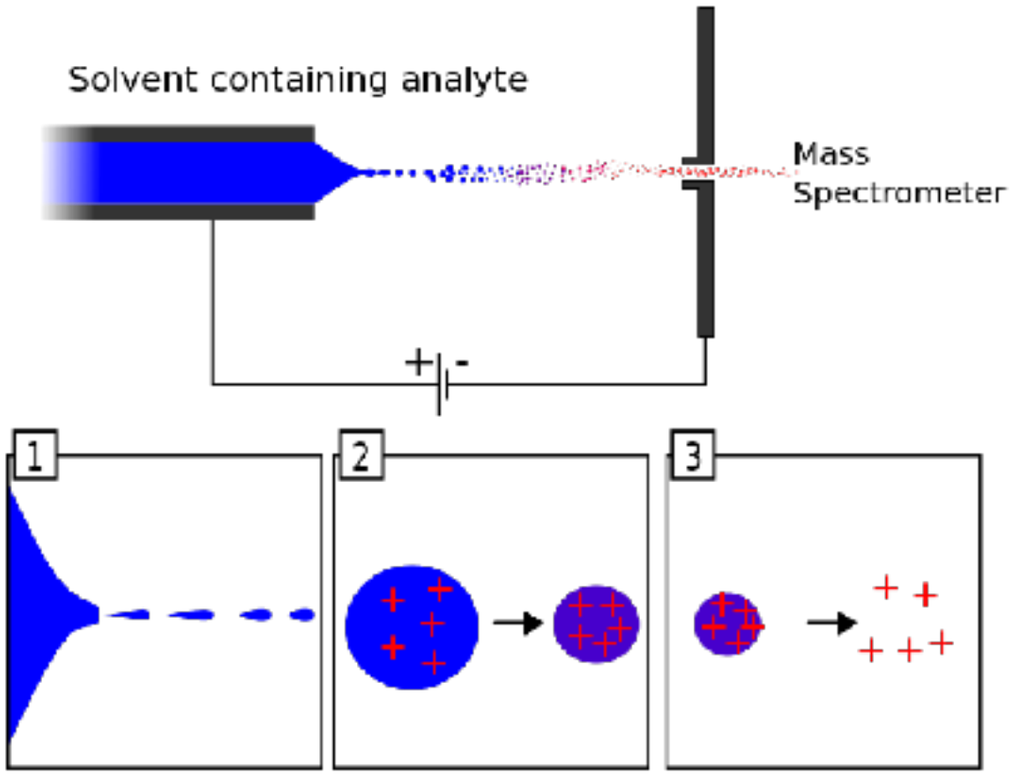
Result: "Stick" diagram



Spectrum is compared with structure database

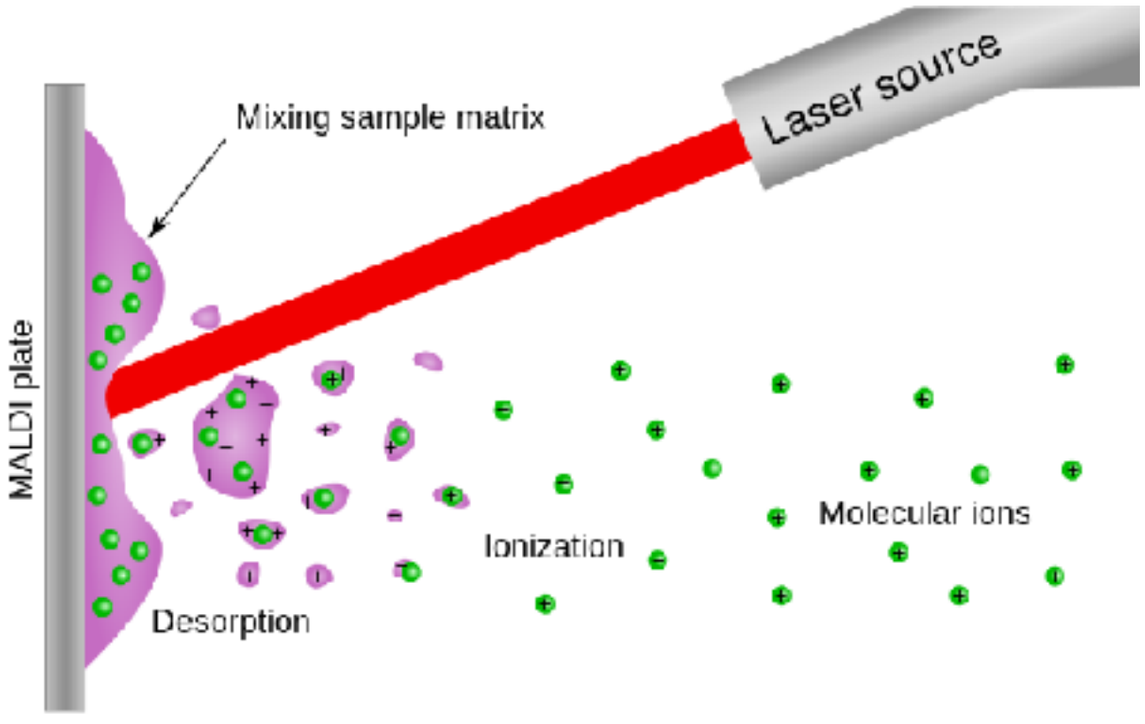
Ionization of biological samples

Electrospray ionization



(2) solvent evaporation → smaller droplet → greater surface charge → (3) Coulomb repulsion → droplets explode → ionized, accelerated molecules

MALDI: “matrix-assisted laser desorption/ionization”

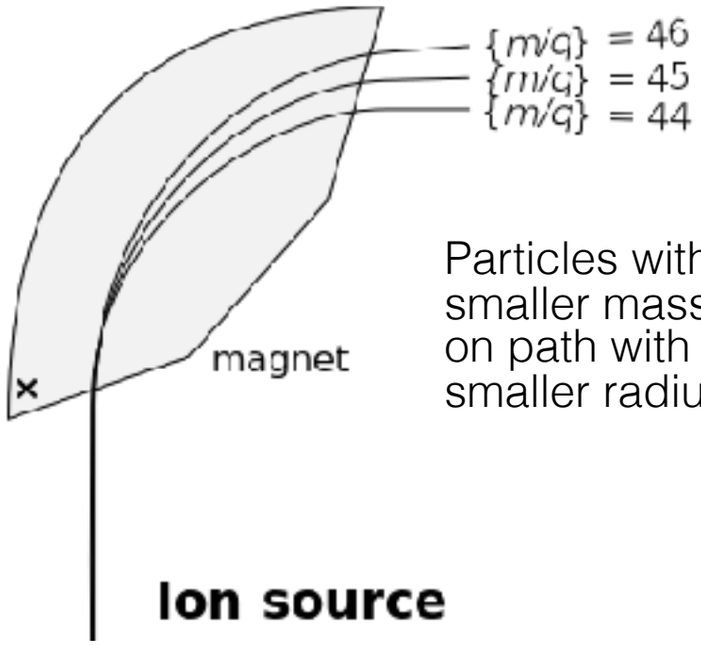


Laser light is absorbed by the atoms/ molecules of the matrix.

Ideal for investigating large molecules.

Methods of mass analysis

Magnetic method



Particles with smaller mass are on path with smaller radius.

Lorentz force accelerates (a) particles of mass m and charge q:

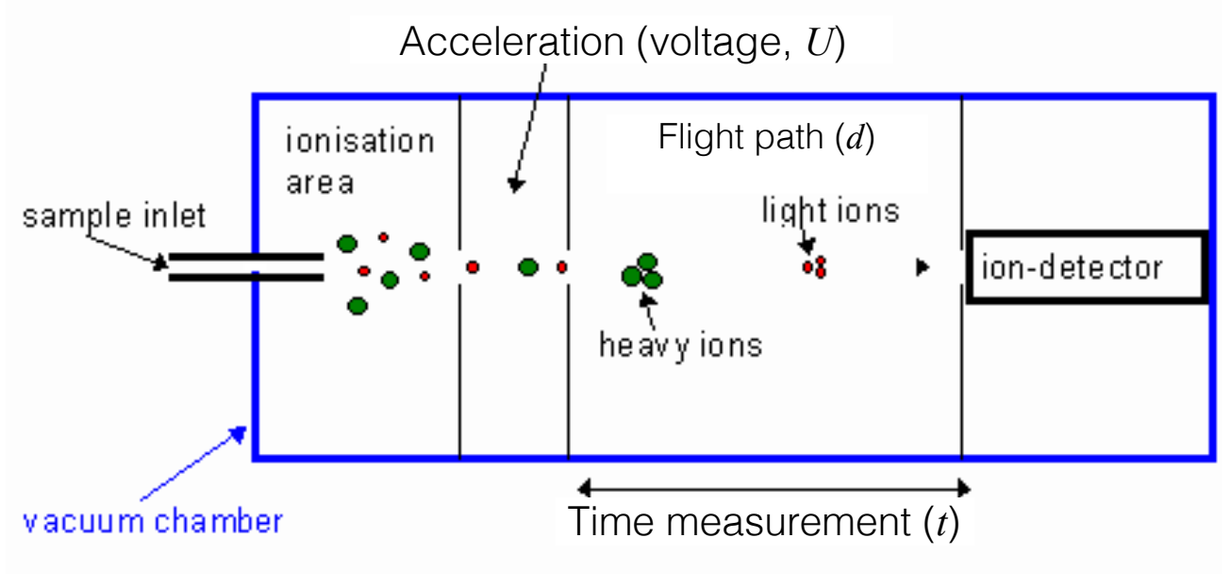
$$q\left(E + v \times B\right) = ma$$

E=electric field
vxB=vectorial product of speed and magnetic induction

from which the mass-charge ratio (m/q) can be determined:

$$\frac{m}{q} = \frac{E + v \times B}{a}$$

"Time-of-flight" method



Potential energy of charged particle (qU) is converted into kinetic energy:

$$qU = \frac{1}{2}mv^2 = \frac{1}{2}m\left(\frac{d}{t}\right)^2$$

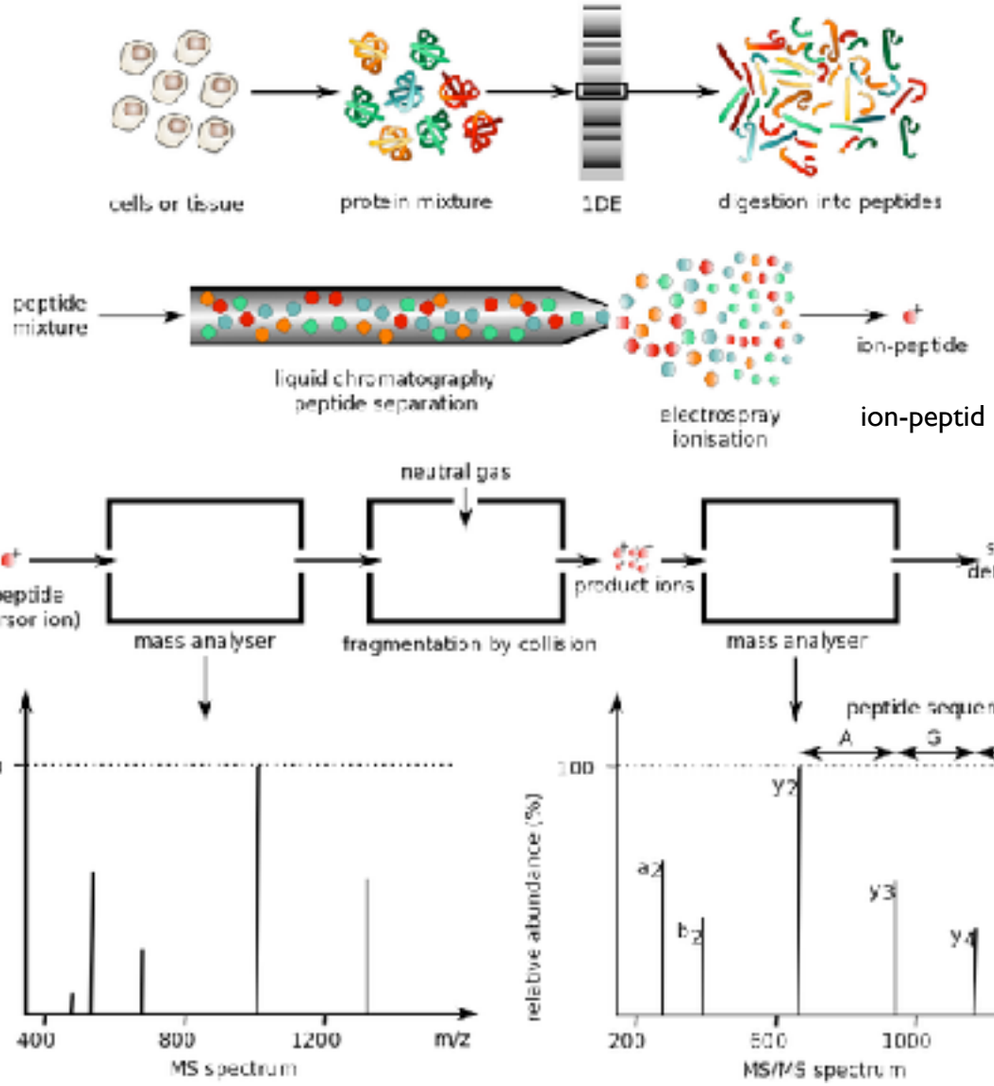
from which time (t) and hence m/q can be calculated:

$$t = \frac{d}{\sqrt{2U}}\sqrt{\frac{m}{q}} = k\sqrt{\frac{m}{q}}$$

instead of m/q, usually m/z is used, where $z=q/e$ (dimensionless number).

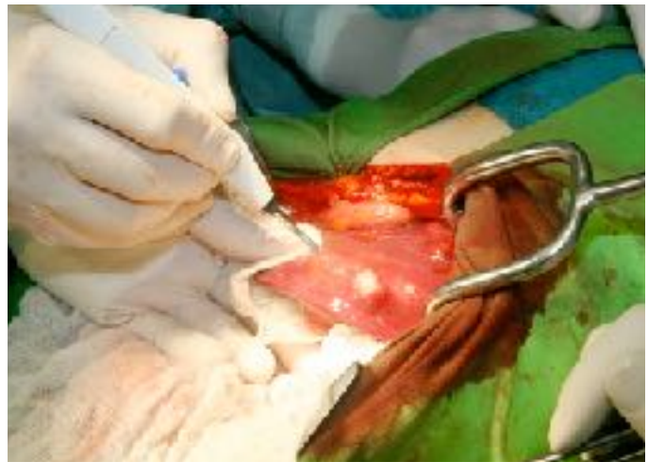
Mass spectrometry applications

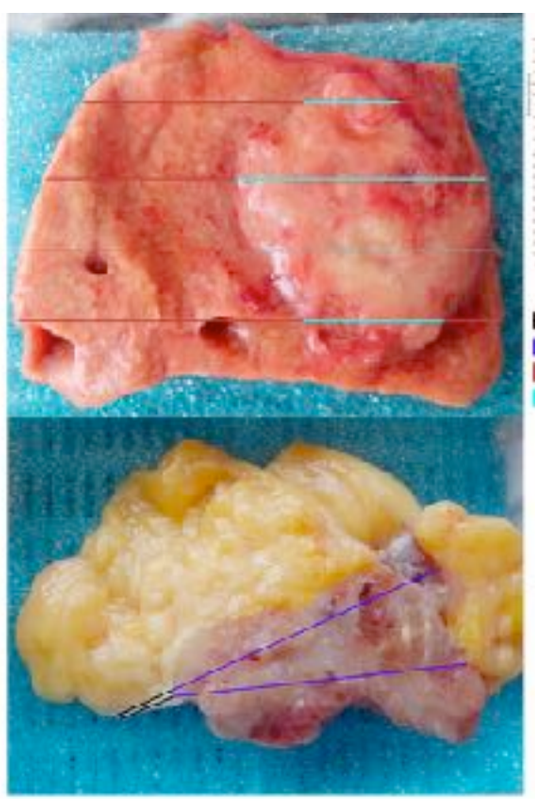
1. **Protein analytics** (proteomics)

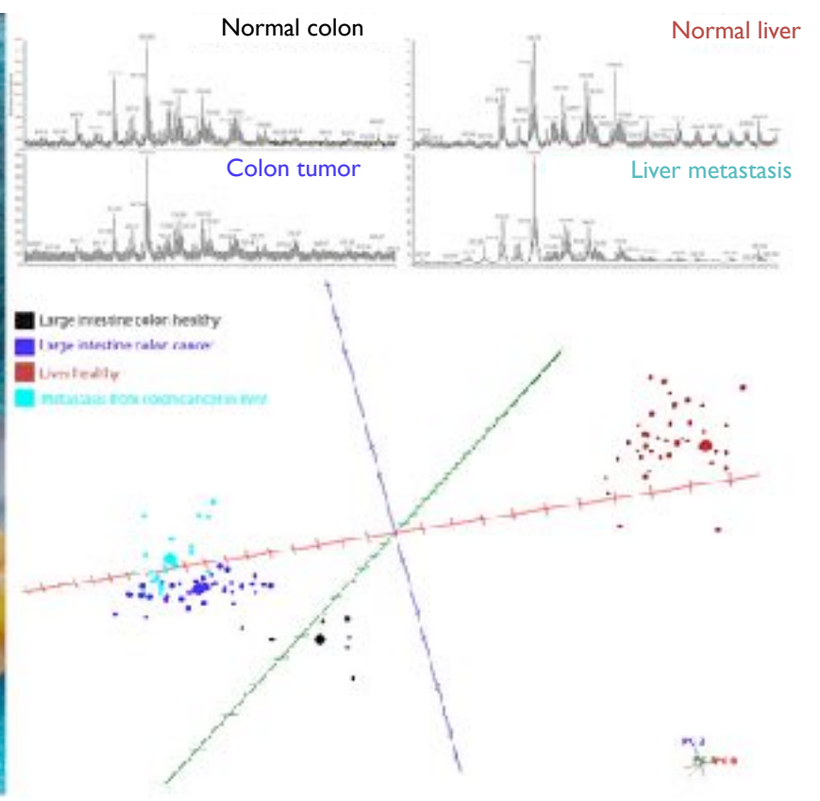


2. **Diagnostic screening:**
Metabolic diseases (from 1 drop of blood)
e.g., phenylketonuria (PKU)

3. **Real-time tissue analysis** (“onco-knife”)







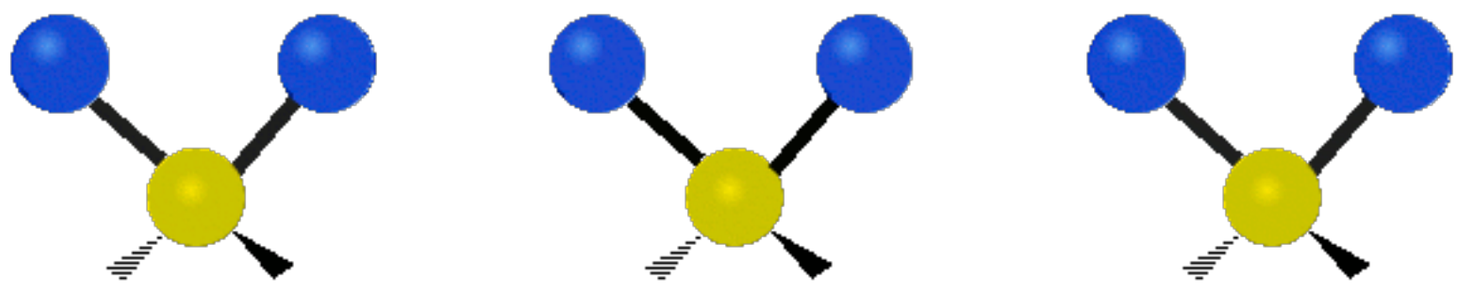
IR spectroscopy

Provides insight into chemical composition and vibrational/rotational **dynamics**

Molecules **vibrate** and **rotate**

Vibration: periodic motion **along** the axis of the covalent bond
Rotation: periodic motion **around** the axis of the covalent bond

Examples of vibrational motion in the triatomic methylene group (-CH_2-):



Asymmetric stretching *Symmetric stretching* *Scissoring*

Scales of transition energies between different states are different:

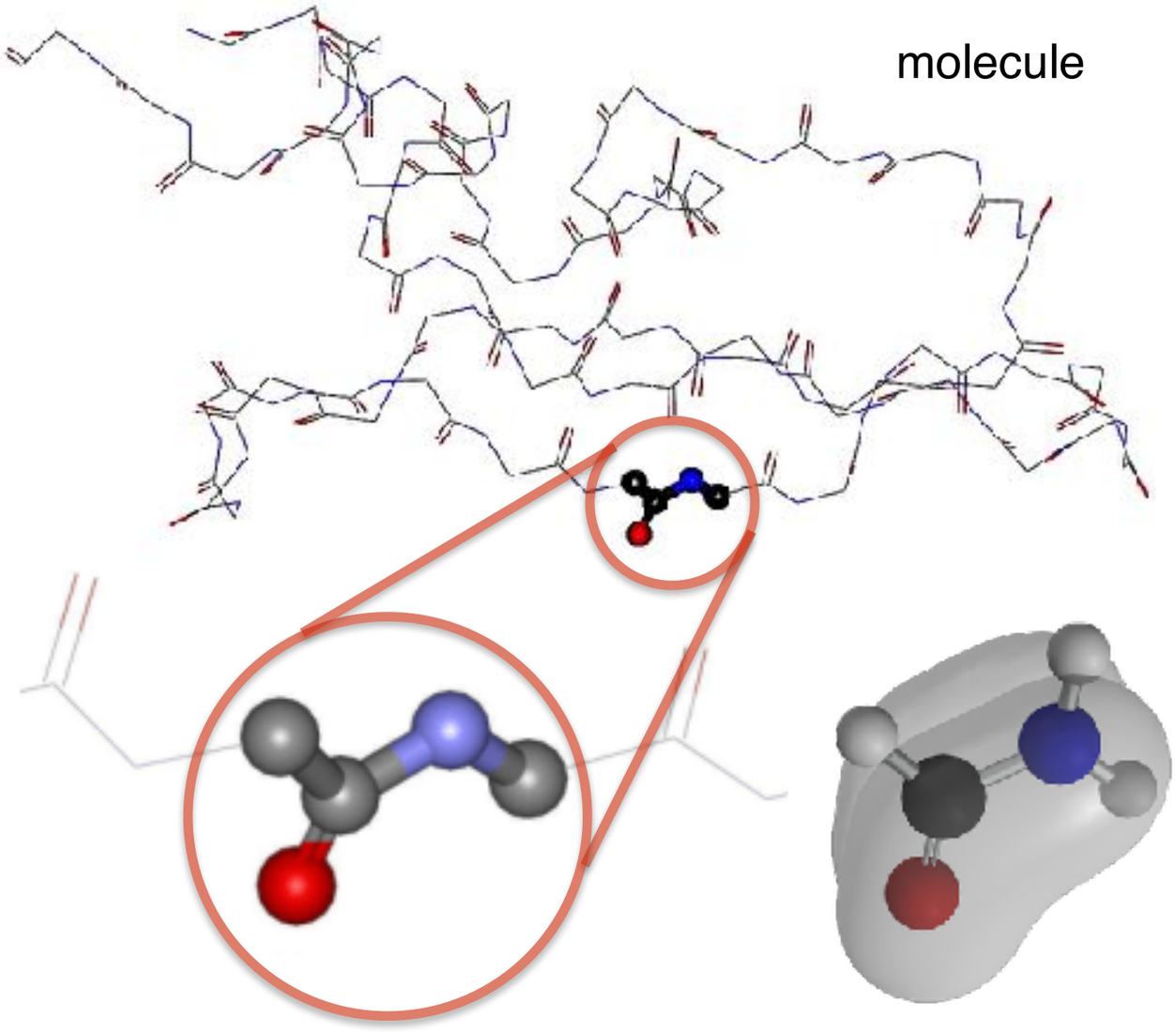
$$E_e \overset{\sim 100x}{>} E_v \overset{\sim 100x}{>} E_r$$

$$\sim 3 \times 10^{-19}\ \text{J}\ (\sim 2\ \text{eV}) > \sim 3 \times 10^{-21}\ \text{J} > \sim 3 \times 10^{-23}\ \text{J}$$

(“Rule of thumb”: ultraviolet > visible > infrared)

IR spectroscopy

IR spectrum: very rich information about molecular structure and vibrational-rotational propertries

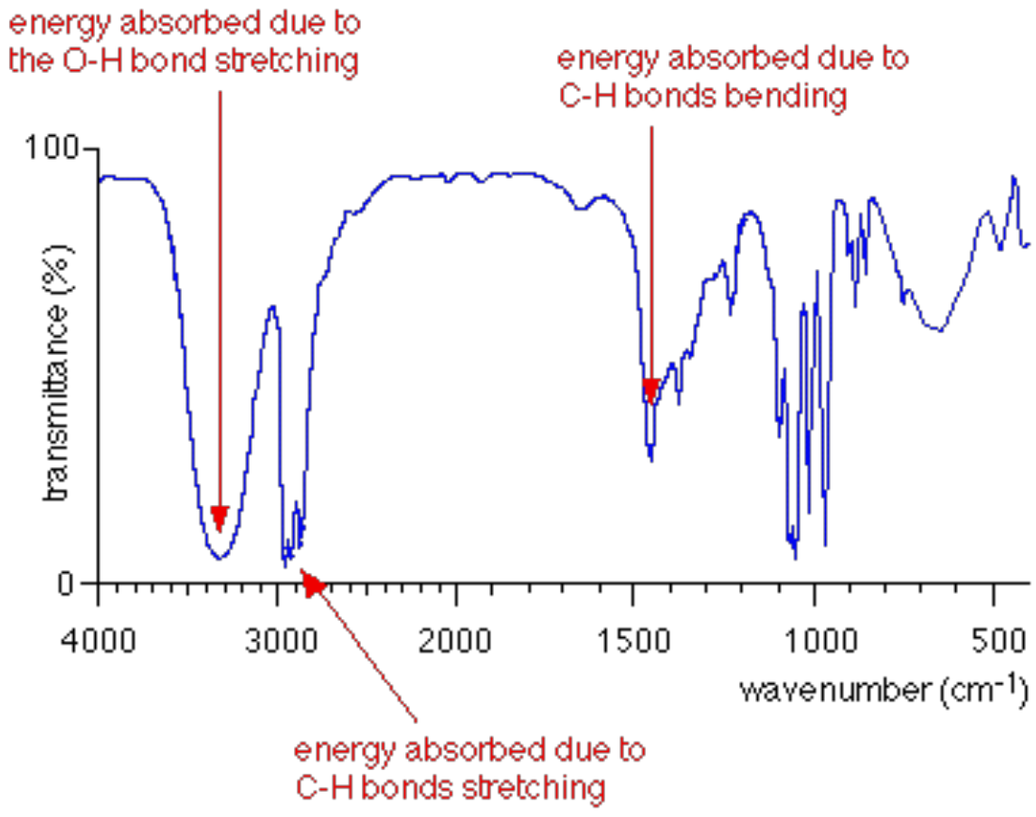


molecule

amide I bond
stretching vibration

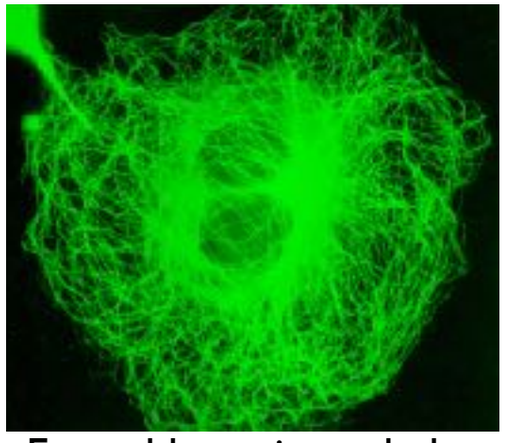
amide I bond
conjugate electron
system

Rich IR spectrum:
transmittance vs. wavenumber
(e.g., propanol)

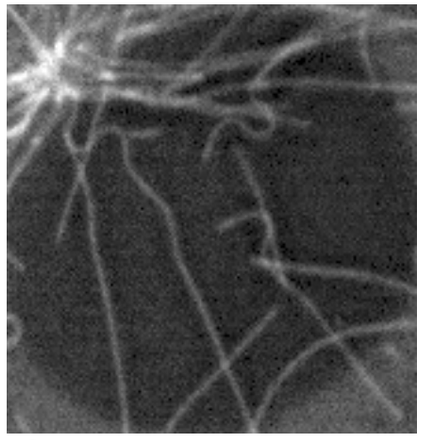


Single-molecule biophysics

1. Individuals (spatial and temporal trajectories) can be idenditfied in an ensemble

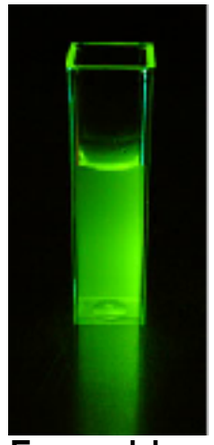


Ensemble - microtubular system

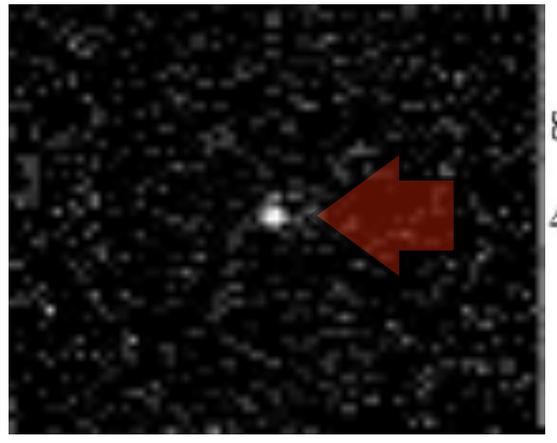


Single microtubules - treadmilling

2. Stochastic events may be discovered

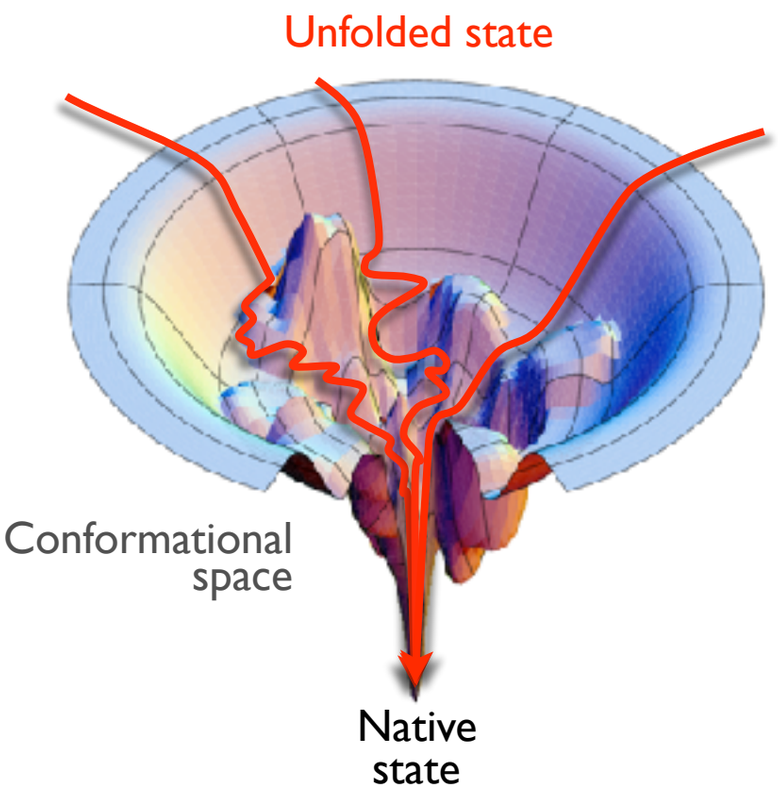


Ensemble - intensity

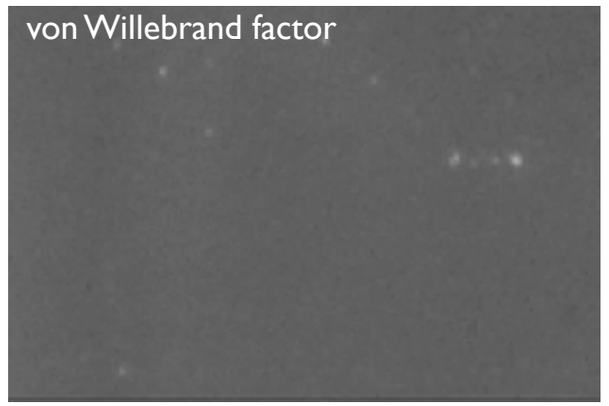


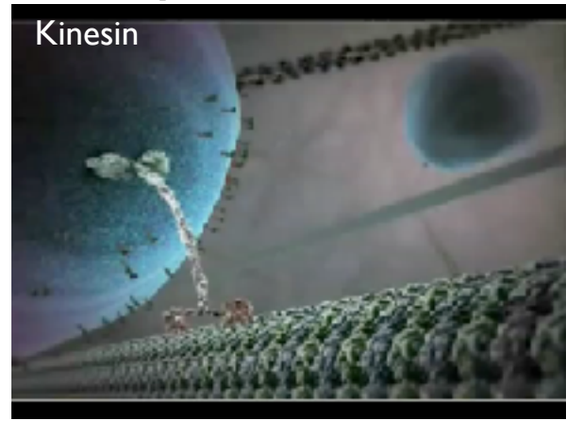
Single quantum dot - blinking

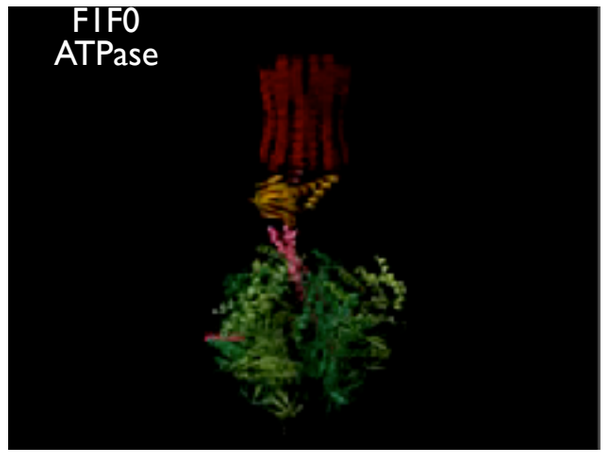
3. Parallel-pathway processes may be described



4. Mechanics of biomolecules may be characterized

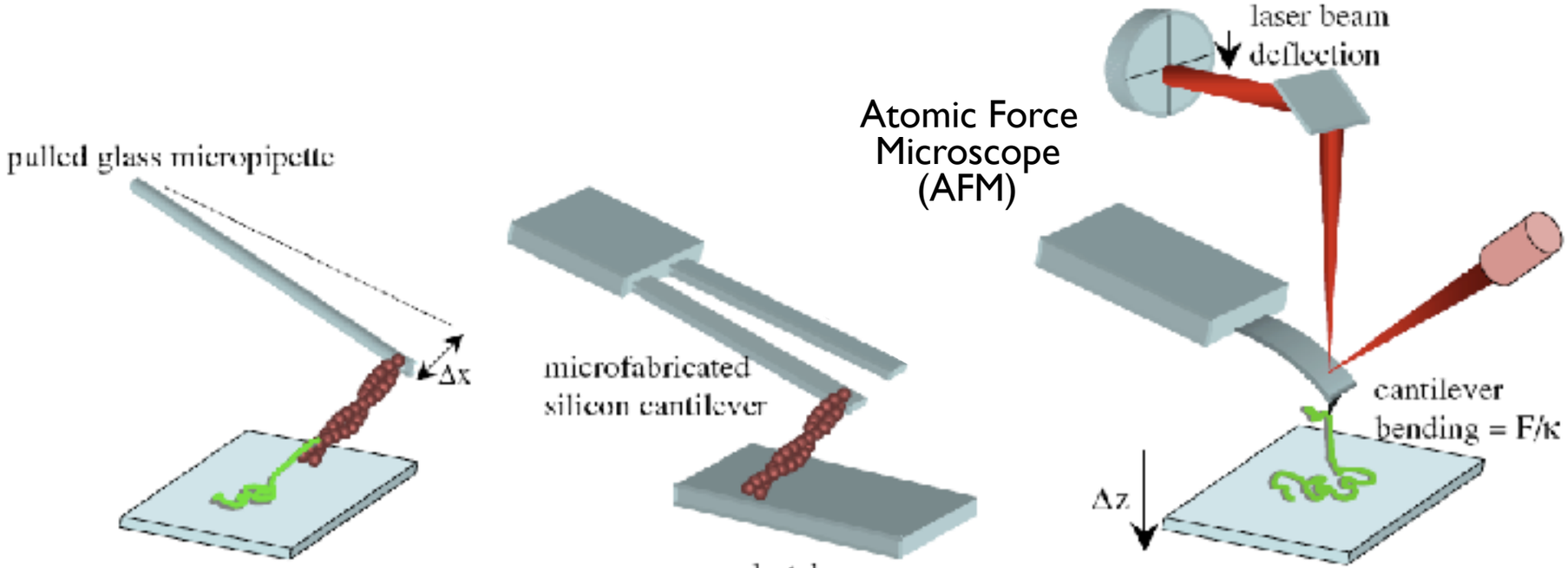




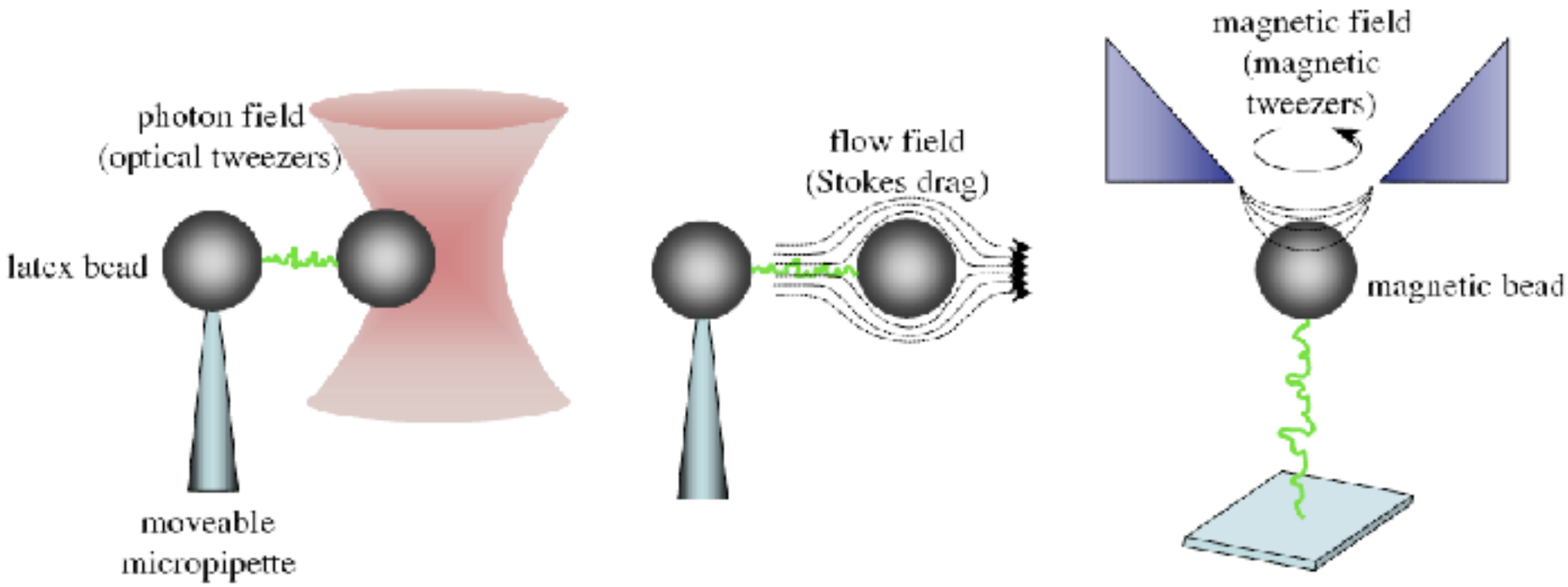


Manipulation of single molecules

Cantilever methods

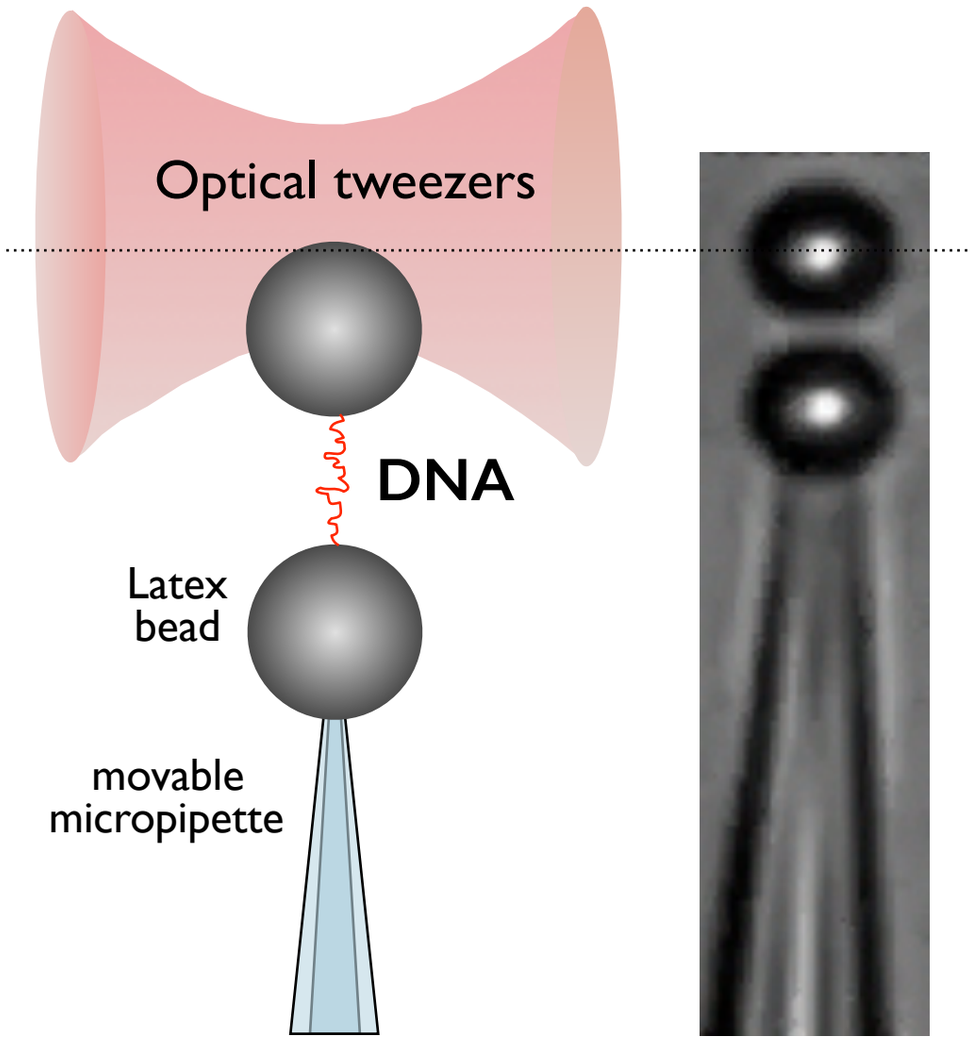


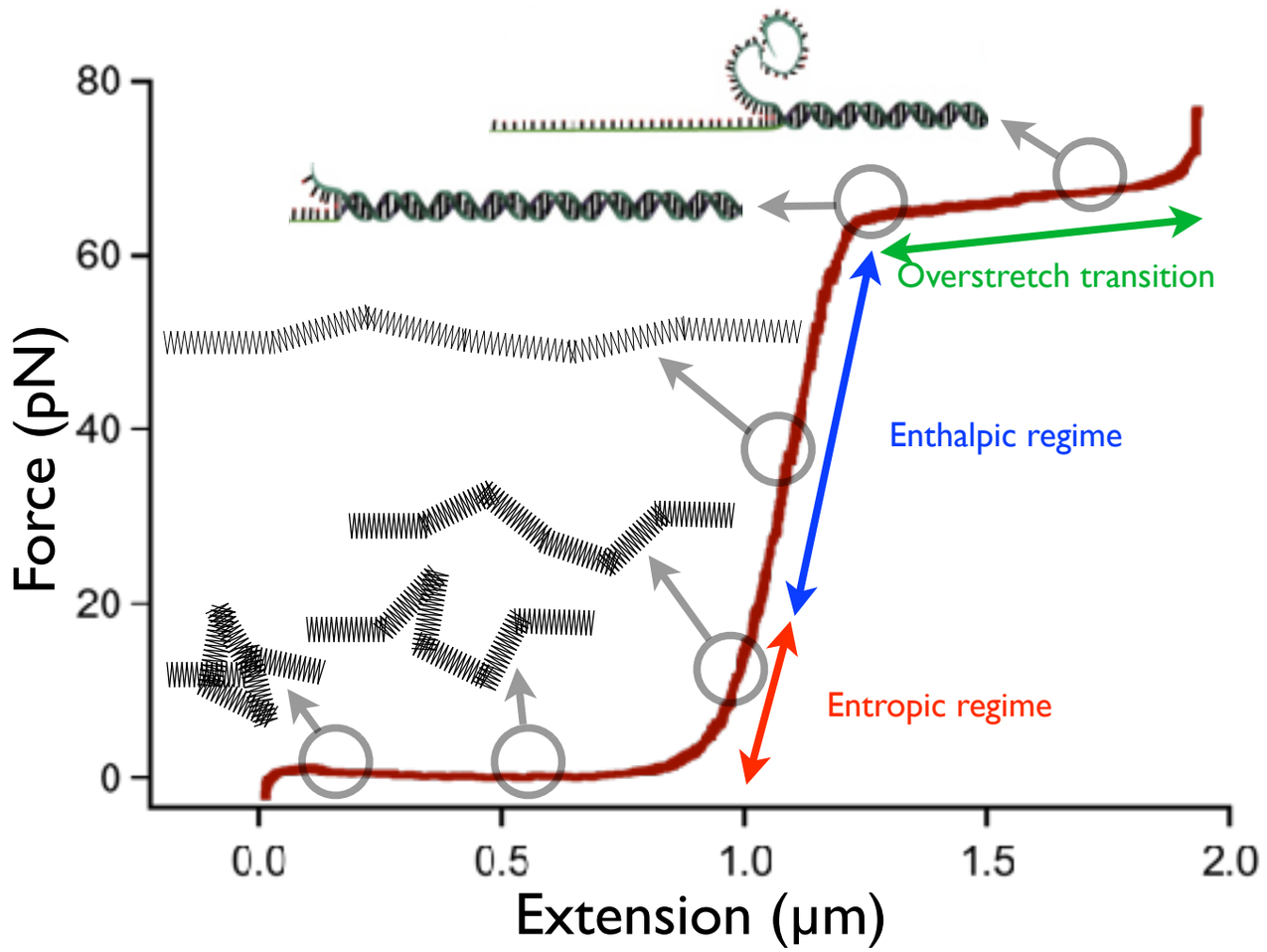
Field-based methods



Measurable parameters I. Force

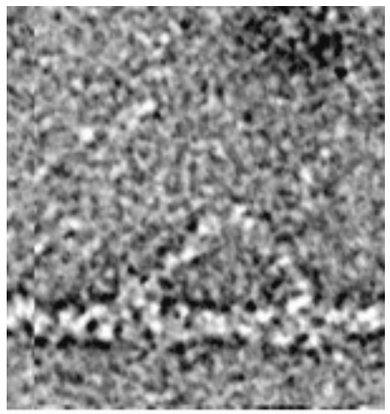
How much force develops during the stretch of a single dsDNA molecule?





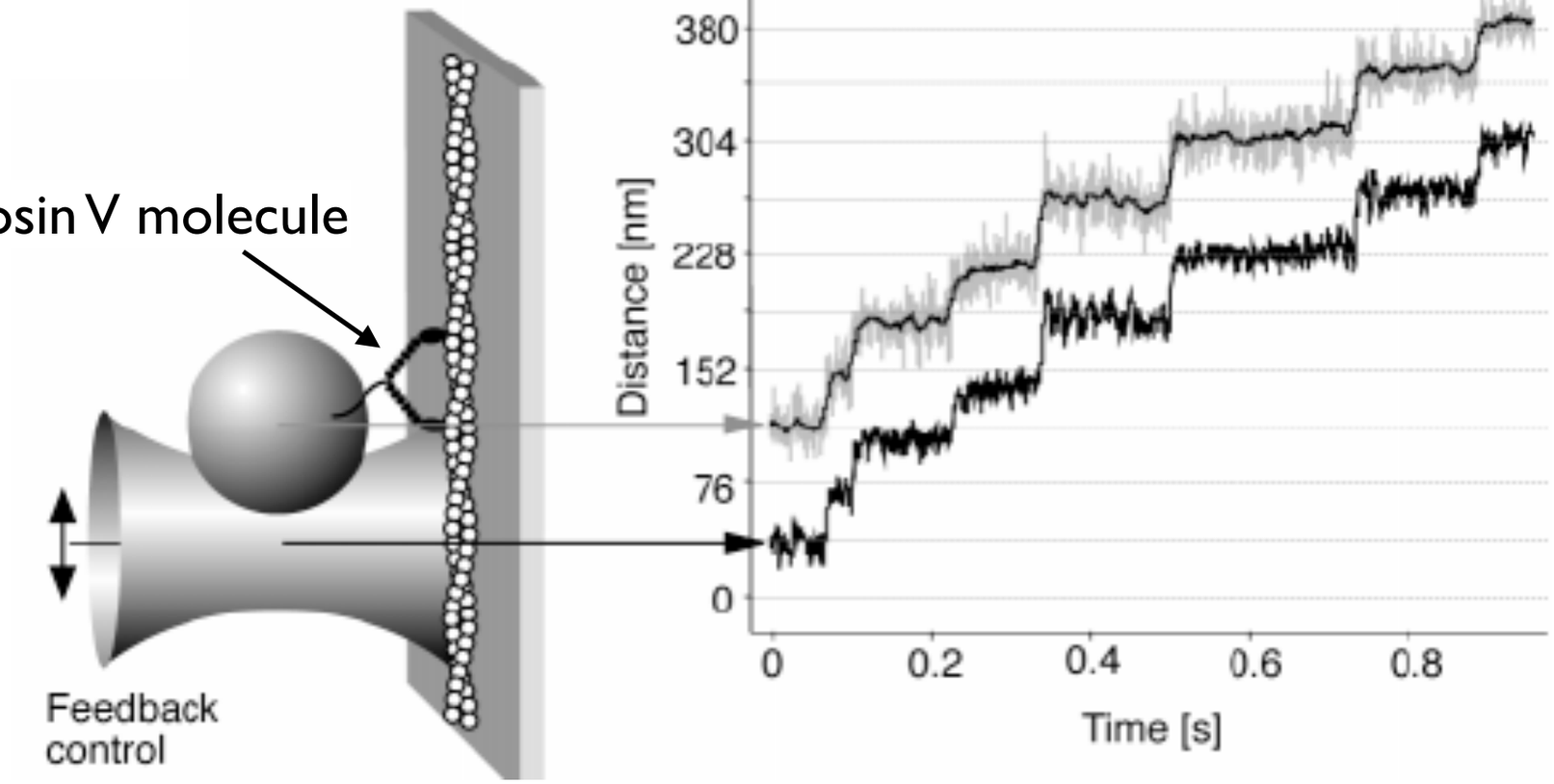
Measurable parameters II. Distance

What is the step size of a motor protein?



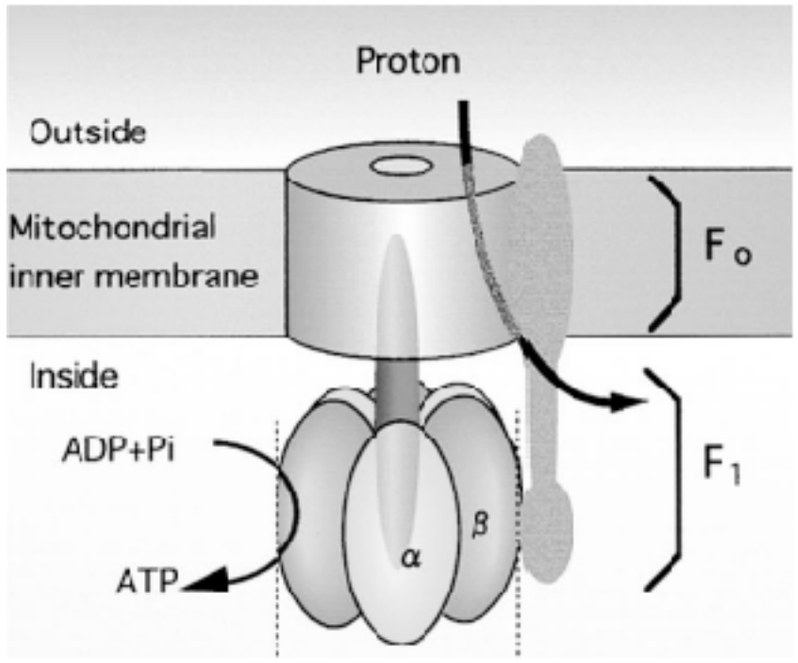
The Muscle Group, Leeds 2000

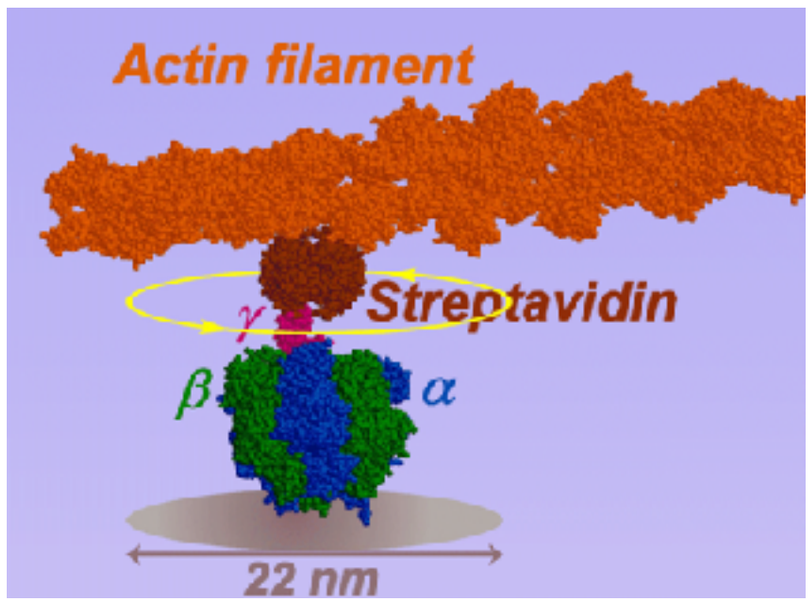
Myosin V cryoelectron-microscopic image series

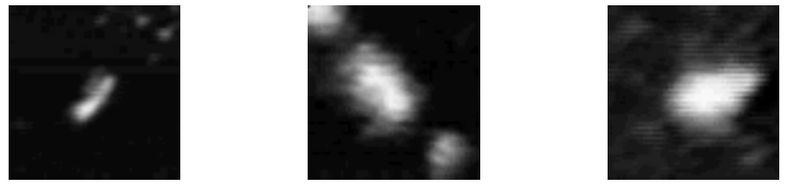


Measurable parameters III. Rotational angle

How does the ATP synthase work?

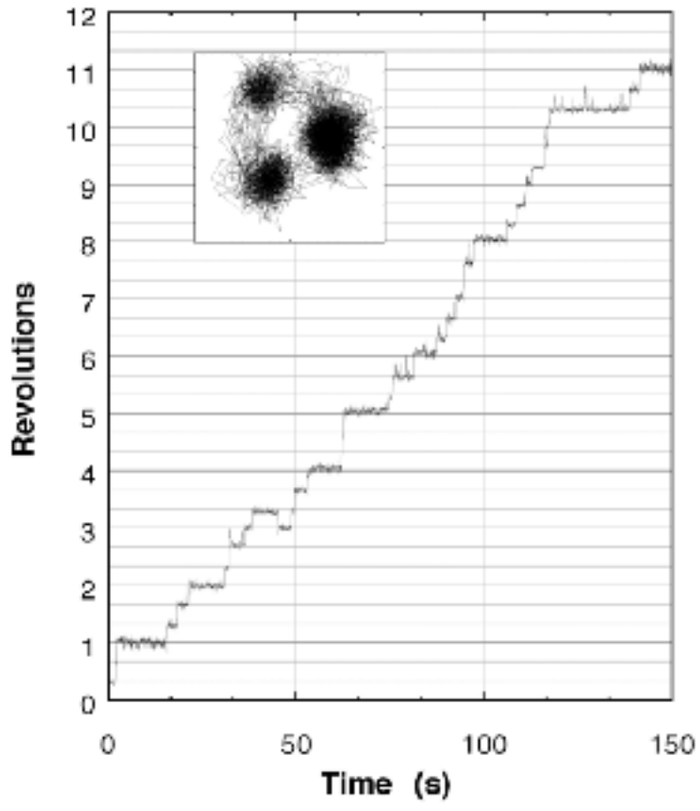






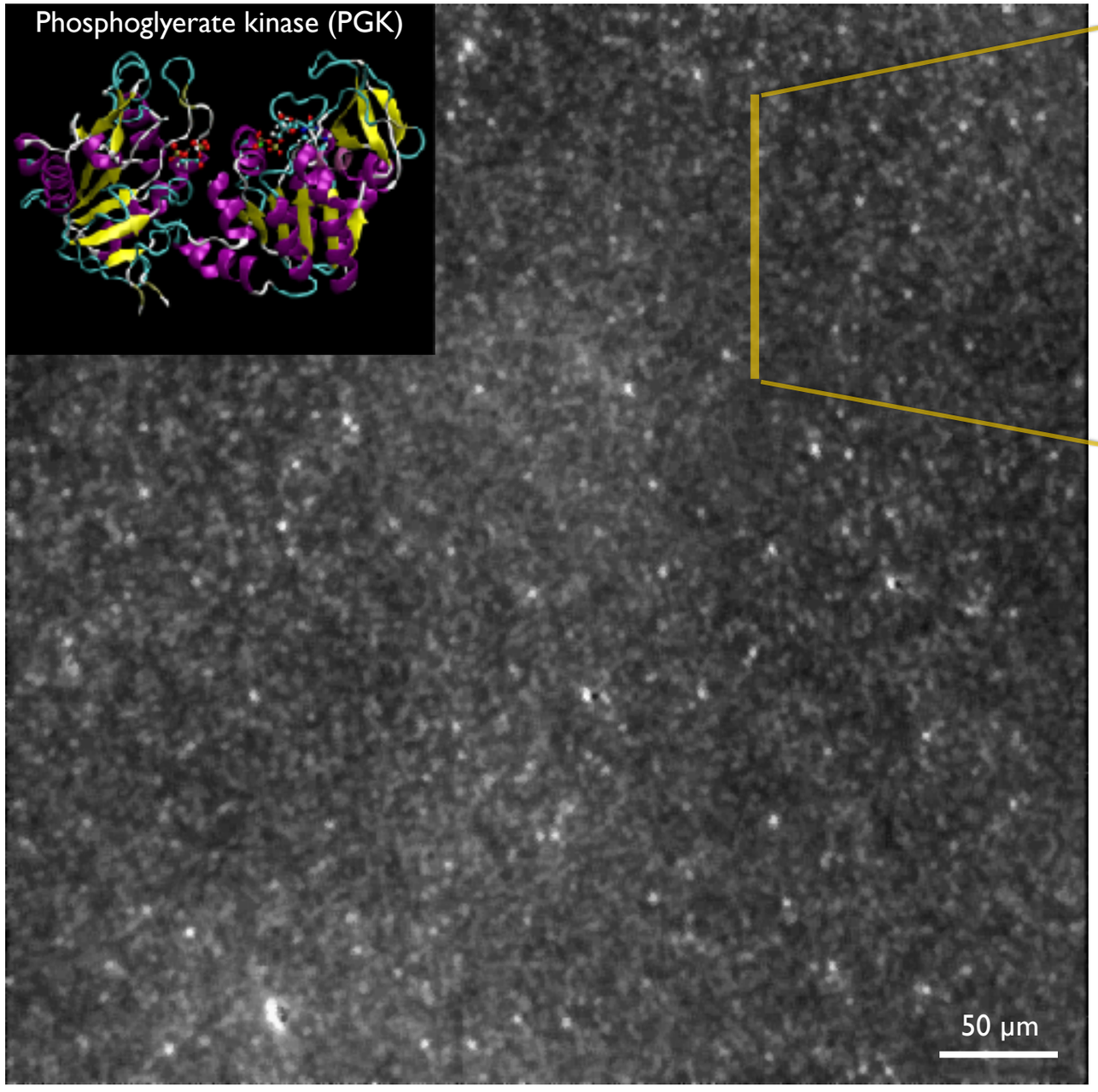
1 mM ATP 200 nM ATP 20 nM ATP

Discrete rotational steps of 120°

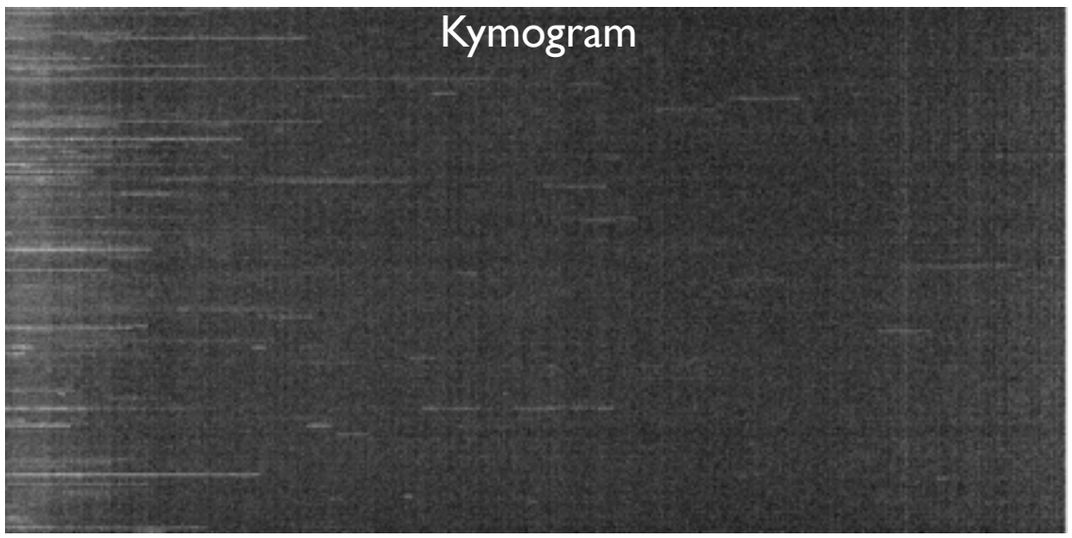


Measurable parameters IV. Fluorescence

What are the conformational states of a molecule?

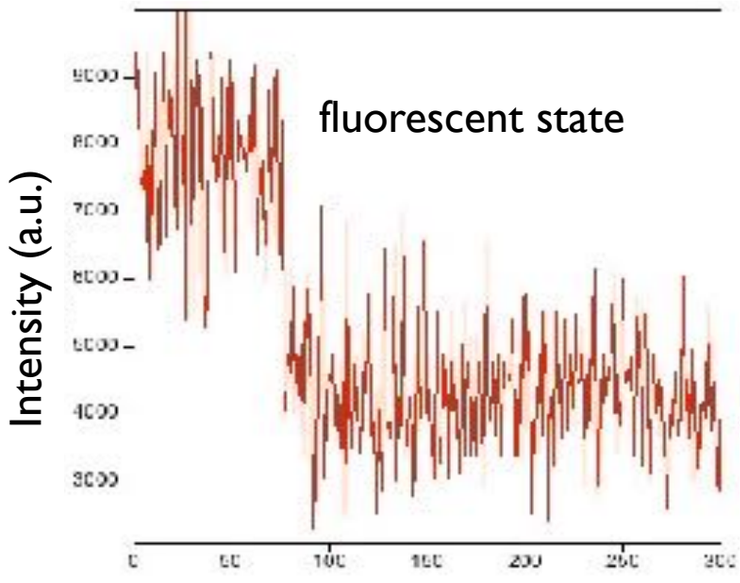


PGK molecules labeled with Alexa488, TIRF microscopy



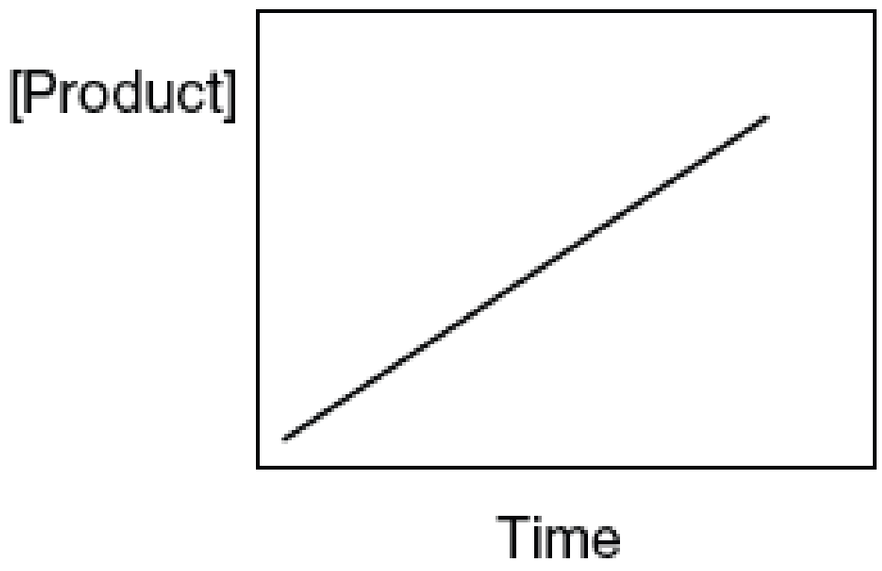
Time

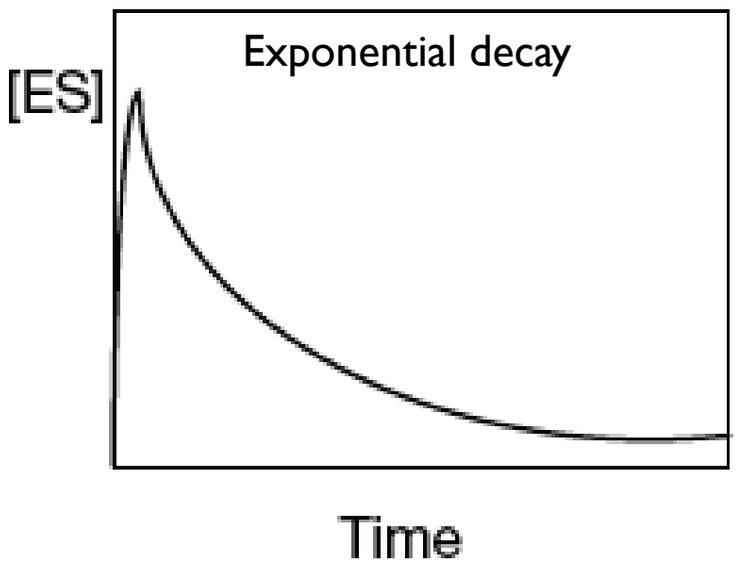
Single-step photobleaching (exponential function in an ensemble!)



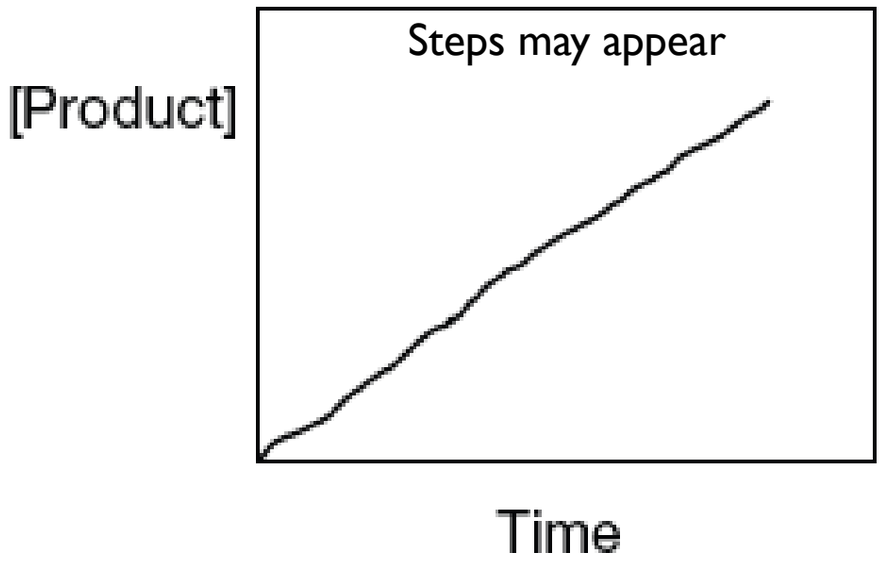
Ensemble versus single molecule behavior

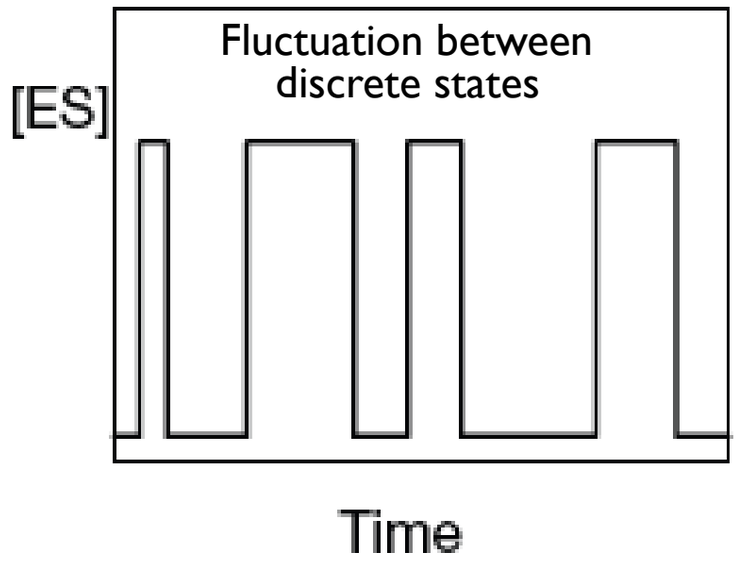
Ensemble:

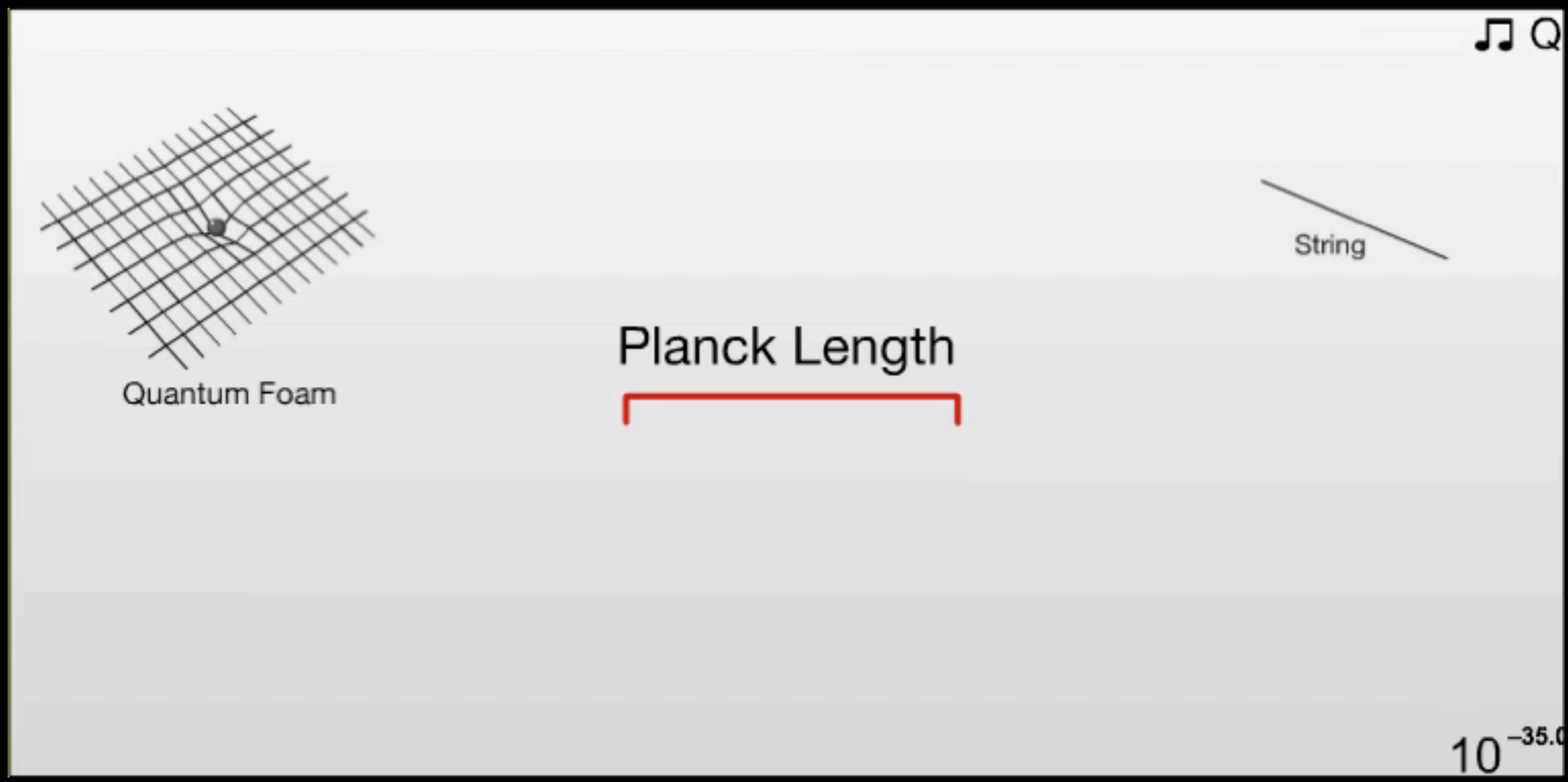




Single molecule:







Cary and Michael Huang (http://htwins.net)