Nanobiotechnology (CH-413)

Spring Semester 2025

Prof. Angela Steinauer

Course material:

- Lecture slides & videos
- Articles for the lecture
- Exercises & papers

} Moodle page

- Textbooks:

1. Nanobiotechnology: concepts, applications and perspectives (Niemeyer & Mirkin), available as PDF from EPFL Library
2. Nanobiotechnology Handbook (Xie)
3. The Handbook of Nanomedicine (Jain), available as PDF from EPFL library

- **Contact**:
  - Email: angela.steinauer@epfl.ch

Course information

EPFL Moodle page:

https://moodle.epfl.ch/course/view.php?id=18417

Slides:

Will be uploaded to the Moodle page shortly before the course

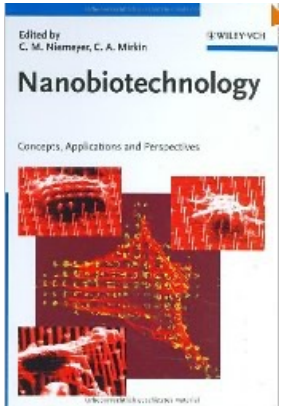
Questions:

1. Moodle Forum
2. Address to me (Angela Steinauer, angela.steinauer@epfl.ch) and the teaching assistants:

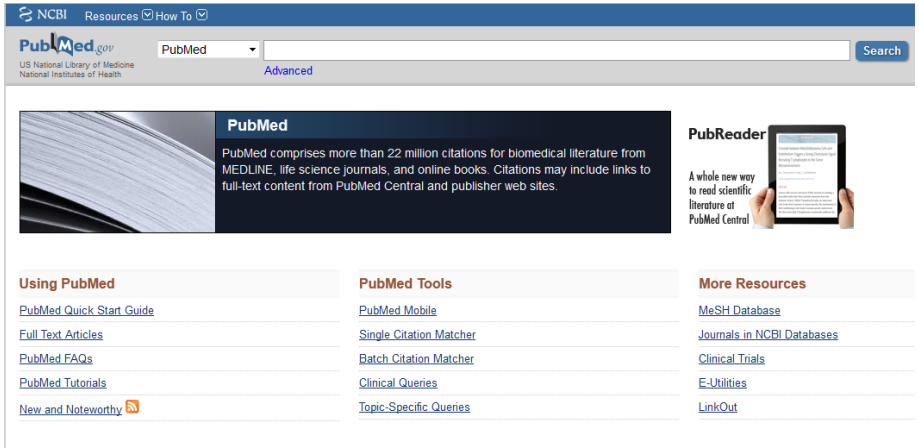
Georges Barnikol (georges.barnikol@epfl.ch)

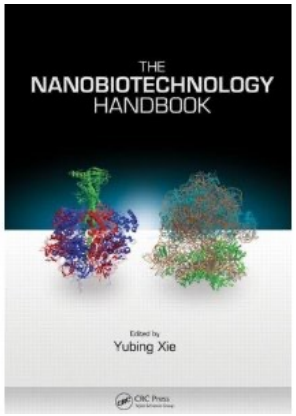
Oliver Dennis (oliver.dennis@epfl.ch)

Textbooks & materials



Nanobiotechnology
Niemeyer & Mirkin,
Wiley VCH





Nanobiotechnology
Handbook
Xie, CRC Press

Scientific Literature:
www.ncbi.nlm.nih.gov
www.webofscience.com

Goals of this course

1. **Develop a comprehensive understanding** of the fundamental principles underlying nano-biotechnological and biophysical methods explored in this course.

2. **Critically analyze and compare** the advantages and disadvantages of various biophysical and nano-biotechnological methods.

3. **Evaluate the limitations and applicability** of different techniques in terms of spatial and temporal resolution and suitability for addressing specific biophysical questions.

4. **Develop the ability to critically review and interpret** current scientific literature in nano-biotechnology and biophysics, identifying key findings and methodologies.

5. **Cultivate collaborative research skills** by engaging in team-based projects, fostering communication, coordination, and joint problem-solving abilities.

6. **Write an original research proposal**

Continuous assessment

Exercises: Read and understand the literature.

- Four times over the semester, we will upload a problem set on the Moodle for you to solve.
- Answers should be uploaded by the following Thursday (23:59).
- Each person is responsible to submit their own answers for an individual grade. **If you do work together in a study group, please declare who you worked with.**

Paper review debate.

- Form groups of 3 people.
- Choose a recent paper from our curated list: **group choice opens tomorrow, Friday, February 21, at 10:00 am.**
- For each paper, we will randomly assign one team to be favorable reviewers and one team to be critical reviewers.
- **More info in today's exercise session.**

Final project: research proposal

What?

- Develop an original research proposal on any **nanobiotechnology** topic, following the **SNSF Spark Grant** structure.

Why?

- Improve skills in **formulating research questions, designing experiments, and presenting ideas**.

A chance to propose **novel and unconventional** ideas in the field.

How?

- **1-page summary** + **3-page project plan** + references (no page limit).
- Must include **at least one original figure** explaining the idea.
- Follow good **scientific practice and proper citations**.

Scaffolded Submission Process:

- Outline/Idea Submission: March 20
- First Draft: April 17
- Final Proposal: May 30

https://www.snf.ch/en/8EWqXT6CZ7wuAJUq/funding/programmes/spark

No exam

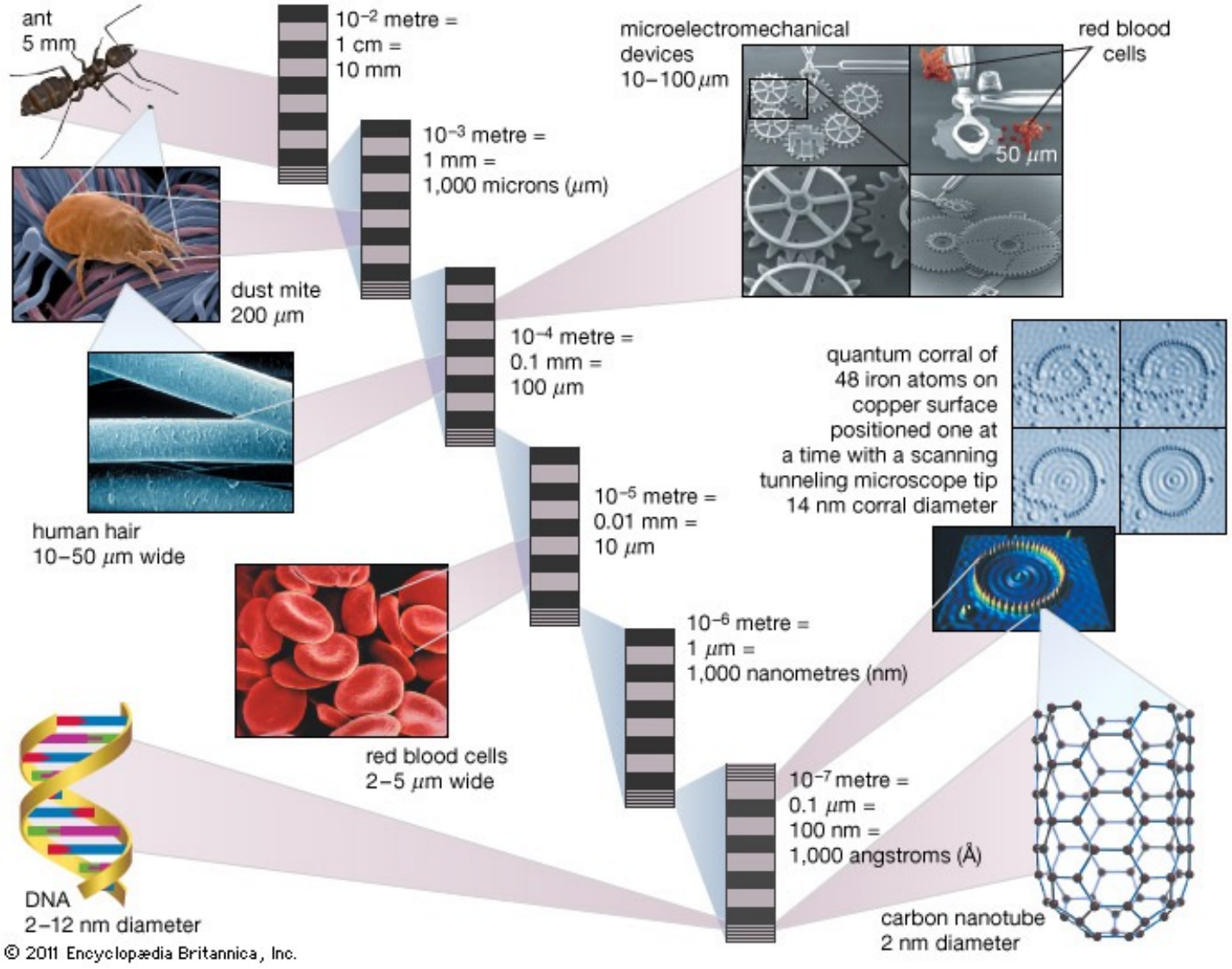
- Graded exercises (15%)
- Paper review debate (35%)
- Individual research proposal (50%)

Learning goals for week 1

- Be able to answer the question: **what is nanobiotechnology** and **what type of molecules/structures/devices are part of nanobiotechnology?**

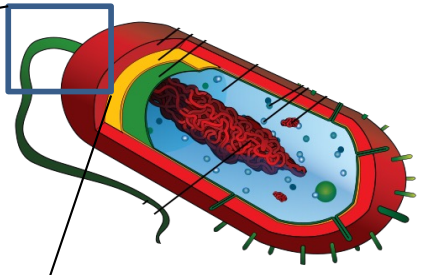
- Understand, be able to explain and apply the concepts of **single molecule vs. ensemble approaches**, **fluorescence** and **FRET**.

Length Scales in Biology and Technology

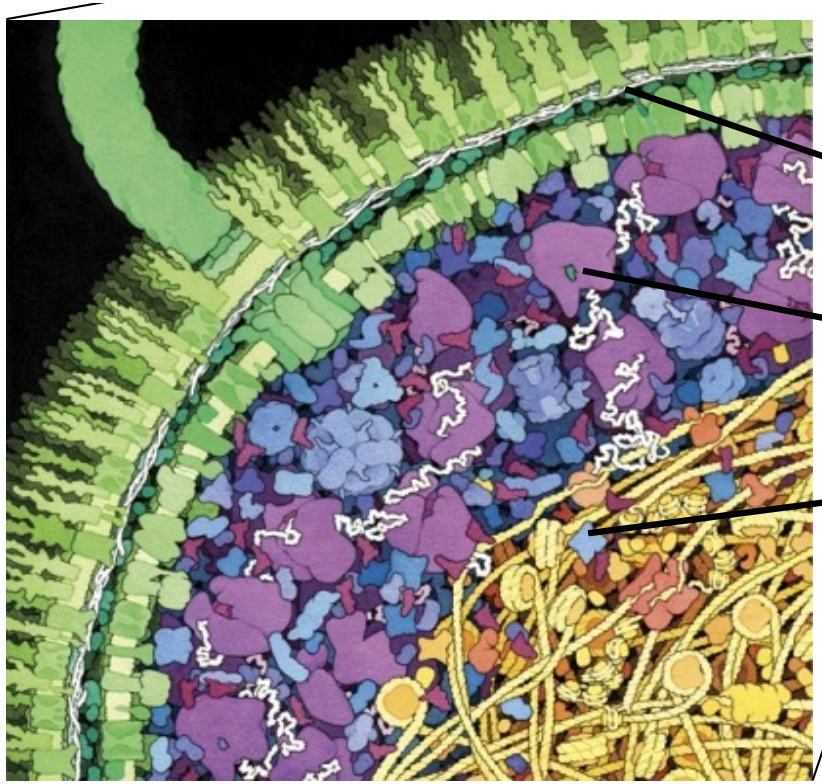


The nanoscale dimension of nanomaterials is **crucial for their function**!

Motivation: Nature functions on the Nanoscale



Procaryotic cell



Membrane proteins

Ribosomes & mRNA

DNA, genetic material

Source: http://www.bio.aps.anl.gov/scihi/BIO080111.pdf

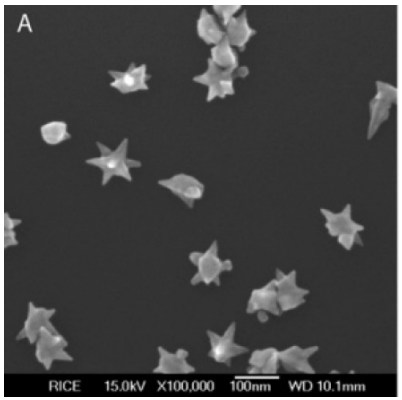
20 nm

To understand nature we require tools to investigate & manipulate the molecules of life in their native environment

What is Nanobiotechnology?

Nanotechnology:

Man-made objects or features on the 10-100 nm scale, e.g. metallic or semiconducting nanoparticles, silica nanodevices



Nano Lett. 6:683-688

Biotechnology:

DNA and protein design, high resolution structural and functional analysis

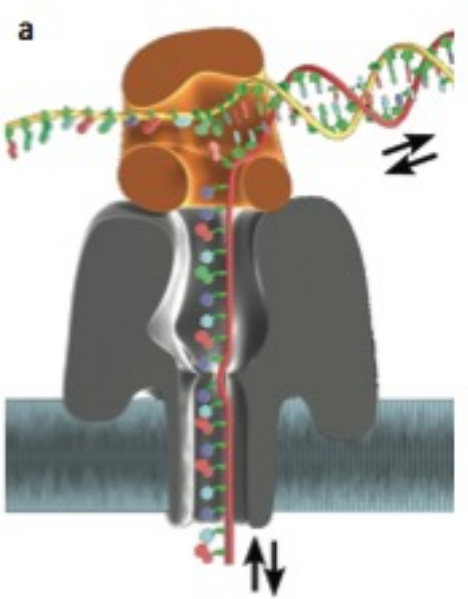
Definition of Terms

Nanobiotechnology:
Interdisciplinary field at the intersection of **nanotechnology** and **biotechnology**

Use of nanotechnology devices for biological and biochemical applications

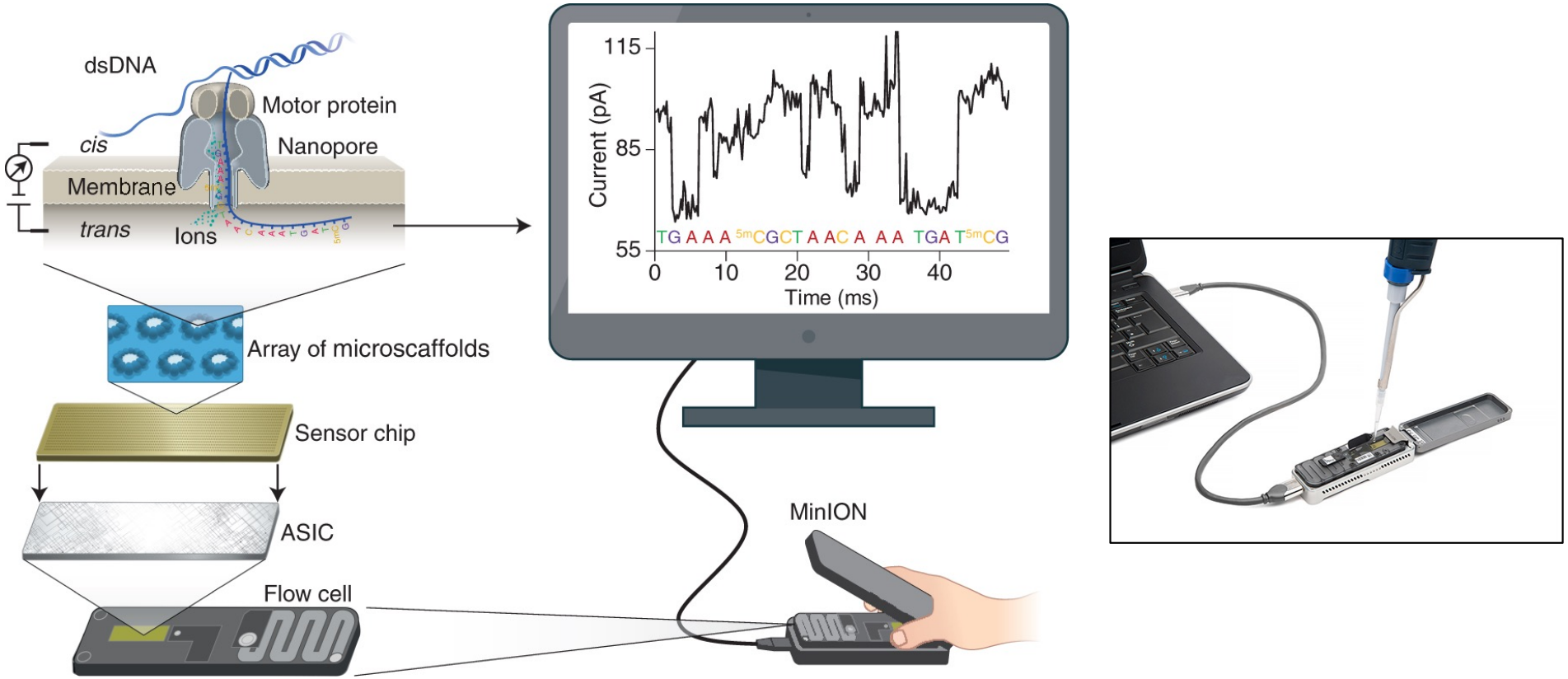
Nano-technology

Biological Systems



Manrao, E. A., Derrington, I. M., et al. (2012). *Nature Biotechnology, 30(4), 349–353.*

Nanopore Sequencing



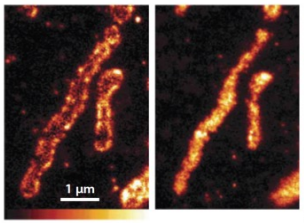
Wang, Y., Zhao, Y., et al. (2021). *Nature Biotechnology, 39, 1348–1365*.

Unique effects at the nanoscale

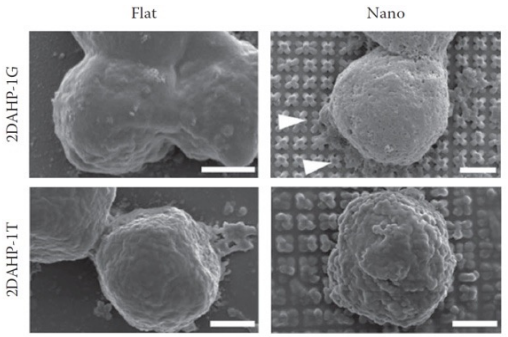
1. **Quantum Effects**: quantum dots with size-dependent properties due to quantum confinement
2. **Surface Area to Volume Ratio**: beneficial for catalysis, drug delivery to facilitate better interaction with biological systems
3. **Chemical Reactivity**: effective catalysts (see point 2)
4. **Mechanical Properties**: e.g. carbon nanotubes, exceptional strength and stiffness for their weight
5. **Optical Properties**: e.g. gold nanoparticles exhibit different colors depending on their size due to localized SPR effects
6. **Thermal Properties**: nanomaterials can be distinct compared to bulk counterparts, applications in thermal management in electronics
7. **Biological Interactions**: biology takes place at the nanoscale, nanoparticles can penetrate cell membranes
8. **Tailorability**: we can engineer nanomaterials!

Nanobiotechnology

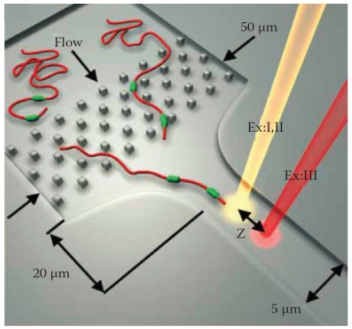
Watch molecular biology happen and **manipulate** the processes



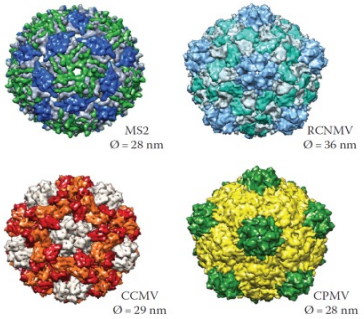
Grow cells and complex tissues *in vitro*



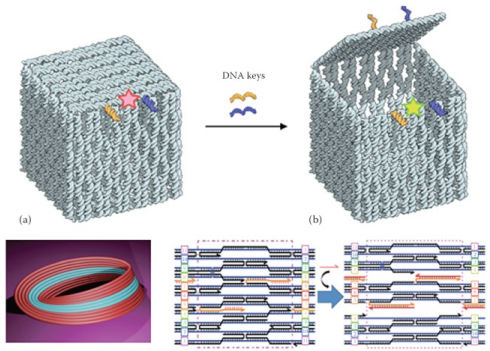
Diagnose diseases from single molecules or cells cells



Target drugs to individual cancer cells



Craft new biomaterials

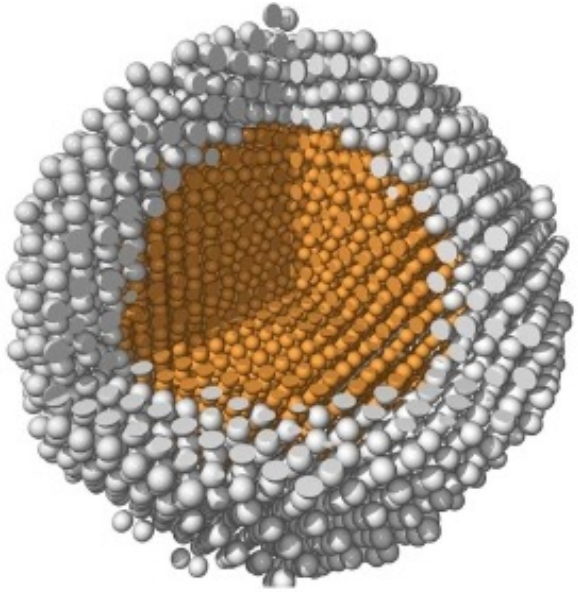


Nanomaterials

- Nanoparticles
- Carbon allotropes
- Biomolecules

Nanoparticles – New Chemical and Physical Properties

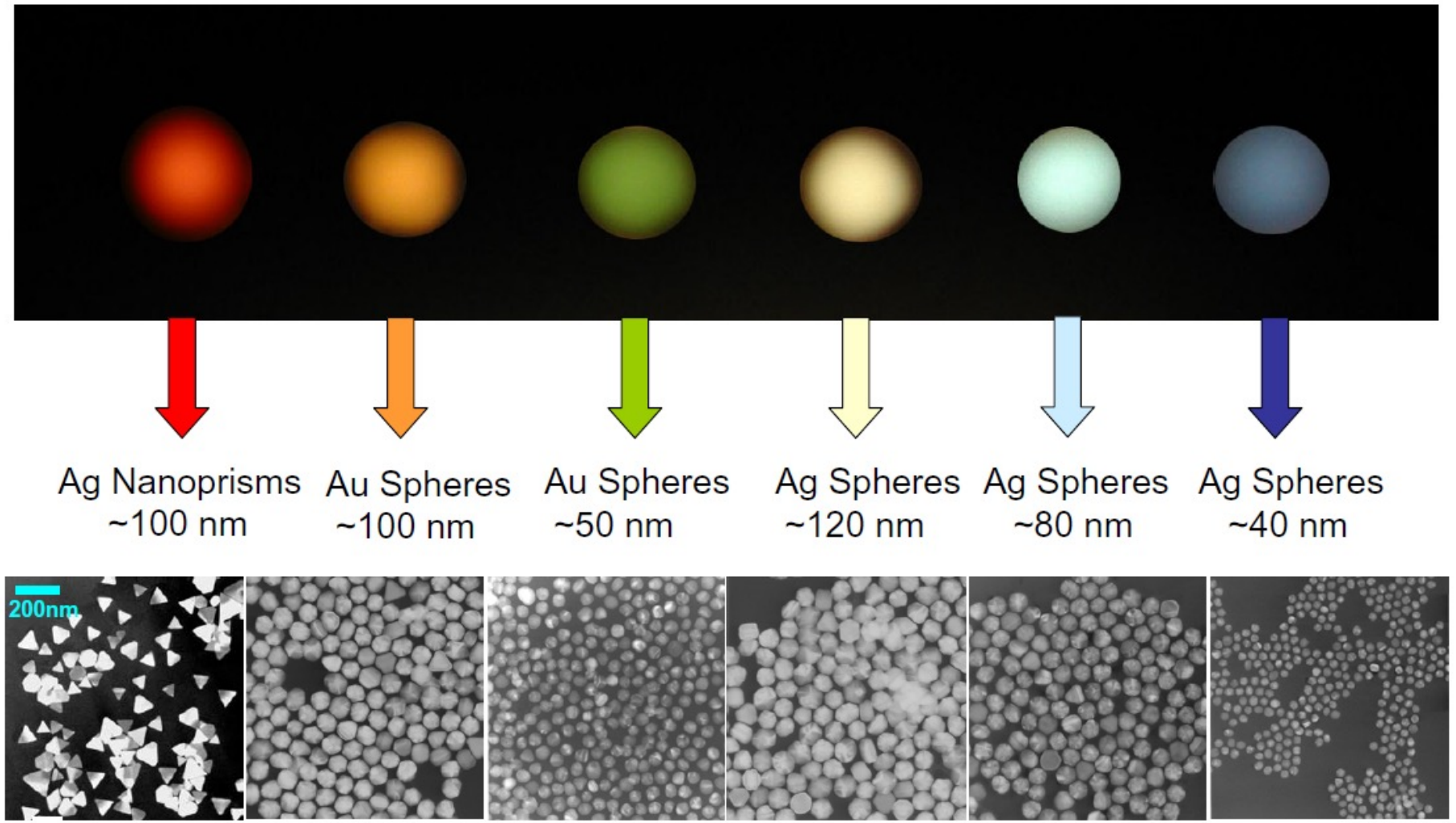
30 Å Au-Ag nanoparticle



J Daniel Gezelter,
University of Notre Dame

- Clusters of 100-1000s of atoms
- Properties are determined by size and surface
- Chemical tuning through surface chemistry

Size Dependent Properties



4th century AD: Nanoparticles!

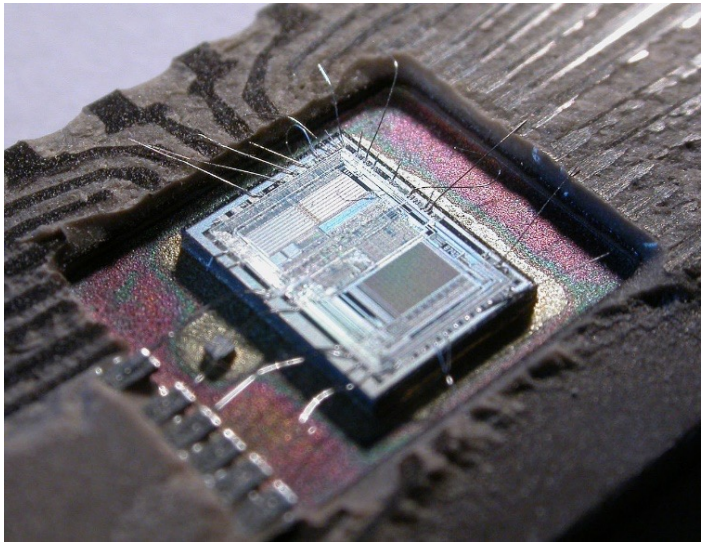
Lycurgus cup

330 ppm silver, 40 ppm gold added to molten glass

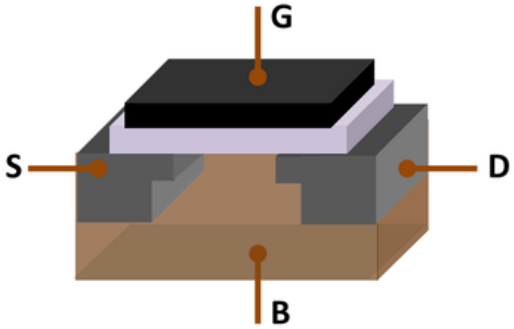
70nm Ag/Au nanoparticles embedded in the glass yield color effect: green in reflected light, deep red light when light is transmitted through it due to surface plasmon resonance effect

Use of metal colloids widespread

Integrated Circuits



images: *wikipedia.org*

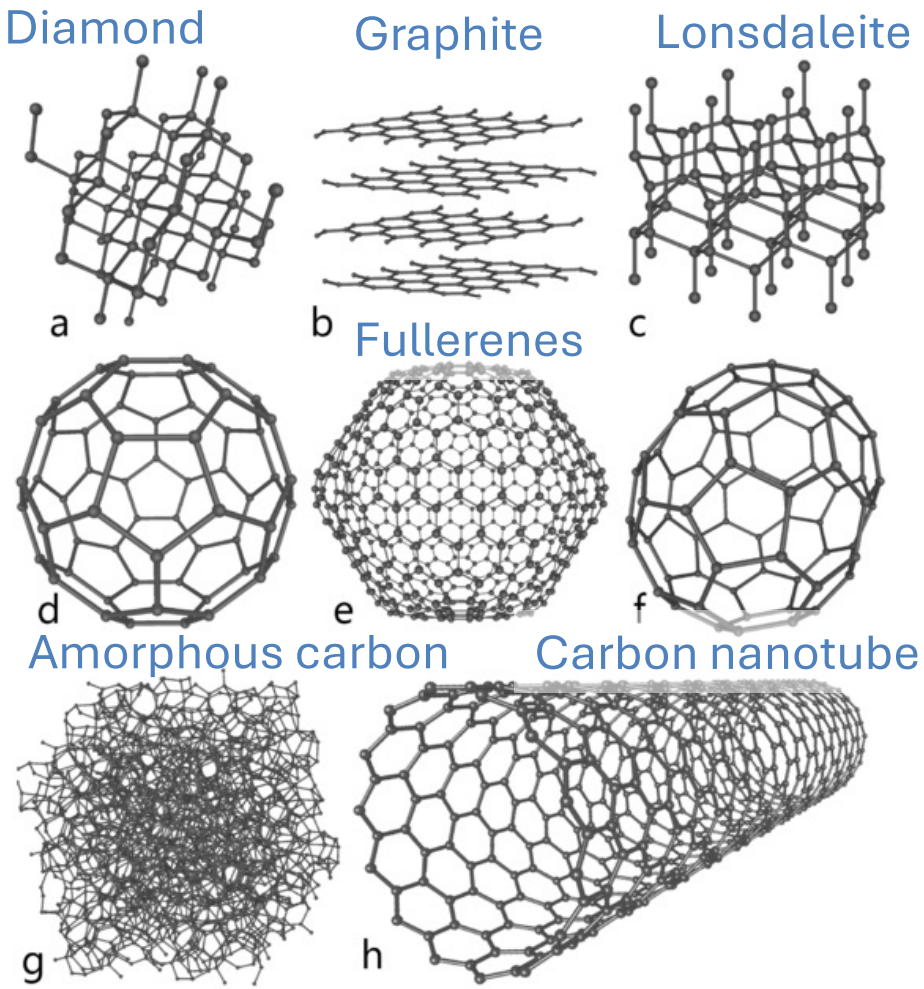


transistor

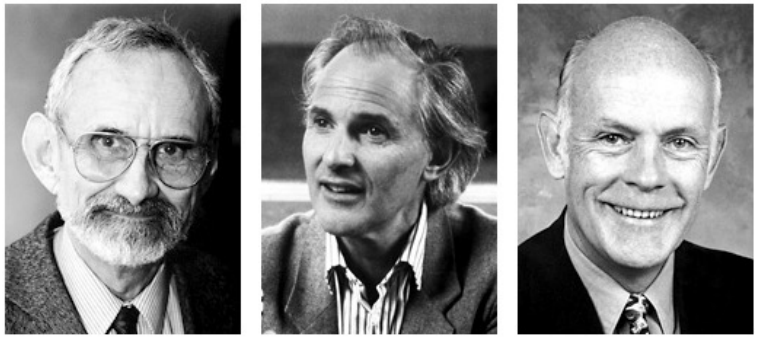
Production
photolithography, deposition, etching
Silicon based
low cost

10 microns transistor size (1970)
10 nm transistor size (2017)

Carbon Allotropes



Allotropy: Property of chemical elements to exist in different forms / structures.



Robert F. Curl Jr. Sir Harold W. Kroto Richard E. Smalley

1996 Nobel Prize for Chemistry for Kroto, Curl and Smalley for their discovery of new carbon allotropes in 1985

A. Geim K. Novoselov

2010 Nobel Prize for Physics for Geim and Novoselov for for groundbreaking experiments regarding the two-dimensional material graphene

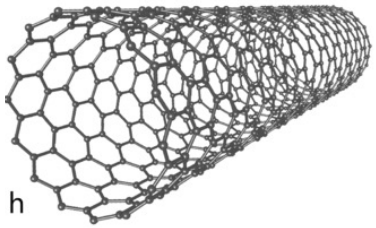
Carbon Nanotubes

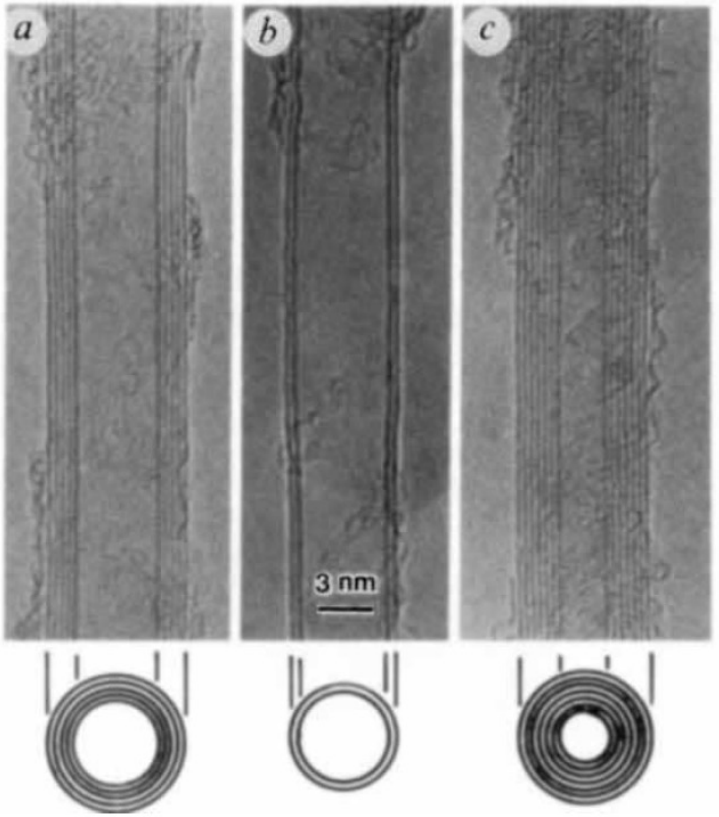
Helical microtubules of graphitic carbon

“rolled up graphene sheets”

2 basic types of nanotubes:

1. Single-wall SWNT
2. Multi-wall MWNT





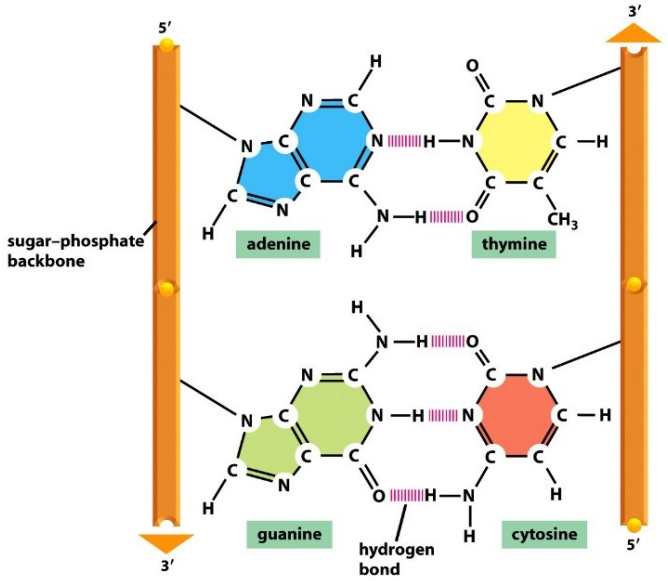
Iijima, S. Nature 1991

Uses:

- Electronics: conducting properties
- Structure: high tensile strength
- Nano-devices: surface features
- Functionalized as drug carriers

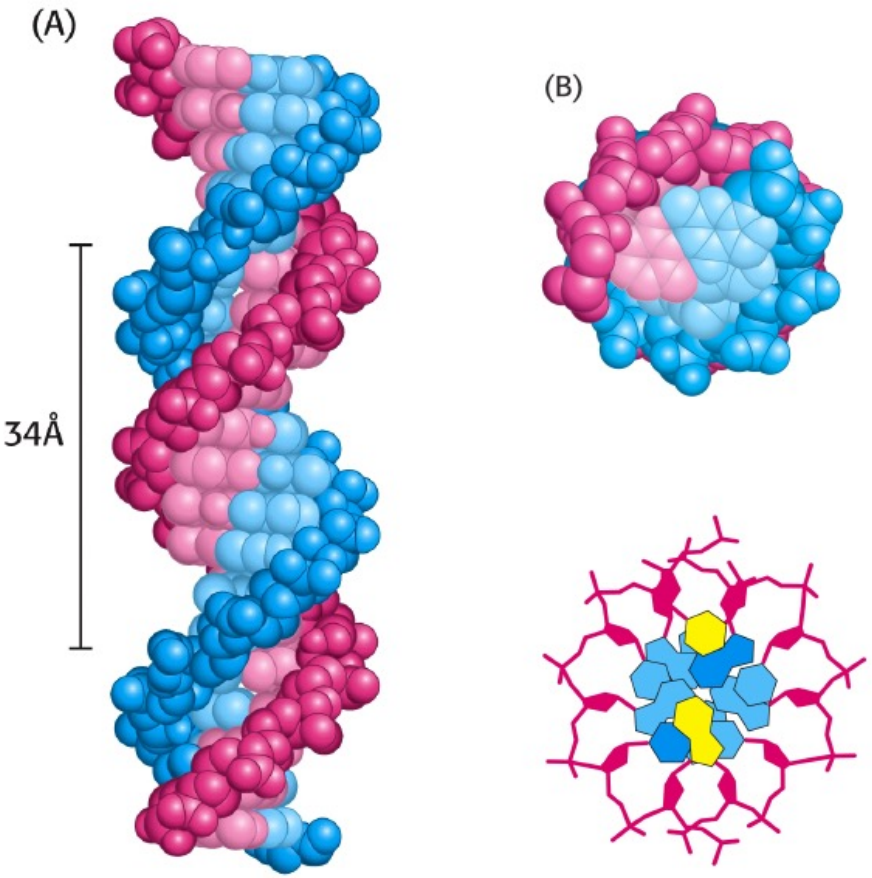
DNA: Information and Structure

DNA base pairing



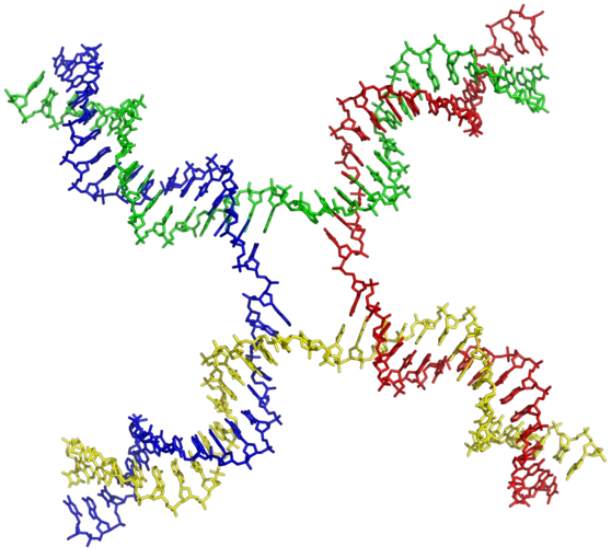
Alberts et al., Molecular Biology of the Cell

DNA double helix



Stryer, Biochemistry

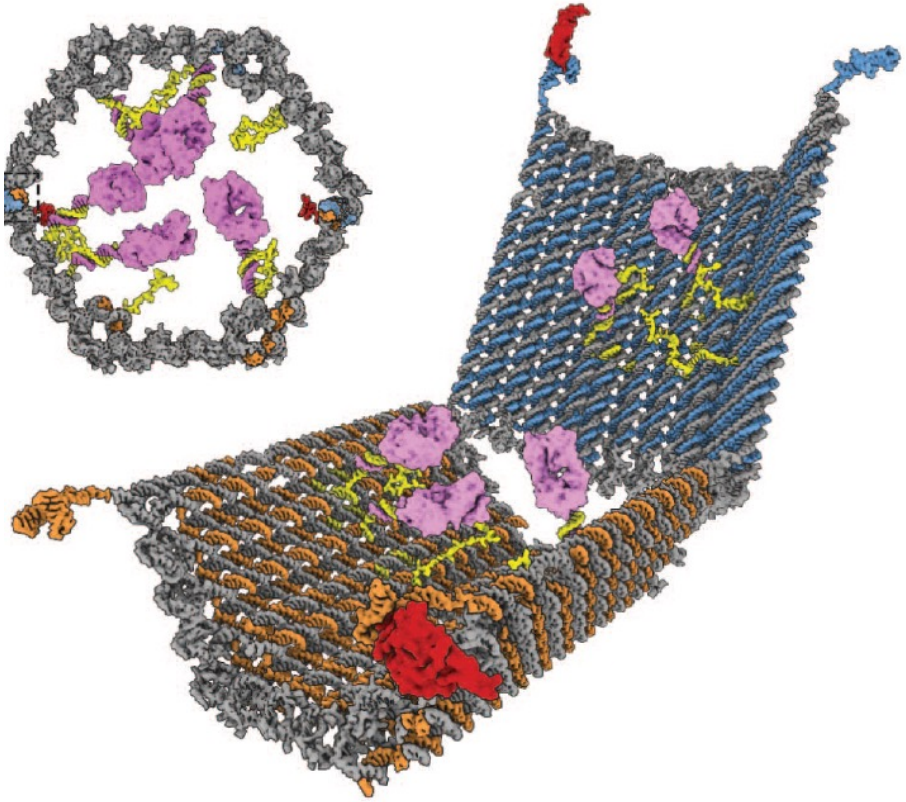
DNA Self Assembly



PDB: 3CRX

Sequence-dependent strand annealing allows construction of complex objects

Logic-gated drug delivery nanorobot



Douglas et al. Science 2012

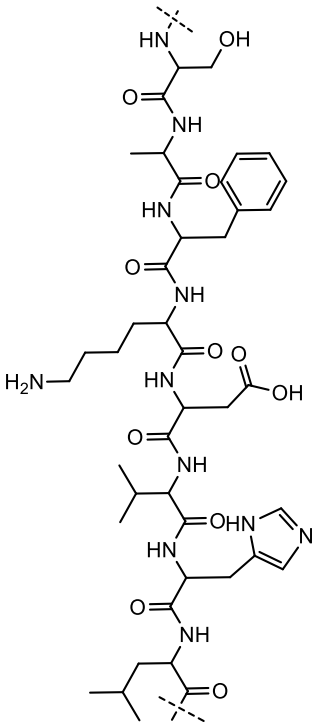
Proteins: Self-Assembling Nanoparticles with a Defined Function

Proteins:

1. Adopt a defined **3-dimensional structure**
2. Interact with each other to self assemble into defined **complexes**
3. Fulfill a defined **biological function**

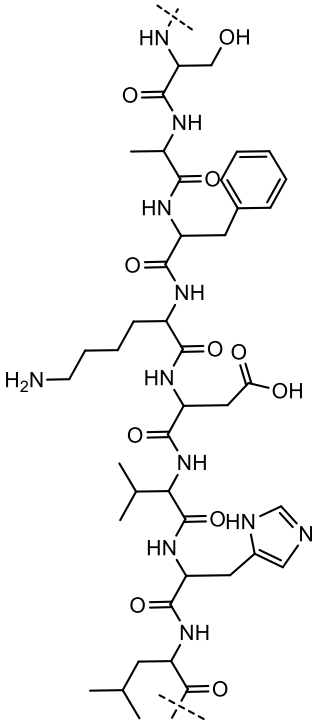
Chemical reactions:	Enzymes
Cell structure:	Architectural proteins
Movement of cargo:	Motor proteins
Information transfer:	Signaling proteins

Proteins – Structural hierarchy



Amino acid chain
primary structure

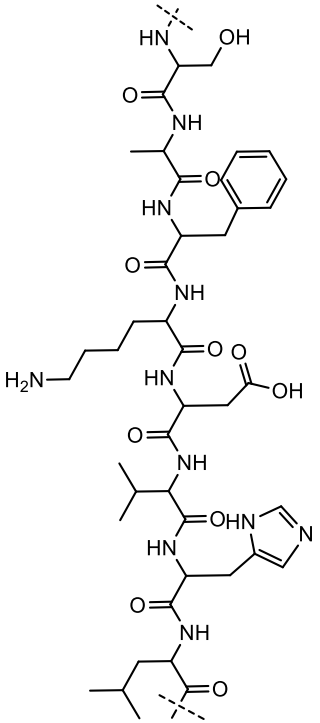
Proteins – Structural hierarchy



Amino acid chain
primary structure

α-helix
secondary
structure

Proteins – Structural hierarchy



Amino acid chain
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α-helix
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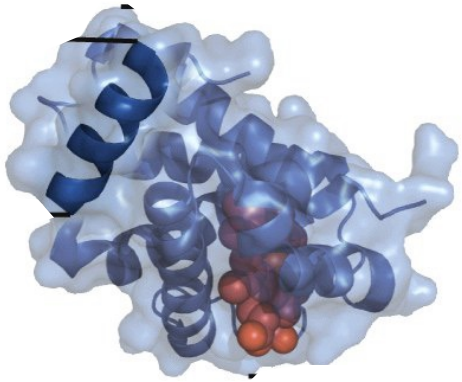
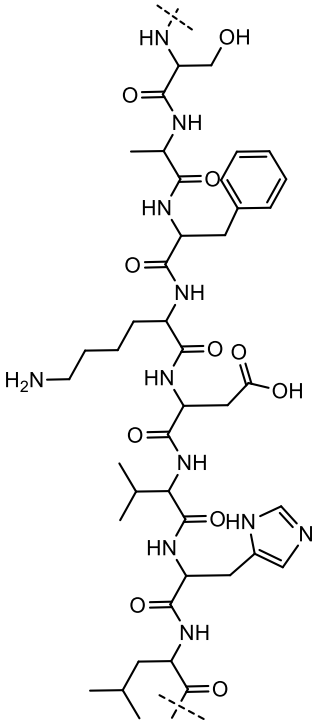
structure



Protein domain
Tertiary structure

Proteins – Structural hierarchy



Amino acid chain
primary structure

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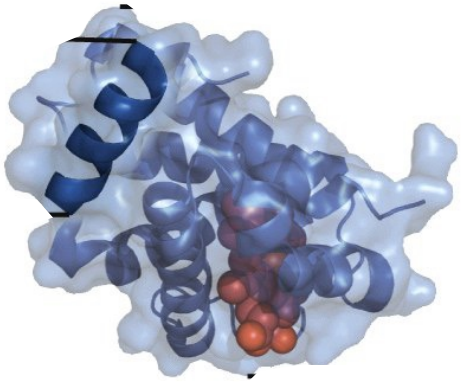
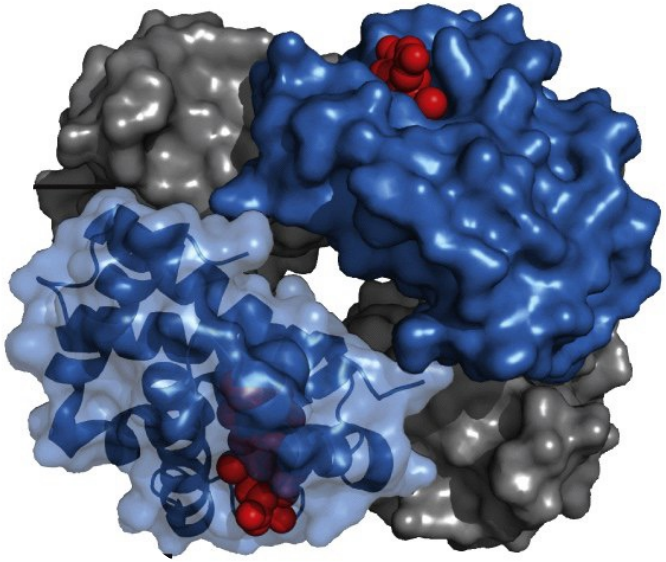


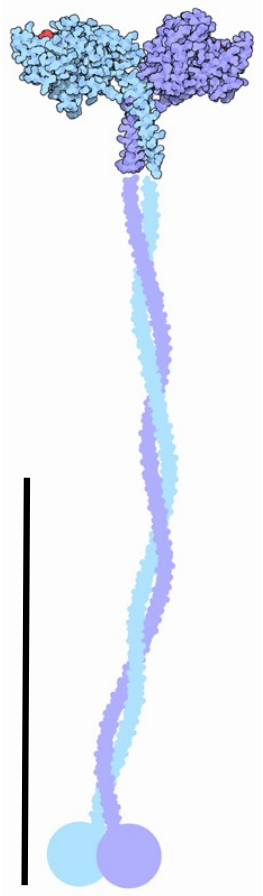
Protein domain
Tertiary structure



Protein complex
Quarternary structrue

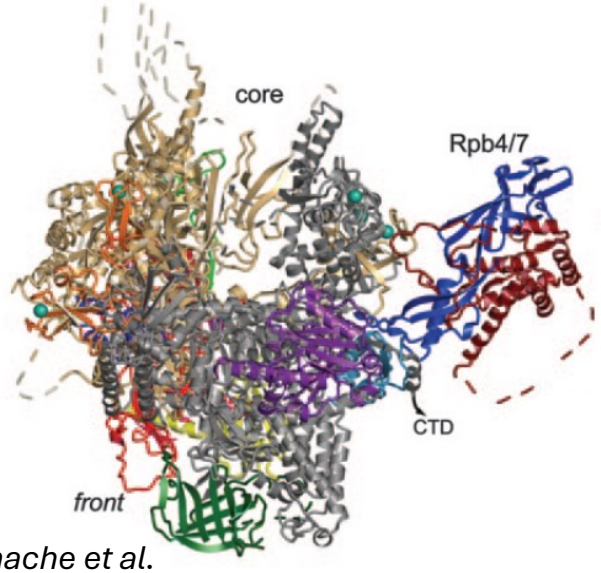
Protein Nanomachines

Motors:
Kinesin



pdb.org

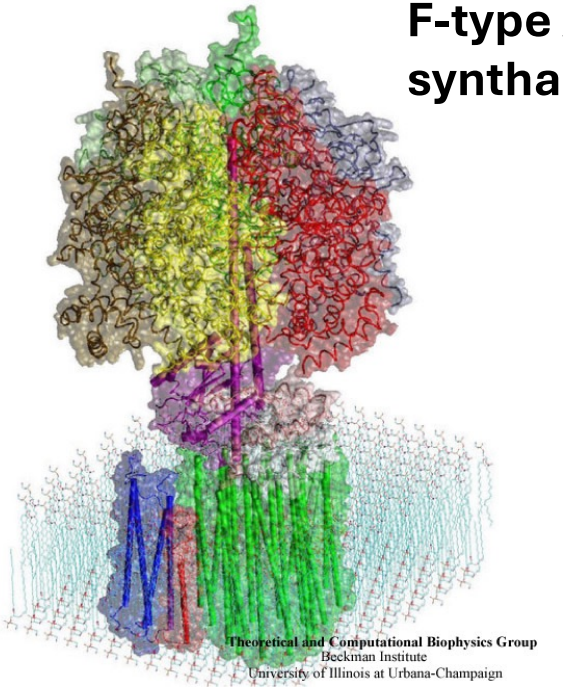
Complex processive Enzymes:
RNA polymerase



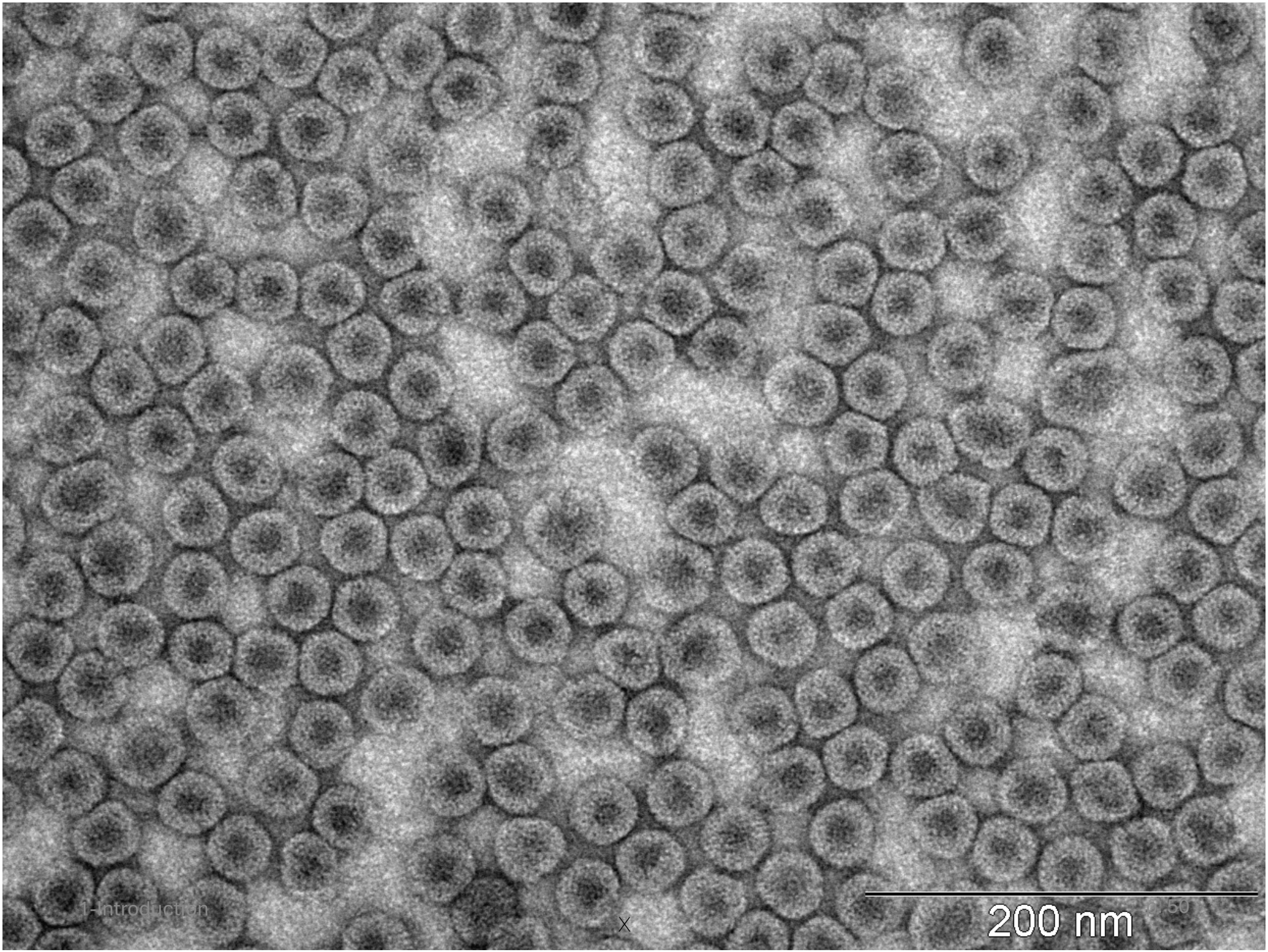
Armache et al.
JBC 2005

10 nm

F-type ATP synthase

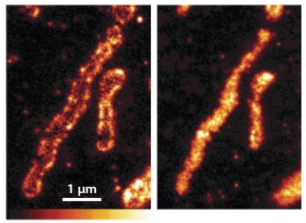


10 nm

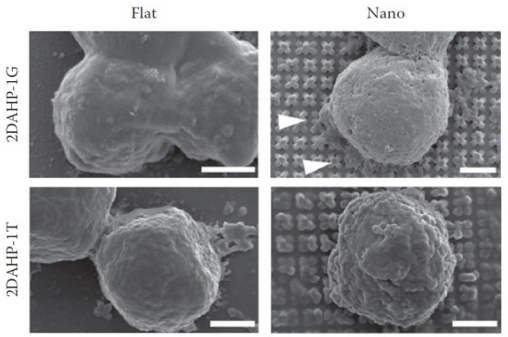

200 nm

Nanobiotechnology

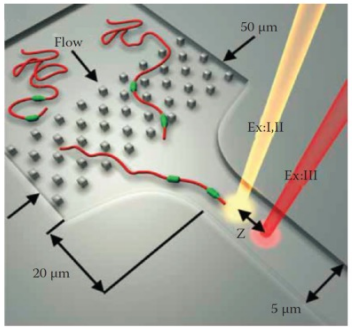
Watch molecular biology happen and **manipulate** the processes



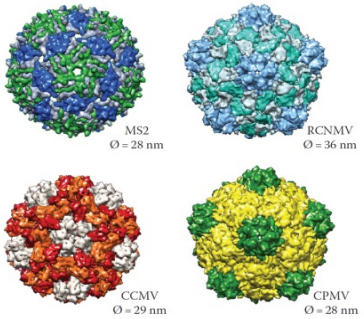
Grow cells and complex tissues *in vitro*



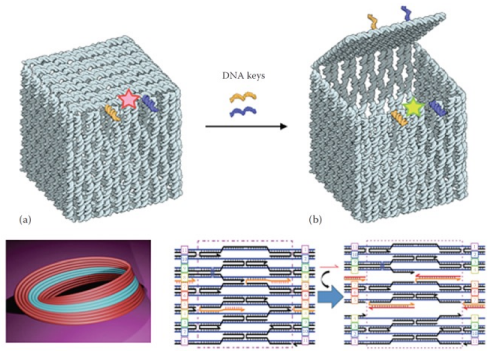
Diagnose diseases from single molecules or cells cells



Target drugs to individual cancer cells



Craft new biomaterials



The Ensemble vs. the Single Molecule

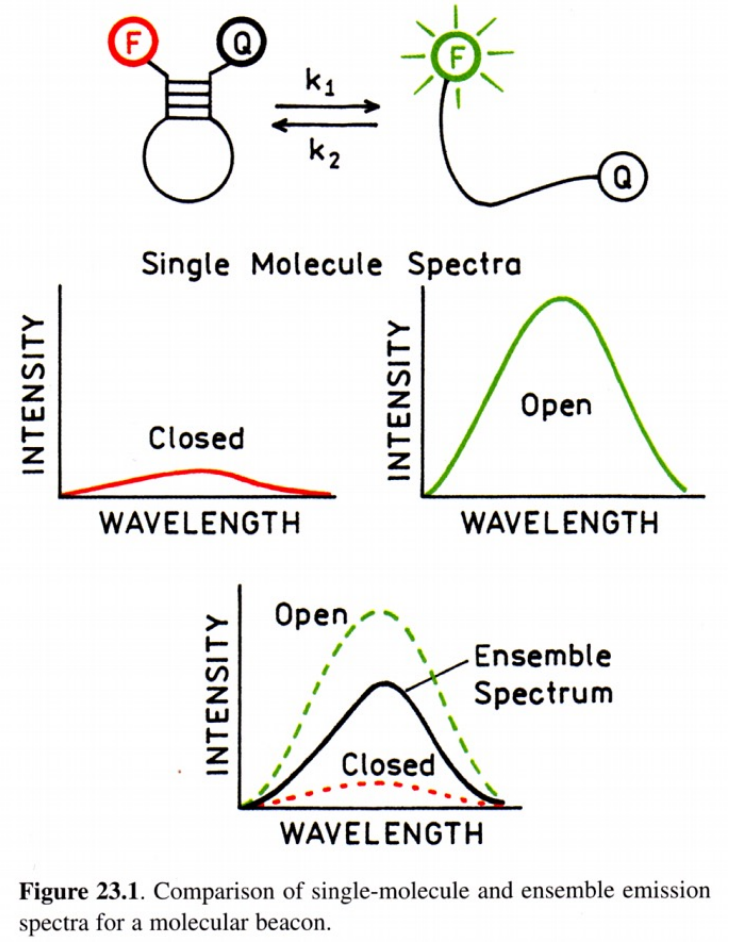


Figure 23.1. Comparison of single-molecule and ensemble emission spectra for a molecular beacon.

Conformational heterogeneity in a sample population:

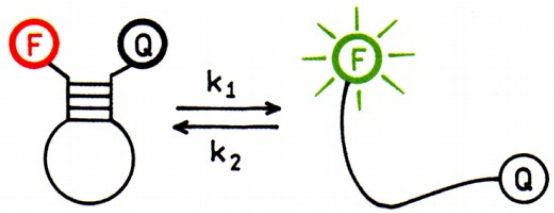
Ensemble methods detect the average fluorescence emission.

E.g. In a system with a fluorescent dye and a quencher, only 50% emission is detected compared to the free dye.

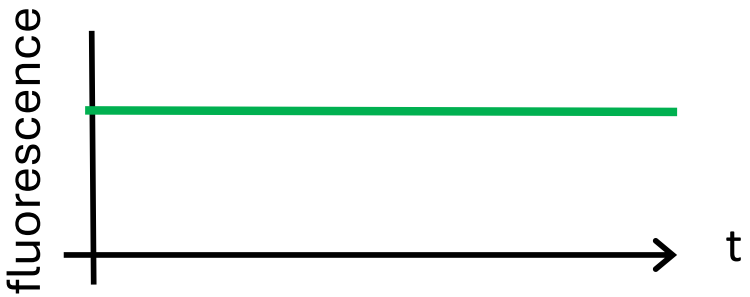
Possible reasons for half maximal emission:

1. Two static populations
2. One population with all hairpins half-closed
3. More than two populations with a variety of intermediate states

Dynamics in the Ensemble



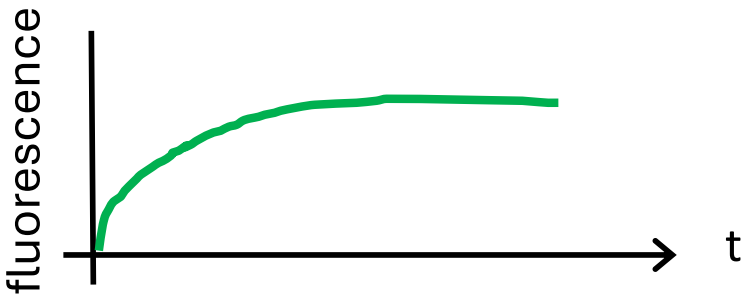
Ensemble:



Equilibrium fluctuations invisible

Ensemble and time average of fluorescence observed

Ensemble, relaxation:

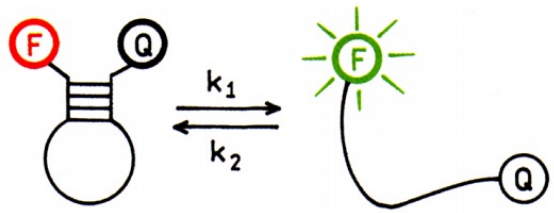


Equilibrium fluctuations are invisible

Rate of system re-equilibrium can be observed following synchronisation of the ensemble.

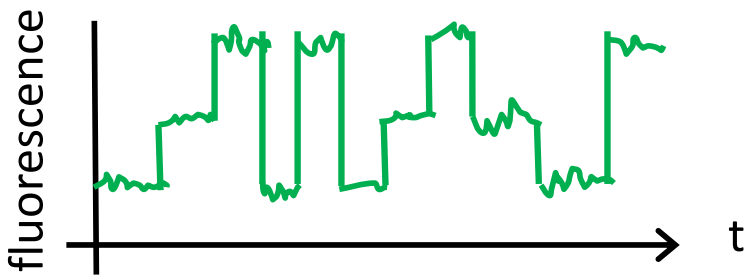
Modeling → microscopic rate constants

Dynamics in Single Molecule Measurements



Single molecule, 2-states:

Single molecule, 3-states:



Statistics:

Equilibrium constant

Rates of equilibrium fluctuations

Numbers of states populated in equilibrium

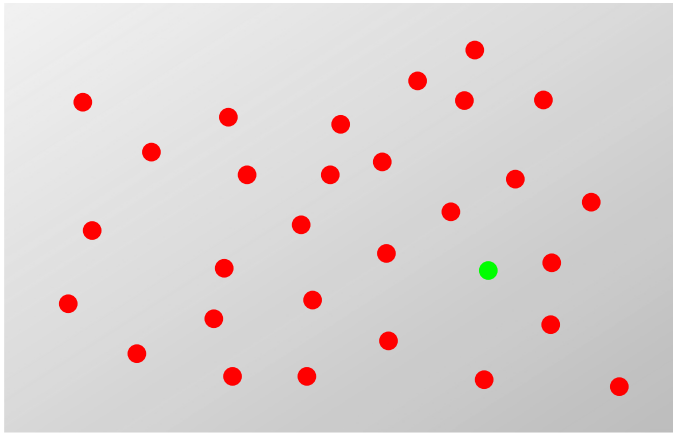
Invisible equilibrium intermediate revealed

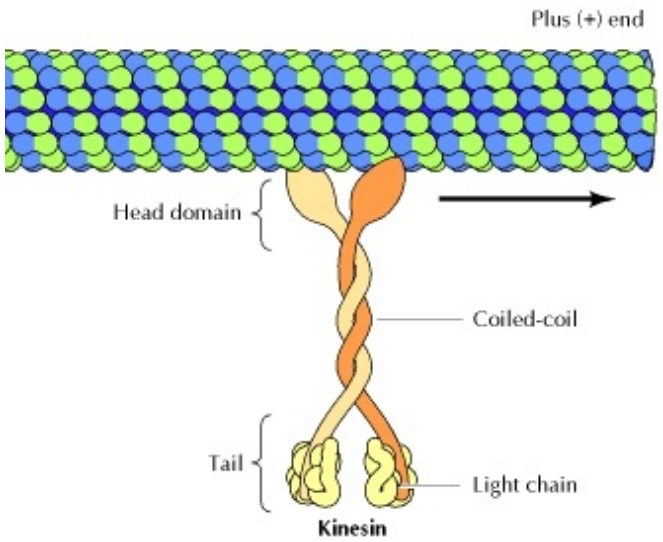
Fewer assumptions about the mechanism required as more parameters observed

Observations Possible with Single Molecule Experiments

No ensemble averaging!
this allows detection of:

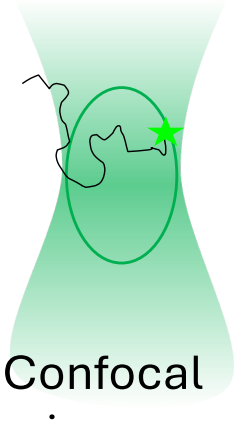
- Heterogeneity in a molecule population
- Rare events → requires large statistics
- Equilibrium excursions from the ground state
- Kinetic intermediates of multistep reactions
- Directional motor action of protein machines





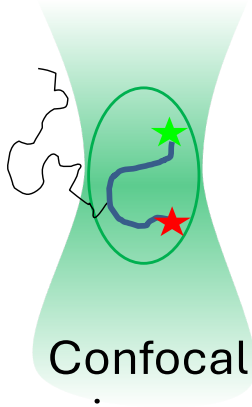
Different Single Molecule Approaches

Fluorescence

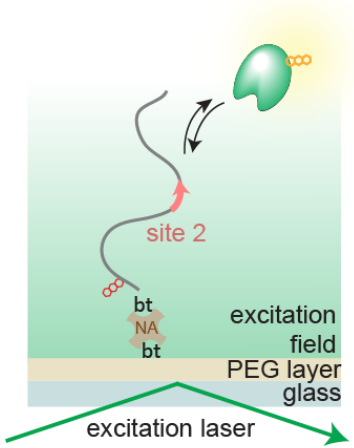


Confocal microscopy

FRET

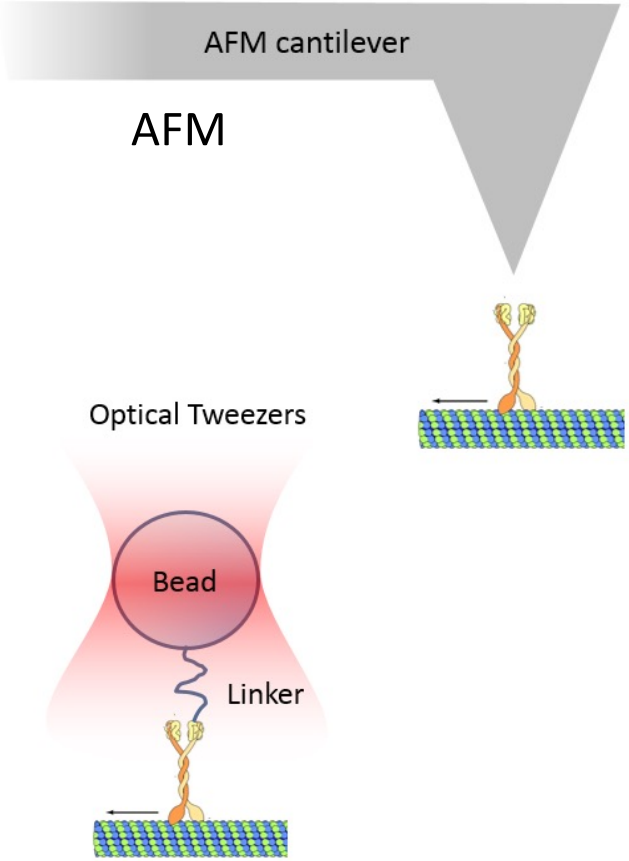


Confocal microscopy

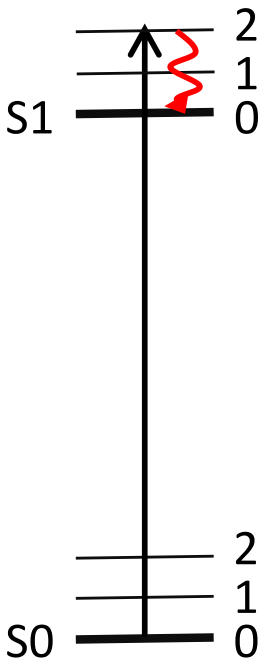


Total internal reflection microscopy (TIRF)

Force spectroscopy



Review: Fluorescence



Interaction of light with molecules: Absorption

Excitation of a molecule

An electron in a bonding (or non-bonding) orbital is elevated to an antibonding orbital (higher in energy).

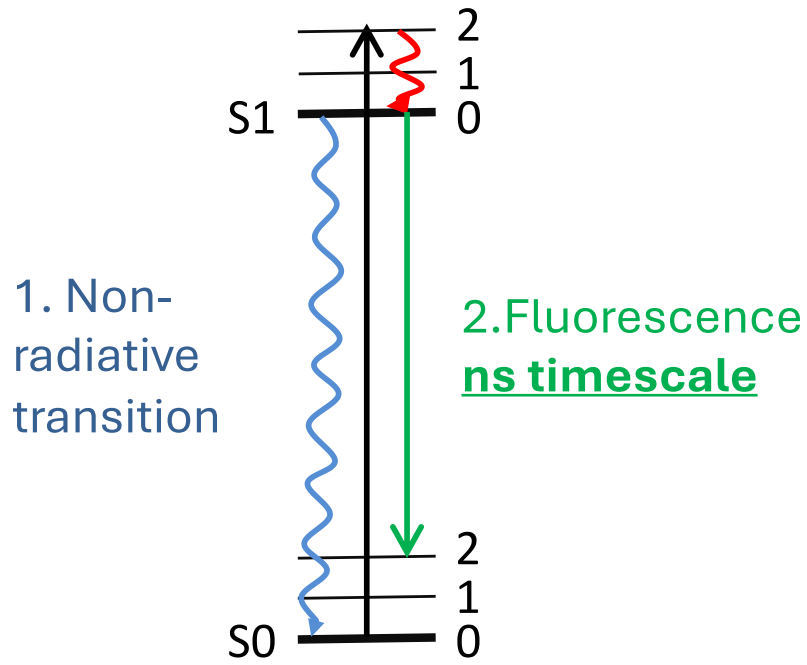
e.g. $\pi \rightarrow \pi^*$ (HOMO → LUMO) in an aromatic system

Wavelength of light a molecule can absorb is elated to the energy gap between HOMO and LUMO:

$\Delta E = h\nu = h \cdot c/\lambda$ Planck's equation

Review: Fluorescence

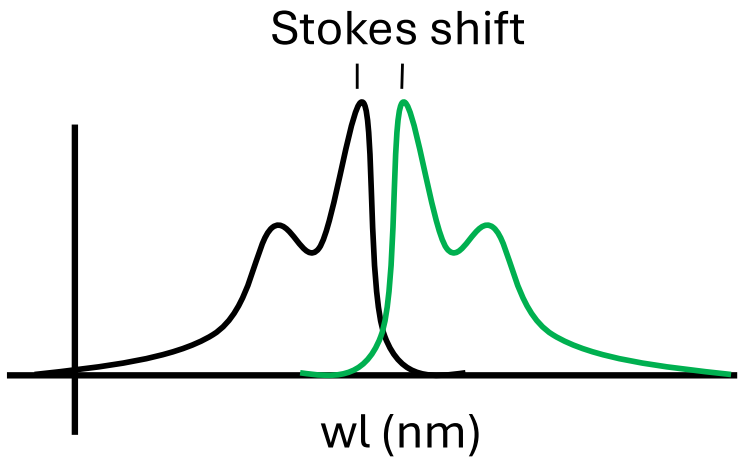
Paths from the excited state



Fluorescence is the emission of a photon from the lowest singlet vibrational state to the ground state

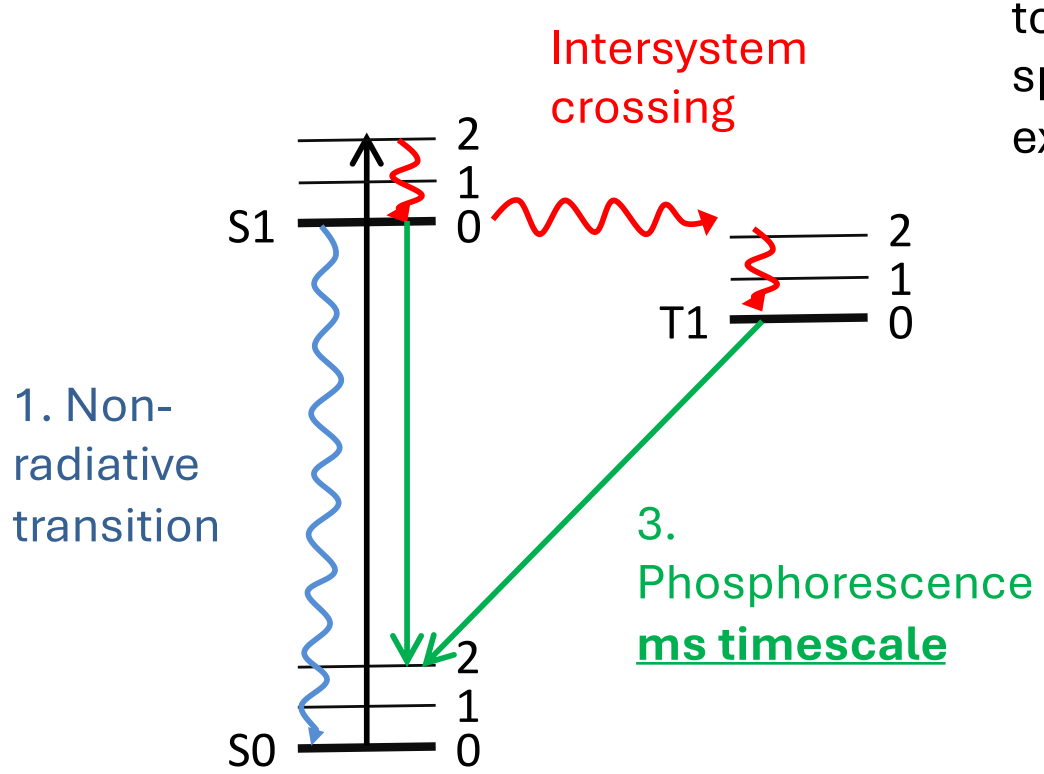
Fluorescence spectra are **mirror** images of the absorption spectra

The energy difference between vibrational states creates a spectral shift, the Stokes shift



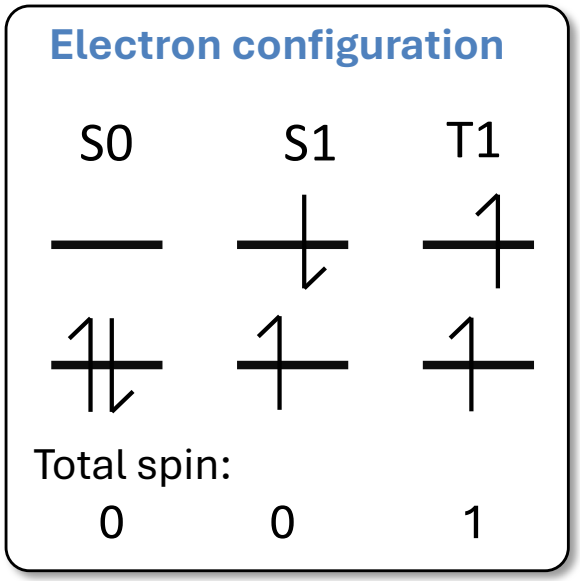
Review: Phosphorescence

Paths from the excited state



An excited singlet state can undergo a spin-flip to a triplet state with a non-zero total spin.

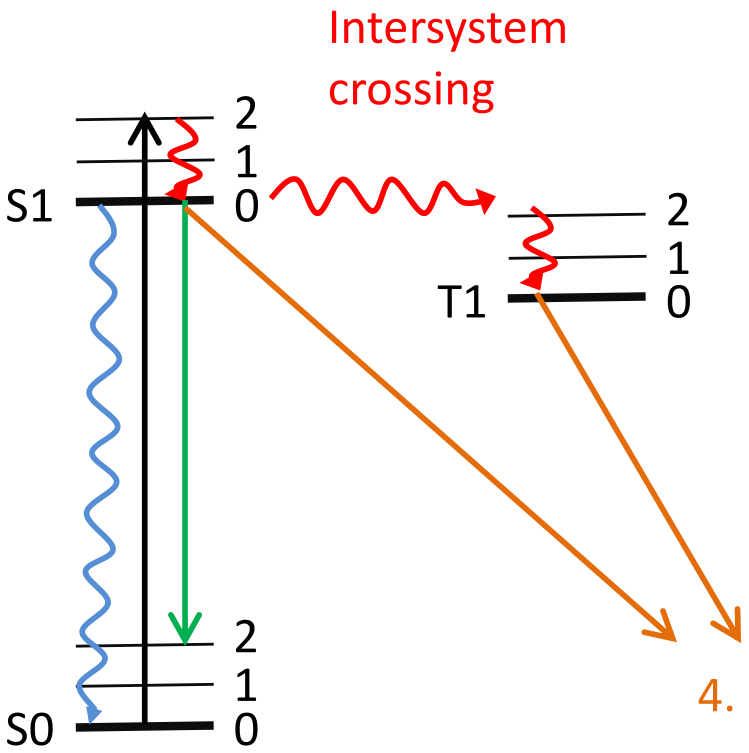
Triplet states are long lived, as relaxation to the ground state involves a forbidden spin flip back to a singlet state (see Pauli exclusion principle).



Triplet states are common reason for temporary loss of fluorescence (**blinking of fluorophores**)

Review: Bleaching

Paths from the excited state



1. Non-radiative transition

4. Photochemistry -> Bleaching

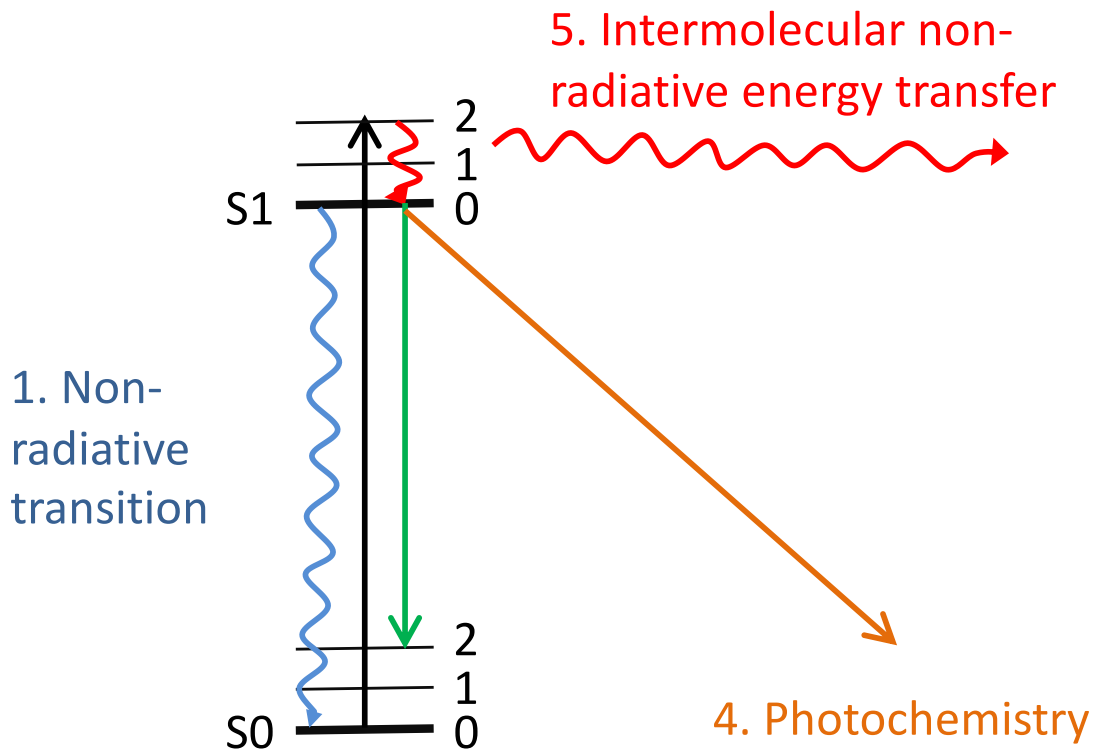
Bleaching of the fluorophore through a photochemical reaction:
Molecular structure of the fluorescent dye or molecule is altered or destroyed, rendering it permanently non-fluorescent

e.g. reaction with molecular oxygen

→ Single molecule experiments often require oxygen free conditions

Review: Förster Resonance Energy Transfer (FRET)

Paths from the excited state



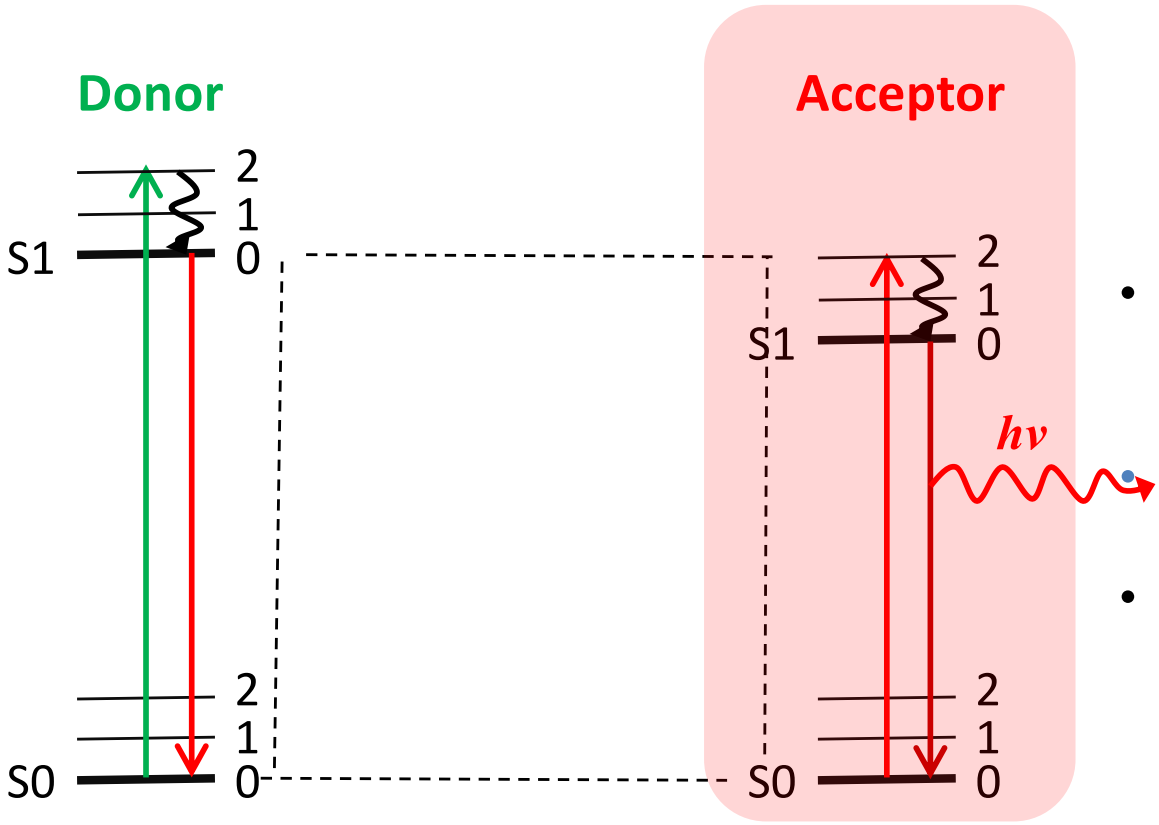
FRET: a mechanism of energy transfer between **two light-sensitive molecules**

Theodor Förster
1910 - 1974

Described and theory developed by Theodor Förster, 1946 at MPI Göttingen for ET reactions in photosynthesis

FRET Mechanism

When the donor molecule absorbs light, it goes to an excited state, creating a dipole moment.



- **Dipole-dipole interaction through space.**
- this results in **energy transfer: overlap allows excited state energy of donor to resonate with acceptor**
- fluorescence emission is detected from the **acceptor**

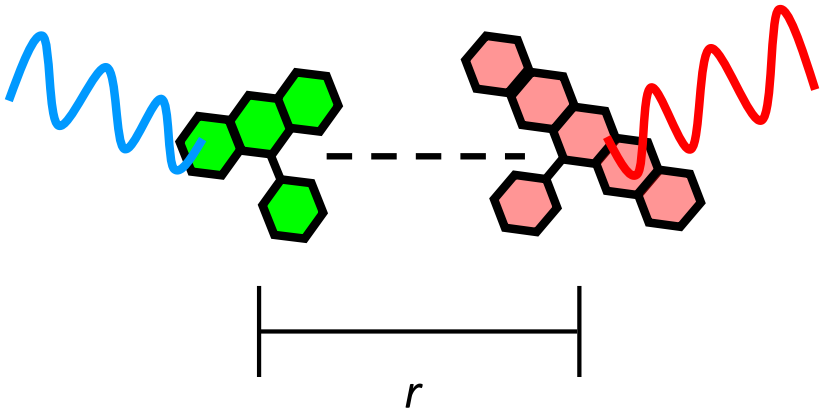
Mechanism:

- No orbital overlap, no electrons exchanged, no photons emitted or absorbed

Efficiency of Energy Transfer

Rate of energy transfer:

$$k_{FRET} = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6$$

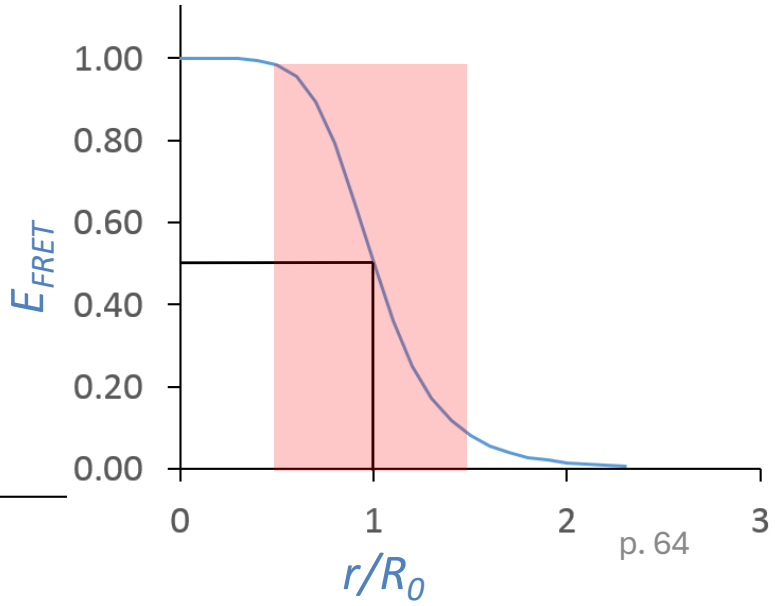


τ_D: fluorescence lifetime of the donor
Exponent of r^{-6} arises from the square of the dipole-dipole coupling (scales with exponent of -3)
R_o: Förster radius, distance at which the energy transfer efficiency is 50%
r: actual distance between fluorophores

Transfer efficiency: $E_{FRET} = \frac{R_0^6}{R_0^6 + r^6}$

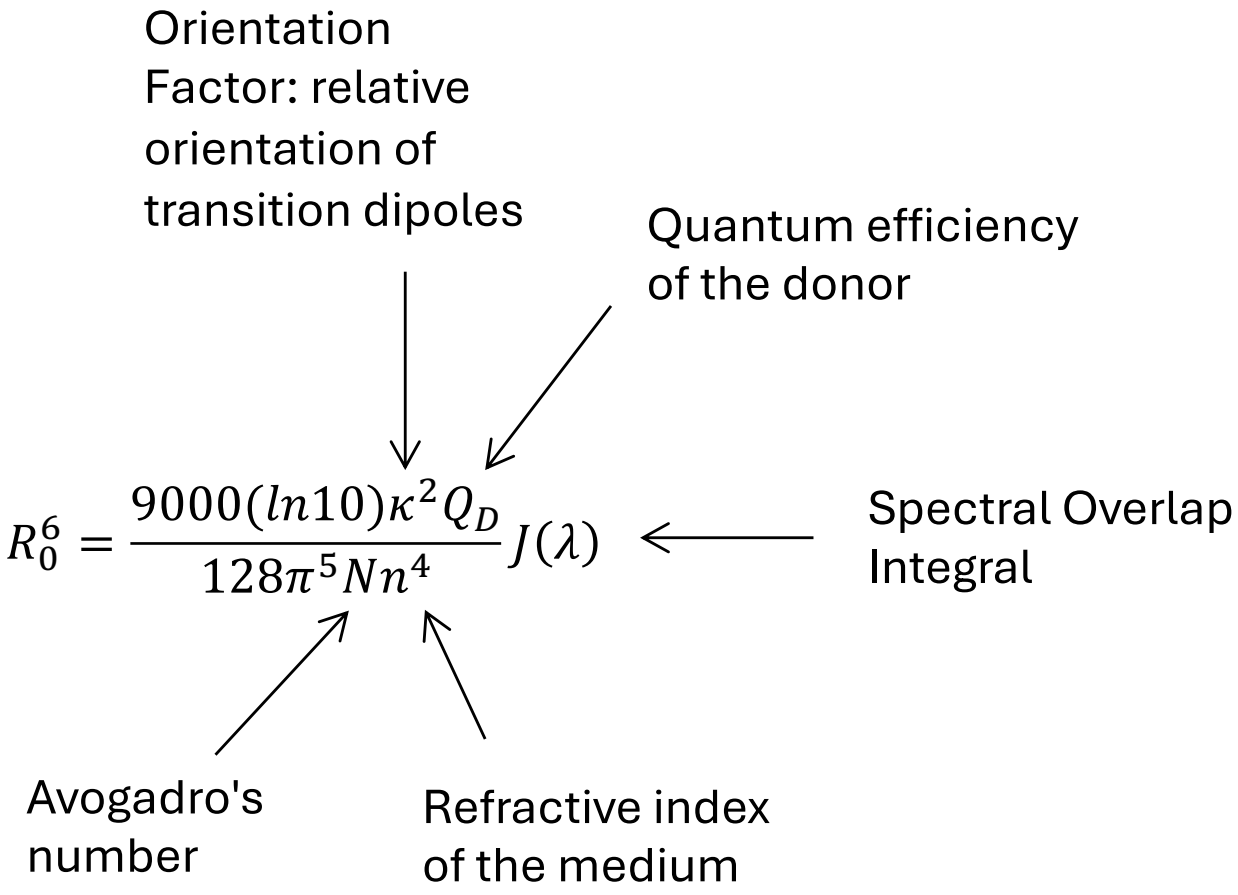
FRET can be used as a **«molecular ruler»**

high sensitivity region



Förster radius R_0

Distance of half-maximal FRET efficiency



Förster radius R_0

$$R_0^6 = \frac{9000(ln10)\kappa^2 Q_D}{128\pi^5 N n^4} J(\lambda)$$

The Förster radius is a property of:

- the fluorophore pair: J(λ), Q_D
- the labeled proteins: κ^2, Q_D

It has thus to be determined for each new protein sample!

Which of the two dye pairs is expected to have a larger R_0:
Cy3 - Cy5 or **Cy3 - Cy7** ?

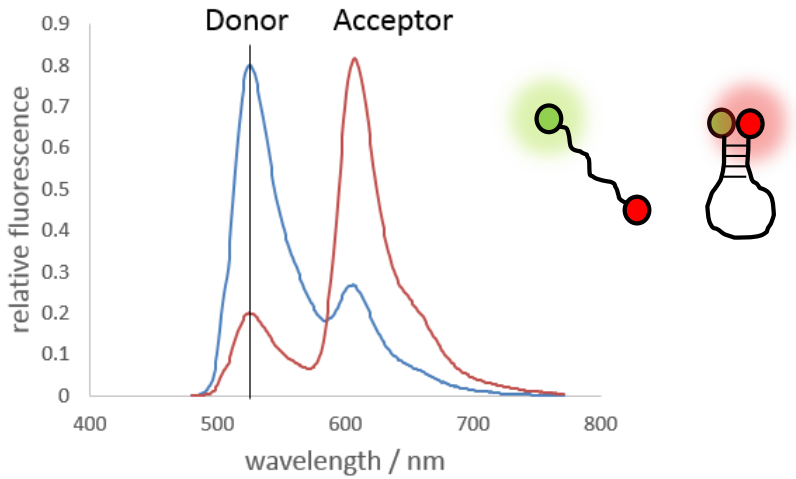
Table 13.3. Representative Förster Distances for Various Donor–Acceptor Pairs[a]

Donor	Acceptor	R_0 (Å)
Naphthalene[14]	Dansyl	22
Dansyl[95]	FITC	33–41
Dansyl[14]	ODR	43
ε-A[14]	NBD	38
IAF[14]	TMR	37–50
Pyrene[14]	Coumarin	39
FITC[14]	TMR	49–54
IAEDANS[14]	FITC	49
IAEDANS[14]	IAF	46–56
IAF[14]	EIA	46
CF	TR	51
Bodipy[25]	Bodipy	57
BPE[14]	Cy5	72
Terbium[96]	Rhodamine	65
Europium[94]	Cy5	70
Europium[97]	APC	90

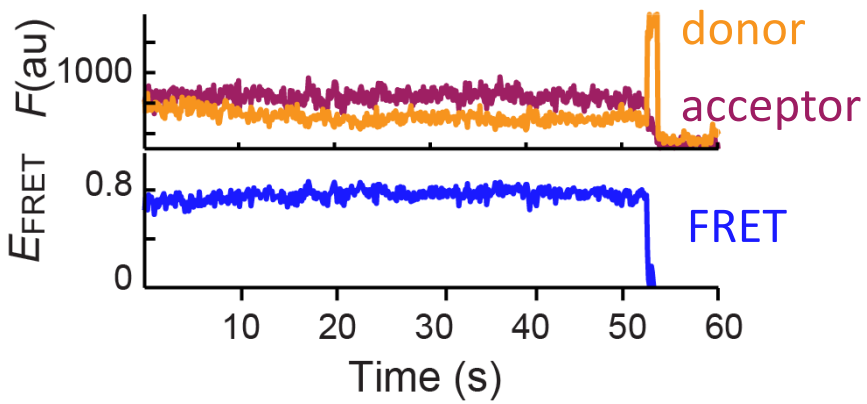
[a]Dansyl, 5-dimethylamino-1-napthalenesulfonic acid. ε-A, 1-N⁶-ethenoadenosine; APC, allophycocyanin; Bodipy, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; BPE, B-phycoerythrin; CF, carboxylfluorescein, succinimidyl ester; Cy5, carboxymethylindocyanine-N-hydroxysuccinimidyl ester; EIA, 5-(iodoacetamido) eosin; FITC, fluorescein-5-isothiocyanate; IAEDANS, 5-(2-0((iodocetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid; IAF, 5-iodoacetamidofluorescein; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; ODR, octadecylrhodamine; TMR, tetramethylrhodamine; TR, Texas Red.

Lakowicz, Principles of fluorescence spectroscopy

Measuring the efficiency of energy transfer



Blue: open state
Red: closed state



FRET efficiency:

$$E_{FRET} = I_A / (I_A + I_D)$$

I_D : intensity of donor emission

I_A : intensity of acceptor emission

Both upon excitiation of donor only

corrected:

$$E_{FRET} = I_A - \beta I_D / ([I_A - \beta I_D] + \gamma I_D)$$

β : leakage of donor emission into acceptor channel ($\beta < 1$)

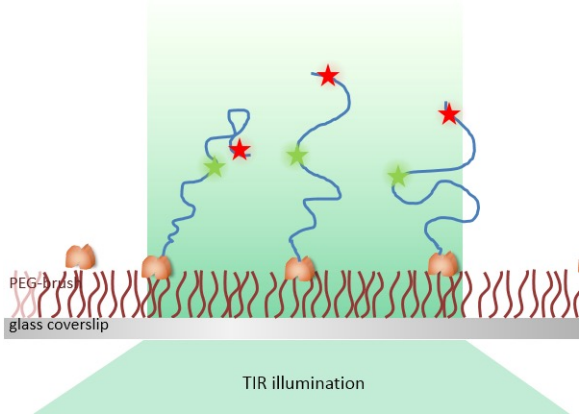
γ : detection efficiency of photons in donor and acceptor channels

Single molecule fluorescence techniques

Immobilized molecules:

molecules are immobilized on a surface (coverslip) and can be observed for a long time, e.g. by total internal reflection fluorescence microscopy

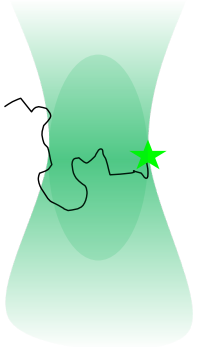
- Imaging and counting single molecules
- internal dynamics
- interaction dynamics
- Colocalization analysis



Freely diffusing molecules:

fluorescently labeled molecules are observed at very high dilution free in solution, using a confocal microscope:

- FRET statistics
- Burst analysis
- Photon counting histograms
- only fast dynamics are monitored (ms)



Next week: Readings

- **Single molecule FRET of immobilized samples**
  - Vafabakhsh et al., Extreme Bendability of DNA Less than 100 Base Pairs Long Revealed by Single-Molecule Cyclization, Science 2012

- **Single molecule FRET in freely diffusing proteins**
  - Schuler B, Lipman EA, Eaton WA. Probing the free-energy surface for protein folding with single-molecule fluorescence spectroscopy. Nature 2002, 419(6908):743-7.

Let’s play connections

https://connections.swellgarfo.com/game/-NrCaTHe5L5xxRF7bgbG

How to Play

Find groups of four items that share something in common.

- Select four items and tap '**Submit**' to check if your guess is correct.
- Find the groups without making 4 mistakes!

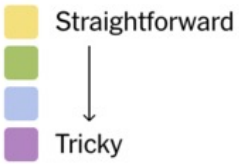
Category Examples

- FISH: Bass, Flounder, Salmon, Trout
- FIRE ___: Ant, Drill, Island, Opal

Categories will always be more specific than "5-LETTER-WORDS," "NAMES" or "VERBS."

Each puzzle has exactly one solution. Watch out for words that seem to belong to multiple categories!

Each group is assigned a color, which will be revealed as you solve:



Last five minutes

Week 1: February 22 This week

Did a question arise during the lecture that you don't have an answer to yet?