Nanomaterials for biology and medicine

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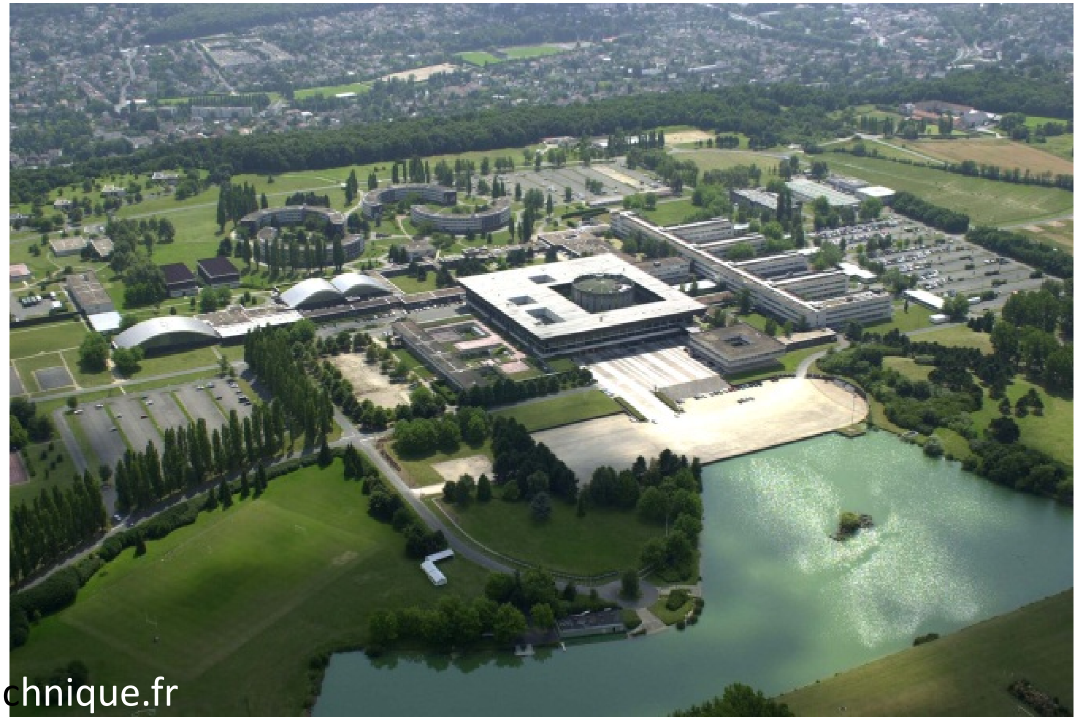
LOB



http://www.lob.polytechnique.fr

Nanomaterials for biological and biomedical applications

At the molecular and cell level: **visualize, understand, and model functioning**
-> towards **quantitative biology**

At the cell, tissue and organism level: **understand functioning** and devise **treatment/healing strategies**

Nanomaterials for biology and medicine

Outline

- Nano-objects for bioapplications (quantum dots, lanthanide nanoparticles, metallic nanoparticles, carbon nanotubes, polymeric nanoparticles, ...): properties, characterization, advantages/disadvantages

- Short introduction to biology

- Biological applications: single-molecule imaging in cells, sensing of cell parameters (Ca^{2+}, ROS, pH)

- Biomedical applications (*in vitro* diagnostics, peroperative imaging, drug delivery, nanoparticle-sensitized radiation therapy)

Use nanoparticles for luminescence labeling and imaging to avoid photobleaching problems of organic fluorophores

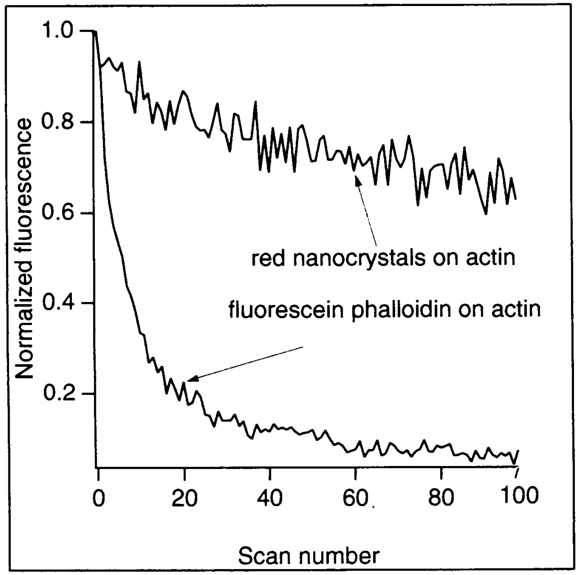
The beginning
1998

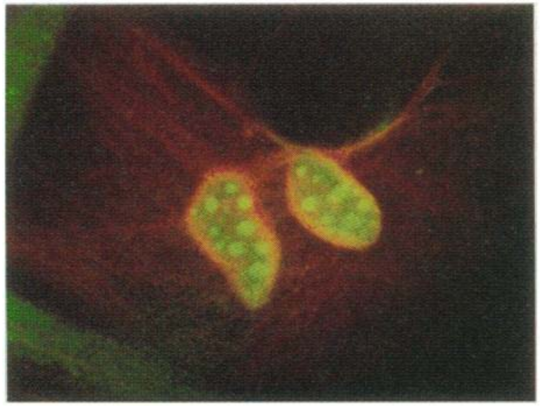
Semiconductor Nanocrystals as Fluorescent Biological Labels

Marcel Bruchez Jr., Mario Moronne, Peter Gin, Shimon Weiss,*
A. Paul Alivisatos*

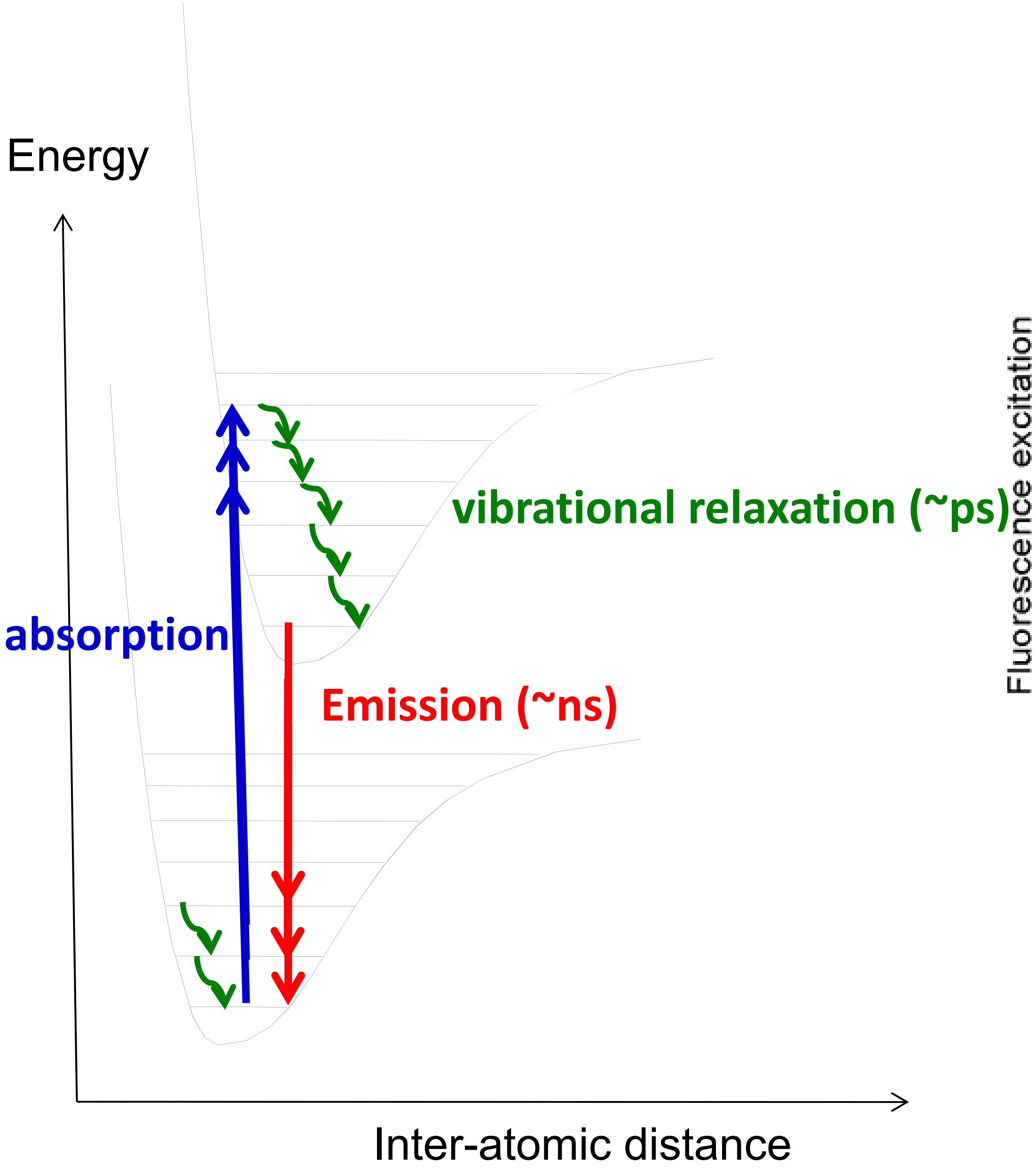
Semiconductor nanocrystals were prepared for use as fluorescent probes in biological staining and diagnostics. Compared with conventional fluorophores, the nanocrystals have a narrow, tunable, symmetric emission spectrum and are photochemically stable. The advantages of the broad, continuous excitation spectrum were demonstrated in a dual-emission, single-excitation labeling experiment on mouse fibroblasts. These nanocrystal probes are thus complementary and in some cases may be superior to existing fluorophores.

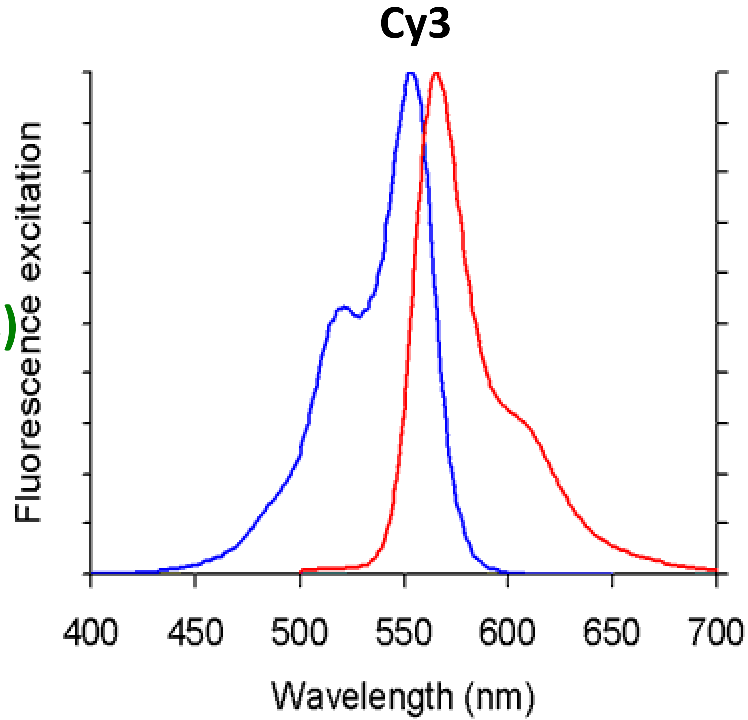
SCIENCE VOL 281 25 SEPTEMBER 1998 **2013**





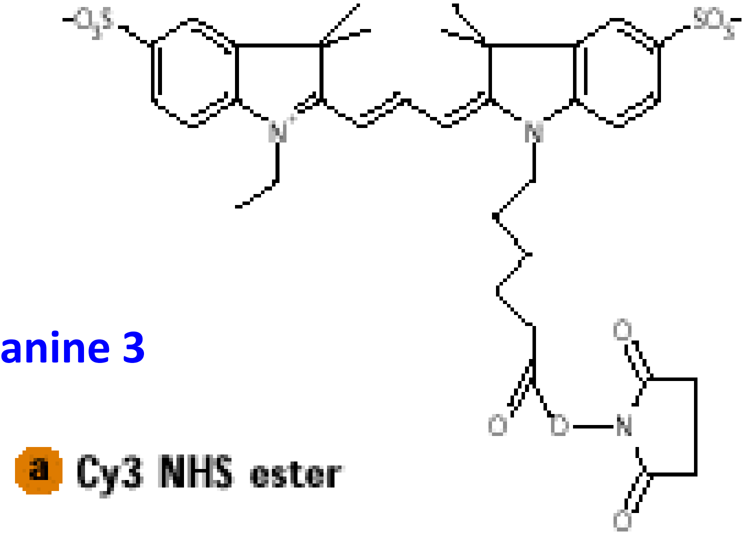
Fluorescence





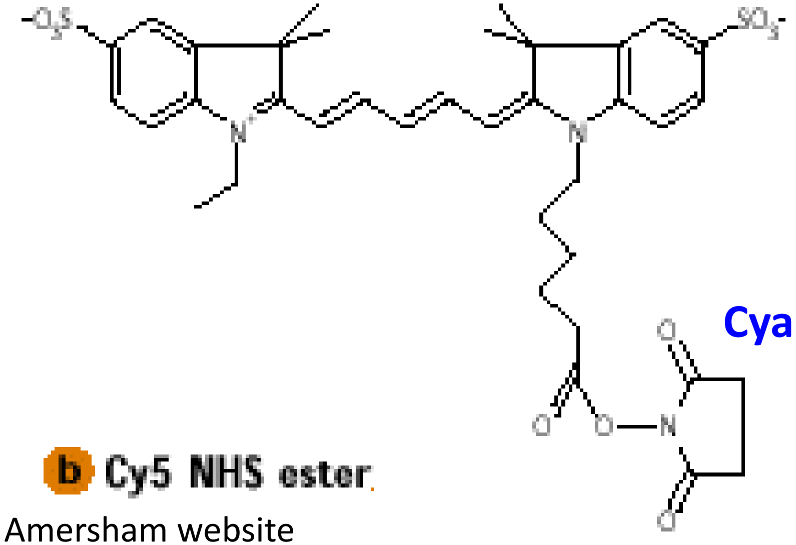
Invitrogen website

Organic fluorescent labels



Cyanine 3

a Cy3 NHS ester



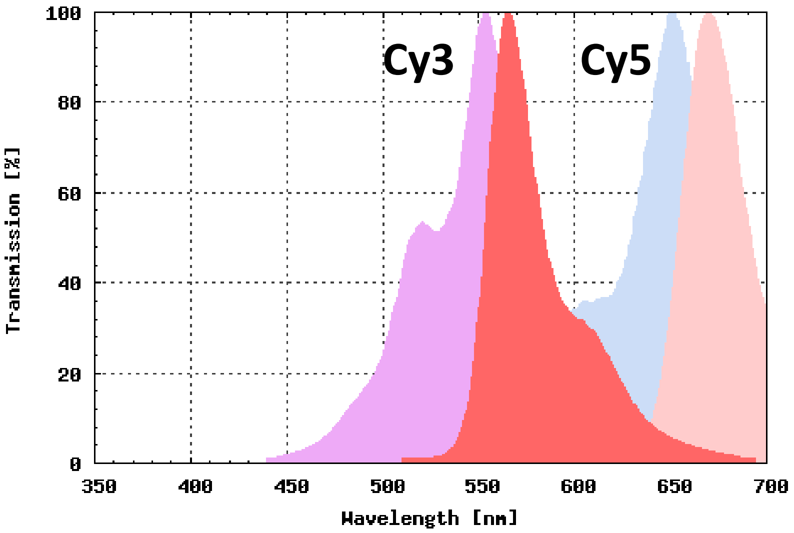
Cyanine 5

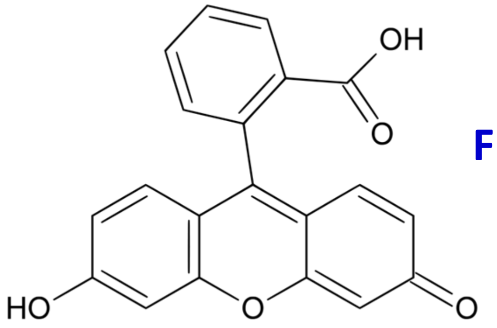
b Cy5 NHS ester

Amersham website

Electrons delocalized across parallel aligned p-orbitals of atoms involved in alternating single and multiple bonds (conjugated system).

The larger the delocalization region, the longer the transition wavelength.

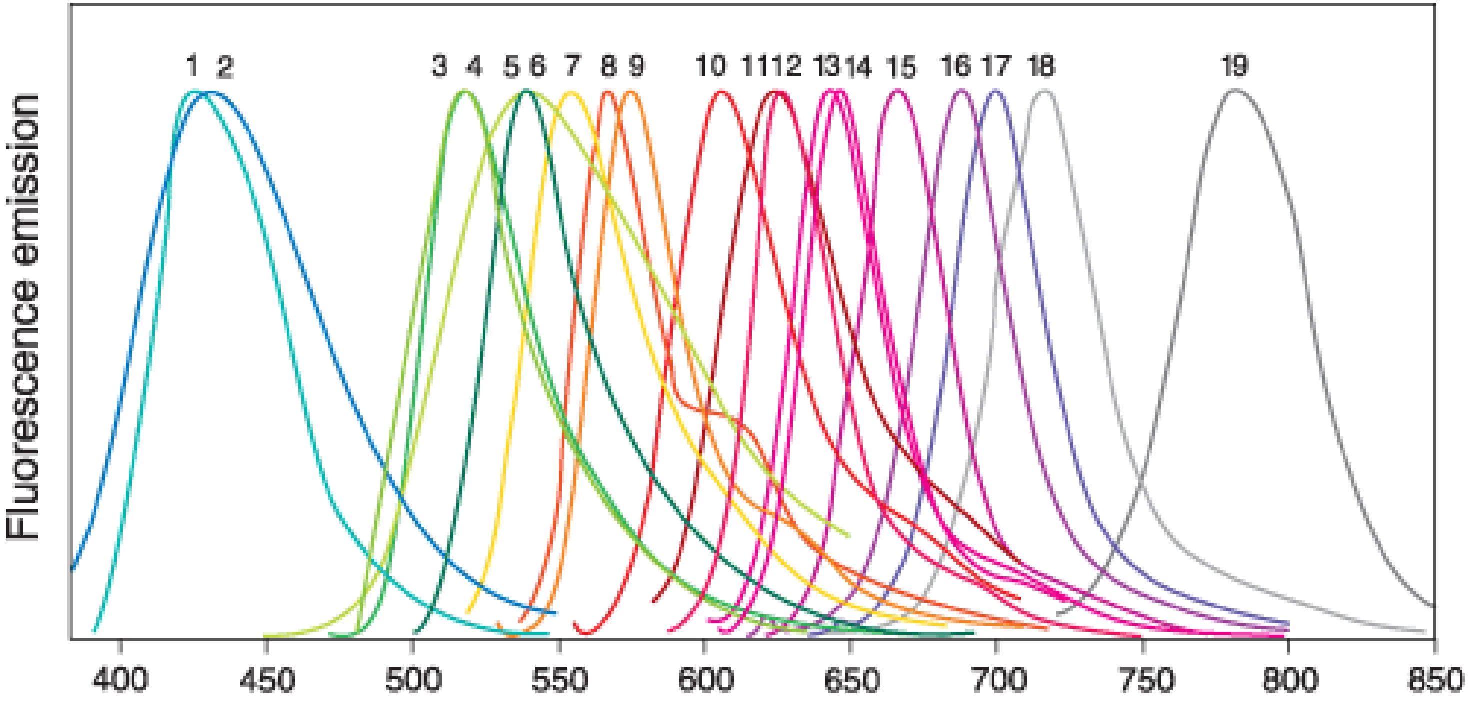




Fluorescein

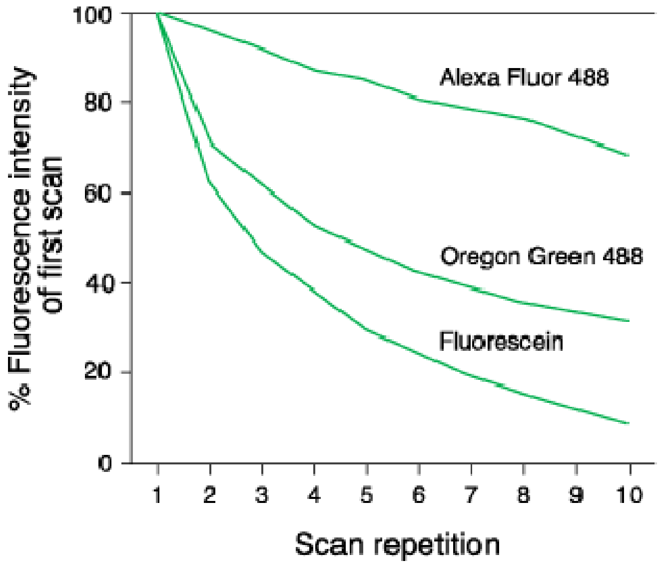
Organic labels

Alexa Fluor Dyes



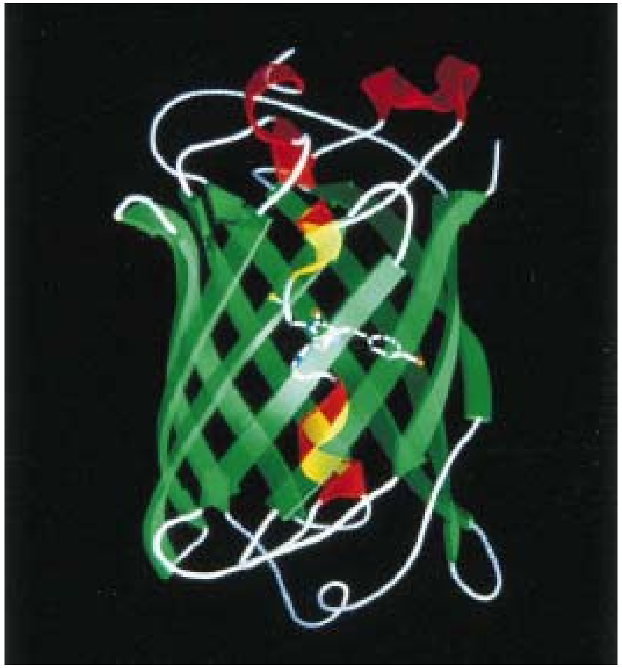
Invitrogen website (Molecular Probes)

Optimize
brightness
photostability
color selection
pH insensitivity
water solubility



Fluorescent proteins

Green fluorescent protein (GFP)

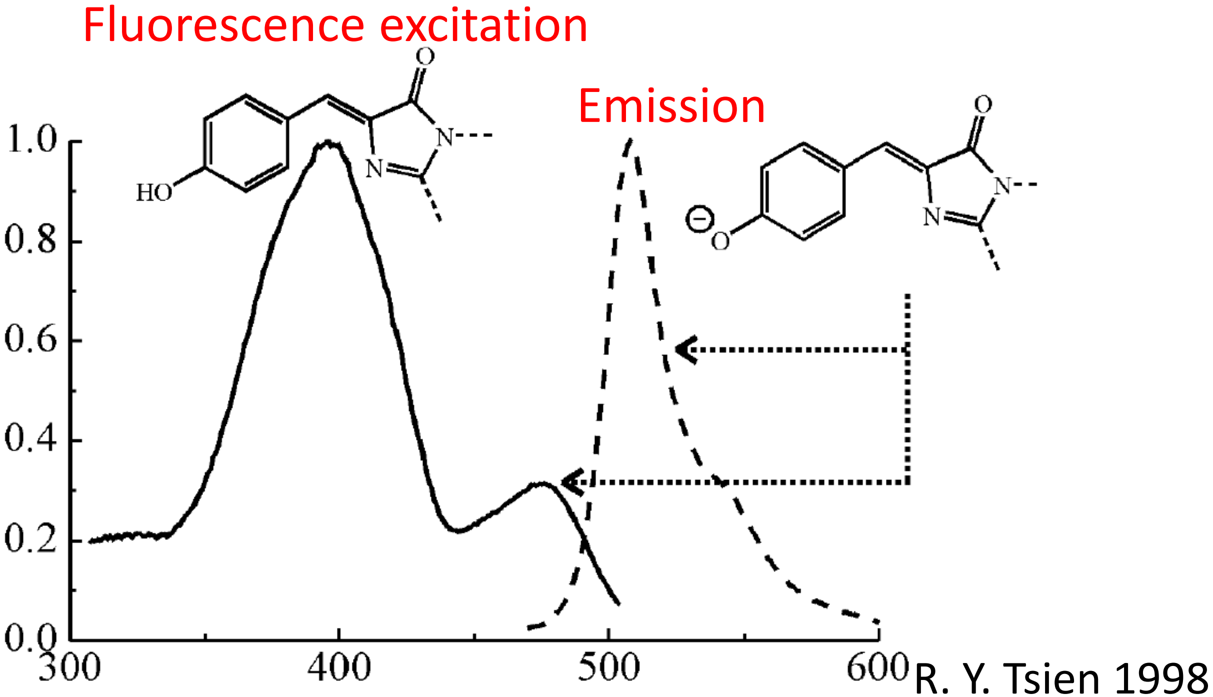


Nobel prize website

2008 Nobel Prize in Chemistry shared by Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien

The green fluorescent protein was first observed in the jellyfish *Aequorea victoria* in 1962.
Osamu Shimomura first isolated GFP from *Aequorea victoria.*
Martin Chalfie demonstrated its use as a fluorescent genetic label
Roger Y. Tsien introduced mutations to optimize the fluorescence properties and obtain emission at different wavelengths.

- GFP consists of an eleven-stranded β-barrel with an α-helix running along the axis of the cylinder.
- Three amino acids in the α-helix close to the center of the cylinder (Ser65-Tyr66-Gly67) form the chromophore.

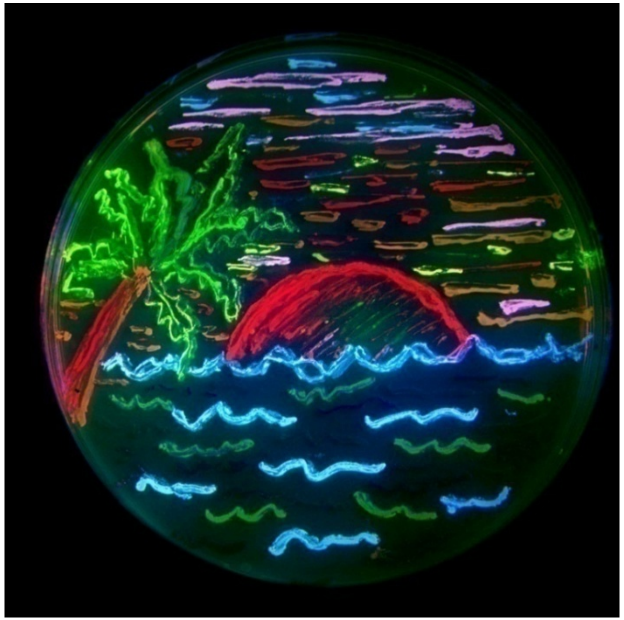


R. Y. Tsien 1998

Fluorescent proteins

Mutations improved the spectral characteristics of GFP to give **eGFP** :
ε=55,000 M^{-1}cm^{-1}, quantum yield=0.60

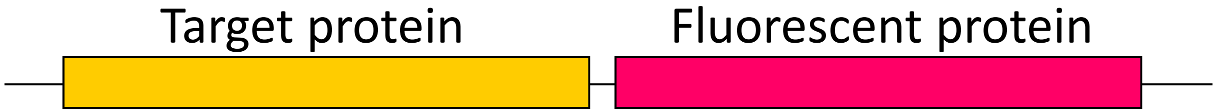
Other mutations produced color mutants: blue (BFP), cyan (CFP), yellow (YFP) fluorescent proteins, ….



Agar Plate of Fluorescent
Bacteria Colonies

Roger Y. Tsien website

Labeling by generating fusion proteins



Target protein

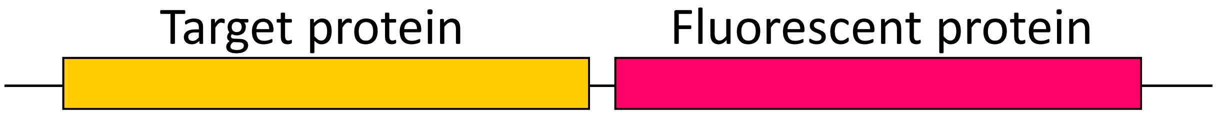
Fluorescent protein

Advantages: coupling specificity
1:1 stochoimetry

R. Y. Tsien lab, Univ. California San Diego
B. Giepmans et al. Science 312, 217 (2006).

Fluorescent proteins
The GFP-rabbit

Labeling by generating fusion proteins

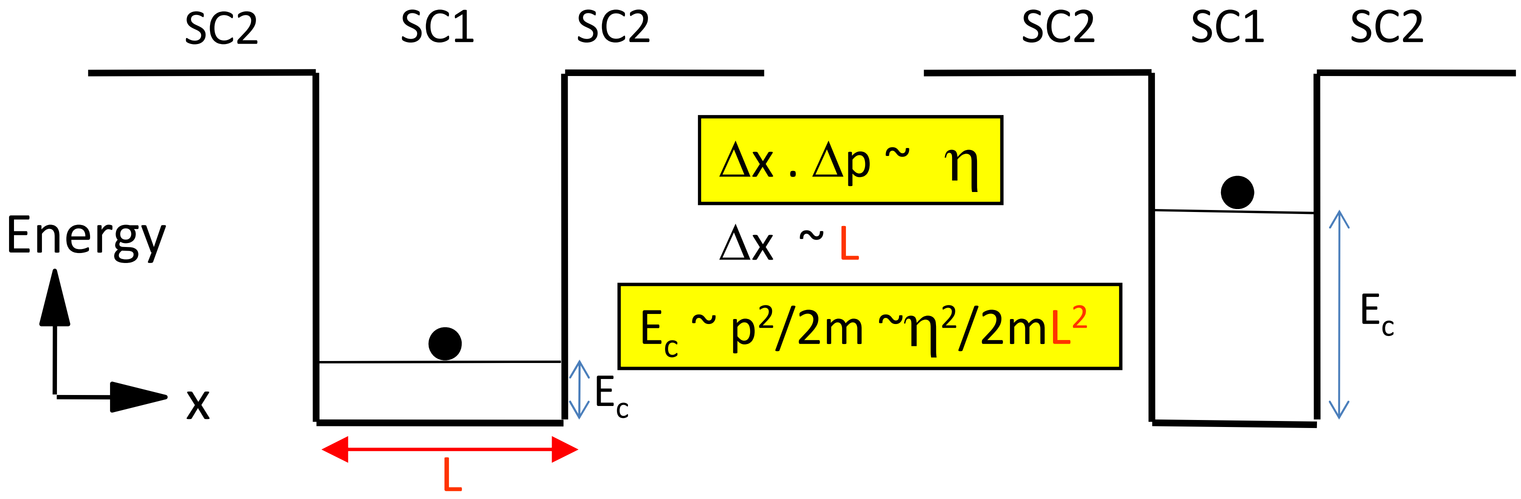


Louis-Marie Houdebine and Patrick Prunet, Jouy-en-Josas, France, 2000

Semiconductor nanocrystals: Quantum dots

0D semiconductors

The confinement energy depends on the size L of the quantum dot.

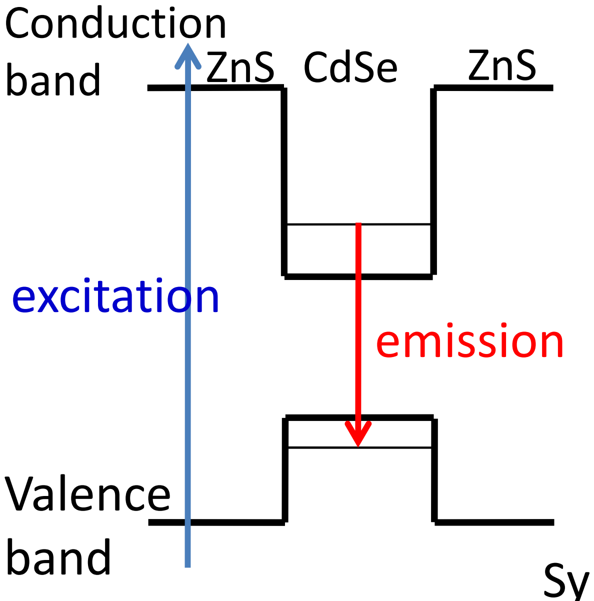


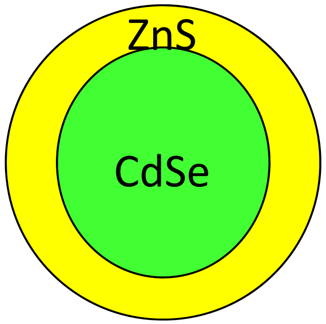
$\Delta x \,.\, \Delta p \sim \eta$

$\Delta x \sim L$

$E_c \sim p^2/2m \sim \eta^2/2mL^2$

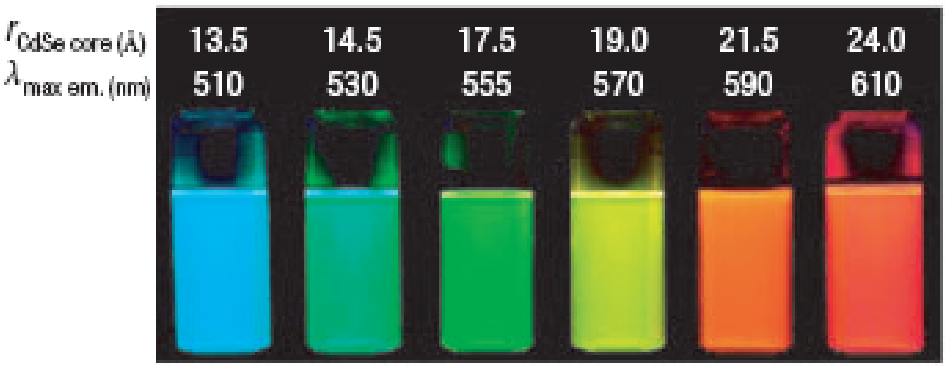
Different emission colors can be obtained for the same excitation wavelength.





Synthesis in organic solvents.

Excitation with a 365 nm UV source

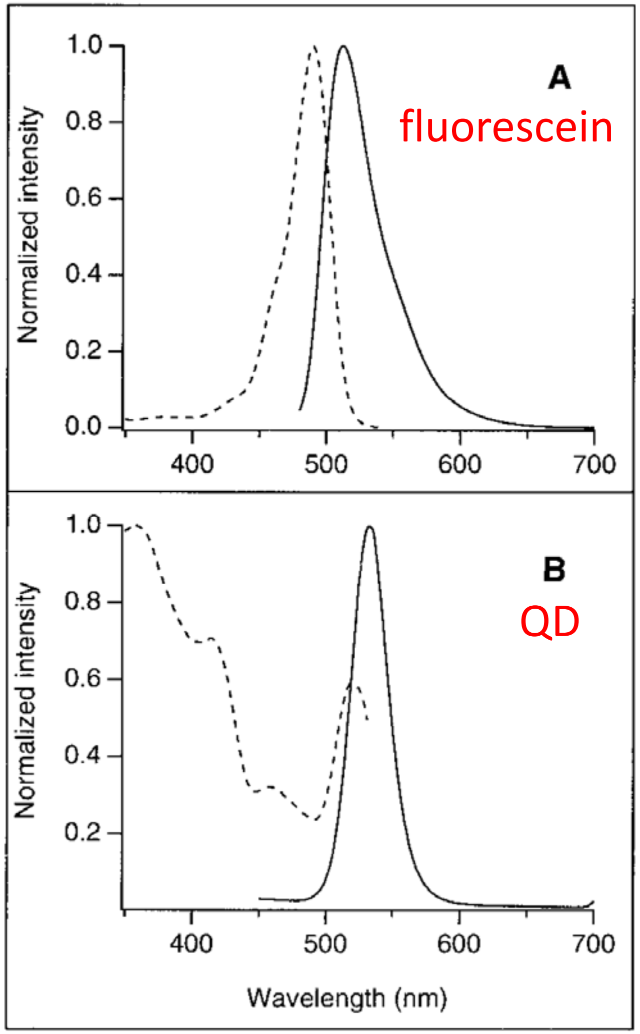


H. Mattoussi group, Naval Research Lab
I. Medintz et al., Nat. Mat. 4, 435 (2005)

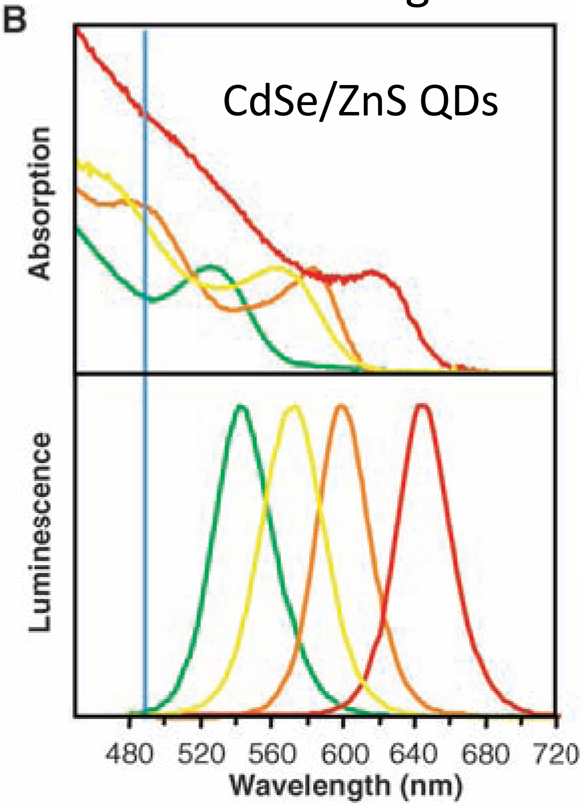
Quantum dots

Advantages

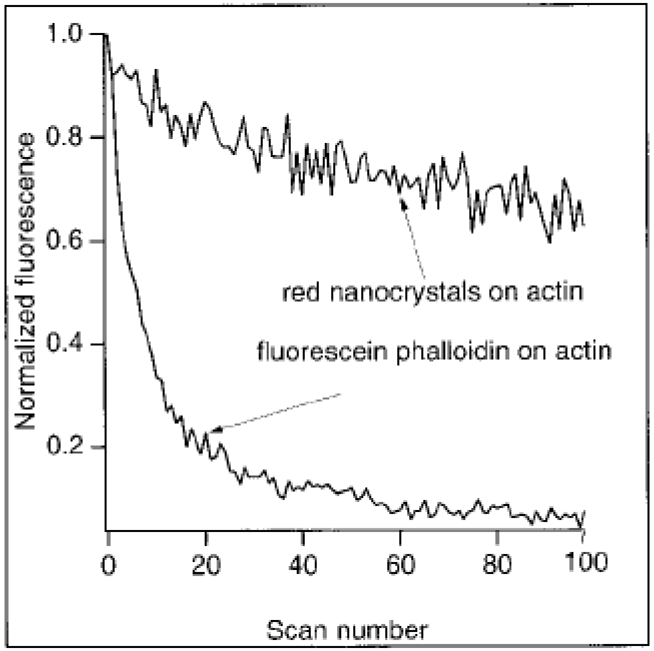
Large Stokes shift
Narrowband,
symmetric emission



Broadband absorption spectrum
Variable emission wavelength for the same excitation wavelength



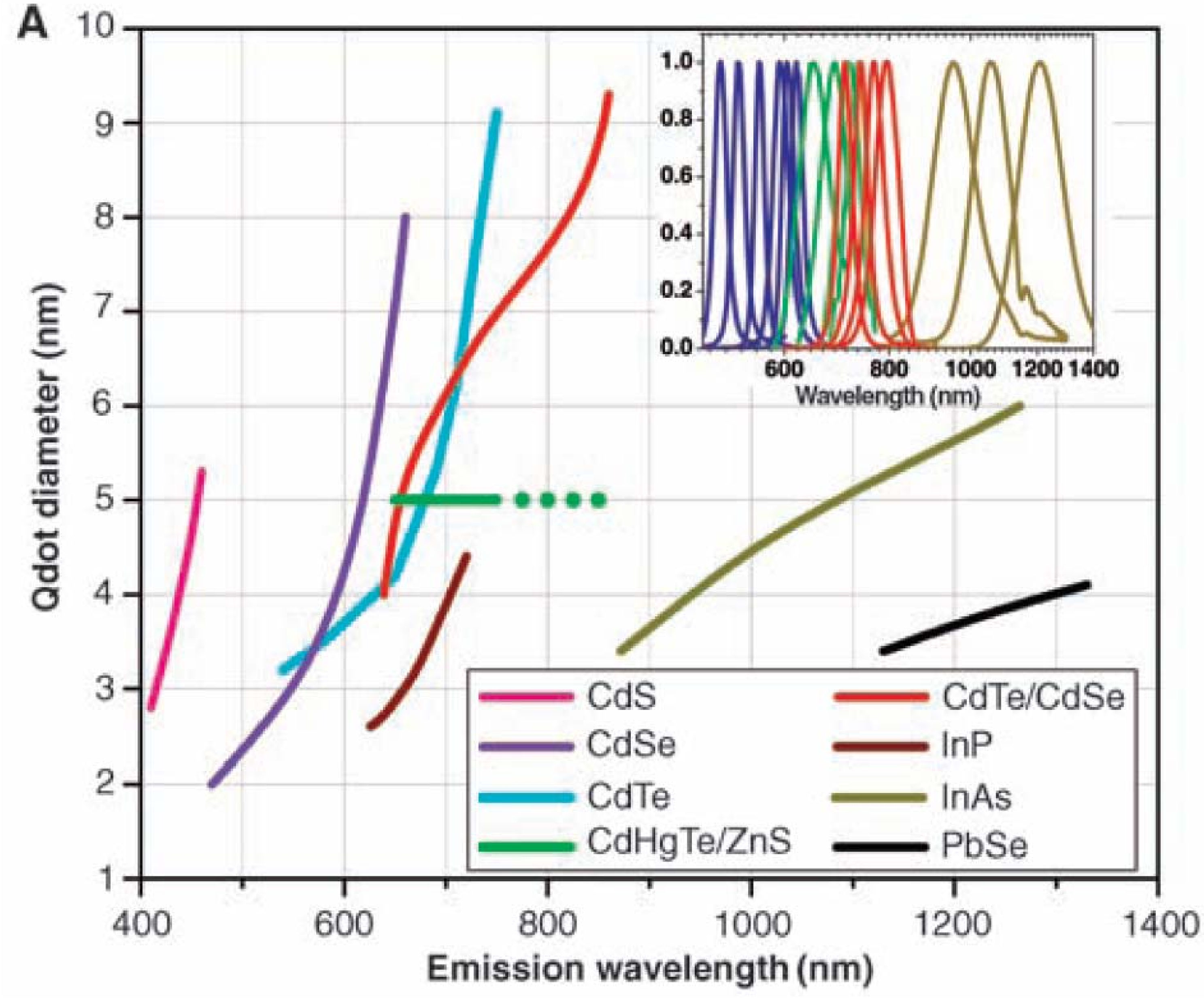
High photostability



P. Alivisatos and S. Weiss groups
M. Bruchez et al., Science 281, 2013 (1998)
X. Michalet et al., Science 307, 538 (2005)

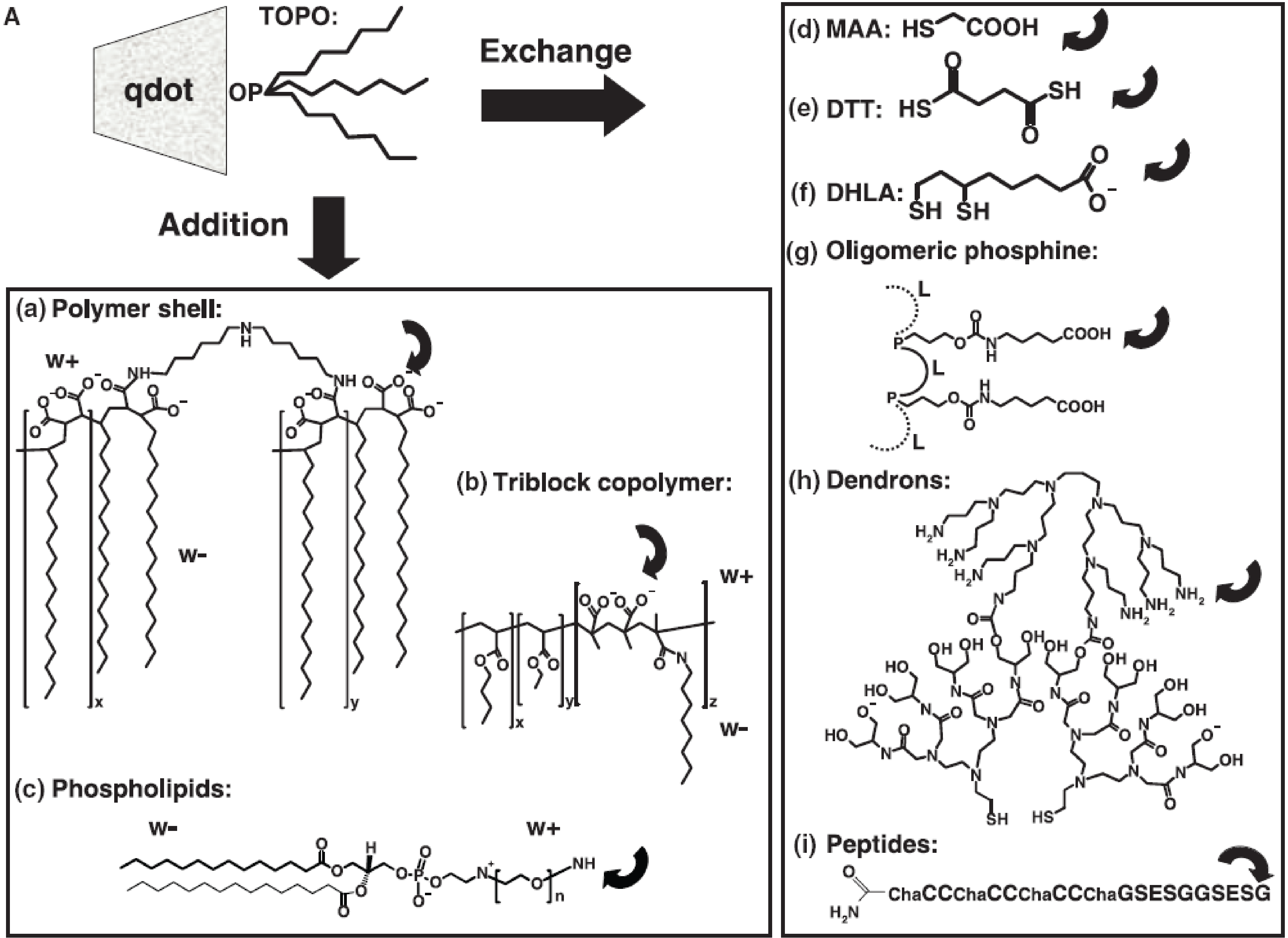
Quantum dots

Emission extending to the near IR



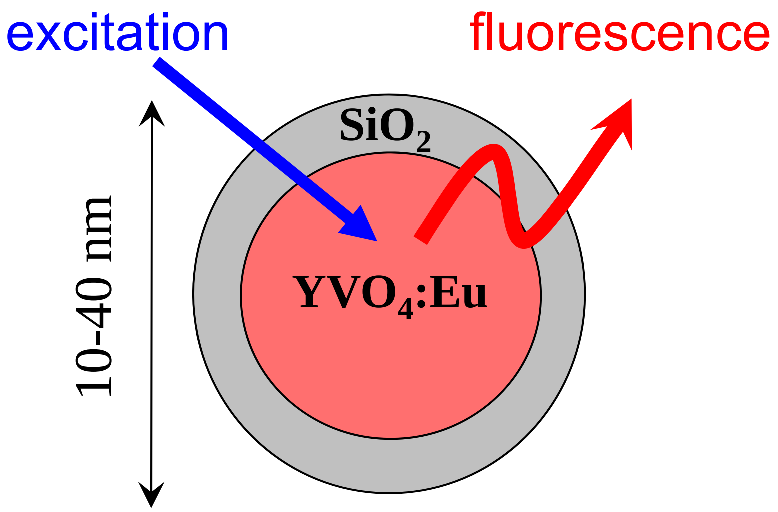
S. Weiss group, UCLA, X. Michalet et al., Science 307, 538 (2005)

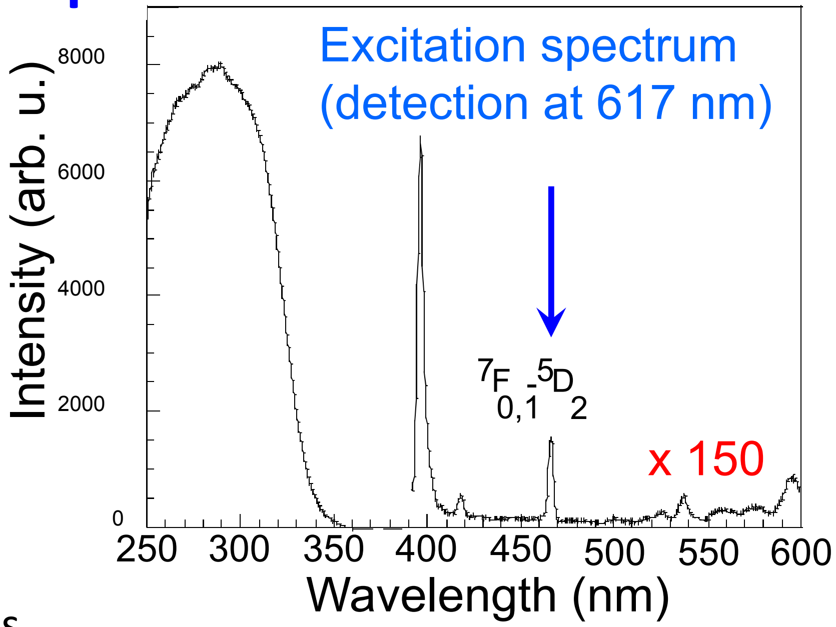
Quantum dots: Solubilization in water and functionalization



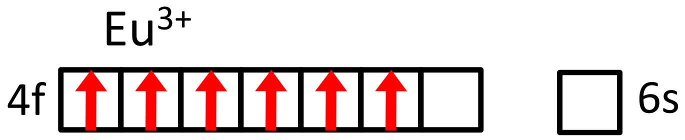
S. Weiss group, UCLA, X. Michalet et al., Science 307, 538 (2005)

Lanthanide-doped nanoparticles





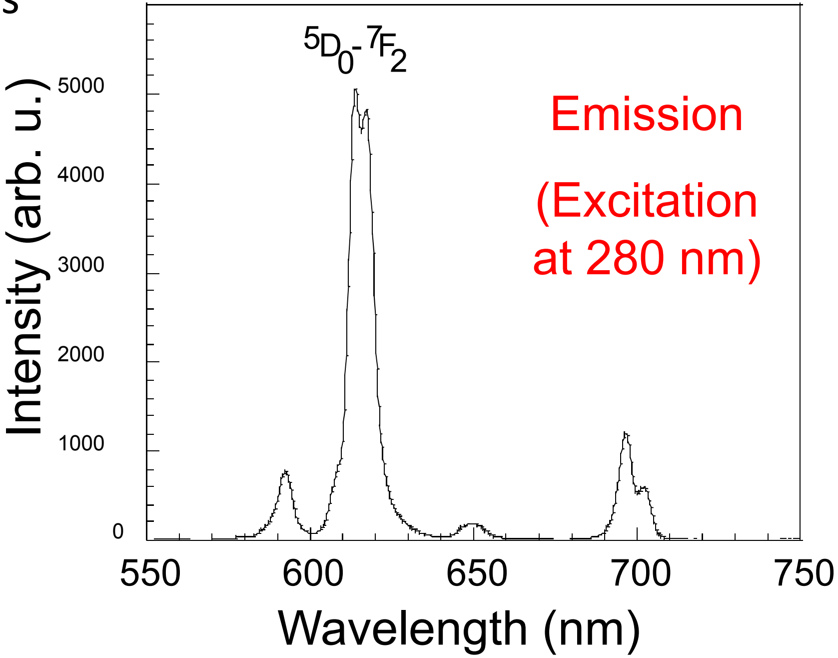
Oxide matrix (insulator) containing lanthanide dopants



Electronic transitions are 4f-4f transitions

Atomic-like transitions (small influence of the matrix)

Forbidden transitions (small admixture of d orbitals)

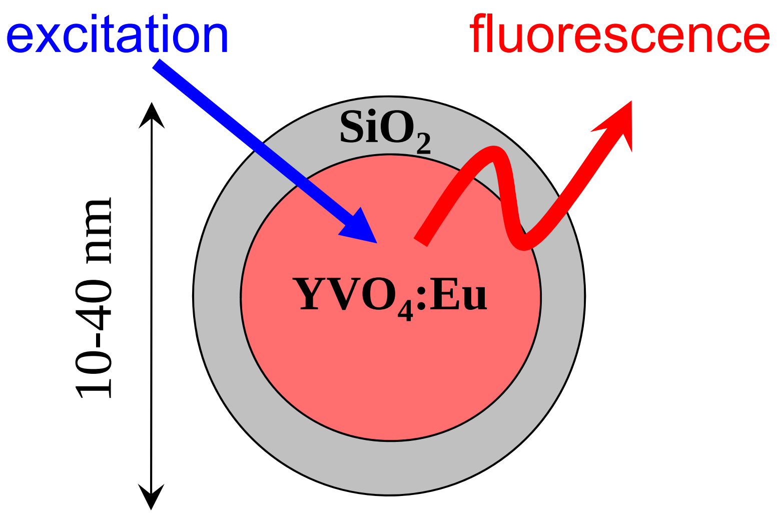


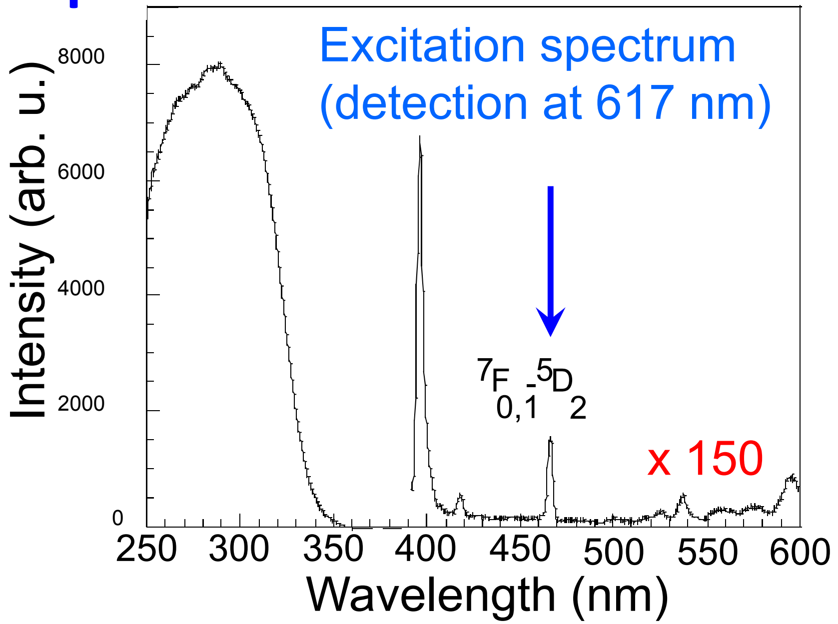
LOB&PMC, Ecole Polytechnique

E. Beaurepaire et al, Nano Lett. 4, 2079 (2004)

Also: Persistant fluorescence nanoparticles

Lanthanide-doped nanoparticles





Advantages:

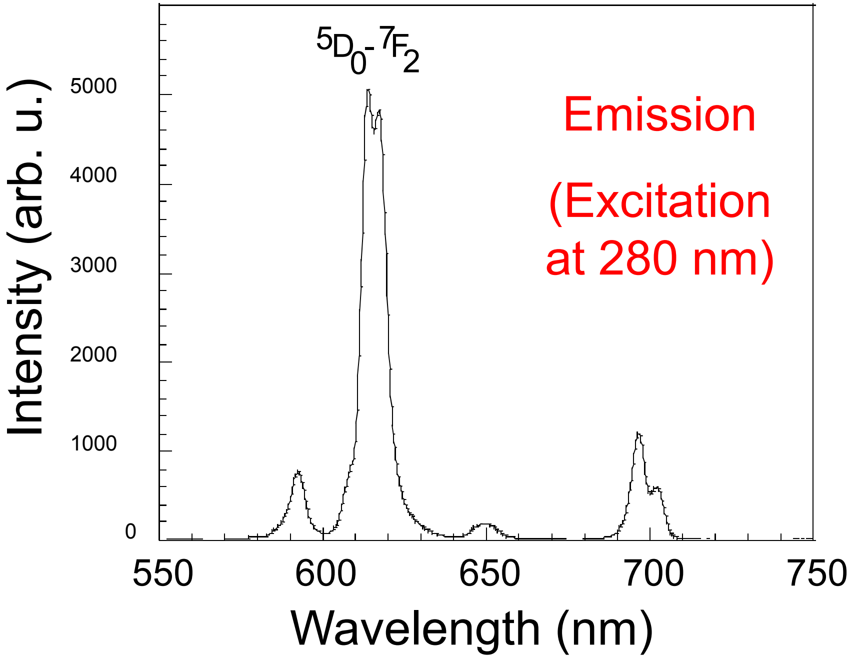
- Synthesized in water
- Good photostability
- No blinking (9000 Eu ions in a 20 nm 20%-doped nanoparticle)
- Narrow emission bandwidth (<10 nm)

  => possibility of multi-color experiments
- Long excited-state lifetime (~0.7 ms)

  => possibility of time-gated detection

-> attractive labels for long-term single-molecule tracking experiments

Also: Persistant fluorescence nanoparticles

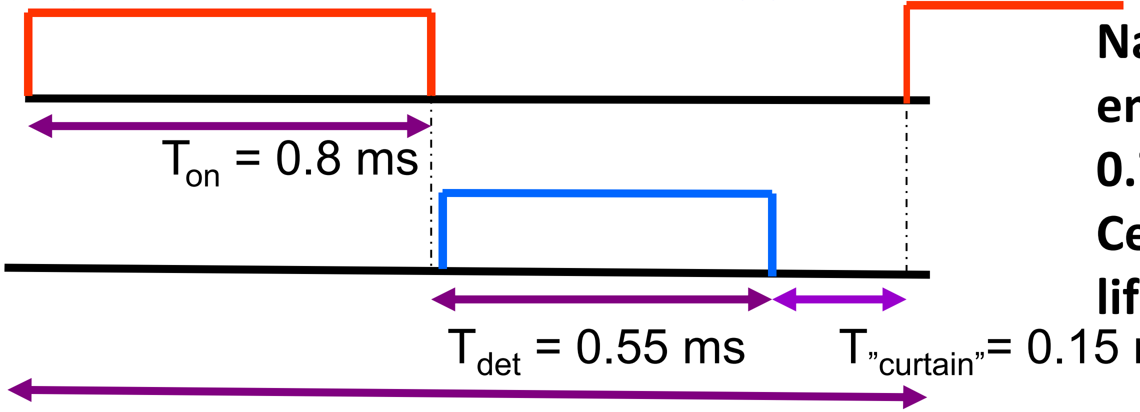


LOB&PMC, Ecole Polytechnique

E. Beaurepaire et al, Nano Lett. 4, 2079 (2004)

Time-gated detection of rare-earth doped nanoparticles

Synchronized mechanical choppers



excitation chopper

T_{on} = 0.8 ms

detection chopper

T_{det} = 0.55 ms

$T_{\text{"curtain"}}$= 0.15 ms

Period T = 1.5 ms

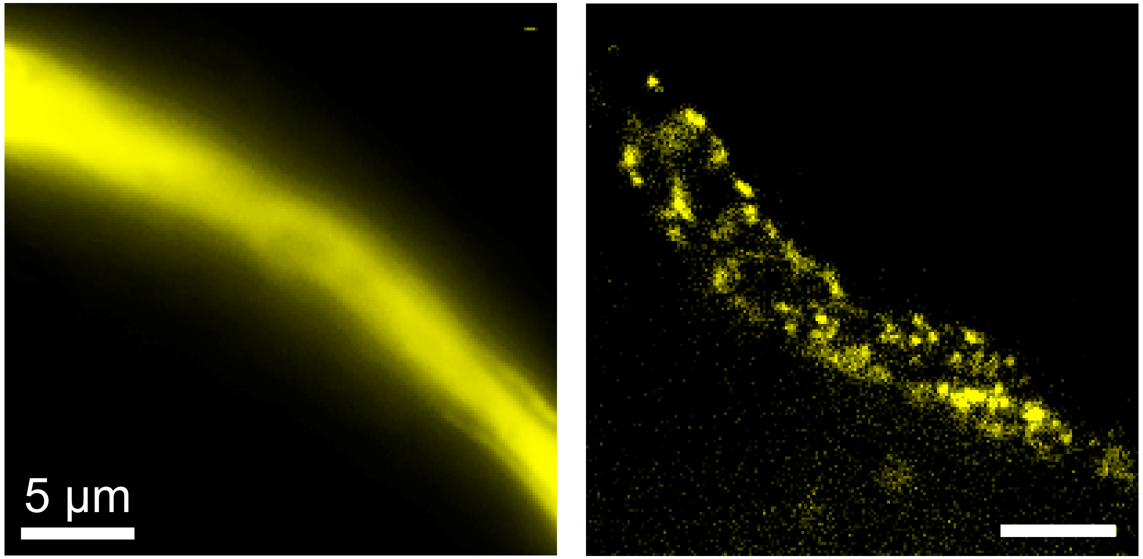
Nanoparticle emission lifetime: 0.7 ms

Cell fluorescence lifetime: ~ns

Without time-gating

With time-gating

Cell fluorescence: 15000-50000 ph/s



200 ph/s

Excellent discrimination against cell fluorescence

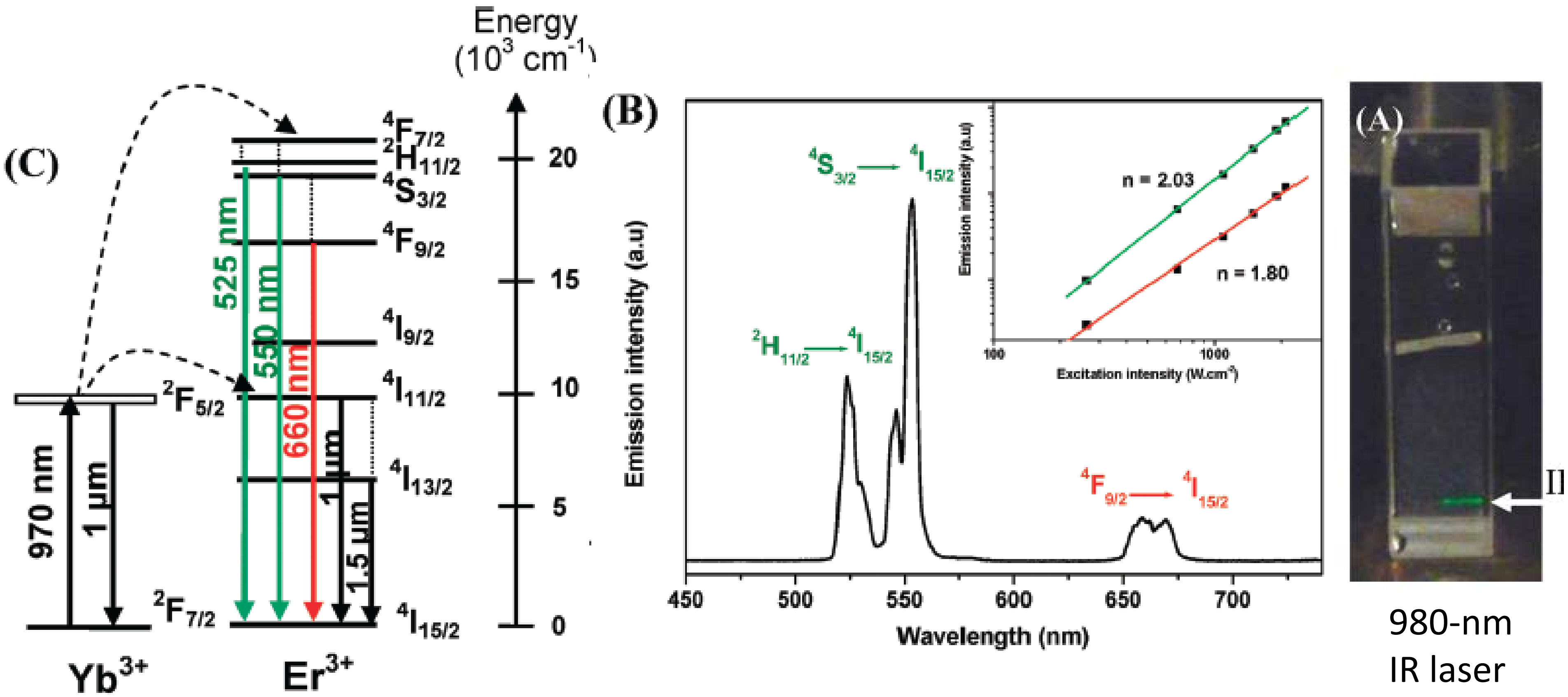
For QDs (lifetime ~ 10 ns), electronic gating is necessary

M. Dahan et al., Opt. Lett. **26**, 825 (2001)

LOB&PMC, Ecole Polytechnique

E. Beaurepaire et al, Nano Lett. 4, 2079 (2004)

Up-conversion lanthanide-doped nanoparticles



980-nm
IR laser

Mialon et al., J. Phys. Chem. 114, 22449 (2010)

C. Mirkin group, Chem. Rev. (2015)

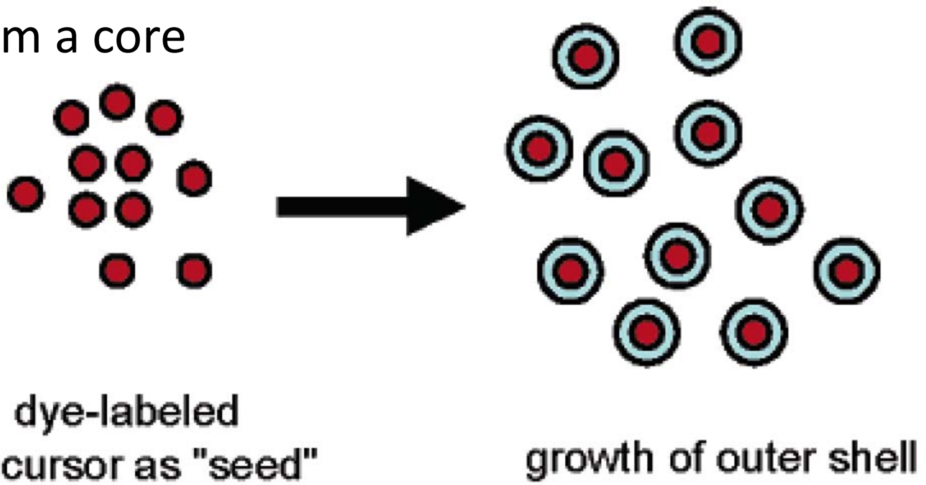
Advantages:
Near-IR excitation
Lower absorption in tissue
-> Low cytotoxicity
-> Higher penetration depth

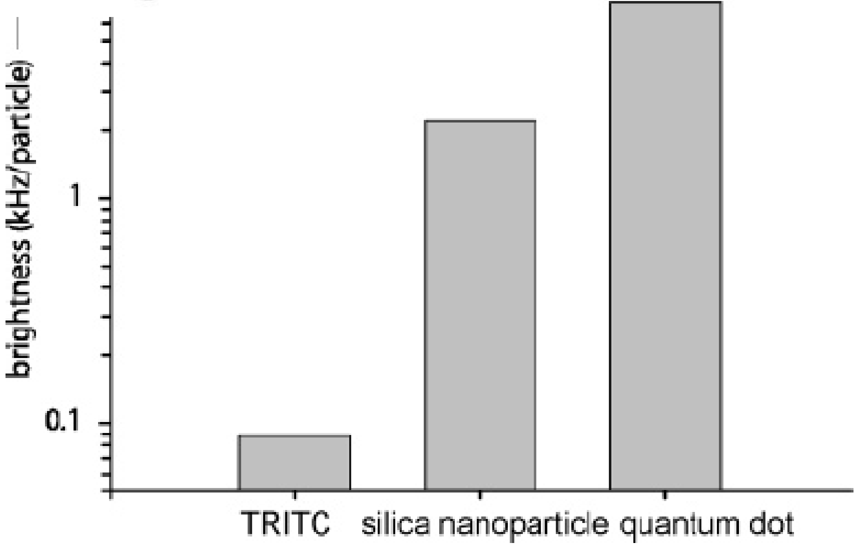
Fluorescent silica nanoparticles incorporating dye molecules

Size: 20-30 nm
20 times brighter and
More photostable
than individual dyes
(the silica shell provides shielding from interactions with the solvent thus suppressing non radiative de-excitation pathways)

First step: covalent attachment of dyes to a silica precursor to form a core

Second step:
growth of a silica shell





U. Wiesner group, Cornell University
H. Ow et al., Nano Lett. 5, 113 (2005).

Metallic (Au, Ag) nanoparticles and surface plasmons

Propagation of electromagnetic waves in metals: Brief reminder

Maxwell equations

$$\begin{aligned}
\nabla \times \vec{E} &= -\frac{\partial \vec{B}}{\partial t} \\
\nabla \times \vec{H} &= \vec{J} + \frac{\partial \vec{D}}{\partial t} \\
\nabla \cdot \vec{B} &= 0 \\
\nabla \cdot \vec{D} &= \rho \qquad \vec{D}(\vec{r},t) = \epsilon_0 \epsilon_R \vec{E}(\vec{r},t)
\end{aligned}$$

Propagation in a homogeneous medium

Helmholtz equations

$$\begin{aligned}
\triangle \vec{E}(r) + k^2 \vec{E}(r) &= 0 \\
\triangle \vec{B}(r) + k^2 \vec{B}(r) &= 0
\end{aligned}$$

with $k^2 = \omega^2 \epsilon_{eff} \mu_0 = \epsilon_R \frac{\omega^2}{c^2}$ $\quad \epsilon_{eff} = \epsilon = \epsilon_R \epsilon_0$

$\epsilon_{eff}, \epsilon_R, \epsilon_0$ effective, relative, vacuum permittivity

Solution for a wave propagating in z-direction:

$$\vec{E}(r) = \vec{E_0} e^{-ikz}$$

In metals:

$$\epsilon_{eff} = \underbrace{\epsilon_R \epsilon_0}_{\text{Real part}} + \underbrace{\frac{\sigma}{i\omega}}_{\text{Imaginary part}}$$

Metal conductivity σ :

$$\sigma = \frac{-Ne\dot{x}}{\vec{E}}$$

N charge density

$\dot{x}$ electron velocity

Drude model:

$$\vec{E}(t) = \vec{E_0} e^{i\omega t}$$

$$m\ddot{x} + m\frac{\dot{x}}{\tau} = -eE_0 e^{i\omega t}$$

Damping force due to collisions

Force acting on the electron

τ characteristic time between collisions

Propagation of electromagnetic waves in metals: Brief reminder

Solution of the equation of motion:

$$\dot{x} = \frac{-eE_0 e^{i\omega t}}{m(\frac{1}{\tau} + i\omega)}$$

New expression for the conductivity:

$$\sigma = \frac{Ne^2}{m\left(\frac{1}{\tau} + i\omega^2\right)}$$

Effective permittivity:

$$\epsilon_{eff} = \epsilon + \frac{\sigma}{i\omega} = \epsilon - \frac{\epsilon_0 \omega_p^2}{\omega^2 - \frac{i\omega}{\tau}}$$

with plasma frequency: $\omega_P = \frac{Ne^2}{m\epsilon_0}$

For: $\tau \longrightarrow \infty$ $\quad \epsilon_{eff,r} = 1 - \frac{\omega_p^2}{\omega}$

with:

$$\epsilon_{eff,r} = \frac{\epsilon_{eff}}{\epsilon_0}$$

When: $\omega_p < \omega$

$\epsilon_{eff,r} > 0 \qquad \epsilon_{eff,r} = n^2$

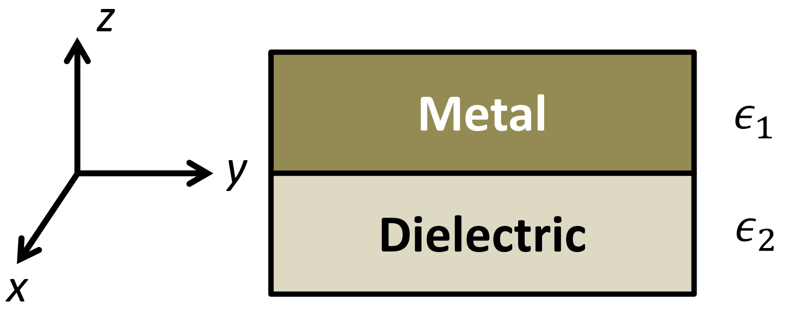
When: $\omega_p > \omega$

$\epsilon_{eff,r} < 0. \qquad n^2 < 0$

n imaginary -> absorption

-> Visible and infrared electromagnetic waves do not propagate in metals

Surface plasmons



$$H_x = H_z = E_y = 0$$

Continuity conditions at the interface between metal and dielectric:

$$H_{y1} = H_{y2}$$

$$E_{x1} = E_{x2}$$

+ Maxwell equations

$$\frac{\vartheta H_y(z)}{\vartheta z} = D_x = \epsilon E_x$$

$$\rightarrow \frac{\vartheta H_{y1}(z)}{\vartheta z} = \frac{\vartheta H_{y2}(z)}{\vartheta z}$$

For surface waves:

$H_{y1}(z) = Ae(-\gamma_1 z)$ for z > 0

$H_{y2}(z) = Be(\gamma_2 z)$ for z < 0

$$\frac{k_{z1}}{\epsilon_1} + \frac{k_{z2}}{\epsilon_2} = 0$$

and

$$k_x{}^2 + k_{z1}{}^2 = \epsilon_1 \left(\frac{\omega}{c}\right)^2$$

$$k_x{}^2 + k_{z2}{}^2 = \epsilon_2 \left(\frac{\omega}{c}\right)^2$$

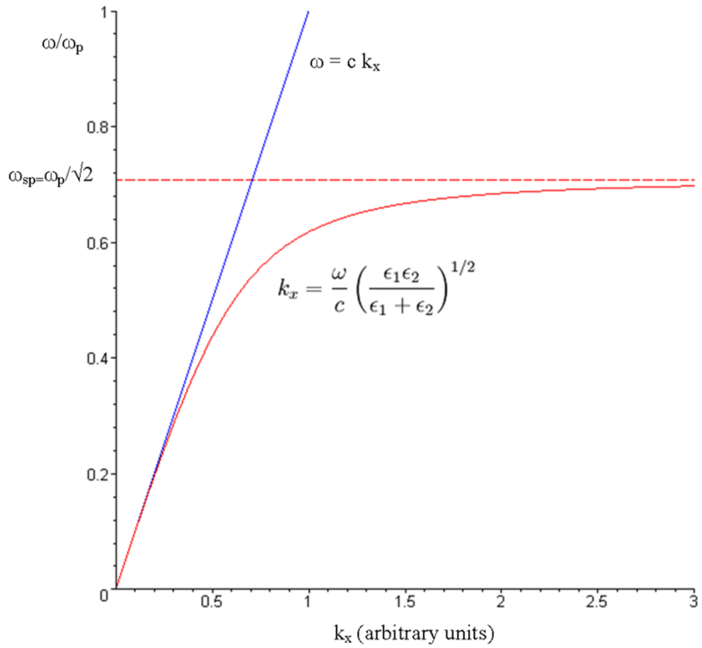
$$\rightarrow \quad k_x = \frac{\omega}{c}\left(\frac{\epsilon_1 \epsilon_2}{\epsilon_1 + \epsilon_2}\right)^{\frac{1}{2}}$$

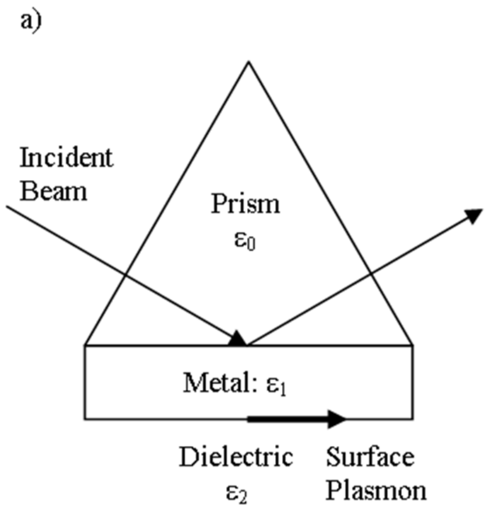
$$\epsilon_1 = 1 - \left(\frac{\omega_P}{\omega}\right)^2 \qquad \omega_{SP} = \frac{\omega_P}{\sqrt{1 + \epsilon_2}}$$

Surface plasmons

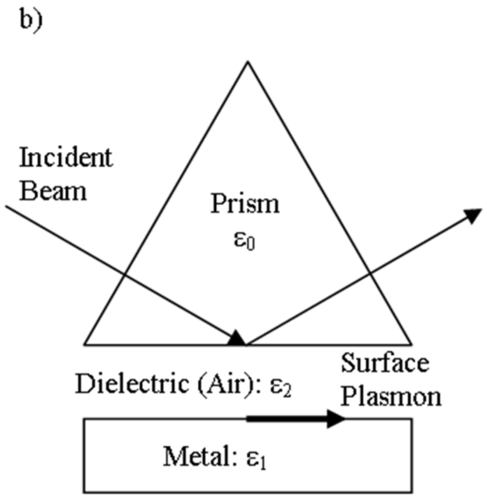
If the dielectric is air:

$$\epsilon_2 = 1$$





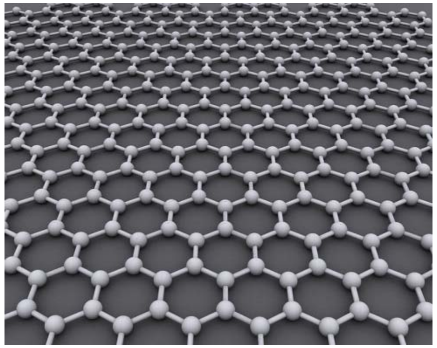
Kretschmann configuration



Otto configuration

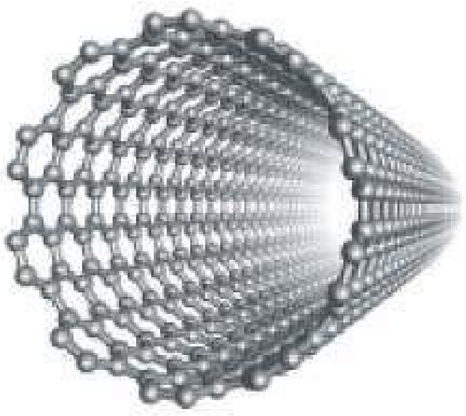
Carbon nanotubes

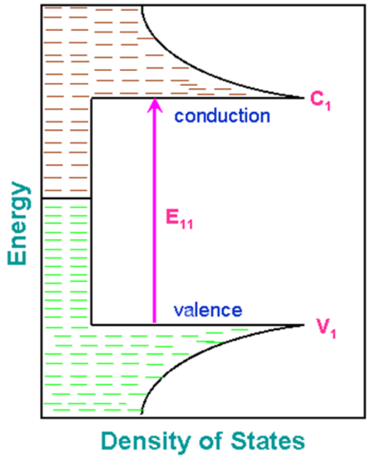
Graphene: carbon sheet



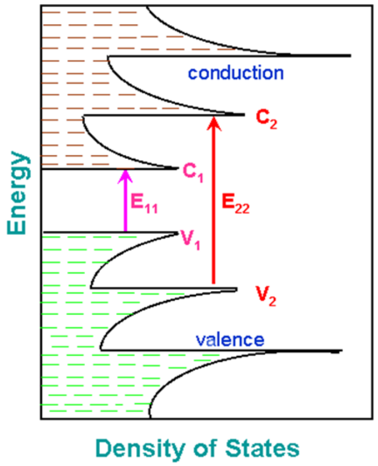
Rolling for certain discrete « chiral » angles

Single-walled carbon nanotube





Metallic SWNT



Semiconducting SWNT

$v_1 \rightarrow c_1$ corresponds to the "first van Hove" optical transition

Combination of **rolling angle** and **radius** determine whether the single-walled nanotube is a **metal** or a **semiconductor**

Strong chemical bonding involves sp^2 hybridized carbon orbitals

M. S. Strano et al., Carbon 95, 767 (2015)

Extraordinary mechanical, electrical, optical, and thermal properties

Stringest and stiffest materials

Characteristic 1D properties

Very good thermal conductivity (ballistic)

Nanoparticle characterization

Nanoparticle characterization:
Which techniques ?
What information do we obtain ?

- Structural characterization
  electron microscopy, X-ray diffraction -> size, shape, cristallinity

- Optical properties
  absorption, emission, luminescence excitation -> electronic transitions

- Magnetic properties

- Coating characterization
  infrared absorption, thermogravimetric measurements, elementary characterization -> chemical nature of the coating

- Stability (colloidal stability, particle degradation)

Examples for metallic oxide nanoparticles doped with rare-earth ions :
YVO_4:Eu and **$GdVO_4$:Eu**

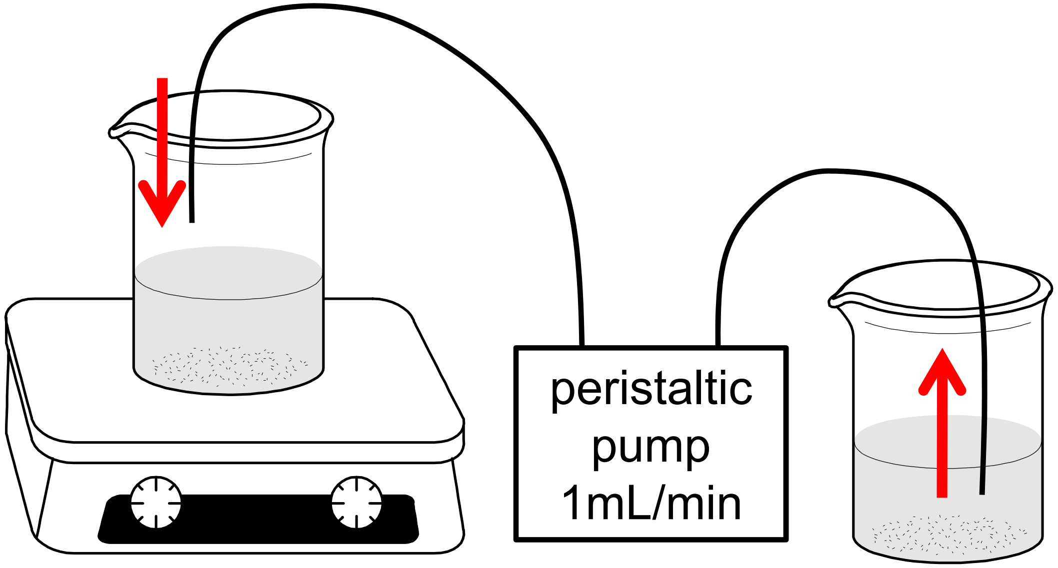
Synthesis of $Y_{0.6}Eu_{0.4}VO_4$ nanoparticles

Soft chemistry

(1980s J. Livage)

Room temperature

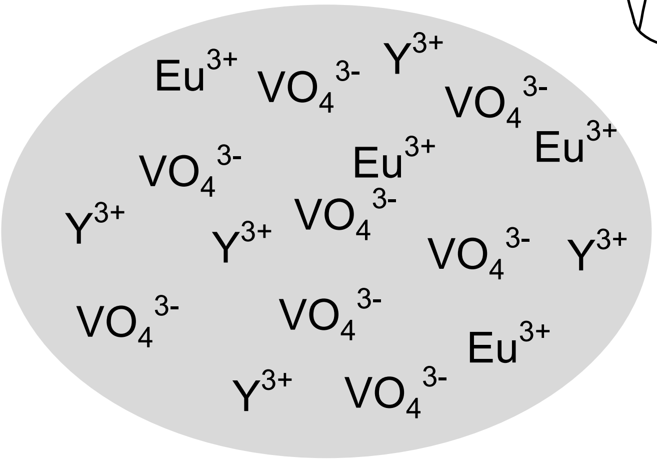
Open reaction vessel



peristaltic pump
1mL/min

0.1 M Na_3VO_4
12.5 ≤ pH ≤ 13.0

0.1 M $RE(NO_3)_3$
(RE: rare earth)
here: RE = 0.6 Y + 0.4 Eu
or 0.6 Gd + 0.4 Eu



Huignard *et al.*, Chem. Mater. (2000) **12**, 1090

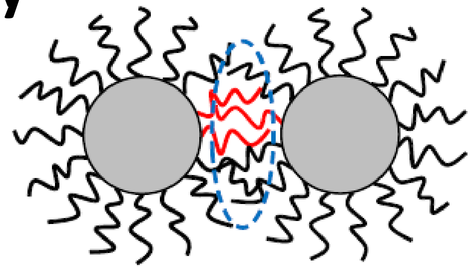
Colloid: insoluble particles suspended in another substance (dispersed+continuous phase)

Colloidal solution: insoluble particles suspended in a solvent

► Nanoparticle
Colloidal solution ->

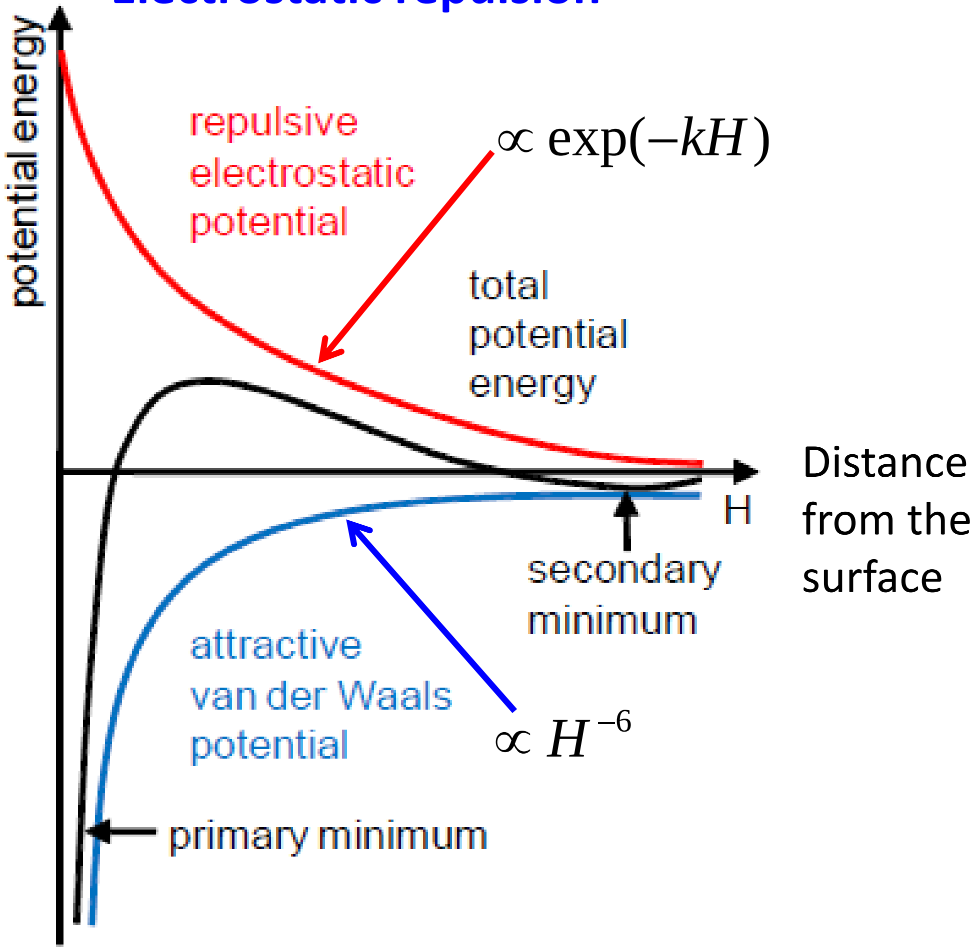
Optical properties Eu
Y->Gd Magnetic properties

Colloidal nanoparticle stability

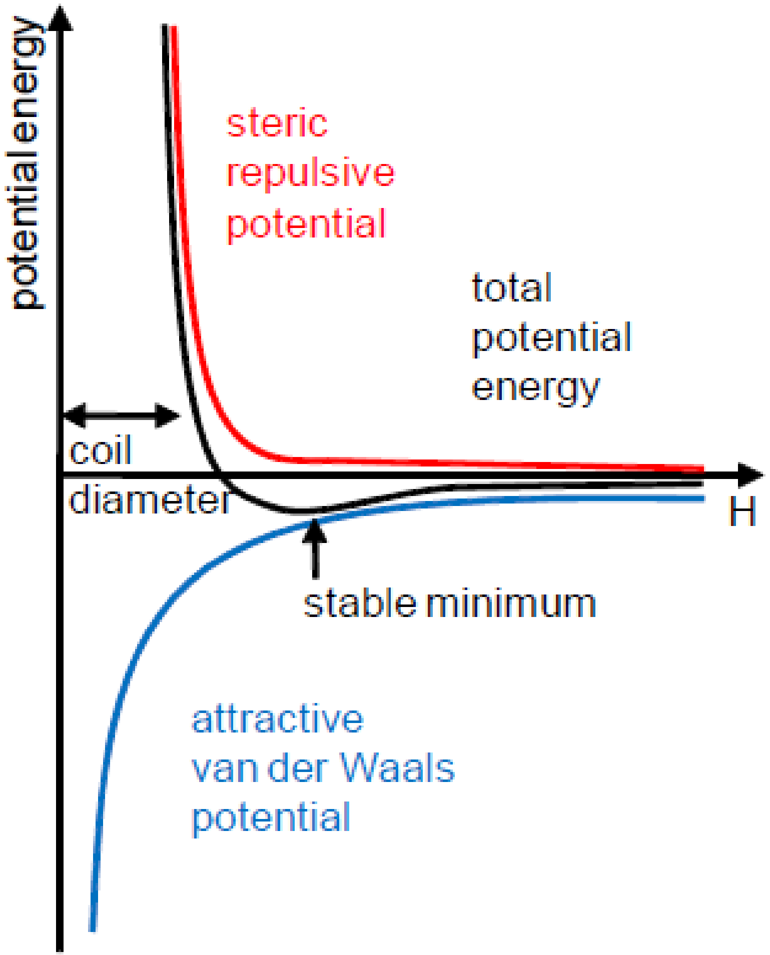


Van der Waals attraction

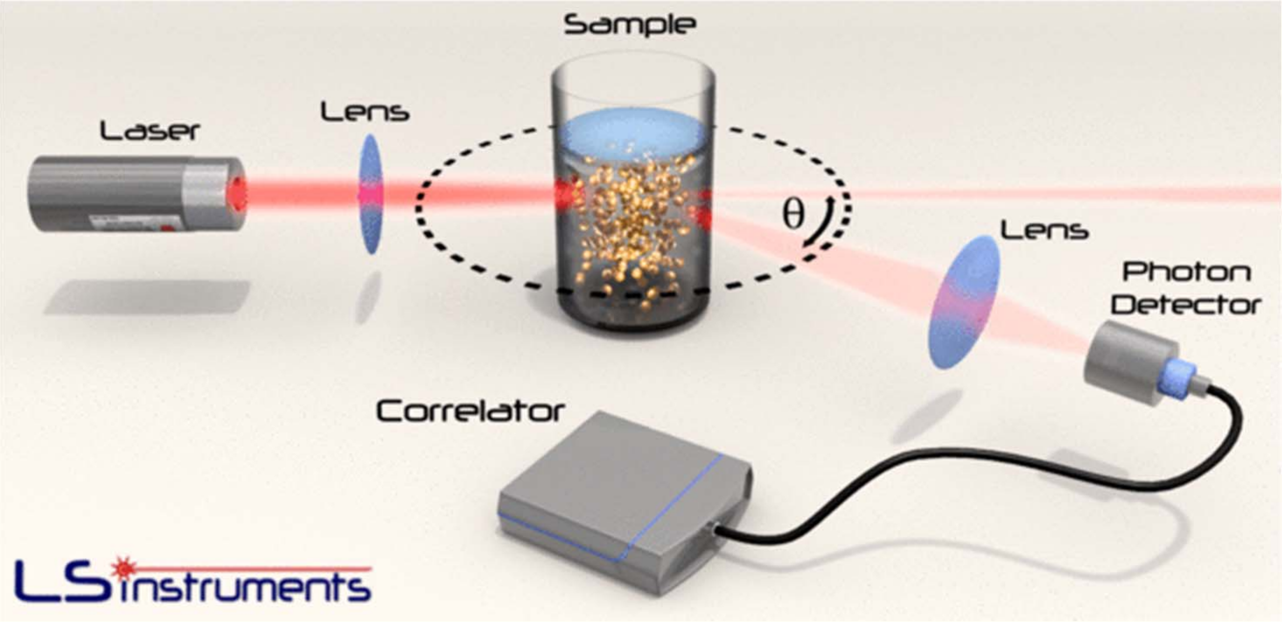
Electrostatic repulsion



Steric hindrance



Dynamic light scattering (DLS)
-> nanoparticle hydrodynamic diameter



Does my sample agreggate?

Autocorrelation of scattered light intensity

<u>Approximate</u> information on:

Nanoparticle size

Polydispersity

Agreggation

$GdVO_4/SiO_2$
Hydrodynamic diameter:

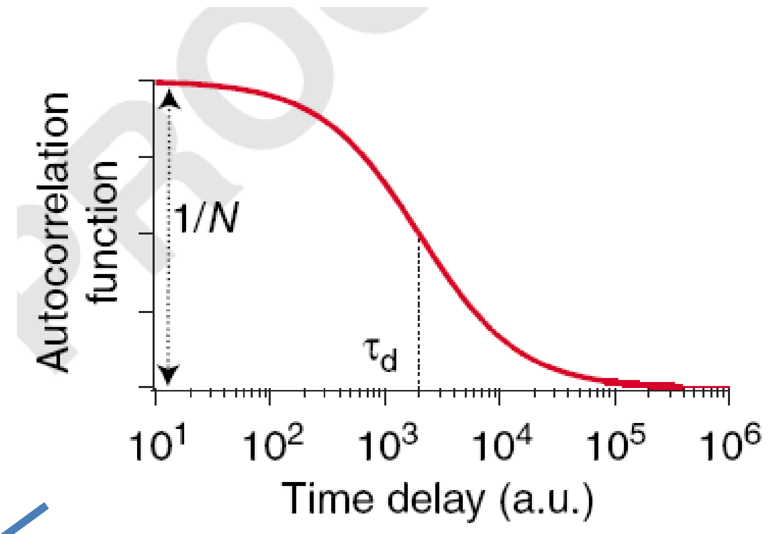
- $d_{<n>}$ = 72 nm
- $\Delta d_{<n>}$ = 21 nm
- Well dispersed nanoparticles

- DLS signal dominated by largest particles in dispersion
- Hydrodynamic diameter
- No information on particle shape

Abdesselem, Schöffel, …Alexandrou, ACS Nano **8**, 11126–11137 (2014)

Dynamic light scattering
Correlation of scattered light

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



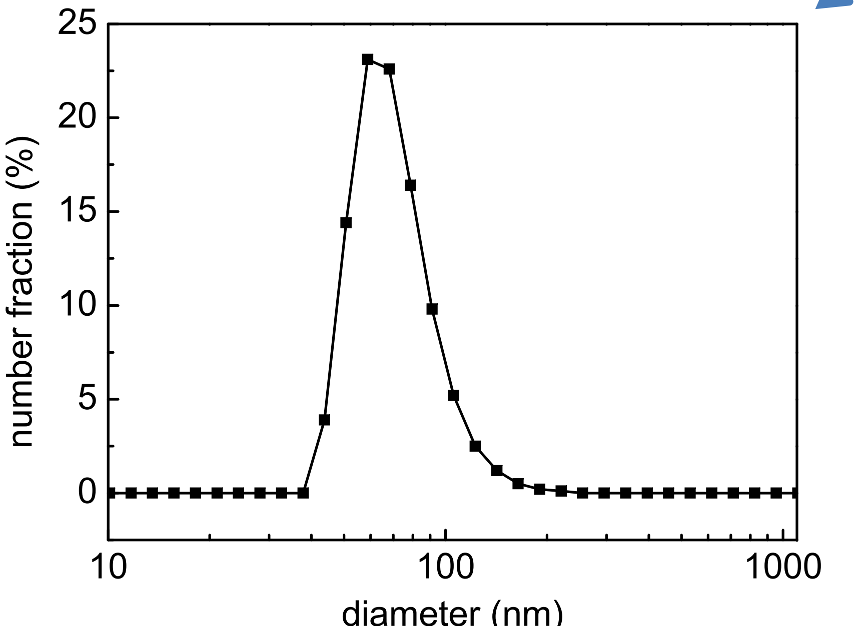
Extract the autocorrelation decay rate Γ

$$\Gamma = q^2 D$$

Wavevector

Depends on the angle at which the detector is placed

Diffusion coefficient



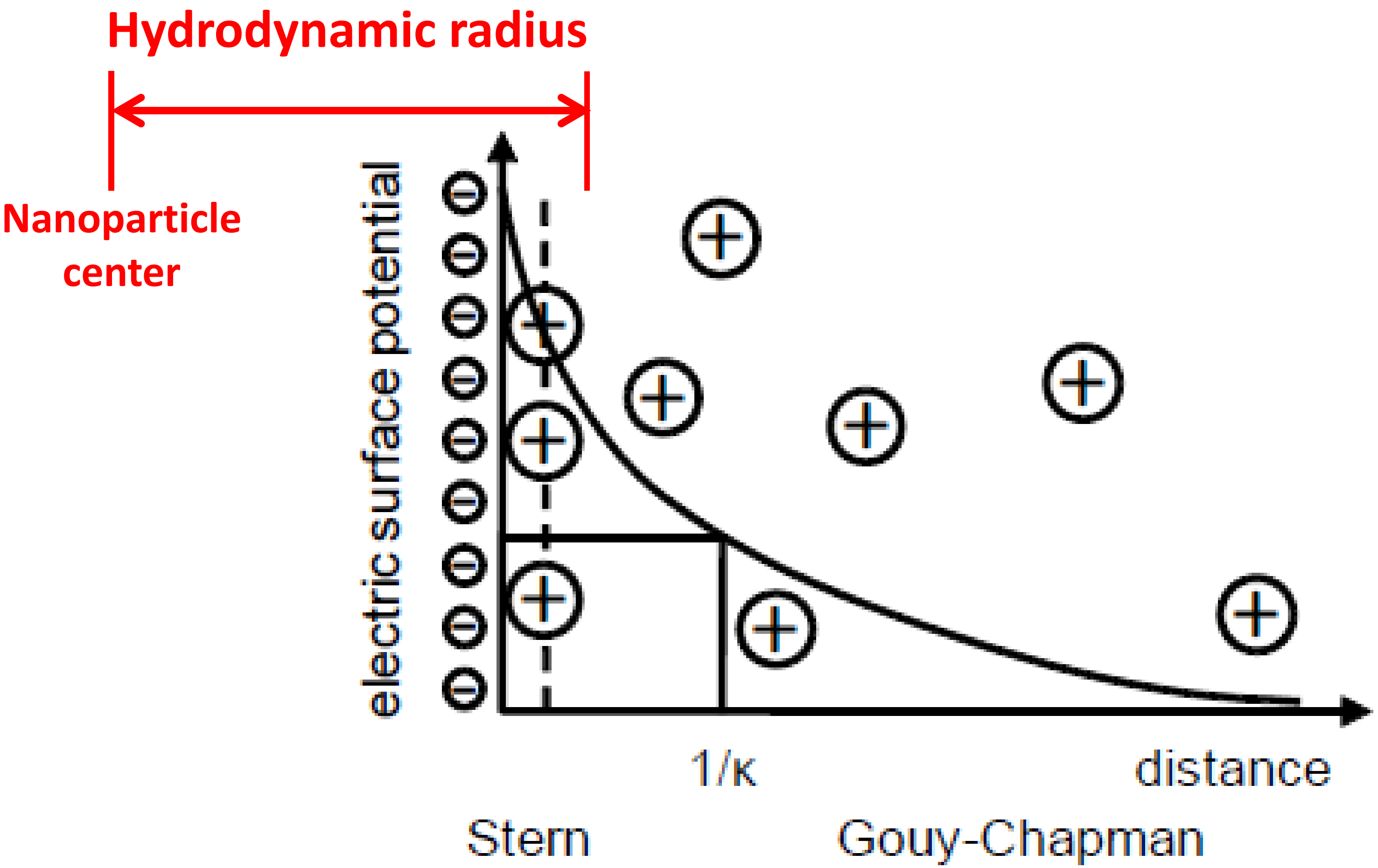
Stokes-Einstein equation

$$D = \frac{k_B T}{6\pi\eta R_h}$$

viscosity

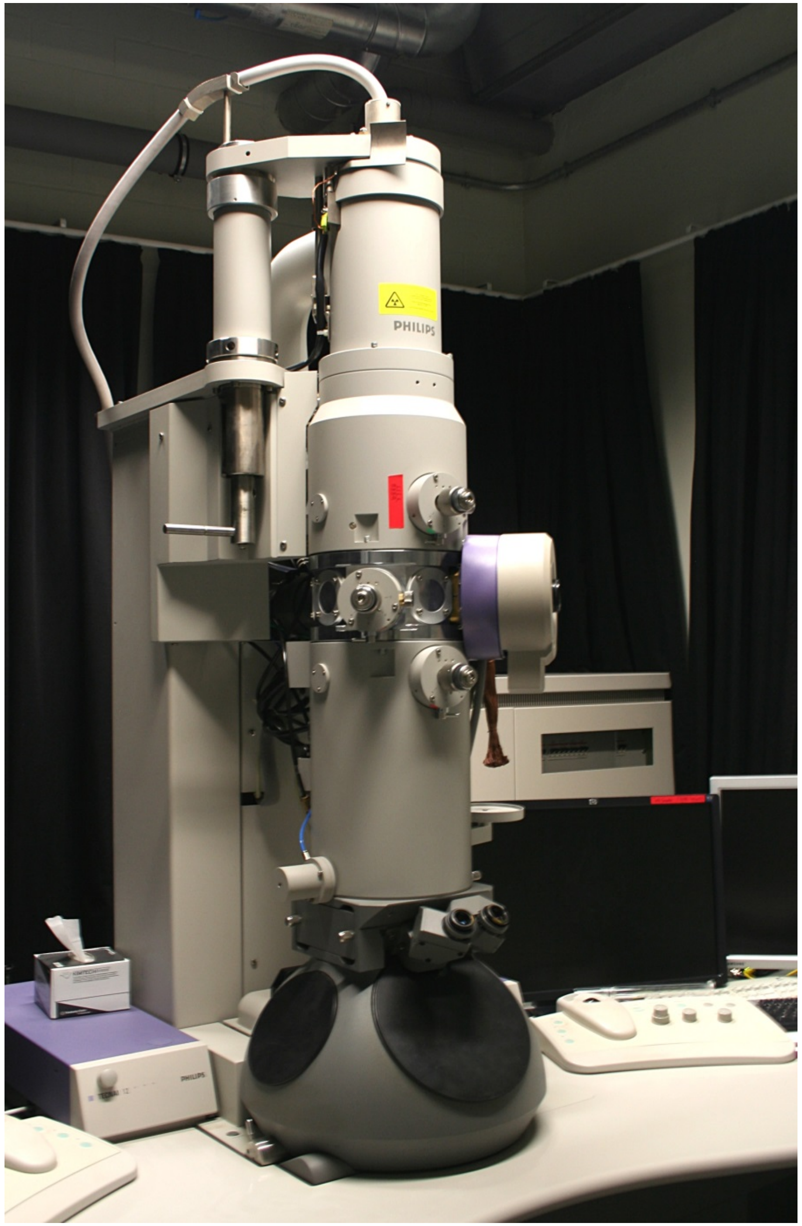
Hydrodynamic radius

Dynamic light scattering (DLS)
-> nanoparticle hydrodynamic diameter



- Hydrodynamic diameter not physical diameter
- No information on particle shape
- Very crude approximative characterization

Electron Microscopy



Philips Transmission Electron Microscope

Optical microscope resolution :

$$\Delta R = 1.22 \frac{\lambda_{photon}}{2\ N.A.}$$

$$N.A. = n \sin\theta$$

Typically : 200 nm

Electron microscope resolution :

$$\Delta R = 1.22 \frac{\lambda_{electron}}{2\ N.A.}$$

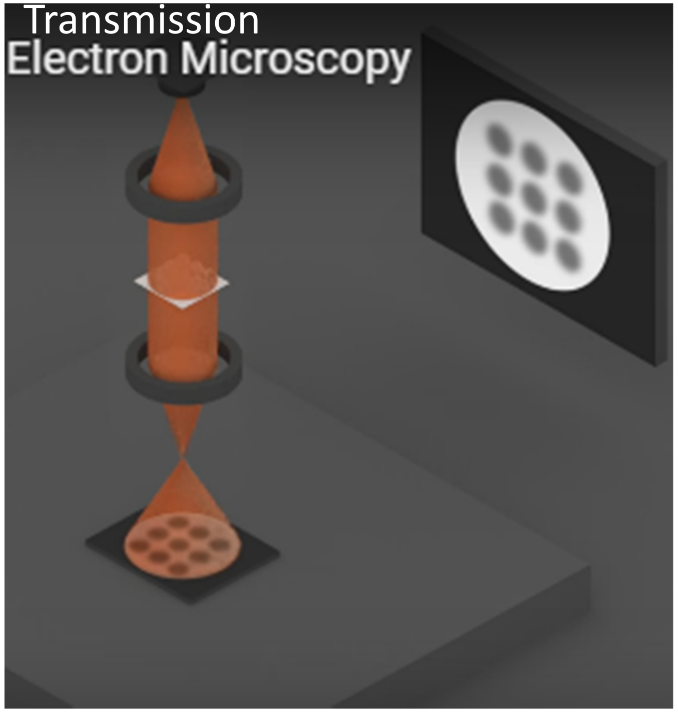
The higher the electron acceleration (5-400 kV), the shorter the electron wavelength $\lambda_{electron}$, the better the electron microscope resolution

Down to 50 pm

Optical lenses -> electromagnetic lenses

Transmission electron microscopy

-> morphological information



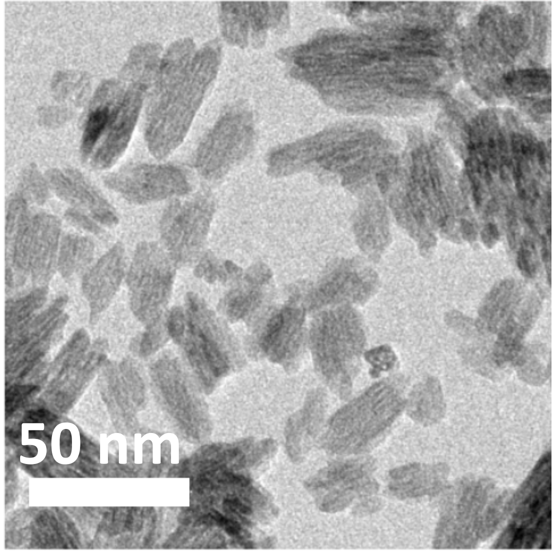
www.quantummadesimple.com
Transmission Electron Microscope

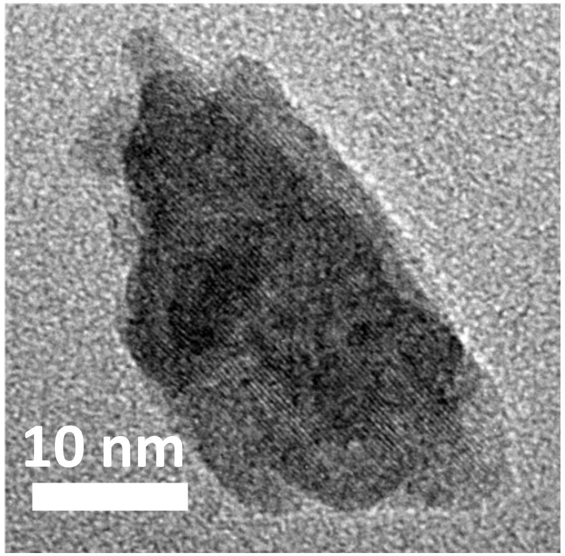
Detection of electrons transmitted through the sample

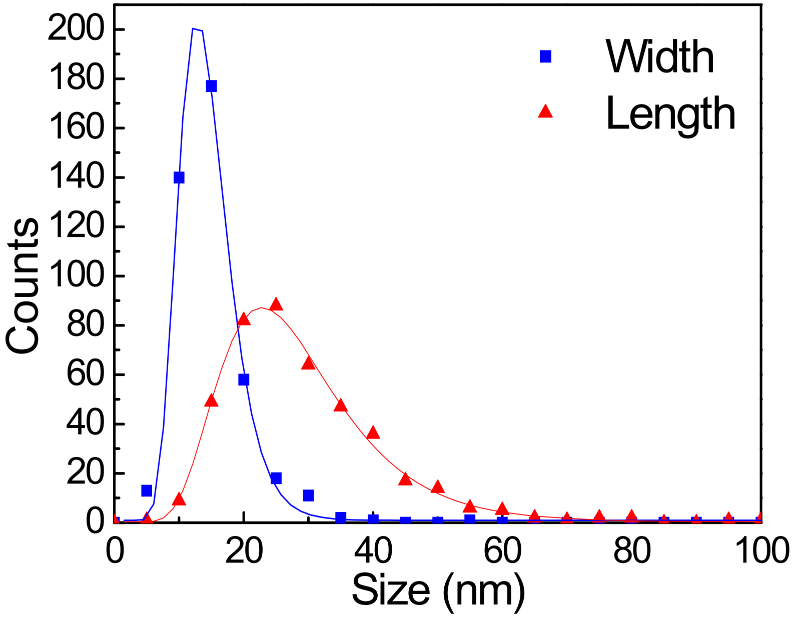
Thin samples required : ~ 100 nm

A solution of the
nanoparticle
solution is deposited
on a carbon grid.

GdVO$_4$ nanoparticles







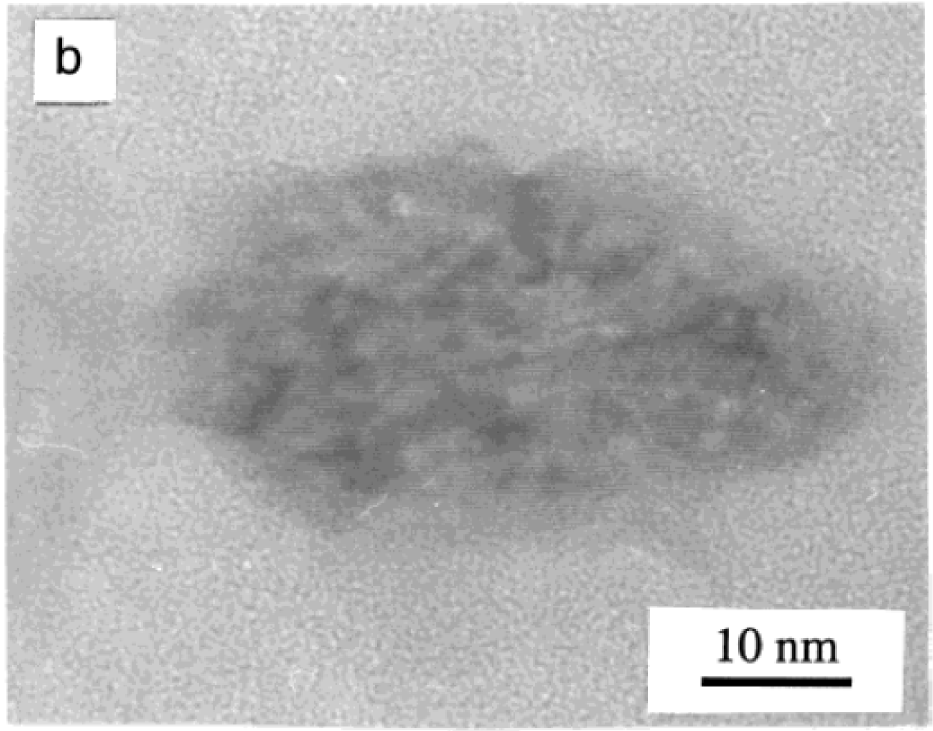
At high resolution, atomic positions can be determined

Transmission electron microscopy

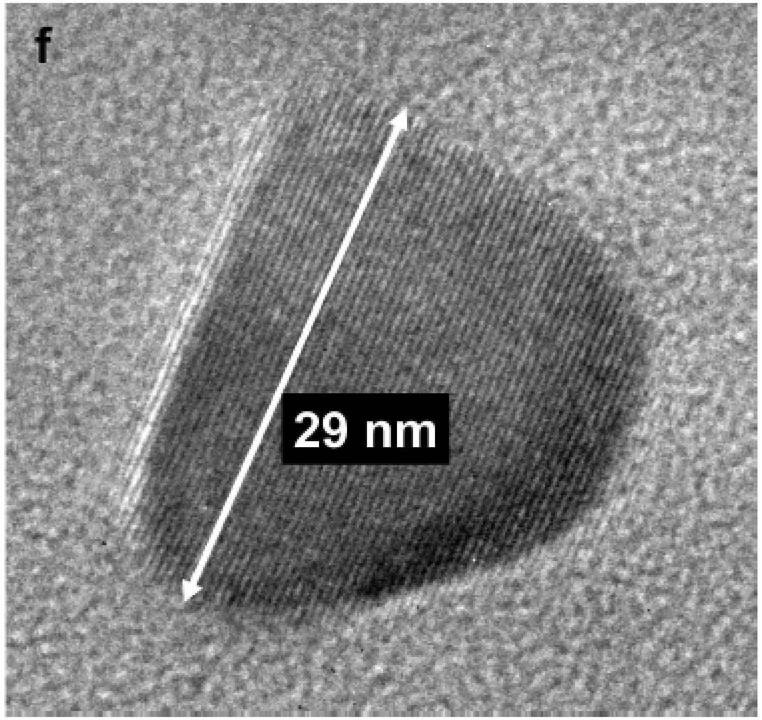
-> structural information

Is my sample cristalline?

Before annealing
YVO_4:Eu (15%)



After annealing at 1000°C
YVO_4:Eu (10%)



-> At high resolution, atomic positions can be determined

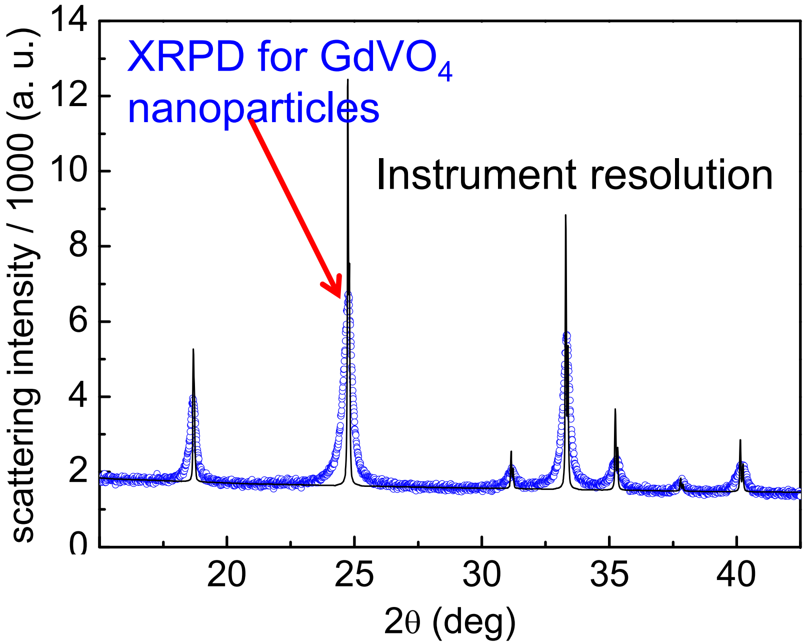
Mialon et al., ACS Nano 2, 2505 (2008)

X-ray powder diffraction (XRPD)

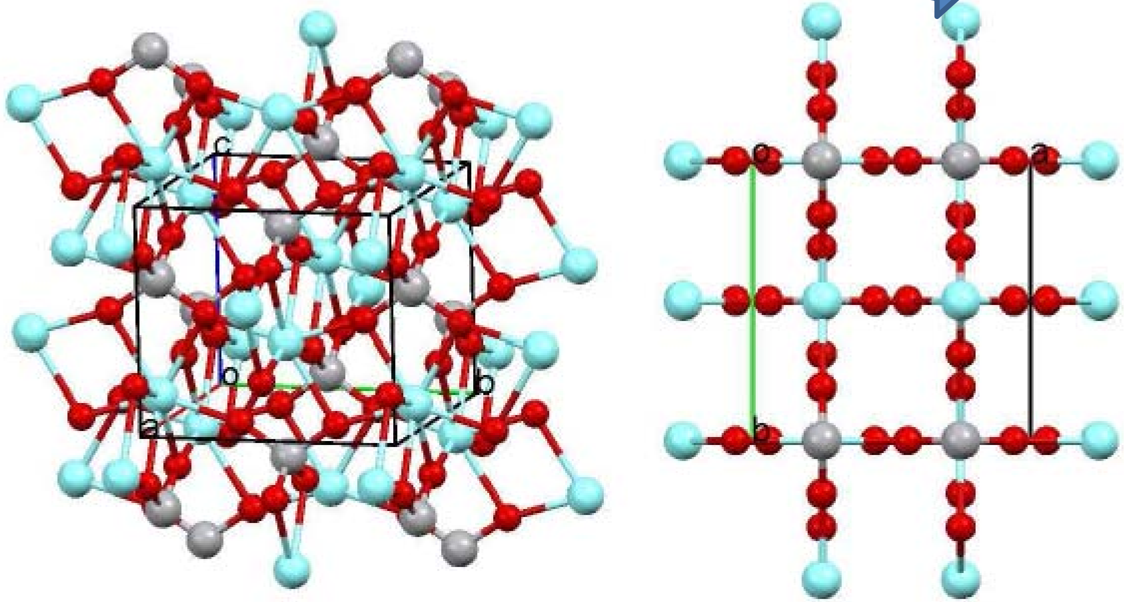
What is the crystal structure of my nanomaterial?

Diffraction peaks from nanoparticle powder

Peak positions -> crystal structure
Peak width -> crystalline coherence length



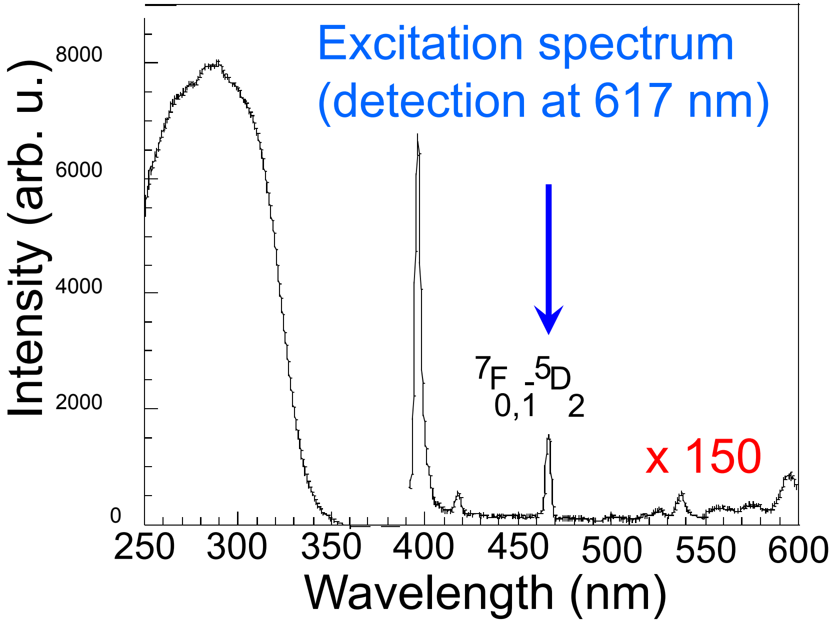
M. Schöffel

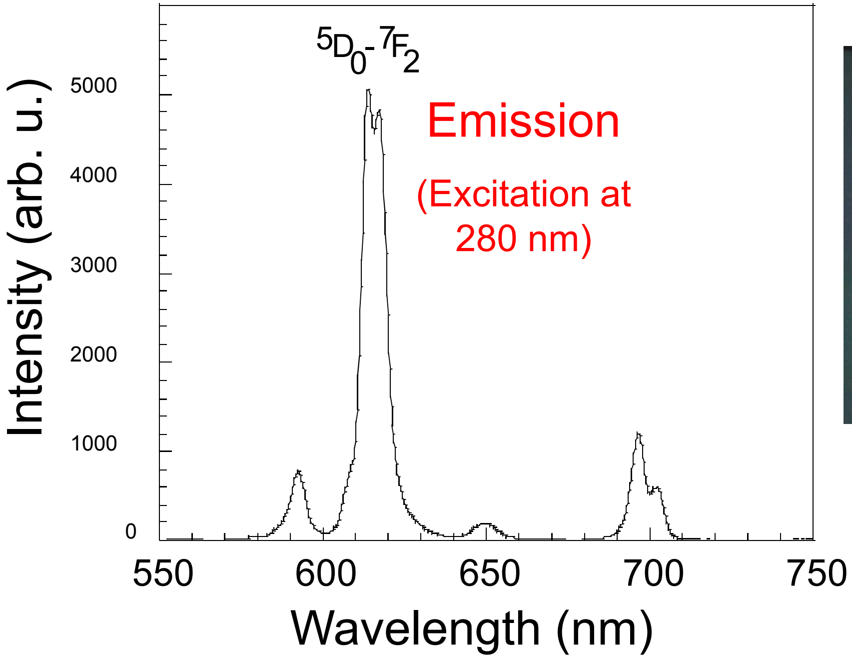


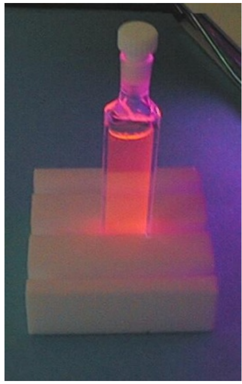
Tetragonal zircon structure
4 Y/Eu and 4 VO_4 per unit cell

Optical properties

Excitation and emission spectra of $Y_{0.8}Eu_{0.2}VO_4$ nanoparticles (20-30 nm)







Excitation:

Argon-ion line 465.8 nm

Solid-state laser Institut d'Optique

Diode laser at 396 nm or at 466 nm

Quantum yield 10-20%

Beaurepaire, …, AA, Nano Lett. **4**, 2079 (2004)

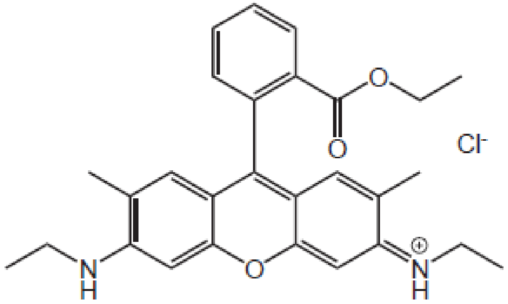
Quantum yield measurements

Radiative recombination rate

Quantum yield $$q = \frac{k_r}{k_r + k_{nr}} = \frac{\text{Emitted photon number}}{\text{Absorbed photon number}}$$

Non-radiative recombination rate

Comparison with a fluorophore of known quantum yield



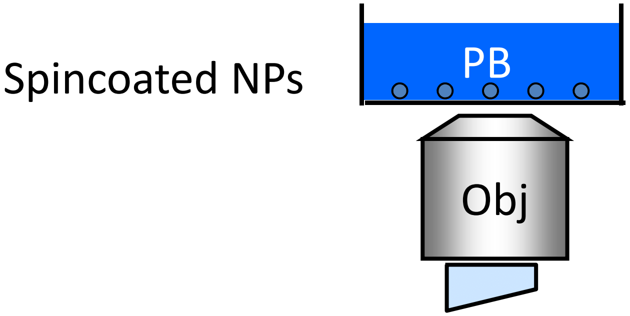
Rhodamine 6G (R6G)
q=90% at room temperature in water

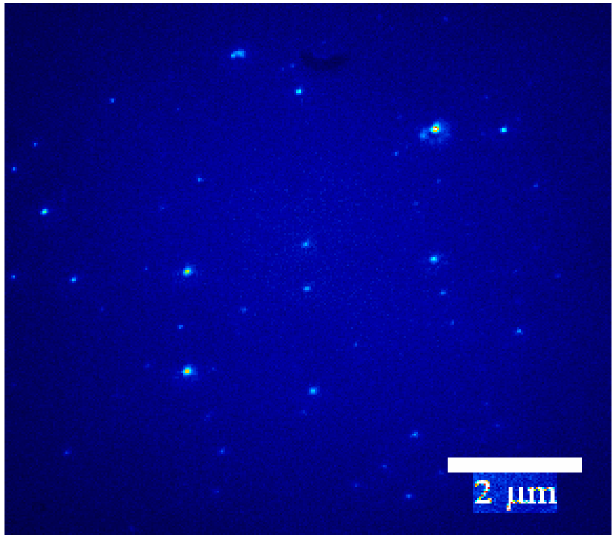
$$\left.\begin{aligned} \frac{q_{NP}}{q_{R6G}} &= \frac{\mathrm{S}_{\mathrm{NP}} / A_{NP}^{280\mathrm{nm}}}{\mathrm{S}_{\mathrm{R6G}} / A_{R6G}^{280\mathrm{nm}}} \\ \mathrm{S}_{\mathrm{R6G}} &= cA_{R6G}^{280\mathrm{nm}} \end{aligned}\right\} \quad q_{NP} = q_{R6G} \frac{\mathrm{S}_{\mathrm{NP}}}{cA_{NP}^{280\mathrm{nm}}}$$

The concentration of the R6G sample does not need to be known.

Optical properties

Single-particle detection with a wide-field fluorescence microscope





Advantages:

- **Synthesized in water** (salt coprecipitation)
- Photostability
- **No blinking** (18000 Eu ions in a 20-nm 40%-doped nanoparticle)
- **Narrow emission bandwidth** (<10 nm)
- Possibility of multi-color experiments
- **Long excited-state lifetime** (~0.7 ms)

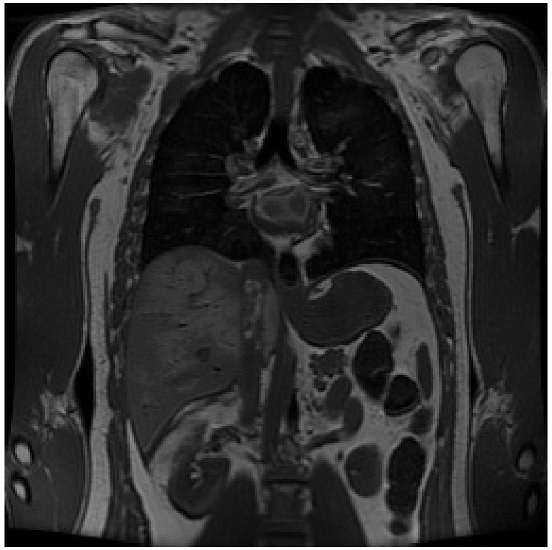
=> time-gated detection

-> single particle detection

ε(466 nm) = 50000 $M^{-1}.cm^{-1}$ for 30-nm 40%-Eu nanoparticles

Magnetic resonance imaging (MRI)

www.medical.siemens.com

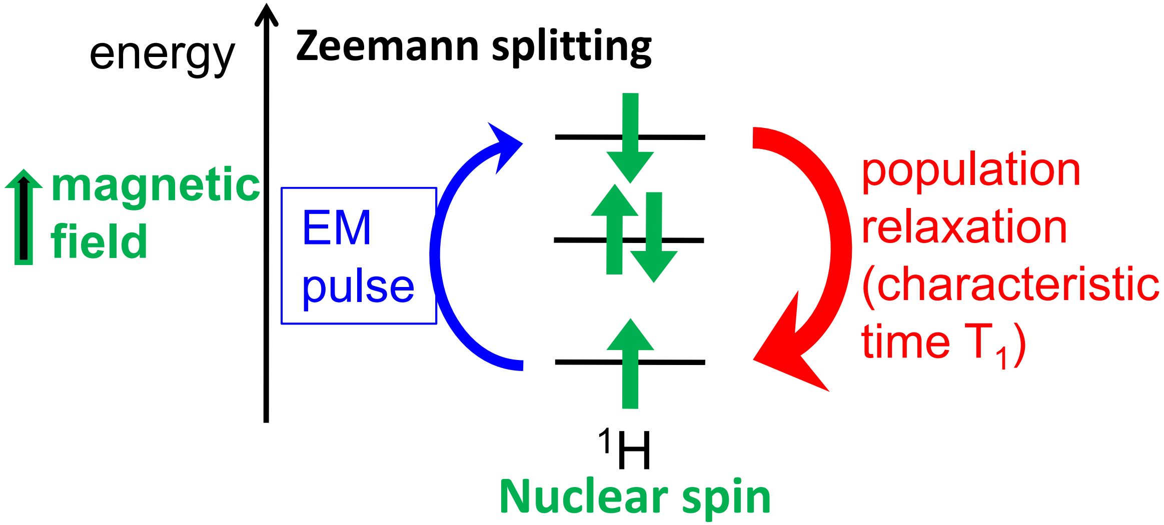


Human male abdomen, T_1 weighted,
U. S. National Library of Medicine

Imaging types

- Angiography
- Organ imaging
- Tumor imaging
- …

Water proton in a magnetic field



energy

Zeemann splitting

magnetic field

EM pulse

population relaxation (characteristic time T_1)

^1H

Nuclear spin

- Measurement of ^1H relaxation time
  - T_1: population relaxation
  - T_2: coherence relaxation
- $T_{1,2}$ vary with environment

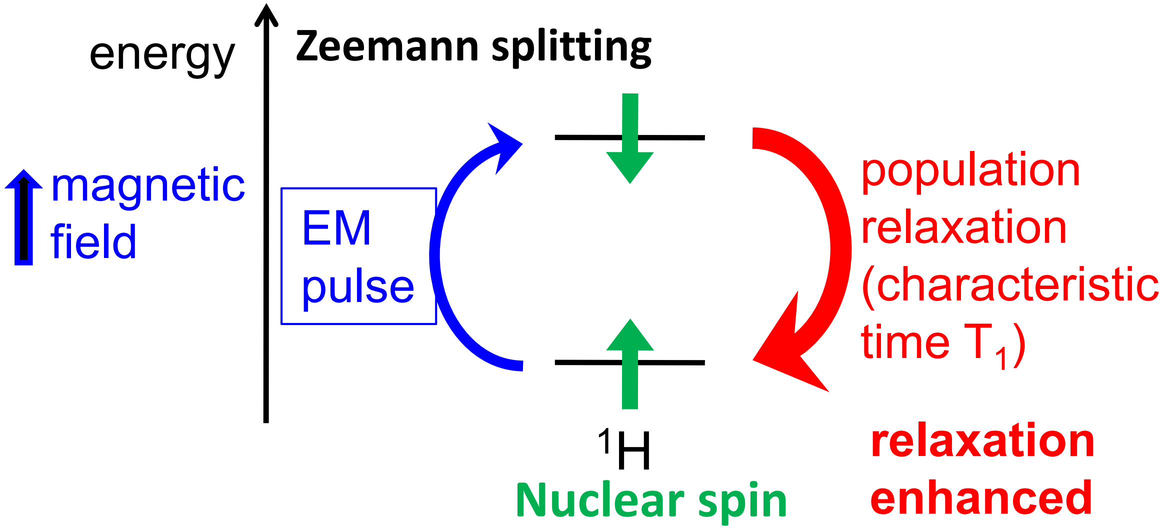
- **Different ^1H relaxation times in water and lipids**

► Contrast

► Image

Principle of action of a contrast agent for MRI

Water proton in a magnetic field



energy

Zeemann splitting

magnetic field

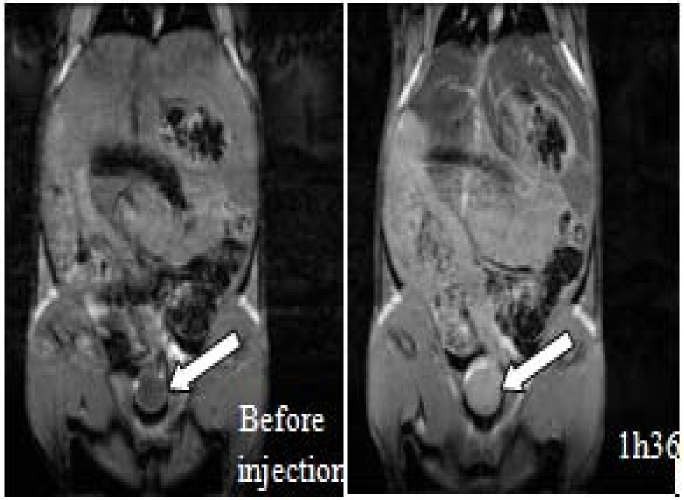
EM pulse

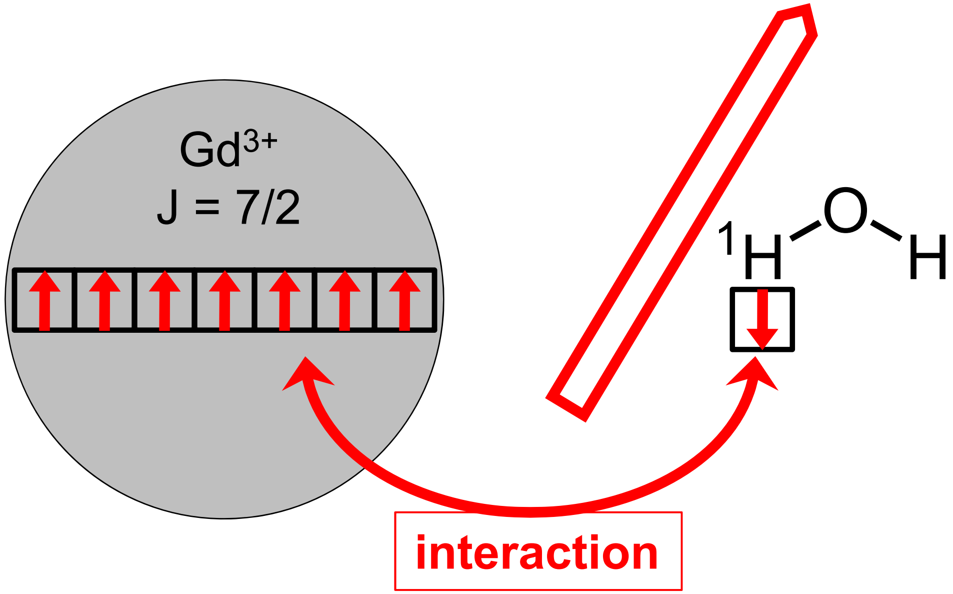
population relaxation (characteristic time T_1)

^1H

Nuclear spin

relaxation enhanced





Gd^{3+}

J = 7/2

^1H–O–H

interaction

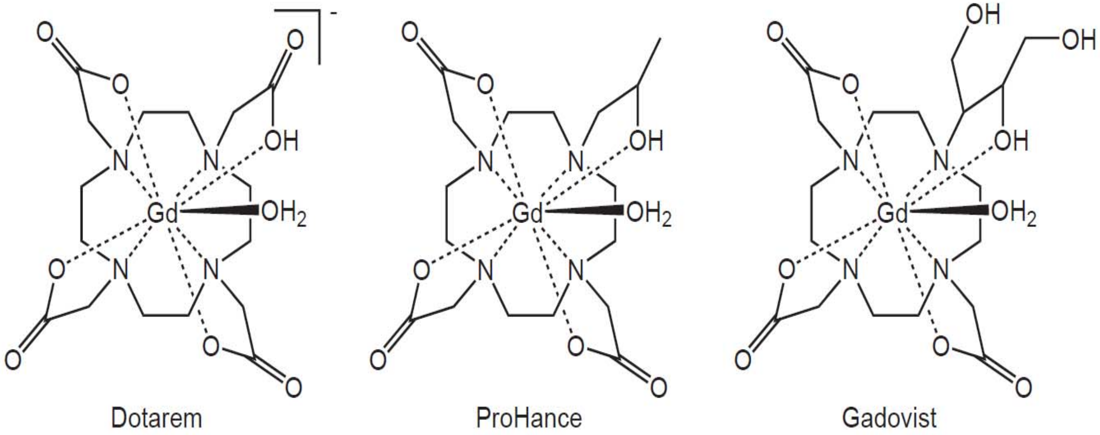
Interaction of water protons with paramagnetic material enhances relaxation

Material of choice: Gd^{3+}

- ► 7 unpaired electrons
- ► High magnetic moment in a magnetic field

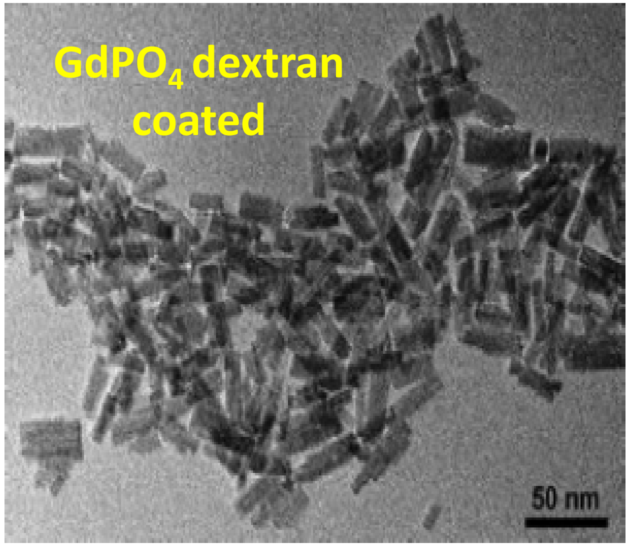
Gd-based MRI contrast enhancers

Gadolinium-based MRI contrast enhancers:



FDA approved

Caravan *et al.* Chemical reviews (1999)9;2293-2352



Hifumi *et al.*, J. Mater. Chem. (2009) **19**, 6396

Advantages of Gd^{3+} nanocrystals over Gd^{3+} chelates

- Higher contrast enhancement
- Long blood circulation time
- Multiple functionalizations
- Less toxic Gd^{3+} leaching

BUT !

Possible accumulation in the body

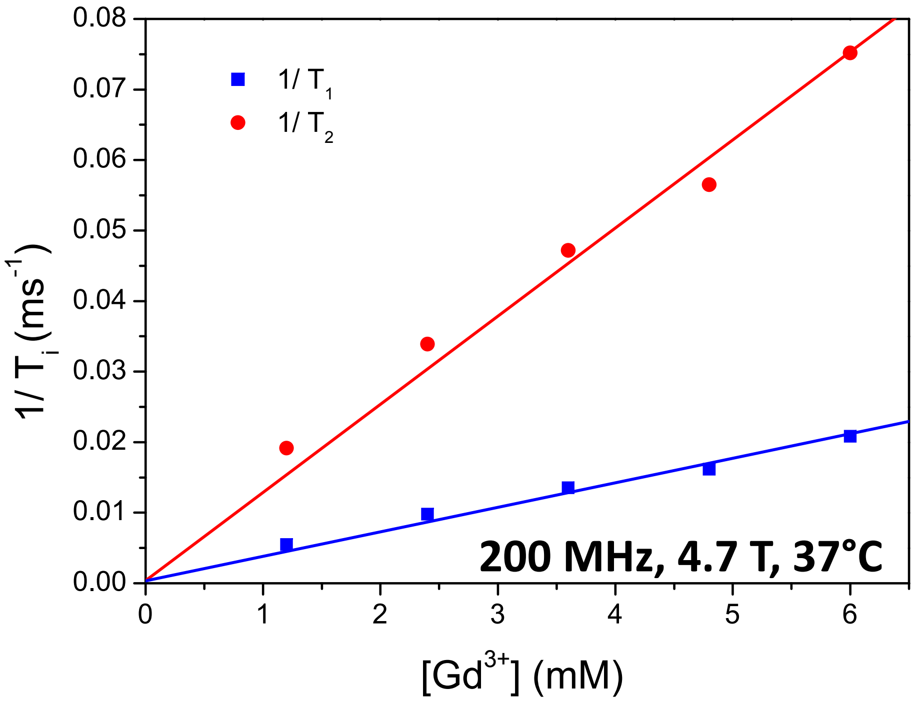
Abdesselem, …, Bouzigues, AA, ACS Nano **8**, 11126–11137 (2014)

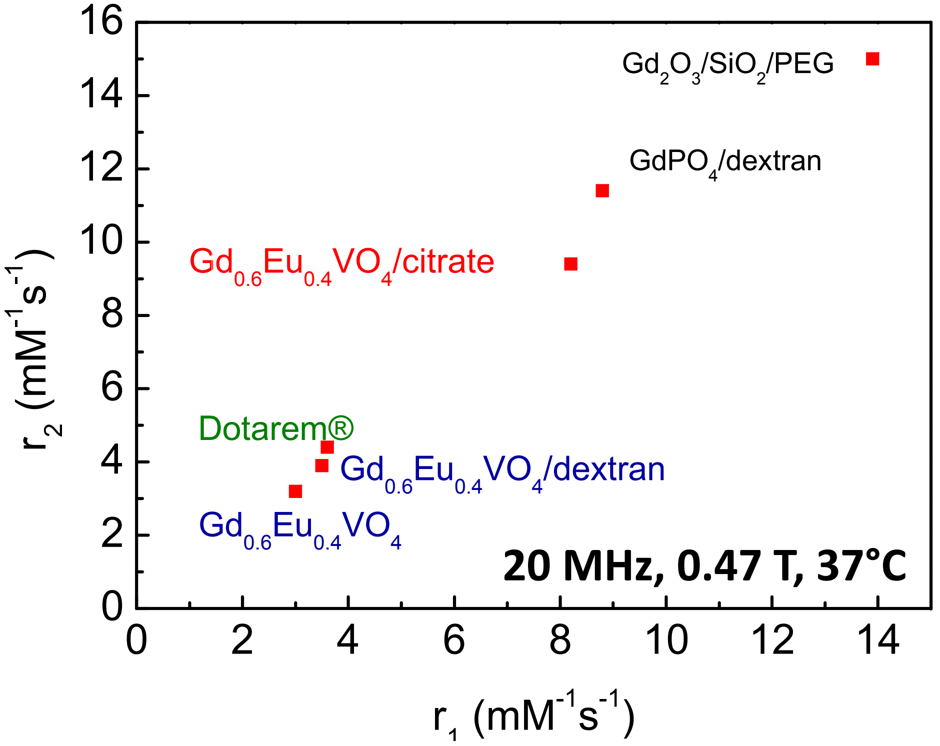
Efficiency of the nanoparticles as contrast agents for MRI

Relaxivity

$$r_{1,2} = \frac{1}{[Gd]} \times (\frac{1}{T_{1,2}} - \left(\frac{1}{T_{1,2}}\right)_{eau})$$

Higher relaxivities than commercial contrast agents (DOTAREM)





Abdesselem, …, Bouzigues, AA, ACS Nano **8**, 11126–11137 (2014)

Important issues
Coupling nanoparticles to biological molecules

Step 1
Functionalize the nanoparticle/nanomaterial surface with active groups like -COOH, -NH_2, -maleimide, …

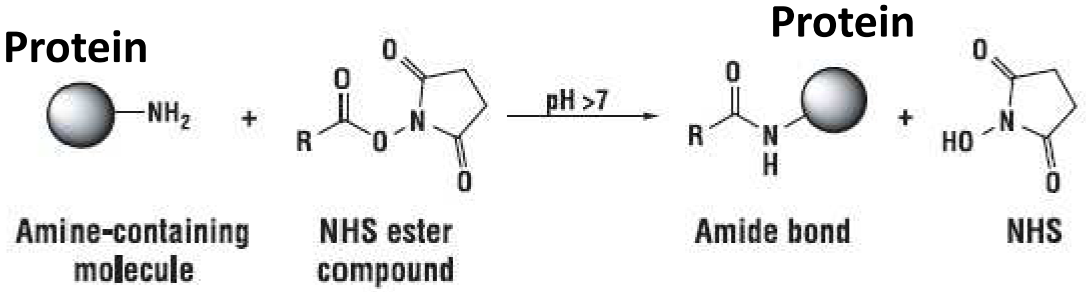
Step 2
Adapt coupling schemes between active groups and biomolecules

Colloïdal suspensions must remain stable !

Coupling luminescent labels to proteins

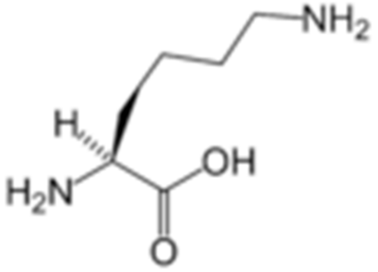
R is the labeling nanoparticle

Coupling to amine groups



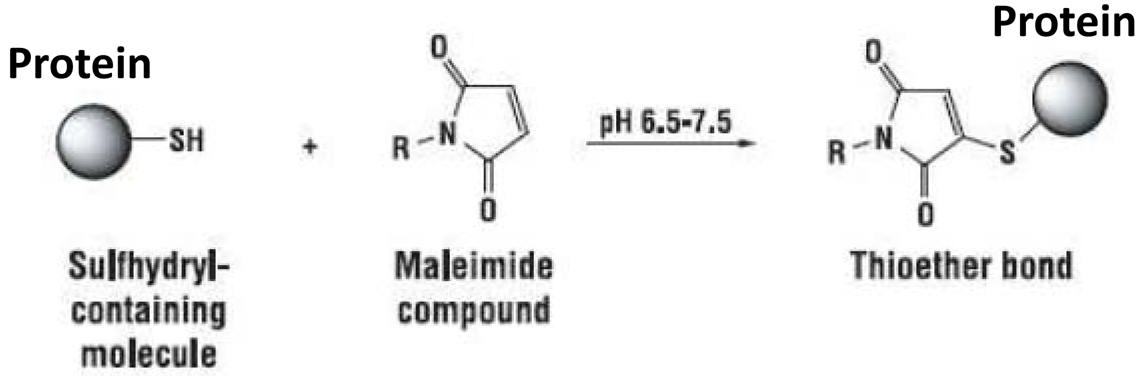
NHS: N-hydroxysuccinimide (activating reagent for carboxylic acids)
Water-soluble analog: sulfo-NHS

Five amino acids contain amine groups. NHS-esters react mainly with Lysine

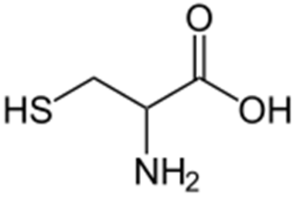


Terminal –NH_2 group can also react.

Coupling to thiol groups



Only one amino acid contains a thiol group Cysteine

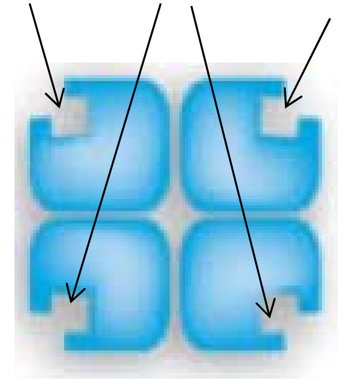


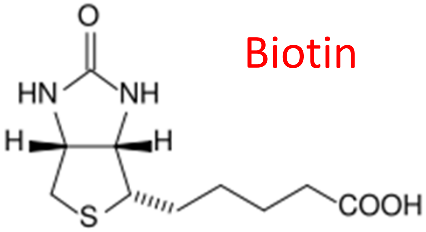
Coupling strategies

Streptavidin-biotin interaction

The egg-white protein, avidin, and its bacterial analog, streptavidin, are tetrameric proteins with four binding sites for the vitamin biotin.

Biotin binding sites





Biotin

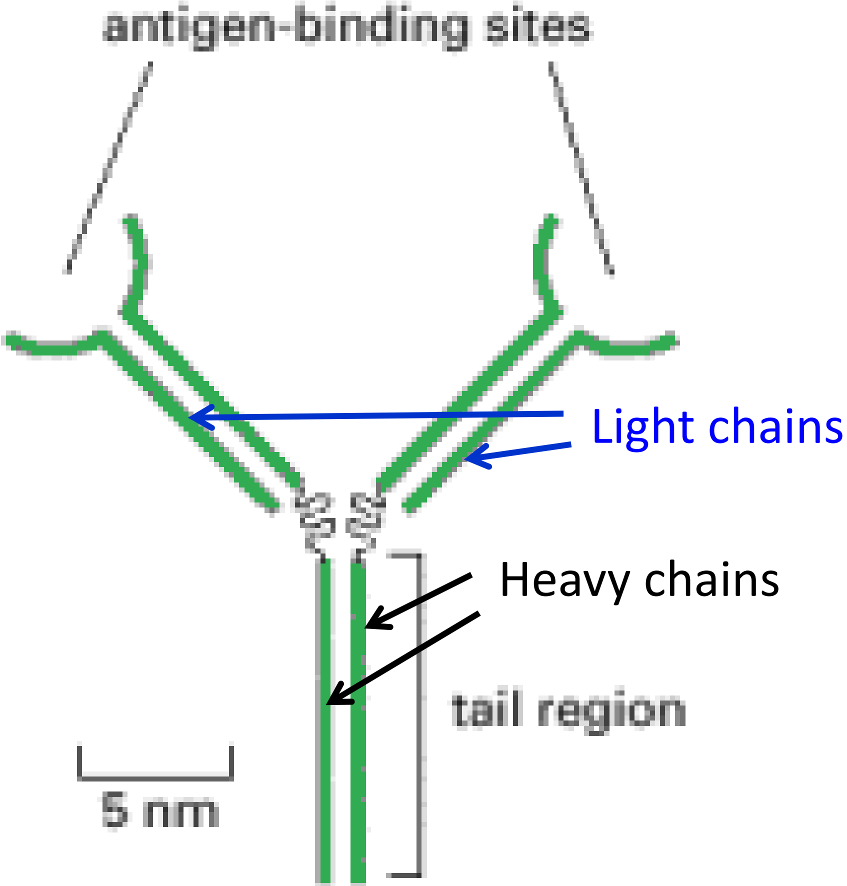
The streptavidin-biotin interaction is one of the strongest known non-covalent interactions: dissociation constant K_d=10^{-15} M

$$[P] + [L] \rightleftarrows [C] \qquad K_d = \frac{[P][L]}{[C]}$$

Protein **L**igand protein-ligand **C**omplex

Coupling strategies

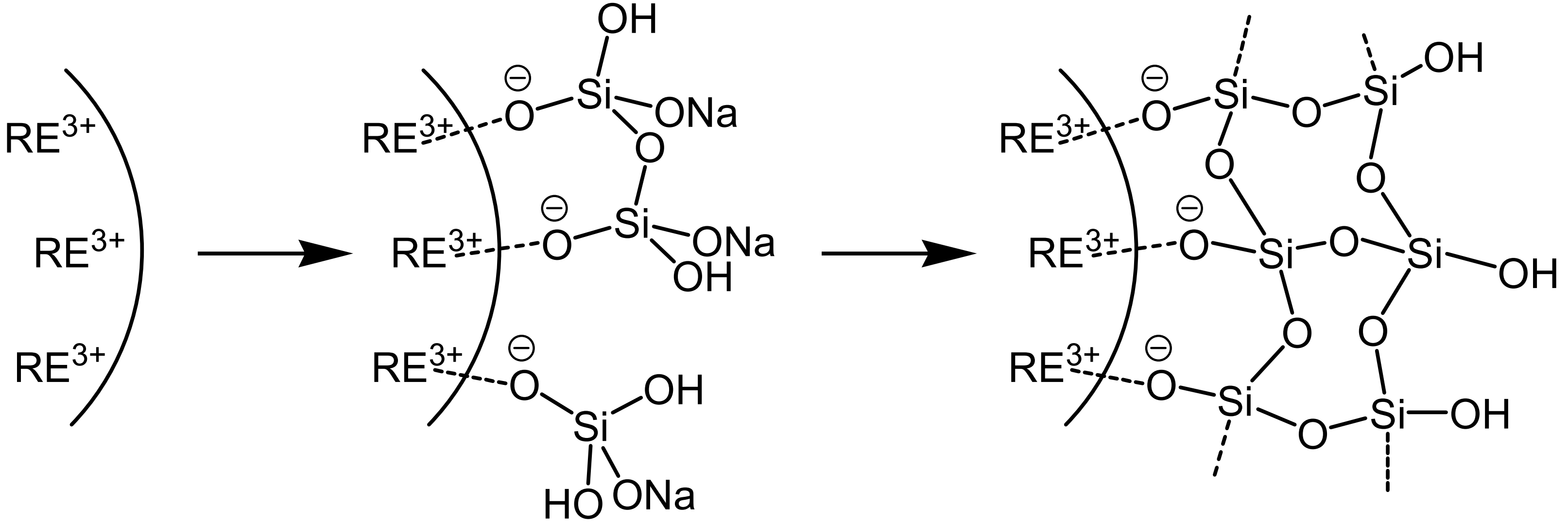
Antigen-antibody interaction



Antibody

<u>Important</u>: verify that the biomolecule remains functional after coupling.

Coating the nanoparticles
Silica coating of $Y_{0.6}Eu_{0.4}VO_4$ nanoparticles

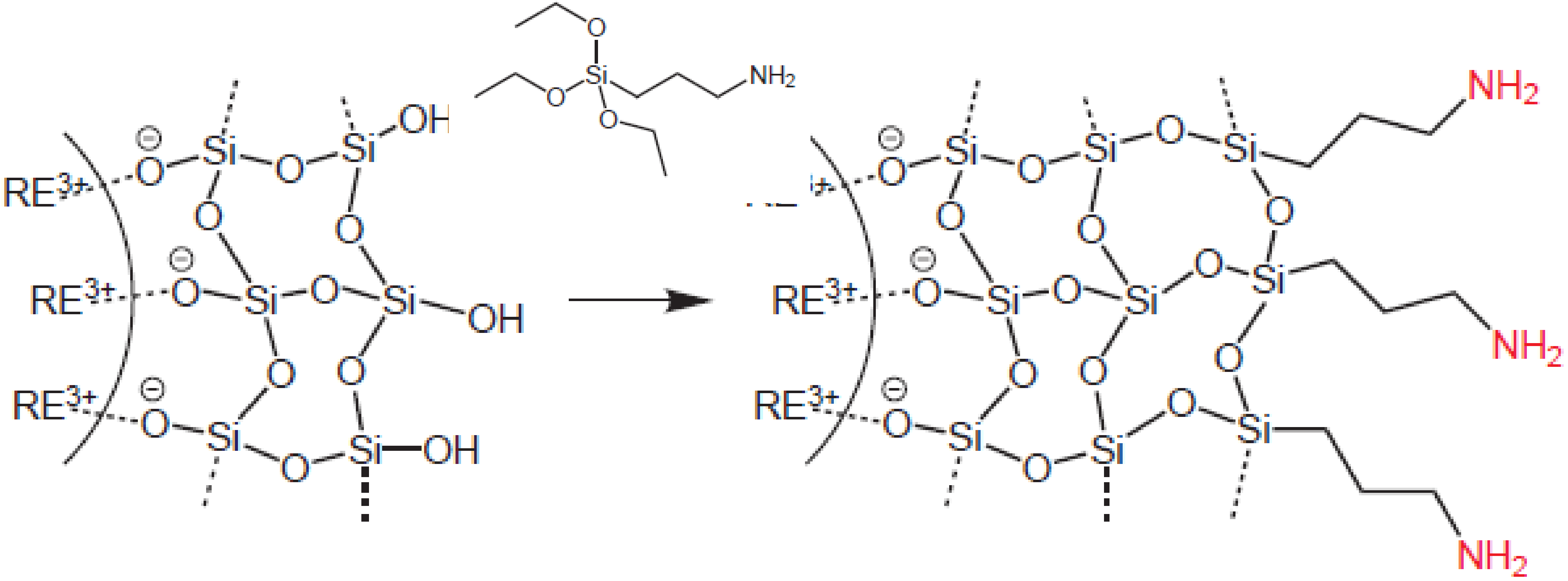


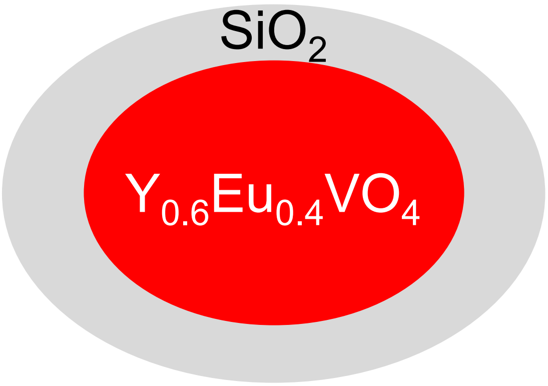
►Silica-coated nanoparticles

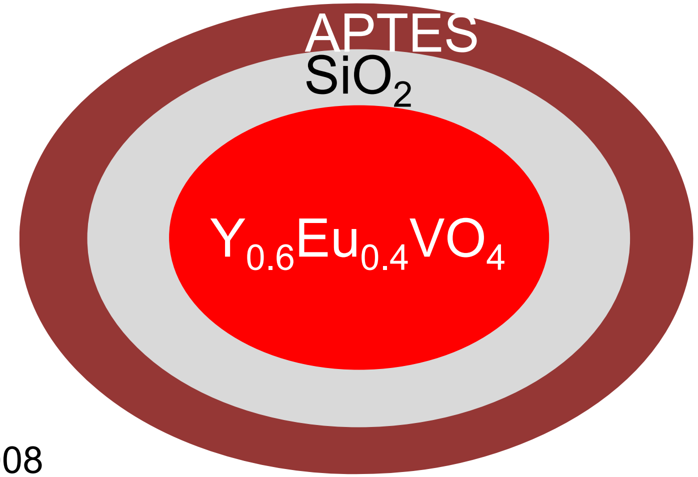
Giaume et al., Langmuir 2008

APTES coating of $Y_{0.6}Eu_{0.4}VO_4/SiO_2$ nanoparticles

APTES: aminopropyltriethoxysilane

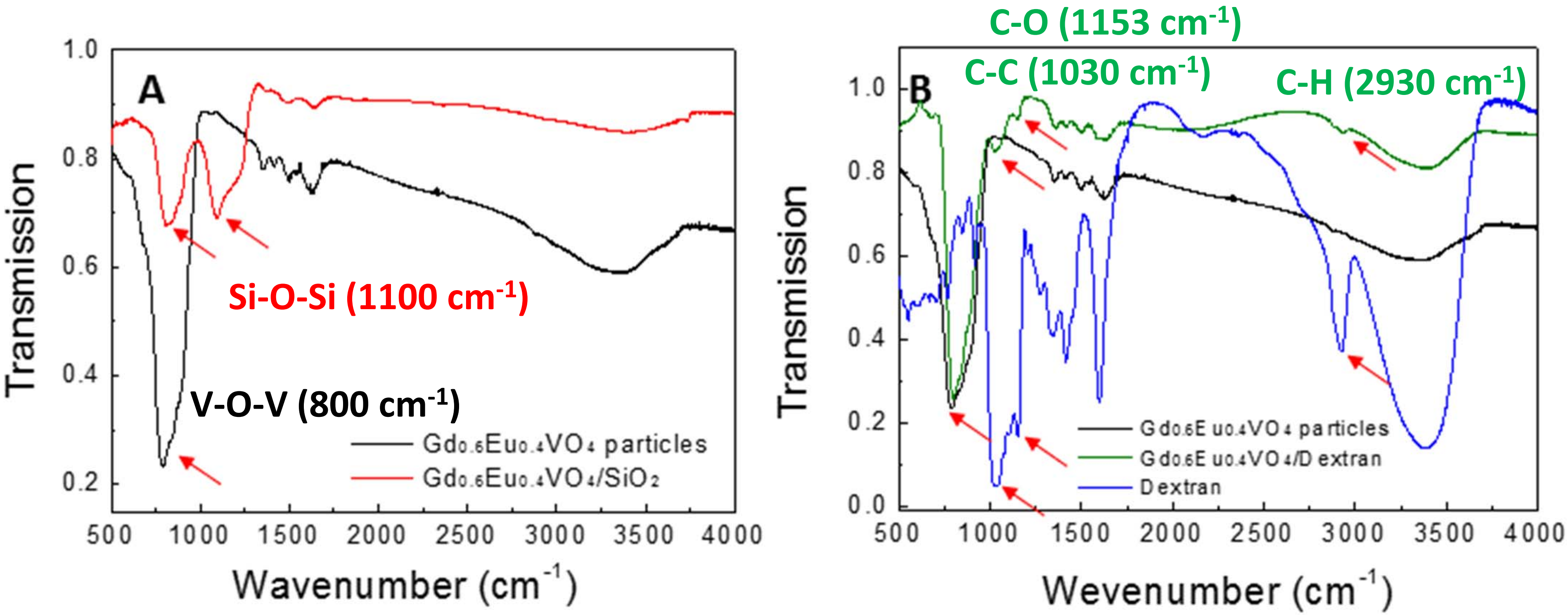






Giaume et al., Langmuir 2008

Nanoparticle and coating composition
Infrared absorption characterization



Si-O-Si (1100 cm^{-1})

V-O-V (800 cm^{-1})

C-O (1153 cm^{-1})

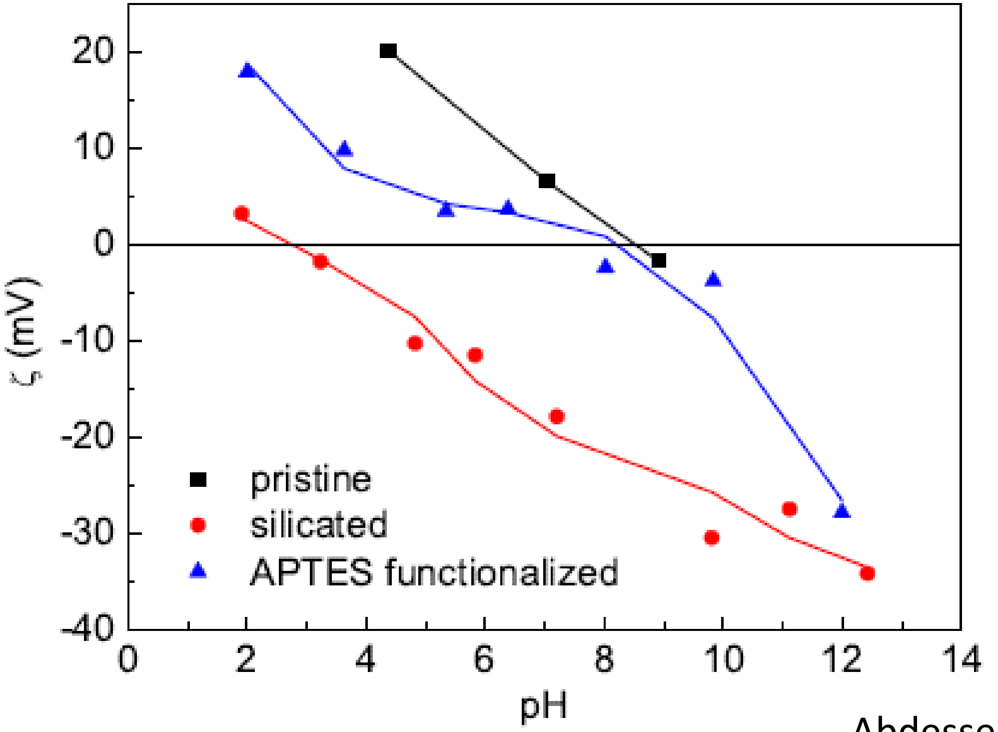
C-C (1030 cm^{-1})

C-H (2930 cm^{-1})

Abdesselem et al. ACS Nano **8**, 11126–11137 (2014)

Surface characterization
ζ-potential

Composition	d <n> (nm)	PdI	Zeta Potential (mV)
$Gd_{0.6}Eu_{0.4}VO_4$	54	0.12	8.6
$Gd_{0.6}Eu_{0.4}VO_4/SiO_2$	69	0.13	-31
$Gd_{0.6}Eu_{0.4}VO_4$/Dextran	72	0.11	-31



Isoelectric point:
pH for which ζ=0

Abdesselem et al. ACS Nano **8**, 11126–11137 (2014)

Introduction to biology

DNA (deoxyribonucleic acid), RNA (ribonucleic acid) and their building blocks: nucleotides

DNA contains the genetic code of each organism.

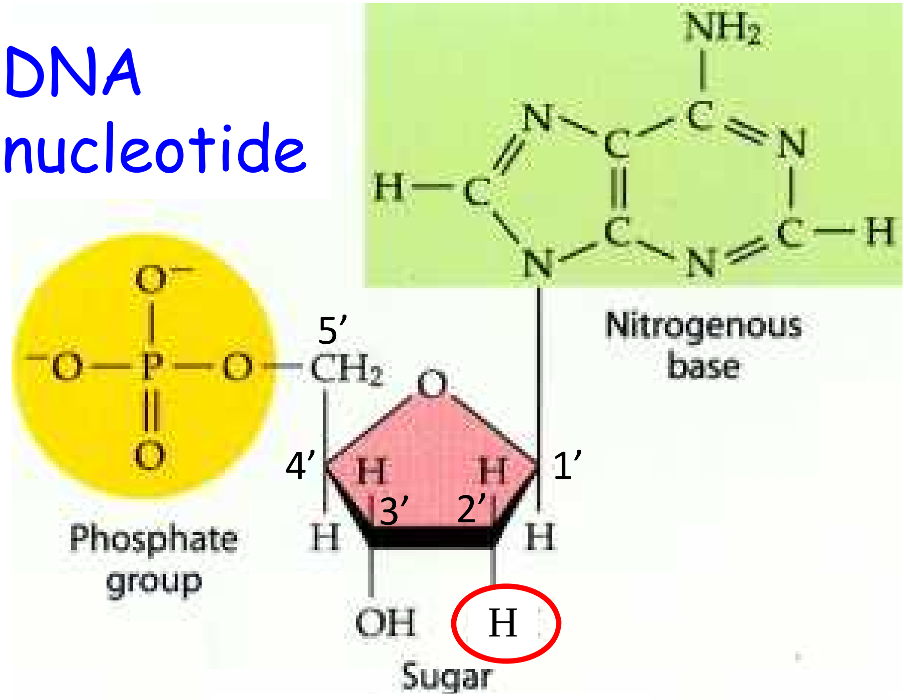
DNA and RNA are made of a sequence of nucleotides.

Nucleotides consist of 3 parts :

- a 5-carbon sugar (pentose)
- a nitrogenous base (four types)
- one or more phosphate groups (DNA and RNA nucleotides have one phosphate)

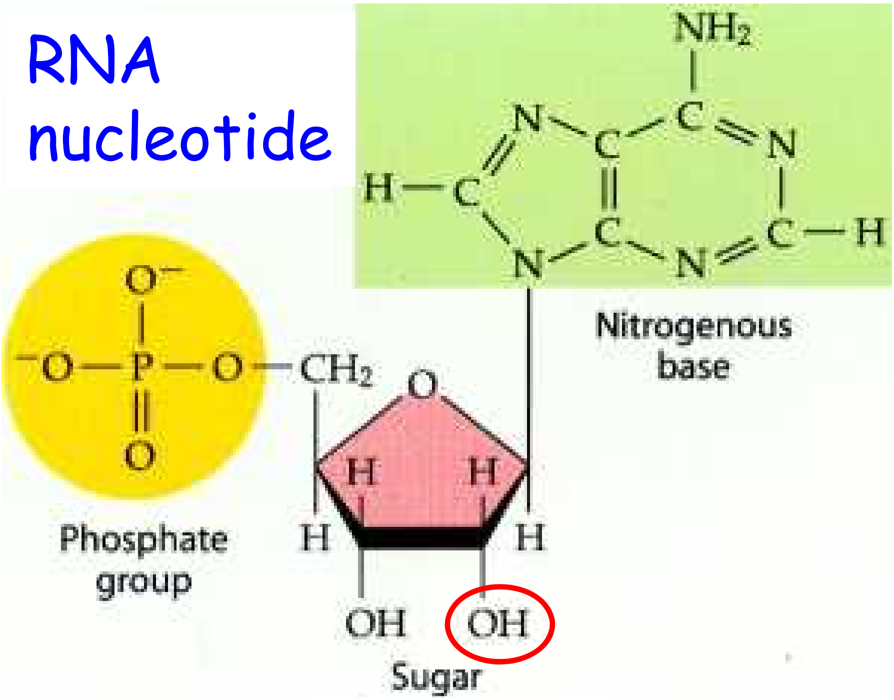
Nucleotides are joined to each other by phosphoester bonds to form strands.

DNA nucleotide



2′-deoxy-D-ribose

RNA nucleotide

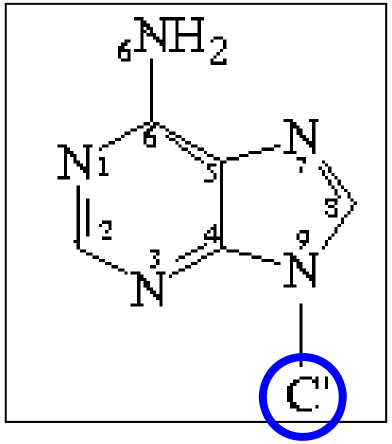


2′-D-ribose

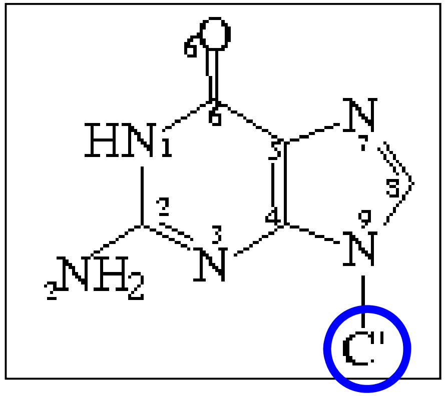
The genetic information is encoded by the sequence of bases.

Nitrogenous bases

purines



Adenine (A)



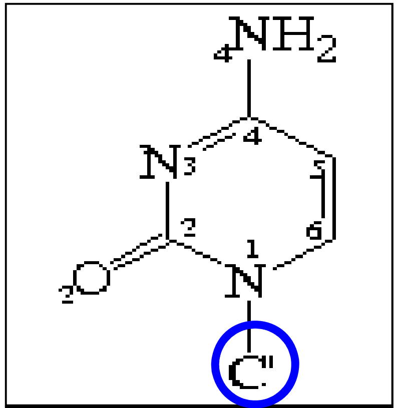
Guanine (G)

Note:

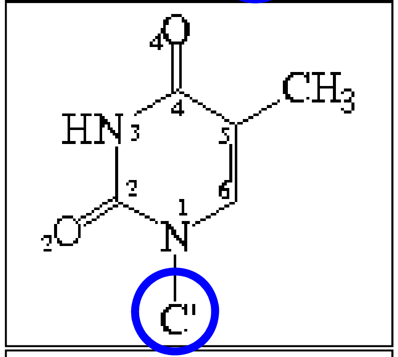
In bases: carbon atoms are noted 1,2 etc

In sugars: carbon atoms are noted 1',2' etc

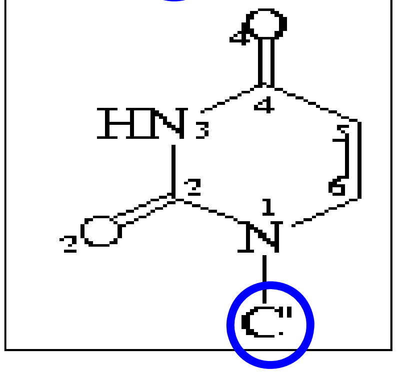
pyrimidines



Cytosine (C)



Thymine (T)



Uracil (U) (in RNA instead of thymine)

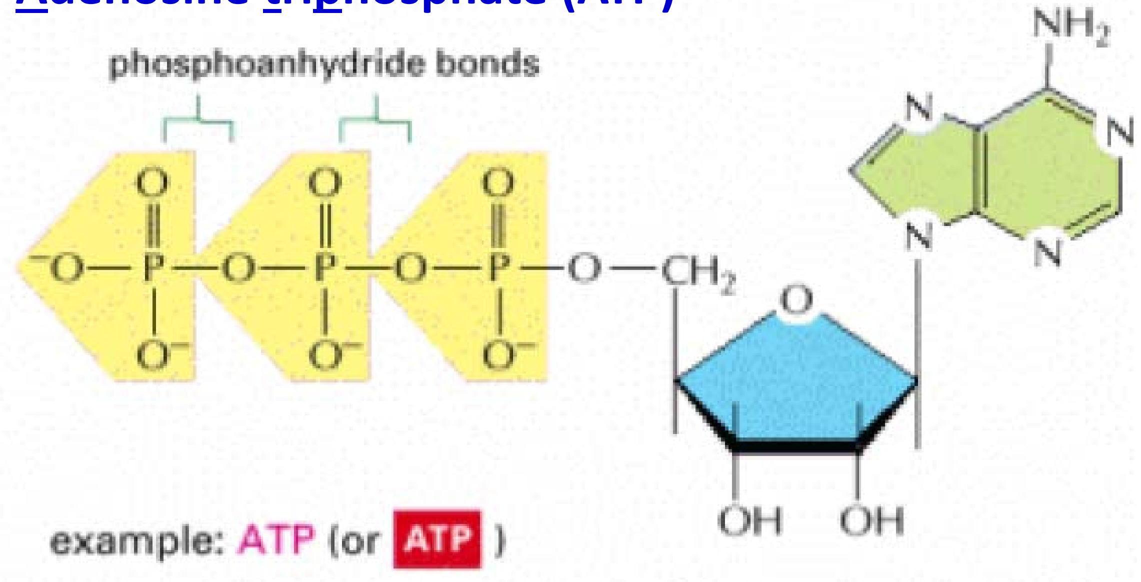
In DNA: bases A, C, G, T **In RNA: bases A, C, G, U**

The genetic information is encoded by the sequence of the four bases.

Nucleotides also have many other functions

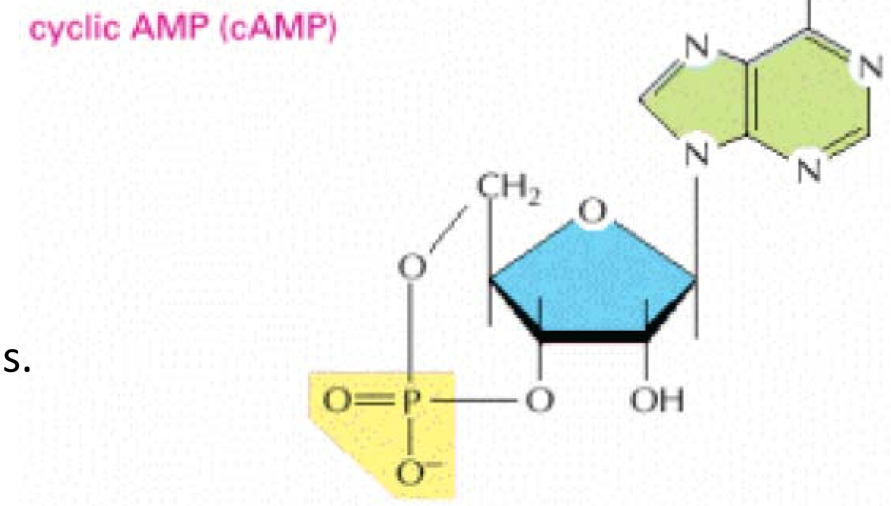
Chemical energy released by hydrolysis of their phosphoanhydride bonds is used to drive energetically unfavorable reactions.

Adenosine triphosphate (ATP)



They are used as signaling molecules.

Cyclic adenosine monophosphate (cAMP)

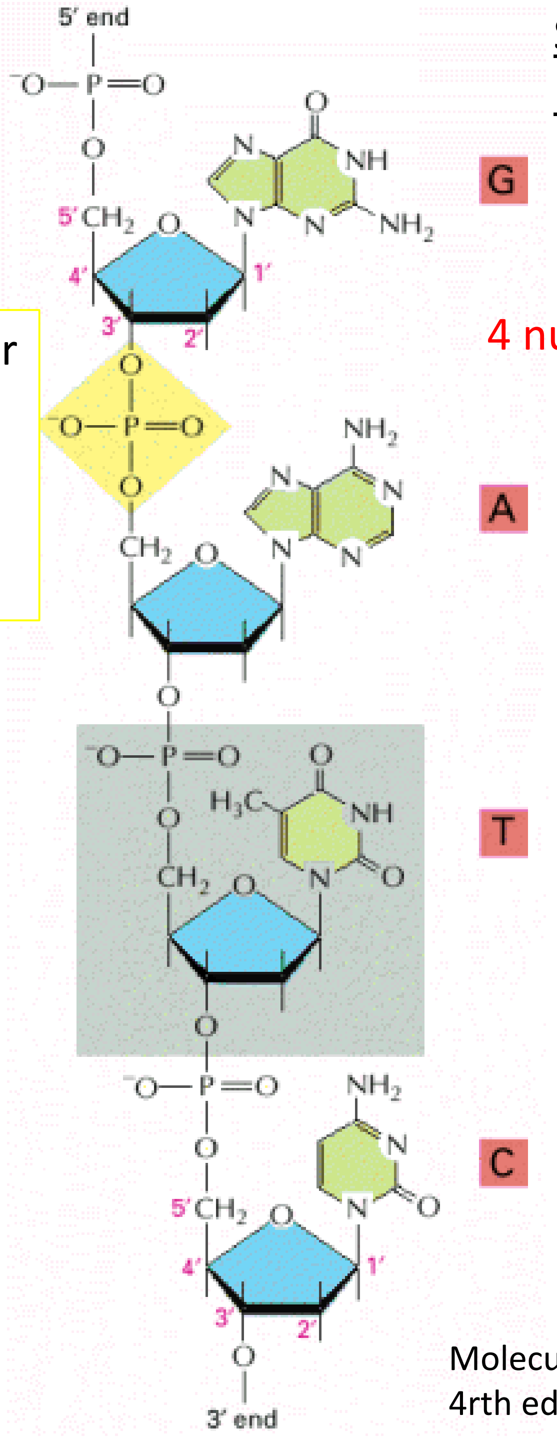


They combine with other groups to form enzymes.

Sugar + base = nucleoside
Sugar + base + phosphate = nucleotide

Molecular Biology of the Cell, 4rth edition, B. Alberts et al.

Nucleotides are linked to each other with phosphodiester bonds to form polynucleotide chains (DNA or RNA chains)



Small part of a DNA chain

4 nucleotides: GATC

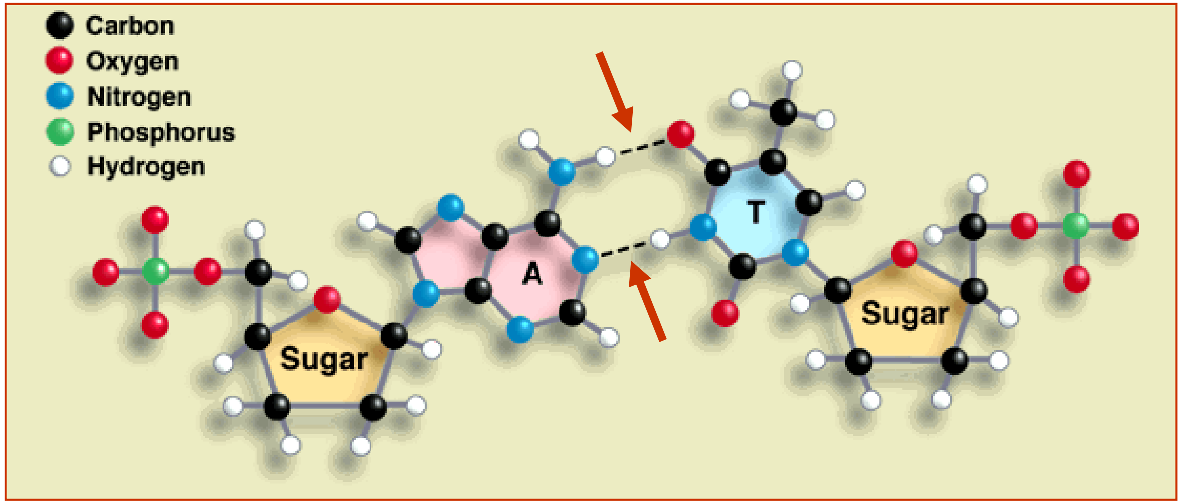
Phosphodiester bond between 5’ and 3’ carbon atoms of the ribose

One letter code for polynucleotide chains read from the 5’ end to the 3’ end
In this case: GATC

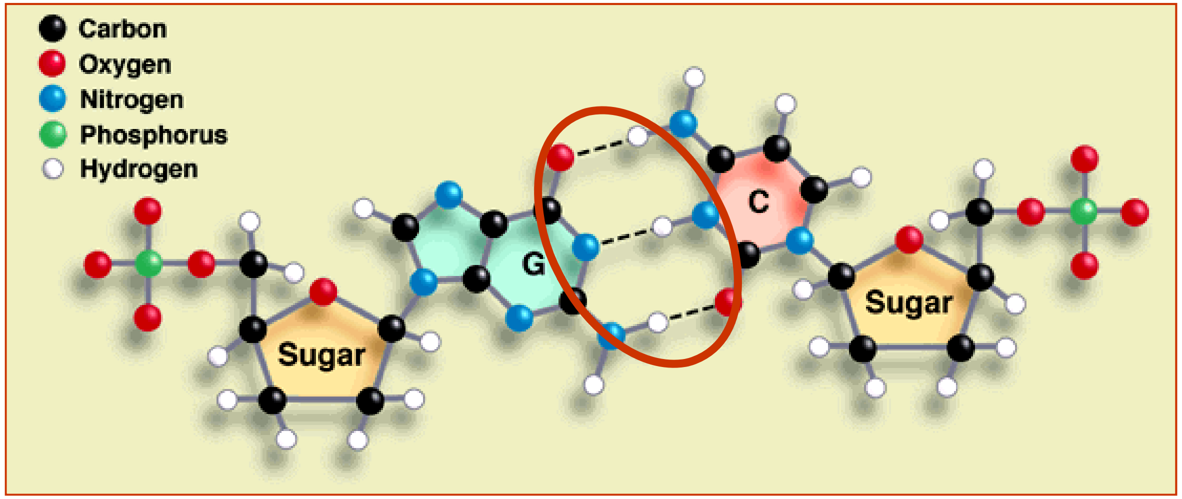
Molecular Biology of the Cell, 4rth edition, B. Alberts et al.

Hydrogen bonding between nucleotides

Formation of base paires : based on hydrogen bonding between bases



A with T : deux hydrogen bonds

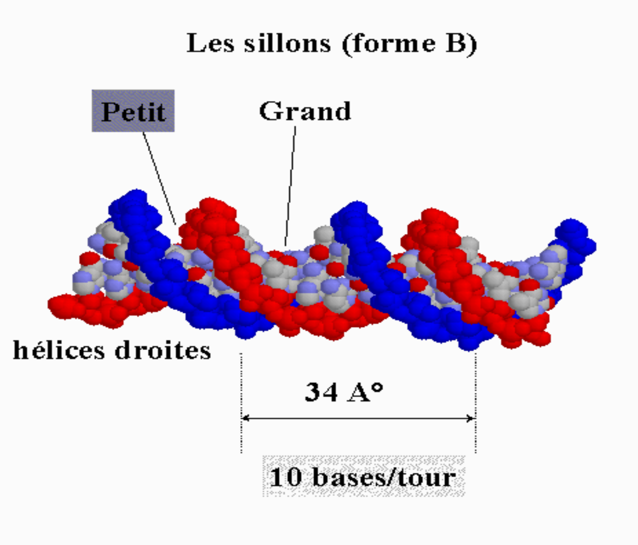


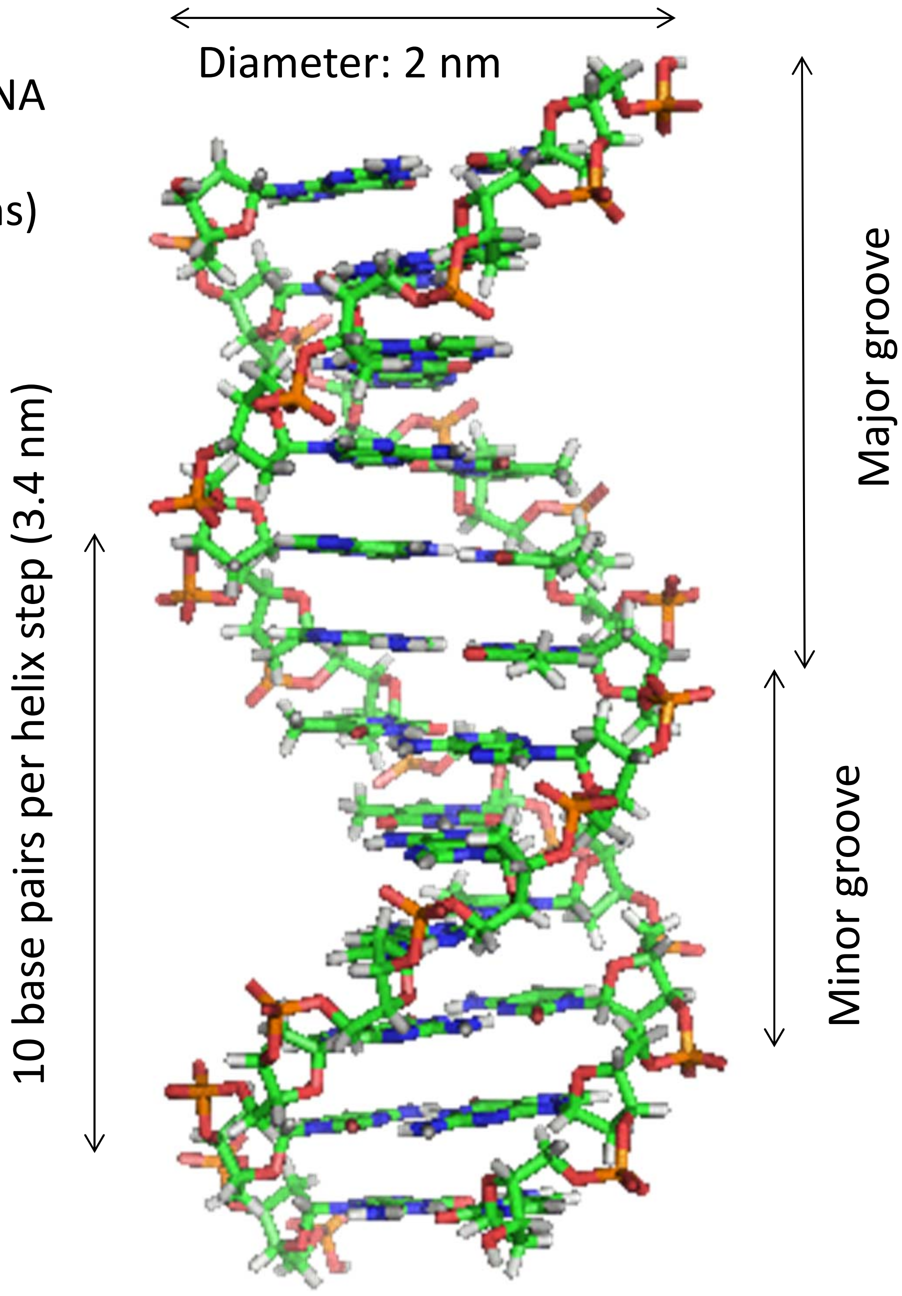
C with G : three hydrogen bonds

DNA helical structure

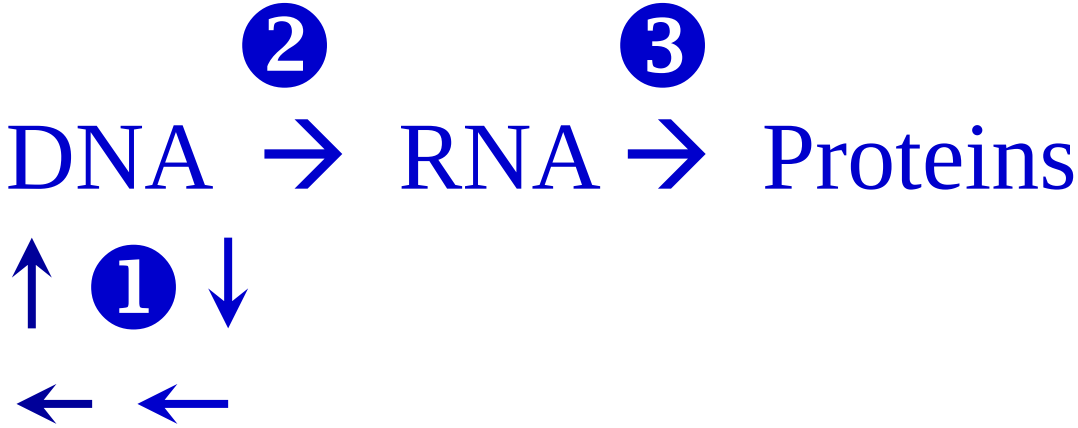
Two polynucleotide branches form a DNA molecule. The two branches are:

- Antiparallel (run in opposite directions)
- Complementary (A-T, C-G)
- Helical





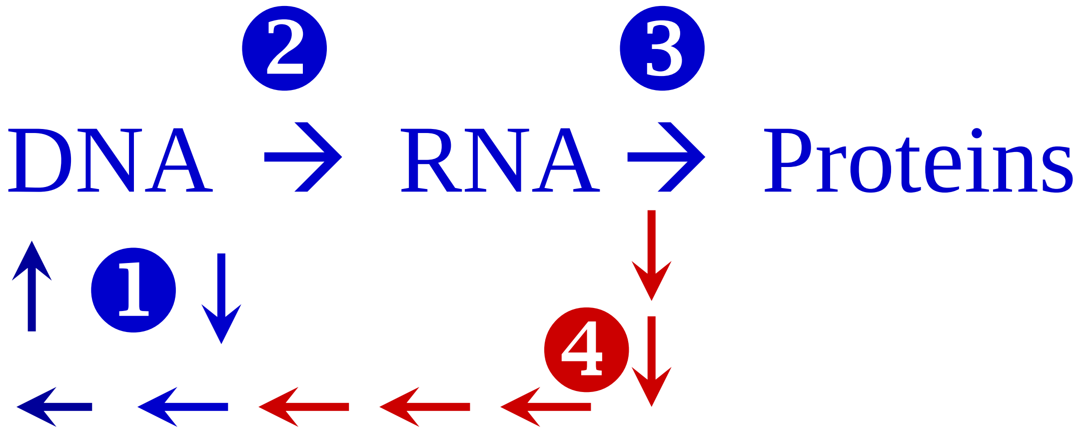
1953 : The central dogma of molecular biology



❶ replication ❷ transcription ❸ translation

We now know that only a small percentage of the human genome is transcribed into RNA
-> dark matter of the genome

1970 : inverse transcription in retroviruses



❷ ❸

DNA → RNA → Proteins

❶ ❹

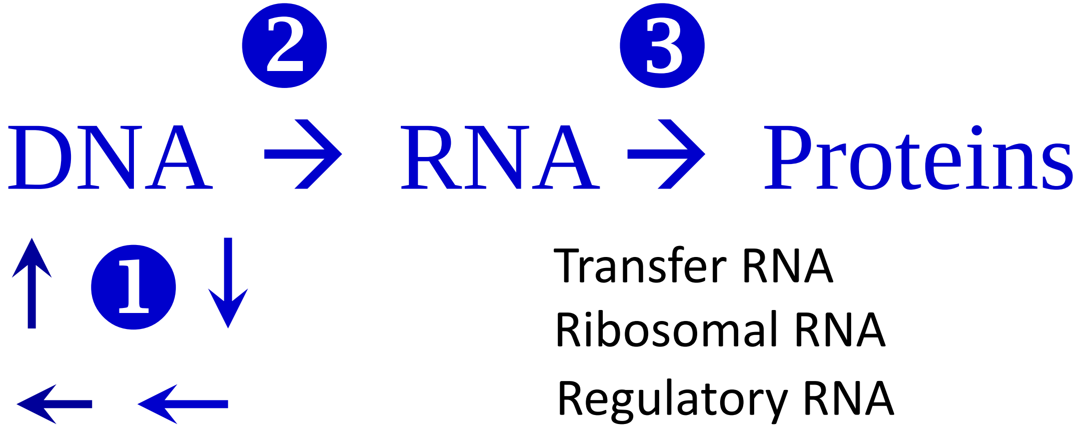
❶ réplication

❷ transcription

❸ translation

❹ inverse transcription

TODAY



❷ ❸

DNA → RNA → Proteins

❶

Transfer RNA
Ribosomal RNA
Regulatory RNA

❶ replication ❷ transcription ❸ translation

Non-coding DNA $\cancel{\rightarrow}$ « Junk » DNA

Non-coding DNA : Transfer RNA
Ribosomal RNA
Regulatory RNA
Centromeres
Telomeres

Fraction of functional DNA:

- 80%
- 8-15%

Proteins are the main actors in the life of a cell

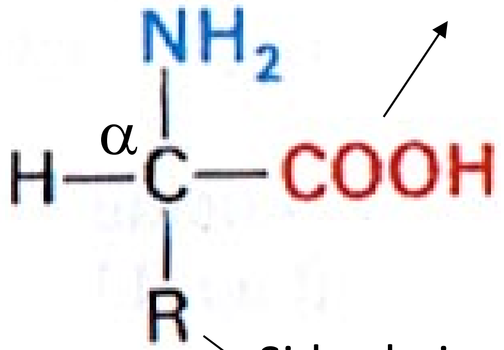
Structural components: actin, tubulin (cytoskeleton)
Metabolism: enzymes
Defense: antibodies
Transport: hemoglobin
Storage: ferritin
Regulation: transcription factors
….

They are made of a sequence of 20 different amino acids.
The sequence of amino acids is determined by the sequence of DNA nucleotides and is characteristic of each protein.

Amino acid structure

Amine group

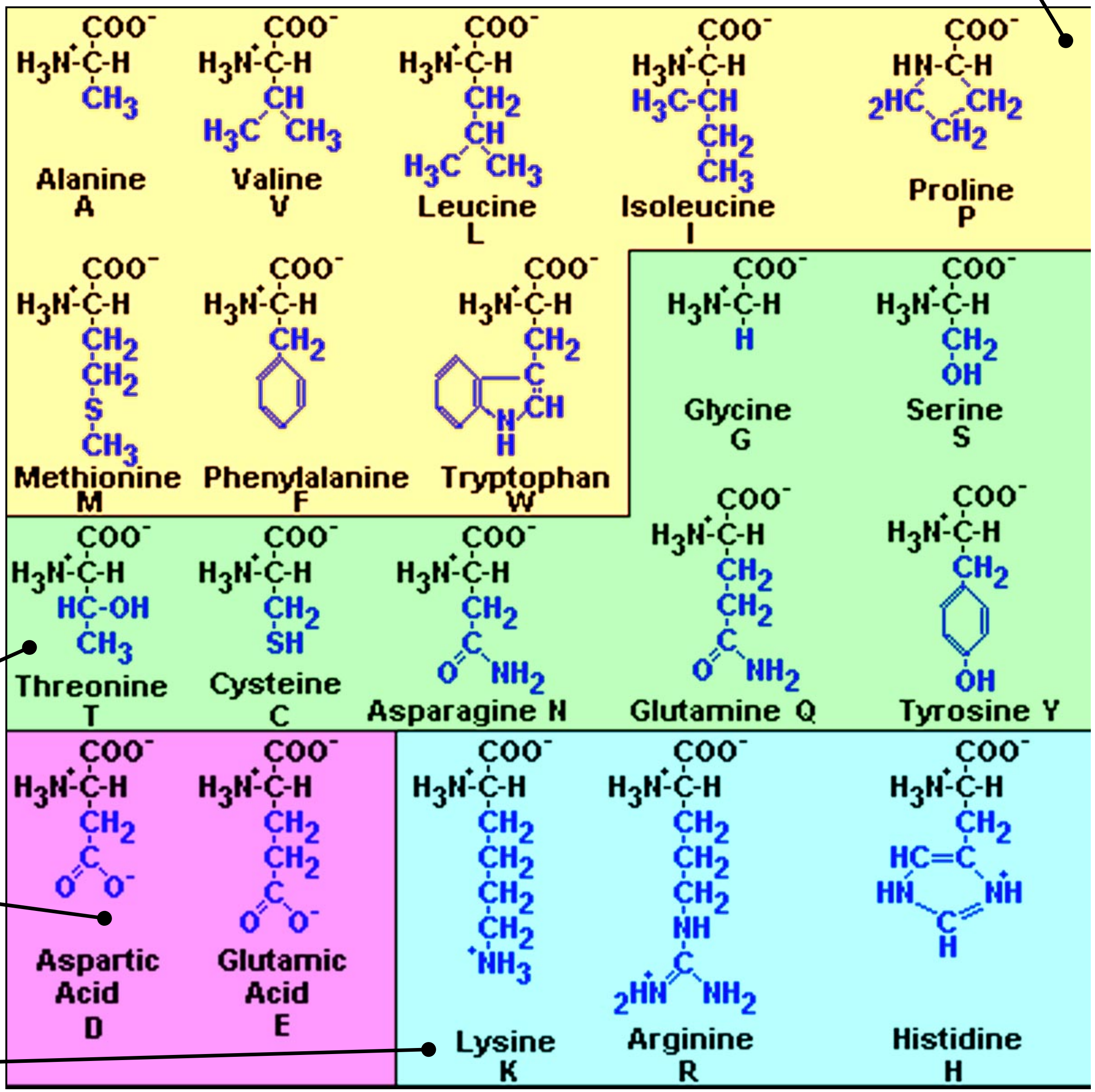
Carboxylic acid group



Side chain

Group R leads to different physico-chemical properties for each amino acid (electric charge, polarity, acidic or basic group)

Non polar chain



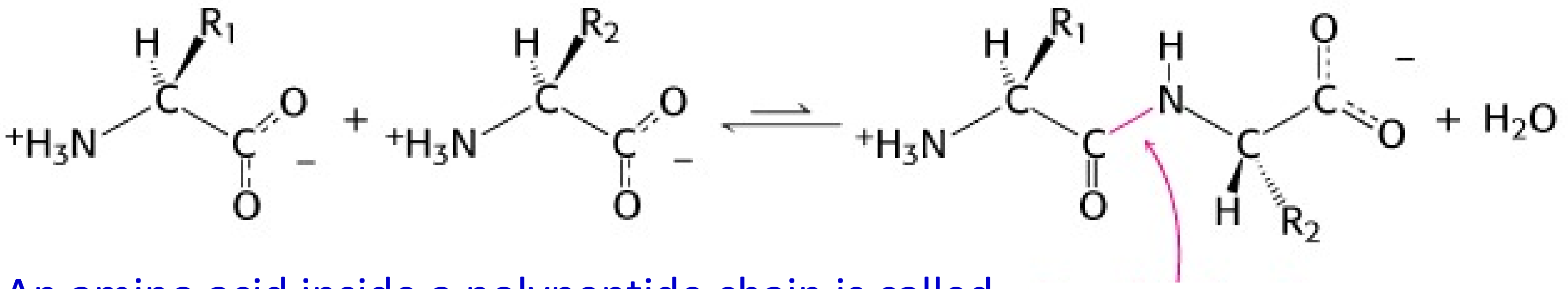
Polar uncharged chain

Acidic chain

Basic chain

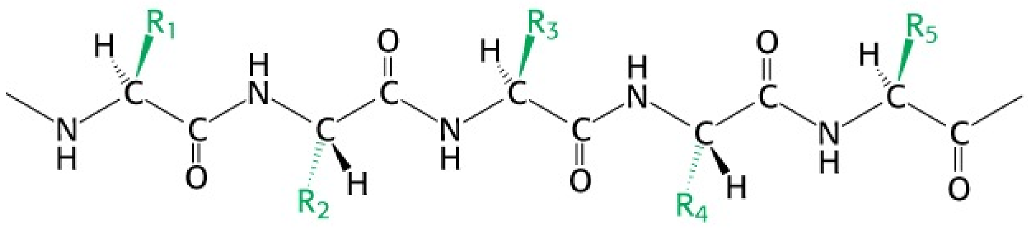
The peptidic bond – the polypeptide backbone

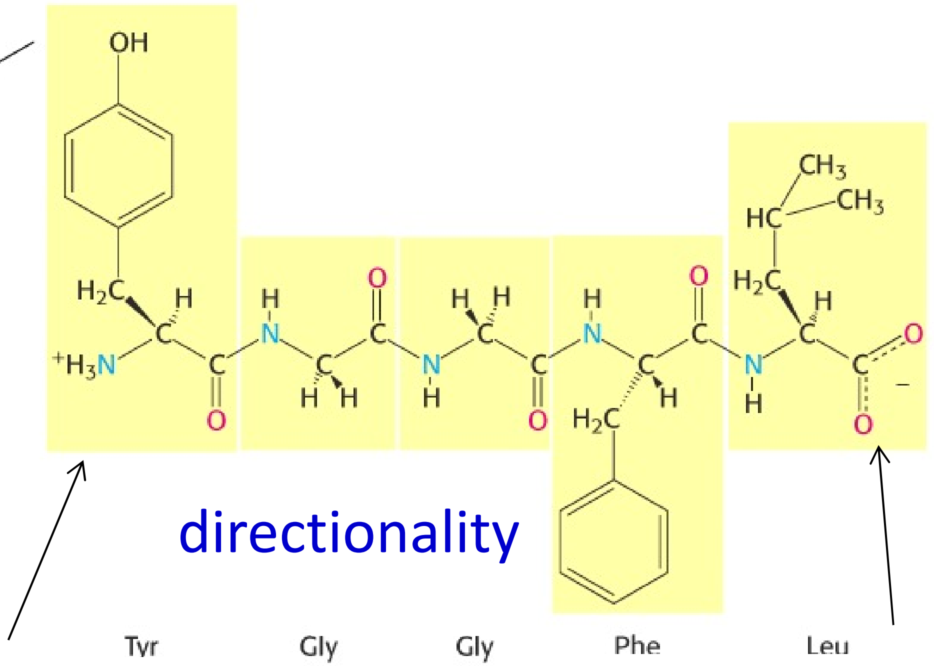
- Condensation of 2 amino acids to form an amide bond (also called peptide bond)



Peptide bond

- An amino acid inside a polypeptide chain is called a residue





directionality

amino-terminal (or N-terminal) residue

carboxyl (or C-terminal) residue

Protein structure

Several levels of organization:

- primary structure (covalent bonds between amino acids)
- secondary structure (α helices, β sheets, …)
- tertiary structure
- quaternary structure

Secondary, tertiary and quaternary structures involve mainly hydrogen bonds (in some cases covalent bonds like disulfur bonds).

Protein structure

Secondary structure

Structures stabilized by **hydrogen bonds** between the C=O and NH groups of the protein backbone (independent of the lateral chains R_i).

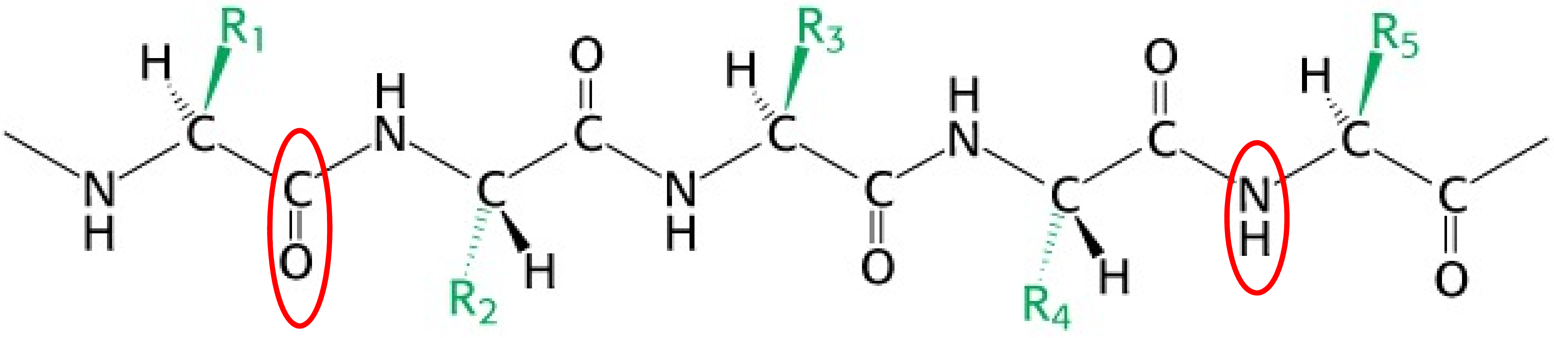
Categories:

α helices

β sheets

β turns

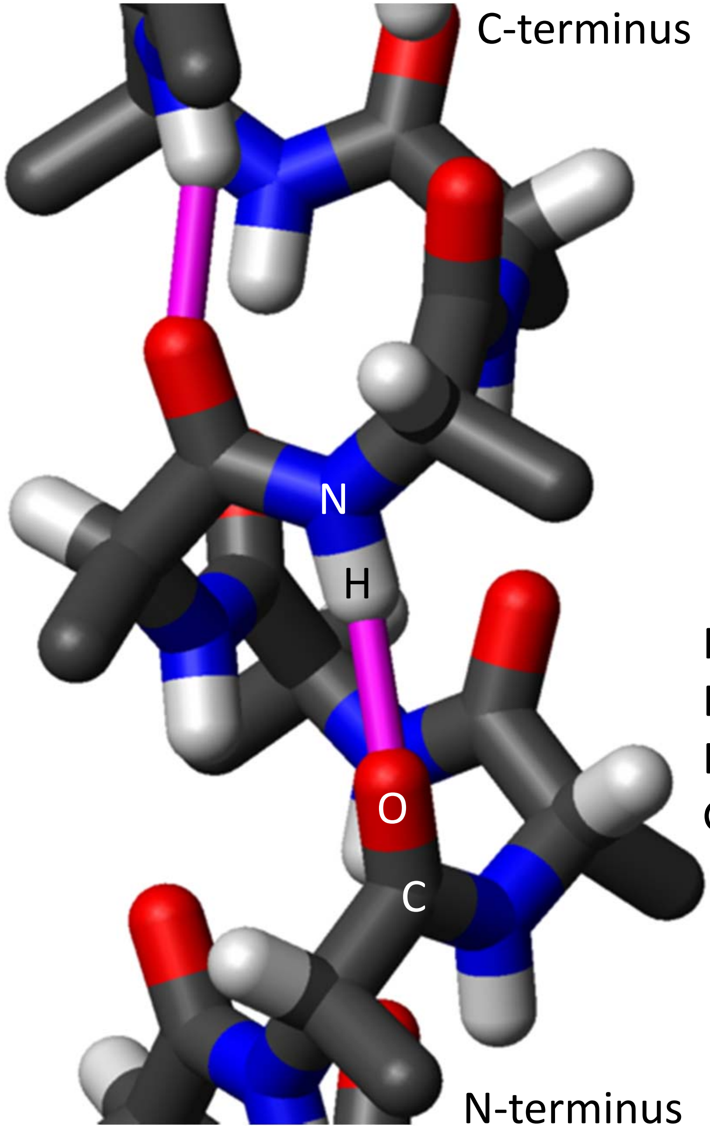
.....



Nucleic acids also have secondary structure (see transfer RNAs).

α helices

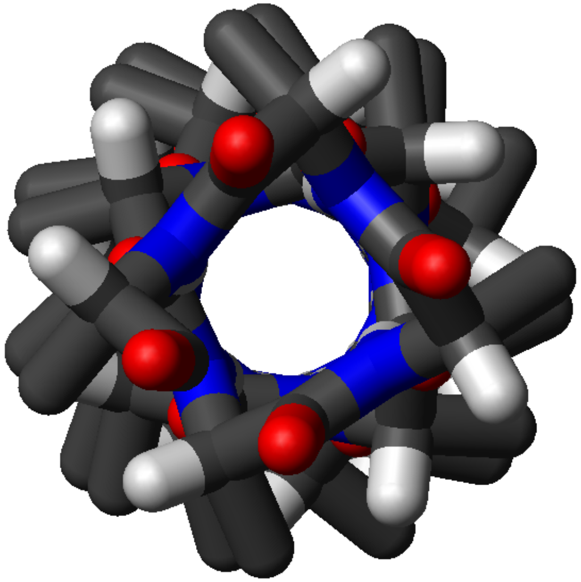
Side view



α-helix of alanine residues

- ➢ Right-handed coiled conformation resembling a spring
- ➢ Structure stabilized by hydrogen bonds between residues n and n+4.
- ➢ 3.6 residues per turn of the helix (one turn: 5.4 Å).

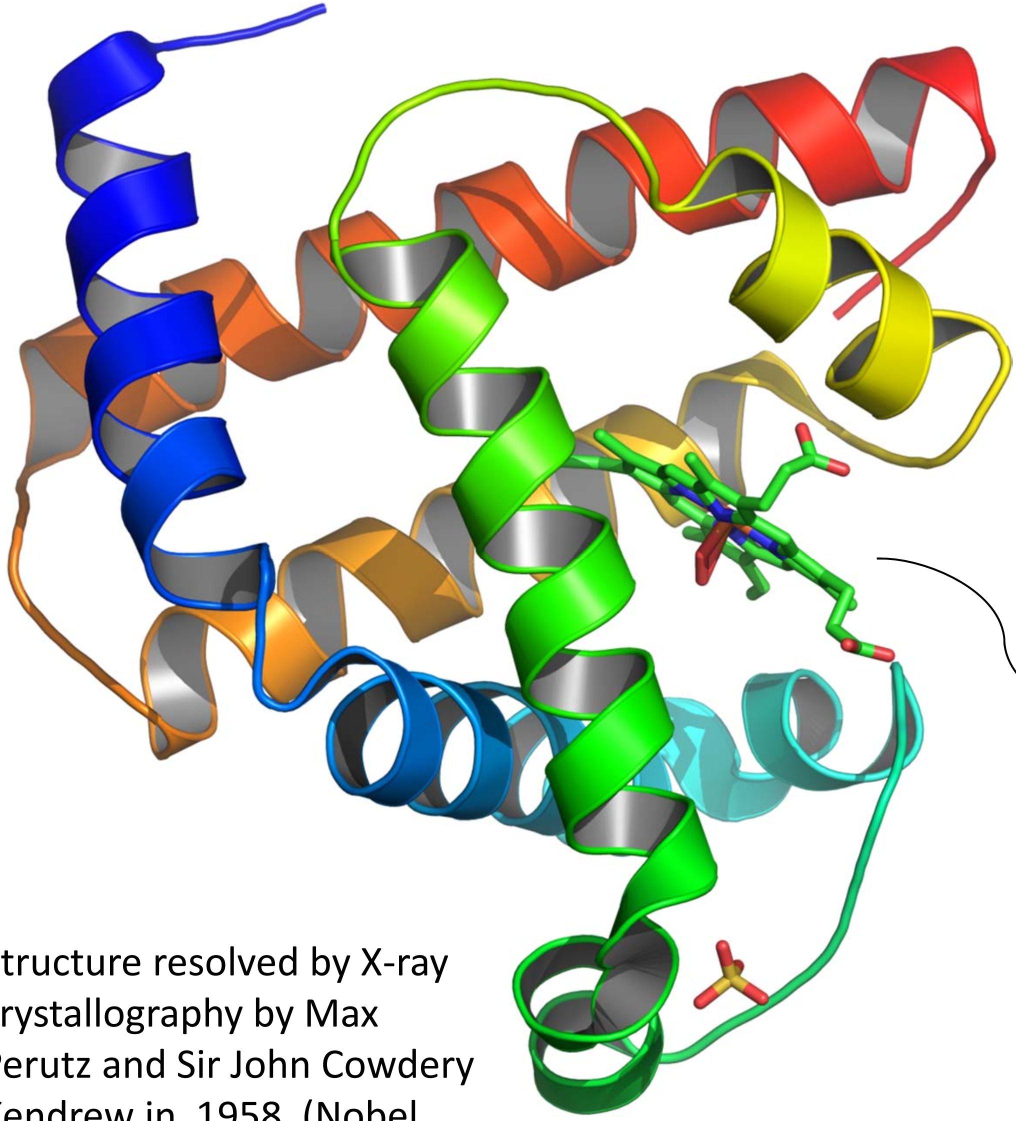
Red: O
Blue: N
Black: C
Grey: H



Amino acids that prefer to adopt helical conformations in proteins include methionine, alanine, leucine, glutamate and lysine ("MALEK" in amino-acid 1-letter codes)

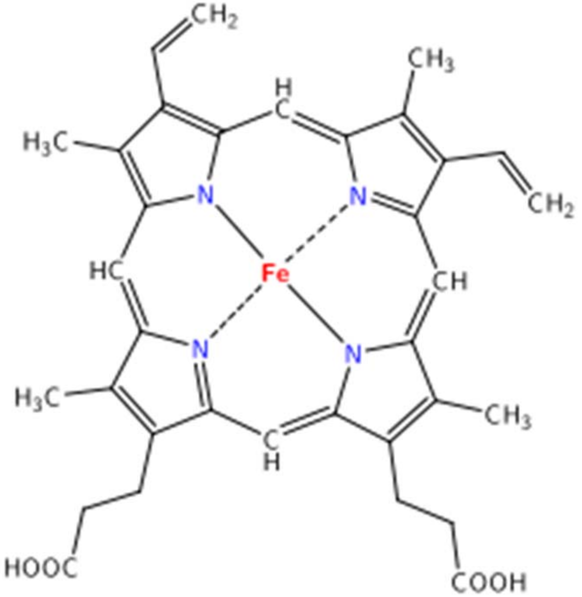
Example of a protein consisting mainly of α helices: myoglobin

Ribbon model: a ribbon is drawn along the polypeptide backbone



Myoglobin has eight α helices and binds a heme prosthetic group.
The heme is an iron-containing porphyrin.
The iron atom binds O_2, CO, NO.
Myoglobin function: storage of oxygen in muscle cells.

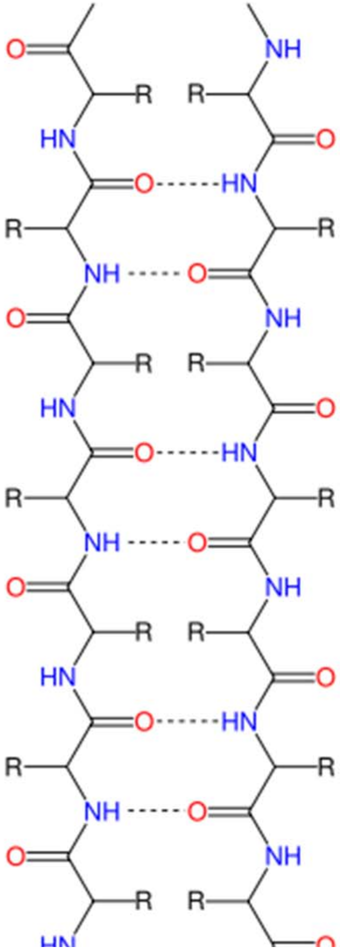
heme



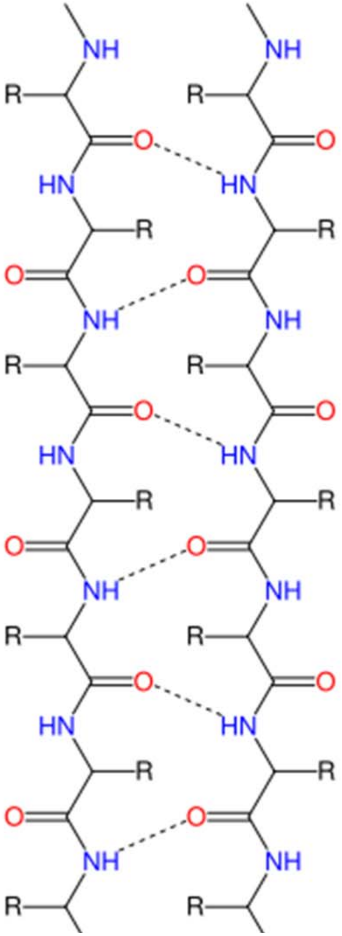
Structure resolved by X-ray crystallography by Max Perutz and Sir John Cowdery Kendrew in 1958 (Nobel Prize in Chemistry in 1962)

β sheets

Parallel

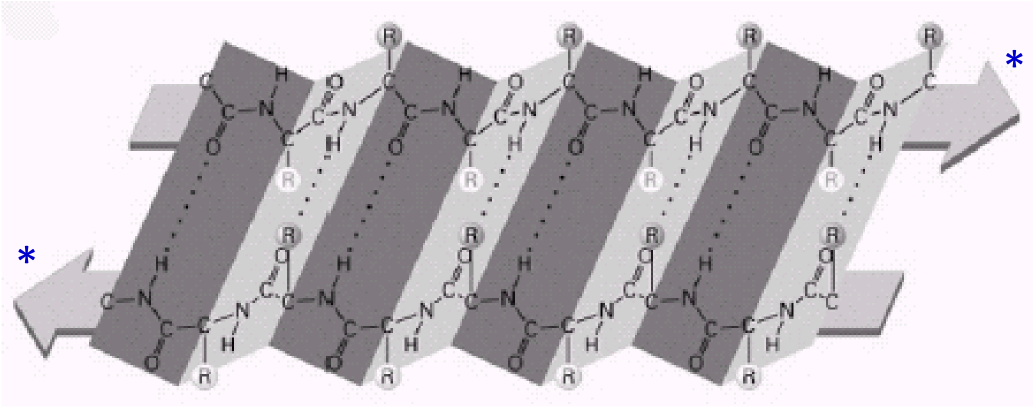


Antiparallel



β strand: single continuous stretch of amino acids adopting an extended conformation
β sheet: assembly of β strands joined by hydrogen bonds between C=O and NH groups.

Due to the tetrahedral chemical bonding at the C_α atom , β strands and β sheets are pleated.



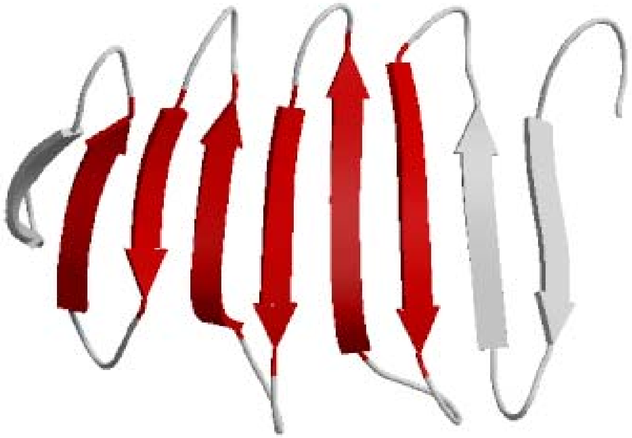
Side chains lie alternatively above and below the sheet.

*Arrows point towards the C-terminus side.

The large aromatic residues (tryptophan, tyrosine, and phenylalanine) and C^β-branched amino acids (isoleucine, valine, threonine) prefer to adopt β-strand conformations.

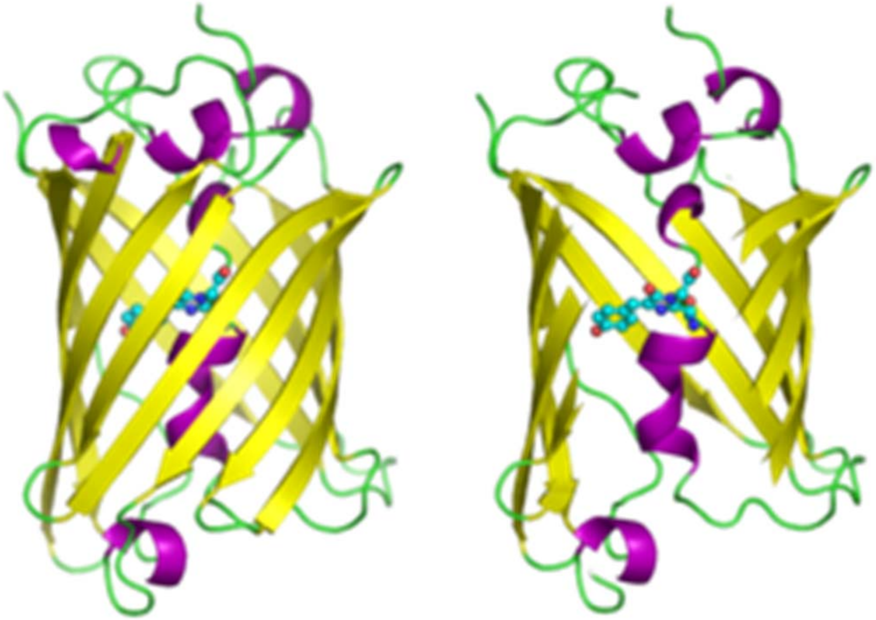
Examples of β–sheet proteins

β meander: antiparallel β strands linked together by hairpin loops (2-5 residues)



Portion of outer surface Protein A of *Borrelia burgdorferi*

β barrel: the last strand is hydrogen bonded to the first strand



Green Fluorescent Protein (GFP)

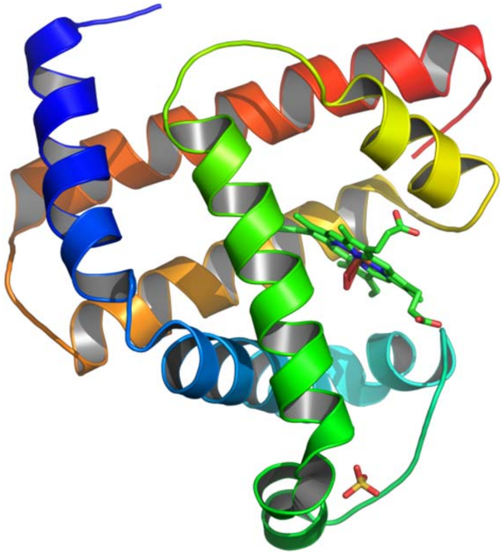
Orientation of hydrophobic residues on one side and hydrophilic residues on the other side of a β sheet can be useful to form a boundary between polar and nonpolar environments.
In GFP the barrel interior forms a hydrophobic core that prevents quenching of the chromophore.

Tertiary structure

Globular proteins

- Hydrophobic residues are located in the protein core, hydrophilic residues are located at the surface
- Soluble in water
- Flexible structure
- May contain an internal cavity
- Diverse roles (enzymes, signal transduction, …)

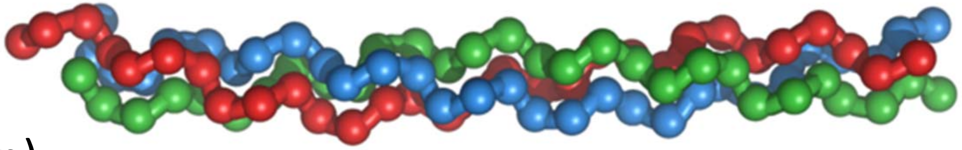
Example: myoglobin



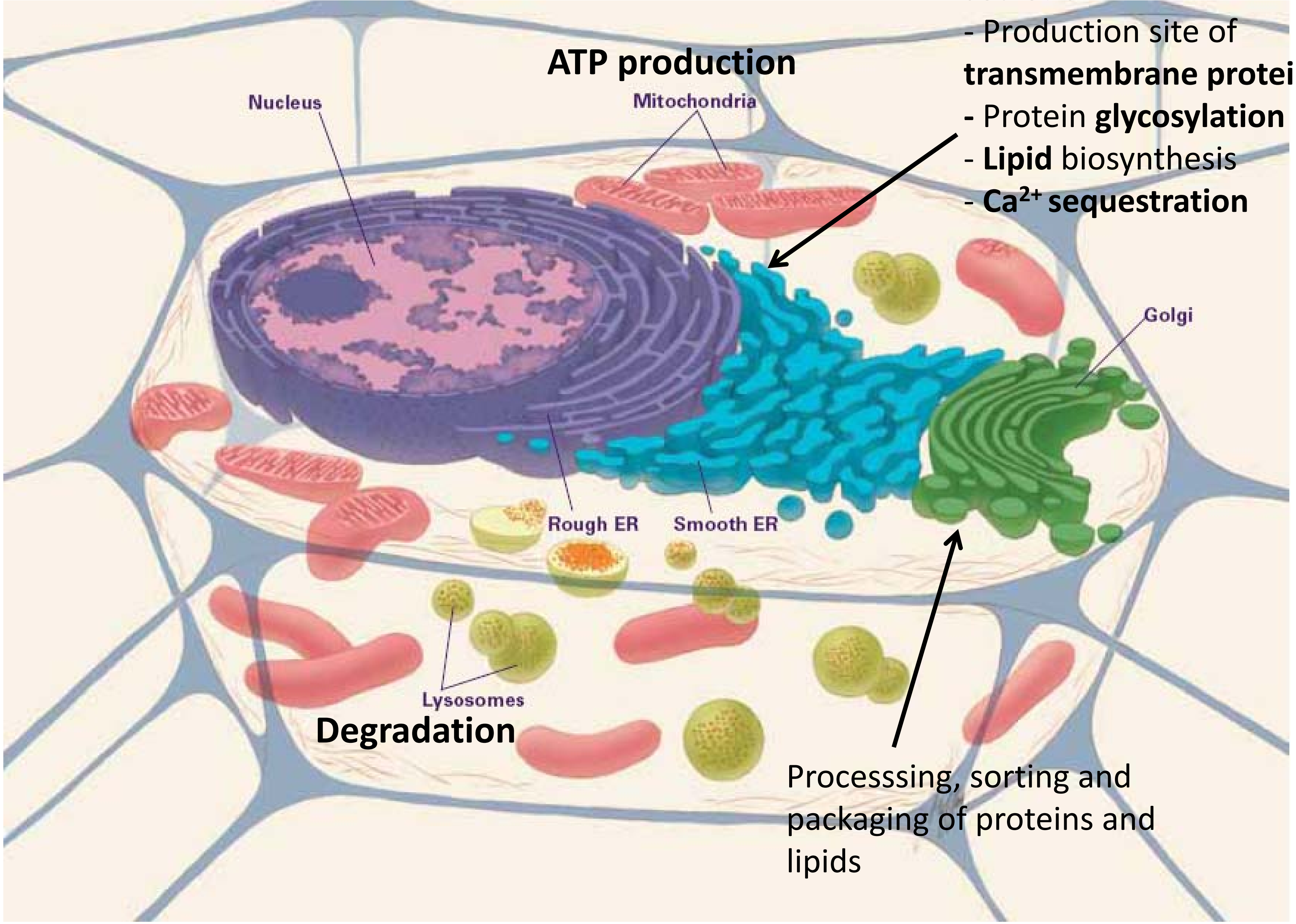
Fibrous proteins

- Made of long peptidic chains
- Generally not soluble in water
- Rigid structure
- Usually have a structural role
- Found in the extracellular matrix (collagen), bone matrix, muscle fiber
- Examples: collagen, keratin, elastin

Example: collagen triple helix



The eucaryotic cell



ATP production

- **Proteins destined for secretion**
- Production site of **transmembrane proteins**
- Protein **glycosylation**
- **Lipid** biosynthesis
- **Ca^{2+} sequestration**

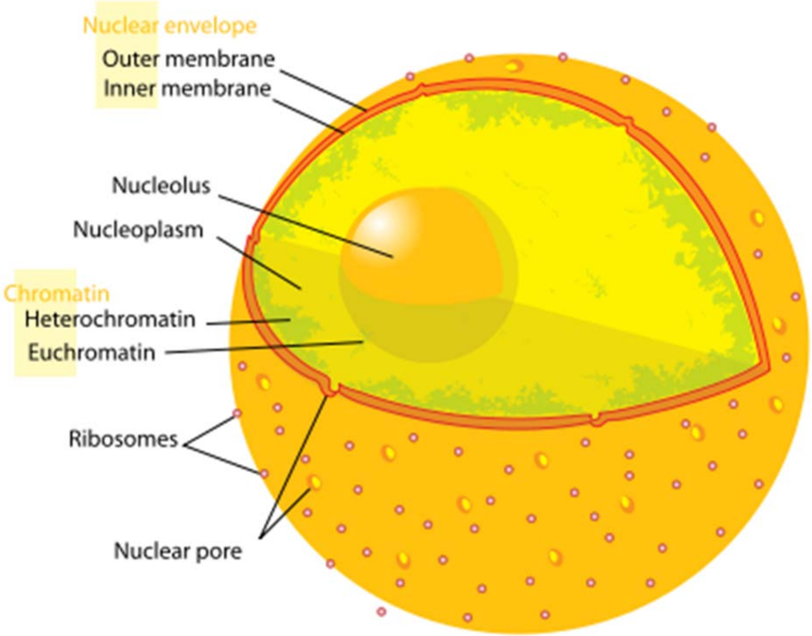
Processsing, sorting and packaging of proteins and lipids

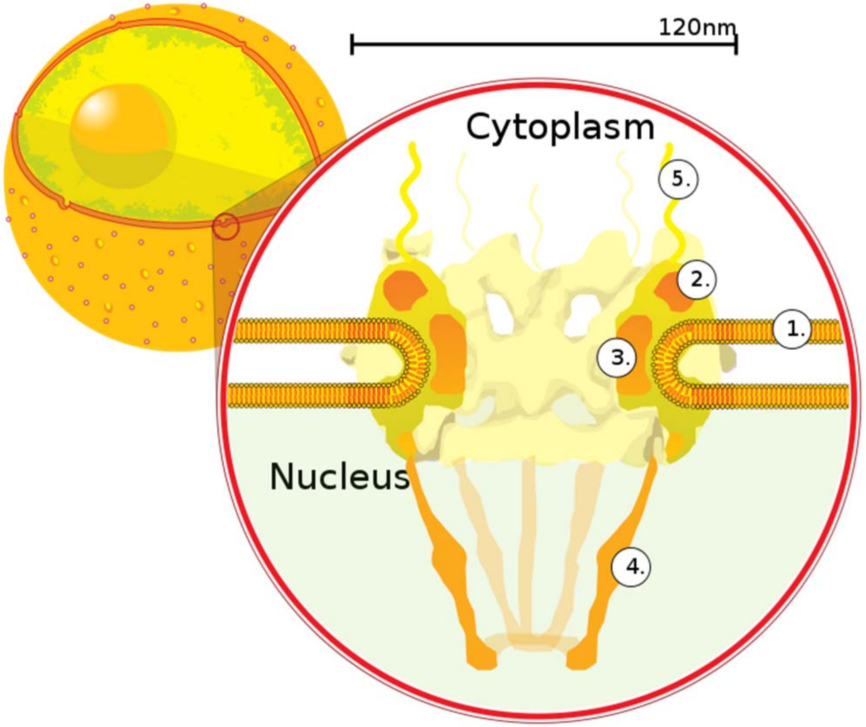
Degradation

« Inside the cell », 2005, NIH Publication No. 05-1051, http://www.nigms.nih.gov

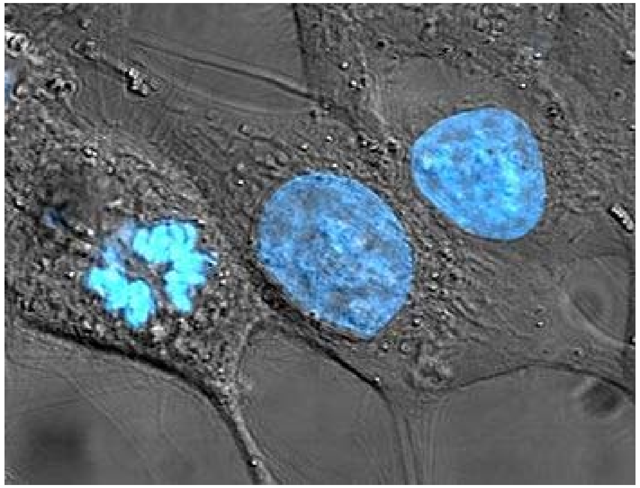
The nucleus

The nucleus contains most of the cell’s genetic material (the rest is found in mitochondria). It maintains the integrity of the nuclear genome and controls the activity of the cell by regulating gene expression.





Nuclear pores allow the movement of molecules across the nuclear envelope.

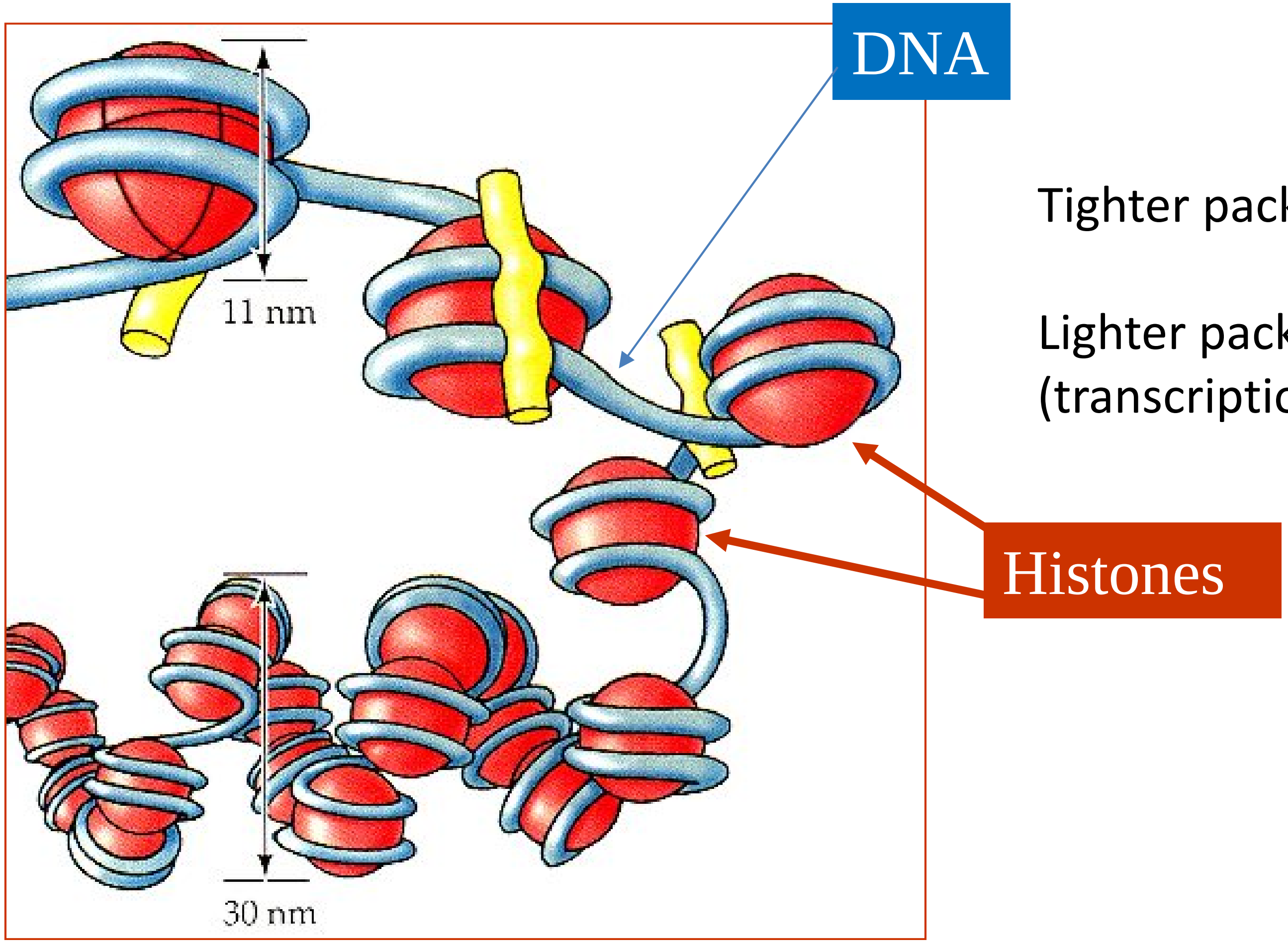


HeLa cells
DNA labeling with the dye Blue Hoechst

DNA packaging in the nucleus

In a human cell: 46 DNA molecules

Each DNA molecule winds around proteins (histones) to form a compacted molecule. The ensemble (DNA+proteins) is called a chromosome.



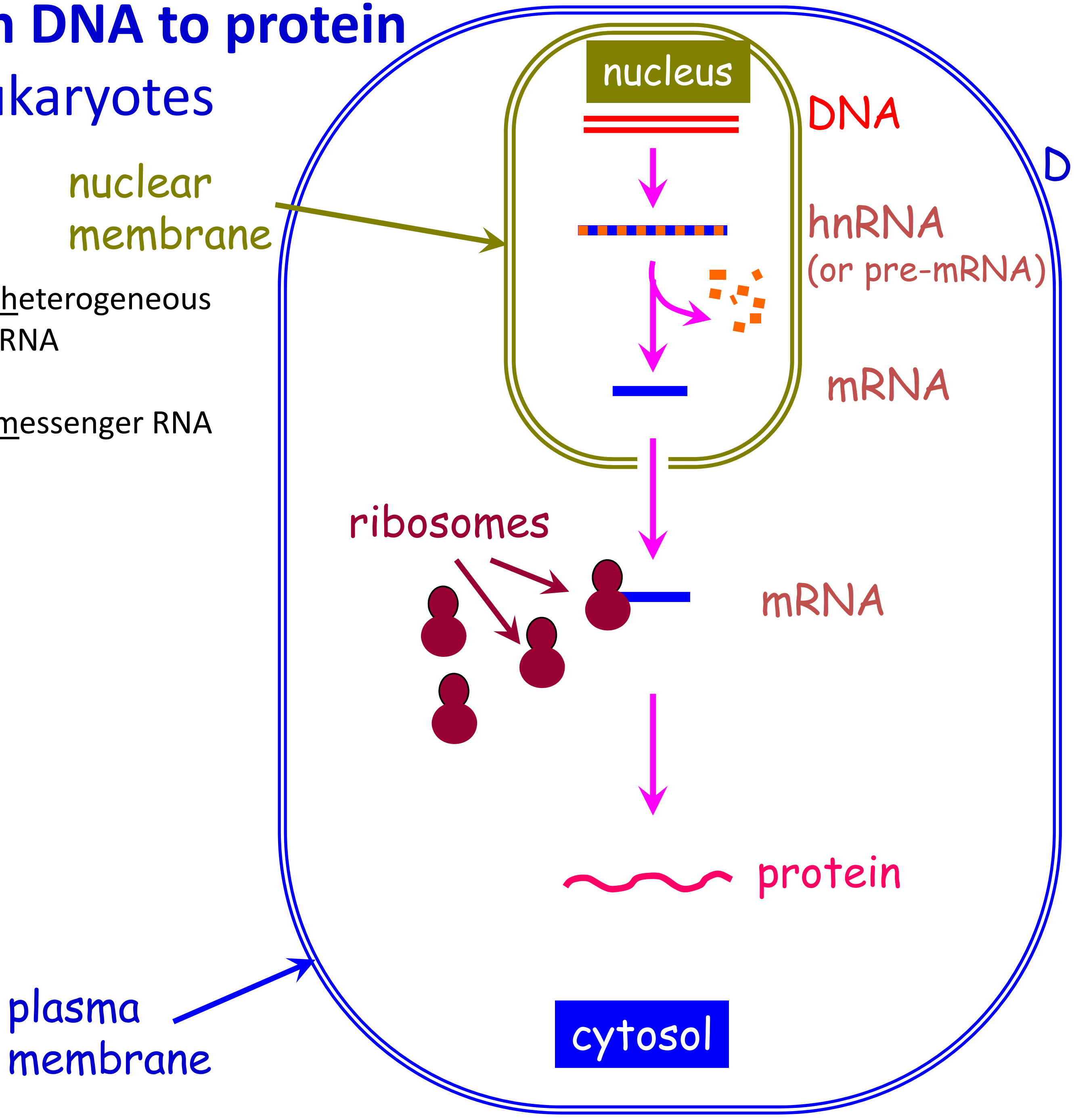
Tighter packaging: heterochromatin

Lighter packaging: euchromatin
(transcription is active)

From DNA to protein
in eukaryotes

hnRNA: heterogeneous nuclear RNA

mRNA: messenger RNA



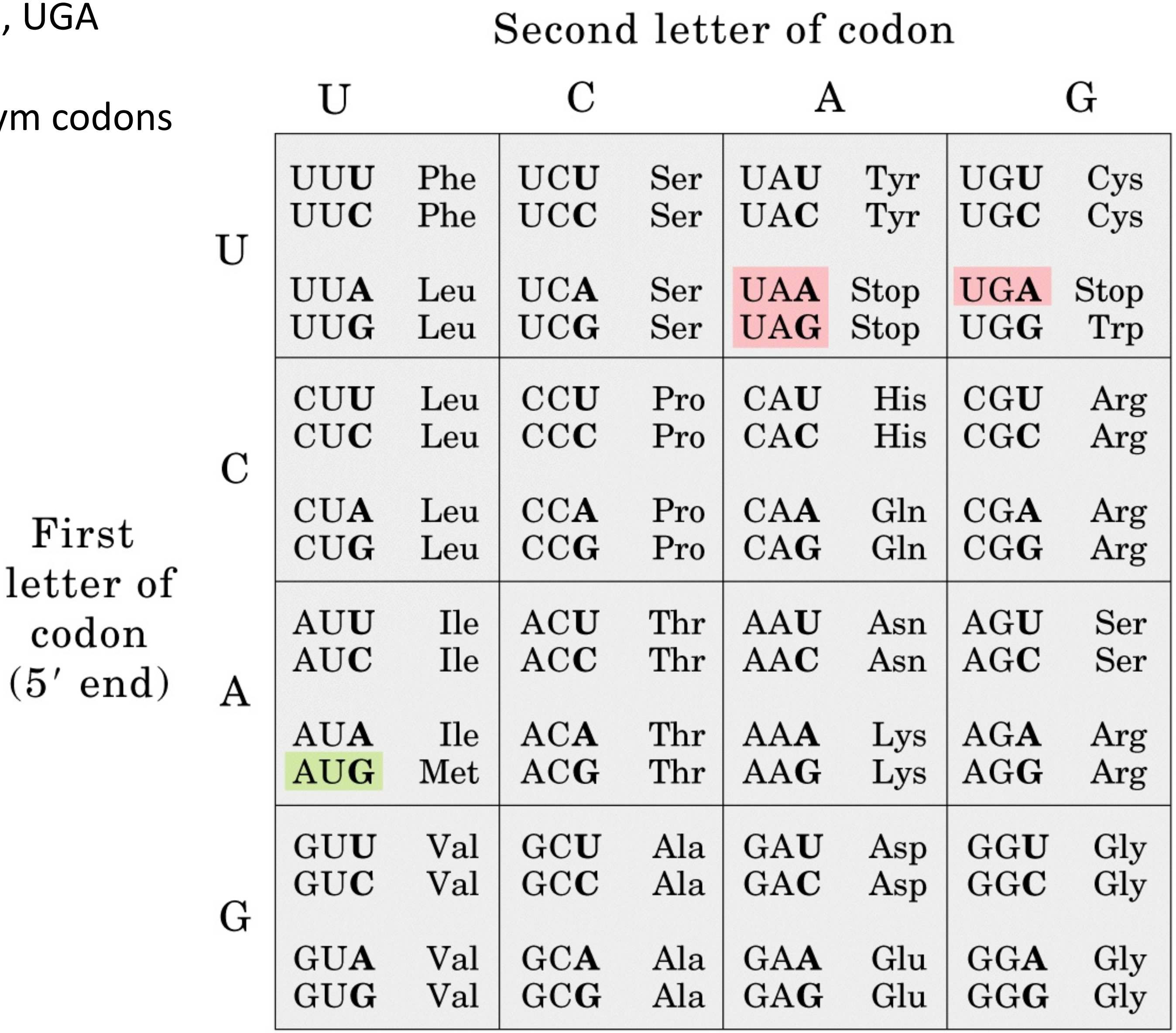
DNA transcription
↓
RNA splicing
↓
RNA transport
↓
RNA translation

RNA translation: the genetic code

RNA translation: protein biosynthesis using the code given by the messenger RNA (mRNA).

- A three-letter code: a sequence of 3 bases, a **codon**, codes for one amino acid
  3 stop codons: UAA, UAG, UGA
  1 initiation codon: AUG
- Degenerate code: synonym codons

61 codons coding for an amino acid

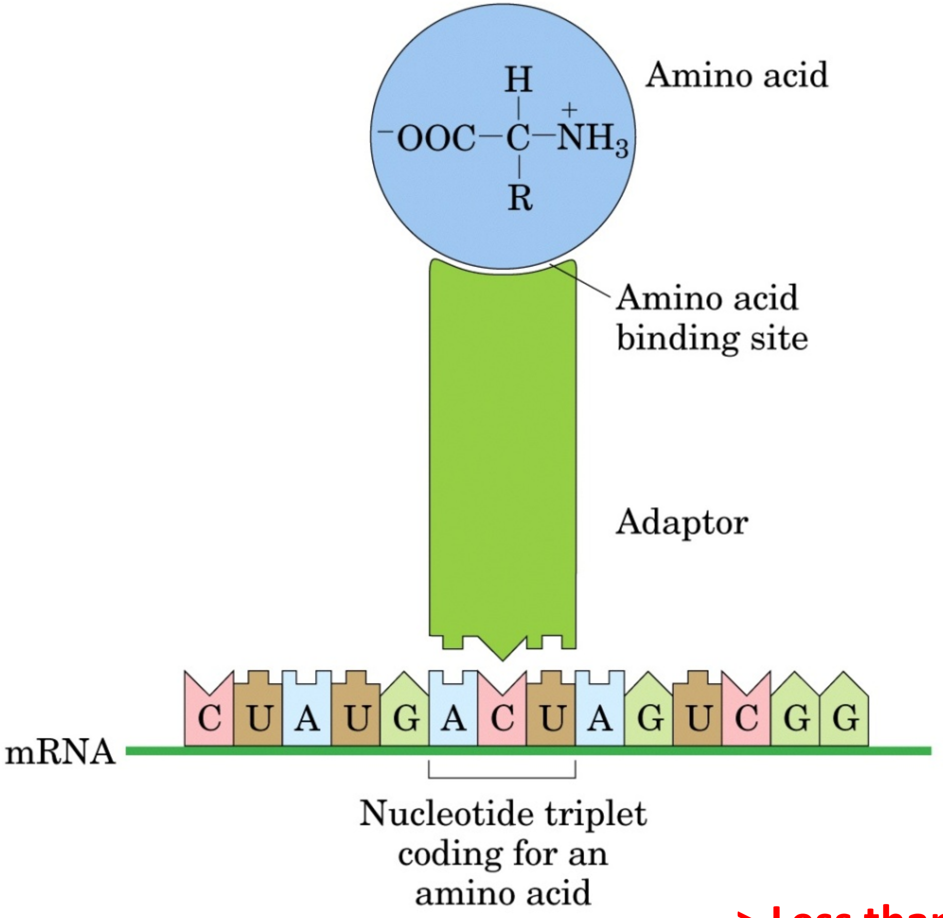


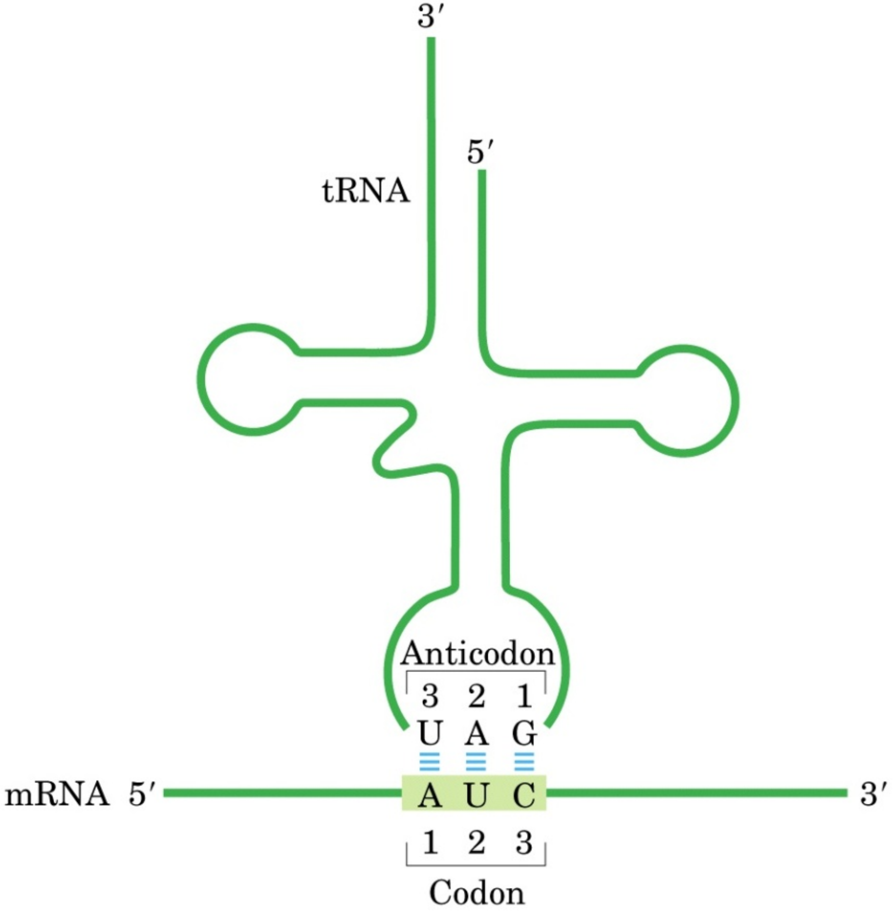
Second letter of codon

First letter of codon (5′ end)	U		C		A		G	
U	UU**U**	Phe	UC**U**	Ser	UA**U**	Tyr	UG**U**	Cys
	UU**C**	Phe	UC**C**	Ser	UA**C**	Tyr	UG**C**	Cys
	UU**A**	Leu	UC**A**	Ser	UA**A**	Stop	UG**A**	Stop
	UU**G**	Leu	UC**G**	Ser	UA**G**	Stop	UG**G**	Trp
C	CU**U**	Leu	CC**U**	Pro	CA**U**	His	CG**U**	Arg
	CU**C**	Leu	CC**C**	Pro	CA**C**	His	CG**C**	Arg
	CU**A**	Leu	CC**A**	Pro	CA**A**	Gln	CG**A**	Arg
	CU**G**	Leu	CC**G**	Pro	CA**G**	Gln	CG**G**	Arg
A	AU**U**	Ile	AC**U**	Thr	AA**U**	Asn	AG**U**	Ser
	AU**C**	Ile	AC**C**	Thr	AA**C**	Asn	AG**C**	Ser
	AU**A**	Ile	AC**A**	Thr	AA**A**	Lys	AG**A**	Arg
	AU**G**	Met	AC**G**	Thr	AA**G**	Lys	AG**G**	Arg
G	GU**U**	Val	GC**U**	Ala	GA**U**	Asp	GG**U**	Gly
	GU**C**	Val	GC**C**	Ala	GA**C**	Asp	GG**C**	Gly
	GU**A**	Val	GC**A**	Ala	GA**A**	Glu	GG**A**	Gly
	GU**G**	Val	GC**G**	Ala	GA**G**	Glu	GG**G**	Gly

RNA translation: the role of tRNA

Transfer RNA plays the role of an adaptor molecule.
On one side it contains a three nucleotide region called the anticodon which recognizes and pairs through hydrogen bonding with a corresponding three nucleotide codon region on the RNA.
On the other side it can be charged with a specific amino acid corresponding to the codon three nucleotide sequence.





61 codons coding for an amino acid

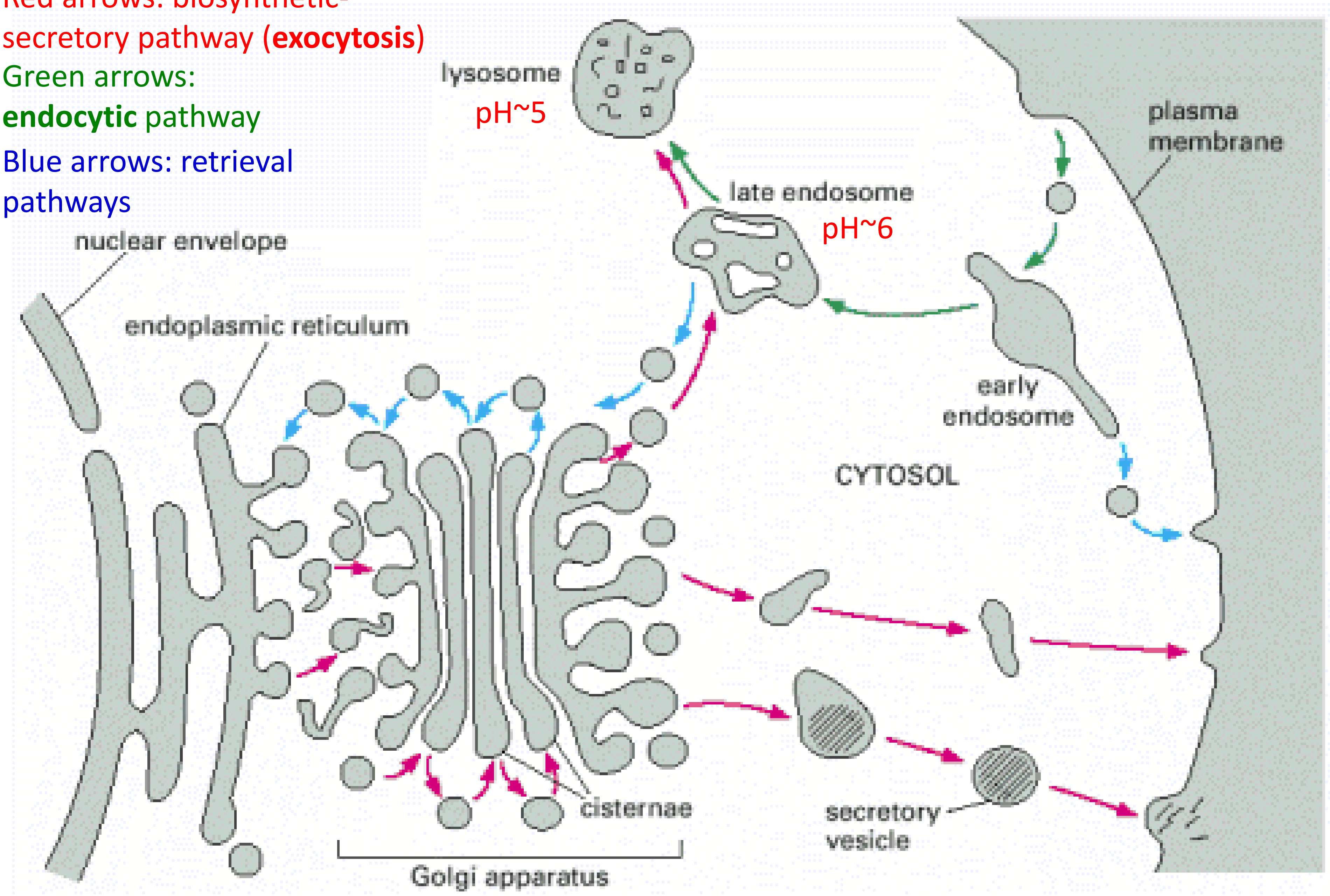
-> Less than 61 tRNAs are necessary thanks to the use of nucleotides that can hydrogen bond to more than one base

Communication between intracellular compartments and the cell membrane

Red arrows: biosynthetic-secretory pathway (**exocytosis**)

Green arrows: **endocytic** pathway

Blue arrows: retrieval pathways



Molecular Biology of the Cell, 4rth edition, B. Alberts et al.

Cell membrane