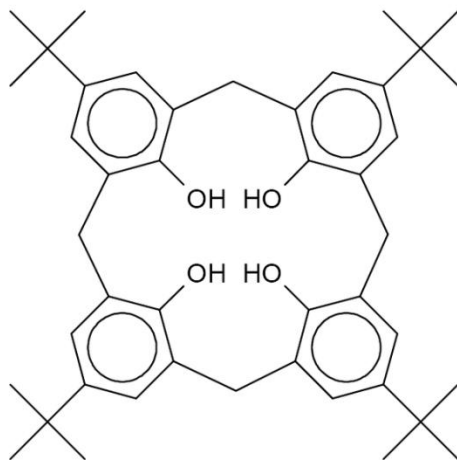
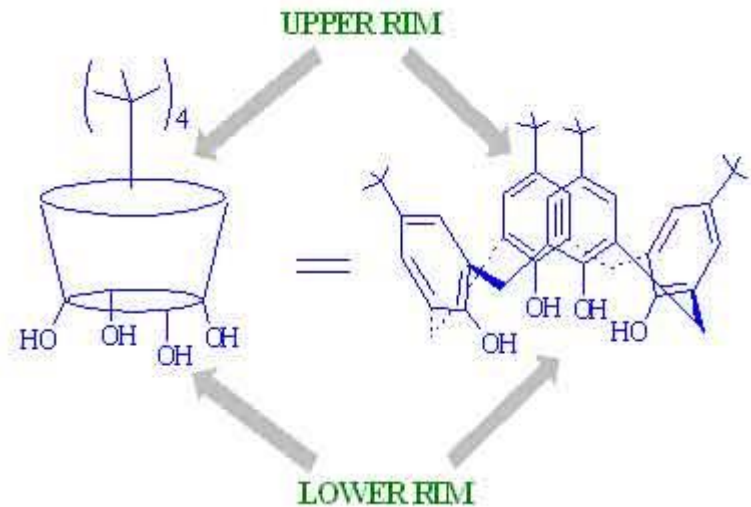
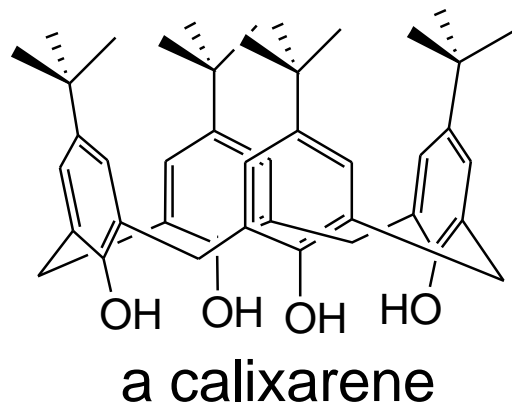


Sensor development using existing scaffolds

- Biological molecules (peptides, oligonucleotides, enzymes, antibodies)
- Synthetic macrocycles (crown ethers, cryptands)
- Cavitands: container shaped molecules (cyclodextrins, calixarenes, cucurbiturils)

Calixarenes

- Calixarenes (cyclic oligophenols)



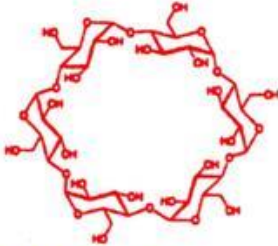
- hydrophobic cavity
- easy modification

Cyclodextrines

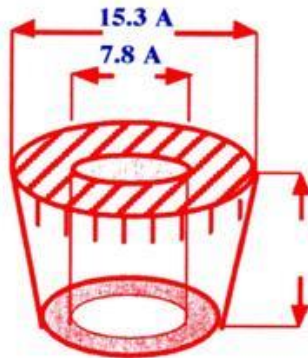
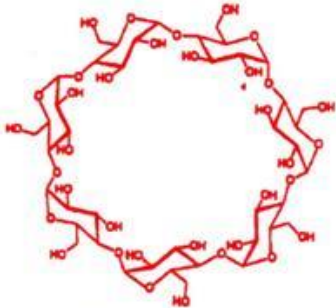
- Cyclodextrines (cyclic oligosugars - oligoglucose); CDs
- Cavity size depends on the number of monomers
- α -cyclodextrin: 6-membered
- β -cyclodextrin: 7-membered
- γ -cyclodextrin: 8-membered

Cyclodextrines

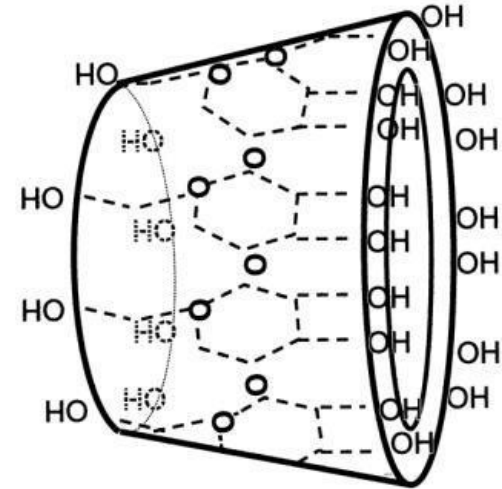
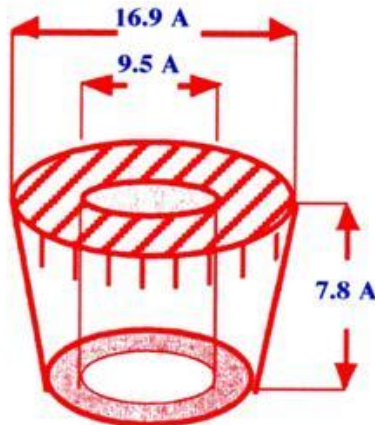
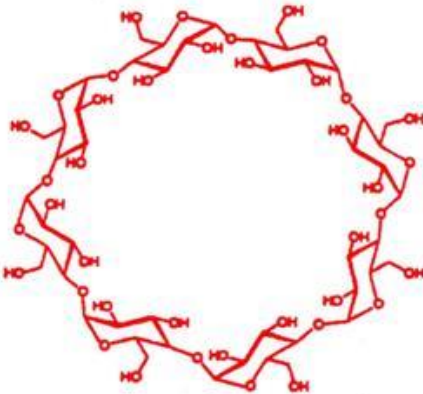
α -Cyclodextrin



β -Cyclodextrin



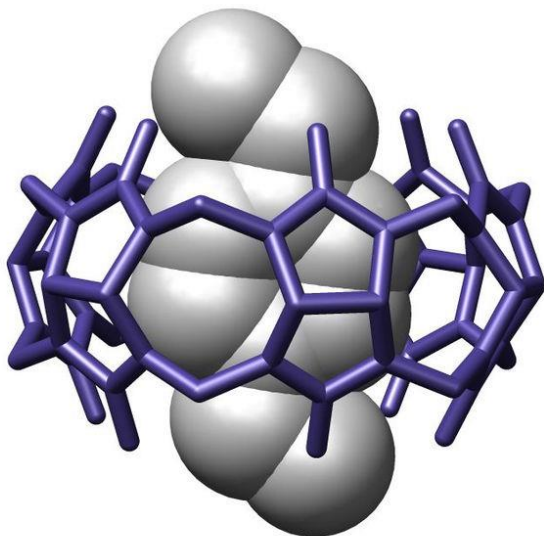
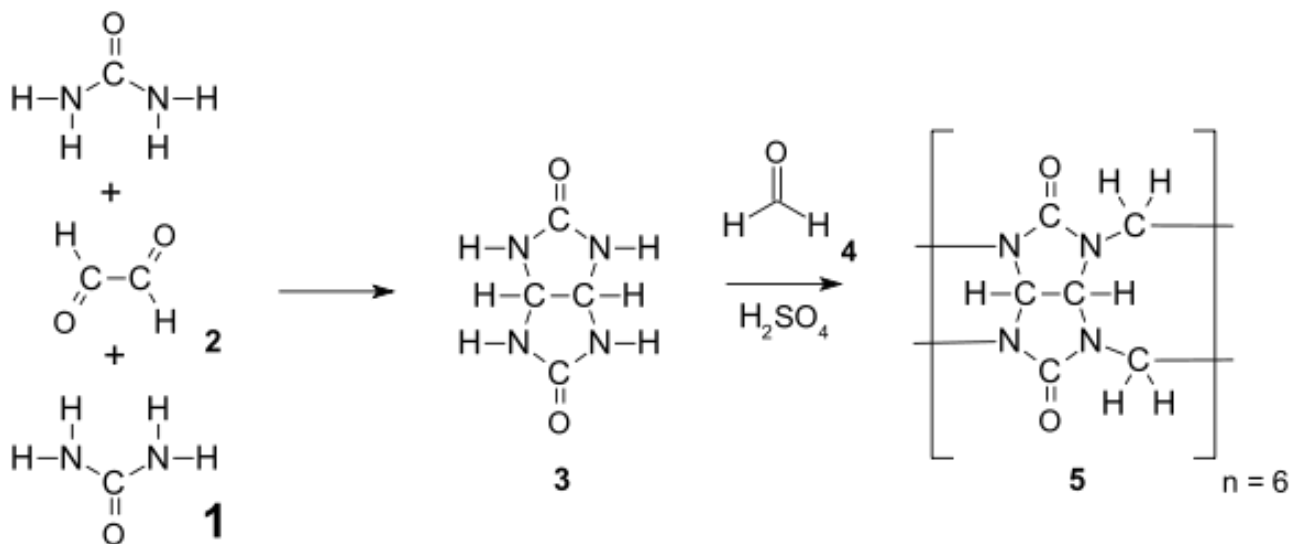
γ -Cyclodextrin



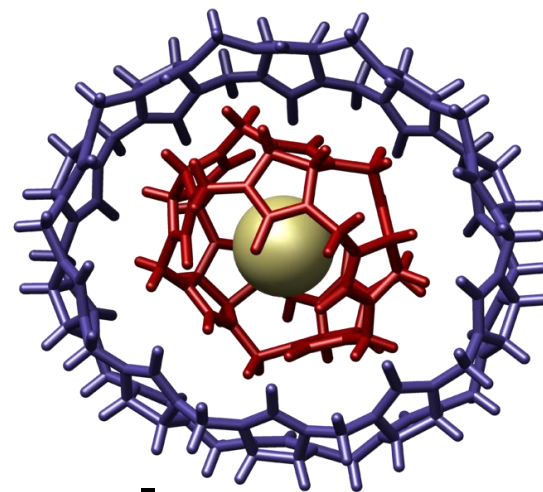
Cucurbiturils

- Methylene linked macrocyclic oligomers of glycouryl
- Cucurbit[n]uril n = number of monomers
- CB[n] or CBn
- 5, 6, 7, 8, and 10 repeat units have cavity volumes of 82, 164, 279, 479, and 870 Å³

Cucurbiturils

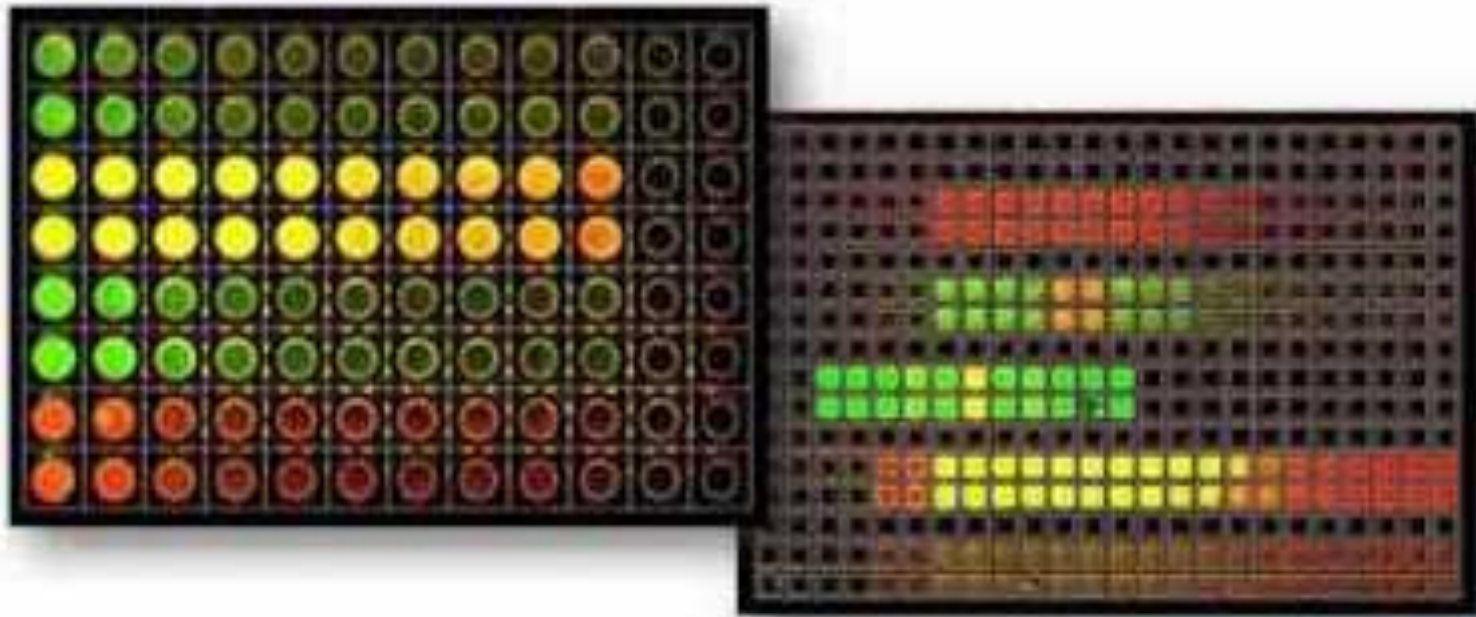


Cucurbit[6]uril .guest complex



CB[10].CB[5].Cl⁻ complex

Detection methods in chemosensing



General issues of detection

- Sensing : detection of an analyte
- Reception of guests is followed by signal generation by means of *signal transduction*
- Receptor unit : specificity, selectivity
- Signaling unit : sensitivity
- Signal : different means of energy

Signals

- Fluorescence

- Very sensitive (micro-, nano-, femtomolar, one molecule)
- Temporal resolution (nanosecondum)
- Spatial resolution (nm scale)
- Relatively easy and cheap detection

-
- Sensitive to contaminants, environment (pH, solvent temperature etc)
 - Stability
 - Toxicity

Signals

- Absorption (Colorimetric detection)
 - Cheap
 - Wider selection of chromophores
 - less sensitive (milli-, micro)
- Electrochemical detection
 - Sensitive
 - Cheap
 - Requires charged entities (electrochemically active species)

Signals

- Label free methods
 - Quartz crystal microbalance (QCM)
 - Surface plasmon resonance (SPR)
 - Both methods are sensitive
 - Provide information on affinity, kinetic and conformational data
 - Both requires immobilization on surfaces
 - Rather expensive instrumentation
 - Skilled operators

Signals

- Selecting the proper transduction strategy
 - Environment
 - Aims
 - Sensitivity
 - Limitations
 - Budget

Fluorescence detection

Basic principles of fluorescence

- Fluorescence is one way of energy dissipation (relaxation) from excited states
- Lowest energy electronic configuration defines the Ground state energy
- Statistically (Boltzmann) most molecules are at their ground state (S_0)
- S refers to singlet state (zero unpaired electron)
- T refers to triplet states (one unpaired electron)

Basic principles of fluorescence

- Excitation of molecules from ground state to excited states
- Excitation energy : light, electricity, heat, chemical/biological reaction etc.
- Absorption of energy quanta
- Different electron configurations (transitions can be e.g. $\pi-\pi^*$; $n-\pi^*$ etc. (HOMO-LUMO))
- Absorption process is very fast (10^{-15} s)

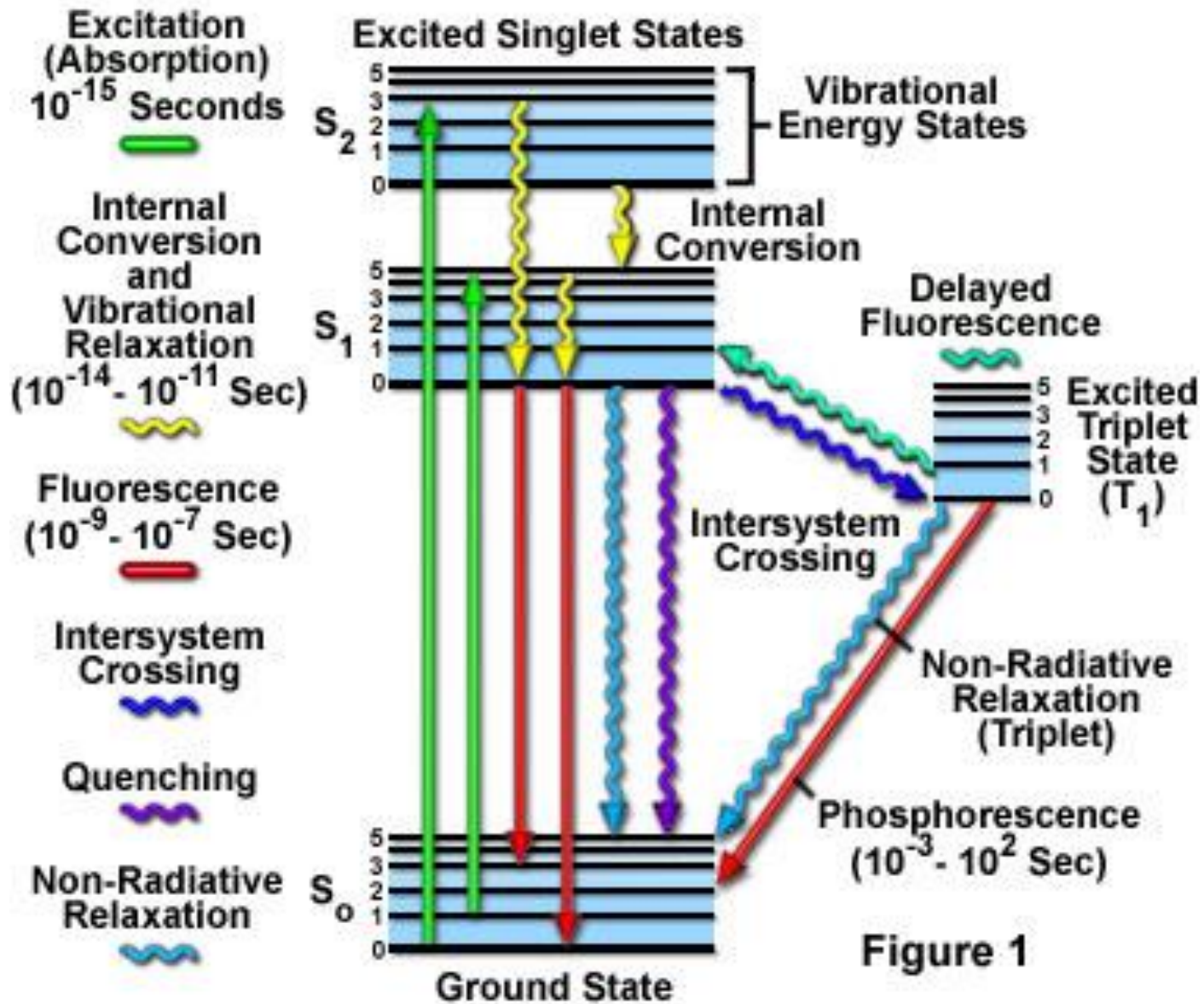
Basic principles of fluorescence

- From higher energy excited states energy dissipates very fast (10^{-13} - 10^{-12} s): *deactivation, relaxation, quenching* of excited states (S_{1-n})
 - Vibrational relaxation (VR) (relaxation via transfer of vibrational energy)
 - Internal conversion (IC) (collisional decay, heat emission)
 - *Non-radiative* decays
- The lowest energy excited state (S_1) has a relatively long life-time ($\sim 10^{-9}$ s) – excitation-relaxation process *stops* here for a while

Basic principles of fluorescence

- From S_1^0 there are several options for relaxation:
 - Vibrational relaxation to S_0^X
 - Vibrational relaxation – in very special cases (overlapping vibrational levels)
 - Intersystem crossing to T_1^X (forbidden transition)
 - Chemical reaction (= photochemistry – photobleaching)
 - Electron transfer (PET), proton transfer
 - Charge transfer (ICT)
 - Emission of a photon = *FLUORESCENCE*

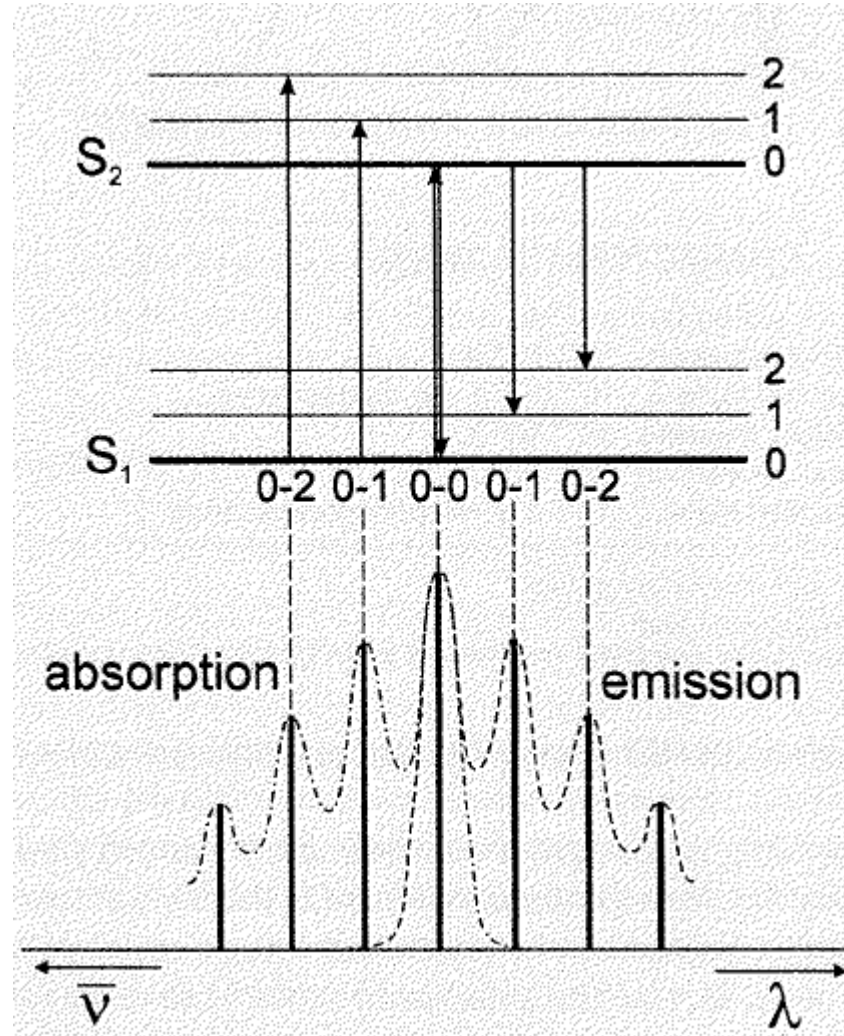
Jablonski Energy Diagram



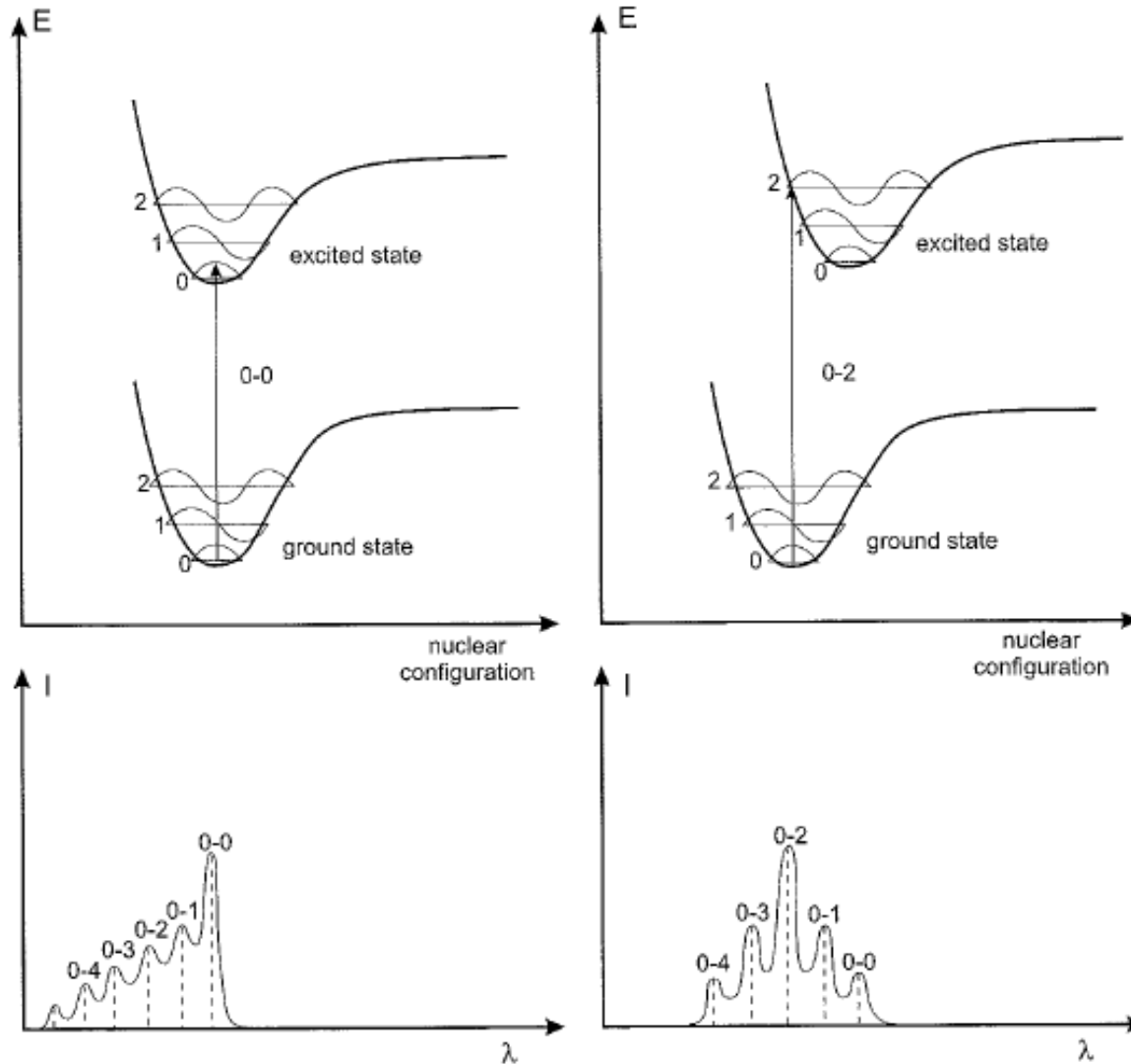
Radiative relaxation pathways

- Relaxation via emission of a photon
- Fluorescence : $S_1^0 \rightarrow S_0^X$
- Phosphorescence : $T_1^0 \rightarrow S_0^X$

Absorption and emission – mirror image principle



Intensity of bands – Franck-Condon principle



Terms related to fluorescence

- *Kasha's rule* : The emission spectrum observed is independent of the excitation wavelength (fast relaxations)
- *Stokes type fluorescence*: excitation is greater in energy than emission energy
- *Stokes shift* : the difference between the wavelength of absorption and emission maxima
 - The larger the clearer the spectrum (mega-Stokes dyes)

Terms related to fluorescence

- *Fluorescence lifetime, τ* (lifetime of excited state)
 - 1/ e of original intensity after excitation
 - Length vs. ISC
 - possibility for O₂ sensing
- *Quantum yield, Φ_F*
 - Photons emitted / photons absorbed
 - non-radiative vs. radiative relaxation

$$\Phi \approx \frac{k_{\text{radiative}}}{k_{\text{radiative}} + \sum k_{\text{non-radiative}}} \leq 1$$

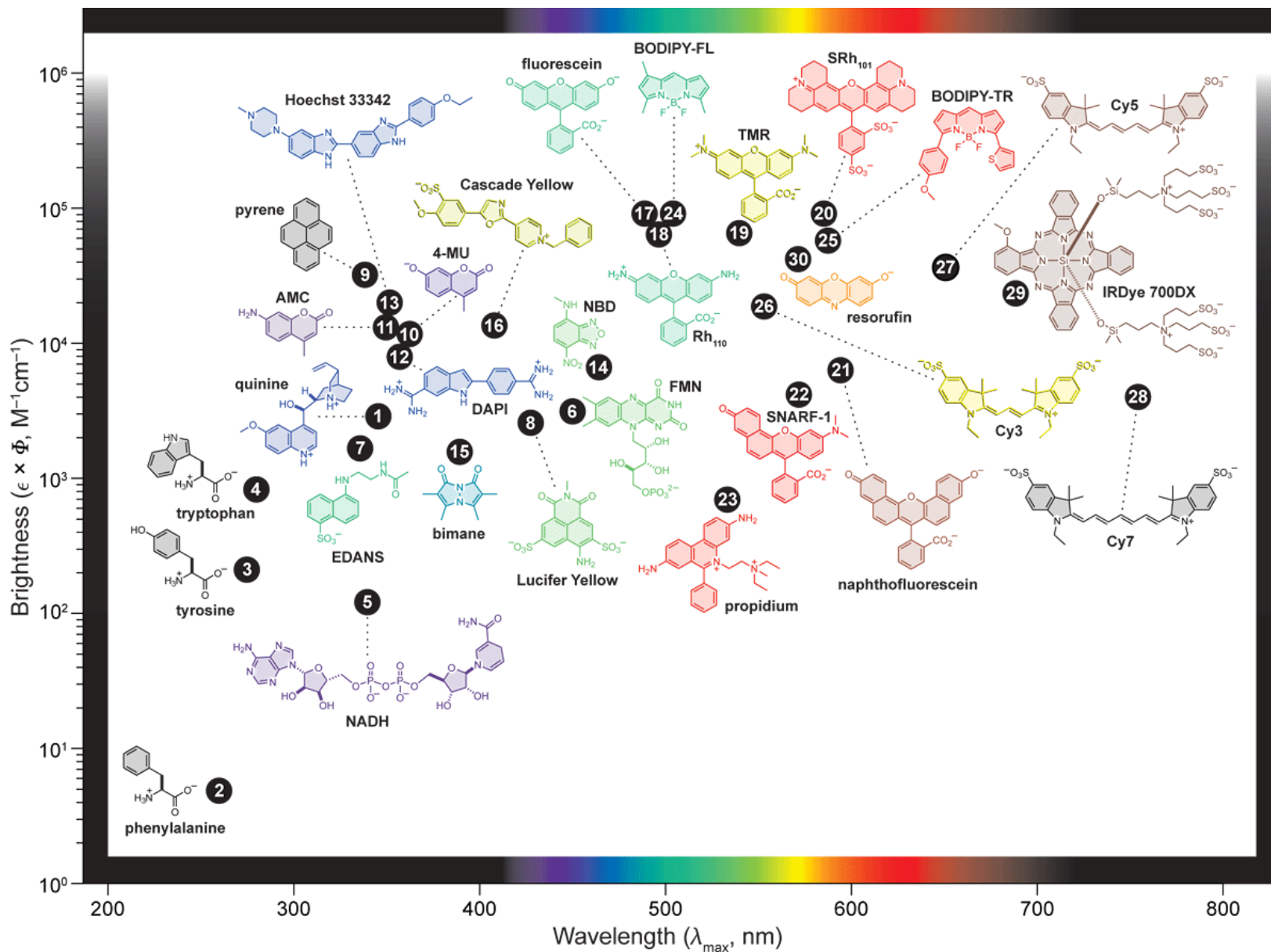
Detecting fluorescence *in vivo*

- Water and oxygen as quenchers
- Photobleaching
- Autofluorescence (Trp, nucleotides, porphyrins etc.) – mega Stokes dyes

Fluorophores

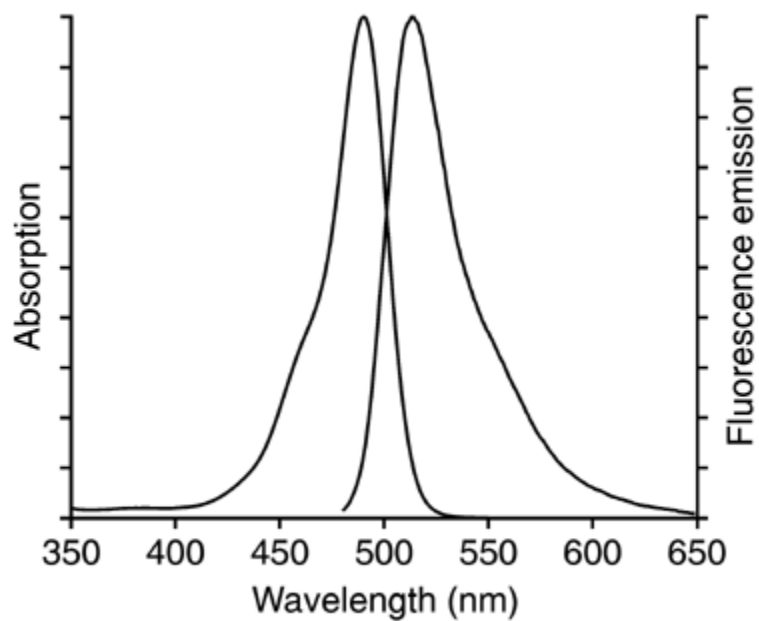
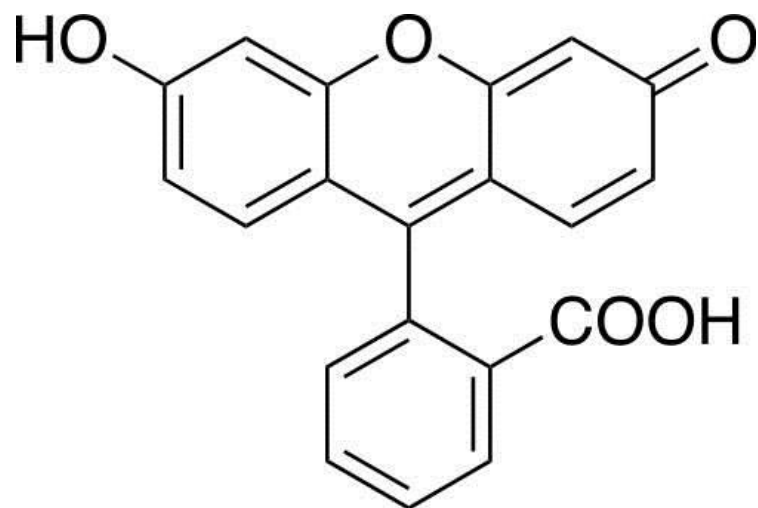
- Organic
 - aromatic compounds
 - metal complexes
 - fluorescent proteins (e.g. GFP)
- Inorganic
 - Semi conductor quantum dots (QDs)
 - Upconverting nanoparticles (UCNPs)

Organic fluorophores



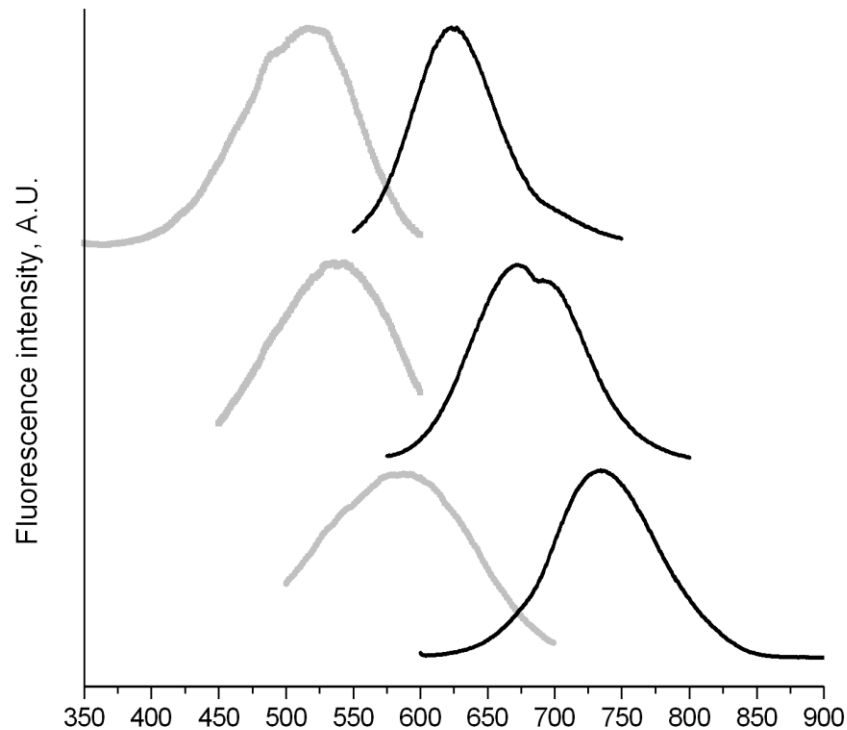
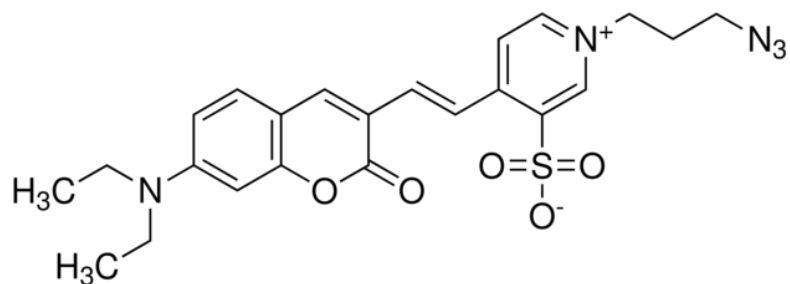
The Stokes shift issue

Fluorescein

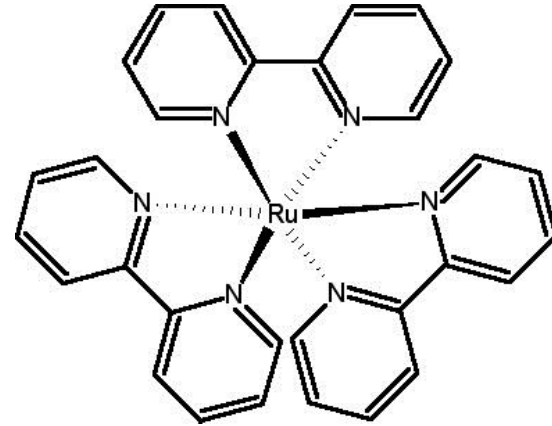


The Stokes shift issue

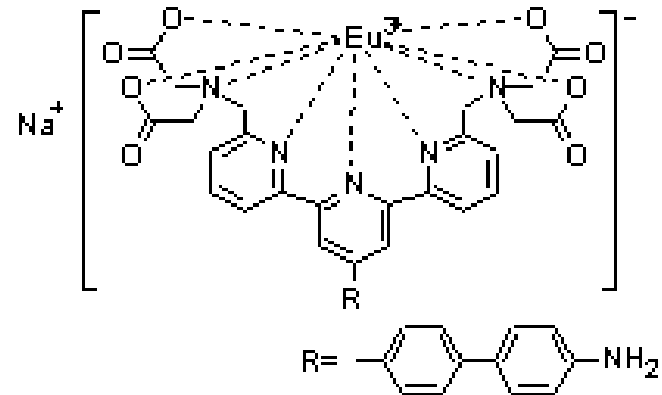
Mega Stokes dyes



Metal complexes



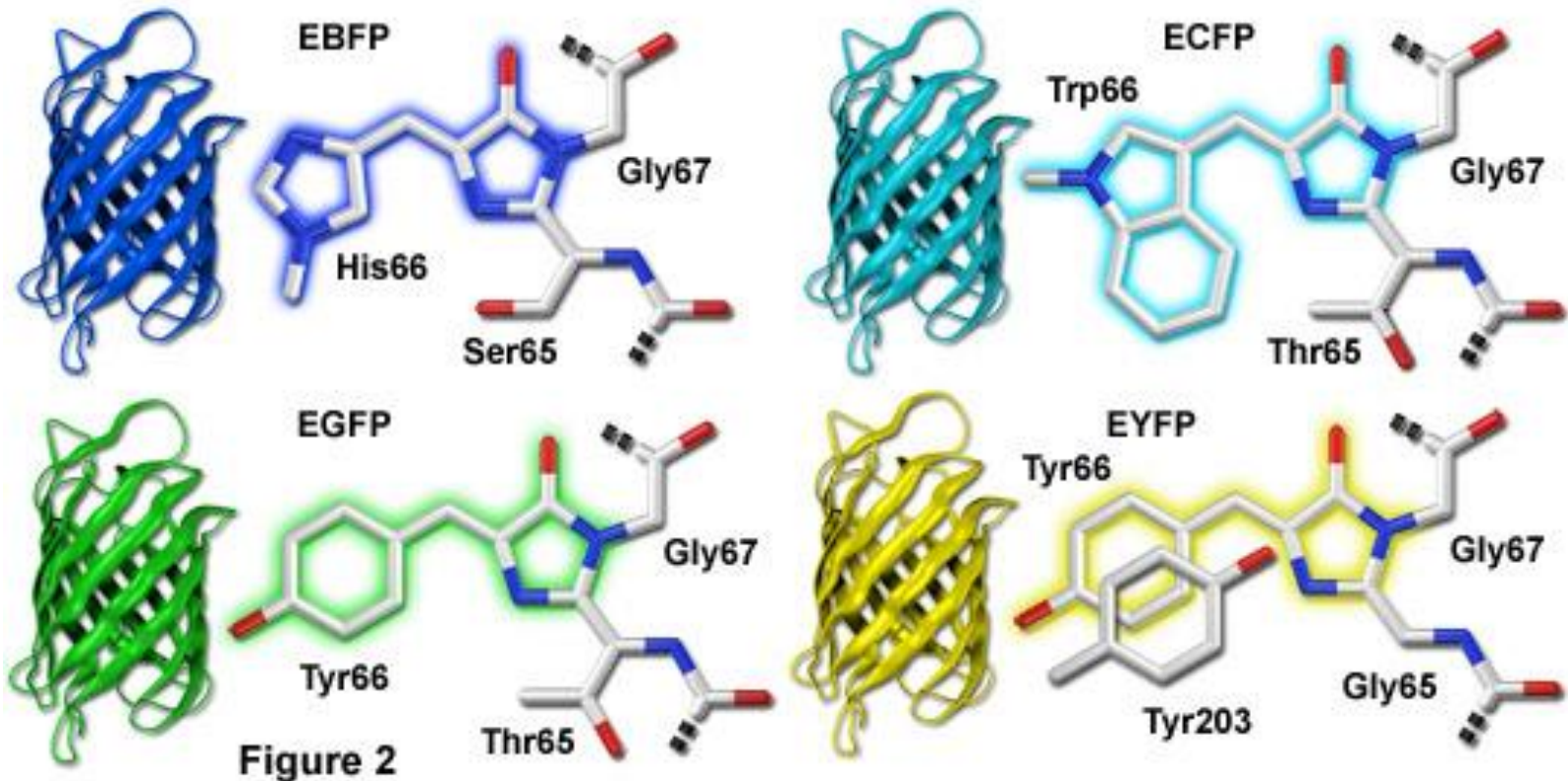
Eu-complexes



A2083 ATBTA-Eu³⁺

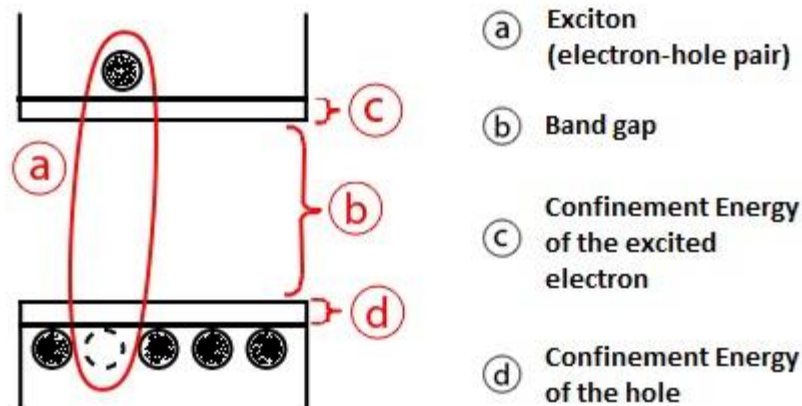
Fluorescent proteins

Chromophore Structural Motifs of Green Fluorescent Protein Variants

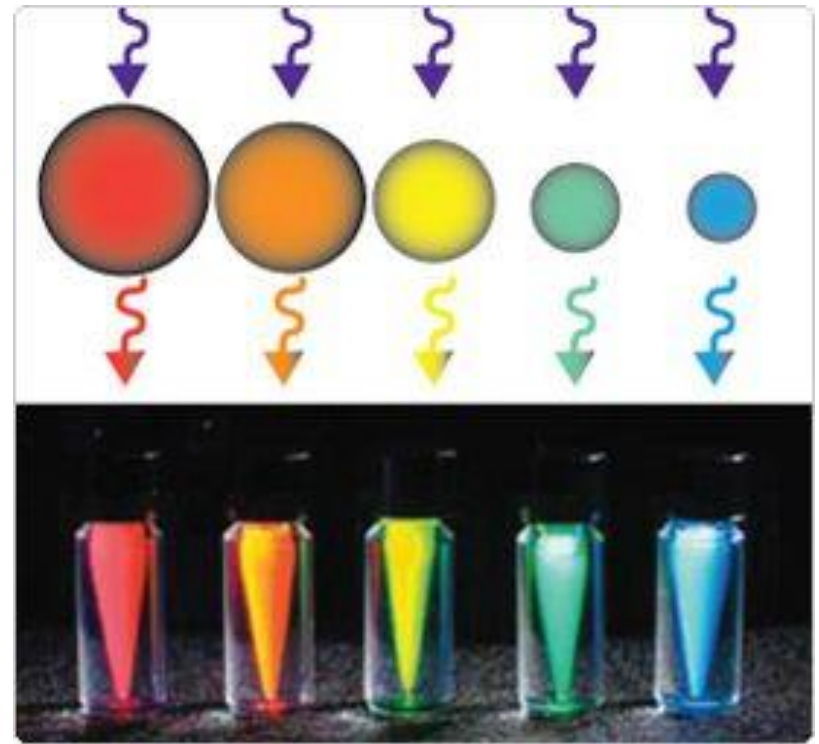
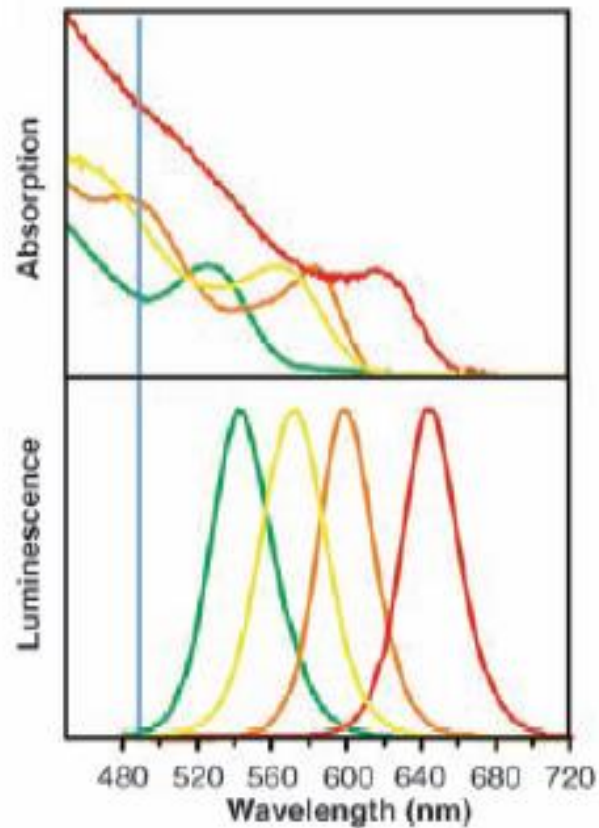


Semi-conductor quantum dots (QDs)

- Semiconductor nanocrystals of e.g. CdSe, CdS, ZnS etc.
- The band-gap between valence and conductive bands is inversely related to their size
- The smaller, the higher the energy of the emitted photon



Semi-conductor quantum dots (QDs)



Semi-conductor quantum dots (QDs)

One excitation wavelength can excite all ranges of size



Semi-conductor quantum dots (QDs)

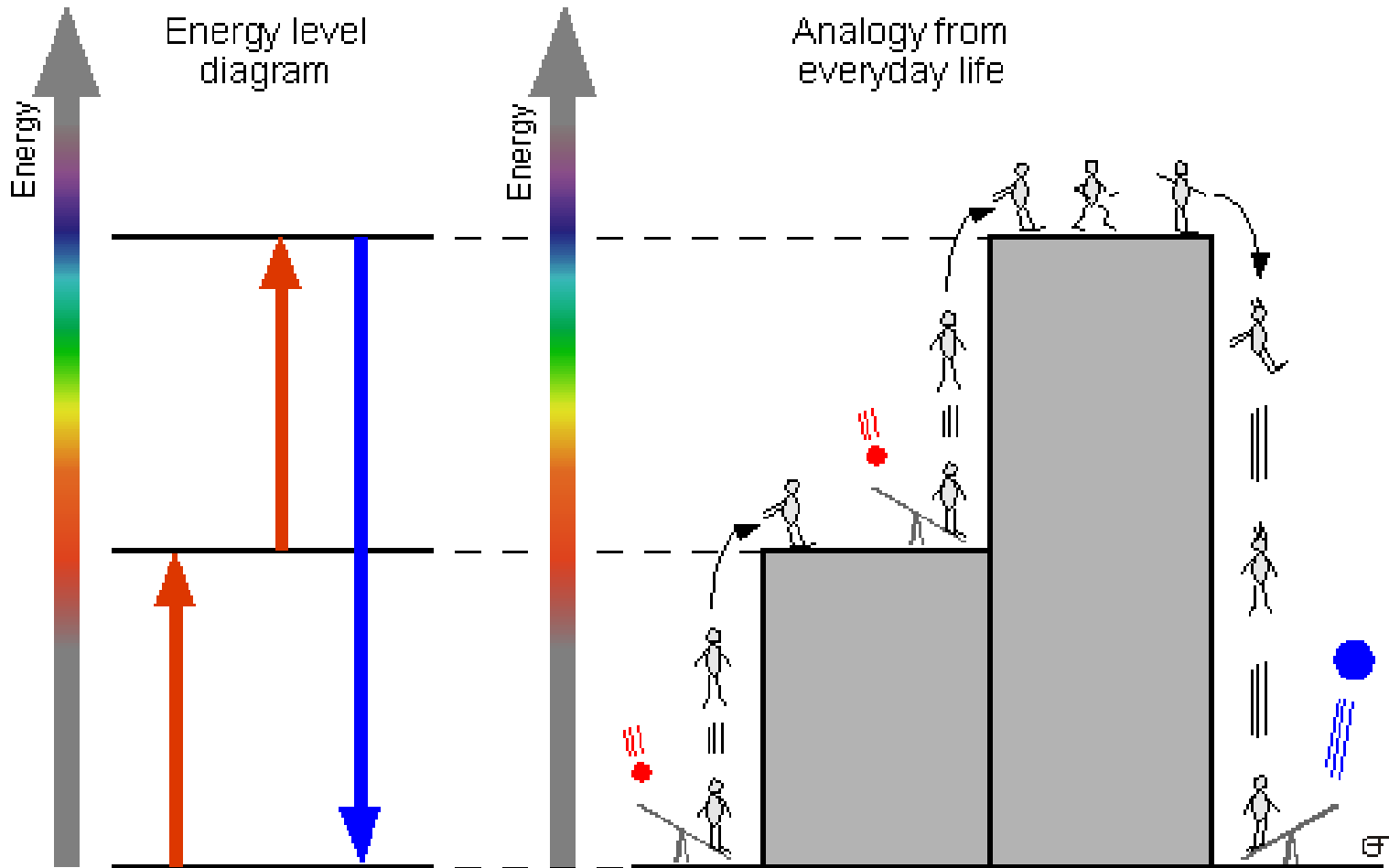
- Very high quantum yield (close to unity)
- Excellent photostability
- Narrow emission bands
- Toxicity issues - need to be coated
- Difficult signal transduction

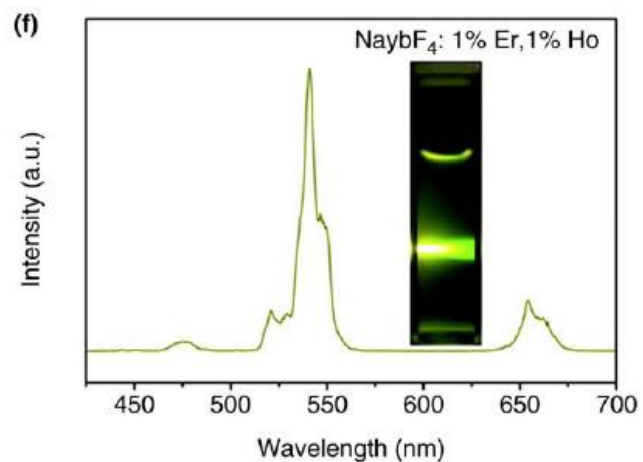
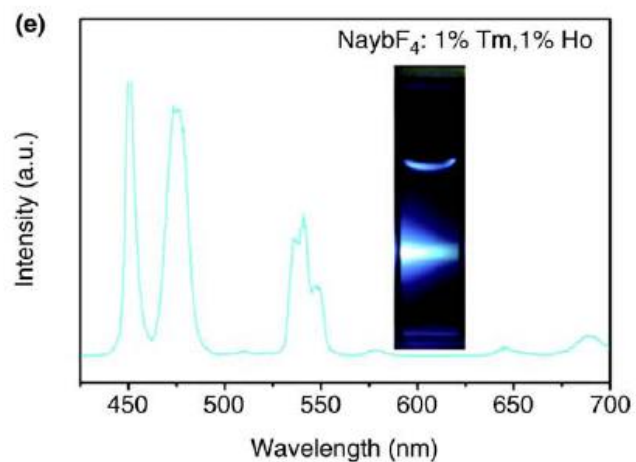
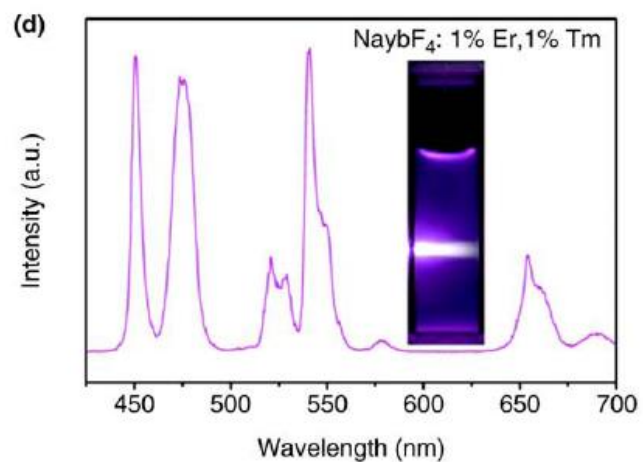
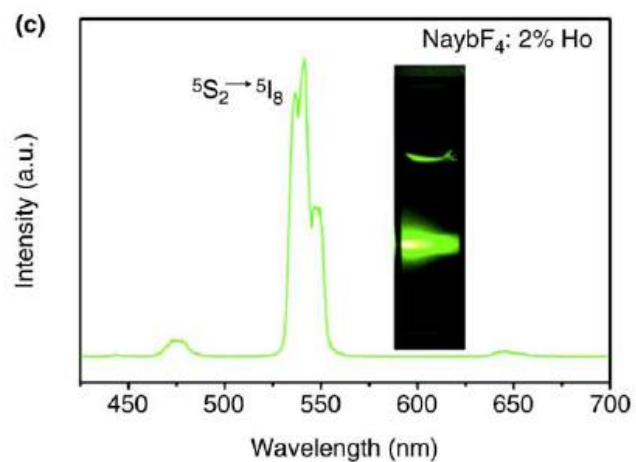
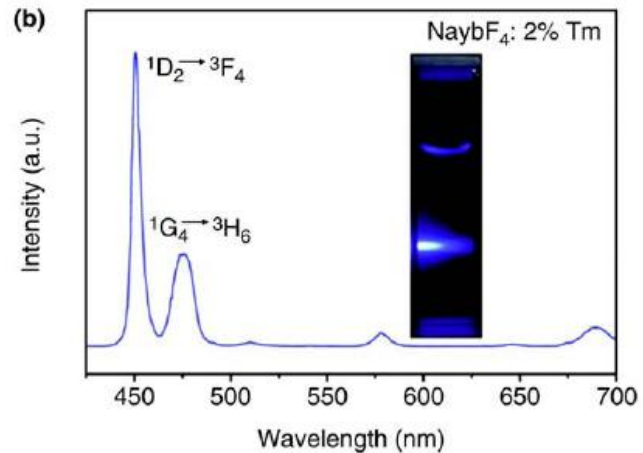
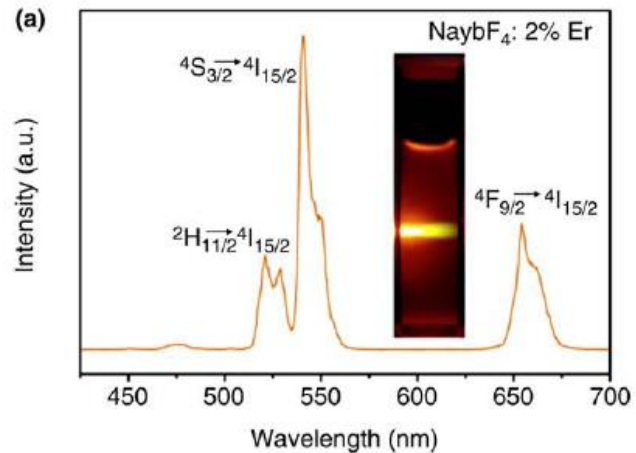
Upconverting nanocrystals

- Rare-earth metals doped into crystal lattice of e.g. NaYF_4 or Y_2O_3
- At least two types of RE metals are needed as dopants
- Anti-Stokes fluorescence
- Low energy excitation (e.g. at 980 nm), visible, higher energy emission
- Sequential two photon excitation followed by energy transfer between the dopants
- RE metals have numerous closely spaced ladder-like relatively long stable energy levels – can be excited again from an excited state

Upconversion

Making 1x blue out of 2x red





Upconverting nanocrystals

- Excitation in the NIR region (water window)
- Enable deep tissue imaging
- Photostable
- Autofluorescence-free fluorescence (higher signal-to-noise)
- Sequential two photon excitation followed by energy transfer between the dopants
- RE metals have numerous closely spaced energy levels
- Non-toxic
- Coating needed for functionalization
- Difficult signal transduction
- Size should be below ~ 40 nm for sensor applications

