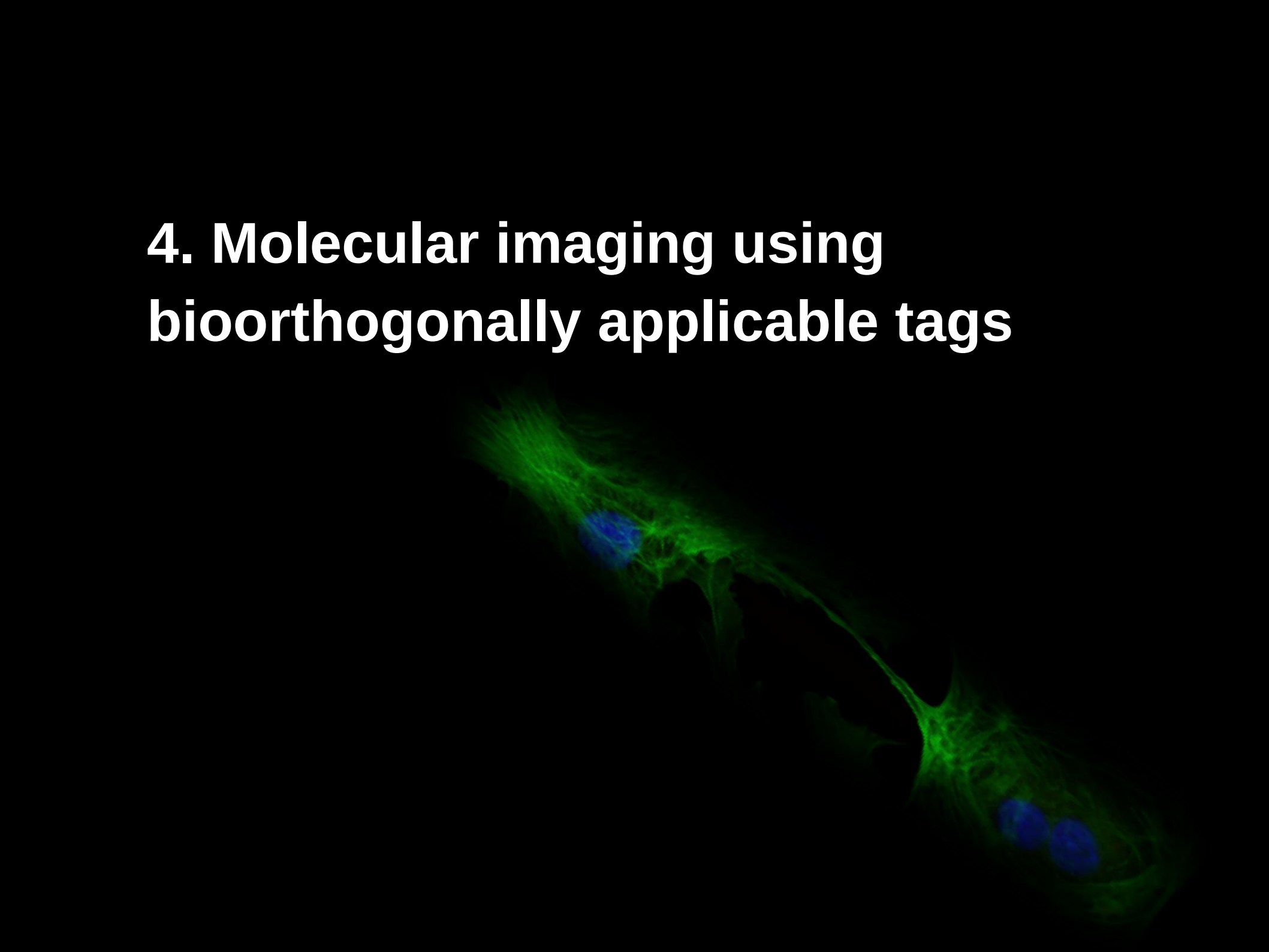
4. Molecular imaging using bioorthogonally applicable tags



4.1. Overview of imaging modalities

- Aims of chemical biology
- Visualize, follow molecular biology processes
- Target biomolecule+chemical reporter
- Exogeneously delivered probes (*Signaling unit)* : fluorescent, radioactive, NMR active nuclides, PET etc.

R. Weissleder, U. Mahmood (2001), *Radiology 219,* 316.

Probes

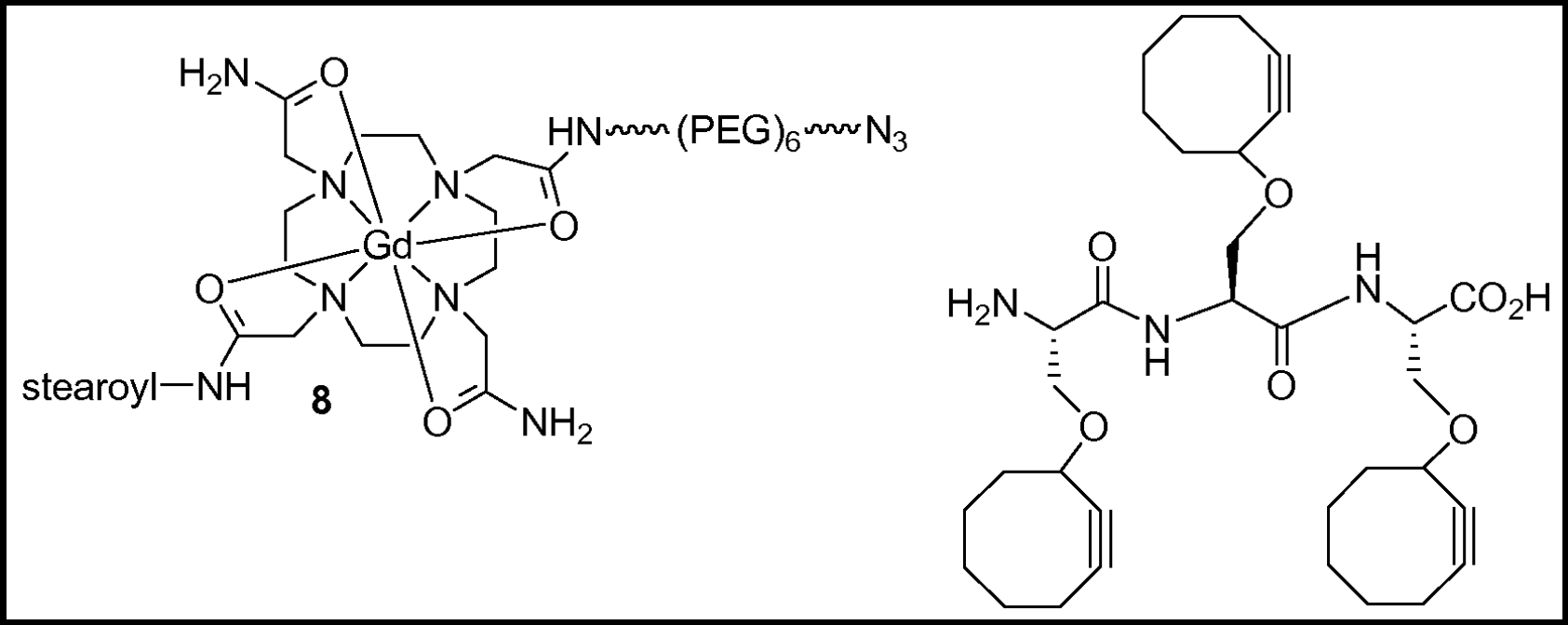
- Magnetic resonance imaging (MRI)
- Single photon emission computed tomography (SPECT)
- Positron emission tomography (PET)
- Optical imaging (fluorescence, luminescence)

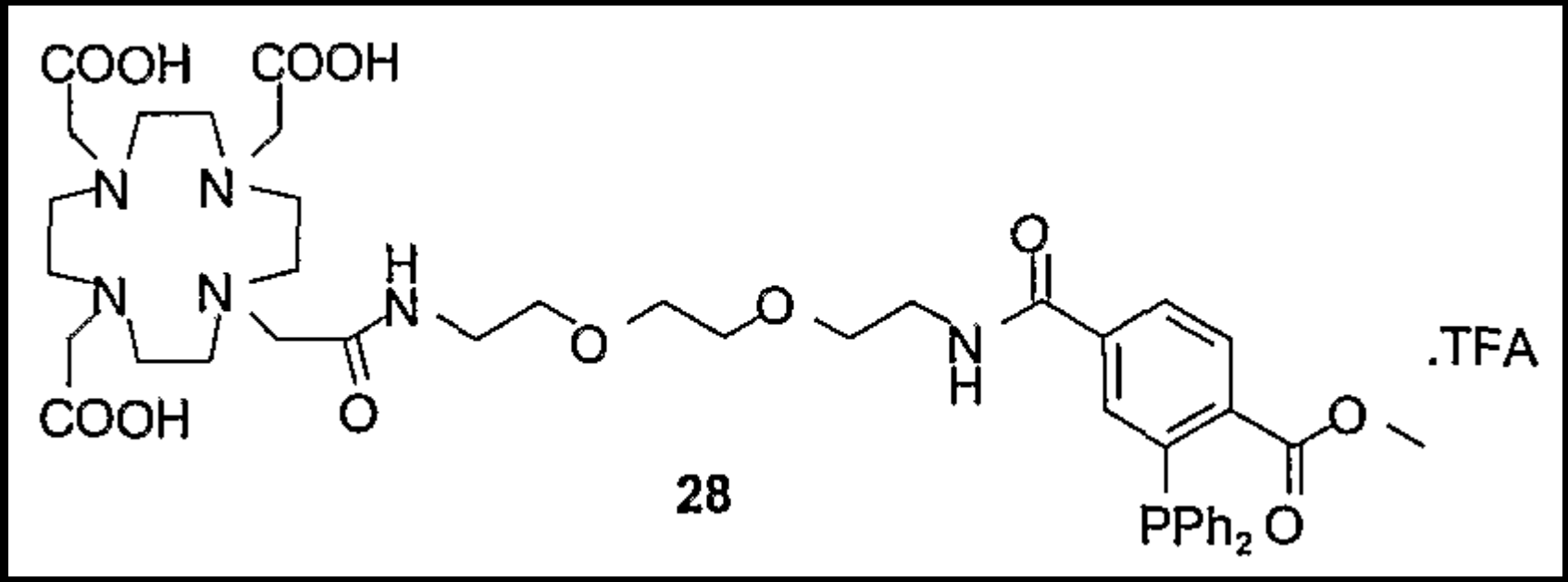
Magnetic resonance imaging

- NMR active nuclei (mainly hydrogen, Gd-complexes)
- Image is generated using the relaxation parameters (T1, T2)
- Each tissue has different characteristics
- Very good spatial resolution (good contrast image)
- Low sensitivity (mmol/L)
- No need for ionizing radiation

- X. Shenghui (2013), *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* **5** (2).

MRI probes


H2N
O
HN
(PEG)6
N3
N
N
Gd
O
O
N
N
stearoyl—NH
8
O
NH2
H2N
O
N
H
O
H
N
CO2H
O
O
O

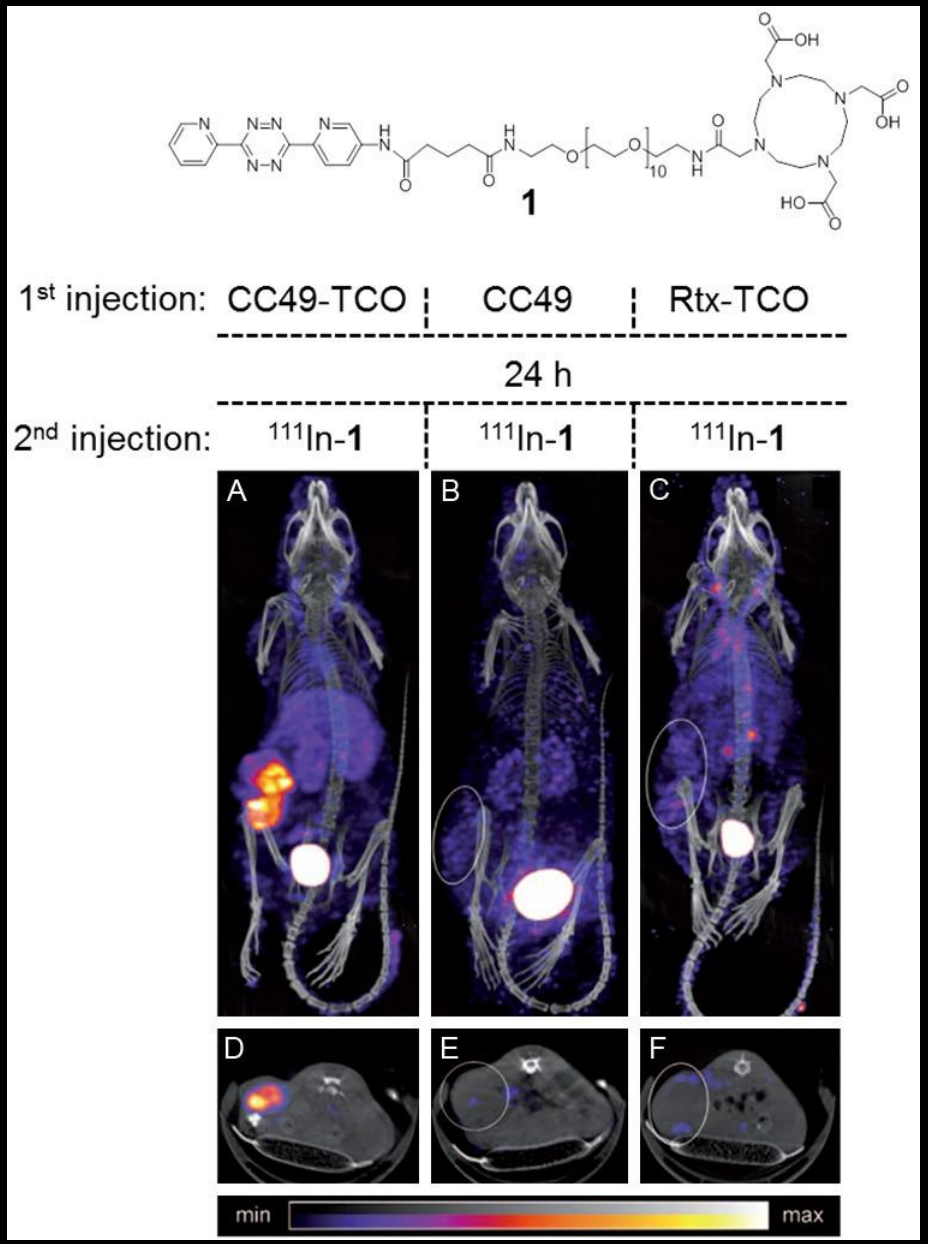

COOH
COOH
N
N
N
N
COOH
H
N
O
O
O
N
H
O
.TFA
O
PPh2
O
28

Single photon emission computed tomography

- Radioactive (γ emitting) active nuclei (^{99m}Tc, ^{111}In, ^{123}I, ^{201}Tl)
- Rotating gamma-camera creates 3D image
- Cheaper than PET
- Low spatial and temporal resolution
- Radioactivity concerns
- No need for ionizing radiation

- X. Shenghui (2013), *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* **5** (2).

SPECT imaging using pre-targeting antibodies

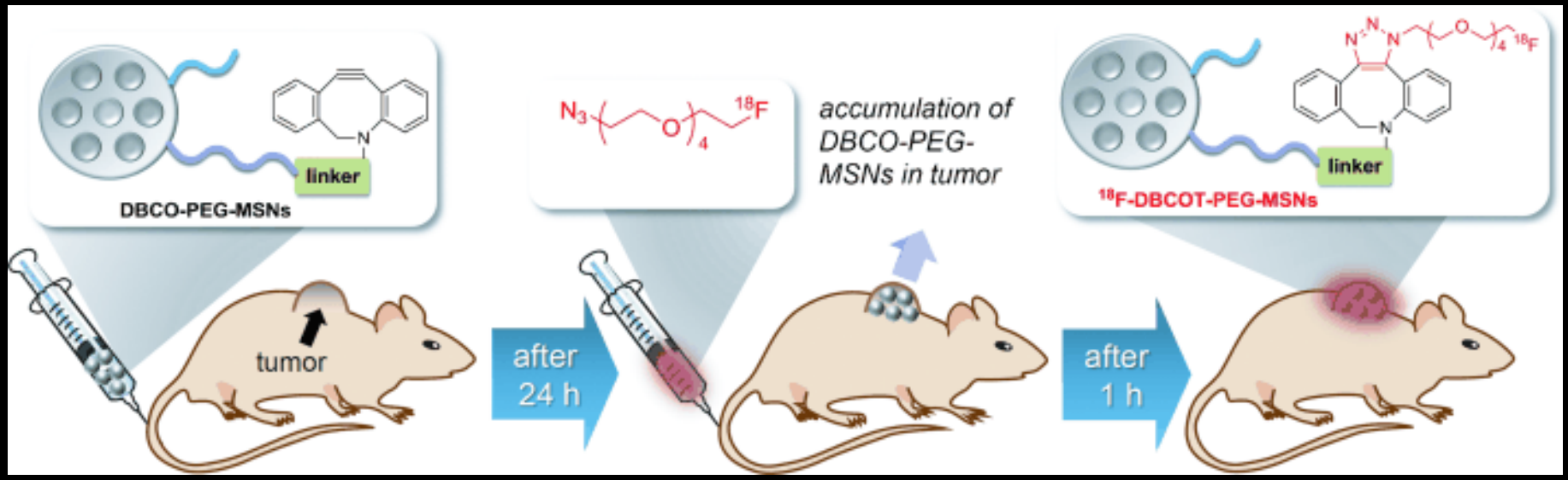


Rossin R, Renart Verkerk P, van den Bosch SM, Vulders RCM, Verel I, Lub J, Robillard MS. Angew Chem Int Ed Engl. 2010, 122, 3447

Positron emission tomography

- Uses positron emitting isotopes
- Annihilation creates two high energy γ-photons
- ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{64}Cu, ^{124}I, ^{76}Br, ^{82}Rb
- Very expensive (isotopes have short lifetime)
- Very sensitive (10^{-11} mol/L to 10^{-12} mol/L)

PET-Imaging



Lee, S. B., Kim, H. L., Jeong, H.-J., Lim, S. T., Sohn, M.-H. and Kim, D. W. (2013), Angew. Chem. Int. Ed., 52: 10549

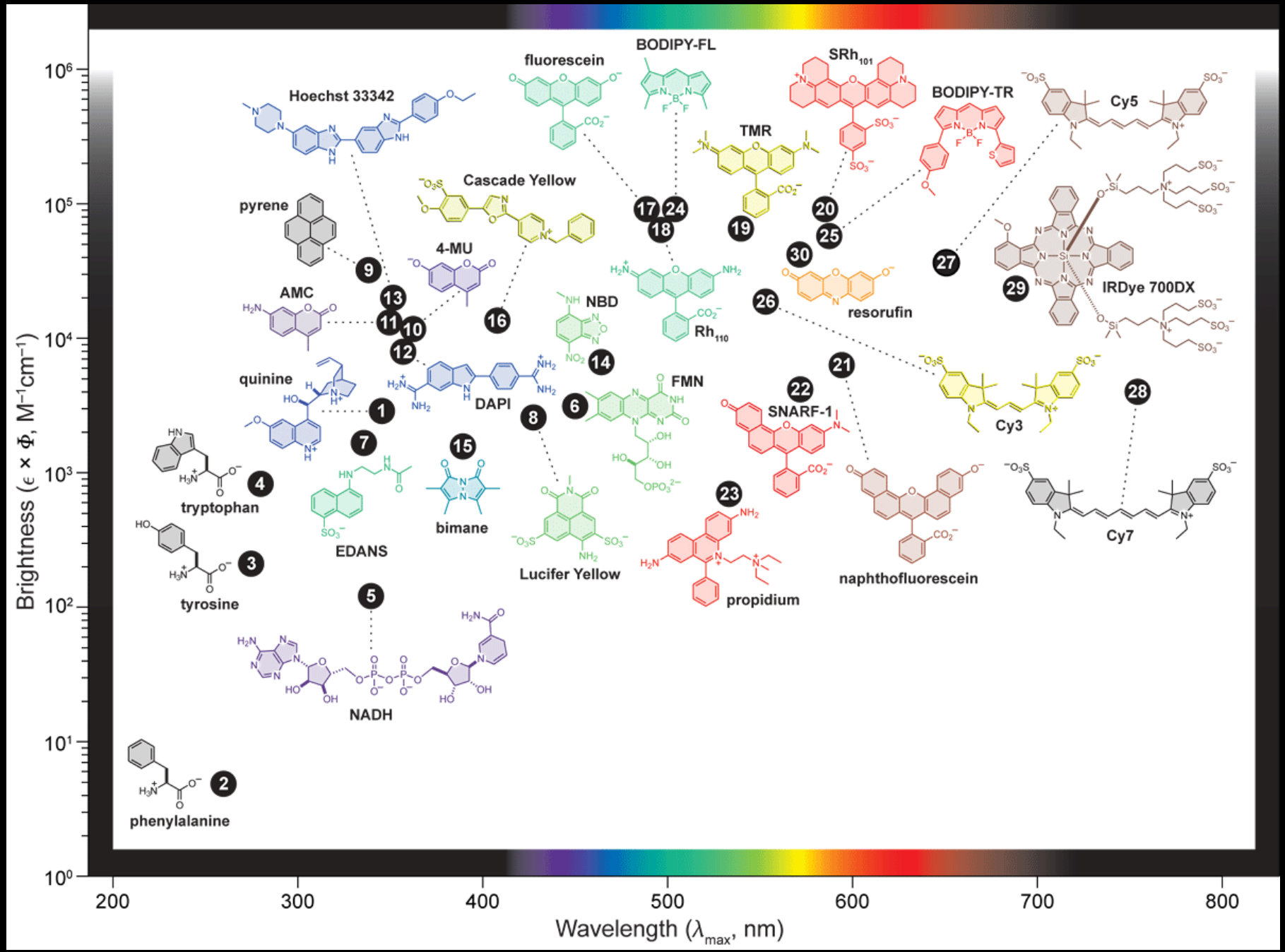
4.2. Optical Imaging

- Fluorescence, luminescence
- Sensitive, good temporal and spatial resolution
- Cheap
- Lower contrast due to diffraction (super-resolution methods)
- Penetration depth, autofluorescence, backgroundfluorescence, photobleaching

Fluorophores

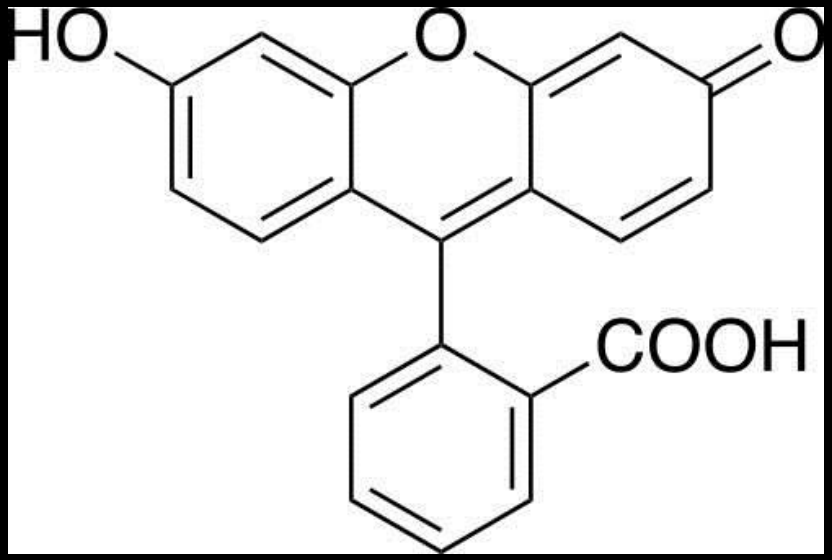
- Organic
  - aromatic compounds
  - metal complexes
  - fluorescent proteins (e.g. GFP)
- Inorganic
  - Semi conductor quantum dots (QDs)
  - Upconverting nanoparticles (UCNPs)

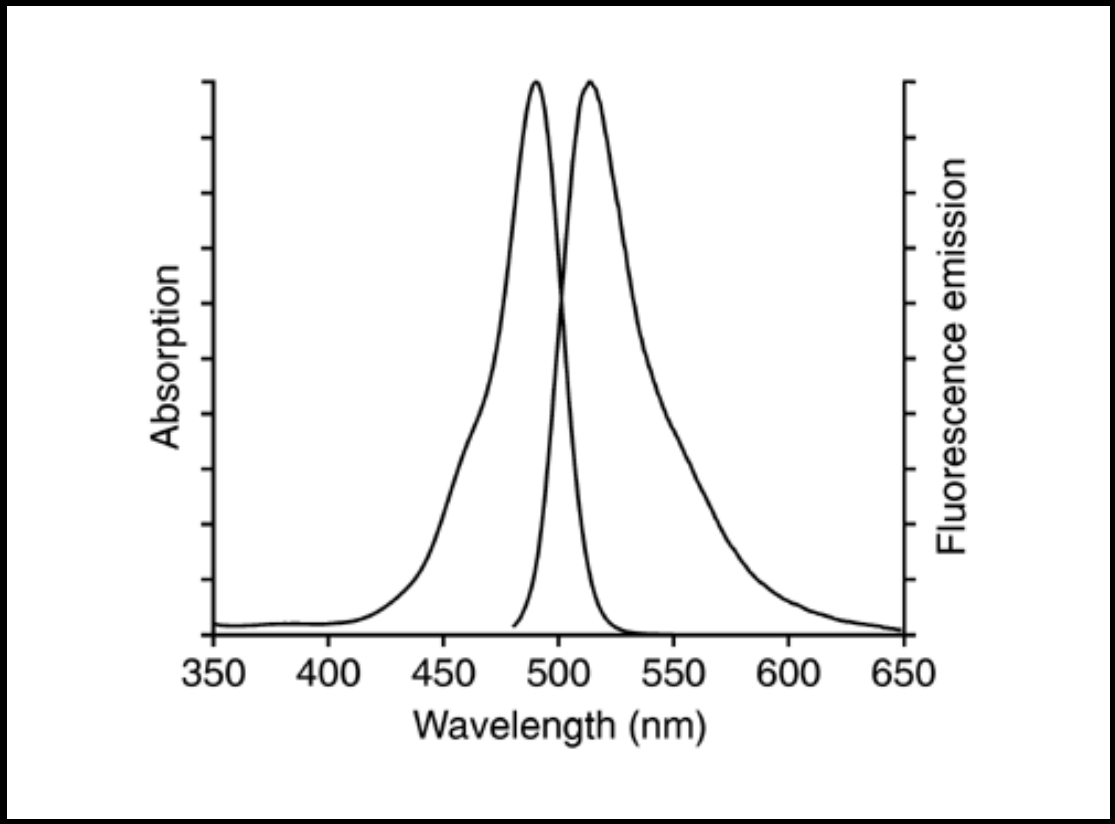
Organic fluorophores



The Stokes shift issue

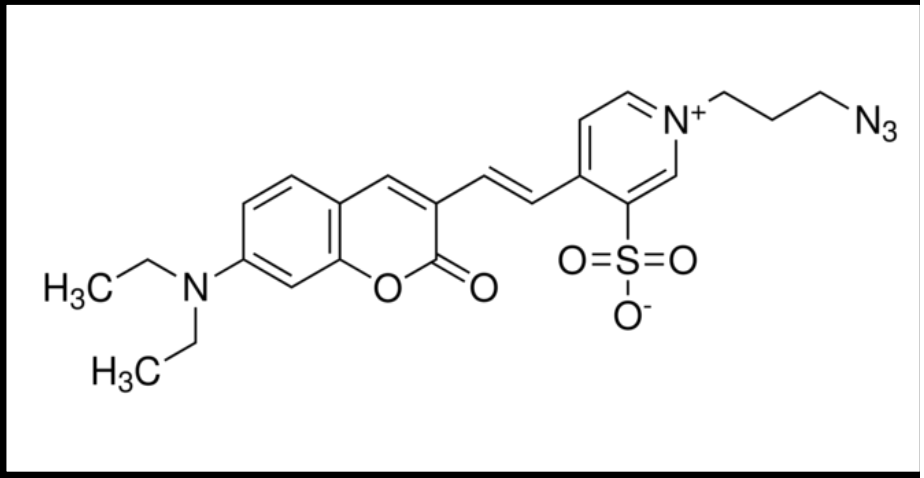
Fluorescein

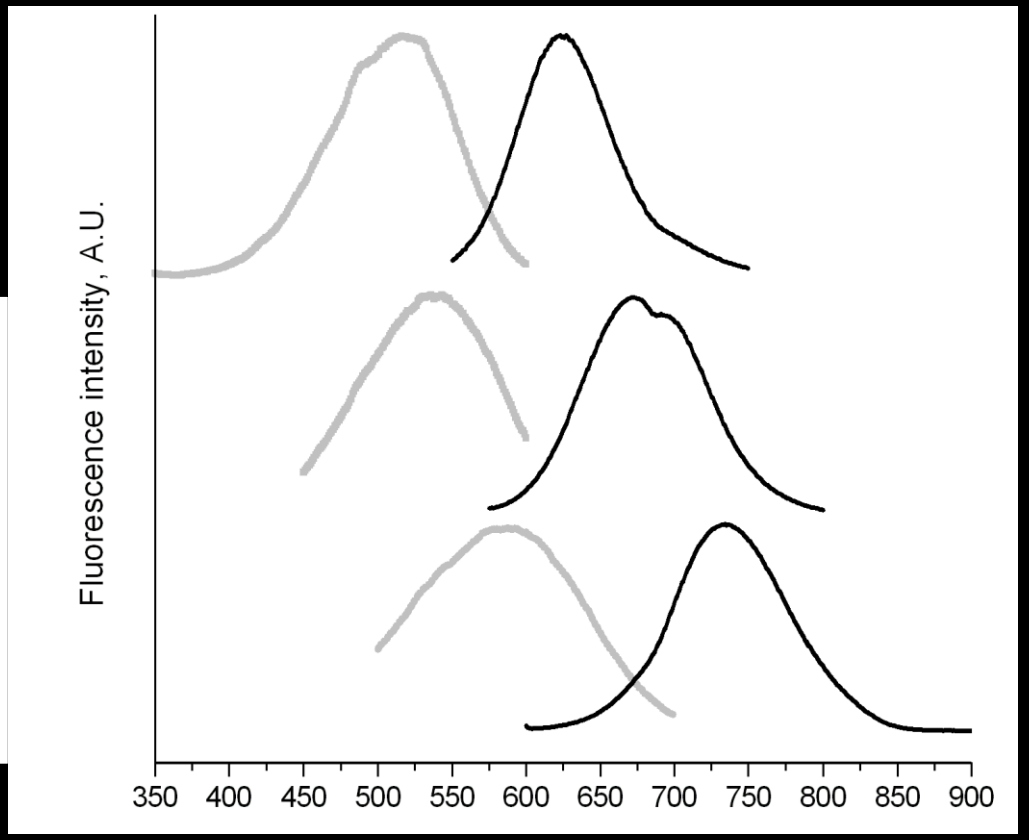




The Stokes shift issue

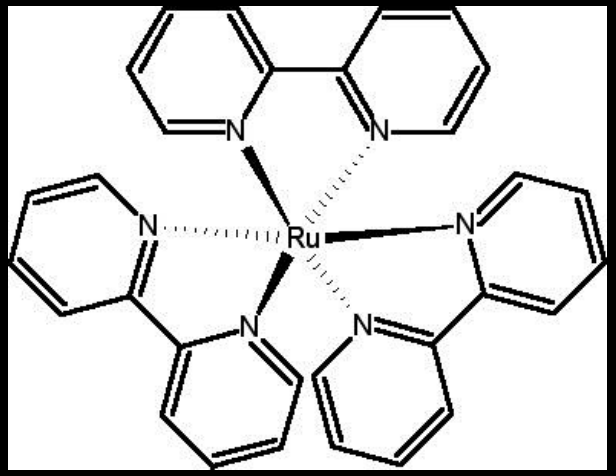
Mega Stokes dyes



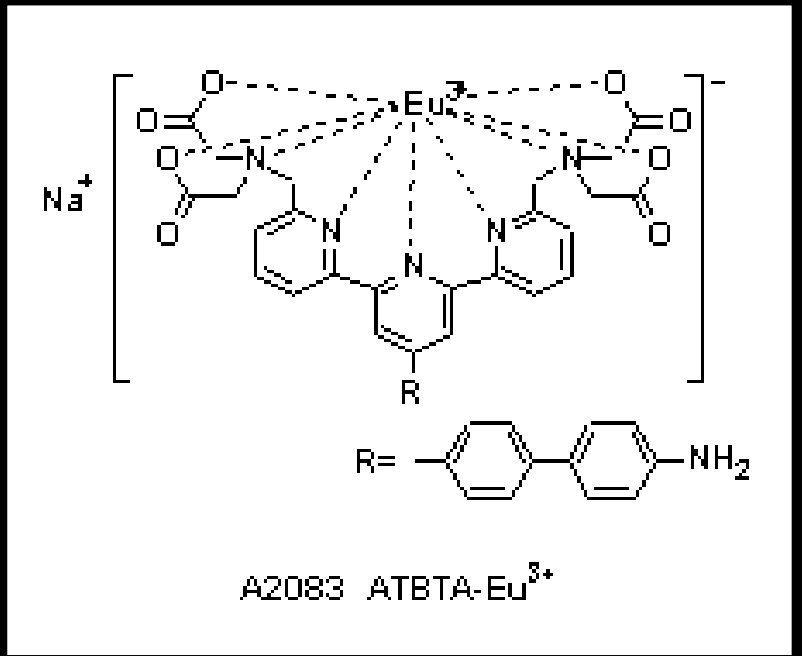


Metal complexes

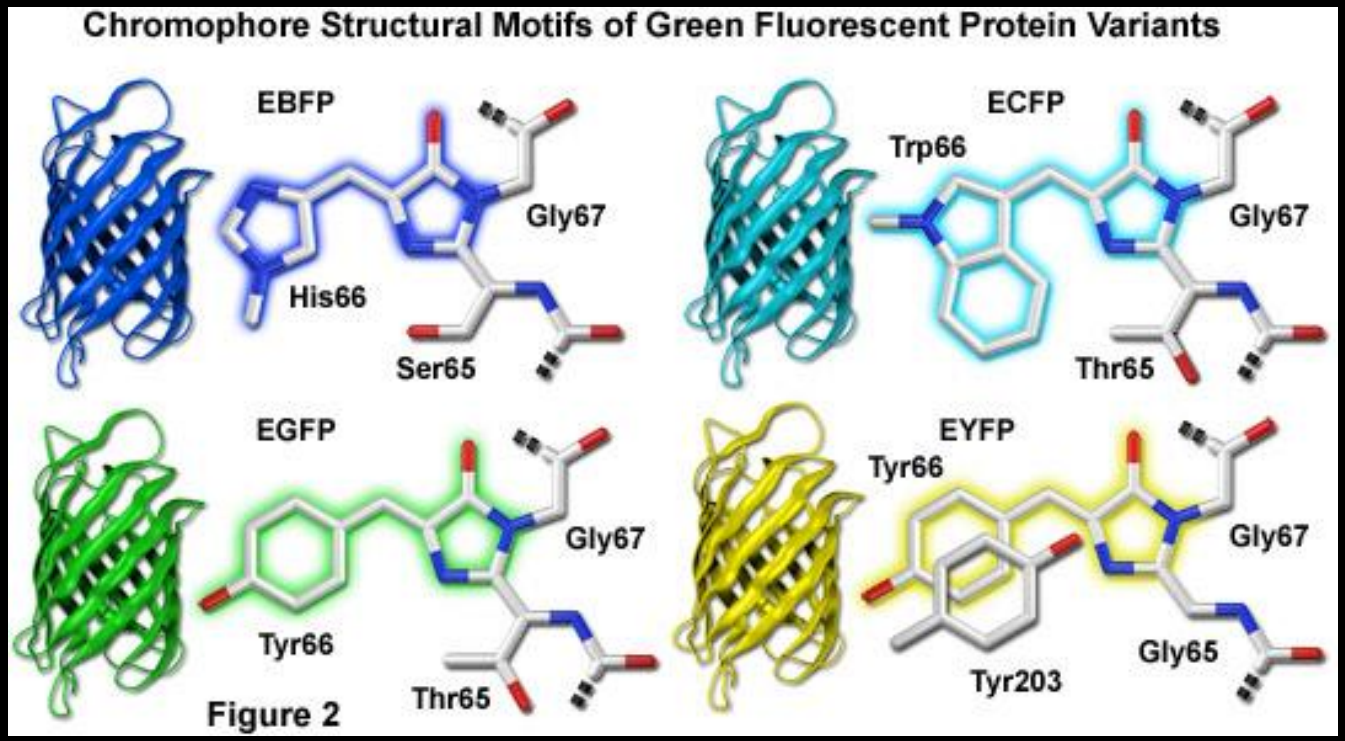
$Ru(Bpy)_3$

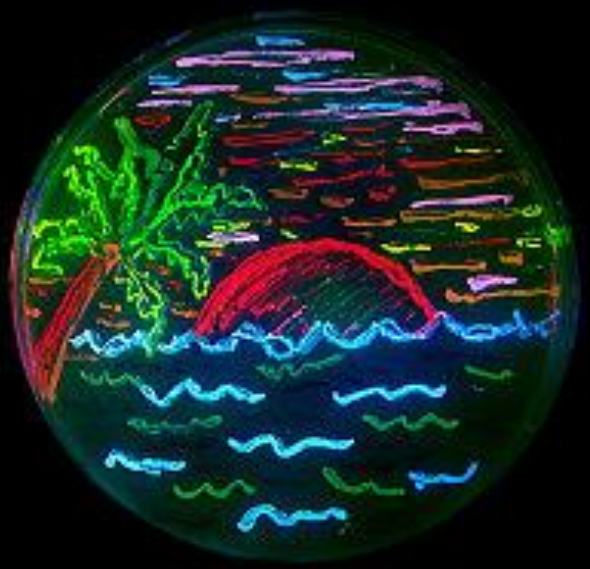


Eu-complexes



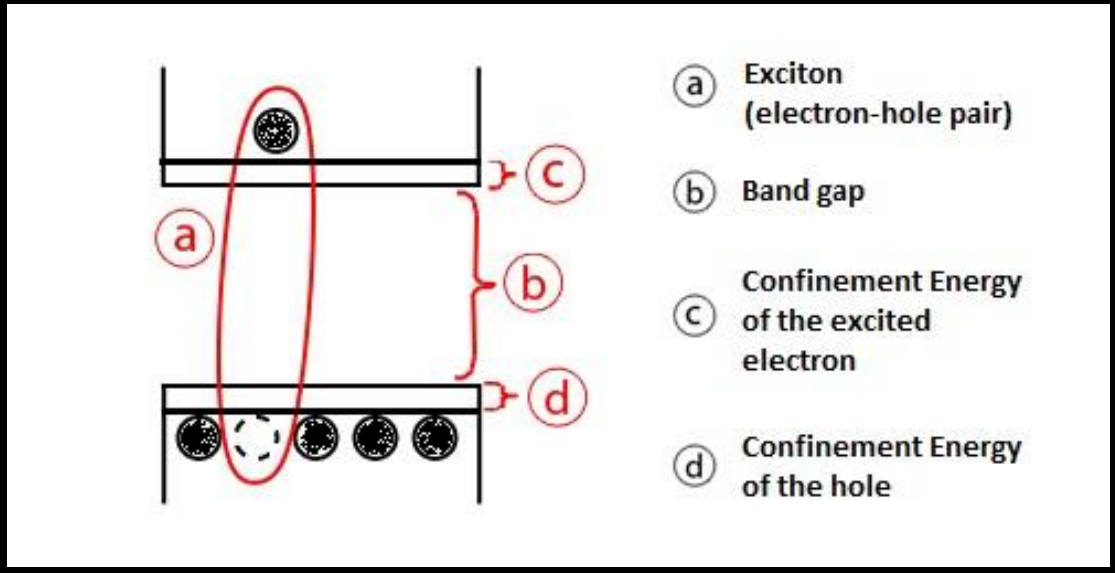
Fluorescent proteins



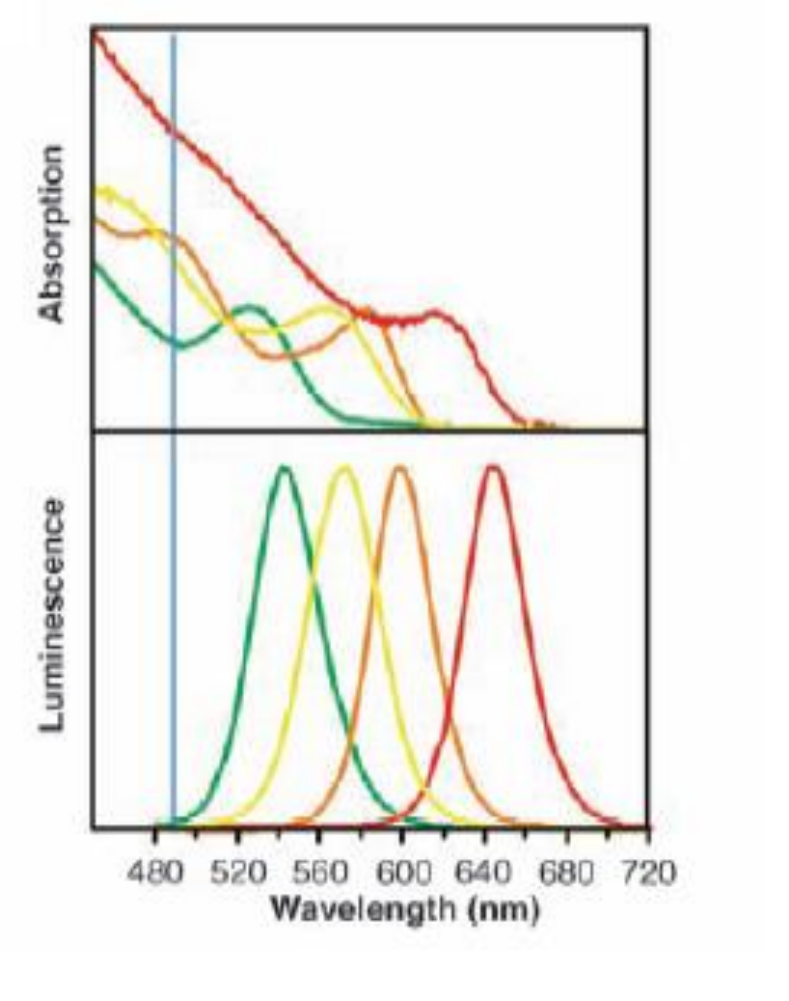


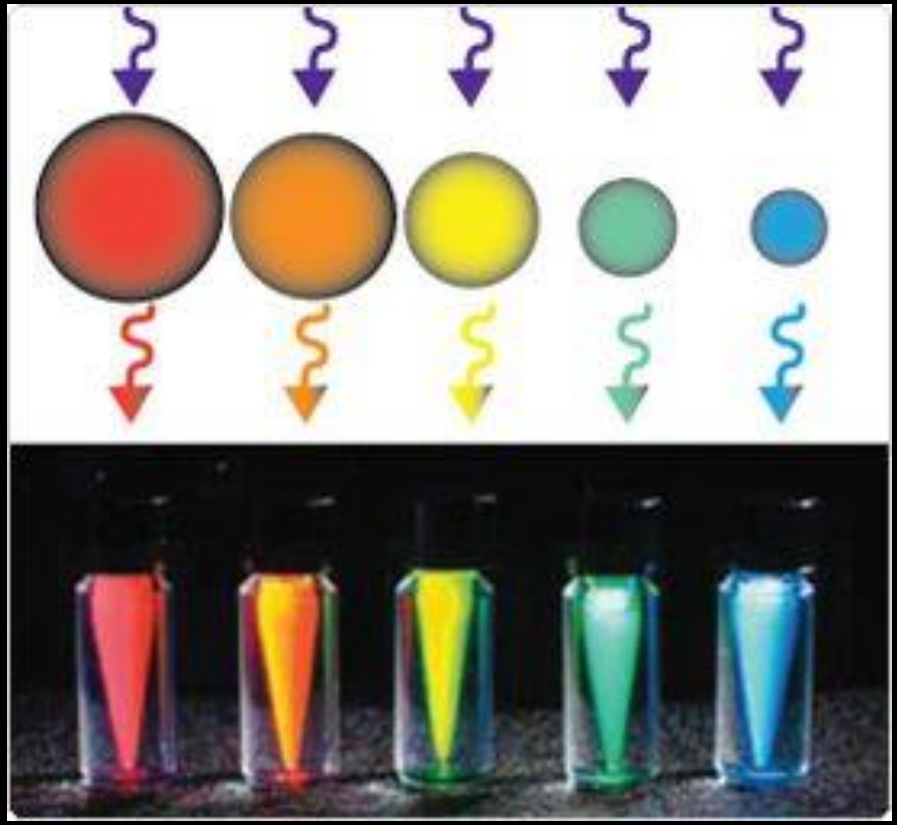
Semi-conductor quantum dots (QDs)

- 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)

Semi-conductor quantum dots (QDs)

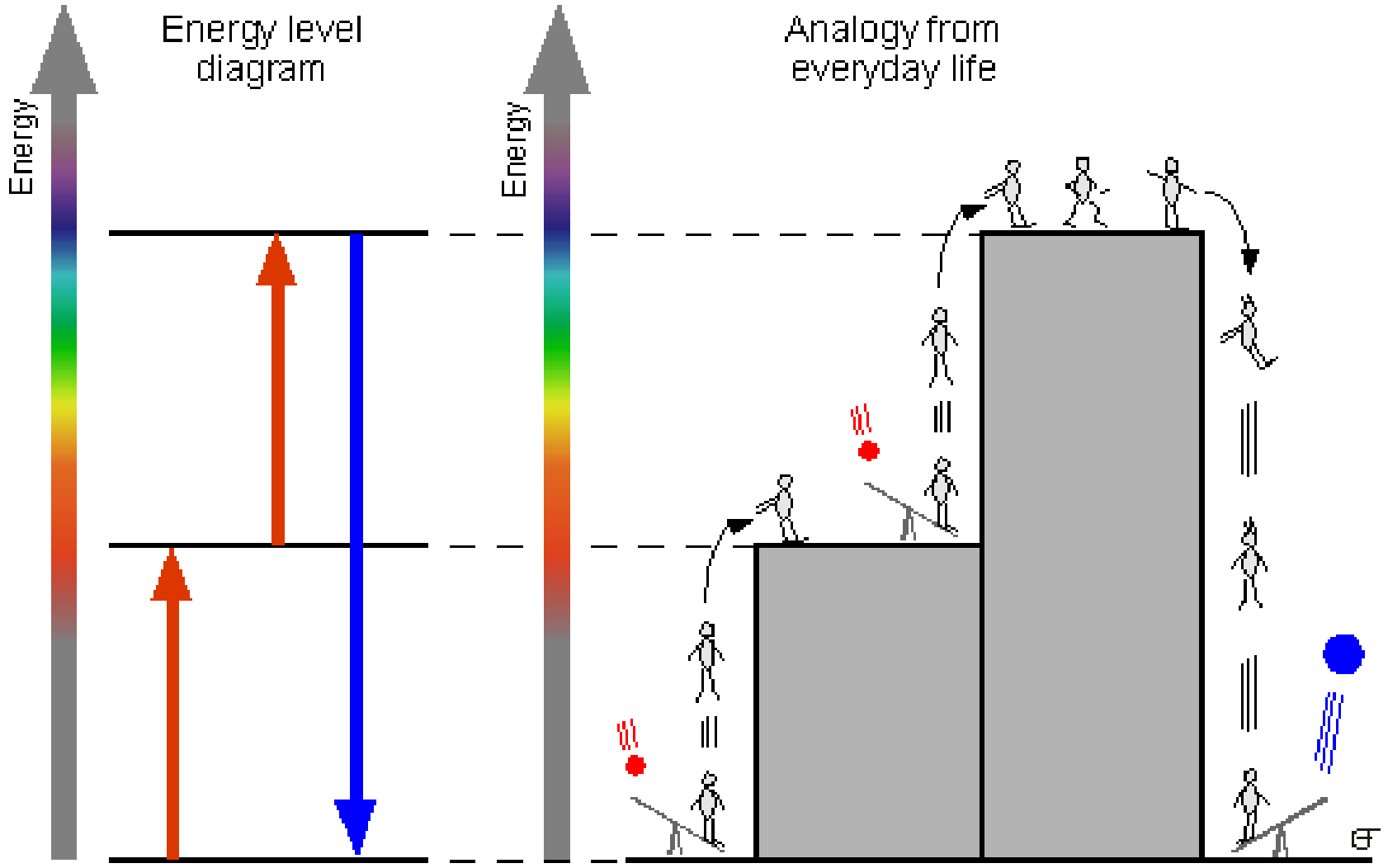
- Very high quantum yield (close to unity)
- Excellent photostability
- Narrow emission bands
- Toxicity issues - need to be coated
- Difficult signal transduction

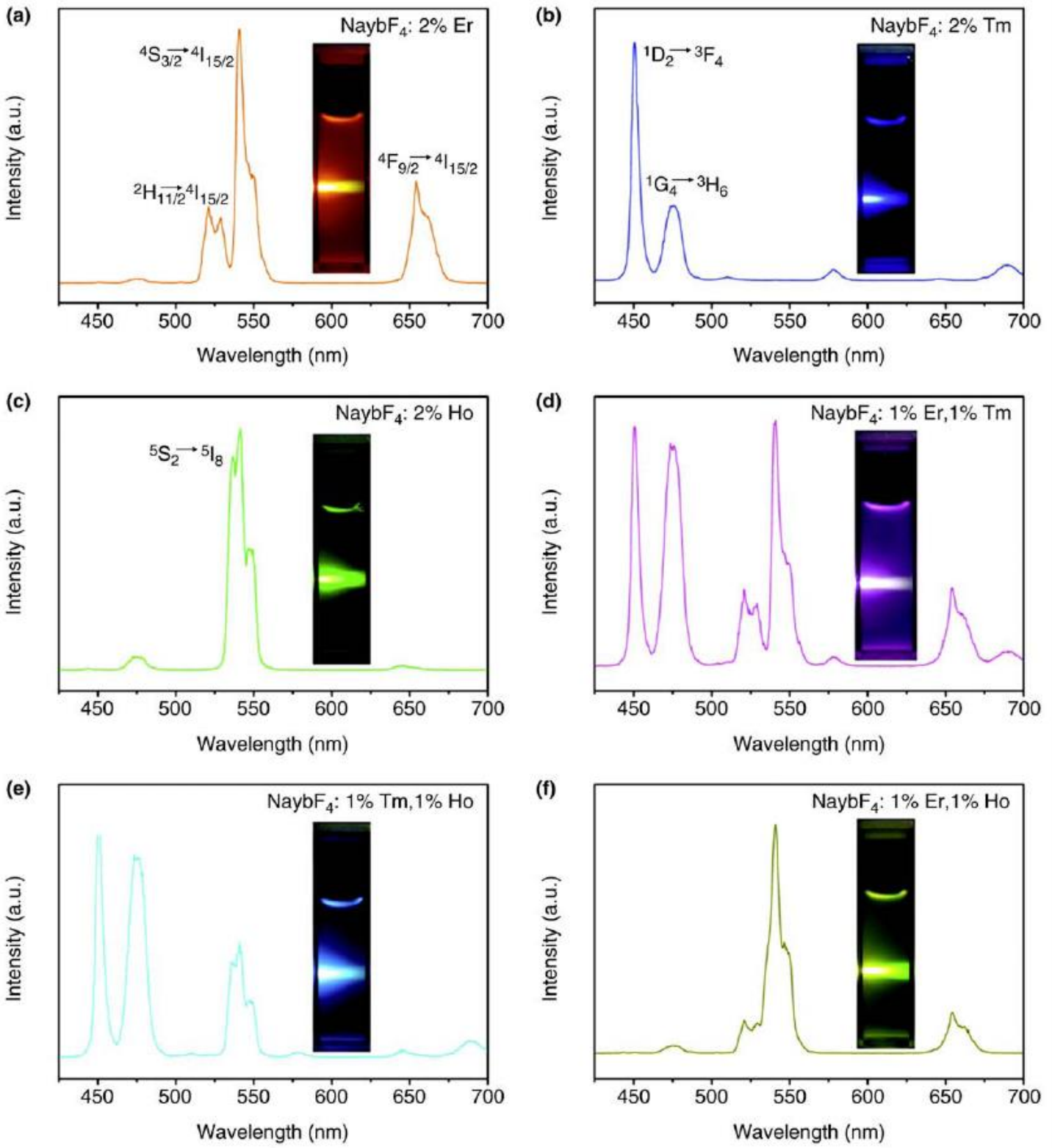
Upconverting nanocrystals

- 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 energy levels

Upconversion

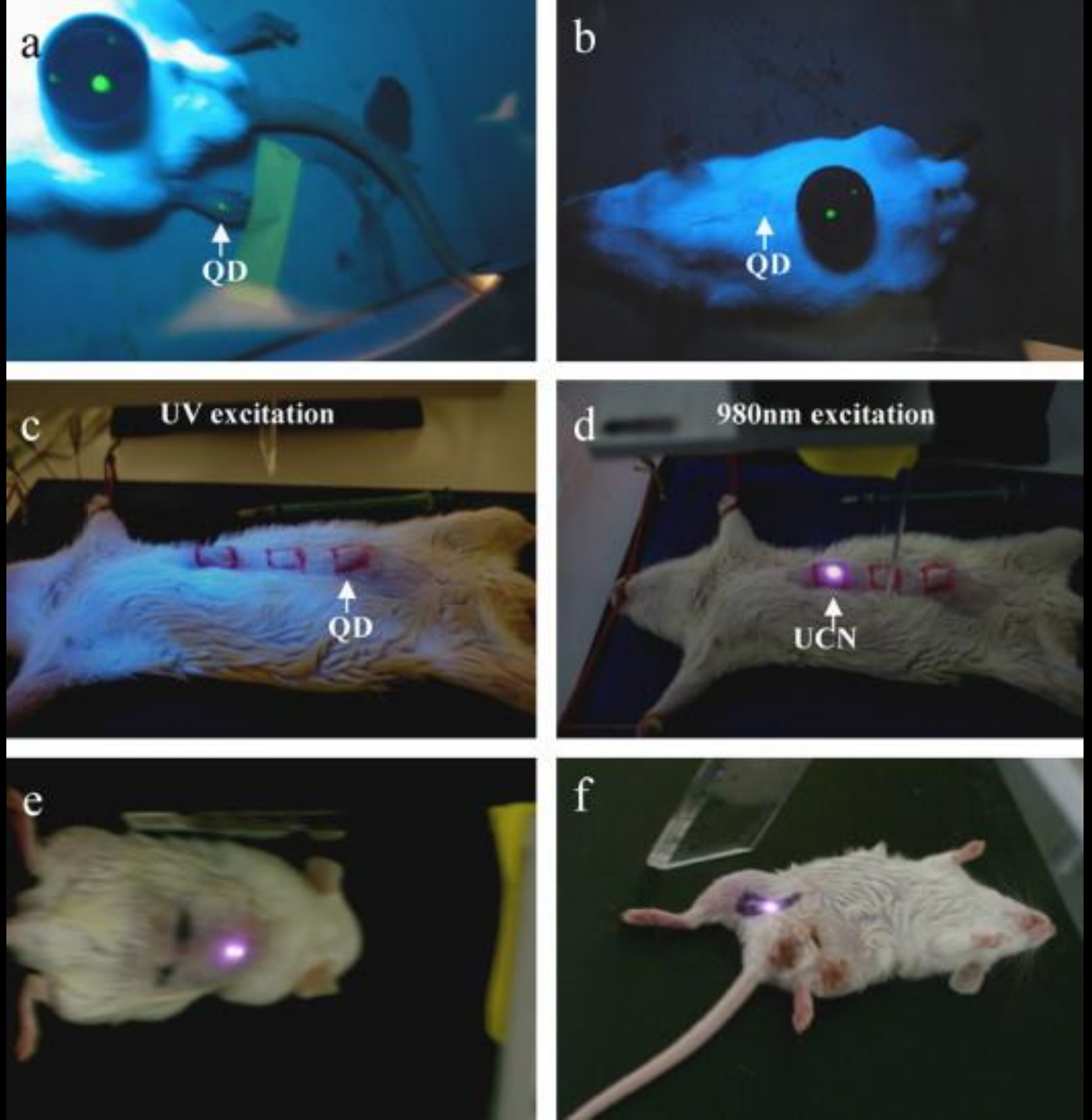
Making 1x blue out of 2x red




(a)
NaybF4: 2% Er
4S3/2→4I15/2
2H11/2→4I15/2
4F9/2→4I15/2
Intensity (a.u.)
450 500 550 600 650 700
Wavelength (nm)
(b)
NaybF4: 2% Tm
1D2→3F4
1G4→3H6
Intensity (a.u.)
450 500 550 600 650 700
Wavelength (nm)
(c)
NaybF4: 2% Ho
5S2→5I8
Intensity (a.u.)
450 500 550 600 650 700
Wavelength (nm)
(d)
NaybF4: 1% Er,1% Tm
Intensity (a.u.)
450 500 550 600 650 700
Wavelength (nm)
(e)
NaybF4: 1% Tm,1% Ho
Intensity (a.u.)
450 500 550 600 650 700
Wavelength (nm)
(f)
NaybF4: 1% Er,1% Ho
Intensity (a.u.)
450 500 550 600 650 700
Wavelength (nm)

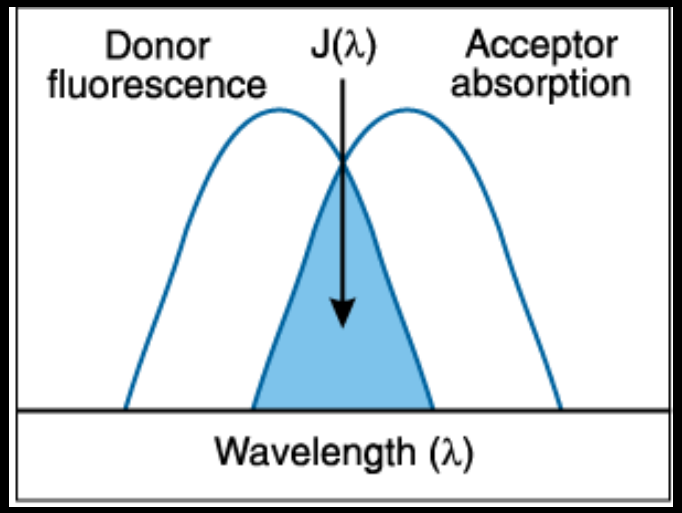
Upconverting nanocrystals

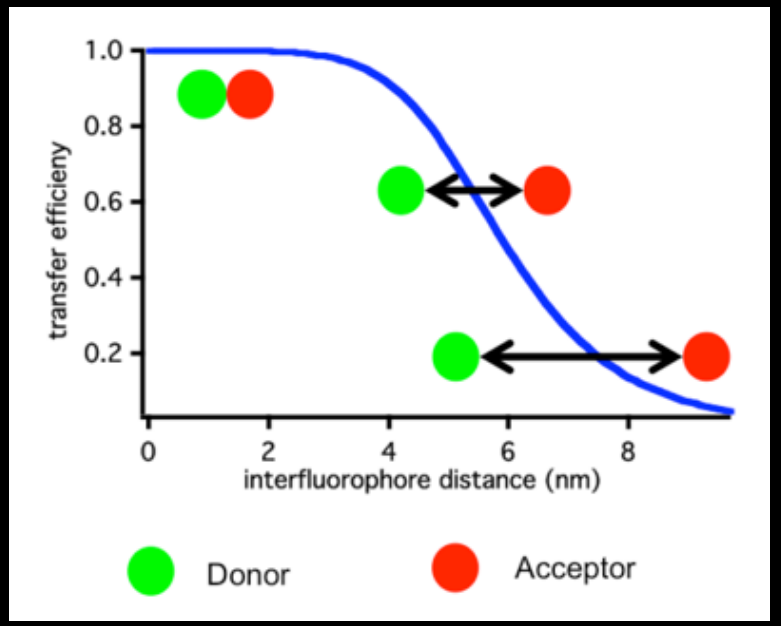
- Excitation in the NIR region (water window)
- Enable deep tissue imaging
- Photostable
- Autofluorescence free fluorescence (higher signal-to-noise)
- Sequential two photon excitation followed by energy transfer between the dopants
- RE metals have numerous closely spaced energy levels
- Non-toxic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


a
QD
b
QD
c
UV excitation
QD
d
980nm excitation
UCN
e
f

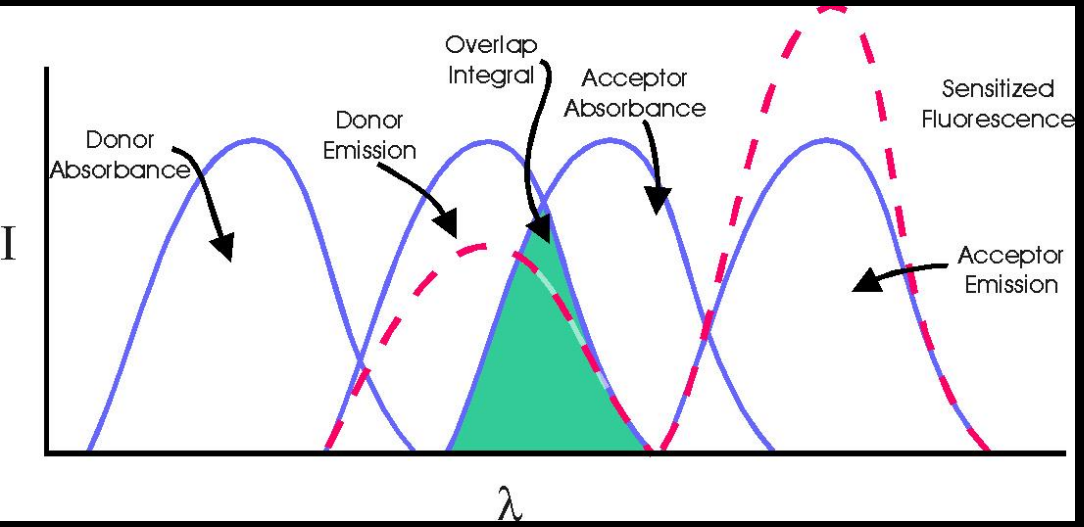
4.3. Optical techniques – Energy transfer systems

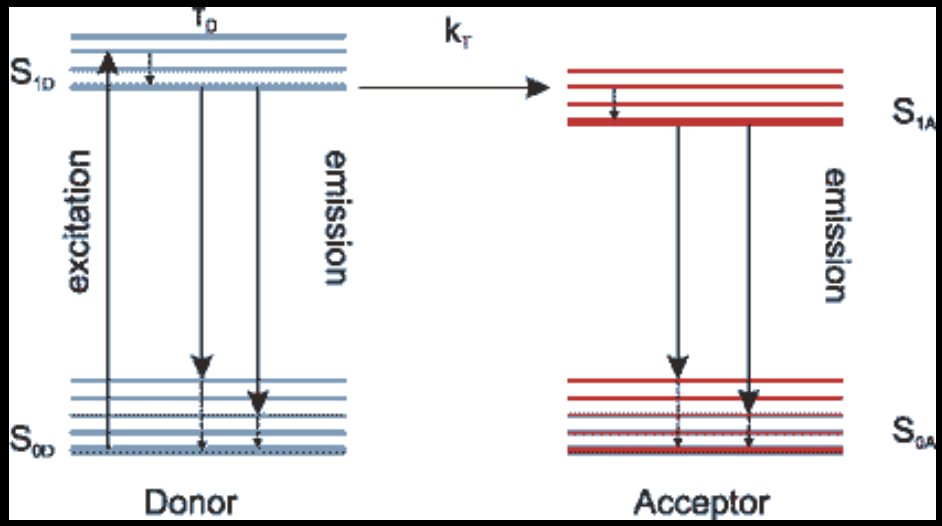
- Two or more fluorophores are needed
- A + D* → A* + D (vs. Inner filter effect)
- Spectral overlap, transition moments
- Distance (<100 Å)





Energy transfer systems





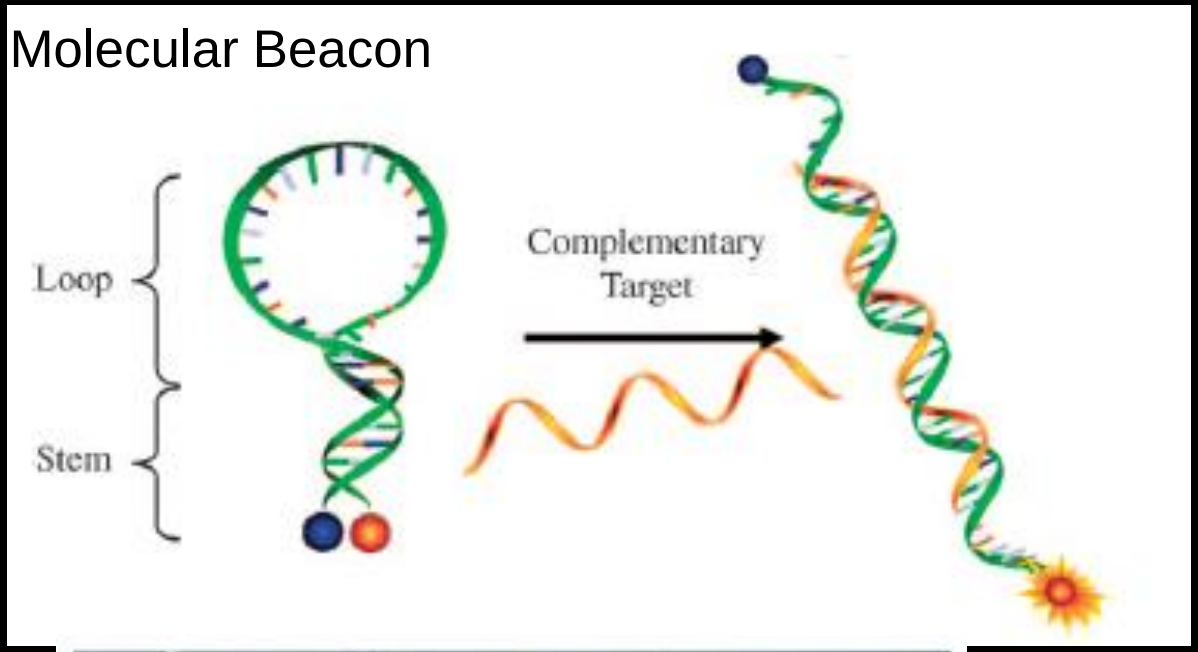
- Short range (Dexter) mechanism : electron exchange between overlapping molecular orbitals (<10 Å)
- Long range (Förster) mechanism : coulombic interactions between opposed dipole moments
- ET depends on $E = \frac{1}{1 + (r/R_0)^6}$

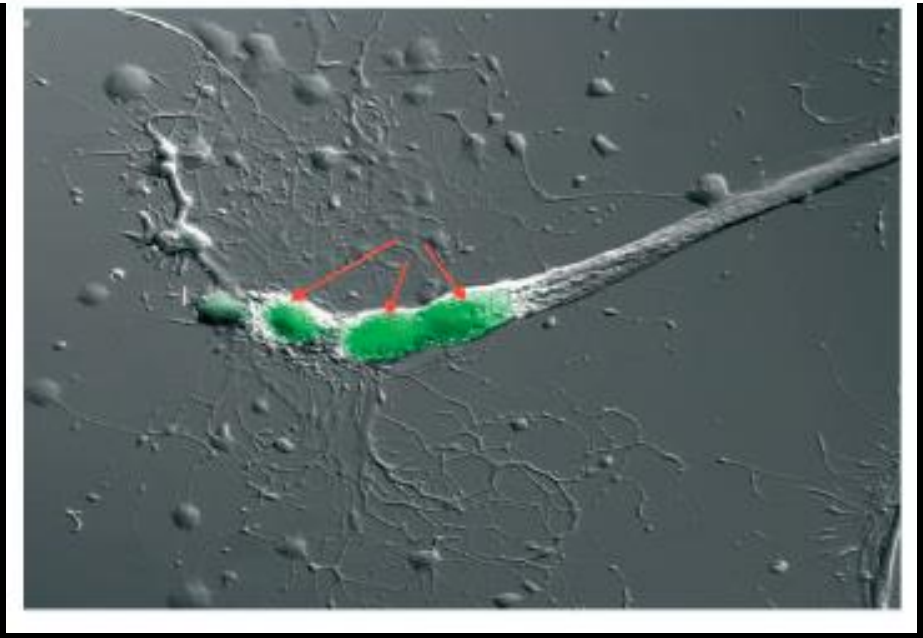
Energy transfer systems

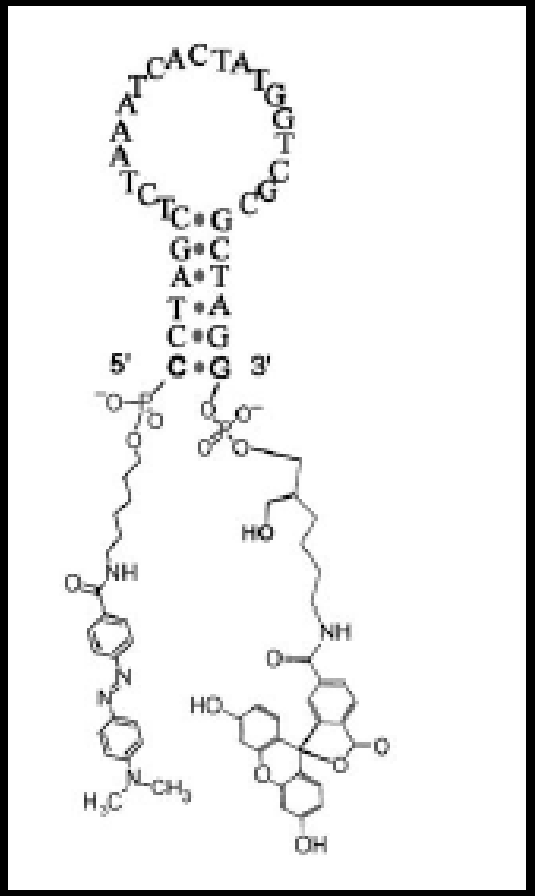
- Förster type of energy transfer (FRET)
- Strongly distance dependent (molecular ruler)
- Enzyme kinetic measurements
- HOST-GUEST (receptor-ligandum)
- Membrane diffusion / fusion
- Conformational changes
- Colocalisation
- Imaging techniques (resolution increase)

Examples

Molecular Beacon





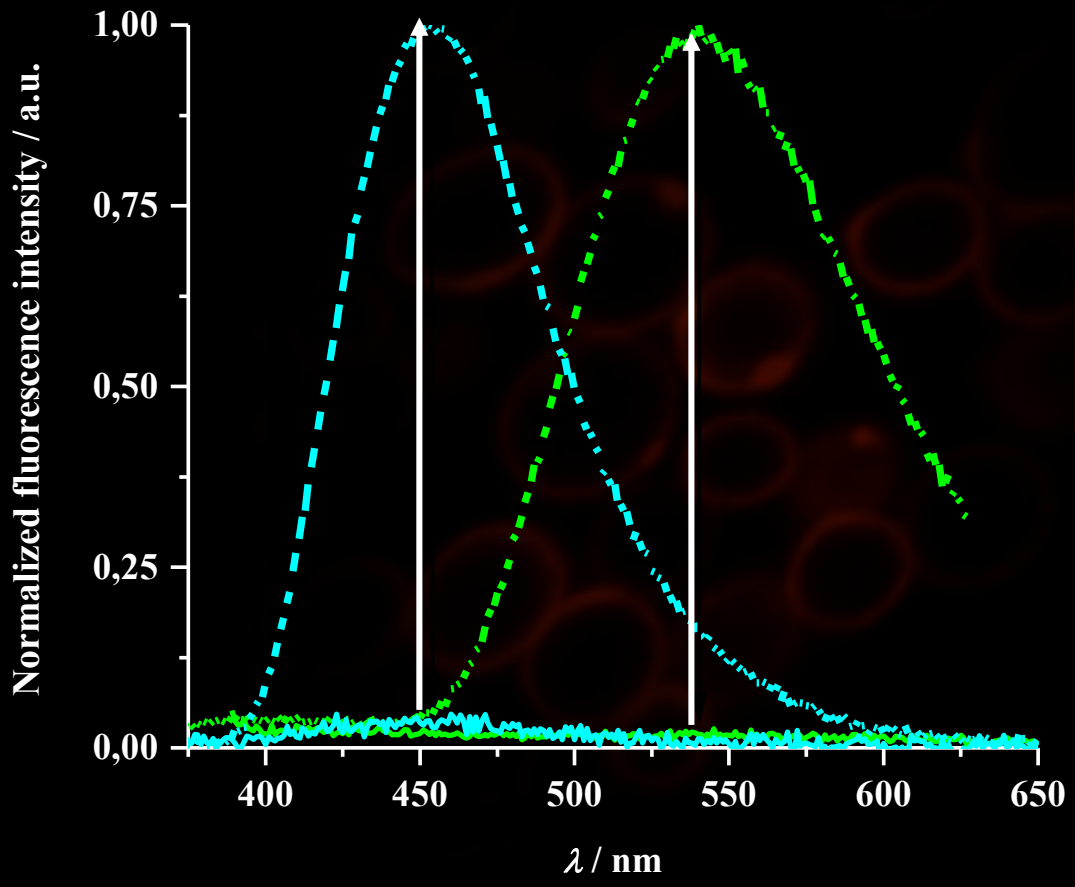


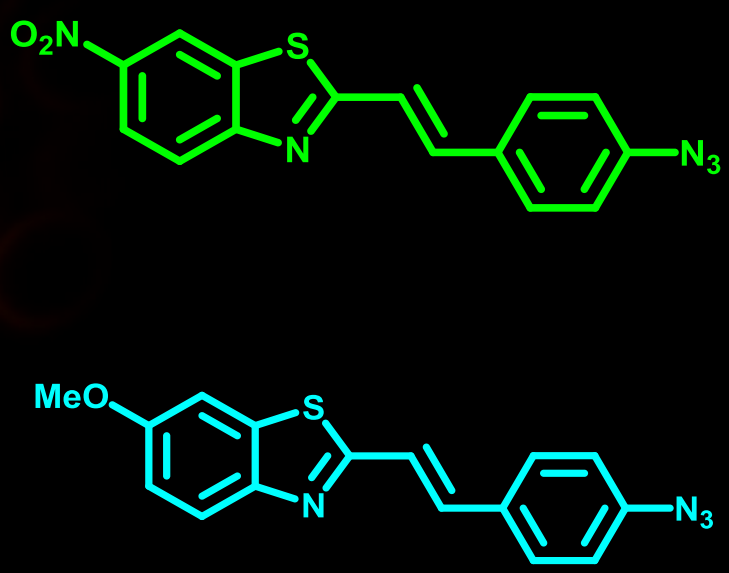
Fluorogenic probes

- Pre-fluorophore form – not fluorescent
- Reaction changes electronics – fluorescent
- Low background fluorescence
- Azide – quencher

- A. Herner (2013) Org. Biomol. Chem. *11*, 3297.

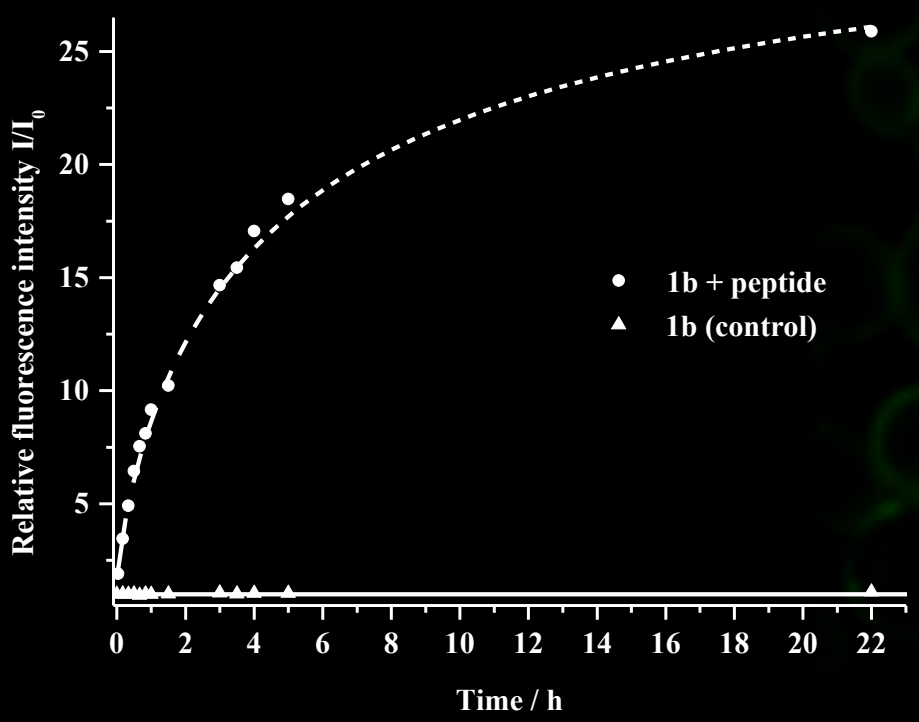
Enhancement upon reacting with alkynes

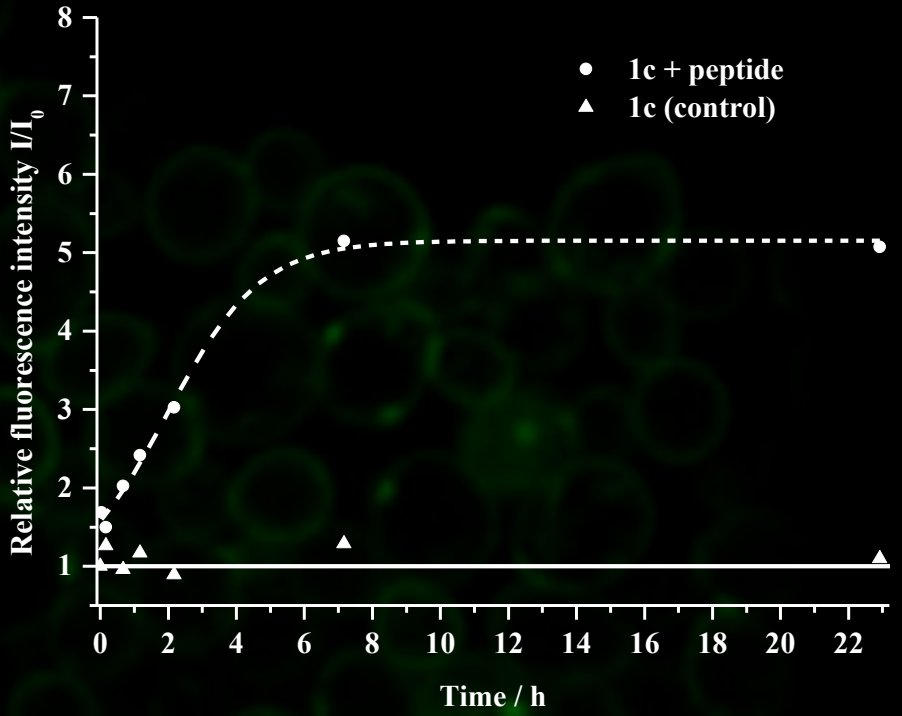


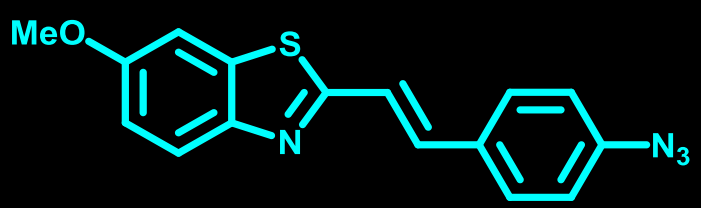


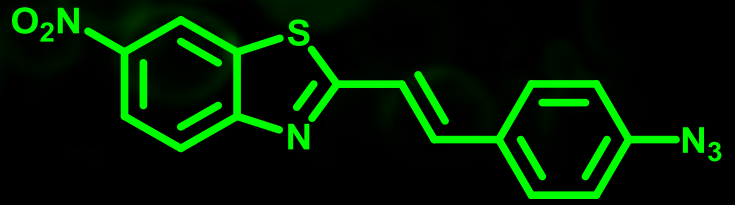
A. Herner, I. Nikic, M. Kállay, E. A. Lemke, P. Kele (2013) *Org. Biomol. Chem. 11*, 3297.

Reaction with a cyclooctynylated peptide







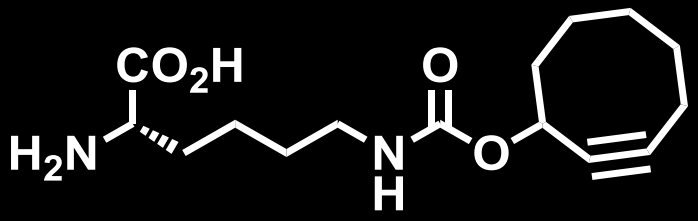


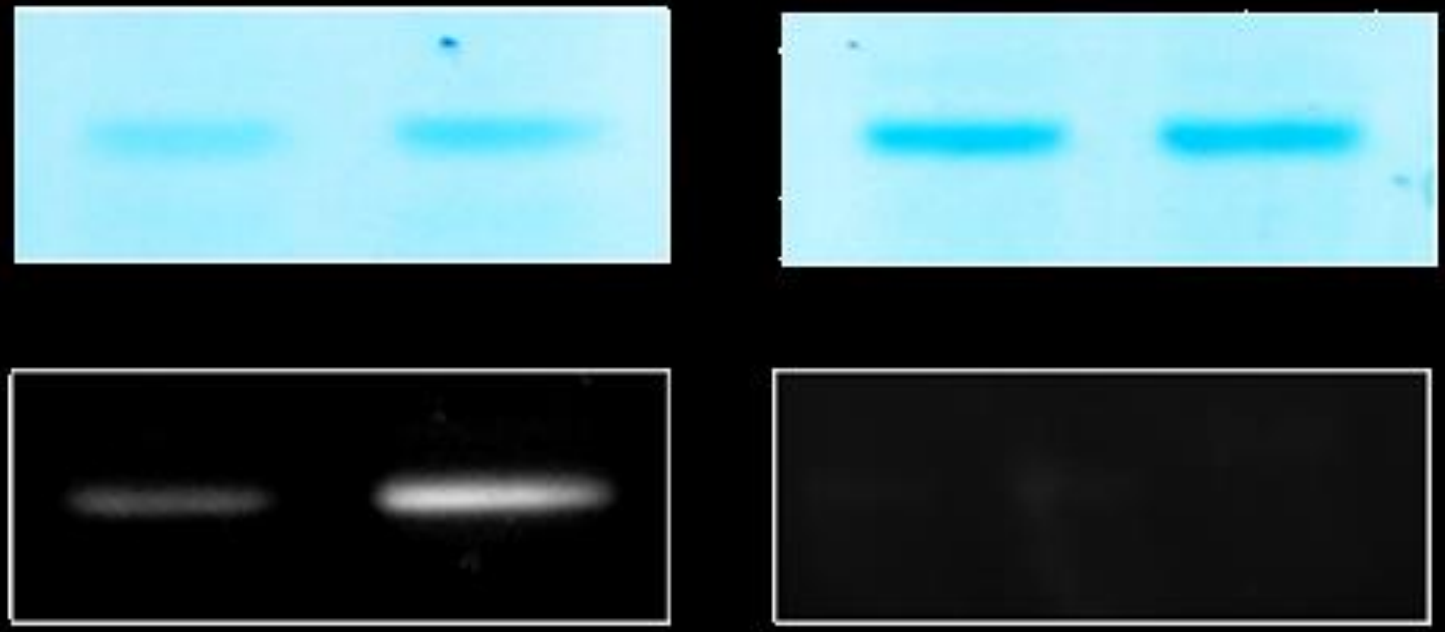
λ_{exc} = 355 nm λ_{em} = 453 nm

λ_{em} = 540 nm

A. Herner, I. Nikic, M. Kállay, E. A. Lemke, P. Kele (2013) *Org. Biomol. Chem. 11*, 3297.

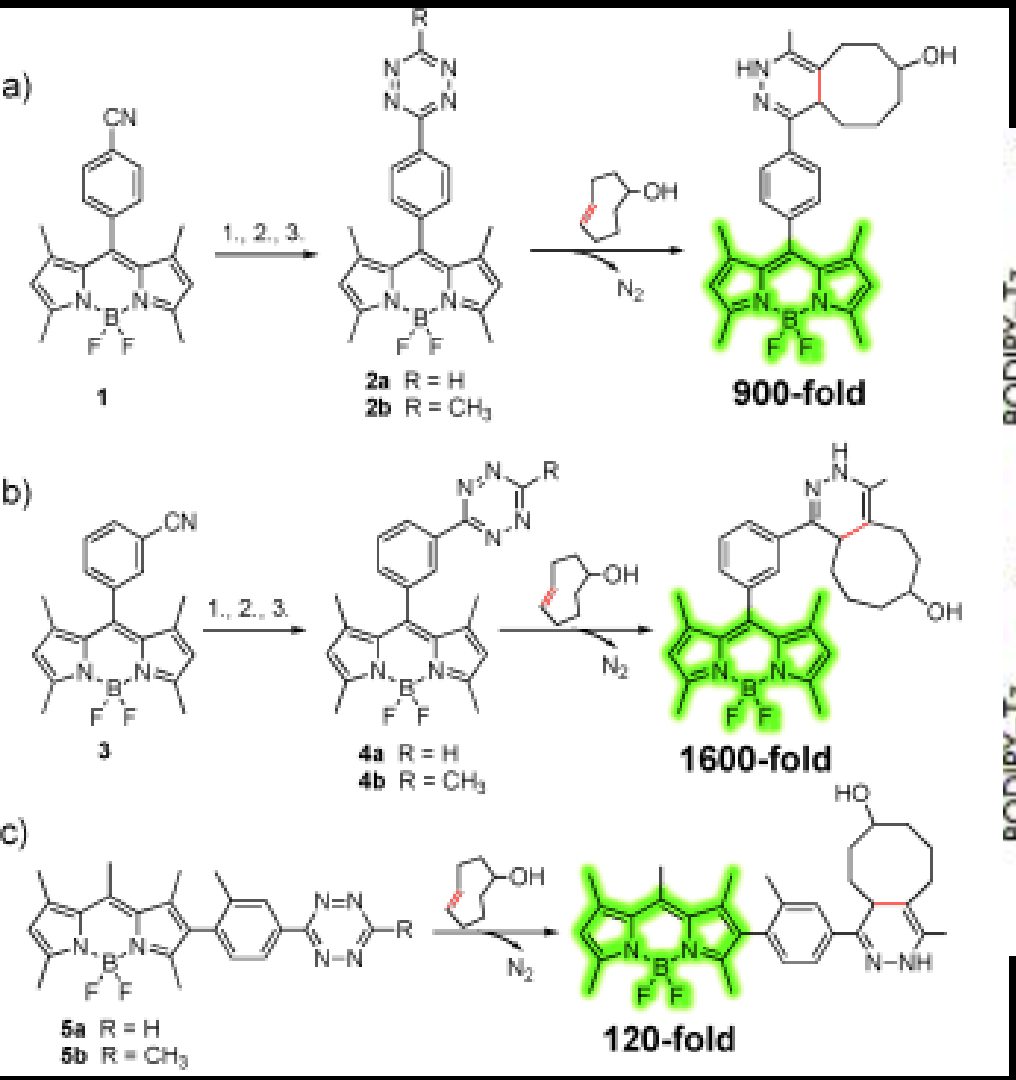
In vivo staining and analysis of lysates

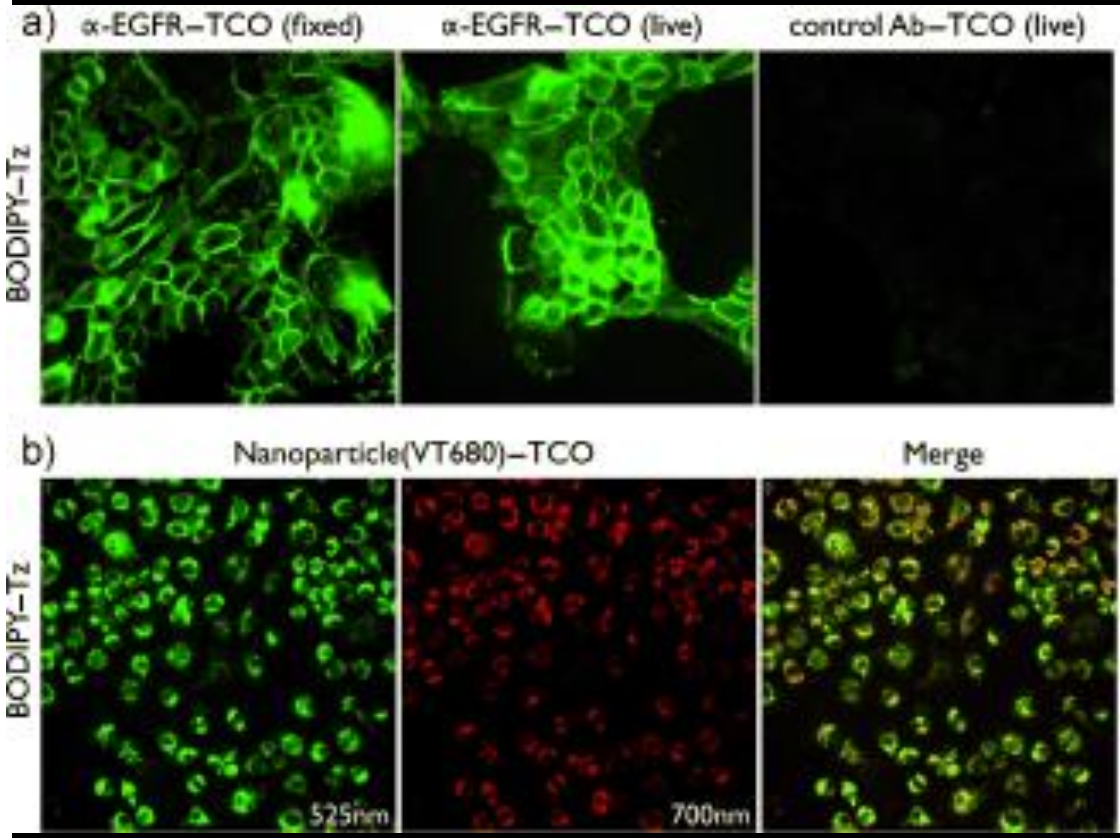




A. Herner, I. Nikic, M. Kállay, E. A. Lemke, P. Kele (2013) *Org. Biomol. Chem. 11*, 3297.

Through-bond-energy-transfer quenching



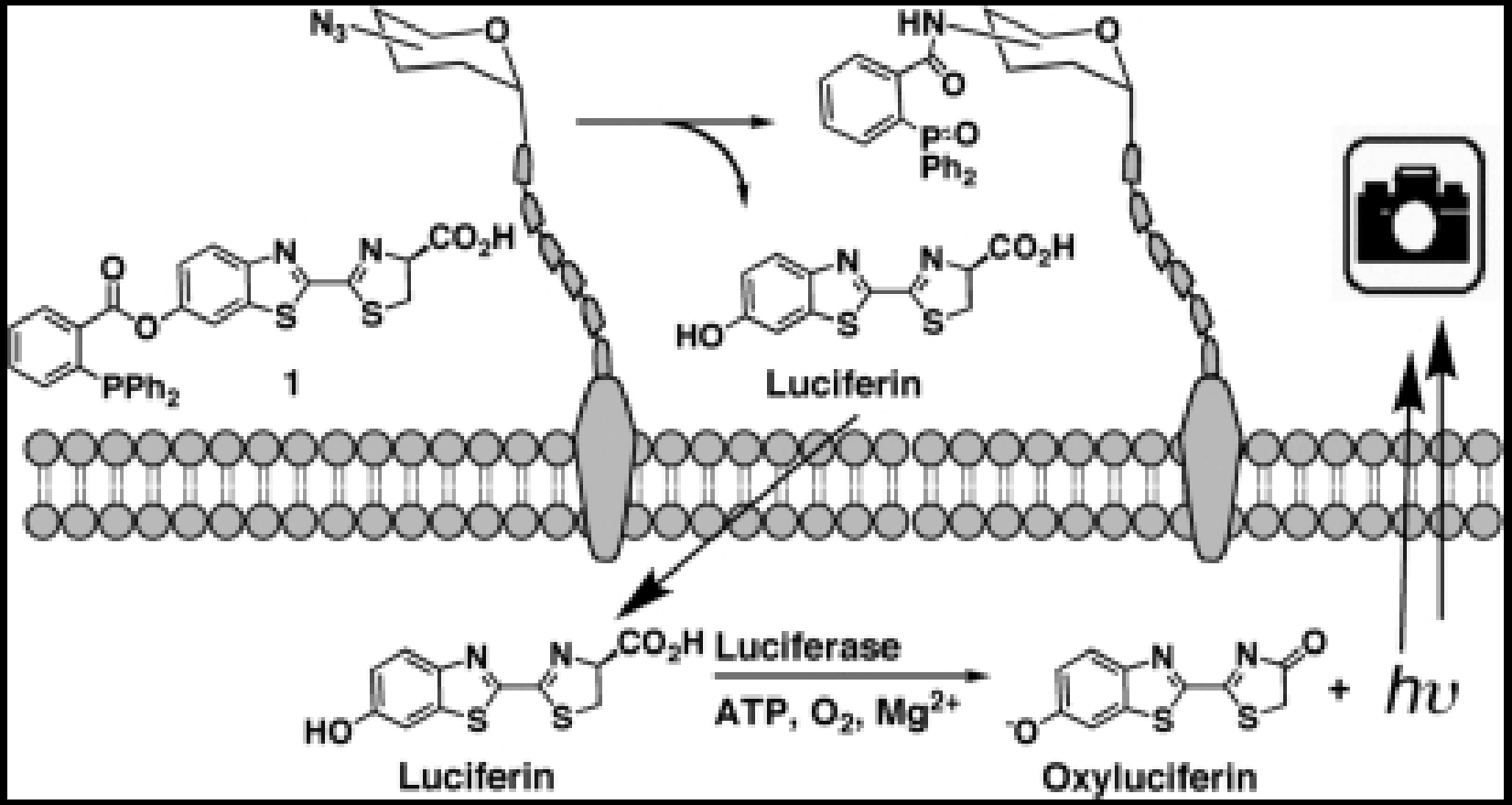


Carlson, J. C. T., Meimetis, L. G., Hilderbrand, S. A. and Weissleder, R. (2013), Angew. Chem. Int. Ed., 52: 6917.

Bioluminescence imaging

- Luciferin-luciferase
- Enzymatic reaction creates an excited state product
- No need for external excitation
- No autofluorescence, no photobleaching

Bioluminescence imaging

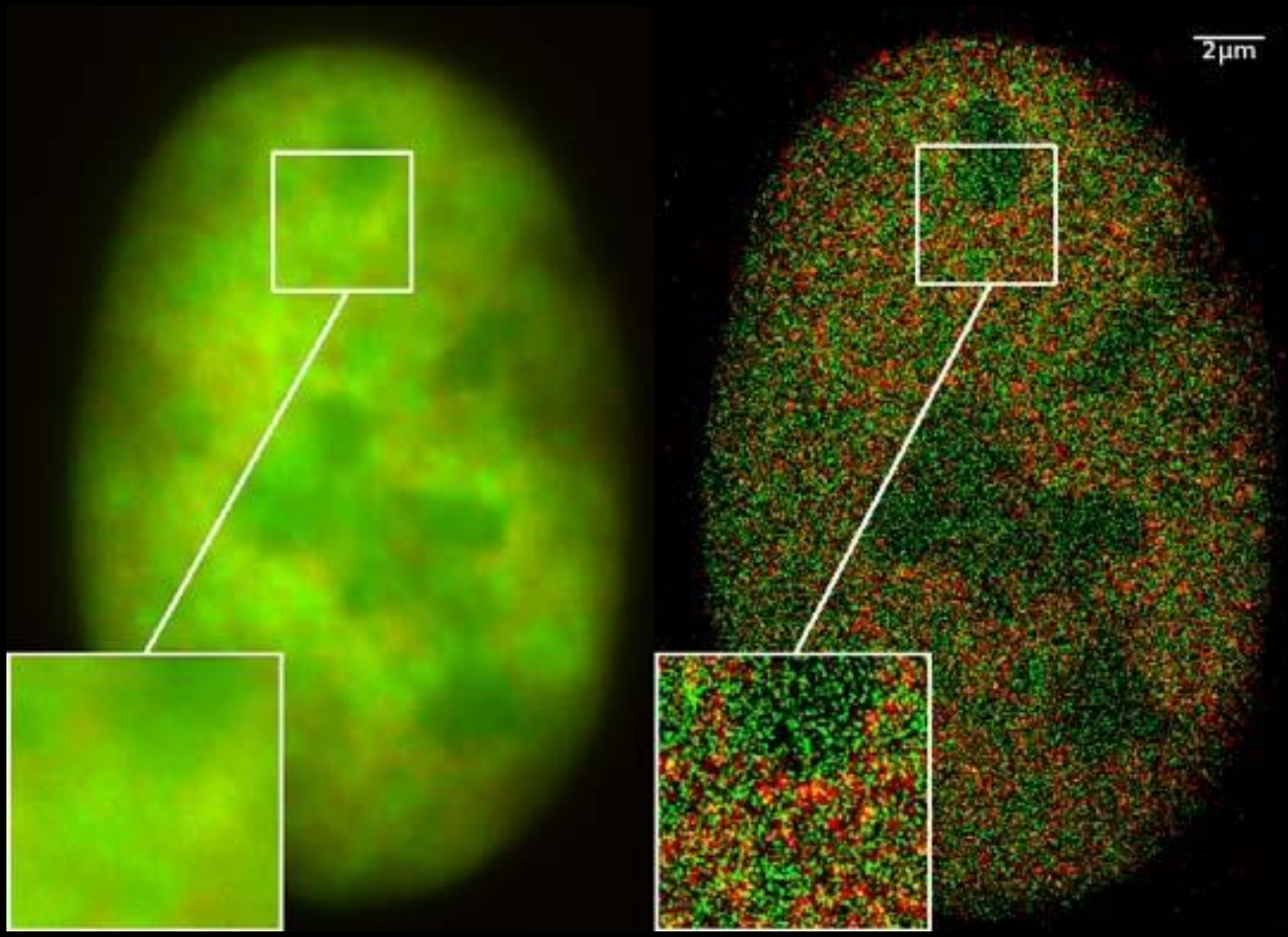


Cohen AS, Dubikovskaya EA, Rush JS, Bertozzi CR - J. Am. Chem. Soc. (2010)

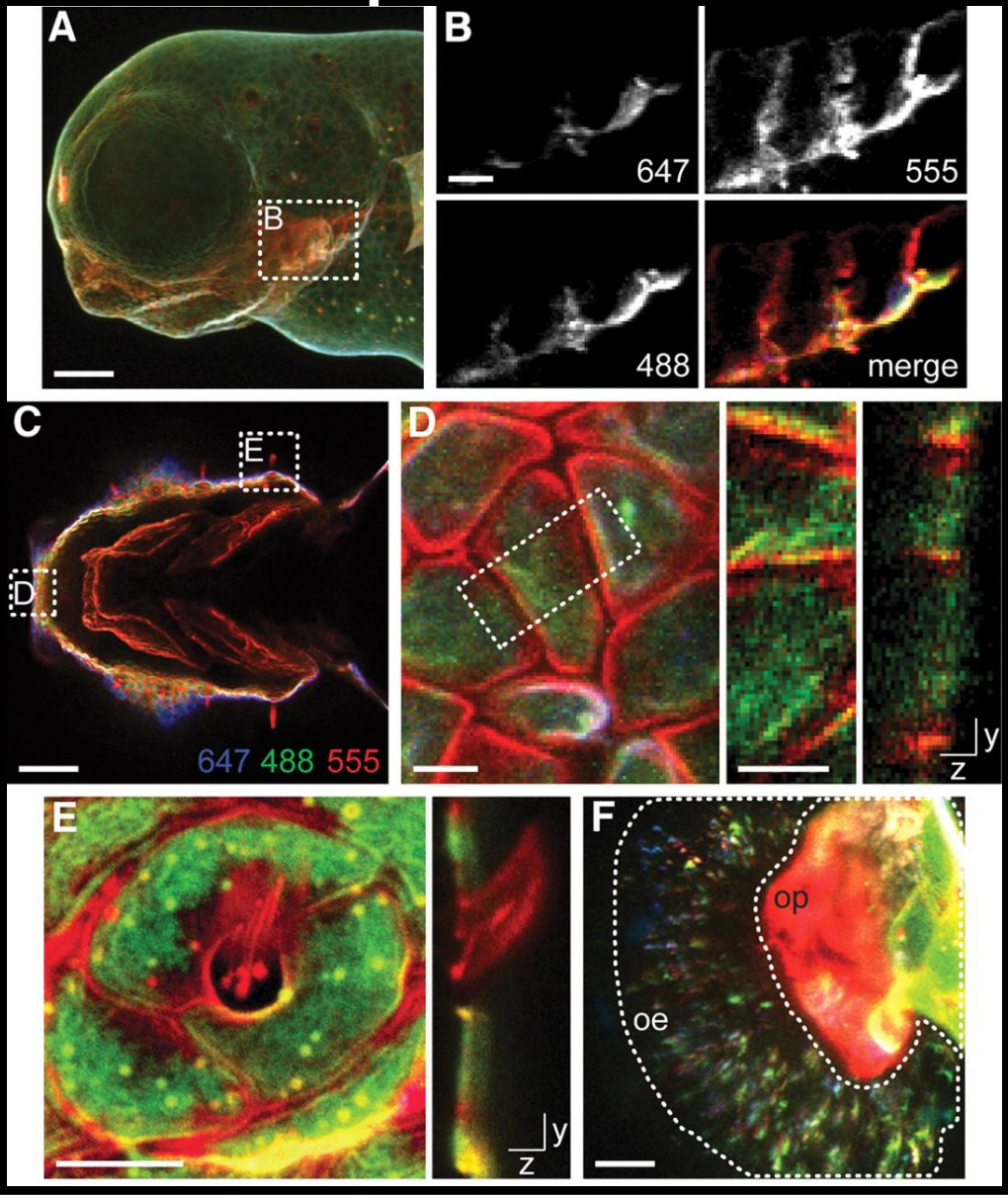
Super resolution microscopy

- Normal fluorescence microscopy is limited by diffraction (half of wavelength)
- SRM techniques reduce it to 10-60 nm
- Structured illumination microscopy (SIM)
- Stimulated depletion microscopy (STED)
- Photoactivation localization microscopy (PALM)
- Stochastic optical reconstruction microscopy (STORM)

SRM imaging



Spatiotemporal imaging of glycan development



$Ac_4GalNAz$ added at different times reacted with DIFO-647, DIFO-488, DIFO-555

Laughlin et al. (2008) Science, 320, 664