

Fluorophores

Jan Sýkora, Martin Hof

Department of Biophysical chemistry

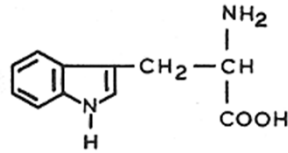


Outline

- Naturally occurring fluorophores
- Fluorescent dyes
- Fluorescent labelling of proteins
- Fluorescent labelling of DNA/RNA
- Labelling lipid membranes
- Fluorescent proteins
- Multicolor experiments
- Fluorescent nanoparticles

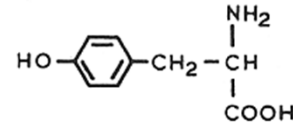
Naturally fluorescent biomolecules

aminoacids:



Tryptophan

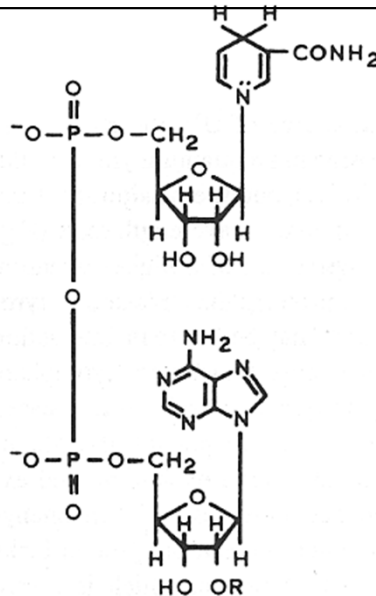
Ex. 280, 296 nm, Em. 330-350 nm



Tyrosine

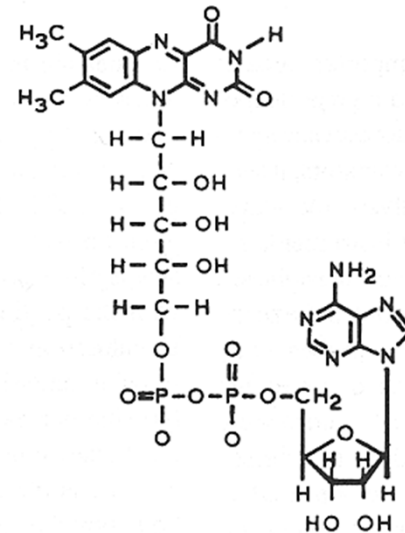
Ex. 275 nm, Em. 305 nm

cofactors:



NADH

reduced form fluorescent
Ex. 340 nm, Em. 460 nm

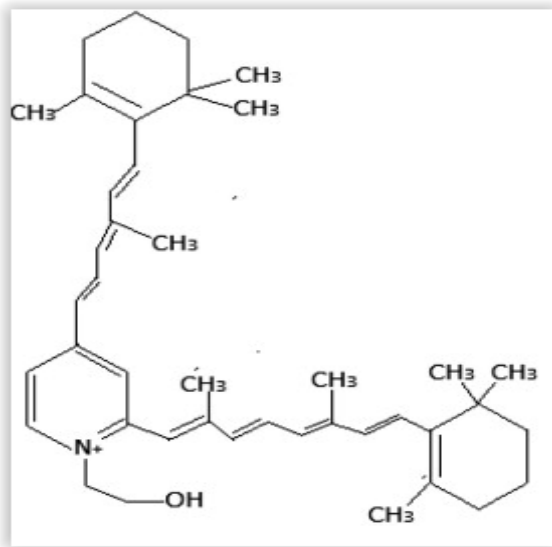


FAD

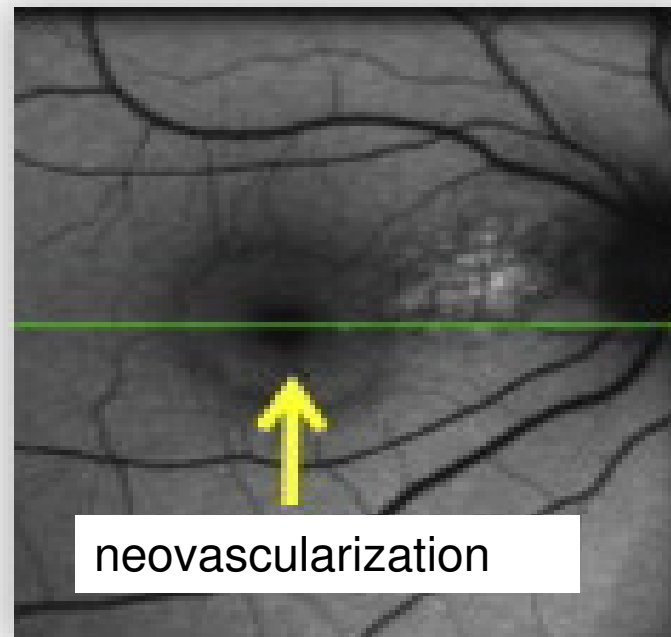
oxidized form fluorescent
Ex. 450 nm, Em. 525 nm

Fundus autofluorescence imaging:

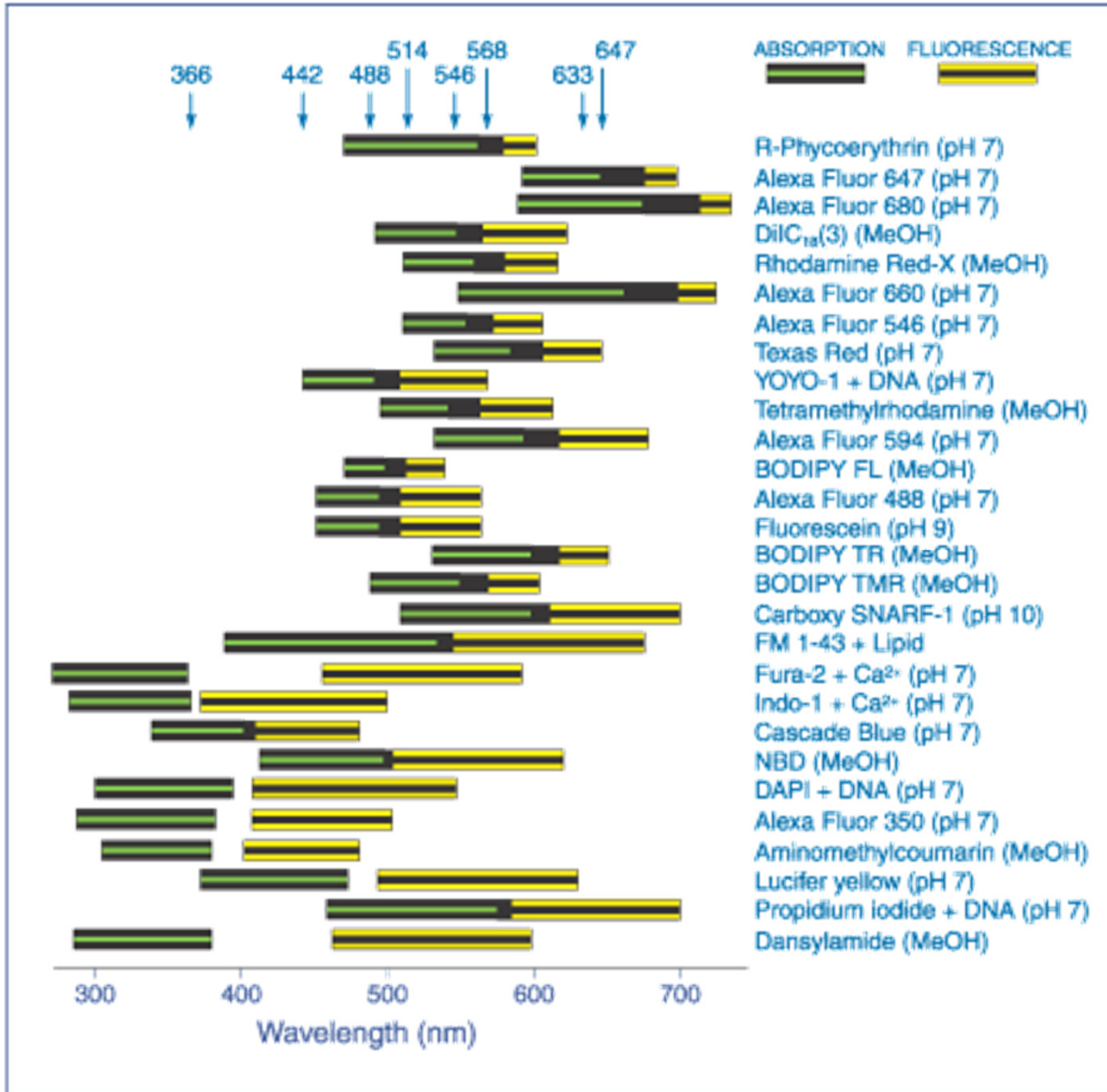
- ❖ Used for eye diagnostics
- ❖ Understanding the pathophysiological mechanisms
- ❖ Example: atrophy of the retinal pigment epithelial layer caused by neovascularization (formation of tangle of blood vessels)



A2E (N-retinyl-N-retinylidene ethanolamine) ~ component of *retinal pigment*



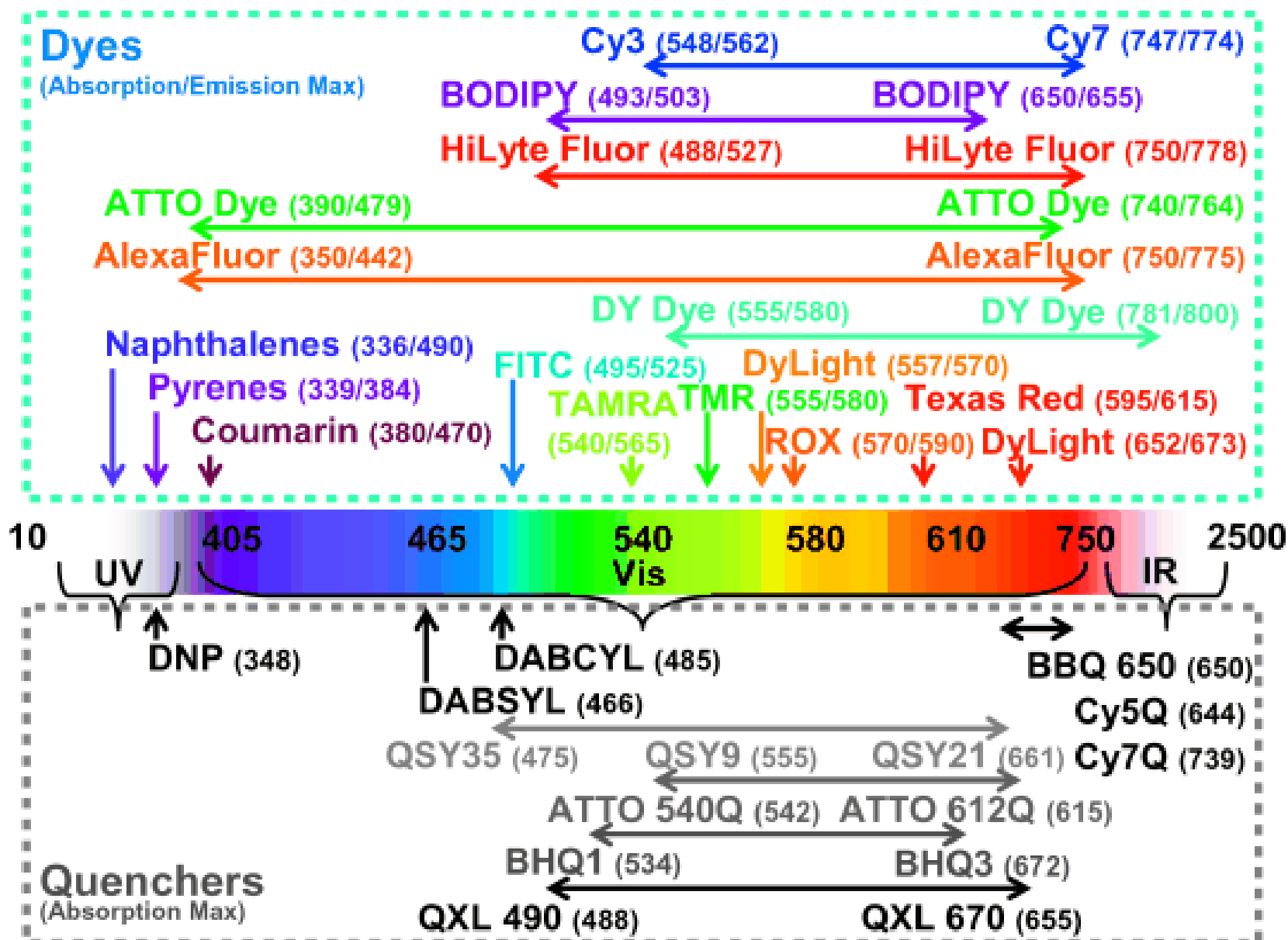
Fluorescent dyes



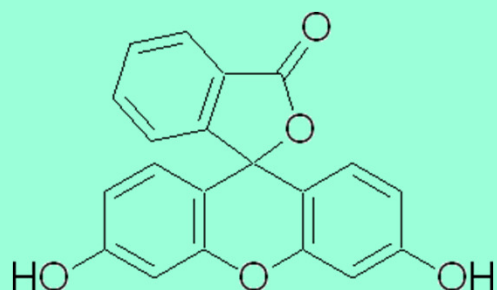
Important parameters:

- colour
- compatibility with light sources
- Stokes shift
- extinction coeff.
- hydrophobicity
- spectral width
- photostability
- environmental sensitivity

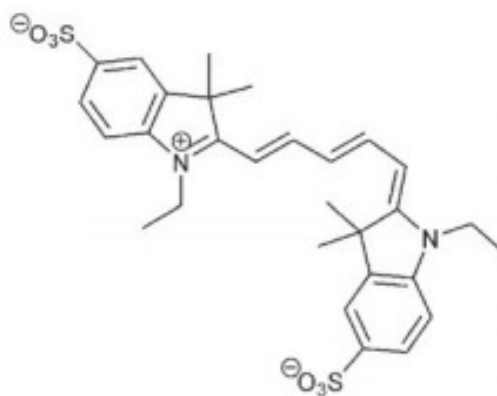
Dye families span the light spectrum



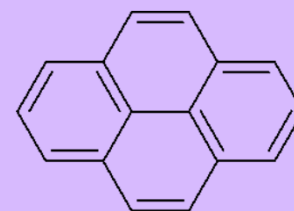
Everything is determined by chemical structure



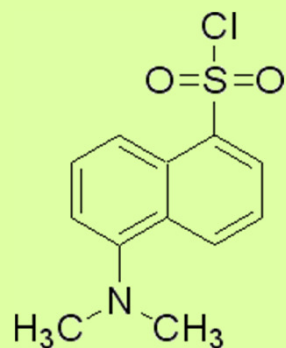
Fluorescein (460:515)
pH sensitive



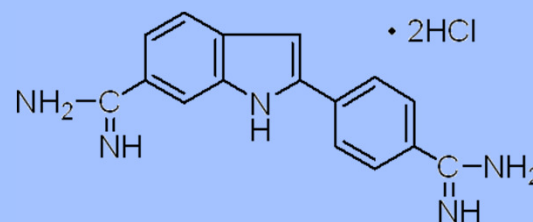
Cy5 (590:610)
distinct isomers



Pyrene (345:395/480)
forms excimers



Dansyl (340:510-560)
Environment sensitive



DAPI (360:460)
Emission upon dsDNA binding

Fluorescent labelling of proteins

Pros

- covalent linkage
- low MW probes
- brightness, exc. in visible range
- attachment at specific location
- fluorescent antibodies

Cons

- *in vivo* applications are limited
- Sometimes difficult to achieve specificity of labelling
- biological activity may be impaired

Labelling chemistries

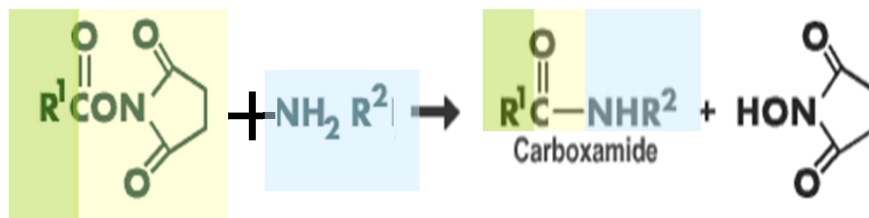
Amine modification

(Lys, N-term.)

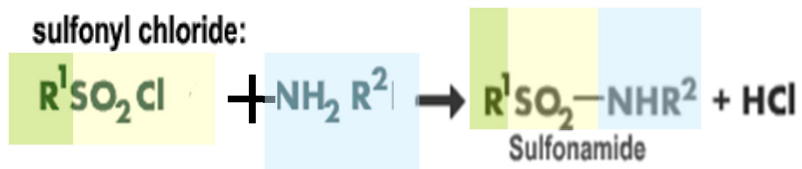
isothiocyanate:



succinimidyl ester:

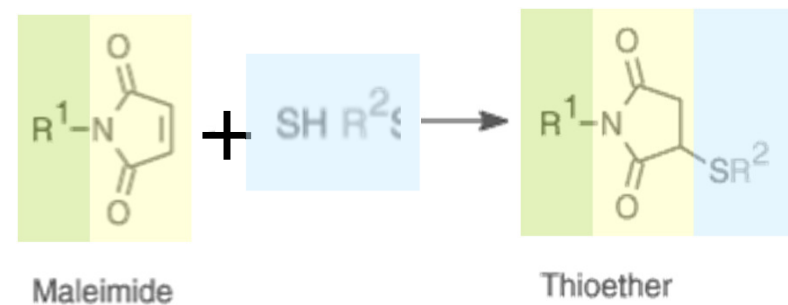


sulfonyl chloride:



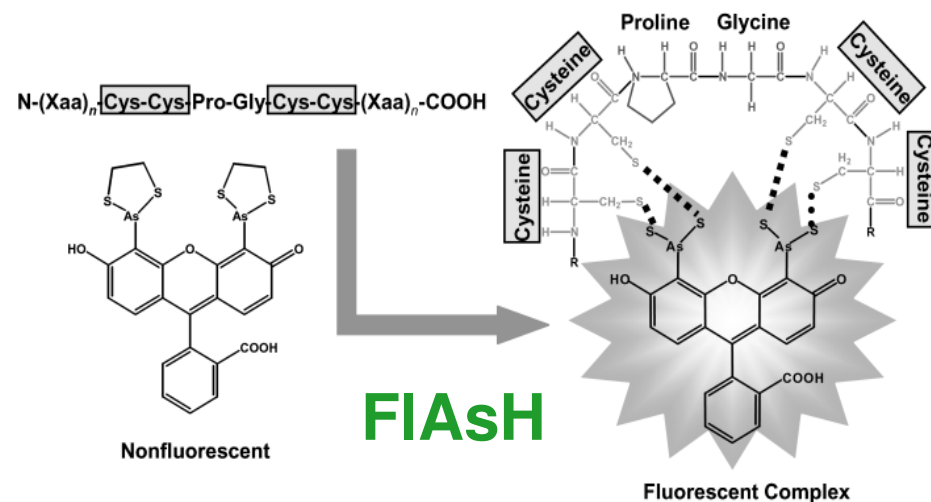
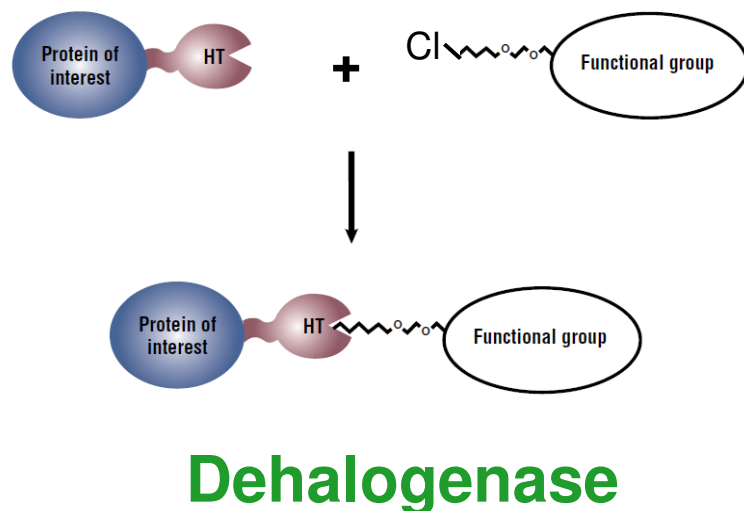
Thiol modification

(Cys)



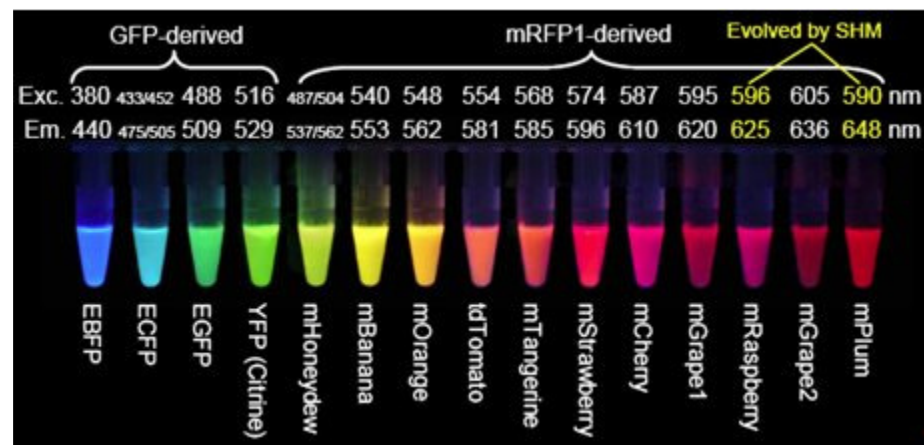
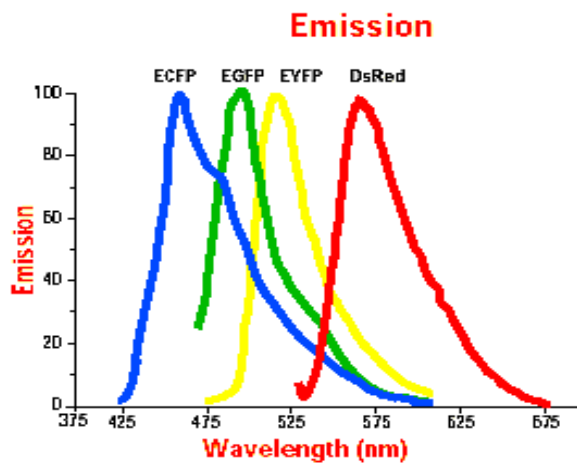
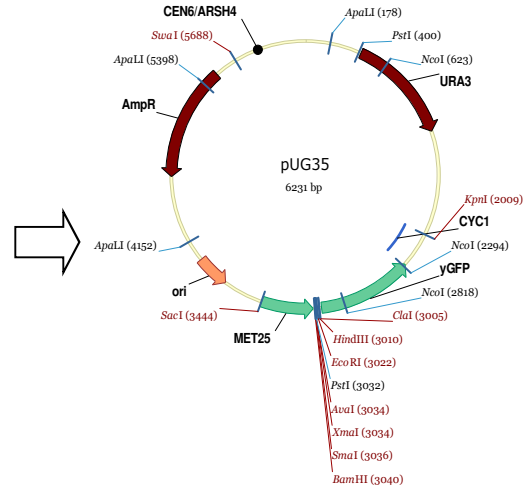
Examples of Specific Labelling *in vivo*

Labeling method	Fluorescent label(s)	Acceptor	Acceptor size (aa)	Enzyme
HaloTag	TMR, fluorescein	Halogenase	210	Halogenase
Biarsenical	Fluorescein (FIAsH), resorufin (ReAsH)	...CCPGCC...	6	NA
NTA [*] -His	Ni ²⁺ -NTA-I, NTA-II, NTA-'lysine'+dye,	Oligohistidine	6	NA



*Nitrilotriacetic acid

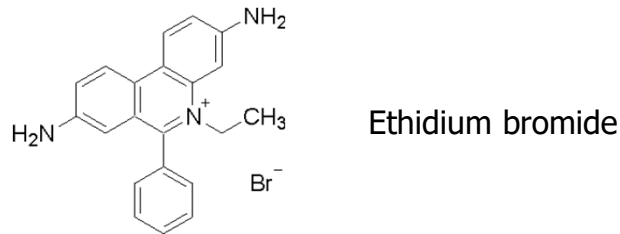
Fluorescent proteins



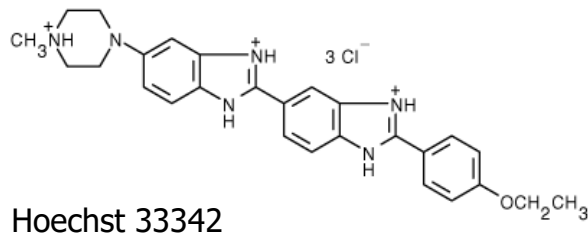
Fluorescent labelling of DNA/RNA

Staining (non-covalent)

Intercalators: EthBr, Acridine Orange

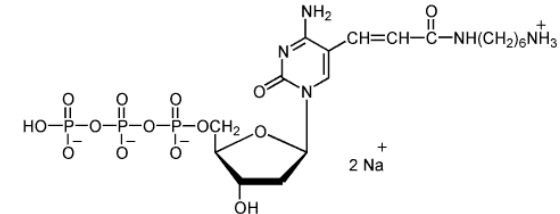


Minor groove-binding: DAPI, Hoechst



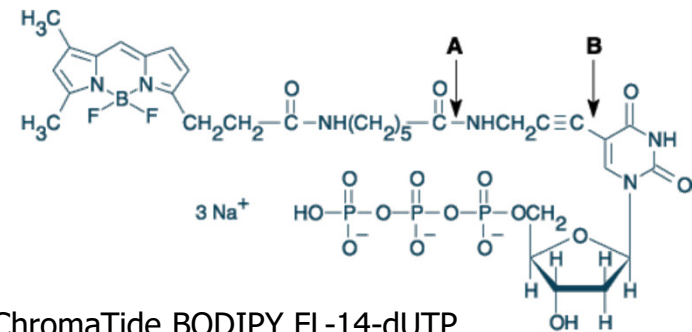
Covalent labelling

Amine-modified nucleotides



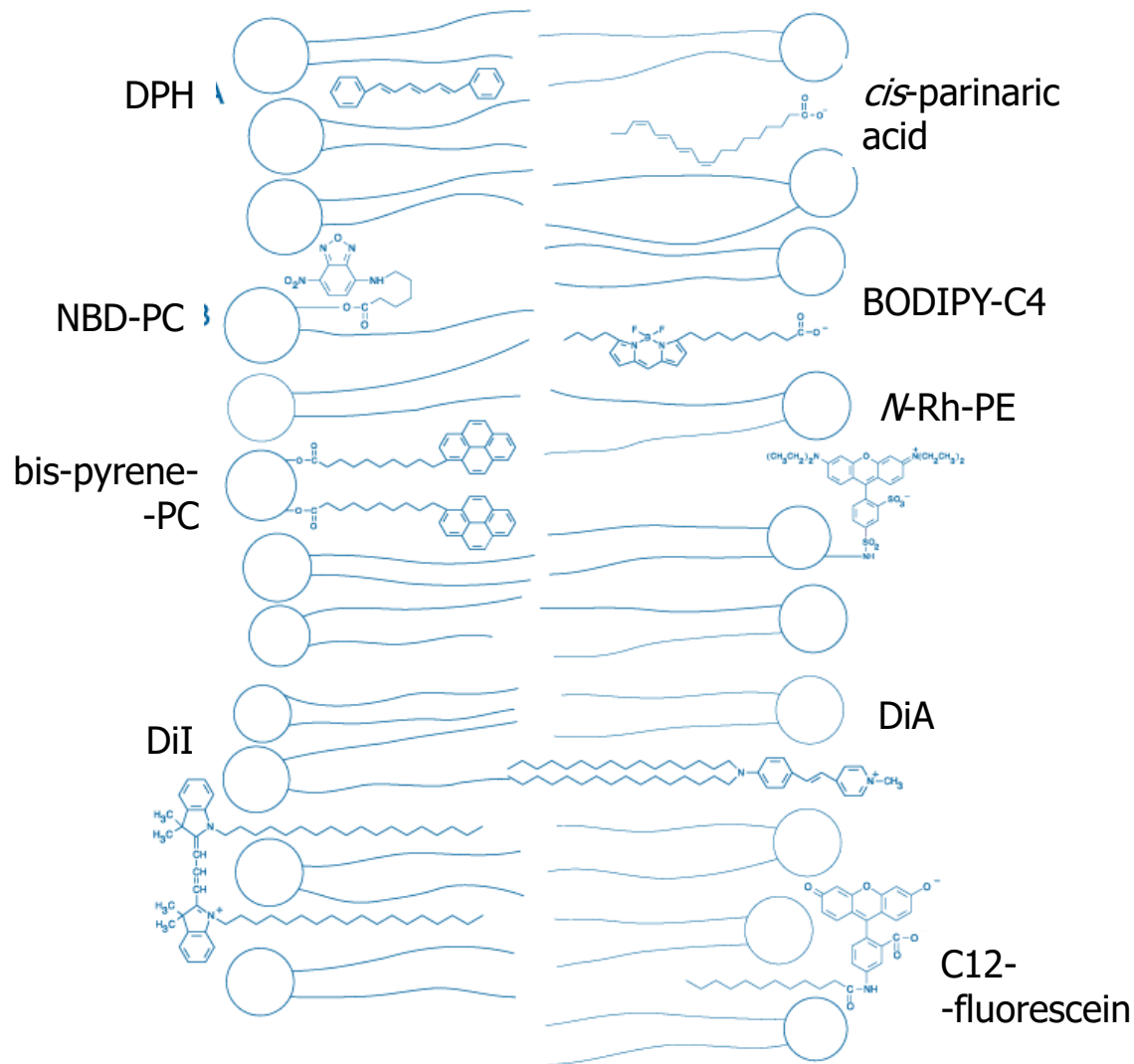
aha-dCTP

Ready-labelled nucleotides



ChromaTide BODIPY FL-14-dUTP

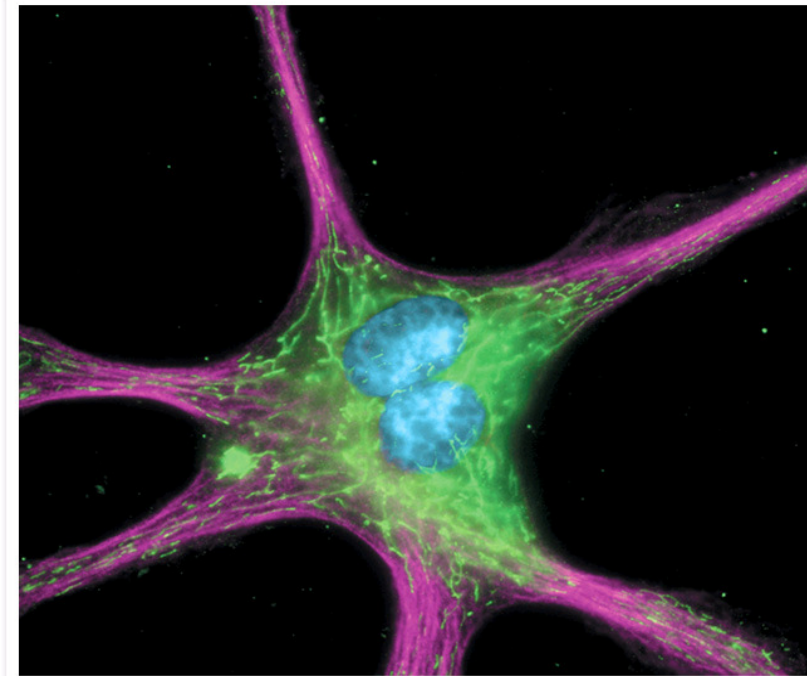
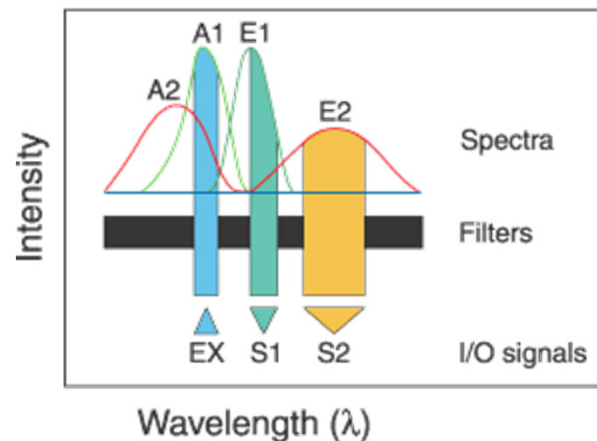
Labelling lipid membranes



- Phospholipids, sphingolipids, FAs, TAGs, sterols
- Inter-membrane transfer
- Depth in the bilayer
- Membrane potential
- Tracing, fusion
- Fluorogenic substrates

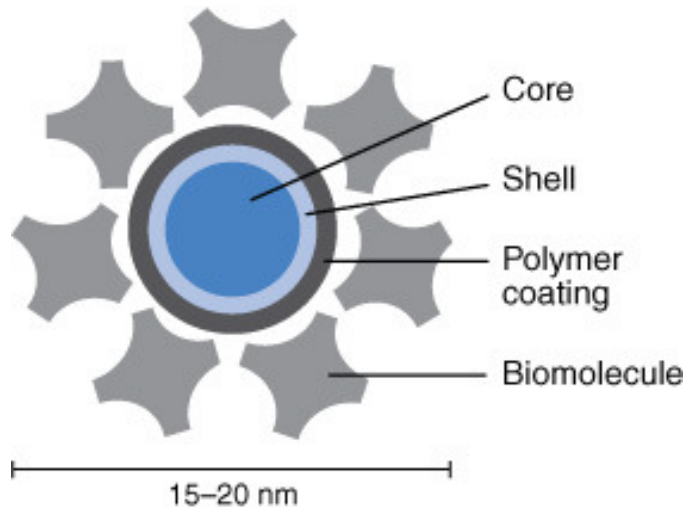
Multicolor labelling experiments

Colocalization Correlation Tracing FRET

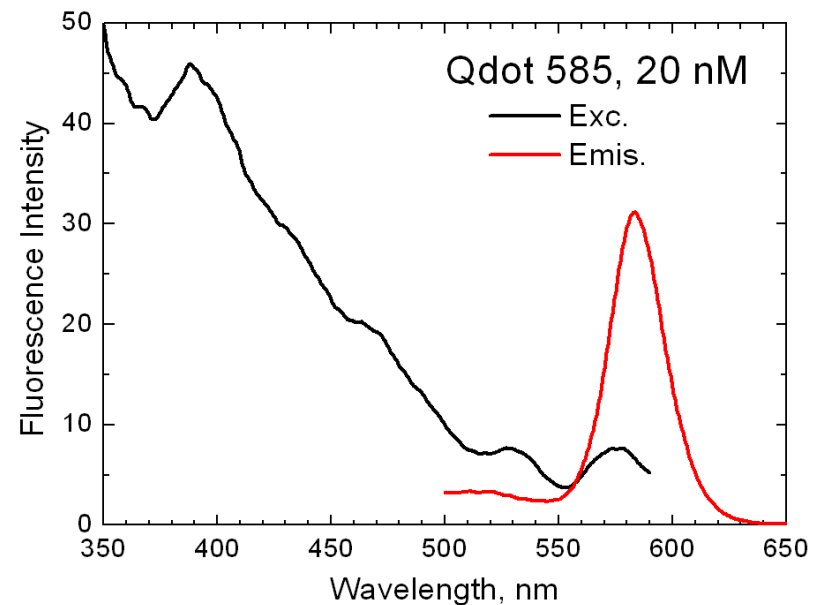
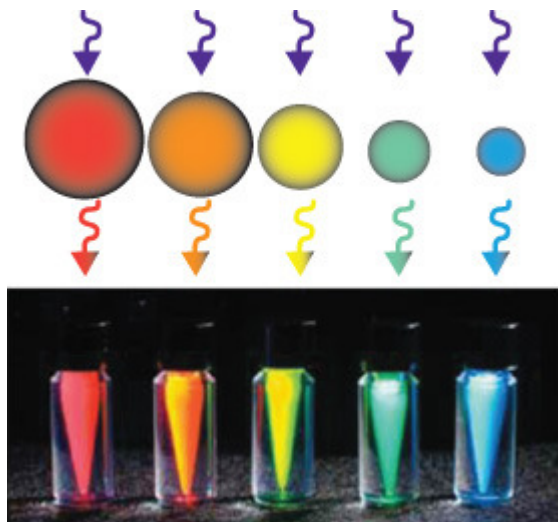


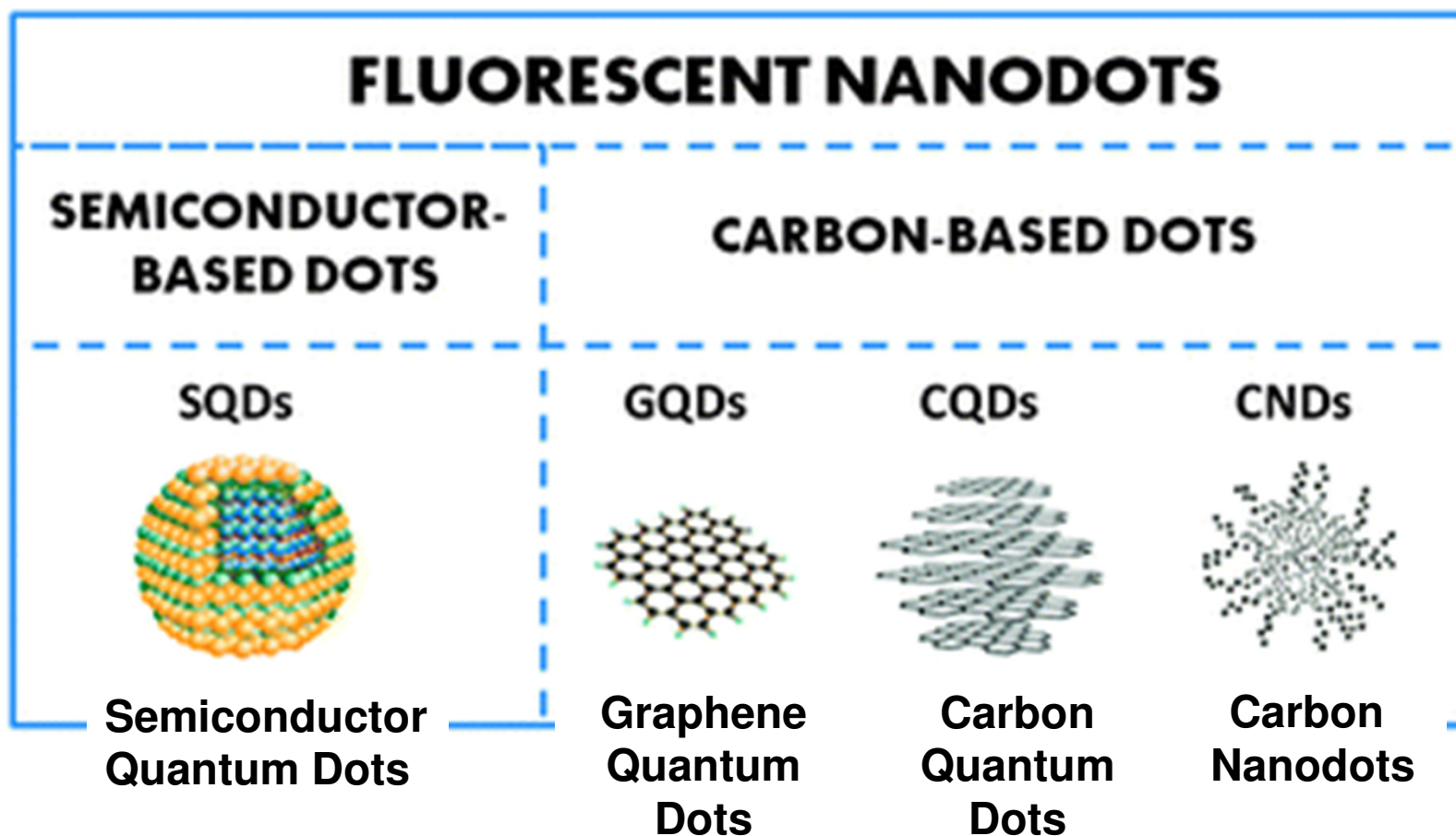
Cytoskeleton (tubulin antibody-Alexa647)
Mitochondria (streptavidin-Alexa488)
Nucleus (Hoechst-DNA intercalator)

Quantum dots



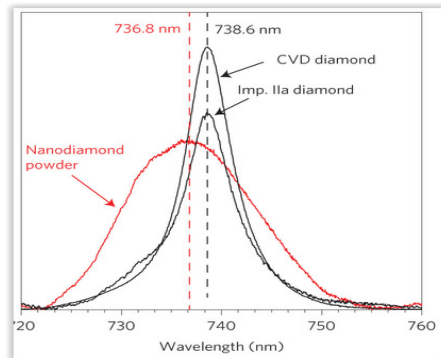
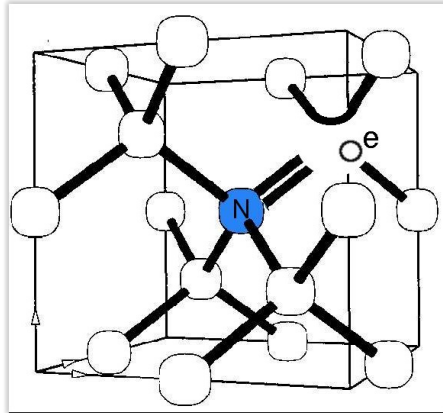
- ❖ Semiconductor nanocrystals
- ❖ Multicolour
- ❖ Extremely stable, but blinking
- ❖ High extinction coeff.
- ❖ Conjugated to streptavidin, antibodies, etc.



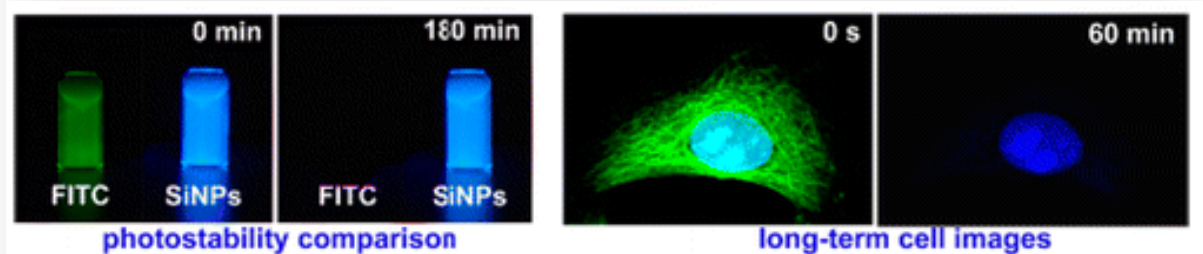


Nanodot Type	SQDs	CQDs / GQDs	CNDs
Characteristics	Size-dependent PL	Size-dependent PL (no clear)	Size-independent PL
	Excitation-independent PL	Excitation-dependent PL	Excitation-dependent PL
	Narrow PL band	Broad PL band	Very broad PL band
	Long lifetimes	Medium lifetimes	Short lifetimes

Nanodiamonds, Silicon Nanoparticles



- ❖ Nitrogen-vacancy (NV, or N-V-N) in nanodiamonds (dimensions: ~ tens of nm)
- ❖ Nanodiamonds are cytocompatible
- ❖ Non-bleaching and non-blinking
- ❖ Dimmer than organic fluorophores
- ❖ NDs can be derivatized
- ❖ Similar behavior as nanodiamonds – *silica nanoparticles* (SiNPs)
- ❖ Dimensions: 2 – tens of nanometers



**Thank you for
your attention**

