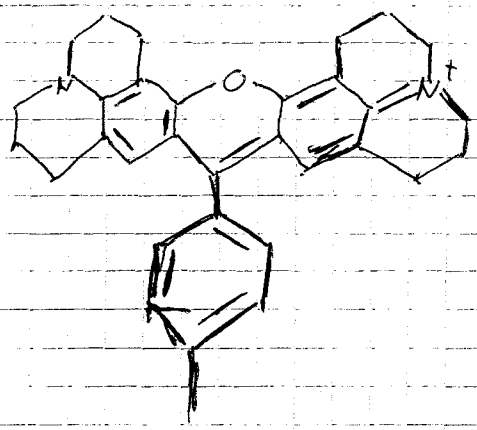
R. P. Haugland

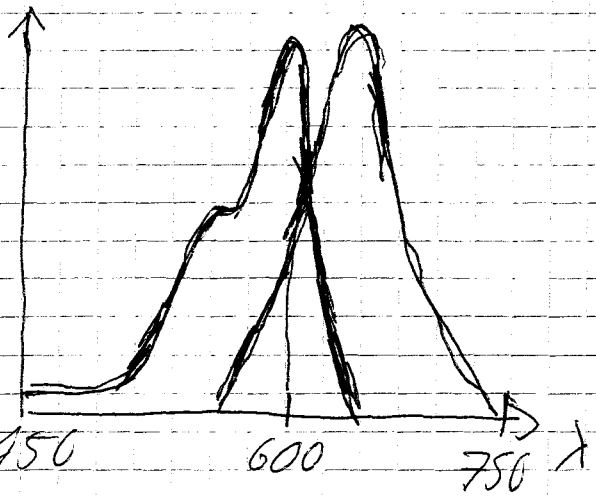
3) Fluorescent Probes

Lit: Handbook of Fluorescent Probes and Research Products, Molecular Probes

(i) normal fluorophores:

e.g. Texas Red





coupling to target

- direct coupling through amine-reative derivatives
  high specificity, requires isolated molecules
- Antibody-coupling
  can be nicely done in fixed cells,
  requires specific monoclonal or polyclonal antibody, expensive, secondary fluorescent antibody directed against IgGs of the species of the first antibody
- Protein-specific drug (phalloidin, taxol etc.)
  peptides, and DNA-Aptameres
  can be often used in live cell
  genetic evolution allows to use
  peptides and aptamers of
  high specificity

- cell fixation and permeation:

  fixation: conserves cells, increases resolution since all cellular motion is stopped by crosslinking cell content,
  adding formaldehyde or glutaraldehyde

  permeation of plasma membrane: transport through membrane has to be possible, has to retain all content
  adding methanol or detergents such as Triton

- bleaching:
  irreversible destruction of excited fluorophores due to photochemistry (intersystem crossing, oxidation)
  reduce light, add anti-fade reagents

- live imaging only possible through microinjection

(ii) caged fluorophores:

Lit.: J. E. Cells, Cell Biology, Vol 2, p. 65
Academic Press

→ Tim J. Mitchison, Julie A. Theriot

- idea: local photoactivation of fluorescence
- labeling with caged fluorophores, local irradiation with 360 nm light triggers an efficient photocleavage reaction generating the active fluorochrome!
- probes use photocleavage of O-nitrobenzyl ~~deriv~~ derivates in the long-wavelength UV for activation

(iii) Green Fluorescent Protein (GFP)

Lit.: D.L. Spector et al, Cells - Light Microscopy and Cell Structure, Vol 2 p. 78.1, Cold Spring Harbor

GFP: 238-amino-acid protein from jellyfish, emit green fluorescent light,
expression of the cDNA encoding GFP is sufficient to produce the characteristic fluorescence of GFP in cells
mutations of the protein have produced new fluorophores

- GFP as a reporter molecule
  expressed in various cells and animals
  examples: - inject GFP-labeled cancer cells
  in nude mice to follow tumor
  development
  - olfactory neurons of a transgenic mouse
  - astrocytes of a transgenic mouse
  - monitor directly transfection
  with another gene
- GFP in fusion constructs:
  fusion constructs that join GFP to another
  coding region can provide important insights
  into the localization of a protein of interest.
  This is largely because GFP itself is not
  localized within a cell.
- all can be done in living cells

(iv) Quantum dots

Lit.: Tuan Vo-Dinh, Biomedical Photonics, p. 58-1, CRC press

• electron in box potential:

$$V(x) = 0 \quad |x| < 0$$

$$= \infty \quad \text{otherwise}$$

$$-\frac{\hbar^2}{2m}\frac{d^2u(x)}{dx^2} + V(x)u(x) = E(u(x))$$

$$u(x \geq |a|) = 0$$

$$|x| < 0 \Rightarrow \frac{d^2u(x)}{dx^2} + \frac{2mE}{\hbar}u(x) = 0$$

anoymmetric solutions:

$$u_n^{(-)}(x) = \frac{1}{\sqrt{a}}\sin\frac{n\pi x}{a} \qquad E_n^{(-)} = \frac{n^2\pi^2\hbar^2}{2ma^2}$$

symmetric solutions:

$$u_n^{(+)}(x) = \frac{1}{\sqrt{a}}\cos\frac{[n-\frac{1}{2}]\pi x}{a} \qquad E_n^{(+)} = \frac{(n-\frac{1}{2})^2\pi^2\hbar^2}{2ma^2}$$