Luminiscencia

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Základné pojmy

Luminiscenciou nazývame emisiu svetla atómu, alebo molekuly, ktorá sa odohráva z excitovaného stavu elektrónu

Luminiscencia sa formálne delí na fluorescenciu a fosforescenciu v závislosti na charaktere elektronového excitovaného stavu.

Fluoroforom nazývame látku schopnú absorbovať a emitovať svetlo.

Objav Fluorescencie


John Herschel

XXX. *On the Change of Refrangibility of Light.* By G. G. Stokes, *M.A., F.R.S., Fellow of Pembroke College, and Lucasian Professor of Mathematics in the University of Cambridge.*

Received May 11,—Read May 27, 1852.

1. THE following researches originated in a consideration of the very remarkable phenomenon discovered by Sir John Herschel in a solution of sulphate of quinine, and described by him in two papers printed in the Philosophical Transactions for 1845, entitled 'On a Case of Superficial Colour presented by a Homogeneous Liquid internally colourless,' and 'On the Epipolic Dispersion of Light.' The solution of quinine, though it appears to be perfectly transparent and colourless, like water, when viewed by transmitted light, exhibits nevertheless in certain aspects, and under certain incidences of the light, a beautiful celestial blue colour. It appears from the experiments of Sir John Herschel that the blue colour comes only from a stratum of fluid of small but finite thickness adjacent to the surface by which the light enters.

* I confess I do not like this term. I am almost inclined to coin a word, and call the appearance *fluorescence*, from fluor-spar, as the analogous term *opalescence* is derived from the name of a mineral.

Fluorescenciou nazývame jav pri ktorom je svetlo emitované atómom alebo molekulou po ohraničenej dobe nasledujúcej po absorpcii elektromagnetického žiarenia

Stokesův posun

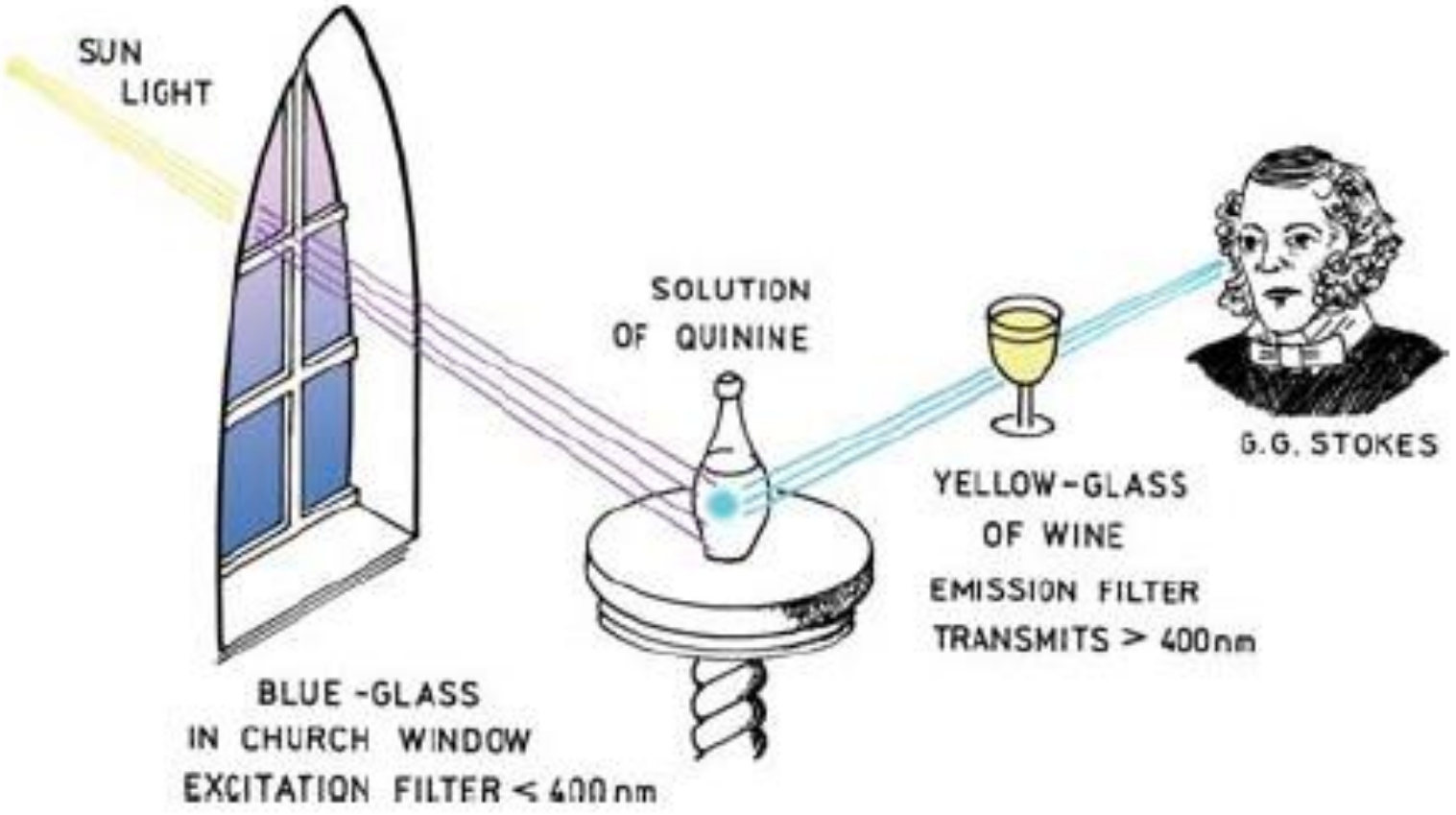
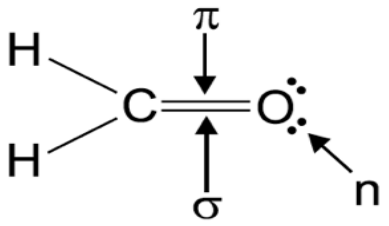
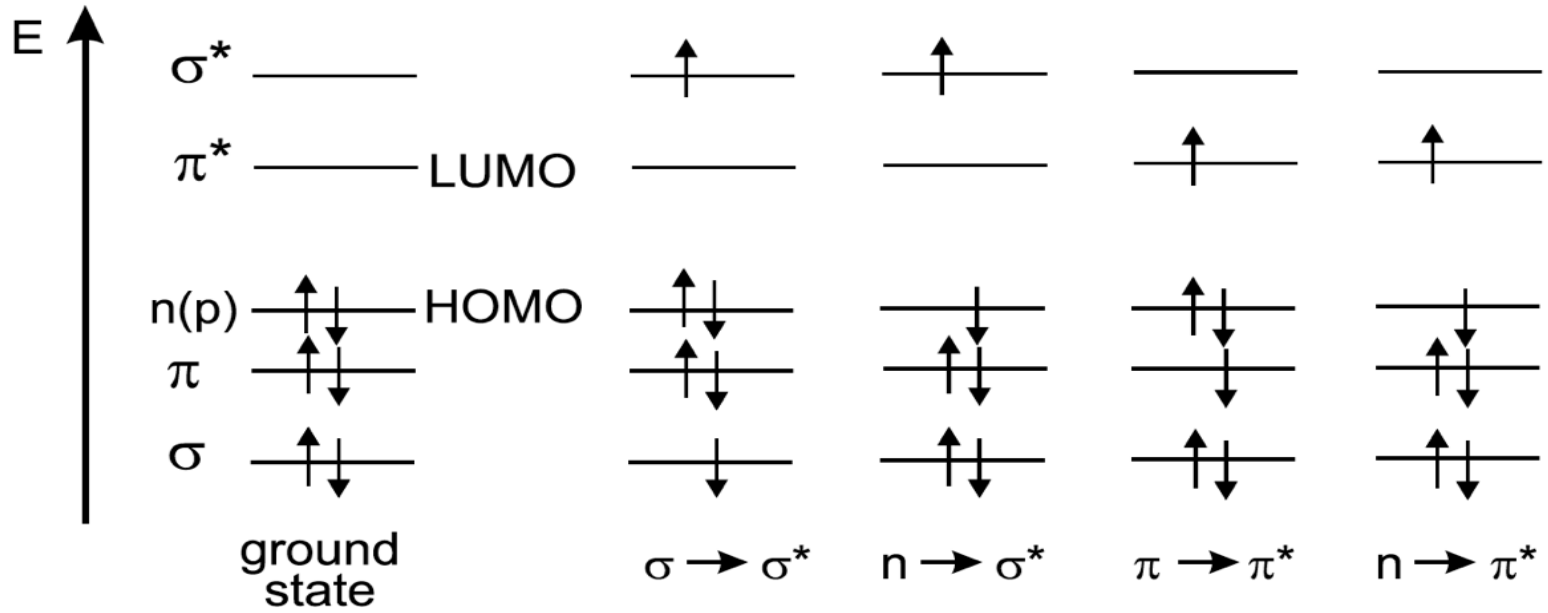


Figure 1.6. Experimental schematic for detection of the Stokes shift.



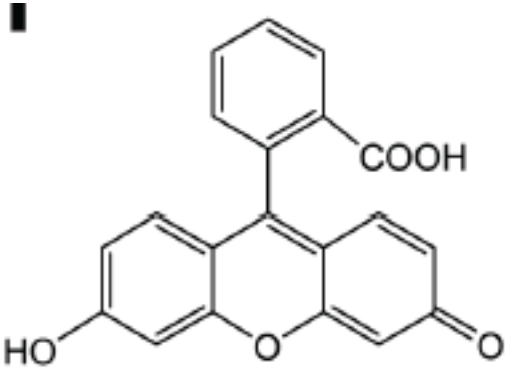


σ^*-σ malokedy vedú ku luminiscencii. Veľmi vysoká energia (λ<250nm) vedie zväčša ku disociácii molekúl

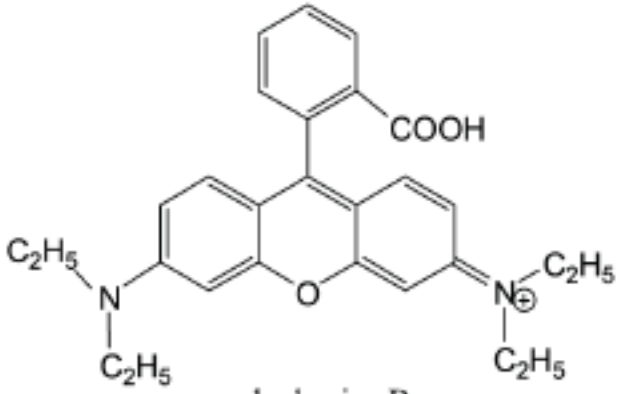
Emisie sú najčastejšie pre prechody π^*-π, niekedy aj π^*-n

Typické fluorofóry

- Molekuly obsahujúce konjugované π elektróny



fluorescein



rhodamine B

Energetické hladiny v molekule

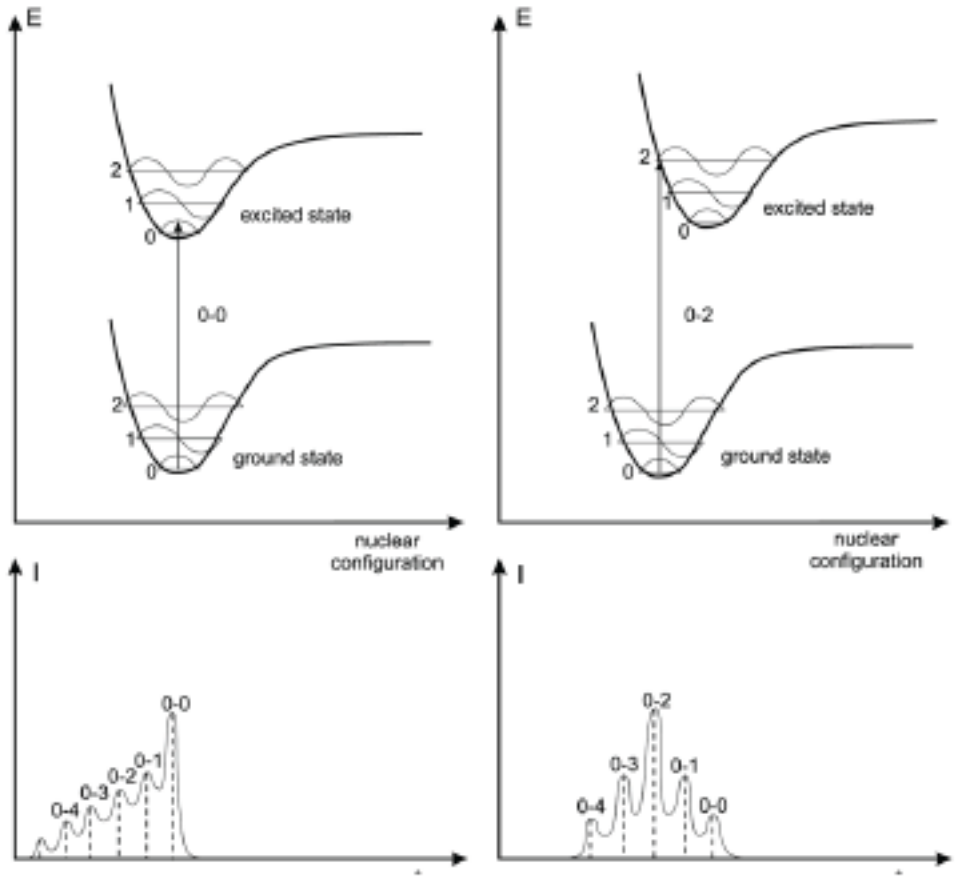
$E_{n,v,J}=E_n+E_v+E_J$
E_n-elektrónová energia
E_V-vibračná energia
E_J-rotačná energia

Kvalitatívne porovnanie jednotlivých energetických komponent:
E_n(UV-VIS) >> E_V (IR) >> E_J(MW)

Čo určuje tvar absorbčného spektra?

Frank-Condonov princíp:
Vertikálne prechody elektrónu, ktoré sa odohrávajú na omnoho rýchlejšej časovej škále ako pohyb jadier atómov

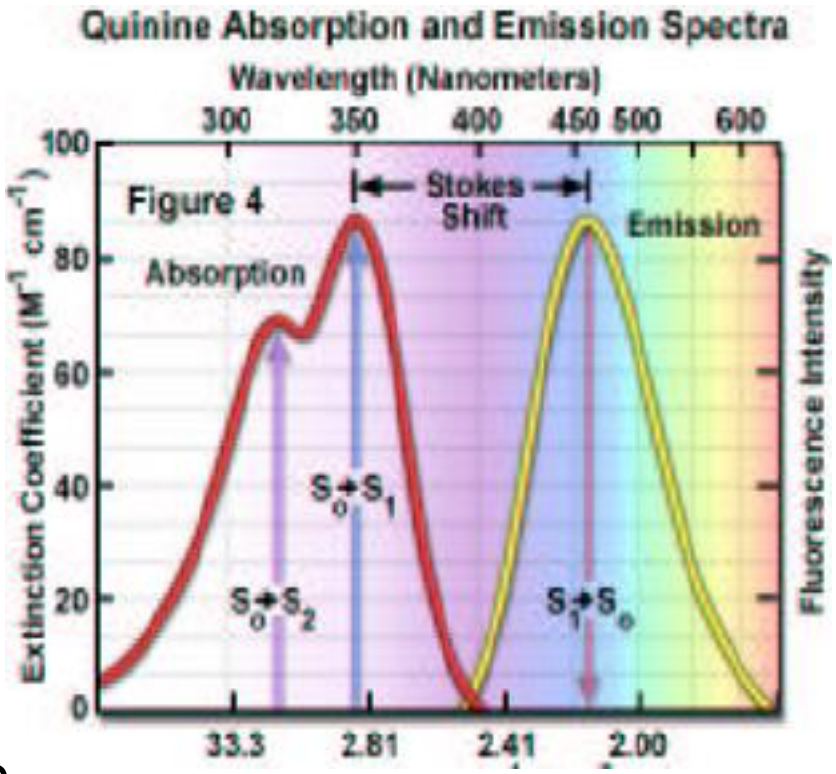
Absorbcia 10^{-15}s
Vibrácie atómov 10^{-12}s-10^{-10}s
Termálna relaxácia 10^{-15}s-10^{-12}s
Fluorescenčná emisia 10^{-9}s



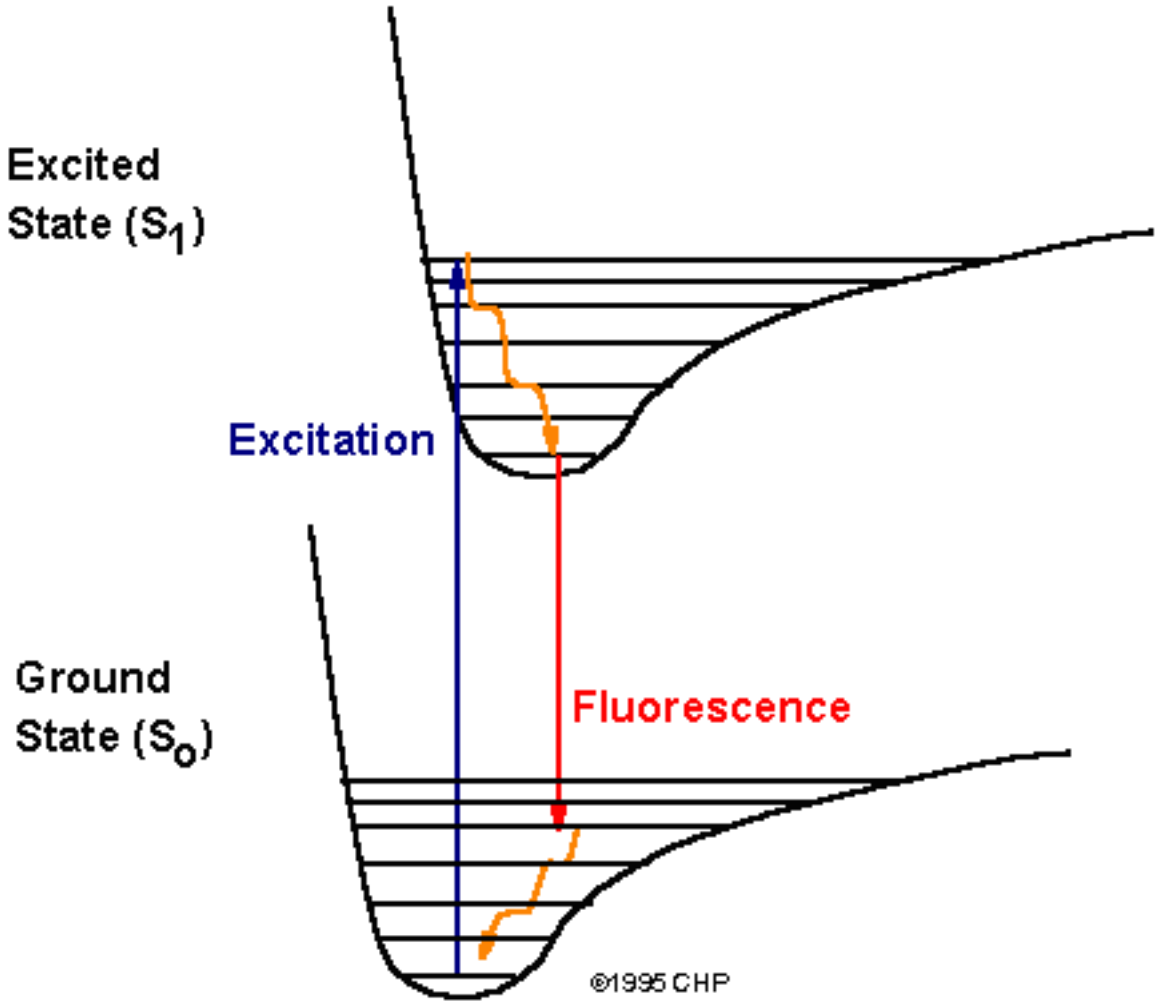
Valeur, B. (2002) Molecular Fluorescence, Wiley-VCH, Ch. 2 Pg 32

Typické charakteristiky fluorescenčných spektier

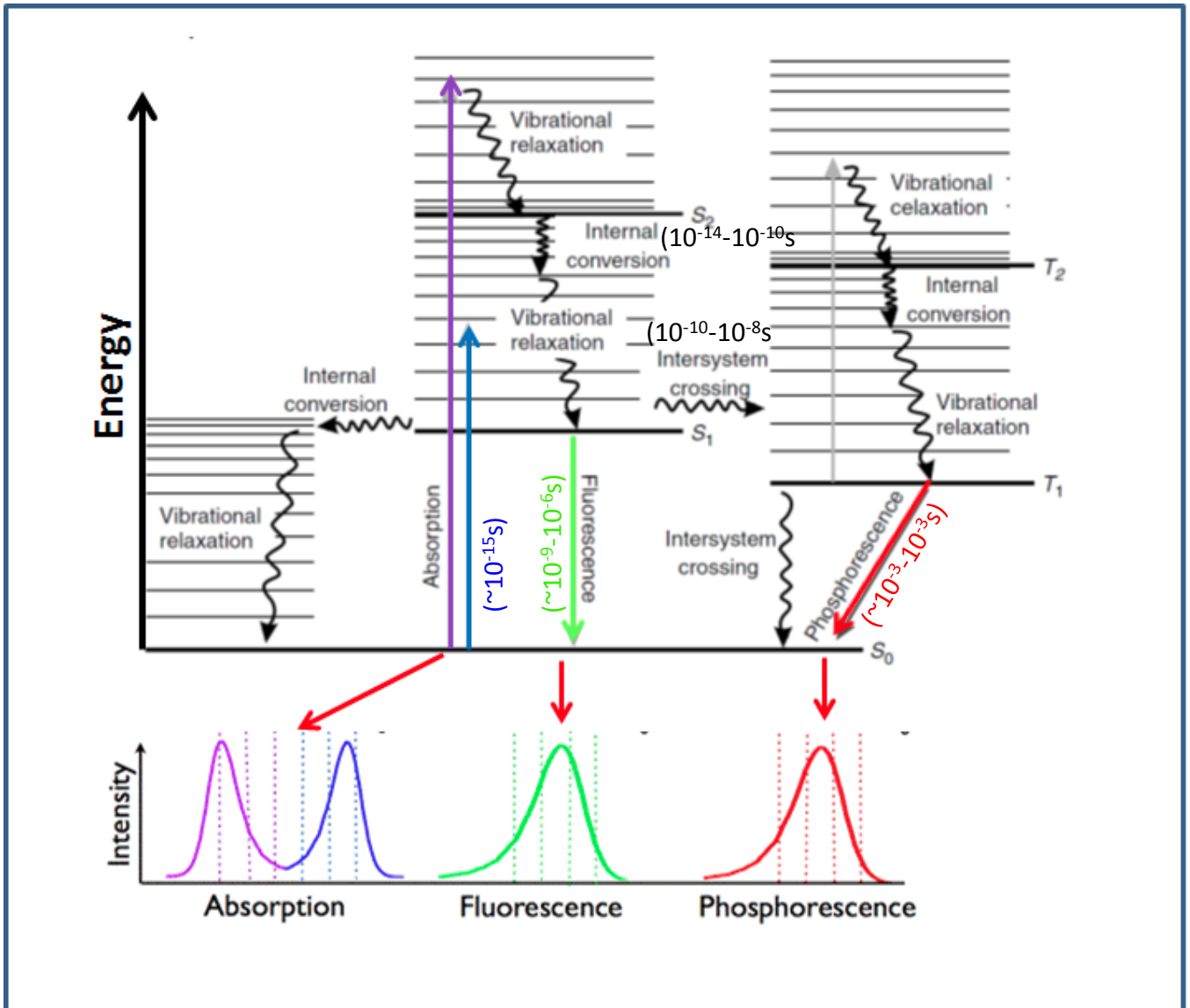
- Stoksov posun
- Nezávislosť emisného spektra na excitačnej vlnovej dĺžke
- Excitačné spektrum “rozumne sa správajúcich molekúl” je prakticky totožné s absorbčným spektrom
- Emisné spektrum je vo väčšine prípadoch takmer zrkadlovým obrazom absorbčného/excitačného spektra



Kasha's Rule


Excited
State (S_1)
Excitation
Ground
State (S_o)
Fluorescence
©1995 CHP

Jablonského diagram



Kvantový výťažok

$$\Phi = \frac{počet\ emitovaných\ fotónov}{počet\ absorbovaných\ fotónov}$$

$$\phi = \frac{k_f}{k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d}$$

k_f = fluorescent rate constant

k_i = intersystem crossing rate constant

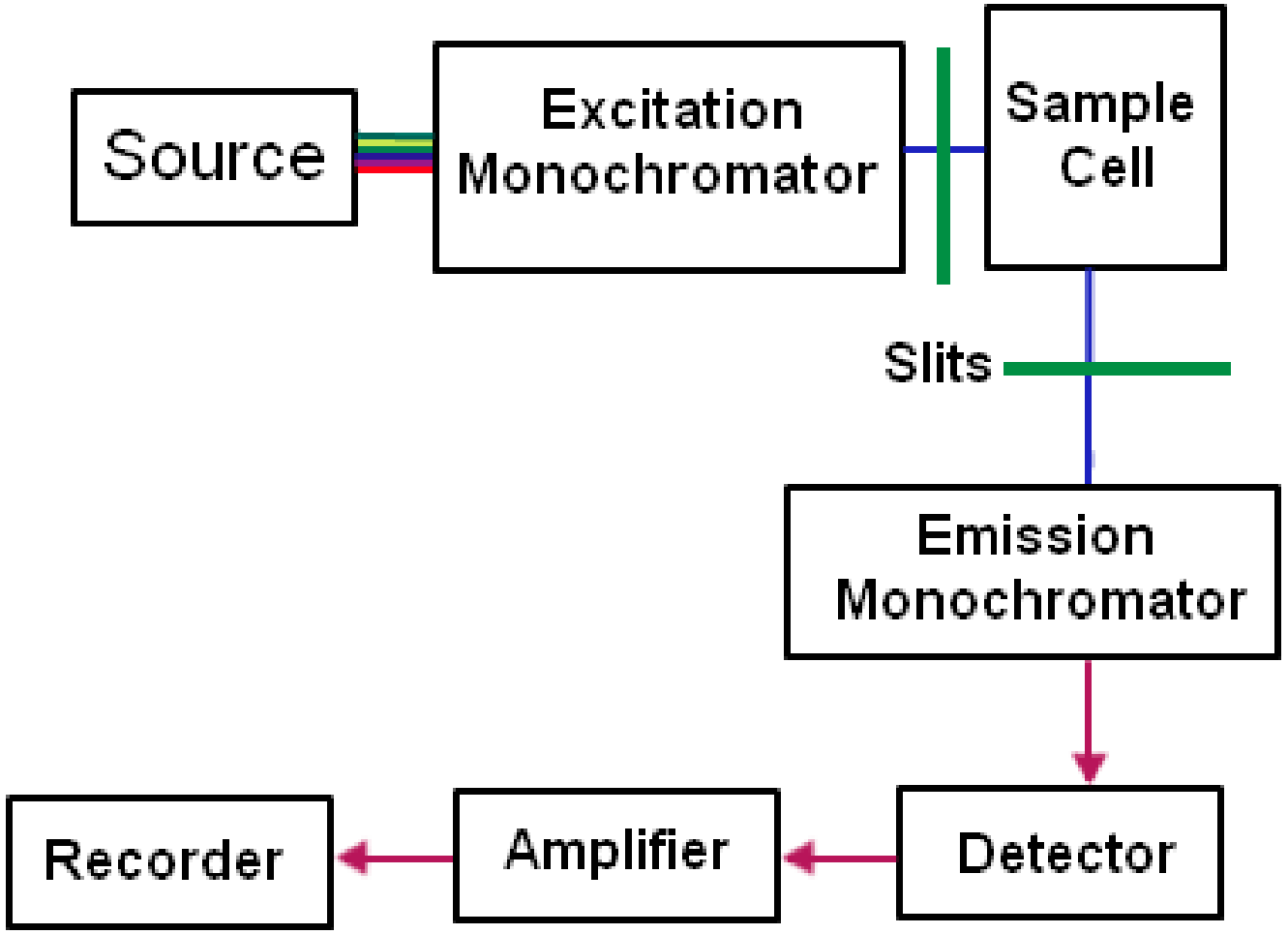
k_{ec} = external conversion rate constant

k_{ic} = internal conversion rate constant

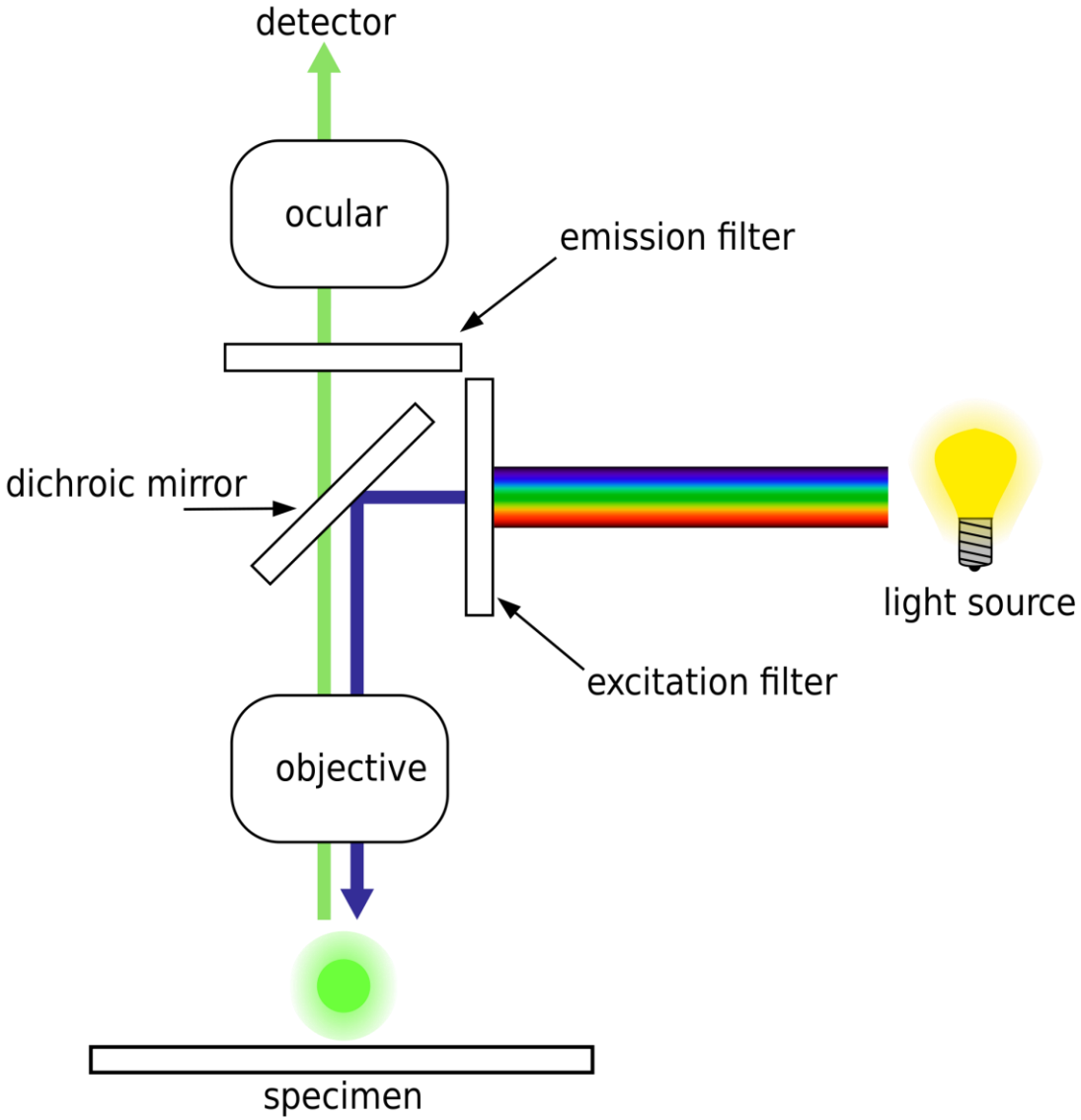
k_{pd} = predissociation rate constant

k_d = dissocation rate constant

Fluorescenčný spektrofotometer

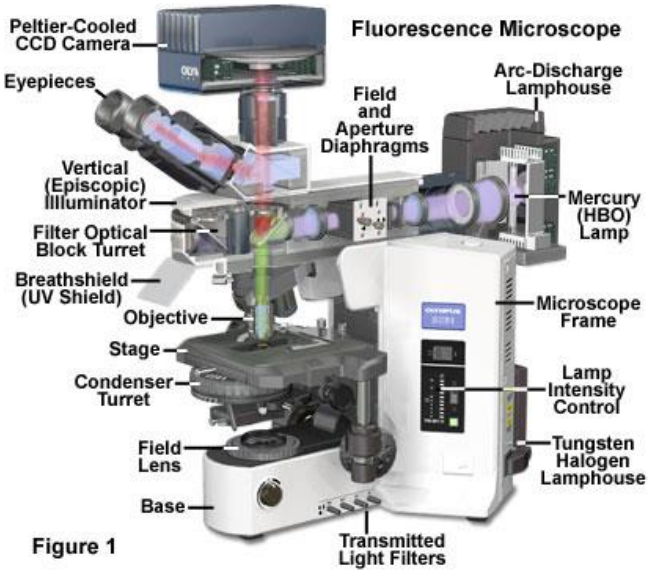


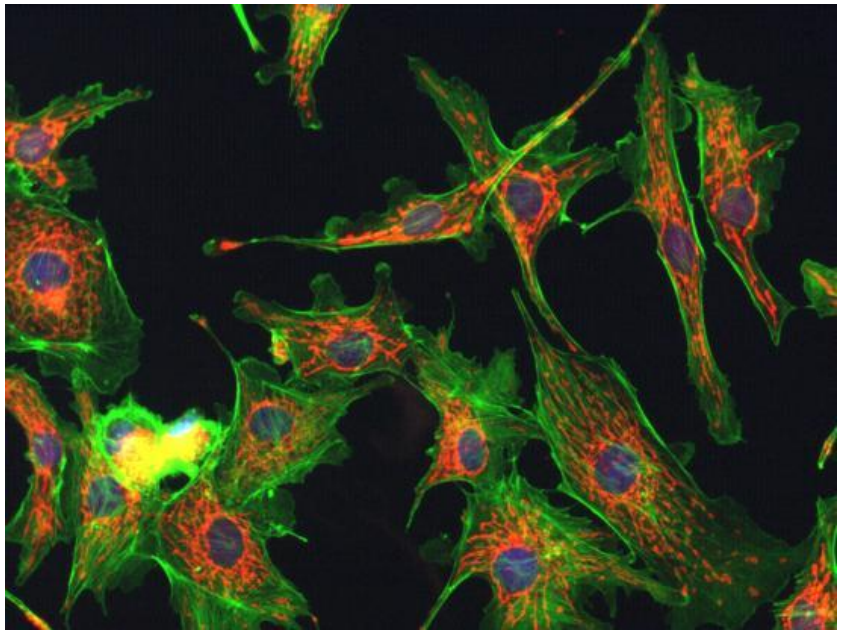
Fluorescenčný mikroskop



Fluorescence microscopy

- Subcellular fluorescence imaging
  - Combined with recombinantly-expressed fluorophores (GFP, etc.—Roger Tsien) has revolutionized biology.

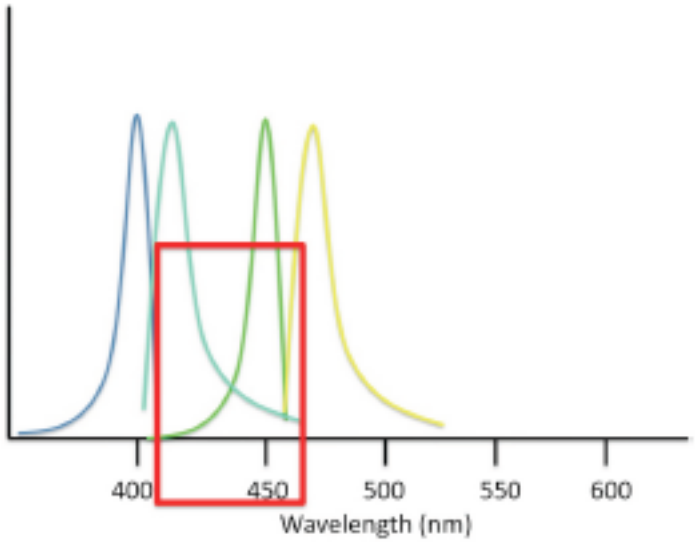


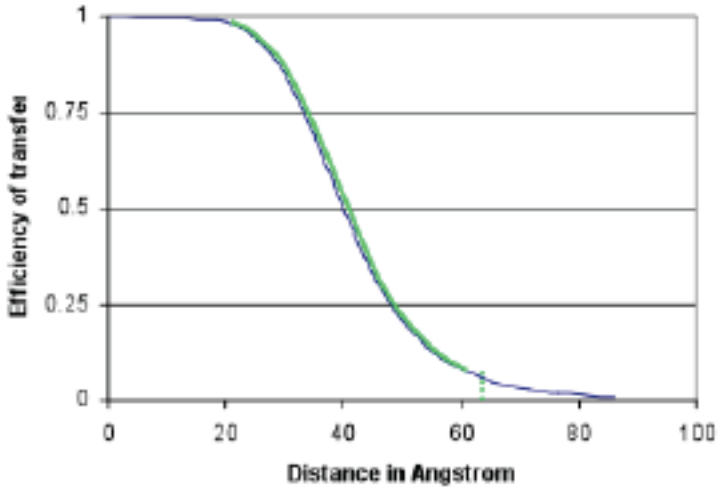


http://micro.magnet.fsu.edu/primer/techniques/fluorescence/anatomy/fluoromicroanatomy.html

http://www.rp-photonics.com/img/kahn_fl_image.jpg

Försterov Rezonančný Transfer Energie (FRET)





$$E = \frac{R_0^6}{R_0^6 + r^6}$$

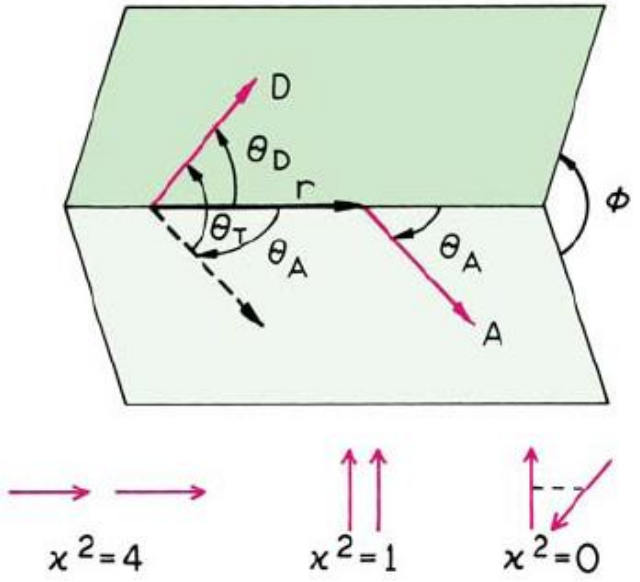
R_0-Försterov polomer/vzdialenosť

Efektívnosť FRET

$$E = \frac{R_0^6}{R_0^6 + r^6} \qquad R_0^6 = \frac{9000(\ln 10)\kappa^2 Q_D}{128\pi^5 N n^4} \int_0^\infty F_D(\lambda)\,\varepsilon_A(\lambda)\,\lambda^4\,d\lambda$$

r – vzdialenosť medzi akceptorom a donorom

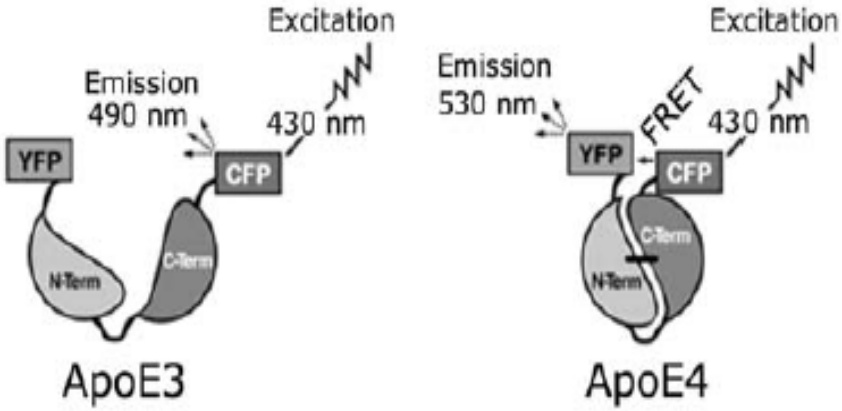
κ^2 – popisuje vzájomnú relatívnu orientáciu tranzitných dipólov donoru a akceptora.
Pre flexibilný náhodný pohyb akceptora a donora sa $\kappa^2=2/3$

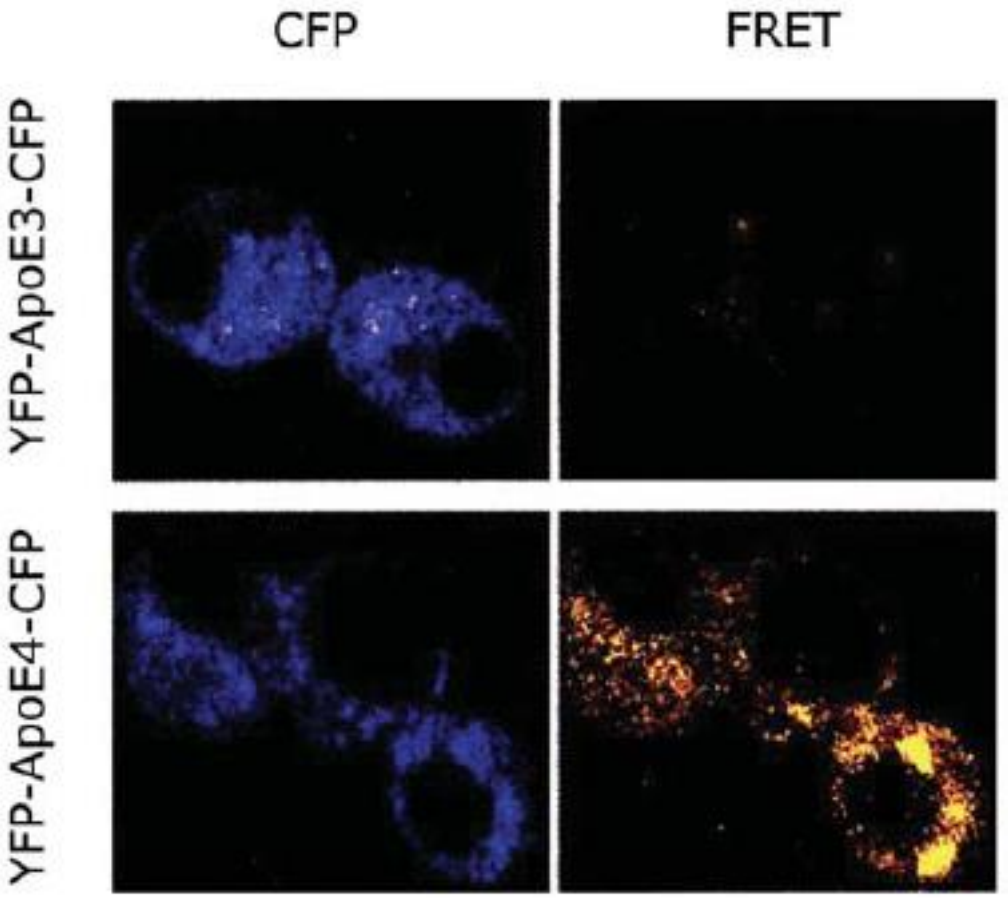


$$x^2 = (\cos\theta_T - 3\cos\theta_D \cos\theta_A)^2$$

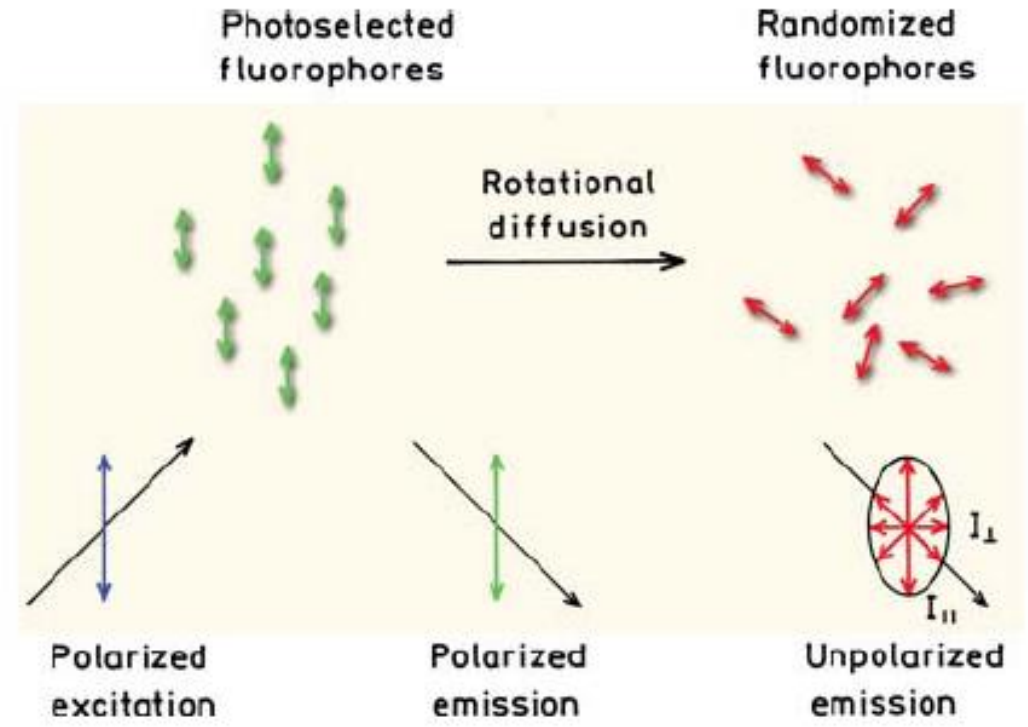
$$x^2 = (\sin\theta_D \sin\theta_A \cos\Phi - 2\cos\theta_D \cos\theta_A)^2$$

Monitorovanie skladania proteínov in vivo





Fluorescenčná anizotropia



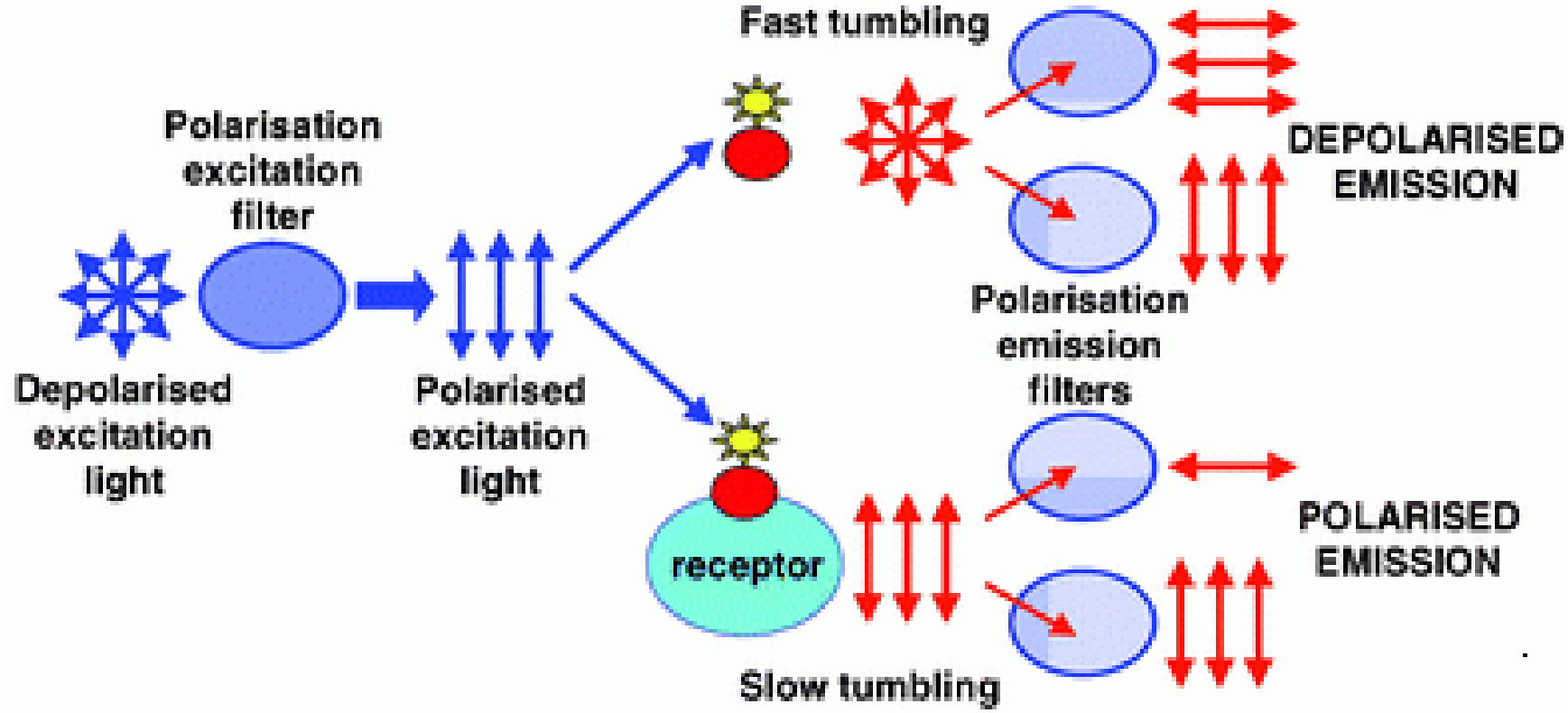
Anizotrópia

$$r = \frac{I_\parallel - I_\perp}{I_\parallel + 2I_\perp}$$

Polarizácia:

$$P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp}$$

Viazanie ligandu do receptoru



Ďalšie čítanie

- Lakowicz, J. R. (2006) Principles of Fluorescence Spectroscopy

http://xibalba.lcg.unam.mx/~rgalindo/bioquimica/BQPosgrado2011/V PurificacionEspeectroscopia/PrincipiesofFluorescenceSpectroscopy3rd.pdf)