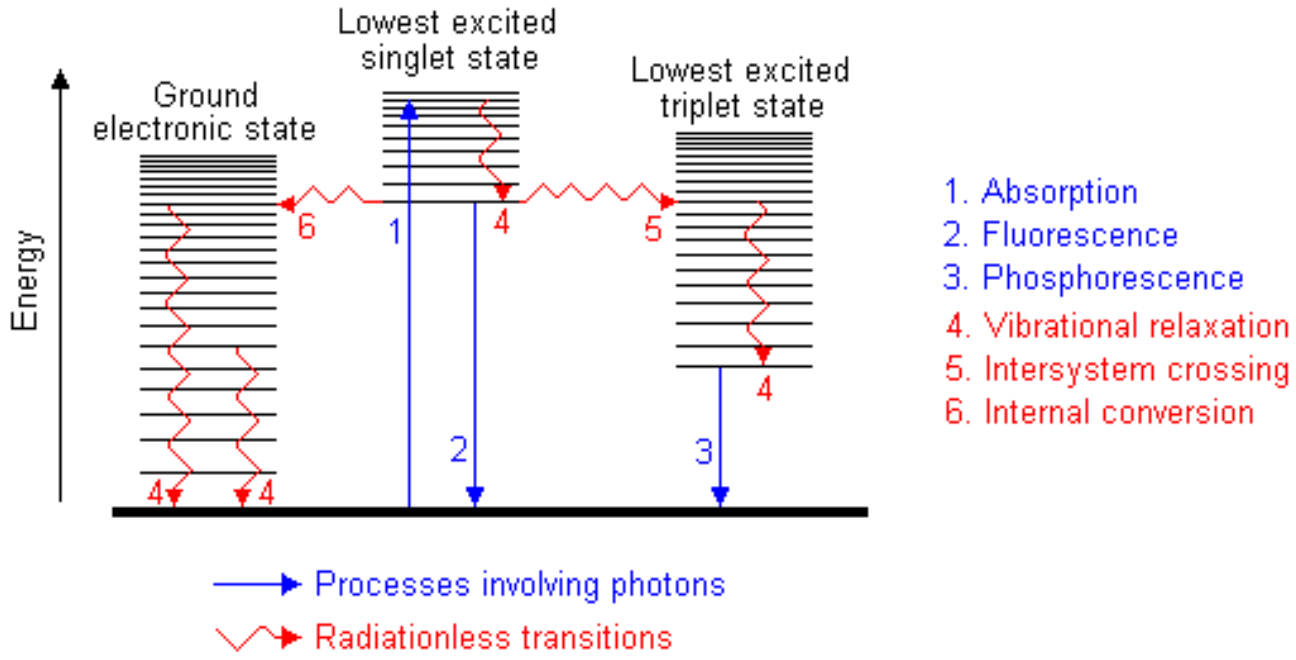
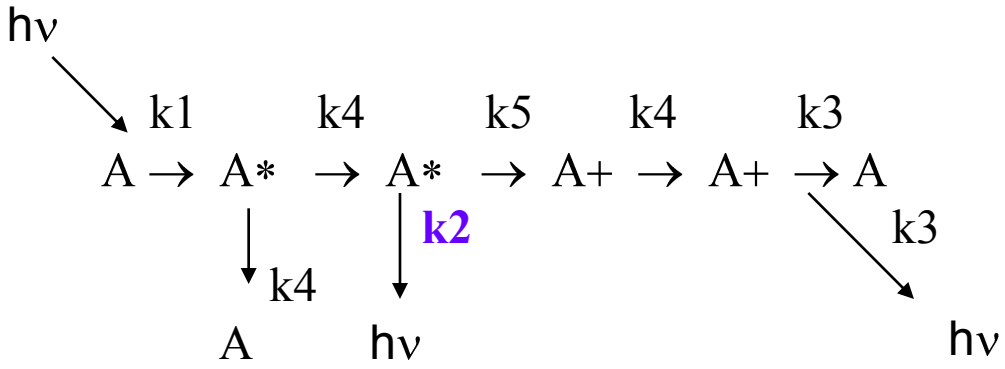
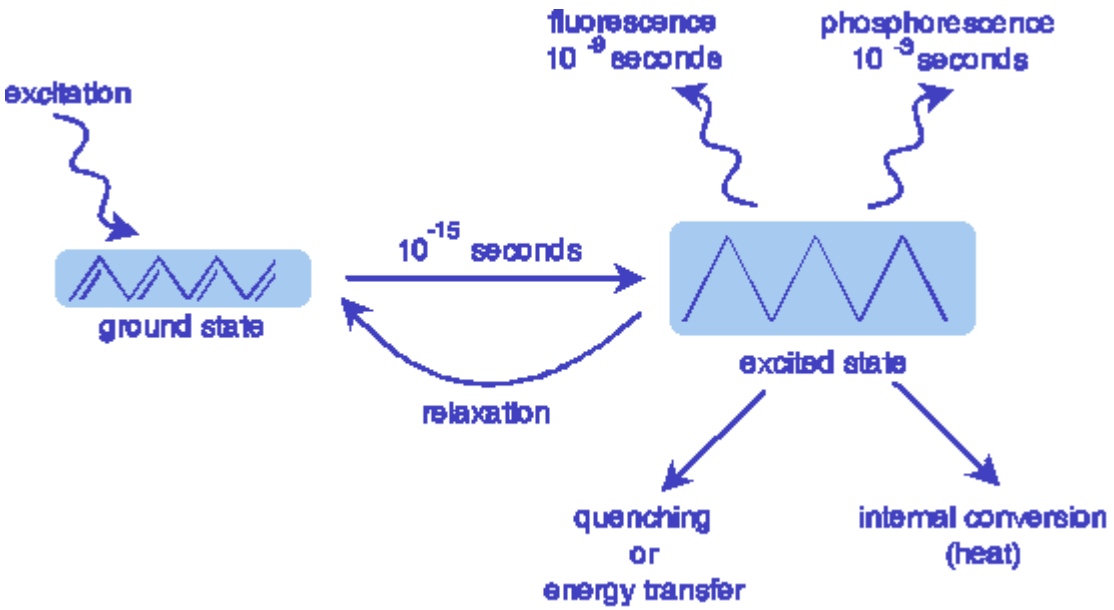
Luminiscenční spektroskopie





Luminiscenční spektroskopie

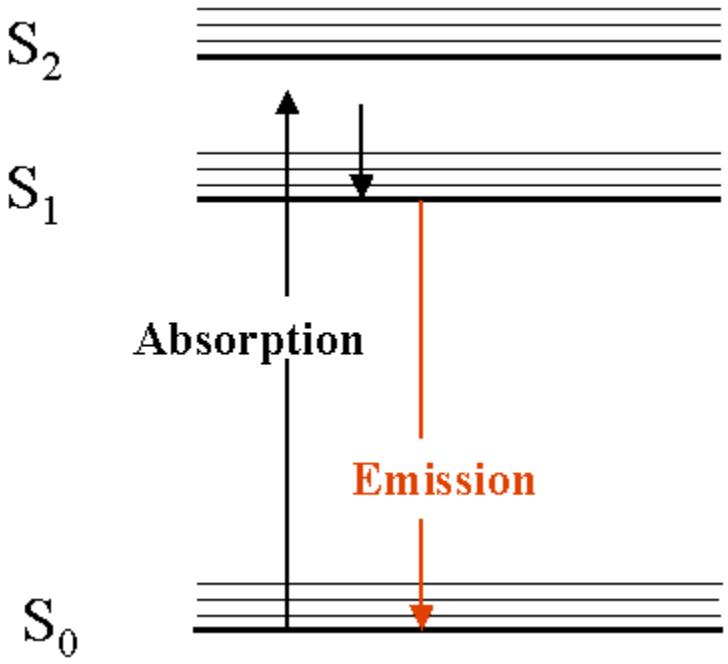


Luminiscenční spektroskopie

Fluorescenční spektroskopie
Fosforescenční spektroskopie
Chemiluminiscenční spektroskopie

Základní pojmy

Excitace a emise

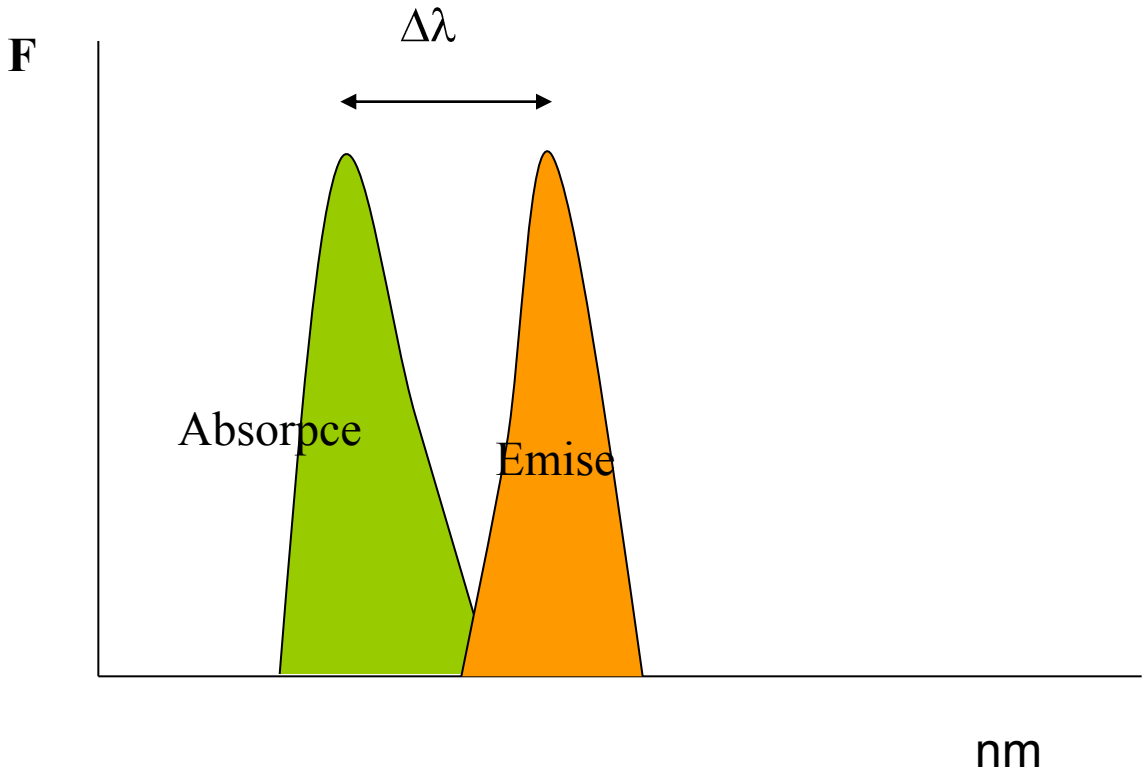


Interakce s rozpouštědlem
Singletový excitovaný stav

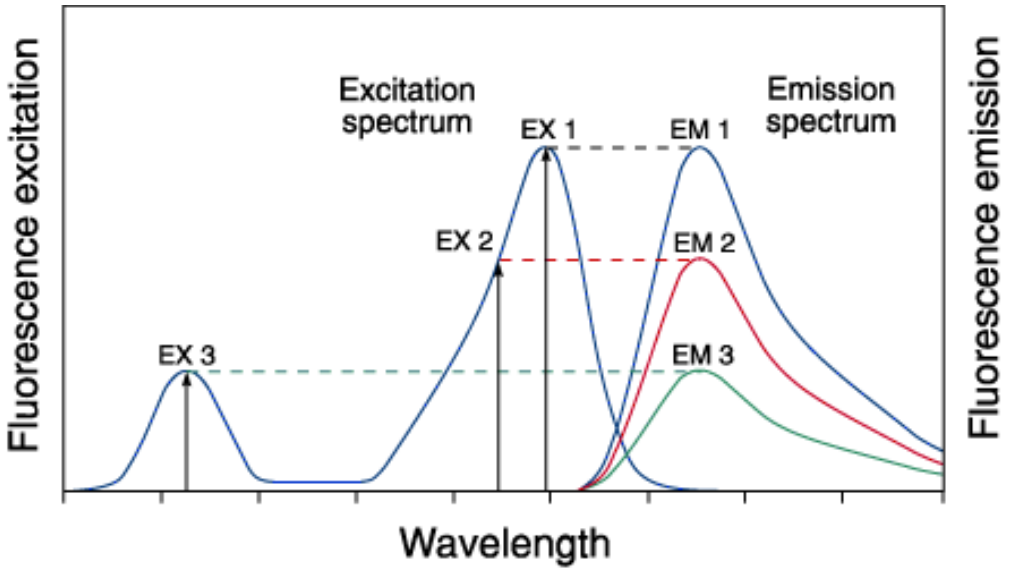
Singletový základní stav

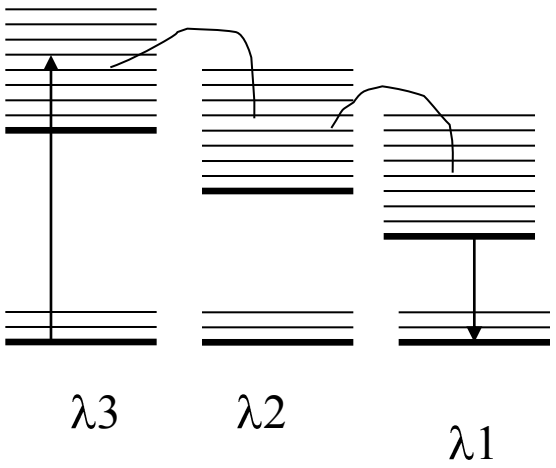
Základní pojmy

Stockesův posun – ztráty energie po dobu excitovaného stavu



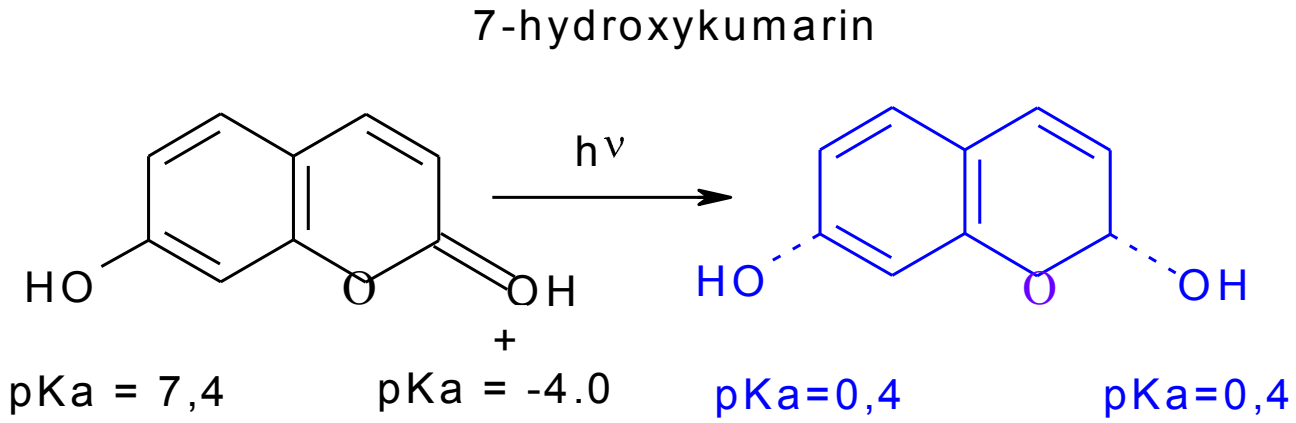
Základní pojmy





Základní pojmy

Excitovaný stav – střední doba života 10^{-7} – 10^{-9} s.



Základní pojmy

Kvantový výtěžek fluorescence

Φ = počet kvant emitovaných/počet kvant absorbovaných

$\Phi = k_e/(k_e + \Sigma\, k_k)$

k_e = rychlost emise
k_k = rychlost konverzních procesů

Intenzita fluorescence látky = f (ε, Φ, N)

Základní pojmy

$$\Phi = \tau/\tau_0$$

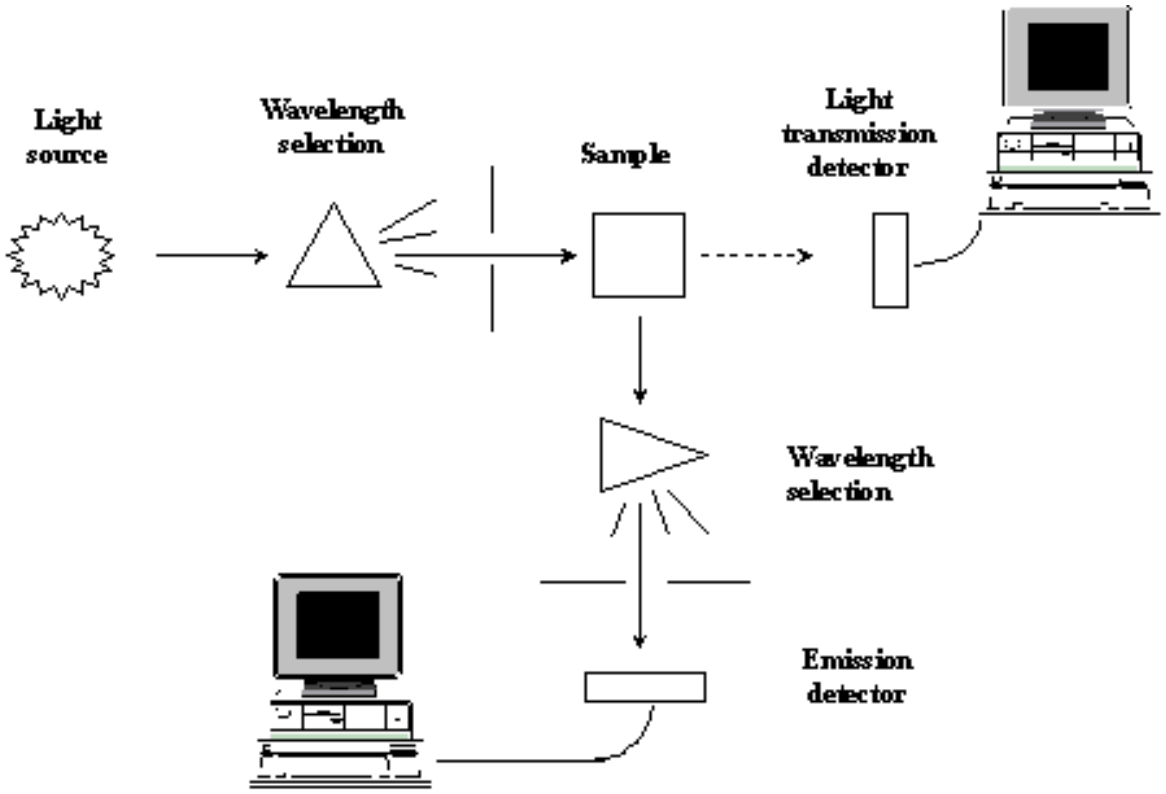
Střední doba života fluorescence

Fluorescein	4,6 ns
Chininsulfát	15 – 40 ns
NADH	0,5 ns

Biochemicky významné fluorofory

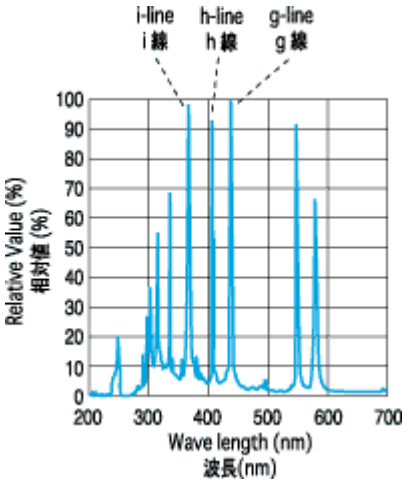
	λ_{exc}	λ_{em}	Q (25°C)
Tyrosine	275	303	0.14
Tryptophane	287	348	0.13
Indole	287	348	0.45
NADH	350	460	0.03
Riboflavine	450	535	-
Chlorophylle	436	670	0.30 (acétone)
Quinine	250	450	0.51 (1M H_2SO_4)
Pyridoxamine	324	392	0.11 (pH=8.2)
Vitamine A	325	470	- (ethanol)
Aminobenzoate	294	345	-

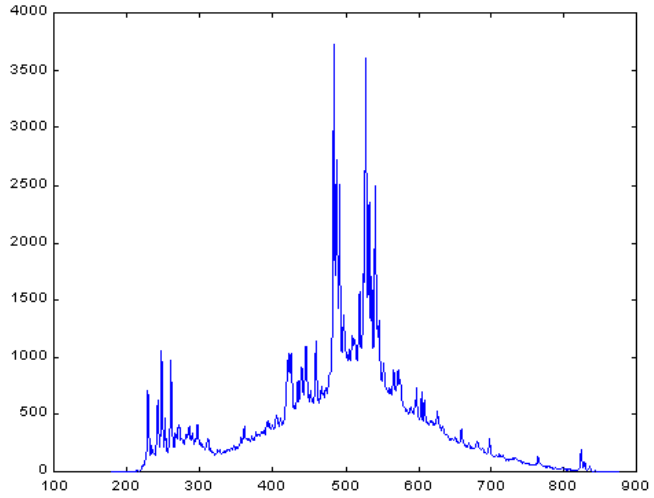
Instrumentace

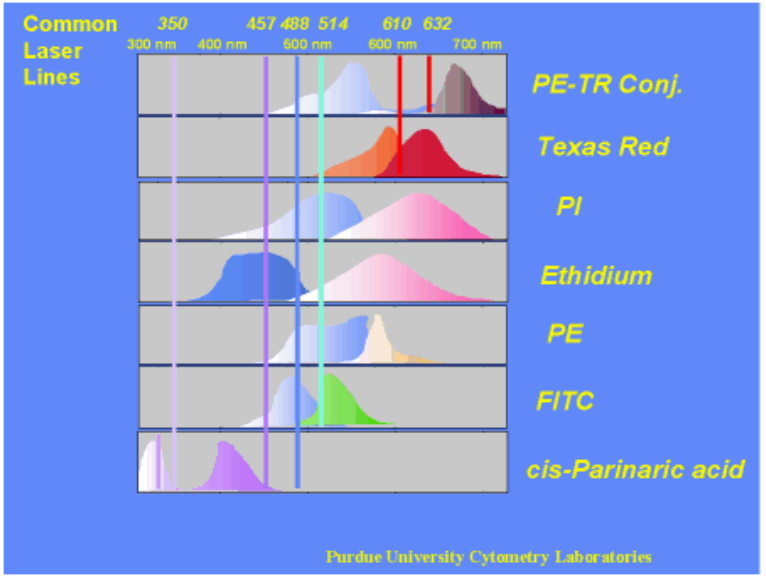


Instrumentace

Zdroj:
Xenonová lampa
Rtuťová výbojka
Laser
Světelné diody - LED (430, 450, 505, 592, 612 and 637 nm)



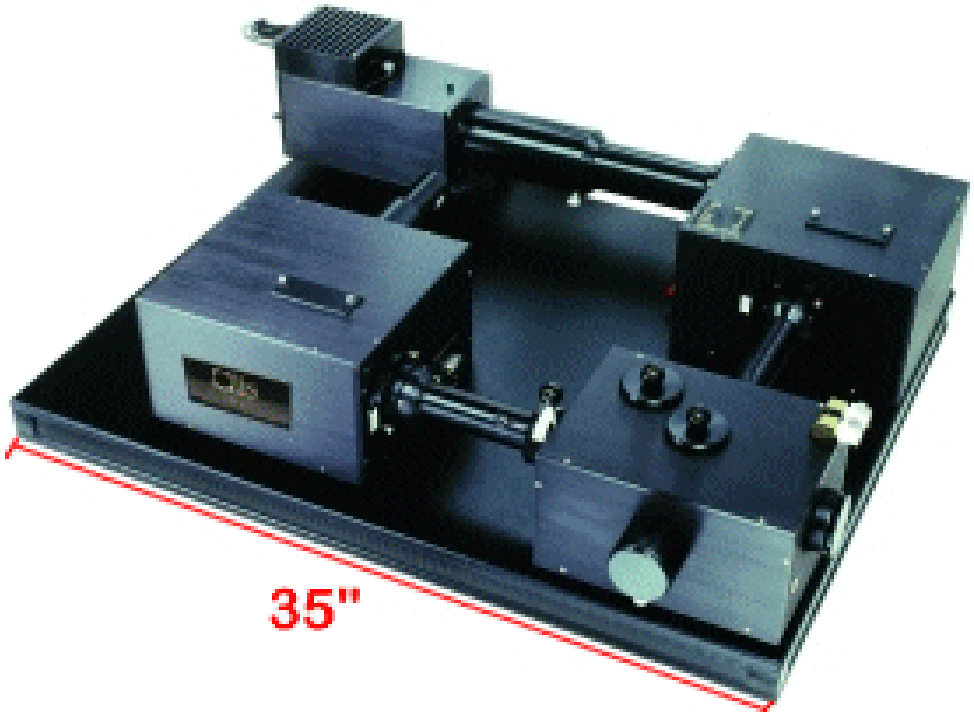




Instrumentace

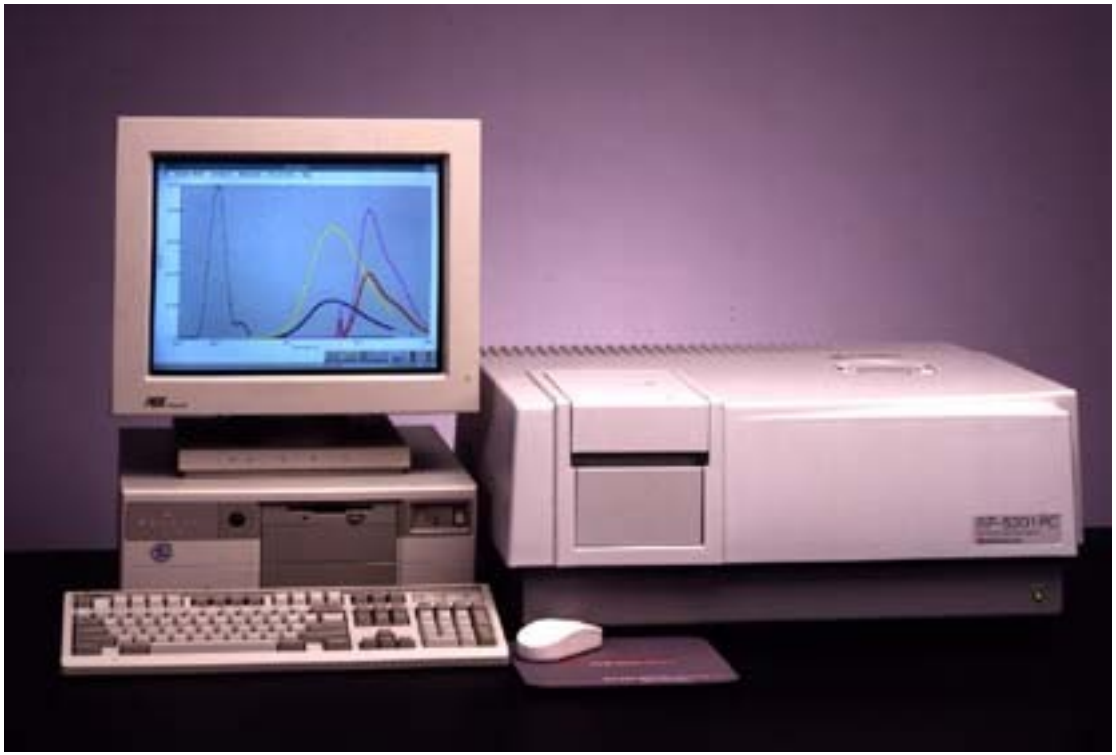
Monochromátor
- mřížka
- filtry

Instrumentace



Instrumentace

RF-5301PC – spektrofluorimetr Shimadzu



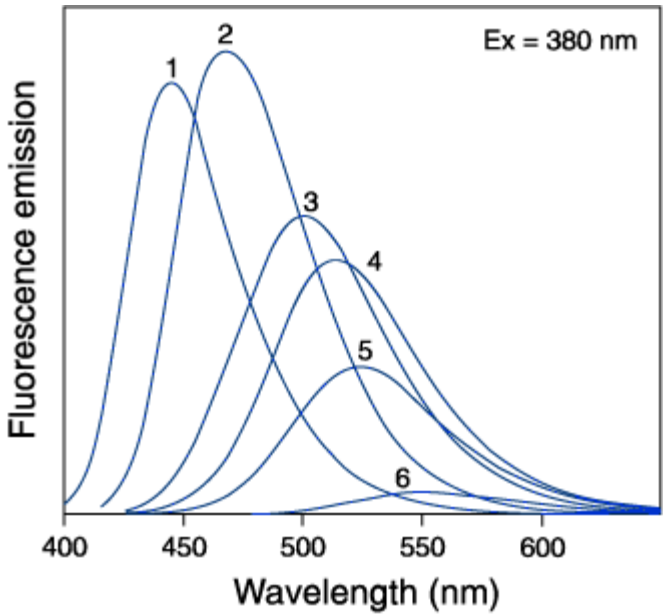
Podmínky fluorescence

Závislost na polaritě a viskozitě

Nitrobenzoxadiazol

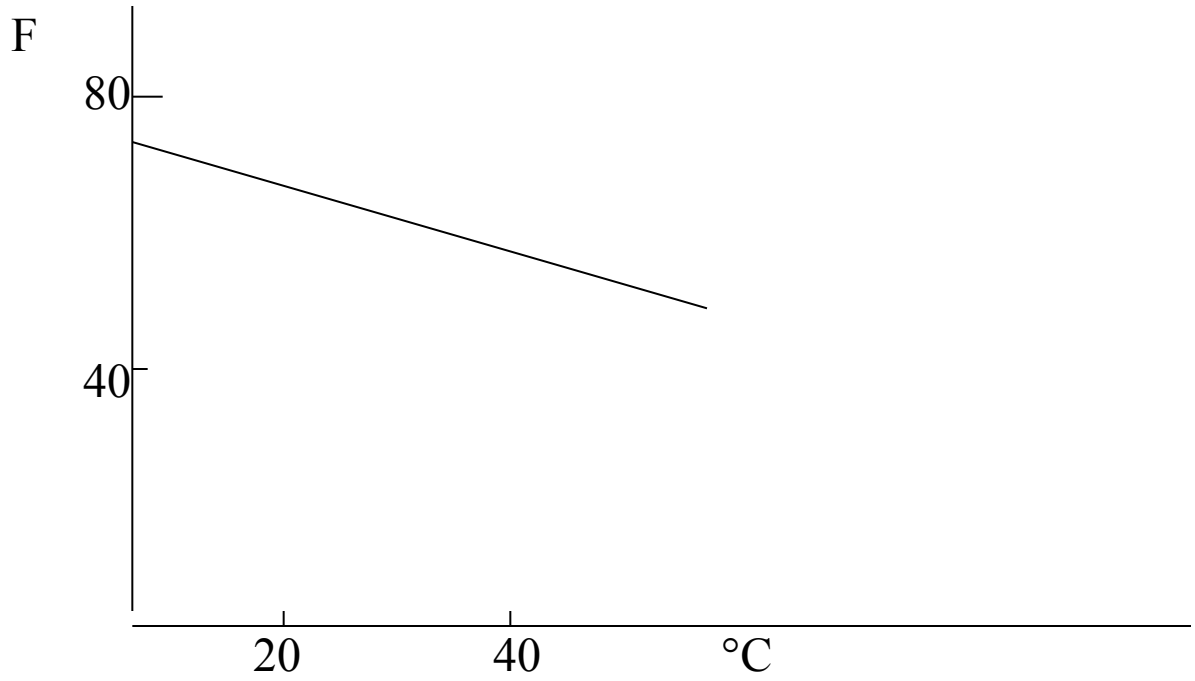
Solvent	Freq. domain (ns)	TCSPC (ns)	Literature (ns)
H_20	0.92	0.97	0.93
Methanol	5.35	5.31	5.64
DMSO	7.15	7.54	7.48
Ethyl acetate	10.93	nd	10.5

Pokles polarity 6 – 1.



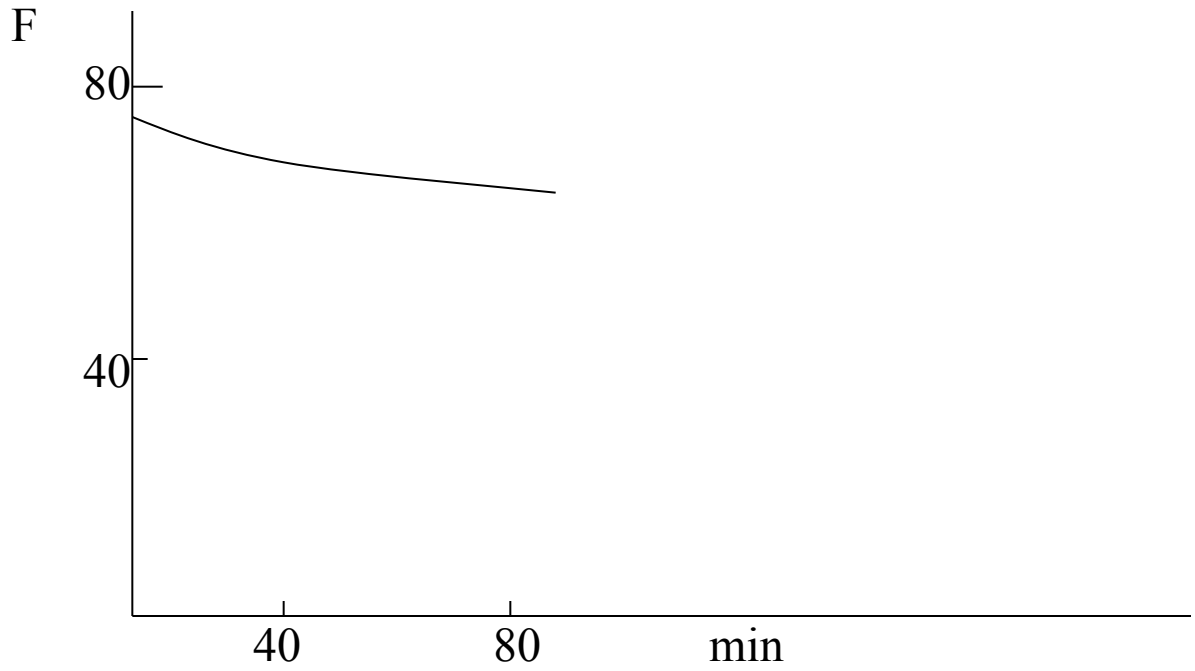
Podmínky fluorescence

Závislost fluorescence na teplotě

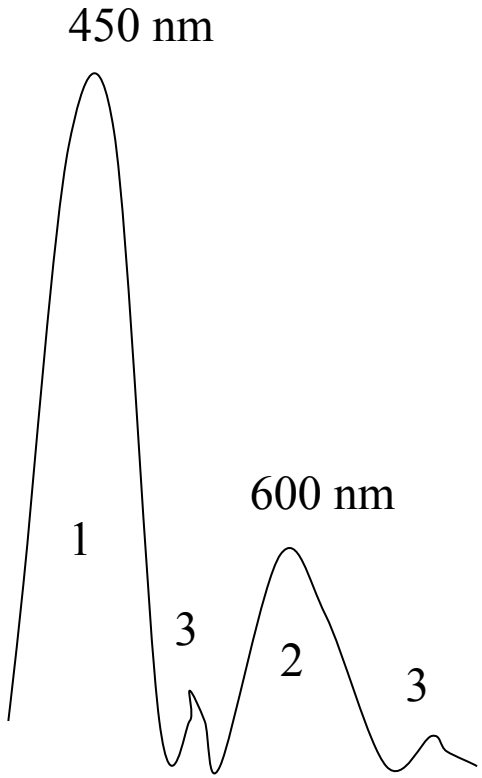


Podmínky fluorescence

Stabilita fluorescenčního signálu chininsulfátu



Podmínky fluorescence



Emisní spektrum

1 Rayleighův rozptyl (Tyndalův rozptyl)
2 Fluorescenční emise
3 Ramanův rozptyl

Excitace 450 nm

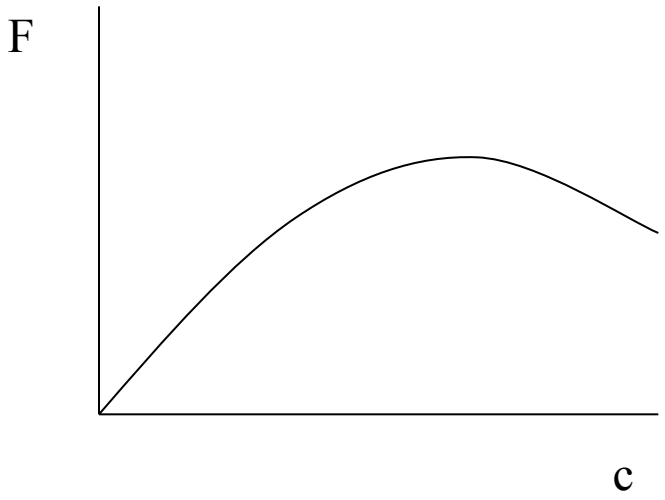
Kvantitativní fluorimetrie

Závislost intenzity fluorescence na koncentraci látky

$F = f(I, \varepsilon, c, \Phi)$

$F = Io\ \Phi\ [1 - 10^{-\varepsilon cd}]$ jestliže $c \rightarrow 0$

$F = Io\ \Phi.2,3.\varepsilon d.c$



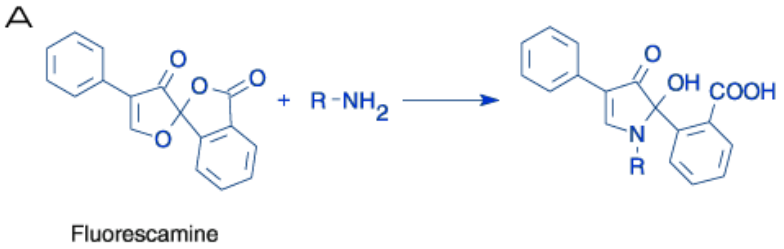
Kvantitativní fluorimetrie

Stanovení koncentrace aminokyselin

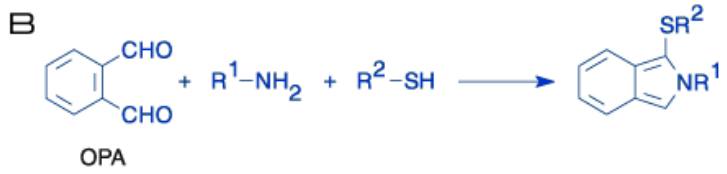
390/464 nm

340/455 nm

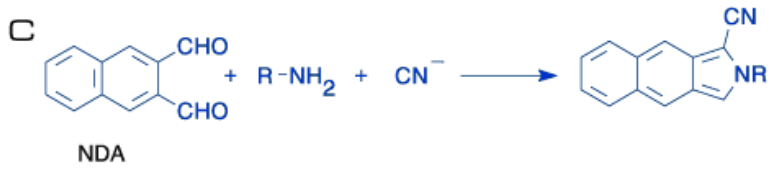
450/550 nm



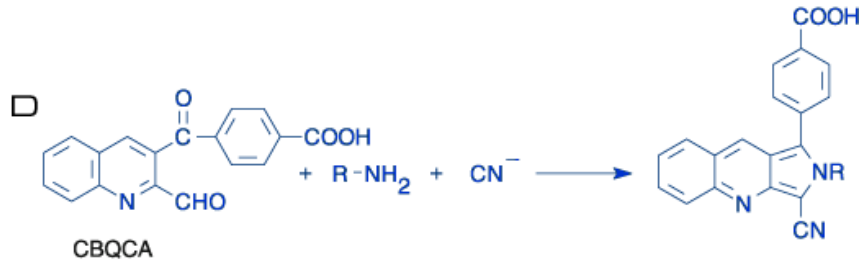
Fluorescamine



OPA



NDA

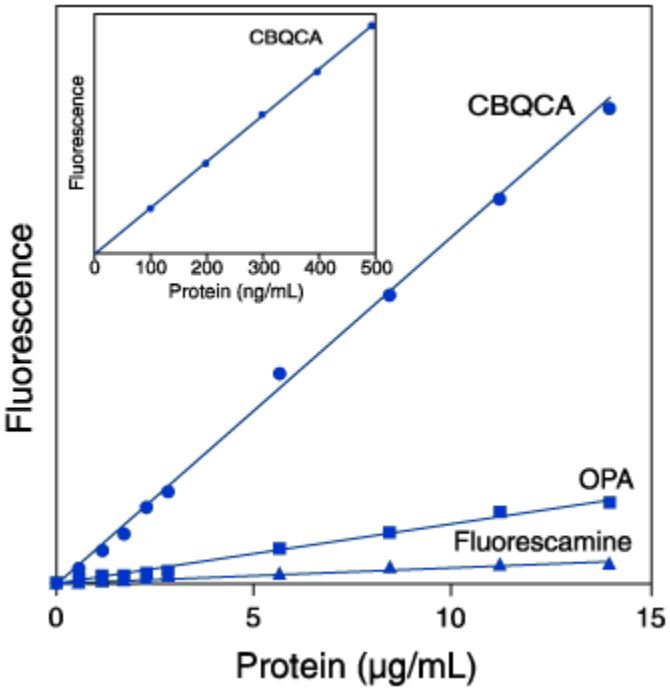


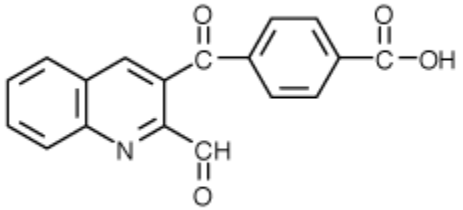
CBQCA

Kvantitativní fluorimetrie

Stanovení bílkovin

CBCQA





Kvantitativní fluorimetrie

Detekce bílkovin v gelu

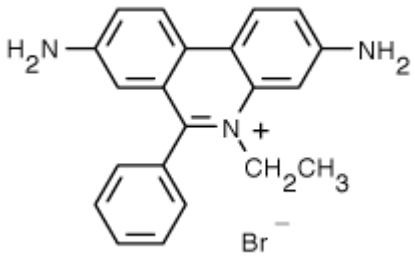
(barevně: Coomasie blue, stříbrné barvení)

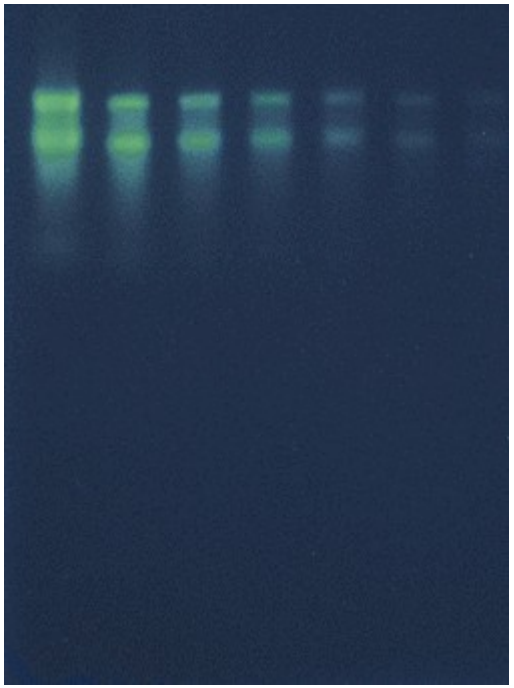
Fluorimetricky: SYPRO Orange (Molecular probes) – citlivost 1 – 2 ng

Kvantitativní fluorimetrie

Detekce nukleových kyselin

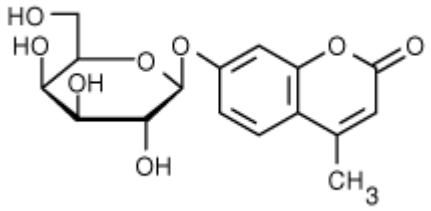
Ethidium bromid





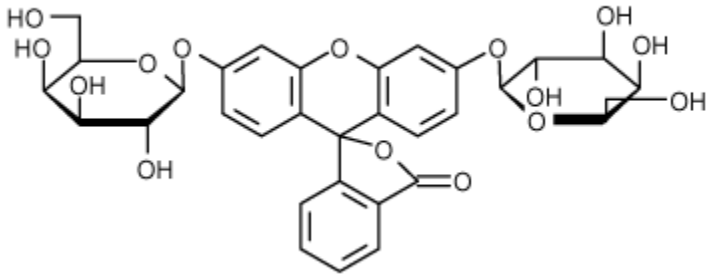
rRNA 16 a 23s barvená SYBR Green II
Molecular Probes

Fluorogenní substráty



Galaktosidasy

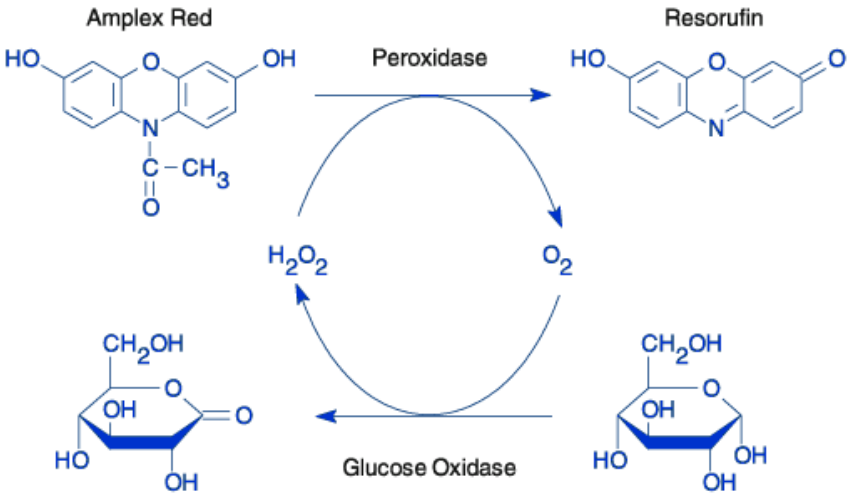
4-methylumbelliferyl-α-galaktosid

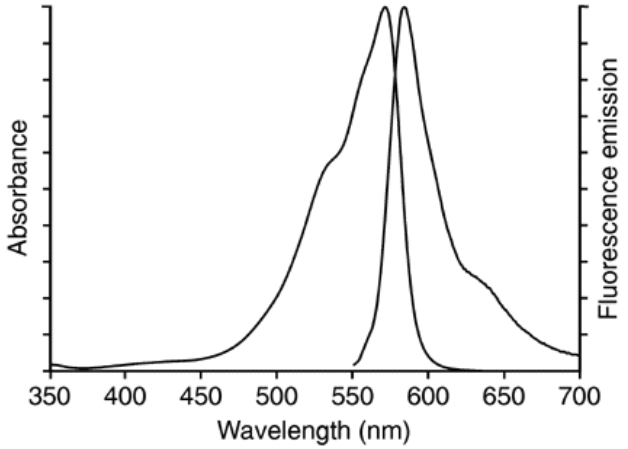


Fluorescein-digalaktosid

Fluorogenní substráty

Peroxidasy – amplex red, vznik resorufinu

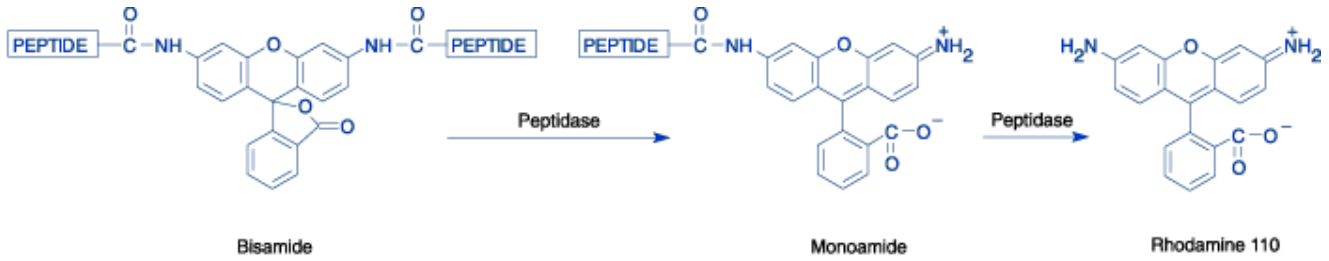


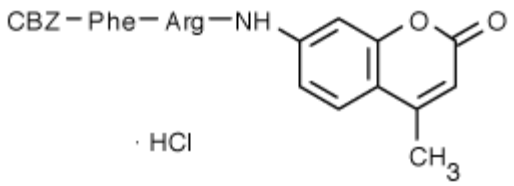


Fluorogenní substráty

Proteinasy, peptidasy

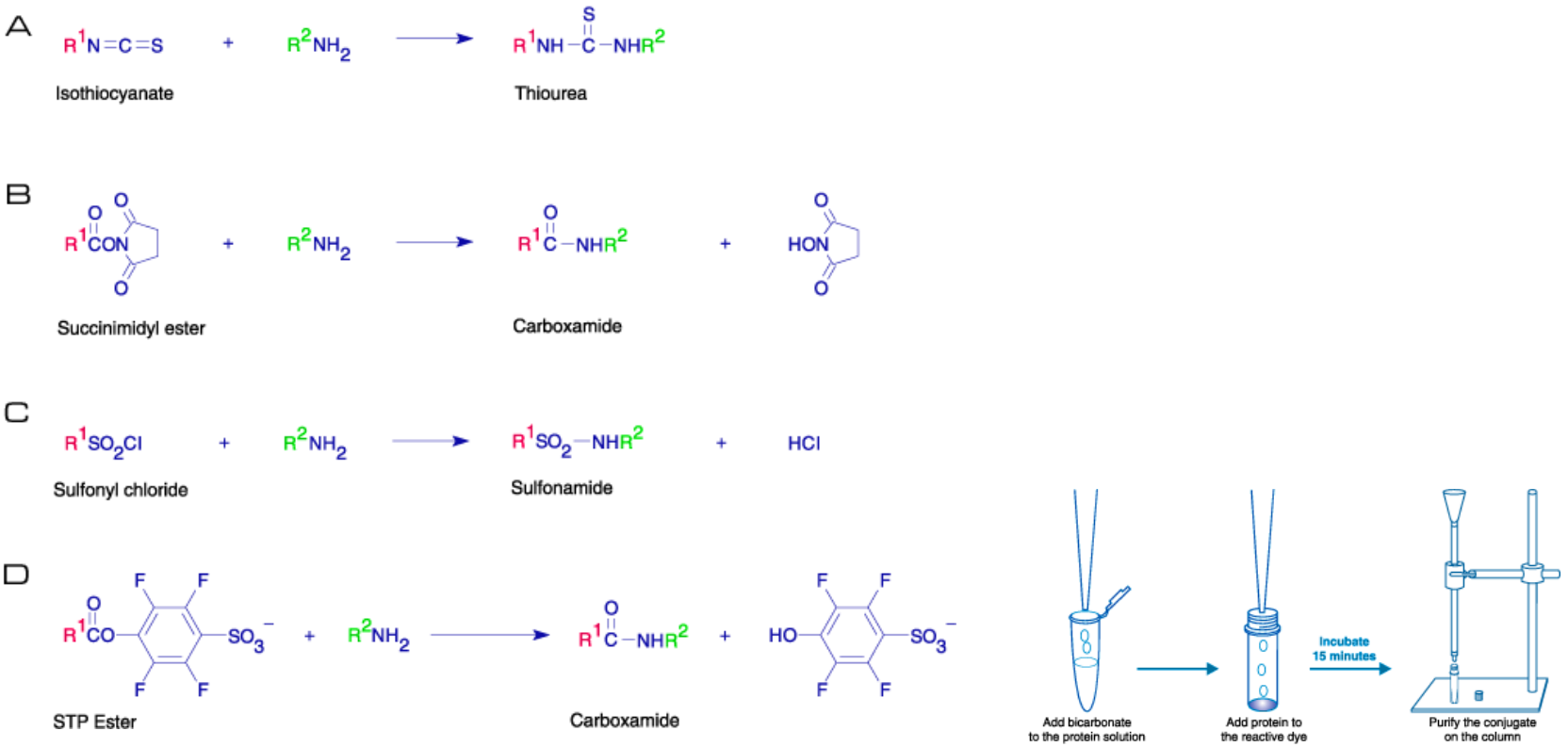
1) Fluorescenční konjugáty proteinů a peptidů





Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

Fluorescenční konjugáty

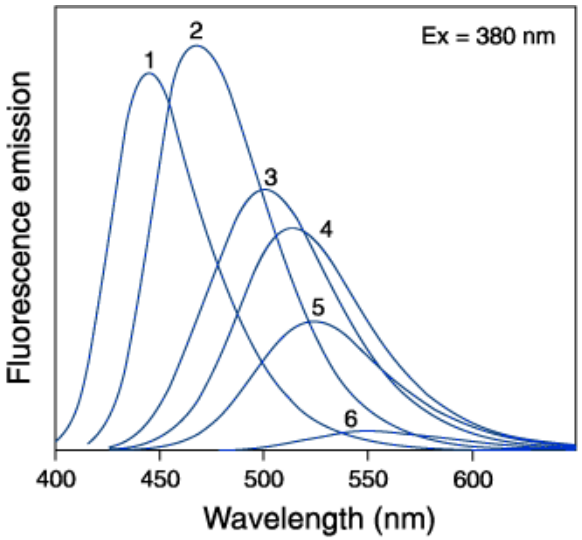


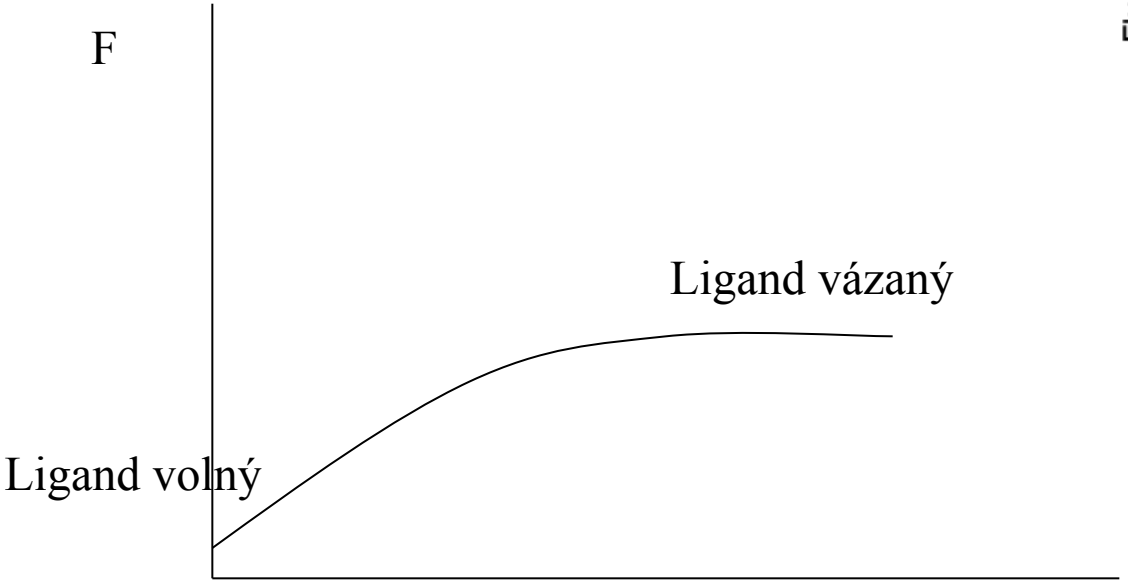
Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

$$L + M \leftrightarrow LM$$

$$Kd = L_f.M_f/LM$$

Interakce makromolekul s ligandy





Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

$F = F_f + F_b$

$F = Cf \,.\Phi_f + C_b.\ \Phi_b$

$F = (C\text{-}C_b)\ \Phi_f + C_b.\ \Phi_b$

$F = C\Phi_f - Cb\Phi_f + C_b.\ \Phi_b$

$F = Fo + C_b\ (\Phi_b - \Phi_f)$

$Cb = (F - Fo)/\ (\Phi_b - \Phi_f)$

Ff, Fb – fluorescence volné, vázané frakce

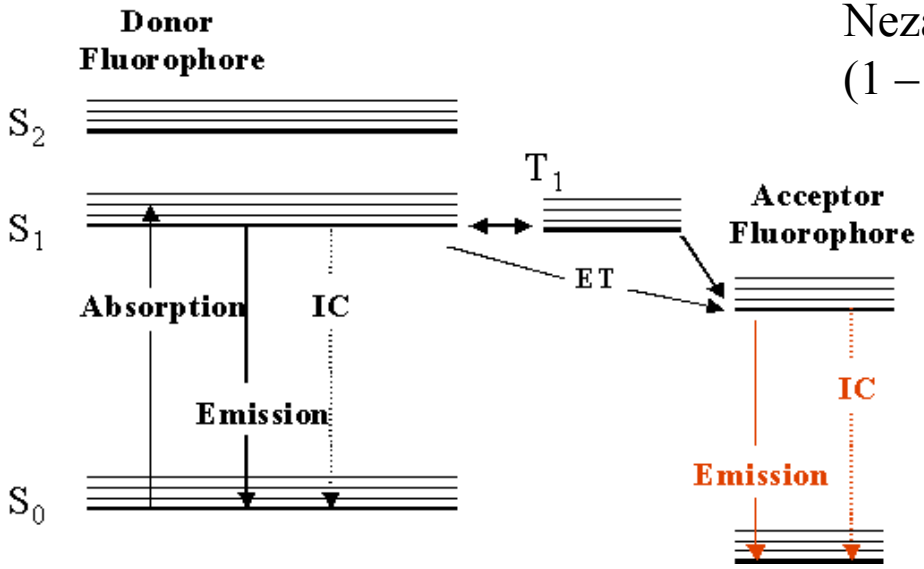
Φb, Φf –kvant. Výtěžek fluorescence vázaného, volného ligandu

Cb,Cf – koncentrace vázaného, volného Ligandu

C – celková koncentrace ligandu

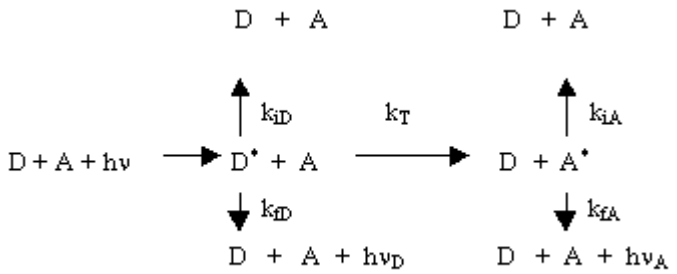
F – celková fluorescence

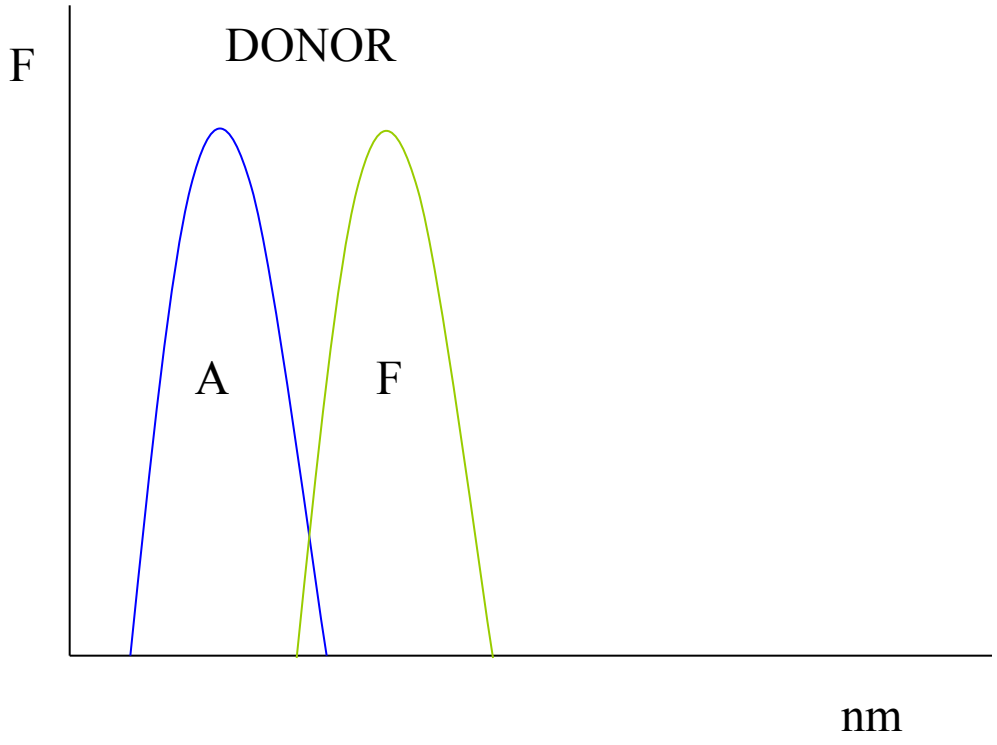
Fluorescenční rezonanční transfer energie (Försterův přenos)



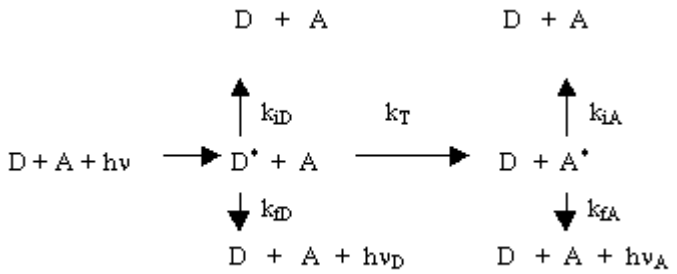
Nezářivý přenos energie z donoru na akceptor (1 – 10 nm)

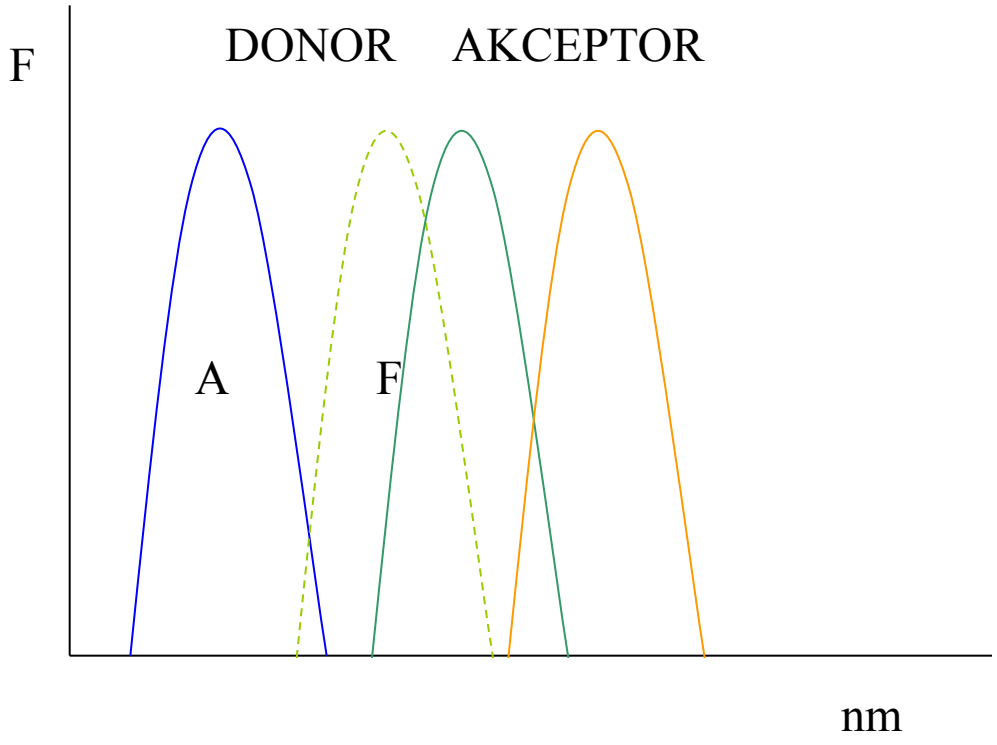
Fluorescenční rezonanční transfer energie



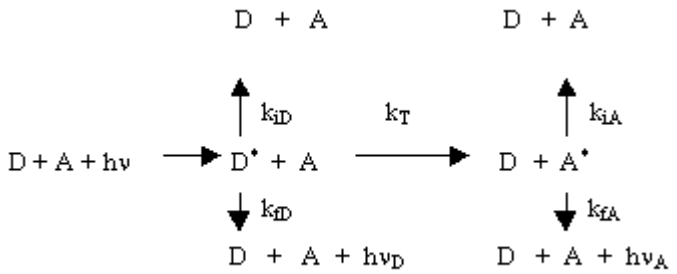


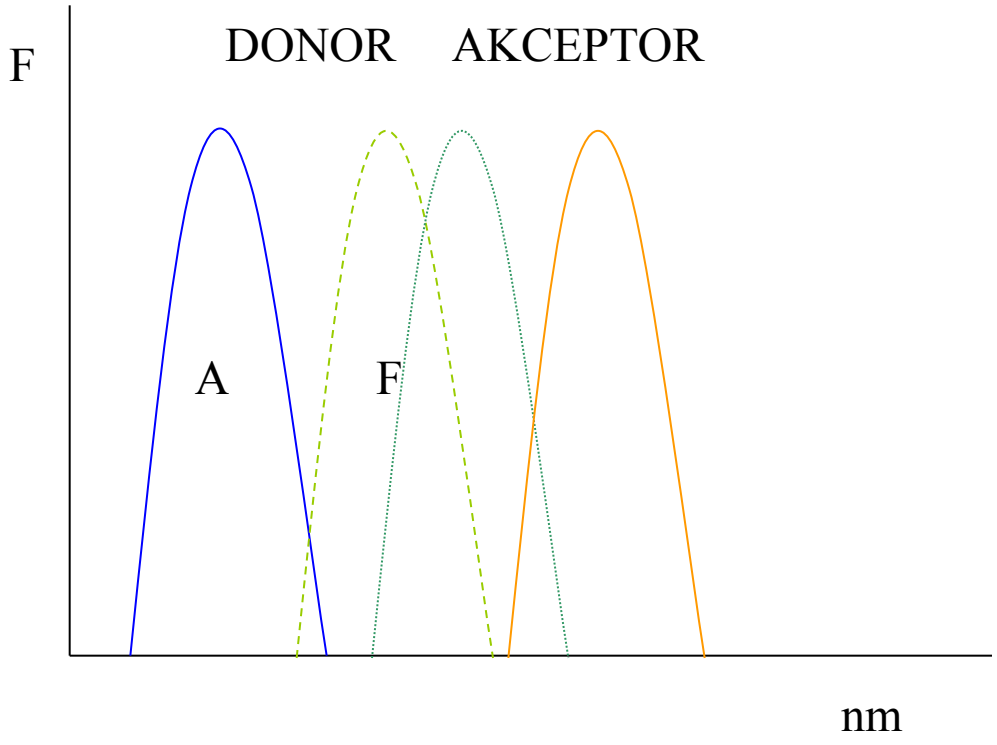
Fluorescenční rezonanční transfer energie



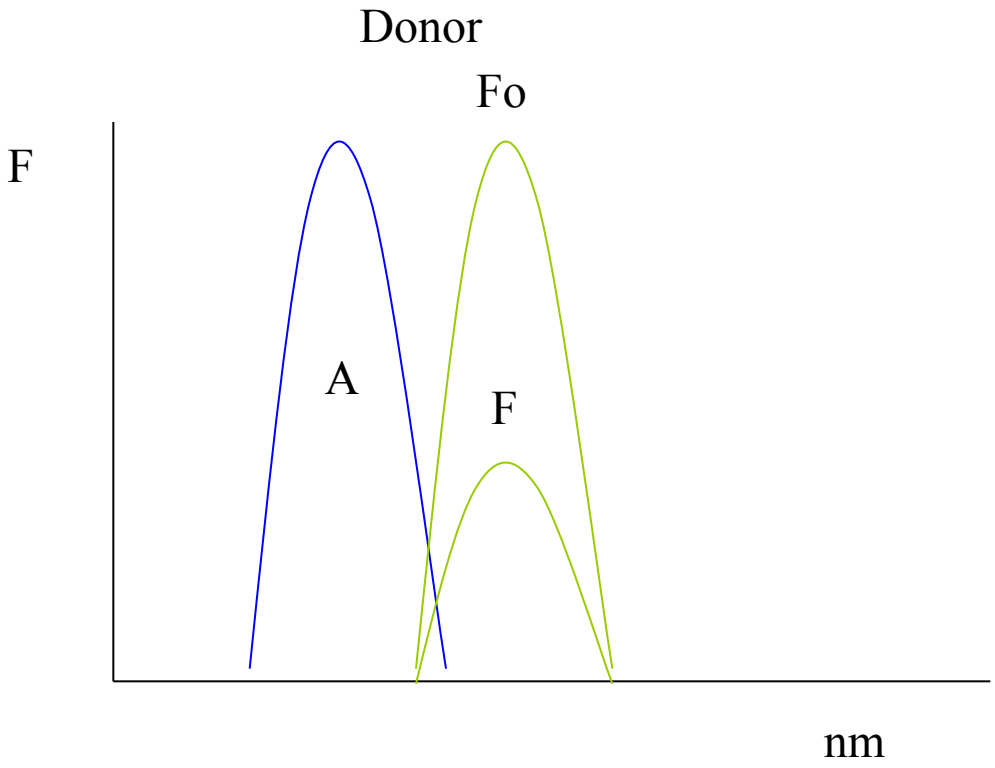


Fluorescenční rezonanční transfer energie





Fluorescenční rezonanční transfer energie



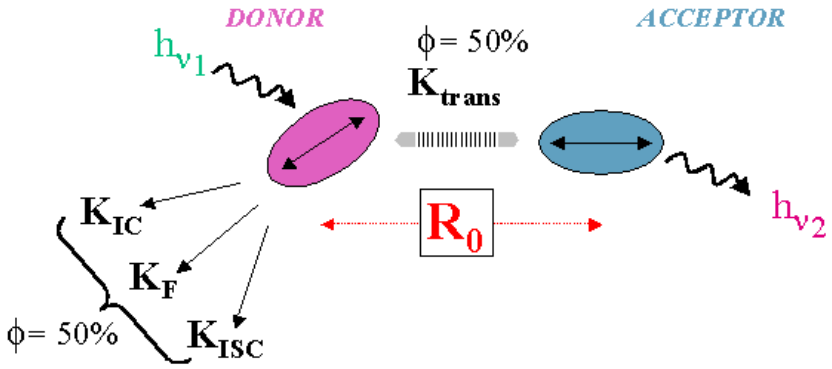
$\eta = 1 - F/Fo$

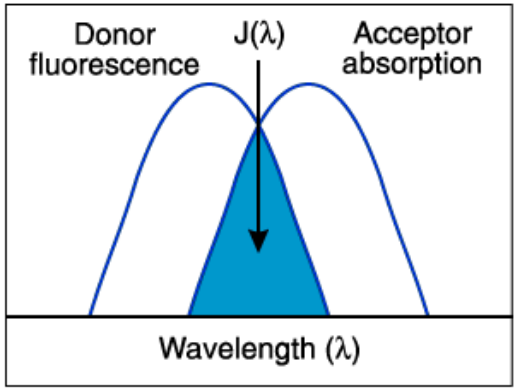
Fluorescenční rezonanční transfer energie

$$\eta = Ro^6/(Ro^6 + R^6)$$

$$Ro^6 = \sqrt{1{,}66.10^{-33}.\tau.J/n^2\nu o^2}$$

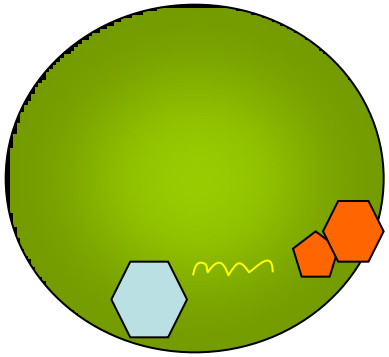
τ – doba života exc. stavu
J – překryvový integrál
n – refraktivní index rozpuštědla
ν – vlnočet emise donoru

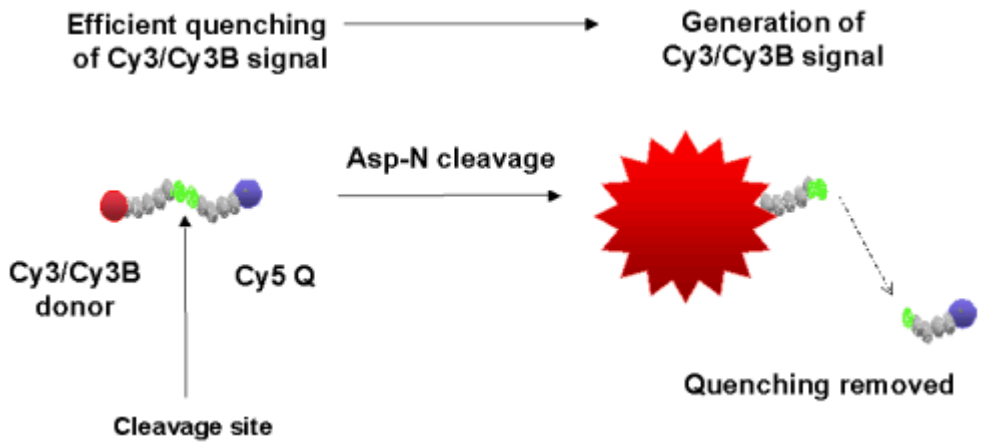




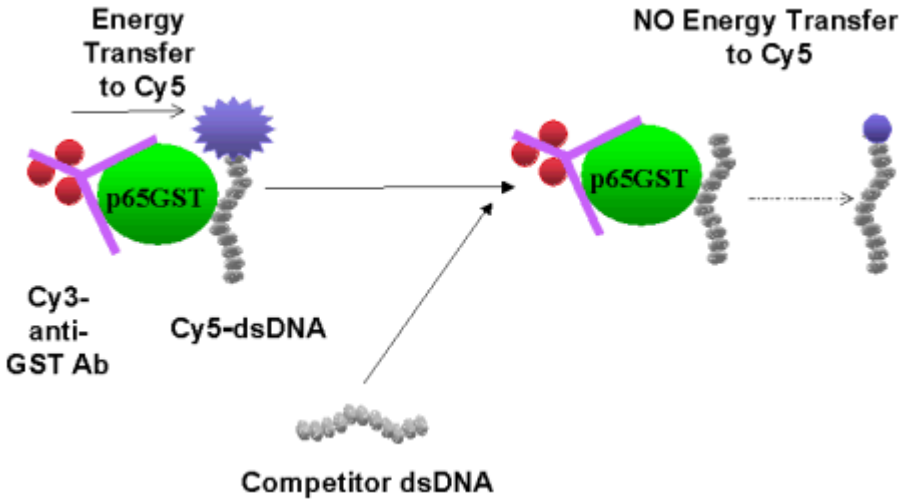
Fluorescenční rezonanční transfer energie

Použití – změření vzdálenosti mezi dvěma molekulami v bílkovině
Tryptofan (290/340) vs. NADH (340/450 nm)

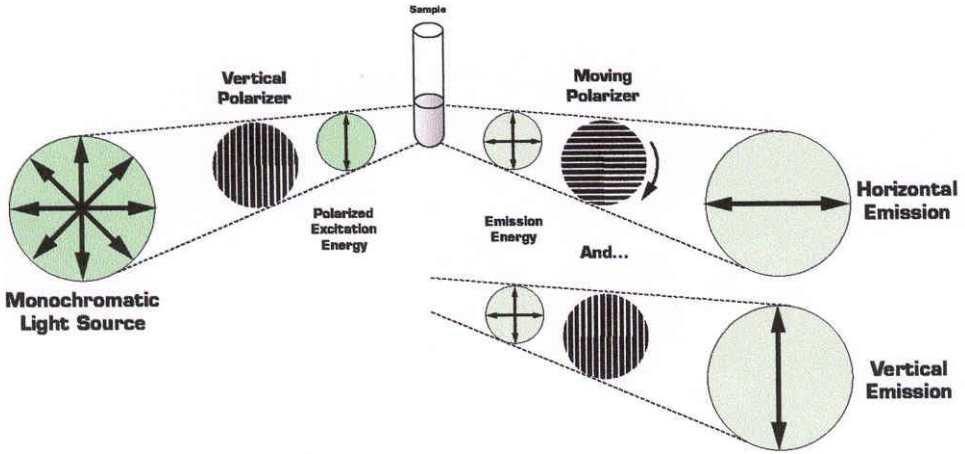




Fluorescenční rezonanční transfer energie



Fluorescenční anizotropie

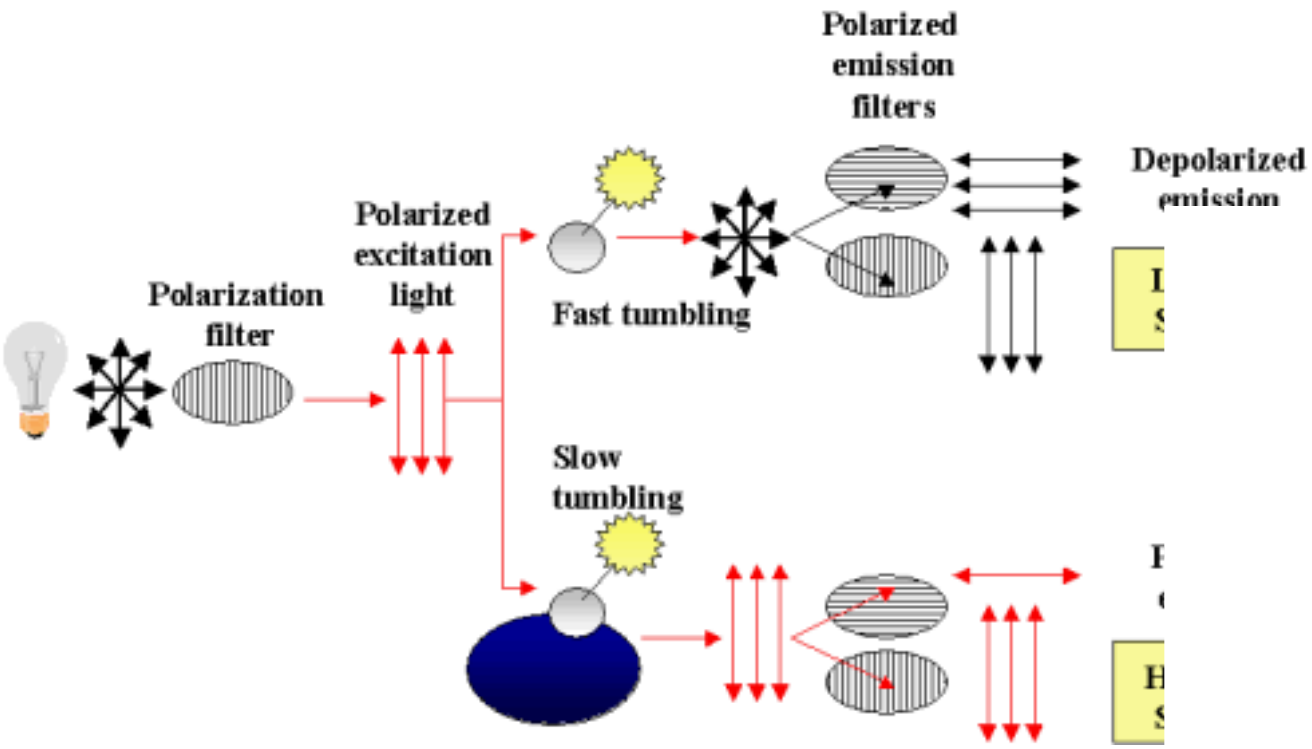


Fluorescenční anizotropie

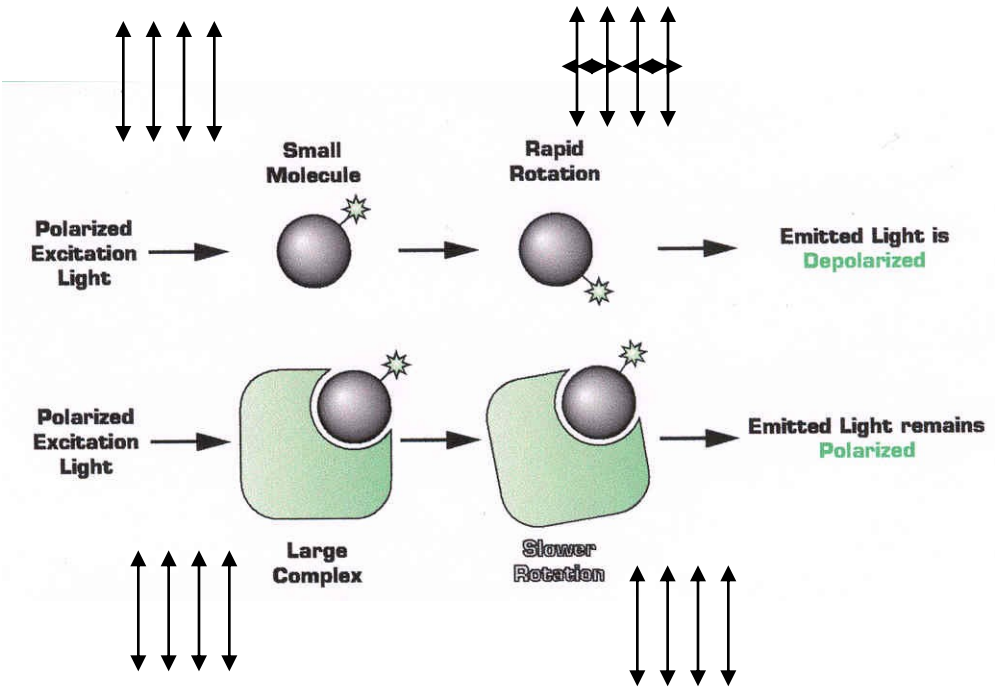
Polarizační filtry

Auto-Polorizer Accessory

Fluorescenční anizotropie



Fluorescenční anizotropie

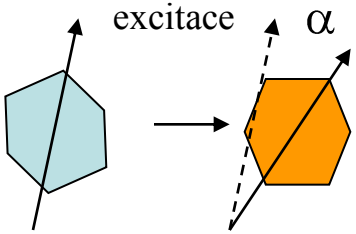


Fluorescenční anizotropie

Fluorescenční anizotropie $\mathbf{r = \frac{I_v - I_h}{I}}$

$I = I_v + 2I_h$

Rotační relaxační čas



$r_o = (3\cos^2\alpha - 1)/5$

$\mathbf{ro/r = 1 + 3\tau/\rho}$

τ, střední doba života fluorescence
ρ, rotační relaxační čas molekuly
ro – anizotropie nepohyblivé molekuly

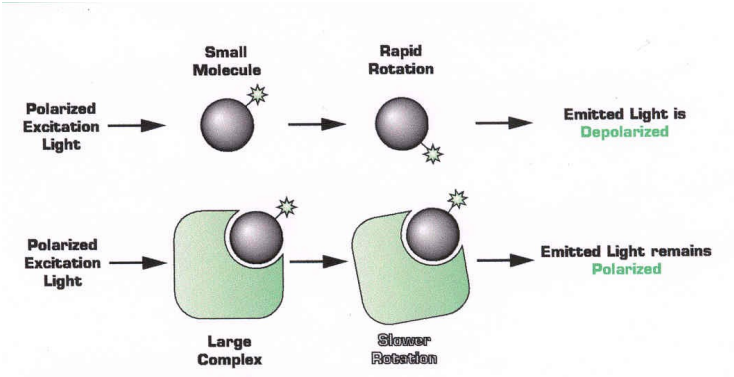
$\rho = V\eta/RT$

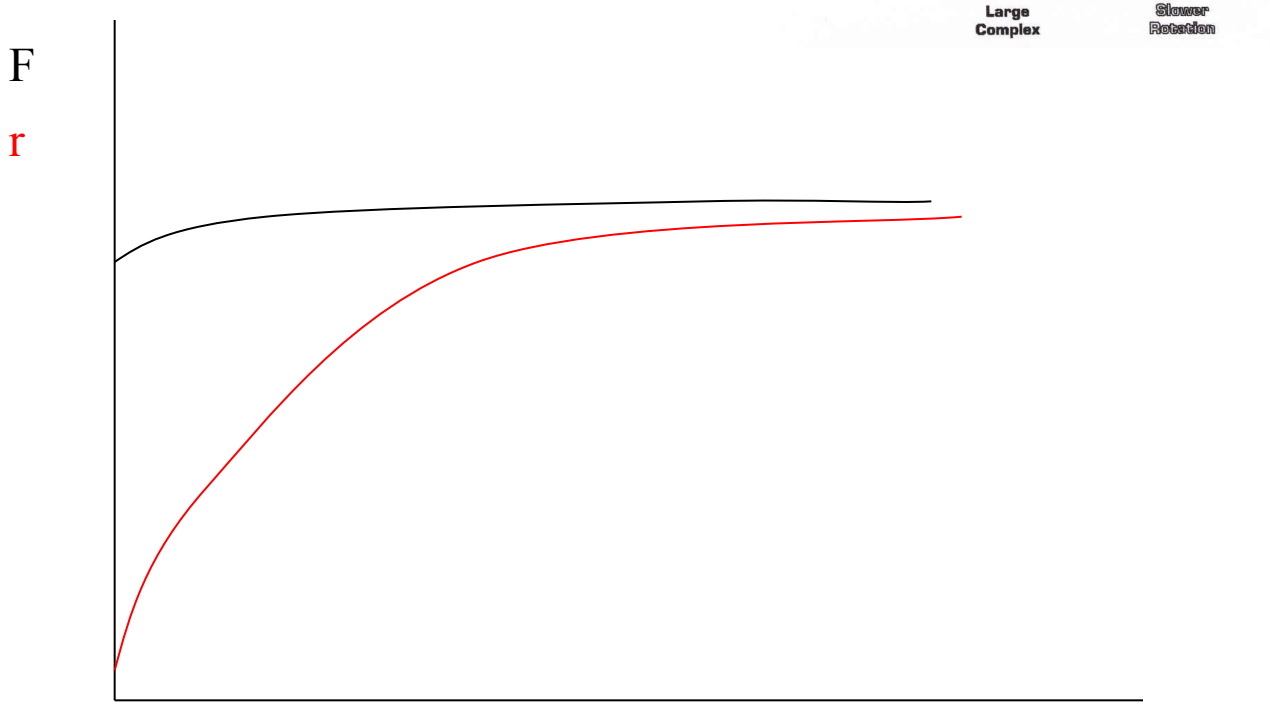
V objem
η viskozita

$\mathbf{ro/r = 1 + 3\tau RT/V\eta}$

Fluorescenční anizotropie

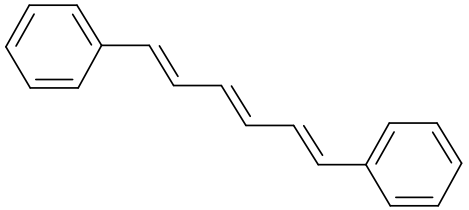
Využití:
Interakce makromolekuly s ligandem





Fluorescenční anizotropie

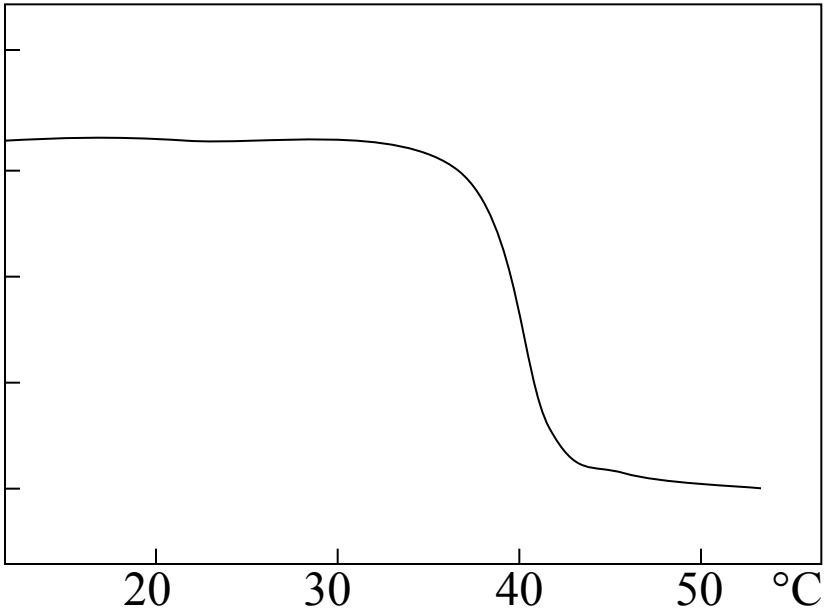
Využití:
Měření viskozity prostředí



$$ro/r = 1 + 3\tau RT/V\eta$$

$$\eta = 2{,}4r/(0{,}362 - r)$$

$$ro/r = 1 + K/\eta$$



Fl. anizotropie DPHT
Vázaného v liposomech
DPPC

Chemiluminiscence

Luminol