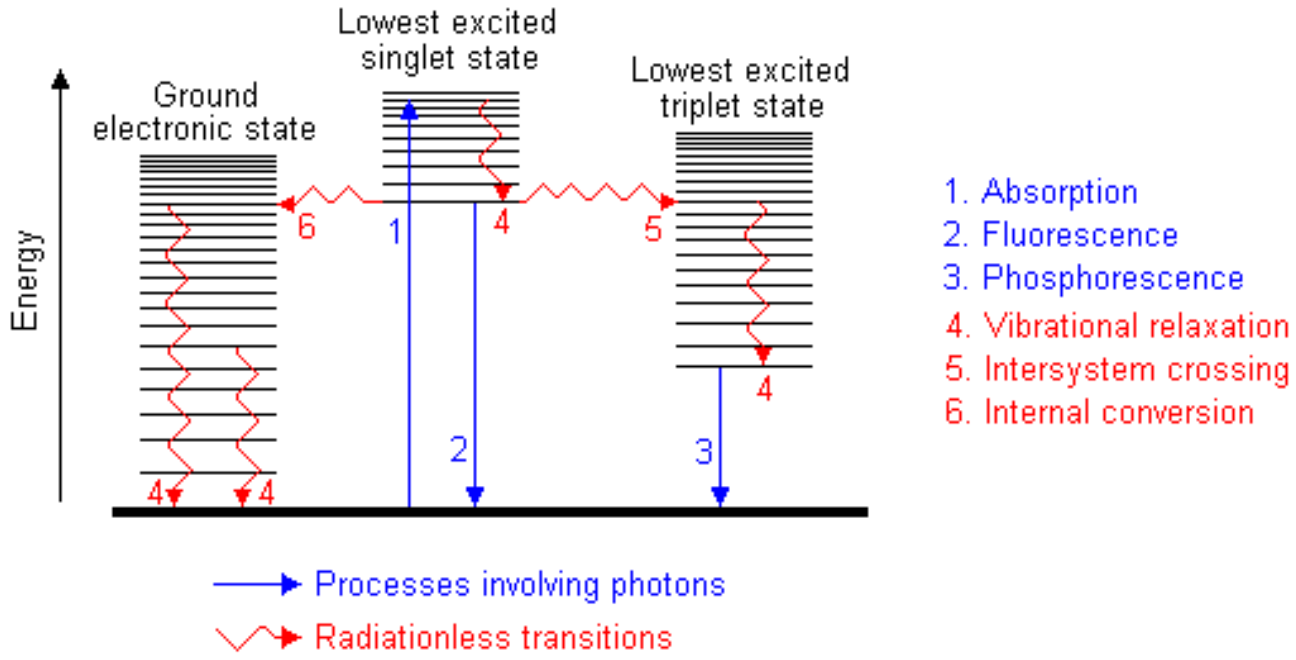
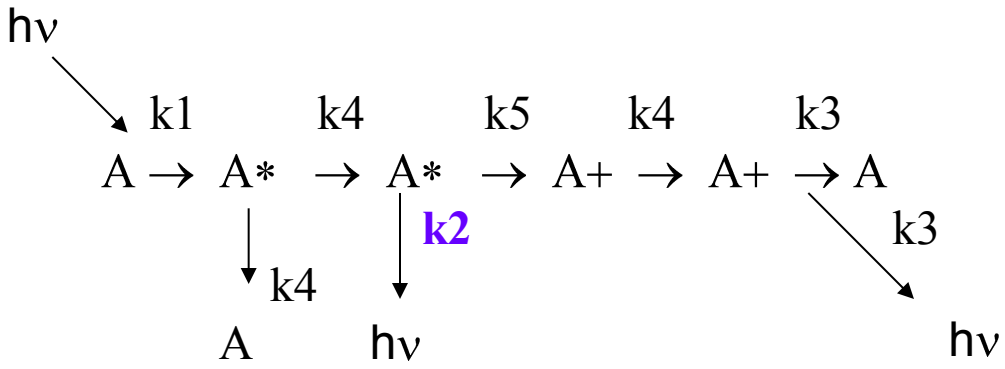
Luminiscenční spektroskopie

- Fotoluminiscence
  - Fluorescence
  - Fosforescence
- Chemiluminiscence
  - Bioluminiscence
- Elektro-, termo-, radio- aj.

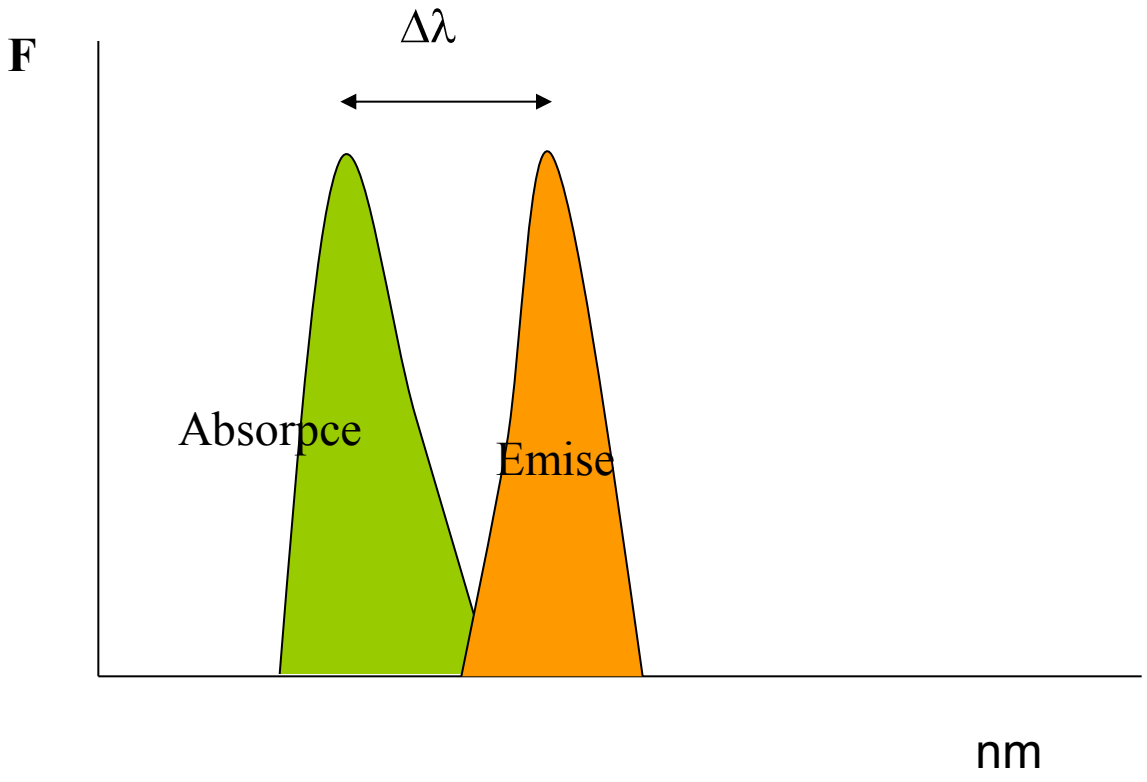
Luminiscenční spektroskopie



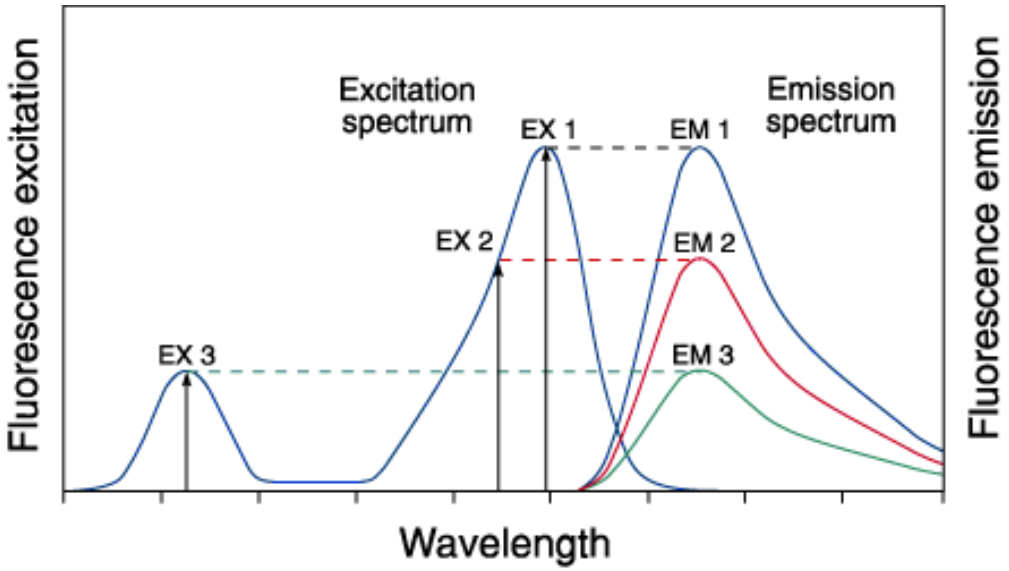


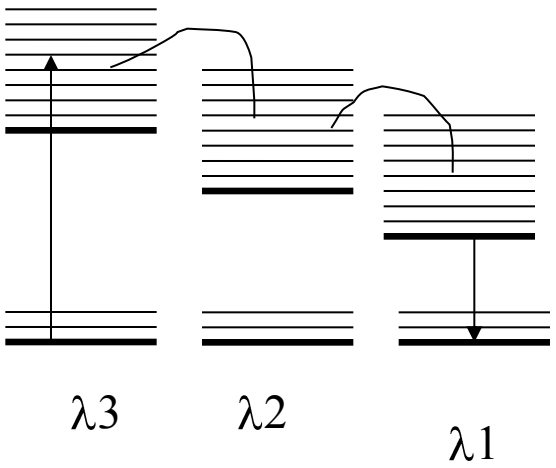
Základní pojmy

Stockesův posun – ztráty energie po dobu excitovaného stavu



Základní pojmy

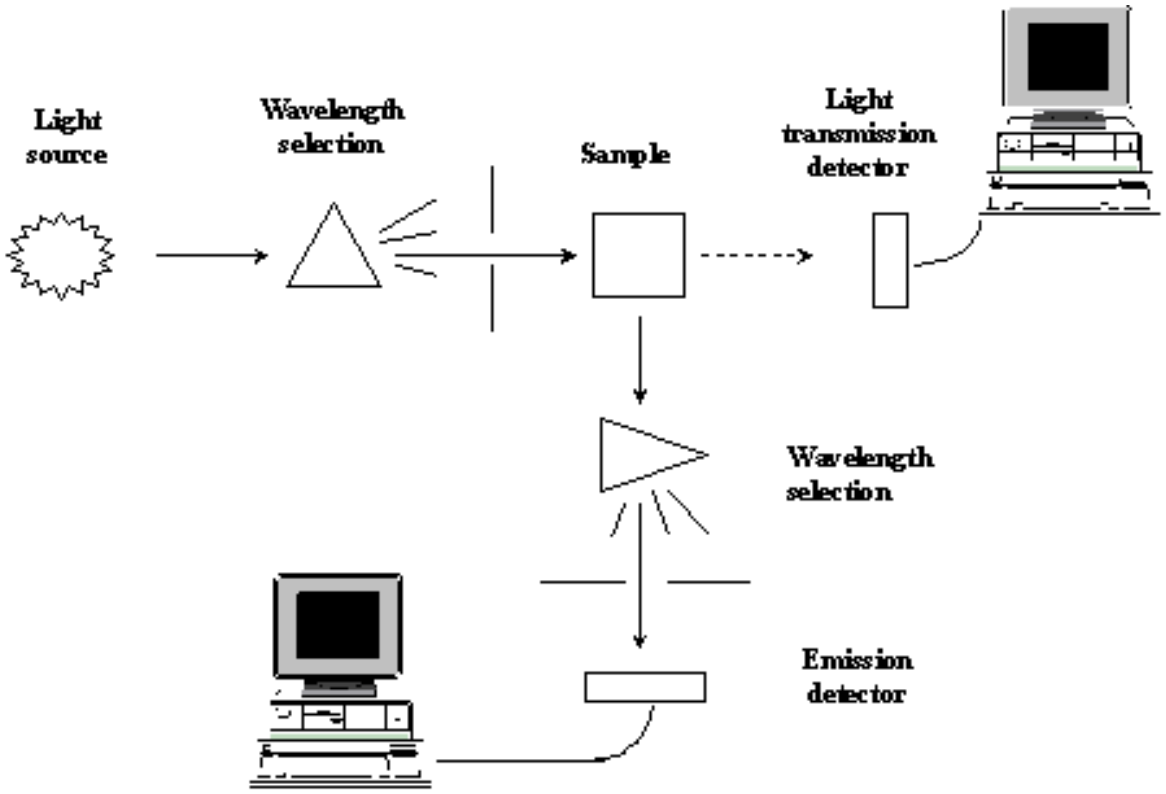




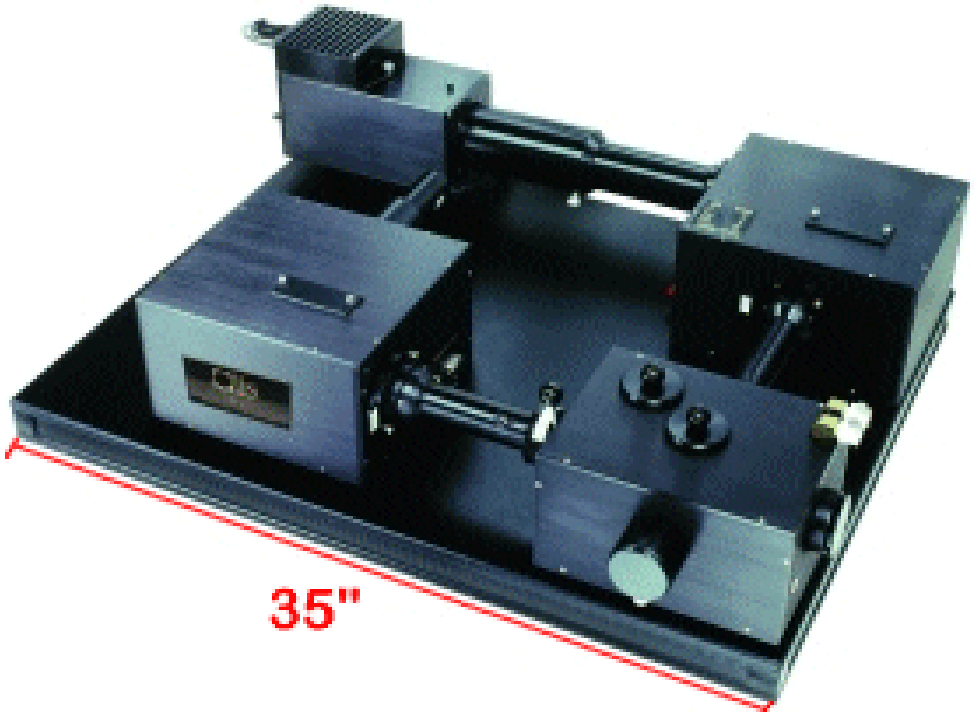
Biochemicky významné fluorofory

	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

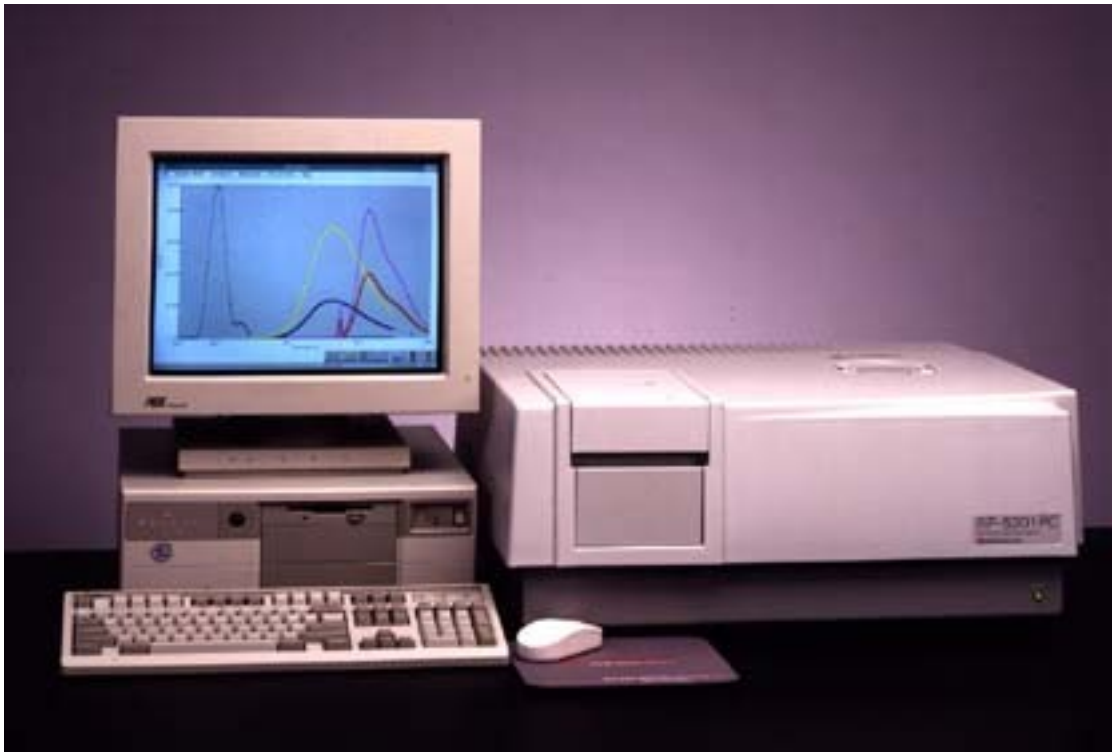


Instrumentace



Instrumentace

RF-5301PC – spektrofluorimetr Shimadzu



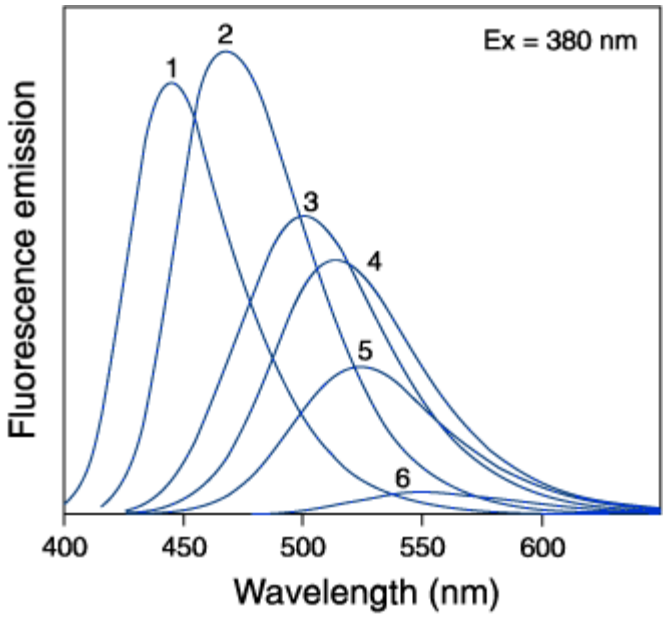
Podmínky fluorescence

Závislost na polaritě

Nitrobenzoxadiazol

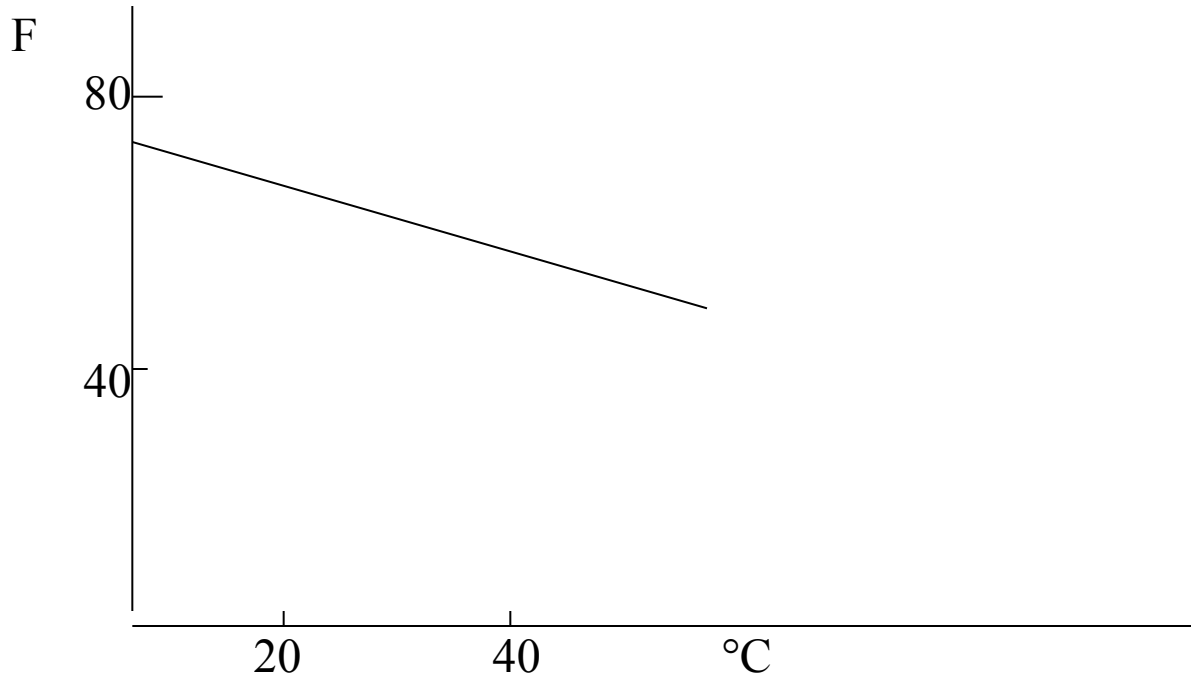
Solvent	Freq. domain (ns)	TCSPC (ns)	Literature (ns)
H_20	0.92	0.97	0.93
Methanol	5.35	5.31	5.64
DMSO	7.15	7.54	7.48
Ethyl acetate	10.93	nd	10.5

Pokles polarity 6 – 1.



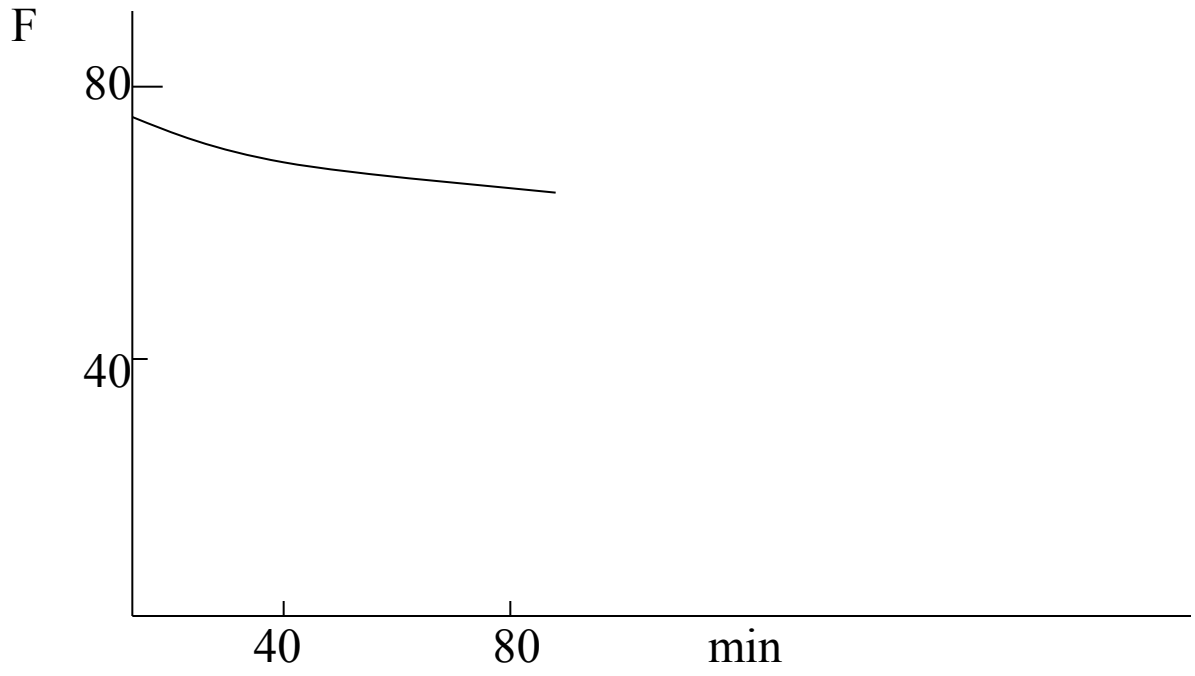
Podmínky fluorescence

Závislost fluorescence na teplotě – též viskozitě

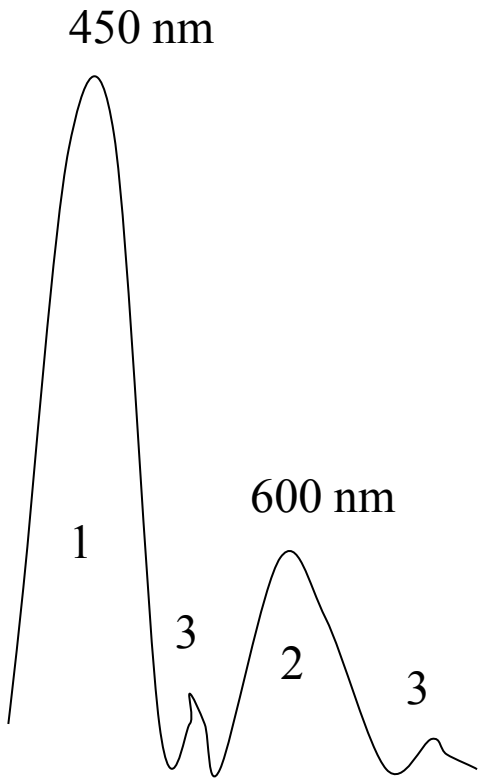


Podmínky fluorescence

Stabilita fluorescenčního signálu chininsulfátu



Podmínky fluorescence



Emisní spektrum

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

Excitace 450 nm

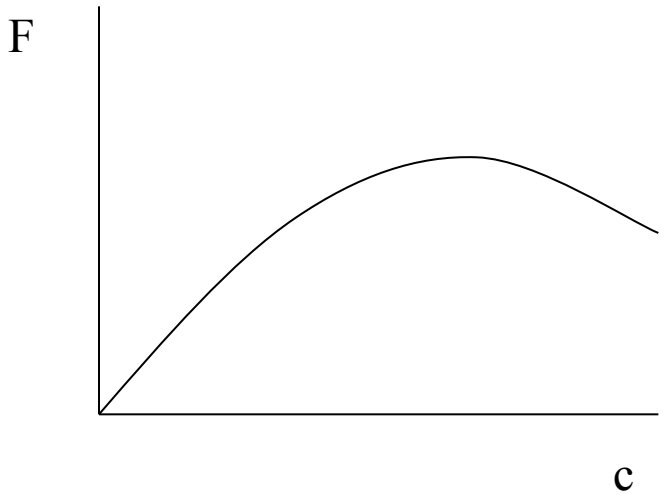
Kvantitativní fluorimetrie

Závislost intenzity fluorescence na koncentraci látky

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

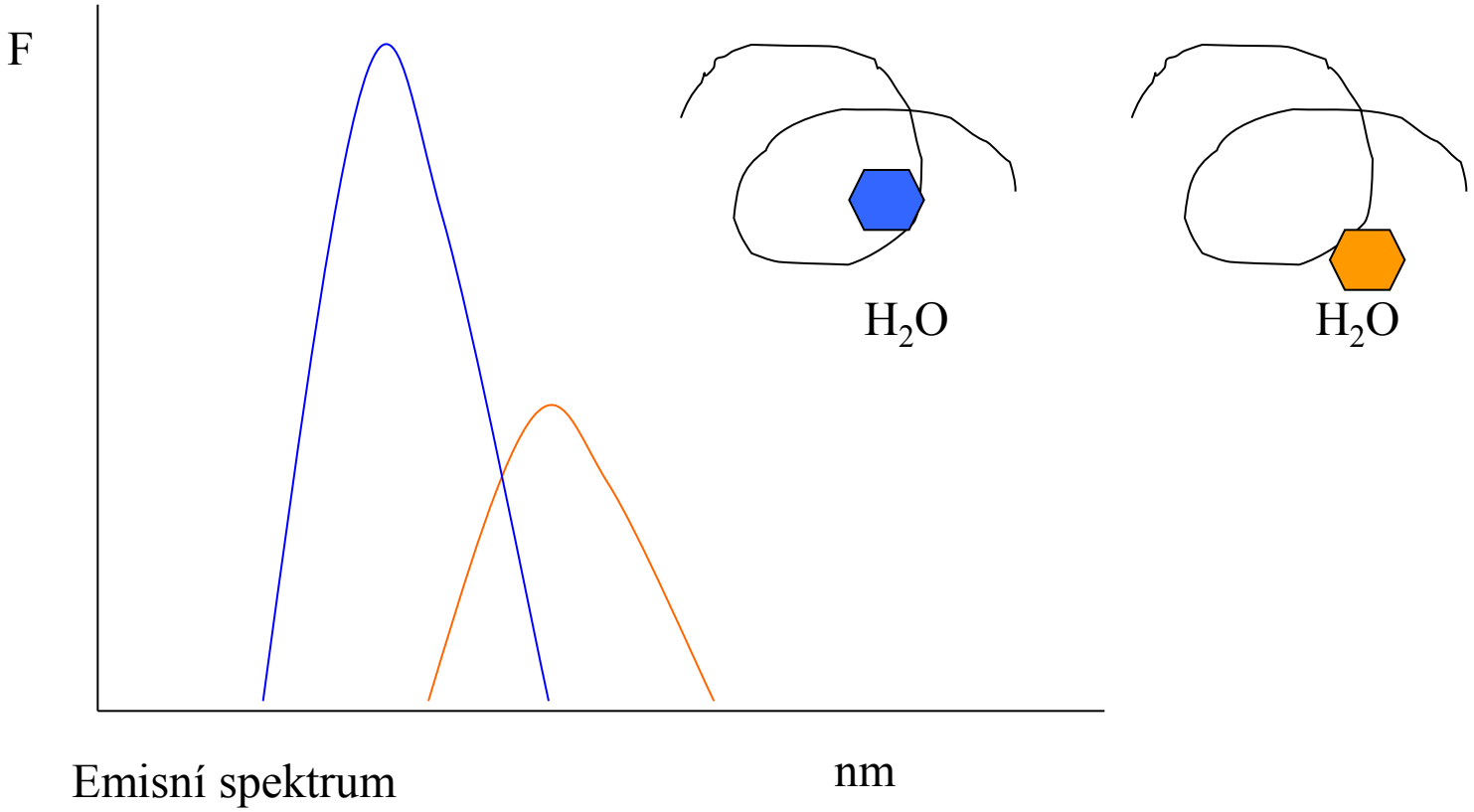
$F = Io\ \Phi\ [1 - 10^{-\varepsilon c d}]$ jestliže $c \rightarrow 0$

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

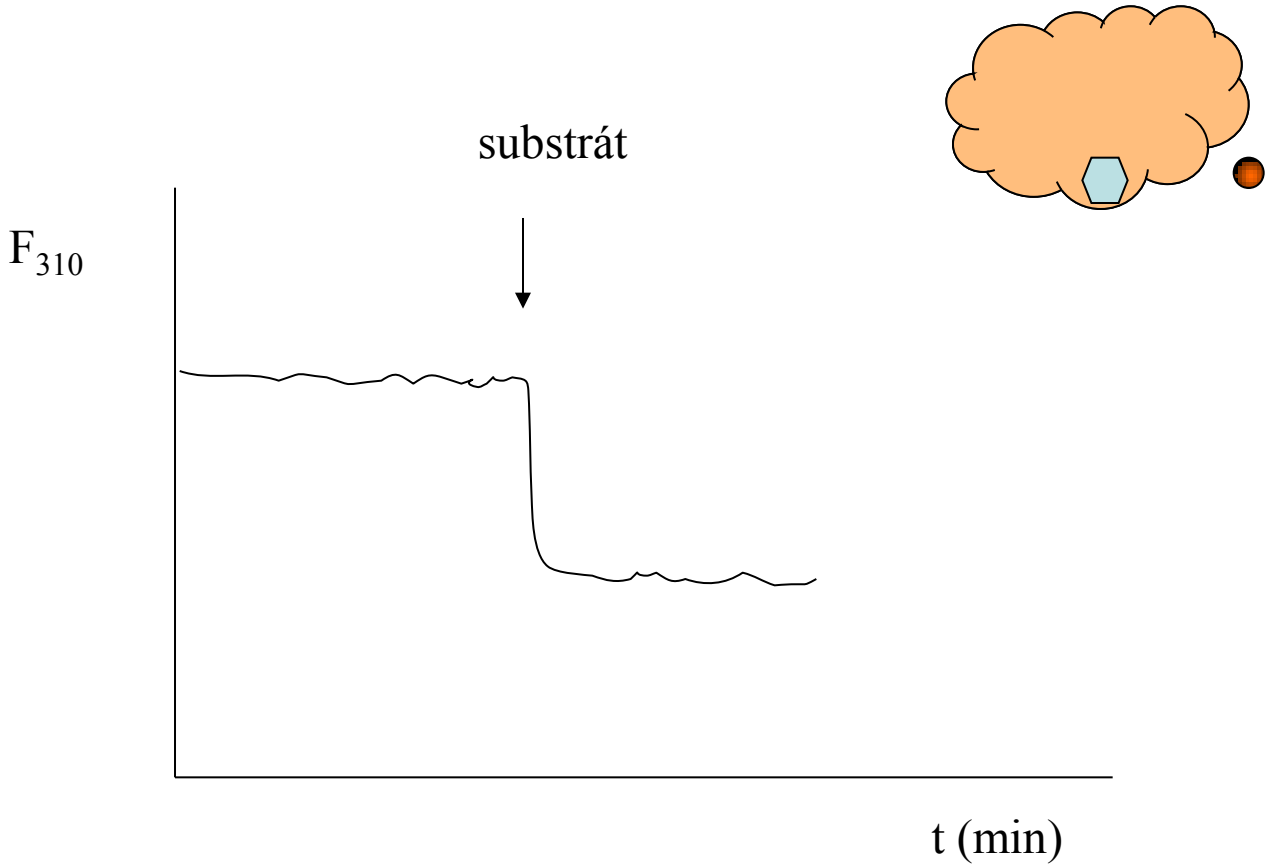


Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

Přirozené fluorofory (Tyr, Try) – fluorescence závislá na polaritě prostředí obklopující fluorofor

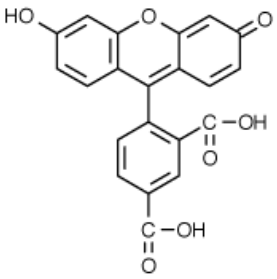


Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

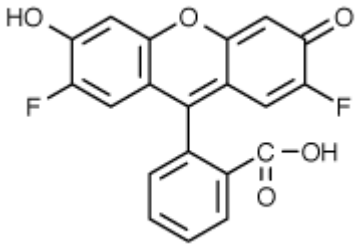


Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

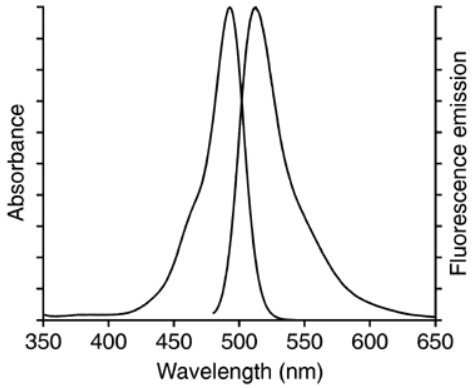
Fluorescenční konjugáty



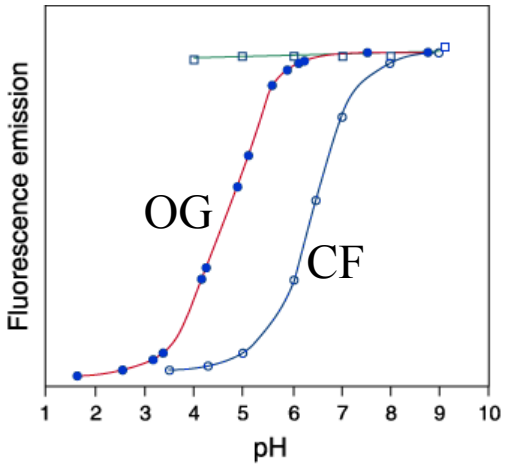
Karboxyfluorescein – (494/520 nm)



Oregon Green - (496/524 nm)



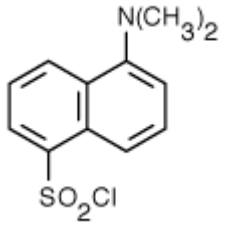
Absorpce/emise fluoresceinu při pH 9



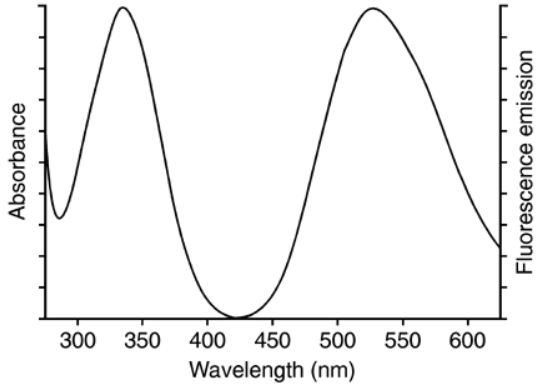
CF-karboxyfluorescein
OG oregon green

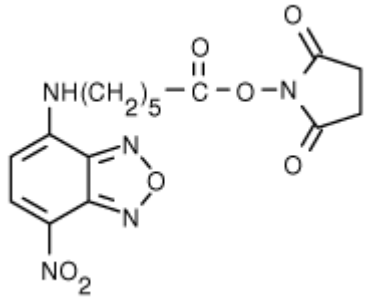
Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

Fluorescenční konjugáty

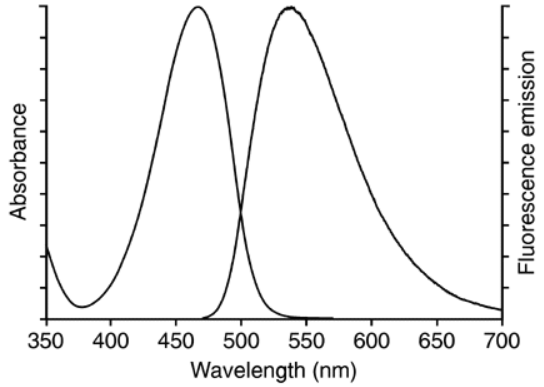


Dansylchlorid



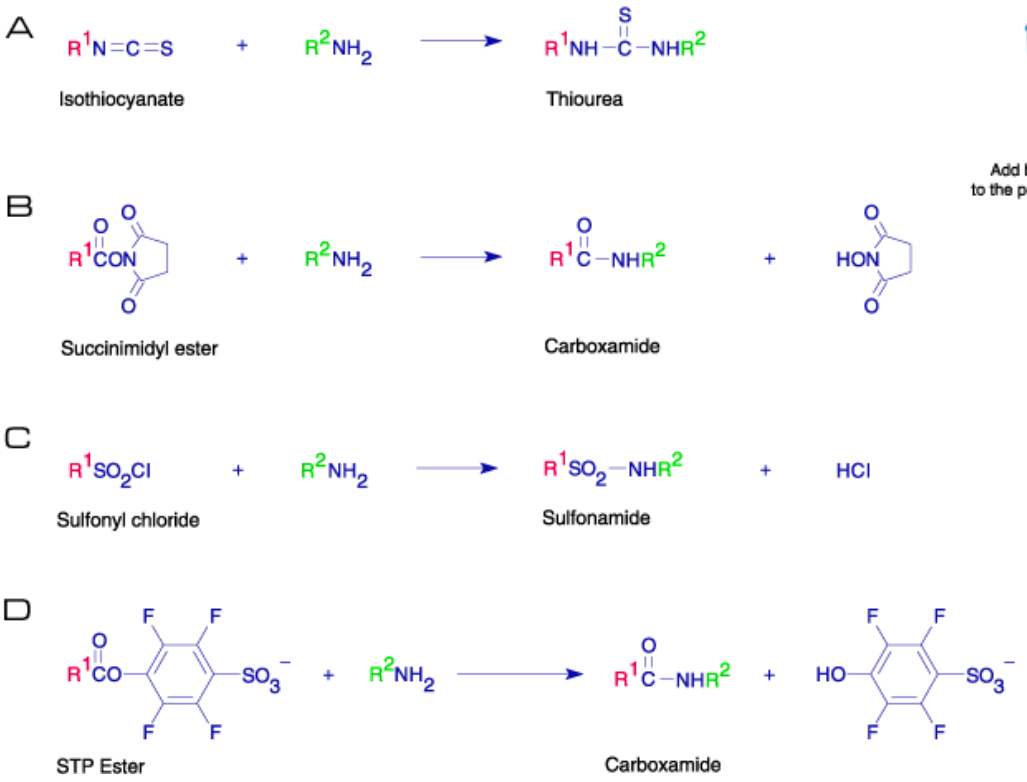


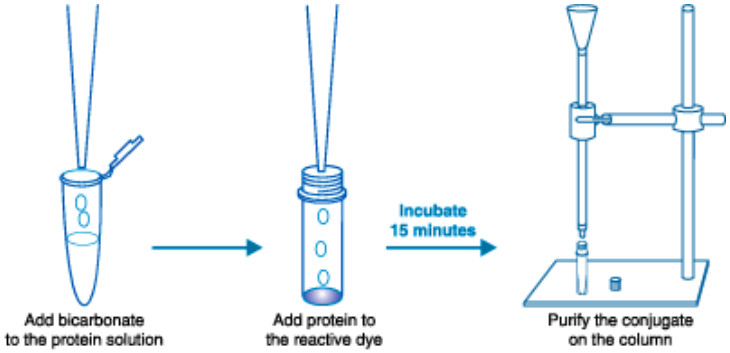
Benzoxadiazol –N-sukcinimid



Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

Fluorescenční konjugáty



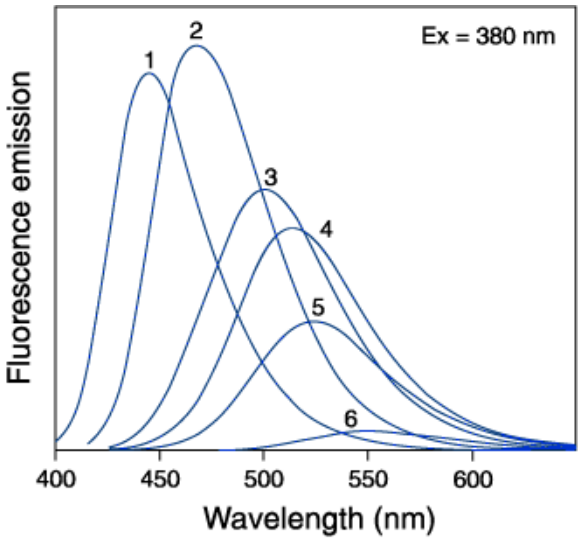


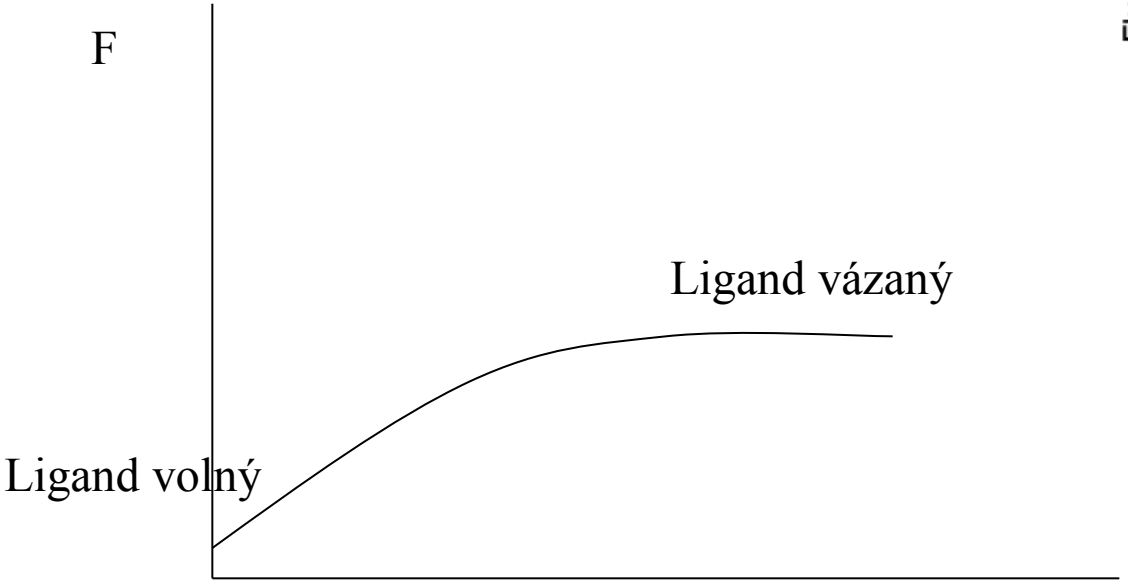
Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

$$L + M \leftrightarrow LM$$

$$K_d = [L]_f.[M_f]/[LM]$$

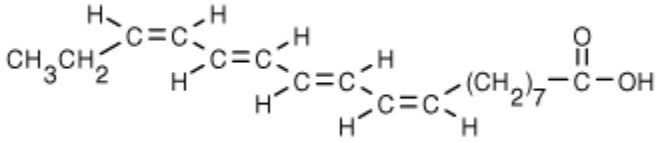
Interakce makromolekul s ligandy



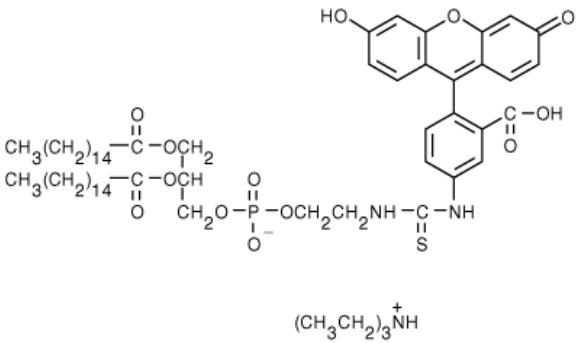


Použití fluorimetrie ke sledování struktury biopolymerů a interakcí

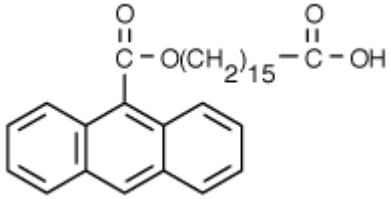
Interakce makromolekul s ligandy
Použití fluorescenčních analogů



Kys. cis-parinarová

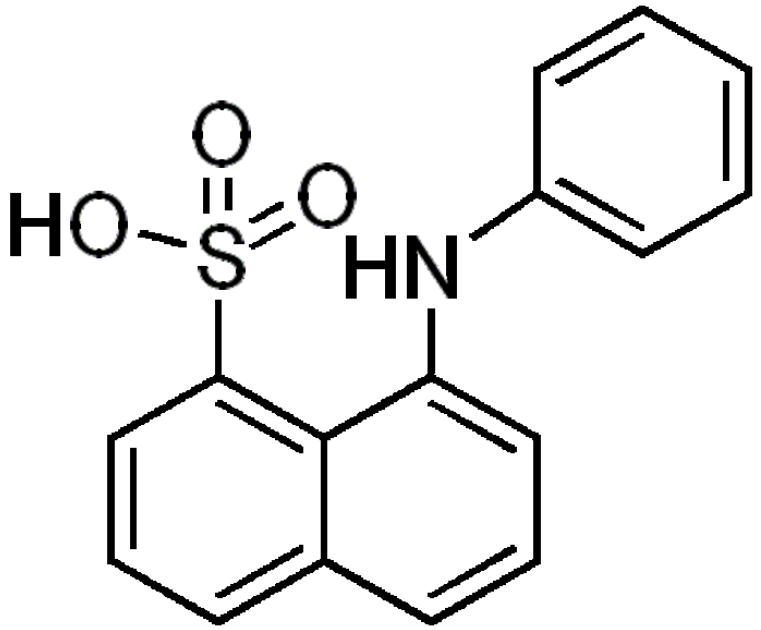


Fluorescein-PE



Anthroyloxypalmitát

Fluorescenční sondy



ANS – 1,8-anilinonaftalensulfonát

(zde kyselina)

Vazba ligandu - Scatchardův model

$$M_f + L_f \longleftrightarrow ML\ (= L_b)$$

$$K_{as} = [L_b] / ([M_f] \,.\, [L_f]) = [L_b] / ([M_t](n-\nu) \,.\, [L_f])$$

$$[L_b] / [M_t] = \nu, \quad \mathbf{[L_b]} = \nu \,.\, [M_t], \quad K_{as} = \nu / (n-\nu) \,.\, \mathbf{[L_f]})$$

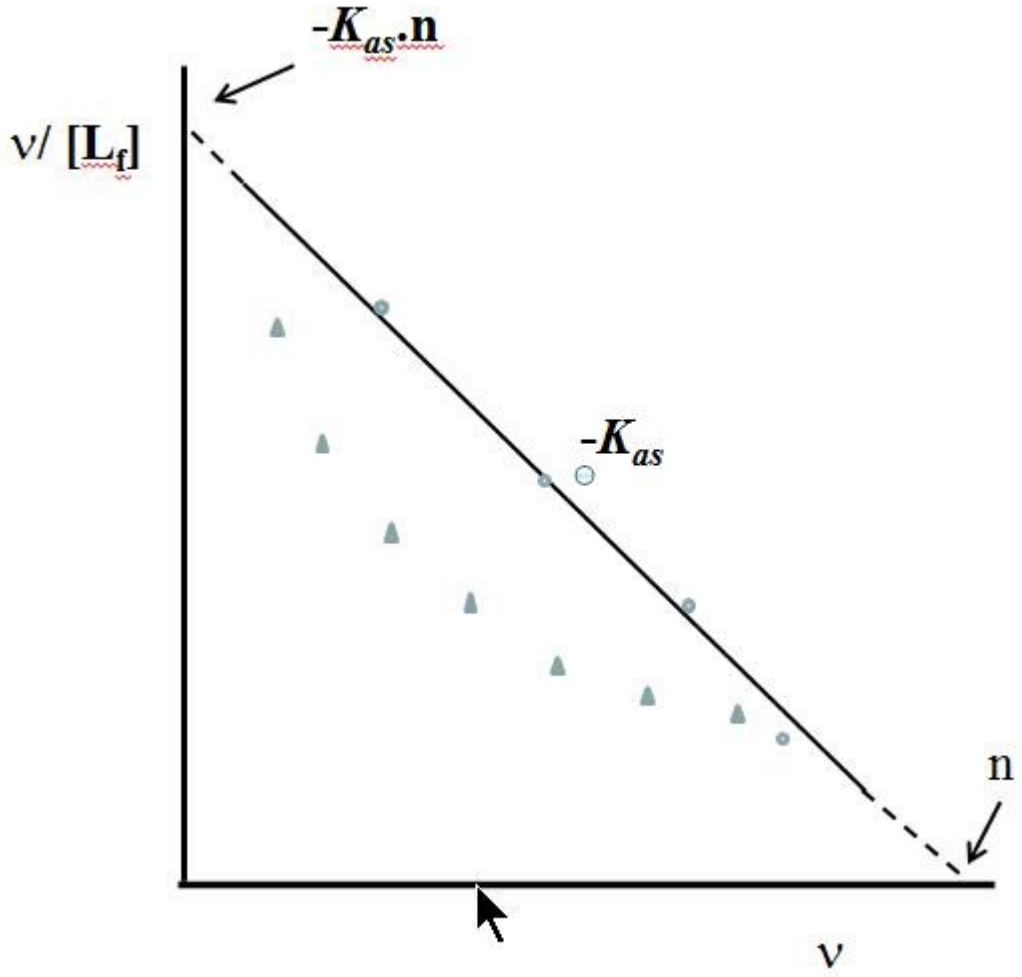
$$\mathbf{K_{as} \,.\, (n-\nu) = \nu / [L_f] \qquad K_{as} \,.\, n - K_{as} \,.\, \nu = \nu / [L_f]}$$

Scatchardův model

Grafické Znázornění

odpovídá ○

nesedí △



Použití fluorimetrie ke sledování vazby

$$F = F_f + F_b$$

$$F = \mathbf{[L_f]} .\Phi_f + \mathbf{[L_b]}.\Phi_b$$

$$F = (\mathbf{[L_t]} - \mathbf{[L_b]}).\Phi_f + \mathbf{[L_b]}.\Phi_b$$

$$F = \mathbf{[L_t]}.\Phi_f - \mathbf{[L_b]}.\Phi_f + \mathbf{[L_b]}.\Phi_b$$

$$F = F_o + \mathbf{[L_b]}.(\Phi_b - \Phi_f)$$

$$\mathbf{[L_b] = (F - Fo)/ (\Phi_b - \Phi_f)}$$

F_f, F_b – fluorescence volné, vázané frakce

Φ_b, Φ_f –kvant. výtěžek fluorescence vázaného, volného ligandu

$\mathbf{[L_b]}$, $\mathbf{[L_f]}$ – koncentrace vázaného, volného ligandu

$\mathbf{[L_t]}$ – celková koncentrace ligandu

F – celková fluorescence

Použití fluorimetrie ke sledování vazby

Určení $\Phi_b - \Phi_f$

$F = [L_f] . \Phi_f + [L_b] . \Phi_b$ – pro $[L_b] = [L_t]$, $[L_f] = 0$