

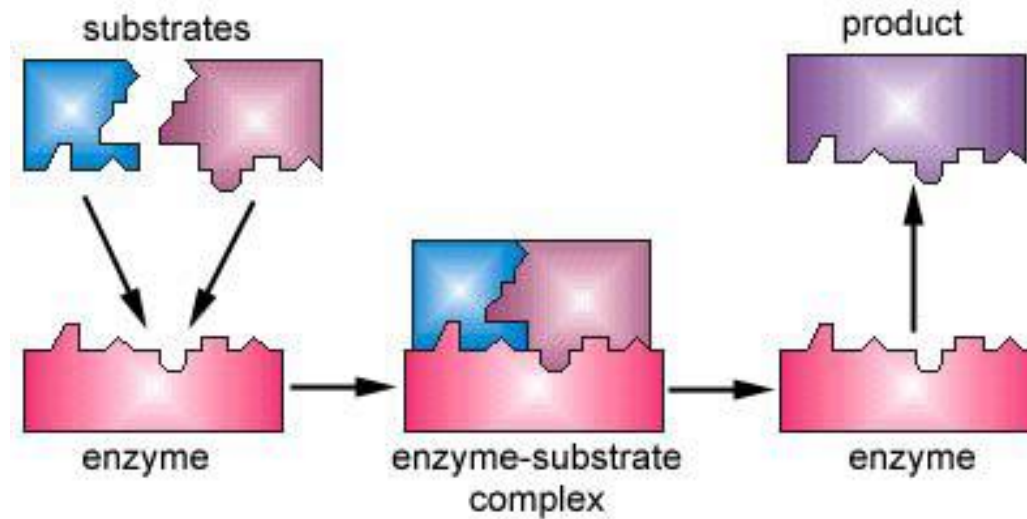


CO NÁM ŘEKNE KAPILÁRNÍ ELEKTROFORÉZA OHLEDNĚ INTERAKCÍ BIOMOLEKUL

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INTERAKCE VS. CHEMICKÁ REAKCE



TYPY VAZEB

- **Kovalentní** – sdílí elektrony
 - Polární vs. nepolární
 - Silné / pevné
 - Ireverzibilní nebo jen velmi obtížně reverzibilní
 - 150 – 450 kJ/mol

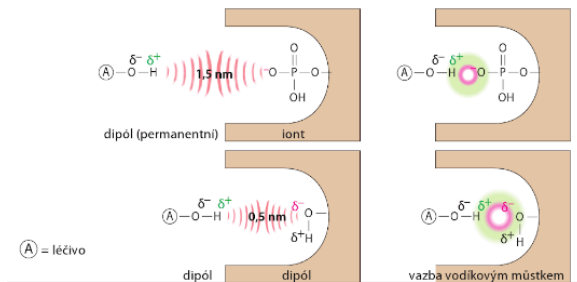
 - Alkylující cytostatika, organofosfáty

- **Nekovalentní** – nevytváří společný elektronový obal
 - Slabé interakce
 - Reverzibilní interakce – typické pro farmaka
 - Účastní se dějů – rozpouštění, krystalizace sublimace, desublimace, vypařování

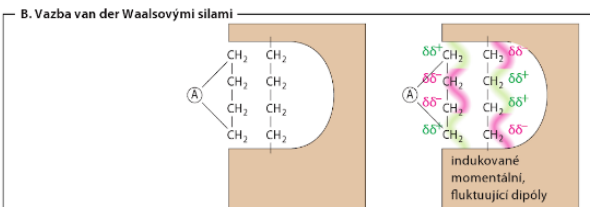
 - iont-iont, dipól-iont, dipól-dipól, Wan der Waals, Coulombické int., H-můstky, hydrofóbní interakce

SÍLA VAZBY

- Intramolekulární vs. intermolekulární

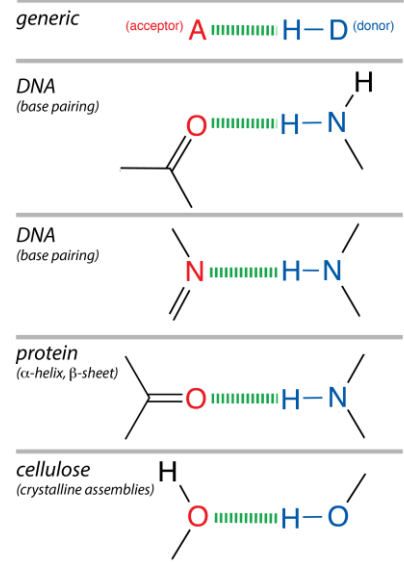


3 – 50 kJ/mol

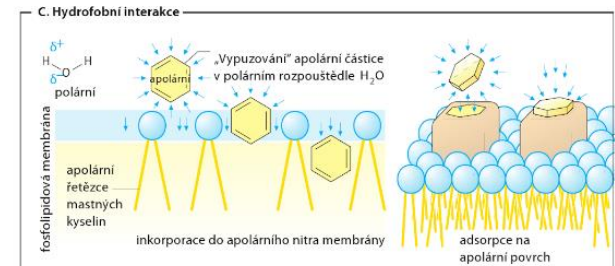


0,1 - 10 kJ/mol

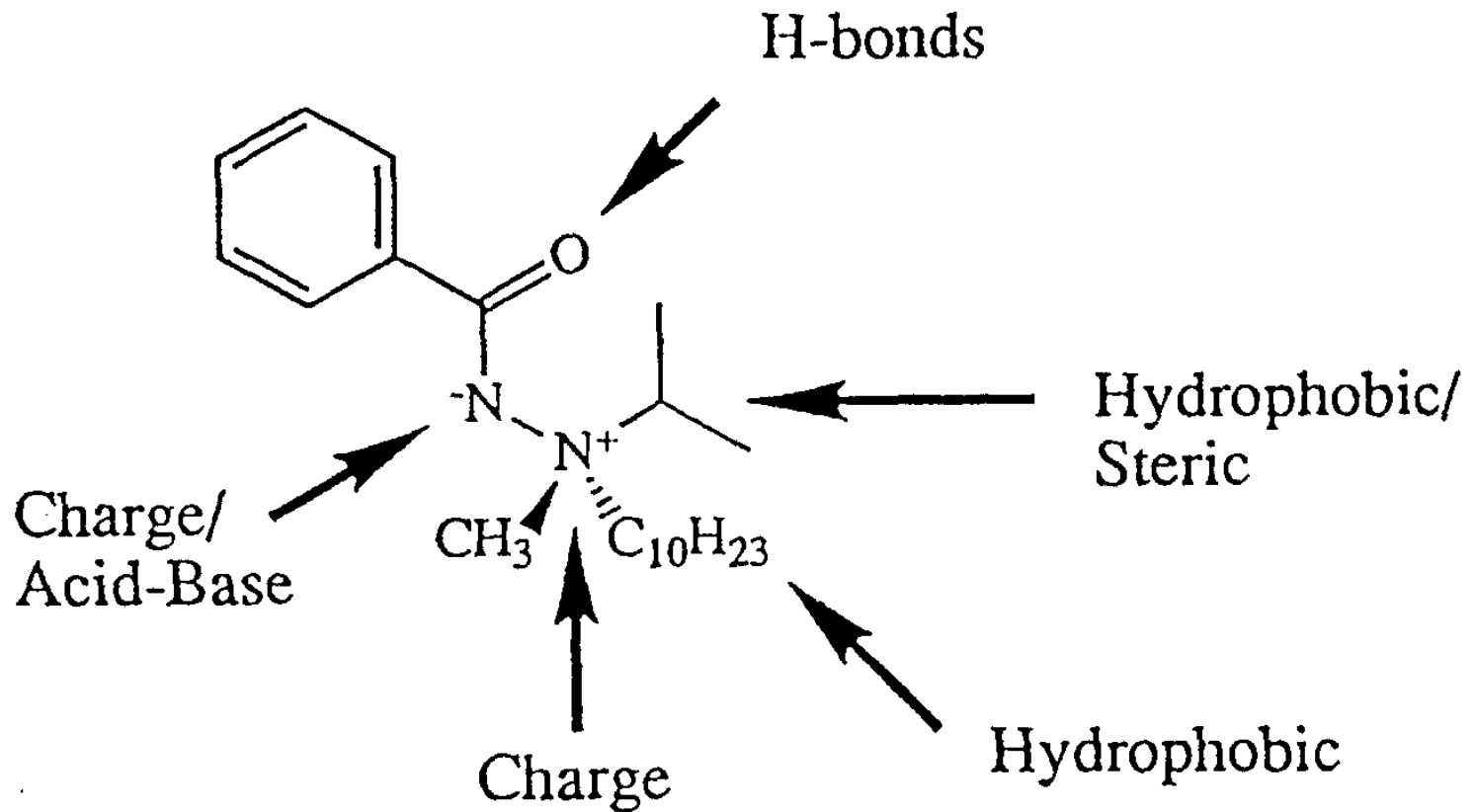
Hydrogen Bonding in Biological Systems



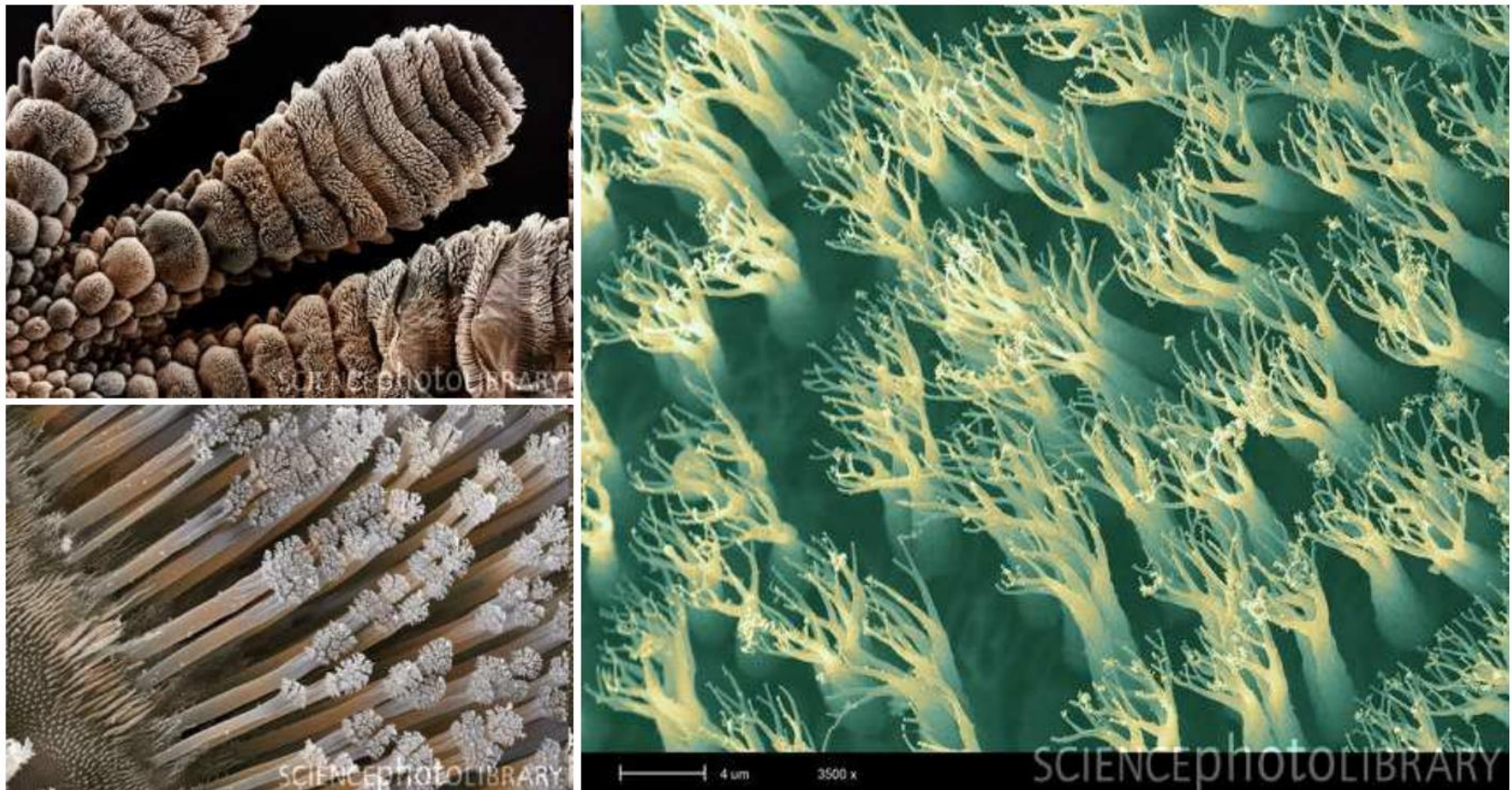
H-můstek 10 – 40 kJ/mol



2 kJ/mol



Gekon



BIOMAKROMOLEKULY

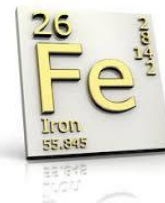
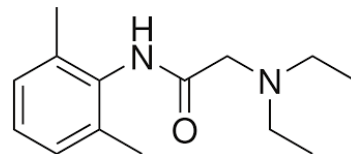
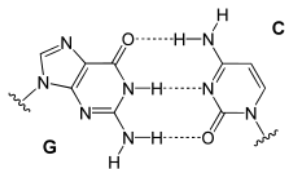
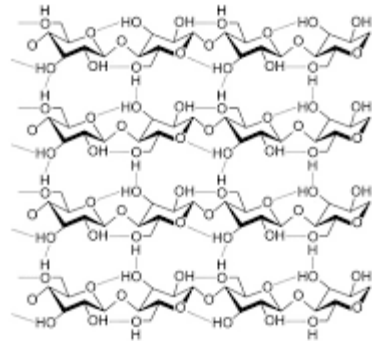
- Struktura všech biomolekul je dána kombinací kovalentních a nekovalentních vazeb
- **Kovalentní vazby** – statické, málo ovlivněny prostředím
- **Nekovalentní vazby** (hydrofobní interakce, vodíková vazba a elektrostatické přitahování) – dynamická rovnováha, ovlivnitelné faktory jako je teplota, obsah iontů a pH
- Biomolekuly musí být – „flexibilní a tuhé“ pro dosažení řádného fungování, a proto síly, které drží molekulární tvar, musí dosahovat rovnováhy s prostředím

BIOMOLEKULY – SLABÉ INTERAKCE

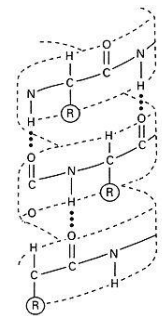
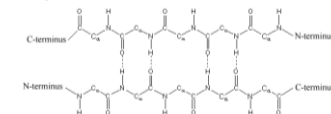
- Rozpoznání
- Struktura
- Funkce

BIOMOLEKULÁRNÍ INTERAKCE

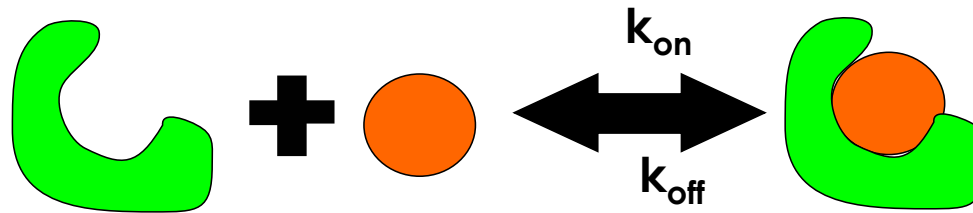
- Typy látek
- **Protein – ligand**
- **Protein – Protein**
- **Protein – NK**
- NK– ligand
- atd.
- Sacharidy, lipidy, proteiny, NK



Antiparallel β Sheet



INTERAKCE RECEPTOR-LIGAND



$$P + D \xrightleftharpoons[k_{off}]{k_{on}} PD$$

$$K_b = \frac{[PD]}{[P][D]} = \frac{k_{on}}{k_{off}} = \frac{1}{K_d}$$

$$\Delta G^\circ = -RT \ln K_b = RT \ln K_d$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

$\Delta G < 0$ exergonické
 $\Delta G > 0$ endergonické

$\Delta H < 0$ exothermické
 $\Delta H > 0$ endothermické

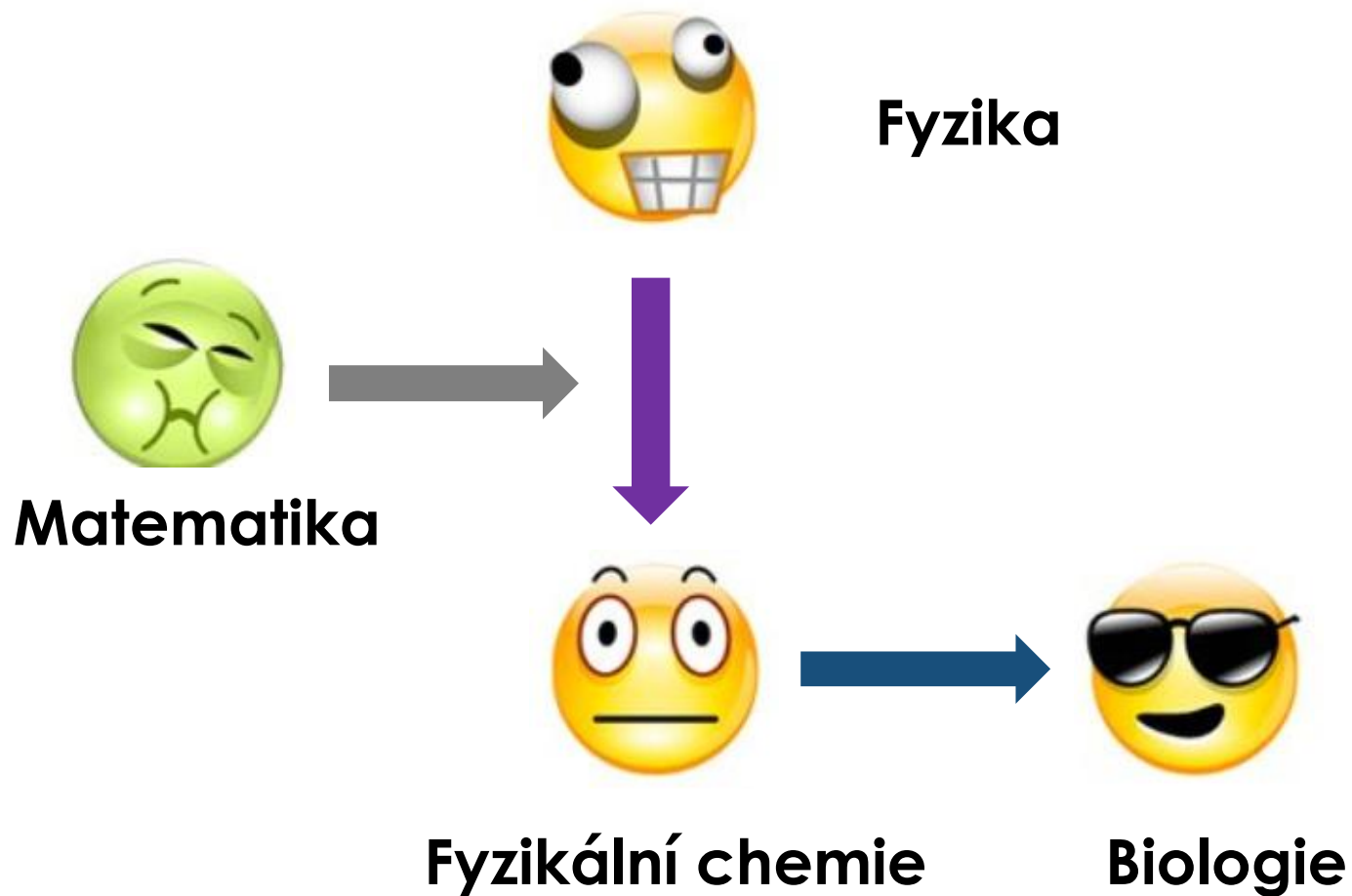
K_B VS. K_D VS. ΔG°

	K_d (M)	K_b (M^{-1})	ΔG° (kJ/mol)	ΔG° (kcal/mol)
No affinity (high millimolar)	$>10^{-1}$	$<10^1$	$>(-5.9)$	$>(-1.4)$
Very weak affinity (low millimolar)	$10^{-3} - 10^{-1}$	$10^1 - 10^3$	$(-18)-(-5.9)$	$(-4.3)-(-1.4)$
Low affinity (high micromolar)	$10^{-5} - 10^{-3}$	$10^3 - 10^5$	$(-30)-(-18)$	$(-7.1)-(-4.3)$
Moderate affinity (low micromolar)	$10^{-6} - 10^{-5}$	$10^5 - 10^6$	$(-36)-(-30)$	$(-8.5)-(-7.1)$
High affinity (nanomolar)	$10^{-9} - 10^{-6}$	$10^6 - 10^9$	$(-53)-(-36)$	$(-13)-(-8.5)$
Very high affinity (pico/femtomolar)	$10^{-14} - 10^{-9}$	$10^9 - 10^{14}$	$(-83)-(-53)$	$(-20)-(-13)$
Effectively irreversible (low femtomolar)	$<10^{-14}$	$>10^{14}$	$<(-83)$	$<(-20)$

PROČ STUDOVAT INTERAKCE?

- **Analýza povahy intermolekulárních interakcí** – typ interakce (hydrofobní, H-můstky,...)
- **Pochopení biologických procesů** - dochází k interakci? jak silná? co vše jí ovlivňuje?
- **Aplikace poznatků** - ve vědě / lékařství
 - Vývoj léčiv
 - Biotechnologie
 - Objev podstaty biologické děje / onemocnění

INTERAKCE



TECHNIKY MĚŘENÍ

- Fyzikálně-chemické vlastnosti látek a prostředí
- Rychlost analýz
- Studovaný systém



- Dostupnost
- Komplementarita
- „In,,



- Moderní vs. klasický přístup



INFORMACE MĚŘENÍ

Kvalitativní



Semi-kvantitativní

KVALITA vs. KVANTITA



Kvantitativní

Metody

Experimentální

Separační

Ultracentrifugace
Ultrafiltrace
Rovnovážná
dialýza
LC, CE

Bez separace

Spektrofotometrické
metody
Kalorimetrické
metody
SPR, AFM, MST

Výpočetní

Databáze
Virtualní
screening
Molekulární
dokování

Technique	High affinity K_D (nM-pM)	Intermediate affinity K_D (10^{-4} - 10^{-5} M)	Low affinity K_D (mM-M)	Low sample amount (<10 μ g)	Online detection	Solution based	Fast analysis (min-h)	Label free	Insensitive to contaminants
Surface plasmon resonance	✓	✓	✗	✓	✓	✗	✓	✓	✗
Calorimetry	✓	✓	✗	✗	✓	✓	✓	✓	✓
Ultracentrifugation	✓	✓	✗	✗	✓	✓	✗	✓	✗
Ultrafiltration	✗	✓	✓	✗	✗	✓	✗	✓	✗
Equilibrium dialysis	✓	✓	✓	✗	✗	✓	✗	✓	✗
Electrospray mass spectrometry	✓	✓	✗	✓	✗	✓	✓	✓	✗
Fluorescence	✓	✓	✗	✗	✓	✓	✗	✓	✗
Circular dichroism	✗	✓	✗	✗	✓	✓	✓	✓	✗
Affinity chromatography	✓	✓	✗	✗	✓	✗	✓	✓	✗
Affinity capillary electrophoresis	✓	✓	✓	✓	✓	✓	✓	✓	✓

Red cross: not applicable

Green tick: applicable

Yellow tick: possible in modified version of the application

ELEKTROFORÉZA - PRINCIP

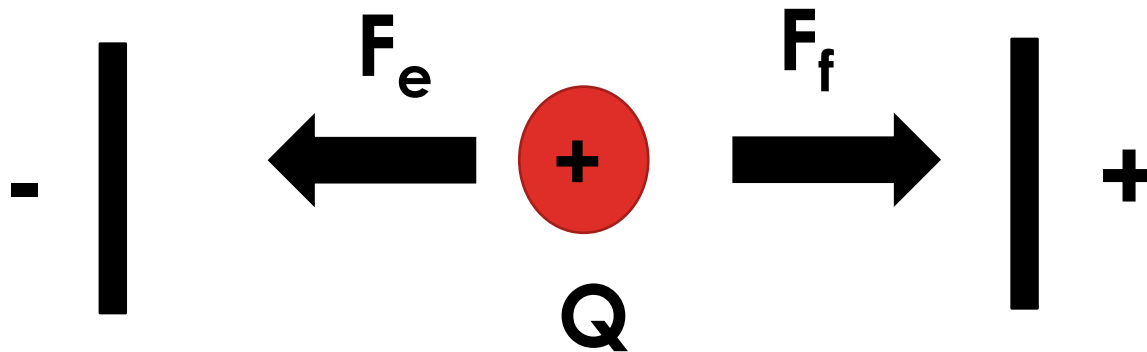
- Elektromigrační separační metoda
- Rozdílná pohyblivost nabitých částic ve stejnosměrném el. poli

Pohyblivost částic závisí na:

- náboj
- velikosti a tvaru molekuly
- hmotnosti částice
- vlastnostech prostředí

PRINCIP

Na nabitou částici o náboji Q působí v elektrickém poli o intenzitě E dvě síly:



$$F_e = E \times Q$$

E = intenzita elektrického pole [V/m]

Q = náboj částice = $z_i \times e$

$$F_f = v \times f$$

v = rychlost částice

f (frikční koeficient) = $6\pi \cdot \eta \cdot r$

$$F_e = F_f$$
$$EQ = vf$$



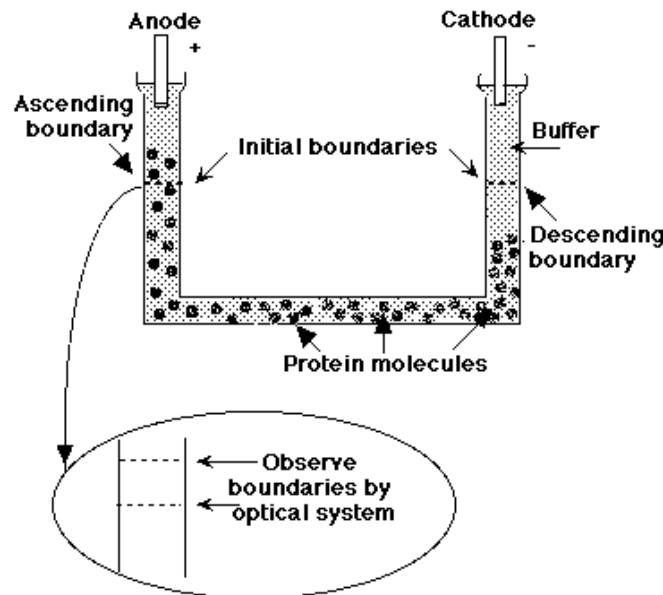
$$v = \frac{EQ}{f}$$

$$\mu = \frac{v}{E} = \frac{Q}{f}$$

$$[\text{m}^{-2}\text{V}^{-1}\text{s}^{-1}]$$

ELEKTROFORÉZA - HISTORIE

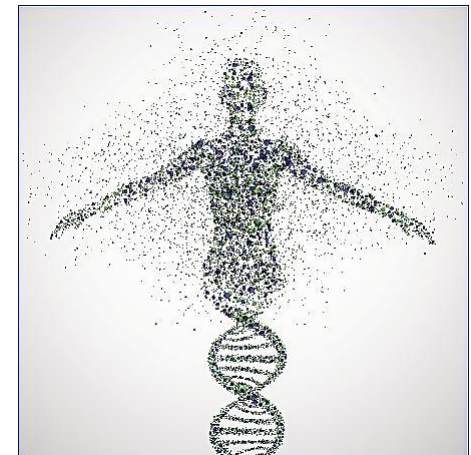
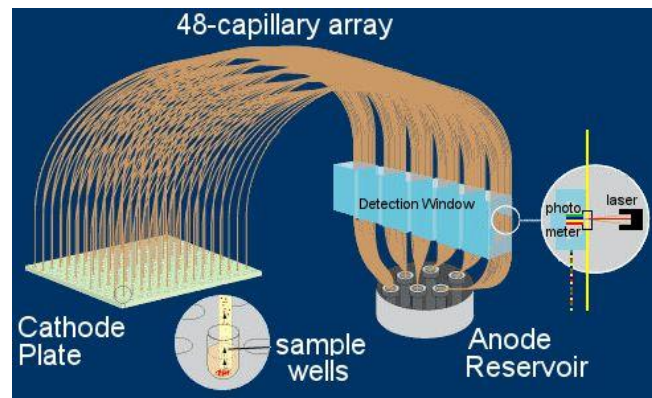
- Poprvé publikoval metodu elektroforézy sérových proteinů v r. **1937** švédský chemik Arne Tiselius.
- v. r. **1948 získal Nobelovu cenu** za práci o separaci koloidů (tzn. proteinů) pomocí elektroforézy.



Tiseliov přístroj

ELEKTROFORÉZA - HISTORIE

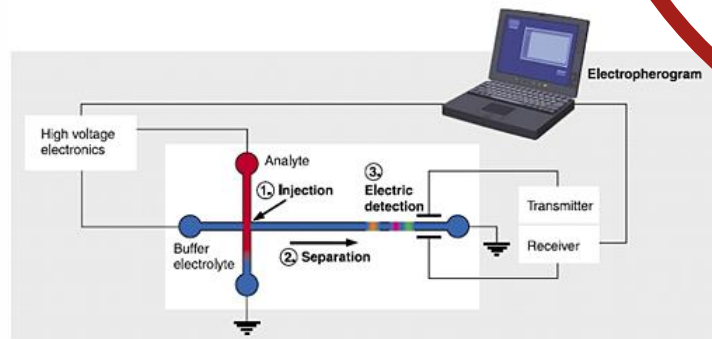
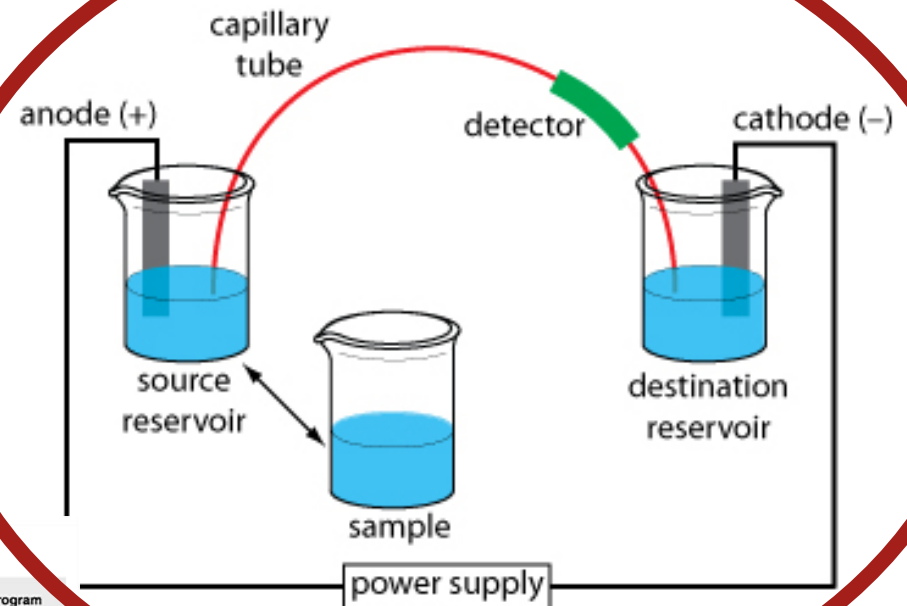
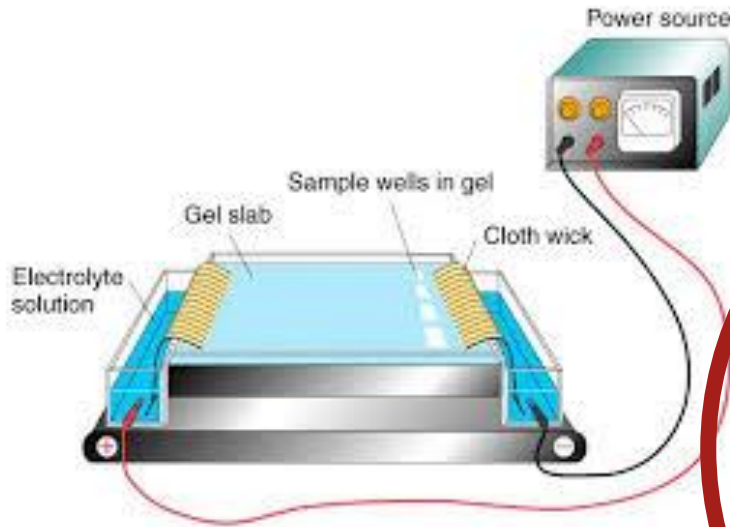
- Kapilární elektroforézu v rotující kapiláře (3 mm) představil v r. **1967** švédský chemik Hjerten.
- v r. **1981** byla provedena separace v 75 μm křemenné kapiláře (Jorgenson, Lukacsová).
- V r. **1989** byl k dispozici první komerční zařízení pro CZE Beckmann
- V r. **2003** - projekt lidského genomu



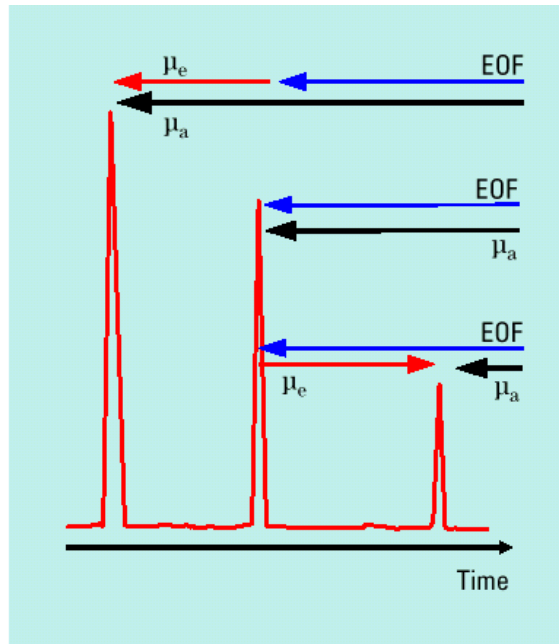
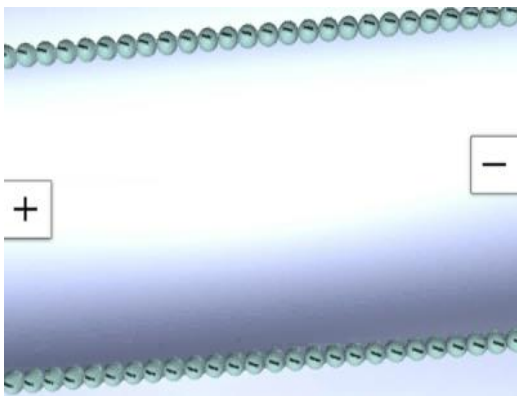
ELEKTROFORÉZA – **AFINITA** - HISTORIE

- Během vývoje CE metod
- **1950** – Tiselius – charakterizování v „U-tube“ interakce Ag-Ab
- Do **1990 - interakce v gelu** – gel shift assay NK-protein
- **1992** – využití elektroforézy ve **volném roztoku** pro stanovení vazebné konstanty

ELEKTROFORÉZA- DNES



KAPILÁRNÍ ZÓNOVÁ ELEKTROFORÉZA



cation — positive μ_e

neutral — zero μ_e

anion — negative μ_e

$$\mu_a = \mu_{\text{EOF}} + \mu_e$$

μ_a = apparent mobility

μ_e = effective mobility

GE VS. CE

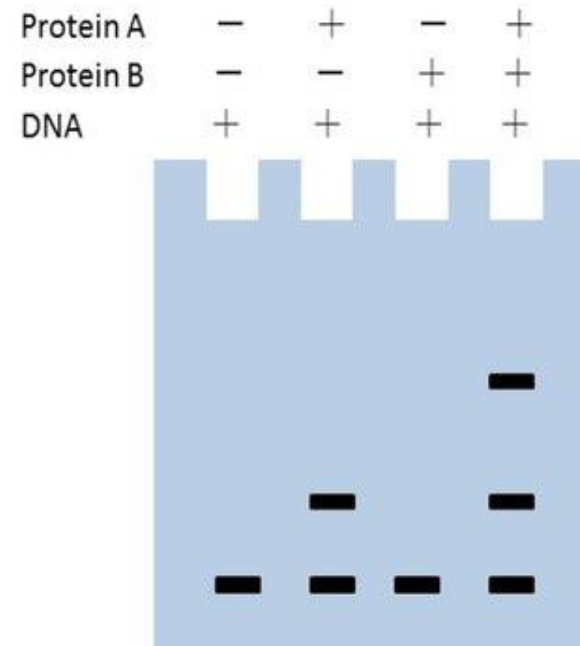
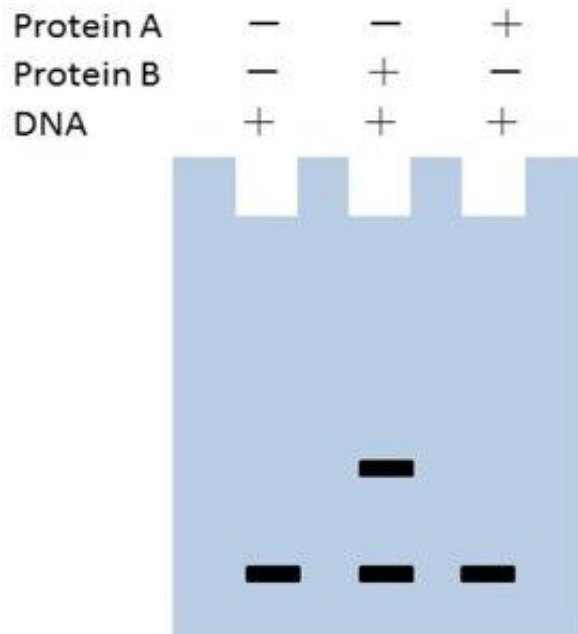
GE

- Silné interakce
- Dostatečný rozdíl ve velikosti či náboji partnerů
- Nelze studovat teplotní závislosti – ne termodynamika

CE

- Slabá interakce nebo rychlá disociace
- Elektroforetická mobilita – mnoho faktorů
- Lze studovat termodynamiku

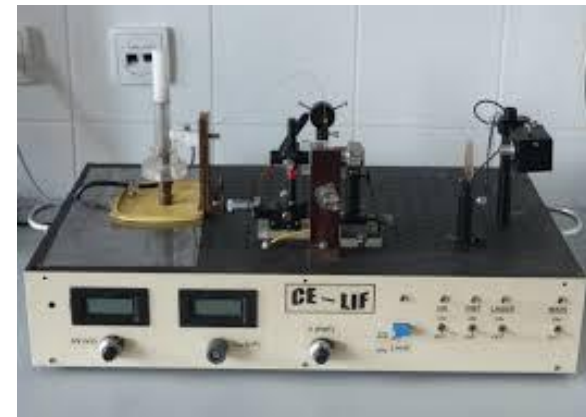
EMSA



Gel mobility Shift Assay - Electrophoretic Mobility Shift Assay (EMSA)

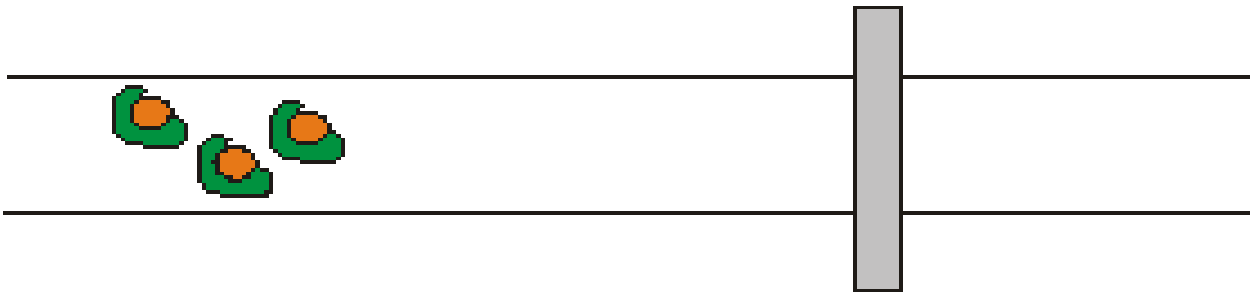
OBECNÉ VÝHODY CE

- Vysoká účinnost a separační selektivita
- Malá spotřeba vzorku a reagentů
- Rychlost analýz
- Jednoduchá automatizovatelnost
- Možnost práce v prostředí blízkém prostředí fyziologickému



ANALÝZA ROVNOVÁŽNÉ SMĚSI

detektor



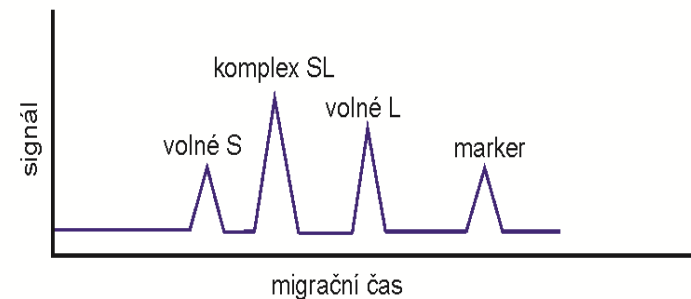
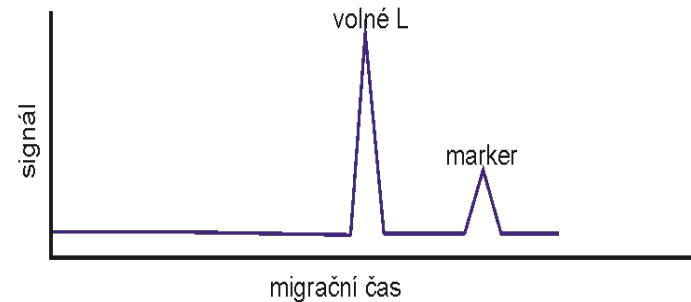
detektor



CZE

- BGE čisté
- Vzorek – směs interakčních partnerů
- Podmínky – vznik stabilního komplexu, odlišná mobilita složek

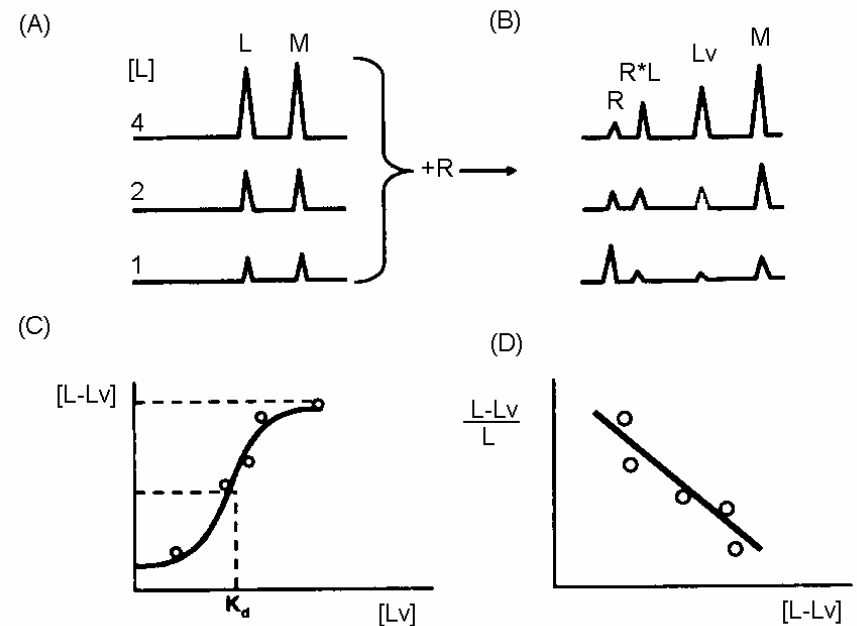
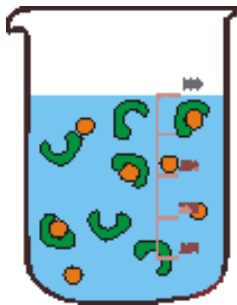
- + bez purifikace
- + stechiometrie
- + % vazby
- + ligand a receptor nemusí mít rozdílnou velikost
- kalibrace
- disociace komplexu



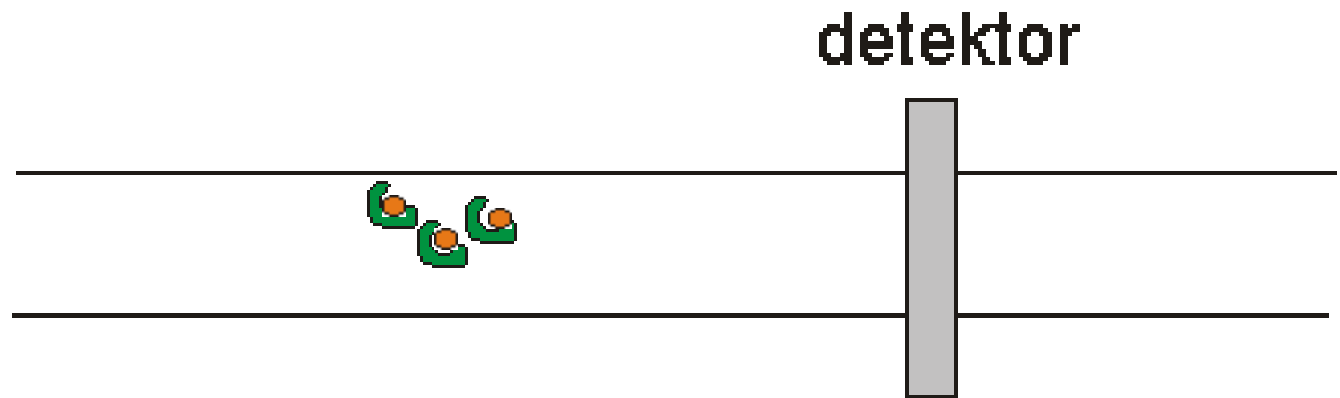
CZE

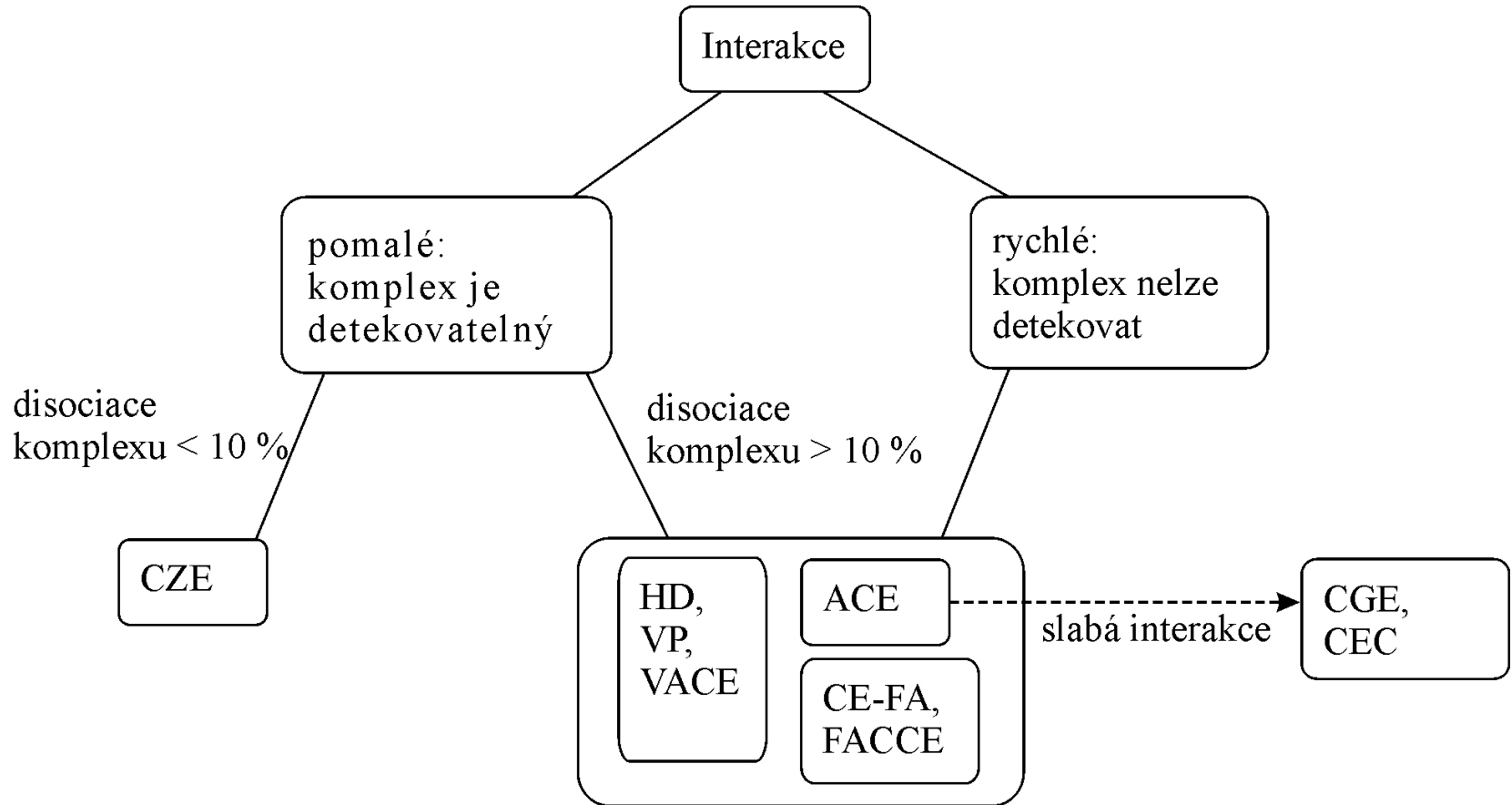
Oddělení komplexu od rovnovážné směsi a jeho detekce

System s pomalou kinetikou, tj. s vysokou afinitou







ANALÝZA ROVNOVÁŽNÉ SMĚSI










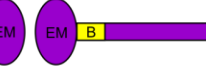


MÓDY CE

- frontal analysis (CE-FA)
- frontal analysis electrophoresis capillary continual (FACCE)
- zonal capillary electrophoresis (CZE)
- affinity capillary electrophoresis (ACE)
- Hummel-Dreyer (HD)
- vacancy peak (VP)
- vacancy affinity capillary electrophoresis (VACE)

	Frontal Analysis (large-plug injection)		Zonal Elution (small-plug injection)				
	CE/FA	FACCE	CZE	ACE	HD	VP	VACE
Setup							
	Injection of a large plug Continuous injection						
How to obtain K_a	$[D_{free}]$: plateau height	$[D_{free}]$: plateau height	$[D_{free}]$: peak area	Change in μ of the injected compound	$[D_{bound}]$: peak area	$[D_{free}]$: peak area	Change in μ of one of the species
Main Advantages and Drawbacks	<ul style="list-style-type: none"> - slow and fast kinetics systems: analysis possible - multiple equilibria easily studied - stoichiometric information - binding percentage easily obtained 		<ul style="list-style-type: none"> - slow kinetics systems ($K_a > 10^7 M^{-1}$) required - multiple equilibria easily studied - stoichiometric information - binding percentage easily obtained - highly purified samples not required 	<ul style="list-style-type: none"> - knowledge of the exact [D] not needed - highly purified samples not required - enantiomeric separation $-[P] \gg [D]$ - stoichiometric information not obtainable - multiple equilibria : difficult to deal with 	<ul style="list-style-type: none"> - multiple equilibria easily studied - stoichiometric information - binding percentage easily obtained - highly purified samples not required 	<ul style="list-style-type: none"> - require higher amount of P and D than other CE set-ups - BGE absorption too low : poor sensitivity - BGE absorption too strong : detector saturation - binding percentage easily obtained by VP but not by VACE - highly purified samples not required 	

	CEFA	FACCE	NECEEM
Setup			
Measured parameters	peak height, $[L_{free}]$		peak area and shape, migration time
Information estimated	K_1, n_1, K_2, n_2		K, k_{on}, k_{off}
Conditions	$\mu_{SL} = \mu_S$ $\mu_L \neq \mu_S, \mu_{SL}$	$\mu_{SL} = \mu_S$ $\mu_L > \mu_{SL}$	$\mu_S \neq \mu_L$

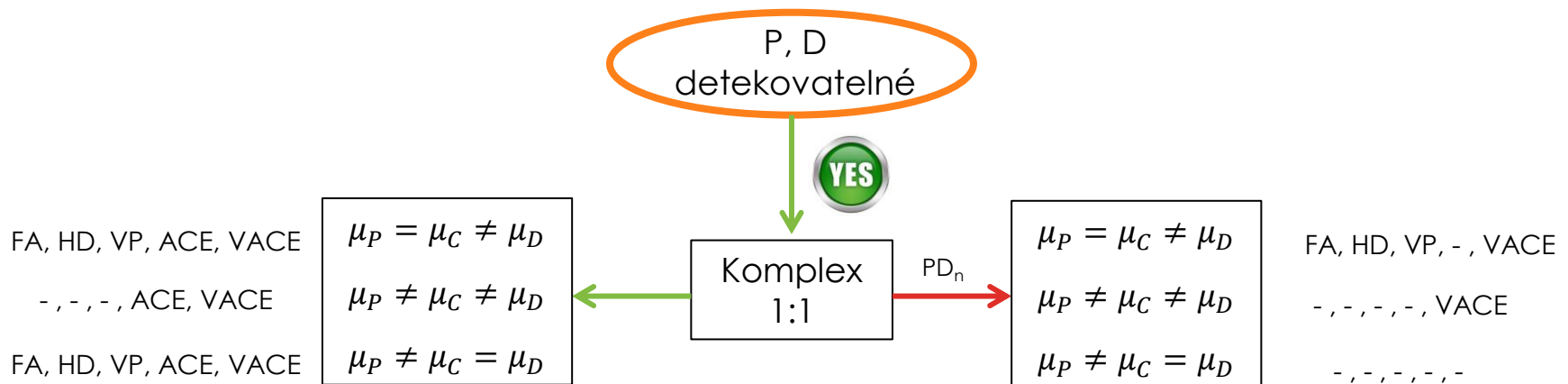
	CZE	ACE	HD	VP	VACE
Setup					
Measured parameters	peak area, $[L_{free}]$	migration time, $\Delta \mu$	peak area, $[L_{bound}]$	peak area, $[L_{free}]$	migration time, $\Delta \mu$
Information estimated	K_1, n_1, K_2, n_2	K_1	K_1, n_1, K_2, n_2	K_1, n_1, K_2, n_2	S-vacancy: K_1 L-vacancy: K_1, n_1, K_2, n_2
Conditions	$\mu_L \neq \mu_S, \mu_{SL}$	$\mu_{SL} \neq \mu_L$	$\mu_{SL} = \mu_S$	$\mu_{SL} = \mu_S$	S-vacancy: $\mu_{SL} \neq \mu_S$ L-vacancy: $\mu_{SL} \neq \mu_L$

CHARAKTERISTICKÉ ELEKTROFOREGRAMY

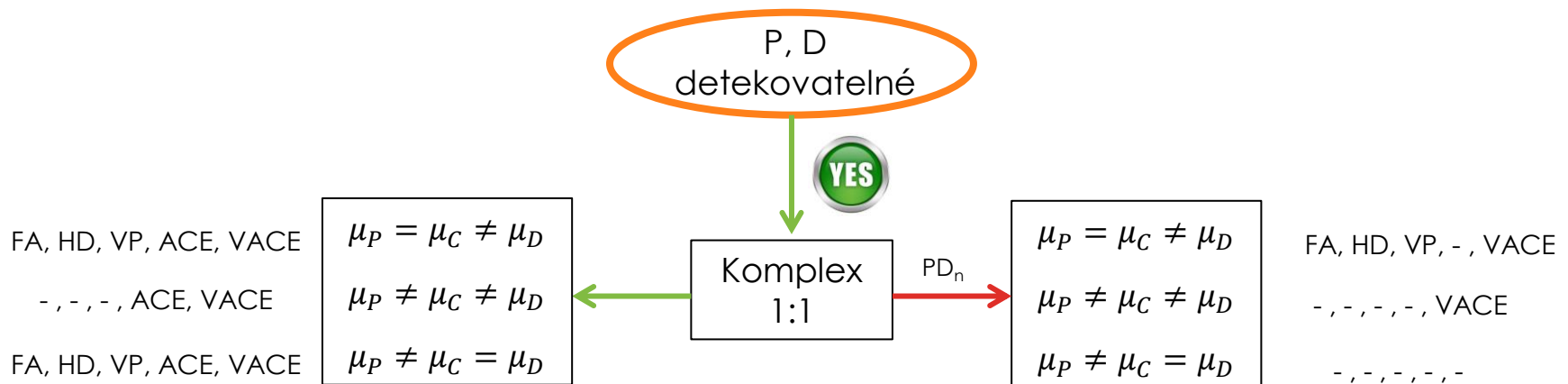
	záznam z měření	záznam z kalibrace
ACE	<p>BGE bez interačního partnera</p> <p>signál</p> <p>marker</p> <p>migrační čas</p>	
	<p>BGE s interakčním partnerem</p> <p>signál</p> <p>marker</p> <p>migrační čas</p>	
HD	<p>komplex SL+ volné S</p> <p>signál</p> <p>migrační čas</p>	<p>externí kalibrace</p> <p>signál</p> <p>migrační čas</p>
		<p>interní kalibrace</p> <p>signál</p> <p>migrační čas</p>

	záznam z měření	záznam z kalibrace
VP	<p>komplex SL+ volné S</p> <p>volné L</p> <p>migrační čas</p>	<p>komplex SL+ volné S</p> <p>volné L</p> <p>přechod z negativního do pozitivního piku pomocí postupných přidavků složky L</p> <p>migrační čas</p>
VACE	<p>marker</p> <p>BGE s minimální koncentrací jednoho z partnerů</p> <p>$\Delta\mu$</p> <p>migrační čas</p>	
	<p>marker</p> <p>BGE s maximální koncentrací jednoho z partnerů</p> <p>$\Delta\mu$</p> <p>migrační čas</p>	
CE-FA	<p>komplex SL+ volné S</p> <p>volné L</p> <p>migrační čas</p>	<p>volné L</p> <p>migrační čas</p>
FACCE	<p>volné L</p> <p>migrační čas</p>	<p>volné L</p> <p>migrační čas</p>

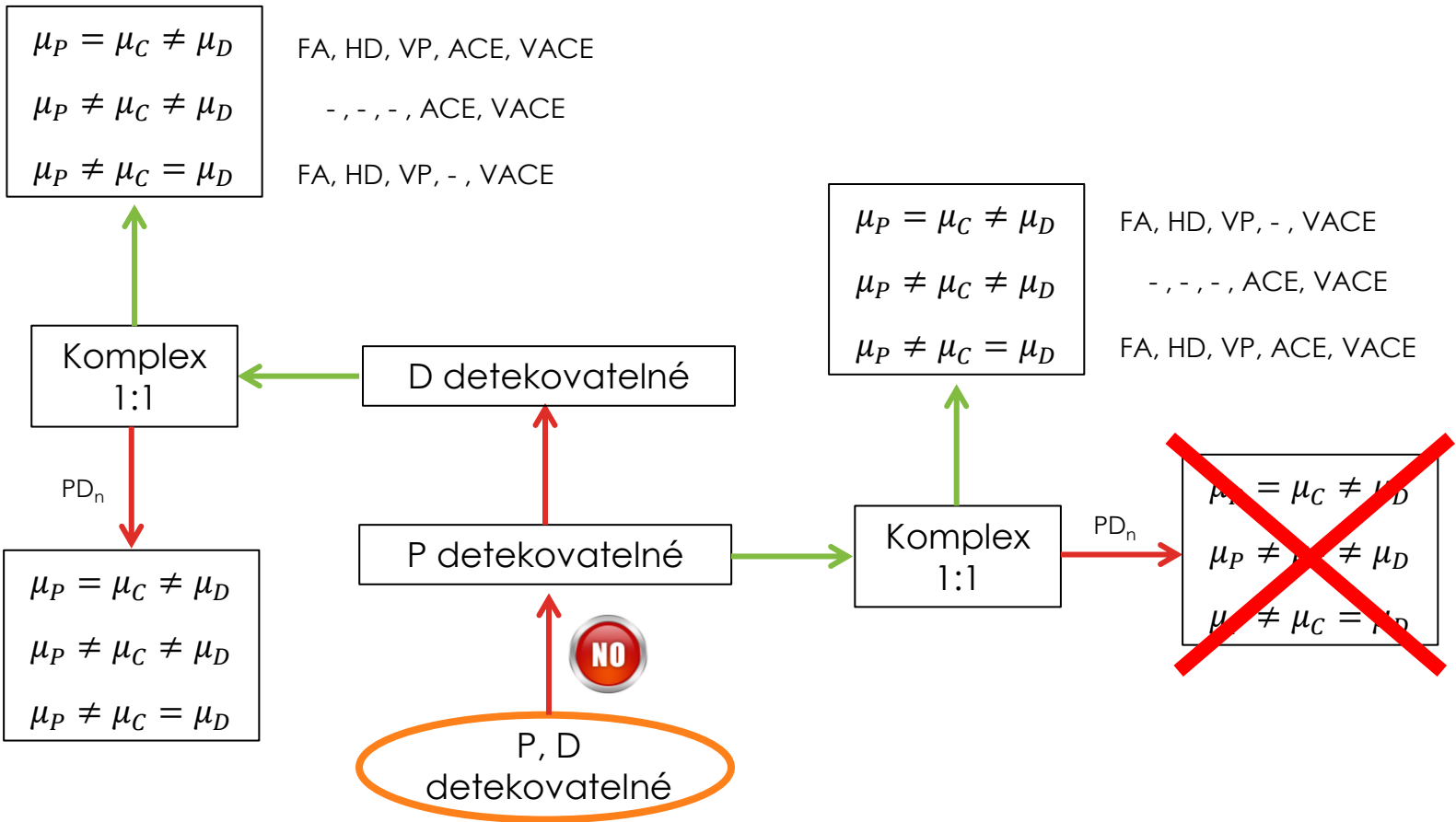
JAKÁ METODA NA CO?



JAKÁ METODA NA CO?

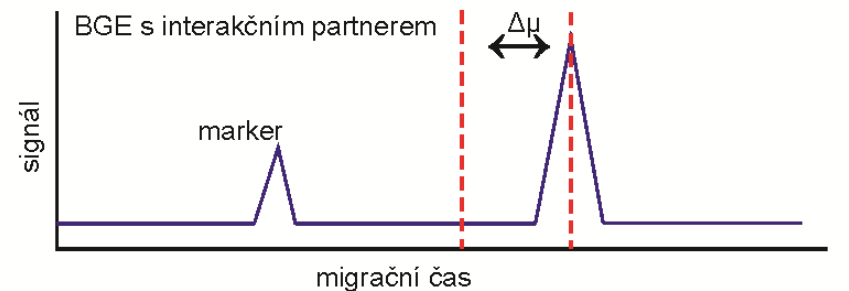
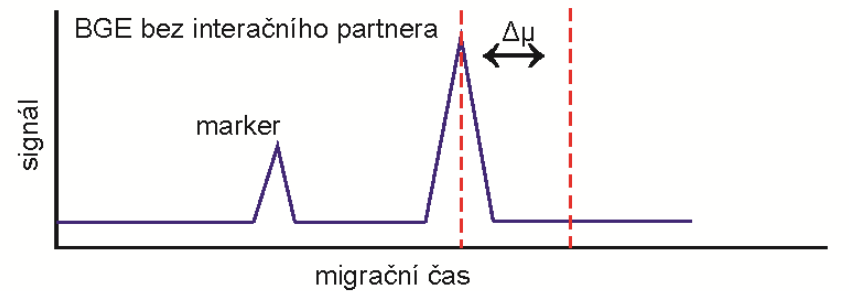


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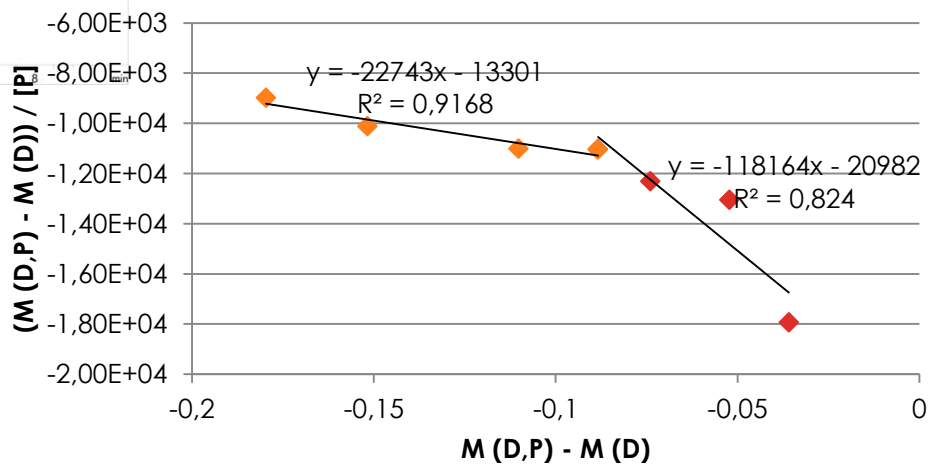
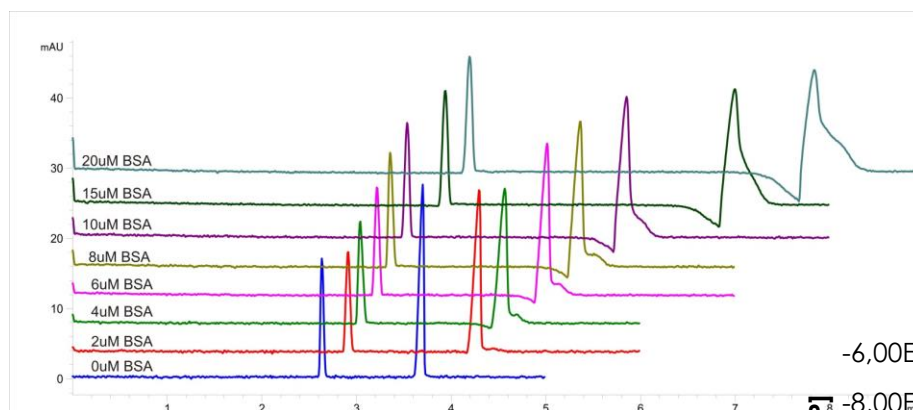
ACE

- Jeden z interakčních partnerů v BGE
- Vzorek – druhý interakční partner
- Podmínky – různé mobility
 - + bez purifikace
 - + enantiomery
 - + bez kalibrace
- stechiometrie 1:1
- enantiomery



ACE

Interakce BSA-diklofenak



- Literatura – CD, kovy, kompetice, multi-assay....

ANALYTICA CHIMICA ACTA 603 (2007) 101–110



available at www.sciencedirect.com



journal homepage: www.elsevier.com/locate/aca



Study of the interactions between fluoroquinolones and human serum albumin by affinity capillary electrophoresis and fluorescence method

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ARTICLE INFO

ABSTRACT

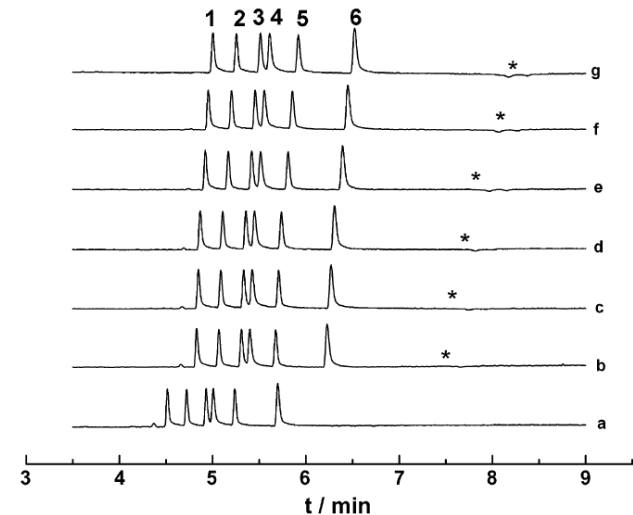
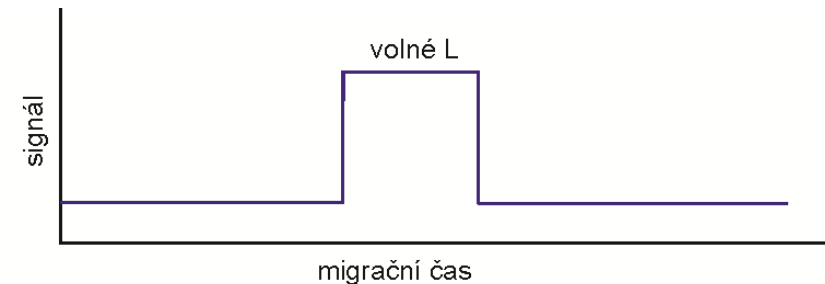
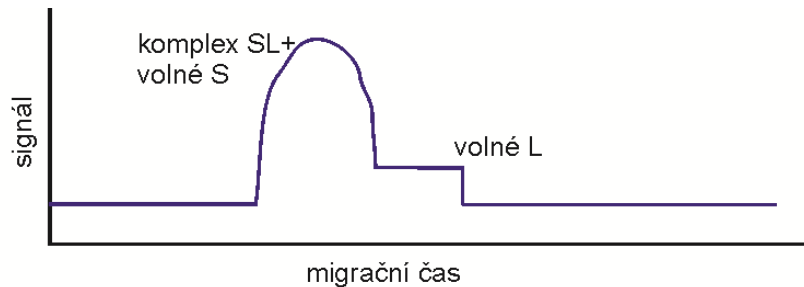


Fig. 6 – Representative set of electropherograms of the migration time shift of the fluoroquinolones vs. HSA concentration change. *a, b, c, d, e, f, g* represent HSA concentration of 0, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1×10^{-5} M, respectively. 1 = sparfloxacin, 2 = lomefloxacin, 3 = levofloxacin, 4 = norfloxacin 5 = pefloxacin, 6 = fleroxacin.

CE-FA

- BGE bez interagujících partnerů
 - Vzorek – oba interakční partneri, dávkování široké zóny vzorku
 - Podmínky – „stejná“ mobilita komplex a jeden z partnerů
- + bez purifikace
- + % vazby
- + stechiometrie
- + nízká spotřeba
- kalibrace

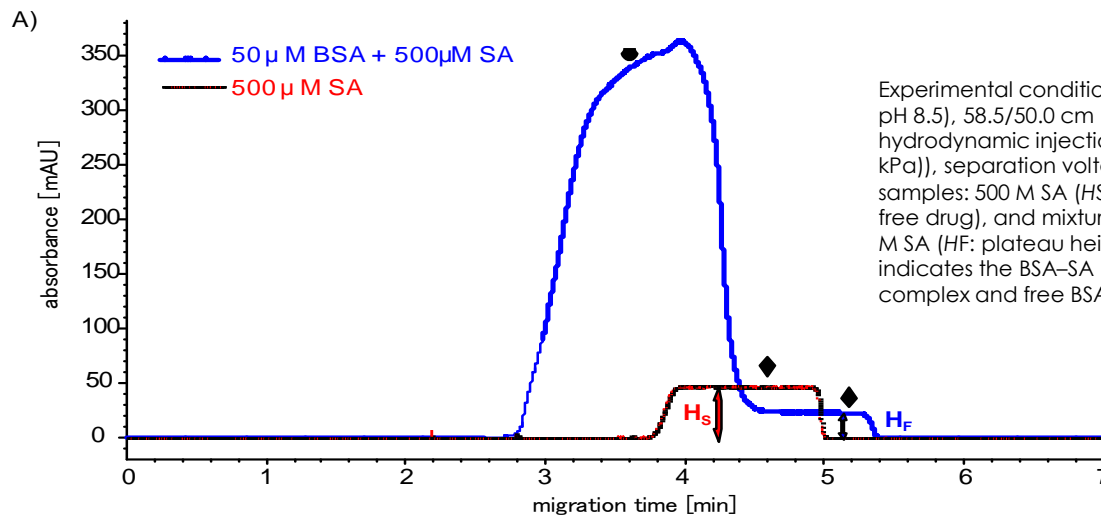


CE-FA



• HSA
• drug

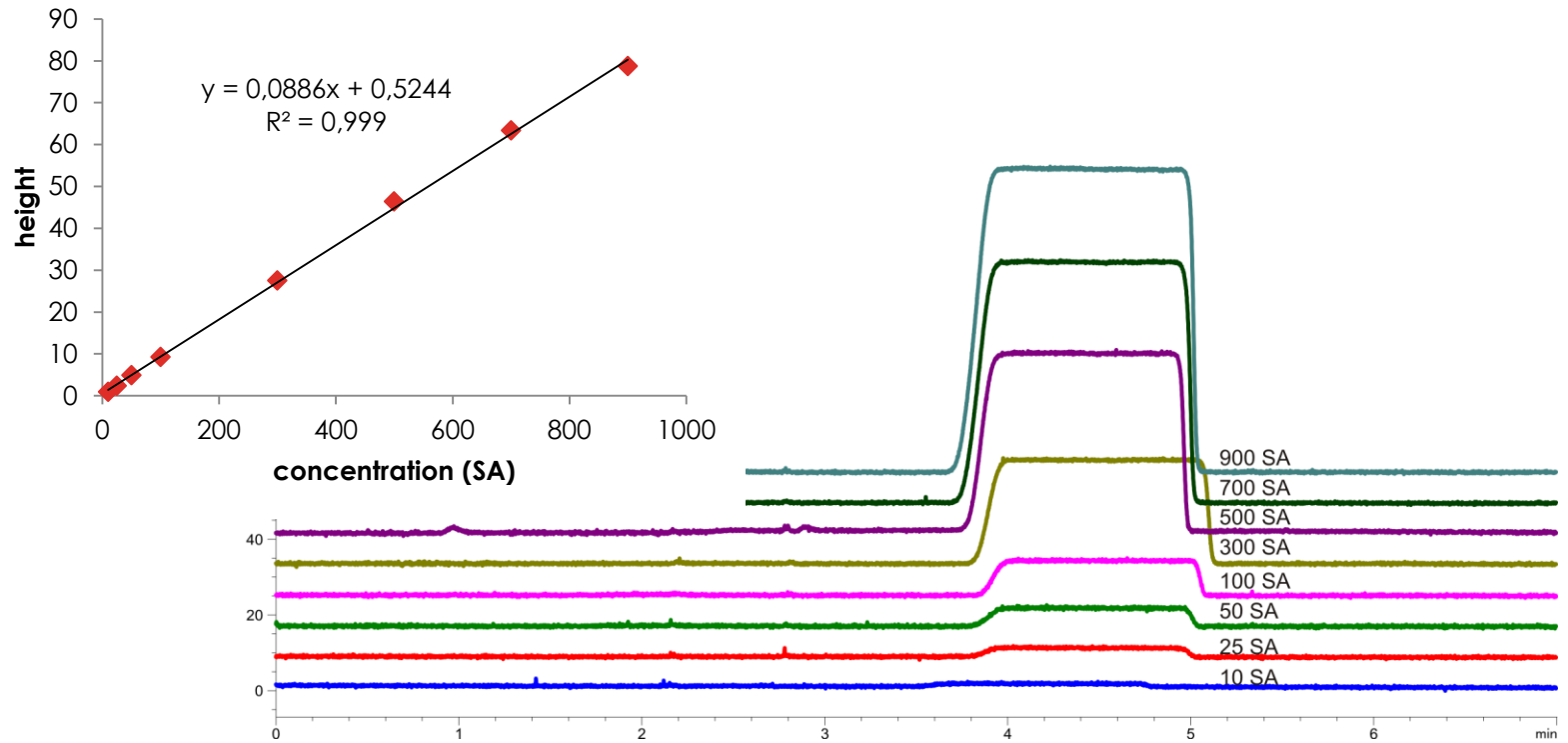
$$r = \frac{[D_{bound}]}{[P_{tot}]} = \sum_{i=1}^m \frac{n_i \cdot K_{bi} \cdot [D_{free}]}{1 + K_{bi} \cdot [D_{free}]}$$



Michalcova, L., Glatz, Z.: Comparison of various capillary electrophoretic approaches for the study of drug-protein interaction with emphasis on minimal consumption of protein sample and possibility of automation, *J. Sep. Sci.* (2015) 38, 325-331.

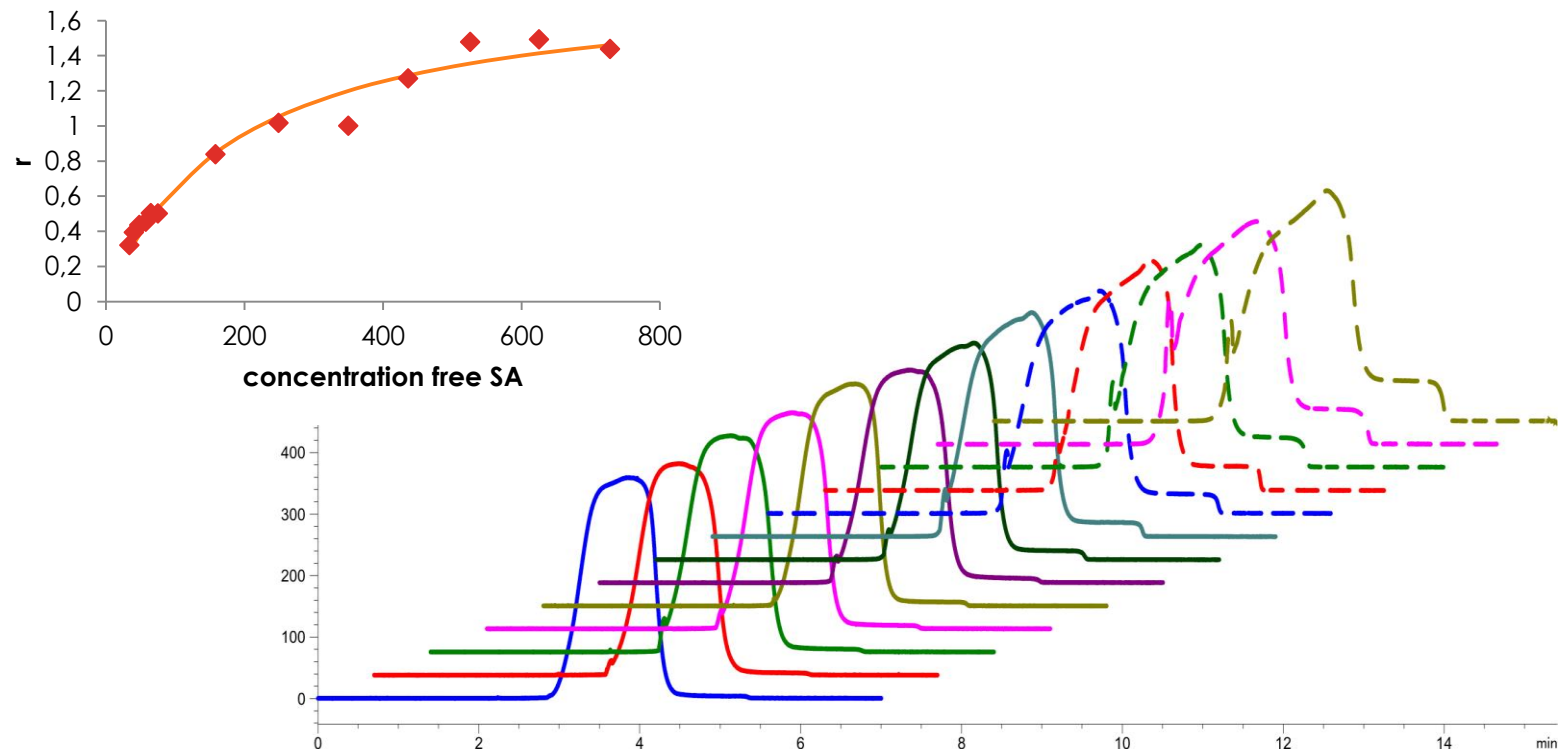
CE-FA

- Interakce BSA-SA



CE-FA

- Interakce BSA-SA



TERMODYNAMIKA

- Jaký druh vazeb hraje roli???

Table 2. Binding constants K_b , number of binding sites n_i , and thermodynamic parameters of BSA–SA system at different temperatures

T (K)	$(K_b \pm SD) 10^{-3}$ (L/mol)	$n_i \pm SD$	R^2	ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (J/mol·K)	R^2
293.15	12.20 ± 2.28	0.93 ± 0.29	0.9646	-22.9	-130.3	-366	0.9969
298.15	5.60 ± 0.28	1.85 ± 0.40	0.9855	-21.3			
303.15	2.10 ± 0.29	0.86 ± 0.30	0.9838	-19.3			

All data were obtained by CE-FA.

- Interakce je spontánní
- H-můstky
- Van der Waals vazby

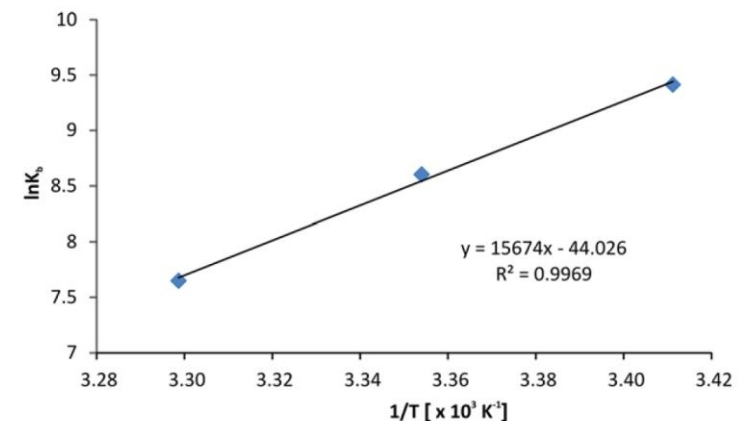


Figure 3. Van't Hoff plot of BSA–SA interaction; T : absolute temperature, K_b : binding constant.

RŮZNÉ CE METODY

- Podmínka: malá spotřeba vzorku (proteinu)

ACE

HD

CE-FA

VP / VACE

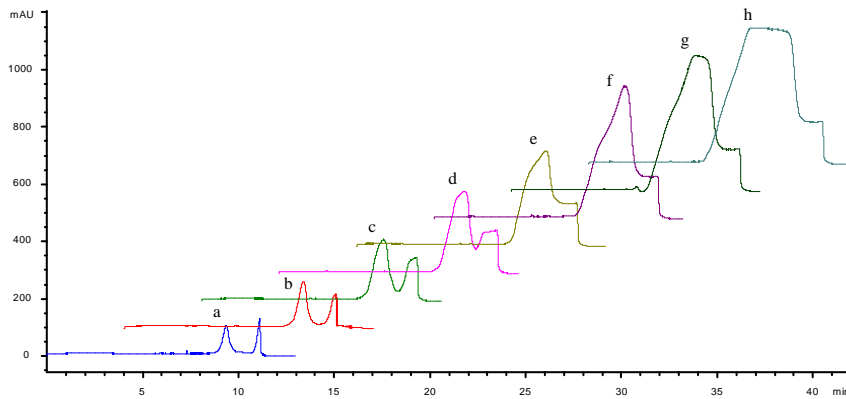
Table 1. Summary of binding parameters obtained by different methods

	$n_i \pm SD$	$(K_b \pm SD) 10^{-3} \text{ (L/mol)}$
CE-FA	1.79 ± 0.31	5.66 ± 0.19
HD-ex	1.71 ± 0.27	3.55 ± 1.34
HD-in	2.22 ± 0.35	5.26 ± 1.74
ED	5.32 ± 1.11	3.72 ± 0.82

Data are expressed as mean values of triplicate measurements.

CE-FA A JINÉ METODY

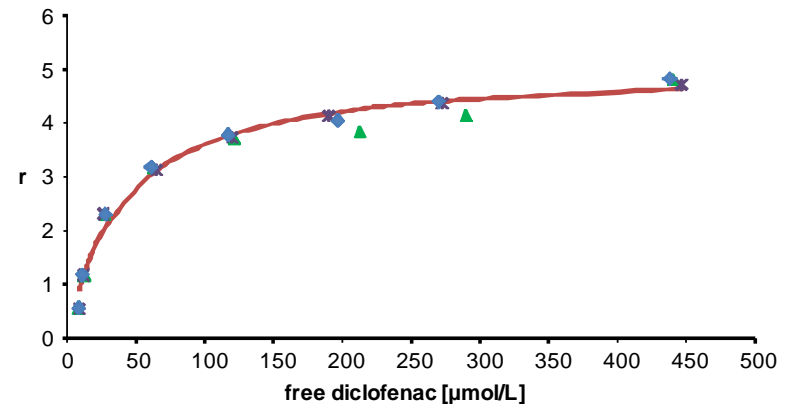
- Interakce HSA-DCF



$$\text{Log } K_b \ 4.41 \pm 0.01$$

$$K_b \ (2.56 \pm 0.07) \cdot 10^4 \text{ L/mol}$$

Experimental conditions: BGE (borate buffer, pH 8.5), 58.5/50.0 cm ($l_{\text{tot}}/l_{\text{eff}}$), 25°C, hydrodynamic injection (40 s, 35 mbar (3.5 kPa)), separation voltage: 14 kV, $\lambda = 276$ nm, samples: mixture of 75 μ M HSA with 50-800 μ M DCF. (a – 5s, b – 10s, c – 20s, d – 30s, e – 40s, f – 60s, g – 80s, h – 120s)

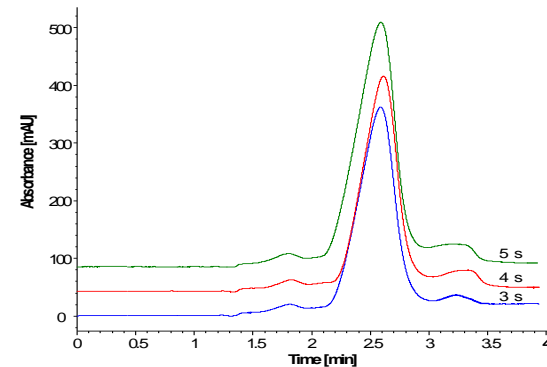
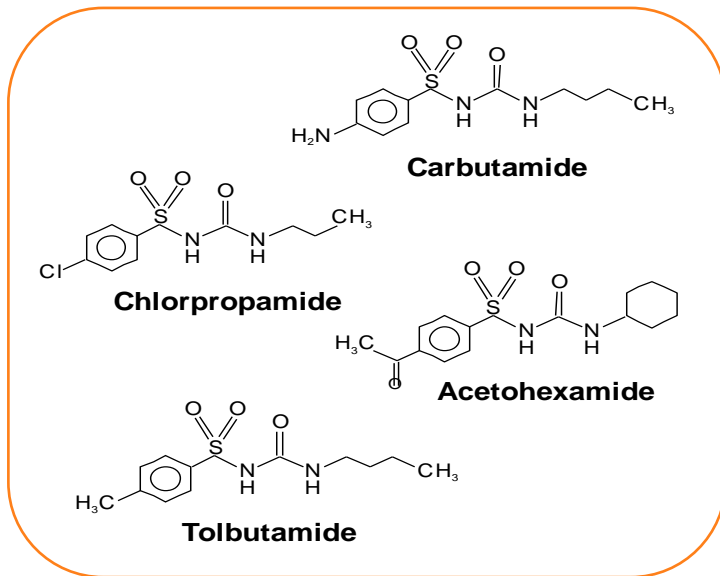


4 METODY

metoda	léčivo	n	K_b	Log K_b
CE-FA	DCF	5,00±0,12	(2,56±0,07)E+04	4,41±0,01
ITC	DCF	2,00±0,11	(1,22±0,09)E+04	4,09±0,03
CD	DCF		(7,91±0,12)E+04	4,90±0,01
ED	DCF	5,05±0,04	(3,55±0,26)E+04	4,55±0,03



CITLIVOST: HSA / GHSA



Experimental conditions: BGE (67 mM phosphate buffer, pH 7.4,) 48.5/8.5 cm (I_{tot}/I_{eff}), 37°C, hydrodynamic injection (5 s, 35 mbar (3.5 kPa)), separation voltage: -10 kV, $\lambda = 214$ nm, samples: mixture of 75 M HSA with 500 M drug

Table 1. Summary of the validation parameters

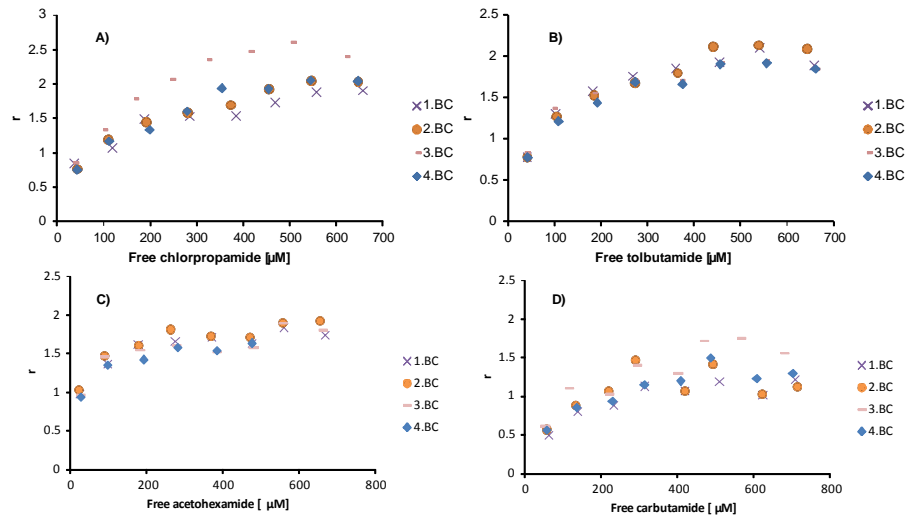
Parameters	Tolbutamide	Chlorpropamide	Acetohexamide	Carbutamide
Detection wavelength (nm)	214	200	250	250
Linearity range ^{a)} (μ M)	20–800	50–800	20–800	50–800
Regression equation	$y = 0.0835x + 0.3148$	$y = 0.1853x + 1.4402$	$y = 0.0874x - 0.1300$	$y = 0.1105x - 1.8760$
Correlation coefficient	0.9997	0.9991	0.9999	0.9989
Repeatability of plateau height – intraday ^{b)} (%)	< 3.37	< 2.56	< 3.53	< 3.08
Repeatability of plateau height – interday ^{c)} (%)	< 3.06	< 3.08	< 2.41	< 3.06
LOD (μ M)	5.4	11.2	5.2	11.7
LOQ (μ M)	16.3	33.9	15.7	35.5

a) All points were measured in triplicate ($n = 3$).

b) RSD for intraday ($n = 3$).

c) RSD for interday ($n = 6$).

CITLIVOST: HSA / GHSA

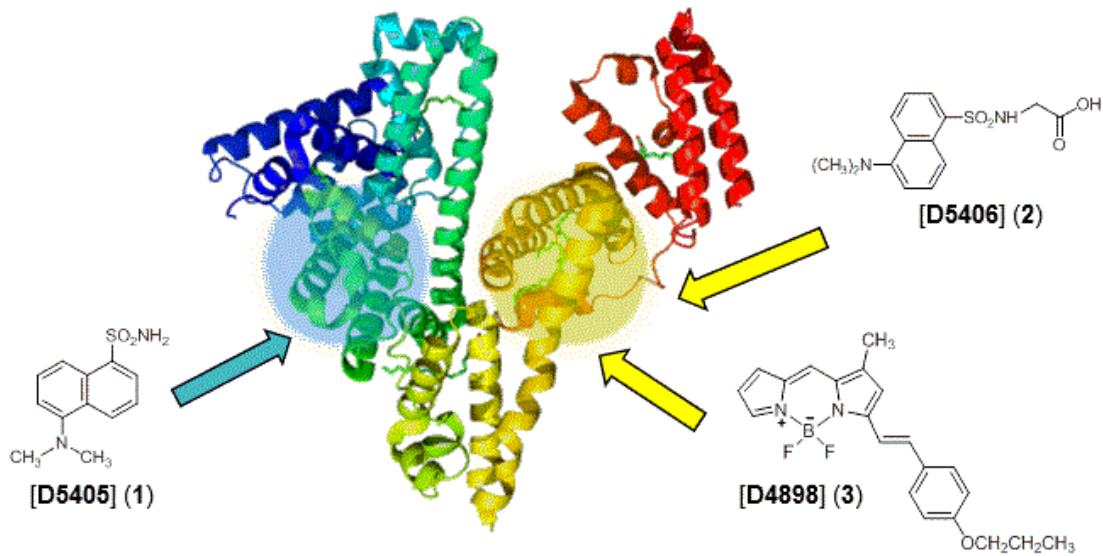


Repeatability of binding curves (BC) obtained for A) chlorpropamide-HSA, B) tolbutamide-HSA, C) acetohexamide-HSA, D) carbutamide-HSA systems with the CE-FA method.

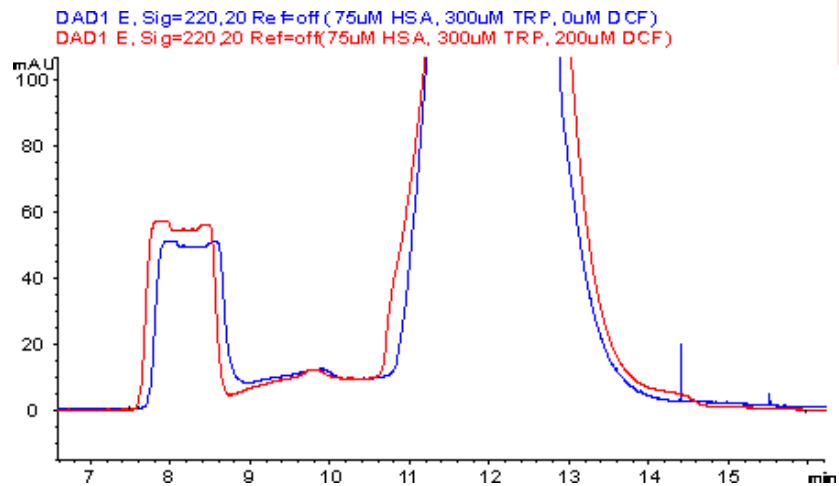
Table 2. Summary of binding parameters for HSA and gHSA

	HSA		gHSA	
	$K_b \pm \text{SD}$ (L/mol)	$n_i \pm \text{SD}$	$K_b \pm \text{SD}$ (L/mol)	$n_i \pm \text{SD}$
Tolbutamide	$(1.34 \pm 0.36) 10^4$	2.22 ± 0.17	$(4.84 \pm 1.59) 10^3$	2.60 ± 0.42
Chlorpropamide	$(9.71 \pm 2.42) 10^3$	2.47 ± 0.43	$(2.93 \pm 1.06) 10^3$	2.84 ± 0.41
Acetohexamide	$(4.18 \pm 0.16) 10^4$	1.78 ± 0.09	$(1.95 \pm 0.59) 10^4$	1.26 ± 0.07
Carbutamide	$(9.53 \pm 1.07) 10^3$	1.54 ± 0.27	$(2.27 \pm 0.06) 10^3$	3.08 ± 0.50

KOMPETICE



léčivo	Log K _b
DCF	4,41±0,01
TRP	3,95±0,01



A NENÍ TO JEN O MOLEKULÁCH...

938

Electrophoresis 2017, 38, 938–941

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Short Communication

Evaluation of interactions between RAW264.7 macrophages and small molecules by capillary electrophoresis

In this study, the affinity interactions between RAW 264.7 macrophages and three small molecules including naringin, oleuropein and paeoniflorin were evaluated by affinity capillary electrophoresis (ACE), partial filling affinity capillary electrophoresis (PFACE) and frontal analysis capillary electrophoresis (FACE), respectively. The result indicated that ACE (varying concentrations of cell suspension were filled in the capillary as receptor) may not be suitable for the evaluation of interactions between cell and small molecules due to the high viscosity of cell suspension; PFACE can qualitatively evaluate the interaction, but the difference in viscosity between RAW264.7 suspension and buffer effects on the liner relationship between filling length and injection time, which makes the calculation of binding constant difficult. Furthermore, based on the PFACE results, naringin showed stronger interaction with macrophages than the other two molecules; taking advantage of the aggregation phenomenon of cell induced by electric field, FACE was successfully used to determine the stoichiometry ($n = 5 \times 10^9$) and binding constant ($K_b = 1 \times 10^4$ L/mol) of the interaction between RAW264.7 and naringin.

Keywords:

Affinity interaction / Cell aggregation / Frontal analysis capillary electrophoresis / RAW264.7 macrophage
DOI 10.1002/elps.201600345

Chromatographia (2018) 81:509–516
<https://doi.org/10.1007/s10337-018-3476-6>

ORIGINAL



Evaluation of the Interactions Between Platelets and Alkaloids by Frontal Analysis Capillary Electrophoresis Using Polyvinyl Alcohol-Coated Capillary

Qiao-Qiao Li¹ · Su-Ying Li² · Feng-Qin Wang¹ · Hua Chen¹ · Yuan-Jia Hu³ · Zhi-Ning Xia¹ · Feng-Qing Yang¹

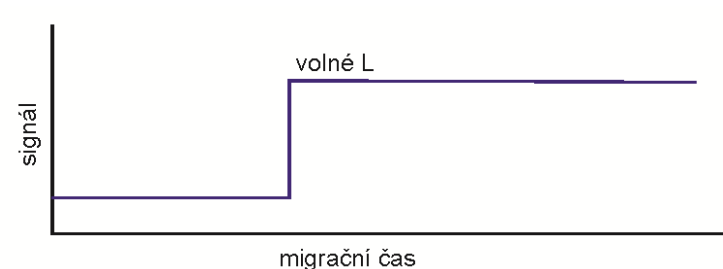
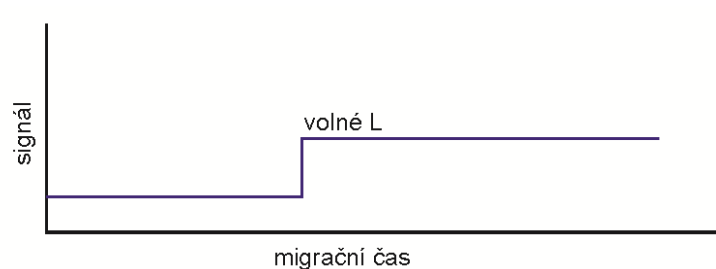
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Abstract

Capillary electrophoresis (CE) has been widely used in the study of the interactions between targets (biological macromolecules) and ligands due to its good biocompatibility. However, the separation and analysis of platelets, which play a pivotal role in thrombotic diseases, were limited owing to platelets adhesion in inner wall of capillary during CE analysis. In this paper, polyvinyl alcohol-coated capillaries were simply prepared to prevent the adhesion of platelet. Then, a frontal analysis CE (FACE) method was developed to evaluate the interactions between platelets and eight alkaloids include glaucine, magnoflorine, dehydrocorydaline, palmatine, berberine, isorhynchophylline, rhynchophylline, and brucine. The binding constants and stoichiometries of the interactions were calculated by Scatchard equation for strong and specific interaction, and the non-specific binding equation for weak interaction. The results indicated that glaucine, dehydrocorydaline, and isorhynchophylline showed relatively high affinity interaction with platelets. The developed FACE method may be further applied in the evaluation of interactions between platelets and other small molecular compounds.

FACCE

- BGE bez interagujících partnerů
 - Vzorek – oba interakční partneri, kontinuální dávkování
 - Podmínky – „stejná“ mobilita komplexu a jeden z partnerů, druhý partner (ligand) má mobilitu větší
- + % vazby
 - + stechiometrie
 - + nízká spotřeba
 - kalibrace
 - vyšší spotřeba vzorku
 - LOD



FACCE

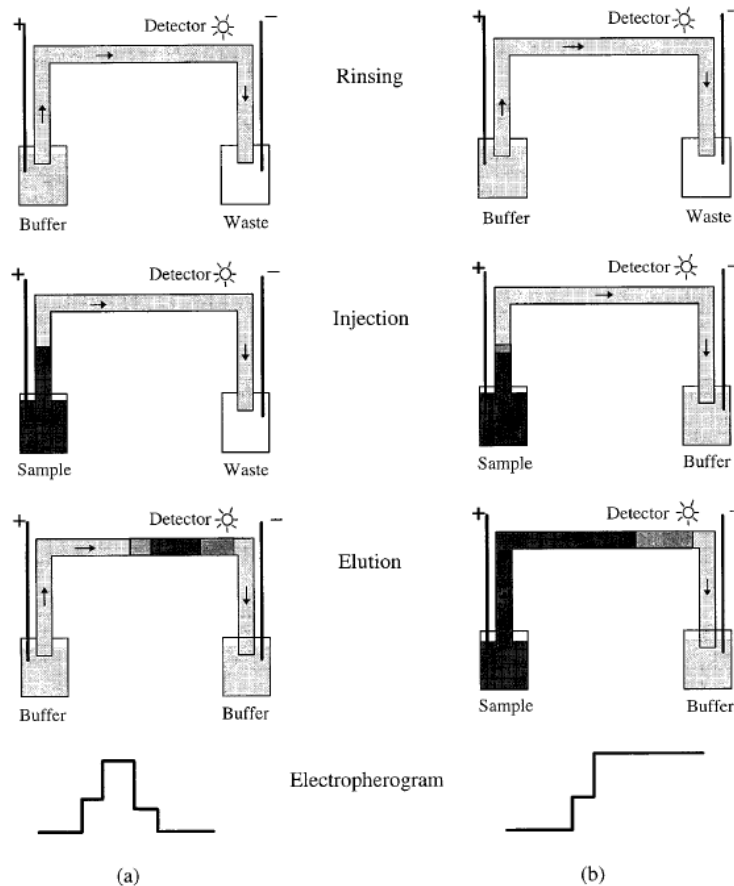


Figure 1. Schematics of CFC in CE (a) and FACCE (b).

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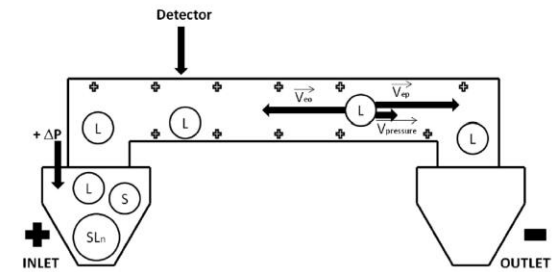
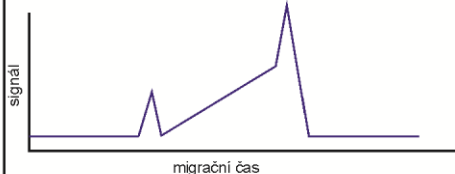


Fig. 1. Schematic representation of the separation occurring during the FACCE experiments. V_{eo} represents the velocity of the electroosmotic flow, $V_{pressure}$ the velocity due to the applied pressure, V_{ep} the velocity due to the effective electrophoretic motion of the corresponding compound.

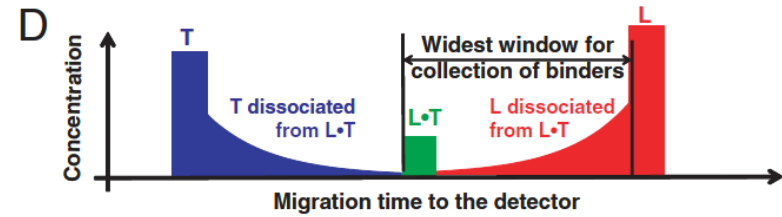
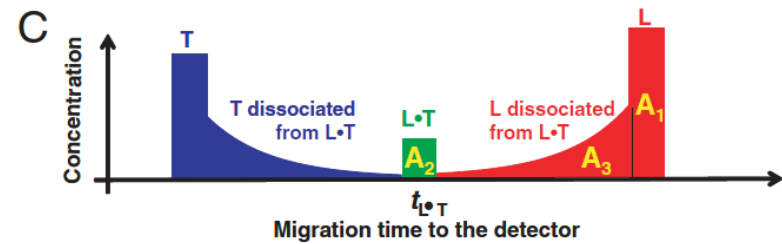
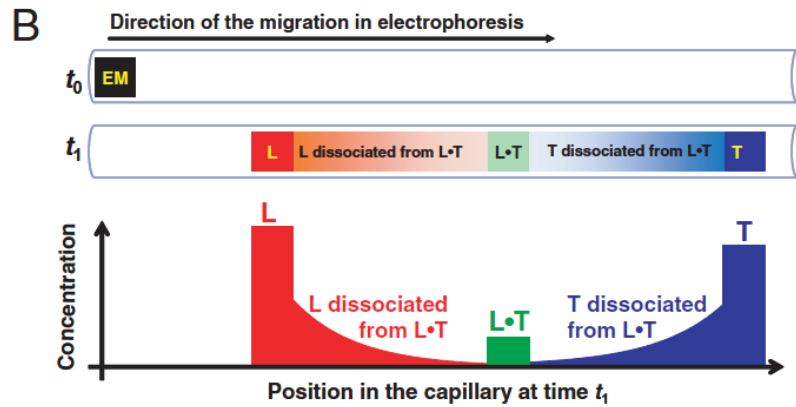
KINETICKÉ MÓDY CE

- Prof. Krylov – 2002
- Několik variant KCE
- K_b , K_d , k_{on} , k_{off}
- NECEEM – Non-Equilibrium Capillary Electrophoresis of Equilibrium Mixtures

Metoda	Základní elektrolyt	Vzorek	Hodnotící parametry	Získané informace	Podmínky	Typický záznam
NECEEM	BGE	S + L	plocha píku. migrační čas	K_d , k_{on} , k_{off}	$\mu_L \neq \mu_S$	

KCE

Krylov



KCE

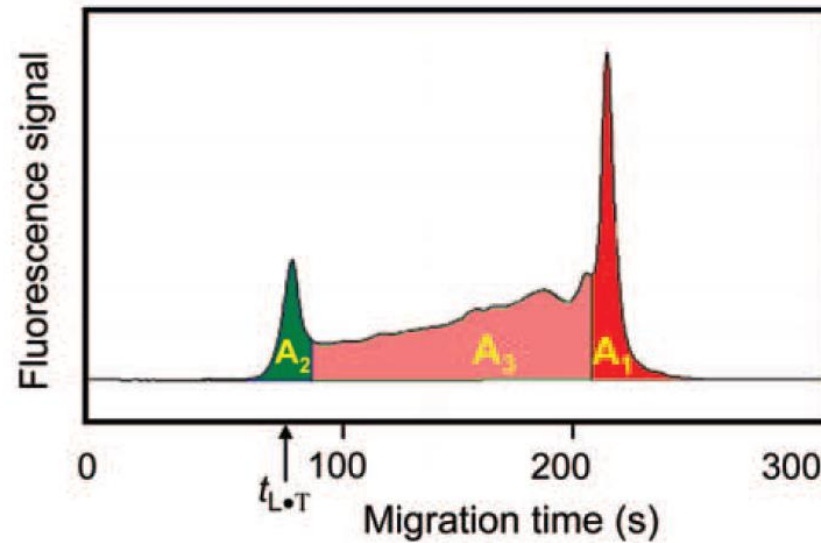


FIG. 4. Example of a nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) electropherogram for the interaction between ssDNA and an ss-binding protein. The colors do not exactly correspond to those in **Figure 3C**: area A_3 is shown by a lighter shade of red to better distinguish between A_1 and A_3 .

PROČ CE RESP. CE-FA?

1. Nevyžaduje vysoce purifikované vzorky, značení ani imobilizaci
2. Nízká spotřeba vzorku (nL) a dalších reagentů
3. Rychlost analýz
4. Možná simulace různých prostředí
5. Velmi robustní metoda – díky platu
6. Využitelnost metody pro systémy s rychlou i pomalou kinetikou
7. Možnost fitování dat s využitím vícevazných modelů a charakterizace vazebné stechiometrie
8. Separace volného ligandu a jeho analýza je prováděna v jednom kroku – integrované řešení
9. Přímé stanovení volného ligandu ve vzorku



TAKE HOME MESSAGE

- Výběr z množství technik - různé principy, požadavky na vzorky, detekční limity, ...
- Neexistuje jediný ideální způsob
- Znalost metod je zásadní pro dosažení nejlepších výsledků
- Důležité je metody kombinovat

DĚKUJI ZA POZORNOST

