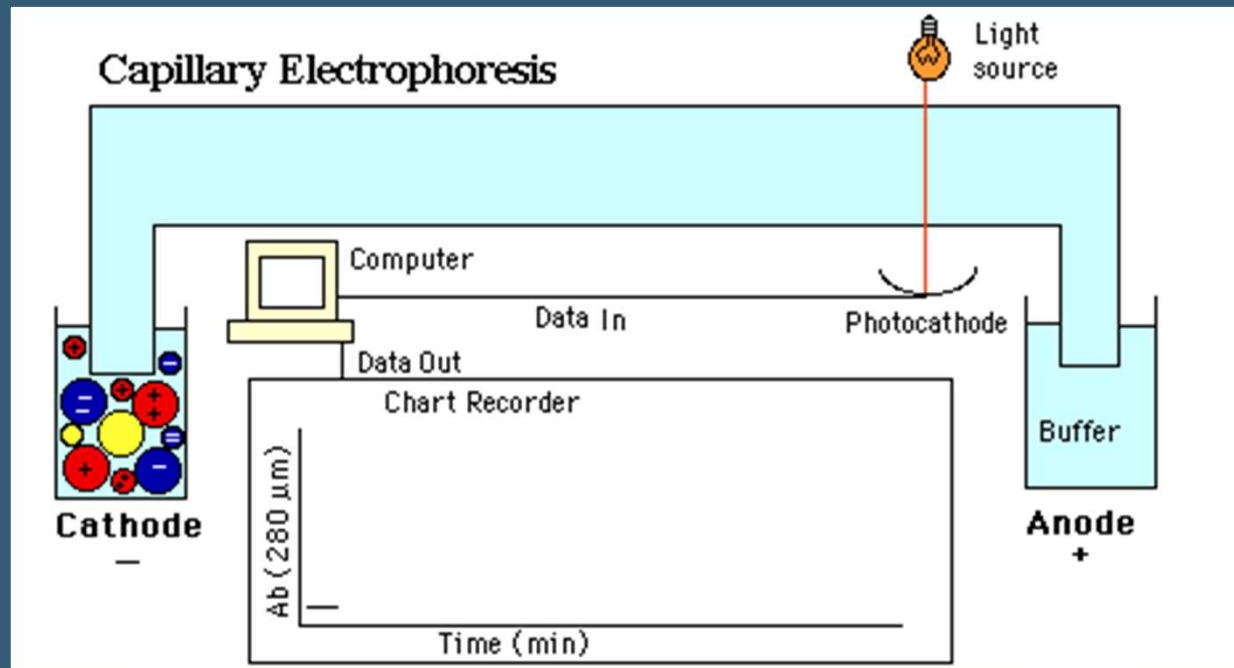
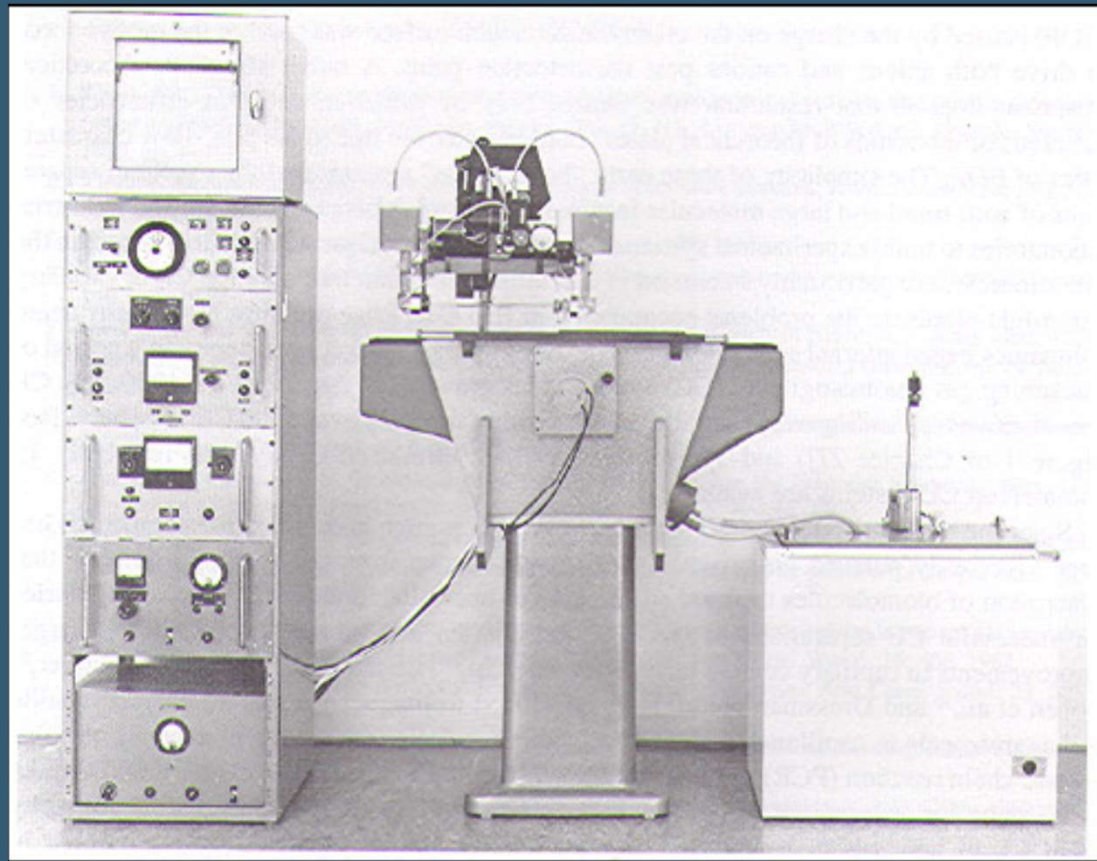


KAPILÁRNÍ ZÓNOVÁ ELEKTROFORÉZA CZE



HJERTEN 1967



1981 - JORGENSON LUKACSOVÁ



CLIN. CHEM. 27/9, 1551-1553 (1981)

Free-Zone Electrophoresis in Glass Capillaries

James W. Jorgenson and Kryn DeArman Lukacs

A simple theory of zone electrophoresis in open-tubular capillaries is developed. According to this theory, to achieve the highest resolution of zones, tubes with as small an inside diameter as possible should be used in combination with as high an applied voltage as feasible. To test this approach, we performed electrophoresis in glass capillaries with an internal diameter of $75\ \mu\text{m}$ and a length of 100 cm. A special fluorescence detector was used to detect fluorescent zones while they migrated inside the capillary. With the application of 30 kV potentials to this system, rapid and efficient separations of amino acids, peptides, and urinary amines were demonstrated. In all cases fluorescent derivatives were necessary for detection. Preliminary results are encouraging, and with further development of sensitive detection devices, applications in clinical analyses may be feasible.

small diameter simultaneously facilitates heat transfer as well as stabilizes the medium. Regardless of the diameter, some radial temperature gradient will persist. However, this temperature gradient is undesirable only to the extent that a significant fraction of the solute molecules making up a zone spend longer than average times migrating within "cool" or "hot" regions of the tube radius. Here a more subtle effect of reduced tube diameters comes into play. If the diameter is small enough that solute molecules diffuse back and forth across the tube many times during their migration, then the probability that a significant fraction of molecules will spend excessive amounts of time in any one portion of the tube radius is greatly reduced. Thus the solute molecules have an excellent chance of traveling throughout all portions of the tube radius, and any variations in migration velocity will tend to average out.

To summarize, the possible advantages of performing zone electrophoresis in open tubes of small diameter are:

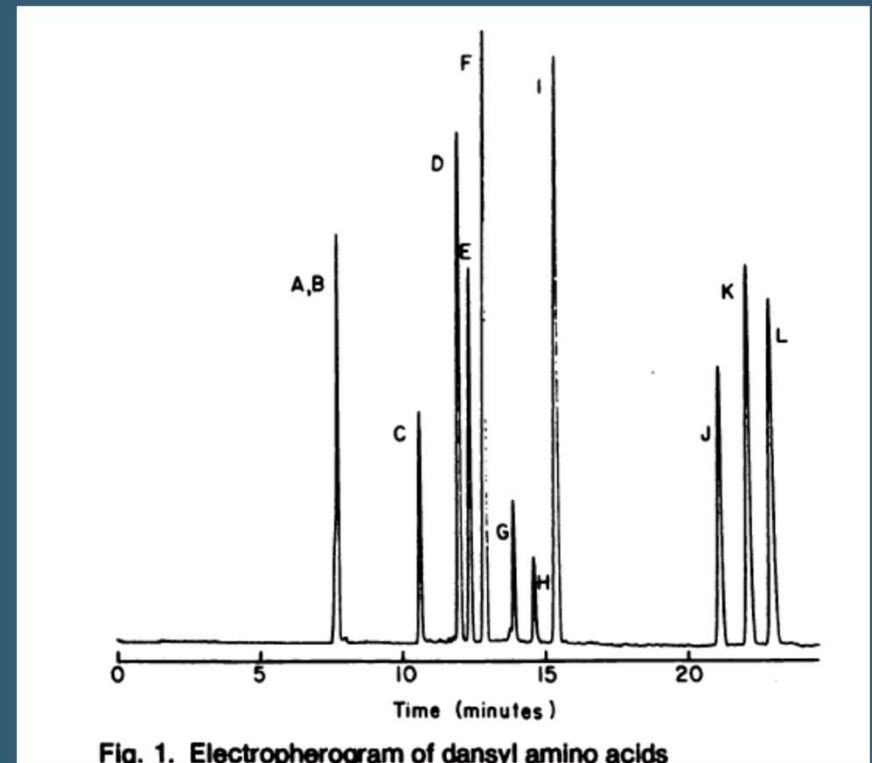


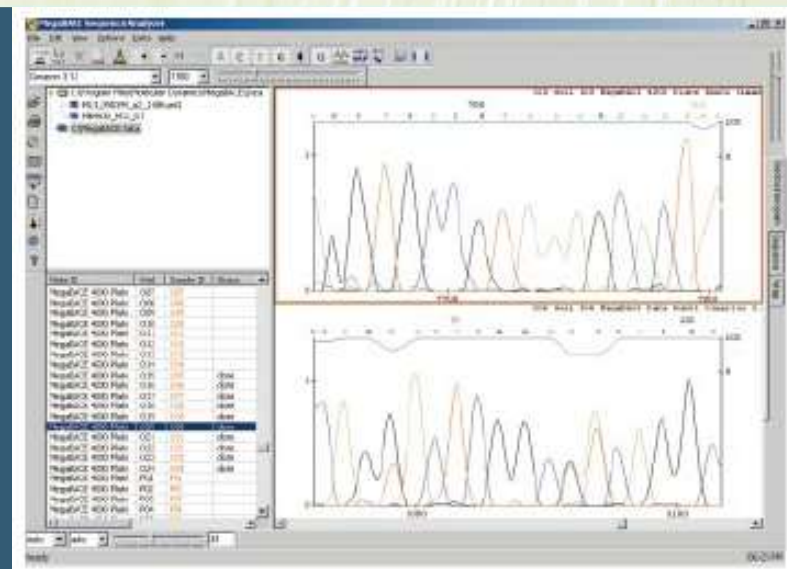
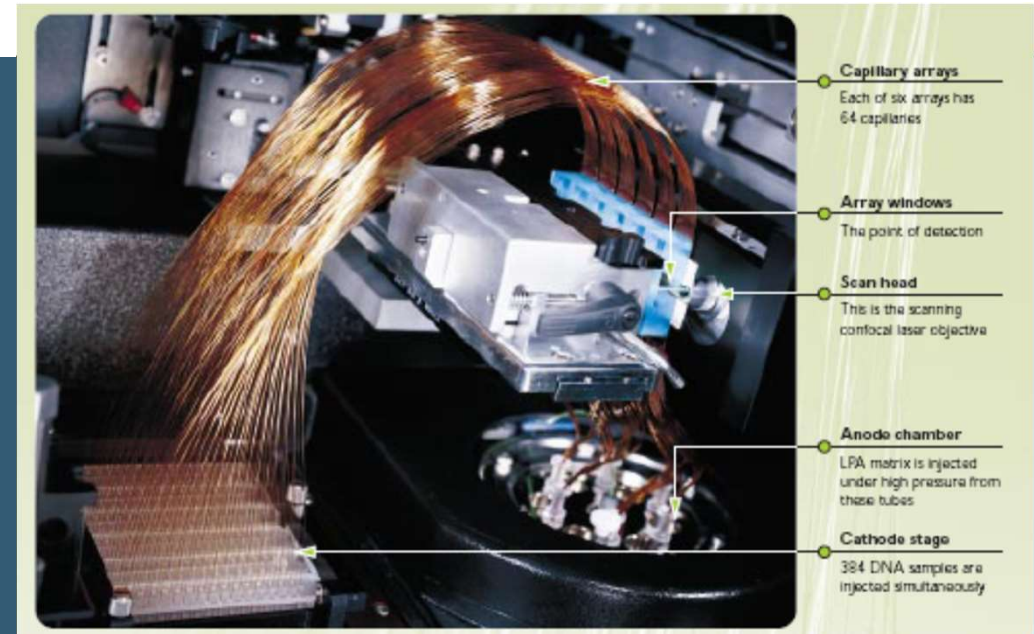
Fig. 1. Electropherogram of dansyl amino acids

BECKMAN 1987



MUNI
SCI

2003 - PROJEKT LIDSKÉHO GENOMU



PROČ CE A BIOCHEMIE ?

VÝHODY CE

- **Aplikační diverzita**
 - nabité i neutrální látky
 - nízkomolekulární i vysokomolekulární látky
 - chirální i achirální látky
 - bakterie i viry

VÝHODY CE

- Aplikační diverzita
- Jednoduchá instrumentace

CZE, MEKC,
CIEF, CITP
NACE, MEEKC,
CGE, ChCE



CEC

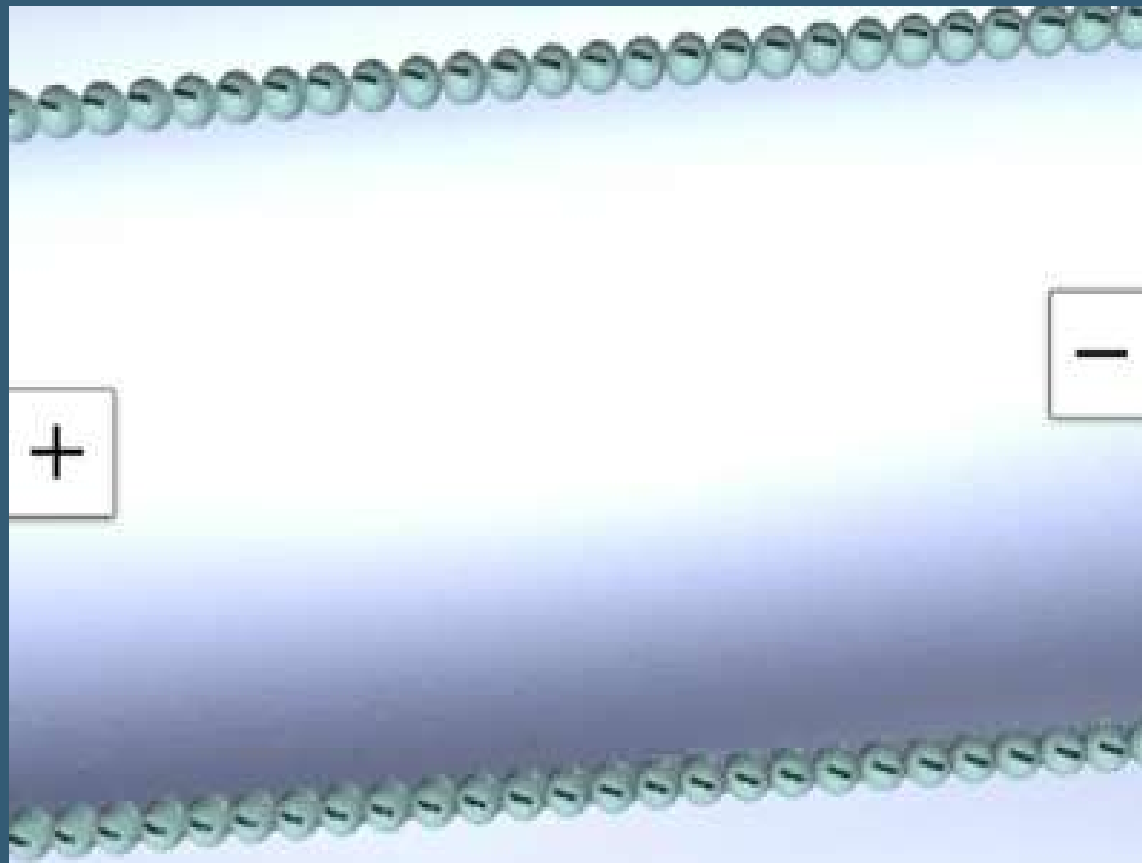
VÝHODY CE

- Aplikační diverzita
- Jednoduchá instrumentace
- Vysoké rozlišení a účinnost separací
- Malá spotřeba vzorku
- Rychlost analýzy
- Malá spotřeba chemikálií a malé množství odpadů

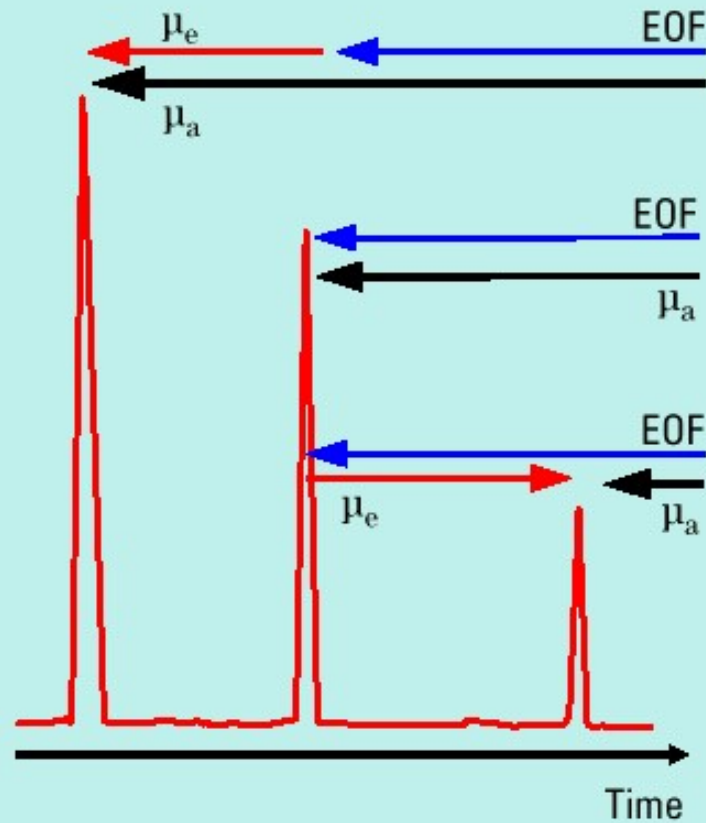
MÓDY CZE

Use CE Mode...	For Analysis of...
Capillary zone electrophoresis (CZE)	Ions, etc.
Micellar electrokinetic chromatography (MEKC)	Neutral and ionic analytes
Chiral capillary electrophoresis (CCE)	Chiral molecules
Capillary electrochromatography (CEC)	Small molecules
Capillary gel electrophoresis (CGE/SDS-PAGE)	DNA/RNA size/protein MW
Capillary isoelectric focussing (CIEF)	Protein/peptide isoelectric point
Capillary isotachopheresis (CITP)	Ions

KAPILÁRNÍ ZÓNOVÁ ELEKTROFORÉZA VE VOLNÉ KAPILÁŘE



VÝSLEDNÁ MOBILITA ČÁSTIC PŘI CZE



cation — positive μ_e

neutral — zero μ_e

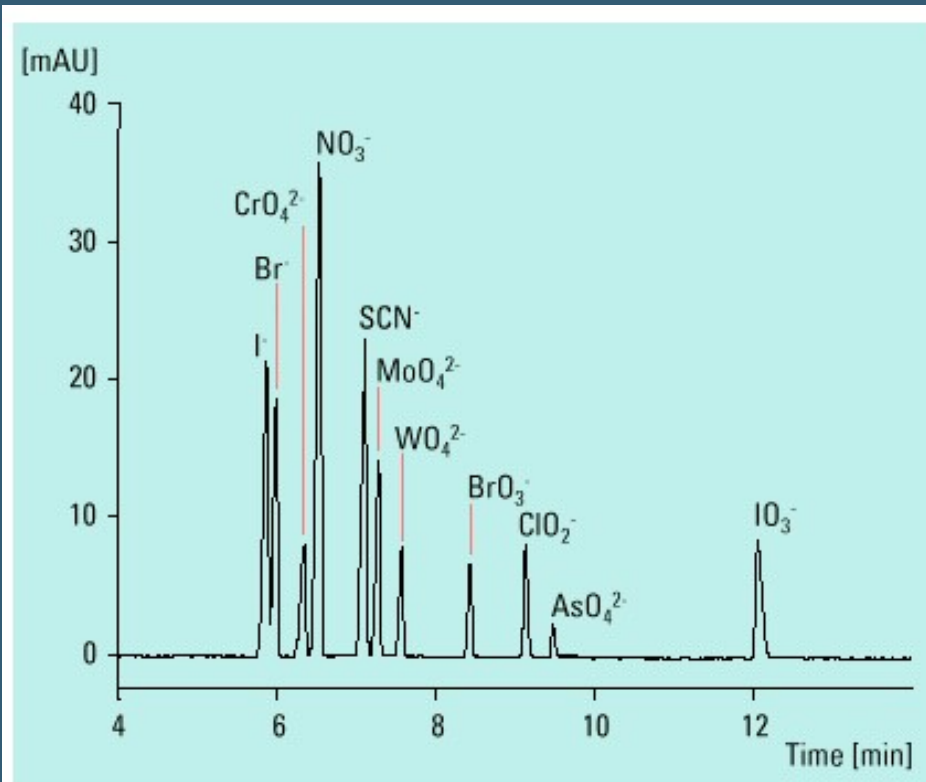
anion — negative μ_e

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

μ_a = apparent mobility

μ_e = effective mobility

SEPARACE ANIONTŮ POMOCÍ CZE



Sample

100 mg/l each analyte

Buffer

20 mM phosphate, pH 8.0

Capillary

DB WAX (J&W)

l = 56 cm

L = 64.5 cm

id = 50 μm

Injection

200 mbar · s

Temperature

20 °C

Electric Field

230 V/cm, reversed polarity

Detection

Signal 200, 10 nm

Reference 450, 80 nm

STANOVENÍ AKTIVITY HD POMOCÍ CZE

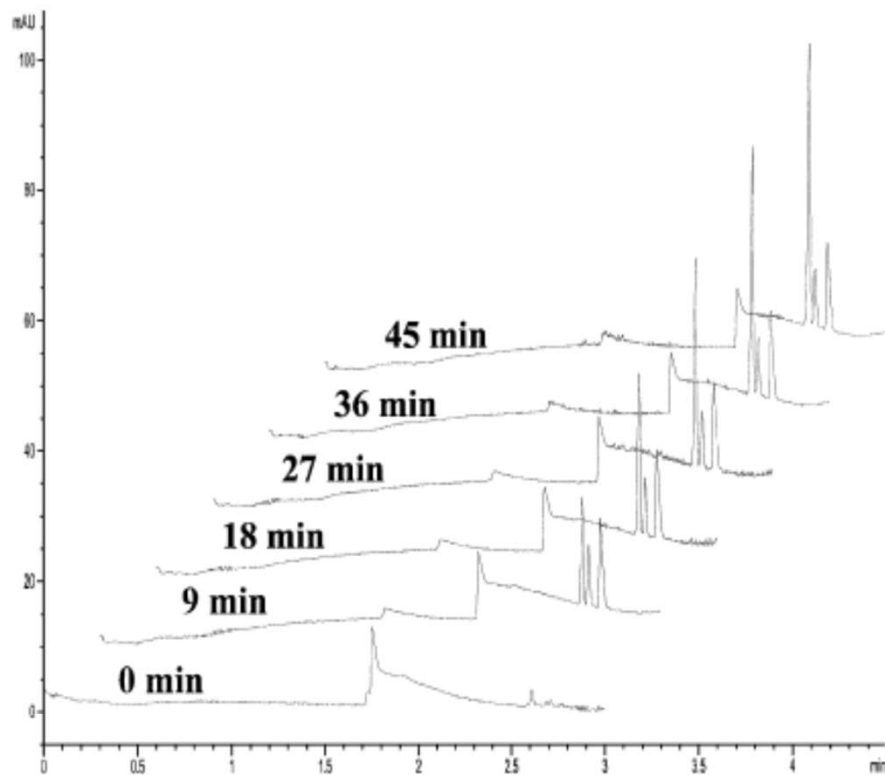


Fig. 1. CZE analysis of haloalkane dehalogenase catalysed reaction using indirect detection. Overlaid electropherograms show the reaction mixture of 10 mM 1,3-dibromopropane in 50 mM glycine buffer (pH 9.0) at 30°C before and 9, 18, 27, 36 and 45 min after addition of 10 μ l of enzyme solution. The separation conditions are the same as in Table 1.

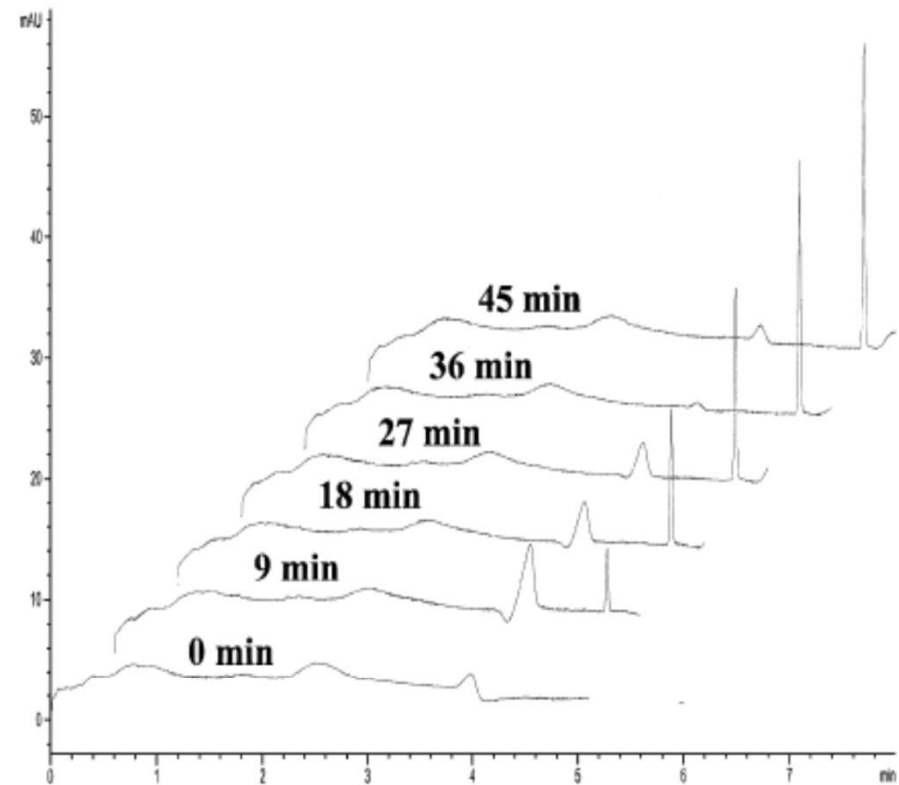
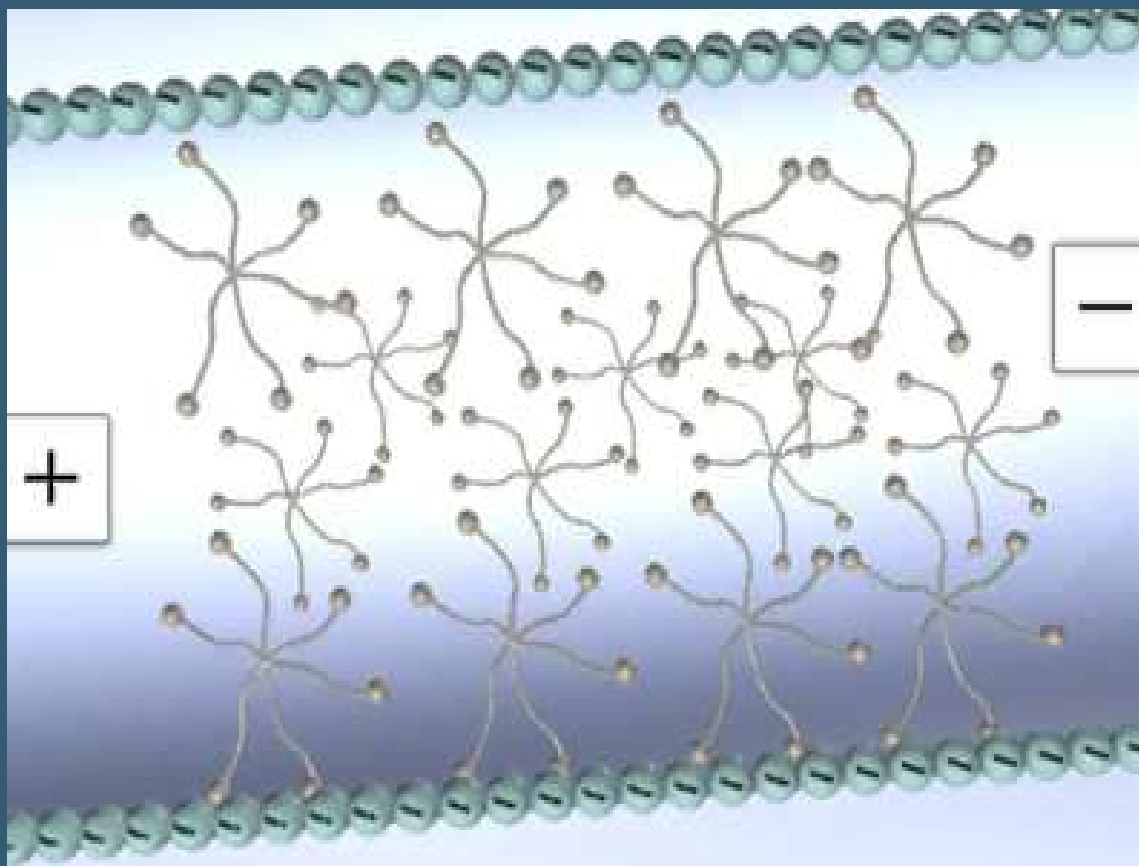


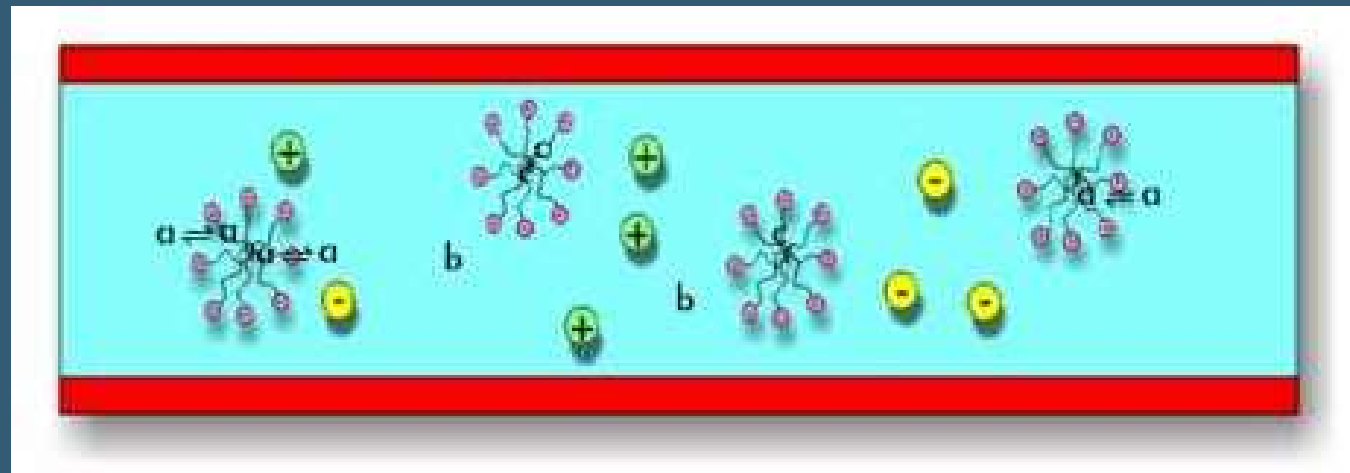
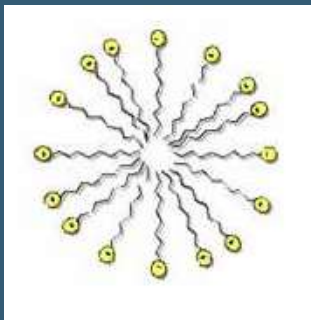
Fig. 2. CZE analysis of haloalkane dehalogenase catalysed reaction using long-end injection in combination with direct detection. The enzyme preparation was 4 times diluted with 50 mM phosphate buffer (pH 7.5). Separation conditions are the same as in Table 2. The other conditions are the same as in Fig. 1.

MICELÁRNÍ ELEKTROKINETICKÁ CHROMATOGRAFIE



PRINCIP MEKC

MICELA

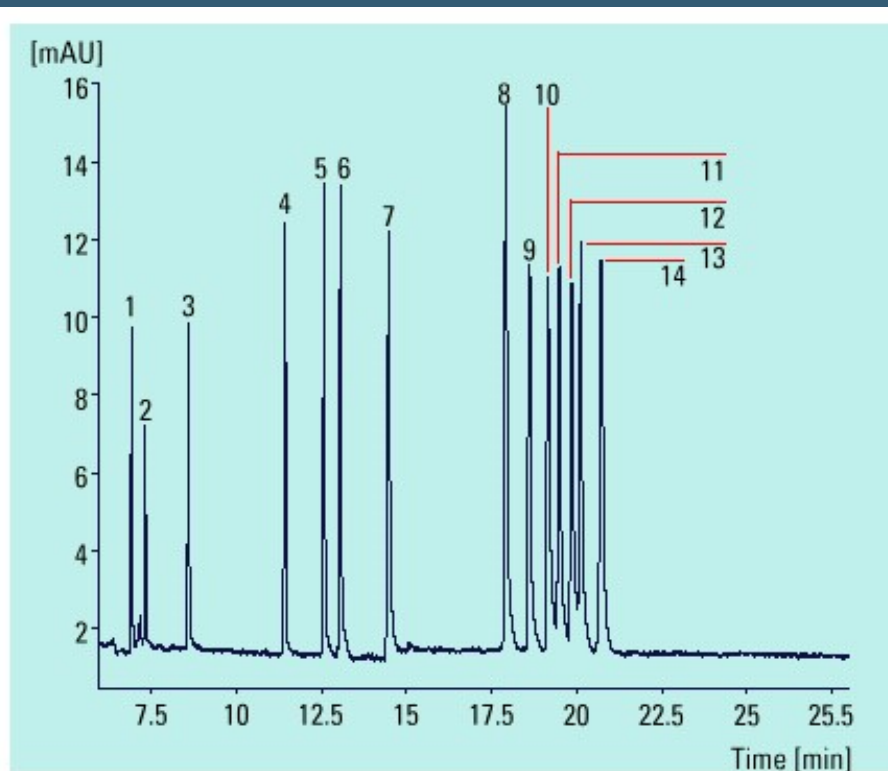


a – střed – rozpustná v obou

b – silně hydrofilní – nerozpustná
v micelle

c – silně hydrofóbní – nerozpustná
ve vodné fázi

SEPARACE FENOLŮ A ALKOHOLŮ POMOCÍ MEKC



- 1 4-hydroxybenzyl alcohol (18 ppm)
- 2 3-hydroxybenzyl alcohol (9 ppm)
- 3 Phenol (26 ppm)
- 4 2-hydroxybenzyl alcohol (18 ppm)
- 5 m-cresol (19 ppm)

- 6 p-cresol (32 ppm)
- 7 2-chlorophenol (22 ppm)
- 8 2,6-xylenol (36 ppm)
- 9 o-ethylphenol (23 ppm)

Buffer

90 mM borate, pH 8.6, 70 mM SDS

Capillary

PVA-coated (no EOF)

l = 56 cm

L = 64.5 cm

id = 50 μ m BF 3

Injection

20 mbar · s

Electric Field

465 V/cm

Temperature

Capillary 12 °C

Detection

Signal 200/10 nm

Reference 350/80 nm

- 10 2,3-xylenol (20 ppm)
- 11 2,5-xylenol (22 ppm)
- 12 3,4-xylenol (23 ppm)
- 13 3,5-xylenol (19 ppm)
- 14 2,4-xylenol (27 ppm)

STANOVENÍ AKTIVITY CYP 2C9 POMOCÍ MEKC

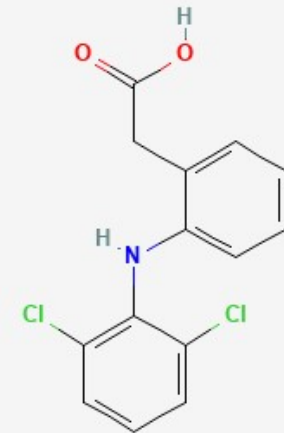
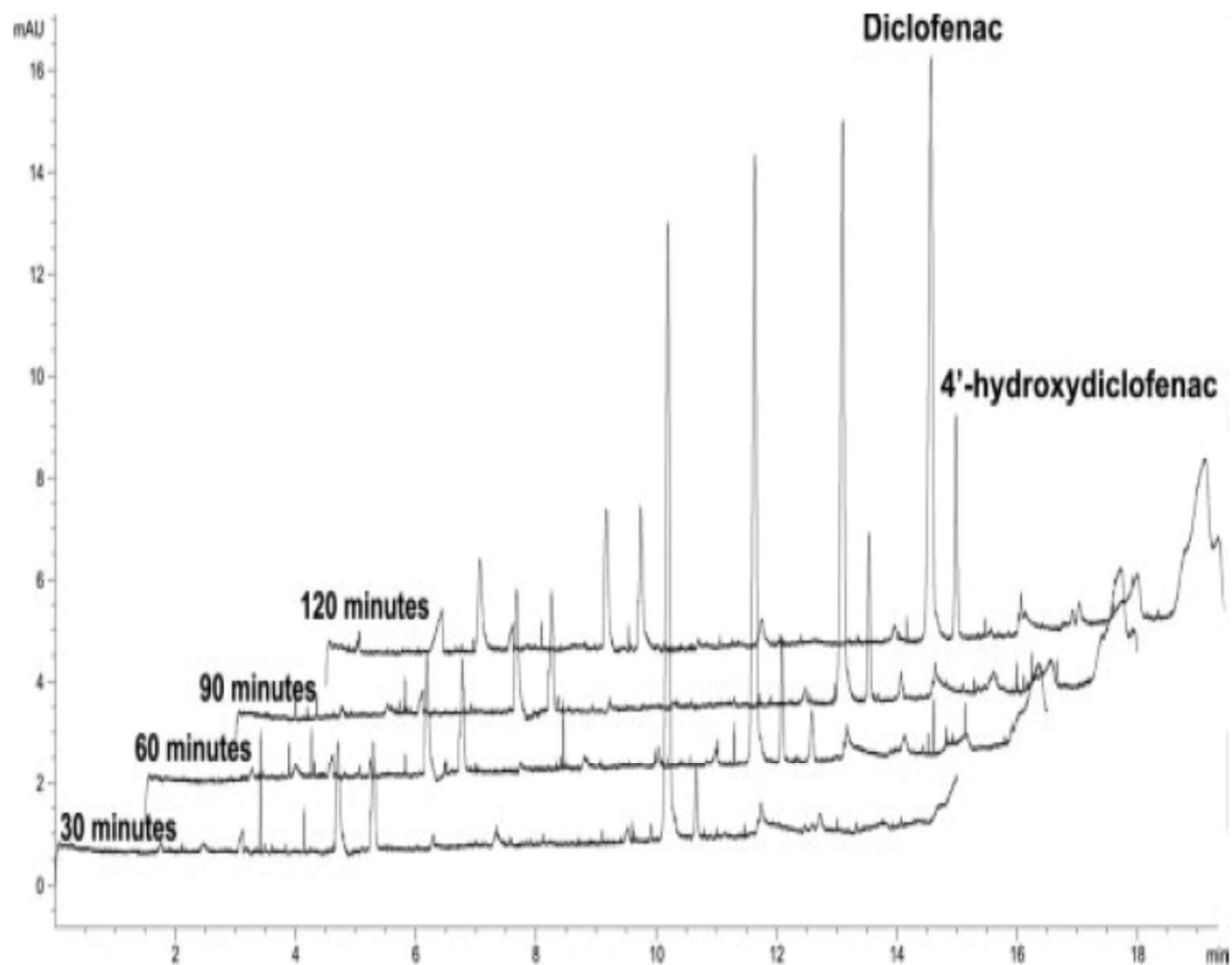
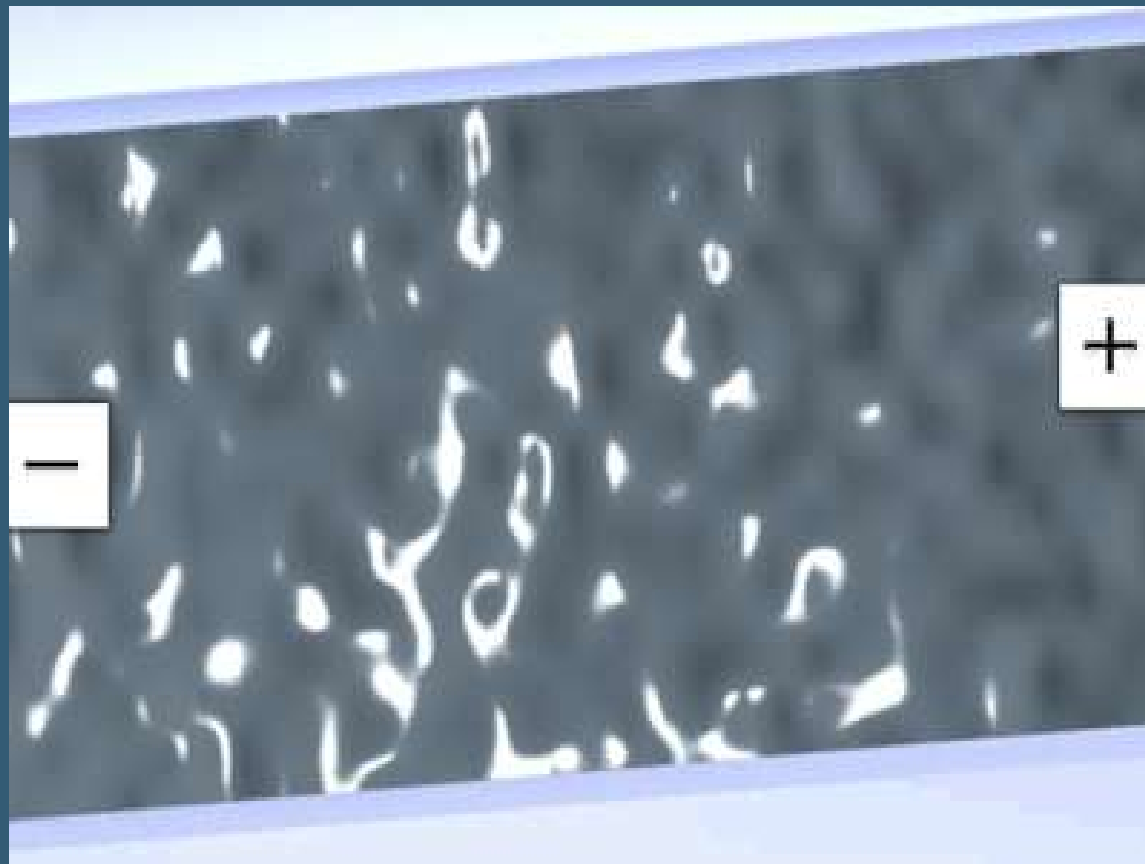
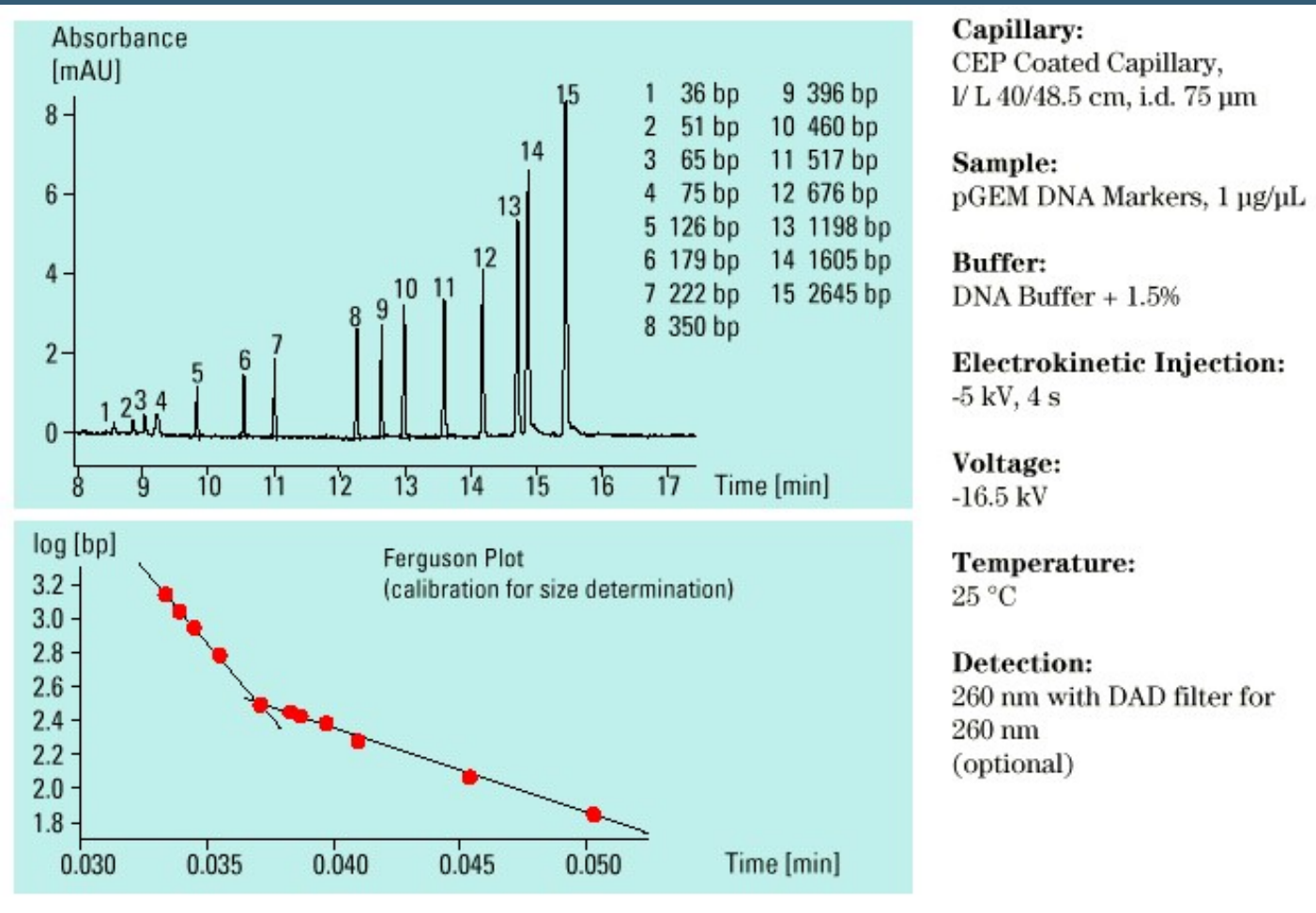


Figure 3. MEKC analysis of CYP2C9 reaction. Overlaid electropherograms show the enzymatic reaction under standard conditions as described in Section 2; the final concentrations of CYP2C9 and diclofenac in reaction mixture were 9.7 nM and 100 μ M, respectively. MEKC conditions as in Fig. 2.

KAPILÁRNÍ GELOVÁ ELEKTROFORÉZA

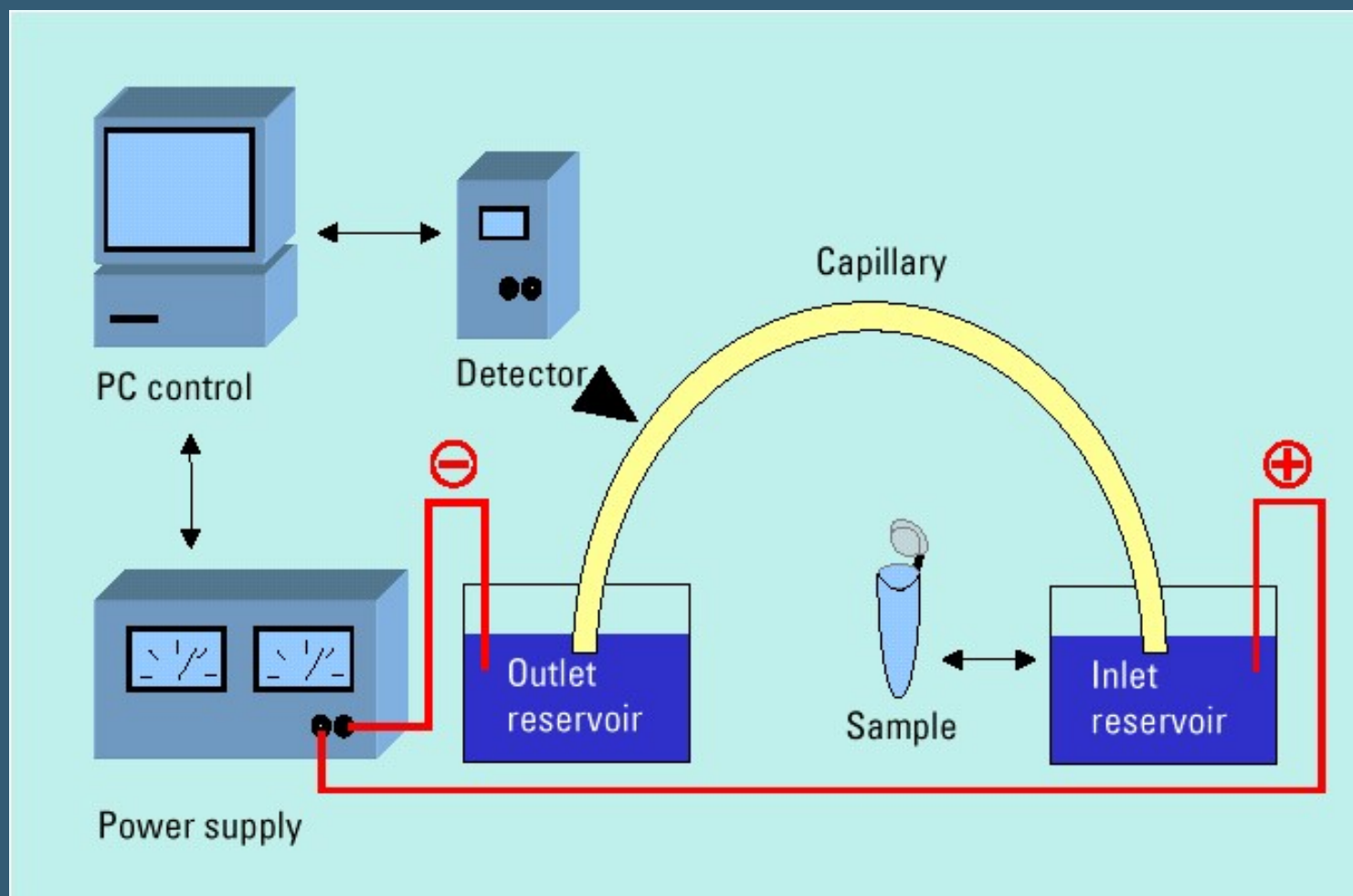


CGE FRAGMENTŮ dsDNA



INSTRUMENTACE CZE

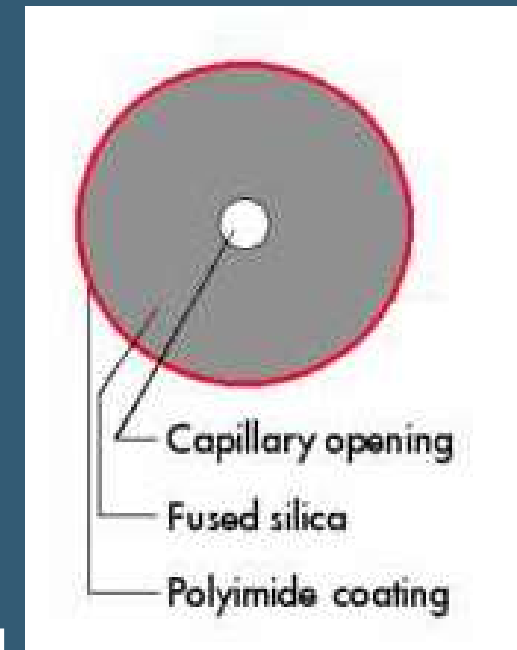
SCHÉMA ZAŘÍZENÍ PRO CZE



NAPÁJECÍ ZDROJ

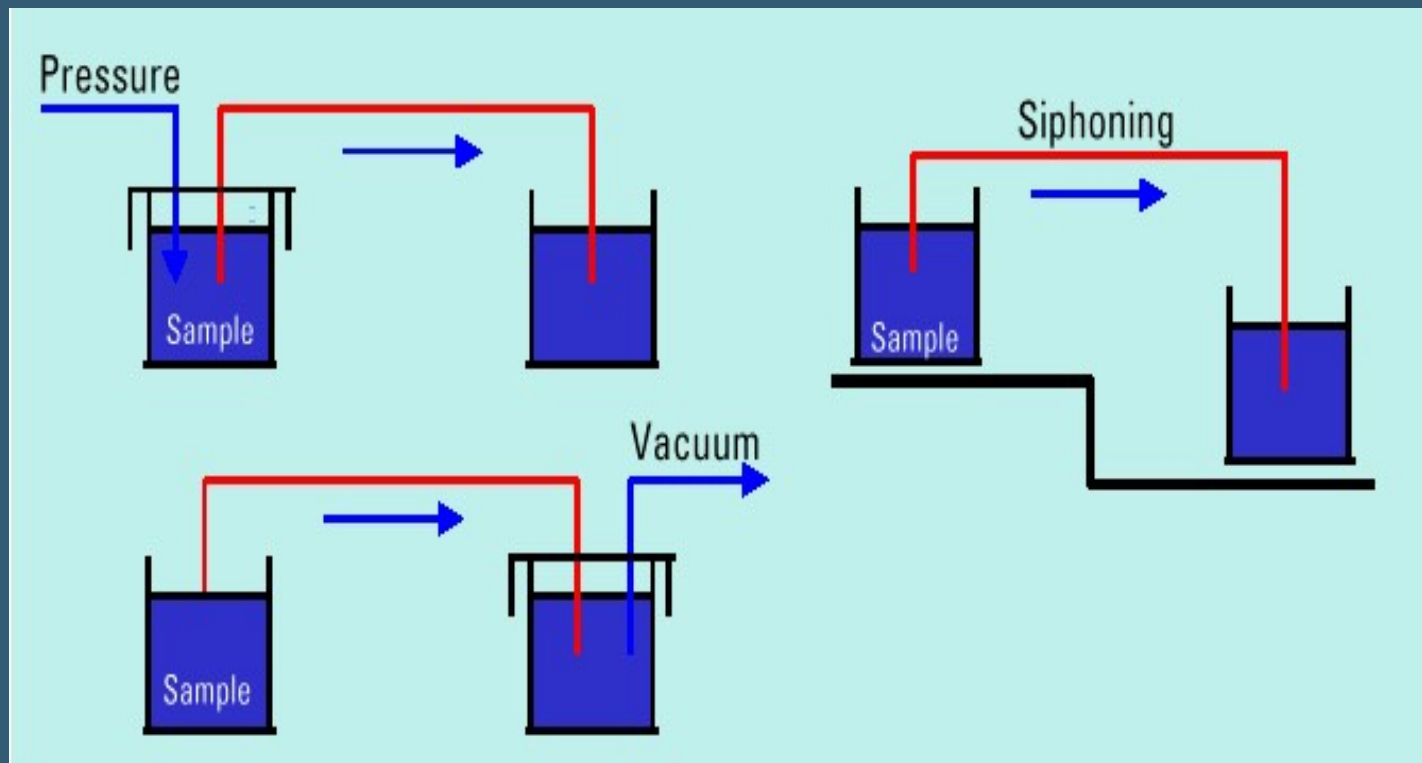
KAPILÁRA

- stabilizovaný $\pm 30 \text{ kV } 300 \text{ } \mu\text{A}$
- konstantní napětí nebo proud
- obojí polarita
- ochrana obsluhy

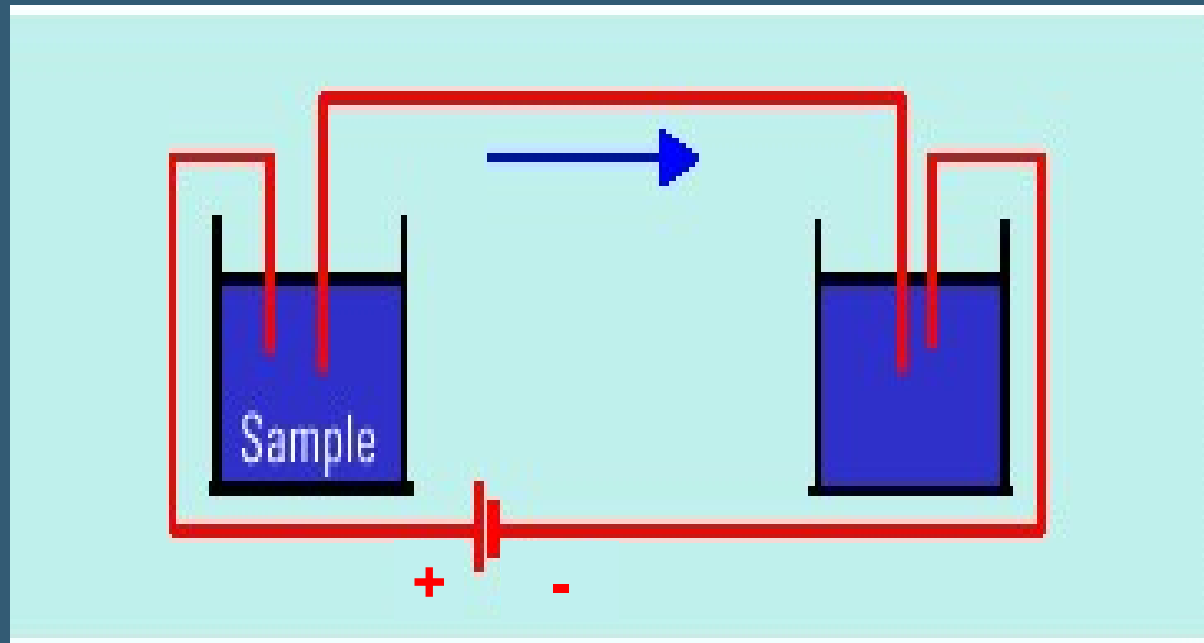


- křemenná - 25 - 100 μm i.d.
- 350 μm o.d.
- délka 10 až 100 cm
- polyimidové vnější pokrytí

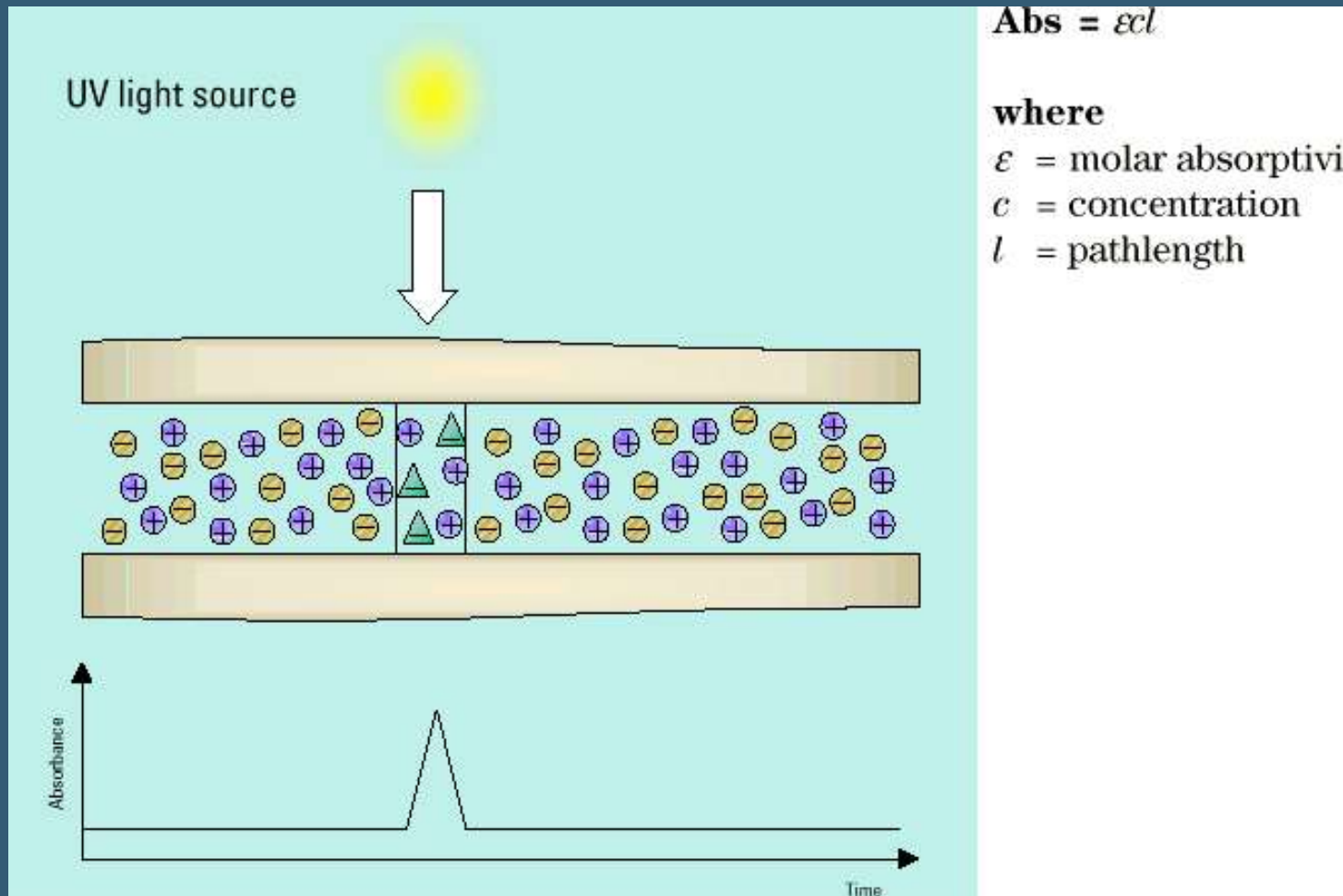
DÁVKOVÁNÍ - HYDRODYNAMICKÉ



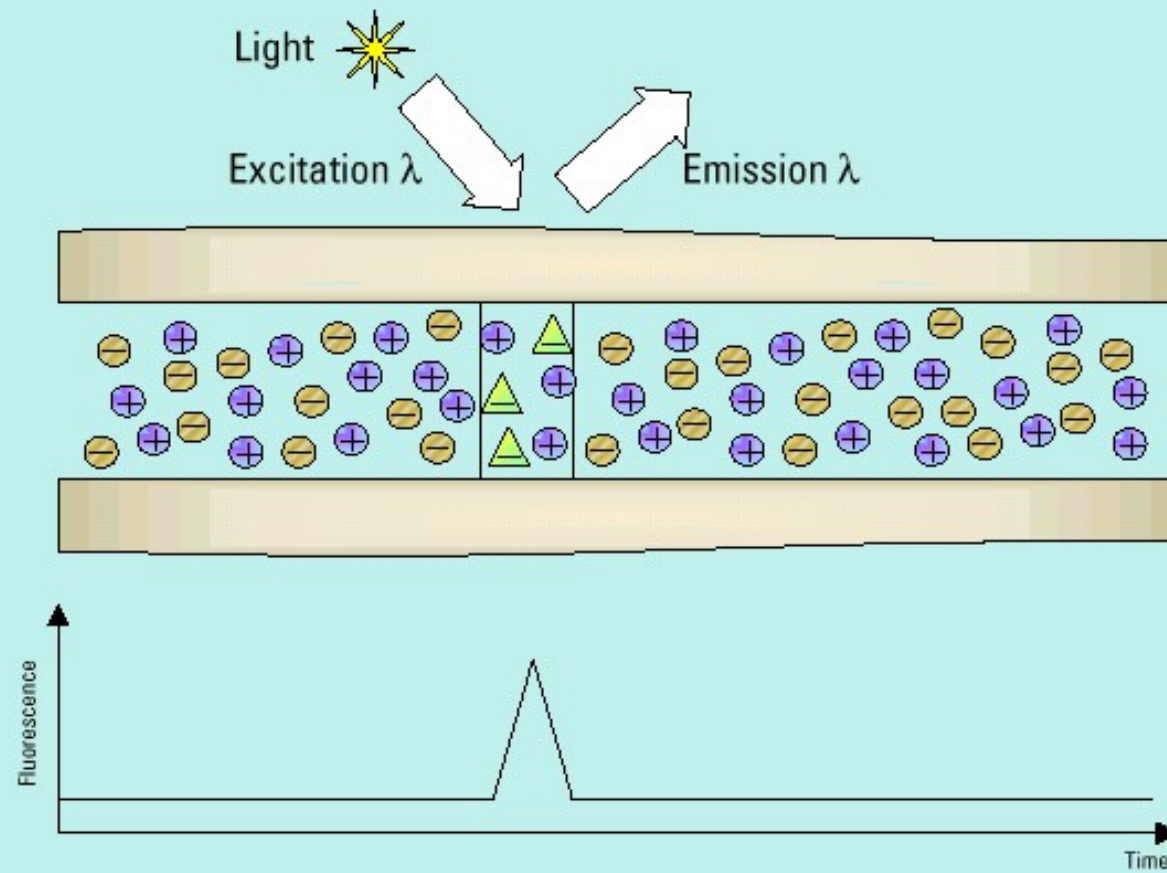
DÁVKOVÁNÍ - ELEKTROKINETICKÉ



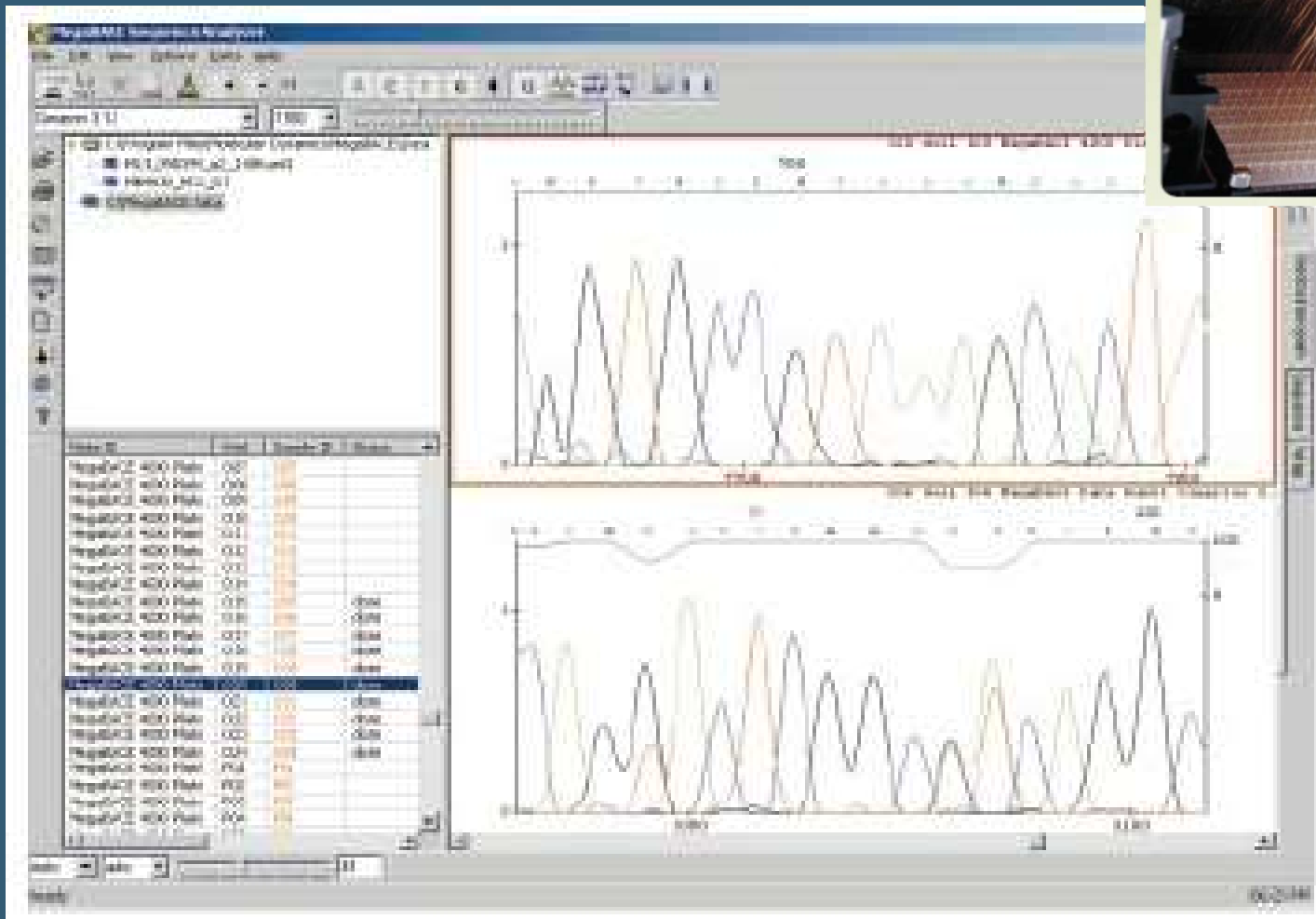
DETEKCE SPEKTROFOTOMETRICKÁ



DETEKCE FLUORESCENČNÍ



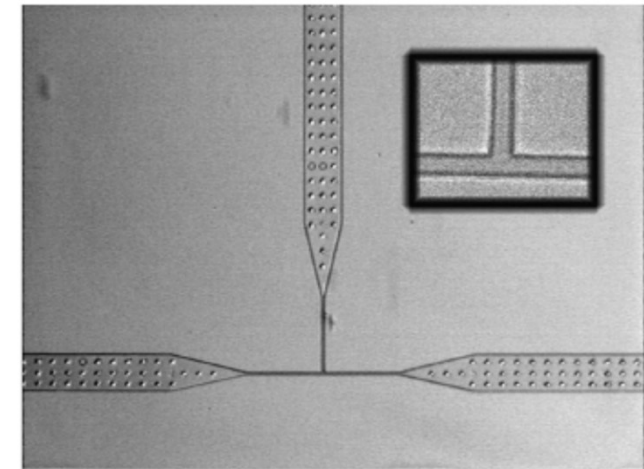
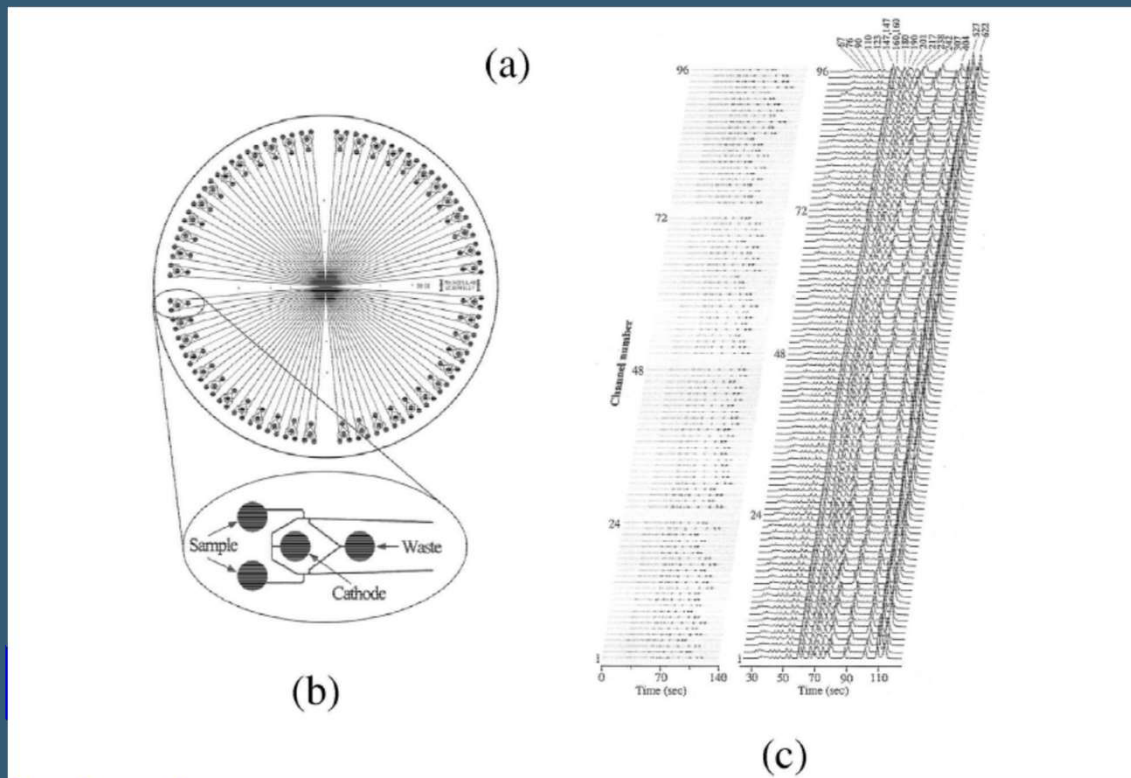
2003 - PROJEKT LIDSKÉHO GENOMU



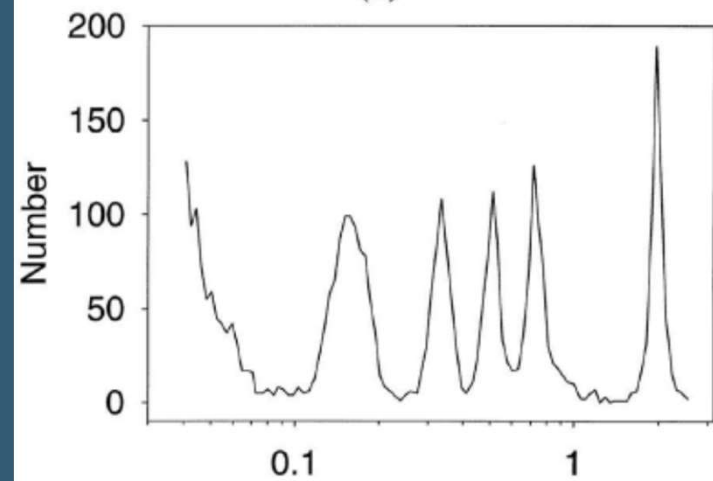
3730XL DNA ANALYZER APPLIED BIOSYSTEMS



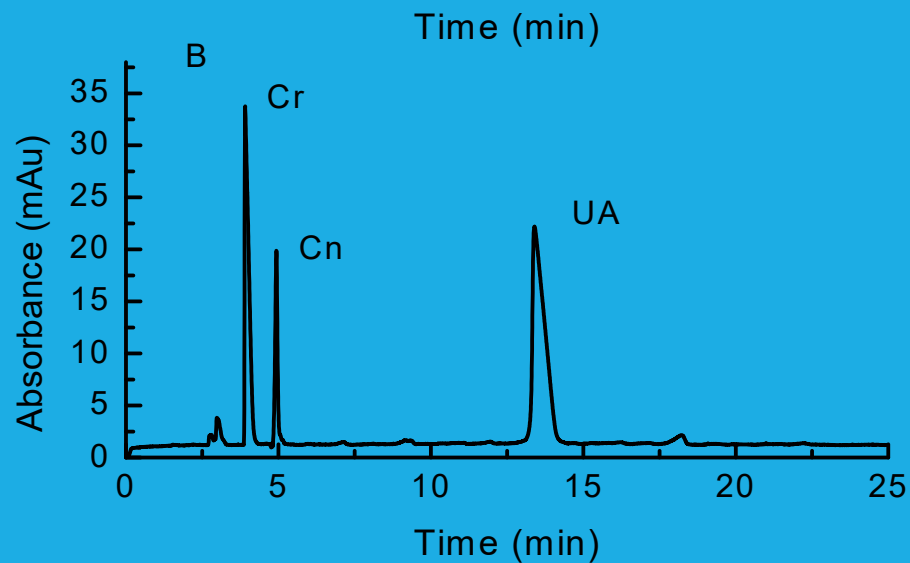
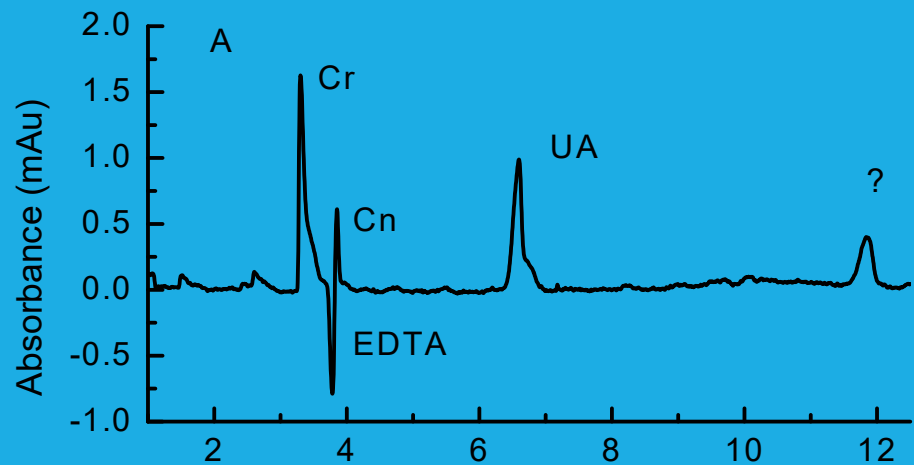
1997 μ CE



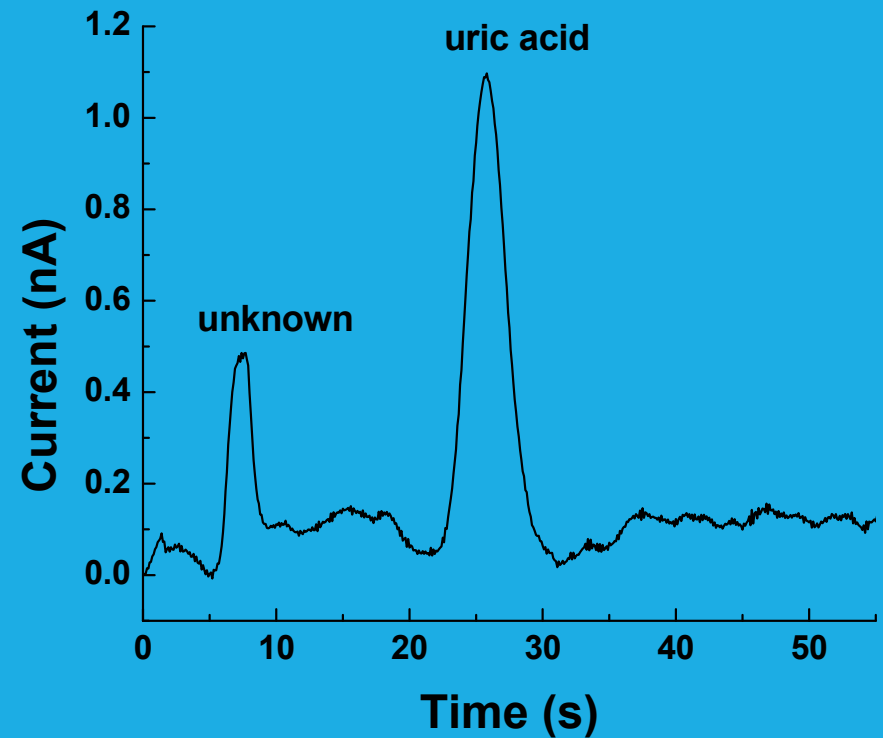
(a)



KLASICKÁ CZE



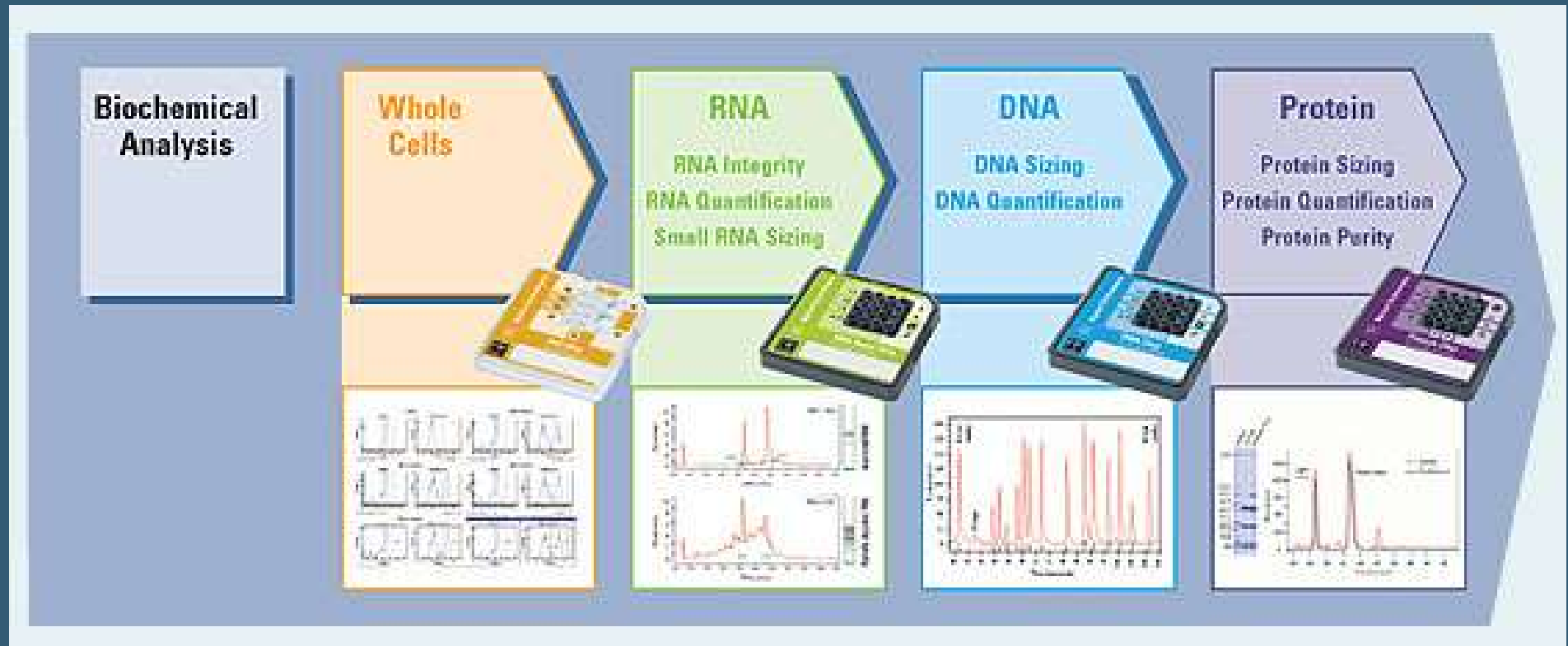
MICROCHIP CZE

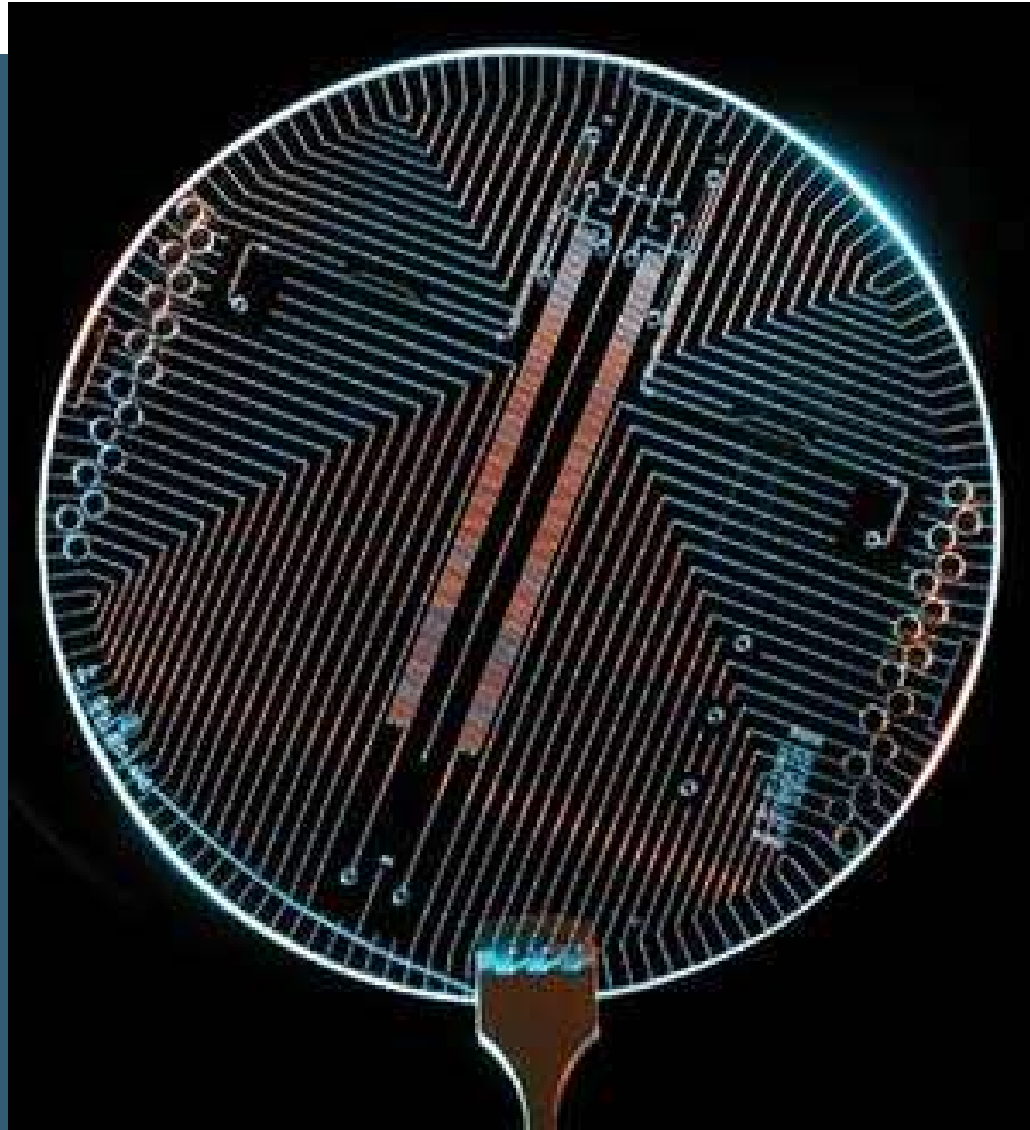


Bioanalyser Agilent 2100



Bioanalyser Agilent 2100

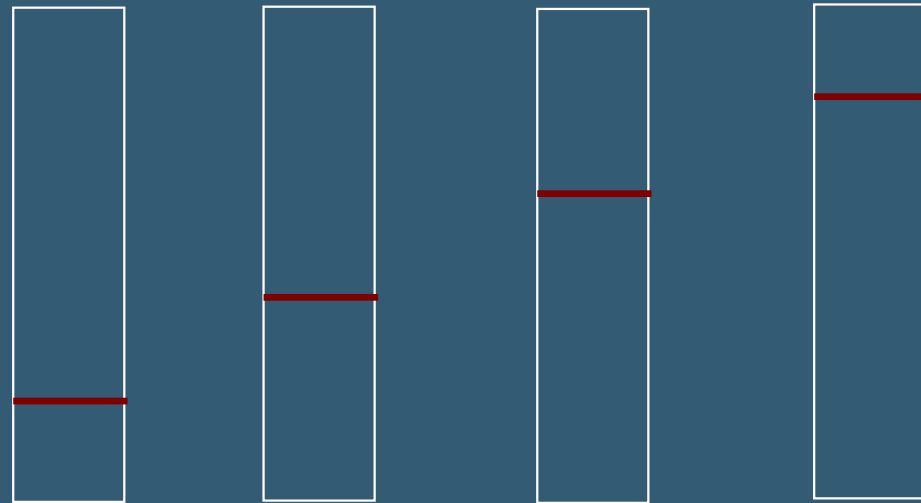




MUNI
SCI

AFINITNÍ ELEKTROFORÉZA

„V inertní matrici je imobilizován ligand, se kterým specificky interaguje separovaný biopolymer“



$$c_L = 0$$

$$c_L = n$$

$$c_L = 2n$$

$$c_L = 4n$$

LIGANDY

Biopolymery	Ligandy
Enzymy	substrát, koenzym, inhibitor
Hormony	receptory
Lektiny	sacharidy
NK	NK

IMOBILIZACE LIGANDU

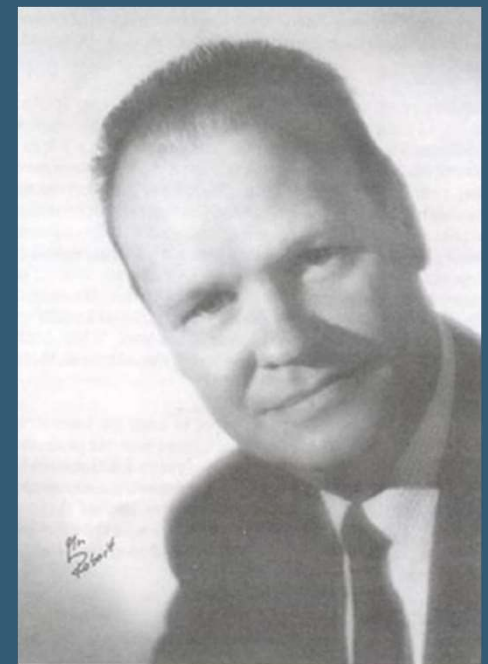
- Chemická – kovalentní vazba
- Fyzikální – ligand vázán na makromolekulu

Použití :

Studium interakce mezi ligandem a biomakromolekulou

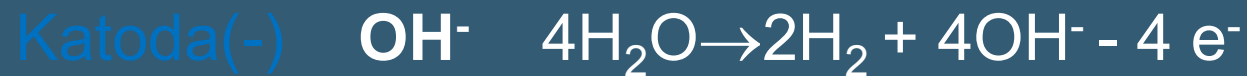
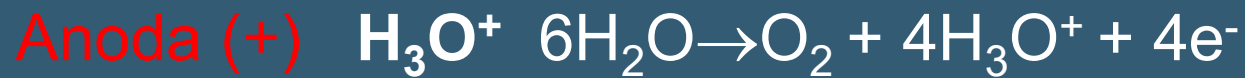
IZOELEKTRICKÁ FOKUSACE

*„Elektroforéza v gradientu pH,
částice jsou separovány podle
svých pI“*

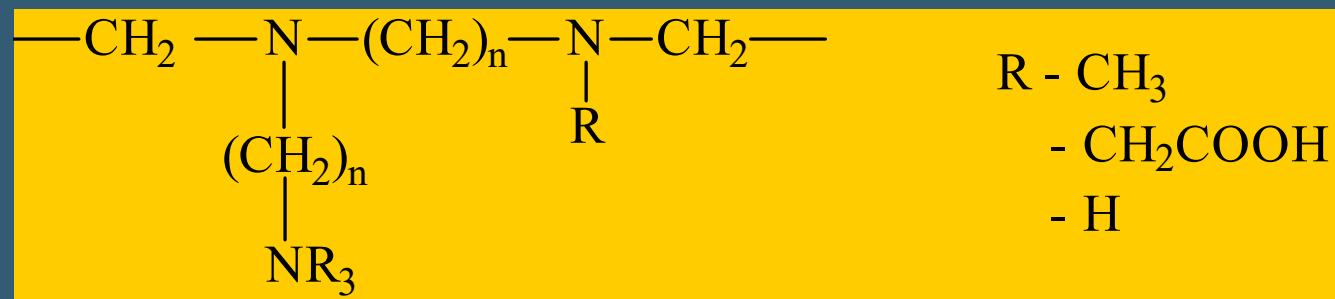


1961 Svensson – Rilbe (1968)

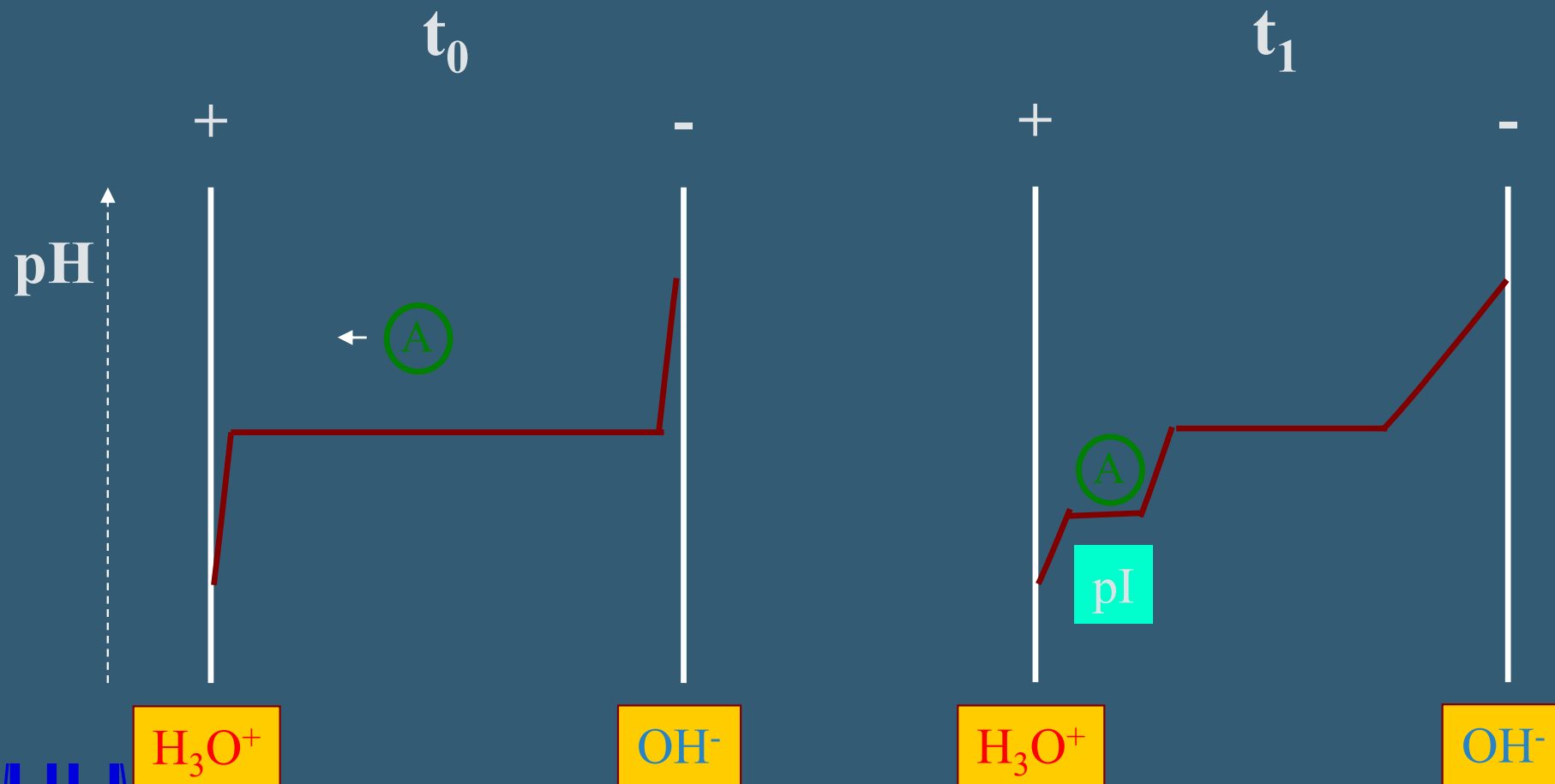
TVORBA GRADIENTU



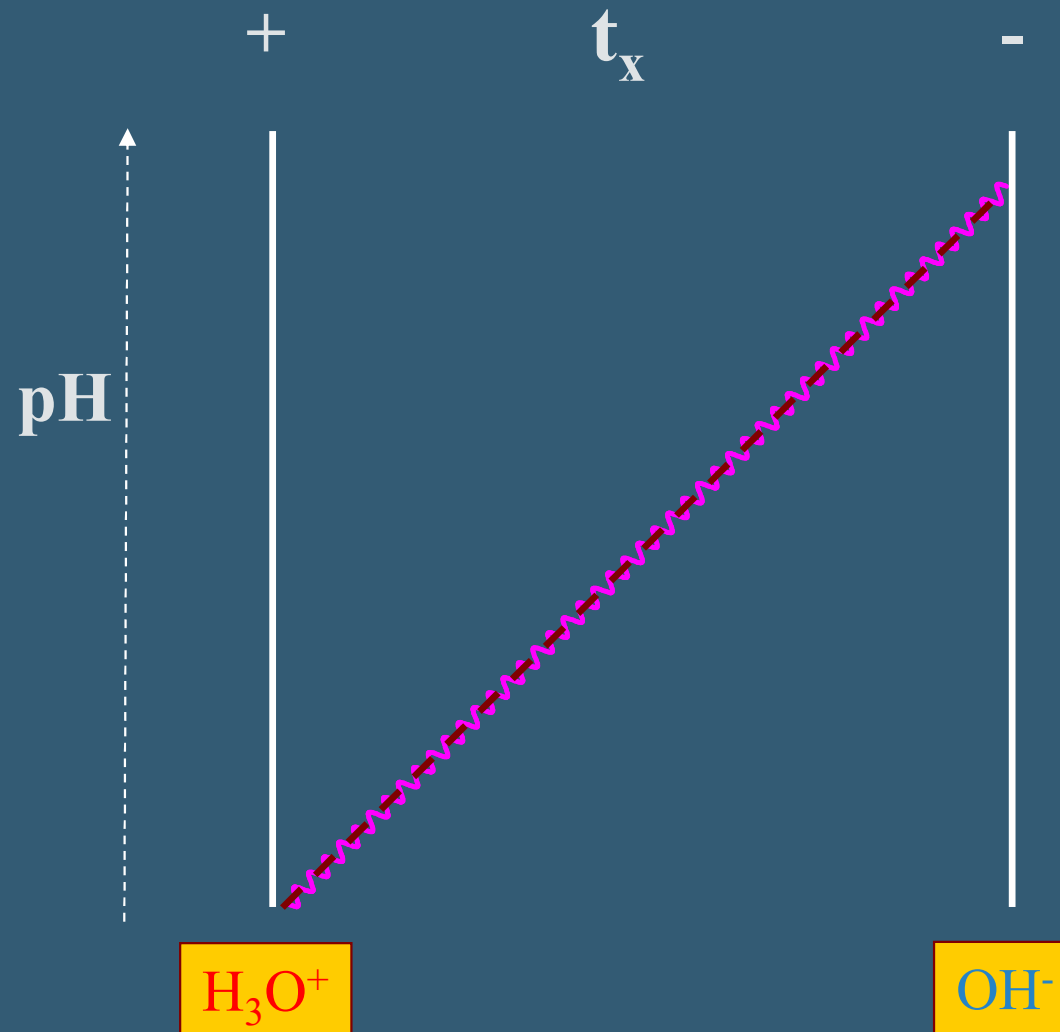
Ampholyty



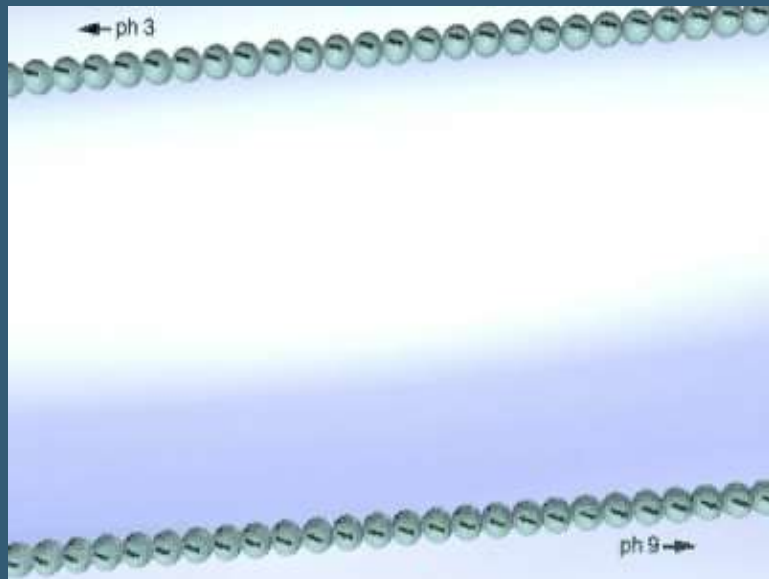
IZOELEKTRICKÁ FOKUSACE



IZOELEKTRICKÁ FOKUSACE



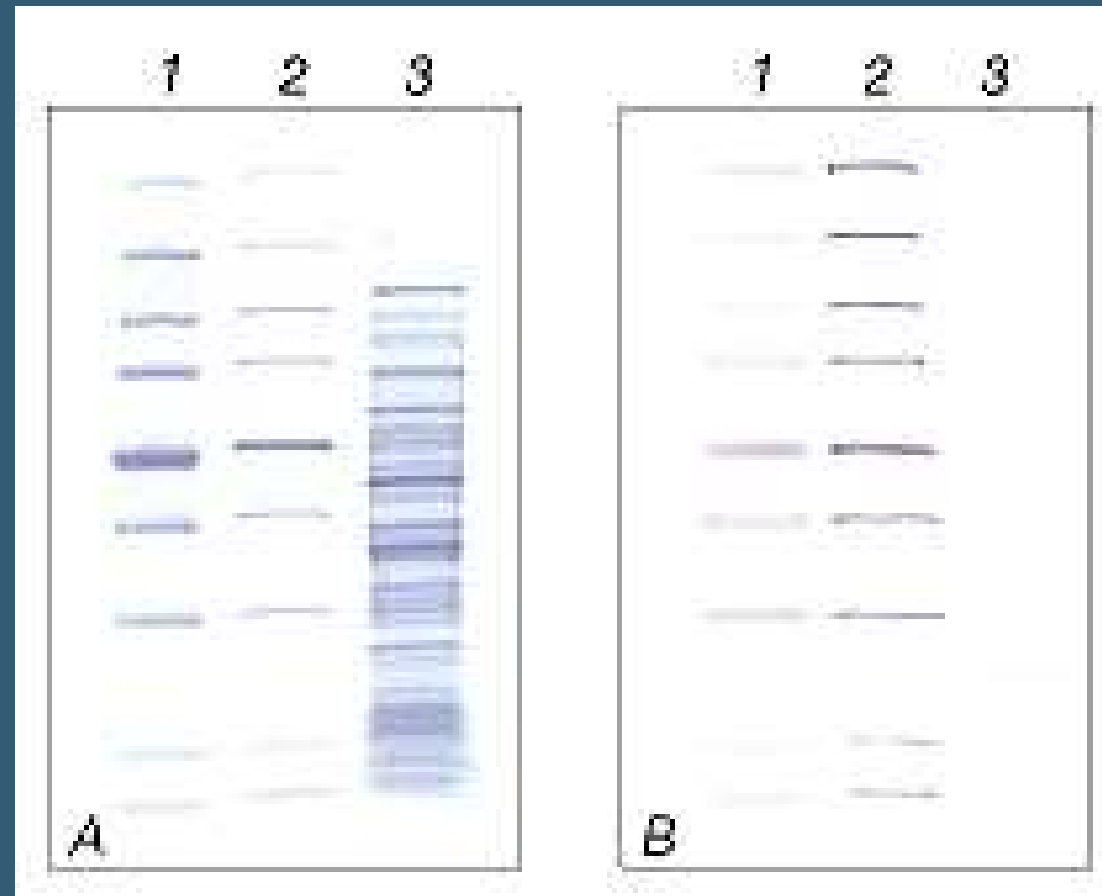
KAPILÁRNÍ IZOELEKTRICKÁ FOKUSACE



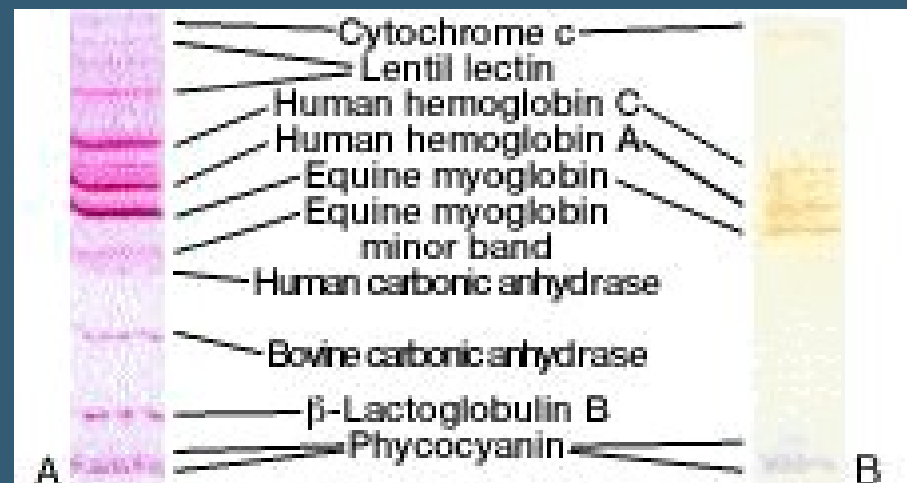
IZOELEKTRICKÁ FOKUSACE ANALYTICKÁ

- Provedení - v gelech – PAGE, agarosa
- Použití - sledování komplexních směsí
 - izoenzymové složení
 - stanovení pI – rozřezání a eluce
 - μpH elektrody
 - pI standardy

IZOELEKTRICKÁ FOKUSACE ANALYTICKÁ

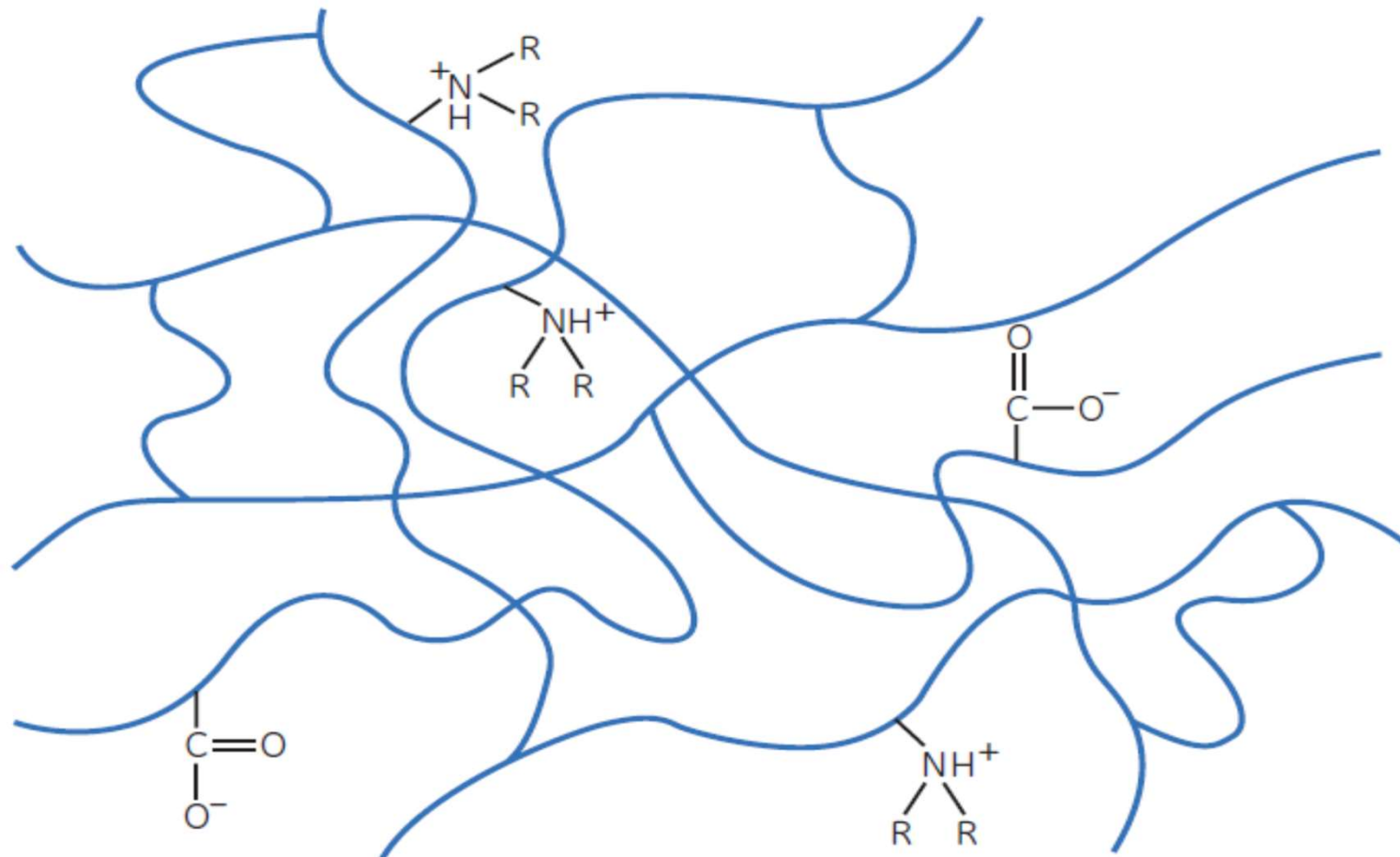
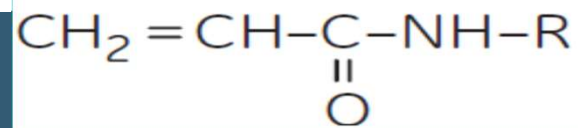


IZOELEKTRICKÁ FOKUSACE ANALYTICKÁ - STANDARDY

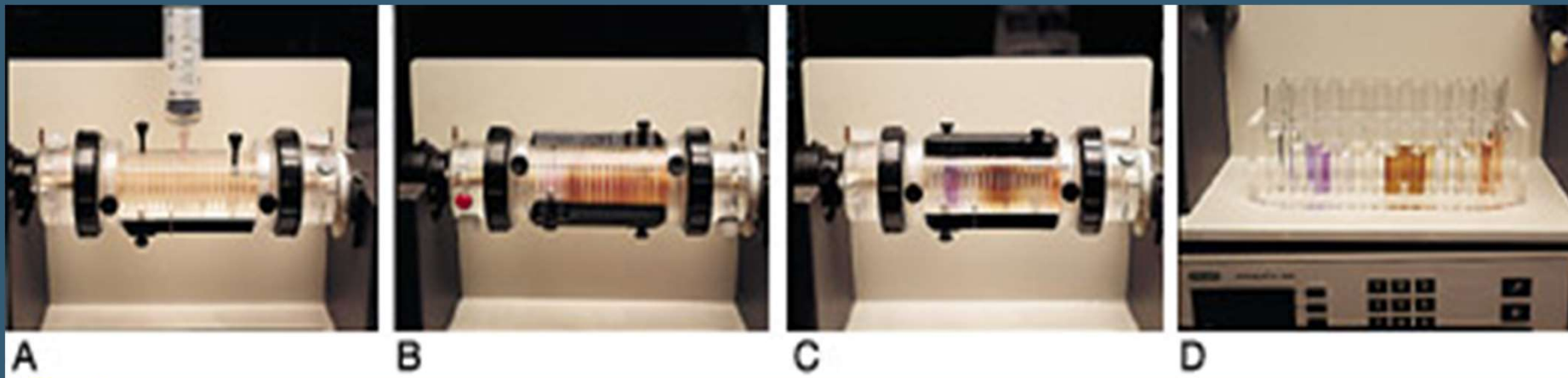


IEF Standards for accurate pI calibration of native IEF gels. A. 5 μ l of the standards were stained with Coomassie blue R-250 dye and crocein scarlet. B. 5 μ l of the standards were left unstained.

IZOELEKTRICKÁ FOKUSACE IMOBILINE



IZOELEKTRICKÁ FOKUSACE PREPARATIVNÍ



IZOELEKTRICKÁ FOKUSACE PREPARATIVNÍ

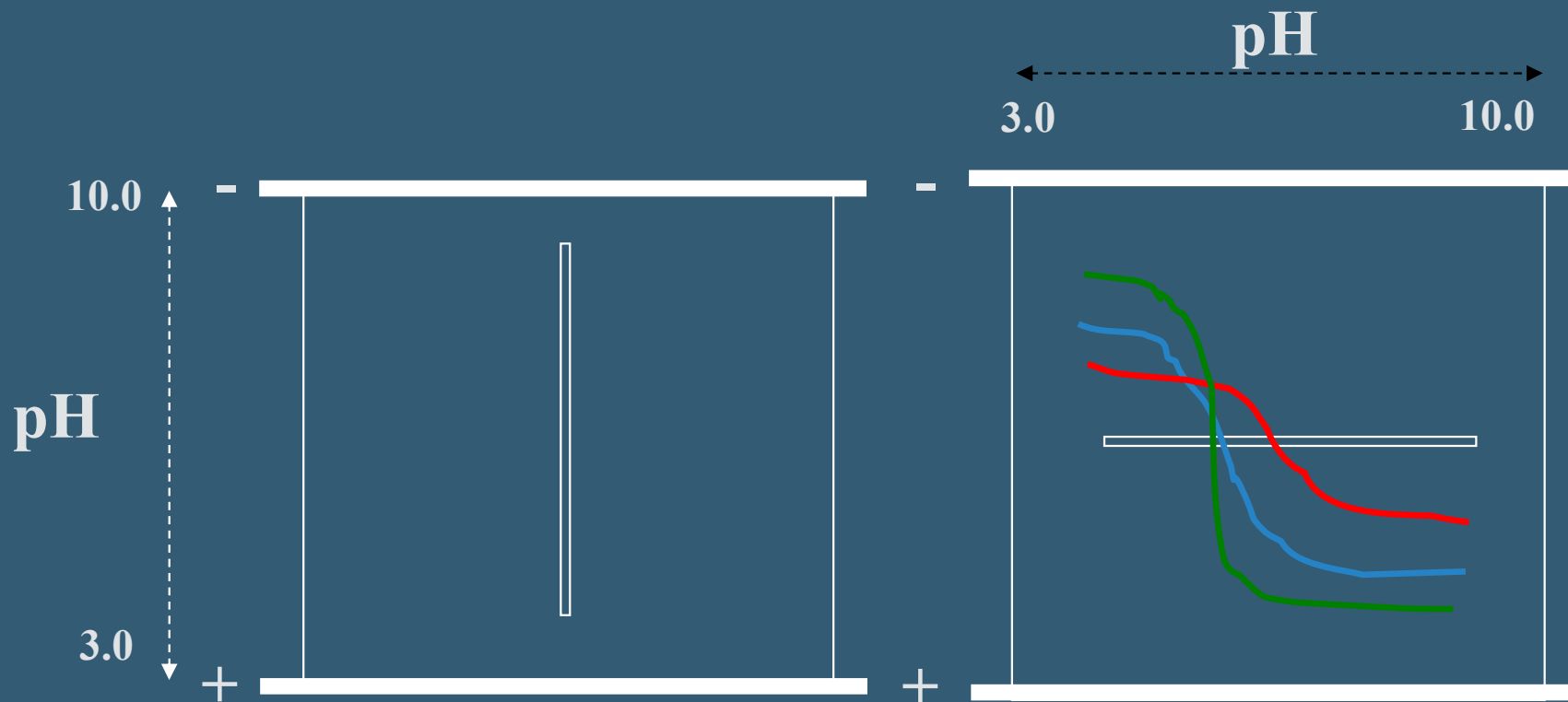
- Provedení - v sypaných vrstvách (Sephadex)
 - v gradientech hustoty (sacharoza)

Použití – izolace bílkovin

DVOUROZMĚRNÉ METODY

- Metoda titračních křivek
- Dvourozměrná elektroforéza

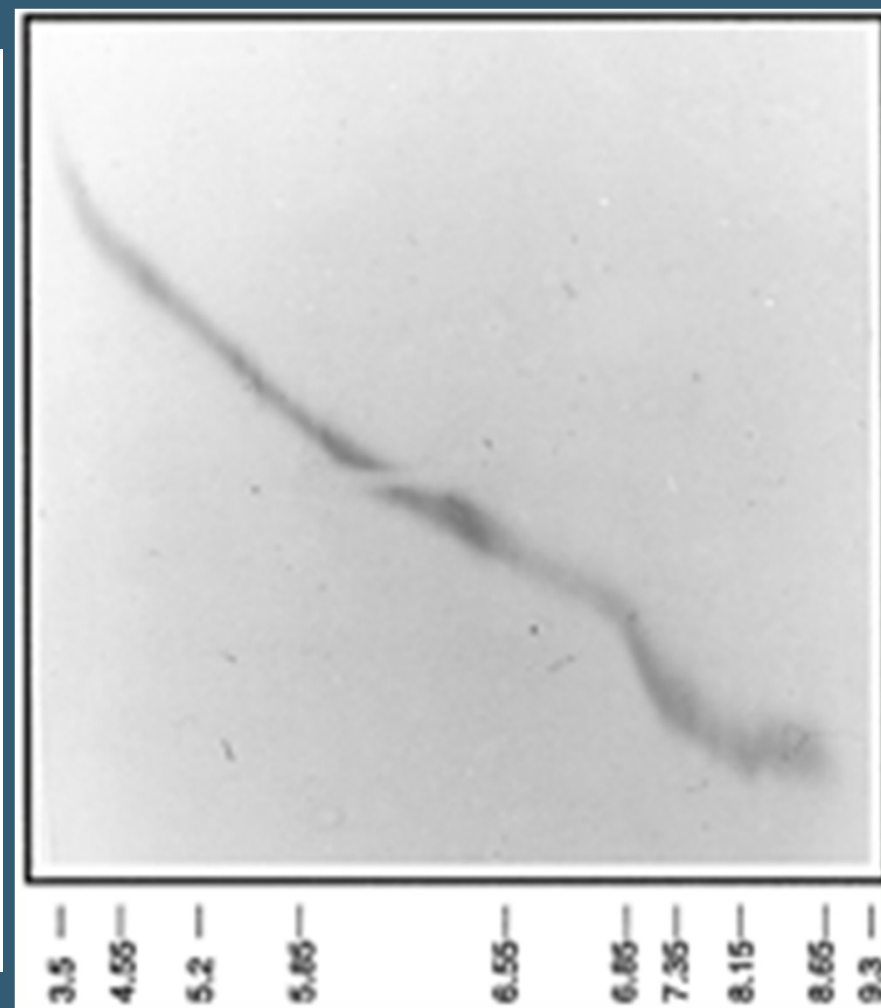
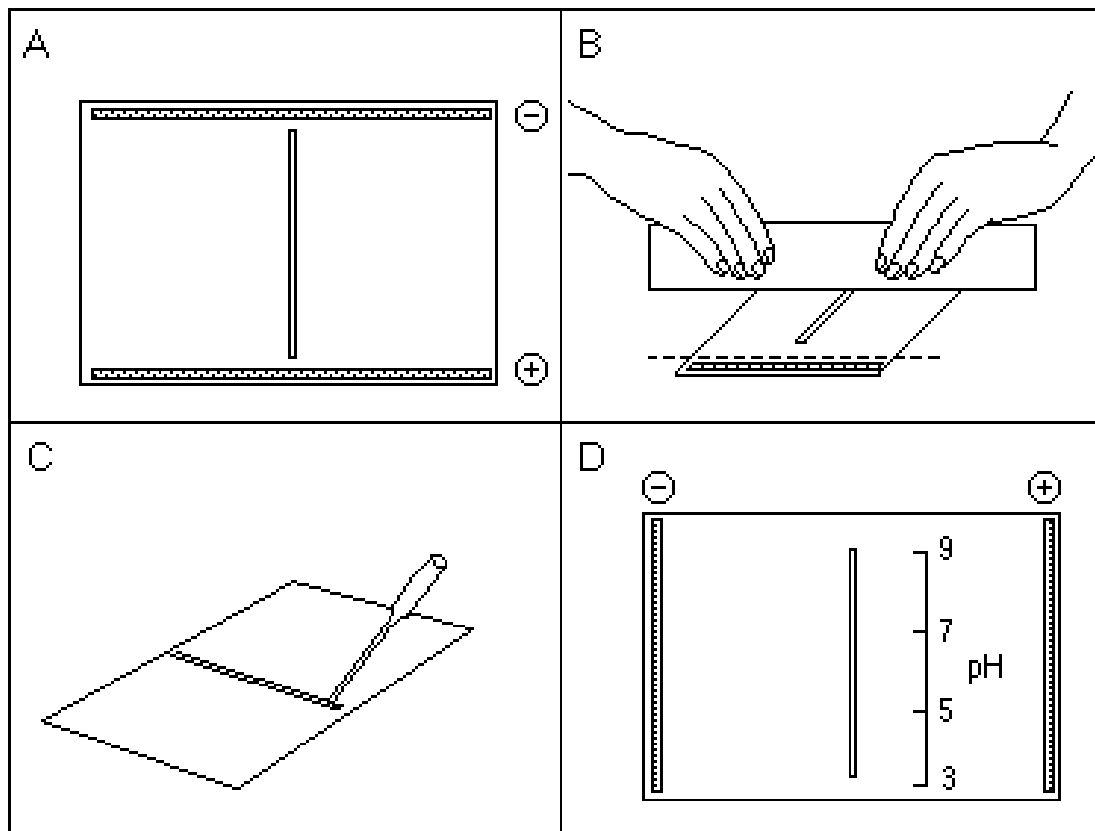
METODA TITRAČNÍCH KŘIVEK



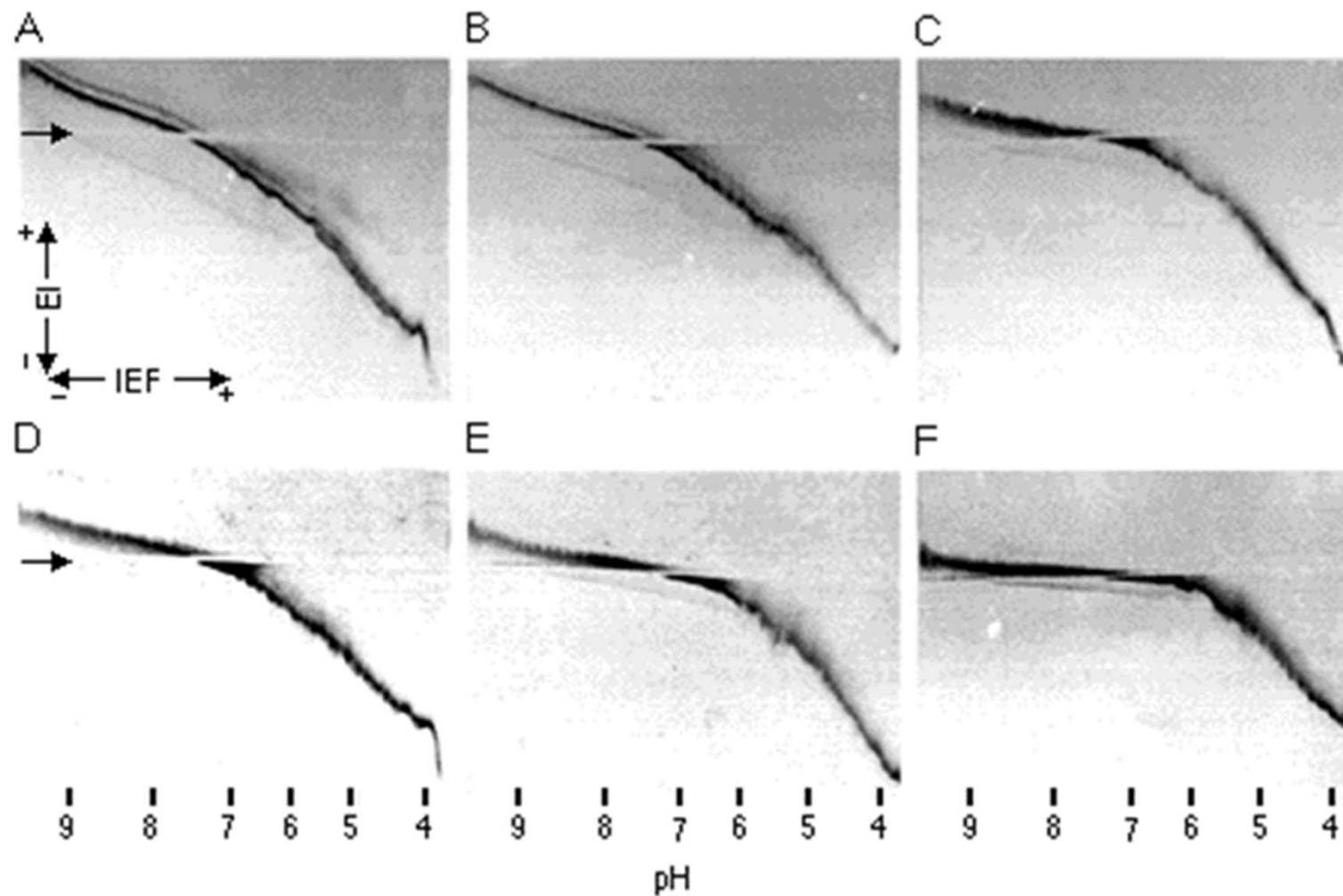
I. rozměr – IEF bez vzorku

II. rozměr – elektroforéza se vzorkem

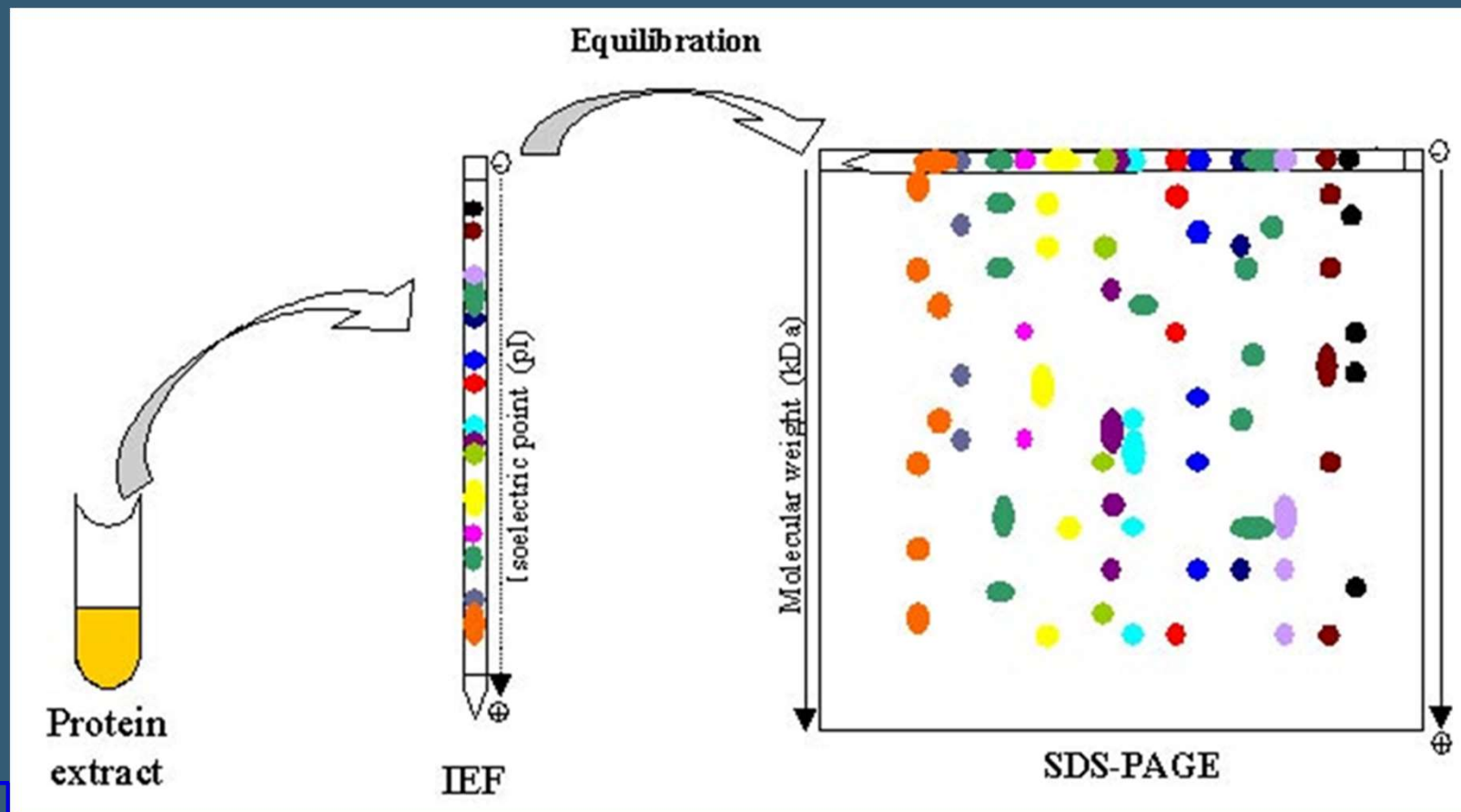
METODA TITRAČNÍCH KŘIVEK



METODA TITRAČNÍCH KŘIVEK

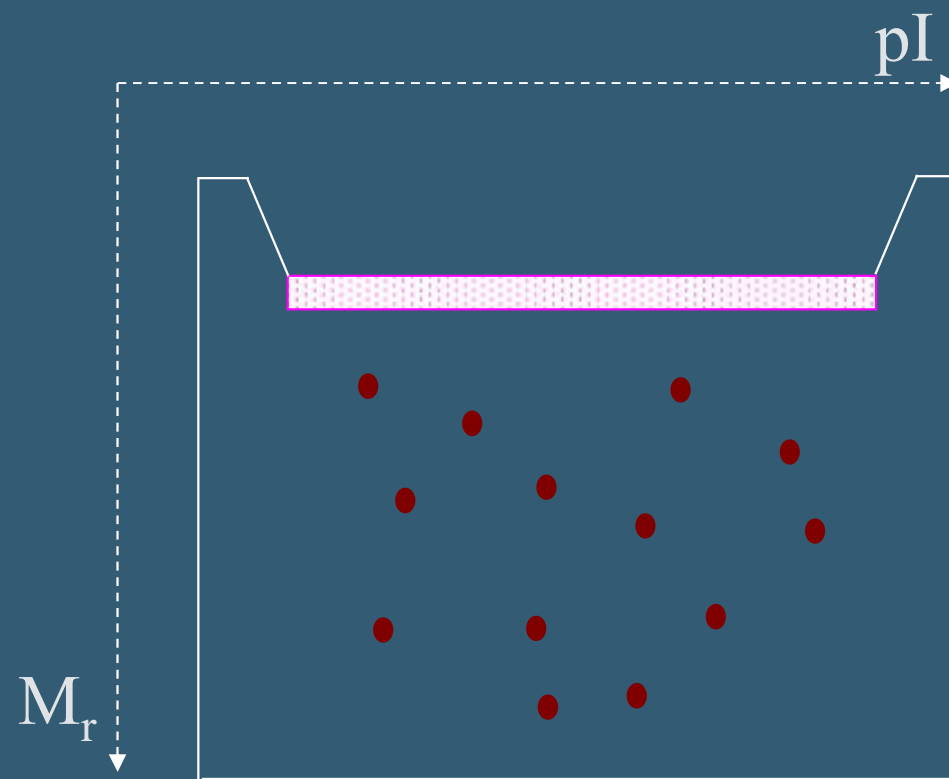
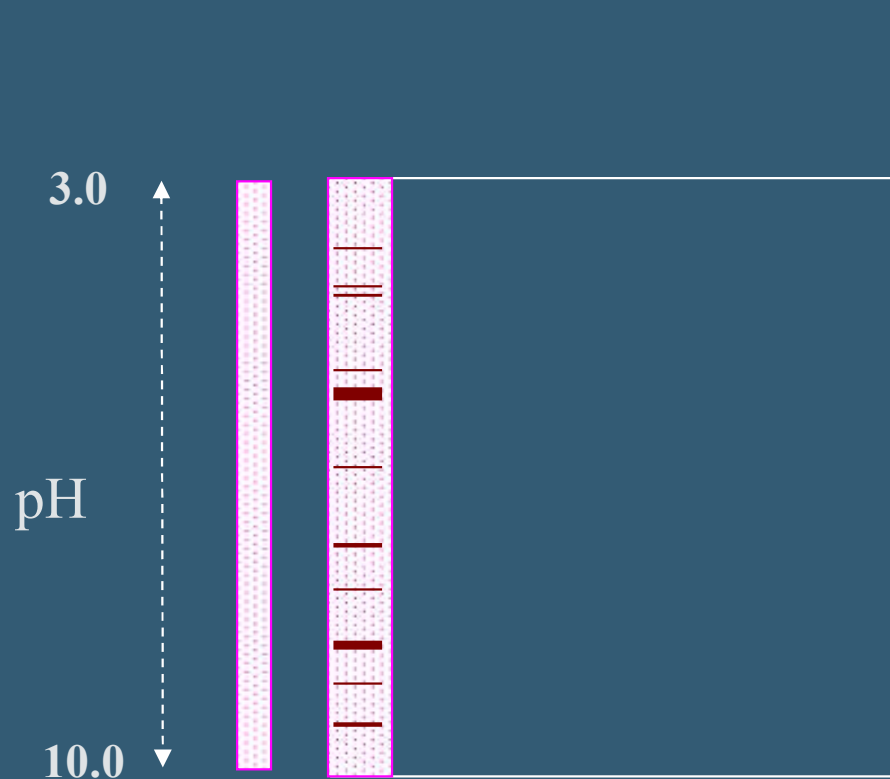


DVOJROZMĚRNÁ ELEKTROFORÉZA



DVOJROZMĚRNÁ ELEKTROFORÉZA

1975 O'FARRELL

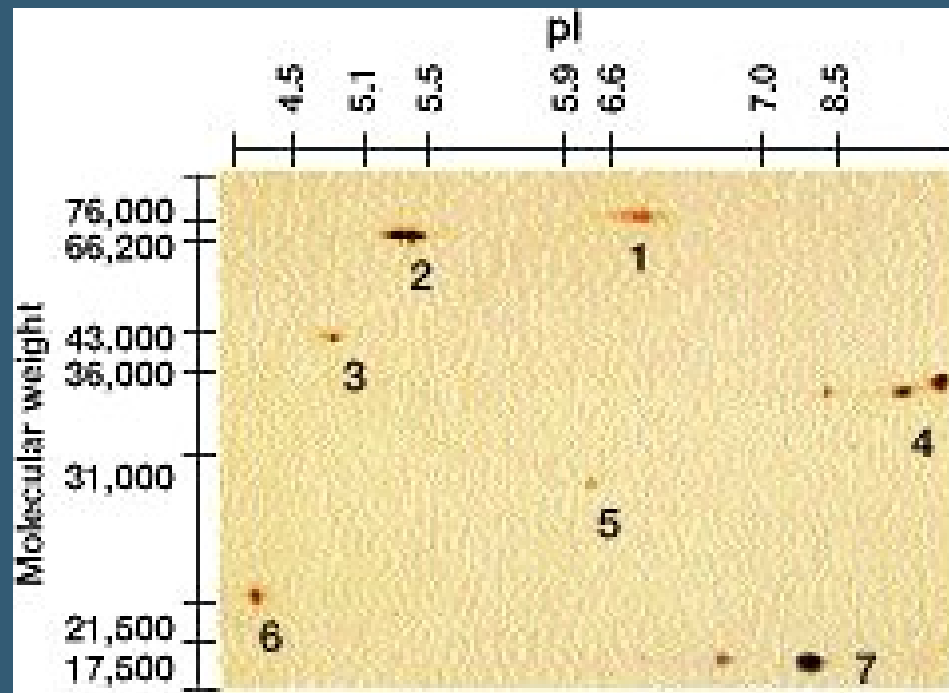


MUNI
SCI

I. rozměr IEF

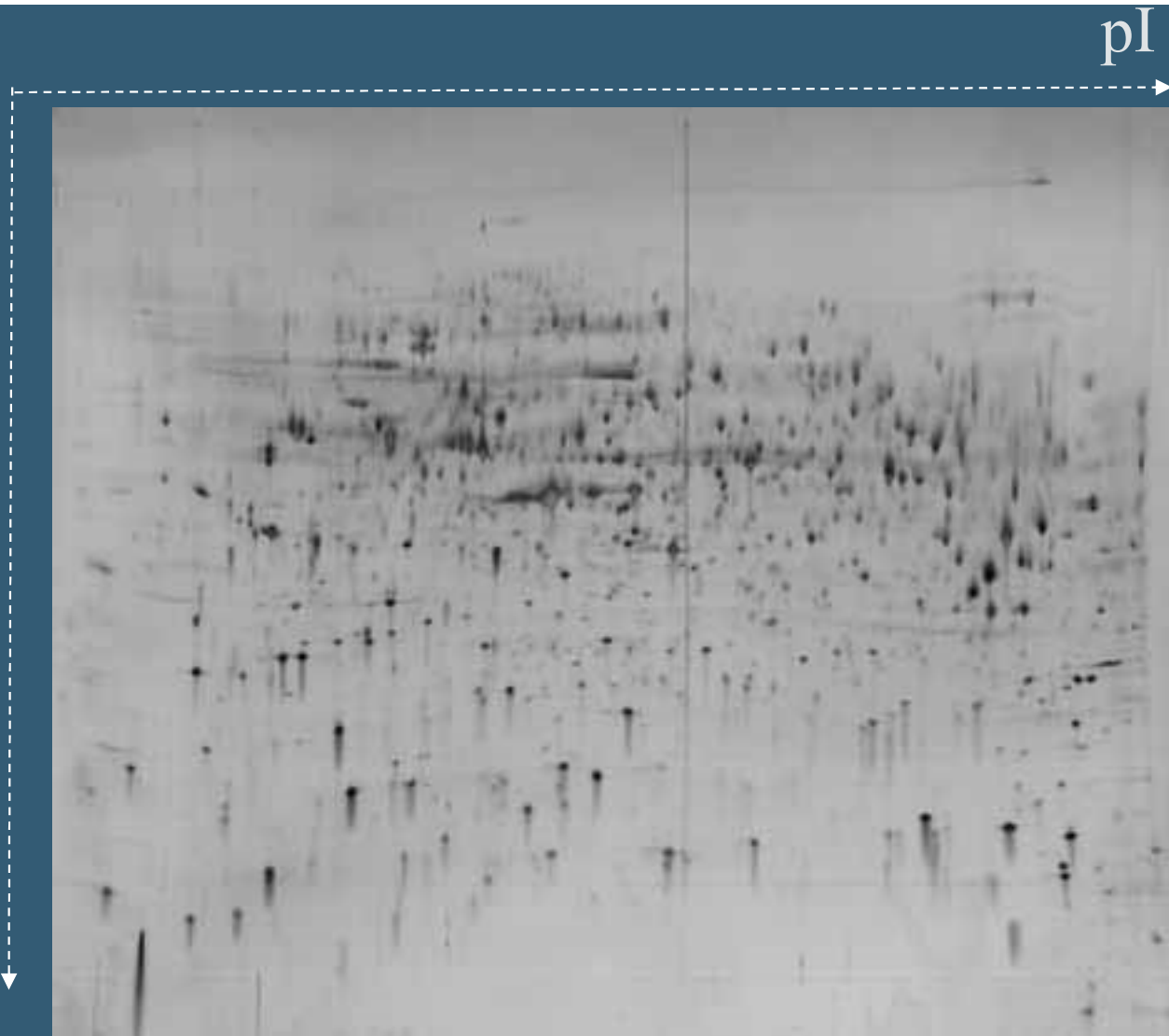
II. rozměr SDS-PAGE

DVOJROZMĚRNÁ ELEKTROFORÉZA

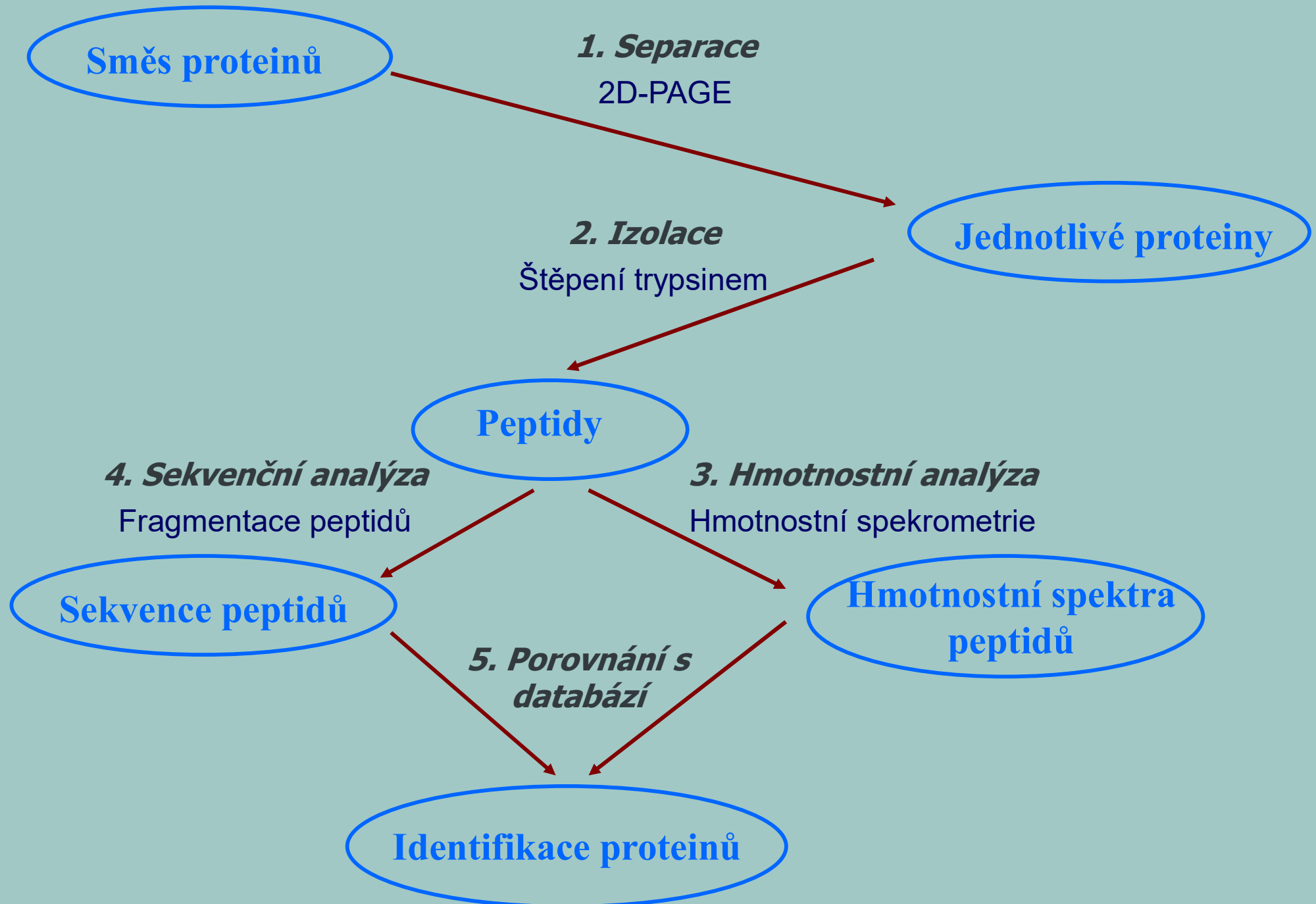


Two-dimensional electrophoretic pattern of the 2-D SDS-PAGE Standards separated on the Mini-PROTEAN II cell. 2.5 μ l were applied to a 3.5% tube gel crosslinked with piperazine diacrylamide containing 9 M urea and 2% Bio-Lyte ampholytes (1 part 3/10, 2 parts 5/7). The tube gel was run on a 15% continuous slab gel crosslinked with bis and silver stained. For details, see references 21, 22, and 23.

DVOJROZMĚRNÁ ELEKTROFORÉZA



Základní schéma analýzy užívané v proteomice



CompuGen Z3 DeskTop v.1.11 Beta

File Edit Image Spots Align View Analysis Window Log Help

[1st] 2small [2nd] 1small

Matching Data Table

The gels in the table are enumerated as follows:

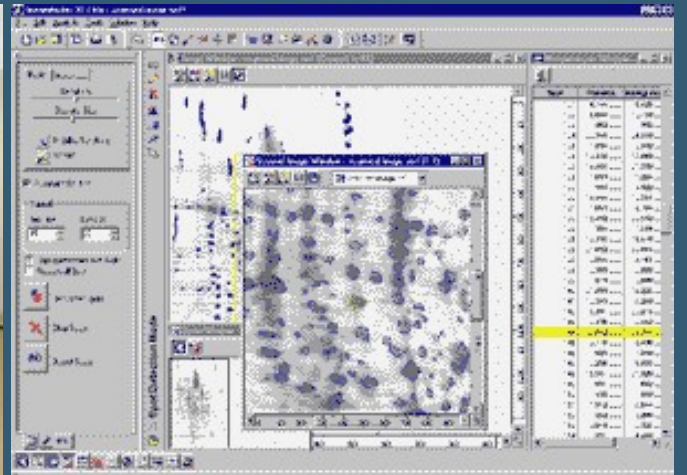
1. 2small
2. 1small

ID	X1	Y1	RE1	X2	Y2	RE2	std	trendline
1016	346	39	6031	337	43	11053	2511.0	0.588
1022	255	45	3712	250	51	3745	16.5	0.009
1023	242	48	2206	237	53	2921	357.5	0.279
1024	119	49	584	125	53	1076	246.0	0.593
1025	100	50	3290	108	54	4738	724.0	0.361
1027	183	50	2104	183	53	3515	705.5	0.502
1029				95	52	1150		
1030	221	52	5409	217	56	4496	456.5	-0.184
1032				264	52	4896		
1033				275	52	1422		
1034	147	53	3078	151	59	1281	898.5	-0.625
1038	290	56	1992					
1040				35	57	1006		

Layered View: 2small vs. 1small

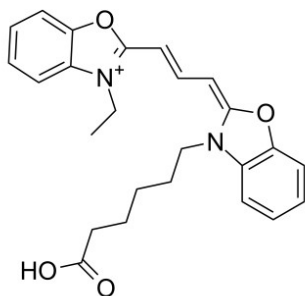
[142, 129] -> [1st: 127 2nd: 184]

MOBI
SCI



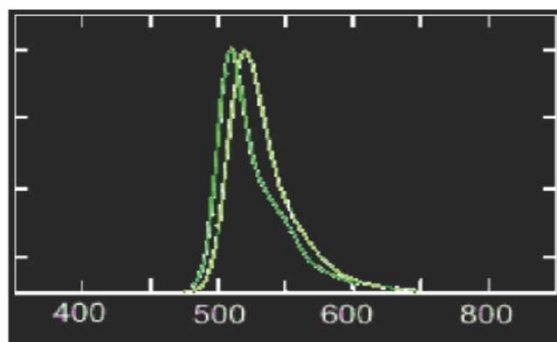
DIGE – DIFERENČNÍ GELOVÁ ELEKTROFORESA

Cy2

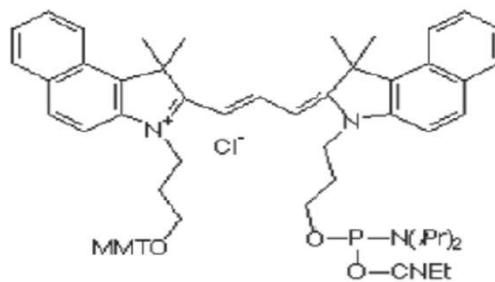


I⁻

excitace 488 nm
emise 520 nm

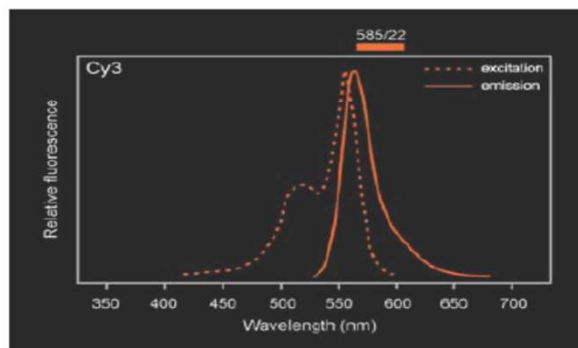


Cy3

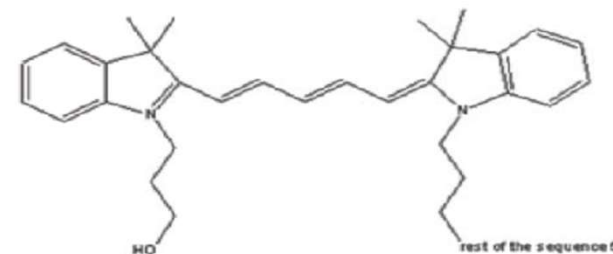


Cl⁻

excitace 532 nm
emise 580 nm

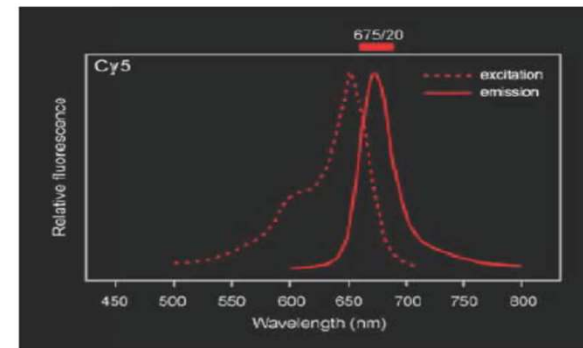


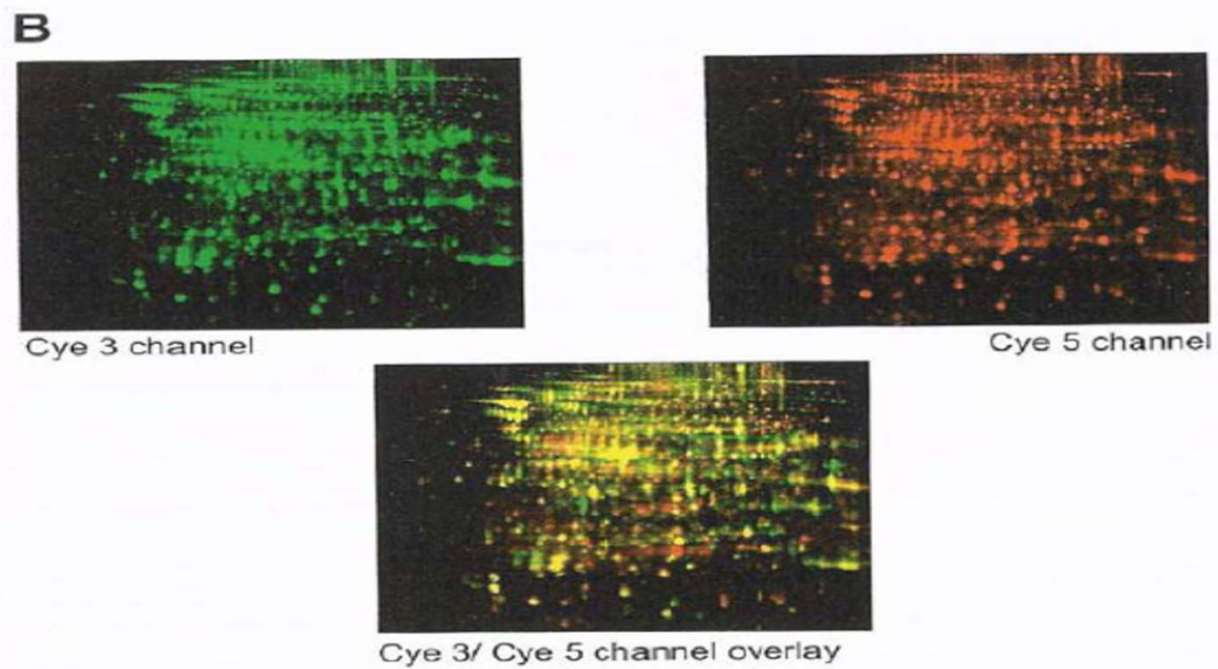
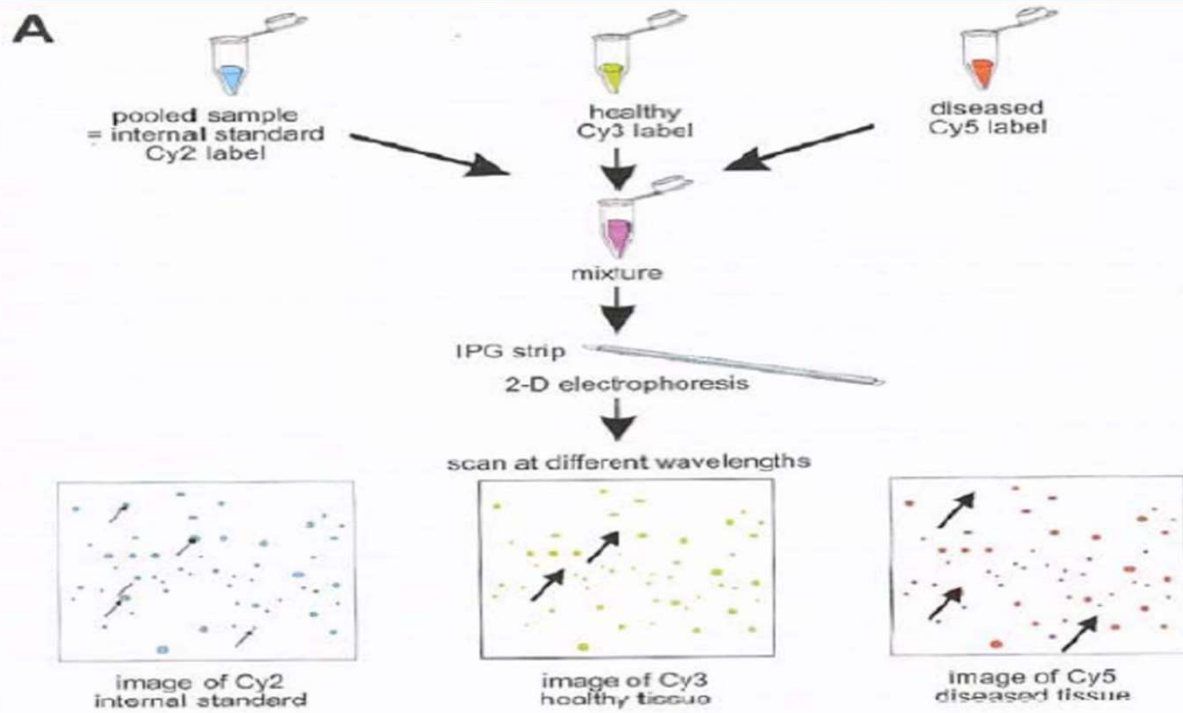
Cy5



rest of the sequence^o

excitace 633 nm
emise 670 nm





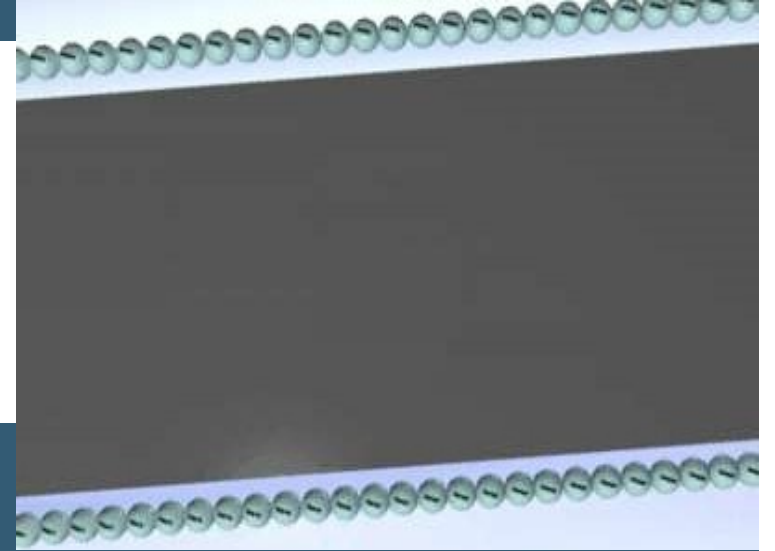
IZOTACHOFORÉZA

1976 EVERAERTS



„Vzorek je umístěn mezi dva elektrolyty: vedoucí L (leading) s nejvyšší mobilitou a - uzavírající T (terminating) s nejmenší mobilitou“

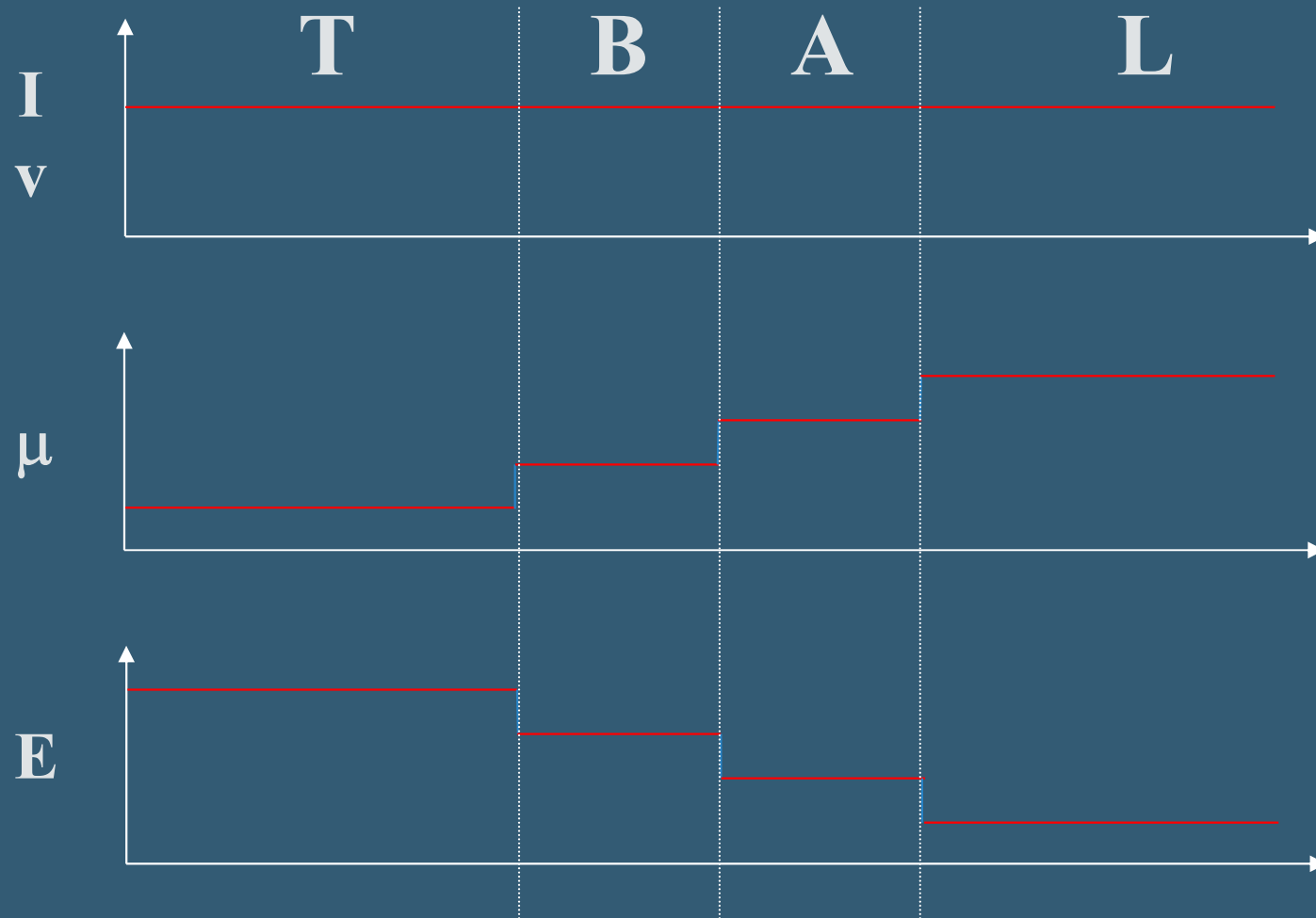
IZOTACHOFORÉZA



IZOTACHOFORÉZA

$$\mu_L > \mu_A > \mu_B > \mu_T$$

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

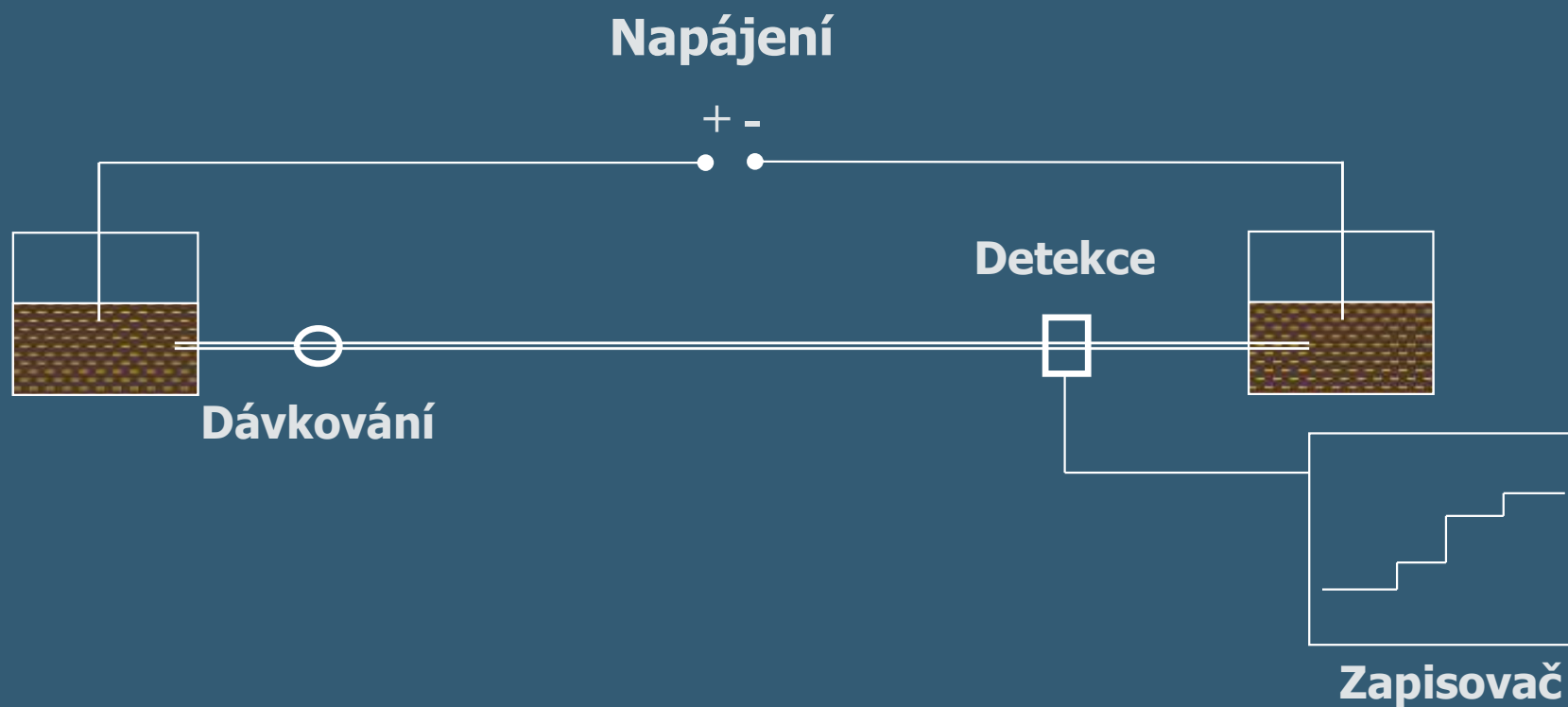


KOHLRAUSCHOVA REGULAČNÍ FUNKCE

$$\sum_i \sum_z \frac{|z| c_i}{\mu_i} = K_x$$

$$c_i = c_L \cdot \frac{\mu_L + \mu_R}{\mu_L} \cdot \frac{\mu_i}{\mu_i + \mu_R} \cdot \frac{z_L}{z_i}$$

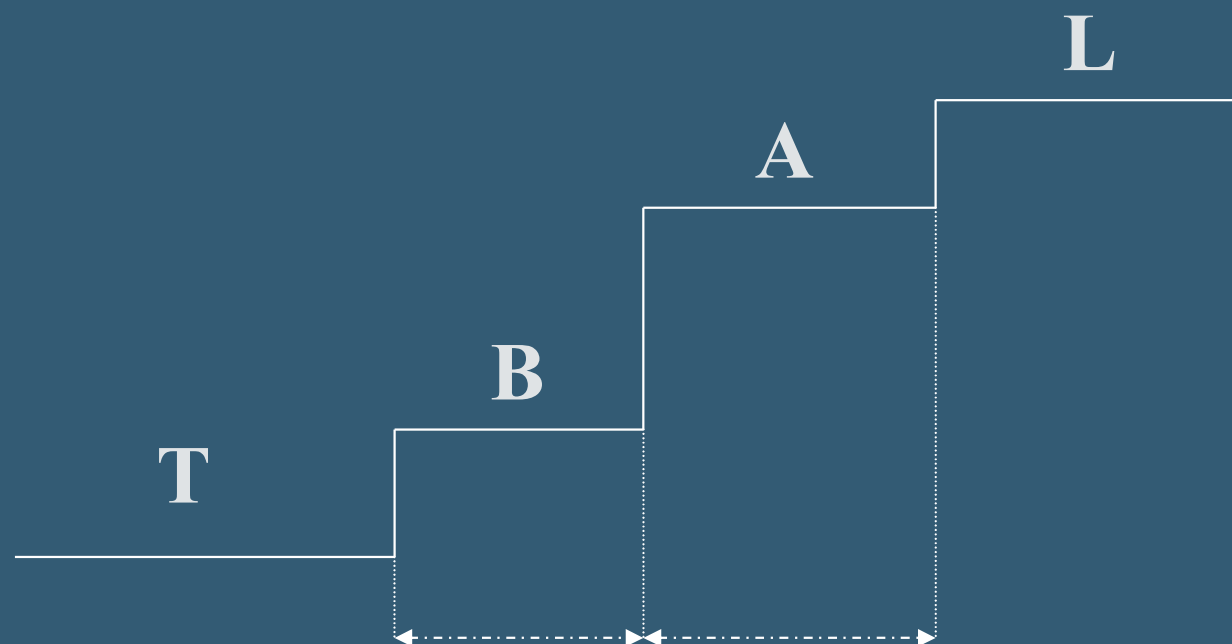
ANALYTICKÁ ITP



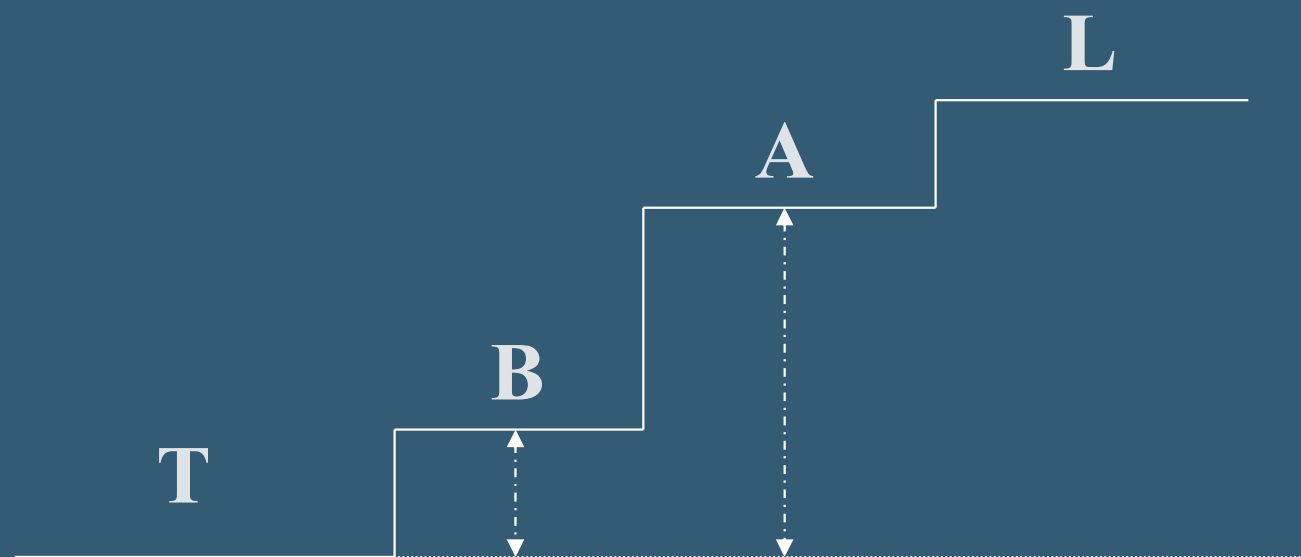
ANALYTICKÁ ITP INSTRUMENTACE

- Napájení – stejnosměrné 3 kV 0,2 – 0,5 mA
- Kapilára 0,1 – 2 mm
- Dávkování - dávkovací ventil
- Detekce - universální – konduktometrická
 - potencialově gradientová
 - selektivní – UV-VIS

IZOTACHOFORETICKÝ ZÁZNAM KVANTITA



IZOTACHOFORETICKÝ ZÁZNAM KVALITA



IZOTACHOFORETICKÝ ZÁZNAM

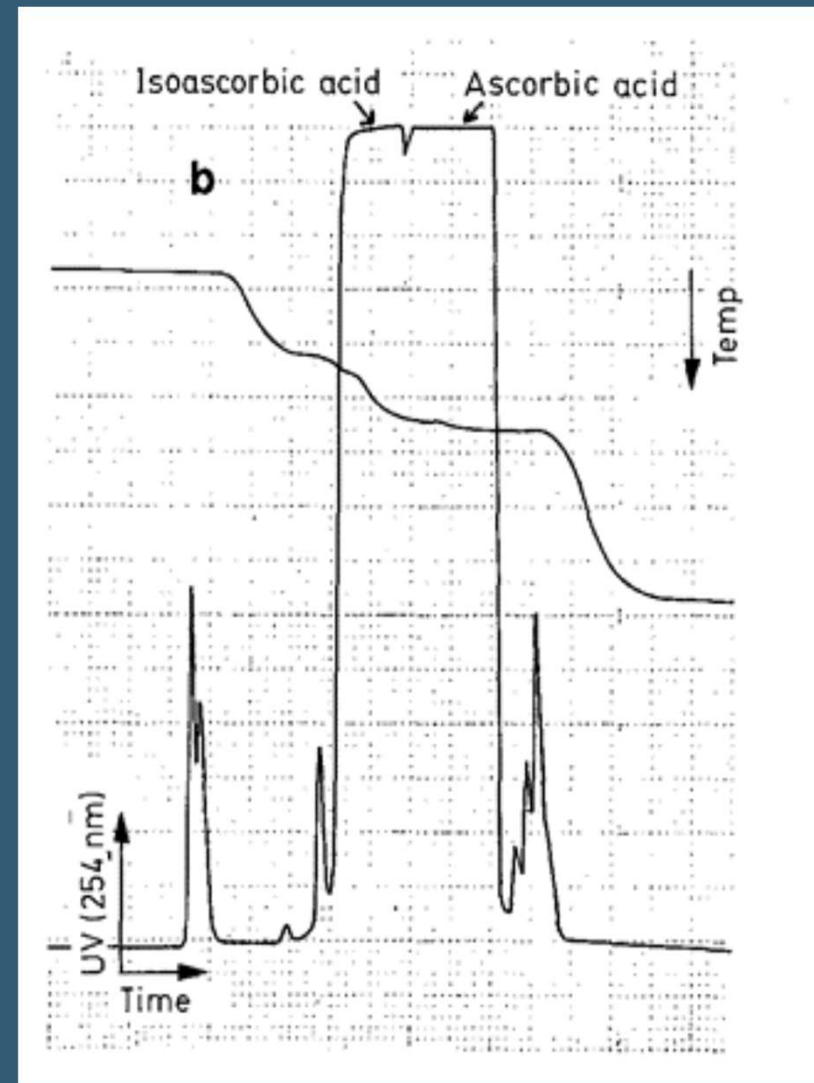
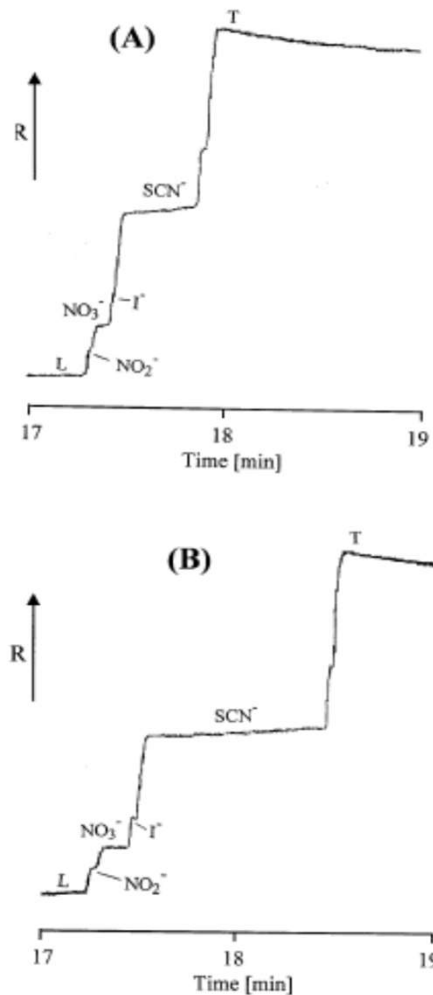


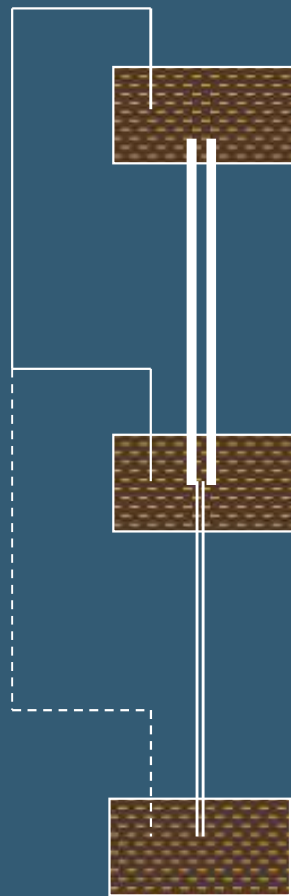
Fig. 2. Isotachopherograms of non-smoker saliva (A) and smoker saliva (B). L, leading ion (chloride); T, terminating ion (acetate); R, increasing resistance.

ANALYTICKÁ ITP VILLA LABECO



ANALYTICKÁ ITP

METODA SPOJOVÁNÍ KAPILÁR



Předseparační kapilára – 2 mm



Analytická kapilára – 0.1 mm



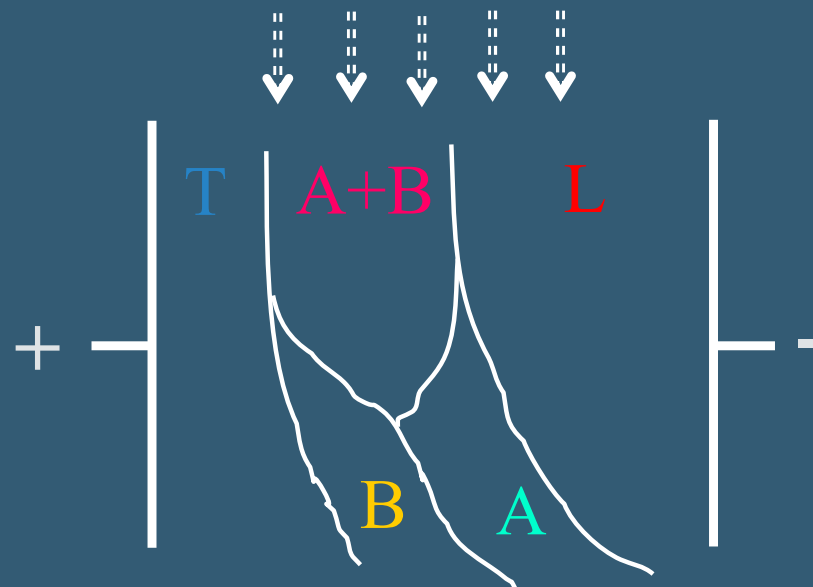
PREPARATIVNÍ ITP

V gelech - Sephadex

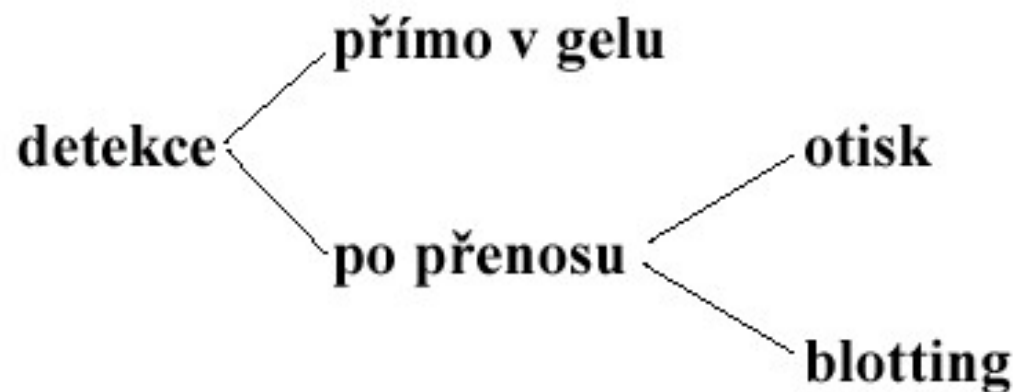


PREPARATIVNÍ ITP

Kontinuální plošná



DETEKCE PO ELEKTROFORÉZE A IZOELEKTRICKÉ FOKUSACI



NESPECIFICKÁ DETEKCE

Reakce s barvičkou

Postup : **A. Fixace**
 B. Barvení
 C. Odbarvování

Používané barvičky

Amido Black 10 B

Coomassie Brilliant Blue R 250

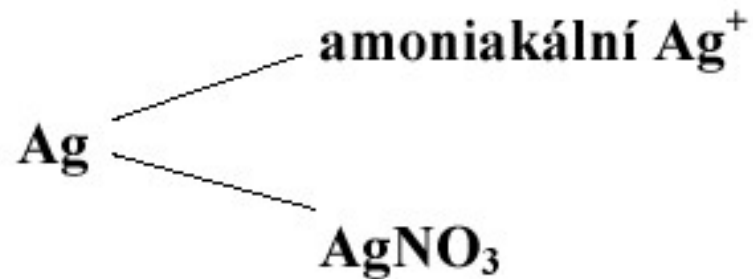
Coomassie Brilliant Blue G 250

Fast Green

Panceau S

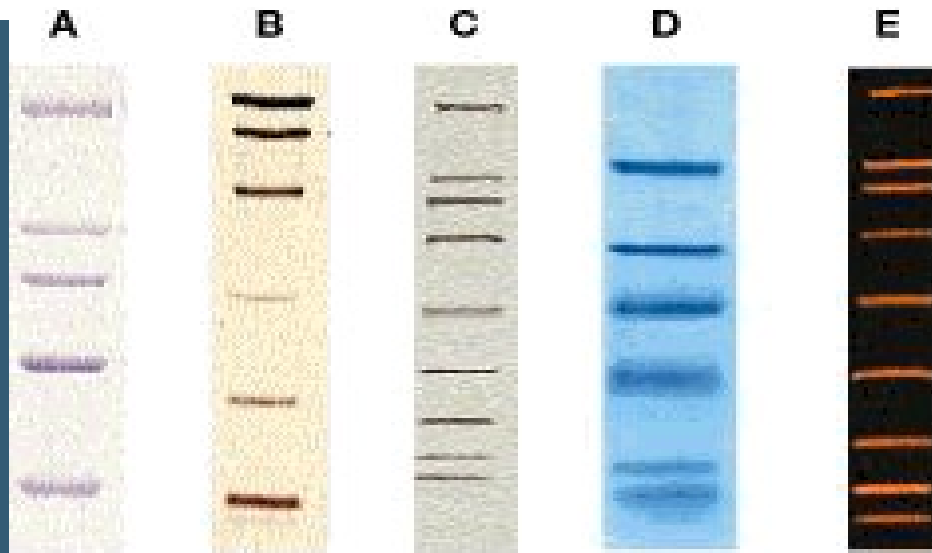
NESPECIFICKÁ DETEKCE

Barvení Ag



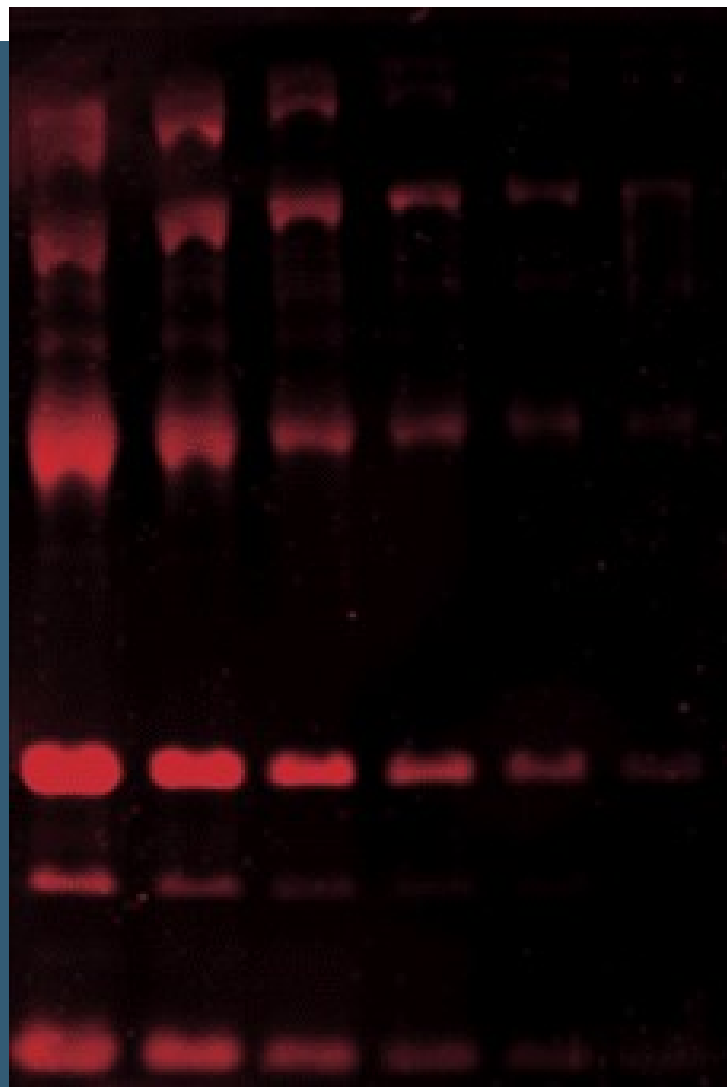
Postup : A. Navázání Ag^+ na bílkovinu
B. Redukce Ag^+ na Ag^0

NESPECIFICKÁ DETEKCE



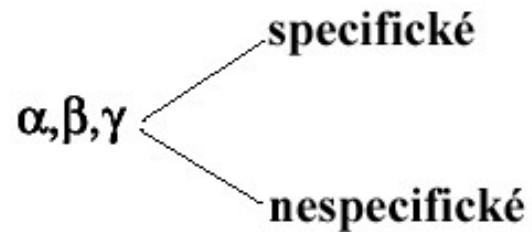
SDS-PAGE Standards provide accurate molecular weight determinations. A. High range SDS-PAGE Standards run on a 7.5% gel and stained with Coomassie blue R-250 dye, B. Low range Silver Stain SDS-PAGE Standards run on a 12% gel and stained with Bio-Rad's Silver Stain Kit, C. Broad range Biotinylated SDS-PAGE standards run on a 4-20% gradient gel, blotted to nitrocellulose, and detected with Avidin-AP. D. Polypeptide SDS-PAGE Standards run on a 16.5% tricine gel and stained with Coomassie blue G-250 dye. E. Broad range SDS-PAGE standards for SYPRO Orange staining run on a 4-20% gel and stained with SYPRO Orange.

FLUORESCENČNÍ DETEKCE



SPECIFICKÁ DETEKCE

Radioaktivní značení



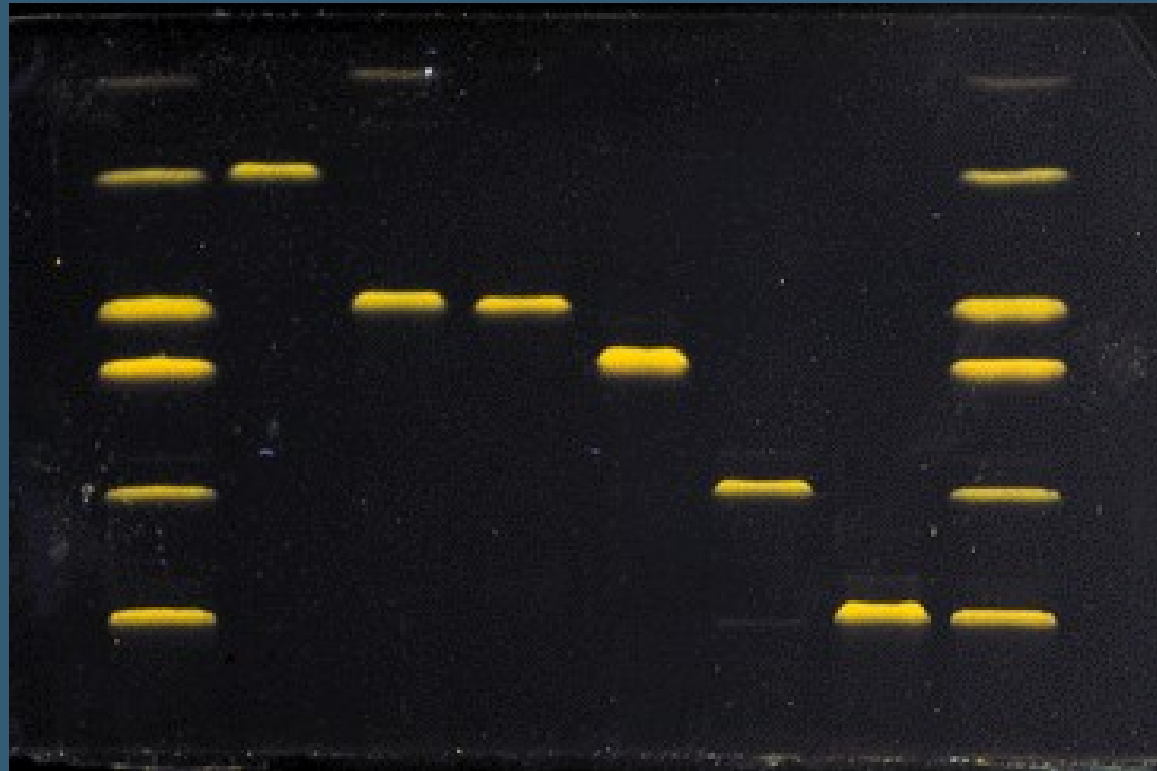
A. Autoradiografie $^{32}\text{P}, ^{125}\text{I}$

značený protein \longrightarrow rentgenový film

B. Fluorografie $^3\text{H}, ^{14}\text{C}, ^{35}\text{S}$

značený protein \longrightarrow fluorofor \longrightarrow rentgenový film

AUTORADIOGRAFIE

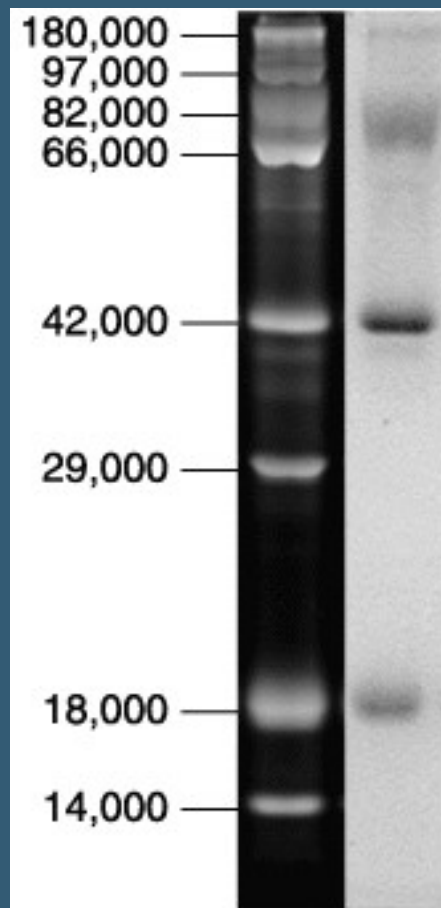


SPECIFICKÁ DETEKCE

Detekce různých skupin bílkovin

glykoproteiny	-	Schiffova reakce (oxidace s HJO_4 , reakce s Schiffovým činidlem - bazický fuchsin)
lipoproteiny	-	Sudan B
hemoproteiny	-	peroxidázová aktivita (hem + benzidin + H_2O_2)
Cu^{2+}	-	alizarin
Fe^{3+}	-	fenantrolin

DETEKCE GLYKOPROTEINŮ



DETEKCE NA ZÁKLADĚ BIOLOGICKÉ AKTIVITY

Detekce na základě enzymové aktivity

Rozdělení

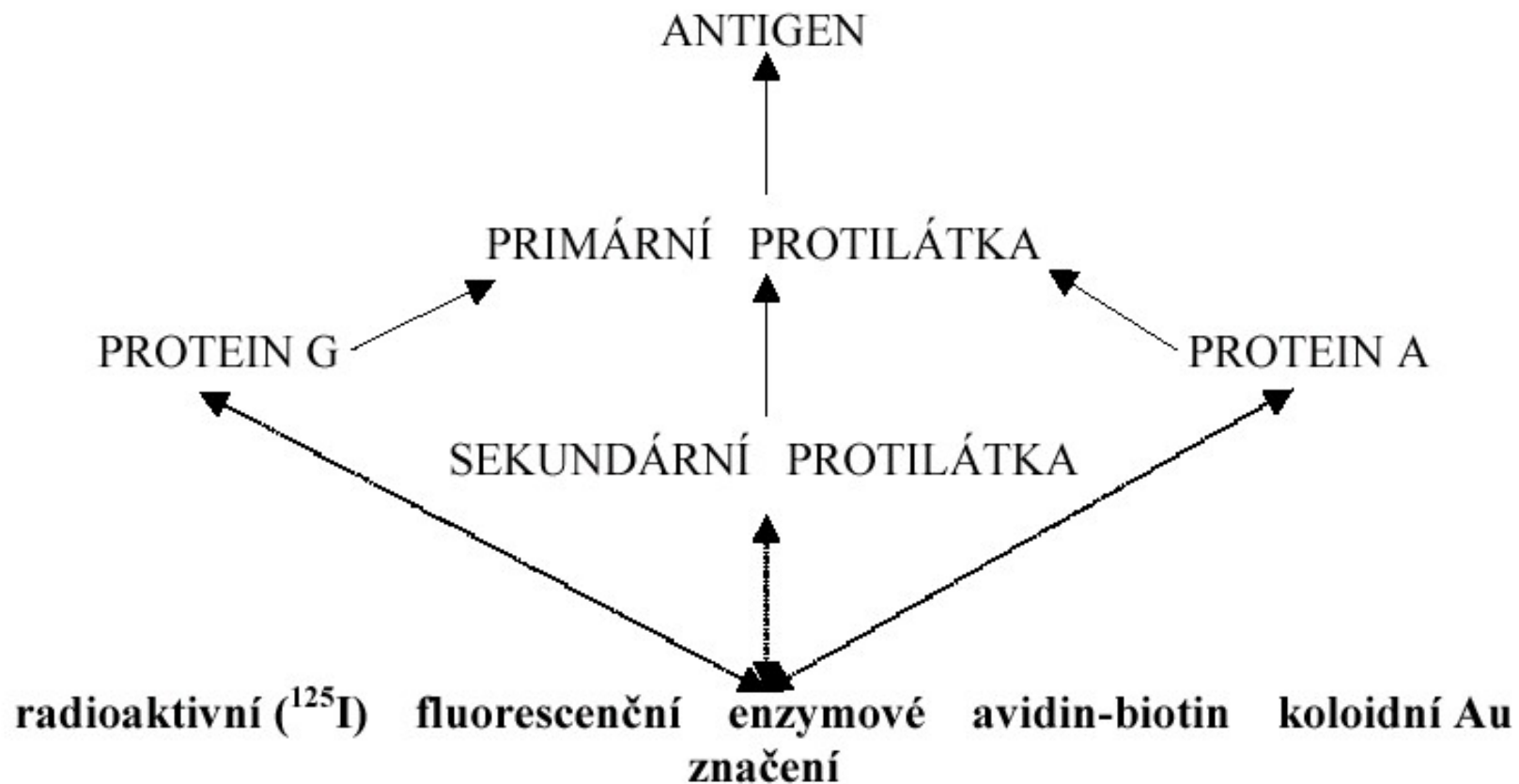
- A. bezbarvý substrát → barevný produkt
- B. barevný substrát → bezbarvý produkt
- C. bezbarvý substrát → bezbarvý produkt
→ barevný produkt
- D. auxiliární enzymy → barevný produkt

Detekce

- přímo v gelu
- přímo v gelu se zapolymerovanými substráty
- otisk - zymogram
- indikátorové gely

DETEKCE NA ZÁKLADĚ BIOLOGICKÉ AKTIVITY

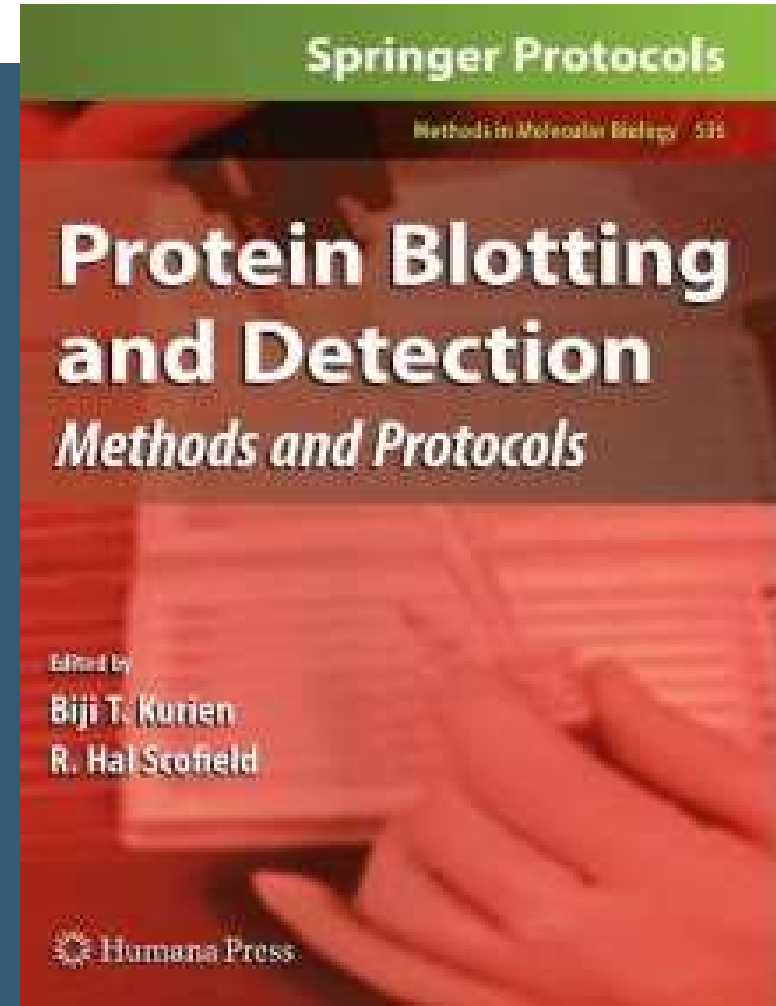
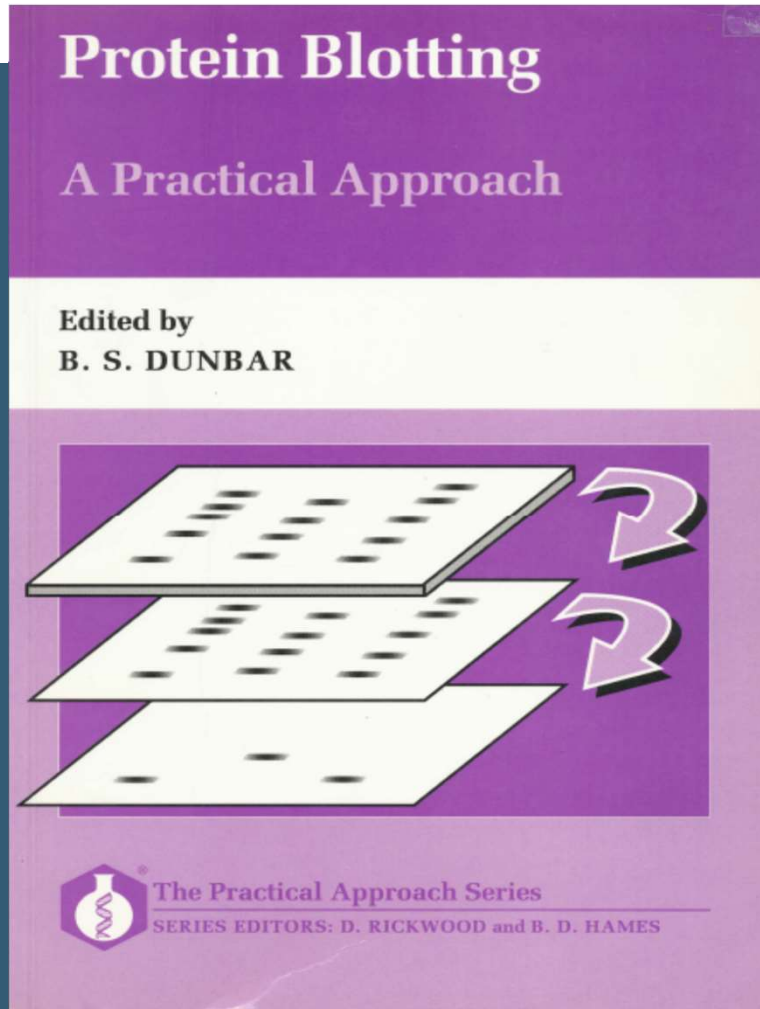
Imunodetekce



BLOTTING

- Southern – DNA
- Northern – RNA
- Western - bílkoviny

BLOTTING



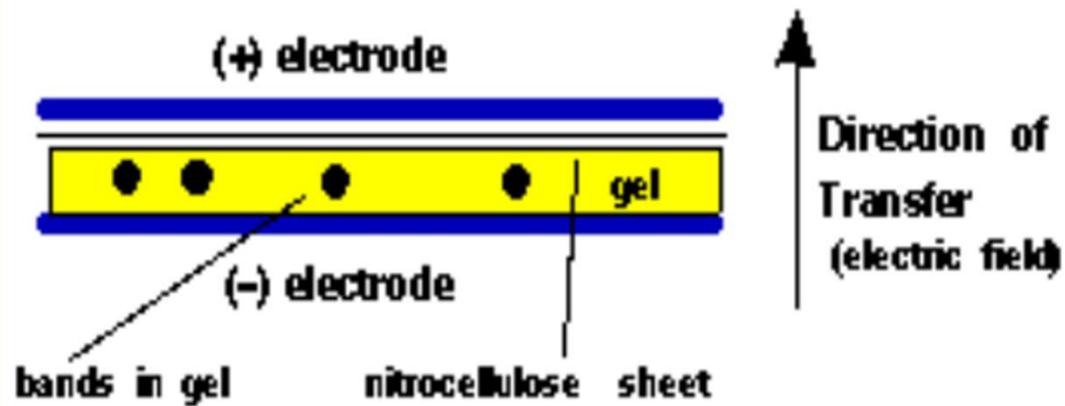
MEMBRÁNY

- nitrocelulosa - nejběžnější
- polyviniliden difluorid – vysoká vazebná kapacita
- diazobenzoyloxymethyl – chemická aktivace
- ionexové membrány - preparativní
- aktivovaná skleněná vlákna – pro přímou sekvenaci

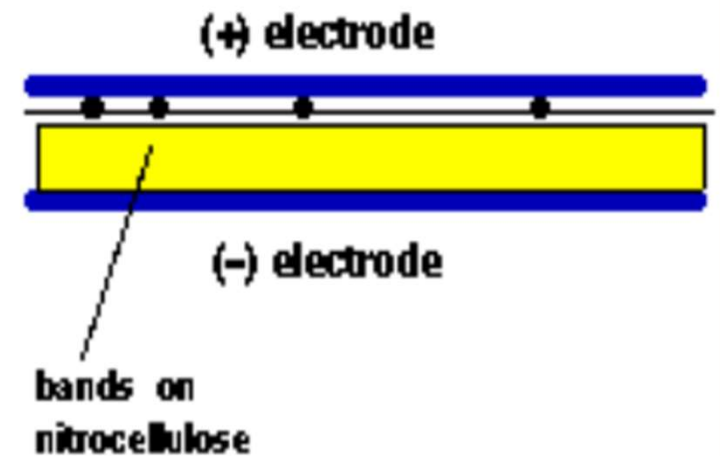
BLOTTING

Side View:

Before Transfer:



After Transfer:

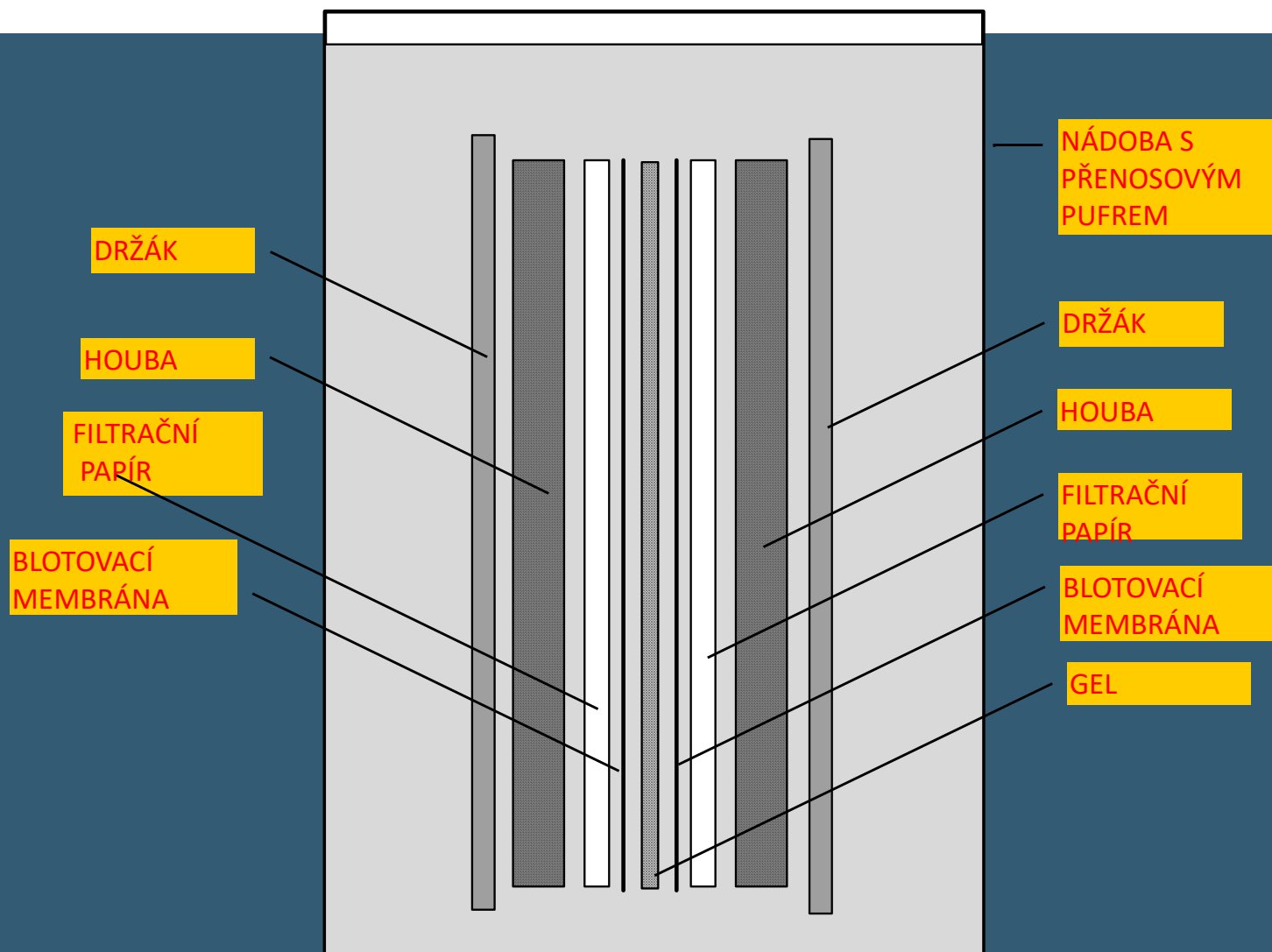


Note: All the layers are pressed tightly together.

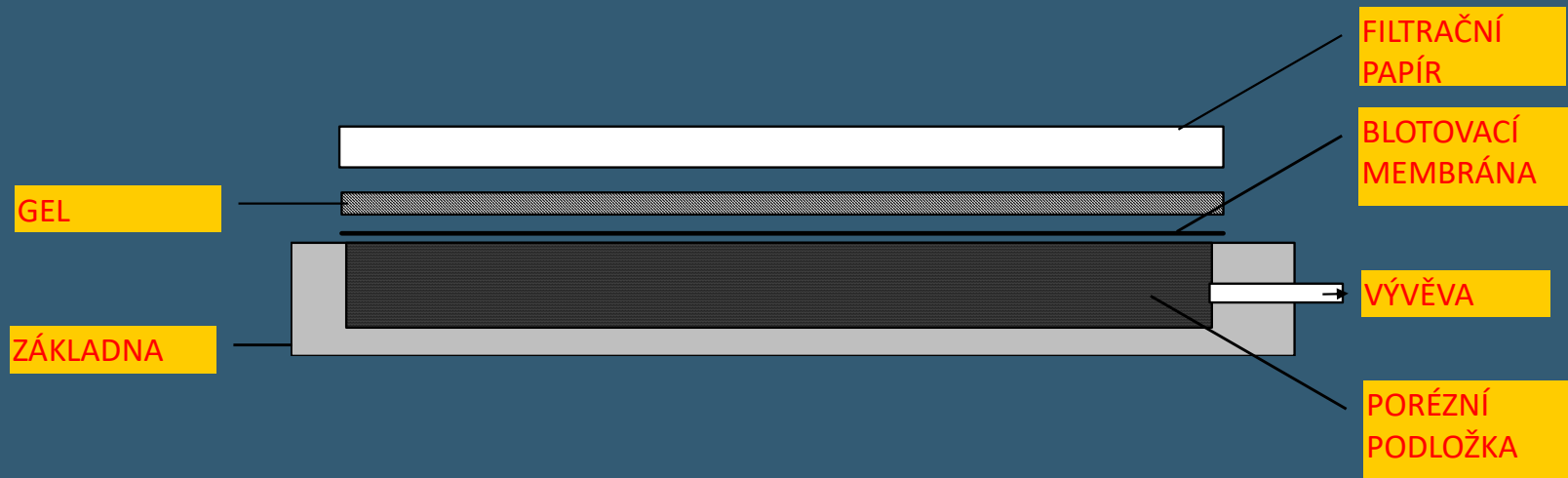
VÝHODY BLOTTINGU

- Dostupnost biomakromolekul
- Zakoncentrování biomakromolekul
- Redukce doby a množství potřebných chemikálií
- Imobilizace biomakromolekul – možnost uchovávání
- Možnost vícenásobné detekce
- Mechanická stabilita

DIFUZNÍ BLOTTING



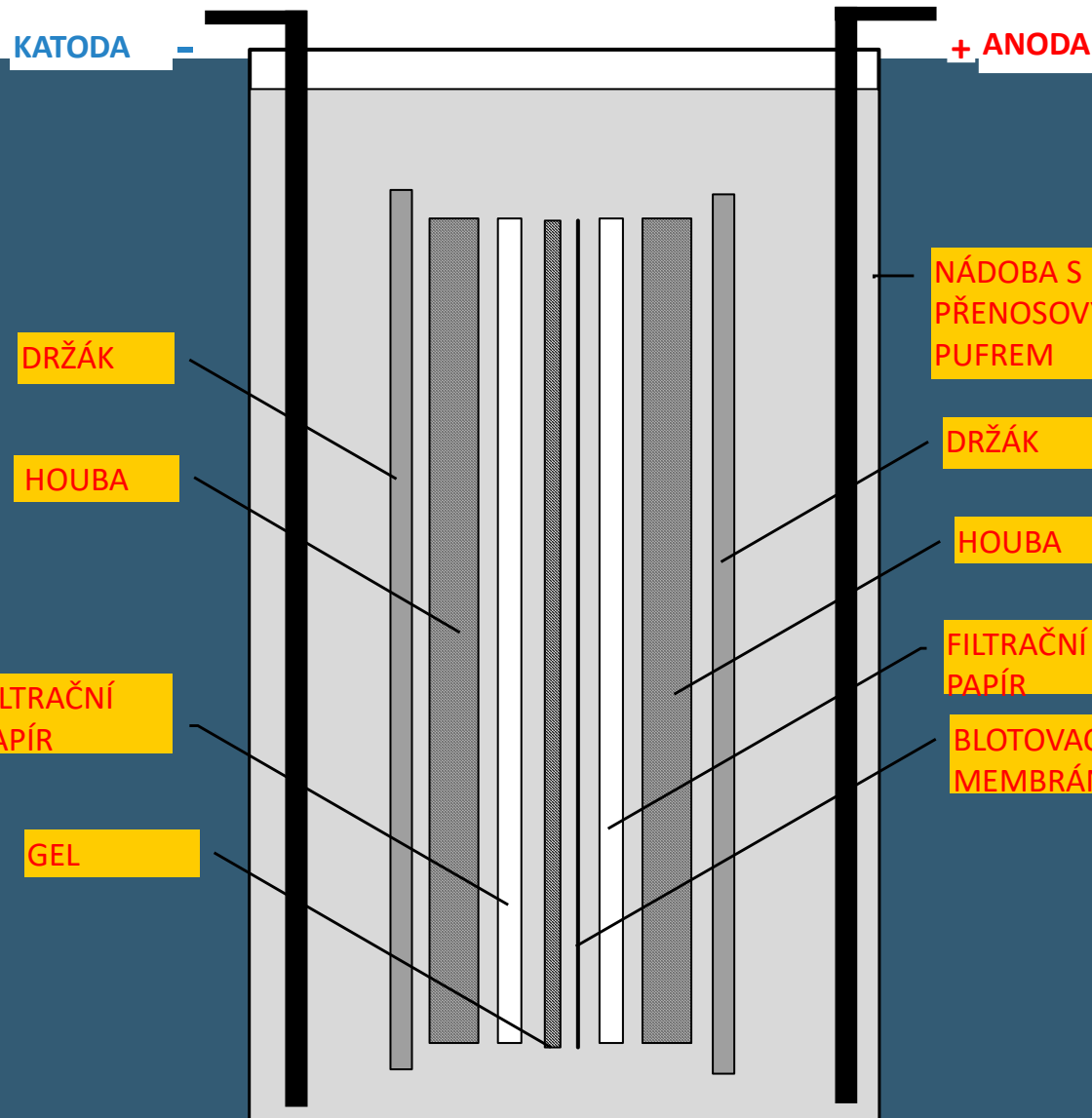
VAKUOVÝ BLOTTING



KAPILÁRNÍ BLOTTING



TANKOVÝ ELEKTROBLOTTING



TANKOVÝ ELEKTROBLOTTING



TANKOVÝ ELEKTROBLOTTING

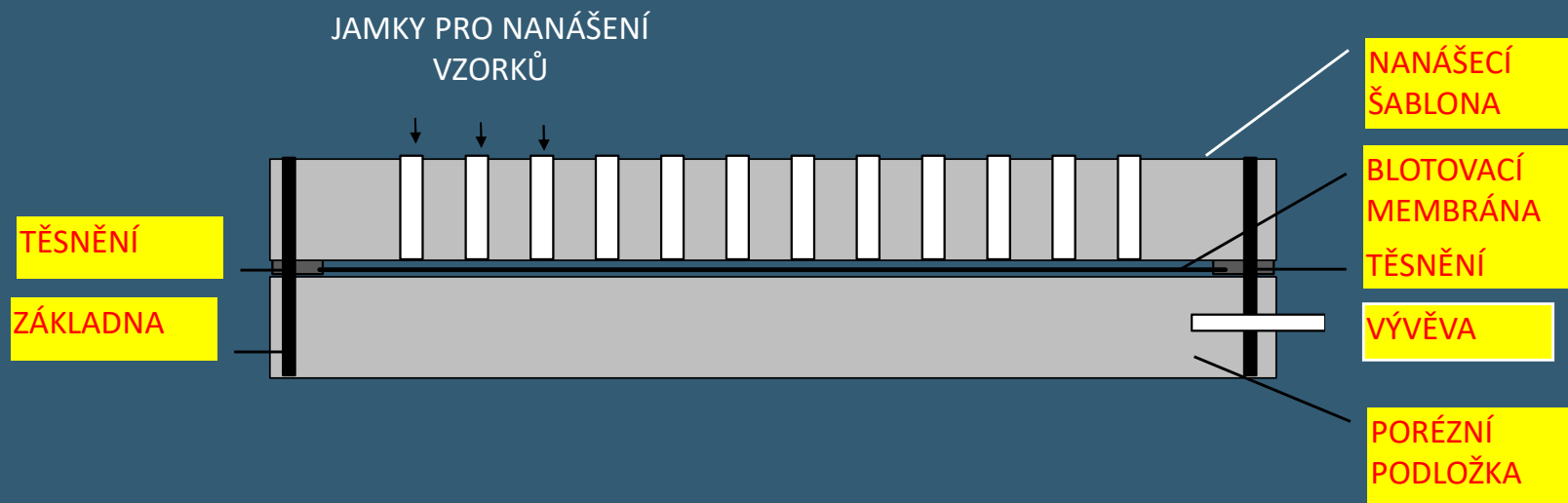
MINI PROTEAN TRANS BLOT CELL



„SEMI DRY“ BLOTTING



KAPKOVACÍ DOT BLOTTING



DETEKCE

HYBRIDIZACE

- radioaktivní proba – vysoká senzitivita, Southern blot
- neradioaktivní proba – biotin – streptavidin, dioxigenin

REAKCE SE SUBSTRÁTEM

nativní enzym, nedifundující substrát

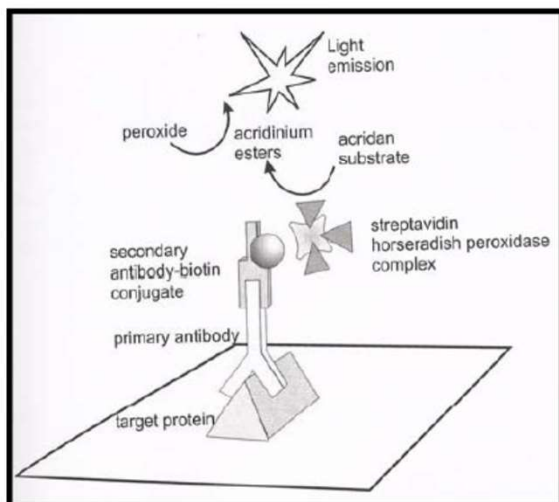
“IMUNOBLOTTING“

^{125}I -protein A

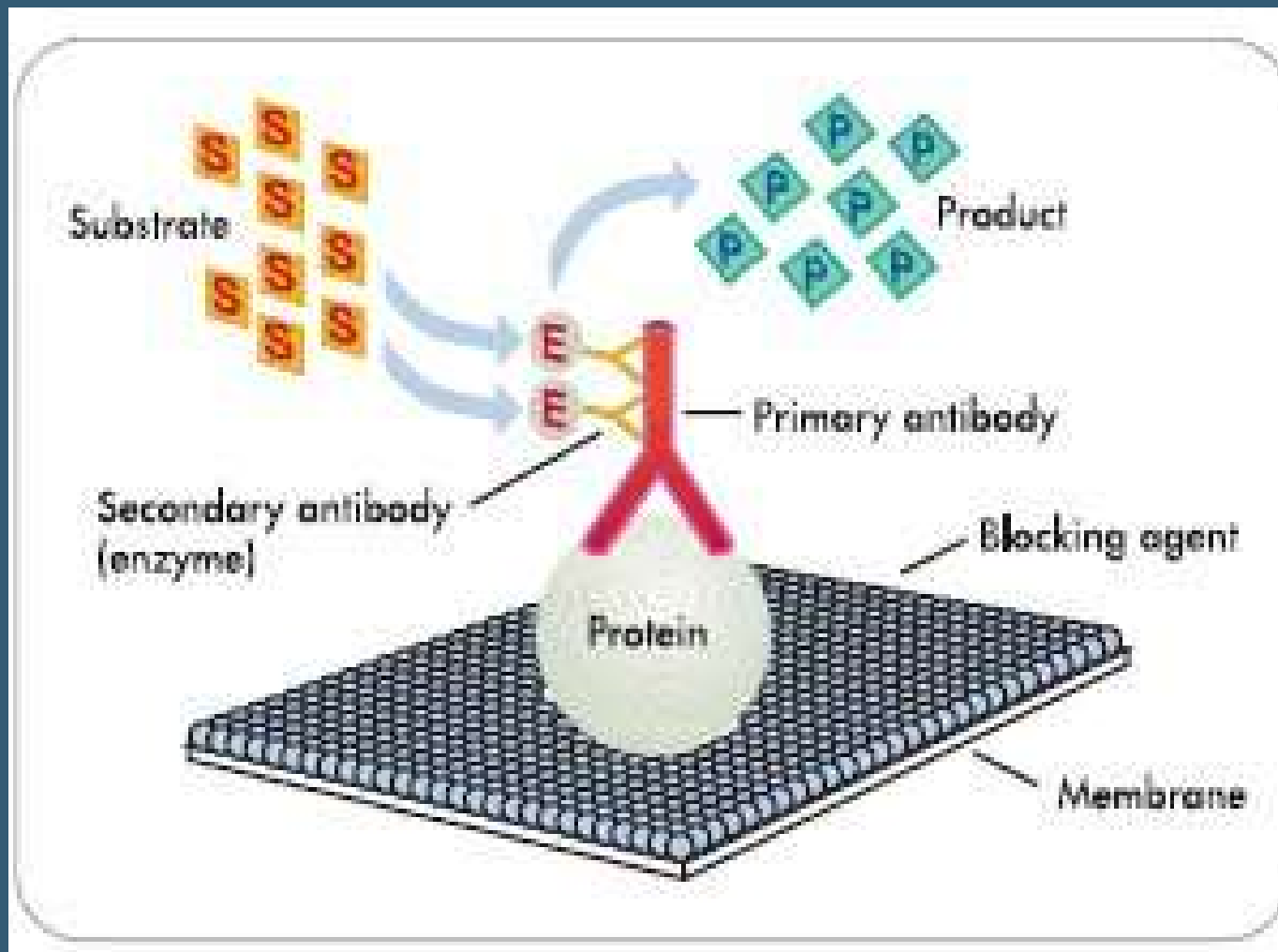
enzymem značená sekundární protilátka – konjugace s peroxidasou (tetrazoliová sůl), alkalickou fosfatasou

zlatem značená sekundární protilátka (100 pg)

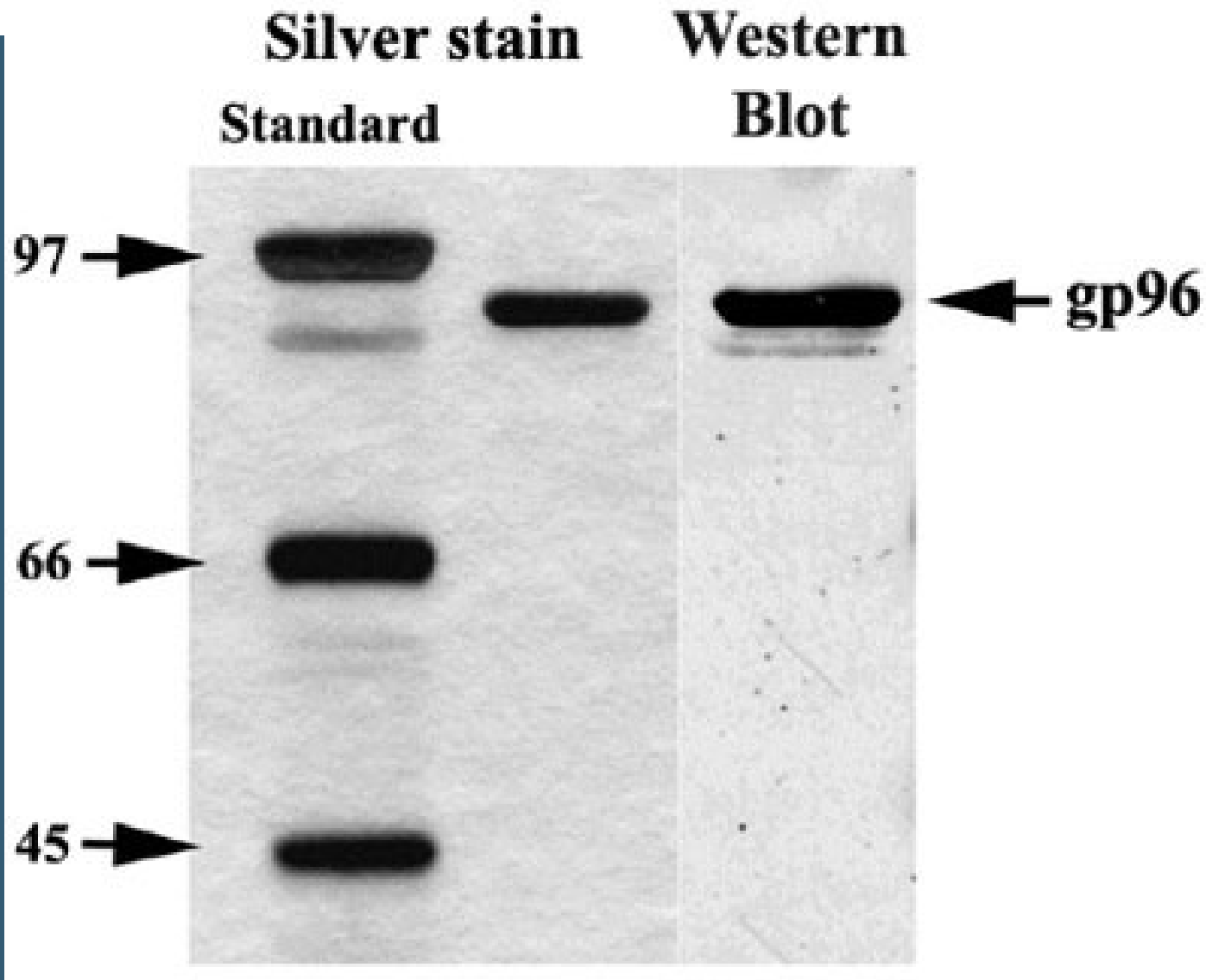
chemiluminiscence – nejcitlivější



DETEKCE



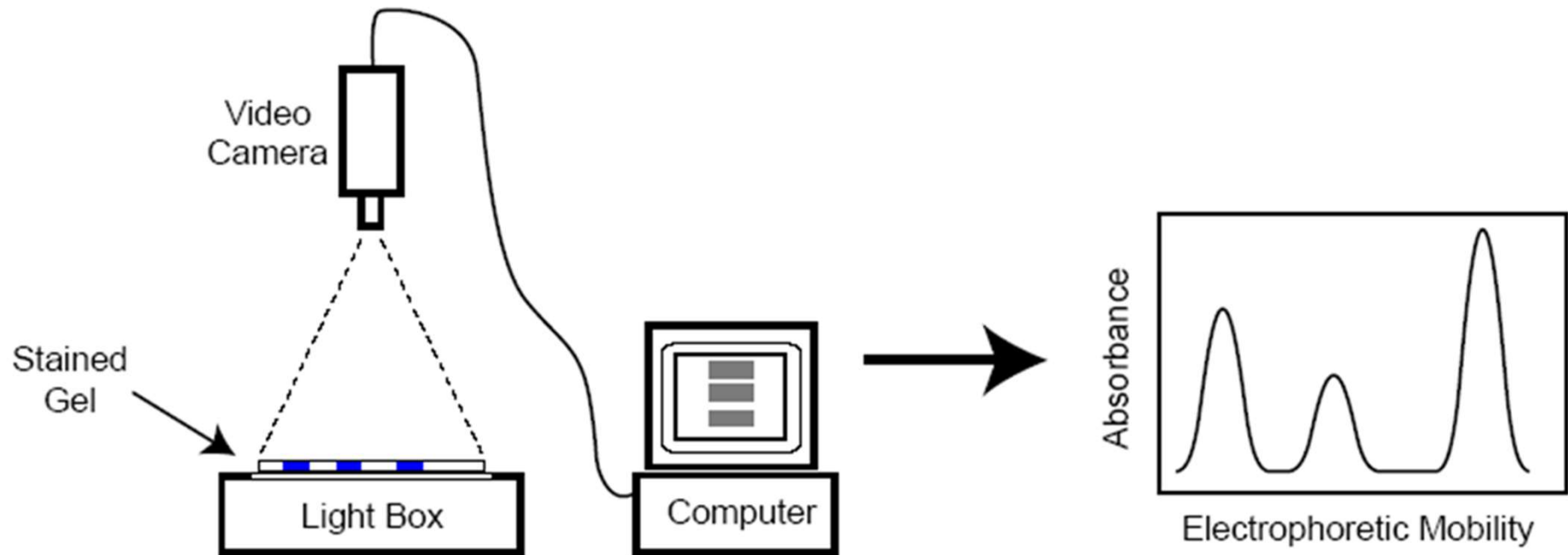
DETEKCE



SPECIÁLNÍ APLIKACE BLOTTINGU

- Získání homogenních preparátů
- Studium složení a struktury bílkovin
- MS bílkovin – MALDI, DESI
- Příprava protilátek
- Purifikace protilátek

DETEKCE



DETEKCE



MUNI
SCI