MASARYKOVA UNIVERZITA

Dvoudimenzionální elektroforéza

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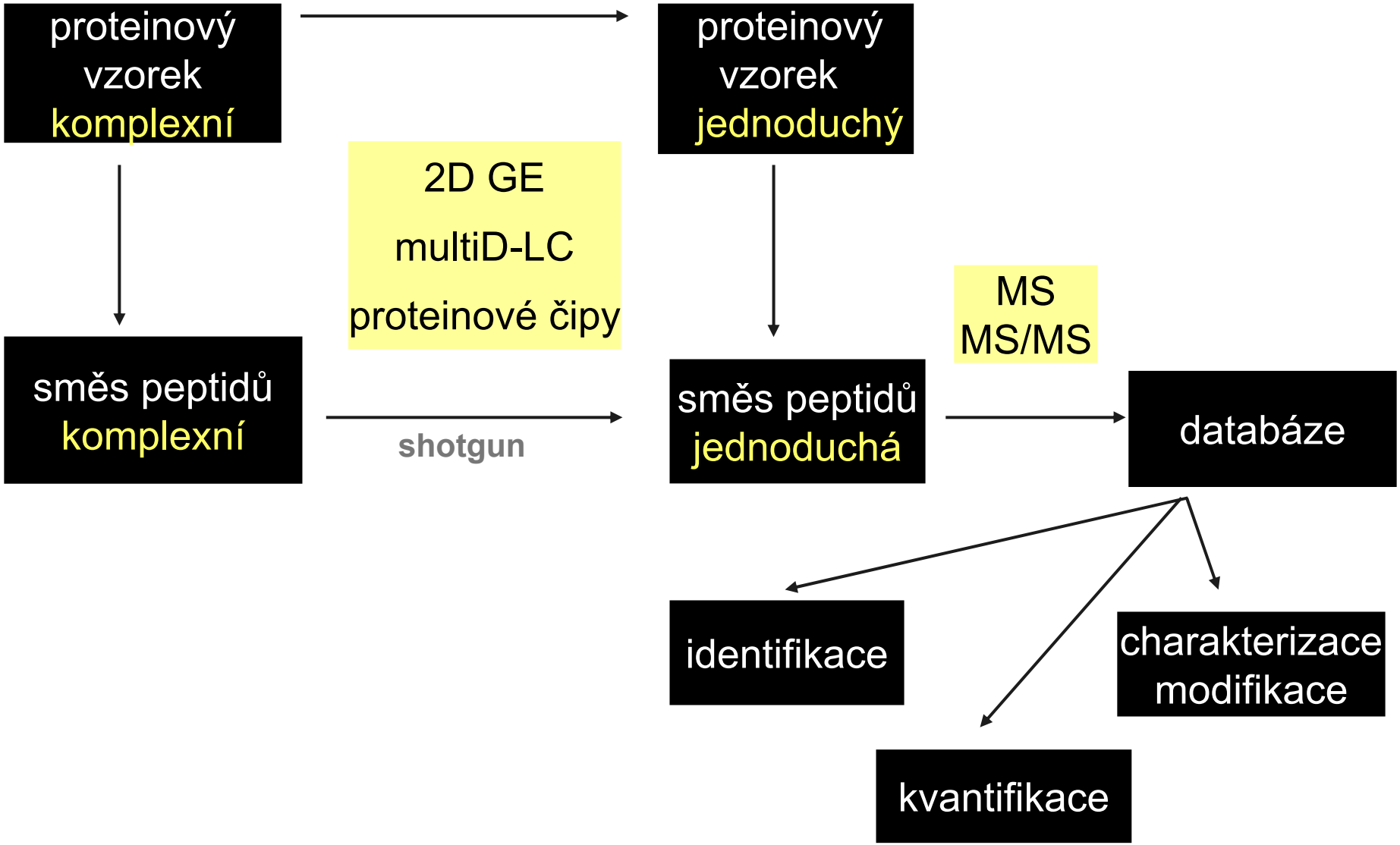
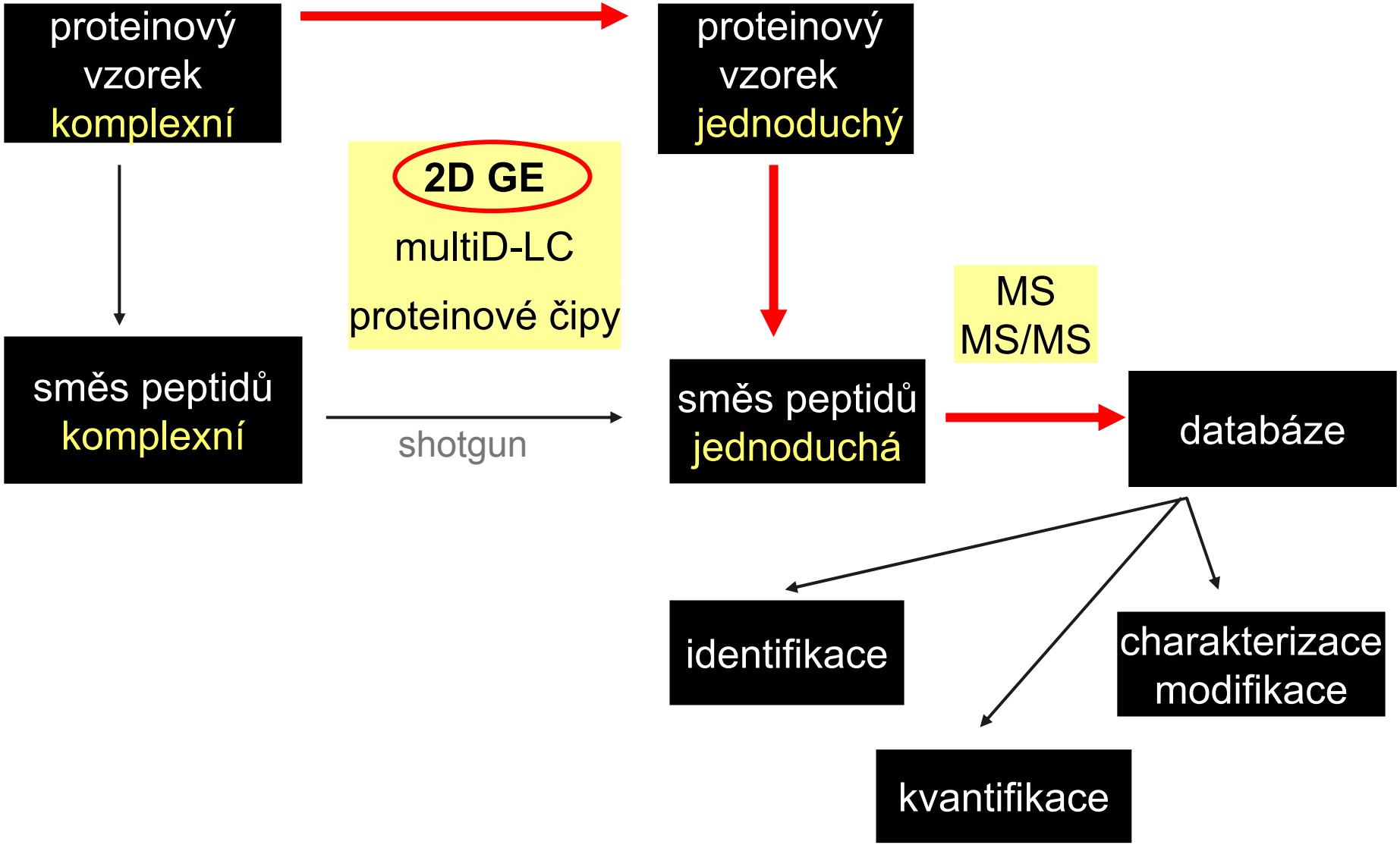
NCBR – Národní centrum pro výzkum biomolekul PřF

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ


proteinový vzorek komplexní
proteinový vzorek jednoduchý
2D GE
multiD-LC
proteinové čipy
směs peptidů komplexní
shotgun
směs peptidů jednoduchá
MS
MS/MS
databáze
identifikace
charakterizace modifikace
kvantifikace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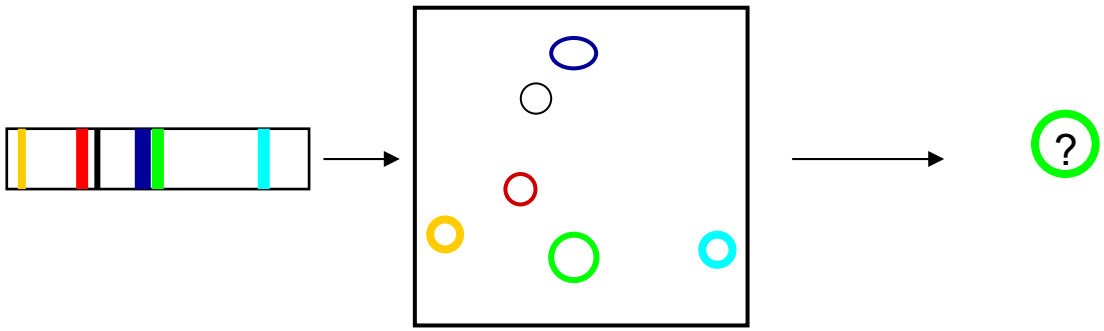
I. SEPARACE

II. PREFRAKCIONACE

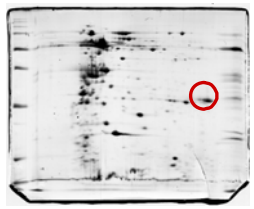
Dvoudimenzionální elektroforéza
2-DE

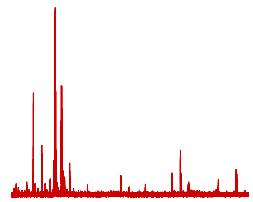
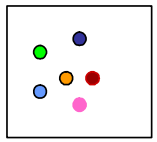


Proteomický experiment

extrakce **fokusace** **SDS-PAGE** **digest** **MS**





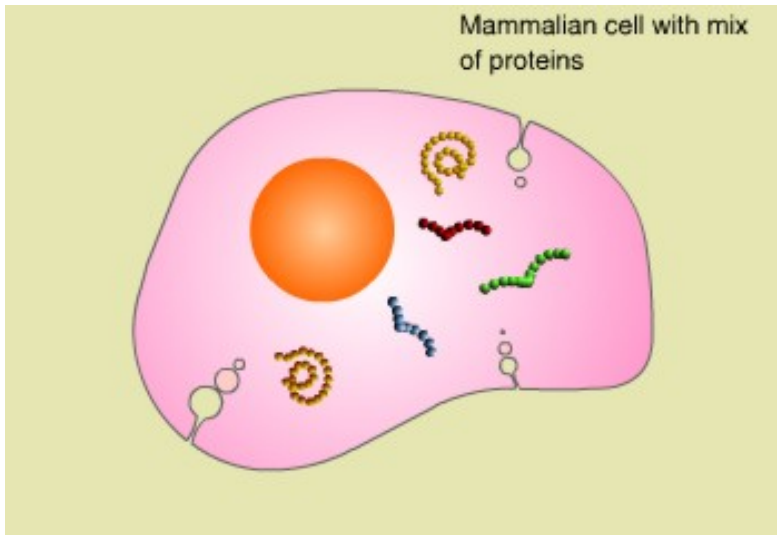



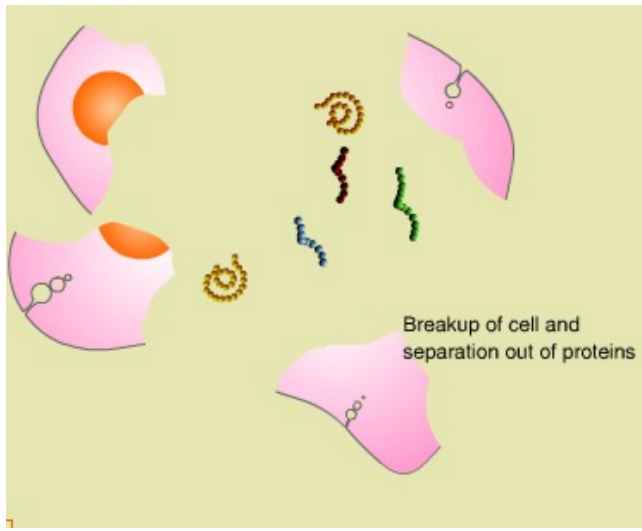
identifikace

neznámý protein

HOMOGENIZACE

- mechanicky
- ultrazvukem
- tlakem
- zmražením / rozmražením
- detergentovou lyzí





PŘÍPRAVA VZORKU

solubilizace močovina, thiomočovina, detergenty

redukce DTT, TBP

inhibice proteáz, fosfatáz, glykosyláz

odstranění kontaminant

MĚŘENÍ KONCENTRACE PROTEINU

DETERGENTY

- žádný celkový náboj
- 0.5 – 4%
- použitelné ve vysokých koncentracích močoviny

- neionogenní
- zwitterointové

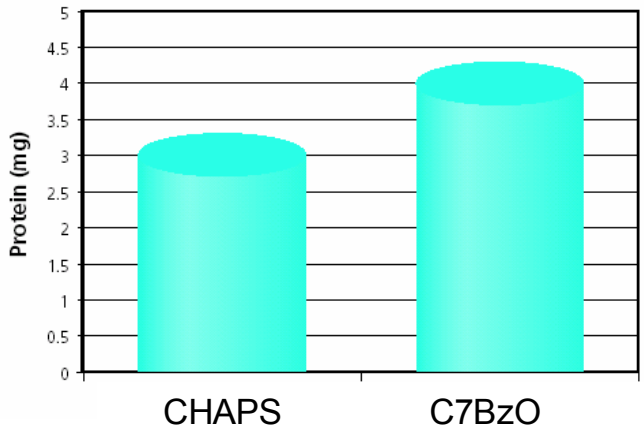
- SDS jen v nízkých koncentracích (do 0.25%)

C7BzO

3-(4-Heptyl)phenyl-3-hydroxypropyl)dimethylammoniopropanesulfonate

CHAPS

3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate



E.coli

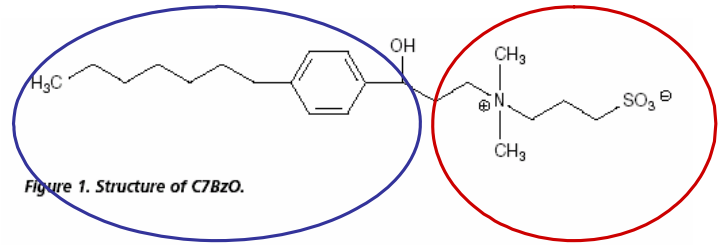
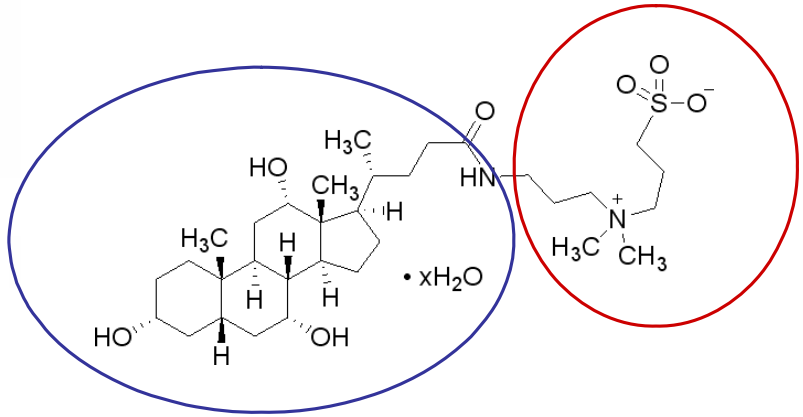


Figure 1. Structure of C7BzO.



ZÁKLADNÍ PRAVIDLA

- zabránit proteolýze
- jednoduchý postup
- čerstvé reagencie
- čerstvý vzorek
- odstranit pevné částice - centrifugace
- odstranit kontaminanty

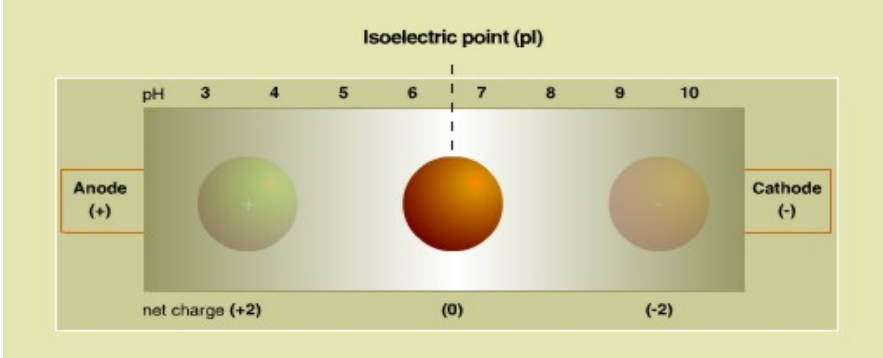
KONTAMINANTY

- soli, zbytky pufrů
- malé endogenní molekuly
- iontové detergenty
- nukleové kyseliny
- polysacharidy
- lipidy
- fenolické látky

2-DE

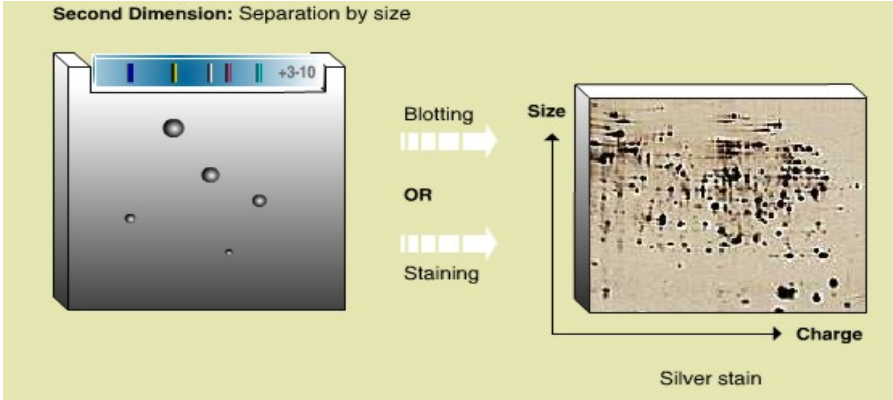
- první rozměr

IEF



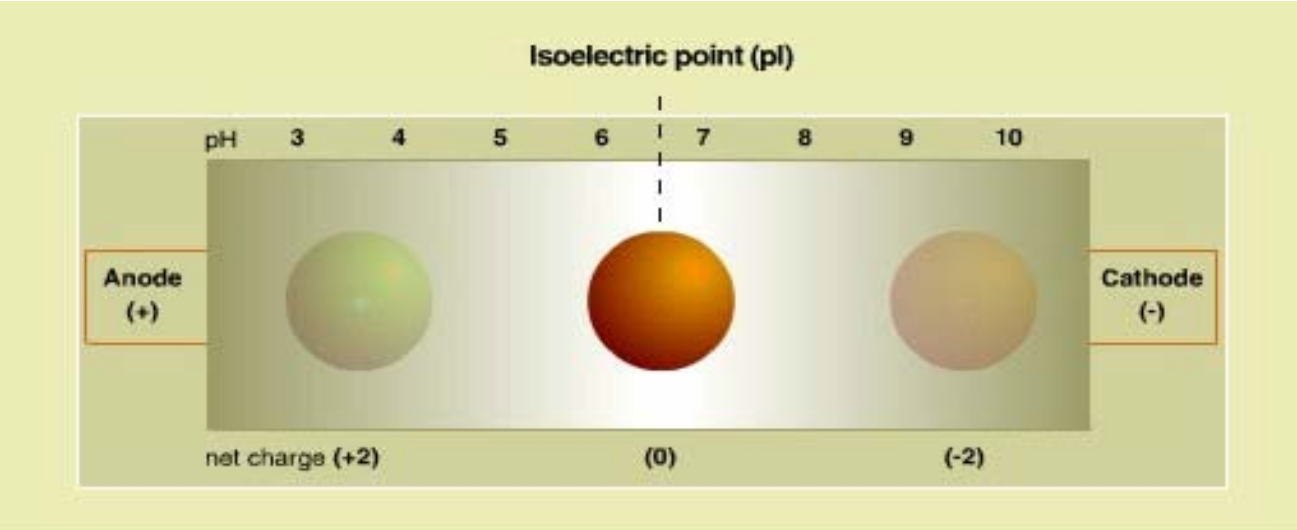
- druhý rozměr

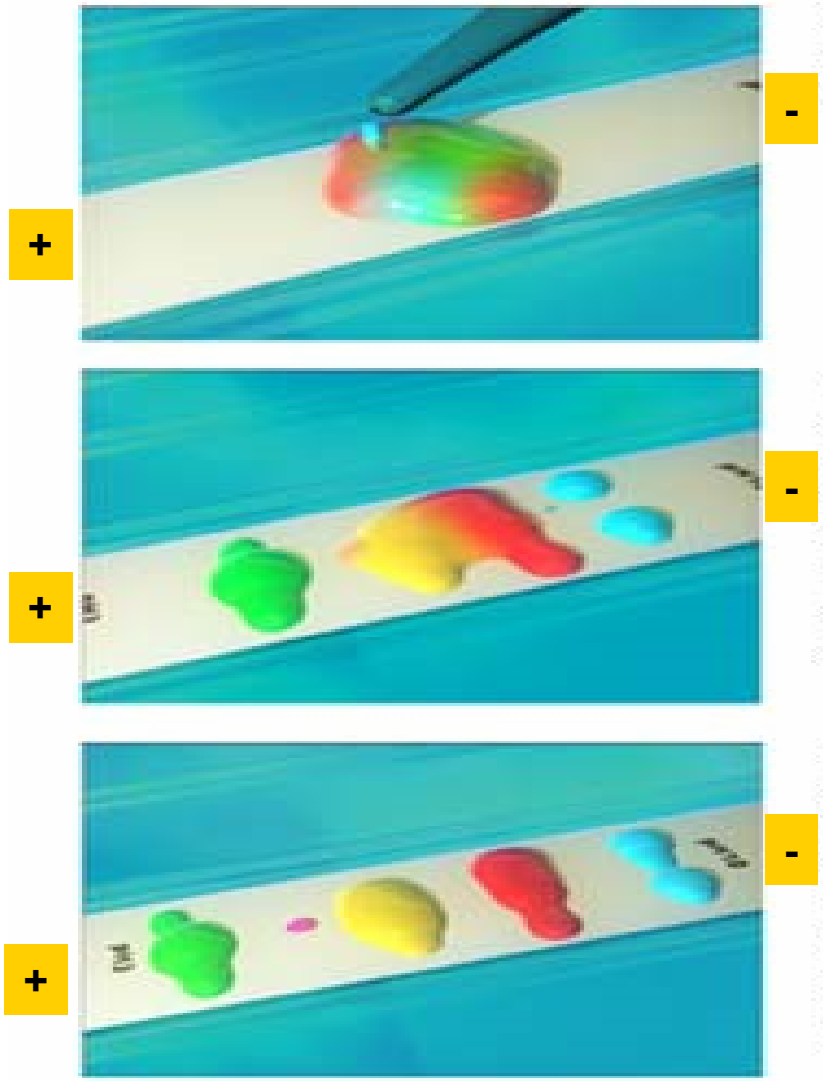
SDS-PAGE



1. ROZMĚR IZOELEKTRICKÁ FOKUSACE

migrace nabitých částic v gradientu pH v elektrickém poli

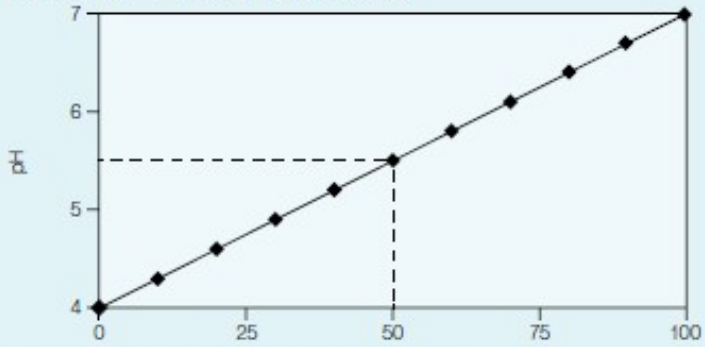


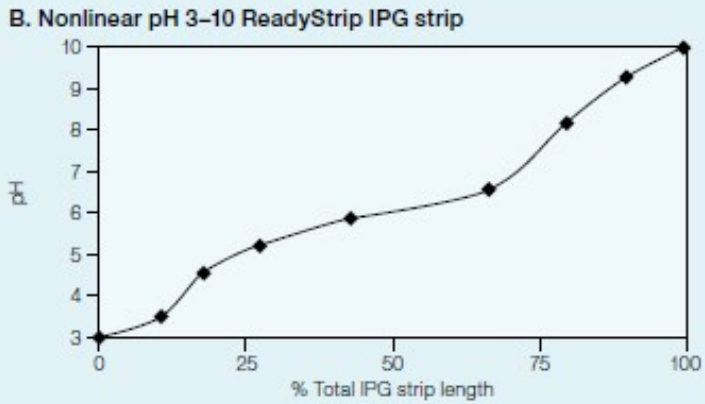


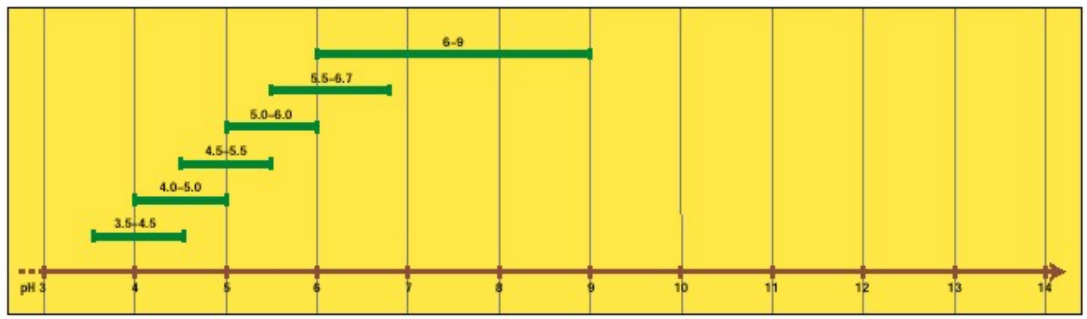
IZOELEKTRICKÁ FOKUSACE

- imobilizovaný pH gradient
- amfolyty

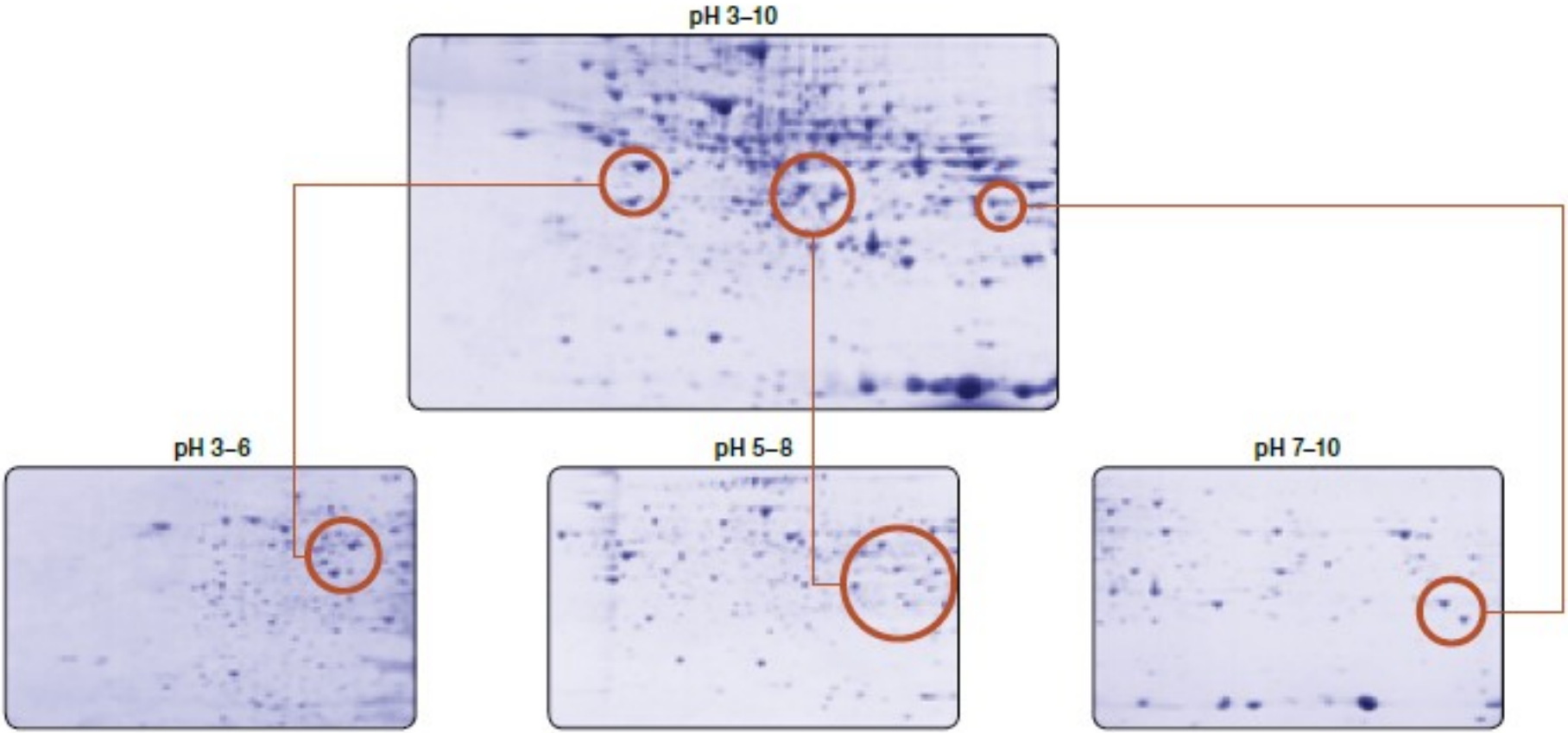
ROZSAH STRIPU
ROZMĚR STRIPU





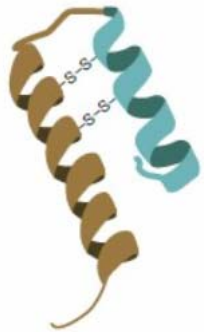


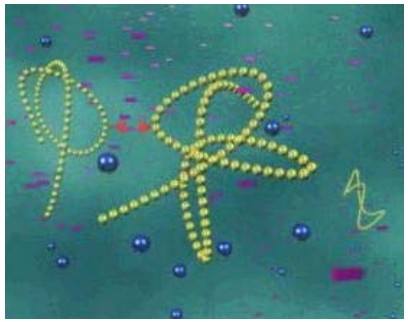
ROZSAH STRIPU

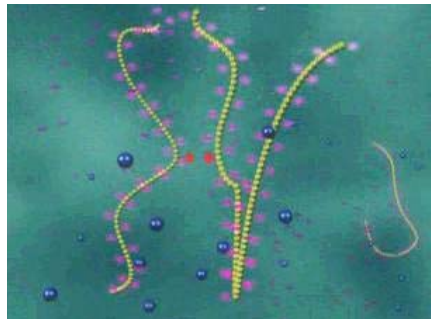


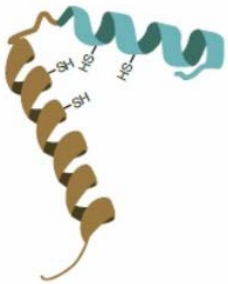
EKVILIBRACE STRIPU

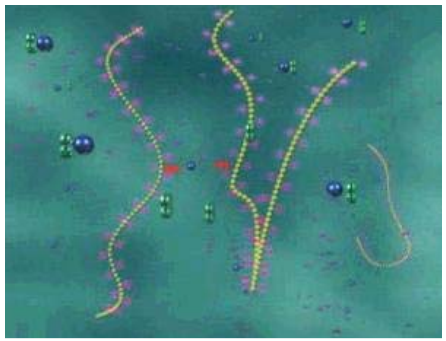
denaturace **SDS** •

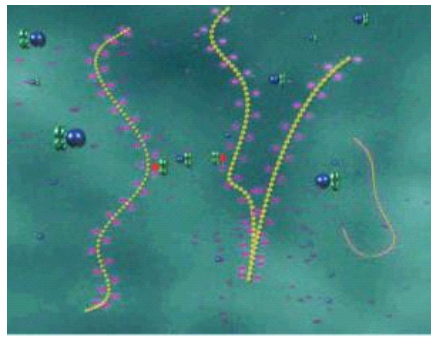


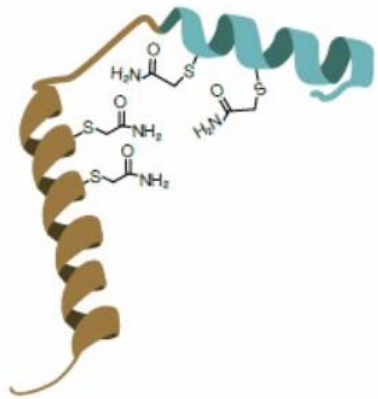








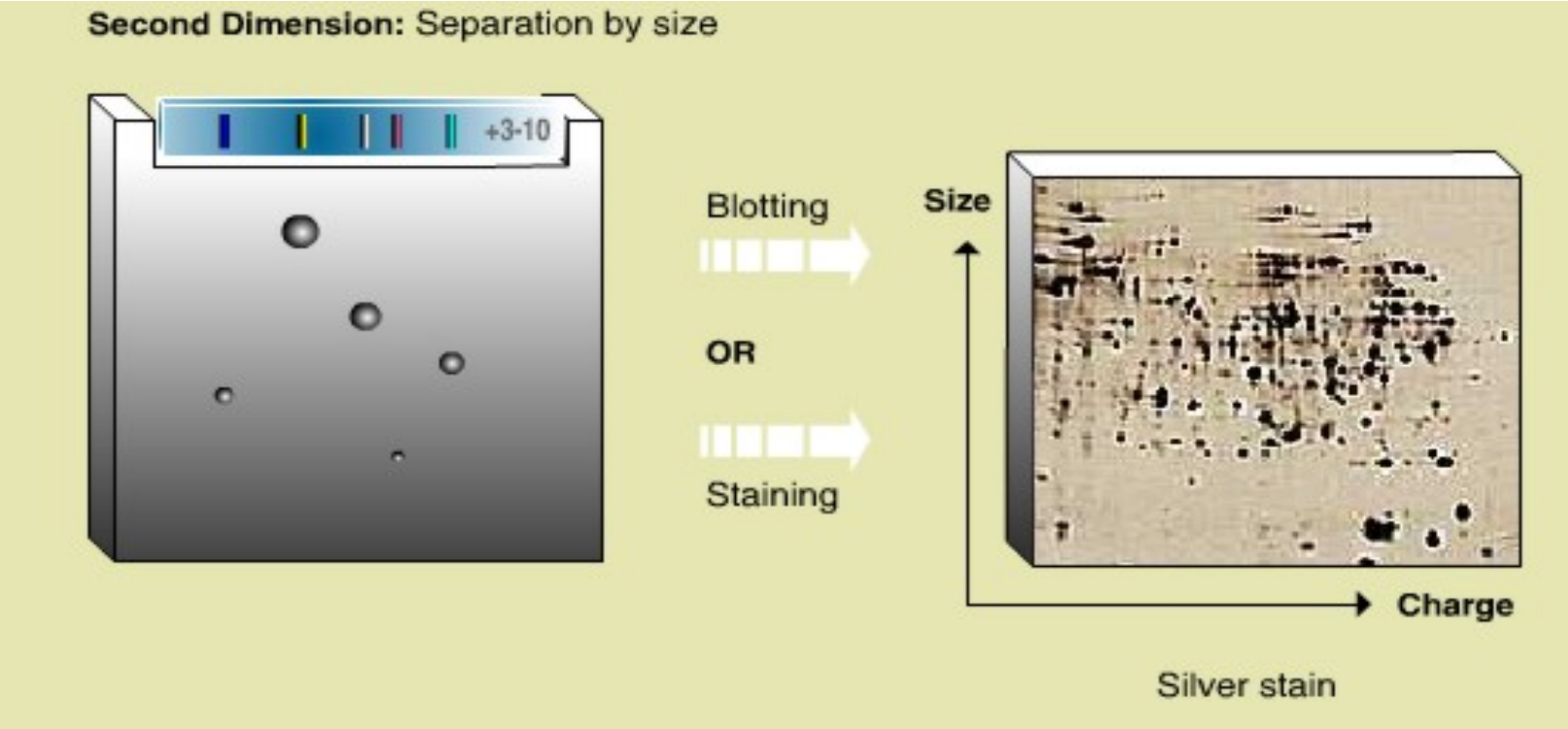




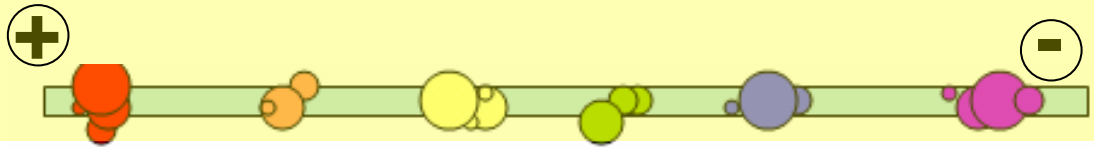
redukce **DTT** ● alkylace **IAA** •

2. ROZMĚR SDS-PAGE

migrace aniontů v elektrickém poli podle MW



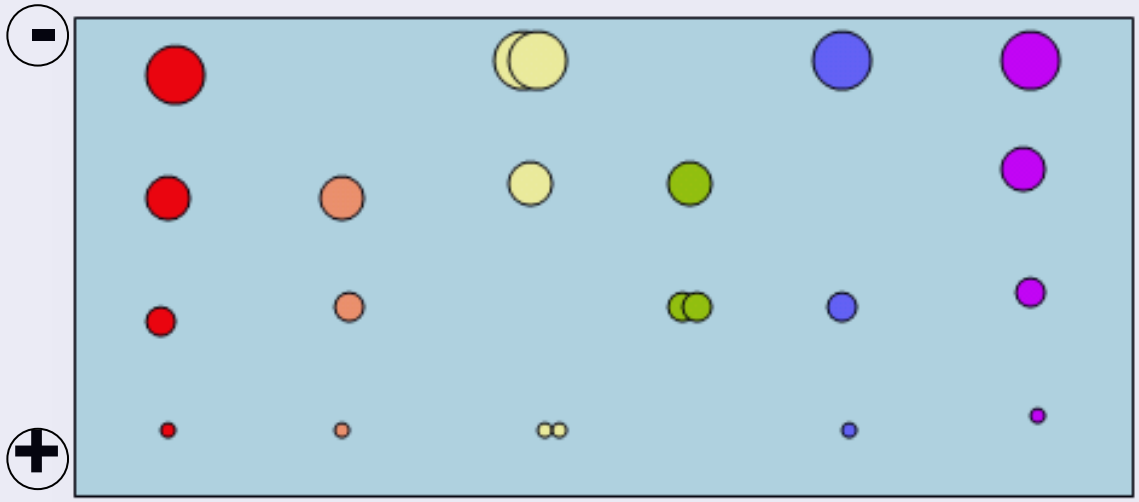
FOKUSACE



STRIP

ekvilibrace

SDS-PAGE



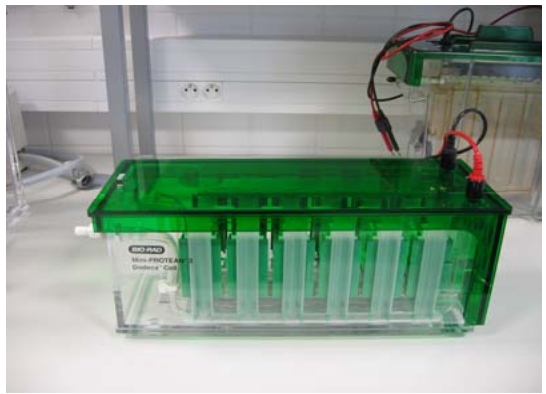
GEL

2-DE INSTRUMENTACE

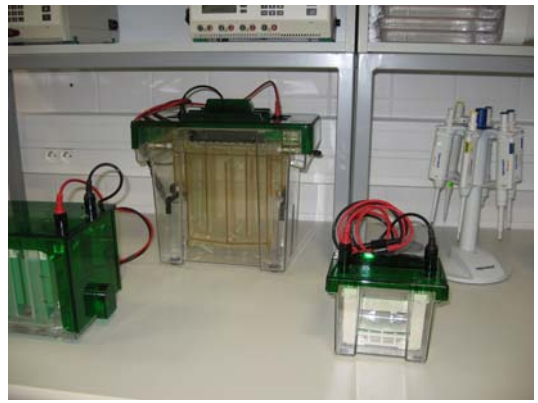
- Protean IEF
- Protean Dodeca Cell
- Densitometer GS-800
- FLA-7000, STORM

PDQuest, Quantity One

Protean Plus Dodeca Cell



Mini-Protean 3 Dodeca Cell

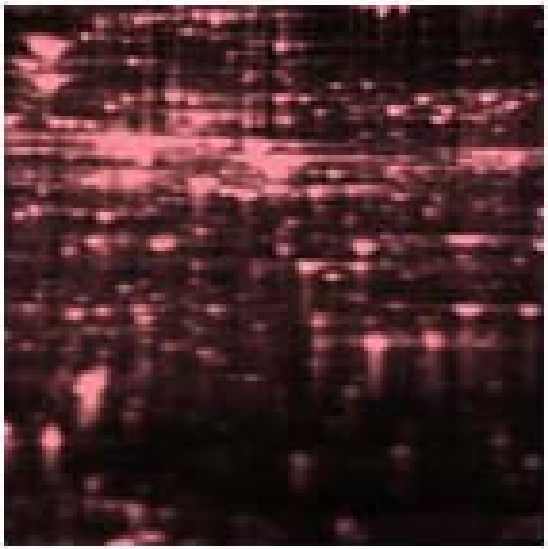


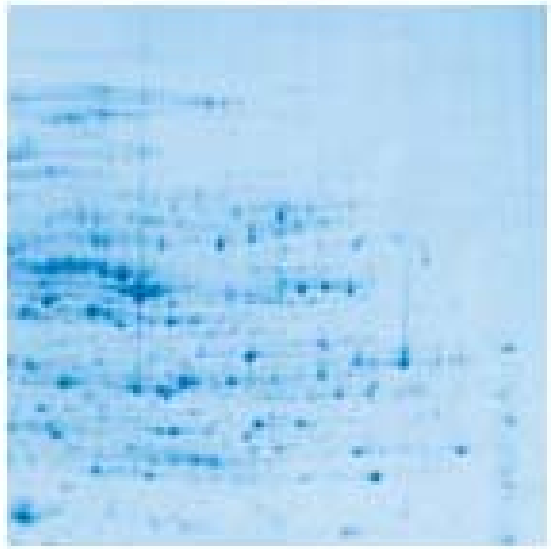
Protean II xi Cell

DETEKCE PROTEINU

- gel x blot
- visualizace → barvení
  → radioaktivita
  → imunodetekce
- barvení v gelu
  - po elektroforéze
  - před elektroforézou
  - specifické pro protein
  - specifické pro PTM
  - viditelné spektrum
  - fluorescence

DETEKCE PROTEINU V GELU



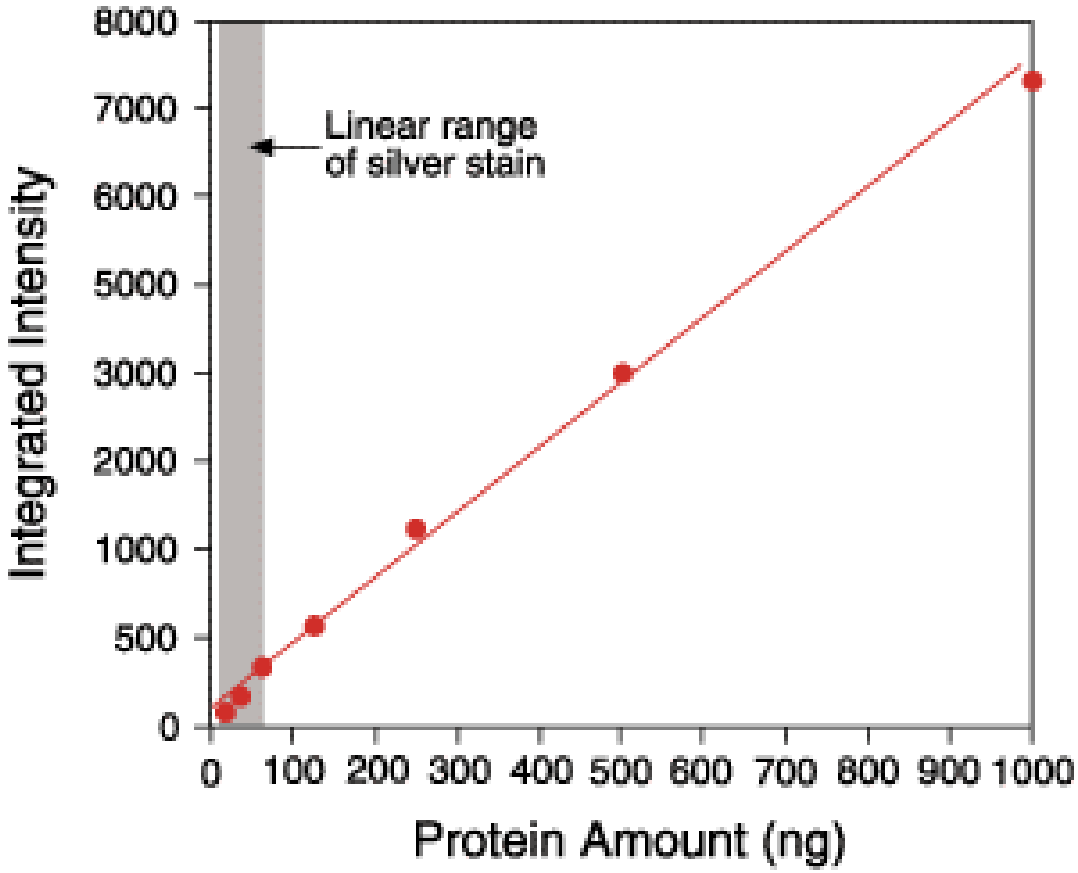


Sypro Ruby	Coomassie	silver
1.4 ng	36 ng	0.6 ng

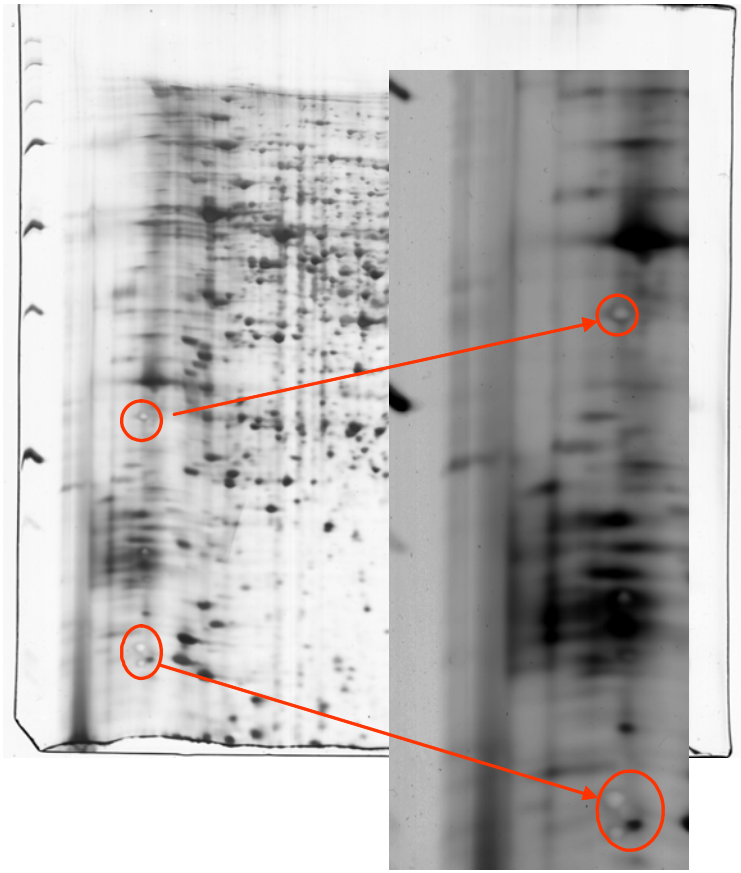
PTM specifická barvení *Pro-Q Diamond* *Pro-Q Emerald*

BARVENÍ PROTEINU – LINEARITA

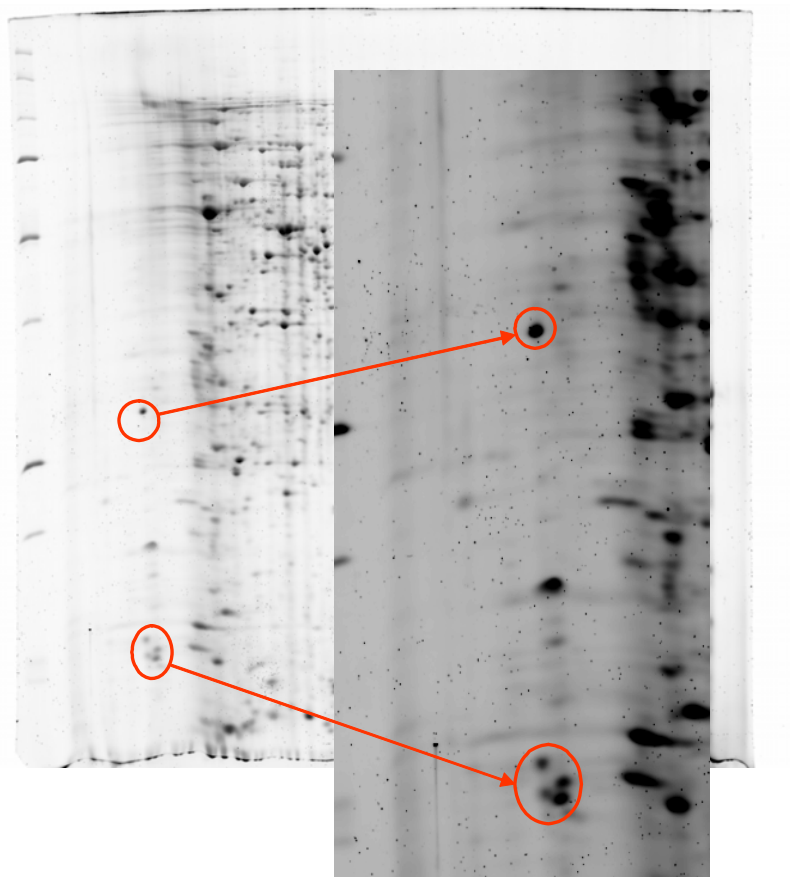
Sypro Ruby



Ag

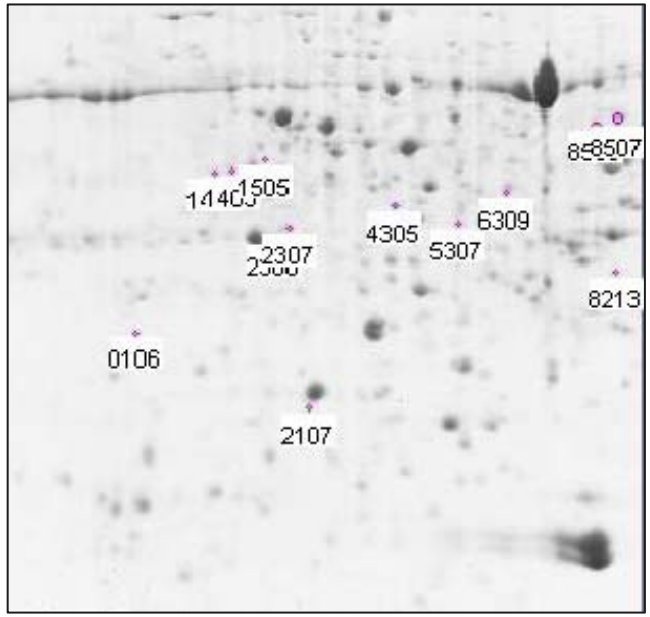


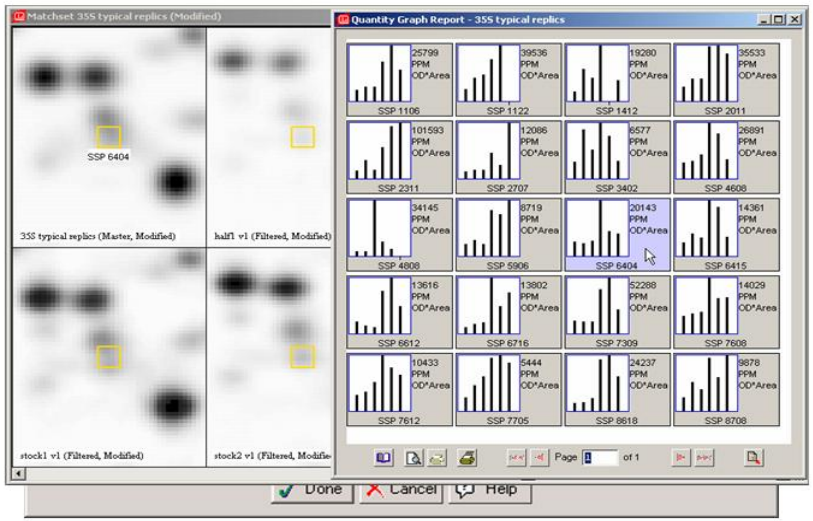
Sypro Ruby



ANALÝZA OBRAZU

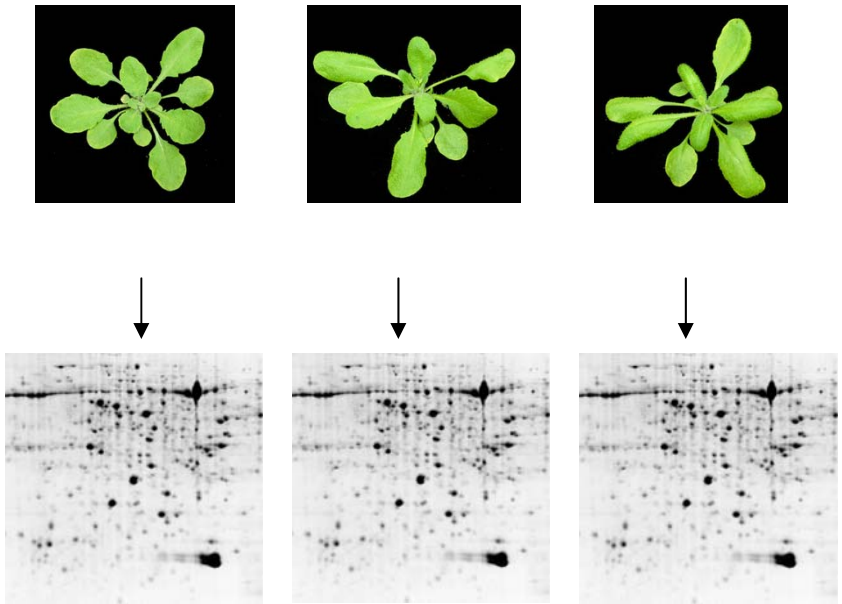
- kvalitativní
- kvantitativní





biologická variabilita

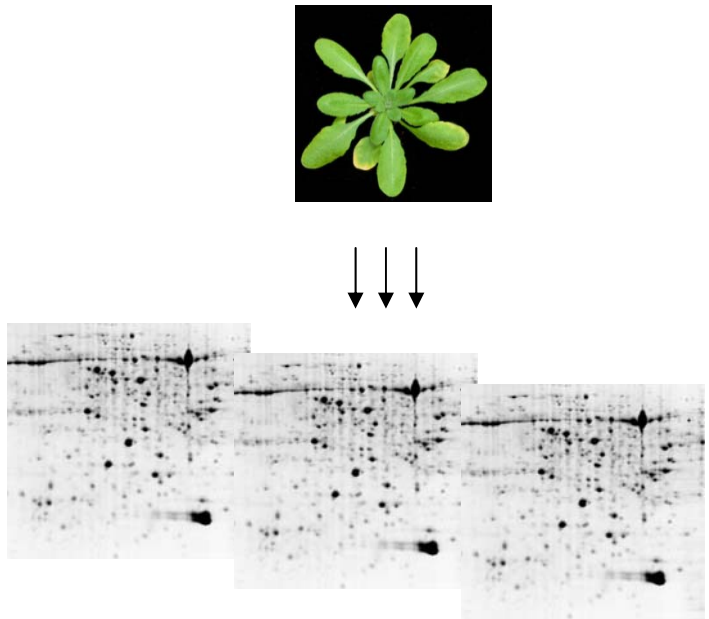
stejný organismus za stejných podmínek



biologické replikáty

technická variabilita

stejný vzorek stejně



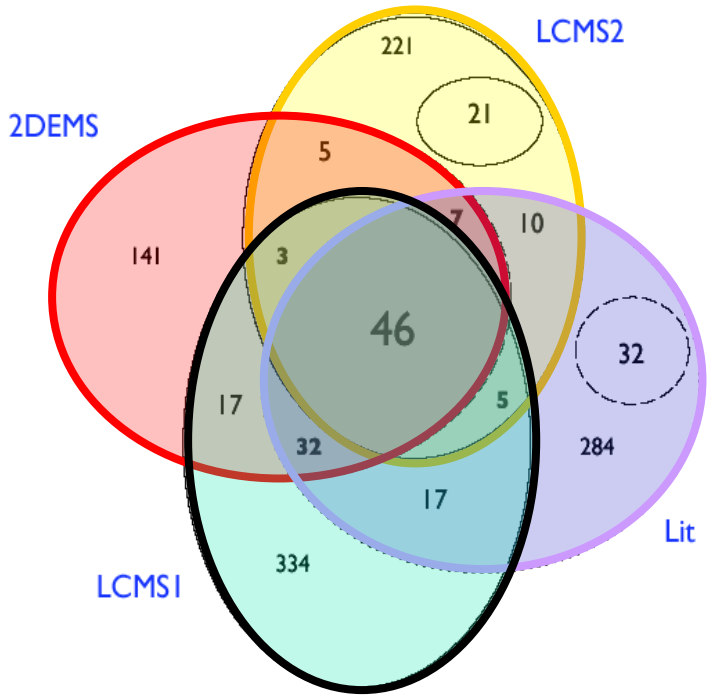
technické replikáty

2D or not 2D ?

- vizuální aspekty
- reprodukovatelnost
- dynamický rozsah
- extrémní proteiny (membránové,basické...)
- nesnadná automatizace
- postdigesční extrakce

Different Platforms See Different Plasma Proteomes: Small Overlap of Four Plasma Proteome Datasets

(Number of NR proteins)



- 46 proteins in all four lists
- 195 proteins in 2 or more lists
- 1175 NR proteins total

From: The Human Plasma Proteome: A Non-Redundant List Developed by Combination of Four Separate Sources, N. L. Anderson et al, Molec. Cell Proteomics, 3: 311-326 (2004).

PPI

MULTIDIMENZIONÁLNÍ CHROMATOGRAFIE

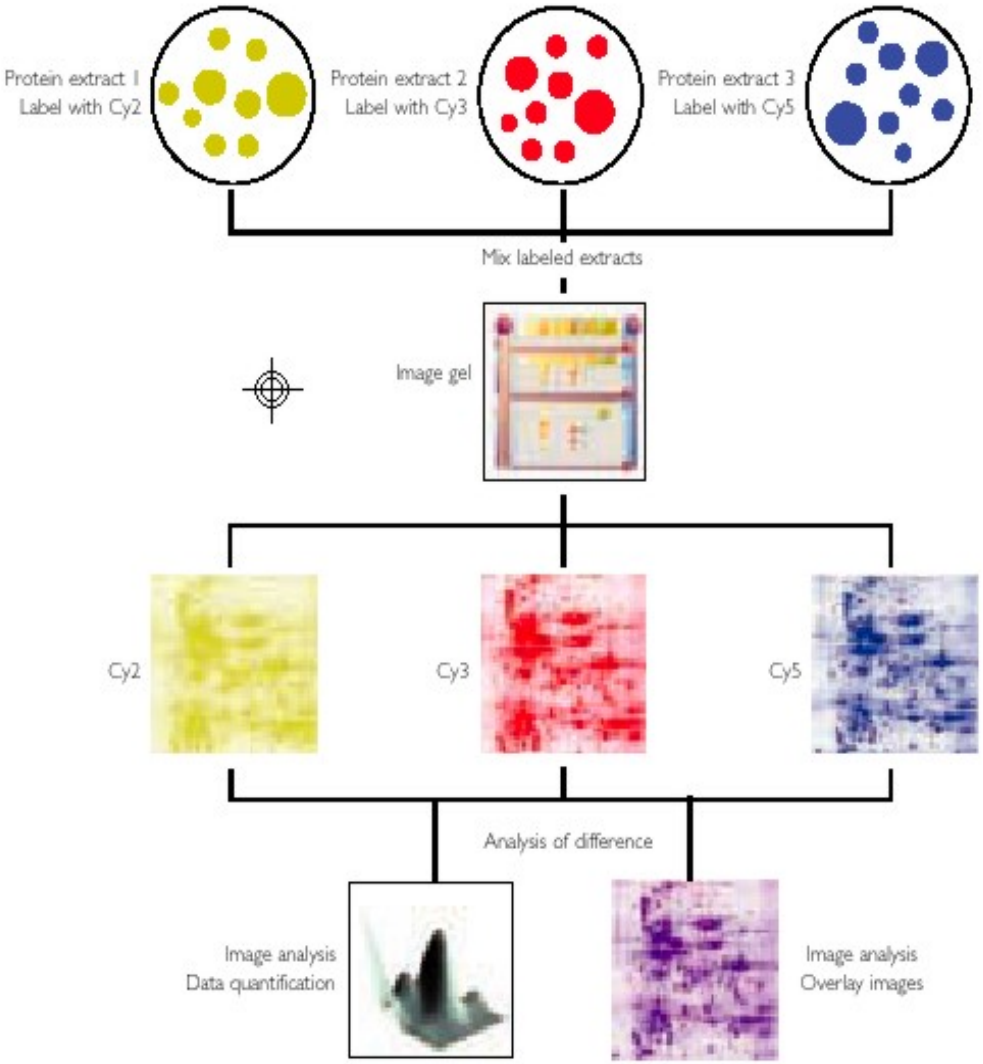
PRO

- velké objemy vzorku
- možnost koncentrace na koloně
- membránové proteiny, basické proteiny
- není nutno barvit
- peptidy – přímo na MS
- automatizace

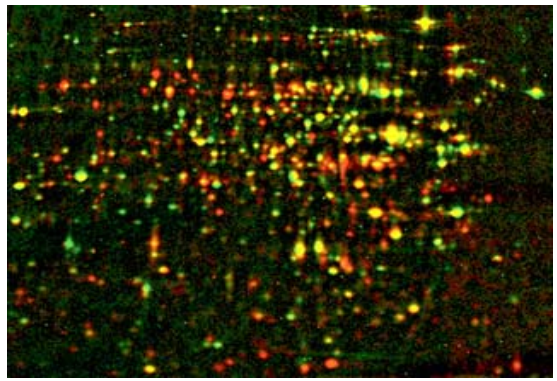
PROTI

- vizuální aspekty ztraceny: pI a Mr
- LC - sériová analýza
- GE současně pro více vzorků

Difference Gel Electrophoresis



DIGE



BIOMARKERY

… jehly v kupce sena

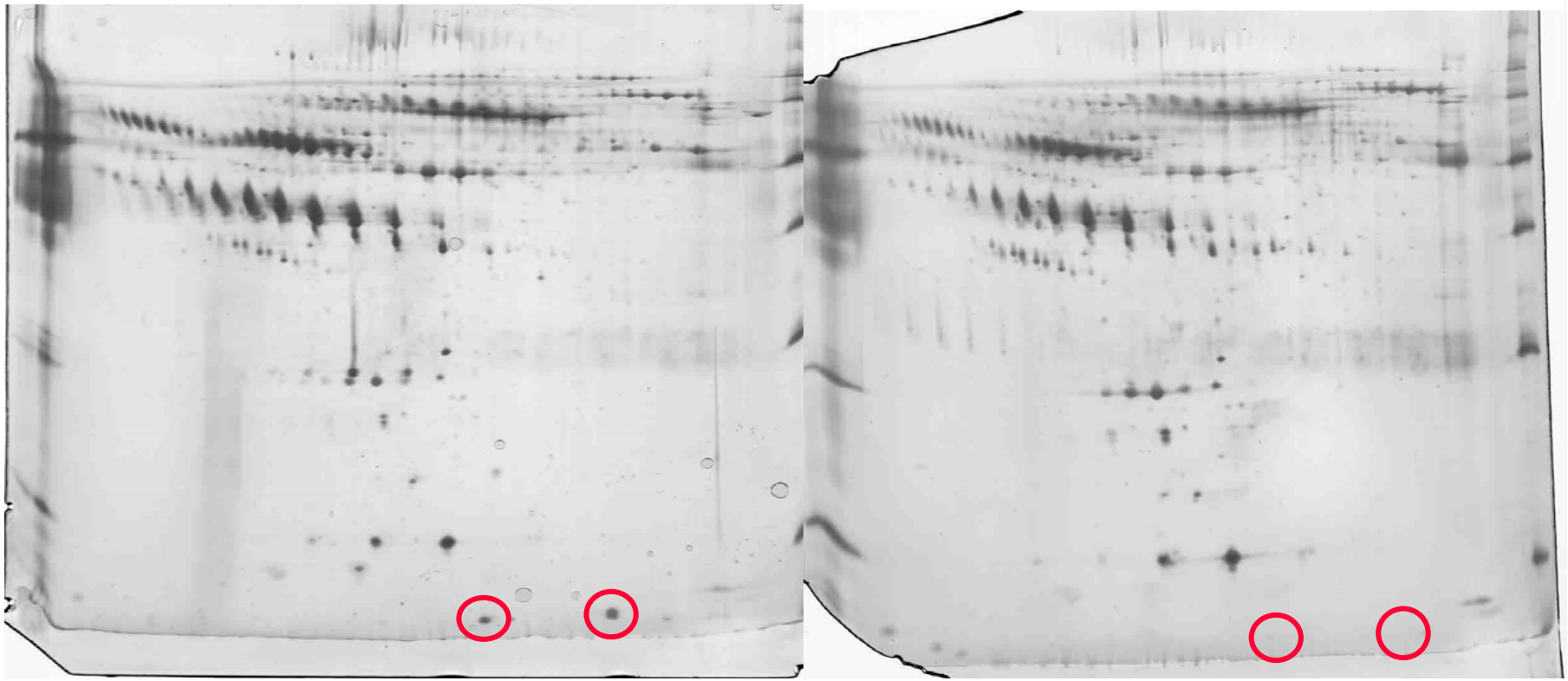
prefrakcionace ▪ separace ▪ identifikace ▪ srovnání kontrola vs.vzorek

- **seno** - proteiny bez vztahu k onemocnění
- **jehly** - specifické proteiny pro onemocnění
- potenciální jehly **obtížně validovatelné** biologická variabilita!
- které jehly dále zkoumat?
- často **PTM,** neidentifikovány MS

Biomarkery v lidské plasmě

Den 21 – před klinickým projevem

Den 44 – po klinickém projevu



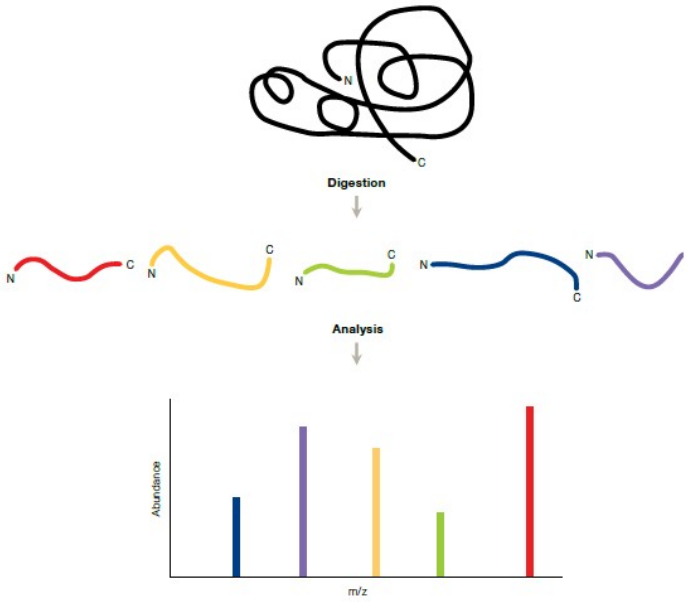
separace → identifikace

DIGESCE

trypsin Glu-C Asp-N thermolysin

MAVEPFPRRPITRPHASIEVDTSGTGGSAGSSE
KVFCLIGQAEGGEPNTVYELRNYAQAKRLFR
SGELLDAIELAWGSNPNYTAGRILAMRIEDAK
PASAEIGGLKITSKIYGNVANNIQVGLEKNTLS
DSLRLRVIFQDDRFNEVYDNIGNIFTIKYKGEE
ANATFSVEHDEETQKASRLVLKVGDQEVKSY
DLTGGAYDYTNAIITDINQLPDFEAKLSPFGD
KNLESSKLDKIENANIKDKAVYVKAVFGDLE
KQTAYNGIVSFEQLNAEGEVPSNVEVEAGEES
ATVTATSPIKTIEPFELTKLKGGTNGEPPATWA
DKLDKFAHEGGYYIVPLSSKQSVHAEVASFV
KERSDAGEPMRAIVGGGFNESKEQLFGRQAS
LSNPRVSLVANSGTFVMDDGRKNHVPAYMV
AVALGGLASGLEIGESITFKPLRVSSLDQIYESI
DLDELNENGIISIEFVRNRTNTFFRIVDDVTTFN
DKSDPVKAEMAVGEANDFLVSELKVQLEDQF
IGTRTINTSASIIKDFIQSYLGRKKRDNEIQDFP
AEDVQVIVEGNEARISMTVYPIRSFKKISVSLV
YKQQTLQA

- IN-GEL
- IN-SOLUTION



MS

G I G O

G I G O

GARBAGE IN - GARBAGE OUT

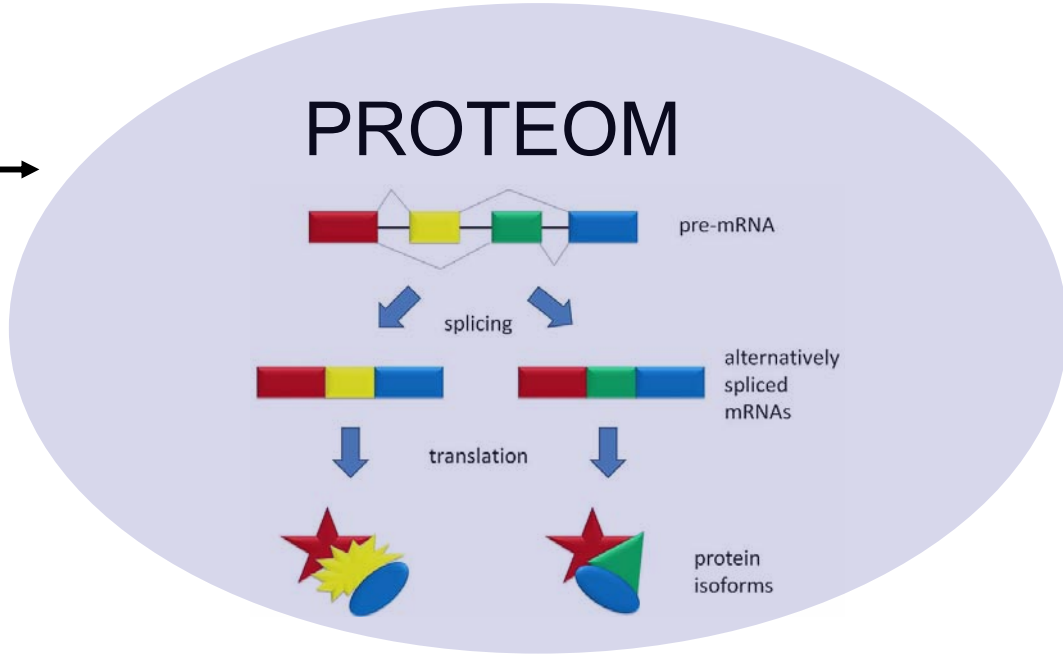
LITERATURA

- R.M. Twyman: Principles of Proteomics
- R.Westermeier, T.Naven, H-R Höpker: Proteomics in Practice
- A.J.Link: 2D Proteome Analysis Protocols
- Current Protocols in Protein Science
- R.J.Simpson: Proteins and Proteomics
- T.Rabilloud: Proteome Research: Two-dimensional Gel
- Electrophoresis and Identification Methods
- A. Görg, W. Weiss, M.J.Dunn: Proteomics 2004, 4, 3665, rev.
- I. Miller, J. Crawford, E. Gianazza: Proteomics 2006, 6, rev.
- F.Chevalier: Proteome Science 2010, 8:23, review
- R. Burgess, M. Deutscher: Guide to Protein Purification

I. SEPARACE

II. PREFRAKCIONACE

GENOM →



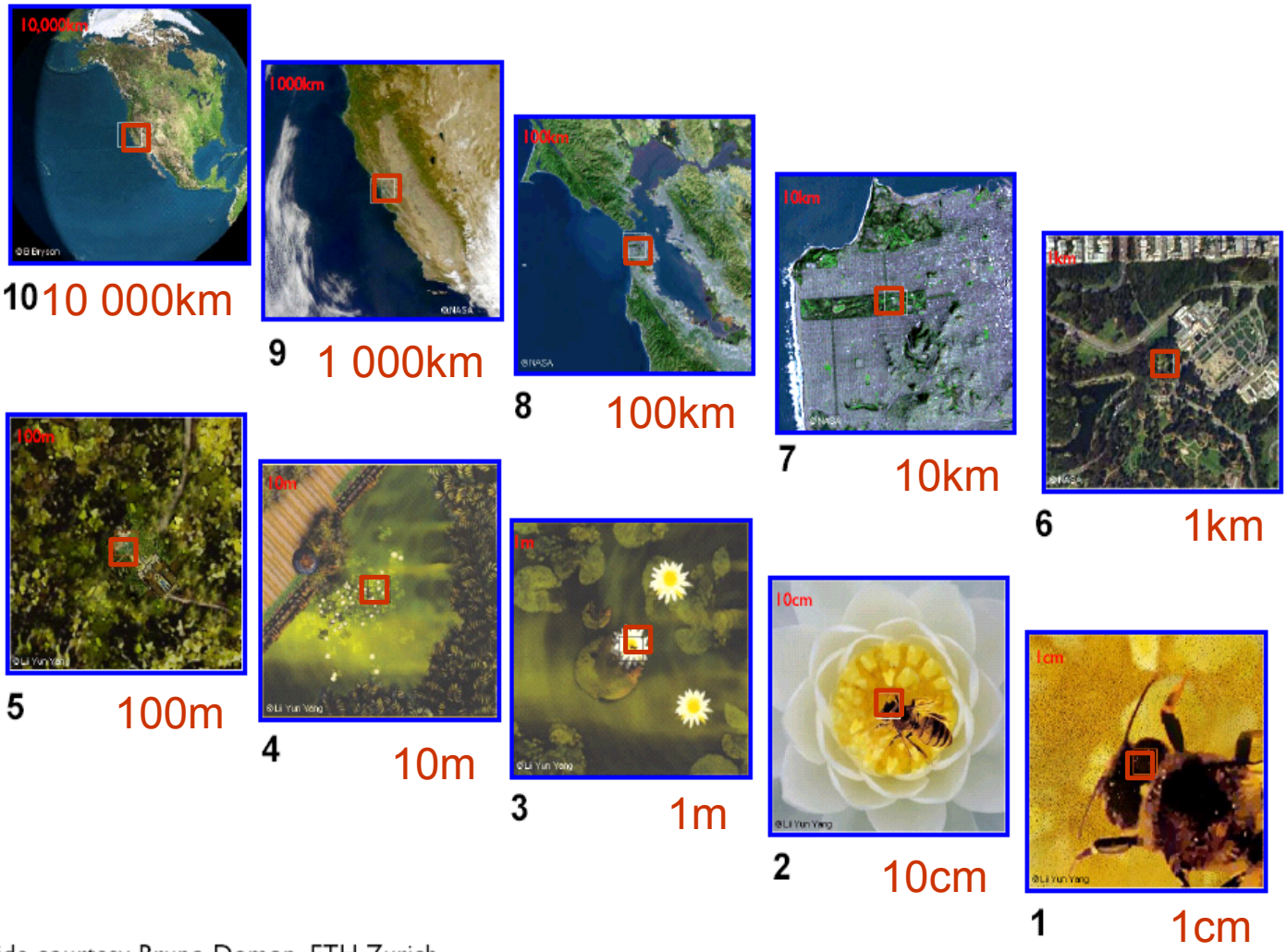
IZOFORMY

PTM asi 200 typů (fosforylace, glykosylace, acylace, methylace…)

KONCENTRAČNÍ ROZSAH asi deset řádů ↓

PREFRAKCIONACE → MS

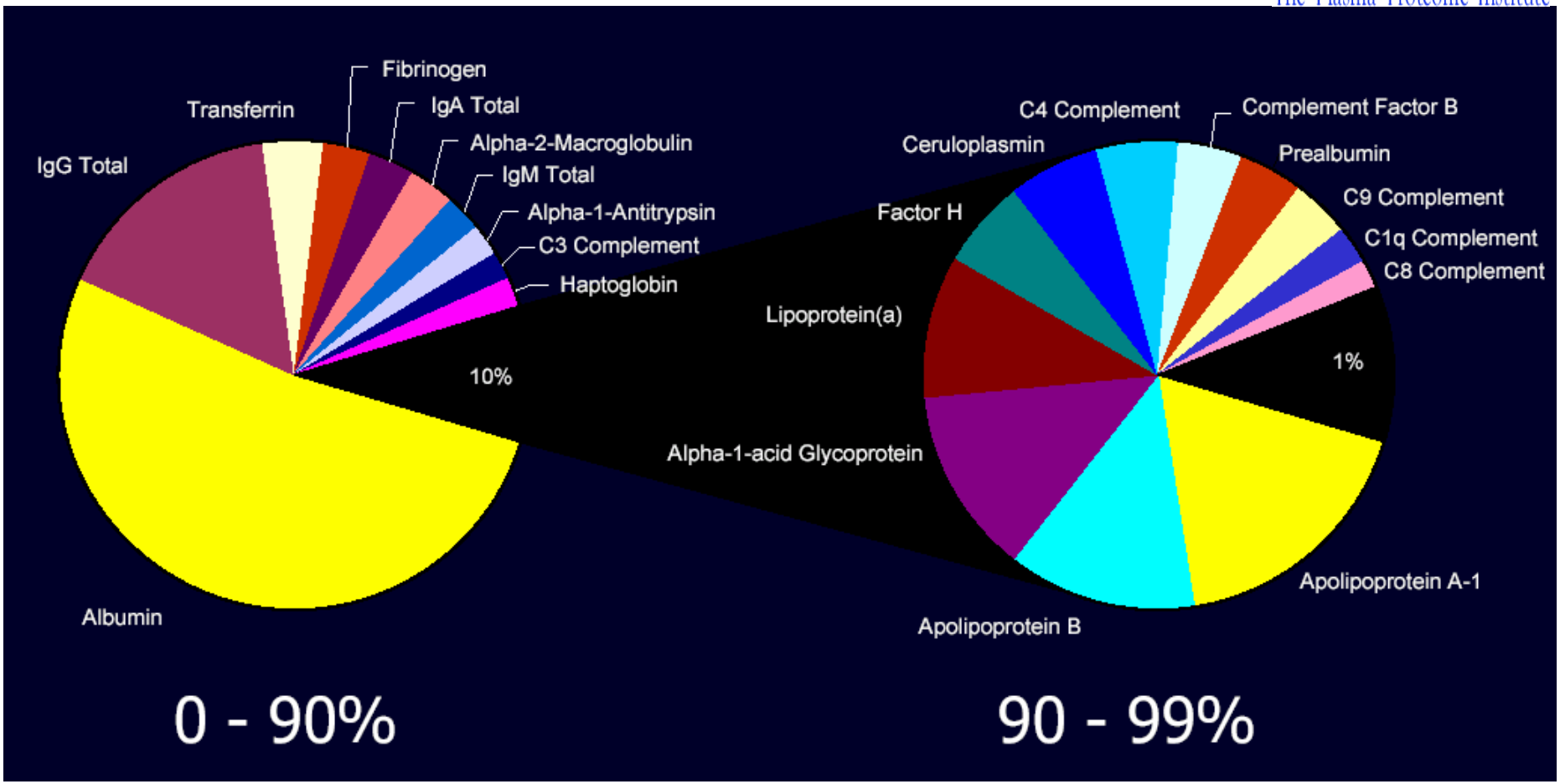
10^{10} Really Is Wide Dynamic Range



Slide courtesy Bruno Domon, ETH Zurich

Abundantní proteiny v lidské plazmě

The Plasma Proteome Institute

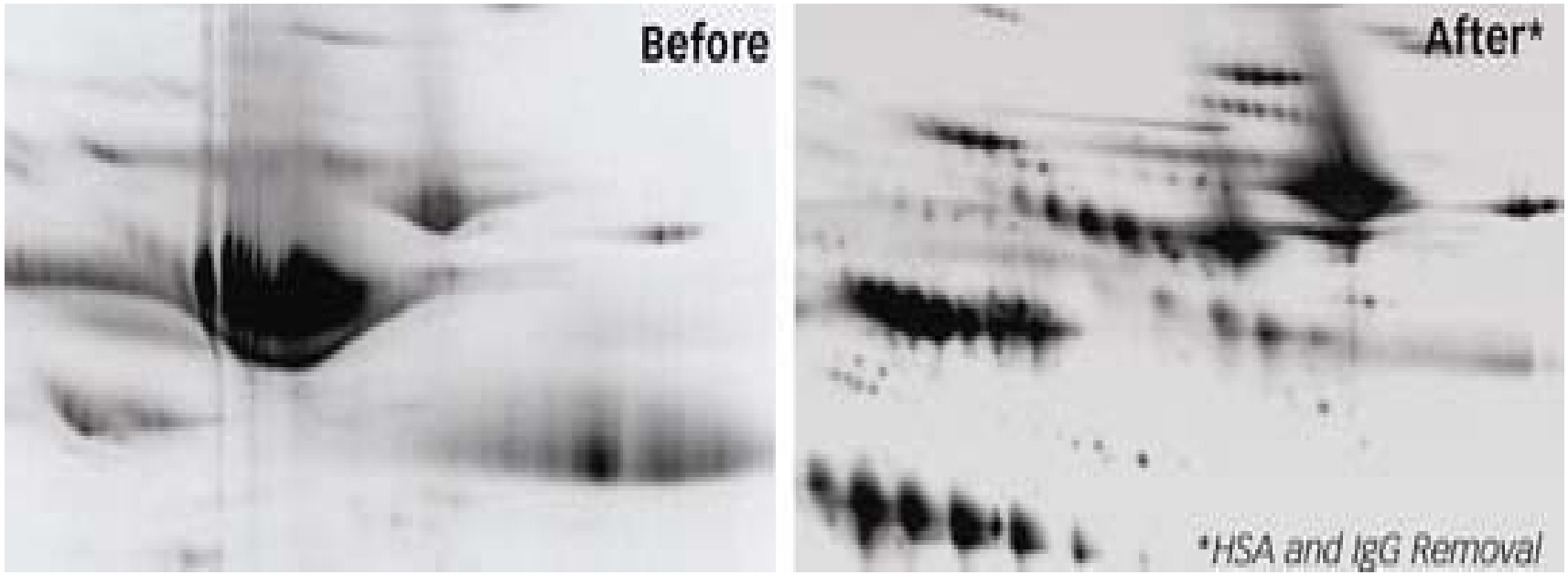


AFINITNÍ DEPLECE

odstranění abundantních proteinů afinitní chromatografií

HSA

IgG

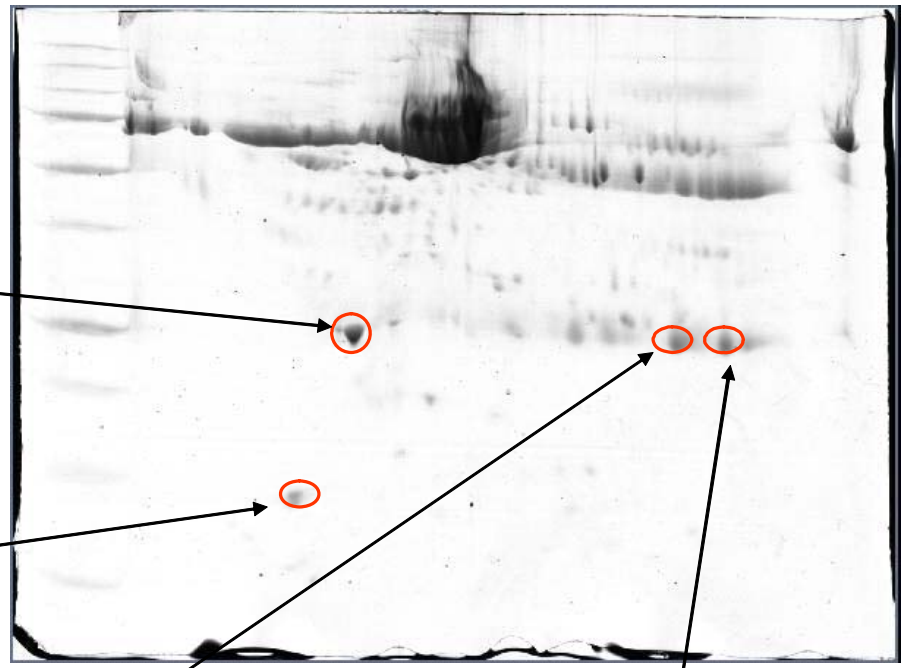


Lidská plazma - vázaná frakce po afinitní depleci

ALBUMIN

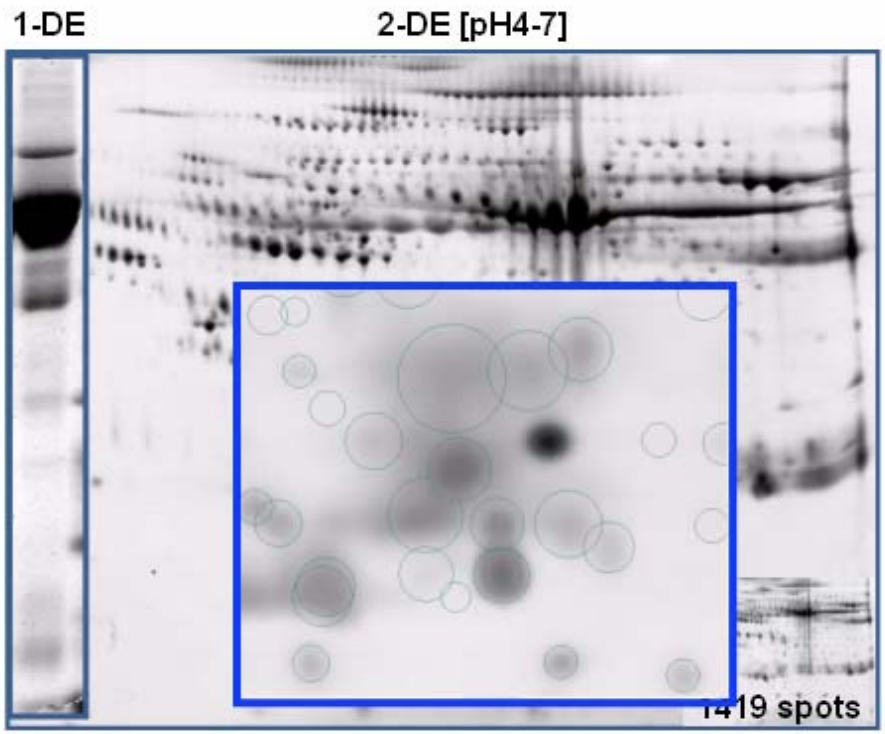
IgG

Barvení: CB G-250

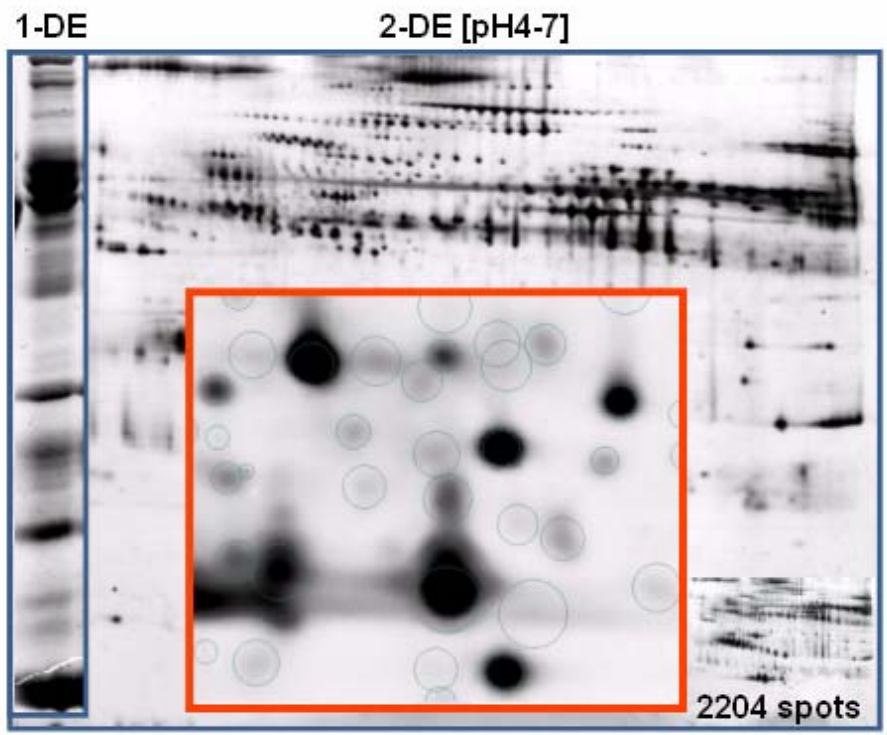


CPPL
Combinatorial Peptide Ligand Library

Native Human Serum

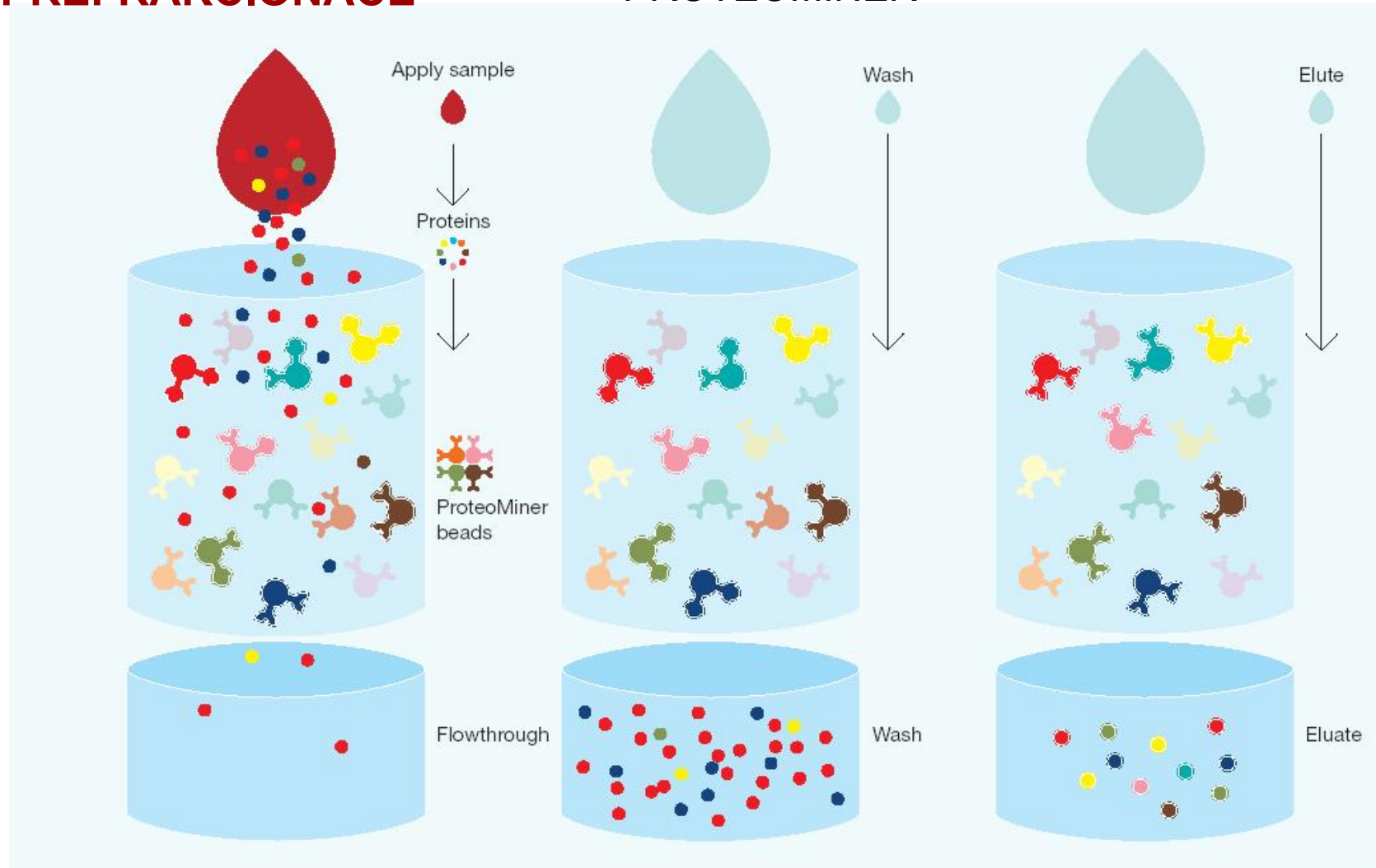


Human Serum Fractionated by ProteoMiner



PREFRAKCIONACE

PROTEOMINER



IEF PREFRAKCIONACE

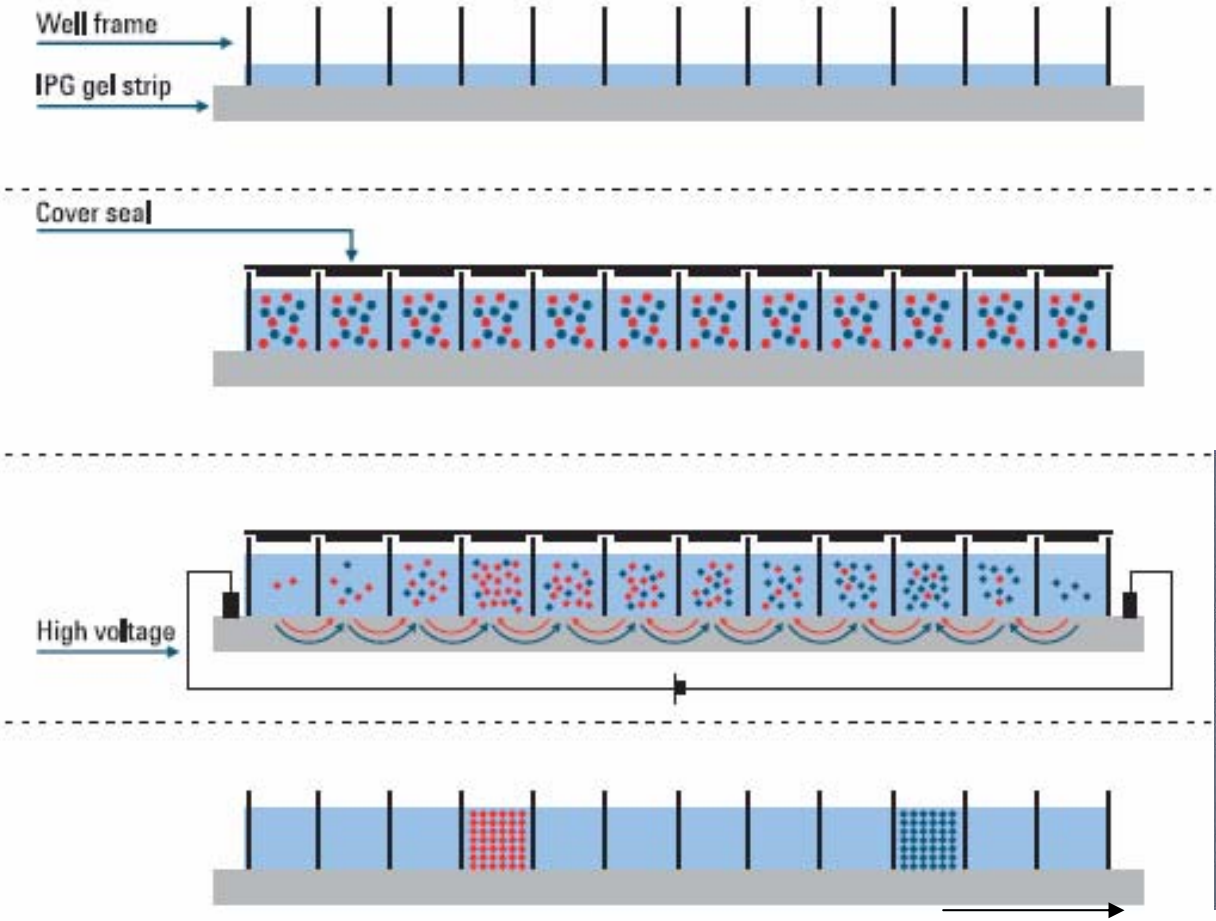
MicroRotofor

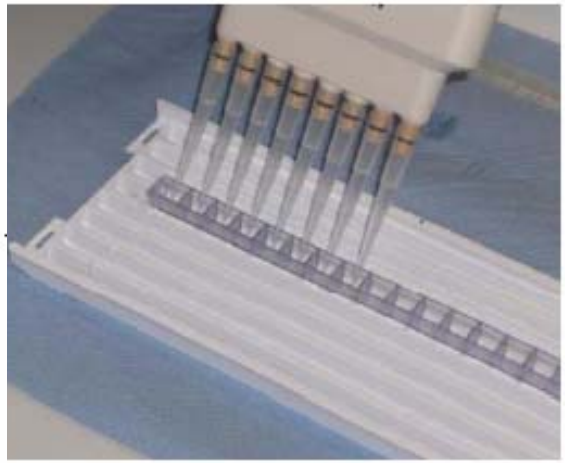
- prefrakcionace v roztoku

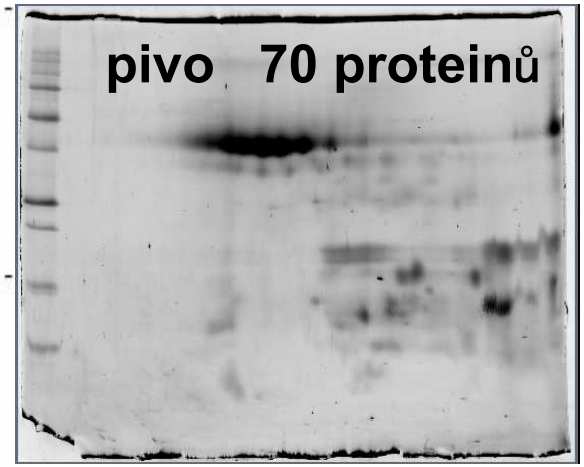
OffGel Fractionator

- prefrakcionace v roztoku na IPG stripu

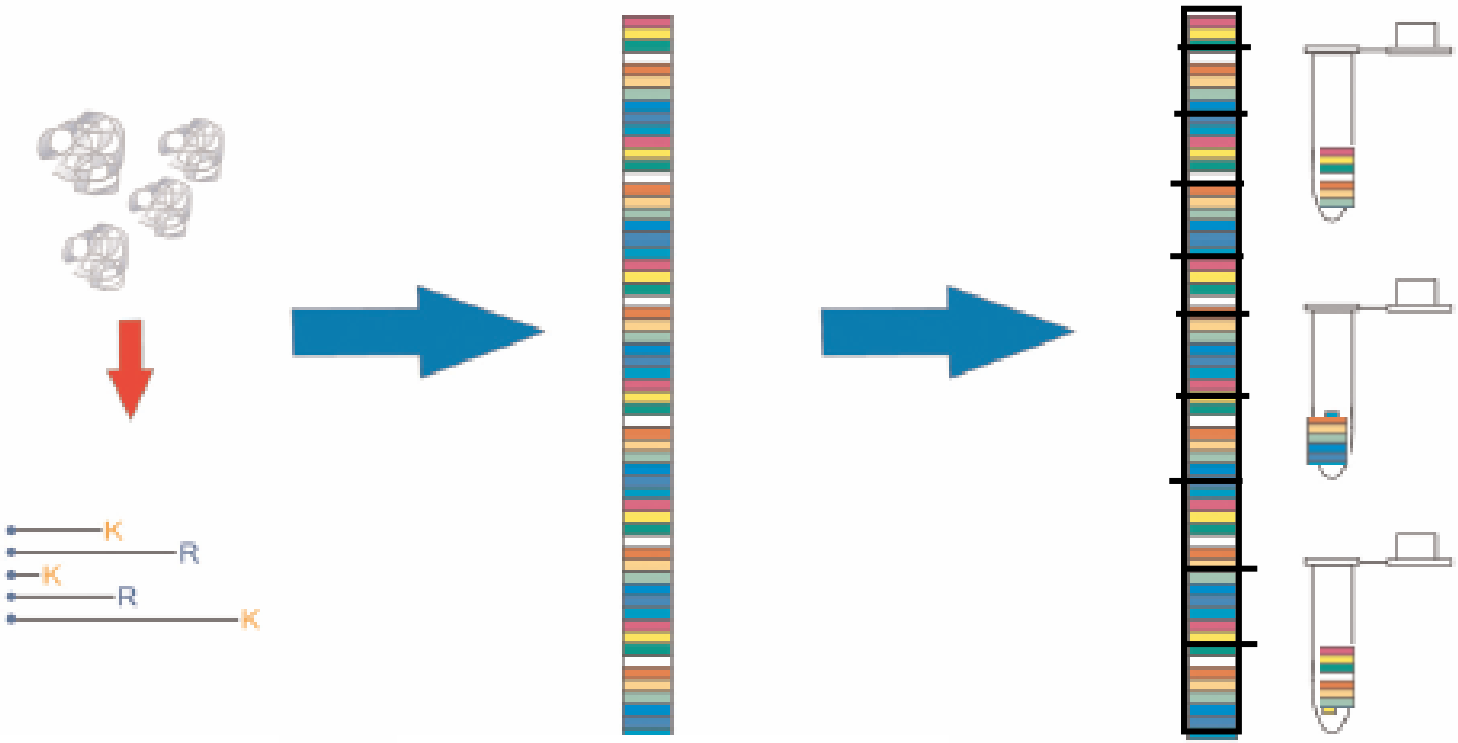
OFFGEL IEF prefrakcionace proteinů nebo peptidů







IPG-IEF



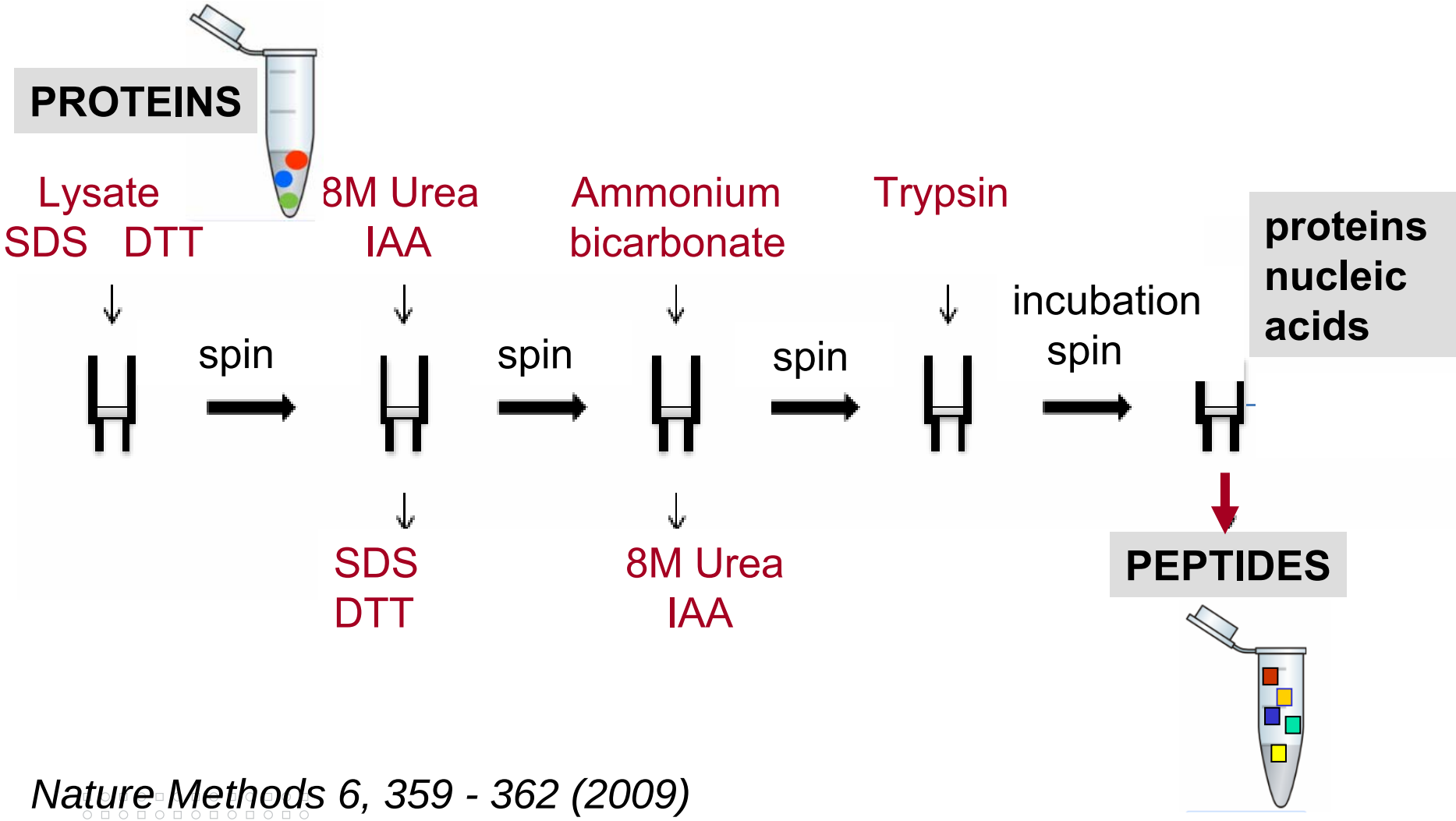
digest směsi proteinů

IEF směsi peptidů na IPG stripu

frakce stripu

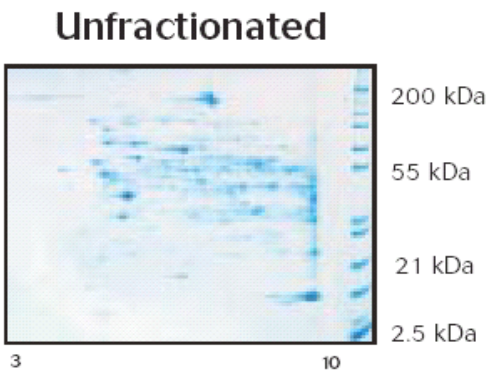
JOURNAL OF BIOMOLECULAR TECHNIQUES, VOLUME 16, ISSUE 3, SEPTEMBER 2005

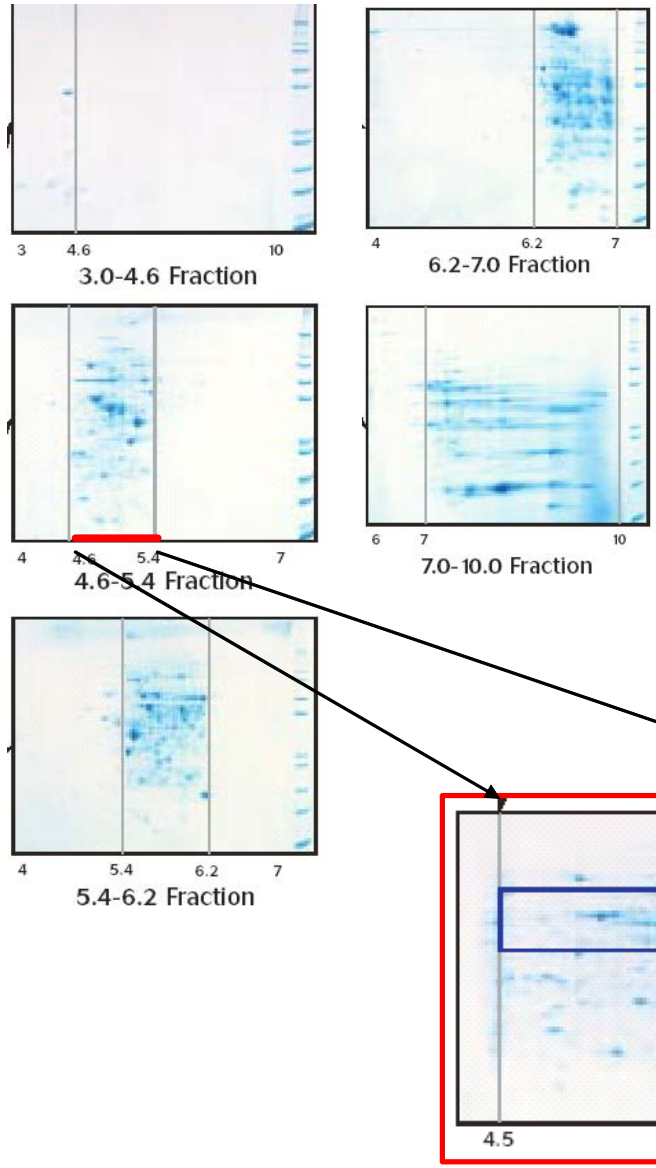
FASP Filter aided sample preparation



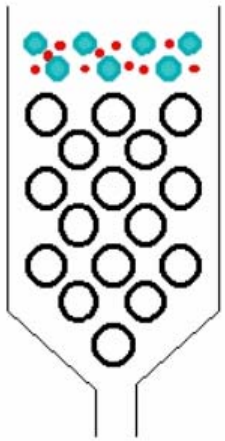
Nature Methods 6, 359 - 362 (2009)

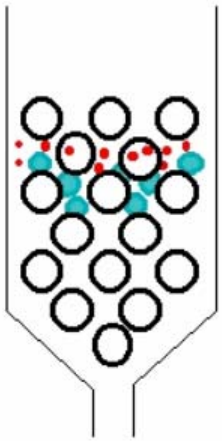
PREFRAKCIONACE MIKRO ROZSAHY

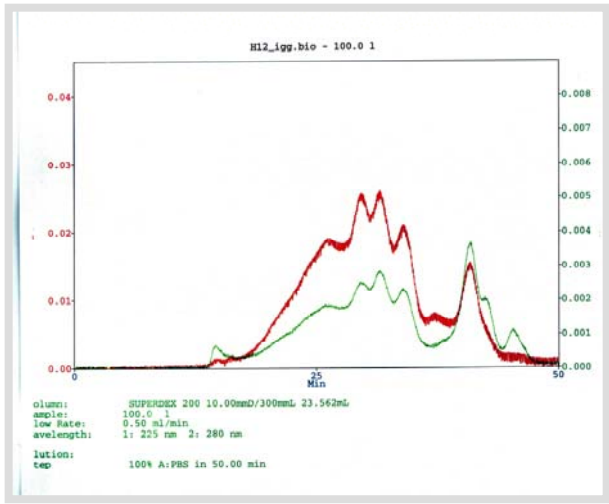




GELOVÁ CHROMATOGRAFIE







INSPIRATIVNÍ LITERATURA PRO MÍRNĚ POKROČILÉ

Two-dimensional gel electrophoresis in proteomics: A tutorial
Thierry Rabilloud et al. *Journal of Proteomics* **2011**

Two-dimensional gel electrophoresis in proteomics: past, present and future
Thierry Rabilloud et al. *Journal of Proteomics* **2010**

Proteomic biomarker discovery: It's more than just mass spectrometry
Josip Blonder et al. *Electrophoresis* **2011**

For all the complex problems and difficult questions there is always one simple, easily comprehensible **w r o n g** answer.

esf evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ