MASARYKOVA UNIVERZITA

Dvoudimenzionální elektroforéza

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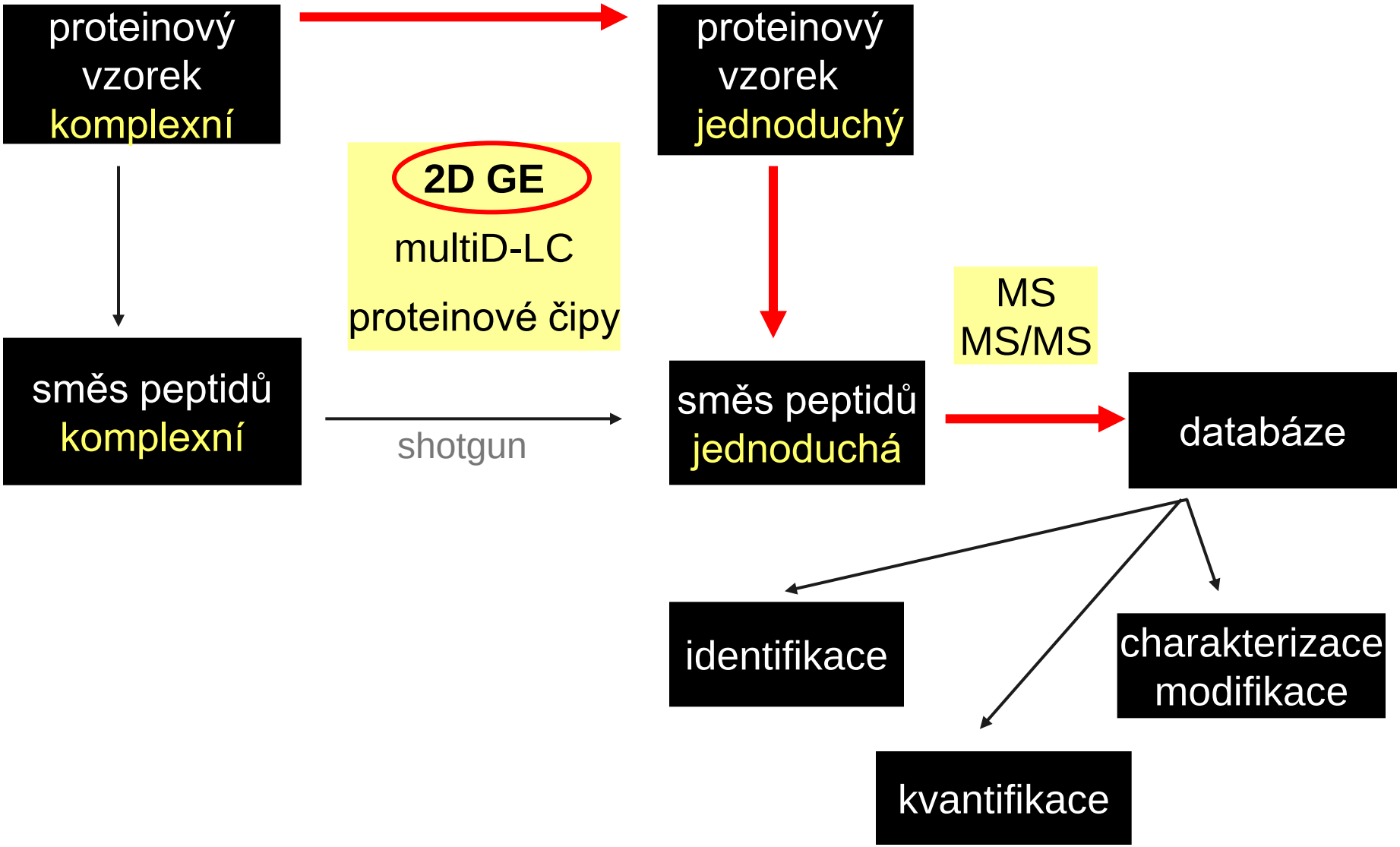
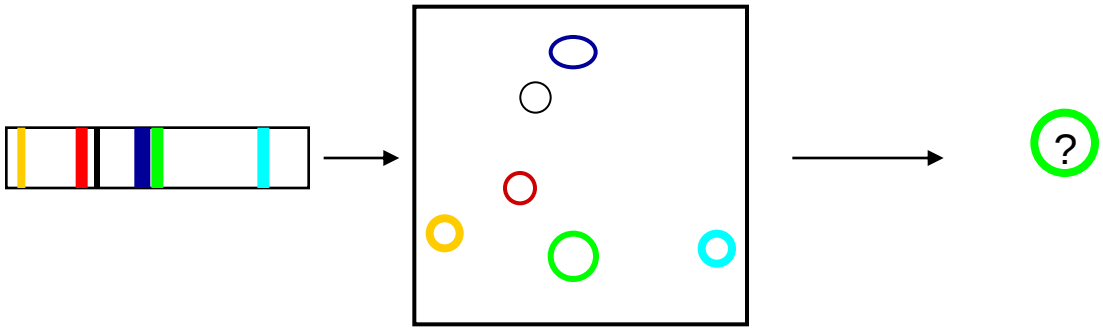
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ


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

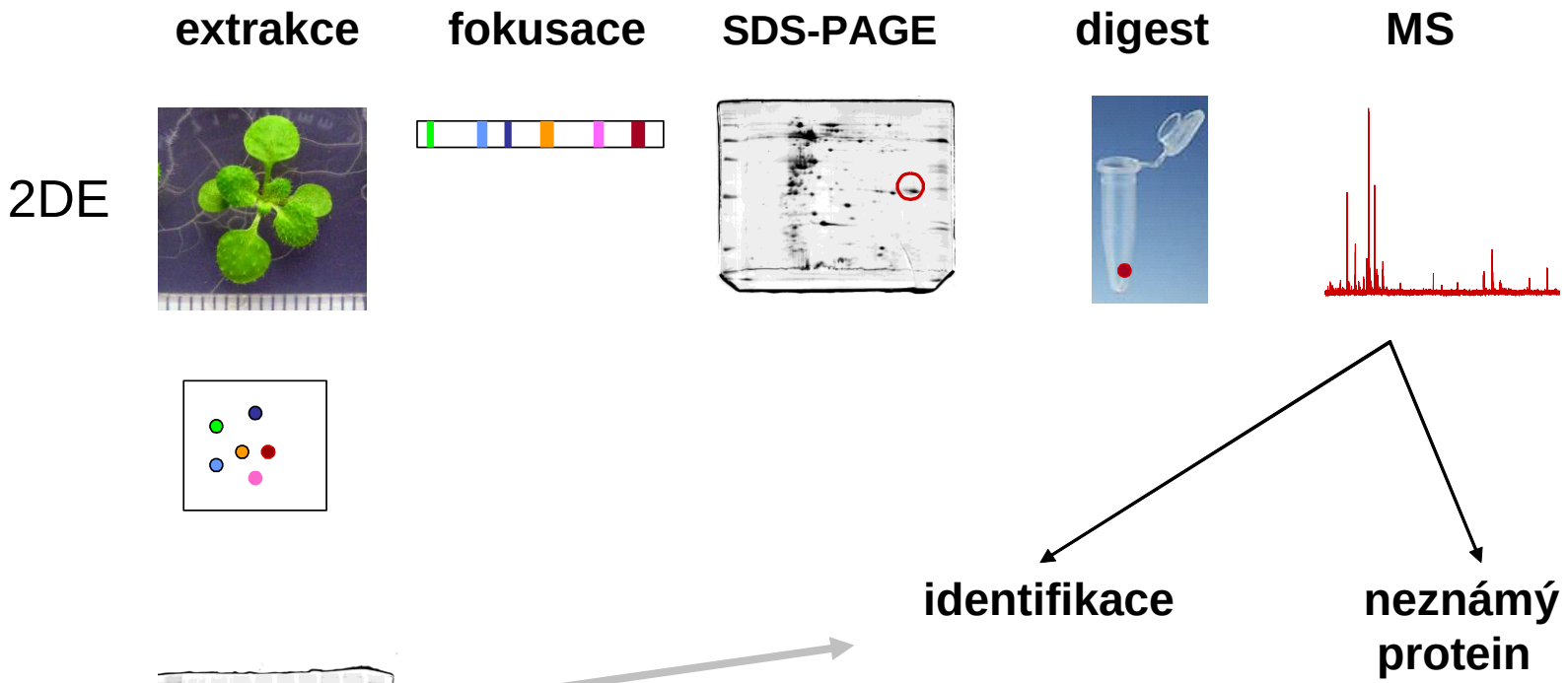
I. SEPARACE

II. PREFRAKCIONACE

Dvoudimenzionální elektroforéza
2-DE

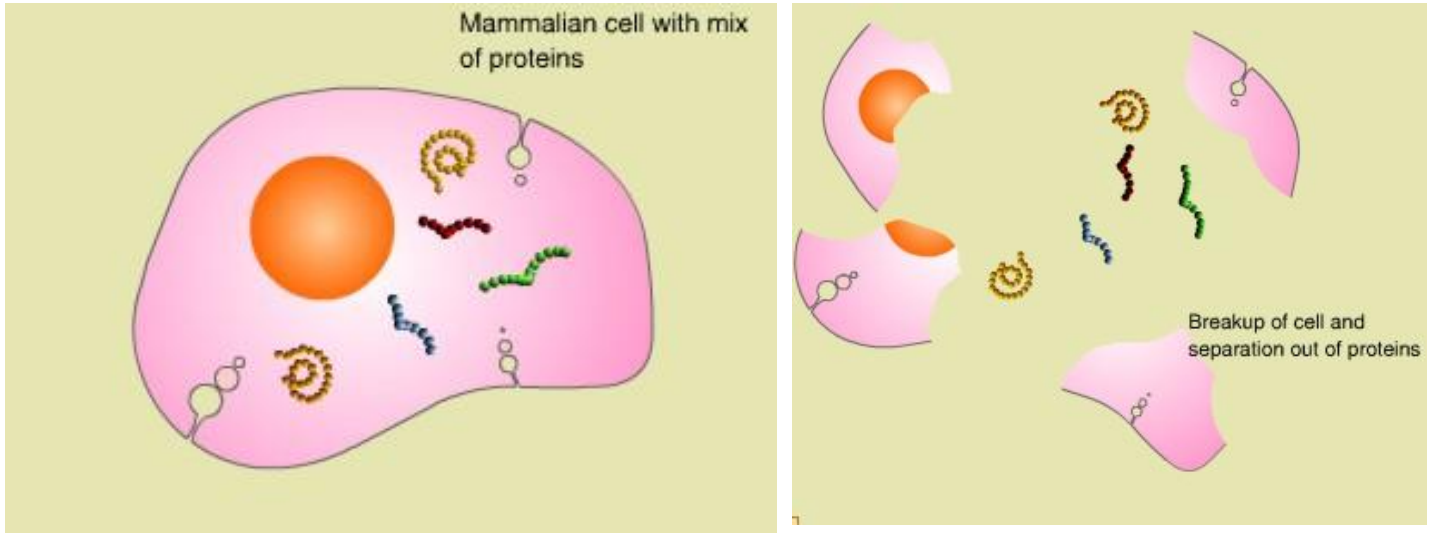


Proteomický experiment



HOMOGENIZACE

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



PŘÍPRAVA VZORKU

!

solubilizace močovina, thiomočovina, detergenty

redukce DTT, TBP

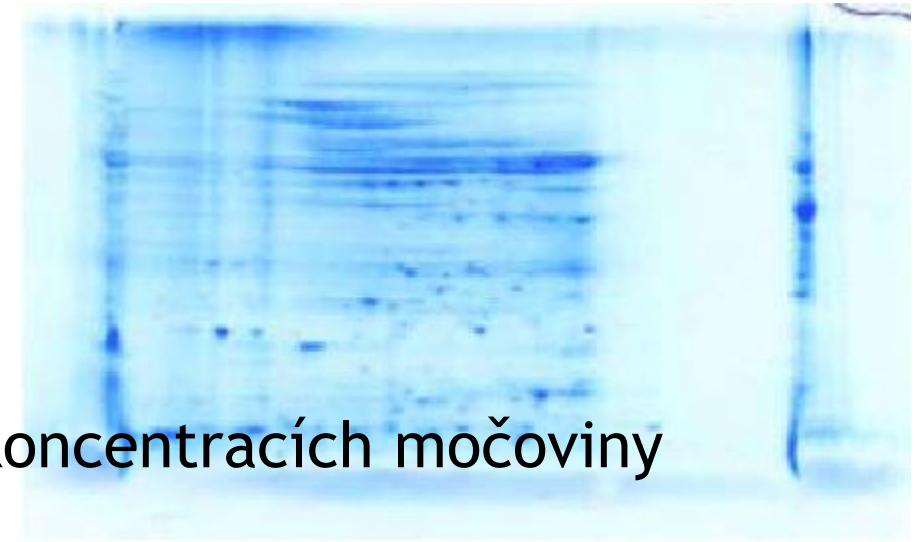
inhibice proteáz, fosfatáz, glykosyláz

odstranění kontaminant

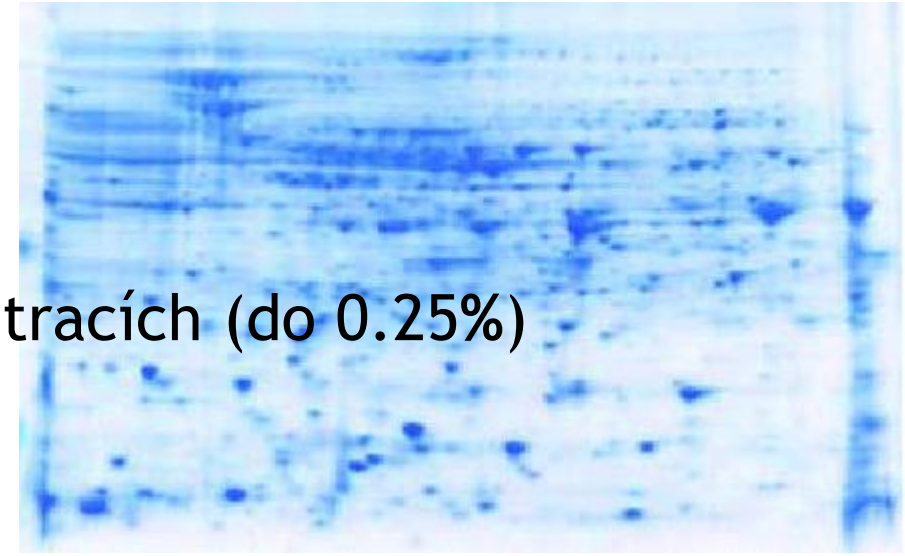
MĚŘENÍ KONCENTRACE PROTEINU

lyzační pufr vs. hydratační pufr

DETERGENTY



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



- neionogenní
- zwitterointové

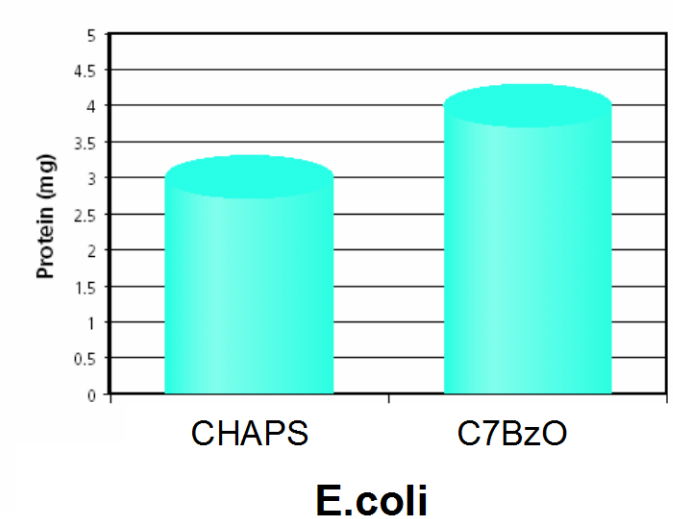
- SDS jen v nízkých koncentracích (do 0.25%)

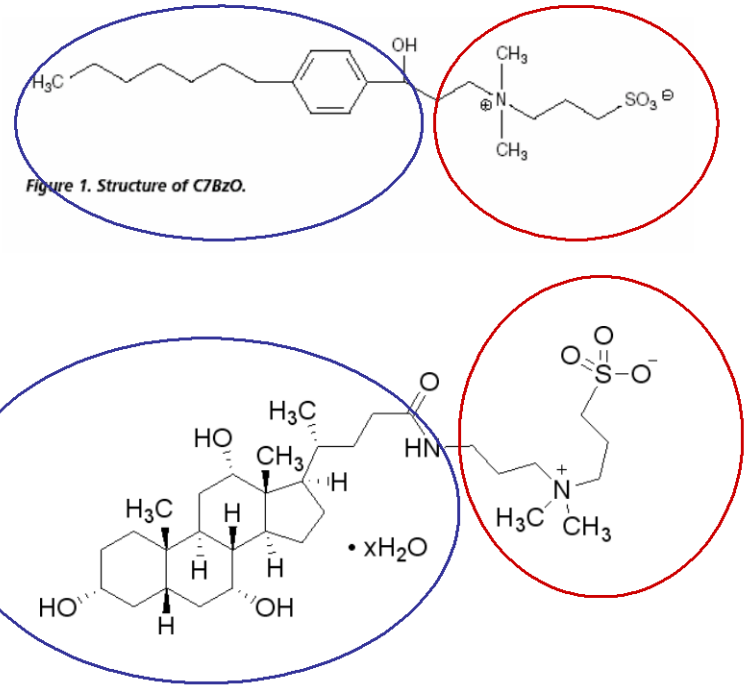
C7BzO

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

CHAPS

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





ZÁKLADNÍ PRAVIDLA

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

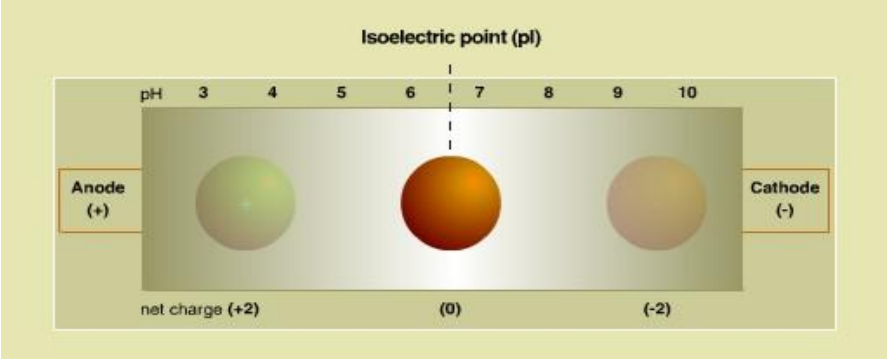
KONTAMINANTY

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

2-DE

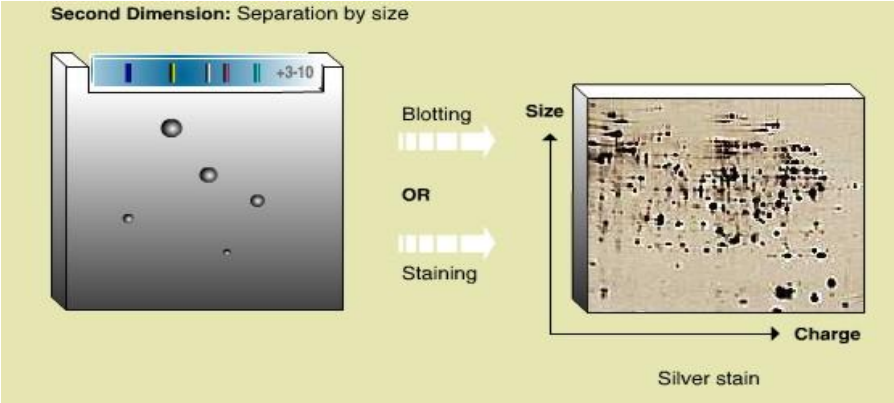
- první rozměr

IEF



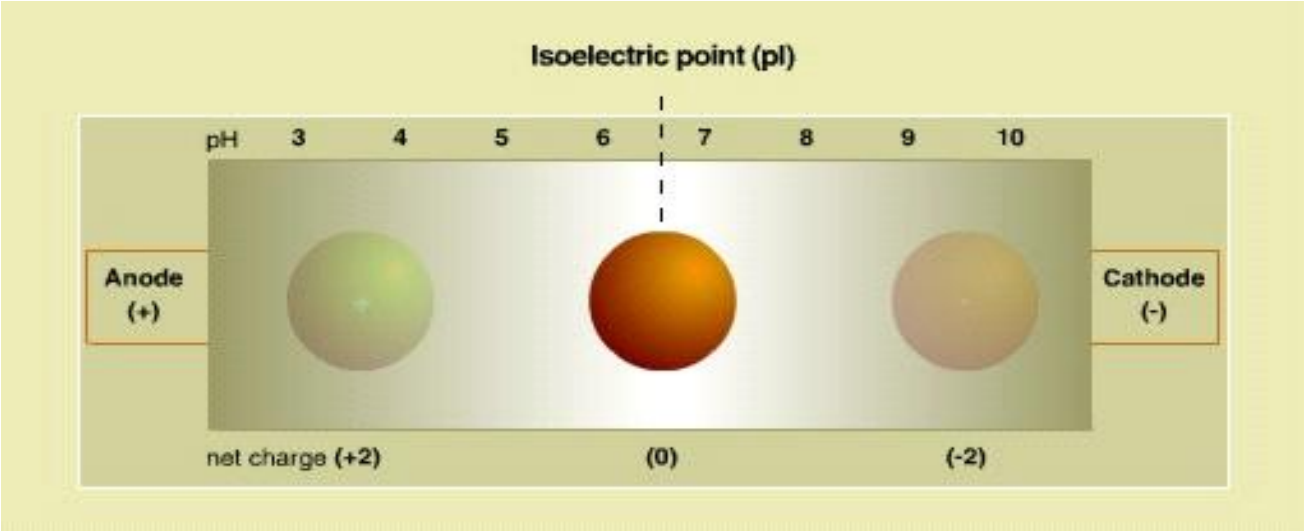
- druhý rozměr

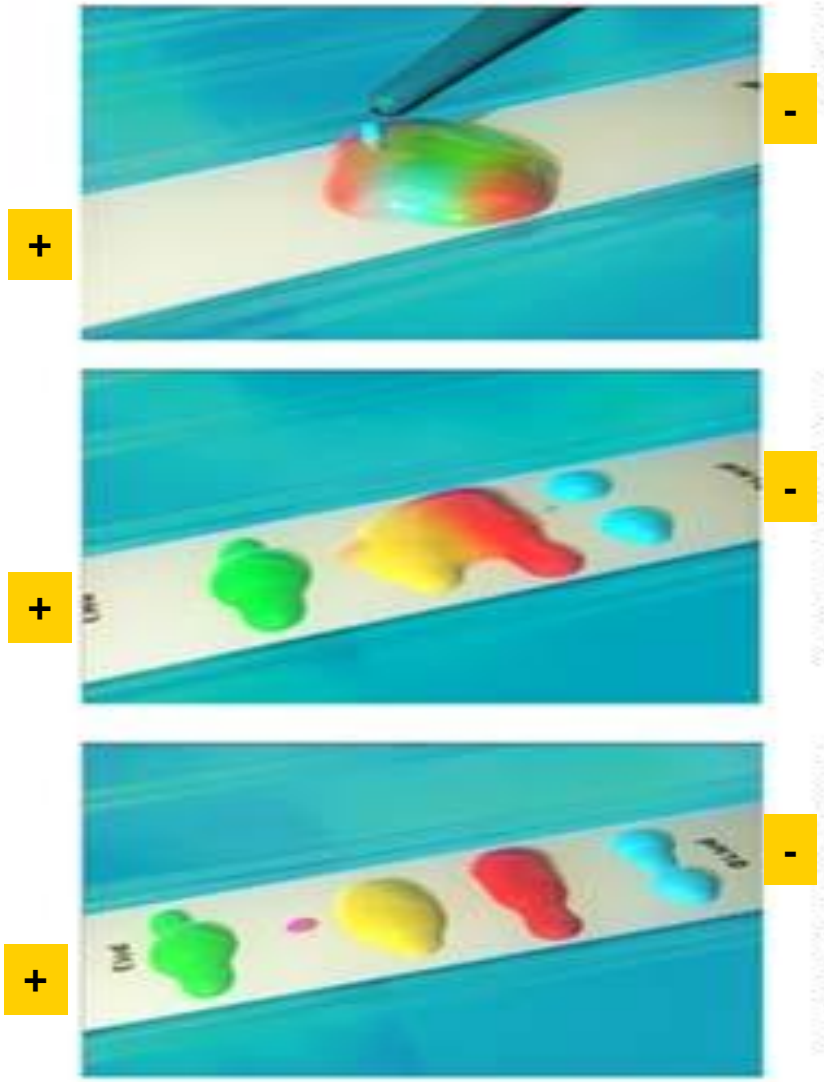
SDS-PAGE



1. ROZMĚR IZOELEKTRICKÁ FOKUSACE

migrace nabitých částic v gradientu pH v elektrickém poli

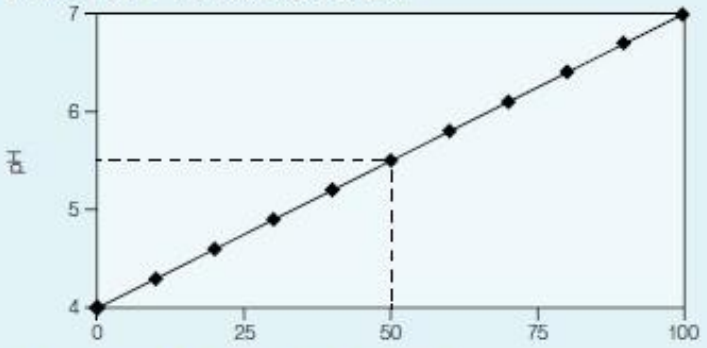


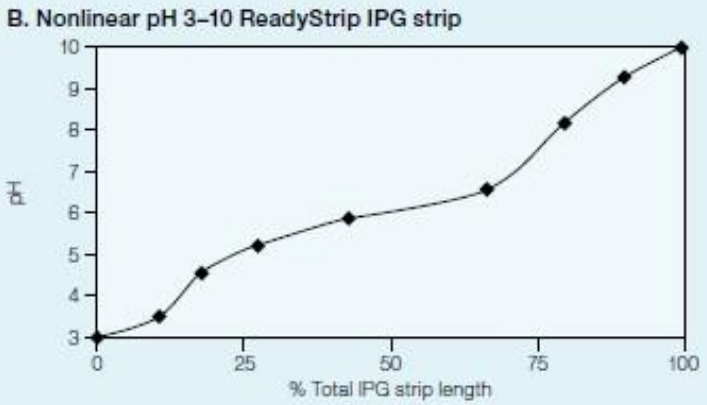


IZOELEKTRICKÁ FOKUSACE

- imobilizovaný pH gradient
- amfolyty

ROZSAH STRIPU
ROZMĚR STRIPU





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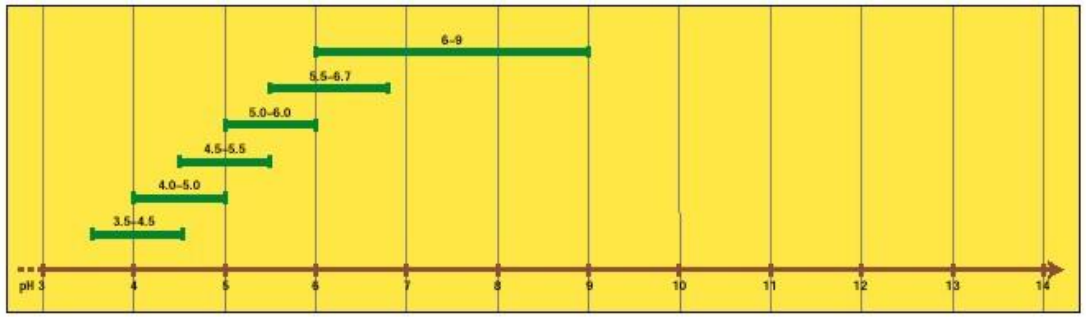
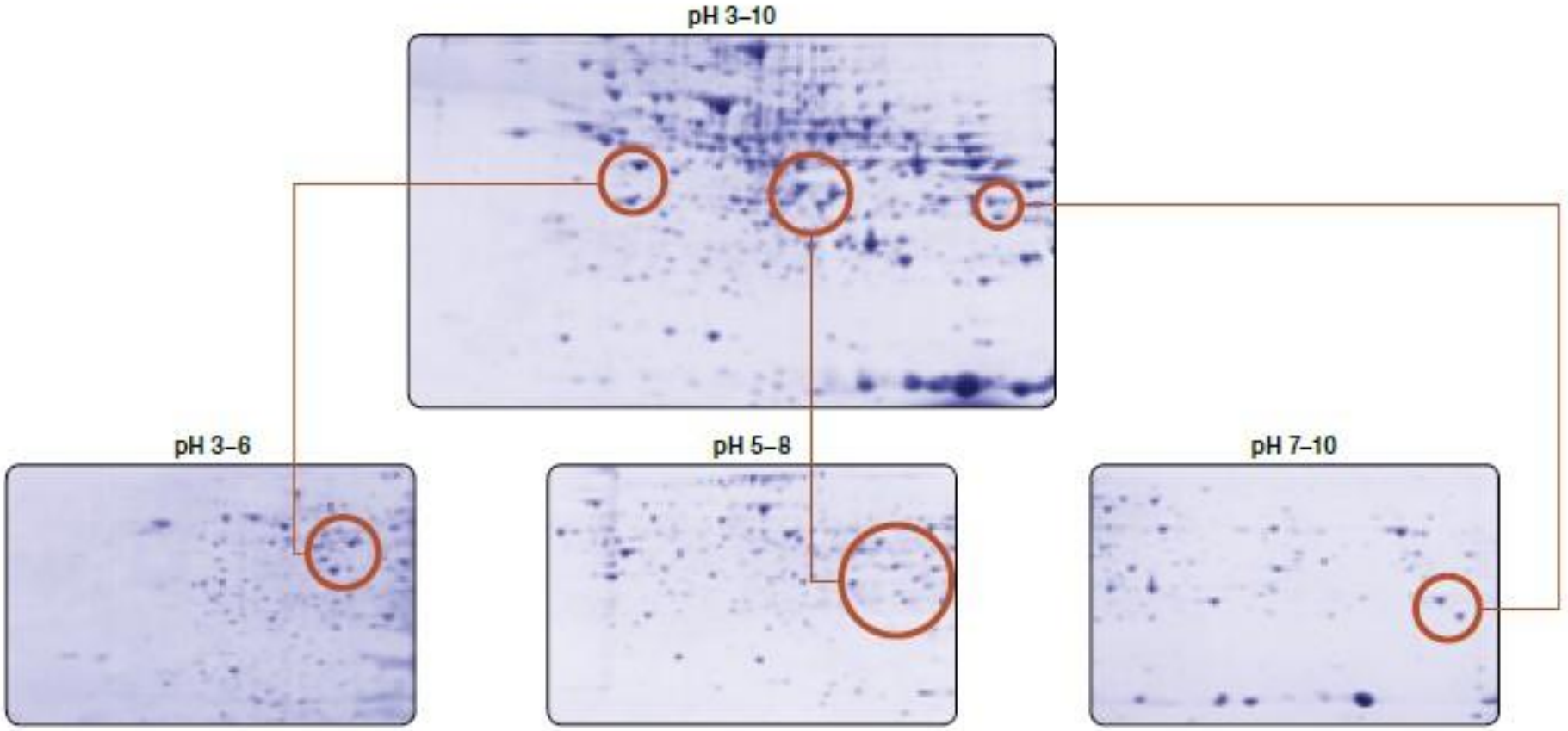
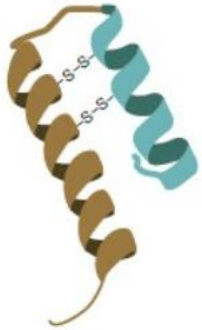


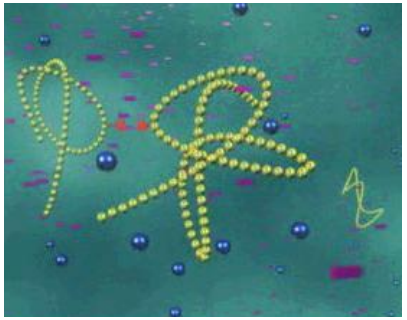
ROZSAH STRIPU

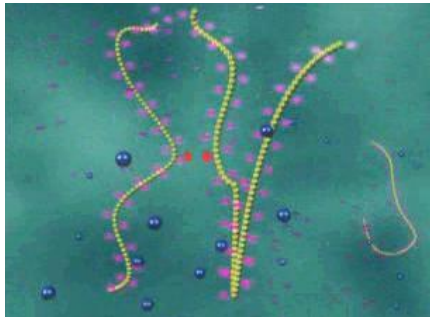


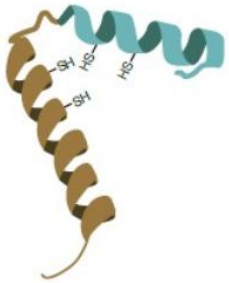
EKVILIBRACE STRIPU !

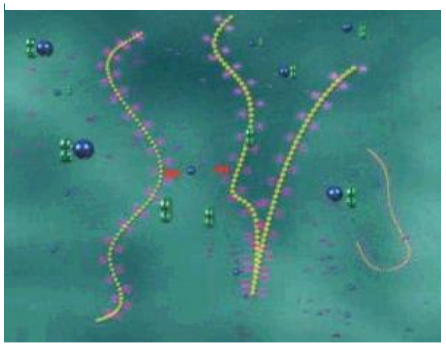
denaturace **SDS** •

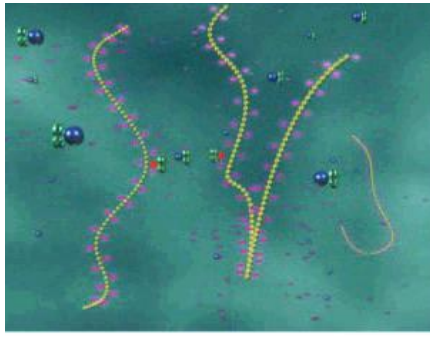


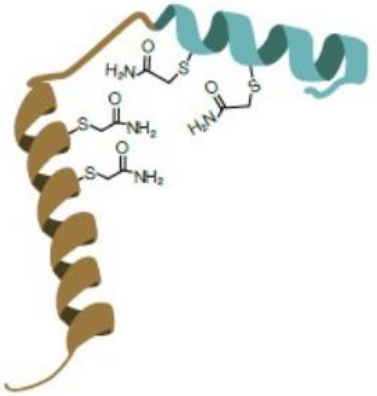








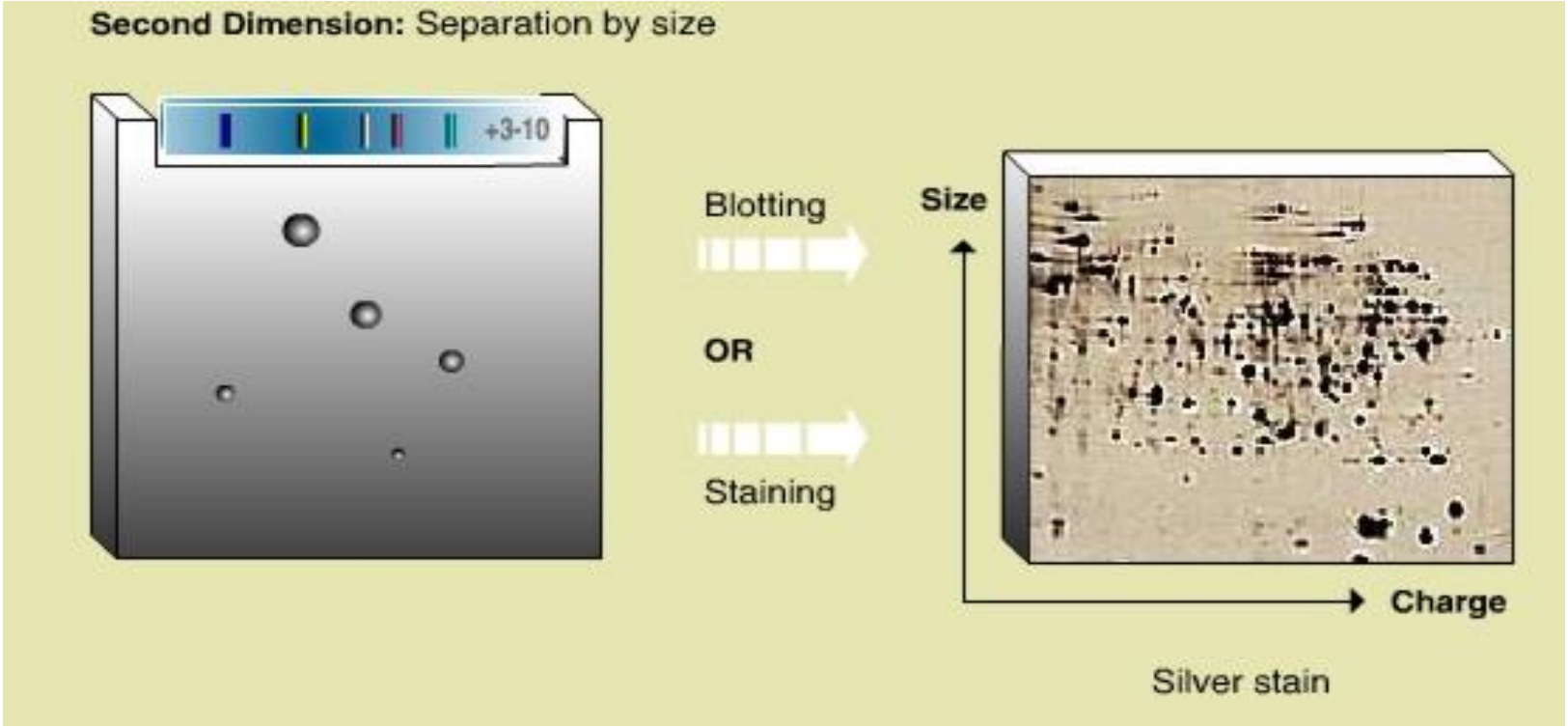




redukce **DTT** ● alkylace **IAA** •

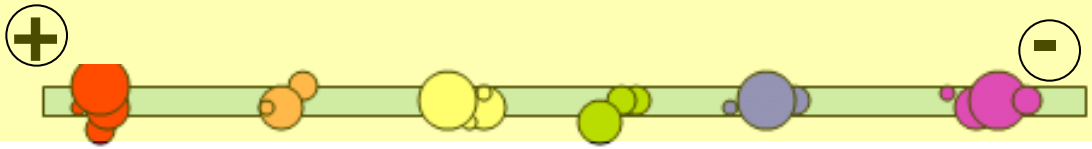
2. ROZMĚR SDS-PAGE !

migrace aniontů v elektrickém poli podle MW



FOKUSACE

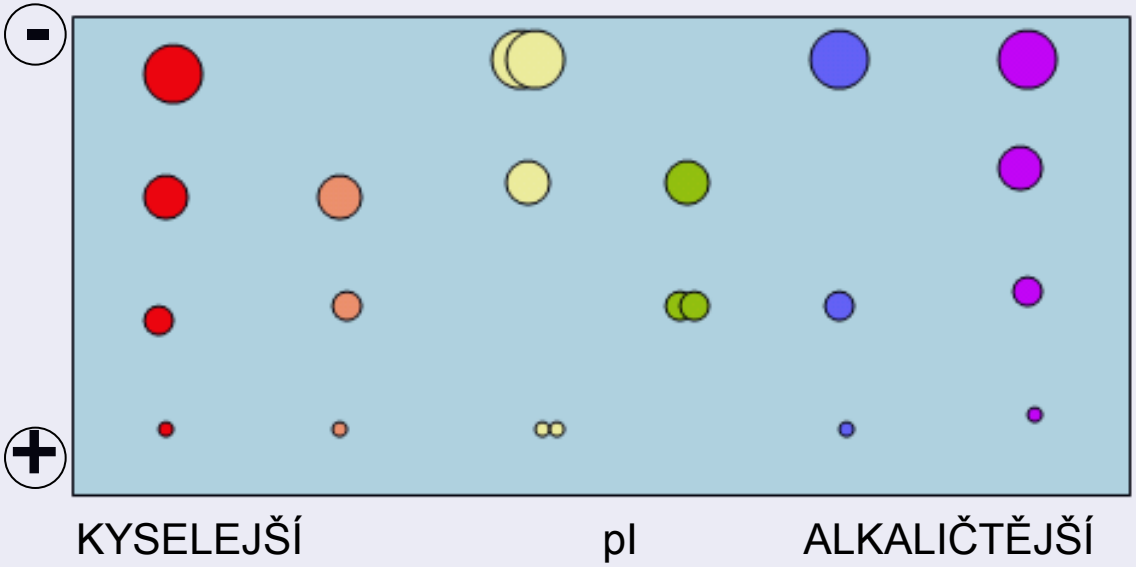
!



STRIP

ekvilibrace

SDS-PAGE



GEL

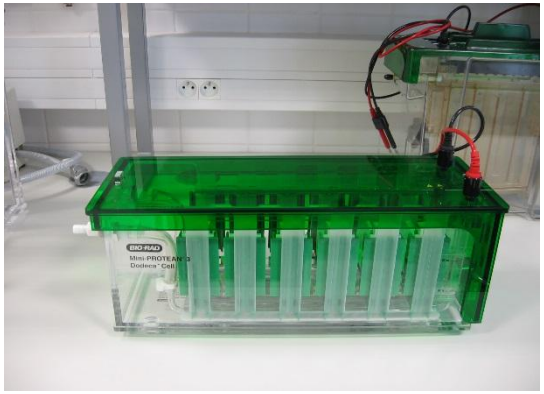
orientace gelu

2-DE INSTRUMENTACE

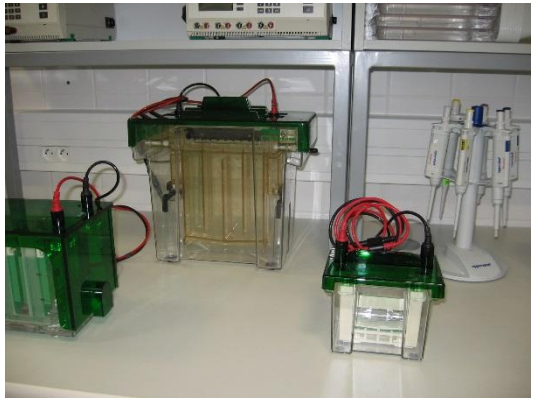
- Protean IEF
- Protean Dodeca Cell
- Densitometer GS-800
- FLA-7000, STORM

PDQuest, Quantity One

Protean Plus Dodeca Cell



Mini-Protean 3 Dodeca Cell



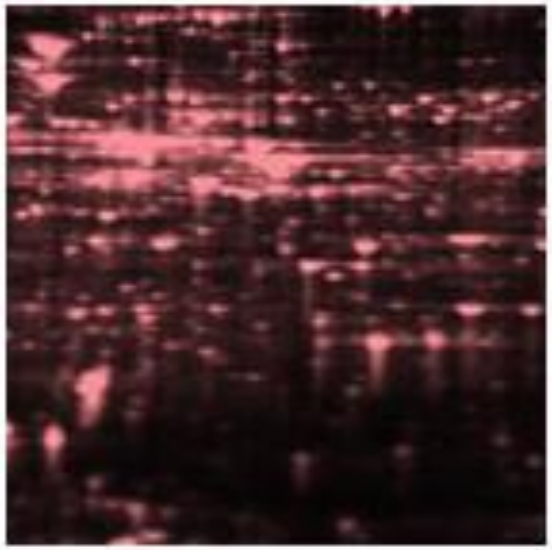
Protean II xi Cell

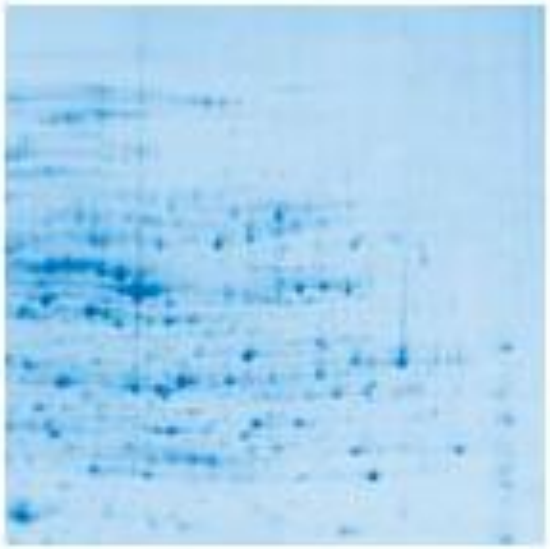
DETEKCE PROTEINU

- 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



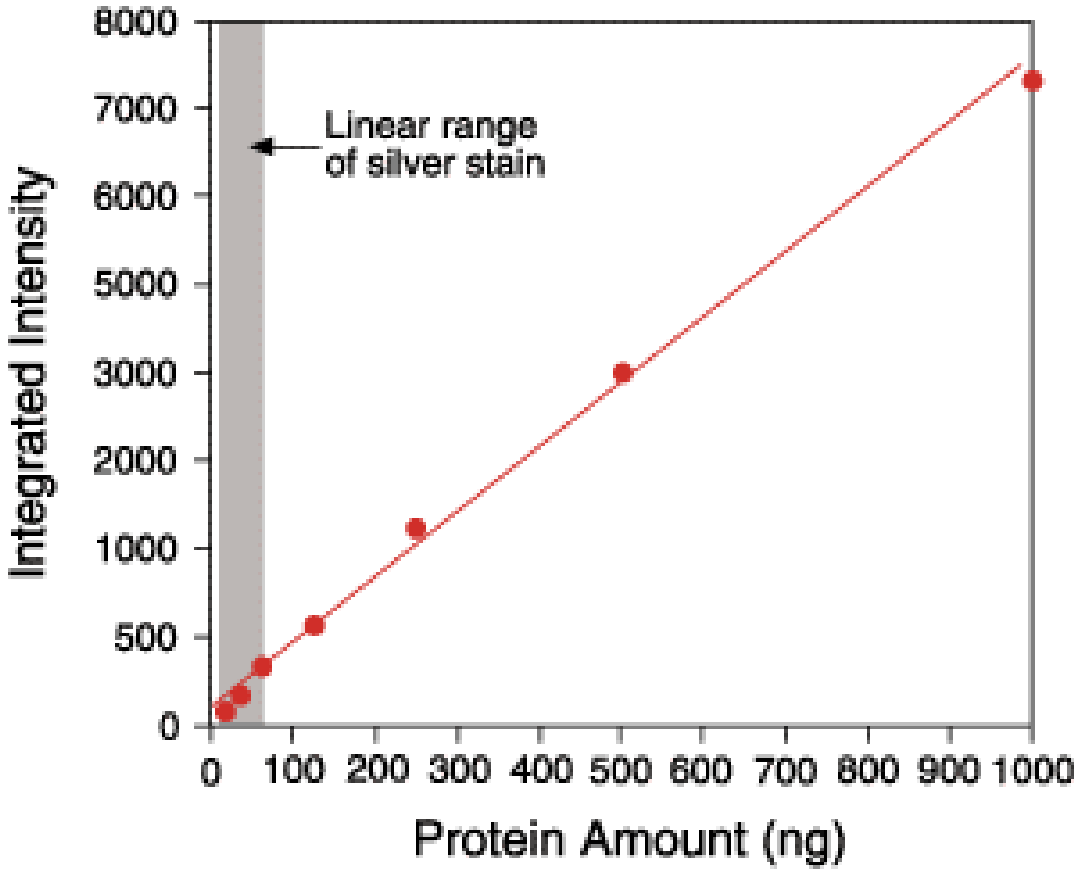


Sypro Ruby	Coomassie	silver
1.4 ng	36 ng	0.6 ng

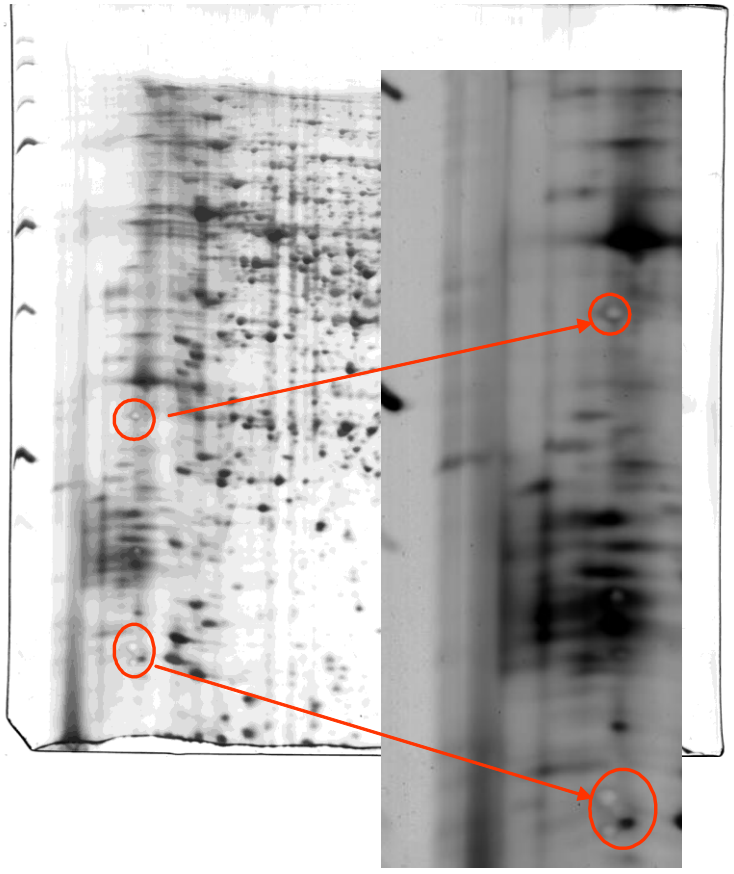
PTM specifická barvení	*Pro-Q Diamond*	*Pro-Q Emerald*

BARVENÍ PROTEINU – LINEARITA

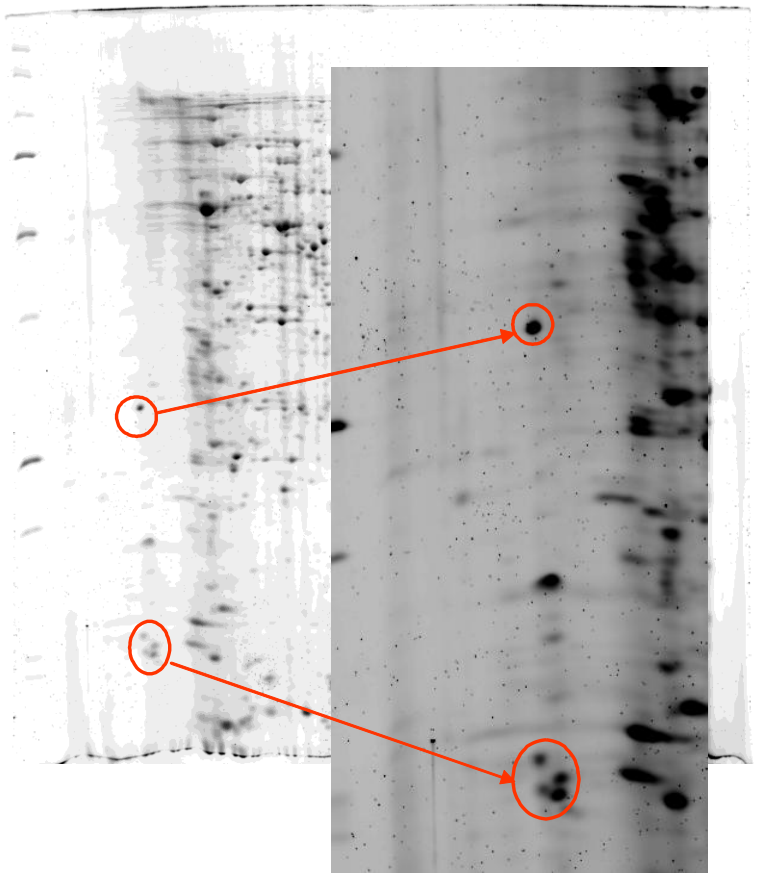
Sypro Ruby



Ag

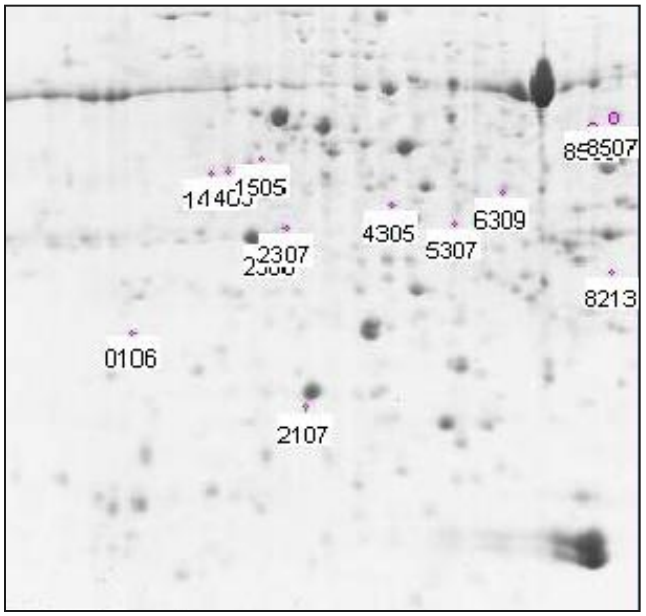


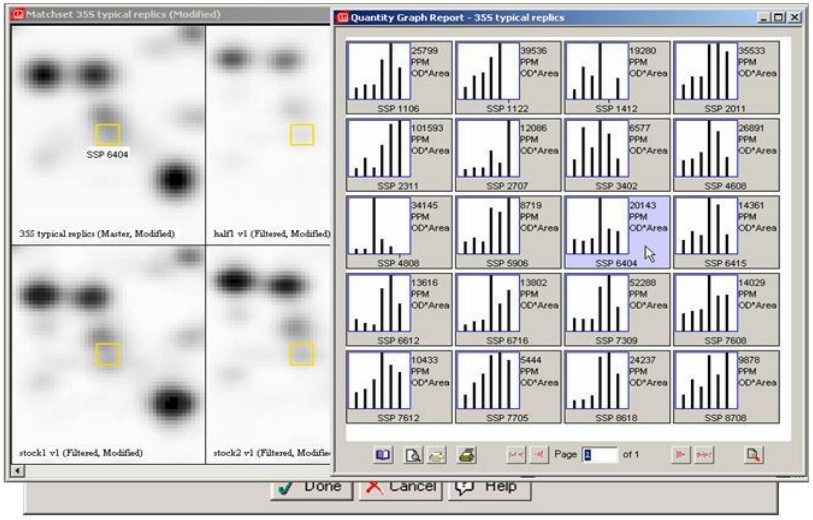
Sypro Ruby



ANALÝZA OBRAZU

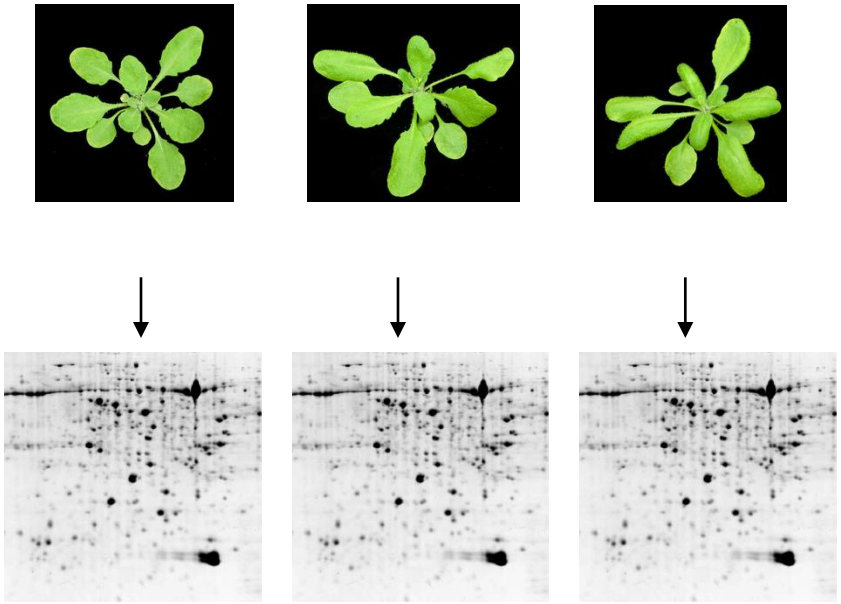
- kvalitativní
- kvantitativní





biologická variabilita

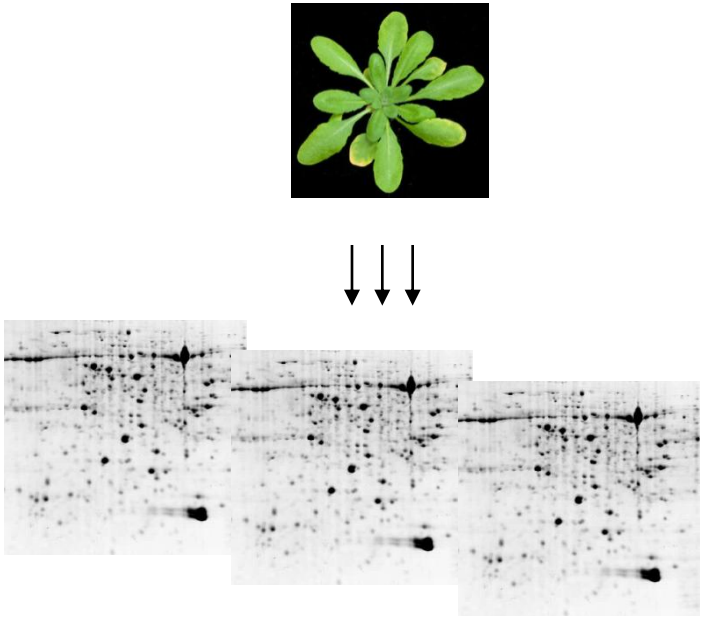
stejný organismus za stejných podmínek



biologické replikáty

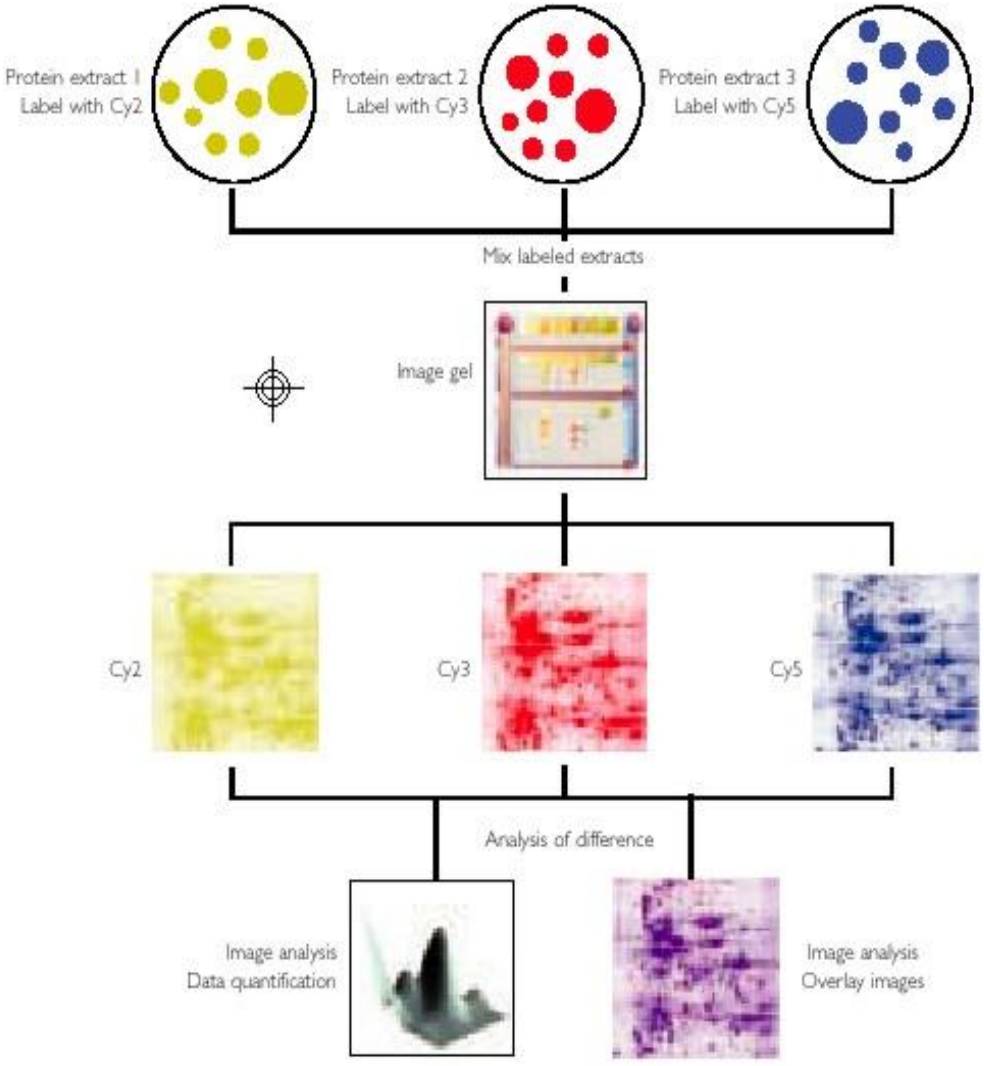
technická variabilita

stejný vzorek stejně

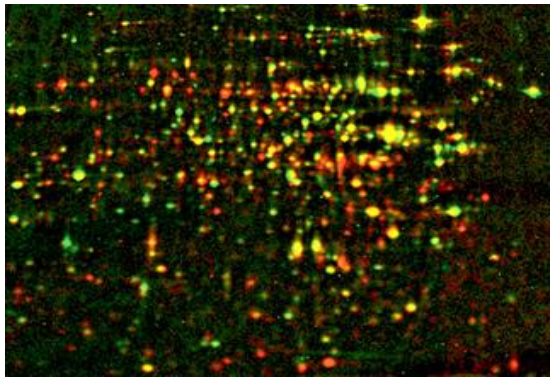


technické replikáty

Difference Gel Electrophoresis



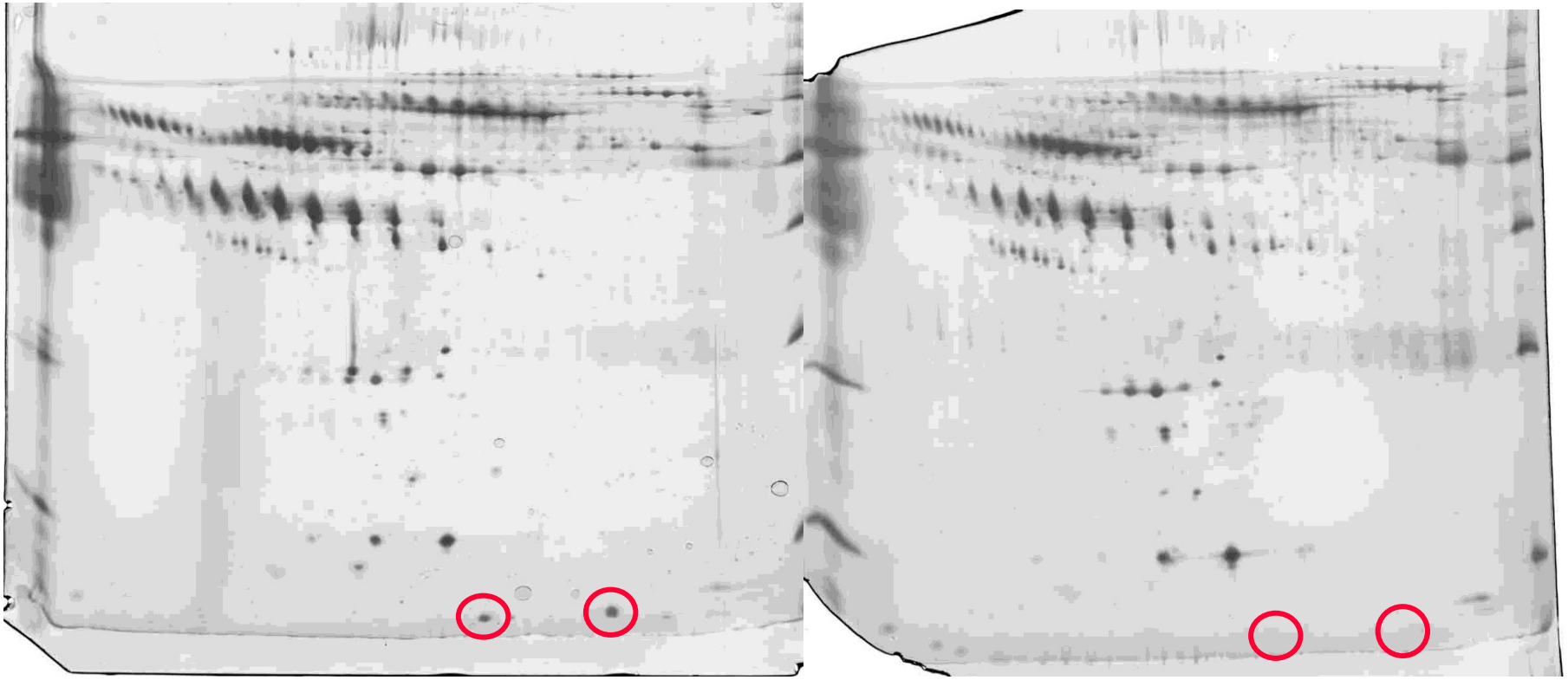
DIGE



Biomarkery v lidské plasmě

Den 21 – před klinickým projevem

Den 44 – po klinickém projevu



separace → identifikace

DIGESCE

trypsin Glu-C Asp-N thermolysin

MAVEPFPRRPITRPHASIEVDTSGTGGSAGSSE
KVFCLIGQAEGGEPNTVYELRNYAQAKRLFRS
GELLDAIELAWGSNPNYTAGRILAMRIEDAKP
ASAEIGGLKITSKIYGNVANNIQVGLEKNTLSD
SLRLRVIFQDDRFNEVYDNIGNIFTIKYKGEEA
NATFSVEHDEETQKASRLVLKVGDQEVKSYD
LTGGAYDYTNAIITDINQLPDFEAKLSPFGDKN
LESSKLDKIENANIKDKAVYVKAVFGDLEKQT
AYNGIVSFEQLNAEGEVPSNVEVEAGEESATV
TATSPIKTIEPFELTKLKGGTNGEPPATWADKL
DKFAHEGGYYIVPLSSKQSVHAEVASFVKERS
DAGEPMRAIVGGGFNESKEQLFGRQASLSNPR
VSLVANSGTFVMDDGRKNHVPAYMVAVALGG
LASGLEIGESITFKPLRVSSLDQIYESIDLDELN
ENGIISIEFVRNRTNTFFRIVDDVTTFNDKSDPV
KAEMAVGEANDFLVSELKVQLEDQFIGTRTIN
TSASIIKDFIQSYLGRKKRDNEIQDFPAEDVQVI
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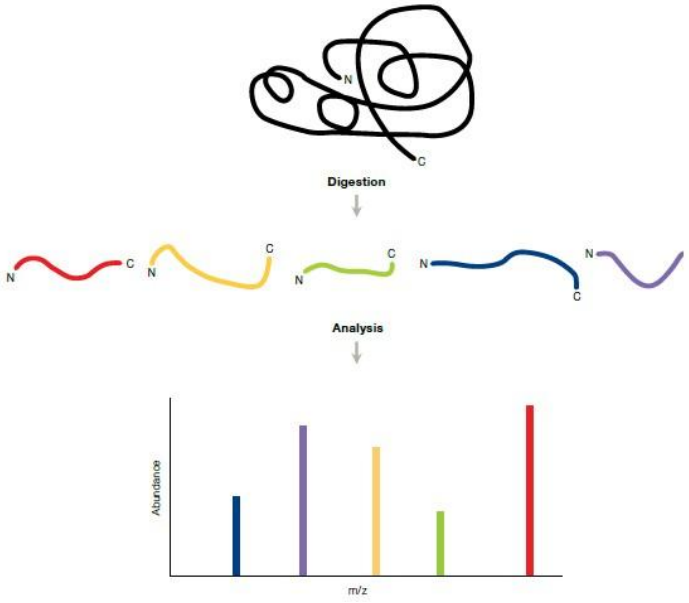
A

- IN-GEL
- IN-SOLUTION



MS

2D or not 2D ?

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

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

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**

Basics and recent advances of two dimensional – polyacrylamide gel electrophoresis
Sameh Magdeldin et al. *Clinical Proteomics* **2014**

MULTIDIMENZIONÁLNÍ CHROMATOGRAFIE

PRO

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

PROTI

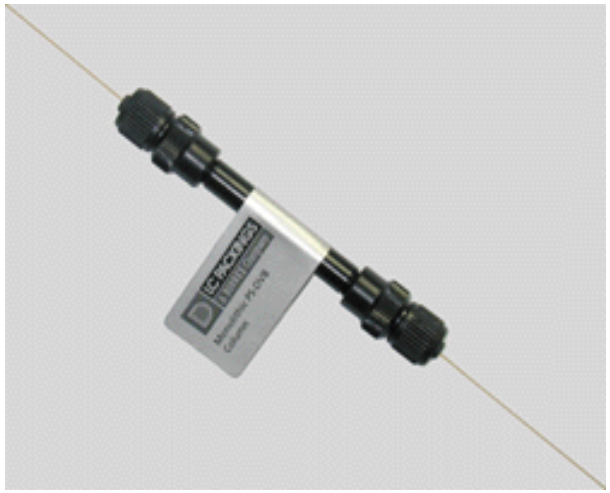
- vizuální aspekty ztraceny: pI a Mr
- LC - sériová analýza
- GE současně pro více vzorků

TYPY LC SEPARACE

CO ROZHODUJE →	KOLONA
▪ náboj	ionex
▪ hydrofobicita	reverzní fáze
▪ biospecifická afinita	afinitní
▪ velikost molekuly	gelová

… a kombinace

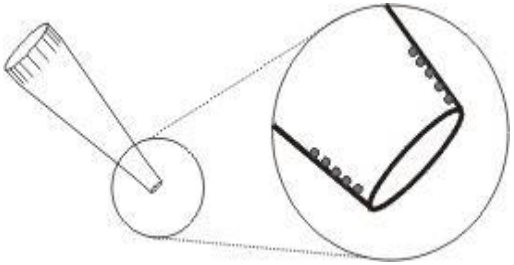
Kolona může vypadat různě . . .



TopTip

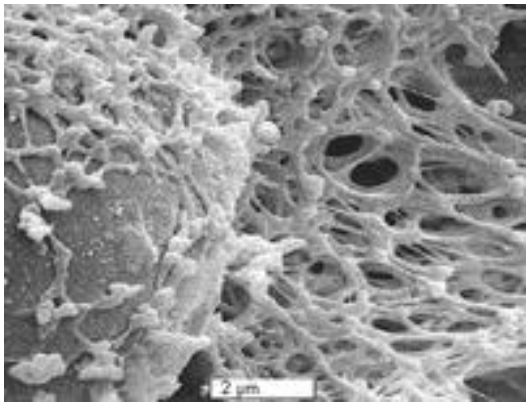
NuTip

Glygen



Zip Tip
Millipore

C18
C4
MC
SCX



LITERATURA - MULTIDIMENSIONÁLNÍ CHROMATOGRAFIE

Hyphenated dimensions in separation science
P.Q.Tranchida et al. *Journal of Chromatography* 2012

Multi-dimensional Liquid Chromatography in Proteomics
Xiang Zhang et al. *Anal Chim Acta* 2010

Multidimensional separation of peptides for effective proteomic analysis
H.J.Issaq et al. *Journal of Chromatography* 2005

Multi-dimensional liquid phase based separations in proteomics
Hong Wang, et al. *Journal of Chromatography* 2003

G I G O

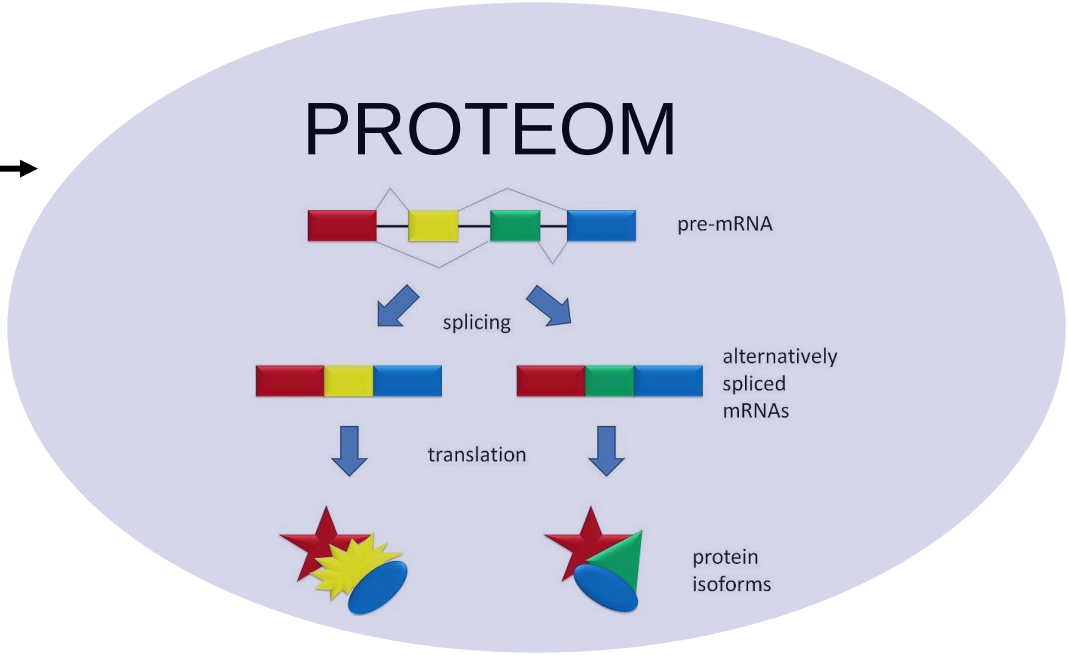
G I G O

GARBAGE IN - GARBAGE OUT

I. SEPARACE

II. PREFRAKCIONACE

GENOM →



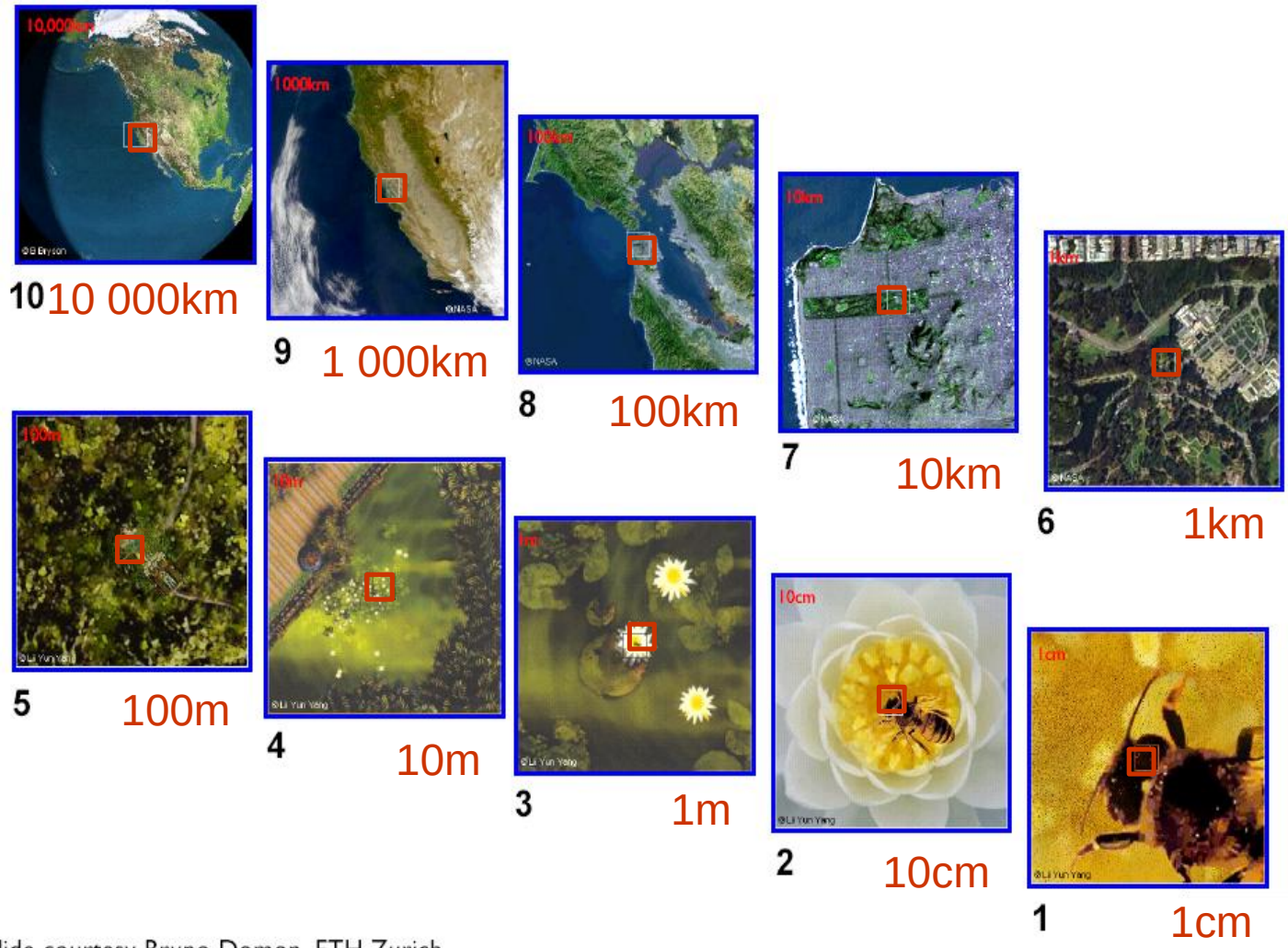
IZOFORMY

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

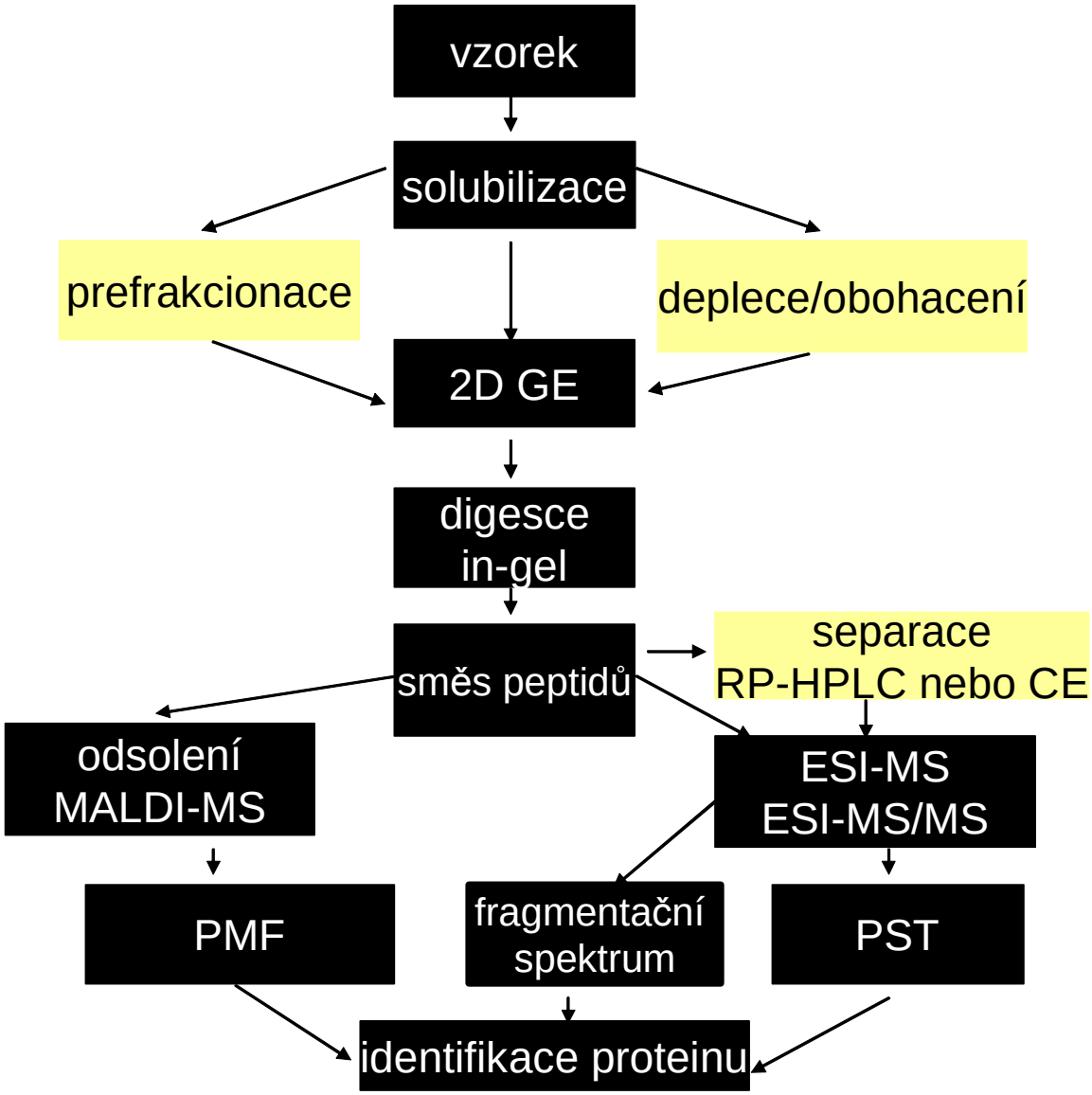
KONCENTRAČNÍ ROZSAH asi deset řádů ↓

PREFRAKCIONACE → MS

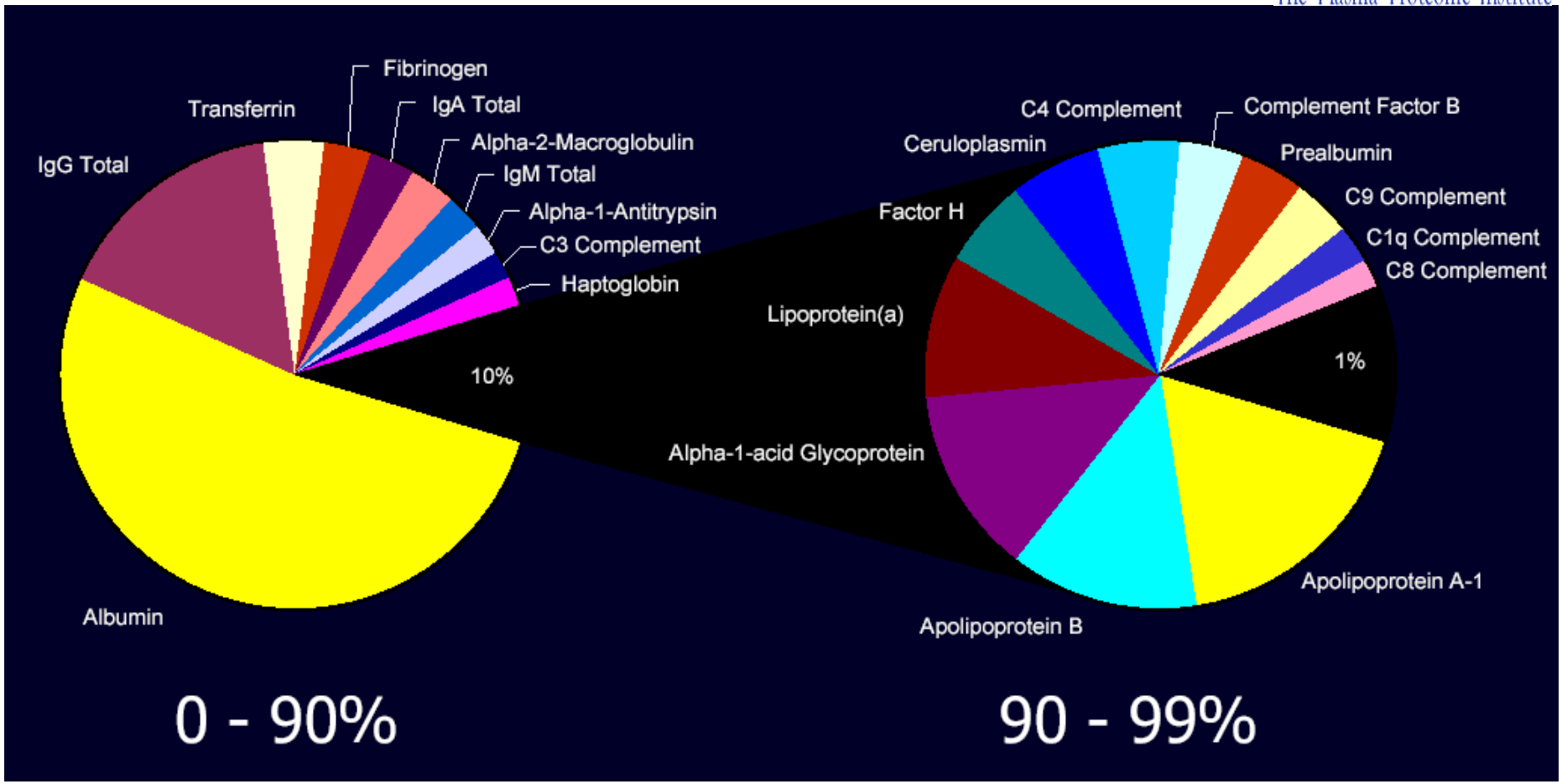
10^{10} Really Is Wide Dynamic Range



Slide courtesy Bruno Domon, ETH Zurich


vzorek
solubilizace
prefrakcionace
deplece/obohacení
2D GE
digesce
in-gel
směs peptidů
separace
RP-HPLC nebo CE
odsolení
MALDI-MS
ESI-MS
ESI-MS/MS
PMF
fragmentační
spektrum
PST
identifikace proteinu

Abundantní proteiny v lidské plazmě



AFINITNÍ DEPLECE

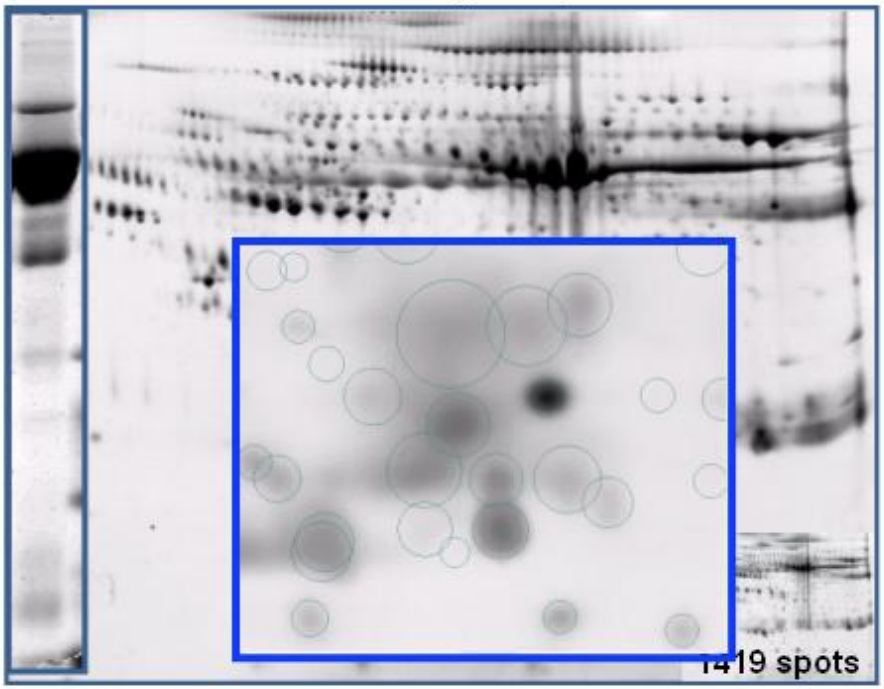
odstranění abundantních proteinů afinitní chromatografií

HSA

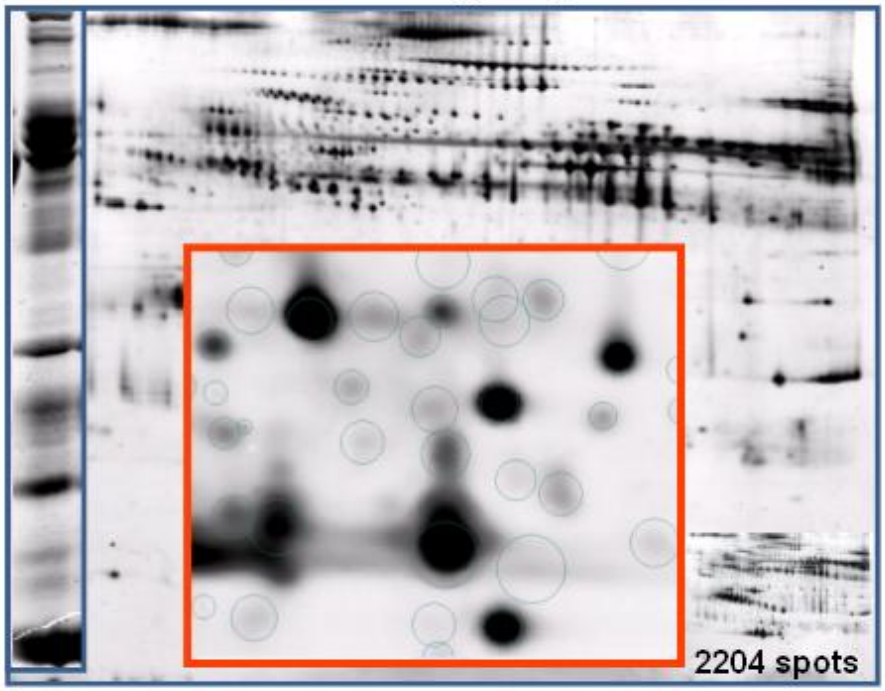
IgG

CPPL
Combinatorial Peptide Ligand Library

Native Human Serum

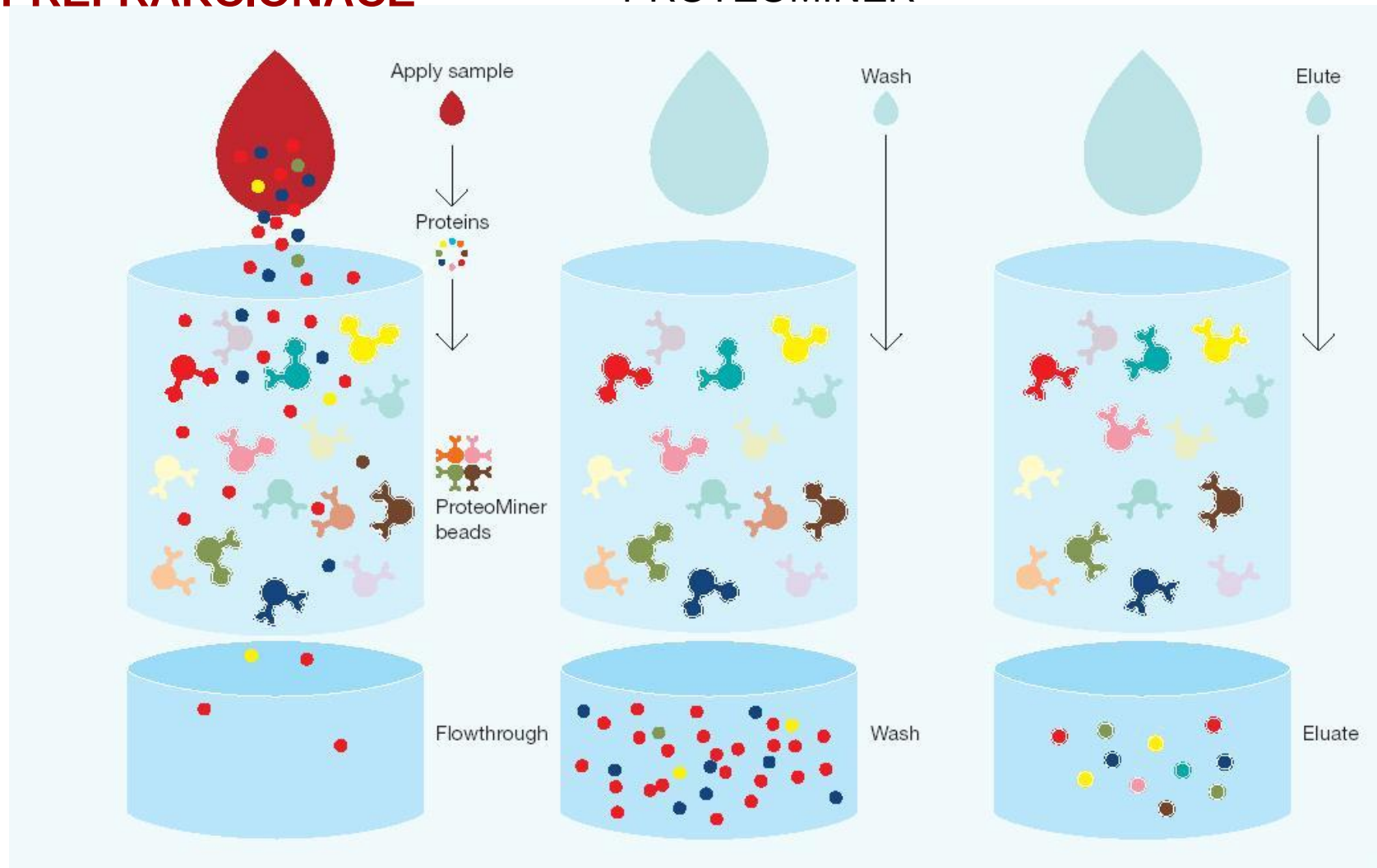


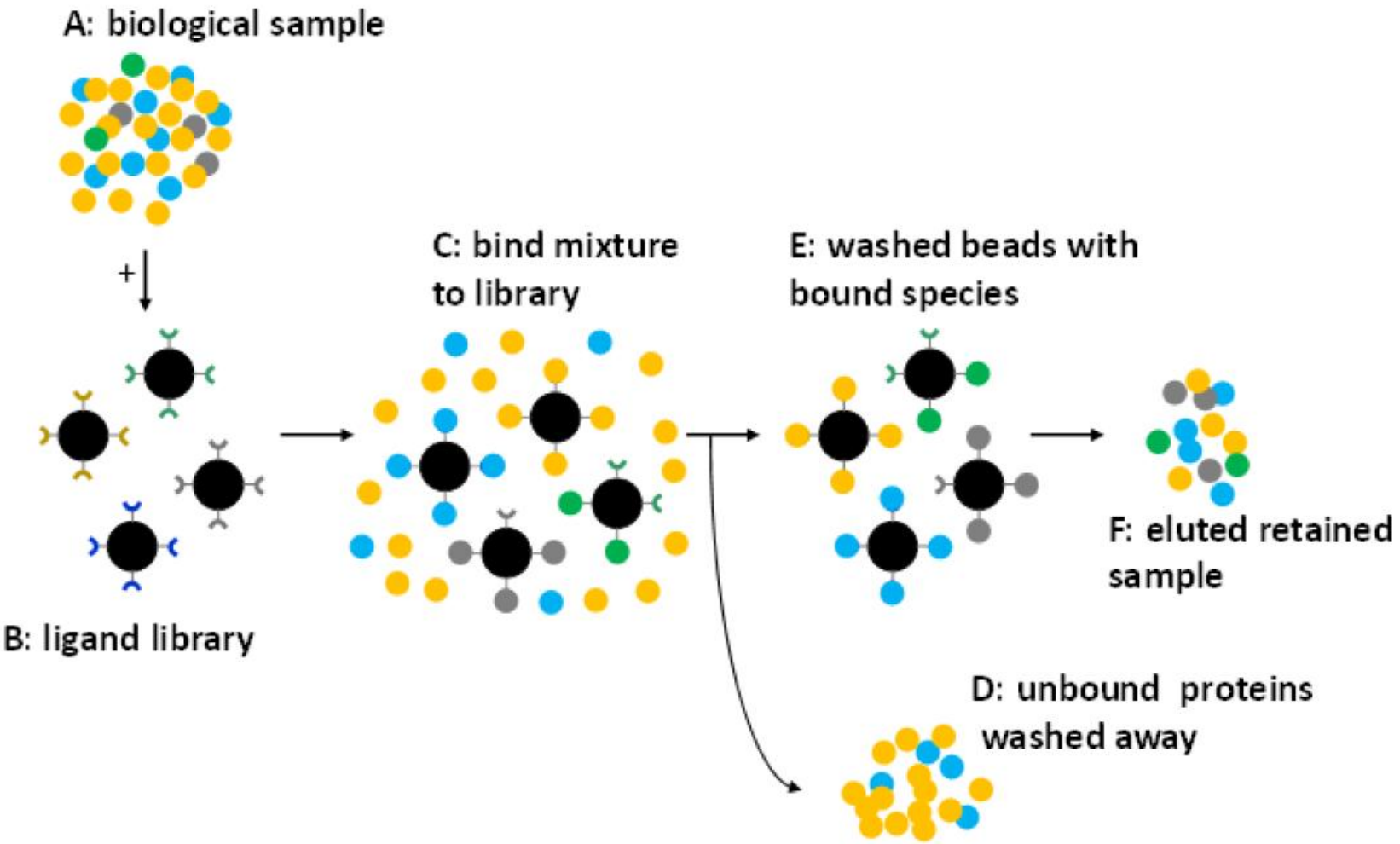
Human Serum Fractionated by ProteoMiner



PREFRAKCIONACE

PROTEOMINER




A: biological sample
+
B: ligand library
C: bind mixture to library
D: unbound proteins washed away
E: washed beads with bound species
F: eluted retained sample

IEF PREFRAKCIONACE

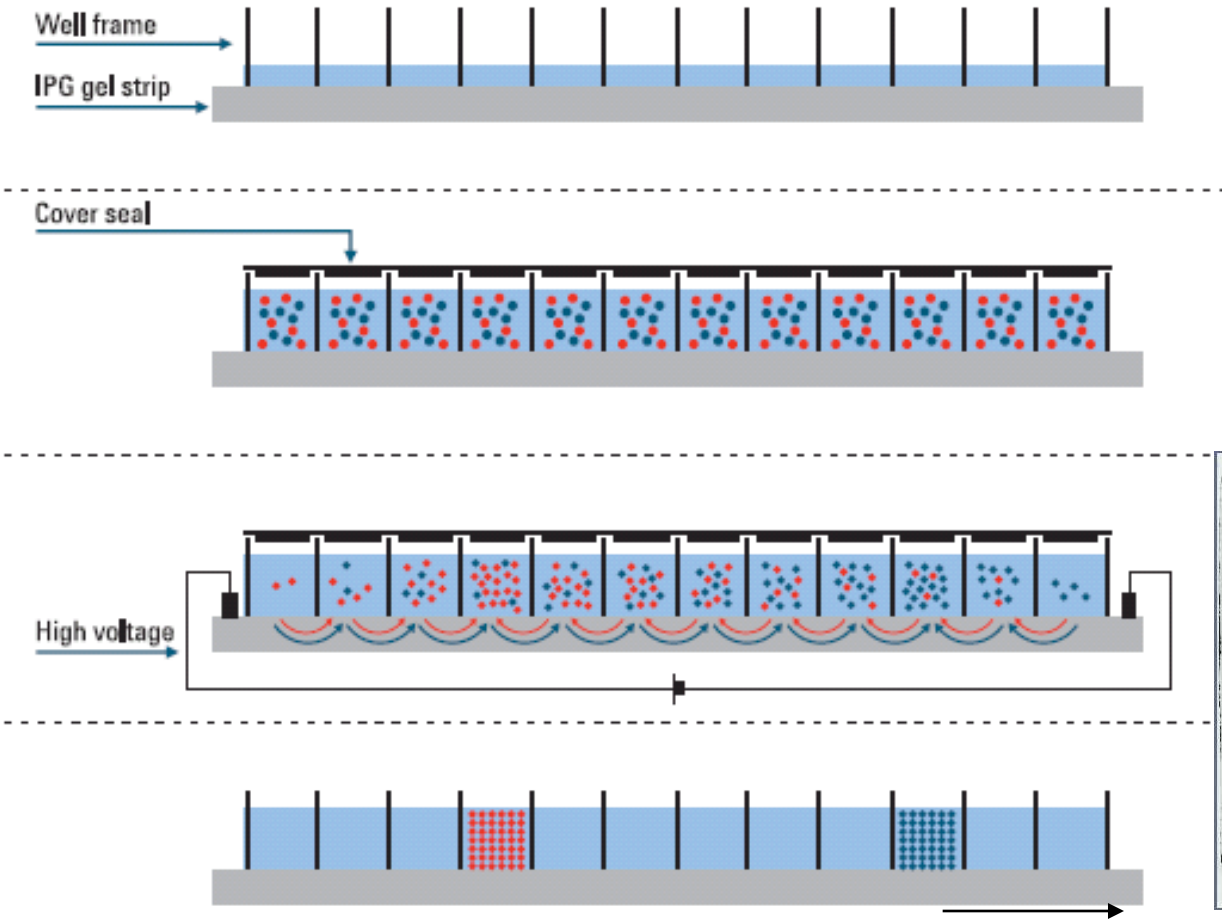
MicroRotofor

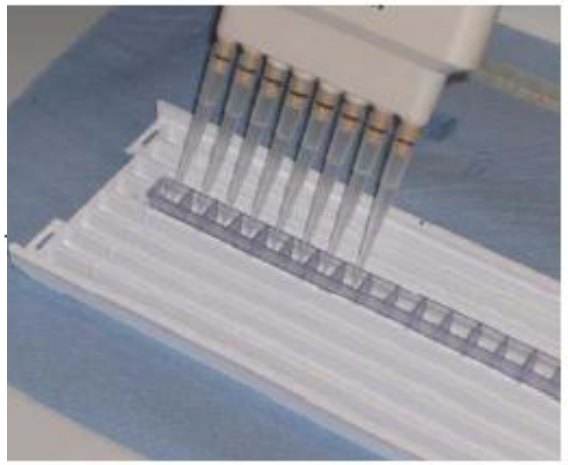
- prefrakcionace v roztoku

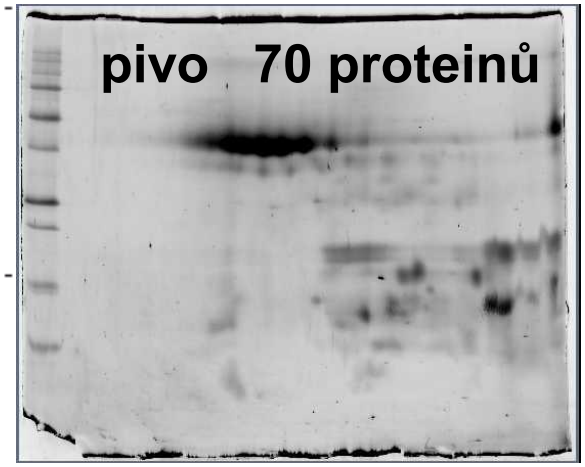
OffGel Fractionator

- prefrakcionace v roztoku na IPG stripu

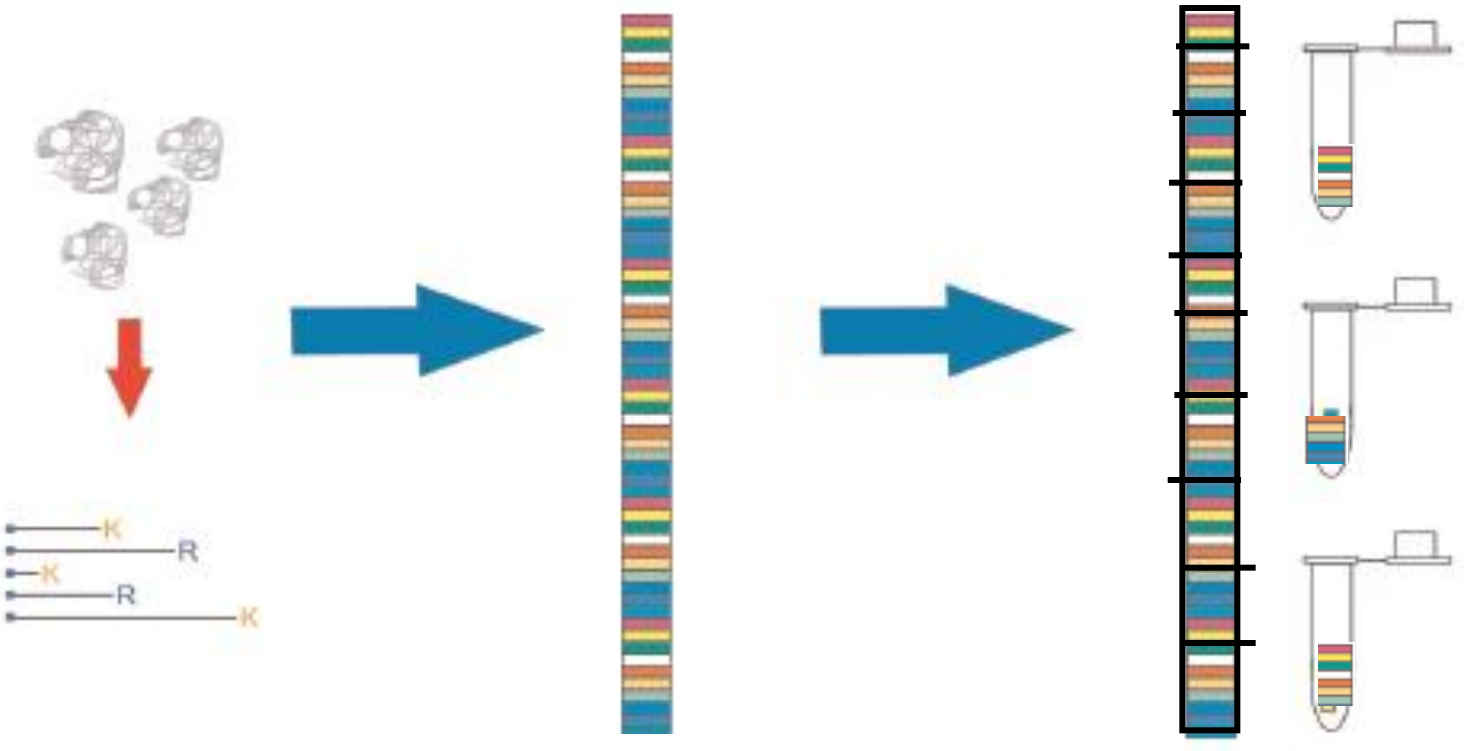
OFFGEL IEF prefrakcionace proteinů nebo peptidů







IPG-IEF



digest směsi proteinů

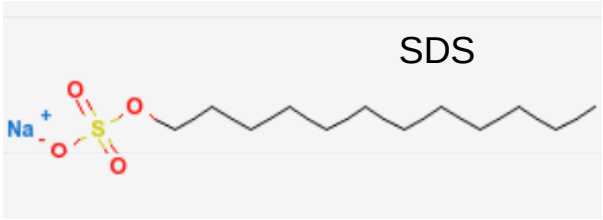
IEF směsi peptidů na IPG stripu

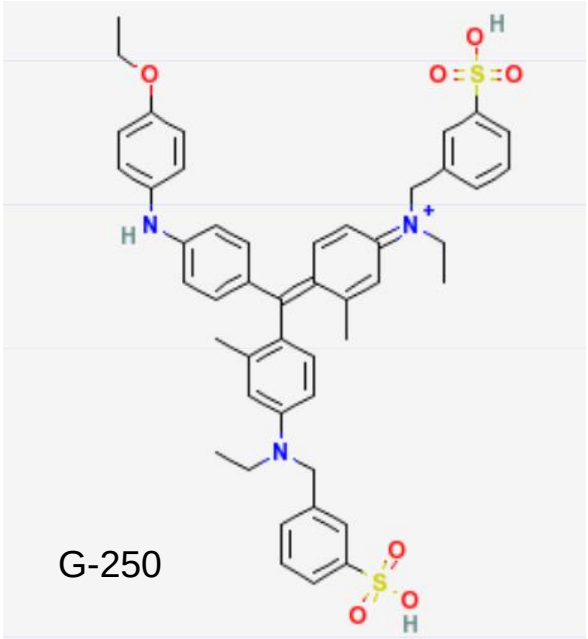
frakce stripu

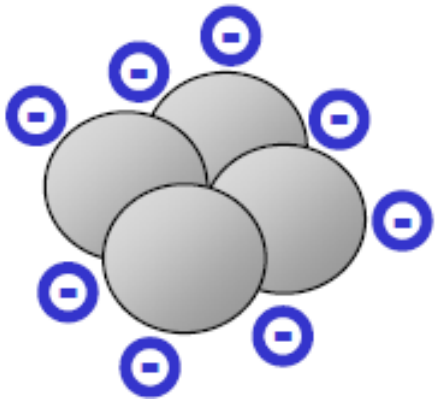
JOURNAL OF BIOMOLECULAR TECHNIQUES, VOLUME 16, ISSUE 3, SEPTEMBER 2005

Blue Native Electrophoresis BNE

- separace proteinů v nativním stavu
- separace membránových komplexů
- solubilizace neionogenními detergenty
- náboj udělen Coomassie G-250
- BN PAGE gel (strip/band) dále zpracován

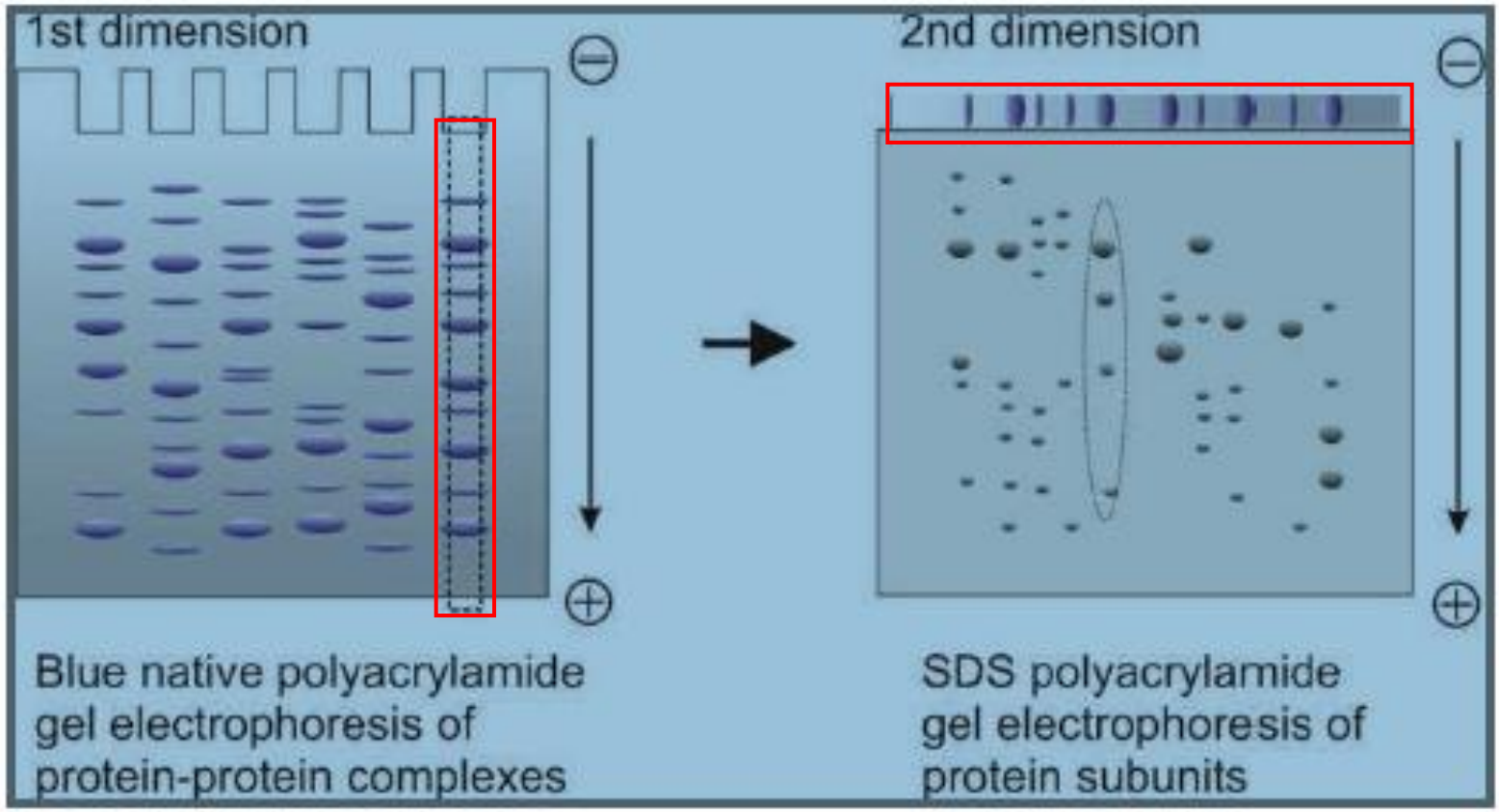




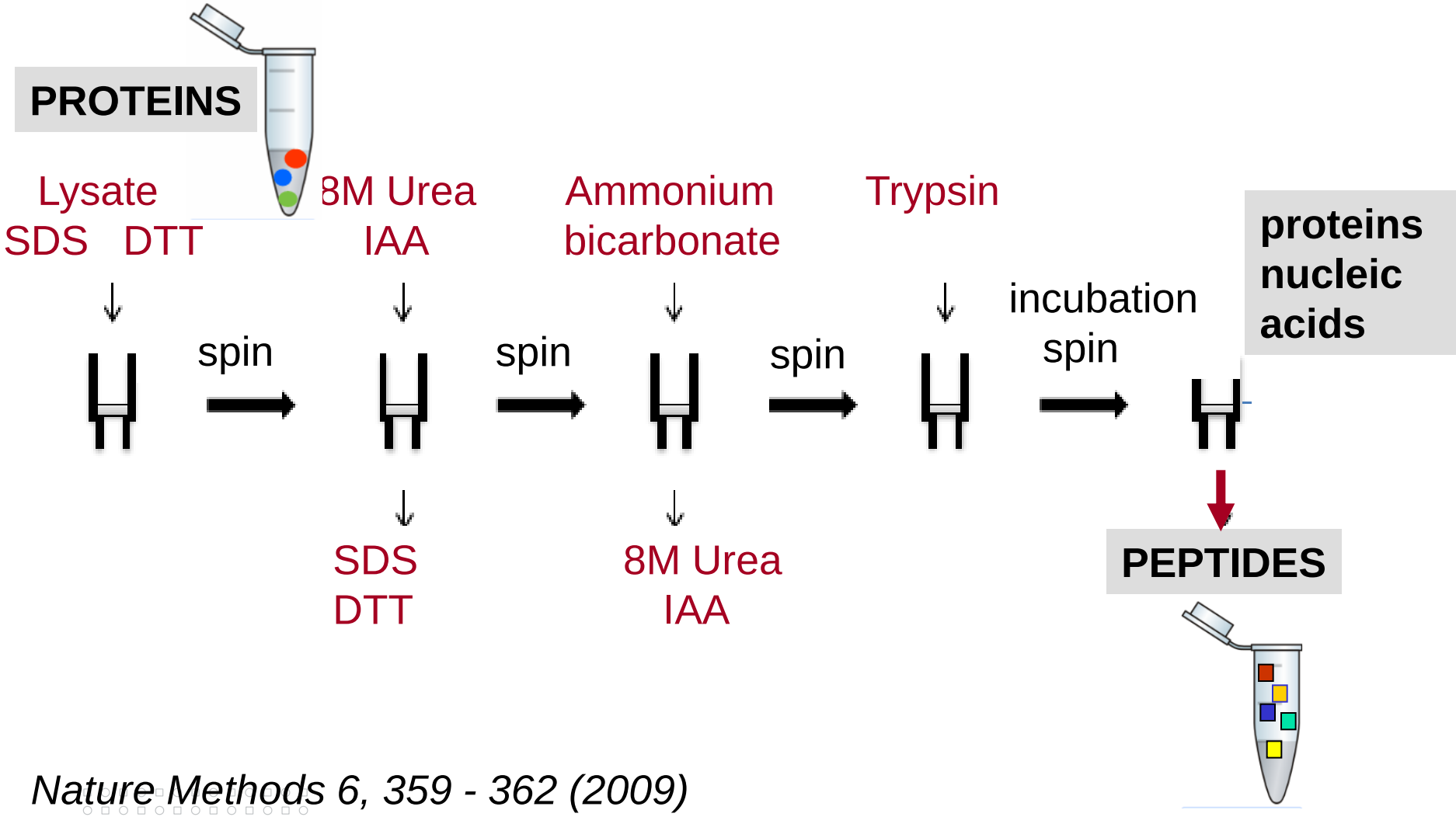


2DE

BNE SDS-PAGE

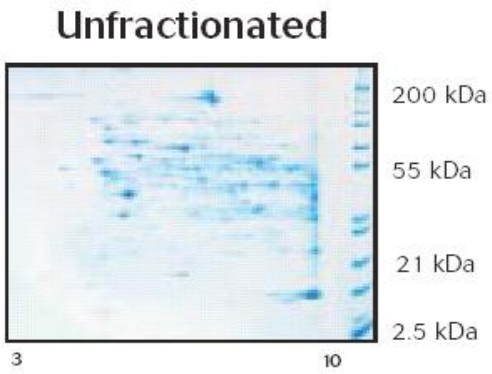


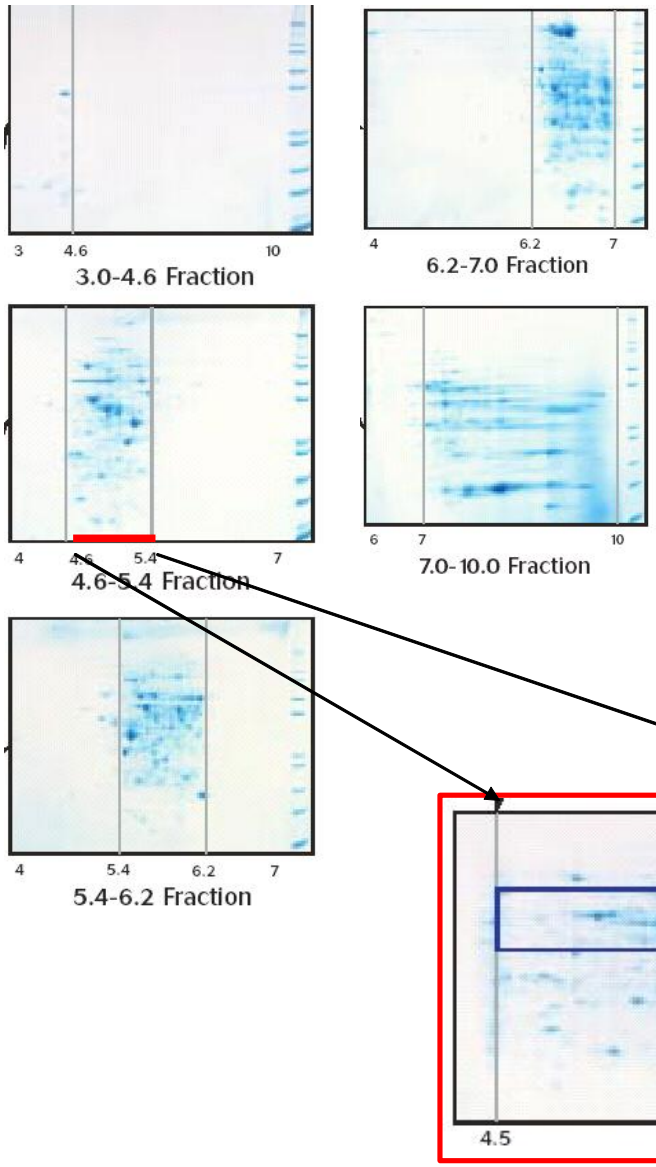
FASP Filter aided sample preparation



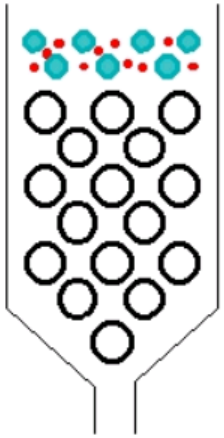
Nature Methods 6, 359 - 362 (2009)

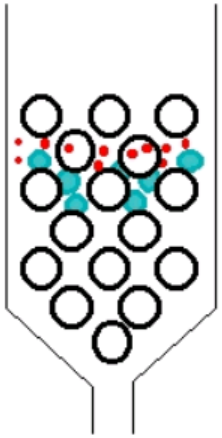
PREFRAKCIONACE MIKRO ROZSAHY

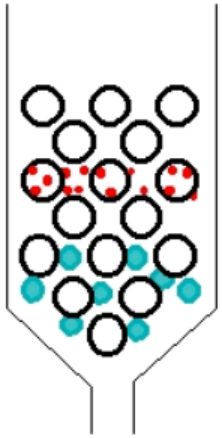


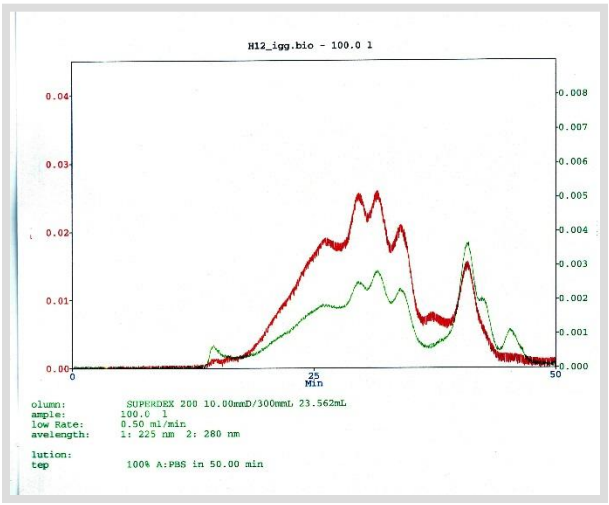


GELOVÁ CHROMATOGRAFIE



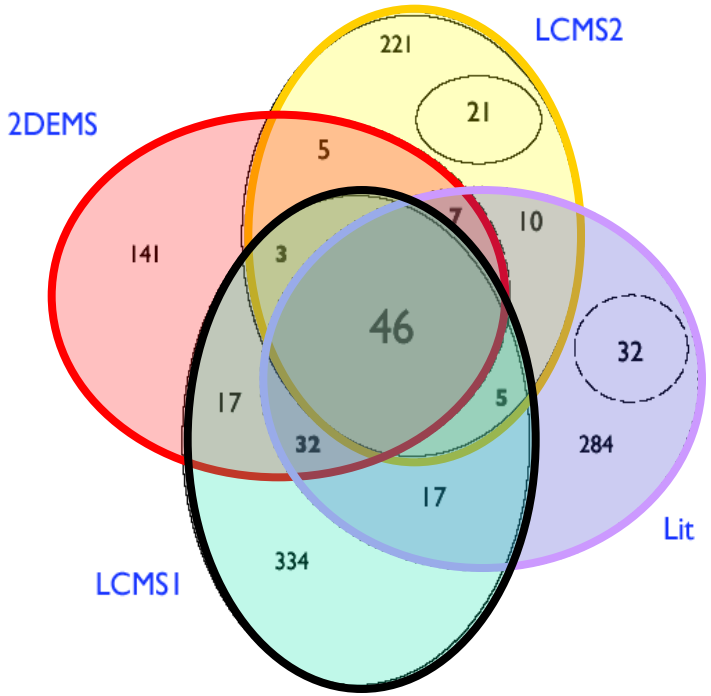






Different Platforms See Different Plasma Proteomes: Small Overlap of Four Plasma Proteome Datasets

(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

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

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ