



MASARYKOVA UNIVERZITA

Dvoudimenzionální elektroforéza

Hana Konečná

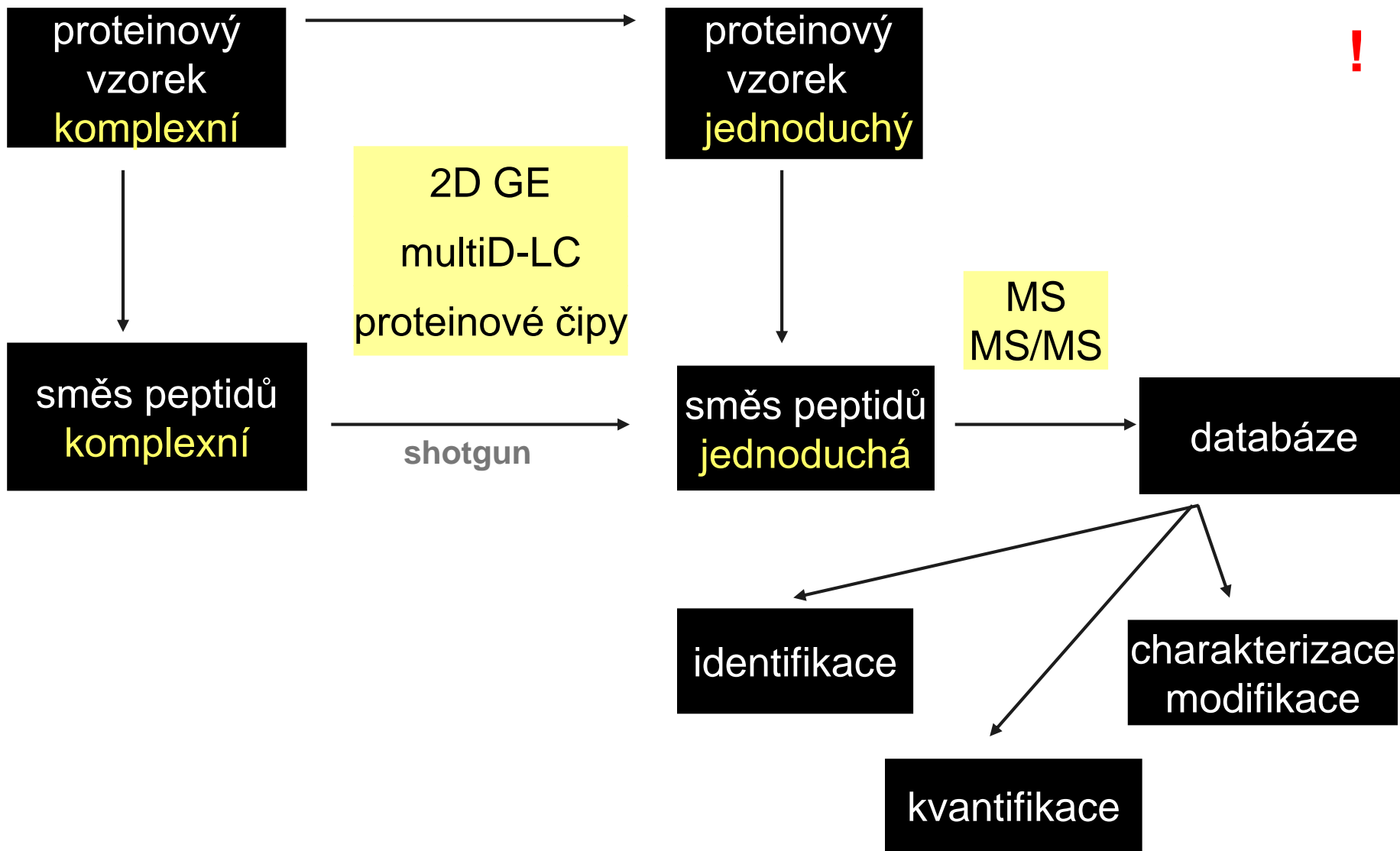
CENTRÁLNÍ LABORATOŘ - PROTEOMIKA

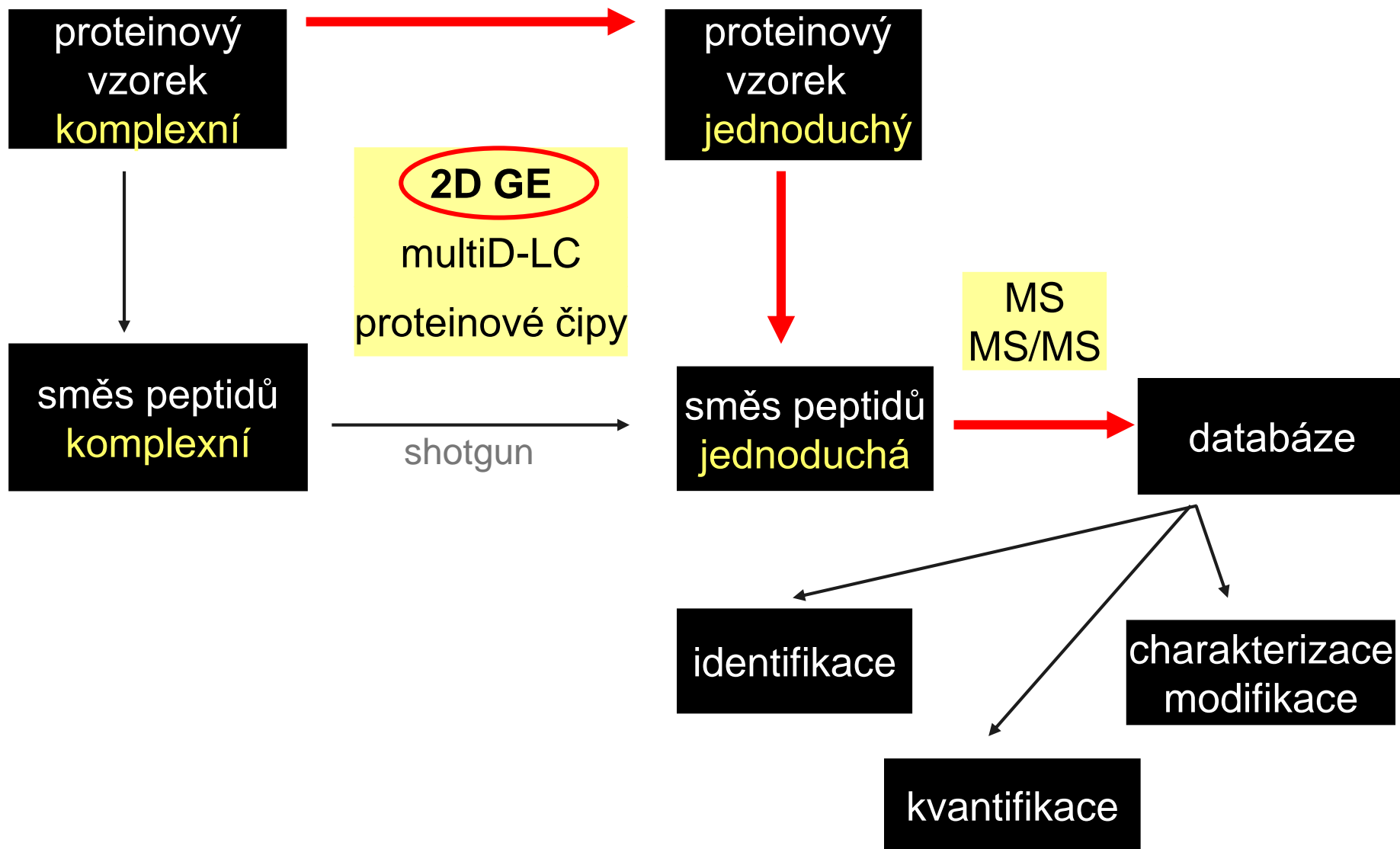
CEITEC – Středoevropský technologický institut

NCBR – Národní centrum pro výzkum biomolekul PŘF



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

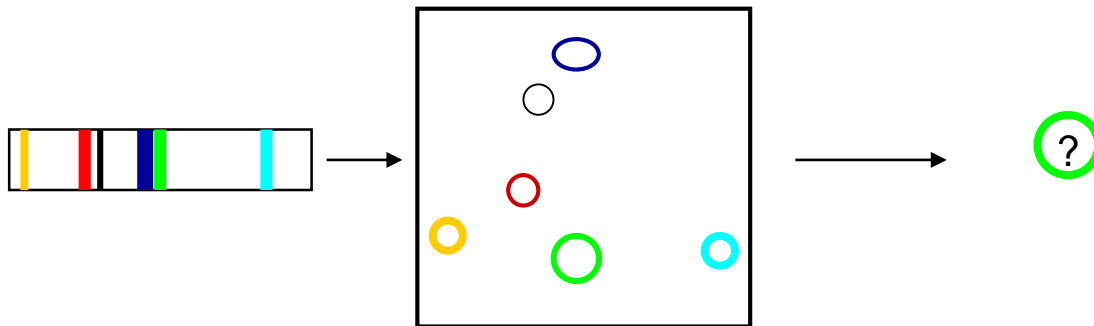




I. SEPARACE

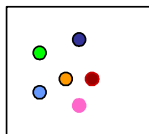
II. PREFRAKCIONACE

Dvoudimenzionální elektroforéza 2-DE



Proteomický experiment

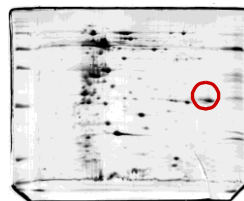
extrakce



fokusace



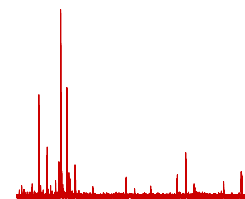
SDS-PAGE



digest



MS

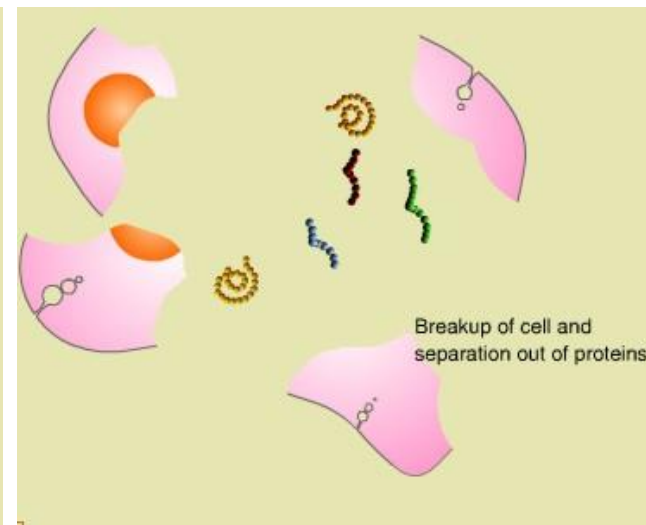
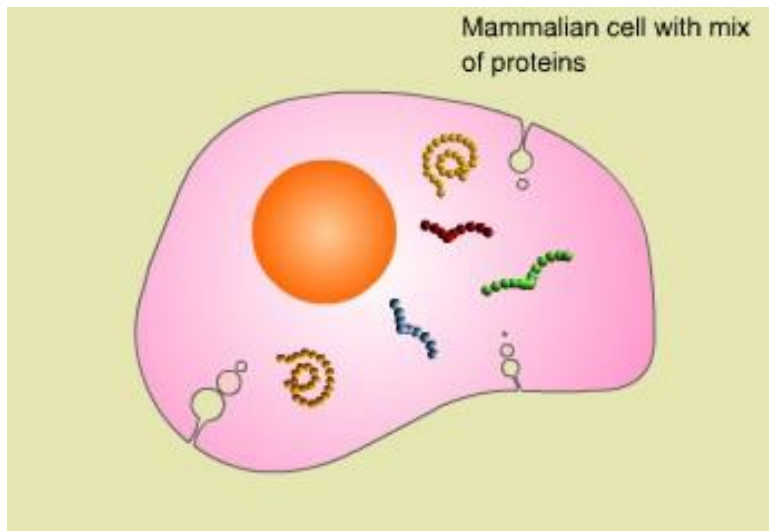


identifikace

neznámý protein

HOMOGENIZACE

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



PŘÍPRAVA VZORKU



solubilizace močovina, thiomčovina, detergenty

redukce DTT, TBP

inhibice proteáz, fosfatáz, glykosyláz

odstranění kontaminant

MĚŘENÍ KONCENTRACE PROTEINU

lyzační pufr vs. hydratační pufr

DETERGENTY



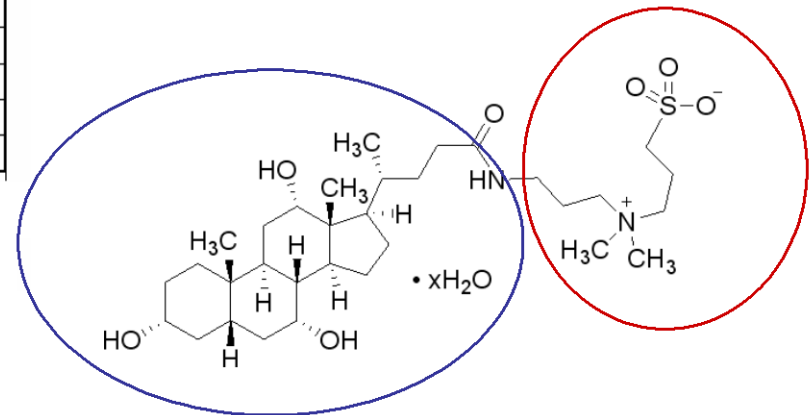
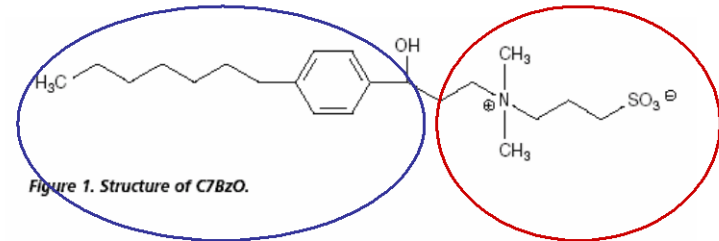
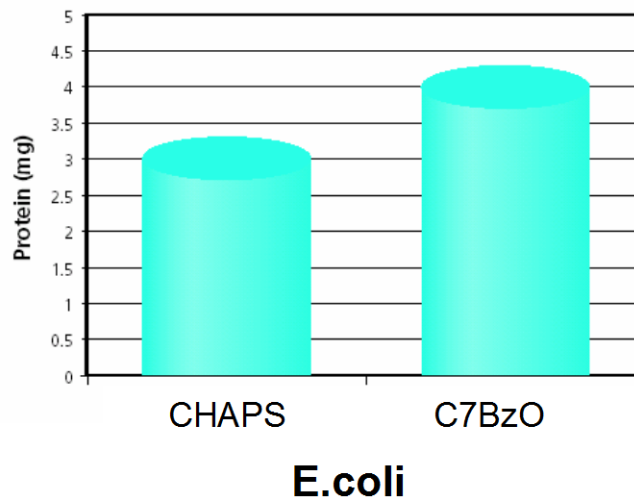
- žádný celkový náboj
- 0.5 - 4%
- použitelné ve vysokých koncentracích močoviny
- neionogenní
- zwitterointové
- SDS jen v nízkých koncentracích (do 0.25%)

C7BzO

3-(4-Heptyl)phenyl-3-hydroxypropyl)dimethylammonio)propanesulfonate

CHAPS

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



ZÁKLADNÍ PRAVIDLA

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

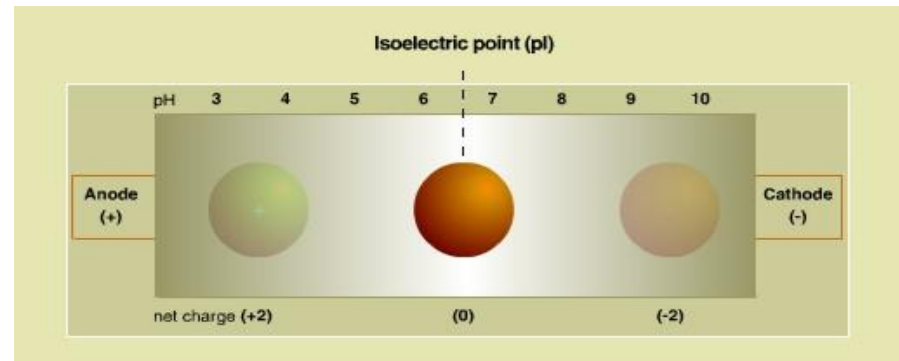
KONTAMINANTY

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

2-DE

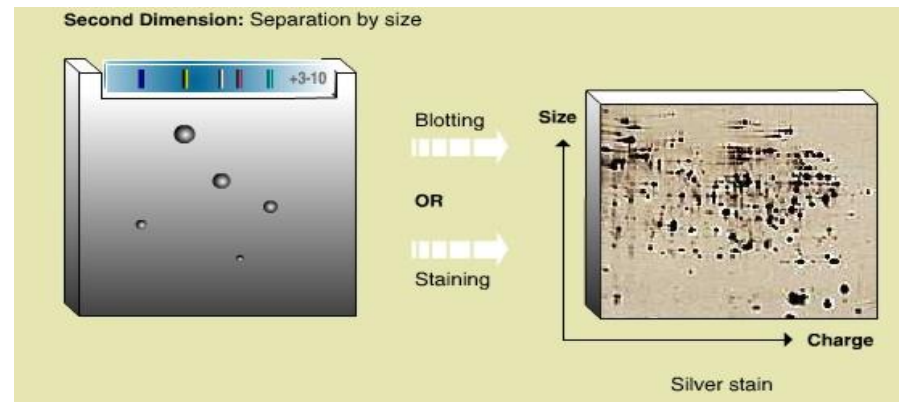
- první rozměr

IEF



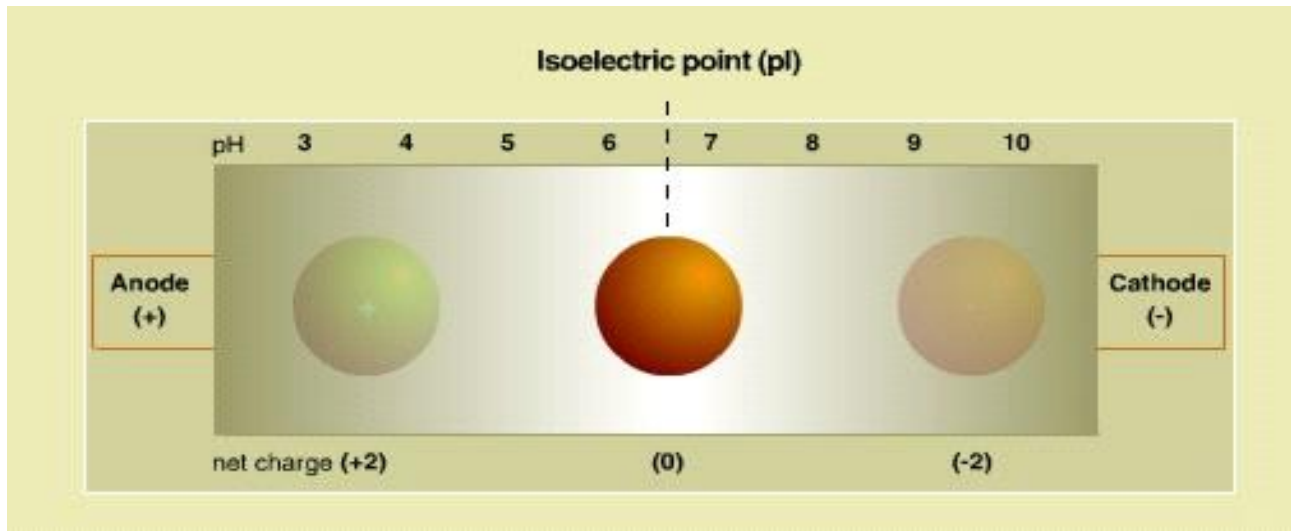
- druhý rozměr

SDS-PAGE

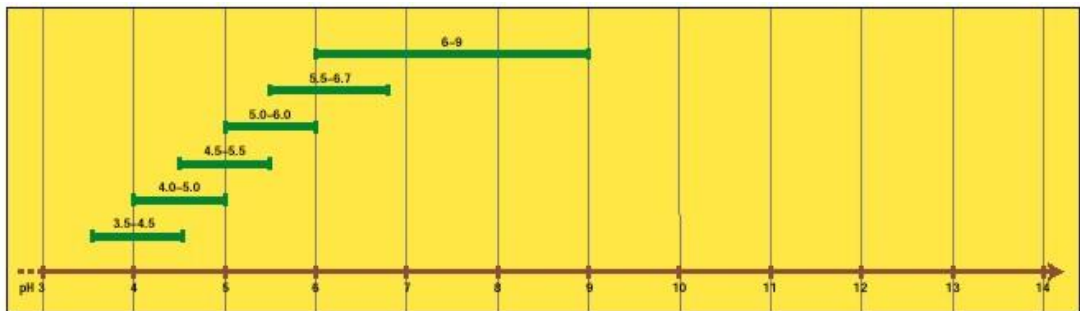
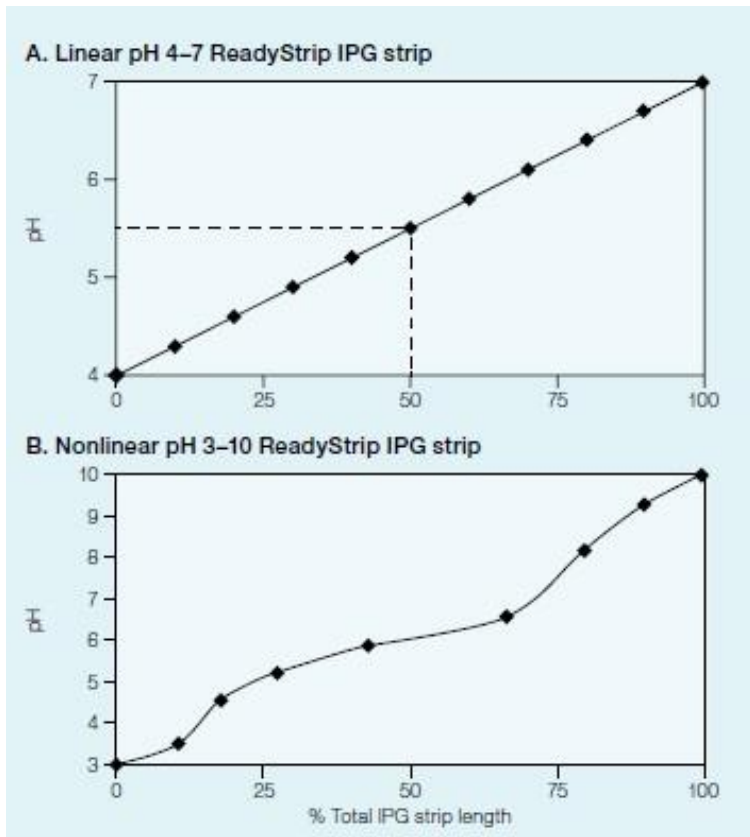


1. ROZMĚR **IZOELEKTRICKÁ FOKUSACE**

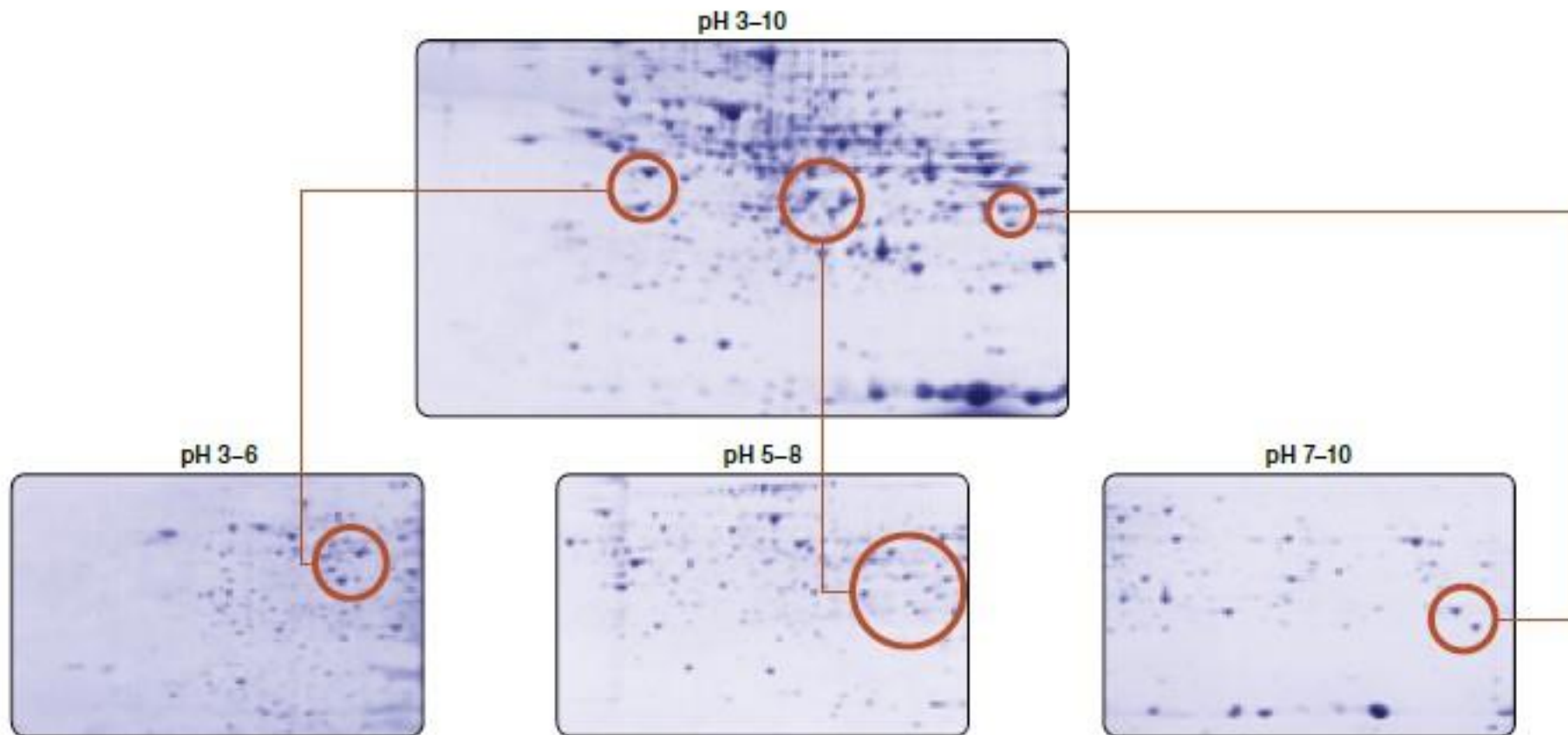
migrace nabitých částic v gradientu pH v elektrickém poli



ROZSAH STRIPU ROZMĚR STRIPU



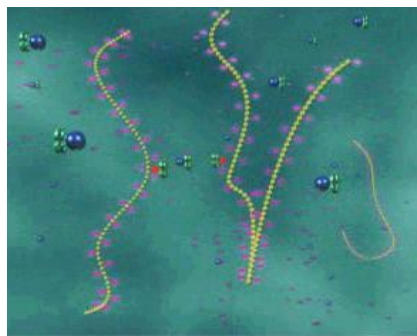
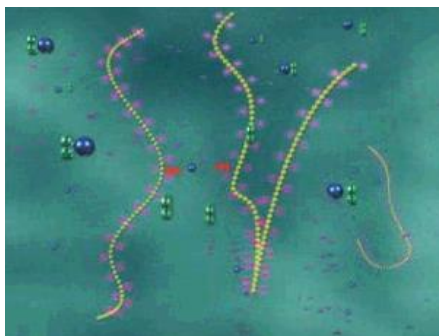
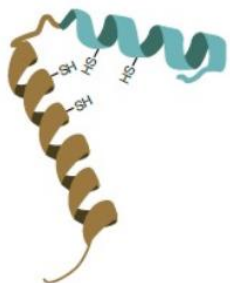
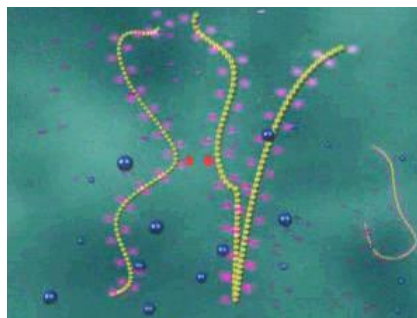
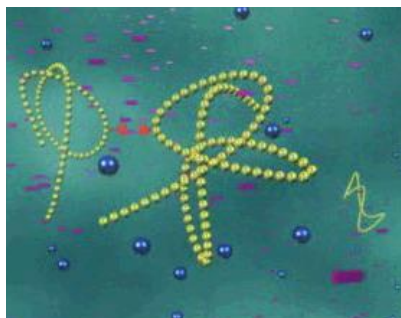
ROZSAH STRIPU



EKVILIBRACE STRIPU

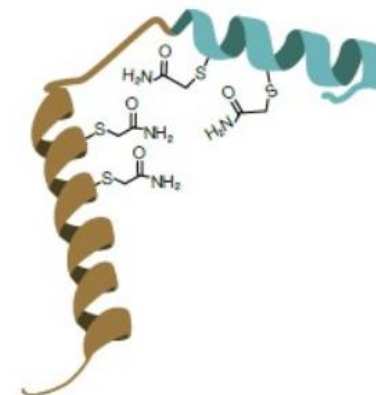


denaturace SDS ●



redukce DTT ●

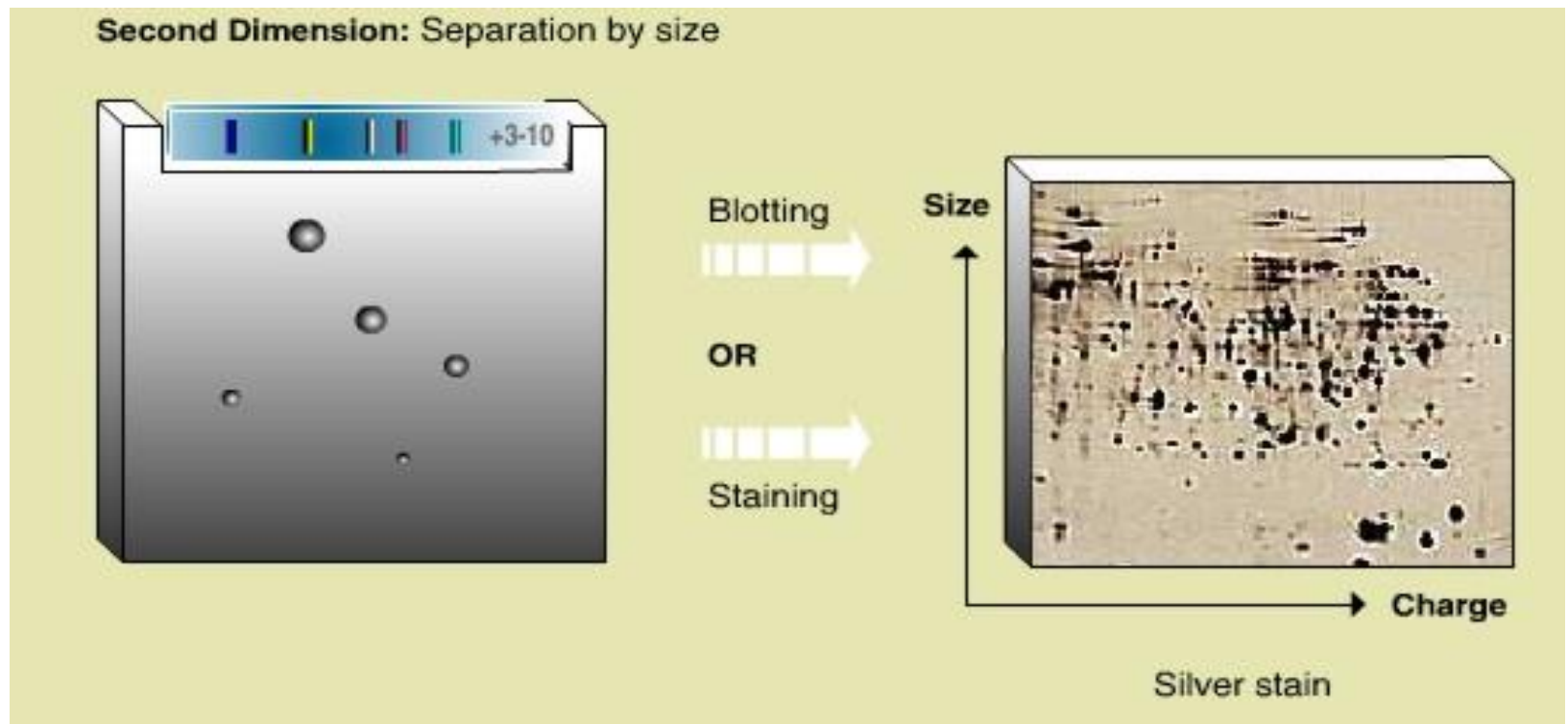
alkylace IAA ●



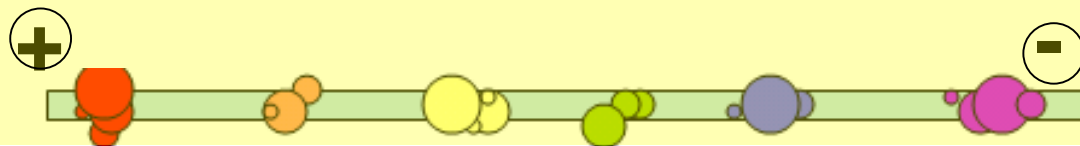
2. ROZMĚR **SDS-PAGE**



migrace aniontů v elektrickém poli podle MW



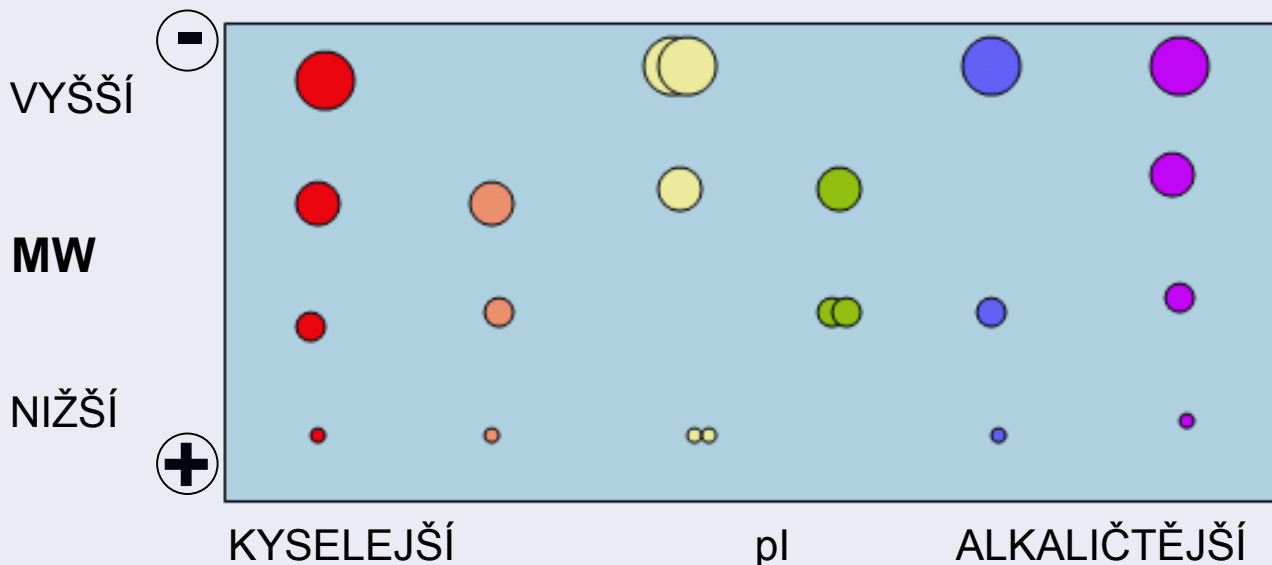
FOKUSACE



STRIP

↓
ekvilibrace

SDS-PAGE



GEL

orientace gelu

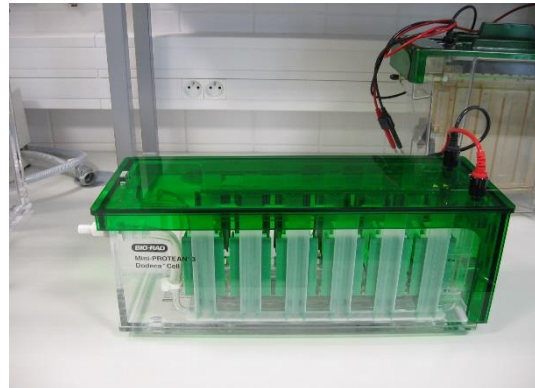


2-DE INSTRUMENTACE

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



Protean Plus Dodeca Cell



Mini-Protean 3 Dodeca Cell



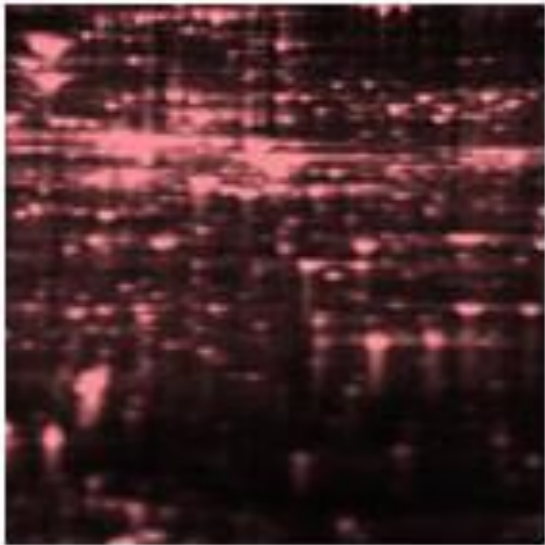
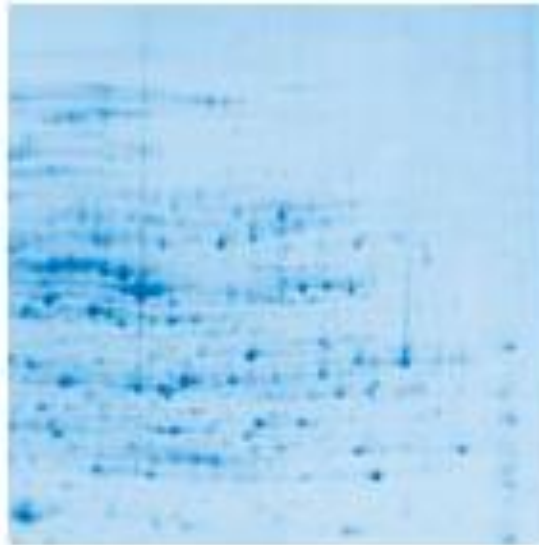
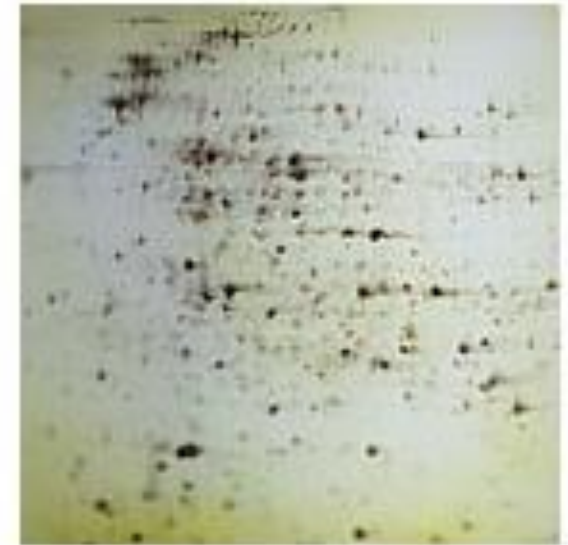
Protean II xi Cell



DETEKCE PROTEINU

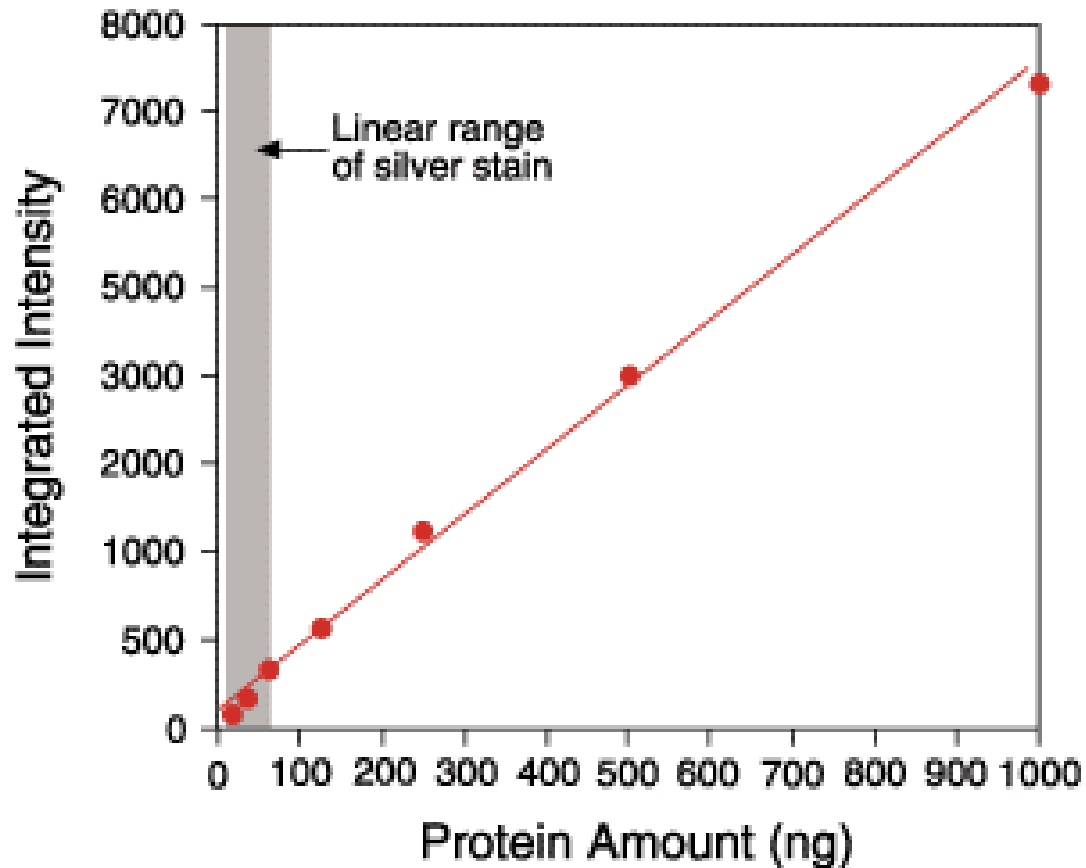
- gel x blot
- visualizace →
 - barvení
 - radioaktivita
 - imunodetekce
- barvení v gelu
 - po elektroforéze
 - před elektroforézou
 - specifické pro protein
 - specifické pro PTM
 - viditelné spektrum
 - fluorescence

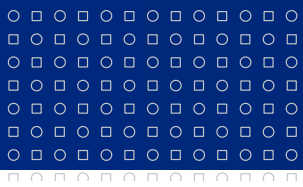
DETEKCE PROTEINU V GELU

**Sypro Ruby****1.4 ng****Coomassie****36 ng****silver****0.6 ng****PTM specifická barvení***Pro-Q Diamond**Pro-Q Emerald*

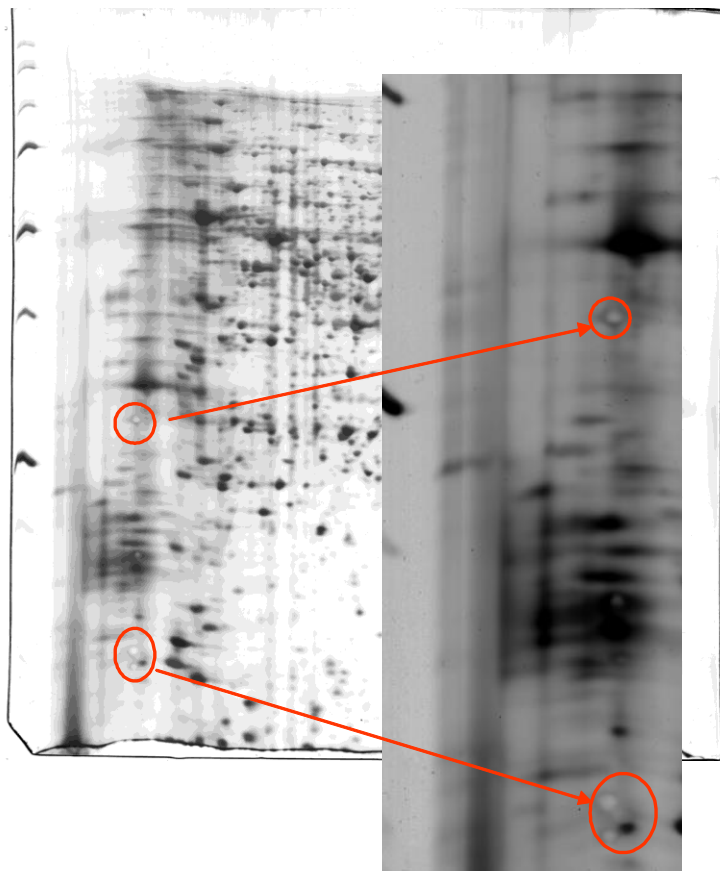
BARVENÍ PROTEINU – LINEARITA

Sypro Ruby

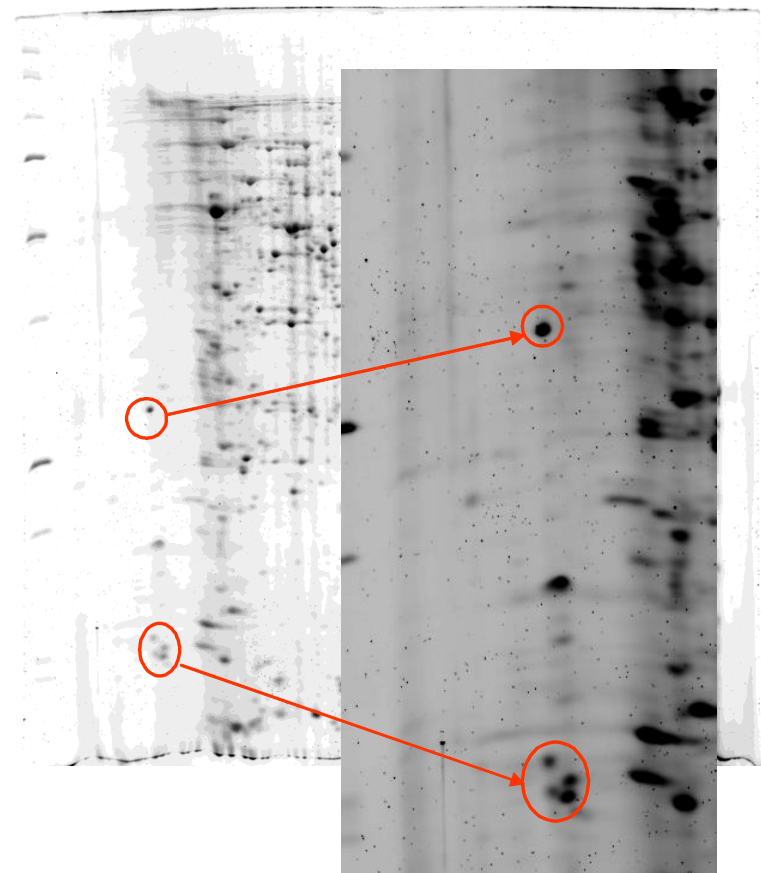




Ag

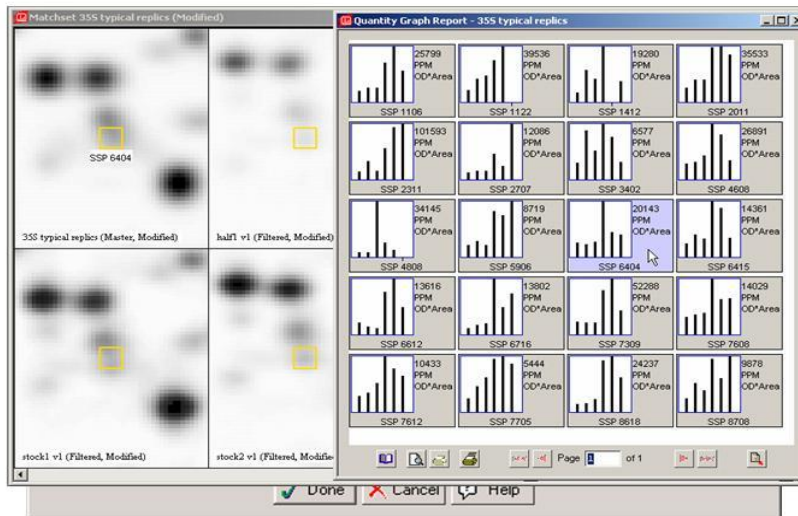
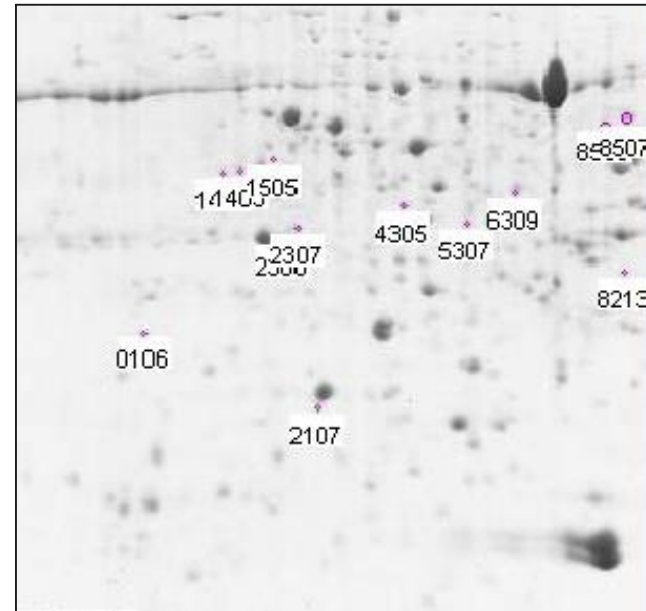


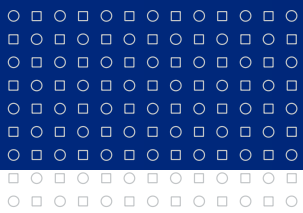
Sypro Ruby



ANALÝZA OBRAZU

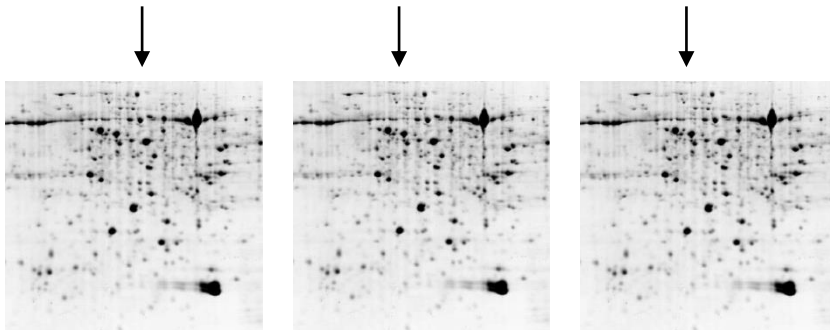
- kvalitativní
- kvantitativní





biologická variabilita

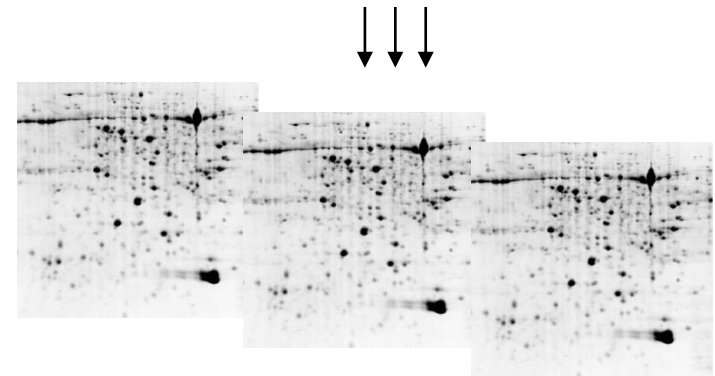
stejný organismus za stejných podmínek



biologické replikáty

technická variabilita

stejný vzorek stejně



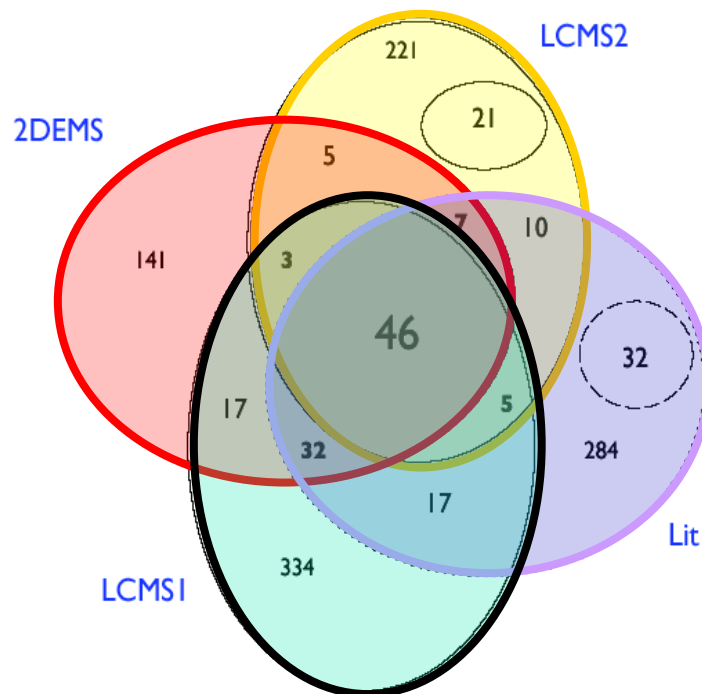
technické replikáty



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

MULTIDIMENZIONÁLNÍ CHROMATOGRRAFIE

PRO

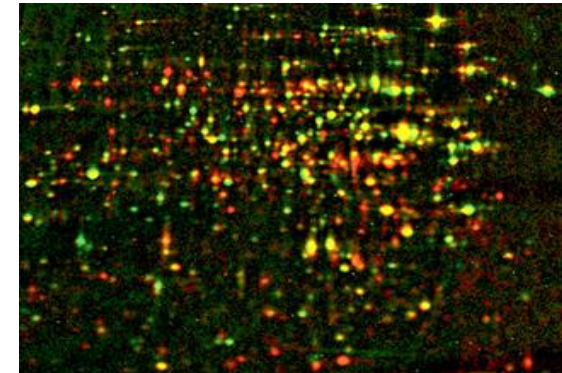
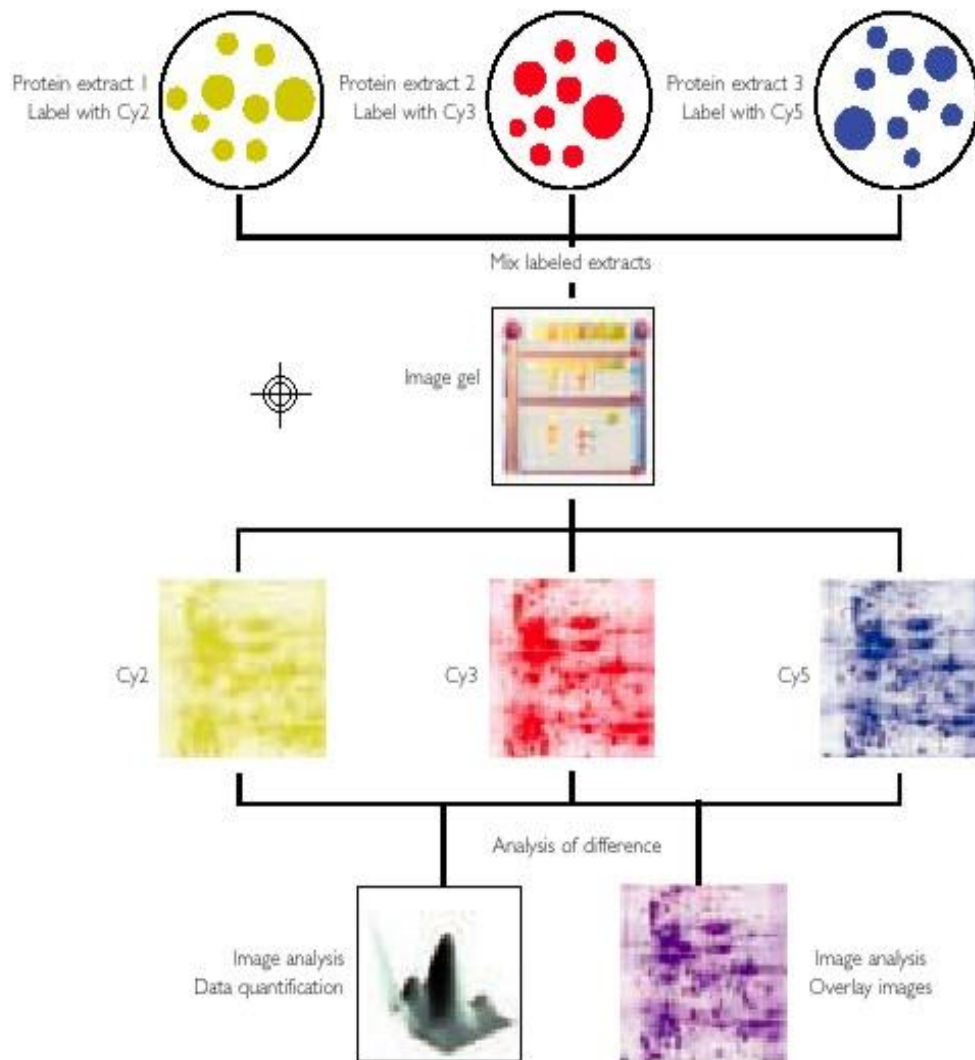
- velké objemy vzorku
- možnost koncentrace na koloně
- membránové proteiny, basické proteiny
- není nutno barvit
- peptidy – přímo na MS
- automatizace

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

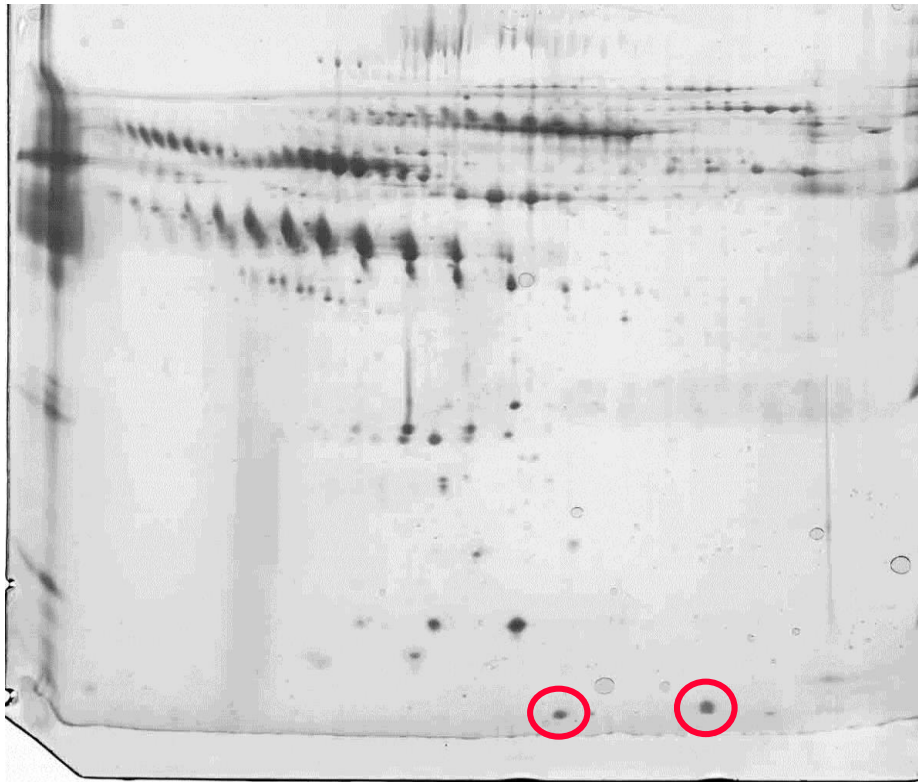
- **seno** - proteiny bez vztahu k onemocnění
- **jehly** - specifické proteiny pro onemocnění
- potenciální jehly **obtížně validovatelné**
- které jehly dále zkoumat?
- často **PTM**, neidentifikovány MS

biologická variabilita!

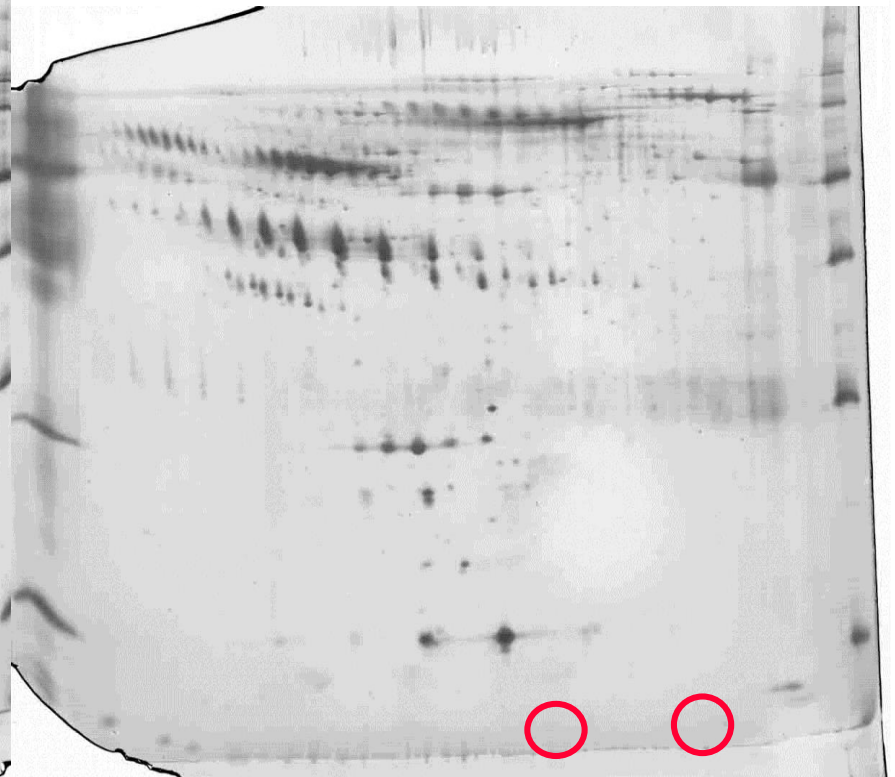


Biomarkery v lidské plasmě

Den 21 – před klinickým projevem



Den 44 – po klinickém projevu



separace



identifikace



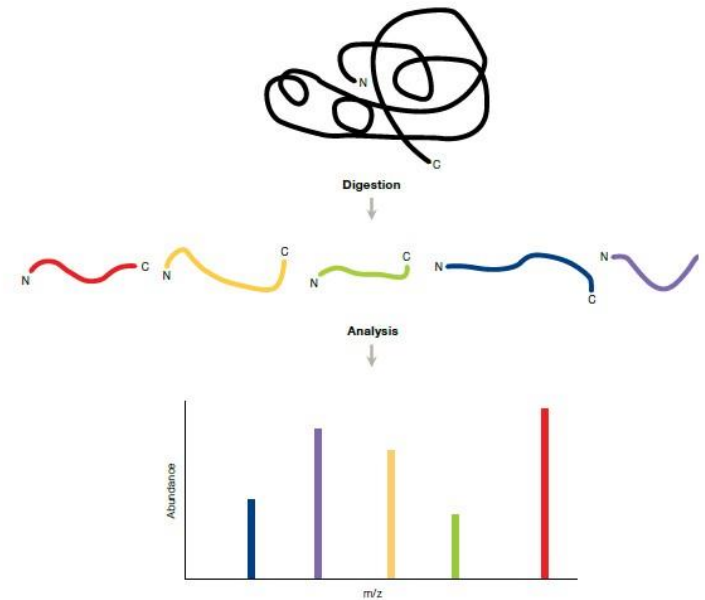
↓ **DIGESCE**

trypsin Glu-C Asp-N thermolysin

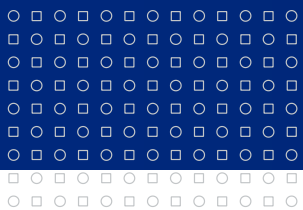
MAVEPFRRPITRPHASIEVDTS GTGG SAGSSE
KVFCLIGQAEGGEPNTVYELRNYAQAKRLFRS
GELLD AIELAWGSNP NYTAGRILAMRIEDAKP
ASAEIGGLKITSKIYGNVANNIQVGLEKNTLSD
SLRLRVIFQDDRFNEVYDNIGNIFTIKYKGEEA
NATFSVEHDEETQKASRLV LKVG DQEVKSYD
 LTGGAYDYTNAIITDINQLPDFEAKLSPFGDKN
 LESSKLDKIENANIKDKAVYVKAVFGDLEKQT
 AYNGIVSFEQLNAEGEVPSNVEVEAGEESATV
 TATSPIK**TIEPFELTKLKGGTNGEPPATWADKL**
DKFAHEGGYYIVPLSSKQSVHAEVASFVKERS
DAGEPMRAIVGGGFNESKEQLFGRQASLSNPR
VSLVANS GTFVMDDGRKNHVPAYMVAVALGG
 LASGLEIGESITFKPLRVSSLDQIYESIDLDELN
 ENGIISIEFVRNRTNTFFRIVDDVTTFNDKSDPV
KAEMAVGEANDFLVSELKVQLEDQFIGTRTIN
TSASI KDFIQSYLGRKKRDNEIQDFPAEDVQVI
VEGNEARISMTVYPIRSFKKISVSLVYKQQTLQ

A

- IN-GEL
- IN-SOLUTION



MS



G I G O



G I G O

GARBAGE IN - GARBAGE OUT

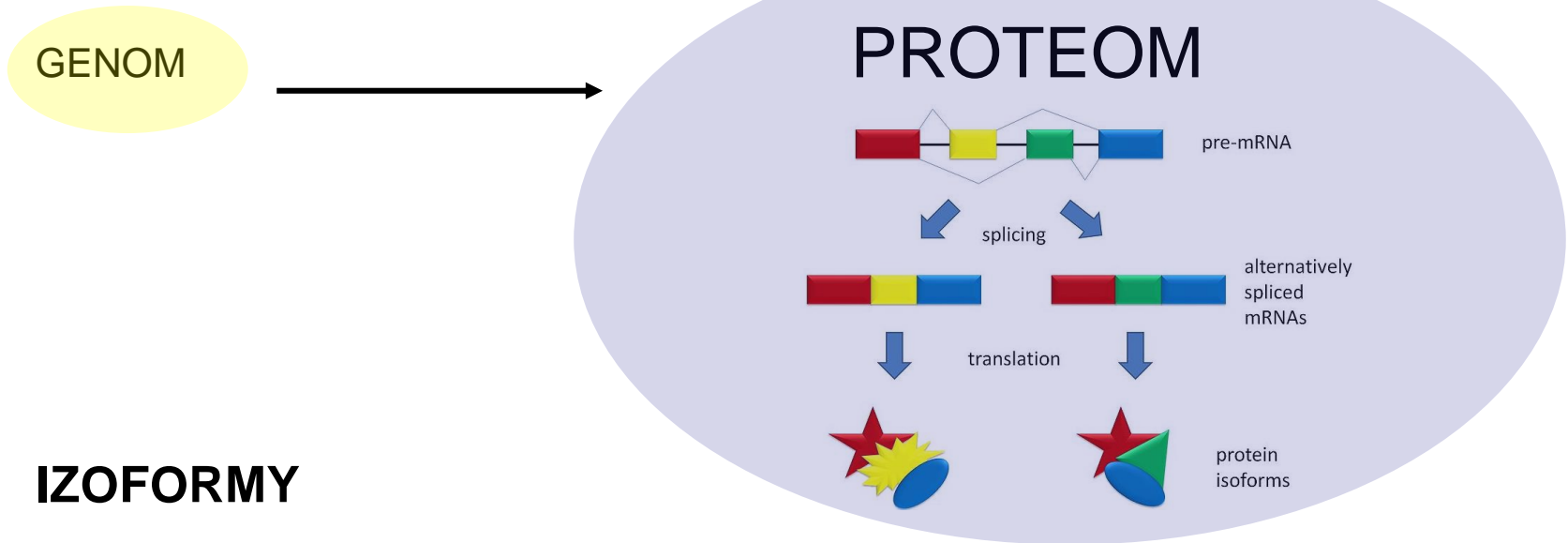


LITERATURA

- 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





IZOFORMY

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

KONCENTRAČNÍ ROZSAH asi deset řádů

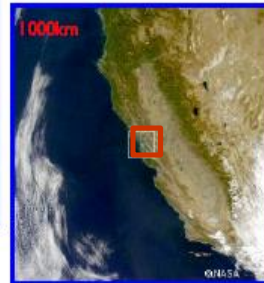
↓
PREFRAKCIONACE → MS



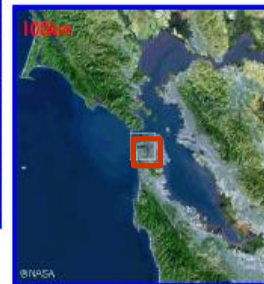
10^{10} Really Is Wide Dynamic Range



10 10 000km



9 1 000km



8 100km



7 10km



6 1km



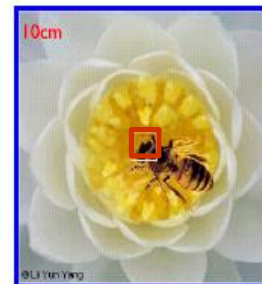
5 100m



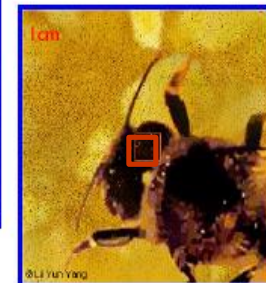
4 10m



3 1m

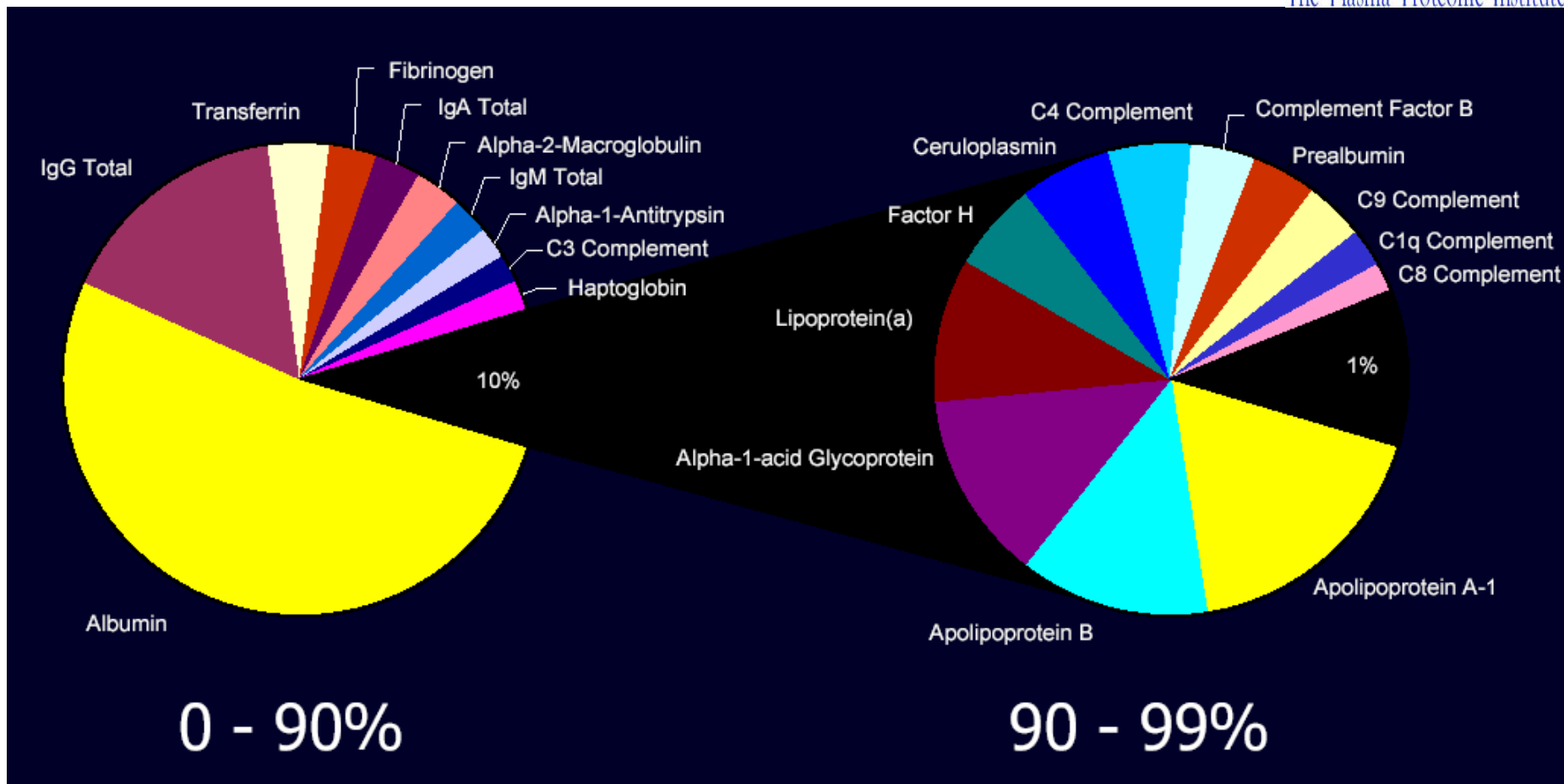
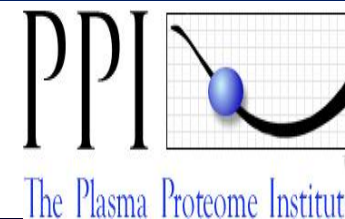


2 10cm



1 1cm

Abundantní proteiny v lidské plazmě

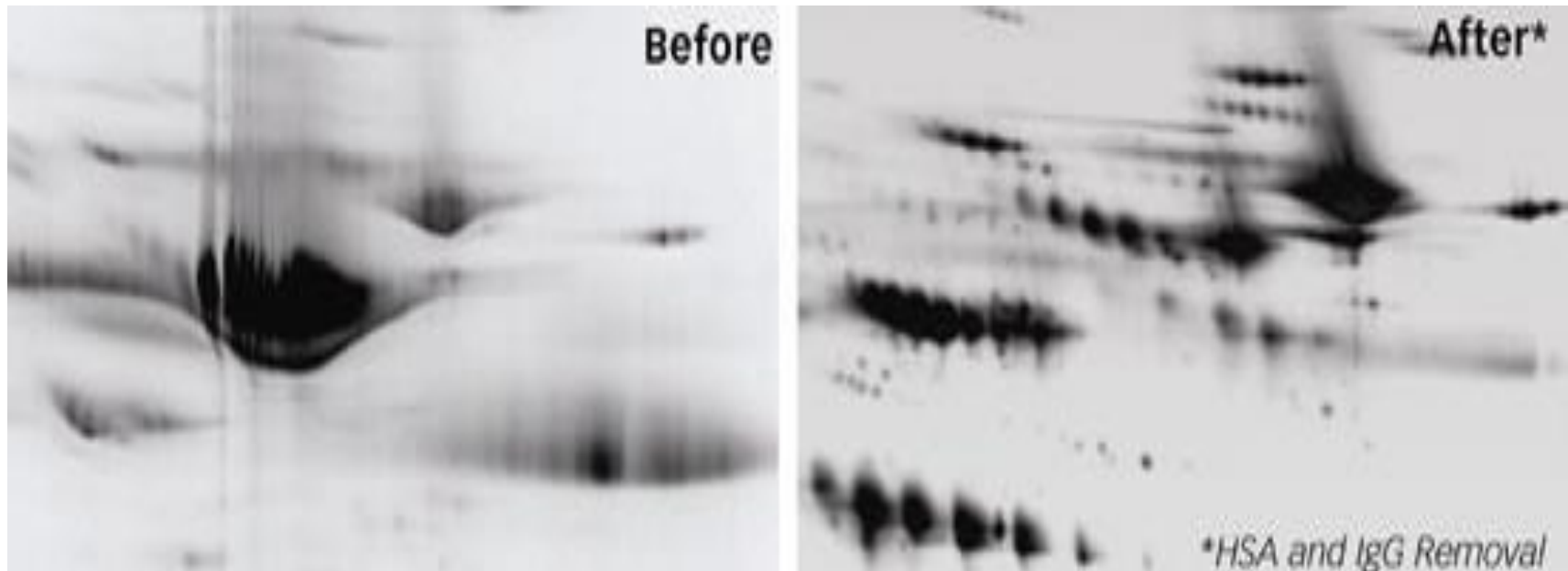


AFINITNÍ DEPLECE

odstranění abundantních proteinů afinitní chromatografií

HSA

IgG



Lidská plazma - vázaná frakce po afinitní depleci

ALBUMIN

IgG

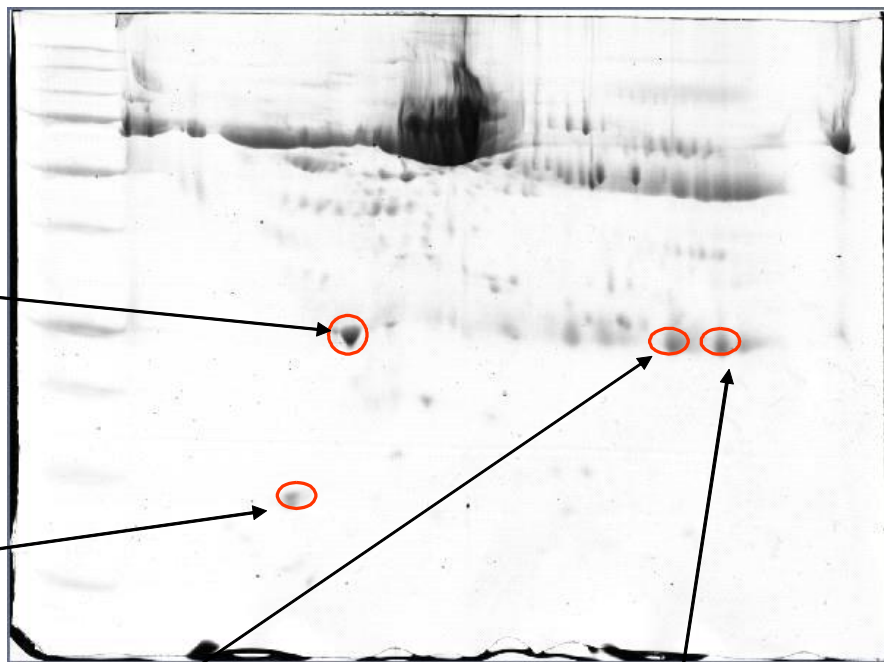
Barvení: CB G-250

Apolipoprotein

albumin

Immunoglobulin kappa light chain

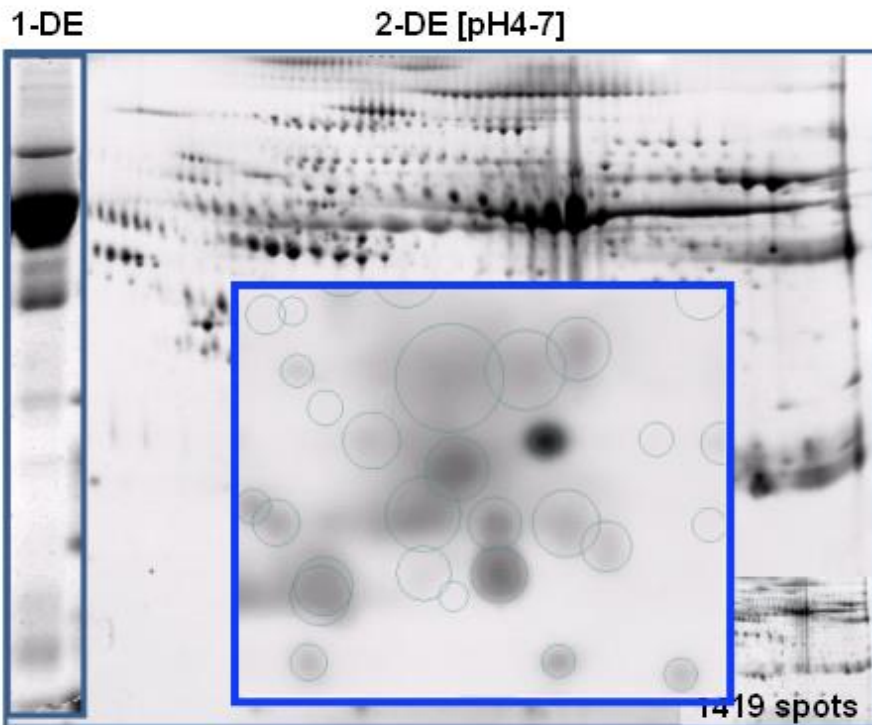
Immunoglobulin light chain



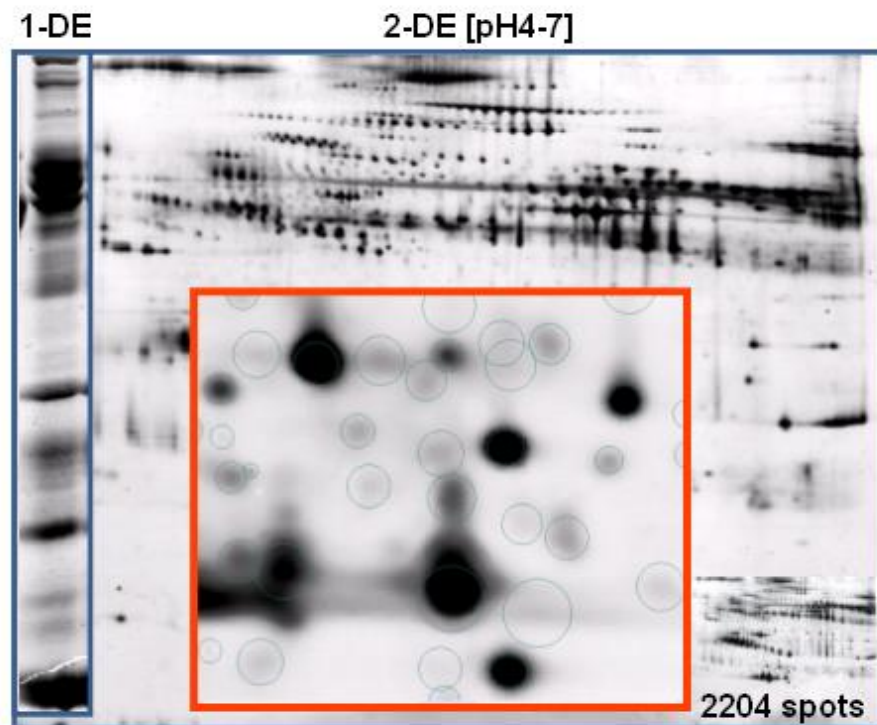
CPPL Combinatorial Peptide Ligand Library



Native Human Serum

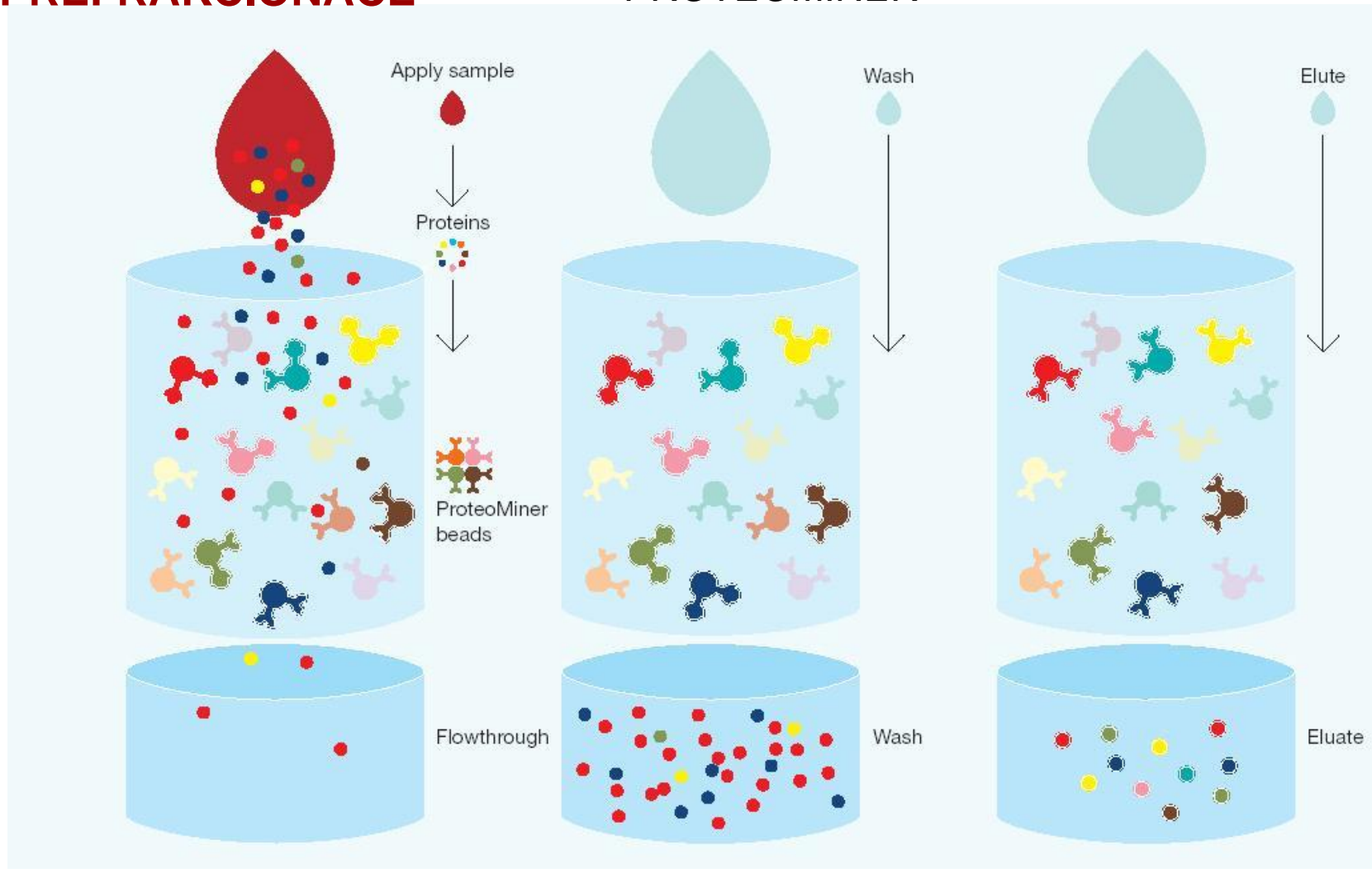


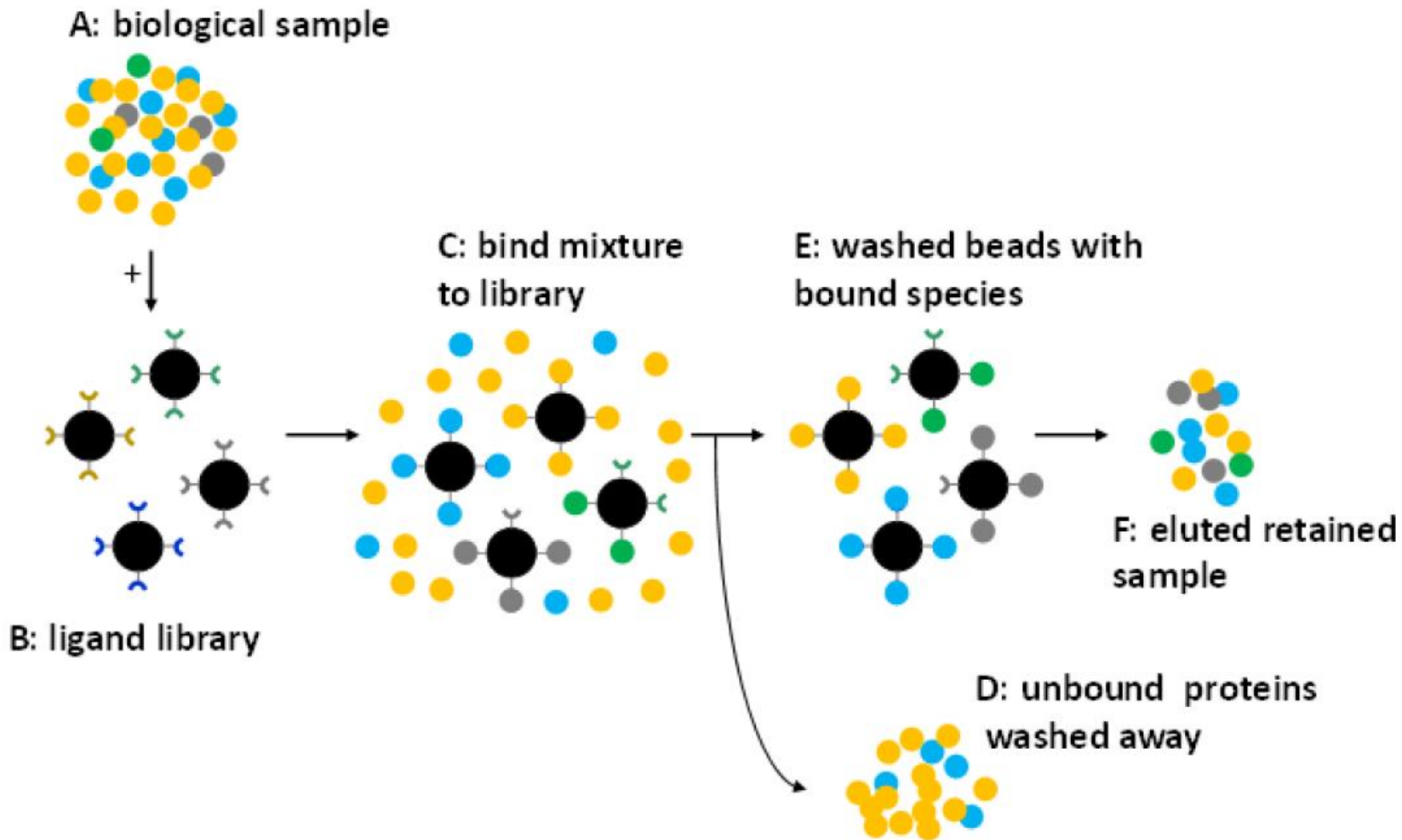
Human Serum Fractionated by ProteoMiner

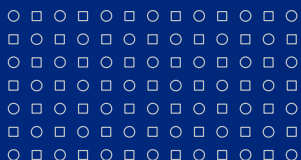


PREFRAKCIONACE

PROTEOMINER







IEF PREFRAKCIONACE



MicroRotor

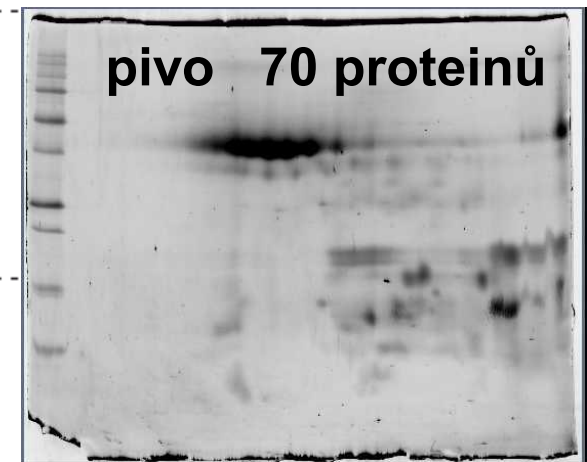
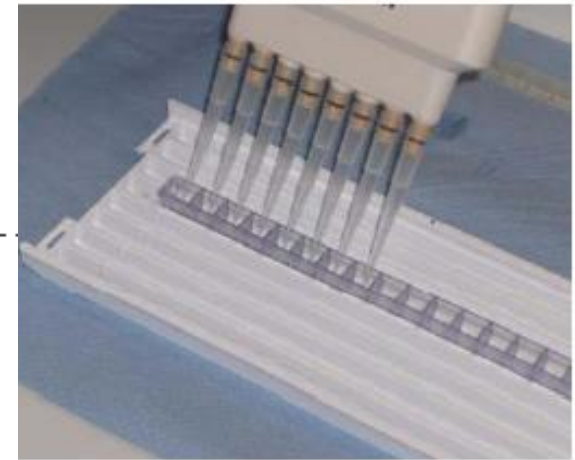
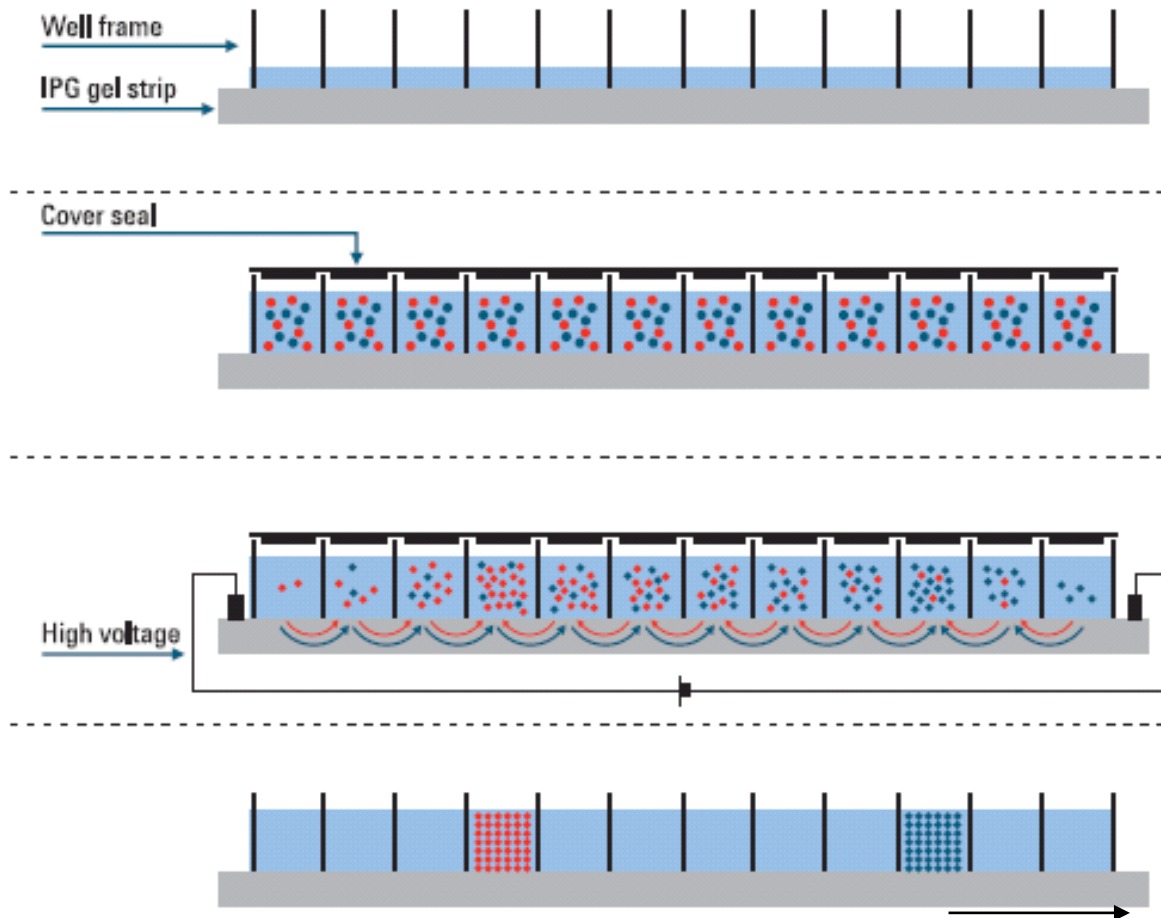
- prefrakcionace v roztoku

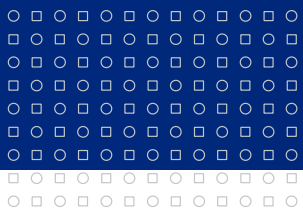


OffGel Fractionator

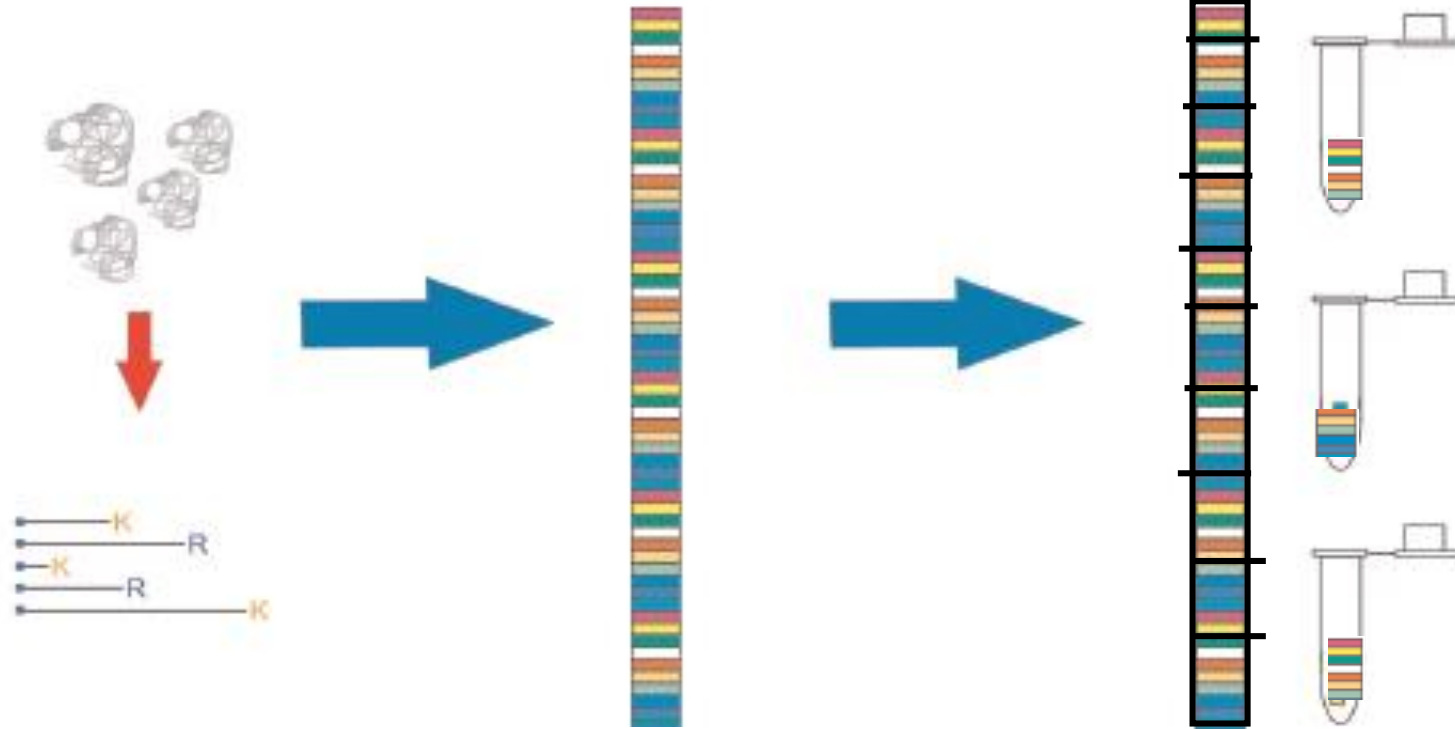
- prefrakcionace v roztoku na IPG stripu

OFFGEL IEF prefrakcionace proteinů nebo peptidů





IPG-IEF

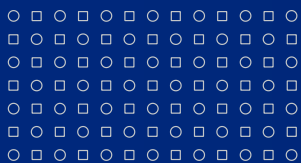


digest směsi
proteinů

of

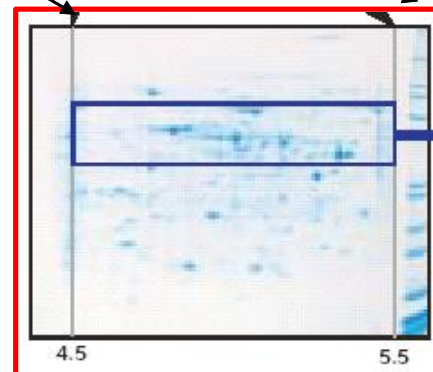
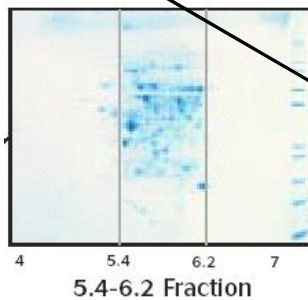
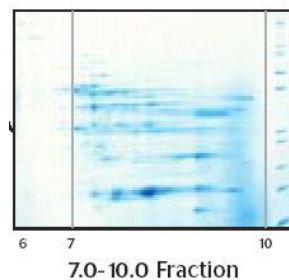
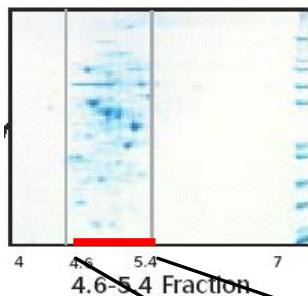
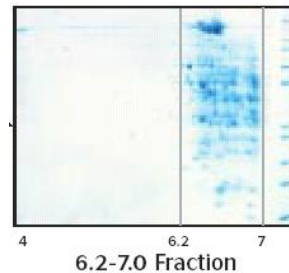
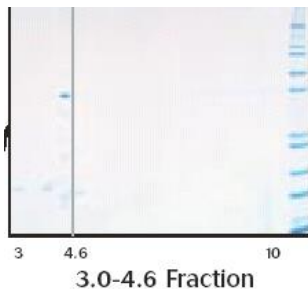
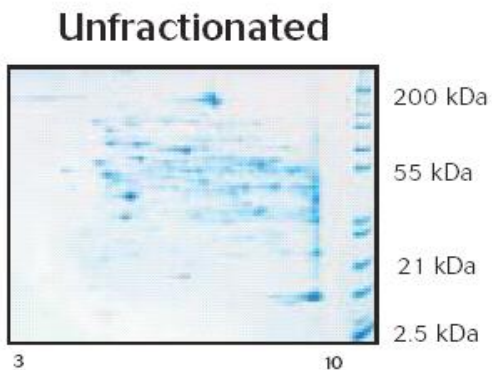
IEF směsi peptidů
na IPG stripu

frakce stripu

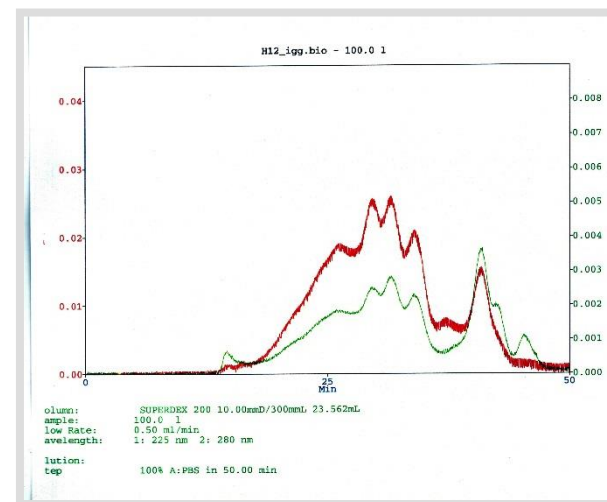
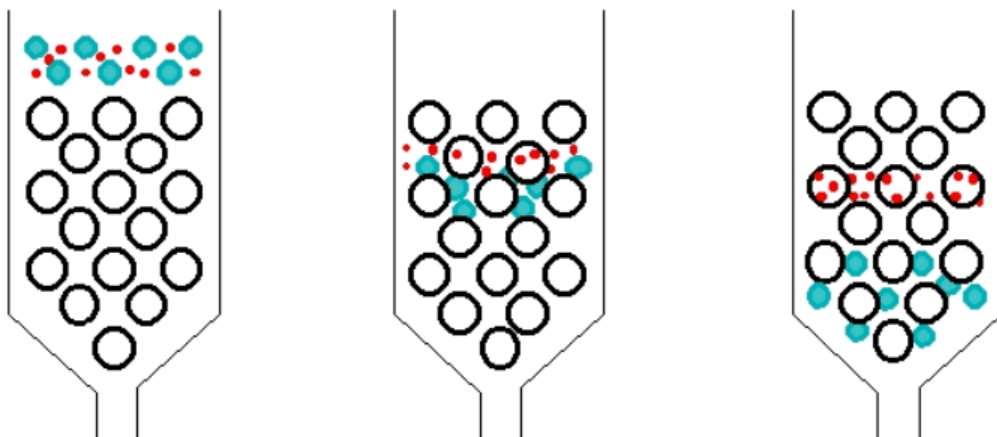
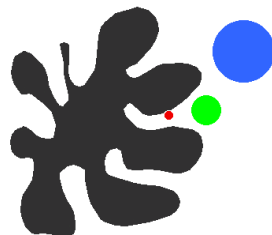


PREFRAKCIONACE MIKRO ROZSAHY

pl



GELOVÁ CHROMATOGRRAFIE



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

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

