



MASARYKOVA UNIVERZITA

Dvoudimenzionální elektroforéza

Hana Konečná

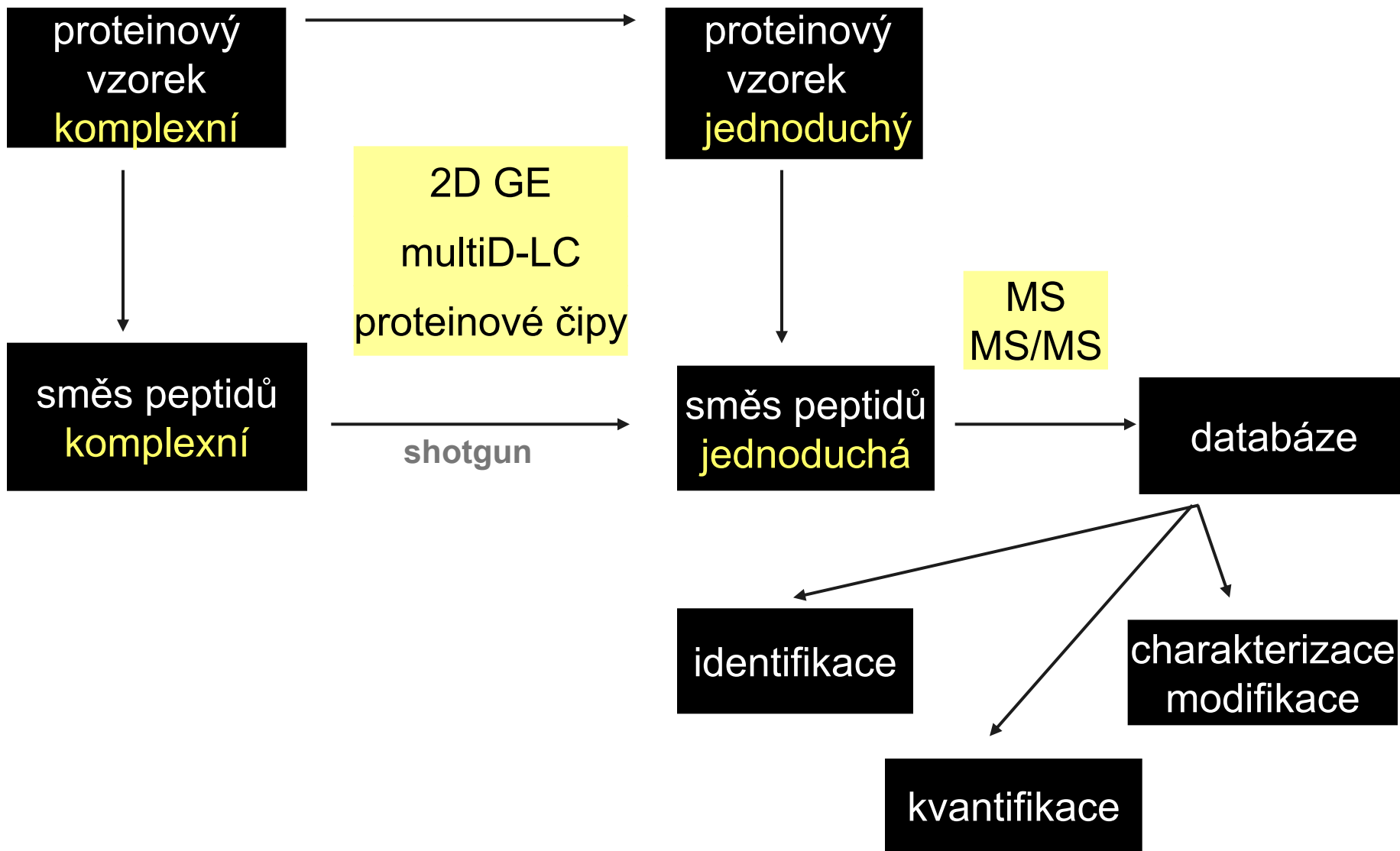
CENTRÁLNÍ LABORATOŘ - PROTEOMIKA

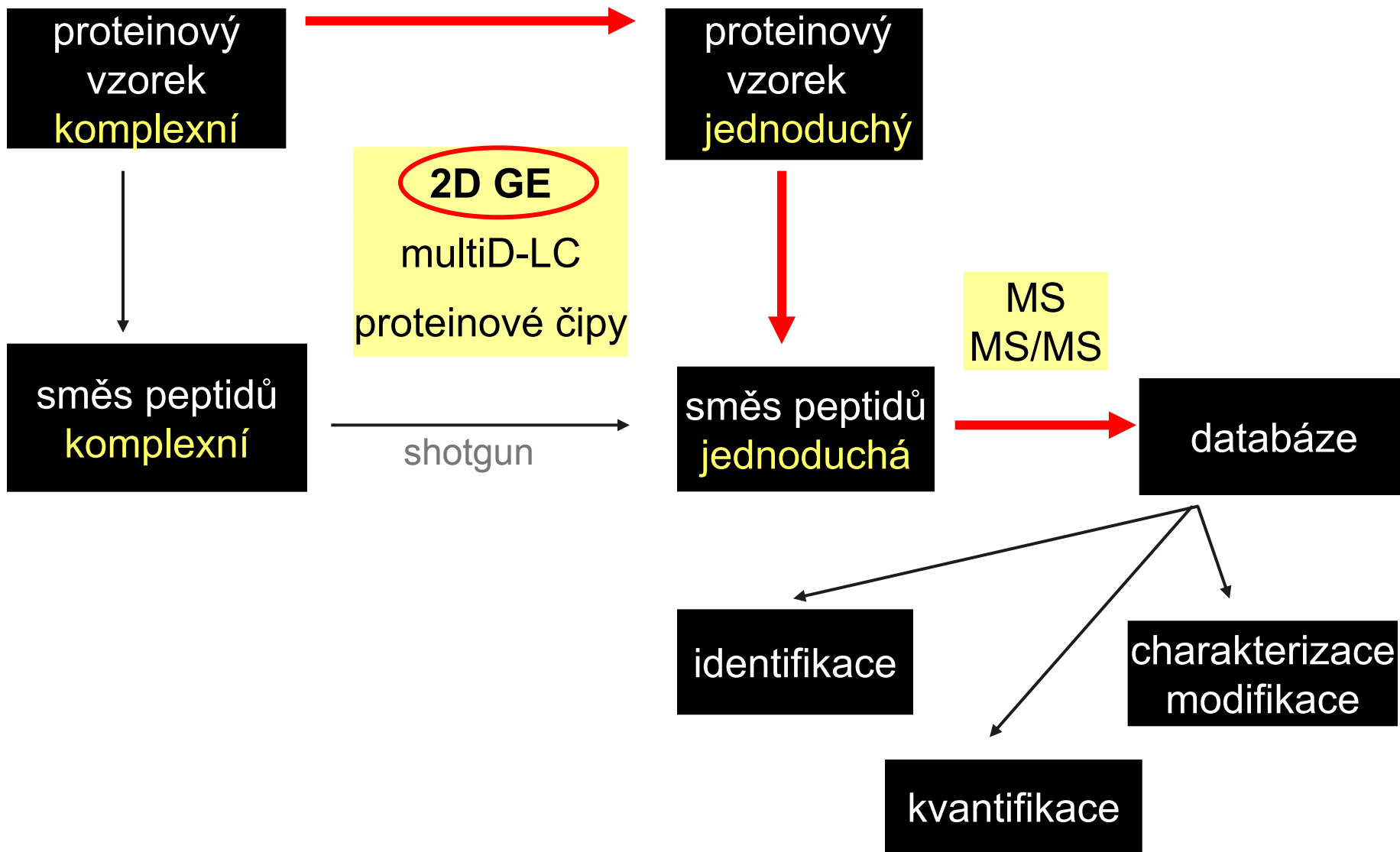
CEITEC – Středoevropský technologický institut

NCBR – Národní centrum pro výzkum biomolekul PŘF



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

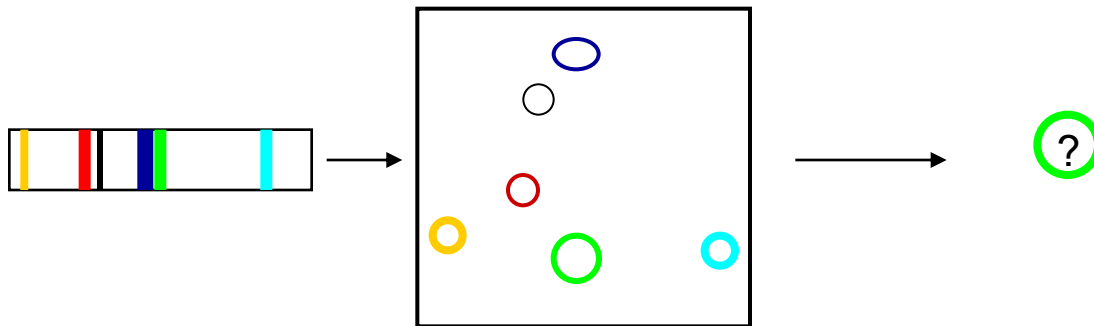




I. SEPARACE

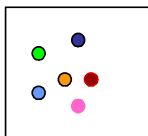
II. PREFRAKCIONACE

Dvoudimenzionální elektroforéza 2-DE



Proteomický experiment

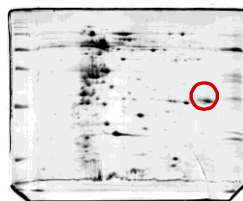
extrakce



fokusace



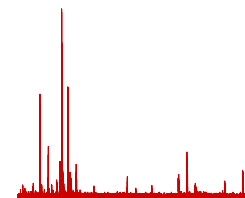
SDS-PAGE



digest



MS

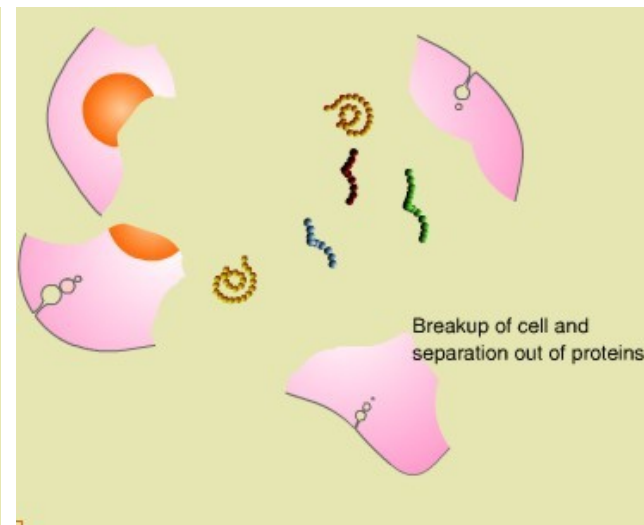
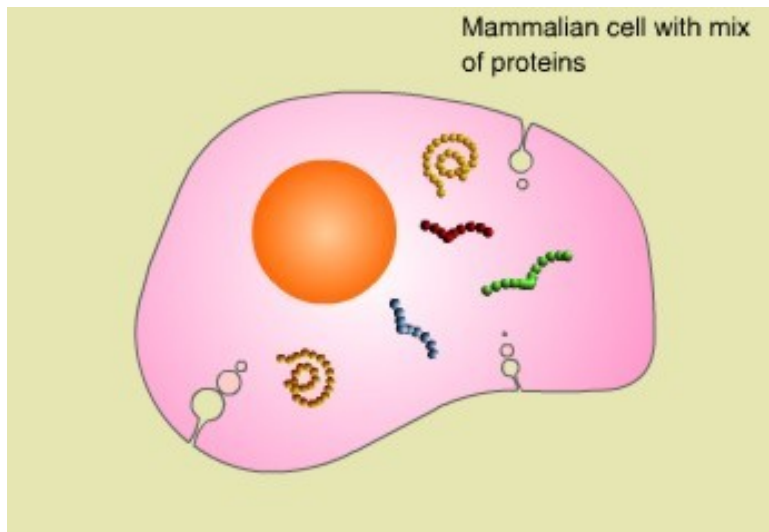


identifikace

neznámý protein

HOMOGENIZACE

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



PŘÍPRAVA VZORKU

solubilizace močovina, thiomočovina, detergenty

redukce DTT, TBP

inhibice proteáz, fosfatáz, glykosyláz

odstranění kontaminant

MĚŘENÍ KONCENTRACE PROTEINU

DETERGENTY

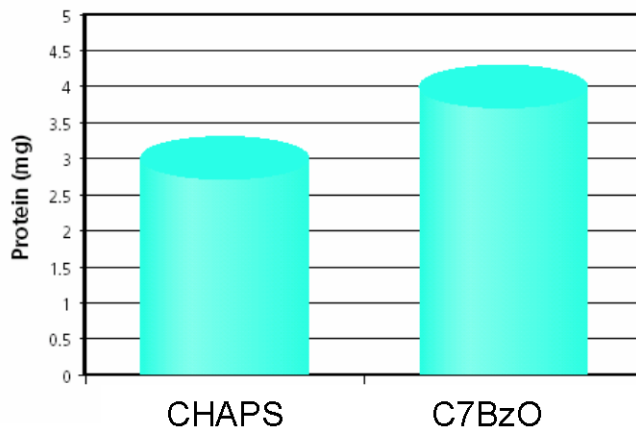
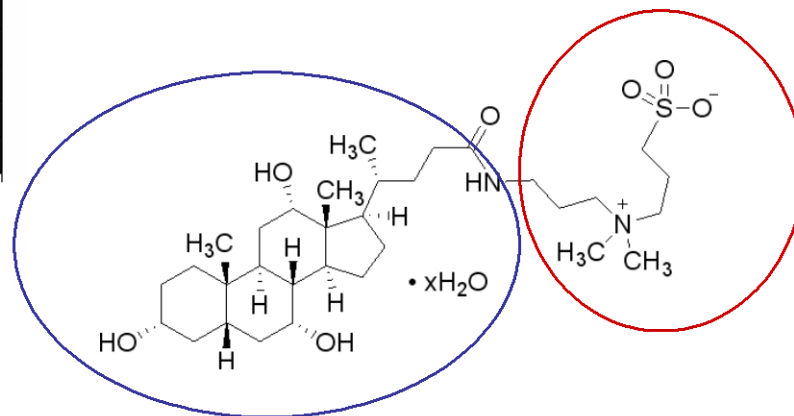
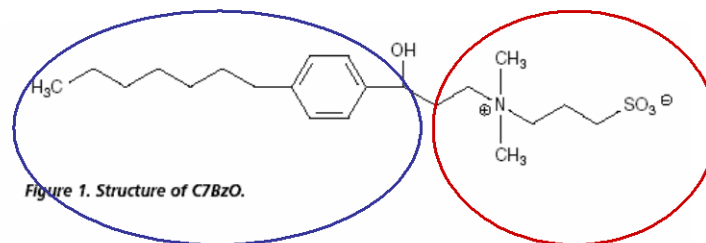
- žádný celkový náboj
- 0.5 - 4%
- použitelné ve vysokých koncentracích močoviny
- neionogenní
- zwitterointové
- SDS jen v nízkých koncentracích (do 0.25%)

C7BzO

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

CHAPS

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

**E.coli**

ZÁKLADNÍ PRAVIDLA

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

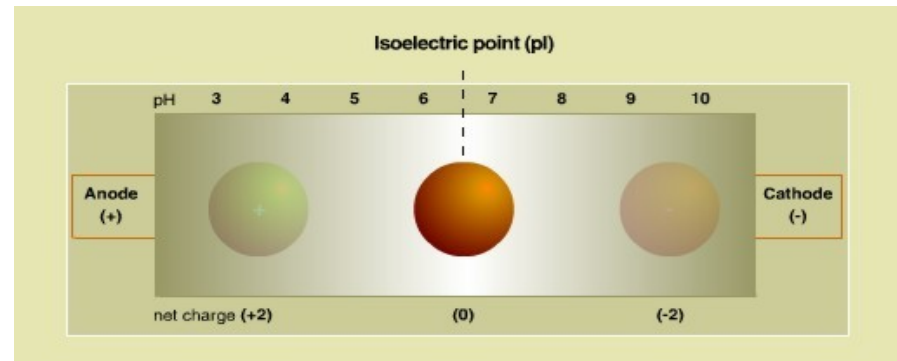
KONTAMINANTY

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

2-DE

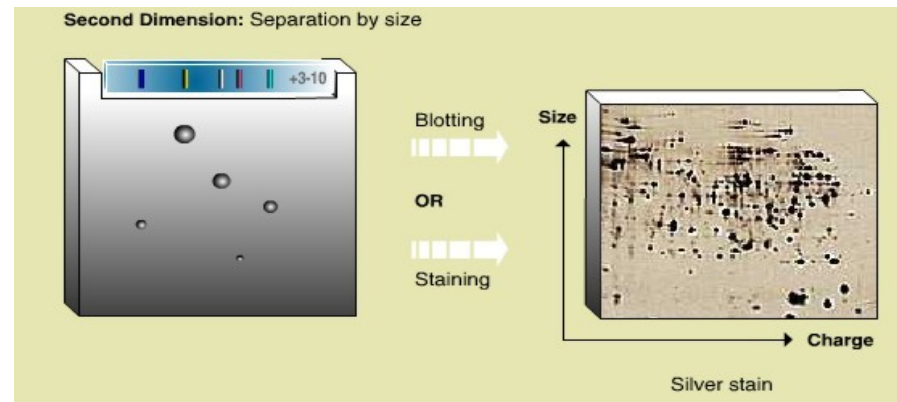
- první rozměr

IEF



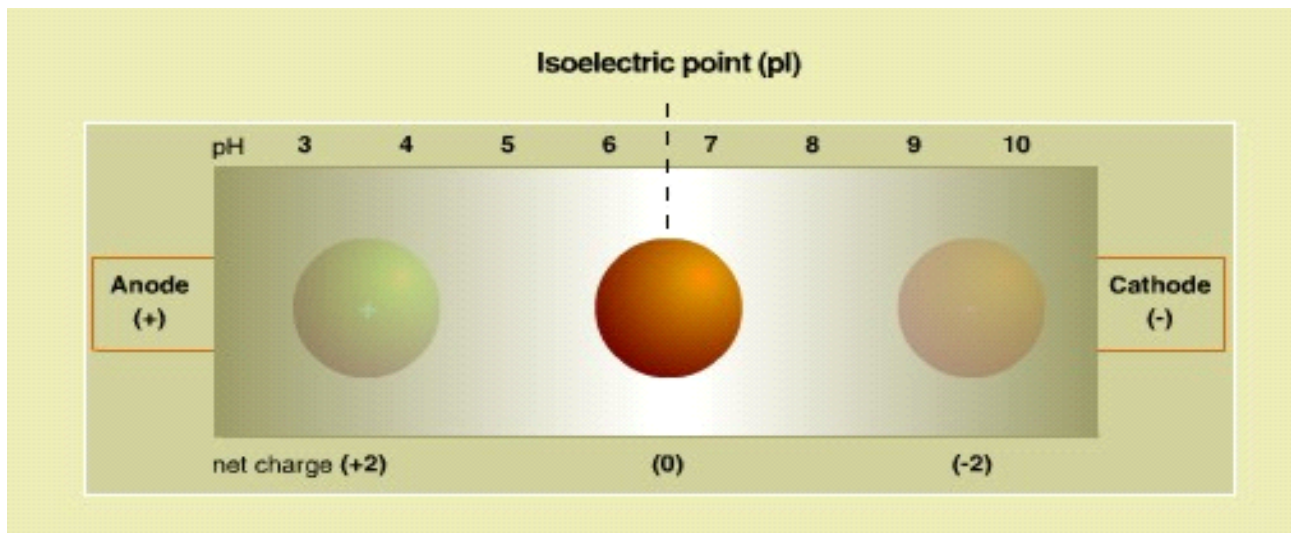
- druhý rozměr

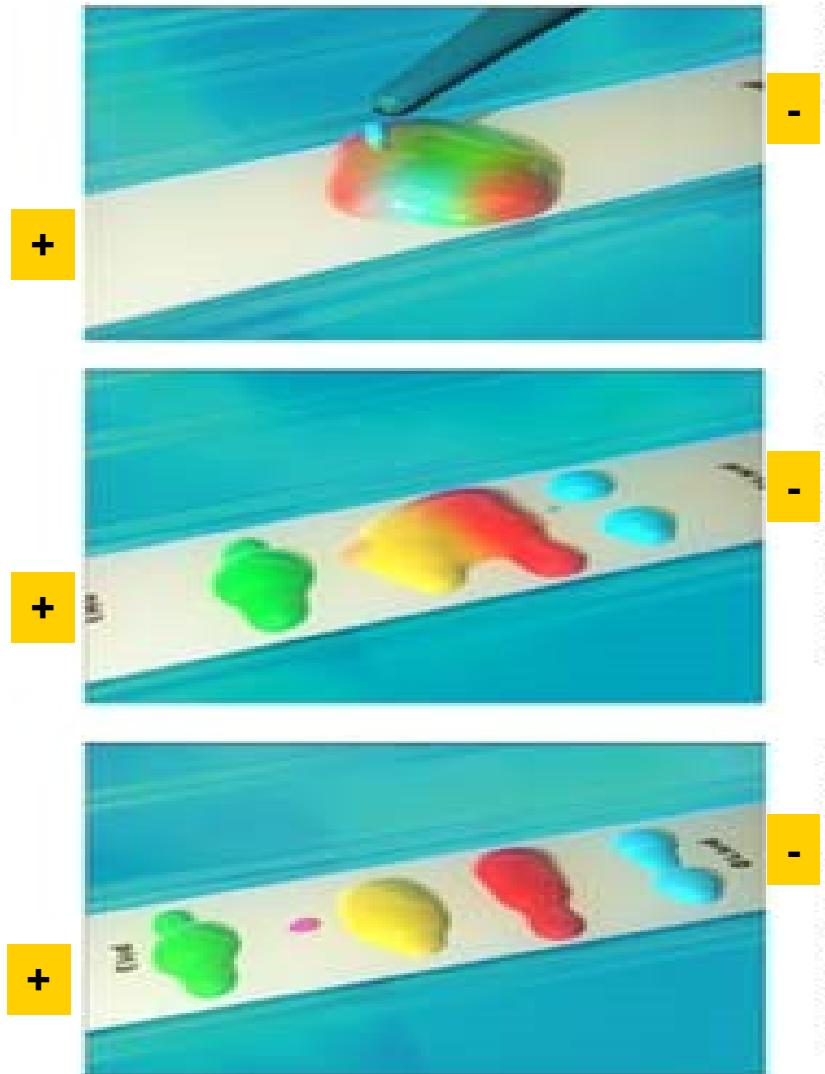
SDS-PAGE



1. ROZMĚR **IZOELEKTRICKÁ FOKUSACE**

migrace nabitých částic v gradientu pH v elektrickém poli

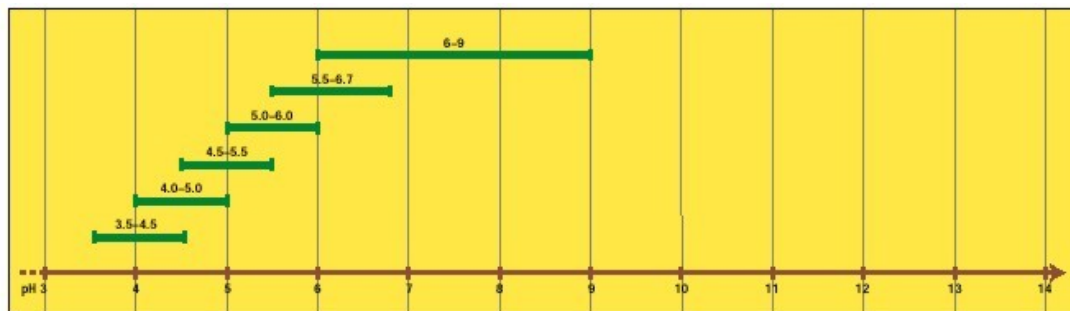
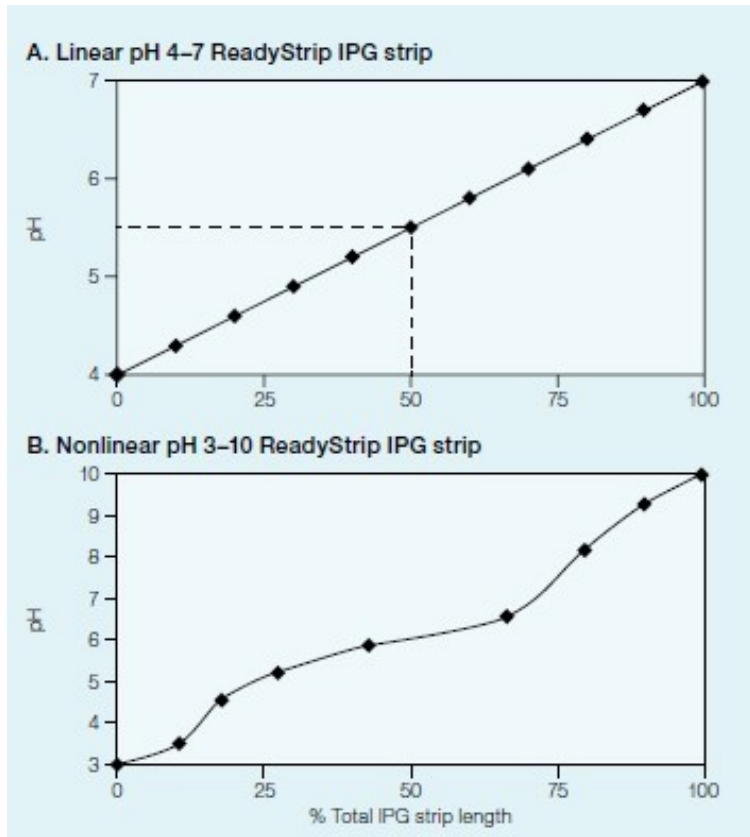




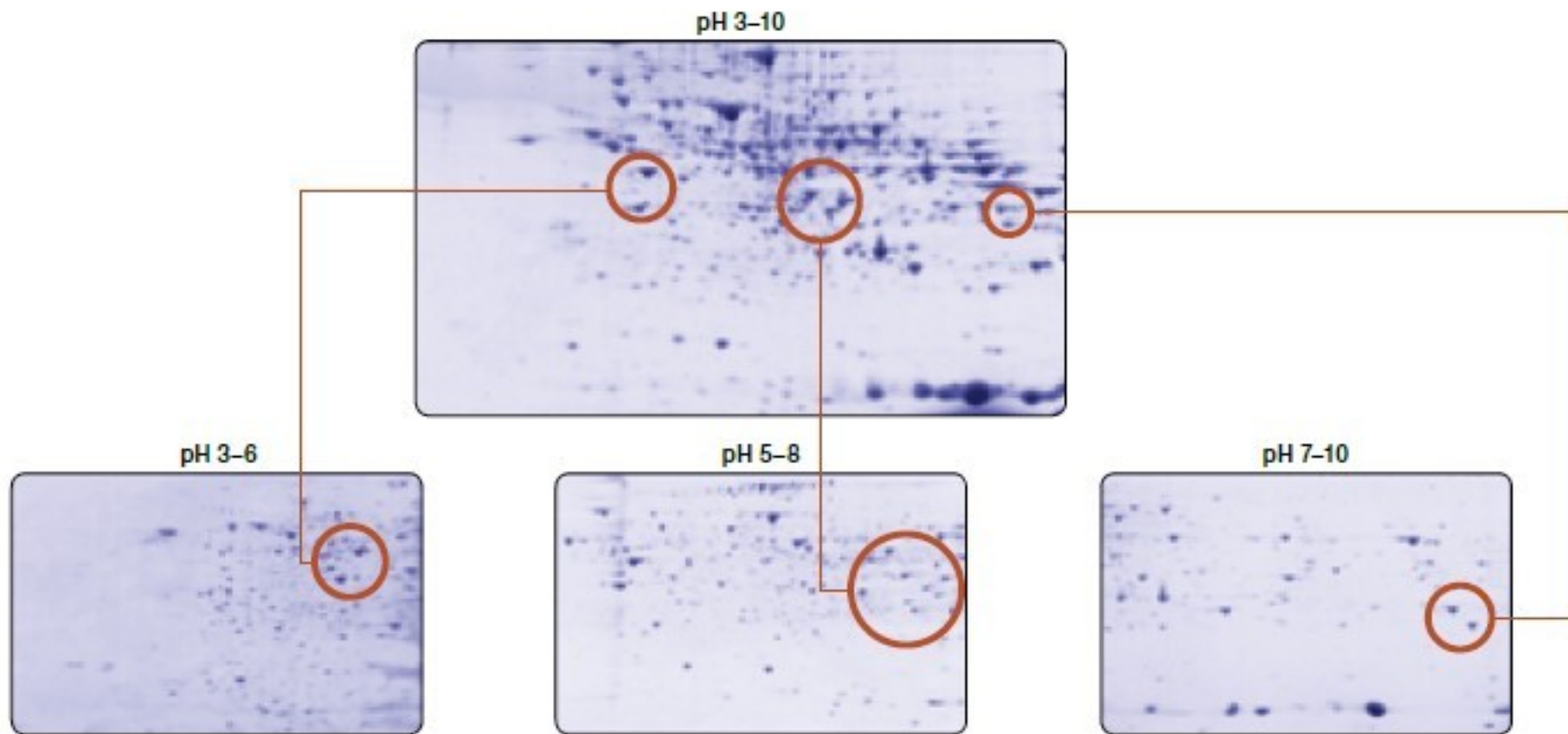
IZOELEKTRICKÁ FOKUSACE

- imobilizovaný pH gradient
- amfolyty

ROZSAH STRIPU ROZMĚR STRIPU

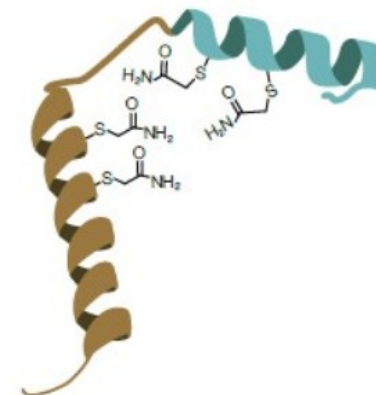
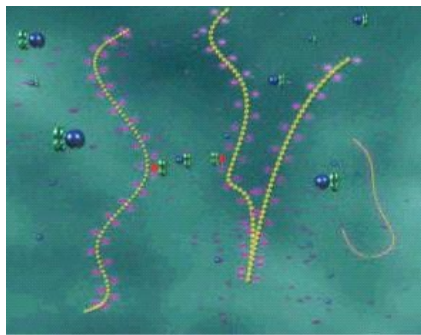
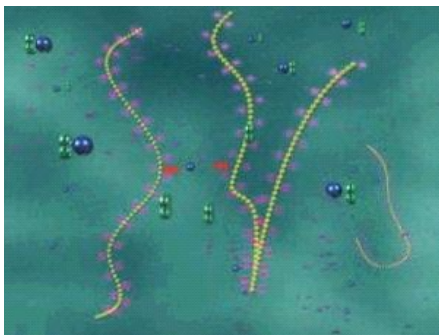
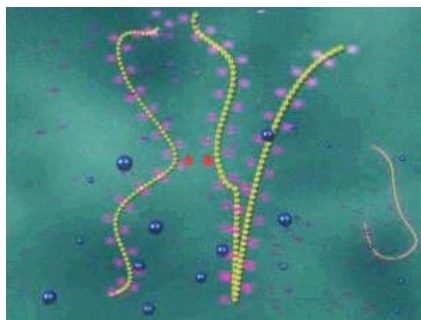
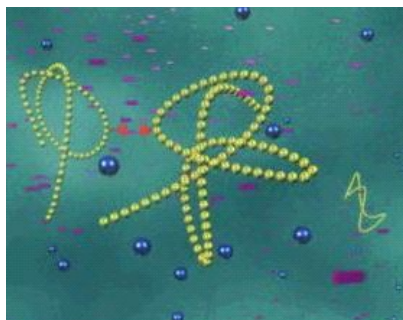


ROZSAH STRIPU



EKVILIBRACE STRIPU

denaturace SDS ●



redukce

DTT ●

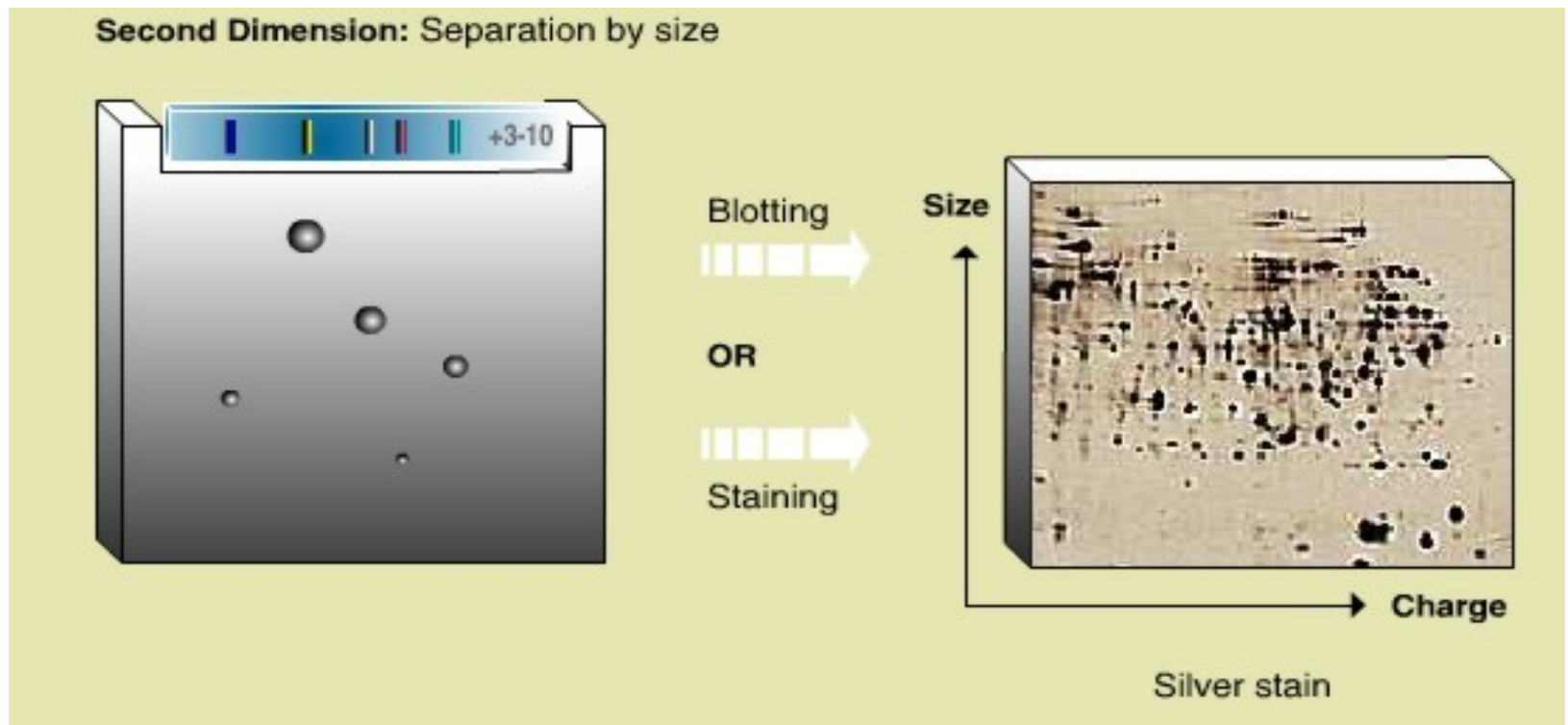
alkylace

IAA ●



2. ROZMĚR **SDS-PAGE**

migrace aniontů v elektrickém poli podle MW





STRIP

ekvilibrace



GEL

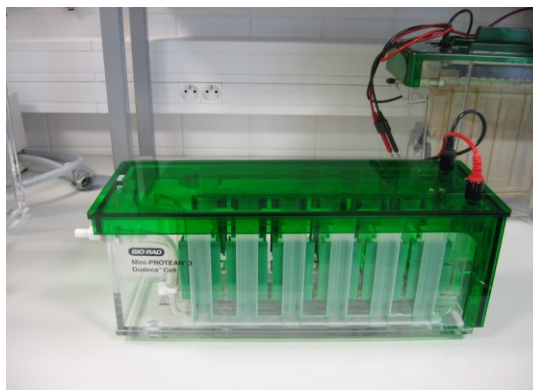


2-DE INSTRUMENTACE

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



Protean Plus Dodeca Cell



Mini-Protean 3 Dodeca Cell

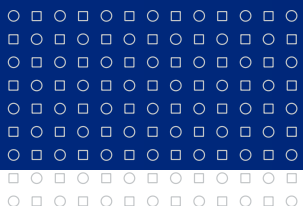


Protean II xi Cell

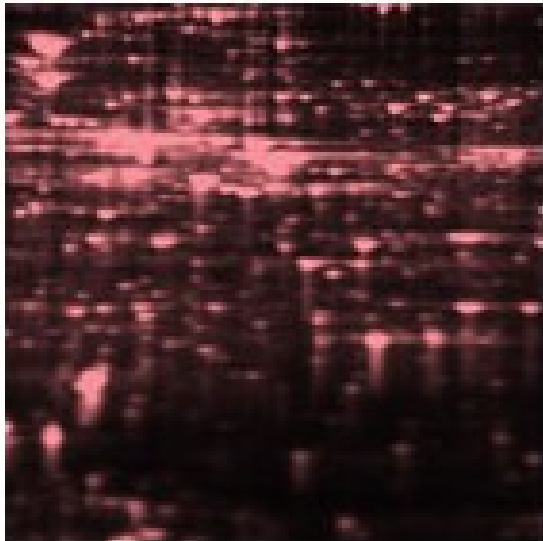


DETEKCE PROTEINU

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

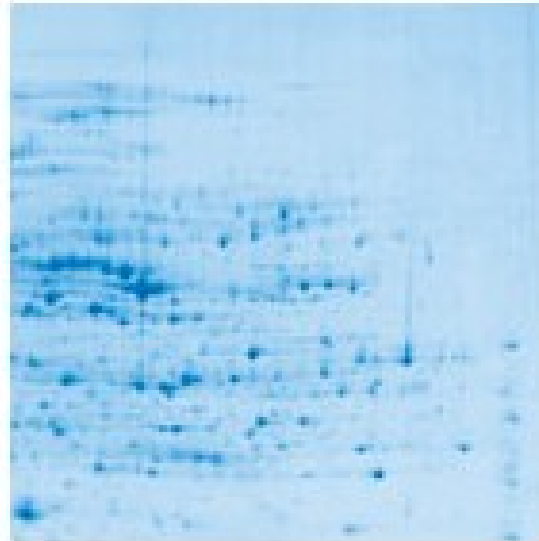


DETEKCE PROTEINU V GELU



Sypro Ruby

1.4 ng



Coomassie

36 ng



silver

0.6 ng

PTM specifická barvení

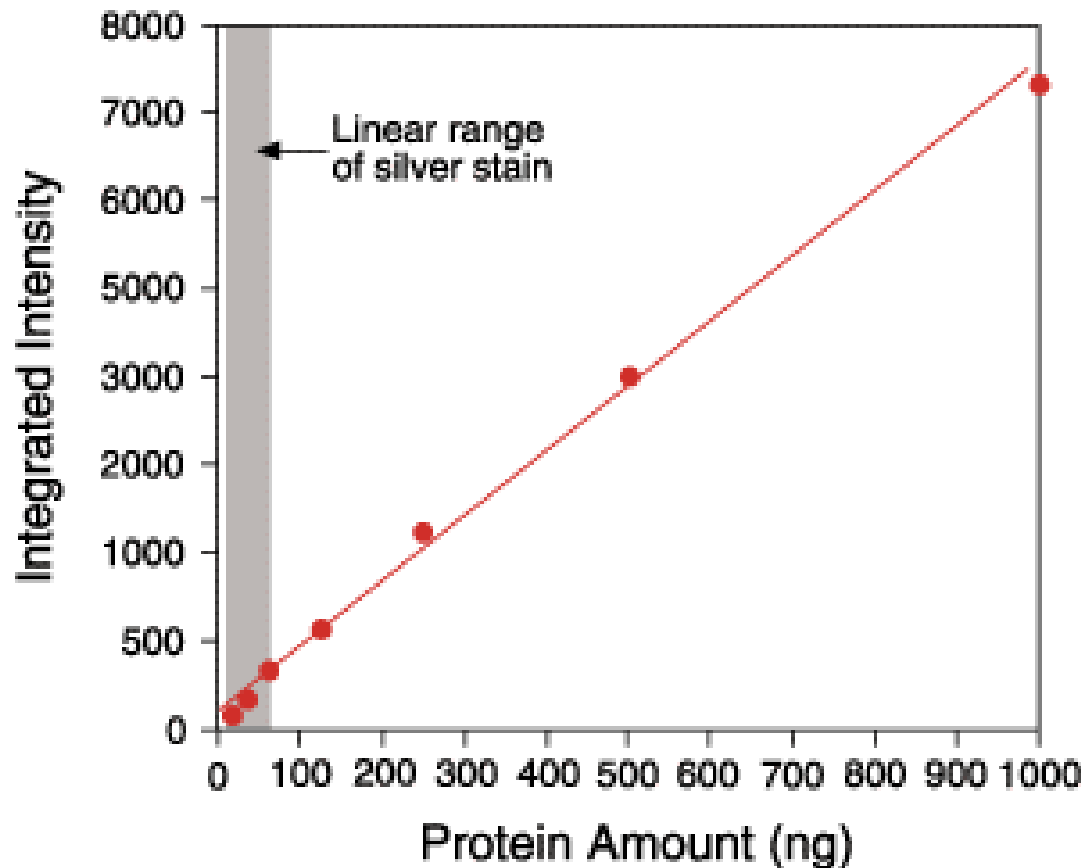
Pro-Q Diamond

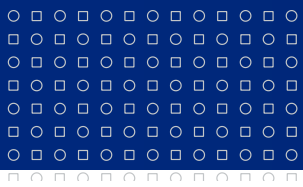
Pro-Q Emerald



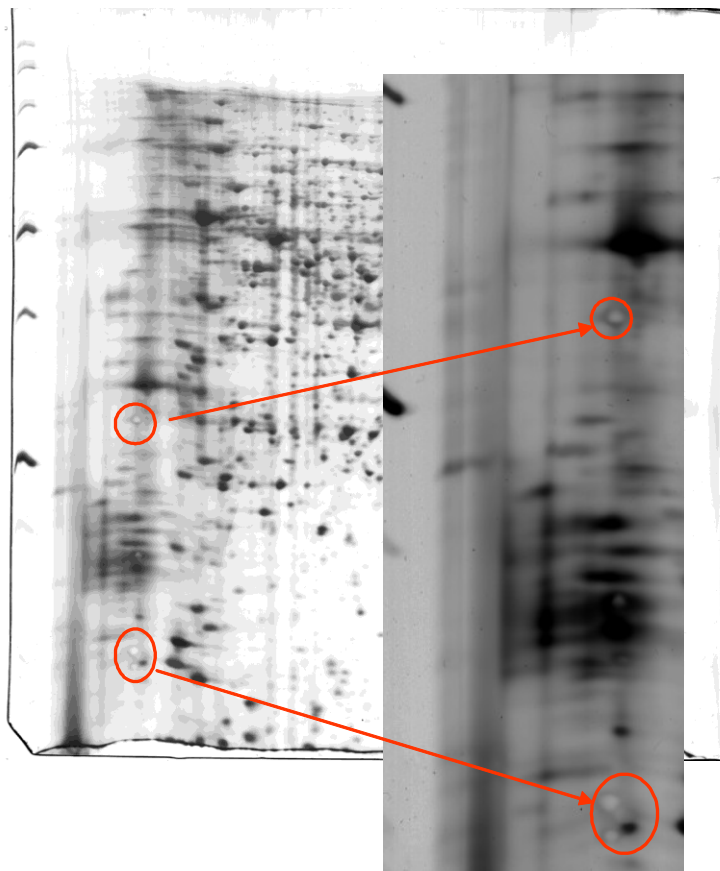
BARVENÍ PROTEINU – LINEARITA

Sypro Ruby

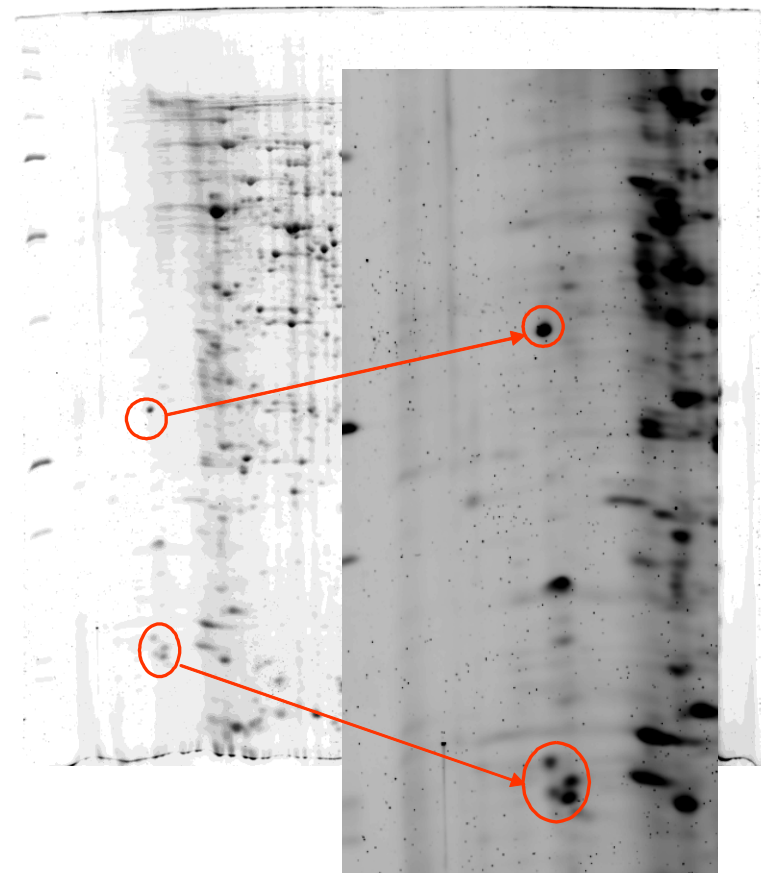




Ag

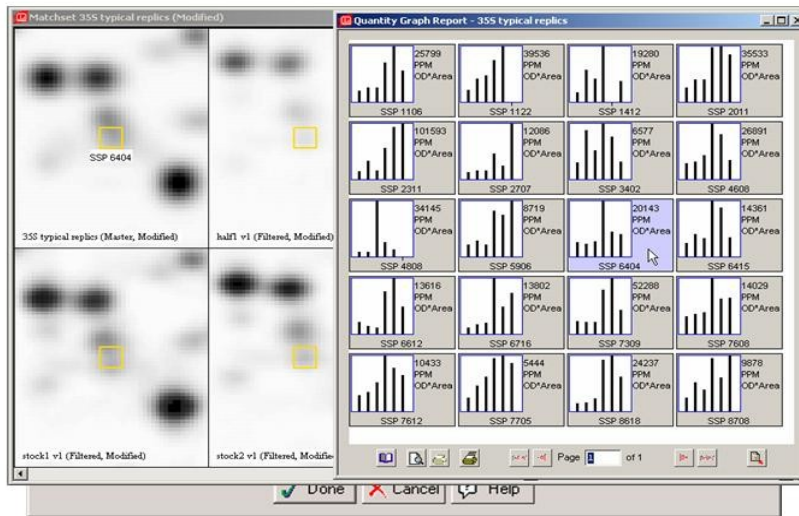
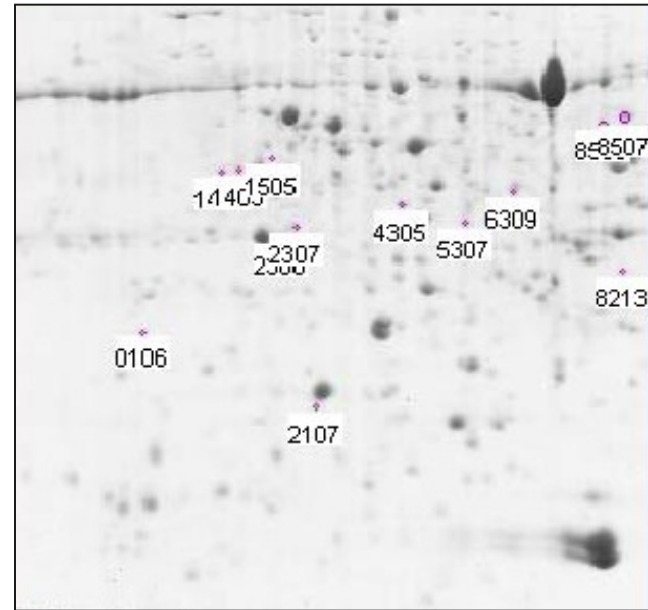


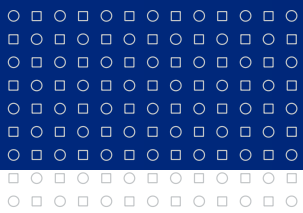
Sypro Ruby



ANALÝZA OBRAZU

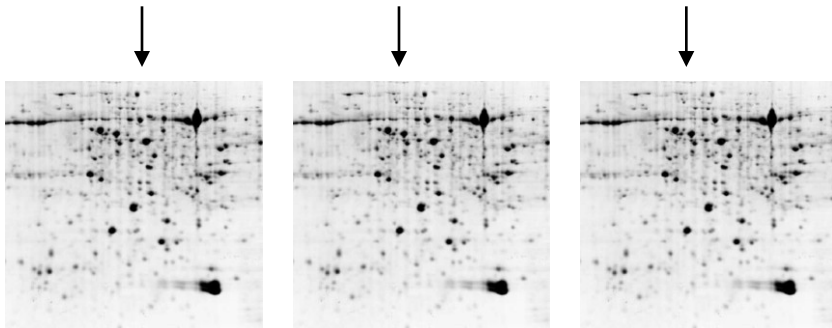
- kvalitativní
- kvantitativní





biologická variabilita

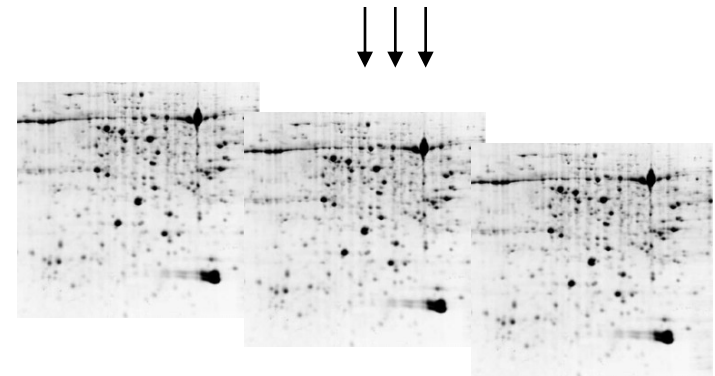
stejný organismus za stejných podmínek



biologické replikáty

technická variabilita

stejný vzorek stejně



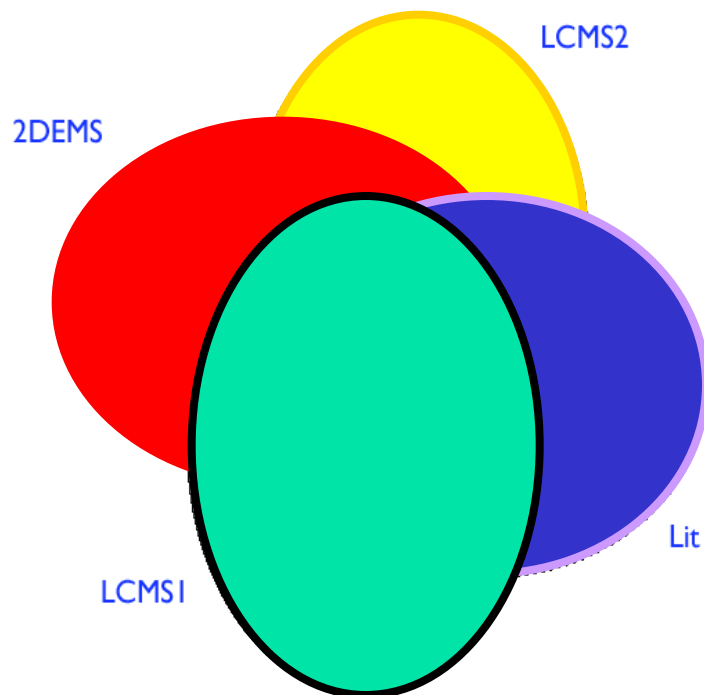
technické replikáty



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: pl a Mr
- LC - sériová analýza
- GE současně pro více vzorků

BIOMARKERY

... jehly v kupce sena

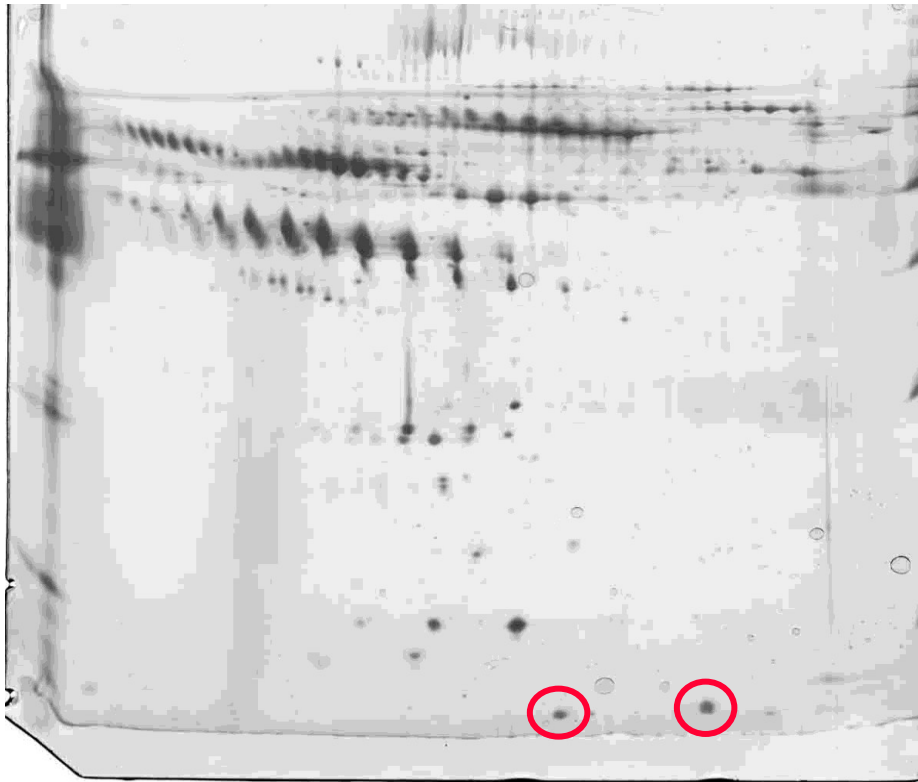
prefrakcionace separace identifikace srovnání kontrola vs.vzorek

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

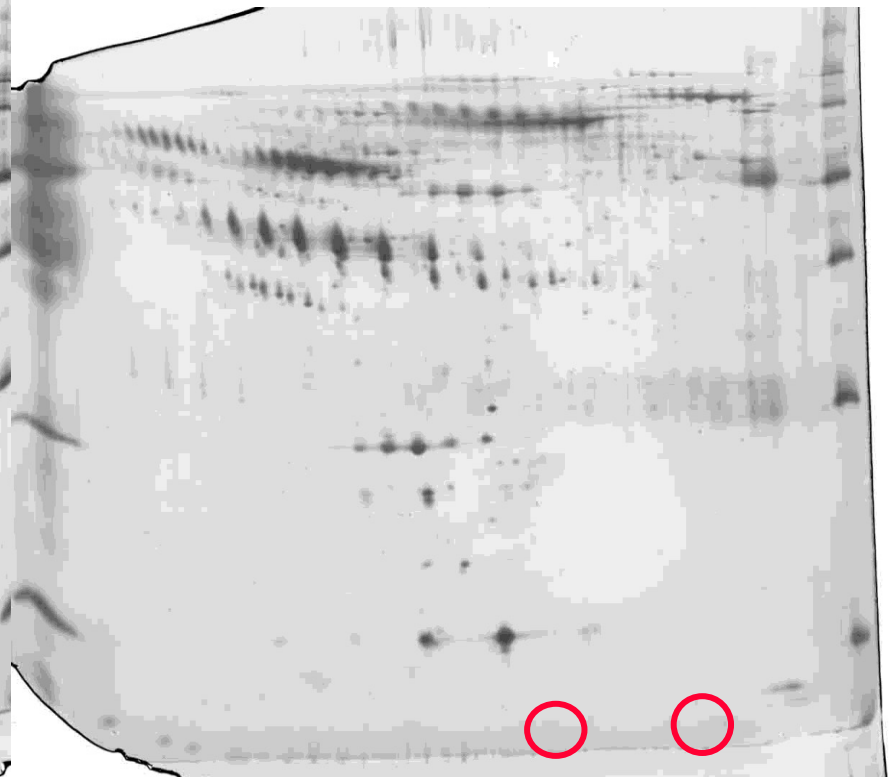


Biomarkery v lidské plasmě

Den 21 – před klinickým projevem



Den 44 – po klinickém projevu



separace



identifikace

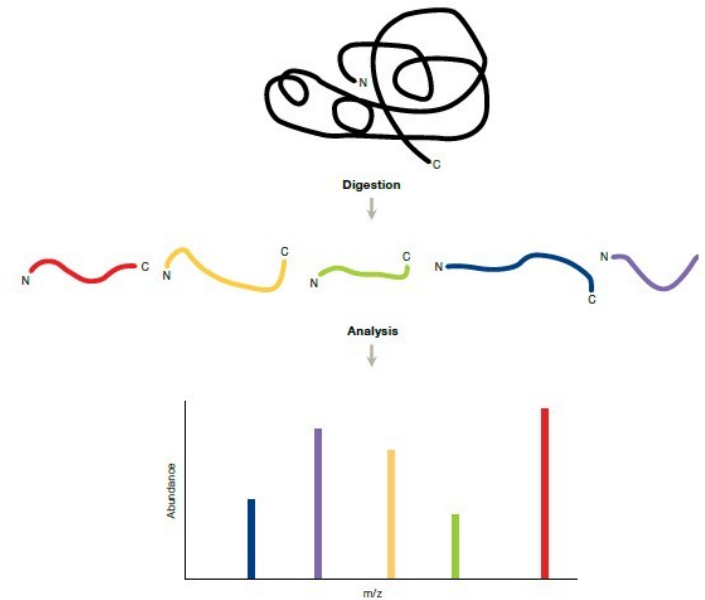
↓ **DIGESCE**

trypsin Glu-C Asp-N thermolysin

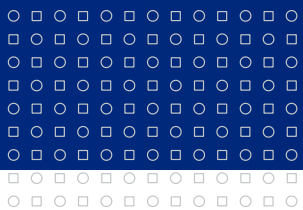
MAVEPFRRPITRPHASIEVDTS GTGG SAGSSE
 KVFCLIGQAEGGEPNTVYELRNYAQAKRLFRS
 GELLD AIELAWG SNP NYTAGRILAMRIEDAKP
 ASAEIGGLKITSKIYGNVANNIQVGLEKNTLSD
 SLRLRVIFQDDRFNEVYDNIGNIFTIKYKGEEA
 NATFSVEHDEETQKASRLVLKVG DQEVKSYD
 LTGGAYDYTNAIITDINQLPDFEAKLSPFGDKN
 LESSKLDKIENANIKDKAVYVKAVFGDLEKQT
 AYNGIVSFEQLNAEGEVPSNVEVEAGEESATV
 TATSPIKTI EPFELTKLKGGTNGEPPATWADKL
 DKFAHEGGYYIVPLSSKQSVHAEVASFVKERS
 DAGEPMRAIVGGGFNESKEQLFGRQASLSNPR
 VSLVANS GTFVMDDGRKNHVPAYMVAVALGG
 LASGLEIGESITFKPLRVSSLDQIYESIDLDELN
 ENGIISIEFVRNRTNTFFRIVDDVTTFNDKSDPV
 KAEMAVGEANDFLVSELKVQLEDQFIGTRTIN
 TSASIIKDFIQSYLGRKKRDNEIQDFPAEDVQVI
 VEGNEARISMTVYPIRSFKKISVSLVYKQQTLQ

A

- IN-GEL
- IN-SOLUTION

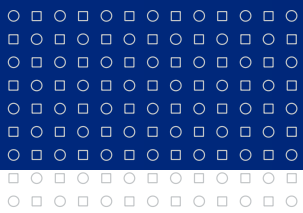


MS



G I G O





G I G O

GARBAGE IN - GARBAGE OUT

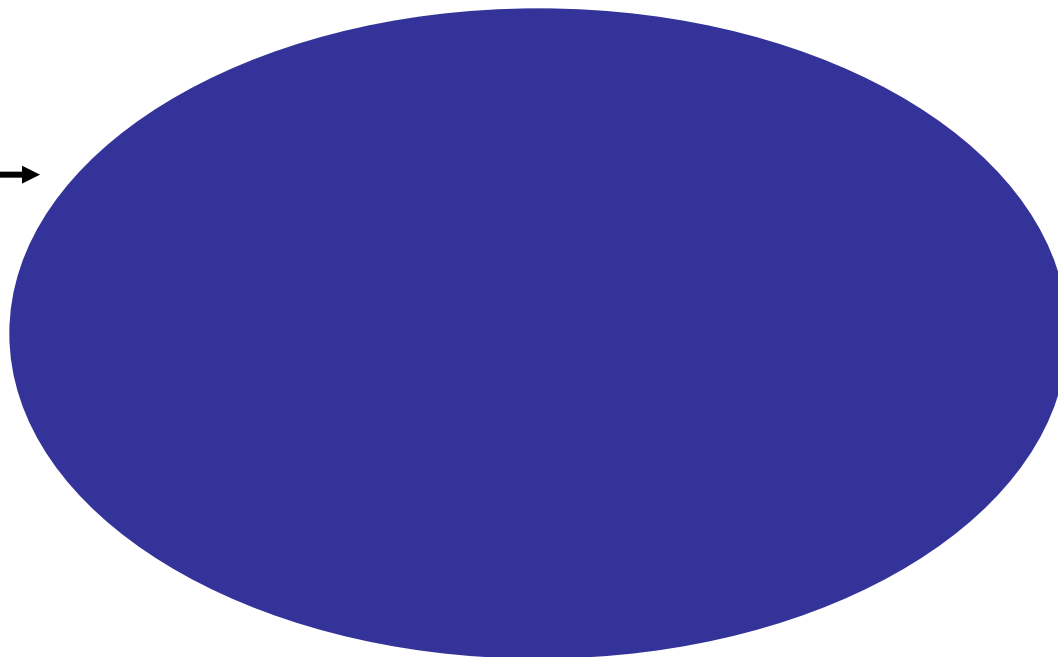
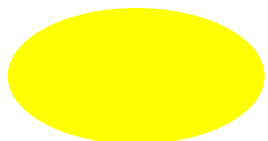


LITERATURA

- 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



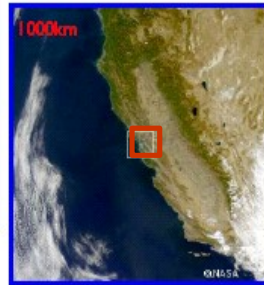
PREFRAKCIONACE → MS



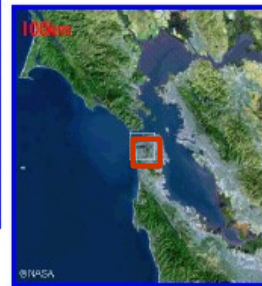
10^{10} Really Is Wide Dynamic Range



10 10 000km



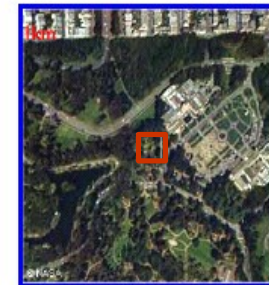
9 1 000km



8 100km



7 10km



6 1km



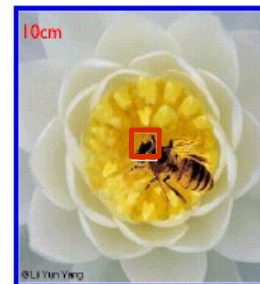
5 100m



4 10m



3 1m

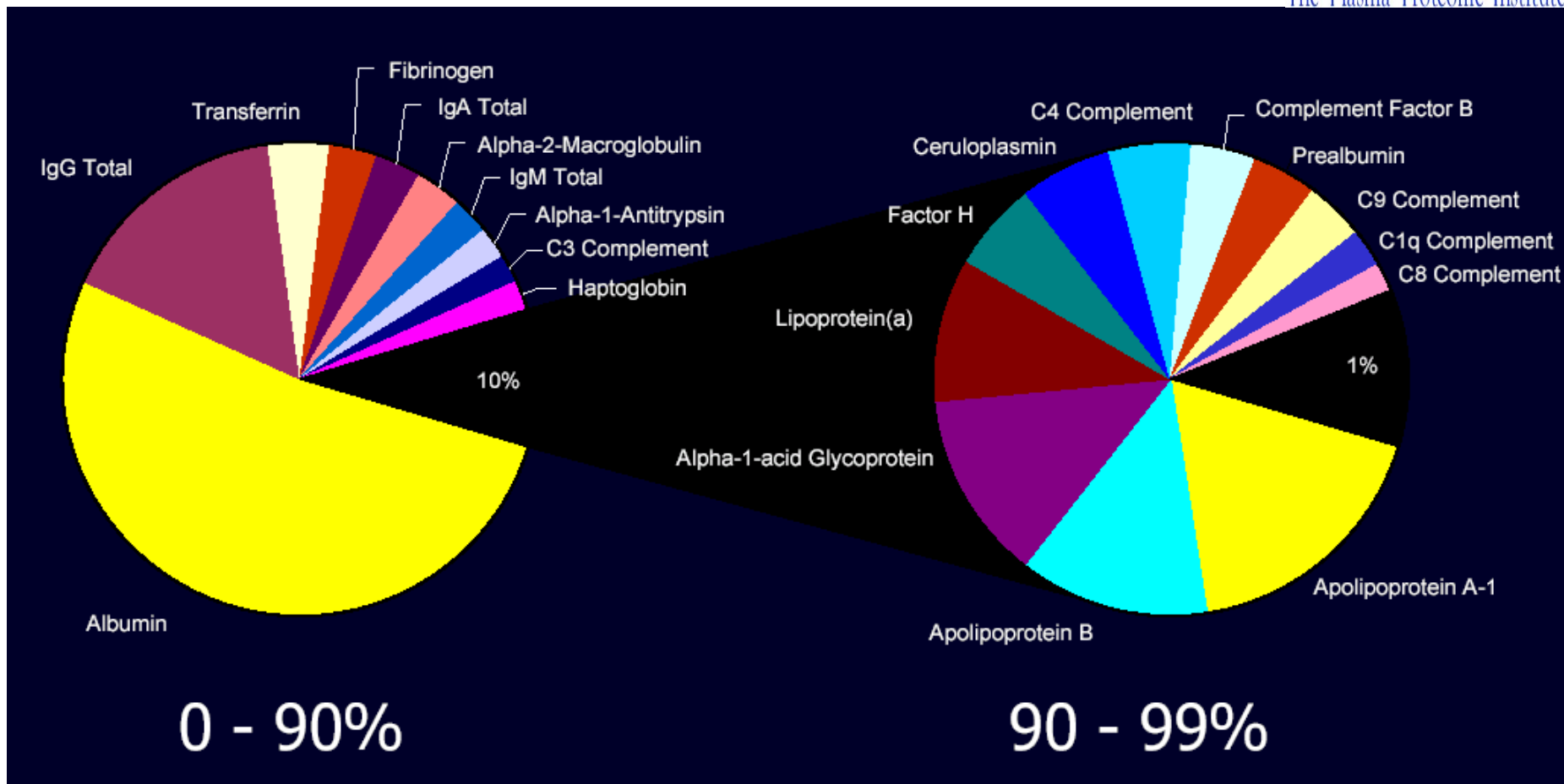
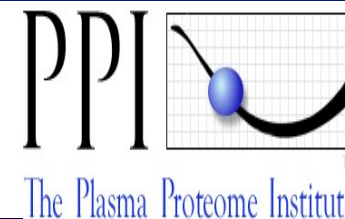


2 10cm



1 1cm

Abundantní proteiny v lidské plazmě

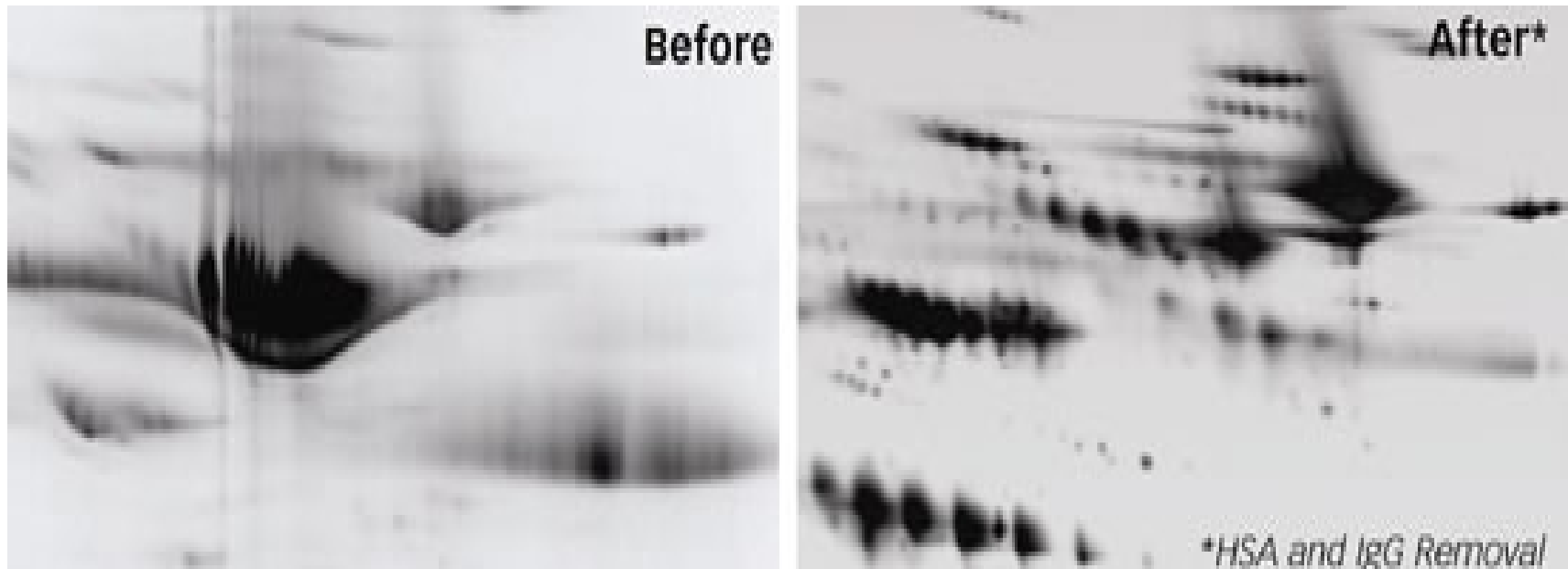


AFINITNÍ DEPLECE

odstranění abundantních proteinů afinitní chromatografií

HSA

IgG



Lidská plazma - vázaná frakce po afinitní depleci

ALBUMIN

IgG

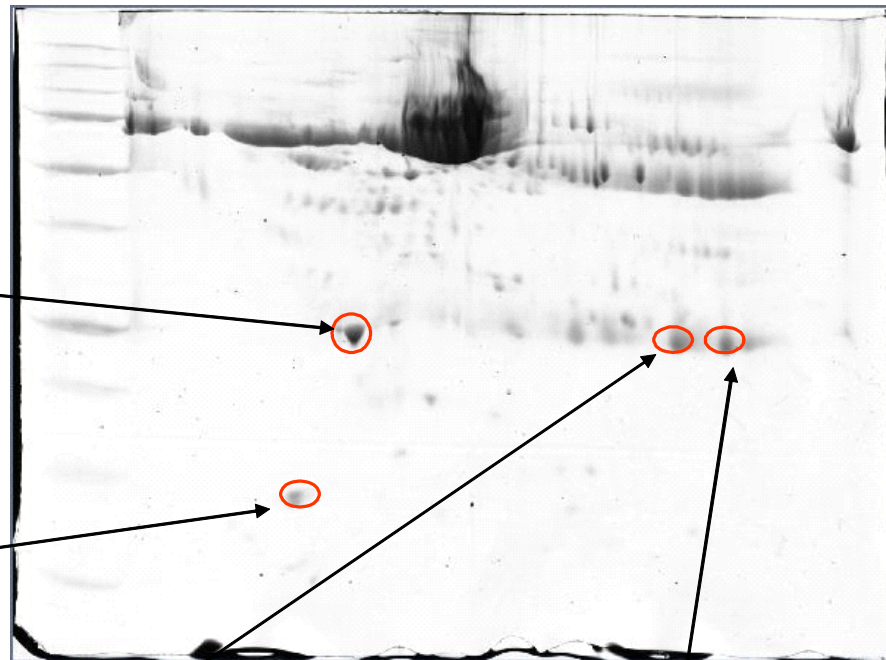
Barvení: CB G-250

Apolipoprotein

albumin

Immunoglobulin kappa light chain

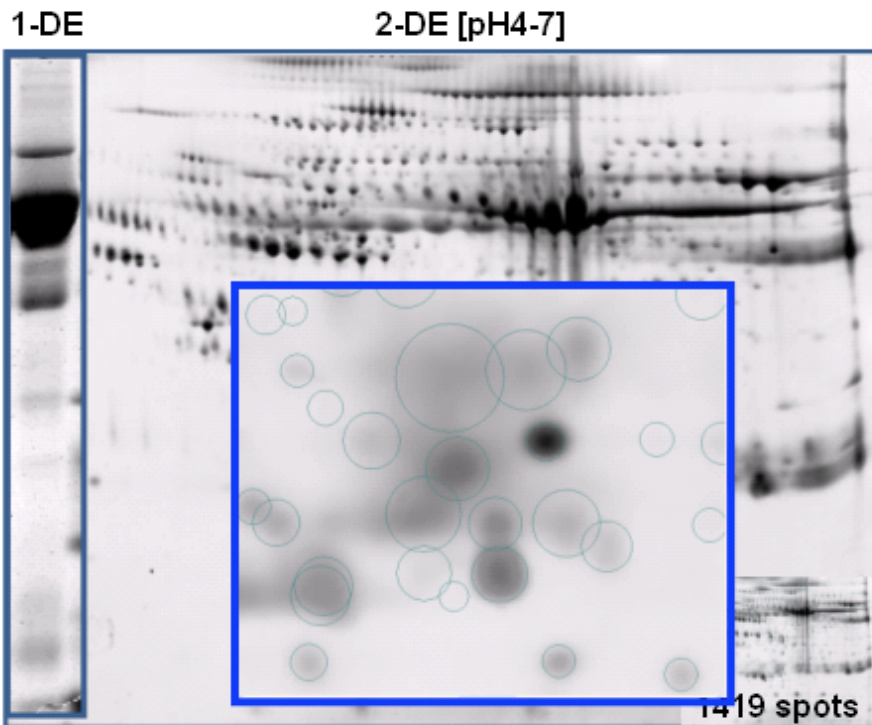
Immunoglobulin light chain



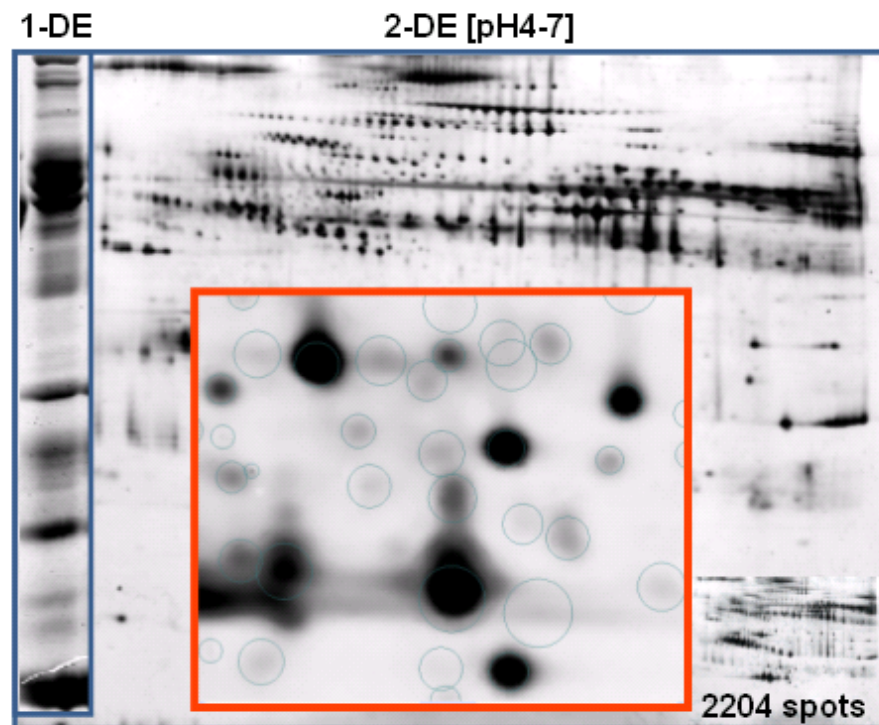
CPPL Combinatorial Peptide Ligand Library



Native Human Serum

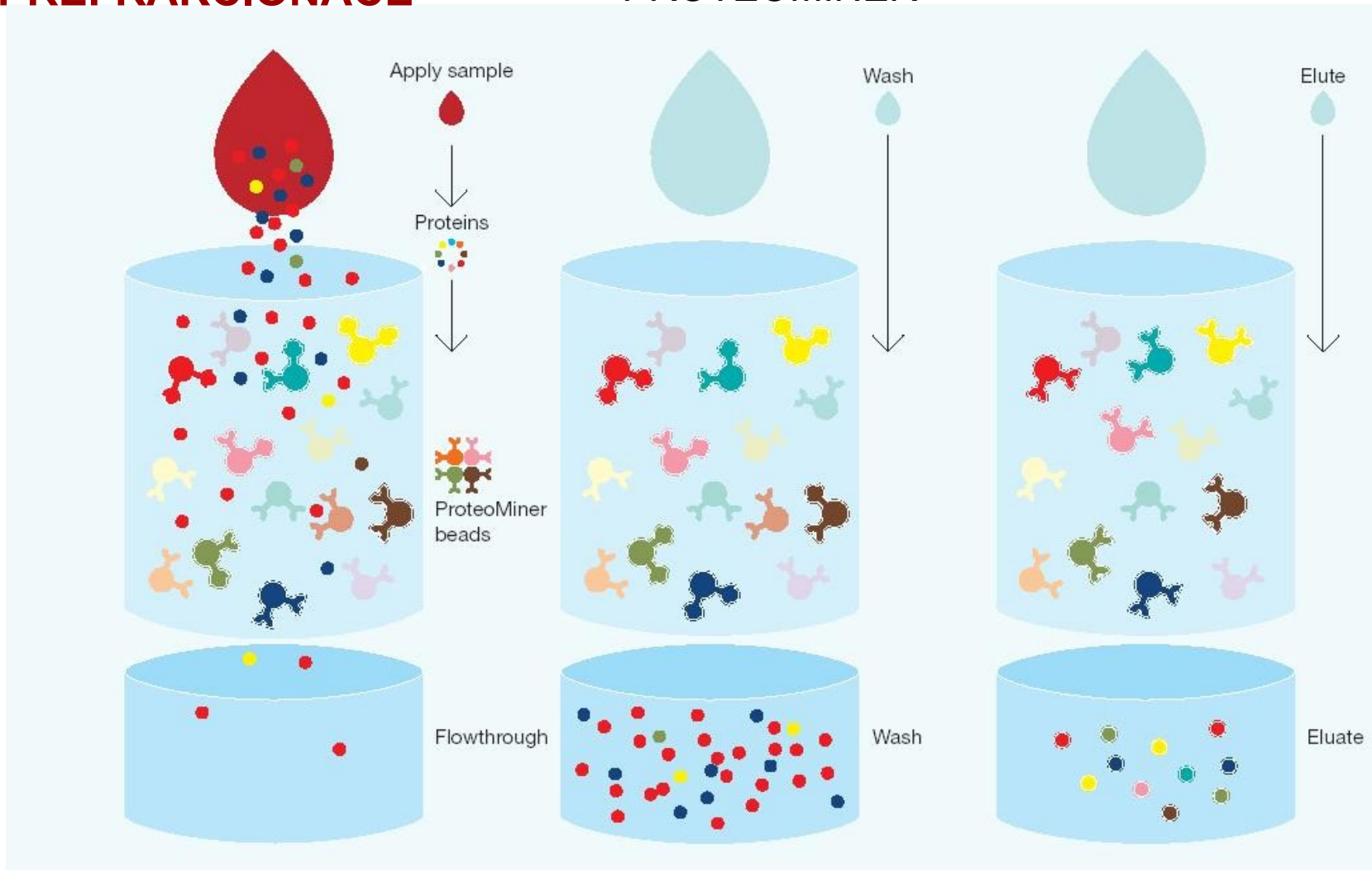


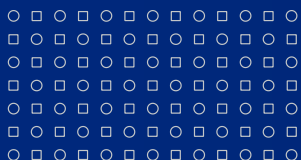
Human Serum Fractionated by ProteoMiner



PREFRAKCIONACE

PROTEOMINER





IEF PREFRAKCIONACE



MicroRotor

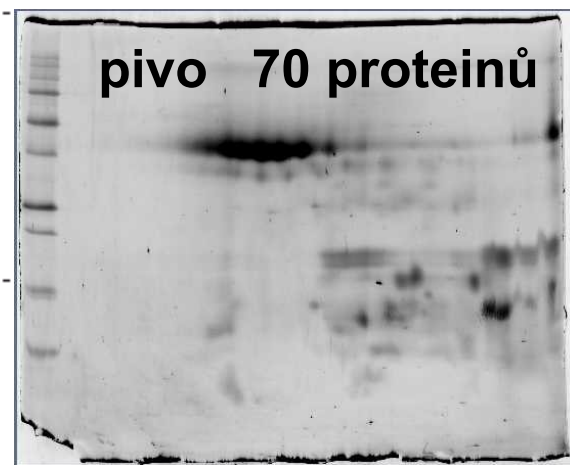
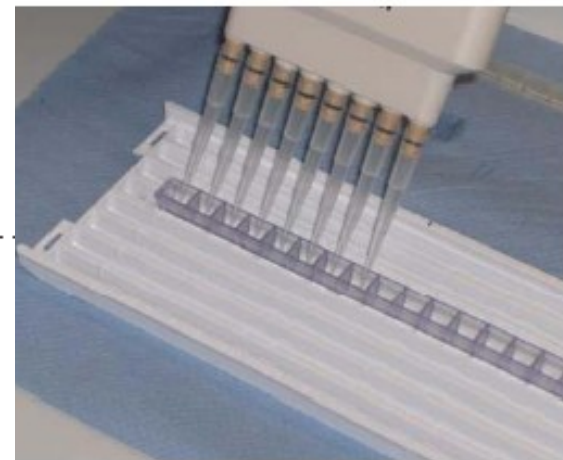
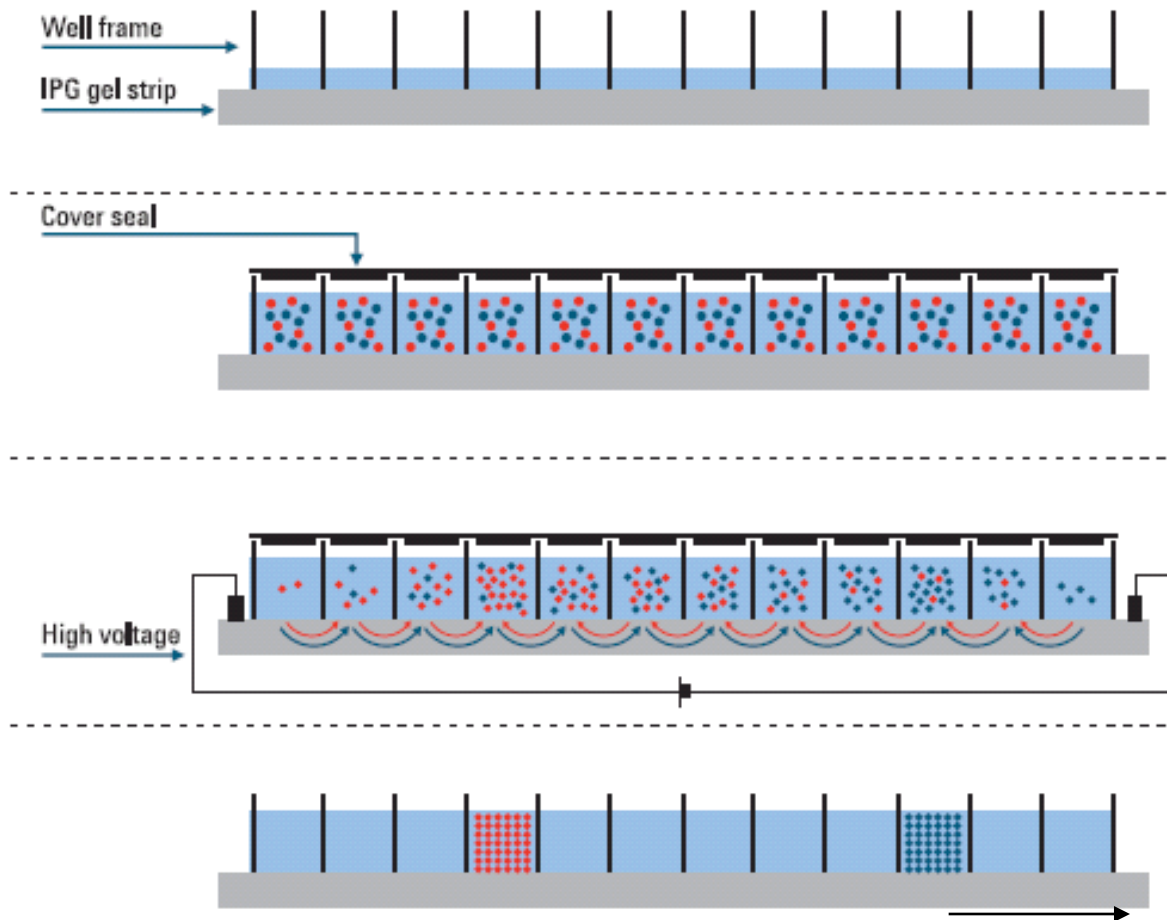
- prefrakcionace v roztoku

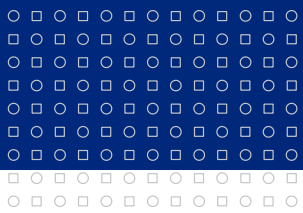


OffGel Fractionator

- prefrakcionace v roztoku na IPG stripu

OFFGEL IEF prefrakcionace proteinů nebo peptidů





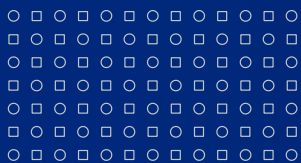
IPG-IEF



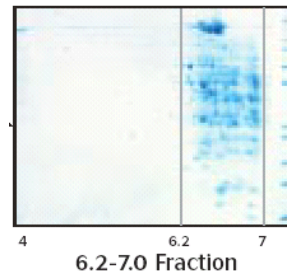
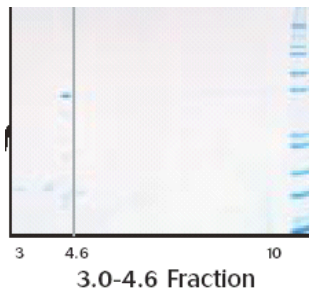
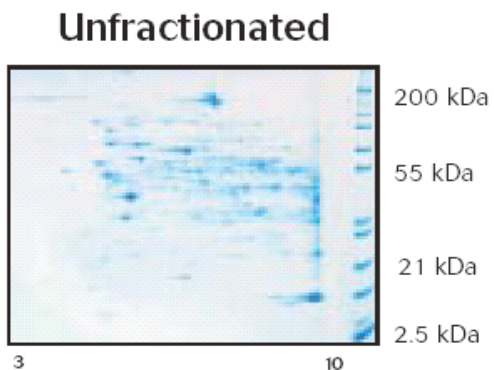
digest smění proteinů of

IEF smění peptidů na IPG stripu

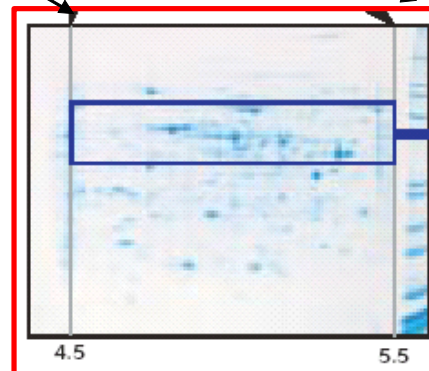
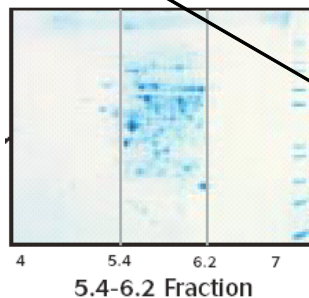
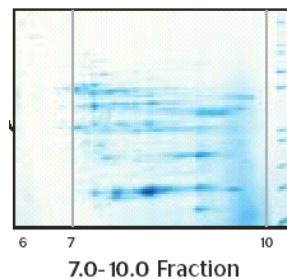
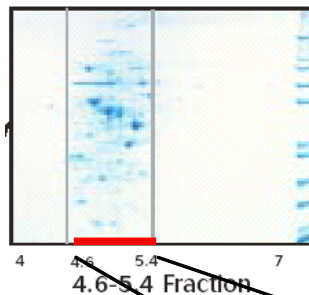
frakce stripu



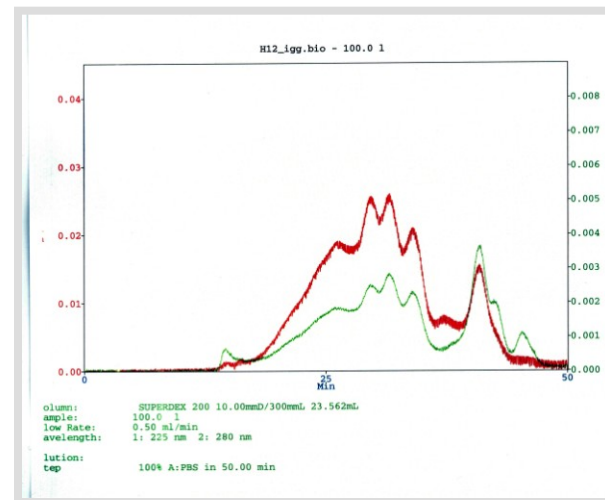
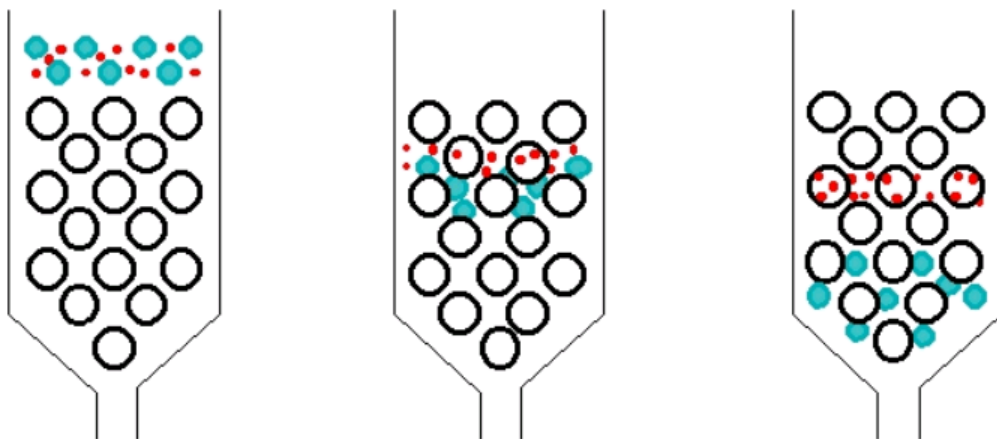
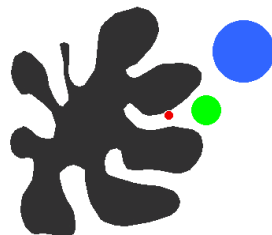
PREFRAKCIONACE MIKRO ROZSAHY



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

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

