

PROTEOMIKA 2024

- Proteomika, Metody práce s bílkovinami (Petrák 7/10)
- Separační metody, digesce a principy ID bílkovin pomocí MS (Petrák 14/10)
- Principy hmotnostní spektrometrie, instrumentace (Man 4/11)
- Hmotnostní spektrometrie v proteomice, analýza PTM (Man 11/11)
- ID proteinů, DDA, DIA, databáze, FDR (Talacko 18/11)
- Kvantifikace, isotopy, LFQ, cílená proteomika (Harant 25/11)
- Design experimentu, zpracování dat, bioinformatika...(Harant 2/12)
- **Proteomika membránových proteinů, proteinové komplexy (Petrák 9/12)**
- **Klinická proteomika, speciální metody (Petrák 16/12)**

Příprava vzorků pro proteomické experimenty

Proteomika membránových proteinů

Analýza proteinových komplexů

Současná proteomika

- **DDA a DIA**
- **až 10 000 proteinů v jednom experimentu**
- **izotopická nebo label-free kvantifikace**
- **náročnost na instrumentaci, statistiku a (bio)informatiku**
- **problém s inferencí proteinu (stejně peptidy v různých proteinech)**
- **analýza PTM je možná**
- **ztráta většiny informace o proteoformách**
- **některé typy proteinů stále unikají**

- **Cílená proteomika – kvantifikace desítek vybraných proteinů**

Kvalita biologického materiálu a příprava vzorků!



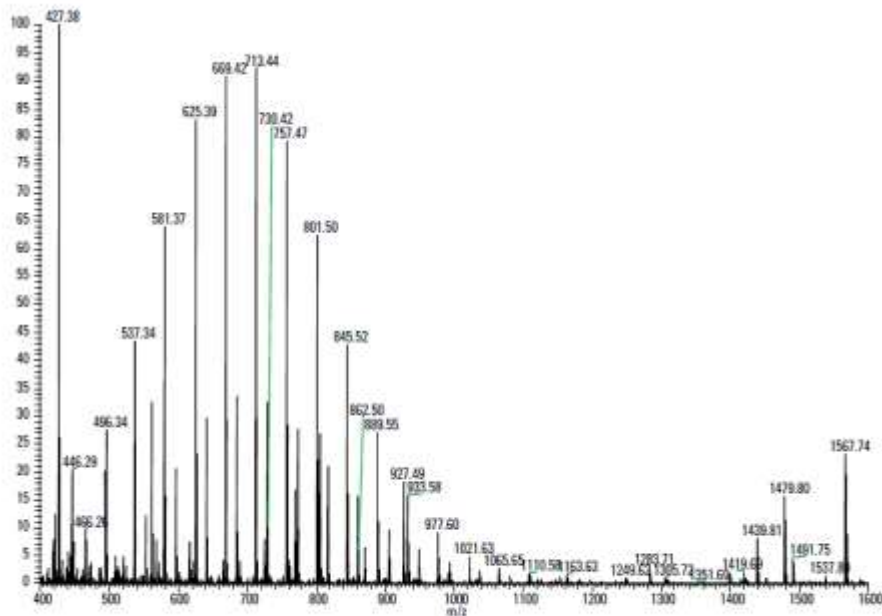
Filozofie přípravy vzorků v roztoku:

Dosáhnout rozbití buněk a maximální rozpustnosti všech bílovin/peptidů při zachování kompatibility se separační metodou a MS analýzou.

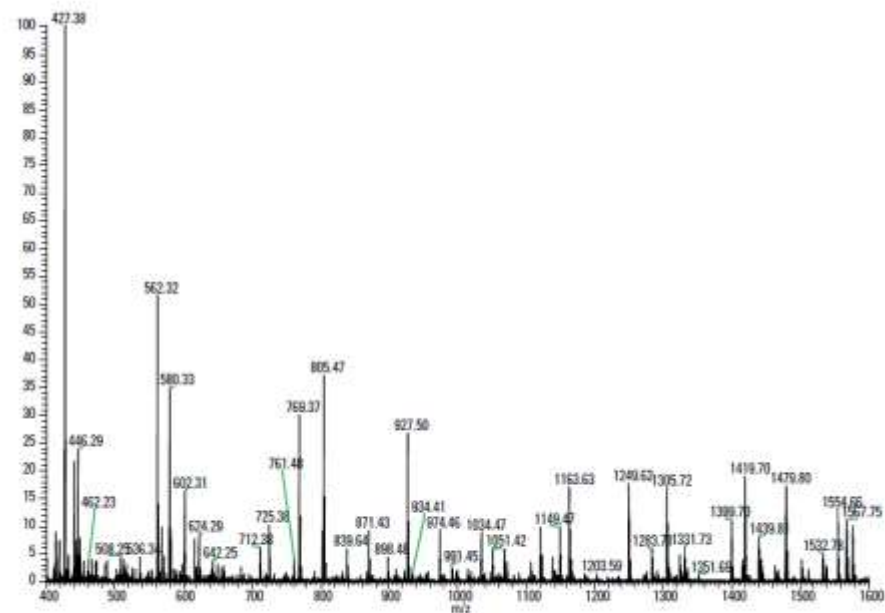
Detrgenty a močovina umožňují solubilizovat a denaturovat vzorek ale jsou nekompatibilní s digescí a/nebo LC-MS analýzou

Jak je využít a jak se jich zase rychle zbavit?

Odstranění detergentů



Triton X-100, Unprocessed



Triton X-100, Processed

Piercenet.com

Odstranění detergentů

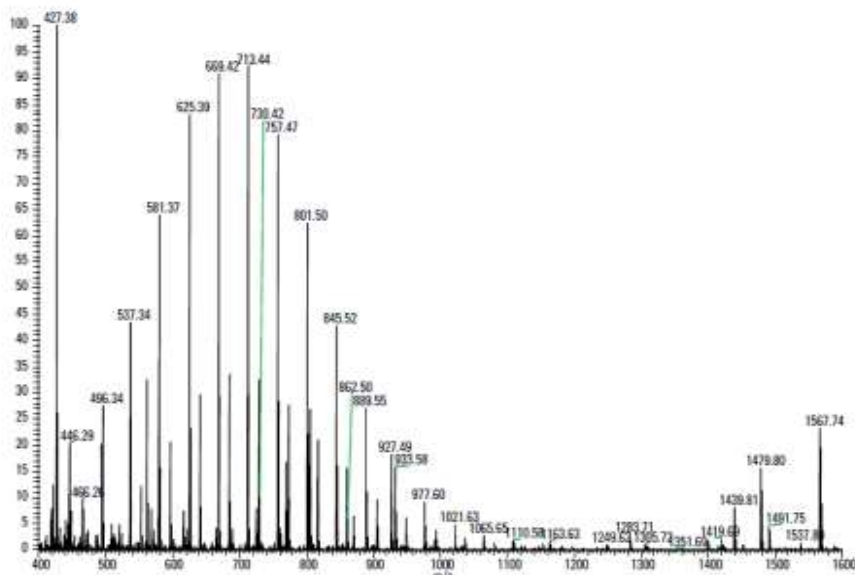
Deoxycholate (SDC)

SDS

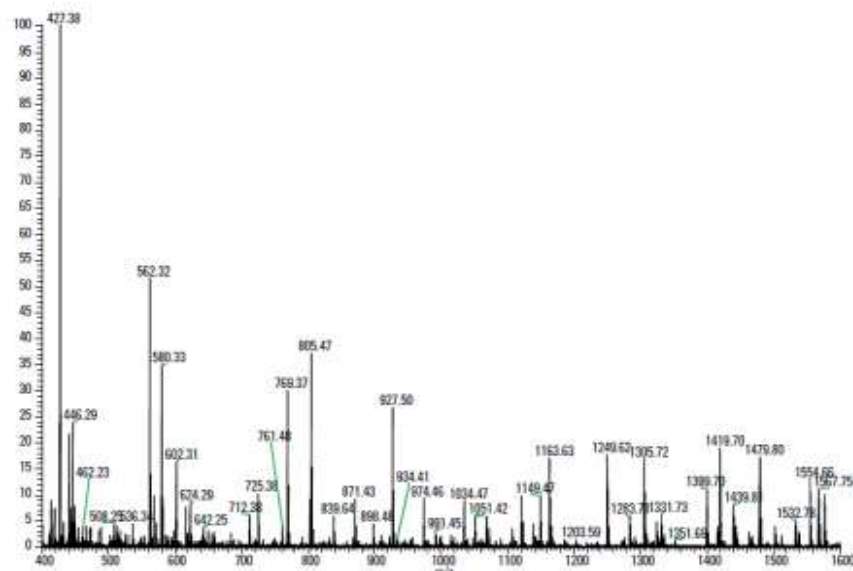
Triton X100

Rapigest

Kyselinou štěpitelné detergenty * Fázová separace * výměna pufru * SP3



Triton X-100, Unprocessed



Triton X-100, Processed

Odstranění detergentů

Deoxycholate (SDC) - Fázová separace, výměna pufru (FASP), SP3

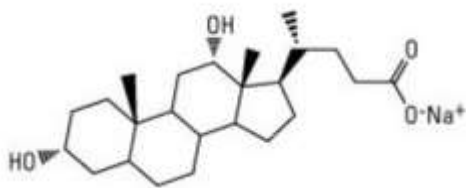
SDS- Výměna pufru (FASP)

Triton X100- SP3

Odstranění detergentů

Deoxycholát sodný (SDC)

Fázová separace * výměna pufru * SP3



Deoxycholát sodný (SDC)

Silný anionický detergent,
tolerovaný trypsinem až do 1%

Lze snadno odstranit po štěpení:

- 1) Okyselení (pH ~ 2)
- 2) Přídavek octanu etylnatého 1:1
- 3) Fázový transfer (vytřepání) do octanu etylnatého
- 4) Odstranění horní fáze (octanu) a následné odsolení peptidů

Filter Assisted Sample Preparation - FASP

Odstranění SDS, SDC, ale nefunguje pro Triton

Výměna pufru, koncentrace vzorku, zbavení se detergentu, digesce

TÉMĚŘ UNIVERZÁLNÍ ŘEŠENÍ - FASP



Vhodné filtry s cut off 10-30 kDa

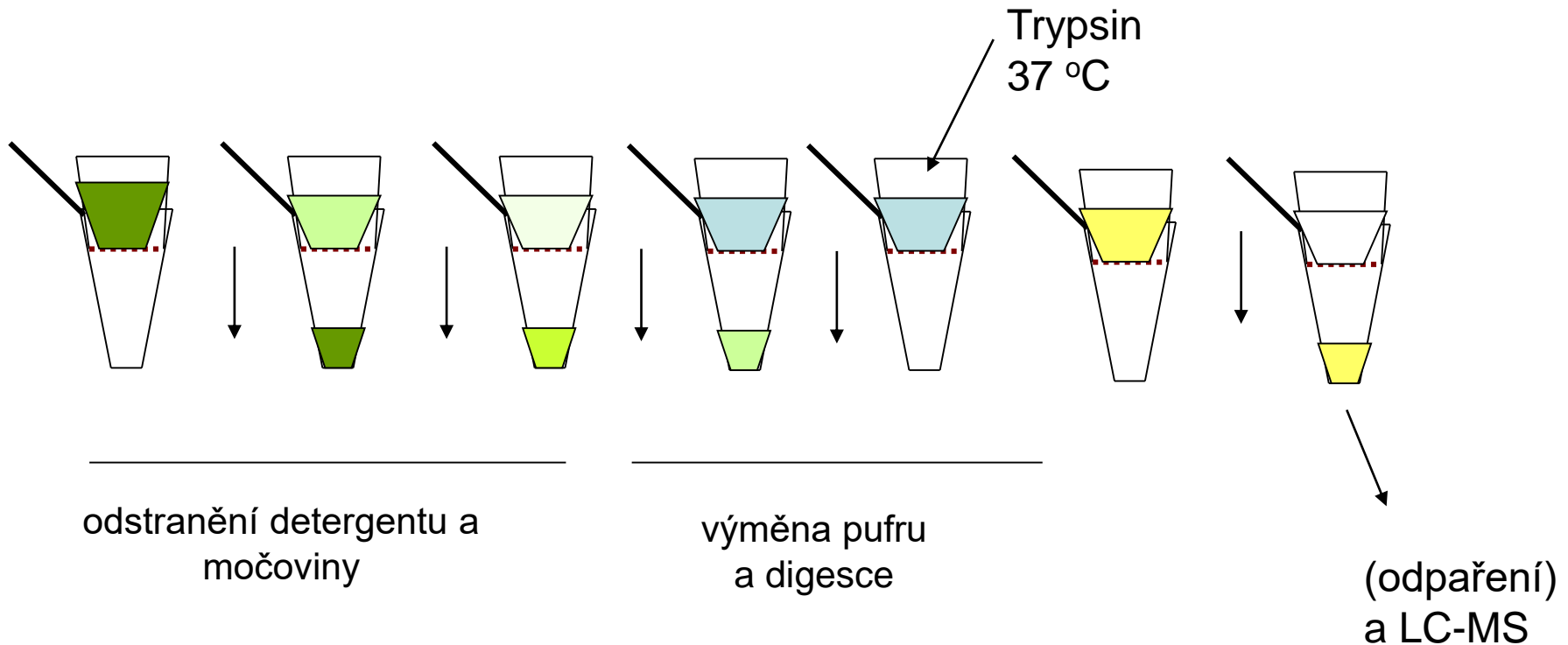
Manza LL, et al. Proteomics. 2005 May;5(7):1742-5.

Wiśniewski JR, et al. Nat Methods. 2009 May;6(5):359-62.

Filter Assisted Sample Preparation - FASP

Vzorek proteinů s vysokou koncentrací močoviny a/nebo detergentu
(nelze štěpit trypsinem)

Vhodné filtry s cut off 10-30 kDa

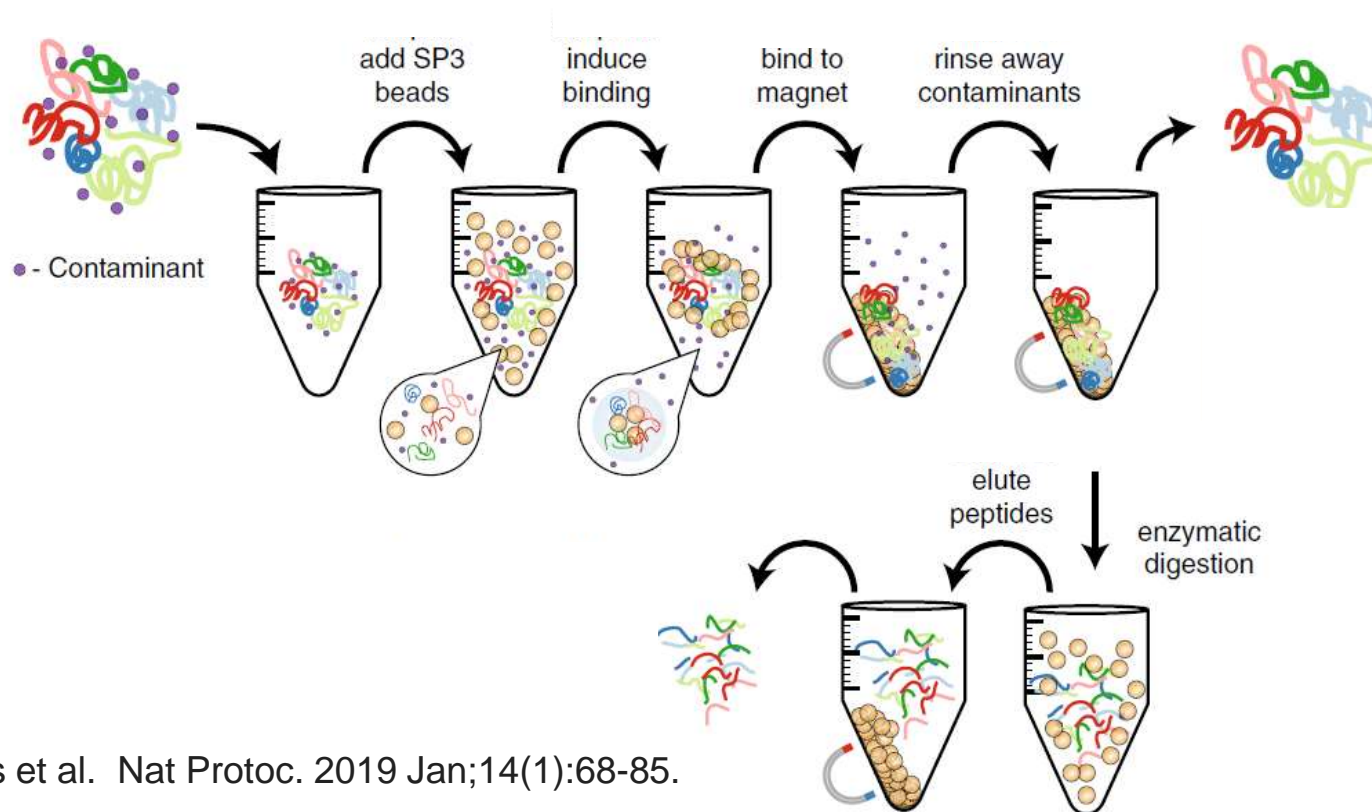


Single-pot, solid-phase-enhanced sample preparation for proteomics experiments

SP3

Odstranění SDS, SDC, Tritonu X100

- Zachycení proteinů pomocí HILIC chromatografie na magnetických kuličkách
- odmytí detergentu
- štěpení na kuličkách
- eluce peptidů

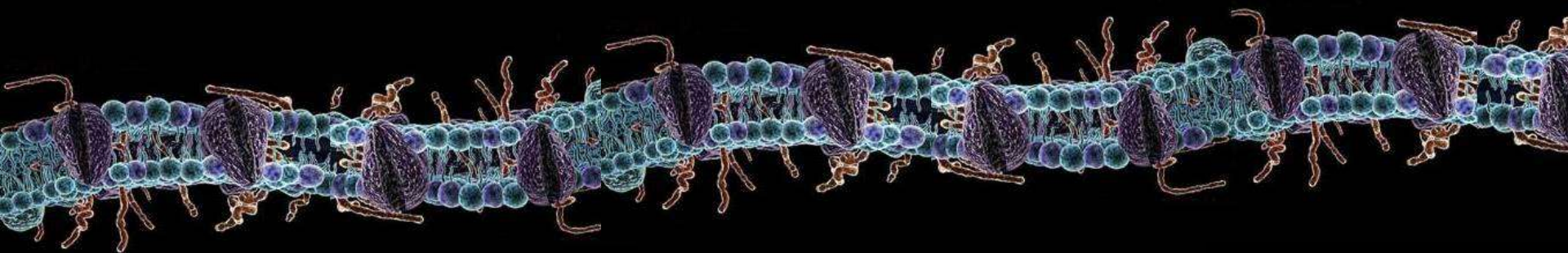


Současná proteomika

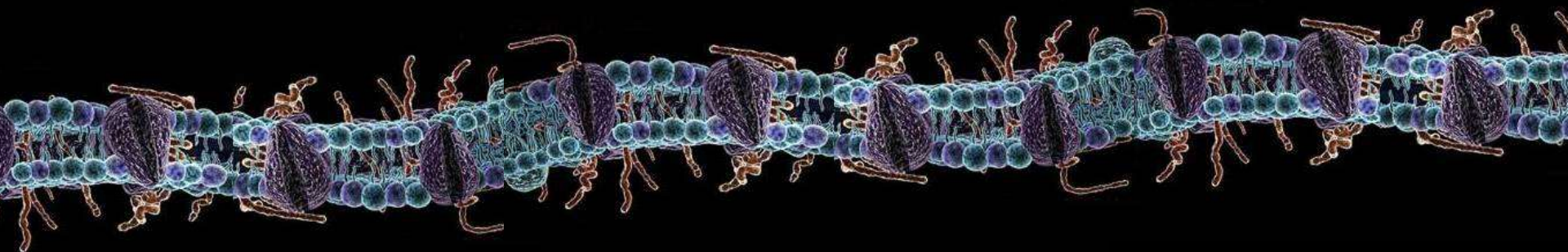
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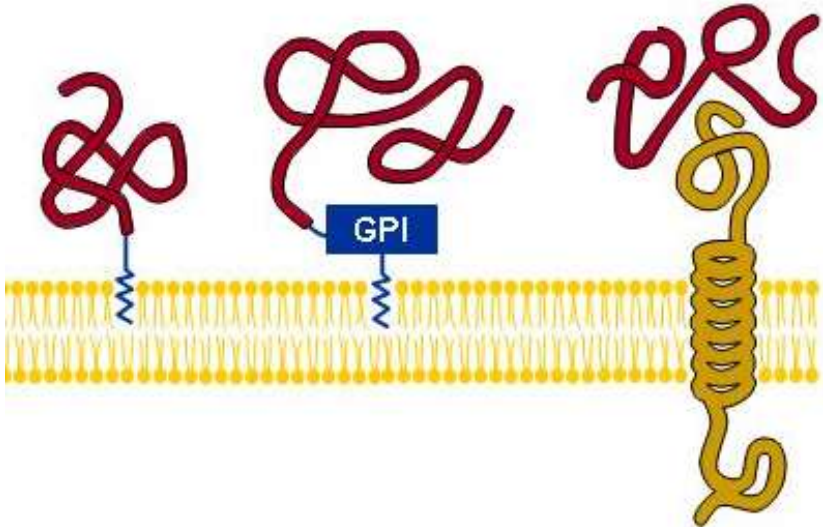
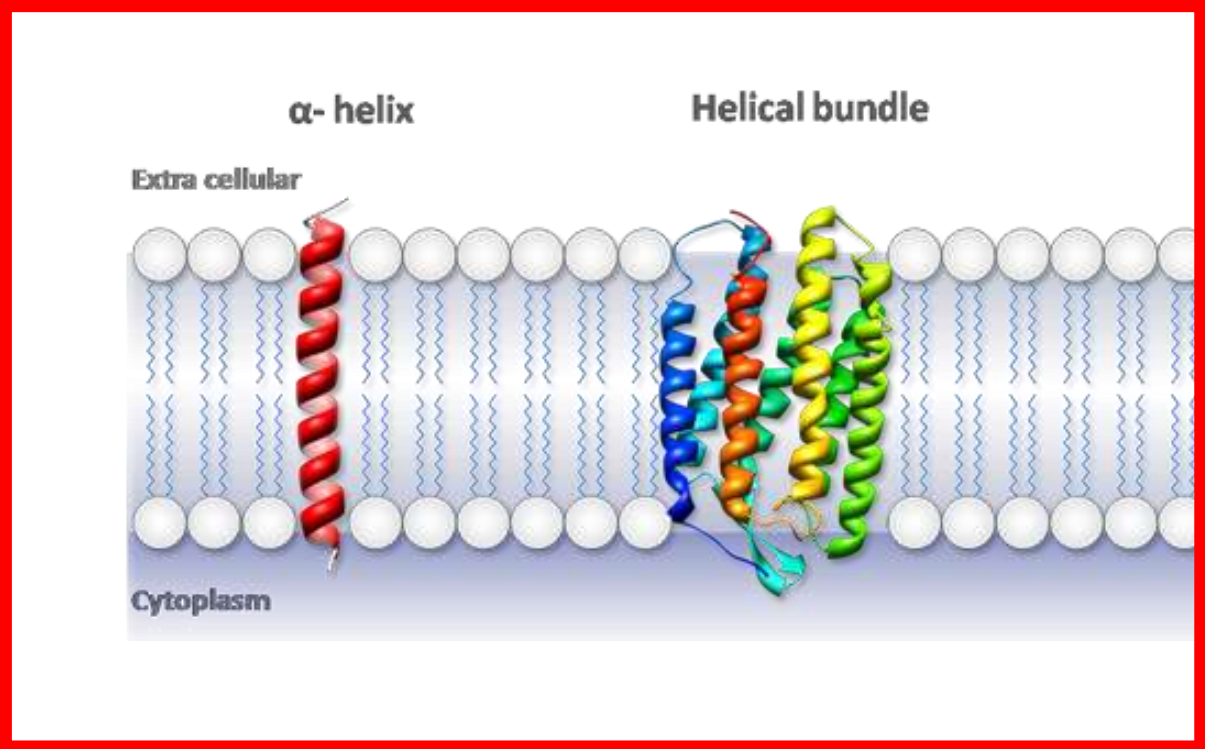
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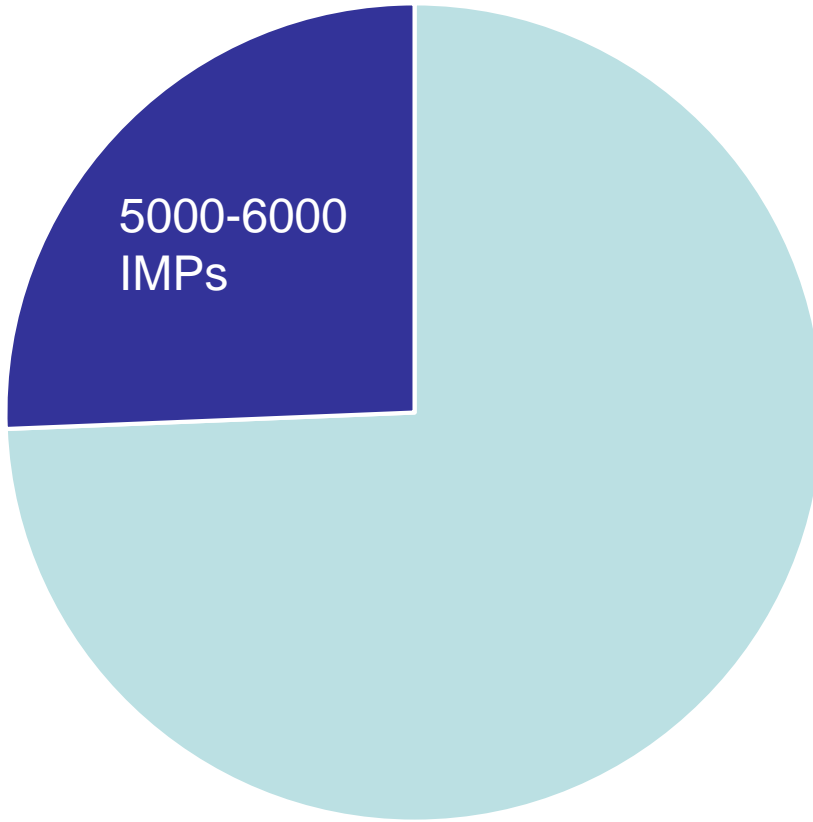


MEMBRÁNOVÉ PROTEINY

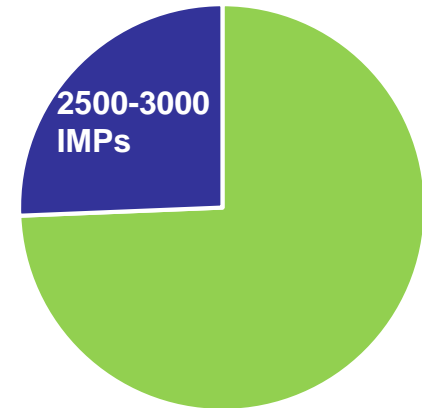


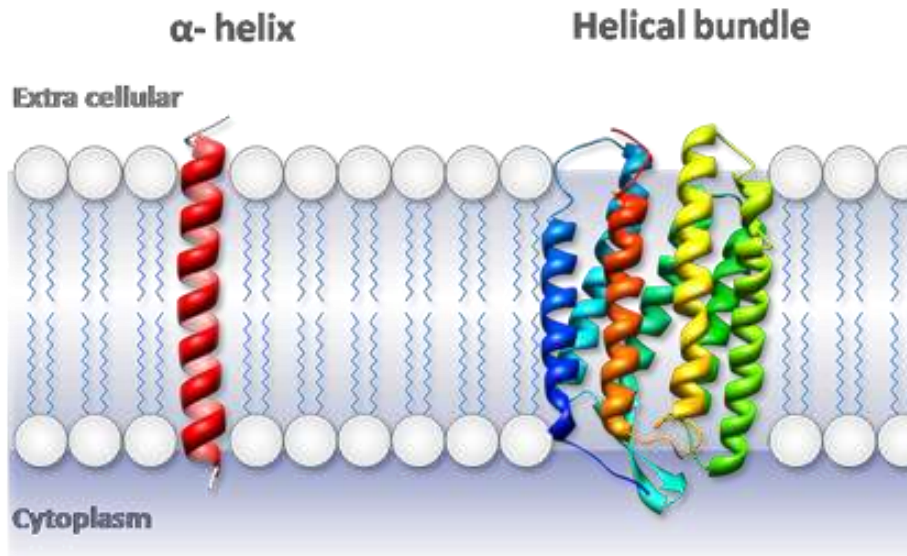


~ 20 000 human protein coding genes



10 000 genes actively expressed by an average cell type

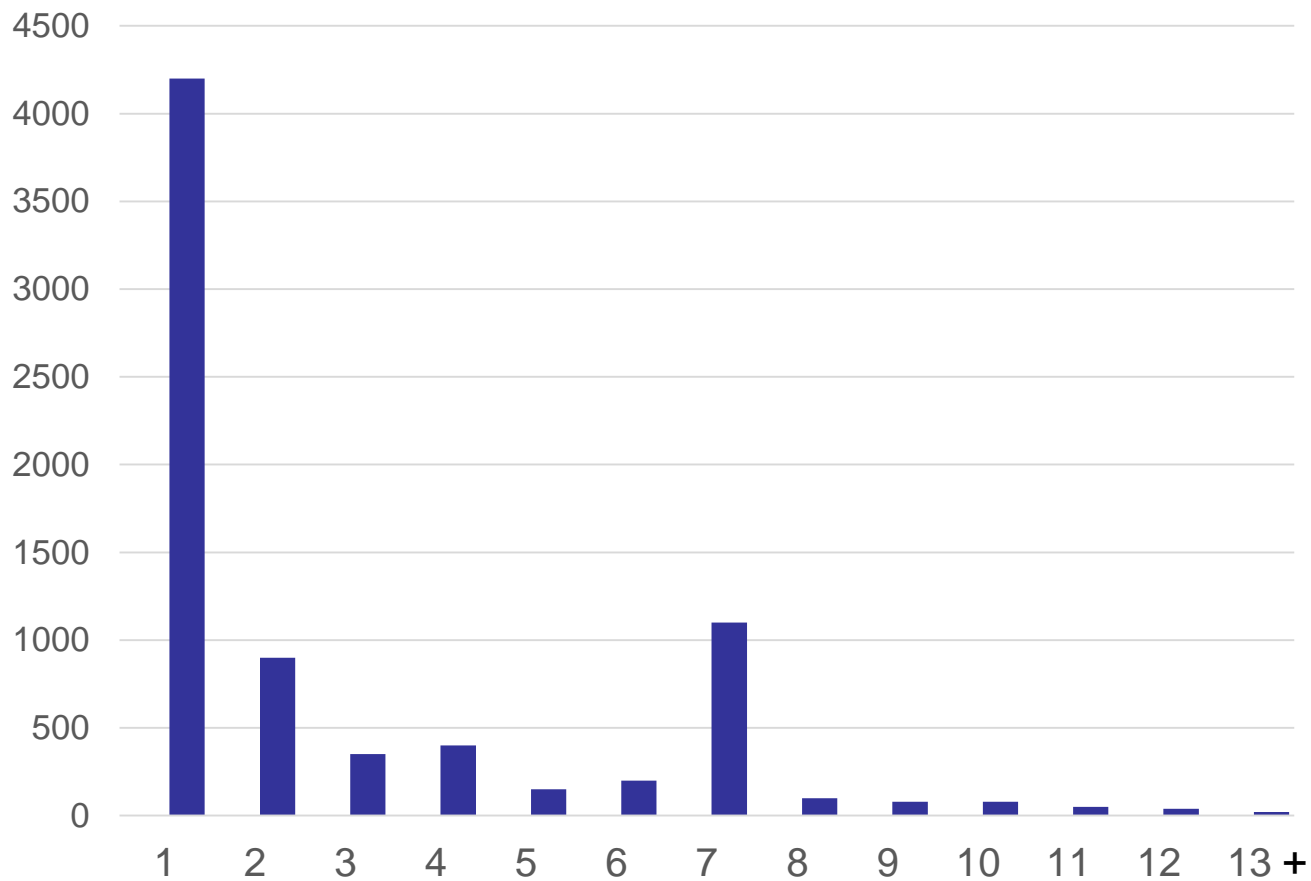


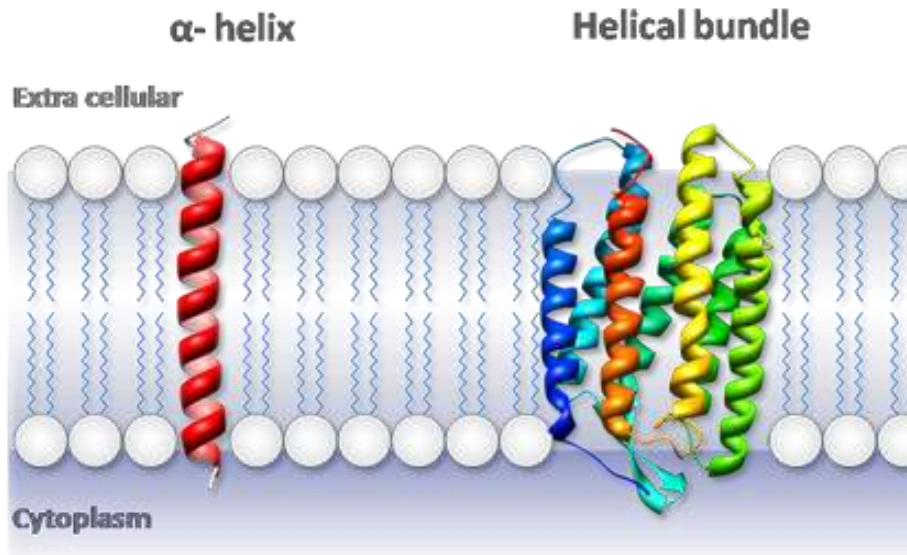


INTEGRAL MEMBRANE PROTEINS

- alpha helix TM domain(s) (20-25 AA) + soluble domains

Number of predicted TM segments in human integral membrane proteins



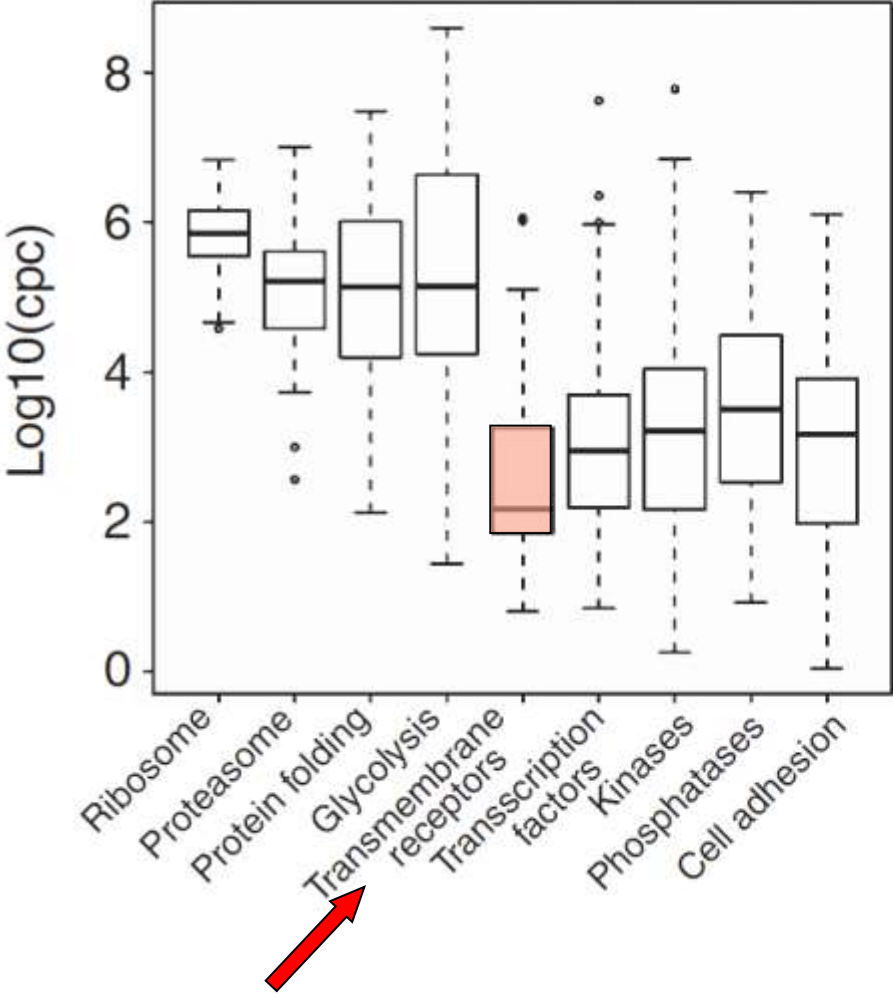


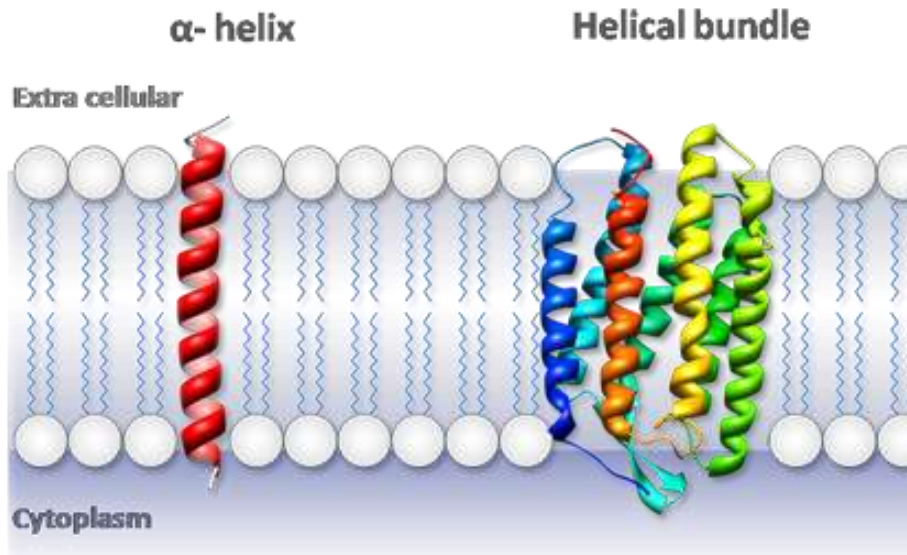
INTEGRAL MEMBRANE PROTEINS

- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression

Cellular abundance of transmembrane proteins is LOW

(100-1000 copies/cell)





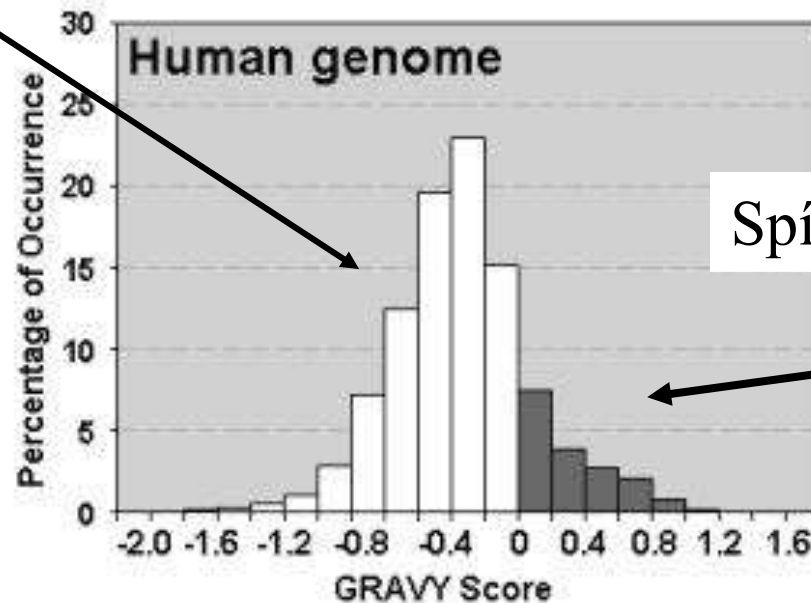
INTEGRAL MEMBRANE PROTEINS

- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression
- hydrophobic/amphipathic nature

Hydrofobicita proteinu

GRAVY SCORE – Grand average hydropathy
(součet „hydrofobicity“ (-4.6 až 4.6) jednotlivých aminokyselin
dělený počtem aminokyselin)

Spíš rozpustné



Spíš transmembránové

Kyte, J., and Doolittle, R.F. (1982) J.Mol.Biol. 157, 105-132

Hydrofobicita proteinu

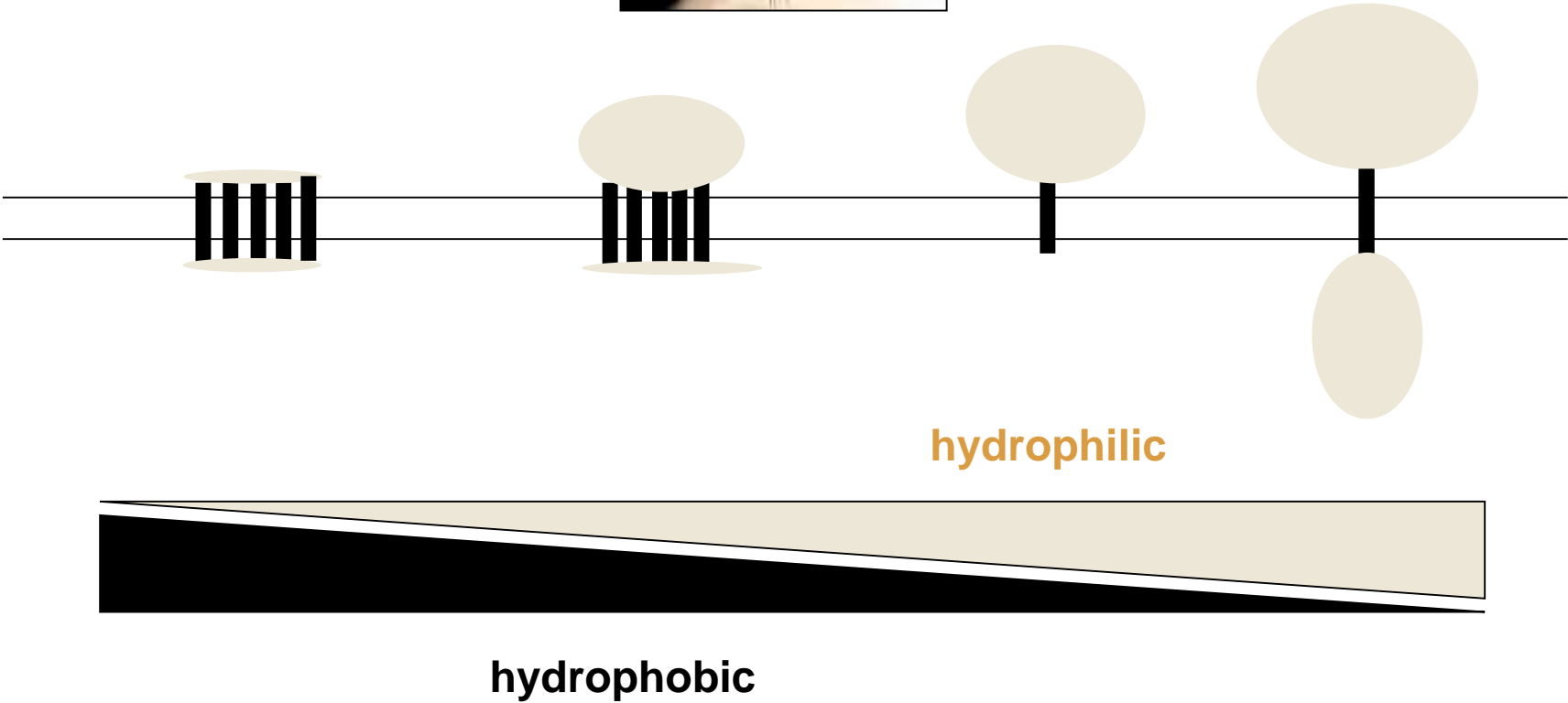
Amino Acid Name	One Letter Code	Hydropathy Score
Isoleucine	I	4.5
Valine	V	4.2
Leucine	L	3.8
Phenylalanine	F	2.8
Cysteine	C	2.5
Methionine	M	1.9
Alanine	A	1.8
Glycine	G	-0.4
Threonine	T	-0.7
Tryptophan	W	-0.9
Serine	S	-0.8
Tyrosine	Y	-1.3
Proline	P	-1.6
Histidine	H	-3.2
Glutamic acid	E	-3.5
Glutamine	Q	-3.5
Aspartic acid	D	-3.5
Asparagine	N	-3.5
Lysine	K	-3.9
Arginine	R	-4.5

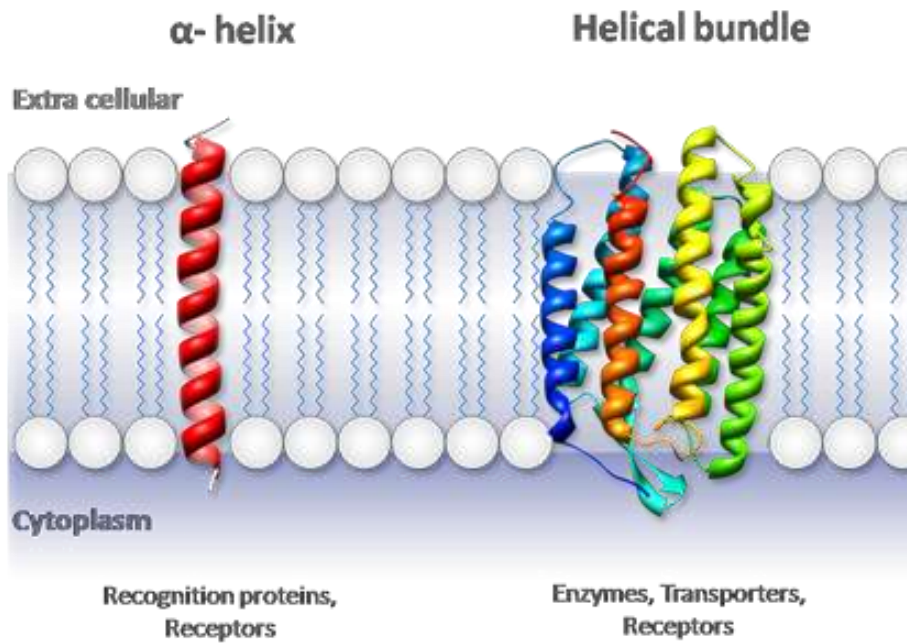
GRAVY SCORE – Grand average
hydropathy
(součet „hydrofobicity“ (-4.5 až 4.5)
jednotlivých aminokyselin dělený
počtem aminokyselin)

**Hydrofobní aminokyseliny
typické pro α -helixy:**

„**FAMILY VW**“ (+S)

IMPs - molecules with split personalities

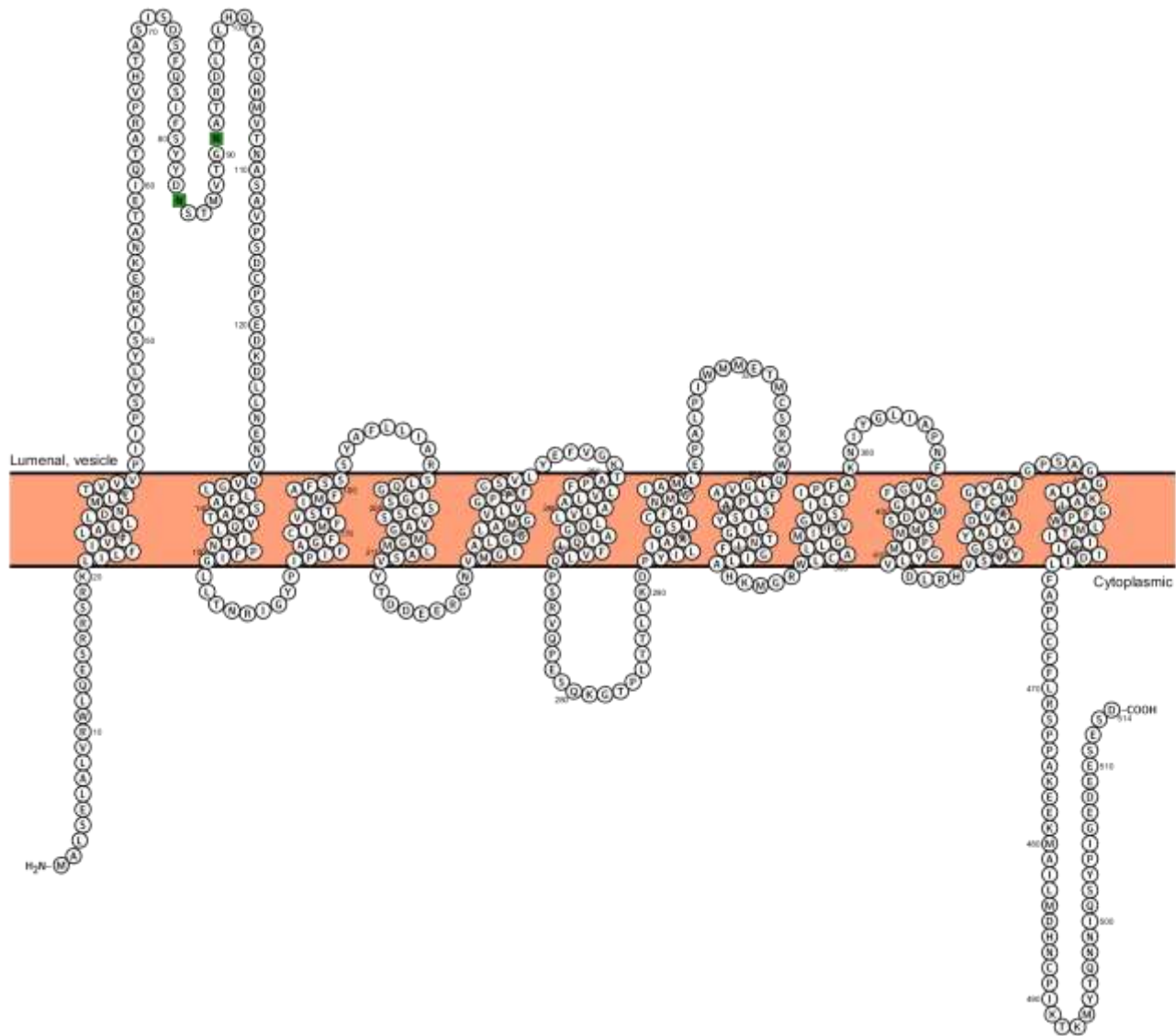




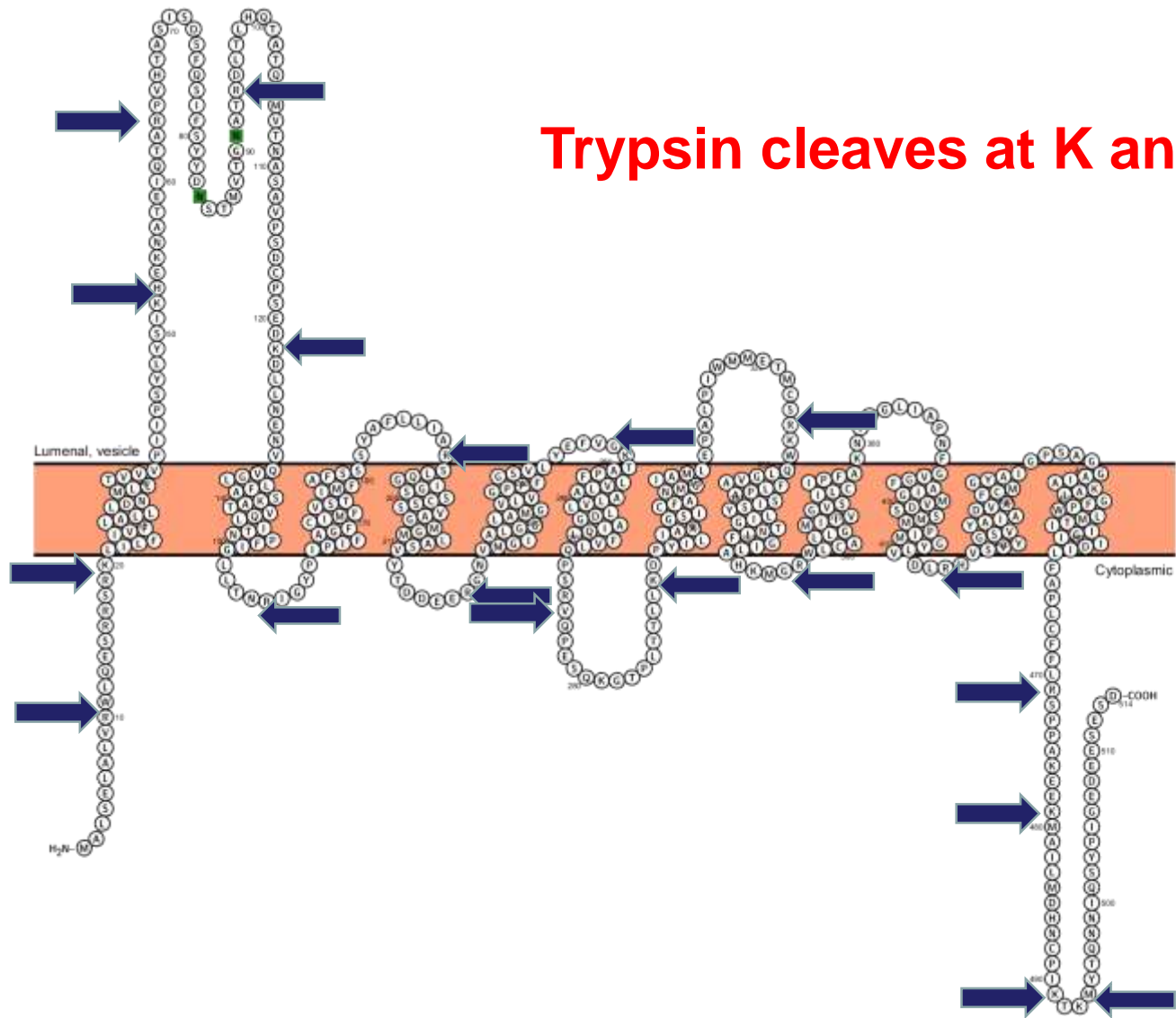
INTEGRAL MEMBRANE PROTEINS

- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression
- hydrophobic/amphipathic nature
- no Arg, Lys in TM domains

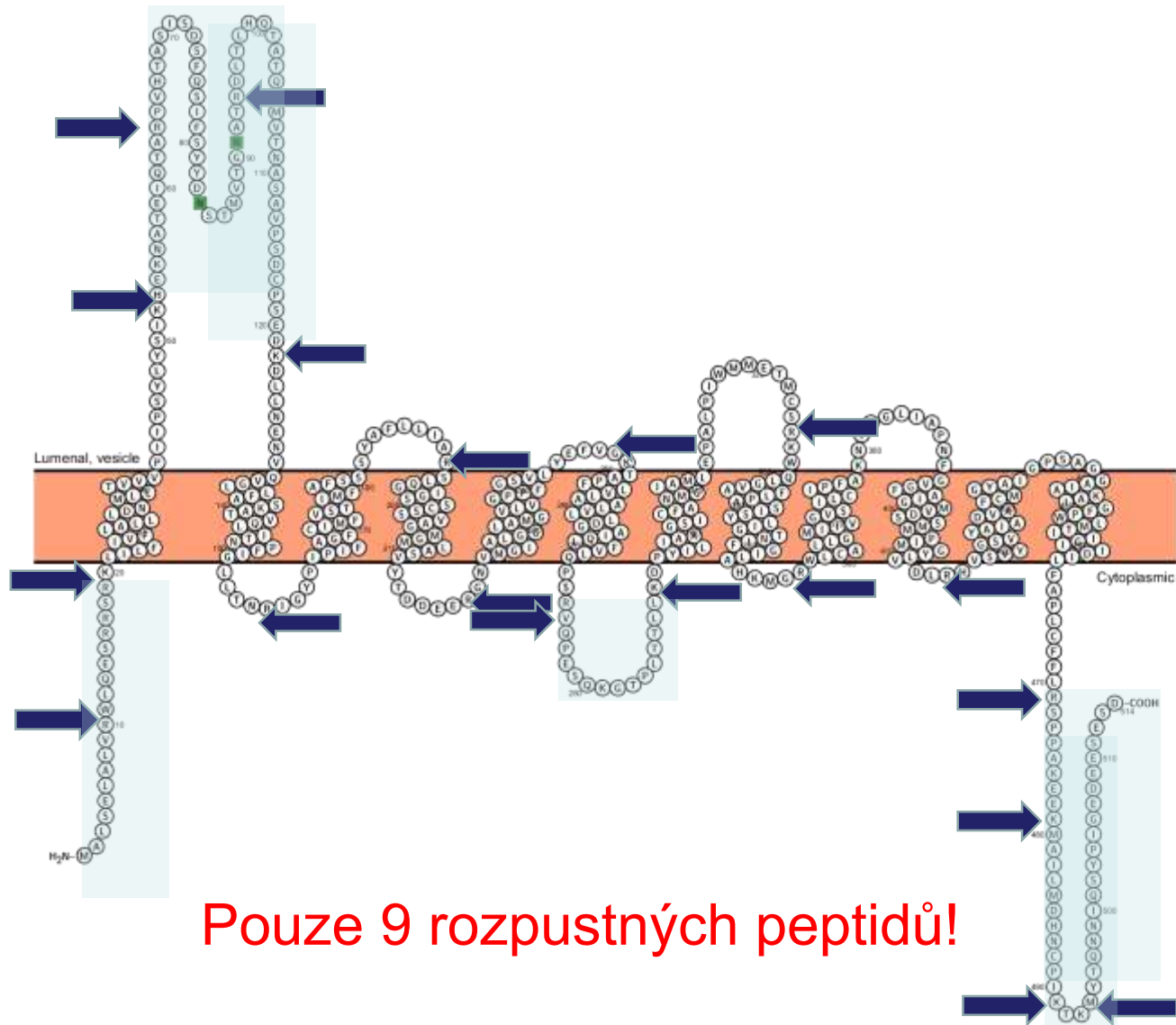
Synaptic vesicular amine transporter (Slc18a2)



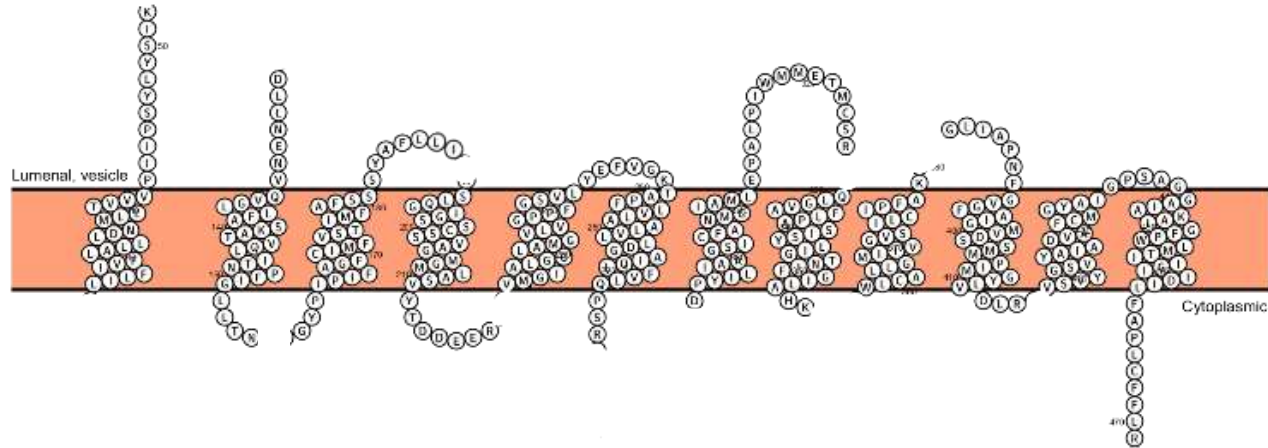
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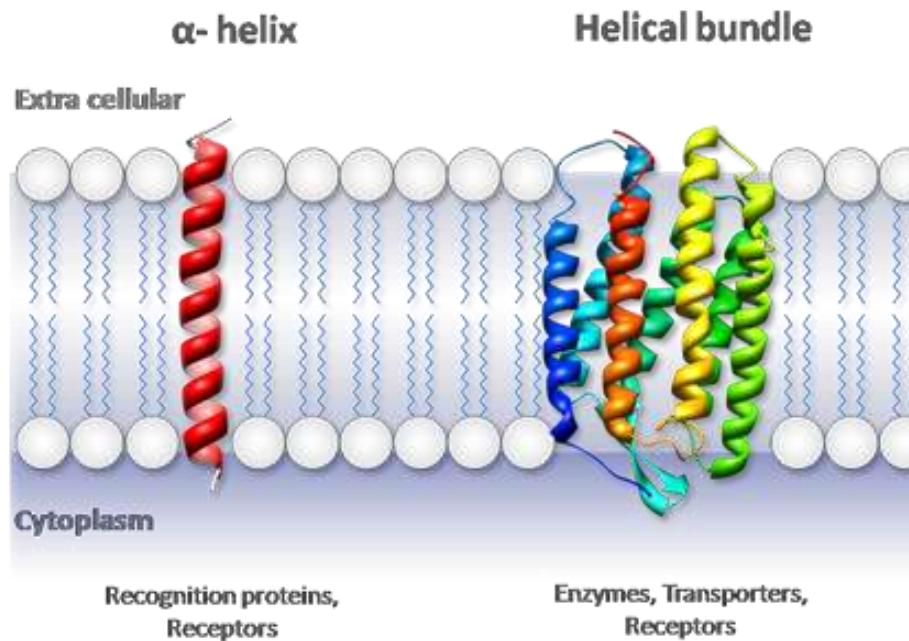
Synaptic vesicular amine transporter (Slc18a2)



Synaptic vesicular amine transporter (Slc18a2)



Pouze 9 tryptických rozpustných peptidů
81% celé sekvence je problematických



INTEGRAL MEMBRANE PROTEINS

- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression
- hydrophobic/amphipathic nature
- no Arg, Lys in TM domains
- detergents interfere with digestion and/or LC-MS
- **UNDER-REPRESENTED IN PROTEOMIC ANALYSES**

Překonávání nízké abundance a amfipatie TM proteinů

Izolace (obohacení) membrán

- centrifugace

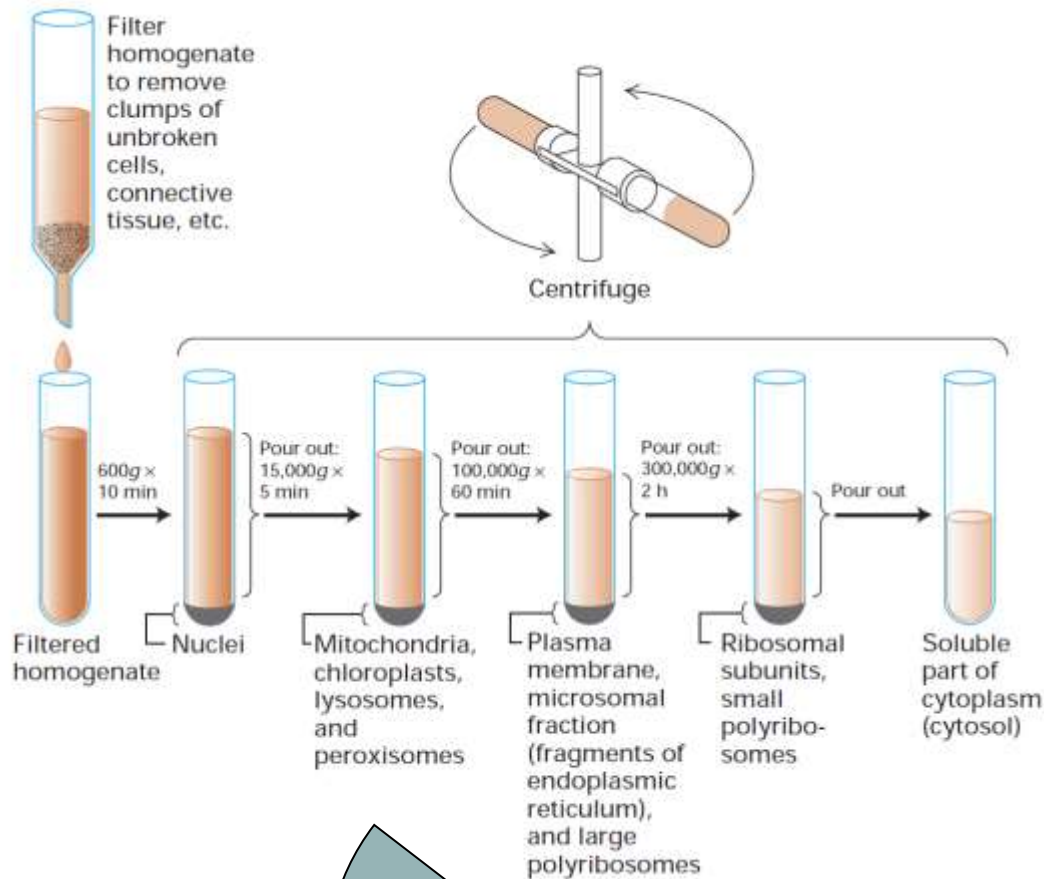
Obohacení/izolace TM proteinů

- „carbonate stripping“ - uhličitan sodný, vysoké pH
- delipidace (MetOH/chloroform)
- izolace povrchově značených (Ab, biotin, hydrazid, lektiny)

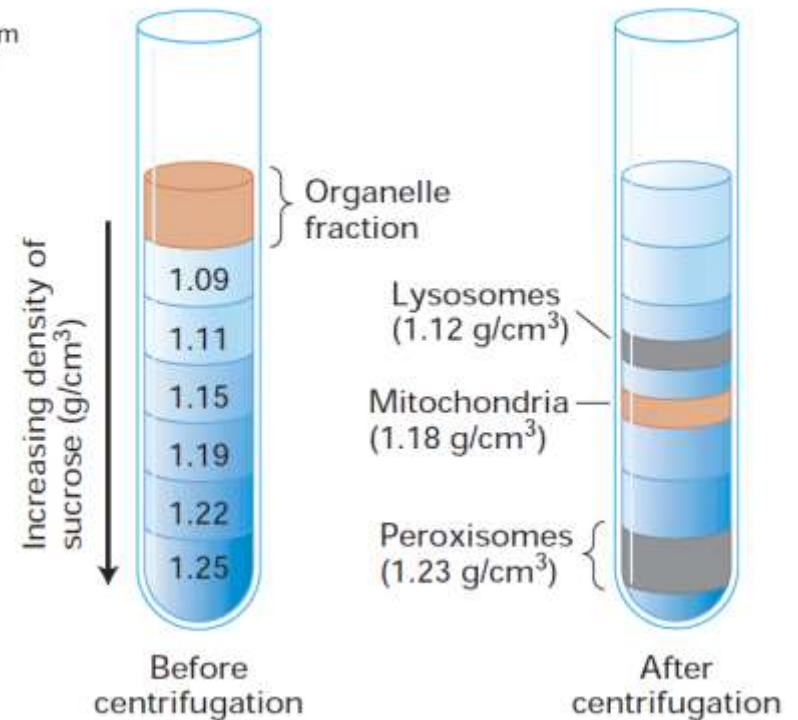
Solubilizace TM

- chaotropy
- detergenty (častá nekompatibilita s digescí, LC a MS!)
- organická rozpouštědla

Obohacení „membránových“ frakcí



- + majoritní cytosolické proteiny
- + cytoskelet
- + proteiny asociované s membránou





**Standard strategy
(targets intact proteins)**

Membrane fraction



Solubilization



Tryptic digestion



LC-MS/MS

**LOW ENRICHMENT
(10-15%)**

„Divide and conquer“
metody



**Standard strategy
(targets intact proteins)**



**Only the hydrophilic
segments**

Membrane fraction



Solubilization

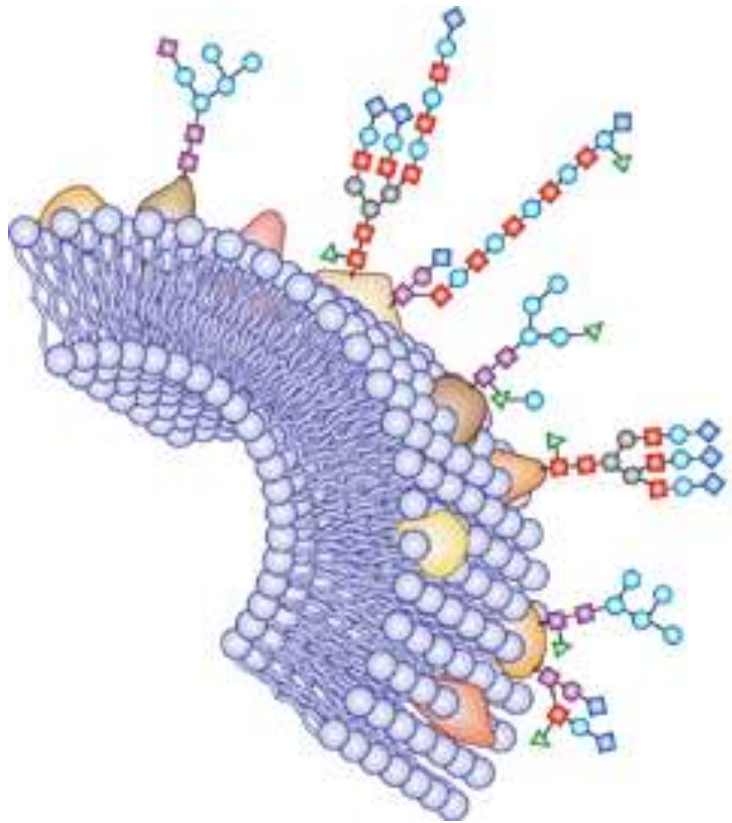


Tryptic digestion

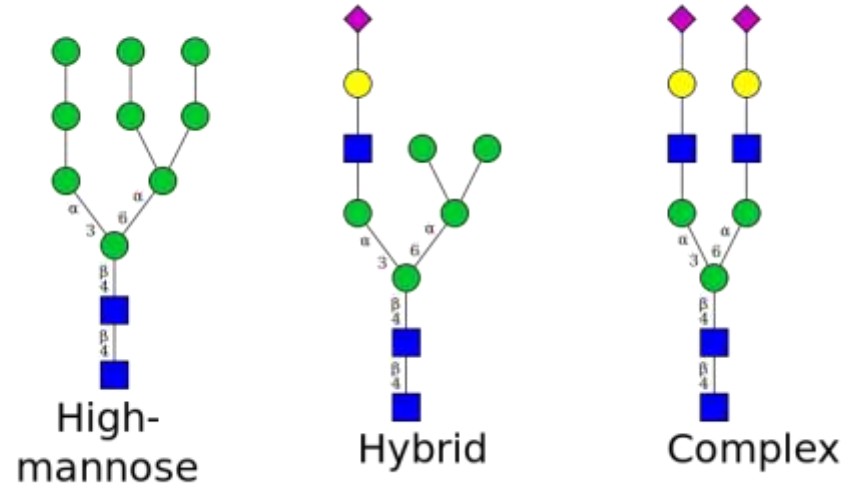


LC-MS/MS

**LOW ENRICHMENT
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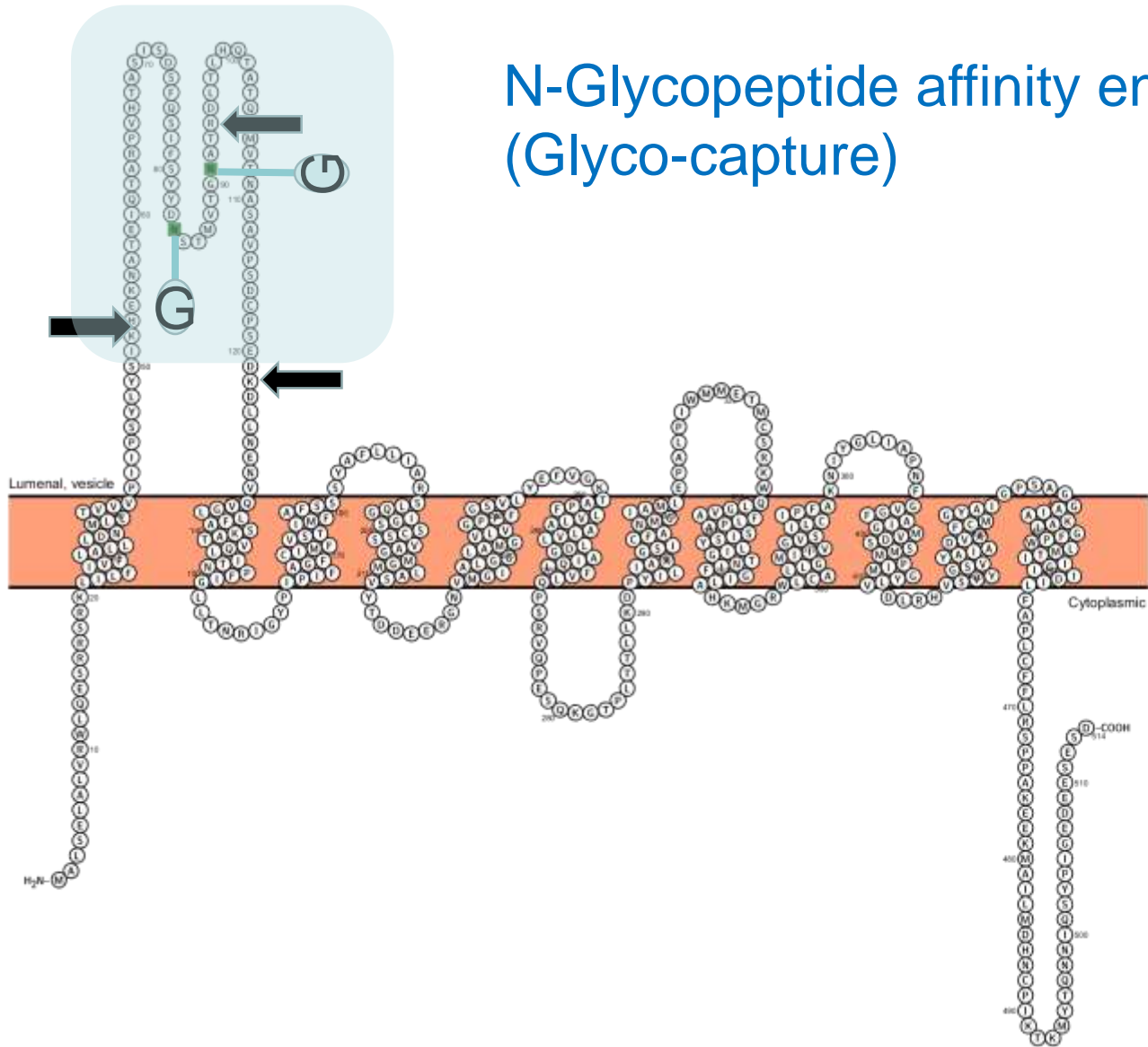
Three major types of N-Glycans



Asn-Xaa-Ser/Thr

“DIVIDE AND CONQUER“ METHODS

N-Glycopeptide affinity enrichment
(Glyco-capture)



N-GLYCOCAPTURE

Capture of N-glycopeptides
by immobilized **LECTINS**



Peptides released
by PNGase F
N-Glyco-FASP

Zielinska et al. Cell, 2010

Capture of N-glycopeptides
using **hydrazide chemistry**



Peptides released
by PNGase F
SPEG

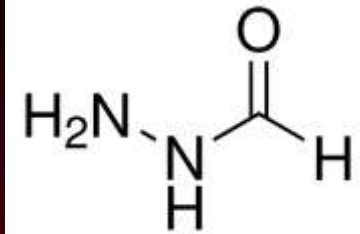
Zhang et al.
Nature Biotechnology, 2003

Glyco-capture

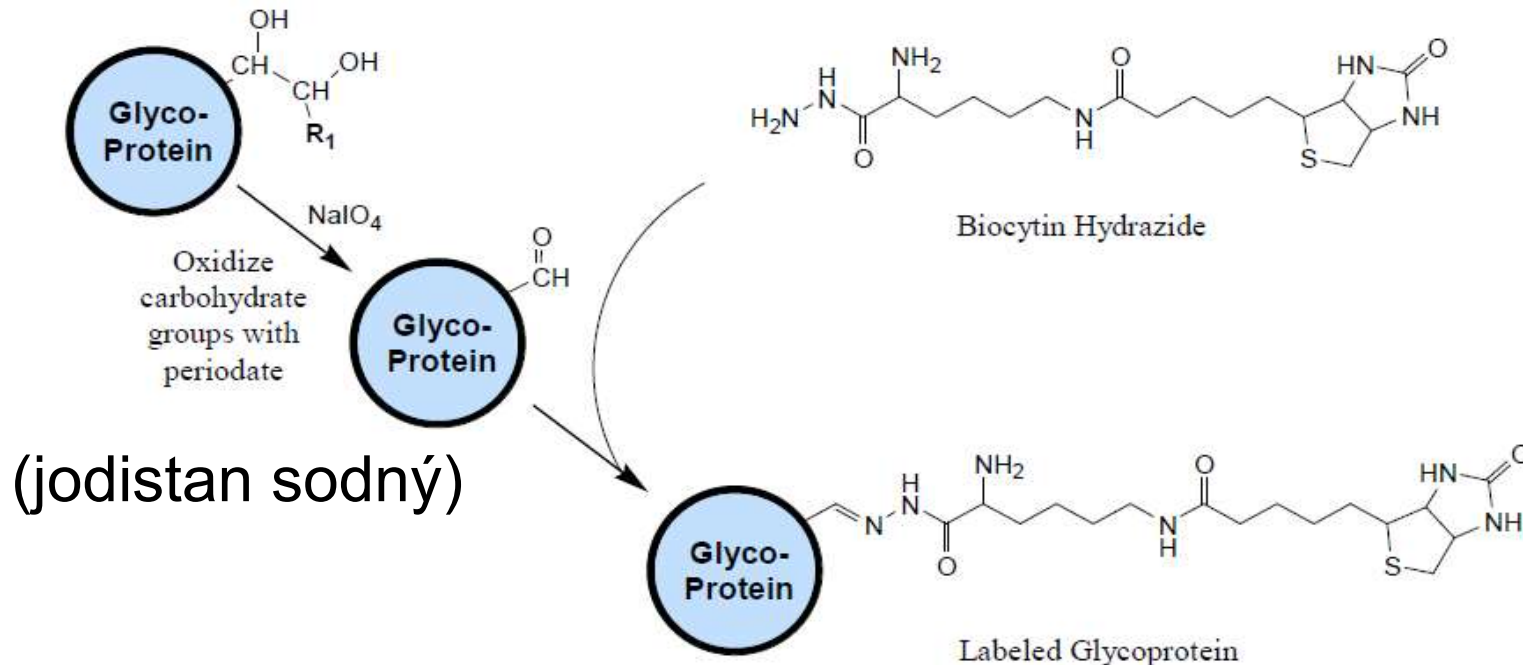
záchyt glykopeptidů z lyzátu

GLYCO-FASP — po digesci vzorku pomocí FASP jsou peptidy smíchány na filtru s **lektiny**. Neglykosylované peptidy jsou odmyty a glykopeptidy následně uvolněny PNGázou (**concanavalin A, WGA (wheat germ agglutinin), RCA (Ricinus communis agglutinin)**)

Cukry



hydrazid



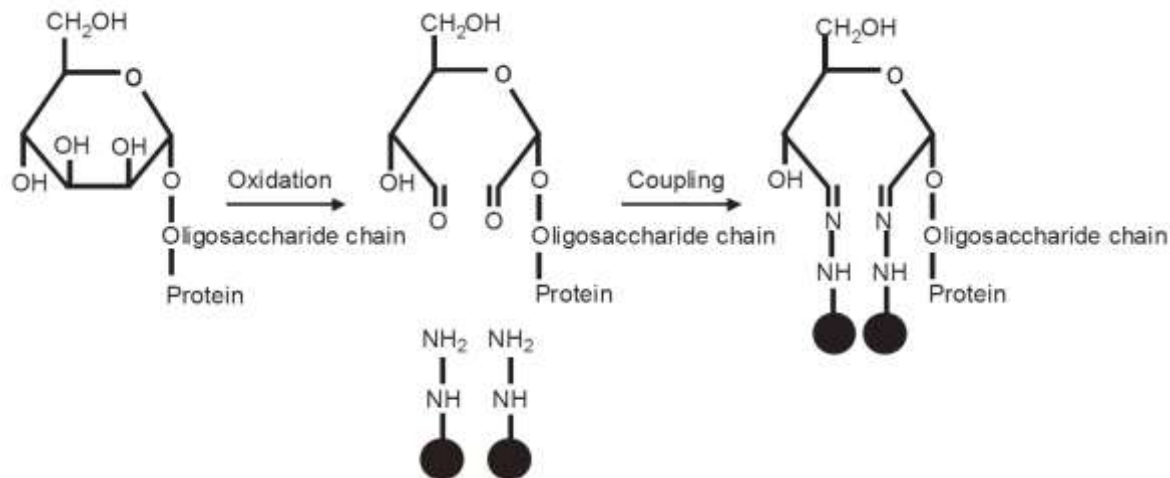
Biotin Hydrazide bind to oxidized carbohydrates through the hydrazide group ($-\text{NH}-\text{NH}_2$), forming a hydrazone linkage. Oxidation of glycoproteins generates reactive aldehydes that react specifically with hydrazide groups.

Glyco-capture

záchyt glykopeptidů z lyzátu

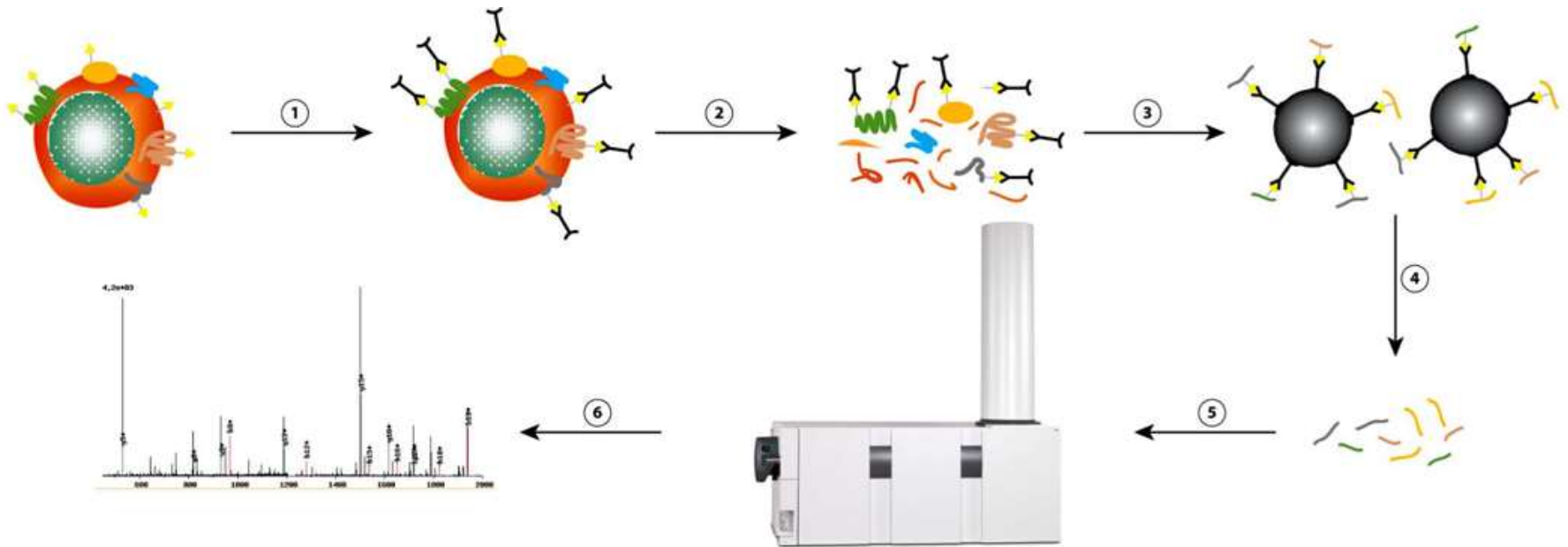
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(**concanavalin A**, **WGA (wheat germ agglutinin)**, **RCA (Ricinus communis agglutinin)**)

SPEG — vychytání **oxidovaných** glykopeptidů na **kuličky s hydrazidem**, odmytí neglykosylovaných peptidů a následná eluce PNGázou



Cell Surface Capturing

biocytin hydrazide and avidin beads



200-800 identified cell surface membrane proteins



Classic strategy

Membrane fraction



Solubilization



Tryptic digestion



LC-MS/MS



Only the hydrophilic segments (GLYCOCAPTURE)

Solubilization



Digestion



Isolation of N-glycopeptides



LC-MS/MS



Only the hydrophobic segments

?



Classic strategy

Membrane fraction



Solubilization



Tryptic digestion



LC-MS/MS



**Only the hydrophilic segments
(GLYCOCAPTURE)**

Solubilization



Digestion



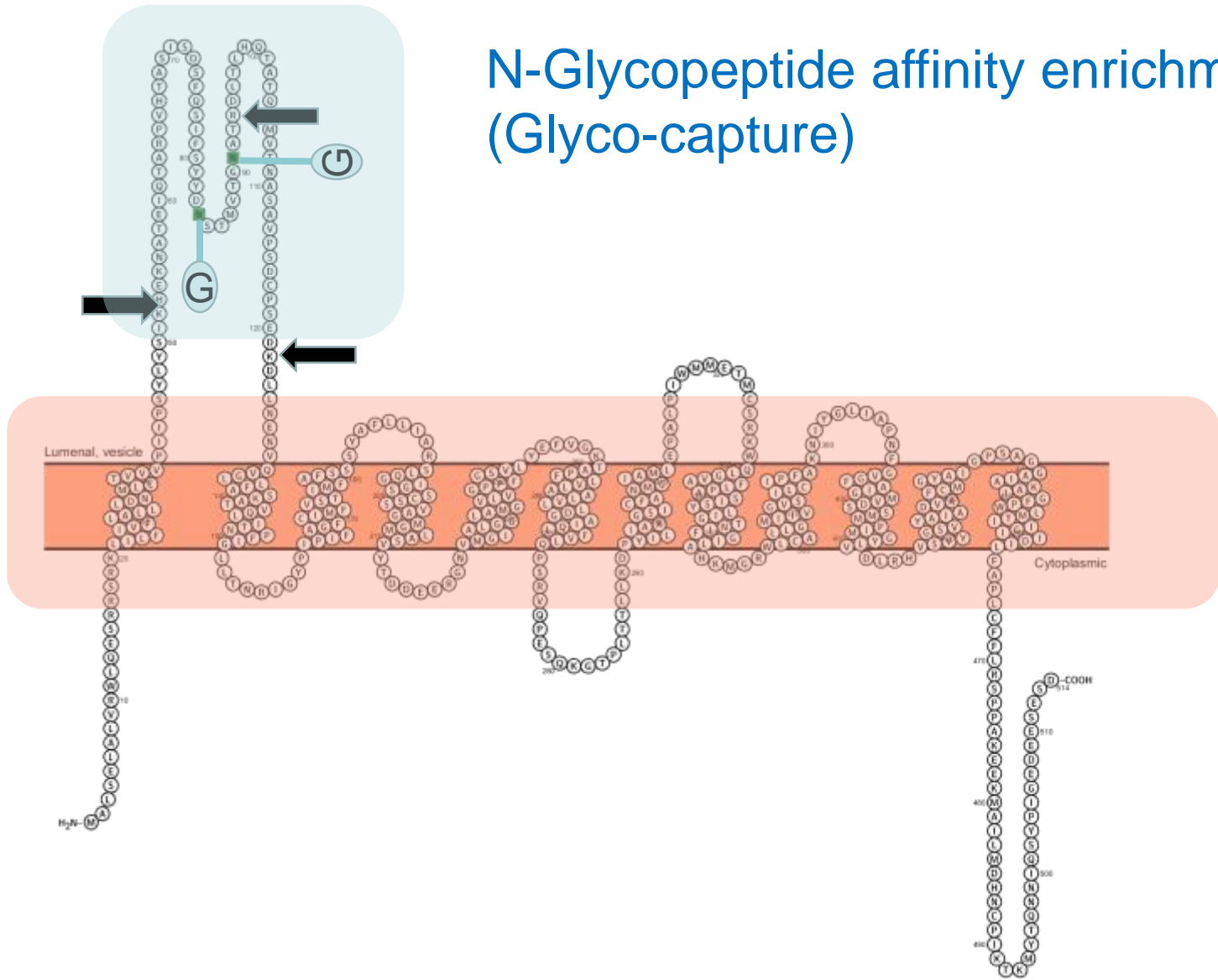
Isolation of N-glycopeptides



LC-MS/MS

“DIVIDE AND CONQUER“ METHODS

N-Glycopeptide affinity enrichment
(Glyco-capture)



Identification of IMPs via
enrichment of membrane-embedded segments
hpTC method
(High pH-Trypsin-CNBr)

Vit O, et al. J Proteomics. 2016, 21;149:15-22.

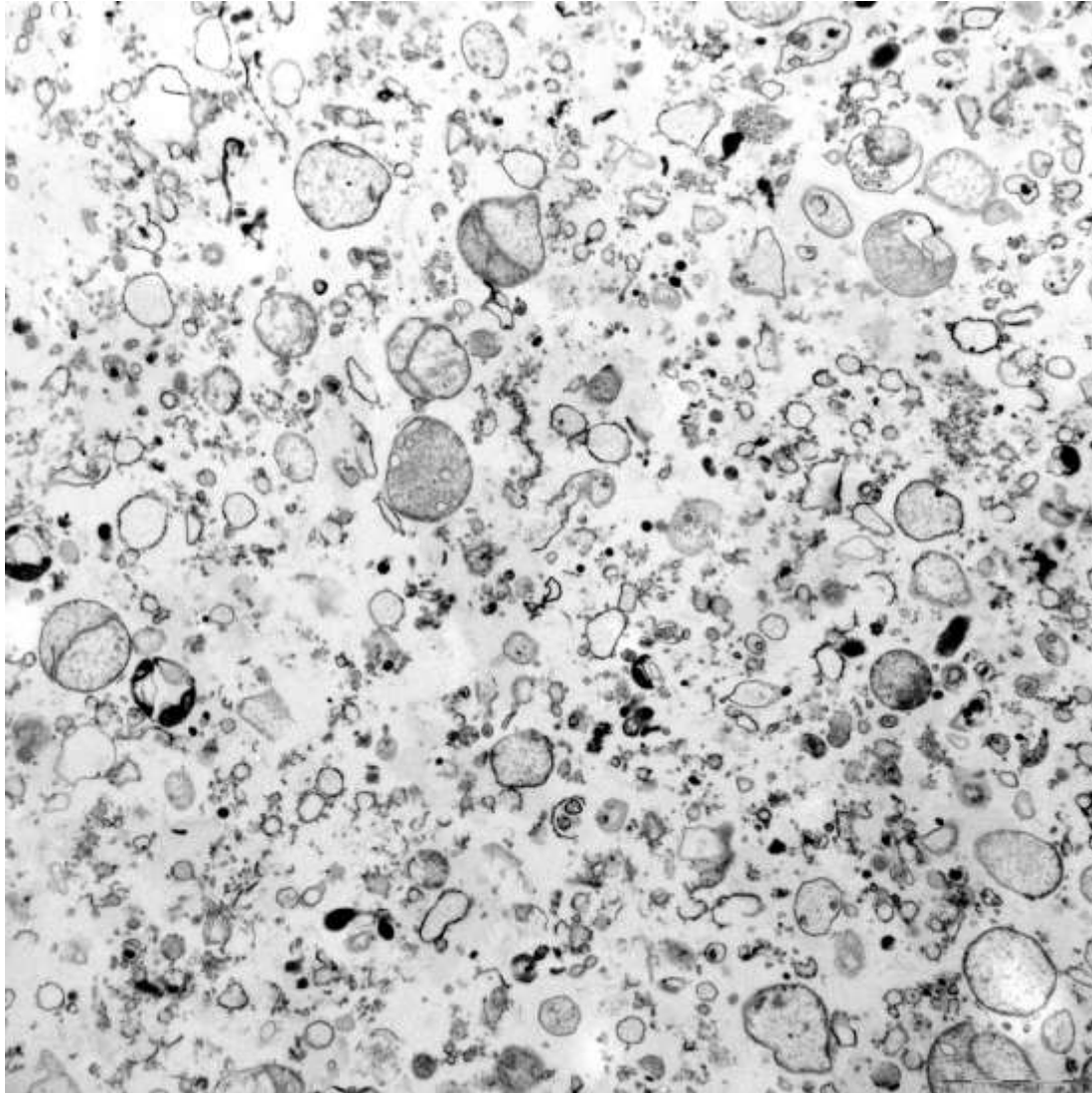
Blackler AR, et al. J Proteome Res. 2008, 7(7):3028-34.

hpTC method (high pH-Trypsin-CNBr)



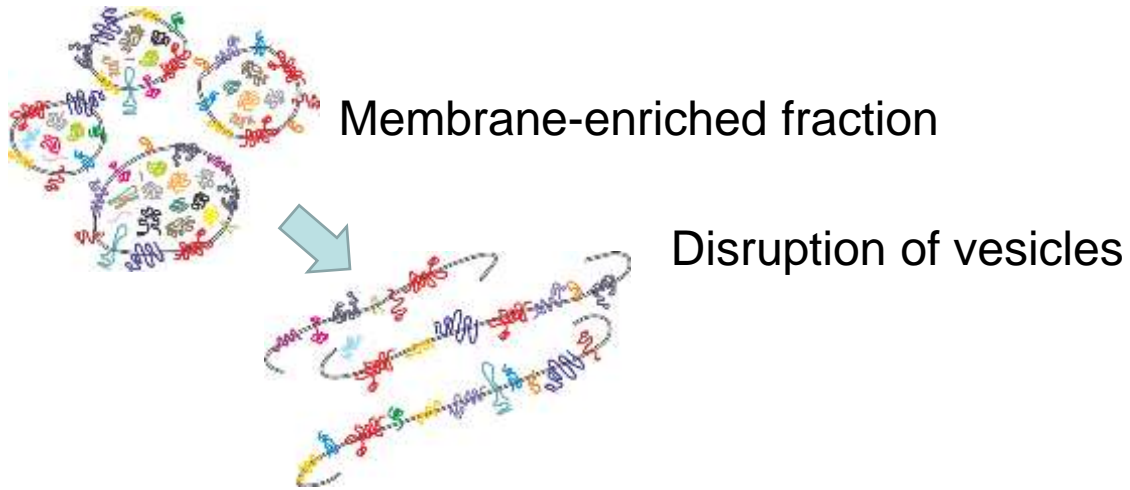
Membrane-enriched fraction

Vit O. et al., *Journal of Proteomics* 2016
Blackler A et al., *J Proteome Res.* 2008

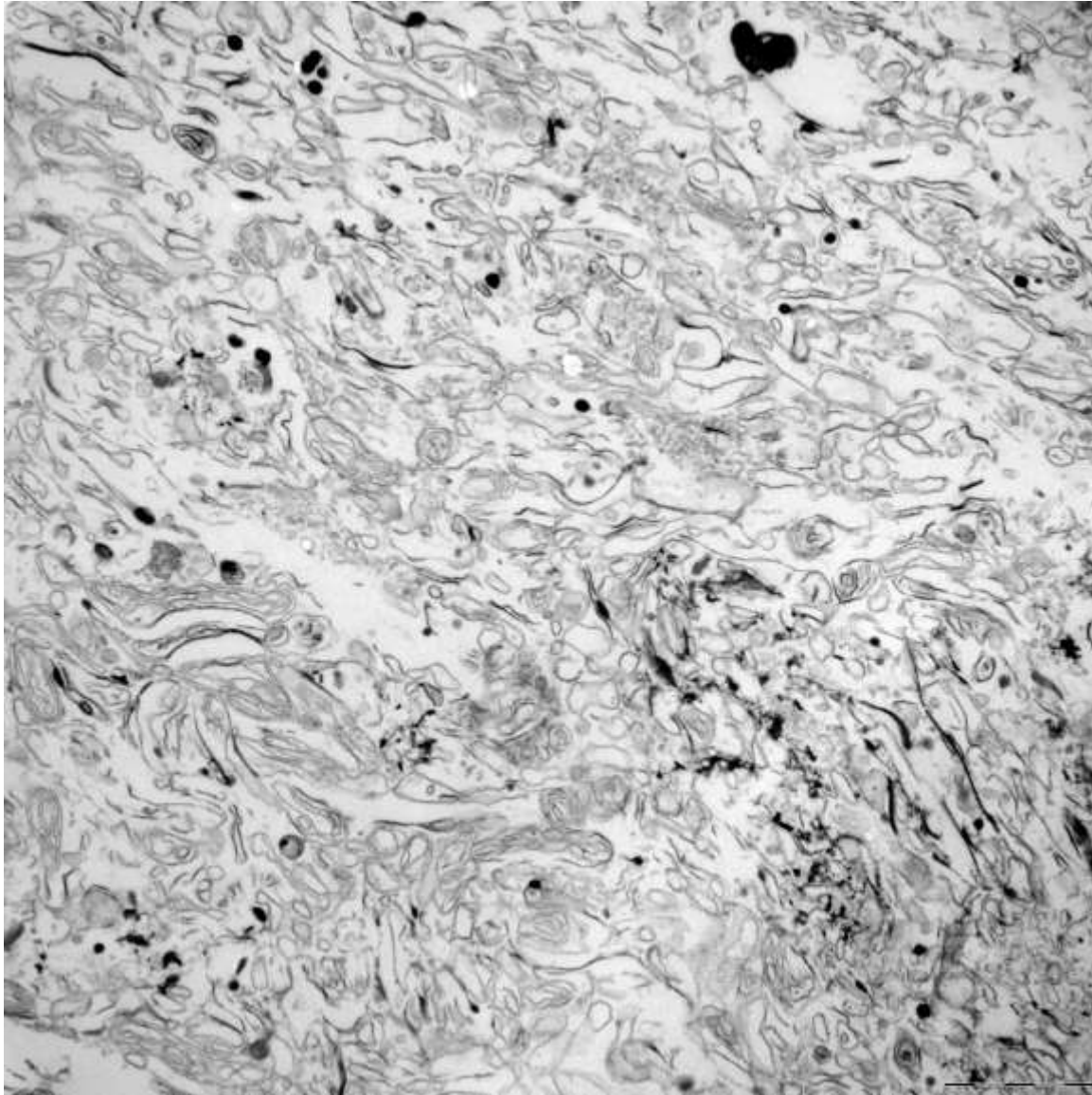


pH 7.4

hpTC method (high pH-Trypsin-CNBr)

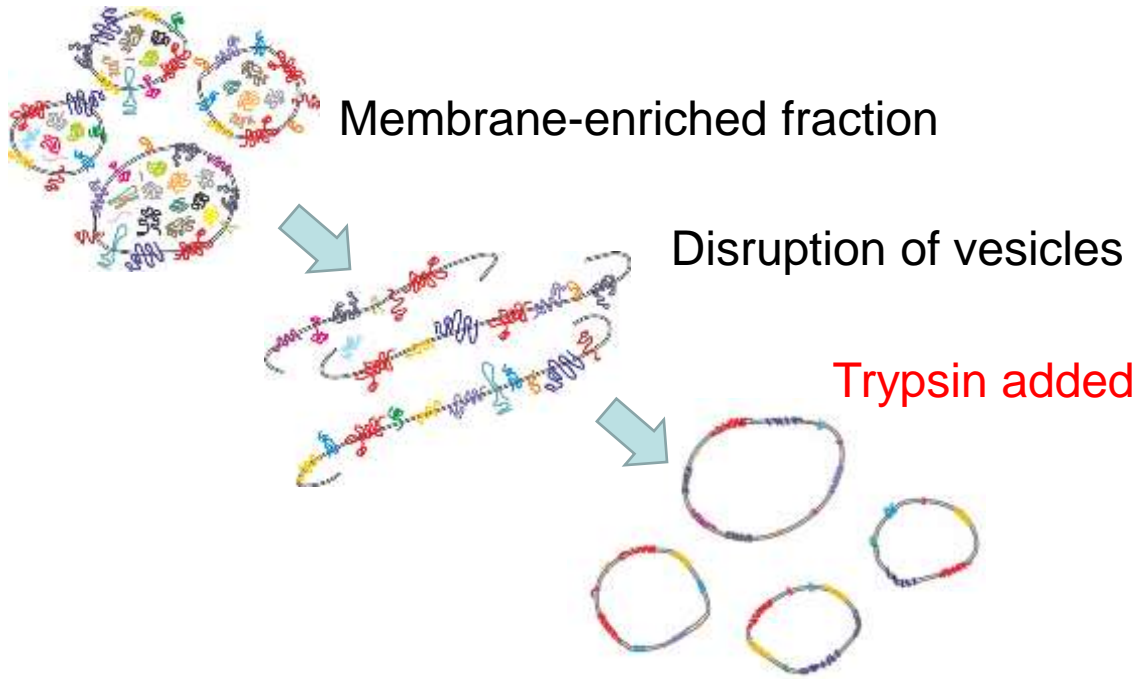


Vit O. et al., *Journal of Proteomics* 2016
Blackler A et al., *J Proteome Res.* 2008



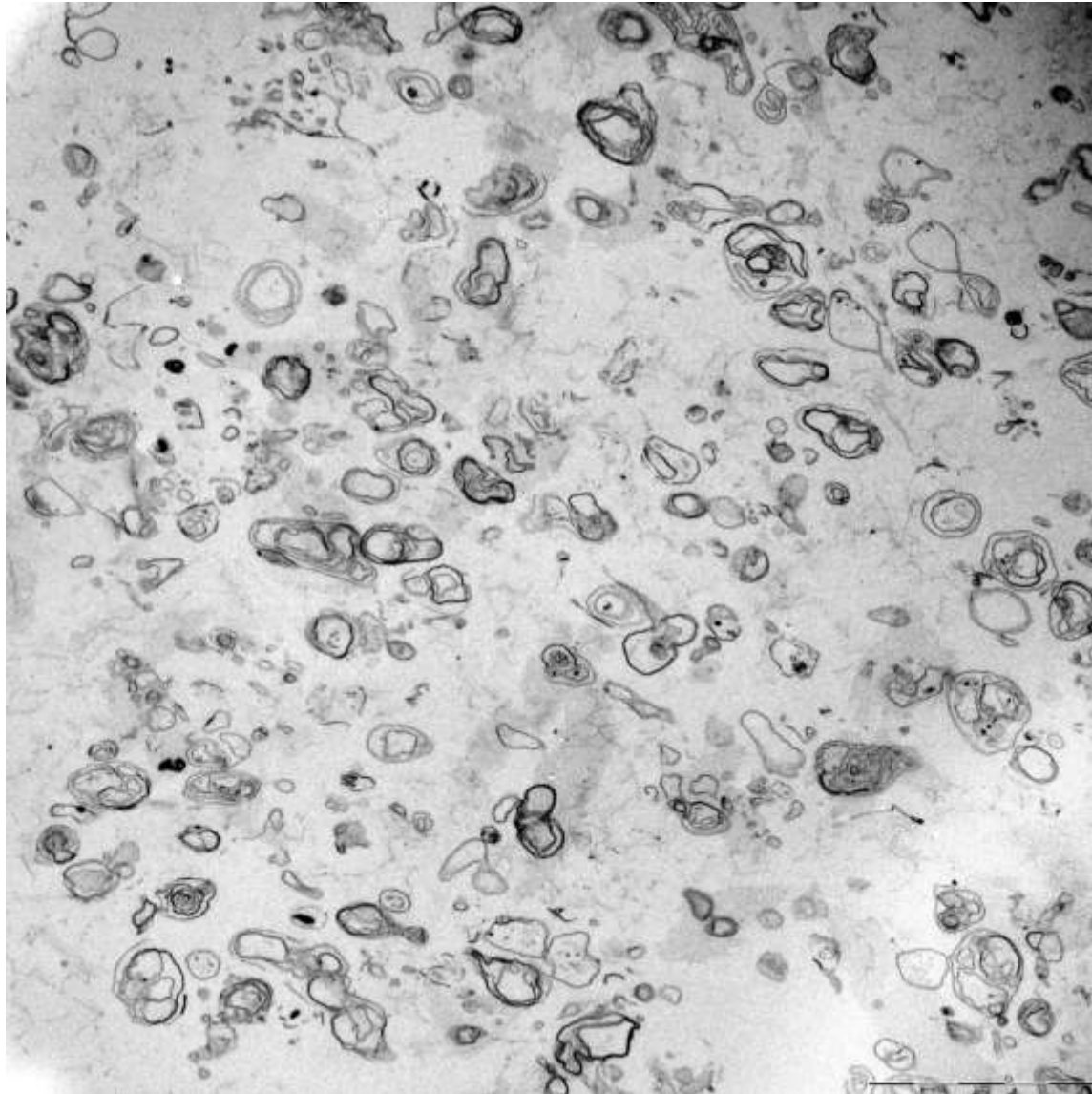
Na ledu
 Na_2CO_3
pH 11

hpTC method (high pH-Trypsin-CNBr)



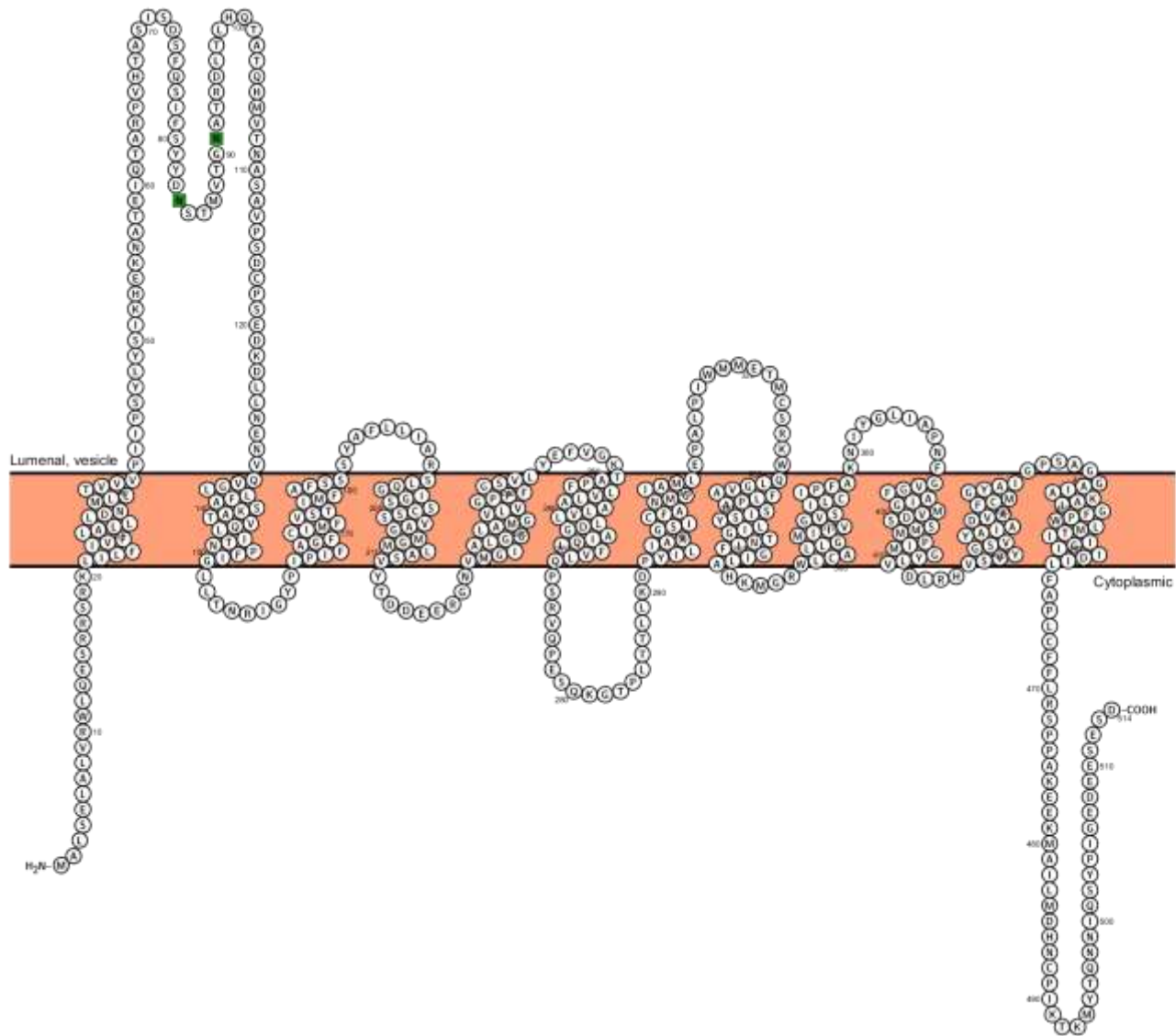
Vit O. et al., *Journal of Proteomics* 2016
Blackler A et al., *J Proteome Res.* 2008



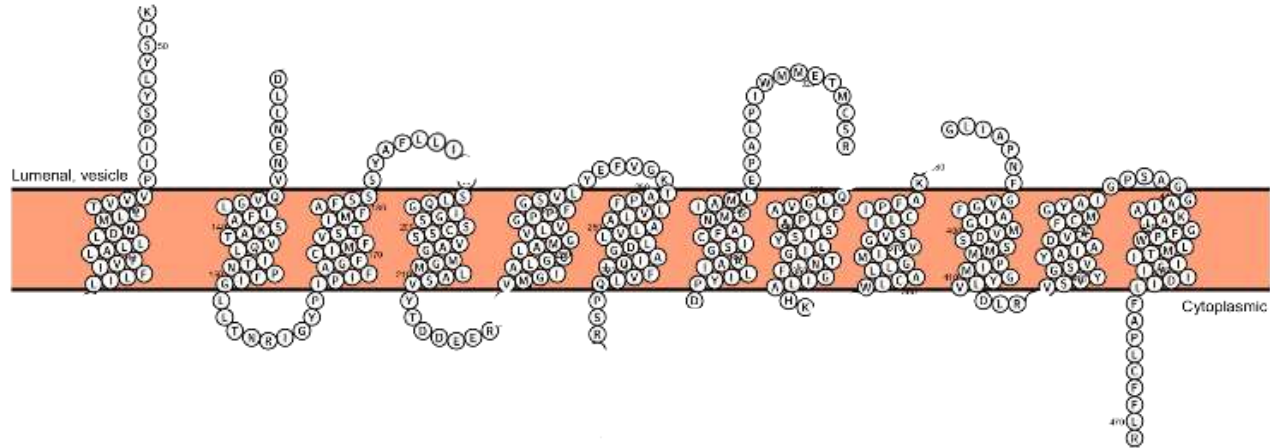


37 °C
trypsin

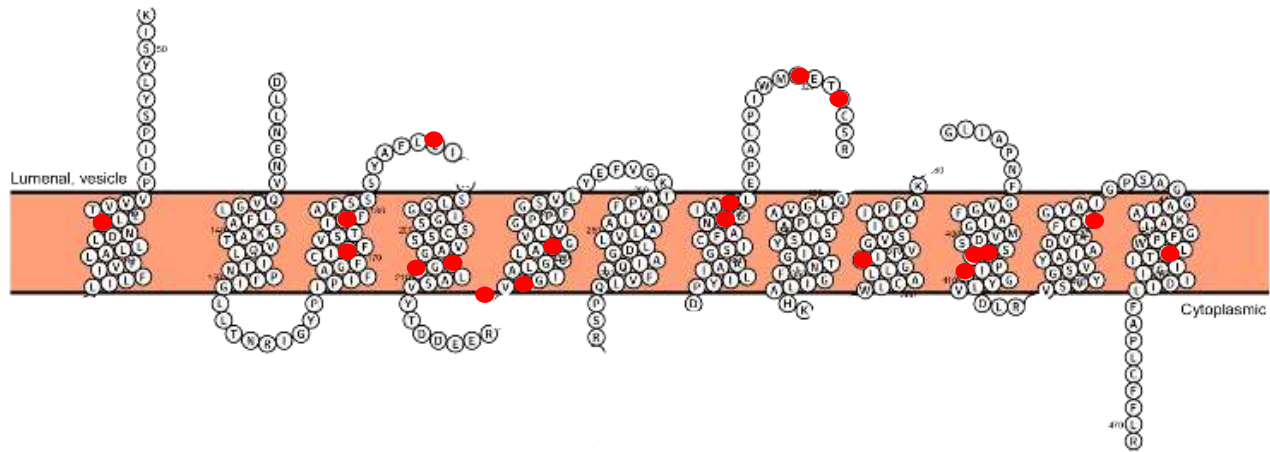
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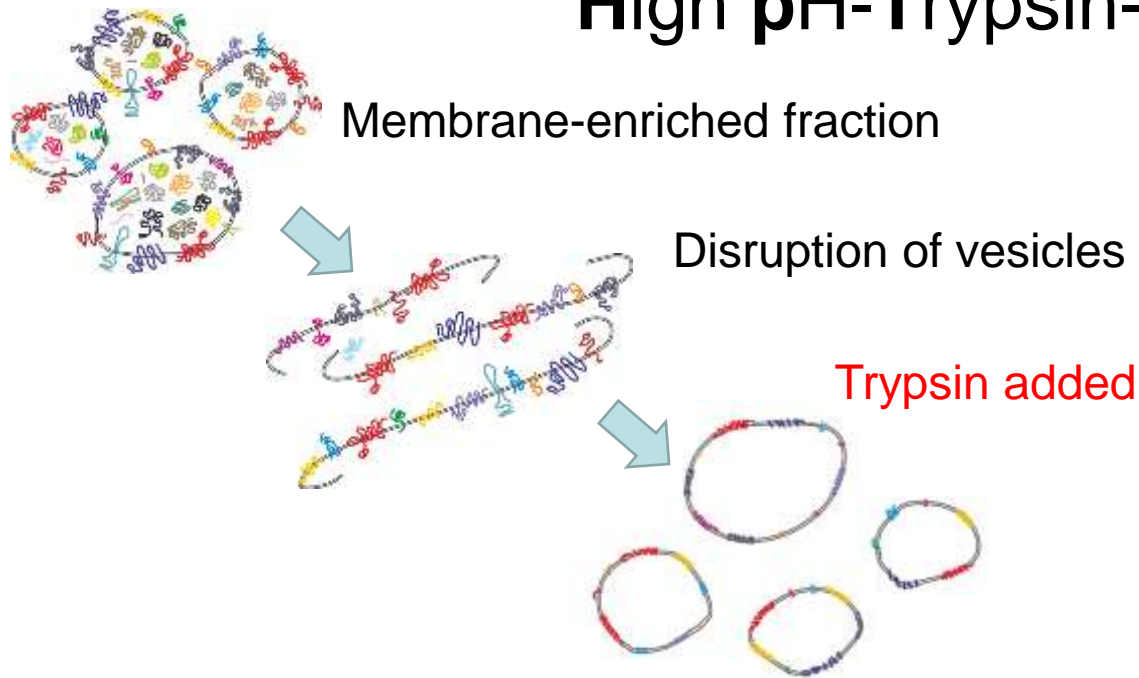
Synaptic vesicular amine transporter (Slc18a2)



Chemical cleavage of peptides by **CNBr** at **Met**

HpTC method

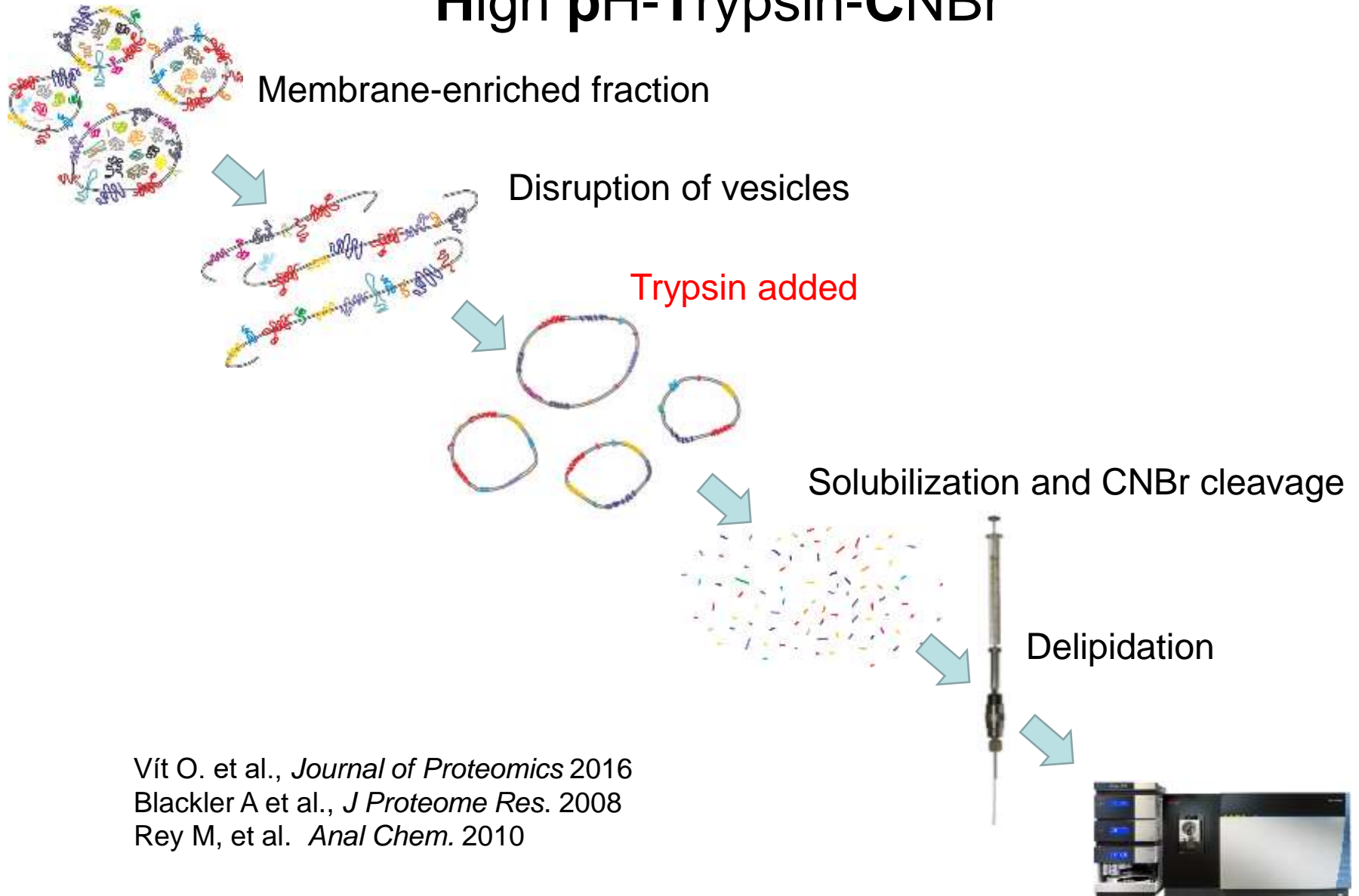
High pH-Trypsin-CNBr



Vit O. et al., *Journal of Proteomics* 2016
Blackler A et al., *J Proteome Res.* 2008

HpTC method

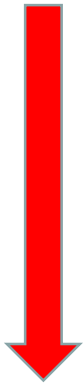
High pH-Trypsin-CNBr



Vit O. et al., *Journal of Proteomics* 2016
Blackler A et al., *J Proteome Res.* 2008
Rey M, et al. *Anal Chem.* 2010

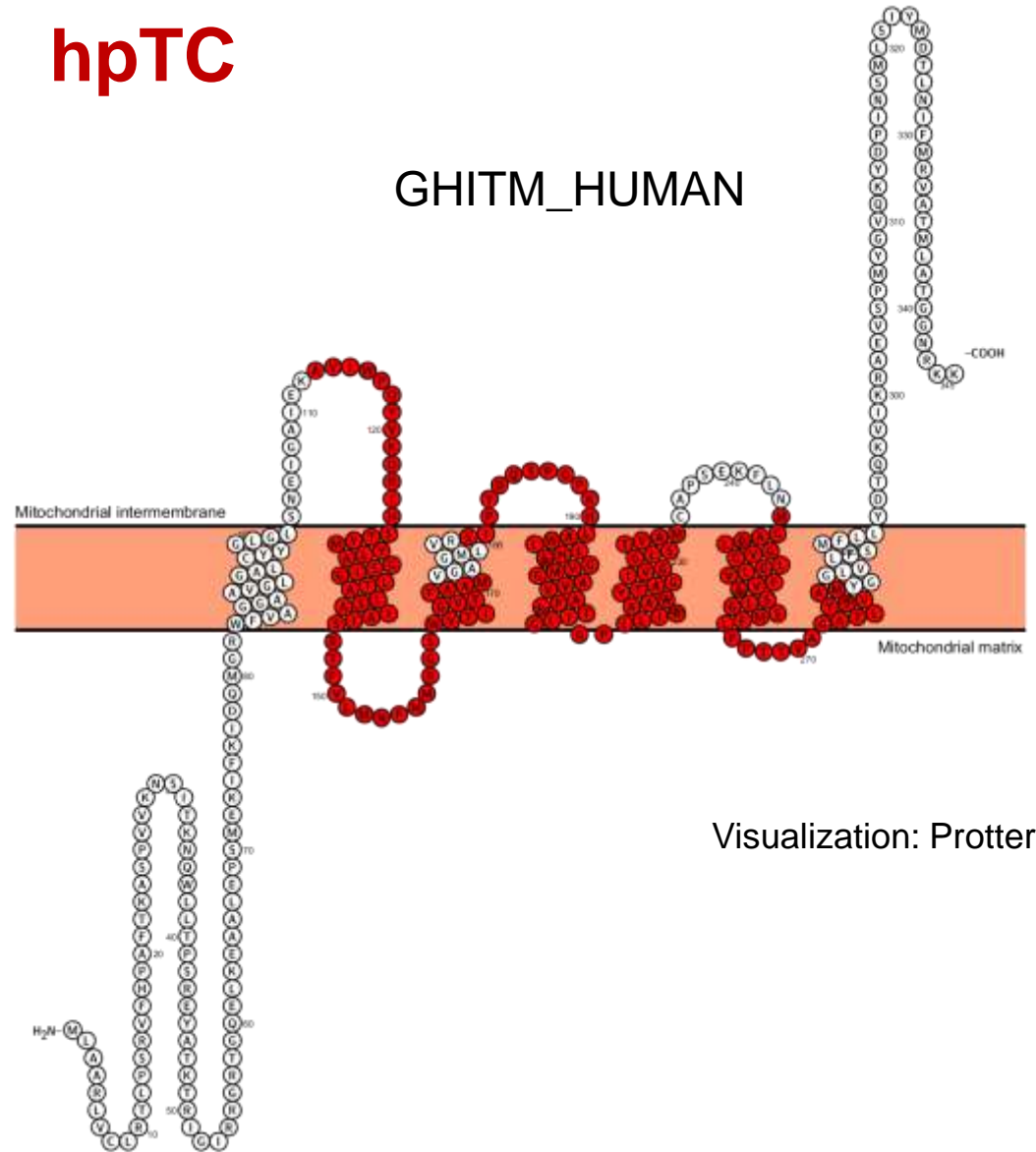
hpTC

Only the hydrophobic segments
(hpTC)



600-1000 IMPs
in various tissues

IMP Enrichment
40-60%

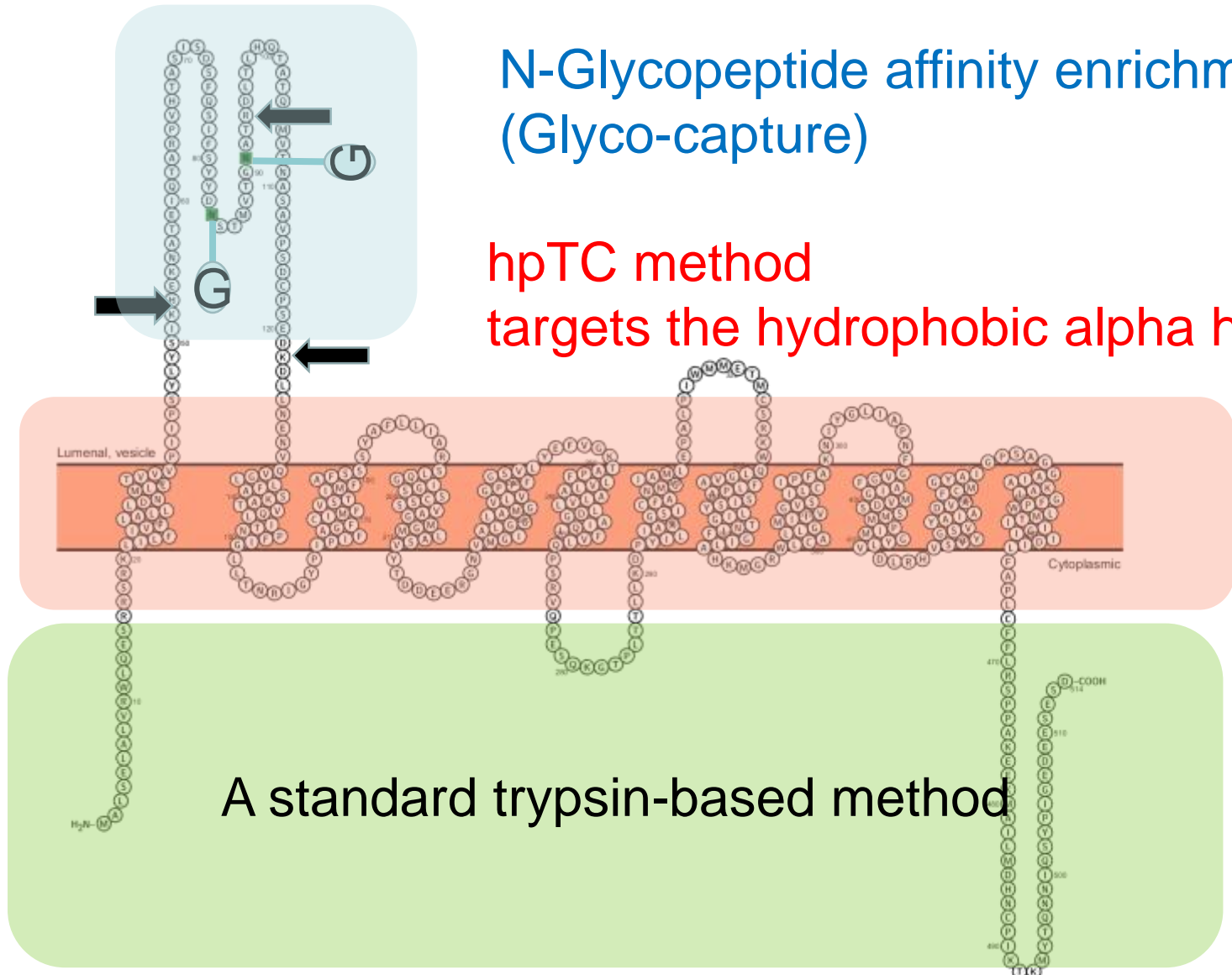


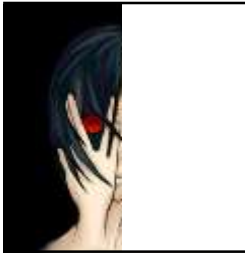
Visualization: Protter

“DIVIDE AND CONQUER“ METHODS

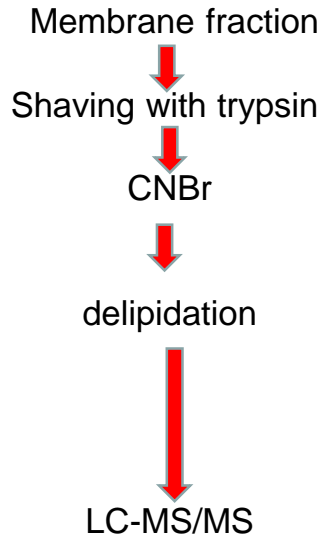
N-Glycopeptide affinity enrichment
(Glyco-capture)

hpTC method
targets the hydrophobic alpha helices

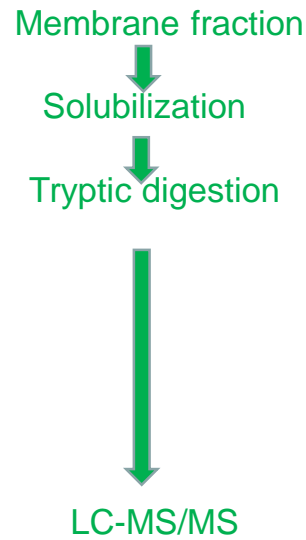




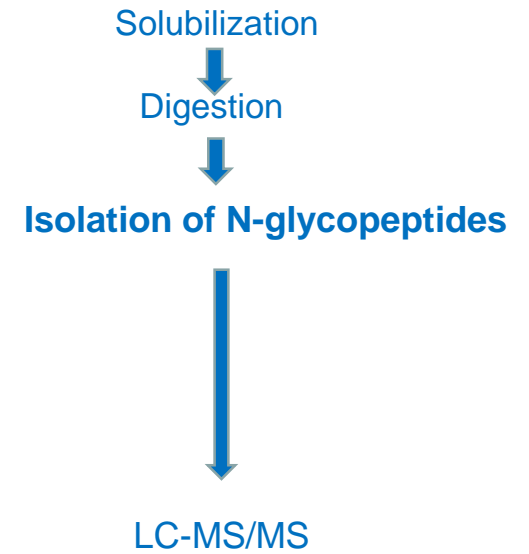
Only the hydrophobic segments



Classic strategy



**Only the hydrophilic segments
(GLYCOCAPTURE)**



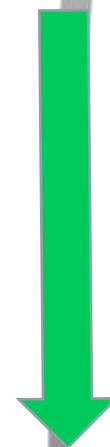
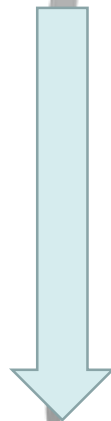
THE PITCHFORK STRATEGY

(Vit et al, J. Proteomics, 2019)

Hydrophilic/glycopeptides
(GLYCO-CAPTURE)

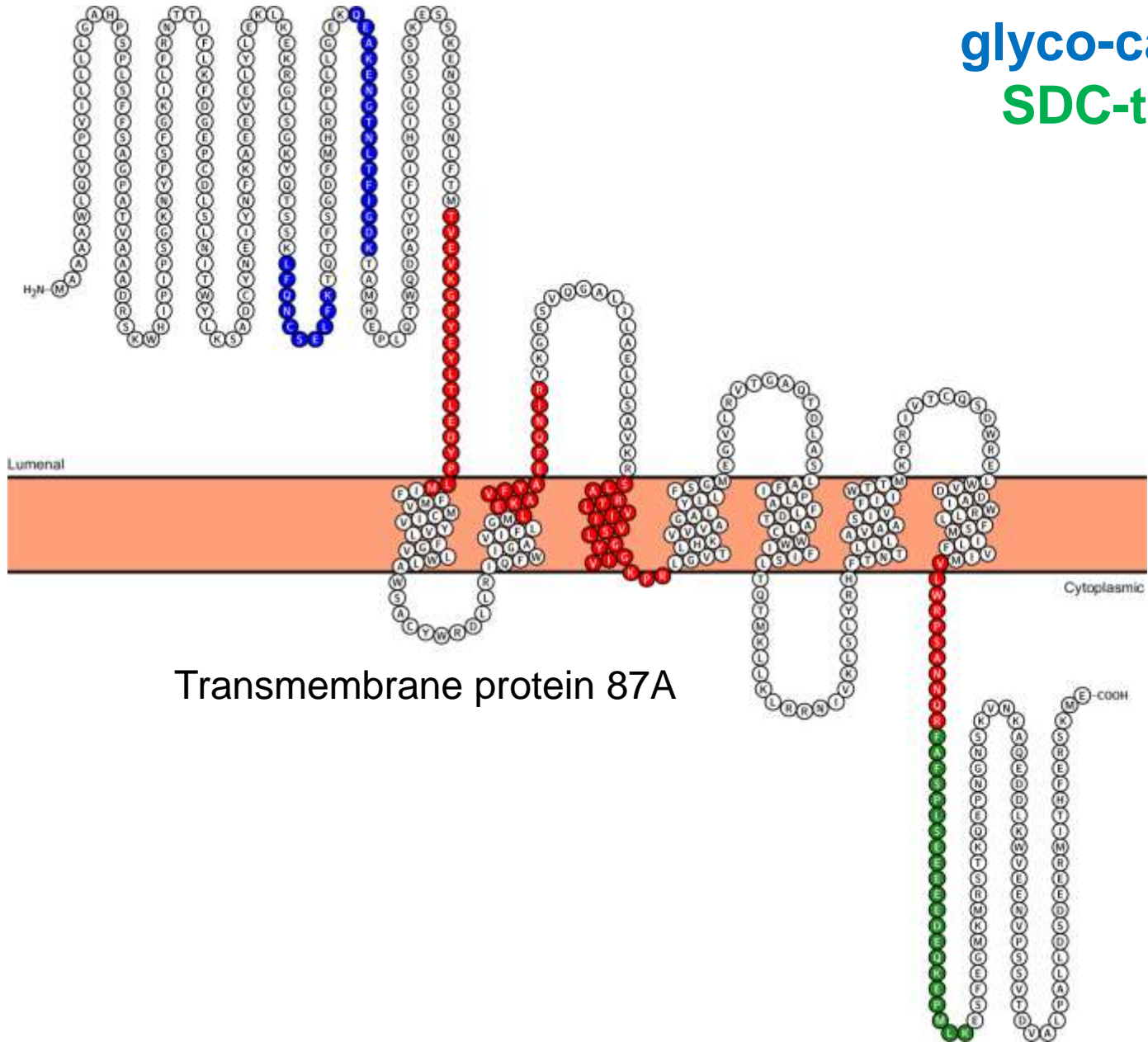
Hydrophobic
segments
(hpTC)

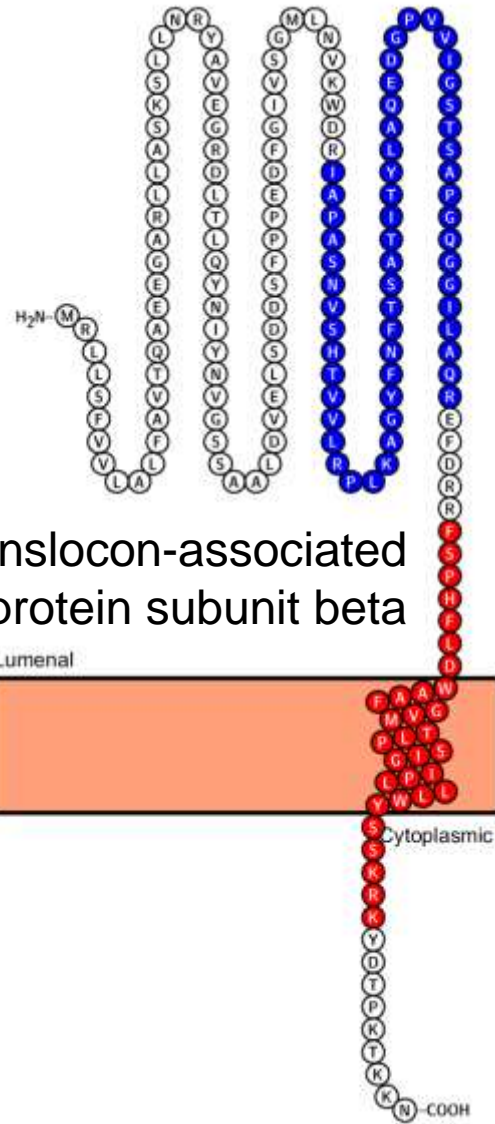
Non-glycosylated
hydrophilic peptides
“Classic strategy”
(trypsin and detergent)



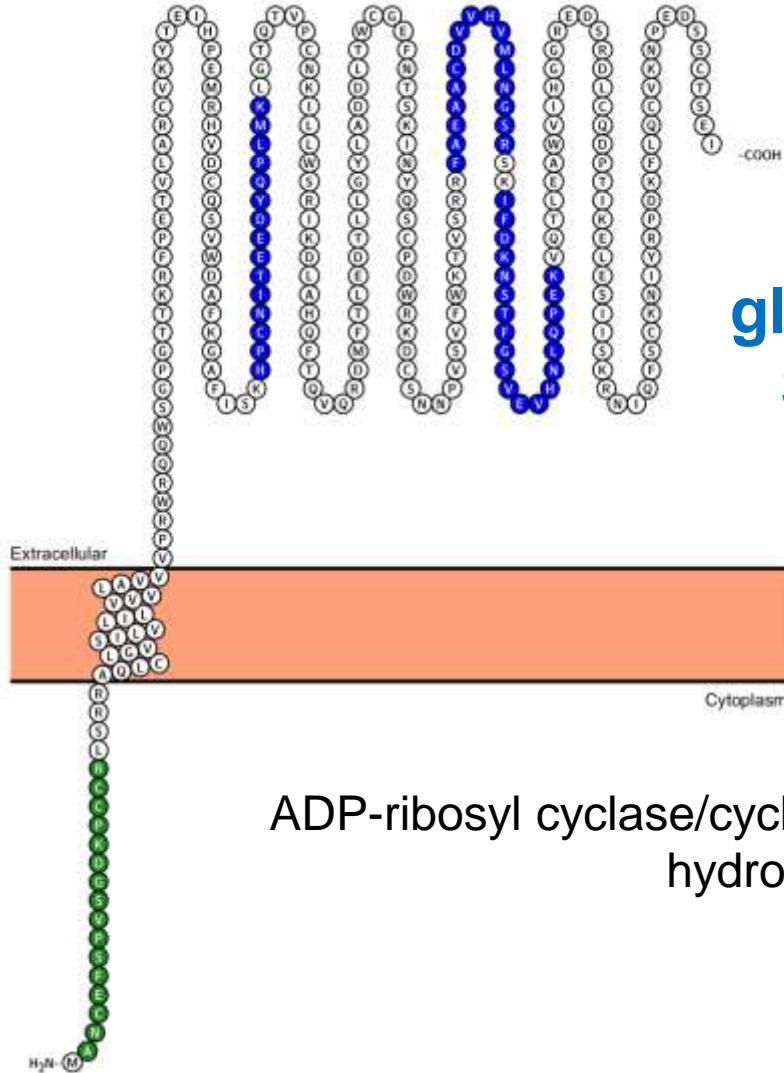
LC-MS/MS

hpTC
glyco-capture
SDC-trypsin





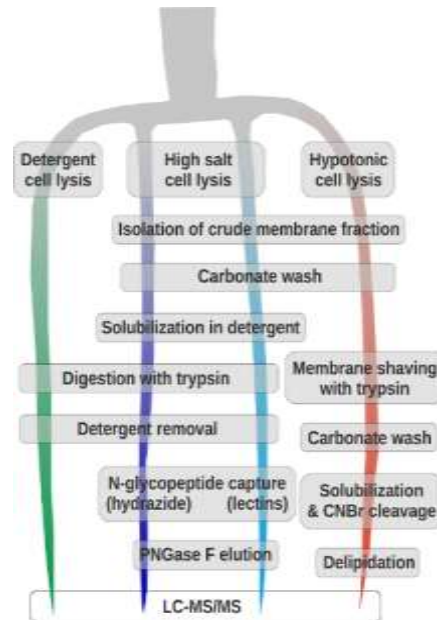
Translocon-associated protein subunit beta



ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1 (CD38)

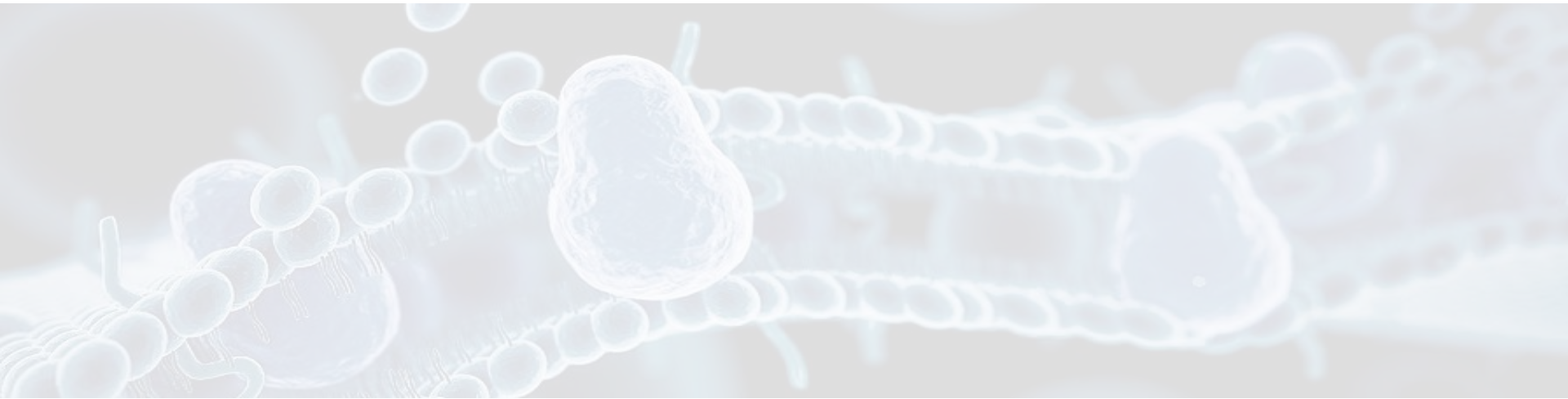
hpTC
glyco-capture
SDC-trypsin

The Pitchfork strategy



- 800-1300 IMPs identified in various human tissue samples
- IMPs from all compartments
- Applicable to any cellular material, fresh or frozen
- No bias toward number of TM domains

Looking for new theranostic targets in human Pheochromocytoma and Paraganglioma



BIOCEV

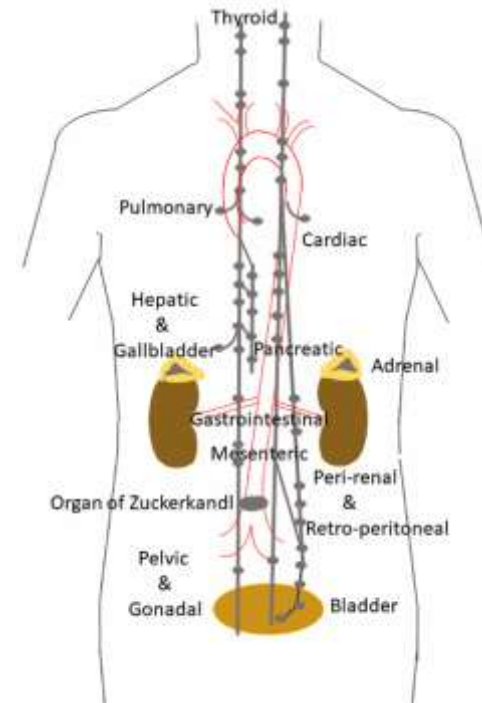
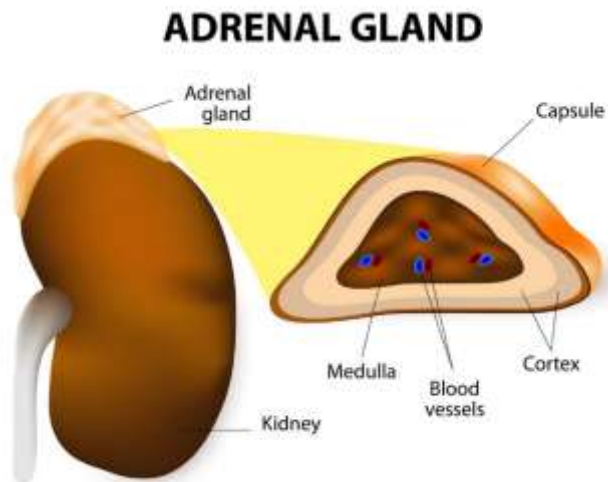
Biotechnology and Biomedicine Centre of the Academy
of Sciences and Charles University in Vestec



Eunice Kennedy Shriver National Institute
of Child Health and Human Development

PHEOCHROMOCYTOMA and PARAGANGLIOMA

- Rare neuro-endocrine tumors (0.8/100,000)
- From chromaffin tissue of adrenal medulla (PHEO) or sympathetic ganglia (PGL)
- From parasympathetic ganglia (PGL)



PHEOCHROMOCYTOMA and PARAGANGLIOMA

- Rare **neuro-endocrine tumors**
- From **chromaffin tissue of adrenal medulla (PHEO) or sympathetic ganglia (PGL)**
- From parasympathetic ganglia (PGL)
- Catecholamine producing tumors (dopamine, noradrenaline, adrenaline)
- Up to 25 % are malignant, even benign disease has high mortality
- **Therapy is limited for patients with metastatic disease**

PHEOCHROMOCYTOMA and PARAGANGLIOMA

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NEW DRUG TARGETS ARE NEEDED

INTEGRAL MEMBRANE PROTEINS ARE EXCELLENT DRUG TARGETS

PHEOCHROMOCYTOMA and PARAGANGLIOMA

Distinct molecular subtypes of based on mutations, mRNA expression...

Cluster 1.	Pseudohypoxia (<i>SDHx, VHL, FH, HIF2A, EGLN1...</i>)
Cluster 2.	Kinase signaling (<i>RET, MAX, NF1, HRAS, TMEM127</i>)
Cluster 3.	Wnt altered (<i>UBTF-MAML3, CSDE1</i>)
Unassigned	Patients with no mutation in the PPGL susceptibility genes



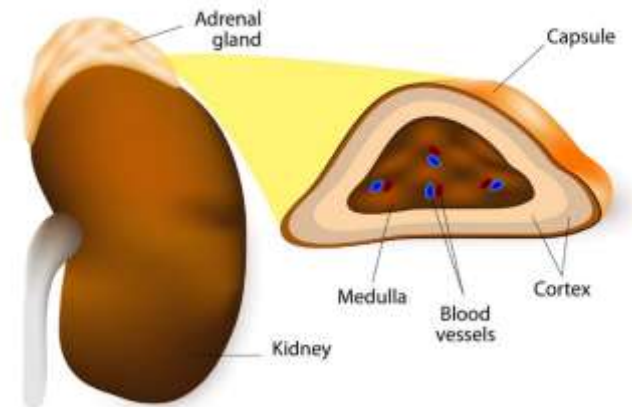
No mutation
Cluster 2
Cluster 1

Patient No.	Sex	Tumor Type	PPGL Cluster	Mutated Gene
1	F	PGL	1	<i>SDHB</i>
2	F	PGL	1	<i>SDHB</i>
3	M	PGL	1	<i>SDHB</i>
4	M	PGL	1	<i>SDHB</i>
5	M	PGL	1	<i>SDHB</i>
6	F	PGL	1	<i>SDHB</i>
7	M	PGL	1	<i>SDHB</i>
8	F	PGL	1	<i>SDHB</i>
9	F	PGL	1	<i>SDHB</i>
10	M	PHEO	1	<i>VHL</i>
11	F	PHEO	1	<i>VHL</i>
12	F	PHEO	1	<i>VHL</i>
13	M	PGL	1	<i>VHL</i>
14	M	PGL	1	<i>VHL</i>
15	F	PHEO	1	<i>EPAS1</i>
16	M	PHEO	2	<i>RET</i>
17	F	PHEO	2	<i>RET</i>
18	M	PHEO	2	<i>RET</i>
19	F	PHEO	2	<i>RET</i>
20	F	PHEO	NA	Sporadic
21	F	PHEO	NA	Sporadic
22	M	PHEO	NA	Sporadic

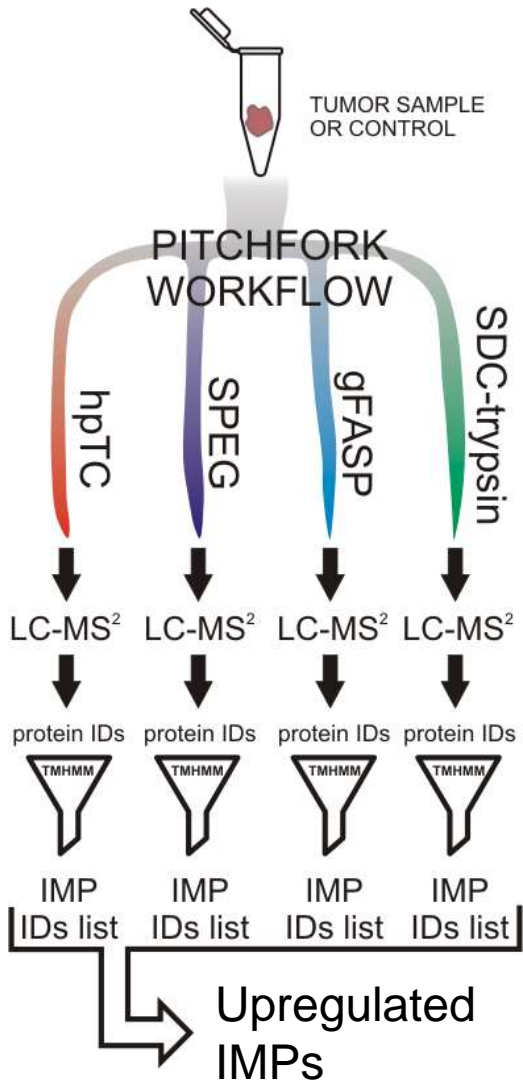
ADRENAL MEDULLA – THE CONTROL CHROMAFFIN TISSUE



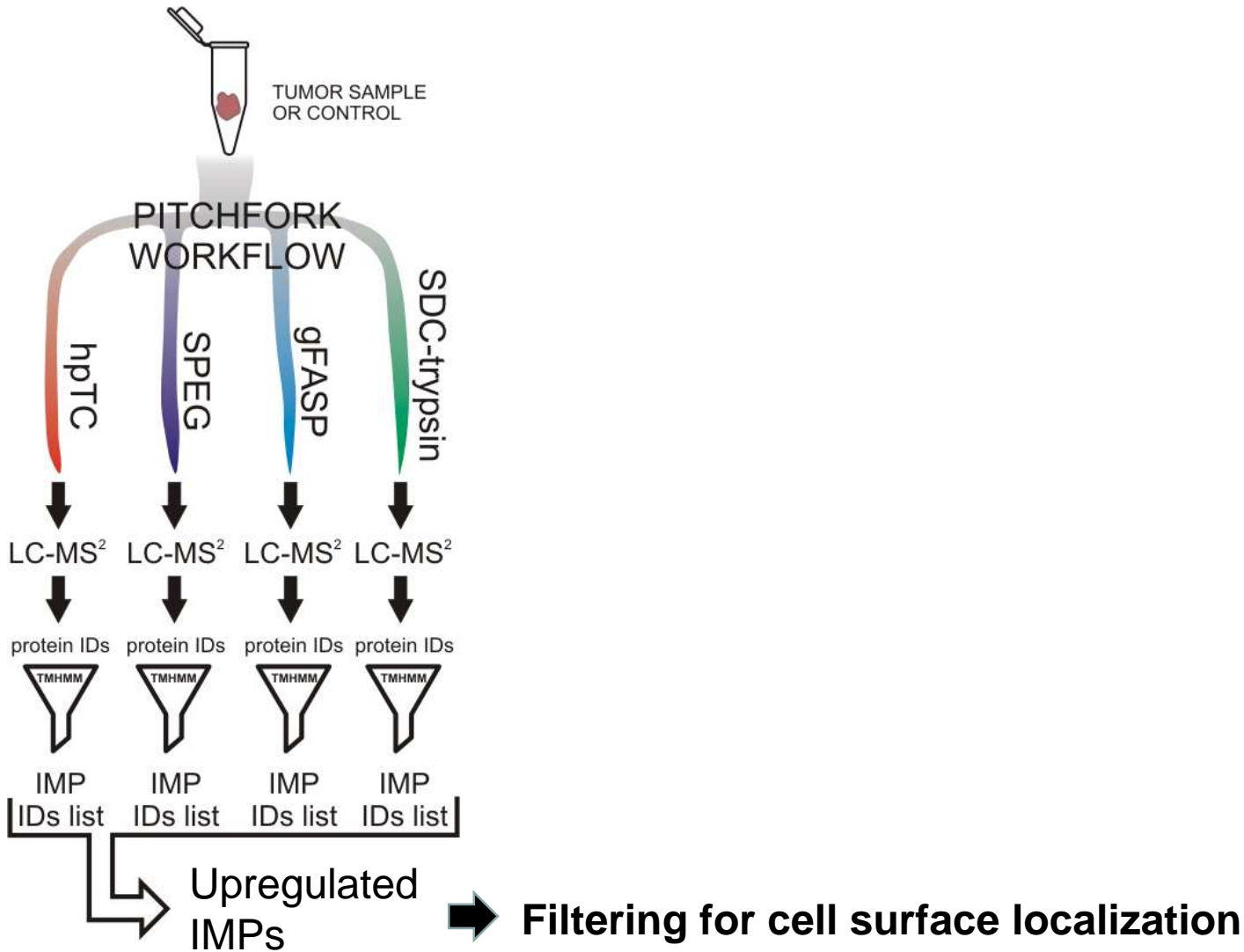
ADRENAL GLAND



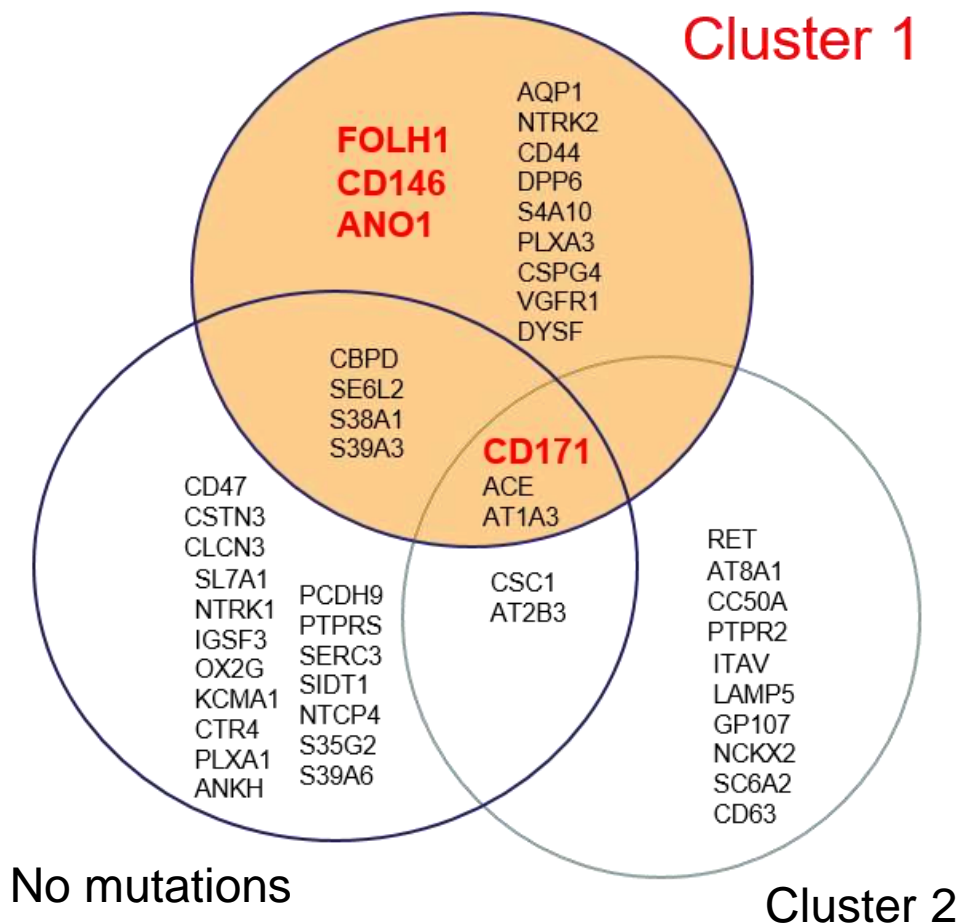
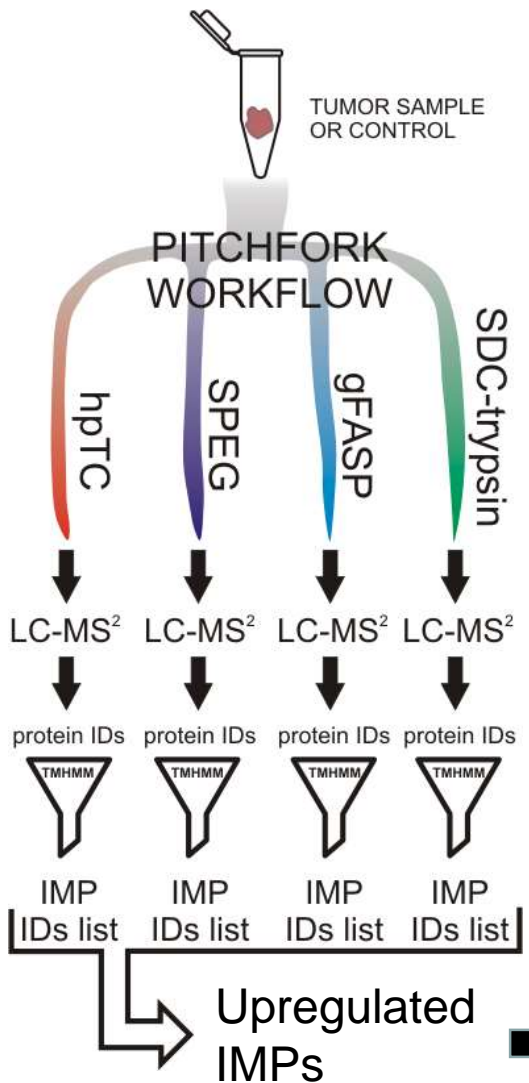
PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME

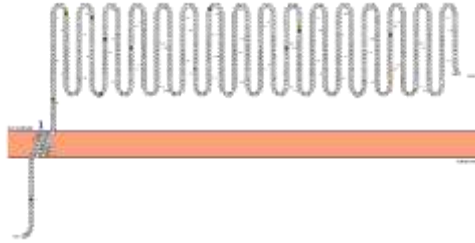


PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME

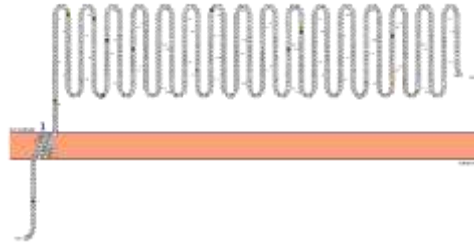


Filtering for cell surface localization

GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1)

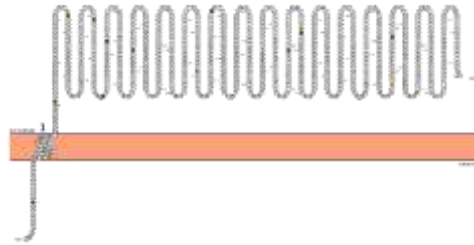


GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1)
PROSTATE-SPECIFIC MEMBRANE ANTIGEN (PSMA)



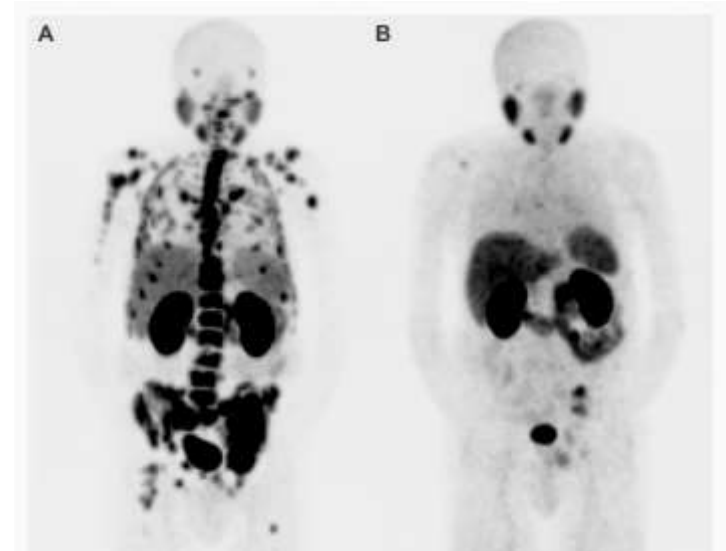
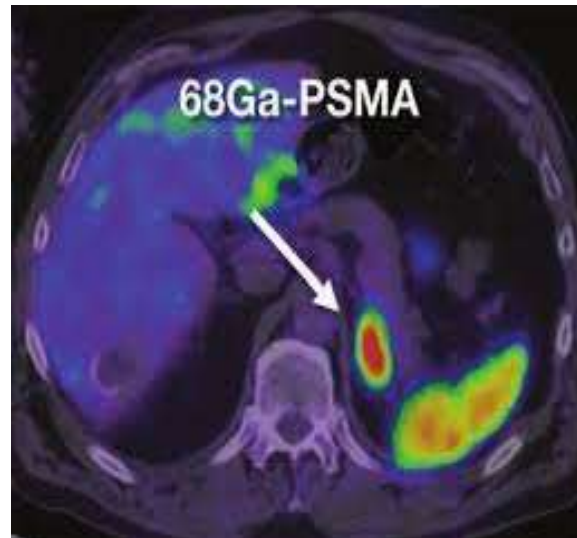
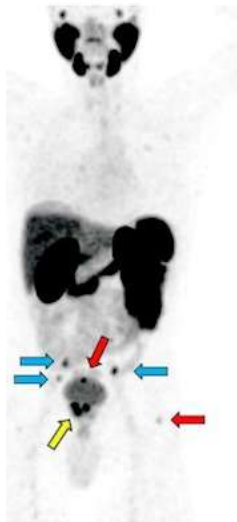
OVEREXPRESSED IN PROSTATE CANCER CELLS

GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1) PROSTATE-SPECIFIC MEMBRANE ANTIGEN (PSMA)



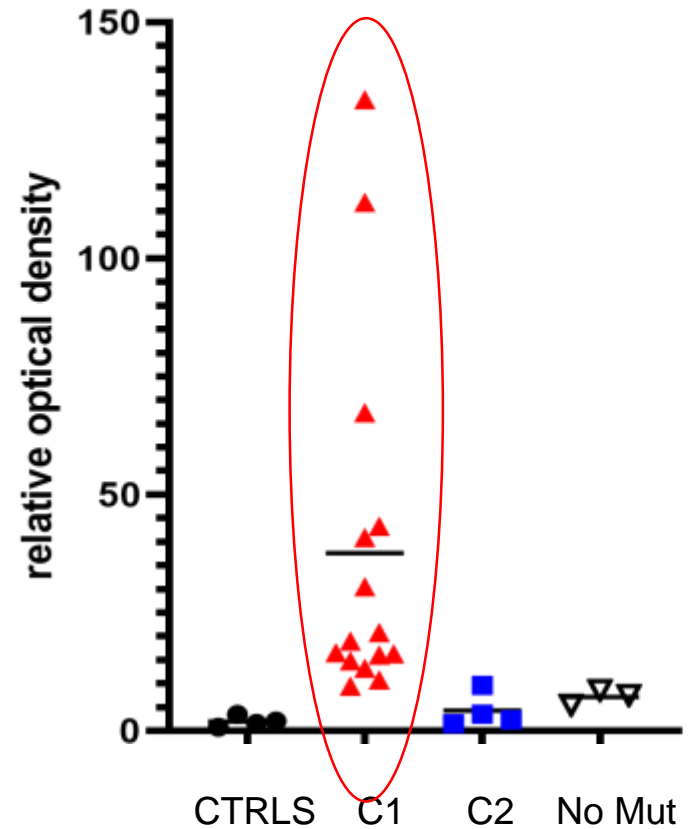
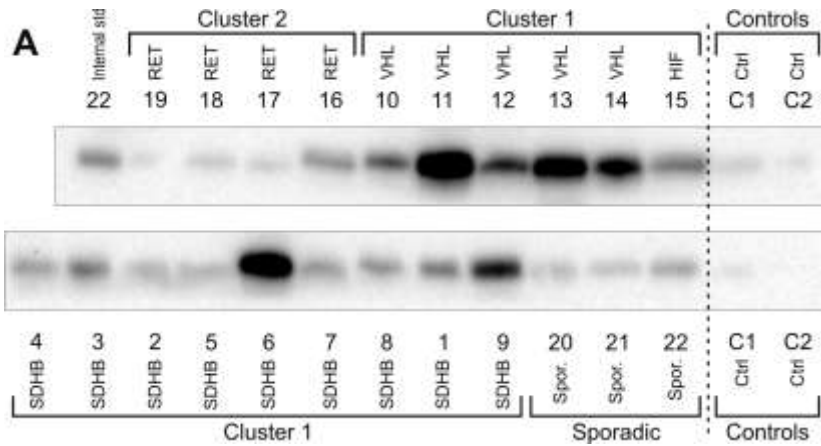
OVEREXPRESSED ON PROSTATE CANCER CELLS

Anti-PSMA radio-conjugates approved for PC tumor imaging
and therapy of advanced PC

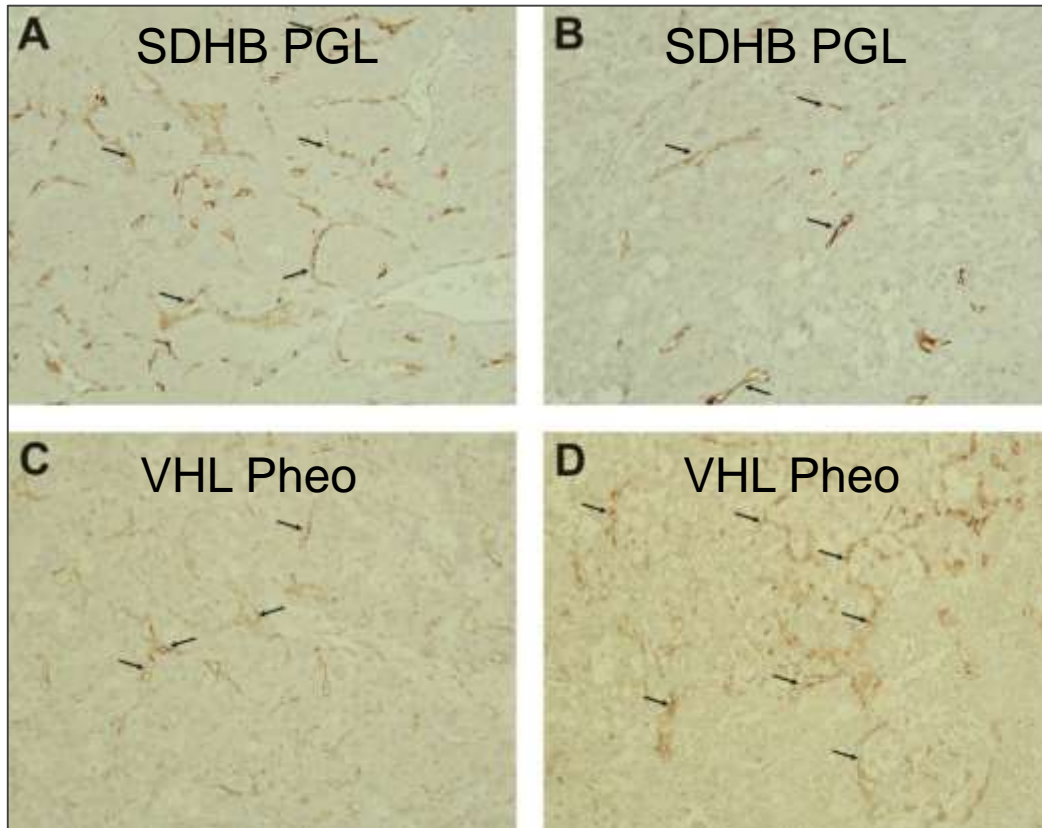


PSMA EXPRESSION IN HUMAN PPGL

PSMA relative expression in PPGL

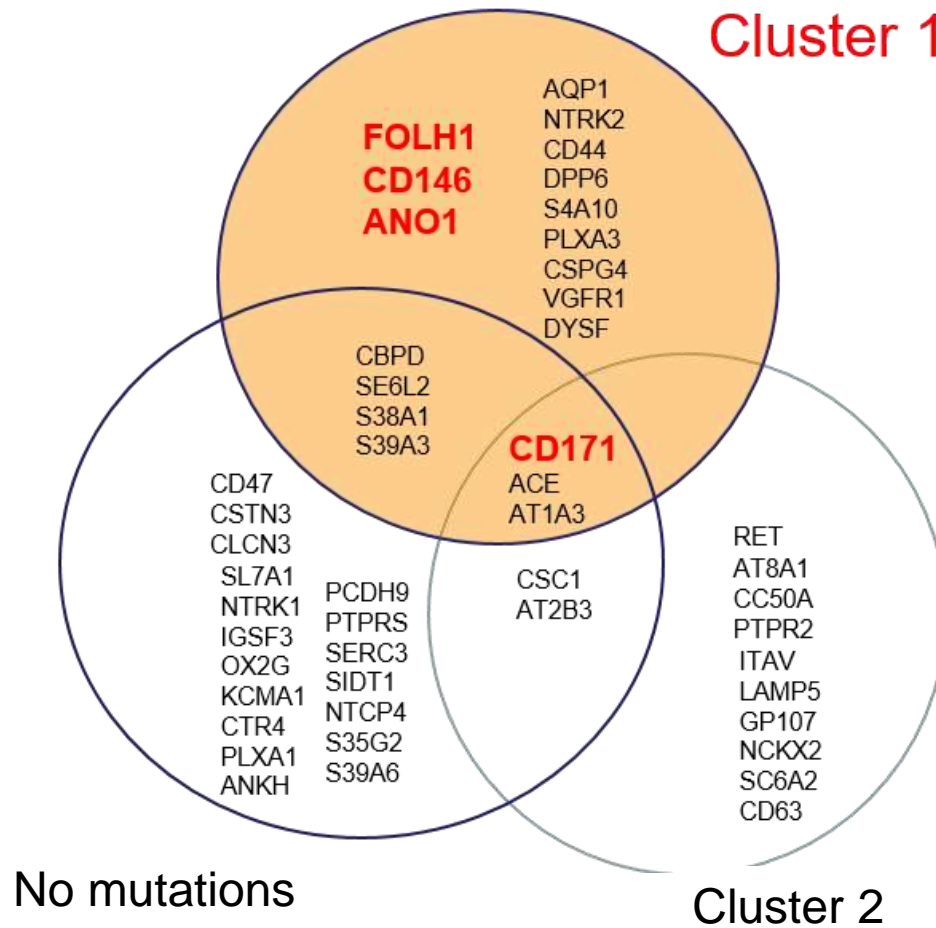


PSMA EXPRESSION IN TUMOR VASCULATURE IN CLUSTER 1 PPGL

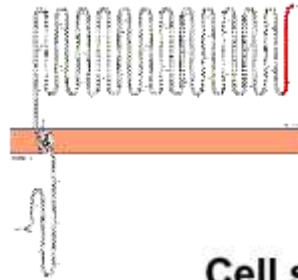


- PPGL imaging using ^{68}Ga -PSMA?
- PPGL therapy with ^{177}Lu -PSMA?

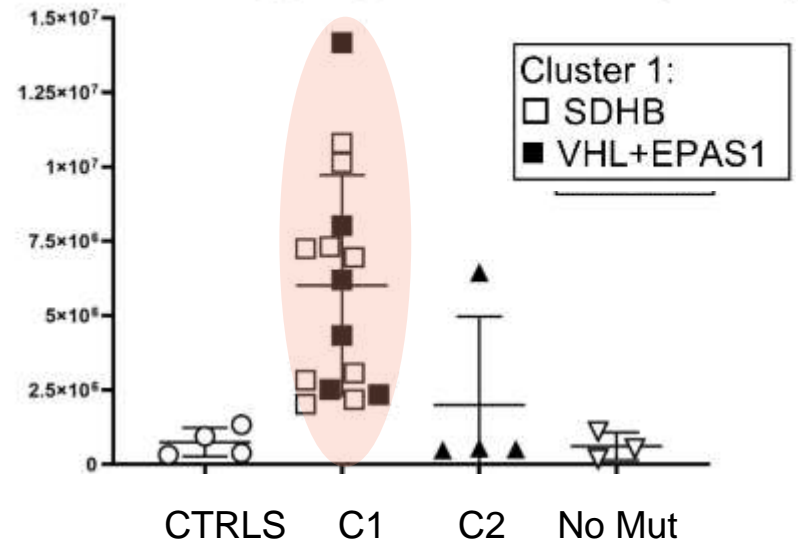
PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



CD146 (Melanoma cell adhesion molecule, MCAM, MUC18)

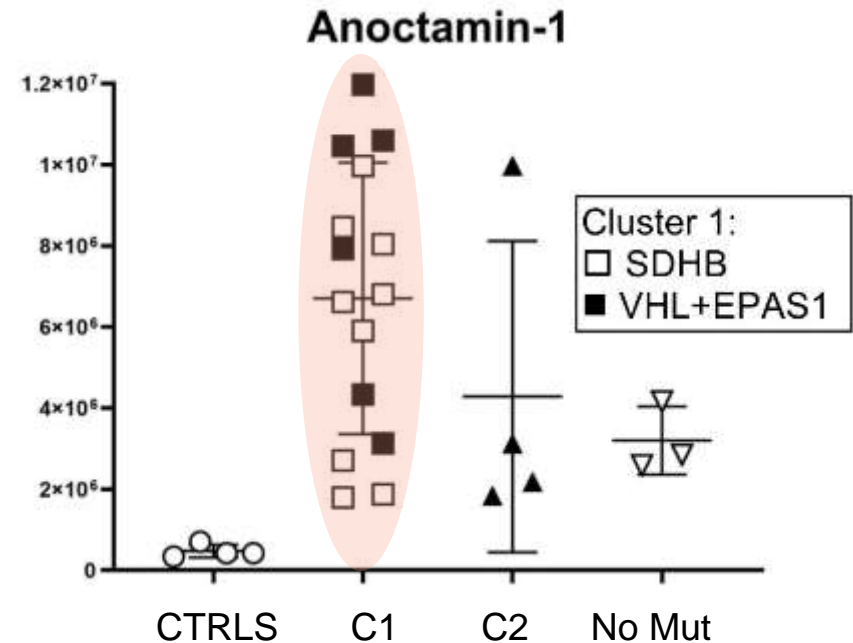
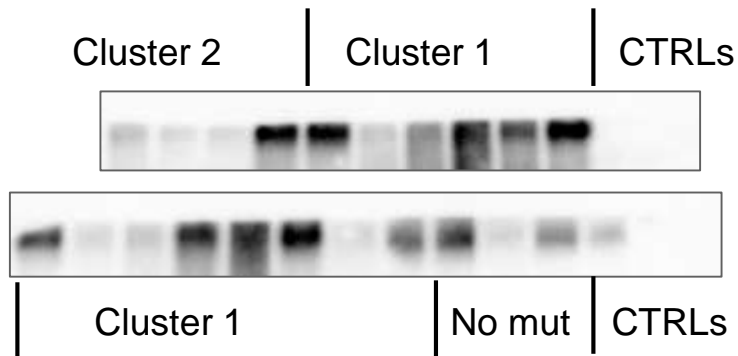
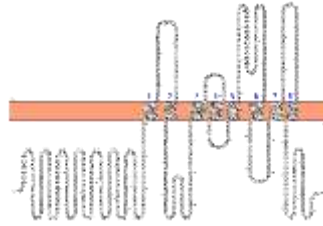


Cell surface glycoprotein MUC18 (CD146)



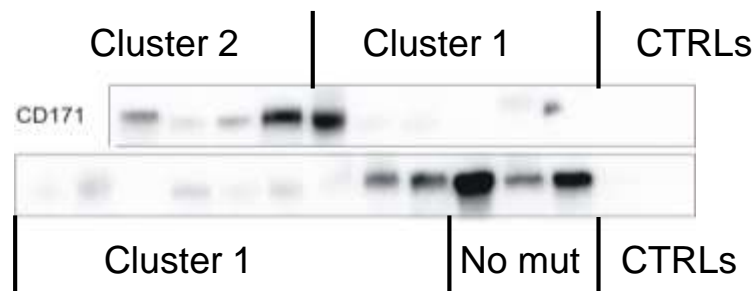
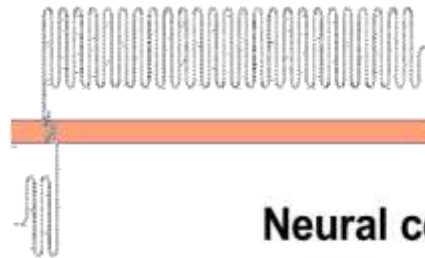
- A cell adhesion molecule, role in endothelial permeability
- Overexpressed in several cancers, expression correlates with progression
- Anti-CD146 antibody inhibited tumor growth in mouse xenograft models
- Tested as a drug target and imaging target for several tumors in preclinical studies

Anoctamin-1 (DOG1, TMEM16A)

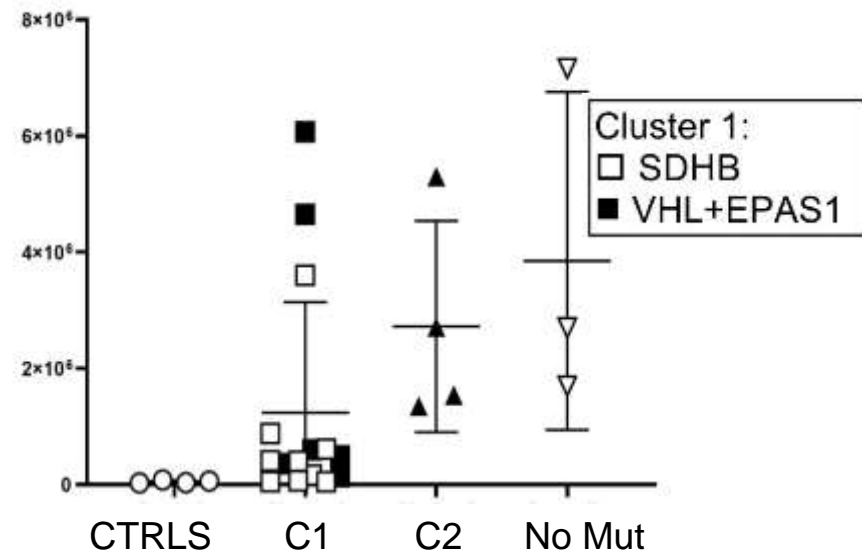


- A Ca^{2+} -activated Cl^- channel
- Overexpressed in several cancers, correlates with poor prognosis
- Function in cancer unknown
- Inhibition reduced growth of cancer cells
- Inhibitors in preclinical studies
- NIH-approved anti-asthma drug zafirlucast is ANO-1 inhibitor

CD171 (Neural cell adhesion molecule L1, NCAM-L1)



Neural cell adhesion molecule L1 (CD171)



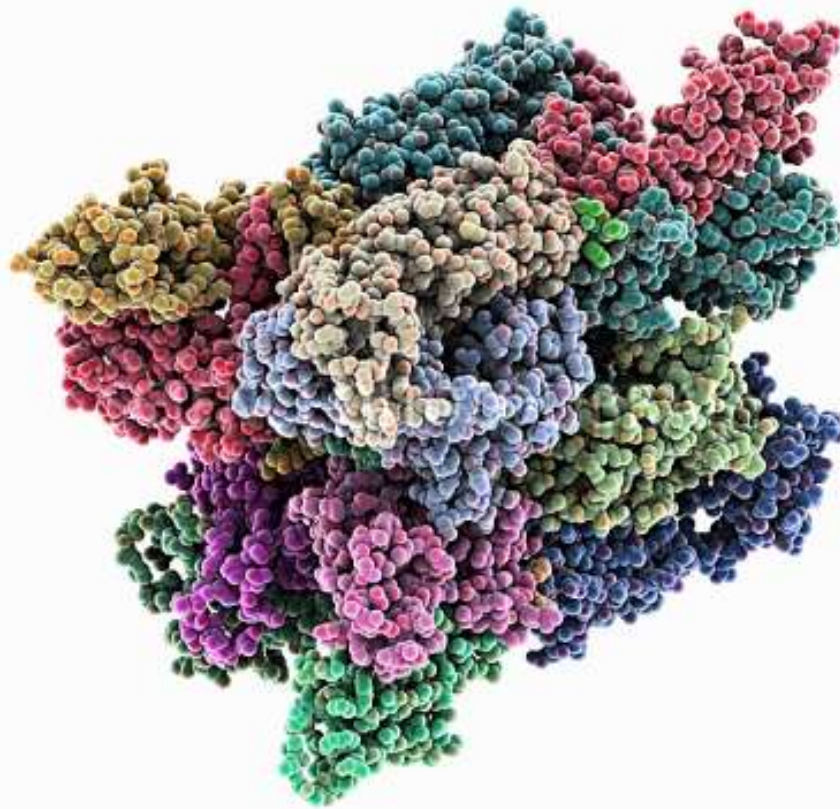
- Cell adhesion molecule, essential for neural development and regeneration
- Overexpressed in numerous cancers, expression correlates with disease progression
- Pro-angiogenic roles in the endothelial cells of tumor-associated vessels
- Anti-CD171 antibody decreased tumor vascularization and progression
- CAR-T cells recognizing CD171 in clinical trials for neuroblastoma

Příprava vzorků pro proteomické experimenty

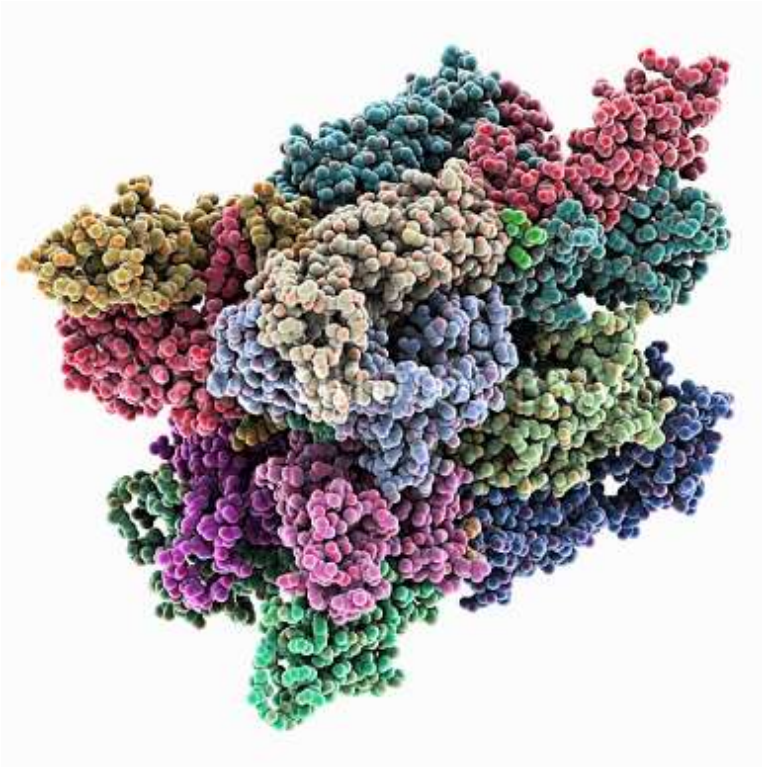
Proteomika membránových proteinů

Analýza proteinových komplexů

ANALÝZA PROTEINOVÝCH KOMPLEXŮ



ANALÝZA PROTEINOVÝCH KOMPLEXŮ



Afinitní purifikace komplexů

- s pomocí protilátky
- přes „tagované“ proteiny
- identifikace pomocí LC-MS/MS

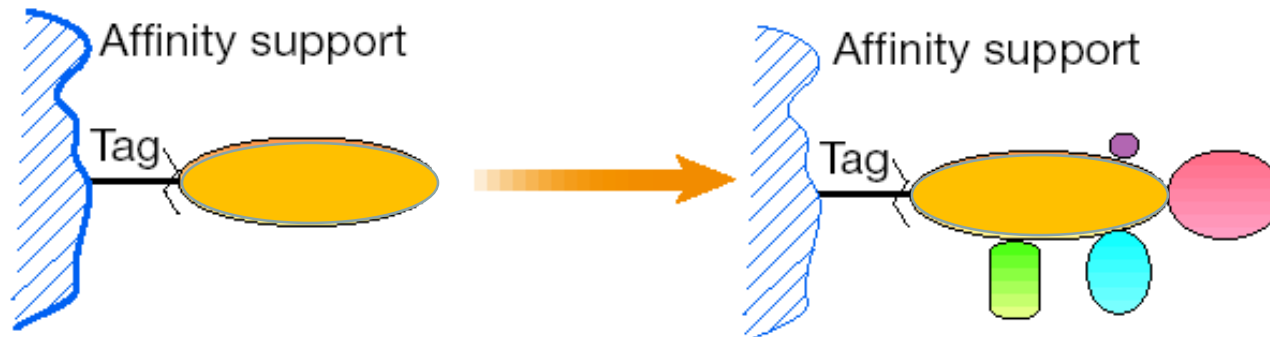
Proximity labeling

Nativní (vícerozměrné) separace

- Blue native/2D elektroforéza
- Clear native/2D elektroforéza

IMUNOAFINITNÍ IZOLACE PROTEINOVÝCH KOMPLEXŮ

- 1) matrix s **protilátkou** proti jedné složce komplexu
- 2) matrix s **rekombinantním proteinem** (složkou komplexu) nebo jinou „návnadou“



AFINITNÍ MATRIX

Aktivované matrice:

NHS Sepharose.....lze vázat za aminoskupinu (succinimid)

CNBr Sepharose.....lze vázat za aminoskupinu

EAH Sepharoselze vázat protein za karboxyl (karbodiimid)

Thiol sepharose.....lze vázat za SH cysteinu

Matrice s afinitou pro IgG (Fc fragment)

Protein G Sepharose

Protein A Sepharose

Protein A, G magnetic beads

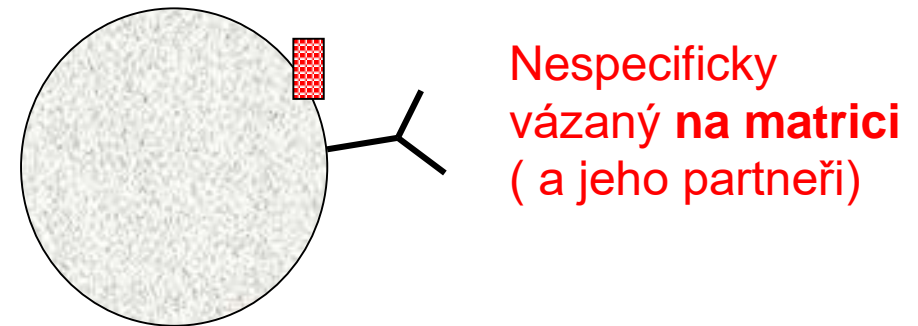
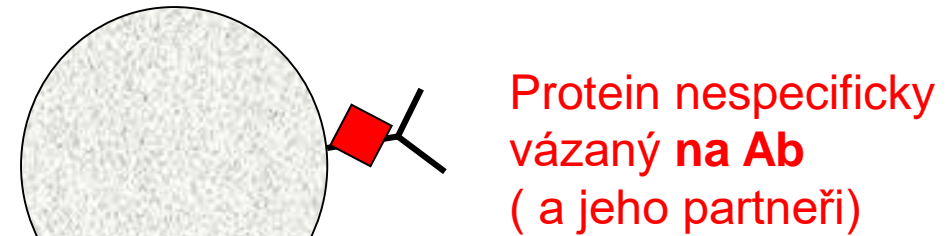
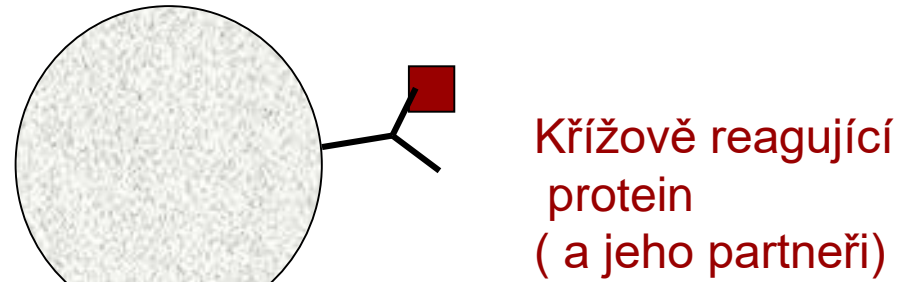
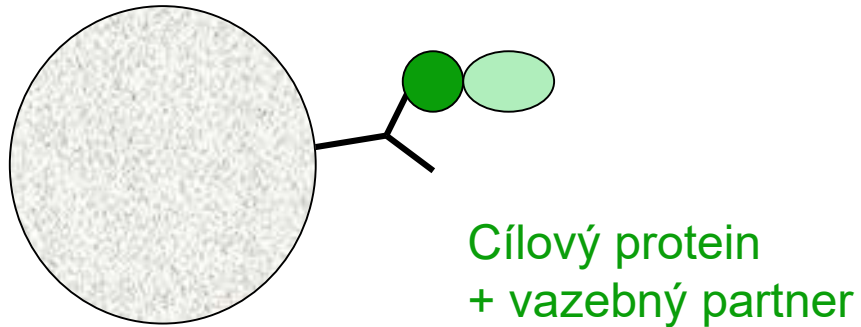
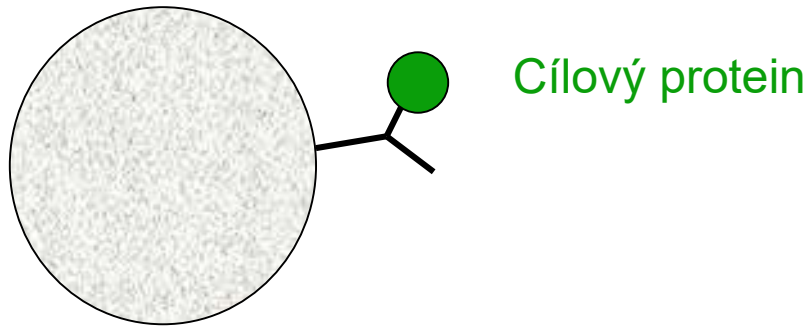
Matrice s afinitou pro glykoproteiny

ConA Sepharose

Velké ligandy (DNA, protein) lze vázat přímo na matrix.

Malé ligandy (nukleotid, NADP, hormon...) se váží přes inertní „spacer arm“.

Typy možných interakcí při imunoprecipitaci

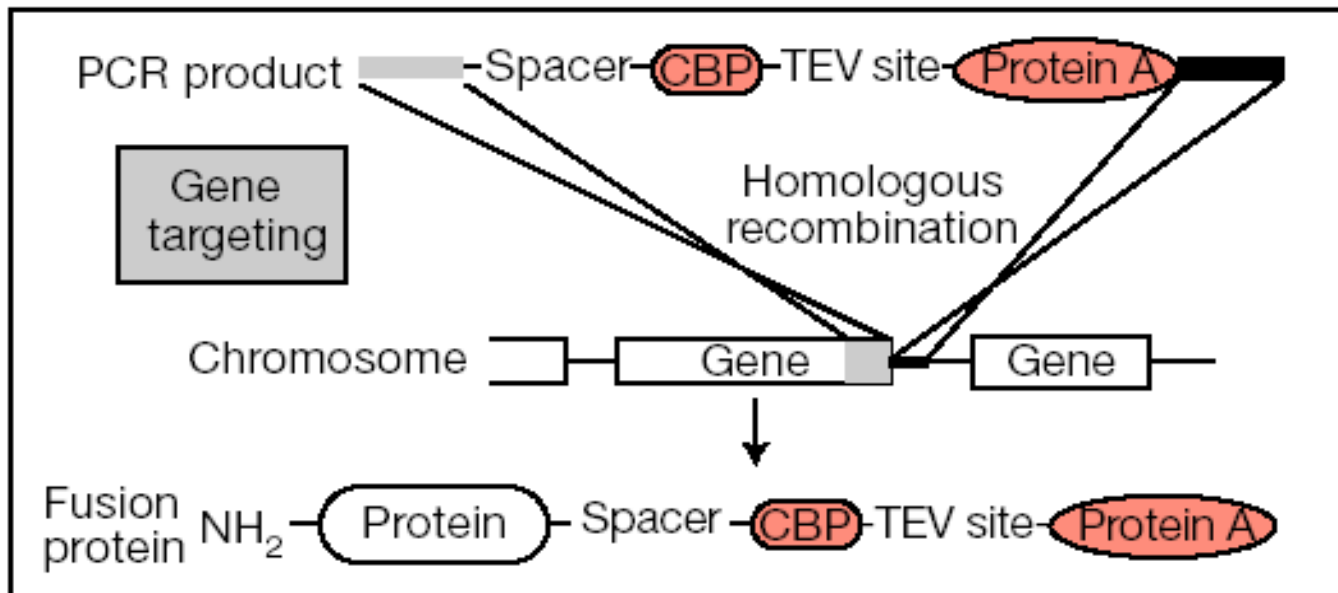


TANDEMOVÁ AFFINITNÍ PURIFIKACE (TAP)

Anne-Claude Gavin et. al., (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 415, 142-147

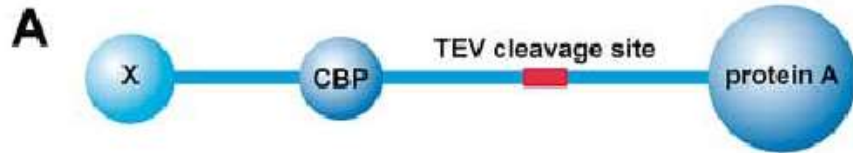
CBP- calmodulin-binding protein

TEV – štěpné místo virové proteázy TEV



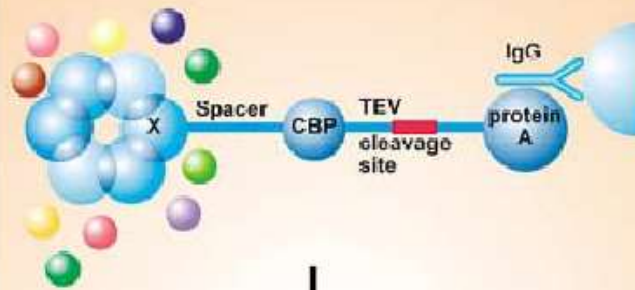
1739 ORF

TANDEMOVÁ AFFINITNÍ PURIFIKACE (TAP)



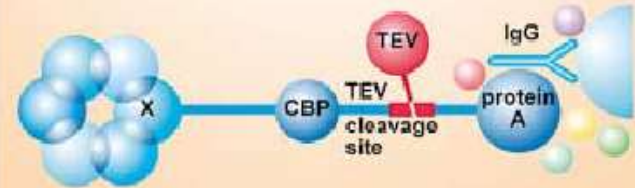
B First affinity purification

Protein A-IgG Interaction



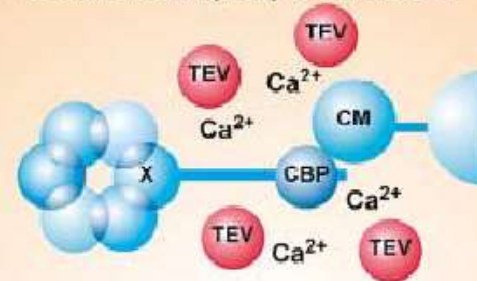
Wash

TEV cleavage



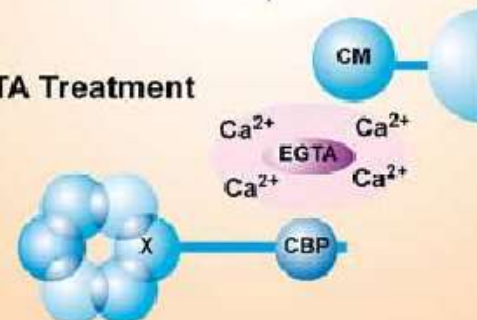
Second affinity purification

CBP-Calmodulin (CM) Interaction



Wash

EGTA Treatment



TANDEMOVÁ AFFINITNÍ PURIFIKACE (TAP)

Strategy	Failed	Success rate		
PCR of the TAP cassette	ORFs processed: 1,739		4562	6466
Transformation of yeast cells (homologous recombination)	Positive homologous recombinations: 1,548	191	89%	
Selection of positive clones	Expressing clones: 1,167 (membrane protein 293)	381	75%	
Large-scale cultivation				
Cell lysis Tandem affinity purification	TAP purifications: 589	285	62%	2357
One-dimensional SDS-PAGE				
MALDI-TOF protein identification				
Bioinformatic data interpretation	Identified complexes: 232		547	491

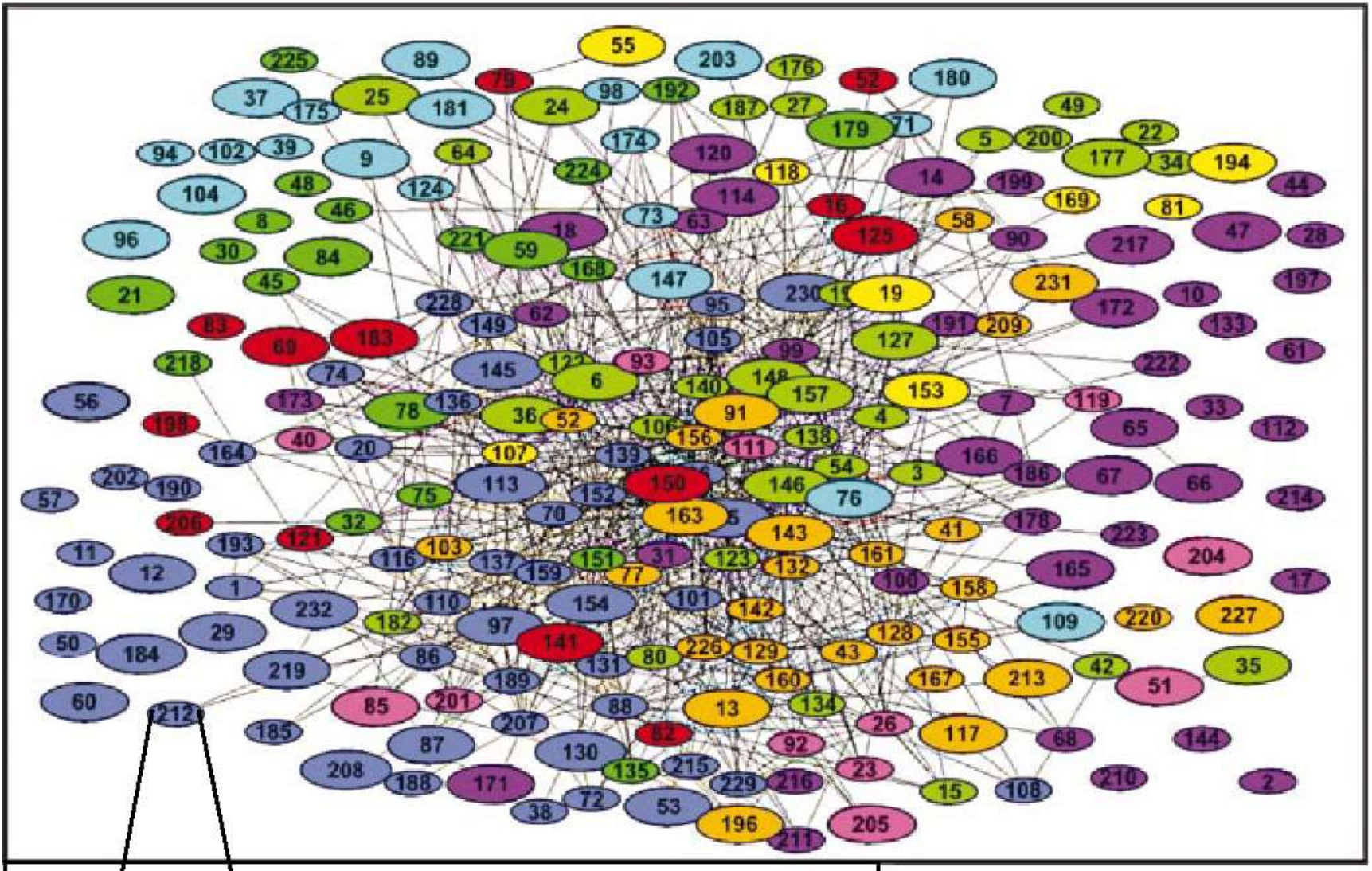
Komplexy (v průměru 5-7 komponent)

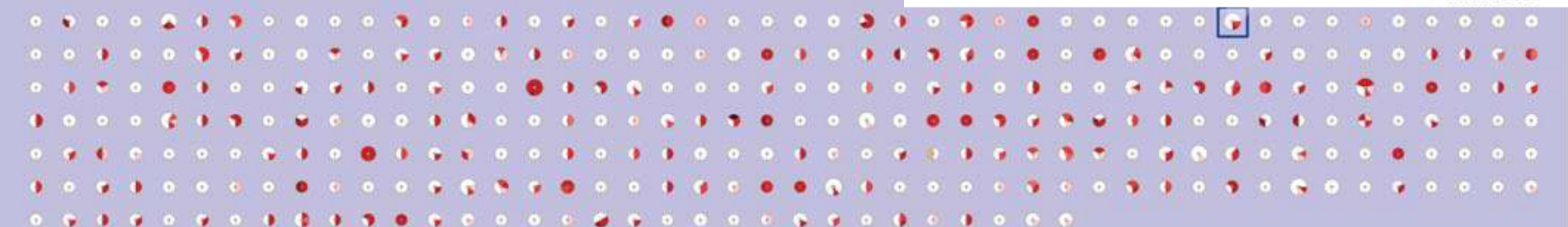
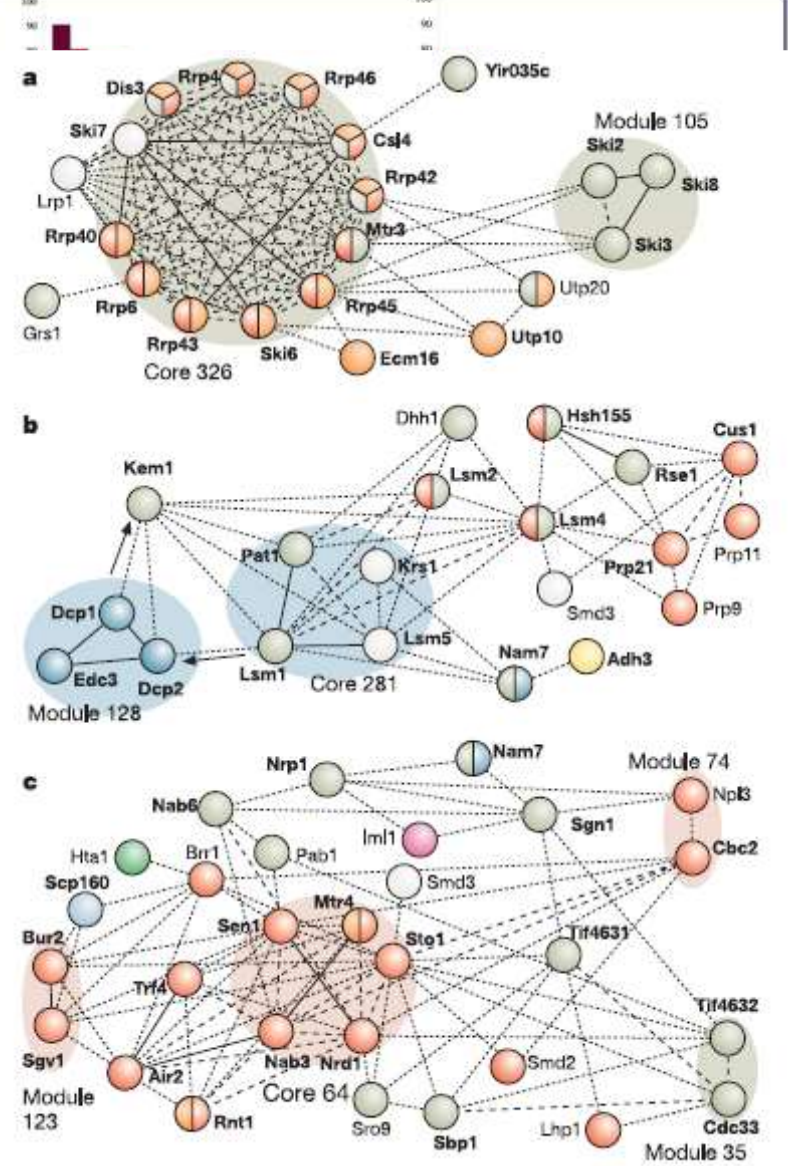
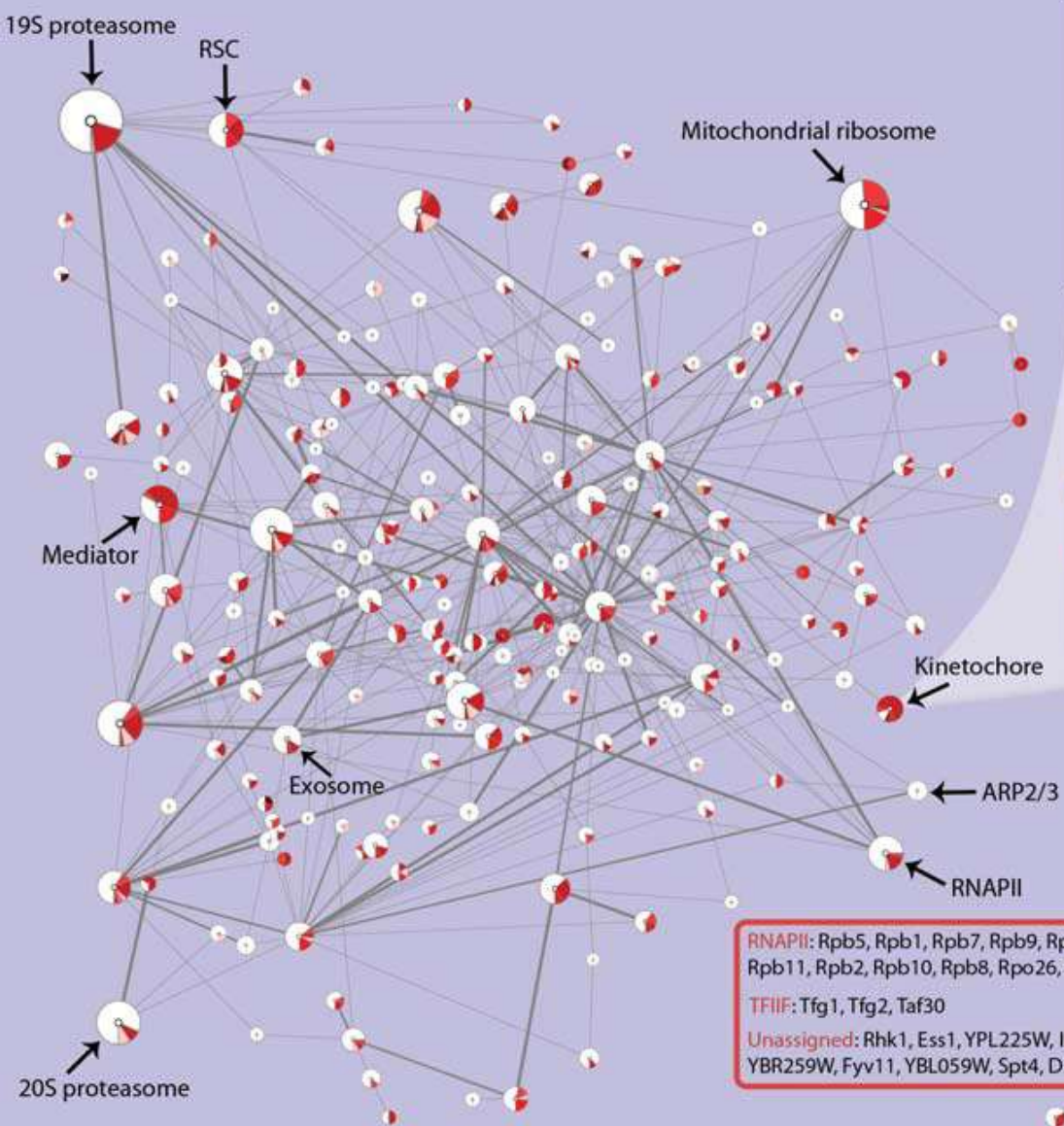
Gavin 2002
Nature

Gavin 2006
Nature

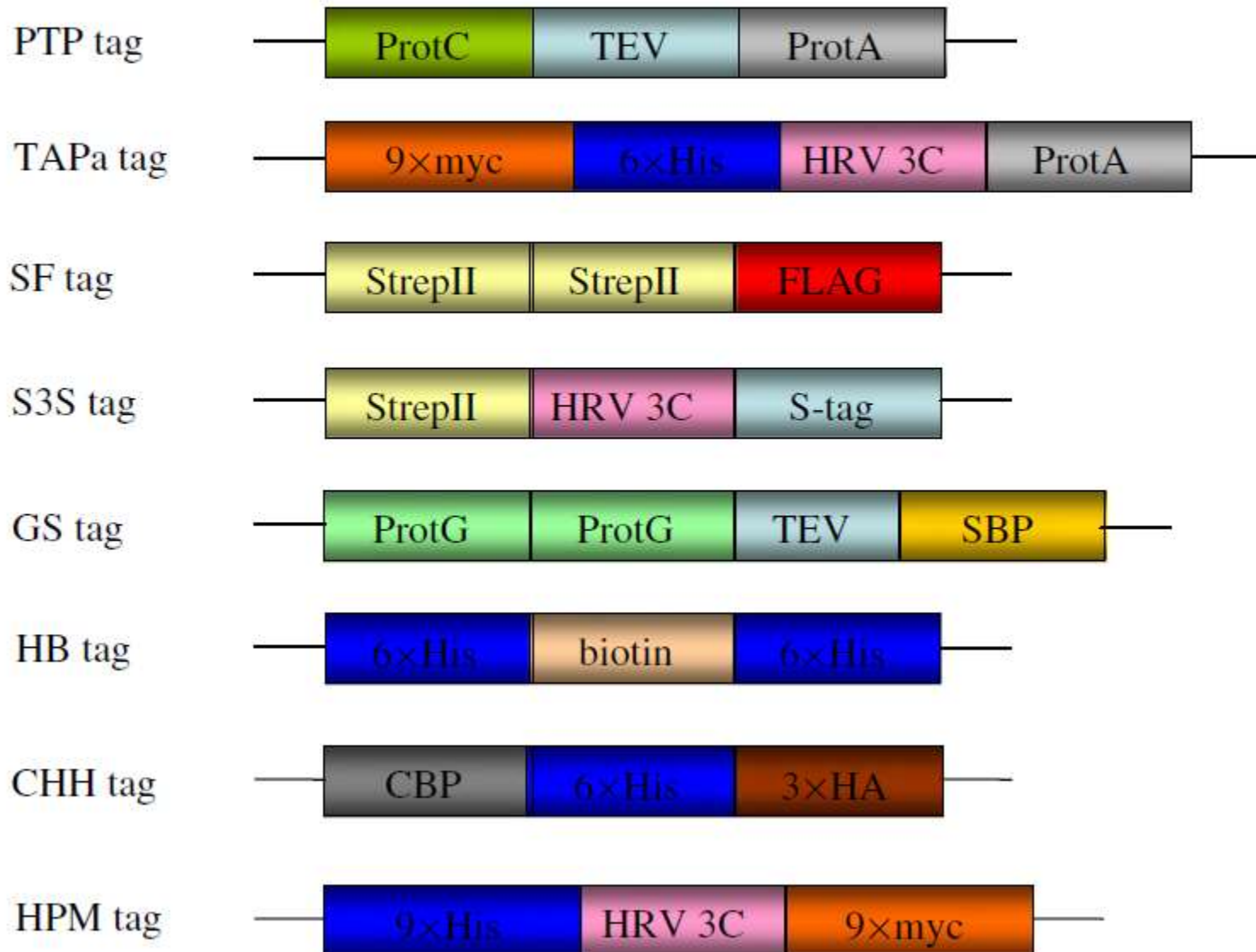
Krogan 2006
Nature

TANDEMOVÁ AFFINITNÍ PURIFIKACE (TAP)

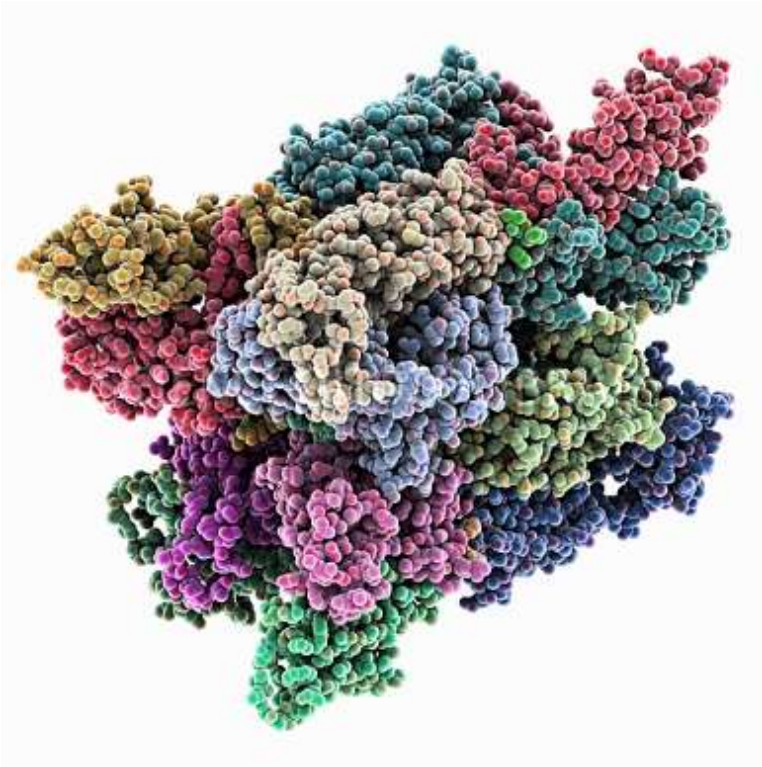




TANDEMOVÁ AFFINITNÍ PURIFIKACE (TAP)



ANALÝZA PROTEINOVÝCH KOMPLEXŮ



Afinitní purifikace komplexů

- s pomocí protilátky
- přes „tagované“ proteiny

Proximity labeling

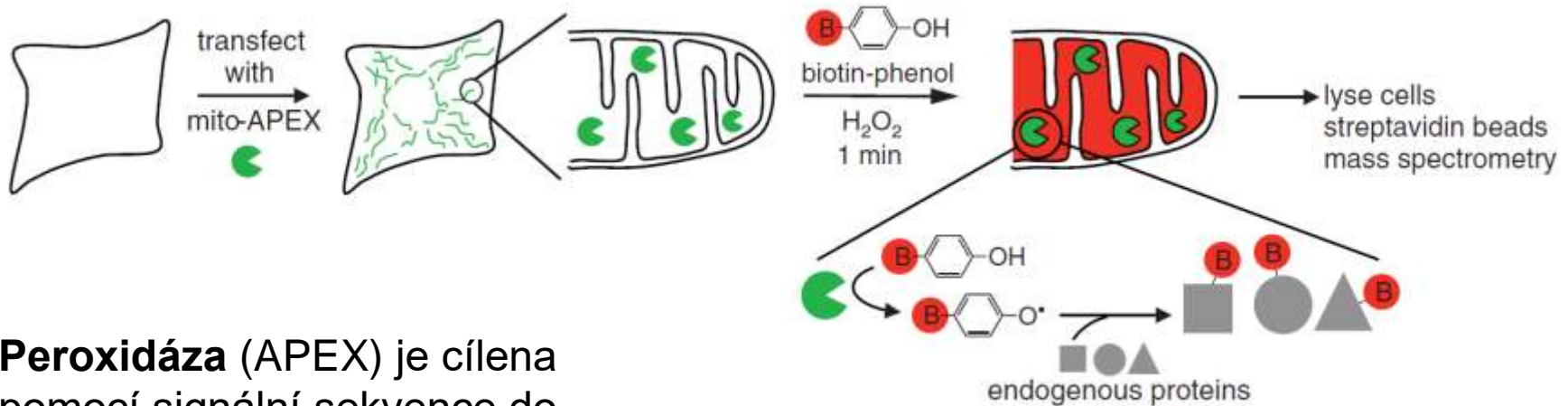
Nativní (vícerozměrné) separace

- Blue native/2D elektroforéza
- Clear native/2D elektroforéza

Proximity labeling

(k určení lokalizace proteinů, mapování kompartmentu)

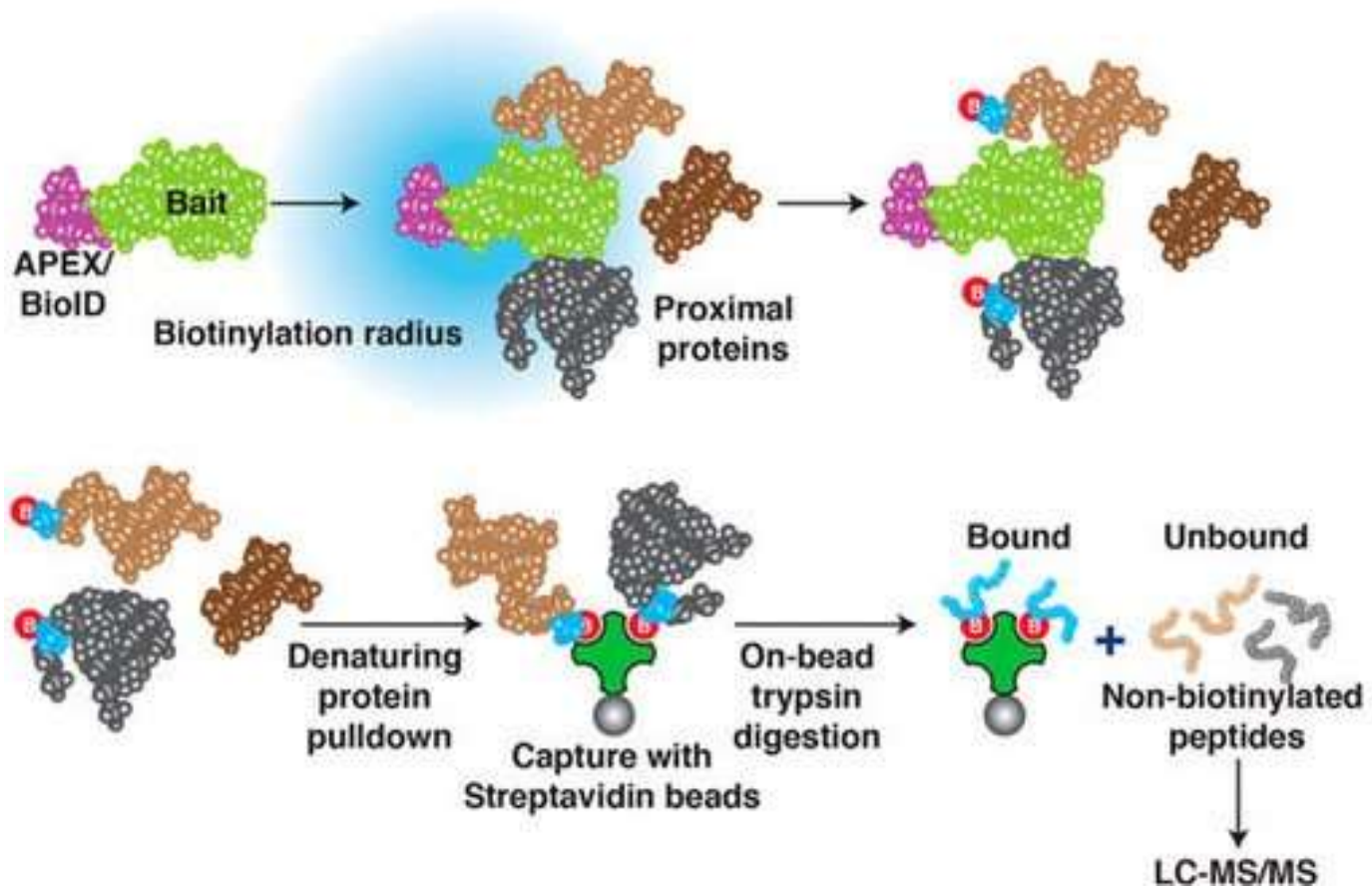
APEX APEX2



Peroxidáza (APEX) je cílena pomocí signální sekvence do cílového kompartmentu (vnitřní membrána MT, ER....a pod) kde v přítomnosti **biotinofenolu** „označí“ proteiny v okolí (Tyr). Označené proteiny se izolují streptavidinem.
Toxicita peroxidu, problematické zajištění substrátu

Proximity labeling

BioID



Protein zájmu je exprimován jako **fuzní protein s bakteriální biotin ligázou** která v přítomnosti biotinu „označí“ proteiny v nejbližším okolí. Označené proteiny se izolují streptavidinem. **Pomalá rekční kinetika**, dostatečná biotinylace v řádu hodin.

Proximity labeling

TurboID a split-TurboID

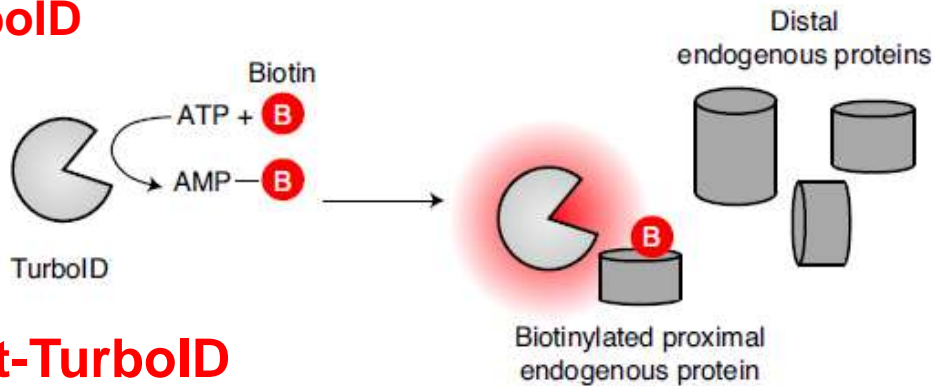
Mutantní kvasničné biotin ligázy – cílená mutagenese a selekce

Aktivovný biotin difunduje z aktivního místa do bezprostředního okolí

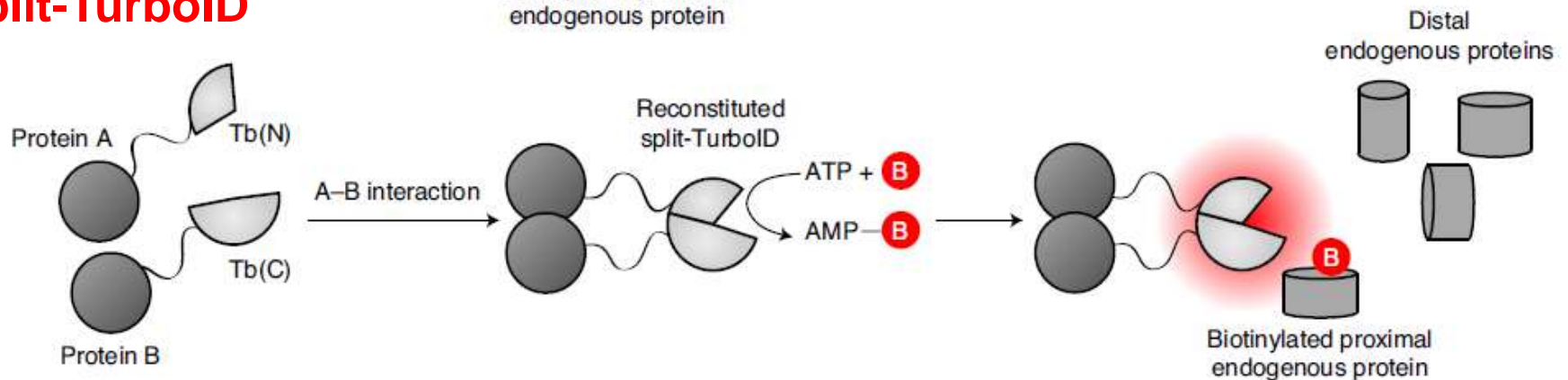
Jeden nebo dva fúzní proteiny

s rychlou kinetikou v řádu minut, nižší toxicita

TurboID



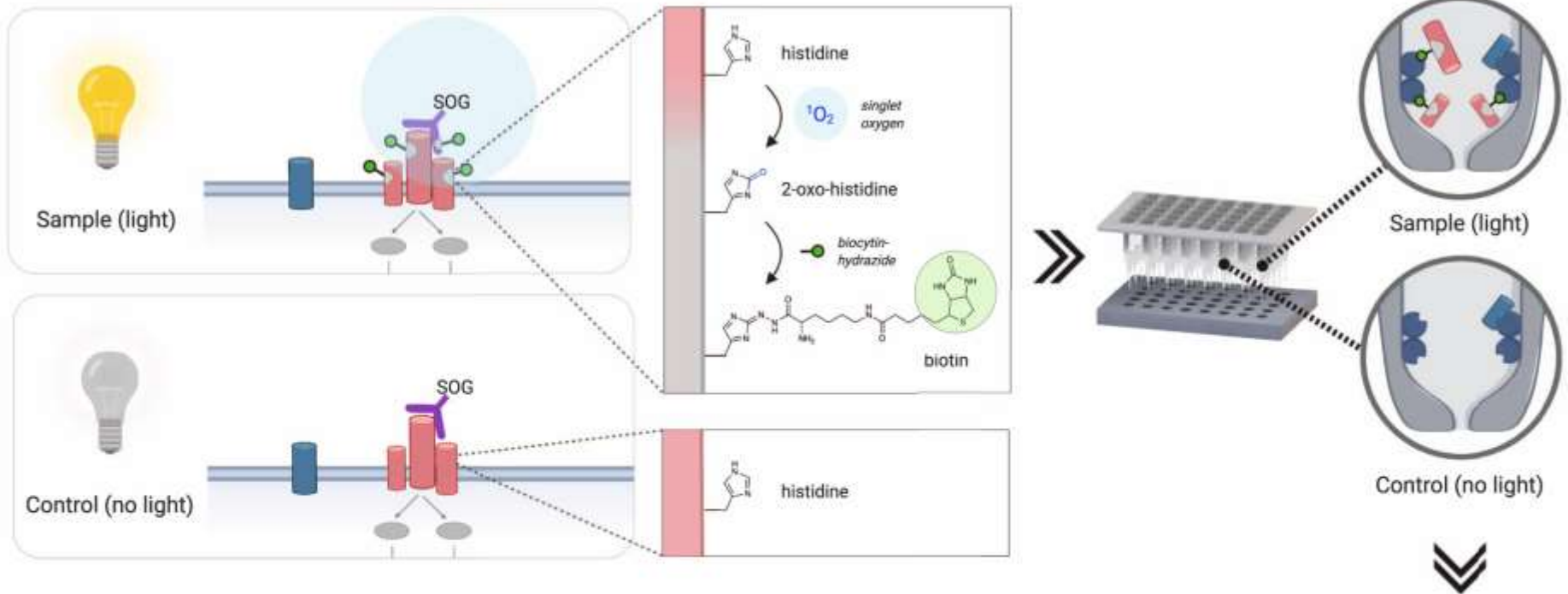
split-TurboID



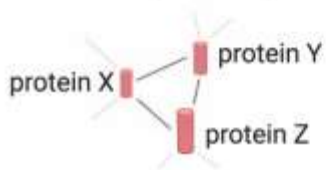
LUX-MS značení okolních histidinů po jejich oxidaci
 (v okolí generátoru singletového kyslíku - SOG)
 Pro mapování proteinových komplexů na povrchu buňky

ligand-guided and light-activated protein proximity labeling

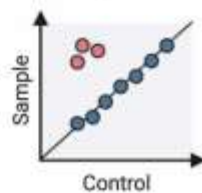
high-throughput protein capture & processing



cell surface proximity network



relative quantification



DDA / DIA LC-MS/MS



release of proteolytic peptides

