PROTEOMIKA
2025

- Proteomika, Metody práce s bílkovinami
- Separační metody, digesce a principy ID bílkovin pomocí MS
- Principy hmotnostní spektrometrie, instrumentace
- Hmotnostní spektrometrie v proteomice, analýza PTM
- ID proteinů, DDA, DIA, databáze, FDR
- Kvantifikace, isotopy, LFQ, cílená proteomika
- Design experimentu, zpracování dat, bioinformatika
- **Příprava vzorků, Proteomika membránových proteinů,** (Petrák 1/12)
- **Proteinové komplexy, klinická proteomika, speciální metody** (Petrák 8/12)

Current proteomic workflow
LC-MS/MS

proteins

trypsin…

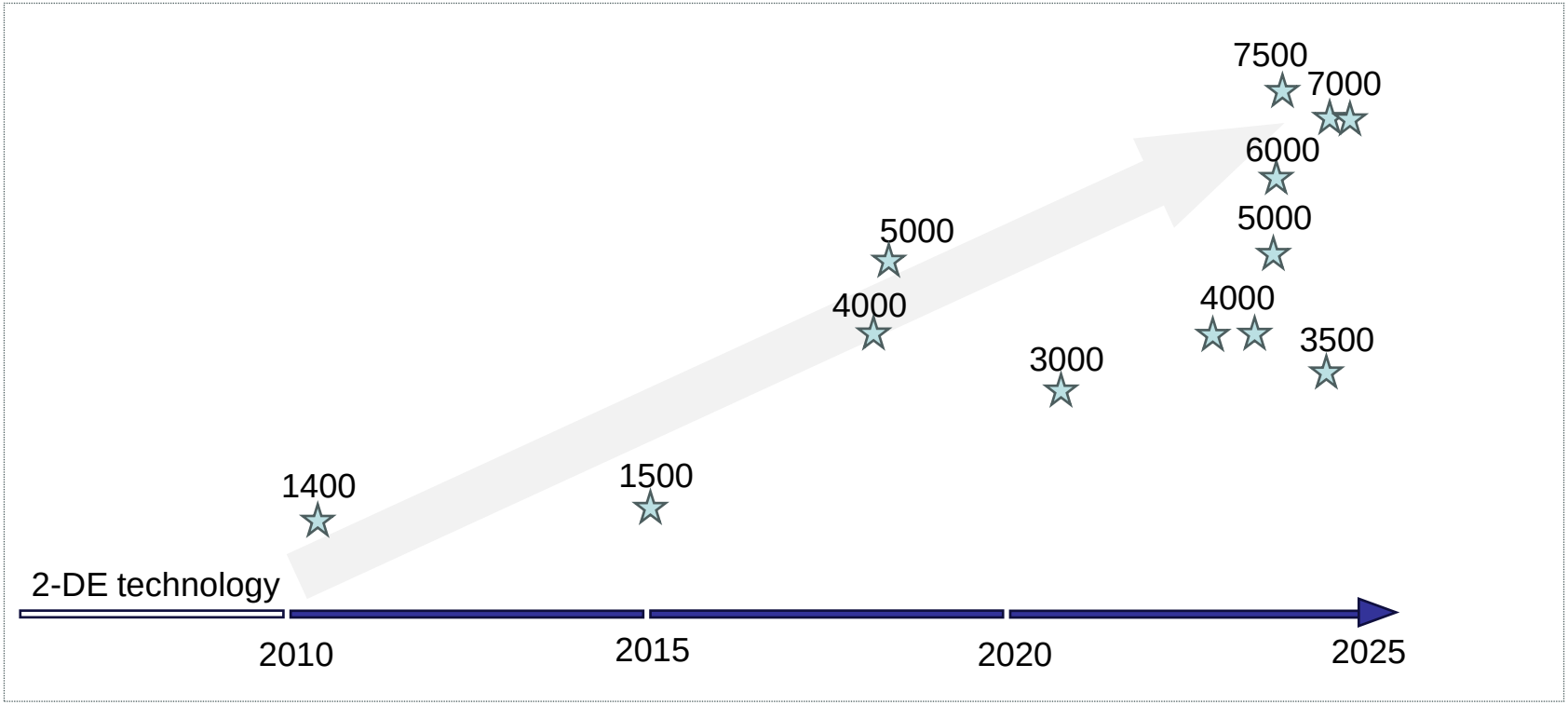
peptides

Micro or nano-LC
1D or 2D

Mass spectrometry

Peptide/protein identification and quantification

Number of proteins identfied in proteomic experiments



Mass spectrometry

Peptide/protein identification and quantification

Instrumentace: Rychlost a paralelizace, Orbitrap, Astral, TIMS-TOF

Akvizice: DDA a DIA

Identifikace: Unikátní peptidy, inference proteinů, protein groups

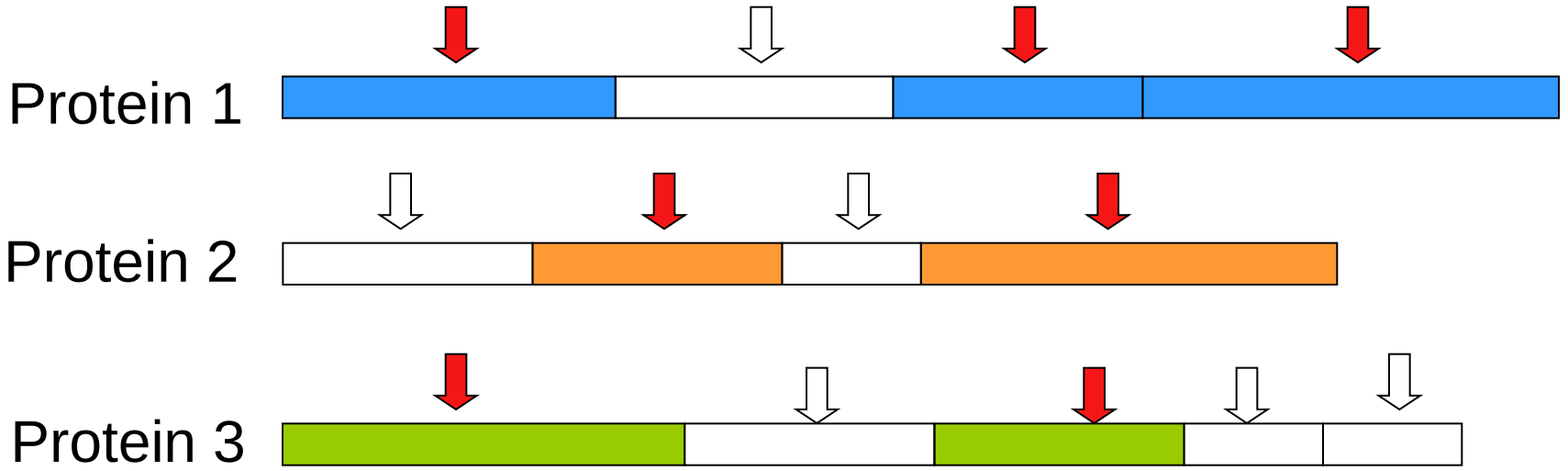
Keratin, type II cytoskeletal 4 (KRT4, P19013)

MIARQQCVRGGPRGFSCGSAIVGGGKRGAFSSVSMSGGAGRCSSGGFGSRSLYNL
RGNKSISMSVAGSRQGACFGGAGGFGTGGFGGGFGGSFSGKGGPGFPVCPAGGI
QEVTINQSLLTPLHVEIDPEIQKVRTEEREQIKLLNNKFASFIDKVQFLEQQNKVLETK
WNLLQQQTTTTSSKNLEPLFETYLSVLRKQLDTLGNDKGRLQSELKTMQDSVEDFK
TKYEEEINKRTAAENDFVVLKKDVDAAYLNKVELEAKVDSLNDEINFLKVLYDAELSQ
MQTHVSDTSVVLSMDNNRNLDLDSIIAEVRAQYEEIAQRSKAEAEALYQTKVQQL
QISVDQHGDNLKNTKSEIAELNRMIQRLRAEIENIKKQCQTLQVSVADAEQRGENA
LKDAHSKRVELEAALQQAKEELARMLREYQELMSVKLALDIEIATYRKLLEGEEYRM
SGECQSAVSISVVSGSTSTGGISGGLGSGSGFGLSSGFGSGSGSGFGFGGSVSGSSS
SKIISTTTLNKRR

Unique tryptic peptides (5-25 AA) represent 38% in KRT4.

Protein identification in shot-gun experiments

Unique peptides **X peptides present in more than 1 protein**



Mass spectrometry

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Instrumentace: rychlost a paralelizace, Orbitrap, Astral, TIMS-TOF

Akvizice: DDA a DIA

Identifikace: Inference proteinů, unikátní peptidy, protein groups

Kvantifikace: Odlišná u DDA a DIA, label-free, TMT nebo SILAC

Statistika identifikace i kvantifikace, Adjusted p-val, missing values

Volcano plot, heatmapy, clustering, DA, GO anotace

Cílená proteomika

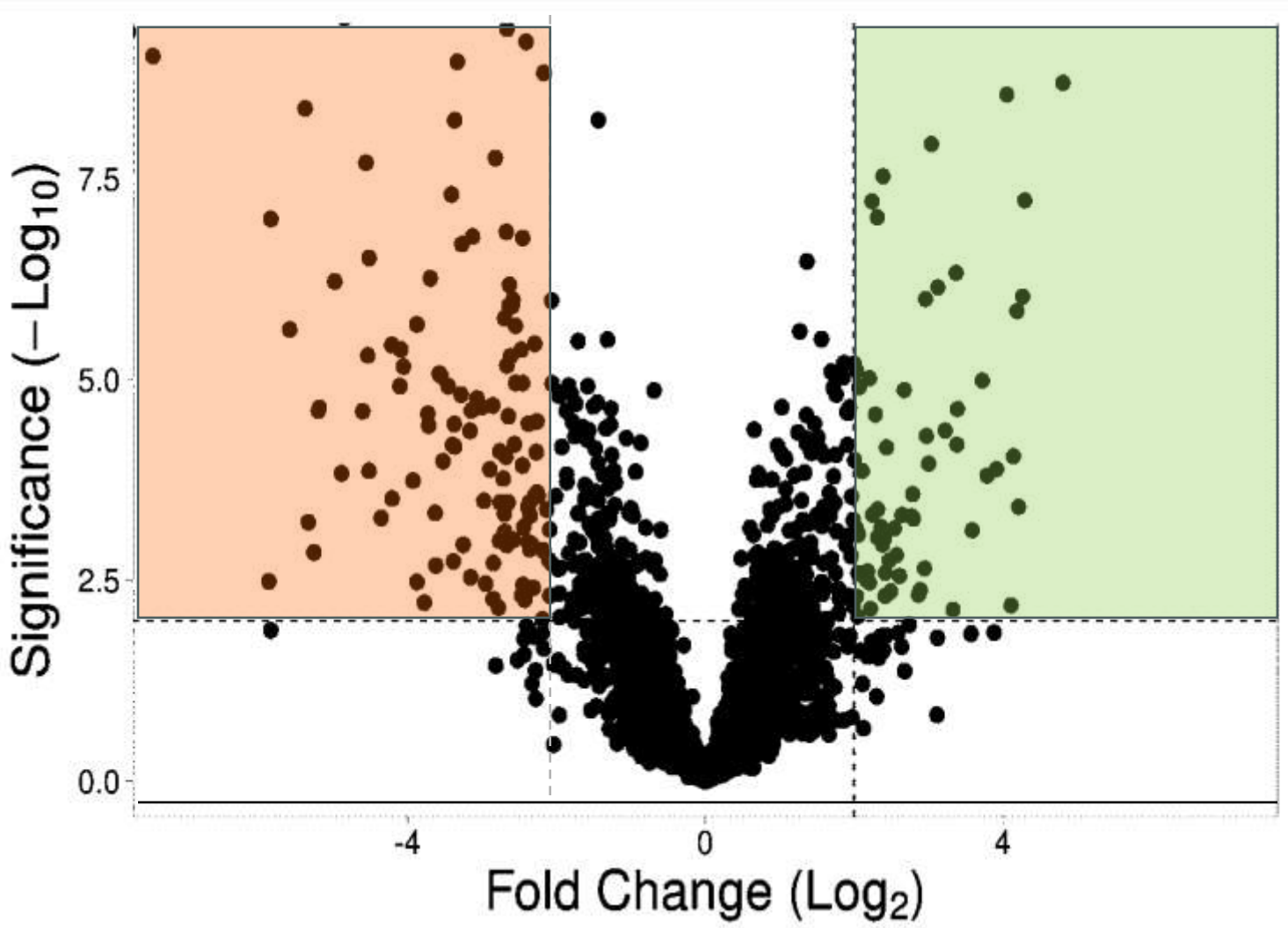
Volcano plot

Fold-change 4x

Adj. P_val 0.01

DOWN-REGULATED

UP-REGULATED



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Cílená proteomika

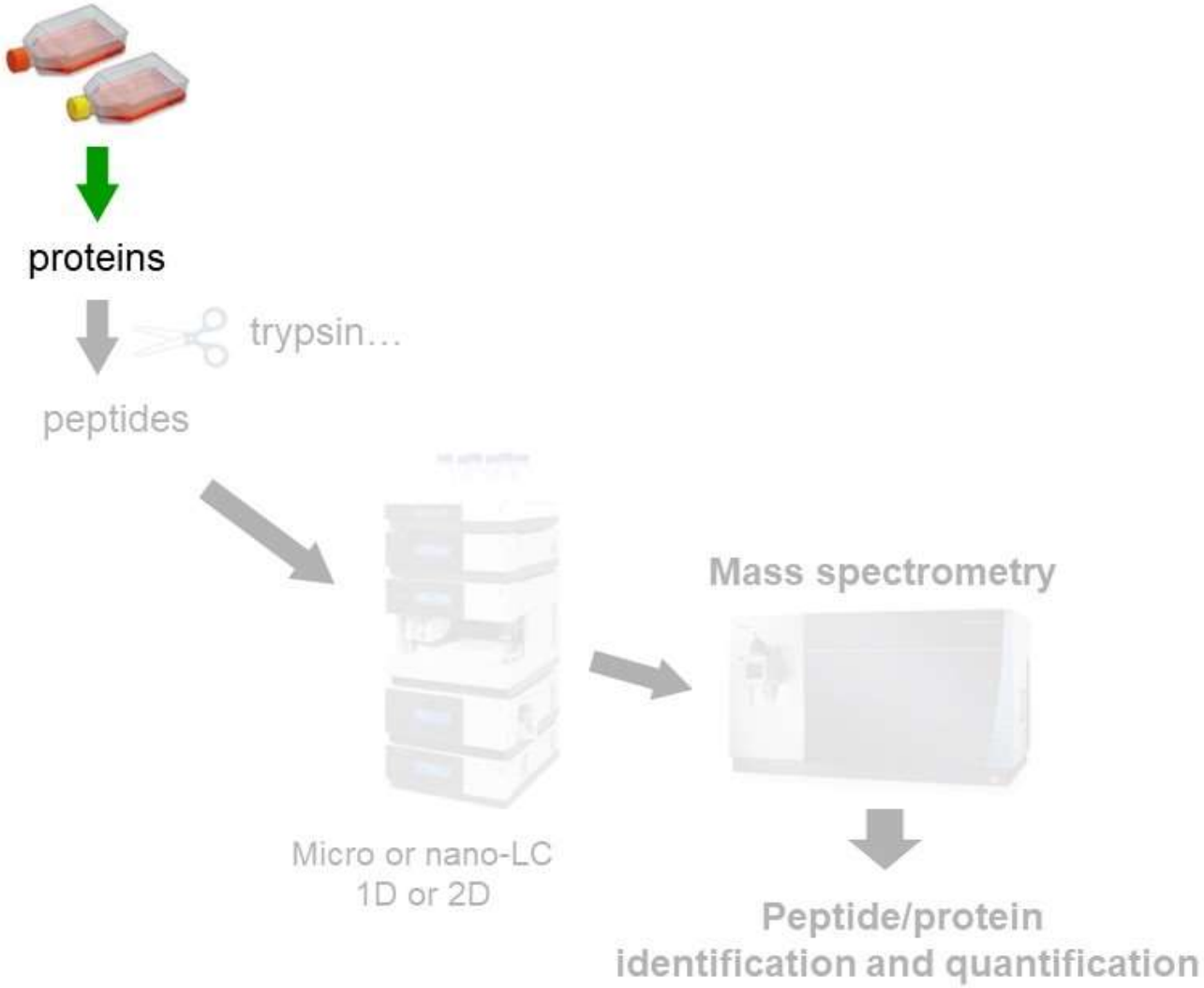
Současná proteomika

- **I více než 10 000 proteinů v jednom experimentu (DIA)**

- **Některé skupiny proteinů (částečně) unikají analýze.**
  - nízká abundance
  - málo unikátních peptidů
  - absence štěpných míst pro trypsin
  - nepříznivé fyzikálně chemické vlastnosti

- **Bottleneck – biologická interpretace naměřených dat**
- **Přehlížený problém** - kvalita biologického materiálu
  - **příprava vzorku**

Příprava vzorku pro proteomickou analýzu



Příprava vzorku pro proteomickou analýzu

Filozofie přípravy vzorků v roztoku:

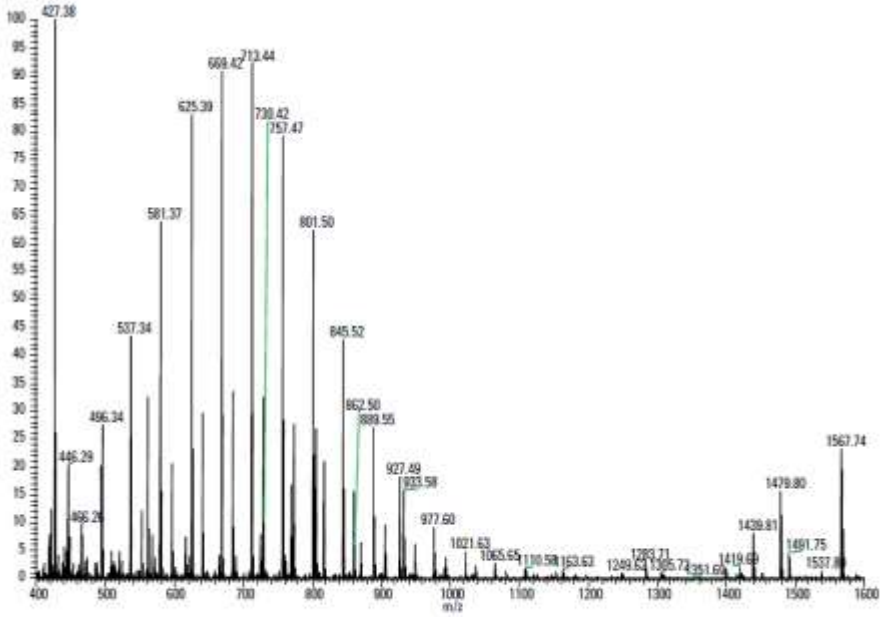
Dosáhnout rozbití buněk a maximální rozpustnosti všech bílovin/peptidů při zachování kompatibility se separační metodou a MS analýzou.

Detrgenty umožňují solubilizovat a denaturovat vzorek

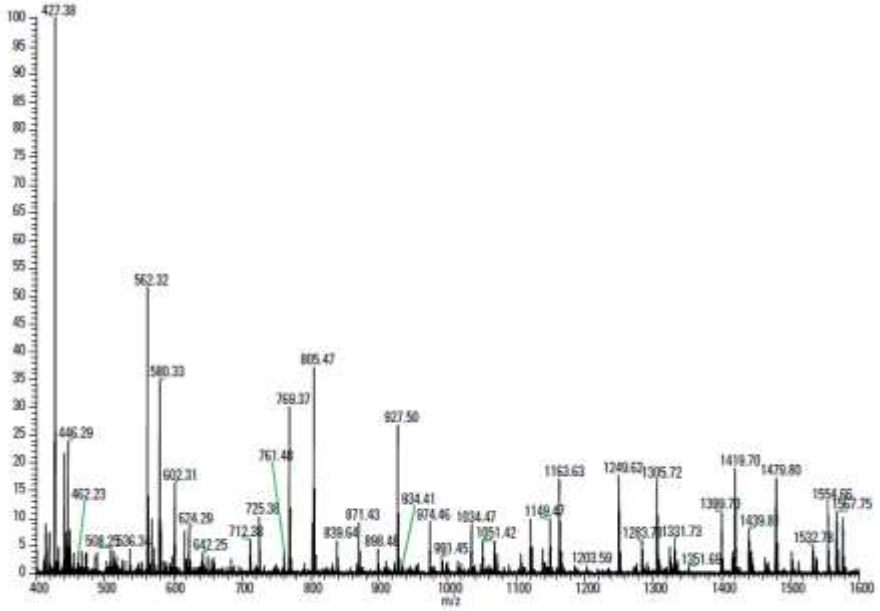
ale jsou nekompatibilní s digescí a/nebo LC-MS analýzou

Jak je využít a jak se jich zase rychle zbavit?

Odstranění detergentů



Triton X-100, Unprocessed



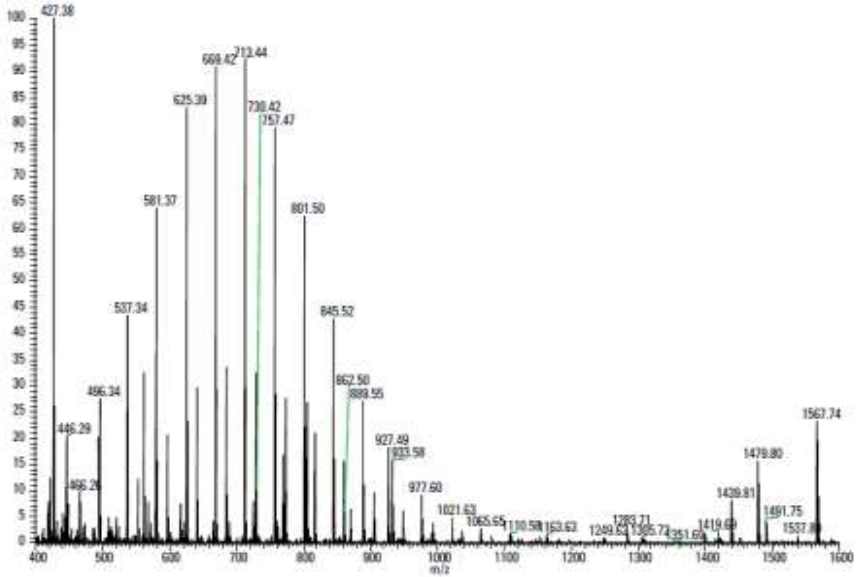
Triton X-100, Processed

Piercenet.com

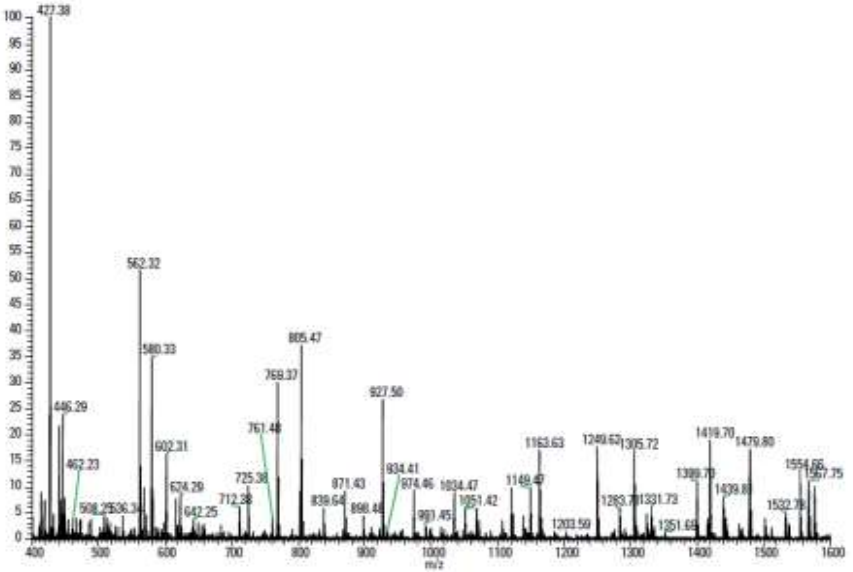
Odstranění detergentů

Deoxycholate (SDC)
SDS
Triton X100

*** Fázová separace * výměna pufru (FASP) * SP3**



Triton X-100, Unprocessed



Triton X-100, Processed

Piercenet.com

Odstranění detergentů

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

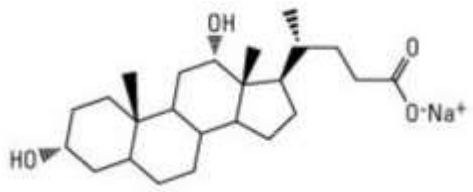
SDS - Výměna pufru (FASP)

Triton X-100 - 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

T. Masuda, et al. J. Proteome Res., 7 (2) (2008), pp. 731-740

Filter Assisted Sample Preparation - FASP

Odstranění SDS, SDC, ale nefunguje pro Triton

Výměna pufru, koncentrace vzorku, zbavení se detergentu, digesce

TÉMĚŘ UNIVERZÁLNÍ ŘEŠENÍ - FASP

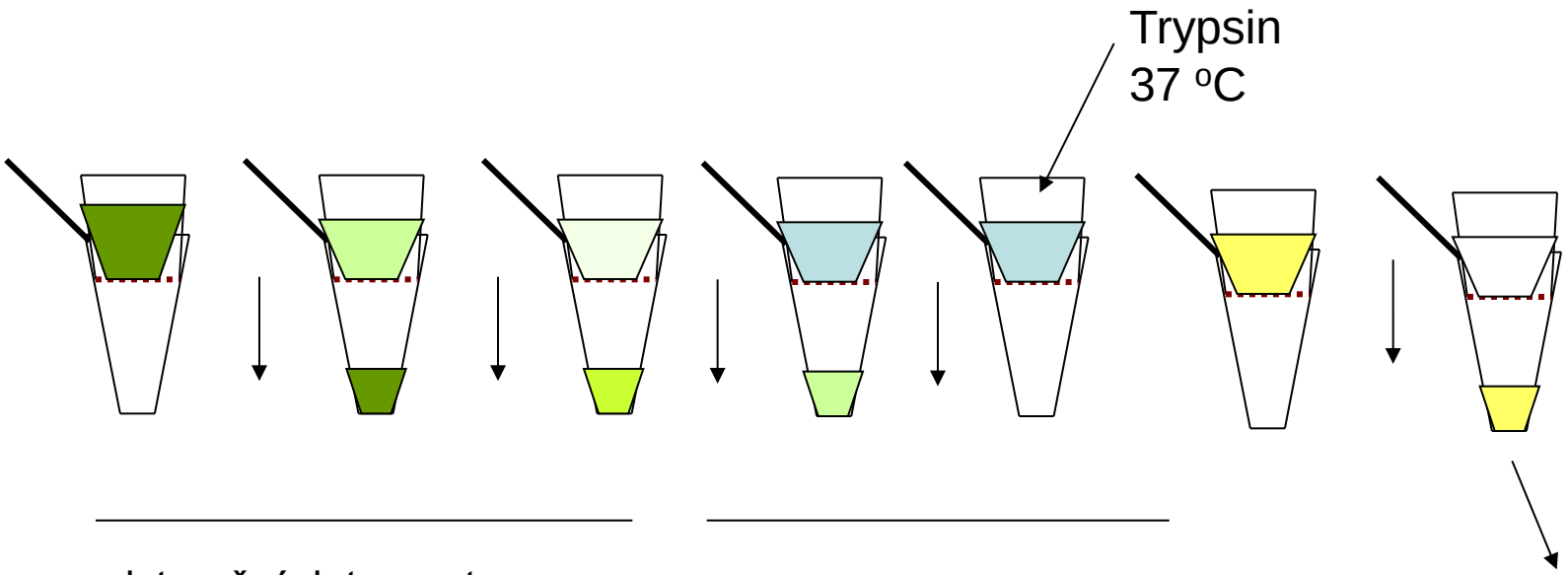
Vhodné filtry s cut off 10-30 kDa

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 detergentu (nelze štěpit trypsinem)

Vhodné filtry s cut off 10-30 kDa

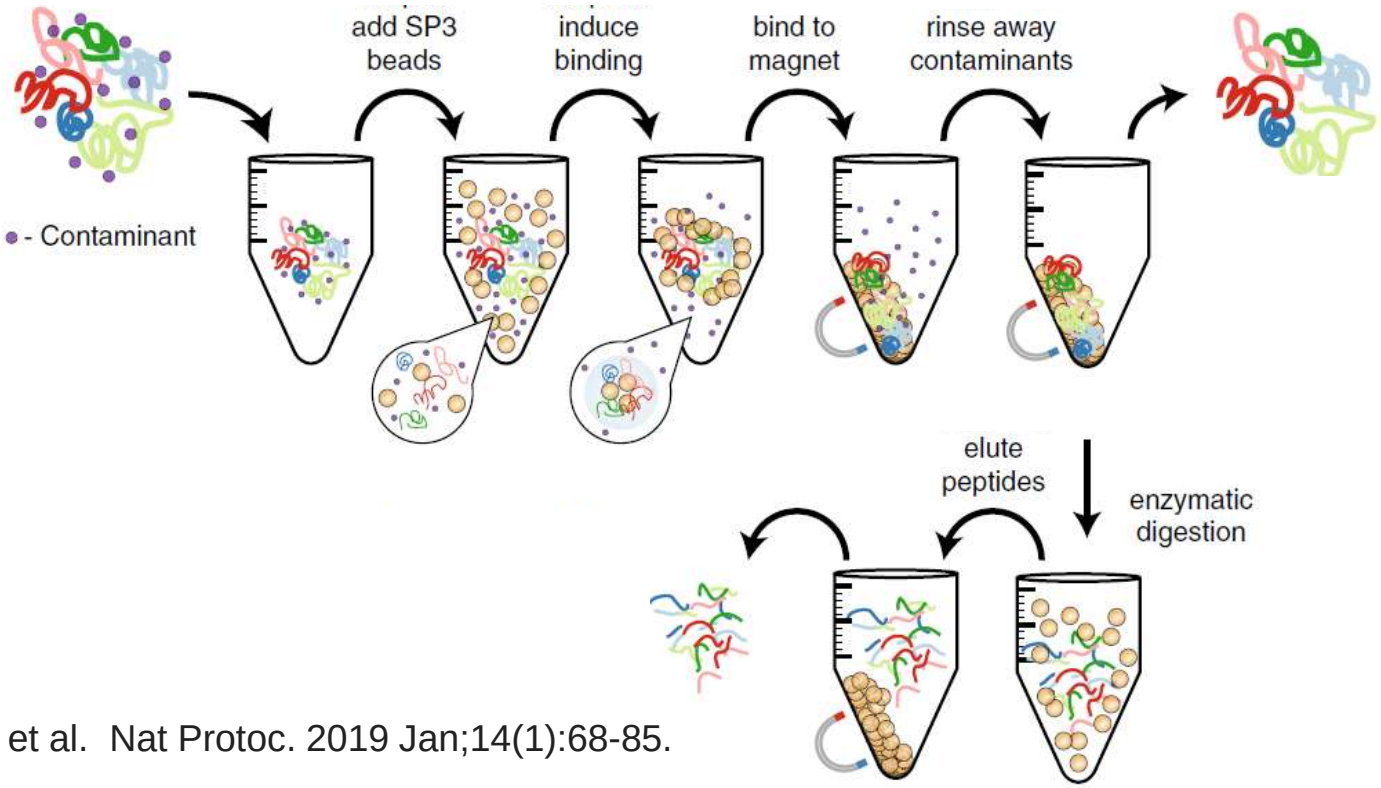


Single-pot, solid-phase-enhanced sample preparation for proteomics experiments

SP3

Odstranění SDS, SDC, **Tritonu X100**

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



Hughes et al. Nat Protoc. 2019 Jan;14(1):68-85.

Současná proteomika

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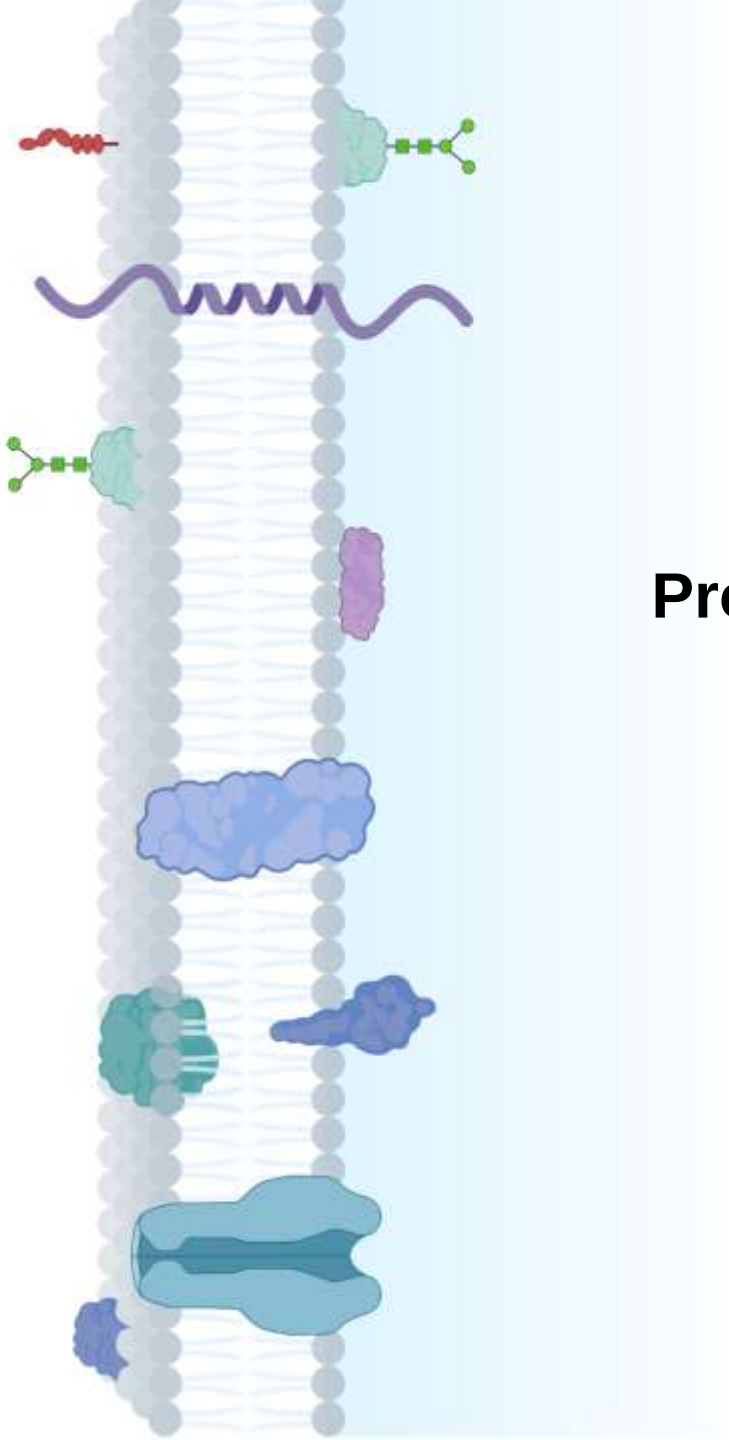
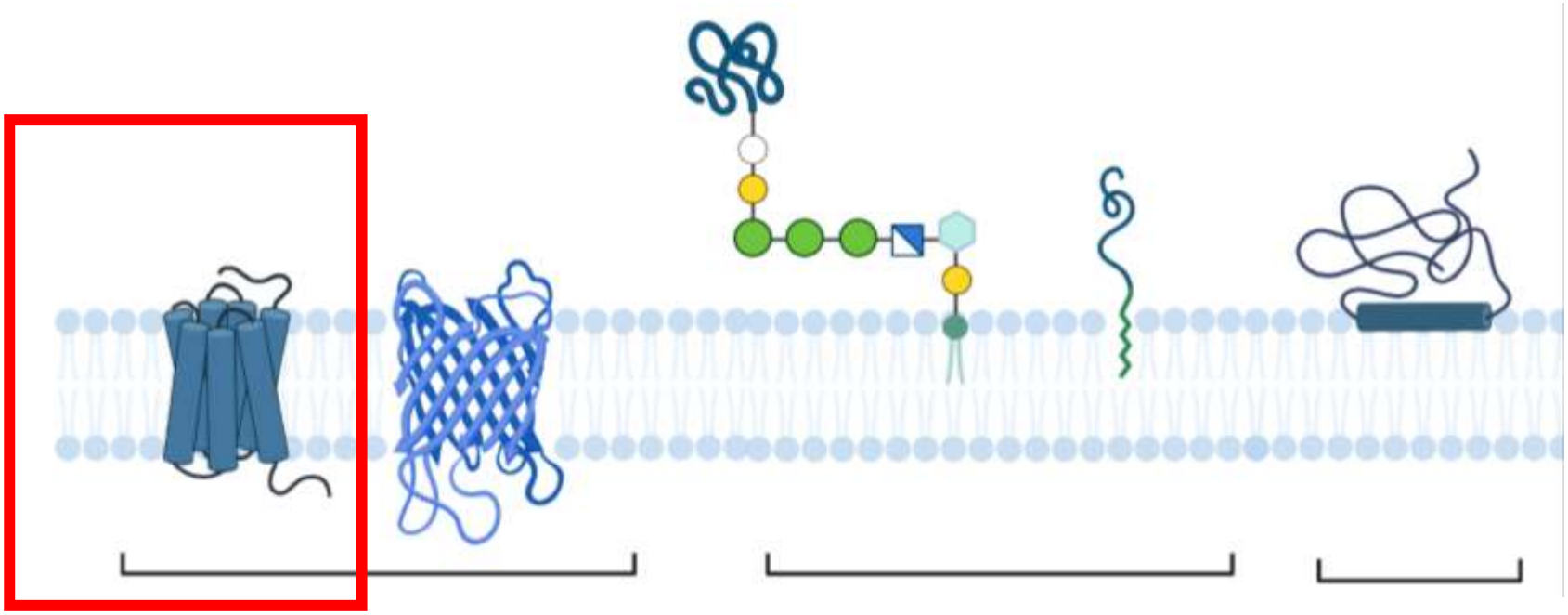


Proteomics of Membrane Proteins

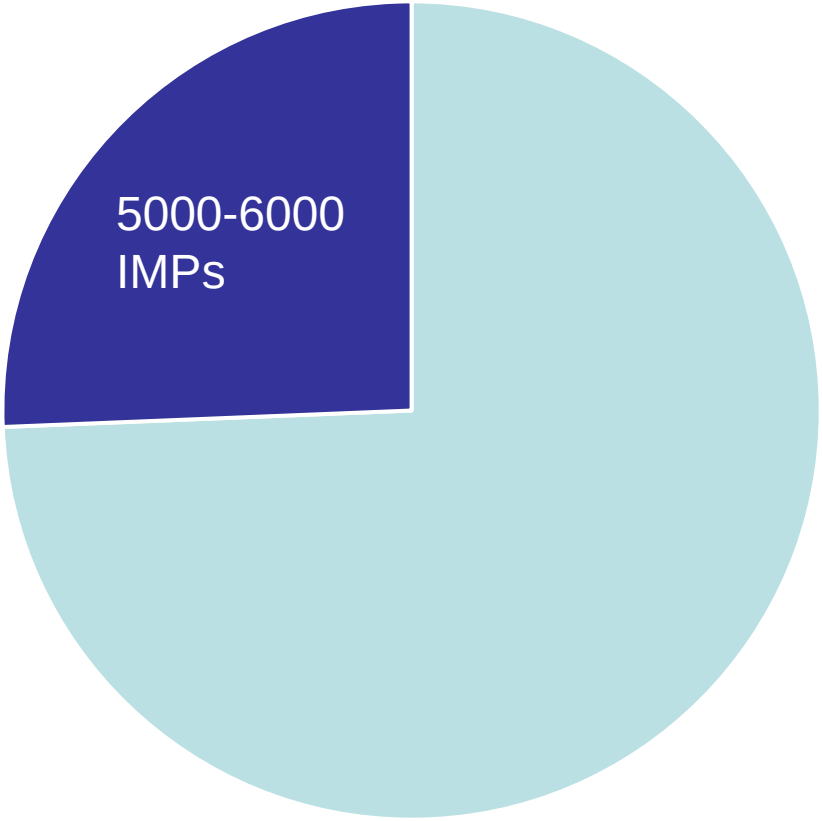


Integral membrane proteins

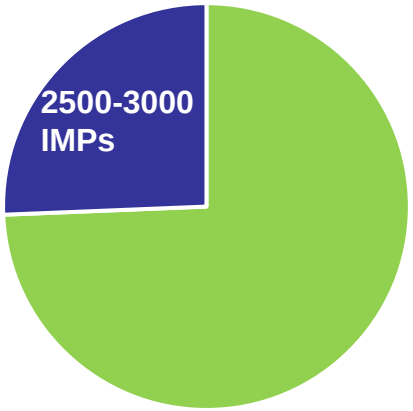
Lipid-anchored

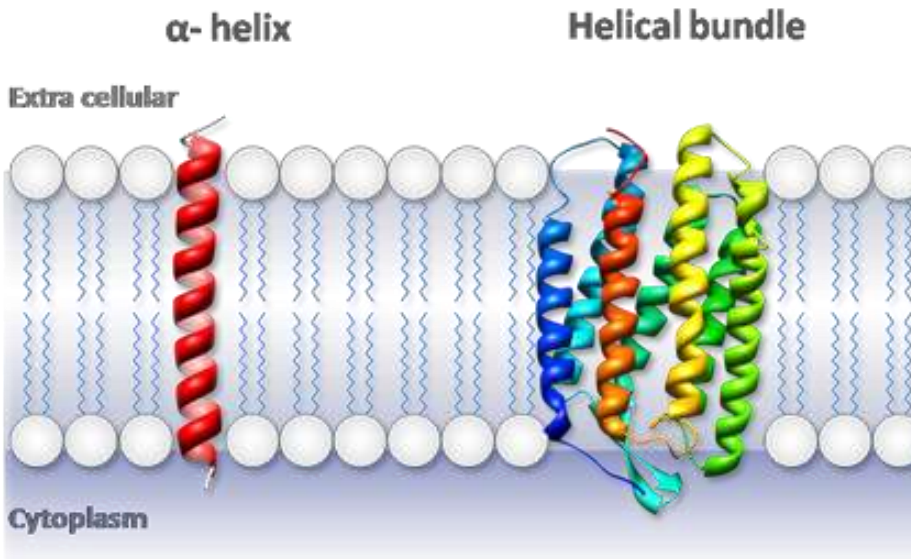
membrane-associated

~ 20 000 human protein coding genes



10 000 genes actively expressed by an average cell type



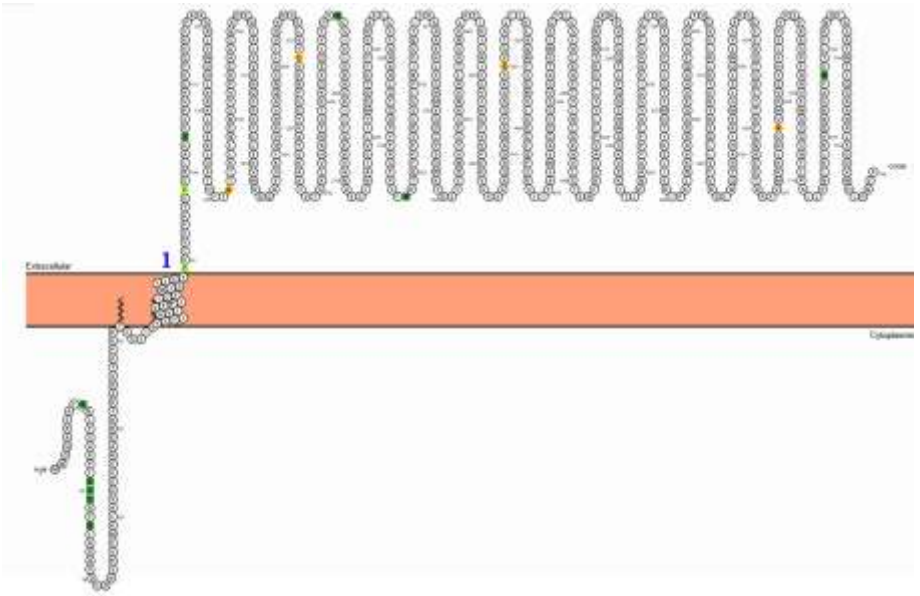


INTEGRAL MEMBRANE PROTEINS

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

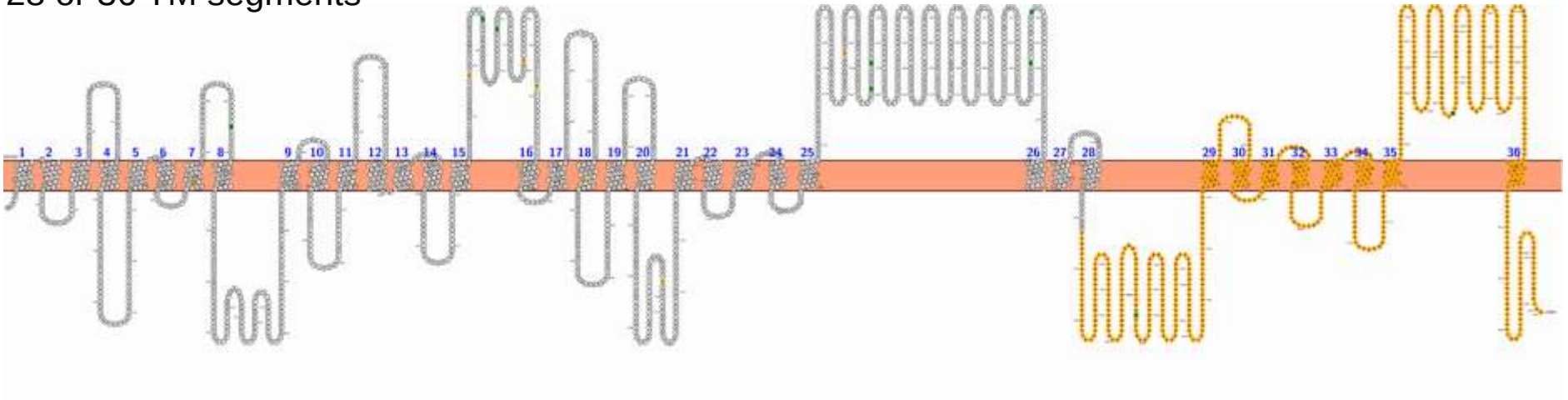
Transferrin receptor protein 1
TFRC

760 AA
1 TM segment

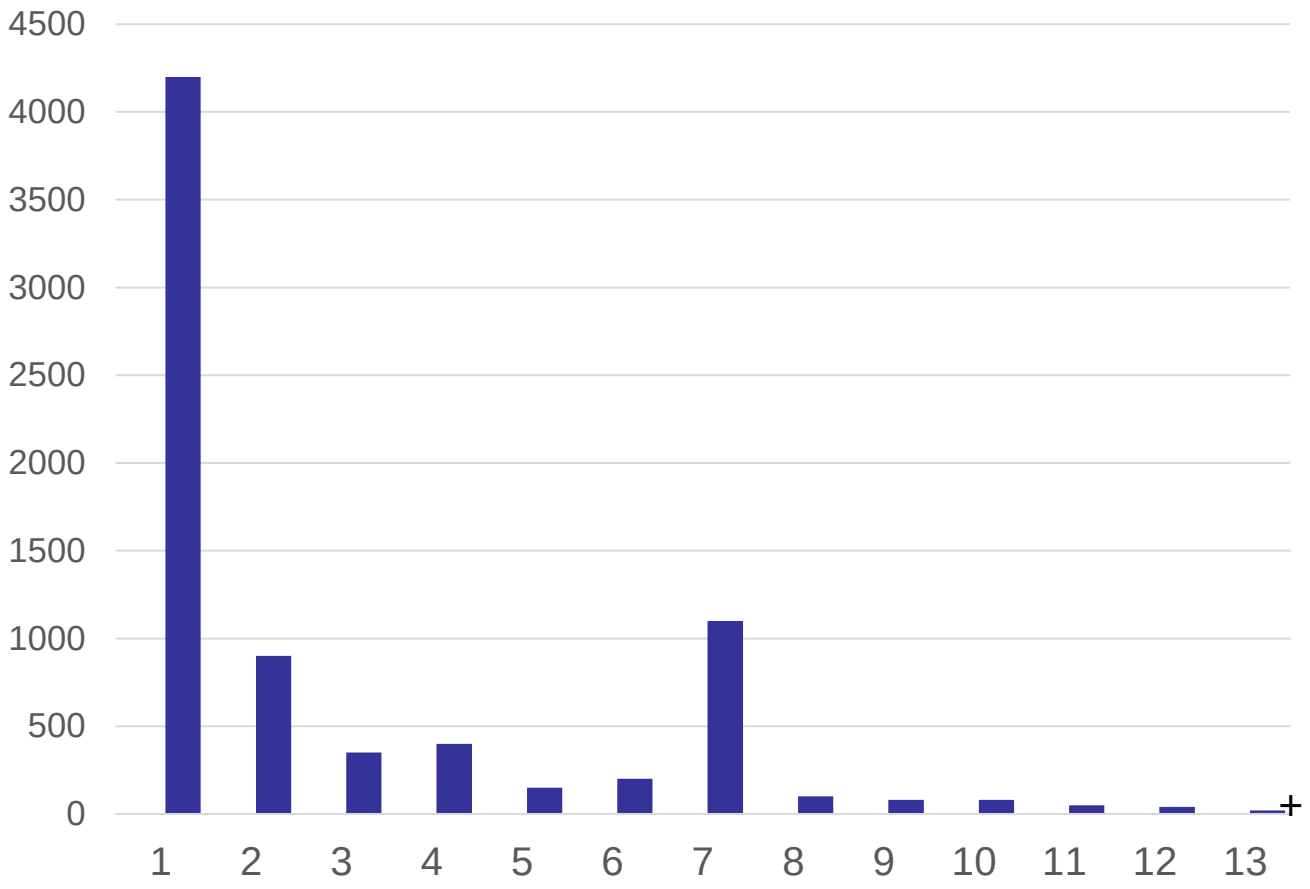


Piezo-type mechanosensitive ion channel component 1
PIEZO4

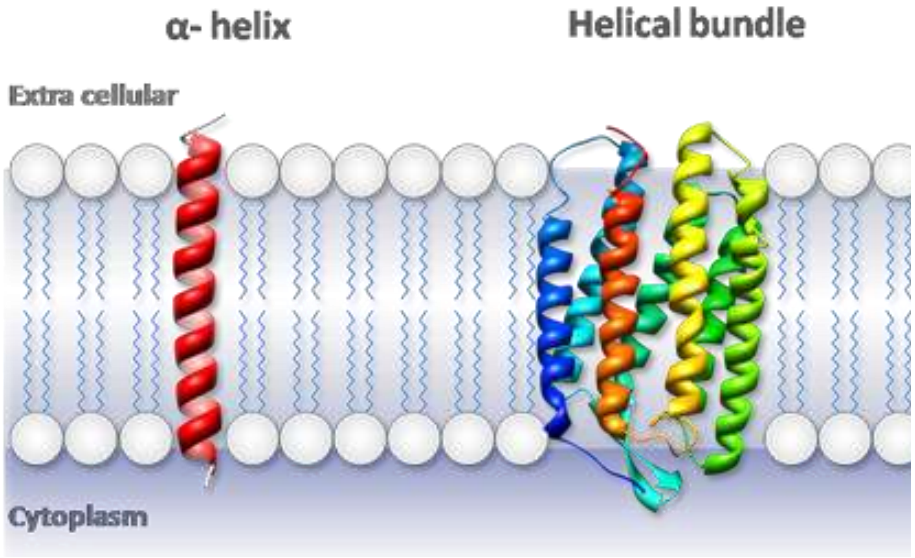
2521 AA
28 or 36 TM segments



Number of predicted TM segments in human integral membrane proteins



Fagerberg et al. Proteomics 2011

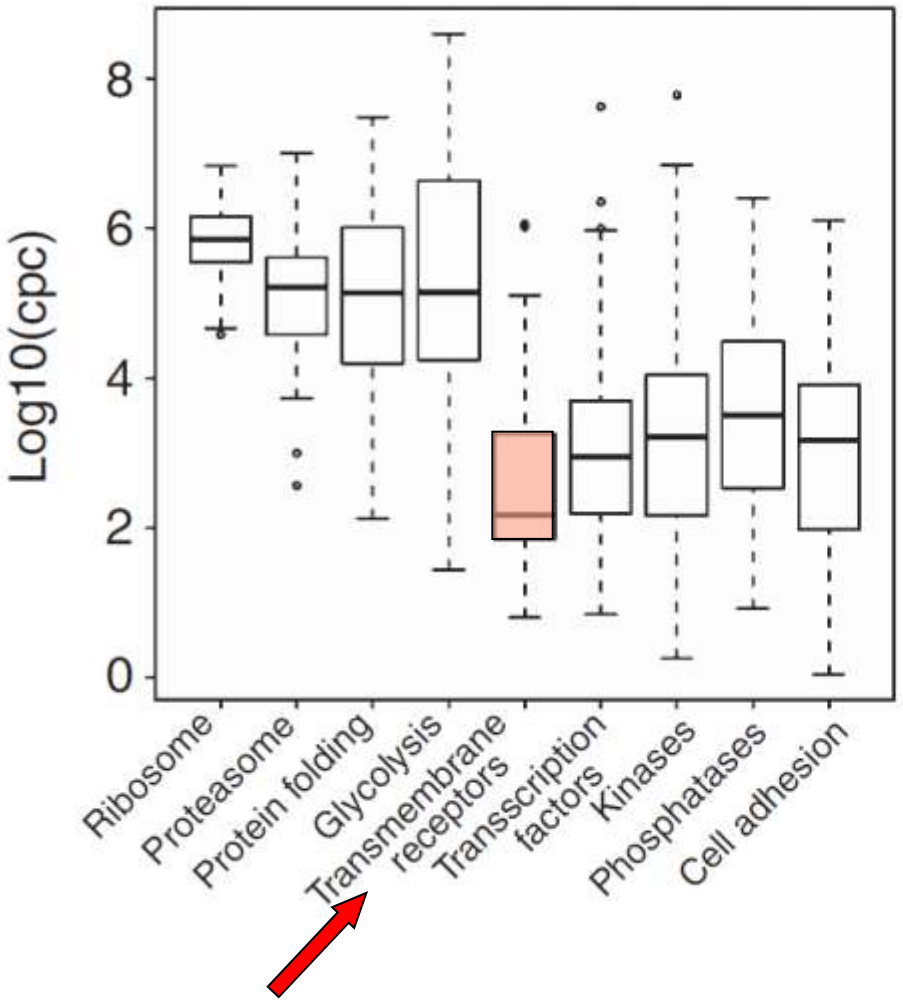


INTEGRAL MEMBRANE PROTEINS

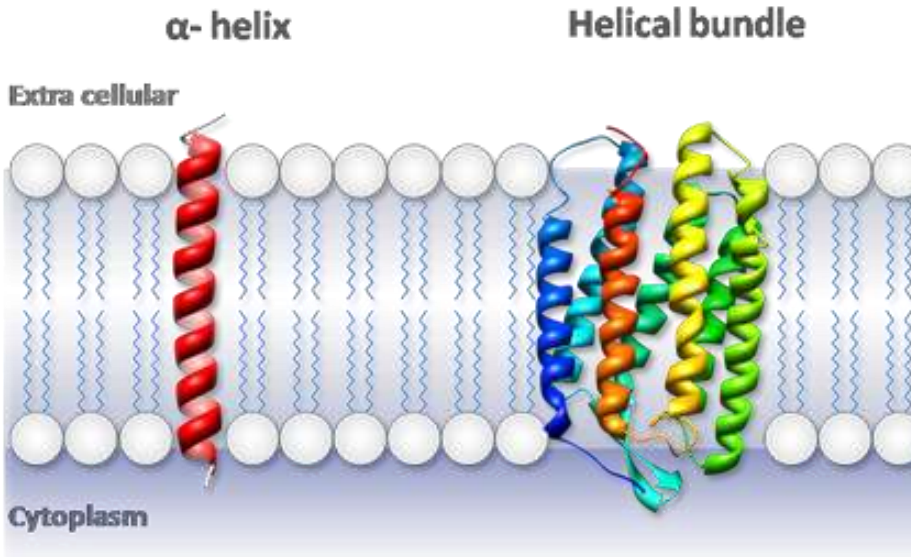
- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression

Cellular abundance of transmembrane proteins is LOW

(100-1000 copies/cell)



Beck et al., Mol Syst Biol. 2011 Nov 8;7:549.



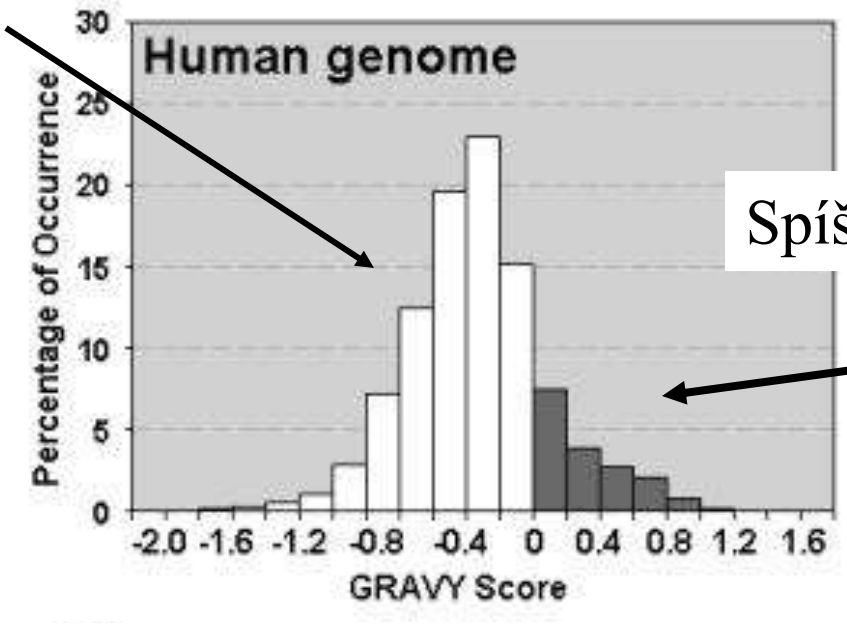
INTEGRAL MEMBRANE PROTEINS

- 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“ (+ G, C, S)

CD171 (P32004)

MVVALRYVWPLLLCSPCLLIQIPEEYEGHHVMEPPVITEQSPRRLVVFPTDDISLKCEASGKPEVQFRWTRDGVHFKPKEELGVTVYQSP
HSGSFTITGNNSNFAQRFQGIYRCFASNKLGTAMSHEIRLMAEGAPKWPKETVKPVEVEEGESVVLPCNPPPSAEPLRIYWMNSKILHIKQ
DERVTMGQNGNLYFANVLTSDNHSDYICHAHFPGTRTIIQKEPIDLRVKATNSMIDRKPRLLFPTNSSSHLVALQGQPLVLECIAEGFPTPTI
KWLRPSGPMPADRVTYQNHNKTLQLLKVGEEDDGEYRCLAENSLGSARHAYYVTVEAAPYWLHKPQSHLYGPGETARLDCQVQGRPQ
PEVTWRINGIPVEELAKDQKYRIQRGALILSNVQPSDTMVTQCEARNRHGLLLANAYIYVVQLPAKILTADNQTYMAVQGSTAYLLCKAFGA
PVPSVQWLDEDGTTVLQDERFFPYANGTLGIRDLQANDTGRYFCLAANDQNNVTIMANLKVKDATQITQGPRSTIEKKGSRVTFTCQASF
DPSLQPSITWRGDGRDLQELGDSDKYFIEDGRLVIHSLDYSDQGNYSCVASTELDVVESRAQLLVVGSPGPVPRLVLSDLHLLTQSQVRV
SWSPAEDHNAPIEKYDIEFEDKEMAPEKWYSLGKVPGNQTSTTLKLSPYVHYTFRVTAINKYGPGEPSPVSETVVTPEAAPEKNPVDVKG
EGNETTNMVITWKPLRWMDWNAPQVQYRVQWRPQGTRGPWQEQIVSDPFLVVSNTSTFVPYEIKVQAVNSQGKGPEPQVTIGYSGED
YPQAIPELEGIEILNSSAVLVKWRPVDLAQVKGHLRGYNVTYWREGSQRKHSKRHIHKDHVVVPANTTSVILSGLRPYSSYHLEVQAFNGR
GSGPASEFTFSTPEGVPGHPEALHLECQSNTSLLLRWQPPLSHNGVLTGYVLSYHPLDEGGKGQLSFNLRDPELRTHNLTDLSPHLRYR
FQLQATTKEGPGEAIVREGGTMALSGISDFGNISATAGENYSVVSWVPKEGQCNFRFHILFKALGEEKGGASLSPQYVSYNQSSYTQWDL
QPDTDYEIHLFKERMFRHQMAVKTNGTGRVRLPPAGFATEGWFIGFVSAIILLLLVLLILCFIKRSKGGKYSVKDKEDTQVDSEARPMKDET
FGEYRSLESDNEEKAFGSSQPSLNGDIKPLGSDDSLADYGGSVDVQFNEDGSFIGQYSGKKEKEAAGGNDSSGATSPINPAVALE

Predicted TM segment

FAMILY VW +C +G +S

The source of protein information (SwissProt/Uniprot)

UniProt
(uniprot.org)

Prediction of TM domains via Hidden Markov Models (using FASTA sequence)

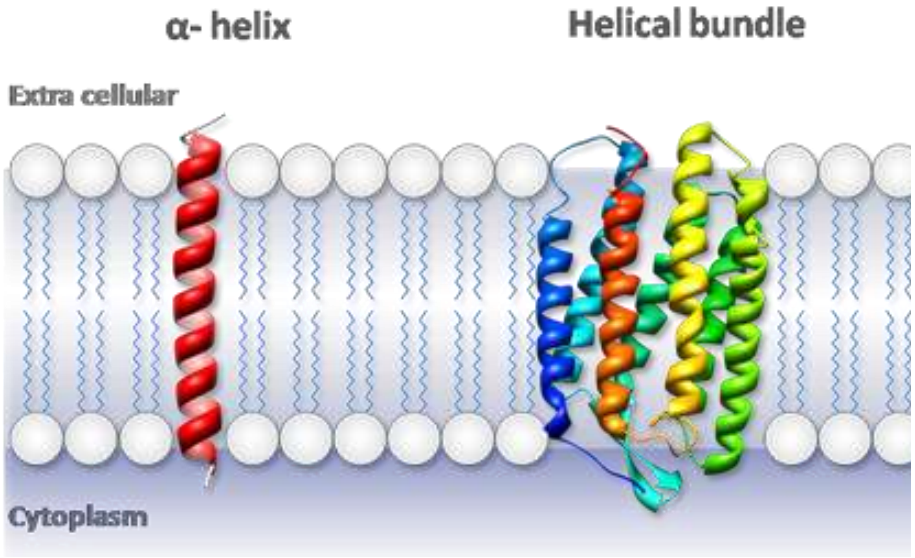
TMHMM 2.0 - DTU Health Tech - Bioinformatic Services
(services.healthtech.dtu.dk/services/TMHMM-2.0)

Visualization of TM domain prediction (using FASTA sequence or accession number)

Protter - interactive protein feature visualization
(wlab.ethz.ch/protter)

Human Protein Atlas

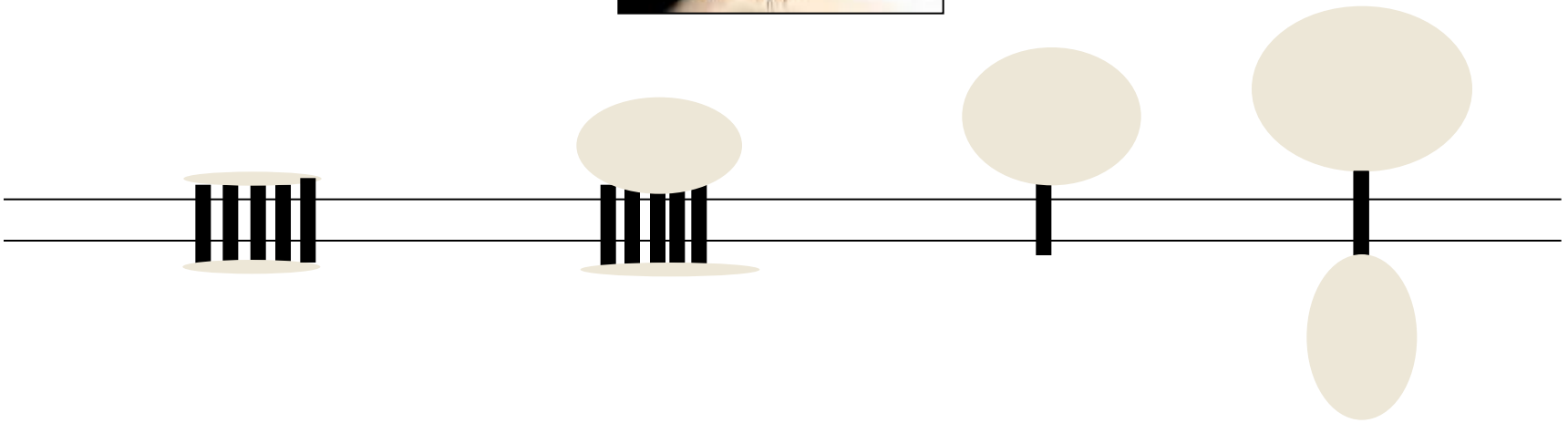
The Human Protein Atlas (www.proteinatlas.org)



INTEGRAL MEMBRANE PROTEINS

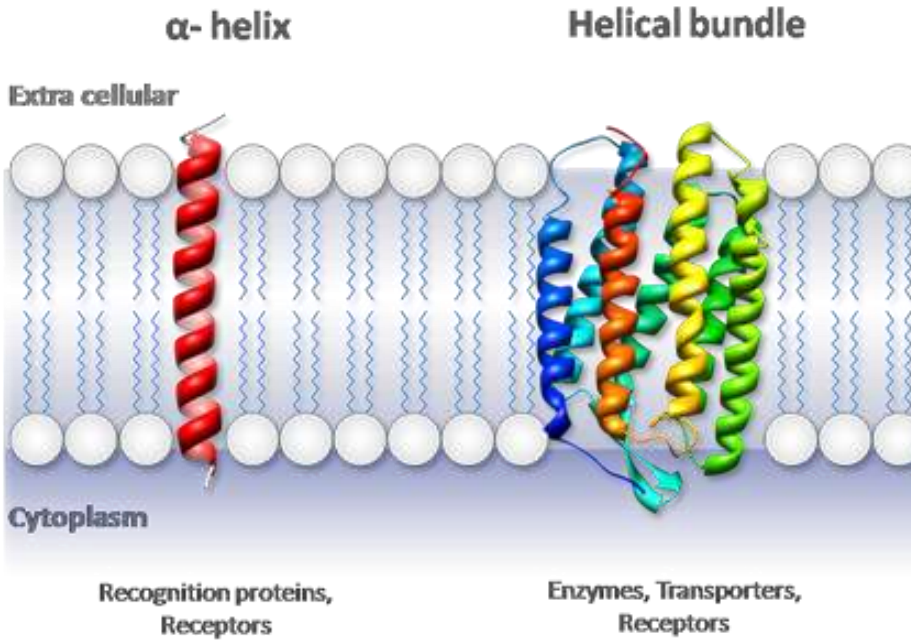
- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression
- hydrophobic/amphipathic nature

IMPs - molecules with split personalities



hydrophilic

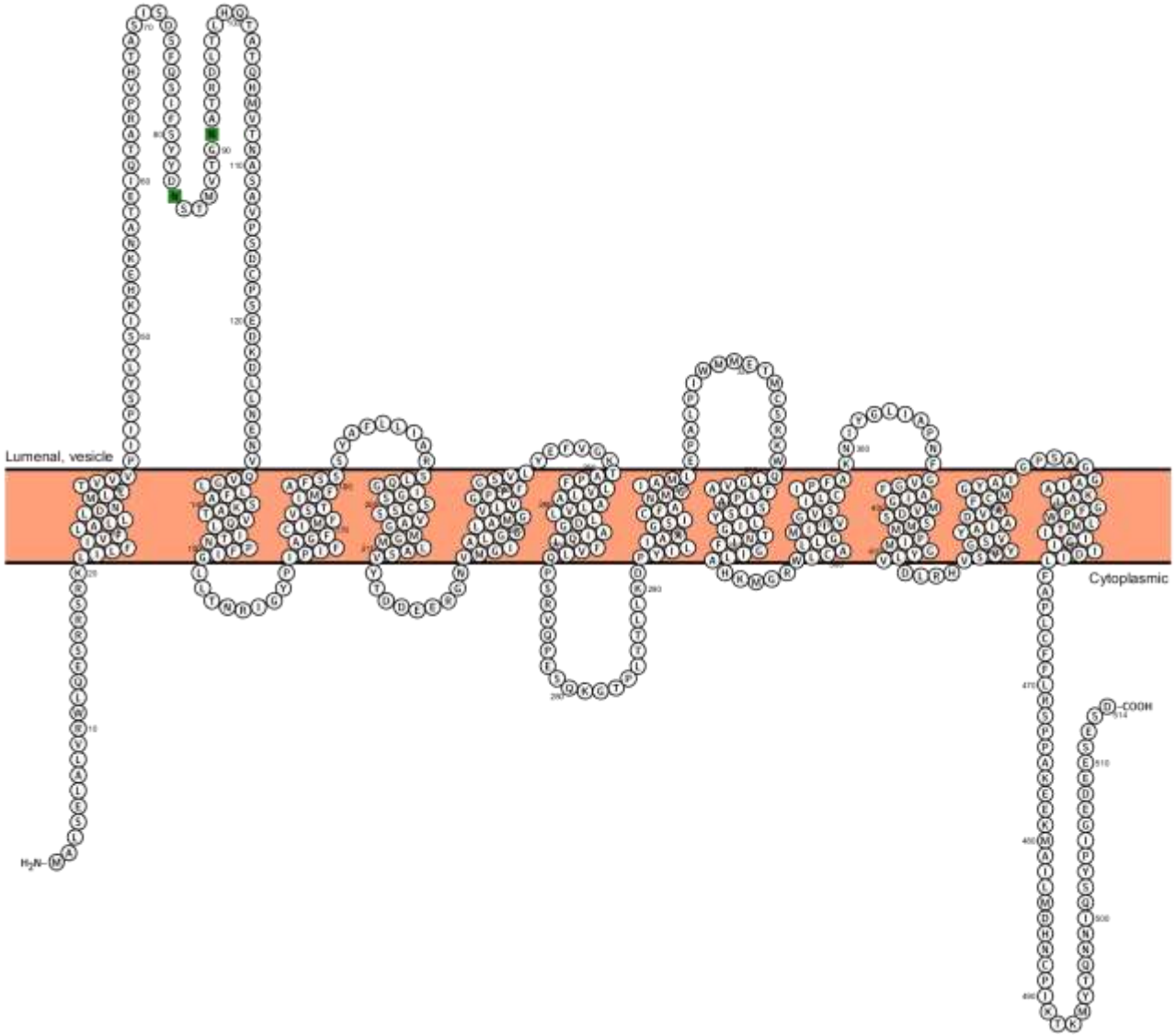
hydrophobic



INTEGRAL MEMBRANE PROTEINS

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

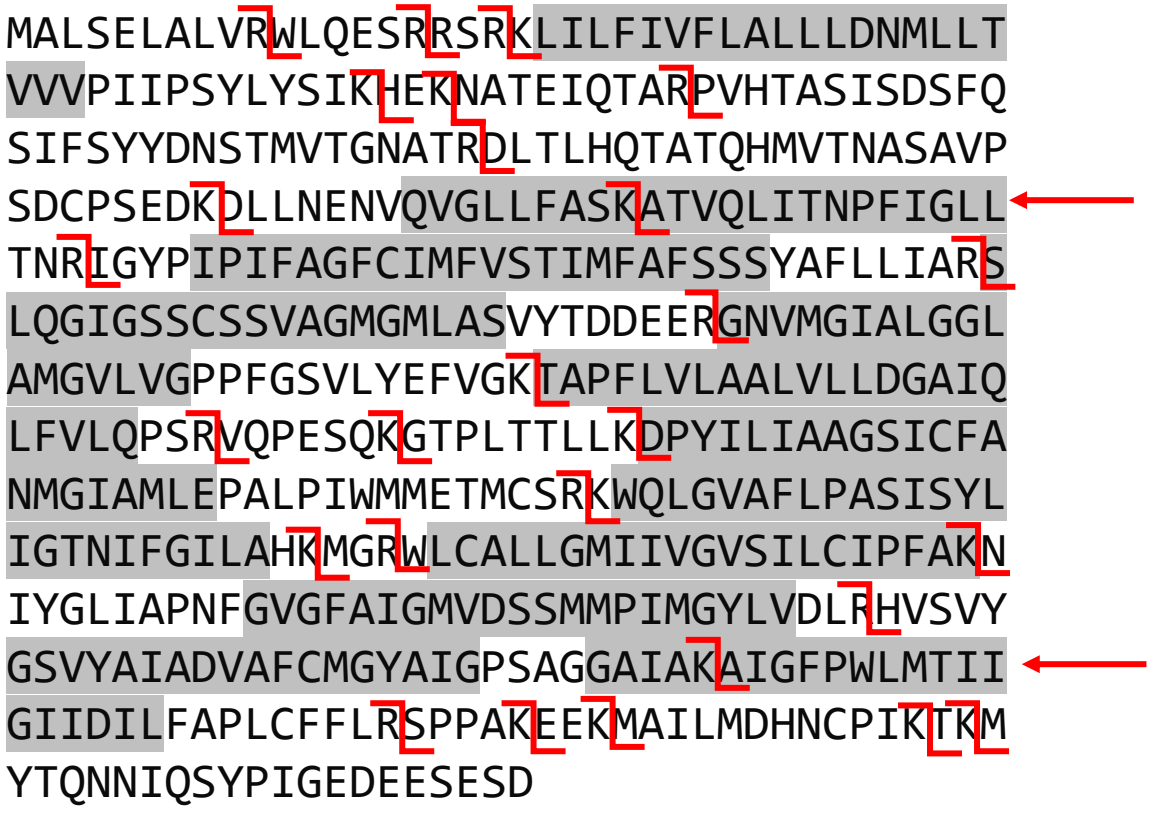
Synaptic vesicular amine transporter (Slc18a2)



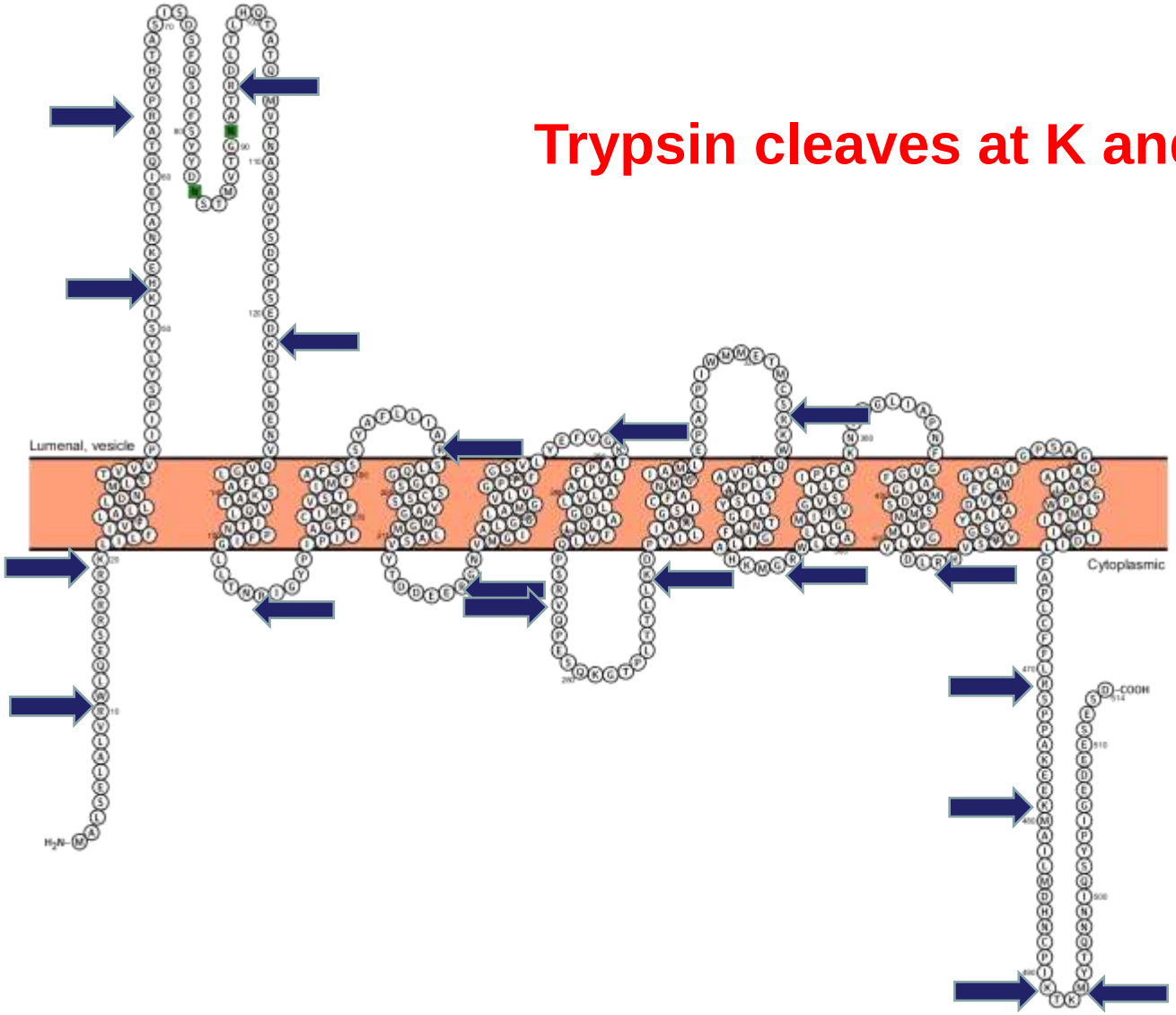
Visualization by Protter (*Omasits et al., Bioinformatics. 2013*)

Synaptic vesicular amine transporter (SLC18A2)

A protein with 12 TM segments



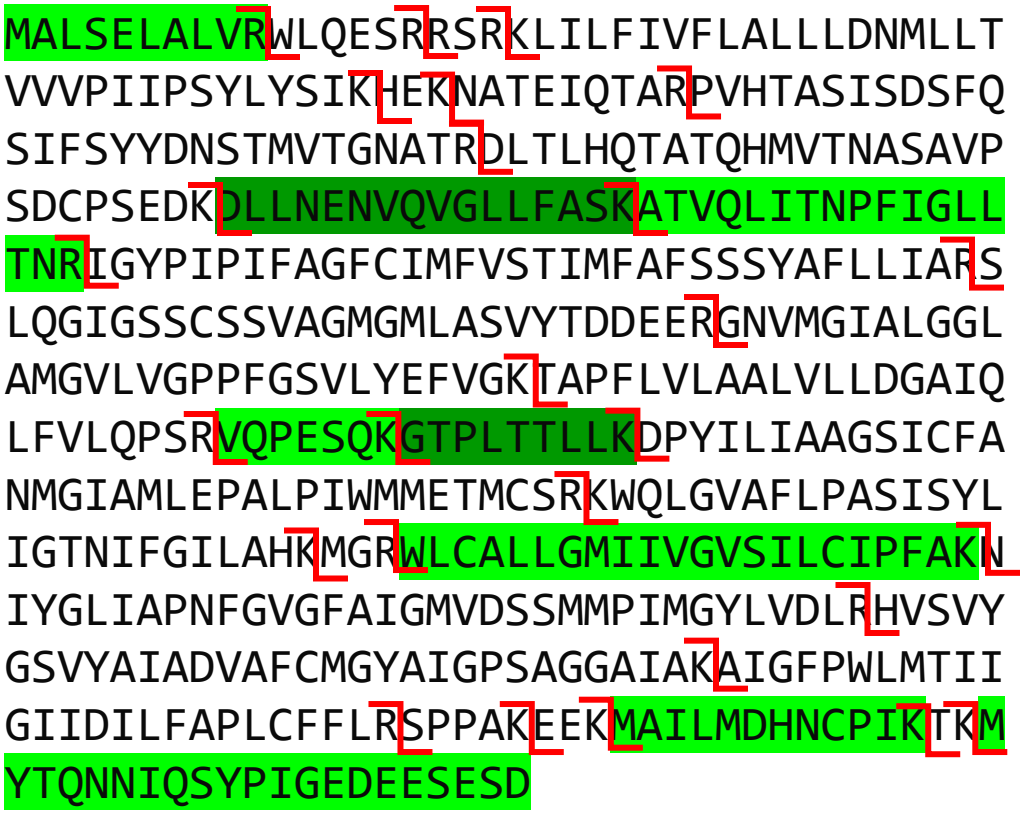
Synaptic vesicular amine transporter (Slc18a2)



Trypsin cleaves at K and R

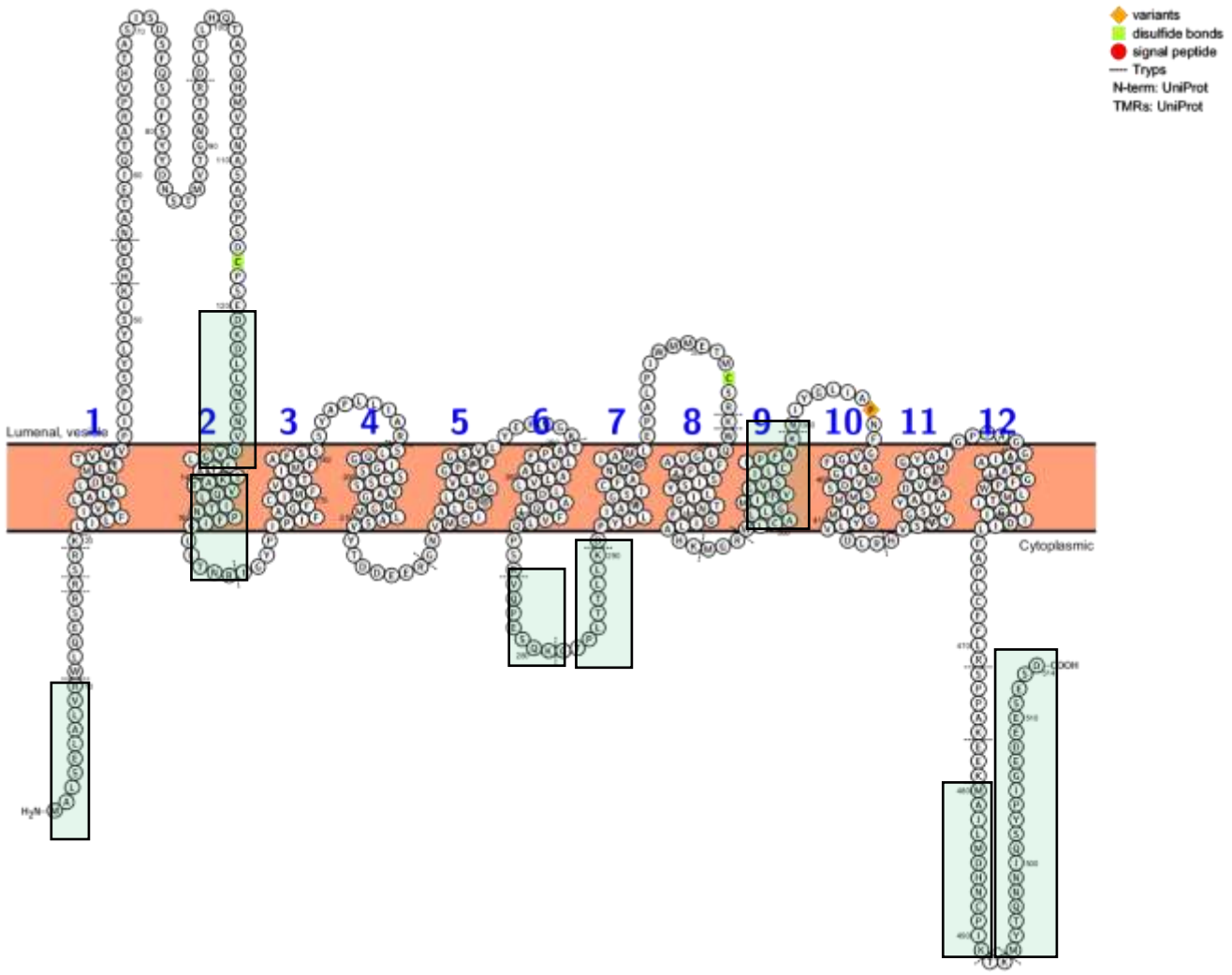
Visualization by Protter (*Omasits et al., Bioinformatics. 2013*)

Synaptic vesicular amine transporter (SLC18A2)



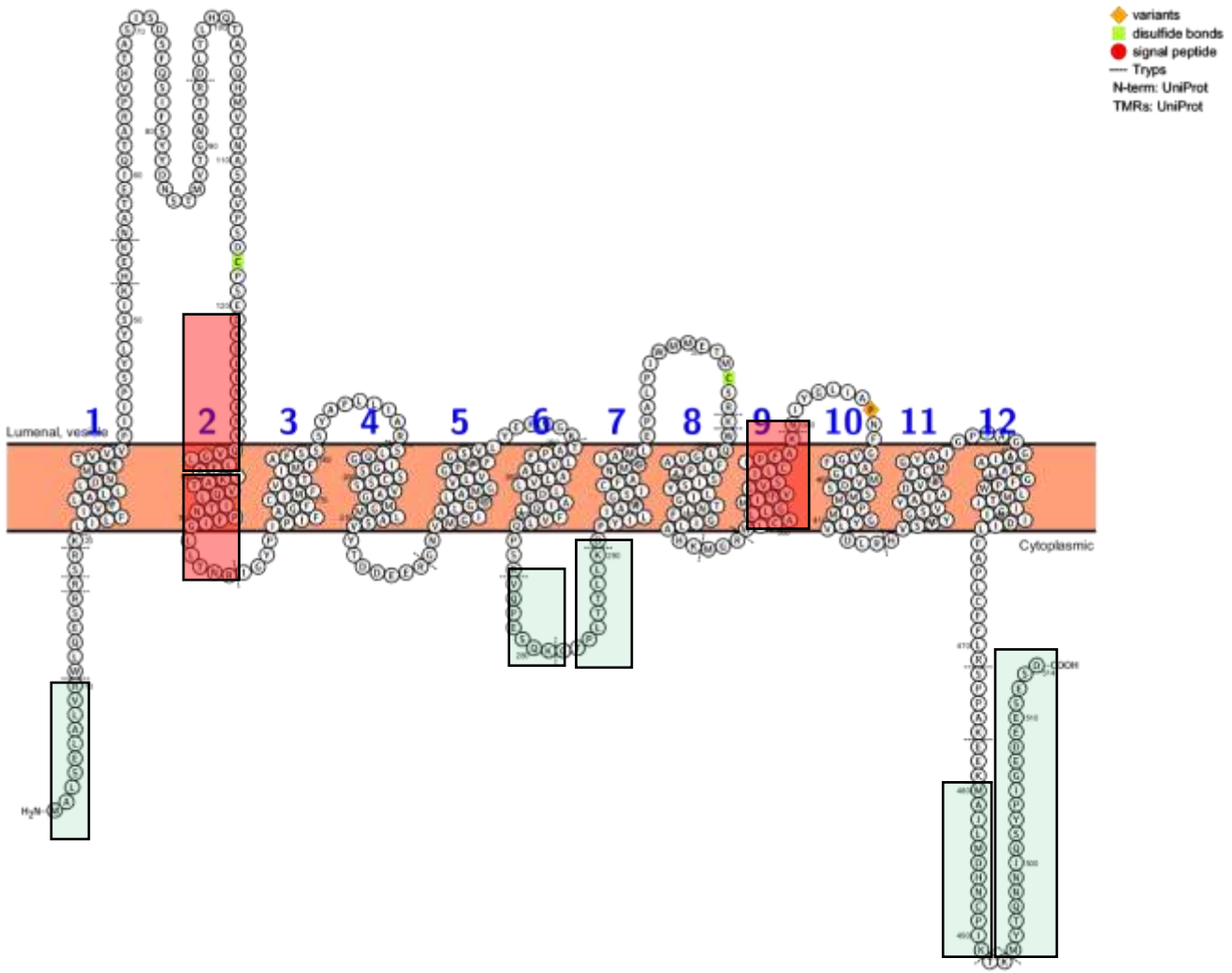
Only 8 unique peptides (5-25AA)

Synaptic vesicular amine transporter (SLC18A2)



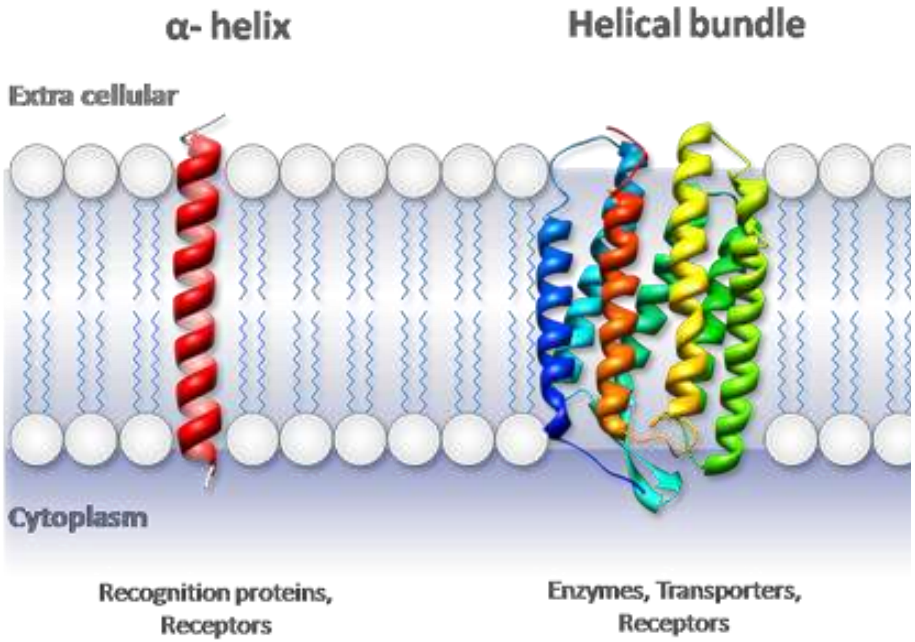
Only 8 unique peptides (5-25AA)

Synaptic vesicular amine transporter (SLC18A2)



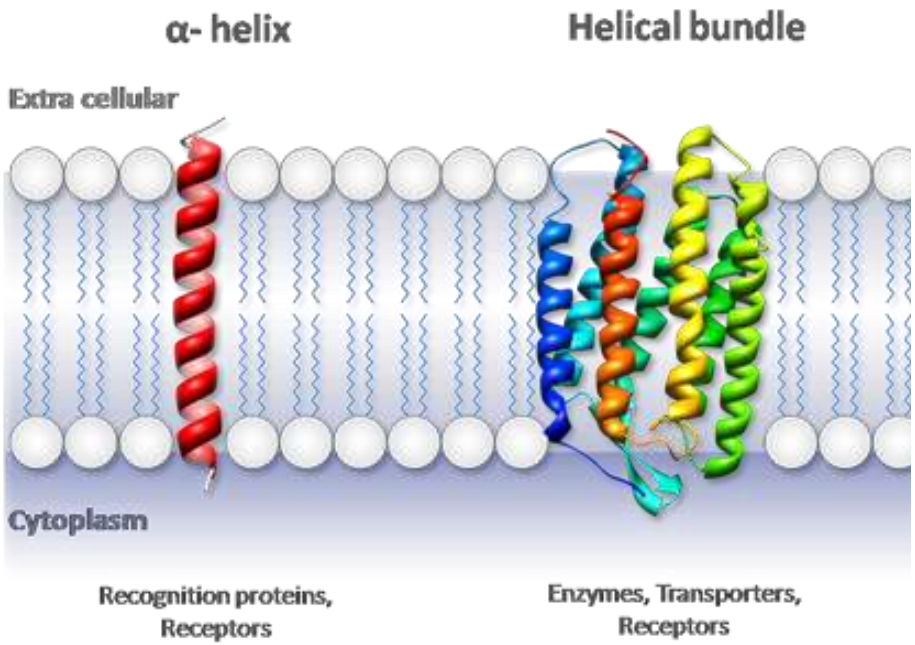
Only 8 unique peptides (5-25AA)

Only 5 unique peptides in the accessible soluble segments



INTEGRAL MEMBRANE PROTEINS

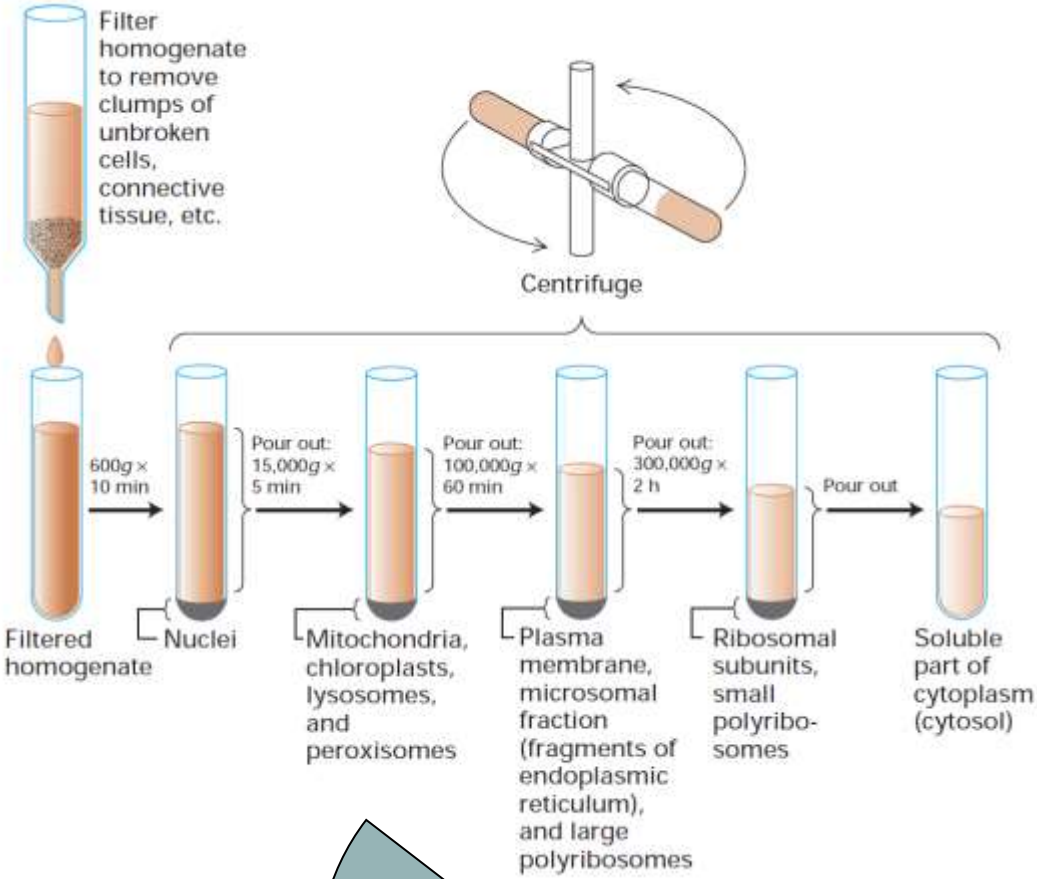
- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression
- hydrophobic/amphipathic nature
- no Arg, Lys in TM domains
- detergents interfere with digestion and/or LC-MS
- UNDER-REPRESENTED IN PROTEOMIC ANALYSES



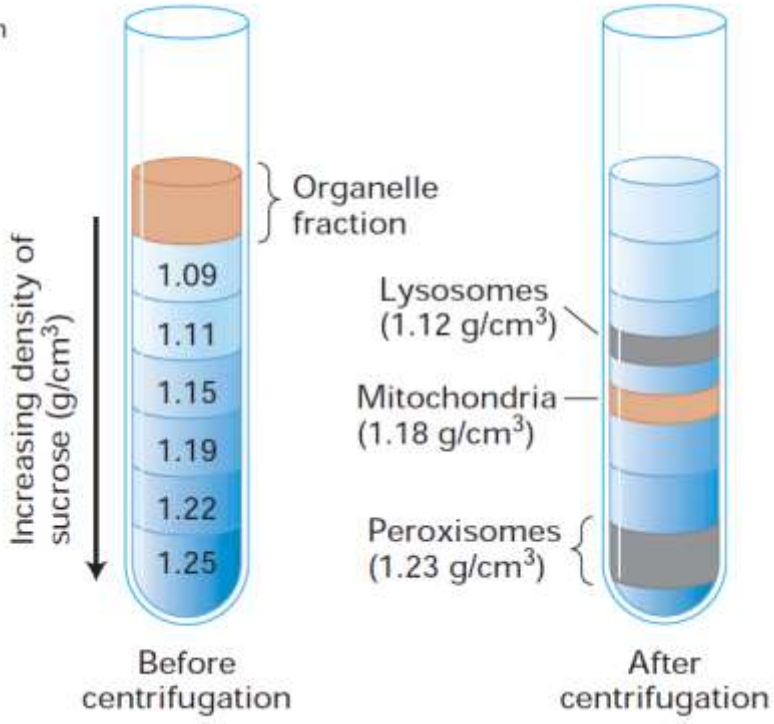
INTEGRAL MEMBRANE PROTEINS

- Increase the enrichment!
- Get acess to the hydrophobic parts of the molecule!
- Avoid or remove detergents!

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

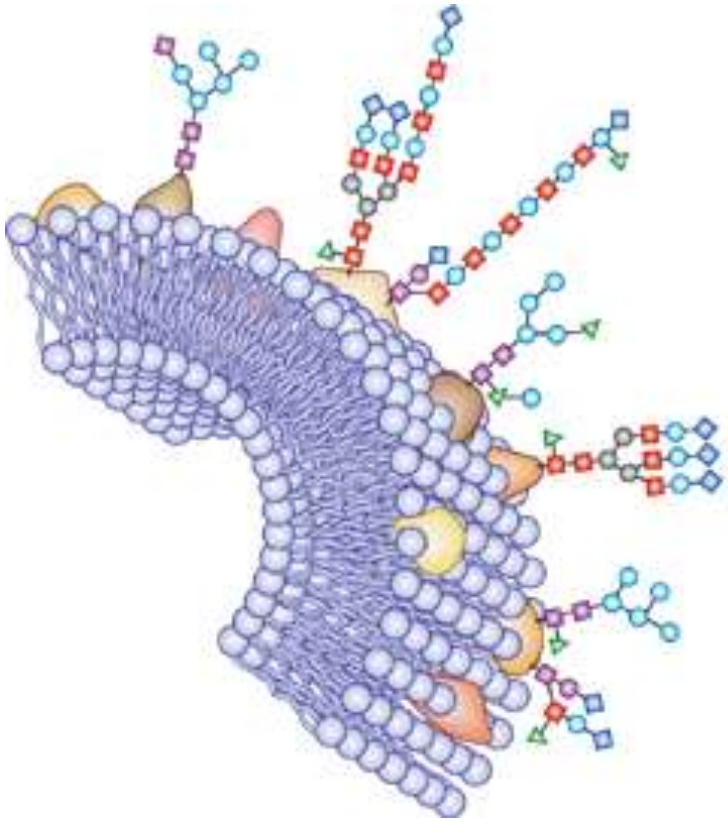
↓

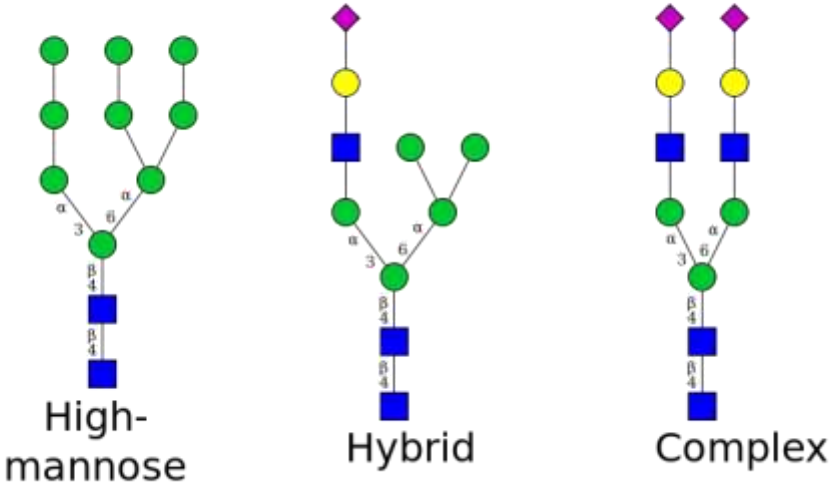
Tryptic digestion

↓

LC-MS/MS

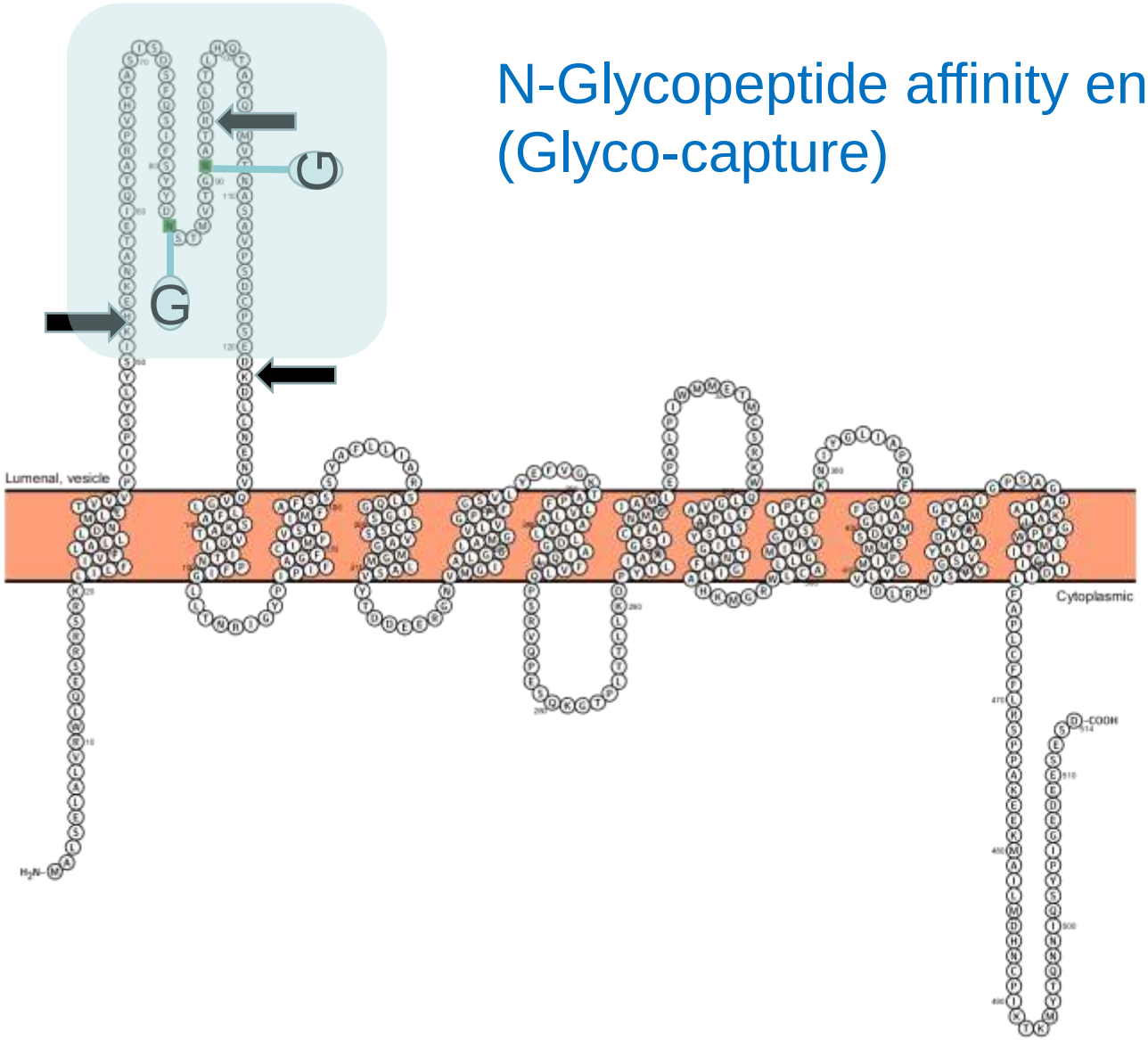
LOW ENRICHMENT
(10-15%)





Asn-Xaa-Ser/Thr

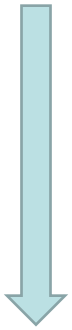
“DIVIDE AND CONQUER“ METHODS



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

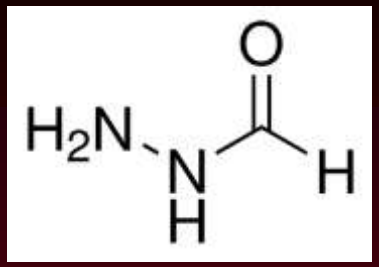
Zhang et al.
Nature Biotechnology, 2003

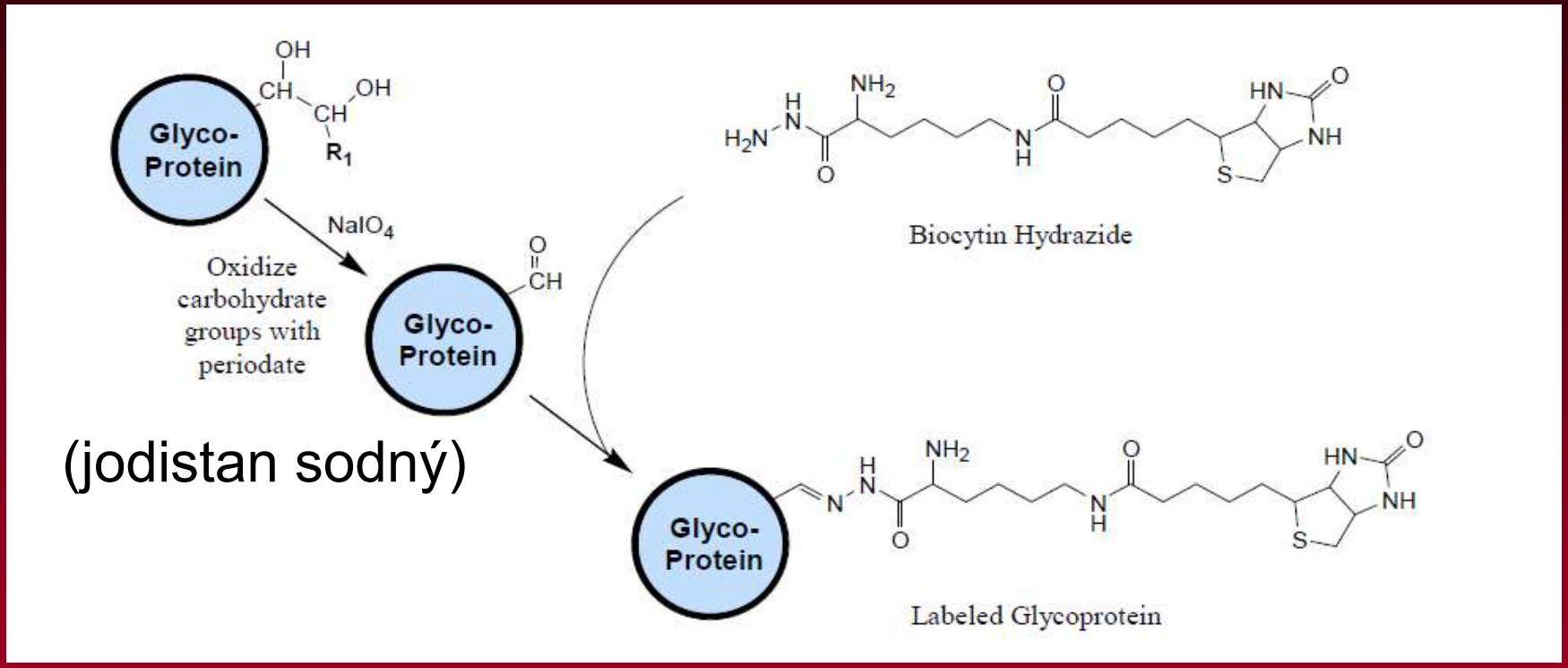
Glyco-capture
záchyt glykopeptidů z lyzátu

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



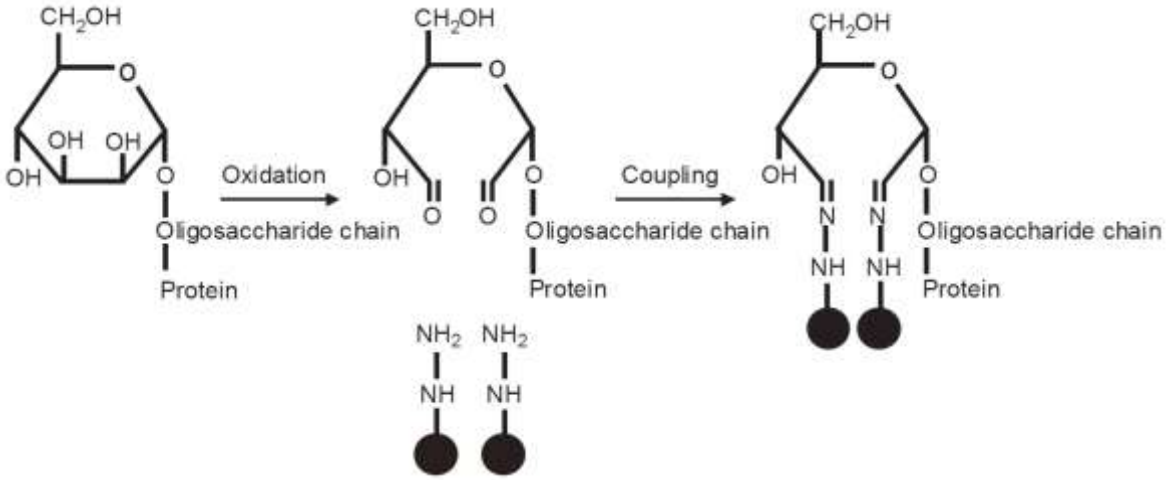


Biotin Hydrazide **bind to oxidized carbohydrates through the hydrazide group (–NH-NH_2), forming a hydrazone linkage.** Oxidation of glycoproteins generates reactive aldehydes that react specifically with hydrazide groups.

Glyco-capture
záchyt glykopeptidů z lyzátu

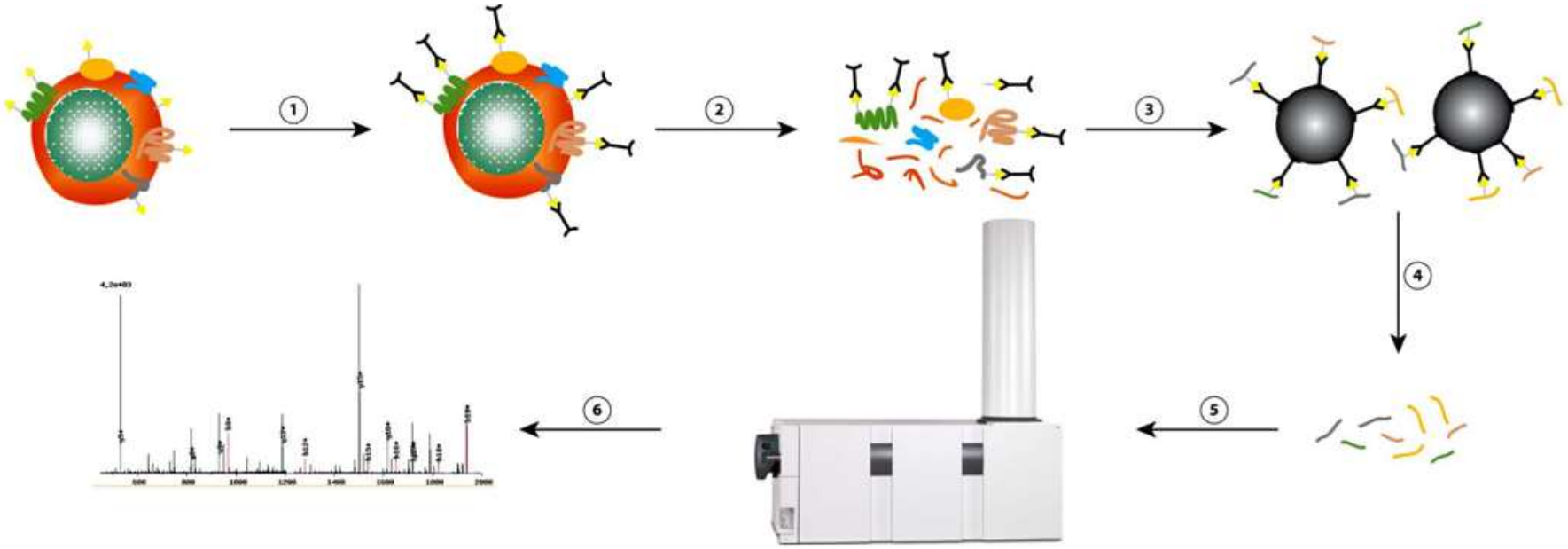
GLYCO-FASP – po digesci vzorku pomocí FASP jsou peptidy smíchány na filtru s lektiny. Neglykosylované peptidy jsou odmyty a glykopeptidy následně uvolněny PNGázou.
(concanavalin A, WGA (wheat germ agglutinin), RCA (Ricinus communis agglutinin))

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

Wollscheid B, et al. Nat Biotechnol. 2009 Apr;27(4):378-86

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

↓

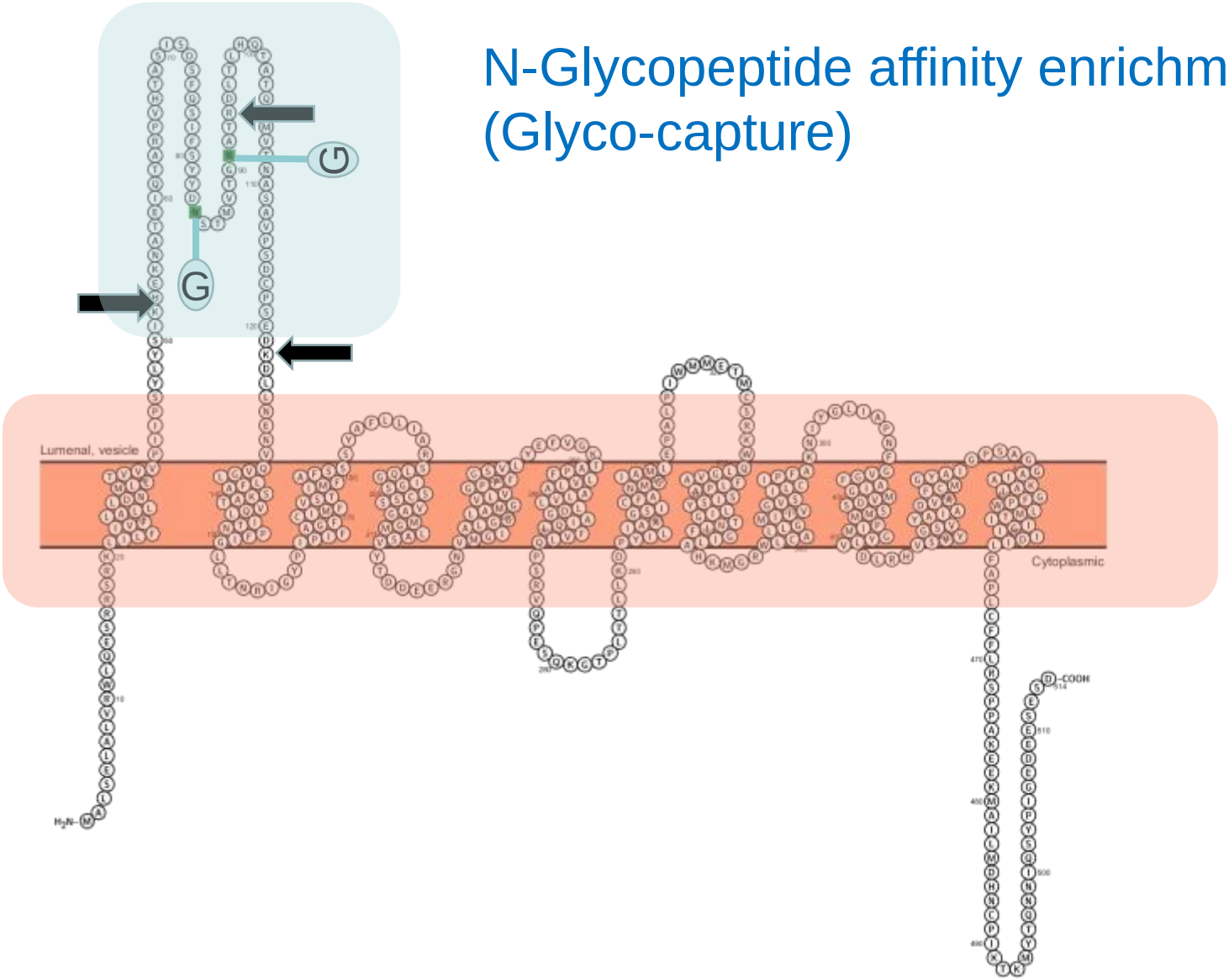
Isolation of N-glycopeptides

↓

LC-MS/MS

“DIVIDE AND CONQUER“ METHODS

N-Glycopeptide affinity enrichment (Glyco-capture)



Identification of IMPs via enrichment of membrane-embedded segments **hpTC method** **(H**igh **p**H-**T**rypsin-**C**NBr)

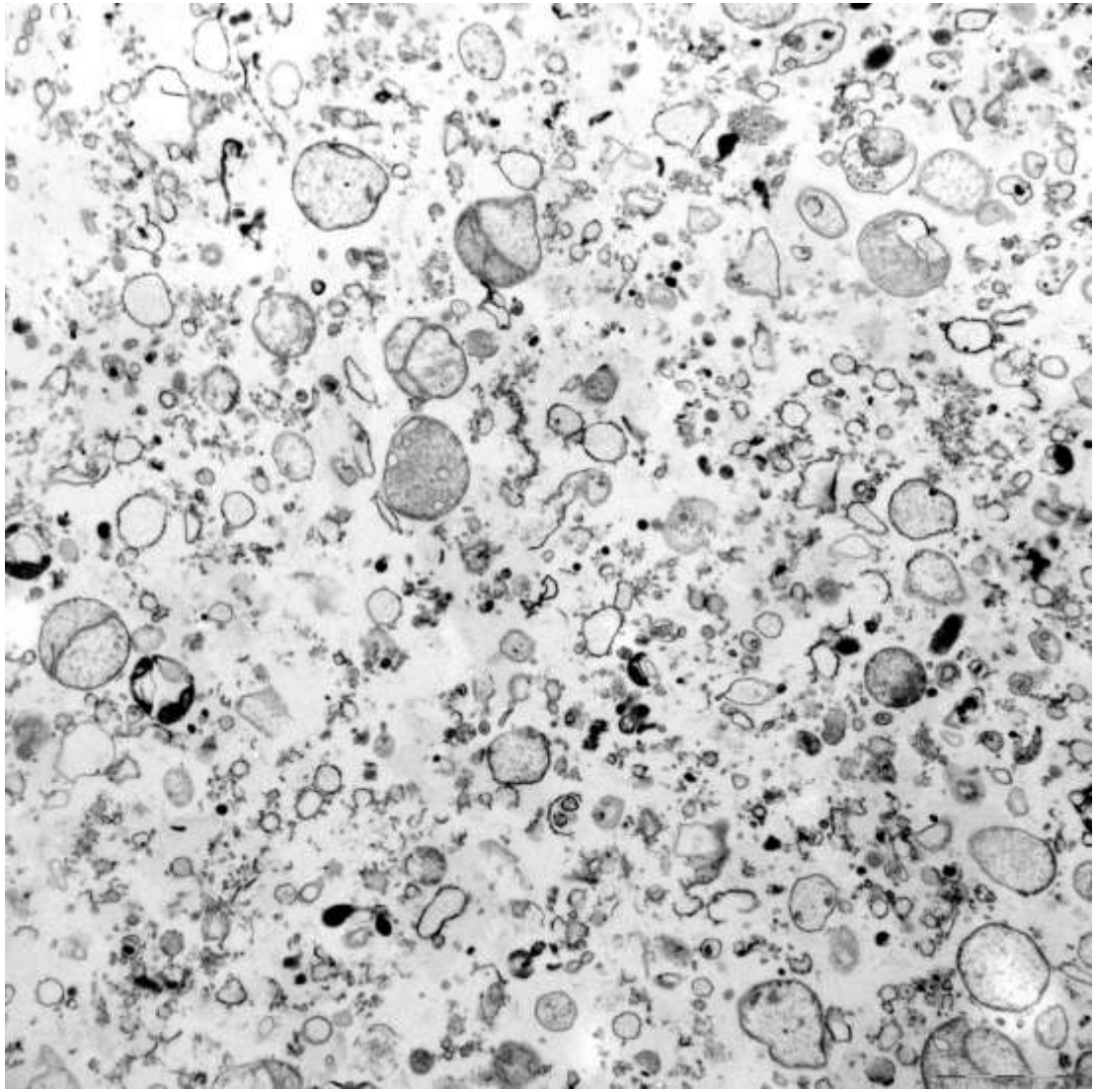
Vit O, et al. J Proteomics. 2016, 21;149:15-22.

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

hpTC method (high **p**H-**T**rypsin-**C**NBr)

Membrane-enriched fraction

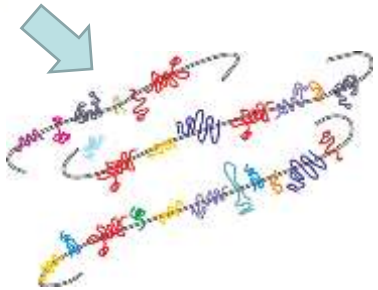
Vít O. et al., *Journal of Proteomics* 2016
Blackler A et al., *J Proteome Res*. 2008



pH 7.4

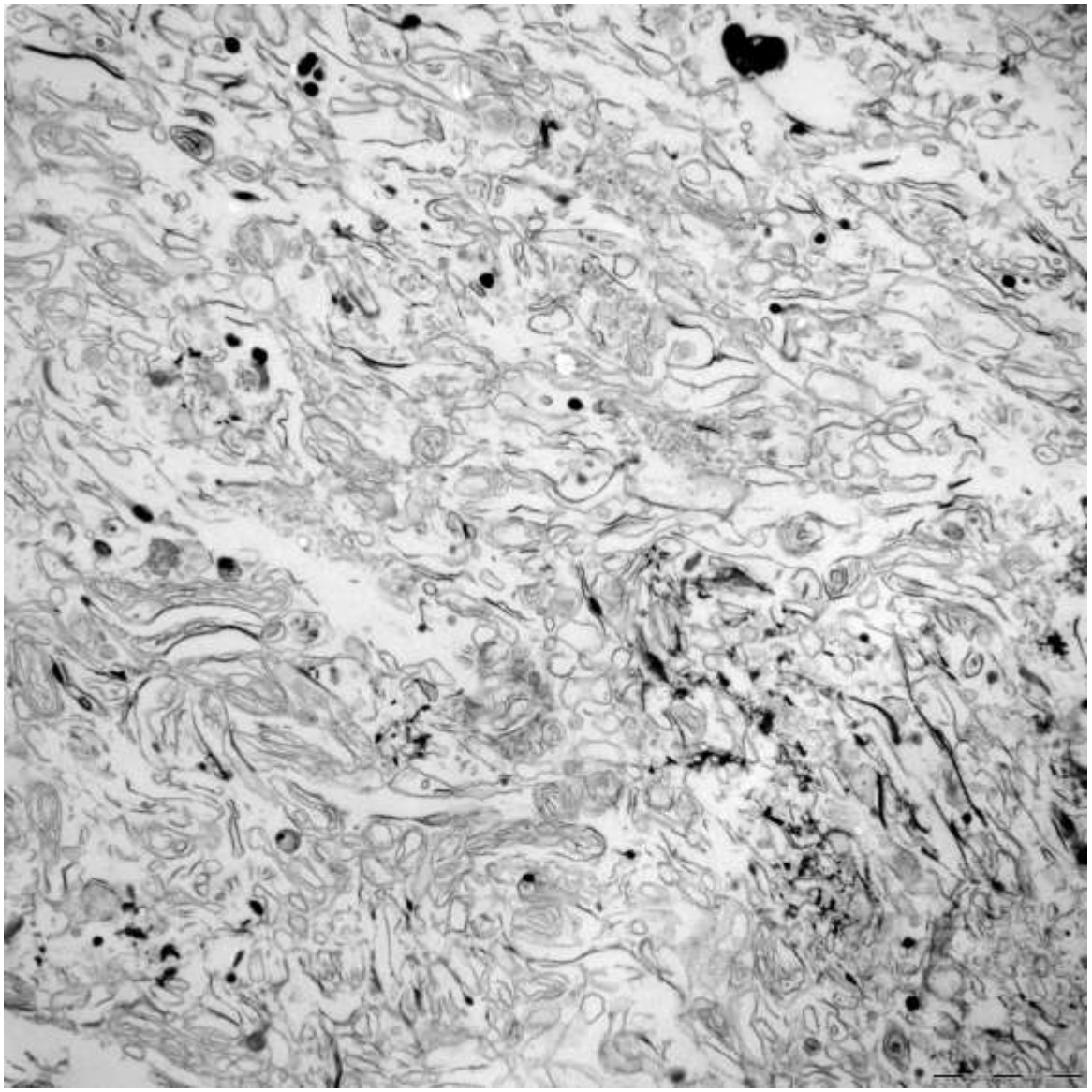
hpTC method (high **p**H-**T**rypsin-**C**NBr)

Membrane-enriched fraction



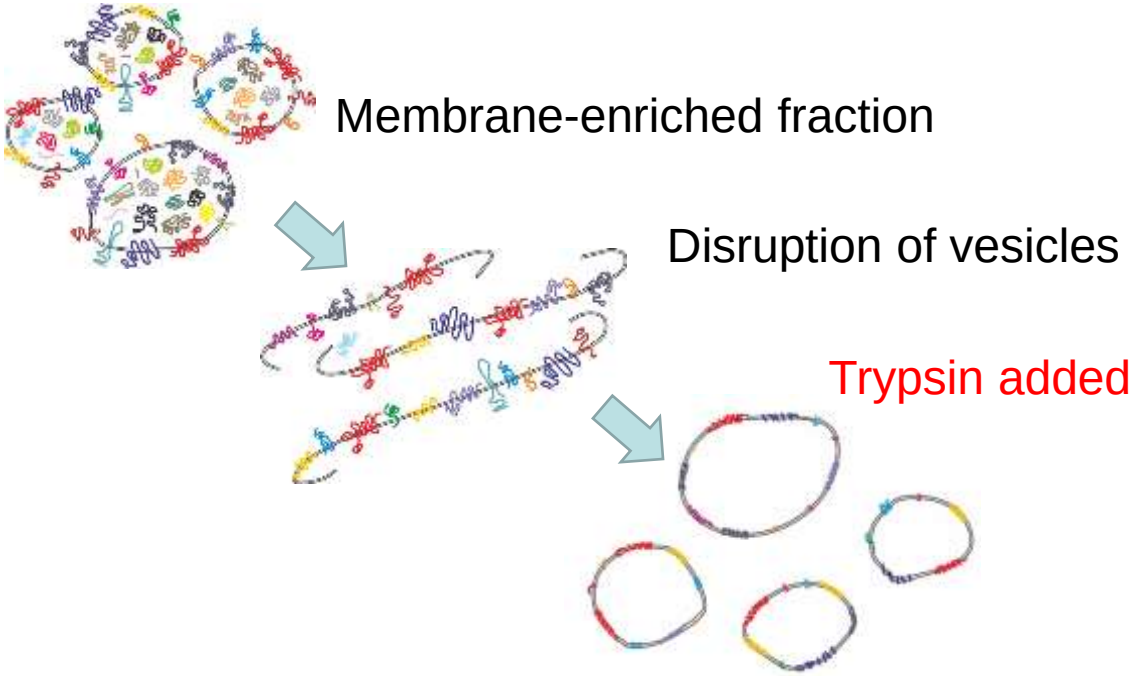
Disruption of vesicles

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

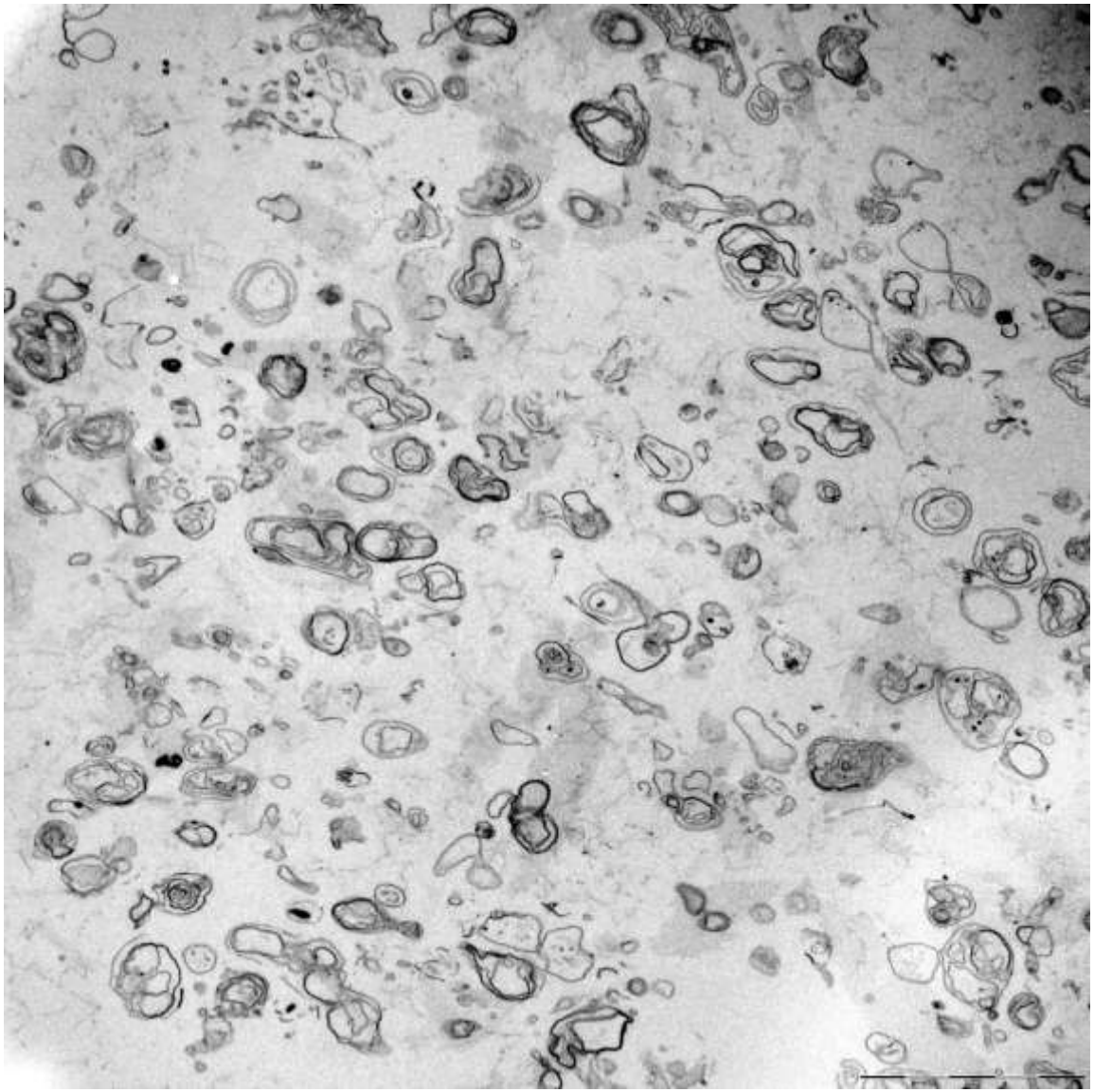


Na ledu
Na_2CO_3
pH 11

hpTC method (high **p**H-**T**rypsin-**C**NBr)

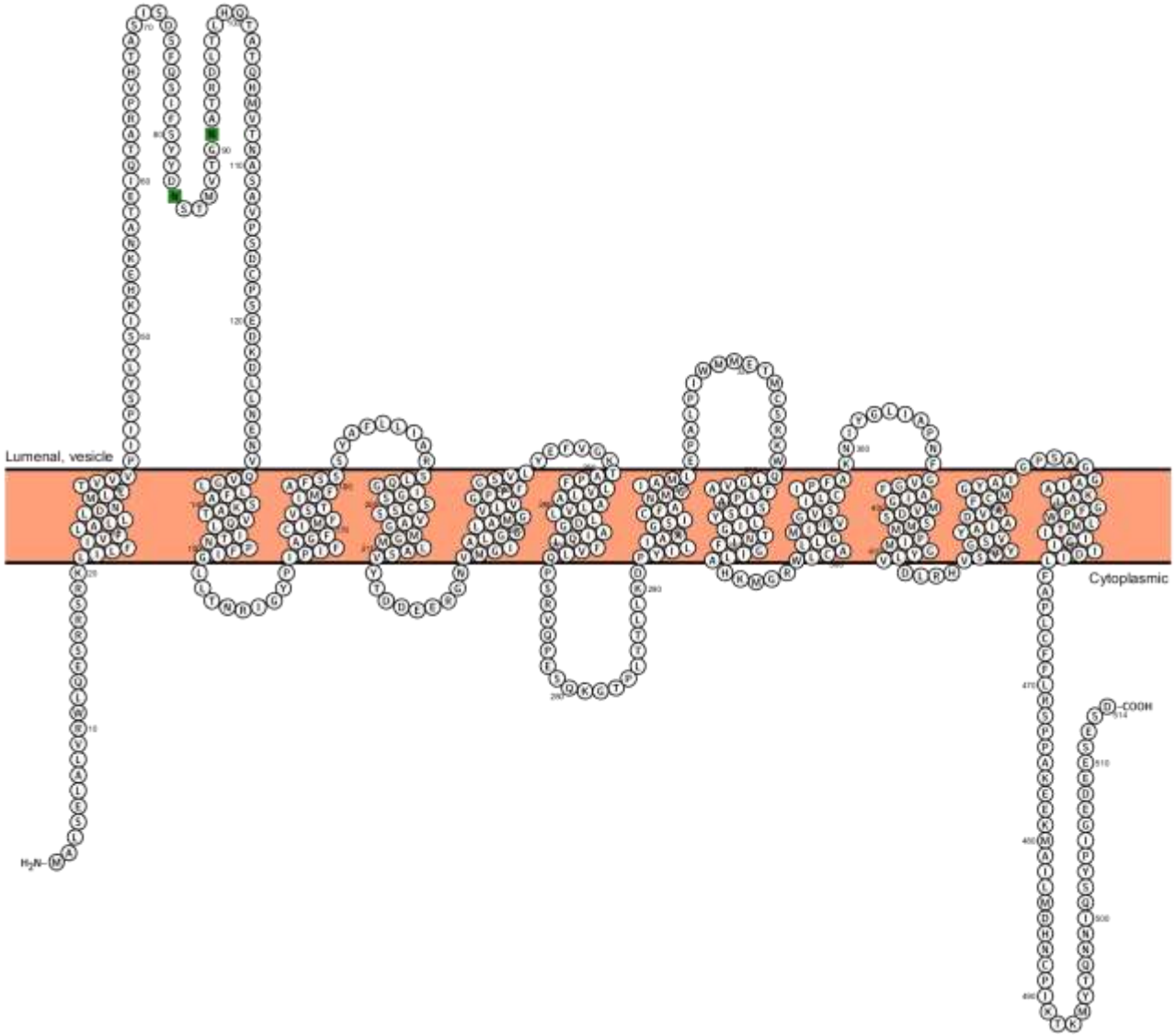


Vít O. et al., *Journal of Proteomics* 2016
Blackler A et al., *J Proteome Res*. 2008



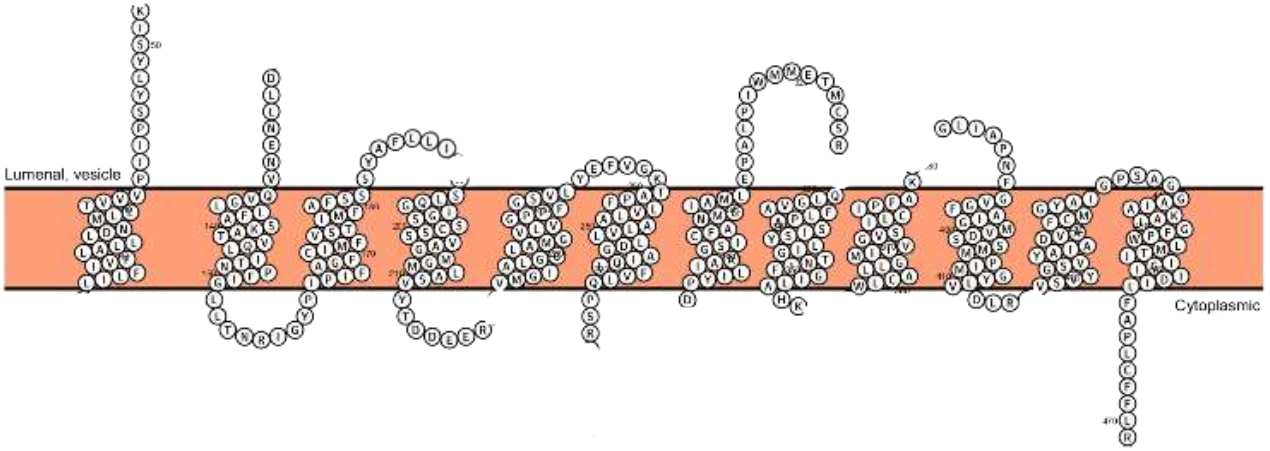
37 °C
trypsin

Synaptic vesicular amine transporter (Slc18a2)

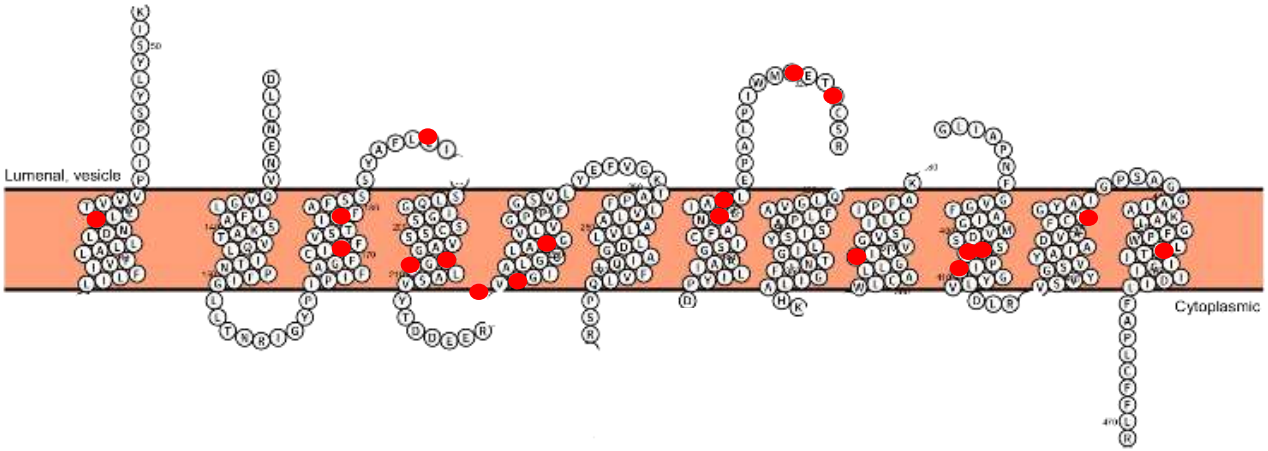


Visualization by Protter (*Omasits et al., Bioinformatics. 2013*)

Synaptic vesicular amine transporter (Slc18a2)

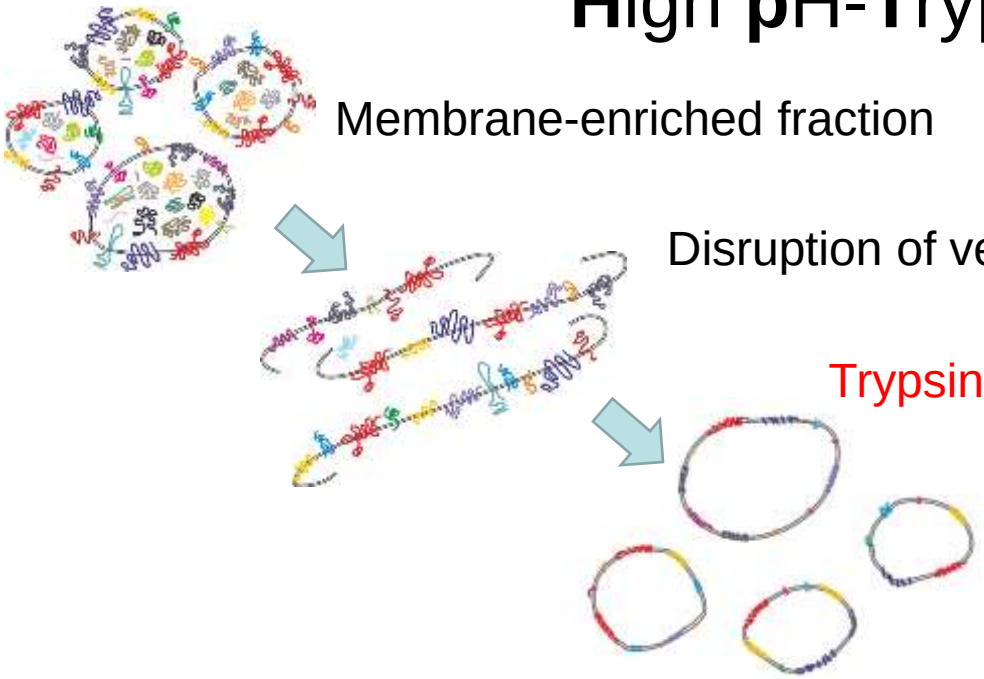


Synaptic vesicular amine transporter (Slc18a2)



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

HpTC method
High **p**H-**T**rypsin-**C**NBr



Membrane-enriched fraction

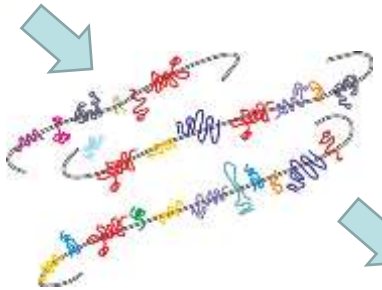
Disruption of vesicles

Trypsin added

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

HpTC method
High pH-Trypsin-CNBr

Membrane-enriched fraction



Disruption of vesicles

Trypsin added

Solubilization and CNBr cleavage

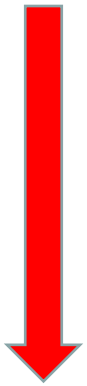
Delipidation

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

hpTC

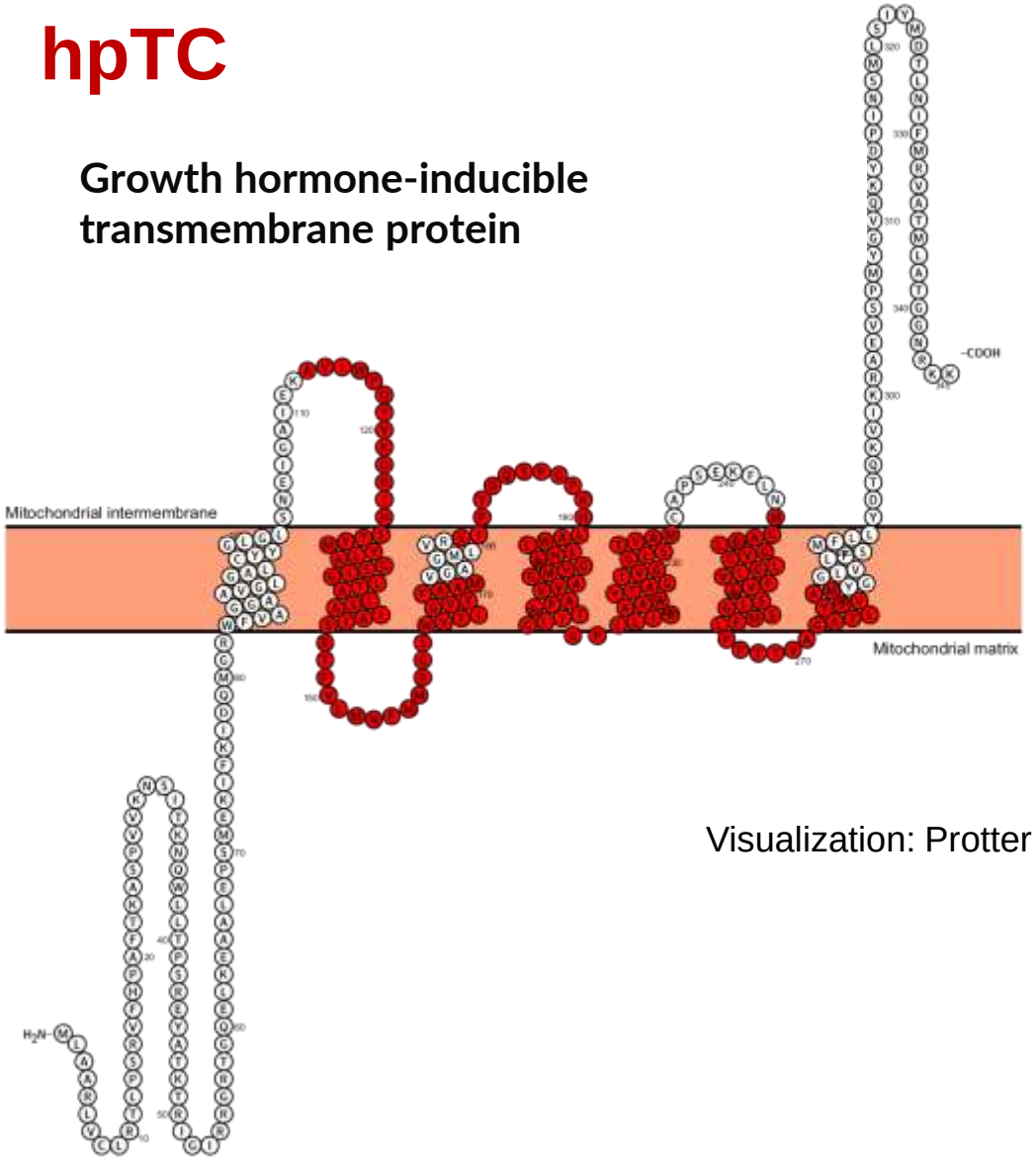
Growth hormone-inducible transmembrane protein

Only the hydrophobic segments
(hpTC)



600-1000 IMPs in various tissues

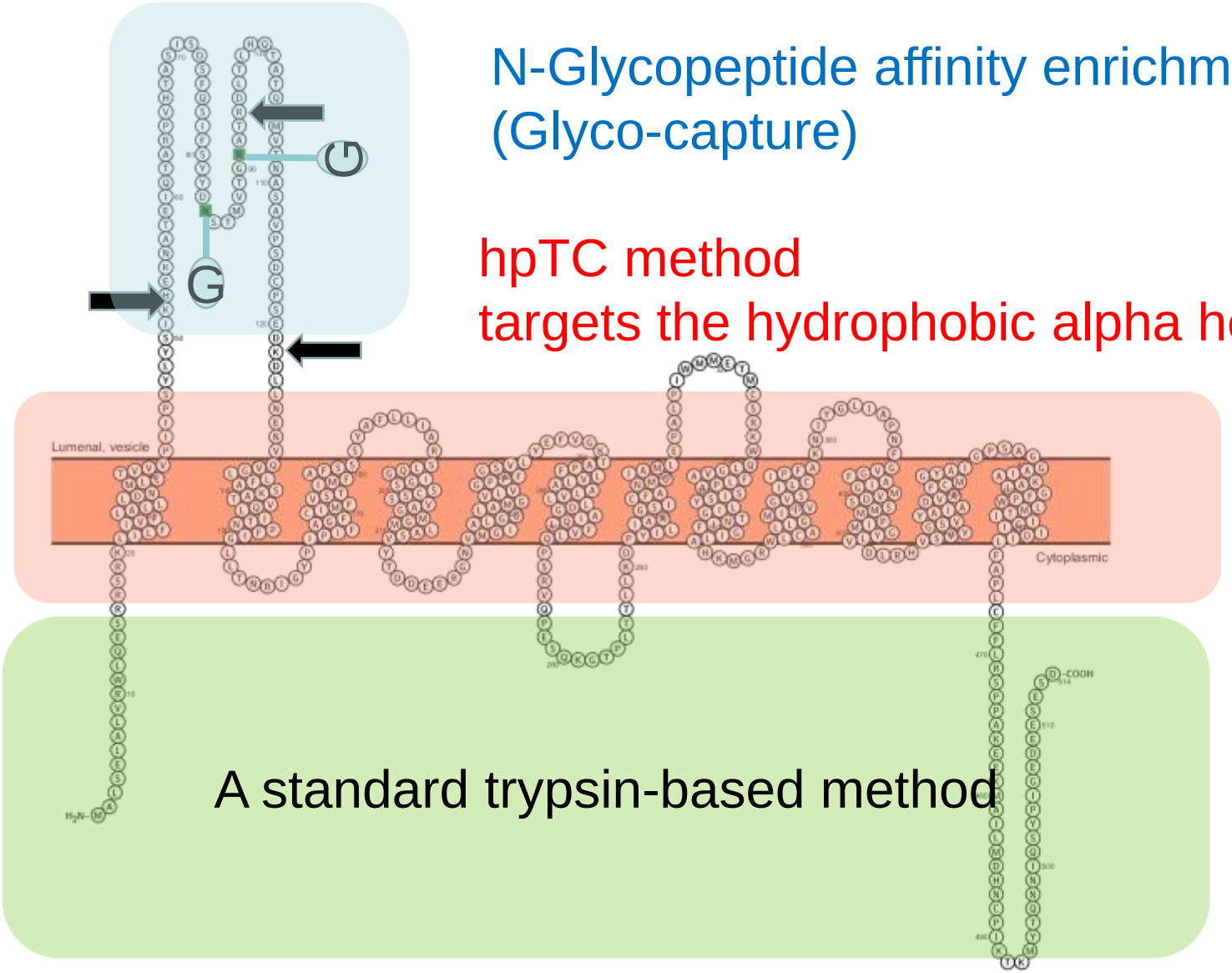
IMP Enrichment 40-60%



Visualization: Protter

Vít O. et al., Journal of Proteomics 2016

“DIVIDE AND CONQUER“ METHODS



N-Glycopeptide affinity enrichment
(Glyco-capture)

hpTC method
targets the hydrophobic alpha helices

A standard trypsin-based method

Only the hydrophobic segments

Membrane fraction

↓

Shaving with trypsin

↓

CNBr

↓

delipidation

↓

LC-MS/MS

Classic strategy

Membrane fraction

↓

Solubilization

↓

Tryptic digestion

↓

LC-MS/MS

Only the hydrophilic segments (GLYCOCAPTURE)

Solubilization

↓

Digestion

↓

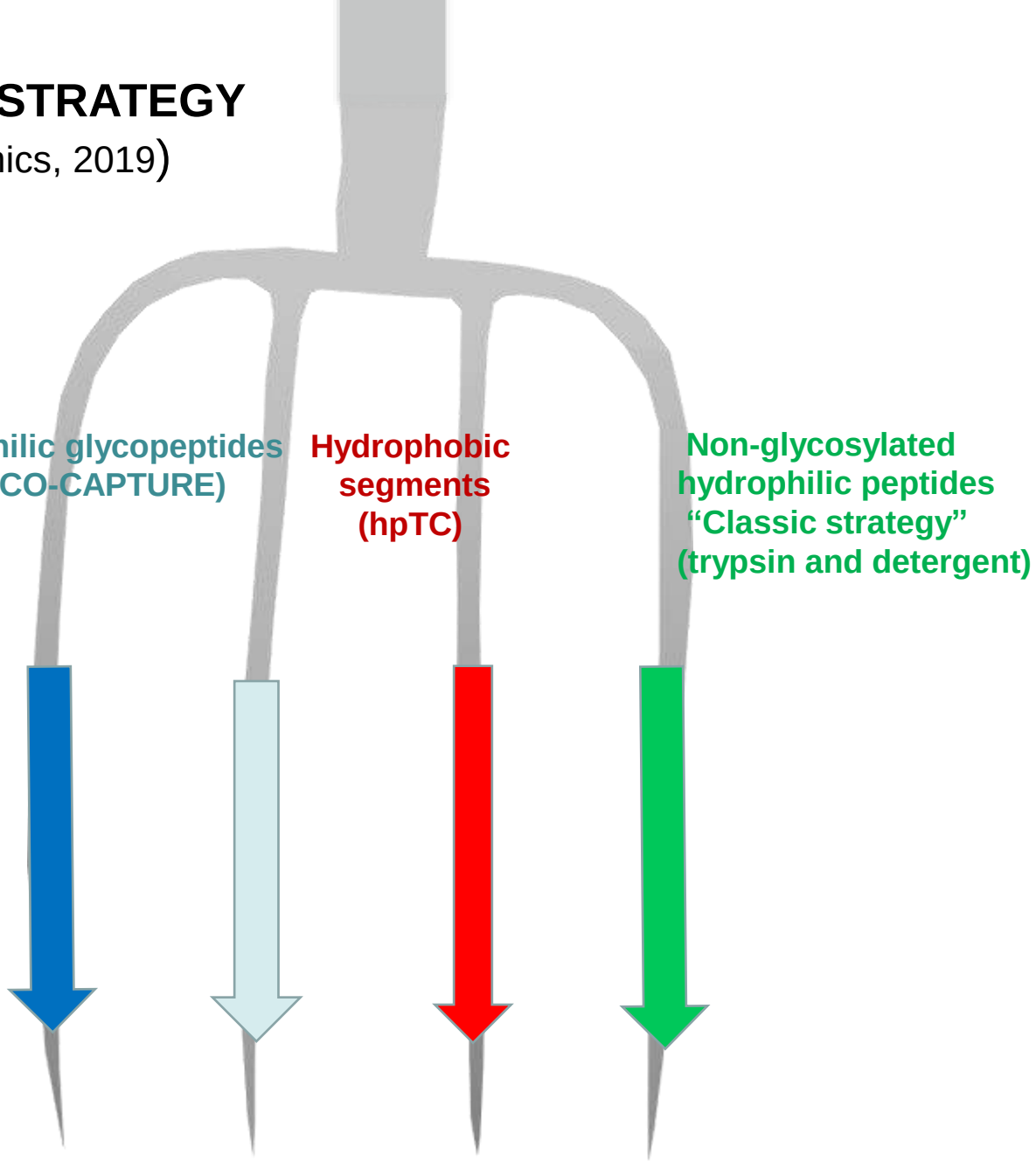
Isolation of N-glycopeptides

↓

LC-MS/MS

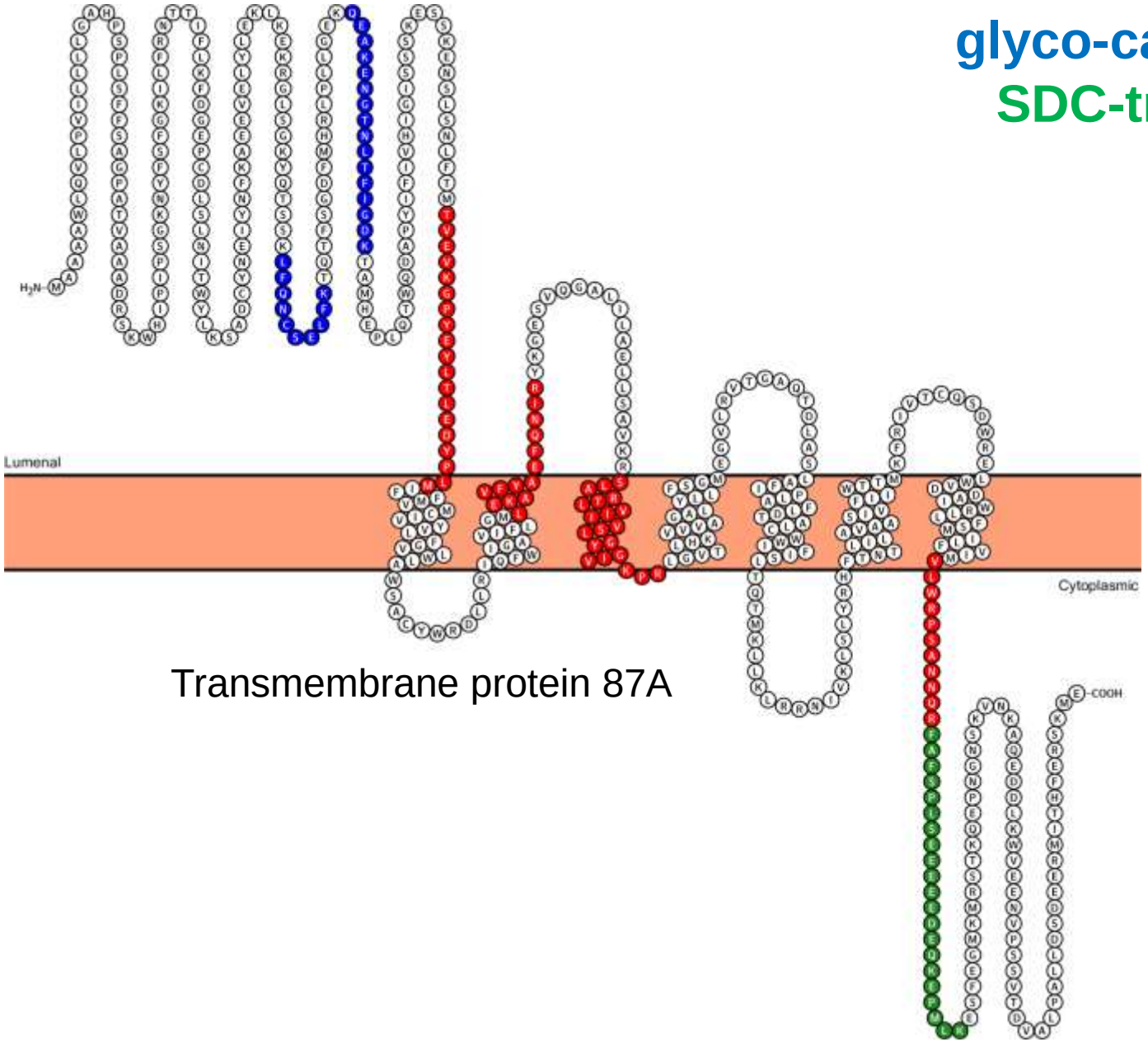
THE PITCHFORK STRATEGY

(Vit et al, J. Proteomics, 2019)

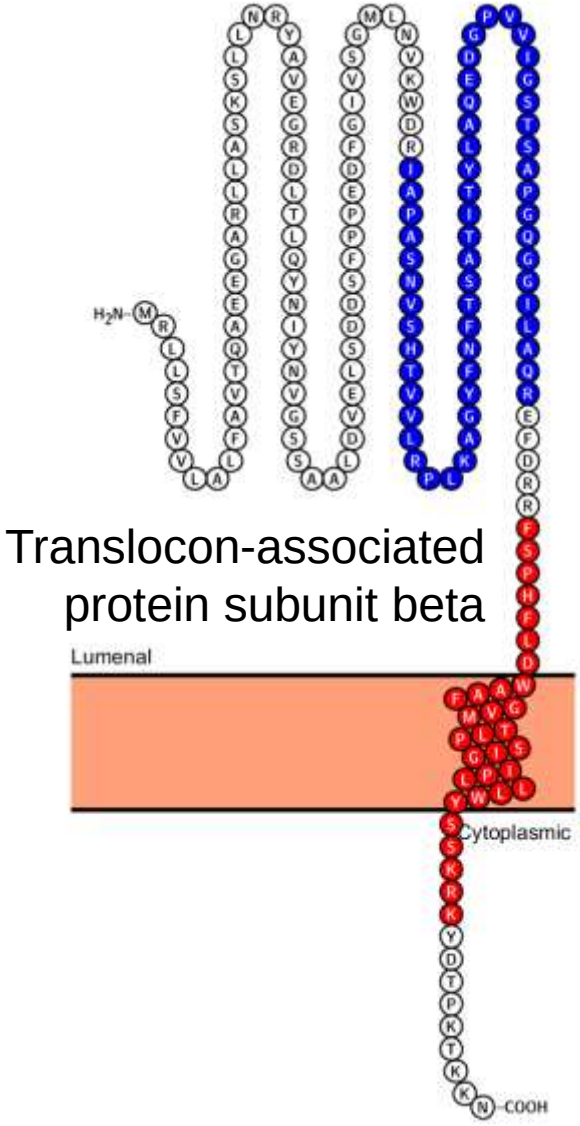


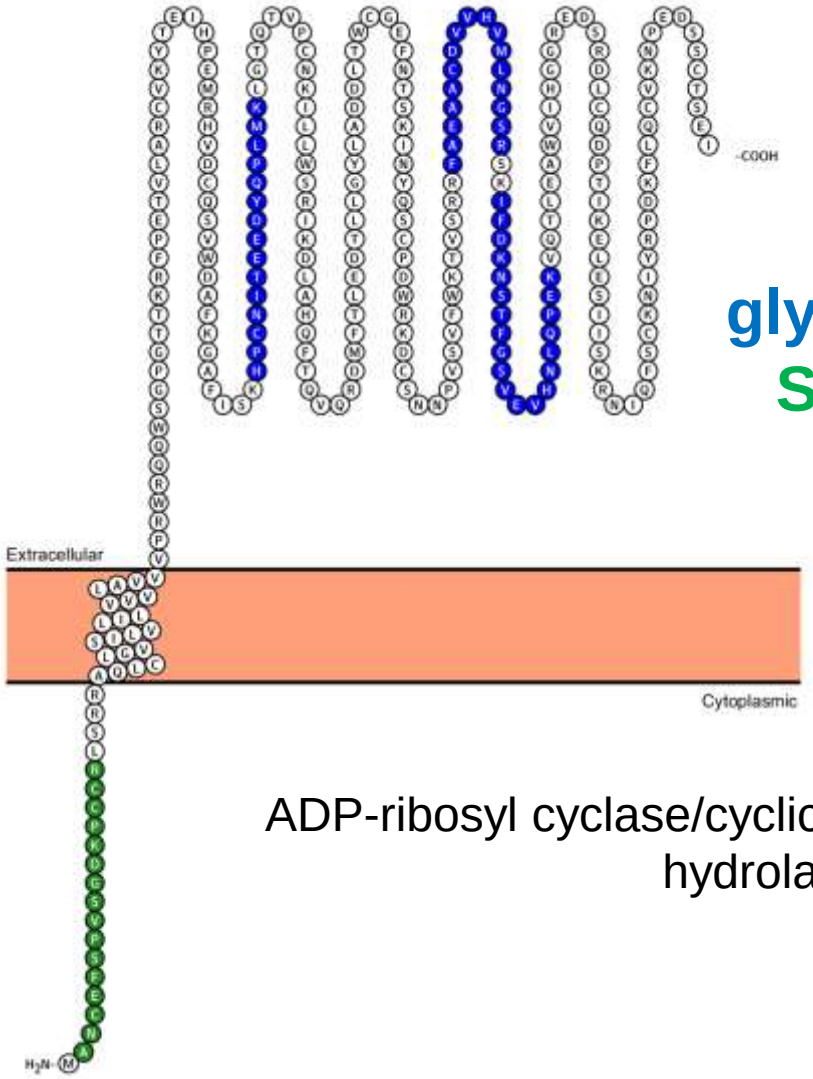
LC-MS/MS

hpTC
glyco-capture
SDC-trypsin



Transmembrane protein 87A

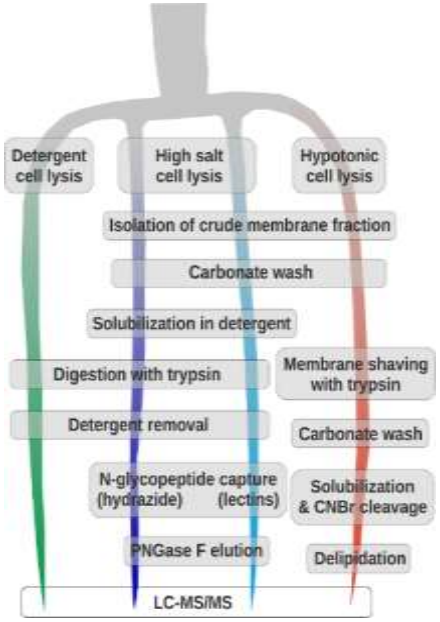




hpTC
glyco-capture
SDC-trypsin

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

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

Vit O. et al. J. Proteomics, 2019
Vít O. et al. Clinical proteomics 2023

Looking for new theranostic targets in human Pheochromocytoma and Paraganglioma

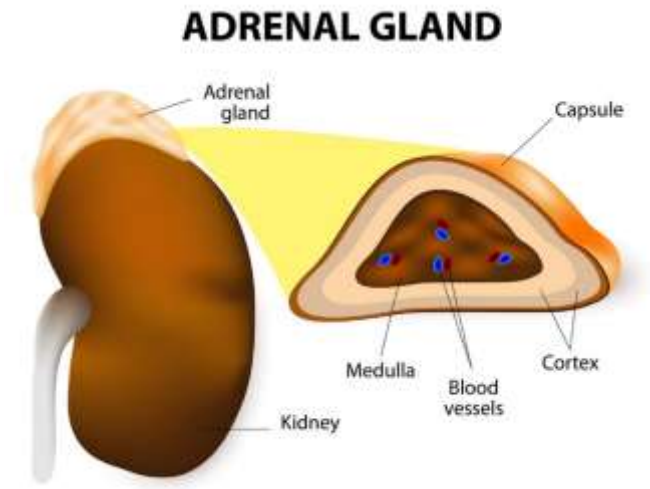
BIOCEV

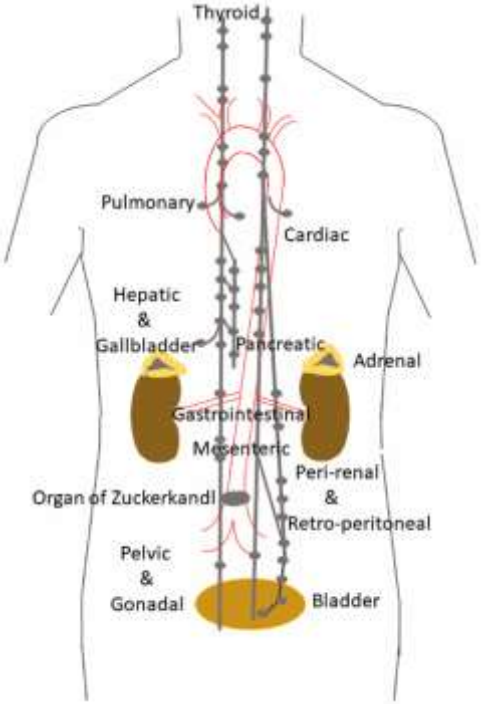
Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University in Vestec

NIH *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 sypathetic ganglia (PGL)**
- From parasympathetic ganglia (PGL)





PHEOCHROMOCYTOMA and PARAGANGLIOMA

- Rare **neuro-endocrine tumors**
- From **chromaffin tissue of adrenal medulla (PHEO) or sypathetic 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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- Catecholamine producing tumors (dopamine, noradrenaline,adrenaline)
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NEW DRUG TARGETS ARE NEEDED

INTEGRAL MEMBRANE PROTEINS ARE EXCELENT DRUG TARGETS

PHEOCHROMOCYTOMA and PARAGANGLIOMA

Distinct molecular subtytypes of based on mutations, mRNA expression…

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

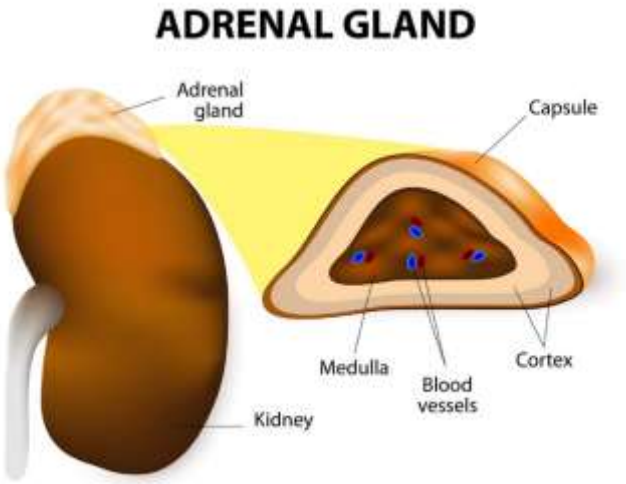
Patient No.	Sex	Tumor Type	PPGL Cluster	Mutated Gene
1	F	PGL	1	*SDHB*
2	F	PGL	1	*SDHB*
3	M	PGL	1	*SDHB*
4	M	PGL	1	*SDHB*
5	M	PGL	1	*SDHB*
6	F	PGL	1	*SDHB*
7	M	PGL	1	*SDHB*
8	F	PGL	1	*SDHB*
9	F	PGL	1	*SDHB*
10	M	PHEO	1	*VHL*
11	F	PHEO	1	*VHL*
12	F	PHEO	1	*VHL*
13	M	PGL	1	*VHL*
14	M	PGL	1	*VHL*
15	F	PHEO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

Cluster 1

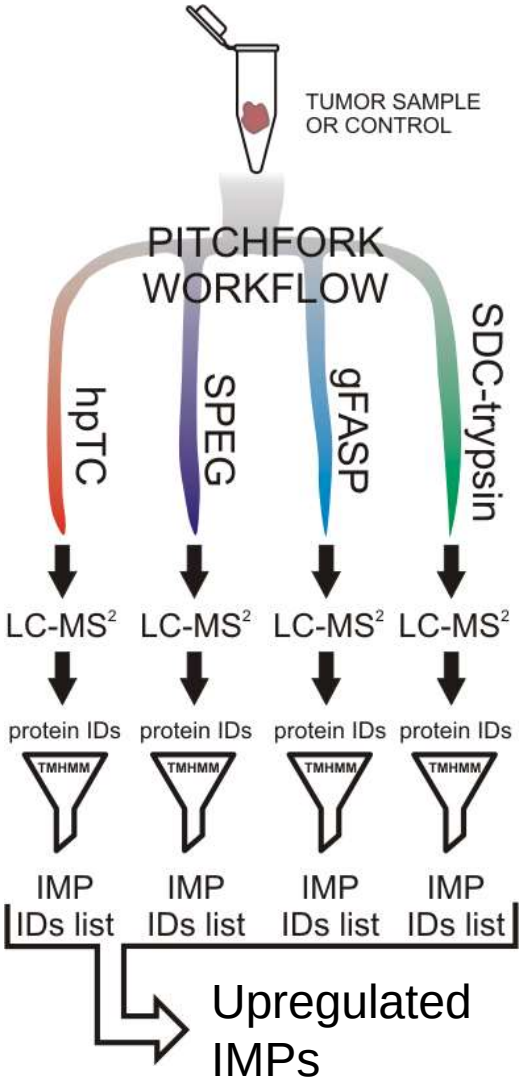
Cluster 2

No mutation

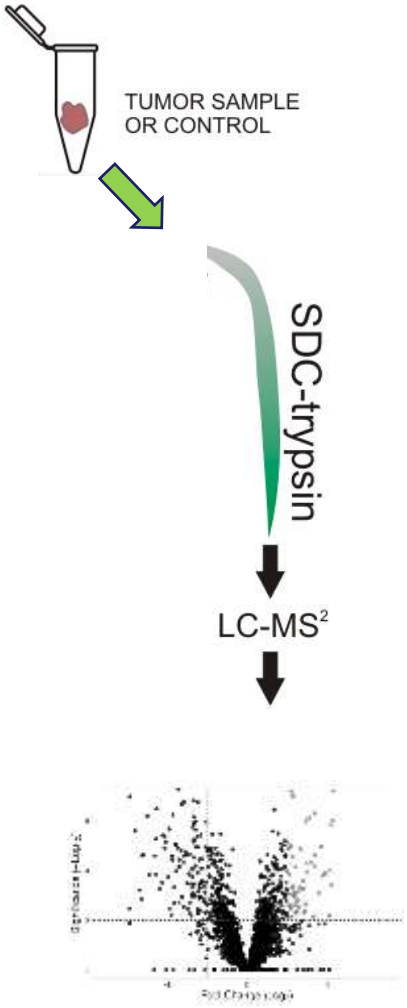
ADRENAL MEDULLA – THE CONTROL CHROMAFFIN TISSUE



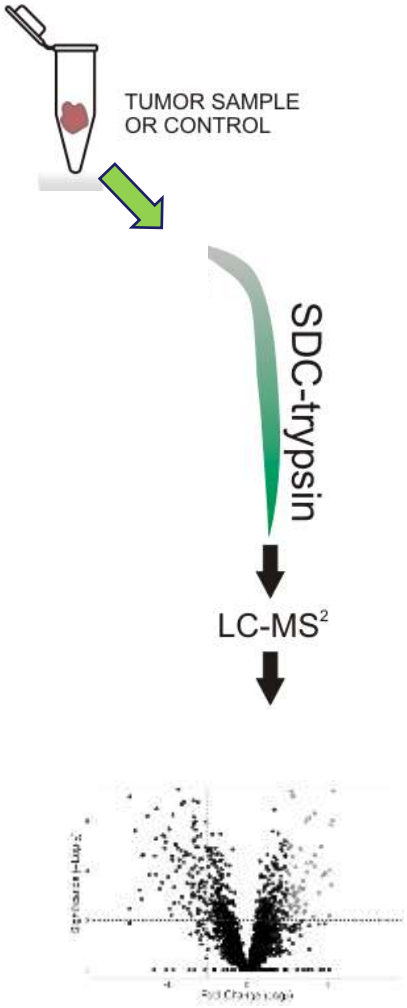
PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME

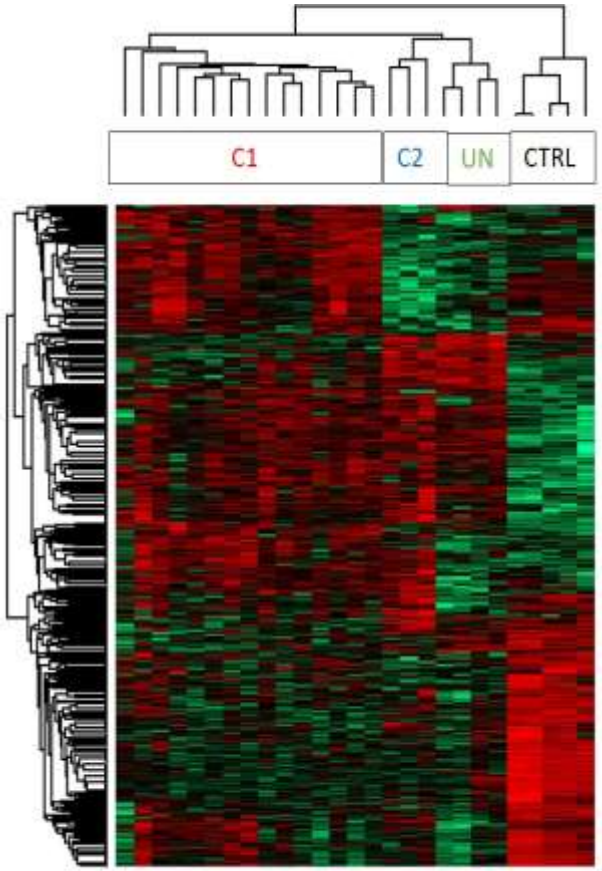


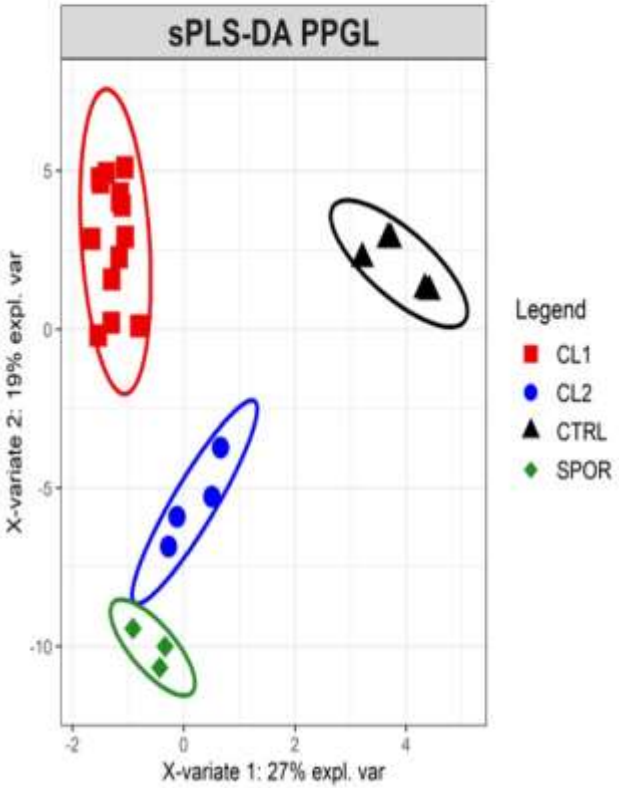
PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME







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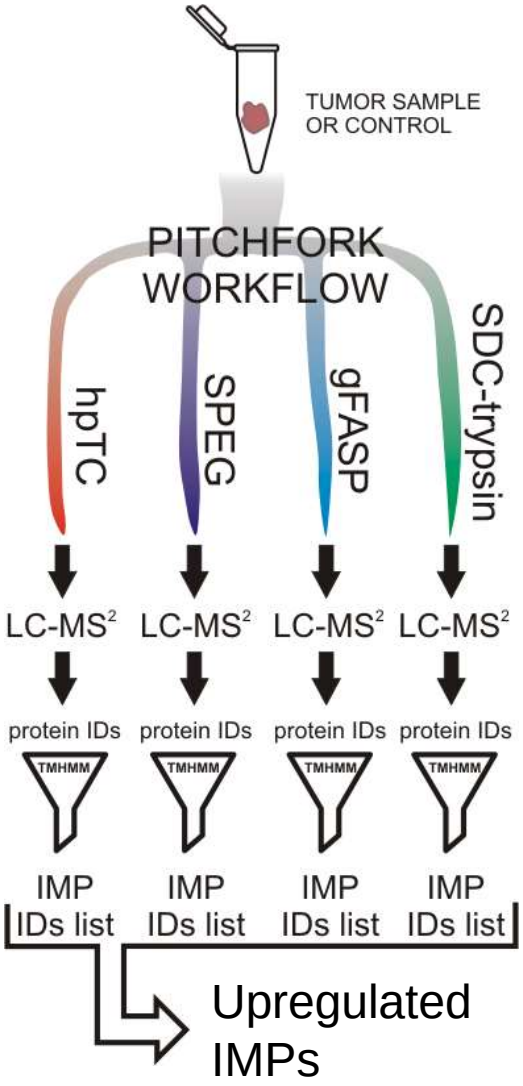
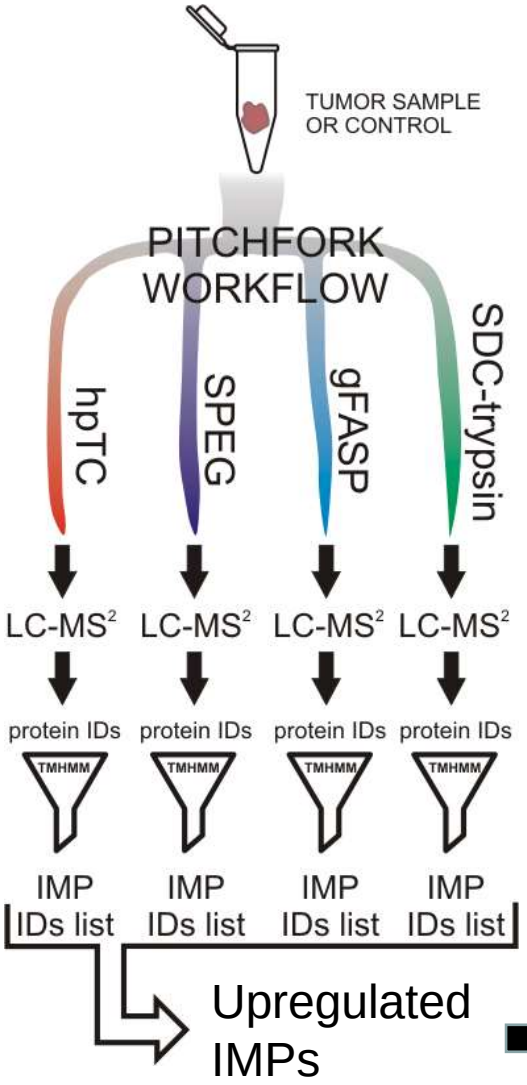
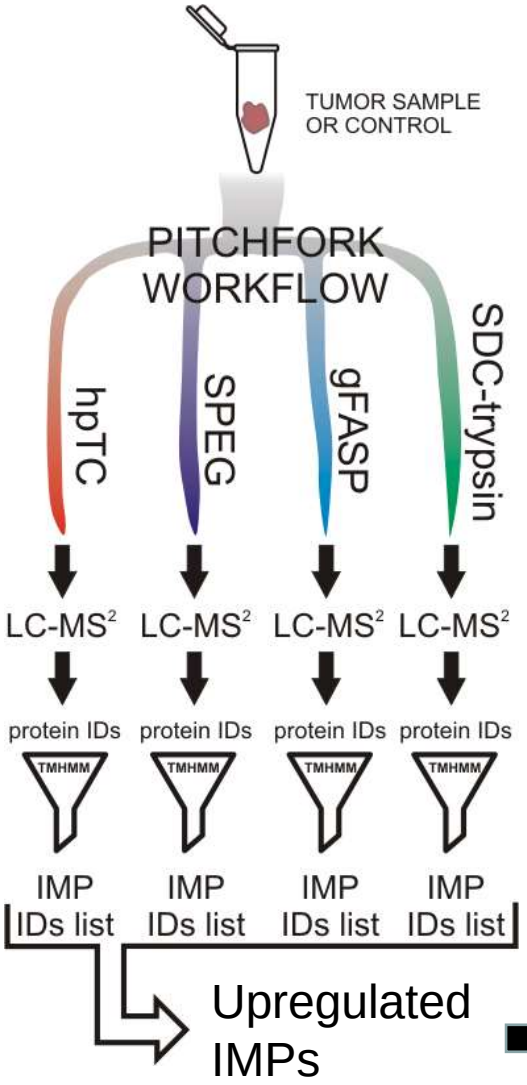


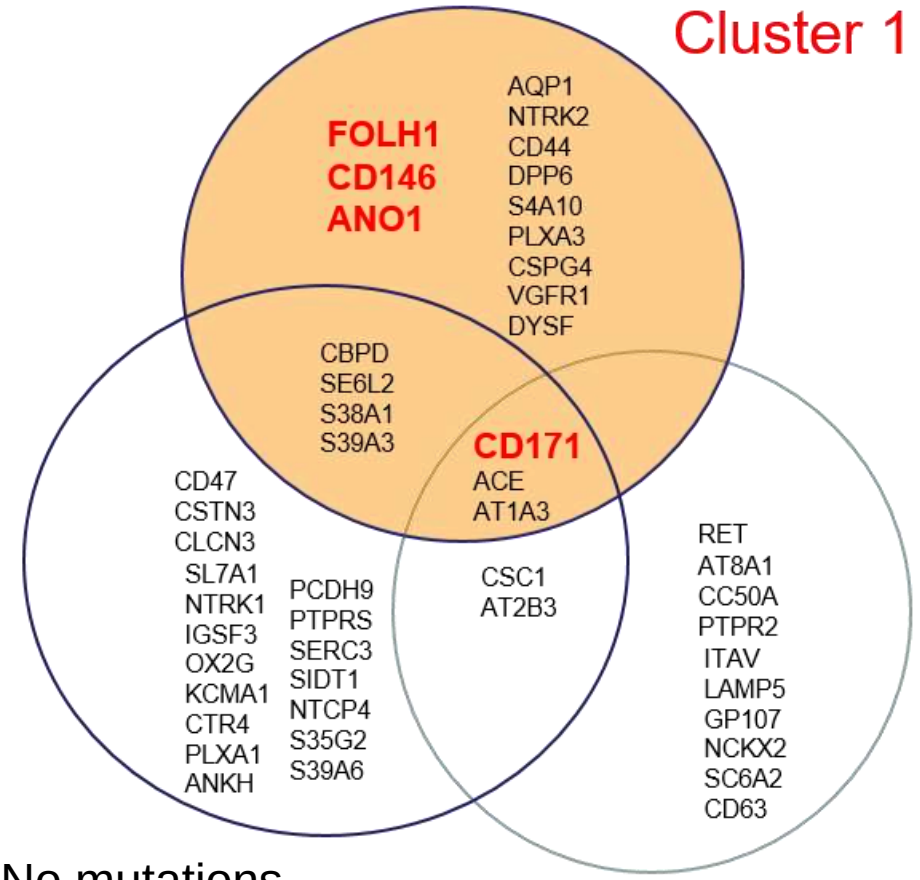
PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



➡ **Filtering for cell surface localization**

PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME





➡ **Filtering for cell surface localization**

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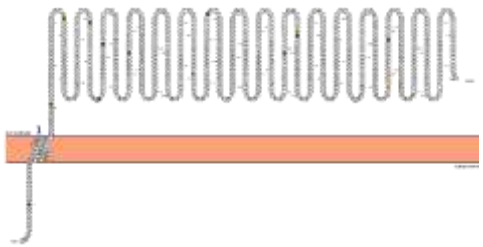
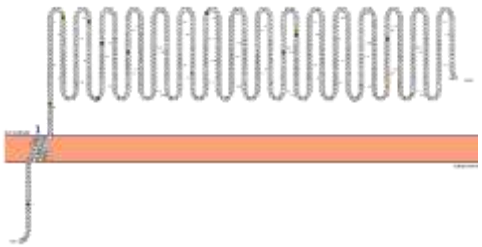
GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1)

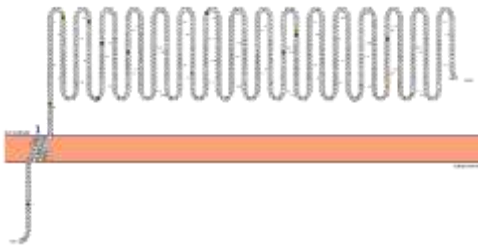


GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1)
PROSTATE-SPECIFIC MEMBRANE ANTIGEN (PSMA)



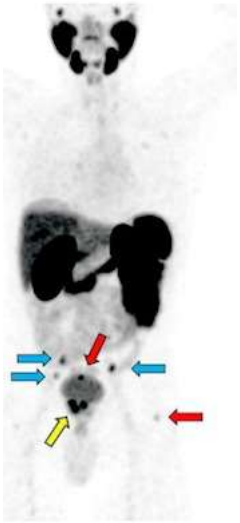
OVEREXPRESSED IN PROSTATE CANCER CELLS

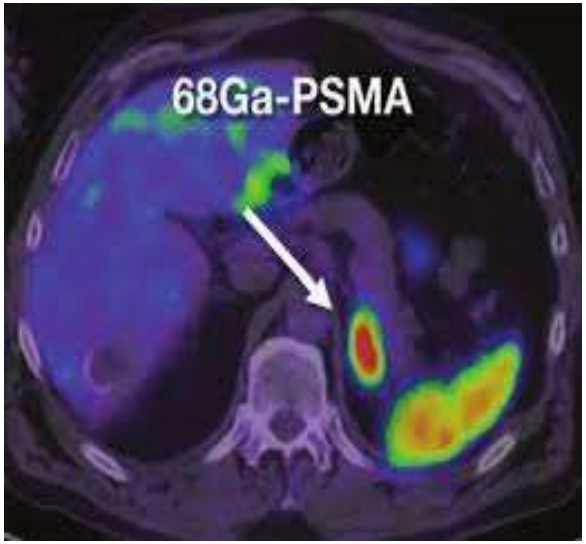
GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1)
PROSTATE-SPECIFIC MEMBRANE ANTIGEN (PSMA)

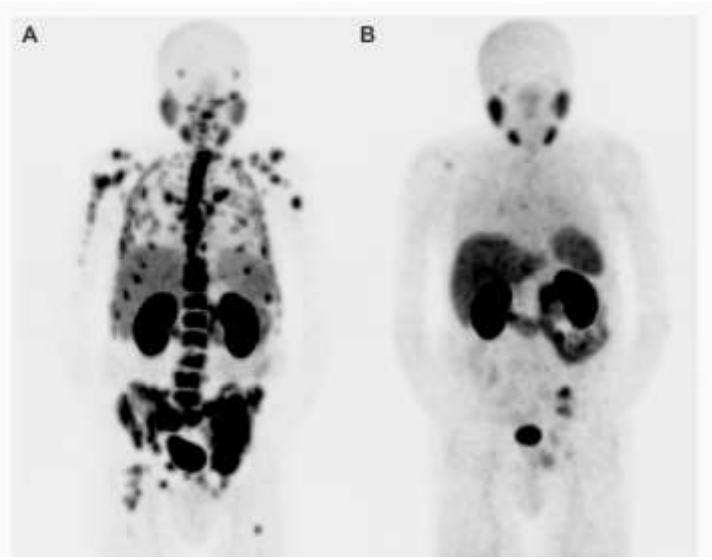


OVEREXPRESSED ON PROSTATE CANCER CELLS

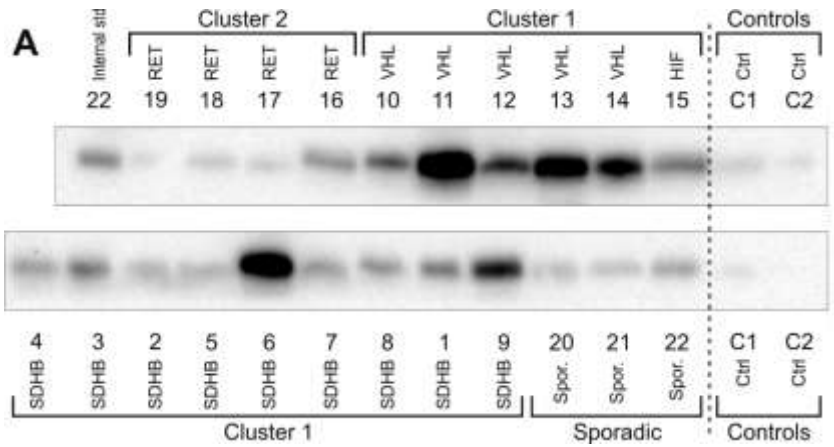
Anti-PSMA radio-conjugates approved for PC tumor imaging and therapy of advanced PC

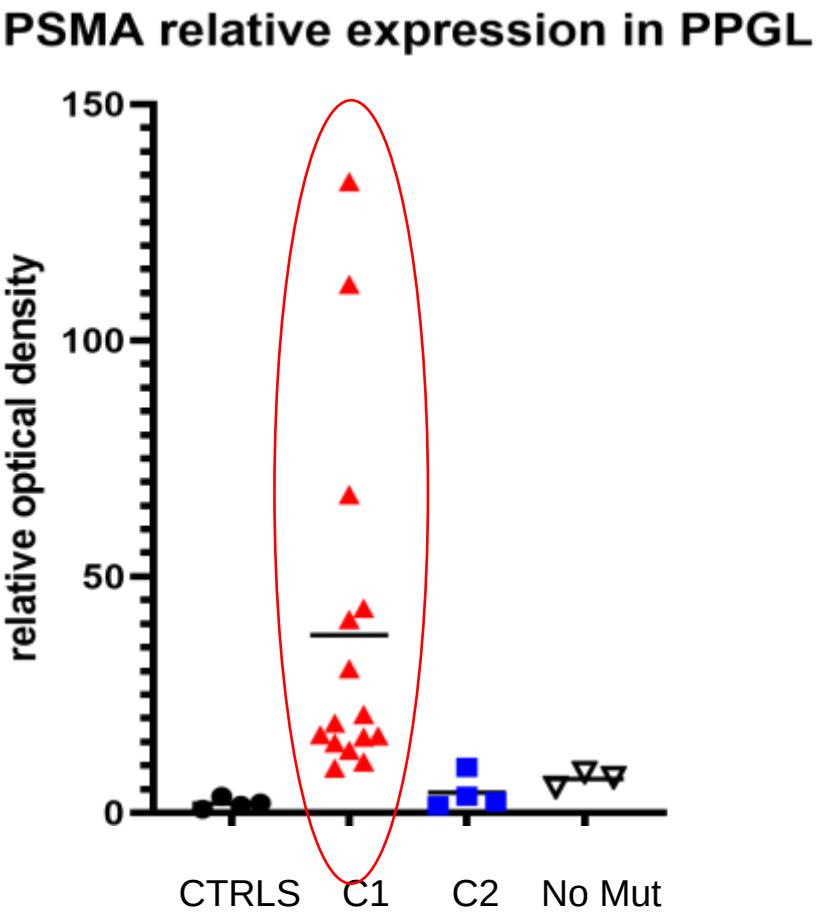






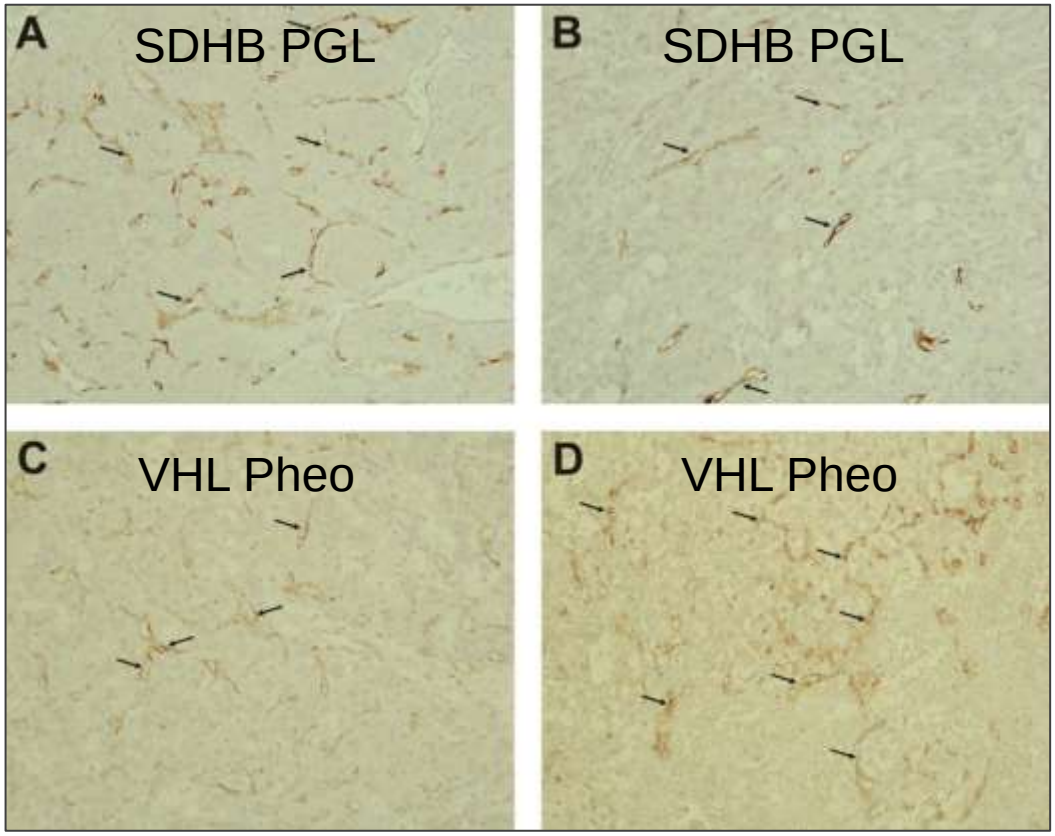
PSMA EXPRESSION IN HUMAN PPGL





*Vit,O. et al. Molecules **2021**, 26, 6567*

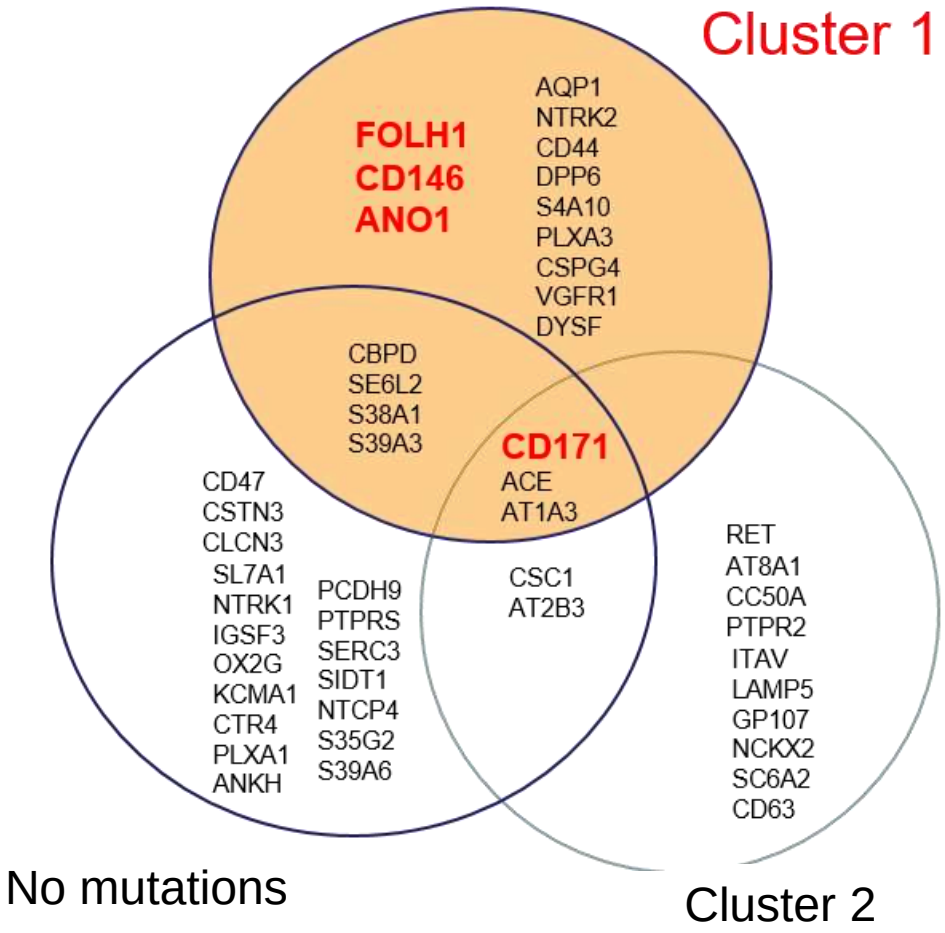
PSMA EXPRESSION IN TUMOR VASCULATURE IN CLUSTER 1 PPGL



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

*Vit,O. et al. Molecules **2021**, 26, 6567*

PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



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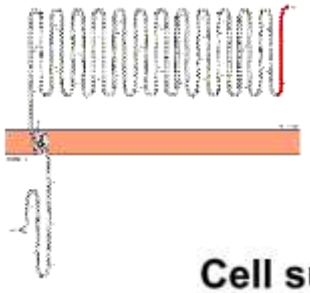
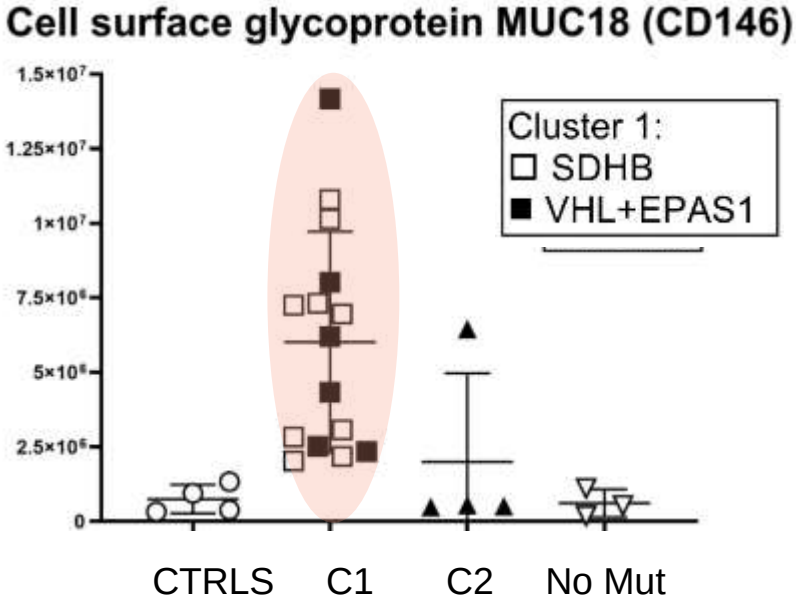
CD146
(Melanoma cell adhesion molecule, MCAM, MUC18)





- 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

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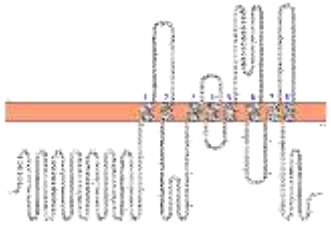
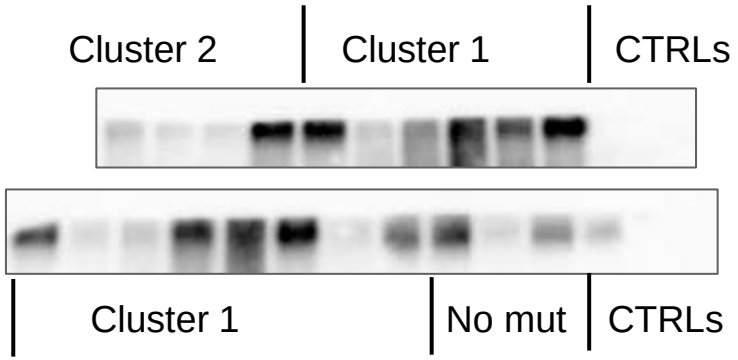
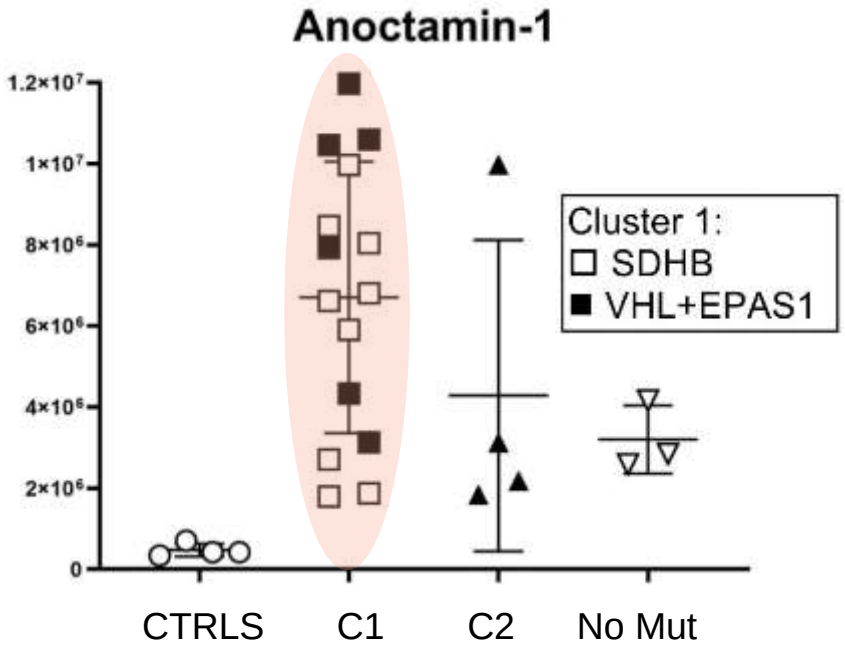
Anoctamin-1 (DOG1, TMEM16A)







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

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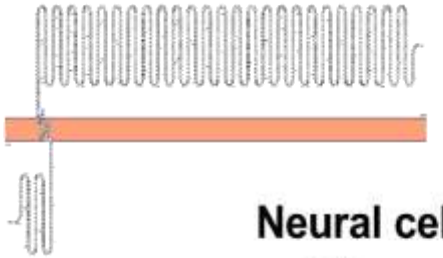
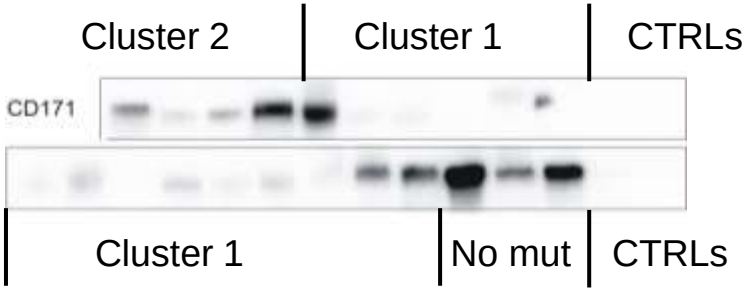
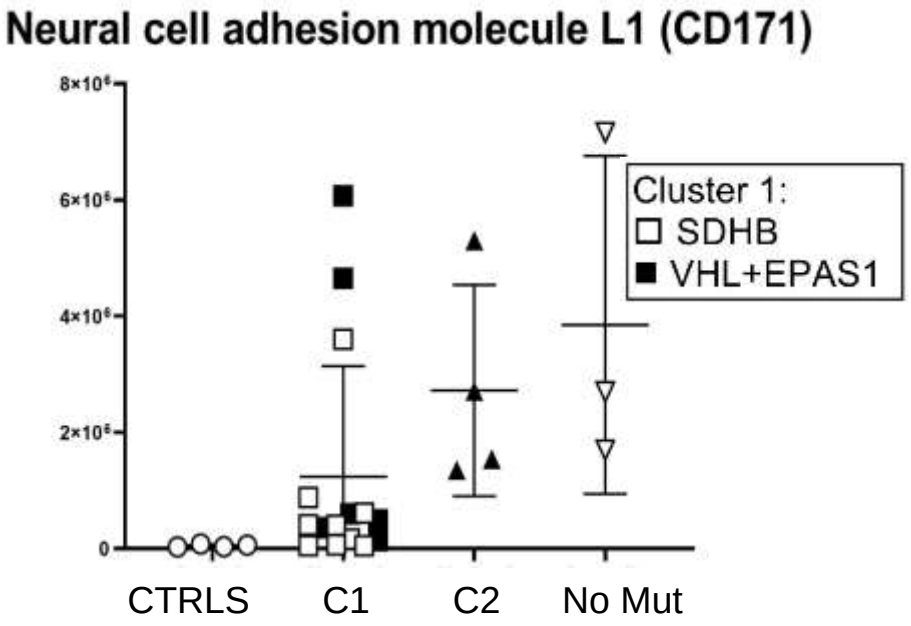
CD171
(Neural cell adhesion molecule L1, NCAM-L1)



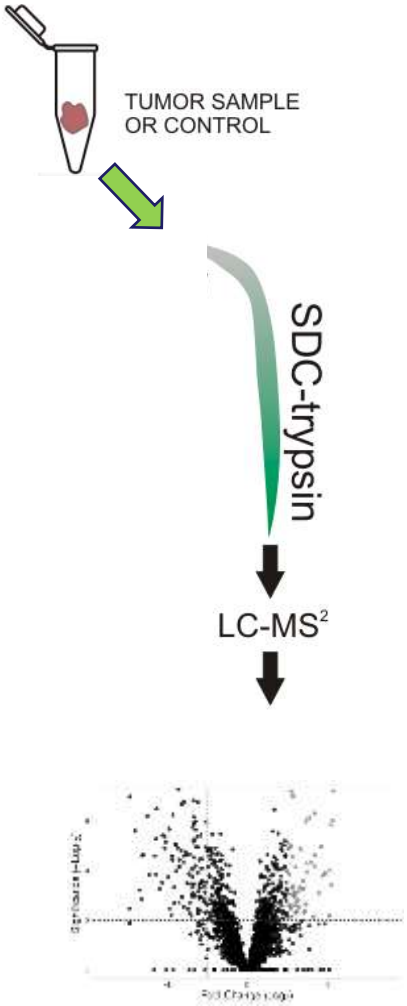




- Cell adhesion molecule, essential for neural developement and regeneration
- Overexpressed in numerous cancers, expression correlates with disease progression
- Pro-angiogenic roles in the endothelial cells of tumor-associated vessels
- Anti-CD171 antibody decreased tumor vascularization and progression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

Vít O. et al. Clinical Proteomics, 2023

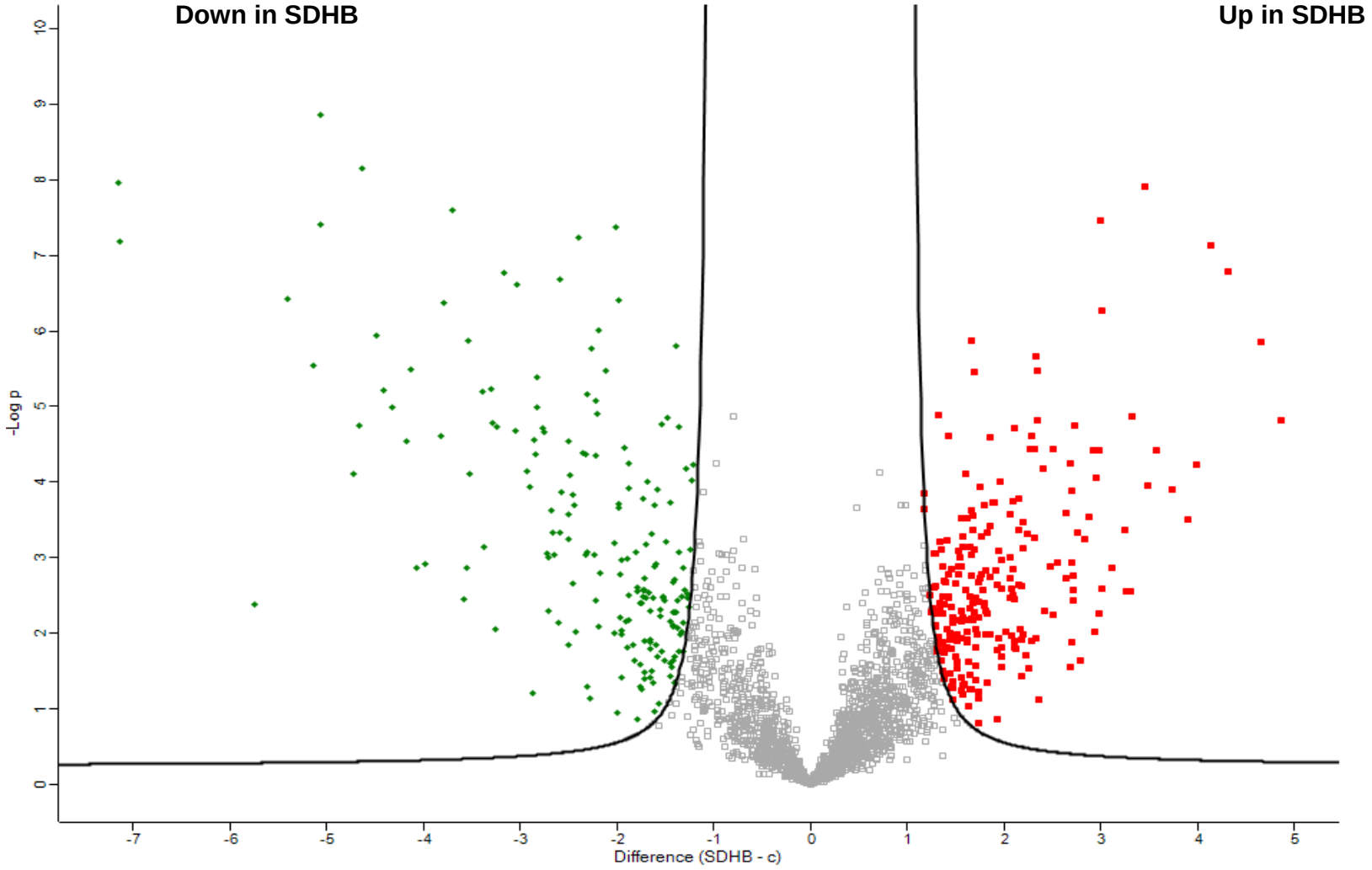
AND WHAT ABOUT THE NON-MEMBRANE PROTEINS?



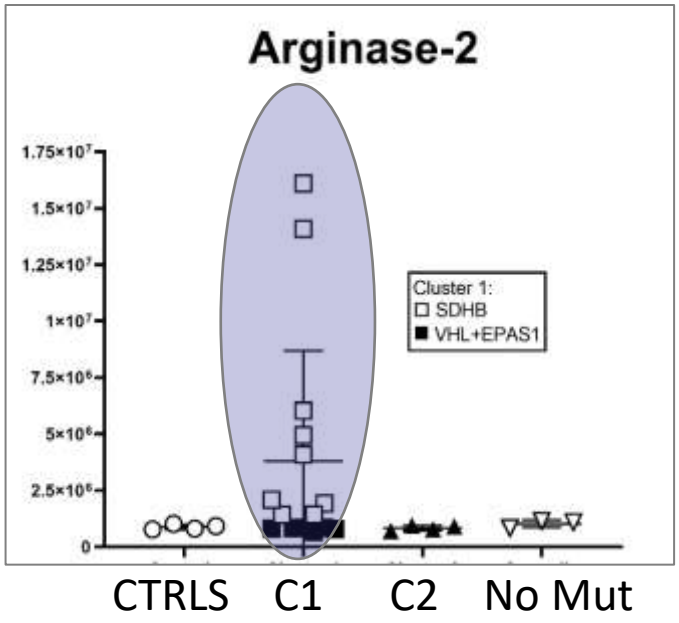
SDHB-specific changes?

Trypsin-based LFQ data

SDHB vs. controls

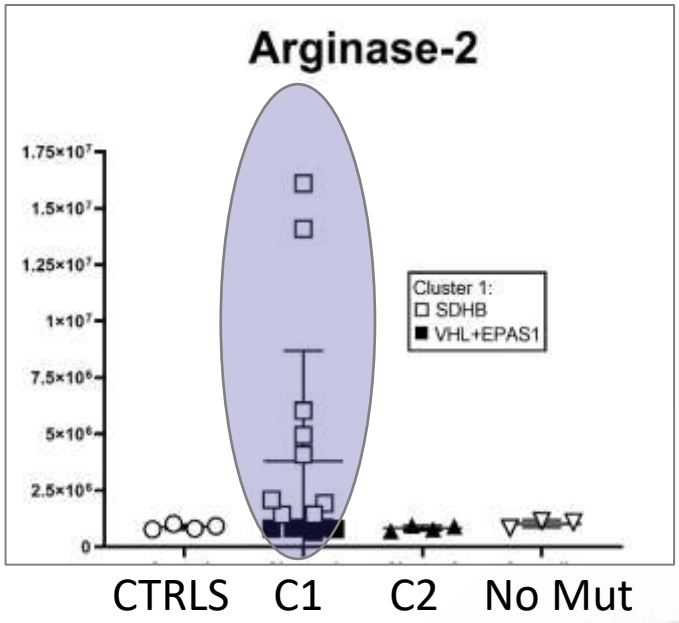


Mitochondrial Arg metabolism



- ARG 2 overexpression in several cancers
- The expression associates with poor prognosis
- Increases the activity of complex II
- LMW inhibitors suppress growth or promotes apoptosis

Mitochondrial Arg metabolism



- ARG 2 overexpression in several cancers
- The expression associates with poor prognosis
- Increases the activity of complex II (Dowling et al, 2021)
- LMW inhibitors suppress growth or promoted apoptosis