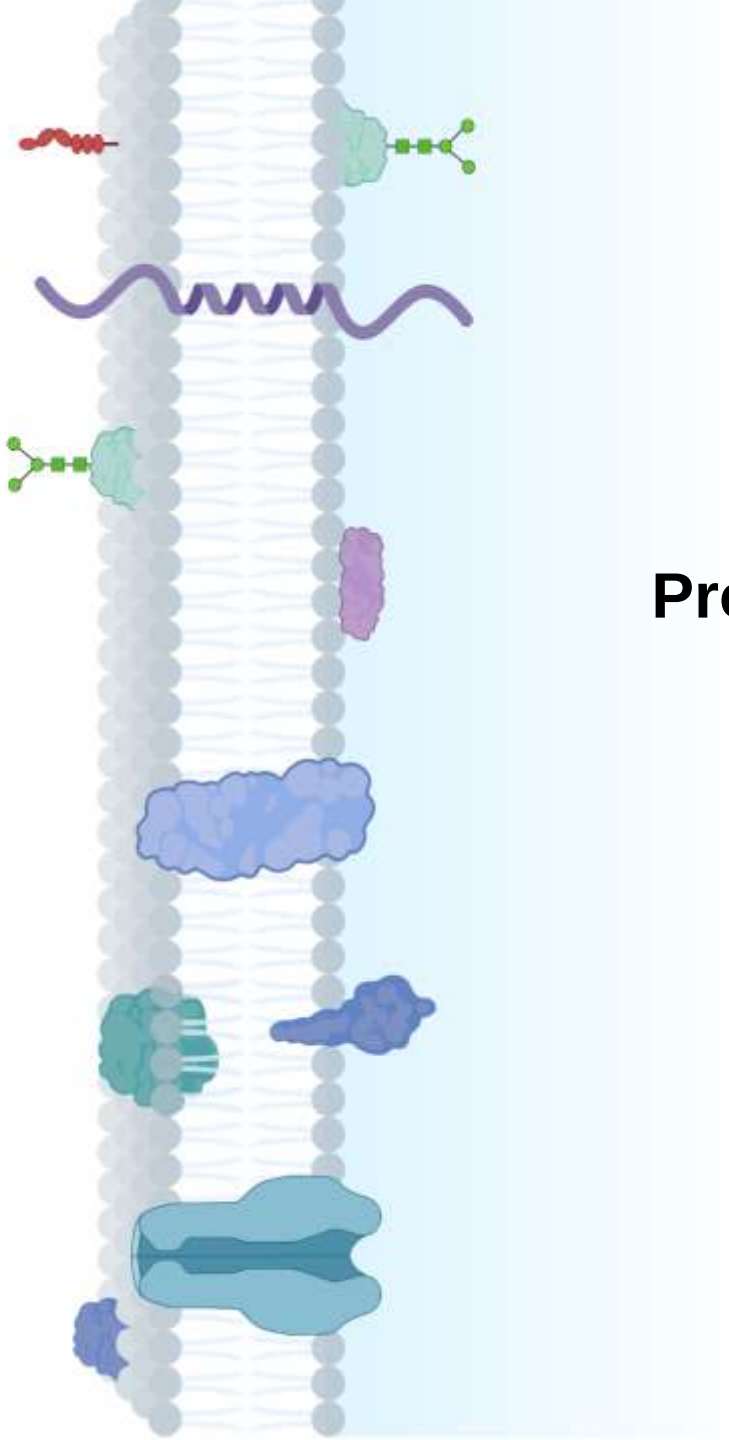
Proteomics wrap-up

- Up to 8000-13000 proteins can be identified/quantified in a human tissue samples
- We are getting closer to comprehensive coverage of human proteome
- MS-based „identification of a protein“ is actually identification of its coding gene
- We can not distinguish between individual protein variants (proteoforms)
- Only micrograms of the peptide sample are needed (mgs of tissue)
- Single-cell analysis is getting reasonable coverage (over 4000 IDs!)
- Specific methods for PTM analysis are available
- Information is semi-quantitative not absolute
- **Some proteins remain under-represented in standard proteomic analyses**



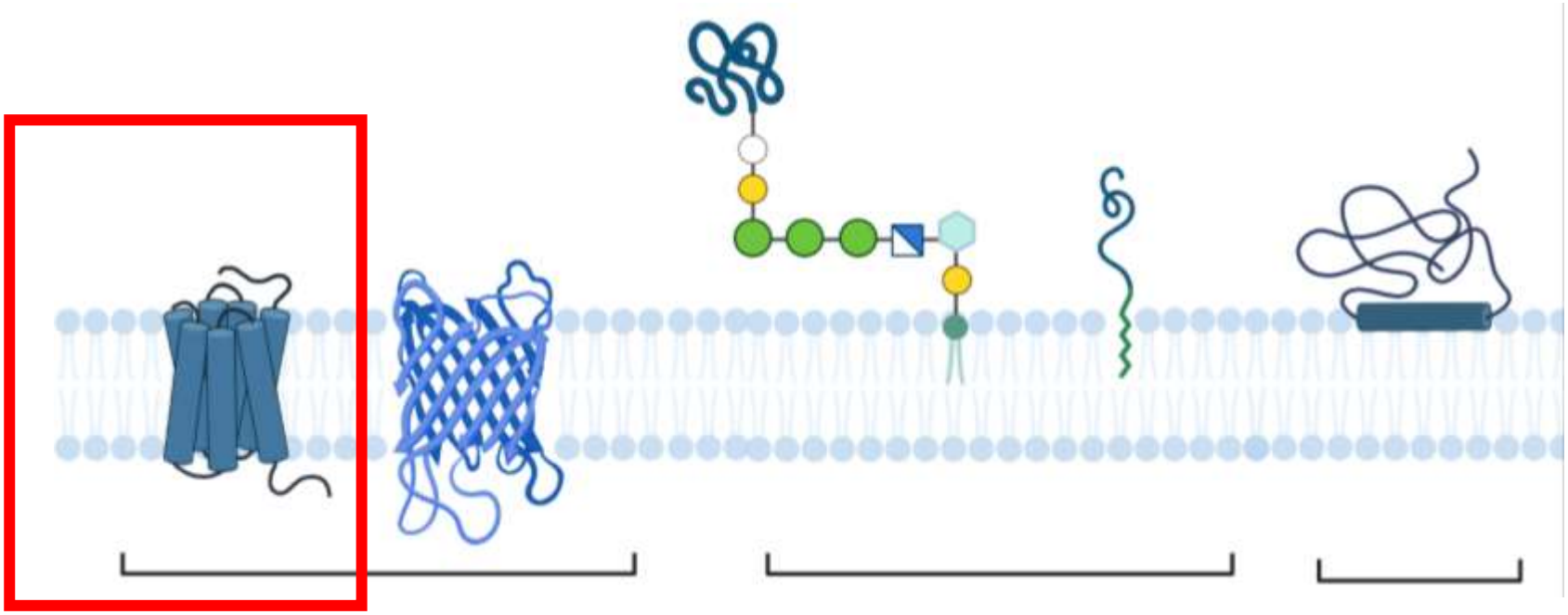
Proteomics of Membrane Proteins

Jiri Petrak, BIOCEV

- Integral membrane proteins – intro and the peculiarities
- Prediction of TM segments, visualization
- Why do membrane proteins escape proteomic analyses?

- Glycopeptide enrichment
- Analysis of the membrane embedded segments
- Pitchfork strategy

- Membrane proteome of neuroendocrine tumors using The Pitchfork strategy

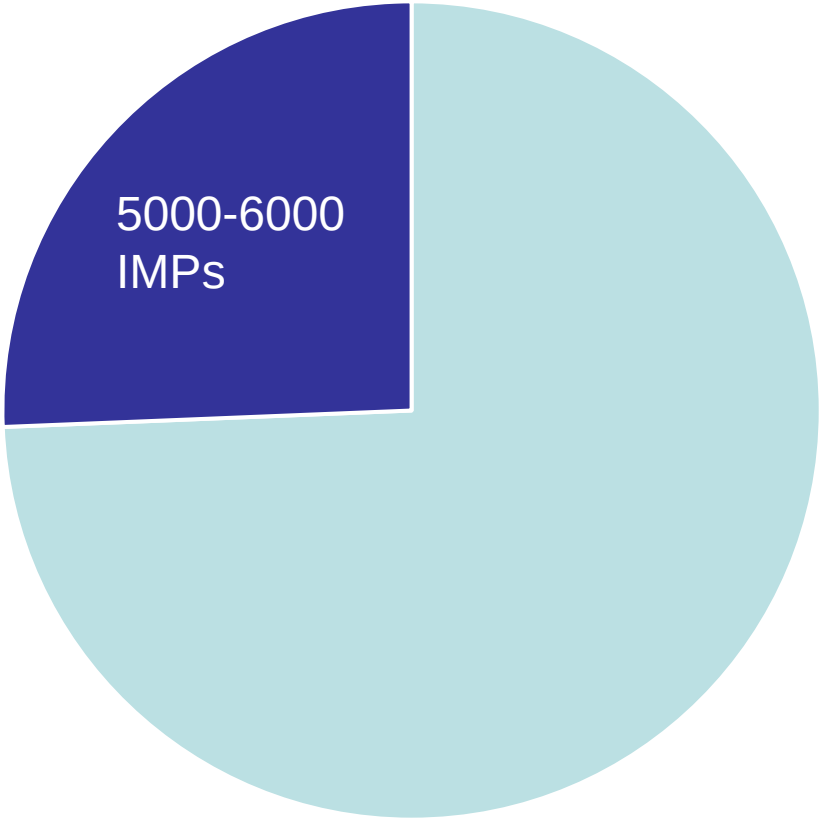


Integral membrane proteins

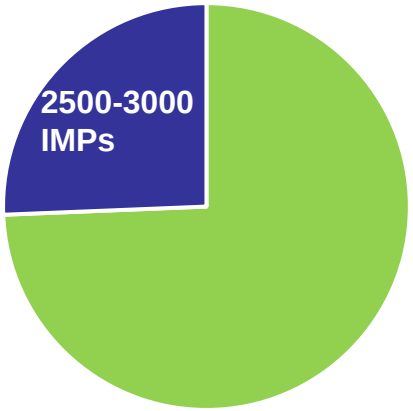
Lipid-anchored

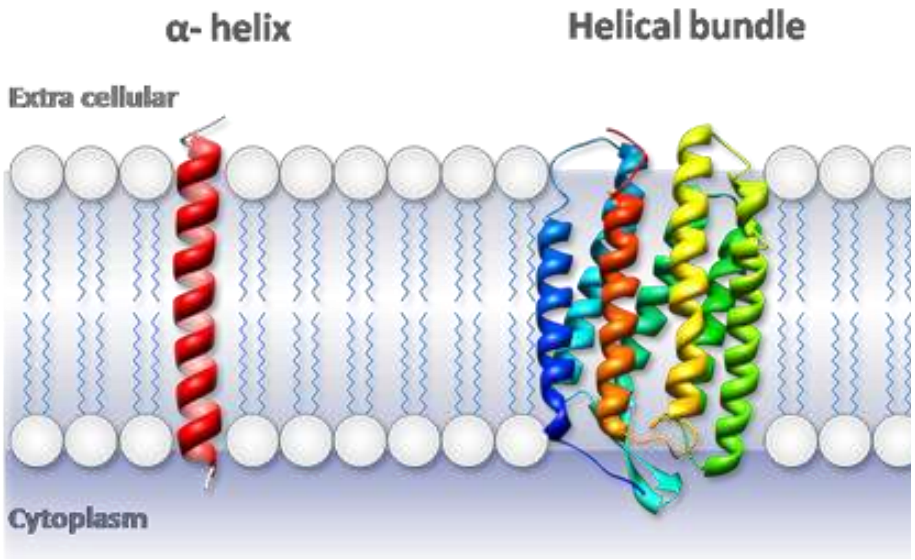
membrane-associated

~ 20 000 human protein coding genes



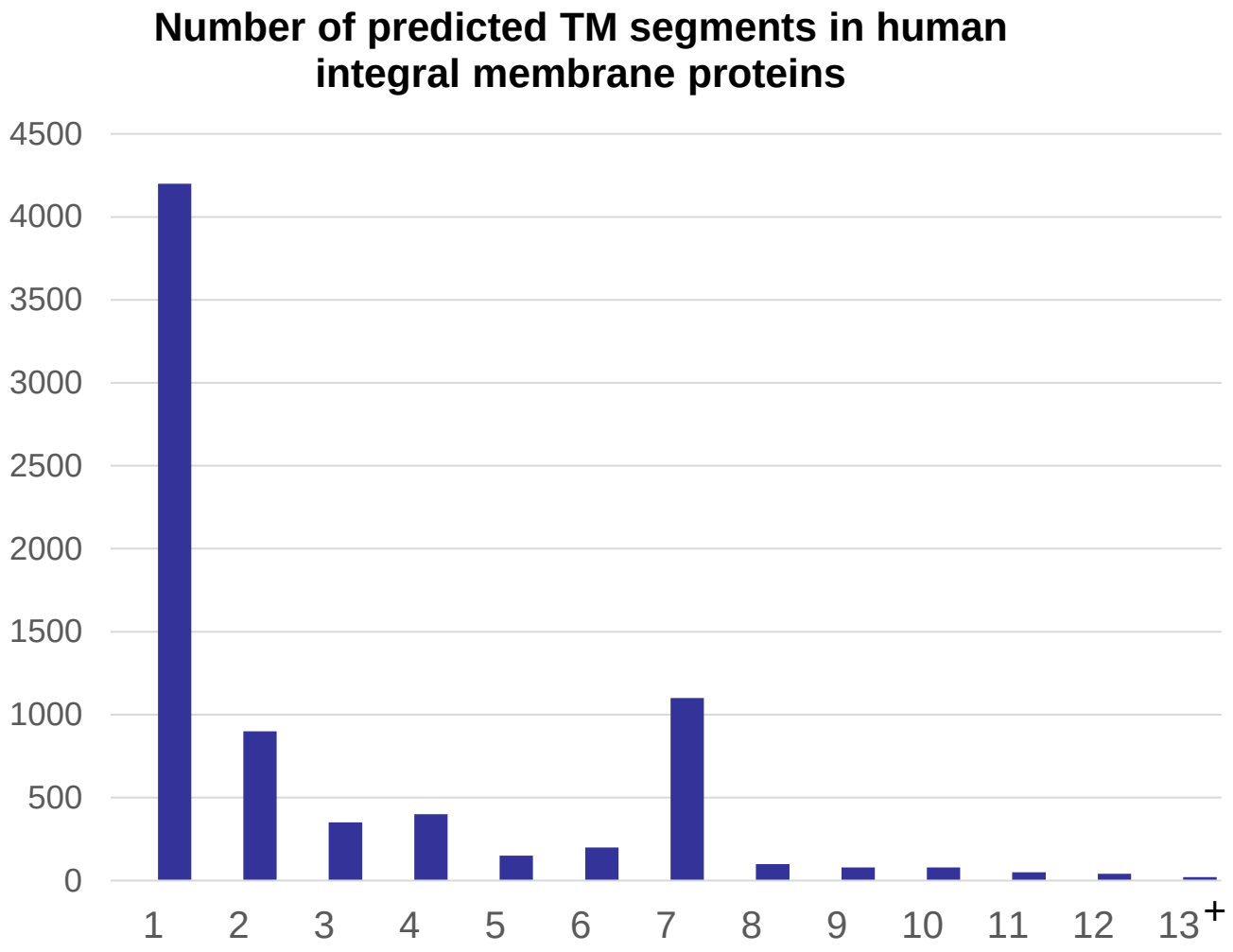
10 000 genes actively expressed by an average cell type





INTEGRAL MEMBRANE PROTEINS

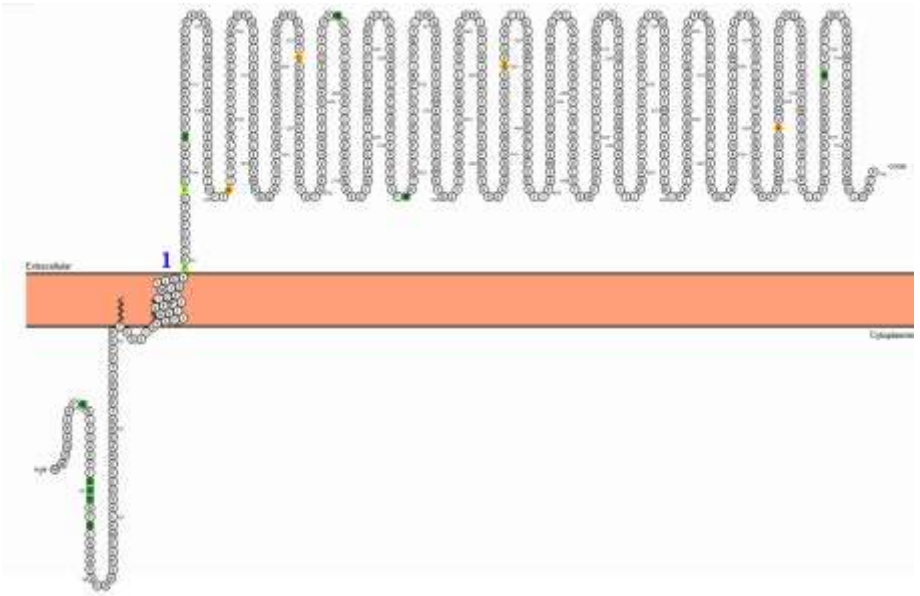
- alpha helix TM domain(s) (20-25 AA) + soluble domains



Fagerberg et al. Proteomics 2011

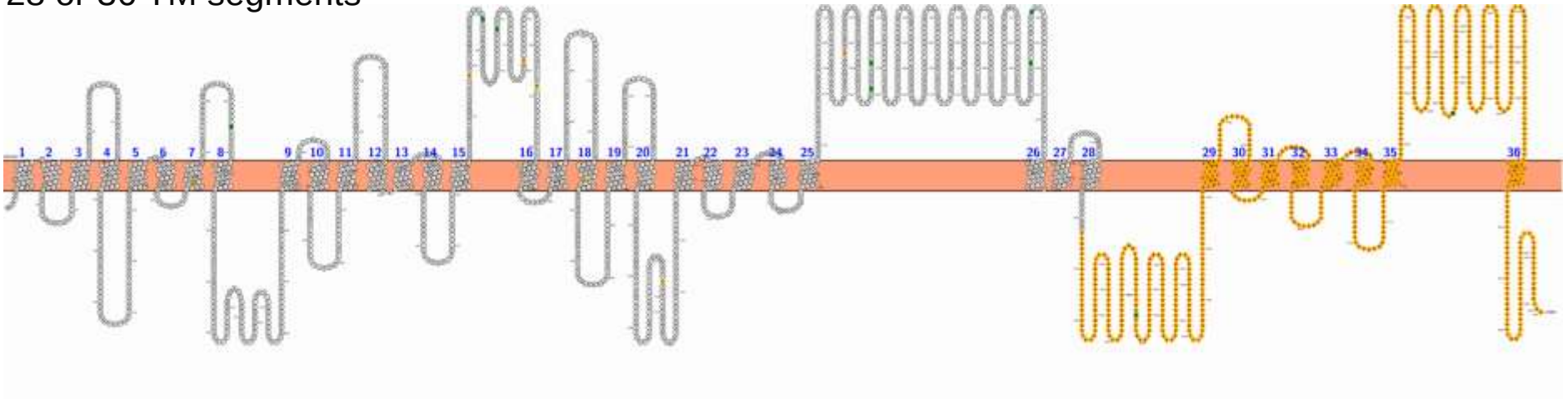
Transferrin receptor protein 1
TFRC

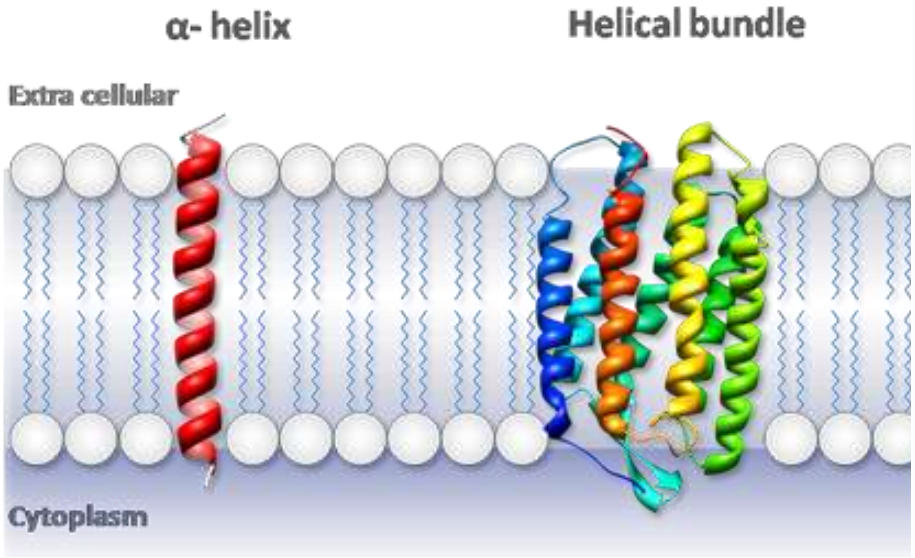
760 AA
1 TM segment



Piezo-type mechanosensitive ion channel component 1
PIEZO4

2521 AA
28 or 36 TM segments



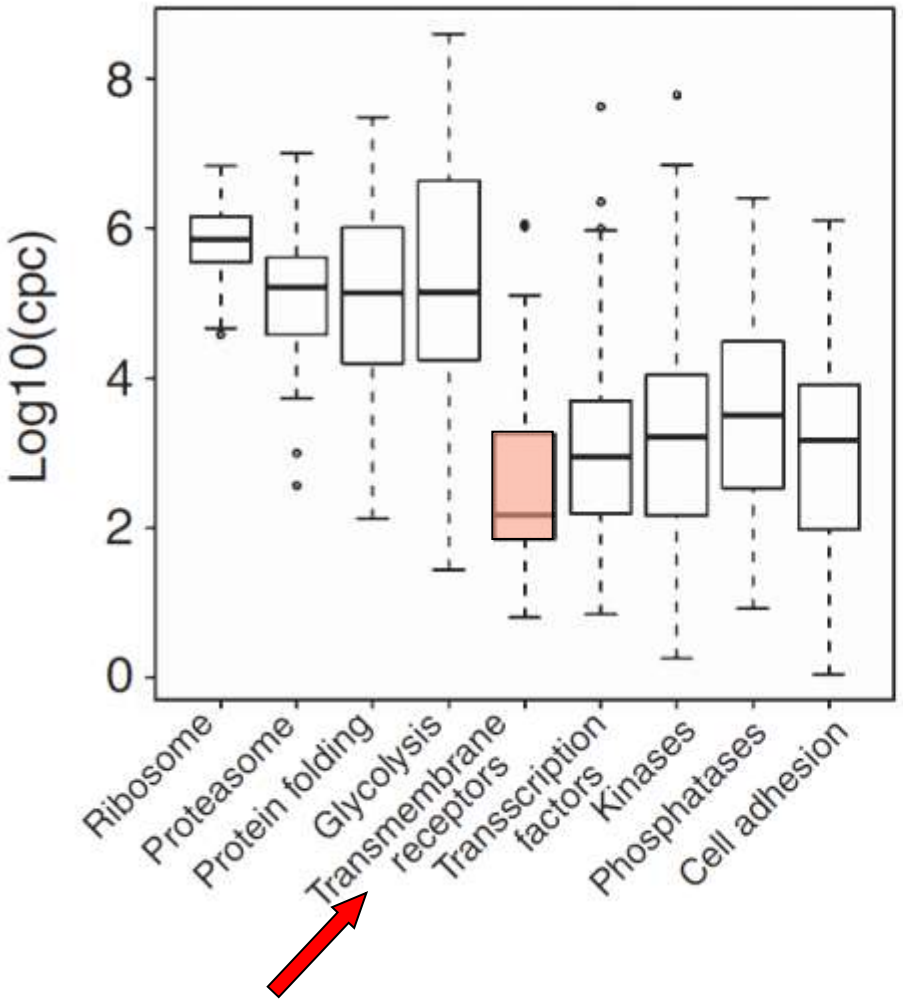


INTEGRAL MEMBRANE PROTEINS

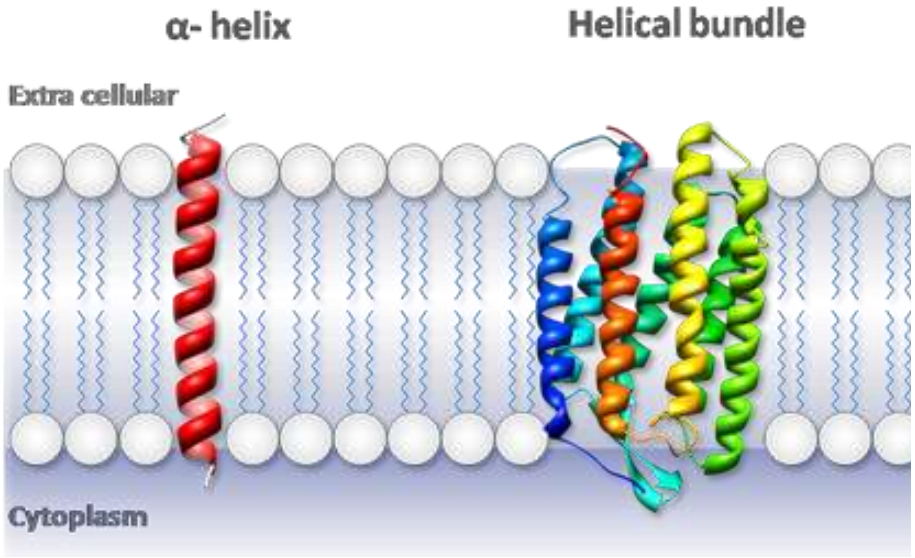
- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression

Cellular abundance of transmembrane proteins is LOW

(100-1000 copies/cell)



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



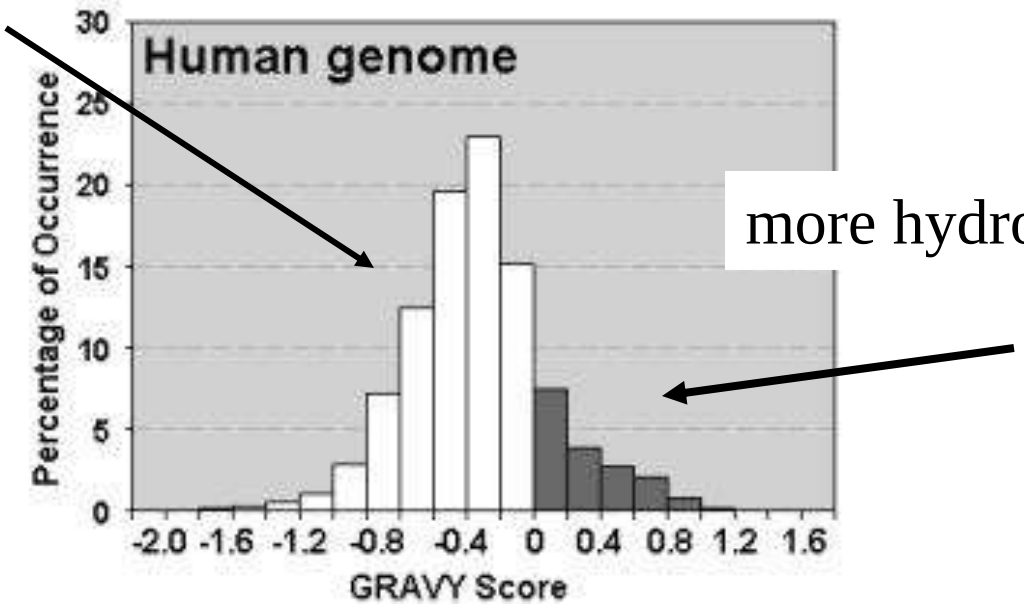
INTEGRAL MEMBRANE PROTEINS

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

Protein hydrophobicity

GRAVY SCORE – Grand average hydropathy
(sum of „hydrofobicities“ (-4.5 to - 4.5) of all AA/number of AA)

more
water-soluble



more hydrophobic

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

Hydrophobicity of AAs

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**

AA typical for transmembrane α-helices:

„FAMILY VW“ (G, S, C…)

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)

CD171 (P32004)

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

Predicted TM segment

FAMILY VW +C +G +S

MVVALRYVWPLLLCSPCLLIQIPEEYEGHHVMEPPVITEQSPRRLVVFPTDDISLKCEASGKPEVQFRWTRDGVHFKPKEELGVTVYQSPHSGSFTITGNNSNFA
QRFQGIYRCFASNKLGTAMSHEIRLMAEGAPKWPKETVKPVEVEEGESVVLPCNPPPSAEPLRIYWMNSKILHIKQDERVTMGQNGNLYFANVLTSDNHSDYI
CHAHFPGTRTIIQKEPIDLRVKATNSMIDRKPRLLFPTNSSSHLVALQGQPLVLECIAEGFPTPTIKWLRPSGPMPADRVTYQNHNKTLQLLKVGEEDDGEYRCLA
ENSLGSARHAYYVTVEAAPYWLHKPQSHLYGPGETARLDCQVQGRPQPEVTWRINGIPVEELAKDQKYRIQRGALILSNVQPSDTMVTQCEARNRHGLLLAN
AYIYVVQLPAKILTADNQTYMAVQGSTAYLLCKAFGAPVPSVQWLDEDGTTVLQDERFFPYANGTLGIRDLQANDTGRYFCLAANDQNNVTIMANLKVKDAT
QITQGPRSTIEKKGSRVTFTCQASFDPSLQPSITWRGDGRDLQELGDSDKYFIEDGRLVIHSLDYSDQGNYSCVASTELDVVESRAQLLVVGSPGPVPRLVLSDLH
LLTQSQVRVSWSPAEDHNAPIEKYDIEFEDKEMAPEKWYSLGKVPGNQTSTTLKLSPYVHYTFRVTAINKYGPGEPSPVSETVVTPEAAPEKNPVDVKGEGNET
TNMVITWKPLRWMDWNAPQVQYRVQWRPQGTRGPWQEQIVSDPFLVVSNTSTFVPYEIKVQAVNSQGKGPEPQVTIGYSGEDYPQAIPELEGIEILNSSAV
LVKWRPVDLAQVKGHLRGYNVTYWREGSQRKHSKRHIHKDHVVVPANTTSVILSGLRPYSSYHLEVQAFNGRGSGPASEFTFSTPEGVPGHPEALHLECQSNT
SLLLRWQPPLSHNGVLTGYVLSYHPLDEGGKGQLSFNLRDPELRTHNLTDLSPHLRYRFQLQATTKEGPGEAIVREGGTMALSGISDFGNISATAGENYSVVSW
VPKEGQCNFRFHILFKALGEEKGGASLSPQYVSYNQSSYTQWDLQPDTDYEIHLFKERMFRHQMAVKTNGTGRVRLPPAGFATEGWFIGFVSAIILLLLVLLILCF
IKRSKGGKYSVKDKEDTQVDSEARPMKDETFGEYRSLESDNEEKAFGSSQPSLNGDIKPLGSDDSLADYGGSVDVQFNEDGSFIGQYSGKKEKEAAGGNDSSG
ATSPINPAVALE

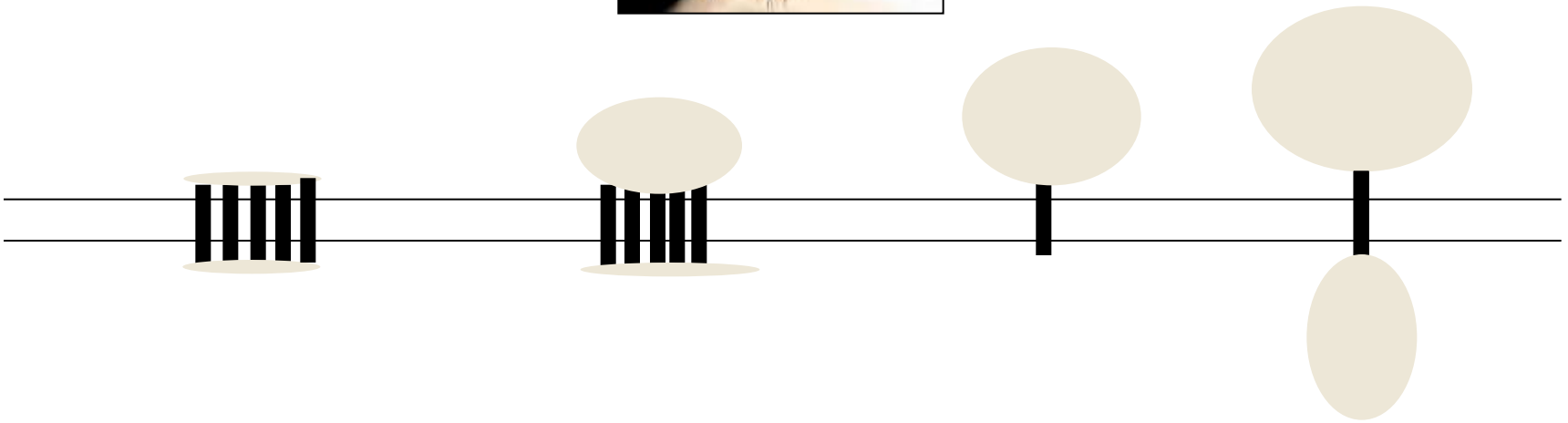
Deletion

Newly added hydrophobic segment

MVVALRYVWPLLLCSPCLLIQIPEEYEGHHVMEPPVITEQSPRRLVVFPTDDISLKCEASGKPEVQFRWTRDGVHFKPKEELGVTVYQSP
HSGSFTITGNNSNFAQRFQGIYRCFASNKLGTAMSHEIRLMAEGAPKWPKETVKPVEVEEGESVVLPCNPPPSAEPLRIYWMNSKILHIKQ
DERVTMGQNGNLYFANVLTSDNHSDYICHAHFPGTRTIIQKEPIDLRVKATNSMIDRKPRLLFPTNSSSHLVALQGQPLVLECIAEGFPTPTI
KWLRPSGPMPADRVTYQNHNKTLQLLKVGEEDDGEYRCLAENSLGSARHAYYVTVEAAPYWLHKPQSHLYGPGETARLDCQVQGRPQ
PEVTWRINGIPVEELAKDQKYRIQRGALILSNVQPSDTMVTQCEARNRHGLLLANAYIYVVQLPAKILTADNQTYMAVQGSTAYLILIVILIFAM
ILIVILICKAFGAPVPSVQWLDEDGTTVLQDERFFPYANGTLGIRDLQANDTGRYFCLAANDQNNVTIMANLKVKDATQITQGPRSTIEKKGS
RVTFTCQASFDPSLQPSITWRGDGRDLQELGDSDKYFIEDGRLVIHSLDYSDQGNYSCVASTELDVVESRAQLLVVGSPGPVPRLVLSDL
HLLTQSQVRVSWSPAEDHNAPIEKYDIEFEDKEMAPEKWYSLGKVPGNQTSTTLKLSPYVHYTFRVTAINKYGPGEPSPVSETVVTPEAA
PEKNPVDVKGEGNETTNMVITWKPLRWMDWNAPQVQYRVQWRPQGTRGPWQEQIVSDPFLVVSNTSTFVPYEIKVQAVNSQGKGPEP
QVTIGYSGEDYPQAIPELEGIEILNSSAVLVKWRPVDLAQVKGHLRGYNVTYWREGSQRKHSKRHIHKDHVVVPANTTSVILSGLRPYSSY
HLEVQAFNGRGSGPASEFTFSTPEGVPGHPEALHLECQSNTSLLLRWQPPLSHNGVLTGYVLSYHPLDEGGKGQLSFNLRDPELRTHNL
TDLSPHLRYRFQLQATTKEGPGEAIVREGGTMALSGISDFGNISATAGENYSVVSWVPKEGQCNFRFHILFKALGEEKGGASLSPQYVSY
NQSSYTQWDLQPDTDYEIHLFKERMFRHQMAVKTNGTGRVRLPPAGFATEGWFIGFVSAIILLLLVLLILCFIKRSKGGKYSVKDKEDTQVD
SEARPMKDETFGEYRSLESDNEEKAFGSSQPSLNGDIKPLGSDDSLADYGGSVDVQFNEDGSFIGQYSGKKEKEAAGGNDSSGATSPIN
PAVALE

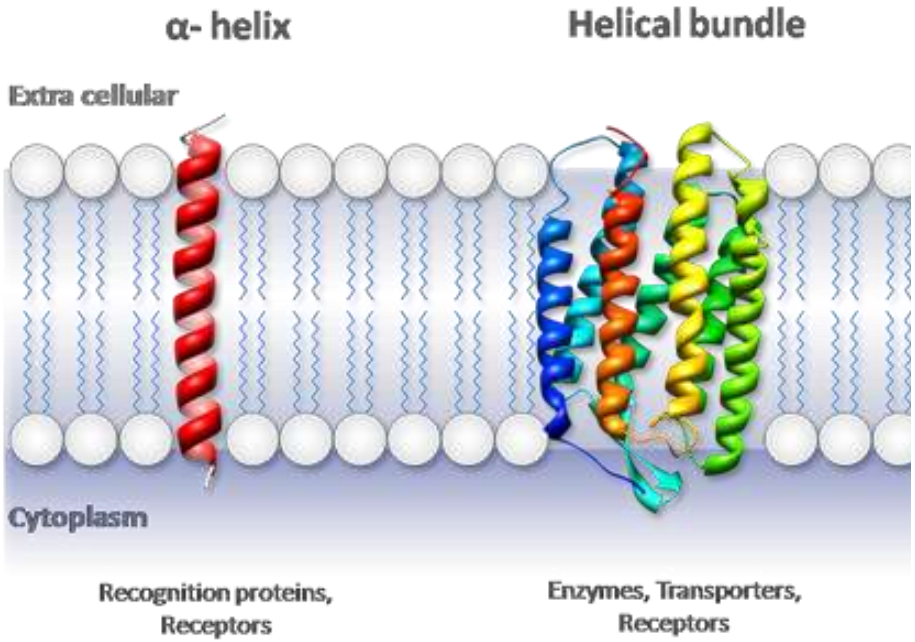
MVVALRYVWPLLLCSPCLLIQIPEEYEGHLILIVILIFAMILIVILIMILIHVMEPPVITEQSPRRLVVFPTDDISLKCEASGKPEVQFRWTRDGVH
FKPKEELGVTVYQSPHSGSFTITGNNSNFAQRFQGIYRCFASNKLGTAMSHEIRLMAEGAPKWPKETVKPVEVEEGESVVLPCNPPPSAE
PLRIYWMNSKILHIKQDERVTMGQNGNLYFANVLTSDNHSDYICHAHFPGTRTIIQKEPIDLRVKATNSMIDRKPRLLFPTNSSSHLVALQGQ
PLVLECIAEGFPTPTIKWLRPSGPMPADRVTYQNHNKTLQLLKVGEEDDGEYRCLAENSLGSARHAYYVTVEAAPYWLHKPQSHLYGPG
ETARLDCQVQGRPQPEVTWRINGIPVEELAKDQKYRIQRGALILSNVQPSDTMVTQCEARNRHGLLLANAYIYVVQLPAKILTADNQTYMA
VQGSTAYLILIVILIFAMILIVILIMILICKAFGAPVPSVQWLDEDGTTVLQDERFFPYANGTLGIRDLQANDTGRYFCLAANDQNNVTIMANLKV
KDATQITQGPRSTIEKKGSRVTFTCQASFDPSLQPSITWRGDGRDLQELGDSDKYFIEDGRLVIHSLDYSDQGNYSCVASTELDVVESRA
QLLVVGSPGPVPRLVLSDLHLLTLILIVILIFAMILIVILIMILIQSQVRVSWSPAEDHNAPIEKYDIEFEDKEMAPEKWYSLGKVPGNQTSTTLKL
SPYVHYTFRVTAINKYGPGEPSPVSETVVTPEAAPEKNPVDVKGEGNETTNMVITWKPLRWMDWNAPQVQYRVQWRPQGTRGPWQE
QIVSDPFLVVSNTSTFVPYEIKVQAVNSQGKGPEPQVTIGYSGEDYPQAIPELEGIEILNSSAVLVKWRPVDLAQVKGHLRGYNVTYWREG
SQRKHSKRHIHKDHVVVPANTTSVILSGLRPYSSYHLEVQAFNGRGSGPASEFTFSTPEGVPGHPEALHLECQSNTSLLILIVILIFAMILIVIL
IMILILLRWQPPLSHNGVLTGYVLSYHPLDEGGKGQLSFNLRDPELRTHNLTDLSPHLRYRFQLQATTKEGPGEAIVREGGTMALSGISDFG
NISATAGENYSVVSWVPKEGQCNFRFHILFKALGEEKGGASLSPQYVSYNQSSYTQWDLQPDTDYEIHLFKERMFRHQMAVKTNGTGRV
RLPPAGFATEGWFIGFVSAIILLLLVLLILCFIKRSKGGKYSVKDKEDTQVDSEARPMKDETFGEYRSLESDNEEKAFGSSQPSLNGDIKPLG
SDDSLADYGGSVDVQFNEDGSFIGQYSGKKEKEAAGGNDSSGATSPINPAVALE

IMPs - molecules with split personalities



hydrophilic

hydrophobic

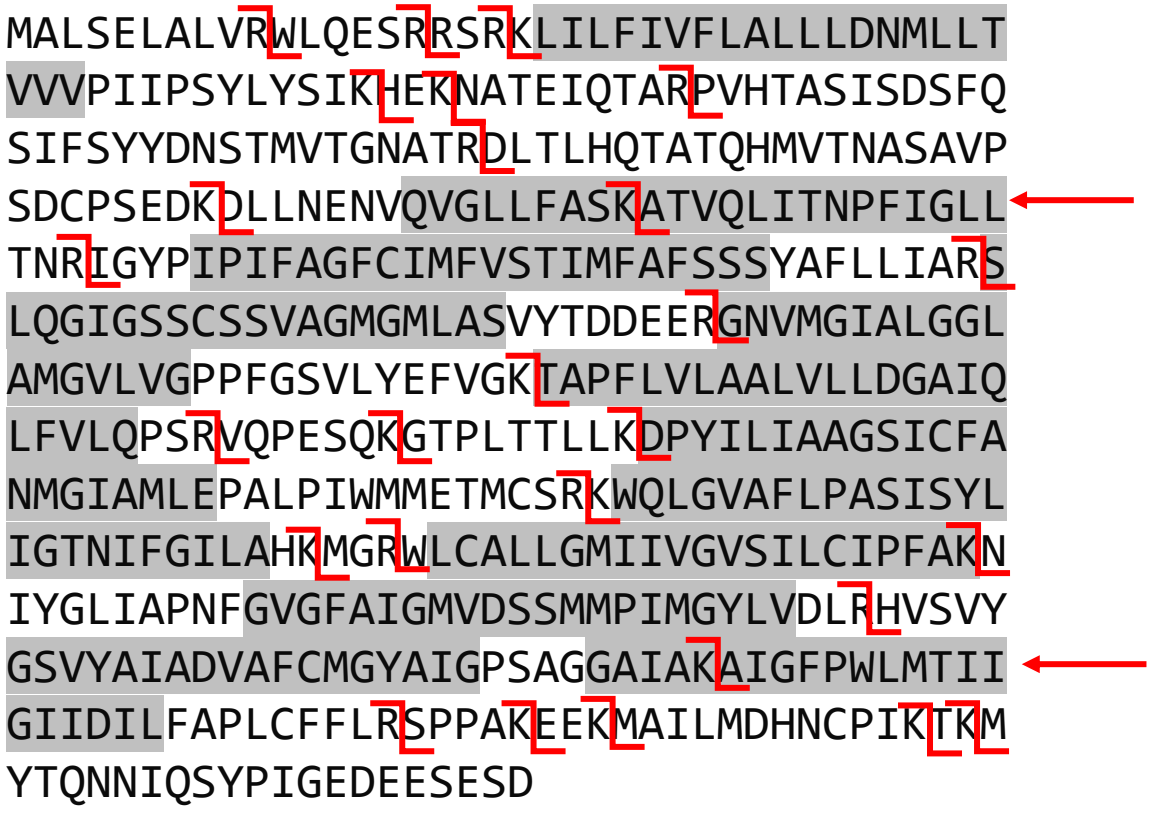


INTEGRAL MEMBRANE PROTEINS

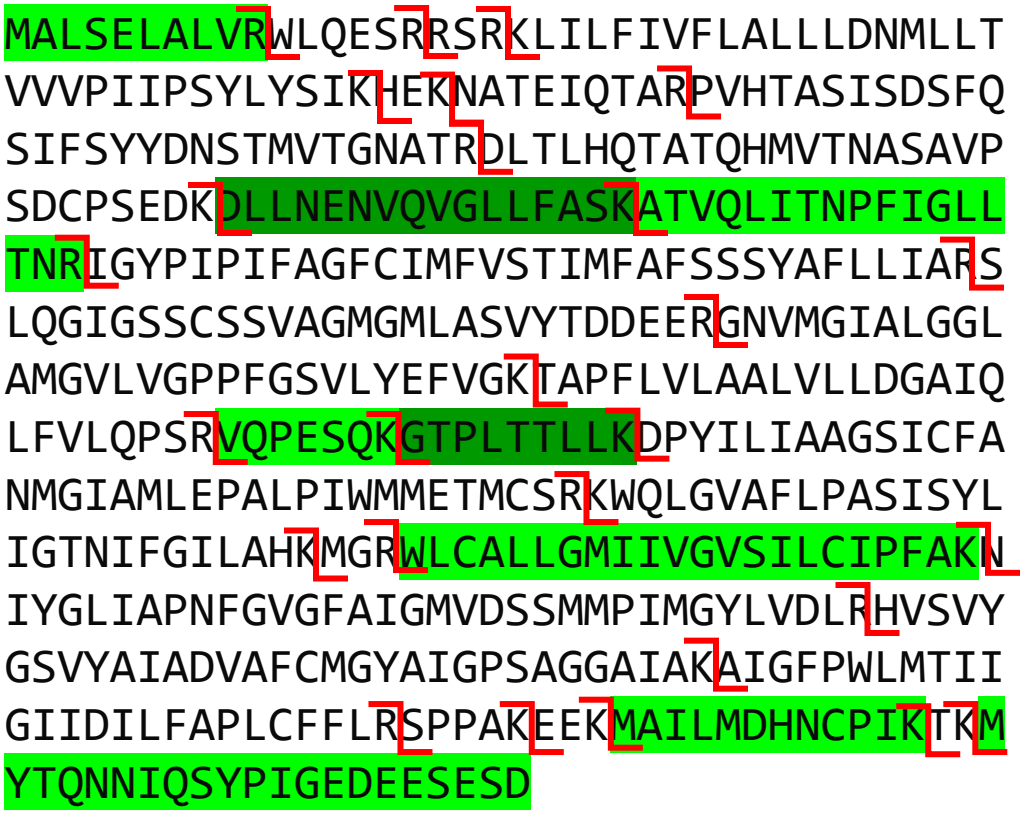
- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression
- hydrophobic/amphipathic nature
- scarcity of Arg, Lys in TM domains

Synaptic vesicular amine transporter (SLC18A2)

A protein with 12 TM segments

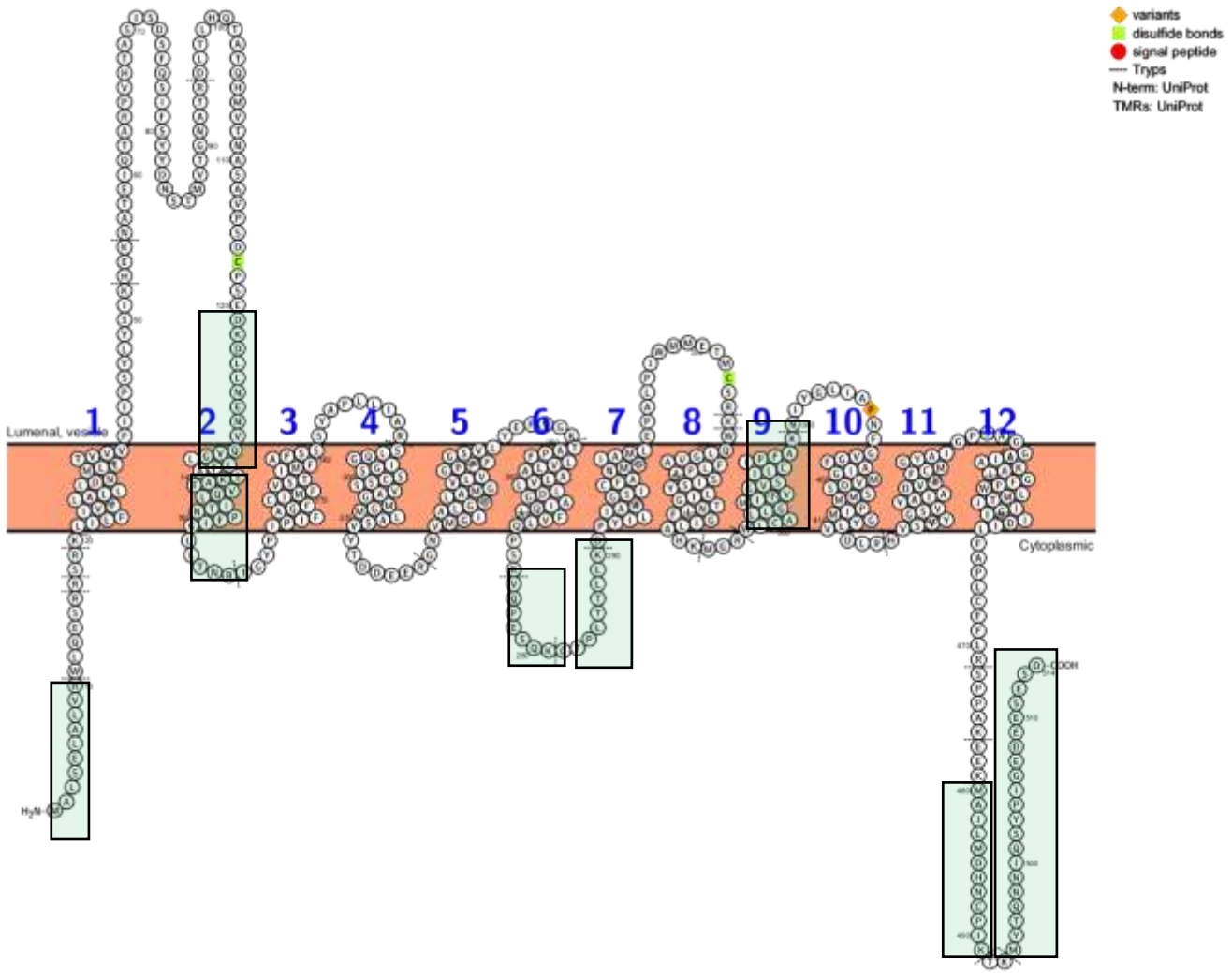


Synaptic vesicular amine transporter (SLC18A2)

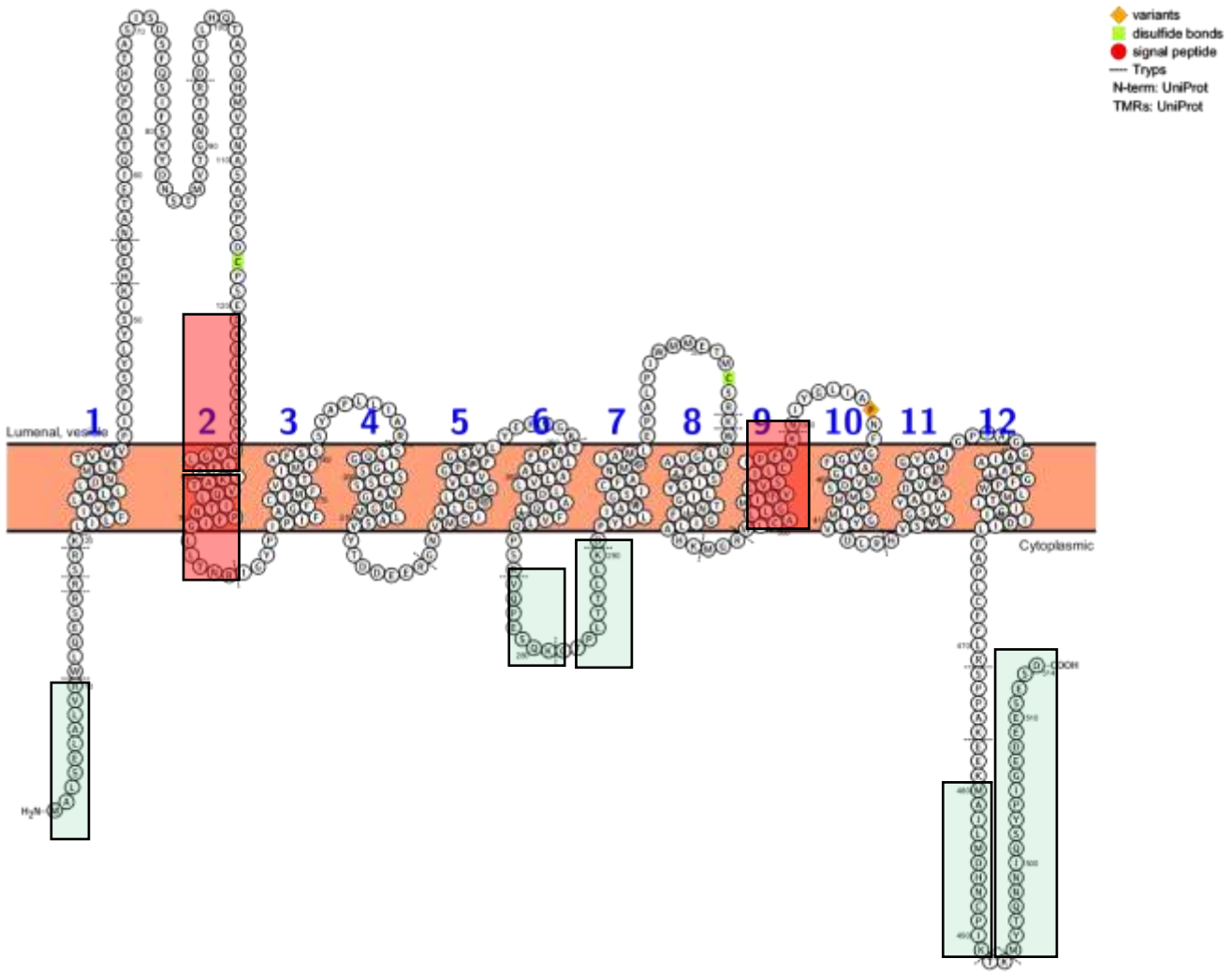


Only 8 unique peptides (5-25AA)

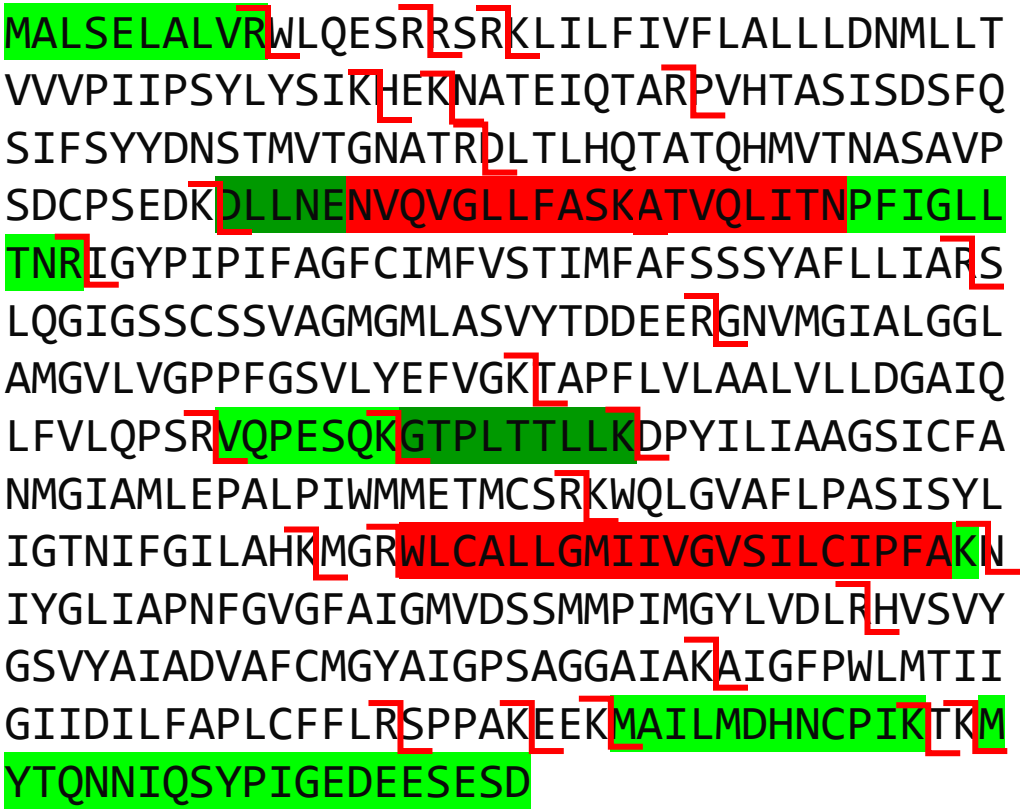
Synaptic vesicular amine transporter (SLC18A2)



Synaptic vesicular amine transporter (SLC18A2)

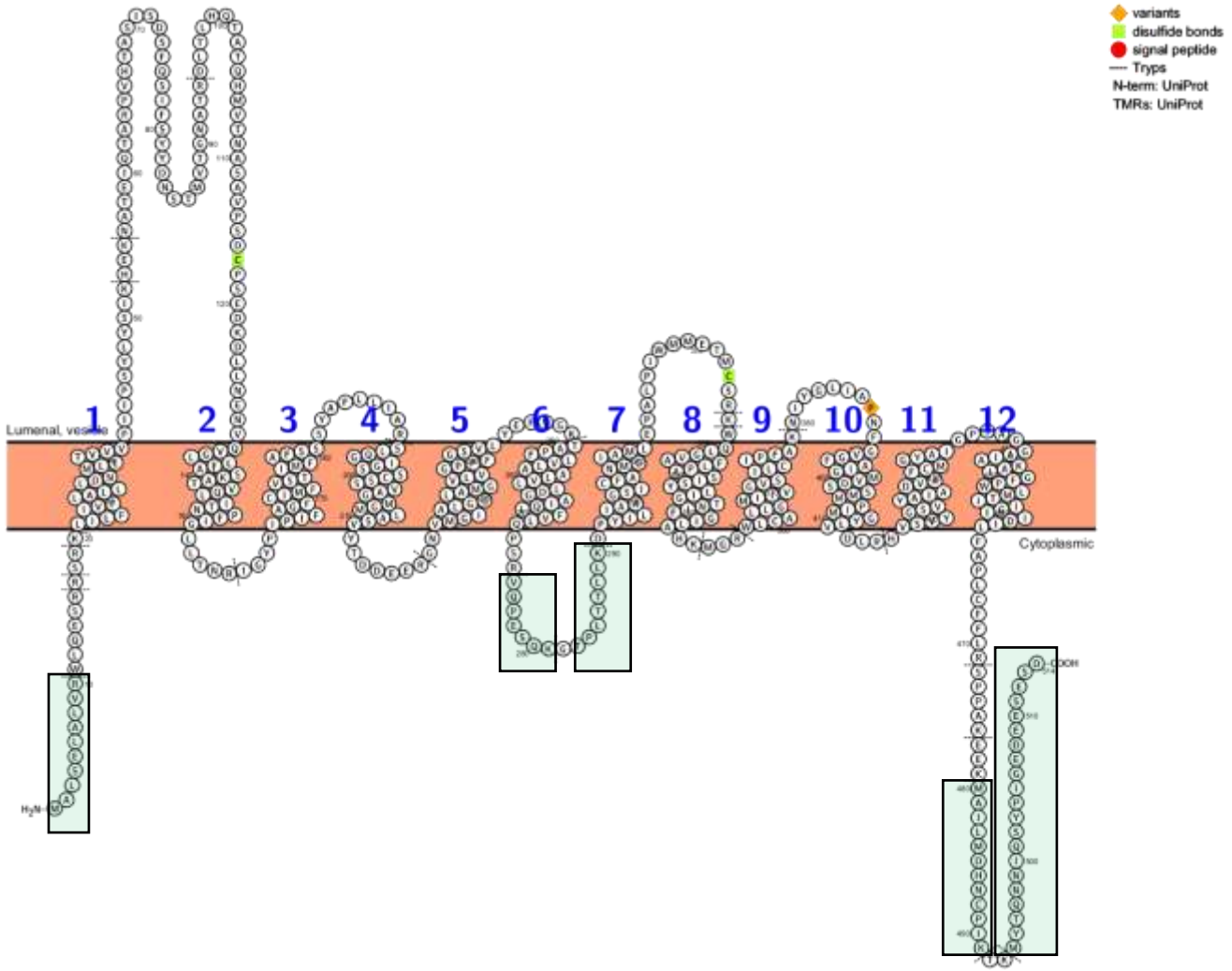


Synaptic vesicular amine transporter (SLC18A2)

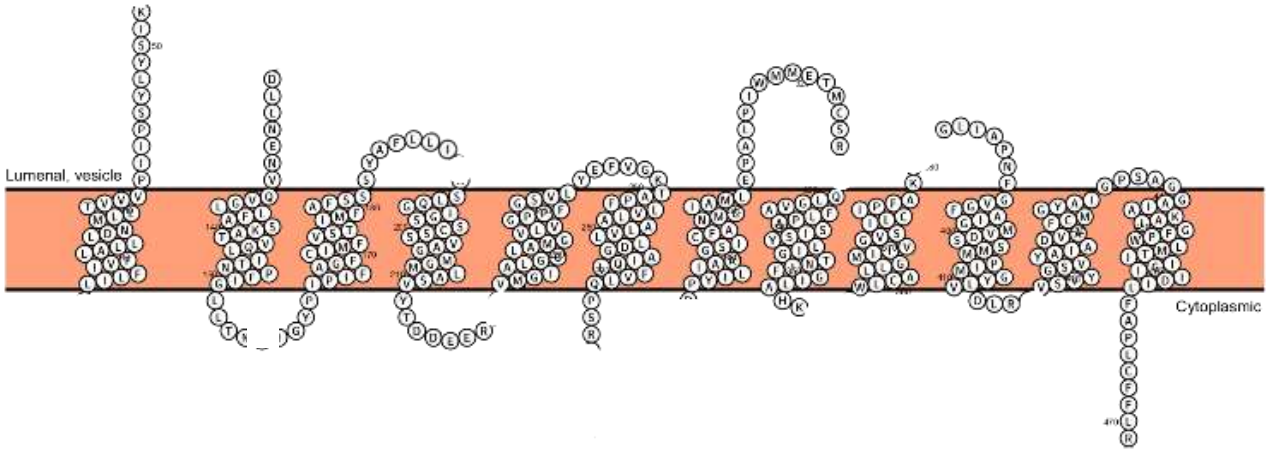


Only 8 unique peptides (5-25AA)

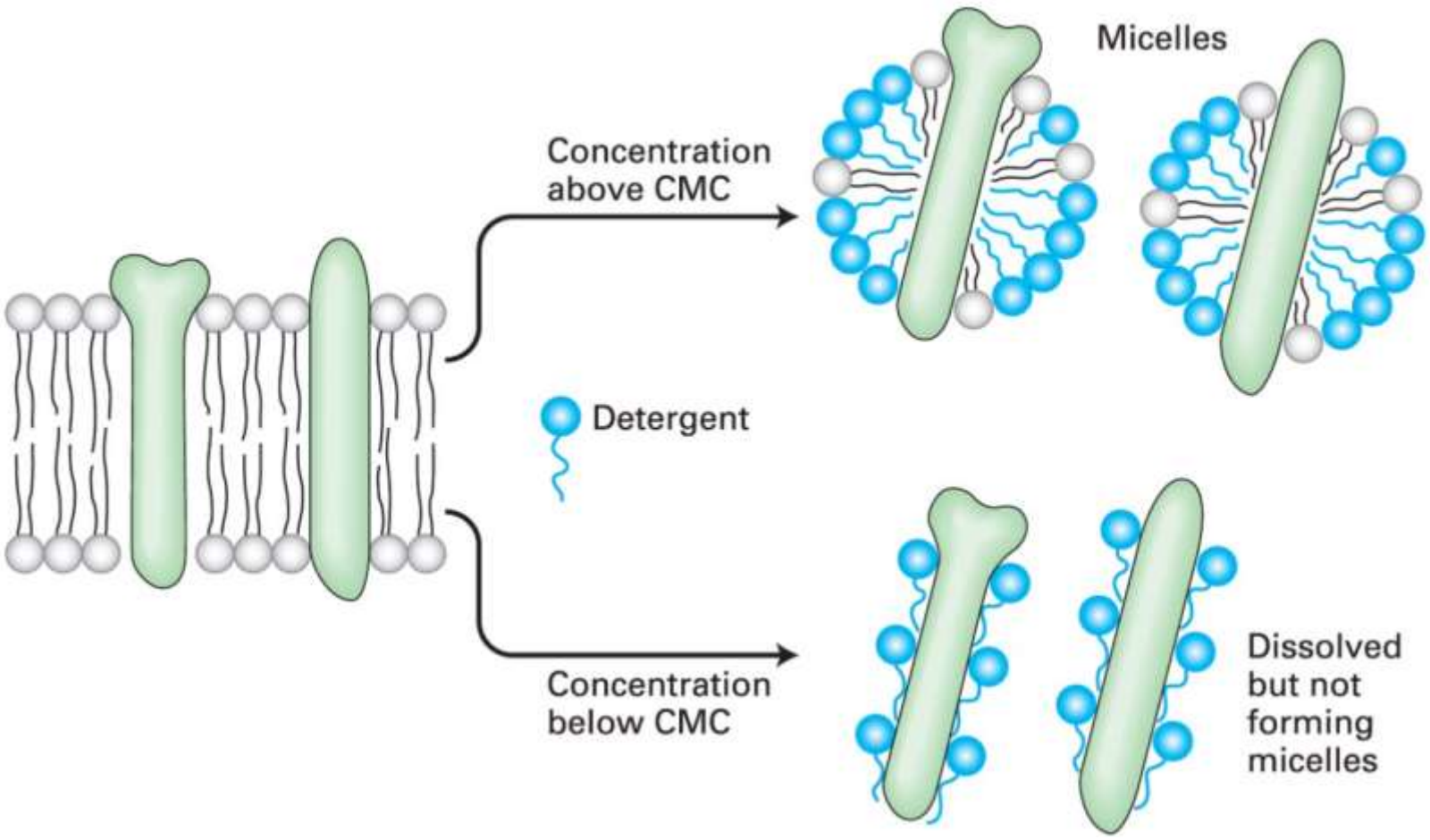
Only 5 unique peptides in the accessible soluble segments


variants
disulfide bonds
signal peptide
Tryps
N-term: UniProt
TMRs: UniProt
1
2
3
4
5
6
7
8
9
10
11
12
Lumenal, vesicle
Cytoplasmic
H2N-
COOH

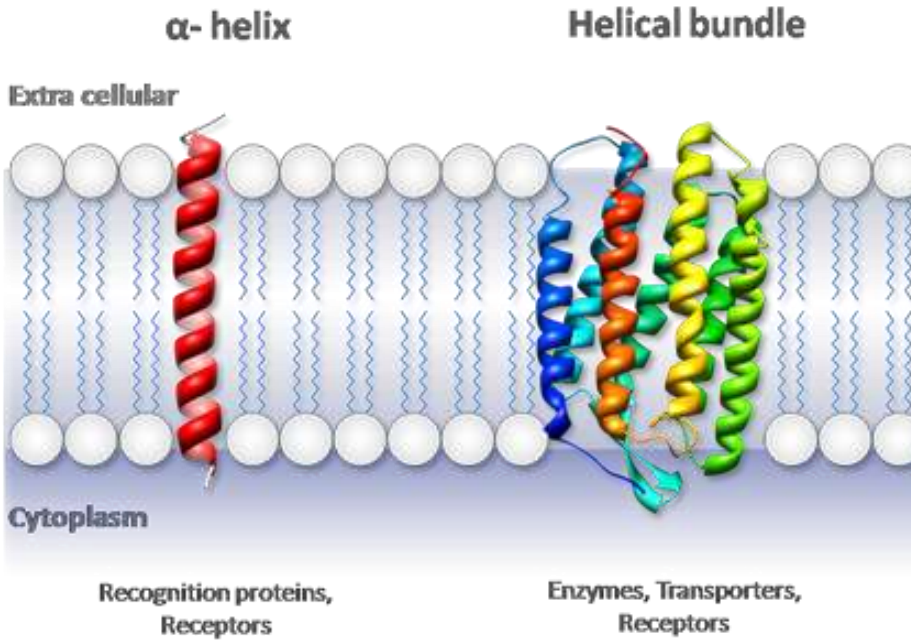
Synaptic vesicular amine transporter (Slc18a2



80% of the sequence of SLC18A2 is represent by **long** tryptic peptides overlapping with TM segments

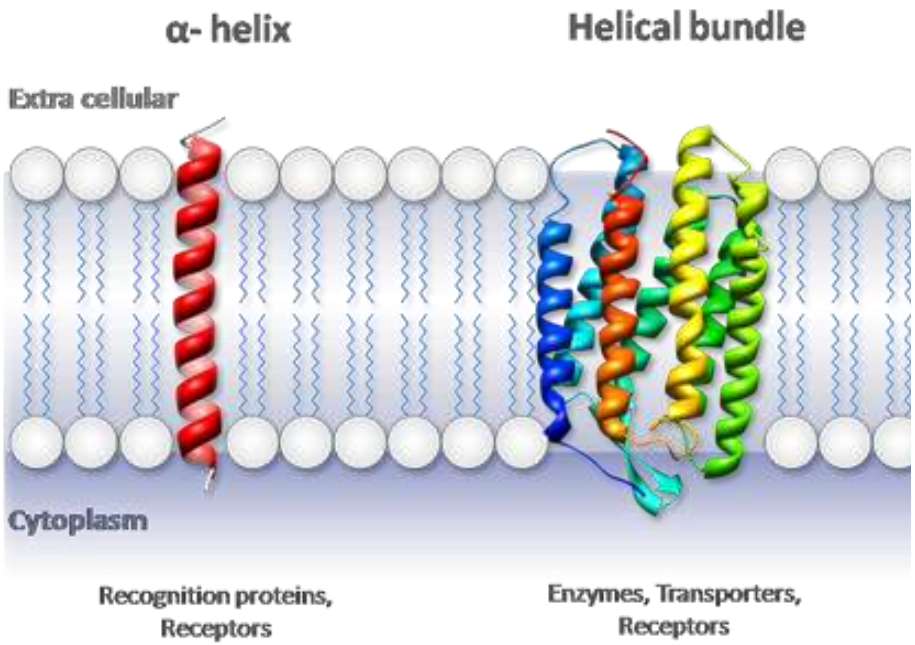


Molecular Cell Biology 8th ed., Lodish and Baltimore



INTEGRAL MEMBRANE PROTEINS

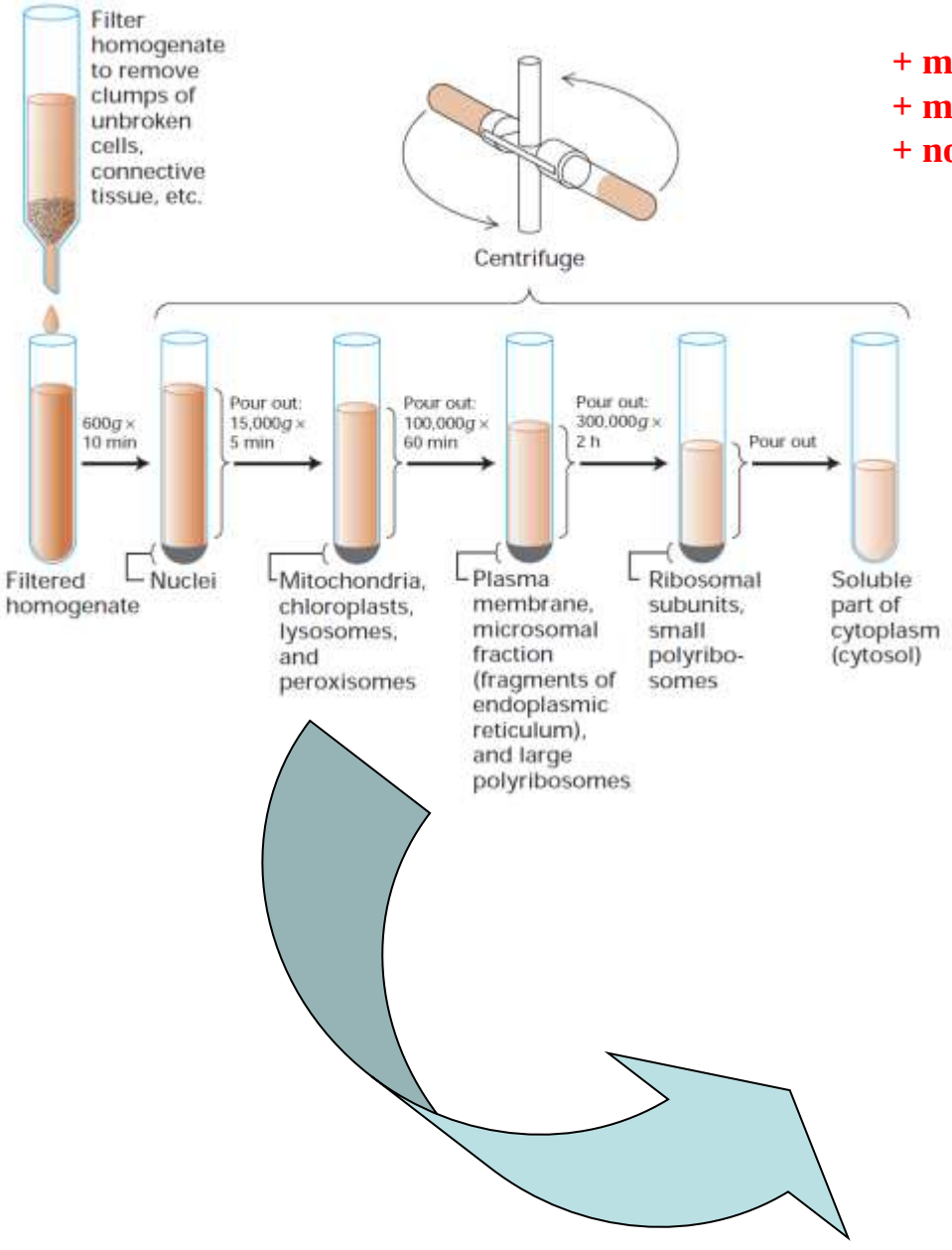
- alpha helix TM domain(s) (20-25 AA) + soluble domains
- low expression
- hydrophobic/amphipathic nature
- no Arg, Lys in TM domains
- detergents interfere with digestion and/or LC-MS
- UNDER-REPRESENTED IN PROTEOMIC ANALYSES



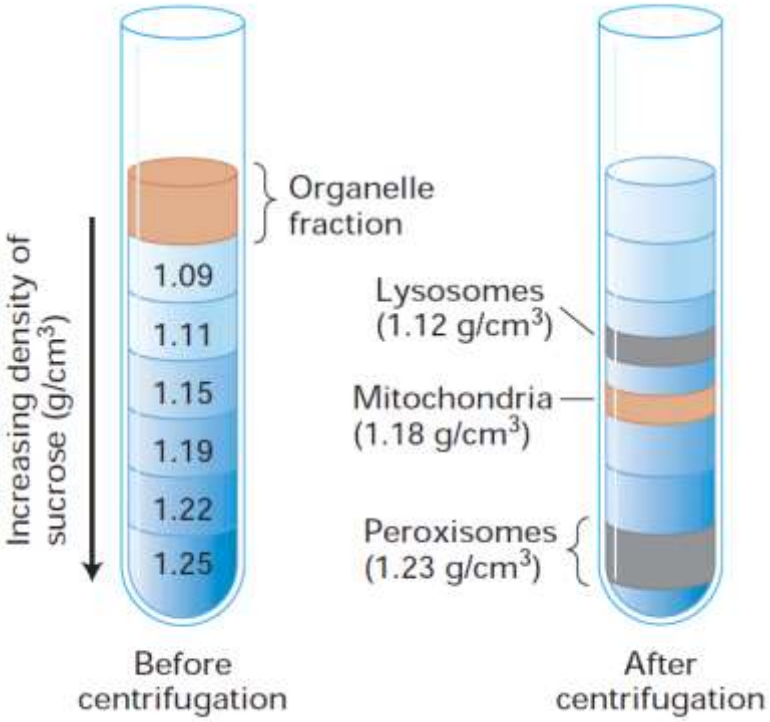
INTEGRAL MEMBRANE PROTEINS

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

„membrane“ fraction enrichment



+ major cytosolic and cytoskeletal contaminants
+ mambrane-associated proteins
+ non-specific adherence to hydrophobic surfaces



Standard strategy
(targets intact proteins)

Membrane fraction

↓

Solubilization

↓

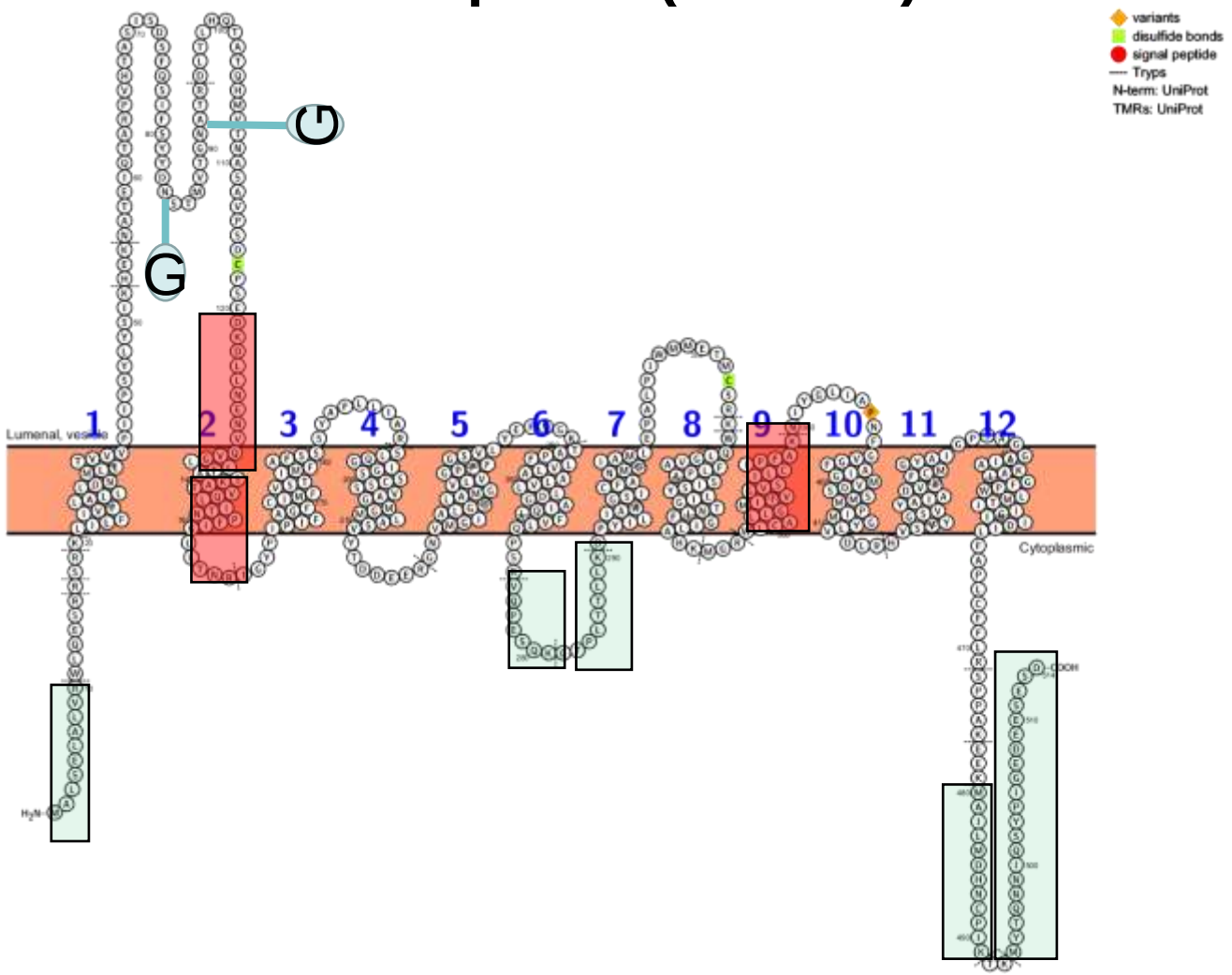
Tryptic digestion

↓

LC-MS/MS

LOW ENRICHMENT
(up to 10-15%)

Synaptic vesicular amine transporter (Slc18a2)



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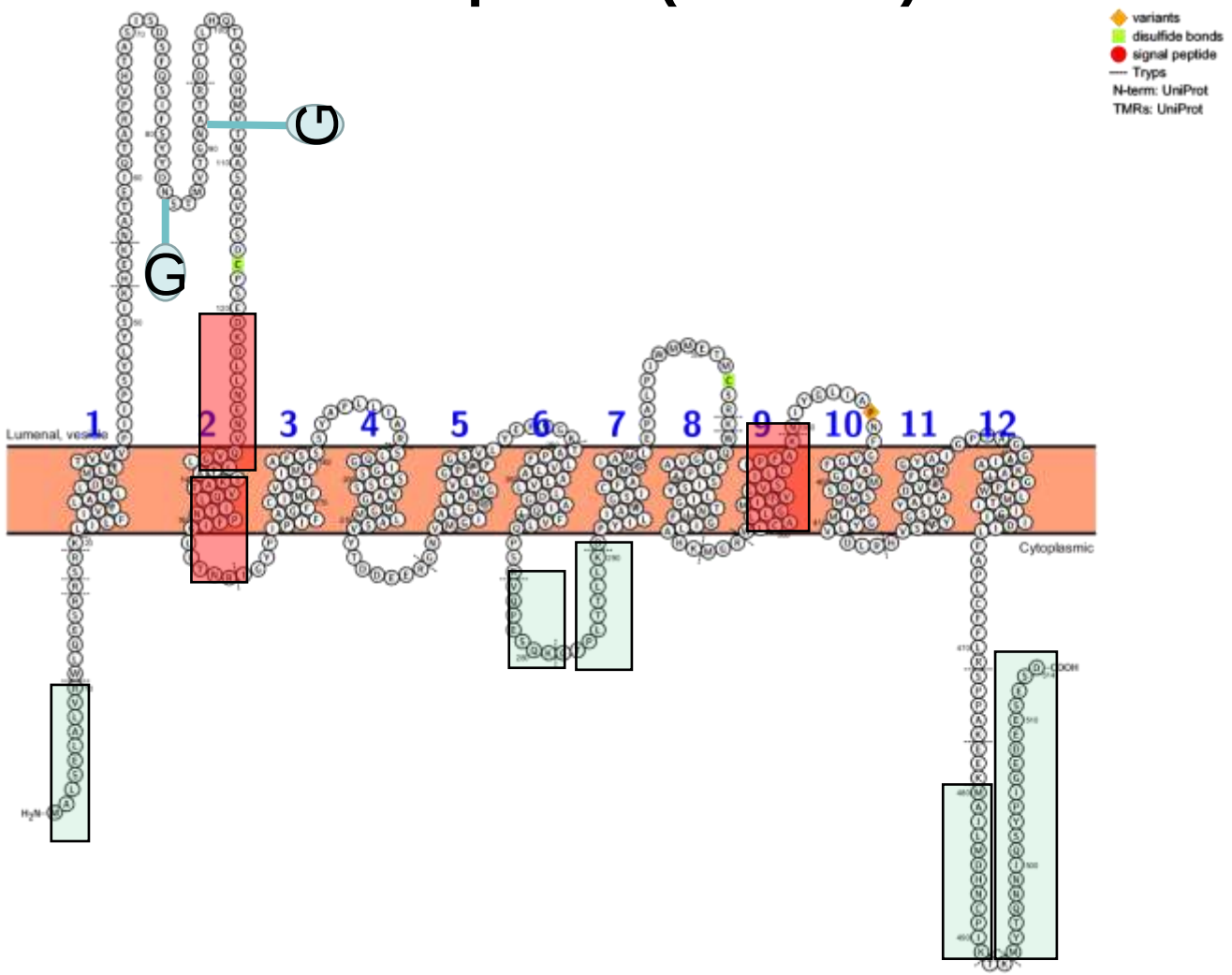

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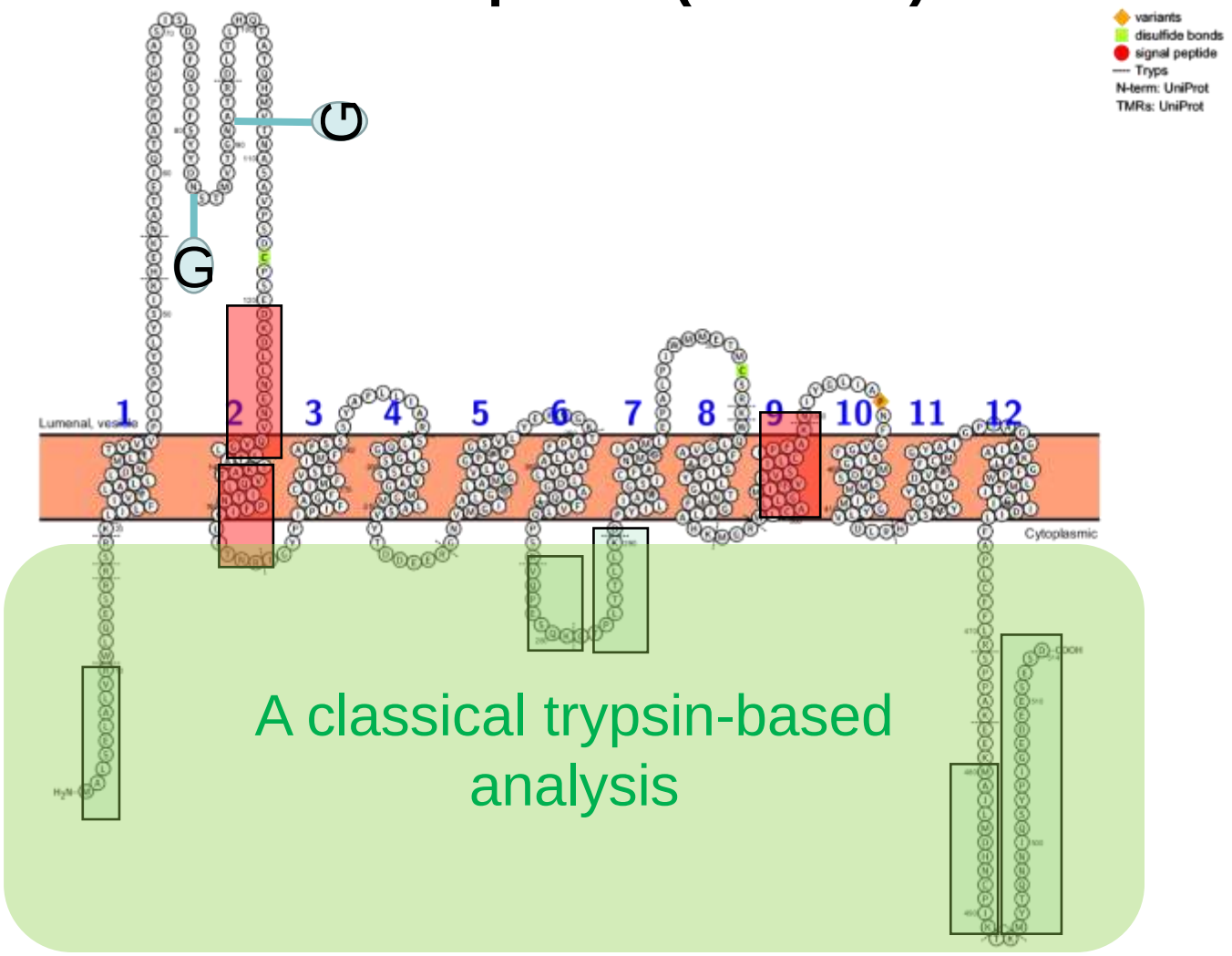


„Divide and conquer“ methods

Standard strategy
(targets intact proteins)

Only the hydrophilic segments

Membrane fraction

↓

Solubilization

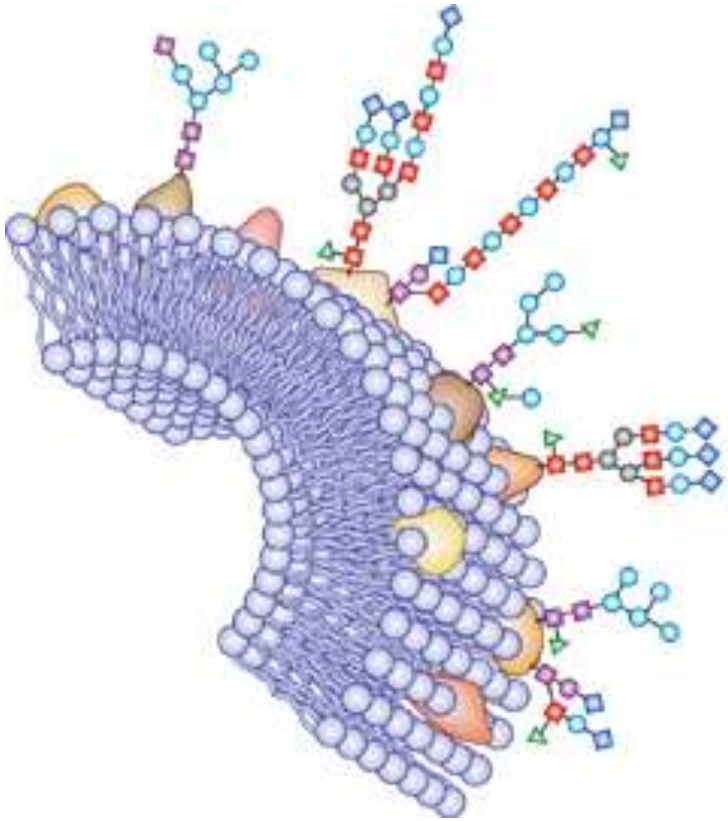
↓

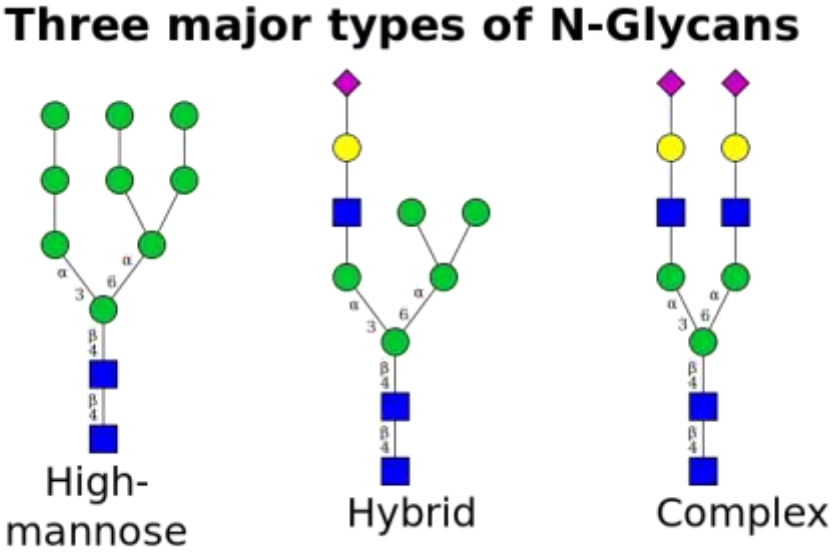
Tryptic digestion

↓

LC-MS/MS

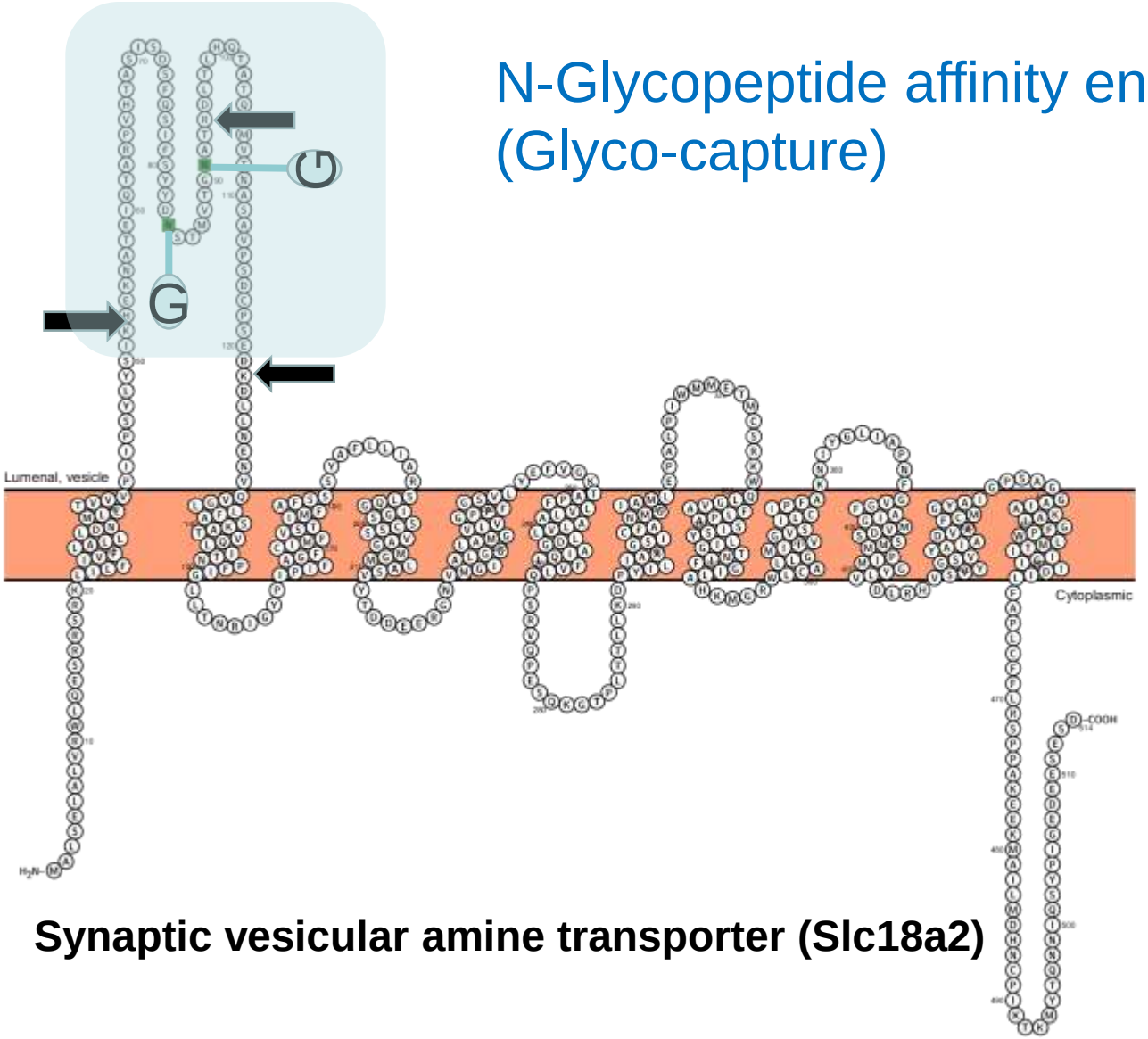
LOW ENRICHMENT
(10-15%)





Asn-Xaa-Ser/Thr

“DIVIDE AND CONQUER“ METHODS



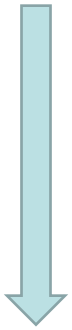
N-Glycopeptide affinity enrichment (Glyco-capture)

Synaptic vesicular amine transporter (Slc18a2)

N-GLYCOCAPTURE

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

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



Peptides released by PNGase F

N-Glyco-FASP

Zielinska et al. Cell, 2010

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

(immobilization on beads via hydrazone linkage)

Peptides released by PNGase F

SPEG

Zhang et al.
Nature Biotechnology, 2003

Classic strategy

Membrane fraction

↓

Solubilization

↓

Tryptic digestion

↓

LC-MS/MS

Only the hydrophilic segments (GLYCOCAPTURE)

Solubilization

↓

Digestion

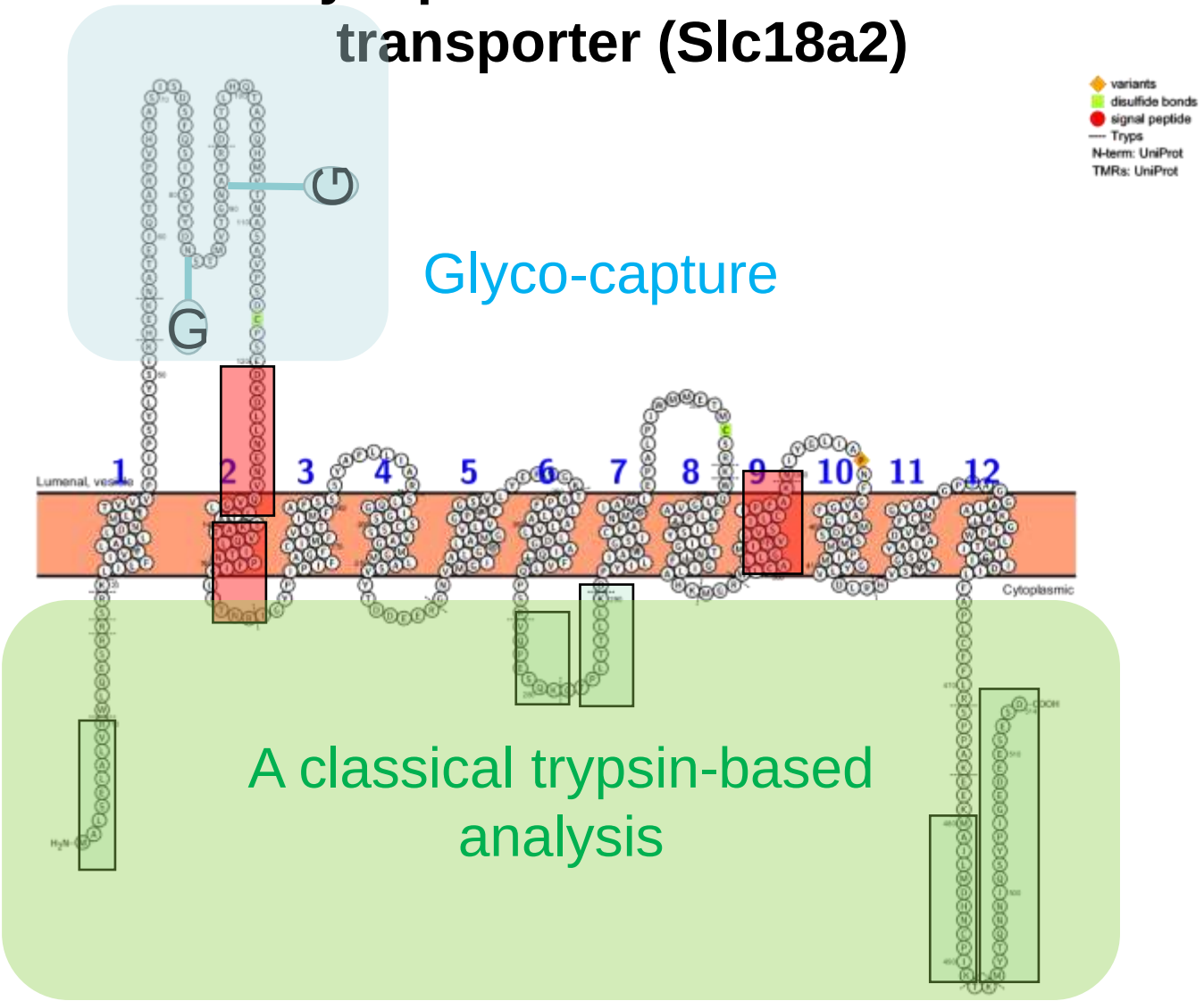
↓

Isolation of N-glycopeptides

↓

LC-MS/MS

Synaptic vesicular amine transporter (Slc18a2)



Only the hydrophobic segments

?

Classic strategy

Membrane fraction

↓

Solubilization

↓

Tryptic digestion

↓

LC-MS/MS

Only the hydrophilic segments (GLYCOCAPTURE)

Solubilization

↓

Digestion

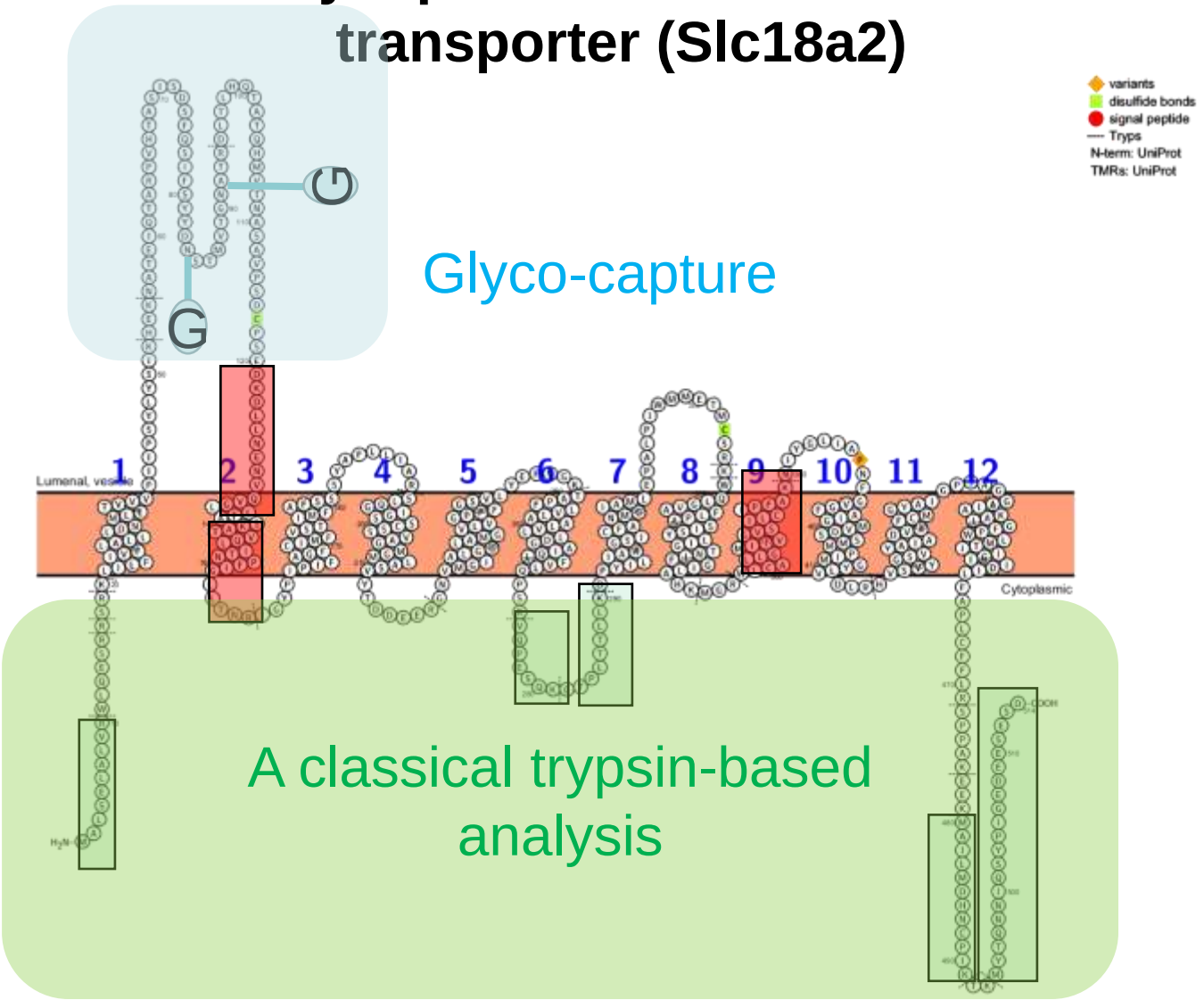
↓

Isolation of N-glycopeptides

↓

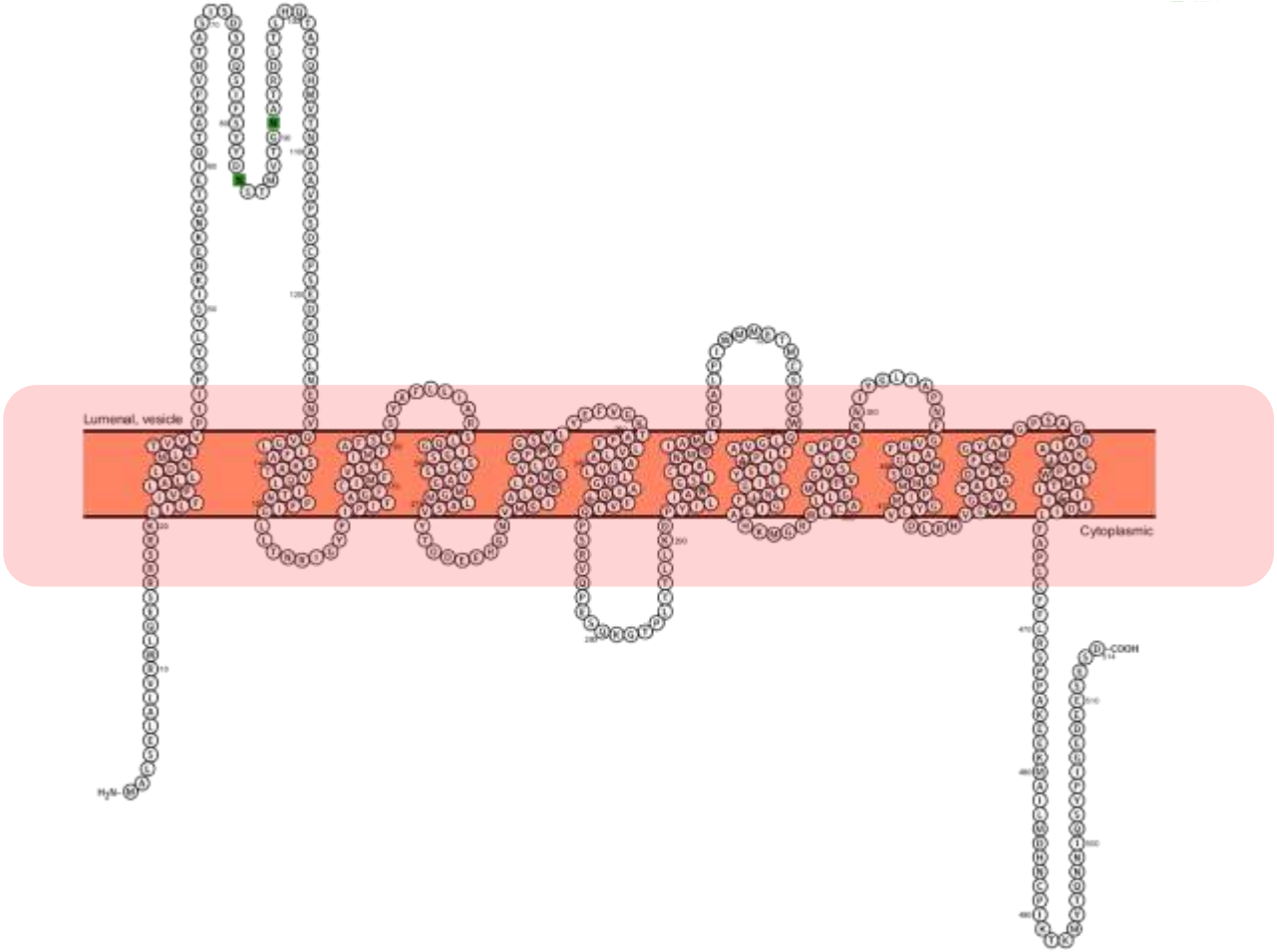
LC-MS/MS

Synaptic vesicular amine transporter (Slc18a2)



Identification of IMPs via enrichment of membrane-embedded segments using **hpTC method.**

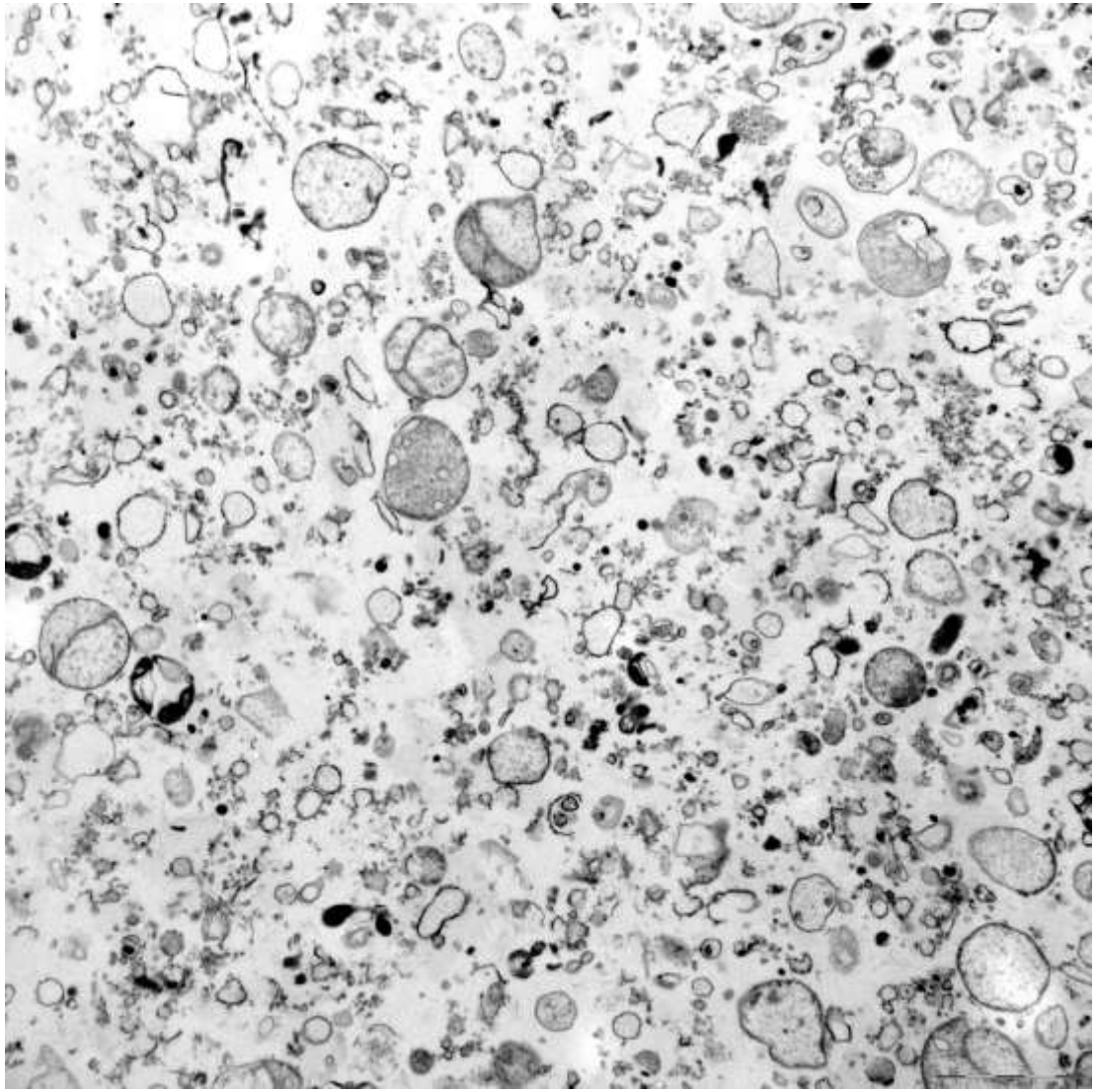
(High **p**H-**T**rypsin-**C**NBr)



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)

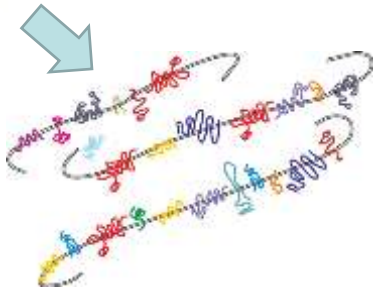
Membrane-enriched fraction



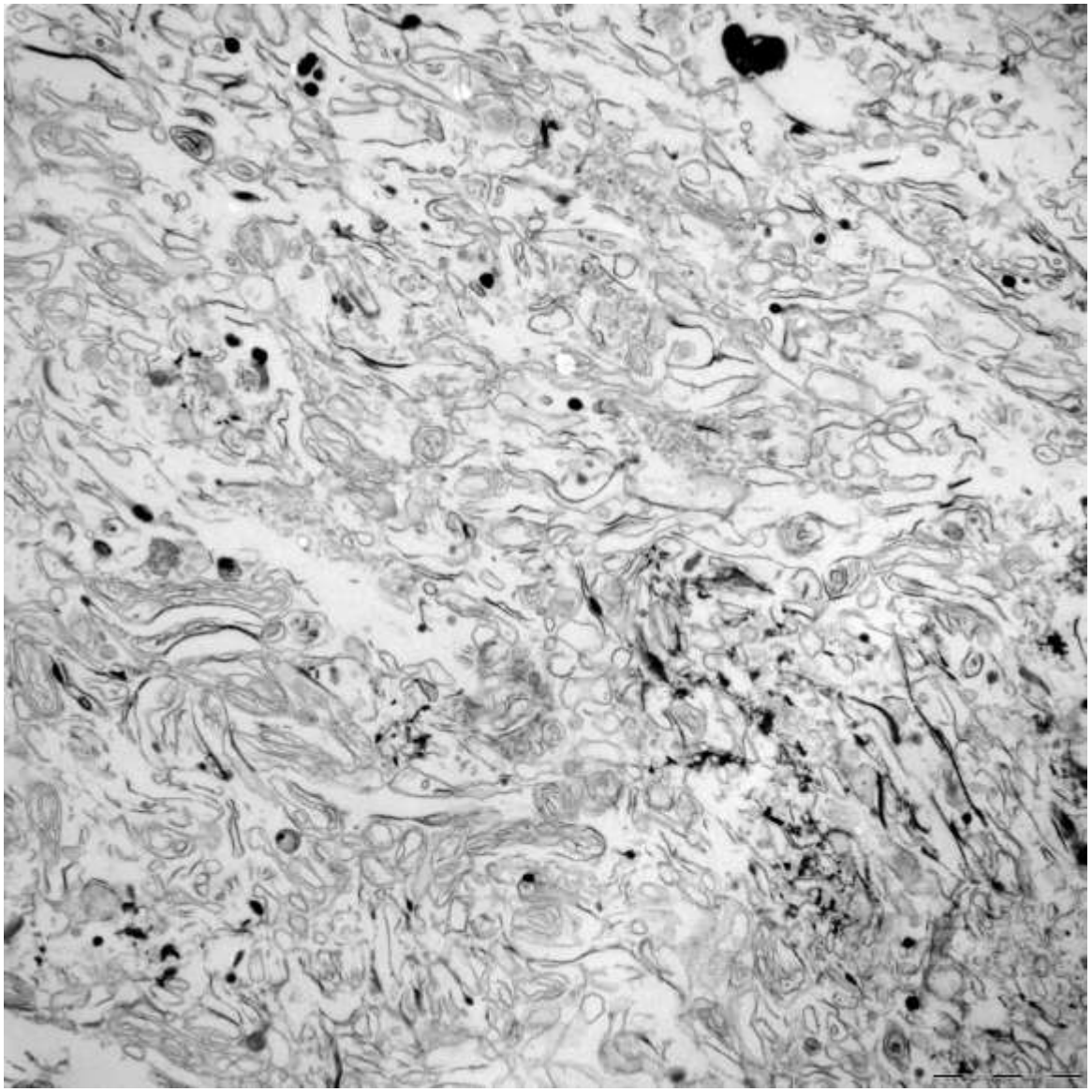
pH 7.4

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

Membrane-enriched fraction

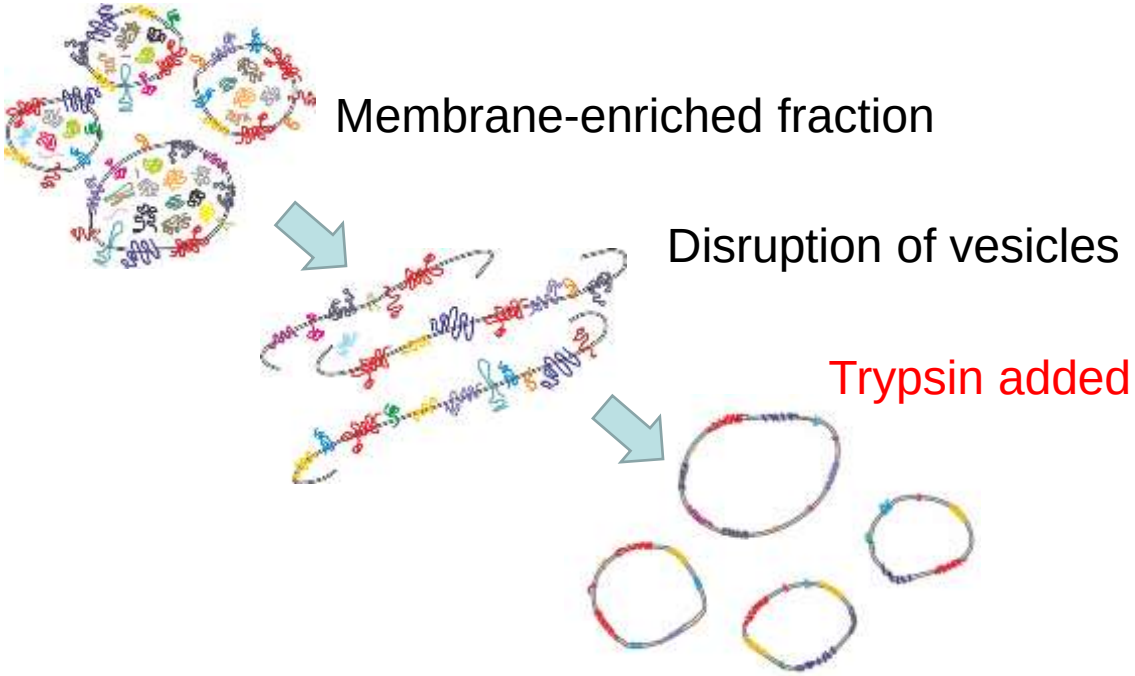


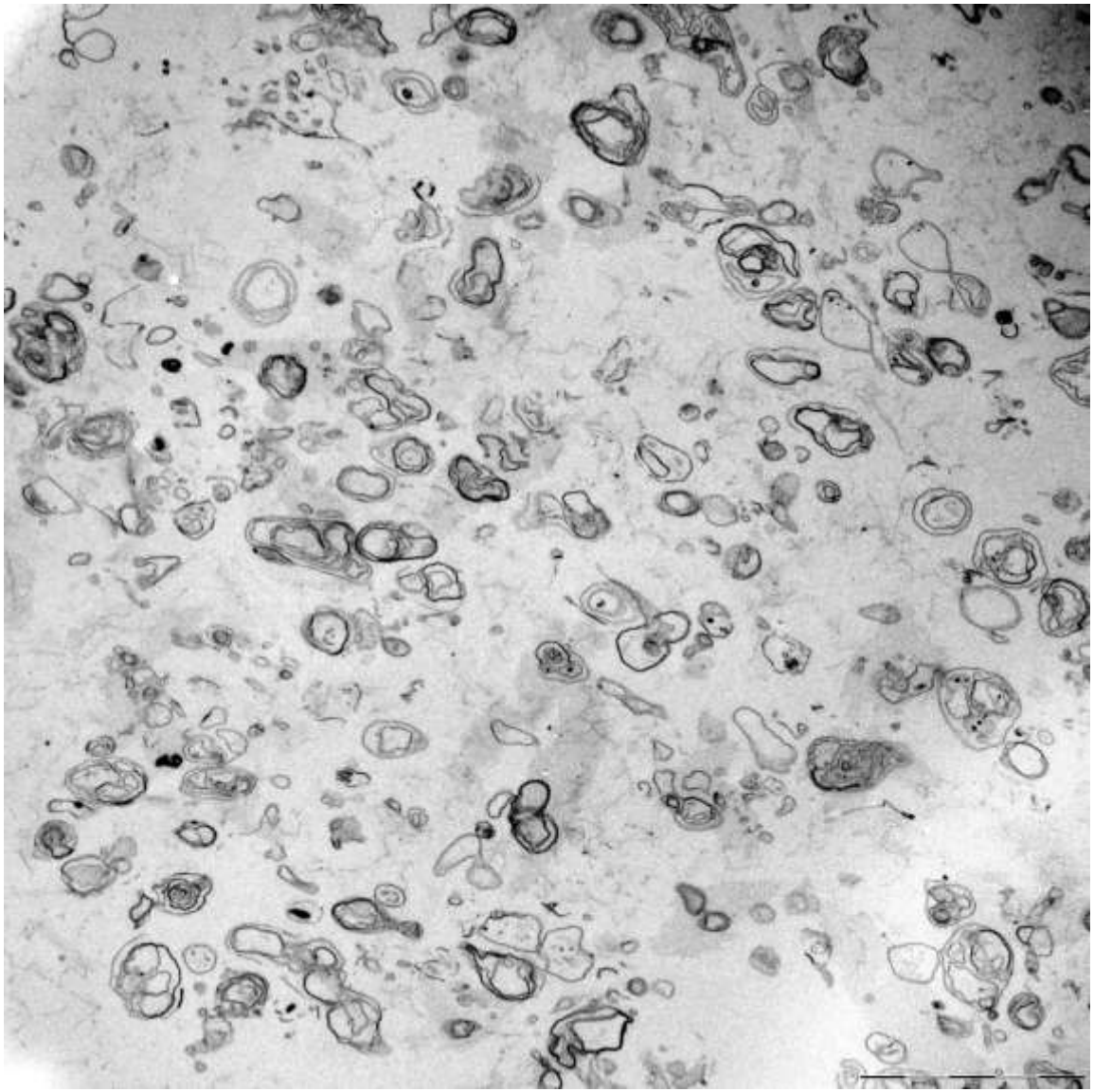
Disruption of vesicles



On ice
Na_2CO_3
pH 11

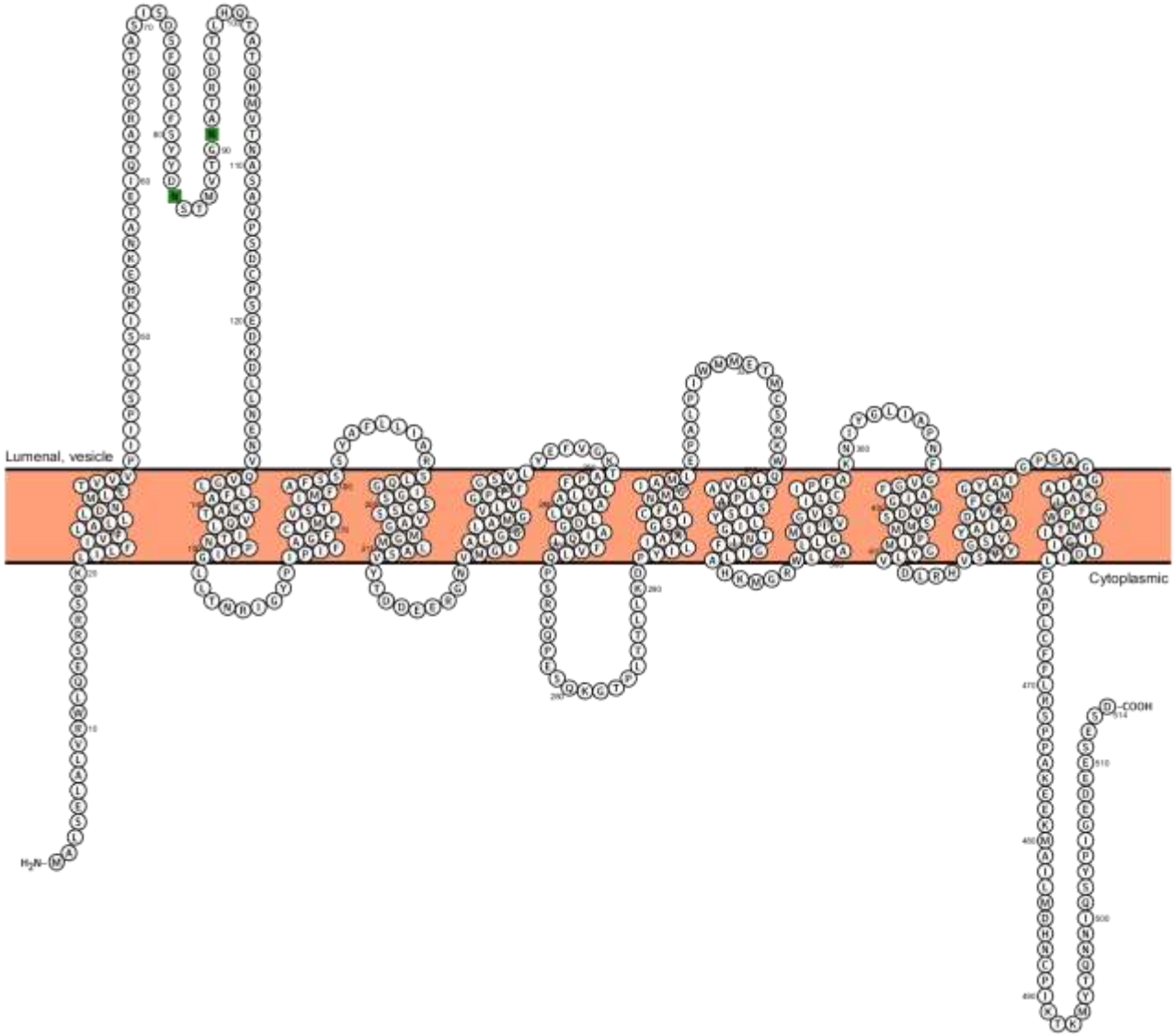
hpTC method (high **p**H-**T**rypsin-**C**NBr)





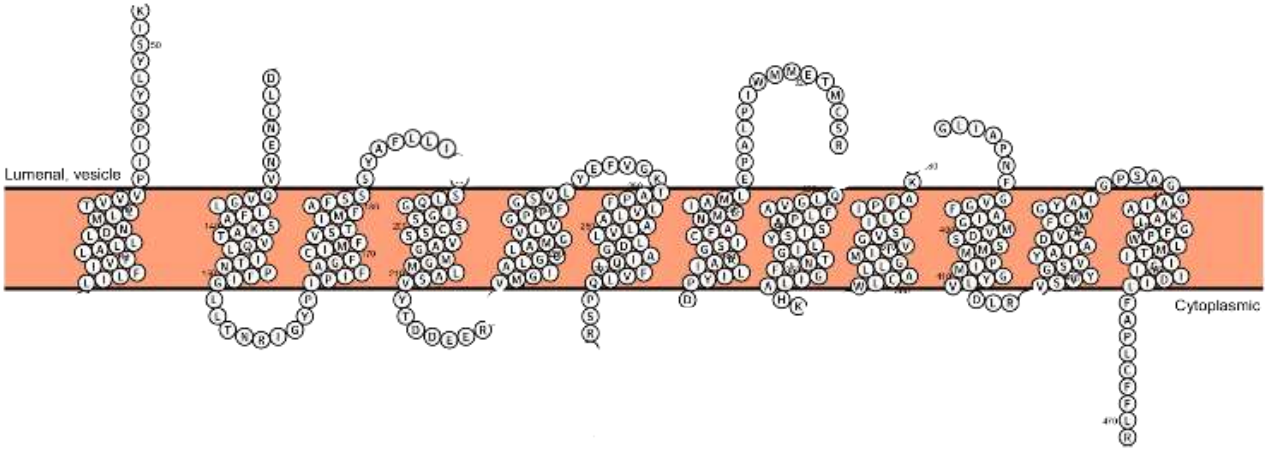
37 °C
trypsin

Synaptic vesicular amine transporter (Slc18a2)

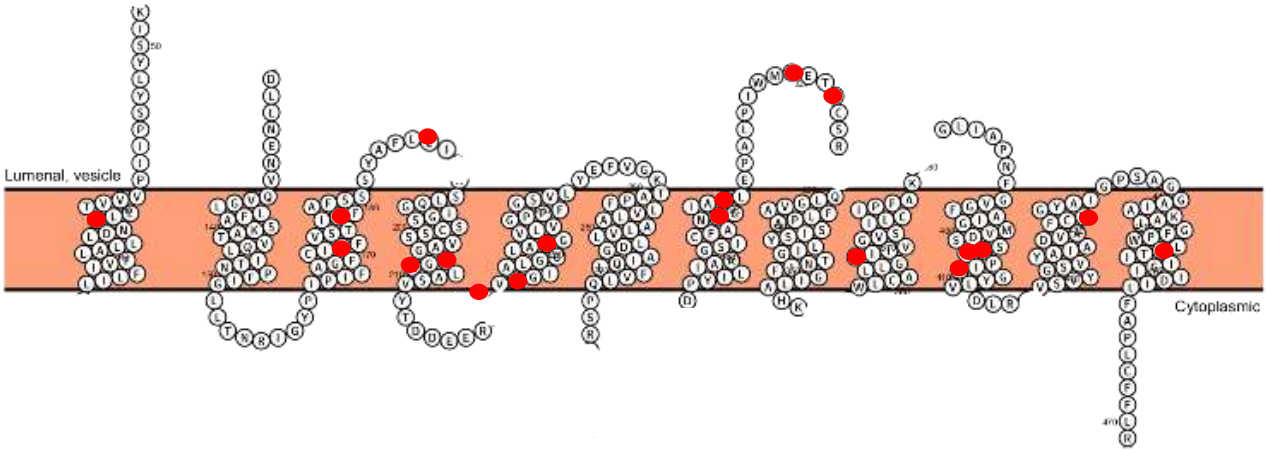


Visualization by Protter (*Omasits et al., Bioinformatics. 2013*)

Synaptic vesicular amine transporter (Slc18a2)

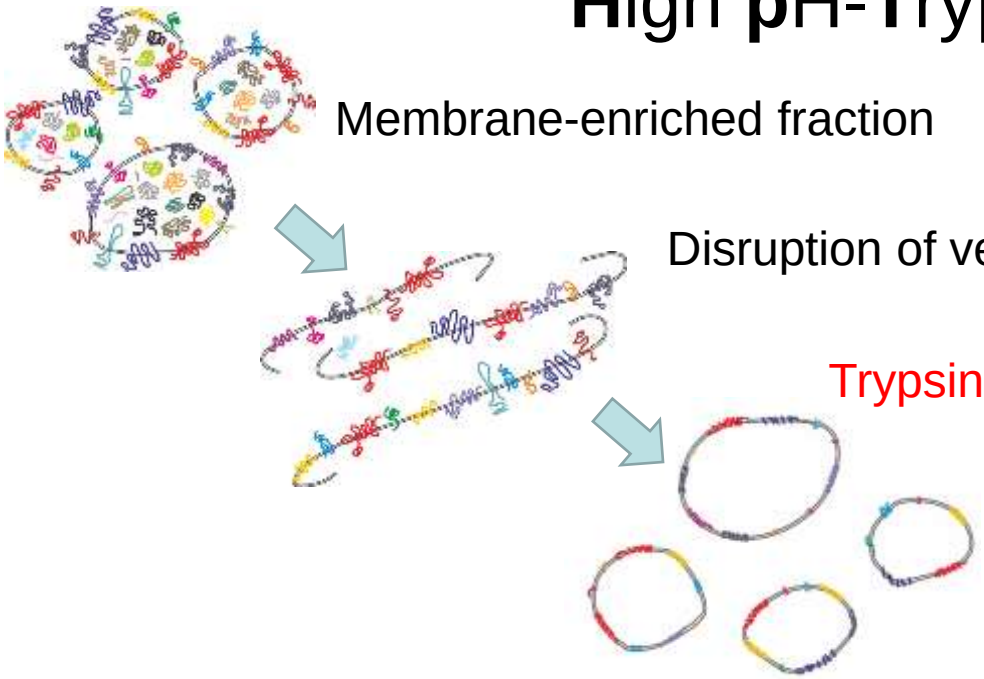


Synaptic vesicular amine transporter (Slc18a2)



Solubilization in organic acid
followed by chemical cleavage of peptides by **CNBr** at **Met**

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



Membrane-enriched fraction

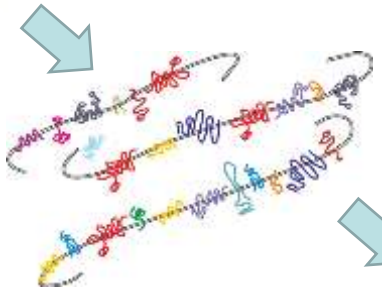
Disruption of vesicles

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

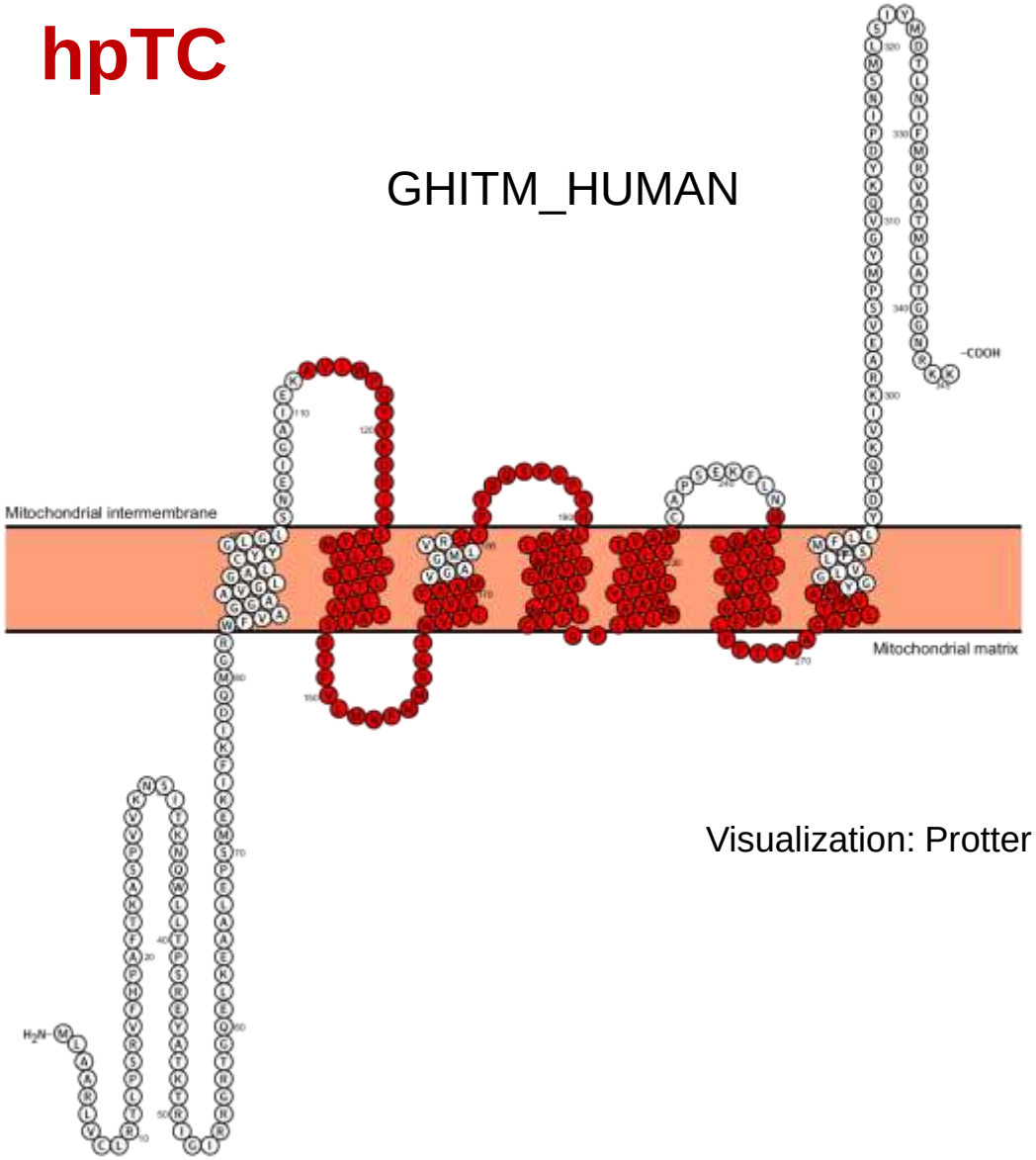
hpTC

Only the hydrophobic segments
(hpTC)

600-900 IMPs
in various tissues

IMP Enrichment
40-60%

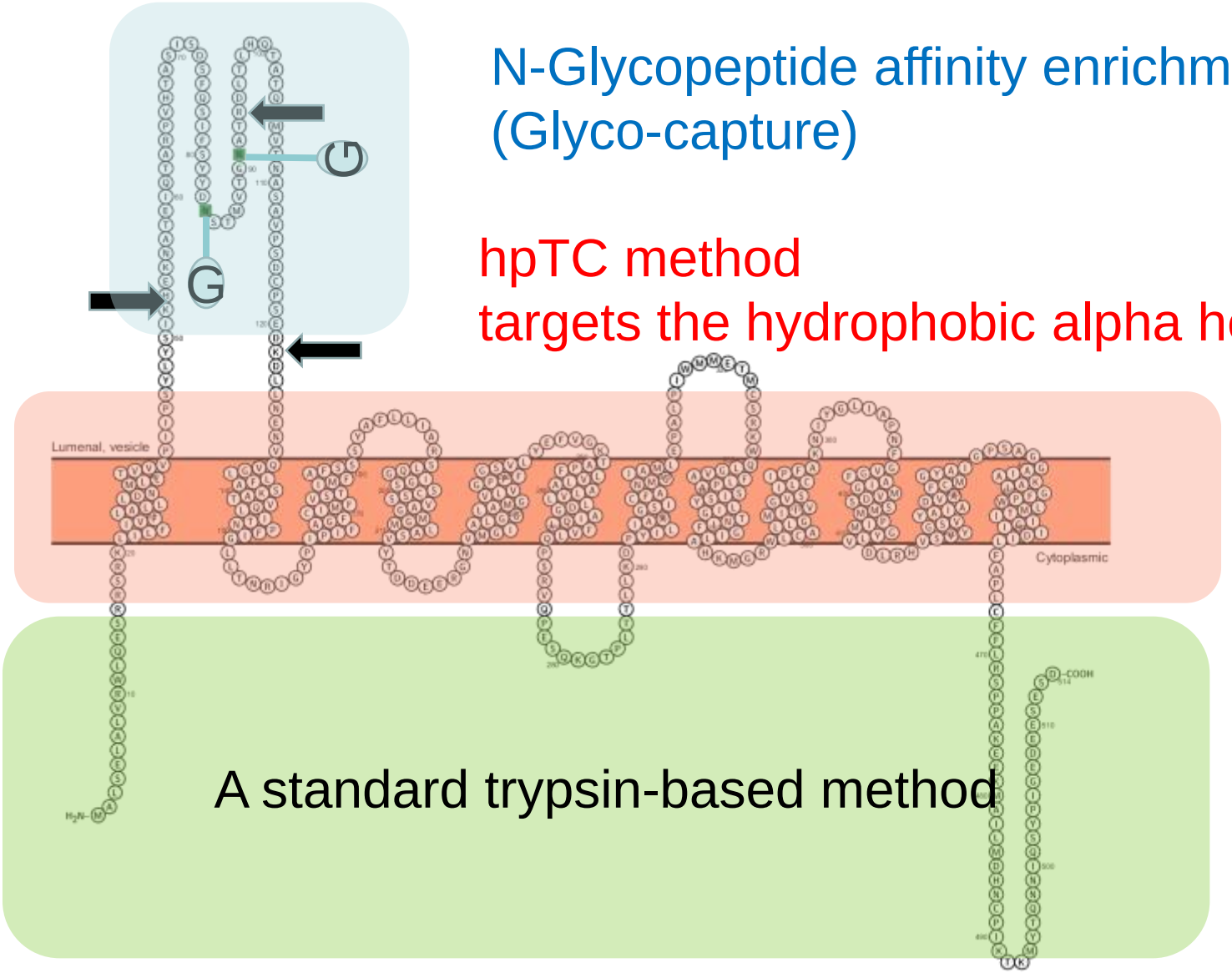
GHITM_HUMAN



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

↓

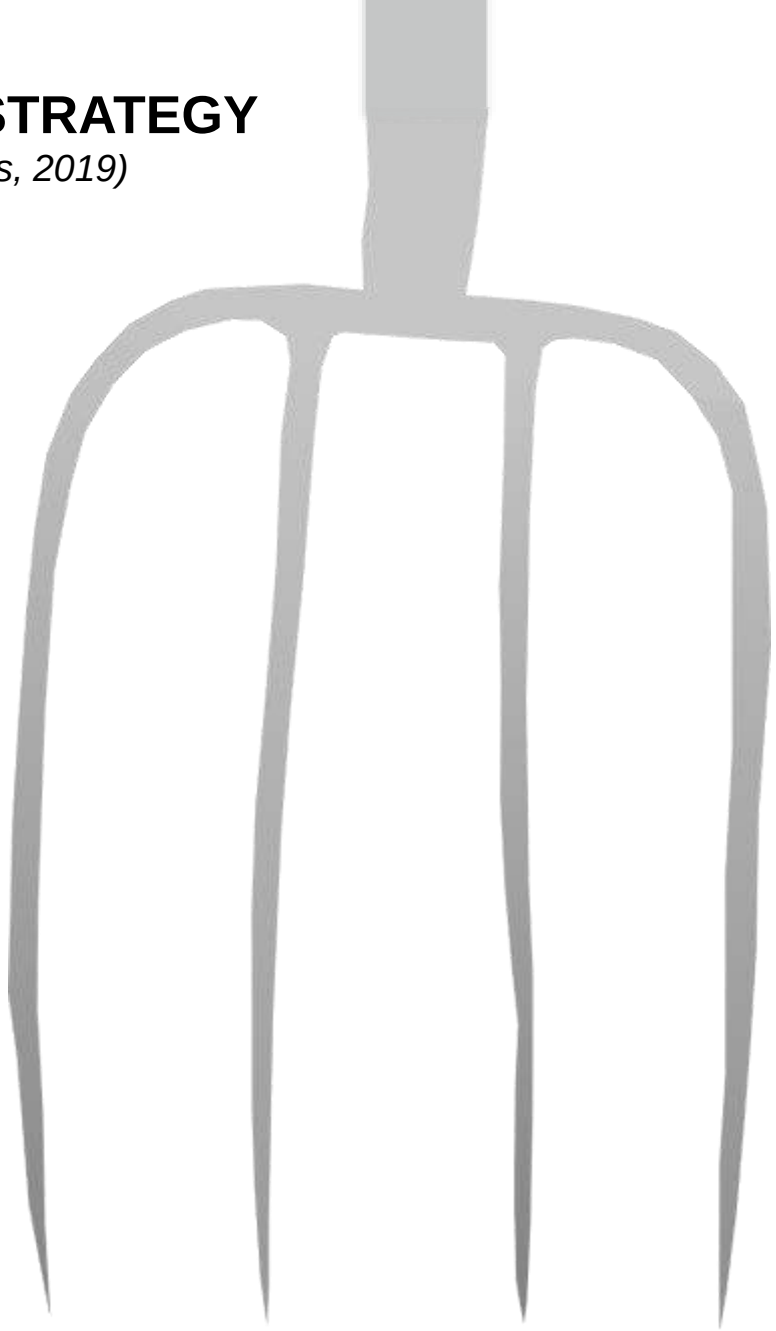
Isolation of N-glycopeptides

↓

LC-MS/MS

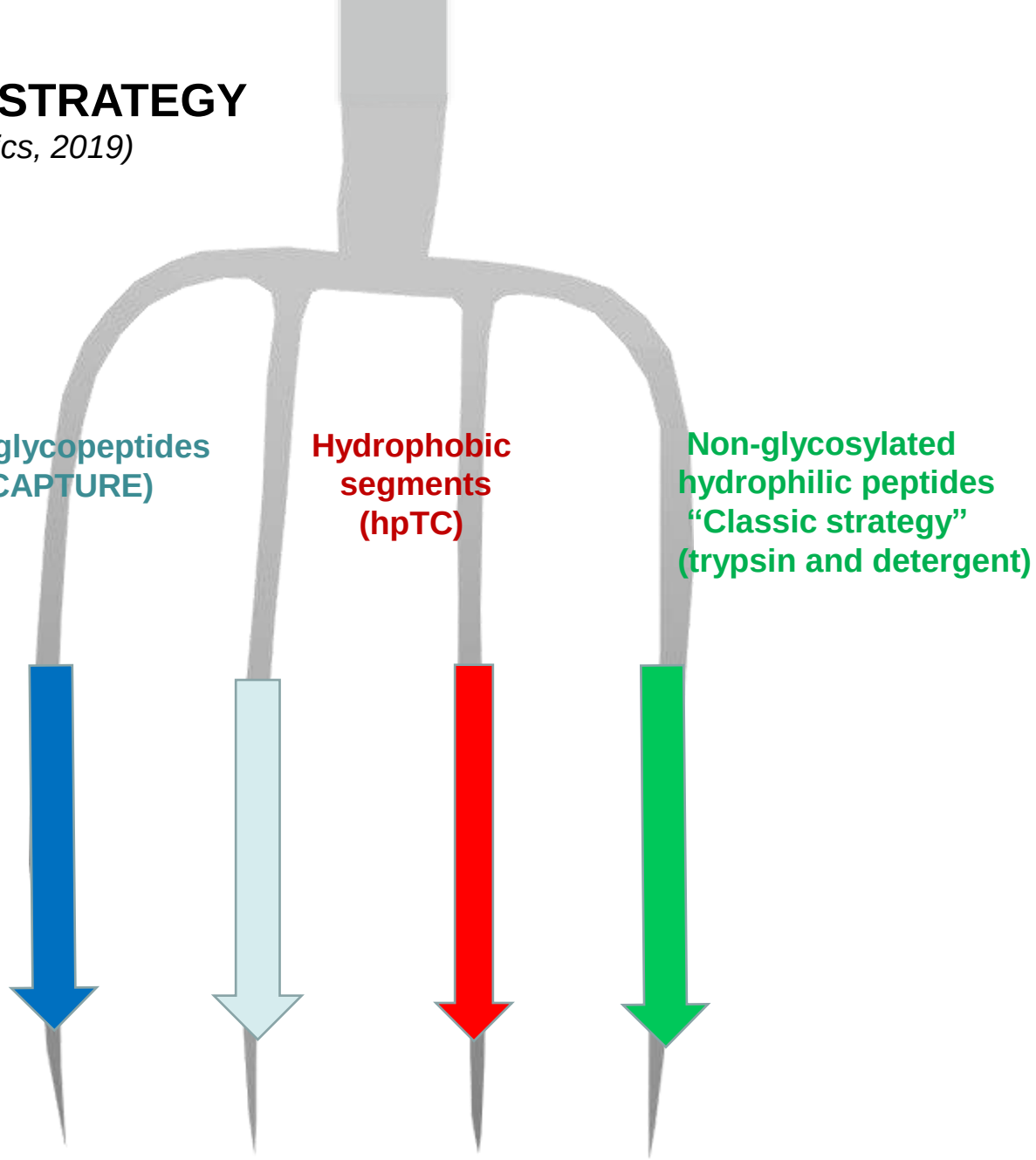
THE PITCHFORK STRATEGY

(*Vit et al, J. Proteomics, 2019)*



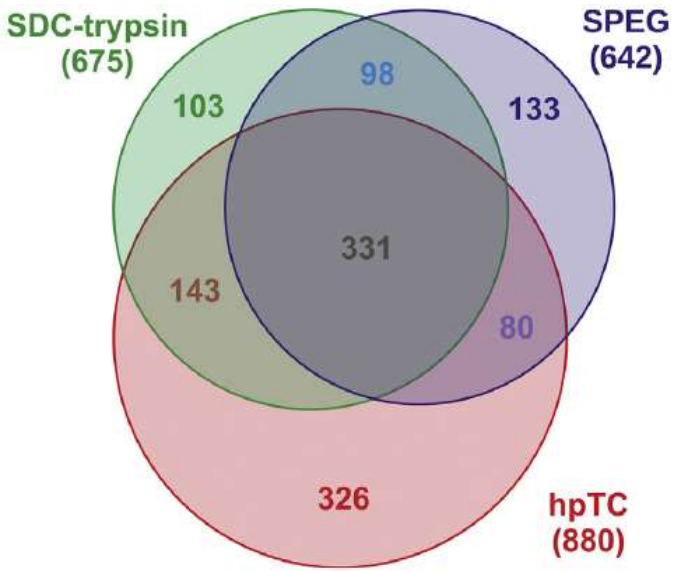
THE PITCHFORK STRATEGY

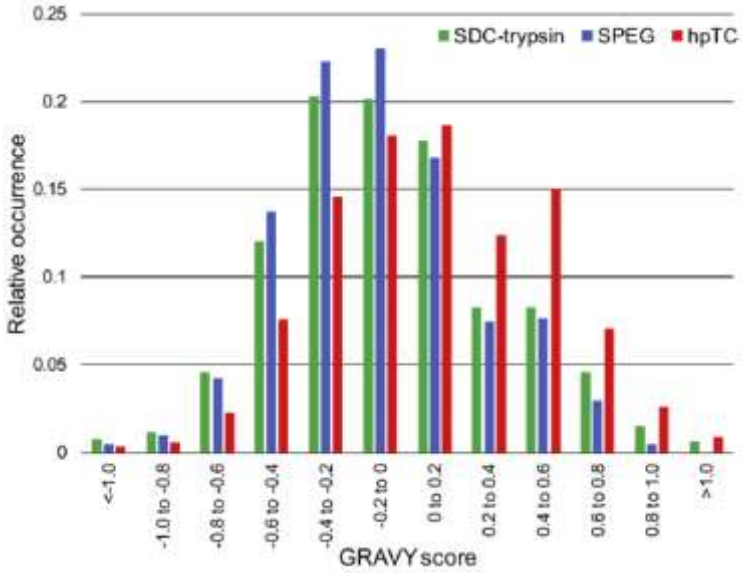
(*Vit et al, J. Proteomics, 2019)*

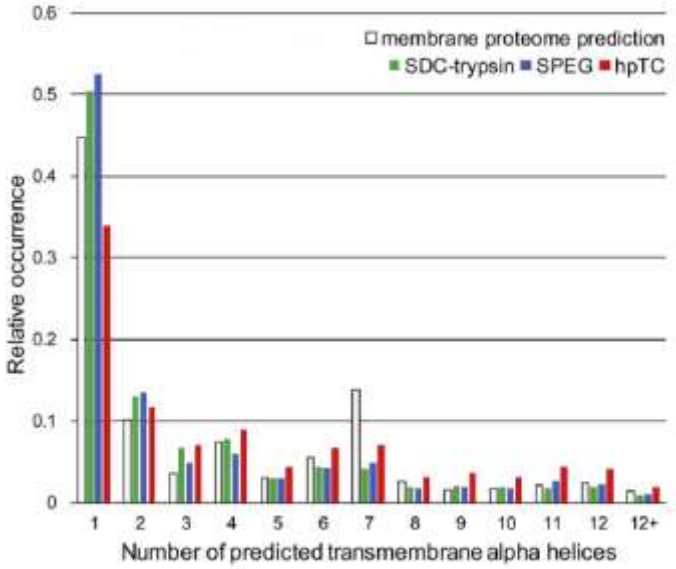


LC-MS/MS

The Pitchfork strategy

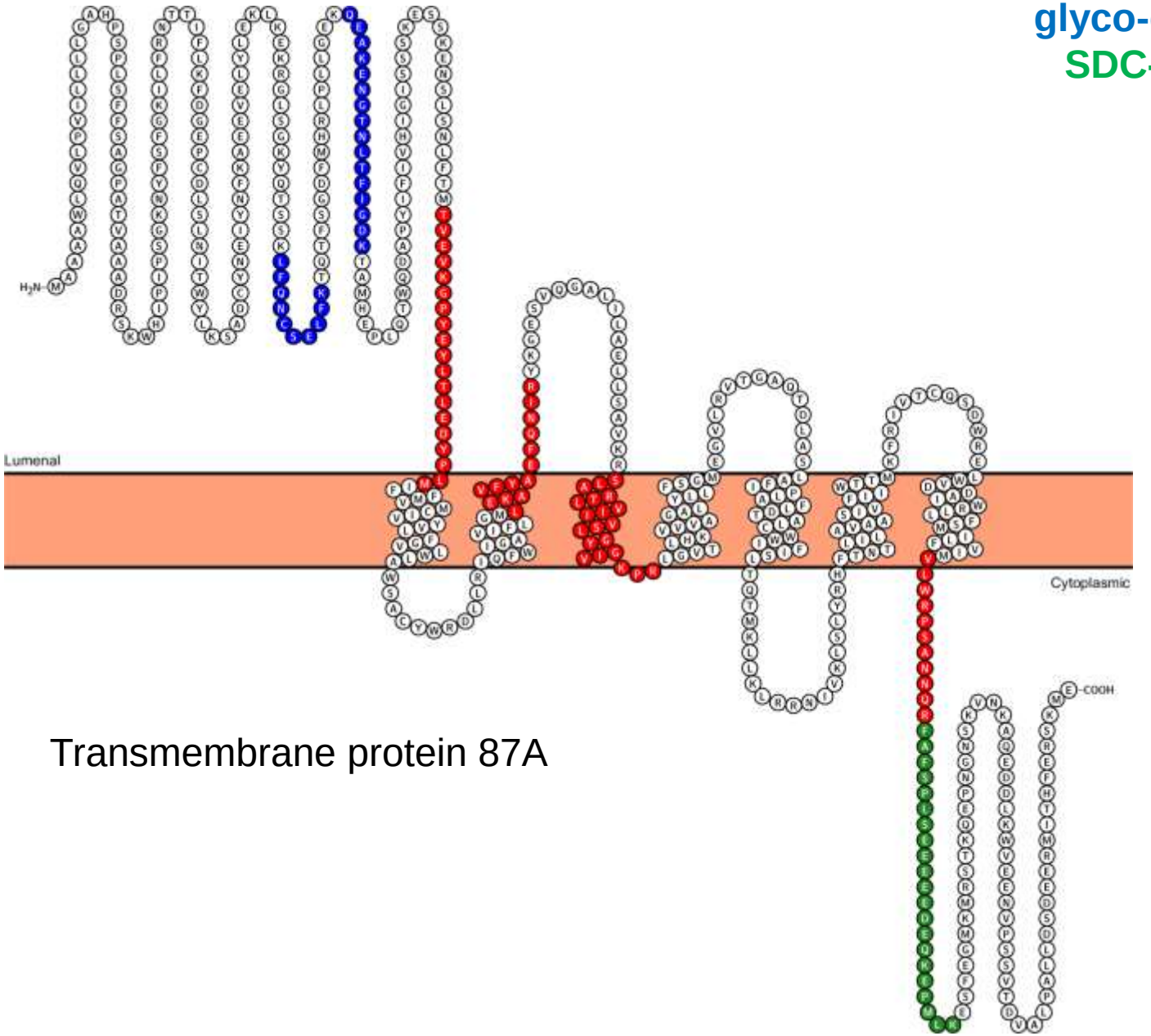






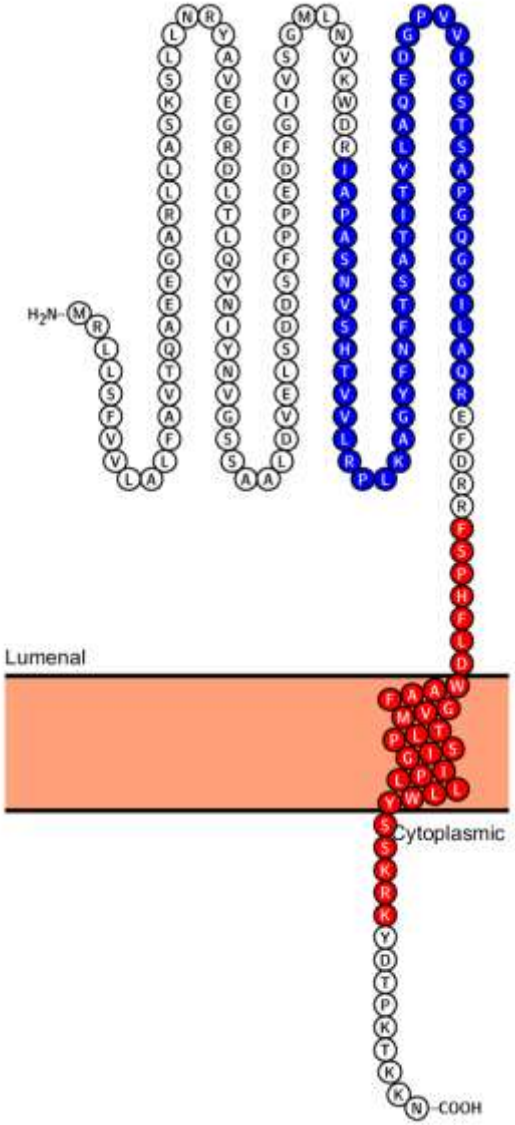
Vit et al, J. Proteomics, 2019

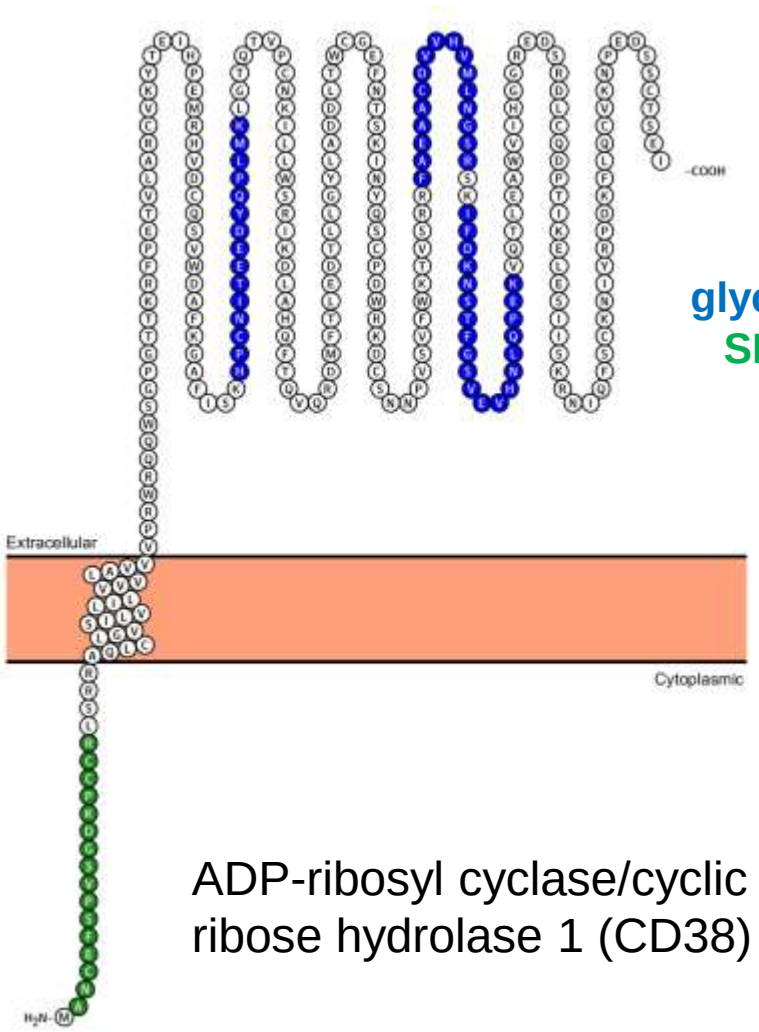
hpTC
glyco-capture
SDC-trypsin



Transmembrane protein 87A

Translocon-associated protein subunit beta

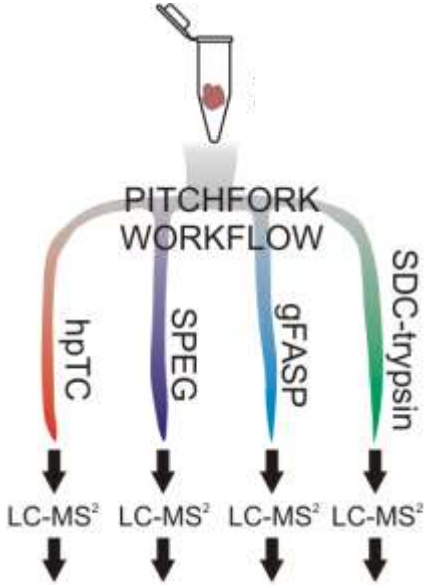




hpTC
glyco-capture
SDC-trypsin

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

The Pitchfork strategy



- 800-1700 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
- Time consuming, high amount of starting material needed

Vit et al, J. Proteomics, 2019

Looking for new theranostic targets in human Pheochromocytoma and Paraganglioma

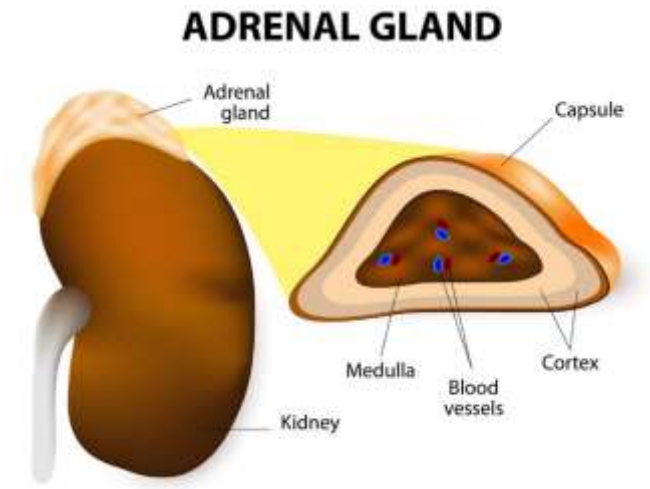
BIOCEV

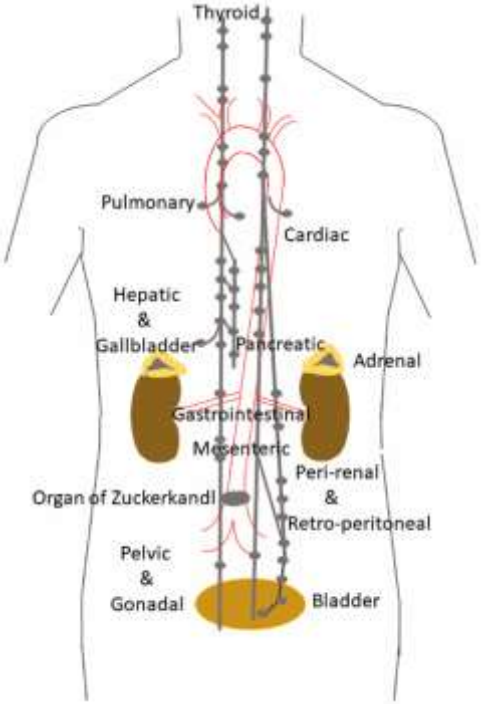
Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University in Vestec

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

- Rare **neuro-endocrine tumors**
- From **chromaffin tissue of adrenal medulla (PHEO) or sypathetic ganglia (PGL)**
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- 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**

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

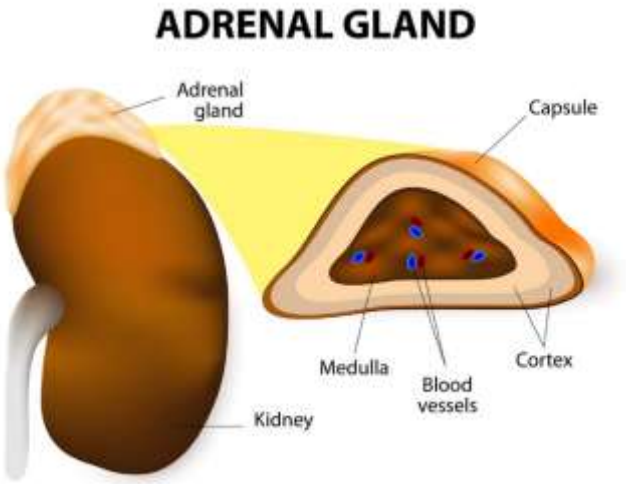
Patient No.	Sex	Tumor Type	PPGL Cluster	Mutated Gene
1	F	PGL	1	*SDHB*
2	F	PGL	1	*SDHB*
3	M	PGL	1	*SDHB*
4	M	PGL	1	*SDHB*
5	M	PGL	1	*SDHB*
6	F	PGL	1	*SDHB*
7	M	PGL	1	*SDHB*
8	F	PGL	1	*SDHB*
9	F	PGL	1	*SDHB*
10	M	PHEO	1	*VHL*
11	F	PHEO	1	*VHL*
12	F	PHEO	1	*VHL*
13	M	PGL	1	*VHL*
14	M	PGL	1	*VHL*
15	F	PHEO	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

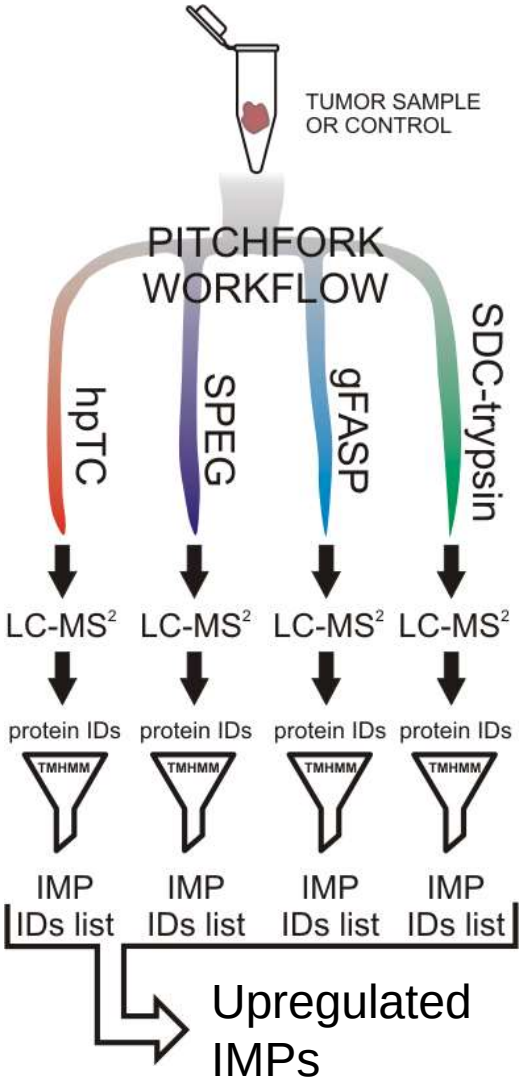
Cluster 2

No mutation

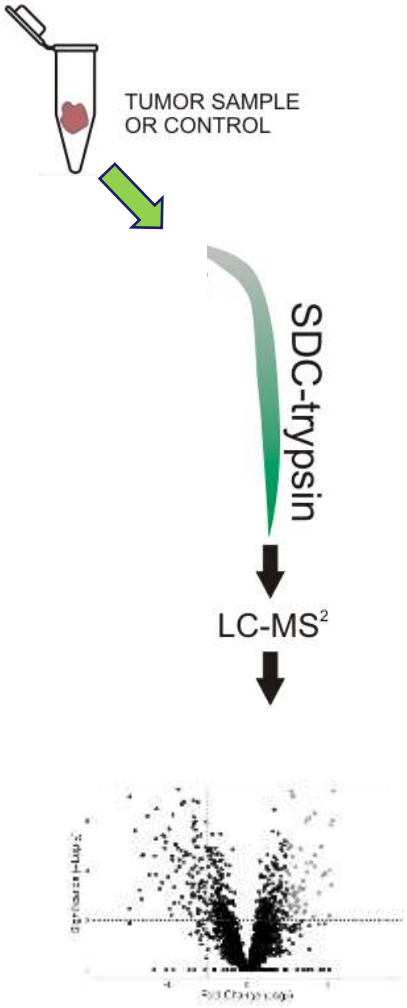
ADRENAL MEDULLA – THE CONTROL CHROMAFFIN TISSUE



PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



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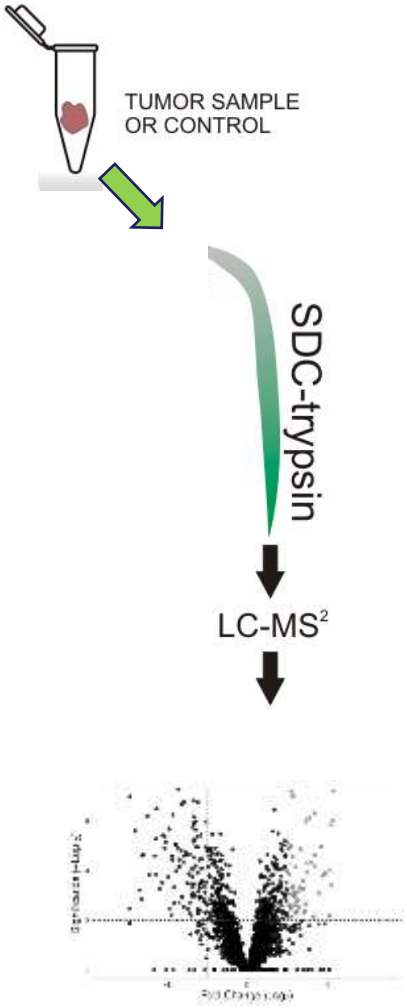
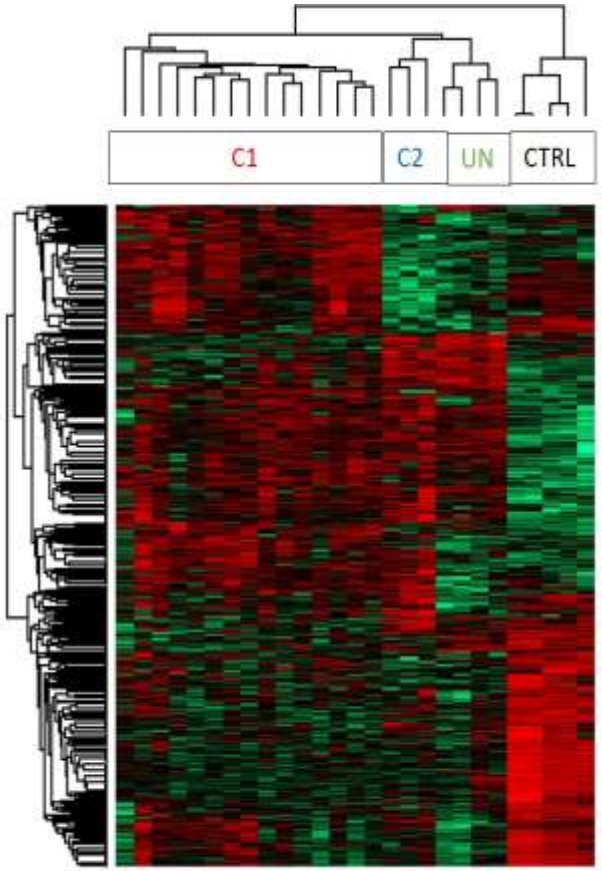
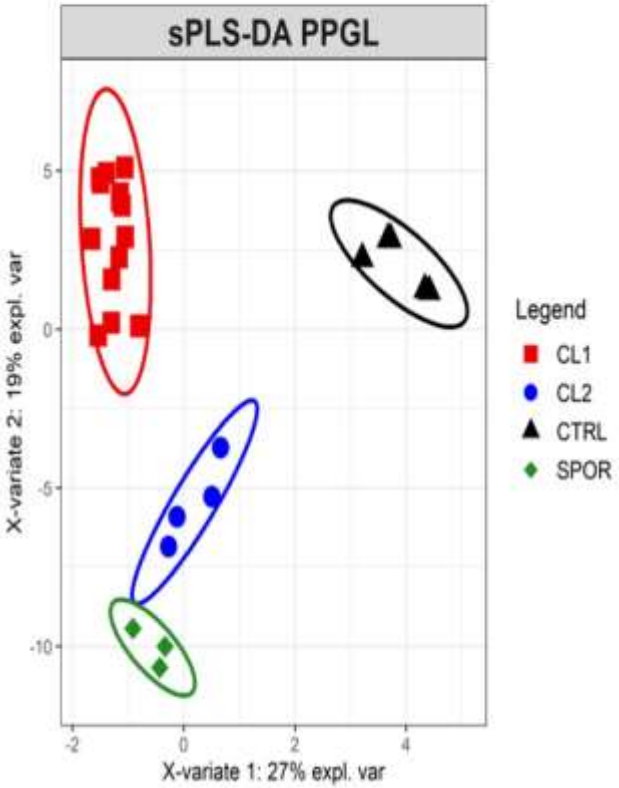
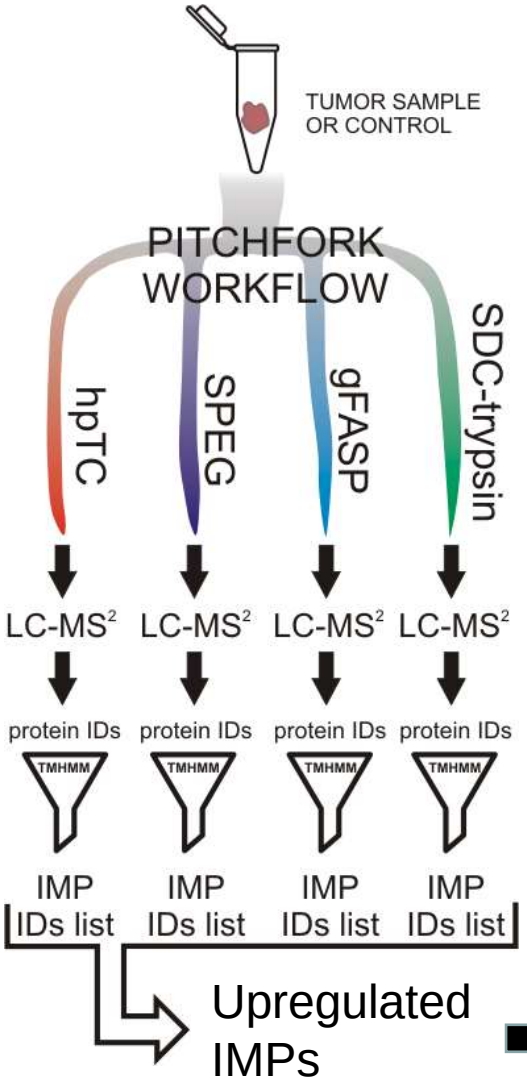




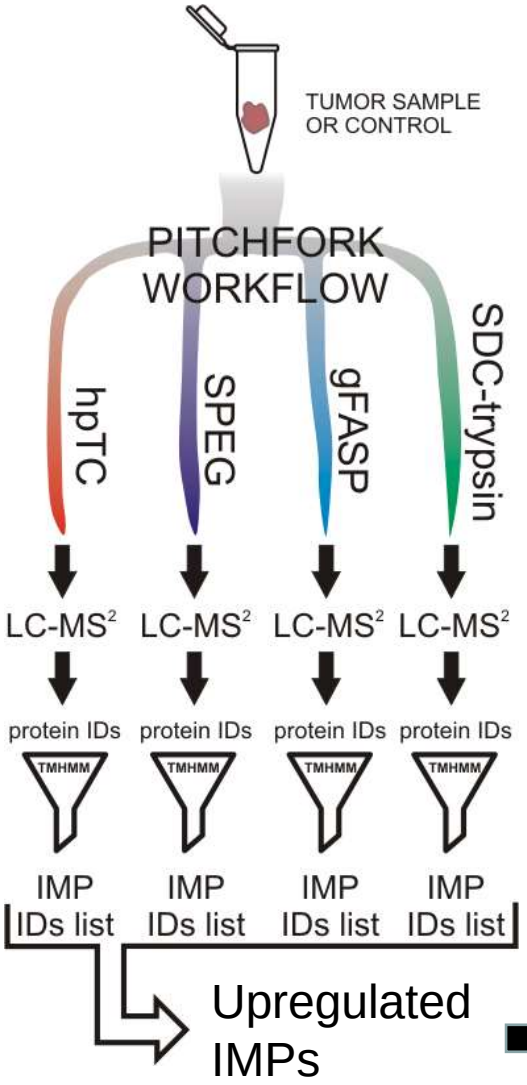


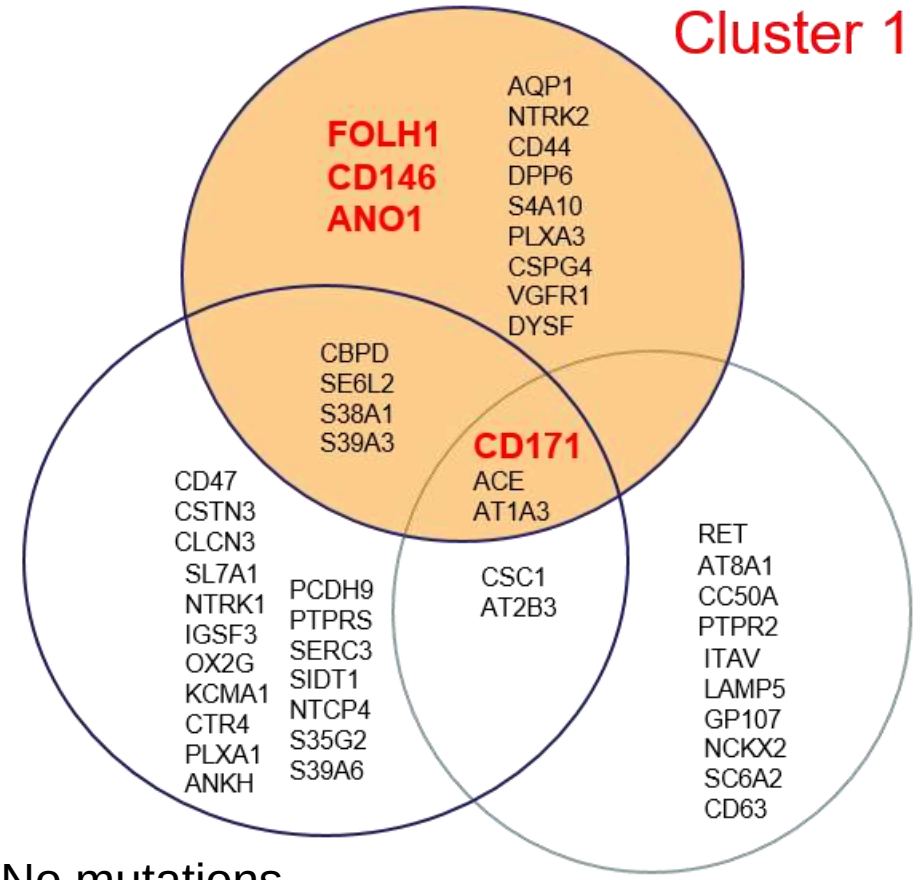
PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



➡ **Filtering for cell surface localization**

PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME

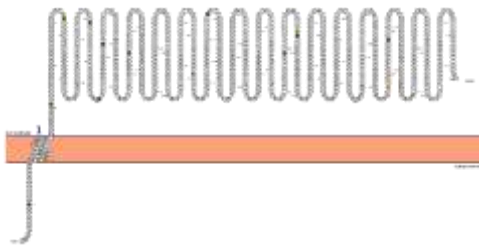




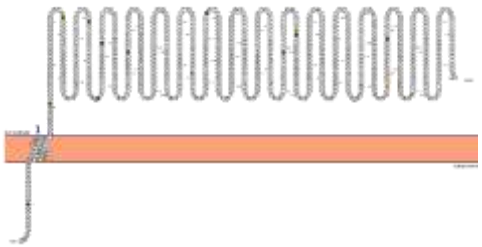
➡ **Filtering for cell surface localization**

Vít O. et al. Clinical Proteomics, 2023

GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1)

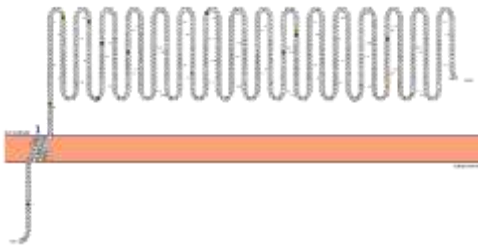


GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1)
PROSTATE-SPECIFIC MEMBRANE ANTIGEN (PSMA)



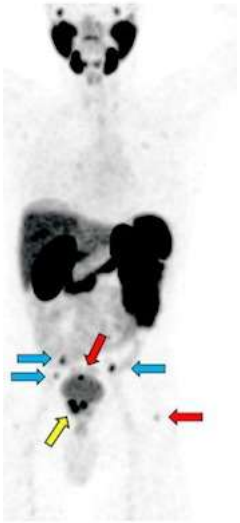
OVEREXPRESSED IN PROSTATE CANCER CELLS

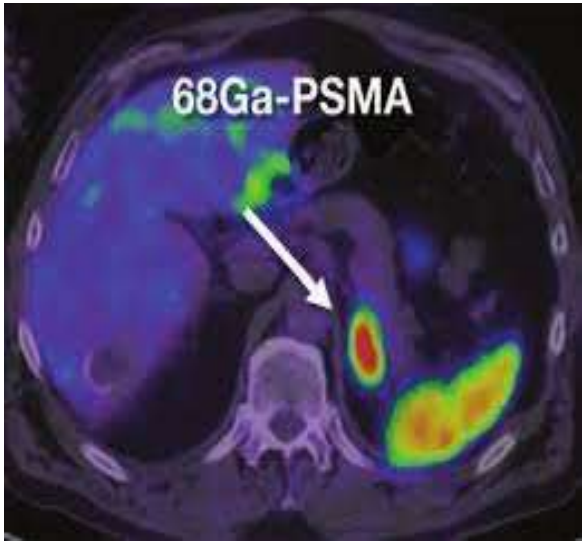
GLUTAMATE CARBOXYPEPTIDASE 2 (FOLH1)
PROSTATE-SPECIFIC MEMBRANE ANTIGEN (PSMA)

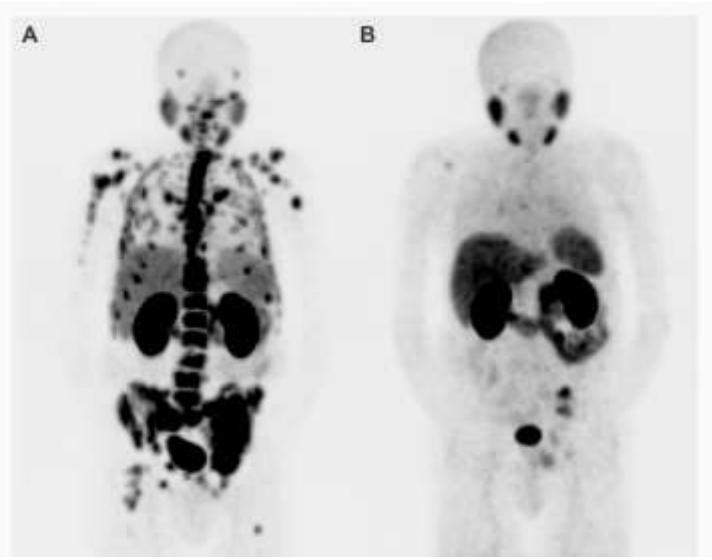


OVEREXPRESSED ON PROSTATE CANCER CELLS

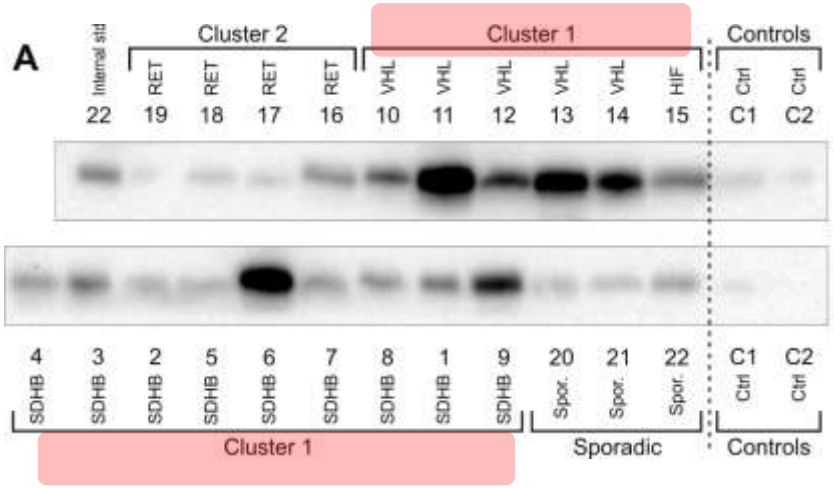
Anti-PSMA radio-conjugates approved for PC tumor imaging and therapy of advanced PC

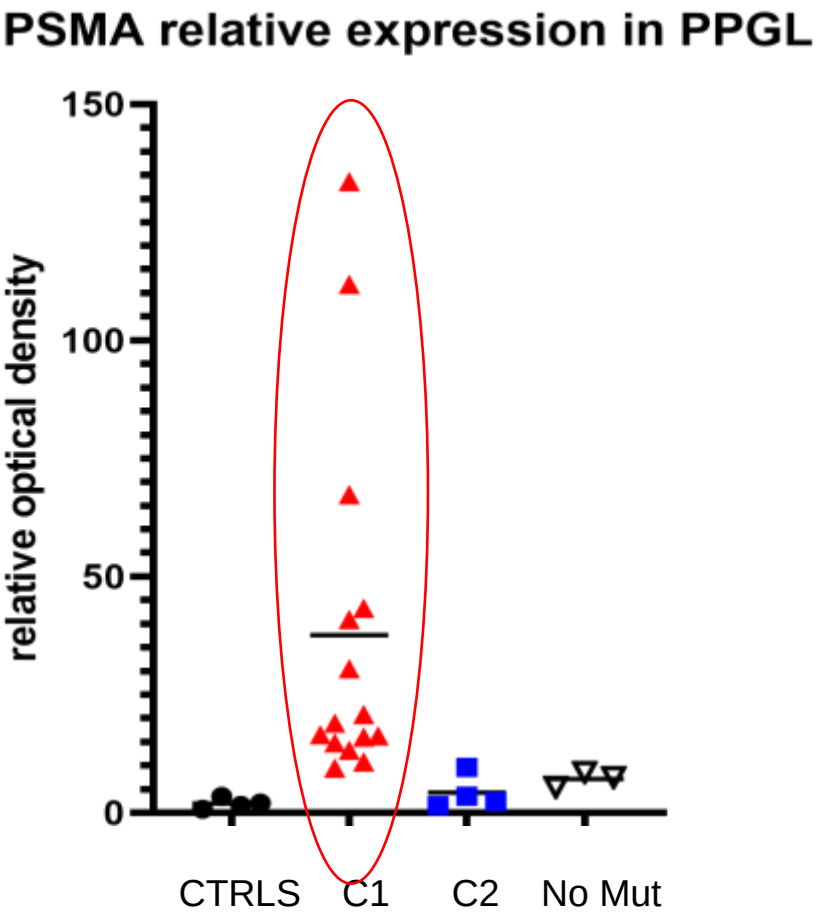






PSMA EXPRESSION IN HUMAN PPGL





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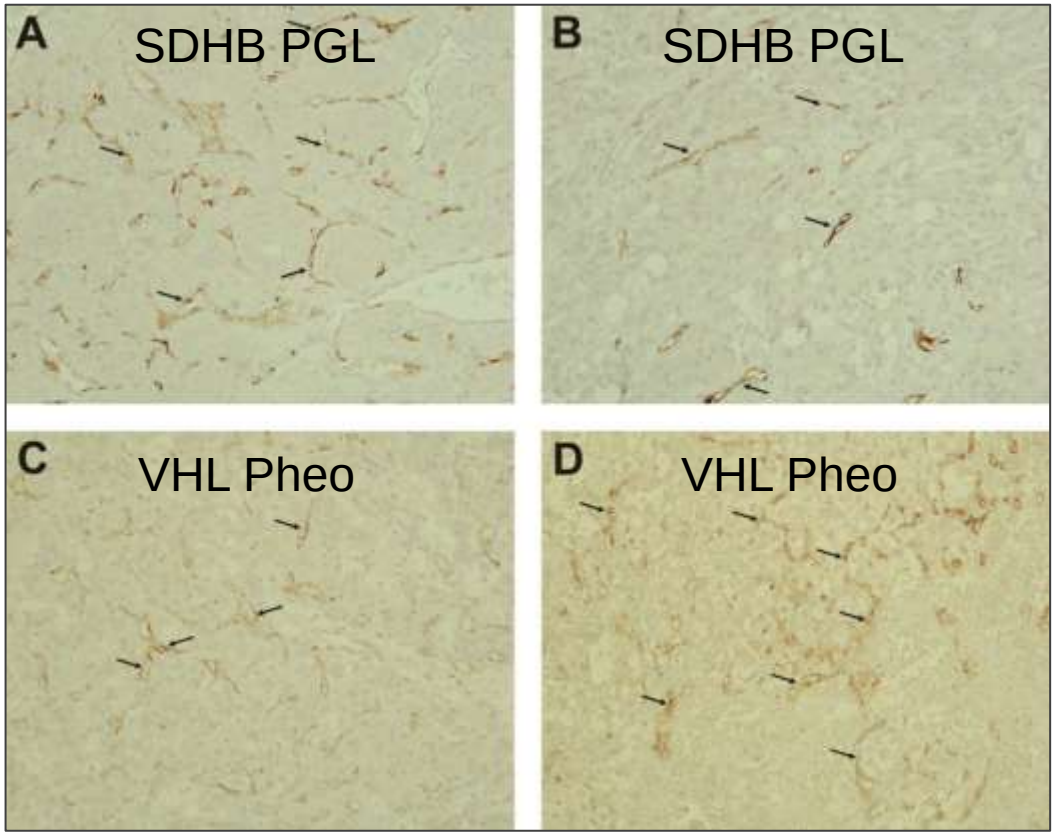
PSMA EXPRESSION IN TUMOR VASCULATURE IN CLUSTER 1 PPGL



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

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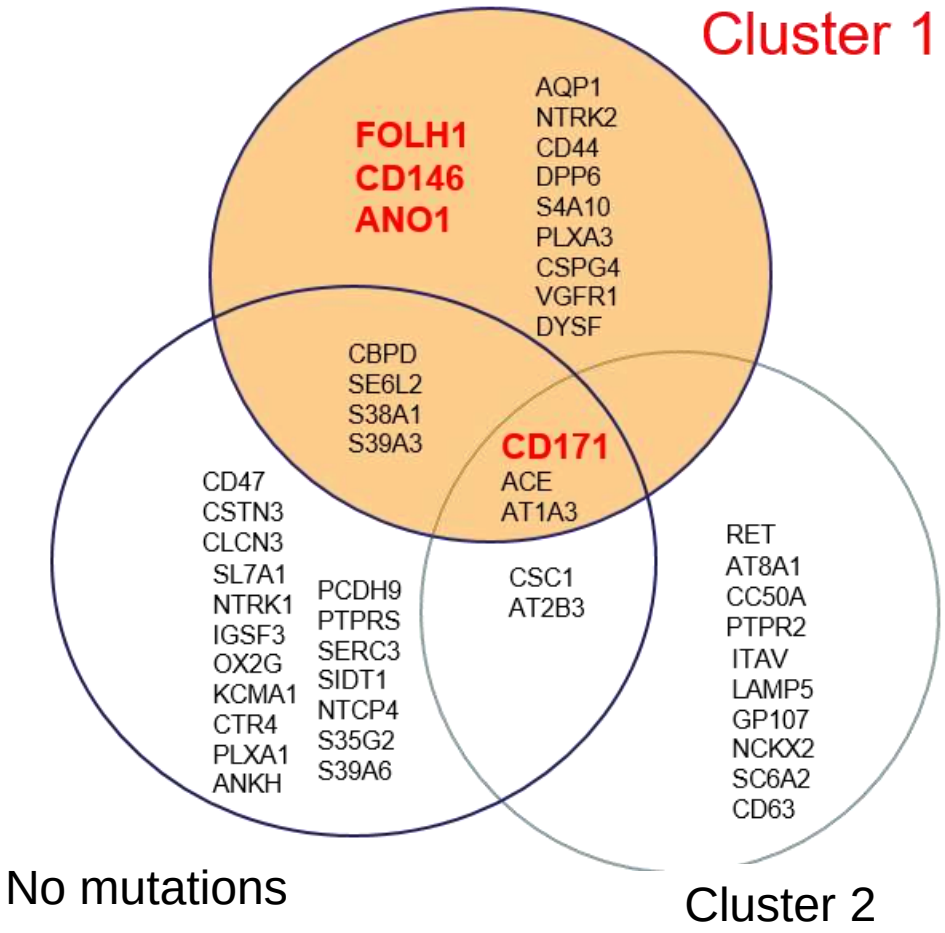
PROTEOMIC ANALYSIS OF PPGL MEMBRANE PROTEOME



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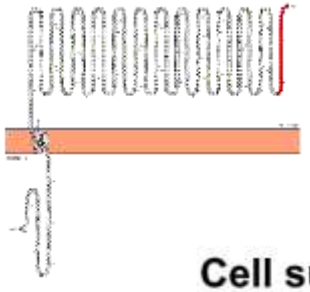
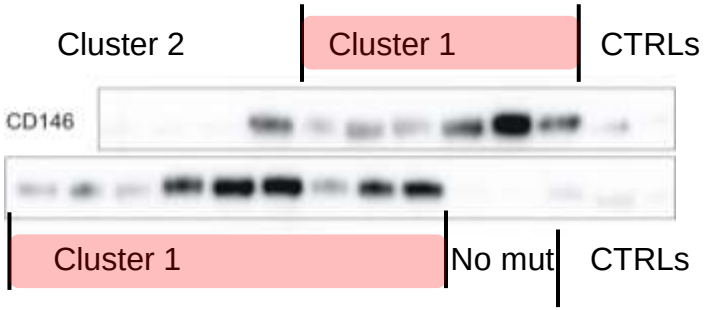
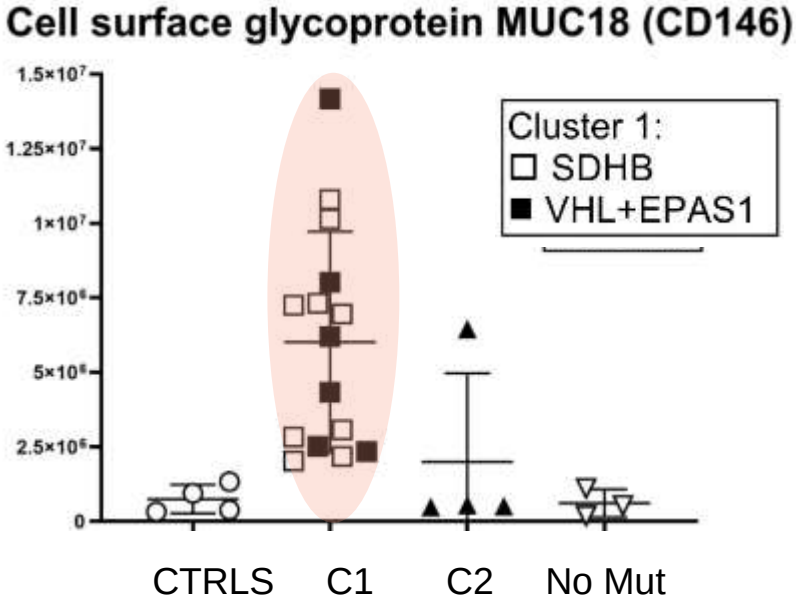
CD146
(Melanoma cell adhesion molecule, MCAM, MUC18)







- 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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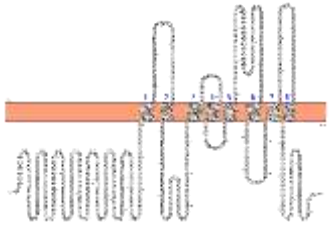
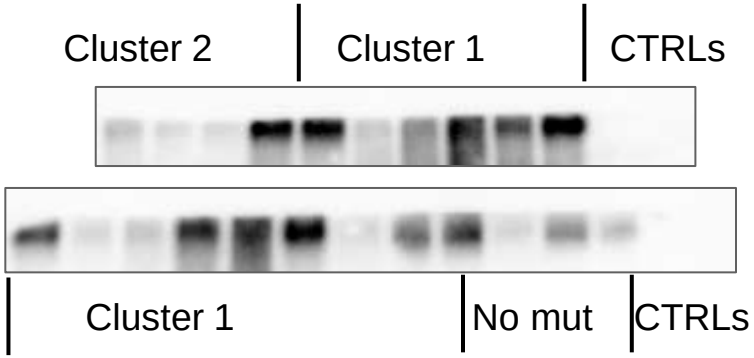
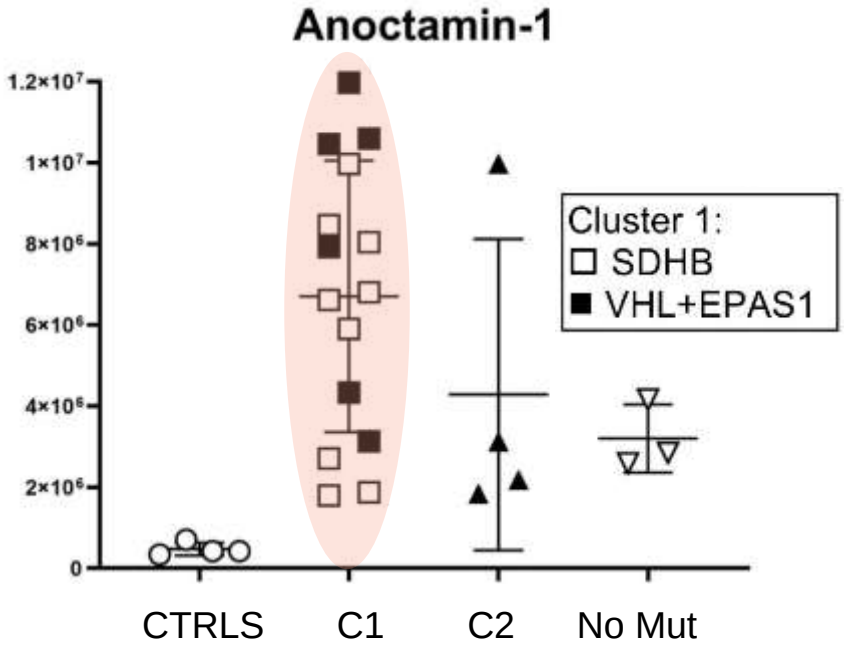
Anoctamin-1 (DOG1, TMEM16A)







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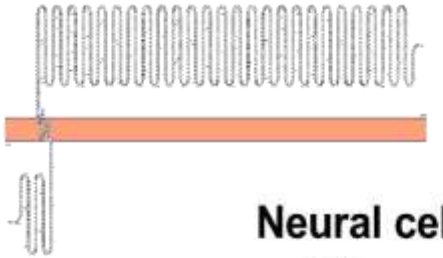
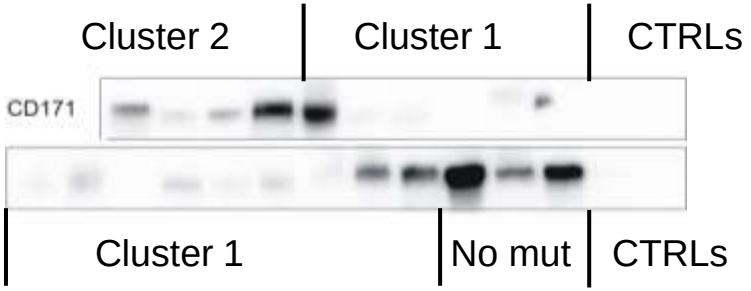
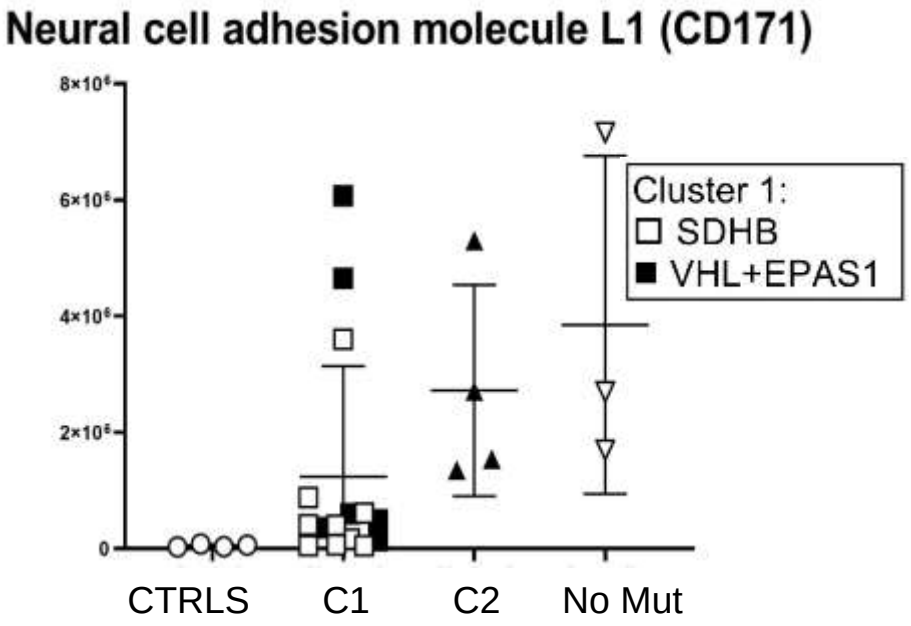
CD171
(Neural cell adhesion molecule L1, NCAM-L1)







- 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

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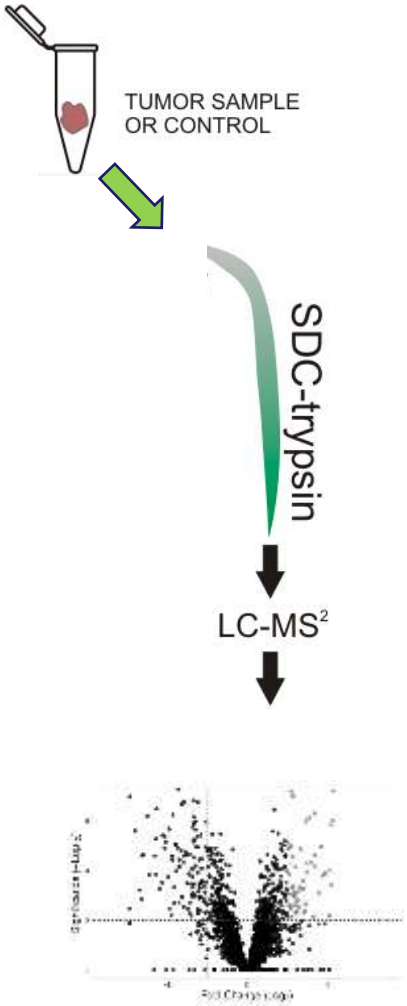
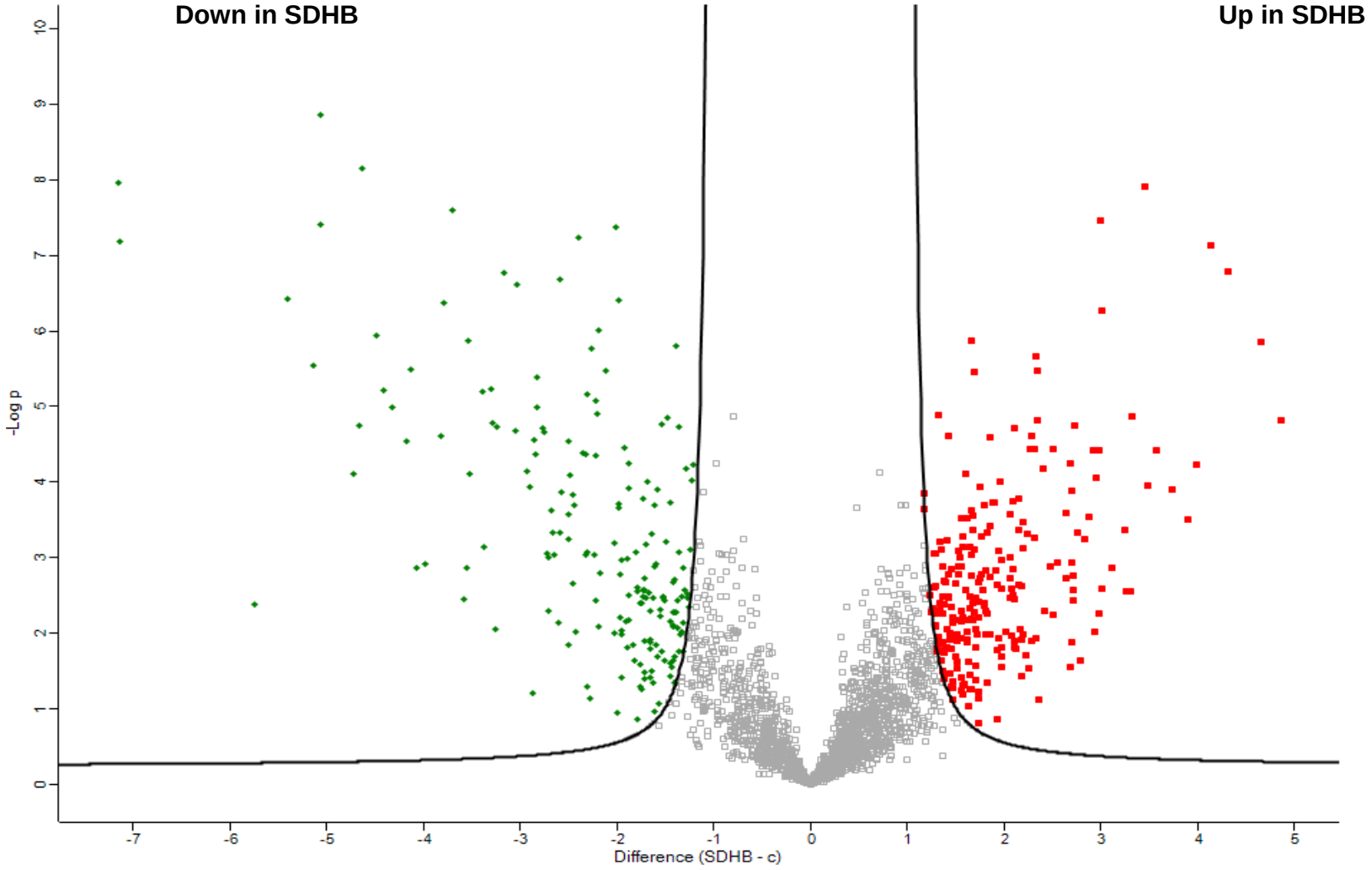
AND WHAT ABOUT THE NON-MEMBRANE PROTEINS?



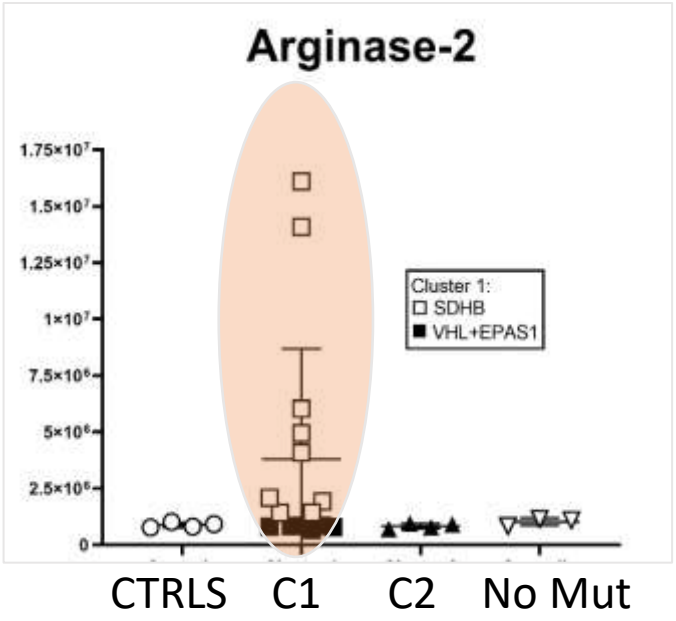
SDHB-specific changes?

Trypsin-based LFQ data

SDHB vs. controls

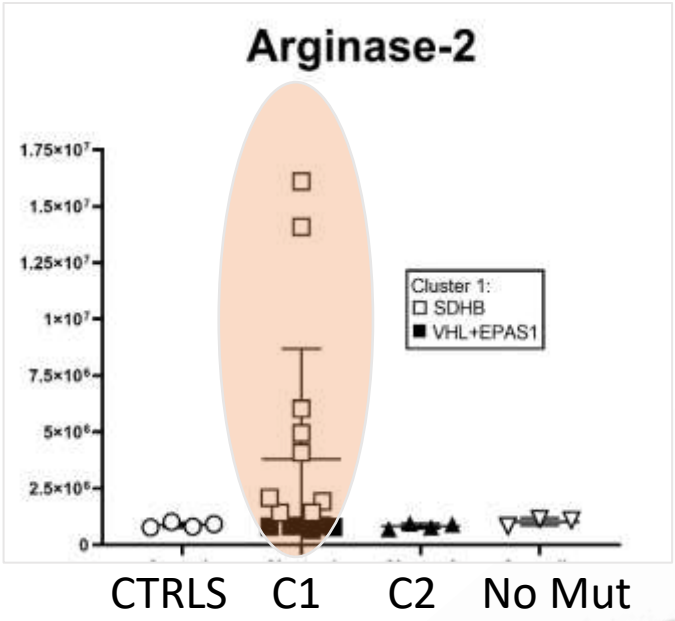


Mitochondrial Arg metabolism



- 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



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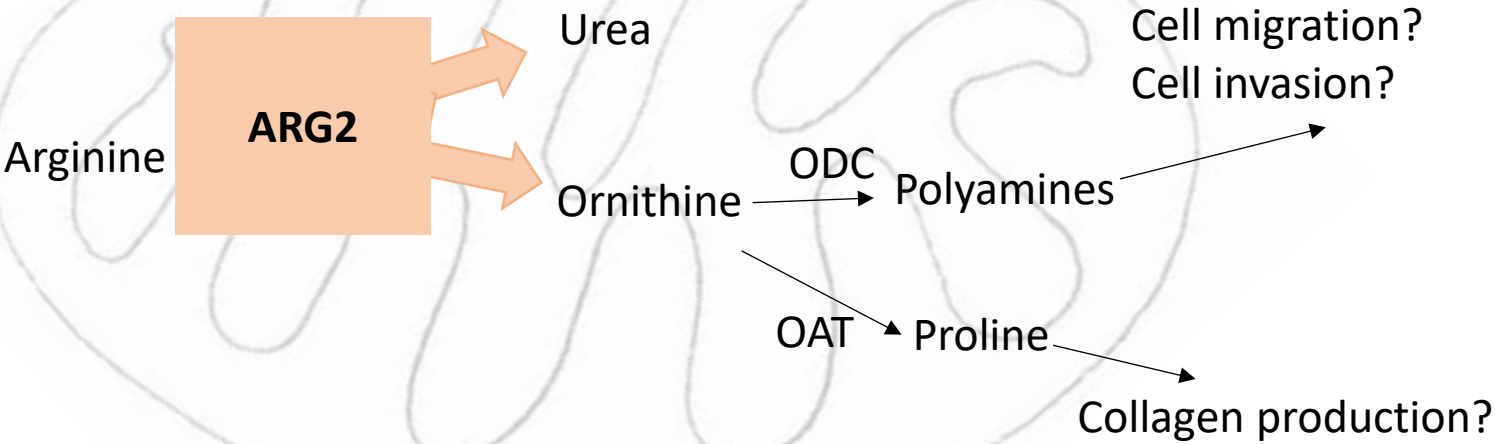
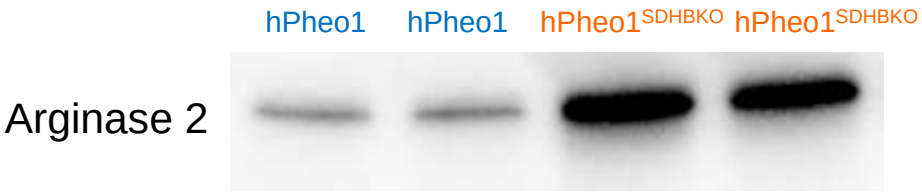
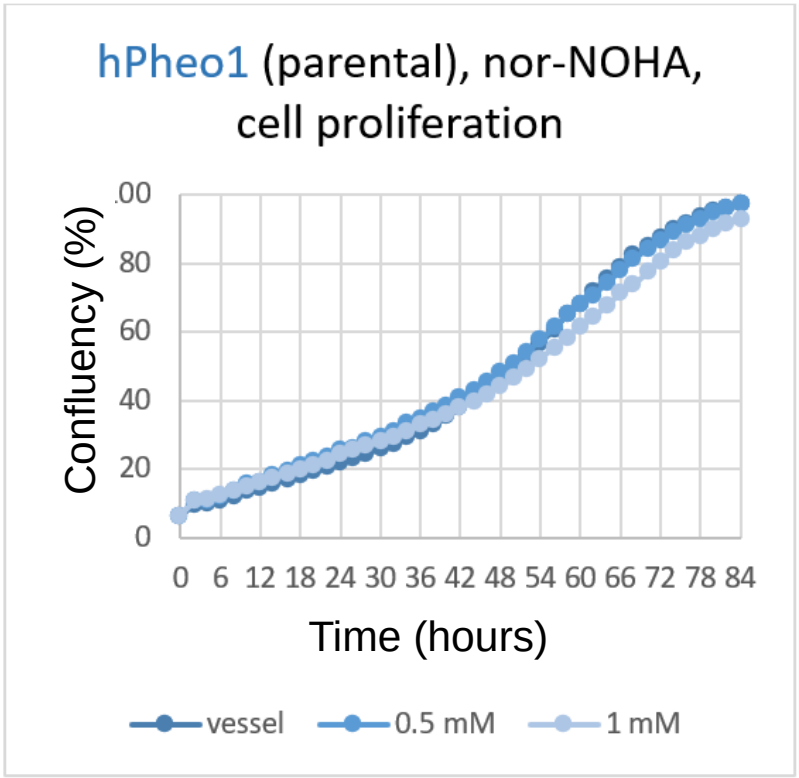
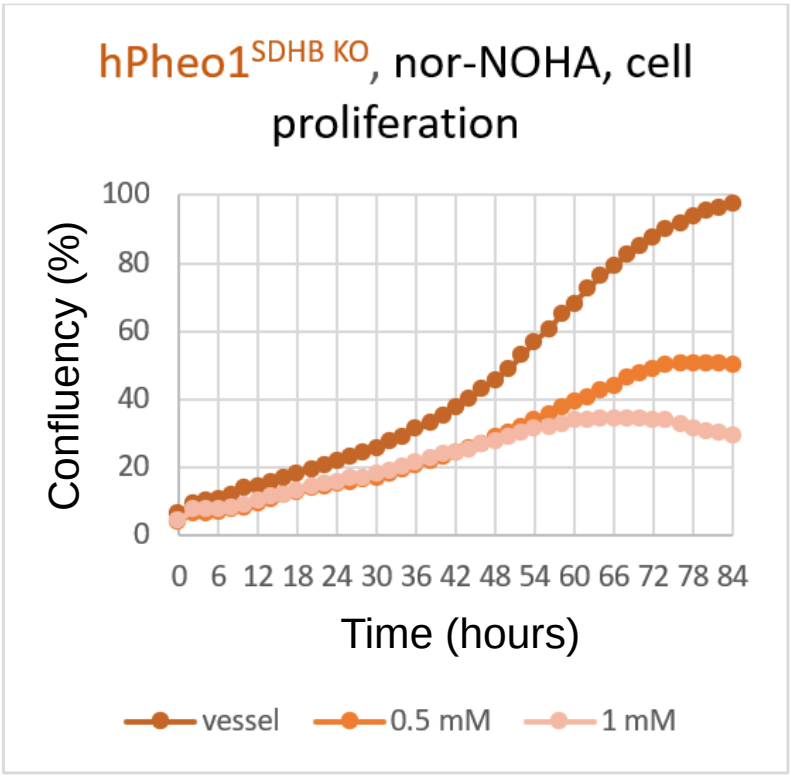


ARG2 inhibitor N-Hydroxy-nor-L-arginine (nor-NOHA)

Inhibitory effect on growth of control and SDHB defficient PPGL cells







Take-home message

- Up to 8000 -12000 of proteins can be identified in a human tissue sample.
- Low-abundance proteins and membrane proteins often escape detection.
- MS-based identification is identification of the coding gene.
- Absence of detection does not mean absence of the peptide/protein
- Most proteomics data are semi-quantitative
- It is just a single snapshot of a spatio-temporal process!
- Design of the study and the sample quality are critical
- The real fun starts after MS/MS analysis
- Proteomics can discover molecular processes, id. biomarkers and drug targets.
- A possible way toward precision medicine and patient-tailored therapy
- Single cell proteomics may open new opportunities….

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