



A Simple Solution-based Method for Targeted and Global Membrane Proteomics Using μ LC-MS/MS Analysis

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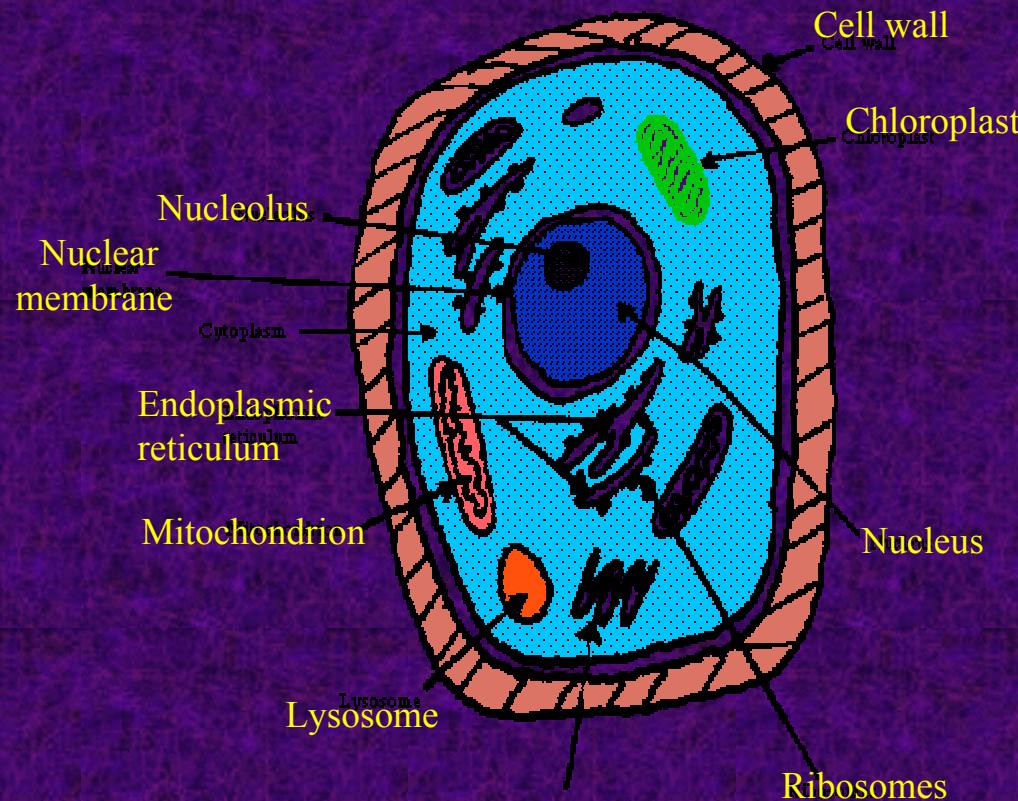
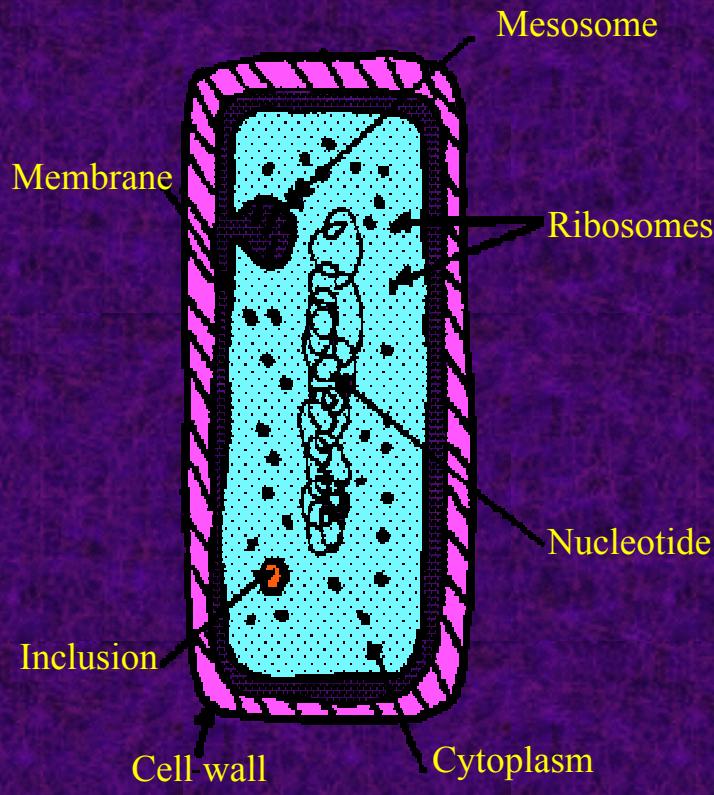


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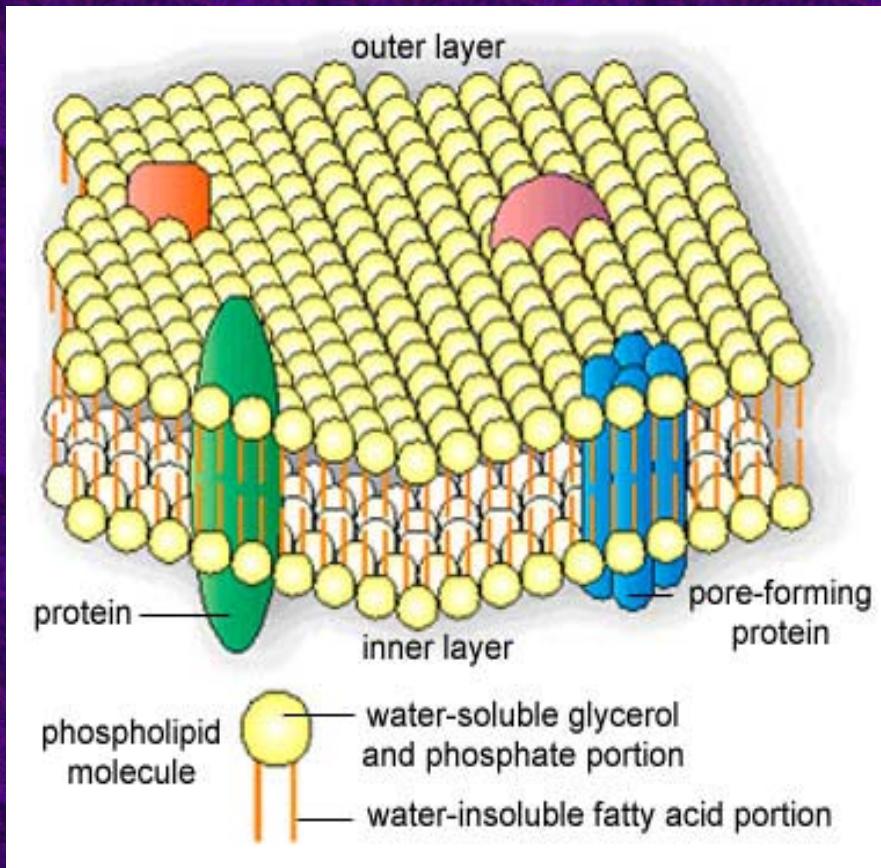
Outline

- Membranes and membrane proteins
- Importance of membrane proteomics
- Current strategies of solution-based membrane proteomics: advantages and limitations.
- Single tube approach for extraction, solubilization and proteolysis of integral membrane proteins.
- Applications

Prokaryote cell vs. eukaryote cell

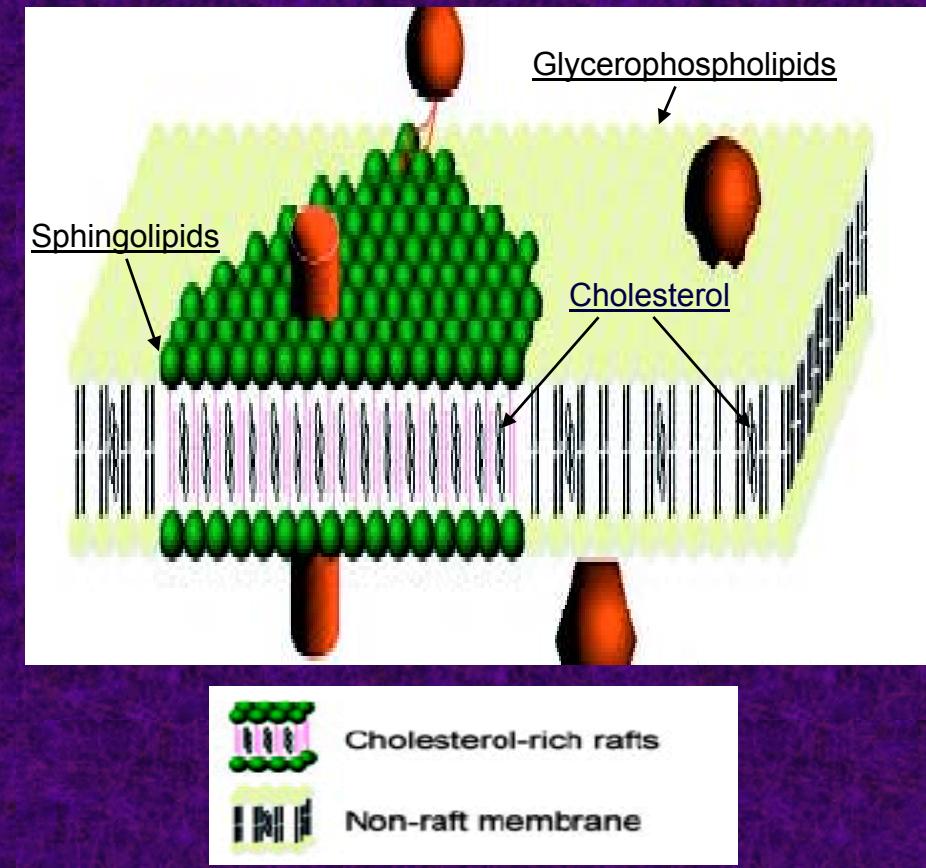


Spatial organization of plasma membrane



Molecular Biology of the Cell, Alberts B *et al* 1994

Fluid-mosaic model



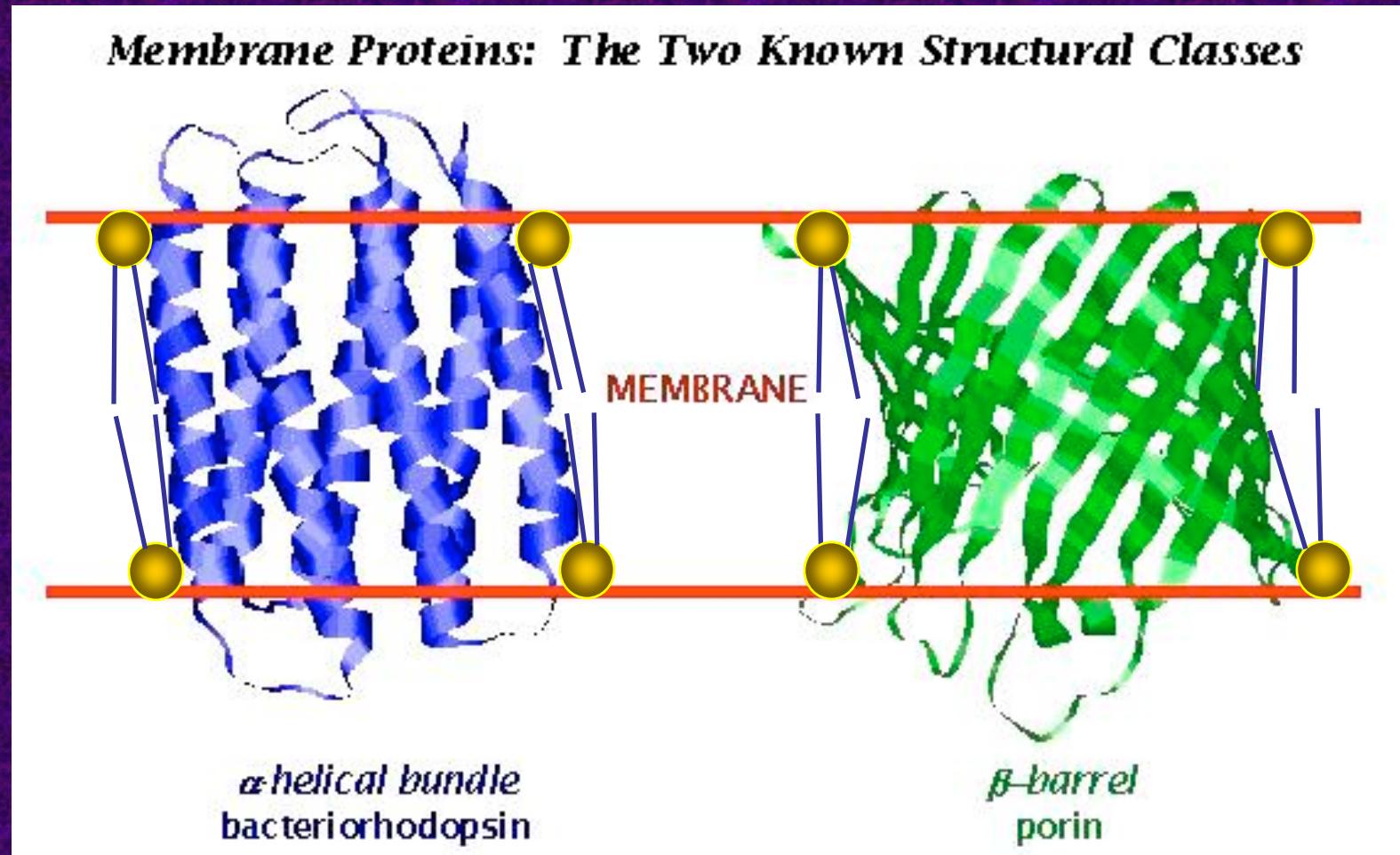
Leonard J. Foster, Carmen L. de Hoog, and Matthias Mann, 2003, PNAS .100: 5813-5818;

Lipid raft model

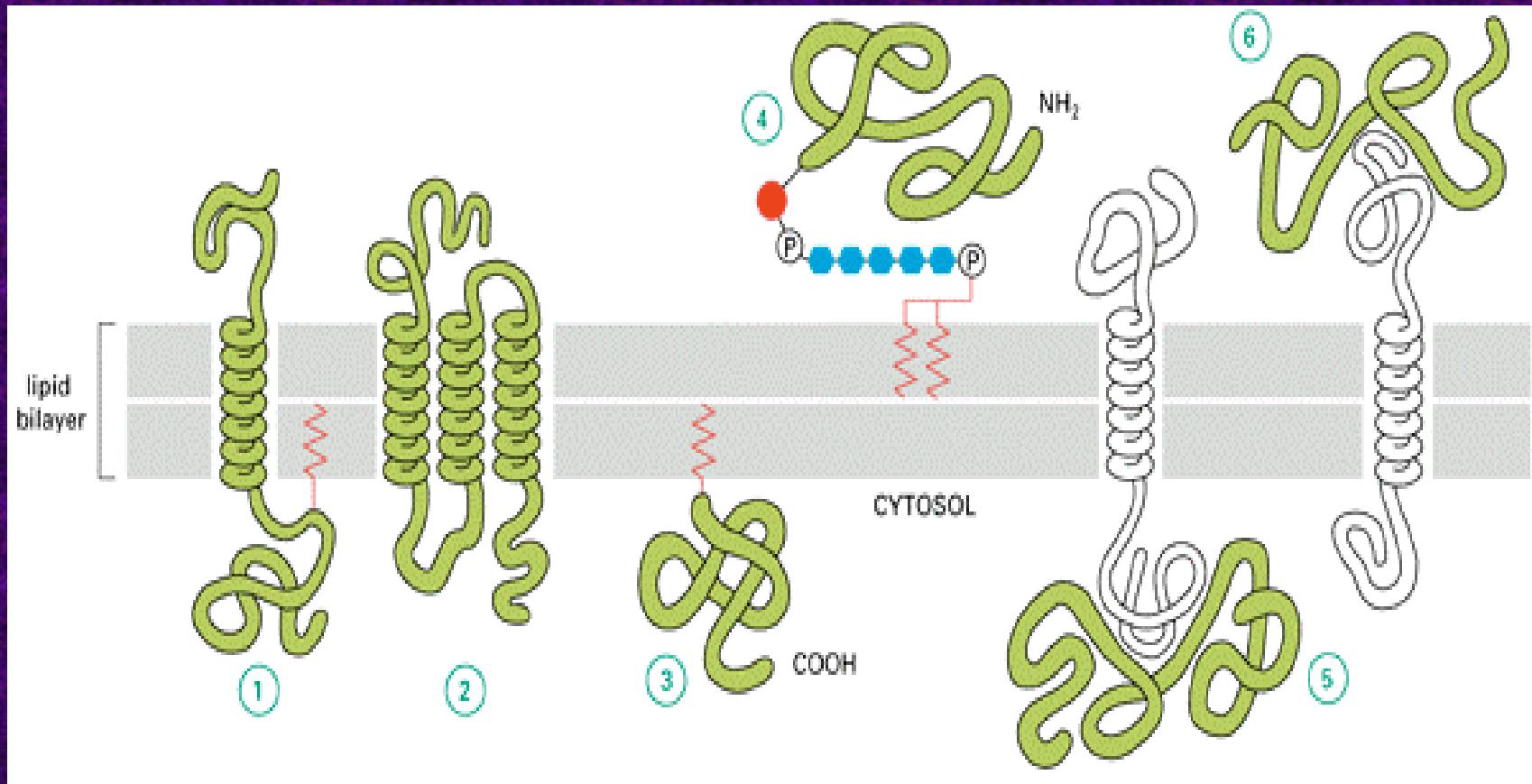
Membrane subproteome of eukaryotic cell



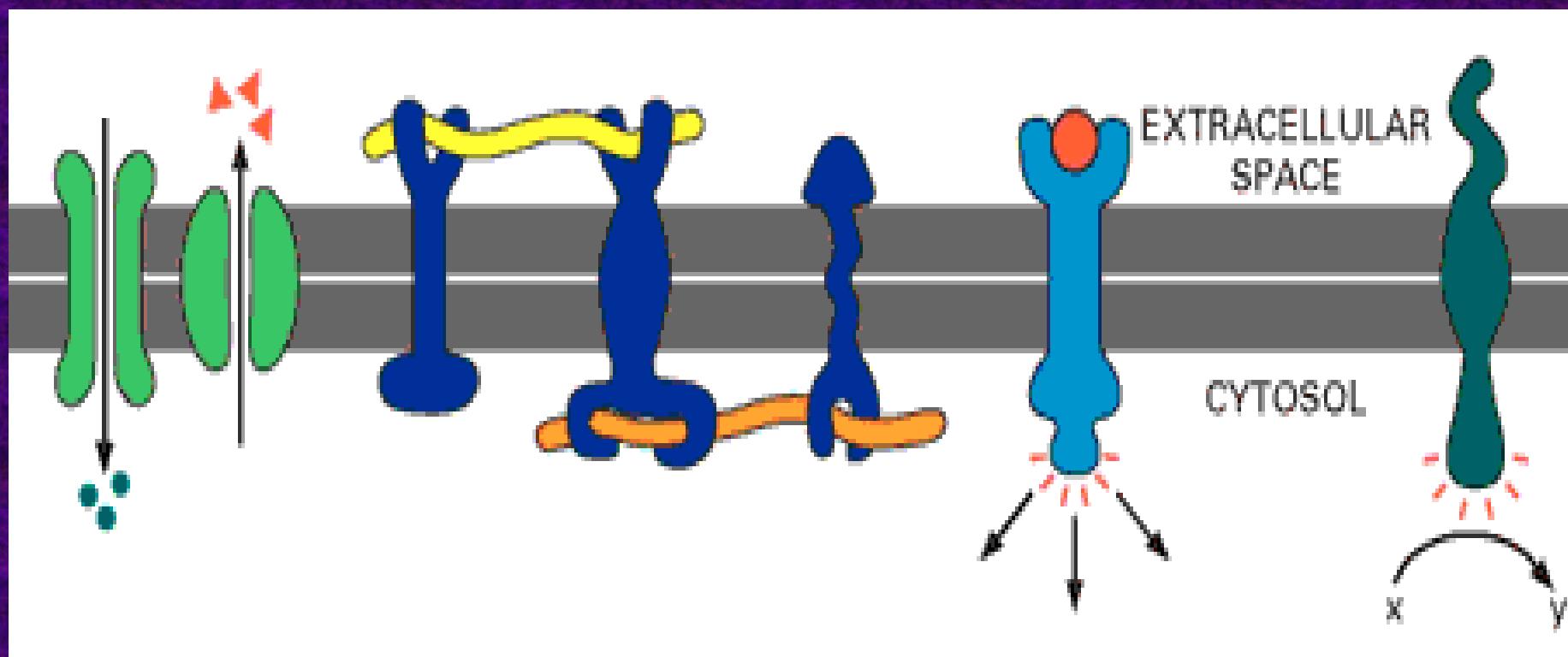
Integral membrane proteins display only two structural motifs: membrane spanning alpha-helix bundles and beta-barrels



The ways in which membrane proteins associate with the lipid bilayer



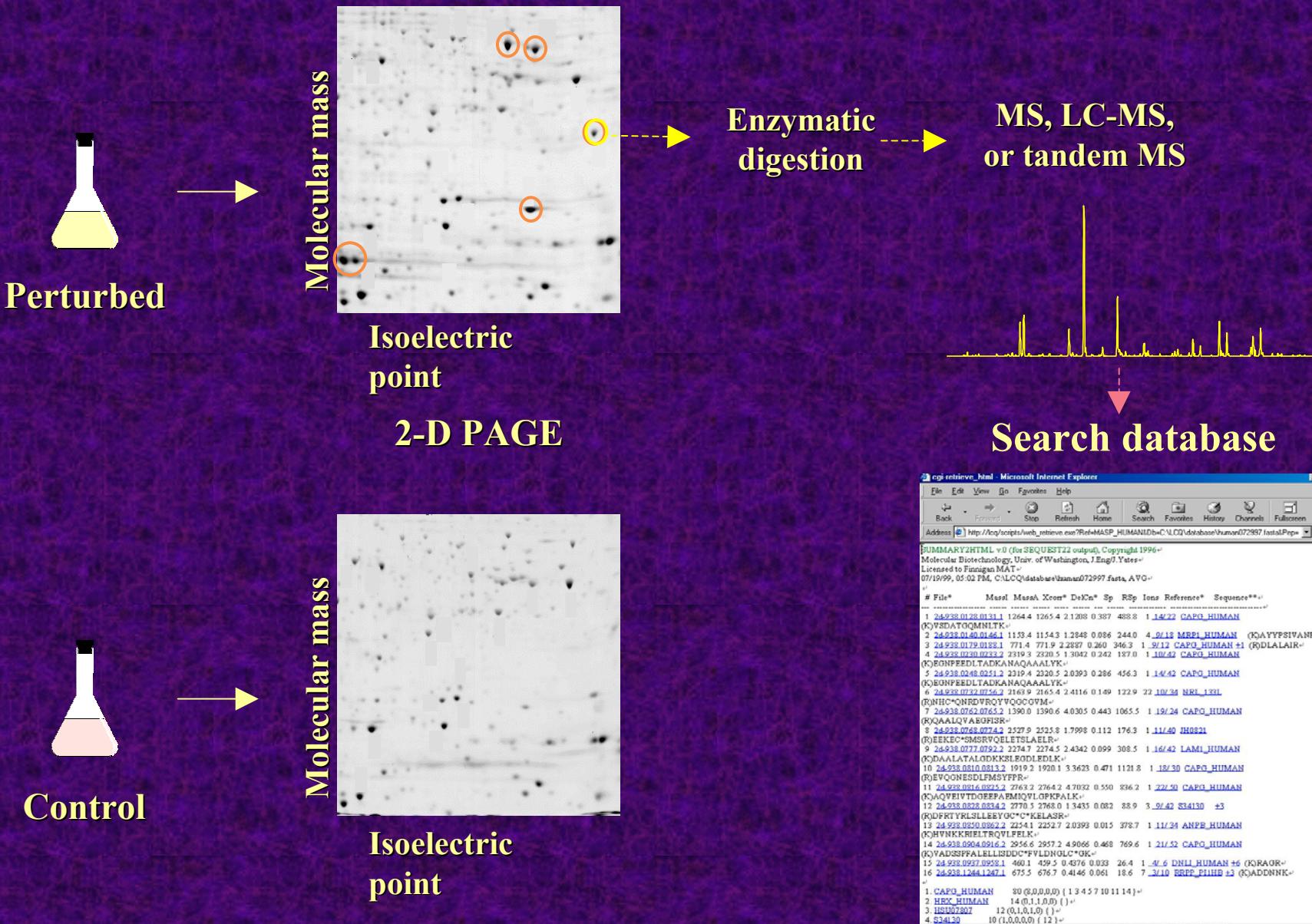
Functional classes of membrane proteins



The need for membrane proteomics

- While the genome determines which membrane proteins *may* be expressed by a cell, the proteome reveals which membrane proteins *are* actually expressed.
- Identical cell types have differently expressed proteomes depending of many factors such as environment, metabolic state, physiological or photophysiological condition.
- mRNA measurements are not always reflective of protein levels.
- Specific membrane protein post-translational modifications play a key role in protein function and can not be predicted solely by genome analysis
- Each membrane of a living cell has unique function and different protein composition.
- As a cell bioeffectors membrane proteins provide membrane function for specific cell and/or cell organelle acting as receptors, transporters, ligands or enzymes.

2-D PAGE-based membrane proteomics



Distinctiveness of membrane proteomics

Membrane proteins, particularly hydrophobic ones are underrepresented in typical proteomic datasets.

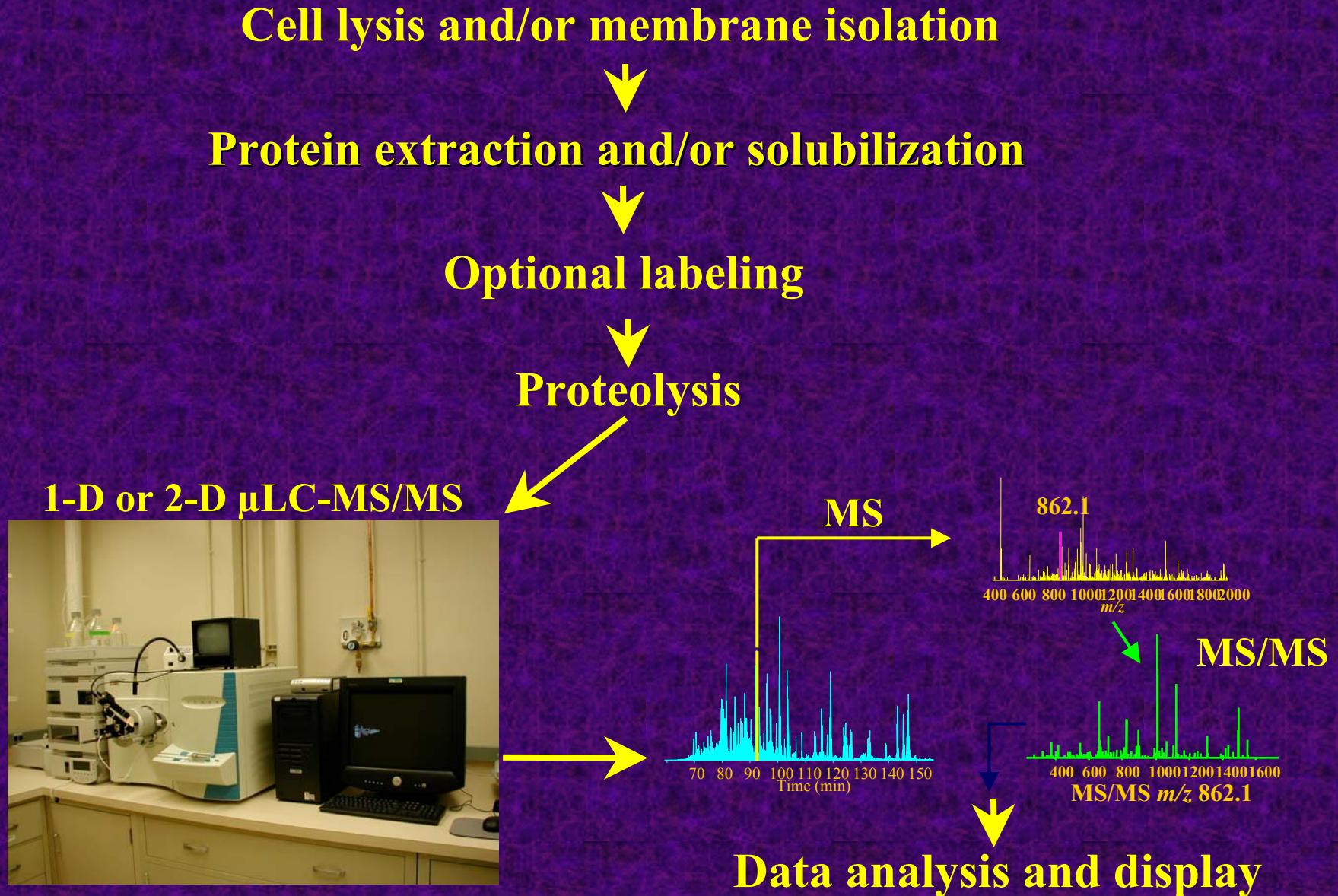
Global and targeted analysis of hydrophobic membrane proteins still presents a significant challenge for MS-based membrane proteomics in terms of extraction, solubilization, separation, and sequence coverage.

The sensitivity of the mass spectrometric analysis of membrane proteins , in terms of sample requirement, is poorer than for a comparable amount of soluble proteins.

Although the phospholipid bilayer is the universal membrane structure its composition varies, as well as its physical state

Membrane proteins provide function. The type and amount of proteins in a membrane varies.

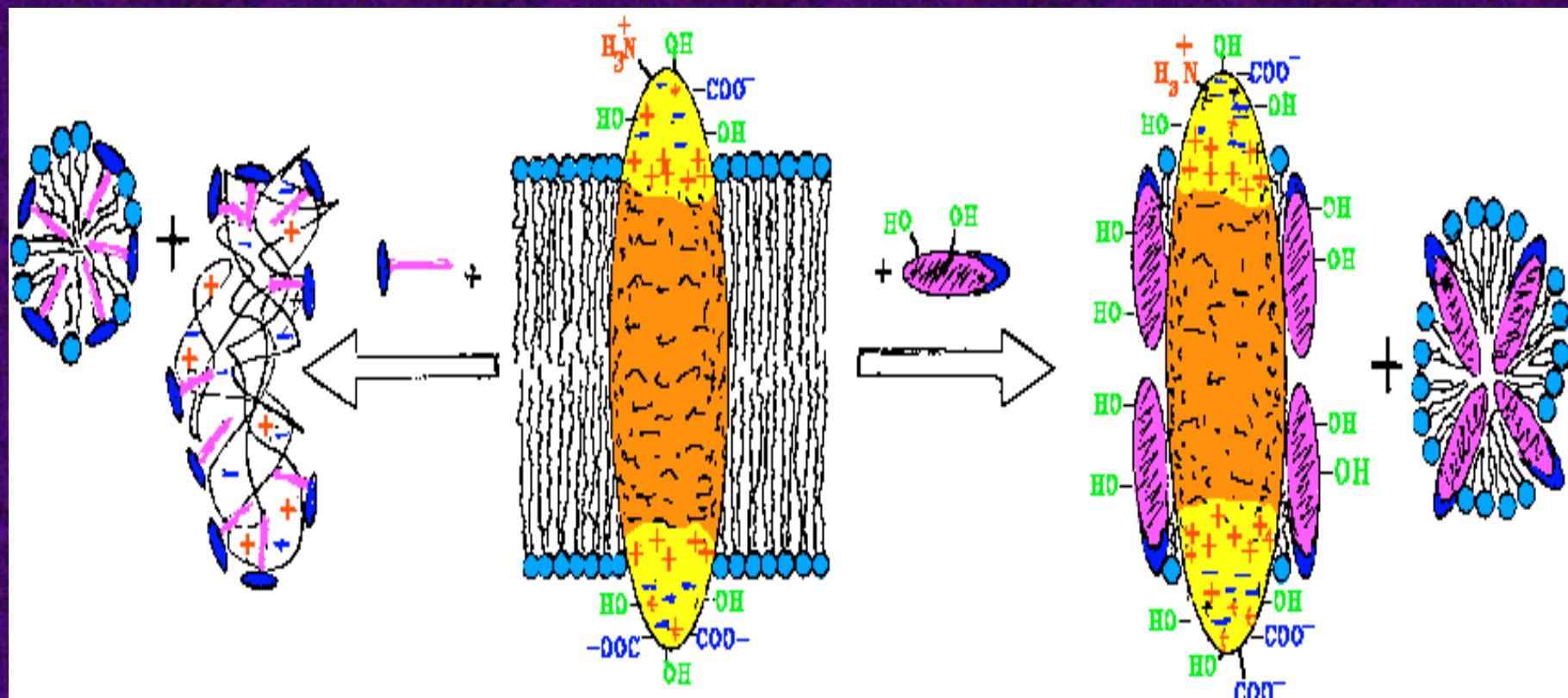
Solution-based membrane proteomics



The significance of sample preparation for solution-based membrane proteomics

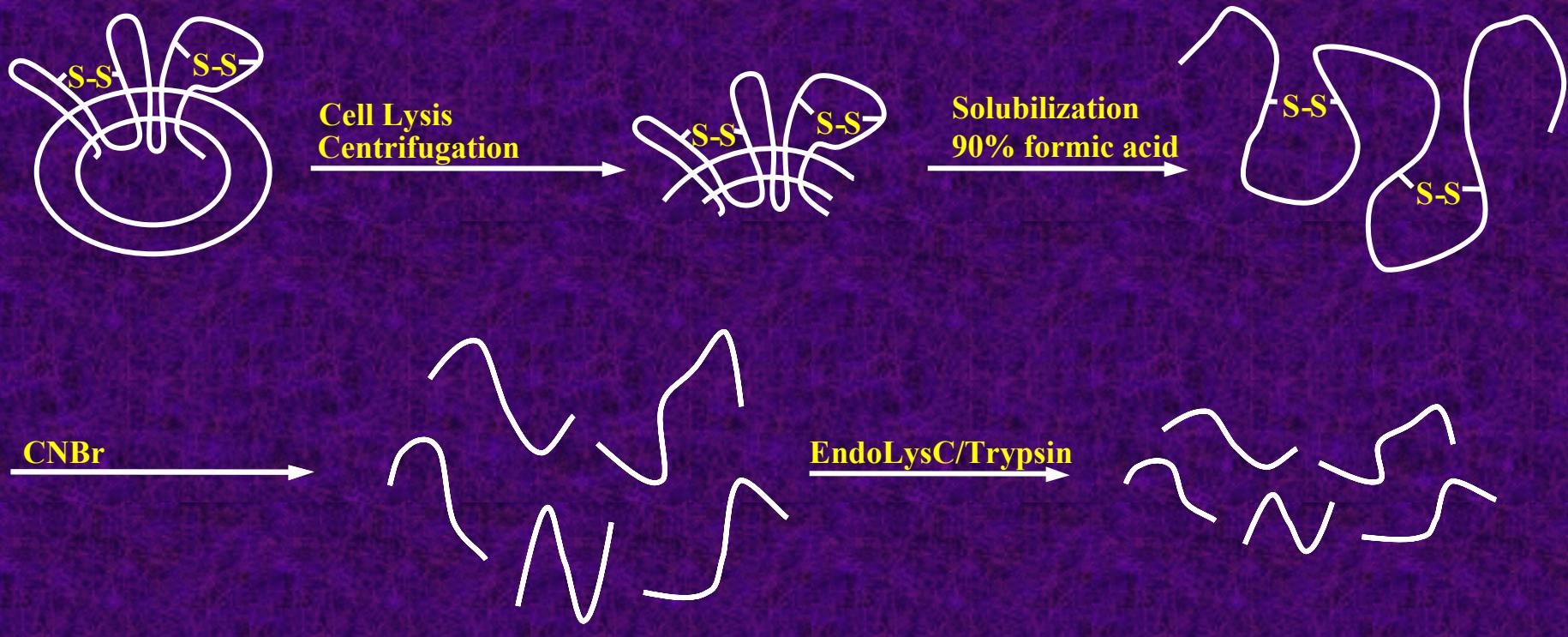
- The sensitivity of the proteomic analysis of membrane proteins is often determined more by the quality of sample preparation and separation strategy than by the sensitivity of the MS instrument.
- Inadequate sample preparation will invalidate any solution-based membrane proteomics strategy.
- Even the most powerful separation technique or MS instrument will not give valid results from poorly prepared membrane samples.
- GIGO (Roger M. Smith, *Journal of Chromatography A*, (2003),

Detergent-based solubilization of membrane proteins



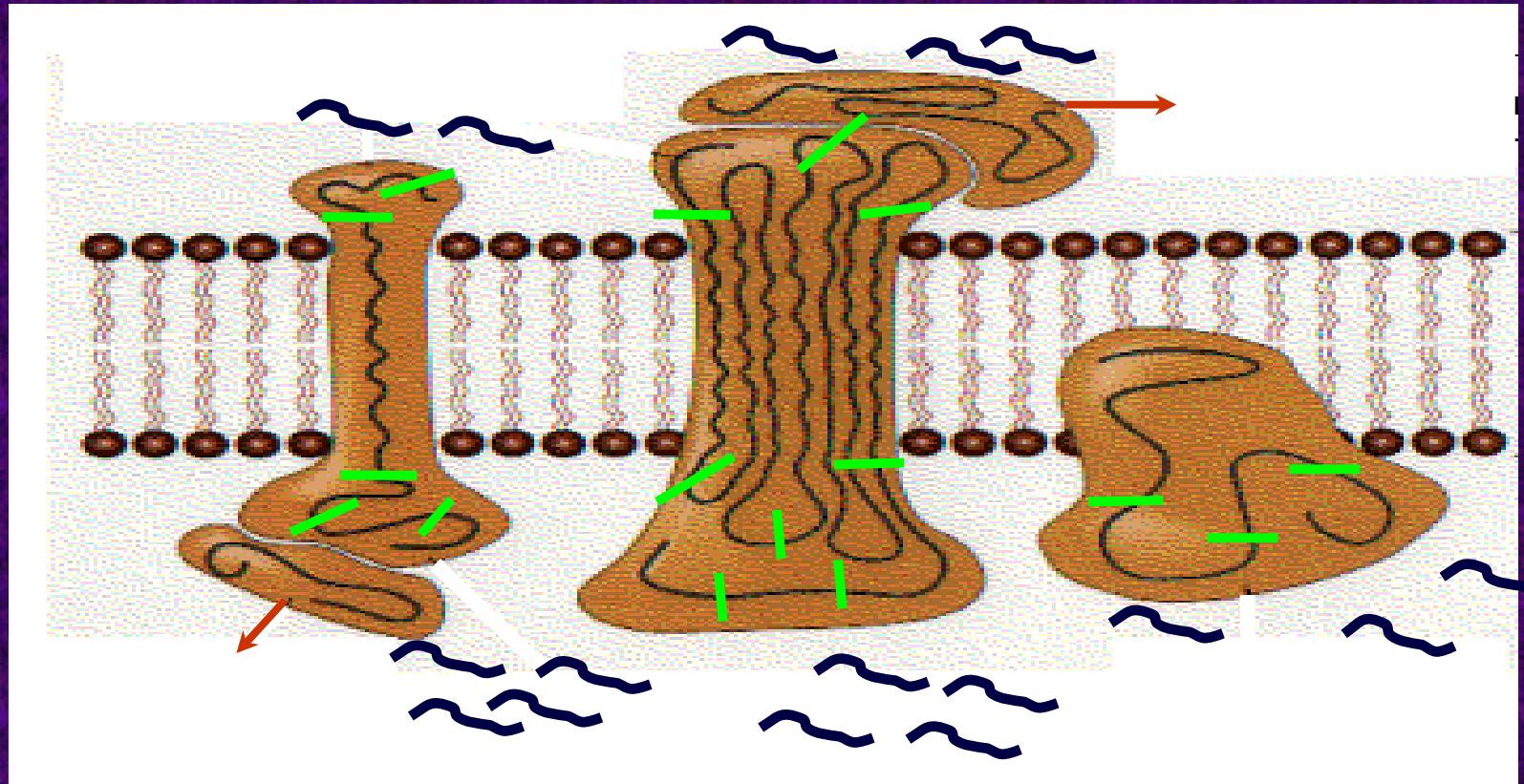
Han DK et al., *Nat Biotechnol.* 2001 Oct;19(10):946-51.

Strong organic acid based solubilization of membrane proteins



Washburn MP et al., *Nat Biotechnol*. 2001 Mar;19(3):242-7.

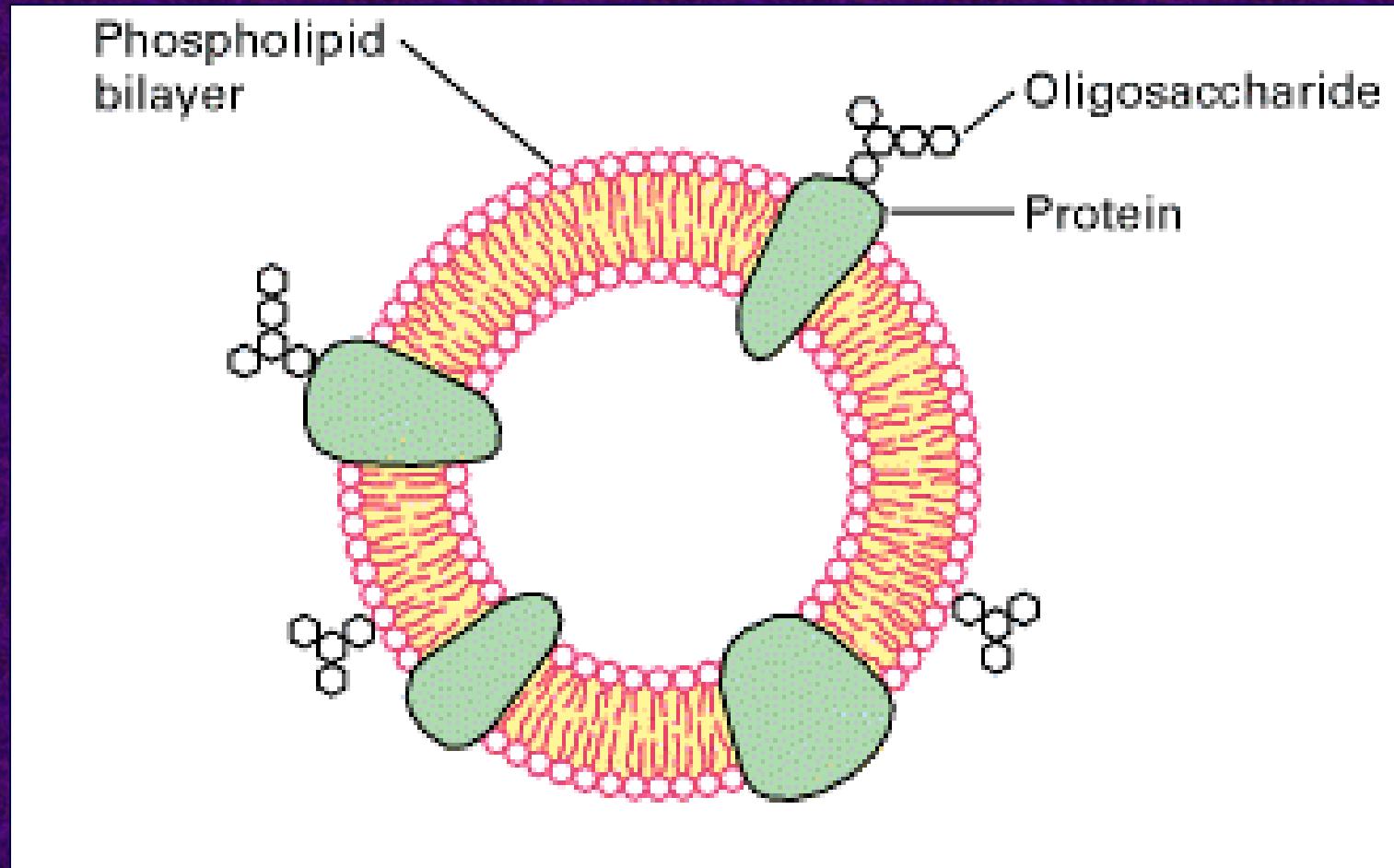
High pH-based membrane proteins pre-fractionation



Wu CC et al., *Nat Biotechnol.* 2003 May;21(5):532-8.

Membrane proteins and proteomics: un amour impossible?

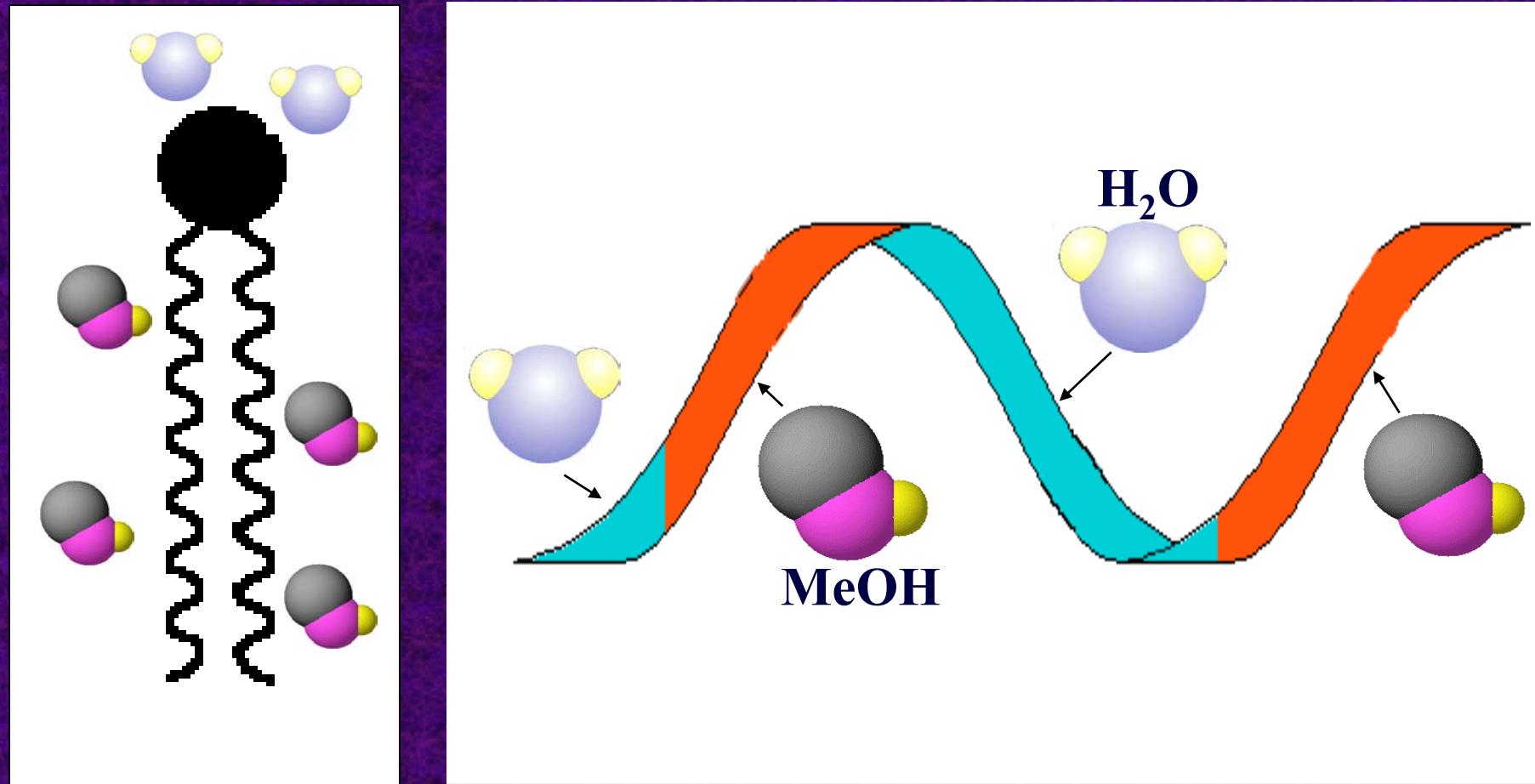
Santoni V, Molloy M, Rabilloud T., *Electrophoresis*, 2000 Apr;21(6):1054-70. Review.



**Is it possible to develop an improved and simple
sample preparation technique amenable for both,
global and targeted membrane proteomics
using μ LC-MS/MS analysis ?**

The answer is YES

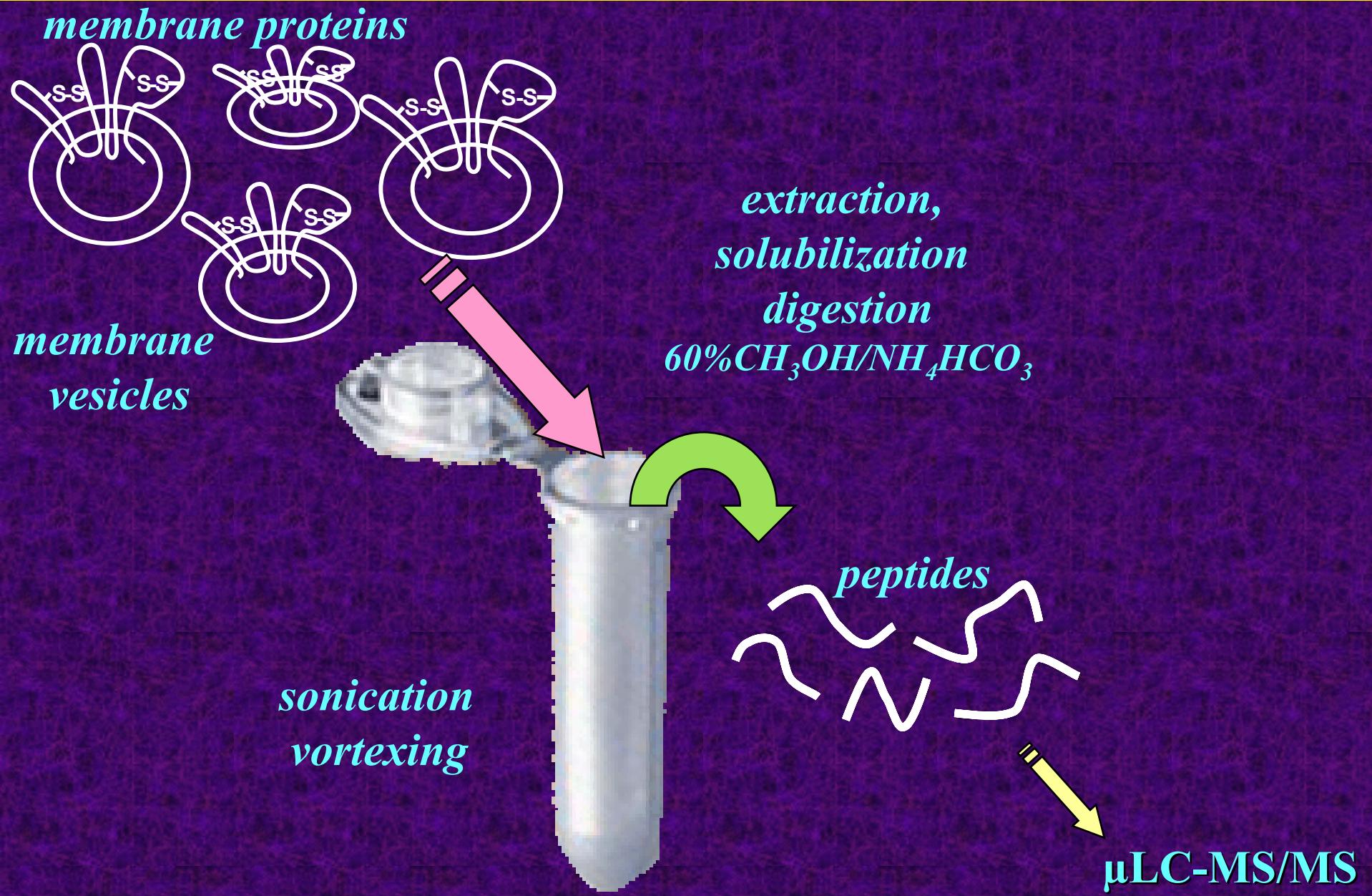
The hypothesis of the single phase miscible extraction and solubilization of integral membrane proteins for targeted and global proteomics



Design by Aaron Lucas, 2003.

Blonder J, Goshe MB, Moore RJ, et al. *J. Proteome Res.* 2002 Jul-Aug;1(4):351-60

Single tube procedure



Targeted μ LC-MS/MS analysis of *Halobacterium halobium* purple membranes

Josip Blonder, Thomas P. Conrads, Li-Rong Yu, Atsushi Terunuma, George M. Janini, Haleem J. Issaq,
Jonathan C. Vogel, and Timothy D. Veenstra, *Proteomics*, 2003.

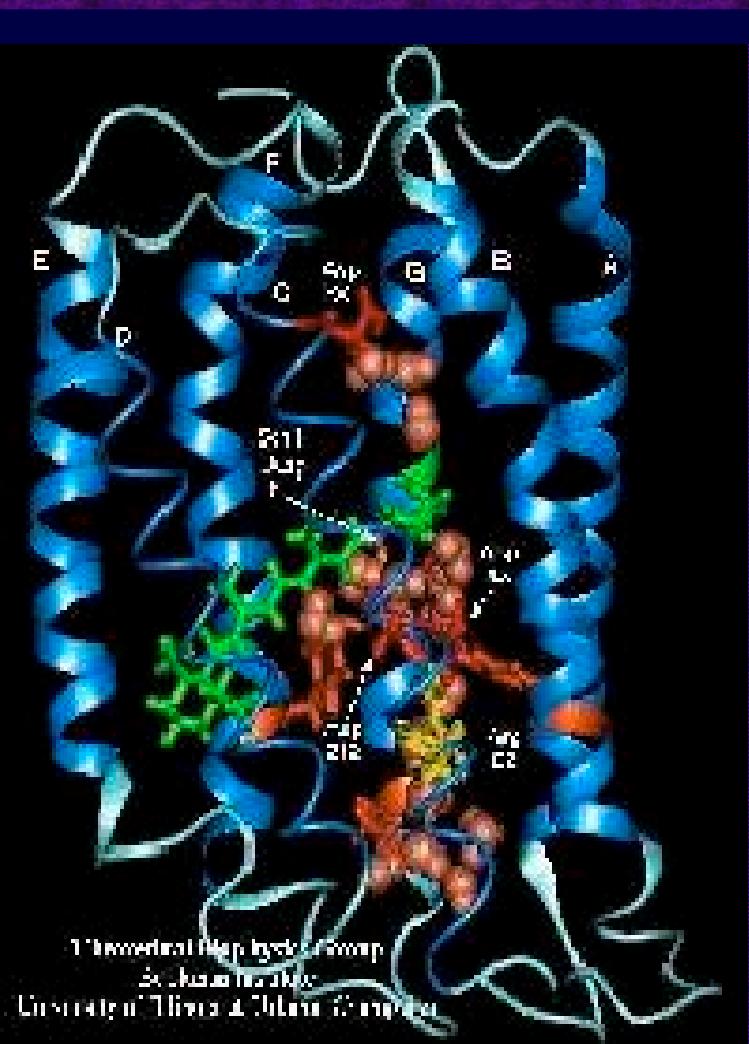
Bacteriorhodopsin and purple membranes

The first integral membrane protein extensively studied using biophysical and proteomics techniques is bacteriorhodopsin from purple membranes of *H. halobium*.



Schematic drawing of the bacterium *Halobacterium halobium* showing the patches of purple membrane that contain bacteriorhodopsin molecules. These bacteria, which live in saltwater pools where they are exposed to a large amount of sunlight, have evolved a variety of light-activated proteins, including bacteriorhodopsin, which is a light-activated proton pump in the plasma membrane.

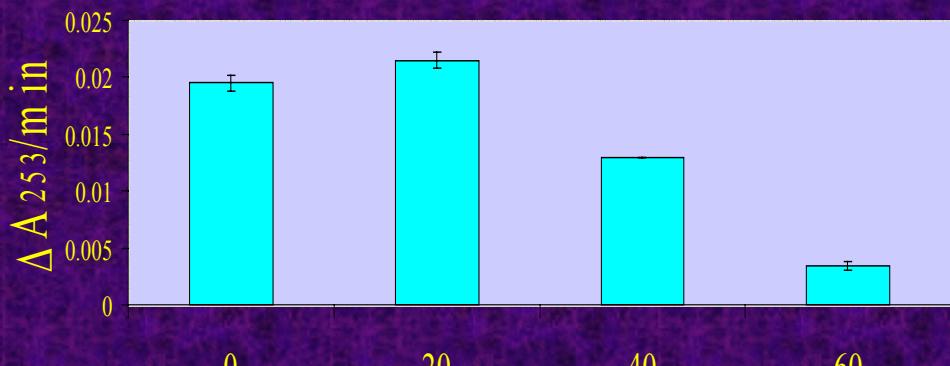
Bacteriorhodopsin



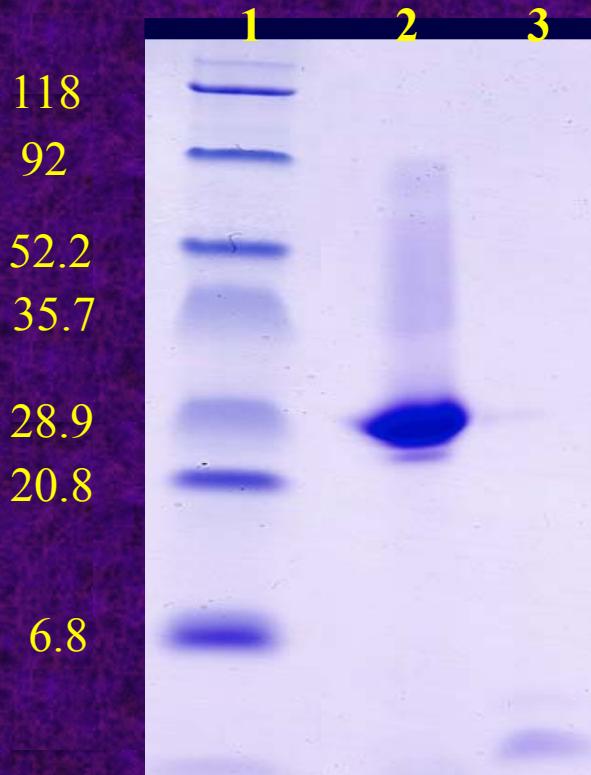
- Bacteriorhodopsin is the best-understood ion transporter and is a recognized model for G protein-coupled receptors .
- It was the first seven trans-membrane helix protein studied by biophysical and structural techniques, and its three-dimensional structure has been determined by X-ray crystallography to a resolution of 1.55 Å .
- The purple membranes in which bacteriorhodopsin is found are, in fact, specialized micro-domains within the plasma membrane and are closely related to eukaryotic lipid rafts.

Determination of trypic activity and its proteolytic efficiency in methanol

Trypsin Activity



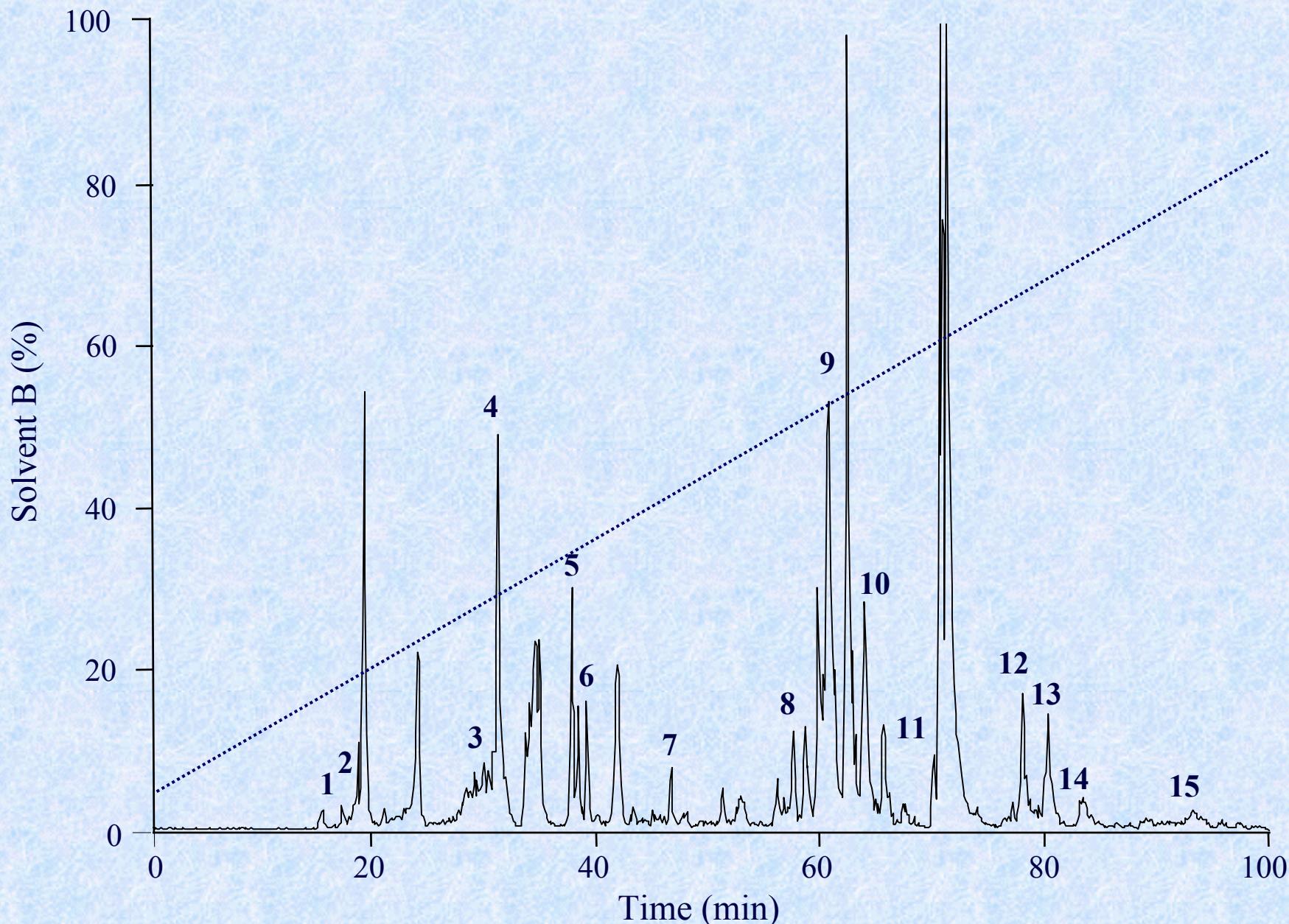
A



B

(A) Determination of trypic activity in 60% methanol using a N-benzoyl-L-arginine ethyl ester (BAEE) assay. The activity of trypsin was assayed in aqueous-organic ratios containing 0%, 20%, 40%, and 60% methanol. (B) The overall trypic digestion efficiency was analyzed using SDS-PAGE. Lane 1: molecular weight markers; Lane 2: 25 μg of intact purple membrane proteins; Lane 3: 25 μg of purple membrane proteins digested in 60% methanol.

Tryptic Peptide	Position	Peptide Sequence	GRAVY
1	1-43	<i>MLELLPTAVEGVSQAQITGRPEWIWLALGTALMGLGTLYFLVK</i>	0.740
2	44-53	GMGVSDPDAK	-0.620
3	54	K	N.A.
4	55-95	FYAITTLVPAIAFTMYLSMLLGYGLTMVPFGGEQNPIYWAR	0.759
5	96-142	YADWLFITPLLLLALLVDADQGTILALVGADGIMIGTG LVGALT	1.119
6	143-147	VYSYR	-0.740
7	148-172	FVWWAISTAAMLYIILYVLFF GFTSK	1.424
8	173-185	AESMRPEVASTFK	-0.523
9	186-188	VLR	N.A.
10	189-229	NVTVVLWSAYPVVWLIGSEG AGIVPLNIETLLFMVLDVSAK	1.154
11	230-238	VGFGLILLR	1.956
12	239-240	SR	N.A.
13	241-262	AIFGEAEAPEPSAGDGAAATSD	-0.195



Base-peak chromatogram of purple membrane analysis. The number labels in the base peak chromatogram present the elution order of Bacteriorhodopsin peptides listed in Table I

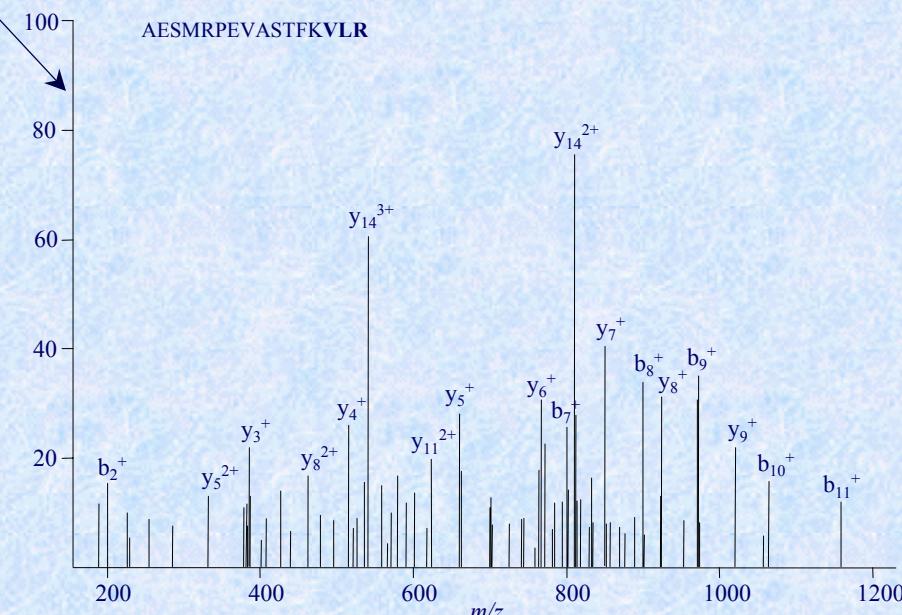
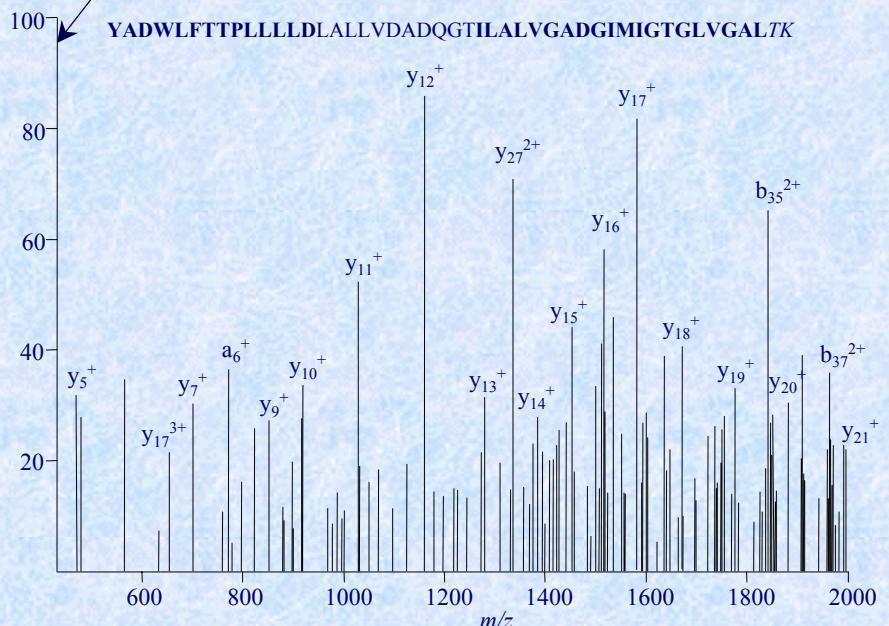
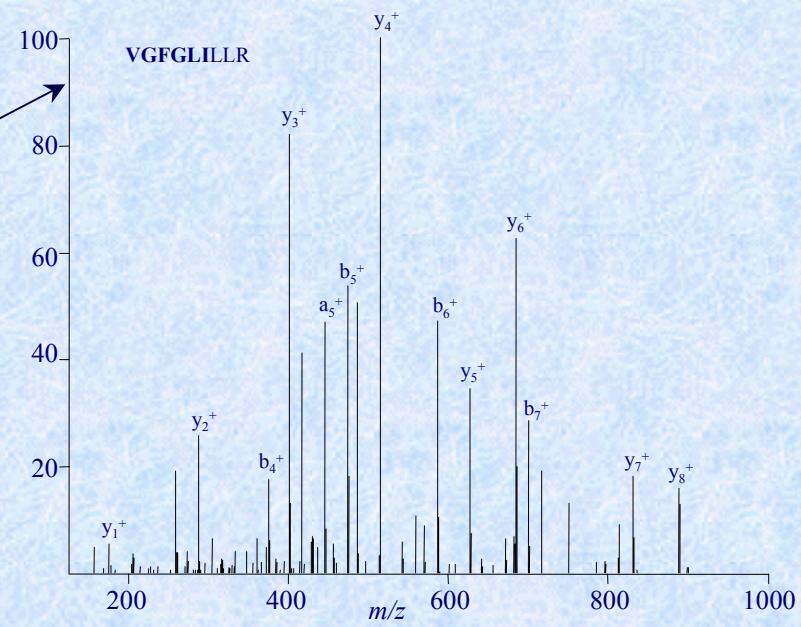
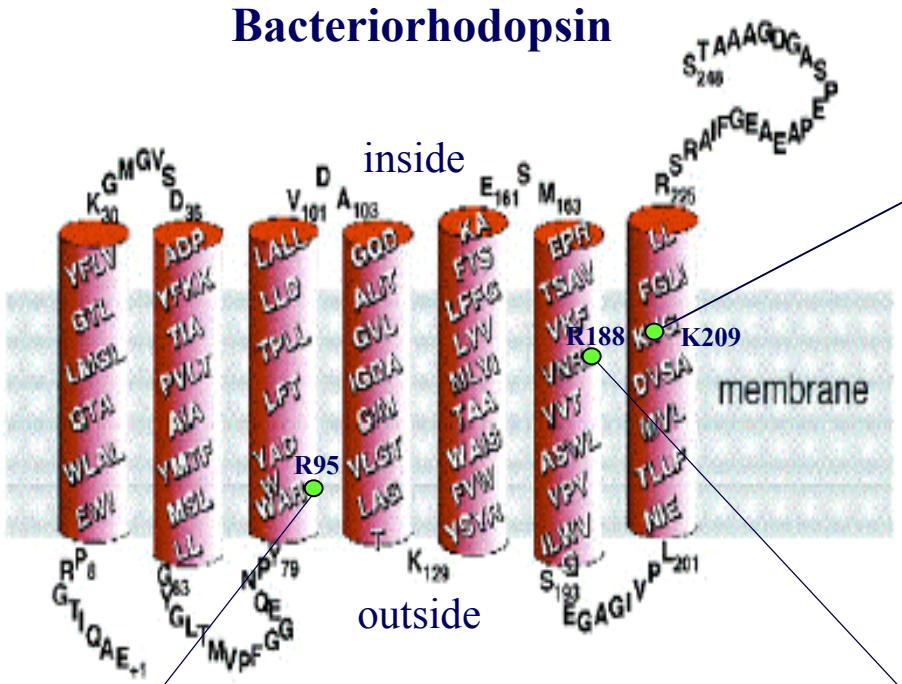
List of bacteriorhodopsin tryptic peptides observed by μLC-ESI-MS/MS.

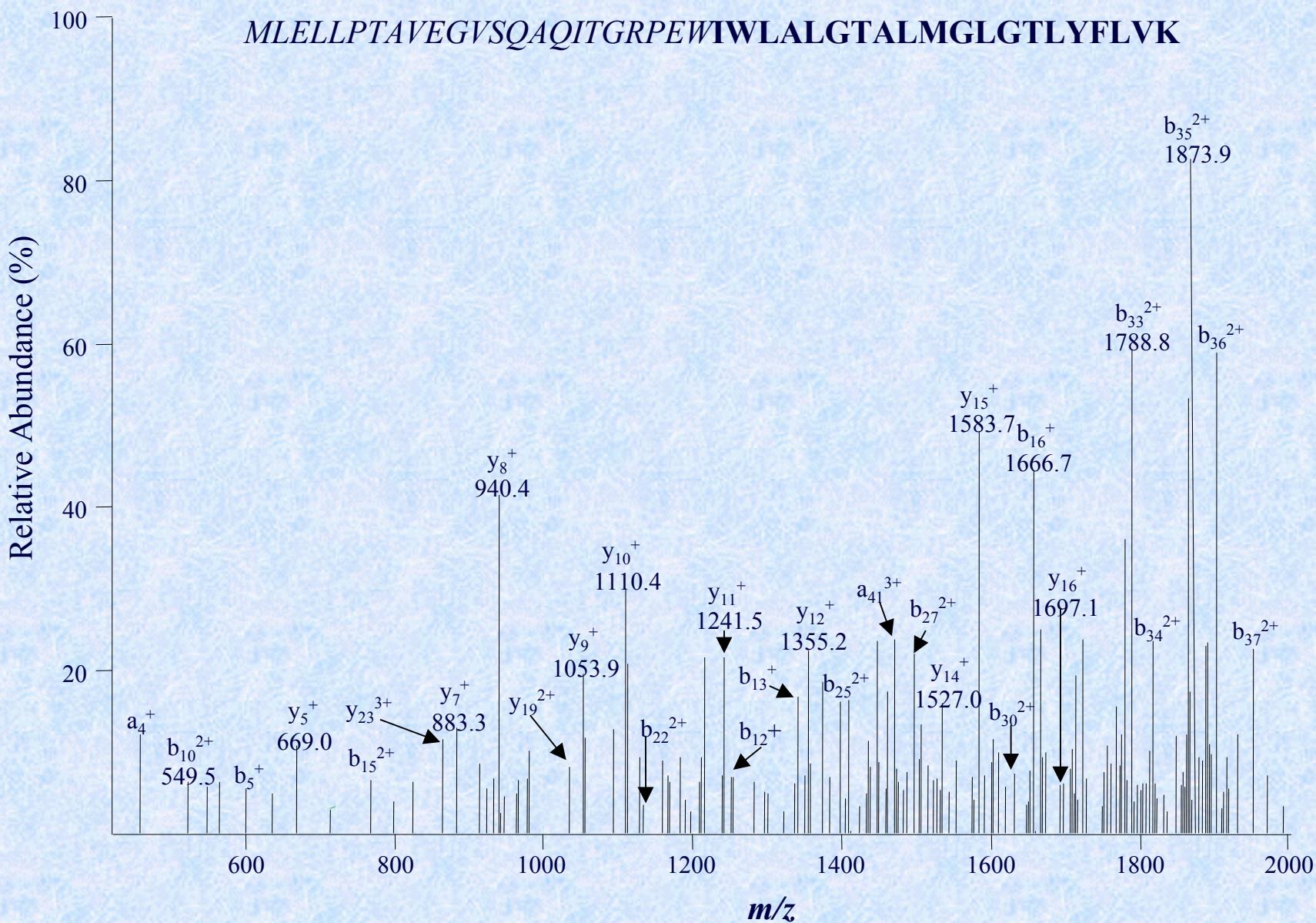
Position	Peptide sequence	#mc ¹	Calculated mass	Charg. state	Xcorr	Retention time	Elution order	GRAVY ²
1-43	MLELLPTAVEGVSQAQITGRP EWIWLALGTALMGLGTLYFLVK	0	4686.544	3	3.900	58.97	8	0.740
1-30	XAQITGRPEWIWLALGTALMG LGTLYFLVK	0	3329.788	2	2.770	62.29	9	0.706
44-53	GMGVSDPDAK	0	976.440	2	3.251	16.48	1	-0.620
44-54	GMGVSDPDAKK	1	1104.535	2	2.363	18.76	2	-0.918
44-95	GMGVSDPDAKKFYAITTLVPAIAFTM YLSMLLGYGLTMVPFGGEQNPIYWAR	2	5700.861	3	3.220	94.08	15	0.404
54-95	KFYAITTLVPAIAFTMYLSMLL GYGLTMVPFGGEQNPIYWAR	0	4744.441	3	4.104	84.84	14	0.648
96-142	YADWLFTTPLLLLDALLVDADQ GTILALVGADGIMIGT LVGALT	0	4841.666	3	5.345	81.13	13	1.119
96-147	YADWLFTTPLLLLDALLVDADQ GTILALVGADGIMIGT GLVGALT <u>KVSYR</u>	1	5509.994	3	4.793	65.15	10	0.940
148-172	FVWWAISTAAMLYILYVLFFGFTSK	0	2974.561	2	3.990	71.63	11	1.424
173-185	AESMRPEVASTFK	0	1452.715	2	3.257	30.67	3	-0.523
173-188	AESMRPEVASTFK VLR	1	1820.969	2	3.505	32.26	4	-0.206
189-238	NVTVVLWSAYPVWLI <u>GSEG</u> <u>AGIVPLN</u> IETLLFMVLDVSAK <u>VGFGLI</u> LLR	1	5381.031	3	2.984	78.35	12	1.298
230-238	VGFGLILLR	0	987.635	2	3.720	46.51	7	1.956
239-262	SRAIFGEAEAPEPSAGDGAAATSD	1	2277.026	2	2.308	36.98	5	-0.400
228-248	AIFGEAEAPEPSAGDGAAATS	0	1918.866	2	4.261	38.87	6	-0.038

¹ Number of missed tryptic cleavages.

² All theoretically predicted tryptic peptides were analyzed using ProtParam software that allows the calculation of the grand average of hydrophobicity (GRAVY). The peptides exhibiting positive GRAVY values are recognized as hydrophobic

Bacteriorhodopsin

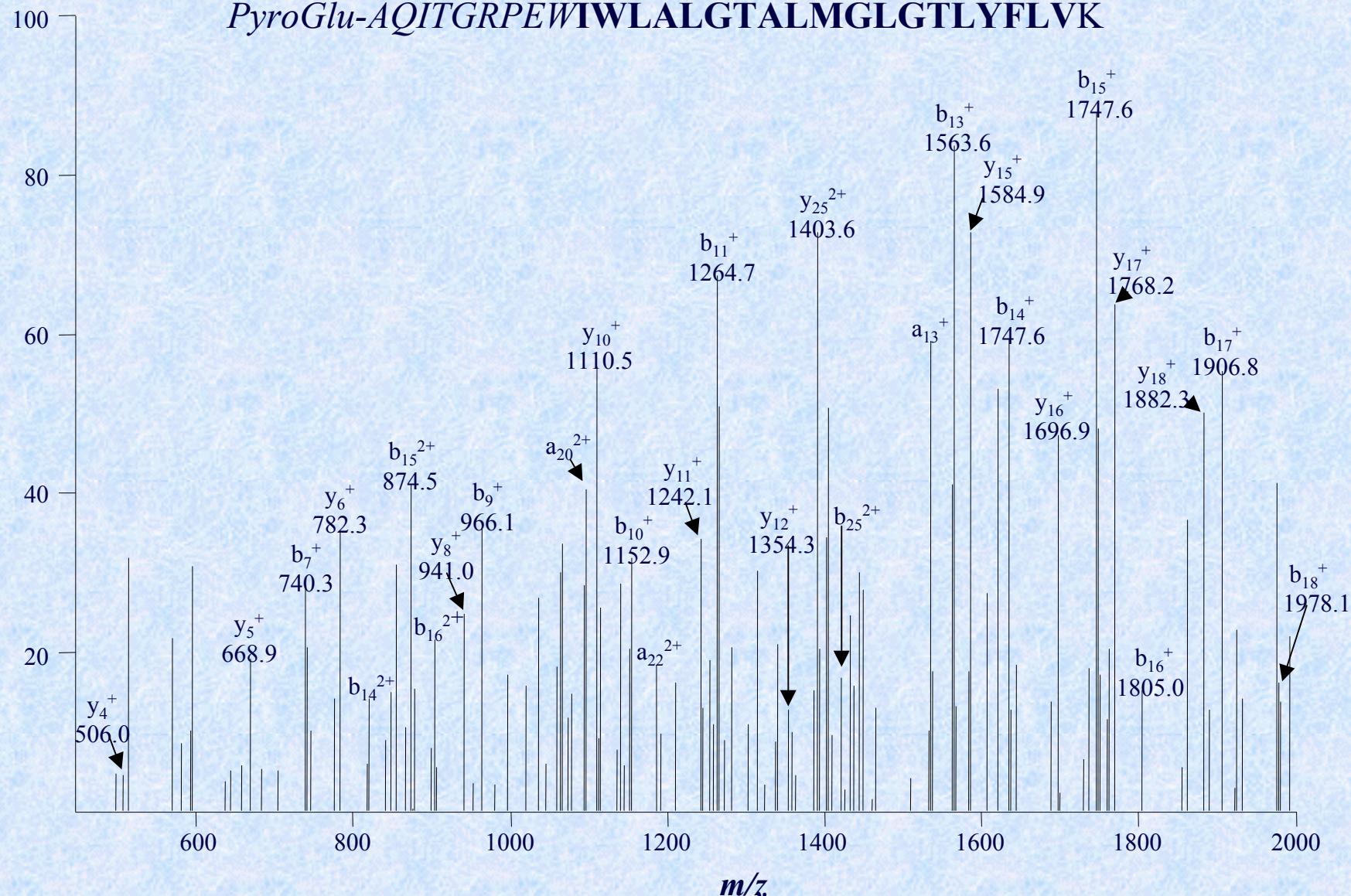




Unmodified N-terminal, 43 amino acid long, peptide completely covers first transmembrane domain of immature bacteriorhodopsin. The MS/MS spectrum is derived from a triply charged molecular ion (calculated mass of 4686.54) and resulted in an Xcorr of 3.9 and DelCn of 0.79 from the SEQUEST analysis.

PyroGlu-AQITGRPEWIWLALGTALMGLGTLYFLVK

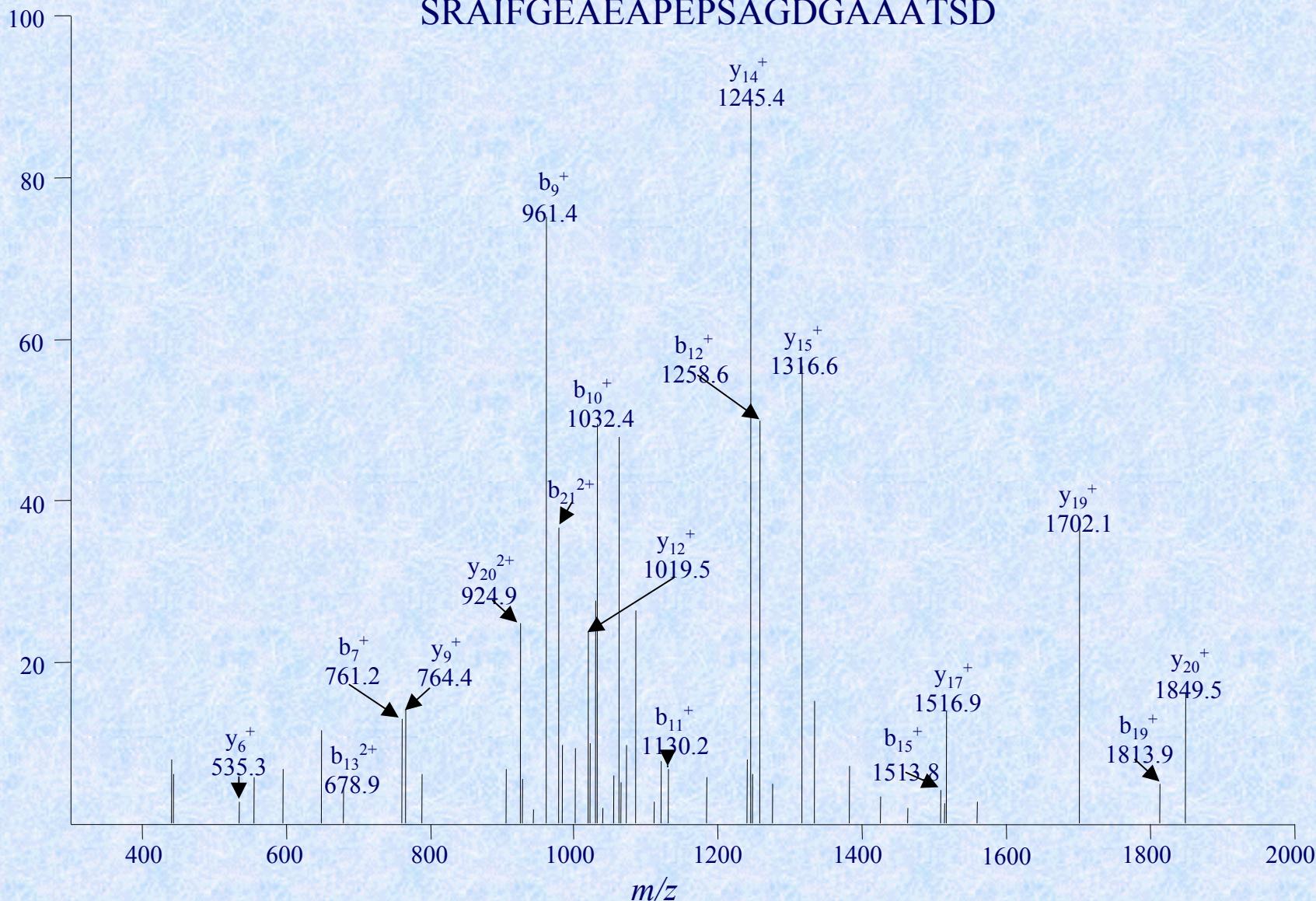
Relative Abundance (%)



Post-translationally modified N-terminal, 30 amino acid long peptide completely traverses the first transmembrane domain of mature bacteriorhodopsin. The MS/MS spectrum is derived from a doubly charged molecular ion (calculated mass of 3329.79) exhibiting an Xcorr of 2.7 and DelCn of 0.7 from the SEQUEST analysis.

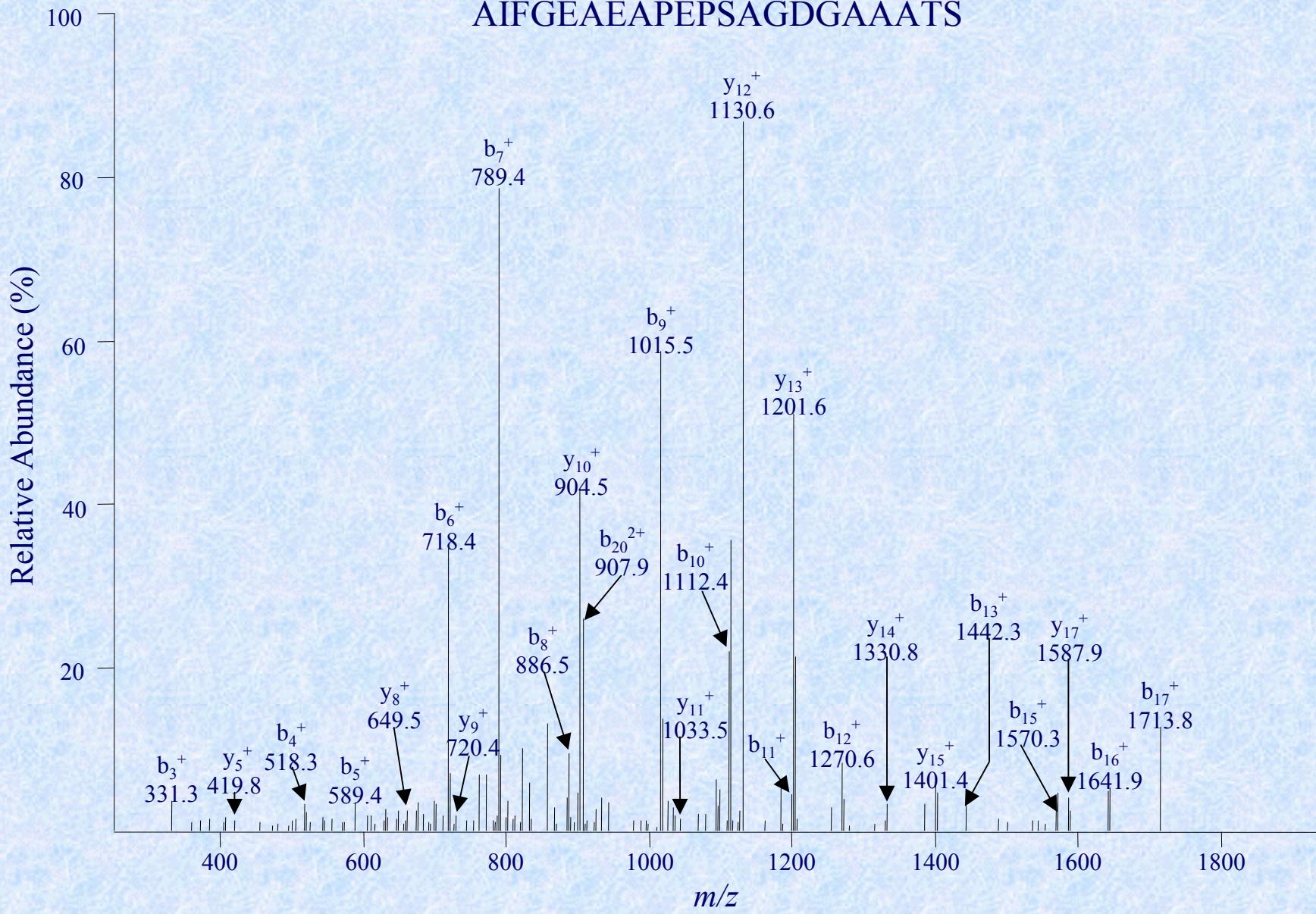
SRAIFGEAEAPEPSAGDGAAATSD

Relative Abundance (%)



Unmodified C-terminal, 24 amino acid long peptide The MS/MS spectrum is derived from a doubly charged molecular ion (calculated mass of 2277.03) exhibiting an Xcorr of 2.3 and DelCn of 0.3 from the SEQUEST analysis.

AIFGEAEAPEPSAGDGAAATS



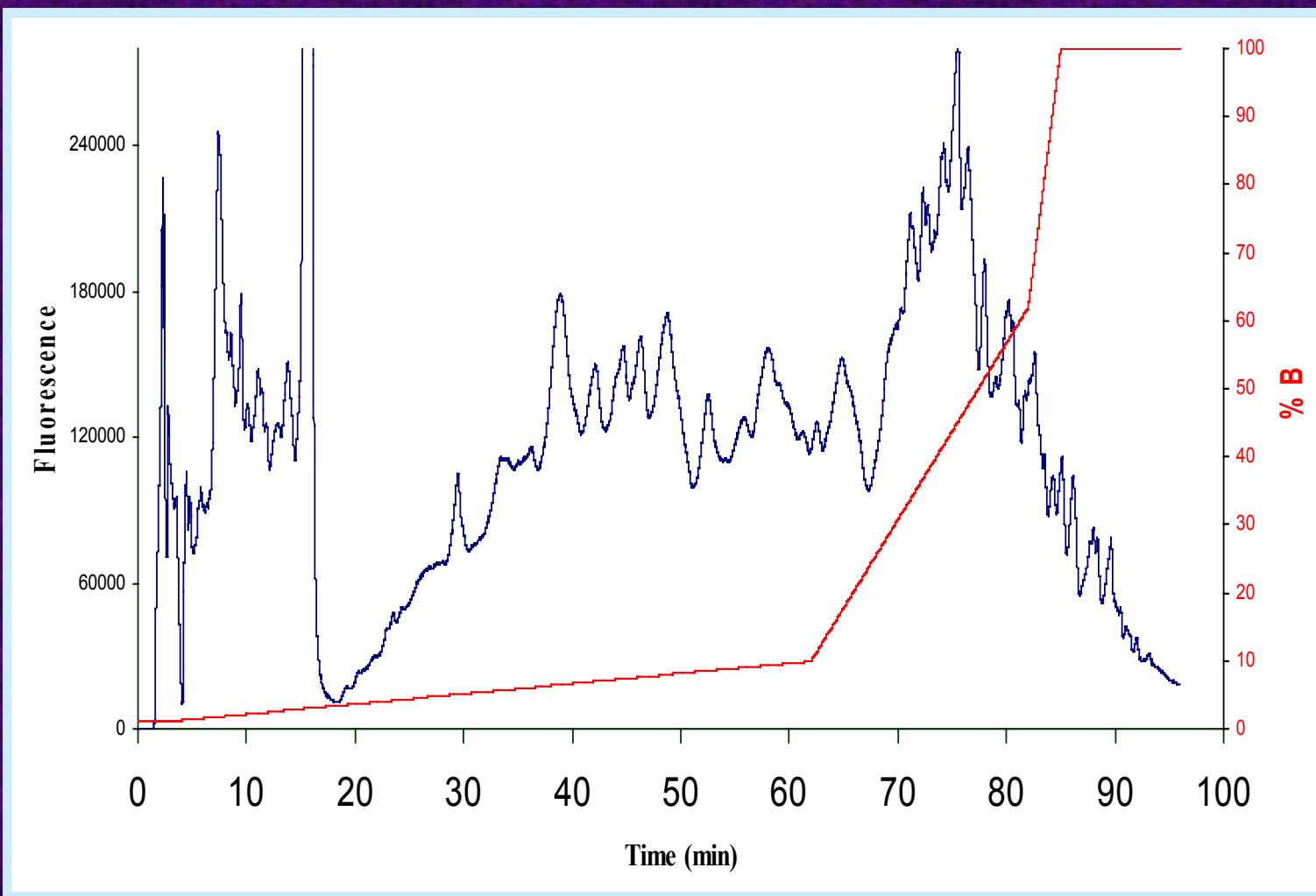
Modified C-terminal, 21 amino acid long peptide. The MS/MS spectrum is derived from a doubly charged molecular ion (calculated mass of 1918.87) exhibiting an Xcorr of 4.2 and DelCn of 0.4 from the SEQUEST analysis.

Accession No	Gene name	Description	Biological process	Pep ¹	TMD ²	GRAVY ³
NP_280801	atpK	H ⁺ -transporting ATP synthase	hydrogen transport	1	1	1.32
NP_280292	bop	Bacteriorhodopsin*	energy production	13	7	0.72
AAG20326	coxA1	cytochrome c oxidase, subunit I	electron transport	2	8	0.68
NP_280847	coxB2	cytochrome c oxidase, subunit II	electron transport	2	0	-0.42
NP_281222	csg	cell surface glycoprotein	unknown	3	1	-0.57
NP_279616	cyb	cytochrome b6	electron transport	2	2	0.42
NP_395927	cydA	cytochrome d oxidase, chain I	electron transport	9	8	0.44
NP_395926	cydB	cytochrome d oxidase, chain II*	electron transport	2	9	0.87
NP_281108	dppD	dipeptide ABC transporter	transport	2	0	-0.45
NP_279779	hcpC	halocyanin precursor-like	copper binding	1	0	-0.31
NP_280948	hdrD	heterodisulfide reductase	electron transport	2	4	-0.20
NP_279271	hsp4	heat shock protein	zinc binding	1	3	0.03
NP_280433	htr1	Htr1 transducer	signal transduction	2	1	-0.40
NP_280332	htr8	Htr8 transducer	signal transduction	1	6	-0.06
NP_279419	ids	isoprenyl diphosphate synthase	isoprenoid biosynthesis	4	1	-0.34
NP_279583	imp	immunogenic protein	signaling	3	0	-0.12
NP_395709	kdpB	K ⁺ -transporting ATPase B chain	cation transport	1	7	0.16
NP_395810	nhaC3	Na ⁺ /H ⁺ antiporter	sodium transport	2	10	0.74
NP_280992	nosY	nitrite/nitrate reduction protein	nitrite/nitrate reduction	2	6	0.83
NP_279665	nuoL	quinone oxidoreductase chain L	oxidative phosphorylation	1	14	0.74
NP_280081	panI	membrane protein	copper binding	1	0	-0.49
NP_280174	sdhC	succinate dehydrogenase subunitC	tricarboxylic acid cycle	1	3	0.77
NP_280173	sdhD	membrane anchor	electron transport	1	2	0.64
NP_280680	secD	protein-export membrane protein	protein-export	2	5	0.22
NP_279456	secE	protein translocase	protein transport	2	1	1.17
NP_280681	secF	protein-export membrane protein	protein transport	2	6	0.72
NP_280434	sopI	sensory rhodopsin*	signal transduction	2	5	0.87
NP_395821	trp6	ABC transporter	transport	1	0	-0.28

Global Proteomic Analysis of Membrane Proteins from Mouse NK Cell Microsomal Fraction

Blonder, J., Rodriguez, C., Young, H., Veenstra, T. D., and Conrads, T. P. Investigation of the Natural Killer Cell Membrane Proteome Employing Gas-phase Fractionation by Mass Spectrometry **2003** (submitted).

SCX chromatogram of 175 µg of mouse NK cell microsomal peptides



Characteristics of NK cells microsomes sample preparation and analysis

Microsomal fraction of NK cells was prepared using differential centrifugation.

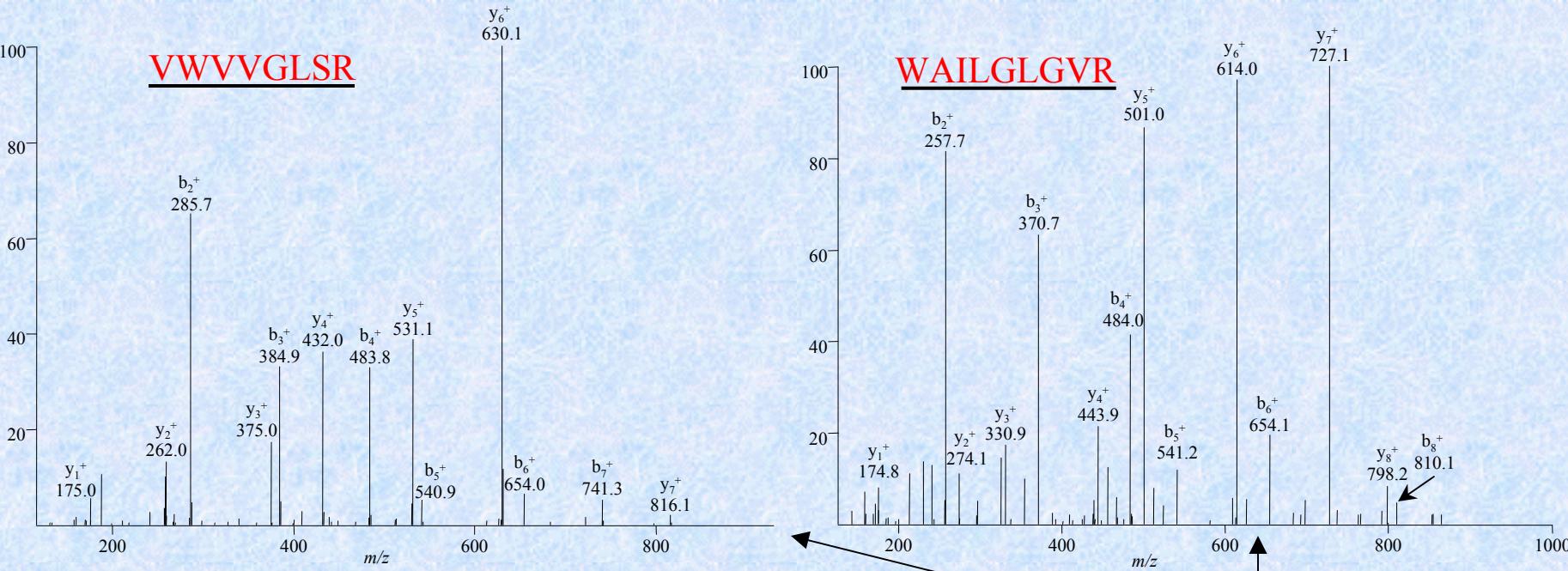
**The extraction, solubilization, and proteolytic digestion of membrane proteins
was identical single tube procedure used for the purple membrane analysis.**

**The resulting peptides were fractionated into 96 fractions by SCX
chromatography prior to μ LC-MS/MS.**

**Microcapillary LC-MS/MS analysis of the 96 SCX fractions resulted in the
detection of 5782 unique peptides and 2563 unique protein.**

**Of these, 768 (30%) have at least one mapped transmembrane domain and are
recognized as α -helical integral membrane proteins.**

Accession #	Description	Pep	TMD	GRAVY
11993939	ATP-binding cassette, sub-family A, member 2	2	12	-0.080715
9506367	ATP-binding cassette, sub-family B, member 10	2	5	0.114266
5902709	ATP-binding cassette, sub-family B, member 7	3	4	-0.032421
22095457	ATP-binding cassette, sub-family B, member 9	2	8	0.230972
7305539	ATP-binding cassette, sub-family B;transporter 1	9	9	0.283012
11038668	ATP-binding cassette, sub-family C, member 9	2	13	0.125227
6671497	ATP-binding cassette, sub-family D, member 1	3	3	-0.008561
6753408	cadherin EGF LAG seven-pass G-type receptor 1	2	7	-0.233191
9790015	coated vesicle membrane protein; Sid394p	2	2	0.007463
280947	complement receptor CR1 precursor	2	2	-0.154091
109889	glucose transport protein GT1 - mouse	4	12	0.519309
7949043	golgi SNAP receptor complex member 1	2	1	-0.542111
12643401	histidine-rich membrane protein Ke4	2	6	-0.238234
6754390	inositol 1,4,5-triphosphate receptor 1	2	6	-0.317026
6680486	integrin alpha V (Cd51),(vitronectin receptor)	6	2	-0.245594
20887691	integrin beta 1 (fibronectin receptor beta)	4	2	-0.369674
6680498	intergral membrane protein 1	4	12	0.231915
6680590	killer cell lectin-like receptor, subfamily A	3	1	-0.57406
6754502	lysosomal membrane glycoprotein 2	3	2	-0.035904
6755244	protein tyrosine phosphatase, receptor type, C	6	2	-0.595052
6678137	signal recognition particle receptor, B subunit	5	1	-0.076952
6678145	signal sequence receptor, delta	4	2	0.140116
20902823	similar to mitochondrial import receptor	3	1	-0.169718
20886541	similar to T-cell receptor alpha chain V regi	5	0	-0.686713
13878806	transmembrane 9 superfamily protein (T9S)	3	9	0.266667

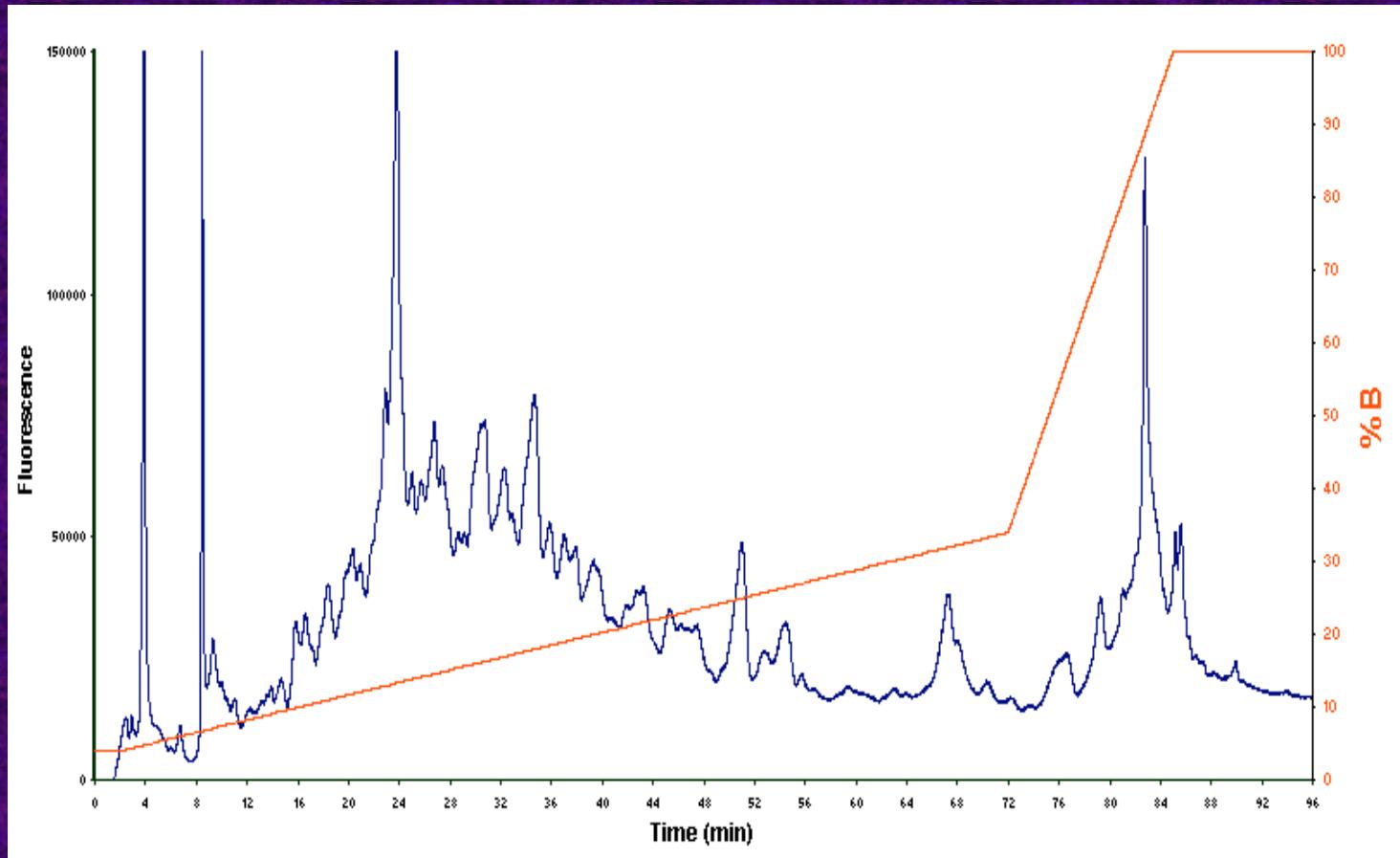


MAAHVWLAAALLLVDWLLLPRMLPGIFSLLVPEVPLLRVWVVGLSRWAILGLGVRGVLGVTAGA
GWLAALQPLVAALSLALPGLALFRELAAWGTLREGDSAGLLYWNSRPDAFAISYVAALPAAALWH
KLGSLWAPSGNRDAGDMLCRMLGFLGPKKRRLYLVLVLLILSCLGEMAIPFFTGRITDWQDKTVPSF
TRNIWLMSILTIASTALEFASDGIYNITMGHMHGRVHREVFRAVLRQETGFFLKNPAGSITSRVTEDT
CESISDTLSLLLWYLGRALCLLVFMFWGSPYLTVTLINLPLLFLPKKLGKVHQSLAVKVQESLAK
STQVALEALSAMPTVRSFANEEGEAQKFRQKLEEMKTLNKKEALAYVAEVWTTSVSGMLLKGILY
LGGQLVIRGTVSSGNLVSFVLYQLQFTQAVQVLLSLYPSMQKAVGSSEKIFYEYLDRTPCSPLSGSLAP
SNMKGLVEFQDVSFAYPNQPKVQVLQGLTFTLHPGTVTALVGPNNGSKSTVAALLQNLYQPTGGQL
LLDGQRLVQYDHHLHTQVAAVGQEPLLGRSFRENIAYGLNRTPTMEEITAVAVESGAHDFSFQPQG
YDTEVGETGNQLSGGQRQAVALARALIRKPLLILDDATSALDAGNQLRVQRLLYESPKRASRTVLL
ITQQQLS LAEQAHHLFLREGSVGEQGTHLQLMKRGGCYRAMVEALA APAD

Transporter 1, a seven transmembrane domain (red) ATP-binding cassette protein identified from 9 peptides (underlined) of which two hydrophobic peptides (MS/MS spectra shown) completely cover first transmembrane domain exhibiting Xcorr values of 2.7 for each peptide.

Large-scale Proteomic Analysis of Keratinocytes Plasma Membrane Enriched from Human Epidermis

SCX chromatogram of 100 µg of keratinocyte plasma membrane digestate



Characteristics of keratinocyte plasma membrane sample preparation and analysis

Evaluation of the applicability of the method for the human tissue global membrane proteomics

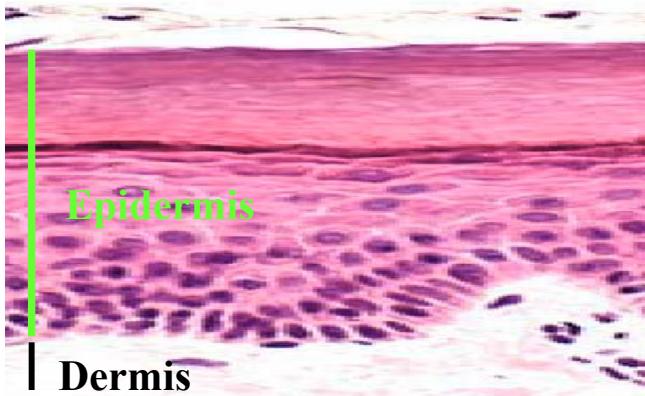
A highly enriched keratinocite plasma membrane fraction was isolated from human epidermis using sucrose gradient centrifugation.

The extraction, solubilization, and proteolytic digestion of membrane proteins was identical single tube procedure used for the purple membrane analysis.

The resulting peptide mixture was fractionated using off-line strong cation-exchange (SCX) chromatography, and analyzed by reversed phase μ LC-MS/MS.

Within the set of 2,875 uniquely detected peptides 1,306 proteins were identified of which 670 (51.3%) were annotated as integral membrane proteins or plasma membrane associated proteins.

A LARGE-SCALE, 2D- μ LC-MS/MS ANALYSIS OF THE PLASMA MEMBRANE SUBPROTEOME OF HUMAN EPIDERMIS



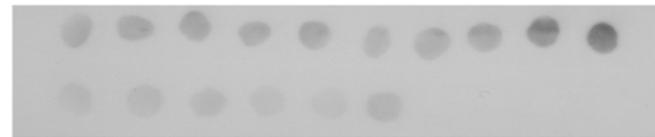
Epidermis Isolation



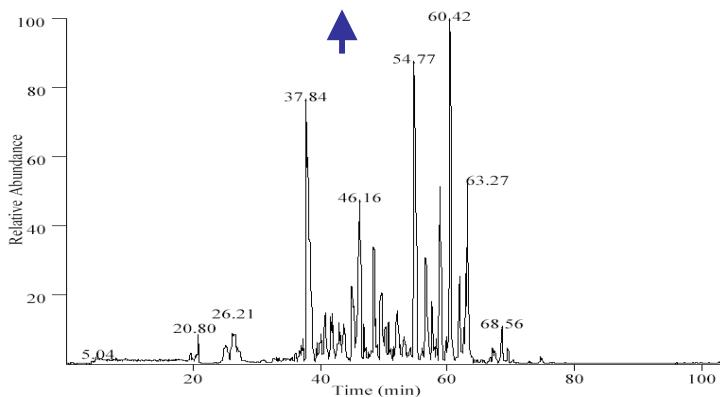
Plasma Membrane Purification



Immunoblot Analysis for $\alpha 6$ -integrin



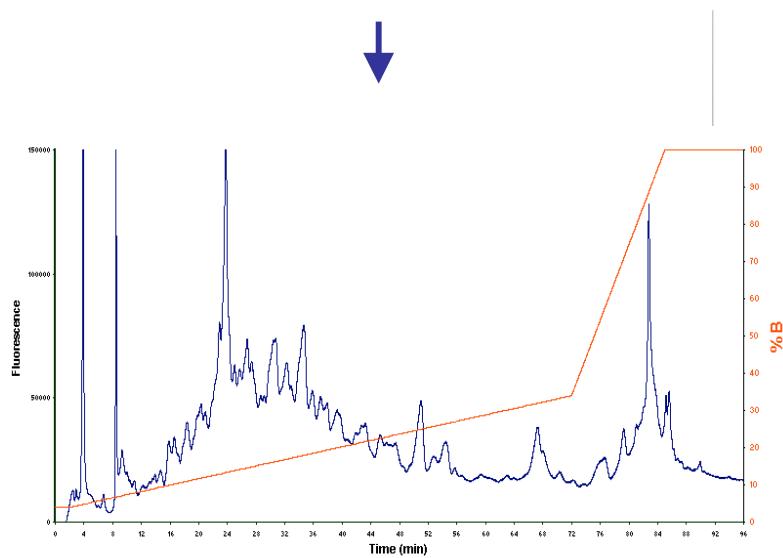
μ LC-MS/MS



A single SCX fraction

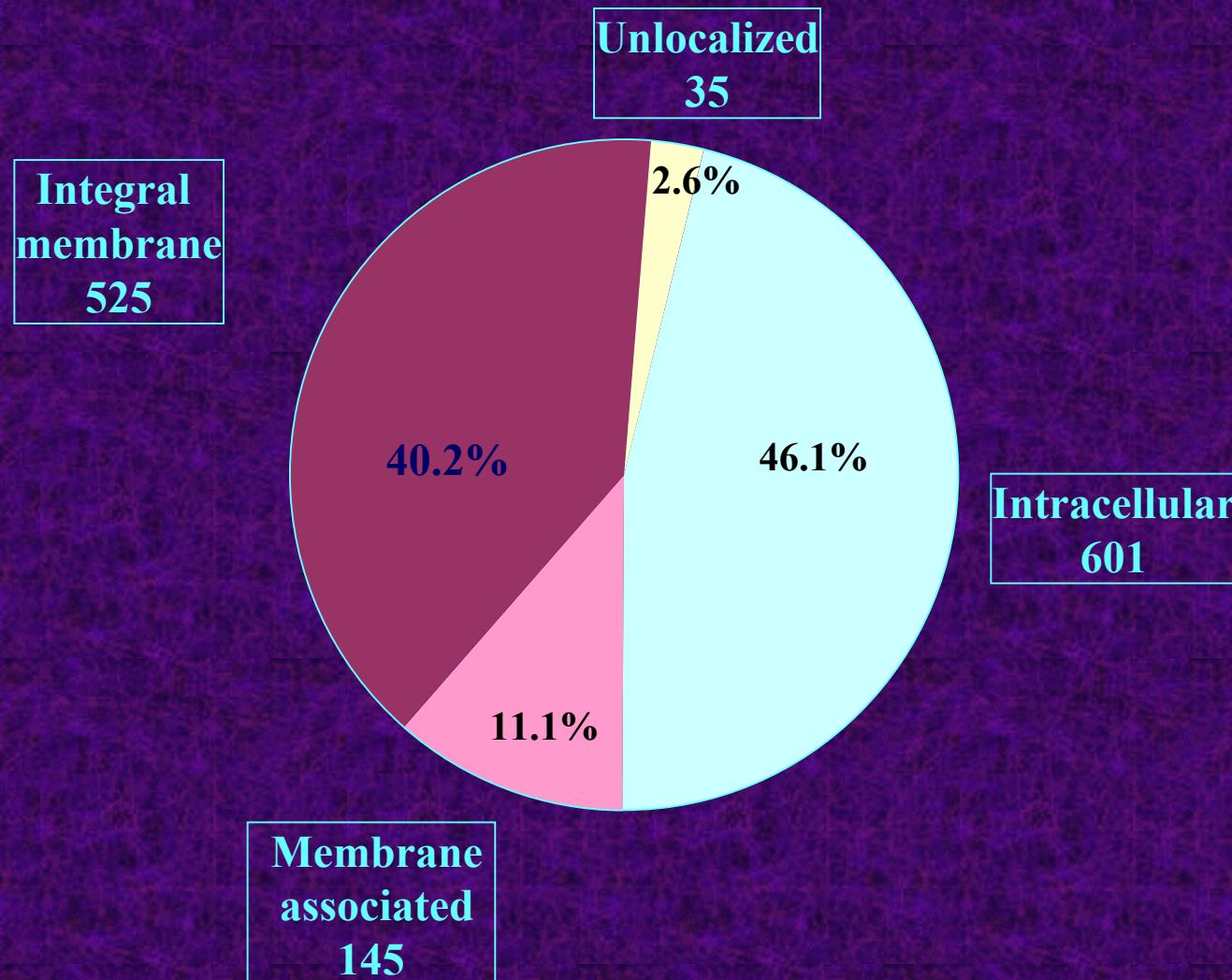


Extraction, Solubilization, and Digestion
of Plasma Membrane Proteins



SCX fractionation

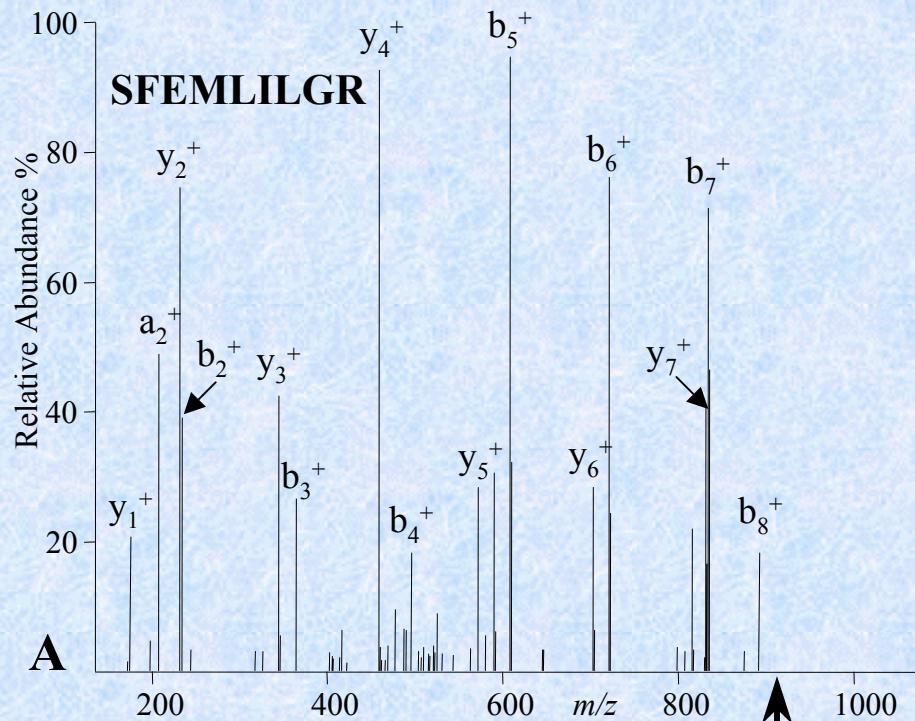
Cellular compartment classification of the 1306 proteins identified in the analysis of the keratinocyte plasma membrane



Selected subset of hydrophobic integral membrane proteins identified from keratinocyte plasma membrane.

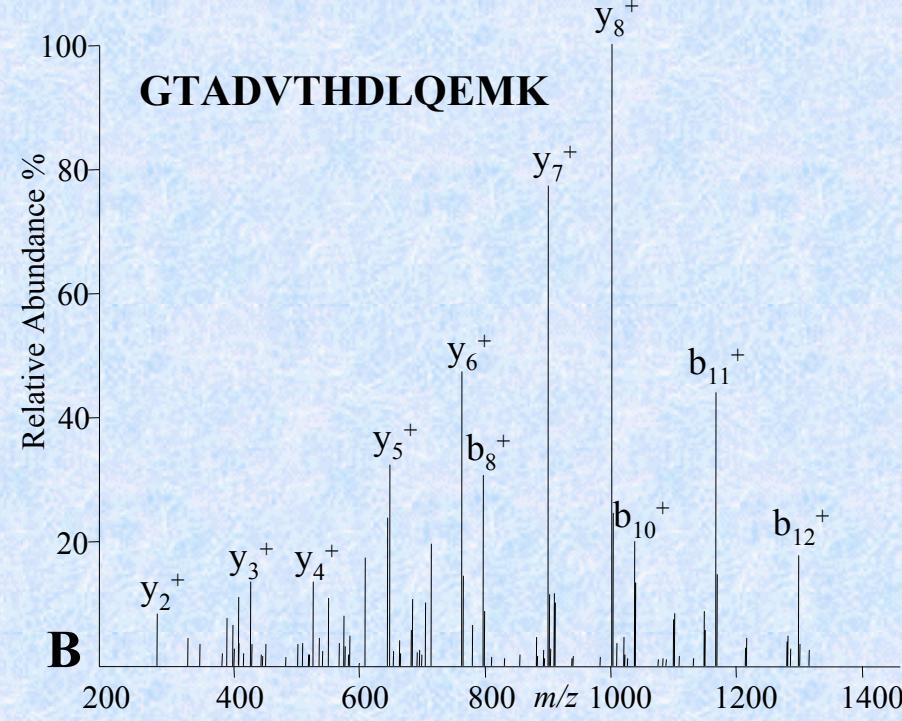
Description	Accession ¹	GRAVY ²	TMD ³	Peptide ⁴	Protein location
Glucose transporter type 1	P11166	0.532114	12	6	Plasma membrane
Sodium/potassium ATPase, alpha-1	P05023	0.011241	10	24	Plasma membrane
Oligosaccharyl transferase	P46977	0.237873	13	6	Plasma membrane
Choline transporter-like protein	Q8WWI5	0.54551	10	3	Plasma membrane
Neutral amino acid transporter	Q15758	0.643807	10	4	Plasma membrane
Large neutral amino acids transporter	Q01650	0.739053	11	4	Plasma membrane
Defender against cell death	P46966	0.824779	3	3	Plasma membrane
CTL2 gene	Q8IWA5	0.397592	10	4	Plasma membrane
Clathrin-coated vesicle proton pump	Q93050	0.019134	8	5	Plasma membrane
Probable cation-transporting ATPase	Q9HD20	0.114037	10	3	Plasma membrane
Transmembrane 9 superfamily member 3	Q9HD45	0.24584	9	4	Plasma membrane
Transmembrane 9 superfamily member 4	Q92544	0.1712	9	4	Plasma membrane
BA207C16.3 -hypothetical protein	Q9NQL6	0.258334	9	4	Plasma membrane

¹ Accession number, Swiss-Prot Release of 08/22/03, ² GRAVY value calculated using ProtParam tool at <http://us.expasy.org/>, ³ Number of mapped transmembrane domains by TMHMM at <http://www.cbs.dtu.dk/services/TMHMM/>, ⁴ Number of peptides detected



MEPSSKKLTG
EEFYFNQQTWVH
FSVGLFVNRF
MLILGRFIIG
QLGIVVGILI
CIVLPFCPES
LQEMKEESRQ
SQQLSGINAV
TVVSLFVER
LPWMSYLSIV
PAAIAVAGES

RIM**L**AVGGAV
RYGESI**L**PTT
GRRN**S**M**I**M**M**N
VY**C**GLTTGFV
AQVFGLDSIM
PRFLLINRNE
MMREKKVTIL
FYYSTSIFEK
AGRRTLHLIG
AIFGFVAFF
NWTSNFIVG
CFOYVEQLC
PYVFIIFTV
Relative Abundance %



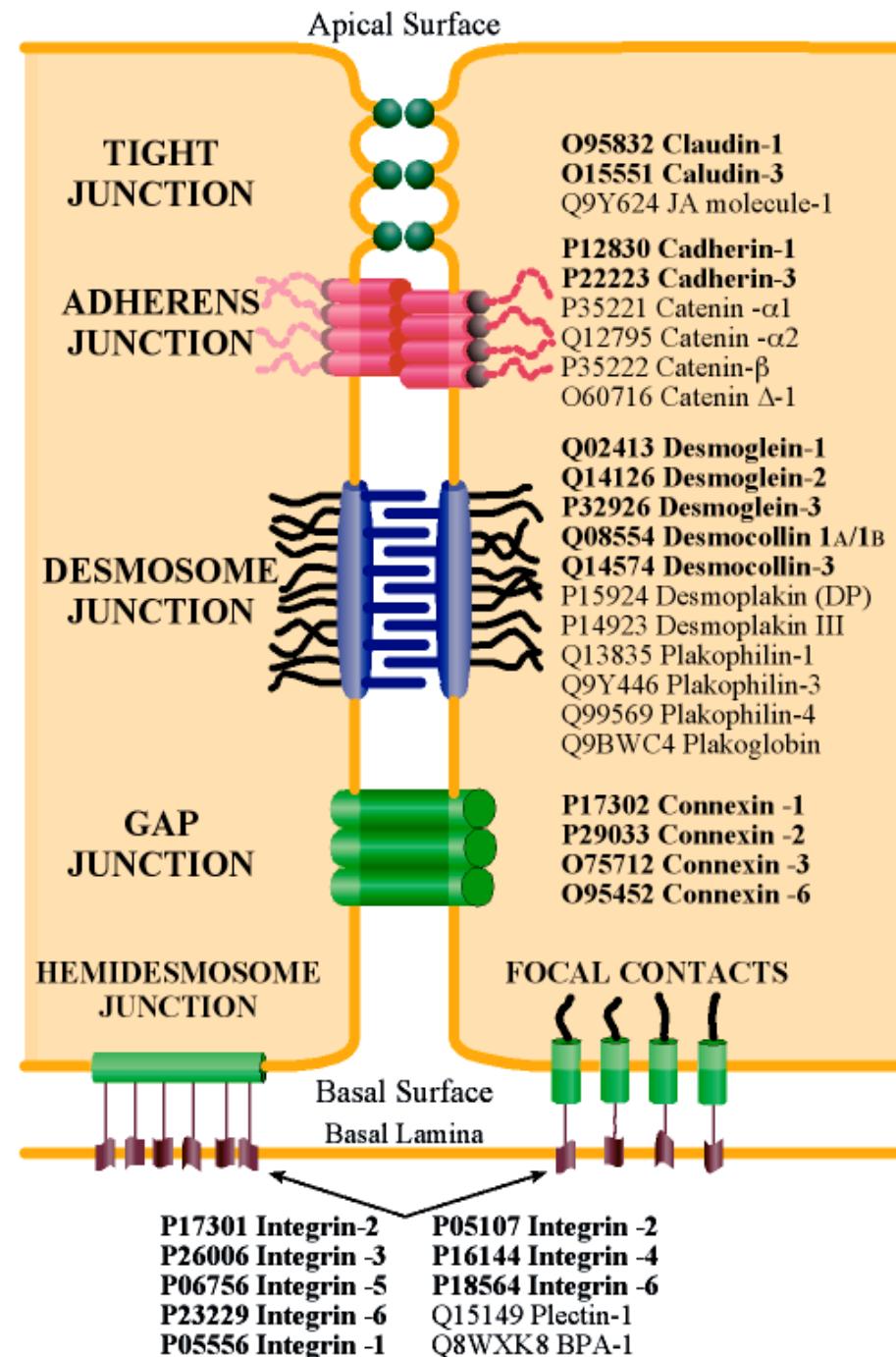
LGSIQFGYN**T**
LTTLWSLSV**A**
LLAFVSAV**L**M
PMYVGEVSPT
GNKDLWPLL
ENRAKSVLKK
ELFRSPAYRQ
AGVQQPVYA**T**
LAGMAGCAI**L**
VGPGPIPWF**I**
CFOYVEQLC
PYVFIIFTV
Relative Abundance %

Subset of confirmed lipid rafts proteins identified from keratinocyte plasma membrane

Description	Accession ¹	Location ²	Peptide ³
Flotillin-1	O75955	LR	5
Flotillin-2	Q14254	LR	6
SNAP-23	O00161	LR	4
Ras-related (RalA)	P11233	LR	6
Ras-like protein (Rac 1)	P15154	LR	3
Stomatin	P27105	LR	5
Junction plakoglobin	P14923	LR	8
Aminopeptidase N (CD13)	P15144	LR	3
Heat shock protein HSP 90-alpha	P07900	LR	5
CD44 antigen	P16070	LR	6
V-ATPase subunit A1	Q93050	LR	5
V-ATPase subunit A2	Q9Y487	LR	2
V-ATPase subunit A3	Q13488	LR	3

¹ Accession number, Swiss-Prot Release of 08/22/03, ² LR, lipid rafts, ³ Number of peptides detected.

The subset of the cell adhesion proteins identified from the keratinocytes plasma membrane displayed in accordance to their junction affiliation (transmembrane linkers printed in bold font and attachment proteins printed in normal font). **(A)** The tight junctions, are localized toward the exterior of the epithelial cell surface. They separate the apical plasma membrane domain from the basolateral one and concomitantly ensure the paracellular sealing preventing apical membrane proteins from moving into basolateral domains. **(B)** Adherens junctions are situated distally from tight junctions. They contain cadherin-type transmembrane linkers, which maintain the tissue structure and its polarity. **(C)** Desmosomes are button-like junctions of the plasma membrane formed of disc-like plaques paired with those of neighboring cells via transmembrane linker proteins. These junctions, unlike adherens, are comprised of two families of cadherin-like proteins: glycosylated integral membrane linker-proteins (desmogleins and desmocollins) and non-glycosylated intracellular attachment proteins (plakins and armadillo proteins). **(D)** Human keratinocytes share inorganic ions and other small water-soluble molecules through clusters of tightly packed intercellular plasma membrane channels known as gap junctions. **(E)** Basal epidermal keratinocytes are attached to an underlying basal membrane via hemidesmosomes and focal contacts, which are formed of different proteins from integrin family. These proteins serve as transmembrane linkers and receptors, which are involved in signaling pathway that control cell migration and tissue spatial structure.



Cell Disruption
Homogenization, sonication



Membrane Isolation and Purification

Differential centrifugation
Sucrose gradient centrifugation



Optional labeling



Single Solution Extraction, Solubilization and Tryptic Digestion of Membrane Proteins

Buffered organic/aqueous system
60% MeOH in 25 mM NH₄HCO₃, pH 7.9



**One-dimensional
Chromatographic
Separation**

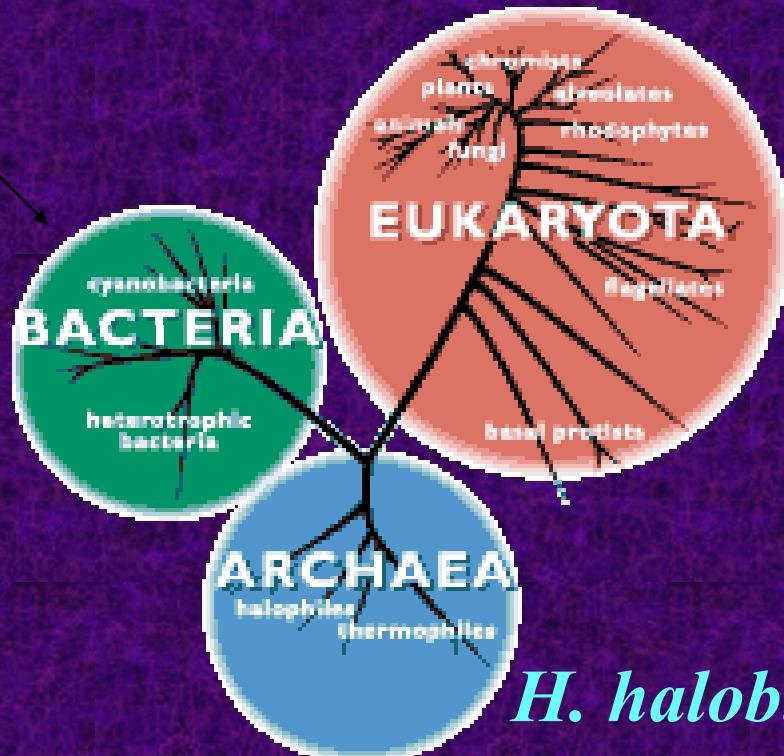
Reversed-phase μLC–MS/MS

**Two-dimensional Chromatographic
Separations**

Strong cation exchange (SCX)
Reversed-phase μLC–MS/MS

General Applicability of Developed Technique

D. radiodurans
P. aeruginosa

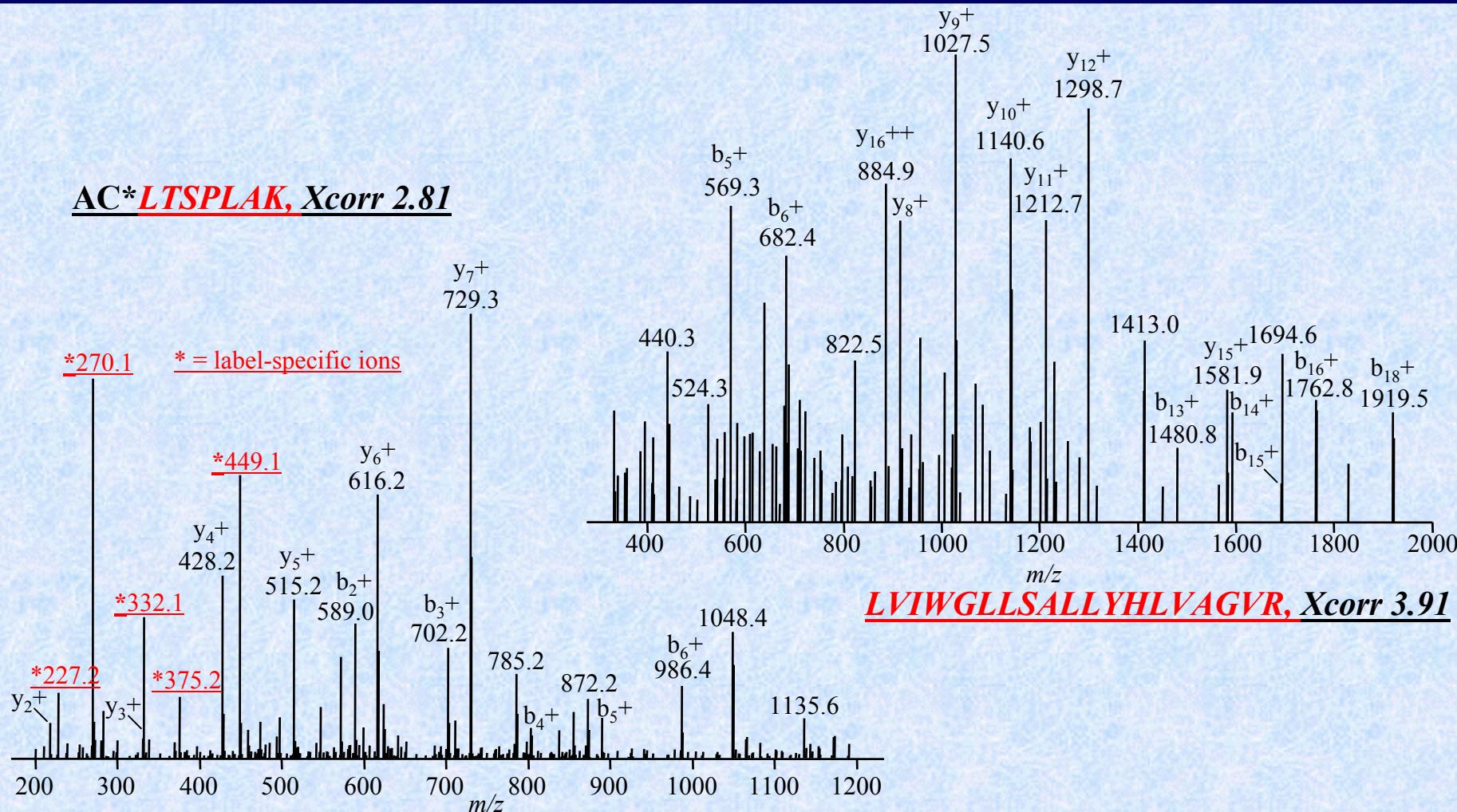


H. halobium

H. Sapiens
M. musculus

Towards Quantitative Membrane Proteomics

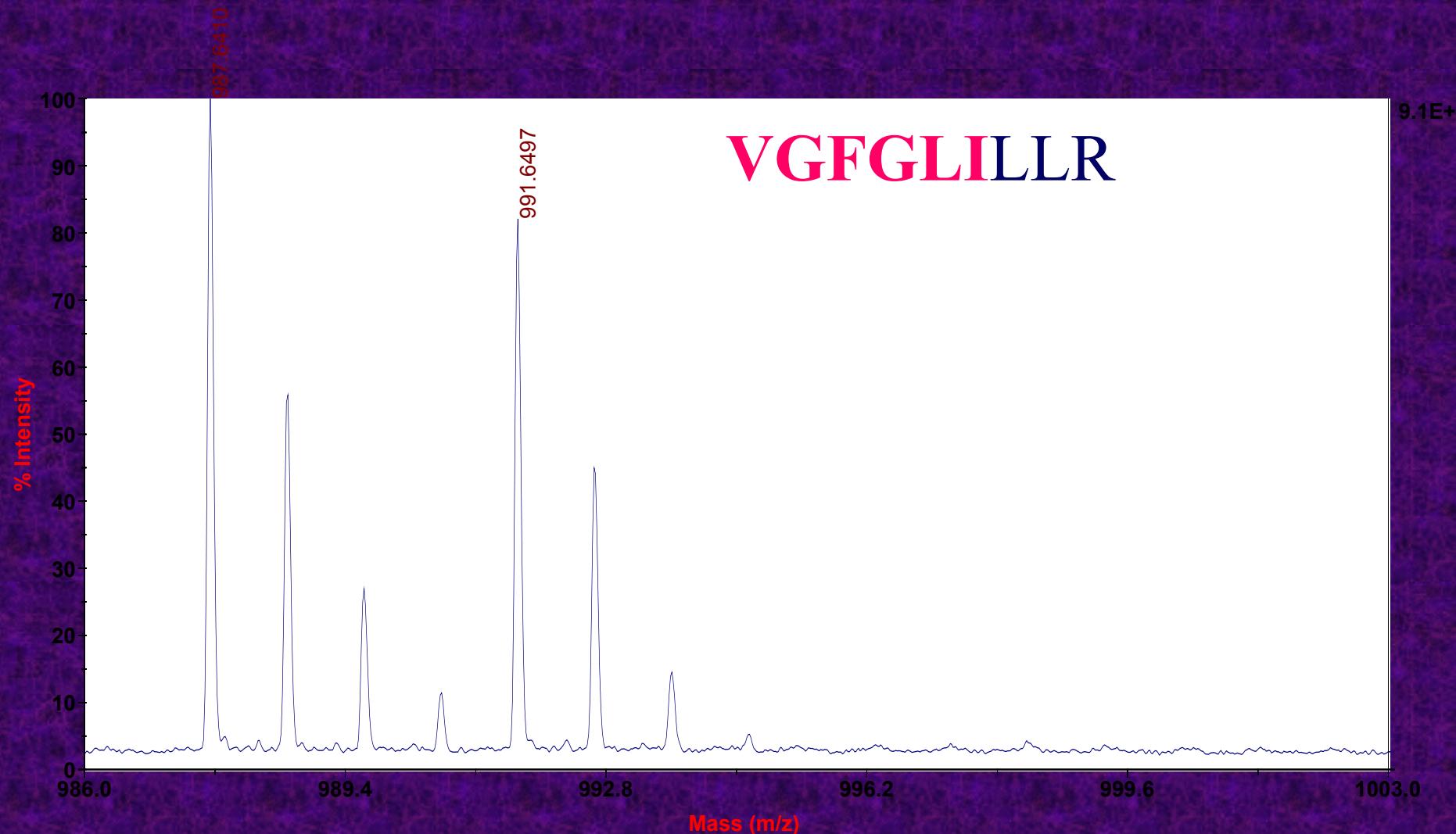
Cysteinyl-specific labeling of membrane spanning peptide of *P. aeruginosa* succinate dehydrogenase (*sdhC*) using iodoacetyl-PEO-biotin.



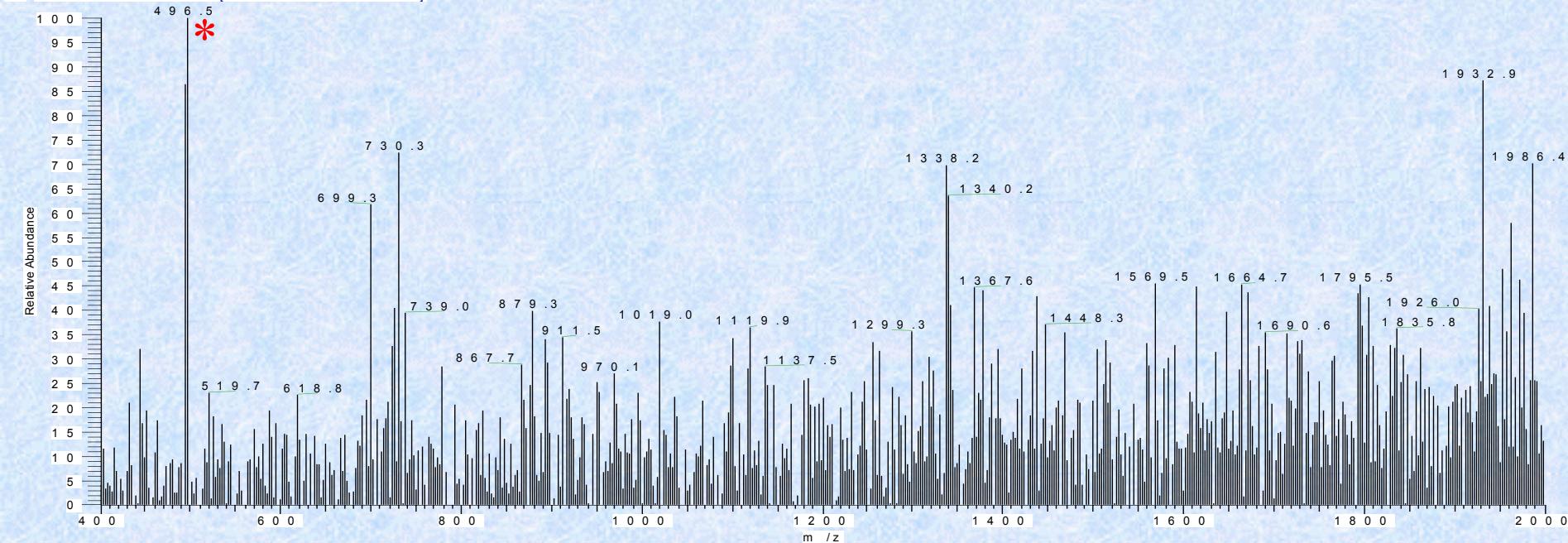
MKKAVNSKRPVNLDLRTIKLPVTAYT**SILHRISGVILFLGIAVL**
LFALDKSISSEEGFEQVK AC**LTSPLAK* **LVIWGLLSALLYHLVA**
GVR HLVMDAGVGETLEGGK**RGSKIVIAIAVVLI VLAGV**WW

Bacteriorhodopsin - O16/O18 labeling

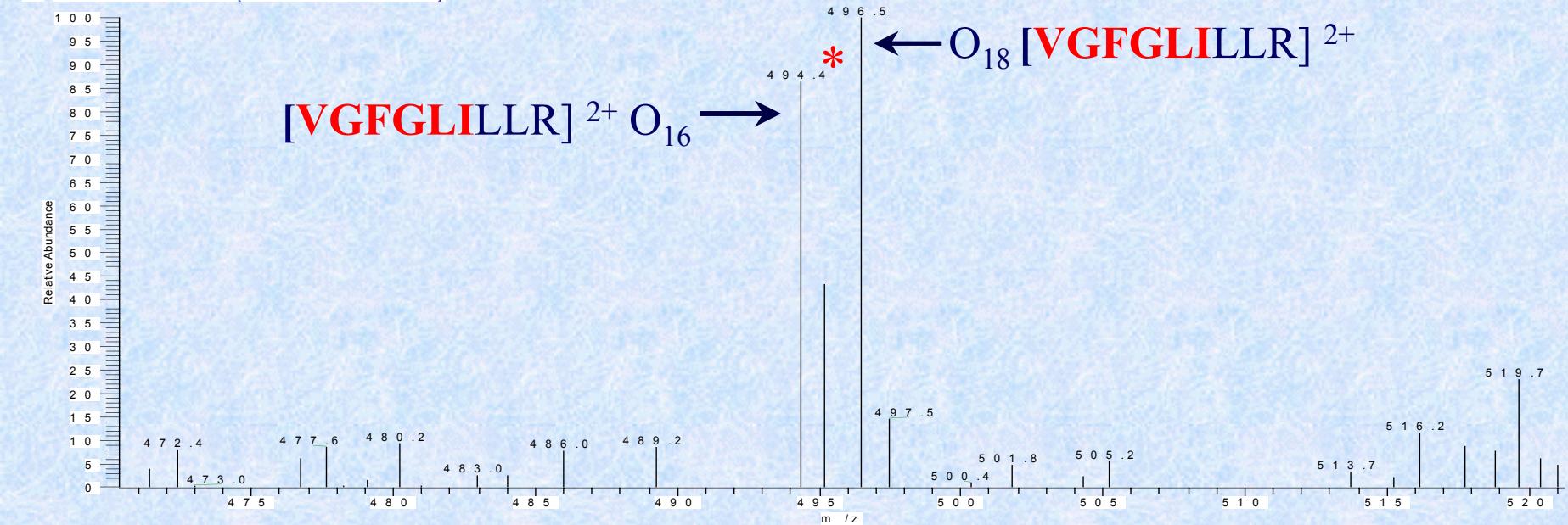
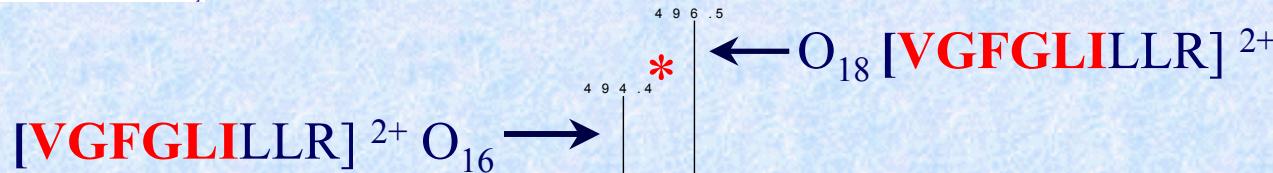
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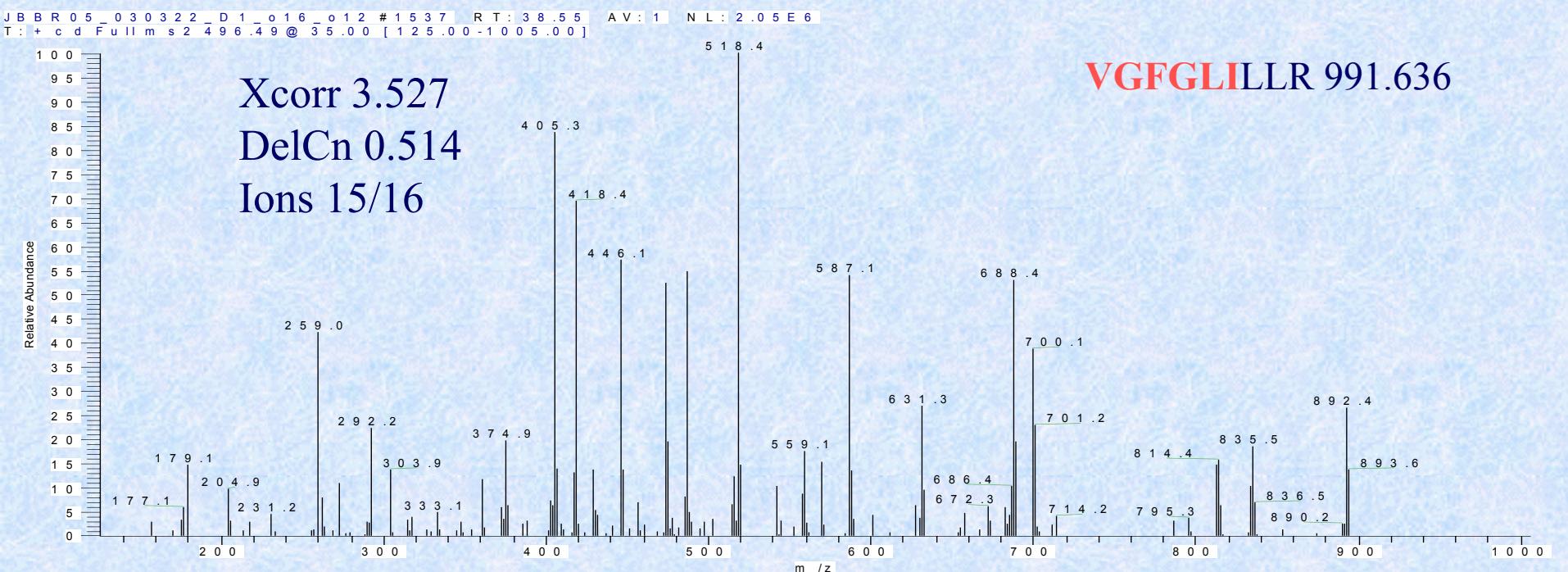
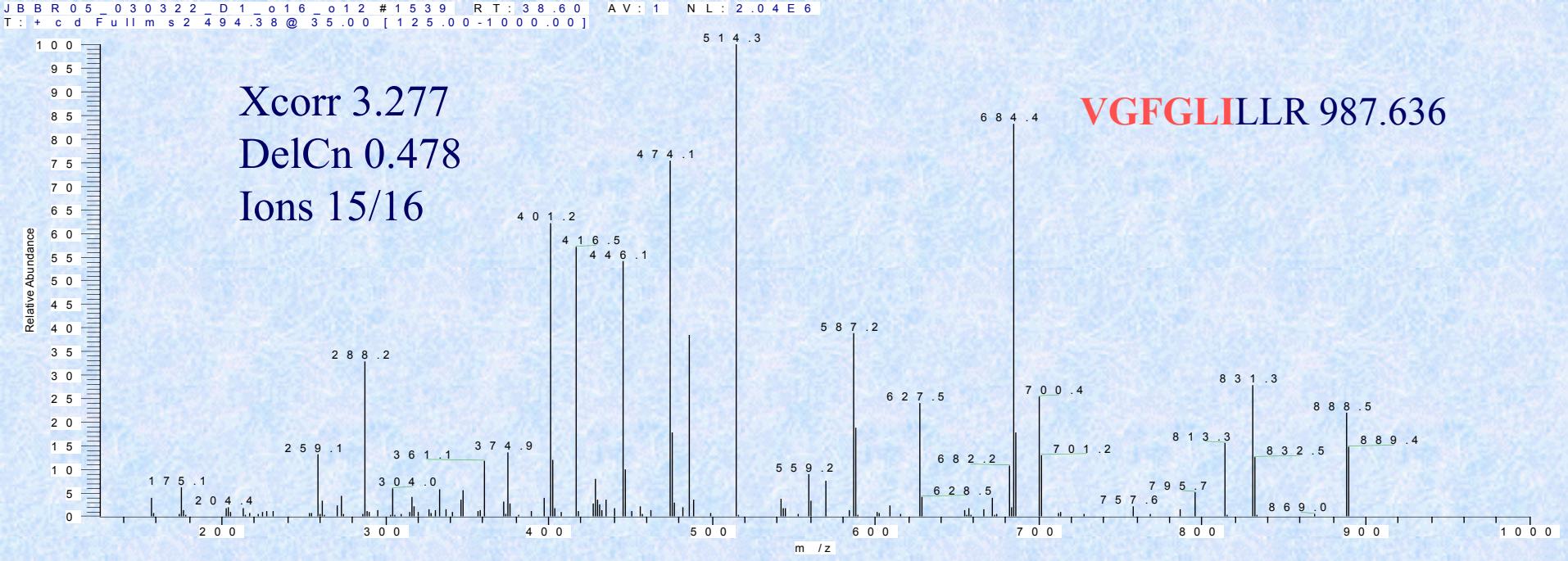


J B B R 0 5 _ 0 3 0 3 2 2 _ D 1 _ o 1 6 _ o 1 2 # 1 5 3 6 R T : 3 8 . 5 2 A V : 1 N L : 1 . 3 7 E 7
T : + c N S I F u l l m s [4 0 0 . 0 0 - 2 0 0 0 . 0 0]



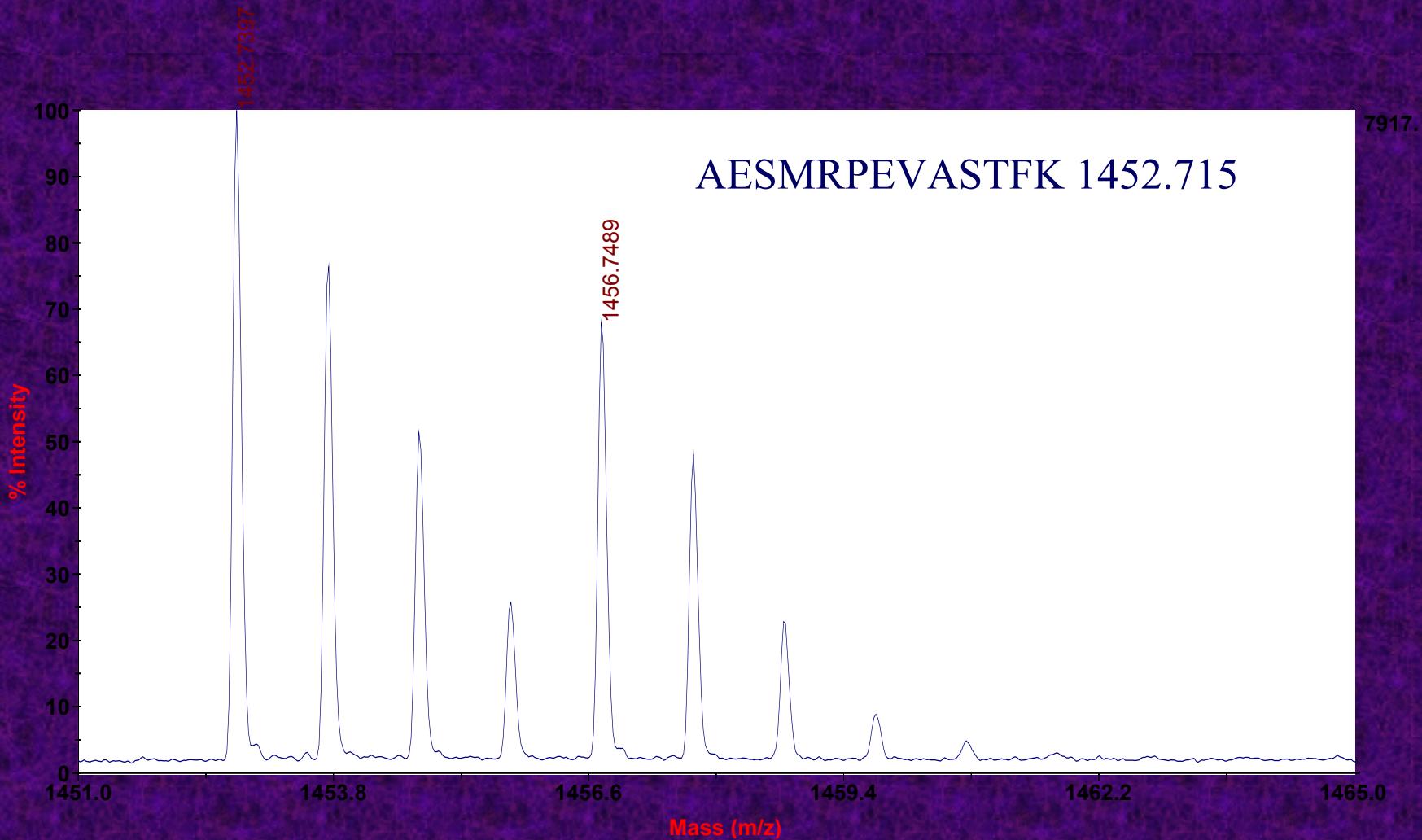
J B B R 0 5 _ 0 3 0 3 2 2 _ D 1 _ o 1 6 _ o 1 2 # 1 5 3 6 R T : 3 8 . 5 2 A V : 1 N L : 1 . 3 7 E 7
T : + c N S I F u l l m s [4 0 0 . 0 0 - 2 0 0 0 . 0 0]





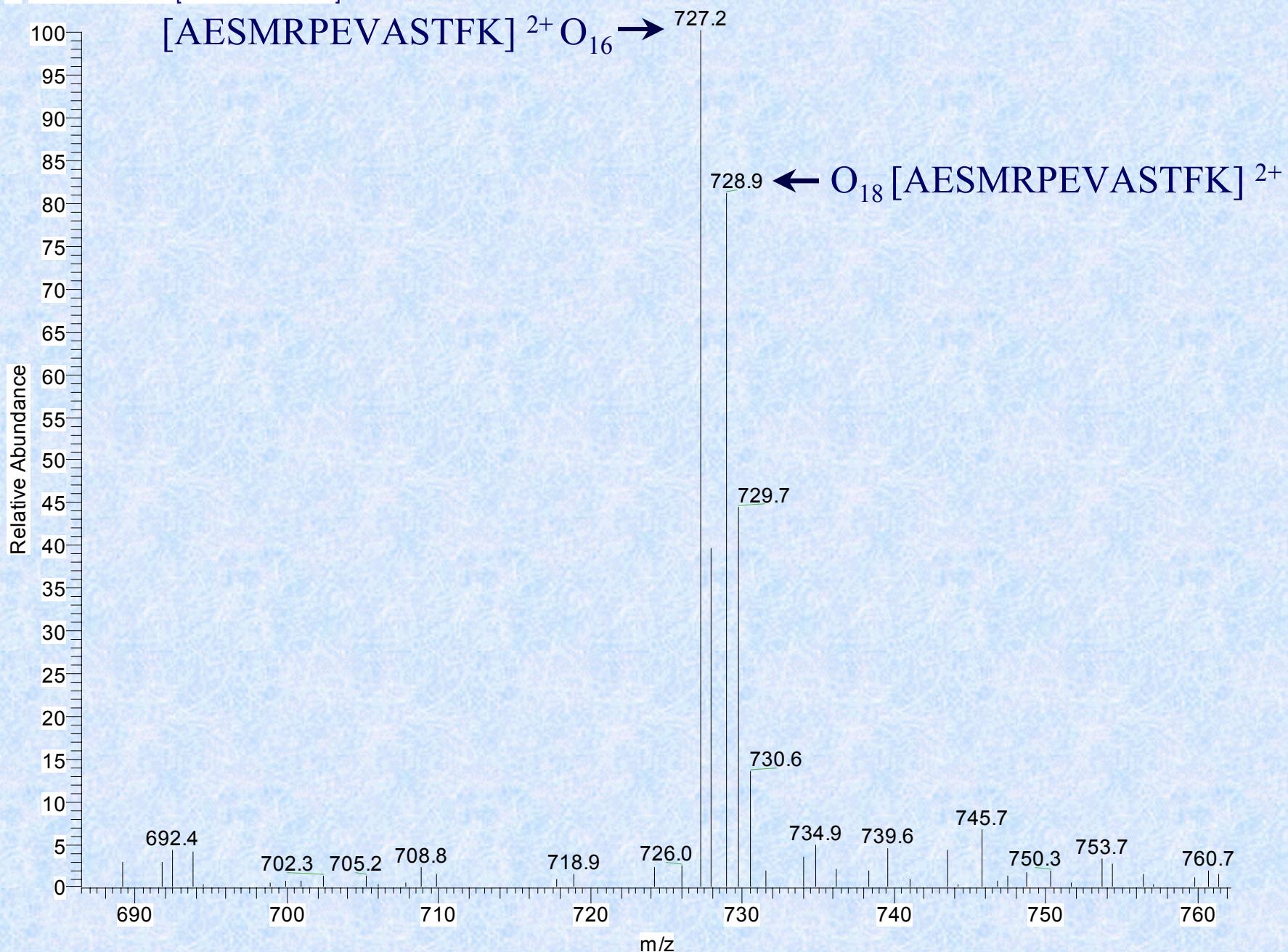
Bacteriorhodopsin - O16/O18 labeling

4700 Reflector Spec #1[BP = 987.6, 9128]



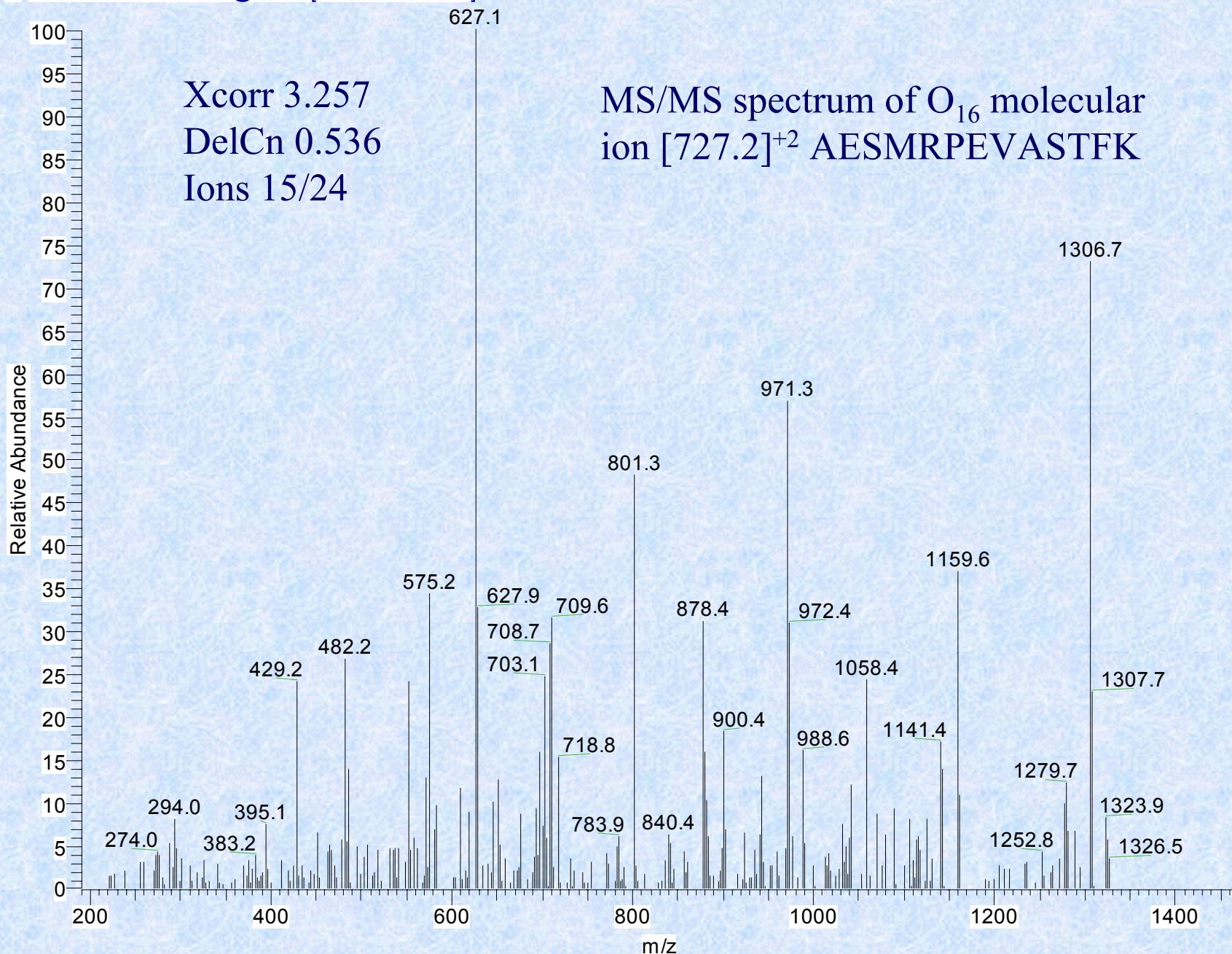
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T: + c NSI Full ms [400.00-2000.00]



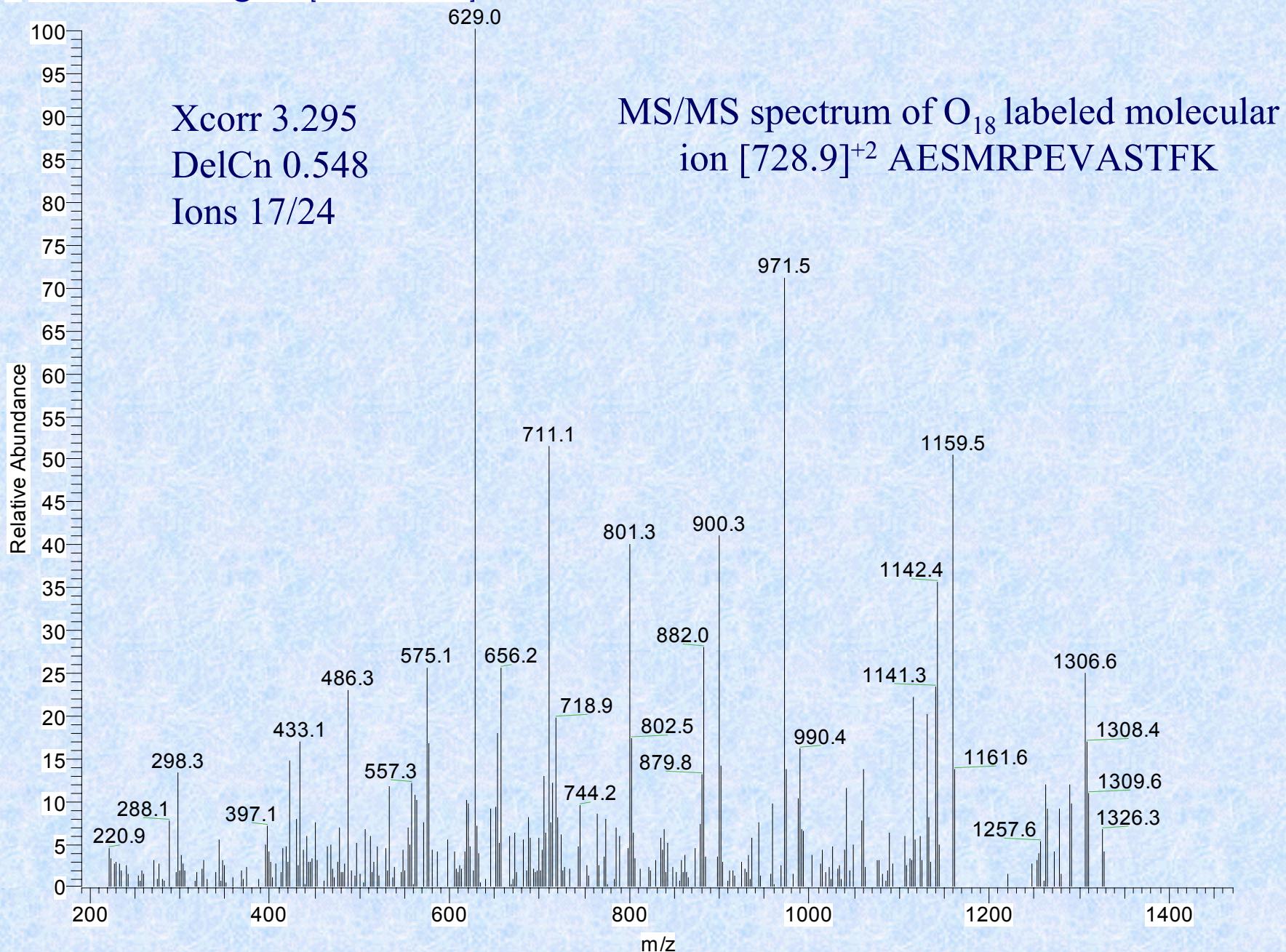
JBBR05_030322_D1_o16_o12 #905 RT: 21.61 AV: 1 NL: 5.74E6

T: + c d Full ms2 727.22@35.00 [190.00-1465.00]



JBBR05_030322_D1_o16_o12 #906 RT: 21.63 AV: 1 NL: 4.80E6

T: + c d Full ms2 728.95@35.00 [190.00-1470.00]



CONCLUSIONS

- An efficient, solution based method using single tube extraction, solubilization, and tryptic digestion of membrane proteins has been developed.
 - This technique is applicable for targeted and global membrane proteomics allowing qualitative and quantitative analysis of integral membrane proteins of different organisms and cell types.
 - The present methodology is amenable for conventional 1D- μ LC-MS/MS analysis or multidimensional μ LC-MS/MS
-

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