

Imaging of Central Nervous System by MALDI Mass Spectrometry

Céline MERIAUX

MALDI Imaging Team
Fundamental & Applied Biological Mass Spectrometry
University Lille 1

Supervised by Prof. M. Salzet

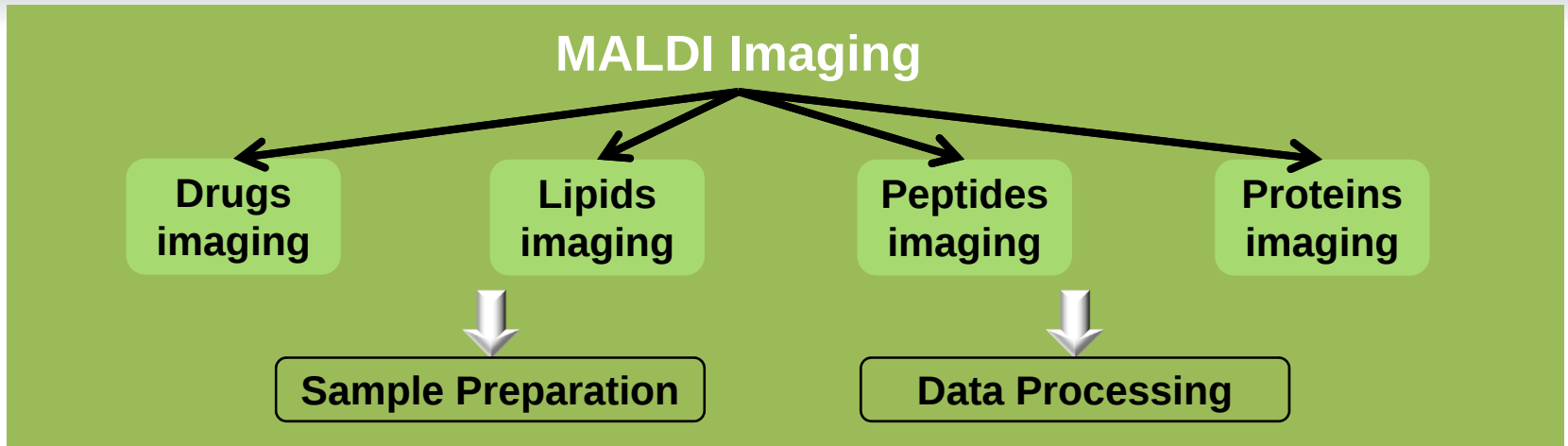


Université Lille Nord de France

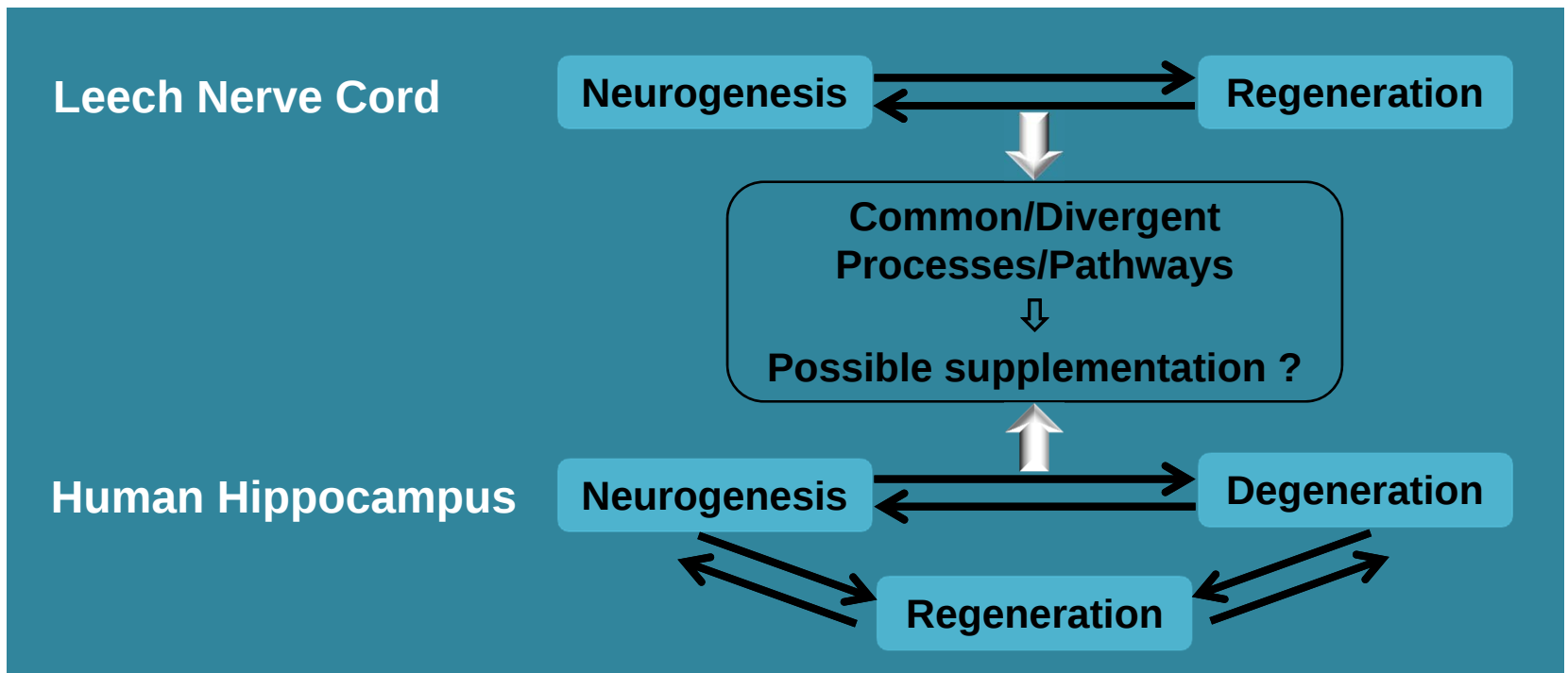


Objectives of thesis

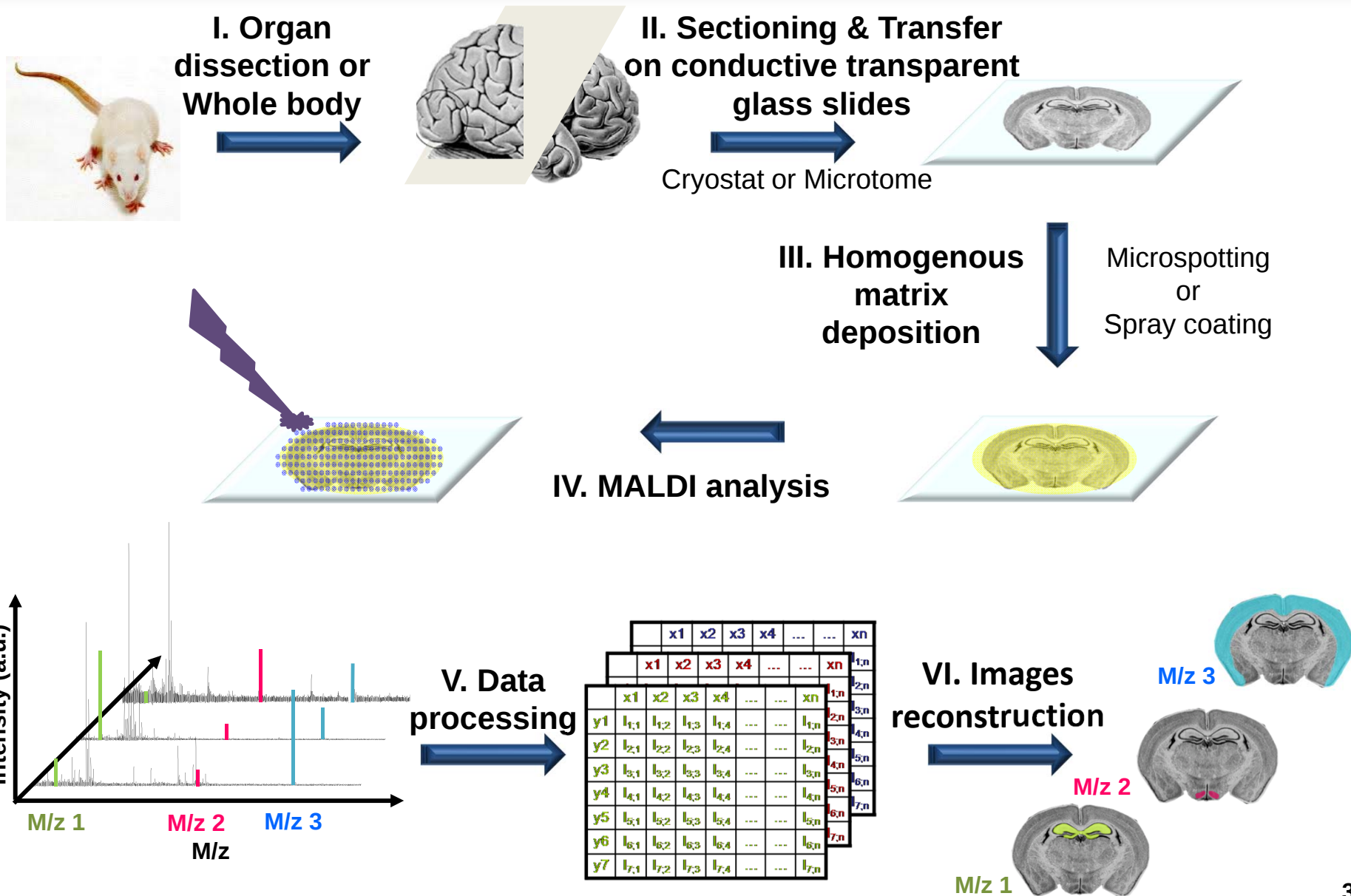
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Principle of MALDI-MSI





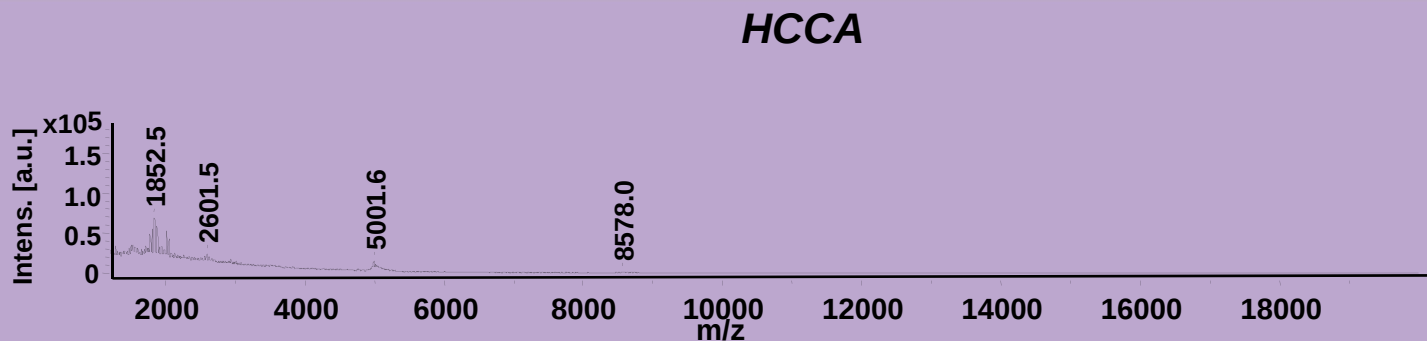
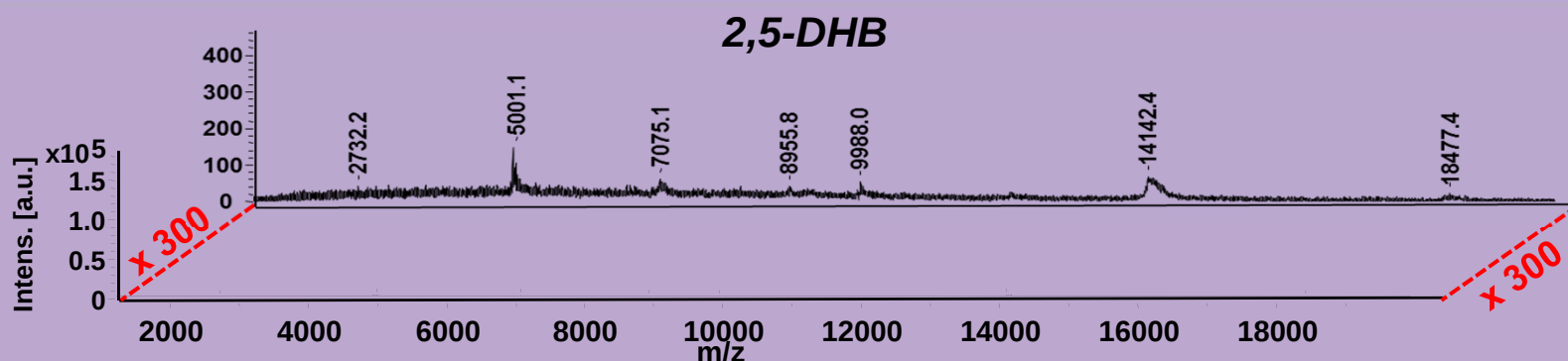
Matrices and Deposits

Spray coating and Conventional Matrices for Peptides

MALDI-MSI of peptides is generally performed using 2,5-DHB or HCCA as matrix

Chemical treatment with chloroform improve peptides analysis ¹

¹ Lemaire R et al. *Anal Chem.* 2006. 78 (20): 7145-53



Solid Ionic Matrices (SIM) based on the use of HCCA were found to be efficient for MALDI-MSI of peptides ²

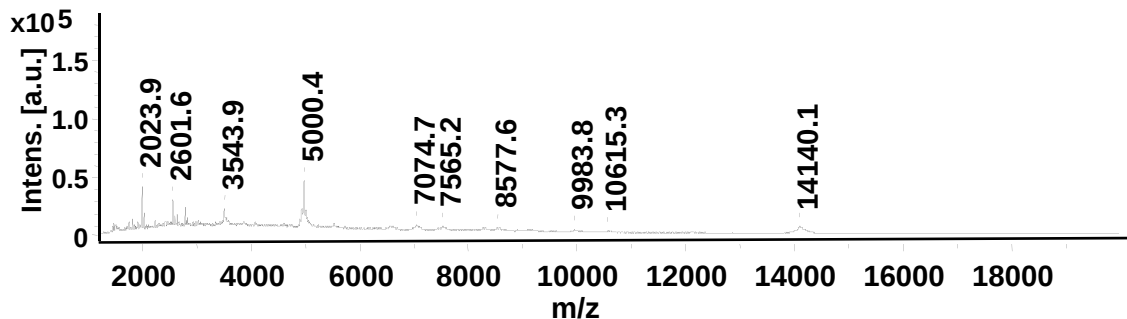
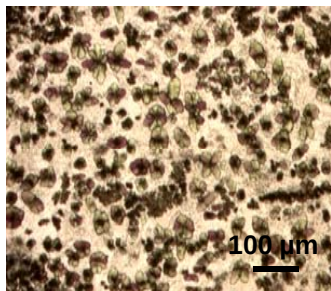
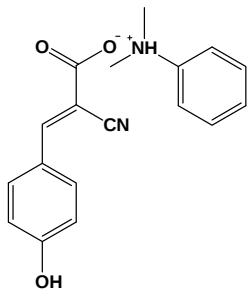
² Franck J et al. *AnalChem.* 2009. 81 (19): 8193-202



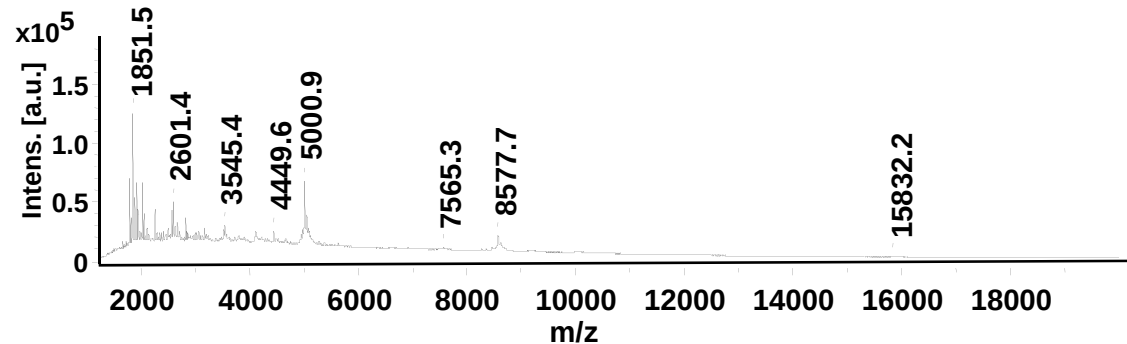
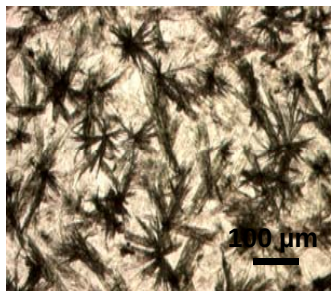
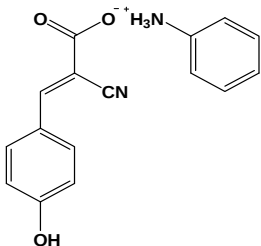
Matrices and Deposits

Spray coating and SIMs for Peptides

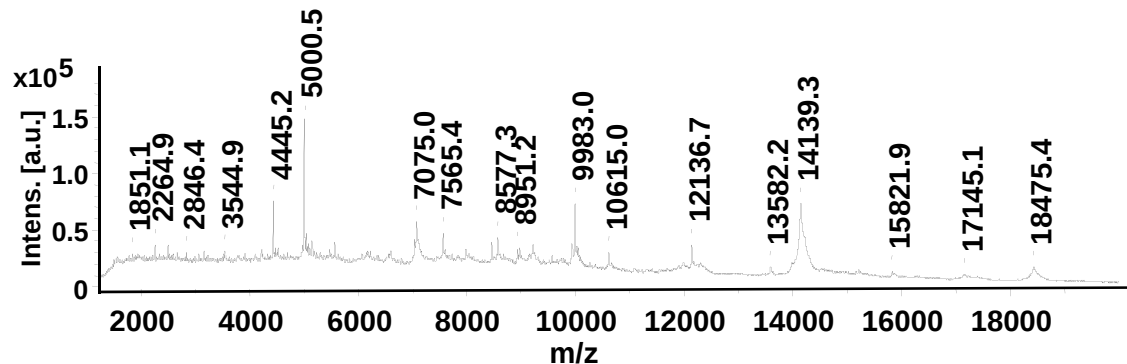
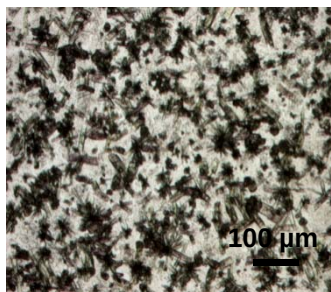
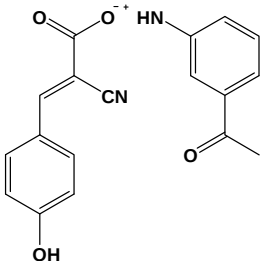
HCCA/DANI



HCCA/ANI



HCCA/3-AP





Matrices and Deposits

Spray coating and SIMs for Peptides



HCCA/ANI

Spatial resolution 150 μm

Lateral 2.40 mm

2 mm

m/z 1392.86
m/z 1493.55
m/z 1784.71
m/z 1839.05

Spatial resolution 40 μm

m/z 1294.42
m/z 1932.81

m/z 1694.12
m/z 3202.84

1 mm



HCCA/ANI can be used as matrix for MALDI-MSI with high spatial resolution



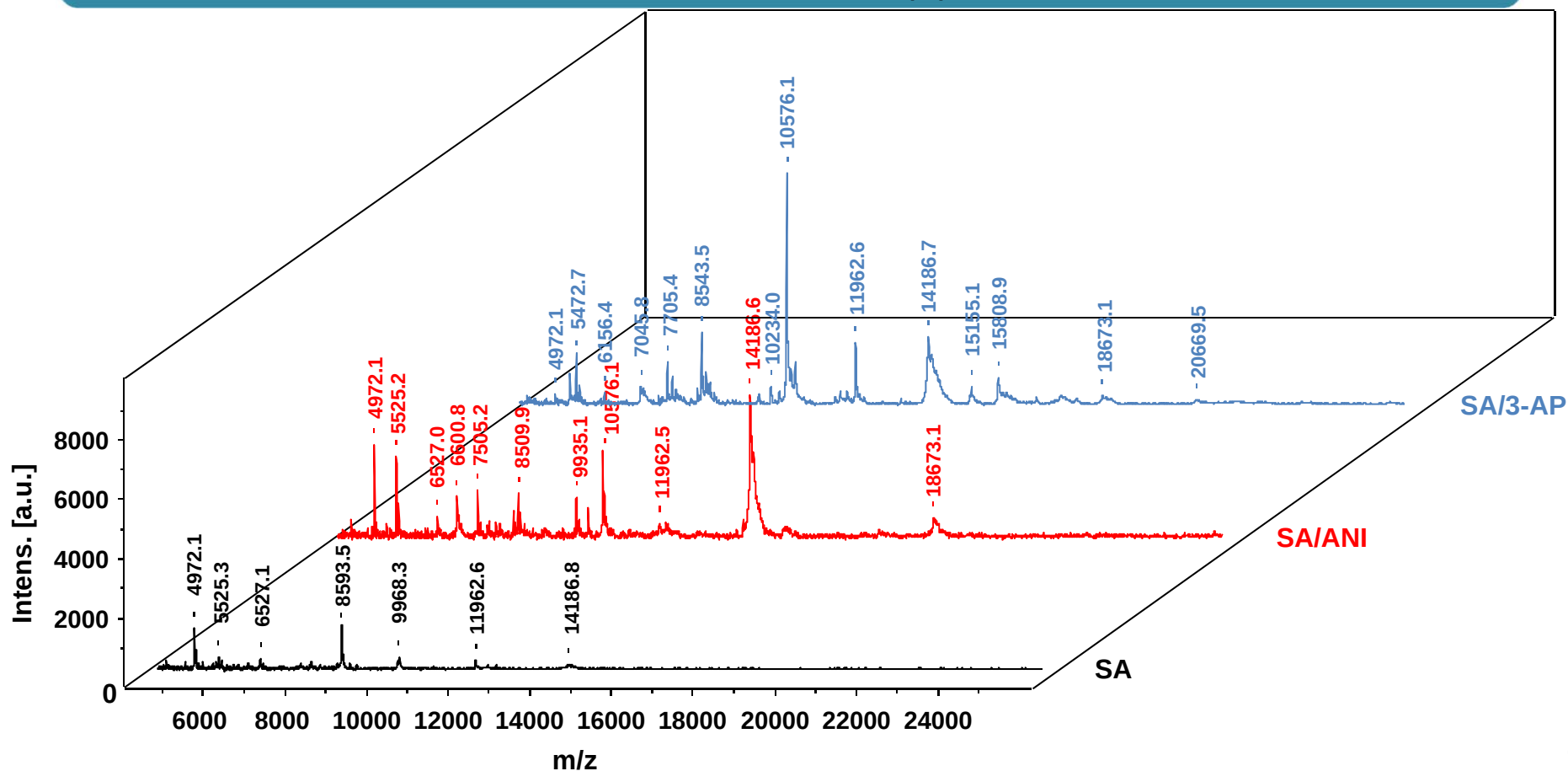
Matrices and Deposits

Spray coating and SIMs for Proteins

MALDI-MSI of proteins is generally performed using Sinapinic acid (SA) as matrix

Solid Ionic Matrices (SIM) based on the use of SA were found to be efficient for MALDI-MSI of proteins ³

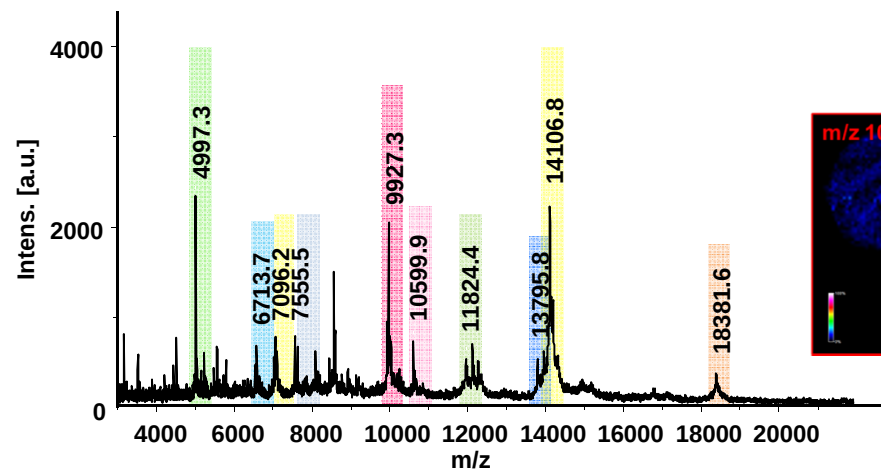
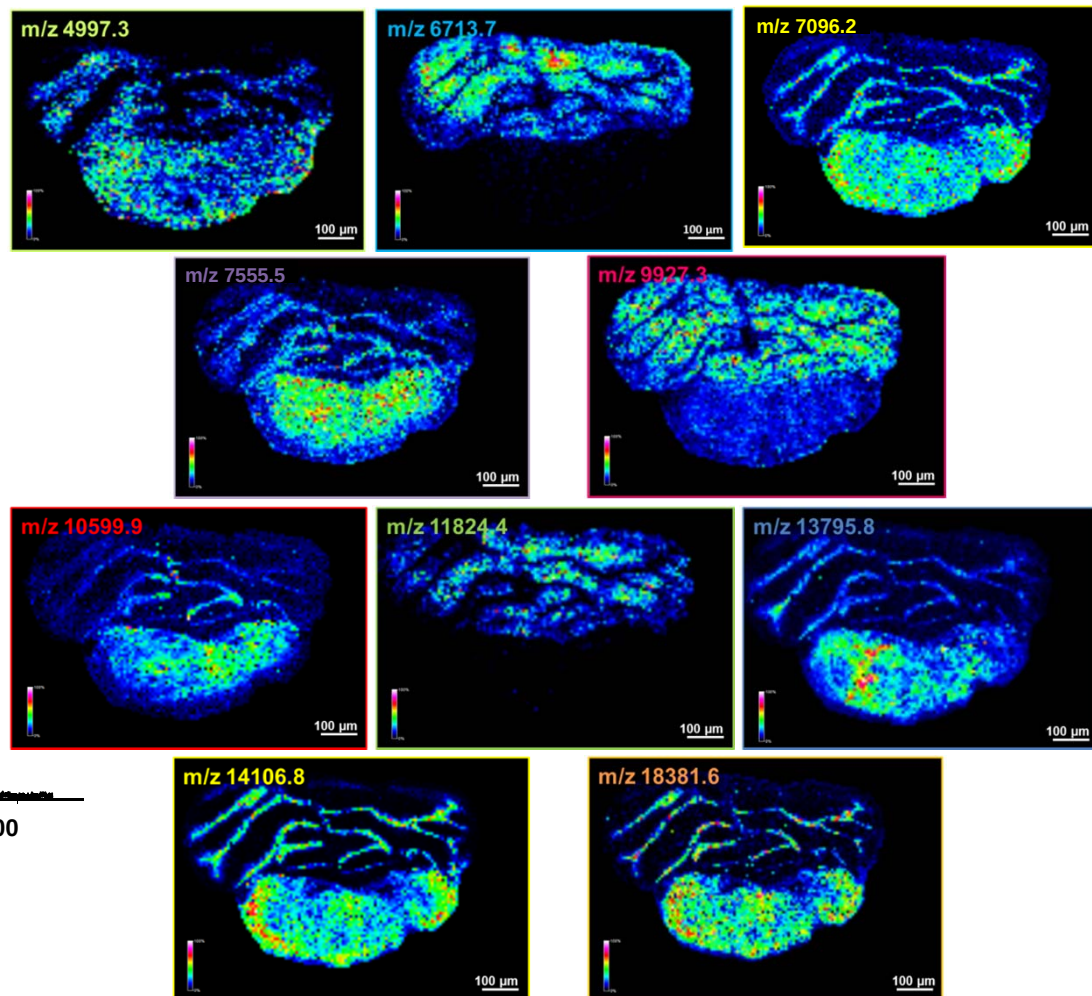
³ Franck J et al. *AnalChem.* 2009. 81 (19): 8193-202





Matrices and Deposits

Spray coating and SIMs for Proteins





Matrices and Deposits

Spray coating and SIMs for Peptides / Proteins

SIMs provide better incorporation into matrix crystals during crystallization

SIMs allow to increase the number of detected peptide and protein ions

SIMs provide higher intensity and S/N ratio

HCCA/ANI and SA/ANI are suitable for MALDI-MSI of peptides and proteins with high spatial resolution



Data processing

Statistical Analysis

Large amount of data generated by MALDI-MSI acquisition

Difficulty to obtain a fine analysis and interpretation

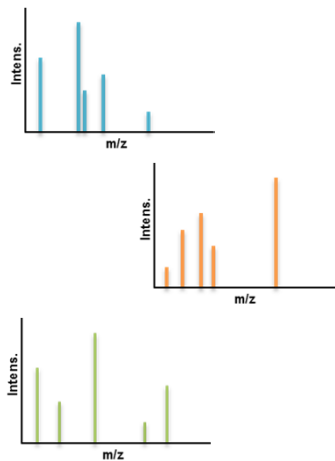
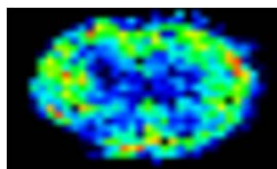
Simplify the dataset



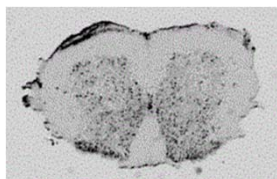
Data processing

Supervised Statistical Analysis

I. Data Acquisition

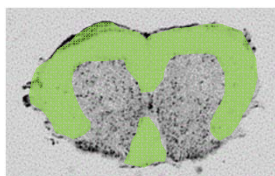


Tissue Section

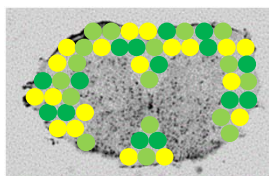


II. Region of Interest (ROI) Definition

mask



query

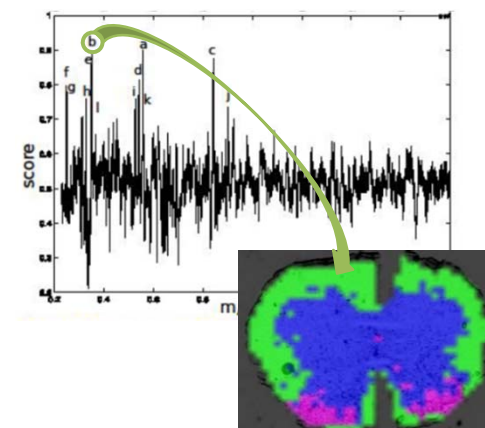


Intensity matrix

	120	201	159	987	589	0	322	222
10	10	202	5	887	684	753	35	1
...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...
311	311	76	249	122	755	357	667	999

intensities

III. Test for Localization of Molecule Expression



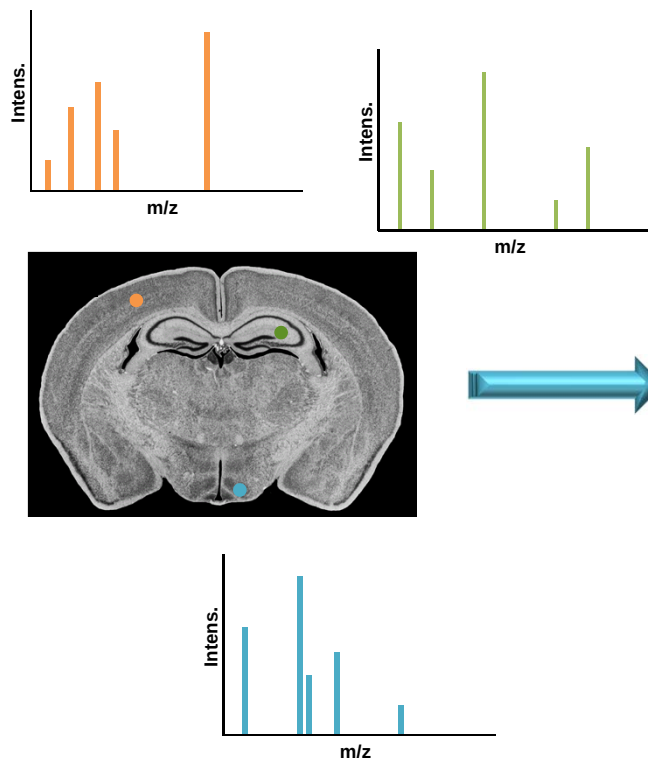
m/z « b » with a specific localization



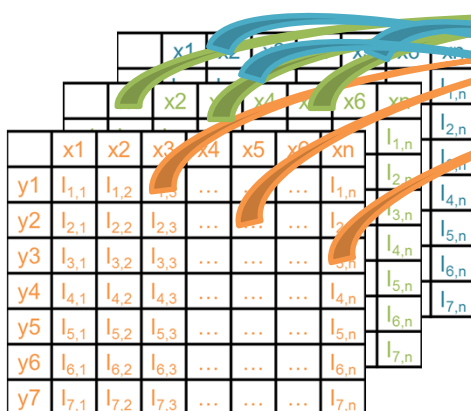
Data processing

Semi-supervised Statistical Analysis

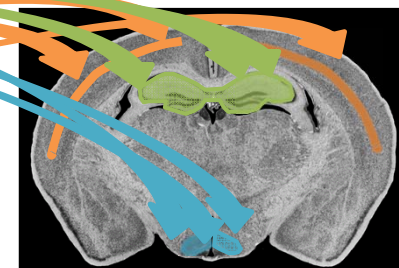
I. Data acquisition and Reference Spectra Selection



II. Dataset Interrogation



III. Cluster creation and associated m/z values





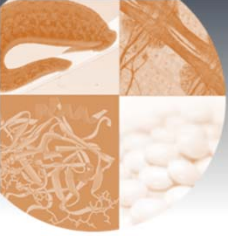
Data processing

Statistical Analysis

Perfect tool to obtain:

molecular signatures

fine research of variable m/z



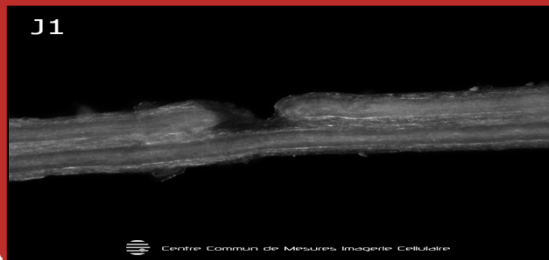
The medicinal leech

Central Nervous System (CNS)



CNS regeneration...

- Structural recovery
1 week
- Functional recovery
1 month



CCMIC, Lille, France

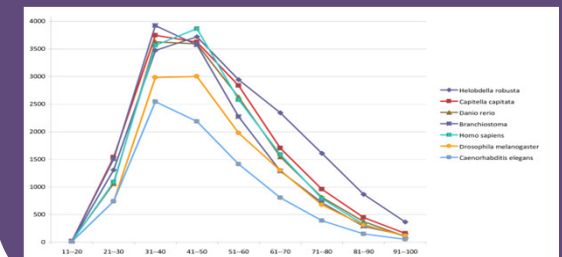
A simple structure...

- Detailed knowledge
- Accessible
- Large neurons

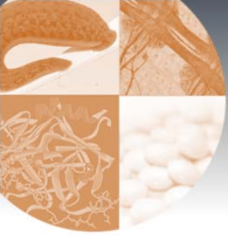


At the genomic level...

H. medicinalis genome
closed to mammals
genomes (80% homology)⁴

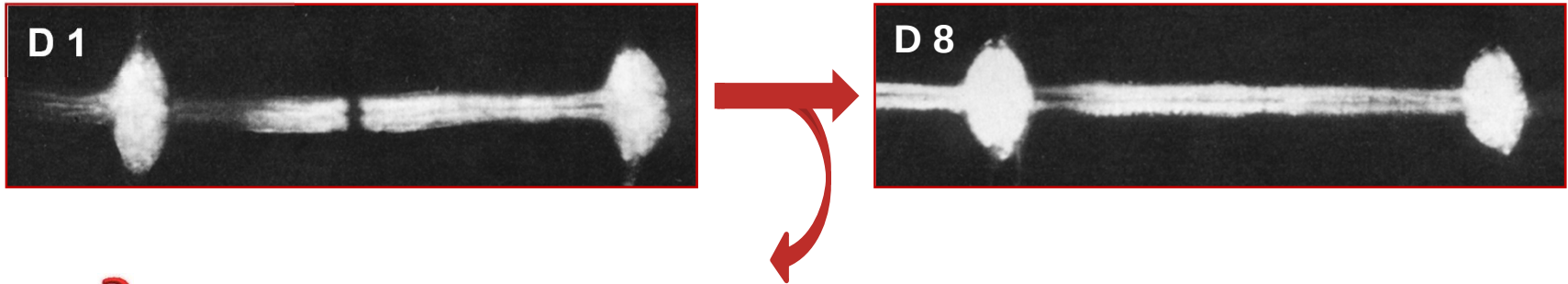


⁴ Salz M et al, 2010, BMC Genomics



The medicinal leech

Problematic



? Molecular changes induced by physical damage to the CNS



Up-regulated Genes during Neural Regeneration

- Leech homologues of mammals genes ^{5,6,7}
(Actin, ATP synthase, Synapsin, Tubulin, Thioredoxin)
- Leech homologues of invertebrates genes ⁸
(LeNa, Myohemerithrin, ReN3)

⁵ Blackshaw SE et al. *J Anat.* 2004. 204:13-24

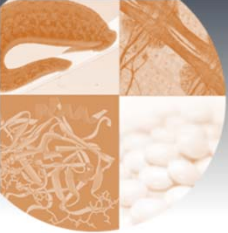
⁶ Emes RD et al. *FEBS Lett.* 2003. 533: 124-28

⁷ Korneev S et al. *Invert Neurosci.* 1997. 3: 185-92

⁸ Vergote D et al. *J Biol Chem.* 2004. 279: 43828-837



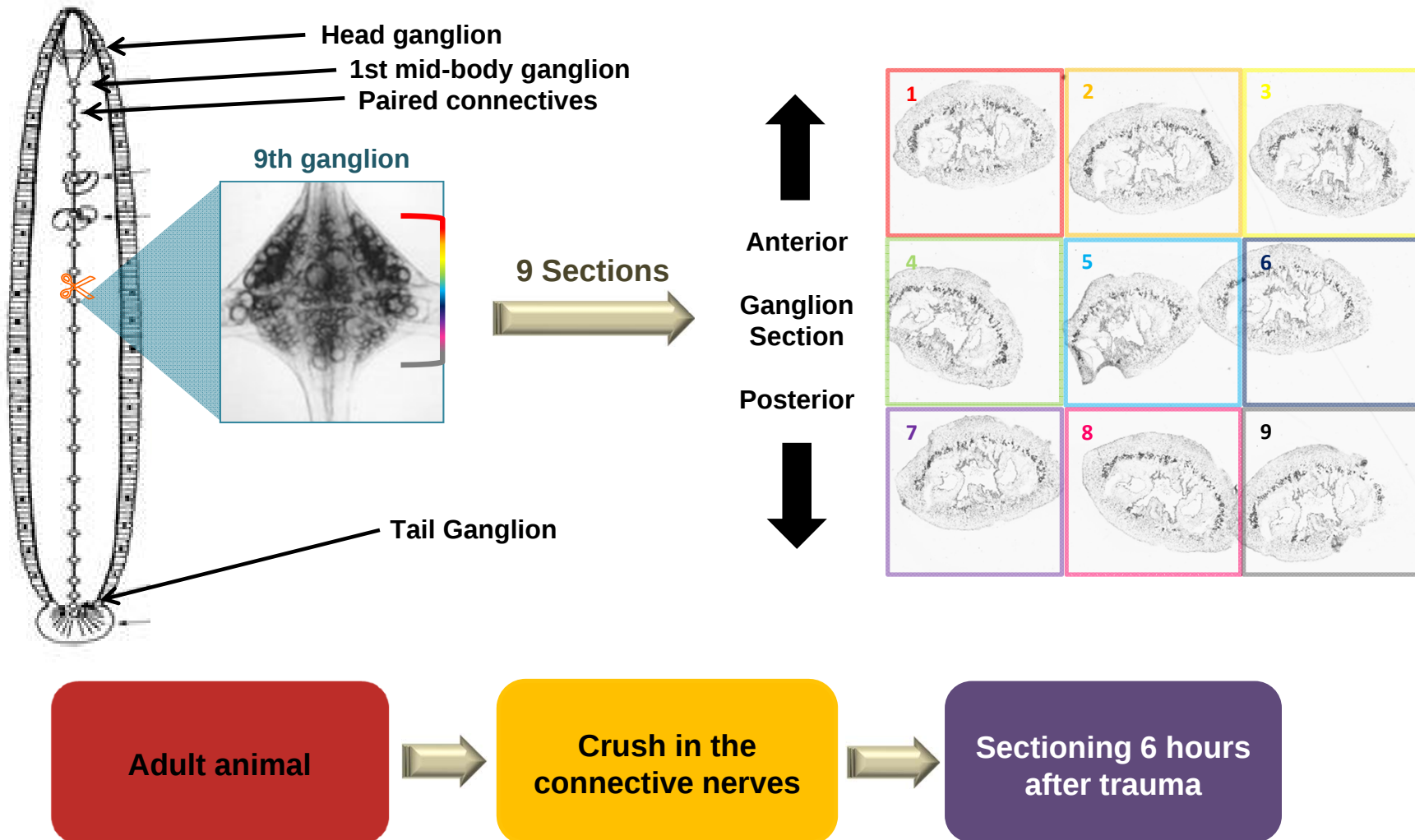
Modifications in protein levels and distributions



The medicinal leech

Strategy

Modulation of Peptide Distributions during Neural Regeneration

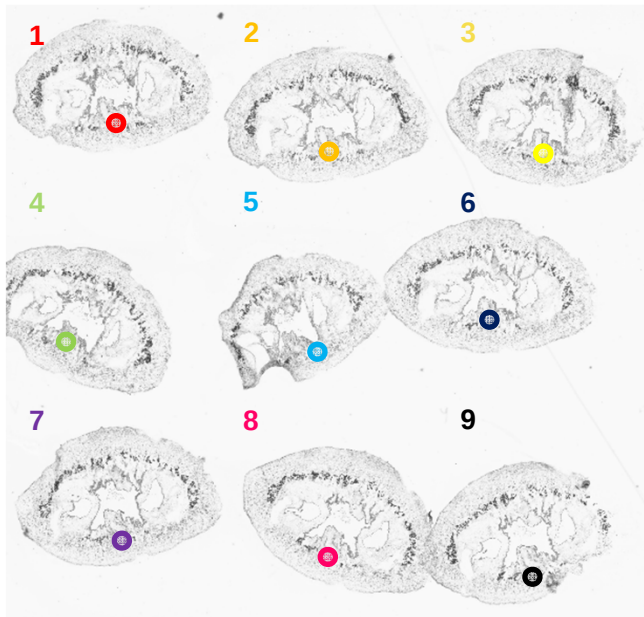




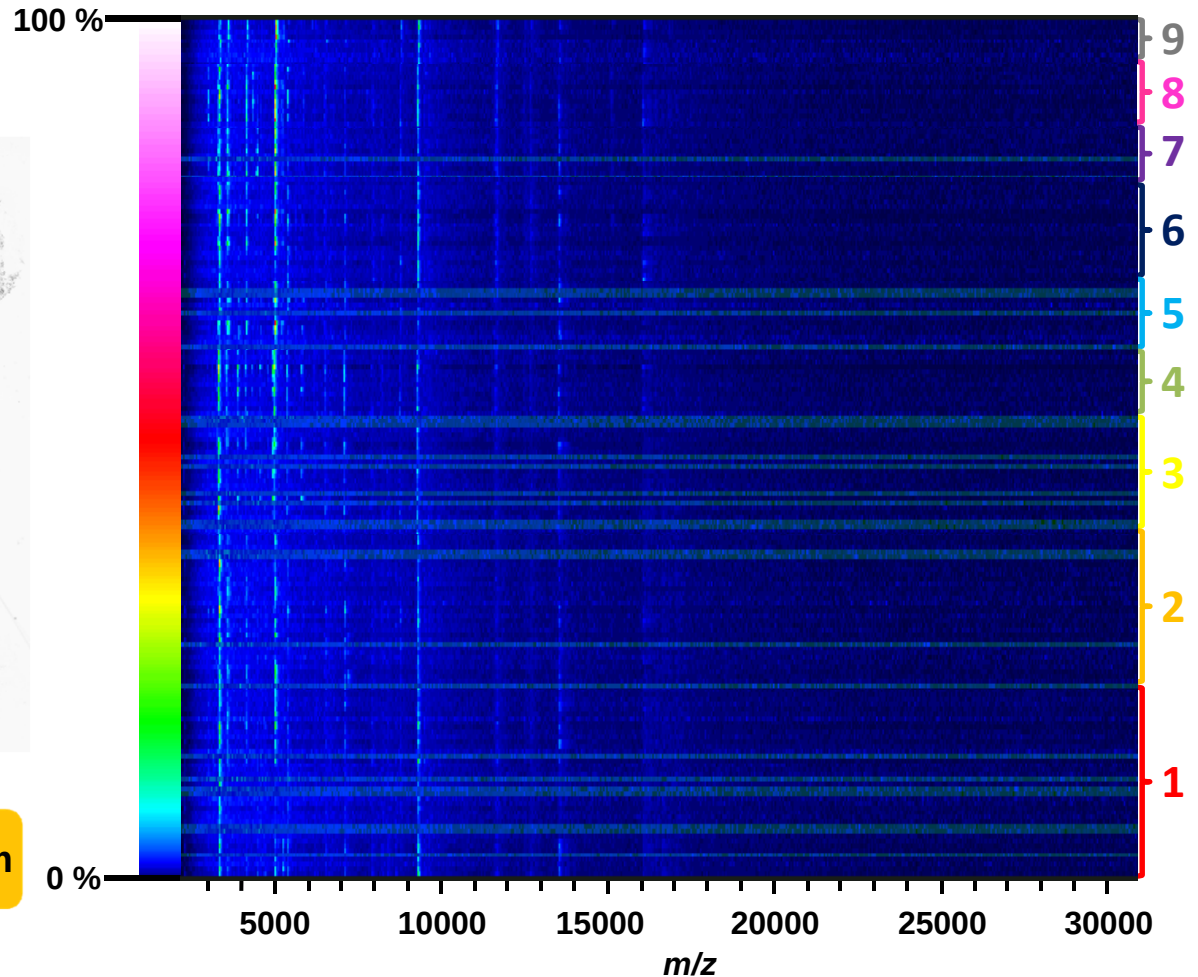
The medicinal leech

Modulation of Peptide Distributions during Neural Regeneration

 MALDI-MSI



Region of interest: Nervous system



Two-dimensional representation of all the spectra

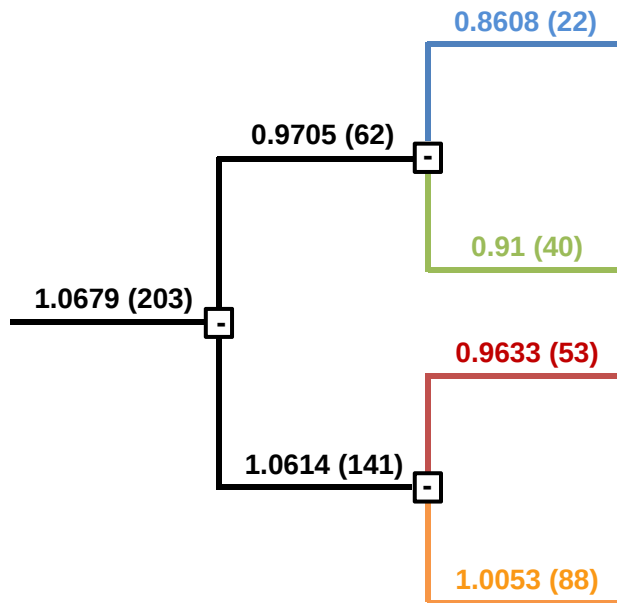


The medicinal leech

Modulation of Peptide Distributions during Neural Regeneration

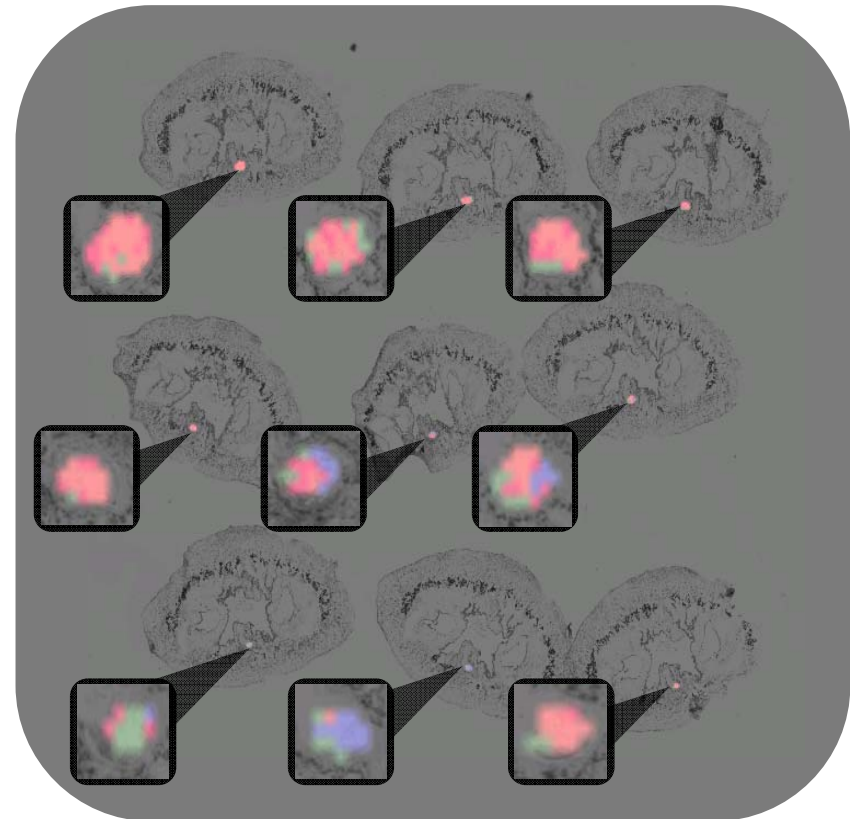


Hierarchical Clustering



Dendrogram of the clustering results

Anterior
↑
Ganglion Section
↓
Posterior



Reconstruction of selected dendrogram branches



The medicinal leech

Modulation of Peptide Distributions during Neural Regeneration

Regenerating vs. Non Regenerating Adult CNS

1st group

m/z 5566

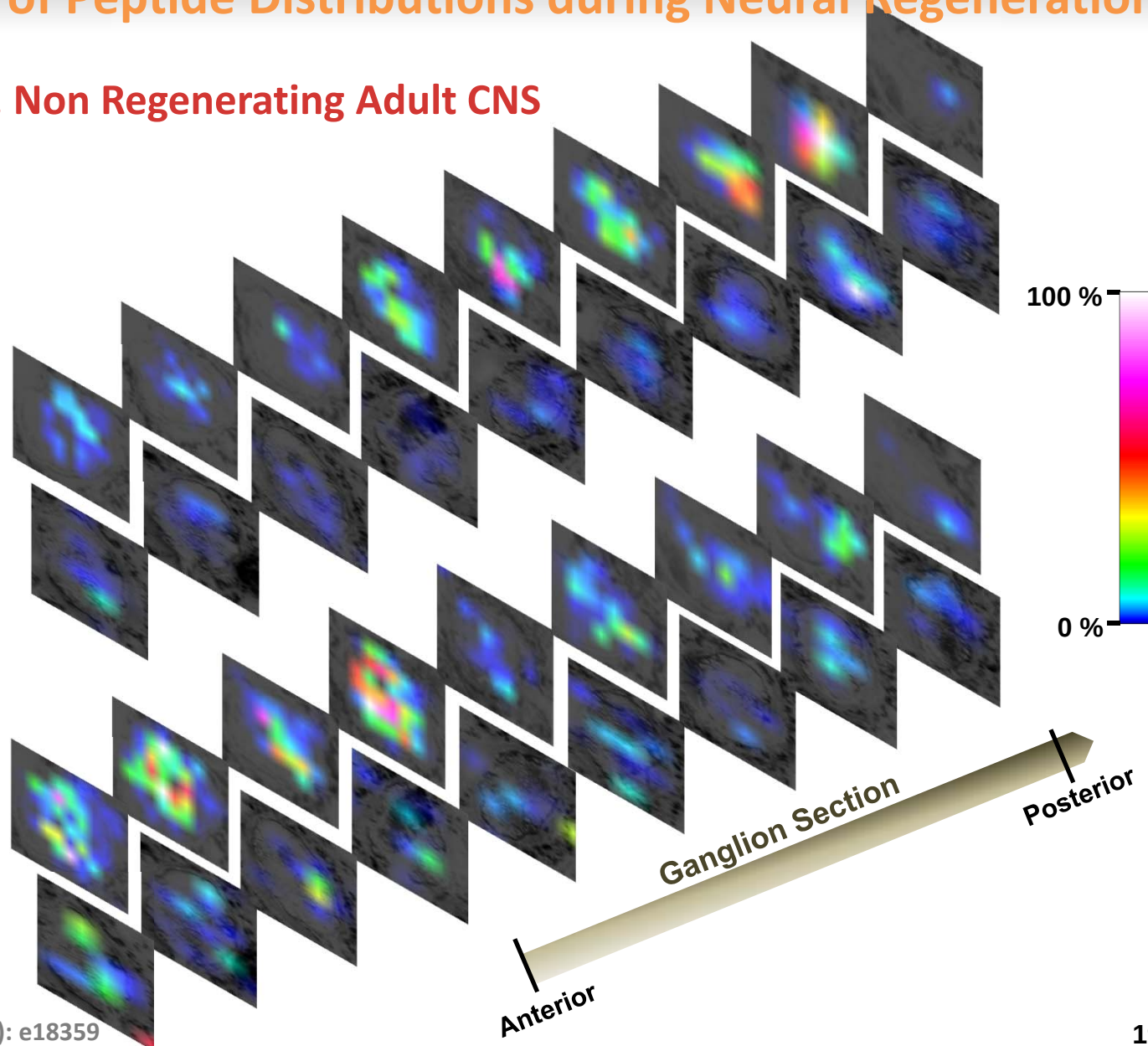
Regenerating

Non Regenerating

m/z 7246

Regenerating

Non Regenerating





The medicinal leech

Modulation of Peptide Distributions during Neural Regeneration

Regenerating vs. Non Regenerating Adult CNS

2nd group

m/z 5415

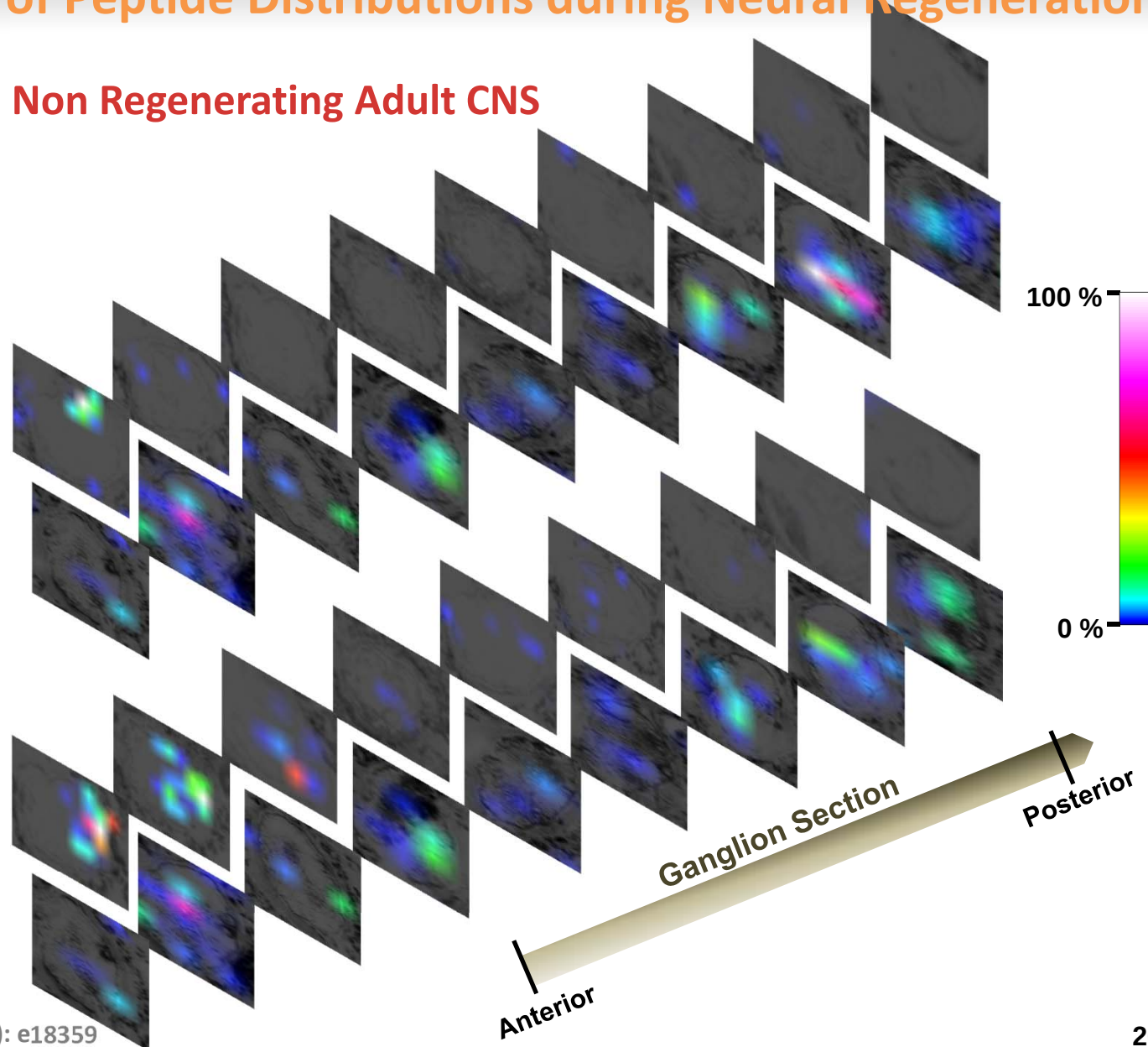
Regenerating

Non Regenerating

m/z 6018

Regenerating

Non Regenerating





The medicinal leech

Modulation of Peptide Distributions during Neural Regeneration

Regenerating vs. Non Regenerating Adult CNS

3rd group

m/z 2475

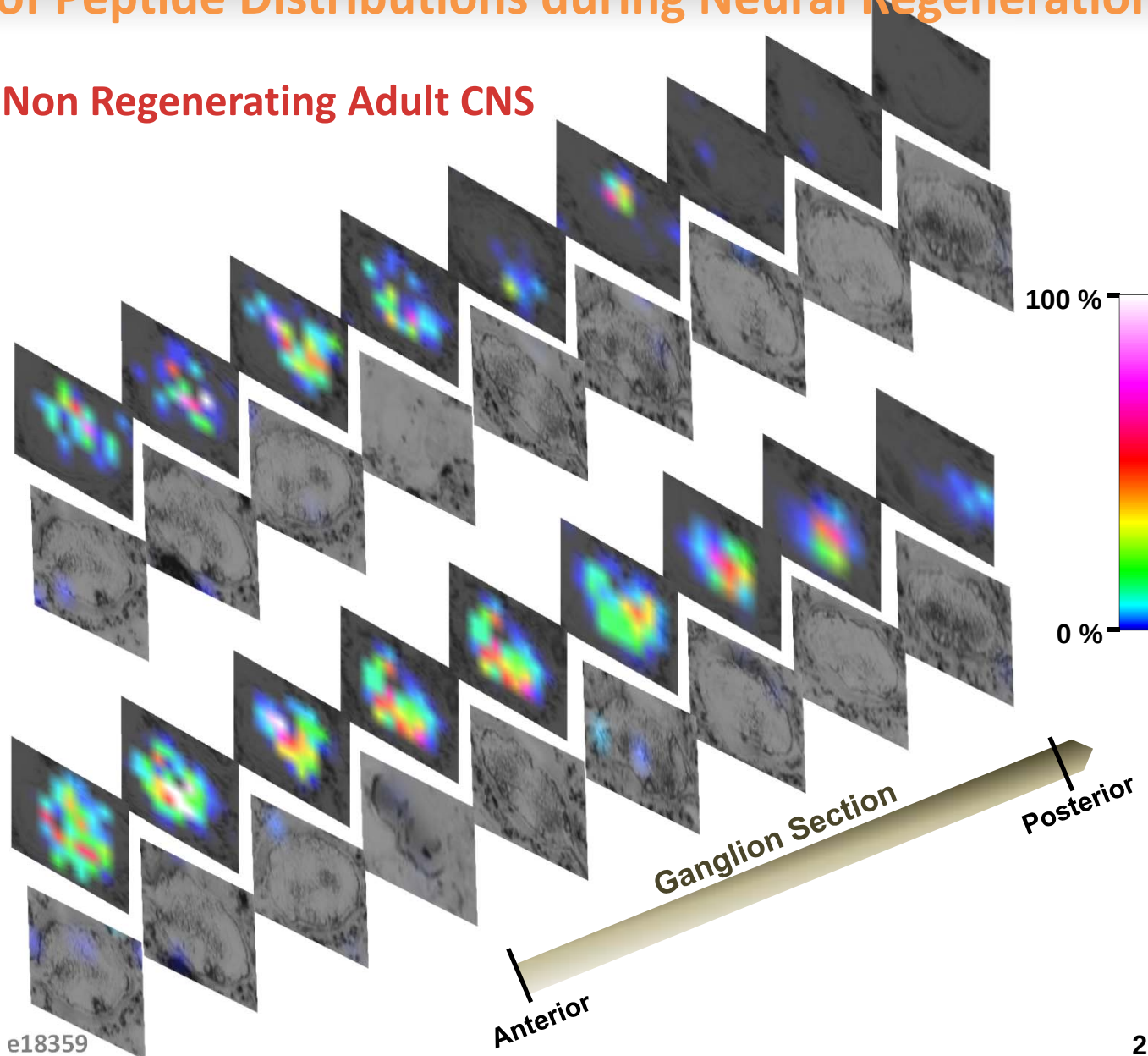
Regenerating

Non Regenerating

m/z 3501

Regenerating

Non Regenerating



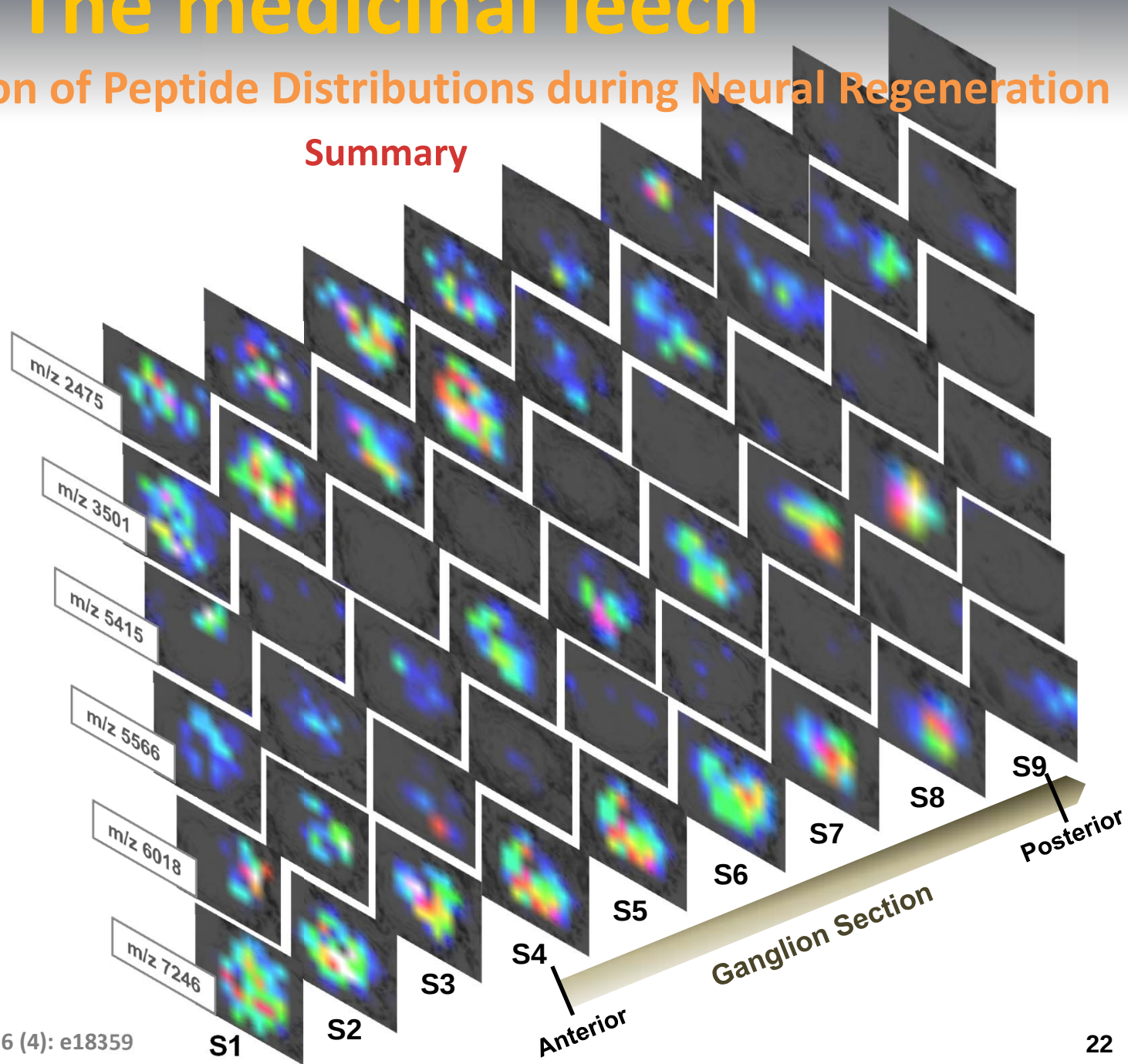


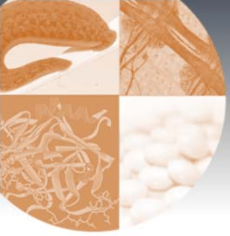
The medicinal leech

Modulation of Peptide Distributions during Neural Regeneration

Summary

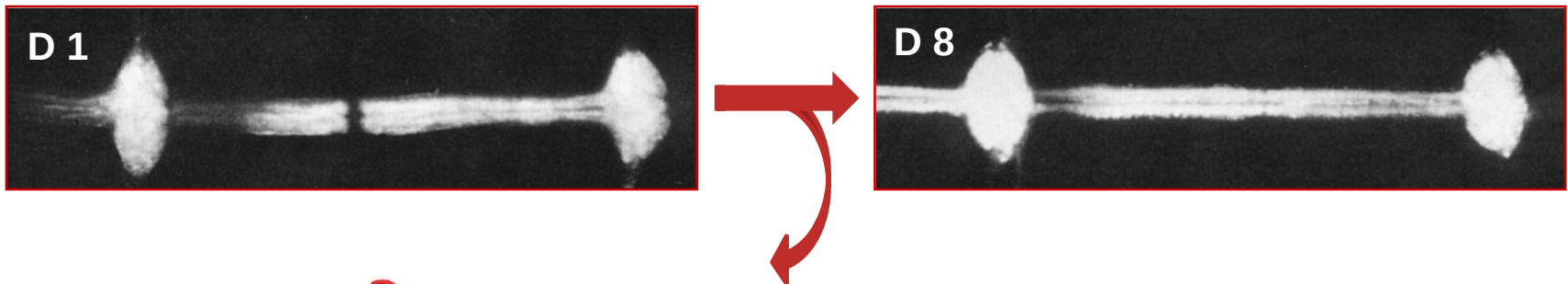
m/z value	Distribution								
	1	2	3	4	5	6	7	8	9
2475									
3300									
3501									
3561									
4074									
4115									
4520									
4733									
5105									
5415									
5566									
5997									
6018									
6694									
7246									
7249									
7305									
11752									
13684									
16221									
16417									





The medicinal leech

Problematic



? Re-expression of embryonic factors

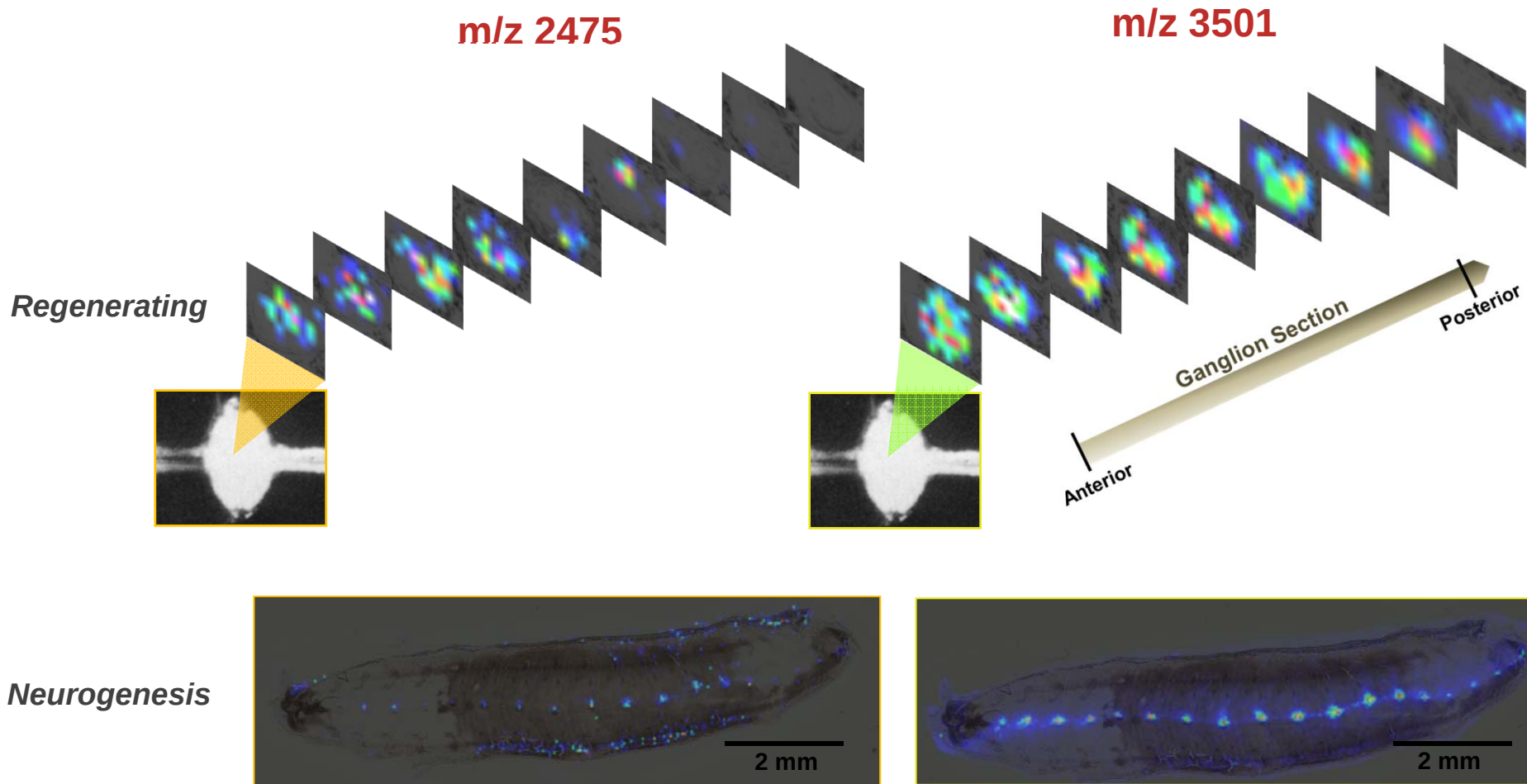


The medicinal leech

Modulation of Peptide Distributions during Neural Regeneration



Neurogenesis vs. Regenerating Adult CNS



Coll. Pr. E. Macagno

Meriaux C *et al.* PLoS One. 2011. 6 (4): e18359



The medicinal leech

Modulation of Peptide Distributions during Neural Regeneration



Identification and Validation

HmIF4

Sequence ¹ GTRTMERSVR TSSQYASGGP MPN ²³

Protein Category Intermediate filaments

Function Axon guidance

Statistical Analysis

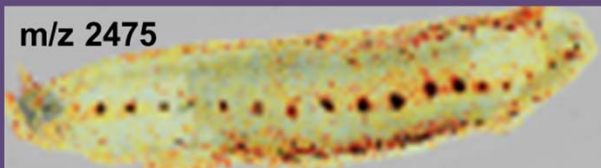
Semi
Supervised

m/z 2475



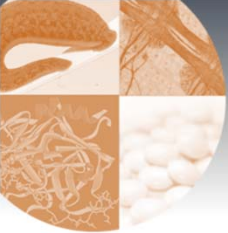
Supervised

m/z 2475



ISH



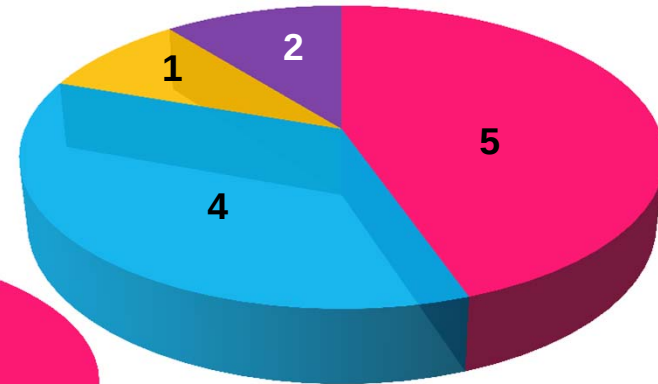


The medicinal leech

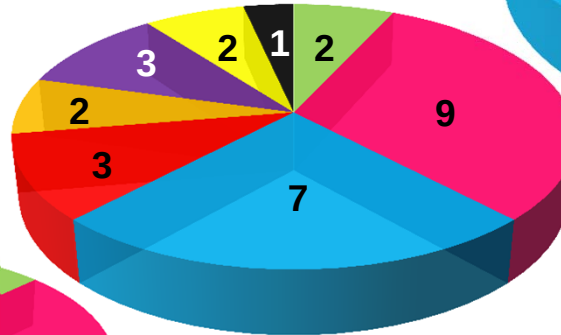
Conclusion

-  Immune factors
-  Cytoskeleton
-  Axon guidance
-  HSP & Chaperones
-  Metal oxydation
-  Metabolism
-  Morphogenesis
-  Calcium sensor

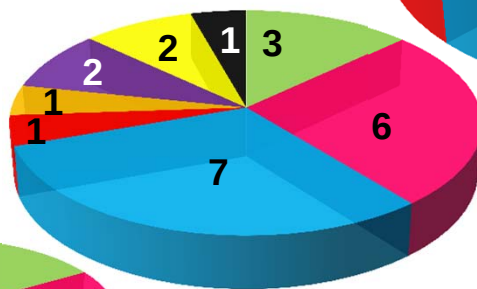
T 48h



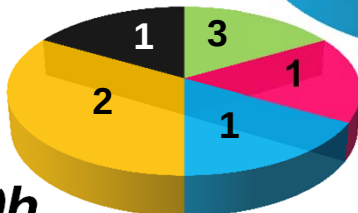
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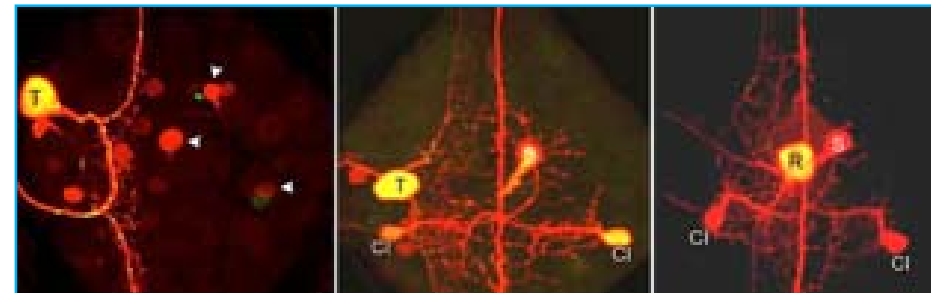
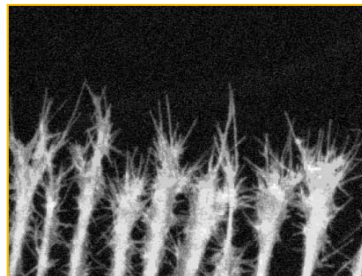
T 6h



T 1h



T 0h



Meriaux C et al. 2011. PLoS One
 Croq F et al. 2010. Glia
 Schikorski D et al. 2009. J Immunol
 Schikorski D et al. 2008. J Immunol

Tatouh M et al. 2009. Mol Immunol
 Tatouh M et al. 2009. Eur Cytokine
 Vergote D et al. 2006. Proteomics
 Vergote D et al. 2004. J Biol Chem



Hippocampus of epileptic patients

Temporal Lobe Epilepsy (TLE)



Epilepsy

- Chronic neurological disorder characterized by recurrent seizures (minimum 2 seizures)⁹
- Abnormal, transient and acute functioning of the electrical activity in an cerebral area

⁹ Fisher R et al. *Epilepsia*. 2005. 46: 470-472



Temporal Lobe Epilepsy

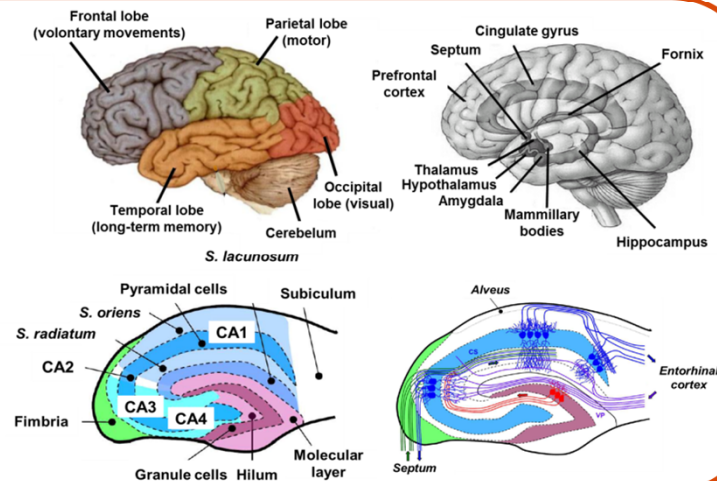
- Most common form of the disease (40%)
- Loss of pyramidal neurons, neurogenesis, changes in the propagation of neuronal circuits¹⁰
- Refractory to drug treatment in most cases
- Abolition of 80-90% of seizures after resective surgery

¹⁰ Jacobs M et al. *Epilepsy Behav*. 2009. 14: 438-445



Hippocampus

- Limbic system (Temporal lobe)
- Pair and symmetric
- 3 structures:
 - Cornu Ammonis (CA)
 - Dentate Gyrus (DG)
 - Subiculum
- 2 types of neurons:
 - Pyramidal cells (—)
 - Granule cells (—)





Hippocampus of epileptic patients

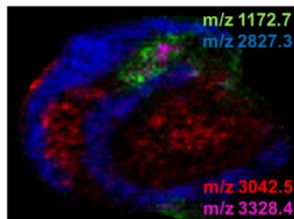
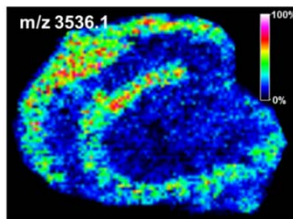
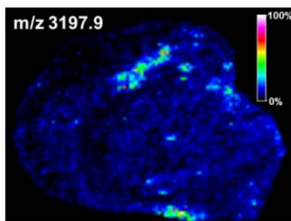
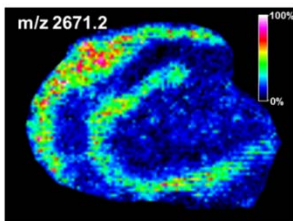
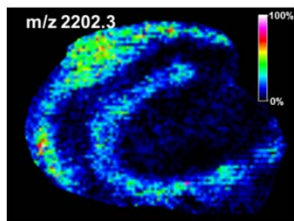
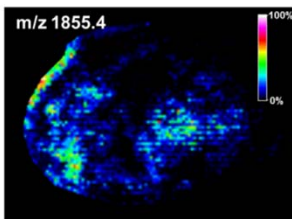
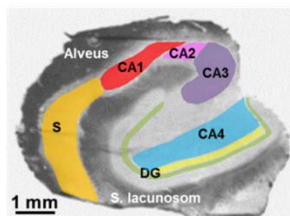
Proteins Distribution



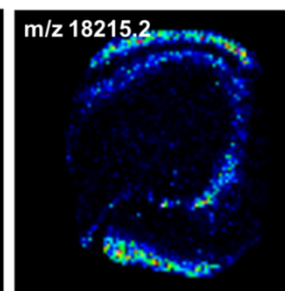
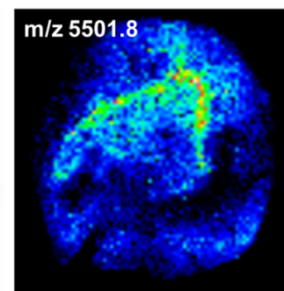
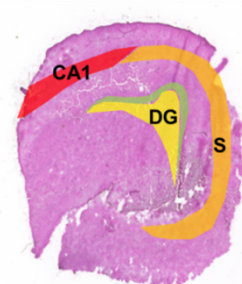
MALDI-MSI



Bottom-Up Strategy:
Micro-nebulisation of Trypsin and
HCCA/ANI



Micro-nebulisation of SA/ANI



No identification of proteins



Hippocampus of epileptic patients

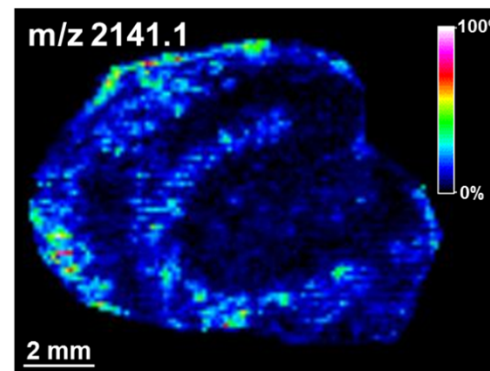
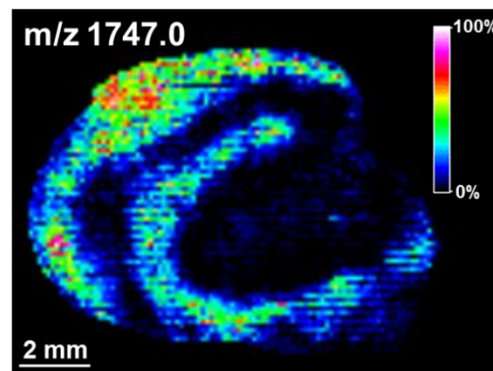
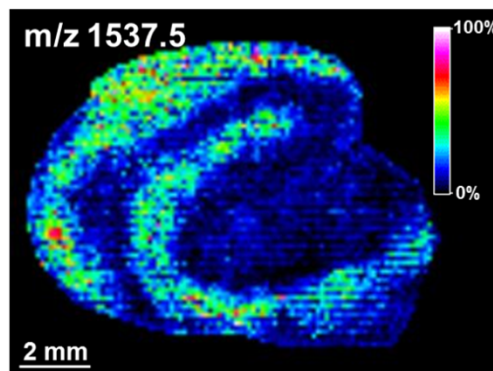
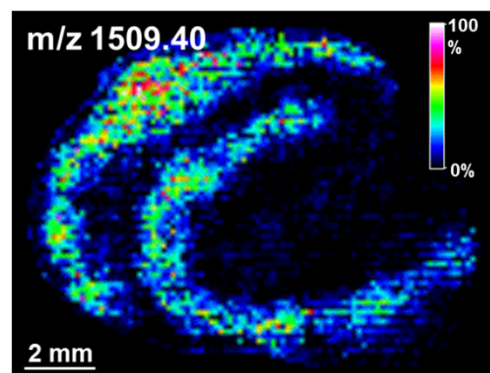
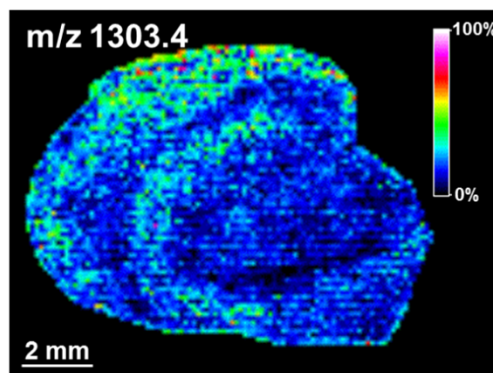
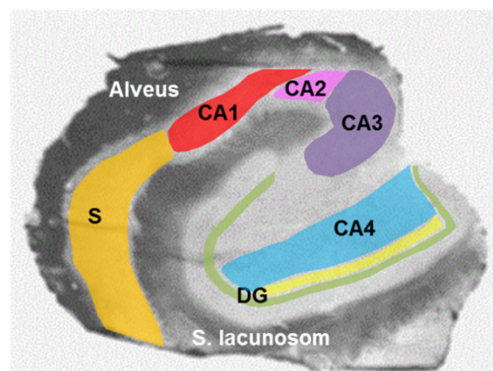
Proteins Distribution



MALDI-MSI



Micro-nebulisation of Trypsin and HCCA/ANI





Hippocampus of epileptic patients

Proteins Distribution

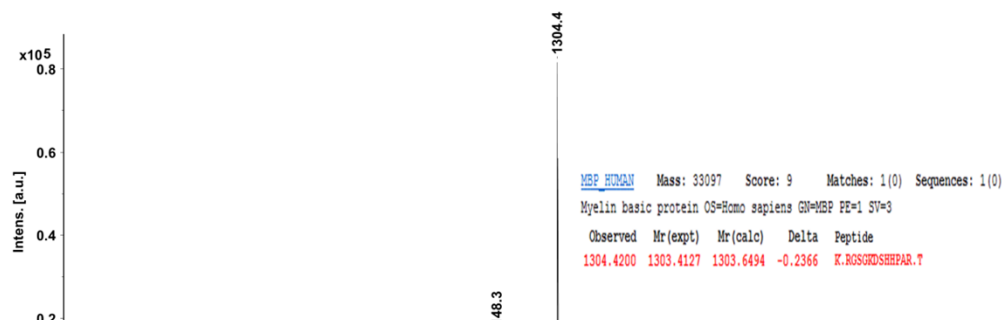


Identification and Validation

Fragments generated from digestion of Myelin Basic Protein

m/z 1147.4
m/z 1362.5
m/z 1509.7
m/z 1747.0
m/z 1303.4
m/z 1459.4
m/z 1537.5
m/z 2141.1

MS/MS m/z 1304



Golli-Myelin Basic Protein Isoform 1



Coverage sequence of Golli MBP-1

1	mgnhagkrel	naekastnse	tnrgesekkr	nlgelsrtts	ednevfgead	anqnngtssq	dtavtdskrt
71	adpknawqda	hpadpgsrph	lirlfsrdap	gredntfkdr	psedelqti	qedsaatses	ldvmasqkrp
141	sqrhgskyla	tastmdharh	gflprhrdtg	ildsigrffg	gdrgapkrqs	gkdshhpart	ahygslpqks
211	hgrrqdenpv	vhffknivtp	rtpppsqqkg	rglsrfsfw	gaegqrpqfg	yggrasdyks	ahkgfkgvda
281	qgtlskifkl	ggrdsrsgsp	marr				



Putative interpretation

- Golli-MBP: Substrate of Matrix Metalloproteases (MMP)
- Demyelination: Loss of myelin sheath insulating the nerves ¹¹
- Demyelination with action of MMP in multiple sclerosis disease ¹²

¹¹ You Y et al. Brain Res. 2011. 1381: 208-216

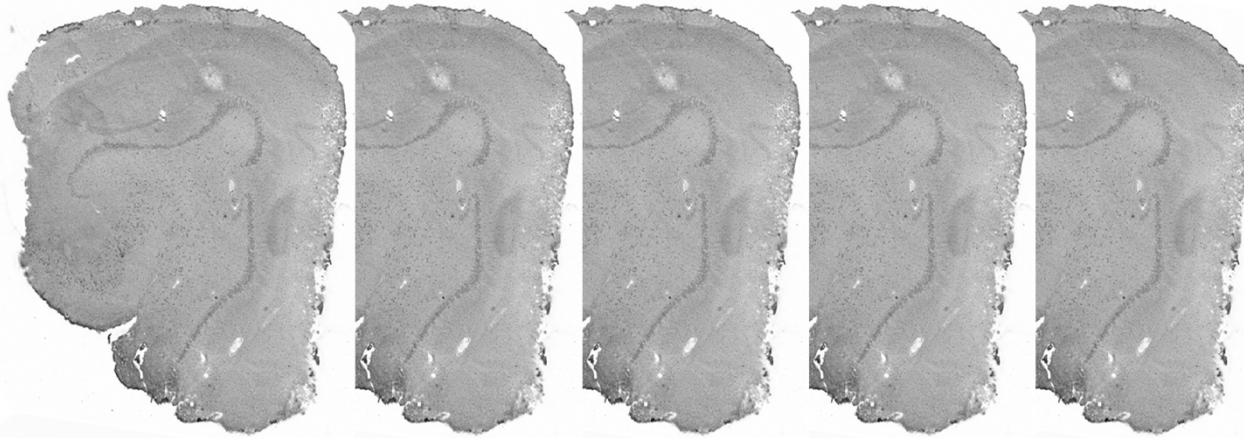
¹² Shiryayev S et al. PLoS One. 2009. 4: e4952



Hippocampus of epileptic patients

Proteins Distribution and Identification

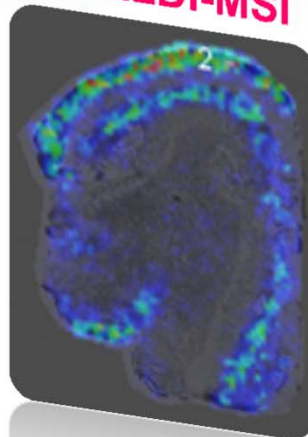
Consecutive Human Hippocampus Sections



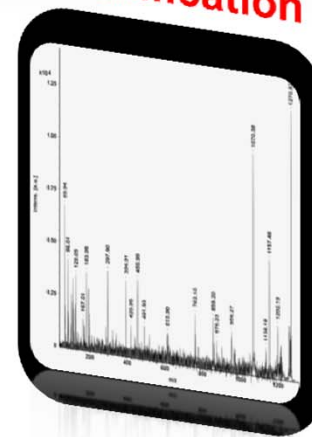
Staining



MALDI-MSI



Identification





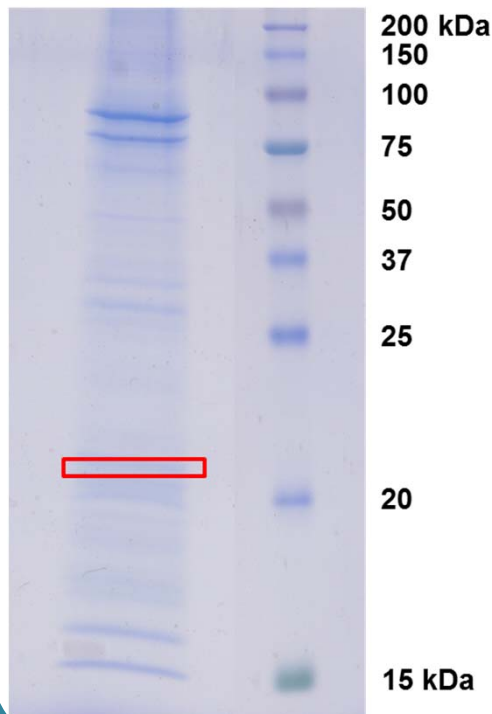
Hippocampus of epileptic patients

Proteins Distribution and Identification

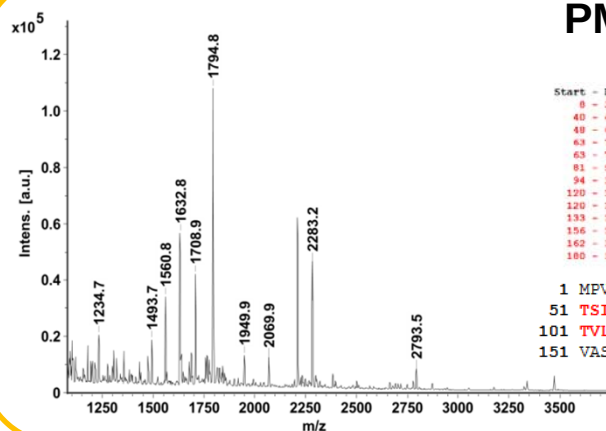


Extraction

SDS-PAGE



PMF

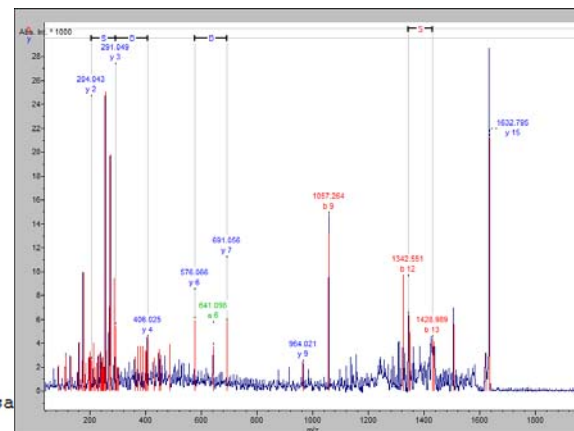


Start - End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence
8 - 39	3472.1178	3471.1105	3470.7106	0.3998	0	K.NSGPLSLQEVDPQHFPLVITYAGAVALDELGE.V
40 - 47	885.5407	884.5335	884.5331	0.0003	0	K.VLPTQVK.N
48 - 62	1632.7776	1631.7703	1631.7903	-0.0200	0	K.NRPTSIWDGLDSGK.L
63 - 76	1560.8102	1559.8030	1559.8190	-0.0160	0	K.LYTLVLTPDAPNR.K
63 - 77	1688.8918	1687.8845	1687.9145	-0.0299	1	K.LYTLVLTPDAPSRG.D
81 - 93	1758.8617	1757.8544	1757.8824	-0.0280	1	K.YKDHKPLVAME.G
94 - 113	1949.9339	1948.9266	1948.9370	-0.0112	0	K.GNDISSQTVLSQVVSOFPE.G
120 - 132	1708.8766	1707.8693	1707.8984	-0.0291	0	K.YVNLVYEQDRFLK.C
120 - 141	2793.5308	2792.5235	2792.5956	0.1279	1	R.YVNLVYEQDRFLKDEFLNR.S
133 - 141	1103.9117	1102.9044	1102.9077	-0.0033	0	K.CDEFLNR.S
156 - 161	836.4933	835.4860	835.4915	-0.0056	2	R.KDYELR.A
162 - 179	2069.9229	2068.9156	2068.9200	-0.0044	0	R.APVAGTCYQAEWDVYVK.L
180 - 187	937.5029	936.4956	936.4916	-0.0040	0	K.LYQLSGK.-

1 MPVDLSKWSG PLSLQEVDEQ PQHPLVITYA GAAVDELGRV LTPQVKNRP
 51 TSISWDGLDS GKLYTLVLTD PDAPSRKDPK YREWHHPLVV NMKGNDISSG
 101 TVLSQVYVSG PPKGTGLHRY VNLVYEQDRP LKCEPILSN RSGDHRGKFK
 151 VASFRKKYEL RAPVAGTCYQ AEWDVYVKL YEQLSGK

MS/MS

PEBP1_HUMAN Mass: 21158 Score: 39
 Phosphatidylethanolamine-binding protein 1 OS=Homo sa
 Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Unique Peptide
 1632.7776 1631.7703 1631.7903 -0.0200 0 39 0.016 1 U K.NRPTSIWDGLDSGK.L



Phosphatidylethanolamine-binding protein 1 (PEBP-1)



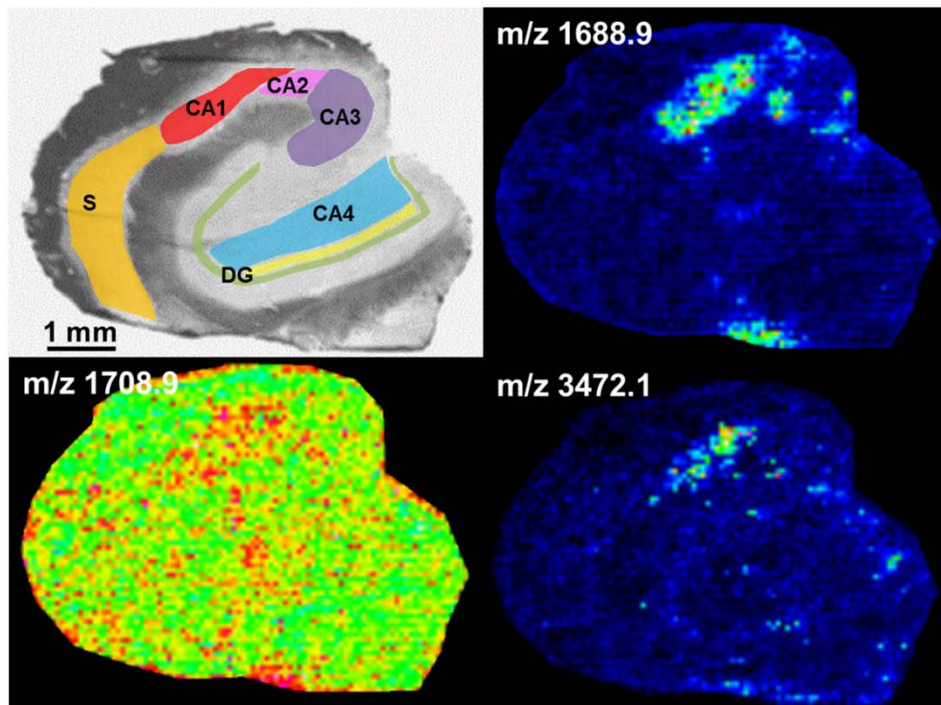
Hippocampus of epileptic patients

Proteins Distribution and Identification



MALDI-MSI

Phosphatidylethanolamine-binding protein 1 (PEBP-1)

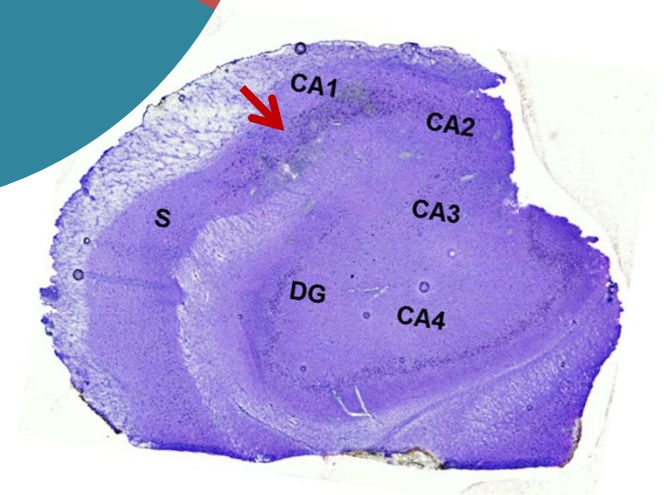
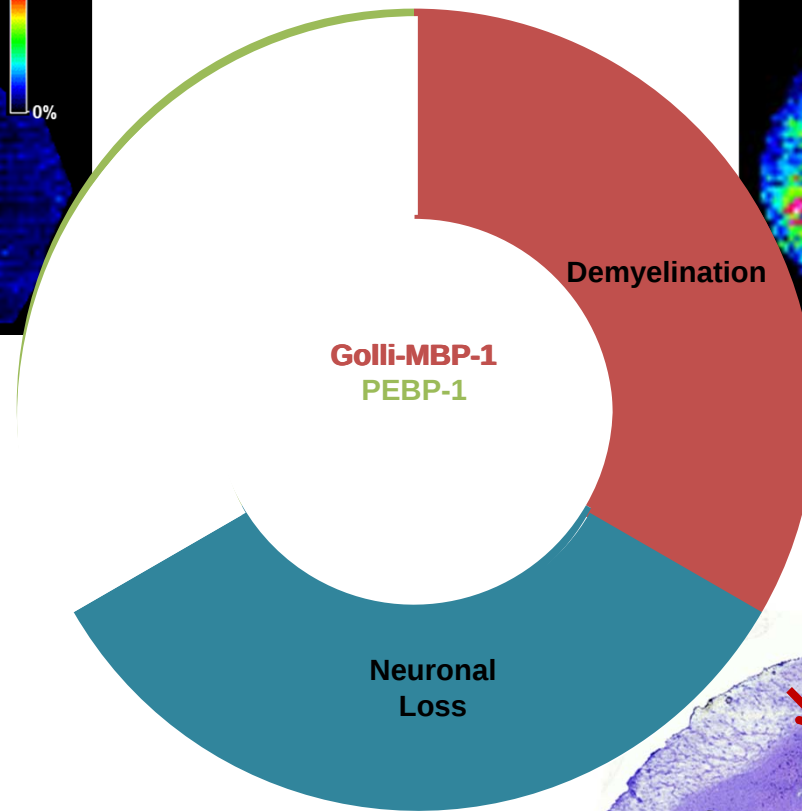
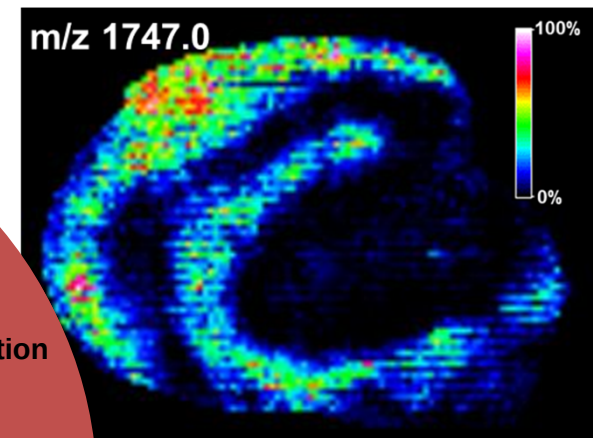
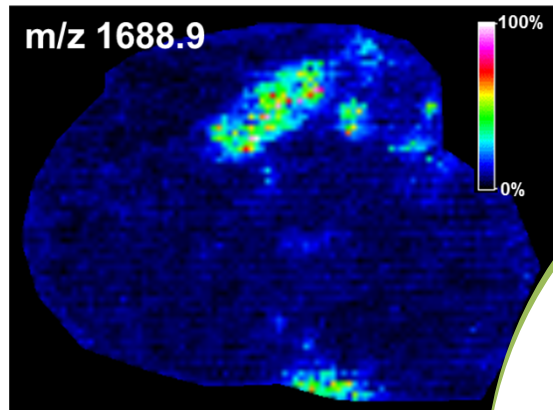


PEBP-1 distribution: CA1



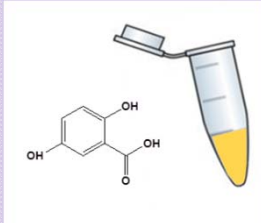
Hippocampus of epileptic patients

Conclusion



Conclusion

Matrices



Matrix deposit modes



Bioinformatic tools

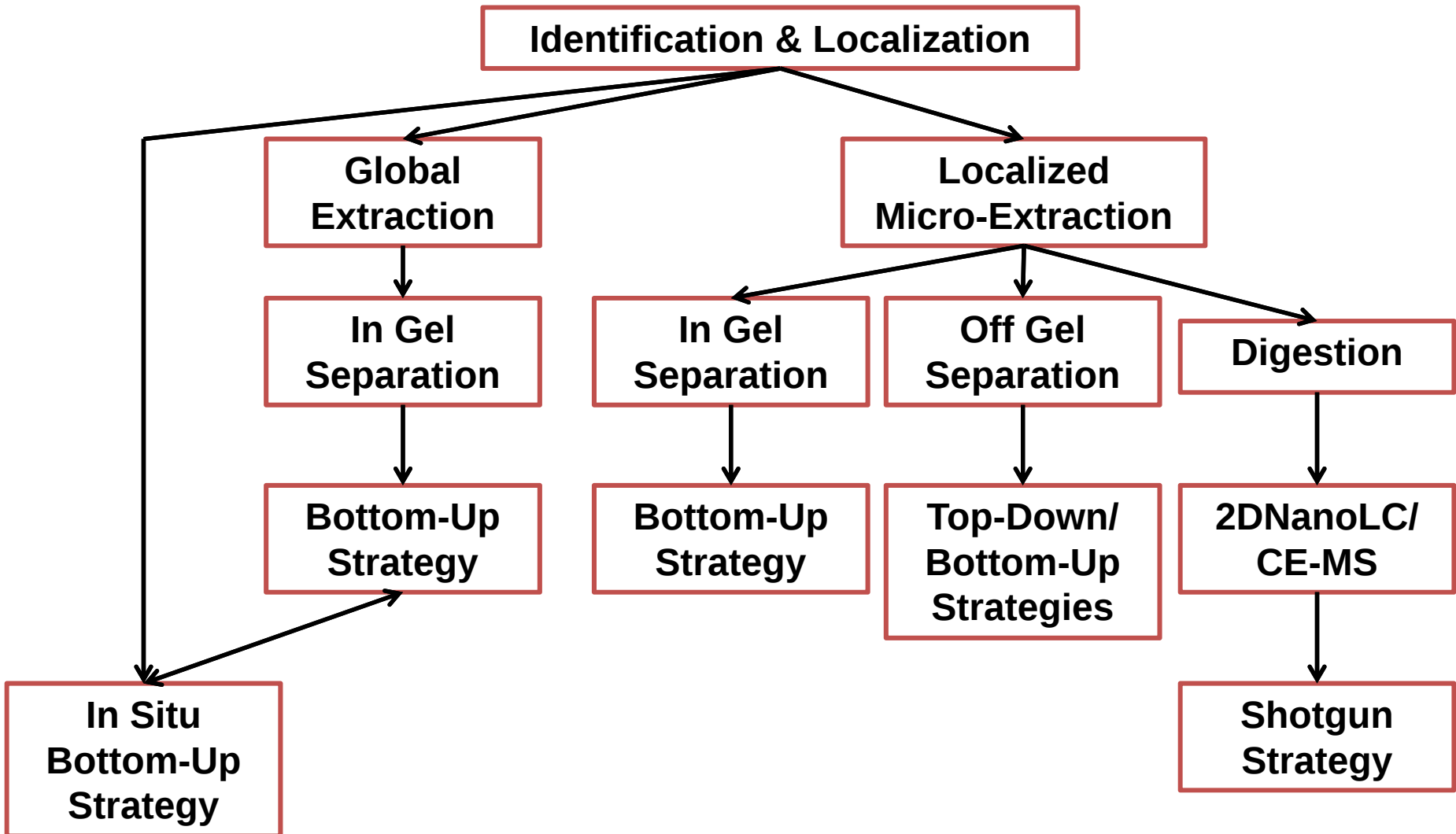


PEPTIDES IMAGING

Spatiotemporal molecular modifications during CNS regeneration in medicinal leech

Molecular pattern specific of processes involved in neural degeneration in the hippocampus of epileptic patients

Perspectives



Acknowledgements

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Pr. Isabelle Fournier

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J. Vizioli, C. Van Camp, AL. Garçon, L. Mylondo-Tso

SFSM

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