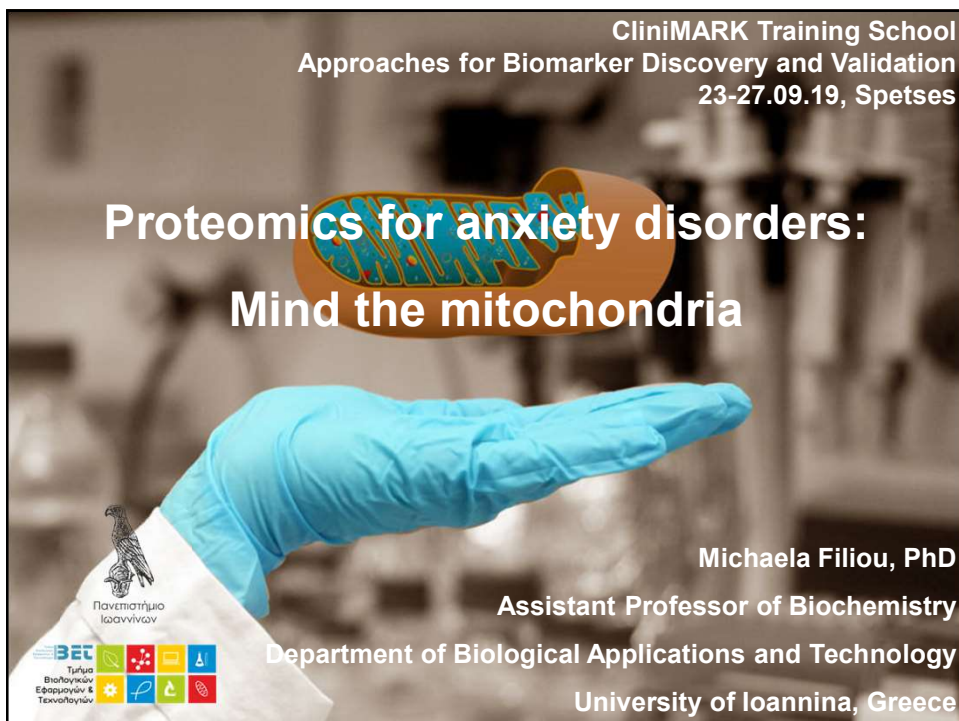




CliniMARK Training School
Approaches for Biomarker Discovery and Validation
23-27.09.19, Spetses

**Proteomics for anxiety disorders:
Mind the mitochondria**

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A hand wearing a blue nitrile glove is shown holding a stylized, 3D-rendered mitochondrion. The mitochondrion is orange with a blue internal structure representing cristae. The background is a blurred laboratory setting with scientific equipment.

 Πανεπιστήμιο
Ιωαννίνων


 BET
Τμήμα
Βιολογικών
Εφαρμογών &
Τεχνολογιών

**Or
how to get a list of differentially
expressed proteins and what to do
with it**






Several levels requiring decisions that need to be made



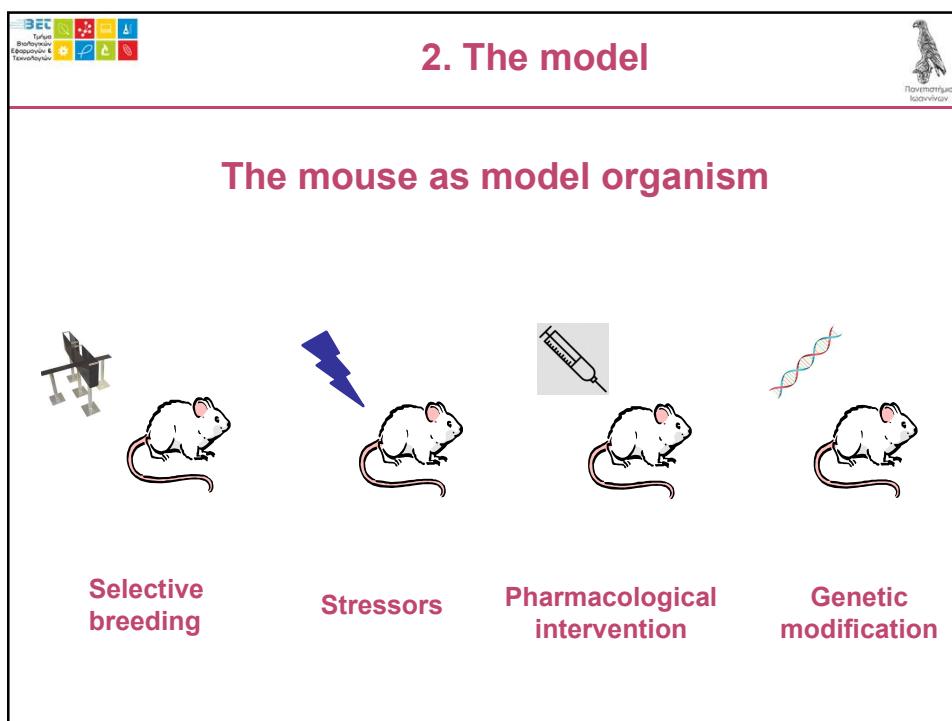
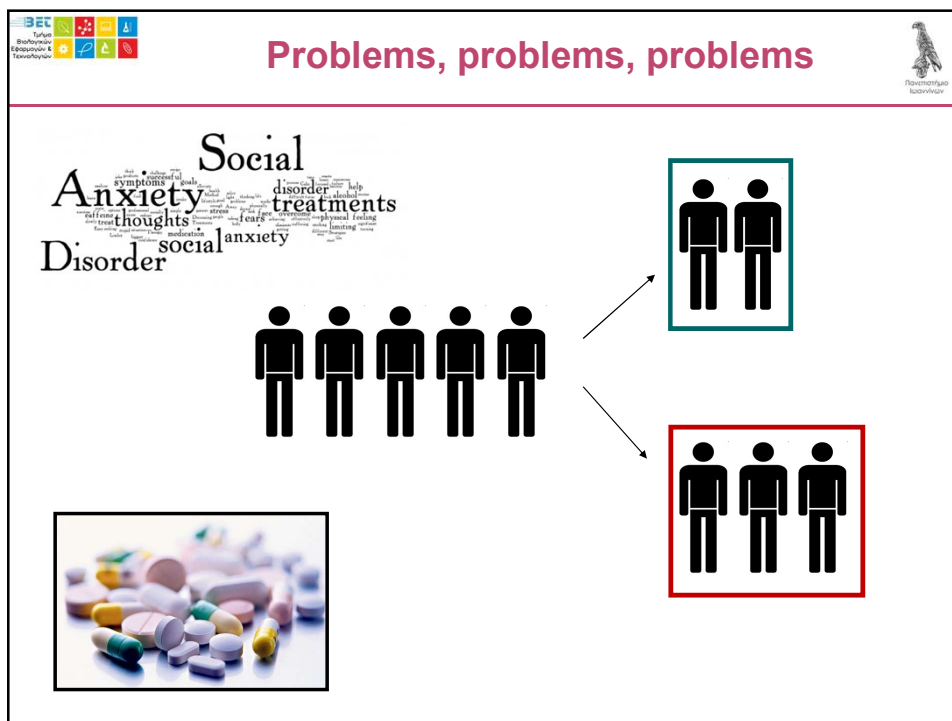
1. The question
2. The model
3. The biological material
4. The subproteome
5. The proteomic method
6. The list, what to make of it
7. How to follow up
8. How to play with pharmacology




[illegible]


1. The question

THERE ARE NO MOLECULAR BIOMARKERS FOR PSYCHIATRIC DISORDERS






3. The biological material




The brain



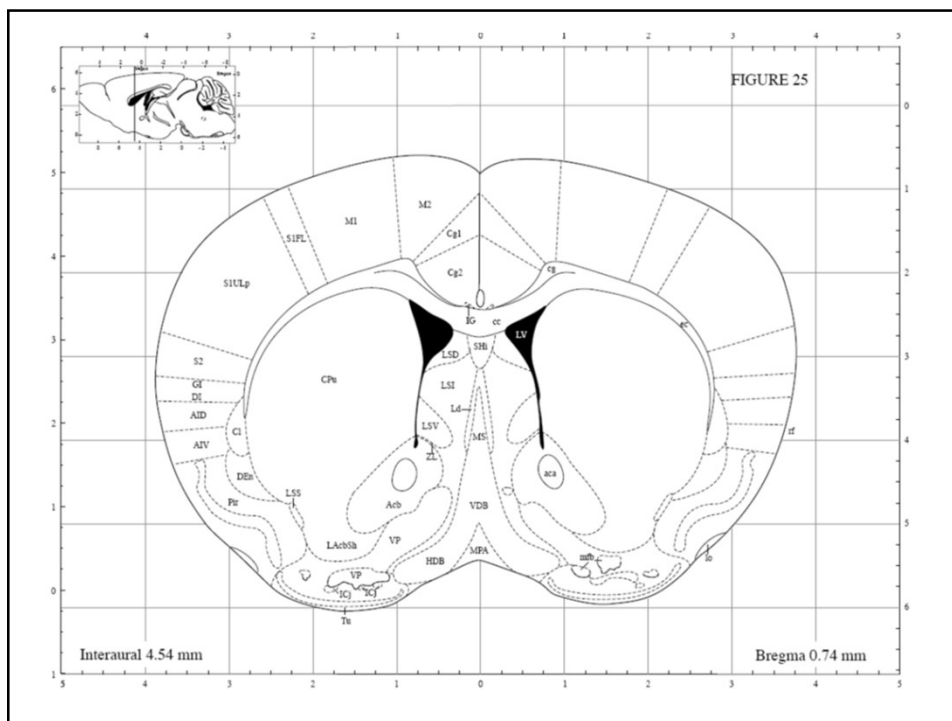
- Causality
- Specificity vs. starting material
- Pooling?


Peripheral material



- Causality?
- Diagnostic value
- What do we see there?


Possibility to combine?






Βιοτεχνολογική
Εταιρεία
Τεχνολογίας


4. The subproteome



Εθνικό Ινστιτούτο
Βιοϊατρικής Έρευνας




- Increase specificity
- Reduce complexity




Βιοτεχνολογική
Εταιρεία
Τεχνολογίας

What shall we keep in mind?




Εθνικό Ινστιτούτο
Βιοϊατρικής Έρευνας

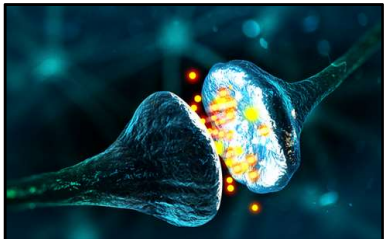
- Do we have a suitable protocol?
- Is the protocol appropriate for our model?
- Is it an enrichment or an isolation protocol?
- Is there a subproteome profiling available?
- How much starting material do we need?
- Should we use fresh tissue?
- Are the buffers compatible with mass spectrometry/the proteomics workflow we will use?



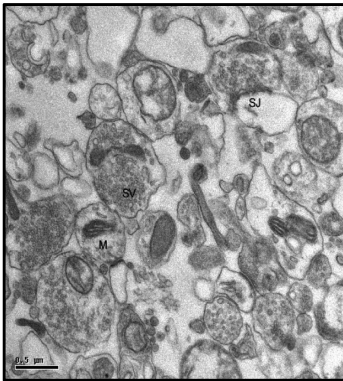
Synaptosomes



Artificially isolated synapses




4% vesicles
8% membranes
24% mitochondria
64% cytoplasm




SV: synaptic vesicles
SJ: synaptic junction
M: mitochondrion

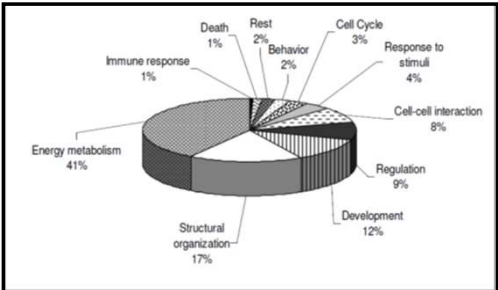
Schrimpf et al. Proteomics 2005



Synaptosome proteome




	C	N	S1	S2	S3	Syn
Gria2						
PSD95						
Slc17a7						
Gap43						
Crym						
Prkc						
Mbp						




Function	Percentage
Energy metabolism	41%
Structural organization	17%
Development	12%
Regulation	9%
Cell-cell interaction	8%
Response to stimuli	4%
Cell Cycle	3%
Rest	2%
Behavior	2%
Death	1%
Immune response	1%

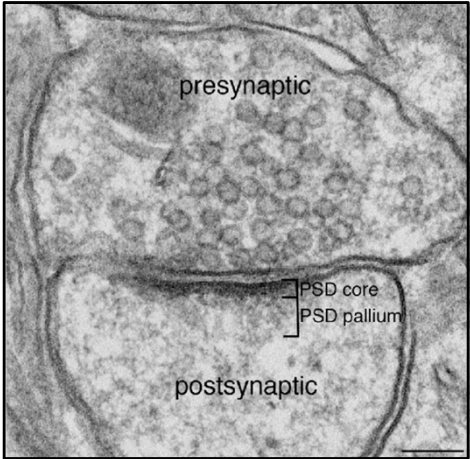
**2980 synaptosomal proteins
from whole mouse brain**

Maccarrone and Filiou Methods Mol Biol 2015
Filiou et al Electrophoresis 2010



Post-synaptic density proteome





presynaptic

postsynaptic


PSD core

PSD pallium


PSD: post-synaptic density

	Total PSD
Channels and receptors	80
MAGUKs/adaptors/scaffolders	54
Serine/threonine kinases	46
Tyrosine kinase	3
Protein phosphatases	18
G proteins and modulators	77
Signalling molecules and enzymes	278
Transcription and translation	119
Cytoskeletal and cell adhesion molecules	153
Synaptic vesicles and protein transport	159
Novel	107
Other	30
Summary	1124


Docemeci et al Frontiers Syn Neurosci 2016



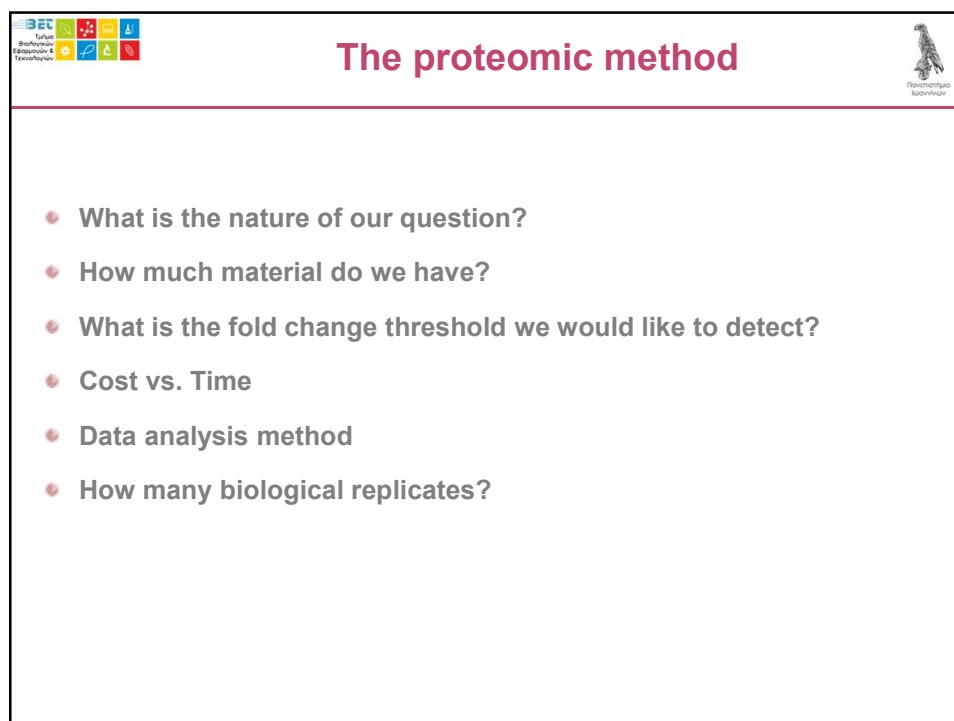
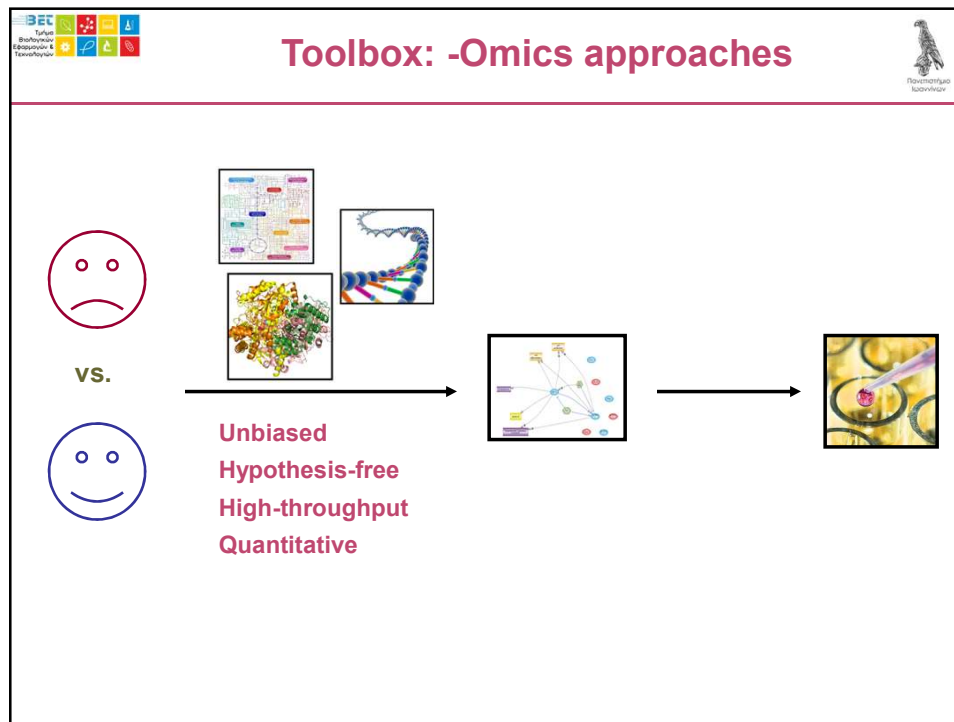
Contaminants

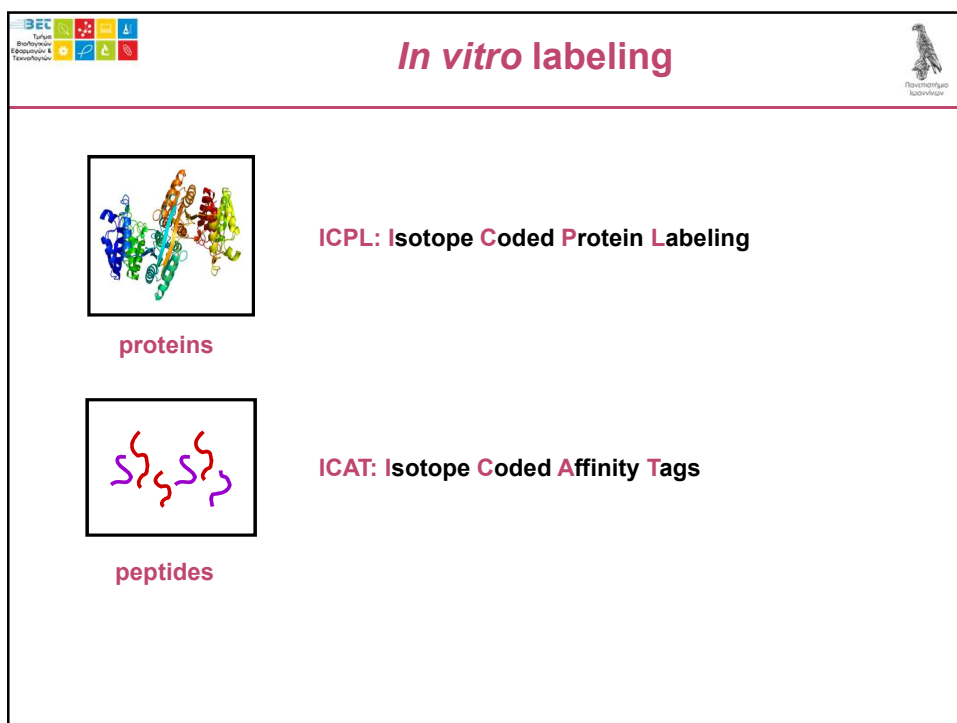
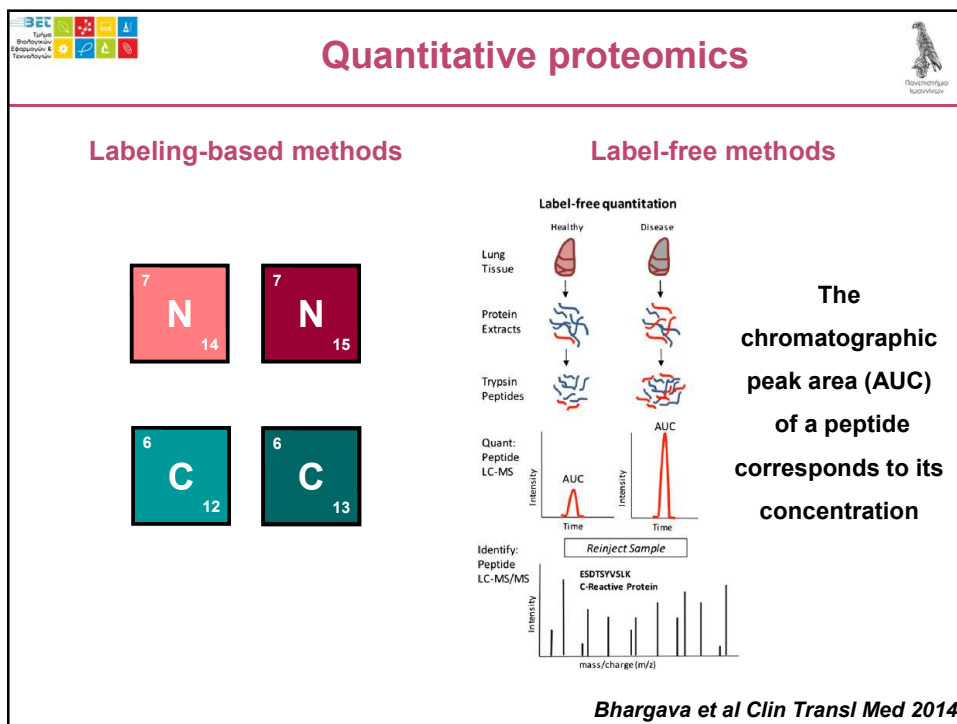


- Non neuronal components → Myelin-related proteins
- Nuclear proteins, Histones
- Keratin




Reproducibility of the subproteome enrichment!







In vivo metabolic labeling



amino acids: ^{13}C Lys, SILAC
isotopes: ^{15}N , SILAM

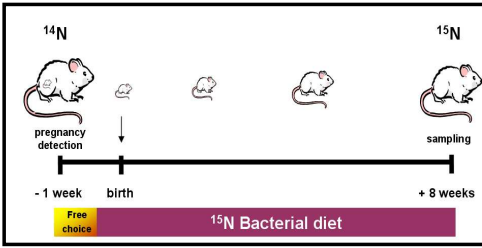
whole organisms

SILAC: Stable Isotope Labeling with Amino acids in Cell culture
SILAM: Stable Isotope Labeling of Mammals

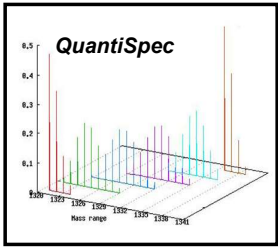
Ong et al Mol Cell Proteomics 2002
McClatchy and Yates Methods Mol Biol 2014

The ^{15}N -labeled mouse

^{15}N metabolic labeling protocol



% ^{15}N incorporation

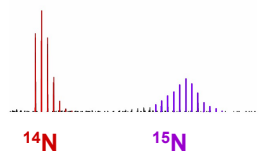


Cerebellum

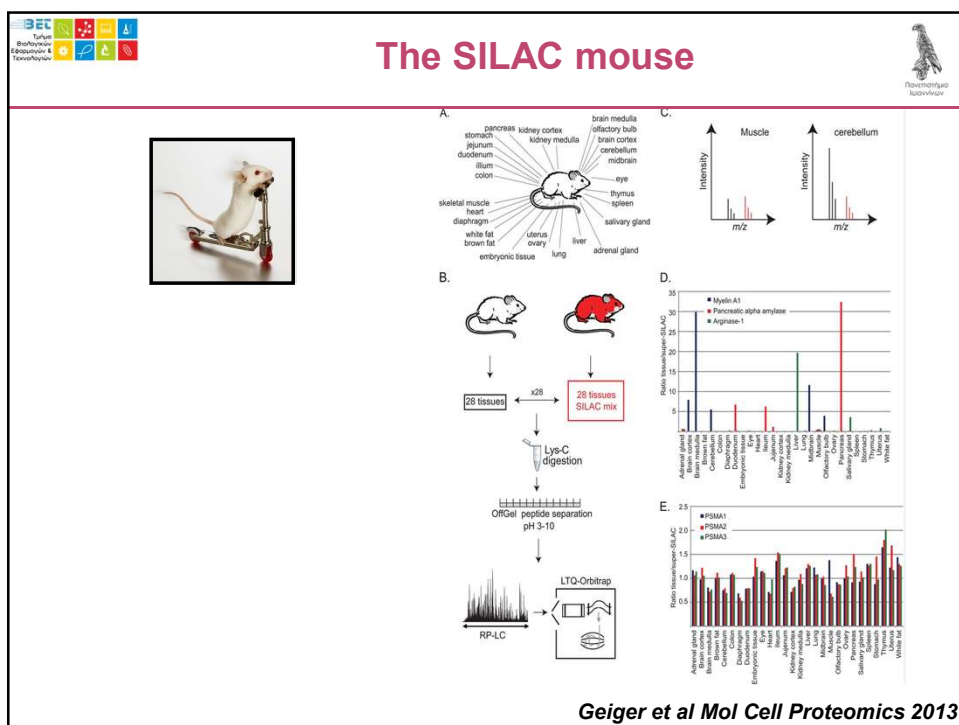
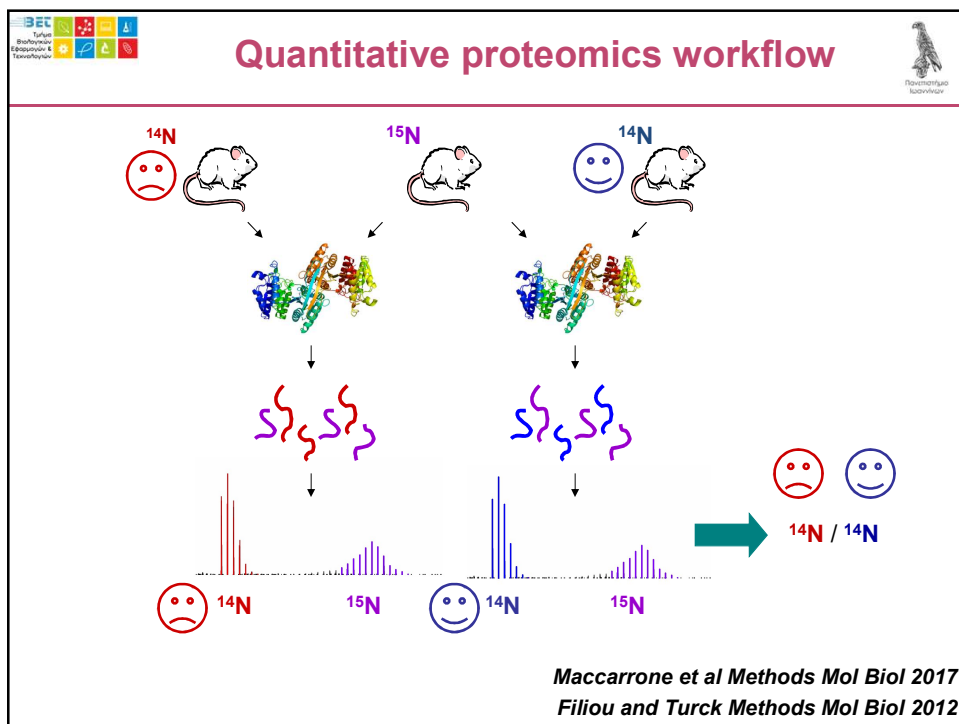
PND	^{15}N incorporation (%)
PND 5	~55
PND 14	~75
PND 28	~80
PND 56	~90

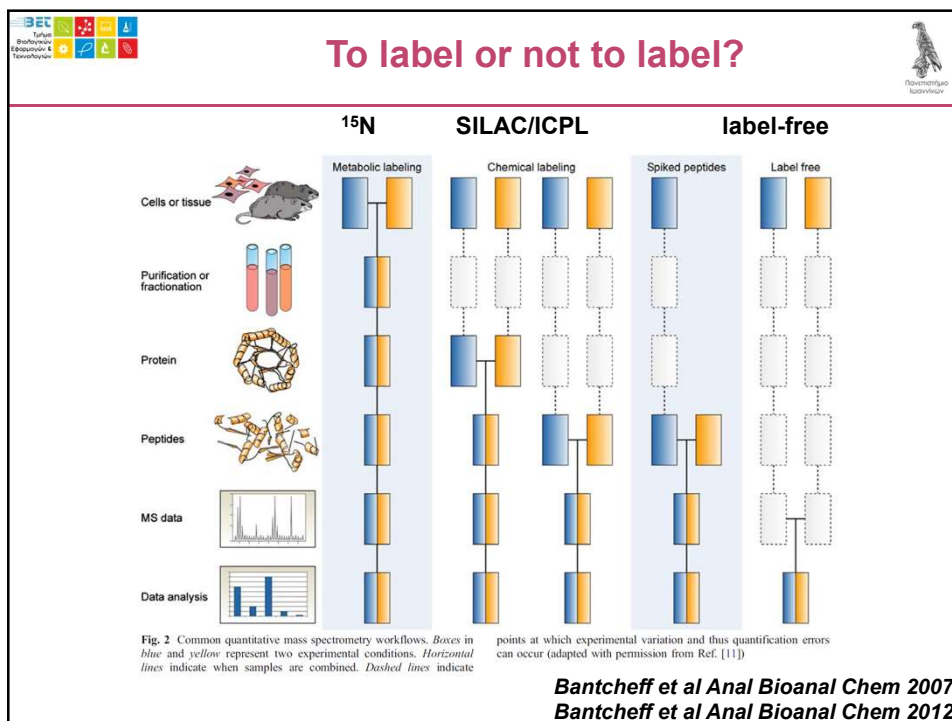
Plasma

PND	^{15}N incorporation (%)
PND 5	~65
PND 14	~80
PND 28	~90
PND 56	~90



Frank et al PLoS ONE 2009





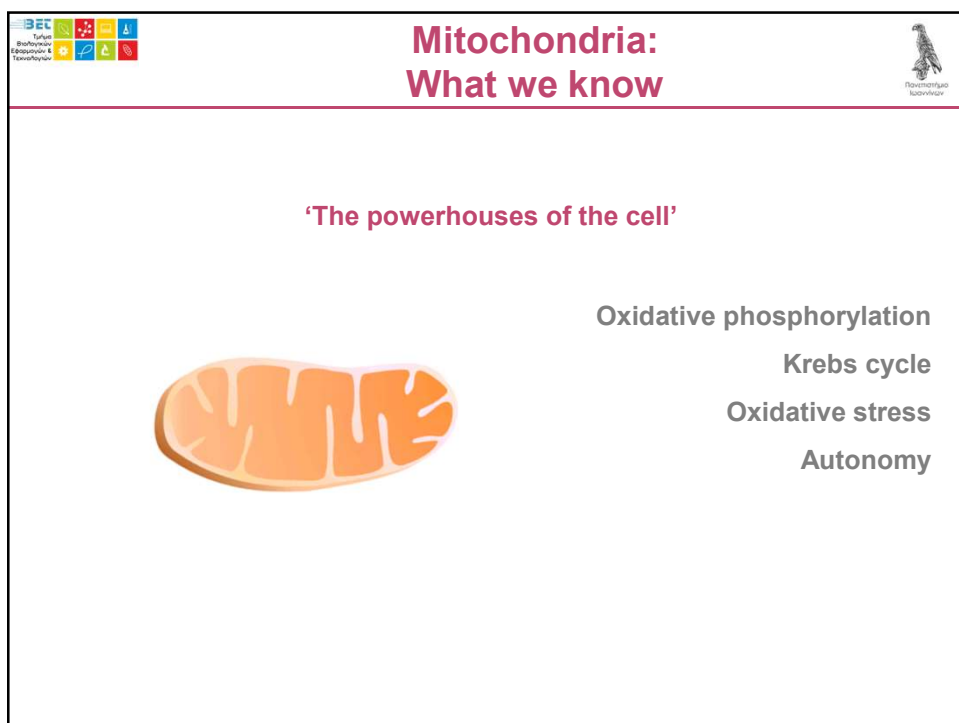
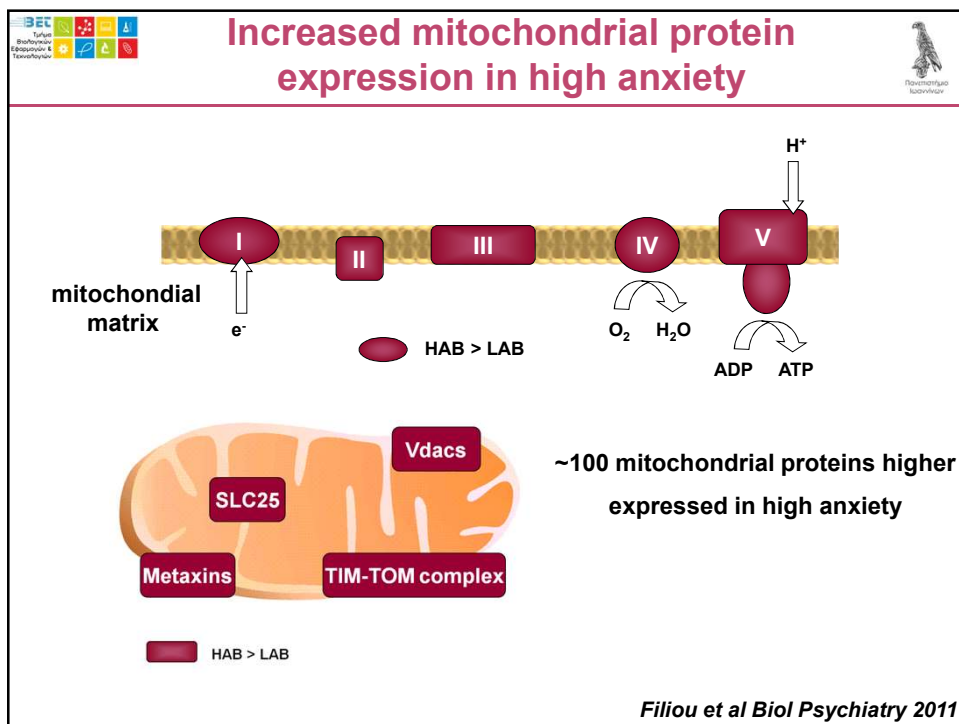
To label or not to label?


Table 1 Characteristics and applications of quantitative mass spectrometry methods

		Application	Accuracy (process)	Quantitative proteome coverage
^{15}N	Metabolic protein labeling	Complex biochemical workflows Comparison of 2–3 states Cell culture systems only	+++	++
SILAC	Chemical protein labeling (MS)	Medium to complex biochemical workflows Comparison of 2–3 states	+++	++
ICPL	Chemical peptide labeling (MS)	Medium complexity biochemical workflows Comparison of 2–3 states	++	++
	Chemical peptide labeling (MS/MS)	Medium complexity biochemical workflows Comparison of 2–8 states	++	++
	Enzymatic labeling (MS)	Medium complexity biochemical workflows Comparison of 2 states	++	++
label-free	Spiked peptides	Medium complexity biochemical workflows Targeted analysis of few proteins	++	+
	Label free (ion intensity)	Simple biochemical workflows Whole proteome analysis Comparison of multiple states	+	+++
	Label free (spectrum counting)	Simple biochemical workflows Whole proteome analysis Comparison of multiple states	+	+++


Overall	
Labeling-based methods	Label-free methods
<ul style="list-style-type: none"> + High accuracy + High resolution + High sensitivity 	<ul style="list-style-type: none"> + Cheap + Fast + Easy
<ul style="list-style-type: none"> - Expensive - Complicated (spectra/software) - Time consuming 	<ul style="list-style-type: none"> - Lower sensitivity (>2 fold) - Lower accuracy - Not optimal for small proteins (<20kDa) or too abundant ones


Practical tips	
Metabolic labeling/SILAC	Label-free
Complex sample preparation (e.g. multistep subproteome enrichment from brain tissue)	Simple sample preparation (e.g. cell culture lysates)
Interested in low fold changes (e.g. mild interventions, chronic effects)	Interested in high fold changes (>2 fold, e.g. acute intervention)
Small datasets to be studied in depth	Large datasets to be screened
Need appropriate organism-specific labeled standard	All organisms






Mitochondria in the brain: What perhaps we do not know







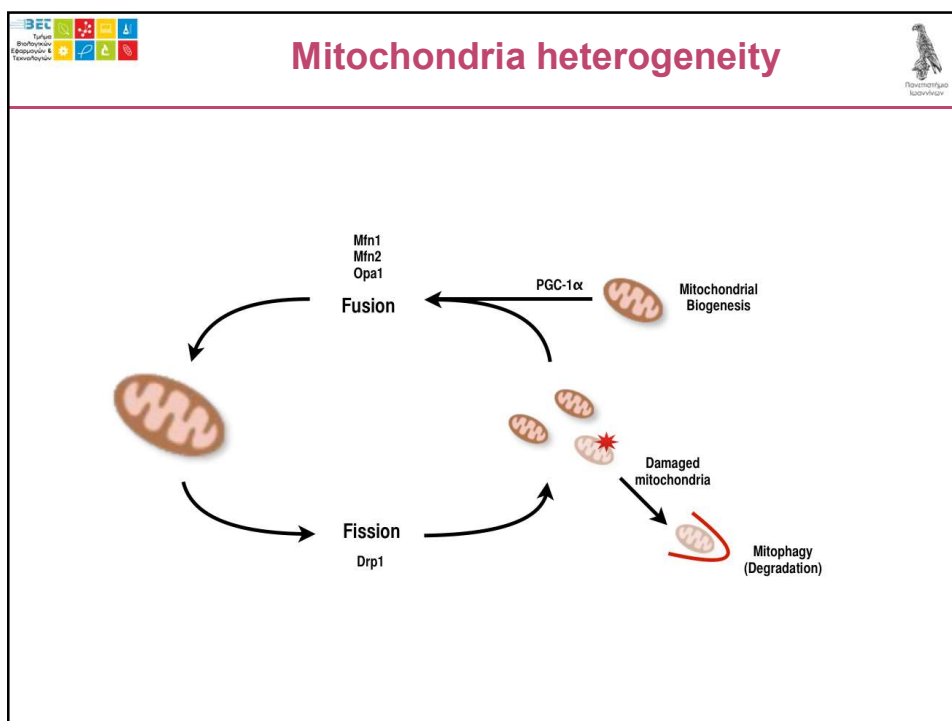
Calcium storages

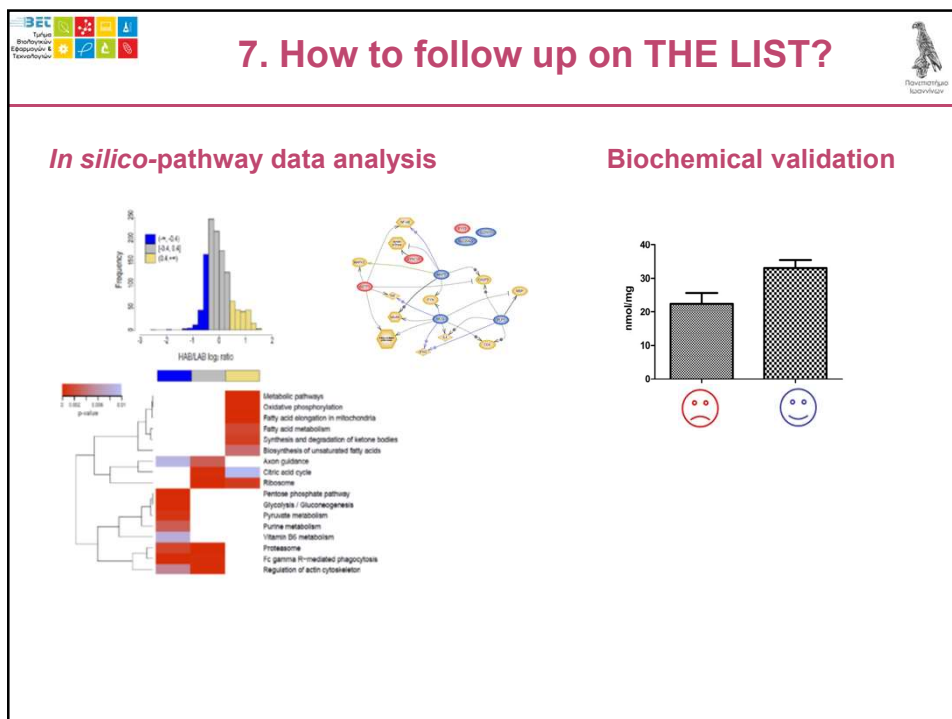
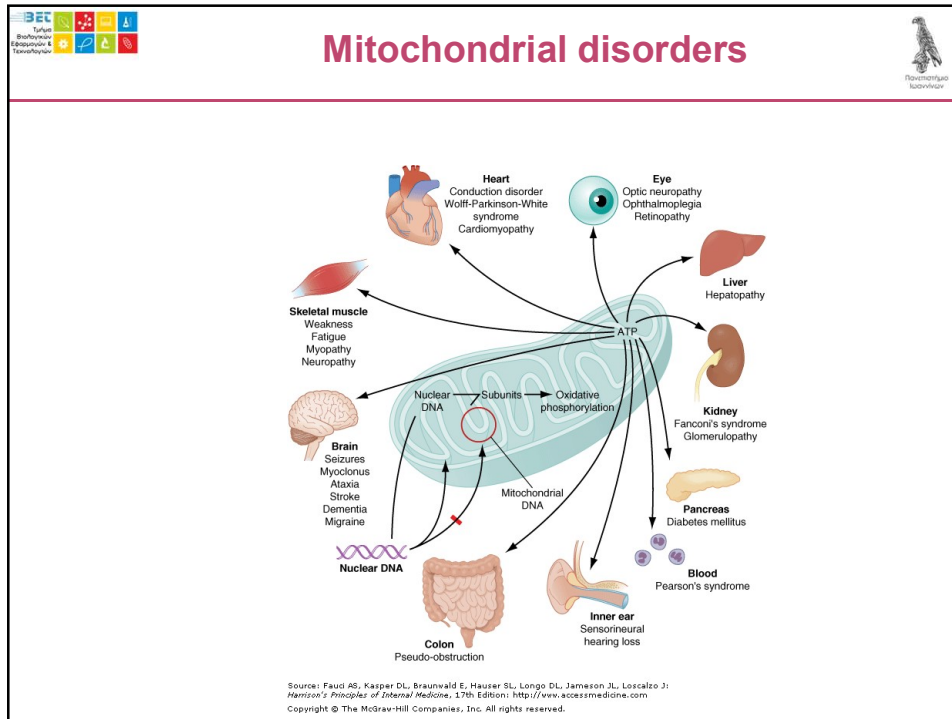
Neurotransmission regulation

Apoptosis

Stress response

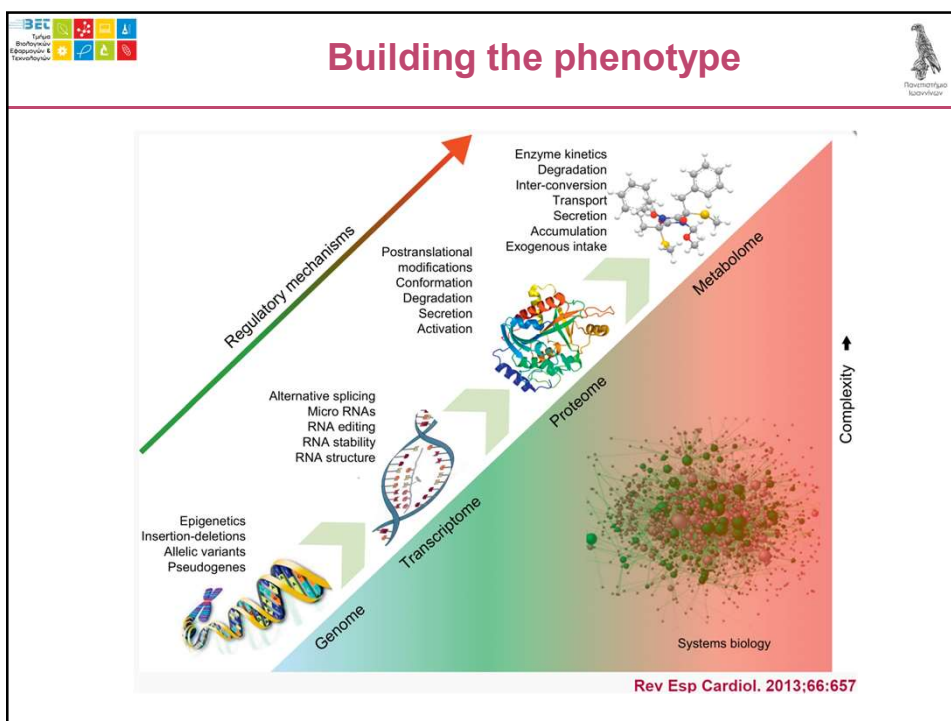
Mitochondrial heterogeneity

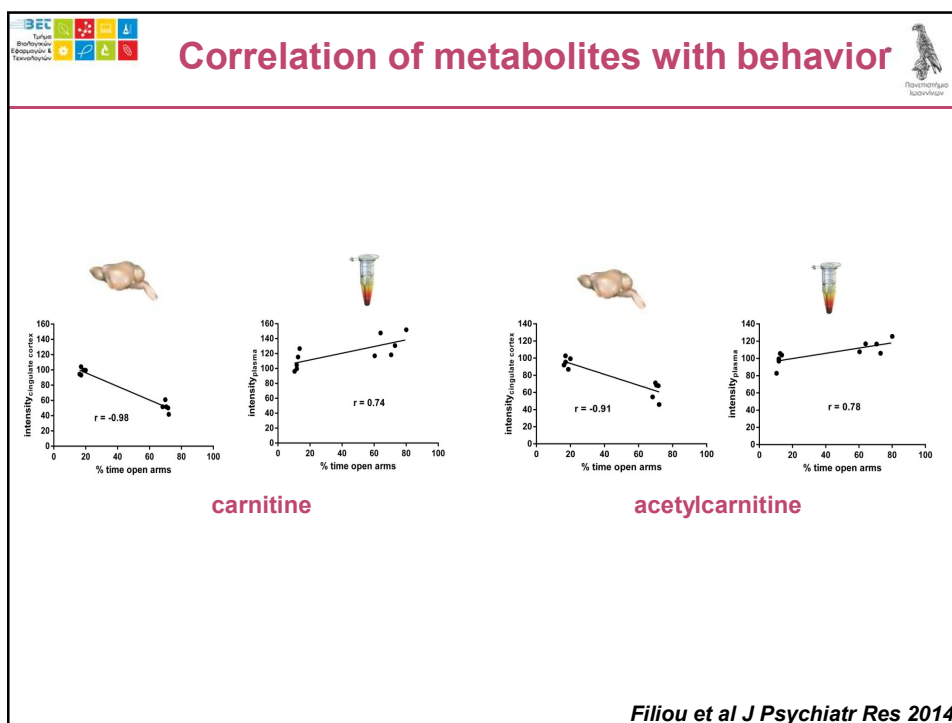
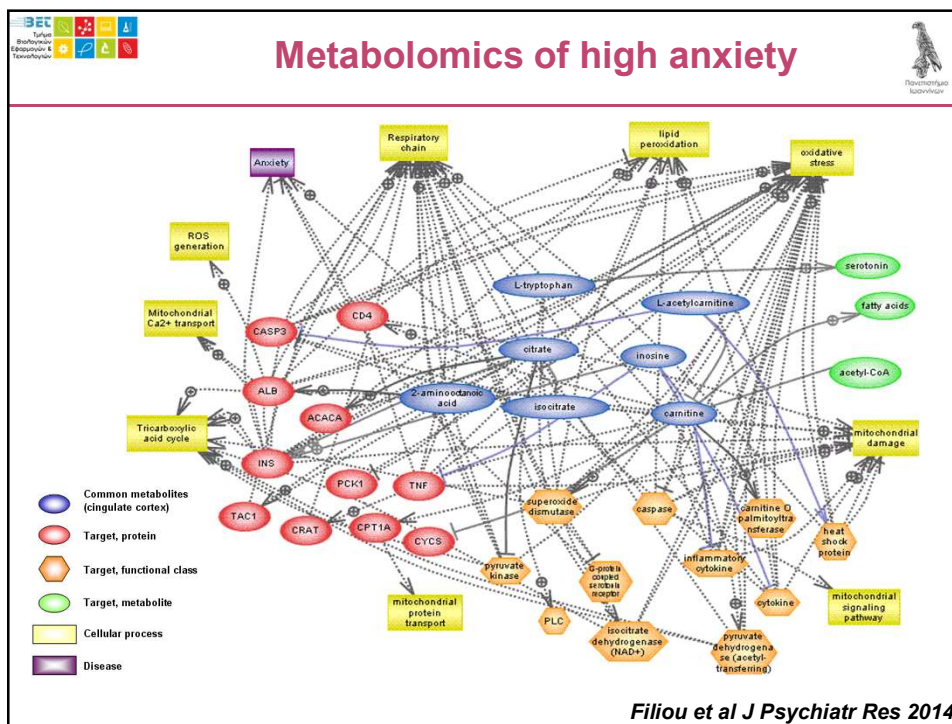


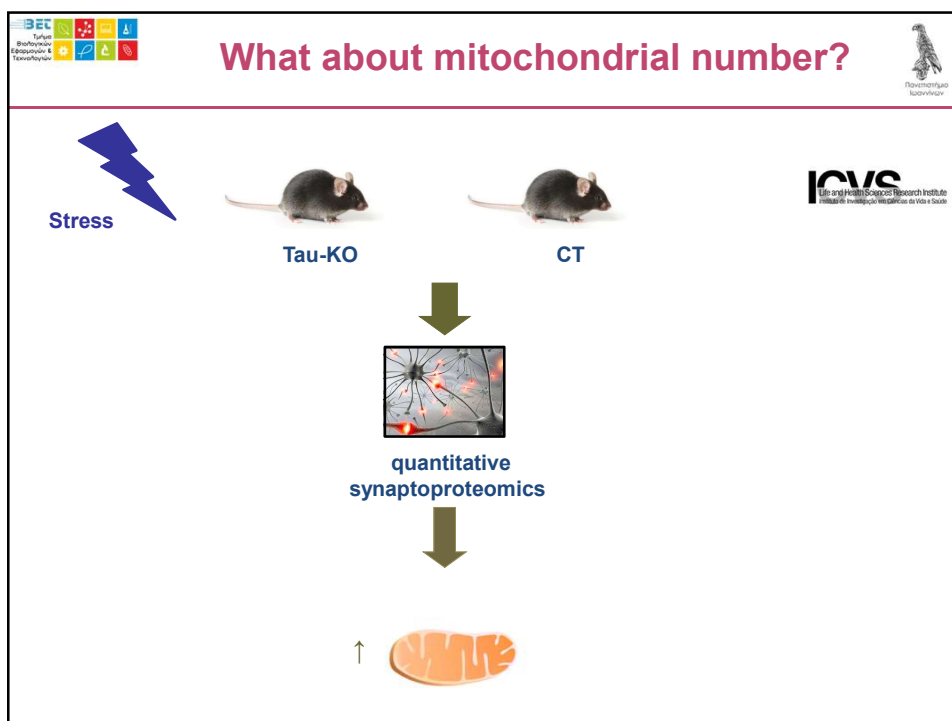
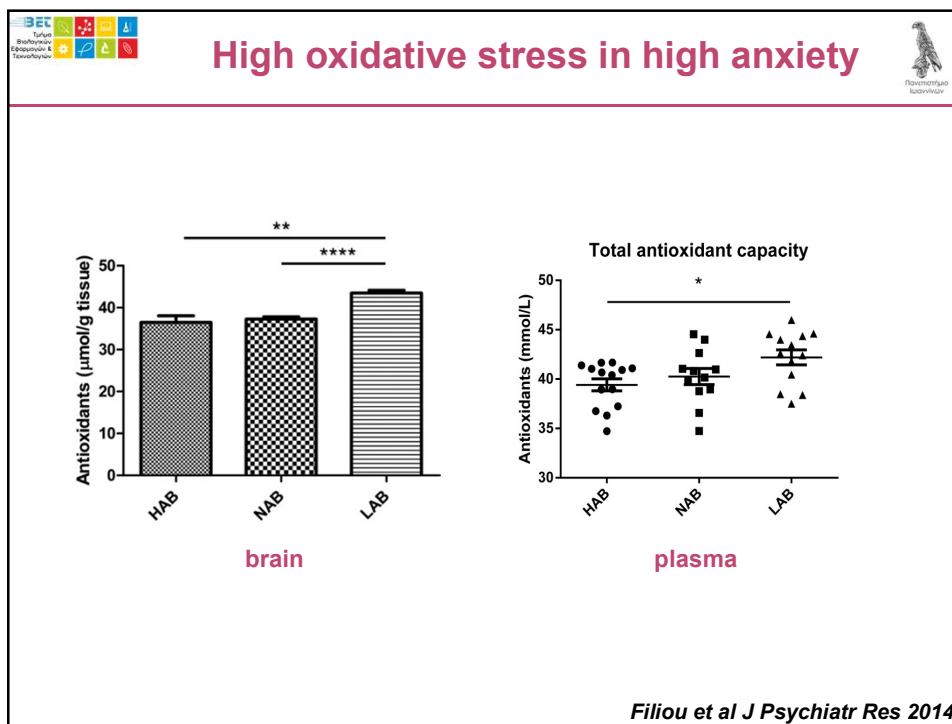


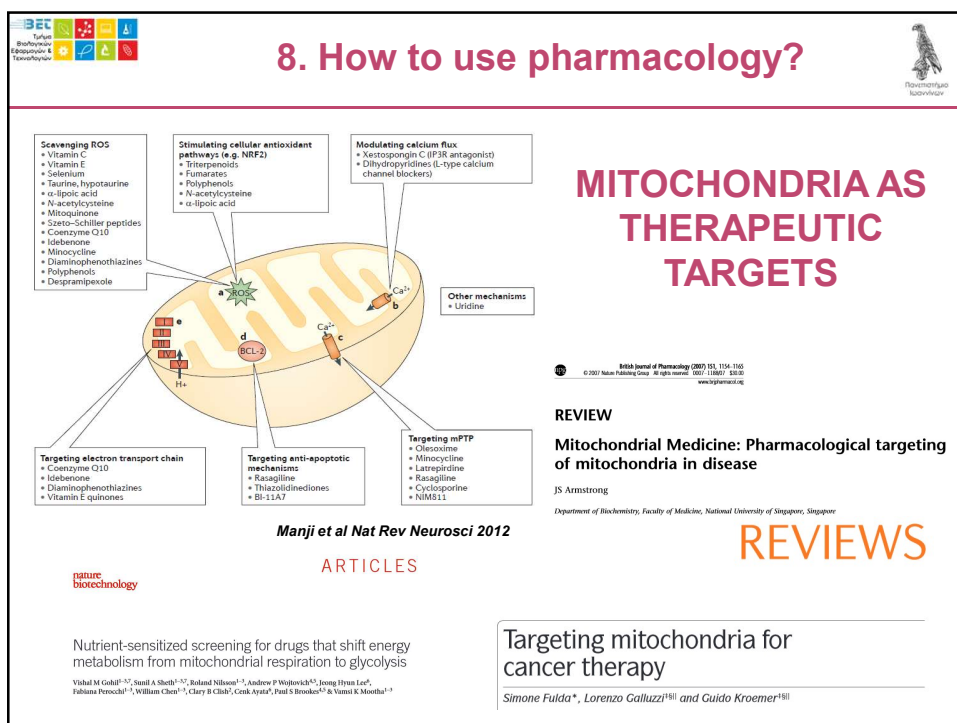
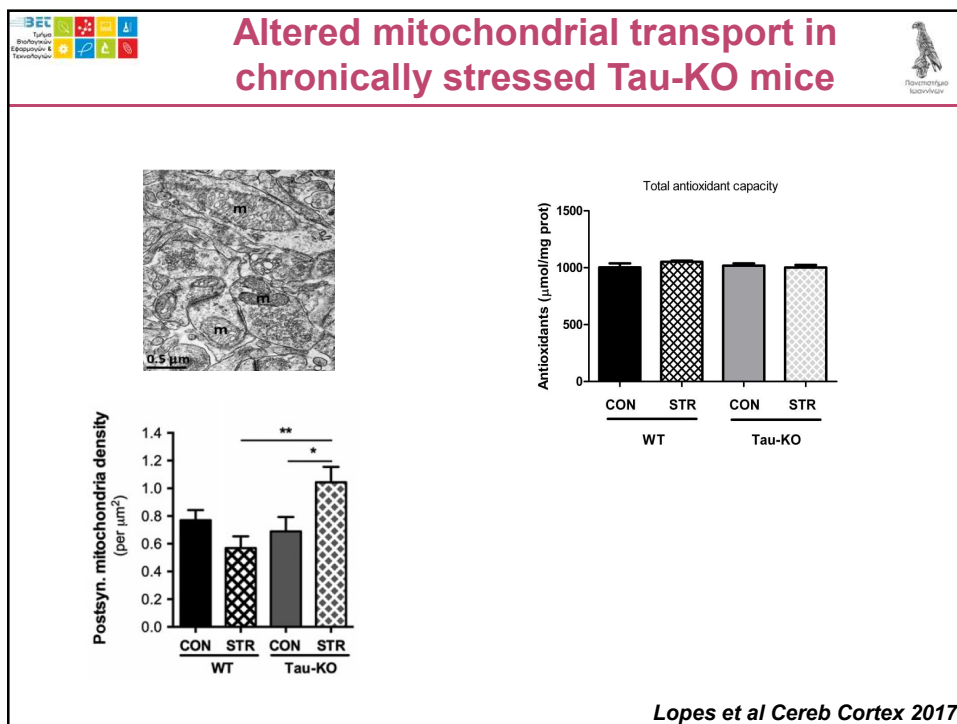
How to follow up on mitochondria as an organellar potential “biomarker”?


- Additional -omics
- Oxidative stress
- Mitochondrial number
- Mitochondrial morphology
- Pharmacological targeting
- What about the periphery?












Türkmen
Bilim
Eğitimi ve
Teknolojisi

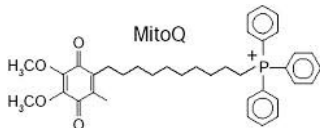
Mitochondria-targeted antioxidants




Türkmenistan
Üniversitesi

MitoQ: mitochondria-targeted antioxidant

quinone + triphenylphosphonium (TPP)



MitoQ



Türkmen
Bilim
Eğitimi ve
Teknolojisi

Experimental approaches

<i>In vitro</i>	<i>In vivo</i>
<p>Test toxicity</p> <p>Verify optimal dosage</p> <p>Quick</p>	<p>Effects on the anxiety phenotype</p> <p>Pathophysiological side-effects</p> <p>Informative</p>

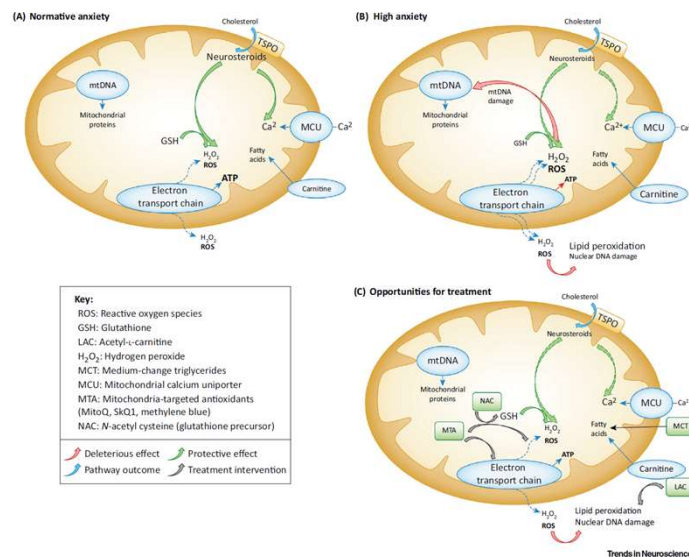


In vivo approach: considerations


- Mode of administration
- How to verify same dosage for all animals?
- Taste?
- Is the substance metabolized before reaching the desired organ?
- Does the substance cross the blood brain barrier?
- Can the substance be administered long-term without side-effects?




Anxiety and mitochondria: A bidirectional crosstalk



Filiou and Sandi Trends Neurosci 2019




Acknowledgments






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Claude Lechene



Mike Murphy



John Sotiropoulos
Nuno Sousa




Marika Syrrou
Zoi Papadopoulou
Daniela Theodoridou





Giannis Kostakis
Vaggelis Gikas
Christina Dalla
Nikos Kokras















Transition to clinical samples

- What type of material? Post-mortem brain? Plasma? Serum?
Blood cells?
- Differential diagnosis
- Drug naive?
- Response to treatment?
- What type of confounding pathologies?