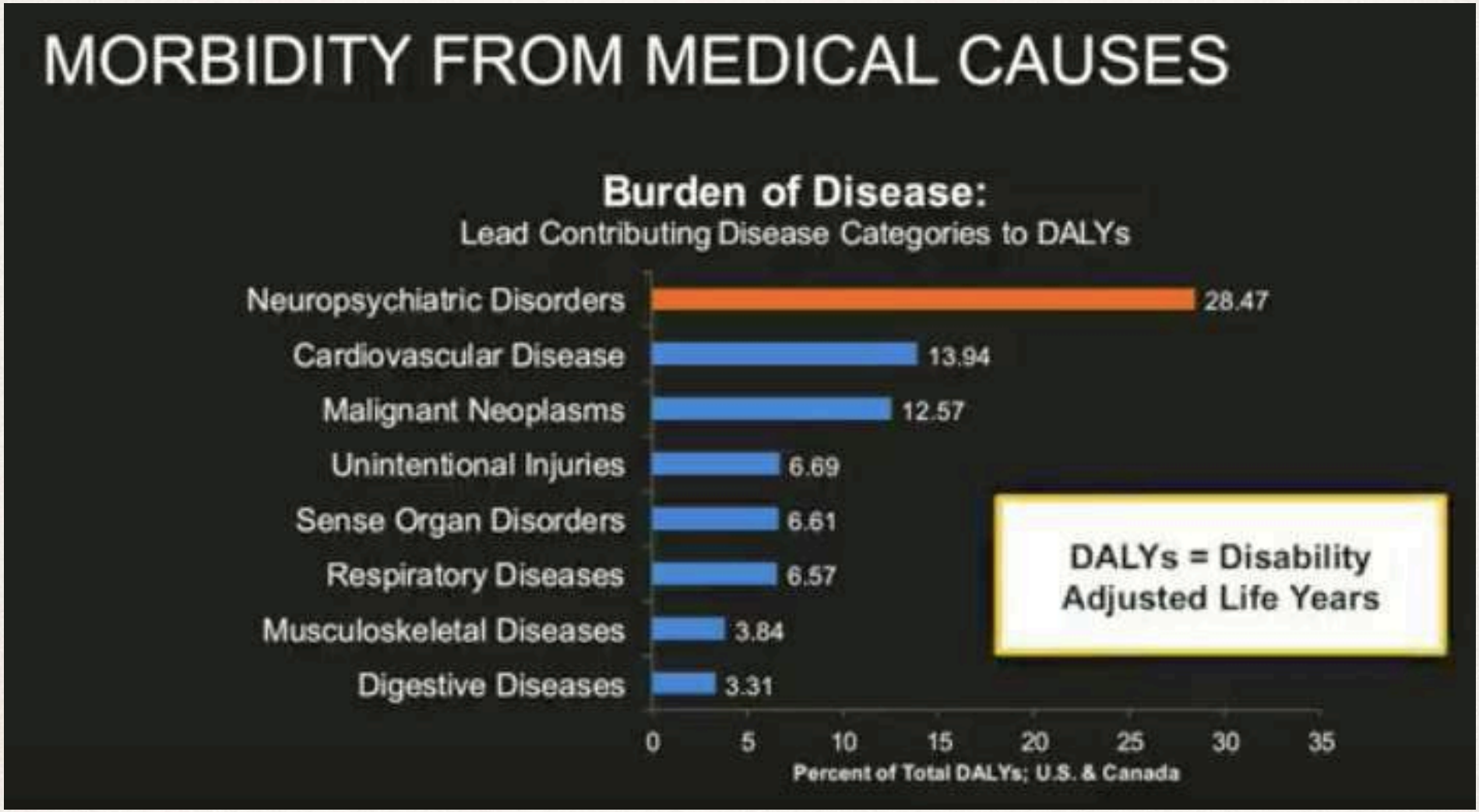
Combining neuroproteomics and mini-brains to understand psychiatric disorders

Prof. Daniel Martins-de-Souza

Lab of Neuroproteomics, Dept of Biochemistry, University of Campinas (UNICAMP), Brazil

Thomas Insel: Toward a new understanding of mental illness



Schizophrenia

* Biochemical comprehension

* Ineffective treatment

* Clinical diagnosis

* Prognosis

*** Translational Strategies ***

'The Scream'
Edvard Munch

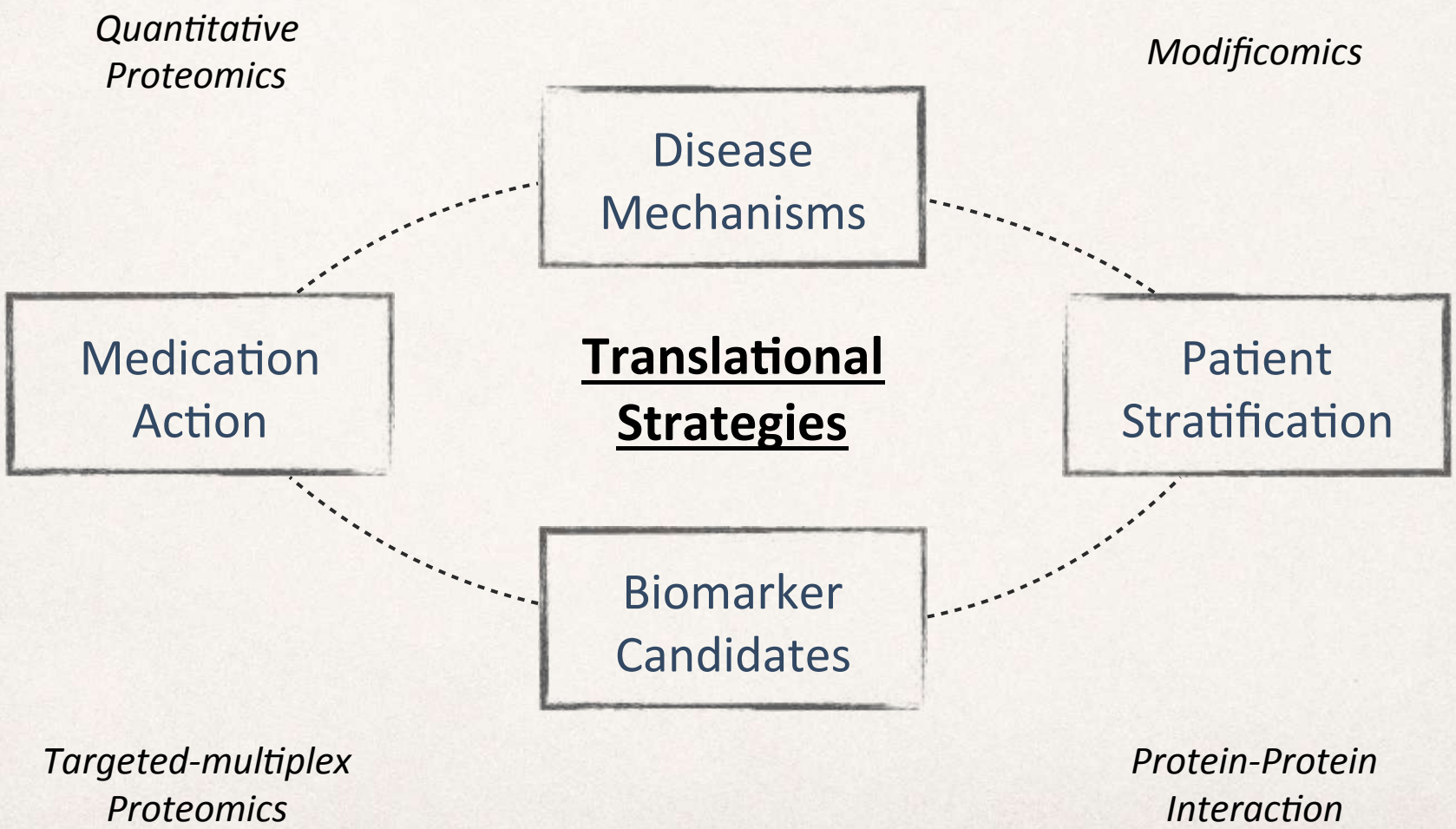
Schizophrenia (and Depression)

Multifactorial disorders

caused by differential gene and protein expression
since the neurodevelopment + **environmental factors**

Proteome → Proteomics

Clinical Proteomics

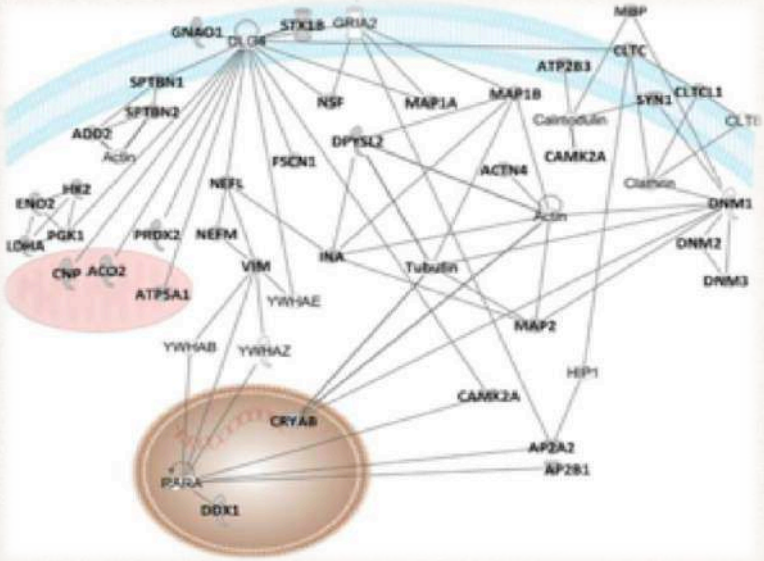


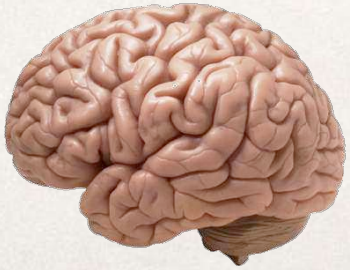
Proteomics Clin Appl. 2016 Dec;10(12):1148-1158. doi: 10.1002/prca.201600021. Epub 2016 Aug 12.

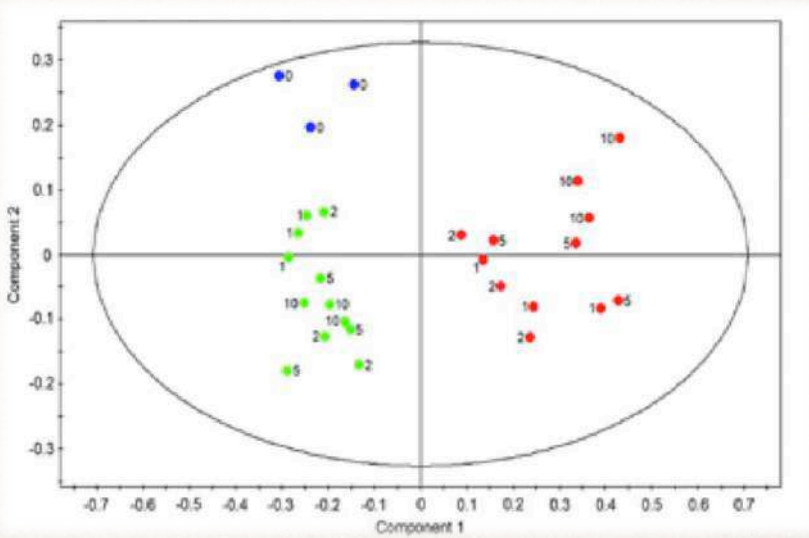
Proteomics and molecular tools for unveiling missing links in the biochemical understanding of schizophrenia.

Nascimento JM[1], Garcia S[1], Saia-Cereda VM[1], Santana AG[1], Brandao-Teles C[1], Zuccoli GS[1], Junqueira DG[1], Reis-de-Oliveira G[1], Baldasso PA[1], Cassoli JS[1], Martins-de-Souza D[1].

Understand Molecular Mechanisms







Potential Biomarker
Candidates

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www.nature.com/npjschz

REVIEW ARTICLE

The proteome of schizophrenia

Juliana M Nascimento[1,2] and Daniel Martins-de-Souza[1]

Corpus Callosum

Mediodorsal thalamus

Wernicke's Area (BA22p)

Cerebrospinal fluid

Dorsolateral Prefrontal Cortex (BA46)

Orbitalfrontal cortex (BA11)

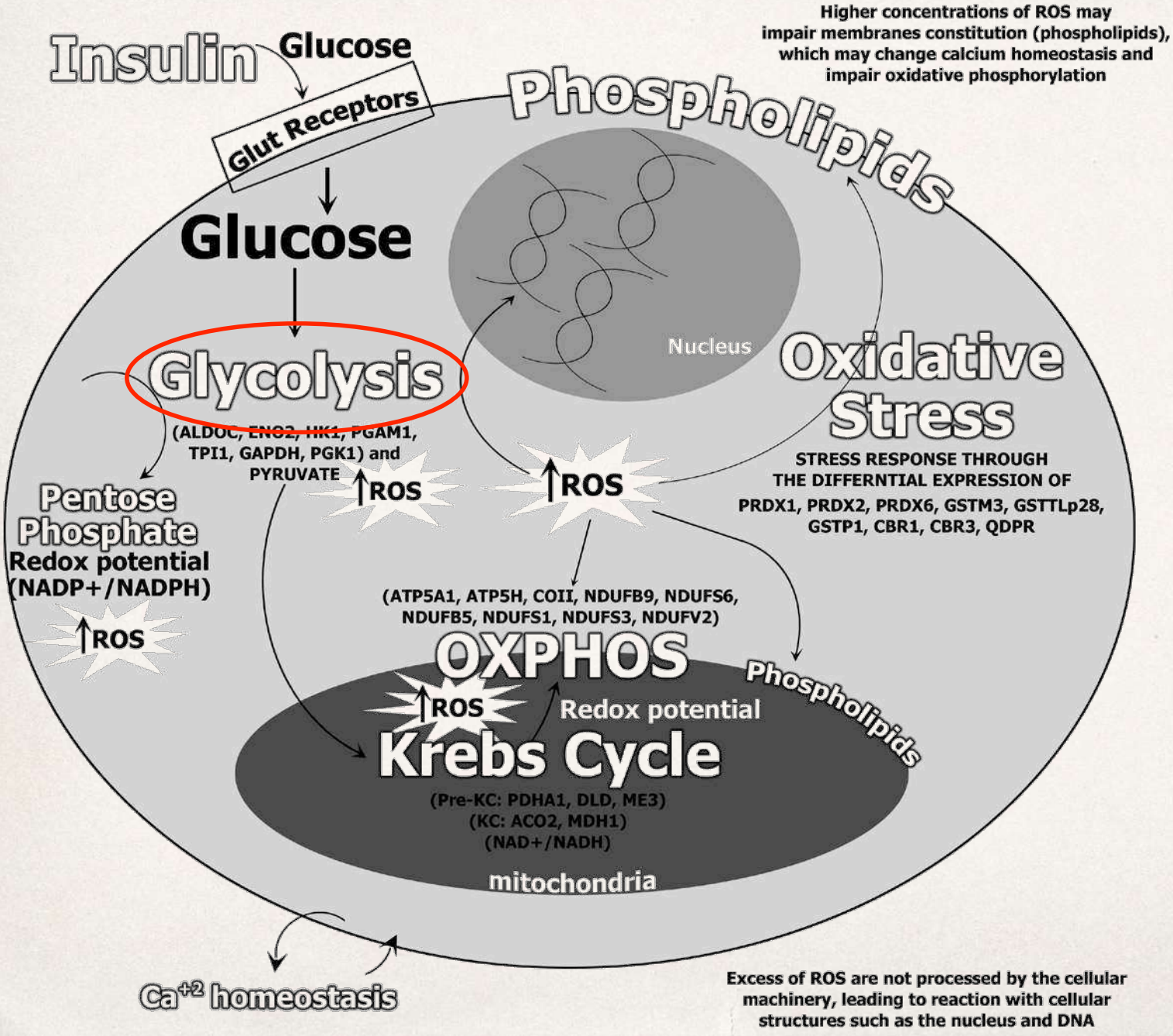
Anterior Cingulate Cortex (BA24a)

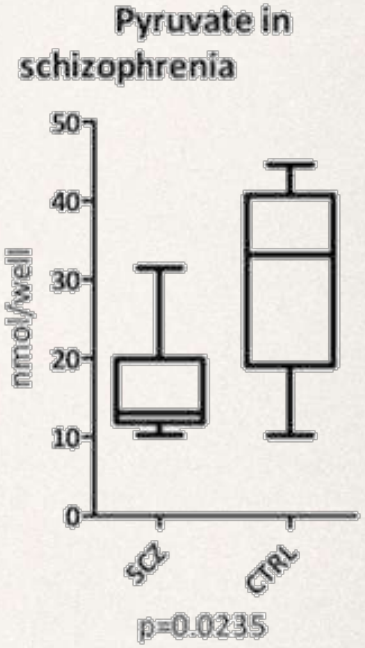
Anterior Temporal Lobe (BA38)

Antioxid Redox Signal. 2011 Oct 1;15(7):2067-79. doi: 10.1089/ars.2010.3459. Epub 2010 Dec 2.

The role of energy metabolism dysfunction and oxidative stress in schizophrenia revealed by proteomics.

Martins-de-Souza D[1], Harris LW, Guest PC, Bahn S.





Glucose and glycolysis in schizophrenia

Hypofrontality

From Wikipedia, the free encyclopedia

Hypofrontality is a state of decreased cerebral blood flow (CBF) in the prefrontal cortex of the brain during tests of executive function (but not at baseline)[1] that is commonly observed in patients suffering from schizophrenia.[2] The condition was first described by Ingvar and Franzén in 1974, who used xenon-enhanced CT scanning to image the

GLUCOSE TOLERANCE IN DISTURBED SCHIZOPHRENIC PATIENTS

C. KNIGHT ALDRICH, M.D.

Arch NeurPsych. 1948;60(5):498-503. doi:10.1001/archneurpsyc.1948.02310050075006.

Cerebral glucography with positron tomography. Use in normal subjects and in patients with **schizophrenia**.

Scans were treated digitally, with a 2.3-cm strip peeled off each slice and ratios to whole-slice activity computed. Patients with schizophrenia showed lower ratios in the frontal cortex, indicating relatively lower glucose use than normal control subjects; this was consistent with previously reported

Arch Gen Psychiatry. 1982 Mar;39(3):251-9.

Leukocyte **glycolysis** in schizophrenic patients.

Smith FL, Ellman GL.

Arch Gen Psychiatry. 1968 Jan;18(1):117-9. No abstract available.

PMID: 5634685 [PubMed - indexed for MEDLINE]

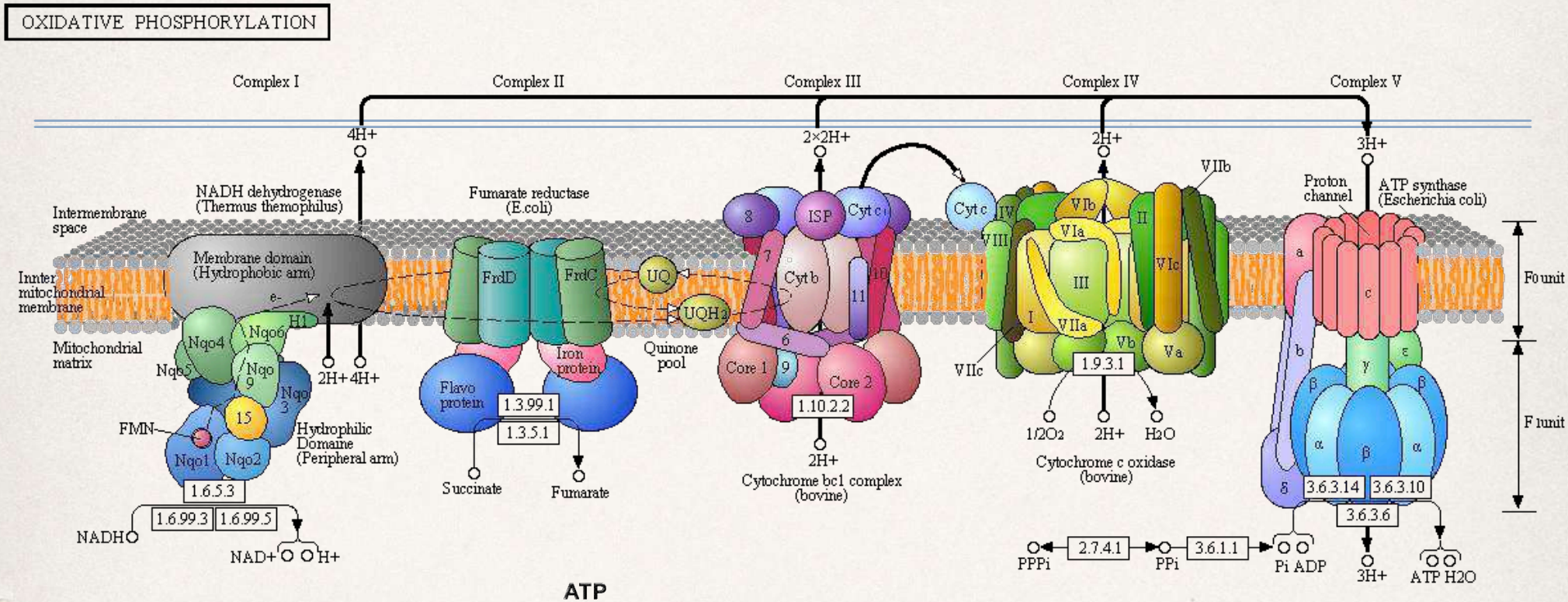
Energy metabolism in psychiatry: How specific is this?

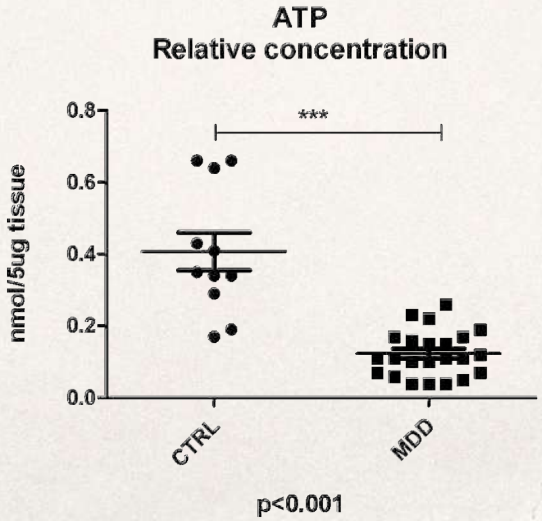
Cytochrome c
Diff. Exp. in MDD

Complex I
45 subunits
4 Diff. Exp. in MDD

Complex III
10 subunits
1 Diff. Exp. in MDD

Complex IV
13 subunits
3 Diff. Exp. in MDD





Complex V
(Mitochondrial ATP synthase)
F0 & F1 units

F0: 9 subunits: 2 upreg. MDD
F1: 5 subunits

Citation: Transl Psychiatry (2012) 2, e87, doi:10.1038/tp.2012.13

www.nature.com/tp

Energy metabolism dysfunction in pre-clincal models

The application of selective reaction monitoring confirms dysregulation of glycolysis in a preclinical model of schizophrenia.
Martins-de-Souza D, Alsaif M, Ernst A, Harris LW, Aerts N, Lenaerts I, Peeters PJ, Amess B, Rahmoune H, Bahn S, Guest PC.
BMC Res Notes. 2012 Mar 15;**5**:**146**. doi: 10.1186/1756-0500-**5**-**146**.
PMID: 22420779 **Free PMC Article**

Proteomic similarities between heterozygous reeler mice and schizophrenia.
Schmitt A, Turck CW, Pilz PK, Malchow B, von Wilmsdorff M, Falkai P, Martins-de-Souza D.
Biol Psychiatry. 2013 Sep 15;74(6):e5-e10. doi: 10.1016/j.biopsych.2013.03.023.
PMID: **23684383**

npj | Schizophrenia
www.nature.com/npjschz

REVIEW ARTICLE

The proteome of schizophrenia

Juliana M Nascimento[1,2] and Daniel Martins-de-Souza[1]

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Orbitalfrontal cortex (BA11)

Anterior Cingulate Cortex (BA24a)

Anterior Temporal Lobe (BA38)

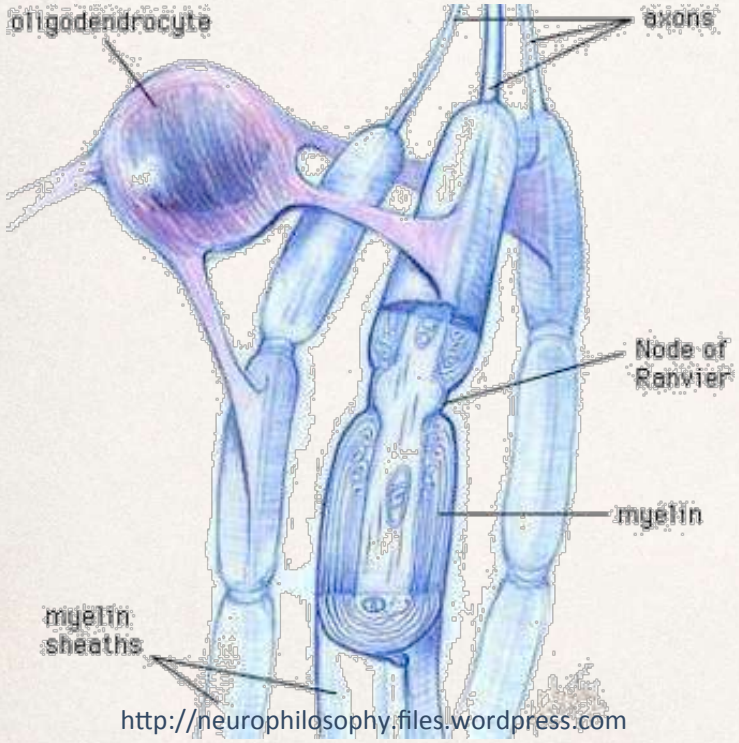
npj | Schizophrenia

www.nature.com/npjschz

Disturbed macro-connectivity in schizophrenia linked to oligodendrocyte dysfunction: from structural findings to molecules

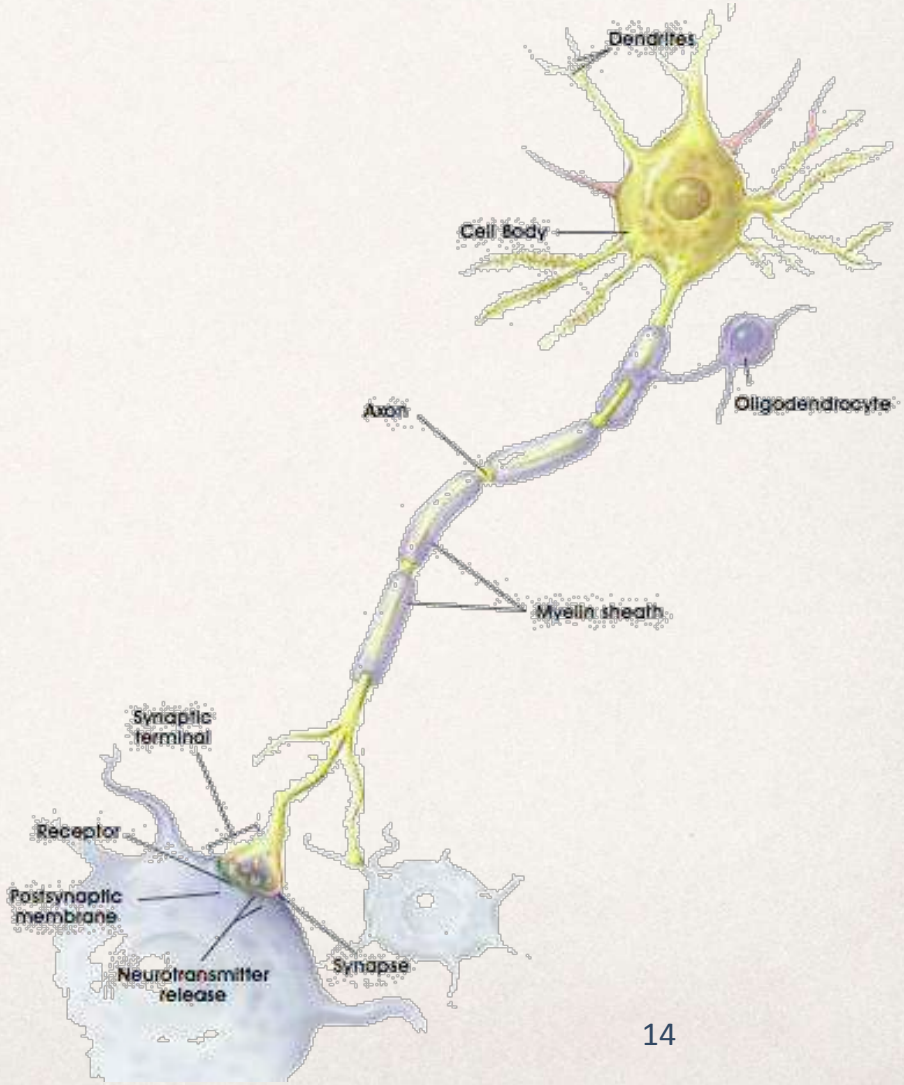
Juliana Silva Cassoli[1], Paul C Guest[1], Berend Malchow[2,3], Andrea Schmitt[1,2,3], Peter Falkai[2,3] and Daniel Martins-de-Souza[1,2,3,4]

MBP & MOG & Ermin

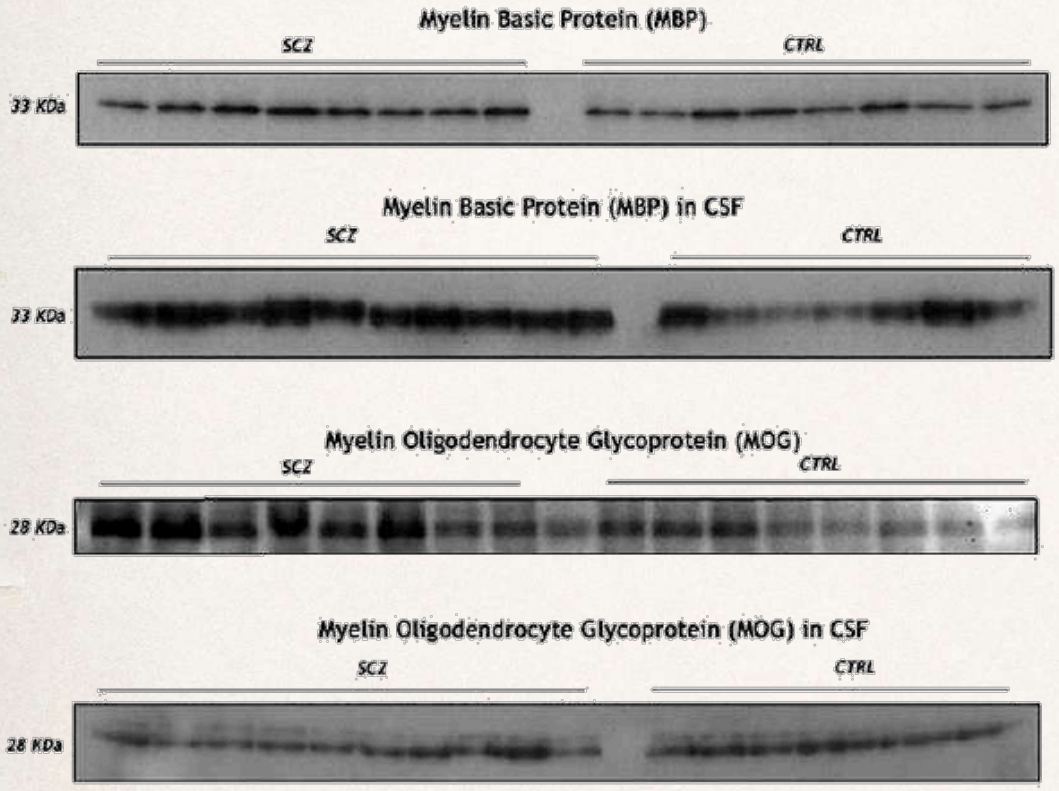


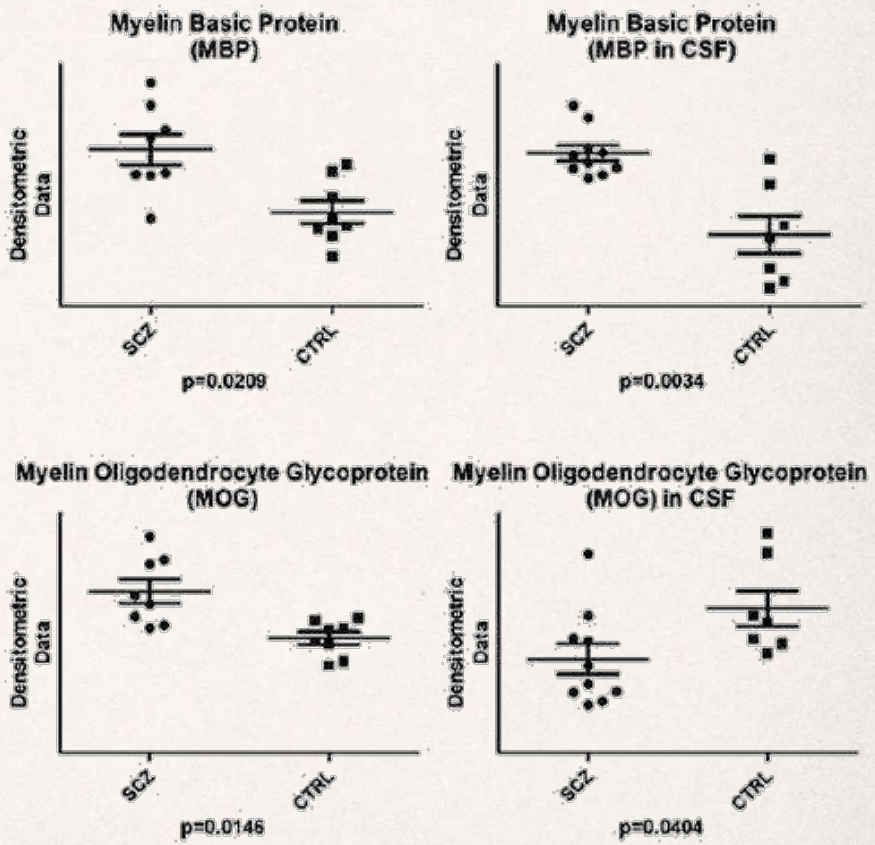
http://neurophilosophy.files.wordpress.com

2',3'-cyclic-nucleotide 3'-phosphodiesterase **(CNP)**



Oligodendrocytes-related proteins in brain and CSF



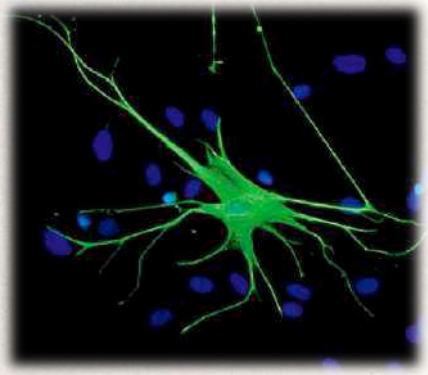


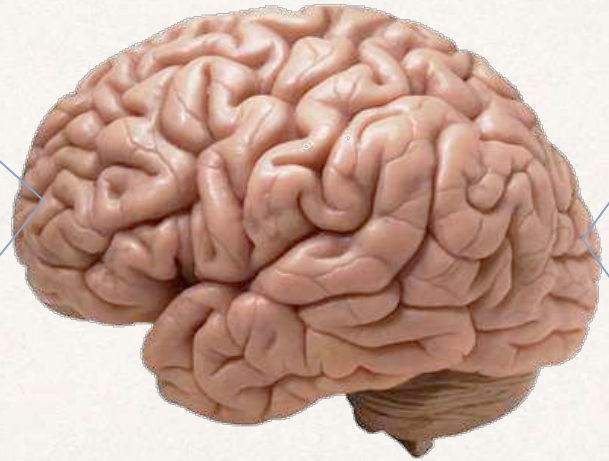
Proteome analysis of the thalamus and cerebrospinal fluid reveals glycolysis dysfunction and potential biomarkers candidates for schizophrenia.

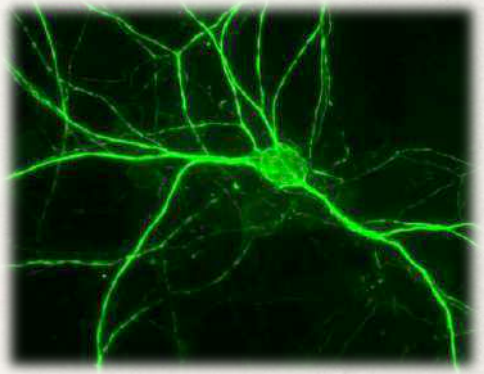
Martins-de-Souza D, Maccarrone G, **Wobrock** T, Zerr I, Gormanns P, Reckow S, Falkai P, Schmitt A, Turck CW.

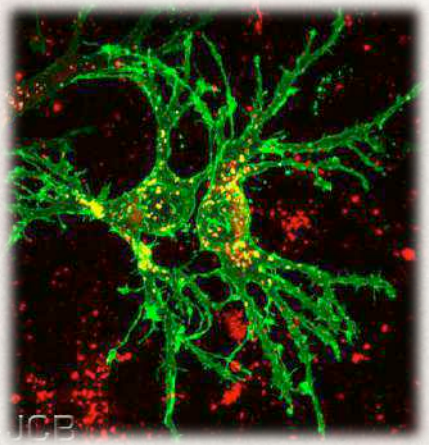
J Psychiatr Res. 2010 Dec;44(16):1176-89. doi: 10.1016/j.jpsychires.2010.04.014. Epub 2010 May 14.

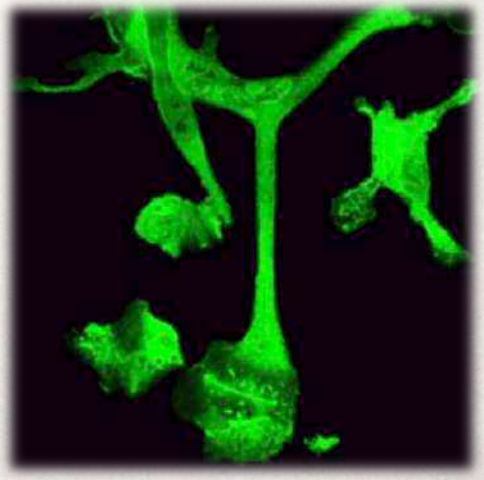
Where are energy metabolism differences?

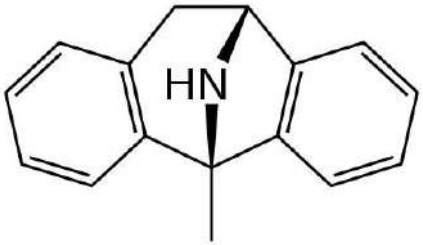










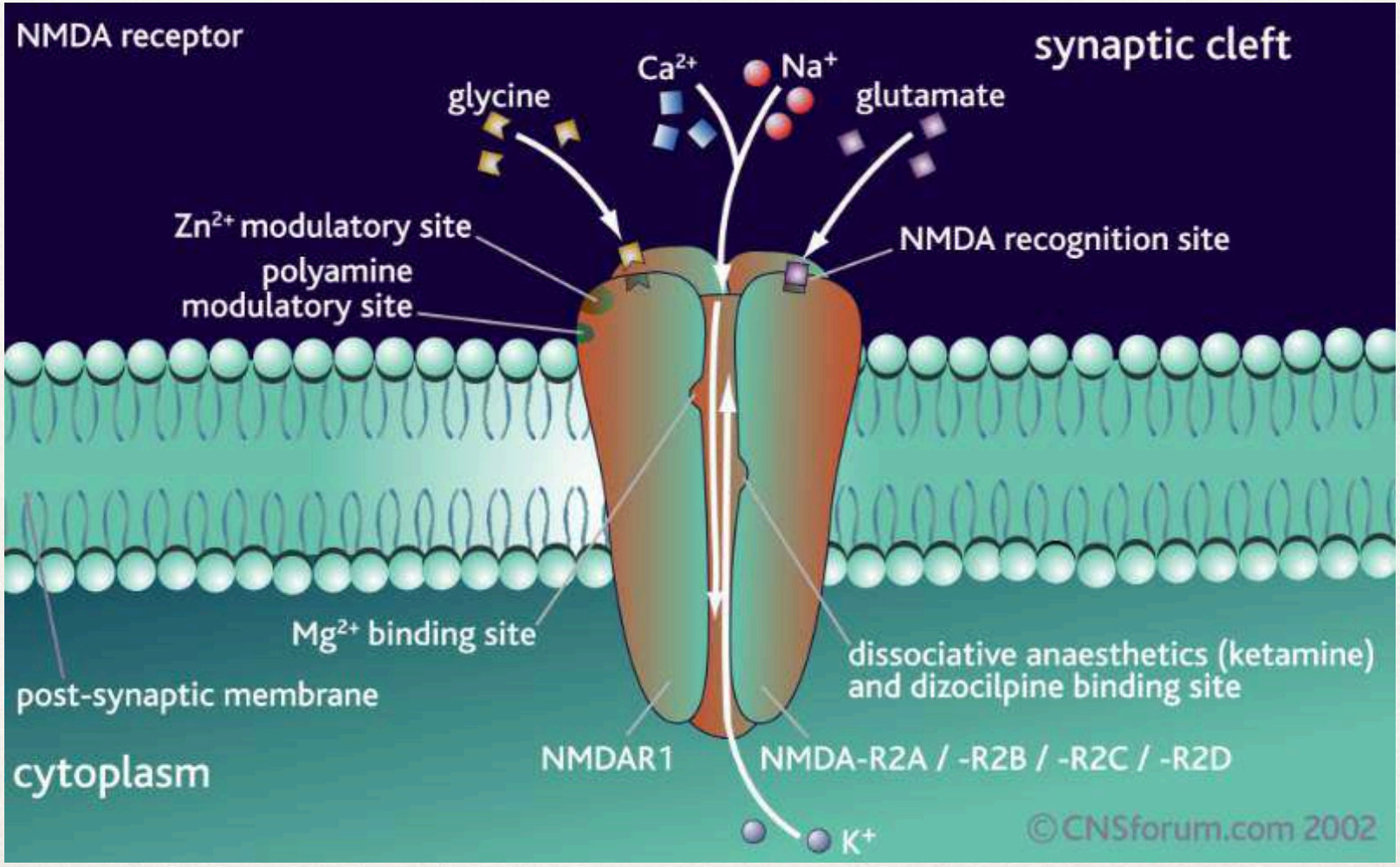


Neurochem Int. 2006 May-Jun;48(6-7):541-6. Epub 2006 Mar 3.

Repeated injection of MK801: an animal model of schizophrenia?

Eyjolfsson EM, Brenner E, Kondziella D, Sonnewald U.

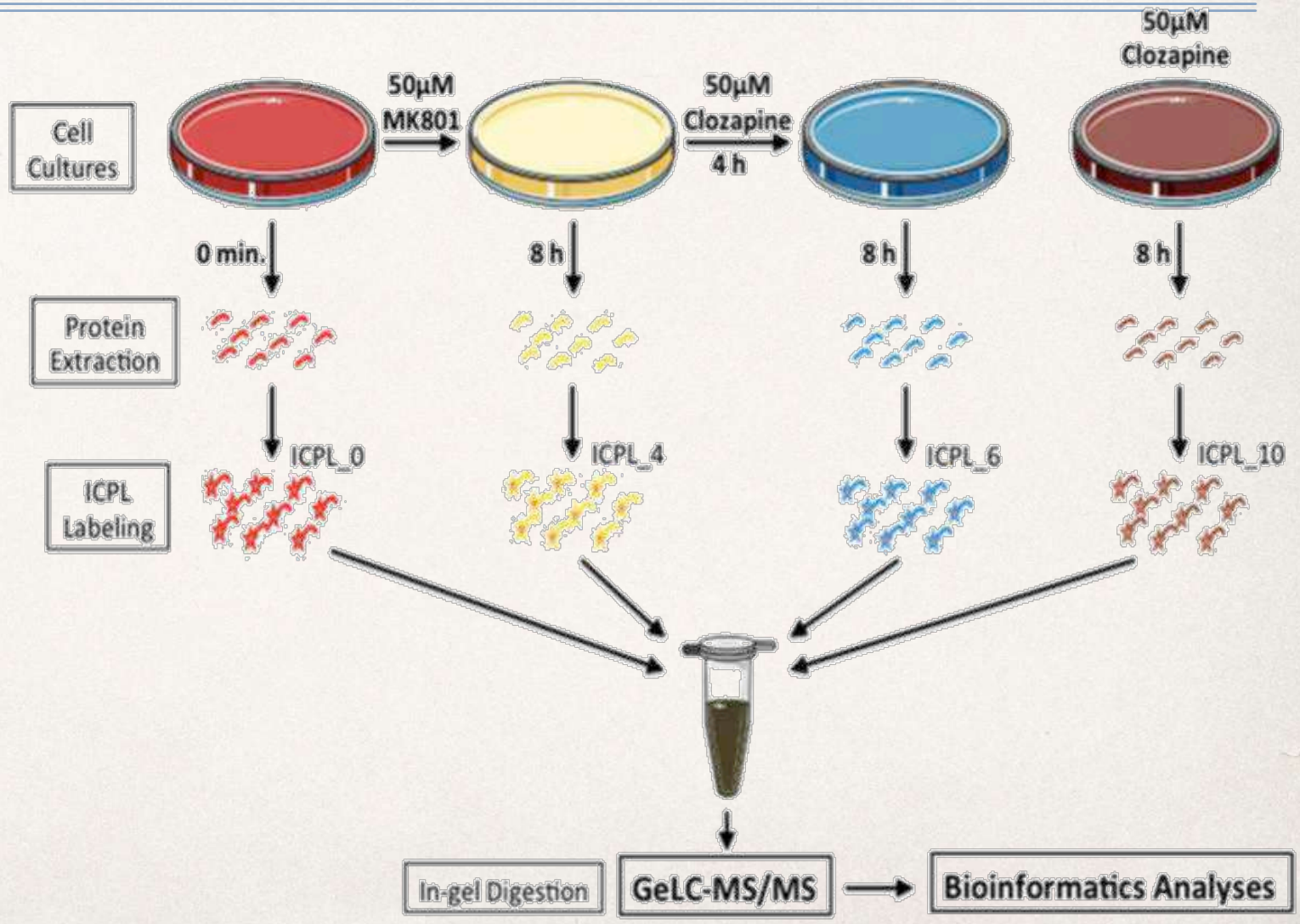
Department of Neuroscience, Norwegian University of Science and Technology, NTNU, N-7489 Trondheim, Norway.



Follow up

Combining energy metabolism and oligodendrocytes findings

Dr. Paul C. Guest



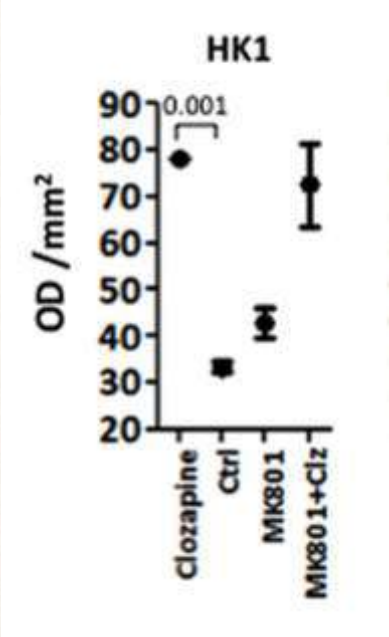
MK-801 treatment affects glycolysis in oligodendrocytes more than in astrocytes and neuronal cells: insights for schizophrenia.

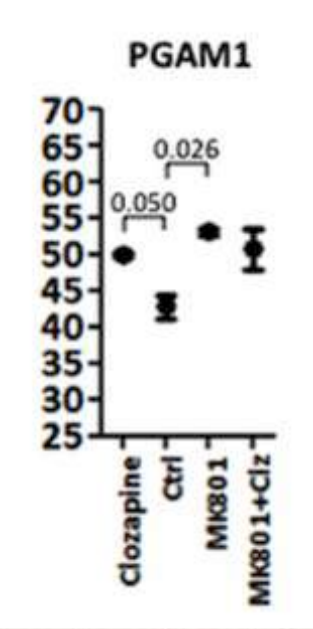
Guest PC, Iwata K, Kato TA, Steiner J, Schmitt A, Turck CW, **Martins-de-Souza D**.

Front Cell Neurosci. 2015 May 12;9:180. doi: 10.3389/fncel.2015.00180. eCollection 2015.

Neuronal cultures:

clozapine-associated differences





Dr. Paul C. Guest

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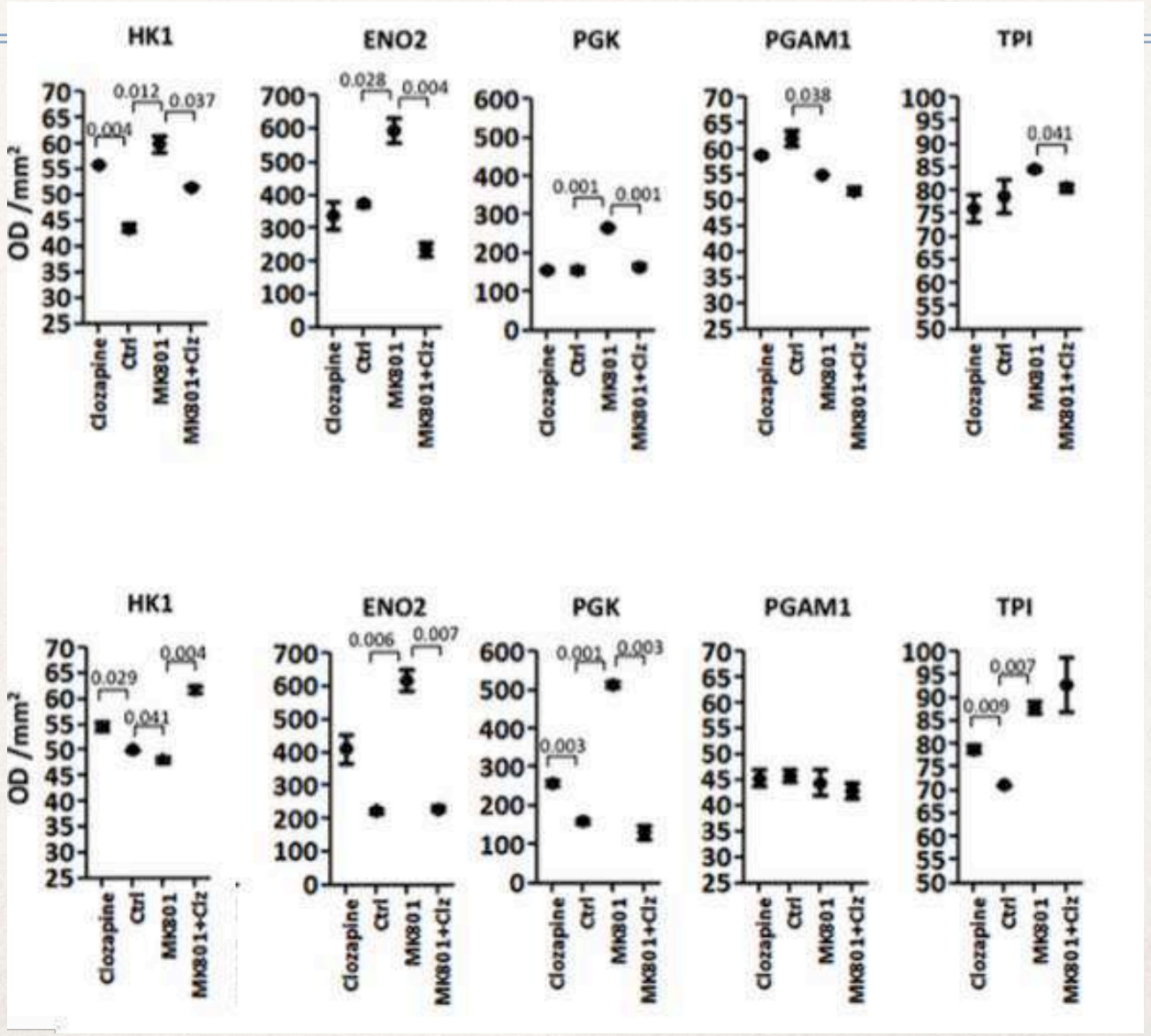
Guest PC, Iwata K, Kato TA, Steiner J, Schmitt A, Turck CW, **Martins-de-Souza D.**

Front Cell Neurosci. 2015 May 12;9:180. doi: 10.3389/fncel.2015.00180. eCollection 2015.

Oligodendrocytes:



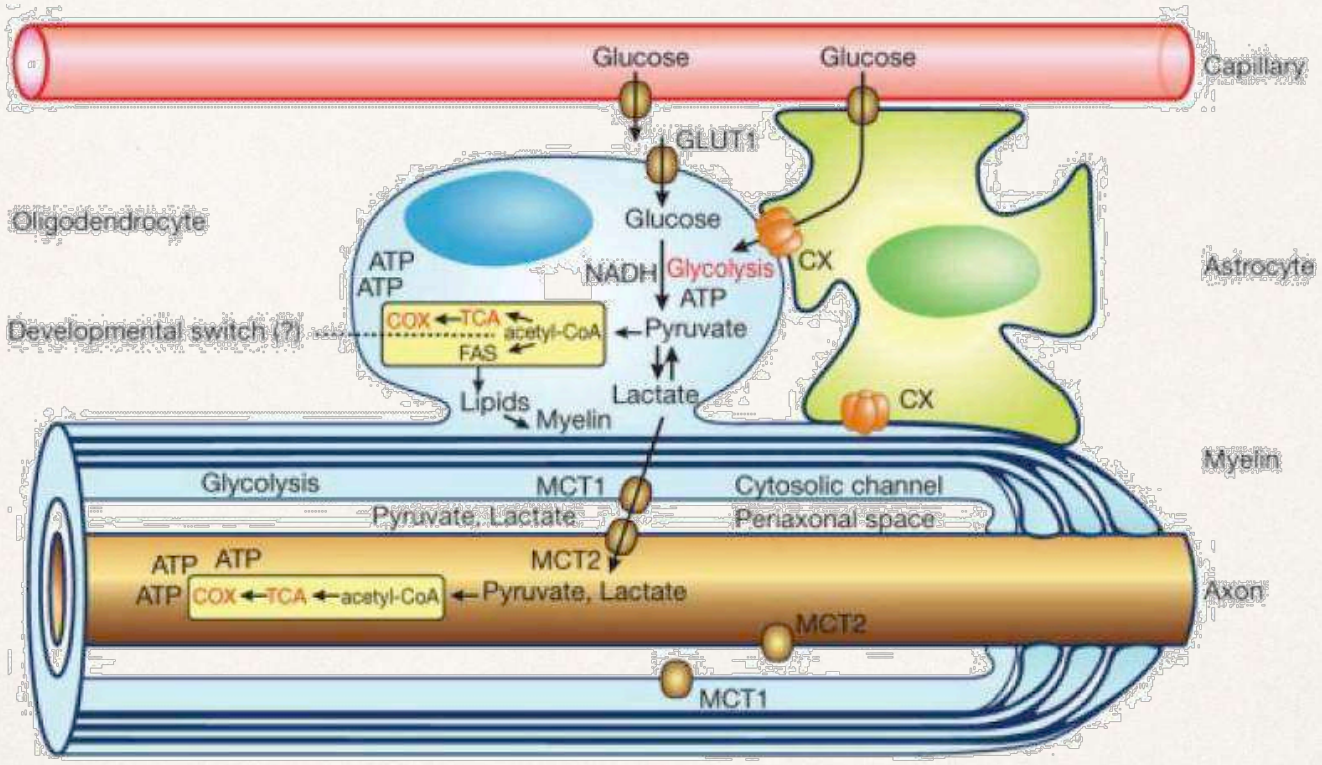
Nature. 2012 Jul 26;487(7408):443-8. doi: 10.1038/nature11314.

Oligodendroglia metabolically support axons and contribute to neurodegeneration.

Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, Liu Y, Tsingalia A, Jin L, Zhang PW, Pellerin L, Magistretti PJ, Rothstein JD.

Department of Neurology, The Johns Hopkins University, Baltimore, Maryland 21205, USA.

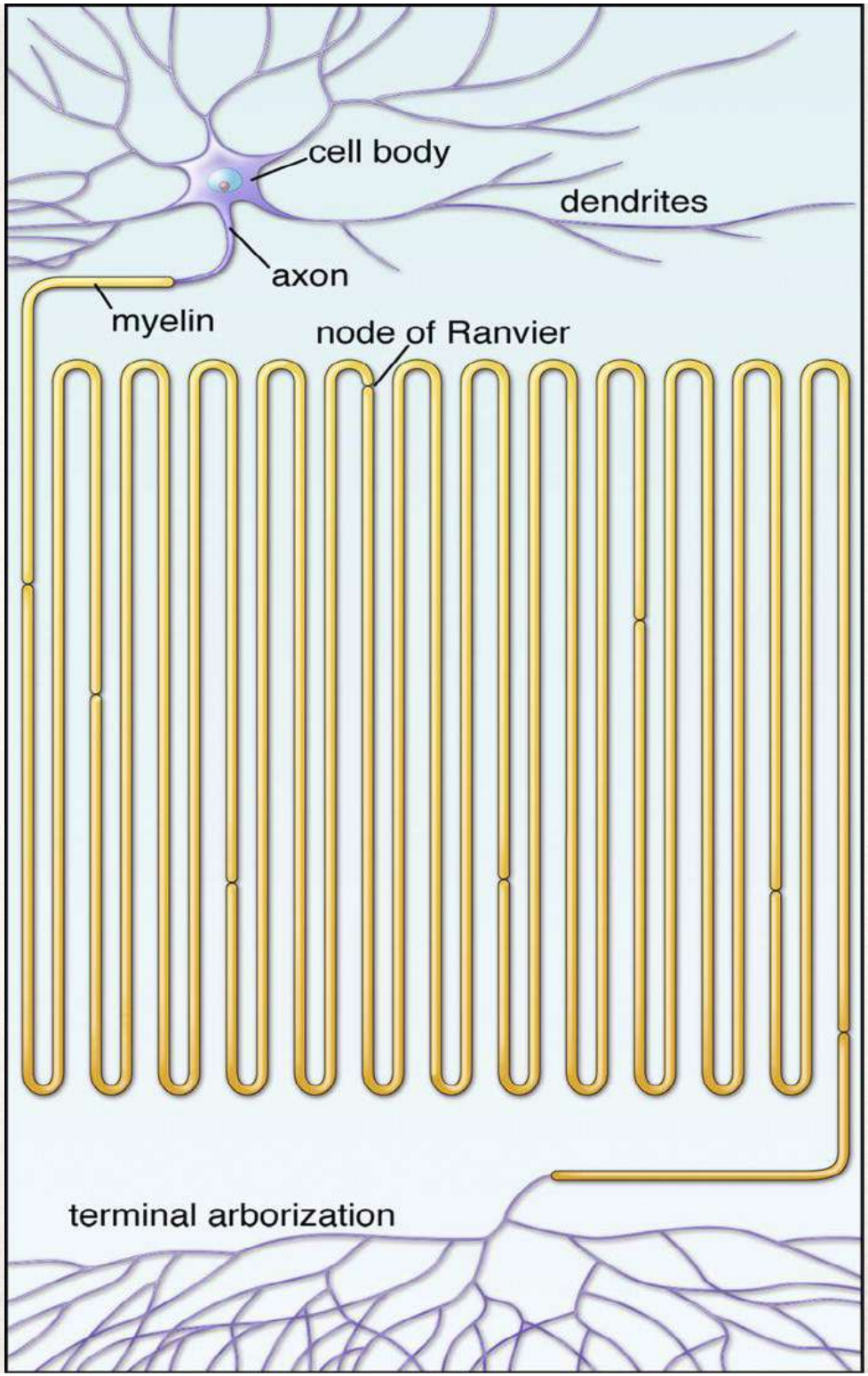
Here we show that the most abundant lactate transporter in the central nervous system, monocarboxylate transporter 1 (MCT1, also known as SLC16A1), is highly enriched within oligodendroglia and that disruption of this transporter produces axon damage and neuron loss in animal and cell



Nature. 2012 Apr 29;485(7399):517-21. doi: 10.1038/nature11007.

Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity.

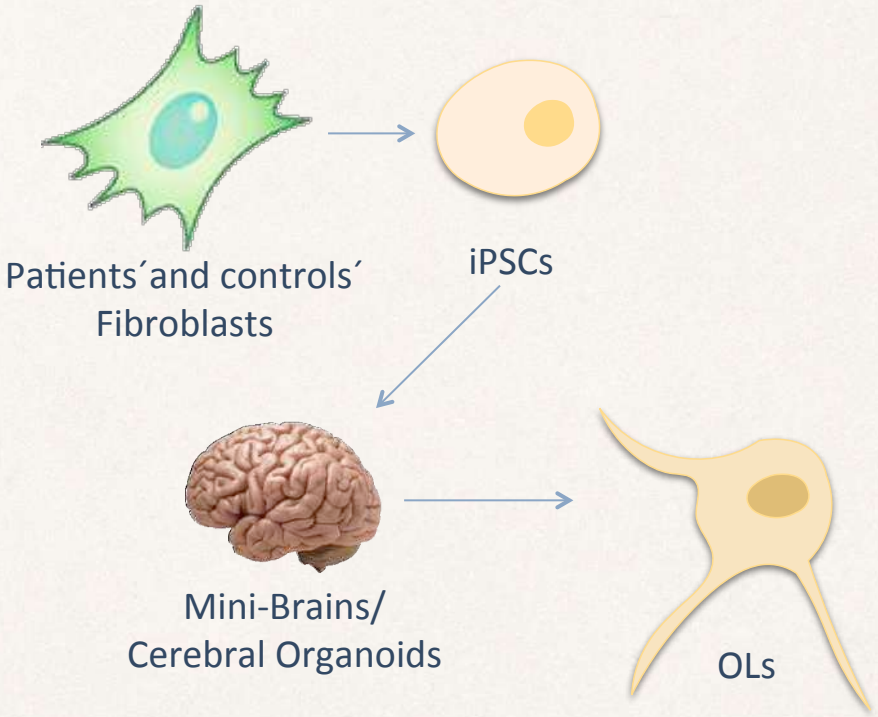
Fünfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Möbius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes CT, Frahm J, Goebbels S, Nave KA.


cell body
dendrites
axon
myelin
node of Ranvier
terminal arborization

Next steps

Combining energy metabolism and oligodendrocytes findings

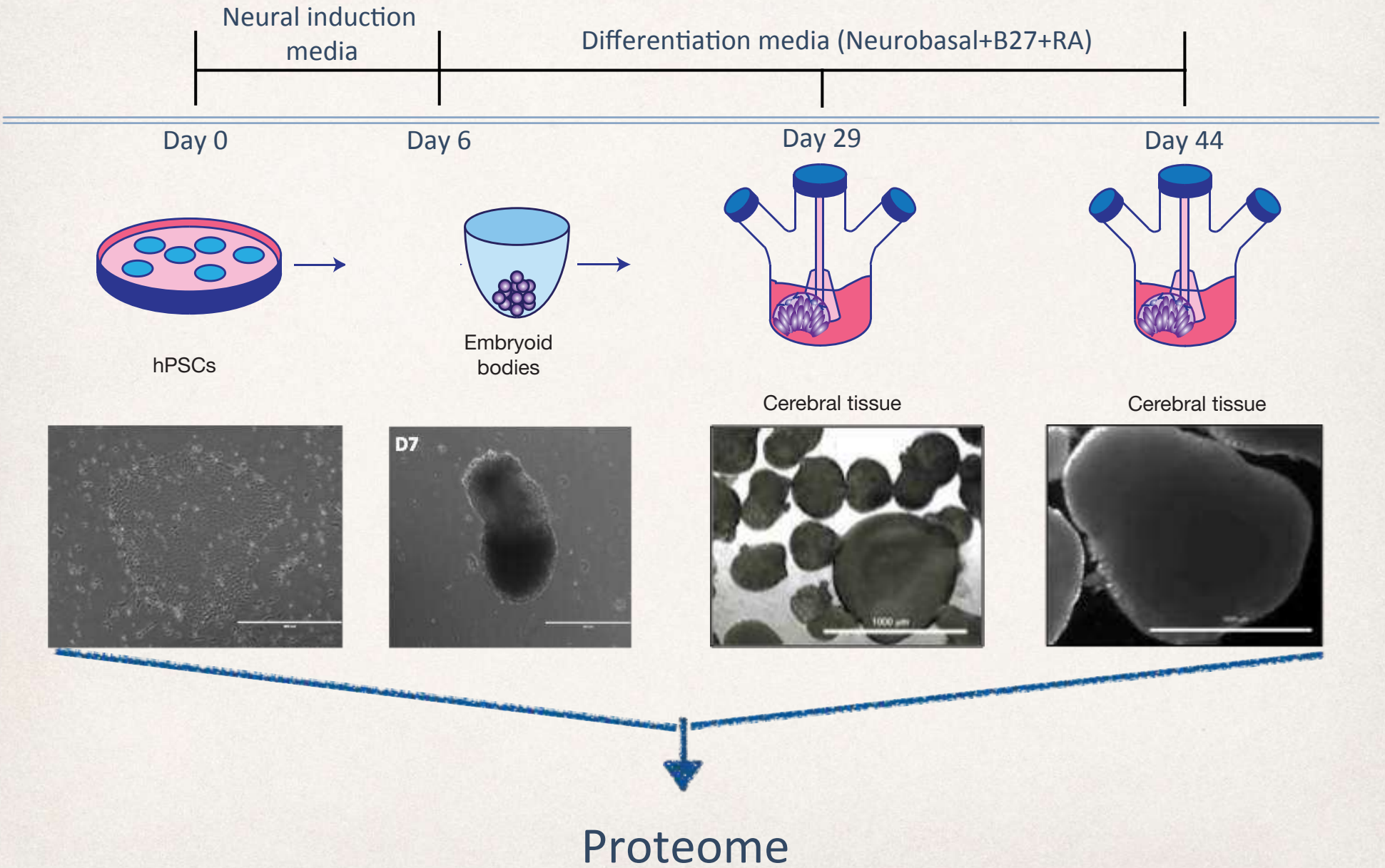
Dr. Juliana Nascimento



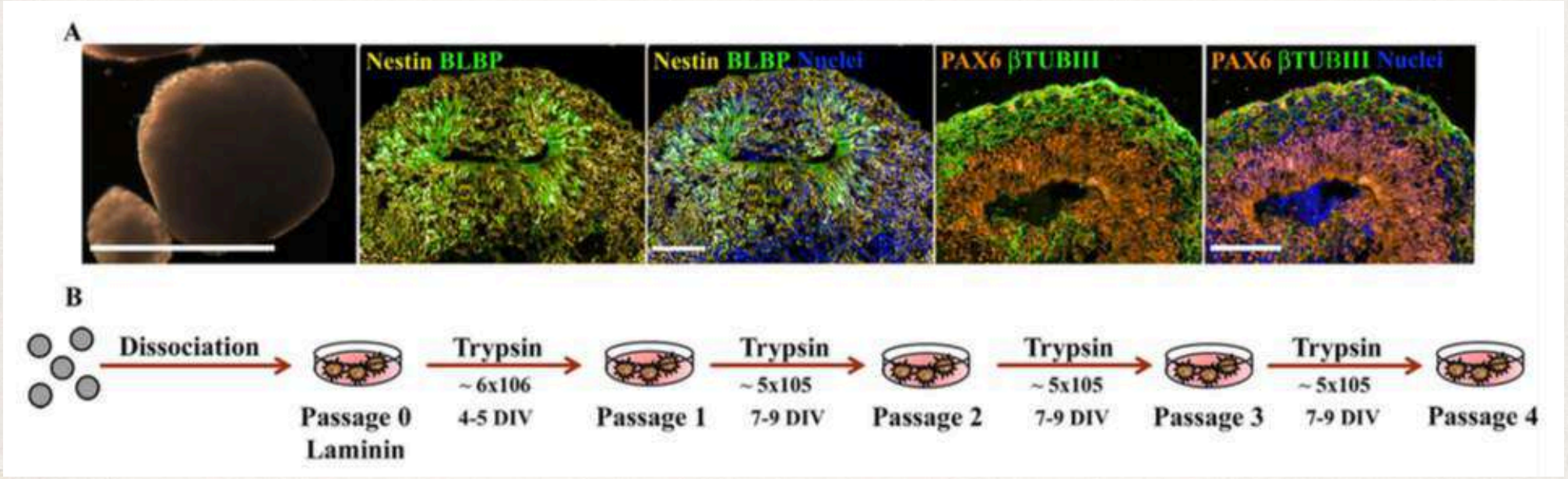
Prof. Stevens Rehen

Mini-brains

Cerebral organoids generated in spinner flasks



iPSCs Cerebral organoids - spinner flasks

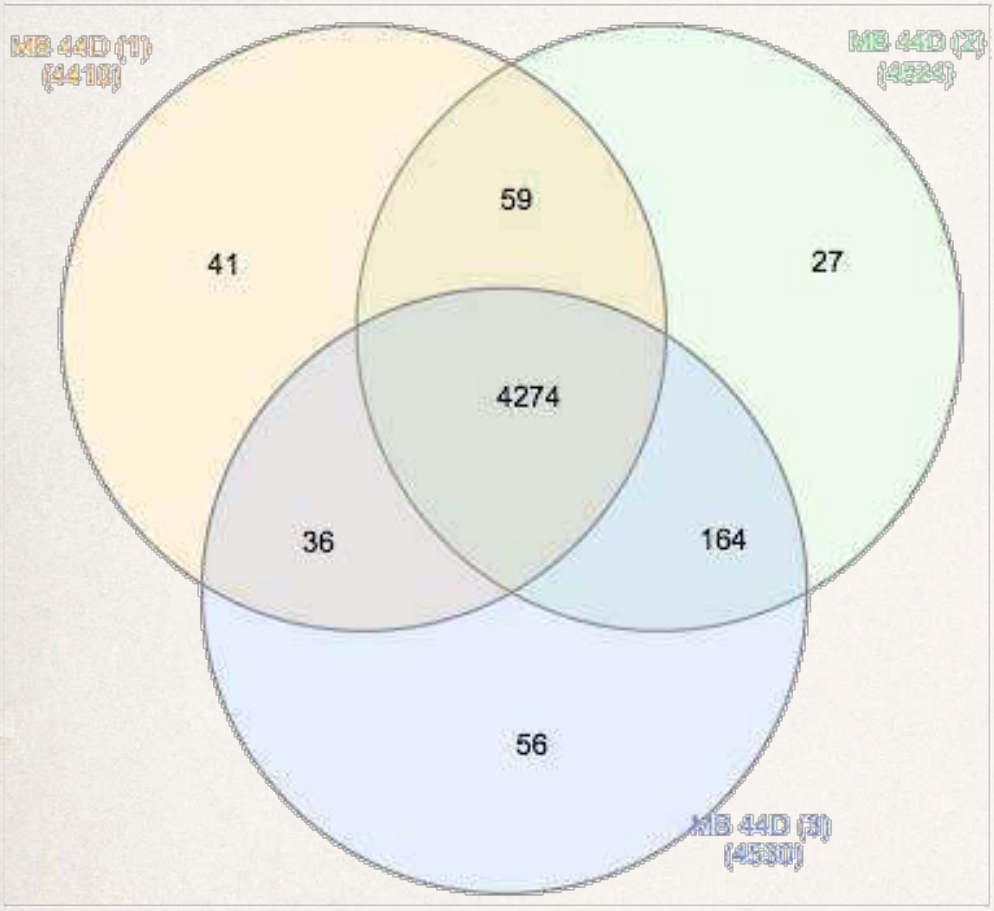


Derivation of Functional Human Astrocytes from Cerebral Organoids.

Dezonne RS, Sartore RC, **Nascimento** JM, **Saia-Cereda** VM, Romão LF, Alves-Leon SV, de Souza JM, **Martins-de-Souza** D, Rehen SK, Gomes FC.

Sci Rep. 2017 Mar 27;7:45091. doi: 10.1038/srep45091.

iPSCs Brain Organoids Proteomics



More than 4,000 proteins IDs using Human Uniprot database

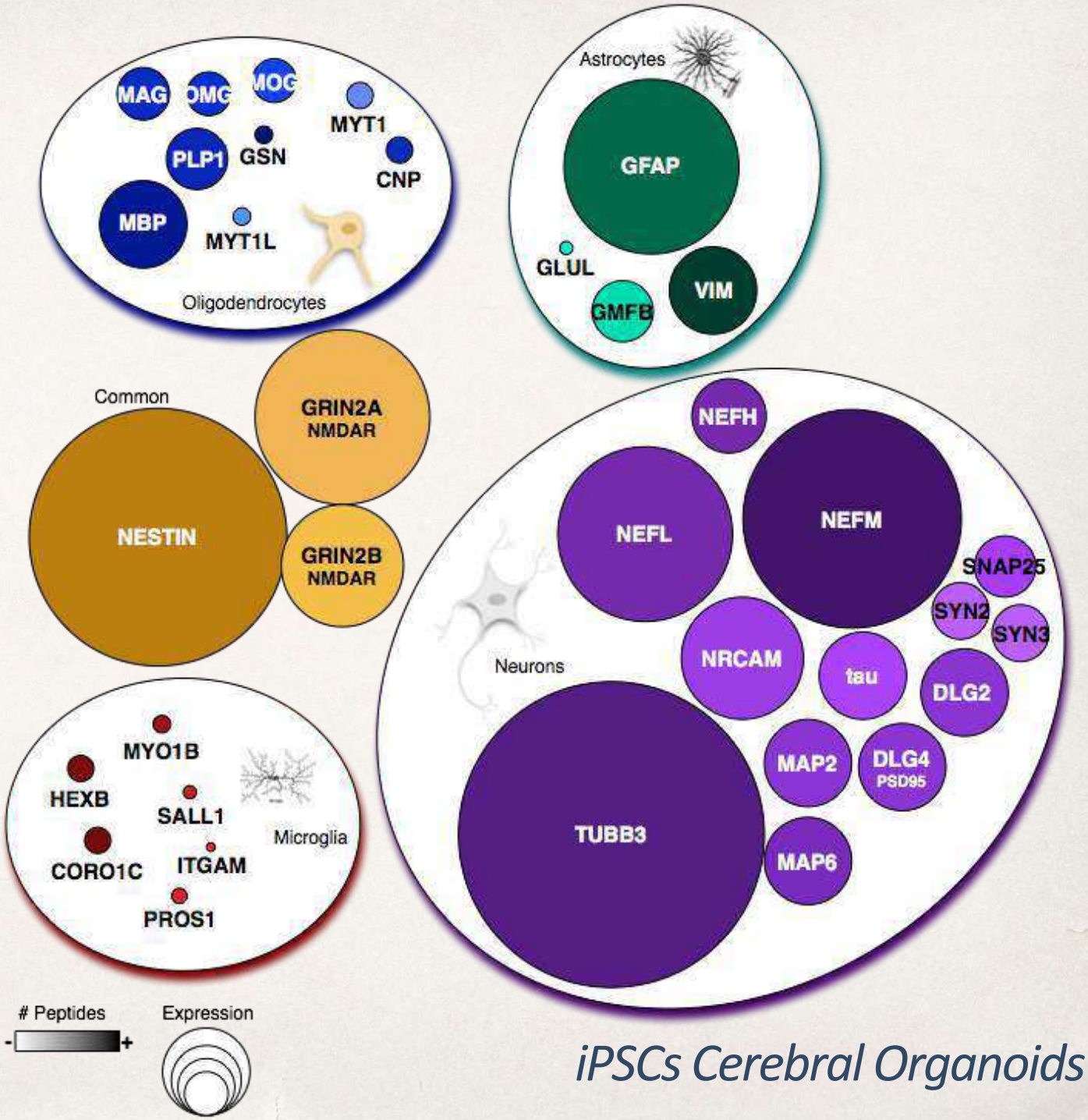
Only ~1% different of proteins identified during each read

More than 94% identical IDs

Filtering at least 2 unique (non-redundant) peptides/protein

2D-HDMSE Synapt G2-Si
technical triplicates

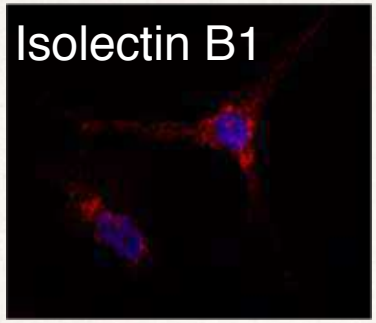
Proteins per cell id

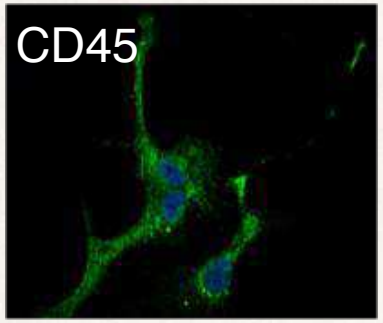


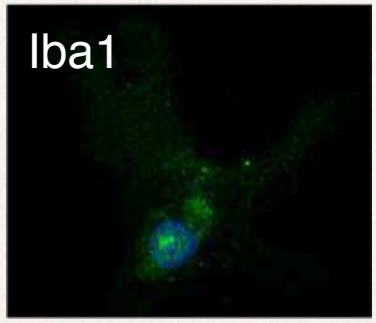
Nascimento et al. in preparation

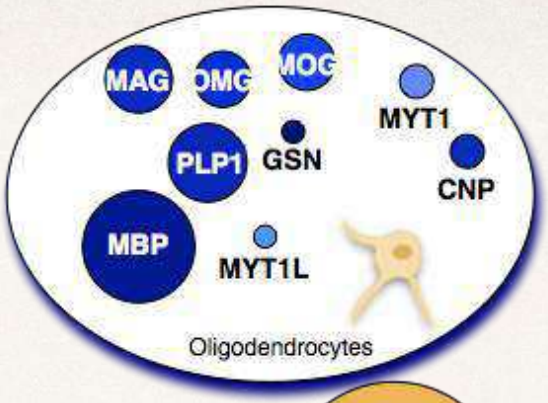
iPSCs Cerebral Organoids

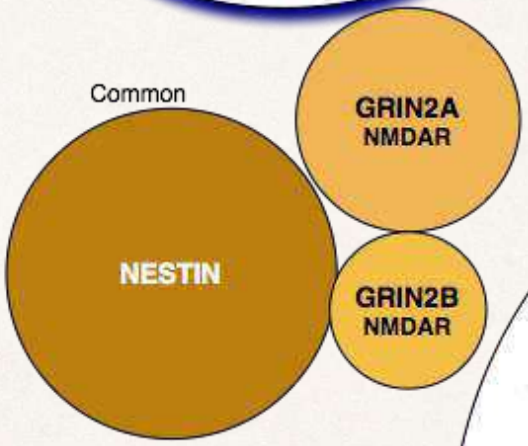
Proteins per cell id

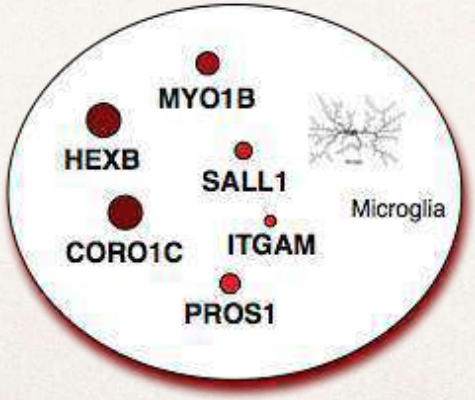


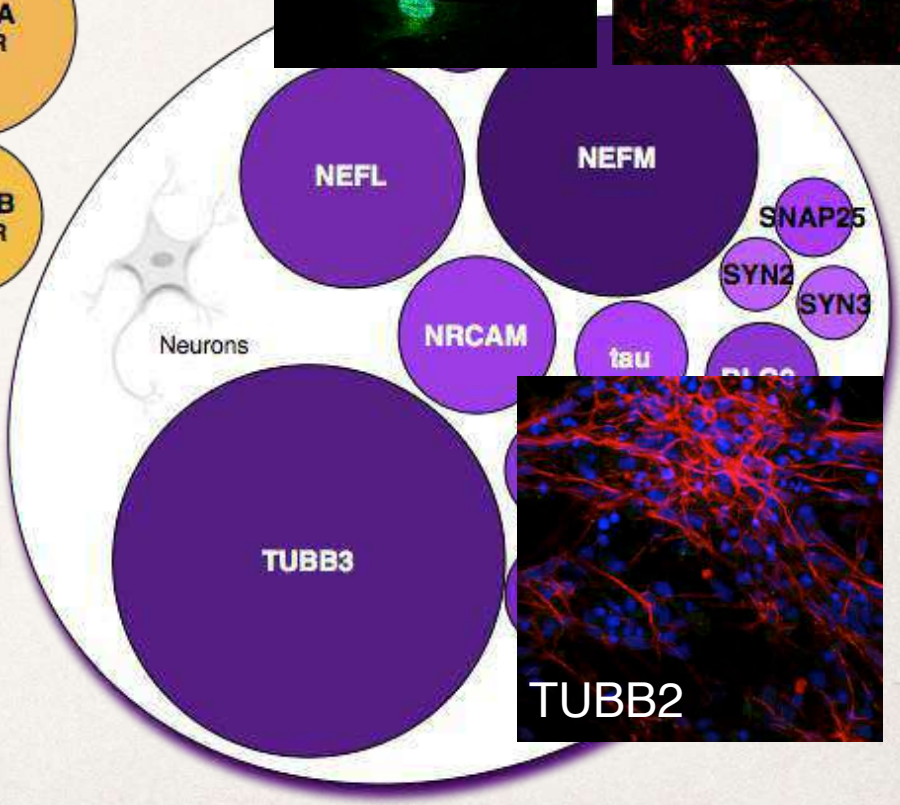


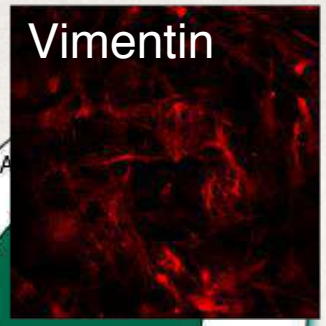


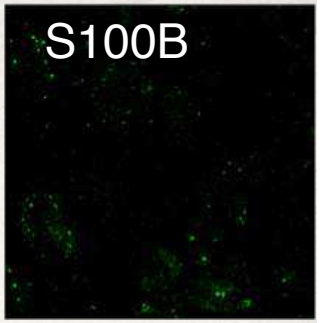


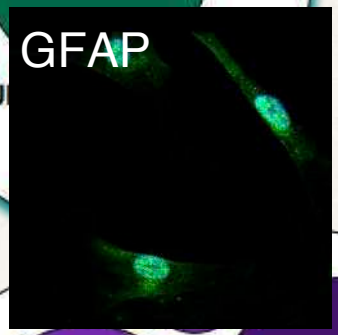


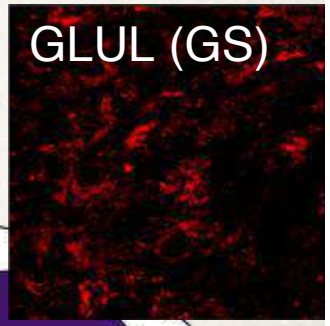


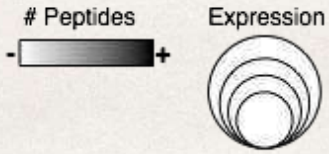












iPSCs Cerebral Organoids

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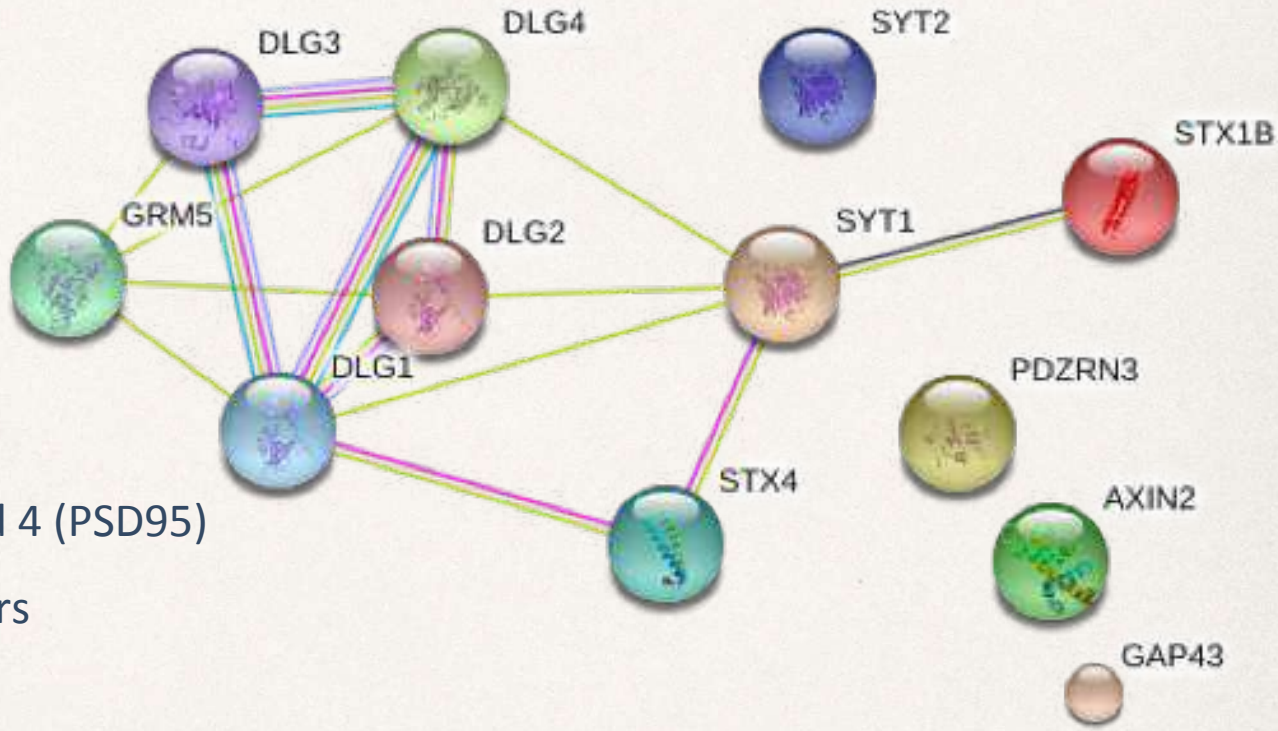
Synapses



DLG1 (SAP97), 2 (PSD93), 3 and 4 (PSD95)

- Clustering of NMDA receptors
- Potassium channels

SYT1 and 2 (Synaptognamins)

- Ca^{2+} sensors in vesicular trafficking and exocytosis

STX1B and 4

- Exocytosis of synaptic vesicles, docking

Myelinating Mini-Brain

Transmission Electron Microscopy
(Dr. Rodrigo Madeiro)

Accession	Gene Name	Description	Absolute Expression	Peptide (unique)	Score
P02686	MBP	Myelin basic protein	9.965900866	18 (10)	157.0157
P60983	GMFB	Glia maturation factor beta	7.740880685	13 (3)	85.1157
P60201	PLP1	Myelin proteolipid protein	7.30485028	13 (7)	71.9899
P20916	MAG	Myelin-associated glycoprotein	6.102421492	11 (5)	57.7011
P23515	OMG	Oligodendrocyte-myelin glycoprotein	5.909651394	10 (6)	78.1343
Q16653	MOG	Myelin-oligodendrocyte glycoprotein	5.235969762	9 (5)	54.0113
Q01538	MYT1	Myelin transcription factor 1	3.735649887	6 (2)	26.2452
Q9UL68	MYT1L	Myelin transcription factor 1-like protein	2.750316464	4 (2)	15.8108

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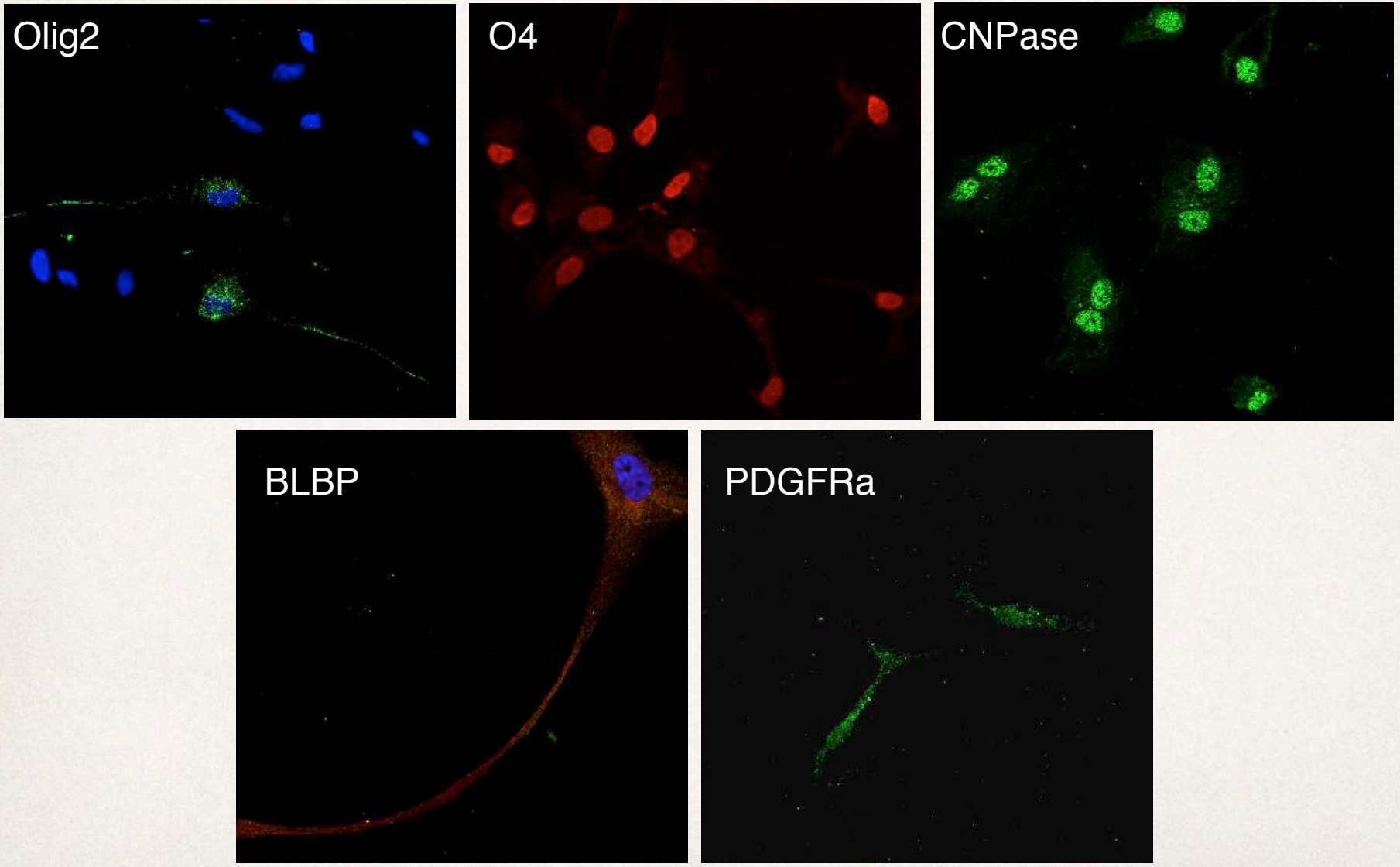
Radial Glia/OPCs and Oligodendrocytes



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