Identifying proteins and metabolic pathways associated to the neuroprotective response mediated by tibolone in astrocytes under an induced inflammatory model

Presented by:

Daniel Camilo Osorio Hurtado

in partial fulfillment of requirements for the degree of

Master in Bioinformatics

Advisors: **Janneth Gonzalez PhD.** and **Andrés Pinzón PhD.**

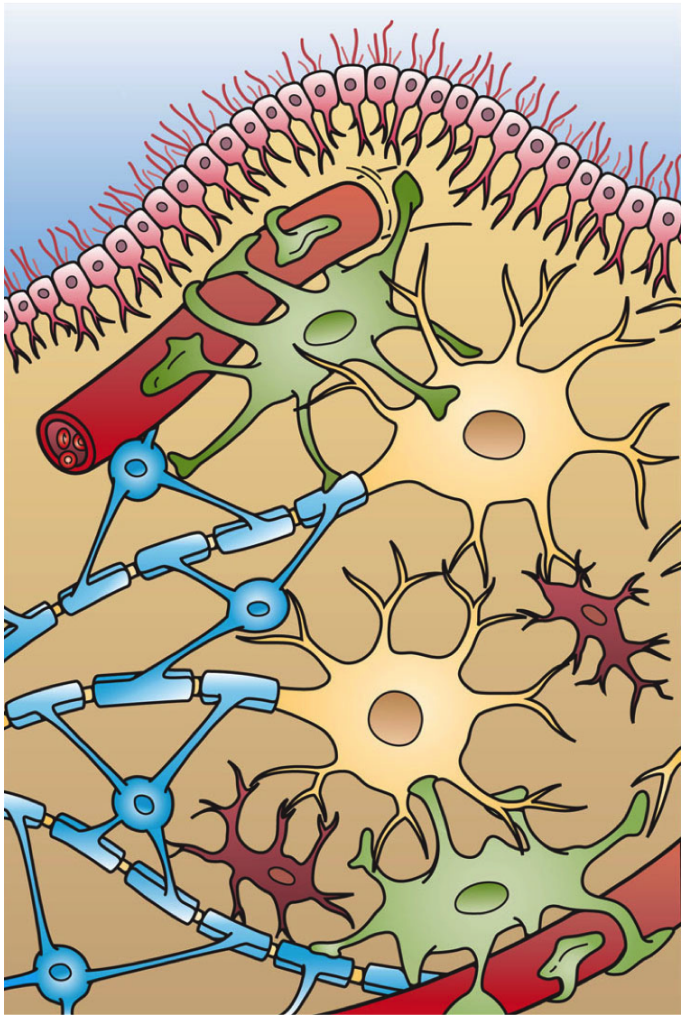
Bioinformatics and Computational Systems Biology Lab

Universidad Nacional de Colombia

Engineering Faculty - Department of Systems and Industrial Engineering

Bogotá, Colombia

CNS: Central Nervous System



© **Holly Fischer** artwork.

Astrocyte - Neuron Metabolic Relationship

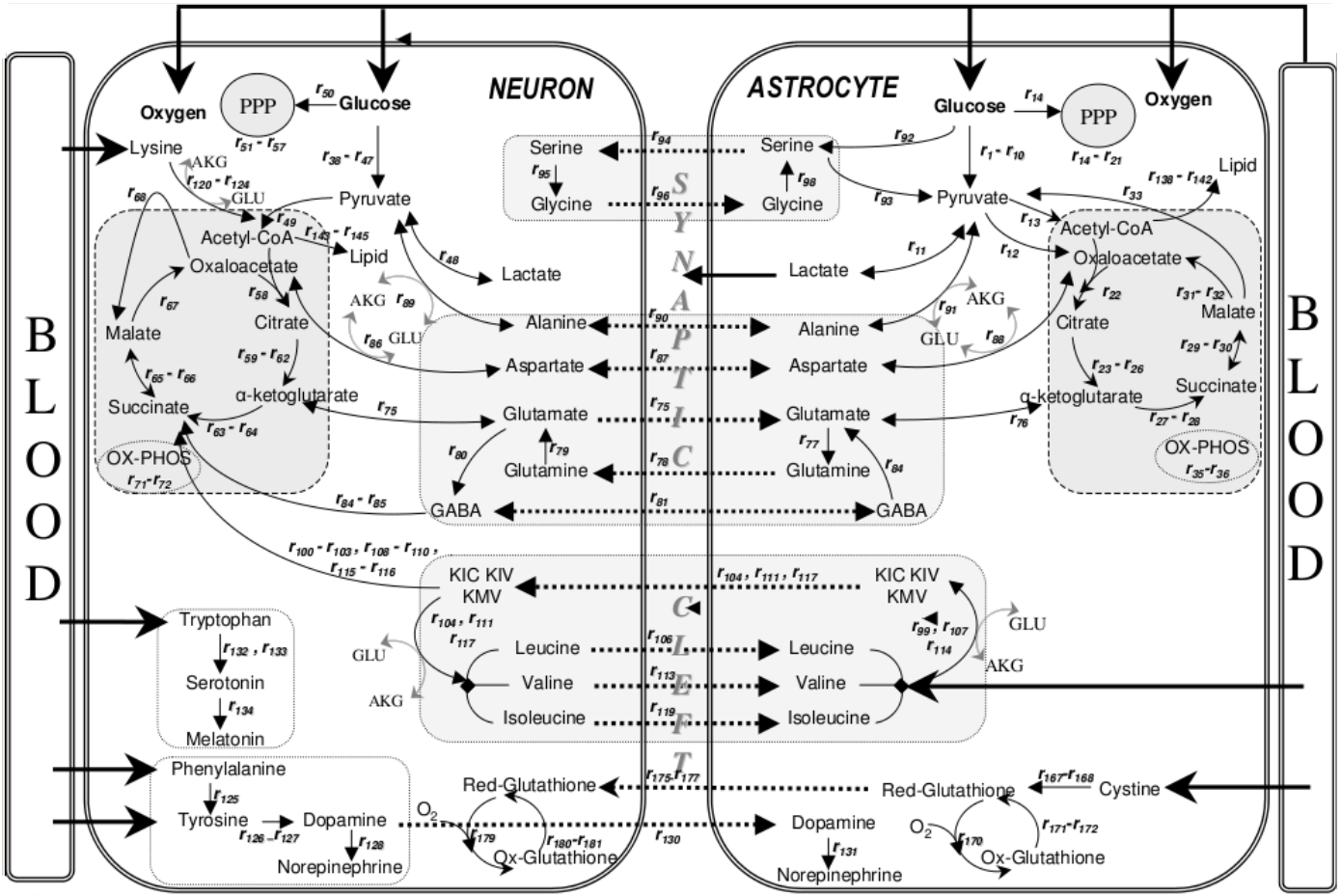
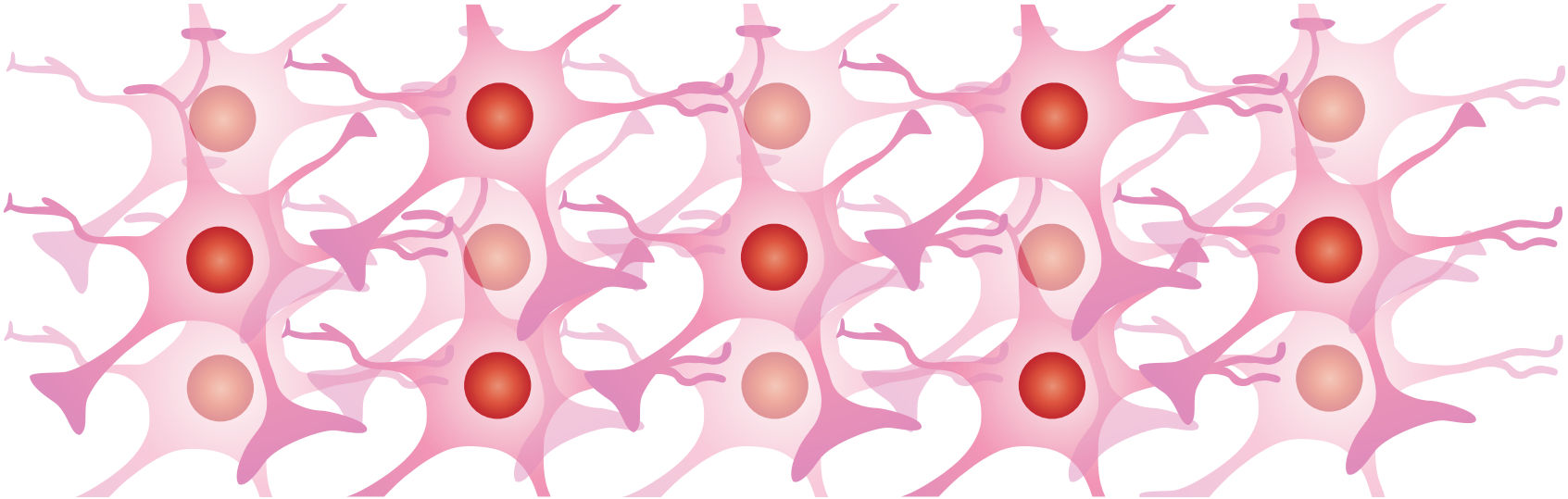


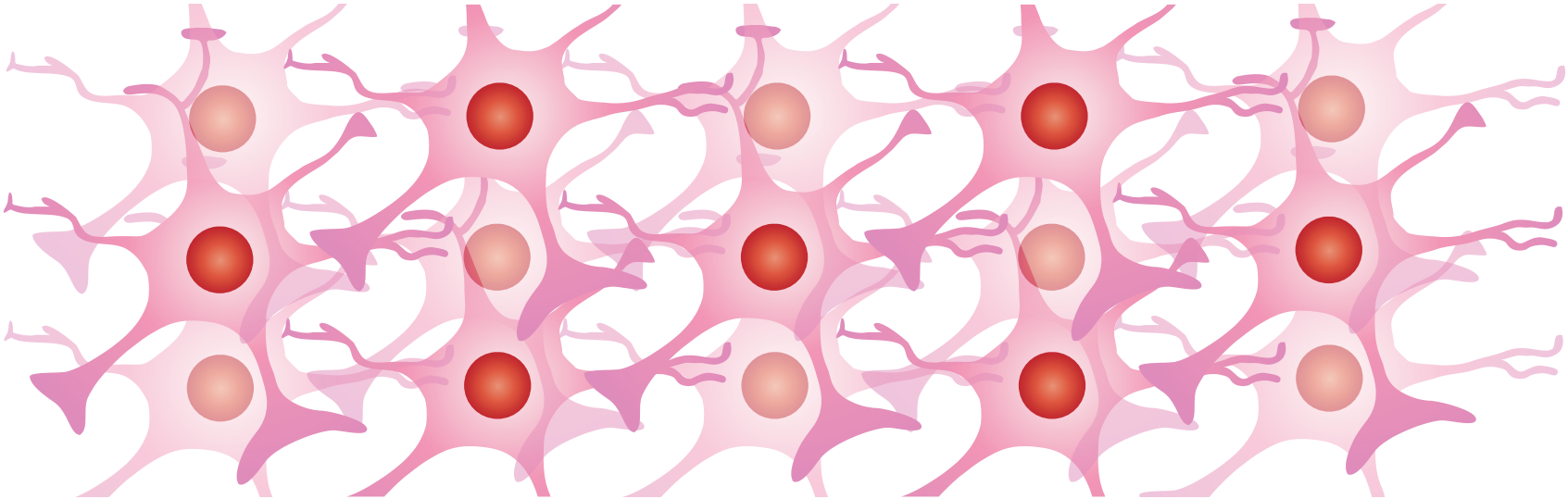
Figure from: Çakir, Tunahan *et al.*, (2007). Reconstruction and flux analysis of coupling between metabolic pathways of astrocytes and neurons: application to cerebral hypoxia.

Astrocytes Metabolic Functions



K^+ Membrane Potential

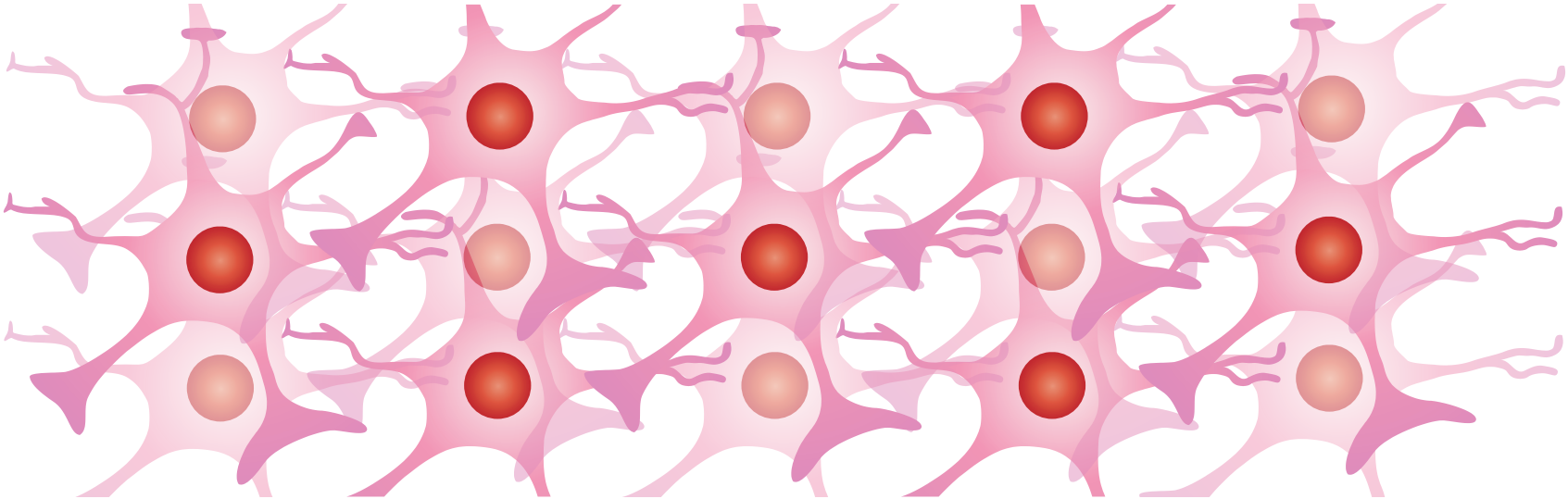
Astrocytes Metabolic Functions



K^+ Membrane Potential

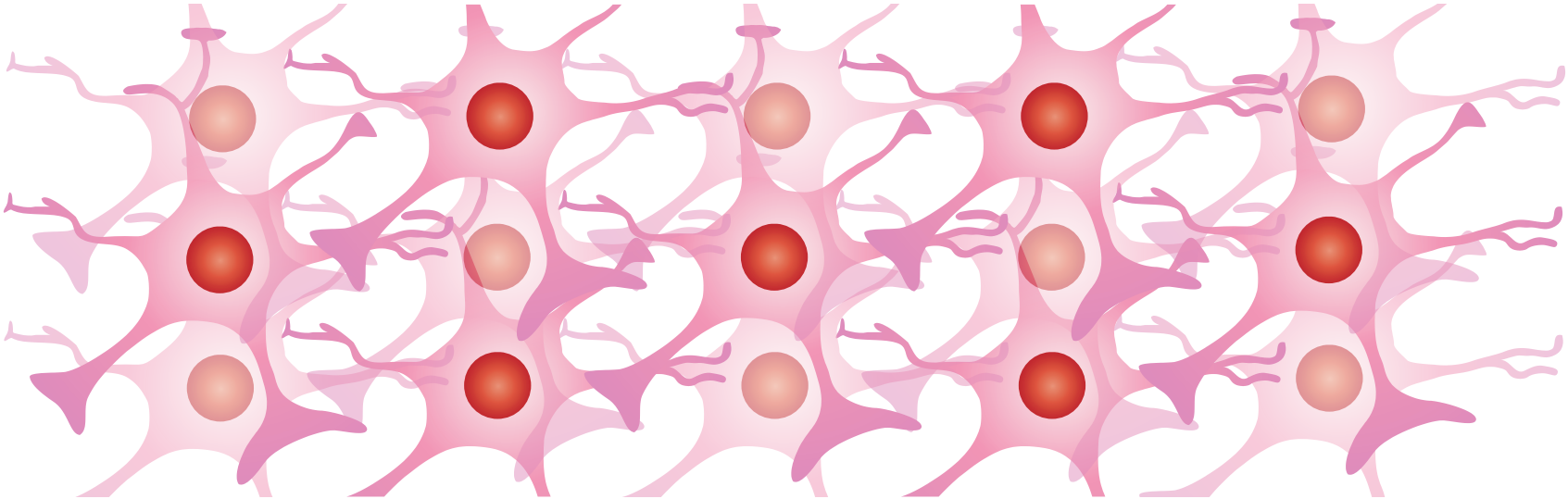
Ca^{+2} signaling

Astrocytes Metabolic Functions



K^+ Membrane Potential | **Ca^{+2}** signaling | **Lactate** release

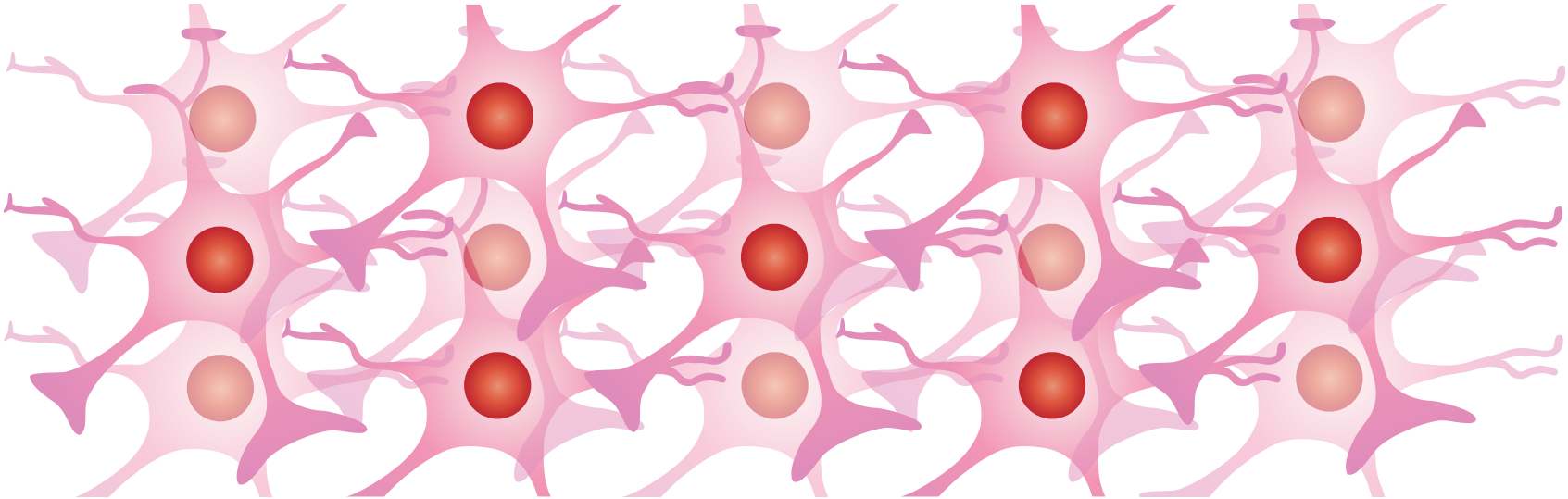
Astrocytes Metabolic Functions



K^{+} Membrane Potential | **Ca**$^{+2}$ signaling | **Lactate** release

[**DOPA**], [**Glu**], [**GABA**], [**Gly**] and [**Cys**] regulator

Astrocytes Metabolic Functions



K^+ Membrane Potential

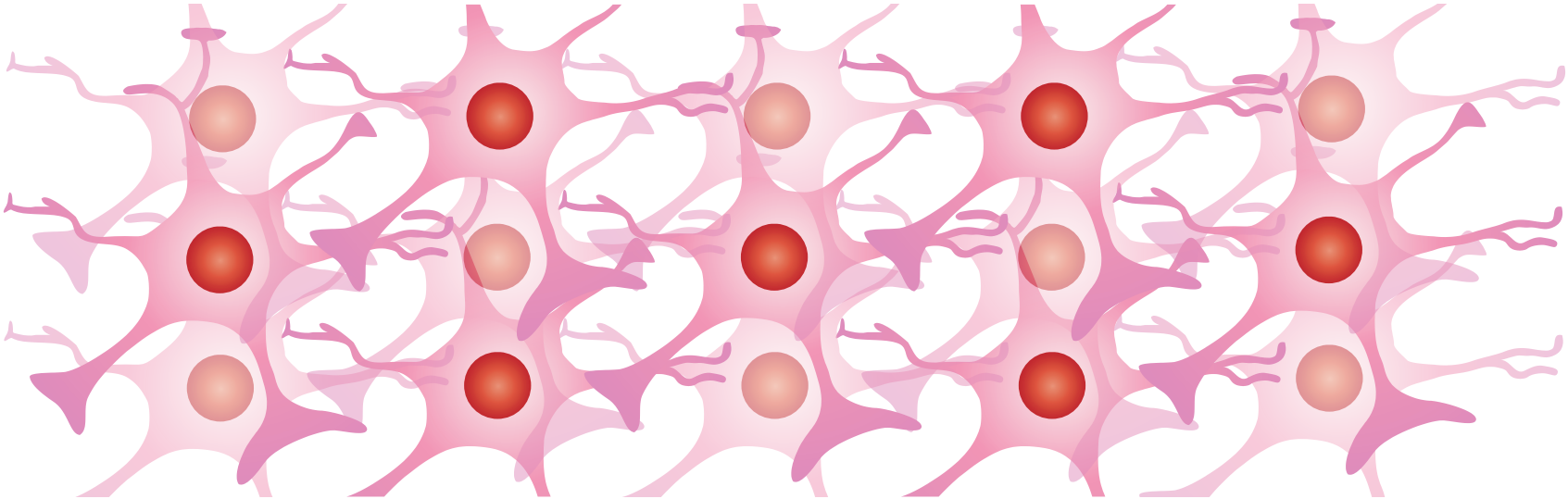
Ca^{+2} signaling

Lactate release

[**DOPA**], [**Glu**], [**GABA**], [**Gly**] and [**Cys**] regulator

pH maintenance

Astrocytes Metabolic Functions

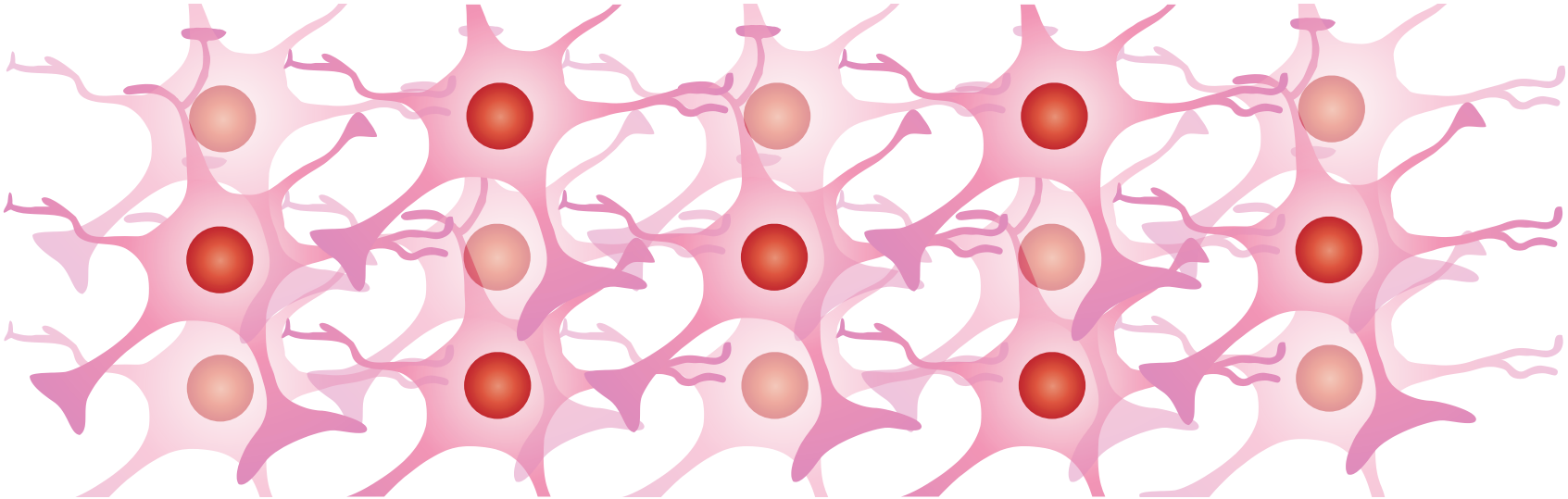


K^+ Membrane Potential | **Ca^{+2}** signaling | **Lactate** release

[**DOPA**], [**Glu**], [**GABA**], [**Gly**] and [**Cys**] regulator

pH maintenance | **ROS** detox

Astrocytes Metabolic Functions



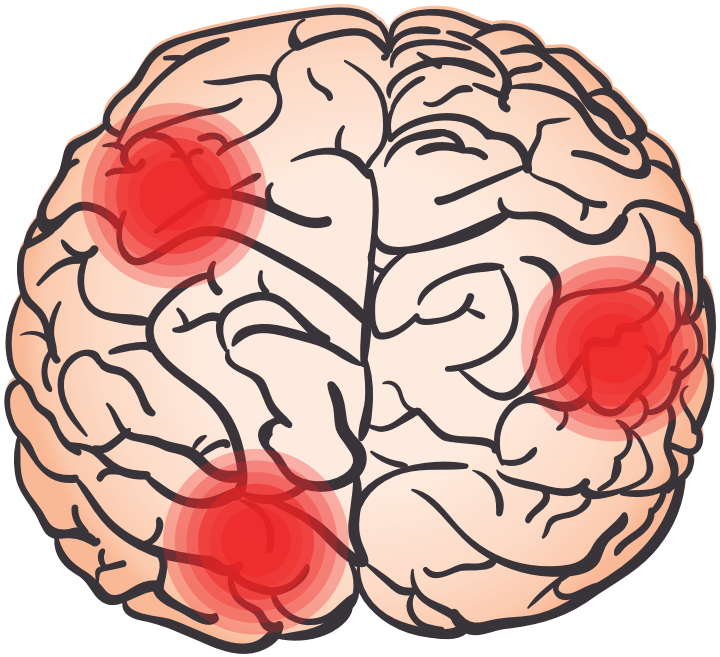
K^+ Membrane Potential | **Ca^{+2}** signaling | **Lactate** release

[**DOPA**], [**Glu**], [**GABA**], [**Gly**] and [**Cys**] regulator

pH maintenance | **ROS** detox | **Gln**, **ATP** and **D-serine** release

What is inflammation about?

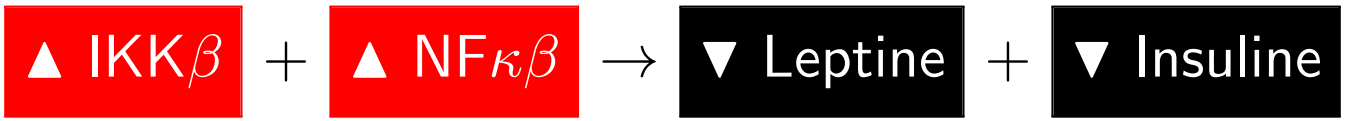
CNS inflammation



Neurodegenerative diseases | Cardiovascular events | Stress

Smoke | Obesity (Over Nutrition or Caloric Excess)

Metabolic Inflammation or Metainflammation



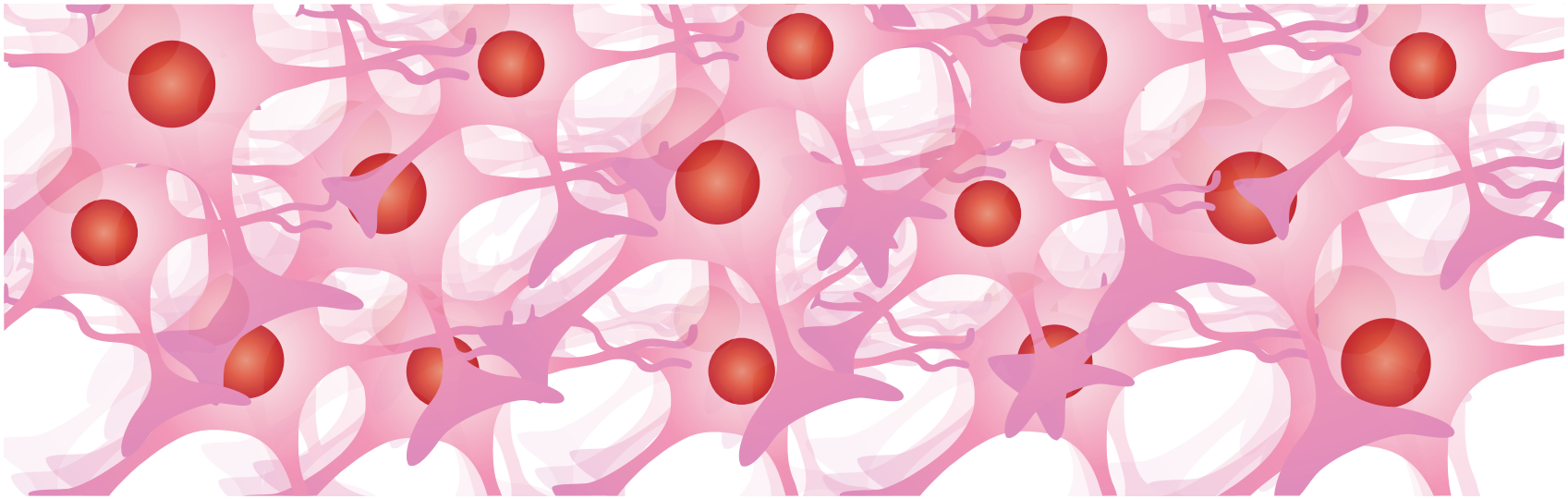
Metabolic Inflammation or Metainflammation

▲ IKKβ + ▲ NF$\kappa\beta$ → ▼ Leptine + ▼ Insuline

▲ Endoplasmic reticulum stress → ▲ UPR

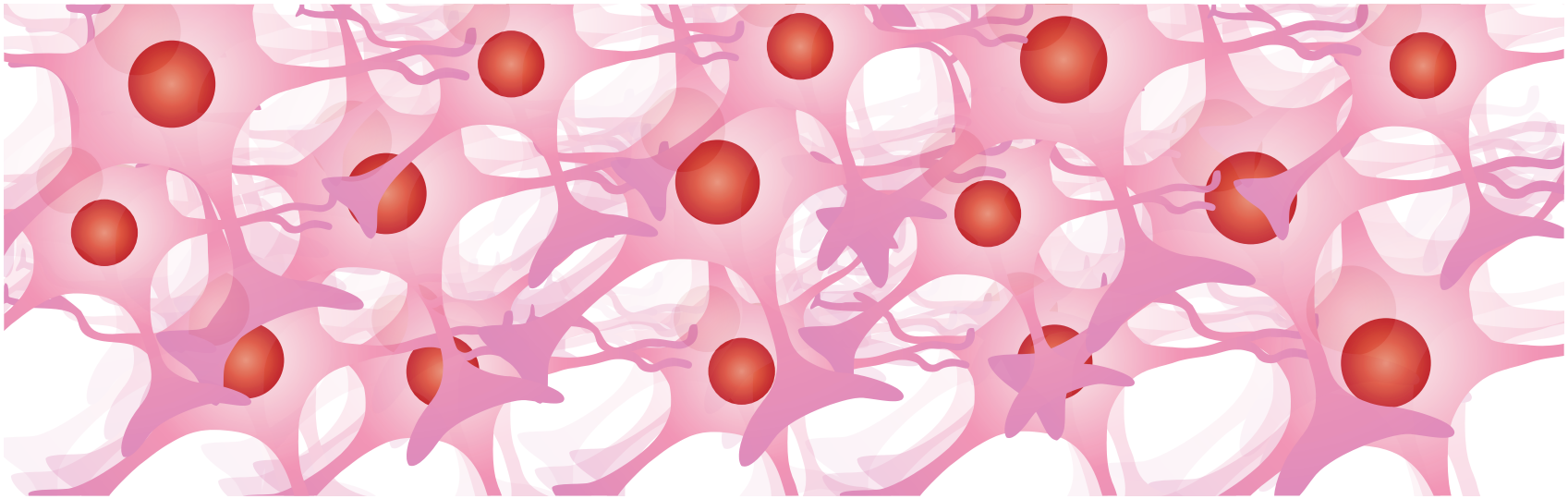
▲ CRP Ligands → ▲ TNFα + ▲ IL6 + ▲ ROS

Astrogliosis or Reactive Astrocytosis



ROS generator $\rightarrow$ **Cytokine** production

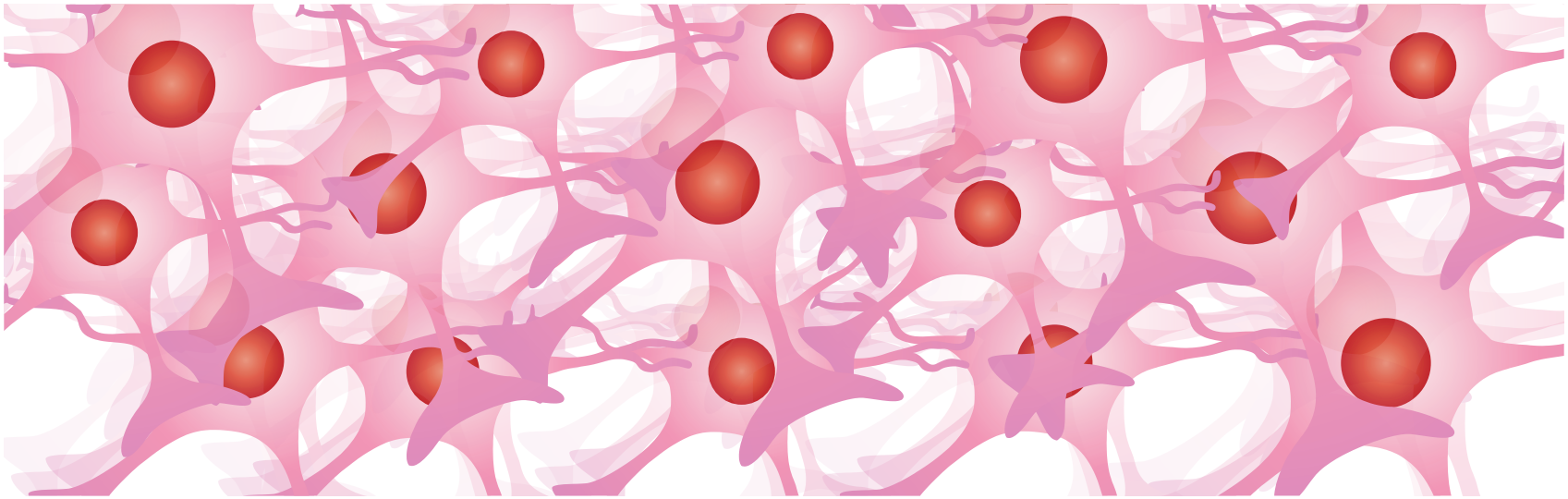
Astrogliosis or Reactive Astrocytosis



ROS generator $\rightarrow$ **Cytokine** production

Glu Excitotoxicity $\rightarrow$ **Ca**$^{+2}$ uptake $+$ **Mitochondrial** failure

Astrogliosis or Reactive Astrocytosis

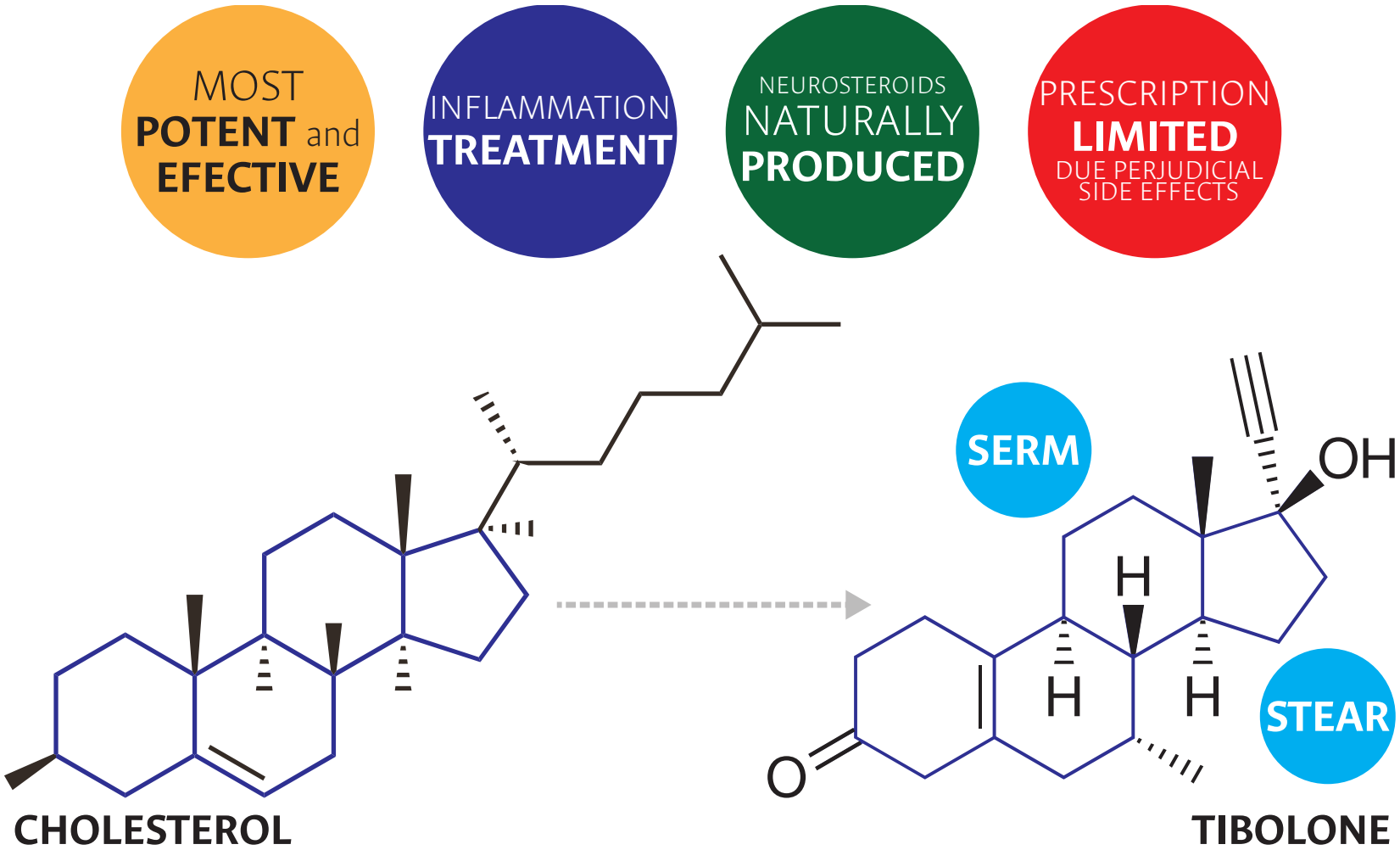


ROS generator $\rightarrow$ **Cytokine** production

Glu Excitotoxicity $\rightarrow$ **Ca**$^{+2}$ uptake $+$ **Mitochondrial** failure

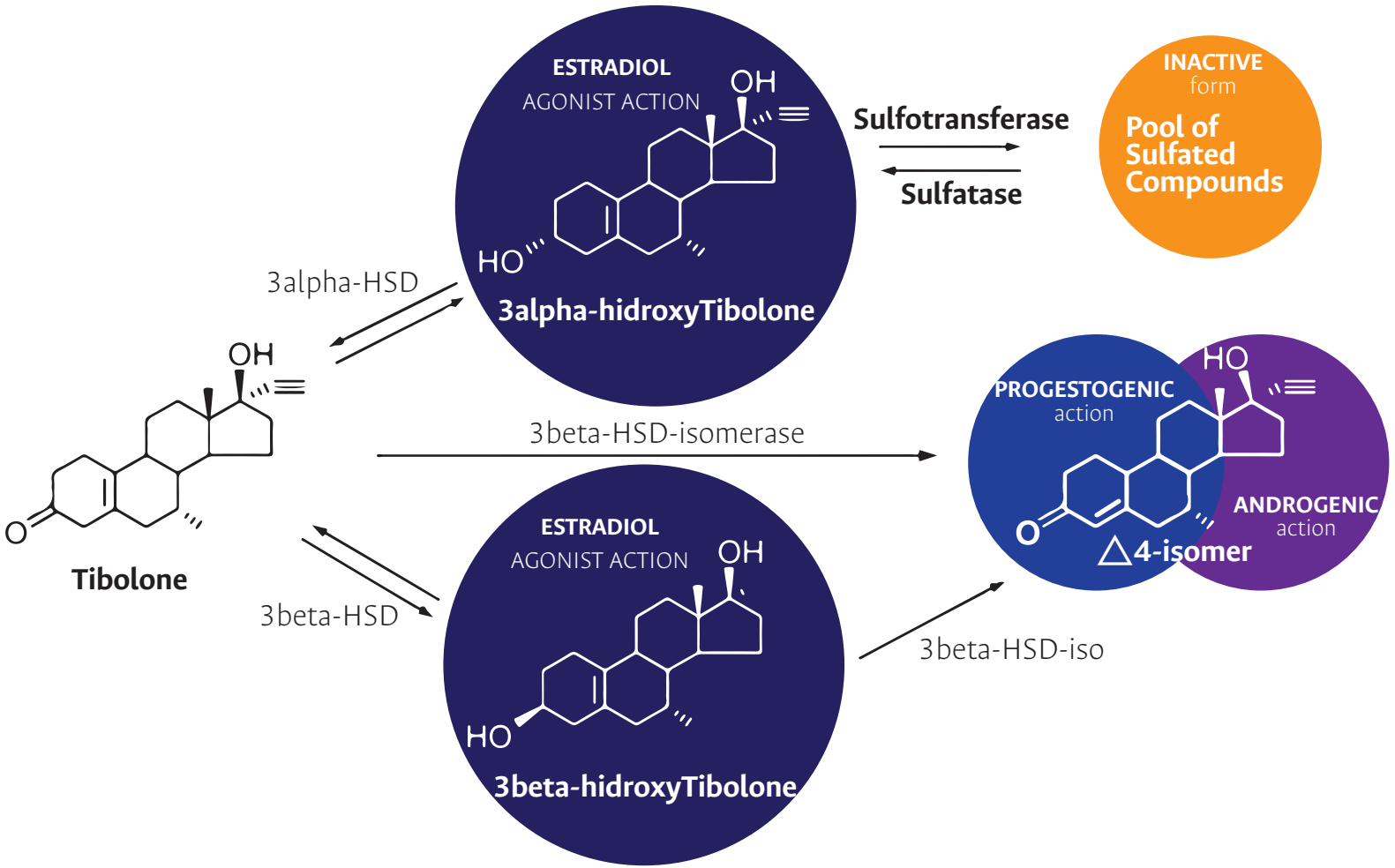
Free Fatty Acids (Palmitate) Increase

Inflammation Treatment: Steroids



Katarzyna Wojtal, Micha l K. Trojnar, and Stanis law J. Czuczwar. (2006).
Endogenous neuroprotective factors: neurosteroids.

Tibolone Metabolism



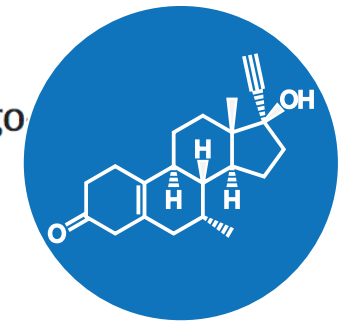
Modified of: Kloosterboer, H. J. (2004). Tissue-selectivity: the mechanism of action of tibolone.

What effects does tibolone have on Astrocytic inflammatory scenarios?

Tibolone protects astrocytic cells from glucose deprivation through a mechanism involving estrogen receptor beta and the upregulation of neuroglobin expression

Marco Avila-Rodriguez [a, c], Luis Miguel Garcia-Segura [b, **], Oscar Hidalgo
Eliana Baez [a], Janneth Gonzalez [a], George E. Barreto [a, d, e, *]

[a] *Departamento de Nutrición y Bioquímica, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, D.C., Colombia*
[b] *Instituto Cajal, CSIC, Avenida Doctor Arce 37, 28002, Madrid, Spain*
[c] *Departamento de Ciencias Clínicas, Facultad de Ciencias de la Salud, Universidad del Tolima, Ibagué, Colombia*
[d] *Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Santiago, Chile*
[e] *Universidad Científica del Sur, Lima, Peru*



Objectives:

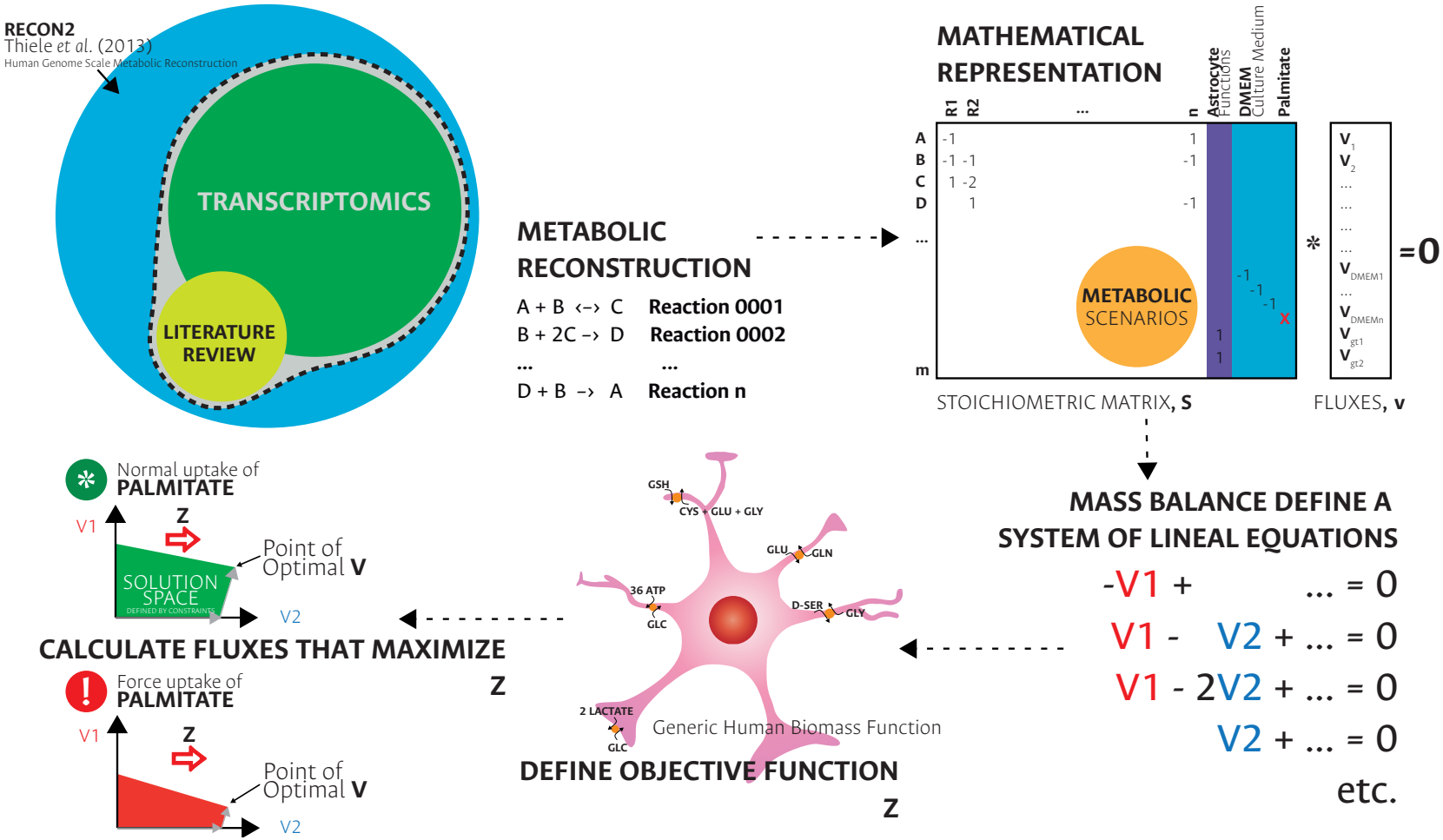
To identify proteins and metabolic pathways involved in the neuroprotective effects of tibolone in human astrocytes based in metabolic scenarios comparation we set:

- Build a tissue specific computational model of astrocytes metabolism using gene expression data integration.
- Evaluate the effects caused by the increase of free fatty acids and tibolone presence in astrocytes metabolism.
- Determine metabolic pathways and relevant functional products in response to steroid tibolone through systems biology approximations.
- Evaluate the importance of proteins and metabolic pathways previously identified on the dynamics of the metabolic model.

OBJECTIVE 1:

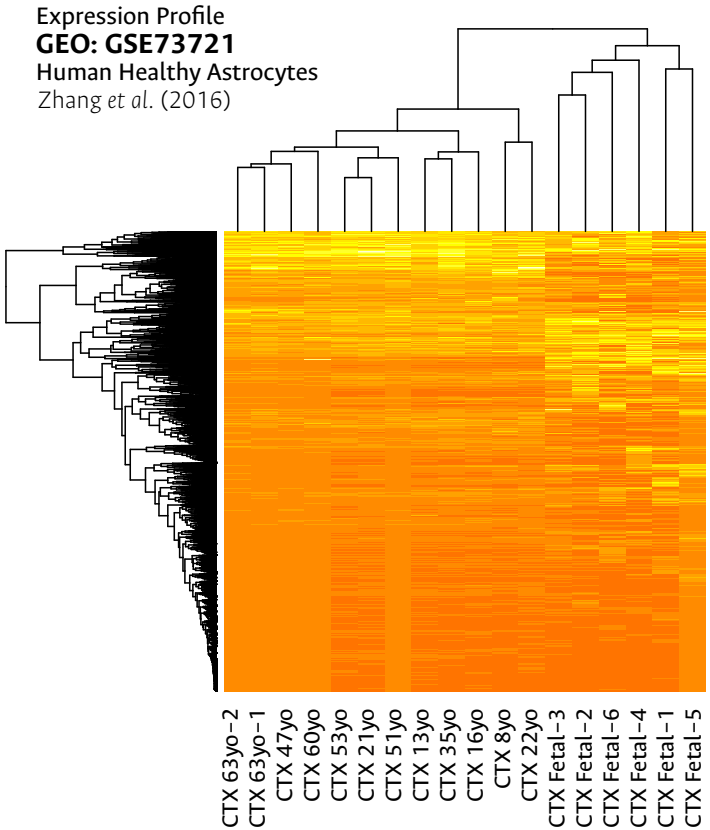
Build a tissue specific computational model of astrocytes metabolism using gene expression data integration.

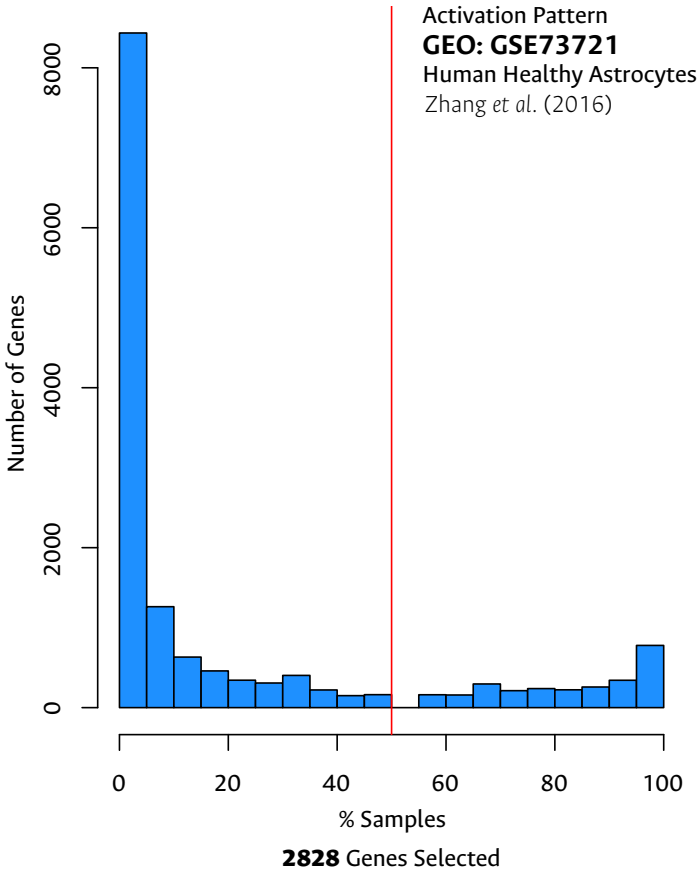
Metabolism Simulation: Flux Balance Analysis (FBA)



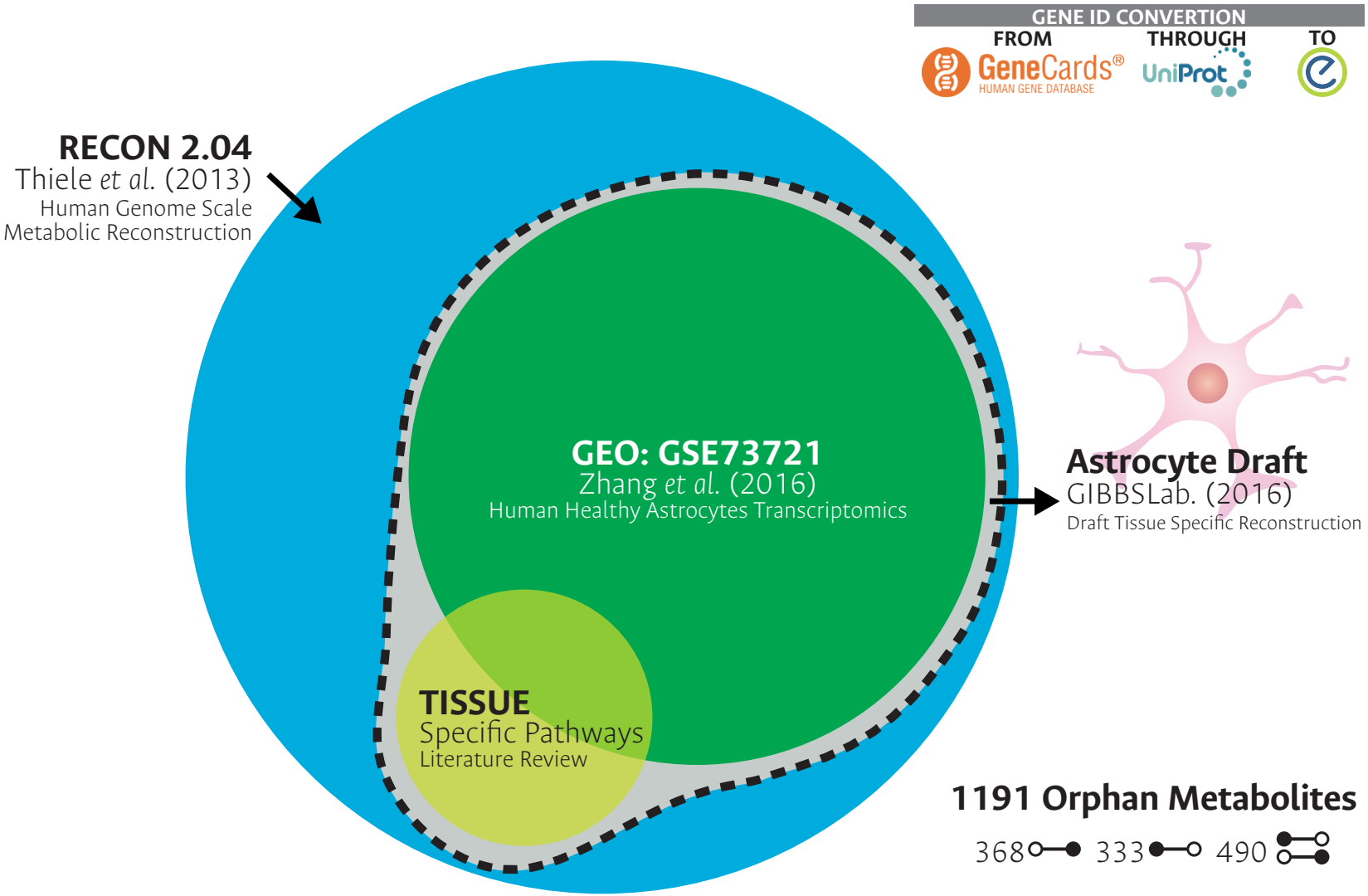
Modified of: Orth, J. D., Thiele, I., and Palsson, B. Ø. (2010). What is flux balance analysis?.

Healthy Human Astrocytes Gene Expression Data





Mapping Reactions



Gap-Find and Gap-Fill Available Algorithms

ALGORITHM	ENVIRONMENT	HOW IT WORKS
SMILEY	Python - OpenSource	· Optimization based. · **Fills one metabolite per time**.
gap-Find/Fill	GAMS - OpenSource	· Optimization based. · **Makes several intra model modifications**.
growMatch	Python - OpenSource	· Optimization based. · **Fills one objective function per time**.
fastGapFill	MATLAB - **Privative**	· Optimization based. · Multiobjective.

Finding and Filling Gaps

'g2f' Package

An R Package to Find and Fill Gaps for genome-scale metabolic networks

*Kelly Botero, **Daniel Osorio**, Janneth Gonzalez and Andres Pinzón-Velasco.*

Language: R
Stable: CRAN
Development: gibbslab/g2f
License: GPL-2
Binaries: Windows - Linux - Mac

h2o[r] + dheas[r] ⇒ h[r] + dhea[r] + so4[r]
uri[e] ⇔ uri[c]
na1[e] + uri[e] ⇒ na1[c] + uri[c]
atp[c] + pi[m] ⇒ pi[c] + atp[m]
na1[e] + gchola[e] ⇒ na1[c] + gchola[c]
ASTROCYTE DRAFT GiBBS Lab (2016)

REACTLIST

getReference( )
DOWNLOAD STOICHIOMETRIC REACTIONS FROM THE KEGG DATABASE

REFERENCE

RECON 2.04 Thiele *et al.* (2013)

gapFill( )
FIND AND FILL GAPS

orphanReactants( ) + orphanProducts( )
IDENTIFY INITIAL GAPS

IDENTIFY REACTIONS THAT INCLUDES ORPHANS AS METABOLITES

additionCost( )
CALCULATE ADITION COST

$$cost = \frac{n(metabolites(newReaction) \notin metabolites(reactionList))}{n(metabolites(newReaction))}$$

IF ADITION COST ≤ GIVEN LIMIT

ADD REACTION TO REACTIONLIST

orphanReactants( ) + orphanProducts( )
IDENTIFY NEW GAPS

IF n(**INITIAL GAPS** ∈ **NEW GAPS**) ≤ n(**INITIAL GAPS**)

REPEAT UNTIL NO MORE INITIAL GAPS COULD BE FILLED

RETURN REACTIONLIST

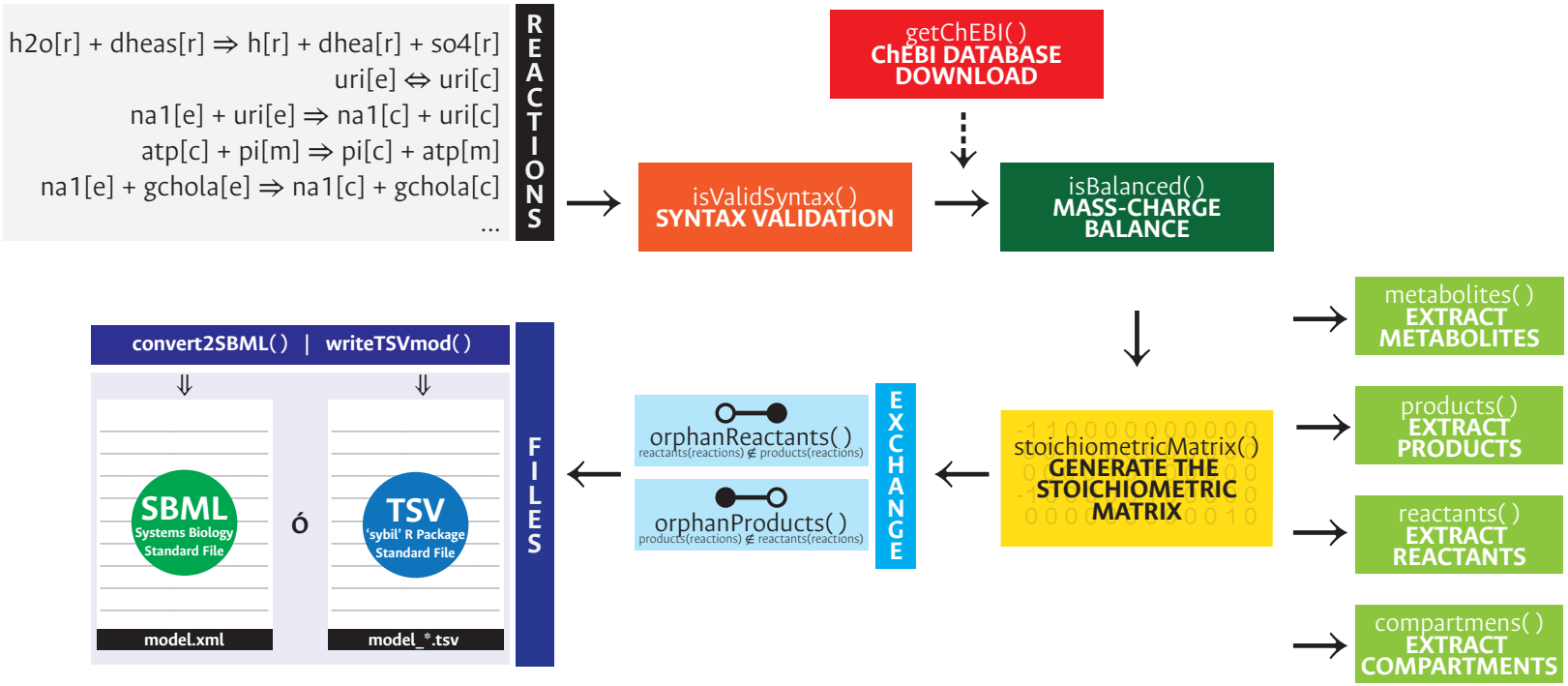
Syntax, Mass-Charge Validation and SBML files

'minval' Package

An R Package for MINimal VALidation of stoichiometric reactions

Daniel Osorio, *Janneth Gonzalez and Andres Pinzón-Velasco.*

Language: R
Stable: CRAN
Development: gibbslab/minval
License: GPL-2
Binaries: Windows - Linux - Mac



Metabolic Model Debugging

'g2f' Package

An R Package to Find and Fill Gaps for genome-scale metabolic networks

*Kelly Botero, **Daniel Osorio**, Janneth Gonzalez and Andres Pinzón-Velasco.*

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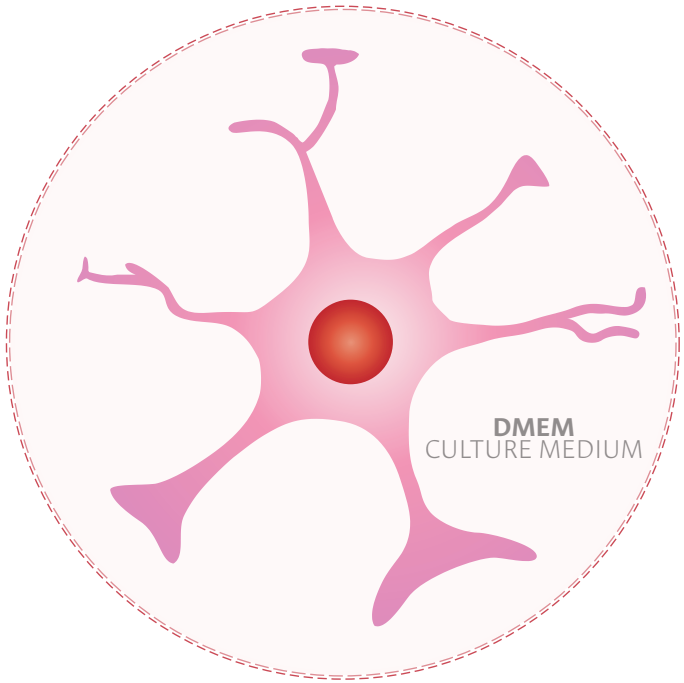
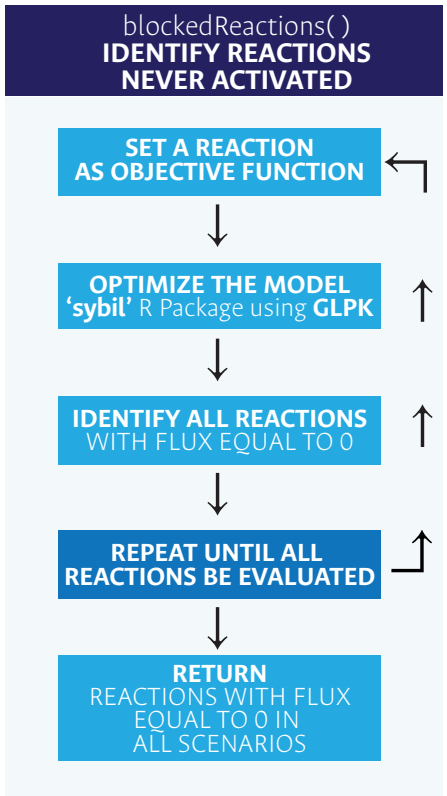




BLOCKED REACTIONS WERE REMOVED

Gene Expression Integration Available Methods

METHOD	ENVIRONMENT	HOW IT WORKS
GIMME	MATLAB **Privative**	·**Binary Disctretization** ·**Ensures flux for a selected objective function**
iMAT	MATLAB **Privative**	· Integration proportional to gene-expression (H, M and L categorization) · Not objective function required
E-FLUX	**Not implemented**	· **Requires a user-given threshold** · Continuous Integration
PROM	MATLAB **Privative**	·**Requires a user-given regulatory network** · Constraints are setting according to the associated transcript. factor

Constraining the Metabolic Model

'exp2flux' Package

An R Package to convert expression data to FBA fluxes

Daniel Osorio, Kelly Botero, Janneth Gonzalez and Andres Pinzón-Velasco.

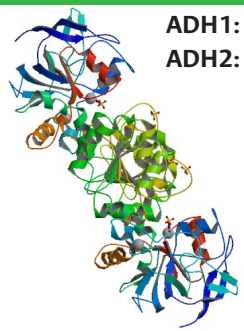
Language: R
Stable: CRAN
Development: gibbslab/exp2flux
License: GPL-2
Binaries: Windows - Linux - Mac

E.C: 1.1.1.1

alcohol[c] + NAD(+)[c] ⇔ aldehyde[c] + NADH[c]

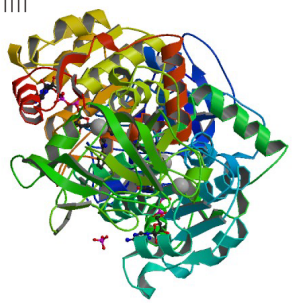
1.1.1.1

ADH2 **ADH1**



ADH1: ||||||||||||||||||||||||||

ADH2: ||||||||||



E.C: 3.4.21.5

polypeptides[e] ⇒ aminoacids[e]

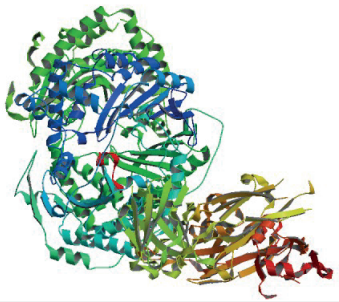
3.4.21.5

IDE.A **IDE.B** **IDE.C**

IDE.A: ||||||||||||||||||||||||||

IDE.B: ||||||||||

IDE.C: ||||||||||||||||||



(ADH2 or ADH1) GPR **(IDE.A and IDE.B and IDE.C)**

sum (ExprADH2 + ExprADH1) min (ExprIDE.A, ExprIDE.B, ExprIDE.C)

GENE EXPRESSION DATA →

METABOLIC MODEL WITH GPR →

exp2flux( )
CONVERT GENE EXPRESSION DATA TO FBA FLUXES →

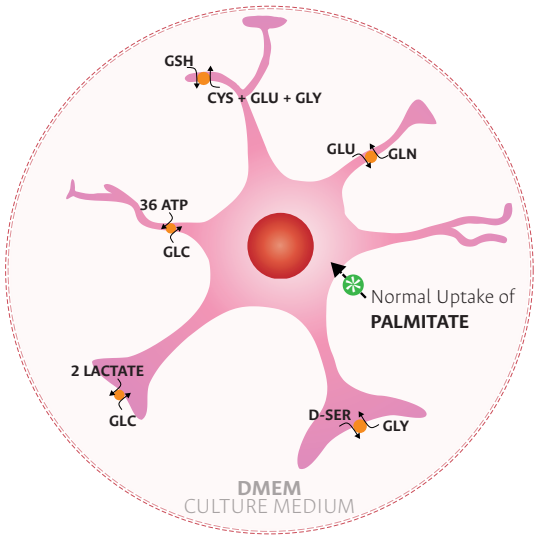
CONSTRAINED METABOLIC MODEL →

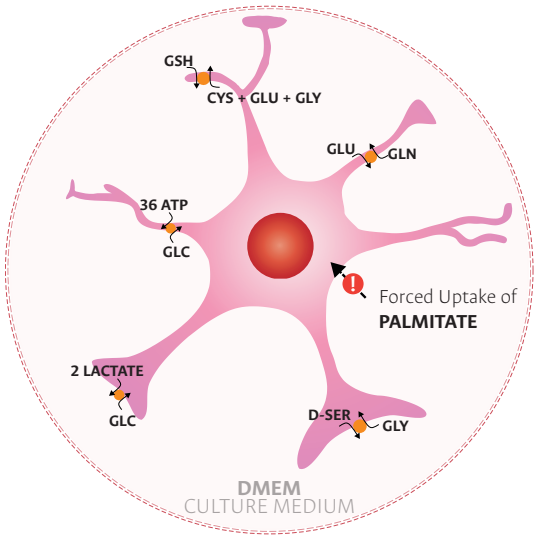
fluxDifferences( )
COMPUTE FOLDCHANGE OF FLUXES BETWEEN METABOLIC SCENARIOS

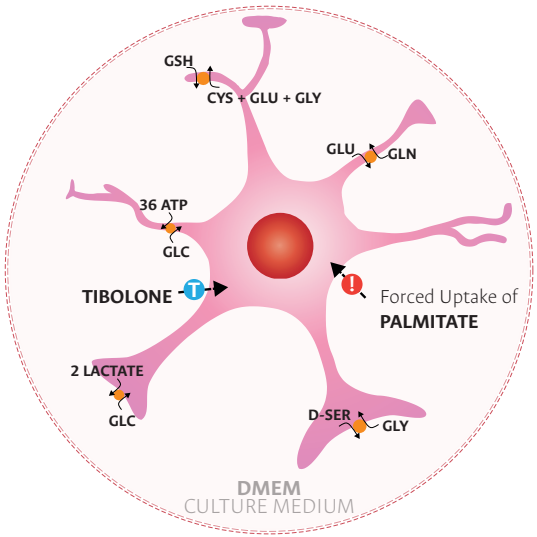
OBJECTIVE 2:

Evaluate the effects caused by the increase of free fatty acids and tibolone presence in astrocytes metabolism.

Metabolic Scenarios





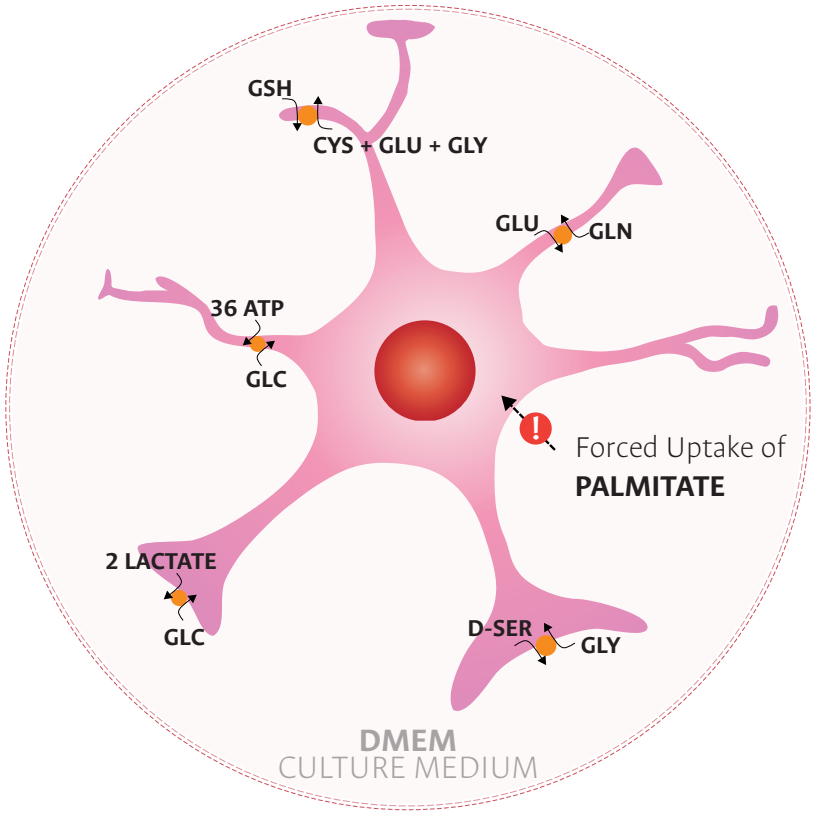


HEALTHY **INFLAMED** **MEDICATED**

MAIN OBJECTIVE FUNCTION:

Generic Human Biomass Reaction included in RECON 2.04 (Thiele *et al.*, 2013)

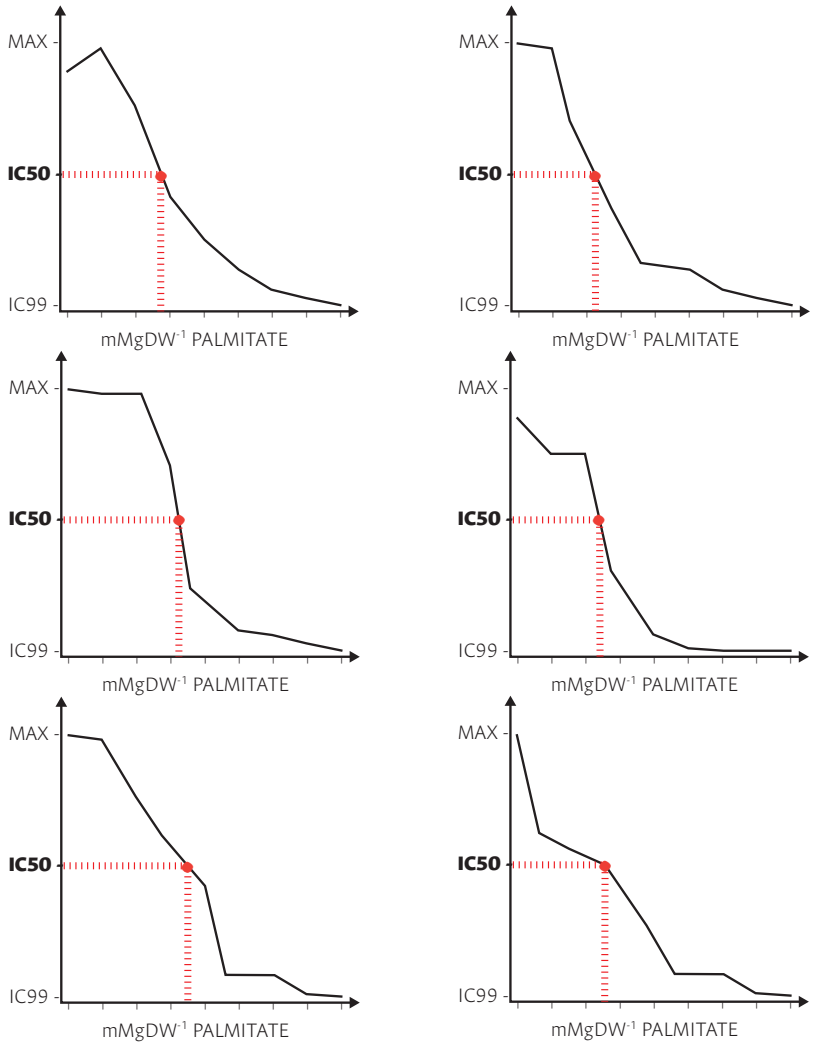
Inflamed Metabolic Scenario



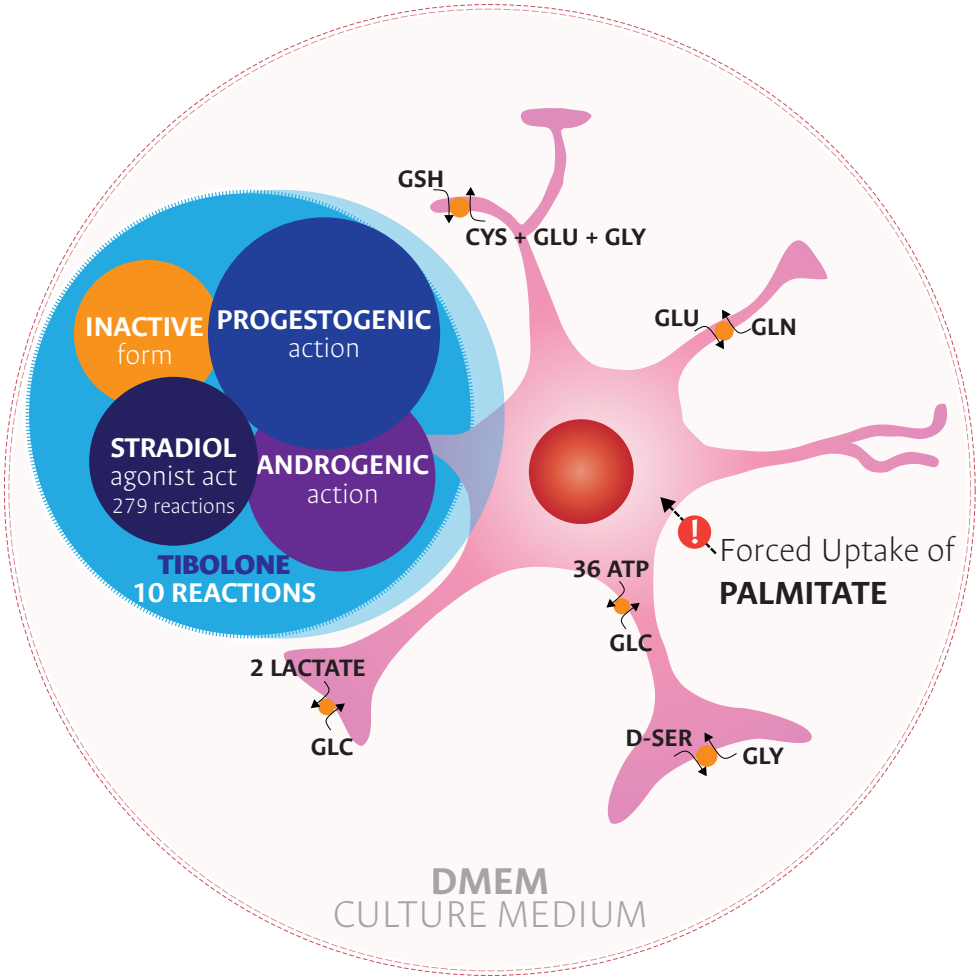
PALMITATE UPTAKE RATE: AVERAGED IC_{50} VALUES

INFLAMED

ROBUSTNESS ANALYSIS



Medicated Metabolic Scenario

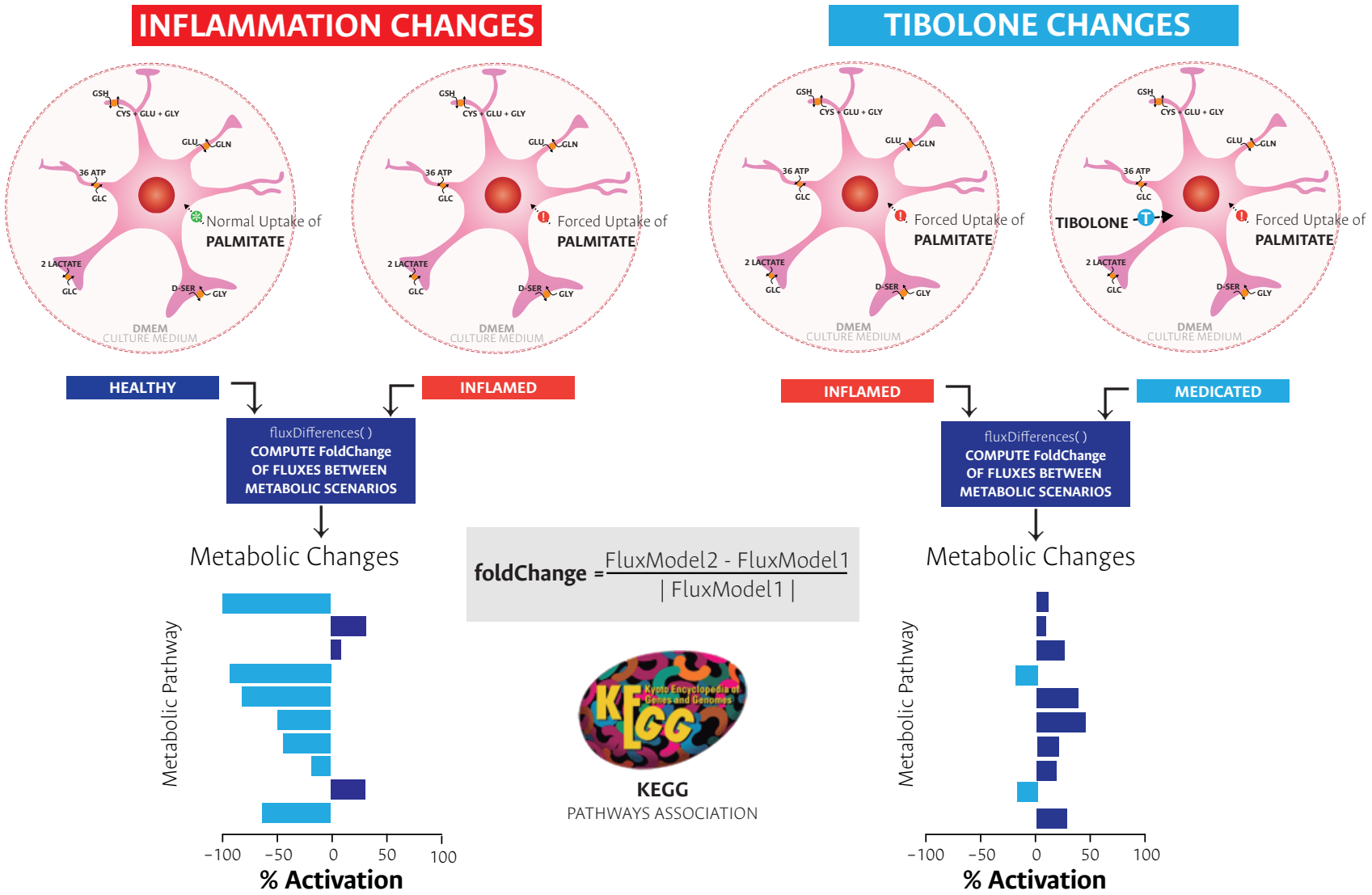


MEDICATED

OBJECTIVE 3:

Determine metabolic pathways and relevant functional products in response to steroid tibolone through systems biology approximations.

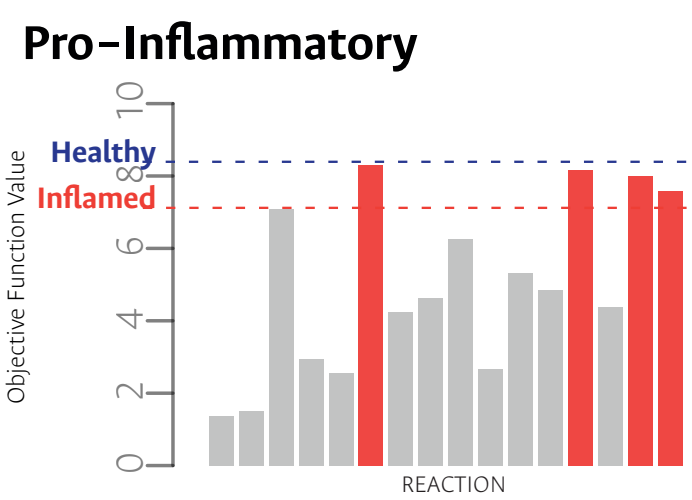
Metabolic Changes

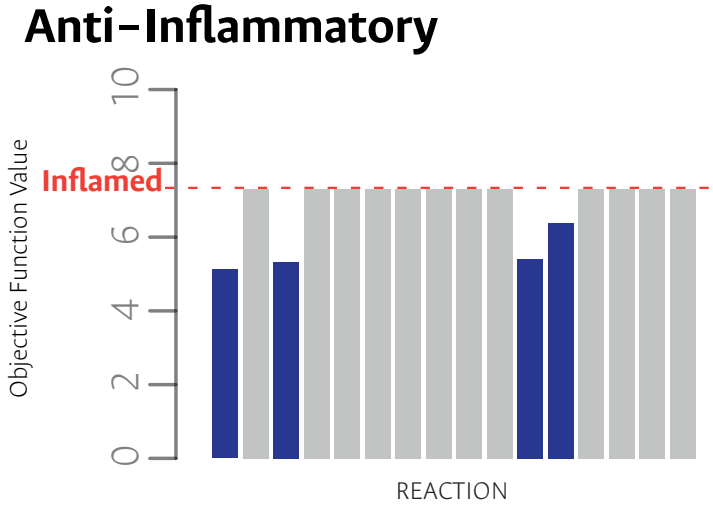


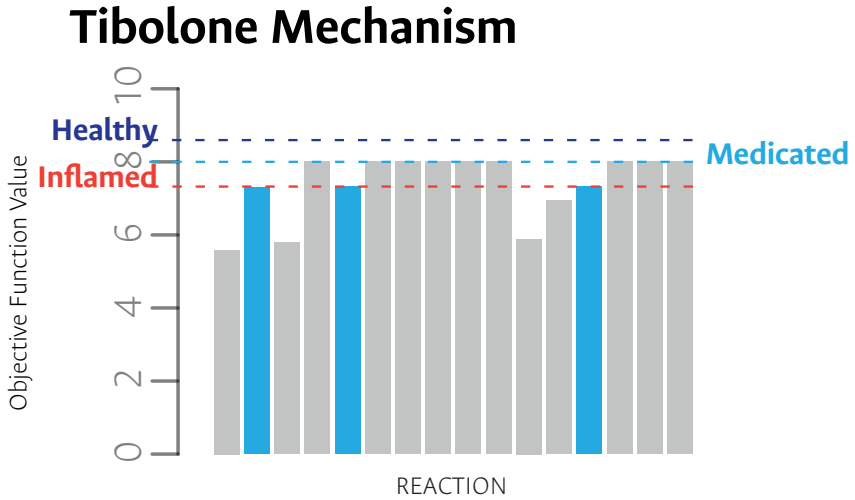
OBJECTIVE 4:

Evaluate the importance of proteins and metabolic pathways previously identified on the dynamics of the metabolic model.

Reaction Knock-out: Protein Importance







Reproducibility

ALL **ANALYSIS** WERE PERFORMED USING

VERSION: **3.3.1**

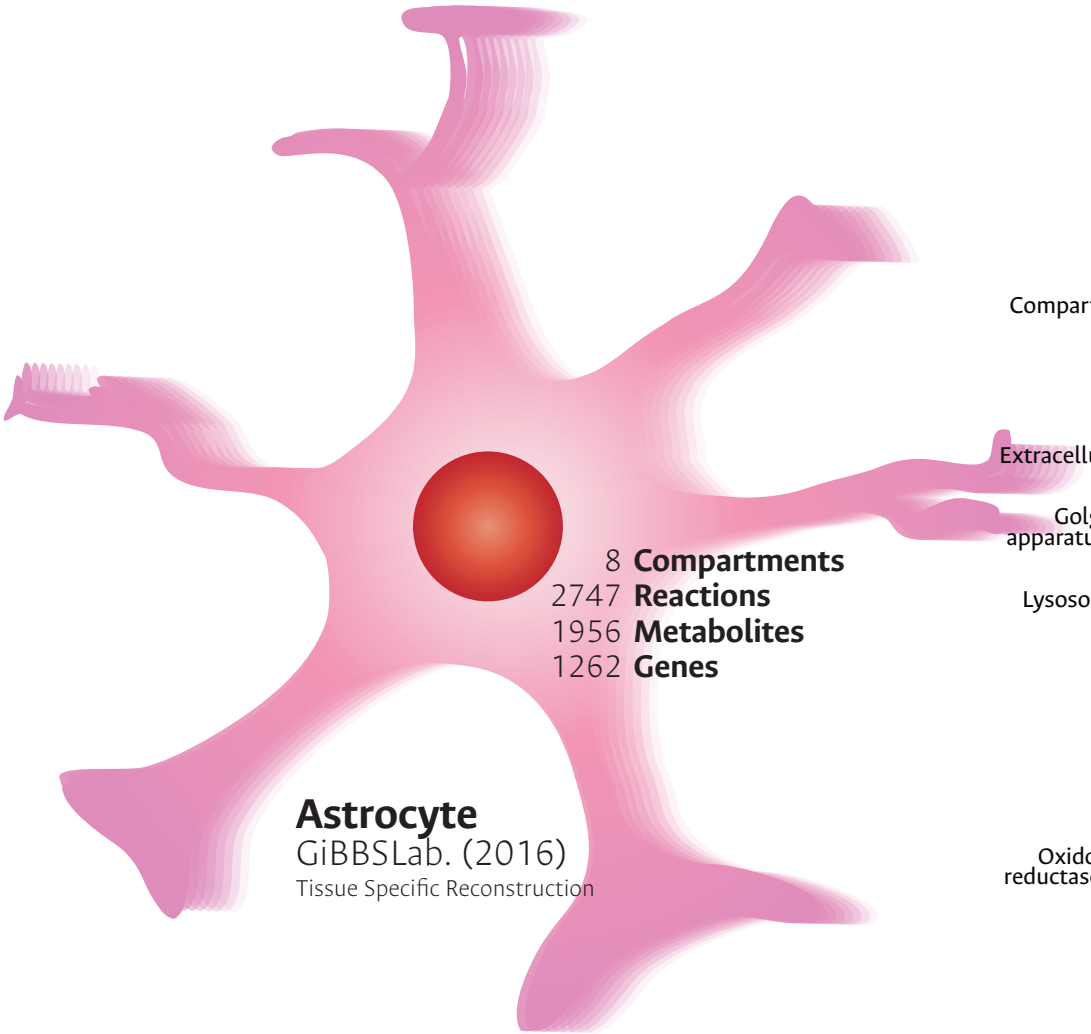
FBA: **SYBIL** (Gelius-Dietrich, G. *et al.*, 2013)
LP SOLVER: **GLPK 4.6**

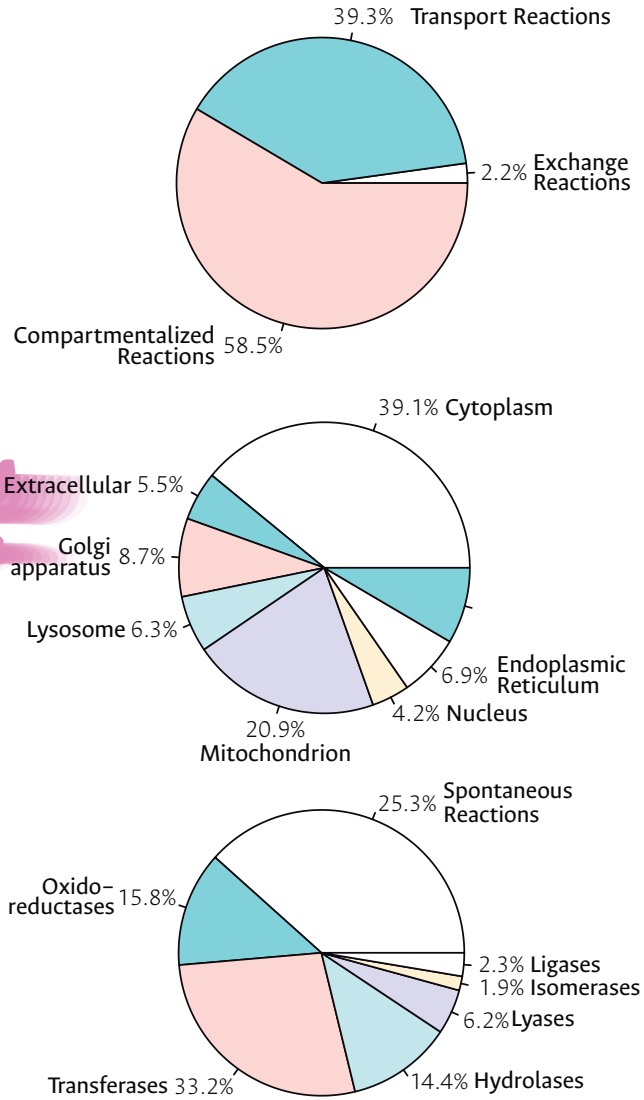
ALL **CODE**, **DATA**, **DOCUMENTS** AND **SLIDES** ARE AVAILABLE AT:

GITHUB: **dosorio/masterThesis**

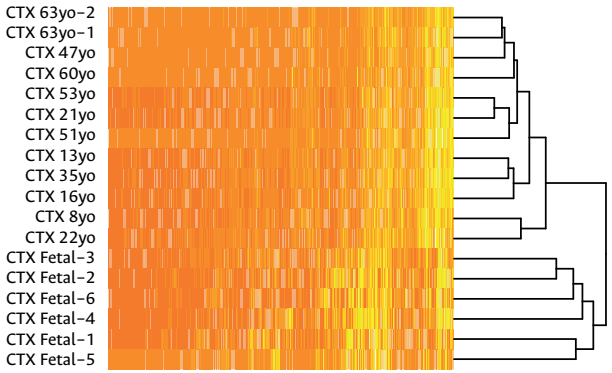
LICENSE: **GNU GPL3**

Tissue Specific Metabolic Reconstruction



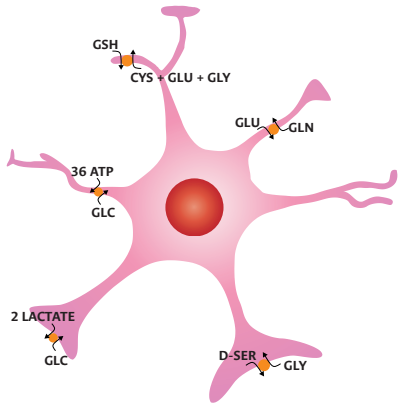


Human Healthy Mature Astrocyte Model



Expression Profile
GEO: GSE73721
Human Healthy Astrocytes
Zhang *et al.* (2016)

SBML
ASTROCYTE
Tissue Specific Model
GiBBSLab. (2016)

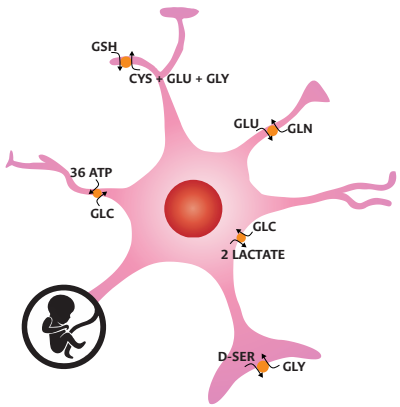


'exp2flux' Package

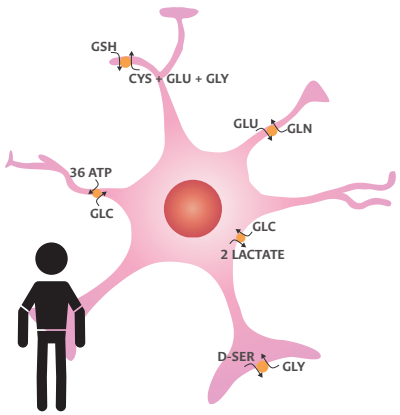
An R Package to convert expression data to FBA fluxes

Daniel Osorio, *Kelly Botero*, *Janneth Gonzalez* and *Andres Pinzón-Velasco*.

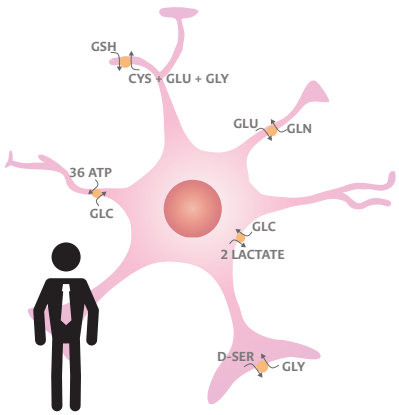
exp2flux( )
CONVERT GENE EXPRESSION DATA TO FBA FLUXES



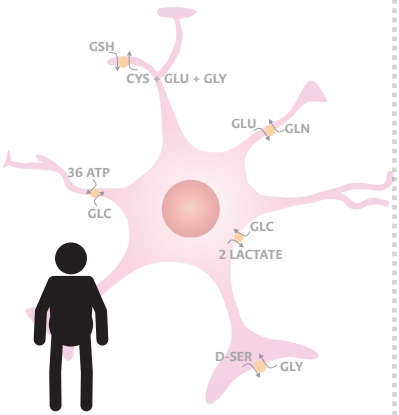
FETAL



YOUNG
8 - 16 yo.



ADULT
21 - 35 yo.

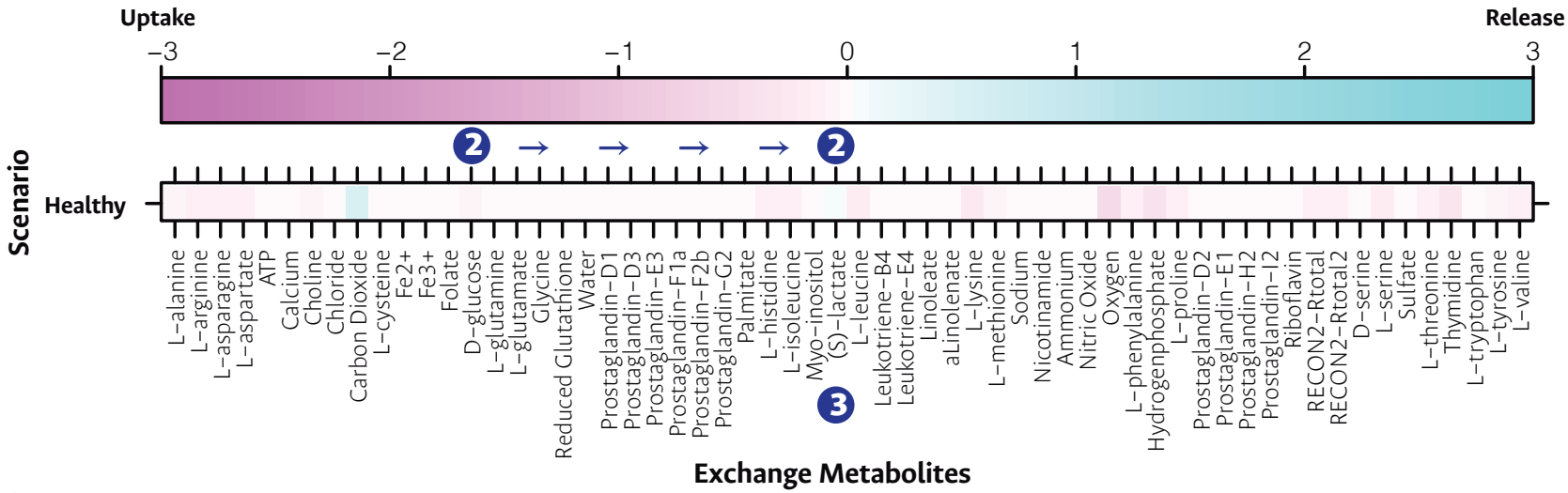


MATURE
47 - 63 yo.

Cellular Maintenance: Healthy Scenario

1 OBJECTIVE FUNCTION: **0.37 mMgWD−1h−1**
DMEM Culture Medium

METABOLISM ACTIVATION: **52%**



(1) Arabinda Das, *et al.* (2010). Flavonoids activated caspases for apoptosis in human glioblastoma T98G and U87MG cells but not in human normal astrocytes.

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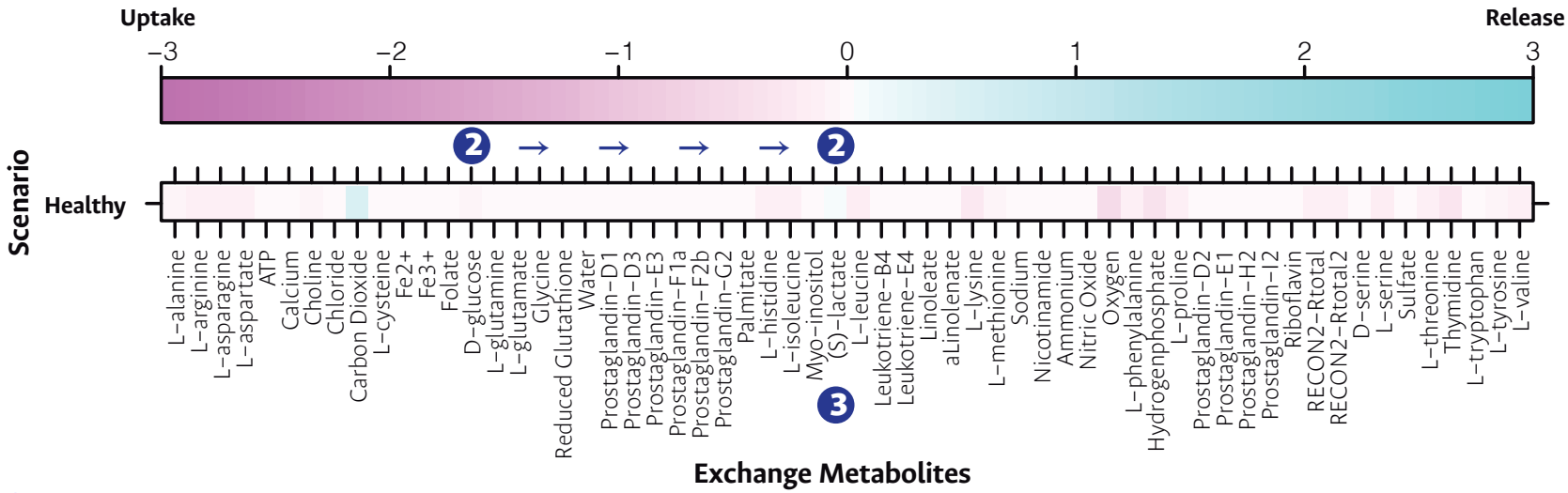

1 Arabinda Das, *et al*. (2010). Flavonoids activated caspases for apoptosis in human glioblastoma T98G and U87MG cells but not in human normal astrocytes.

2 Tunahan Cakir *et al*. (2007). Reconstruction and flux analysis of coupling between metabolic pathways of astrocytes and neurons: application to cerebral hypoxia.

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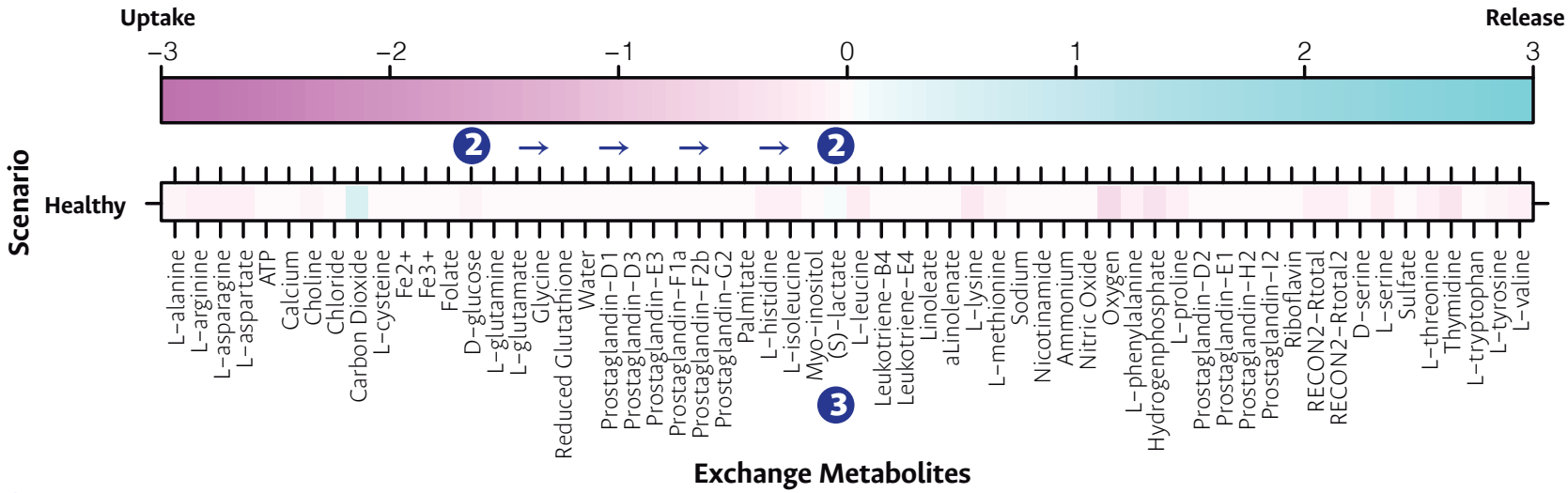

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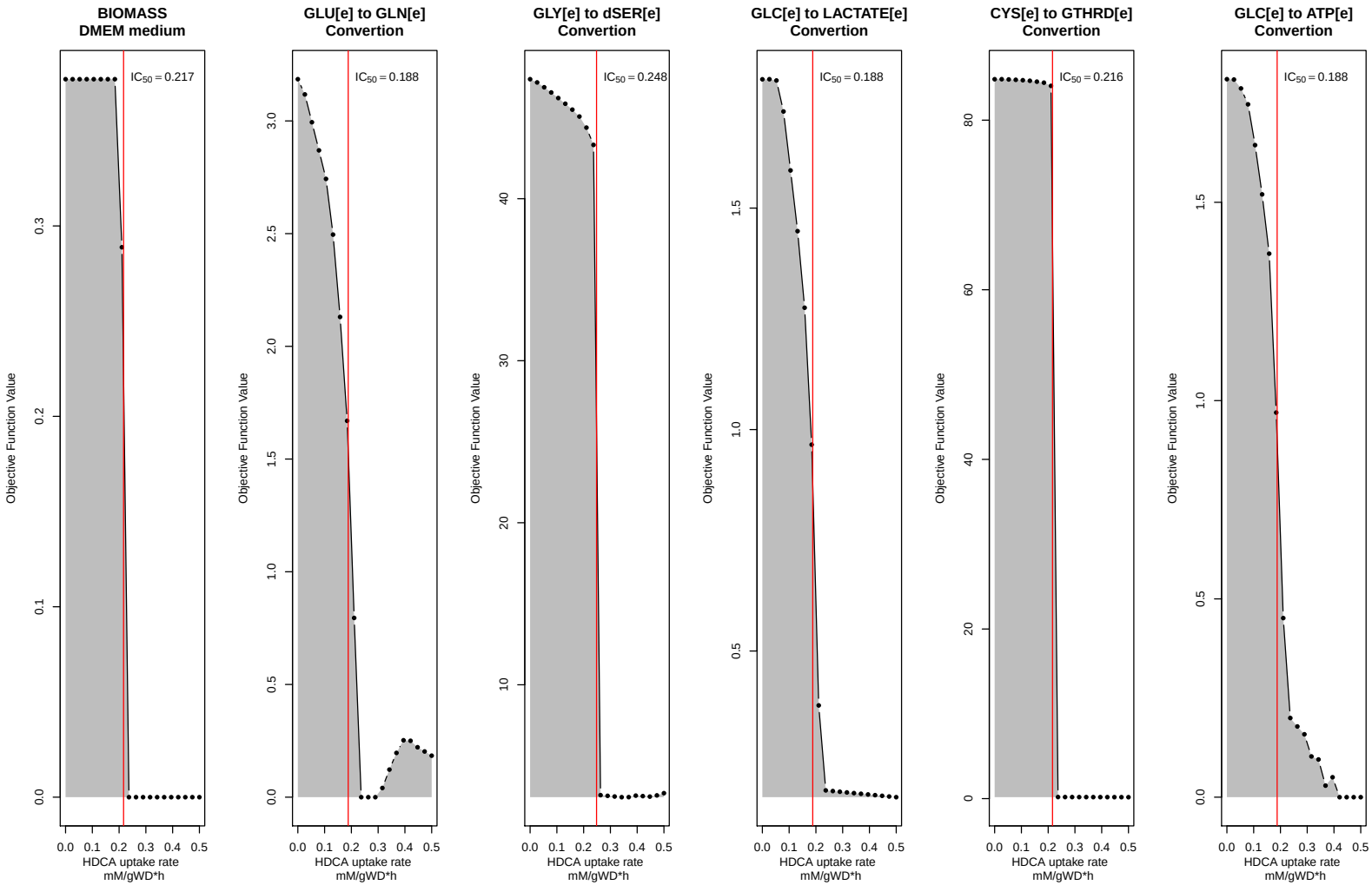
3 Christelle Le Foll, et al. (2010). Fatty acid-induced astrocyte ketone production and the control of food intake.

Averaged IC50: 0.208 ± 0.024 $mMgDW^{-1}h^{-1}$

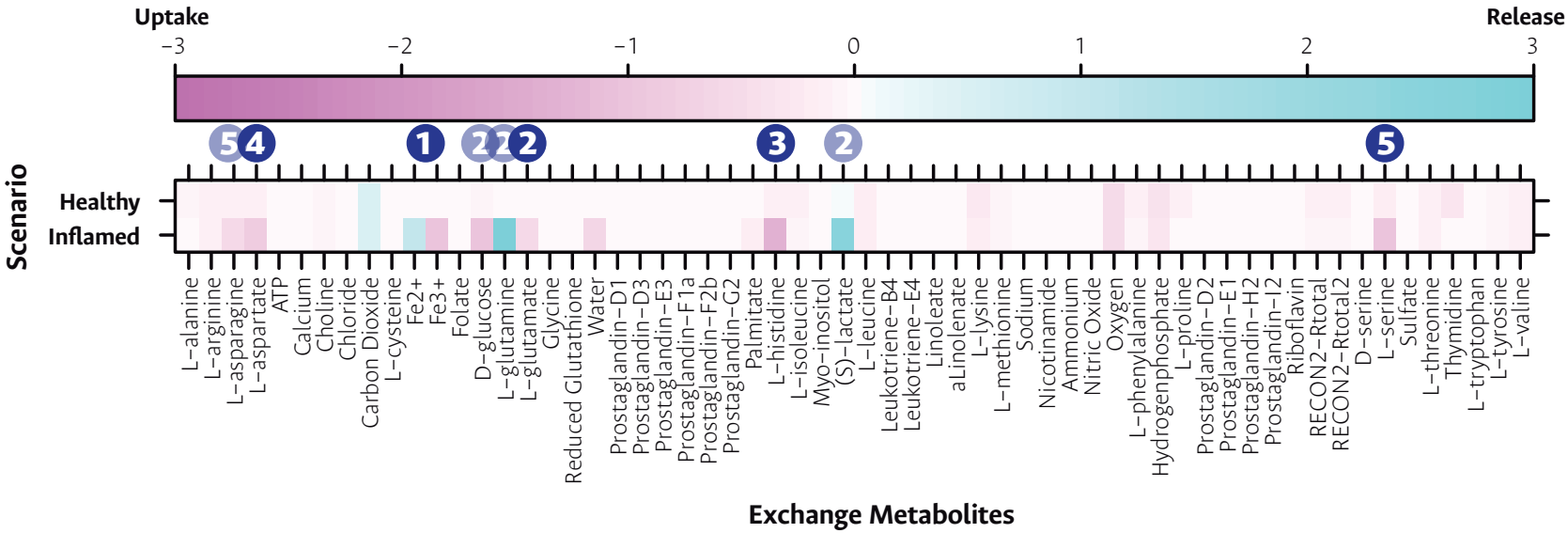


Li Liu *et al*. (2013) Palmitate-activated astrocytes via serine palmitoyltransferase increase BACE1 in primary neurons by sphingomyelinases.

Cellular Maintenance: Inflamed Scenario

OBJECTIVE FUNCTION: **0.31 mMgWD−1h−1** (-15.6%)
DMEM Culture Medium + 0.208 mM Palmitate

METABOLISM ACTIVATION: **46.6%** (-5.6%)



(1) Rachel Williams *et al.* (2012). Pathogenic implications of iron accumulation in multiple sclerosis.

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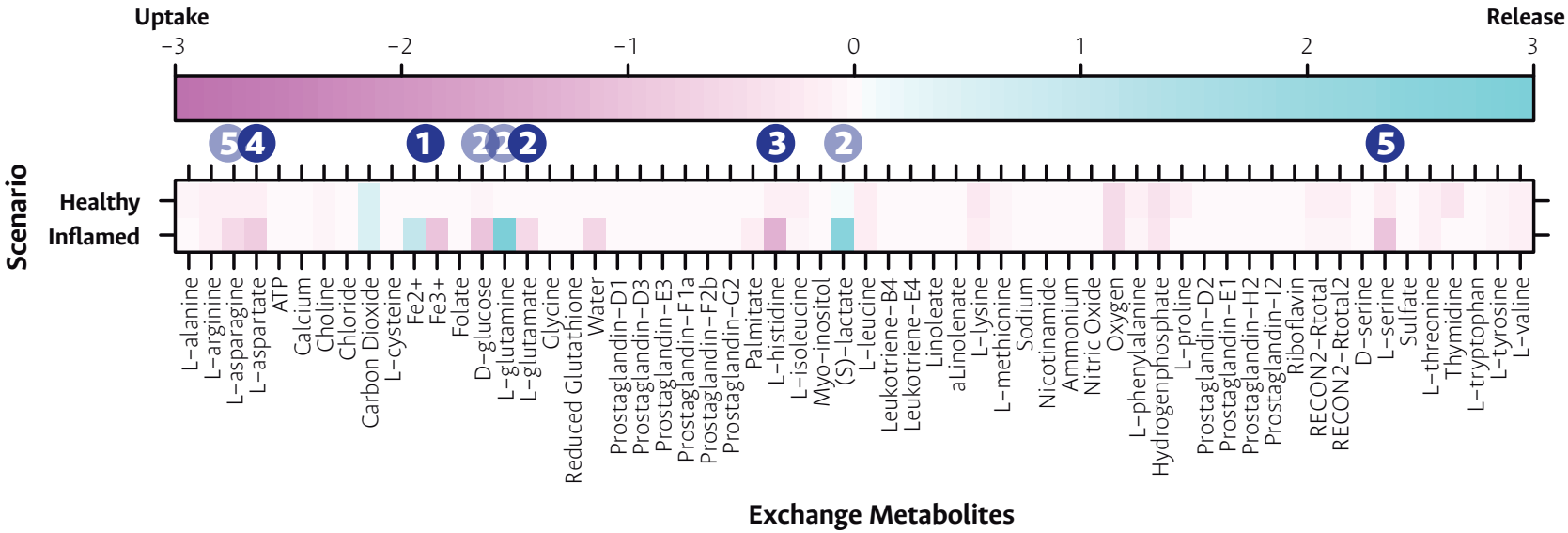

(1) Rachel Williams *et al.* (2012). Pathogenic implications of iron accumulation in multiple sclerosis.

(2) Mithilesh Kumar Jha *et al.* (2016). Metabolic Control of Glia-Mediated Neuroinflammation.

(2) V Parpura and P G Haydon. (2000). Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons.

(2) Leif Hertz *et al.* (1999). Astrocytes: Glutamate producers for neurons.

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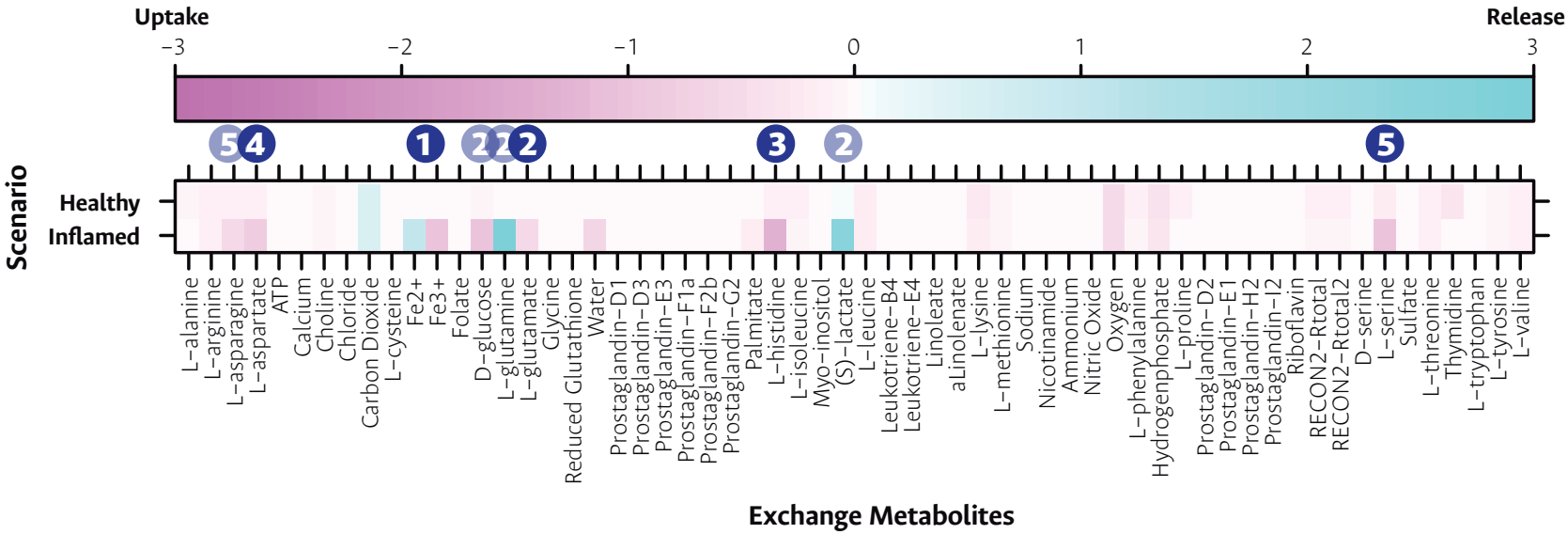
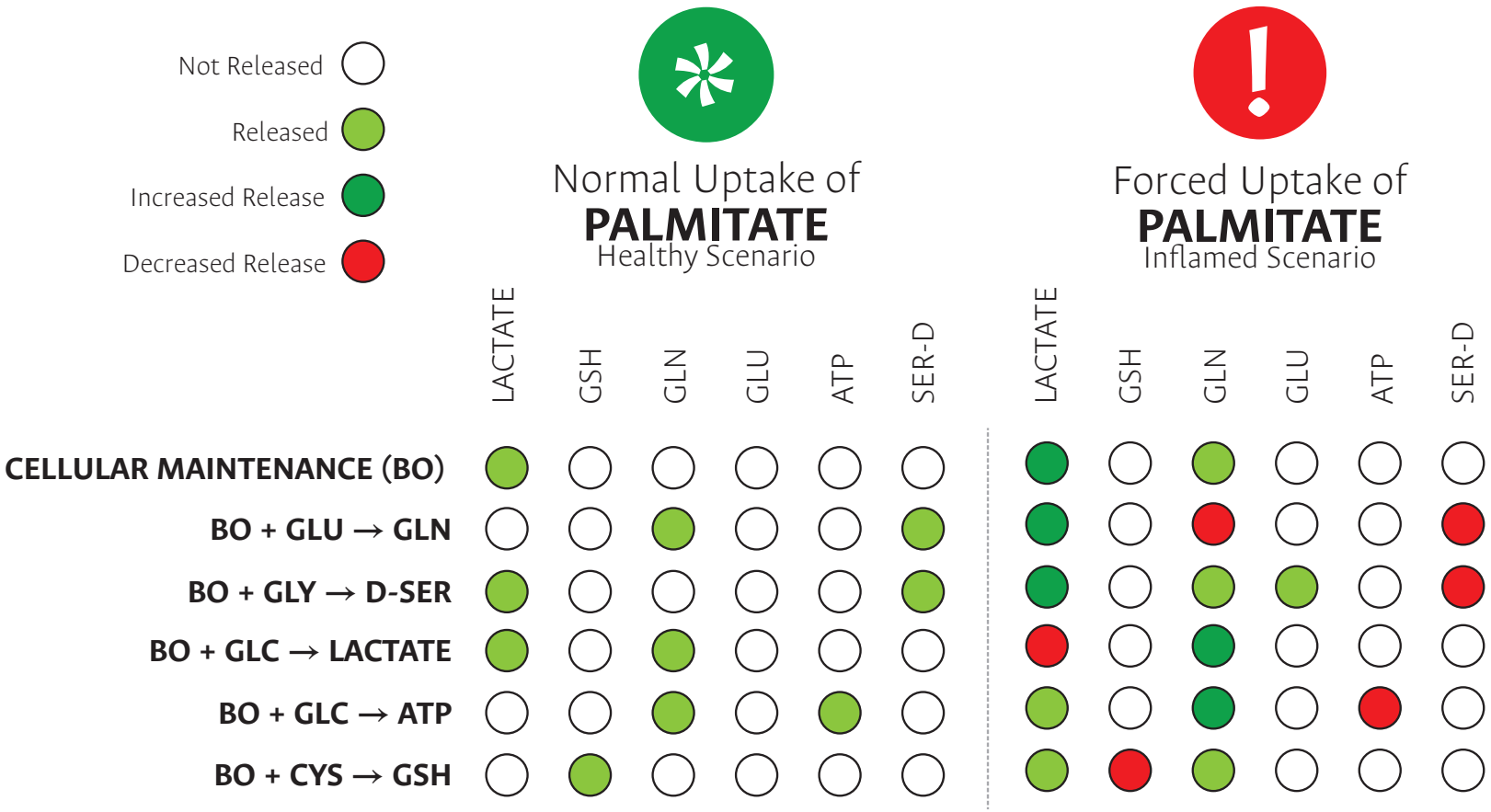


(3) Yu-Cun Niu *et al.* (2012). Histidine and arginine are associated with inflammation and oxidative stress in obese women.

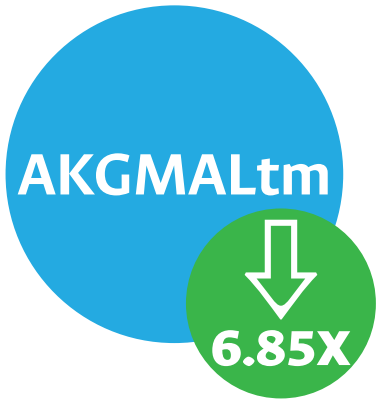
(4) Leonard T. Rael *et al.* (2004). An anti-inflammatory role for N-acetyl aspartate in stimulated human astroglial cells.

(5) D. R. Green *et al.* (2014). Metabolic control of cell death.

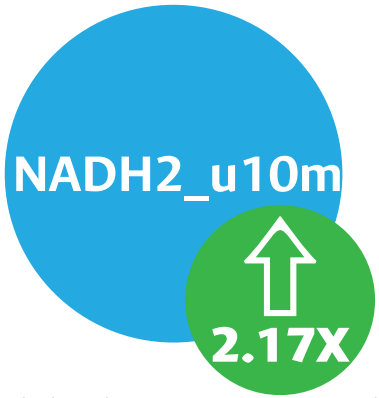
Gliotransmitters



Anti-inflammatory Enzymes: : Innate defence against inflammation



alpha-ketoglutarate/malate transporter



NADH dehydrogenase mitochondrial

MITOCHONDRIAL FATTY ACID ELONGATION

NADH + NADPH + 2H+ + Acetyl-COA + LongChainFattyAcyl-COA

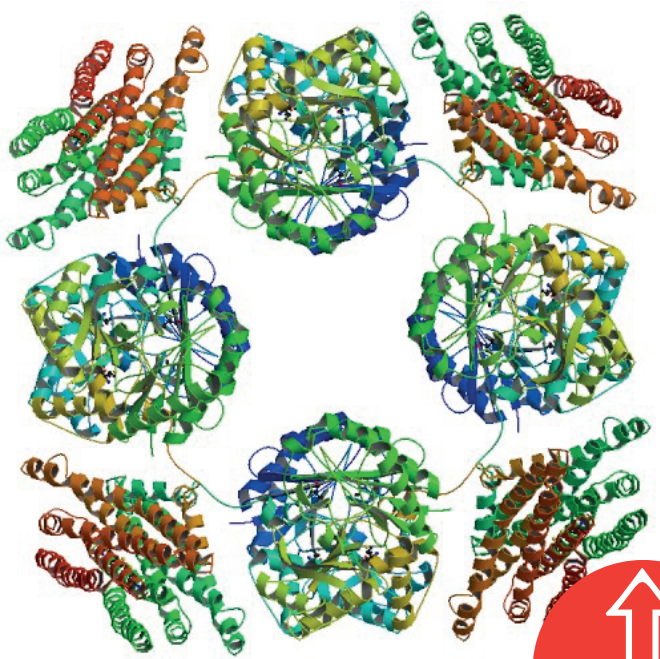
→

FattyAcil-COA Lengthened TwoCarbons

Pro-inflammatory Enzymes

FTCD

FormimidoylTransferase CycloDeaminase



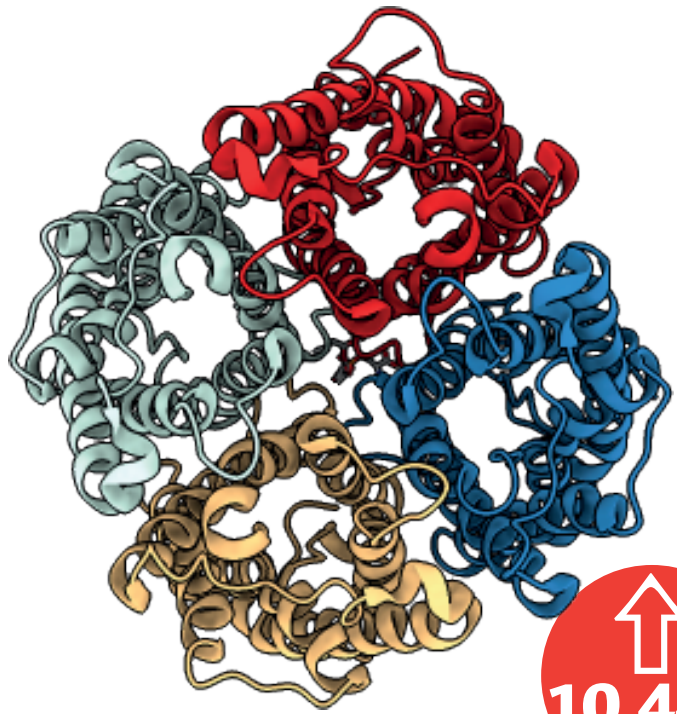
EC: 4.3.1.4 **EC:** 2.1.2.5

2 h[c] + 5forthf[c] ⇔ nh4[c] + methf[c]

2.28X
11.44%

H2Otm

H2O Transport Mitochondrial



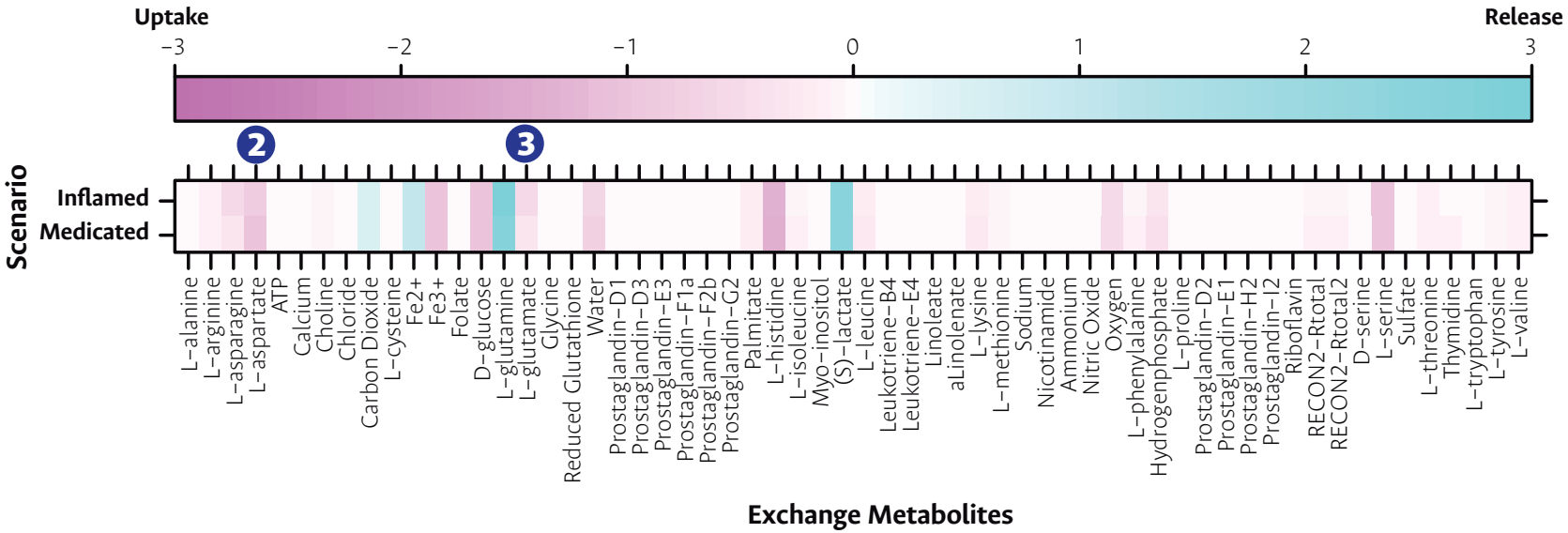
h2o[c] ⇔ h2o[m]

10.44X
5.14%

Cellular Maintenance: Medicated Scenario

1 OBJECTIVE FUNCTION: **0.41 mMgWD−1h−1** (+13.26% than Healthy Scenario)
DMEM Culture Medium + 0.208 mM Palmitate + 289 Tibolone associated reactions

METABOLISM ACTIVATION: **46.6%**



1 Kazuhiro Takuma, Akemichi Baba, and Toshio Matsuda. (2004). Astrocyte apoptosis: Implications for neuroprotection.

1 Graham A., *et al*. (1993). Hormone replacement therapy and risk of breast cancer: Results from epidemiologic studies.

1 Graham A., *et al*. (1995). The Use of Estrogens and Progestins and the Risk of Breast Cancer in Postmenopausal Women

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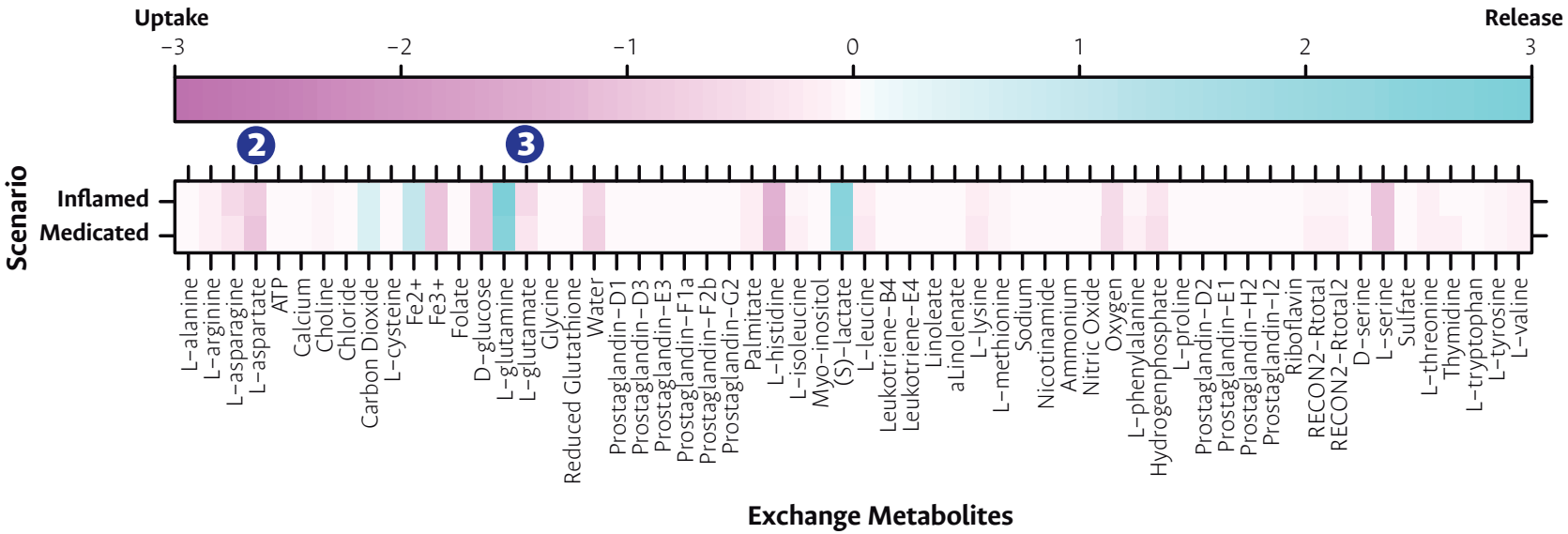

2 Leonard T. Rael *et al.* (2004). An anti-inflammatory role for N-acetyl aspartate in stimulated human astroglial cells.

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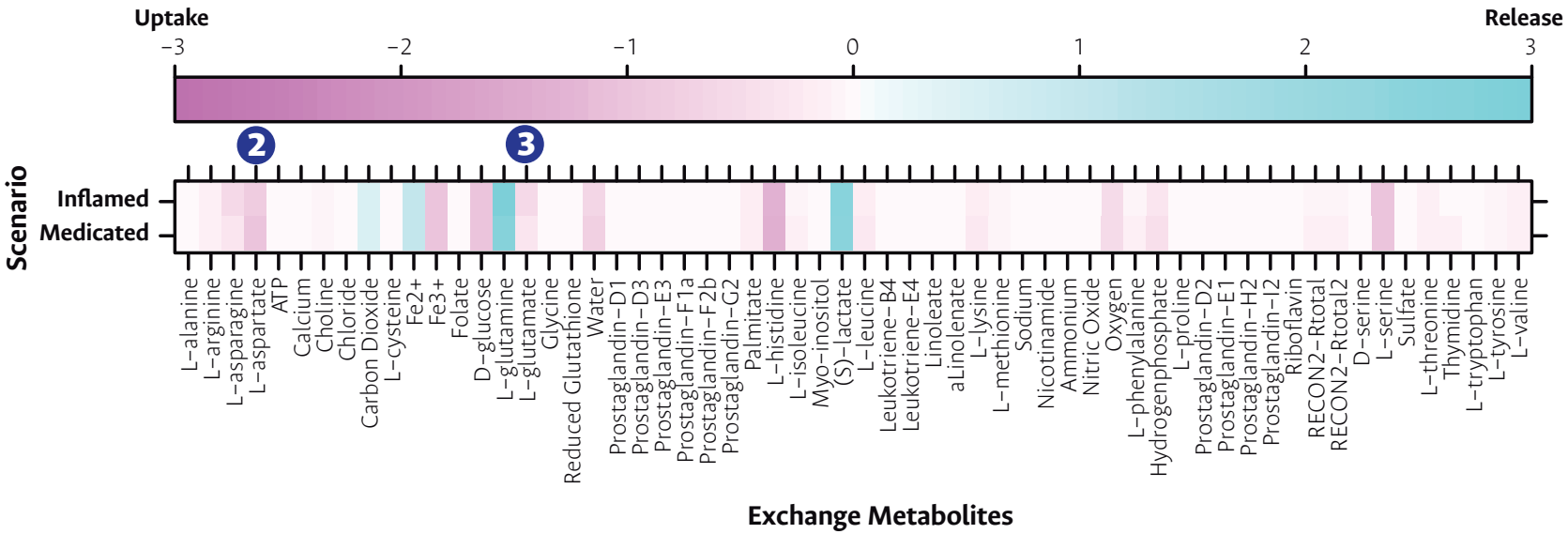
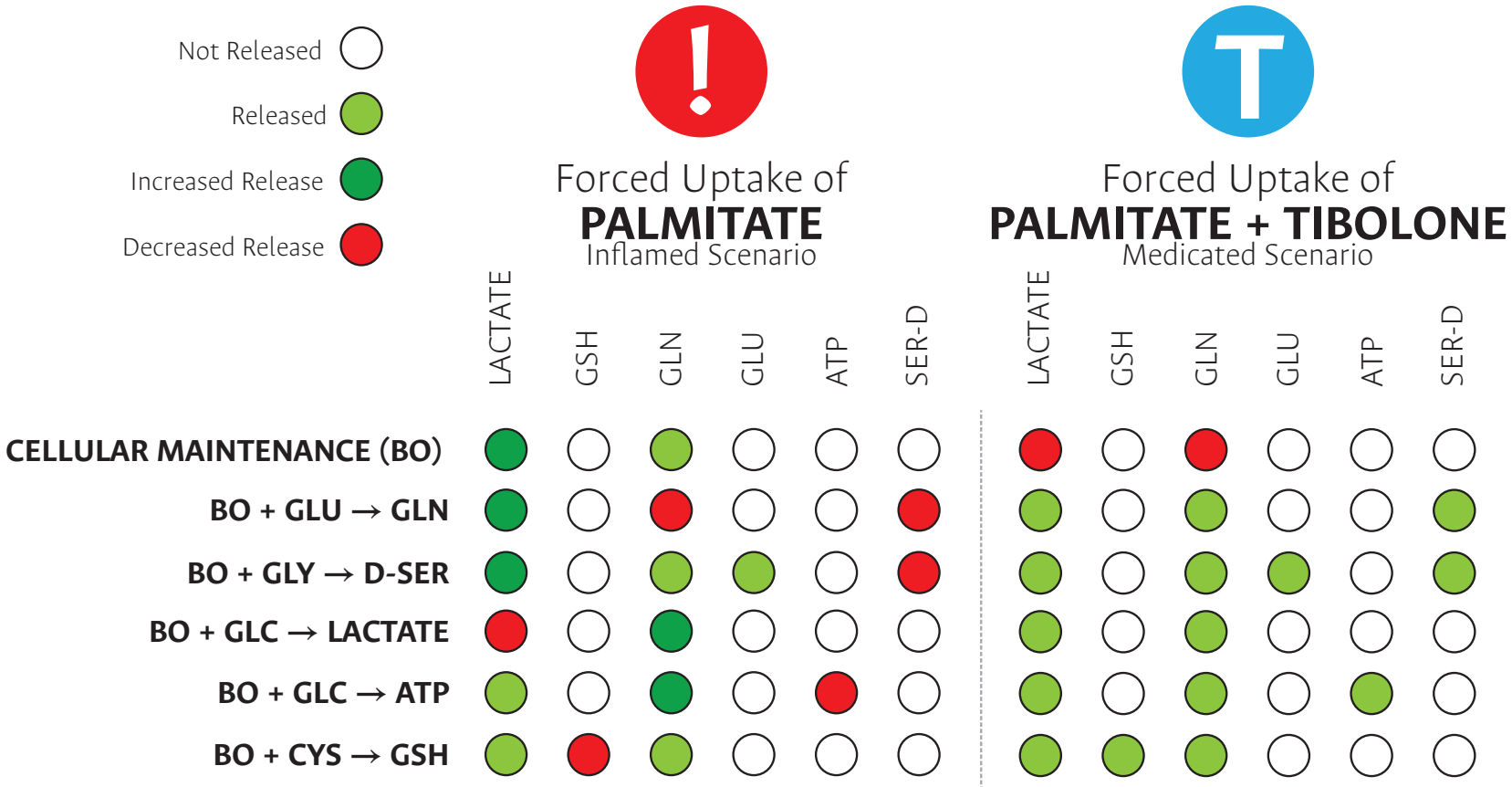


2 Leonard T. Rael *et al*. (2004). An anti-inflammatory role for N-acetyl aspartate in stimulated human astroglial cells.

3 Francesco Petrelli and Paola Bezzi. (2016). Novel insights into gliotransmitters.

3 Barbara Ahlemeyer *et al*. (2002). Increase in glutamate-induced neurotoxicity by activated astrocytes involves stimulation of protein kinase C.

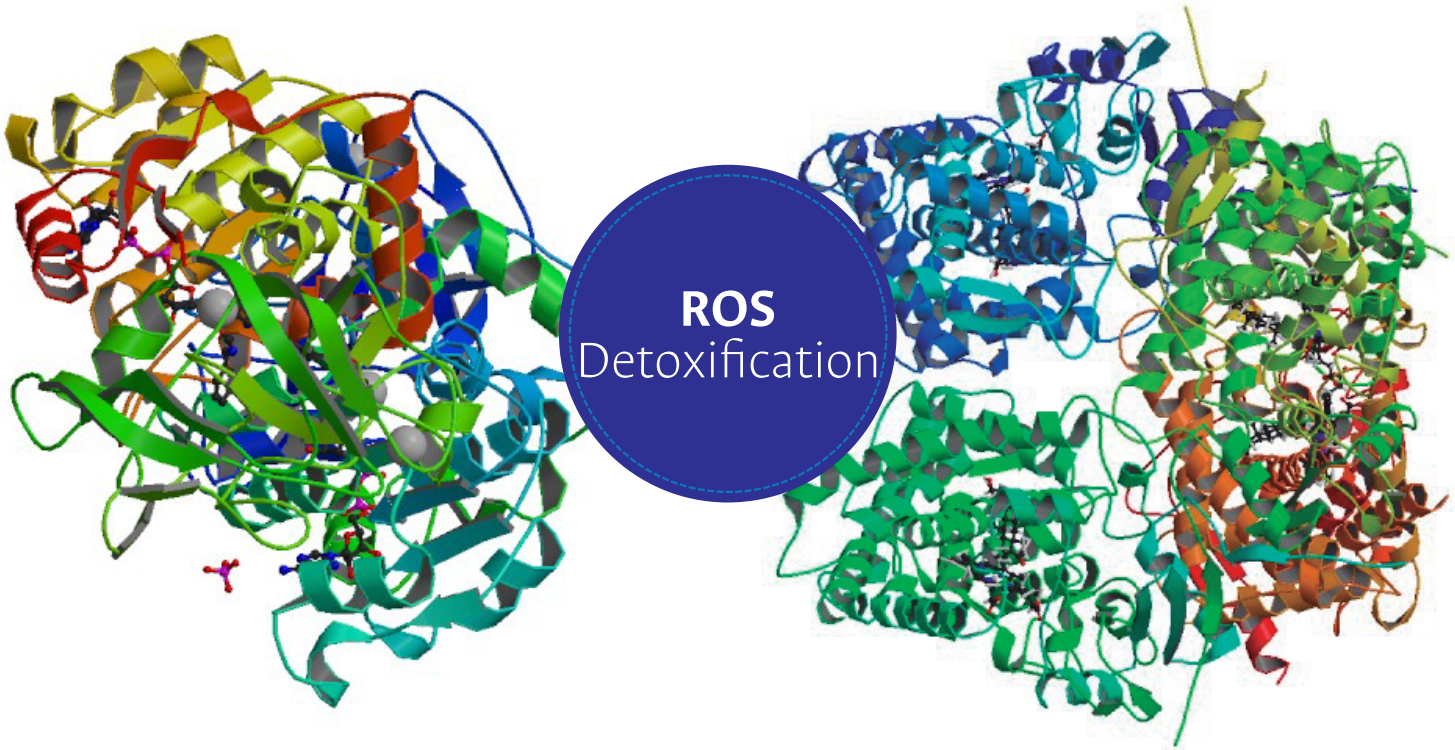
Gliotransmitters



Tibolone Related Enzymes

r0739
Alcohol Dehydrogenase 1 - 7

REM1804m | REM1807m
Cytochrome P450 Family 27 Subfamily A Member 1



EC: 1.1.1.1
nad[c] + xol7ah3[c] ⇔ h[c] + nadh[c] + xol7ah2al[c]

EC:1.14.15.15
nad[c] + xol7ah3[c] ⇔ h[c] + nadh[c] + xol7ah2al[c]

F. Sun *et al.* (2009). Inhibitory effect of osthole on alcohol-induced fatty liver in mice.

Conclusions

1. A tissue-specific metabolic reconstruction for mature astrocytes has been developed and on it, three different metabolic scenarios were modeled.
2. The metabolic model was capable of yielding results which were in correspondence to the experimentally proved metabolic processes.
3. Adverse effects associated with the increase of palmitate uptake were described based on exchange, metabolite production, and metabolic pathways perturbed under the inflammatory response.
4. Two possible reactions and their associated enzymes susceptible to be knocked out to reduce the metabolic inflammation were identified.

Conclusions

5. Based on literature reports a tibolone medicated scenario was modeled and used to identify and describe the neuroprotective effects of this synthetic neurosteroid under an inflamed scenario in mature astrocytes.
6. Our main results suggest that tibolone execute their neuroprotective effects through a reduction of neurotoxicity mediated by L-glutamate in mature astrocytes.
7. We found a tibolone associated increase in growth rate probably in concordance to previously reported side effects of steroids in other human cell types.

Published Software Packages

'g2f' Package
An R Package to Find and Fill Gaps for genome-scale metabolic networks
Kelly Botero, **Daniel Osorio**, *Janneth Gonzalez and Andres Pinzón-Velasco.*

Language: R
Stable: CRAN
Development: gibbslab/g2f
License: GPL-2
Binaries: Windows - Linux - Mac

'minval' Package
An R Package for MINimal VALidation of stoichiometric reactions
Daniel Osorio, *Janneth Gonzalez and Andres Pinzón-Velasco.*

Language: R
Stable: CRAN
Development: gibbslab/minval
License: GPL-2
Binaries: Windows - Linux - Mac

'exp2flux' Package
An R Package to convert expression data to FBA fluxes
Daniel Osorio, *Kelly Botero, Janneth Gonzalez and Andres Pinzón-Velasco.*

Language: R
Stable: CRAN
Development: gibbslab/exp2flux
License: GPL-2
Binaries: Windows - Linux - Mac

This work is a set of four manuscripts

ACCEPTED WITH CORRECTIONS Osorio, D., Gonzalez, J., and Pinzón-Velasco, A. **minval: An R package for MINimal VALidation of stoichiometric reactions.** *The R Journal*.

IN PREPARATION Osorio, D., Botero, K., Gonzalez, J., and Pinzón-Velasco, A. **exp2flux: An R package to convert expression data to FBA fluxes.** To be submitted to *The R Journal*

IN PREPARATION Osorio, D., Gonzalez, J., and Pinzón-Velasco, A. **Exploring the neuroprotective effects of tibolone during astrocytic metabolic inflammation: a flux balance analysis approach.** To be submitted to *Medical Hypotheses*

IN PREPARATION Botero, K., Osorio, D., Gonzalez, J., and Pinzón-Velasco, A. **g2f: An R packafe to find and fill gaps for genome-scale metabolic networks.** To be submitted to *The R Journal*

Advances of this work were presented as:

Metabolic inflammation effects over the gliotransmitters release in mature astrocytes: a network-based approach.

Daniel Osorio MSc., Janneth Gonzalez PhD., Andrés Pinzón-Velasco PhD.
Bioinformatics and Computational Systems Biology Lab, Universidad Nacional de Colombia.

at:

CDMX, México
Short Talk

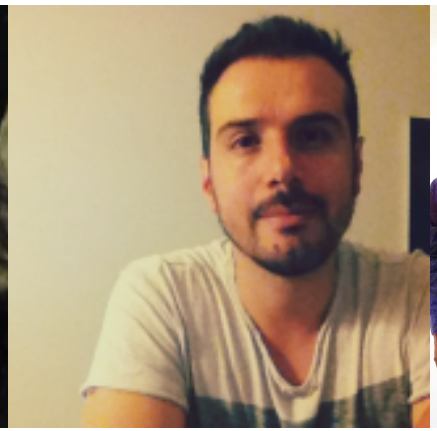
Barcelona, España
Poster

Bogotá, Colombia
Short Talk

Acknowledgements

UNIVERSIDAD NACIONAL DE COLOMBIA

Pontificia Universidad JAVERIANA Bogotá

Thanks to be here!

This study was developed at the:

Bioinformatics and Computational Systems Biology Lab
Institute for Genetics - Universidad Nacional de Colombia

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Andrés Pinzón PhD
ampinzonv@unal.edu.co