

# 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**

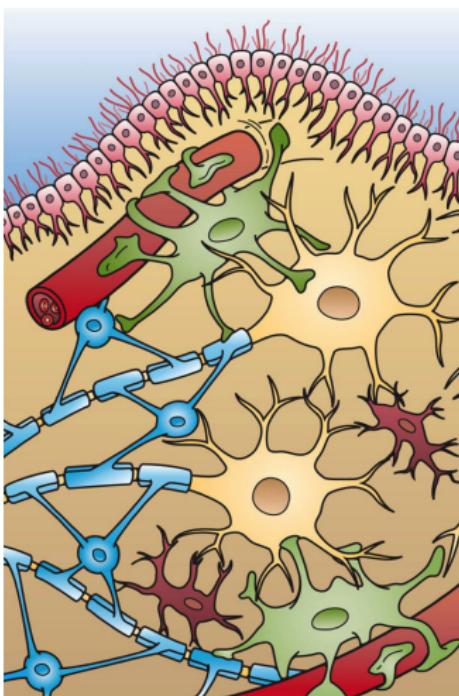
in partial fulfillment of requirements for the degree of  
**Master in Bioinformatics**

Advisors: **Janneth Gonzalez PhD.** and **Andrés Pinzón PhD.**  
Bioinformatics and Computational Systems Biology Lab



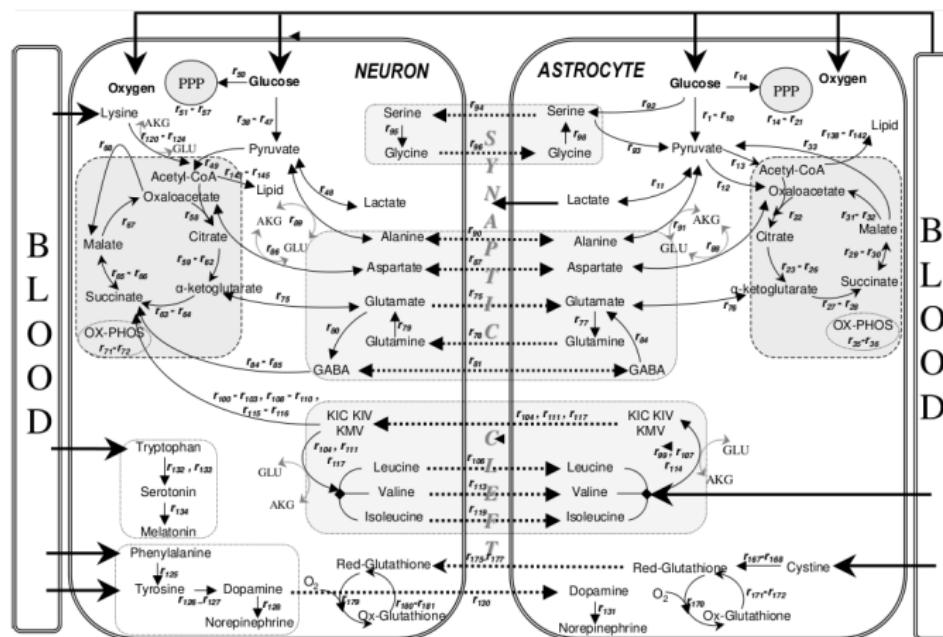
**Universidad Nacional de Colombia**  
**Engineering Faculty - Department of Systems and Industrial Engineering**  
**Bogotá, Colombia**

# CNS: Central Nervous System



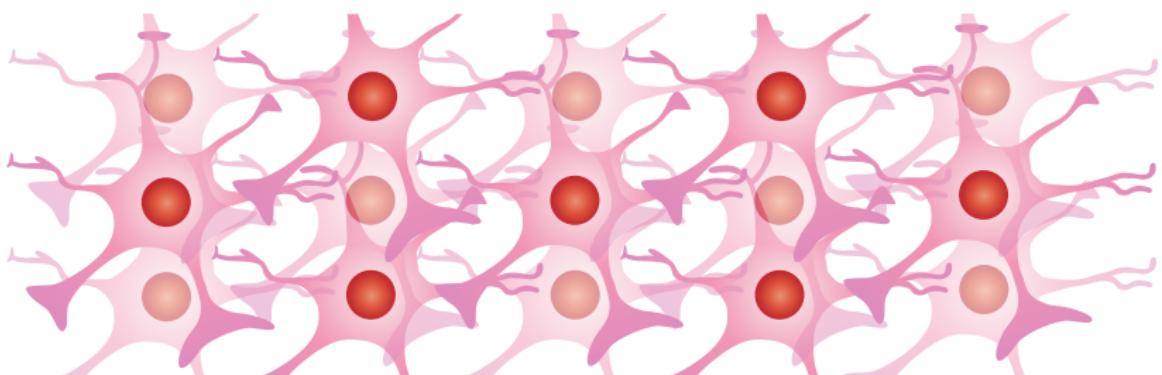
© Holly Fischer artwork.

# Astrocyte - Neuron Metabolic Relationship



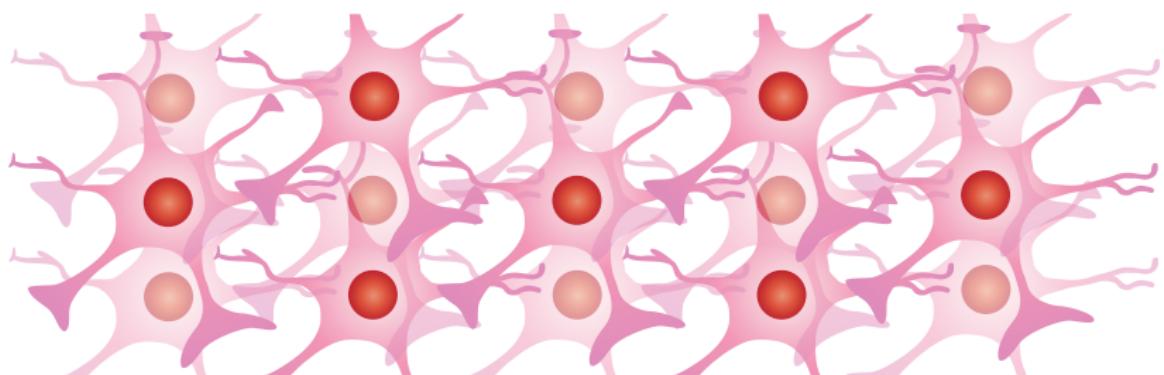
**Figure from:** Çakir, Tunahan *et al.*, (2007). Reconstruction and flux analysis of coupling between metabolic pathways of astrocytes and neurons: application to cerebral hypoxia.

# Astrocytes Metabolic Functions



$K^+$  Membrane Potential

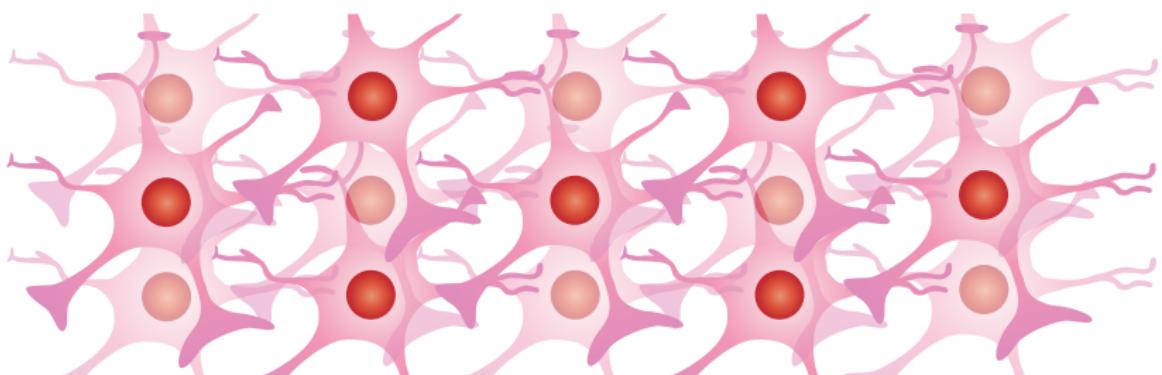
# Astrocytes Metabolic Functions



$K^+$  Membrane Potential

$Ca^{+2}$  signaling

# Astrocytes Metabolic Functions

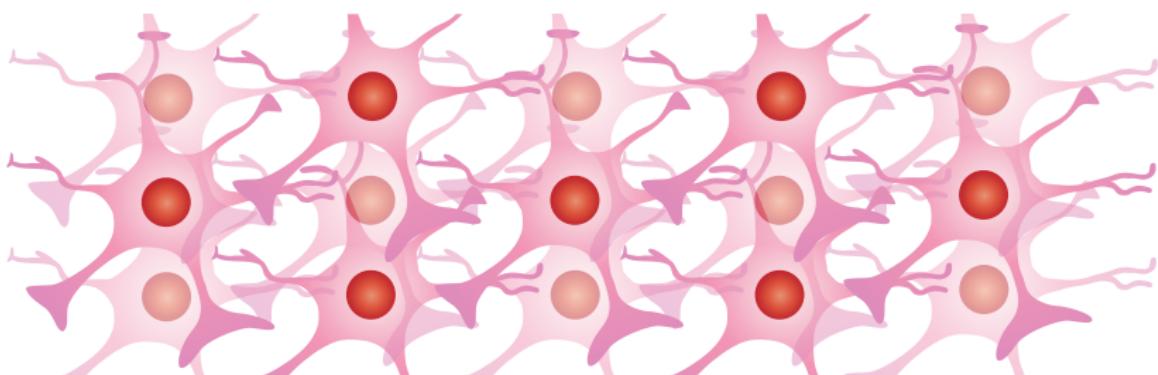


**K<sup>+</sup> Membrane Potential**

**Ca<sup>+2</sup> signaling**

**Lactate release**

# Astrocytes Metabolic Functions



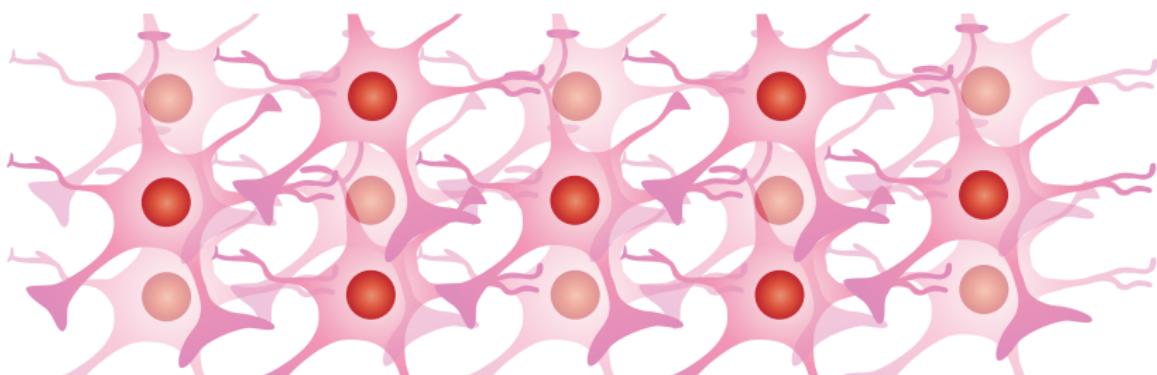
**K<sup>+</sup> Membrane Potential**

**Ca<sup>+2</sup> signaling**

**Lactate release**

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

# Astrocytes Metabolic Functions



**K<sup>+</sup> Membrane Potential**

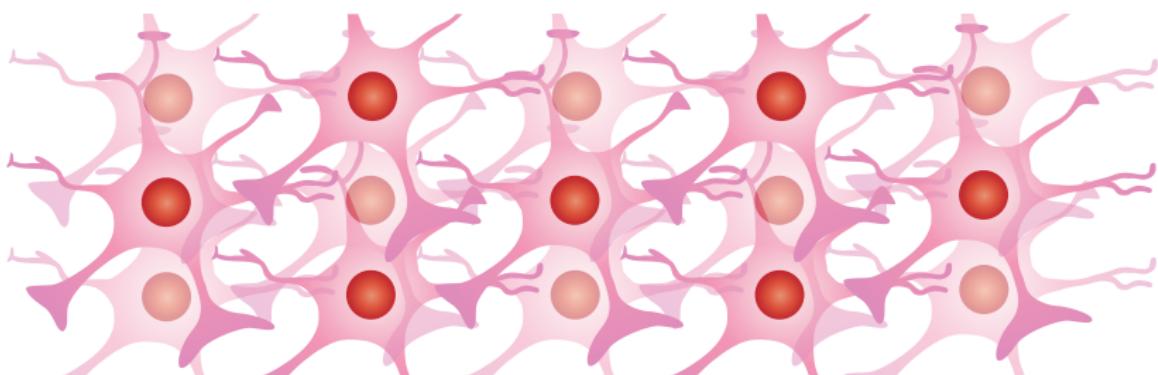
**Ca<sup>+2</sup> signaling**

**Lactate release**

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

**pH maintenance**

# Astrocytes Metabolic Functions



**K<sup>+</sup>** Membrane Potential

**Ca<sup>+2</sup>** signaling

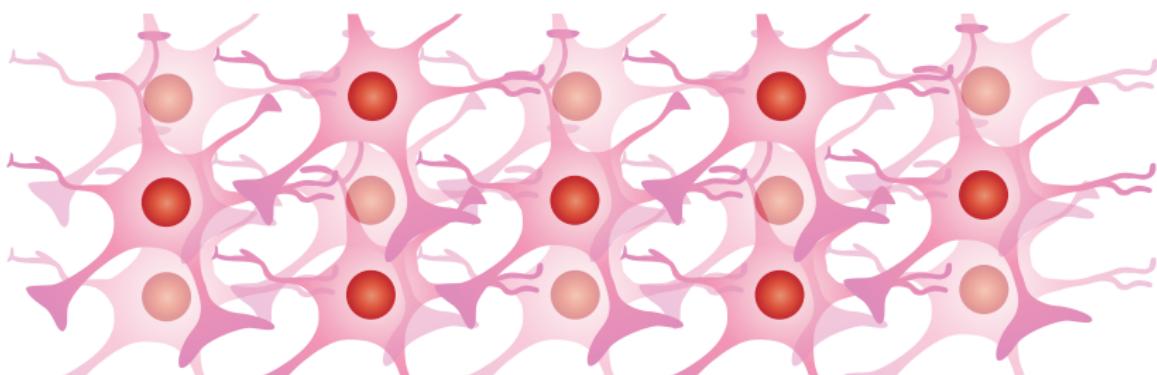
**Lactate** release

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

**pH** maintenance

**ROS** detox

# Astrocytes Metabolic Functions



**K<sup>+</sup>** Membrane Potential

**Ca<sup>+2</sup>** signaling

**Lactate** release

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

**pH** maintenance

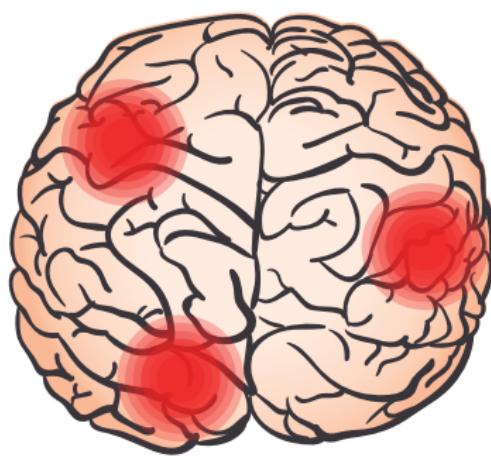
**ROS** detox

**Gln, ATP** and **D-serine** release

## What is inflammation about?

protective redundant response  
interconnected robustness metabolic  
immune pathways fragility  
**Inflammation**  
nonintuitive behaviors complex system  
conserved sensible to initial conditions  
negative feedback positive

# CNS inflammation



Neurodegenerative diseases

Cardiovascular events

Stress

Smoke

Obesity (Over Nutrition or Caloric Excess)

# Metabolic Inflammation or Metainflammation



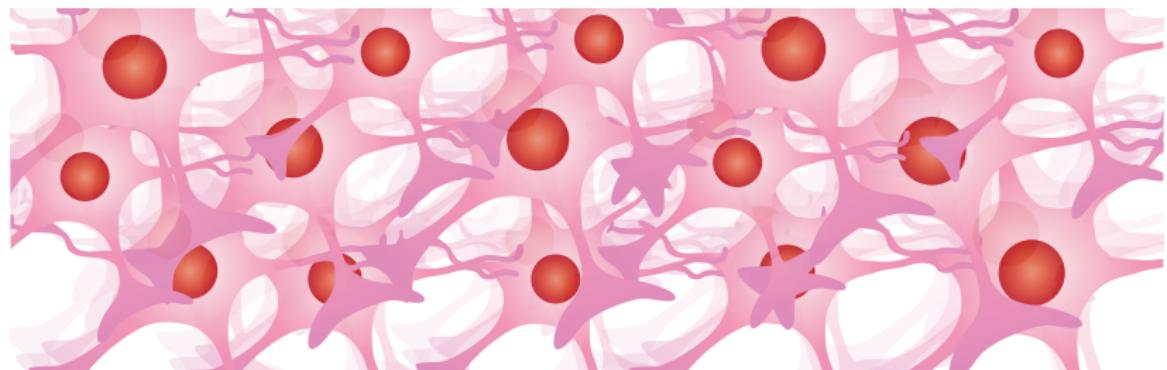
# Metabolic Inflammation or Metainflammation

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

▲ Endoplasmic reticulum stress → ▲ UPR

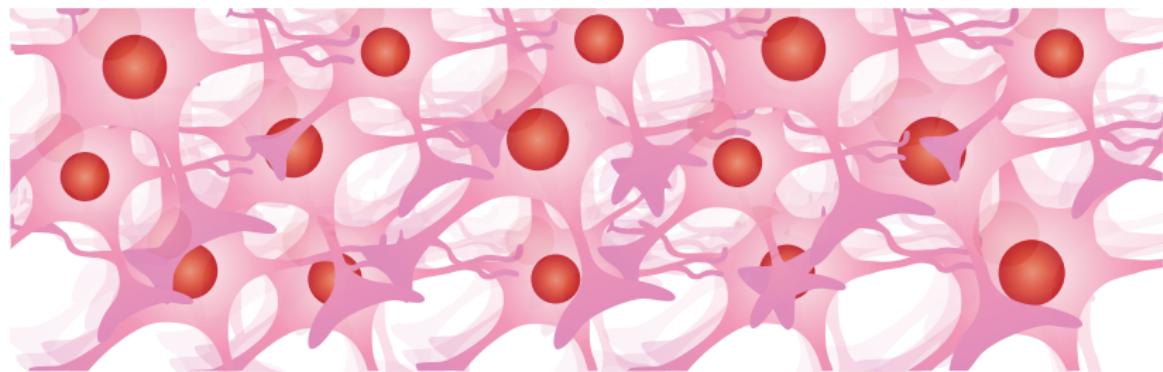
▲ Reactive C Protein Ligands + ▲ TNF $\alpha$  + ▲ IL6 + ▲ ROS

# Astrogliosis or Reactive Astrocytosis



**ROS generator**

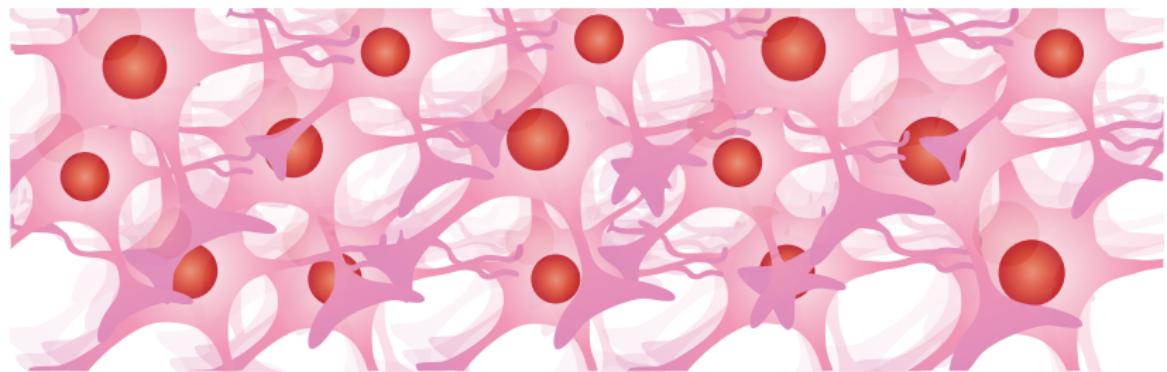
# Astrogliosis or Reactive Astrocytosis



**ROS generator**

**Mitochondrial Failure**

# Astrogliosis or Reactive Astrocytosis

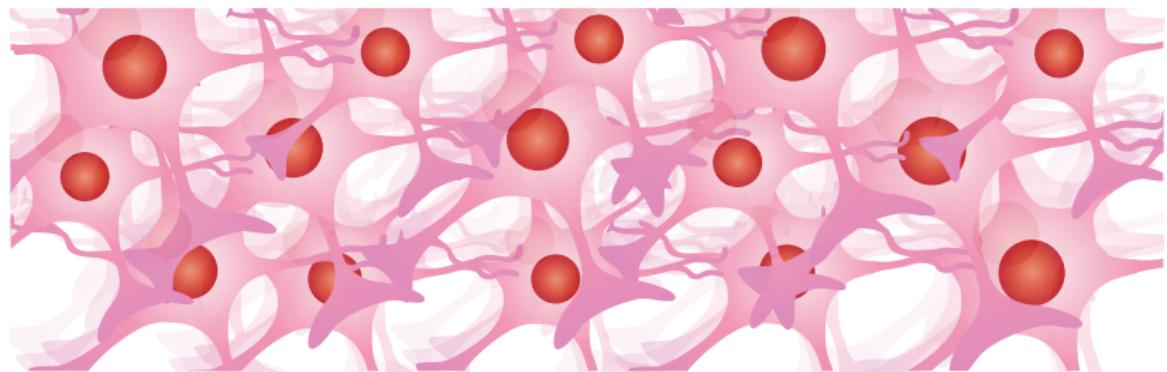


**ROS generator**

**Mitochondrial Failure**

**$\text{Ca}^{+2}$  disregulation**

# Astrogliosis or Reactive Astrocytosis



ROS generator

Mitochondrial Failure

$\text{Ca}^{+2}$  disregulation

Free Fatty Acids (Palmitate) Increase

# Inflammation Treatment

# Tibolone

# Translational Systems Biology

# Metabolism Simulation: Flux Balance Analysis (FBA)

# 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.

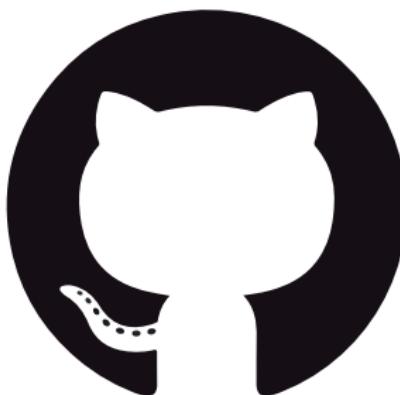
# Reproducibility

ALL ANALYSIS WERE  
PERFORMED USING



VERSION: 3.3.1

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



GITHUB: [dosorio/masterThesis](#)

FBA: **SYBIL** (Gelius-Dietrich, G. et al., 2013)

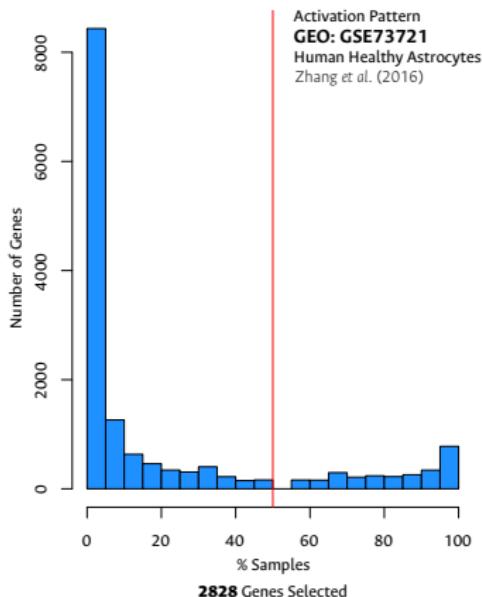
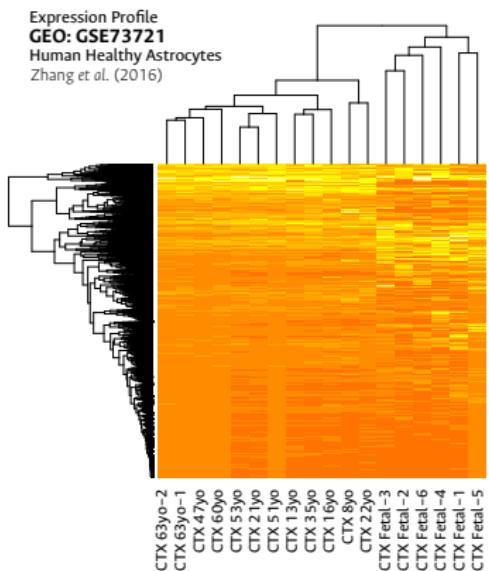
LP SOLVER: **GLPK 4.6**

LICENSE: **GNU GPL3**

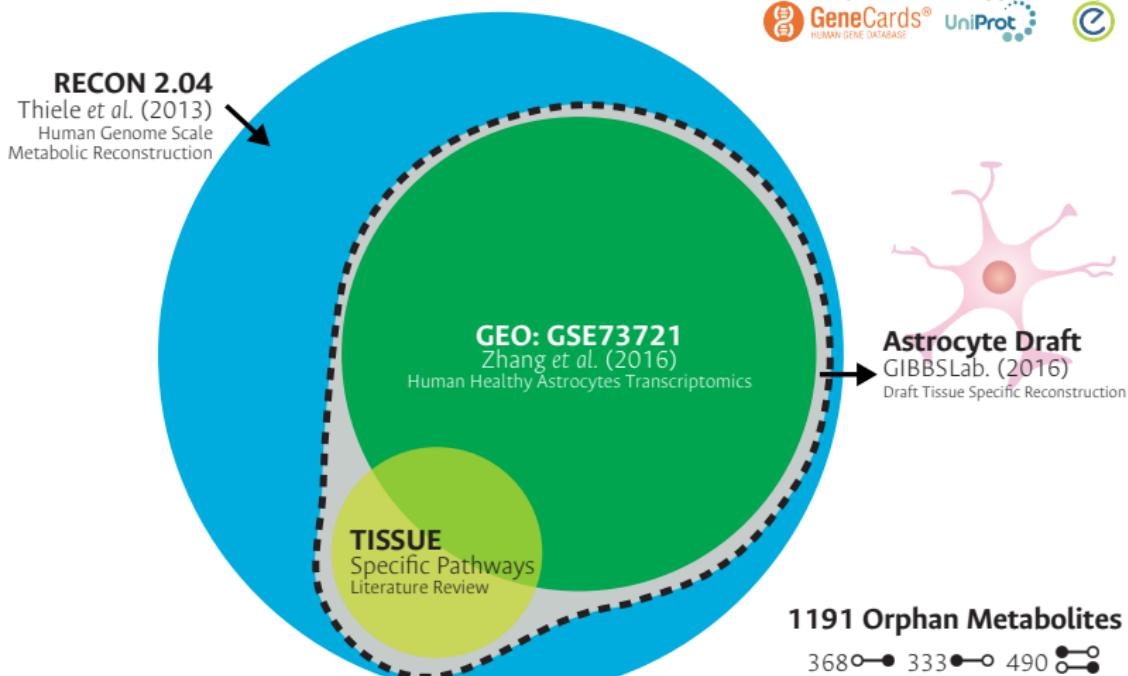
## OBJECTIVE 1:

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

# Healthy Human Astrocytes Gene Expression Data



# Mapping Reactions



# Gap-Find and Gap-Fill Available Algorithms

ALGORITHM	ENVIRONMENT	HOW IT WORKS
SMILEY	Python - OpenSource	<ul style="list-style-type: none"><li>Optimization based.</li><li><b>Fills one metabolite per time.</b></li></ul>
gap-Find/Fill	GAMS - OpenSource	<ul style="list-style-type: none"><li>Optimization based.</li><li><b>Makes several intra model modifications.</b></li></ul>
growMatch	Python - OpenSource	<ul style="list-style-type: none"><li>Optimization based.</li><li><b>Fills one objective function per time.</b></li></ul>
fastGapFill	MATLAB - Privative	<ul style="list-style-type: none"><li>Optimization based.</li><li>Multiobjective.</li></ul>

# 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

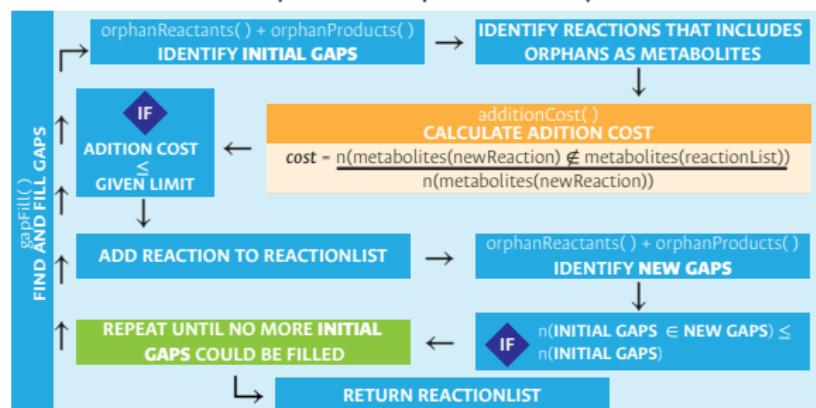
$\text{h2o[r]} + \text{dheas[r]} \Rightarrow \text{h[r]} + \text{dhea[r]} + \text{so4[r]}$   
 $\text{uri[e]} \Leftrightarrow \text{uri[c]}$   
 $\text{na1[e]} + \text{uri[e]} \Rightarrow \text{na1[c]} + \text{uri[c]}$   
 $\text{atp[c]} + \text{pi[m]} \Rightarrow \text{pi[c]} + \text{atp[m]}$   
 $\text{na1[e]} + \text{gchola[e]} \Rightarrow \text{na1[c]} + \text{gchola[c]}$   
**ASTROCYTE DRAFT** GiBBS Lab (2016)

**R**EACTI**L**IST



getReference()  
 DOWNLOAD  
 STOICHIOMETRIC REACTIONS  
 FROM THE KEGG DATABASE

**R**EFERENC**E**



# 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.

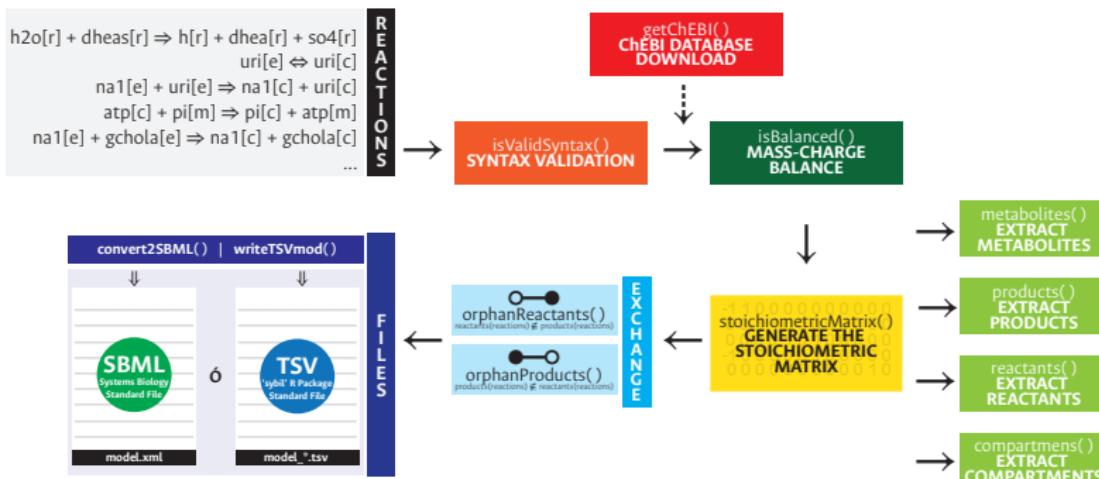
Language: R

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.

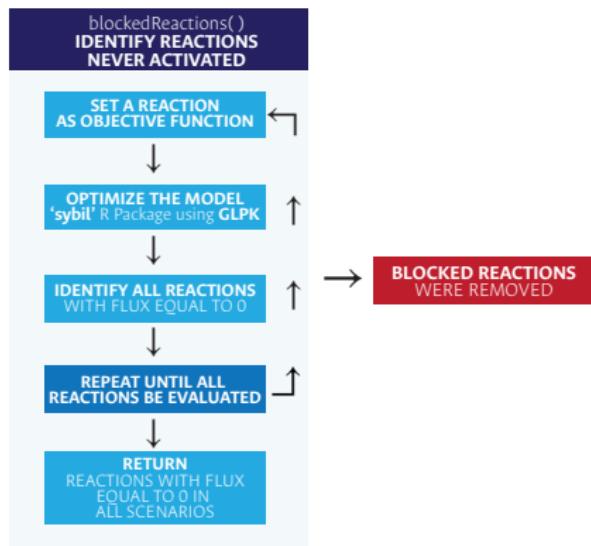
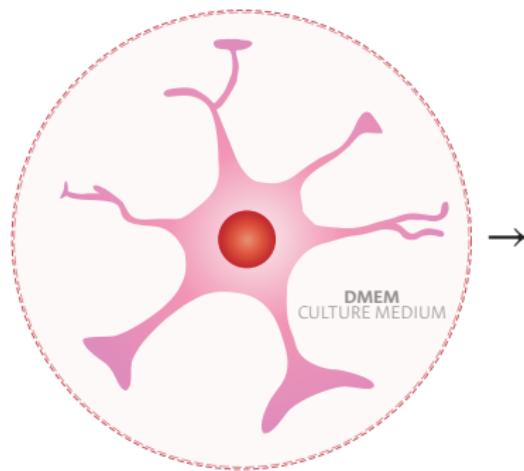
Language: R

Stable: CRAN

Development: [gibbslab/g2f](#)

License: [GPL-2](#)

Binaries: Windows - Linux - Mac



# Gene Expression Integration Available Methods

METHOD	ENVIRONMENT	HOW IT WORKS
GIMME	MATLAB <b>Privative</b>	<ul style="list-style-type: none"><li>· Binary Discretization</li><li>· Ensures flux for a selected objective function</li></ul>
iMAT	MATLAB <b>Privative</b>	<ul style="list-style-type: none"><li>· Integration proportional to gene-expression (H, M and L categorization)</li><li>· Not objective function required</li></ul>
E-FLUX	<b>Not implemented</b>	<ul style="list-style-type: none"><li>· Requires a user-given threshold</li><li>· Continuous Integration</li></ul>
PROM	MATLAB <b>Privative</b>	<ul style="list-style-type: none"><li>· Requires a user-given regulatory network</li><li>· Constraints are setting according to the associated transcript. factor</li></ul>

# 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

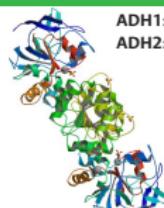
E.C: 1.1.1.1



1.1.1.1

ADH2

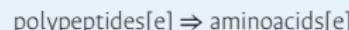
ADH1



(ADH2 or ADH1)

sum (ExprADH2 + ExprADH1)

E.C: 3.4.21.5



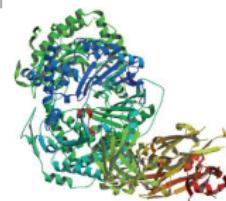
3.4.21.5

IDE.A

IDE.B

IDE.C

IDE.A: IDE.B: IDE.C:



GPR

(IDE.A and IDE.B and IDE.C)

min (ExprIDE.A, ExprIDE.B, ExprIDE.C)

GENE EXPRESSION  
DATA



exp2flux()  
CONVERT GENE  
EXPRESSION DATA  
TO FBA FLUXES



CONSTRAINED  
METABOLIC MODEL



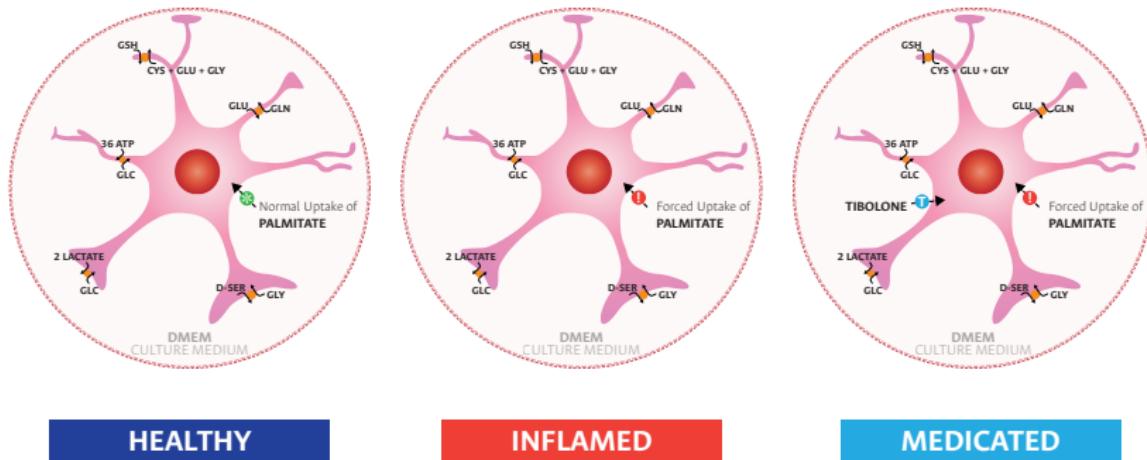
fluxDifferences()  
COMPUTE FOLDCHANGE  
OF FLUXES BETWEEN  
METABOLIC SCENARIOS

METABOLIC MODEL  
WITH GPR

## OBJECTIVE 2:

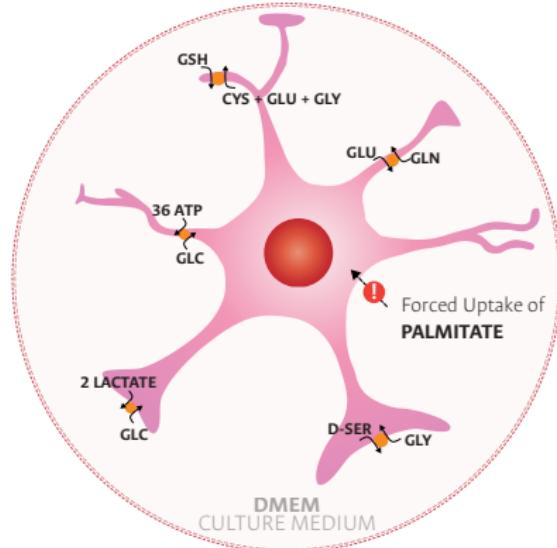
Evaluate the effects caused by the increase of free fatty acids and tibolone presence in astrocytes metabolism.

# Metabolic Scenarios



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

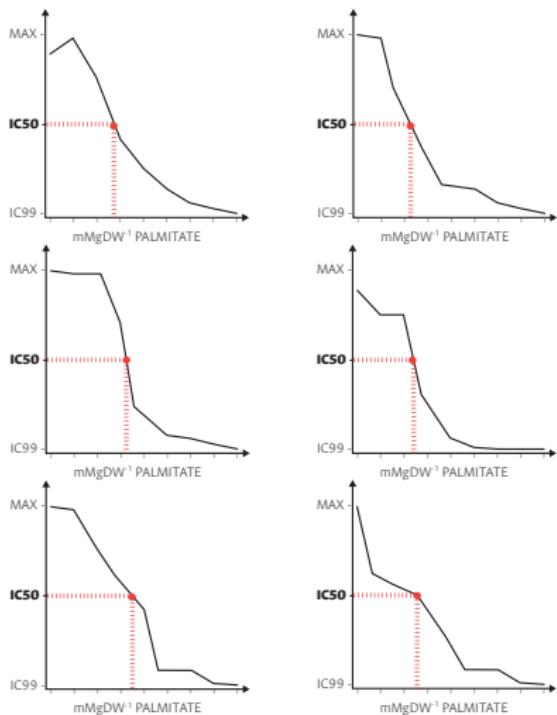
# Inflamed Metabolic Scenario



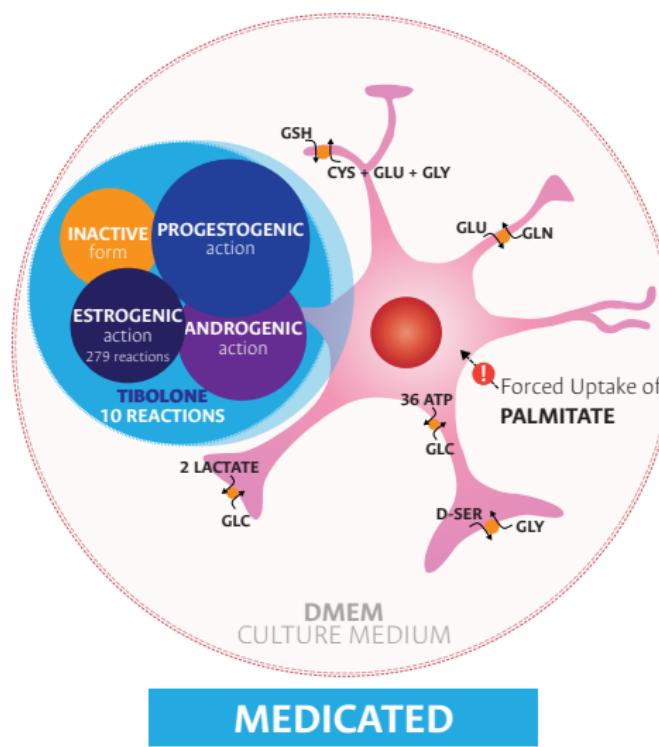
PALMITATE UPTAKE RATE: AVERAGED IC<sub>50</sub> VALUES

**INFLAMED**

## ROBUSTNESS ANALYSIS



# Medicated Metabolic Scenario

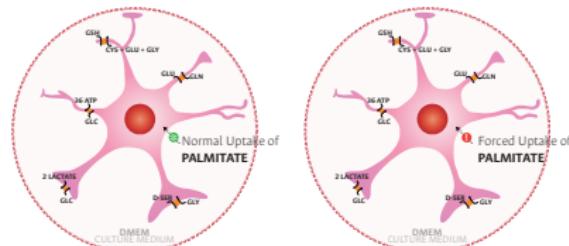


## OBJECTIVE 3:

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

# Metabolic Pathways Activation Pattern Changes

## INFLAMMATION CHANGES

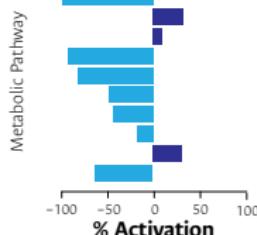


HEALTHY

INFLAMED

```
fluxDifferences()
COMPUTE FoldChange
OF FLUXES BETWEEN
METABOLIC SCENARIOS
```

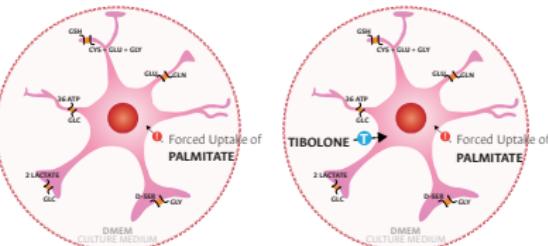
Metabolic Changes



$$\text{foldChange} = \frac{\text{FluxModel2} - \text{FluxModel1}}{|\text{FluxModel1}|}$$



## TIBOLONE CHANGES

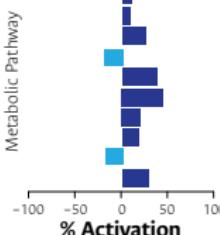


INFLAMED

MEDICATED

```
fluxDifferences()
COMPUTE FoldChange
OF FLUXES BETWEEN
METABOLIC SCENARIOS
```

Metabolic Changes

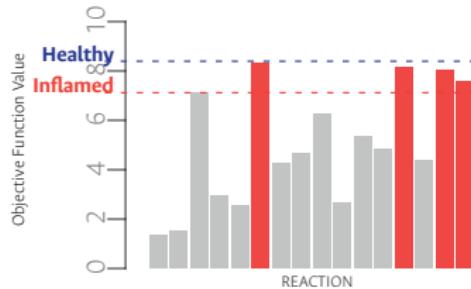


## OBJECTIVE 4:

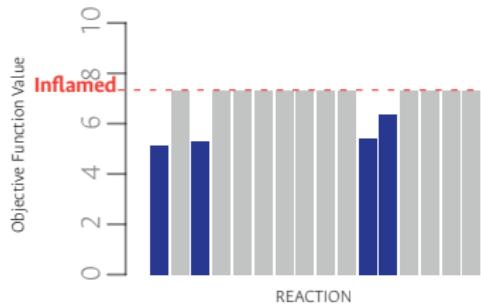
Evaluate the importance of proteins and metabolic pathways previously identified on the dynamics of the metabolic model.

# Reaction Knock-out: Protein Importance

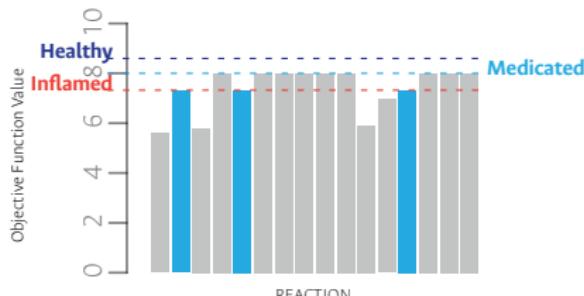
## Pro-Inflammatory



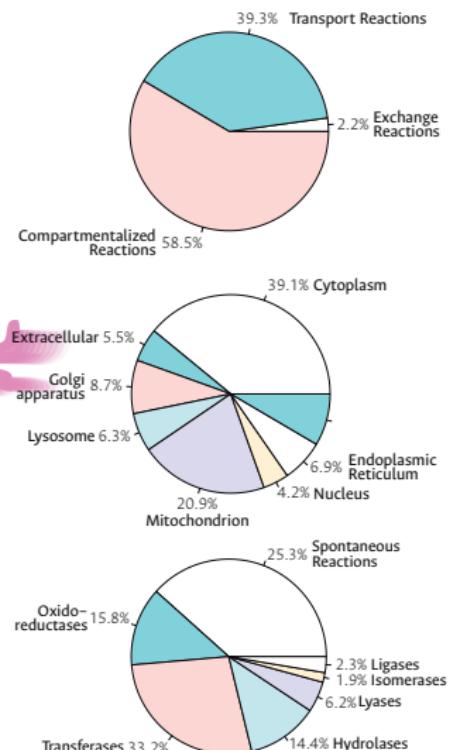
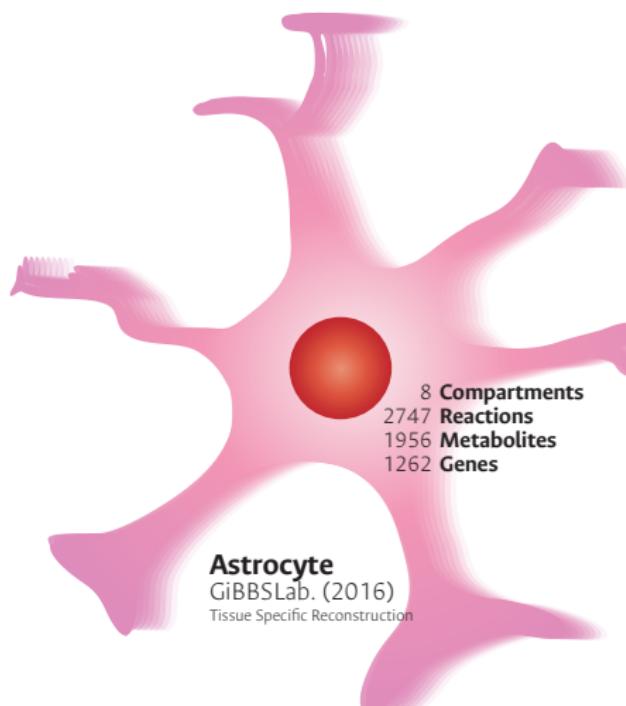
## Anti-Inflammatory



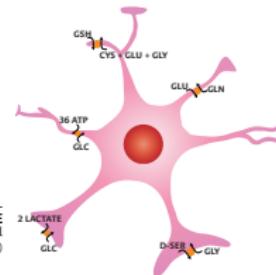
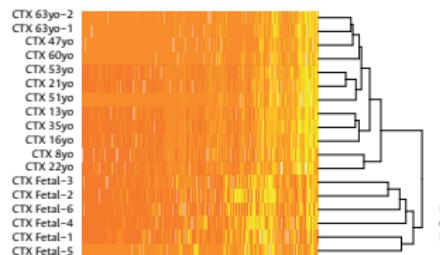
## Tibolone Mechanism



# Tissue Specific Metabolic Reconstruction



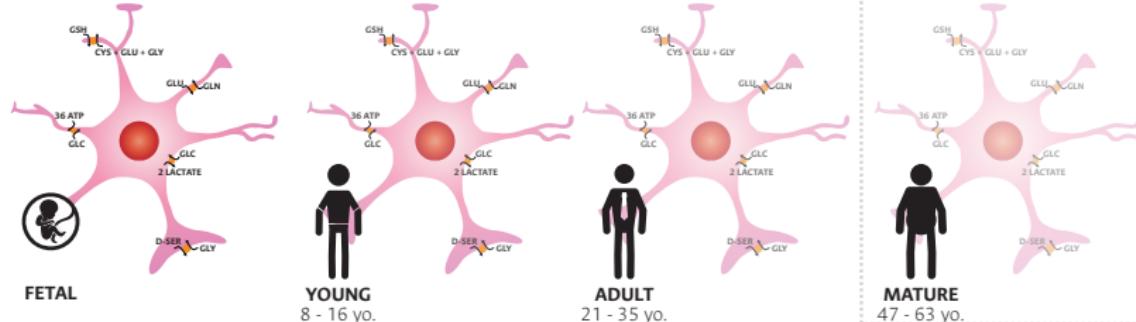
# Human Healthy Mature Astrocyte Model



**'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

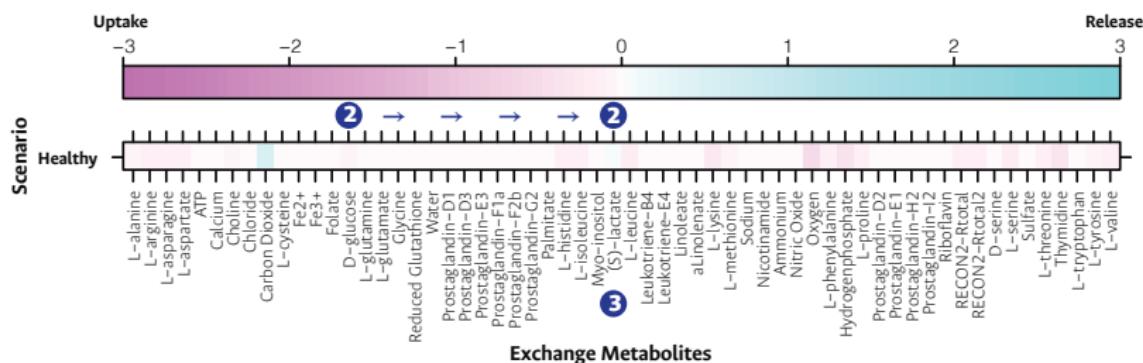


# Cellular Maintenance: Healthy Scenario

**1** OBJECTIVE FUNCTION: **0.37 mMgWD-1h-1**

DMEM Culture Medium

METABOLISM ACTIVATION: **52%**



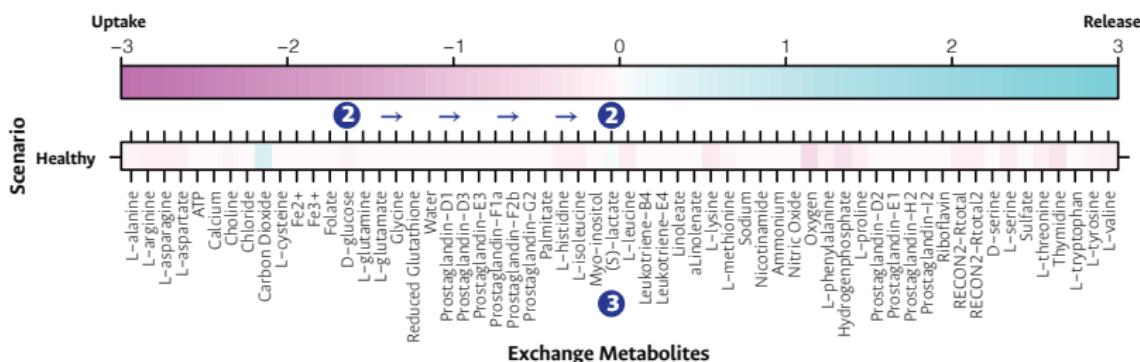
**1** Arabinda Das, et al. (2010). Flavonoids activated caspases for apoptosis in human glioblastoma T98G and U87MG cells but not in human normal astrocytes.

# 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.

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

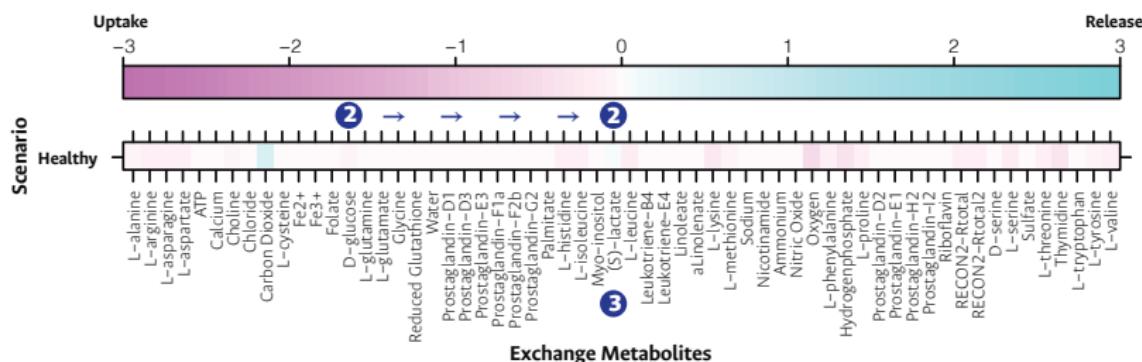
② Rupa Bhowmick, *et al.* (2015). Exploring the differences in metabolic behavior of astrocyte and glioblastoma: a flux balance analysis approach.

# Cellular Maintenance: Healthy Scenario

① OBJECTIVE FUNCTION: **0.37 mMgWD-1h-1**

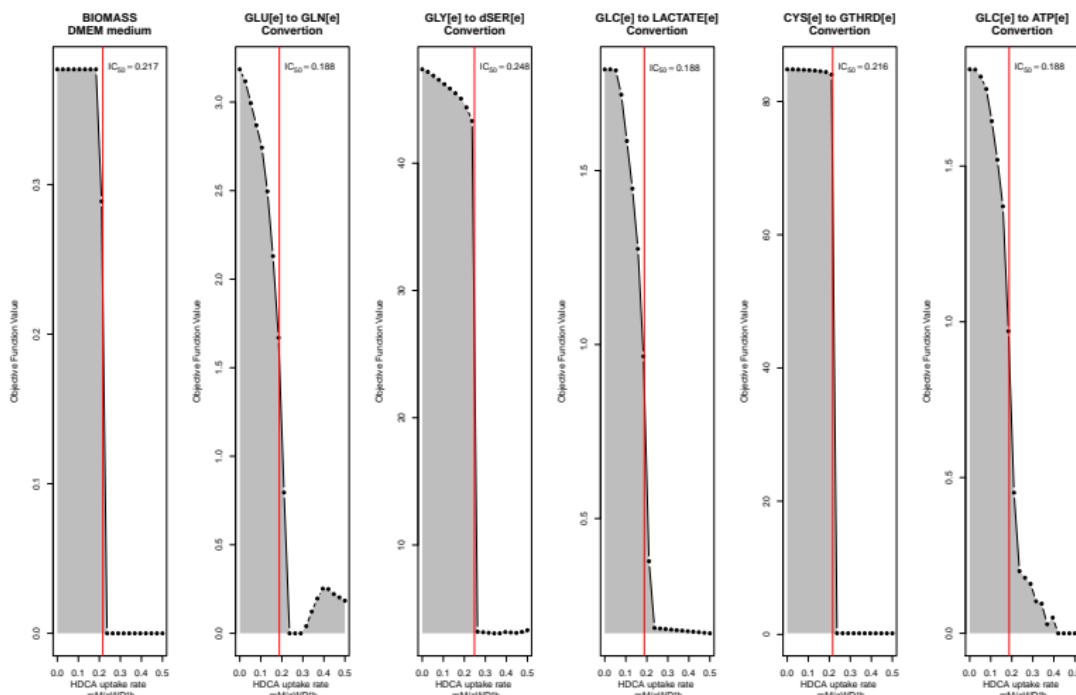
DMEM Culture Medium

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.
- ② Tunahan Cakir *et al.* (2007). Reconstruction and flux analysis of coupling between metabolic pathways of astrocytes and neurons: application to cerebral hypoxia.
- ② Rupa Bhowmick, *et al.* (2015). Exploring the differences in metabolic behavior of astrocyte and glioblastoma: a flux balance analysis approach.
- ③ Christelle Le Foll, *et al.* (2010). Fatty acid-induced astrocyte ketone production and the control of food intake.

# Averaged IC<sub>50</sub>: $0.208 \pm 0.024 \text{ mMgDW}^{-1}\text{h}^{-1}$



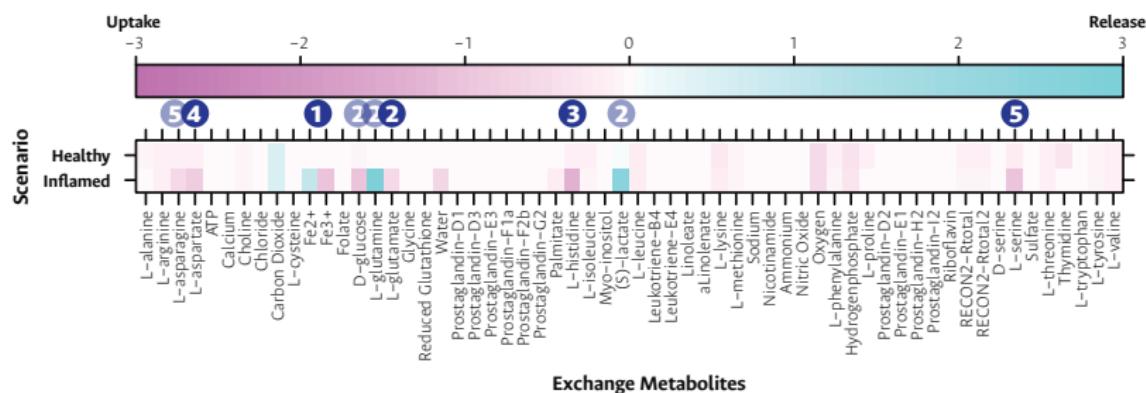
Li Liu et al. (2013) Palmitate-activated astrocytes via serine palmitoyltransferase increase BACE1 in primary neurons by sphingomyelinases.

# 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%)



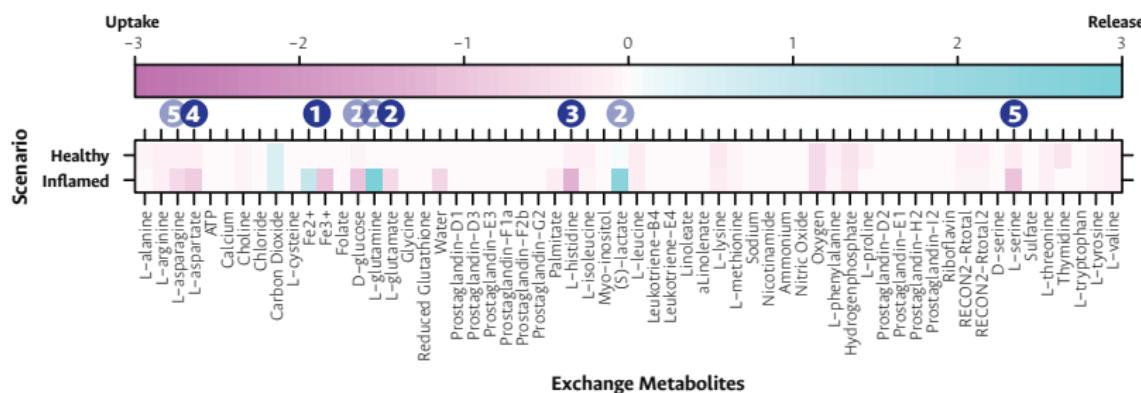
- ① Rachel Williams et al. (2012). Pathogenic implications of iron accumulation in multiple sclerosis.

# 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%)



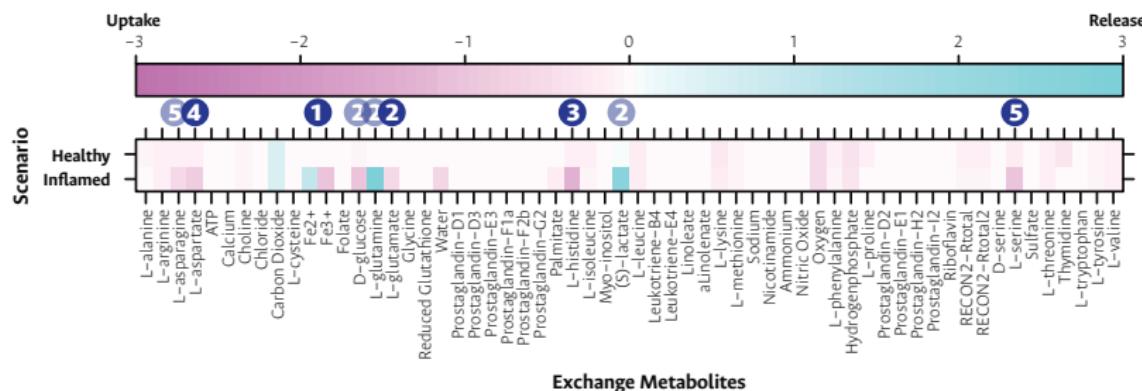
- ① Rachel Williams *et al.* (2012). Pathogenic implications of iron accumulation in multiple sclerosis.
- ② Mithilesh Kumar Jha *et al.* (2016). Metabolic Control of Glia-Mediated Neuroinflammation.
- ② V Pampura and P G Haydon. (2000). Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons.
- ② Leif Hertz *et al.* (1999). Astrocytes: Glutamate producers for neurons.

# 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%)



- 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

- Not Released
- Released
- Increased Release
- Decreased Release



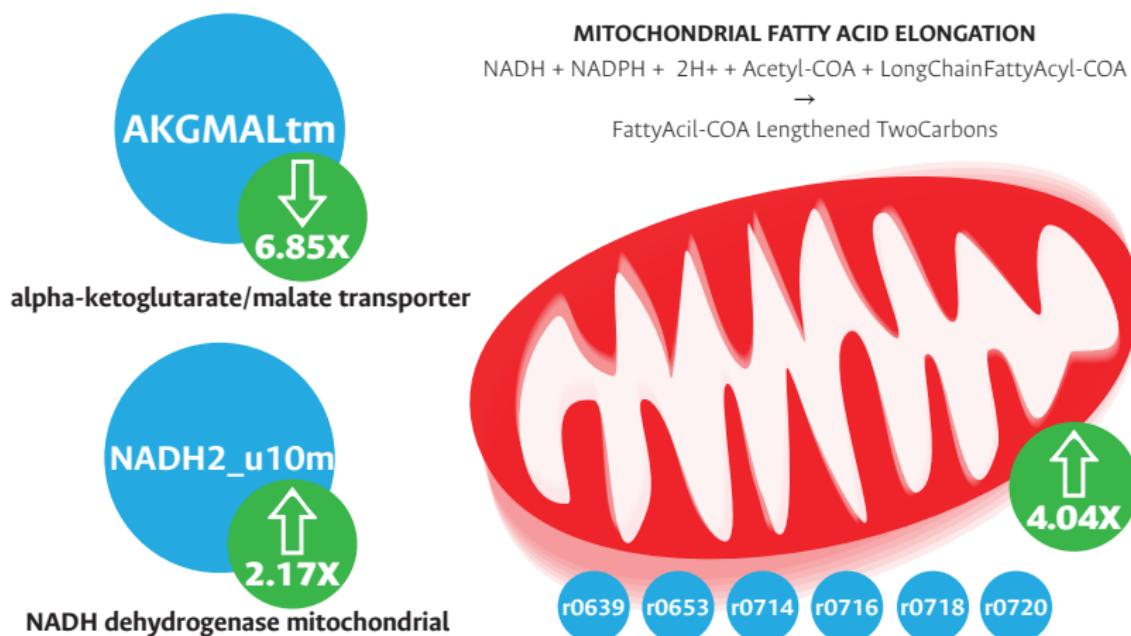
Normal Uptake of  
**PALMITATE**  
Healthy Scenario

	LACTATE	GSH	GLN	GLU	ATP	SERD		LACTATE	GSH	GLN	GLU	ATP	SERD
<b>CELLULAR MAINTENANCE (BO)</b>													
<b>BO + GLU → GLN</b>													
<b>BO + GLY → D-SER</b>													
<b>BO + GLC → LACTATE</b>													
<b>BO + GLC → ATP</b>													
<b>BO + CYS → GSH</b>													



Forced Uptake of  
**PALMITATE**  
Inflamed Scenario

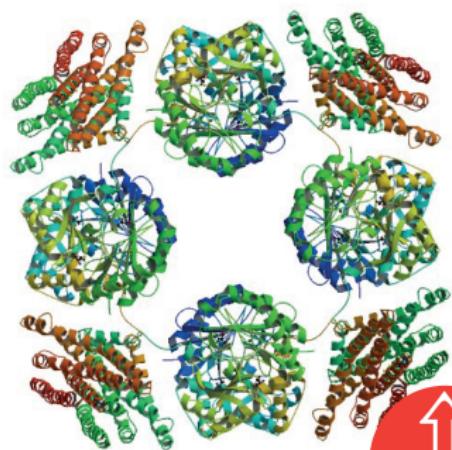
# Anti-inflammatory Enzymes: Innate defence against inflammation



# Pro-inflammatory Enzymes

**FTCD**

FormimidoylTransferase CycloDeaminase

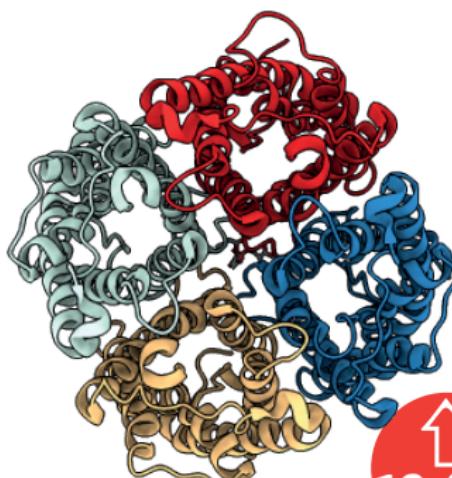


EC: 4.3.1.4

EC: 2.1.2.5 11.44%

 **2.28X****H2Otm**

H2O Transport Mitochondrial

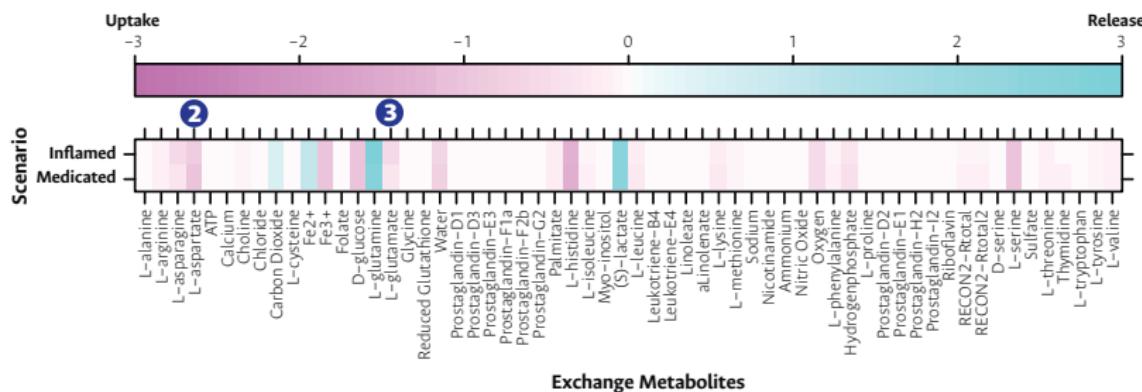
 **10.44X**  
5.14%

# 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.

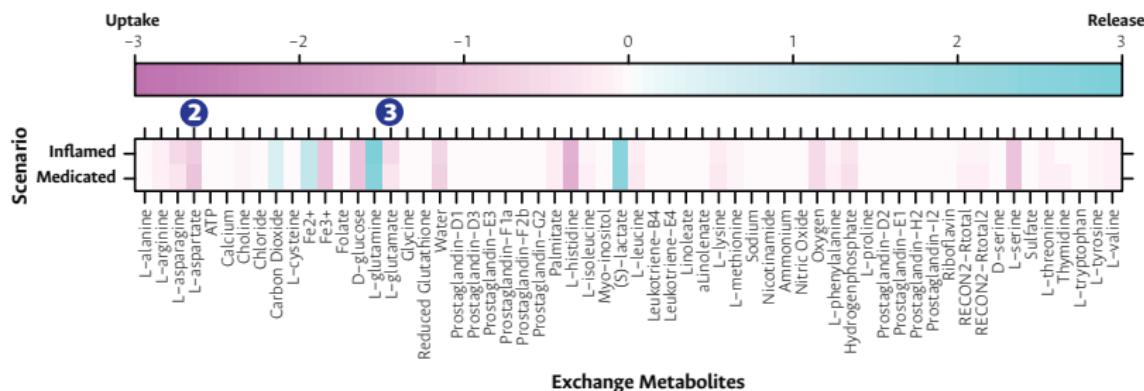
① Graham A., et al. (1995). The Use of Estrogens and Progestins and the Risk of Breast Cancer in Postmenopausal Women

# 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%**



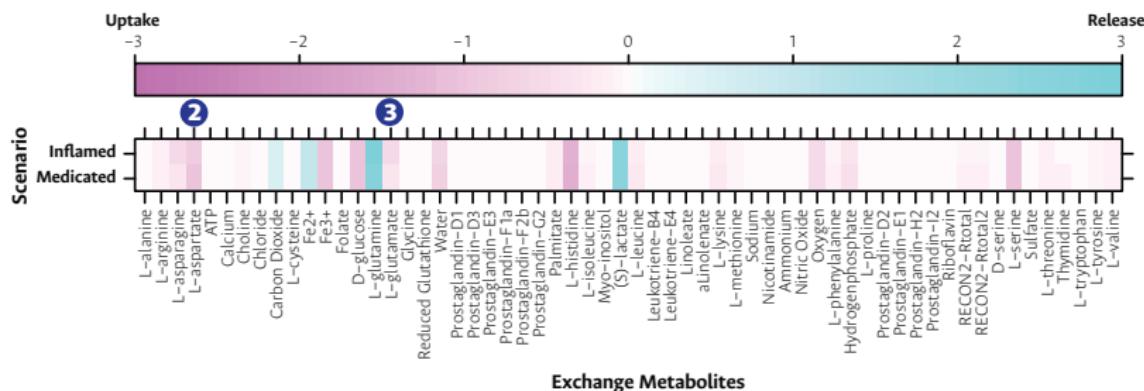
**②** Leonard T. Rael *et al.* (2004). An anti-inflammatory role for N-acetyl aspartate in stimulated human astroglial cells.

# 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%**

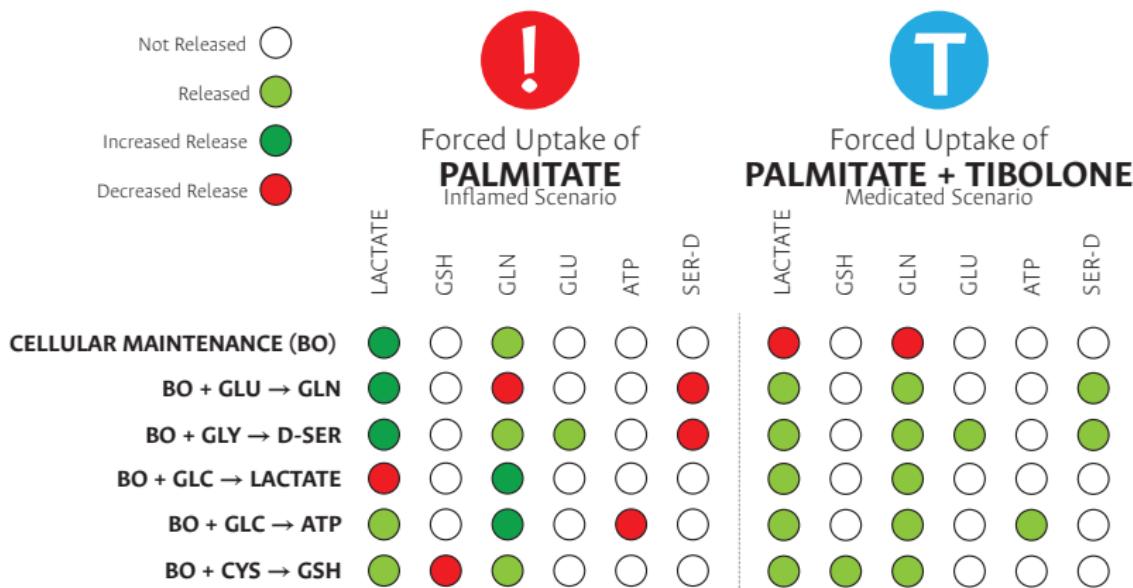


② Leonard T. Rael *et al.* (2004). An anti-inflammatory role for N-acetyl aspartate in stimulated human astroglial cells.

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.

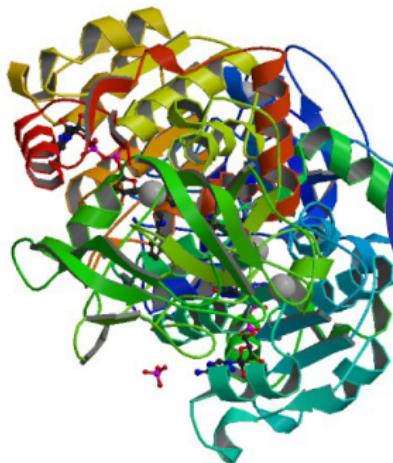
# Gliotransmitters



# Tibolone Related Enzymes

**r0739**

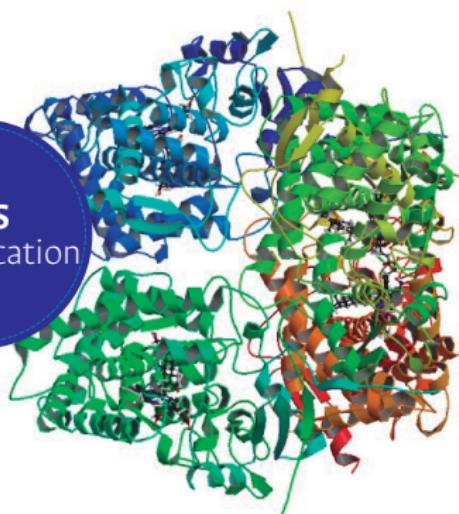
Alcohol Dehydrogenase 1 - 7



EC: 1.1.1.1

**REM1804m | REM1807m**

Cytochrome P450 Family 27 Subfamily A Member 1

ROS  
Detoxification

EC: 1.14.15.15



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

ICGEB Course on Bioinformatics and Computational Neuroscience



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

**Daniel Osorio**

[dcosorioh@unal.edu.co](mailto:dcosorioh@unal.edu.co)

**Andrés Pinzón PhD**

[ampinzonv@unal.edu.co](mailto:ampinzonv@unal.edu.co)