

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

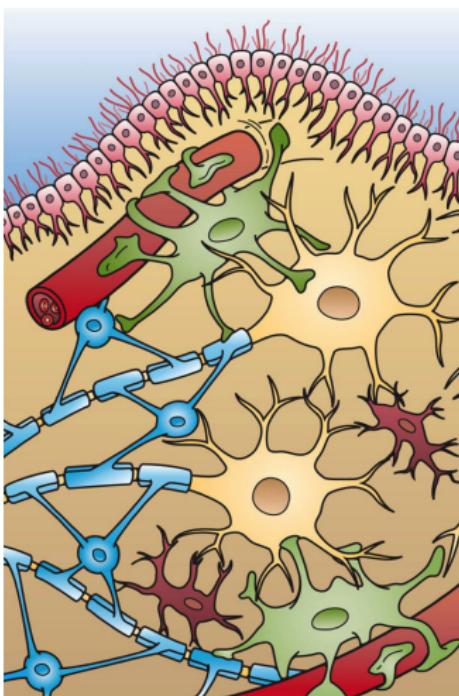
Master in Bioinformatics

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

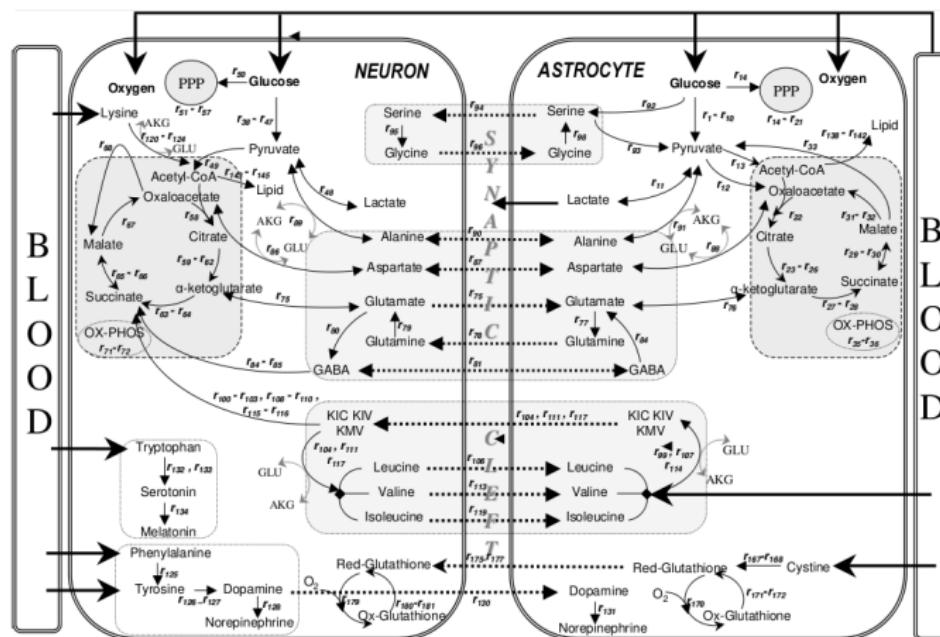
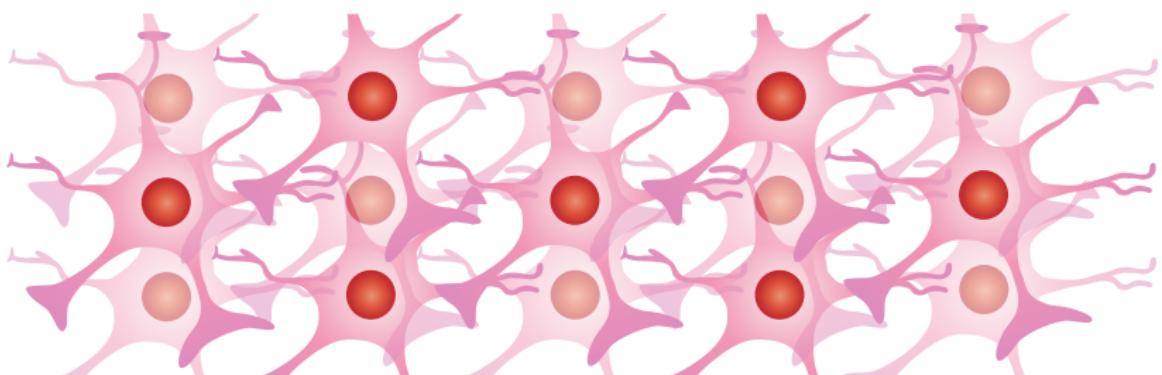


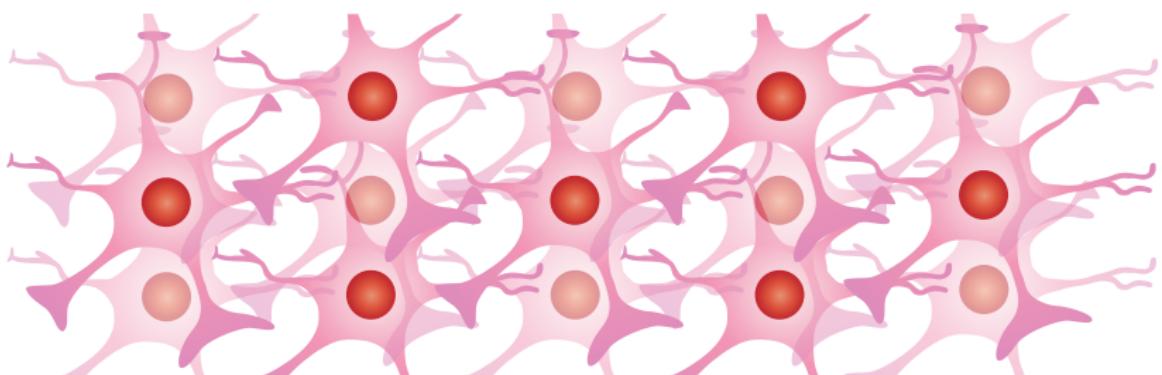
Figure from: Çakir, Tunahan *et al.*, (2007). Reconstruction and flux analysis of coupling between metabolic pathways of astrocytes and neurons: application to cerebral hypoxia.

Astrocytes Metabolic Functions



K^+ Membrane Potential

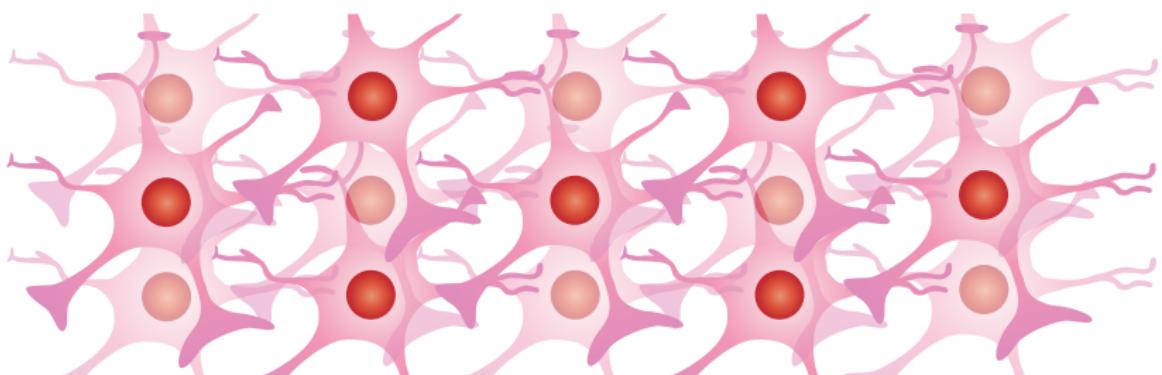
Astrocytes Metabolic Functions



K^+ Membrane Potential

Ca^{+2} signaling

Astrocytes Metabolic Functions

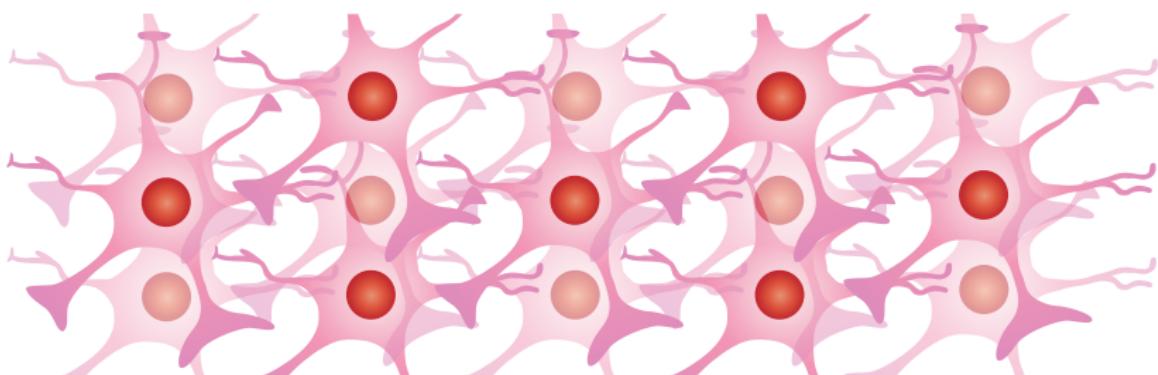


K⁺ Membrane Potential

Ca⁺² signaling

Lactate release

Astrocytes Metabolic Functions



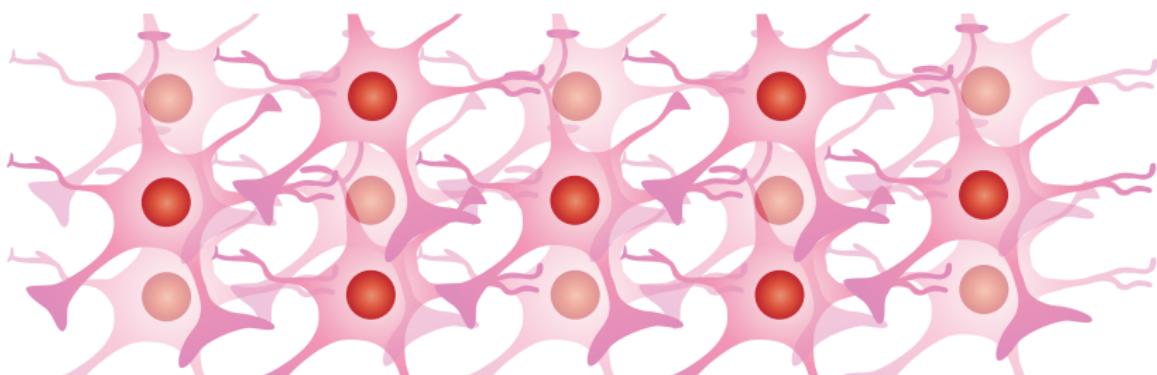
K⁺ Membrane Potential

Ca⁺² signaling

Lactate release

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

Astrocytes Metabolic Functions



K⁺ Membrane Potential

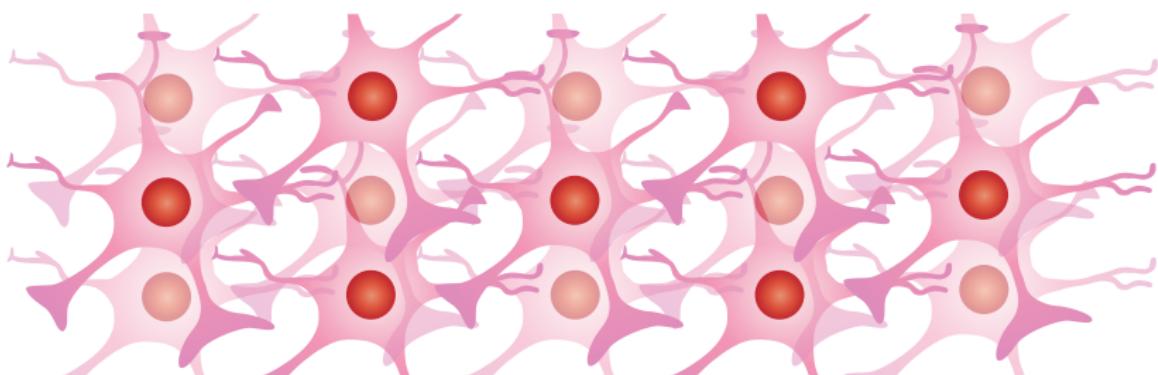
Ca⁺² signaling

Lactate release

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

pH maintenance

Astrocytes Metabolic Functions



K⁺ Membrane Potential

Ca⁺² signaling

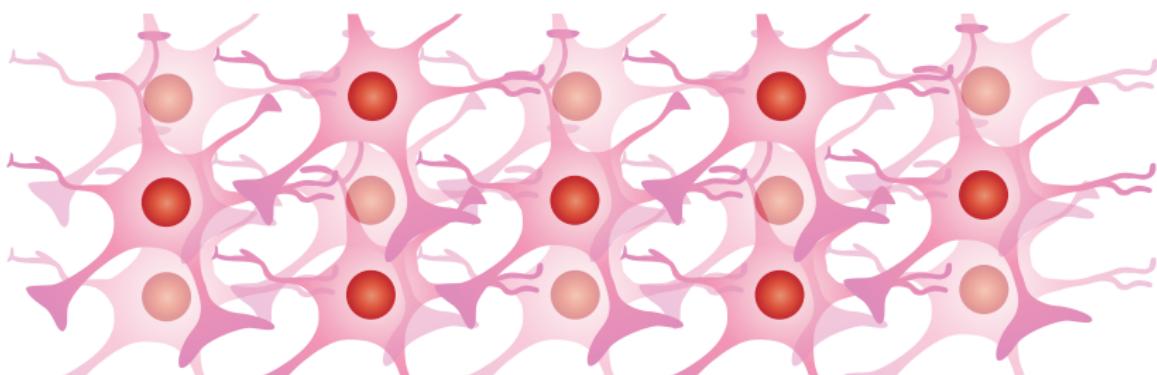
Lactate release

[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

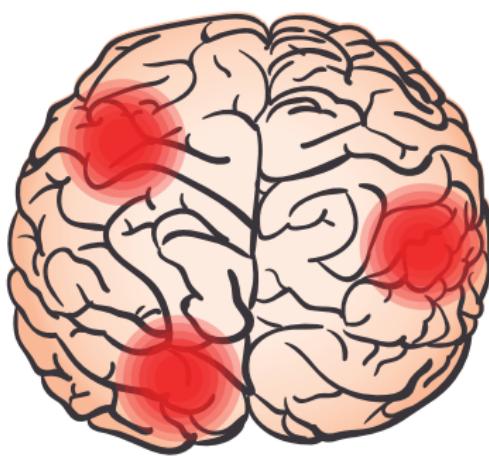
ROS detox

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

What is inflammation about?

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



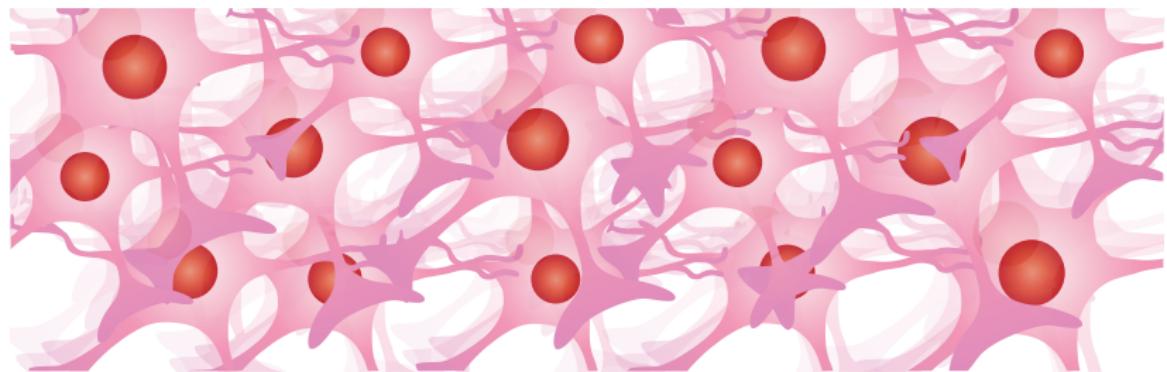
Metabolic Inflammation or Metainflammation

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

▲ Endoplasmic reticulum stress → ▲ UPR

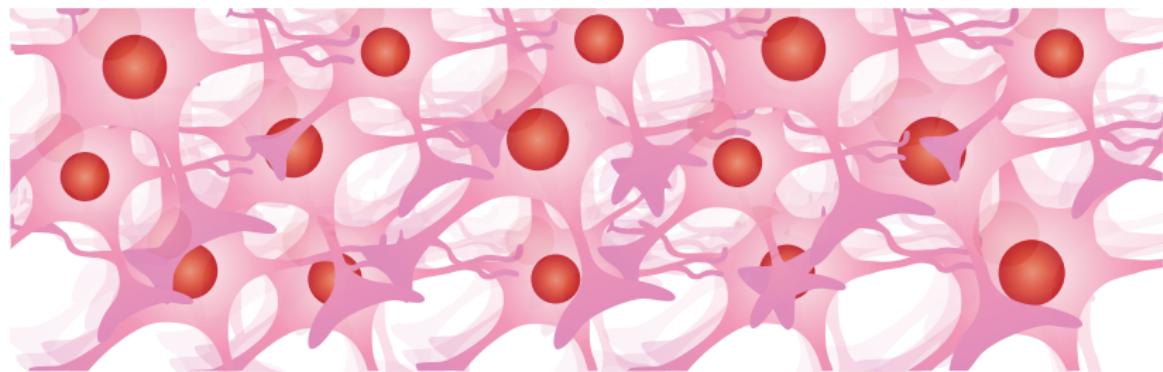
▲ Reactive C Protein Ligands + ▲ TNF α + ▲ IL6 + ▲ ROS

Astrogliosis or Reactive Astrocytosis



ROS generator

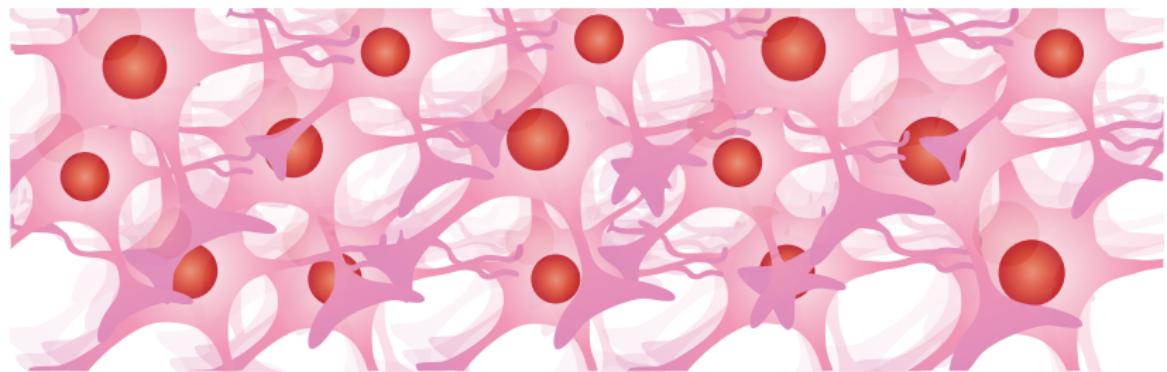
Astrogliosis or Reactive Astrocytosis



ROS generator

Mitochondrial Failure

Astrogliosis or Reactive Astrocytosis

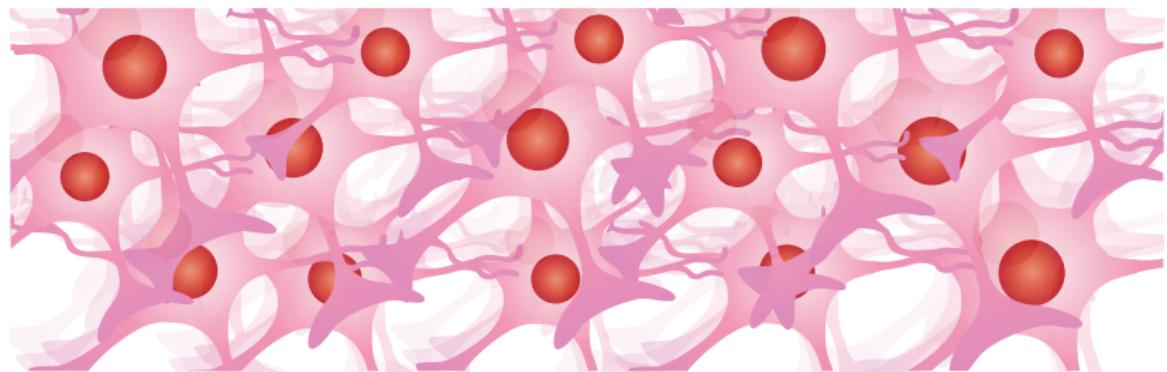


ROS generator

Mitochondrial Failure

Ca^{+2} disregulation

Astrogliosis or Reactive Astrocytosis



ROS generator

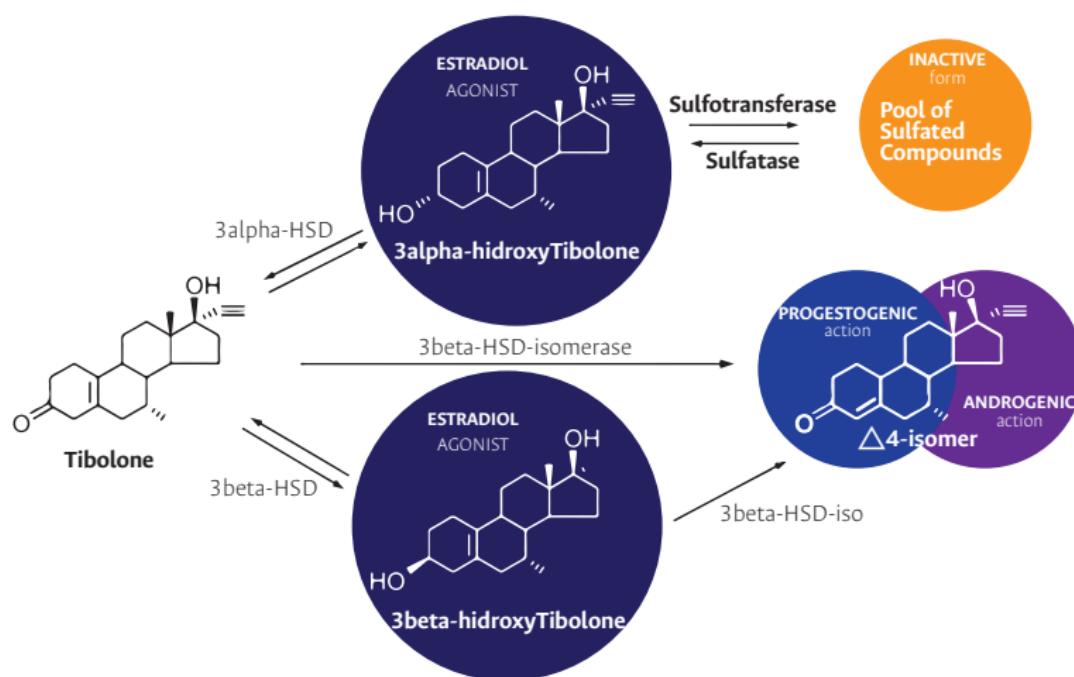
Mitochondrial Failure

Ca^{+2} disregulation

Free Fatty Acids (Palmitate) Increase

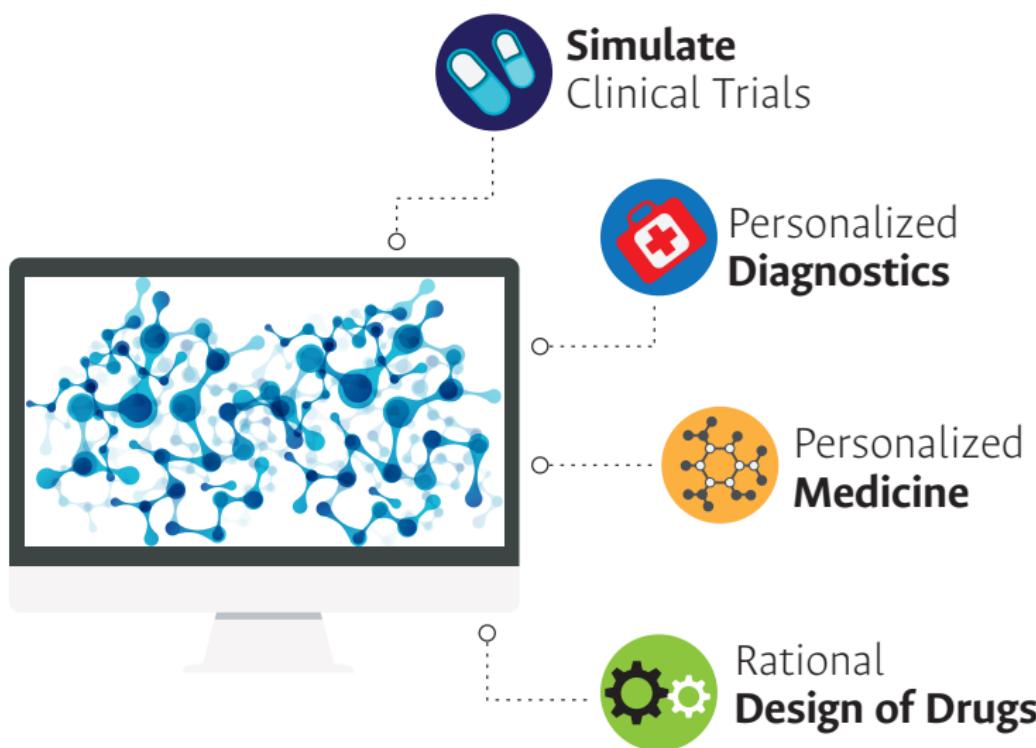
Inflammation Treatment

Tibolone Metabolism



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

Translational Bioinformatics: 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.

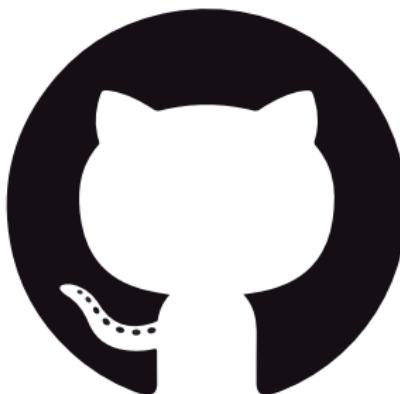
Reproducibility

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)

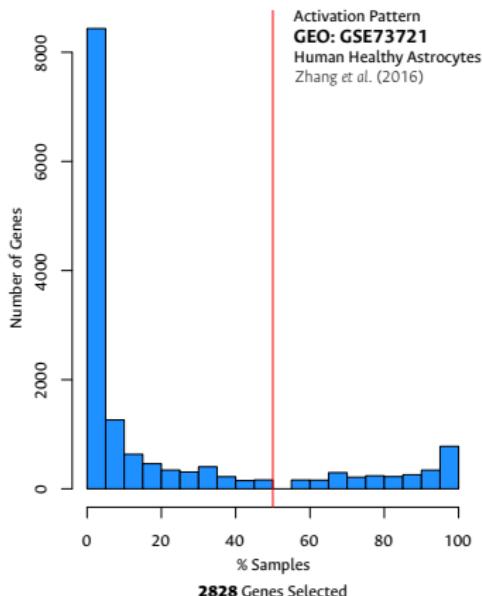
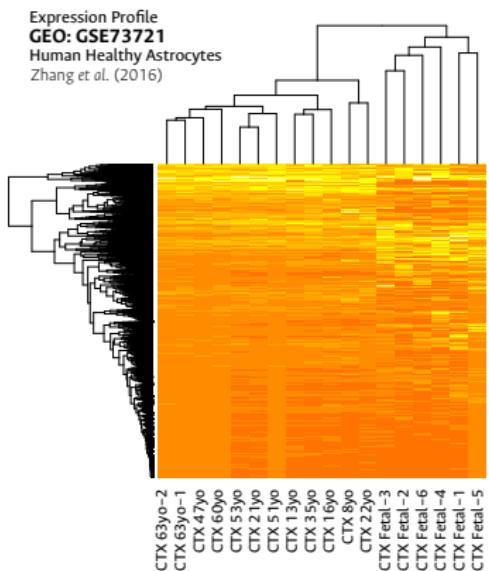
LP SOLVER: **GLPK 4.6**

LICENSE: **GNU GPL3**

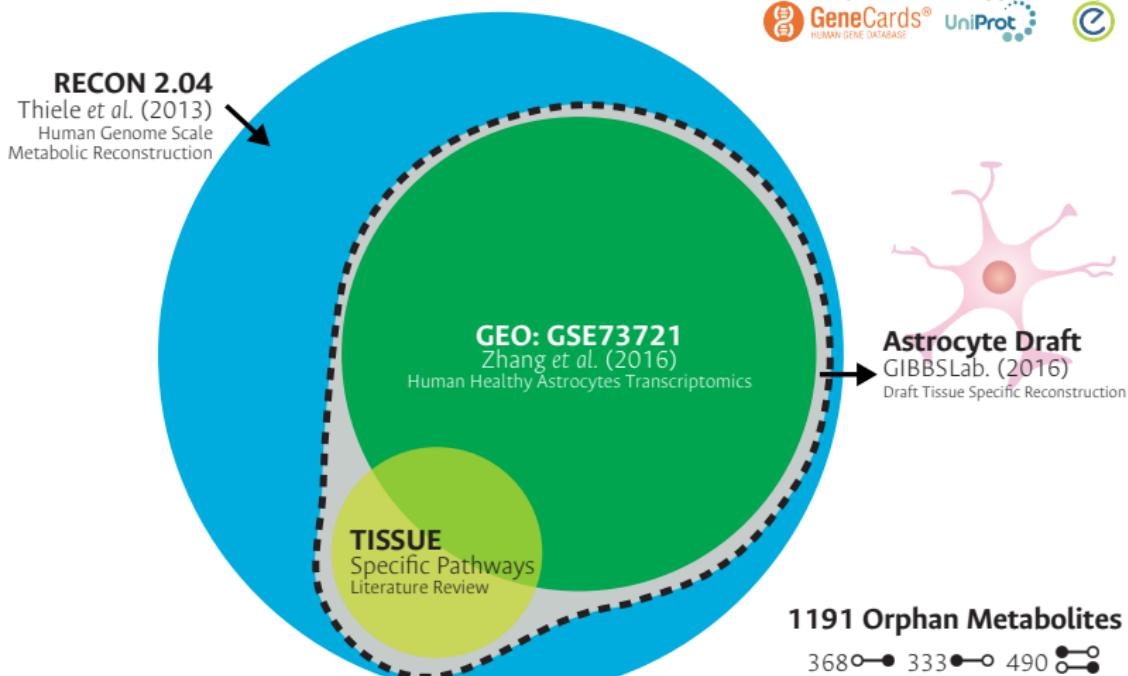
OBJECTIVE 1:

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

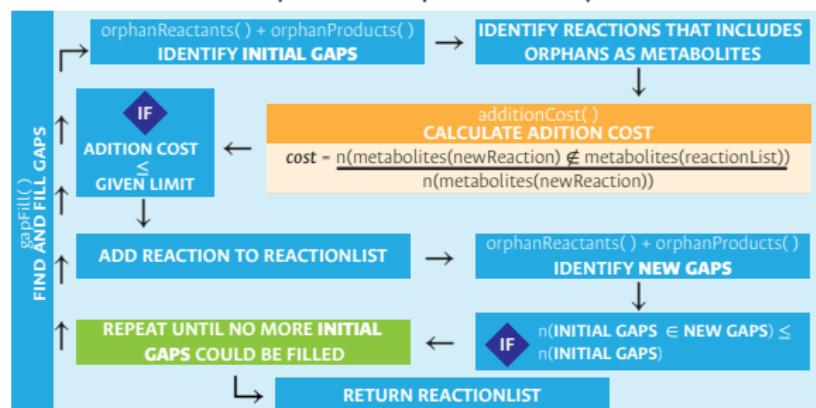
$\text{h2o[r]} + \text{dheas[r]} \Rightarrow \text{h[r]} + \text{dhea[r]} + \text{so4[r]}$
 $\text{uri[e]} \Leftrightarrow \text{uri[c]}$
 $\text{na1[e]} + \text{uri[e]} \Rightarrow \text{na1[c]} + \text{uri[c]}$
 $\text{atp[c]} + \text{pi[m]} \Rightarrow \text{pi[c]} + \text{atp[m]}$
 $\text{na1[e]}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

REACTI**L**IST



getReference()
 DOWNLOAD
 STOICHIOMETRIC REACTIONS
 FROM THE KEGG DATABASE

REFERENC**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.

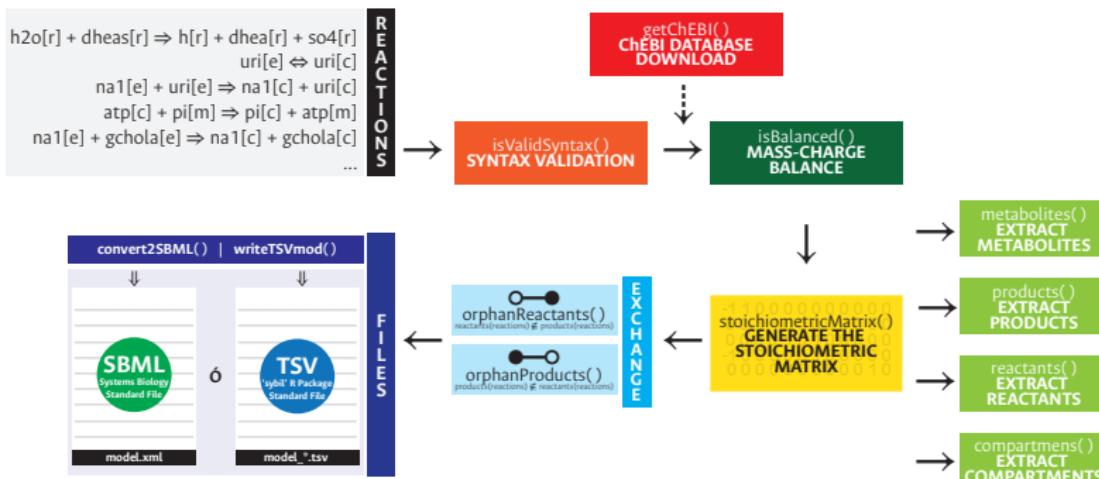
Language: R

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.

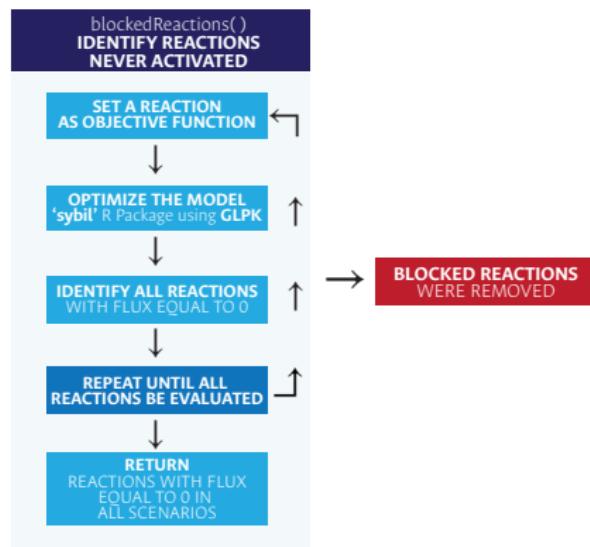
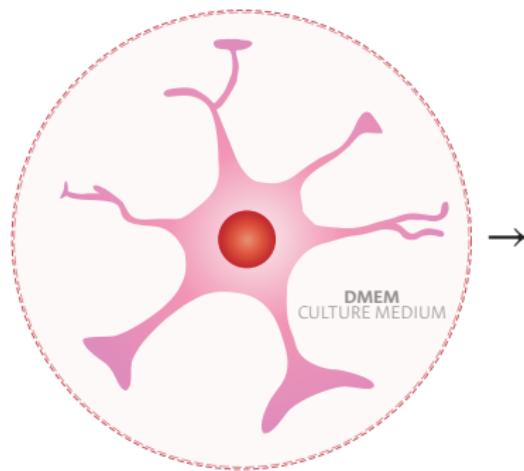
Language: R

Stable: CRAN

Development: [gibbslab/g2f](#)

License: [GPL-2](#)

Binaries: Windows - Linux - Mac



Gene Expression Integration Available Methods

METHOD	ENVIRONMENT	HOW IT WORKS
GIMME	MATLAB Privative	<ul style="list-style-type: none">· Binary Discretization· Ensures flux for a selected objective function
iMAT	MATLAB Privative	<ul style="list-style-type: none">· Integration proportional to gene-expression (H, M and L categorization)· Not objective function required
E-FLUX	Not implemented	<ul style="list-style-type: none">· Requires a user-given threshold· Continuous Integration
PROM	MATLAB Privative	<ul style="list-style-type: none">· 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

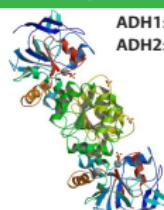
Stable: CRAN

Development: [gibbslab/exp2flux](#)

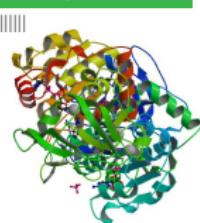
License: GPL-2

Binaries: Windows - Linux - Mac

E.C: 1.1.1.1



ADH1: |||||
ADH2: |||||



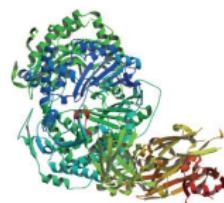
(ADH2 or ADH1)

sum (ExprADH2 + ExprADH1)

E.C: 3.4.21.5



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



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

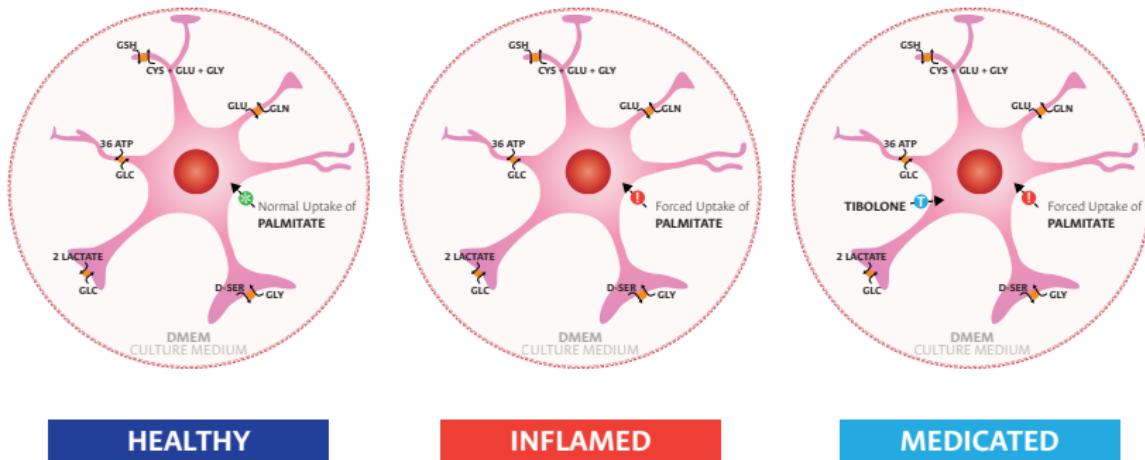
METABOLIC MODEL
WITH GPR



OBJECTIVE 2:

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

Metabolic Scenarios



HEALTHY

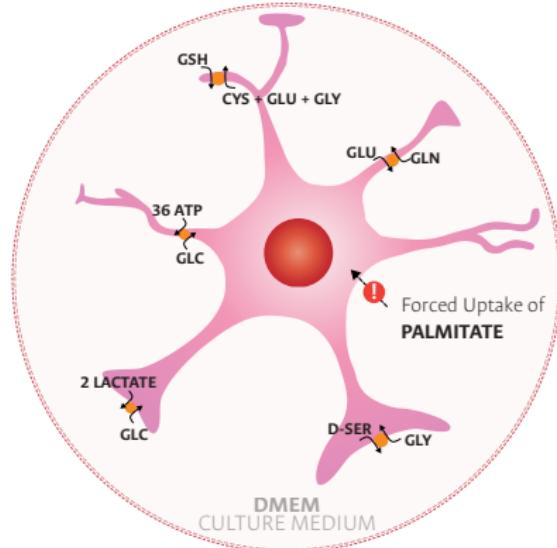
INFLAMED

MEDICATED

MAIN OBJECTIVE FUNCTION:

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

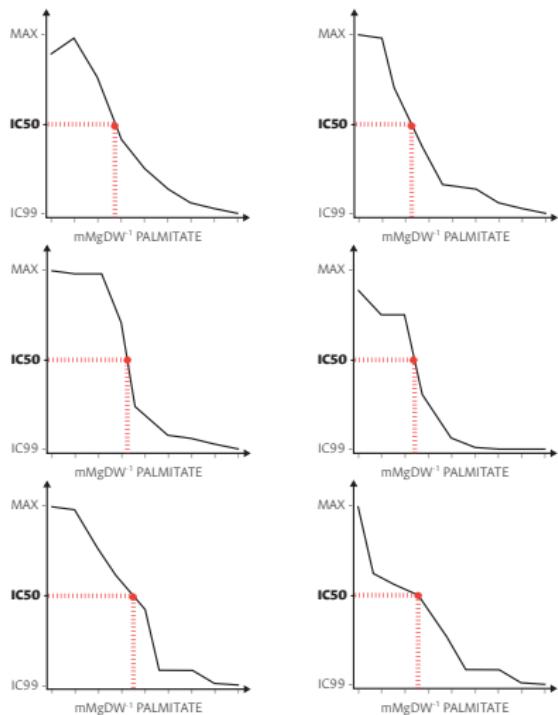
Inflamed Metabolic Scenario



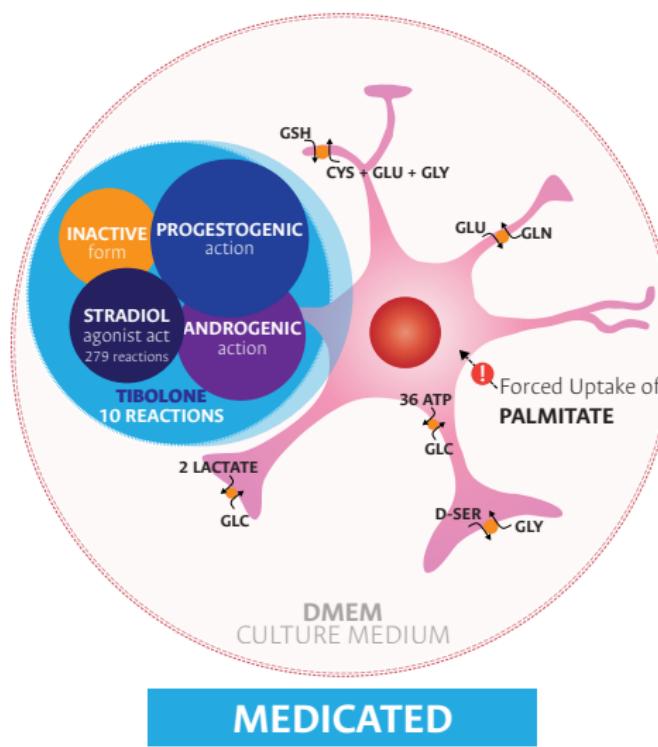
PALMITATE UPTAKE RATE: AVERAGED IC₅₀ VALUES

INFLAMED

ROBUSTNESS ANALYSIS



Medicated Metabolic Scenario

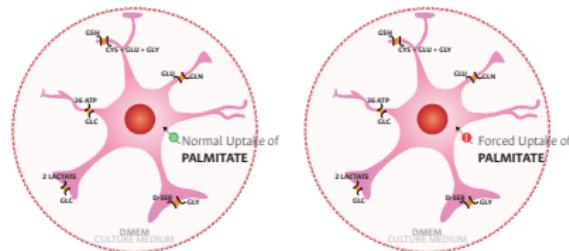


OBJECTIVE 3:

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

Metabolic Pathways Activation Pattern Changes

INFLAMMATION CHANGES

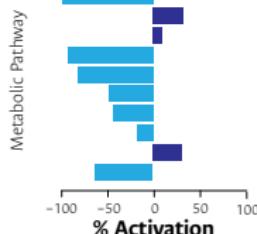


HEALTHY

INFLAMED

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

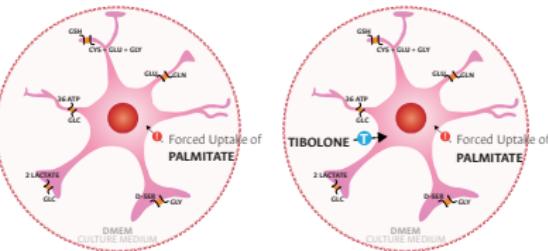
Metabolic Changes



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



TIBOLONE CHANGES

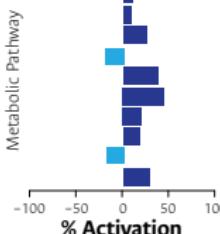


INFLAMED

MEDICATED

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

Metabolic Changes

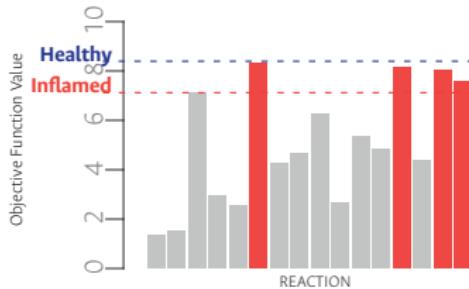


OBJECTIVE 4:

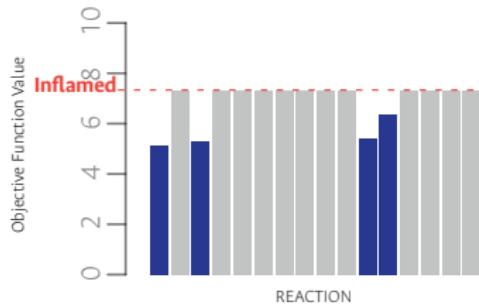
Evaluate the importance of proteins and metabolic pathways previously identified on the dynamics of the metabolic model.

Reaction Knock-out: Protein Importance

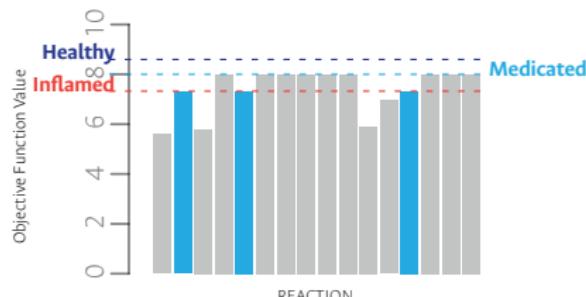
Pro-Inflammatory



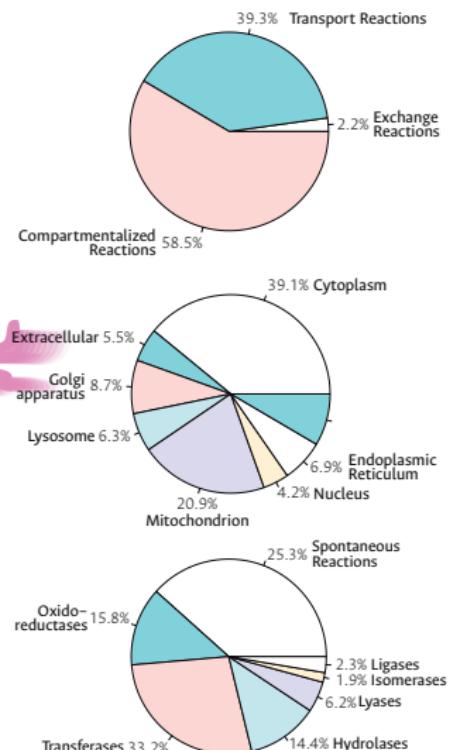
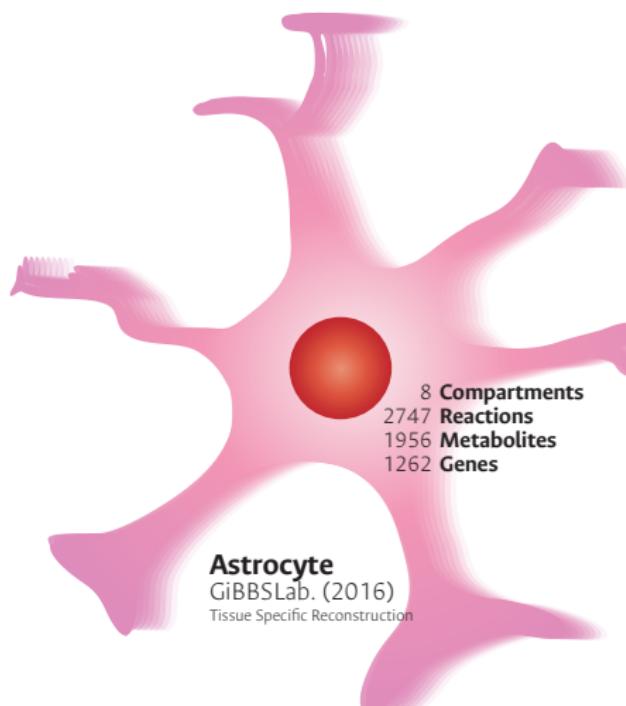
Anti-Inflammatory



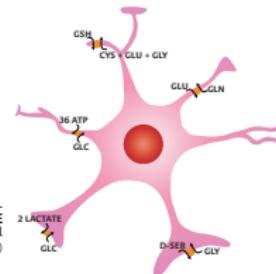
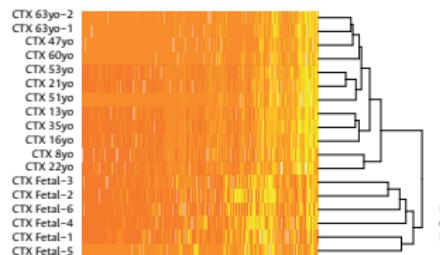
Tibolone Mechanism



Tissue Specific Metabolic Reconstruction



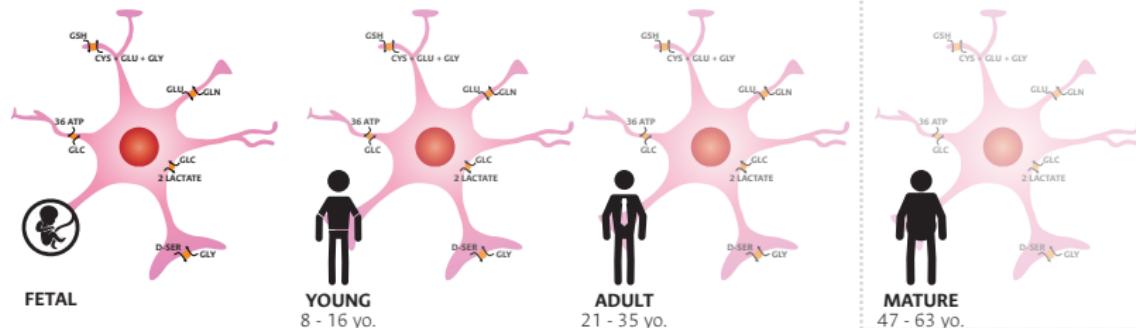
Human Healthy Mature Astrocyte Model



'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

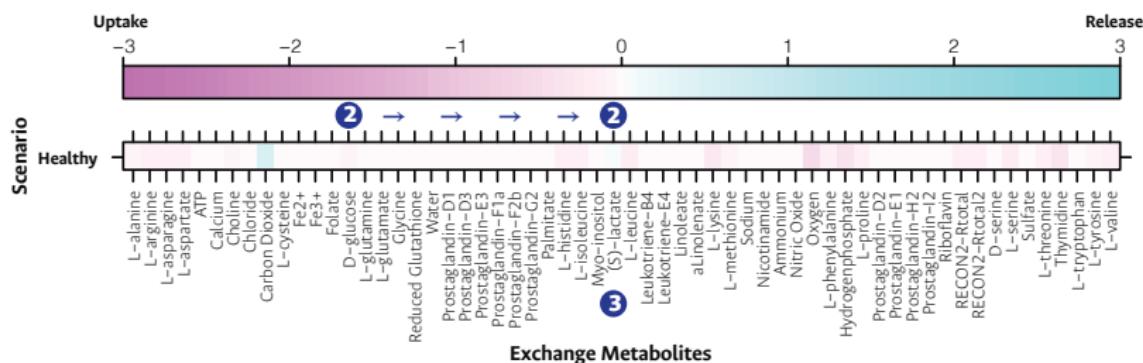


Cellular Maintenance: Healthy Scenario

① OBJECTIVE FUNCTION: 0.37 mMgWD-1h-1

DMEM Culture Medium

METABOLISM ACTIVATION: 52%



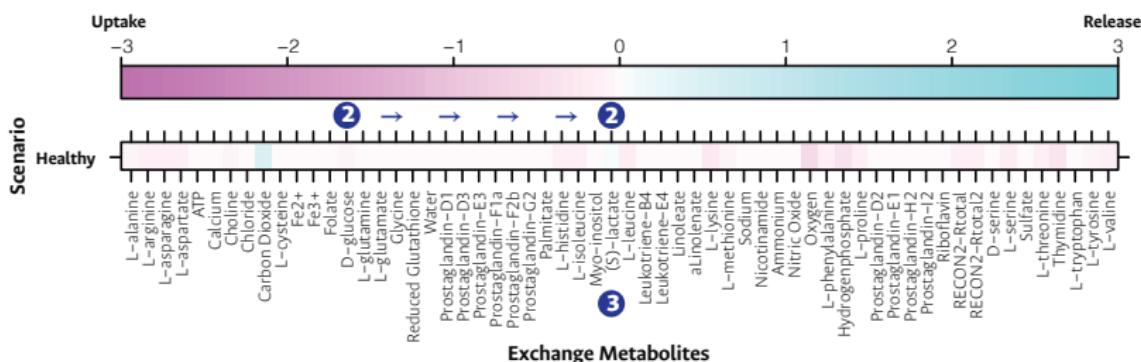
① Arabinda Das, et al. (2010). Flavonoids activated caspases for apoptosis in human glioblastoma T98G and U87MG cells but not in human normal astrocytes.

Cellular Maintenance: Healthy Scenario

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

DMEM Culture Medium

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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.

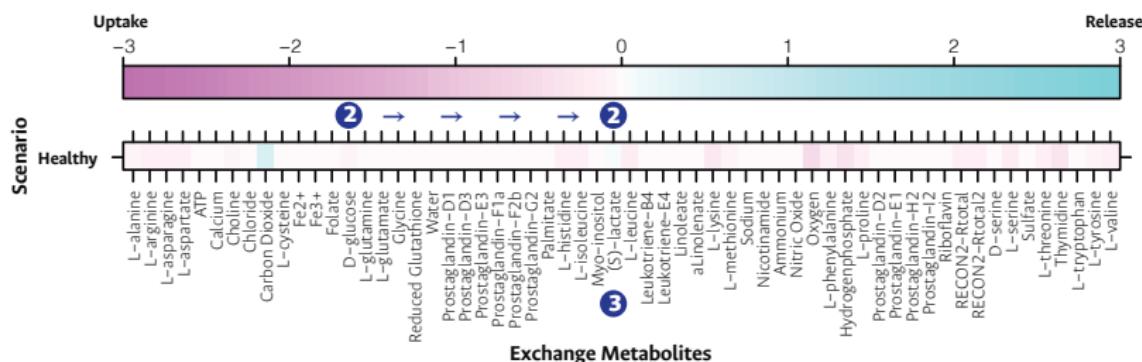
③ Rupa Bhowmick, *et al.* (2015). Exploring the differences in metabolic behavior of astrocyte and glioblastoma: a flux balance analysis approach.

Cellular Maintenance: Healthy Scenario

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

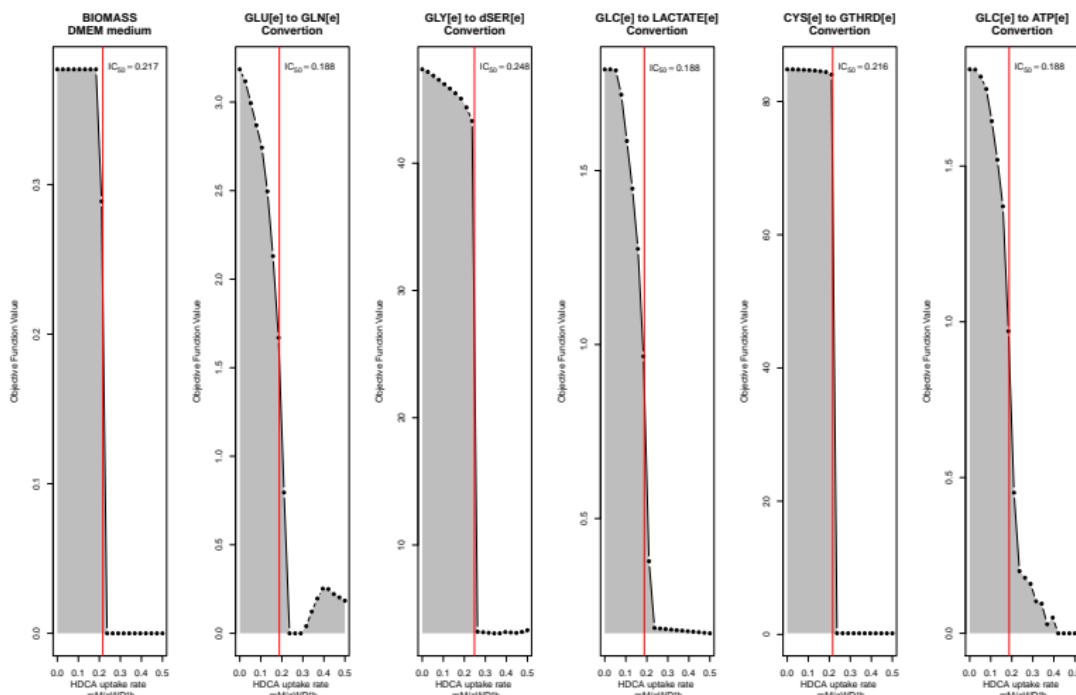
DMEM Culture Medium

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.
- ③ Rupa Bhowmick, et al. (2015). Exploring the differences in metabolic behavior of astrocyte and glioblastoma: a flux balance analysis approach.
- ④ Christelle Le Foll, et al. (2010). Fatty acid-induced astrocyte ketone production and the control of food intake.

Averaged IC₅₀: $0.208 \pm 0.024 \text{ mMgDW}^{-1}\text{h}^{-1}$



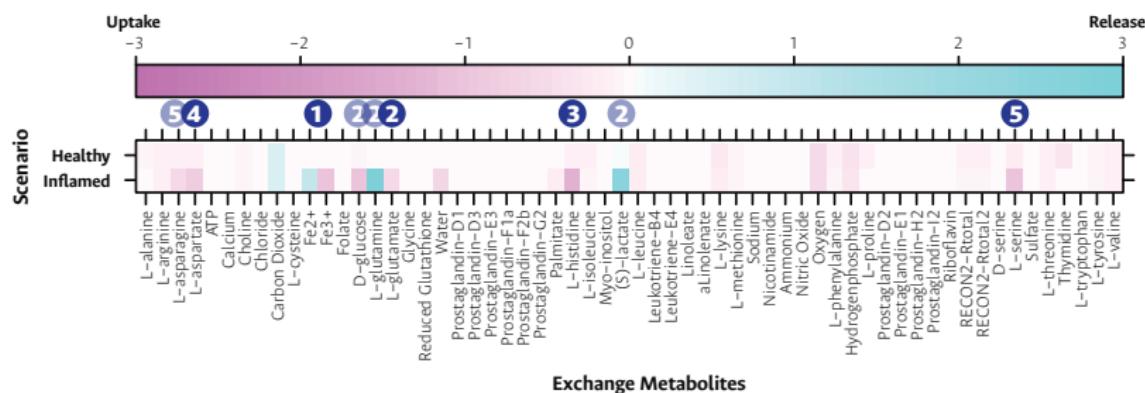
Li Liu et al. (2013) Palmitate-activated astrocytes via serine palmitoyltransferase increase BACE1 in primary neurons by sphingomyelinases.

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%)



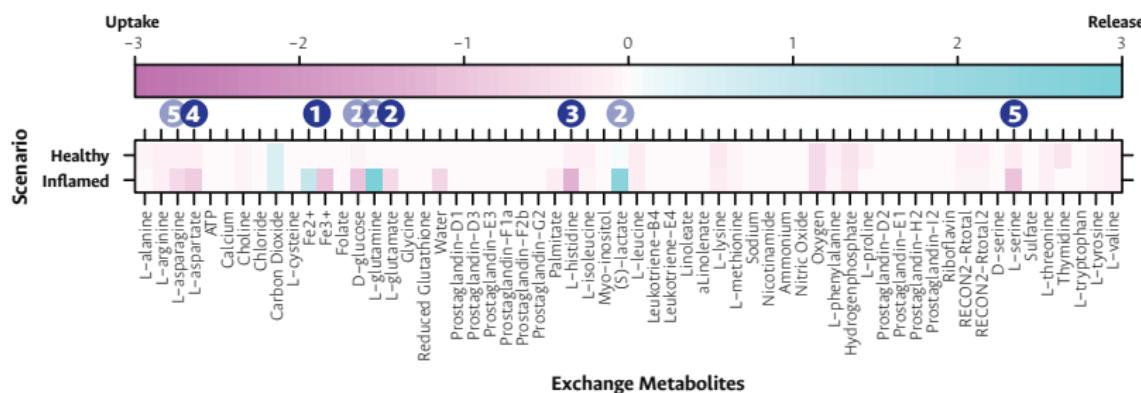
- ① Rachel Williams et al. (2012). Pathogenic implications of iron accumulation in multiple sclerosis.

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%)



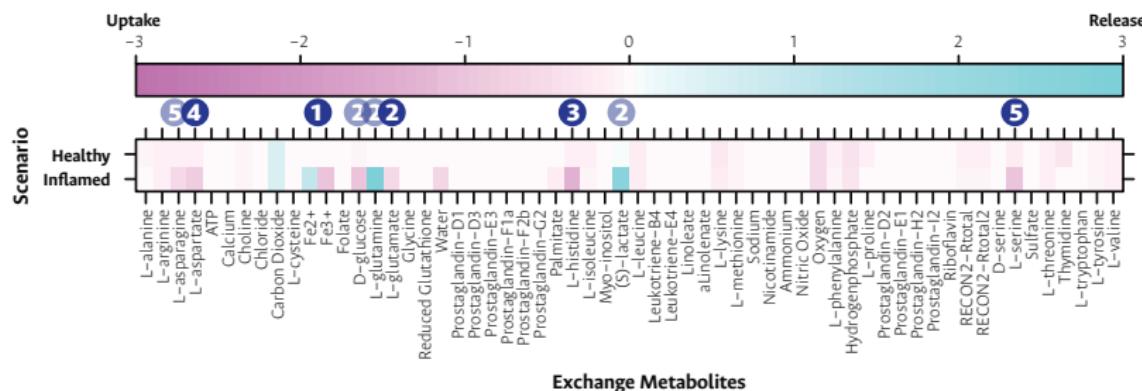
- ① Rachel Williams *et al.* (2012). Pathogenic implications of iron accumulation in multiple sclerosis.
- ② Mithilesh Kumar Jha *et al.* (2016). Metabolic Control of Glia-Mediated Neuroinflammation.
- ② V Pampura and P G Haydon. (2000). Physiological astrocytic calcium levels stimulate glutamate release 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



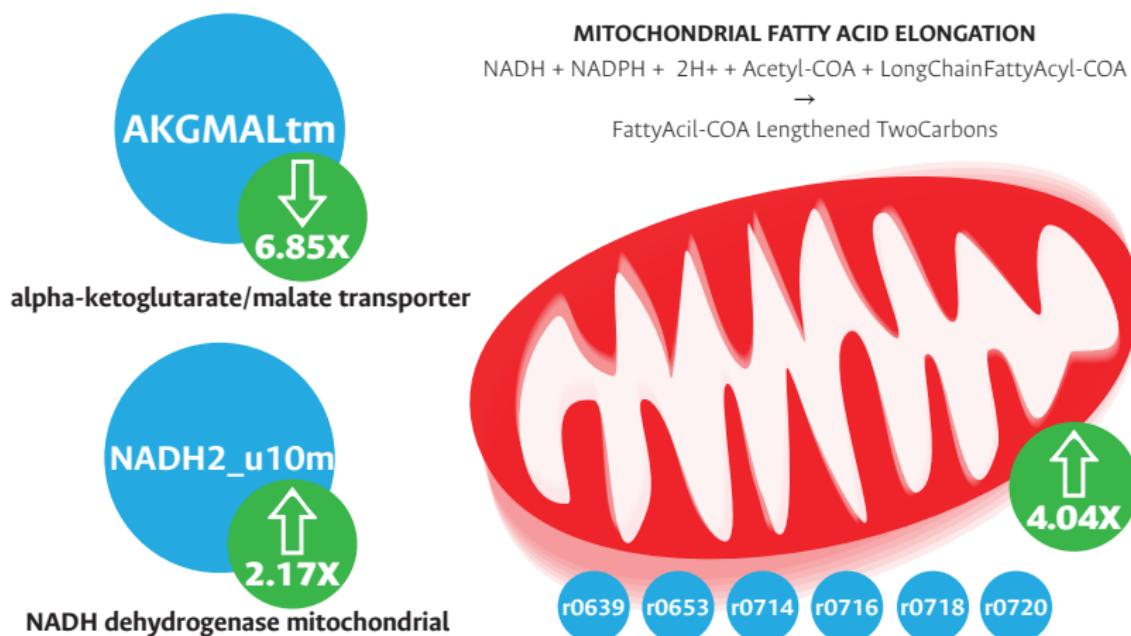
Normal Uptake of
PALMITATE
Healthy Scenario

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



Forced Uptake of
PALMITATE
Inflamed Scenario

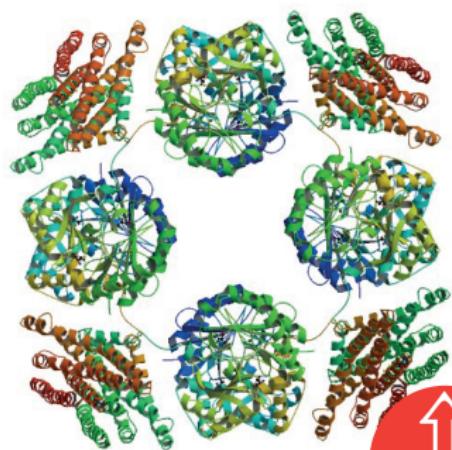
Anti-inflammatory Enzymes: Innate defence against inflammation



Pro-inflammatory Enzymes

FTCD

FormimidoylTransferase CycloDeaminase

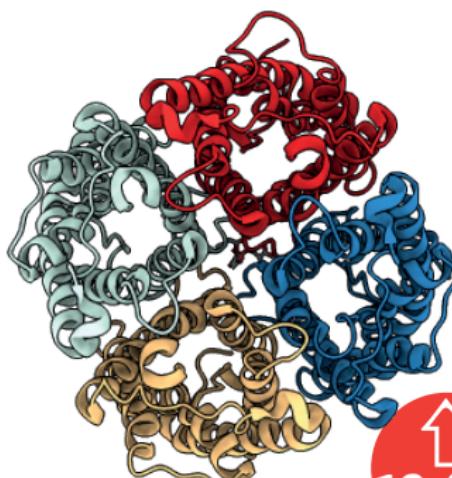


EC: 4.3.1.4

EC: 2.1.2.5 11.44%

**H2Otm**

H2O Transport Mitochondrial

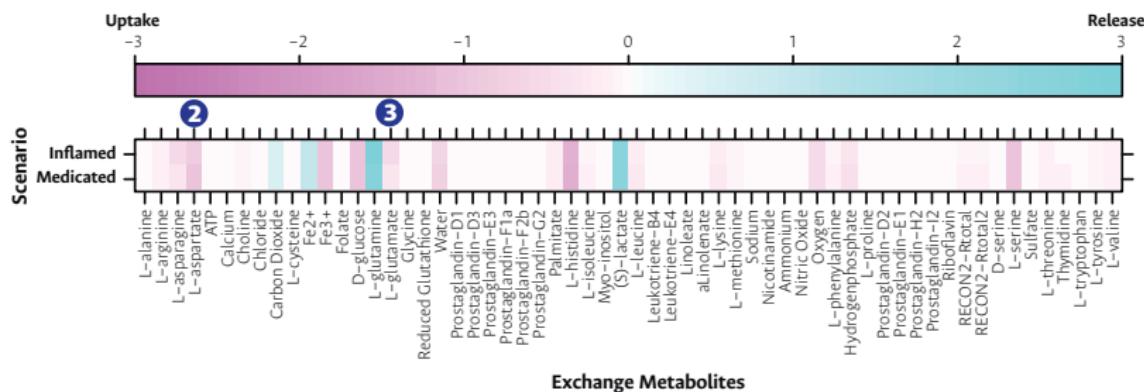


Cellular Maintenance: Medicated Scenario

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.

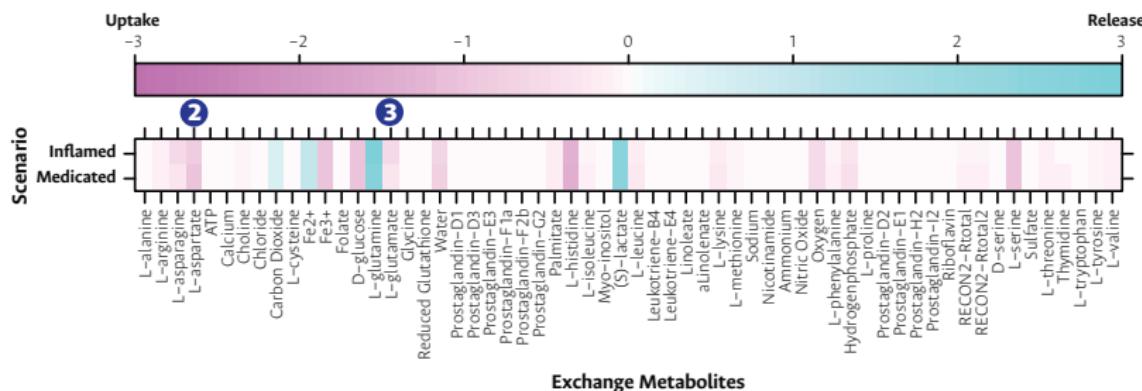
① Graham A., et al. (1995). The Use of Estrogens and Progestins and the Risk of Breast Cancer in Postmenopausal Women

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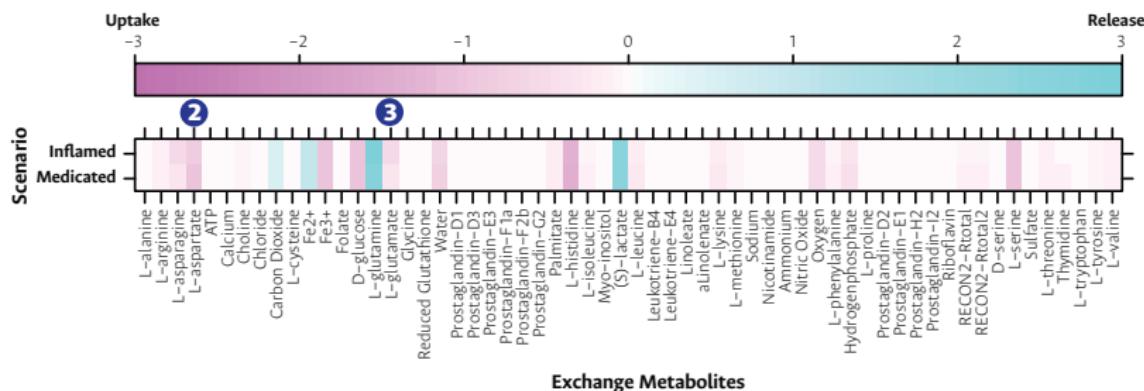
② Leonard T. Rael *et al.* (2004). An anti-inflammatory role for N-acetyl aspartate in stimulated human astroglial cells.

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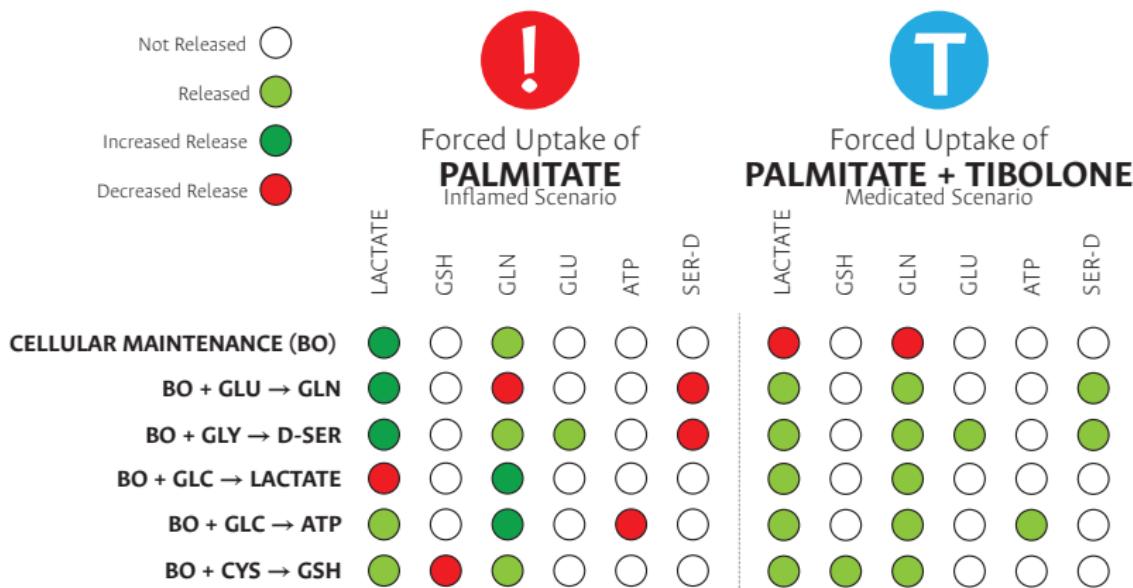


② Leonard T. Rael *et al.* (2004). An anti-inflammatory role for N-acetyl aspartate in stimulated human astroglial cells.

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.

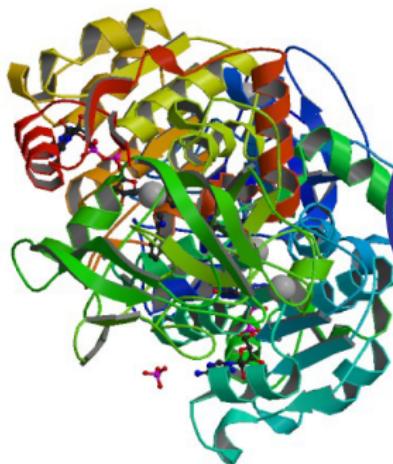
Gliotransmitters



Tibolone Related Enzymes

r0739

Alcohol Dehydrogenase 1 - 7



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



Pontificia Universidad Javeriana
5 - 8 October - Bogotá, Colombia

Bogotá, Colombia
Short Talk

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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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