

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

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.

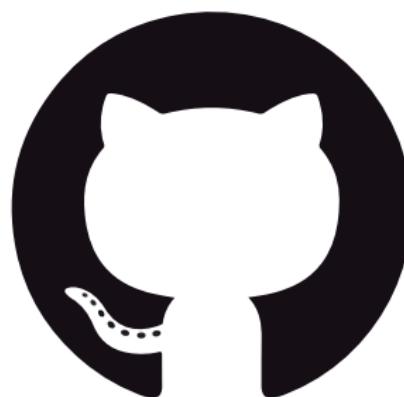
Reproducibility

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)

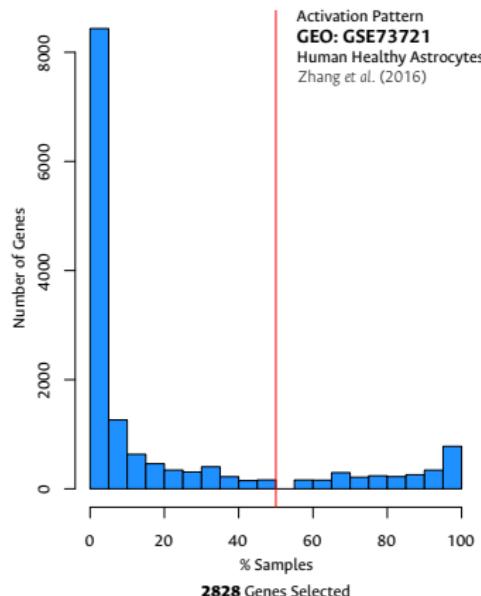
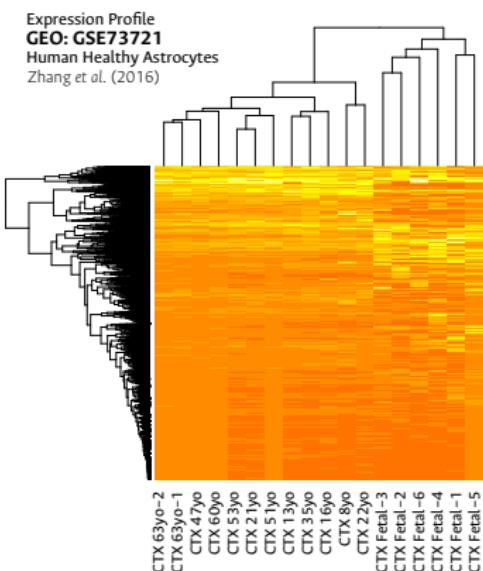
LP SOLVER: **GLPK 4.6**

LICENSE: **GNU GPL3**

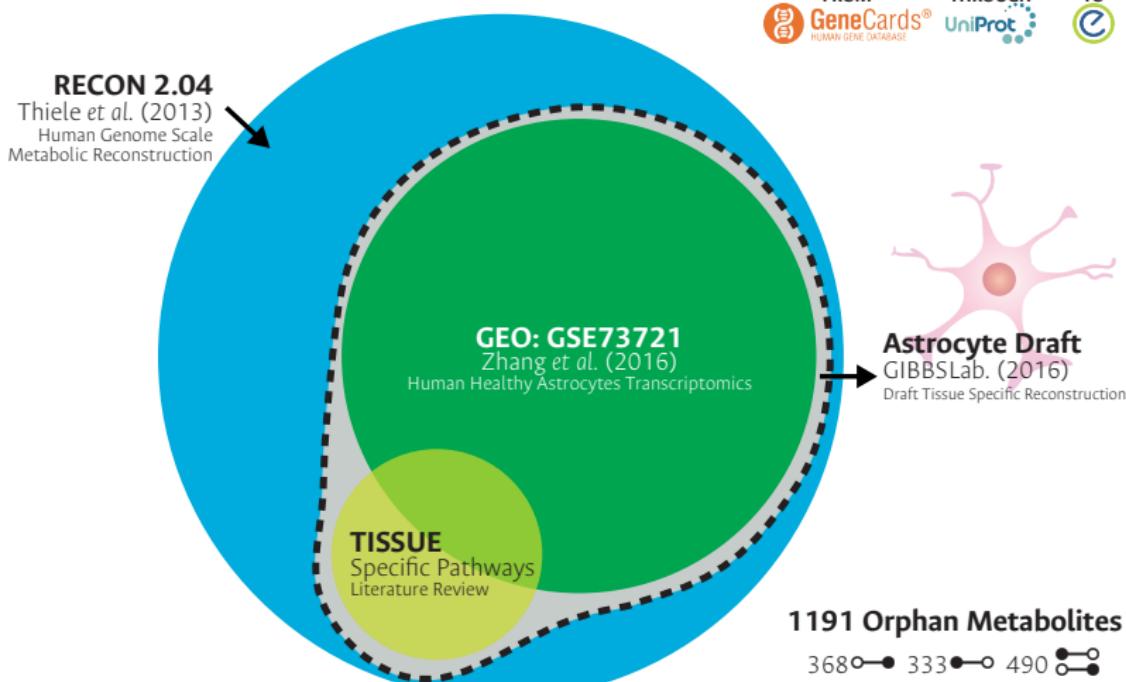
OBJECTIVE 1:

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

$\text{h2o[r]} + \text{dheas[r]} \Rightarrow \text{h[r]} + \text{dhea[r]} + \text{so4[r]}$
 $\text{uri[e]} \Leftrightarrow \text{uri[c]}$
 $\text{na1[e]} + \text{uri[e]} \Rightarrow \text{na1[c]} + \text{uri[c]}$
 $\text{atp[c]} + \text{pi[m]} \Rightarrow \text{pi[c]} + \text{atp[m]}$
 $\text{na1[e]} + \text{gchola[e]} \Rightarrow \text{na1[c]} + \text{gchola[c]}$

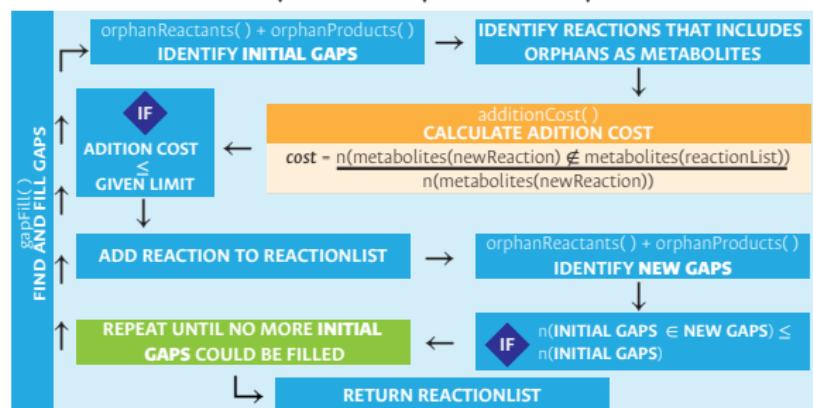
ASTROCYTE DRAFT GiBBS Lab (2016)

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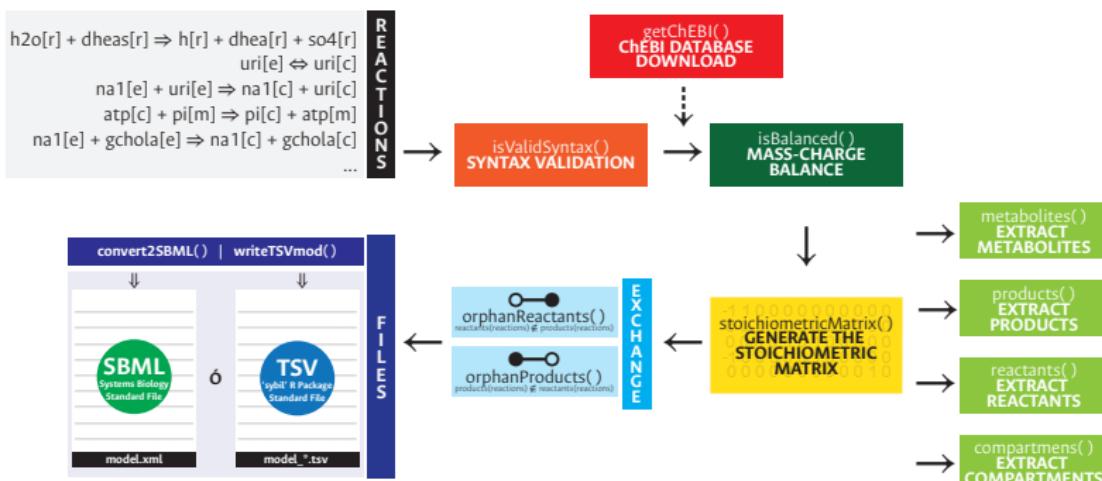


getReference()
DOWNLOAD
STOICHIOMETRIC REACTIONS
FROM THE KEGG DATABASE

R
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R
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Syntax, Mass-Charge Validation and SBML files



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.

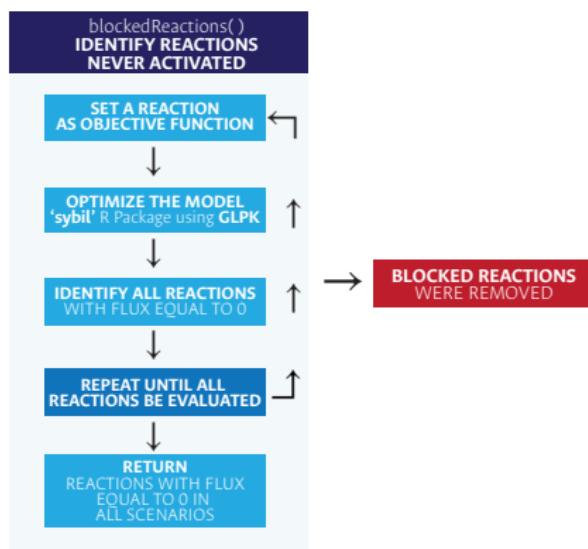
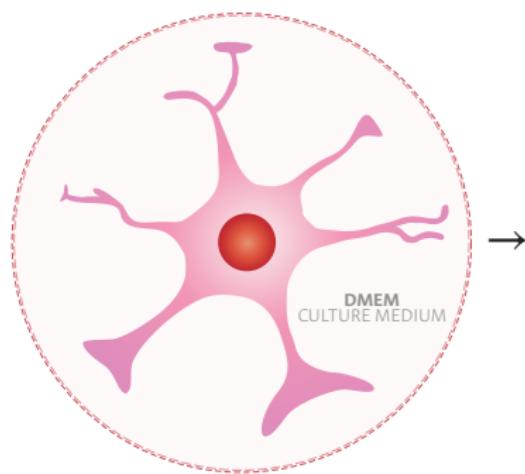
Language: R

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

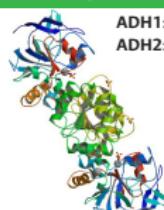
E.C: 1.1.1.1



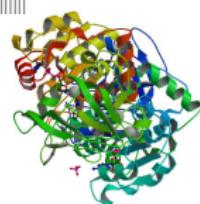
1.1.1.1

ADH2

ADH1



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



(ADH2 or ADH1)

sum (ExprADH2 + ExprADH1)

E.C: 3.4.21.5



3.4.21.5

IDE.A

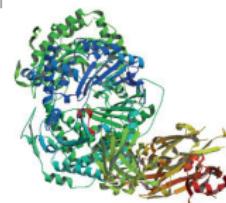
IDE.B

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

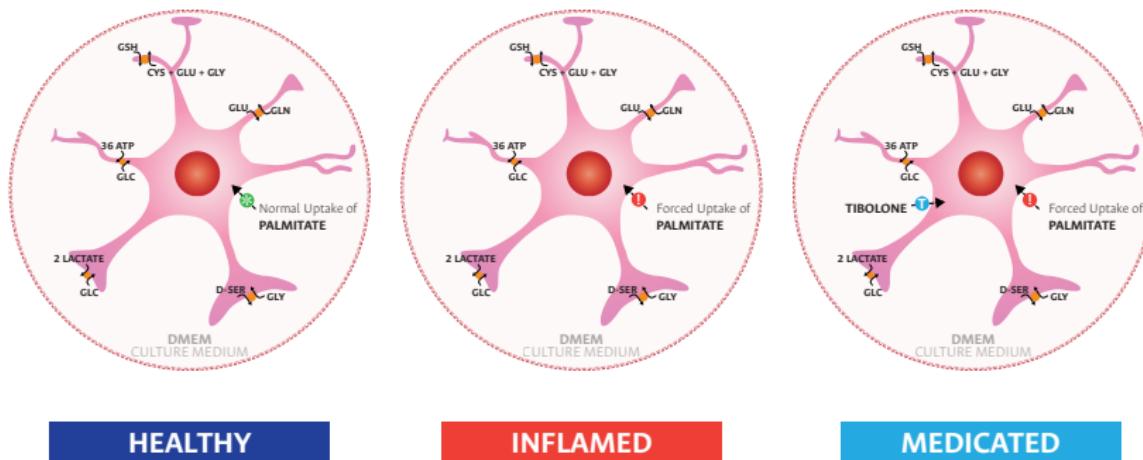
METABOLIC MODEL
WITH GPR



OBJECTIVE 2:

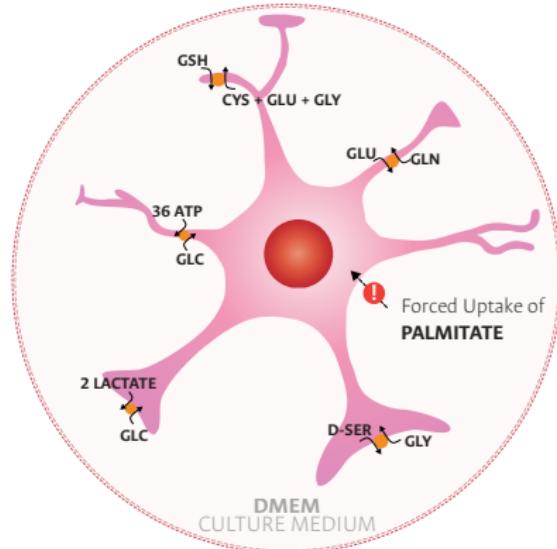
Evaluate the effects caused by the increase of free fatty acids and tibolone presence in astrocytes metabolism.

Metabolic Scenarios



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

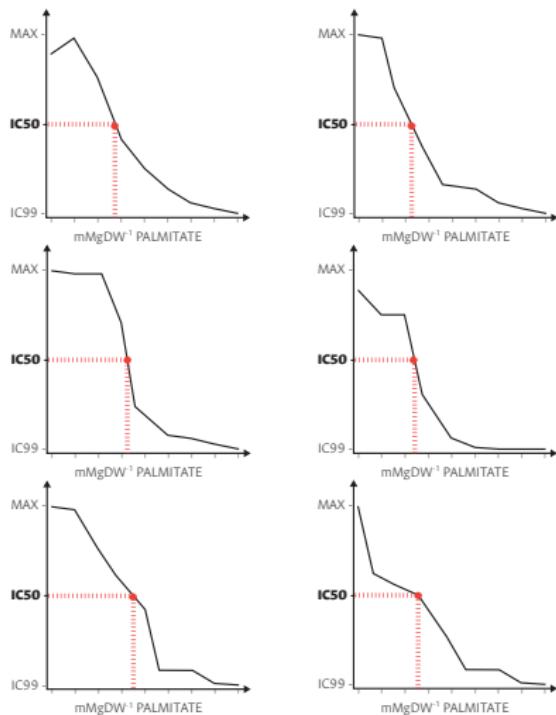
Inflamed Metabolic Scenario



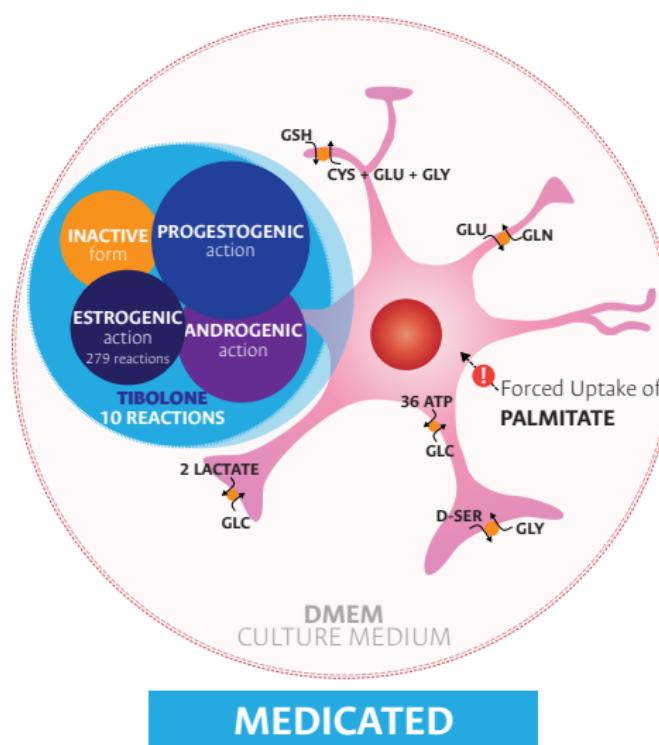
PALMITATE UPTAKE RATE: AVERAGED IC₅₀ VALUES

INFLAMED

ROBUSTNESS ANALYSIS



Medicated Metabolic Scenario



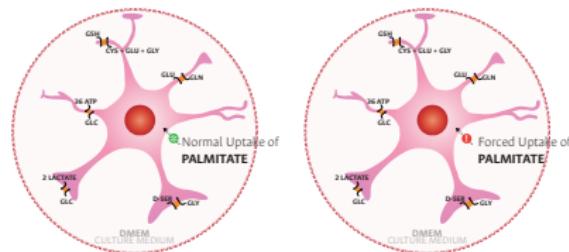
MEDICATED

OBJECTIVE 3:

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

Metabolic Pathways Activation Pattern Changes

INFLAMMATION CHANGES

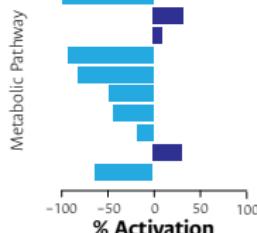


HEALTHY

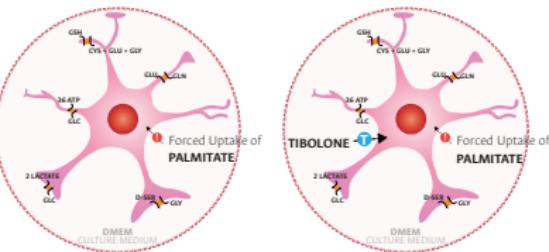
INFLAMED

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

Metabolic Changes



TIBOLONE CHANGES

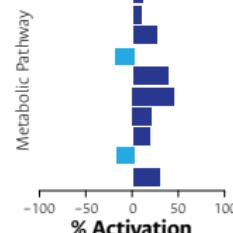


INFLAMED

MEDICATED

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

Metabolic Changes



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

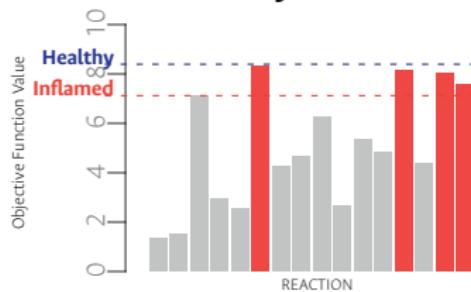


OBJECTIVE 4:

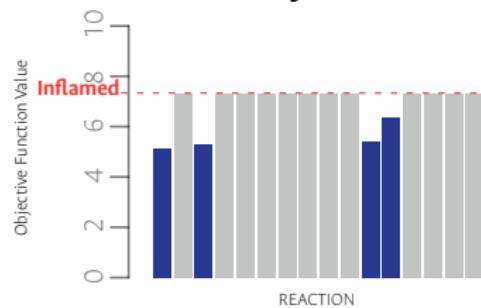
Evaluate the importance of proteins and metabolic pathways previously identified on the dynamics of the metabolic model.

Reaction Knock-out: Protein Importance

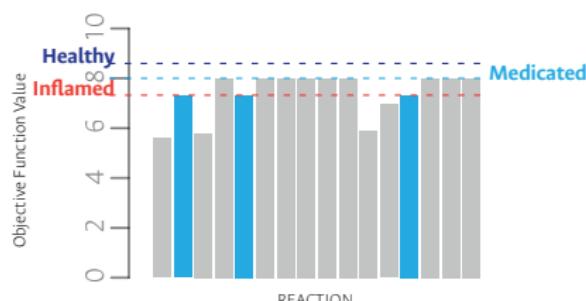
Pro-Inflammatory



Anti-Inflammatory



Tibolone Mechanism



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

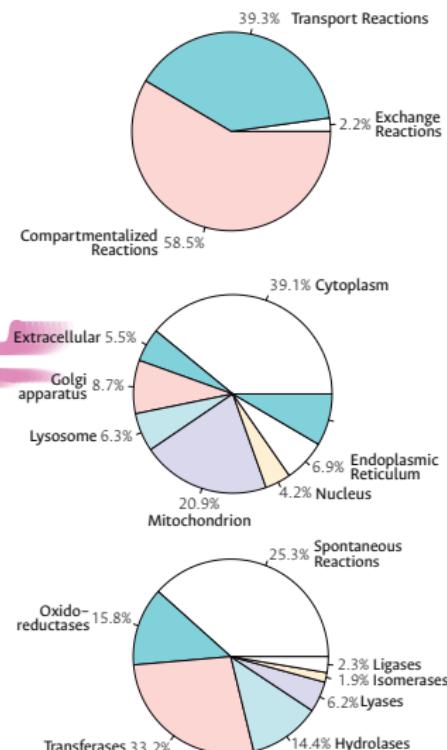
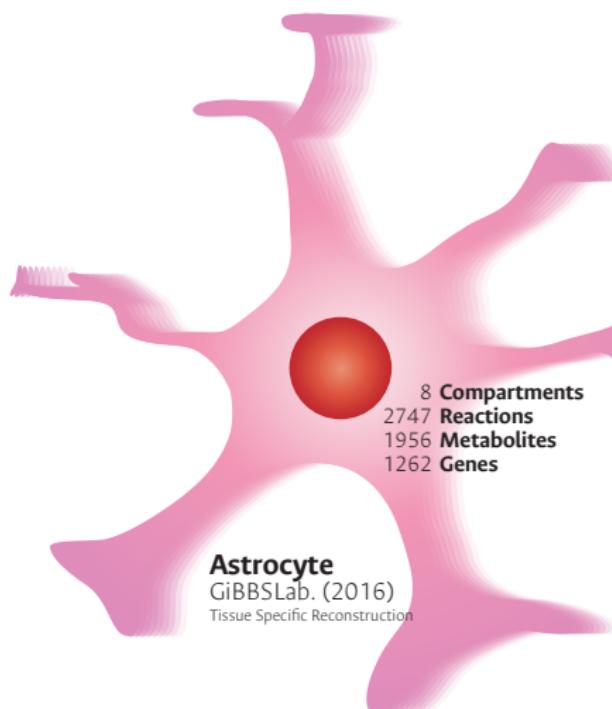


'exp2flux' Package

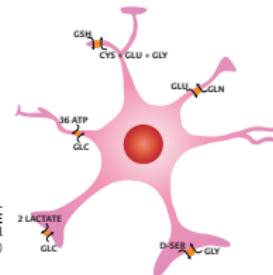
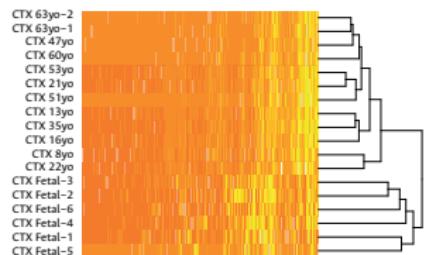
An R Package to convert expression data to FBA fluxes
Daniel Osorio, Kelly Botero, Janneth Gonzalez and Andres Pinzón-Velasco.

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

Tissue Specific Metabolic Reconstruction



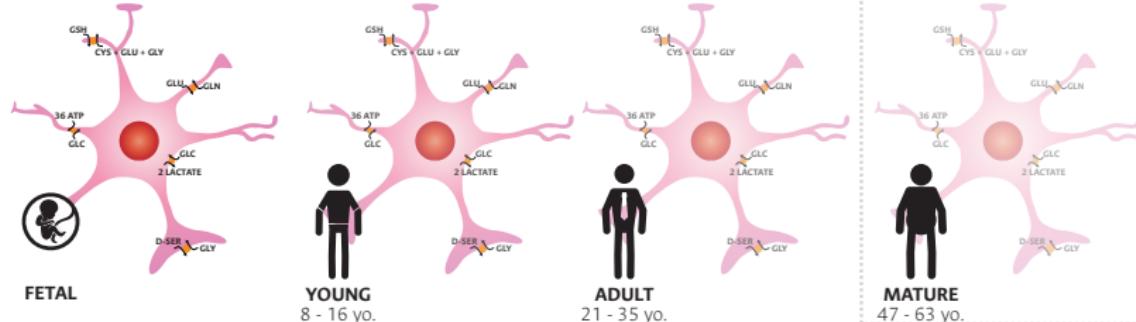
Human Healthy Mature Astrocyte Model



'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

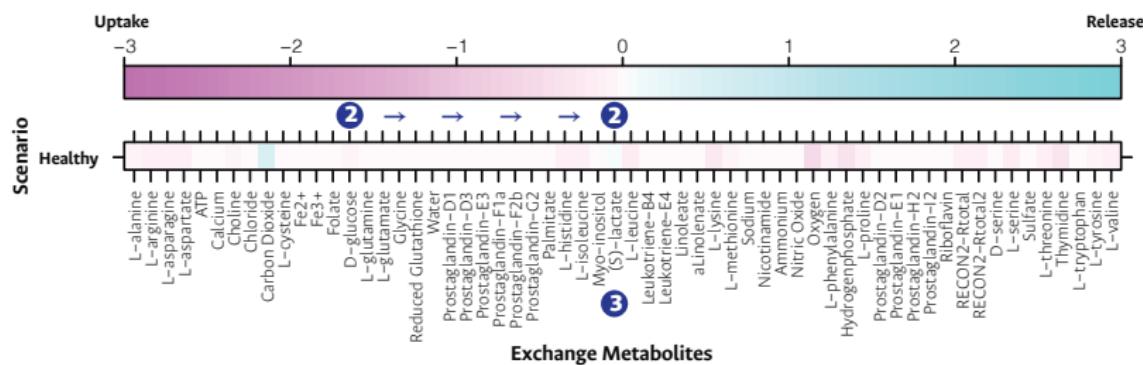


Cellular Maintenance: Healthy Scenario

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

DMEM Culture Medium

METABOLISM ACTIVATION: **52%**



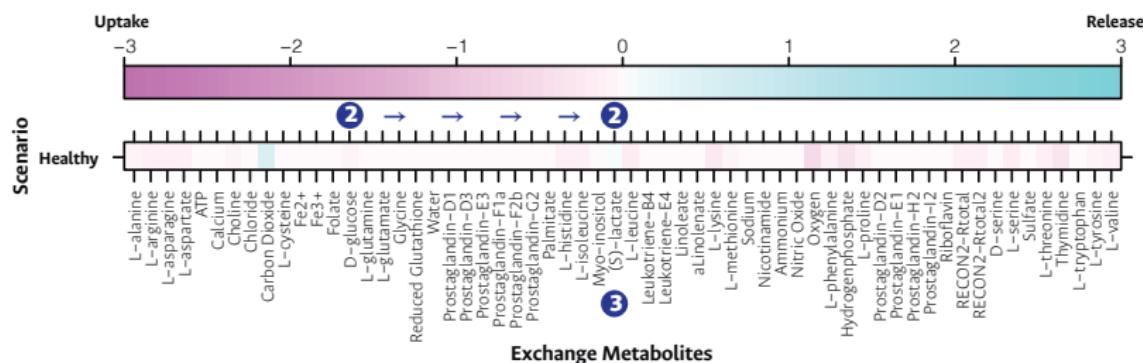
- 1** Arabinda Das, et al. (2010). Flavonoids activated caspases for apoptosis in human glioblastoma T98G and U87MG cells but not in human normal astrocytes.

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.

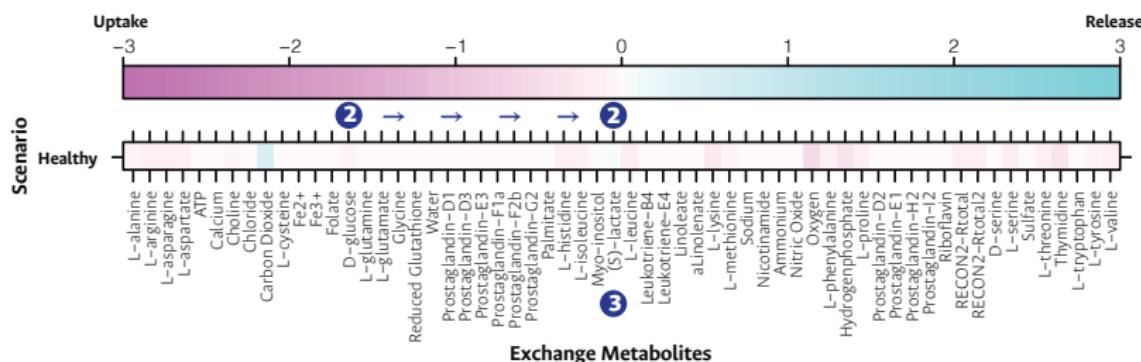
② Rupa Bhowmick, *et al.* (2015). Exploring the differences in metabolic behavior of astrocyte and glioblastoma: a flux balance analysis approach.

Cellular Maintenance: Healthy Scenario

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

DMEM Culture Medium

METABOLISM ACTIVATION: **52%**



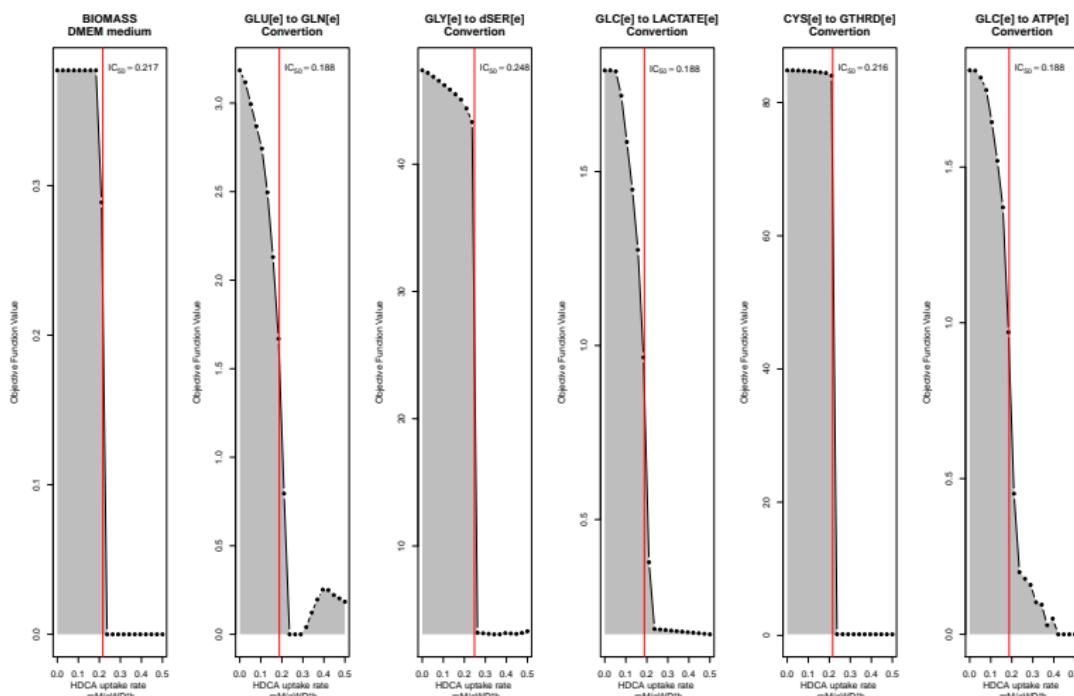
① Arabinda Das, *et al.* (2010). Flavonoids activated caspases for apoptosis in human glioblastoma T98G and U87MG cells but not in human normal astrocytes.

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



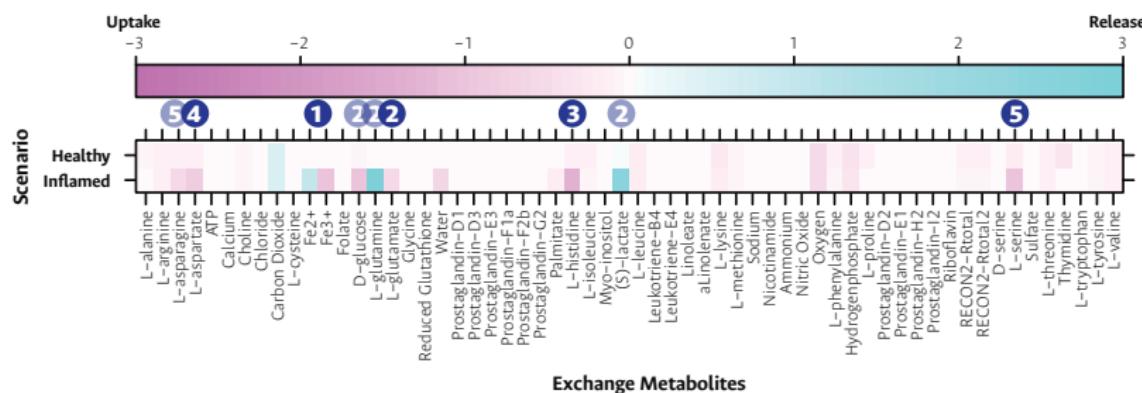
Li Liu et al. (2013) Palmitate-activated astrocytes via serine palmitoyltransferase increase BACE1 in primary neurons by sphingomyelinases.

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%)



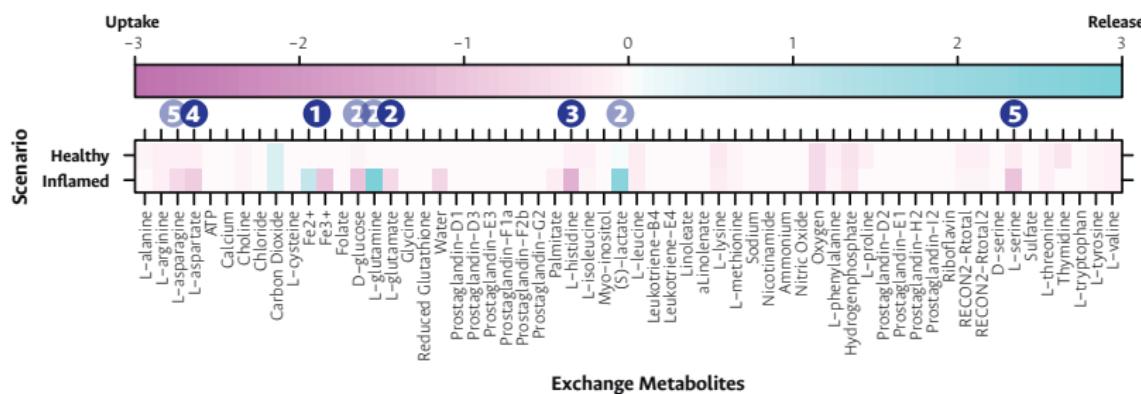
- ① Rachel Williams et al. (2012). Pathogenic implications of iron accumulation in multiple sclerosis.

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%)



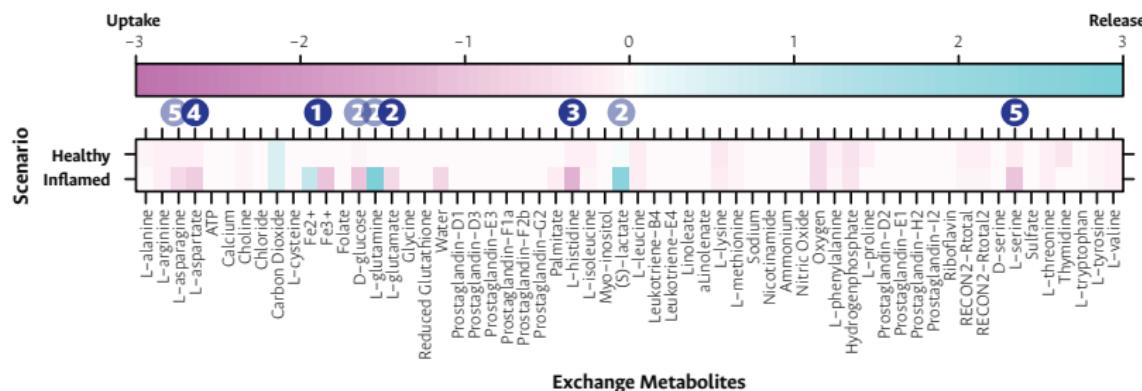
- ① Rachel Williams *et al.* (2012). Pathogenic implications of iron accumulation in multiple sclerosis.
- ② Mithilesh Kumar Jha *et al.* (2016). Metabolic Control of Glia-Mediated Neuroinflammation.
- ② V Pampura and P G Haydon. (2000). Physiological astrocytic calcium levels stimulate glutamate release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

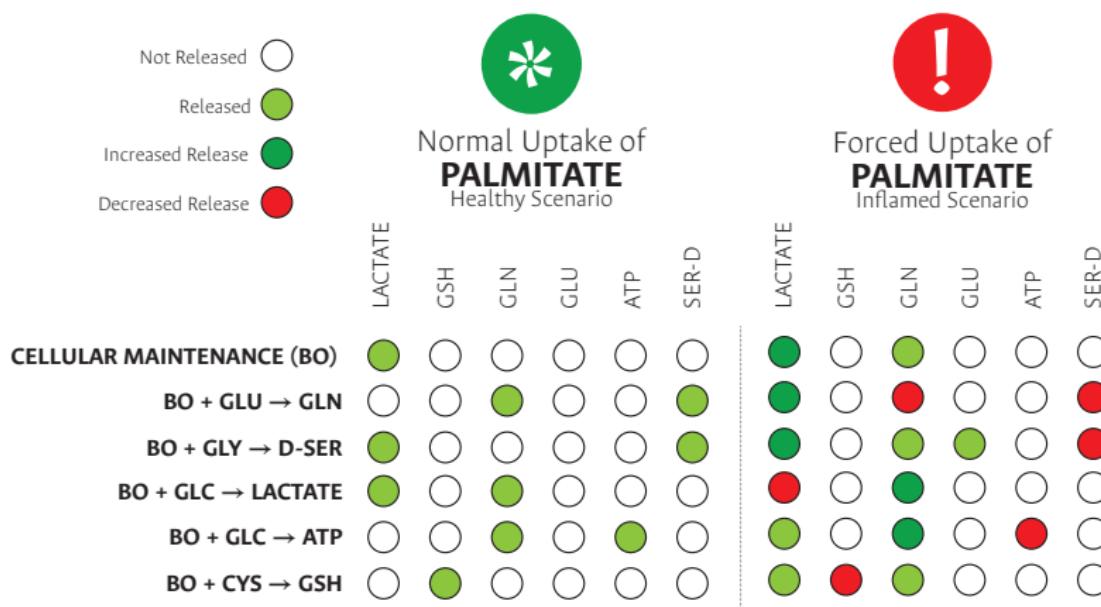
DMEM Culture Medium + 0.208 mM Palmitate

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

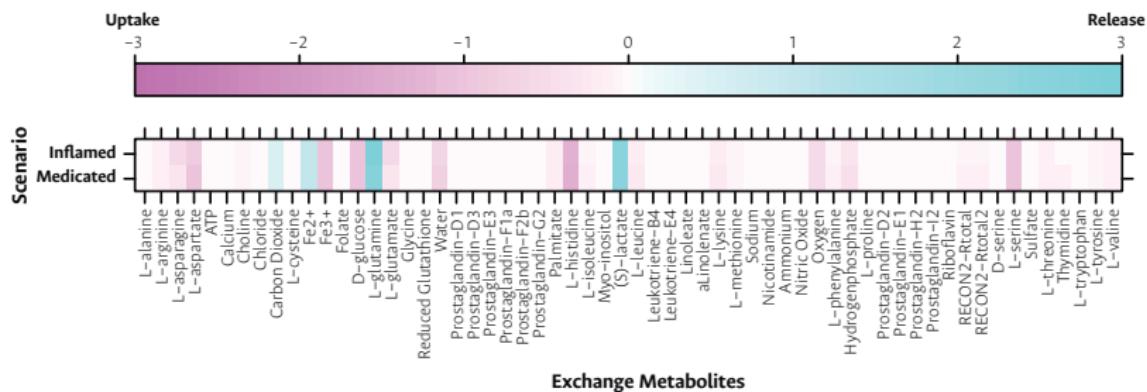


Cellular Maintenance: Medicated Scenario

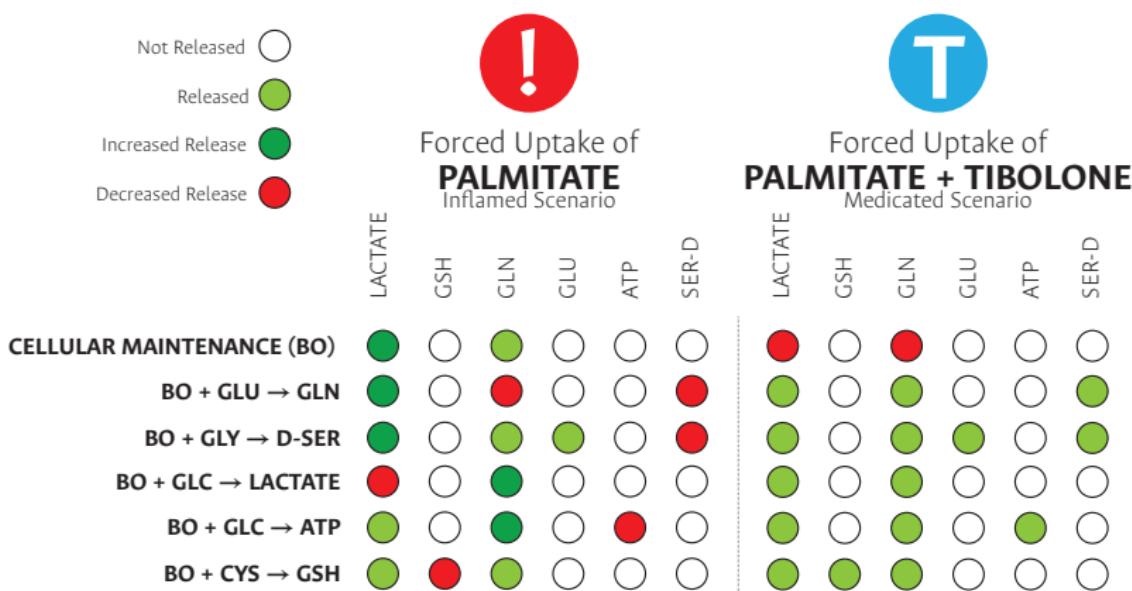
OBJECTIVE FUNCTION: **0.31 mMgWD-1h-1** (-15.6%)

DMEM Culture Medium + 0.208 mM Palmitate

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



Gliotransmitters



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

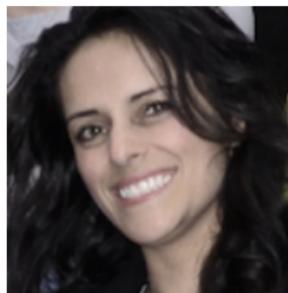
Acknowledgements



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

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Andrés Pinzón PhD

ampinzonv@unal.edu.co