

1 Exploring the neuroprotective effects 2 of tibolone during astrocytic metabolic 3 inflammation: a flux balance analysis 4 approach

5 Abstract:

6 1.1. Introduction

7 Astrocyte-Neuron Metabolic Relationships

8 Astrocytes are the most abundant cells in the human brain and play important roles in
9 the central nervous system (CNS) [1]. They are highly associated to several homeostatic fun-
10 ctions such as glutamate, ion, and water homeostasis, energy storage in the form of glycogen,
11 synapse formation and remodeling, defense against oxidative stress, scar formation, tissue
12 repair and modulation of synaptic activity via the release of gliotransmitters [2]. Astrocytes
13 metabolize glucose in anaerobic way to produce lactate, which is released to neurons through
14 monocarboxylate transporters [3]. Lactate is used in neurons as an energy substrate after
15 its conversion to pyruvate and subsequently to ATP via oxidative phosphorylation [4]. As-
16 trocytes play an important role in glutamate mediated synaptic activity [5]; according to the
17 astrocyte–neuron lactate shuttle model, astrocytes respond to glutamate induced activation
18 by increasing their rate of glucose uptake and the release of lactate into the extracellular spa-
19 ce, increasing the lactate available to be used by neurons to supply their energetic needs [6].
20 Glutamate is uptaked by astrocytes through the glutamate aspartate transporter and glial
21 glutamate transporter-1, inducing events that involves the activation of $\text{Na}^+\text{--K}^+\text{--ATPase}$
22 and maintaining extracellular glutamate at homeostatic levels [7]. Part of incorporated glu-
23 tamate is converted to glutamine through glutamine synthetase, which is only associated to
24 glial cells and released to neurons using electroneutral systems-N transporters coupled to
25 Na^+ and H^+ [8]. In neurons glutaminase enzyme converts glutamine back into glutamate
26 which can be used again for neurotransmission or metabolized into the neuronal Krebs cycle
27 [9]. Astrocytes release many other substances related to synaptic transmission [10]. However
28 D-serine, a neurotransmitter that act as a coagonist with glutamate at NMDA receptors is

one of the most important [5]. Due in brain only glial cells can synthesize serine, all available D-serine at synapsis is associated to be primarily produced and secreted by astrocytes [8]. D-serine is synthesized in astrocytes by serine racemase from L-serine [11]. Additionally to these energy and synaptic support associated functions, astrocytes also play an important role in the reduced glutathione (GSH) metabolism of the brain [12]. GSH is the major cellular antioxidant and plays an important neuroprotective role [13]. Cellular GSH levels are closely correlated with cell survival under adverse conditions [14]. GSH is synthesized from glutamate, cysteine, and glycine and release directly from astrocytes through GSH transporters ion-independent and its net transport is concentration-gradient dependent [15]. This strong metabolic cooperation between astrocytes and neurons allows to predict that even an small astrocytic dysfunction might cause and/or contribute neurodegenerative processes [16]. Homeostatic astrocyte function is required for neuronal survival after different brain insults, such as inflammation, glucose deprivation, traumatic brain injury and ischemia [13, 17]. Astrocytes protect neurons of the most important factors that contribute to neuronal cell death such as glutamate-mediated excitotoxicity leading to disturbances in calcium and sodium intracellular metabolism, mitochondrial dysfunction, oxidative stress, cytokines and toxins [1, 2, 7, 18].

Astrocytes response to Inflammation

Inflammation is a complex biological response to injuries, metabolic disorders or infections and its dysregulation induce many complex diseases through astrocytic dysfunction [13, 19, 20]. In brain, inflammatory response acts as a defense mechanism against any threat to homeostatic state inducing changes in glucose metabolism and release of proinflammatory factors [14]. Inflammation responses in CNS are mediated by glial cells that acquire reactive phenotypes to participate in repair mechanisms [1, 13, 21]. Astrocytes, as glial cells are highly sensitive cells to inflammatory mediators, they respond to inflammation through a complex reaction named astrogliosis [22]. During astrogliosis, glial cells generally associated to several beneficial activities in the CNS, also act as a source of inflammatory mediators and as generators of reactive oxidant species (ROS) that have the potential to damage neurons [23]. Astrogliosis is characterized by a low regulation of mitochondrial dynamics that result in mitochondrial failure [24]. Mitochondrial failure induces the deregulation of Ca^{2+} homeostasis and increased ROS generation, both of which are linked to neurotoxicity [2]. At metabolic level, inflammatory process has been associated to an increase of free saturated fatty acid in comparison with healthy conditions in some brain tissues [25]. The increase of free saturated fatty acid induce metabolic inflammation, a response associated with the induction of diverse intracellular stresses, such as mitochondrial oxidative stress, endoplasmic reticulum stress, and autophagy defects [13]. Lipid excess in metabolic inflammation activates $\text{IKK}\beta$ and $\text{NF-}\kappa\beta$ signaling pathways, which ultimately impairs leptin and insulin hormonal signaling and further triggers the synthesis and release of increased amounts of ROS and proinflammatory

cytokines (TNF- α and IL-6) from glial cells to sustain the neuroinflammatory state [26]. Enhanced ROS generation by reactive glial cells trigger mitochondria dysfunction in neuron, which induces neuronal apoptosis, the prerequisite for a diverse number of neurodegenerative conditions [27].

Systems Biology and Inflammation

Inflammatory pathways are evolutionarily conserved, complex, redundant and interconnected [28]. These characteristics difficult each attempt to understand any disease having inflammation at its core using the traditional reductionism-based scientific method and the current regulatory framework [29]. Traditional methods generally focus on single molecules and genes as the targets of study and potential therapy development, nevertheless mechanistic simulation through a translational systems biology methods allows lead to an understanding of the origin of patterns based in omic data integration in order to facilitate the design of novel therapies [30]. Inflammation is a complex system, which is characterized by sensitivity to initial conditions, positive and negative feedback loops, combined robustness and fragility, and emergence of nonintuitive behaviors [31]. Translational Systems Biology to inflammation is focused on simulated clinical trials, trying to progress toward personalized diagnostics, personalized medicine, and the rational design of drugs [28].

Tibolone

Drugs as steroids compounds are the most potent and effective agents in controlling chronic inflammatory diseases [32]. However, steroids prescription is limited due their adverse side effects [33]. Some steroids synthesized in the nervous system, called ‘neurosteroids’, display beneficial neuroprotective properties, which may be of particular importance in the treatment of diseases where inflammation and neurodegeneration is predominant including age-dependent dementia, stroke, epilepsy, spinal cord injury, Alzheimer’s disease (AD) and Parkinson’s disease (PD) [34]. Neuroprotective actions of molecules that may imitate the neuroprotective actions of estereoids without the perjudicial side effects, such as selective estrogen receptor modulators (SERMs) and selective tissue estrogenic activity regulators (STEARs) have been tested in previous studies [35, 36]. Tibolone is one of these compounds with SERMs and STEARs activities, traditionally used as hormone replacement therapy in post-menopausal women [37]. Tibolone has been shown neuroprotective effects in cultured and under ischemia injury rat neurons [38]. Tibolone is a synthetic steroid drug with estrogenic, progestogenic, and weak androgenic actions; is metabolized in three compounds, two major active metabolites, 3 α -hydroxytibolone and 3 β -hydroxytibolone acting as potent agonists of the estrogen receptor (ER) and its metabolite Δ 4tibolone acting as agonists of the progesterone and androgen receptors [39]. Tibolone and their metabolites have tissue selective action mechanisms (progestogenic, androgenic and estrogenic) reported in liver, bone,

103 breast and brain according to receptor interaction and activation [35]. Nevertheless, actually
 104 is not well know the effects of tibolone over glial cells that allows its neuroprotective ac-
 105 tion [17]. Previous studies have shown that 3-hydroxy-metabolites of tibolone exert agonistic
 106 actions on human astrocytes through the activation of estrogen receptors, indicating that
 107 astrocytes are a target for tibolone [38].

108 In this work we simulate the metabolic inflammatory response in healthy mature astrocytes
 109 caused by the increase uptake of palmitate, the most common free saturated fatty acid. We
 110 model and simulate the metabolic response using a translational system biology approach
 111 called Flux Balance Analysis (FBA) described in methods. We focused in identification of
 112 changes in metabolic pathways activation, functional products, gliotransmitter release and
 113 the neuroprotective effects mediated by tibolone in the inflamed scenario.

114 1.2. Material and Methods

115 Tissue Specific Model Construction

116 The tissue specific model construction process started with the identification of all enzyme-
 117 coding genes expressed over the mean in at least 50 % of samples for healthy human astrocy-
 118 tes indexed in the GEO database [40] as GSE73721 [41]. Gene identifiers conversion from
 119 GeneCards[42] to ENTREZ [43] was performed through ‘UniProt.ws’ R Package [44]. Reac-
 120 tions associated with the identified genes were mapped from the Human Genome Scale Me-
 121 tabolic Reconstruction RECON 2.04 downloaded from the VMH Lab (<https://vmh.uni.lu>)
 122 [45]. The R package ‘g2f’ [46] was used to identify and fill the gaps using all no gene as-
 123 sociated reactions included in RECON 2.04, as well as to identify and remove all blocked
 124 reactions from the reconstruction. All reactions involved in the conversion of extracellular
 125 glutamate, glycine, cysteine and glucose to extracellular glutamine, glycine, serine-D, redu-
 126 ced glutathione, lactate and ATP respectively were added. Exchange reactions were limited
 127 to components of the Dulbecco’s Modified Eagle Medium (DMEM) as input and gliotrans-
 128 mitters (glutamine, D-serine, ATP, glutamate), reduced glutathione, lactate, glucose, nitric
 129 oxide, prostaglandins and leukotrienes as output. Finally, syntax, mass-charge validation and
 130 creation of SBML files were carried out through the ‘minval’ R Package [47]. Reaction limits
 131 (upper and lower bounds) were constrained proportional to the mean gene expression repor-
 132 ted for genes included in Gene-Protein-Reaction (GPR) [48] associated to each reaction in
 133 samples of 47 to 63 years old using ‘exp2flux’ R package [49]. All analysis were done by the
 134 ‘sybil’ [50] R Package running under R 3.3.1 [51].

135 Flux Balance Analysis

136 Flux Balance Analysis (FBA) is a linear optimization method for simulating metabolism that
 137 allows to identify the set of reactions involved in the production of a biological response within

a metabolic model [52]. The metabolic reactions are represented internally as a stoichiometric matrix (S), of size $m * n$, where m represents the compounds and n the reactions; the entries in the matrix are the stoichiometric coefficients of the metabolites participating in a reaction [53]. The flux through all of the reactions in a network is represented by the vector v , which has a length of n . The concentrations of all metabolites are represented by the vector x , with length m . The systems of mass balance equations at steady state, $\frac{d_x}{d_t} = 0$ or $S * v = 0$. FBA seeks to maximize or minimize an objective function which can be any linear combination fluxes, to obtain a flux for each reaction, indicating how much each reaction contributes to the objective function [52]. FBA for healthy, inflamed and medicated scenarios was resolved using GLPK 4.60, setting the generic human biomass reaction included in RECON 2.04 and each one of reactions described in table **1-1** as objective functions. Models were analyzed by comparing fluxes between scenarios, metabolites production rate and sensitivity analysis.

Table 1-1: Main metabolic capabilities associated to astrocytes represented as the set of objective functions used to evaluate neuroprotective effects of Tibolone under inflamed scenarios

ID	FORMULA REACTION	DESCRIPTION
Glu2Gln	1 glu_L[e] \Rightarrow 1 gln_L[e]	Glutamate - Glutamine Cycle
Gly2SerD	1 gly[e] \Rightarrow 1 ser_D[e]	Glycine to D-serine conversion
Glc2Lac	1 glc_D[e] \Rightarrow 2 lac_L[e]	Lactate production from Glucose
Glc2ATP	1 glc_D[e] \Rightarrow 36 atp[e]	ATP production from Glucose
Cys2GTHRD	1 cys_L[e] + 1 glu_L[c] + 1 gly[c] \Rightarrow 1 gthrd[e]	Catch of Cysteine to produce reduced Glutathione

Metabolic Scenarios

To test neuroprotective effects of tibolone during astrocytic metabolic inflammation we define three different metabolic scenarios. A ‘healthy’ scenario, where palmitate uptake rate was freely set by optimizer; an ‘inflamed’ scenario, where uptake rate of palmitate was forced to be stable in the mean of the half maximal inhibitory concentration (IC50) value for all objective functions included in table **1-1**. IC50 values were calculated through a robustness analysis performed using uptake of palmitate (‘EX_hdca(e)’ in RECON 2.04) as control reaction and a 1000 points in the range from 0 to 1 mMgDW⁻¹h⁻¹ for each objective function. Uptake value where each objective function reached IC50 was selected and subsequently averaged. Finally, a medicated scenario, defined as an inflamed scenario that include 279 reactions associated with tibolone and estradiol-derived compounds metabolism. Ten

162 specific reactions described in table 1-2 associated to specific Tibolone action mechanism non included in RECON 2.04 were added to medicated scenario.

Table 1-2: Set of reactions associated to tibolone specific action mechanism in brain reported by Kloosterboer, H. J. (2004) added to medicated scenario model.

ID	FORMULA REACTION	DESCRIPTION
T1	tibolone[e] \Leftrightarrow	Tibolone exchange reaction
T2	tibolone[e] \Leftrightarrow a3OHTibolone[e]	3 α hydroxytibolone interconversion
T3	tibolone[e] \Leftrightarrow b3OHTibolone[e]	3 β hydroxytibolone interconversion
T4	tibolone[e] \Rightarrow d4tibolone[e]	Δ^4 tibolone isomer formation
T5	b3OHTibolone[e] \Rightarrow d4tibolone[e]	Δ^4 tibolone isomer formation from 3 β -hydroxytibolone
T6	a3OHTibolone[e] \Rightarrow estradiol[c]	Estradiol receptor agonist action mechanism of 3 α -hydroxytibolone
T7	b3OHTibolone[e] \Rightarrow estradiol[c]	Estradiol receptor agonist action mechanism of 3 β -hydroxytibolone
T8	d4tibolone[e] \Rightarrow prgstrn[c] + tststerone[c]	Progesterone and androgen receptor activation by tibolone Δ^4 isomer
T9	a3OHTibolone[e] \Leftrightarrow a3SOTibolone[e]	3 α hydroxytibolone interconversion to sulfated inactive compounds
T10	a3SOTibolone[e] \Rightarrow	Tibolone inactive form in blood

163

164 Metabolic Changes

Metabolic changes across metabolic scenarios were measured through two different approximations. Flux differences for each reaction between optimized scenarios were measured using the fold change calculated as described in equation 1-1.

$$foldChange = \frac{valueModel2 - valueModel1}{|valueModel1|} \quad (1-1)$$

165 Additionally, to obtain a full perspective about inflammation effects in metabolites pro-
 166 duction, the production of each metabolite was set as objective function in each metabolic
 167 scenario and differences were evaluated as well as flux differences.

Proinflammatory, Antiinflammatory and Tibolone Action Mechanism Associated Enzymes

Identification of enzymes involved in proinflammatory and antiinflammatory responses as well as in the tibolone action mechanism were identified through several sensitivity analysis as follows: Proinflammatory enzymes, are those that catalyze reactions that being knocked out allows an increase of objective function value. Antiinflammatory enzymes, are those associated to reactions that being knocked out reduce even more the objective function value. Tibolone action mechanism associated enzymes are those that catalyze reactions that being knocked out inhibit entirely the metabolic effect of tibolone.

1.3. Results

Tissue Specific Metabolic Model

Astrocyte tissue-specific metabolism model has a total of X reactions, with X exchange reactions and X transport reactions. Reactions included in astrocytes metabolism model can be classified on the basis of enzymatic activity (E.C. Number), sub-cellular locations (Compartments), and metabolic pathways (Fig. ??).

Based in the associated enzyme to each reaction X % of them are catalyzed by an oxidoreductase enzyme, X % by an enzyme, X % by a

A large number of the reactions in the model belonged to the class 1 category of enzyme classification i.e., the oxidoreductases (22 %). These set of enzymes catalyze the oxidation of one chemical species and the simultaneous reduction of the other by transfer of electrons from one species to another. The other classes of enzymes in this classification scheme were the transferases (14 %) followed by lyases (10 %), hydrolases (4 %), isomerases (2 %), and ligases (2 %). Another 28 % of the reactions belonged to transport reactions and 16 % to extracellular exchange reactions, which occurred spontaneously in the system (Fig. 1a). The reactions can also be classified on the basis of their association with genes to understand gene reaction associations (Fig. 1b). 60 % of the model reactions were gene-associated, out of which 6 % were transport reactions. The rest of the reactions were classified as: Non-Gene associated Exchange Reactions (16 %), Non-Gene associated Intracellular Reactions (2 %) and Non-Gene associated Transport Reactions (22 %). In the classification shown in Fig. 1c, the cytosolic and mitochondrial reactions contributed to 54 % of the total reactions in the model. 2 % of the reactions belonged to the mitochondrial intermembrane space model compartment that specifically accounted for oxidative phosphorylation. The transport reactions were categorized according to the membrane to which it is associated. Transports accounted for 30 % of the total reactions: Mitochondrial membrane spanning (11 %), Nuclear membrane spanning (2 %) and Plasma Membrane spanning (17 %). With reference to the metabolic processes, 23 % of the reactions belonged to fatty acid metabolism inclusive of

both biosynthesis and beta oxidation of palmitic acid. The rest of the pathways contributed to 30 % of the total count of which 14 % belonged to Glycolytic, PPP, TCA cycle and Oxidative phosphorylation pathway and 2 % were contributed each by Glycine–Serine Metabolism, Cysteine Metabolism, Methionine Metabolism and Glutamate Metabolism, without taking into account the transport and exchange reactions. Another set of reactions, namely, cytosolic ATPase (ATPS), cytoplasmic malate dehydro- genase (MDH(Cyto)), Phosphoenolpyruvate carboxykinase (GTP) (PEP_CarbK_1), mitochondrial pyruvate carboxy- lase (Pyr_Carbm) which could not be assigned strictly under any particular pathway, were categorized as ‘Others’ which contributed 2 % of reactions to the (Fig. 1d).

213 **Healthy Scenario**

214 **Inflamed Scenario**

215 **Medicated Scenario**

216 **1.4. Conclusion**

Bibliography

- 218 [1] Kazuhiro Takuma, Akemichi Baba, and Toshio Matsuda. Astrocyte apoptosis: Impli-
219 cations for neuroprotection. *Progress in Neurobiology*, 72:111–127, 2004.
- 220 [2] Sofie C. Lange, Lasse K. Bak, Helle S. Waagepetersen, Arne Schousboe, and Michael D.
221 Norenberg. Primary cultures of astrocytes: Their value in understanding astrocytes in
222 health and disease. *Neurochemical Research*, 37:2569–2588, 2012.
- 223 [3] Harold K Kimelberg. Functions of Mature Mammalian Astrocytes: A Current View.
224 *The Neuroscientist*, 16(1):79–106, feb 2010.
- 225 [4] Nicola J Allen and Ben Barres. Neuroscience: Glia - more than just brain glue. *Nature*,
226 457(7230):675–677, 2009.
- 227 [5] Michael M Halassa and Philip G Haydon. Integrated brain circuits: astrocytic networks
228 modulate neuronal activity and behavior. *Annual review of physiology*, 72(2):335–355,
229 2010.
- 230 [6] Christian Giaume, Annette Koulakoff, Lisa Roux, David Holcman, and Nathalie Rouach.
231 Astroglial networks: a step further in neuroglial and gliovascular interactions. *Nature*
232 *reviews. Neuroscience*, 11(fEbRuARy):87–99, 2010.
- 233 [7] Cora H. Nijboer, Cobi J. Heijnen, Vincent Degos, Hanneke L M Willemsen, Pierre Gres-
234 sens, and Annemieke Kavelaars. Astrocyte GRK2 as a novel regulator of glutamate
235 transport and brain damage. *Neurobiology of Disease*, 54:206–215, 2013.
- 236 [8] Ben Barres. The mystery and magic of glia: a perspective on their roles in health and
237 disease. *Neuron*, 60(3):430–40, nov 2008.
- 238 [9] Jun Shen. Modeling the glutamate-glutamine neurotransmitter cycle. *Frontiers in*
239 *Neuroenergetics*, 5(JAN):1–13, 2013.
- 240 [10] Francesco Petrelli and Paola Bezzi. Novel insights into gliotransmitters. *Current Opinion*
241 *in Pharmacology*, 26(Table 1):138–145, 2016.
- 242 [11] Andrea R Durrant and Uriel Heresco-Levy. D-Serine in Neuropsychiatric Disorders:
243 New Advances. *Advances in Psychiatry*, 2014:1–16, 2014.

- [12] Stephen P. Raps, James C K Lai, Leif Hertz, and Arthur J L Cooper. Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons. *Brain Research*, 493(2):398–401, 1989.
- [13] Mithilesh Kumar Jha, Dong Ho Park, Hyun Kook, In-Kyu Lee, Won-Ha Lee, and Kyoungso Suk. Metabolic Control of Glia-Mediated Neuroinflammation. *Current Alzheimer research*, 13(4):387–402, 2016.
- [14] Igor Allaman, Mireille Bélanger, and Pierre J. Magistretti. Astrocyte–neuron metabolic relationships: for better and for worse. *Trends in Neurosciences*, 34(2):76–87, feb 2011.
- [15] Xue Feng Wang and Max S. Cynader. Astrocytes provide cysteine to neurons by releasing glutathione. *Journal of Neurochemistry*, 74(4):1434–1442, 2000.
- [16] Nicholas J Maragakis and Jeffrey D Rothstein. Mechanisms of Disease: astrocytes in neurodegenerative disease. *Nature clinical practice. Neurology*, 2(12):679–689, 2006.
- [17] Marco Avila-Rodriguez, Luis Miguel Garcia-Segura, Ricardo Cabezas, Daniel Torrente, Francisco Capani, Janneth Gonzalez, and George E. Barreto. Tibolone protects T98G cells from glucose deprivation. *The Journal of Steroid Biochemistry and Molecular Biology*, 144(8):294–303, 2014.
- [18] Ghulam Hussain, Florent Schmitt, Jean-Philippe Loeffler, and Jose-Luis Gonzalez de Aguilar. Fattening the brain: a brief of recent research. *Frontiers in cellular neuroscience*, 7(September):144, 2013.
- [19] Brent E Masel and Douglas S DeWitt. Traumatic brain injury: a disease process, not an event. *Journal of neurotrauma*, 27(8):1529–1540, 2010.
- [20] Qing Yan. *Systems Biology in Drug Discovery and Development*, volume 662 of *Methods in Molecular Biology*. Humana Press, Totowa, NJ, 2010.
- [21] Michael T. Fitch and Jerry Silver. CNS injury, glial scars, and inflammation: Inhibitory extracellular matrices and regeneration failure. *Experimental Neurology*, 209(2):294–301, 2008.
- [22] James a. Dowell, Jeffrey a. Johnson, and Lingjun Li. Identification of astrocyte secreted proteins with a combination of shotgun proteomics and bioinformatics. *Journal of Proteome Research*, 8(8):4135–4143, 2009.
- [23] Anna V. Molofsk, Robert Krenick, Erik Ullian, Hui Hsin Tsai, Benjamin Deneen, William D. Richardson, Ben. Barres, and David H. Rowitch. Astrocytes and disease: A neurodevelopmental perspective. *Genes and Development*, 26:891–907, 2012.

- [24] Marta Sidoryk-Wegrzynowicz and Michael Aschner. Role of astrocytes in manganese mediated neurotoxicity. *BMC pharmacology & toxicology*, 14:23, 2013.
- [25] Sunita Gupta, Alecia G. Knight, Shruti Gupta, Jeffrey N. Keller, and Annadora J. Bruce-Keller. Saturated long-chain fatty acids activate inflammatory signaling in astrocytes. *Journal of Neurochemistry*, 120(6):1060–1071, 2012.
- [26] Sudarshana Purkayastha and Dongsheng Cai. Neuroinflammatory basis of metabolic syndrome. *Molecular Metabolism*, 2(4):356–363, 2015.
- [27] Kyoungcho Suk. Proteomics-based discovery of biomarkers and therapeutic targets in neurodegenerative diseases: perspective of microglia and neuroinflammation. *Expert Opinion on Therapeutic Patents*, 16(3):237–247, mar 2006.
- [28] Yoram Vodovotz. Translational systems biology of inflammation and healing. *Wound Repair and Regeneration*, 18(1):3–7, 2010.
- [29] Yoram Vodovotz, Yoram Vodovotz, Marie Csete, Marie Csete, John Bartels, John Bartels, Steven Chang, Steven Chang, Gary An, and Gary An. Translational Systems Biology of Inflammation. *PLoS Comput Biol*, 4(4), 2008.
- [30] Gary An, John Bartels, and Yoram Vodovotz. In silico augmentation of the drug development pipeline: Examples from the study of acute inflammation. *Drug Development Research*, 72(2):187–200, 2011.
- [31] Qi Mi, Nicole Yee-Key Li, Cordelia Ziraldo, Ali Ghuma, Maxim Mikheev, Robert Squires, David O Okonkwo, Katherine Verdolini-Abbott, Gregory Constantine, Gary An, and Yoram Vodovotz. Translational systems biology of inflammation: potential applications to personalized medicine. *Personalized Medicine*, 7(5):549–559, 2010.
- [32] Durgaprasad Laveti, Manoj Kumar, R Hemalatha, Ramakrishna Sistla, V G M Naidu, Venu Talla, Vinod Verma, Navrinder Kaur, and Ravinder Nagpal. Anti-inflammatory treatments for chronic diseases: a review. *Inflammation & allergy drug targets*, 12(5):349–61, 2013.
- [33] Paola Albertazzi, Raffaele Di Micco, and Ettore Zanardi. Tibolone: A review, 1998.
- [34] Katarzyna Wojtal, Michał K. Trojnar, and Stanisław J. Czuczwar. Endogenous neuroprotective factors: Neurosteroids, 2006.
- [35] H. J. Kloosterboer. Tibolone: A steroid with a tissue-specific mode of action. In *Journal of Steroid Biochemistry and Molecular Biology*, volume 76, pages 231–238, 2001.
- [36] Sudhaa Sharma, Annil Mahajan, Sudesh Kumar, and Vishal R. Tandon. Tibolone: A selective tissue estrogenic activity regulator, 2006.

- [37] Cees J. Timmer, H. A M Verheul, and D. P. Doorstam. Pharmacokinetics of tibolone in early and late postmenopausal women. *British Journal of Clinical Pharmacology*, 54(2):101–106, 2002.
- [38] M A Altinoz, S B Albayrak, A Karasu, P A Sabanci, M Imer, and A Bilir. The effects of tibolone on the human primary glioblastoma multiforme cell culture and the rat C6 glioma model. *Neurol Res*, 31(9):923–927, 2009.
- [39] Helenius J. Kloosterboer. Tissue-selectivity: the mechanism of action of tibolone. *Maturitas*, 48(SUPPL. 1):30–40, aug 2004.
- [40] Ron Edgar, Michael Domrachev, and Alex E Lash. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res*, 30(1):207–210, 2002.
- [41] Ye Zhang, Steven A. Sloan, Laura E. Clarke, Christine Caneda, Colton A. Plaza, Paul D. Blumenthal, Hannes Vogel, Gary K. Steinberg, Michael S B Edwards, Gordon Li, John A. Duncan, Samuel H. Cheshier, Lawrence M. Shuer, Edward F. Chang, Gerald A. Grant, Melanie G Hayden Gephart, and Ben A. Barres. Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse. *Neuron*, 89(1):37–53, 2016.
- [42] Michael Rebhan, Vered Chalifa-Caspi, Jaime Prilusky, and Doron Lancet. GeneCards: integrating information about genes, proteins and diseases. *Trends in Genetics*, 13(4):163, 1997.
- [43] Donna Maglott, Jim Ostell, Kim D Pruitt, and Tatiana Tatusova. Entrez Gene: gene-centered information at NCBI. *Nucleic acids research*, 33(suppl 1):D54—D58, 2005.
- [44] Marc Carlson. *UniProt.ws: R Interface to UniProt Web Services*, 2016.
- [45] Ines Thiele, Neil Swainston, Ronan M T Fleming, Andreas Hoppe, Swagatika Sahoo, Maike K Aurich, Hulda Haraldsdottir, Monica L Mo, Ottar Rolfsson, Miranda D Stobbe, and Others. A community-driven global reconstruction of human metabolism. *Nature biotechnology*, 31(5):419–425, 2013.
- [46] Kelly Botero, Daniel Osorio, Janneth Gonzalez, and Andres Pinzon. *g2f: Find and Fill Gaps in Metabolic Networks*, 2016.
- [47] Daniel Osorio, Janneth Gonzalez, and Andres Pinzon. minval: MINimal VALidation for Stoichiometric Reactions, 2016.
- [48] Ines Thiele and Bernhard Ø Palsson. A protocol for generating a high-quality genome-scale metabolic reconstruction. *Nature Protocols*, 5(1):93–121, jan 2010.

-
- 342 [49] Daniel Osorio, Kelly Botero, Janneth Gonzalez, and Andres Pinzon. *exp2flux: Convert*
343 *Gene EXPression Data to FBA FLUXes*, 2016.
- 344 [50] Gabriel Gelius-dietrich, Abdelmoneim Amer Desouki, Claus Jonathan Fritzscheier, and
345 Martin J Lercher. *sybil – Efficient constraint-based modelling in R*. 2013.
- 346 [51] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation
347 for Statistical Computing, Vienna, Austria, 2016.
- 348 [52] Jeffrey D Orth, Ines Thiele, and B O Palsson. What is flux balance analysis? *Nature*
349 *Biotechnology*, 28(3):245–248, 2010.
- 350 [53] Karthik Raman and Nagasuma Chandra. Flux balance analysis of biological systems:
351 Applications and challenges. *Briefings in Bioinformatics*, 10(4):435–449, 2009.