University at Buffalo
School of Pharmacy and Pharmaceutical Sciences

Expanding From Basic Towards Systems Pharmacodynamic Models for Methylprednisolone

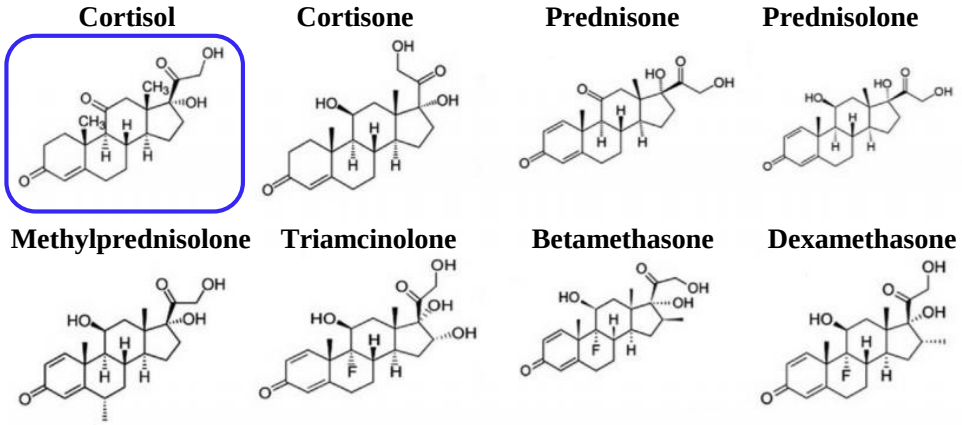
Vivaswath S. Ayyar, Ph.D.

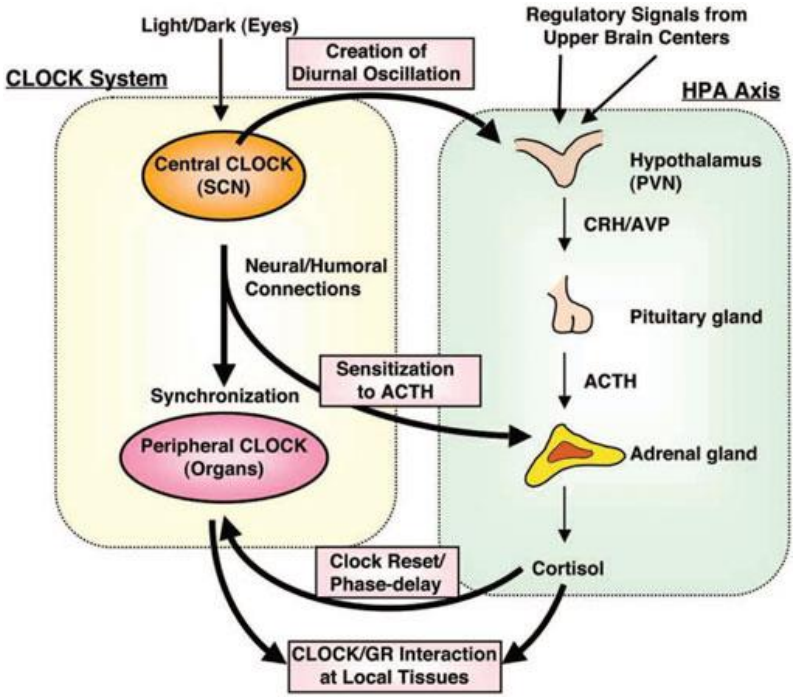
Adjunct Assistant Professor of Pharmaceutical Sciences, UB

PK/PD Scientist, Janssen BioTherapeutics

The Glucocorticoids (GC)

- Steroid hormones that regulate development, metabolism, and immunity
- Produced endogenously from adrenal cortex; regulated by the HPA axis
- Exert biological effects upon binding the ubiquitously expressed glucocorticoid receptor (GR)

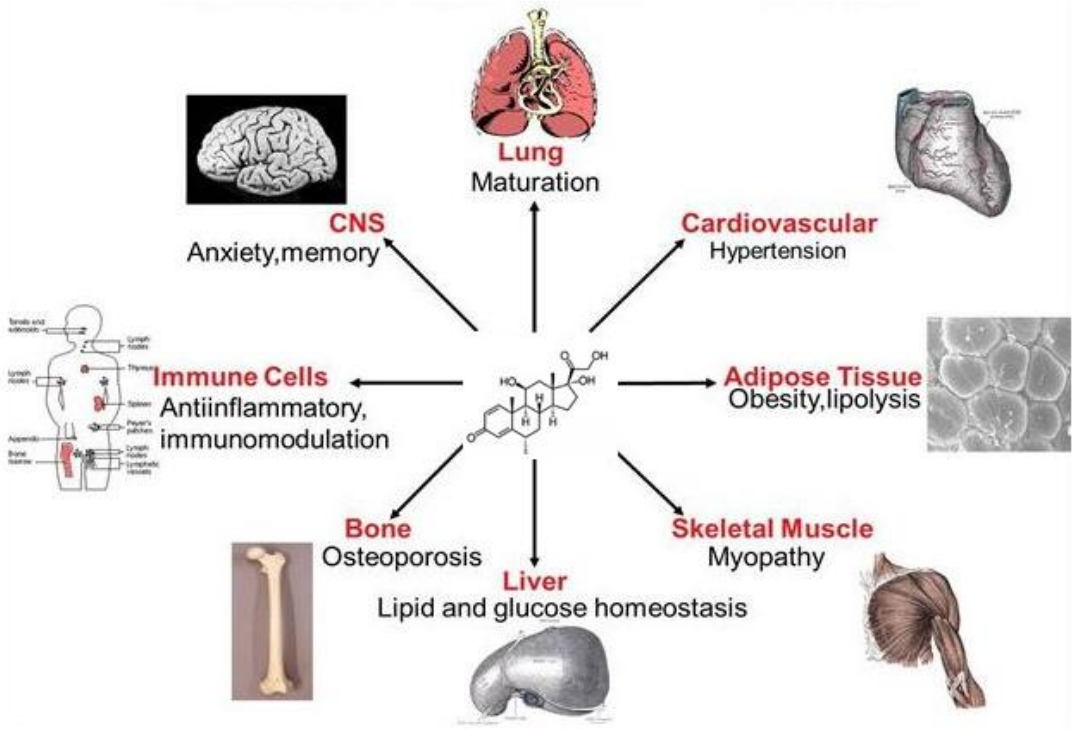




Kassi and Chrousos, *Hormones* 12: 172-191 (2013).
Burns, CM, *Rheum Dis Clin N Am* 42: 1-14 (2016).

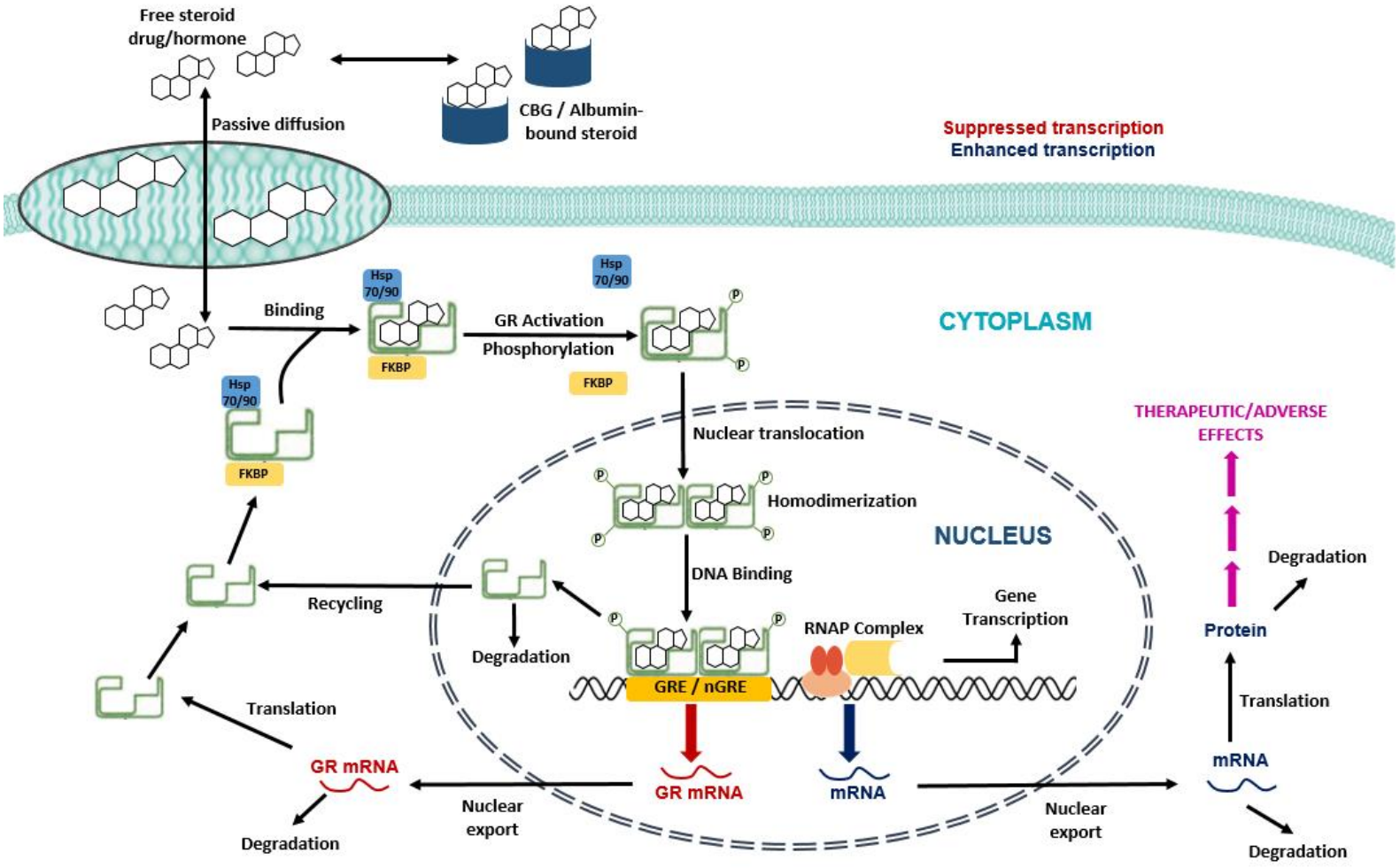
The Corticosteroids (CS)

- Synthetic analogues of the endogenous GC hormone
- Possess potent immunosuppressive efficacy - widely prescribed
- Elicit adverse effects in multiple organs - magnify normal GC functions



Hench PS et al. *Proc Staff Meet Mayo Clin* 24:181-97 (1949).
Kassi and Chrousos, *Hormones* 12: 172-191 (2013).
Burns, CM, *Rheum Dis Clin N Am* 42: 1-14 (2016).

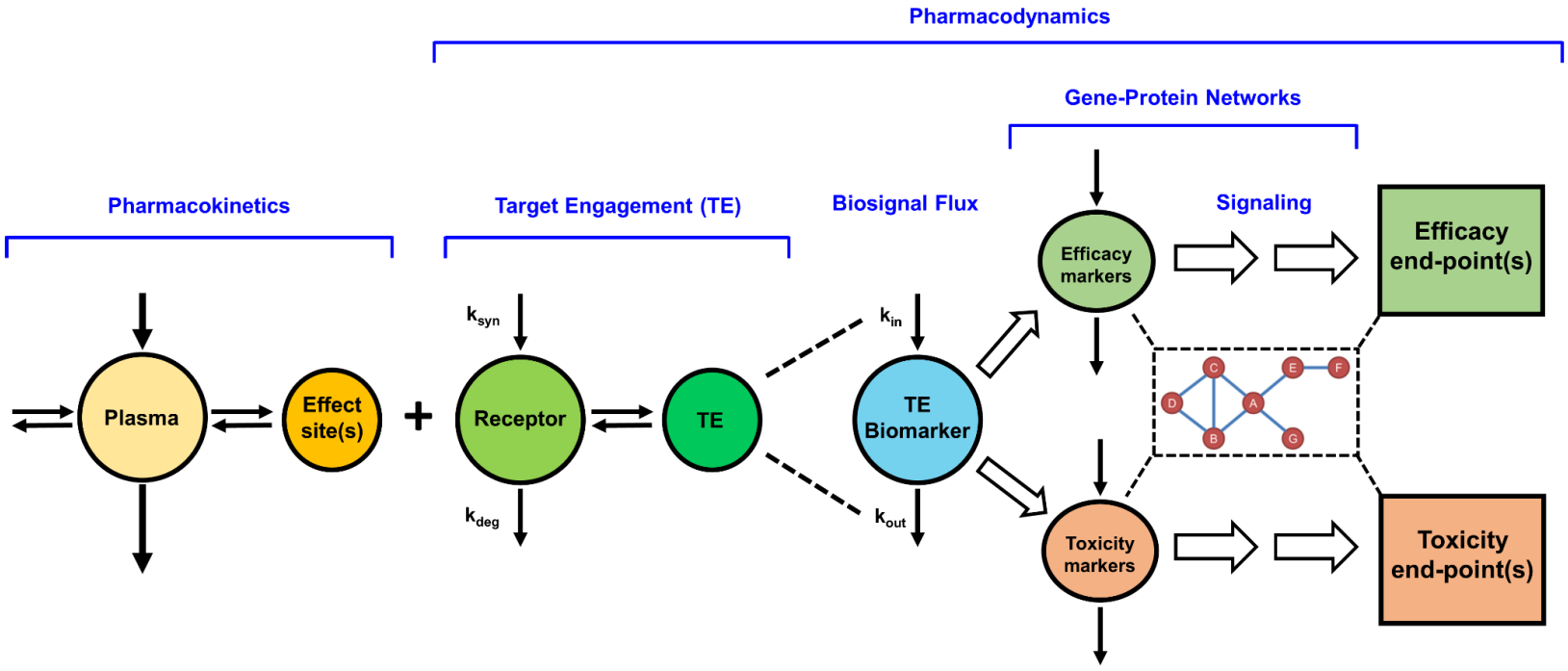
Pharmacogenomic Mechanisms of CS Action



Jusko WJ, *Toxicology* 102: 189-196 (1995).
Almon RR, DuBois DC, Jusko WJ, *Endocrinol* 148: 2209-2225 (2007).
Ayyar VS, Almon RR, DuBois DC, Sukumaran S, Qu J, Jusko WJ, *J Proteomics* 160: 84-105 (2017).
Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Qu J, Jusko WJ, *J Pharmacokinet Pharmacodyn* 45: 557–575 (2018).
Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* 367: 168-183 (2018).

Components of Systems Models for Genomic Drug Actions

Quantitative Systems Pharmacology (QSP) aims to understand drugs at the levels of **target engagement**, changes in **cellular biochemistry**, impact on **human pathophysiology**, and **optimal clinical use** (Sorger et. al. 2011, NIH QSP Whitepaper).



Circadian Rhythms (Non-stationarity)

Tolerance and rebound

System variability (cell, tissue specificity)

Biomarker dynamics (mRNA, protein, activity)

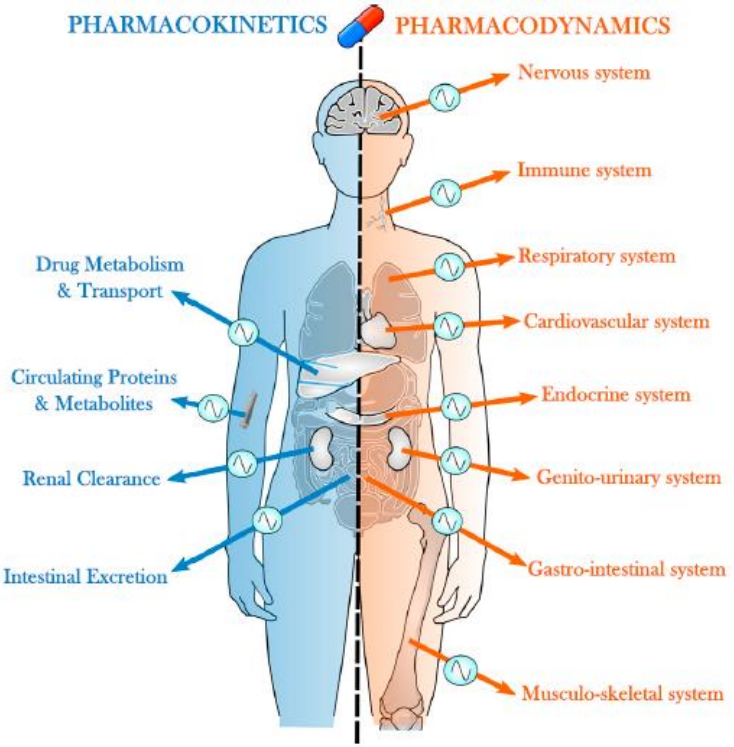
Unbound drug at site of action

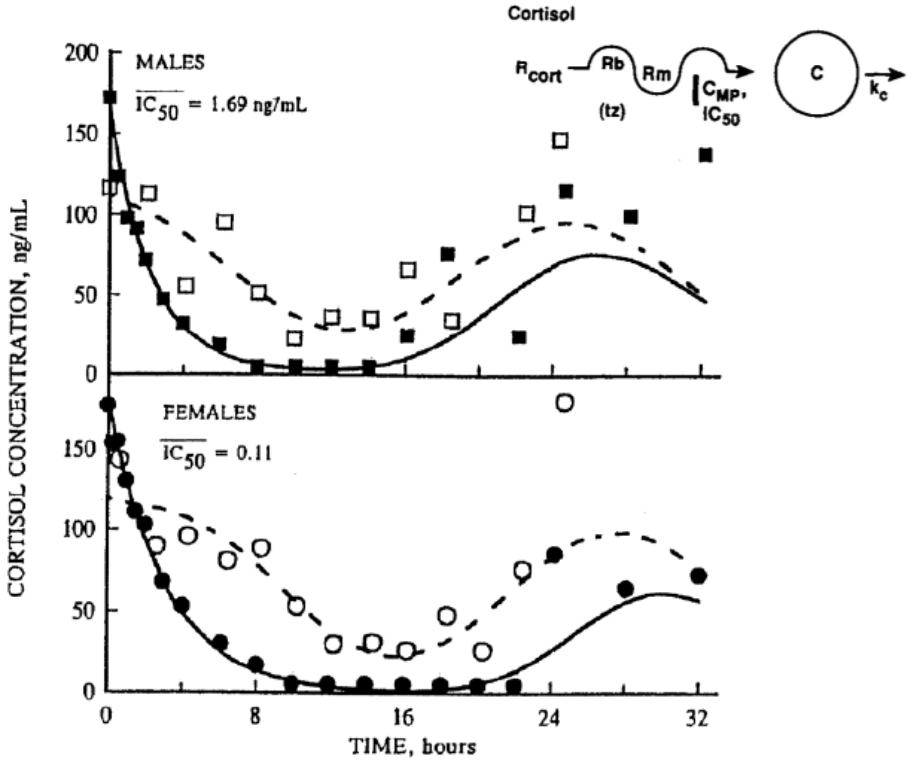
Sex differences

Others (disease, aging, DDI, genetic polymorphisms, epigenetics)

Circadian Rhythms – Relevance to PK/PD

- Occur in physiology at macroscopic (whole-body) to molecular (gene) levels
- Can affect the availability of target and/or effector molecules
- Add time-dependent complexities in PK and PD responses





Jusko WJ, *Toxicology* 102: 189-196 (1995).
Sukumaran S, Almon RR, DuBois DC, Jusko WJ *Adv Drug Deliv Rev* 62: 904-917 (2010).
Ballesta A, Innominato PF, Dallman R, Rand DA, Levi FA *Pharmacol Rev* 69: 161-199 (2017).

GILZ as a Genomic Biomarker & Mediator of CS

Identified in steroid-treated thymocytes (1997)

Factors making GILZ a favorable genomic marker:

- **Expressed in multiple tissues**
- **Correlates, in part, to CS efficacy**
- **Exquisite sensitivity to regulation by CS**

The FASEB Journal • Review

Glucocorticoid-induced leucine zipper (GILZ): a new important mediator of glucocorticoid action

Emira Ayroldi[1] and Carlo Riccardi[1]

Department of Clinical and Experimental Medicine, Section of Pharmacology, University of Perugia, Perugia, Italy

ARTHRITIS & RHEUMATISM
Vol. 62, No. 9, September 2010, pp 2651–2661
DOI 10.1002/art.27566

Glucocorticoid-Induced Leucine Zipper Is an Endogenous Antiinflammatory Mediator in Arthritis

Elaine Beaulieu,[1] Devi Ngo,[1] Leilani Santos,[1] Yuan Hang Yang,[1] Malcolm Smith,[2] Christian Jorgensen,[3] Virginie Escriou,[4] Daniel Scherman,[4] Gabriel Courties,[5] Florence Apparailly,[3] and Eric F. Morand[1]

The Journal of Clinical Investigation http://www.jci.org Volume 117 Number 6 June 2007

GILZ mediates the antiproliferative activity of glucocorticoids by negative regulation of Ras signaling

Emira Ayroldi, Ornella Zollo, Alessandra Bastianelli, Cristina Marchetti, Massimiliano Agostini, Rosa Di Virgilio, and Carlo Riccardi

Department of Clinical and Experimental Medicine, Section of Pharmacology, University of Perugia, Perugia, Italy.

Neurotherapeutics (2012) 9:210–225
DOI 10.1007/s13311-011-0084-7

Glucocorticoid-Induced Leucine Zipper (GILZ) Over-Expression in T Lymphocytes Inhibits Inflammation and Tissue Damage in Spinal Cord Injury

Emanuela Esposito · Stefano Bruscoli · Emanuela Mazzon · Irene Paterniti · Maddalena Coppo · Enrico Velardi · Salvatore Cuzzocrea · Carlo Riccardi

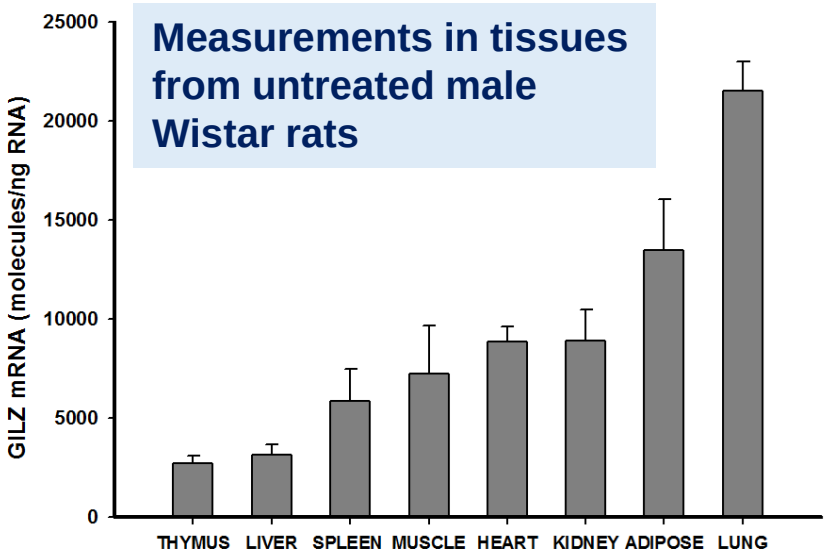
Cell Death and Differentiation (2015) 22, 118–130

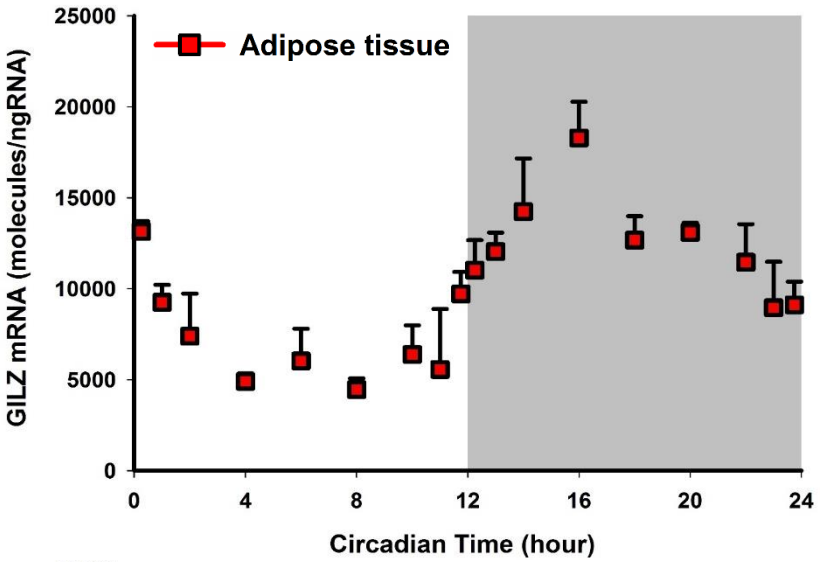
L-GILZ binds p53 and MDM2 and suppresses tumor growth through p53 activation in human cancer cells

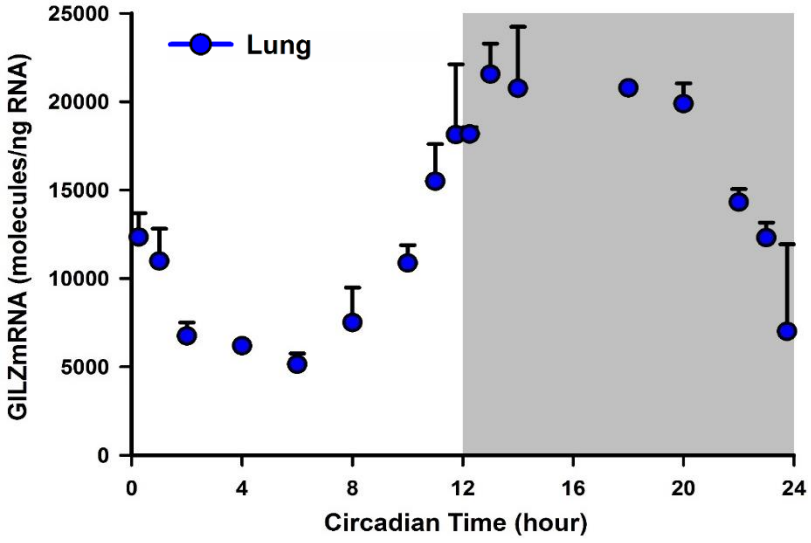
E Ayroldi*[,1], MG Petrillo[1], A Bastianelli[1], MC Marchetti[1], S Ronchetti[1], G Nocentini[1], L Ricciotti[1], L Cannarile[1] and C Riccardi*[,1]

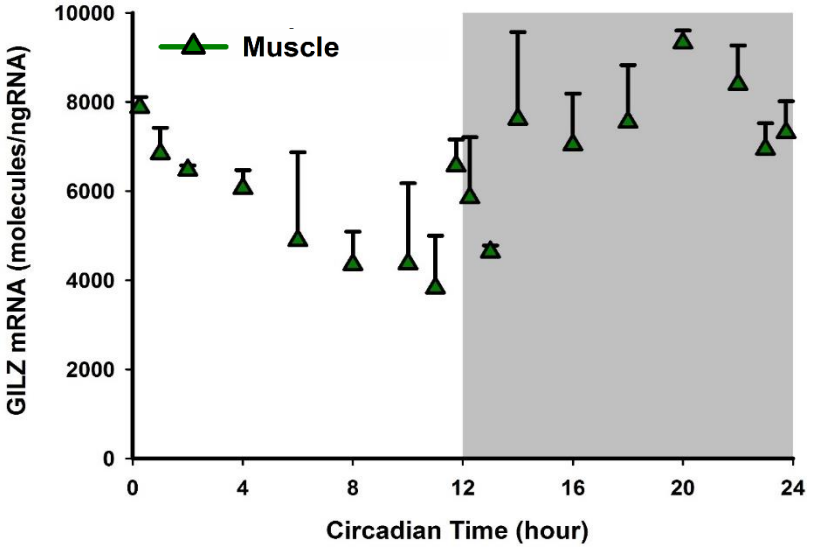
[1]Department Medicine, Section of Pharmacology, University of Perugia Medical School, Perugia, Italy

Basal GILZ Expression and Circadian Regulation







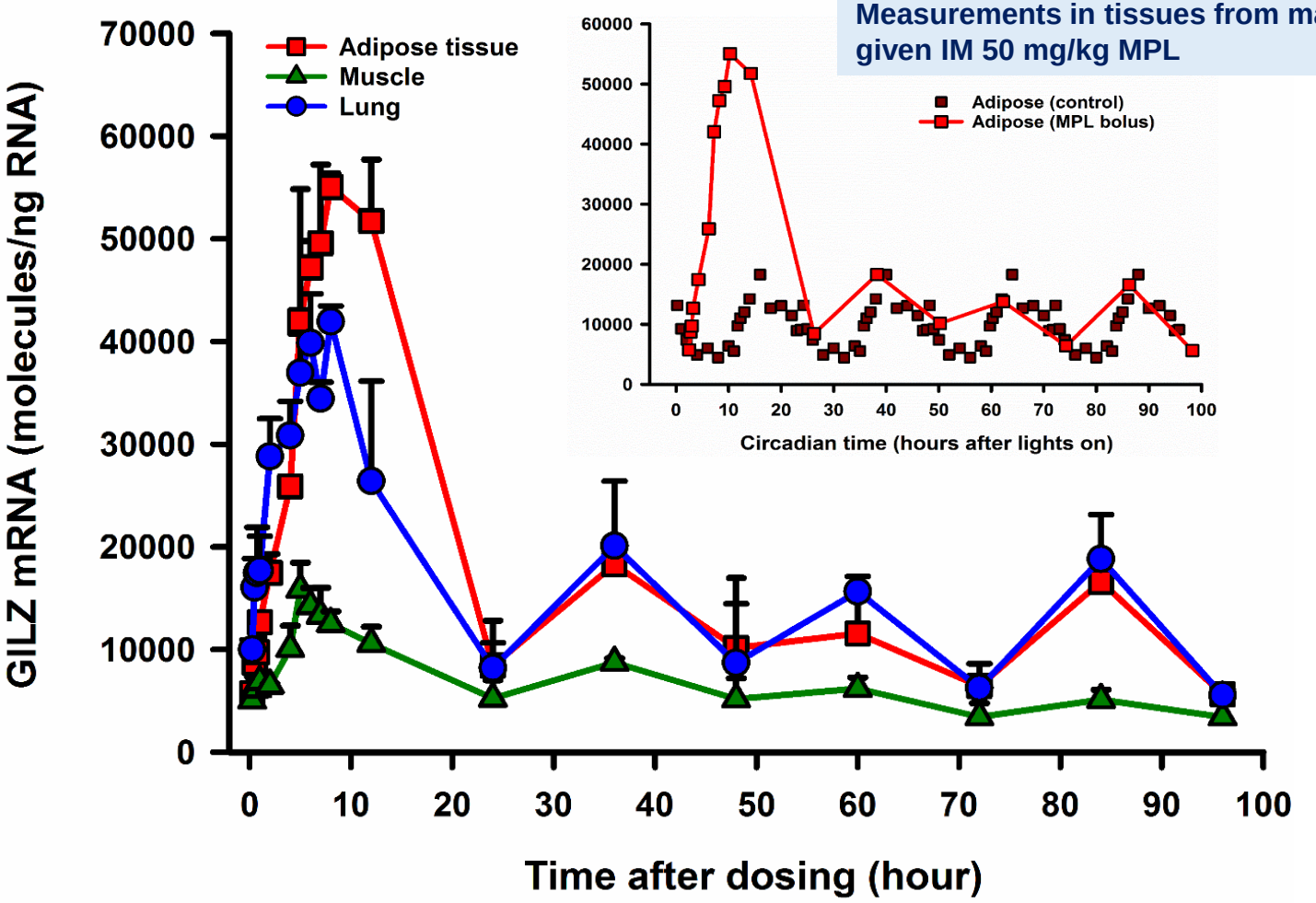


Ayyar VS, Almon RR, Jusko WJ, DuBois DC, *Am J Physiol - Physiol Rep* 6: e12382 (2015).

Enhancement of GILZ mRNA by MPL (IM dosing)

Hypothesis: A mechanistic PK/PD model of transactivation can characterize the multi-tissue dynamics of GILZ regulation upon MPL dosing

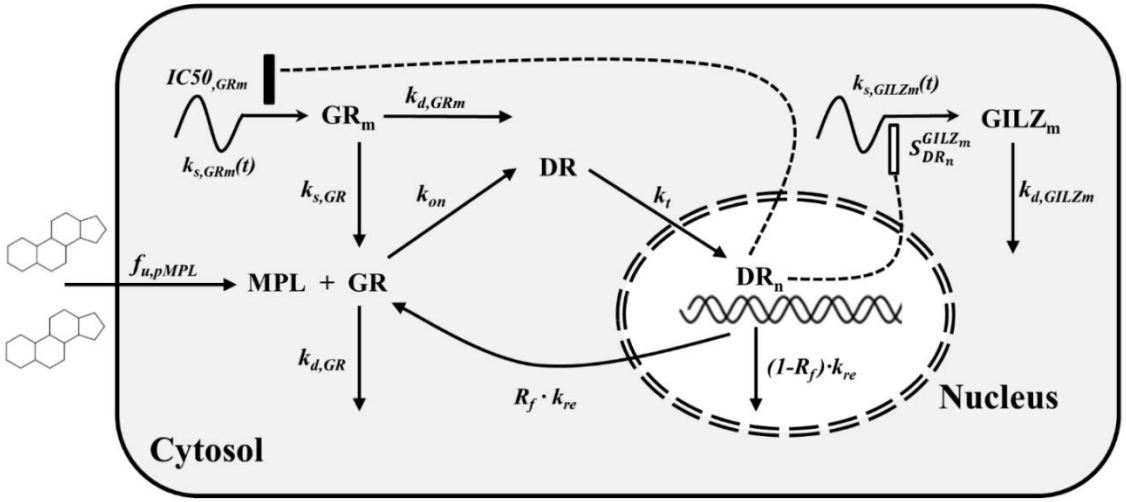
Measurements in tissues from male Wistars given IM 50 mg/kg MPL

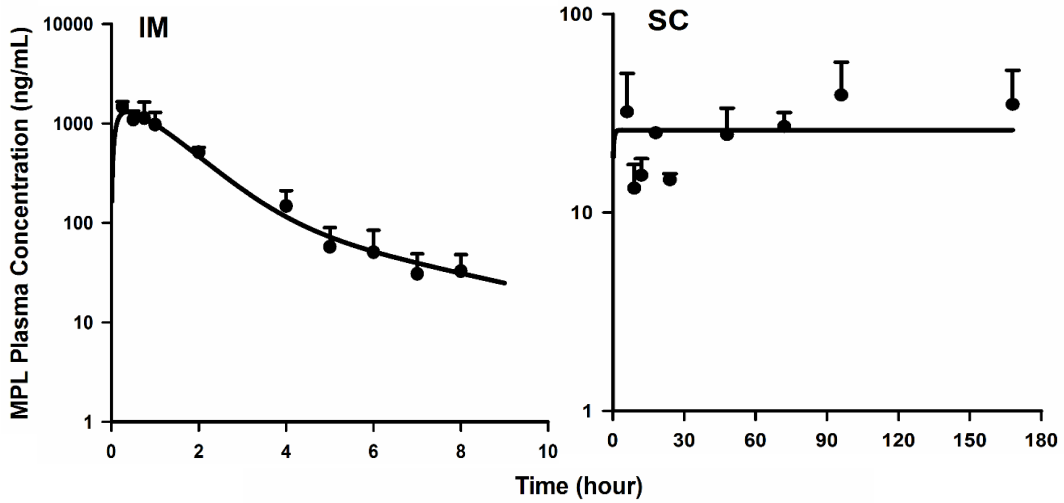


Ayyar VS, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* 363: 45-57 (2017).

Pharmacokinetic/Pharmacodynamic Model of Transactivation

Ramakrishnan R, DuBois DC, Almon RR, Pyszczynski NA, Jusko WJ, *J Pharmacokinet Pharmacodyn* 29: 1-24 (2002).

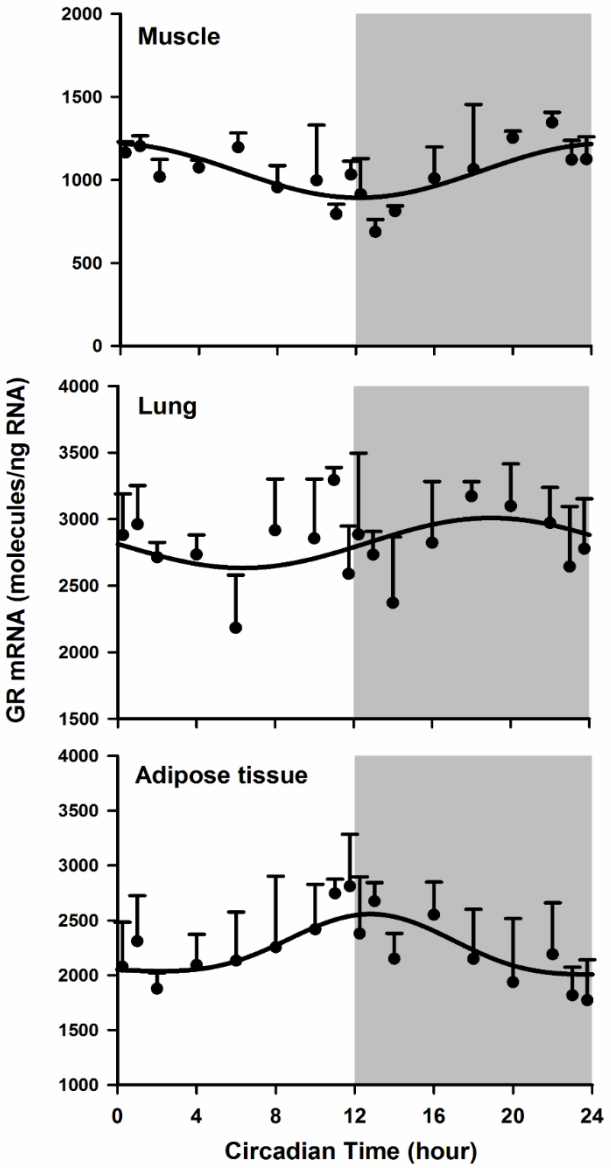


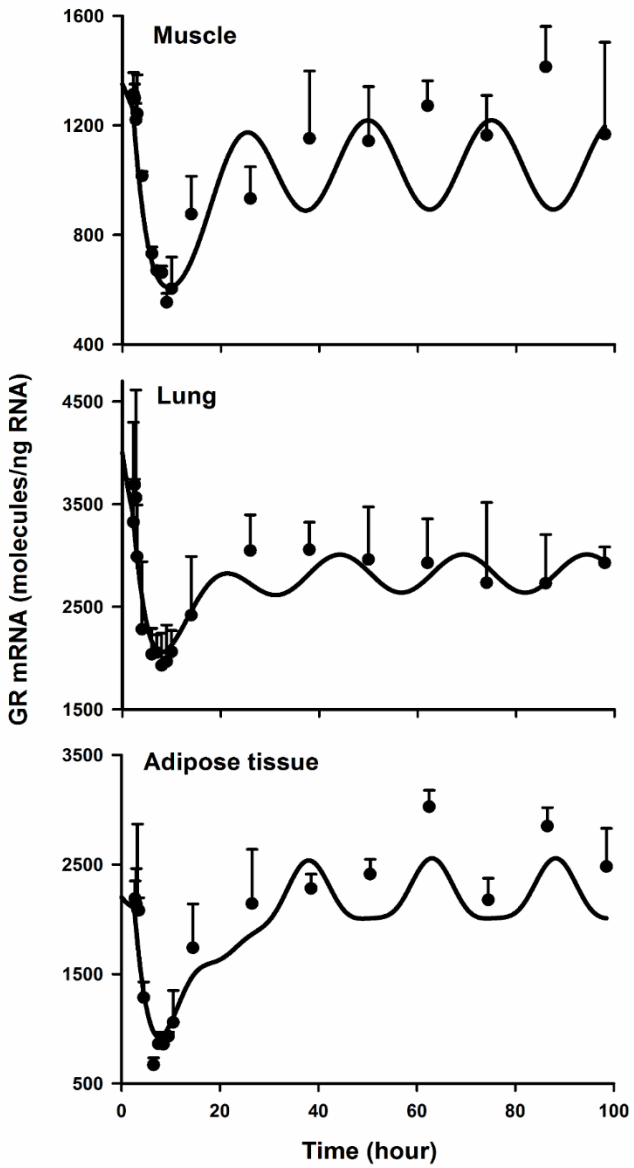


- Implemented using ADAPT 5 and FOURPHARM
- Control and IM bolus data fitted simultaneously
- Model externally validated using chronic infusion study

Krzyzanski W, Chakraborty A, Jusko WJ, *Chronobiol Int* 17: 77-93 (2000).
Hazra A, Pyszczynski N, DuBois DC, Almon RR, Jusko WJ, *Biopharm Drug Dispos* 28: 263-73 (2007).

Glucocorticoid Receptor mRNA (Control & IM MPL)





Glucocorticoid Receptor mRNA (Control & IM MPL)

Parameter	Definition	Estimate (CV%)
$a_{0,GRm}$	Fourier coefficient for GR mRNA	2824[c] / 1055.9[d] / 2216[a,e]
$a_{1,GRm}$	Fourier coefficient for GR mRNA	6.8[c] / 162.2[d] / -273.2[a,e]
$a_{2,GRm}$	Fourier coefficient for GR mRNA	65.9 [a,e]
$b_{1,GRm}$	Fourier coefficient for GR mRNA	185.6[c] / -19.9[d] / -10.9[a,e]
$b_{2,GRm}$	Fourier coefficient for GR mRNA	10.1 [a,e]
$k_{d,GRm}$ (h^{-1})	Degradation rate constant for GR mRNA	0.26[c] (15.4) / 0.28[d] (29.9) / 0.31[a,e]
$k_{s,GR}$ (nM/h)(mol/ng)$^{-1}$	Synthesis rate constant for receptor	0.00025[c] (5.3) / 0.00121[d] (34.5) / 0.00196[a,e]
$IC_{50,GRm}$ (nM^{-1})	Inhibition of GR mRNA production	15.6 [b]
$k_{d,GR}$ (h^{-1})	Degradation rate constant for receptor	0.05 [b]
k_{on} ($nM^{-1}\cdot h^{-1}$)	Association rate constant	0.016 [b]
f_{mpl}	Unbound fraction of MPL in plasma	0.23 [b]
k_{re} (h^{-1})	DR_n loss rate constant	1.31 [b]
R_f	Fraction recycled	0.93 [b]
k_T (h^{-1})	Translocation rate constant	58.3 [b]
$GR_{m,MPL}(0)$ (mol/ng RNA)	GR mRNA initial concentration (treatment)	3995[c] / 1350[d] (10.7) / 2200[a,e]
$GR(0)$ (nM)	Free cytosolic receptor initial concentration	19.7[c] (5.3) / 32.7[d] (34.5) / 86.2[a,e]
$DR(0)$ (nM)	Drug-receptor complex initial concentration	0 (fixed)
$DR_n(0)$ (nM)	Nuclear complex initial concentration	0 (fixed)

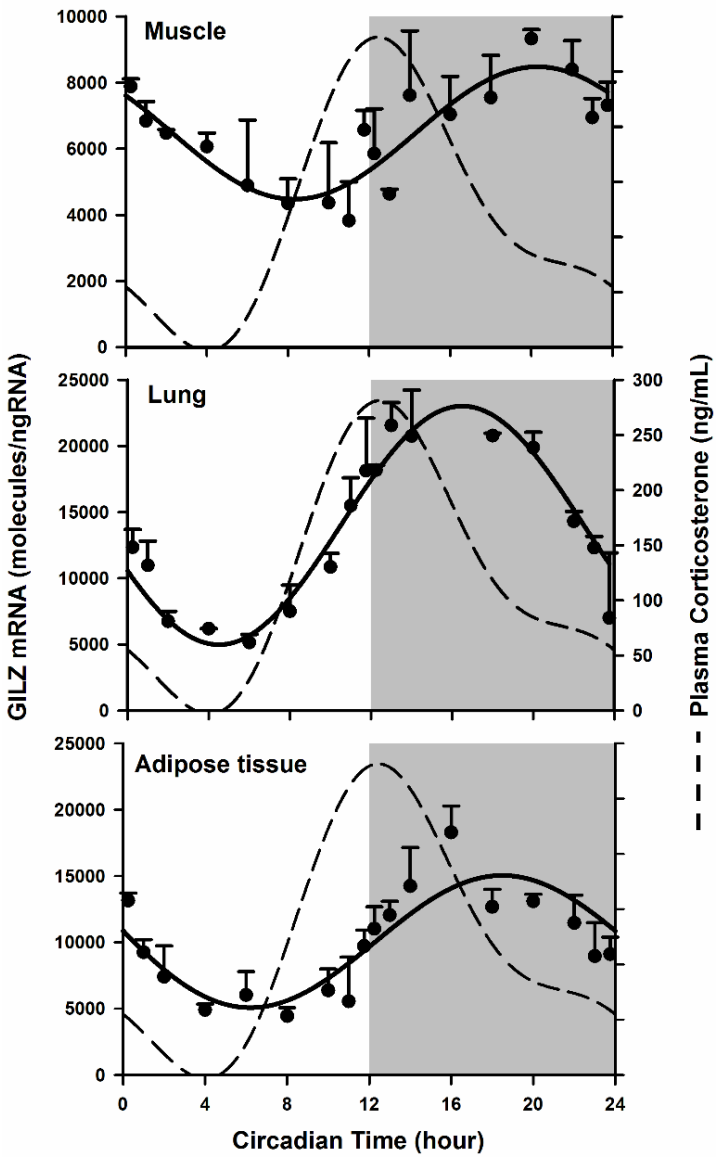
GR mRNA half-life: 2 – 3 hours

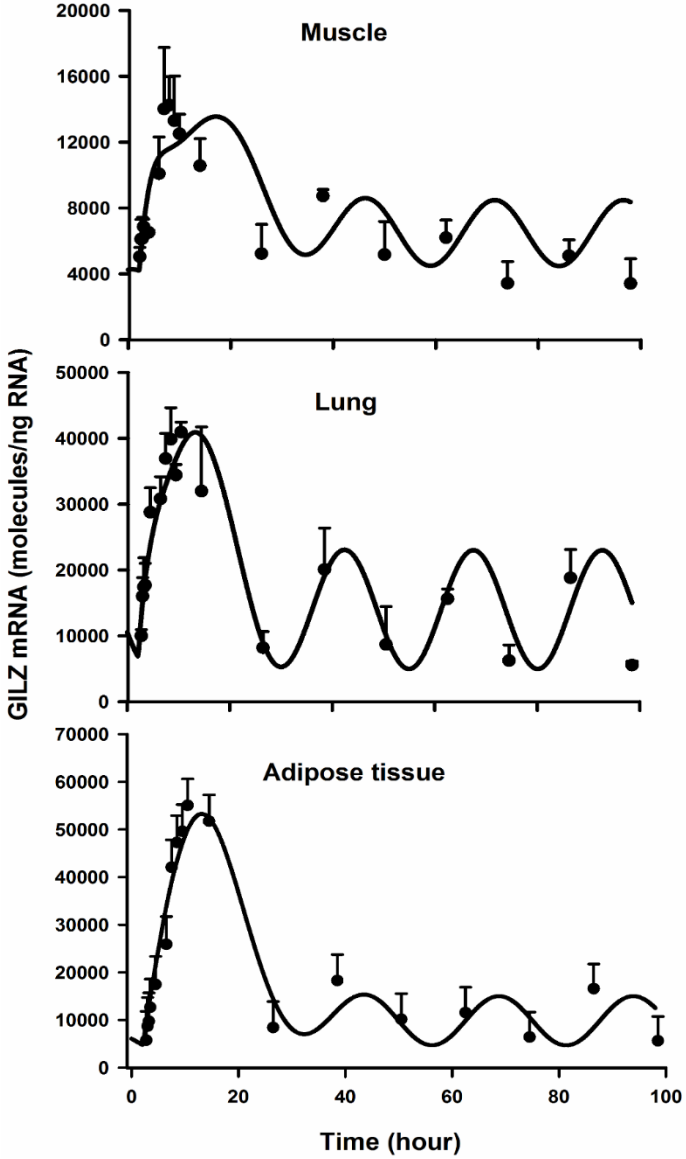
[a] Parameter values fixed from Sukumaran et al. 2011

[b] Parameter values fixed from Hazra et al. 2007a

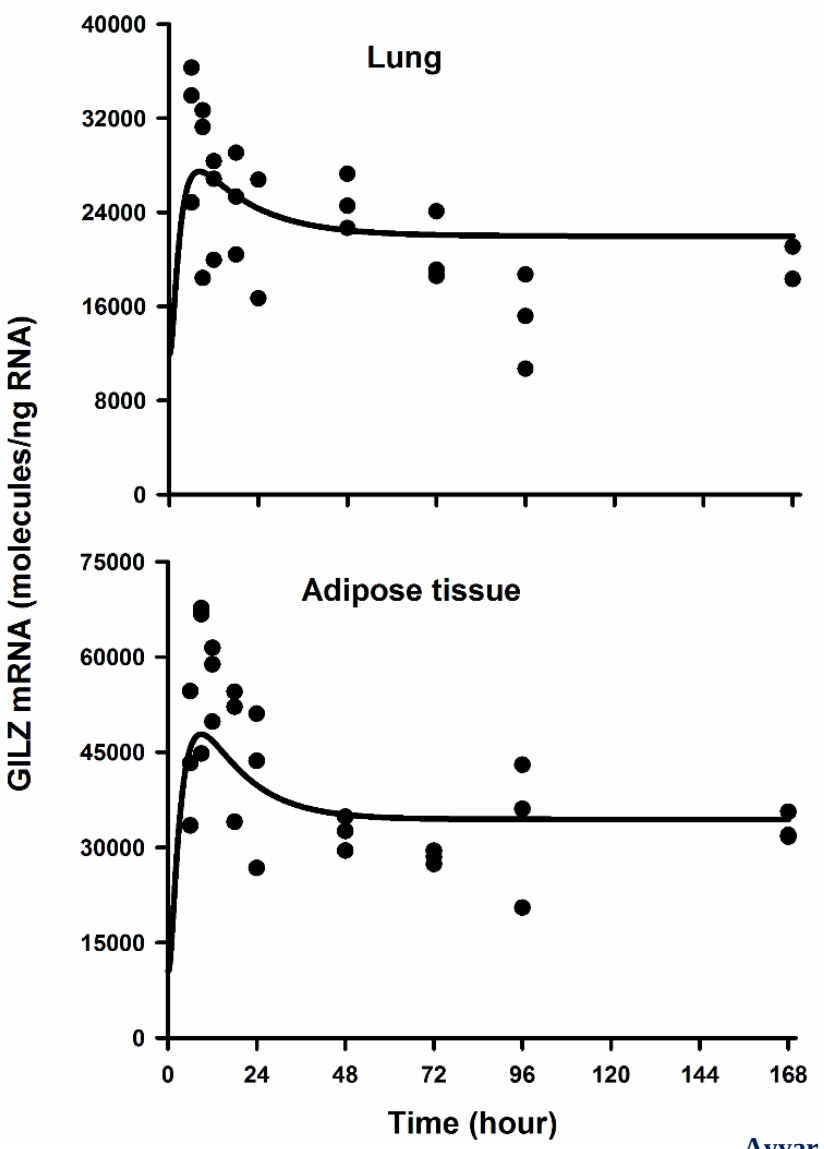
[c] Lung; [d] Muscle; [e] Adipose tissue

GILZ mRNA (Control & IM MPL)





Simulation of Tissue-specific GILZ – SC Infusion



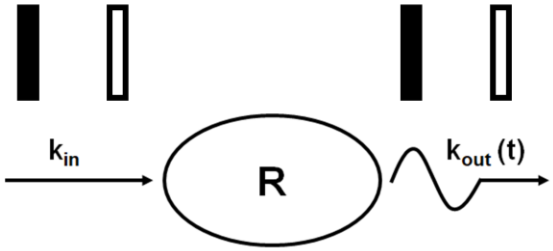
Chronic MPL PK and tissue-specific parameters for GR and GILZ employed to predict the dynamic behavior of GILZ under chronic dosing

Measurements in tissues from male Wistars given 0.3 mg/kg/h SC infusion of MPL for 7 days

Ayyar VS, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* 363: 45-57 (2017).

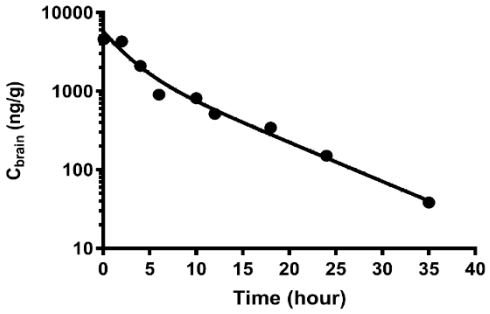
Modeling Circadian Removal of PD Responses

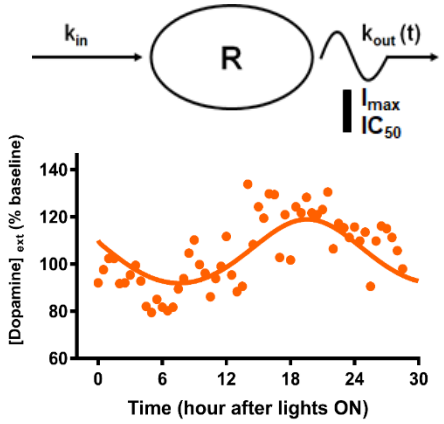
Ayyar VS, Krzyzanski W, Jusko WJ, *J Pharmacokinet Pharmacodyn* 46: 89-101 (2019).

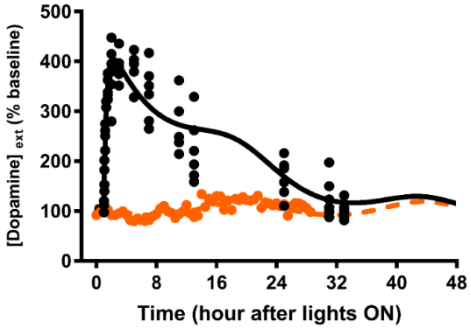


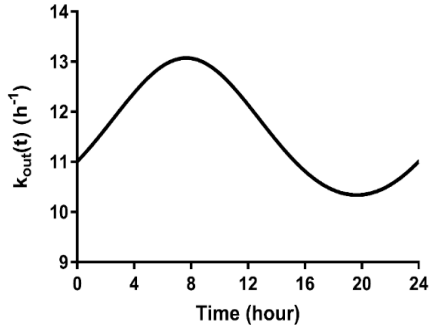
$$\frac{dR}{dt} = k_{in} \cdot \left(1 + H_1(t)\right) - k_{out}(t) \cdot \left(1 + H_2(t)\right) \cdot R(t)$$

$$k_{out}(t) = \frac{k_{in} + \frac{2\pi}{T} R_a sin\left(\frac{2\pi}{T}(t - t_p)\right)}{R_m + R_a cos\left(\frac{2\pi}{T}(t - t_p)\right)}$$





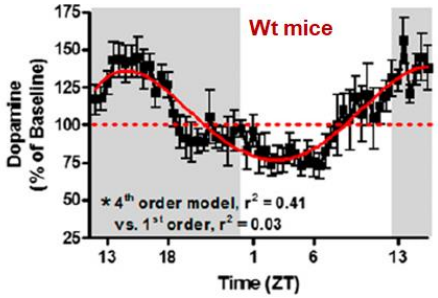


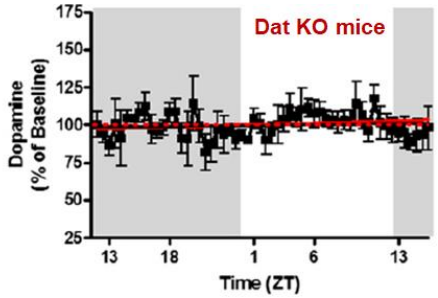


Applicable physiology and PD biomarkers:

- Renal excretion (GFR) – Uric acid
- Transporter activity – [Dopamine] brain, ECF
- Glymphatic clearance – [Amyloid-β] brain

Ferris, MJ et al., *Proc Nat Acad Sci* 111: E2751-E2759 (2014).

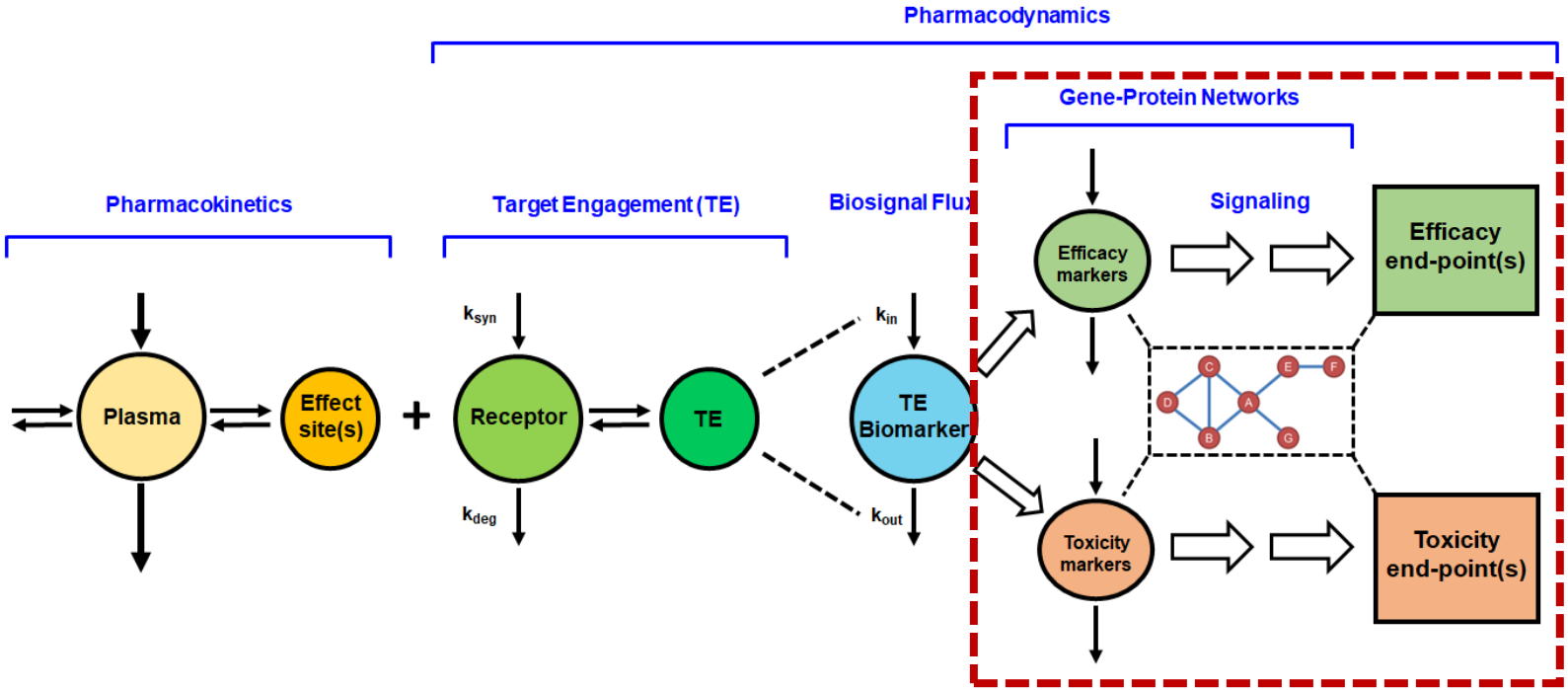




Modeling Receptor/Gene/Protein-Mediated MPL Signaling

Overarching goals:

- **Characterize temporal dynamics of MPL-regulated transcripts and proteins in liver**
- **Examine quantitative relationships between hepatic transcripts and proteins**
- **Develop a mechanistic model that connects gene/protein-mediated signaling to physiological PD endpoints**



Study Design – 'omics' Measures and Bioinformatics Analyses

Male Wistar rats

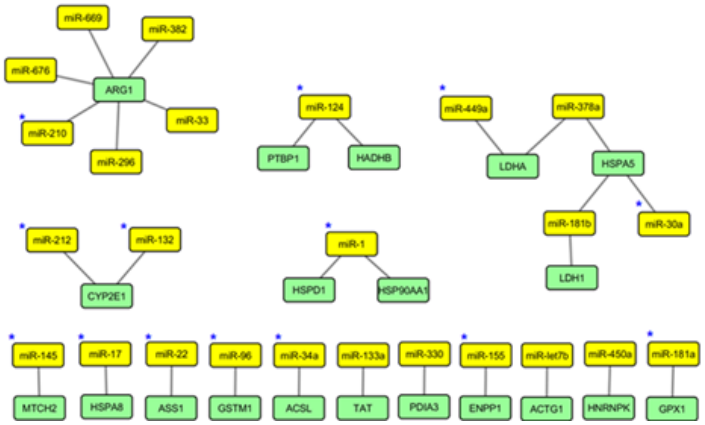
50 mg/kg MPL

0.25 0.5 0.75 1 2 4 5 5.5 6 7 8 12 18 30 48 72 hours

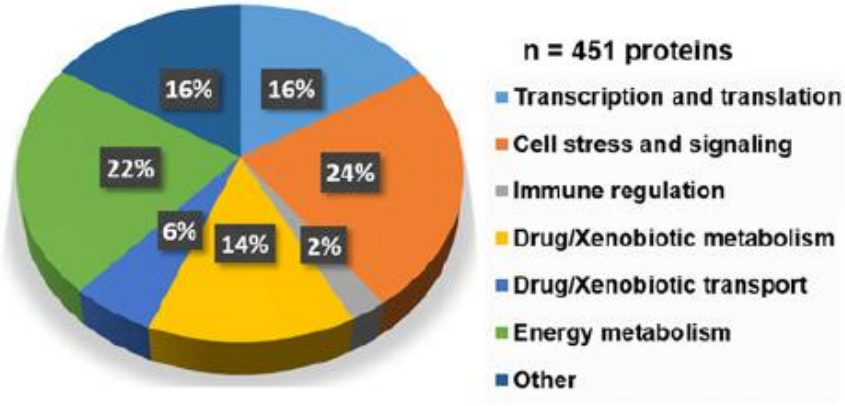
MICROARRAYS: euthanized, non-perfused livers excised (n = 3 per time-point)

PROTEOMICS: perfused, euthanized, livers excised (n = 5 per time-point)

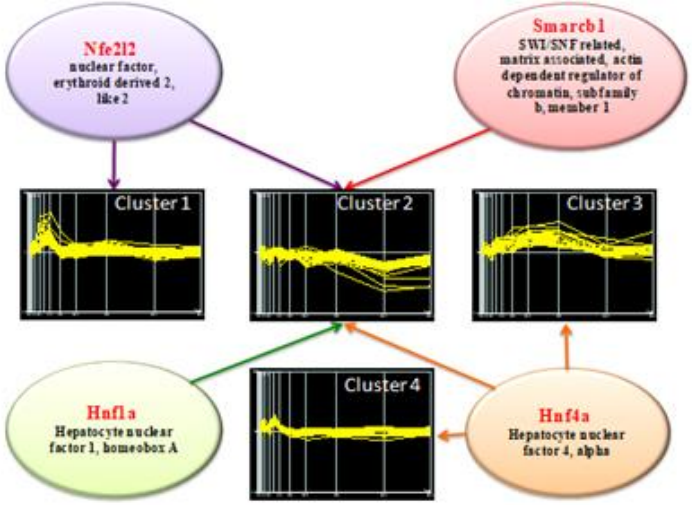
miRNA-gene interactions (MiRTarBase®)



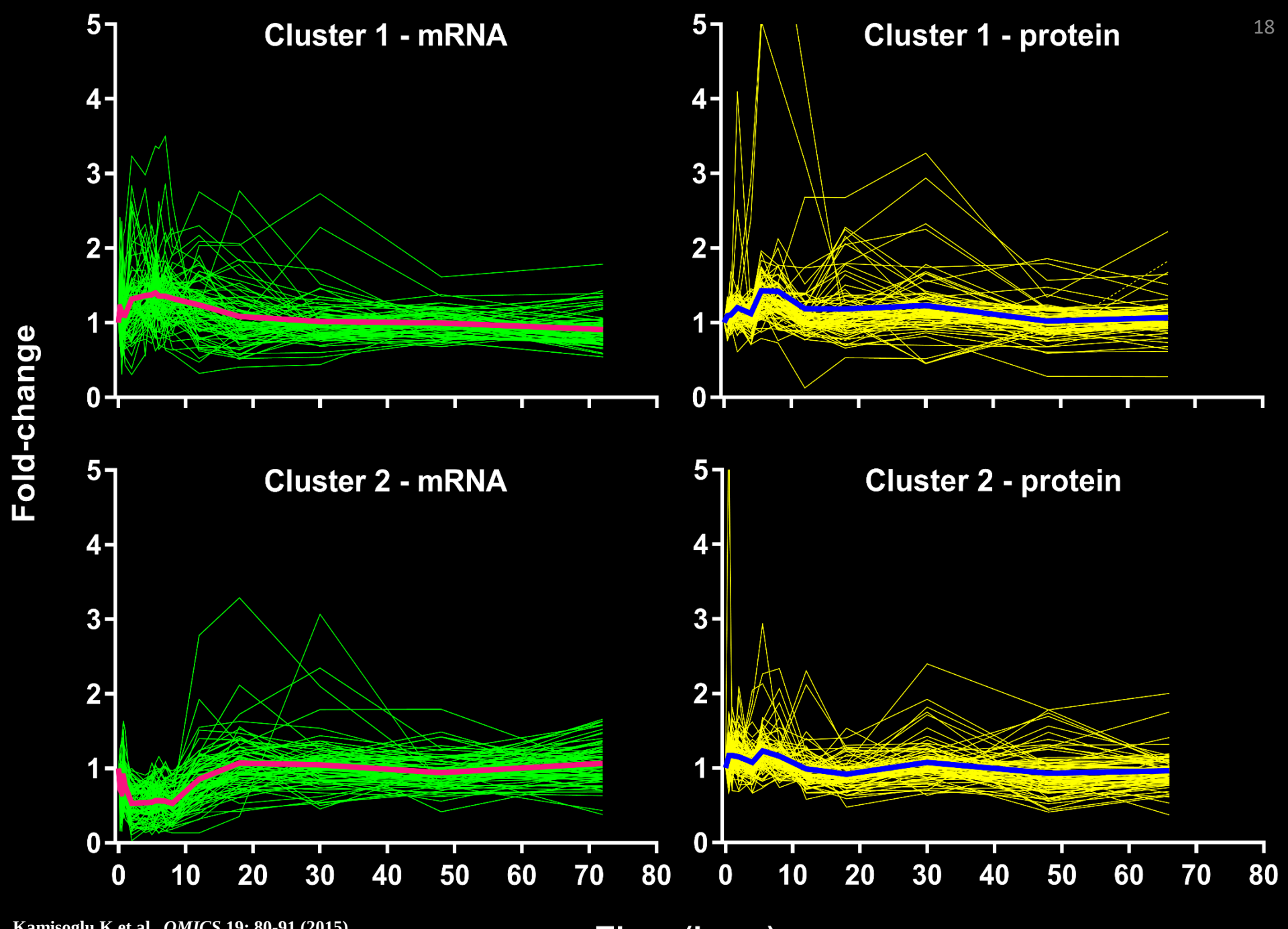
Functional proteomics



Transcription factor identification (IPA®)



Jin JY et al., *J Pharmacol Exp Ther* 307: 93-109 (2003).
Nouri-Nigjeh E et al., *Anal Chem* 86: 8149-57 (2014).
Ayyar VS, Almon RR, DuBois DC, Sukumaran S, Qu J, Jusko WJ, *J Proteomics* 160: 84-105 (2017).
Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Jusko WJ J *Pharmacol Exp Ther* 367: 168-183 (2018).



Kamisoglu K et al., *OMICS* 19: 80-91 (2015).
Ayyar VS et al., *J Pharmacol Exp Ther* 367: 168-183 (2018).

General PK/PD Paradigm for Genomic Drug Effects

Jin JY, DuBois DC, Almon RR, Jusko WJ , *J Pharmacol Exp Ther* 307: 93-109 (2003).
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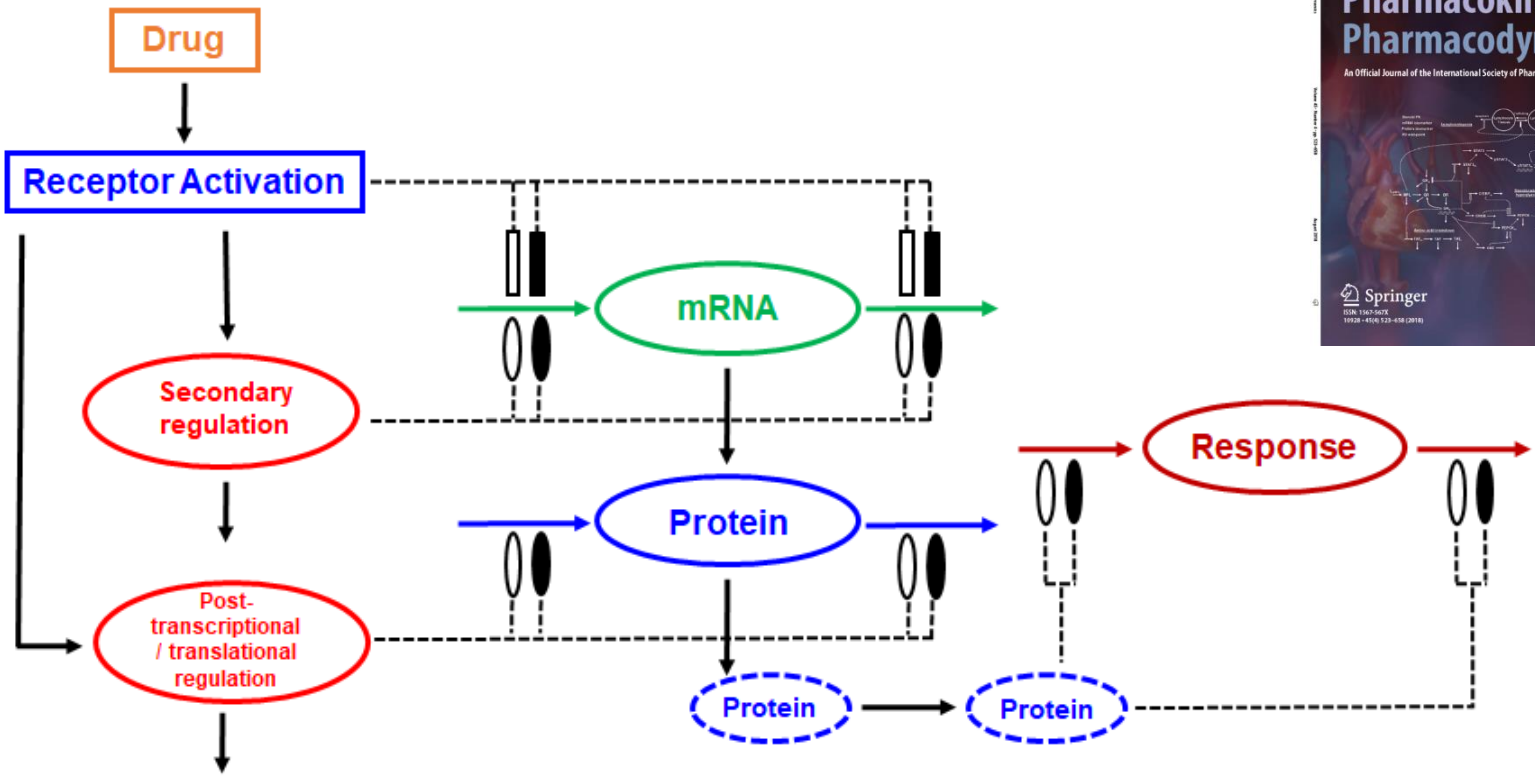
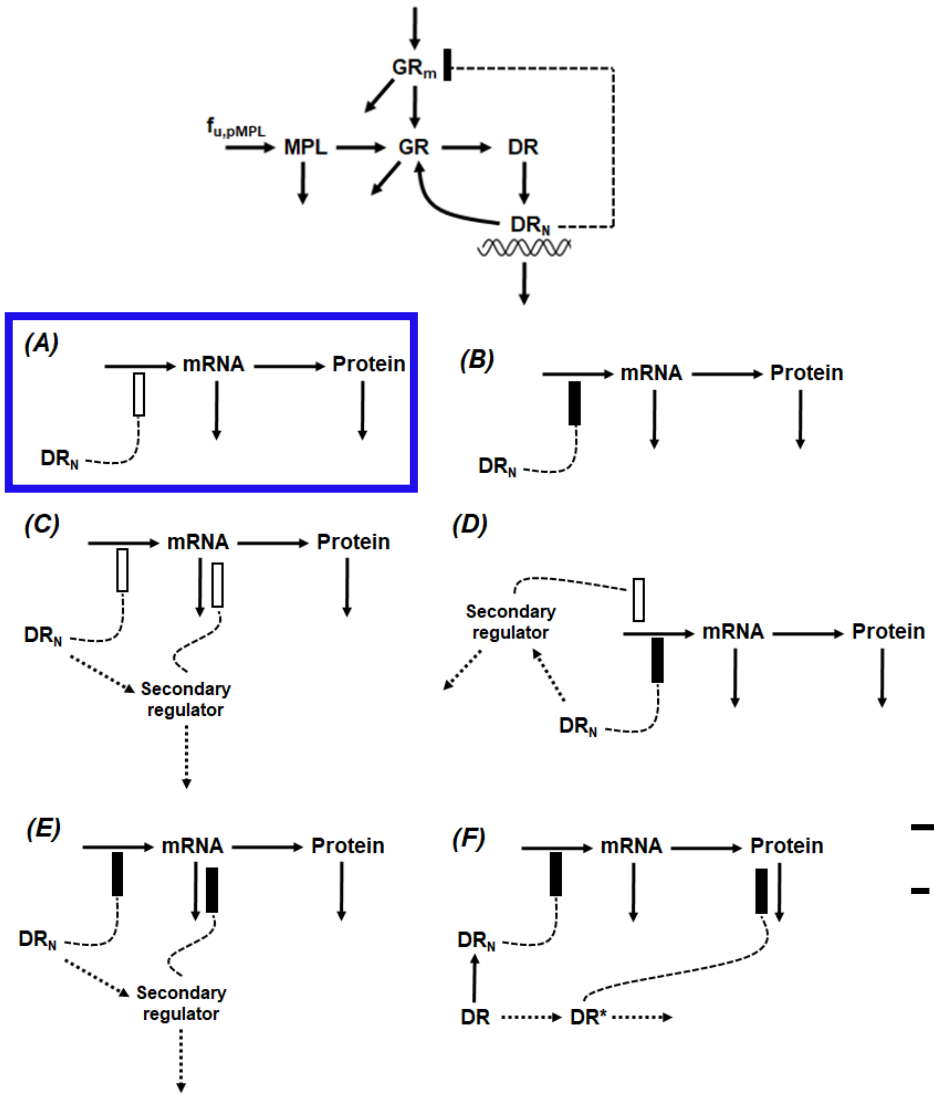


General Hypothesis: Genomic regulation by CS occurs at the mRNA and protein levels via mechanisms affecting key turnover processes.

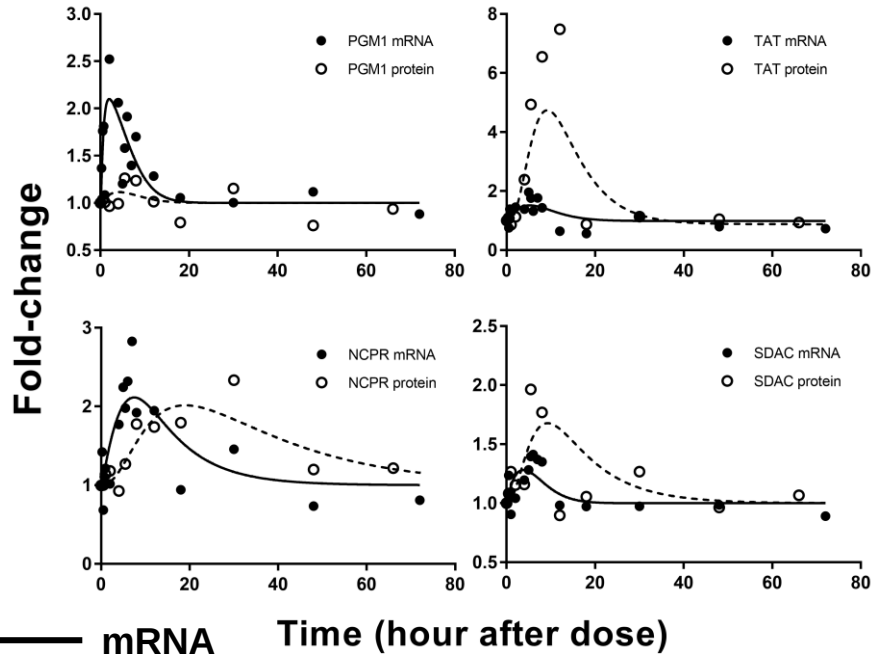
Temporal Modeling of Transcriptomic and Proteomic Patterns

Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* 367: 168-183 (2018).



Largest cluster – 64 mRNA/proteins

Up-regulated genes

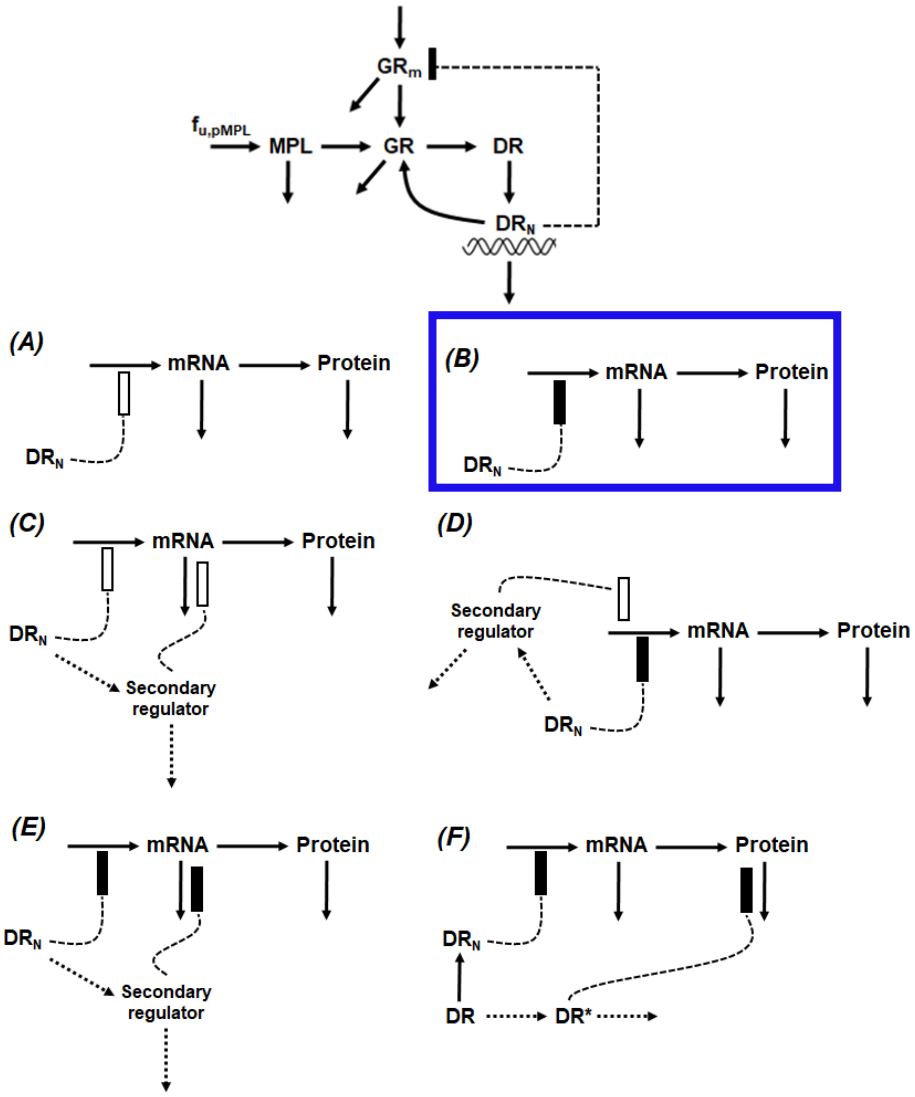


mRNA half-lives: 1 – 8 hours

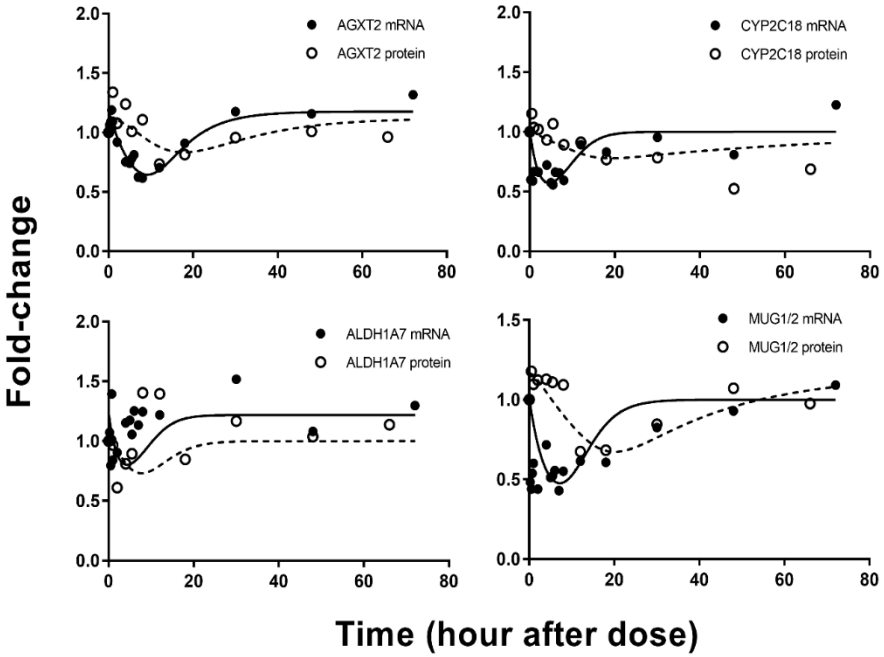
Protein half-lives: 1 – 86 hours

Temporal Modeling of Transcriptomic and Proteomic Patterns

Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* 367: 168-183 (2018).

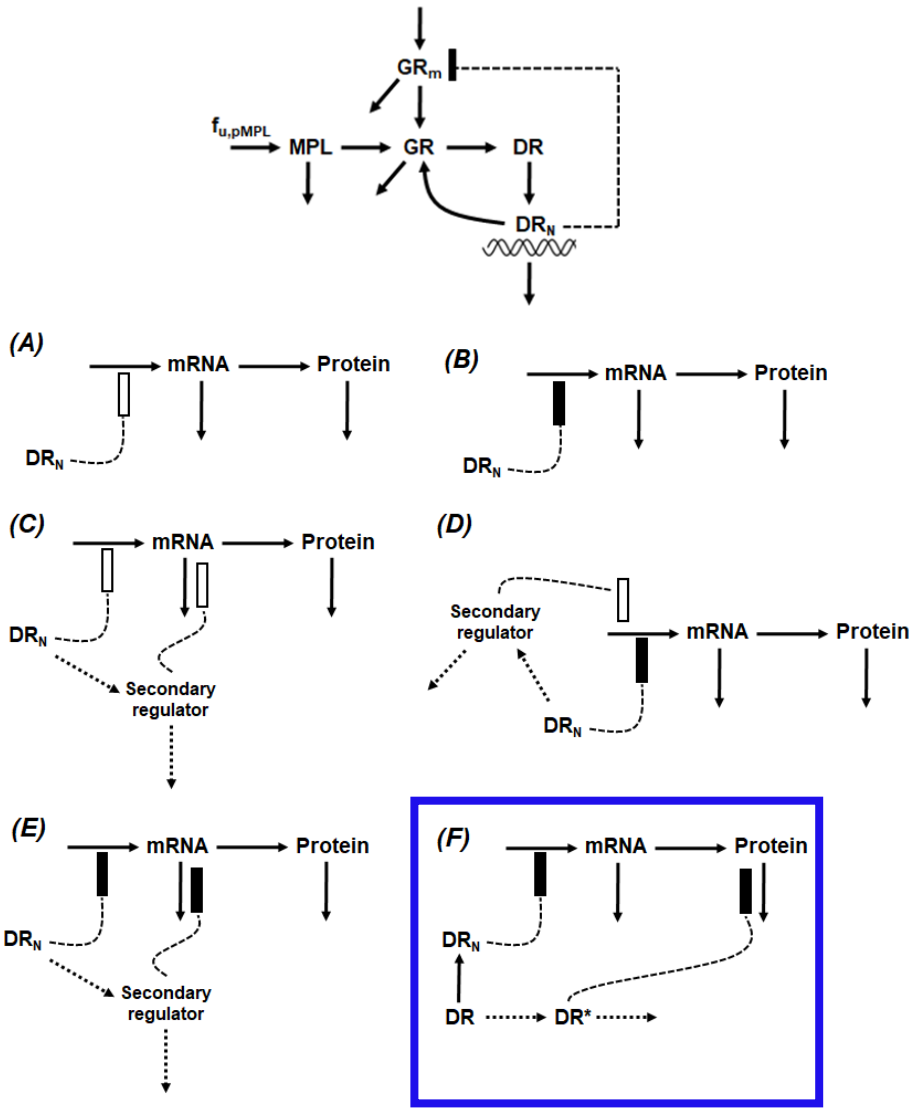


Down-regulated genes

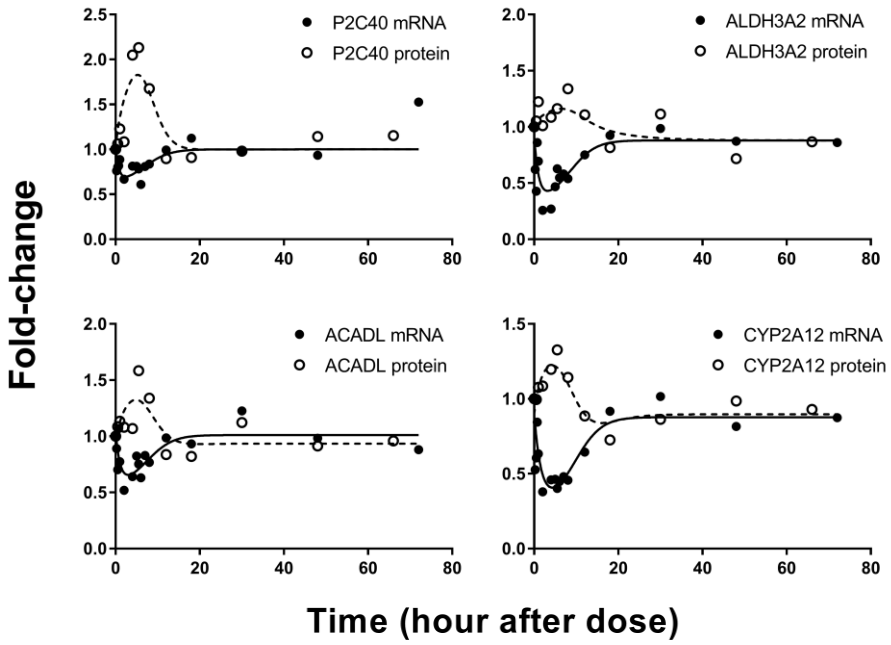


Temporal Modeling of Transcriptomic and Proteomic Patterns

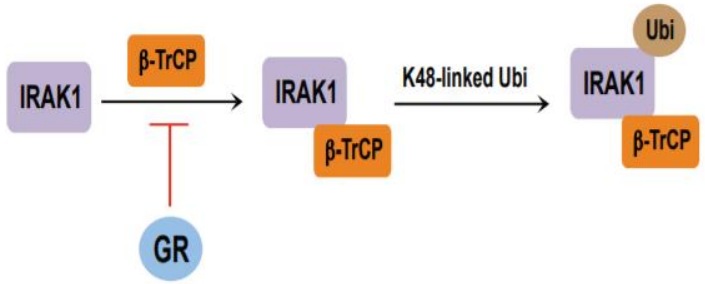
Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* 367: 168-183 (2018).



Discordant mRNA and proteins

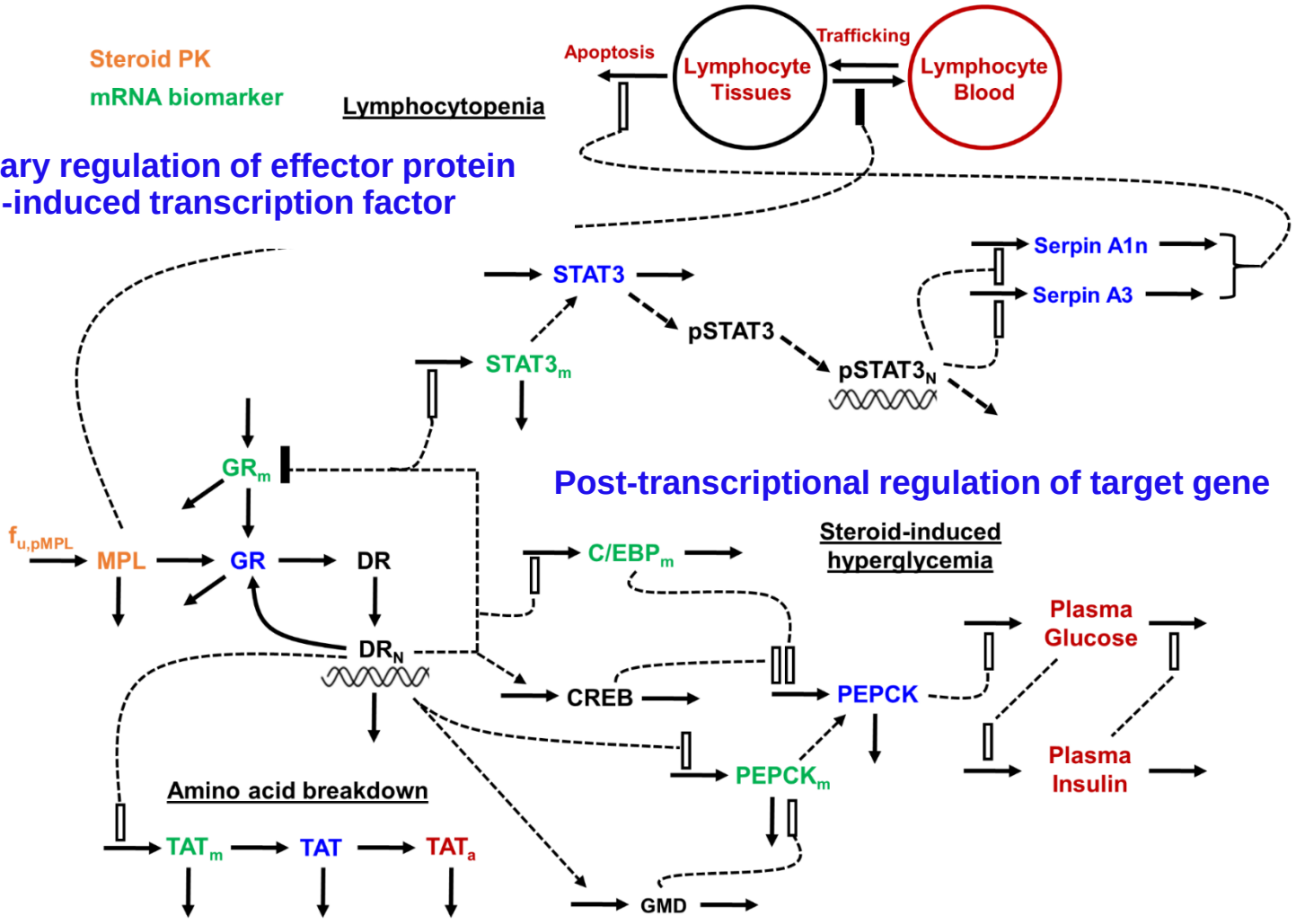


Kong et al., *J Immunol* 199:3654-3667 (2017).

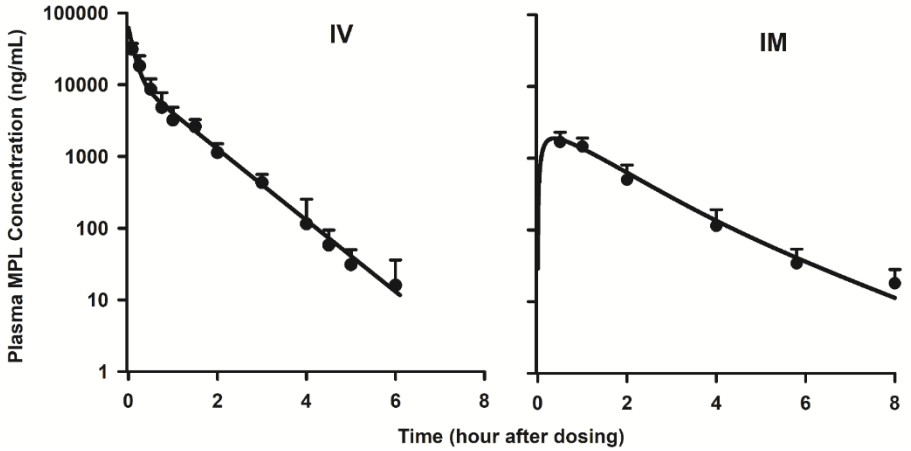


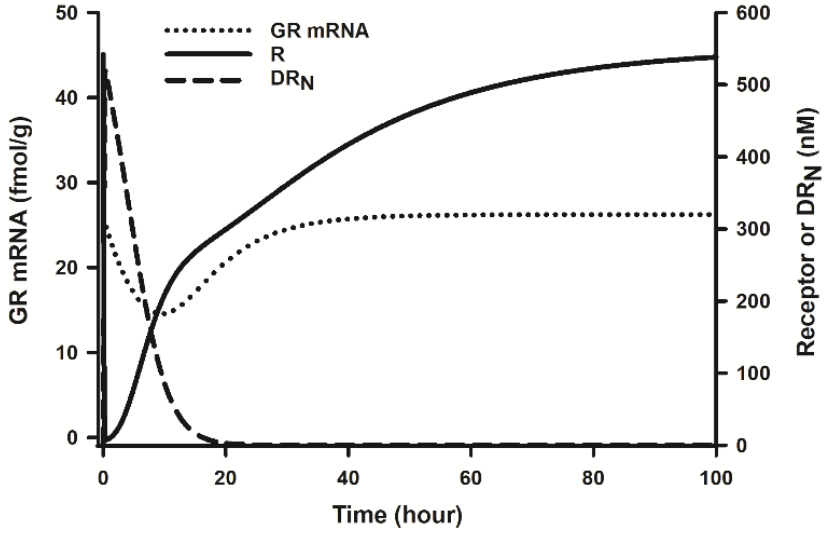
Systems PK/PD Model for MPL in Liver

Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Qu J, Jusko WJ, *J Pharmacokinet Pharmacodyn* 45: 557–575 (2018).



MPL Pharmacokinetics and Receptor Dynamics



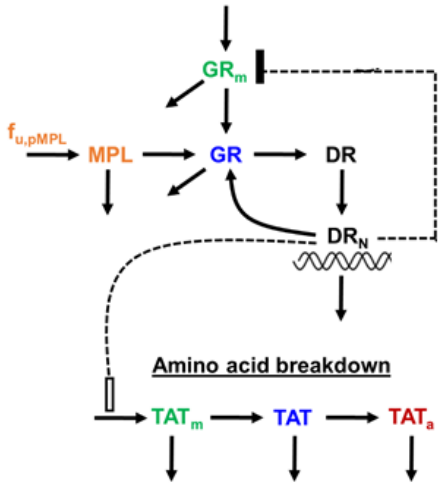


Parameter (units)	Definition	Est (%CV)
MPL Pharmacokinetics		
CL (L/h/kg)	Clearance	2.93 (0.89)
CL_D (L/h/kg)	Distribution clearance	2.51 (1.94)
V_c (L/kg)	Central volume of distribution	0.803 (0.97)
V_T (L/kg)	Peripheral volume of distribution	0.974 (1.51)
F	Bioavailability	0.2 (0.94)
Fr	Fraction absorbed by k_{a1}	0.725 (fixed)
k_{a1} (h^{-1})	Absorption rate constant	1.82 (2.8)
k_{a2} (h^{-1})	Absorption rate constant	0.54 (4.1)
Glucocorticoid Receptor Dynamics [a]		
$k_{s,GRm}$ (fmol/g/h)	Synthesis rate constant for GR mRNA	3.2
$k_{d,GRm}$ (h^{-1})	Degradation rate constant for GR mRNA	0.12
$k_{s,GR}$ (nM/h)(fmol/g)$^{-1}$	Synthesis rate constant for receptor	0.84
$IC_{50,GRm}$ (nM)	DR_n for 50% inhibition of GR mRNA synthesis	123.7
$k_{d,GR}$ (h^{-1})	Degradation rate constant for receptor	0.04
k_{on} ($nM^{-1}\cdot h^{-1}$)	Association rate constant	0.019
f_{mpl}	Unbound fraction of MPL	0.23
k_{re} (h^{-1})	DR_n loss rate constant	0.402
R_f	Fraction recycled	0.69
k_T (h^{-1})	Translocation rate constant	58.2
$GR_m(0)$ (fmol/g)	GR mRNA initial concentration	25.8
$GR(0)$ (nM)	Free cytosolic receptor initial concentration	540.7
$DR(0)$ (nM)	Drug-receptor complex initial concentration	0
$DR_n(0)$ (nM)	Nuclear complex initial concentration	0

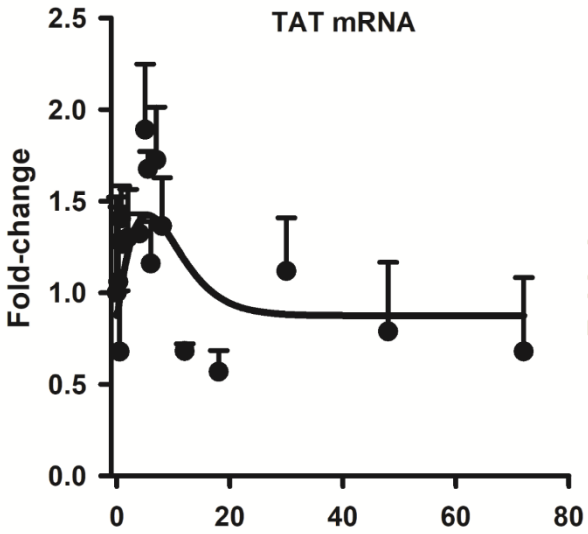
[a] Parameter values obtained from Hazra et al [36]

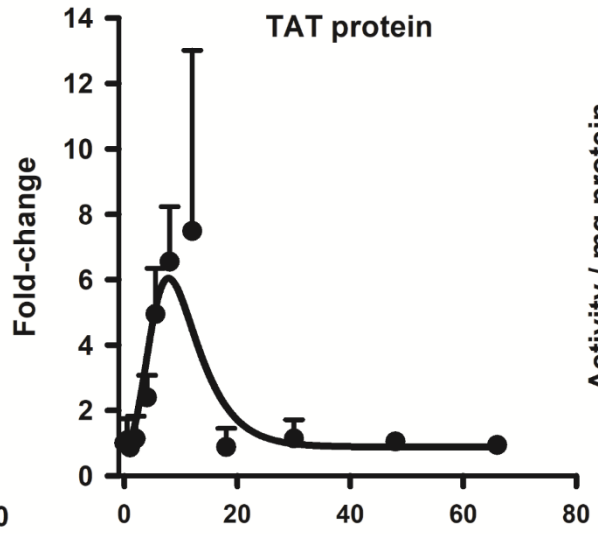
Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Qu J, Jusko WJ, *J Pharmacokinet Pharmacodyn* 45: 557–575 (2018).

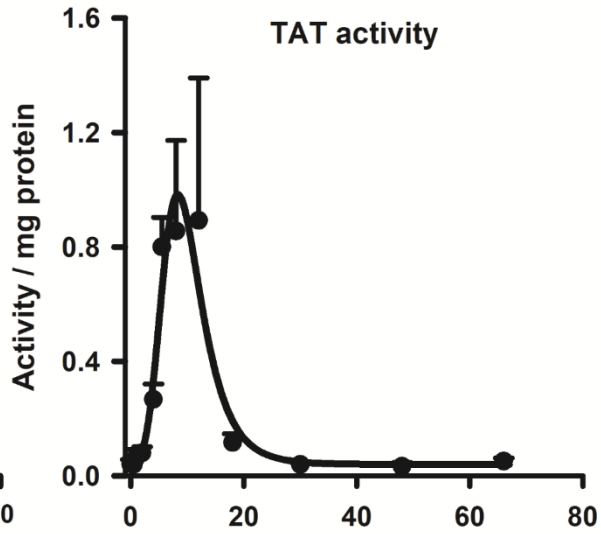
Modeling TAT mRNA, Protein, and Activity



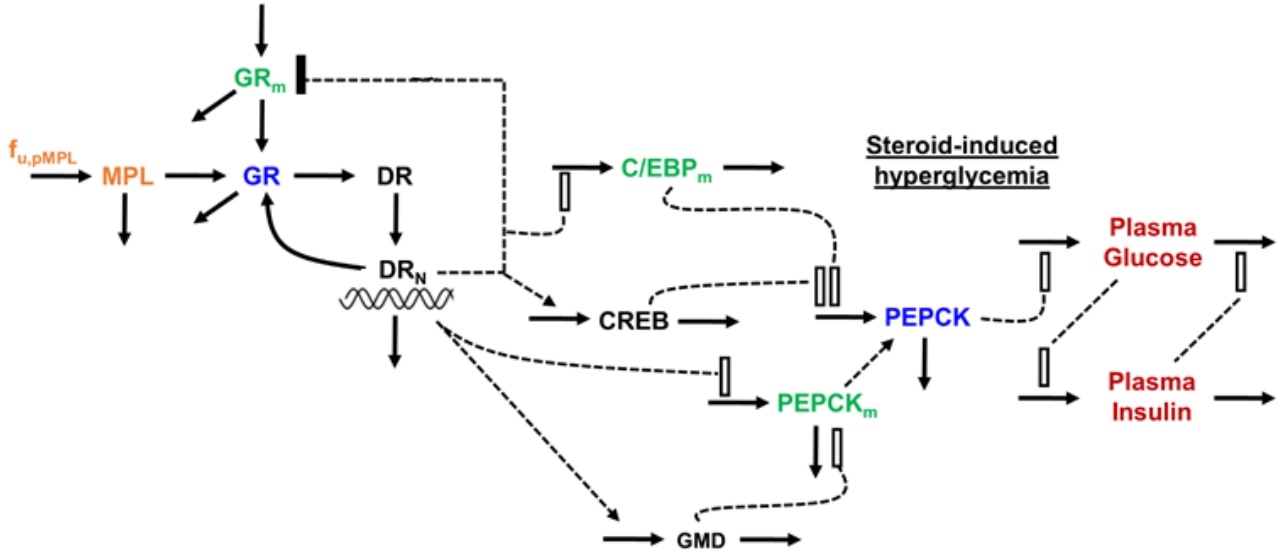
Parameter (units)	Definition	Estimate (% CV)
Tyrosine aminotransferase dynamics		
$k_{d,TATm}$ (h^{-1})	Degradation rate constant for TAT mRNA	**0.22 (66.6)**
S_{DRn}^{TATm} (nM^{-1})	Stimulation constant for TAT mRNA	**0.002 (48.1)**
$k_{d,TAT}$ (h^{-1})	Degradation rate constant for TAT protein	**0.29 (73.6)**
γ_1	Amplification factor for TAT protein	**4.5 (34.9)**
$k_{d,TAT}$ (h^{-1})	Degradation rate constant for TAT activity	**2.0 (96.0)**
γ_2	Amplification factor for TAT activity	**1.67 (11.8)**
TAT_m (0)	Baseline TAT mRNA	**0.87 (12.0)**
TAT (0)	Baseline TAT protein	**0.88 (12.8)**
TAT_a (0) $(activity/mg)$	Baseline TAT activity	**0.041 (7.3)**

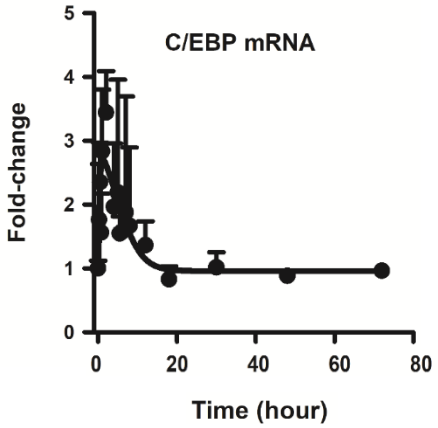


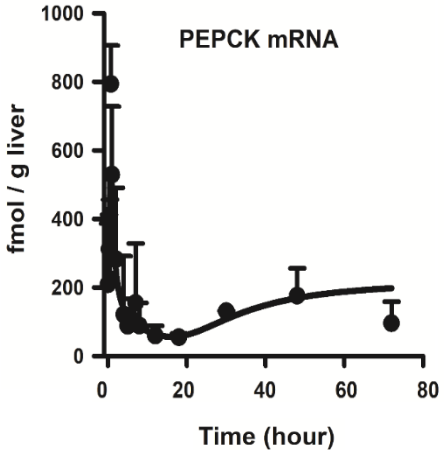


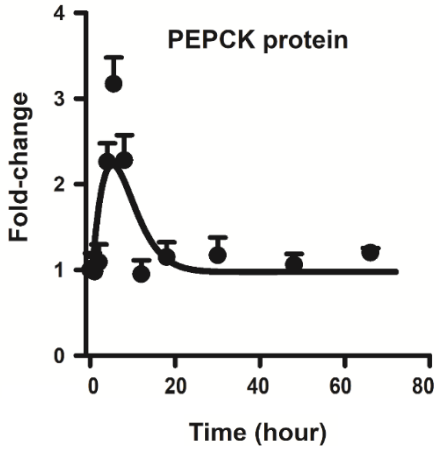


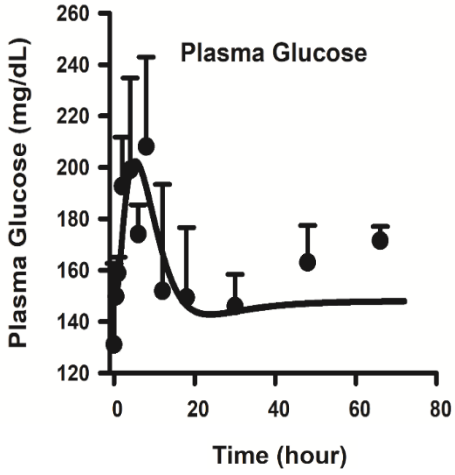
Modeling MPL-Induced Hyperglycemia

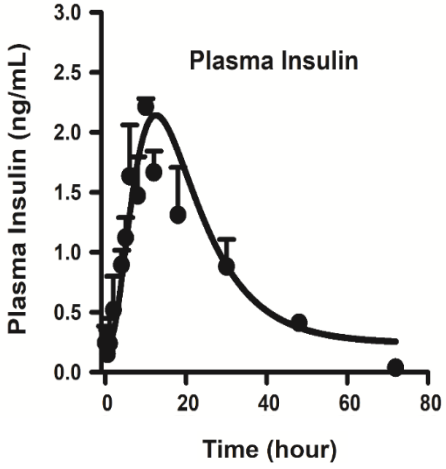






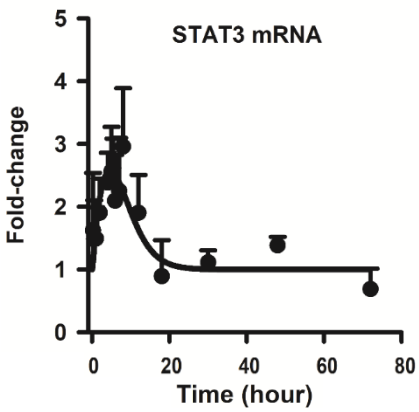


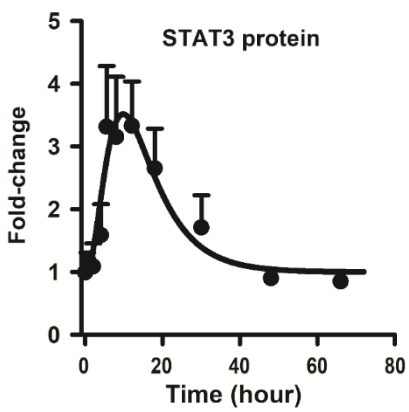


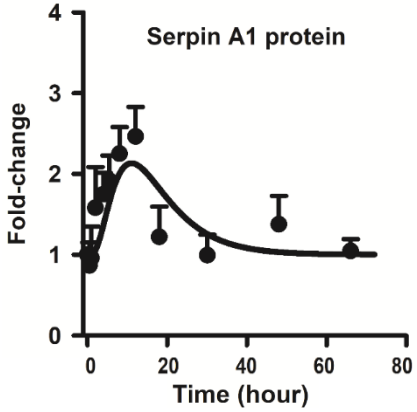


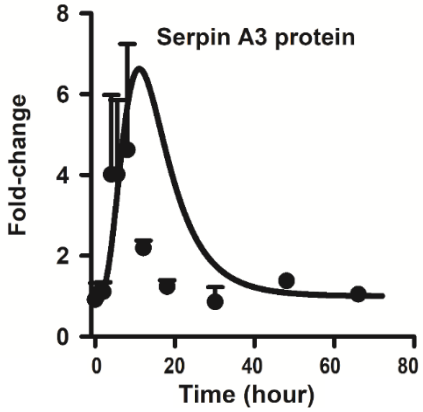
Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Qu J, Jusko WJ, *J Pharmacokinet Pharmacodyn* 45: 557–575 (2018).

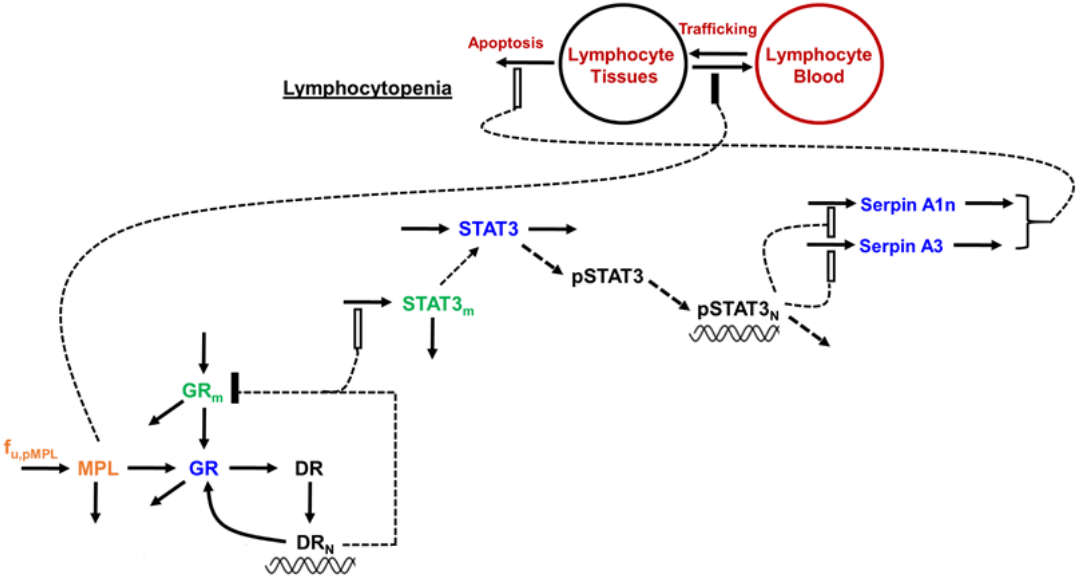
Modeling Combined Lymphocyte Trafficking & Apoptosis

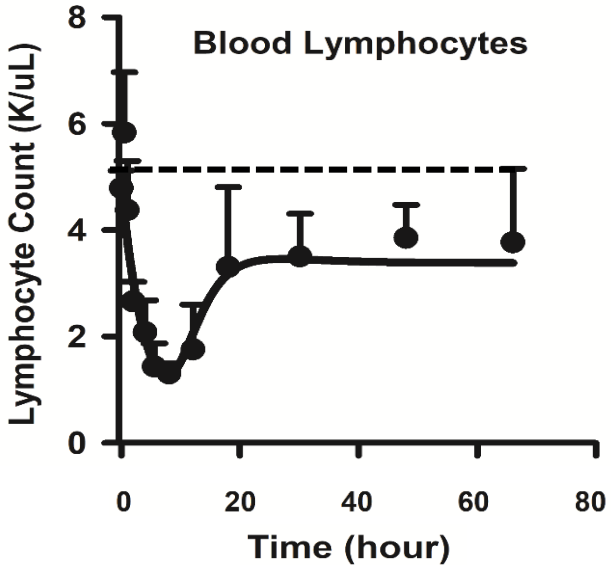










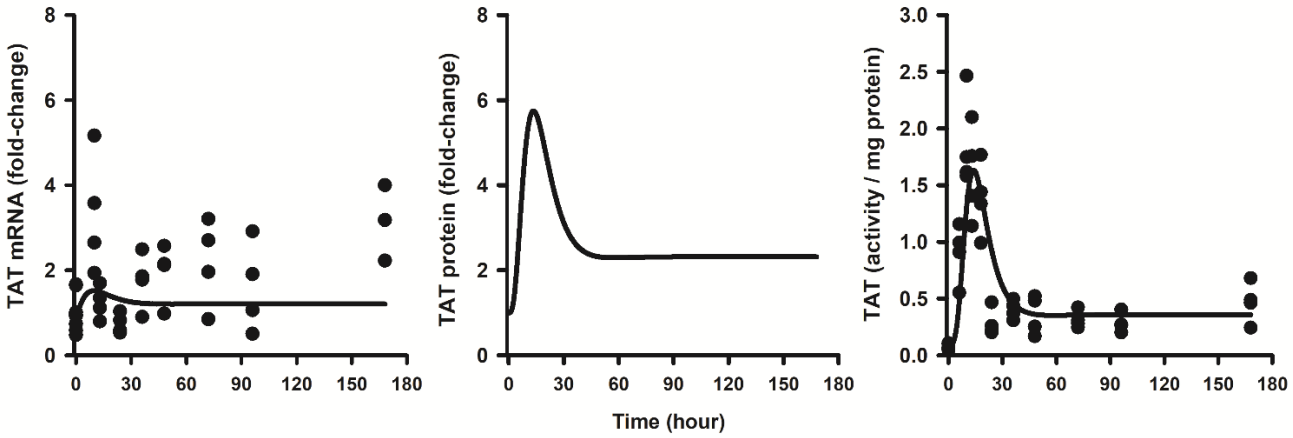


Ayyar VS, Sukumaran S, DuBois DC, Almon RR, Qu J, Jusko WJ, *J Pharmacokinet Pharmacodyn* 45: 557–575 (2018).

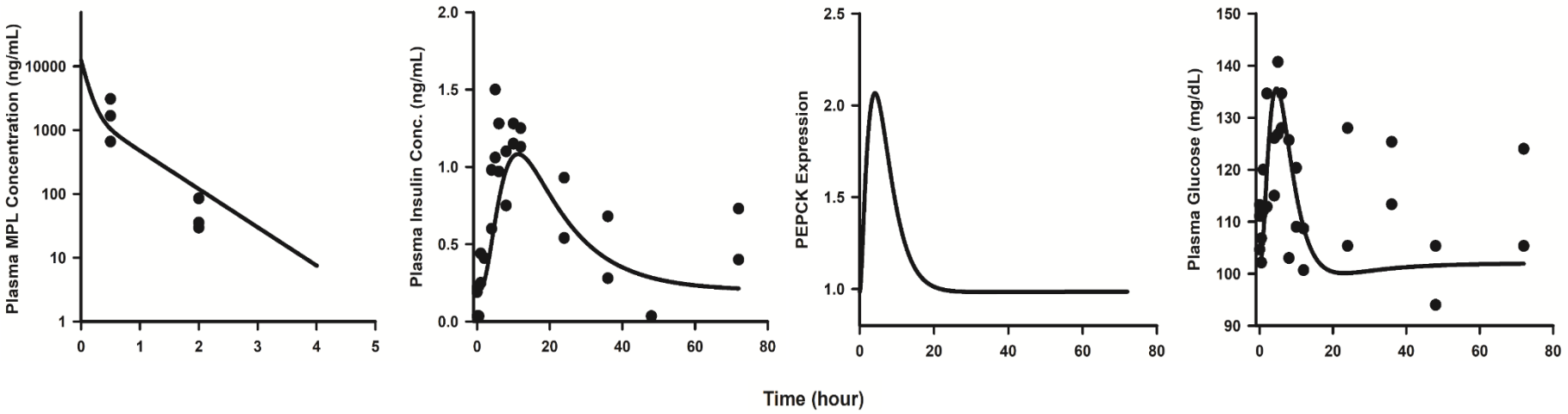
Simulation of MPL Responses

Data from Jin JY and Jusko, WJ, *Biopharm Drug Dispos* 30: 21-34(2009).
Data from Ramakrishnan R et al, *J Pharmacol Exp Ther* 300: 245-256 (2002).

TAT Dynamics – 7-day 0.3 mg/kg/h SC infusion



Insulin / Glucose Dynamics – 5 mg/kg IV bolus



Sex Differences in PK/PD - Why is This Important?

PRECLINICAL RESEARCH

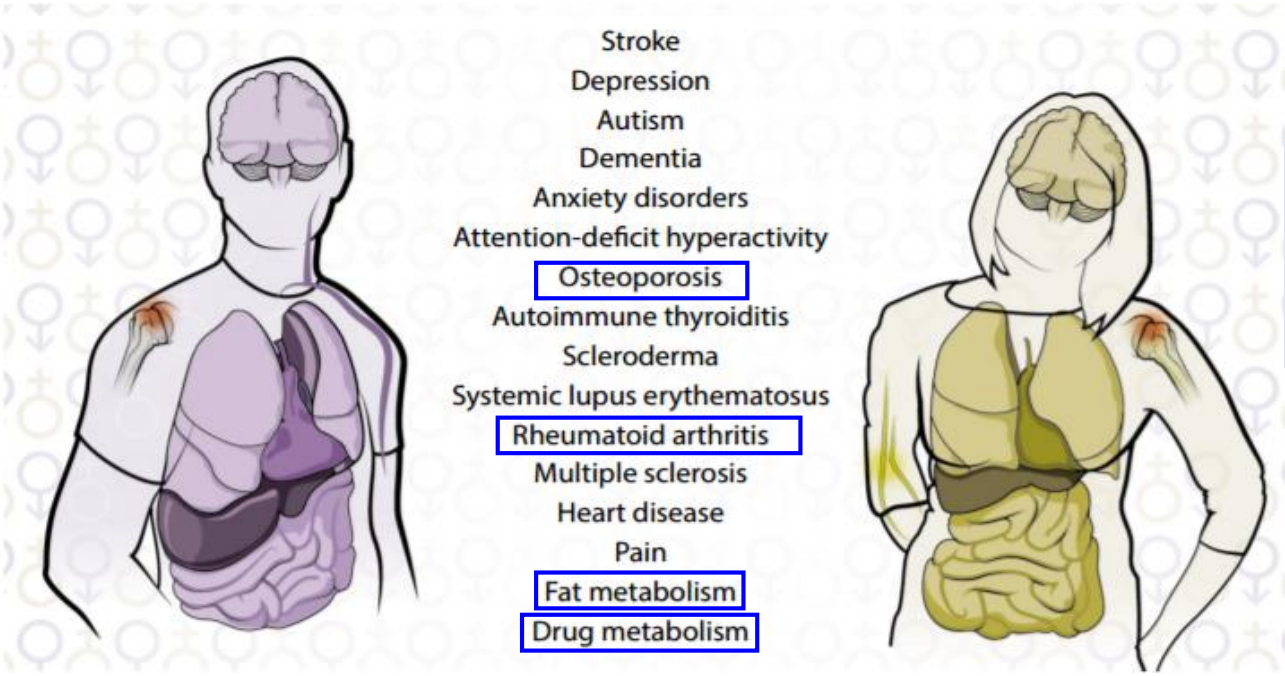
Sex Matters for Mechanism

Jayne S. Danska

Some funding agencies now require consideration of sex and gender in preclinical research, a policy that heralds opportunities and challenges for researchers.

NIH to balance sex in cell and animal studies

Janine A. Clayton and Francis S. Collins unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.



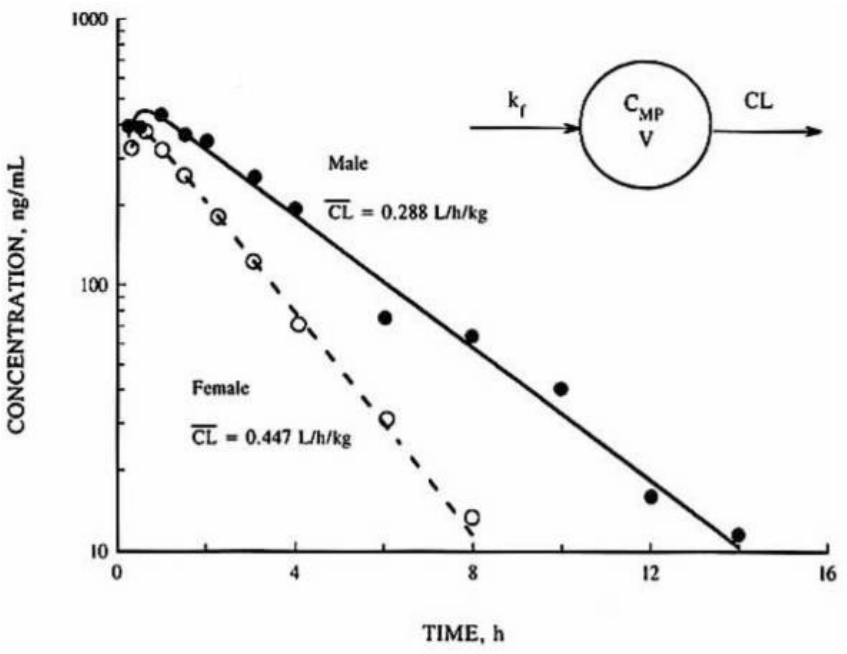
Pharmacology studies include five times as many male animals as females!

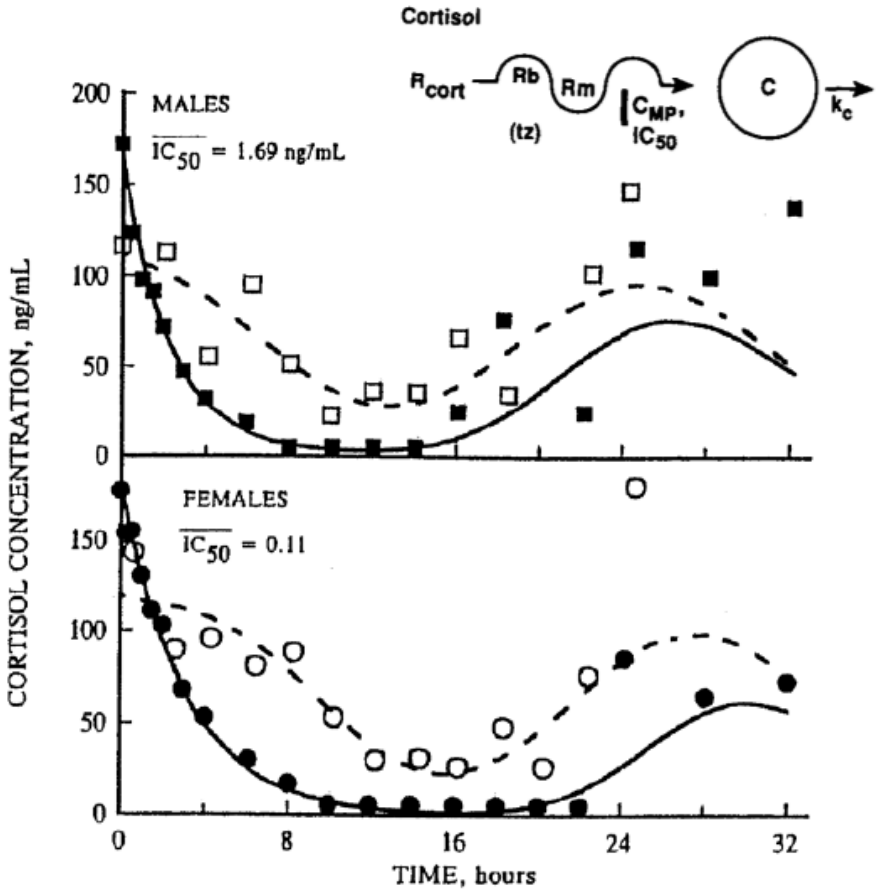
Danska JS, *Sci Transl Med* 6: 258fs40 (2014).

Beery AK and Zucker I, *Neurosci and Biobehav Rev* 35: 565-572 (2011).
Clayton JA and Collins SC, *Nature* 509: 282-283 (2014).

Sex Effects on Methylprednisolone PK/PD in Humans

Methylprednisolone dosed at 0.6 mg/kg ideal body weight

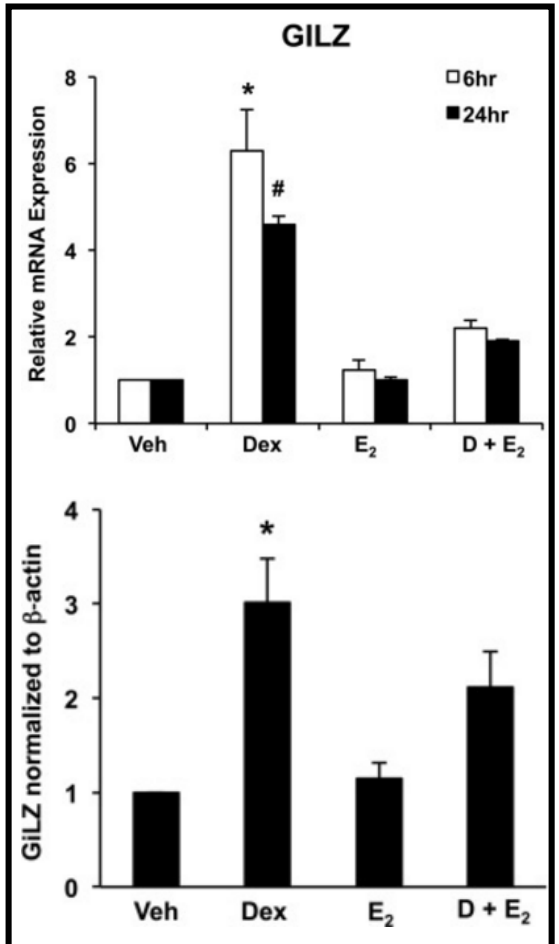




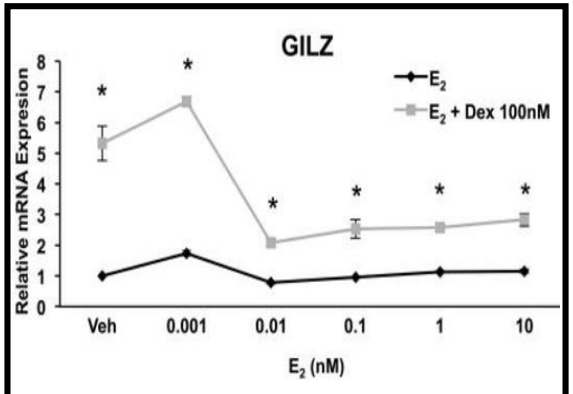
"Although women are more sensitive to methylprednisolone as measured by cortisol suppression, they eliminate the drug more quickly, generally producing a similar net response."

Lew K.H. et. al., *Clin Pharmacol Ther* 54: 402-414 (1993).
Jusko WJ, *Toxicology* 102: 189-196 (1995).

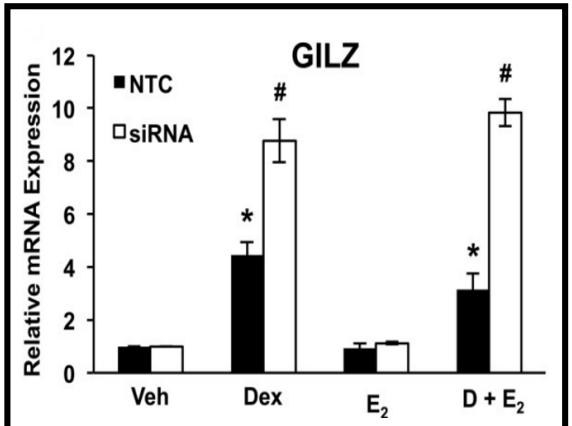
DEX-inducible GILZ is Antagonized by Estradiol *in vitro*



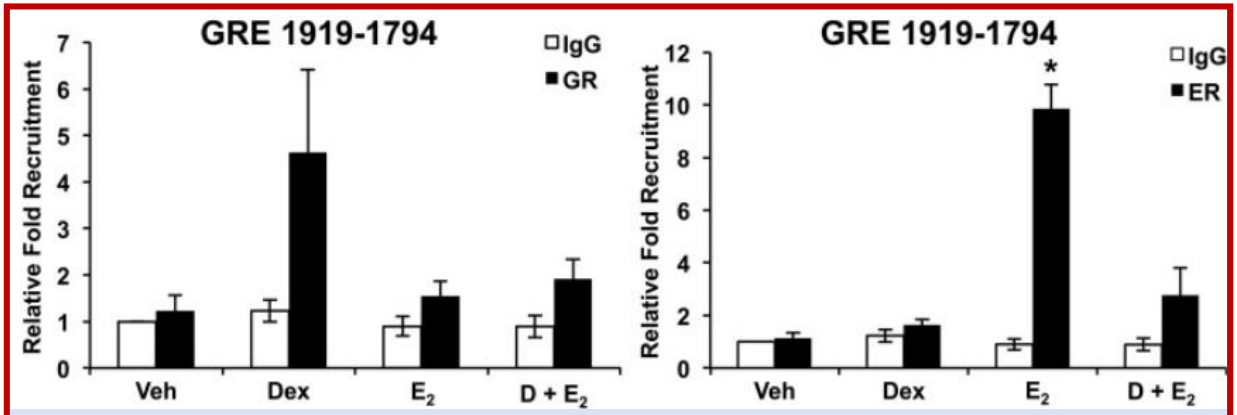
DEX-inducible GILZ mRNA (top) and protein (bottom) in hECC1 cells incubated with 100 nM DEX and 10 nM E_2 is suppressed vs. DEX alone



E_2 potently antagonizes DEX-inducible GILZ mRNA at 1 – 10 pM concentrations in hECC1 cells



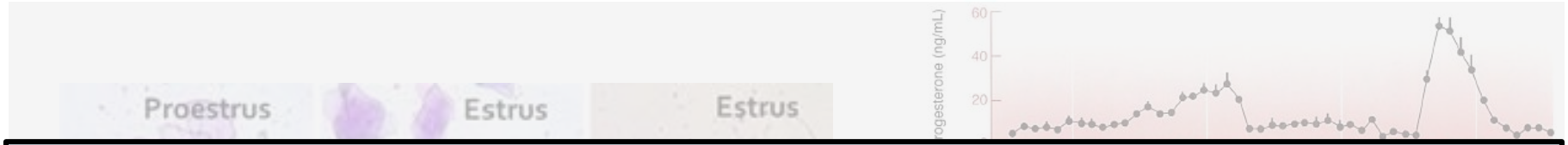
Transfection with ERα siRNA reverses E_2-mediated suppression of DEX-inducible GILZ mRNA



Association of GR and ERα at the promoter of GILZ. Recruitment of GR and ERα to GRE at position −1919 to −1794 as assessed by ChIP assay

Whirledge S and Cidlowski JA, *Endocrinol* 154: 499-510 (2013).

Estrous Cycle & Sex Hormone Regulation in Female Rats



Overall Aims:

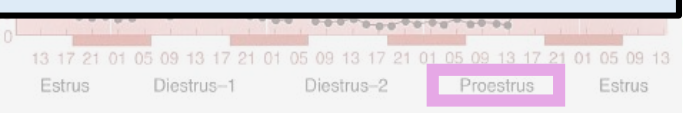
i) To investigate sex differences in PK and PD responses of methylprednisolone in rats.

ii) To quantitatively evaluate the determinants of sex differences in PK and PD of MPL in liver and uterus using mechanistic modeling.

Major Hypothesis:

Elevated estradiol production during **proestrus** in females can **antagonize** corticosteroid-regulated GILZ dynamics in tissues with **estrogen receptors**.

Mastorakos G, Pavlatou MG, Mizamtsidi M, *Pediatr Endocrinol Rev* 3: 172-181 (2006).
Boden MJ and Kennaway DJ, *Reproduction* 132: 379-392 (2006).

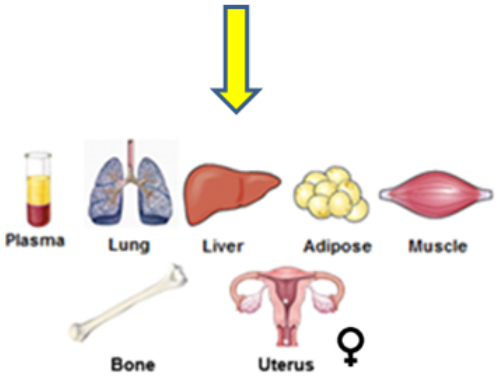


Methylprednisolone PK/PD Studies – Sex and Estrous Stage

Hazra A, Pyszczynski N, DuBois DC, Almon RR, Jusko WJ, *J Pharmacokinet Pharmacodyn* 28: 263-73 (2007).

Male – No MPL	Male – MPL	Female (Estrus) - MPL	Female (Proestrus) - MPL	Male – MPL
♂ 	♂ 	♀ 	♀ 	♂ 
54 male Wistars (7 wk)	54 male Wistars (7 wk)	39 female Wistars (11 wk)	36 female Wistars (11 wk)	30 male Wistars (11 wk)
↓	↓	↓	↓	↓
Normal 12 hours : 12 hours light/dark cycle				
CIRCADIAN CONTROLS	50 mg/kg MPL IM			
↓	↓	↓	↓	↓
Euthanized at 18 time points over 24 h (n = 3)	Euthanized at 18 time points over 96 h after MPL (n = 3)	Euthanized at 12 time points over 24 h after MPL (n = 3-4)	Euthanized at 12 time points over 24 h after MPL (n = 3)	Euthanized at 9 time points over 48 h after MPL (n = 2-3)

All animals dosed between 1.5 – 3 h after lights ON

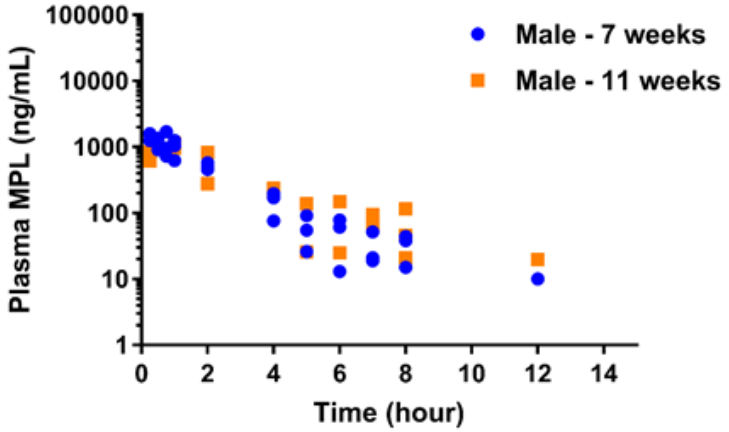


Daily vaginal lavages in females performed from 8 – 11 weeks of age to stage estrous cycle

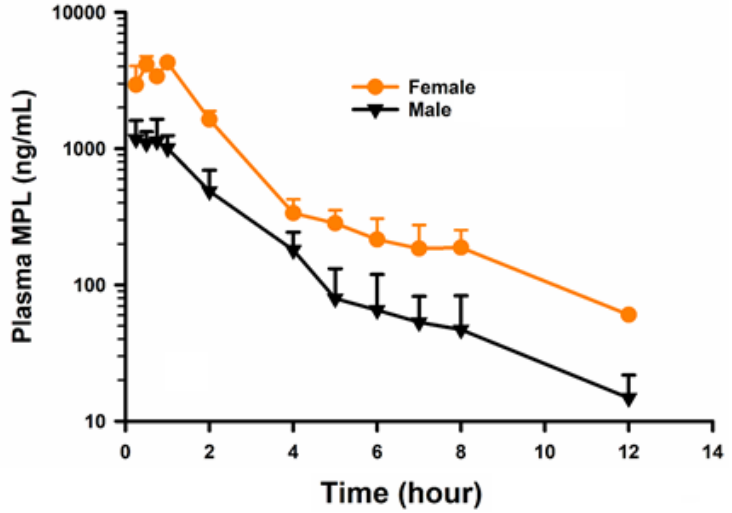
Sex Differences in Plasma and Hepatic PK: 50 mg/kg IM

Ayyar VS, DuBois DC, Nakamura T, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* Accepted (2019b).

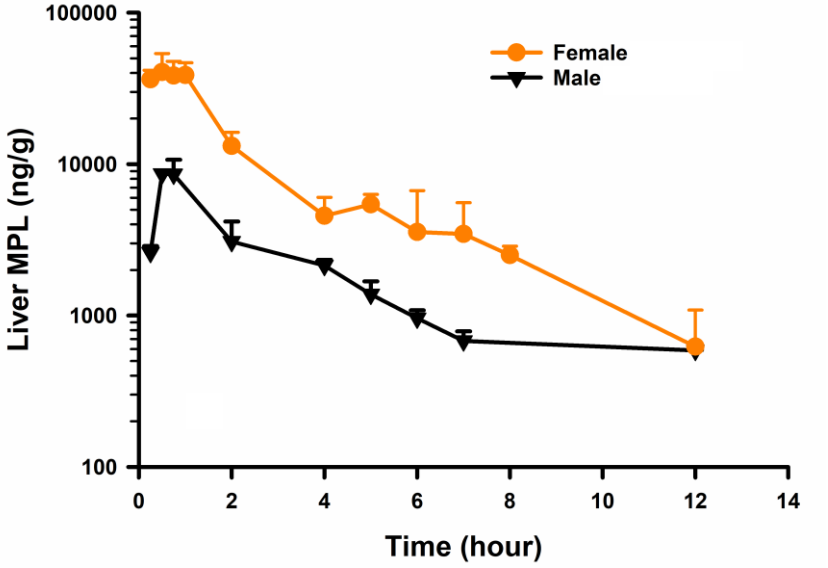
No difference in males by age or body weight



No statistically significant difference found in females per estrous stage



Consistent differences in hepatic exposure



~ 3-fold higher plasma exposure in females

Sex	$AUC_{0-\infty}$ (h·ng/mL)	CL/F (mL/h/kg)
Male	2901 ± 185[b]	17,232 ± 1099
Female	9751 ± 482[b]	5128 ± 252

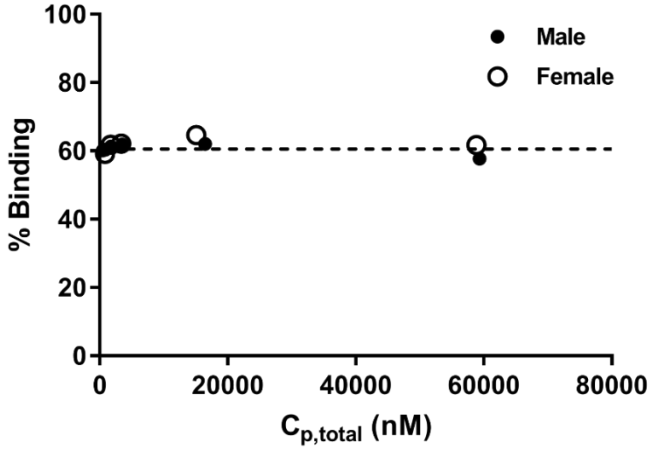
[a] Bailer's Method applied to compute area-under-curve (AUC)

[b] Standard error

Steroid Assay: Normal-phase HPLC

No Sex Differences in Plasma Protein Binding of MPL and Glucocorticoid Receptors in Tissues

Ayyar VS, Song D, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* Accepted (2019a).

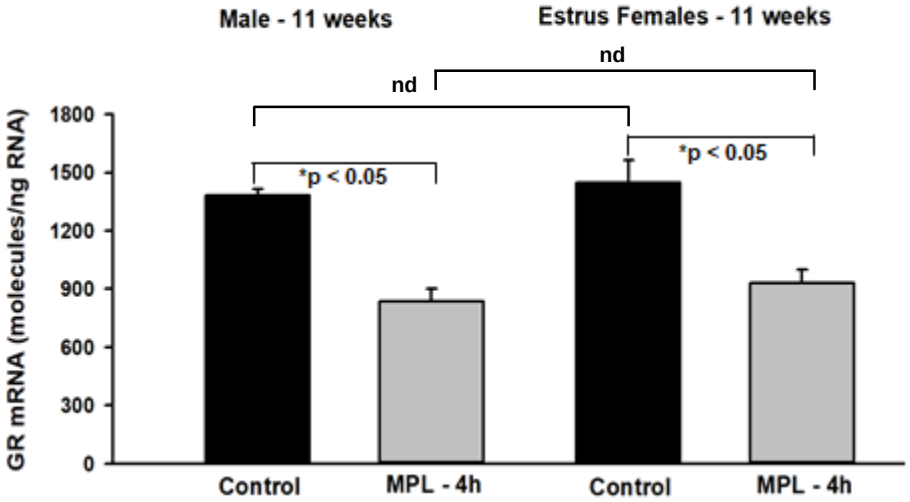


METHOD: Centrifree® ULTRAFILTRATION (25 kDa cut-off)

Similar to value (63 ± 0.8 %) in male rats (Haughey et al, 1991)

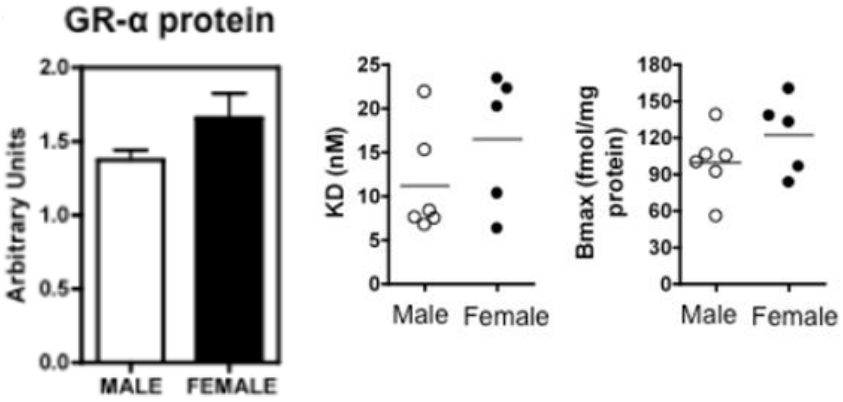
Percent bound in RATS is lower compared to HUMANS and RABBITS (77 – 78 %) (Ebling et al, 1986)

No difference in hepatic GR mRNA in males and E-females



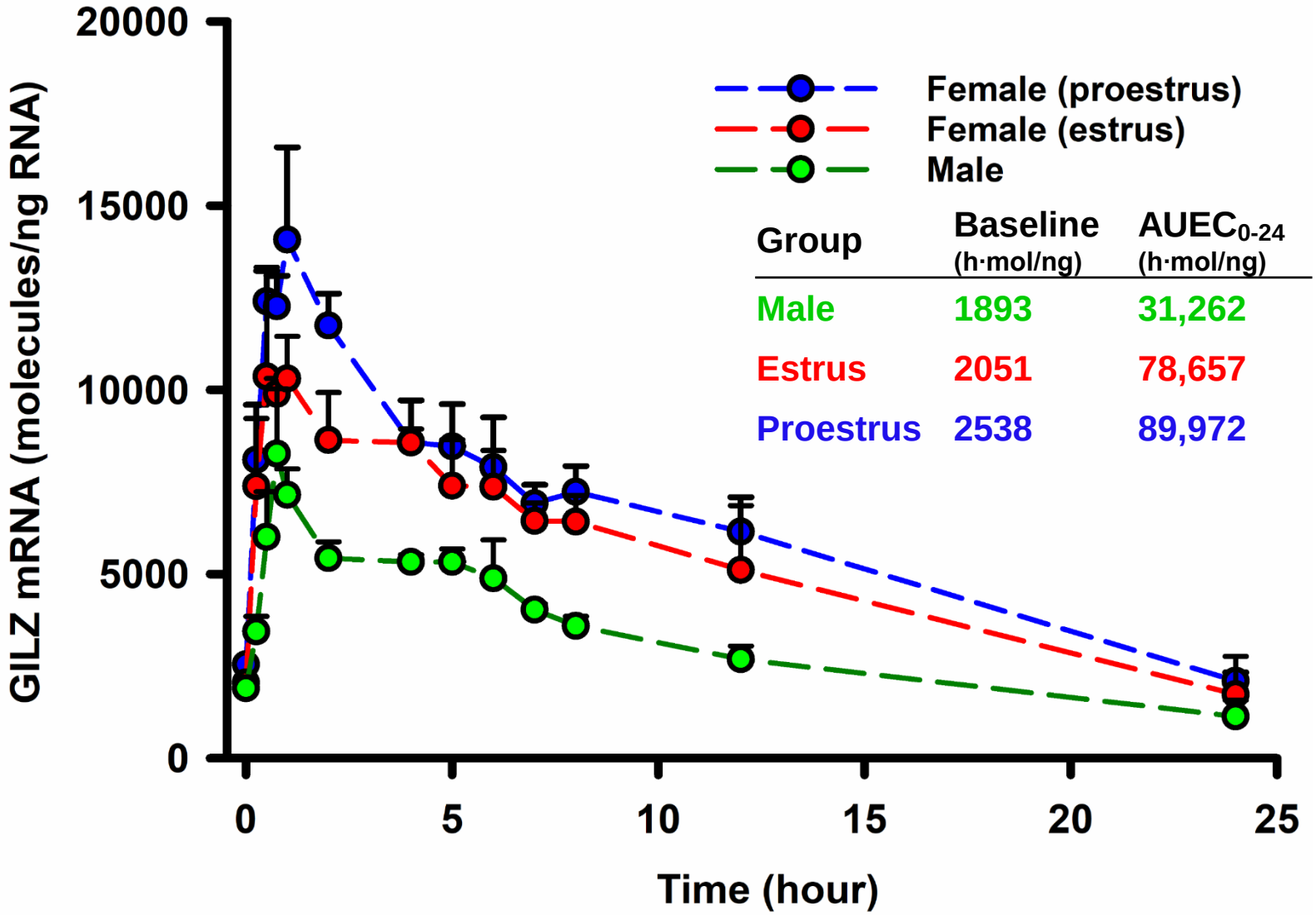
Previous report indicates no difference in hepatic GR protein density and binding in rats

Duma D and Cidlowski JA, *Sci Signaling* 3: ra74 (2010).



Sex Differences in Pharmacodynamics - GILZ in Liver

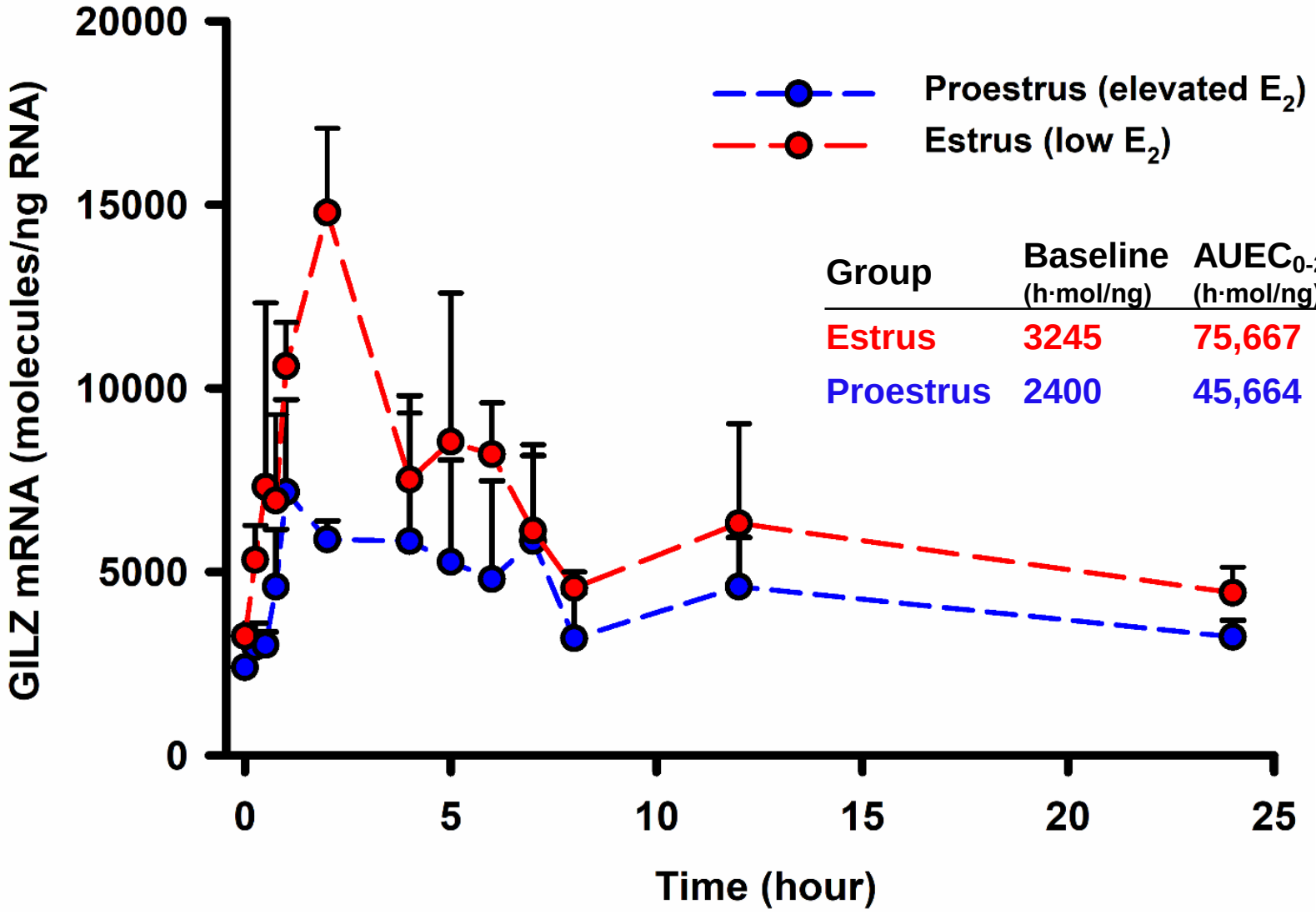
AUEC of hepatic GILZ increased in females (E and PE) compared to males



Group	Baseline (h·mol/ng)	$AUEC_{0-24}$ (h·mol/ng)
Male	1893	31,262
Estrus	2051	78,657
Proestrus	2538	89,972

Sex Differences in Pharmacodynamics - GILZ in Uterus

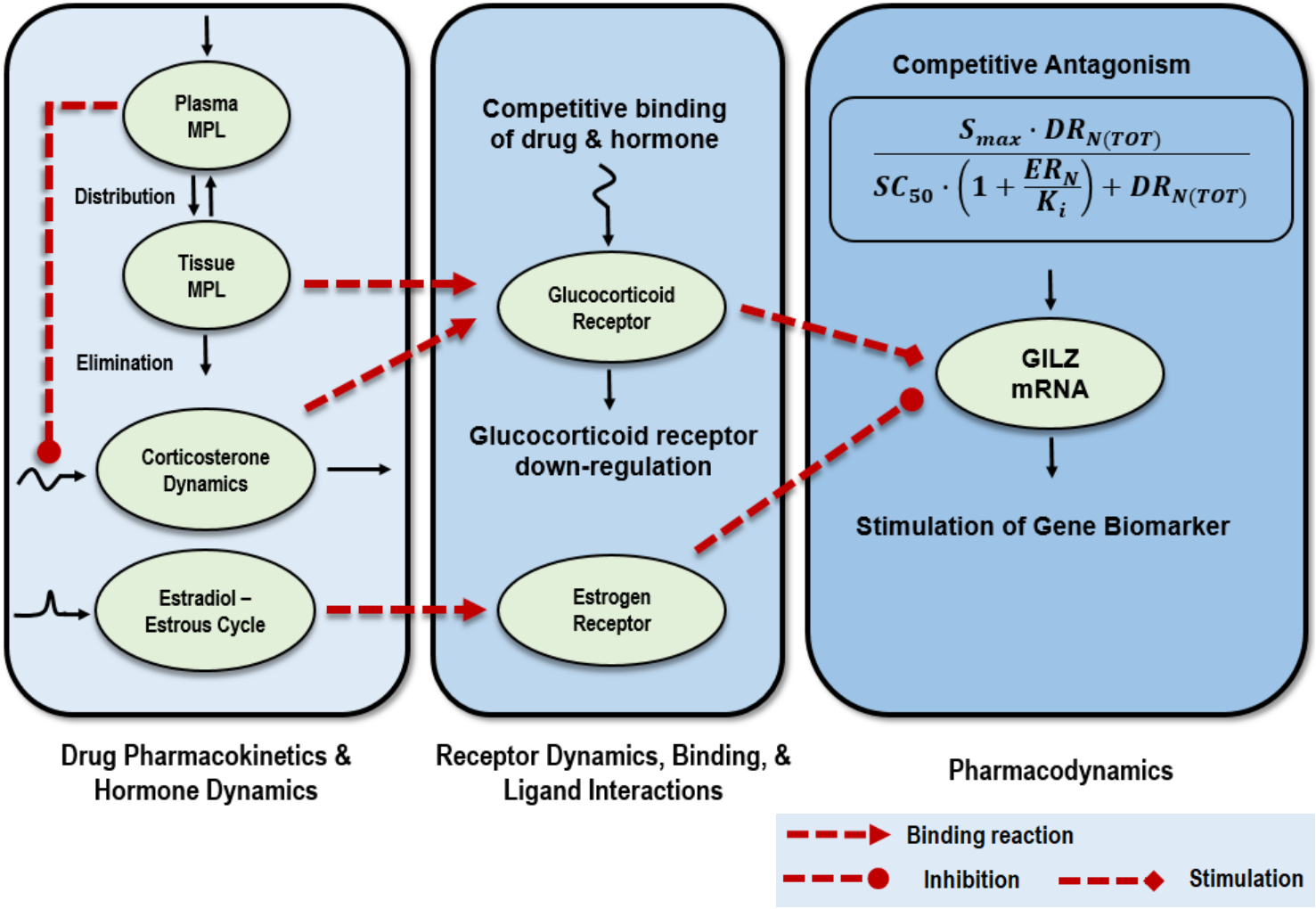
Baseline and AUEC of uterine GILZ in proestrus phase decreased compared to estrus



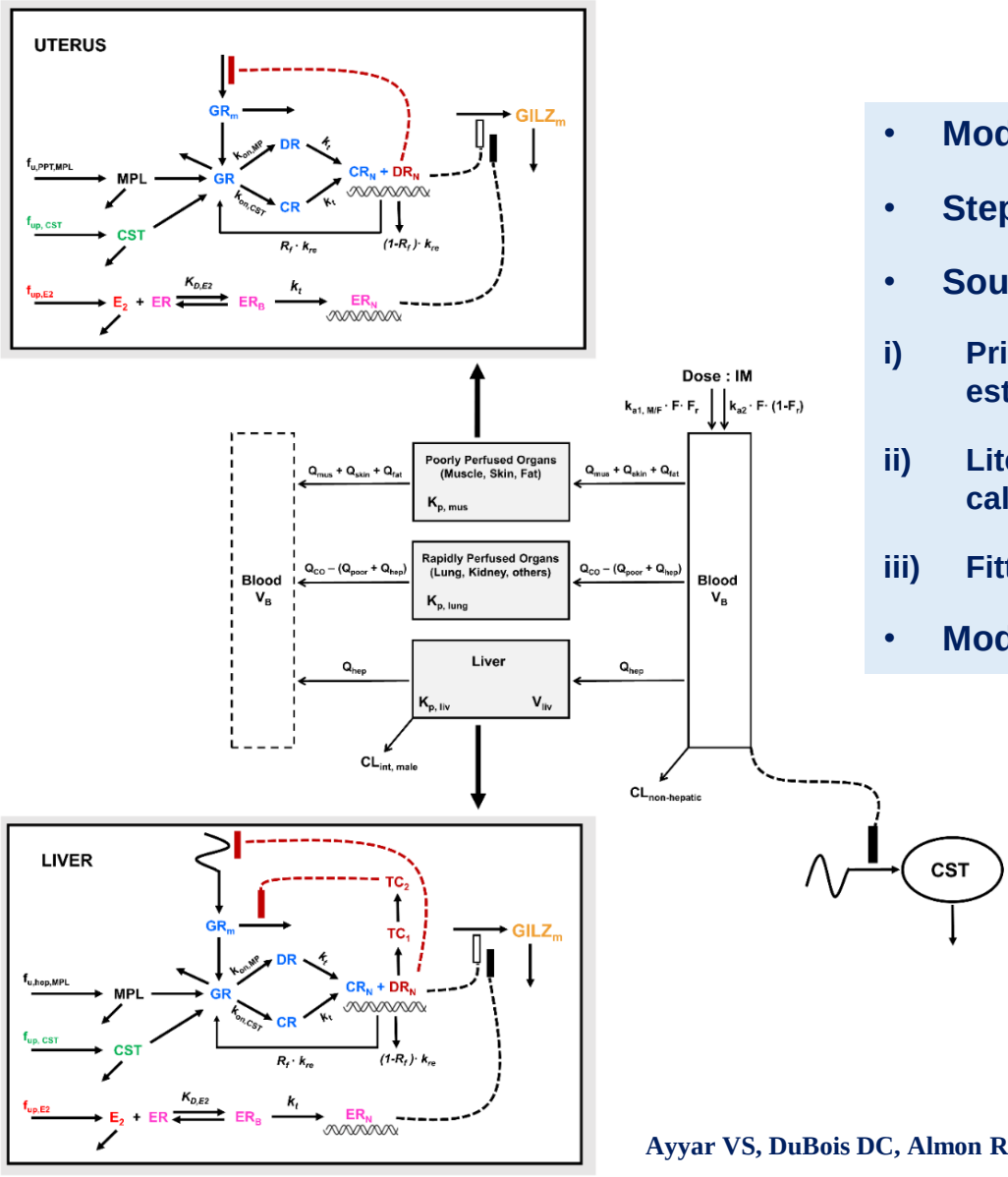
Group	Baseline (h·mol/ng)	$AUEC_{0-24}$ (h·mol/ng)
Estrus	3245	75,667
Proestrus	2400	45,664

General Schematic of Mechanism-Based Modeling Approach

Objective: To develop a quantitative model that integrates mechanisms controlling steroid disposition and pharmacodynamics across sex, reproductive stage, and tissue type



mPBPK / PD Model for MPL in Males and Females



- Model implemented using ADAPT 5
- Stepwise modeling approach employed
- Sources of parameter values:

i) Prior in-house experiments (data re-fit or estimated values fixed)

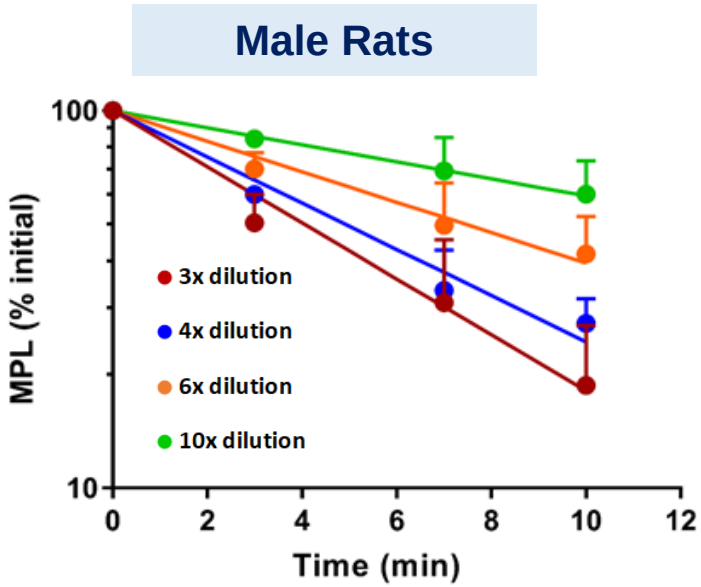
ii) Literature (data fitting, directly obtained, or calculated from *in vitro* studies)

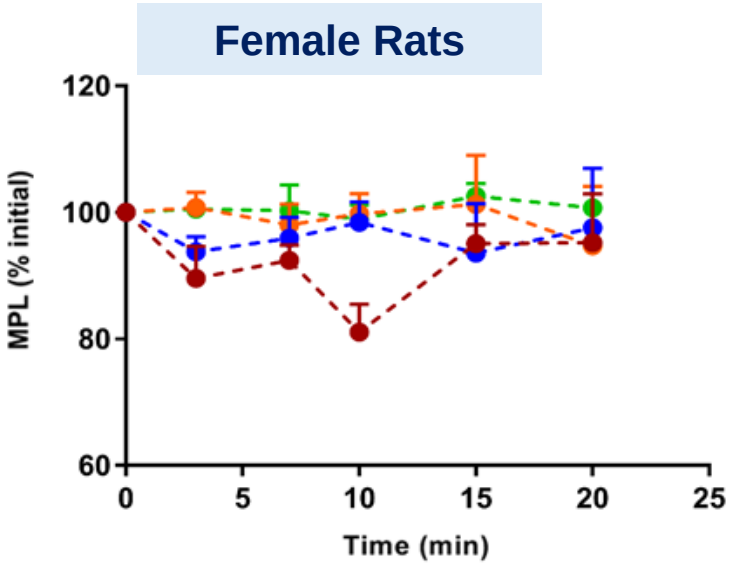
iii) Fitting of obtained *in vivo* data

- Model assessed jointly across all groups

Ayyar VS, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* Accepted (2019c).

Mechanistic Considerations: Sex and Hepatic Metabolism





Tomlinson ES et al, *J Steroid Biochem Molec Biol* 62: 345-352 (1997).

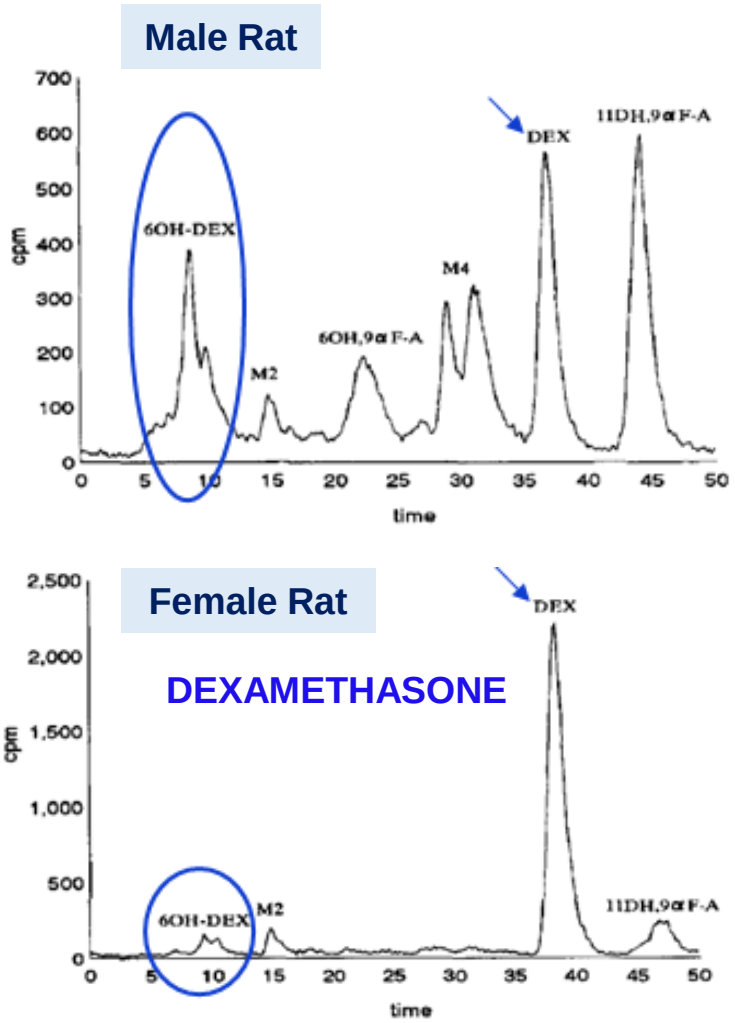
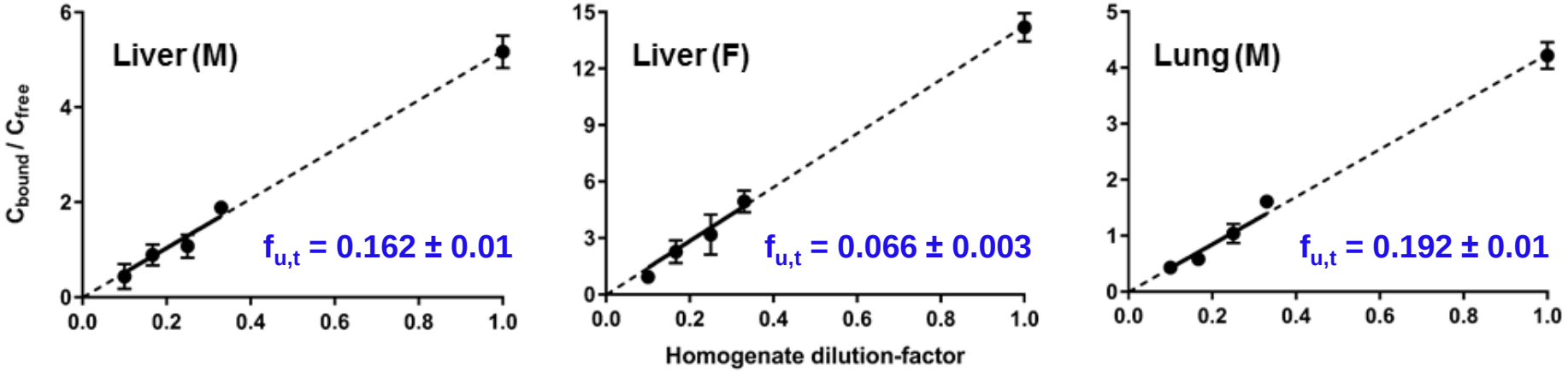


Fig. 4. High performance liquid chromatograms from microsomal incubations of ^{3}HDEX (1 μM) with microsomes from (a) male rat and (b) female rat.

Mechanistic Considerations: Tissue Binding of MPL *in vivo* vs. *in vitro vs. in silico* Differences

Ayyar VS, Song D, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* Accepted (2019a).

Tissue ultrafiltration methods were developed and validated for analysis of MPL binding *in vitro*



$K_{p,u\ (in\ vitro)} = C_{tot} / C_u = (C_b/C_u) + 1 = 1 / f_{u,t}$ $\qquad K_p = K_{p,u\ (in\ vitro)} \cdot f_{u,p}$ (0.4)

Tissue	*In vivo Kp*	*In vitro*	*In silico*[d]
Liver (male)	12.6[a,b]	2.5 ± 0.2	0.8
Liver (female)		6.1 ± 0.4	
Lung (male)	2.3 ± 0.0[b]	2.1	0.9
Muscle (male)	1.8 ± 0.4[b]	1.4[c]	0.6

[a] Model estimated value from IM data (corrected for hepatic CL_{int})

[b] Calculated from steady-state infusion PK in male rats

[c] Unbound fraction corrected for single tissue dilution using Kalvass-Maurer equation

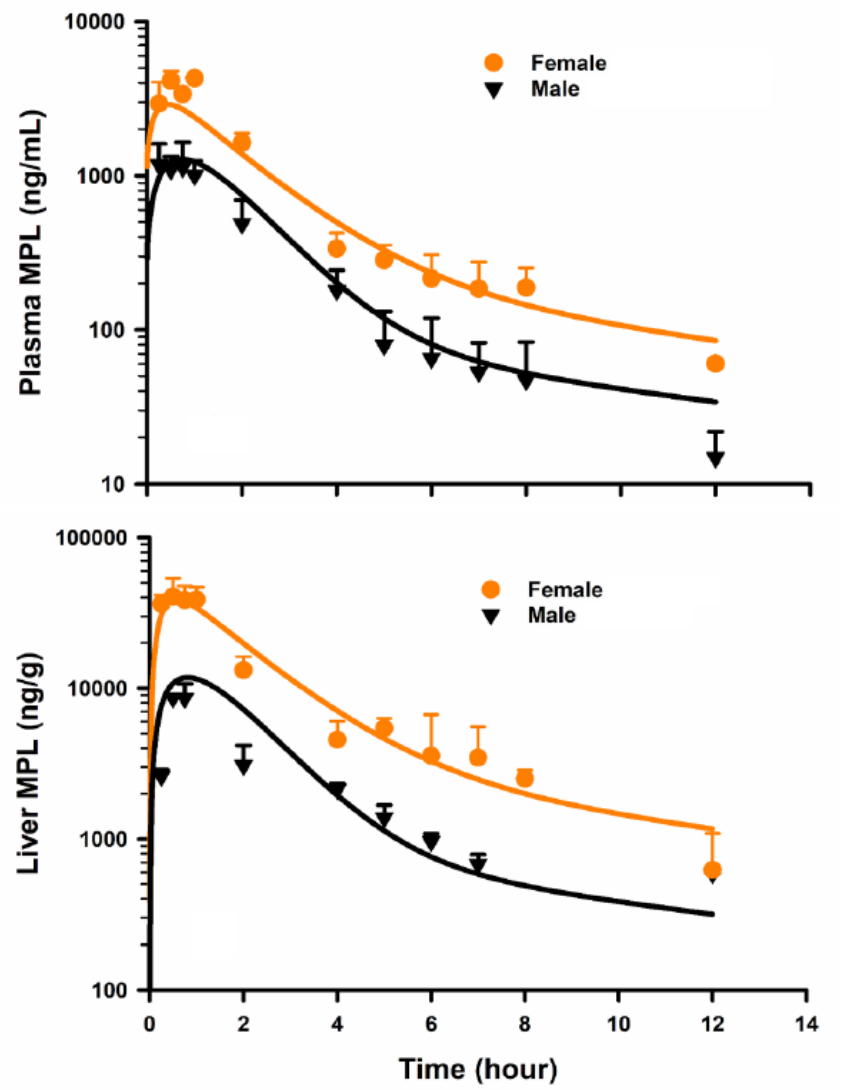
[d] Computed using method of Rogers and Rowland (J Pharm Sci, 2006)

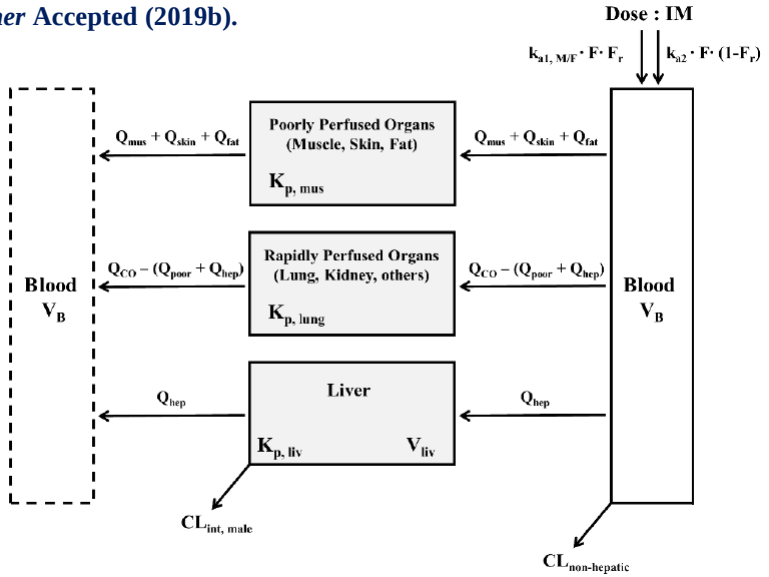
$$Undiluted\ f_u = \frac{1/DIL}{\left(\left(1/f_{u,meas}\right) - 1\right) + 1/DIL}$$

Kalvass JC and Maurer TS, *Biopharm Drug Dispos* 23: 327-38 (2002).

Step 1: MPL Pharmacokinetics (mPBPK Approach)

Ayyar VS, DuBois DC, Nakamura T, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* Accepted (2019b).





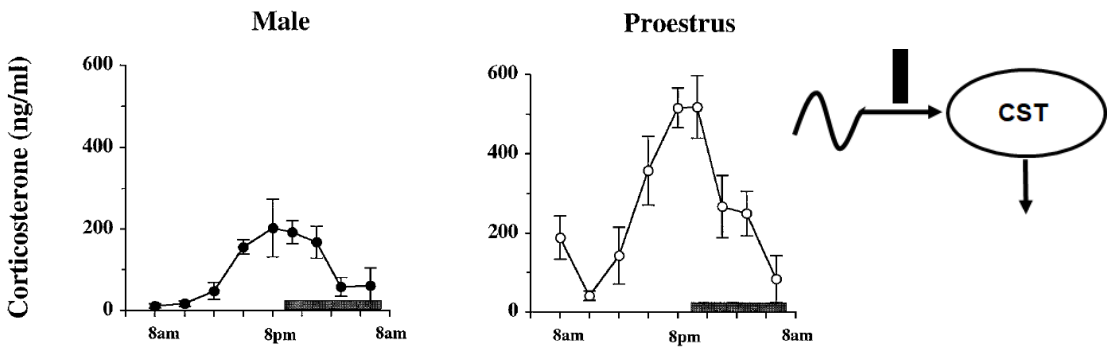
Parameter	Estimate (% CV)
F	0.214[a]
Fr	0.725[a]
k_{a1} (h^{-1})	0.8 (8.8)[b] / 4.1 (24.8)[c]
k_{a2} (h^{-1})	0.17 (17.3)
CL_{EH} (mL/h)	347 (4.2)
$CL_{u,int,male}$ (mL/h)	2987 (12.7)
$K_{P, liver}$	12.6 (7.6)
$K_{P, PPT (muscle)}$	1.4[d]
$K_{P, RPT (lung)}$	2.1[d]
$f_{u,p}$	0.4[d]

[a] Bioavailability fixed from Hazra et al. (2007b)
[b] Male ; [c] Female
[d] Fixed from *in vitro* experiments

Step 2: Dynamics of Corticosterone (CST) Suppression

Sexual dimorphism in diurnal variation of the rat HPA axis

Atkinson HC and Waddell BJ, *Endocrinol* 138: 3842-48 (1997).

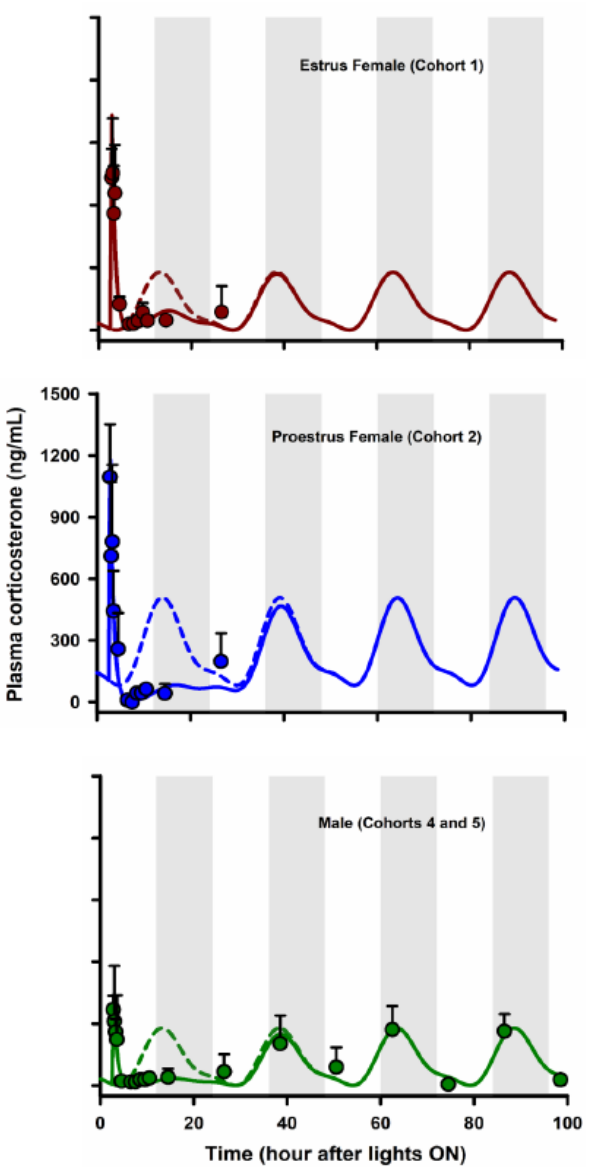


Data incorporated into model to account for varying circadian CST baseline concentrations (Fourier Harmonics)

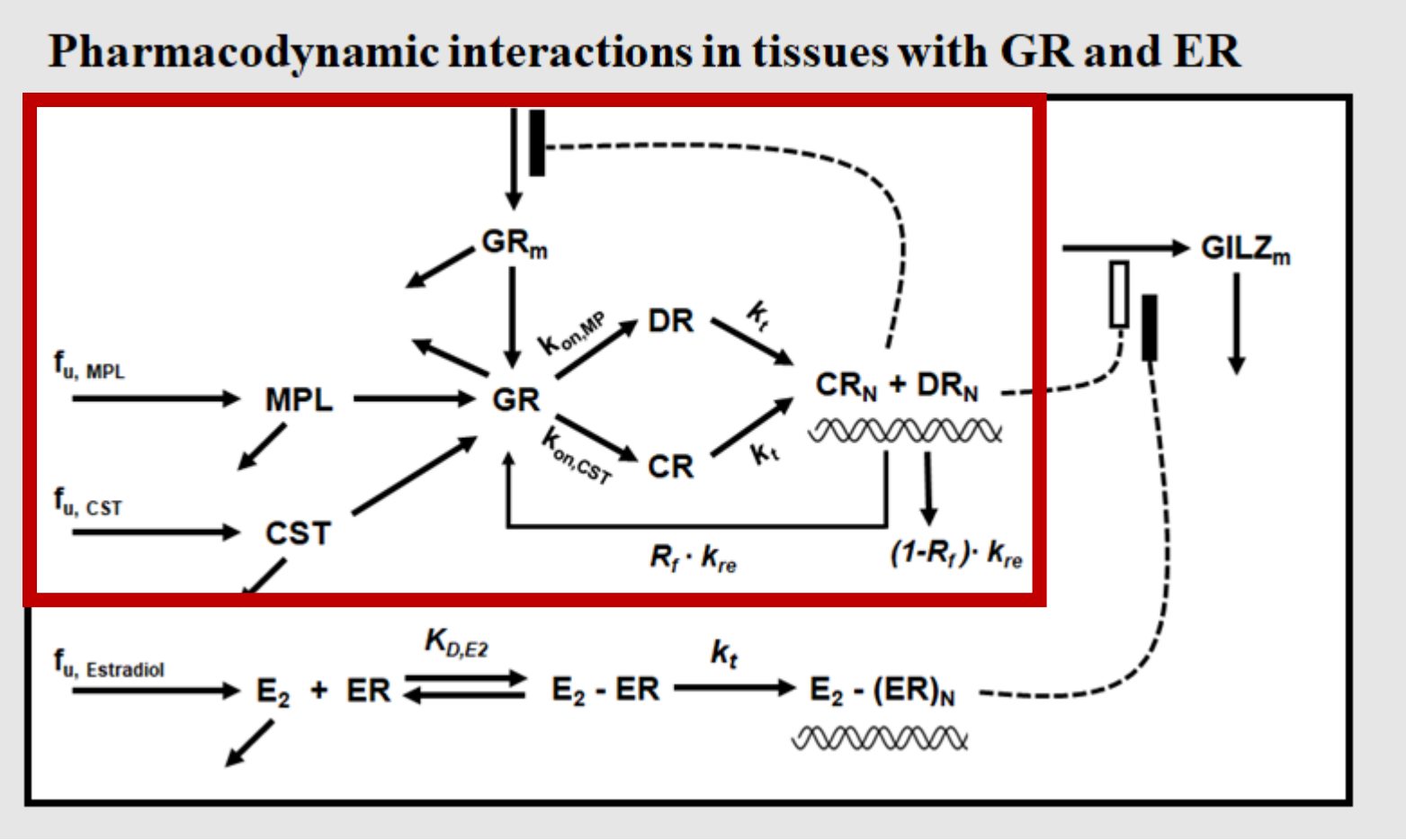
Baseline and IM Bolus data modeled jointly

Parameter	Estimate (% CV)
$k_{deg,CST}$ (h^{-1})	1.38 (12.6)[a,b,c]
τ (h)	0.34 (12.8)[a,b,c]
CST_{stress} (ng/mL)	535 (18.0)[a] / 1292 (13.3)[b] / 1397 (12.9)[c]
$IC_{50,CST}$ (ng/mL)	3.4 (47.6)[a] / 41.6 (48.0)[b] / 12.9 (45.6)[c]

[a] Male ; [b] Estrus female ; [c] Proestrus female

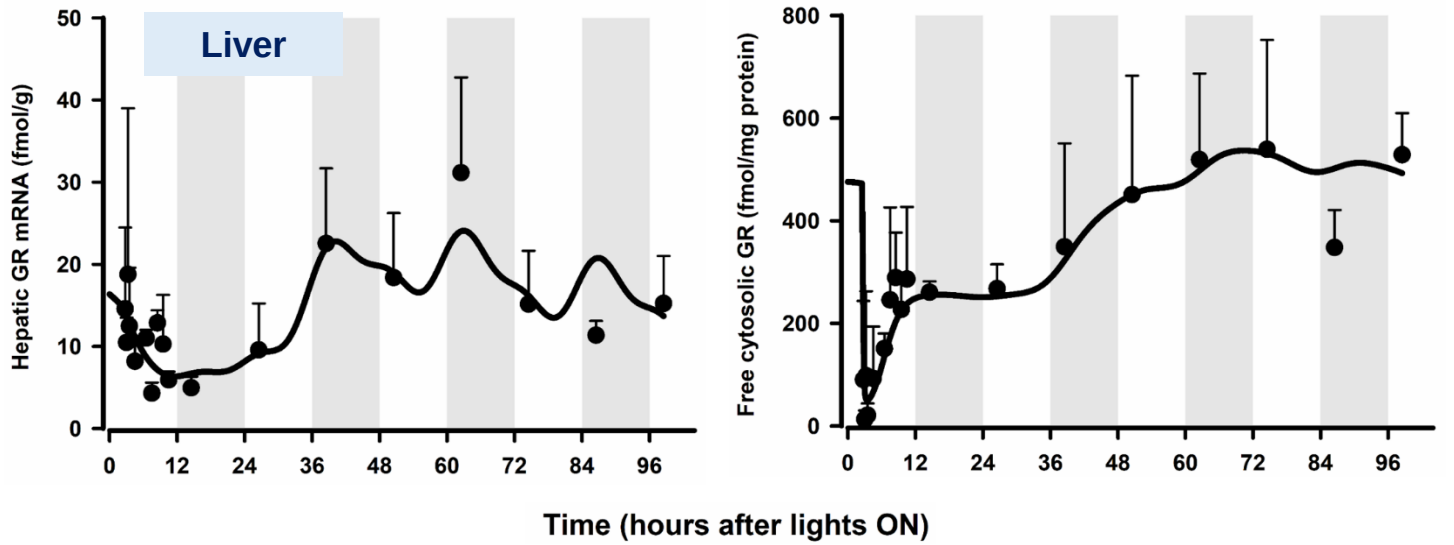


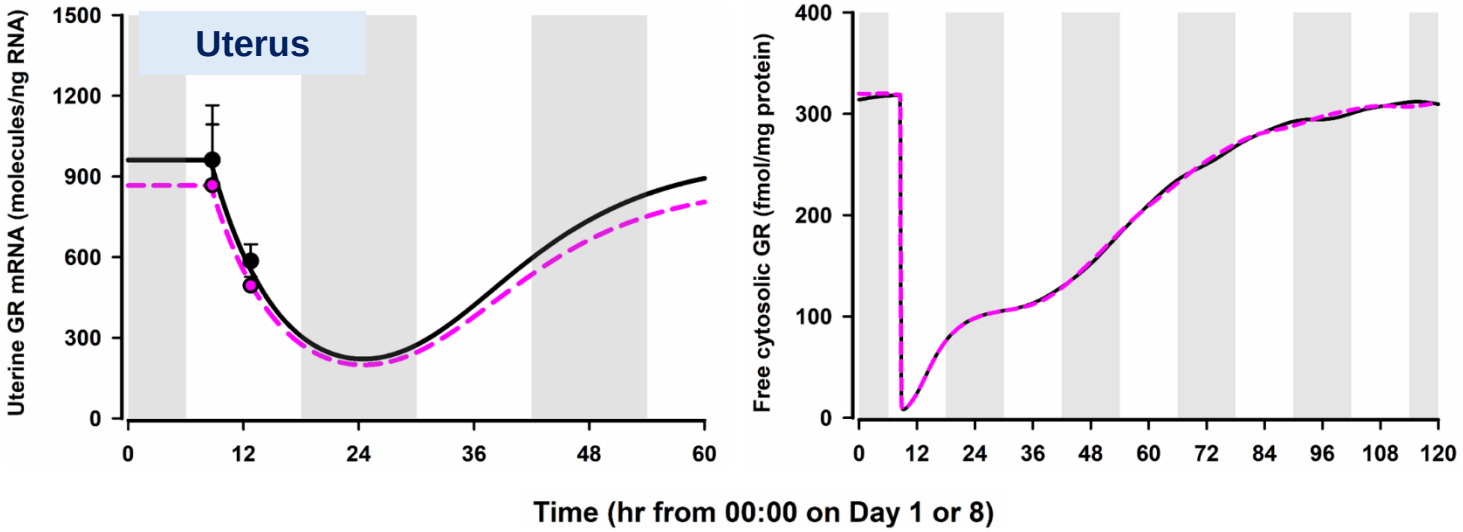
Step 3: Glucocorticoid Receptor Binding and Dynamics



Step 3: Glucocorticoid Receptor Binding and Dynamics

Liver data from Hazra A et al., *J Pharmacokinet Pharmacodyn* 28: 263-73 (2007).





Step 3: Glucocorticoid Receptor Binding and Dynamics

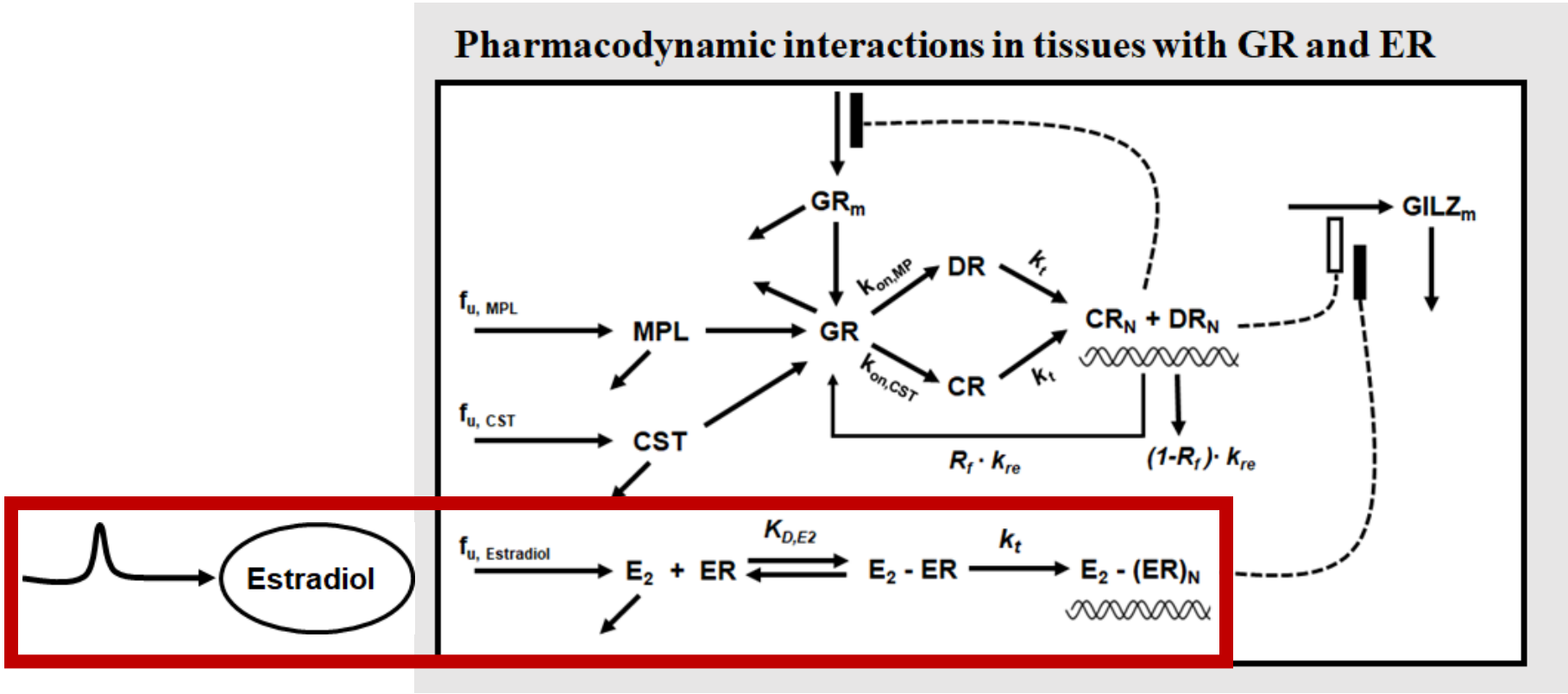
Parameter	Definition	Estimate (CV%)
$a_{0,GRm,liver}$		14.3
$a_{1,GRm,liver}$		-1.53
$a_{2,GRm,liver}$	Fourier coefficient for liver GR mRNA[a]	0.554
$b_{1,GRm,liver}$		-3.04
$b_{2,GRm,liver}$		1.18
$k_{d,GRm}$ (h^{-1})	Degradation rate constant for GR mRNA	0.14 (17.0)
$IC_{50,GRm}$ $(fmol/mg)$	Half-maximal inhibition of GR mRNA production	15.2 [a]
τ_{GRm} (h)	Transduction delay for mRNA rebound	15.6 [a]
$IC_{50,TC2}$ $(fmol/mg)$	Half-maximal inhibition of GR mRNA removal	60.5 [a]
$k_{d,GR}$ (h^{-1})	Degradation rate constant for receptor	0.05 [a]
$k_{on,MPL}$ $(nM^{-1}\cdot h^{-1})$	Association rate constant for MPL	0.016 [a]
$k_{on,CST}$ $(nM^{-1}\cdot h^{-1})$	Association rate constant for CST	0.001 (fixed)
$f_{up,mpl}$	Unbound fraction of MPL in plasma	0.4 [b]
$f_{u,liv,mpl}$	Unbound fraction of MPL in liver	0.032 (calculated as $K_{p,hep} / f_{u,p}$)
$f_{u,cst}$	Unbound fraction of CST in plasma	0.017 [a]
k_{re} (h^{-1})	DR_N loss rate constant	1.31 [a]
R_f	Fraction recycled	0.93 [a]
k_T (h^{-1})	Translocation rate constant	58.3 [a]
$GR(0)$ $(fmol/mg\ protein)$	Free cytosolic receptor initial concentration	476.0 [a] (liver) ; 320.0 (uterus) [c]

[a] Fixed from Hazra et al. (2007)

[b] Fixed from Ayyar et al. (2019a)

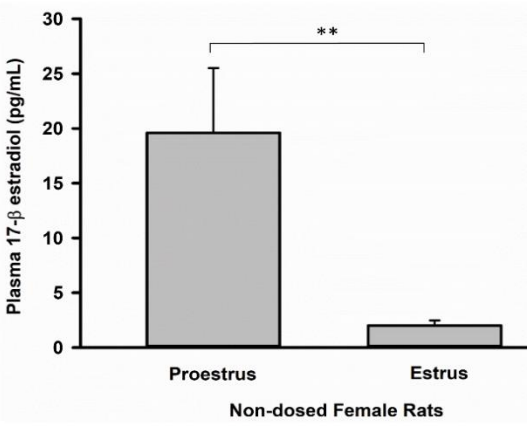
[c] Fixed from Izawa et al. (1984)

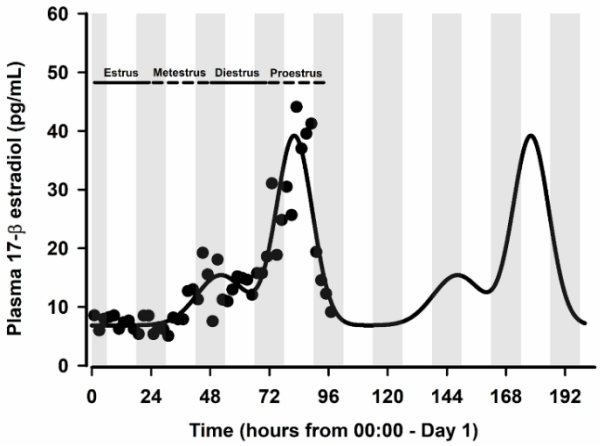
Step 4: Estrous Variation of Plasma 17β-Estradiol in Rats

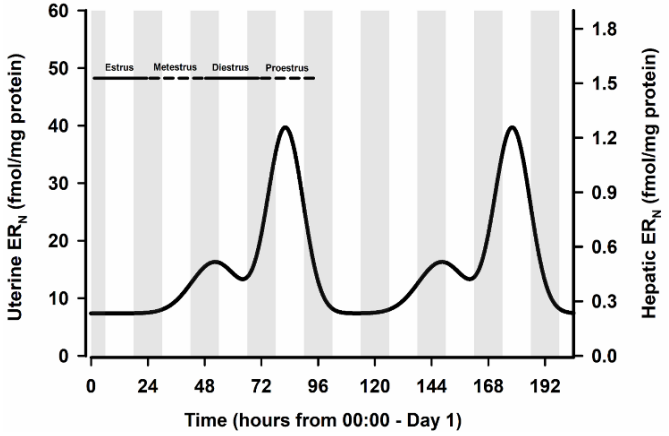


Step 4: Estrous Variation of Plasma 17β-Estradiol in Rats

Smith MS et al., *Endocrinol* 96: 219-226 (1975).





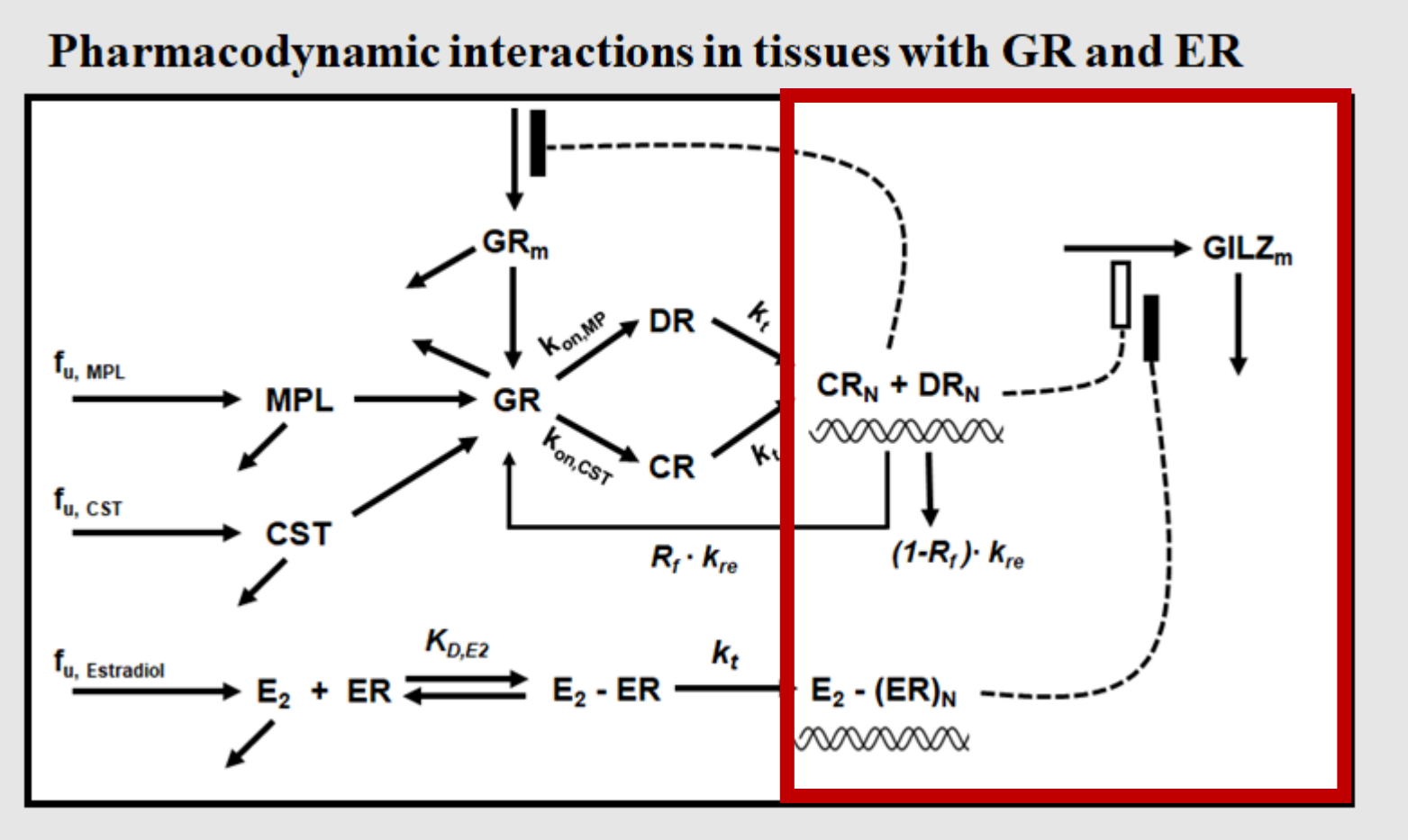


$$E_2(t) = \mathrm{BL} + \mathrm{A} \cdot e^{-\frac{(T-T_{peak1})^2}{\propto}} + B \cdot e^{-\frac{(T-T_{peak2})^2}{\beta}} + \mathrm{A} \cdot e^{-\frac{(T-(\tau+T_{peak1}))^2}{\propto}} + \mathrm{B} \cdot e^{-\frac{(T-(\tau+T_{peak2}))^2}{\beta}}$$

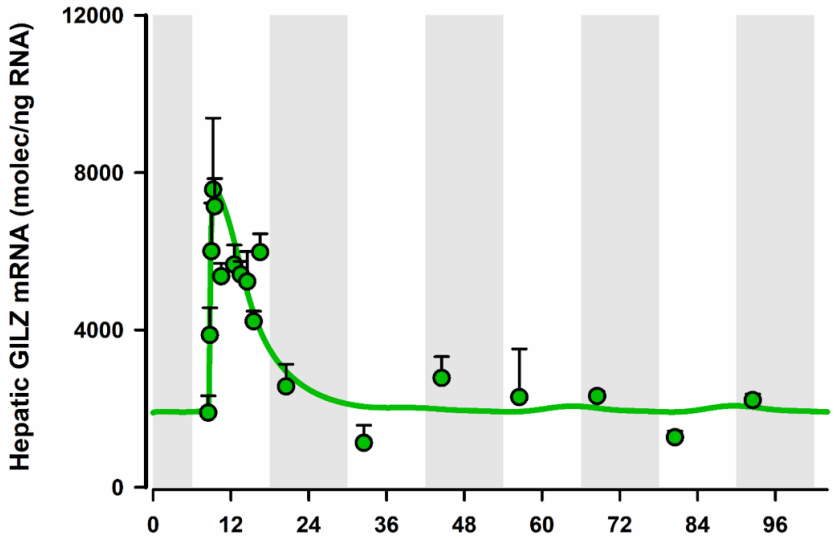
Parameter	Definition	Estimate (%CV) or Value (Source)
Estrogen Receptor Binding & Dynamics		
$f_{up,E2}$	Unbound fraction of estradiol in plasma	0.053 (Plowchalk and Teeguarden, 2002)
$B_{max,ER(liv)}$ (fmol/mg protein)	Estrogen receptor content in liver	24.5 (Dickson and Eisenfeld, 1979; Aten et al., 1978)
$B_{max,ER(uterus)}$ (fmol/mg protein)	Estrogen receptor content in uterus	560 (Notides, 1970)
$K_{D,ER(liv)}$ (pM)	ER Binding Constant in liver	140 (Dickson and Eisenfeld, 1979)
$K_{D,ER(uterus)}$ (pM)	ER Binding Constant in uterus	100 (Branham et al., 2002)
k_t (h^{-1})	Translocation rate constant	58.3 (Assumed equal to GR translocation)

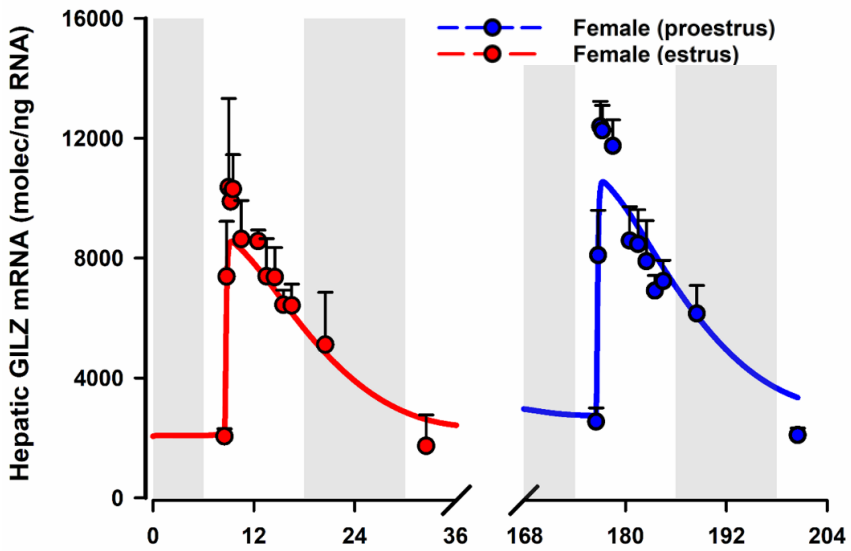
Ayyar VS, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* Accepted (2019c).

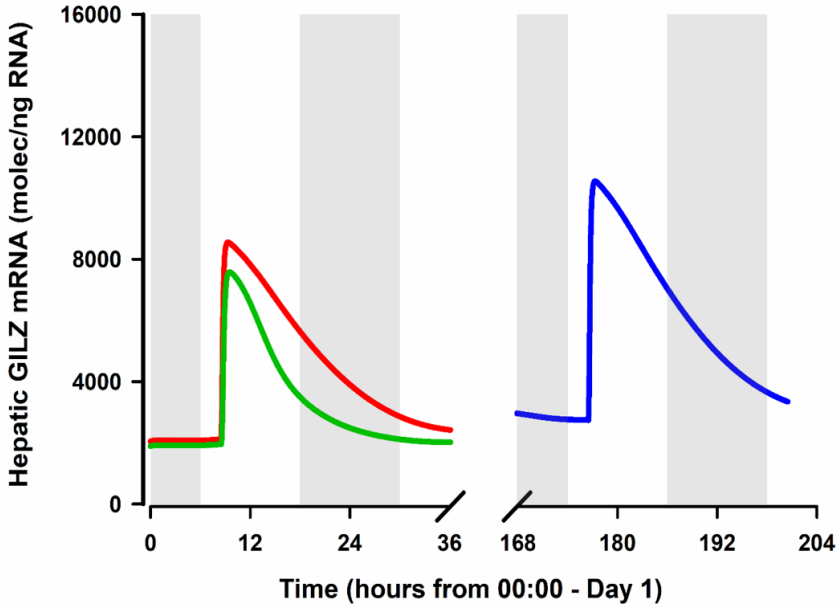
Step 5: Pharmacodynamic Regulation of GILZ

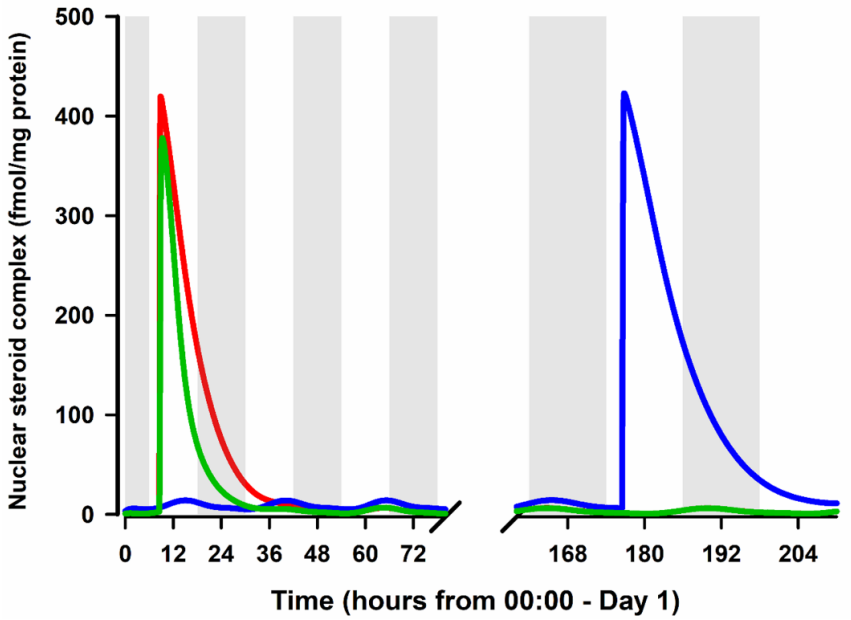


Step 5A: Pharmacodynamic Regulation of GILZ in Liver



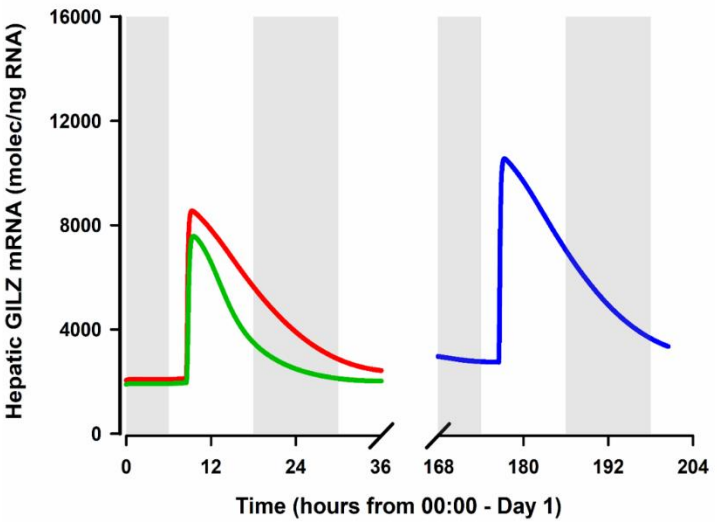


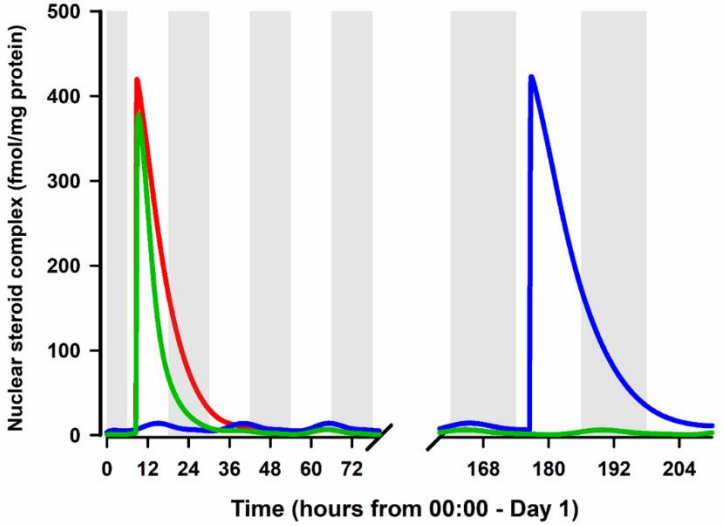




Step 5A: Pharmacodynamic Regulation of GILZ in Liver

$$\frac{dGILZ_m}{dt} = k_{s,GILZm} \cdot \left(1 + \left(\frac{S_{max} \cdot NR_{n\_TOT}}{SC_{50,GILZm} \cdot \left(1 + \frac{ER_n}{K_i}\right) + NR_{n\_TOT}}\right)\right) - k_{d,GILZm} \cdot GILZ_m$$

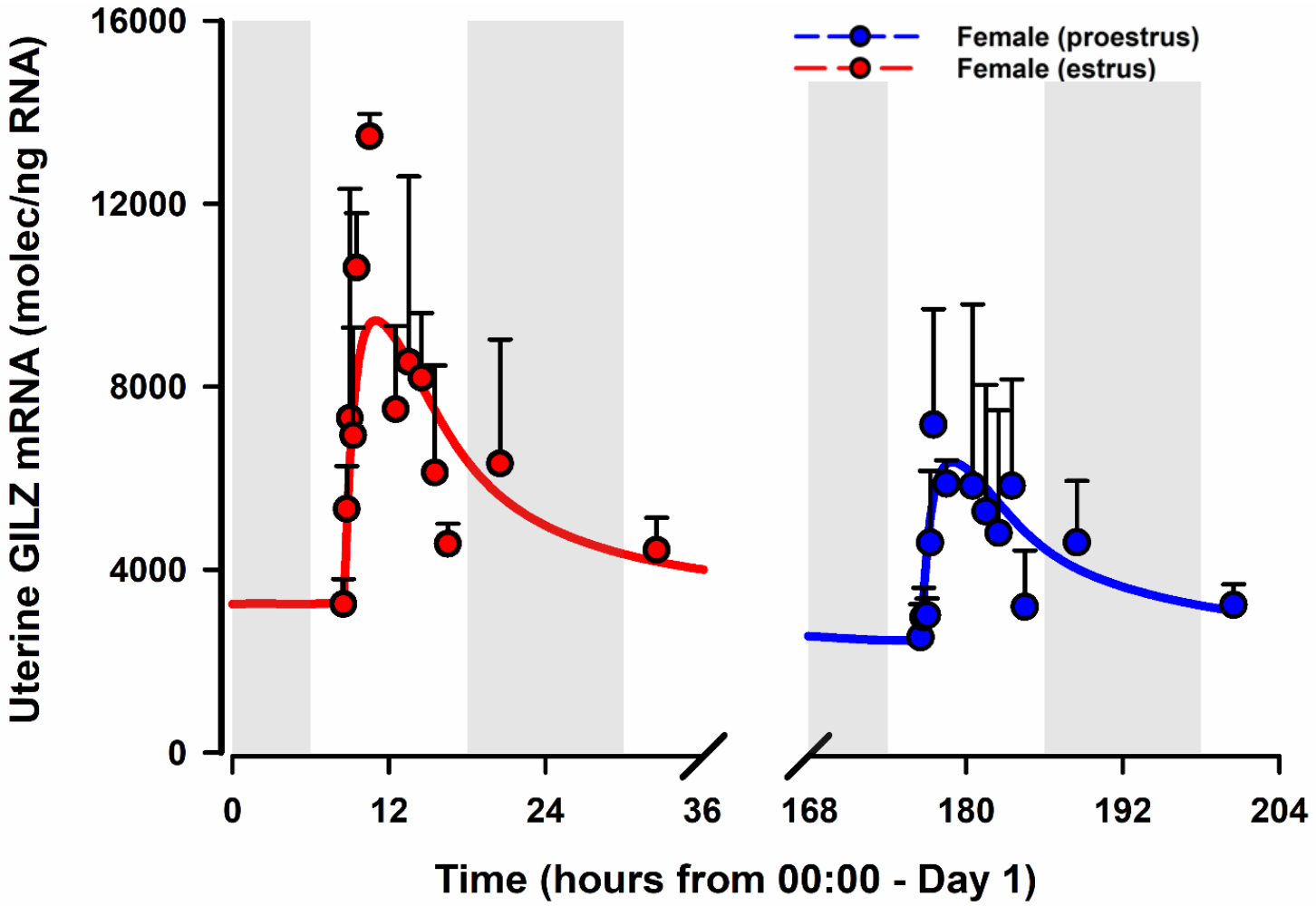




Parameter	Definition	Estimate (% CV)
$k_{d,GILZm}$ **(h^{-1})**	Degradation rate constant for GILZ mRNA	7.5 (21.8)
$S_{max,\ GILZm}$	Maximal stimulatory capacity by DR_N	7.5 (fixed)
$SC_{50,\ DRn,\ GILZm}$ **(fmol/mg)**	DR_N producing half maximal stimulation	558 (5.5)
$K_{i,\ ERn,\ GILZm}$ **(fmol/mg)**	ER_N producing half maximal inhibition of GILZ mRNA	62.1 (fixed based on uterine data)
$GILZ_m\ (0)$ **(molecules/ng RNA)**	GILZ mRNA initial concentration	1893 (M) ; 2051 (E) ; 2538 (PE)

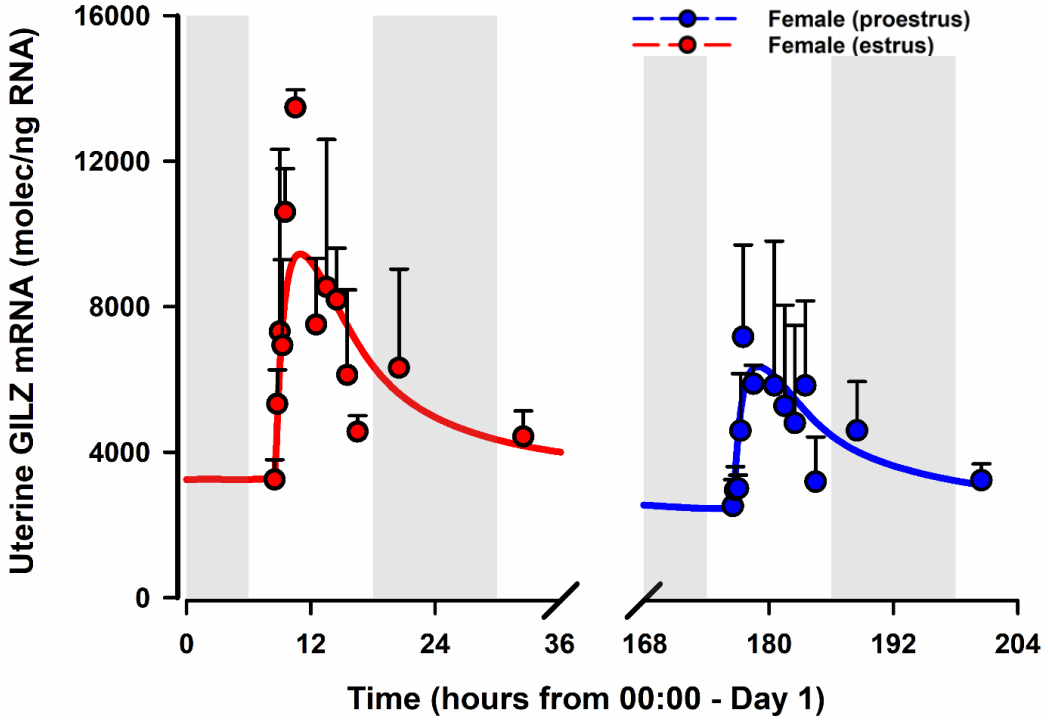
Step 5B: Pharmacodynamic Regulation of GILZ in Uterus

$$\frac{dGILZ_m}{dt} = k_{s,GILZm} \cdot \left(1 + \left(\frac{S_{max} \cdot NR_{n\_TOT}}{SC_{50,GILZm} \cdot \left(1 + \frac{ER_n}{K_i}\right) + NR_{n\_TOT}}\right)\right) - k_{d,GILZm} \cdot GILZ_m$$



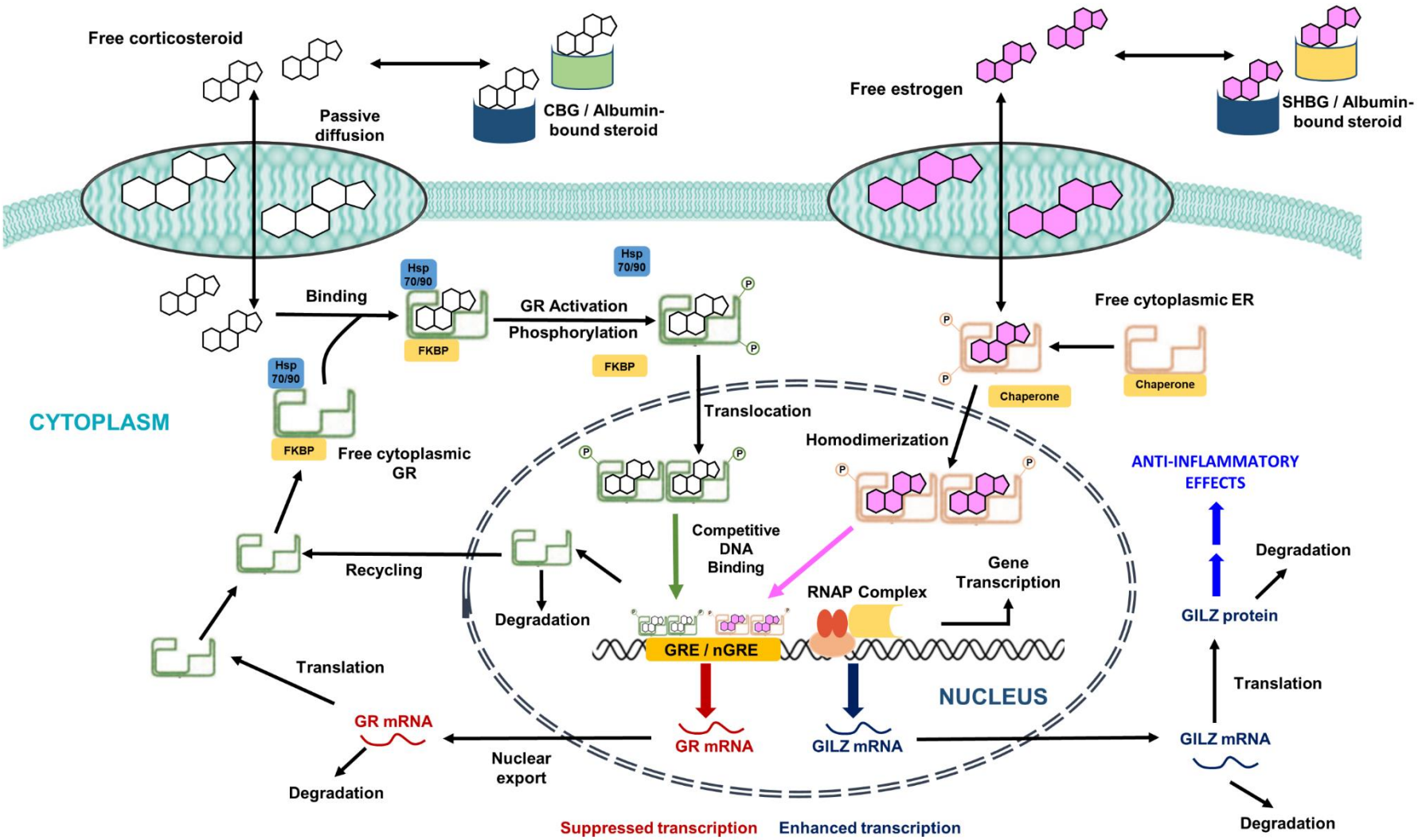
Step 5B: Pharmacodynamic Regulation of GILZ in Uterus

Ayyar VS, DuBois DC, Almon RR, Jusko WJ, *J Pharmacol Exp Ther* Accepted (2019c).



Parameter	Definition	Estimate (% CV)
$k_{d,GILZm}$ (h^{-1})	Degradation rate constant for GILZ mRNA	1.9 (27.5)
$S_{max,\ GILZm}$	Maximal stimulatory capacity by DR_N	7.5 (fixed)
$SC_{50,\ DRn,\ GILZm}$ (fmol/mg)	DR_N producing half maximal stimulation	672 (19.2)
$K_{i,\ ERn,\ GILZm}$ (fmol/mg)	ER_N producing half maximal inhibition of GILZ mRNA	62.1 (68.6)
$GILZ_m(0)$ (molecules/ng RNA)	GILZ mRNA initial concentration	3245 (E) ; 2400 (PE)

Antagonism of Glucocorticoid Signaling by Estrogens: Implications for CS Efficacy and Individualized Therapy?



SUMMARY & CONCLUSIONS

- **Early PK/PD models of methylprednisolone were evolved into more mechanistic and global systems models.**

- **Mechanistic modeling of GILZ integrated circadian rhythms and receptor-mediated steroid pharmacogenomics to capture and predict tissue-specific responses.**

- **Systems modeling offers a mechanistic bridge to connect drug exposure to relevant PD endpoints via indirect (receptor/gene/protein-mediated) mechanisms.**

- **Time- (estrous cycle) and estrogen receptor-dependent antagonistic co-regulation by estradiol explained sex differences in genomic steroid response.**

- **The combined systems (experimental and modeling) approach revealed a unique pharmacodynamic interaction of multi-receptor signaling *in vivo*.**

- **The fundamental array of mechanism-based PK/PD models serve as building blocks for developing global and mechanistic systems pharmacology models.**

ACKNOWLEDGEMENTS

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Dr. Debra C. DuBois

Dr. Richard R. Almon

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NIH National Institutes of Health

University at Buffalo The State University of New York

ISoP Special Interest Group Quantitative Systems Pharmacology

aaps American Association of Pharmaceutical Scientists