Mathematically modelling the induction of xenobiotic-metabolising and transporter enzymes

Mohammed I. Atari, Paul D. Metcalfe and Simon Thomas
Scientific Computing Group
Cyprotex Discovery Ltd.

Presentation outline

- **Introduction**
- **Background biology**
  - Nuclear receptors
  - Cytochrome P450
  - Drug transporters
- **Mathematical model**
  - CYP3A4 induction by phenobarbital
  - Gene expression data
  - Parameter estimation
- **Sensitivity analysis**
- **Model validation**
  - CYP induction
- **Linking *in vitro* results to *in vivo* outcomes**
- **Conclusions**

Introduction

- **Motivation**
  - Living organisms are exposed to numerous foreign and endogenous substances (including drugs and environmental chemicals)
  - Some of these compounds can bind to receptors in the liver and other organs leading to changes in enzyme expression
  - Changes in transporters and drug-metabolising enzymes are a well-studied system, and are important in drug discovery
- **Objective**
  - Investigate the effect of typical nuclear receptor activators on the induction of proteins that govern xenobiotic metabolism and disposition in human liver
- **Method**
  - Developed a **novel** mathematical model for the *in vitro* kinetics of xenobiotics
  - The model describes the expression of cytochrome P450 isoforms and ATP-binding cassette transporters in response to different activators
  - Gene expression time-series data from primary human hepatocytes (parameter estimation and model validation)

Background biology

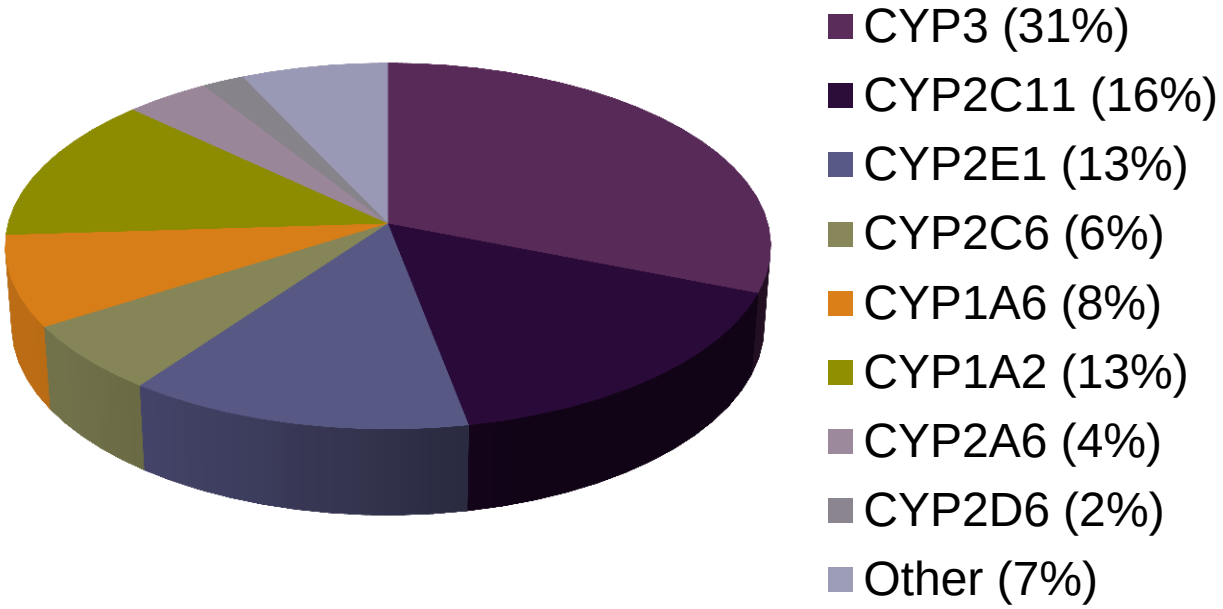
- Xenobiotic-metabolising enzymes and transporter (uptake & efflux) proteins play crucial roles in the metabolism and disposition of xenobiotics
- Metabolism of xenobiotics can result in detoxification and/or toxification (by forming toxic metabolites)
- Transport can either remove chemicals from the body or cause increased chemical concentration in certain tissues (potentially increasing toxic effects)
- The expression of xenobiotic-metabolising enzymes and transporters is up-regulated by a group ligand-activated transcription factors known as the nuclear receptors (NRs)
- Xenobiotics can alter the transcription of a broad array of genes expressed in tissues and vital organs including the **liver**, kidney, intestine and pancreas
- The structural features of NRs include a highly-conserved DNA-binding domain (DBD) and a less conserved ligand-binding domain (LBD)

Background biology

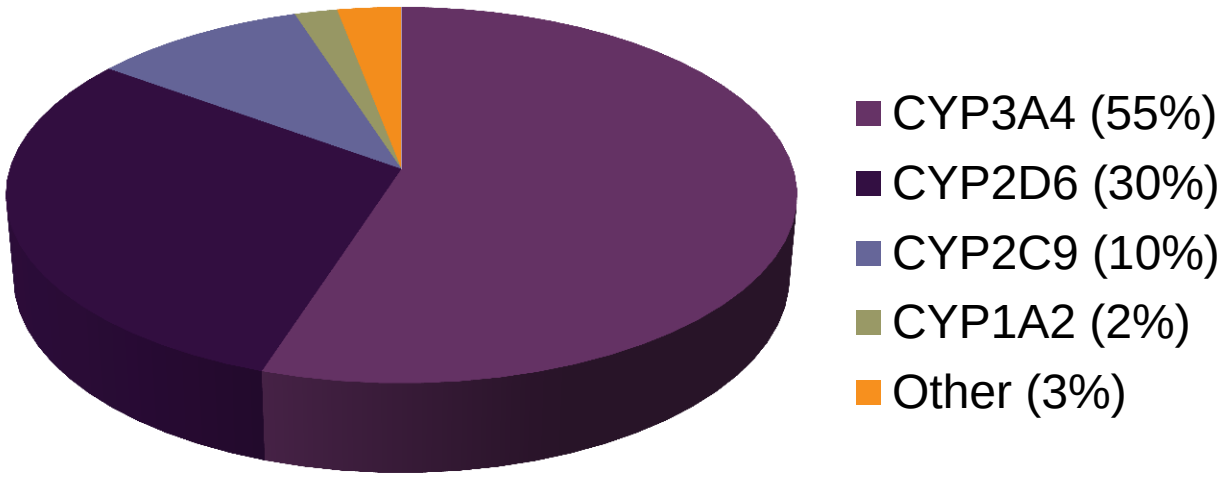
- The majority of known ligands for orphan receptors are xenobiotics including:
  - Drugs
  - Industrial chemicals
  - Environmental pollutants
  - Food additives
- Pregnane X receptor (PXR; NR1I2), an orphan receptor, is one of the best-studied NRs which mediates the induction of cytochrome P450 (CYP) and ABC genes
- The superfamily of CYP enzymes has a pivotal role in the metabolism of xenobiotics
- The highest expression levels of CYPs is found in the liver, however, certain CYPs are present in the wall cells of the intestine, and elsewhere

Background biology

Distribution of CYP isoforms

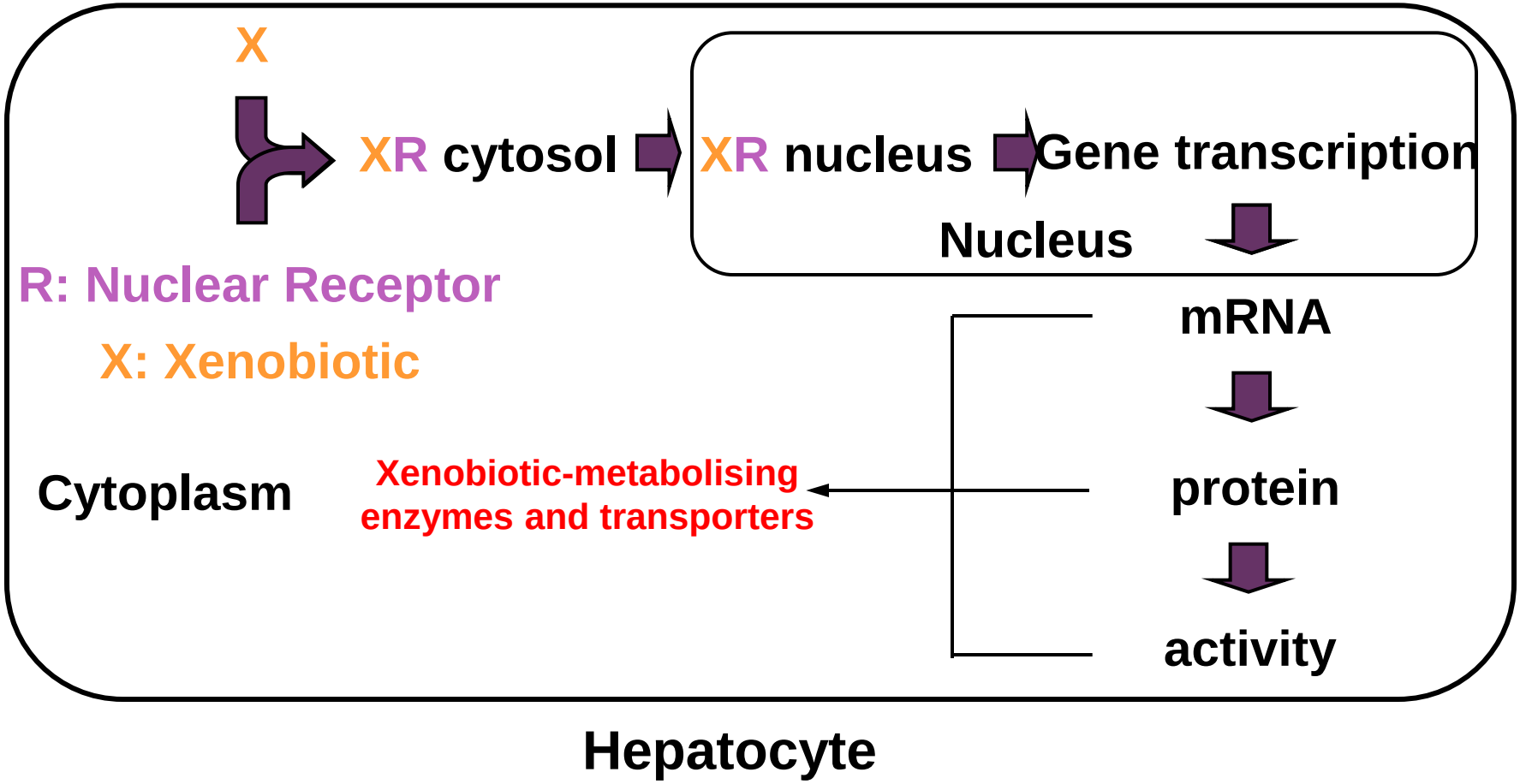


Background biology

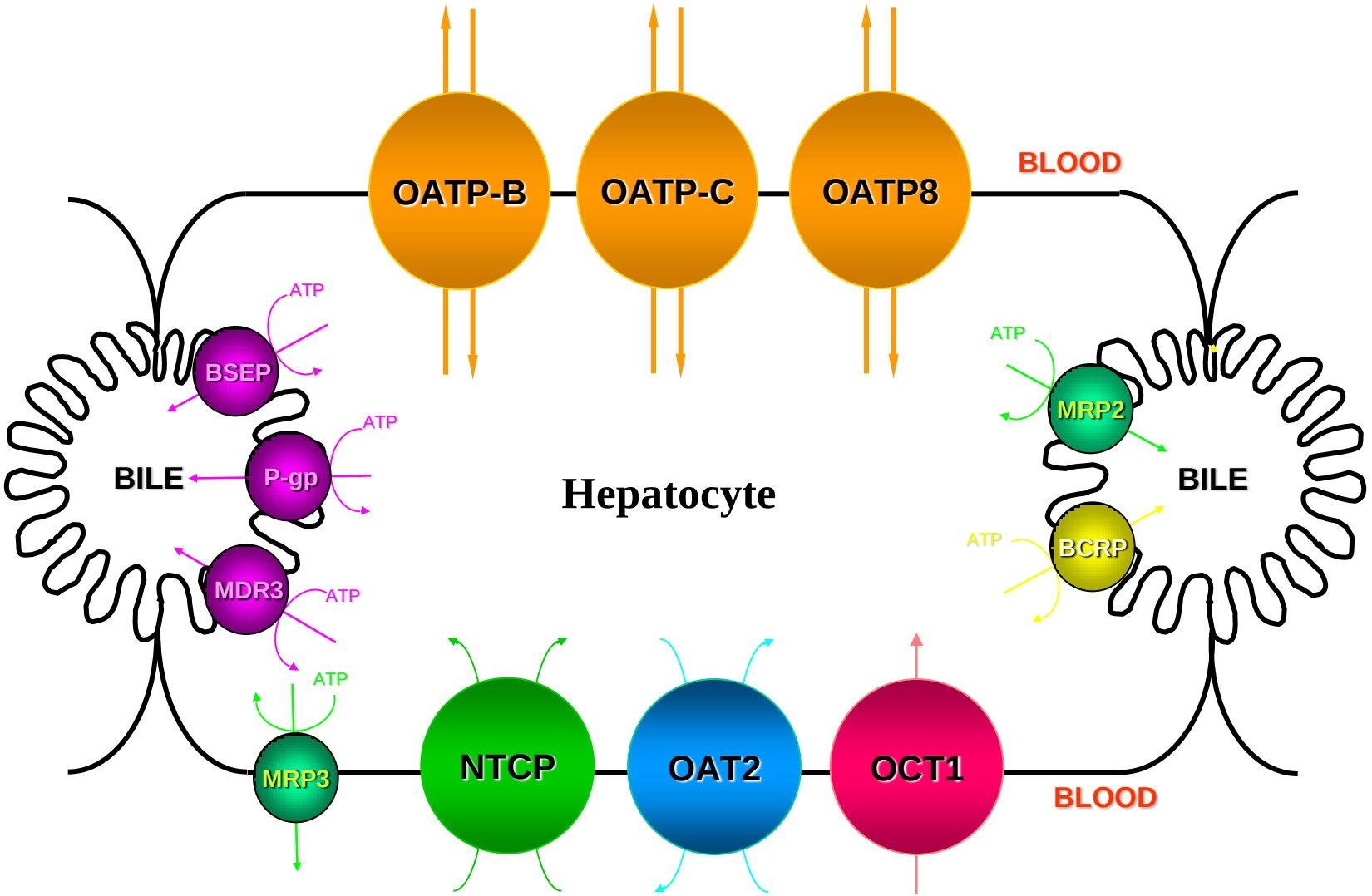


Background biology

General mechanism of induction

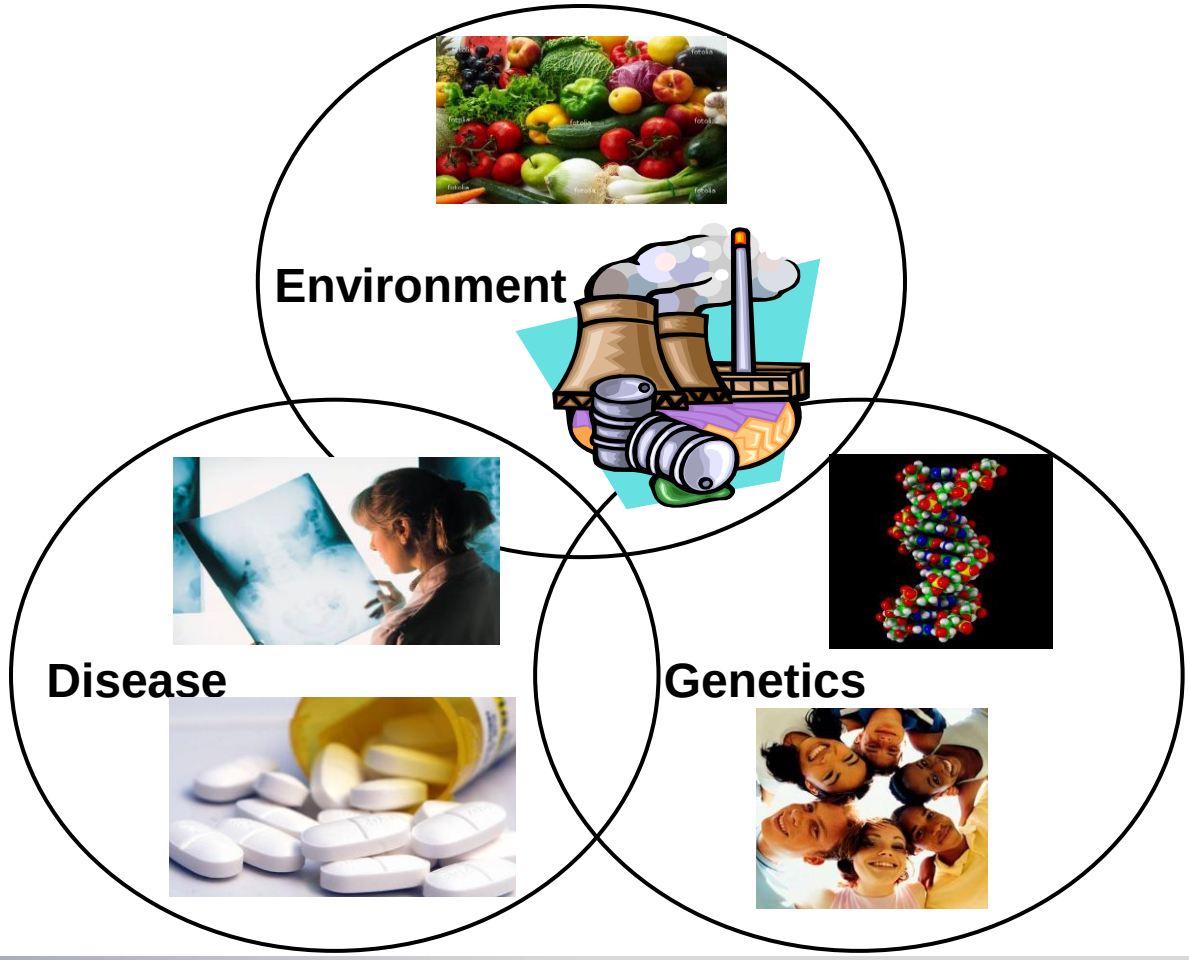


Background biology

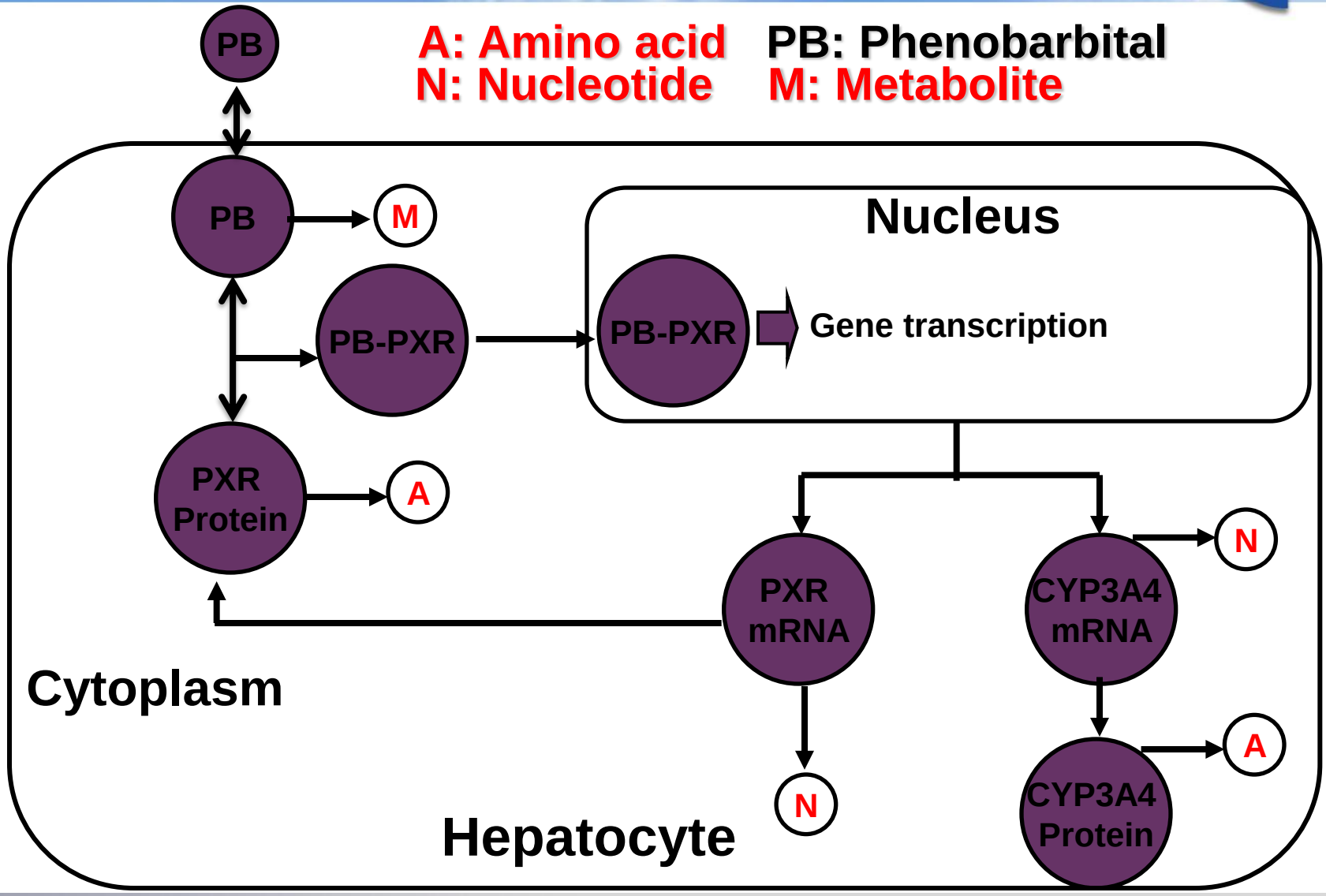


Background biology

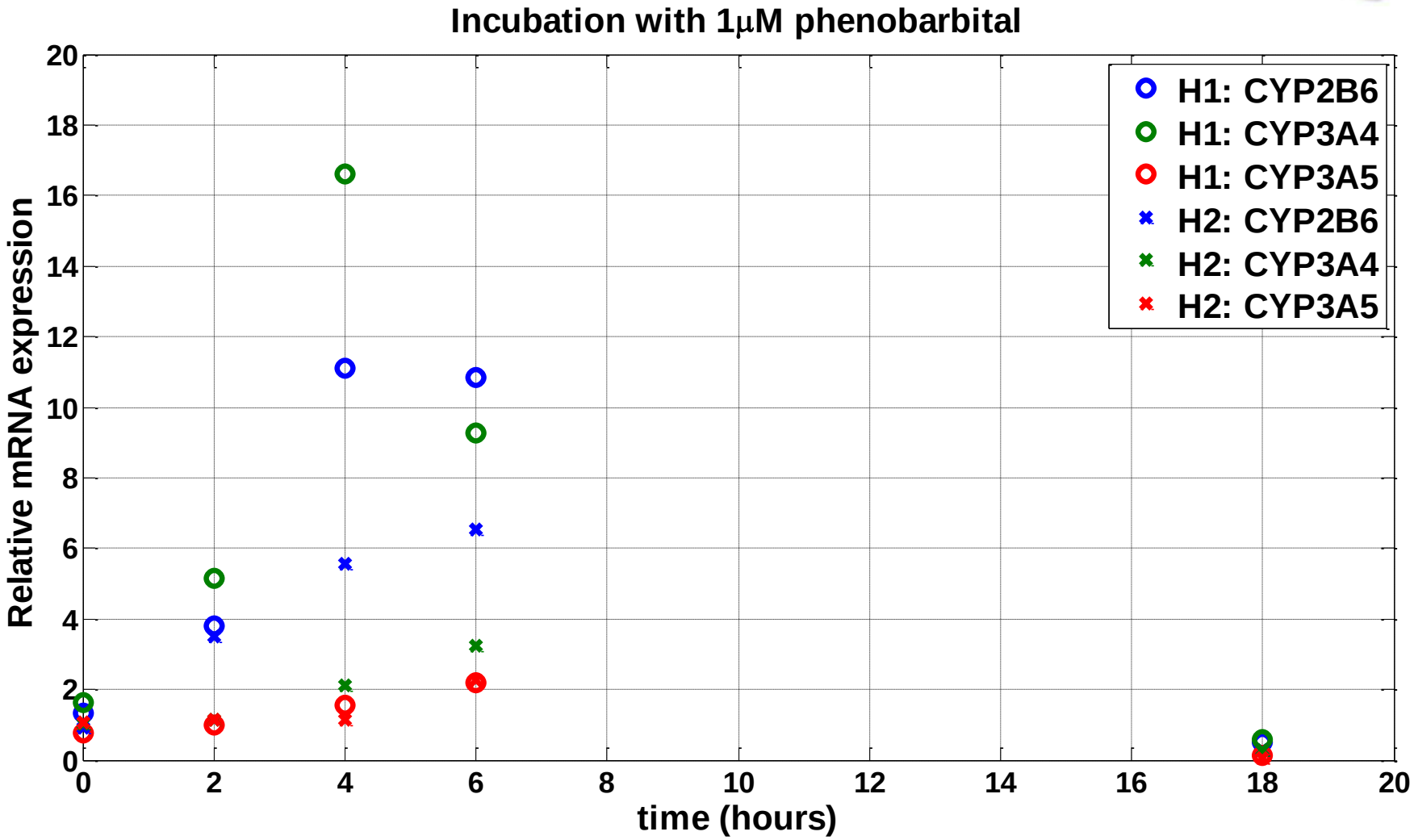
Variability in response to xenobiotics



Mathematical model

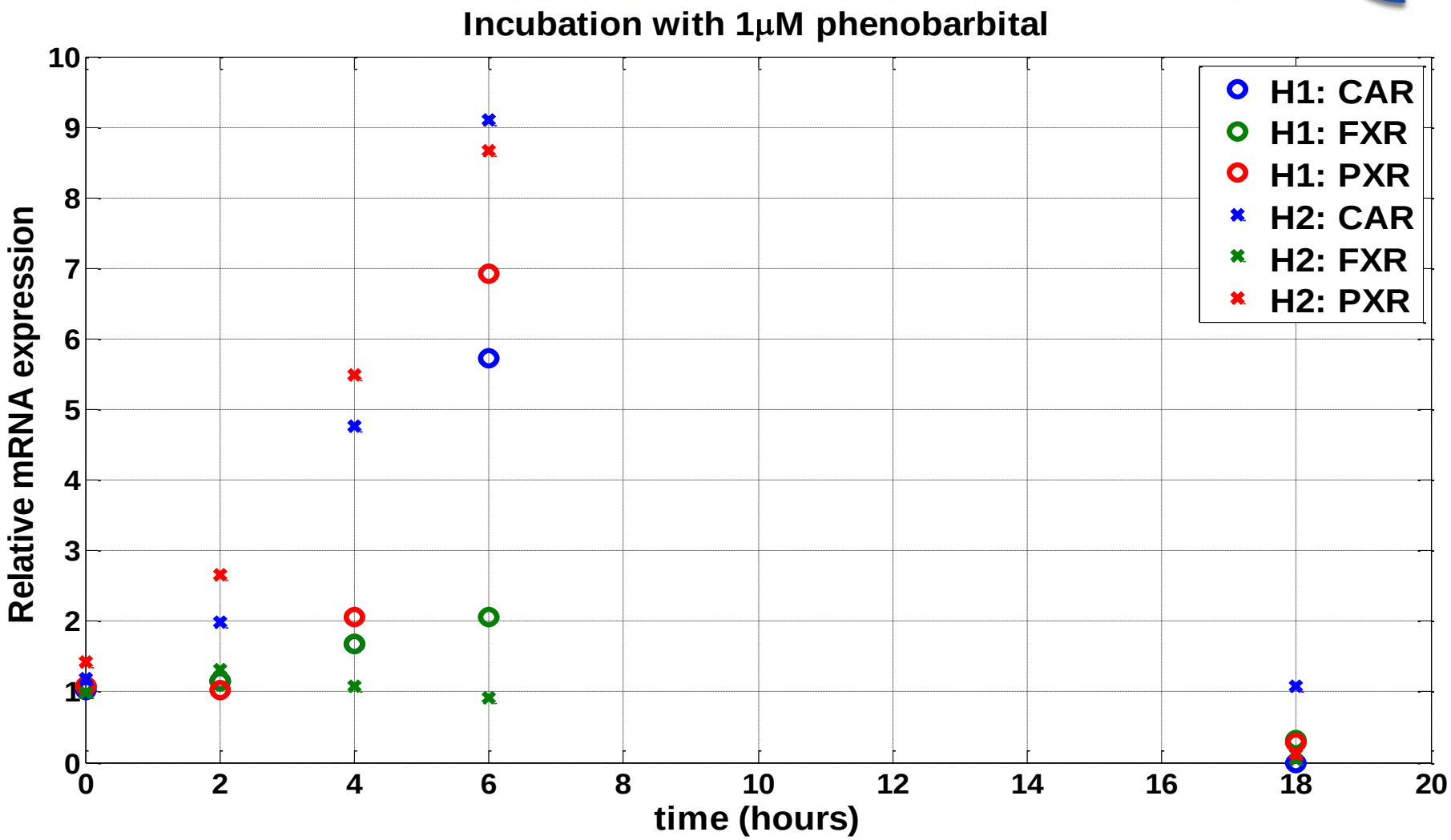


Experimental data



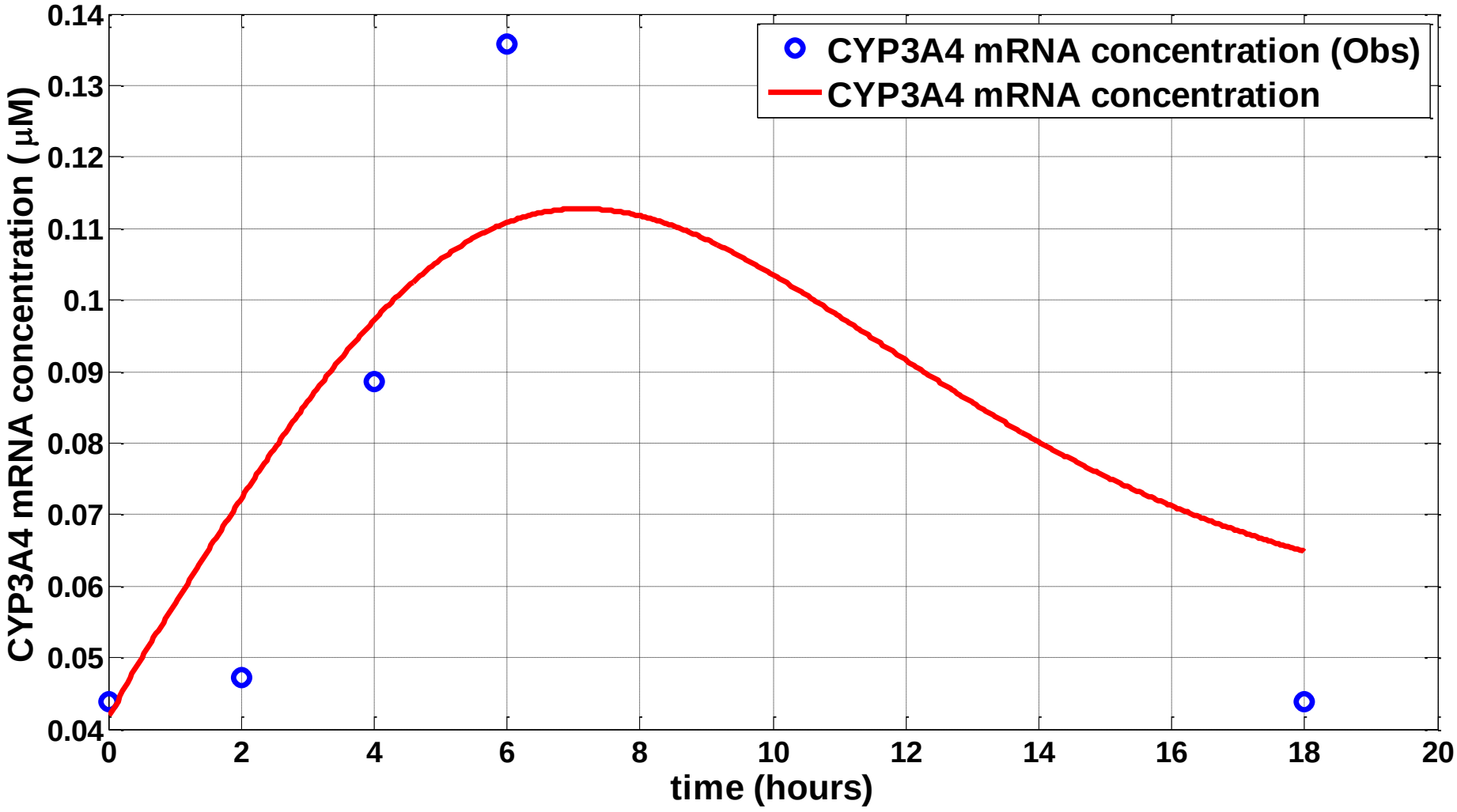
(Data taken from P. Martin *et al.*, Br. J. Pharmacol., Vol. 153, 2008, pp. 805-819)

Experimental data

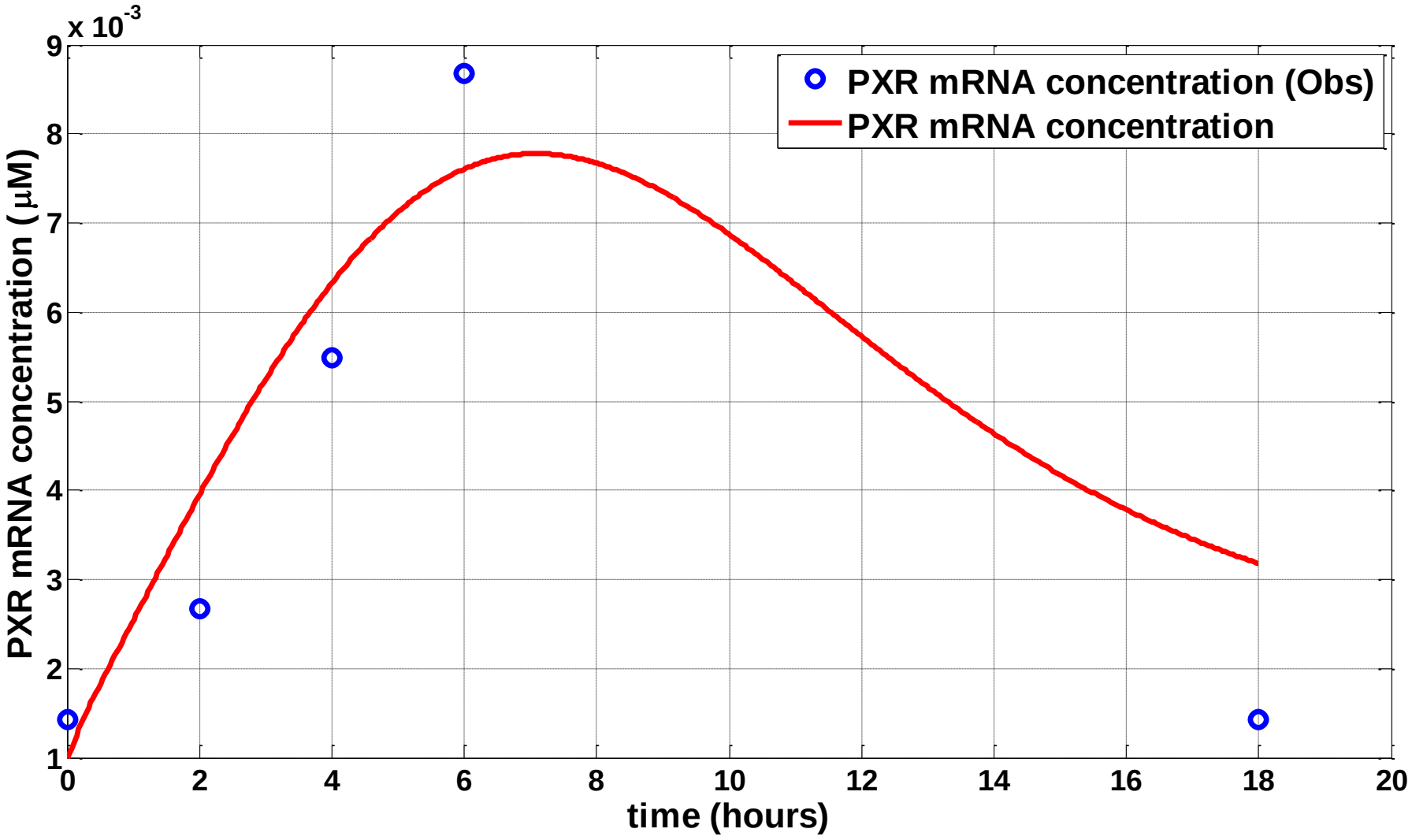


(Data taken from P. Martin *et al.*, Br. J. Pharmacol., Vol. 153, 2008, pp. 805-819)

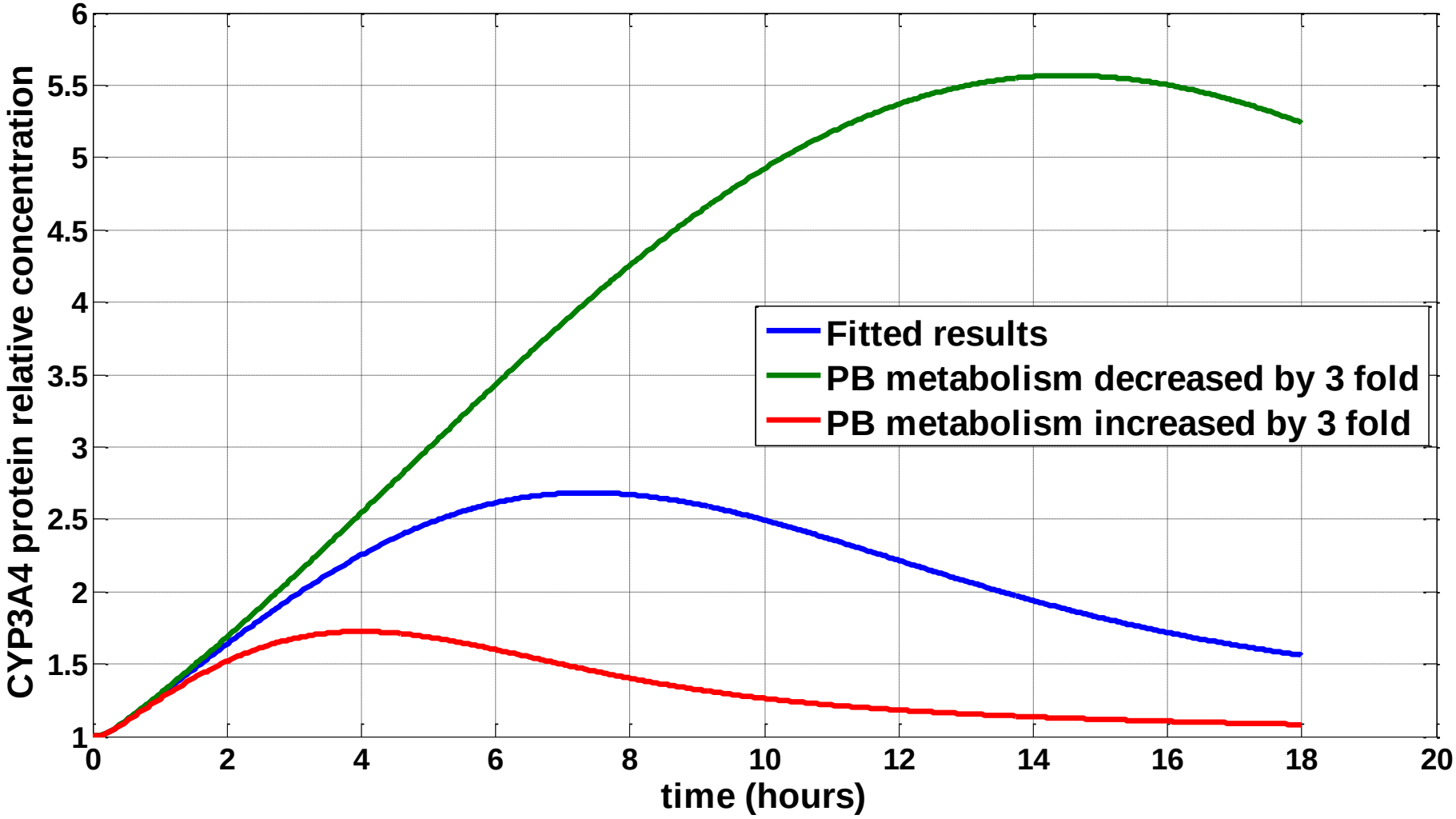
Parameter estimation



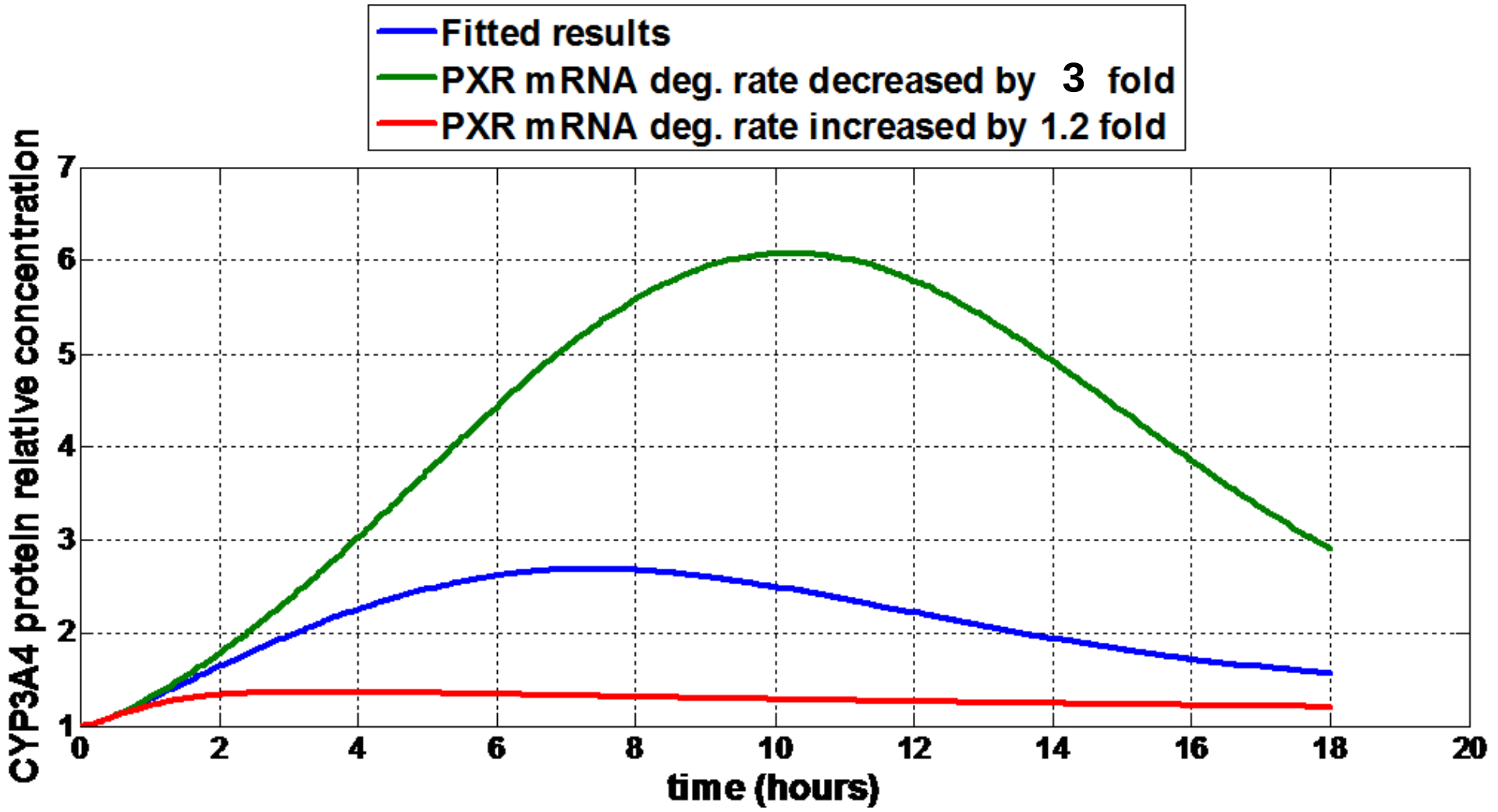
Parameter estimation



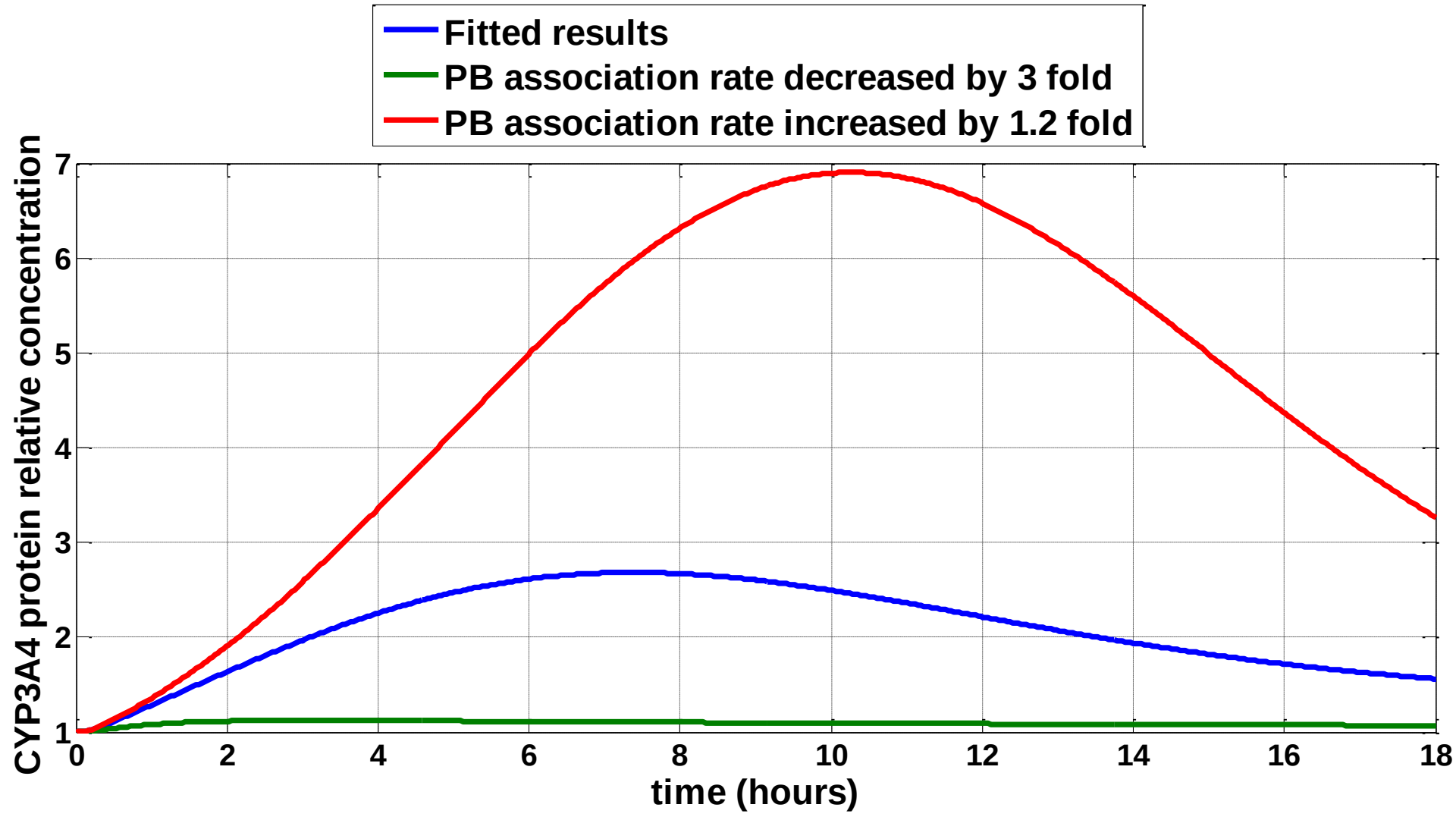
Sensitivity analysis



Sensitivity analysis

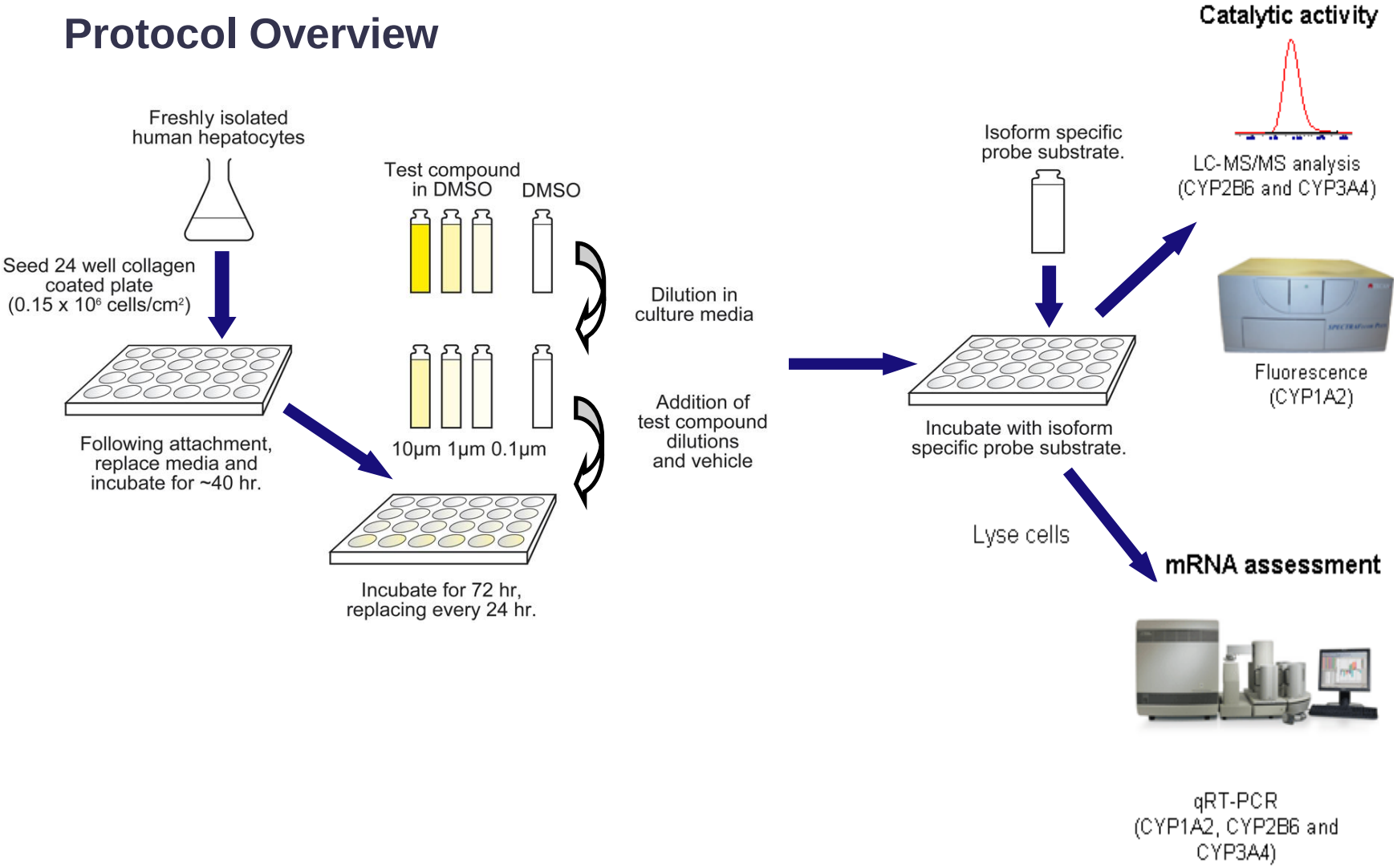


Sensitivity analysis

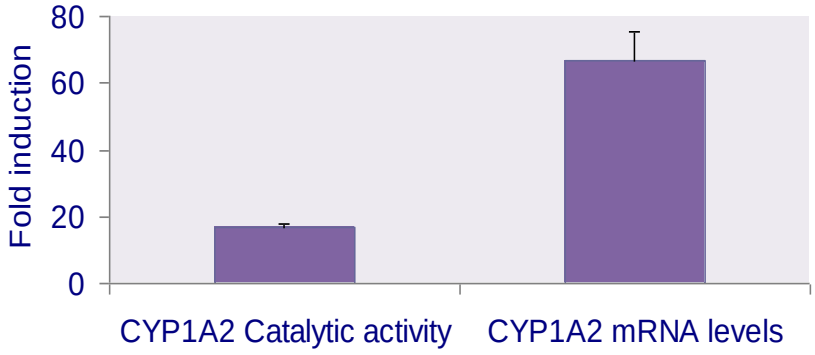


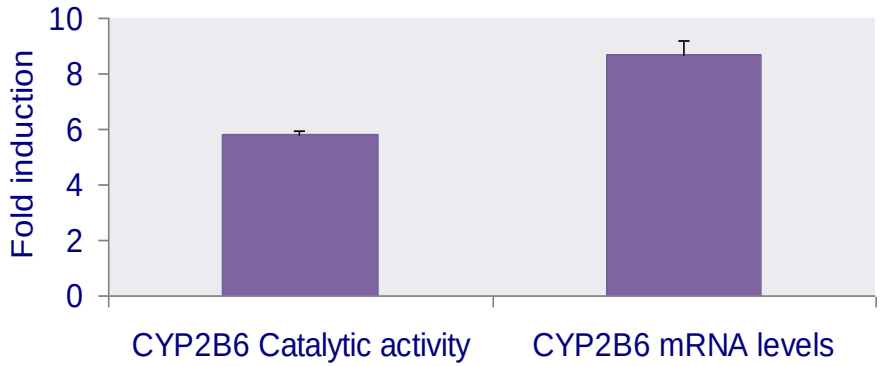
Model validation: CYP Induction

Protocol Overview

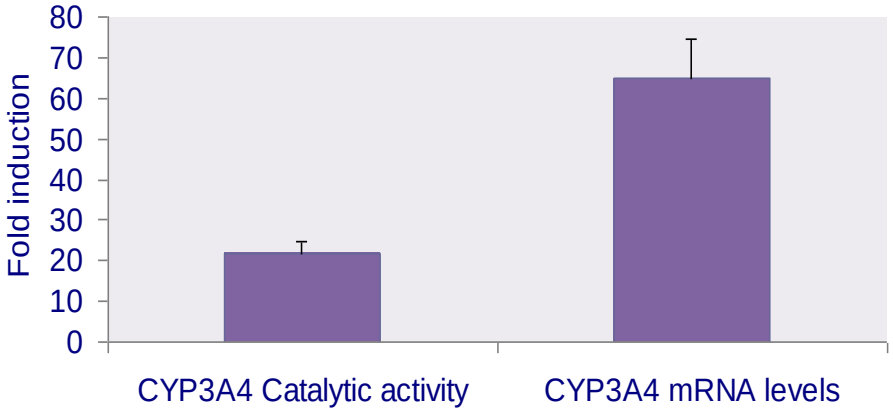


Model validation: CYP Induction



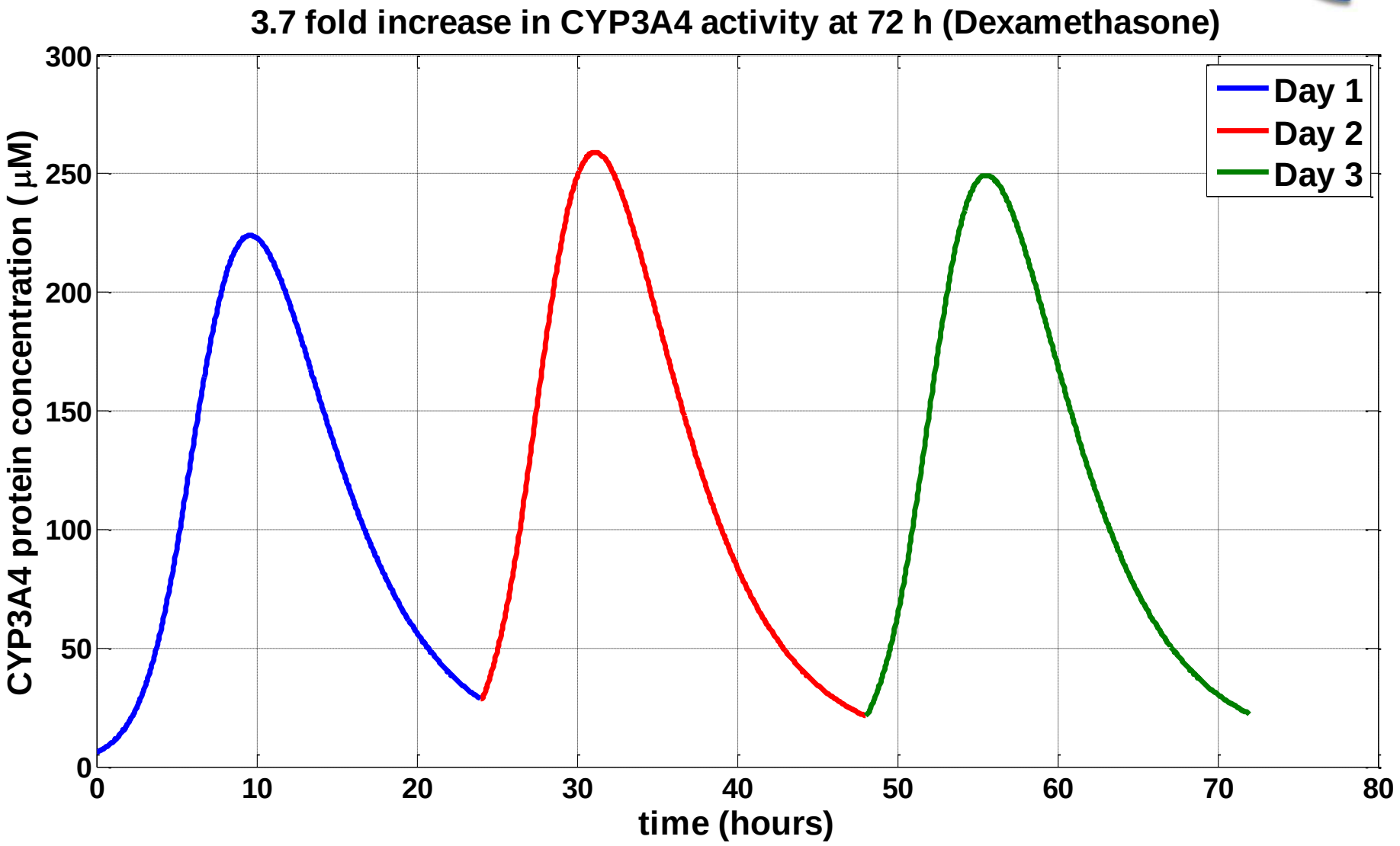


CYP Isoform	Probe substrate	Positive control inducer
CYP1A2	Ethoxyresorufin	Omeprazole
CYP2B6	Bupropion	Phenobarbital
CYP3A4	Midazolam	Dexamethasone Rifampicin



Positive control induction of CYP1A2 (omeprazole), CYP2B6 (phenobarbital) and CYP3A4 (rifampicin)

Model validation: CYP Induction



Model validation: CYP Induction

Parameter increase by 3 fold	Fold over base model
PXR resting protein concentration	2.2
CYP3A4 resting protein concentration	1.5
Drug permeability	0.3
Induced drug metabolism rate	0.5

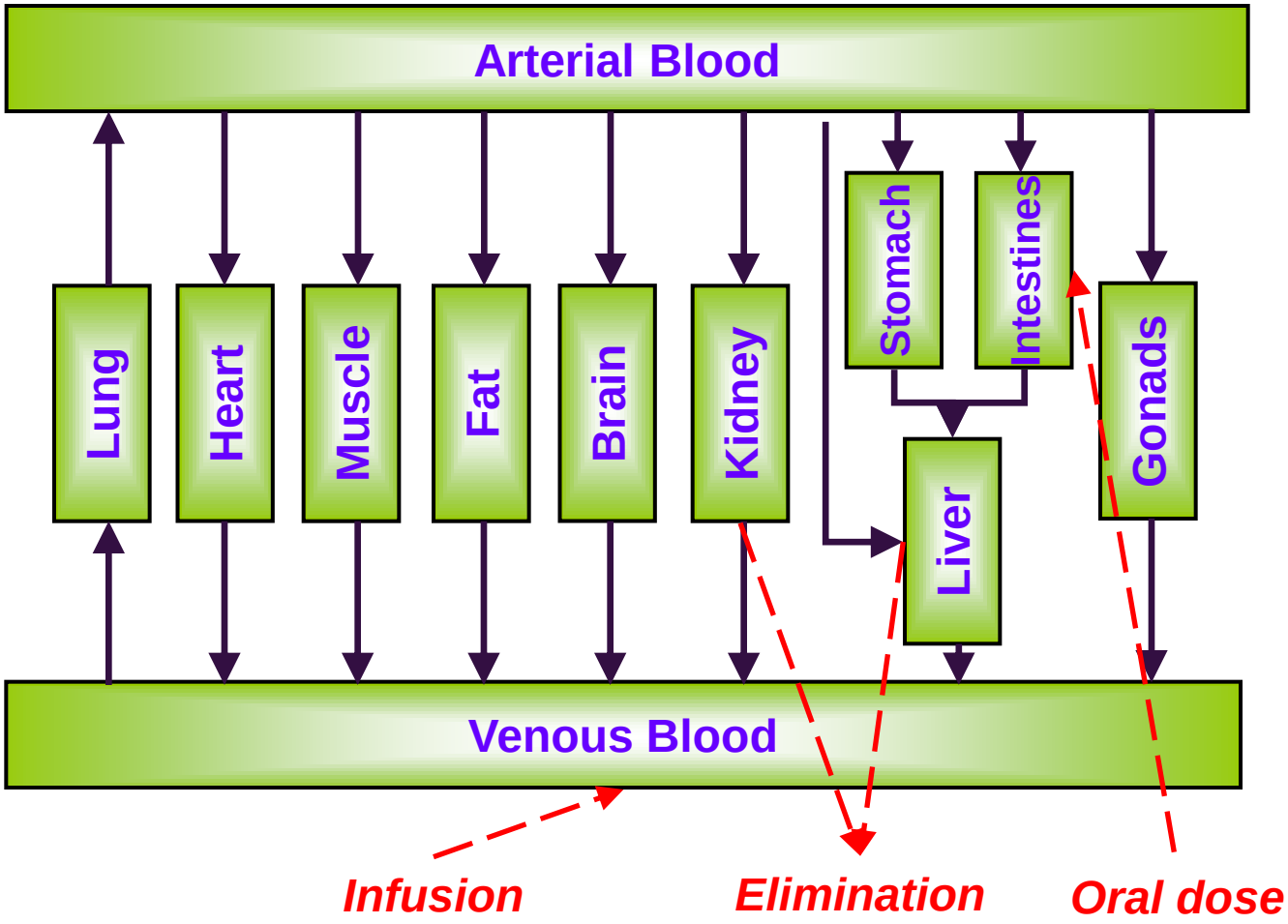
Linking *in vitro* results to *in vivo* outcomes

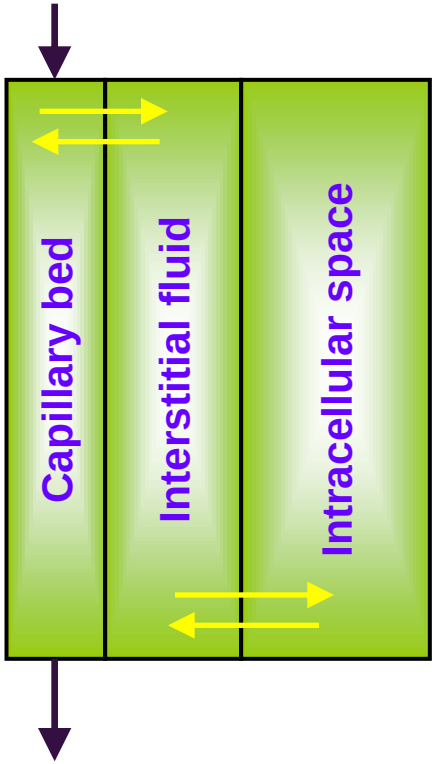
- Predictive models for *in vivo* toxicity require predictive modelling of exposure
- Our ultimate aim is to predict the *in vivo* outcome by linking the hepatocyte model to a whole body model
- Physiologically-based pharmacokinetic (PBPK) models satisfy these requirements.
- PBPK models can predict exposure of hepatocytes *in vivo* for various external exposure scenarios

Linking *in vitro* results to *in vivo* outcomes

- PBPK models predict the fates of compounds in the body
- PBPK models are mathematical simulation models.
- They are devised to predict the fate(s) of compound(s) in the bodies of humans, and other animals.
- Their primary output is the change over time following dosing of relevant quantities. e.g. the concentration of a compound in the plasma and other tissues.
- Simple physchem and *in vitro* ADME data can be used as inputs.

Linking *in vitro* results to *in vivo* outcomes





Linking *in vitro* results to *in vivo* outcomes

PBPK models inputs* for screening in drug discovery

Input Property
Hepatic microsomal intrinsic clearance (species-dependent)
Fraction unbound in plasma (species-dependent)
Blood:plasma ratio (species-dependent)
pKa(s)
logP octanol/water
Caco-2 permeability
Solubility (buffered)

Prediction of i.v. dose, p.o. dose exposure

*Cloe® PK V2.1

Conclusions

- Xenobiotic-metabolising enzymes and transporter (uptake & efflux) proteins play crucial roles in the metabolism and disposition of chemical compounds
- Many xenobiotics affect enzyme induction in liver and other organs
- Hepatic induction of CYP450 isoforms is probably the best-studied facet of this response
- A model of PXR and CYP3A4 in hepatocytes quantitatively predicts the response to dexamethasone (DMSO) *in vitro*
- *In vivo* response should be predictable by incorporating cellular model into a PBPK model