

In vitro-in silico platform for mechanistic investigation of (G)CDCA disposition in primary human hepatocytes



Kristof De Vos¹, Justina Clarinda Wolters², Miao-Chan Huang¹, Pieter Annaert^{1,3}

KU LEUVEN

¹ Drug Delivery and Disposition, KU Leuven Department of Pharmaceutical and Pharmacological Sciences, 3000 Leuven, Belgium

² Section Systems Medicine of Metabolism and Signaling, Department of Pediatrics, University of Groningen, University Medical Center Groningen, Groningen, 9713 AV, The Netherlands

³ BioNotus GCV, 2845 Niel, Belgium

Background information

Drug-induced liver injury (DILI) is a relatively rare hepatic condition but is the leading cause of acute liver failure in North America and Europe. Drug-induced cholestasis (DiCho) accounts for about 50 % of all DILI cases, with altered bile acid disposition as hallmark of DiCho development¹. Using glycochenodeoxycholic acid (GCDCA) as index bile acid, the aim was to compute a quantitative reference model for bile acid disposition in human hepatocytes in a mechanistic manner. This model also incorporates the abundance and activity of the bile acid export pump (BSEP), a key bile acid exporter linked to DiCho progression.

Rationale and objectives

Establishing reference values from parameter predictions obtained under biorelevant conditions provides baseline kinetics for bile acid disposition at the cellular level. This value set serves as benchmark for comparison against estimates derived from datasets reflecting perturbed hepatic conditions. This comparison enables the identification of (subtle) changes in kinetics when hepatocytes are exposed to (cholestatic) xenobiotics. This assessment helps to evaluate the impact of these xenobiotics on bile acid homeostasis and BSEP abundance and activity.

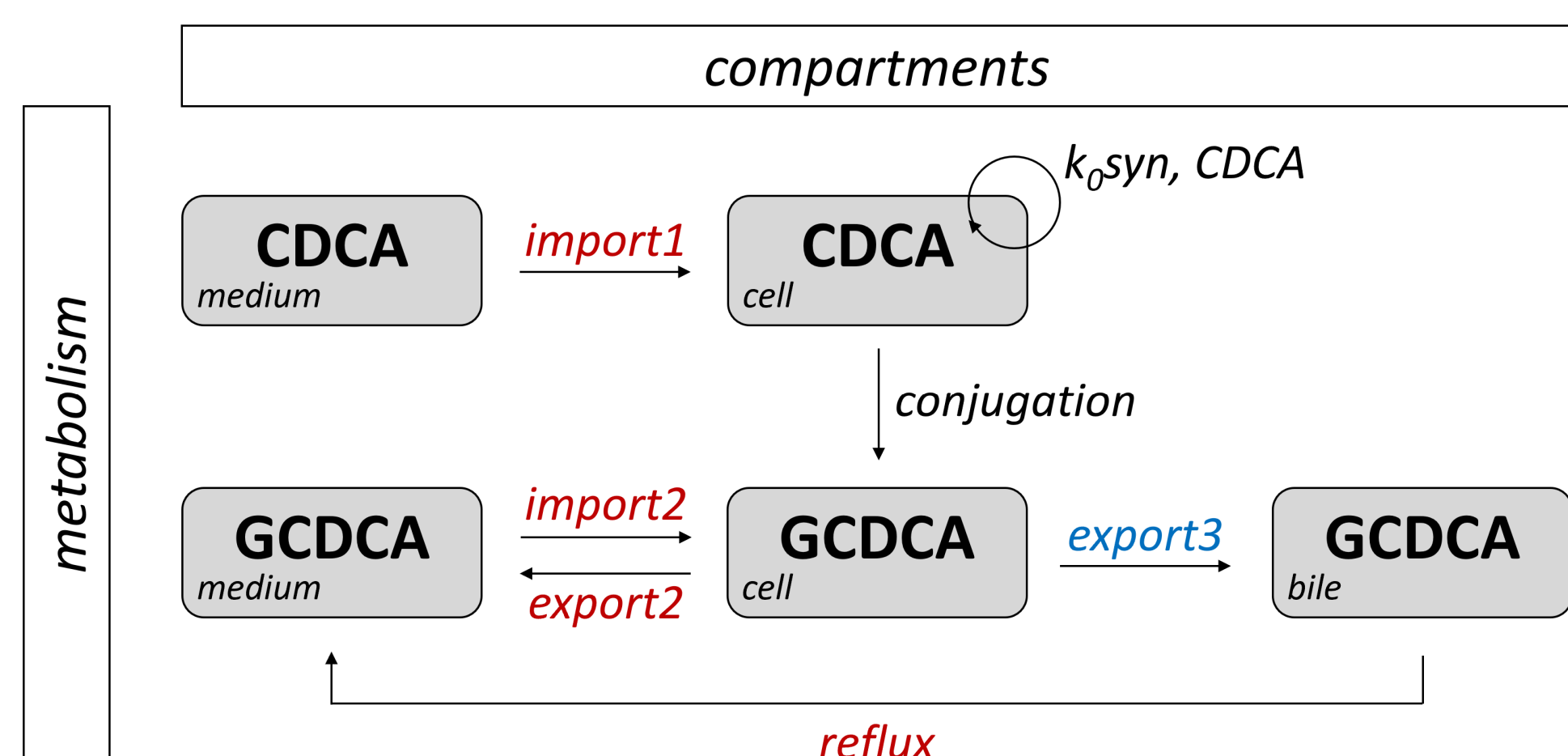
Methods

Exogenous CDCA (0, 5, 10, 25, and 50 μ M) was administered to primary human hepatocytes and the concentrations of (G)CDCA in the medium, cell, and bile compartment were measured using LC-MS. Targeted proteomics quantified the absolute abundance of BSEP. Ordinary differential equations were solved, and sensitivity analysis and diagnostics were performed using IQRtools (version 99.0.0, IntiQuan GmbH).

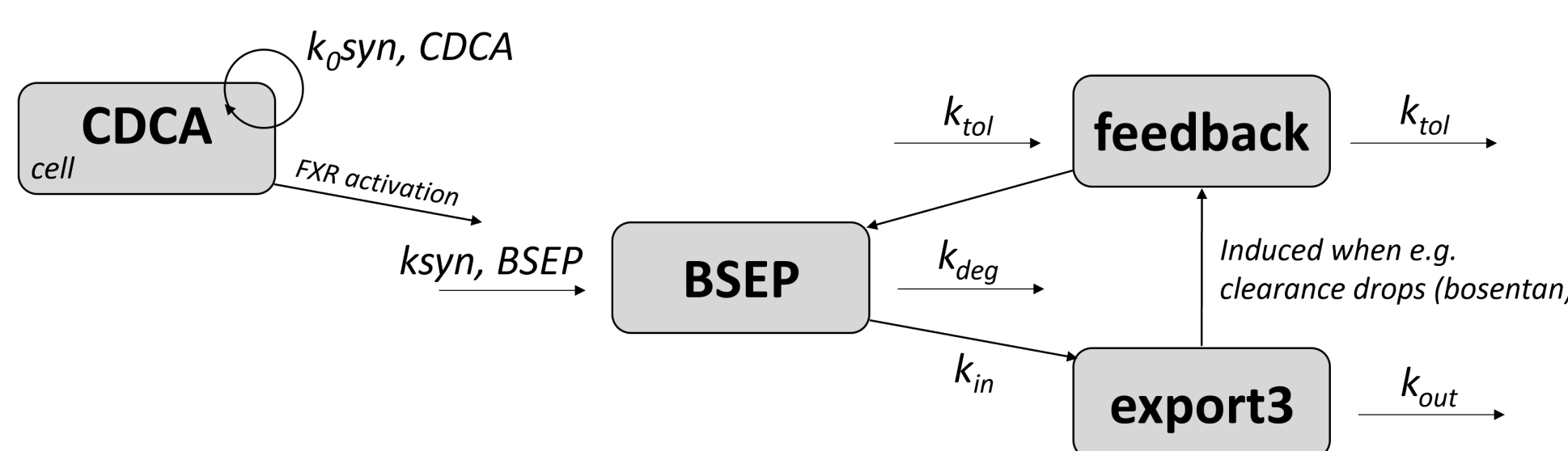
Results

Schematic model structure

The disposition model contains a compartmental dimension and a metabolic dimension. Bile acids can migrate across the different compartments, and can be metabolized within the cell compartment. Processes were parameterized as first-order clearances (red), or time-dependent (blue).

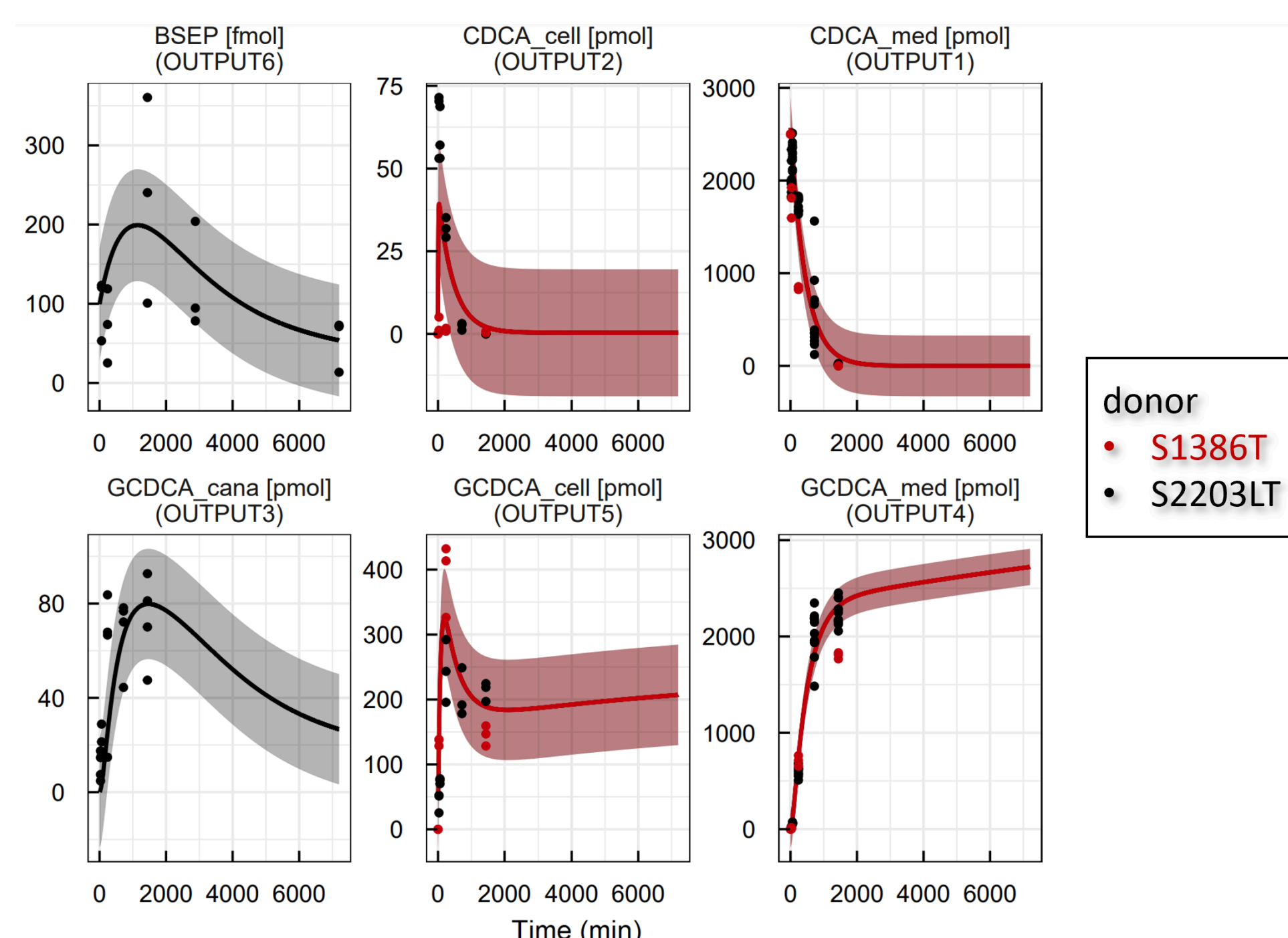


BSEP abundance was modeled as dependent on (i) the intracellular CDCA concentration (through FXR)², and (ii) the “export3” activity. The latter connection is based on an *in vitro* experiment where deliberate inhibition of BSEP with bosentan resulted in elevated BSEP abundances.



Note: The BSEP's synthesis rate (“ k_{syn} , BSEP”), degradation rate (“ k_{deg} ”), activity inputs (“ k_{in} ”), activity outputs (“ k_{out} ”), and feedback constant (“ k_{tol} ”) are all first-order constants. From a simplified version of this model structure, “ k_{out} ” was estimated to be 0.006888/min (RSE = 8%; result not shown).

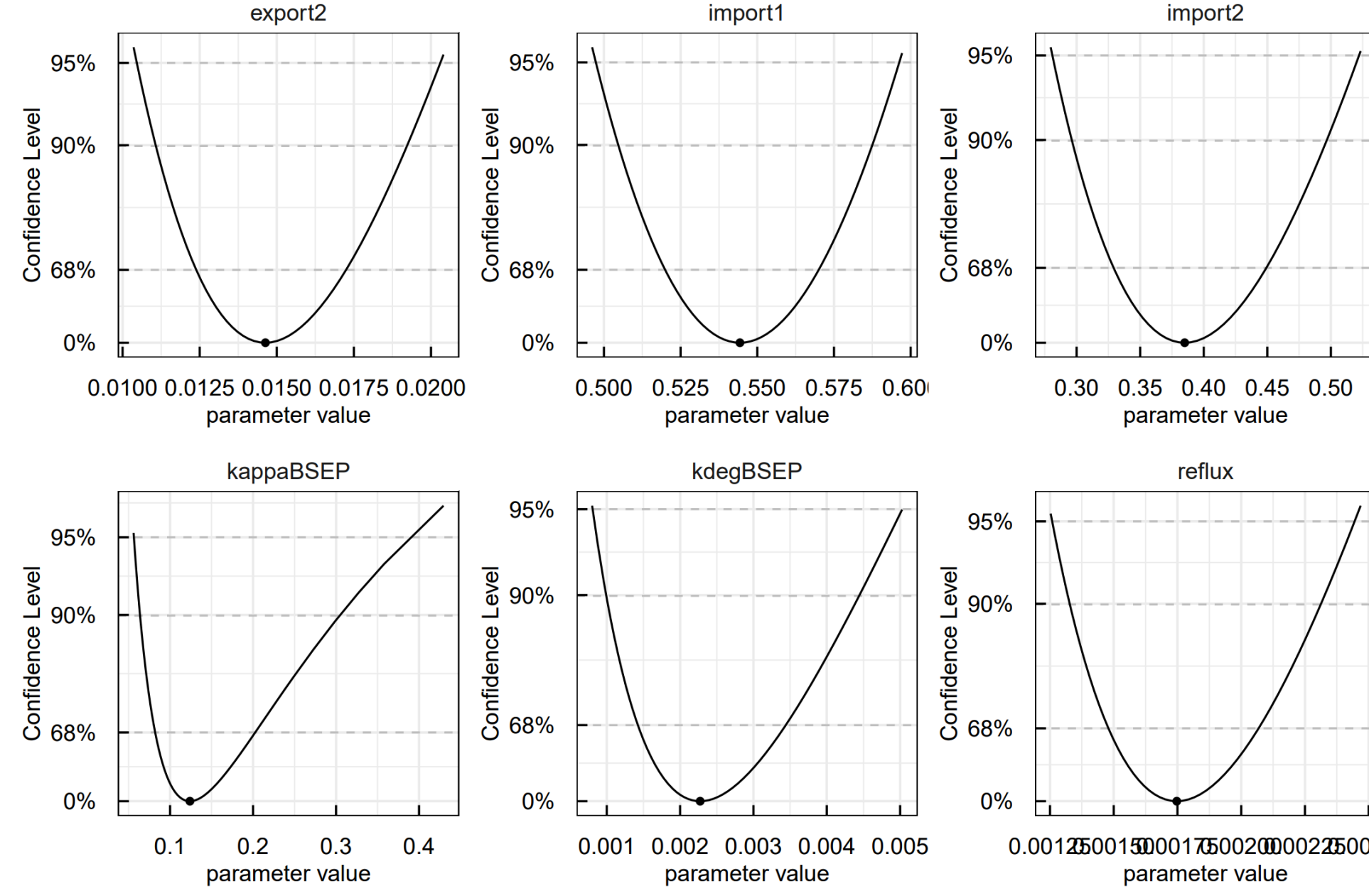
Model distributions, parameter estimations, and sensitivity analysis



Parameter	Value	Units	RSE [%]	CI [95%]
Model error				
error_ADD1	622.2	pmol	8.634	-
error_ADD2	19.2	pmol	13.6	-
error_ADD3	15.65	pmol	15.92	-
error_ADD4	186.5	pmol	8.805	-
error_ADD5	75.69	pmol	14.21	-
error_ADD6	70.6	fmol	18.31	-
Parameter error				
V medium	250	μ L	-	-
V cell	1.37	μ L	-	-
V canaliculi	0.072	μ L	-	-
ksyn	0.046	pmol/min	-	-
conjugation	0.186	μ L/min	-	-
BSEP base	99	fmol	-	-
export3 base	0.0058	μ L/min	-	-
kout BSEP	0.006888	1/min	-	-
import1	0.5443	μ L/min	4.651	[0.4974, 0.5963]
import2	0.385	μ L/min	15.81	[0.2809, 0.5222]
export2	0.01463	μ L/min	15.48	[0.01046, 0.0203]
reflux	0.001747	μ L/min	17.79	[0.001259, 0.002445]
kdeg BSEP	0.002273	1/min	50.67	[0.0008068, 0.005031]
kappa BSEP	0.1239	-	45.7	[0.0564, 0.3893]

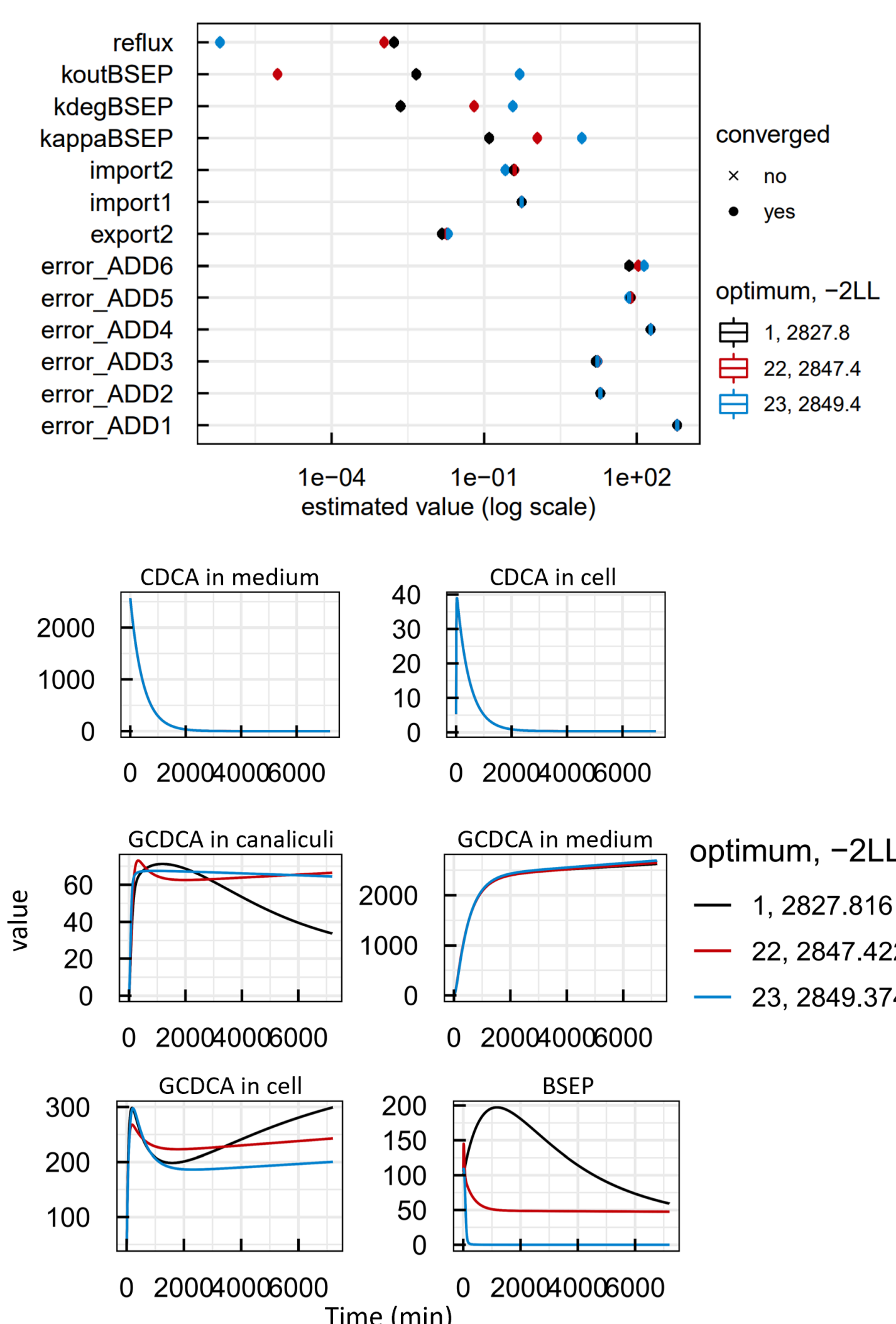
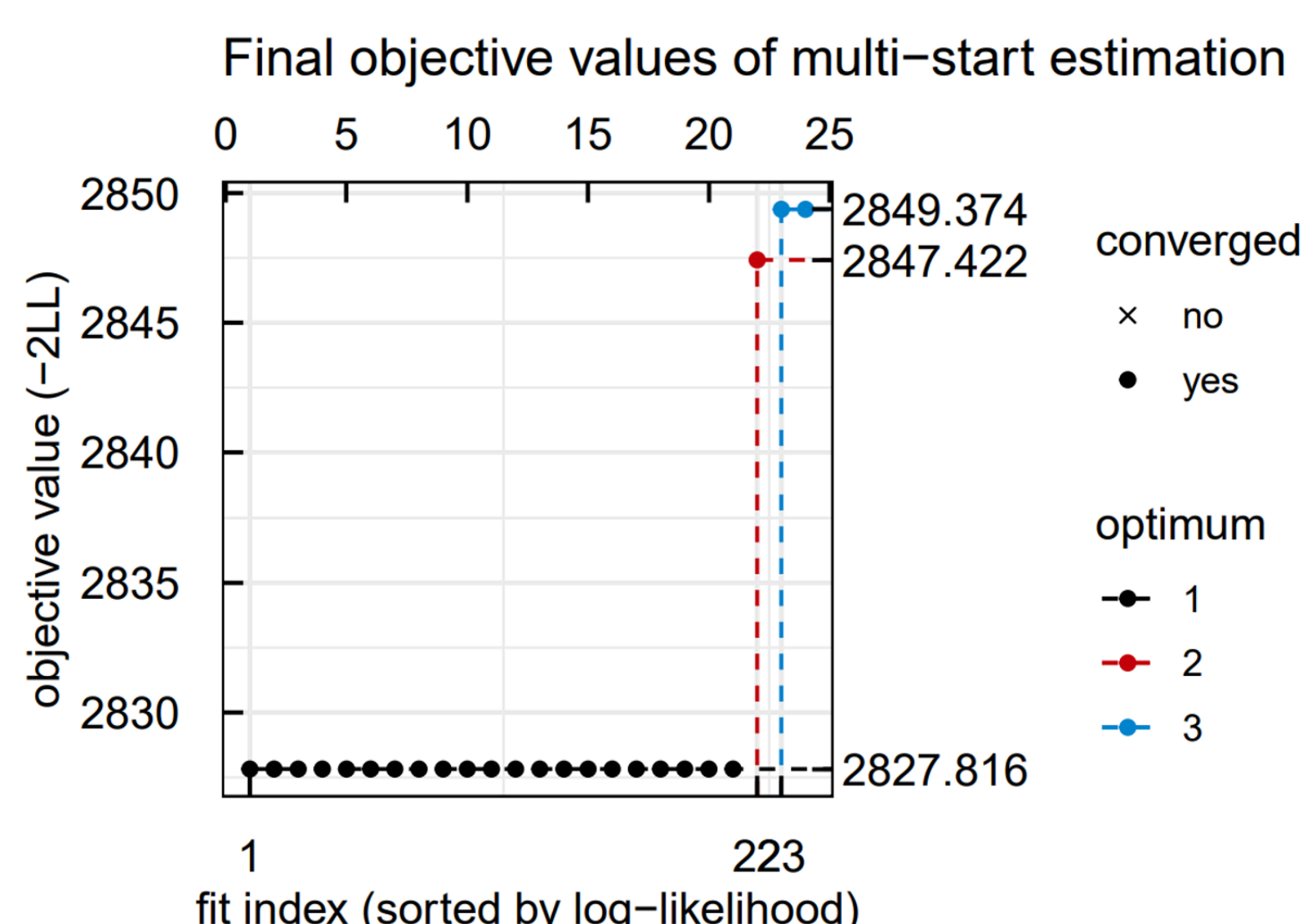
Note: Parameters without RSE were fixed. The additive error term (“error_ADD”) corresponds to the respective output number (OUTPUT*)

Note: The half-life of BSEP was estimated to be 5.1 h under physiological *in vitro* conditions. This is consistent with literature, reporting an half-life of 6 h in kidney cultures³. The “kappa” parameter is defined as k_{tol}/k_{deg} , representing an early indicator of the rebound velocity of BSEP.

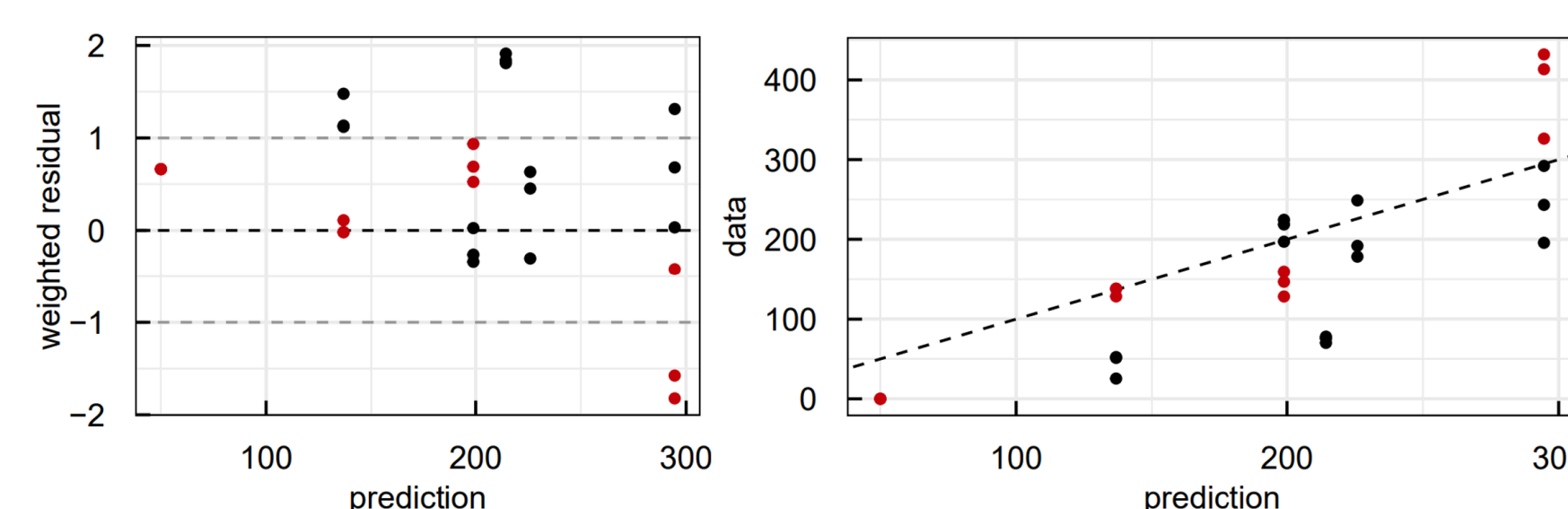


Diagnostic analysis

The optima of the likelihood landscape were analyzed using a waterfall plot, along with the parameter sets and model distributions for each optimum.



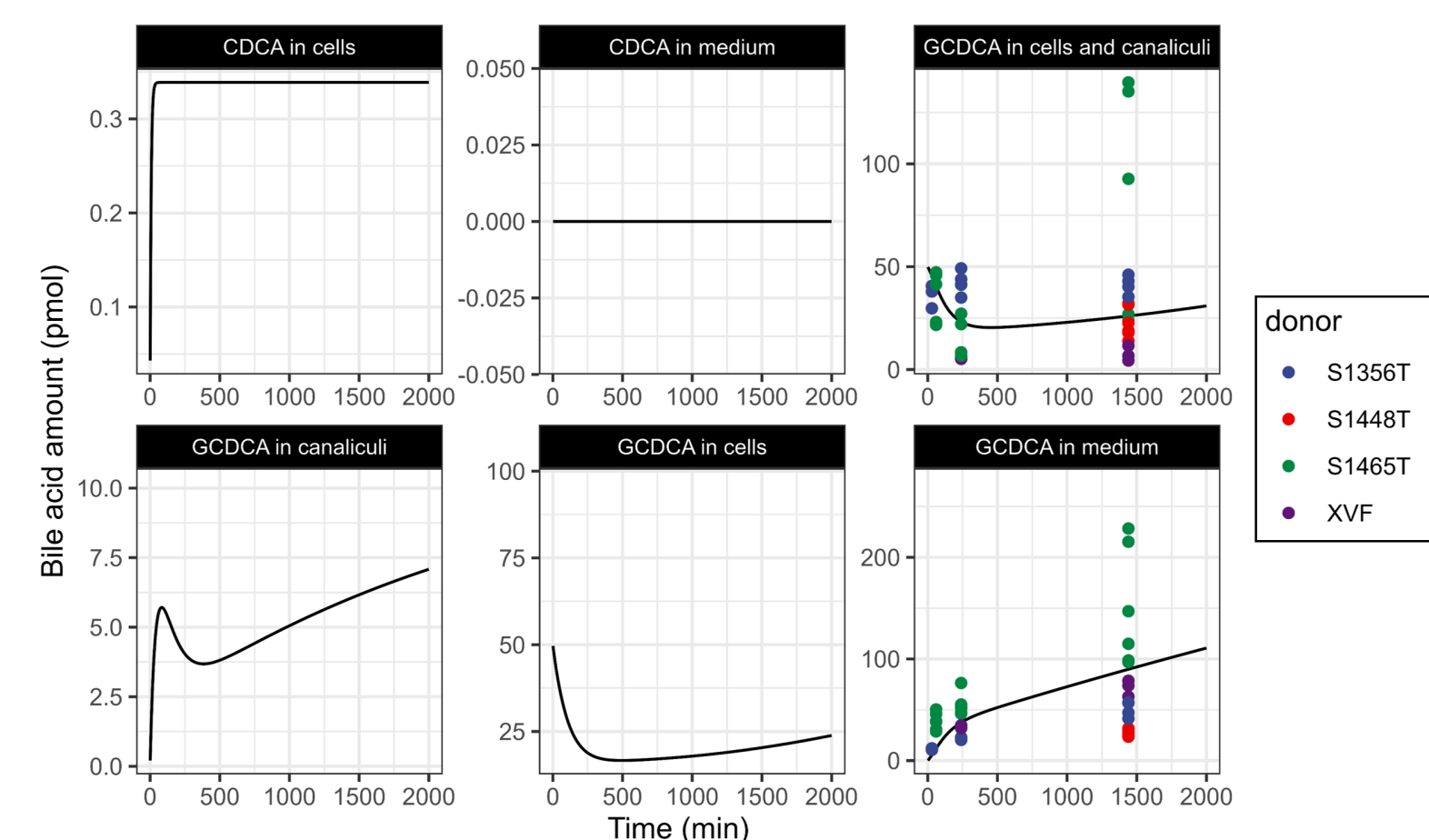
The GOF for intracellular GCDCA is shown as an example:



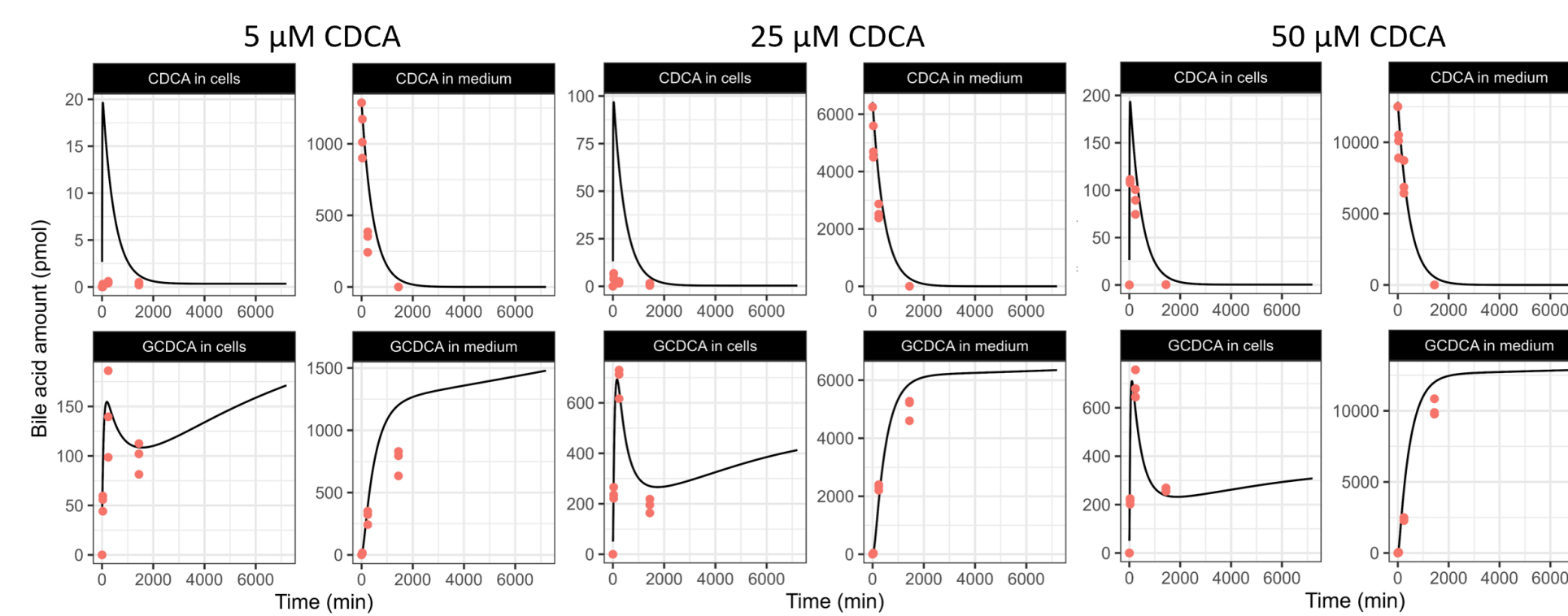
Validation of simulations

To validate the disposition model, different scenarios (0 μ M, 5 μ M, 25 μ M, and 50 μ M CDCA administration) were simulated, and subsequently overlapped with data of two independent (G)CDCA disposition datasets.

Independent dataset 1 (endogenous (G)CDCA)



Independent dataset 2 (donor S2203LT)



Conclusion

The presented *in vitro-in silico* platform effectively describes and predicts (G)CDCA disposition in human hepatocytes. Such a bottom-up methodology can advance the understanding of bile acid disposition, uncover commonalities across different (cholestatic) compounds, and aid in selecting non-toxic drug candidates during drug development.

References

¹Chatterjee S, et al. Curr Drug Metab. 2018 ²Jie L, et al., Toxicol Sci. 2014 ³Ortiz DF, et al. J Biol Chem. 2004