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data from the project partners across different scales**

Work package 3

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## Contributions to deliverable - Internal review procedure

<b>Deliverable produced by:</b>	<b>Date:</b>
Steven Niederer - Partner KCL	September 2016
Lars Kuepfer - Partner RWTH	October 2016
<b>Deliverable internally reviewed by:</b>	<b>Date:</b>
Jos Kleinjans - partner UM	October 2016

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## PUBLISHABLE SUMMARY

We have developed two cardiac modeling workflows for predicting emergent multiscale toxicity phenotypes. The first builds on a model of mitochondria metabolism, reactive oxygen species production and anti-oxidants to simulate the short and long term changes in metabolism associated with changes in protein density measured from mass spectrometry. This model allows us to link protein density and gene expression data with measurements of cellular metabolism. The second model uses detailed biophysical models of contraction embedded with in a patient specific modeling framework to predict changes in whole organ cardiac function from changes in protein density and mass spectrometry data. This framework enables us to link molecular measurements from stem cell preparations through to changes in cardiac function from clinical images.

Recently we have developed PICD, a generic approach for PBPK-based *in vivo* contextualisation of *in vitro* toxicity data (Thiel et al., 2016, see MS 32 report). As a proof of concept, *in vitro* transcriptomics data from the Open TG-GATEs library (Igarashi et al. 2015) have been used. In a complementary approach, PBPK models have been used to design *in vitro* assay concentration profiles mimicking PK exposure in human patients. The integration of specifically generated HeCaToS omics data in the multiscale PBPK models will allow accurate *in vitro-in vivo* extrapolations of high clinical relevance.

## CARDIOTOXICITY

### OBJECTIVES

The cardiac toxicity modelling framework has two objectives:

1. The project aims to model and validate the effects of a two week exposure protocol on pluripotent human cardiac myocyte stem cells. This is achieved by developing a biophysical modelling framework that quantitatively links stem cell proteomic data with physiological measurements of stem cell metabolism exposed to a common pharmacological exposure protocol;
2. Propagate the drug effects on pluripotent stem cells to a whole human heart model that can predict organ scale deformation patterns that can be quantitatively compared with clinical indices of compromised cardiac contraction recorded from routine clinical imaging modalities.

### INTRODUCTION

In the “Integrated cellular model report” (MS.31) we have described a mitochondria model for simulating the metabolic effects of anthracycline toxicity. We demonstrated that the model represented the major pathways implicated in, the prototypical anthracycline: doxorubicin toxicity in both the acute and chronic contexts. This provides a framework that can simulate the effects of experimentally observed changes in protein expression during a two week exposure protocol applied to pluripotent stem cells and at the same time simulate the direct actions of anthracyclines on the electron transport chain, mitochondrial DNA (mtDNA) and direct anthracycline ROS production. Through using models these two effects (direct drug action and changes in protein expression) can be analysed both together and independently.

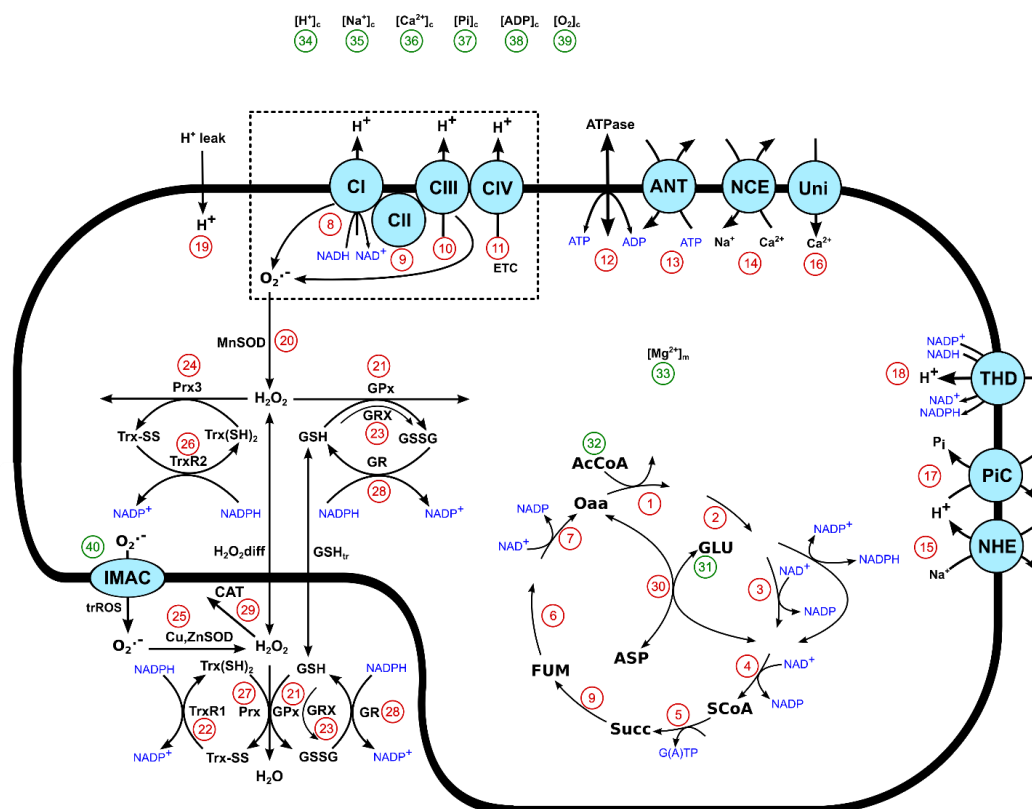
The ability to characterise cardiac mitochondria function *in-vivo* in a clinical context is not routine. With cardiac-oncology clinics patients do routinely receive cardiac MRI and echocardiography. These imaging modalities provide high detail images of tissue properties, motion and anatomy. To link experimentally measured changes in stem cell protein expression levels to whole organ cardiac function in human

hearts we have exploited our multiscale modelling framework for simulating patient hearts. This modelling framework explicitly represents patient anatomy, myocardial microstructure orientation, haemodynamic boundary conditions and cellular contractile function. The cellular model explicitly represents the dynamics and density of proteins required for the regulation and generation of cardiac function. This allows changes in protein expression observed in pluripotent stem cell preparations to be projected into models that can in turn predict how these changes in protein expression would manifest as changes in whole organ cardiac function. These can then be compared to clinical measurements of anatomy and mechanical function.

## RESULTS

### Linking Stem Cell Protein Expression with Physiological Measurements

A computational model of the mitochondria [1] was adapted to study the effects of changes in proteomics associated with drugs in mitochondrial function. Thirty components of the model were identified and their expressions were scaled based on the measured variations of their proteins and ten additional components were also included in the sensitivity analysis simulations. Figure 1 shows a schematic of this model where the components associated to proteins were labelled in red and the ones not associated to proteins were labelled in green.



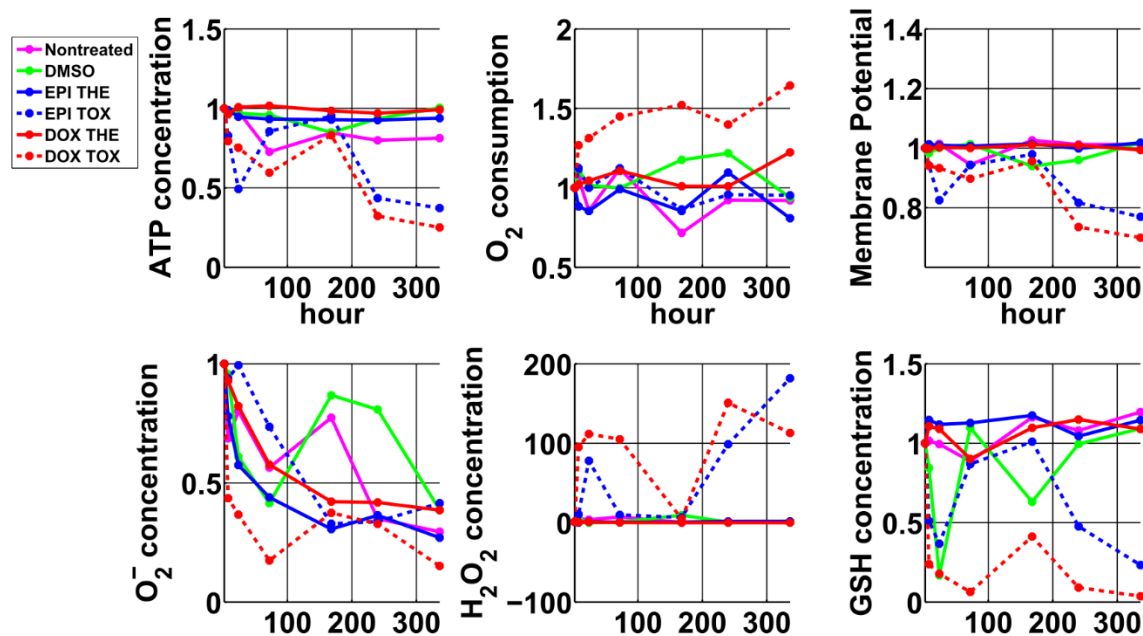
**Figure 1:** Schematic of the mitochondrial model with key components. Parameters associated to proteins are tagged in red. Boundary conditions and parameters not associated to proteins are tagged in green. Note that not all parameters associated to proteins have data available.

Index	Parameter Name	Uniprot Code
1	Citrate synthase	O75390
2	Aconitate hydratase	Q99798
3	Isocitrate dehydrogenase	O43837, P48735, P50213, P51553
4	Oxoglutarate dehydrogenase	Q02218
5	Succinyl-CoA ligase	Q9P2R7, Q96I99, P53597
6	Fumarate hydratase	P07954
7	Malate dehydrogenase	P40926
8	Complex I - (mtDNA)	P49821, O75251, O00217, P19404, O75489, O75306, P28331, P03886, P03891, P03897, P03905, P03901, P03915, P03923
9	Complex II - Succinate dehydrogenase	O14521, Q99643, P21912, P31040
10	Complex III -(mtDNA)	P00156, P08574, P47985, P31930, P22695, P07919, P14927, O14949, Q9UDW1, O14957
11	Complex IV - (mtDNA)	P00395, P00403, P00414, P13073, Q96KJ9, P20674, P10606, P12074, Q02221, P14854, Q6YFQ2, P09669, P24310, P14406, O60397, P24311, P15954, O14548, P10176, Q7Z4L0
12	ATP synthase (mtDNA)	Q5VTU8 , P24539, P05496, Q06055 , P48201, P56381, O75947 , P18859 , P00846, P03928, P25705, P06576, P30049, P36542, P56134, P48047, Q8N5M1, Q5TC12, Q99766, O75964, Q7Z4Y8, P56385, Q9NW81
13	ADP/ATP translocase	P12235, P05141, P12236, Q9H0C2
14	Sodium/calcium exchanger	P32418, Q9UPR5, P57103
15	Sodium/hydrogen exchanger	P19634, P48764, Q6AI14, Q9UBY0, Q14940, Q96T83, Q9Y2E8, Q8IVB4, Q92581
16	Calcium uniporter	Q9H4I9, Q8NE86, Q9NWR8, Q96AQ8, Q9BPX6, Q8IYU8
17	Phosphate carrier	Q00325
18	NAD(P) transhydrogenase, mitochondrial	Q13423
19	Proton Leak	P55851
20	Superoxide dismutase [Mn]	P04179
21	Glutathione peroxidase	P22352
22	Cyto thioredoxin reductase (TrxR1)	Q16881
23	Glutaredoxin	Q9NS18
24	Mitho Peroxiredoxin	P30048, P30044
25	Superoxide dismutase [Cu-Zn]	P00441
26	Mitho thioredoxin reductase (TrxR2)	Q9NNW7
27	Cyto Peroxiredoxin	P30041, Q06830, Q13162, P32119
28	Mitho Glutathione	P00390

	reductase	
29	Catalase	P04040
30	Aspartate aminotransferase, mitochondrial	P00505
31	Glutamate concentration	
32	AcCoA concentration	
33	Matrix Magnesium concentration	
34	Cytoplasmic H <sup>+</sup> concentration	
35	Cytoplasmic Na <sup>+</sup> concentration	
36	Cytoplasmic Ca <sup>2+</sup> concentration	
37	Cytoplasmic Pi concentration	
38	Cytoplasmic ADP concentration	
39	Cytoplasmic O <sub>2</sub> concentration	
40	Inner membrane anion channel	

To demonstrate the efficacy of the modelling proposed modelling framework six protein fold change data sets were analysed corresponding to the following assays: non-treated, DMSO only, therapeutic epirubicin, toxic epirubicin, therapeutic doxorubicin and toxic doxorubicin. Each dataset contains the measured protein expression in seven different time points corresponding to 2h, 8h, 24h, 72h, 168h, 240h and 336h. As no data is available for the initial time, T0, the earliest measurement, T2, was considered to be the baseline. For each dataset, the protein expressions in all time points (T8, T24, T72, T168, T240 and T336) were self-normalized with respect to T2. This data was used to calculate a vector to scale the expression of the 30 components associated to proteins for each time point in each assay.

For the components composed of multiple proteins, the parameters were scaled with respect to the average variation of the proteins associated to them using the uniprot code map in the table above. Simulations were performed until a steady state was reached to estimate how changes in protein expressions affect mitochondrial function. Figure 2 shows the results for some of the mitochondria measurements; ATP concentration, O<sub>2</sub> consumption, Membrane Potential, O<sub>2</sub><sup>-</sup> concentration, H<sub>2</sub>O<sub>2</sub> concentration and reduced glutathione concentration. We can observe a clear difference in the simulations with therapeutic and toxic doses, particularly in the ATP concentrations and mitochondrial membrane potential.

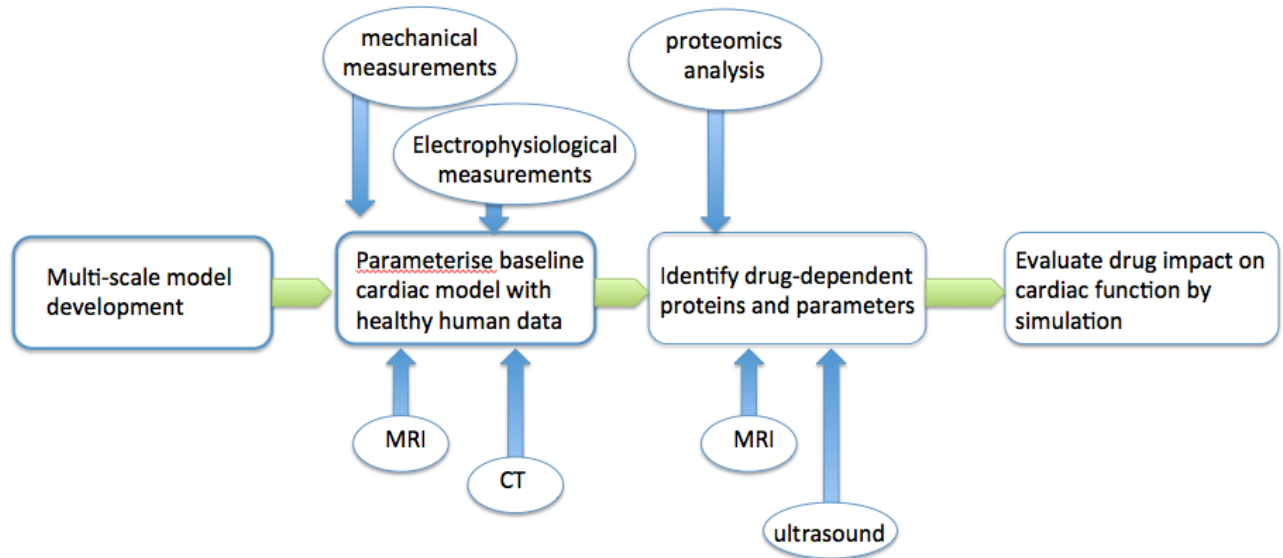


**Figure 2:** Predicted variation in mitochondria function with respect to baseline

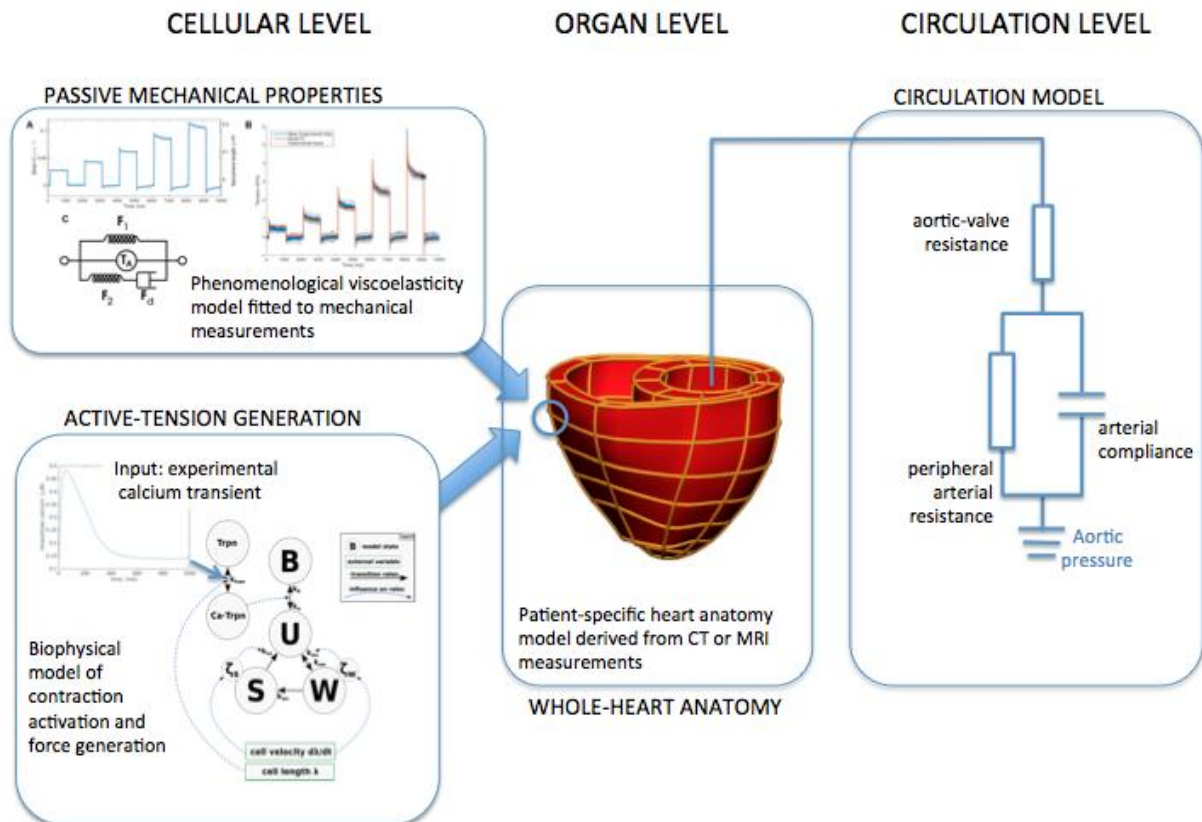
### Linking Changes in Protein Expression to Organ Scale function

We developed a multi-scale computational model of the heart that aims to reproduce measurable clinically relevant features of cardiac function by simulations. The analysis workflow is outlined in Figure 3. In summary, the cardiac model implements heart properties ranging from the cellular level (myocyte mechanical and electrophysiological behaviour) to the whole-heart organ. The model (Figure 4) aims to reproduce real cardiac behaviour under physiological conditions. When the tissue is locally stimulated by an externally imposed signal, contractive forces are generated throughout the tissue mass, eliciting a viscoelastic deformation of the cardiac anatomy and the ejection of blood into the circulation. The model parameters that govern the tension generation and mechanical response are all amenable to fitting, based either on direct measurements or on data in the literature. This provides a setting for investigating cardiac function with maximal consistency, in particular by assessing the impact of specific parameters that are altered by drug exposure.

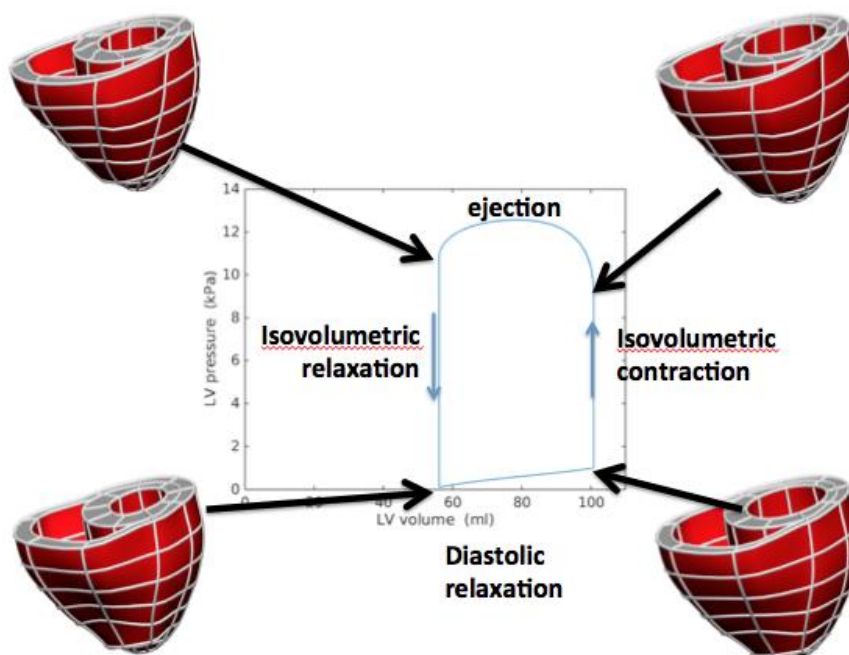
Figure 5 shows a typical simulation output, predicting the left-ventricular ejection fraction and hemodynamic, which are routinely measured in clinical settings.



**Figure 3:** General framework for the analysis. The generic multi-scale model is parameterised based on experimental and clinical data. The simulation outcome is maximally consistent with real cardiac function.



**Figure 4:** Overview of the multi-scale cardiac model, covering the cellular, organ, and wider systemic levels. Parameters at all levels can be set using experimental and clinical data.



**Figure 5:** The cardiac cycle: representative output of the simulation. Morphologies and hemodynamic variables are predicted at all points of the cardiac cycle, allowing direct comparison with measurements.

### Determining the impact of drug exposure: mass-spectrometry data analysis

We analysed the mass spectrometry data provided by N. Selevsek (Partner ETH Zurich) to determine the impact of doxorubicin on cardiac function at therapeutic and toxic dose levels in organ scale models. Our analysis focused on the proteins that feature in our whole heart computational model, in the following categories:

- Intracellular electrophysiology;
- Excitation-contraction coupling;
- Contractile apparatus.

Having identified the measurements for these target proteins in the baseline, therapeutic, and toxic datasets provided, we calculated the time evolution of the mean protein concentrations. The example in Figure shows the results for the sodium-calcium exchanger (NCX), a key electrophysiological protein. No systematic variation is apparent in the NCX concentration, for any of the three dose levels, suggesting that the drug has had no impact on this protein. Other proteins, however, showed clearer signs of variation.

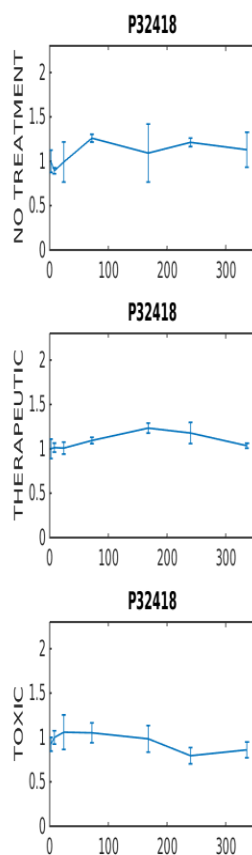
To allow a more reliable assessment of the significance of observed trends above experimental uncertainties, we extended the analysis to all the proteins for which measurements were found at all three dose levels. To quantify this approach, the average rate of change in concentration for a given protein (calculated by linear regression) was taken as a measure of the effect of the drug at each dose level.

$$mgY1 = \text{mean concentration gradient for "no treatment"} / \text{initial concentration}$$

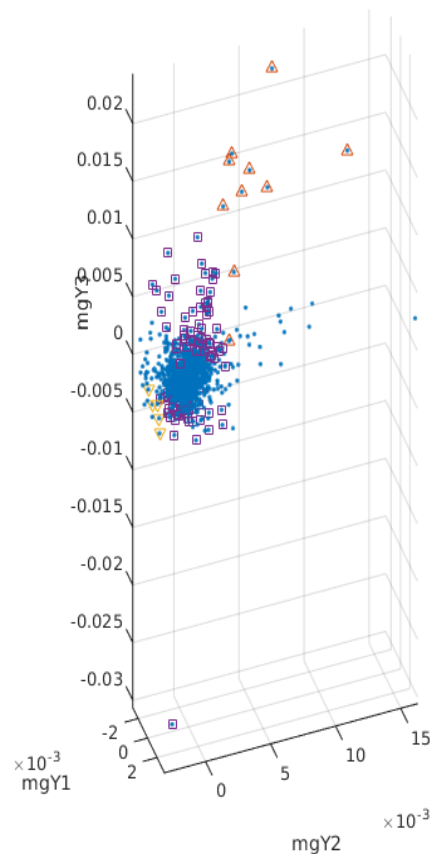
Similarly, *mgY2* and *mgY3* correspond to the “therapeutic” and “toxic” doses, respectively. Figure shows a three-dimensional scatter plot of this metric (*mgY1*, *mgY2*, *mgY3*), with each point representing one protein.

This representation:

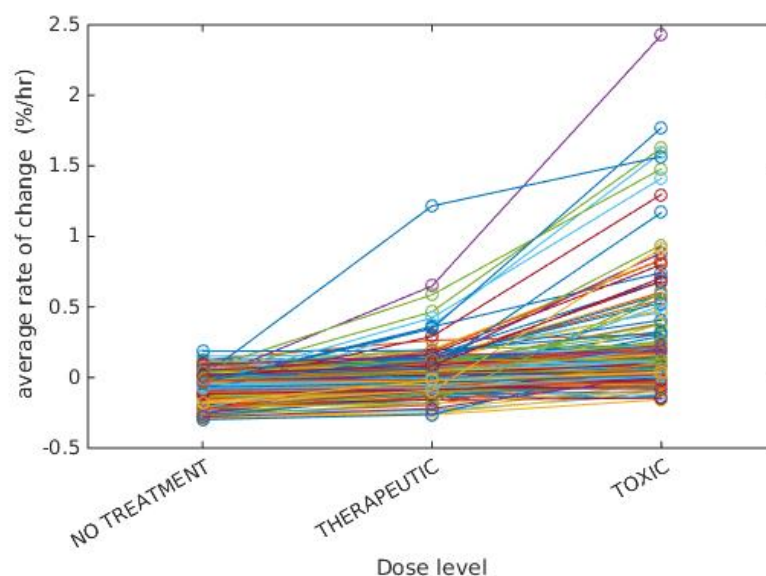
- Provides a global overview of the entire dataset, allowing an estimate of the intrinsic uncertainties (with the central “cloud” representing the drug-insensitive proteins);
- Facilitates the identification of proteins that are significantly affected by the drug, i.e. the points that deviate significantly from the central “cloud”;
- Provides an appreciation of the range of measured variations inherent in the dataset.



**Figure 6:** Normalised time. Dependence of the concentration of the sodium-calcium exchanger (NCX) a key electrophysiological ion channel.



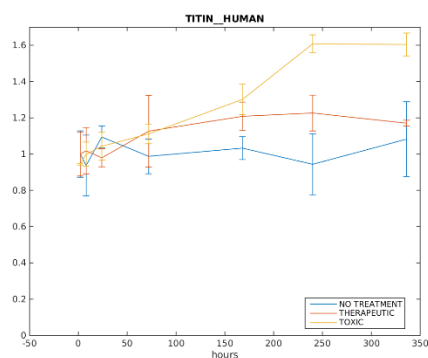
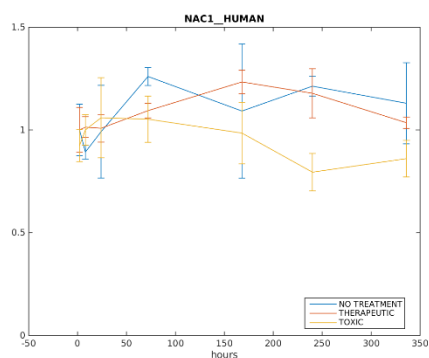
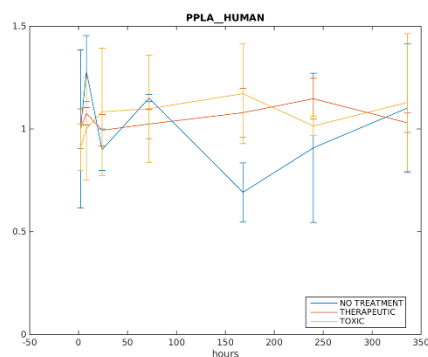
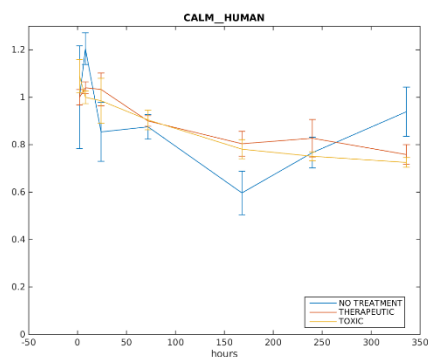
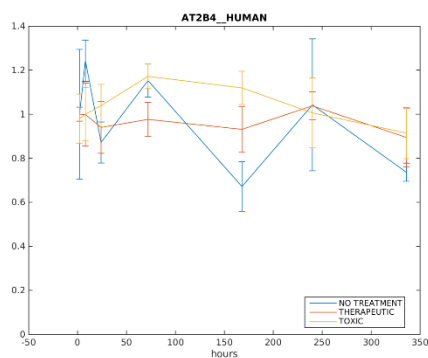
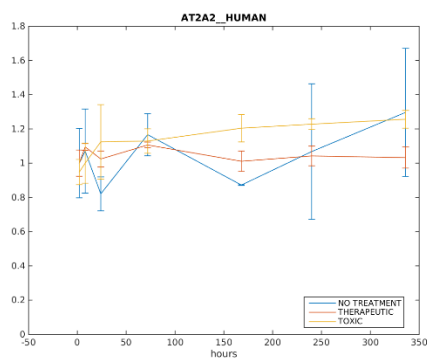
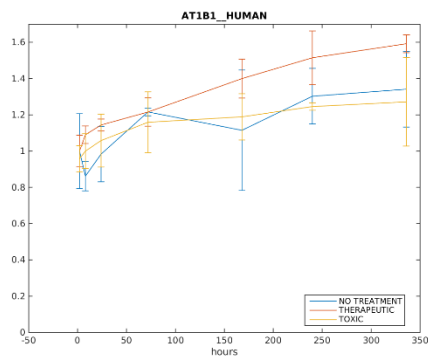
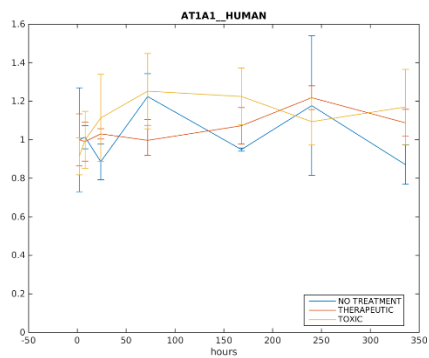
**Figure 7:** Three-dimensional plot of the “mean gradient” metrics (*mgY1* for the “no treatment” data, and *mgY2* and *mgY3* for the “therapeutic” and “toxic” doxorubicin dose levels, respectively)

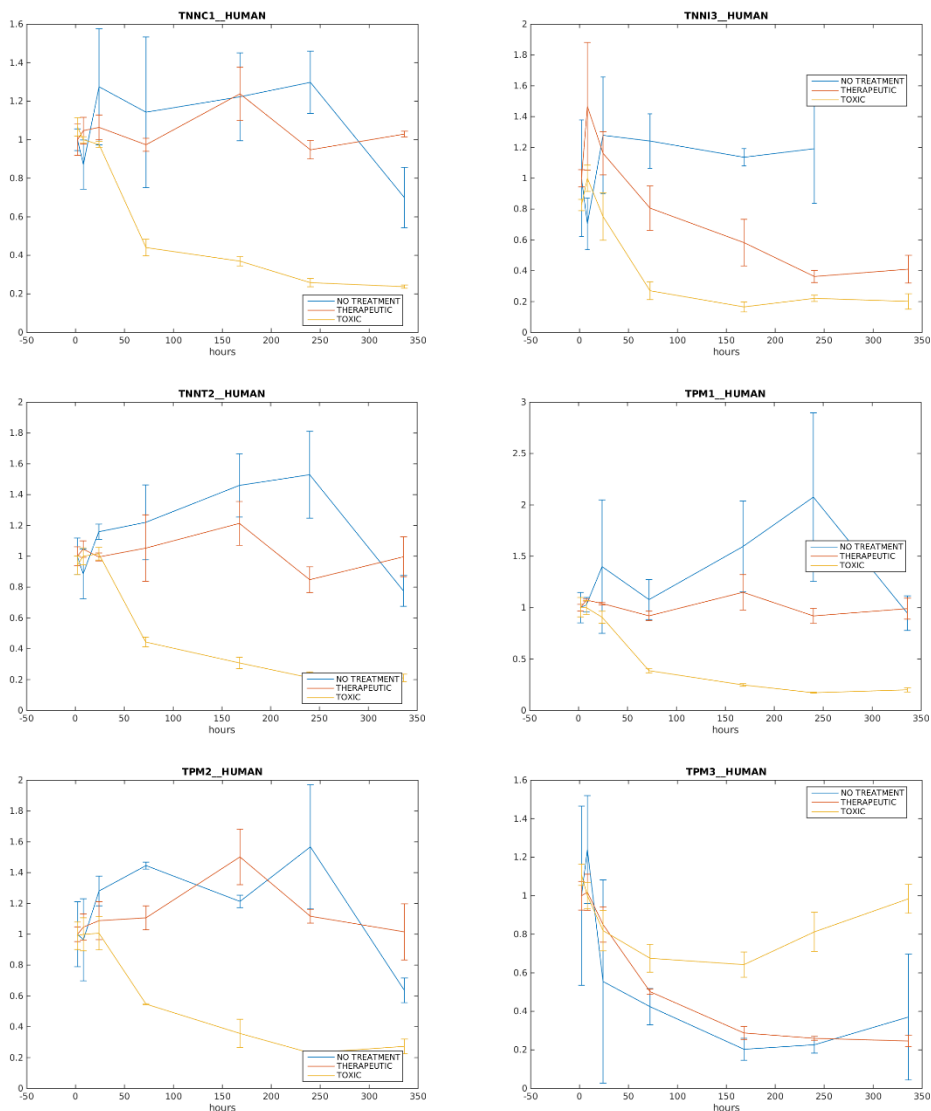


**Figure 8:** The "mean gradient" metric (mgY1,2,3) plotted against the dose level for each protein showing a monotonic increase ("zero dose" < "therapeutic" < "toxic")

The table lists the proteins that are included in the dataset and that have play a role in the contraction model. The time dependences of each protein are plotted (for the "zero", "therapeutic", and "toxic" dose levels) in the figures below.

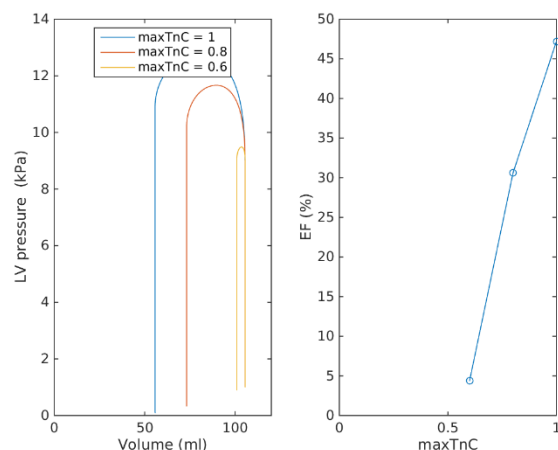
PROTEIN	PROTEIN CODE	EFFECT OF DOXORUBICIN OVER 14 DAYS
Sodium-potassium pump (alpha 1)	AT1A1_HUMAN	No systematic effect above noise level
Sodium-potassium pump (alpha 2)	AT1B1_HUMAN	No systematic effect above noise level
SERCA	AT2A2_HUMAN	No systematic effect above noise level
Calcium pump	AT2B4_HUMAN	No systematic effect above noise level
Calmodulin	CALM_HUMAN	Decrease by ~20% at both therapeutic and toxic doses
Phospholamban	PPLA_HUMAN	No systematic effect above noise level
Sodium-calcium exchanger	NAC1_HUMAN	No systematic effect above noise level
Titin	TITIN_HUMAN	Increase by ~60% at toxic dose
Troponin C	TNNC1_HUMAN	No effect at therapeutic dose; 70% decrease at toxic dose
Troponin I	TNNI3_HUMAN	~50% decrease at therapeutic dose; ~70% at toxic dose
Troponin T	TNNT2_HUMAN	No effect at therapeutic dose; ~70% decrease at toxic dose
Tropomyosin alpha-1 chain	TPM1_HUMAN	No effect at therapeutic dose; ~80% decrease at toxic dose
Tropomyosin beta chain	TPM2_HUMAN	No effect at therapeutic dose; ~70% decrease at toxic dose
Tropomyosin alpha-3 chain	TPM3_HUMAN	Inconclusive





## Doxorubicin: implementation of proteomics measurements into the computational model

Most of the proteins of interest display a consistent response (or are insensitive) to doxorubicin exposure, within measurement error. Notably, the measurements relating to the different troponin complex proteins are quite consistent, showing limited effect at therapeutic doses, and a significant drop (by around 70%) at toxic doses. As a preliminary investigation of this effect, we simulated the drug effect by varying the model parameter corresponding to the amount of active troponin C proteins. Consistently with the clinical effect of the “toxic” dose, the left-ventricular ejection fraction (EF) decreases rapidly with decreasing troponin C level, a common indicator of cardiac dysfunction.



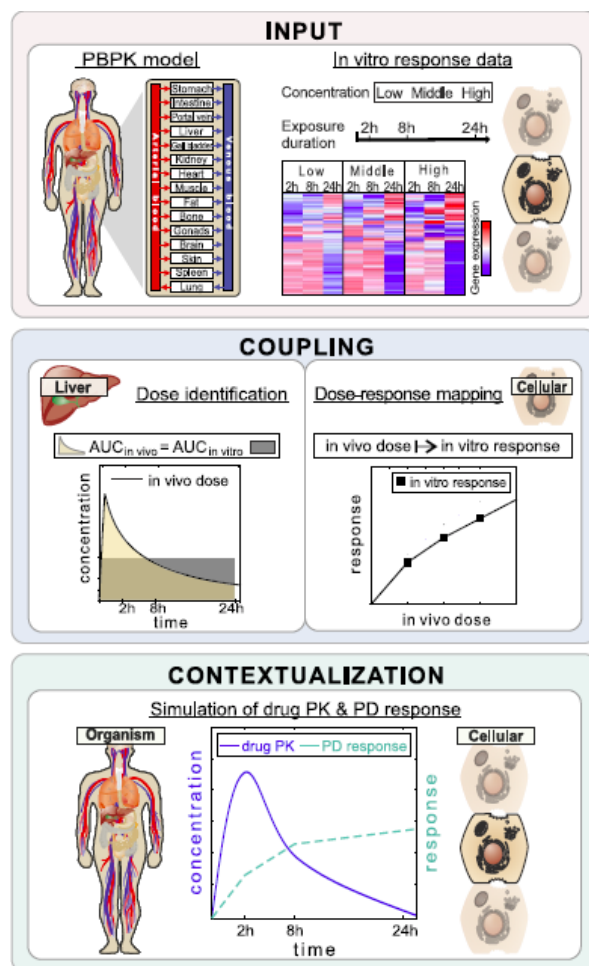
**Figure 9:** Simulation result, showing a rapid decrease in the ejection fraction with decreasing troponin C level, consistent with the effect of a "toxic" dose.

## HEPATOTOXICITY

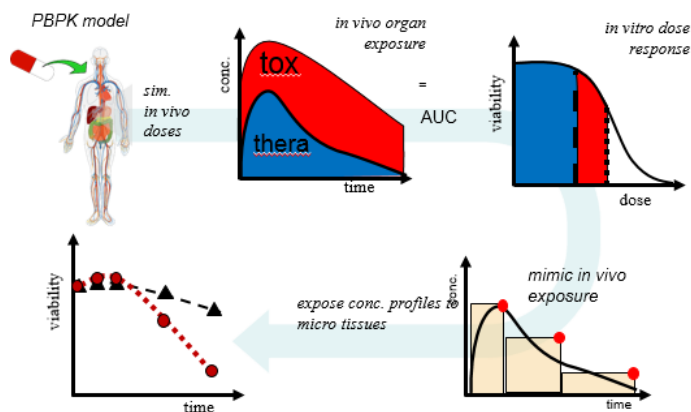
### Results

For the integration of integrating experimental data from the project partners across different scales we have developed PICD (Thiel et al. 2016) as a generic approach for the analysis of cellular data within a patient context. AS such PICD integrates *in vitro* toxicity data into drug-specific whole-body PBPK models to translate drug-induced *in vitro* findings to an actual *in vivo* situation (Figure 10). Thus drug-specific response profiles induced by different dose levels administered *in vivo* can be described. At the cellular level, *in vitro* toxicity data may be correlated with corresponding PBPK-simulated concentration–time profiles at the organism level to allow a quantitative description of time-resolved *in vivo* drug response of key cellular processes at pathway level. Notably, PICD supports the model-based translation of preclinical *in vitro* toxicity data into an *in vivo* context through model-based dose-response correlations.

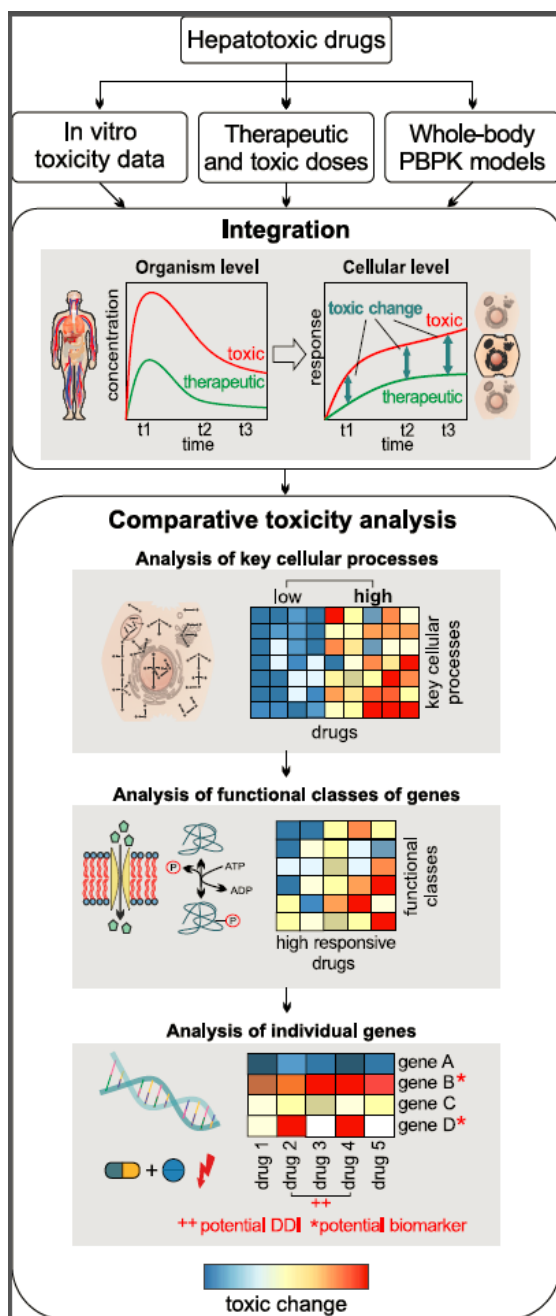
In a proof of concept study, *in vitro* transcriptomics data from the Open TG-GATEs library (Igarashi et al. 2015) have been used. PBPK models have been used for several of the HeCaToS compounds to design *in vitro* setups mimicking PK concentration profiles in human patients (Figure 11). Experimental omics data generated with this novel assay design are much closer to the human *in vivo* situation since they are no longer obtained from stationary *in vitro* cultures. Nevertheless, the various studies which have already been performed with PICD (Thiel et al., 2016; Thiel et al. Submitted a) and b) they can nevertheless be seen as a blueprint for the upcoming. In particular, the escalation from therapeutic to toxic doses has already been performed with Open TG-GATEs library (Thiel et al., 2016). The availability of equivalent, yet largely refined HeCaToS omics data will hence be an important result of the project (Figure 11).



**Fig 10:** PBPK-based *in vivo* contextualization of *in vitro* toxicity data (PICD). **INPUT:** At the organism level, PBPK models are developed for specific drugs. At the cellular level, *in vitro* response data of compound-treated primary hepatocytes are analyzed. **COUPLING:** *In vivo* doses are identified, which are directly related to *in vitro* drug exposure ( $AUC_{in vivo} = AUC_{in vitro}$ ). Time-dependent dose-response curves are built by mapping *in vivo* doses to *in vitro* responses. **CONTEXTUALIZATION:** By use of the time-dependent dose-response curves drug responses over time are predicted for PK profiles simulated for different doses.



**Fig 10:** PBPK-based experimental design.



**Fig 11:** Consideration of therapeutic and toxic doses within a whole-body PBPK context. For a set of hepatotoxic drugs, toxic changes will be measured at different timepoints (2h, 8h, 24h) by comparing cellular response following drug administration of therapeutic and toxic doses and will be subsequently evaluated with regard to key cellular processes, functional classes of genes, and individual genes, respectively (Thiel et al, submitted a).

## DIFFICULTIES

The electrophysiology modelling framework developed for MS 3.1 captured the reported changes in calcium handling and electrophysiology proteins from reported doxorubicin toxicity studies. In the HeCaToS consortium we have developed a model-based approach for experimental planning of the spheroid assay. Notably, compound specific PBPK models are applied in this approach to calculate dynamic drug concentration profiles in the supernatant as such mimicking PK profiles in patients.

So far, the protein expression data generated for HeCaToS did not observe significant changes in these proteins limiting the capacity to use this modelling framework with the current data derived from stem cells, as its predictions are neutral. We expect, however, that the extrapolation of the customized *in vitro* data to the presented modelling frameworks will be further improved when more and more HeCaToS data become available within the consortium.

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