



Whole body physiological based pharmacokinetic pharmacodynamic model for GalNac Conjugated siRNAs



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INTRODUCTION

N-Acetyl-galactosamine small interfering RNAs (GalNac-siRNA) are an emerging class of drugs that have revolutionized RNA interfering drug therapy due to its high target specificity and durable knockdown of disease related protein. Conjugation onto GalNac allows for target specific uptake into hepatocytes via the Asia Glycoprotein Receptor (ASGPR). GalNac-siRNA exhibits their target protein knockdown from a series of events in the cell cytosol by loading into Ago2 proteins and forming the RNA-induced Silencing Complex (RISC) that cleaves the warranted mRNA. With a transient plasma exposure combined with a rapid uptake and prolonged half-life in the liver, GalNac-siRNA exhibit distinct disposition characteristics compared to small molecules and other therapeutic peptides(1).

Physiologically-based pharmacokinetic (PBPK) modeling facilitate for quantitative local disposition description and complex PK-PD relationships and thus can address the needs for investigations of GalNac-siRNAs.

We aimed to build a generic GalNac-siRNAs Whole-Body PBPK-PD (WB-PBPK-PD) model for the means of describing systemic and local disposition and the identification of modality specific knowledge gaps.

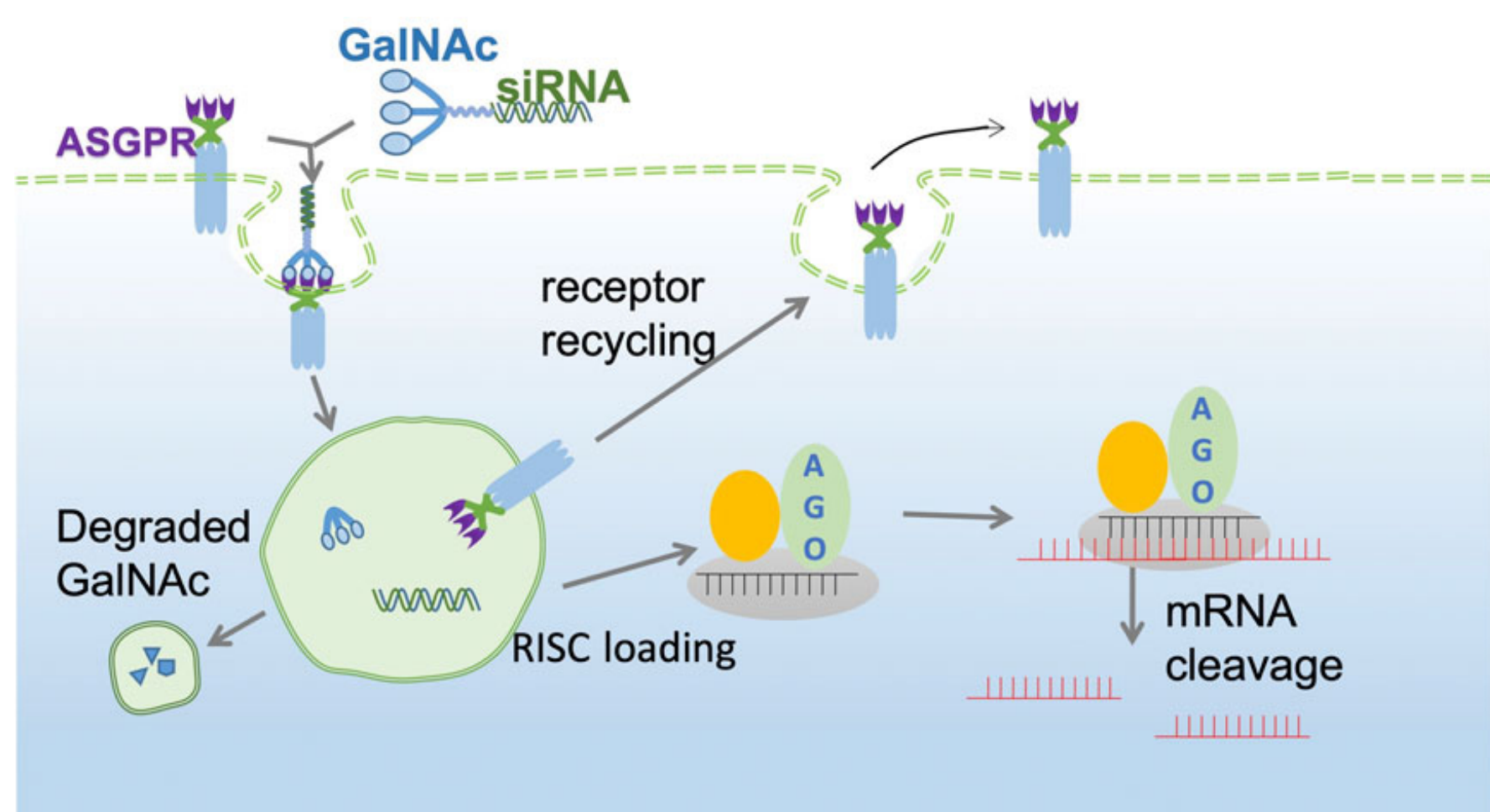


Figure 1 ASGPR mediated uptake of GalNac-siRNA. GalNac-siRNA binds to ASGPR receptor on hepatocytes and undergoes endocytotic uptake. pH drop in the liver endosome decompose GalNac-siRNA complex and 1% siRNA escapes into cell cytoplasm. The ASGPR recycles to cell surface. The RISC is constituted via siRNA loaded Ago2 which cleaves the target mRNA via siRNA guidance(2).

Model Development

For model development a reference data set was compiled from published studies on investigational GalNac-siRNAs in mouse(3-5). The model was implemented using the open source platform Open System Pharmacology Suite, PK-Sim®/MoBi®, Version 11.2. The WB-PBPK model structure leveraged the PK-Sim default implementation for large molecules, e.g., including the two-pore-formalism for extravasation and elimination from vascular endothelial endosomes.

Specification of siRNA and ASGPR/RISC dynamics, e.g., binding, internalization, recycling was described via implementation in MoBi as a TMDD model approach (6). The mRNA silencing and downstream translation were described with an indirect response model. The PBPK-PD model parameters were either derived from the literature (In vitro/ In vivo experiments) or optimized via Monte Carlo simulation towards reference data.

Compound	Target	Administration/Dose	Measurement	Reference
ALN-AT3 (Fitusiran Phase III)	Anti Thrombin	SC 1-5 mg/kg	Plasma, Liver, mRNA, Serum AT	Sehgal et al., 2015; Nair et al. 2017
SIAT-2 (Investigational)	Anti Thrombin	SC 2.5-25 mg/kg	Plasma, Liver, mRNA, RISC	Nair et al. 2017
siF9-1/2 (Investigational)	Factor 9	SC 0.75 - 2.5 mg/kg	RISC, mRNA	Brown et al. 2020
SITTR-1/SITTR-2 (Investigational)	Transferrin Protein	SC 0.5, 1.5 mg/kg SC, IV 10 mg/kg	Plasma, Liver, mRNA (SC)	Nair et al. 2017; Brown et al. 2020

Table 1 Summary of the published data on the GalNac-siRNAs used in the model development including target, doses, administration and their measurements.

CONCLUSION

The WB-PBPK-PD model successfully quantified systemic and target tissue distribution as well as the complex ASGPR mediated uptake. Compound and system specific attributes of importance for the PK-PD relationship were identified and characterized.

This provides a generic WB-PBPK-PD model for the investigation of GalNac-siRNAs implemented in an open-source platform.

RESULTS

- The generic model containing the two pore formalism could not readily describe the extravasation, and the default radius solute of the GalNac-siRNAs were decreased by a 100-fold.
- Endosomal stability of the GalNac-siRNAs were estimated individually for each compound which governed the prolonged half life in the liver tissue.
- Fraction of free siRNA escaping the endosome and forming RISC was individually estimated across compounds.
- RISC dynamics were estimated as global parameters.
- PD parameters were estimated individually for each compound with S_{max} showing the biggest variation between compounds.
- Sensitivity analysis revealed that endosomal stability had the highest impact on liver $AUC_{0-1000h}$ and that K_{DR} governing the RISC half life, would be the most influential PBPK parameter for PK-PD characterization of GalNac-siRNAs.

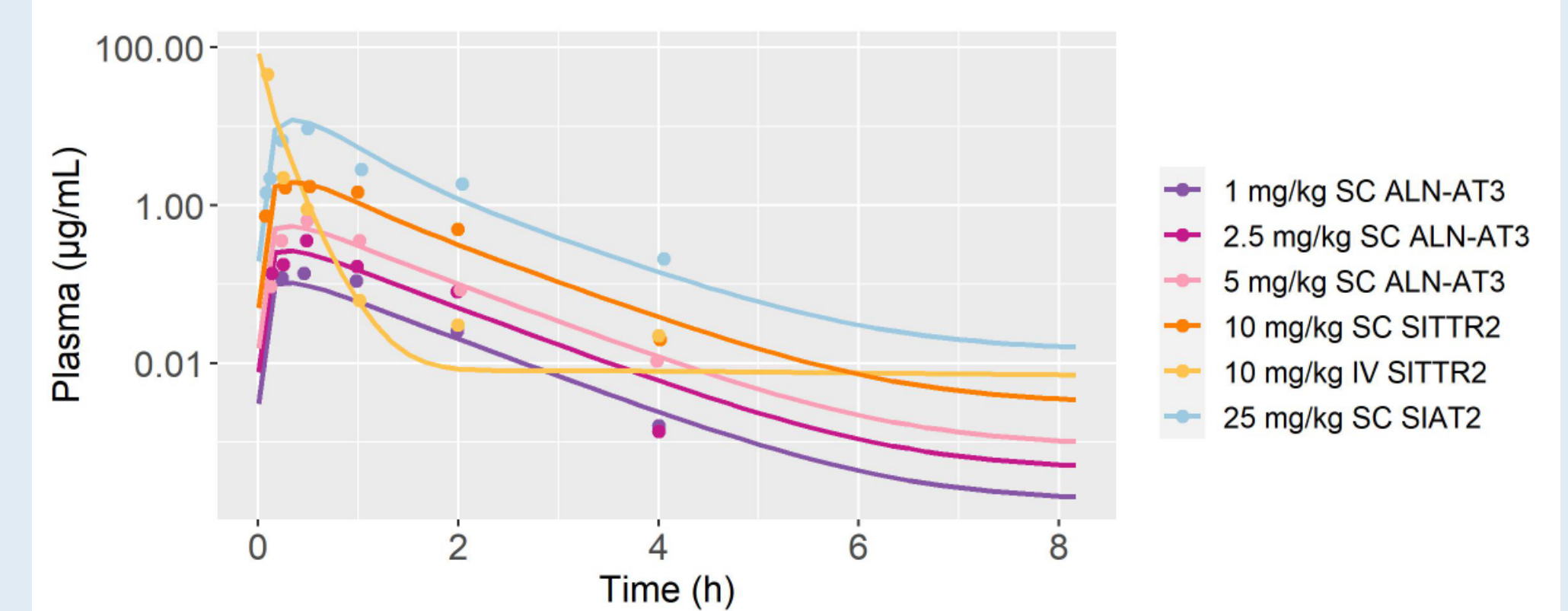


Figure 1 Observed and model simulated plasma concentration profiles for GalNac-siRNAs targeting anti thrombin (ALN-AT3/SIAT-2) and transthyretin protein (SITTR2). Solid lines represents model simulations and dots represents observations. Unique colors are explained in the figure.

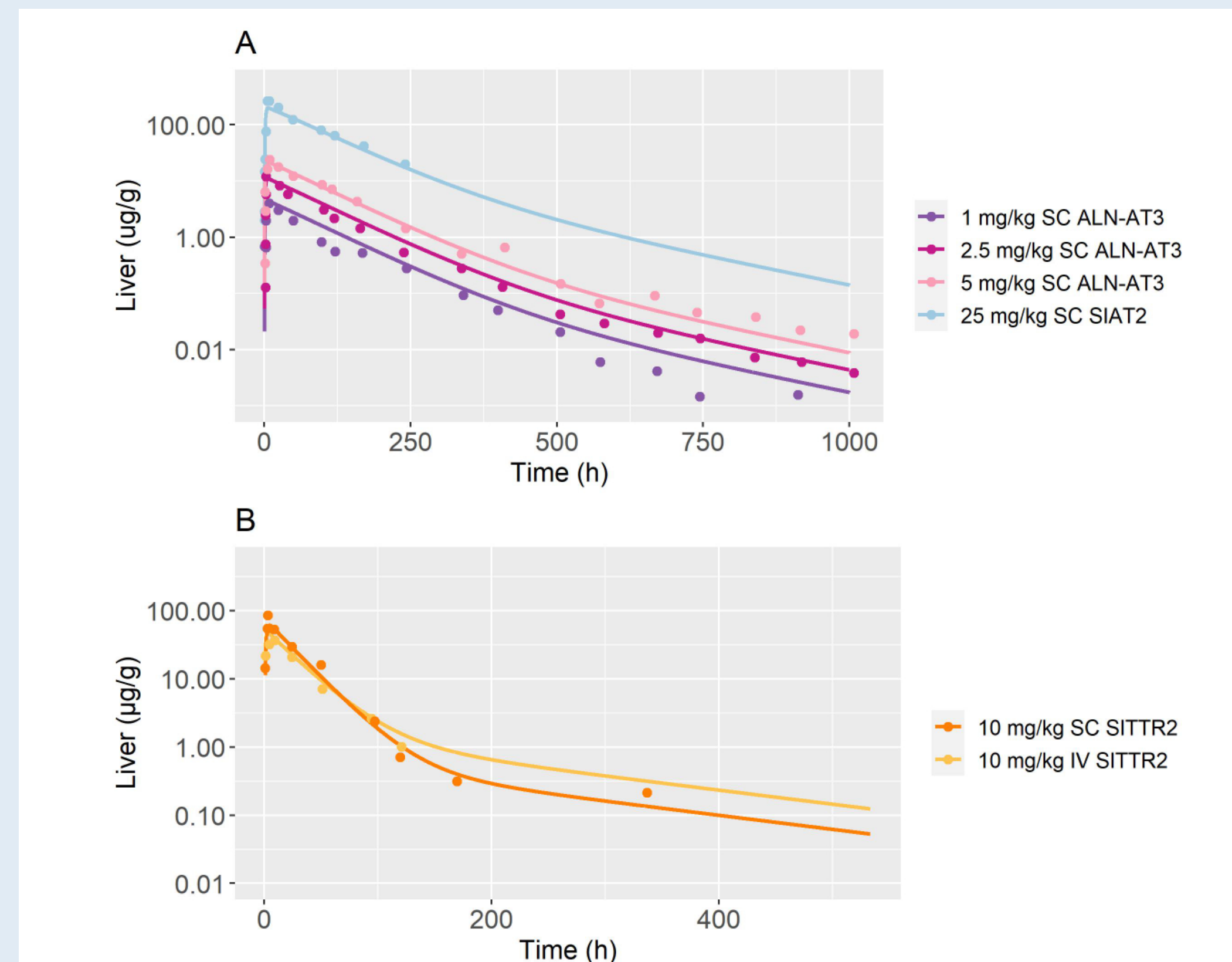


Figure 2 Observed and model simulated liver tissue distribution for GalNac-siRNAs in mouse targeting A) antithrombin (ALN-AT3/SIAT-2), B) targeting transthyretin protein (SITTR2). Solid lines represent model simulations and dots represents observations. Unique colours are explained in the figure.

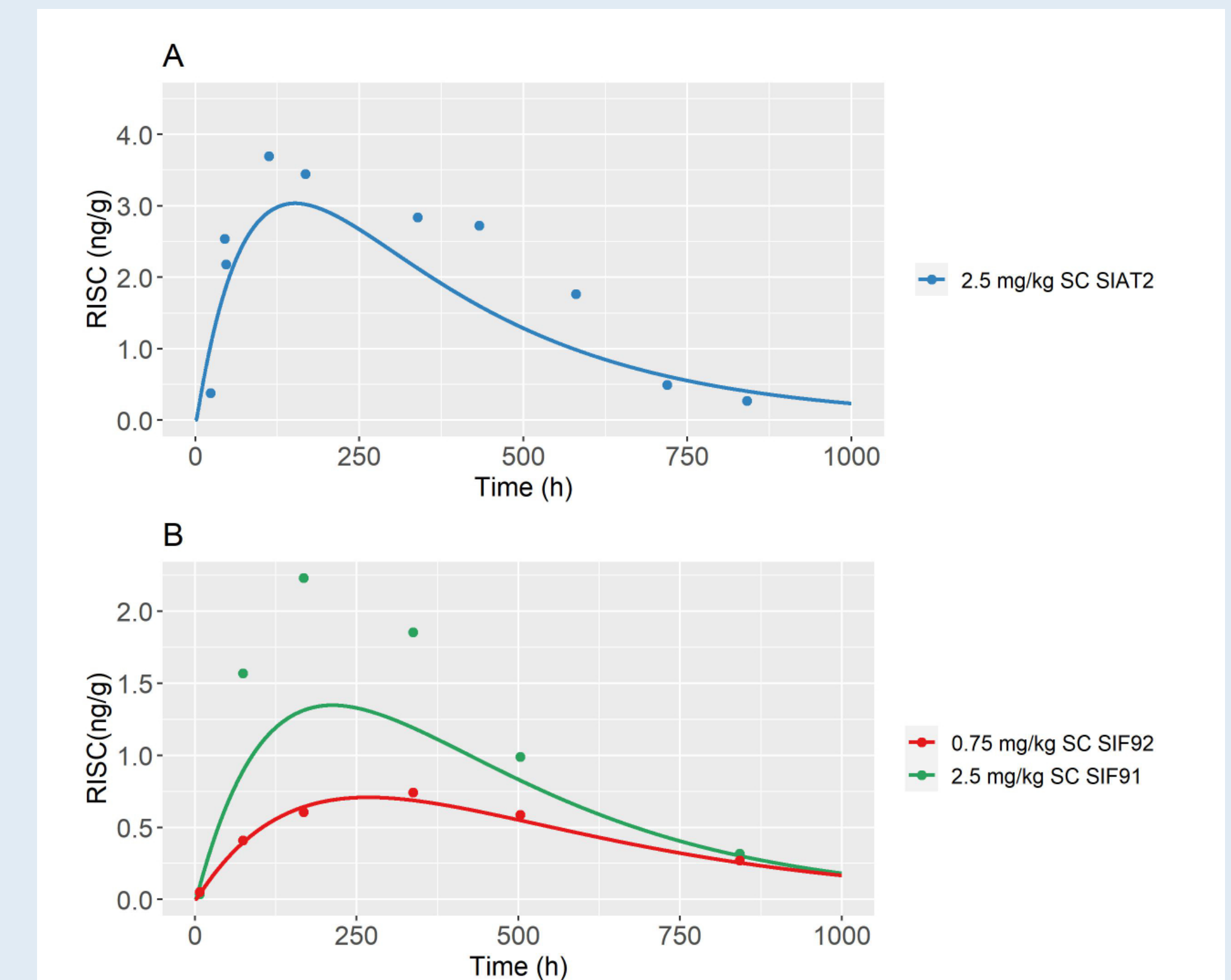


Figure 3 Observed and model simulated siRNA induced RISC formation measured as antisense strand loaded into Ago2 for GalNac-siRNAs in mouse targeting A) antithrombin (SIAT-2) and B) targeting factor 9 (siF9-1/2). Solid line represents model simulation and dots represent observations. Unique colours are explained in the figures

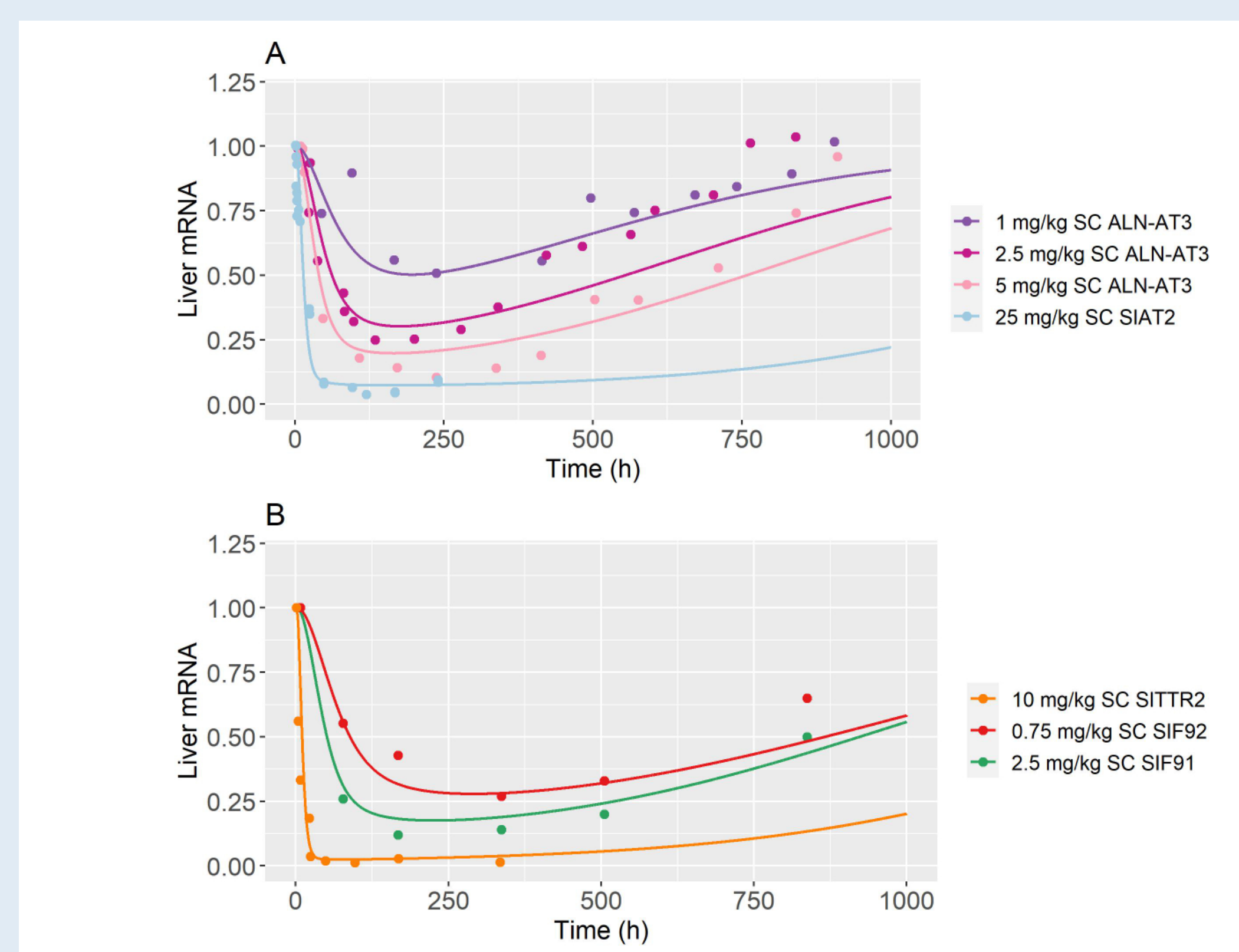


Figure 4 Observed and model simulated liver mRNA silencing for GalNac-siRNAs in mouse targeting A) antithrombin (ALN-AT3/SIAT-2) and B) targeting transthyretin protein (SITTR2) and targeting factor 9 (siF9-1/2). Solid lines represents model simulations and dots represents observations. Unique colours are described in the figures.

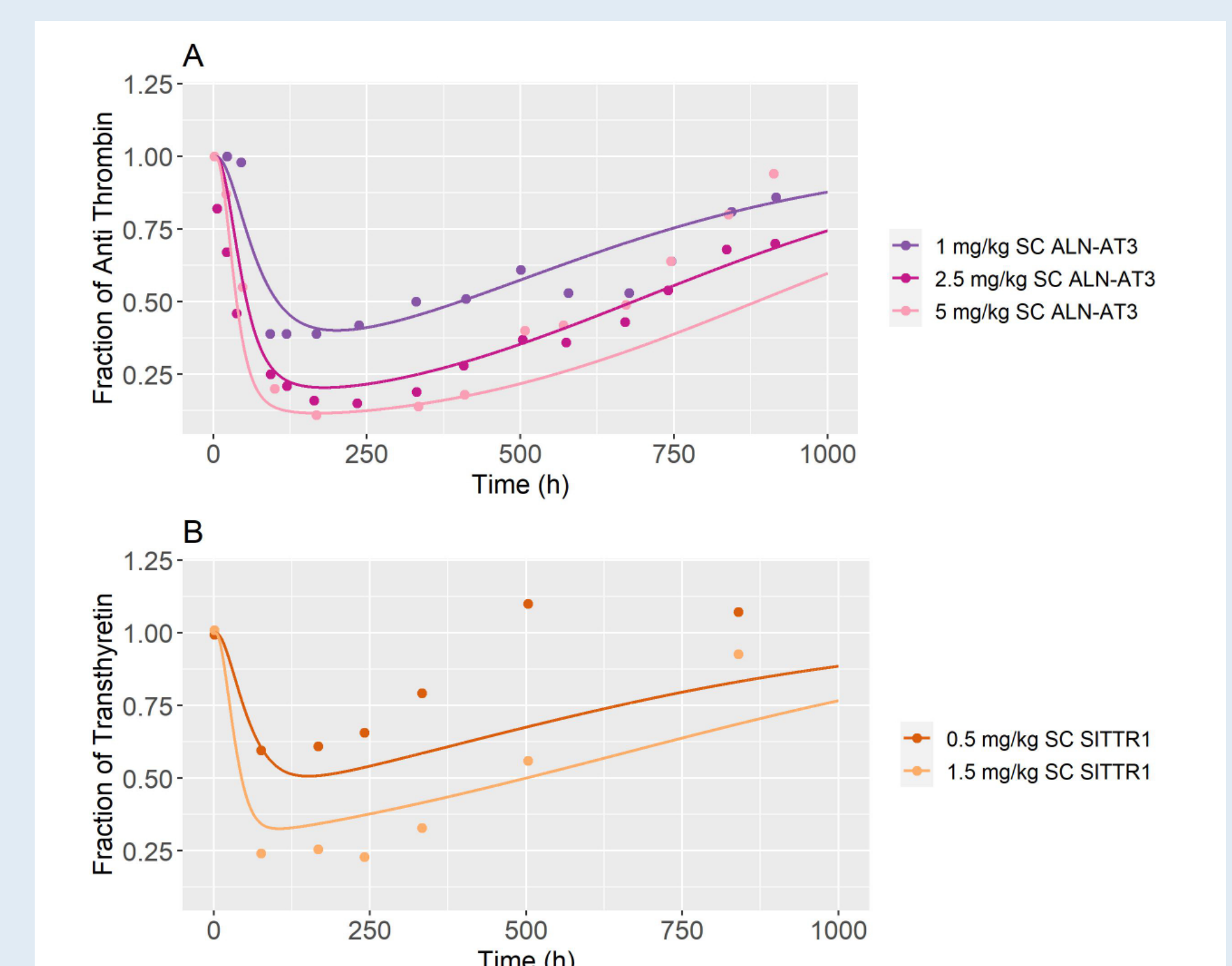


Figure 6 Observed and model simulations of downstream effect on target protein for GalNac-siRNAs in mouse targeting A) antithrombin (ALN-AT3/SIAT-2) and B) targeting transthyretin protein (SITTR1). Solid lines represents model simulations and dots represents observations. Unique colours are described in the figures.

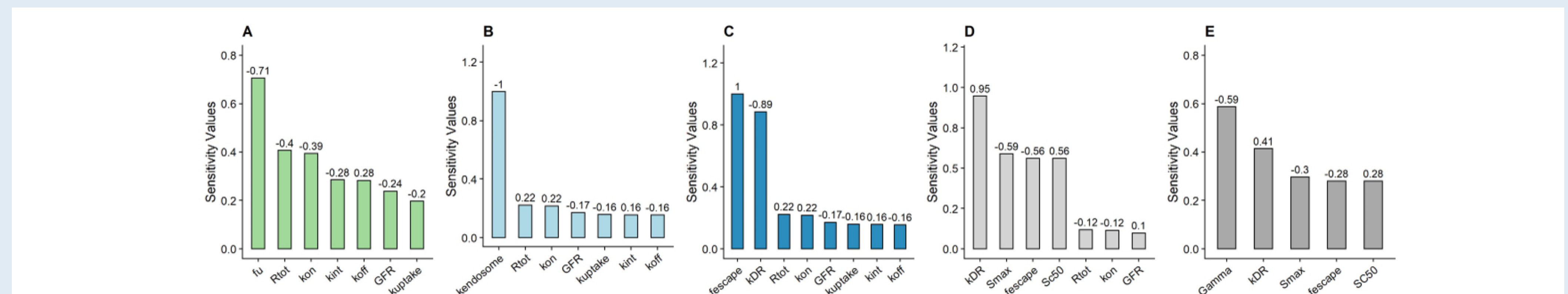


Figure 7 Model sensitivity analysis of PBPK parameters measured as the change in Area Under the Curve (AUC) simulated from time 0h to time 1000h ($AUC_{0-1000h}$) in A) plasma (light green bars), B) liver tissue (light blue bars), C) siRNA induced RISC formation (dark blue bars), D) mRNA silencing (light grey bars) E) downstream effect on target protein (dark grey bars). Positive sensitivity coefficient denotes an increase in AUC when the investigated parameter is increased by 10%. Negative sensitivity coefficients denote a decrease in AUC when the investigated parameter is increased with 10%.

References

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