

An enhanced PBPK model for predicting mRNA-encoded therapeutic trafficking in mice

Elio Campanile^{1,2}, Elisa Pettinà^{2,3}, Lorena Leonardelli², Stefano Giampiccolo^{2,4}, Luca Marchetti^{2,3}

¹Department of Mathematics, University of Trento, Italy.

²Fondazione The Microsoft Research – University of Trento Centre for Computational and Systems Biology (COSBI), Italy.

³Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Italy.

⁴Department of Information Engineering and Computer Science, University of Trento, Italy.

THE MICROSOFT RESEARCH-UNIVERSITY OF TRENTO CENTRE FOR COMPUTATIONAL AND SYSTEMS BIOLOGY (COSBI), ITALY



UNIVERSITY OF TRENTO

Background

Messenger RNA (mRNA)-encoded therapeutics have gained significant attention for cancer treatment due to their ability to transiently express proteins in vivo.

However, several challenges remain:

- The mechanistic understanding of how mRNA-loaded lipid nanoparticles (LNPs) traffic through the body post-injection is still limited.
- The conversion of mRNA into therapeutic proteins,

including the dynamics of uptake, translation, and degradation, is not fully characterized.

- There is a lack of predictive tools to guide formulation development, optimize dose regimens, and tailor treatment protocols across therapeutic classes.

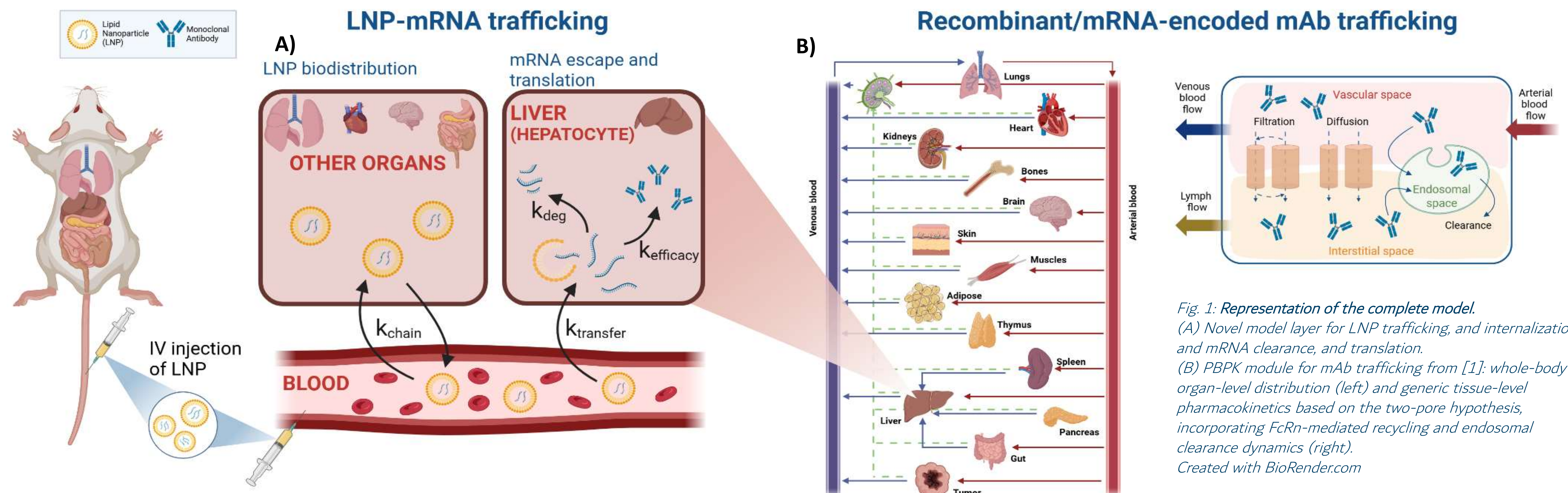


Fig. 1: Representation of the complete model. (A) Novel model layer for LNP trafficking, and internalization and mRNA clearance, and translation. (B) PBPK module for mAb trafficking from [1]: whole-body organ-level distribution (left) and generic tissue-level pharmacokinetics based on the two-pore hypothesis, incorporating FcRn-mediated recycling and endosomal clearance dynamics (right). Created with BioRender.com

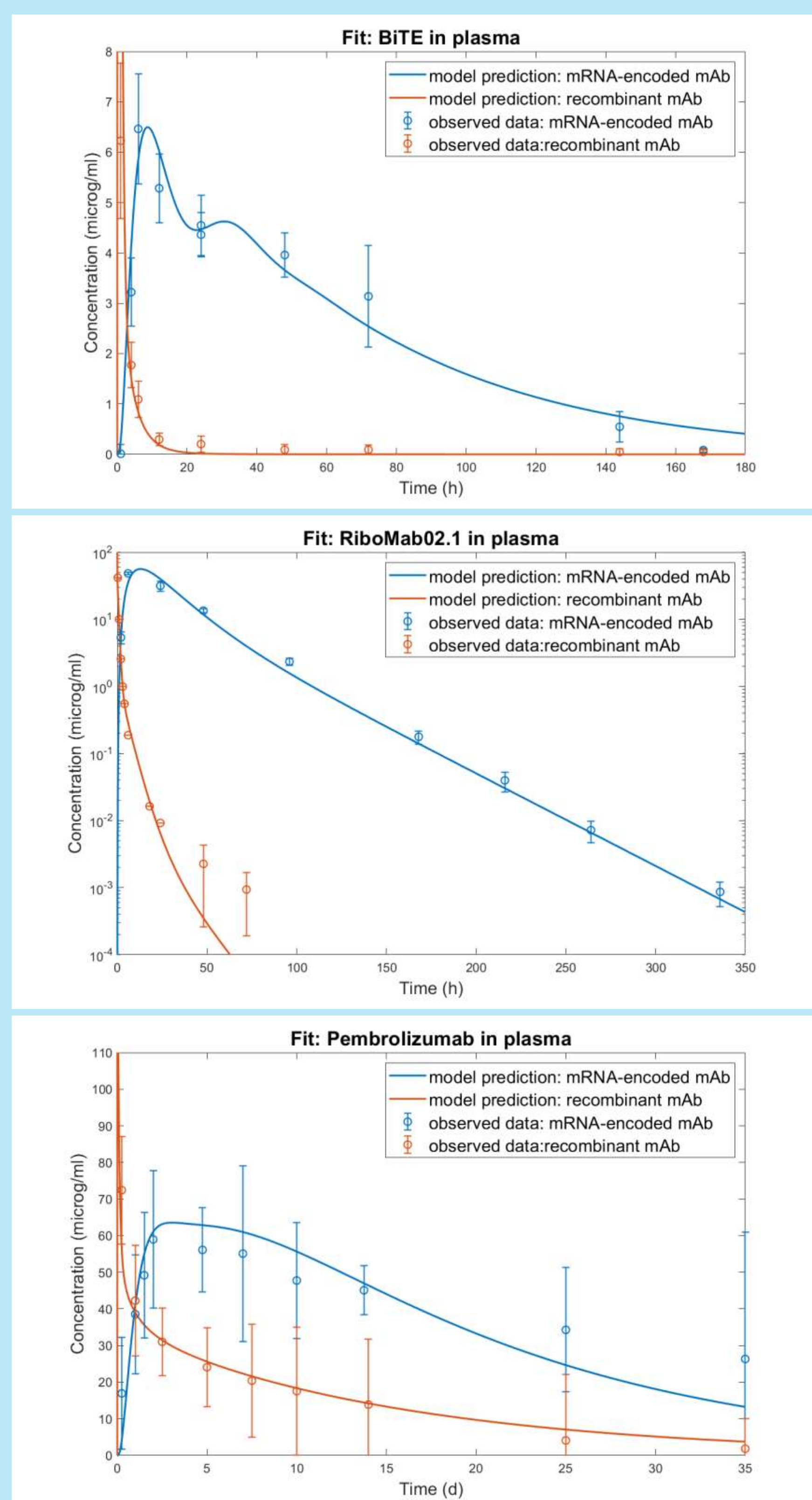


Fig. 2: Model fits for plasma concentration-time profiles in mice of three mRNA-encoded therapeutics (BiTE [3], RiboMab02.1 [4], and Pembrolizumab [5]) and their recombinant counterparts.

The model

We extended a previously published PBPK model [1] by adding a phenomenological LNP trafficking module and mRNA reaction kinetics:

- LNP trafficking is modeled using a transit compartment approach to preserve parsimony and ensure identifiable parameter estimation. mRNA kinetics includes three ODEs for escape, degradation, and protein translation in hepatocytes (Fig. 1A).
- The model accounts for therapeutic-specific features including molecular weight (55–150 kDa), Fc domain presence, and interstitial distribution using the two-pore theory and FcRn-mediated recycling (Fig. 1B).

All mRNA-related parameters underwent identifiability analysis using GENSSI [2], confirming global structural identifiability. Implementation was done in MATLAB2024b Simbiology.

Results: model training and validation

The model was successfully calibrated and validated against six different mRNA-encoded therapeutics in mice using literature data [3–8].

It captured concentration-time profiles with high accuracy for both recombinant and mRNA-encoded versions, across different doses and treatment regimens. In Fig. 2,3, we show simulations with B7H3xCD3 BiTE, RiboMab02.1, and Pembrolizumab.

Attempts to simplify the mRNA module—such as omitting the LNP trafficking in Other Organs—led to a marked loss in predictive accuracy, particularly in capturing peak timing and clearance profiles. In contrast, the full model not only provided excellent fits to experimental data but also generalized across therapeutic classes and formulations, demonstrating strong predictive utility.

Parameter estimates and preliminary sensitivity analysis revealed biologically relevant insights, such as recurrent kinetic patterns among structurally similar LNPs and potential modulation of translation efficiency under multidose regimens.

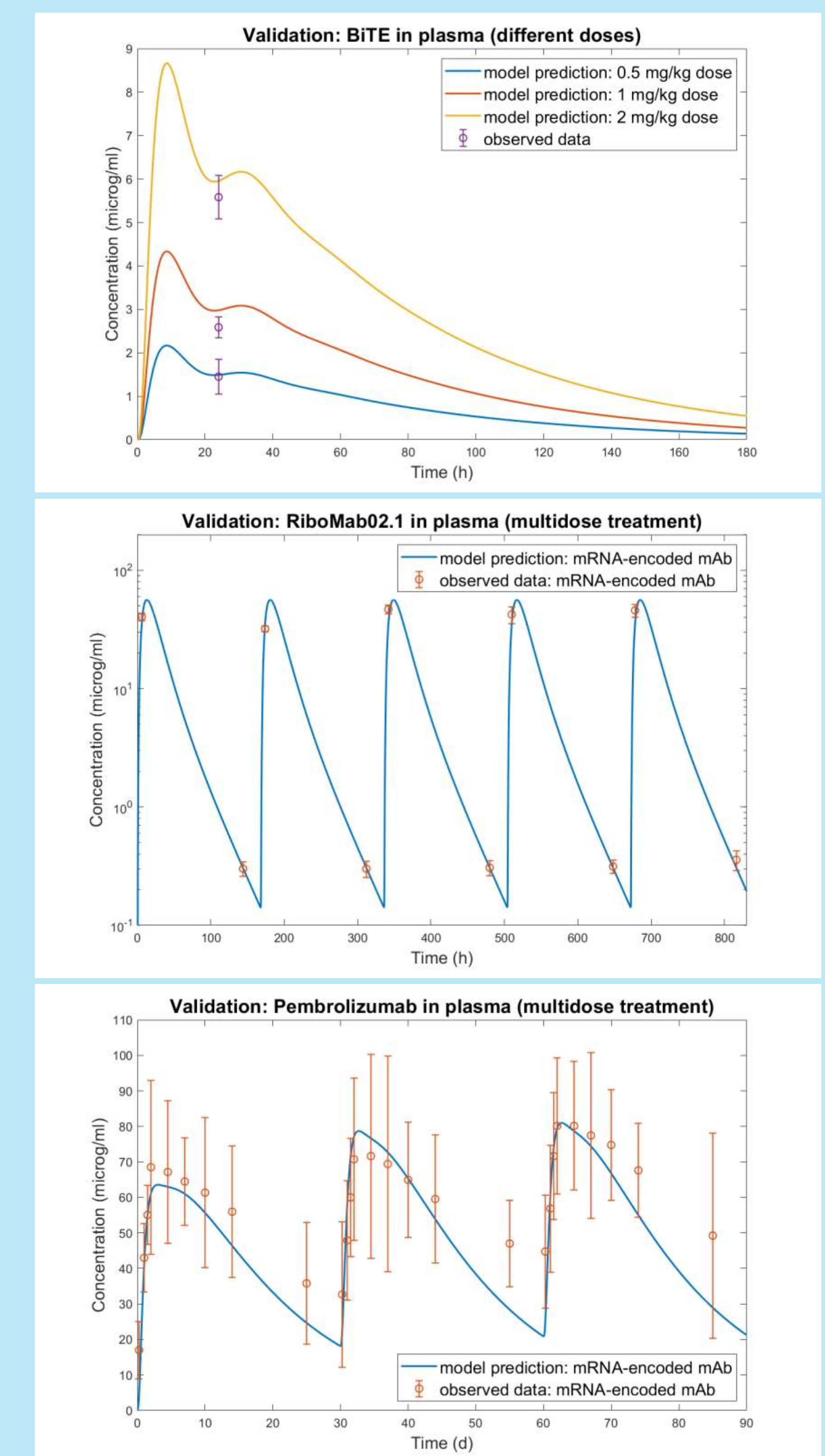


Fig. 3: Model validation for mRNA-encoded therapeutics in mice. Predictions match observed data across dose levels (BiTE [3]) and multidose regimens (RiboMab02.1 [4], Pembrolizumab [5]).

Conclusions and future perspectives

We developed a flexible, multiscale PBPK model that successfully predicts the biodistribution of mRNA-encoded therapeutics in mice across a range of molecules, doses, and regimens.

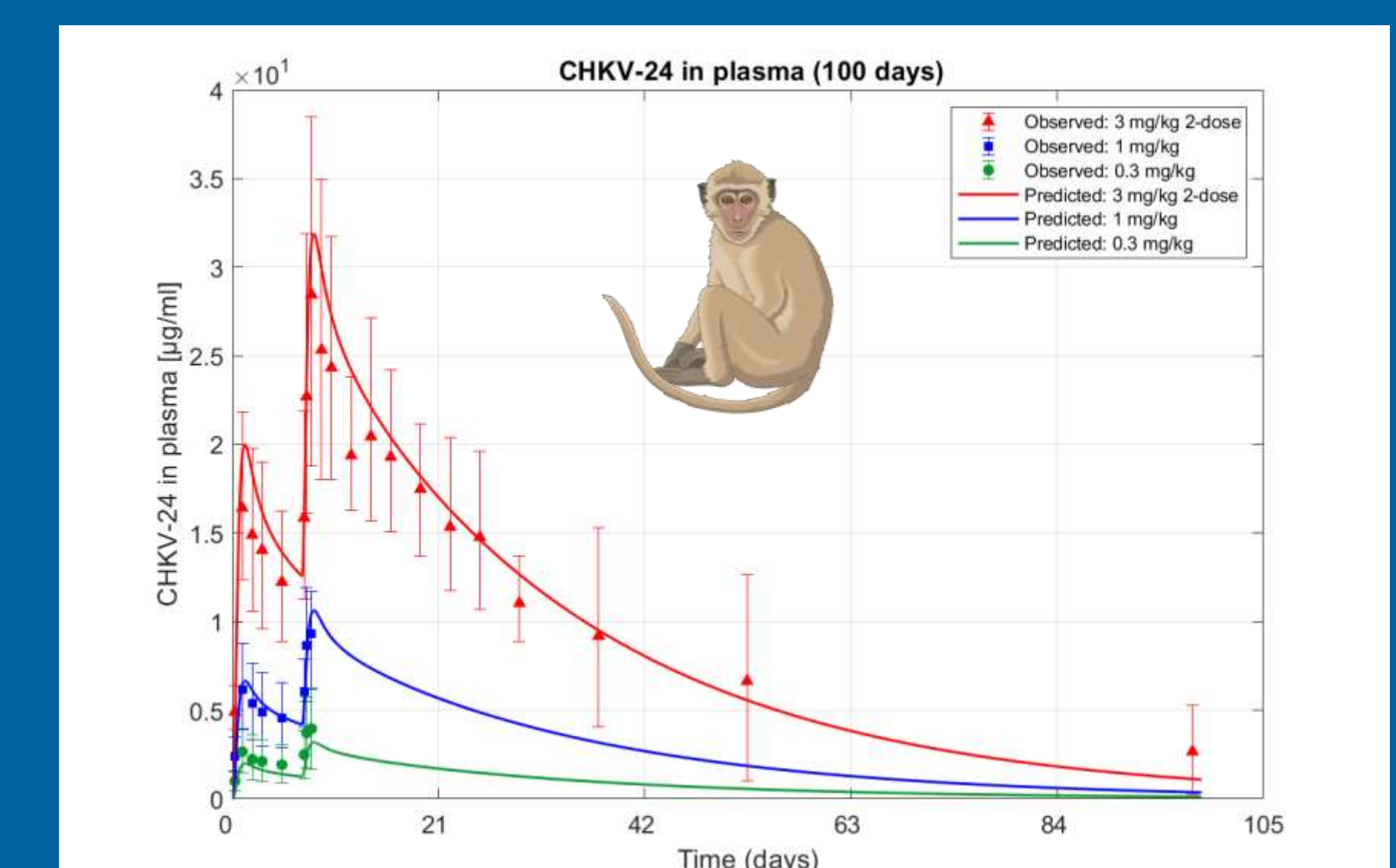
Future work will focus on expanding the model's scope to enhance its translational potential. Key directions include:

- Extension to higher species, such as non-human primates (NHPs), and ultimately humans (preliminary results in Fig. 4);
- Support for alternative administration routes, including subcutaneous and intramuscular delivery;

- Incorporation of organ- and cell-specific tropisms to better reflect targeted therapeutic strategies.

These advancements will enable more accurate predictions in complex biological systems and support the rational design, dosing, and optimization of next-generation mRNA therapeutics.

Fig. 4: Predicted CHKV-24 IgG levels in cynomolgus monkeys after repeated mRNA dosing. Simulations at 0.3, 1, and 3 mg/kg match data from Kose et al. [9], demonstrating cross-species accuracy.



References

- [1] Sepp A, J Pharmacokinet Pharmacodyn, 2019, 46: 339–359.
- [2] Chiş O, Bioinformatics, 2011, 27(18): 2610–2611.
- [3] Huang C, Adv Sci, 2023, 10(3): 2205532.
- [4] Stadler CR, Sci Transl Med, 2024, 16(2720).
- [5] Wu L, J Cancer, 2022, 13(2): 579–588.

- [6] Wu L, Bioengineered, 2021, 12(2): 12383–12393.
- [7] Rybakova Y, Mol Ther, 2019, 27(8): 1415–1423.
- [8] Bähr-Mahmud H, Oncoimmunology, 2023, 12(1).
- [9] Kose N, Sci Immunol, 2019, 4(35).

Contacts

Elio Campanile:
campanile@cosbi.eu



Luca Marchetti:
luca.marchetti@unitn.it