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Introduction

- Staphylococcus aureus*, as a facultative **intracellular pathogen**, is difficult to treat with antibiotics due to **low intracellular exposures**.
- Host-directed therapies** (HDT) instead target the host, but require **(semi-) mechanistic models** to quantify their host effect.

Aim

- Develop a **semi-mechanistic model** that describes the **host-pathogen dynamics** of an intracellular infection.
- Quantify the *in vitro* pharmacodynamics of **vancomycin** and HDT compound **GW296115X** alone, and as a combination against *S. aureus*.

Experimental Method

- HeLa cells were infected by *S. aureus* for one hour and remaining extracellular bacteria were removed with a gentamicin wash (**Figure 1**).
- Infected cells** were treated with a concentration range of vancomycin and GW296115X, and the total bacterial burden was measured by luminescence. ¹

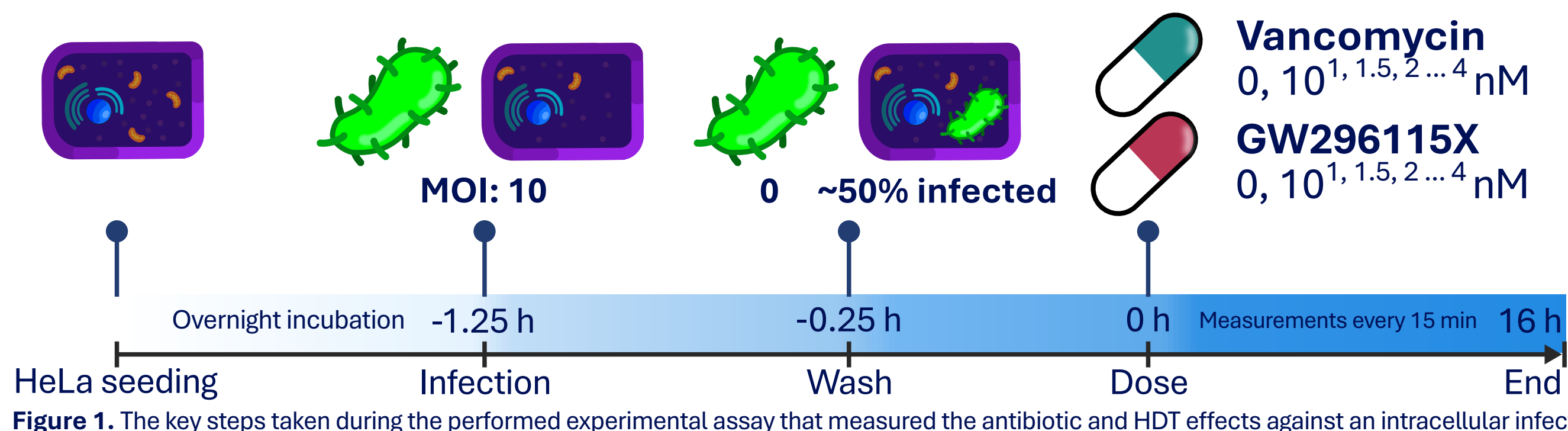


Figure 1. The key steps taken during the performed experimental assay that measured the antibiotic and HDT effects against an intracellular infection.

Modeling Approach

- Different host-pathogen dynamics models were conceptualized (**Figure 2**).^{2,3}

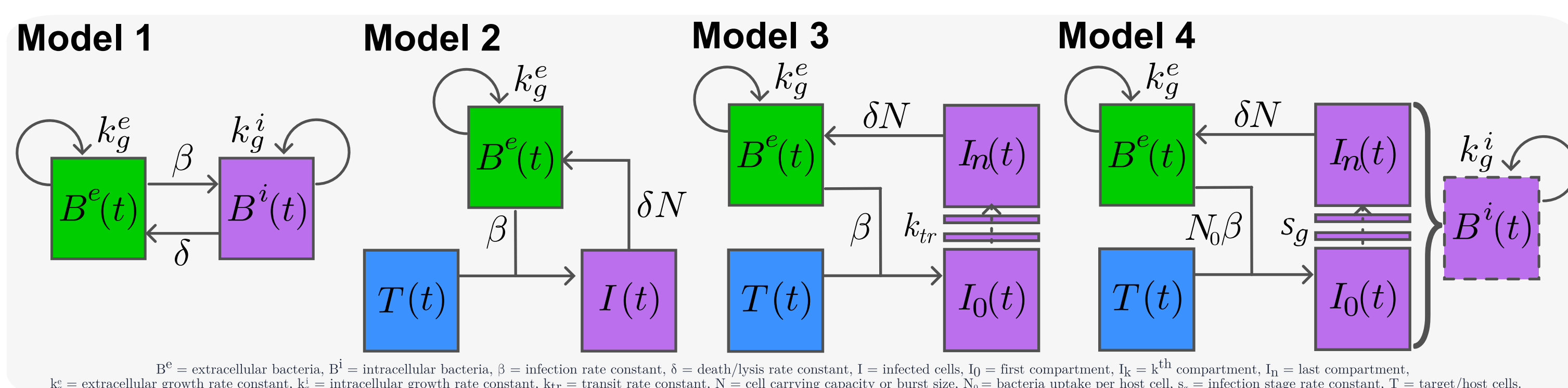


Figure 2. A schematic overview of the structural models. Shown are the included variables for each model and their implementation of host-pathogen interactions.

- The structural models were first simulated with *rxode2* to determine their characteristics.⁴ The *in vitro* data was then modeled in NONMEM.⁵

Attempted model variations included:

- Growth** (exponential, logistic, Gompertz, or time-dependent),
- Effect parameterization** (direct or proportional),
- Concentration-response equations** (linear, Emax, or sigmoid Emax).

- An intracellular growth derived from transit compartment models enabled the quantification of HDT pharmacodynamics (**Figure 3**).

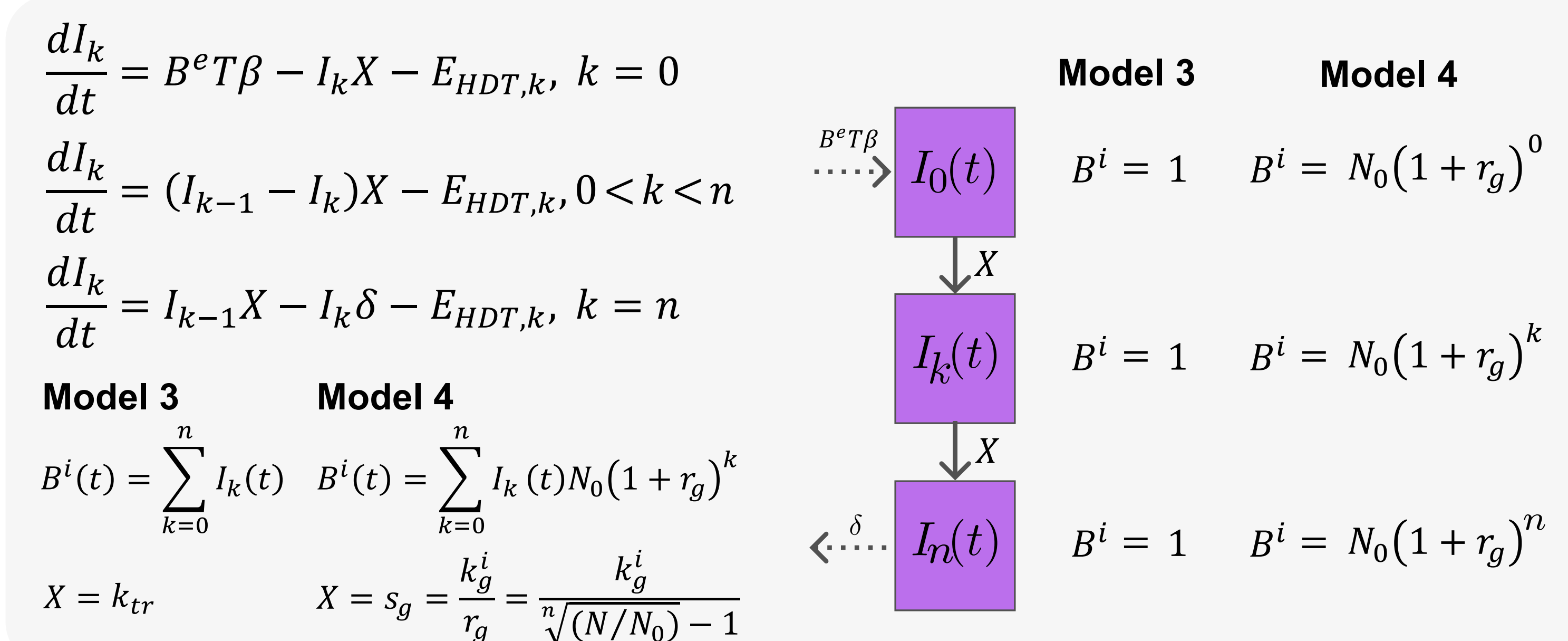


Figure 3. A detailed overview of the transit model structure. Model 4 derived the intracellular growth from an increasing number of bacteria per infected host.

Results

Structural Model Characterization

- From the structural models, only **Model 4** modeled all required components: intra- and extracellular bacteria with growth, and host cells (**Figure 4**).

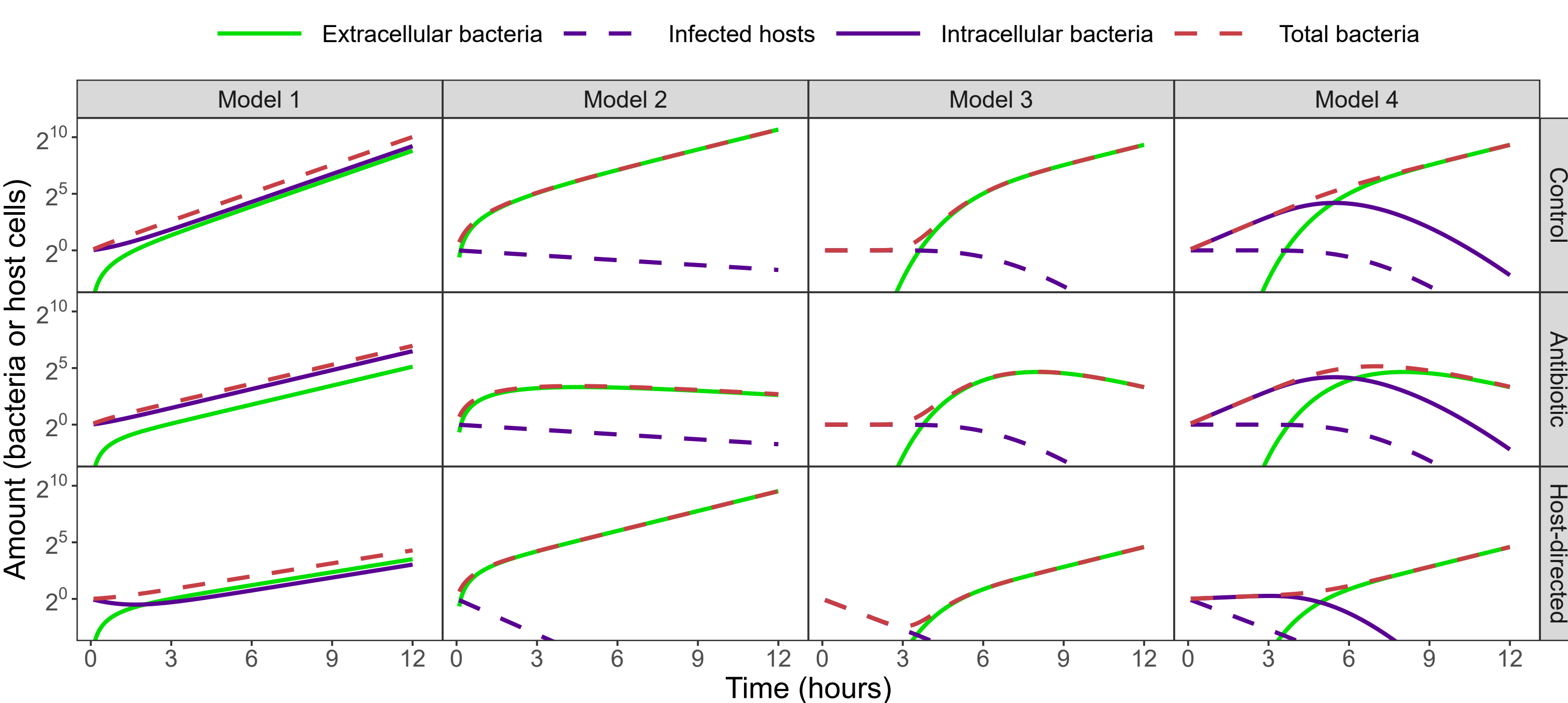


Figure 4. Simulations that were performed with the structural models, showing their description of the bacterial burden over time when starting with one intracellular bacteria or one infected host cell. The resulting changes in the bacterial burden when exposed to an antibiotic or host-directed therapy are compared against the control condition.

Description of Intracellular Growth

- Model 4's intracellular growth reflected **mechanistic bacterial growth** by infected cells transiting across compartments (**Figure 5A-B**).

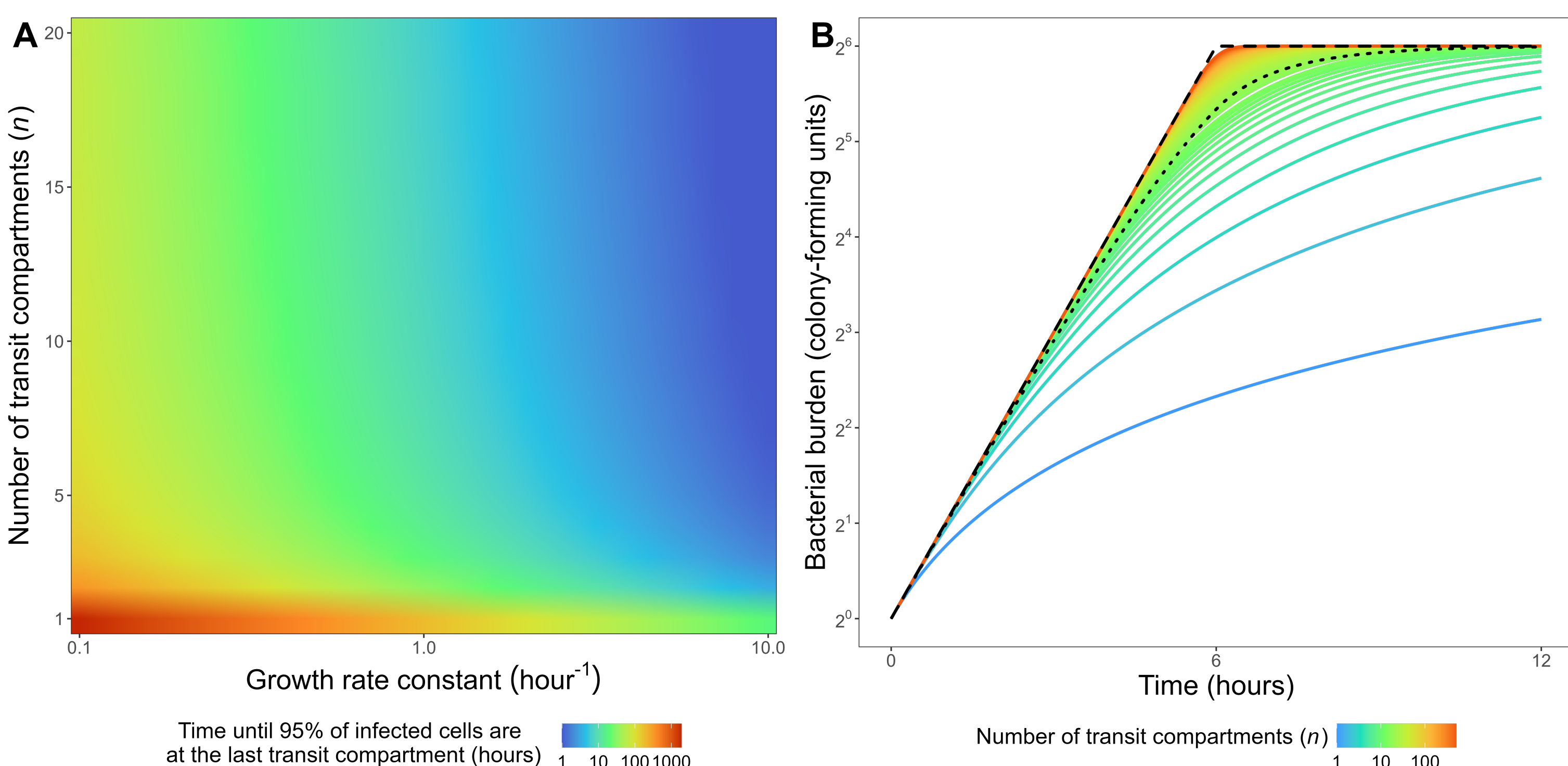


Figure 5. Simulations performed with Model 4, to evaluate its derived description of intracellular growth. (A) Simulations starting with one infected cell at the first compartment with a range of growth rate constants and number of transit compartments. At a low number of transit compartments, the model's behavior in terms of the time until the infected host reached the last compartment (color), was sensitive to changes in the number of compartments. The behavior became consistent after 10 transit compartments. (B) The derived growth curves over time from simulations with one infected cell and one bacterium (color) resembled logistic growth (dotted line) at around 10 transit compartments and exponential growth (dashed line) at a higher number.

Pharmacodynamic Modeling

- The host-pathogen-drug dynamics were described well (**Figure 6A-B**).
- The final model captured the **observed changes** in bacterial burden over time in the control, mono- and combination therapy conditions (**Figure 6C**).

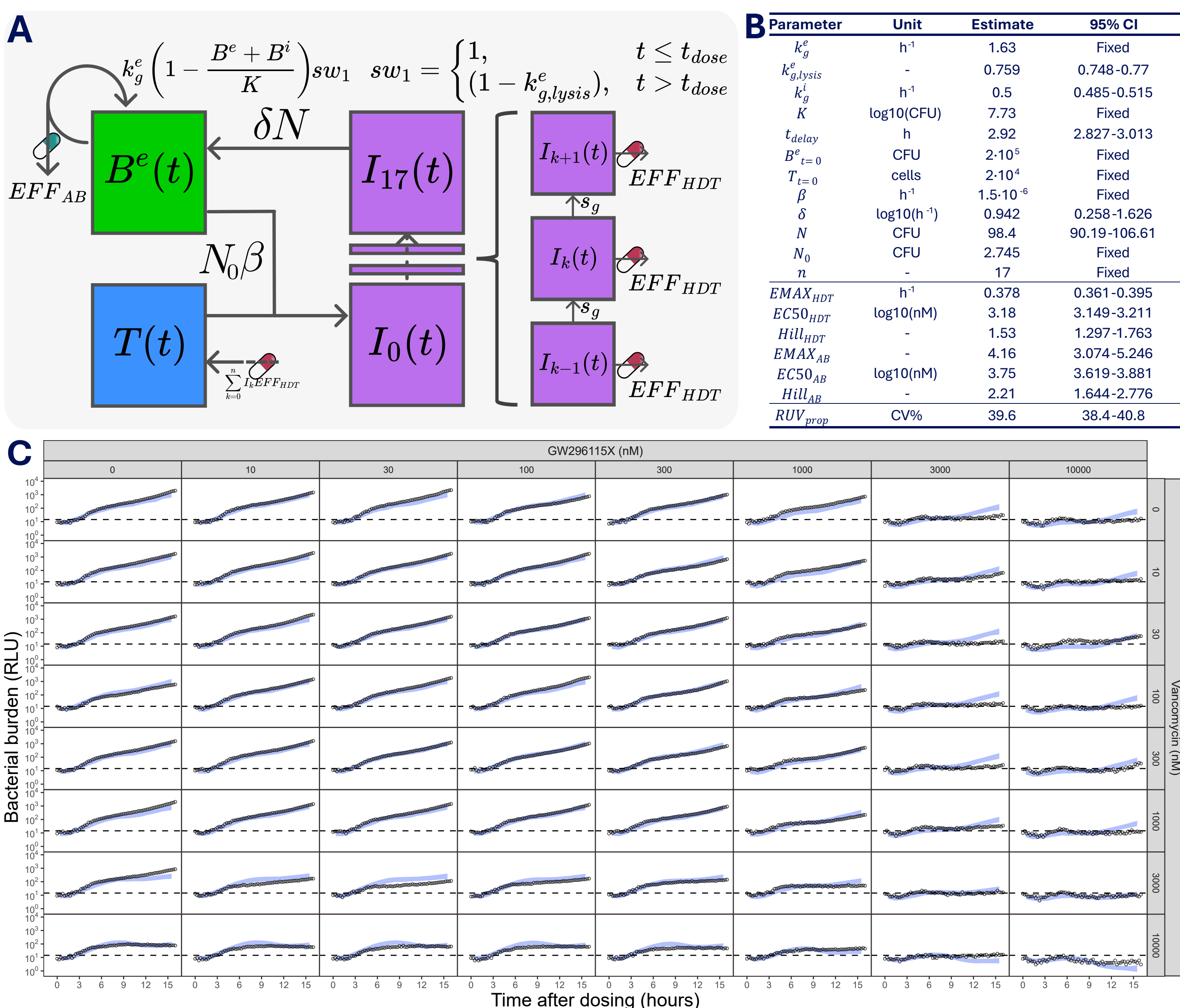


Figure 6. (A) Structure of the final model that best described the data. (B) An overview of key parameter values from the final model. Known parameters from previous experiments were fixed. Estimated parameter values are shown with their 95% confidence intervals (95% CI). (C) Visual predictive check of the final model across different concentrations of the tested compounds. Shown are the observed bacterial burdens over time (open circles) in relative light units (RLU) and the 95% prediction interval of the simulated medians (blue ribbon, $n = 200$). The lower limit of quantification was 14.4 RLU (dashed black line) and modeled with the M3 method.

Conclusions

- A **novel semi-mechanistic model** was developed that described the host-pathogen-drug dynamics of an antibiotic and HDT combination.
- This approach can serve as a **modeling framework for different HDT targets** and intracellular pathogens.