Electronic Supplementary Material (ESI) for Molecular BioSystems

Electronic Supplementary Information

Modeling synergistic drug inhibition of *Mycobacterium tuberculosis* growth in murine macrophages

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S1. Selection of the starting metabolic network model

In order to develop *iNJ*661i, we needed an initial starting representation of *Mycobacterium tuberculosis* metabolism. We selected an initial network among six existing *M. tuberculosis* metabolic network variants[1-4] based on which network best matched gene essentiality data of the bacteria in murine macrophages.

There are two independently derived genome-wide reconstructions of *M. tuberculosis* metabolic networks, GSMN-TB[1] and *iNJ*661.[2] The GSMN-TB network has both *in vitro* and *in vivo* biomass objective functions, where the *in vivo* model is represented as GSMN-TBv. The *iNJ*661 network has been integrated with a human macrophage metabolic network (*iAB-iAMØ*-1410-Mt-661).[3] Furthermore, we have previously extended the *iNJ*661 network by adding reactions and metabolites to form *iNJ*661m and further extensively revised this network to a murine *in vivo*-compatible network, *iNJ*661v.[4]

We used all of the above network models to predict gene essentiality and compared the predicted essentiality with experimental data of *M. tuberculosis* in murine macrophages.[5] Table S1 shows the comparison results. Among these networks, the *iNJ*661v network generated the overall highest agreement between the predicted and experimental essentiality. Therefore, we chose the *iNJ*661v network as a starting point for further development.

Table S1. Comparison of predicted and *ex vivo* experimental gene essentiality using different networks.

A true positive (TP) prediction refers to a gene correctly predicted to be essential, whereas a false negative (FN) prediction refers to a gene incorrectly predicted to be non-essential. A false positive (FP) prediction refers to a gene incorrectly predicted to be essential, whereas a true negative (TN) prediction refers to a gene correctly predicted to be non-essential. Sensitivity = TP/(TP + FN). Specificity = TN/(TN + FP). Matthews correlation coefficient (MCC) = $(TP \times TN - FP \times FN)/[(TP + FP)(TP + FN)(TN + FP)(TN + FN)]^{½}$. The MCC ranges in values from +1 to –1, with +1 indicating a perfect prediction, 0 indicating a random prediction, and –1 indicating an inverse prediction.

Network	The numbers of genes				Sensitivity	Specificity	MCC
	TP	FN	FP	TN			
GSMN-TB	1	7	63	344	0.125	0.845	-0.011
GSMN-TBv	2	6	86	321	0.250	0.789	0.013
*iNJ*661	1	9	79	276	0.100	0.777	-0.048
iAB-iAMØ-1410-Mt-661	0	10	60	295	0.000	0.831	-0.074
*iNJ*661m	1	9	79	277	0.100	0.778	-0.048
*iNJ*661v	5	5	66	290	0.500	0.815	0.130

S2. The procedure used to determine parameter values and reproduce experimental cell concentrations of *Mycobacterium tuberculosis*

In our calculations, we adjusted parameter values in order to match the predicted *M. tuberculosis* cell concentrations with experimental values under eight conditions. The simulation for each condition was used to estimate the value of one or two parameters under a specific set of condition, which allowed us to build up three models and the corresponding parameters. A brief technical description of this procedure was included in Table 3. Here, we provided a more detailed description of this procedure.

In the simulation for *Condition 1*, we adjusted the upper limit of the glycerol uptake U_{Glyc} and the end time of the lag stage τ to obtain maximum agreement between the simulated and experimental cell concentrations of the *Δicl1Δicl2* deletion mutant. Thus, for each set of tested values for U_{Glyc} and τ, we obtained the simulated cell concentrations by calculating the biomass production rate via a flux balance analysis (FBA) of the metabolic network and used this rate to estimate the corresponding cell concentrations through our population growth model. In the FBA of the metabolic network, we placed no constraint on the uptake of fatty acids but blocked the fluxes through the isocitrate lyase (ICL) and methylisocitrate lyase (MCL) reactions to mimic the effect of the *Δicl1Δicl2* deletion mutant.

In the simulation for *Condition 2*, we adjusted the upper limits of fatty acid uptake U_{Fat} and the end time of the lag stage τ until we obtained maximum agreement between the simulated

and experimentally determined wild-type cell concentrations. Similar to the simulation for *Condition 1*, we obtained the simulated cell concentrations by using the metabolic network and the population growth model. In the FBA of the metabolic network commensurate with the wild-type bacterium, we placed no constraint on the ICL and MCL reactions but constrained the glycerol uptake using the upper limit U_{Glyc} obtained in the simulation for *Condition 1*.

In the simulation for *Condition 3*, we adjusted the end time of the lag stage τ until we obtained maximum agreement between the simulated and experimentally determined wild-type cell concentrations. Similar to the simulation for *Condition 2*, we obtained the simulated cell concentrations by using the metabolic network and the population growth model. In the FBA of the metabolic network, we placed no constraint on the ICL and MCL reactions but constrained the uptakes of glycerol and fatty acids using the upper limits U_{Glyc} and U_{Fat} calculated in the simulations for *Condition 1* and *Condition 2*, respectively.

In the simulations for *Conditions 4-7*, we adjusted the intracellular 3-nitropropionate (3-NP) concentration $[3\text{-}NP]_i$ and the end time of the lag stage τ until we obtained maximum agreement between the simulated and experimental cell concentrations in the presence of the 3-NP inhibitor. For each set of tested values for $[3\text{-}NP]_i$ and τ, we first used our inhibition model to determine the constraints to be placed on the ICL and MCL reaction fluxes. By using these constraints and simultaneously constraining the glycerol and fatty acid uptakes (using U_{Glyc} and U_{Fat} from the simulations for *Condition 1* and *Condition 2*, respectively), we

then calculated the biomass production rate via the FBA of the metabolic network. Finally, we used this rate to estimate the corresponding cell concentrations through our population growth model.

In the simulation for *Condition 8*, we again adjusted the end time of the lag stage τ to obtain maximum agreement between the simulated and experimentally determined wild-type cell concentrations. Similar to the simulations for *Condition 3*, we obtained the simulated cell concentrations by using the metabolic network and the population growth model. In the FBA of the metabolic network, we placed no constraint on the ICL and MCL reactions but constrained the uptakes of glycerol and fatty acids using U_{Glyc} and U_{Fat} from the simulations for *Condition 1* and *Condition 2*, respectively.

S3. Derivation of the effective 3-nitropropionate concentration in macrophages

Cellular pharmacodynamics (PD) and pharmacokinetics (PK) aim at describing how drug molecules accumulate in different compartment of the human body, including host cells, and how the body responds to the drug.[6] Here, we were interested in describing how inhibitor molecules penetrate into the macrophage cytosol from the surrounding *in vitro* medium and then enter the phagosome compartments, where *M. tuberculosis* accumulates during the initial stages of infection.[6] Specifically, we studied the relation between the 3-NP concentrations in medium ($[3\text{-}NP]_e$) and the concentrations inside the macrophage phagosomes ($[3\text{-}NP]_i$) by analyzing the mechanisms that drive inhibitor molecules to exchange between the medium and cytosol and between the cytosol and phagosomes.

Medium to cytosol (influx)

The influx of an inhibitor can occur via three basic mechanisms: diffusion across the macrophage membrane, endocytotic processes where the macrophage cells engulf inhibitor molecules, or through inward transport via particular macrophage membrane-bound protein complexes.[6] Based on the prevalence of passive diffusion in small-molecule transport across cell membranes and the lack of evidence for any other mechanism, we assumed that the low-molecular-weight 3-NP molecule entered the macrophage cytosol through passive diffusion.

Because solvated 3-NP has an electric charge (z) = –1, we used the following Nernst-Planck

equation[7] to describe the diffusion flux (J_d) as a function of 3-NP concentrations in the medium ([3-*NP*]$_e$) and the 3-NP concentration in the macrophage cytosol ([3-*NP*]$_c$):

$$J_d = P_d \frac{N}{e^N - 1}([\text{3-}NP]_e - [\text{3-}NP]_c e^N) \quad \text{(S1)}$$

where P_d represents the permeability of the membrane, $N = zEF/(RT)$, E is the membrane potential, F denotes the Faraday constant, R denotes the universal gas constant, and T is the absolute temperature.

Medium to cytosol (efflux)

When considering the efflux of an inhibitor, eukaryotic cells usually have active efflux mechanisms to transport intracellular molecules out of the cells.[8] Hence, we assumed that once the 3-NP concentration in the macrophage cytosol ([3-*NP*]$_c$) was higher than a threshold concentration (C_T), macrophage cells would activate a 3-NP efflux mechanism to flush out 3-NP. The efflux (J_e) then depended on [3-*NP*]$_c$, as follows:

$$J_e = \begin{cases} 0, & [\text{3-}NP]_c < C_T \\ P_e([\text{3-}NP]_c - C_T), & [\text{3-}NP]_c \geq C_T \end{cases} \quad \text{(S2)}$$

where P_e represents the rate constant of the efflux. The threshold mechanism is present in many biological systems. For example, *Escherichia coli* degrades 3-phenylpropionic acid (3-PPA) in the medium only when the 3-PPA concentration is above a threshold of ~3 mg/l.[9]

Equilibrium between medium-to-cytosol influx and efflux

Based on the experimental observation that the concentration of penicillin in the medium and in macrophages reaches equilibrium within hours,[10] we assumed that the 3-NP concentrations in the medium and in the cytosol were also in a state of equilibrium. Thus, the influx of 3-NP

molecules was assumed to be equal to their efflux:

$$J_d = J_e \tag{S3}$$

By replacing J_d and J_e in *Eq. S3* with the expressions of *Eqs. S1* and *S2*, respectively, we obtained the following relation between the 3-NP concentration in the cytosol ([3-*NP*]$_c$) and in the medium ([3-*NP*]$_e$):

$$[3-NP]_c = \begin{cases} A_c[3\text{-}NP]_e, & [3\text{-}NP]_e < H_c \\ K_c[3\text{-}NP]_e + B_c, & [3\text{-}NP]_e \geq H_c \end{cases} \tag{S4}$$

where

$$A_c = \frac{1}{e^N} \tag{S5}$$

$$H_c = C_T e^N \tag{S6}$$

$$K_c = \frac{\frac{P_d N}{e^N - 1}}{P_e + \frac{P_d N e^N}{e^N - 1}} \tag{S7}$$

$$B_c = \frac{P_e}{P_e + \frac{P_d N e^N}{e^N - 1}} C_T \tag{S8}$$

Cytosol to phagosome

We assumed that transport between the cytosol and phagosome was based on similar transport mechanisms between the cytosol and medium. Thus, the relation between 3-NP concentrations in the phagosome ([3-*NP*]$_i$) and concentrations in the cytosol ([3-*NP*]$_c$) can be written similarly to *Eq. S4*, as follows:

$$[3-NP]_i = \begin{cases} A_i[3\text{-}NP]_c, & [3\text{-}NP]_c < H_i \\ K_i[3\text{-}NP]_c + B_i, & [3\text{-}NP]_c \geq H_i \end{cases} \tag{S9}$$

In *Eq. S9*, A_i, K_i, B_i, and H_i are defined analogously as in *Eqs. S5-S8*.

3-NP concentrations in the medium vs. in the phagosome

Finally, we replaced $[3\text{-}NP]_c$ in *Eq. S9* with the right side of *Eq. S4* and obtained the following relation between 3-NP concentrations in the phagosome ($[3\text{-}NP]_i$) and in the medium ($[3\text{-}NP]_e$) under different conditions:

$$[3\text{-}NP]_i = A_i A_c [3\text{-}NP]_e, \qquad [3\text{-}NP]_e < H_c \,\&\, [3\text{-}NP]_e < \frac{H_i}{A_c} \tag{S10}$$

$$[3\text{-}NP]_i = K_i A_c [3\text{-}NP]_e + B_i, \qquad [3\text{-}NP]_e < H_c \,\&\, [3\text{-}NP]_e \geq \frac{H_i}{A_c} \tag{S11}$$

$$[3\text{-}NP]_i = A_i K_c [3\text{-}NP]_e + A_i B_c, \qquad [3\text{-}NP]_e \geq H_c \,\&\, [3\text{-}NP]_e < \frac{H_i - B_c}{K_c} \tag{S12}$$

$$[3\text{-}NP]_i = K_i K_c [3\text{-}NP]_e + (K_i B_c + B_i), \qquad [3\text{-}NP]_e \geq H_c \,\&\, [3\text{-}NP]_e \geq \frac{H_i - B_c}{K_c} \tag{S13}$$

Because *Eqs. S10-S13* all showed linear relations between $[3\text{-}NP]_i$ and $[3\text{-}NP]_e$, we deduced that a linear relation exists between $[3\text{-}NP]_i$ and $[3\text{-}NP]_e$, as follows:

$$[3\text{-}NP]_i = K[3\text{-}NP]_e + B \tag{S14}$$

where K and B are constants independent of extracellular 3-NP concentration $[3\text{-}NP]_e$. This analysis provided an independent theoretical rationale and helped validate the observed linear concentration relationship between $[3\text{-}NP]_i$ and $[3\text{-}NP]_e$ in the growth inhibition simulations of *M. tuberculosis*.

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