Mathematical model

Our ordinary differential equation model is presented below in non-dimensional form. The model describes the dynamics of two populations of bacteria, those containing the luminescent plasmid (n^+) and those who have lost the plasmid (n^-). The two populations grow inside the tumor environment and consume a substrate (S) which is in limited supply.

$$\frac{dn^+}{dt} = (1-\tau)\mu^+ n^+ - \gamma_+ n_+ \quad (1)$$

$$\frac{dn^-}{dt} = \mu^- n^- + \tau\mu^+ n^+ - \gamma_- n_- \quad (2)$$

$$\frac{dS}{dt} = -[\frac{\mu^- n^-}{A^-} - \frac{\mu^+ n^+}{A^+}][\frac{1}{1+q\cdot(n_- + n_+)}] \quad (3)$$

$$\mu^+ = \frac{\mu^+_{max} S}{K+S} \qquad \mu^- = \frac{\mu^-_{max} S}{K+S} \quad (4)$$

The parameters above are τ, the rate at which cells lose plasmid, μ^+_{max} and μ^-_{max}, the maximal growth of plasmid and non-plasmid containing cells respectively. K, the Michaelis-saturation constant of growth rate, A^+ and A^-, the depletion rate constants of substrate S by μ^+ and μ^- cells. γ_+ and γ_-, the death rates of the bacteria, and q, the rate at which tumor substrate depletion is limited by the maximal amount of cells. This last term is to account for the fact that a limited number of bacteria (those on the outward growing rim) can contribute to the decay of the tumor substrate. The model is non-dimensionalized by hours, 1 bacteria, and a substrate concentration of μM.

The total IVIS signal is reflective of the number of luciferase enzymes and hence the number of actively expressing luciferase bacteria. As an approximation to this signal, we modeled the signal to be proportional to the number of plasmid containing cells (dominant contribution to the IVIS signal) minus the first-order decay of the luciferase enzyme. We assume that each bacterium containing a plasmid contributes equally to the IVIS signal although there is likely variability due to the distribution of plasmids per cell in a population as well as contributions from non-plasmid containing bacteria where luciferase is not yet significantly diluted. Another approximation in the IVIS signal arises in that plasmid-containing bacteria initially populate the tumor core and express luciferase, but as bacteria grow radially to a larger population, less nutrients are left for the bacteria in the center to express any of the luxCDABE genes (either luciferase or luciferin substrates), which are the main contributors to the IVIS signal. This results in colony counts reaching a nearly steady-state as a function of time but expression level plateauing, causing an decay in IVIS signal due luciferase instability. We have previously modeled this scenario with a spatial model and a density dependent protein production term [1].

The rate of change of the luciferase enzymes is given by:

$$\frac{dL}{dt} = Bn_+\mu_+(1-\tau-\gamma_+/\mu_+) - \gamma_L L$$

where B represents the number of rate of expression of luciferase per cell and γ_L represents the luciferase decay. The IVIS signal (I) is proportional to the luciferase signal, i.e., I=κL, which scales parameters B and γ_L accordingly. The value of κ contains physical properties

such as the permitivity of skin to luciferase enzymes and number of photons emitted per enzyme. The lumped parameters chosen for the data presented in Figure 4 are: $\tau = 0.2, \kappa B = 10, A^+ = A^- = 0.01, K = 1000, \kappa\gamma_L = 0.3, \gamma_- = 0.001, \gamma_+ = 0.075, q = 1.6$ with initial conditions $n_+ = 3365, n_- = 0, I = 0, S = 1000$. Supplementary Figure 2 shows the substrate (all of Eq 3), growth rate for plasmid containing cells (Eq 4, teal), and substrate limitation function (right bracket Eq 3, green) as a function of time.

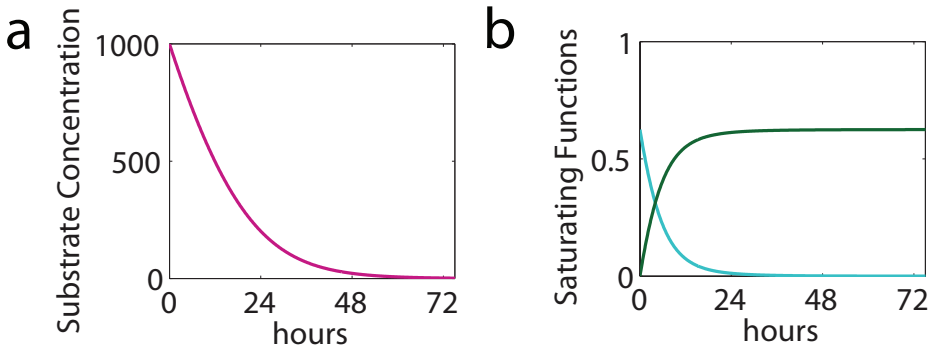

Figure 1: **a** Substrate concentration as a function of time for Figure4. **b** Saturating functions for the model. Green is the consumption of tumor substrate as a function of time. Teal is the growth rate of plasmid-containing cells as a function of time.

Model equations and parameter sets. Initially, an ODE system for plasmid loss was used that is similar to previous models [2]. One difference that arises in the tumor environment are that nutrients are spatially limited, hence consumption of tumor substrate is restricted to a certain number of bacteria. We accounted for this by limiting the substrate decay by total number of bacteria present. Several of the parameters are unknown, thus parameters were chosen to fit the bacterial population curves (Fig 2B) with growth rates and a loss rate close to experimental values. Then IVIS trajectories were generated using these fits. Quantitatively similar fits can be obtained by setting the growth rates equal and modifying the plasmid loss rate. Plasmid loss rate and a growth rate advantage of non-plasmid containing cells can compensate for one another, though experimentally we observe that non-plasmid containing cells grow faster in the tumor. This difference in growth rate may arise from the fact that newly formed non plasmid containing bacteria will occupy a larger percentage of cells on the growing front, allowing them to be in a more nutrient-available environment and grow faster as a population. This is also why we made the choice for non-plasmid containing cells degrading at a lower rate than the plasmid-containing ones.

References

[1] Danino, T., Mondragón-Palomino, O., Tsimring, L. & Hasty, J. A synchronized quorum of genetic clocks. *Nature* **463**, 326–330 (2010, PMID: 20090747).

[2] Ganusov, V., Bril kov, A. & Pechurkin, N. Mathematical modeling of population dynamics of unstable plasmid-bearing bacterial strains under continuous cultivation in a chemostat. *BIOPHYSICS-PERGAMON THEN MAIK NAUKA-C/C OF BIOFIZIKA* **45**, 881–887 (2000).

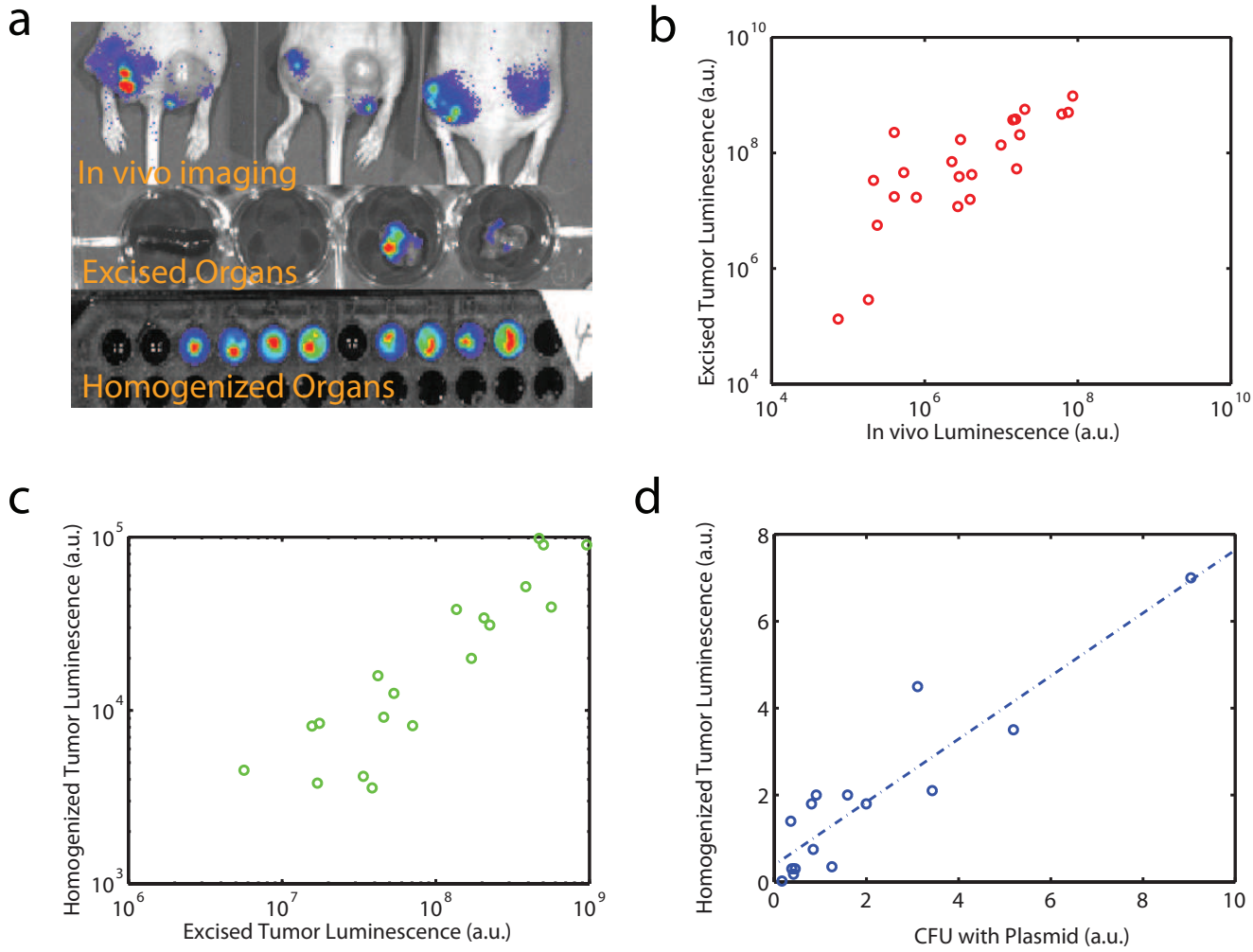

Figure 2: Correlations between in vivo and ex vivo imaging of tumors. High correlations are observed for each step of the process.

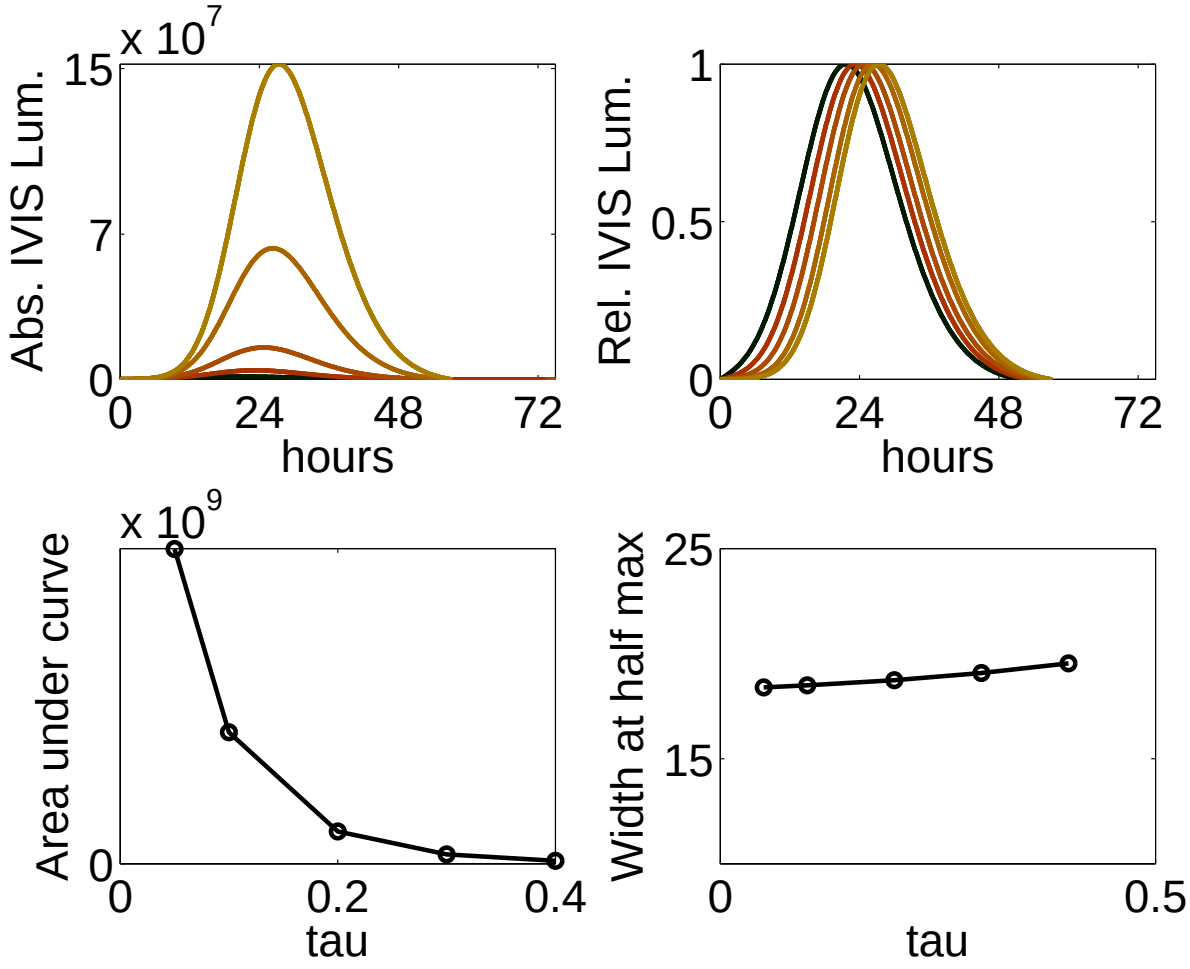

Figure 3: Effect of increasing τ on gene expression dynamics.