Supplementary information

Mathematically modeling the sequential application of a cytotoxic nanoparticle and a PI3K-inhibitor enhances anti-tumor efficacy

Ambarish Pandey[1, 5], Ashish Kulkarni[1†], Bhaskar Roy[1†], Aaron Goldman[1], Sasmit Sarangi[1], Poulomi Sengupta[1], Colin Phipps[2,3], Jawahar Kopparam[1], Michael Oh[1], Sudipta Basu[1], Mohammad Kohandel[2,4], Shiladitya Sengupta[1*].

[1]Laboratory for Nanomedicine, Division of Biomedical Engineering, Brigham and Women's Hospital, Department of Medicine, Harvard Medical School. [2]Department of Applied Mathematics, University of Waterloo, Waterloo, ON, N2L 3G1, Canada. [3]Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, ON, N2L 3G1, Canada. [4]Center for Mathematical Medicine, Fields Institute for Research in Mathematical Sciences, Toronto, ON, M5T 3J1, Canada. [5]Current Affiliation: Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas.

Mathematical Model

Protein expression: We denote the dimensionless concentrations of the three proteins of interest by $P=[\mathrm{pAkt}]/P_0$, $C=[\mathrm{Casp3}]/C_0$, $X=[\mathrm{XIAP}]/X_0$ where P_0, C_0 and X_0 are experiment-dependent scaling factors. Since the intracellular concentration of total protein remains approximately constant between experiments, we assume that concentration for each protein, say [Y] (mol/L), is given by $[\mathrm{Y}]=Y_m\gamma[\mathrm{Z}]/W_Y$, where Y_m is the measured intensity (normalized to actin intensity) ascertained from western blot experiments, γ is the percentage of actin to total protein, total intercellular protein concentration is denoted by [Z] (gr/L), and W_Y is the molecular weight of the protein Y (gr/mol). The system of equations corresponding to the network shown in **Fig. 1a**, described in more detail in the Quick Guide to Equations and Assumptions, is given by

$$\frac{dP}{dt}=\frac{k_p+\lambda_p f_p(t)}{1+\alpha_p C+\gamma_p g(t)}-\delta_p P,$$

$$\frac{dC}{dt}=\frac{k_c+\lambda_c f_c(t)}{1+\alpha_c P}-\beta_c XC-\delta_c C,$$

$$\frac{dX}{dt}=k_x+\lambda_x PX-\beta_x CX-\delta_x X,$$

where the concentration of PI828 is denoted by $g(t)$ and the effects of cisplatin on pAkt and Casp3 production are denoted by $f_p(t)$ and $f_c(t)$ respectively. The models for these treatment effects will be addressed below.

Treatment effects: Since PI828 is not administered in a delivery vehicle, we assume simple decay for their effect on pAkt expression giving

$$\frac{dg}{dt} = -d_g g(t).$$

The treatment effects of cisplatin on pAkt and Casp3 are modeled by

$$\frac{df_p}{dt} = r_c c_i(t - T_i) - d_p f_p(t),$$
$$\frac{df_c}{dt} = r_c c_i(t - T_i) - d_c f_c(t),$$

where $c_i(t)$ is the intracellular cisplatin concentration which will be considered in the next section. In the interest of parameter reduction, we assume that the effect of the drug has the same activation constant r_c and time delay T_i, after the release of cisplatin nanoparticles, that accounts for intracellular diffusion to active sites and the delayed impact of this cisplatin on the apoptosis pathway, attributed to the translational and transcriptional events that occur prior to changes in the expression of the proteins of interest. However, to facilitate the fitting to follow this model development, the delay in the equations above is not a strict time delay. Instead, a linear chain of reactions is used where the delay of a signal is approximated by *N* steps with equal reaction rates *k* [1]. Thus, the value of T_i is not explicitly included in parameter fitting and can be shown to have mean $\bar{T} = N/k$ standard deviation $\sigma_T = \bar{T}/\sqrt{N}$ [2]. The number of intermediary reactions, *N*, which is suggested to be set somewhere between 5 and 15, is set to 10 here since anything larger would promote stiffness in the system and impede the proficiency of the fitting algorithms. The treatment effects do exhibit different decay rates d_p and d_c since the cisplatin-induced production of pAkt is sustained longer than the increased production of caspase (i.e. $d_p < d_c$).

Cisplatin release from nanoparticles: To determine the rate of cisplatin release from the nanoparticle nanoparticles, release experiments were performed over 120 hours in acidic and neutral conditions. In an acidic environment these release profiles can be accurately determined by a one-parameter exponential release profile. We can motivate the functional form by considering a simple ordinary differential equation (ODE) that includes both the constant and sustained release of drug due to encapsulation in the nanoparticle. To facilitate this, we assume that the administered nanoparticles initially consists of a fixed fraction of cisplatin, θ_u, that will be subsequently released upon administration at rate r_u and a fixed fraction of cisplatin, $\theta_i = 1 - \theta_u$, that remains inert and is omitted from the modelling that follows. The fraction of the total amount of contained cisplatin that is released depends primarily on the acidity of the environment. In an acidic environment, just over 60% of the cisplatin is eventually released from the nanoparticle, as opposed to the approximately 40% that is released in a neutral environment. This helps to preferentially target tissues exhibiting an acidic microenvironment as found in solid tumors. The concentration of the cisplatin that is released from the nanoparticle is denoted by $c(t)$ while the unreleased cisplatin concentration remains to be released is denoted by $u(t)$. The initial conditions are $u(t_0) = \theta_u c_T$ and $c(t_0) = 0$ where t_0 is the time of administration and c_T is the administered dosage (μM). The system of ODEs is then:

$$\begin{aligned} \frac{du}{dt} &= -r_u u(t), \\ \frac{dc}{dt} &= r_u u(t), \end{aligned} \qquad (1S)$$

with the solution of interest being: $c(t)$.

The model given above for the release profile experiments must be extended for the in vitro experiments where expression of proteins are measured. The previous model will now correspond to extracellular concentrations of cisplatin (denoted with subscript *e*) that will now be transported inside the cell where the intracellular concentration (denoted with subscript *i*) can then have an effect on the protein pathways. To facilitate this we add a term to these extracellular concentrations to include the mass transport of cisplatin into the cells, a process with some rate consant r_c and time delay T_e. This time delay

accounts for the effects of extracellular diffusion and transmembrane transport. In the interest of avoiding extraneous parameters, and since the computational process to include delay parameters introduces significant stiffness, we will make the assumption that this transport delay (and the rate constant) is the same for nanoparticles and free cisplatin. Once again we assume a linear chain of reactions to obtain a distributed delay for the data fitting procedure. These drug-related reactions are shown schematically in **Fig. 1S.** This leads to the extended system:

$$\frac{du_e}{dt} = -r_u u_e(t) - \varphi_c u_e(t),$$
$$\frac{dc_e}{dt} = r_u u_e(t) - \varphi_c c_e(t),$$
$$\frac{du_i}{dt} = -r_u u_i(t) + \varphi_c u_e(t - T_e),$$
$$\frac{dc_i}{dt} = r_u u_i(t) + \varphi_c c_e(t - T_e).$$

Cell Viability: With the model for protein expression and therapeutic concentrations established, we now propose a model to predict cell viability. Making the simple assumptions that cell survival is proportional to pAkt expression and cell death is proportional to Caspase-3 expression proves to be a sufficient model for this prediction (see **Fig. 1b**). The resulting differential equation for cell viability is then given by

$$\frac{dN}{dt} = [\lambda_N P - \delta_N C] N.$$

Data Fitting

Software and methodology: All of the parameter estimation to follow was performed using the Matlab toolbox PottersWheel (http://www.potterswheel.de). This toolbox is capable of loading multiple experimental data files and then estimating local and global parameters in the ODE model [2]. All parameters were not determined at once. The data fitting process was done step-by-step as shown schematically in **Fig. 2S**. While the best fit for release parameters is likely the unique global minimum of the chi-square error, those determined for protein expression are not guaranteed to be a global minimum due to the lack of experimental estimates of most of the parameters (aside from the decay

rates) and the sheer number of parameters to be estimated. The cell viability parameters are likely the best estimate based on the given set of protein expression parameters.

Cisplatin release: Fitting the parameters to the release profile data yields a suitable fit for the acidic environment (*χ2=0.54,N=15,p=2*) and a mediocre one for the neutral environment (*χ2=1.66,N=15,p=2*), see **Fig. 3S** for these profiles. The release parameters for these fits are given in **Table 1S.** Only those determined for acidic environments are utilized in the calculations to follow so we will not present a detailed model for neutral release properties; however, the release profile for a neutral environment could be suitably fit by the addition of a single parameter that accounts for liposomal erosion (*χ2=0.61,N=15,p=3*); this would have negligible effect on the acidic release (*χ2=0.58,N=15,p=3*).

Table 1S: Release parameters

Parameter	Neutral	Acidic
r_u	0.0471 [1/h]	0.0171 [1/h]
θ_u	0.499	0.614

Protein expression: The data from the *in vitro* protein expression experiments are used to determine the parameters in the model above; these parameters are given in **Table 2S**. The only parameters present in the literature are those that determine decay rates of the proteins [3]. Each experiment is considered to have a scaling factor for each protein which are given in **Table 3S**. This takes into account that the value used to non-dimensionalize the protein concentrations is different for each experiment.

Table 2S: Protein expression parameters

Parameter	Value	Parameter	Value	Parameter	Value
k_p	1.86 [1/h]	α_p	0.13	d_g	0.001* [1/h]
k_c	11.56 [1/h]	α_c	0.56	γ_p	6.42 [1/μM]
k_x	0.29 [1/h]	λ_x	0.01 [1/h]	φ_c	0.26 [1/h]

δ_p	0.36 [1/h]	β_c	2.30 [1/h]	r_c	0.47 [1/h]
δ_c	0.48 [1/h]	β_x	0.040 [1/h]	d_p	0.030 [1/h]
δ_x	0.57 [1/h]	λ_p	0.34 [1/h·nM]	d_c	0.28 [1/h]
		λ_c	11.9 [1/h·nM]		

* Parameter reached minimum allowable value. Indicates PI828 decay is not detected in experiments on this time scale. This is evident from the continued inhibition exhibited in **Fig. 5D**.

Table 3S: Scaling factors

Figure(s)	P_0	C_0	X_0
Figure 5a-c (3μM)	0.514	1.18	1.06
Figure 5a-c (5μM)	0.381	1.36	0.770
Figure 5d	0.812	1.32	0.1*
Figure e-f (Post)	0.510	0.834	1.29
Figure e-f (Control)	0.511	0.529	1.29

Cell viability: Finally, with the release parameters and protein expression parameters approximated from the data, we can introduce two parameters that predict cell viability. For this step of the fitting we must have the same scaling parameters for each experiment. Fitting to the existing cell viability data gives the parameters in **Table 4S**. Using this model, the cell viability associated with other treatment combinations can be determined as shown in **Figs 5a-b**.

Table 4S: Cell viability parameters

Parameter	Value
λ_N	$2.7\text{x}10^{-3}$ [1/h]
δ_N	$1.5\text{x}10^{-3}$ [1/h]

References

1. MacDonald, N., Time delay in simple chemostat models. *Biotechnol Bioeng* **1976**, *165* (6), 805-812.
2. Maiwald, T.; Timmer, J., Dynamical modeling and multi-experiment fitting with PottersWheel. *Bioinformatics* **2008**, *24* (18), 2037-2043.
3. Eissing, T.; Conzelmann, H.; Gilles, E. D.; Allgower, F.; Bullinger, E.; Scheurich, P., Bistability analyses of a caspase activation model for receptor-induced apoptosis. *The Journal of biological chemistry* **2004,** *279* (35), 36892-7.

Fig. 1S: Depiction of drug model for nanoparticle release and delayed transmembrane transport of self-assembling cis-platinum nanoparticles (SACNs) and free cisplatin in an acidic environment.

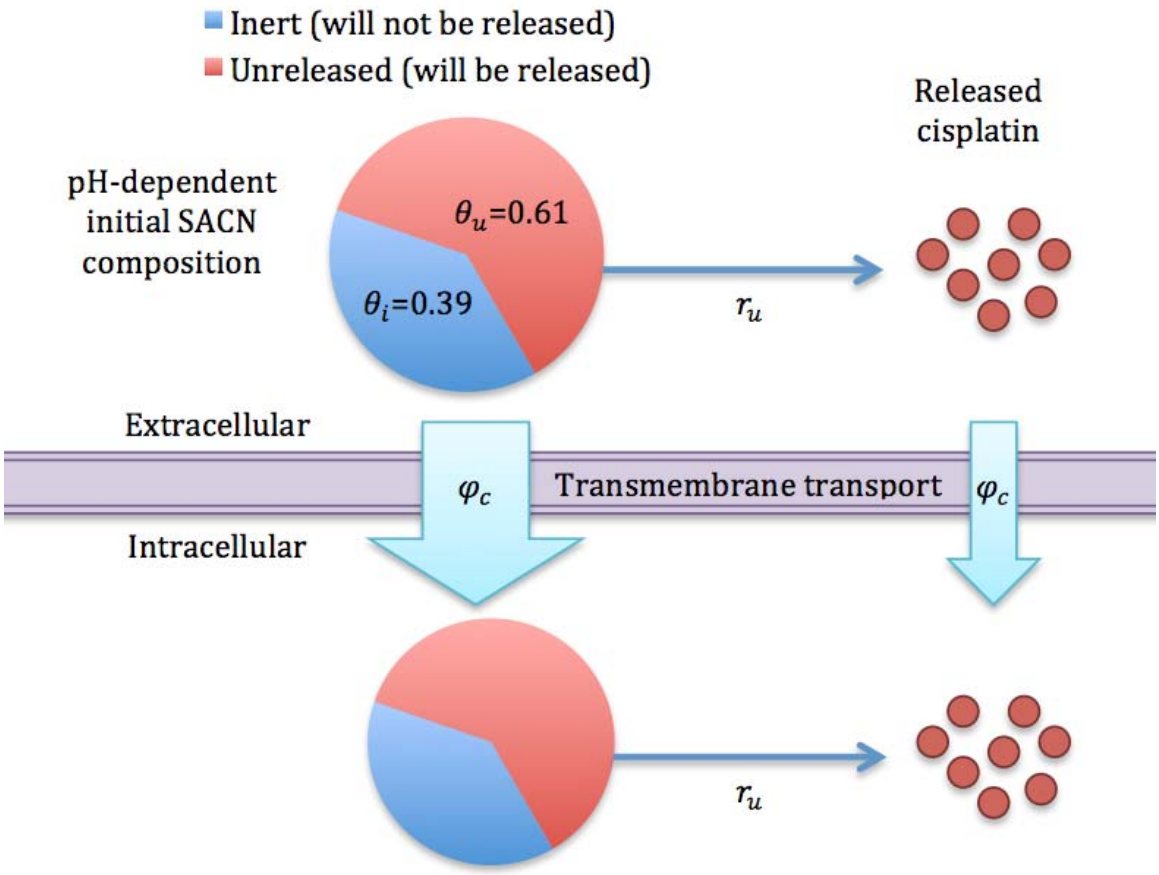

Fig. 2S: Flowchart showing the computational algorithm used to fit parameters in the mathematical model.

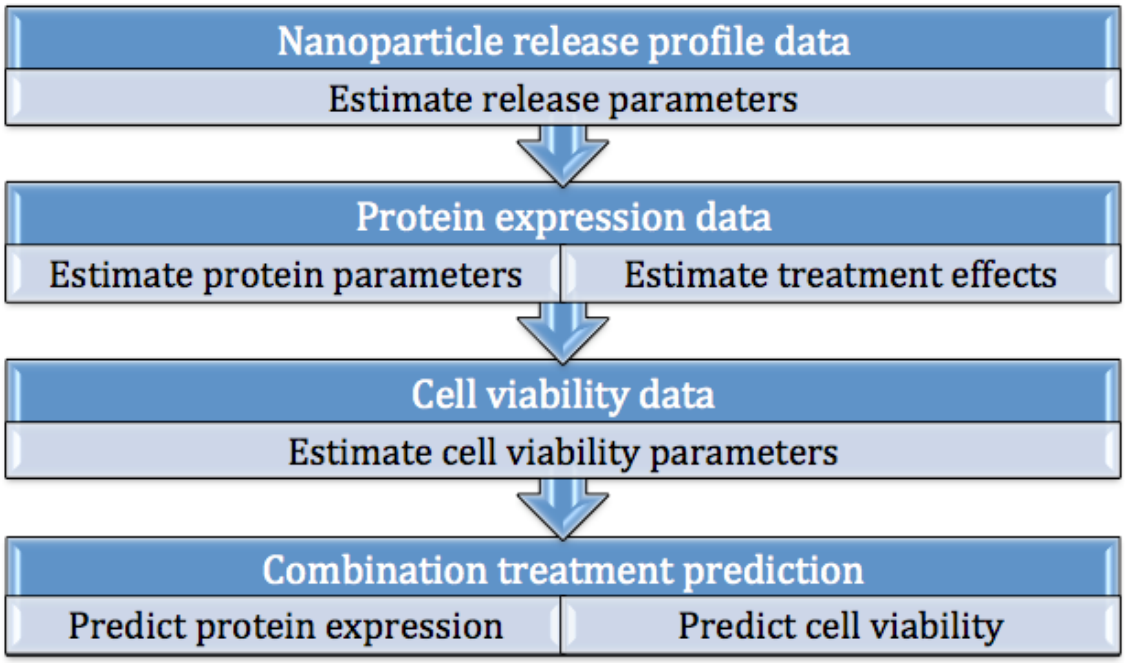

Fig. 3S: (Data points) The release profile of cisplatin nanoparticles was evaluated at 37°C in neutral and acidic (tumor like) pH in triplicates. 500 mwco dialysis bags packed with 16198665 ng nanoparticle were stirred in 30 mL of acidic and neutral pH. 500 µL samples was taken out at predetermined time points from the outer chamber and analyzed by AAS by serial dilution. (Solid lines) Release profiles of cisplatin nanoparticles in acidic ($\chi 2=0.54, N=15, p=2$) and neutral ($\chi 2=1.66, N=15, p=2$) microenvironments determined by fitting solutions of Equations (1S).