

## **SUPPLEMENTARY MATERIAL**

### **Insights into the kinetics of siRNA-mediated gene silencing from live-cell and live-animal bioluminescent imaging**

Derek W. Bartlett and Mark E. Davis

## A Detailed Explanation of the Mathematical Model

The initial conditions for the target mRNA and target protein levels are set to their steady-state values (assuming constitutive expression) while all other species are initially set to 0 except the siRNA being introduced at a selected dose.

The model enables the user to specify whether the experiment will take place *in vitro* or *in vivo*. The *in vitro* version uses the amount of media applied per well as the extracellular volume,  $V_e$ , and only incorporates a single transport step for entry of the siRNA complexes into the cells. The *in vivo* version includes a compartment for the blood plasma volume,  $V_p$ , in addition to the extracellular volume (e.g., the tumor extracellular fluid).  $B_{cf}$  and  $B_{cb}$  represent the unbound and bound complexes, respectively, present in the blood plasma after injection. Presumably, a portion of the delivered dose will partition into the microenvironment of the target cells, giving a pool of complexes,  $E_c$ , present in the extracellular fluid that can be taken up by the target cells. A partitioning parameter, *partition*, allows for a user-defined fraction of the initial applied dose that can reach the vicinity of the target cells, and then a single transport step accounts for entry of the siRNA complexes into the cells. This highly simplified model of *in vivo* delivery was chosen until more extensive biodistribution and pharmacokinetic data are available. Because all species are given in units of #/volume, the ratio  $V_e/V_p$  is included to adjust for the different volumes of the plasma and extracellular fluid compartments. Current work is being done to more accurately determine the biodistribution of delivered complexes, and these data will be used to provide a more accurate model of the *in vivo* transport process leading to uptake by the target cells.

Once the delivered complexes are in the extracellular space immediately surrounding the target cells, they can be transported into the cells. The module of equations accounting for uptake and intracellular trafficking provides the basic framework used in gene delivery, including endocytosis, vector unpacking, and endosomal escape. Again, because of the volume change between the extracellular and intracellular compartments, the ratio  $V_e/V_i$  is included to adjust the concentrations appropriately.

The internalized siRNA molecules can then interact with the RNAi machinery in the cells. The free siRNA molecules,  $C_{na}$ , associate with the RNAi machinery to form the RNA-induced silencing complex (RISC). Previous studies of the RISC enzyme complex in human cells revealed that it follows Michaelis-Menten kinetics in the presence of ATP, with a  $k_{cat}$  of  $1-2 \times 10^{-3} \text{ s}^{-1}$  and a  $K_m$  in the low nanomolar range (42-44). The total amount of RISC proteins available to form activated RISC complexes is assumed to remain constant inside the cell at a concentration of 3 nM (44). The model accounts for siRNA interaction with the pool of free intracellular RISC proteins,  $r_{tot}$ , to form an activated RISC complex,  $R$ . Some recently proposed models of RISC assembly involve intermediate complexes before the activated RISC is formed. Although these intermediate steps were not included in the version of the model described here, such steps could be incorporated as more details about RISC assembly in mammalian cells are discovered. This activated RISC complex can then bind to the target mRNA molecule to form the RISC/mRNA complex,  $C$ . Therefore, the amount of free RISC proteins available for formation of activated complexes is  $r_{tot} - R - C$ . The target mRNA is cleaved enzymatically by an endonuclease present in the RISC/mRNA complex, restoring a free activated RISC complex that can act catalytically to seek out another target mRNA molecule for cleavage. The removal of mRNA by the activated RISC complex is responsible for the gene silencing effect since this

reduces the mRNA levels below the normal steady-state value resulting from the balance between production and degradation as reflected in the *kformmRNA* and *kdegmRNA* parameters.

The final module provides the equations for protein production and cellular growth that reflect the observable phenotype of the system. If a reporter gene such as luciferase is targeted for downregulation by RNAi, the decrease in luciferase protein mRNA transcripts leads to a decreased level of luciferase protein. A logistic growth equation is used to provide a simple model of tumor cell growth since most solid tumors exhibit such sigmoidal growth. Standard exponential growth can be obtained by simply increasing the *max* parameter, representing the maximum sustainable cell population, so that the damping effect is minimized.

Because the measured luciferase bioluminescence is a combination of the signal emitted by both transfected and untransfected cells, the model provides the opportunity to specify a transfection efficiency, corresponding to the fraction of the total target cells that receive siRNA. For example, if the transfection efficiency is 50%, then even complete knockdown of the luciferase protein in transfected cells will lead to an observable phenotype of a 50% reduction in total luciferase bioluminescence relative to an untransfected control. Since total observed bioluminescence is often reduced by >80% after siRNA transfection, the transfection efficiency must be >80%. A transfection efficiency of 90% is used for the in vitro version of the model since this provides the best fit to experimental data and is also consistent with previously reported transfection efficiencies using Oligofectamine (9). To provide the best fit to the in vivo experimental results, transfection efficiencies of 50% and 90%, respectively, are used for in vivo delivery of siRNA to tumors after LPTV injection and to hepatocytes after HPTV co-injection. Nearly 100% effective transfection efficiency is reasonable for HPTV co-injection because only cells that receive the co-injected plasmid contribute to the observed luciferase expression, and most of these cells can be expected to also received the co-injected siRNA.

Model parameters were determined from previous literature reports when available. When not directly available from the literature, parameter values were adjusted such that the model system's response matched the experimental data. For the purposes of this study, the accuracy of the individual rate constants is less important than the ability of the model to mimic the trends observed with siRNA-mediated gene silencing. Order-of-magnitude estimates for rate constants governing the delivery process were made from previously reported data and then adjusted to give the best fit to the experimental data (16,17,54). Determination of rate constants governing the fraction bound in plasma, partitioning to target cells, extracellular/plasma elimination, and intercompartmental transport was guided by characteristic values for related biological processes (e.g., drug pharmacokinetics) but predominantly achieved through variation of the parameters until the model agreed with experimental observations.