

MODEL-BASED THERAPEUTIC TARGET DISCRIMINATION USING STOCHASTIC SIMULATION AND ROBUSTNESS ANALYSIS IN AN INSULIN SIGNALING PATHWAY

Eric Kwei¹, Jason Shoemaker¹, Kevin Sanft², Linda Petzold², and Francis Doyle, III¹

¹Department of Chemical Engineering

²Department of Computer Science
University of California-Santa Barbara
Santa Barbara, CA 93106

Abstract

Insulin resistance, which precedes the onset of type 2 diabetes mellitus (T2DM), is a decreased sensitivity of response to normal insulin levels which has been linked to a number of possible changes in the insulin signal transduction network. In this work, we simulate two hypothesized resistance-inducing perturbations using a published differential equation model of insulin signaling, estimate noise in this signaling model with stochastic simulation, and predict sets of optimal multi-drug targets that are robust against the estimated signaling noise using structured singular value analysis.

Keywords

Insulin signaling, combinatorial drug targeting, robustness analysis.

Introduction

Guided by evolutionary pressures, cellular signaling networks can generally maintain robust performance despite stochastic fluctuations and/or external perturbations. However, when these signaling networks fail to function properly, this inherent network robustness may be a critical obstacle for effective therapeutic intervention.

One such signaling network is mediated by insulin, which regulates, among other processes, glucose uptake in insulin-sensitive tissues. In patients who suffer from type 2 diabetes mellitus (T2DM), glucose uptake into insulin-sensitive tissues in response to insulin is reduced, a phenomenon known as insulin resistance. Many possible changes in signaling pathways have been implicated in insulin resistance (Muoio and Newgard, 2008).

Current drug therapies that are designed to reverse insulin resistance and treat type 2 diabetes mellitus (T2DM) have limited efficacy and significant side effects. We believe that by using a model-based analysis of the networks involved in insulin action, we can select robust

multi-target therapies that should work synergistically and at lower dosages than existing treatments.

In previous work, we have demonstrated the use of a robustness analysis tool, the structured singular value, in selecting optimal drug targets for an insulin resistant signaling model (Kwei et al., 2008b). In this work, we have refined this analysis with a different insulin resistant signaling model and show that different sources of insulin resistance may indeed be treatable by the same therapeutic intervention.

Modeling Insulin Signaling

Glucose transporter 4 (GLUT4) is the primary transporter responsible for insulin-stimulated glucose uptake; upon insulin stimulation, GLUT4 storage vesicles translocate to and fuse with the plasma membrane, and increase the rate of glucose uptake (Watson et al., 2004). It has been shown that insulin resistance significantly reduces GLUT4 translocation and glucose uptake (Shepherd and Kahn, 1999).

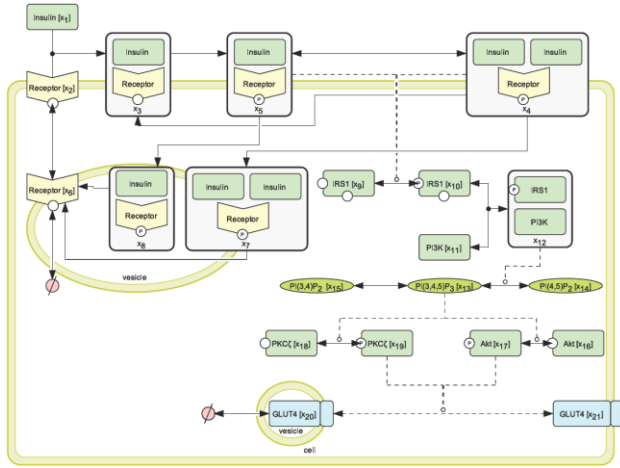


Figure 1. The Sedaghat model of insulin-stimulated GLUT4 translocation (without feedback mechanisms).

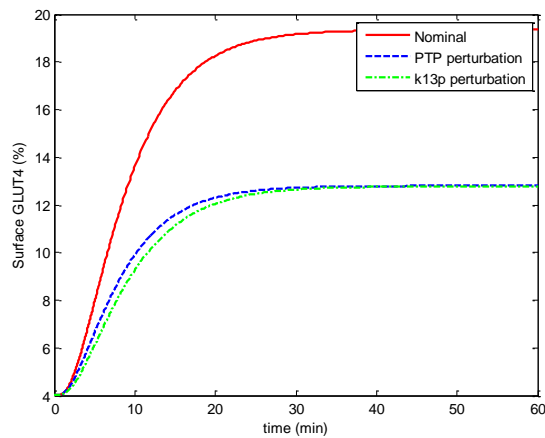


Figure 2. Response of surface GLUT4 percentage to parameter perturbations.

We have selected the model of insulin-stimulated glucose transporter 4 (GLUT4) translocation by Sedaghat et al. (2002) for this robust target selection analysis, as this model is the most detailed published differential equation model of the insulin signaling kinetics (Figure 1).

The model can be decomposed into 3 sub-models. The first sub-model describes insulin receptor dynamics. Insulin receptor can bind insulin (which is treated as an input) causing receptor autophosphorylation and can be recycled or degraded through an endocytic mechanism. The second sub-model describes the signaling cascade downstream from insulin receptor. Phosphorylated insulin receptor activates a signaling cascade consisting of insulin receptor substrate 1 (IRS1), phosphatidylinositol 3-kinase (PI3K), phosphatidylinositol triphosphate (PIP3), followed by protein kinases B (Akt) and C (PKC ζ).

The third sub-model describes the control by Akt and PKC ζ of movement of GLUT4 storage vesicles to the plasma membrane. Of note for this work, the extent of GLUT4 translocation is quantified by the percentage of the

total amount of GLUT4 that exists in the plasma membrane (surface GLUT4); thus, an “insulin resistant” model would have a smaller surface GLUT4 percentage than an “insulin sensitive” model.

Insulin Resistance Modeling

As mentioned previously, a number of signaling changes have been linked to insulin resistant behavior *in vitro*. This work highlights two network perturbations in the Sedaghat model that simulate insulin resistance (Figure 2). The first is a 50% increase in the effect of protein tyrosine phosphatase (PTP), which dephosphorylates insulin receptor and IRS1; this has a net effect of reducing GLUT4 translocation by about 35% at an insulin input of $10^{-9.5}$ M. The other is a 47% decrease in a parameter, k_{13} , that describes the ability of phosphorylated Akt and PKC ζ to stimulate GLUT4 translocation; this value was tuned to match the steady-state surface GLUT4 response from the PTP parameter perturbation for an input of $10^{-9.5}$ M insulin.

Stochastic Simulation

For estimates of noise likely to be encountered in this network as well as testing our hypotheses about robustness, we used stochastic simulation methods. We adapted the differential equations in the Sedaghat model into a stochastic model by converting reaction rate equations into reaction propensity functions (probabilities) as described by the chemical master equation (CME). These probability distributions can rarely be solved exactly. However, using Gillespie's stochastic simulation algorithm (SSA) to run many realizations of the stochastic model, it is possible to get reasonable estimates of the solution of the CME (Gillespie, 1976).

The SSA used to simulate the stochastic Sedaghat model was implemented using the StochKit software package. Modification of the propensity functions was necessary to accommodate non-mass-action kinetics in the deterministic model. Additional custom code was written to allow arbitrary insulin input functions.

To estimate the impact of stochastic fluctuation at the cellular level we used the SSA to generate 100 realizations of the stochastic model. A 15 minute pulse input of insulin was used, and state information was collected at every minute of simulation time up to 60 minutes. Results for the nominal Sedaghat model as well as the resistance-induced models are illustrated in Figure 5.

Structured Singular Value Analysis

In this work, we use structured singular value (SSV) analysis to quantify the range of fluctuation a set of parameters can tolerate while maintaining robust insulin signaling performance (Shoemaker et al., 2009).

Briefly, SSV analysis identifies the smallest structured, but possibly multi-dimensional, perturbation,

Δ , which destabilizes a stable system, N . The largest singular value of Δ , $\bar{\sigma}(\Delta_{calc})$, (which describes the size of the perturbation) is used to calculate the value of μ , defined below.

$$\mu^{-1} = \min_{\Delta_{calc}} \{ \bar{\sigma}(\Delta_{calc}) \mid \det(I - N\Delta_{calc}) = 0 \text{ for structured } \Delta \}. \quad (1)$$

While the value of μ can be calculated precisely for small networks, for large networks, it can only be bounded. We have shown that the “normal” network as modeled by Sedaghat as well as the insulin resistant versions are robustly stable (data not shown).

By extension of the Nyquist stability criterion, it can be shown that for $\mu < 1$, the system N is robustly stable to all Δ within the space of allowed parameter values. Conversely, for $\mu > 1$, we can conclude that the system is destabilized for at least one parameter set within the space of allowed parameter values. Through manipulation of the structure of Δ and N , one can impose performance specifications on a system and treat a robust performance problem as a robust stability problem. For a more detailed treatment of robust performance and SSV, consult Skogestad and Postlethwaite (2005).

This consideration of robust performance is highly relevant in biological systems, which are generally robust to some perturbations but highly sensitive to others. Thus, using SSV analysis, we can design a treatment that is not only effective but robustly effective (Figure 3).

Application to Insulin Resistance Model

For this application, we are interested in restoring our two insulin resistant models (with PTP and $k_{13'}$ parameter perturbations, respectively) to normal function. We limit the number of possible perturbable parameters, i.e. therapeutic targets, to groups of three for computational and practical purposes.

First, we examined the parameter perturbations that move the system outside the estimated noise range where insulin resistant responses are found, e.g. $\mu > 1$. From our stochastic simulation, we find that a reasonable spread around the nominal and insulin resistant models is about 3% (in absolute units) in surface GLUT4 (the model output); this is our performance envelope.

In the linearized Sedaghat model, a set of 17 parameters can be perturbed (PTP and $k_{13'}$ were not included). Allowing all combinations of 3 parameters, we tested 680 combinations for each insulin resistant case. Allowing up to a 10% parameter change, we search for parameter sets for which $\mu > 1$ (e.g. are sensitive enough to push the system outside the expected noise range).

For the PTP-induced insulin resistance case, 34 of the 680 possible parameter combinations have both tight bounds on the value of μ (less than 10% error between the upper and lower bounds) as well as $\mu > 1$ and thus represent feasible therapeutic target sets (Figure 4). For the $k_{13'}$ -induced insulin resistant case, only 3 of the 680 possible

parameter combinations fit these criteria. These 3 parameter sets are all included within the 34 possible therapeutic options for the PTP-induced insulin resistance. This suggests that our proposed therapeutic parameter set for the $k_{13'}$ induced insulin resistant case will also work to reverse PTP-induced insulin resistance.

Of the 34 therapeutic options identified, they each contain at least two out of three of the following parameters: PI3K, k_{-1} , and k_{9stim} , which represent the total amount of PI3K in the system, the dissociation of insulin from insulin receptor, and the formation of PI(3,4,5)P₃ by phosphorylated IRS1/PI3K complex.

Interestingly enough, a standard steady-state parameter sensitivity analysis did not identify k_{-1} as a sensitive drug target (Kwei et al., 2008a). Only when considering noise was k_{-1} found to be an important potential drug target, making a strong case for the SSV approach as compared to a typical sensitivity analysis approach.

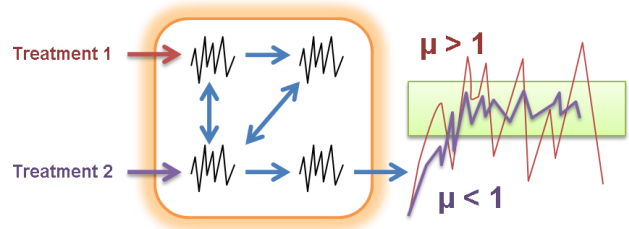


Figure 3. Distinguishing between treatments that pass the robust performance test ($\mu < 1$) and those that fail ($\mu > 1$) (Shoemaker et al., 2009).

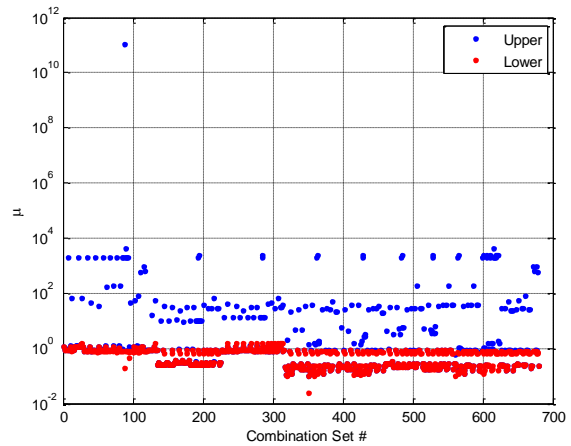


Figure 4. μ value calculation results for the insulin-resistance inducing PTP perturbation for all 680 possible parameter combination sets. The 34 well-bounded combinations with $\mu > 1$ were kept for parameter estimation.

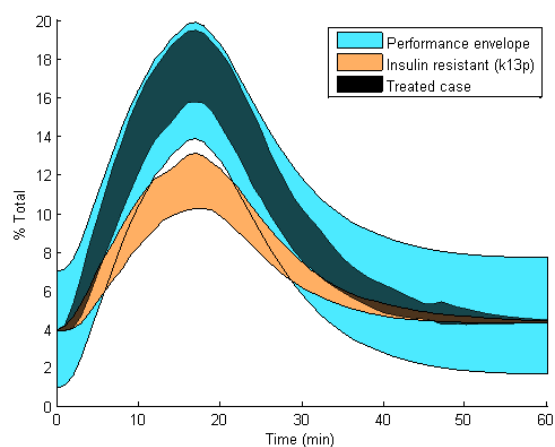


Figure 5. Stochastic simulation of normal performance, k_{13} -induced insulin resistant models, and a predicted treatment, respectively. The width of the colored bands represents one standard deviation up and down from the mean of 100 stochastic realizations.

Restoring Insulin Sensitivity

Simply identifying the parameter perturbation sets that could take the surface GLUT4 value outside the expected *insulin resistant* performance boundaries is not sufficient to restore *normal insulin sensitivity* performance; therefore, we must perform parameter fitting to check whether our treatments can restore the system dose-response performance back to normal. However, because we are guaranteed that perturbations in any parameter set for which $\mu < 1$ cannot restore insulin sensitivity, we need only perform parameter fitting on the remaining 34 “treatments” for the PTP-induced resistance case and on the 3 for the k_{13} -induced case rather than 680 parameter fittings for each case.

Of the 34 therapeutic parameter sets for the PTP-induced resistance case, 15 can be fit to match normal behavior, whereas for the 3 sets for the k_{13} -induced case, only 2 of the sets can be fit back to normal. These parameter sets represent the best *nominal* treatment sets.

To illustrate the robust performance of the nominal treatments themselves, one of the treatment sets for k_{13} -induced resistance (perturbations of PI3K, k_{1} , and k_{9stim} of -54%, -2.5%, and 16%) was simulated stochastically (Figure 5). The output of the simulated treatment (black) remains within the desired performance envelope (cyan).

Conclusions

In this work, two different causes of insulin resistance, PTP upregulation and GLUT4-recruiting dysregulation downstream of Akt and PKC ζ were modeled. It was found that perturbing three specific parameters that represent the total amount of PI3K in the system, the dissociation of insulin from insulin receptor, and the formation of PI(3,4,5)P $_3$ by phosphorylated IRS1/PI3K complex would

be an optimal choice for reversing two very different causes of insulin resistance *in silico*.

Unfortunately, this signaling pathway shares these signaling components with a number of other insulin- and non-insulin-stimulated pathways. This makes perturbing the predicted parameters *in vitro* (and *in vivo*) problematic in terms of creating potential side effects. One way around this is to choose pathway-specific targets in the insulin-stimulated GLUT4 translocation pathway, which currently do not exist in the model. This motivates further identification and modeling of cell-type specific insulin-stimulated signaling components that will allow for identification of drug targets with high effectiveness and minimal side effects.

Acknowledgments

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