Implications of multiple sensitivity analysis techniques in stochastic models of focal adhesion dynamics

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Introduction

Cell motility is motivated by diverse organismal needs, from embryonic development to wound healing. Key to cellular movement is the development of focal adhesions at the leading edge of the cell. Focal adhesions are protein complexes made up of cytoskeletal elements, transmembrane proteins, and extracellular matrix components. Characteristics of focal adhesion assembly and disassembly govern the strength and duration of these contacts. These phenomena are challenging to study experimentally; however, we can gain insight into their development through mathematical models.

Our model investigates the dynamic interactions between three specific proteins in a nascent focal adhesion: a ligand, integrin, and talin. Integrin is a transmembrane receptor that is ligated by an extracellular matrix molecule and bound by the cytoskeletal protein talin (Figure 1). Talin serves as a link between integrin and actin, effectively connecting the outside of the cell to the inside.

The model focuses on the reactions between these proteins (Figure 2) to quantify the number of molecules in a particular state at a given time. Analysis of these equations provides understanding about which molecules are most important at what time for a focal adhesion.

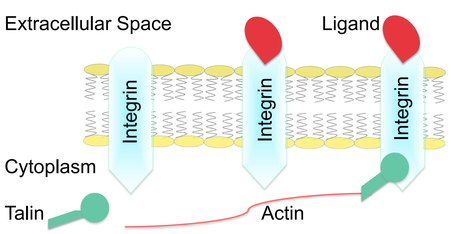

Figure 1. (Above) Three protein components of a focal adhesion.

$$L + I \underset{k_L^-}{\overset{k_L^+}{\rightleftharpoons}} LI$$

$$I + T \underset{k_T^-}{\overset{k_T^+}{\rightleftharpoons}} IT$$

$$IT + L \underset{k_{LT}^-}{\overset{k_{LT}^+}{\rightleftharpoons}} LIT$$

$$LI + T \underset{k_{LIT}^-}{\overset{k_{LIT}^+}{\rightleftharpoons}} LIT$$

$$S \underset{k_D^-}{\overset{k_D^+}{\rightleftharpoons}} I$$

Figure 2. (Right) Ligand (L), integrin (I), and talin (T) reactions (Blucher et al. 2014).

Stochastic Simulation

The model incorporates five rate reactions involving ligand (L), integrin (I), and talin (T) molecules. As exemplified in the reactions listed in Figure 2, these molecules can interact in a variety of ways. For example, free L and I may bind together to form ligand-integrin (LI) complexes; alternatively, LI complexes may unbind to form free ligand and integrin. In the last reaction, S represents integrin diffusing across the plasma membrane. Our main focus is on the fully activated complex LIT.

When the number of molecules in a given biological interaction is small (on the order of tens of molecules), the end product of a reaction will fluctuate over time, even when the initial values and rate constants remain fixed; *stochastic* models incorporate this characteristic by introducing randomness into the outputs of the model (Figure 3). As opposed to a *deterministic* model, where a specific set of inputs will always produce the exact same outputs, stochastic models may have a variety of potential outputs, even with the exact same set of inputs.

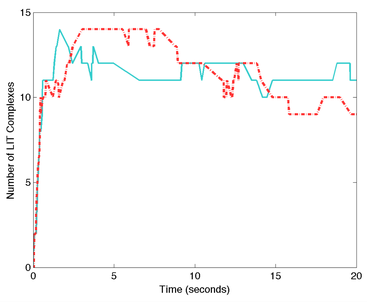

Figure 3. Sample time course of LIT output using the Stochastic Simulation Algorithm (SSA). The number of LIT (ligand-integrin-talin) complexes is plotted from 0 to 20 seconds for two separate simulations of the model without perturbation of any model parameters. This demonstrates the variability of outputs present in stochastic models.

Sensitivity Analysis

Sensitivity analysis (SA) provides a measure of the variability of the outputs of a model due to perturbation of the parameters. Many methods have been developed for deterministic models (Saltelli et al. 2004), but recent work has adapted some of these methods for stochastic models (Degasperi 2008). One way to account for the innate variation of stochastic models in sensitivity analysis is through the incorporation of a measure called *histogram distance*. The histogram distance provides a summary of the differences between two histograms, and is found using the following formula:

$$D_k(X,Y) = \sum_{i=1}^{k} \left| \frac{\sum_{j=1}^{|X|} \chi(x_j, I_i)}{|X|} - \frac{\sum_{j=1}^{|Y|} \chi(y_j, I_i)}{|Y|} \right|$$

where *k* is the number of histogram intervals, *I*. |*X*| and |*Y*| are the number of simulations that were performed and χ refers to the characteristic equation.

The Method of Morris is a screening technique, originally designed for deterministic models (Morris 1991), but more recently adaptation of the method was used on stochastic models (Degasperi 2008). After a comparison of the SA results with both the deterministic and stochastic models, a more rigorous SA technique called Fourier Amplitude Sensitivity Testing (FAST) (Saltelli et al. 2004) was performed on the deterministic model while fixing the non-significant parameters as determined by the screening method.

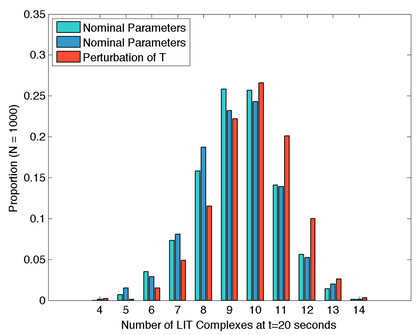

Figure 4. Histograms of the number of LIT (ligand-integrin-talin) complexes at 20 seconds, each depicting 1000 model simulations. Nominal parameters refer to simulations run without perturbation of any model parameters. Thus the histogram difference between the two nominal parameter histograms represents the "self-distance" of LIT. The third histogram was created by increasing the initial number of T (talin) from 15 to 16 molecules.

Results

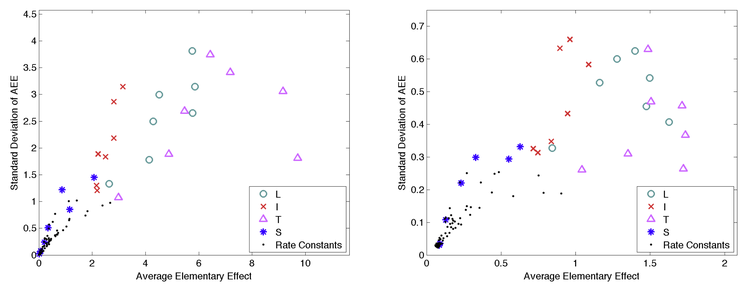

Figure 5. Average elementary effect (AEE) versus standard deviation of AEE obtained using the Method of Morris screening technique. The Method of Morris provides two measures: the *average elementary effect* and the *standard deviation of AEE.* The former provides a sense of the overall strength of the effect of a particular parameter on a given output, and the latter gives information on any nonlinear interactions in the model (Degasperi 2008, Saltelli et al. 2004). Left: The results obtained from the deterministic model using the methods described by Morris (1991) and Saltelli et al. (2004). Right: The results obtained from the stochastic model using the adaptations described by Degasperi (2008), incorporating histogram distance.

Results (continued)

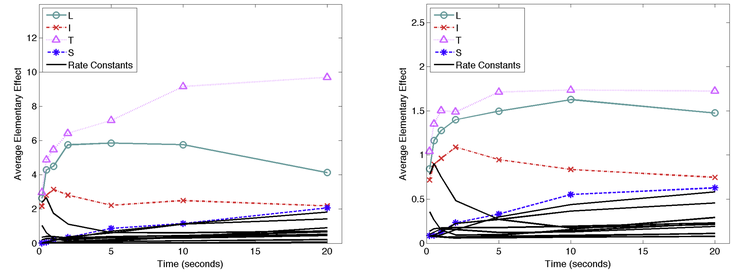

Figure 6. Average elementary effect at t = 0.2, 0.5, 1, 2, 5, 10, and 20 seconds, obtained using the Method of Morris. Left: The results obtained using the deterministic model. Right: The results obtained using the stochastic model.

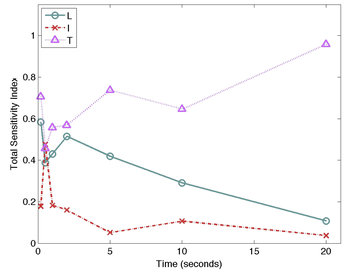

Figure 7. Total sensitivity index at t = 0.2, 0.5, 1, 2, 5, 10, and 20 seconds, obtained using FAST with deterministic model. FAST is a variance-based SA technique that provides quantitative comparisons between the most significant parameters of a model. These results were obtained using the methods described by Saltelli et al. (2004).

Discussion

The results from the Method of Morris were qualitatively similar with both the deterministic and the stochastic models. This suggests that the deterministic model may be viably utilized for future analysis when the stochastic model cannot be used. Based on the results of the screening technique, we found LIT production was less sensitive to the rate constants than to initial conditions of L, I, and T; we therefore fixed the rate constants at the nominal values in order to perform more rigorous FAST.

FAST with the deterministic model indicates that the number of LIT complexes at various times is most sensitive to changes in the initial number of T molecules. Thus, the results of the SA suggest that future biological experimentation might focus efforts on determining an accurate number of talin molecules to further improve the mathematical model, or perhaps the experimentation could even aim to control talin in order to control the process of focal adhesion formation.

The current model incorporates only three types of molecules; in reality a focal adhesion is much more complex with some studies incorporating up to eight different critical components (Lele 2008). For future work, we plan to integrate the protein Focal Adhesion Kinase (FAK) into our model. FAK binds ligated integrin, resulting in the phosphorylation of other complex-associated proteins. Studies have shown that FAK deficient cells migrate poorly in response to stimuli, even when other components of the focal adhesion are present (Parsons 2000). Thus, including interactions of FAK into the model could provide key insights into the dynamics of these focal adhesions.

References

A. Blucher, M. Salas, N. Williams, H.L. Callender. Mathematical Modeling of Integrin Dynamics in Initial Formation of Focal Adhesions. *Involve*. **7.4**, 509-527 (2014).

A. Degasperi and S. Gilmore. Sensitivity analysis of stochastic models of bistable biochemical reactions. *Formal Methods for Computational Systems Biology*. Springer Berlin Heidelberg, 1-20 (2008).

T.P Lele, C.K. Thodeti, J. Pendse, D.E. Ingber. Investigating Complexity of Protein–protein Interactions in Focal Adhesions. *Biochemical and Biophysical Research Communications*. **369.3**, 929-34 (2008).

M.D. Morris, Factorial sampling plans for preliminary computational experiments. *Technometrics*. **33.2**,161-174 (1991).

J.T. Parsons, K.H. Martin, J.K. Slack, J.M. Taylor, S.A. Weed. Focal Adhesion Kinase: A Regulator of Focal Adhesion Dynamics and Cell Movement. *Oncogene*. **19.49**, 5606-613 (2000).

A. Saltelli, et al. *Sensitivity analysis in practice: a guide to assessing scientific models*. (John Wiley & Sons, 2004).