Non-mass-action modeling for the binding of Erk2 phosphorylated Gli1 protein with Sufu

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August 18, 2013

Abstract

Gli1 is a transcription factor of the Hedgehog (Hh) signaling pathway involved in embryonic development and stem cell differentiation. Recent biological studies have found a cross-link between the Erk2 MAP kinase pathway and that of Gli1, in which Erk2 is able to phosphorylate Gli1, resulting in a lower affinity for Gli1-Sufu binding [Bardwell 2010]. When bound to Sufu, Gli1 is sequestered in the cytoplasm of the cell, while when unbound, Gli1 is able to enter and be expressed in the cell nucleus, promoting cell growth. Overexpression of the Gli1 protein has been linked to many cancers, most notably glioblastoma multiforme, the most common and aggressive brain tumor. The exact biochemical interactions resulting in these dynamics are not yet known. In our study we propose the incorporation of Holling Type-II interactions from ecology to explain the variance in saturation levels of bound Sufu, resulting from the phosphorylation of Gli1 by Erk2. We show that neither the traditional mass-action model nor one in which Gli1 may dimerize sufficiently explain the observed dynamics, proposing that the Erk2/Gli1/Sufu system does not follow mass-action dynamics

1 Biological Background

The overall system that we are studying consists of the Hedgehog (Hh) signaling pathway and the MAP kinase pathway. Within these pathways, there are two proteins of interest: the Gli1 protein, an effector protein in the Hh pathway, and the Erk2 protein, a member of the kinase family. The relationship between these two are of interest because of the cross talk of Erk2 with Gli1 affecting the activity of Gli1. The Gli1 protein has been found to be overactive in cancers. In fact, the protein was first isolated from patients with glioblastoma, a brain cancer.

Erk2 is an effector protein in the MAP kinase pathway, and it is also the kinase that phosphorylates Gli1. Phosphorylation of a protein is a common way for a cell to control the level of activity of that protein. However, many proteins have more than one phosphorylation site, meaning that they are not simply activated when phosphorylated and deactivated when not phosphorylated, but are rather more robust, sensing the environment and being able to

express varying degrees of phosphorylation which in turn trigger the protein to switch from deactivated to activated (and vice versa). Gli1 is such an example of a protein with multiple phosphorylation sites. When Gli1 is deactivated (non-phosphorylated), Sufu, an inhibitor protein, will bind to it, sequestering this bound Gli1-Sufu complex in the cell cytoplasm. It has been observed by the Bardwell lab that Gli1 phosphorylation by Erk2 lowers the affinity of Gli1 to Sufu. It has further been observed that the more Erk2 is used to phosphorylate Gli1, the less likely Sufu will bind to it, resulting in active (phosphorylated) Gli1. An active Gli1 protein is able to enter the nucleus of the cell and act as a transcription protein, promoting the growth of the cell. Over-expression of Gli1 has been found present in many cancers, most notably glioblastoma.

2 Experimental Design and Data Collection

To obtain numerical data of the system, the Bardwell Lab conducted several titration and protein binding assay experiments in which different combinations of Gli1 and Erk2 concentrations were mixed, allowing the proteins to interact for 20 or 120 minutes. Erk2 was then extracted, and Sufu added. After 40 minutes, free Sufu was extracted, leaving only free Gli1 and the Gli1-Sufu complex. Separation of these remaining proteins was done via gel electrophoresis, and the visualization of the complex was faciliated by radioactive S-35 methionine tagged onto the Sufu earlier in the procedure. By comparing the density of the Gli1-Sufu complex obtained from the mixtures to a control consisting of 100% of the Sufu concentration initially allowed to bind to Gli1, a numerical value of percent Sufu bound to Gli1 was obtained for each mixture.

3 Mass-Action Modeling

In order to better understand the mechanism for phosphorylation in the Gli1/Erk2/Sufu system, we began our investigation by fitting mass-action, or Michaelis-Menten type, rate kinetics to the data obtained from the binding protein assays done by the Bardwell Lab [Jane], where Erk2 levels were varied and total phosphate incorporation was measured. Here we model the system as S=[Sufu], G=[Gli1], C=[bound Sufu-Gli1 Complex], and k_{on}, k_{off} are the forward and backward reaction rates, respectively, such that we have

$$\mathrm{S} + \mathrm{G} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \mathrm{C} \tag{1}$$

With the ODEs

$$G' = -k_{on}SG + k_{off}C \tag{2}$$

$$S' = -k_{on}SG + k_{off}C \tag{3}$$

$$C' = k_{on}SG - k_{off}C \tag{4}$$

and mass conservation laws

$$S_{total} = S_t = S + C \tag{5}$$

$$G_{total} = G_t = G + C \tag{6}$$

To better observe the amount of the Sufu-Gli1 complex C at steady state, we need only set one of the ODEs to zero, seeing that setting any would give the same result. Using steady state analysis and mass conservation laws (5), we find

$$C = \frac{S_t G}{k_d + G}$$

where

$$k_d = \frac{k_{off}}{k_{on}} \tag{7}$$

By definition, percent Sufu bound takes the form C/S_t. When fitting this graph into our data, a maximum saturation percent of Sufu-Gli1 binding, V_{max}, needed to be identified. This value was held constant for all Erk2 levels. Thus, mass-action dynamics and analysis gave us the following equation and fits [Fig1].

$$C = V_{max} \frac{G}{k_d + G} \tag{8}$$

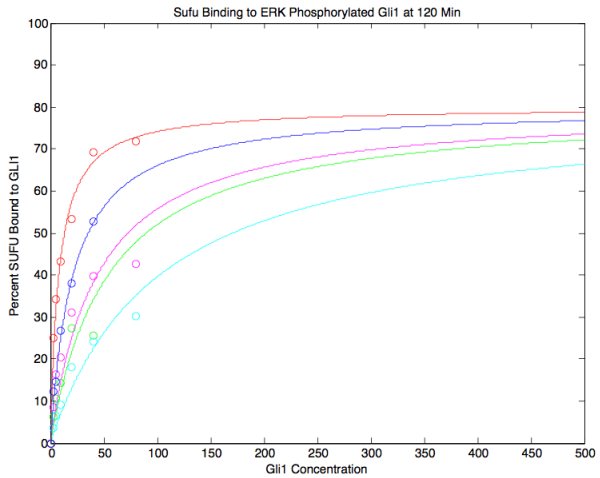

Figure 1: Total percent bound Sufu-Gli1 given varying levels of Gli1 and Erk2, fitted on equation (8). The red line is the fit for [Erk2]=0ul, dark blue is for [Erk2]=10ul, purple is for [Erk2]=20ul, green is for [Erk2]=50ul, and light blue is for [Erk2]=100ul. For these graphs, V_{max} was set at 80%.

4 Two Approaches for Varying Saturation Levels

Our initial results using mass-action dynamics were helpful in directing further experimental research for the Bardwell Lab. In order to examine whether the static maximum saturation was a reasonable assumption, a protein assay experiment was designed and run where Sufu-Gli1 binding was looked at for Gli1 concentrations up to $250nM$. From these experiments, it became clear that saturation levels were changing given Gli1 interaction with Erk2 [Fig2].

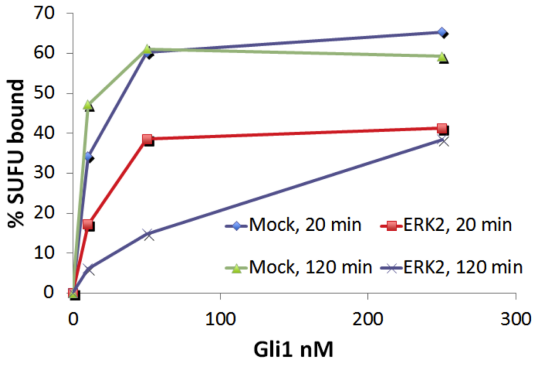

Figure 2: Total percent bound Sufu-Gli1 given varying levels of Gli1 and Erk. We observe that both Erk2 20min and Erk2 120min saturate at a lower total percent Sufu-Gli1 than do the mock trials, and these differing saturations are consistent over time.

Given these experimental results, we worked with Dr. Bardwell and Dr. Enciso to test two approaches for modeling this behavior, one where Gli1 is able to dimerize and one where Gli1 does not follow mass-action kinetics.

4.1 Gli1 Dimerization model

Our model for Gli1 Dimerization allows for the Gli1 protein to be bound with itself, tying up Gli1 and limiting the total amount of Sufu-Gli1 present. The system modeled is defined with the following equations where S=[Sufu], G=[Gli1], C=[bound Sufu-Gli1 Complex], D=[Gli1 dimer], ki_{on}, ki_{off} are the respective forward and backward reaction rates. Accompanying equation (1) is the equation for Gli1 dimerizing, forming a complex that is unable to bind Sufu.

$$\begin{aligned} \mathrm{S} + \mathrm{G} &\underset{k1_{off}}{\overset{k1_{on}}{\rightleftharpoons}} \mathrm{C} \\ 2\mathrm{G} &\underset{k2_{off}}{\overset{k2_{on}}{\rightleftharpoons}} \mathrm{D} \end{aligned} \tag{9}$$

This gives us the ODEs

$$S' = -k1_{on}SG + k1_{off}C \tag{10}$$

$$G' = -k1_{on}SG - k2_{on}G^2 + 2k2_{off}D + k1_{off}C \tag{11}$$

$$C' = -k1_{off}C + k1_{on}SG \tag{12}$$

$$D' = -k2_{off}D + k2_{on}G^2 \tag{13}$$

While this model accounts for lower bound levels of Gli1-Sufu at a given time, when examined for steady state behavior, we find that total Gli1-Sufu binding still reaches 100%.

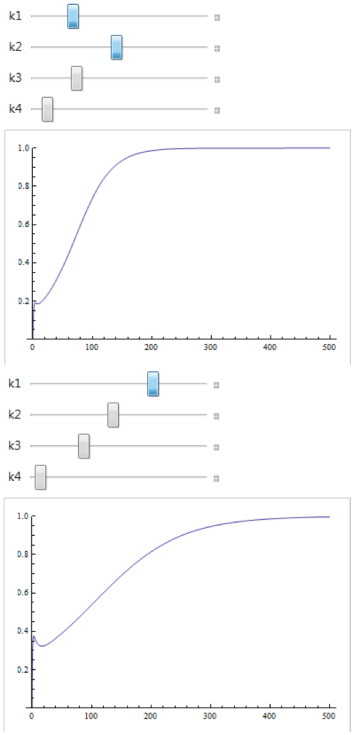

Figure 3: We can see that changing parameter values changes the curvature of the graphs, but the saturation remains at 100% at high [Gli1].

In both the mass-action and dimerization models, a high enough [Gli1] results in 100% saturation at steady state. This is expected, as in mass-action model, equation (8) shows that at $G >> k_d$, $C/S_t \simeq V_{max}$. In the dimerization model, a similar case occurs. At high [Gli1], there is enough free Gli1 available for 100% of the Sufu present to bind to. Therefore, neither mass-action nor dimerization of Gli1 is a good approach to modeling Erk2/Gli1/Sufu dynamics.

4.2 Non-Mass Action Model

Given the results obtained from the dimerization model, we worked with Dr. Enciso to develop a model which allows for dynamic steady-state saturation level. Unlike the two

previous models, in our non-mass action model we take Gli1-Sufu binding to not be instantaneous. We keep the same interaction terms as in (1), but incorporate a time-delay term, τ, such that the number of bindings per Sufu molecule in ΔT becomes

$$k_{on}G(\Delta t - y\tau) \tag{14}$$

where y is the number of bindings per sufu molecule during δt. This results in the Sufu-Gli1 interaction rate term to be

$$k_{on}\frac{G}{1+k_{on}\tau G}S \tag{15}$$

which yeilds the following governing rate equations,

$$S' = -k_{on}S\frac{G}{1+\tau k_{on}G} + k_{off}C \tag{16}$$

$$G' = -k_{on}S\frac{G}{1+\tau k_{on}G} + k_{off}C \tag{17}$$

$$C' = -k_{off}C + k_{on}S\frac{G}{1+\tau k_{on}G} \tag{18}$$

Here we find the steady-state bound-complex percent to be

$$C = \frac{G_t k_{on}+k_{off}+k_{on}S_t+G_t k_{on}k_{off}\tau \pm \sqrt{4G_t k_{on}S_t(-k_{on}-k_{on}k_{off}\tau)+(G_t k_{on}+k_{off}+k_{on}S_t+G_t k_{on}k_{off}\tau)^2}}{2(k_{on}+k_{on}k_{off}\tau)} \tag{19}$$

and the steady-state saturation level to be $\frac{S_{total}}{(1+k_{off}\tau)}$.

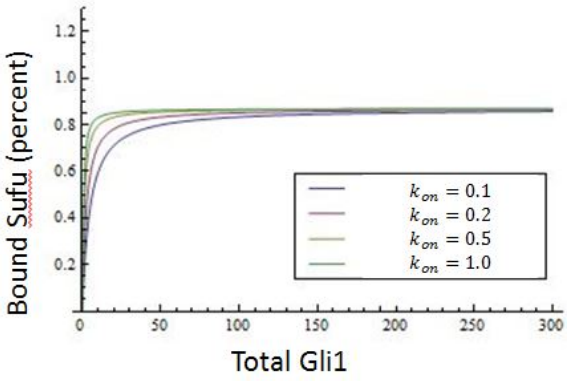

Figure 4: Steady state plots for dynamical model shows that increasing k_{on} results in faster saturation, but does not change final percent bound.

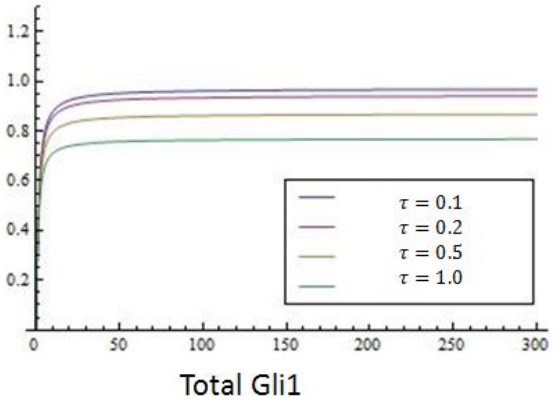

Figure 5: Steady state plots with varying τ shows that increasing τ lowers total saturation level bound Sufu. Similar dynamics occur for k_{off}.

In order to fit our biological data to our proposed non-mass action, we utilize a non-linear least squares regression in Matlab and observe very good fits with $R > 0.94$ for all plots.

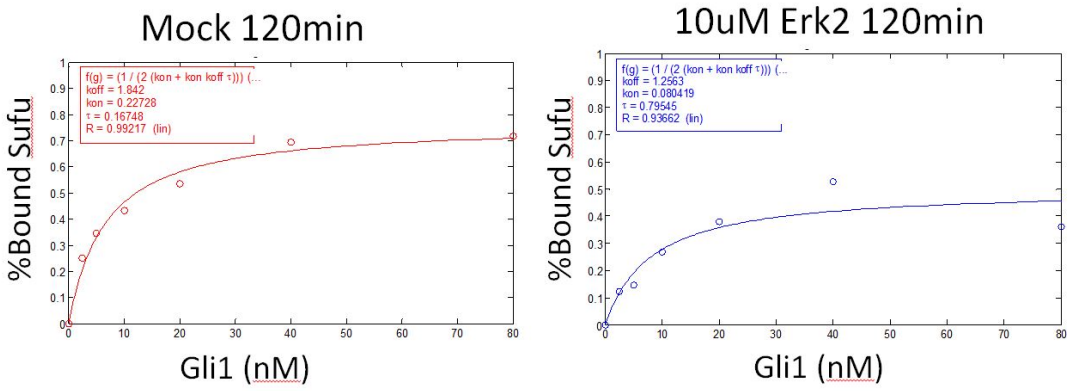

Figure 6: Sample fits of proposed non-linear model to experimental data ($R > 0.94$ for all)

After finding the fits for our data, we are able to analyze the changes in k_{on}, k_{off}, τ values given varying levels of Erk2. We find that, although no significant relation exists between the k_{on}, k_{off} values alone, when we set $k_d = k_{off}/k_{on}$ we find k_d to increase for increasing amounts of Erk2.

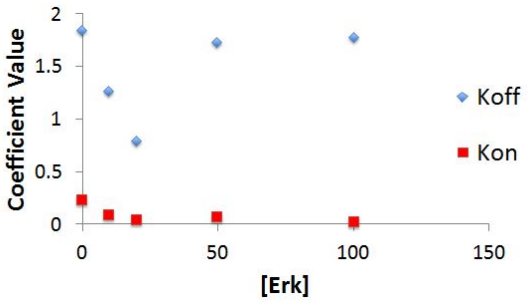

Figure 7: Obtained values for k_{on}, k_{off} given varying levels of Erk2

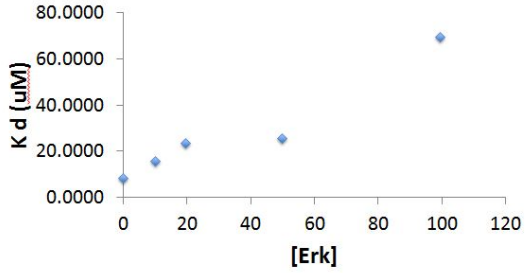

Figure 8: Obtained values for k_d given varying amounts of Erk2, here we see k_d increases as Erk2 increases.

Similarly, we find a general trend for τ with increasing values given increasing amounts of Erk2.

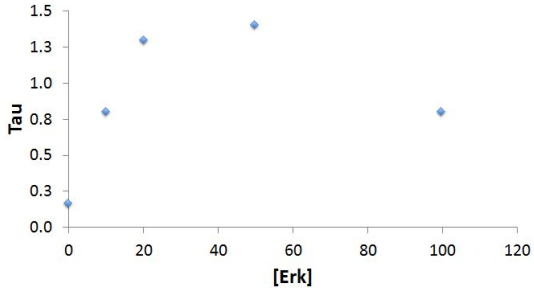

Figure 9: Obtained values for τ given varying amounts of Erk2, here we see τ increases as Erk2 increases for [Erk2] values between 0-80uL. We believe difference in the [Erk]=100ul value to be a residual of the non-linear least squares approximation due to τ never appearing alone in the steady-state equation.

5 Conclusion

Similar to the Holling type-II functional response model from ecology, where predator-prey interactions are dependent on the maximum consumption rate of the predator, our non-mass action model allows for variability in the rate of successful Gli1-Sufu binding interactions. When $\tau << 1$, we see that our non-mass action model simplifies to the classic mass action interaction model (2-4), and as τ increases, we see an exponential relationship arise between the amount of Gli1 present and rate at which Sufu is able to bind. We find that this new non-mass action model is able to model the behavior of Erk2-phosphorylated Gli1 and Sufu binding accurately, which is not able to happen with a traditional mass-action model or even a dimerization model.

From fitting our model to biological data, we find that the time-delay τ increases as greater amounts of Erk2 are used to phosphorylate Gli1 leading us to conclude that phosphorylated Gli1 takes longer to successfully bind to Sufu, with this effect increasing given increasing phosphorylation levels of Gli1. Due to the multi-site nature of Gli1, we believe that this may be a result of "faster" Sufu-binding sites being phosphorylated first. Since the individual-site dynamics of Gli1 are still under investigation by the Bardwell Lab, we can not draw a definite conclusion regarding this matter. However, preliminary data suggests that there are differences in Sufu binding affinity for the multiple sites, which is consistent with our theory.

We plan to continue to analyze our model and hopefully expand to a multi-site phosphorylation model as biological data becomes availible.

6 References

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J.H.P. Dawes, M.O. Souza, A derivation of Holling's type I, II and III functional responses in predatorprey systems, Journal of Theoretical Biology, Volume 327, 21 June 2013, Pages 11-22, ISSN 0022-5193, http://dx.doi.org/10.1016/j.jtbi.2013.02.017.

Whisenant TC, Ho DT, Benz RW, Rogers JS, Kaake RM, et al. (2010) Computational Prediction and Experimental Verification of New MAP Kinase Docking Sites and Substrates Including Gli Transcription Factors. PLoS Comput Biol 6(8)