Non-Mass Action Modeling for the Binding of Phosphorylated Gli1 with Sufu

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What is this?
Hedgehog Pathway!
Hedgehog & MAPK Cross-Talk

Signal cascade

Cross-talk

EGF  Erk

Shh  Gli1
The Biological Question

Cancers (Glioblastoma Multiforme)
Where is the math?

- Propose biological question
- Design an experiment
- Perform experiment / Gather data
- Adjust Theory
- Analyze data
Where is the math?

Propose biological question

Design an experiment

Perform experiment / Gather data

Adjust Theory

Data Analysis
Our Research

In order to understand the Erk2-Gli1-Sufu system we propose a novel method of biochemical modeling using Holling Type-II non-linear interactions from ecology.
Experimental Design – Bardwell Lab

• Want Gli1_{68-232} (has Sufu binding site)
  o CDNA Cloning
  o Radioactive tagging Sufu
• Titrations of Gli1 and Erk1
• Analyze with protein binding assays
  o Gel electrophoresis
Data Collection

Gli\textsubscript{168-232} kinase with Erk2

Gli\textsubscript{168-232} binding to Sufu
**Data Translation**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Treatment</th>
<th>Time</th>
<th>nM GLI1_{68-232}</th>
<th>raw</th>
<th>bkgd adj</th>
<th>% pptd</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLI1_{68-232}</td>
<td>Mock</td>
<td>120 min</td>
<td>10</td>
<td>1950344</td>
<td>1707458</td>
<td>43.12</td>
</tr>
<tr>
<td>GLI1_{68-232}</td>
<td>ERK2 10 u</td>
<td>120 min</td>
<td>20</td>
<td>1743213</td>
<td>1500327</td>
<td>37.89</td>
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<tr>
<td>GLI1_{68-232}</td>
<td>ERK2 20 u</td>
<td>120 min</td>
<td>80</td>
<td>1928816</td>
<td>1685930</td>
<td>42.58</td>
</tr>
<tr>
<td>GLI1_{68-232}</td>
<td>ERK2 50 u</td>
<td>120 min</td>
<td>2.5</td>
<td>487568</td>
<td>244682</td>
<td>6.18</td>
</tr>
<tr>
<td>GLI1_{68-232}</td>
<td>ERK2 100 u</td>
<td>120 min</td>
<td>0</td>
<td>246405</td>
<td>3519</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3959758</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing increased [Gli1] leads to increased Sufu binding](image)
Initial Model: Mass-Action

\[ S + G \xrightarrow{k_{on}} C \xleftarrow{k_{off}} \]

\( S = [\text{Sufu}] \quad G = [\text{Gli1}] \quad C = [\text{Bound Complex}] \)

\( k_{on}, k_{off} = \text{rate constants} \)

\[ G' = -k_{on}SG + k_{off}C' \]

\[ S' = -k_{on}SG + k_{off}C' \]

\[ C' = k_{on}SG - k_{off}C' \]
Steady State Analysis: Mass Action

\[ G' = -k_{on}SG + k_{off}C = 0 \]

\[-k_{on}(S_t - C)G + k_{off}C = 0 \]

\[-(S_t - C)G + k_d C = 0 \]

\[ k_d C = (S_t - C)G \]

\[ k_d C + GC = S_t G \]

\[ C(k_d + G) = S_t G \]

\[ C = \frac{S_t G}{(k_d + G)} \]

\[ \%SuFuBound = \frac{C}{S_t} = \frac{G}{(k_d + G')} \]
Curve fitting: Mass-Action

\[
\frac{G}{(k_d + G)}
\]

This requires forced saturation level, “S_{max}”, to be identified. We tested
- Equal saturation
- Variable saturation

Equal saturation: R>0.64
Variable saturation: R>0.93
Mass-Action Dynamics

• Assumes all curves saturate to the same level, 100% of $S_{\text{max}}$
• Occurs regardless of $k_{\text{on}}$, $k_{\text{off}}$ parameters
Identifying Saturation Levels

Original data: Might all saturate at equal levels

Directed Experiment: find saturation levels decrease when Gli1 phosphorylated by Erk. Consistent through time
Explaining Saturation Levels

- Simple mass-action is not reasonable
- Try dimerization model:
  - Same basis as simple mass-action, however Gli1 is able to form a dimer, D
  - Hope Gli1 dimerization lowers binding with Sufu

\[
S + G \xrightleftharpoons[k_2]{k_1} C \\
2G \xrightleftharpoons[k_4]{k_3} D
\]

\[
S' = -k_1 SG + k_2 S \\
G' = -k_1 SG - k_3 G^2 + 2k_4 D + k_2 C \\
C' = -k_2 C + k_1 SG \\
D' = -k_4 D + k_3 G^2
\]
Steady-State Analysis: Dimerization

- Curve shape changes, but saturation remains at 100%
- Not a sufficient explanation of varying saturation levels
Proposed Solution: Non-Linear Dynamical Model

\[ S + G \xrightarrow[\frac{k_{on}}{k_{off}}]{k_{on}} C \]

\[ G' = S' = -k_{on} \frac{G}{1 + \tau k_{on} G} S + k_{off} C \]

\[ C' = -k_{off} C + k_{on} \frac{G}{1 + \tau k_{on} G} S \]

- General form remains the same
- Gli1 and Sufu binding rate taken to be non-linear
- Binding rate changes based on \( \tau \), time delay between Gli1-Sufu initial interaction and successful binding
Steady-State Analysis:
Non-Mass Action

\[ S' = -k_{on} \frac{G}{1 + \tau k_{on} G} S + k_{off} C \]

\[ C = S_t \frac{G_{k_{on}} + k_{off} + k_{on} S_t + G_{k_{on}} k_{off} \tau \pm \sqrt{4G_{k_{on}} S_t(-k_{on} - k_{on} k_{off} \tau) + (G_{k_{on}} + k_{off} + k_{on} S_t + G_{k_{on}} k_{off} \tau)^2}}}{2(k_{on} + k_{on} k_{off} \tau)} \]

\[ \%SuFuBound = \frac{G_{k_{on}} + k_{off} + k_{on} S_t + G_{k_{on}} k_{off} \tau + \sqrt{4G_{k_{on}} S_t(-k_{on} - k_{on} k_{off} \tau) + (G_{k_{on}} + k_{off} + k_{on} S_t + G_{k_{on}} k_{off} \tau)^2}}}{2(k_{on} + k_{on} k_{off} \tau)} \]
Steady-State Analysis:
Non-Mass Action

\[ S' = -k_{on} \frac{G}{1 + \tau k_{on} G} S + k_{off} C \]

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

\[ \% Sufu Bound = \frac{G_t k_{on} + k_{off} + k_{on} S_t - G_t k_{on} k_{off} \tau}{2} \pm \sqrt{4 G_t k_{on} S_t (-k_{on} - k_{on} k_{off} \tau) + (G_t k_{on} + k_{off} + k_{on} S_t + G_t k_{on} k_{off} \tau)^2} \]

Notice total Sufu becomes important, along with individual \( k_{on}, k_{off} \)
Steady-State Dynamics

- Non-linear system allows for variable saturation levels. Increasing $\tau$ lowers total saturation level bound $S_{ufu}$. Similar dynamics occur for $k_{off}$. Increasing $k_{on}$ results in saturation at lower levels total Gli1.
- Saturation occurs at $\frac{S_{total}}{1+k_{off} \tau}$.
Curve fitting: Non-Mass Action

\[
\frac{G_{tkon} + k_{off} + k_{on}S_t + G_{tkon}k_{off}\tau + \sqrt{4G_{tkon}S_t(-k_{on} - k_{on}k_{off}\tau) + (G_{tkon} + k_{off} + k_{on}S_t + G_{tkon}k_{off}\tau)^2}}}{2(k_{on} + k_{on}k_{off}\tau)}
\]
Validity of Non-Linear Model

- Holling Type-II functional response
- Mass-Action is Holling Type-I
Validity of Non-Linear Model

• Binding time = handling time
• Sufu can only bind and unbind at a certain maximal rate – binding is not instantaneous
• Introduce time-delay to model this

\( \Delta t \): some time interval
\( y \): # binding per sufu molecule in time interval \( \Delta t \)
\( r = \frac{y}{\Delta t} S \)

Non Mass-Action

\( \tau \): binding time per sufu molecule
\( y = k_{on} G (\Delta t - y \tau) \)

\( y = \frac{k_{on} G}{1 + k_{on} G \tau} \Delta t \)

\( r = \frac{1}{\tau \_au} \frac{G}{k_{on} + G} S \)

\( = \frac{1}{\tau \_au} \frac{G}{k_{on} + G} S \)

\( = -k_{on} \frac{G}{1 + k_{on} \tau G} S \)
Binding Rate Comparison

\[ S + G \xrightarrow{k_{on} \quad k_{off}} C \]

Mass-action binding rate:

\[ r = k_{on}SG \quad \rightarrow \quad G' = -k_{on}SG + k_{off}C \]

Proposed non-linear binding rate:

\[ r = k_{on} \frac{G}{1 + k_{on}\tau G}S \quad \rightarrow \quad G' = -k_{on} \frac{G}{1 + k_{on}\tau G}S + k_{off}C \]
Conclusions

- Phosphorylation of Gli1 by Erk2 lowers total Sufu-Gli1 binding
- Biochemical interactions of phosphorylated Gli1-Sufu are more complex than is accounted for by mass action
- Modeling of Sufu-Gli1 binding based on Holling Type-II rate dynamics allows for variable saturation levels at steady-state, consistent with biological data
- Total Gli1-Sufu bound saturation is $\frac{S_{total}}{1+k_{off} \tau}$
- The interaction of Gli1-Sufu is not instantaneous and phosphorylation of Gli1 by Erk2 increases time delay, $\tau$
- Cross-disciplinary studies can bring new insight, even when within a field
Future Direction

• Gli1 is a multisite protein – we believe this is likely important for Erk2/Sufu binding
  ➢ Possible phosphorylation of given sites affects subsequent binding to Sufu

• Multisite dynamics experiments are currently in process [Bardwell Lab]
  ➢ We would like to extend our analysis to a multisite phosphorylation model of Gli1 with Erk2 and Sufu to incorporate this data.
  ➢ In particular, we hope to be able to fit this data to a concerted, redundant activation (CRA) model recently developed by G. Enciso. [submitted June 11, 2013]
Acknowledgments

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G. Enciso, D.R. Kellogg, A. Vargas, Modeling of a yeast bud checkpoint using a novel multisite mechanism, Submitted 11 June 2013 to PLOS Computational Biology
