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

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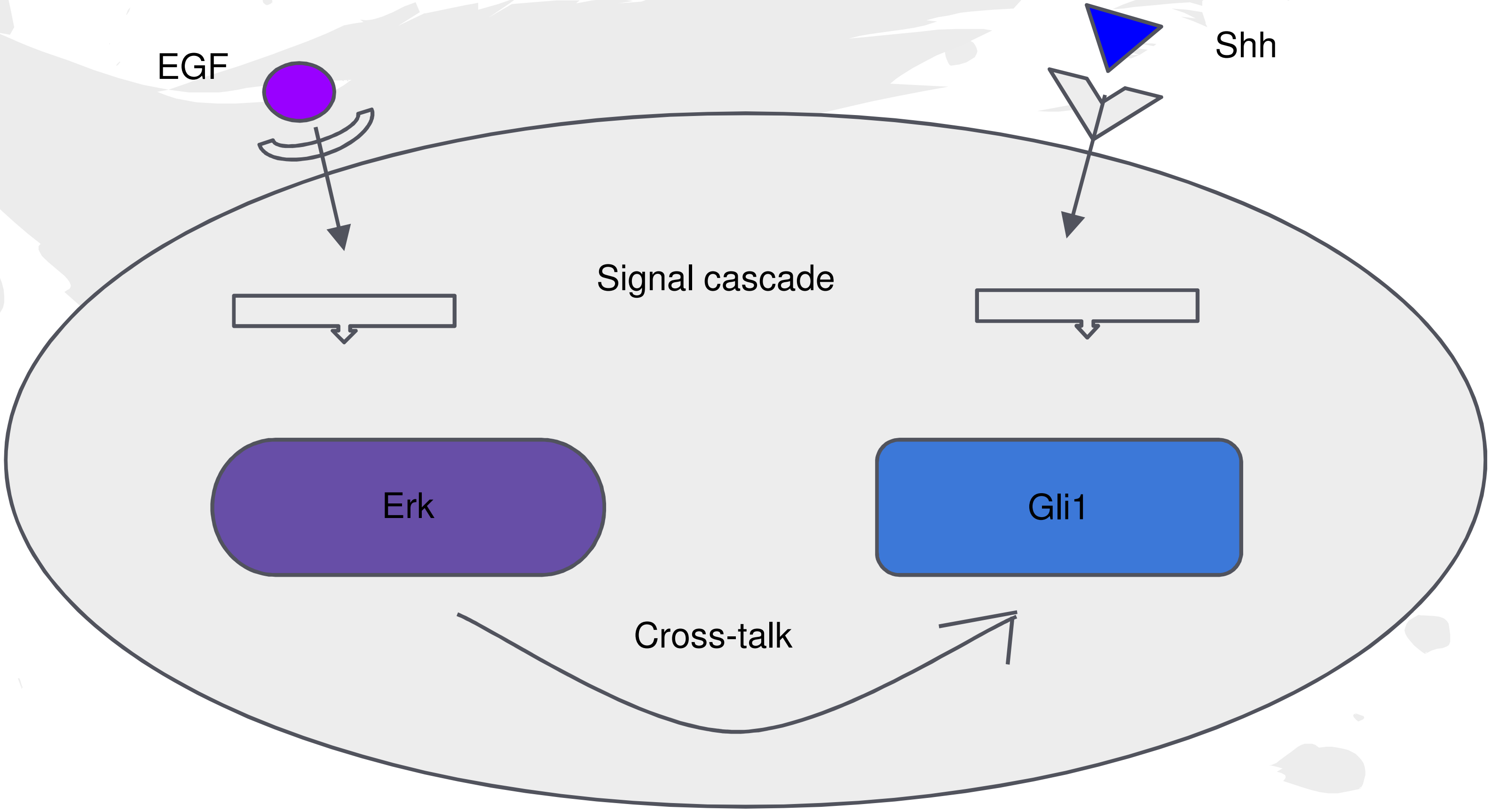
What is this?

Hedgehog Pathway!

Hedgehog & MAPK Cross-Talk



EGF

Shh

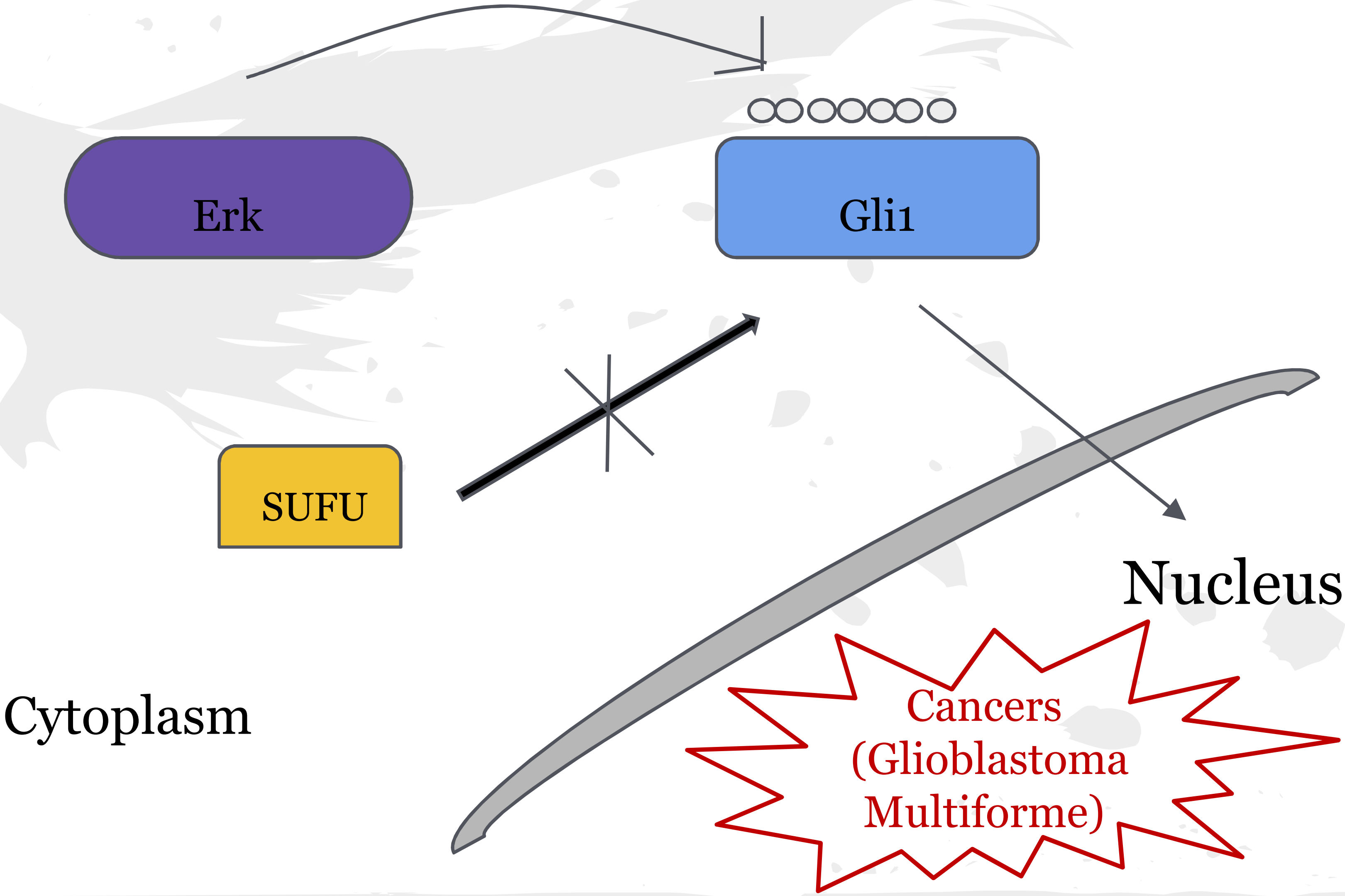
Signal cascade

Erk

Gli1

Cross-talk

The Biological Question



Erk

Gli1

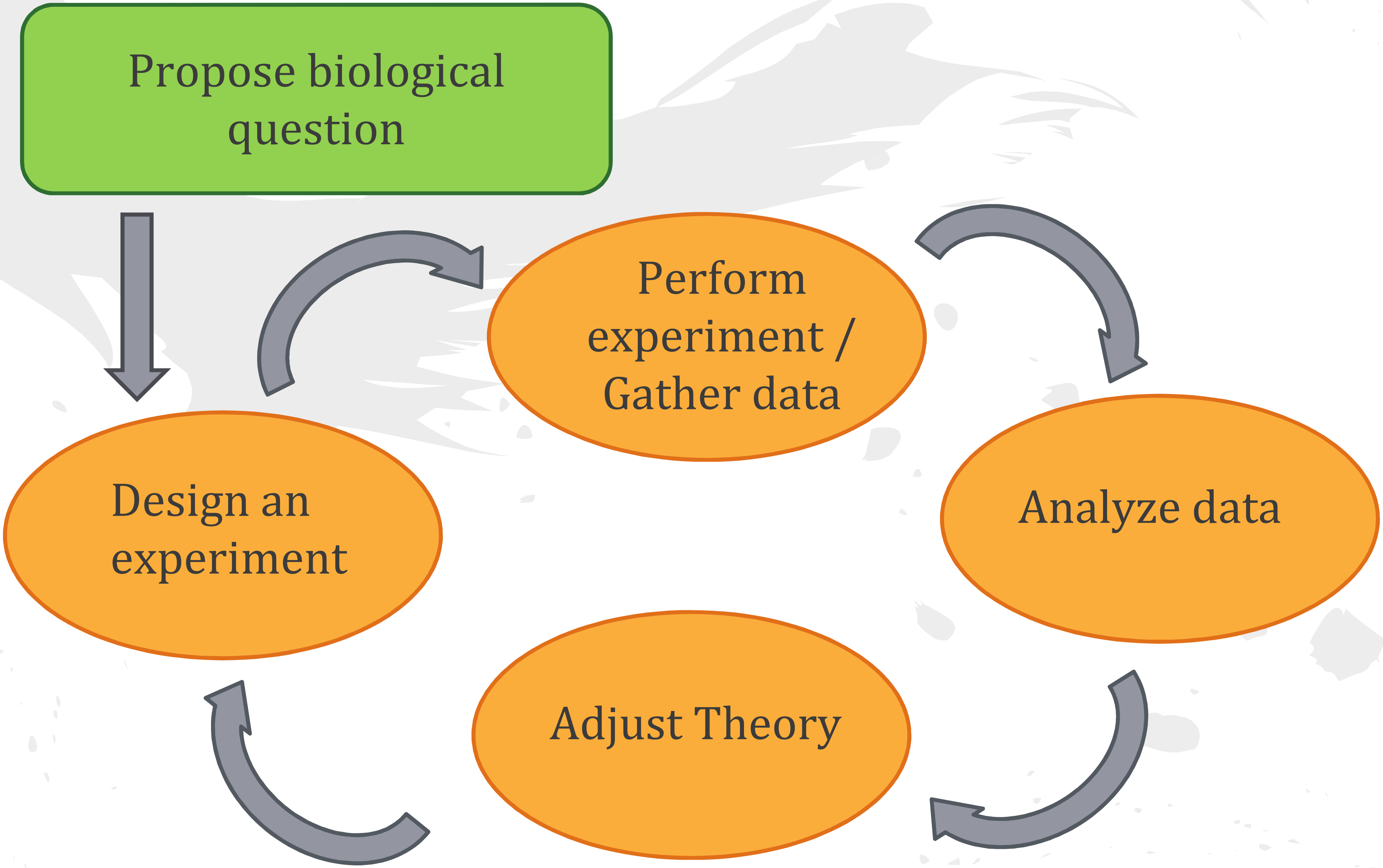
SUFU

Nucleus

Cytoplasm

Cancers
(Glioblastoma
Multiforme)

Where is the math?



Propose biological question

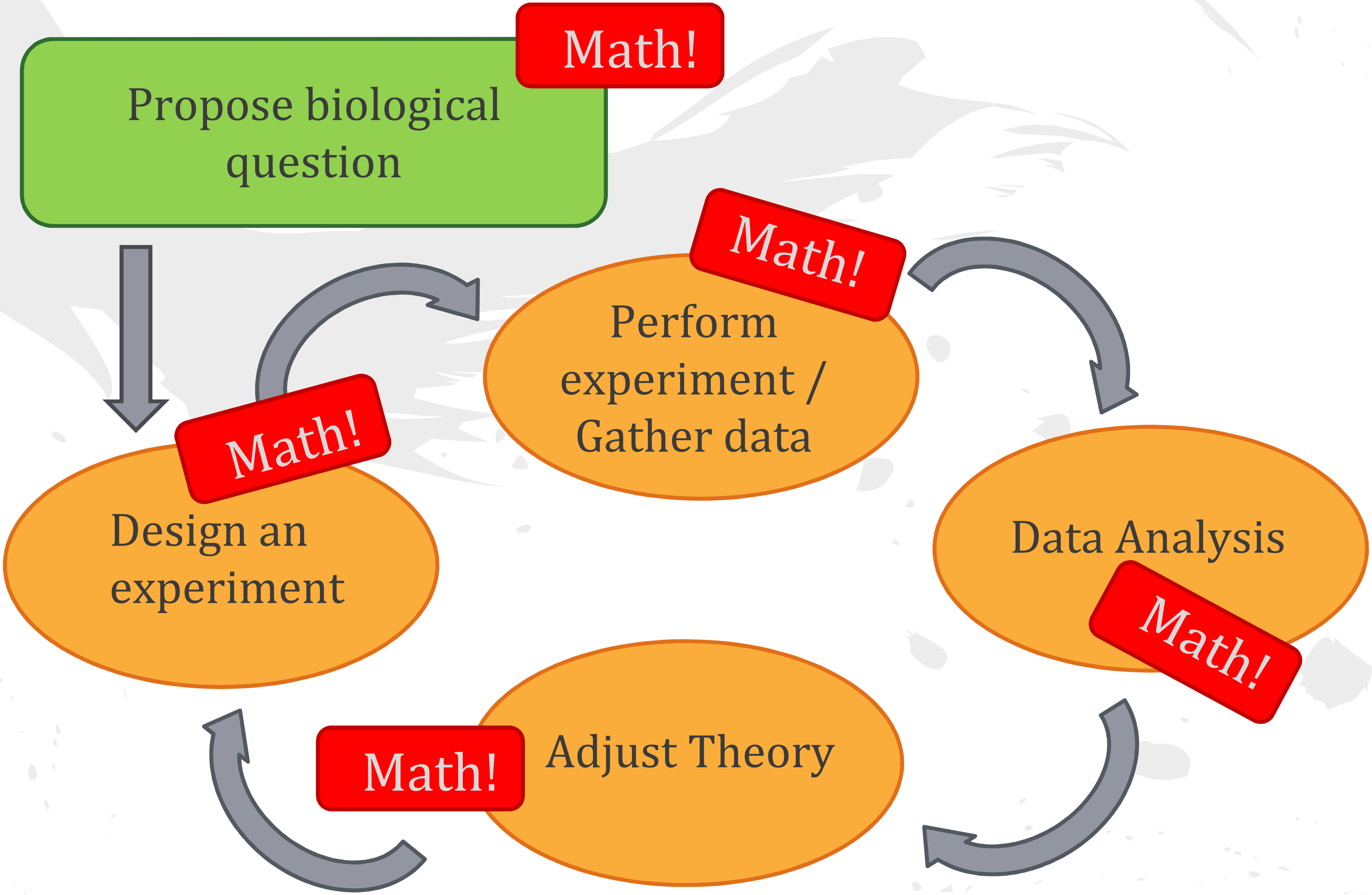
Design an experiment

Perform experiment / Gather data

Analyze data

Adjust Theory

Where is the math?



Propose biological question

Math!

Design an experiment

Math!

Perform experiment / Gather data

Math!

Data Analysis

Math!

Adjust Theory

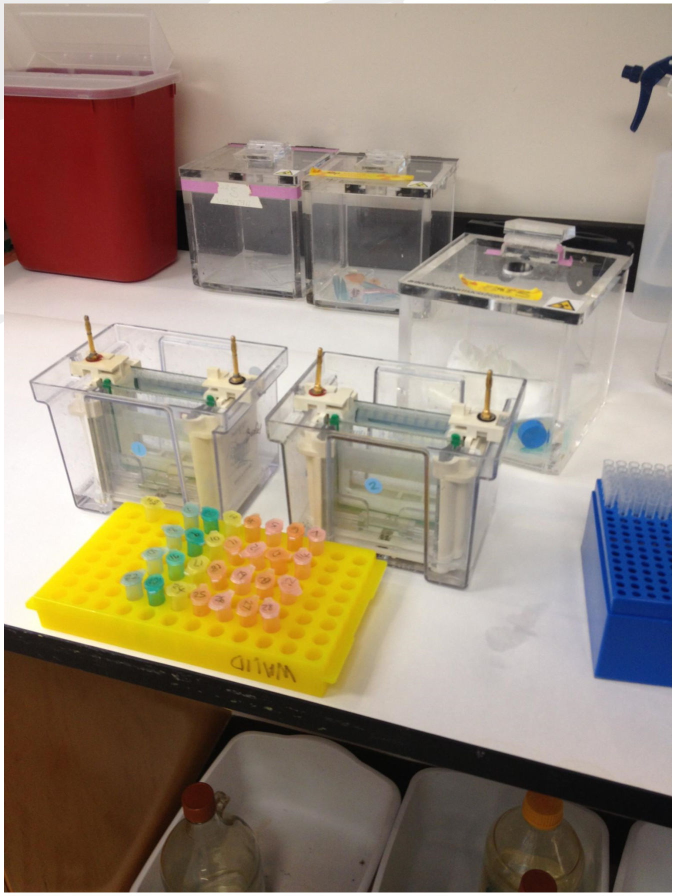
Math!

Our Research

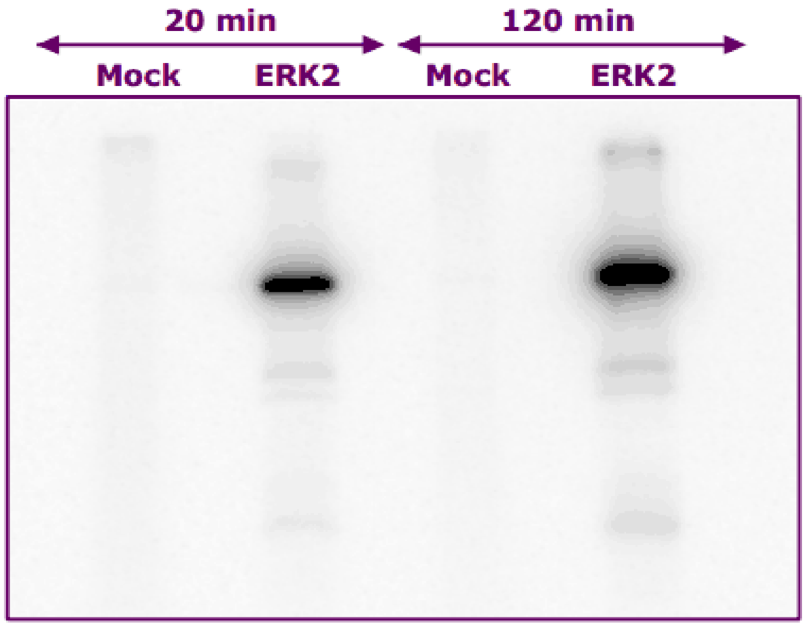
In order to understand the Erk2-Gli1-Sufu system we propose a novel method of biochemical modeling using Holling Type-II non-linear interactions from ecology.

Experimental Design – Bardwell Lab

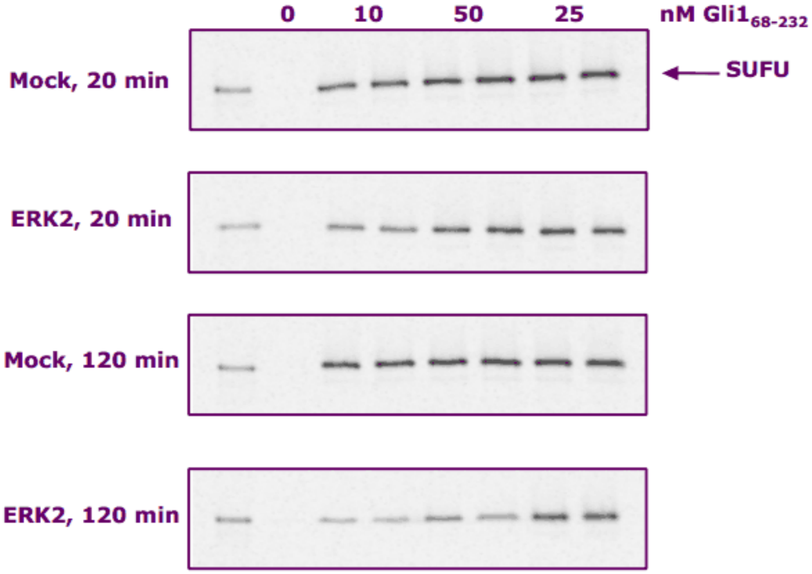
- Want Gli1$_{68\text{-}232}$ (has Sufu binding site)
  - CDNA Cloning
  - Radioactive tagging Sufu
- Titrations of Gli1 and Erk1
- Analyze with protein binding assays
  - Gel electrophoresis



Data Collection

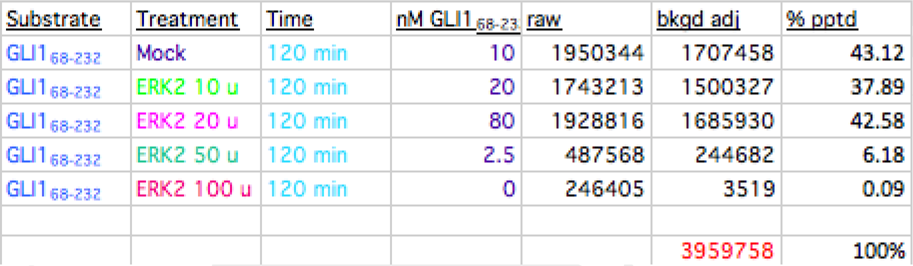


Gli1$_{68-232}$ kinase with Erk2

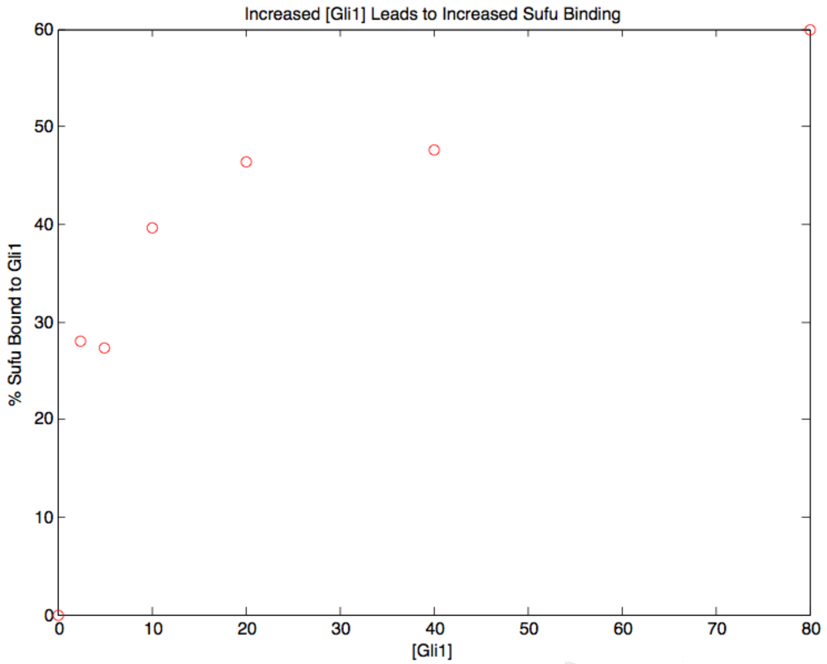


Gli1$_{68-232}$ binding to Sufu

Data Translation



Substrate	Treatment	Time	nM GLI1$_{68-23}$	raw	bkgd adj	% pptd
GLI1$_{68-232}$	Mock	120 min	10	1950344	1707458	43.12
GLI1$_{68-232}$	ERK2 10 u	120 min	20	1743213	1500327	37.89
GLI1$_{68-232}$	ERK2 20 u	120 min	80	1928816	1685930	42.58
GLI1$_{68-232}$	ERK2 50 u	120 min	2.5	487568	244682	6.18
GLI1$_{68-232}$	ERK2 100 u	120 min	0	246405	3519	0.09
					3959758	100%



Initial Model: Mass-Action

$$\mathrm{S} + \mathrm{G} \xleftrightarrow[k_{off}]{k_{on}} \mathrm{C}$$

S = [Sufu]   G = [Gli1]   C = [Bound Complex]

k_{on} , k_{off} = 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

$$G' = -k_{on}SG + k_{off}C = 0$$

$$-k_{on}(S_t - C)G + k_{off}C = 0$$

$$-(S_t - C)G + k_dC = 0$$

$$k_dC = (S_t - C)G$$

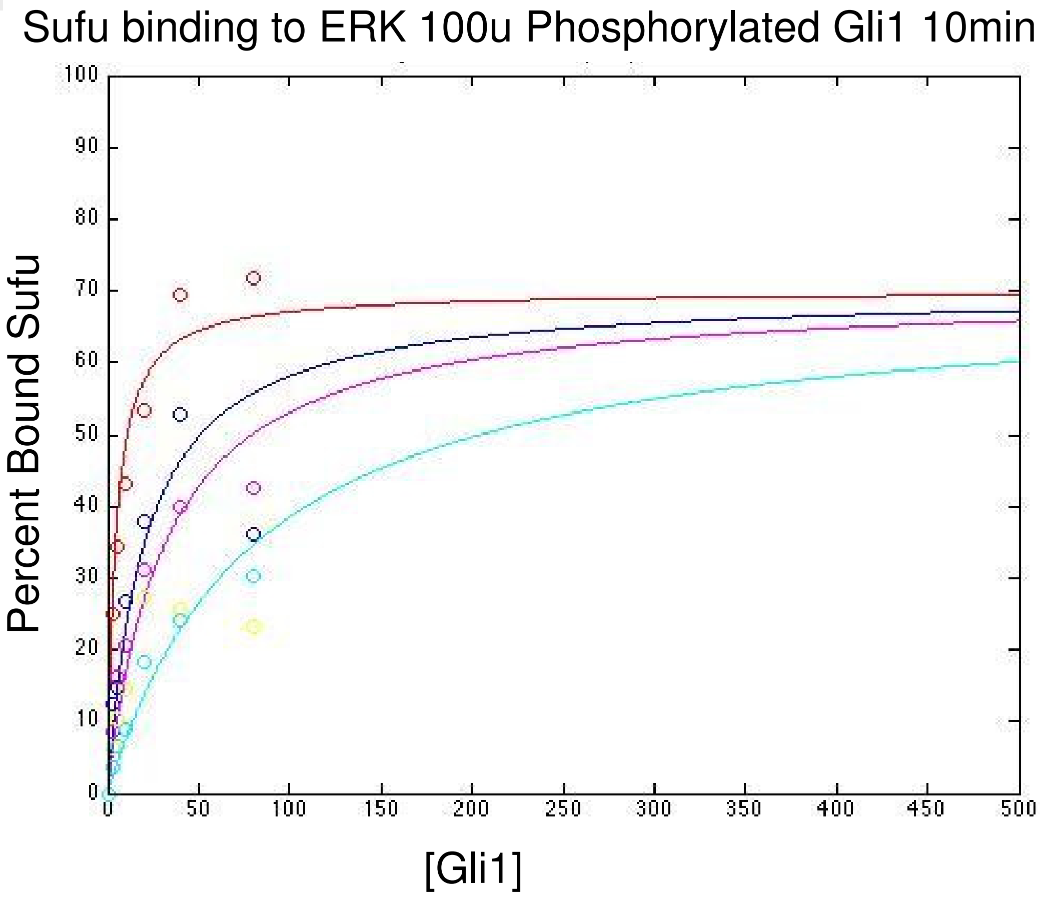
$$k_dC + GC = S_tG$$

$$C(k_d + G) = S_tG$$

$$C = \frac{S_tG}{(k_d + G)}$$

$$\%SufuBound = \frac{C}{S_t} = \frac{G}{(k_d + G)}$$

Curve fitting: Mass-Action



Equal saturation: R>0.64
Variable saturation: R>0.93

Least-squares fit (Matlab) on:

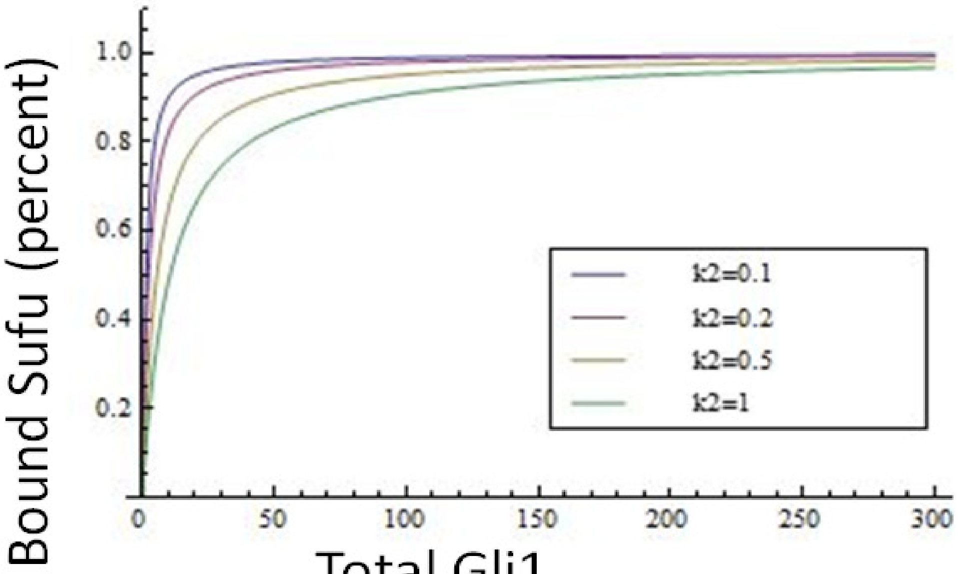
$$\frac{G}{(k_d + G)}$$

This requires forced saturation level, "S_{max}", to be identified. We tested

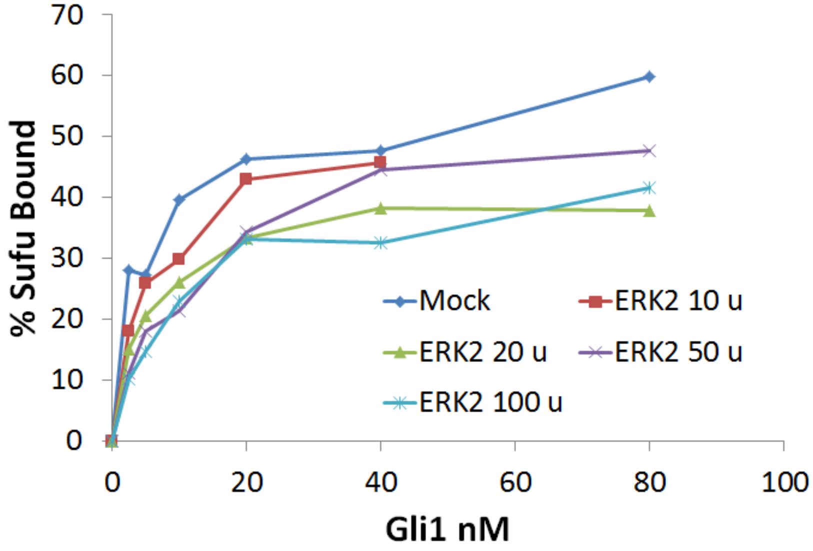
- Equal saturation
- Variable saturation

Mass-Action Dynamics

- Assumes all curves saturate to the same level, 100% of $_{\text{Smax}}$
- Occurs regardless of k_{on}, k_{off} parameters

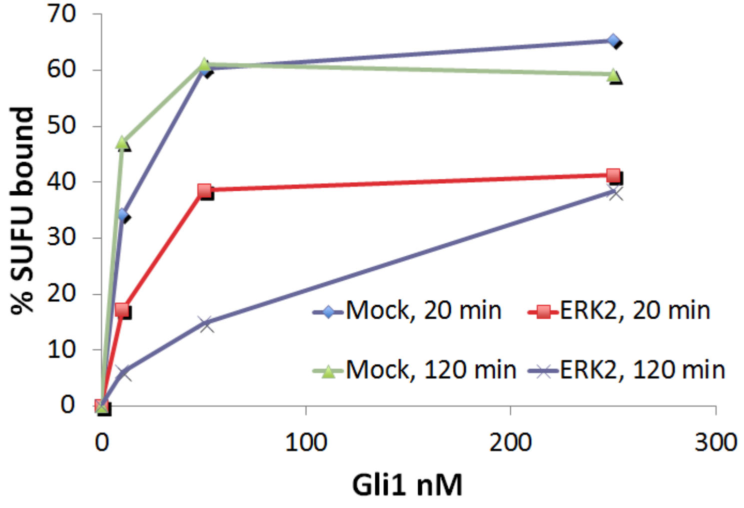


Identifying Saturation Levels



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

$$\mathrm{S} + \mathrm{G} \xrightarrow[k_2]{k_1} \mathrm{C} \qquad\qquad 2G \overset{k_3}{\underset{k_4}{\leftrightarrow}} D$$

$$S' = -k_1 SG + k_2 S$$

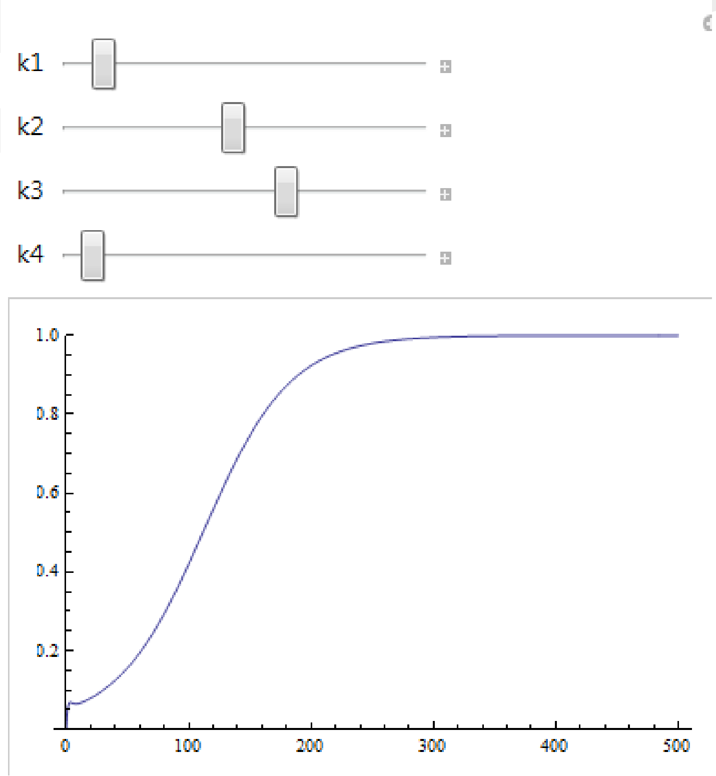
$$G' = -k_1 SG - k_3 G^2 + 2k_4 D + k_2 C$$

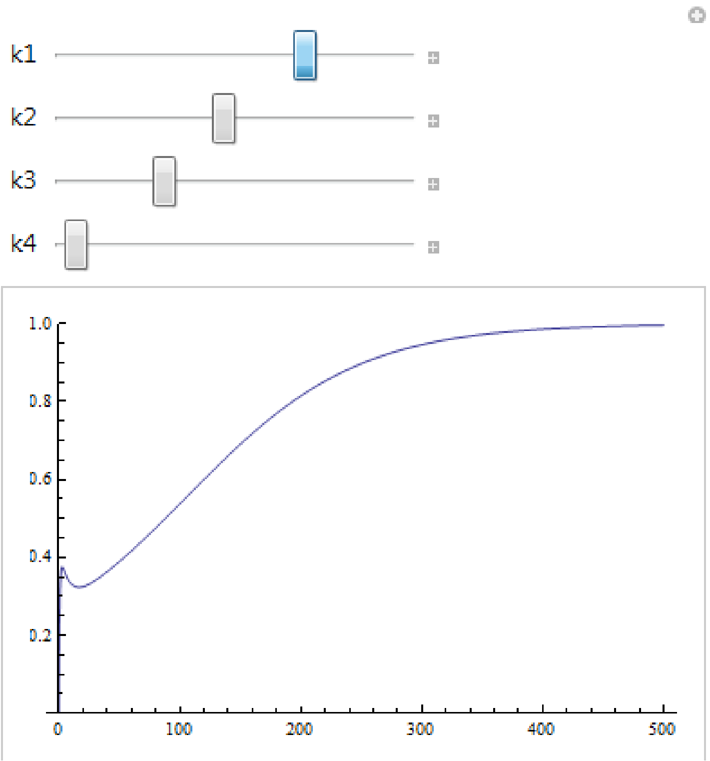
$$C' = -k_2 C + k_1 SG$$

$$D' = -k_4 D + k_3 G^2$$

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

$$\mathrm{S} + \mathrm{G} \xrightleftharpoons[k_{off}]{k_{on}} \mathrm{C}$$

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

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

- General form remains the same
- Gli1 and Sufu binding rate taken to be non-linear
- Binding rate changes based on τ, time delay between Gli1-Sufu initial interaction and successful binding

Steady-State Analysis: Non-Mass Action

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

• • •

Equations are a little bit more complex:

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

$$\%SufuBound = \frac{G_t k_{on} + k_{off} + k_{on}S_t + G_t k_{on}k_{off}\tau + \sqrt{4G_t k_{on}S_t(-k_{on} - k_{on}k_{off}\tau) + (G_t k_{on} + k_{off} + k_{on}S_t + G_t k_{on}k_{off}\tau)^2}}{2(k_{on} + k_{on}k_{off}\tau)}$$

Steady-State Analysis: Non-Mass Action

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

…

Equations are a little bit more complex:

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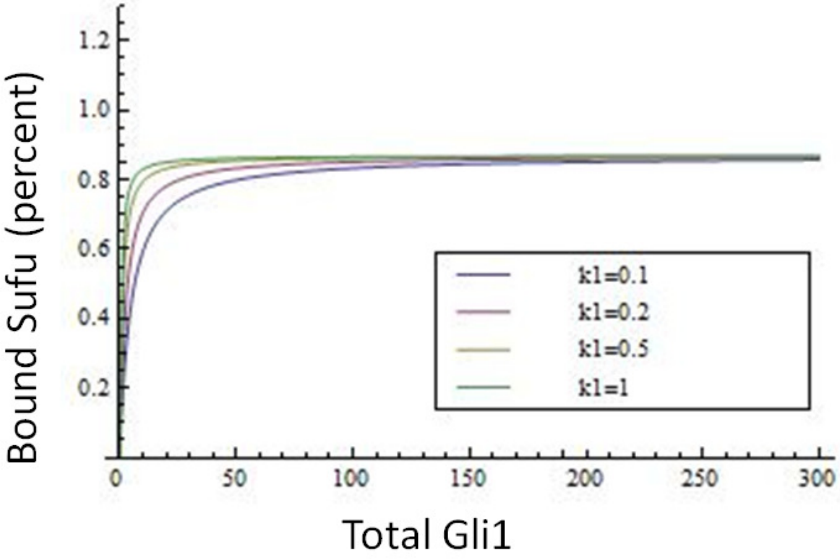
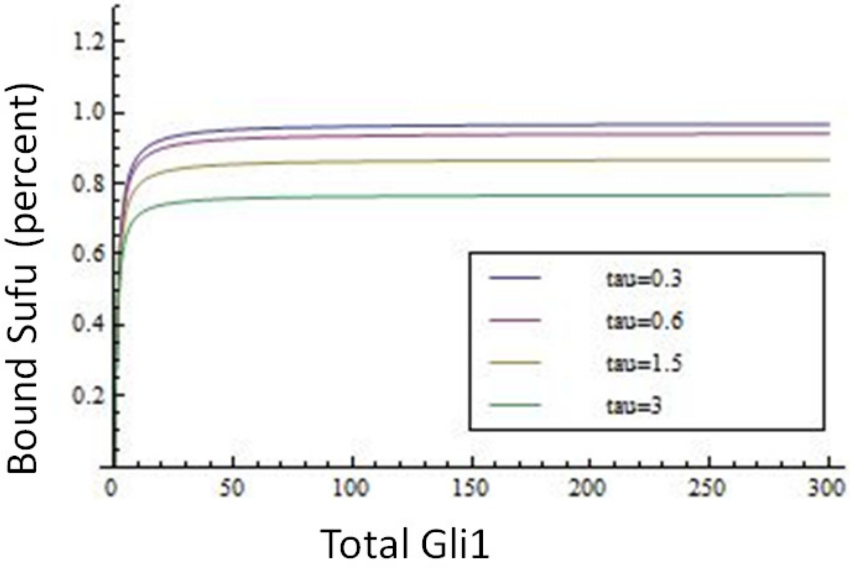
$$\%SufuBound = \frac{G_t k_{on} + k_{off} + k_{on}\boxed{S_t} + G_t k_{on}k_{off}\tau + \sqrt{4G_t k_{on}\boxed{S_t}(-k_{on} - k_{on}k_{off}\tau) + (G_t k_{on} + k_{off} + k_{on}\boxed{S_t} + G_t k_{on}k_{off}\tau)^2}}{2(k_{on} + k_{on}k_{off}\tau)}$$

Notice total Sufu becomes important, along with individual k_{on}, k_{off}

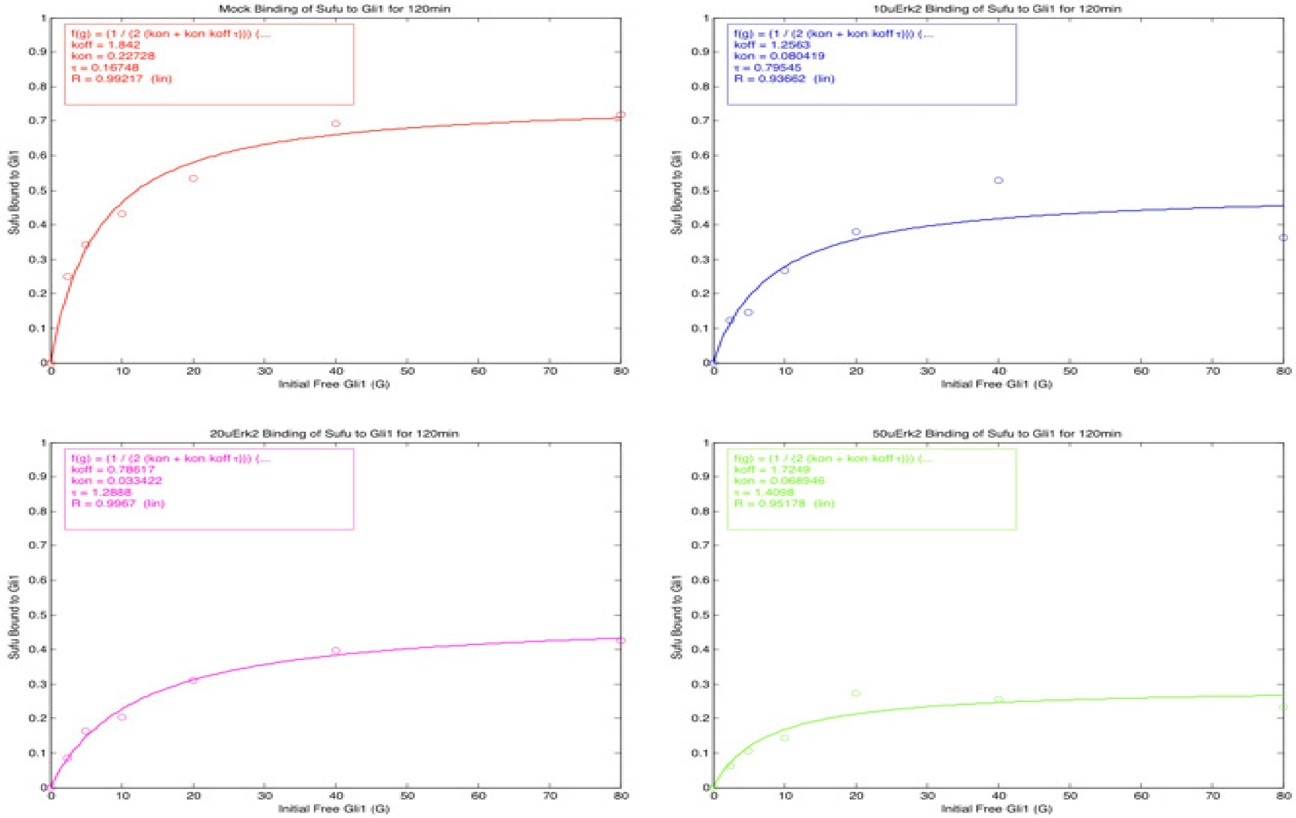
Steady-State Dynamics





- Non-linear system allows for variable saturation levels. Increasing τ lowers total saturation level bound Sufu. 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



Percent bound Sufu

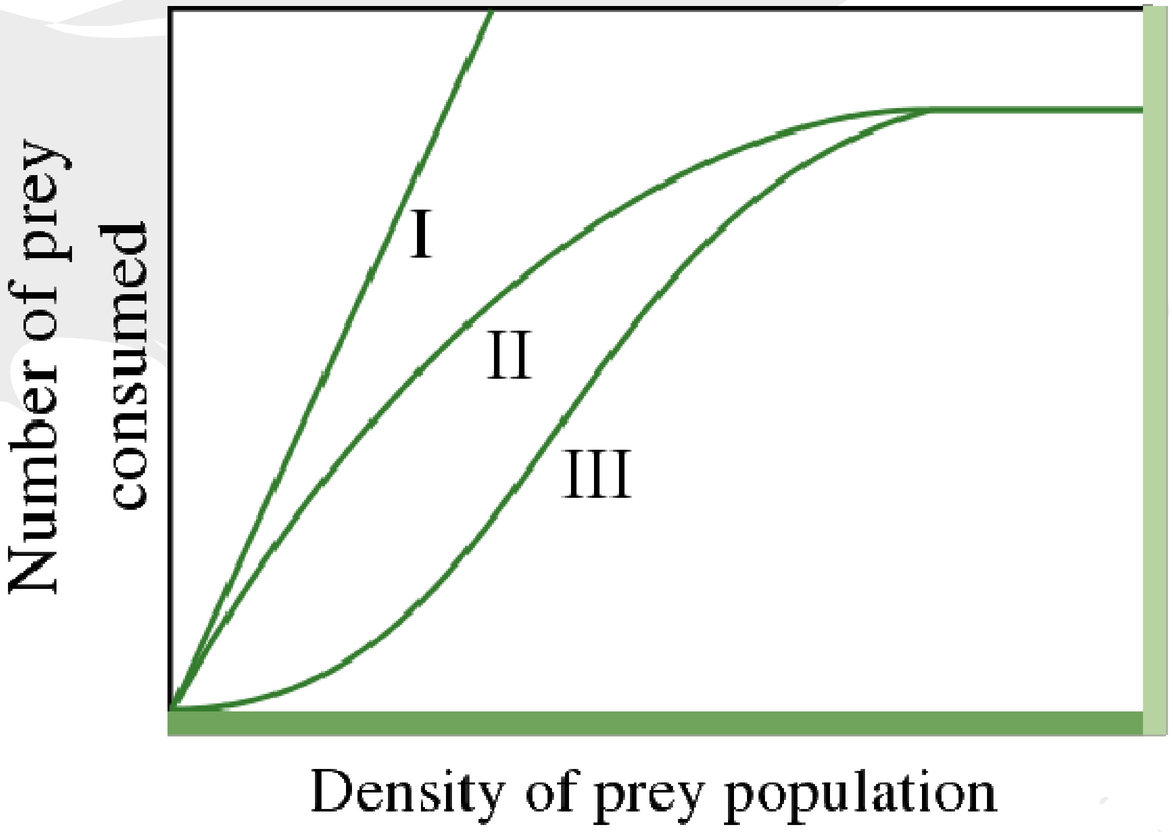
Concentration Gli1 total

(R>0.94
for all)

$$\frac{G_t k_{on} + k_{off} + k_{on} S_t + G_t k_{on} k_{off} \tau + \sqrt{4 G_t k_{on} S_t (-k_{on} - k_{on} k_{off} \tau) + (G_t k_{on} + k_{off} + k_{on} S_t + G_t k_{on} k_{off} \tau)^2}}{2(k_{on} + k_{on} k_{off} \tau)}$$

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

Δt: some time interval

y: # binding per sufu molecule in time interval Δt

$r = \frac{y}{\Delta t} S$

Non Mass-Action

τ: binding time per sufu molecule

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

...

$y = \frac{k_{on1} G}{1 + k_{on} G\tau} \Delta t$

$$r = \frac{1}{tau} \frac{G}{\frac{1}{k_{on}\tau} + G} S$$

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

$$= -k_{on} \frac{G}{1 + k_{on}\tau G} S$$

Binding Rate Comparison

$$\mathrm{S} + \mathrm{G} \xleftrightarrow[k_{off}]{k_{on}} \mathrm{C}$$

Mass-action binding rate:

$$r = k_{on} S G \longrightarrow G' = -k_{on} S G + k_{off} C$$

Proposed non-linear binding rate:

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

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 phosphoryation of Gli1 by Erk2 increases time delay, τ
- 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]

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References

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