# Modeling the G protein signal transduction system

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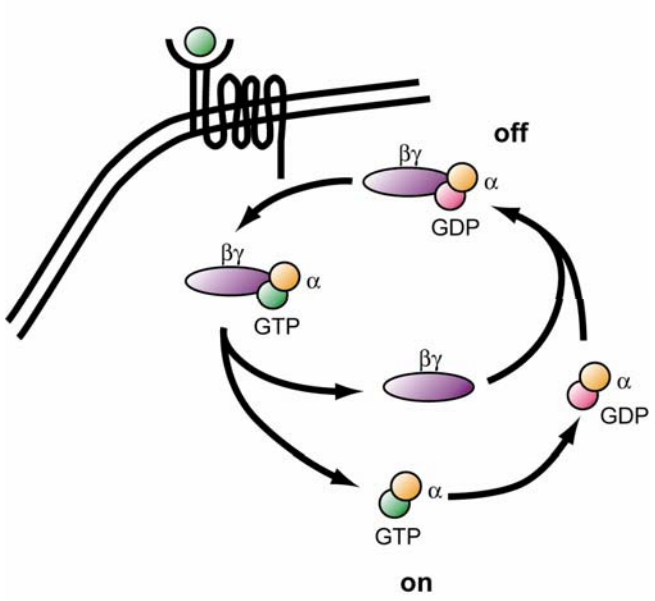
# G protein-coupled receptors

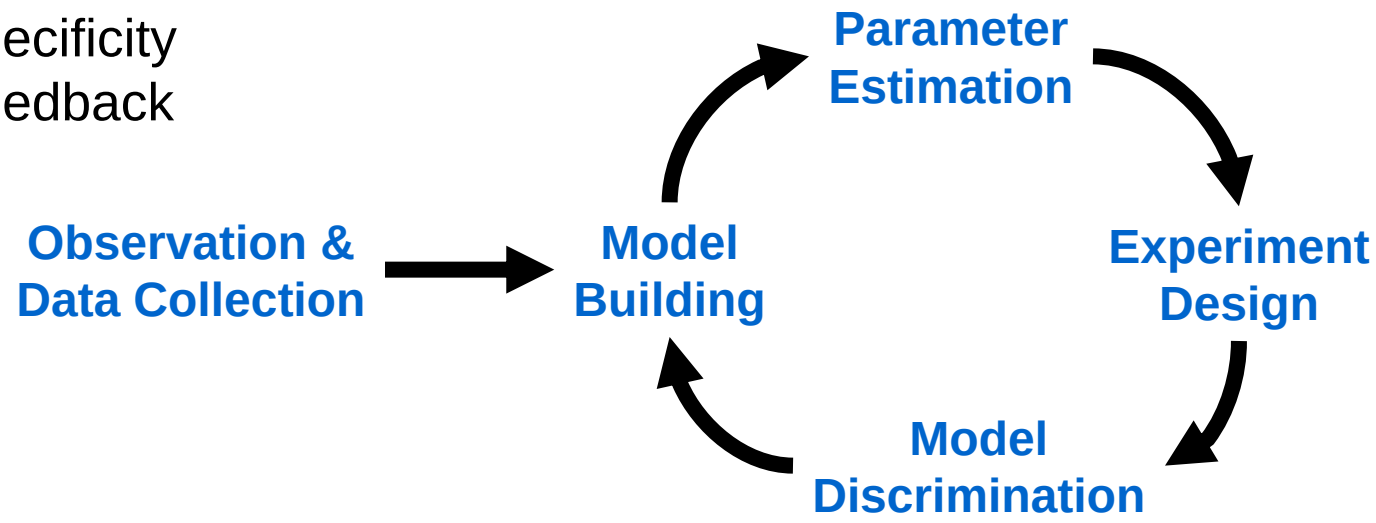
- Pharmacologically important
  - receptor system accounts for 40-50% of modern medicinal drug targets
  - only 10% of the known receptors are targeted by drugs
- GPCRs respond to: light, neurotransmitters, odorants, amino acids, hormones, nucleotides and chemokines
- Macrophage cells use chemokines and the GPCR system to locate and eliminate pathogens.

How does the GPCR system integrate and buffer signals from multiple receptors?



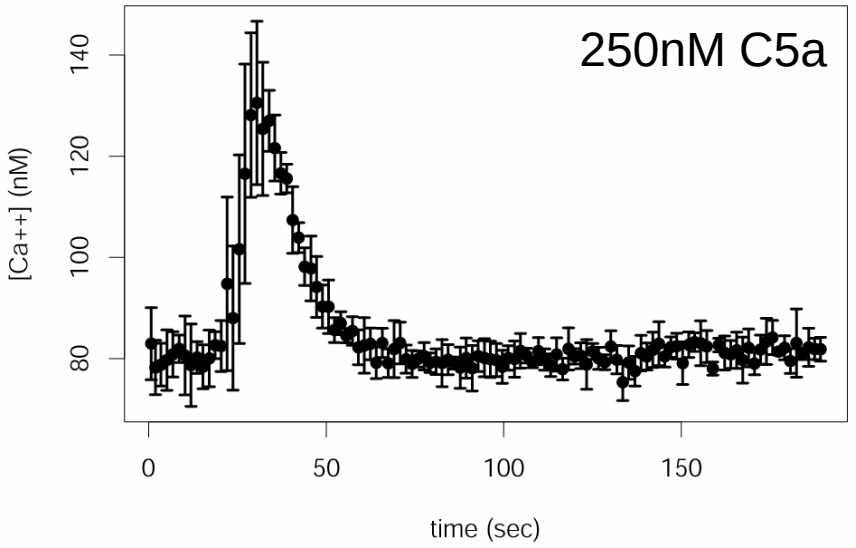
# Today we'll talk about…

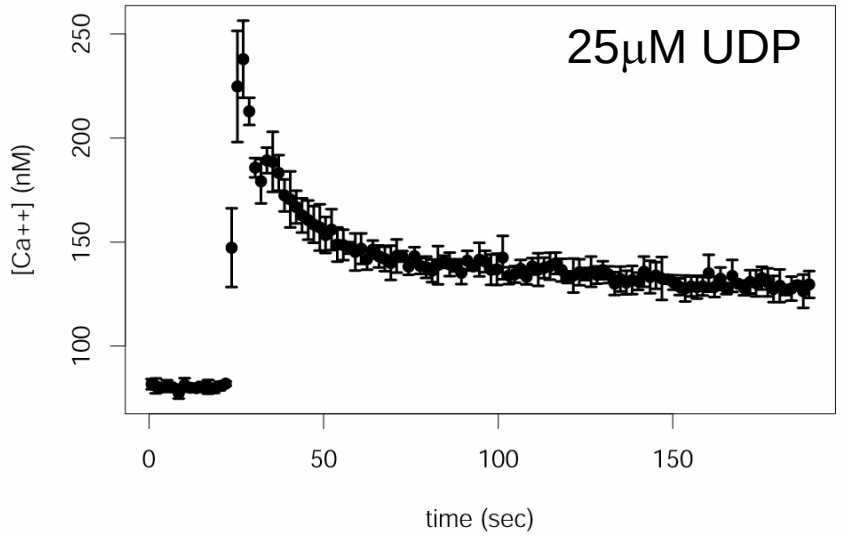
- Single Ligand Experiments (C5a, UDP)
  - wild-type cells
  - knockdown cell lines (GRK, Gαi2, Gαq, PLCβ3, PLCβ4)
- Model Structure & Statistical Methods
  - system components & structure
  - parameter inference
  - posterior prediction intervals
- Double Ligand Experiments
  - synergistic $Ca^{2+}$ release dose response to C5a+UDP
- Signal Transduction Mechanism
  - isoform specificity
  - delayed feedback



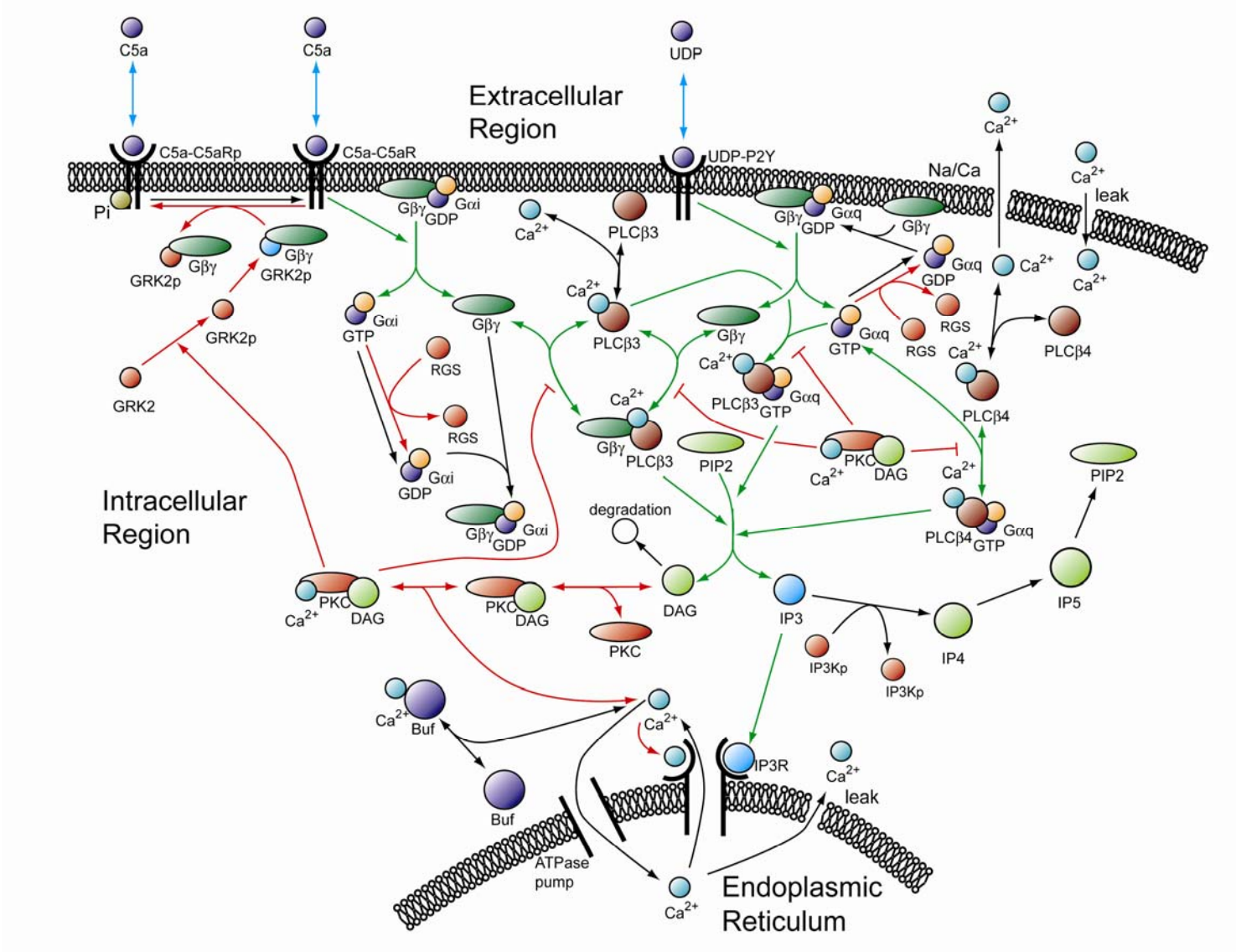
# Single Ligand Experiments

- RAW267.4 macrophage cells respond to C5a or UDP with a pulse of cytosolic calcium – measured with Fura-2.
- The response is qualitatively consistent among different labs and with primary cells.
- Macrophage cells are perturbed by shRNAi to investigate key signaling molecules

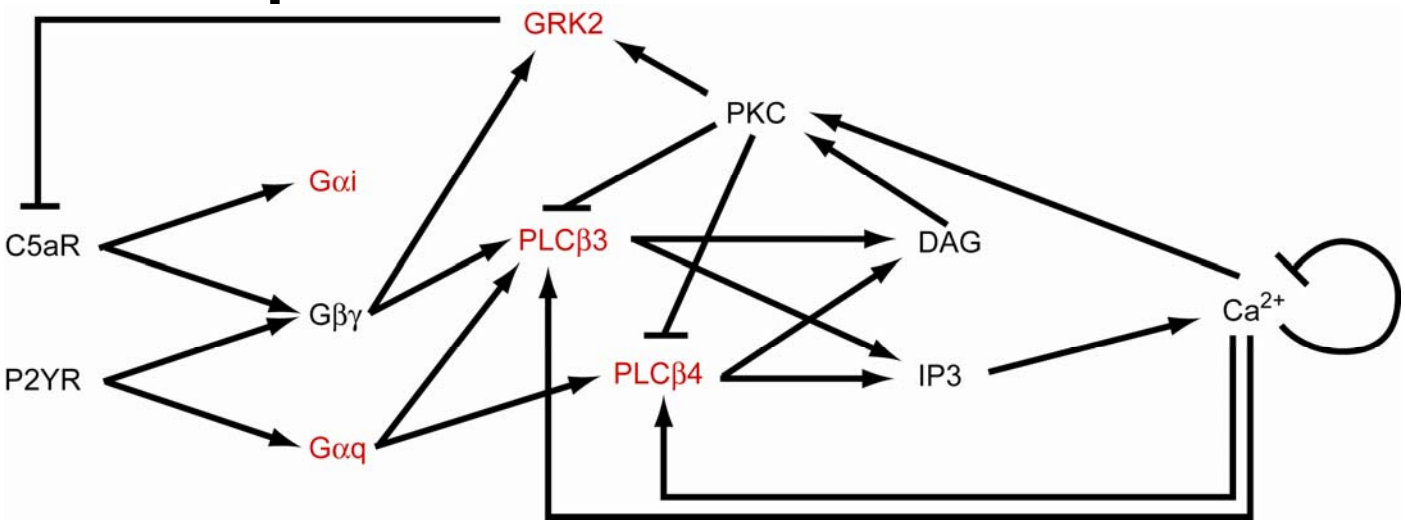


# GPCR Kinetic Model Structure

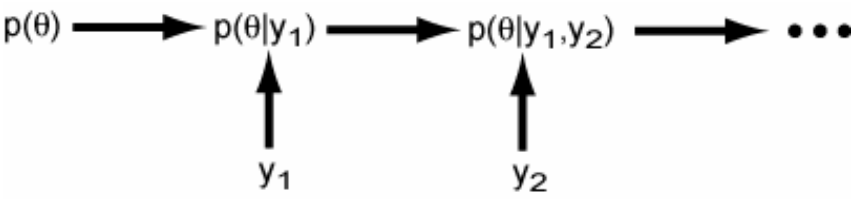


# Simplified Model Structure



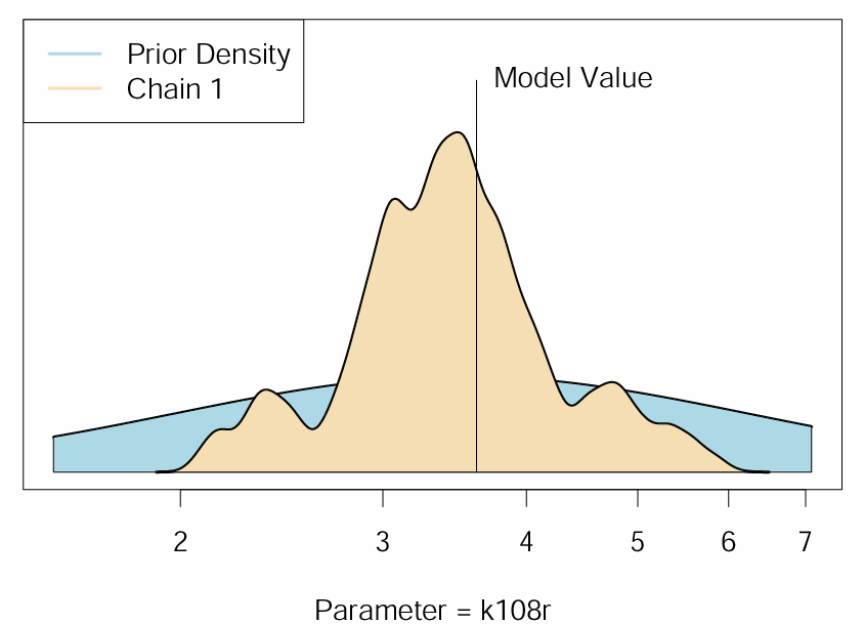

- Parts list: C5a receptor, P2Y6 receptor, Gαi, Gαq, Gβγ, PLCβ3, PLCβ4, GRK2, PKC, PIP2, DAG, IP3, IP3 receptor, $Ca^{2+}$, RGS, SERCA pump, $Na^{+}/Ca^{2+}$ exchanger, $Ca^{2+}$ buffer
- Almost every reaction is mass-action kinetics
- Each reaction is based on some evidence from literature
- 84 kinetic parameters and 50 species states in the system of ODEs

# Bayesian Inference



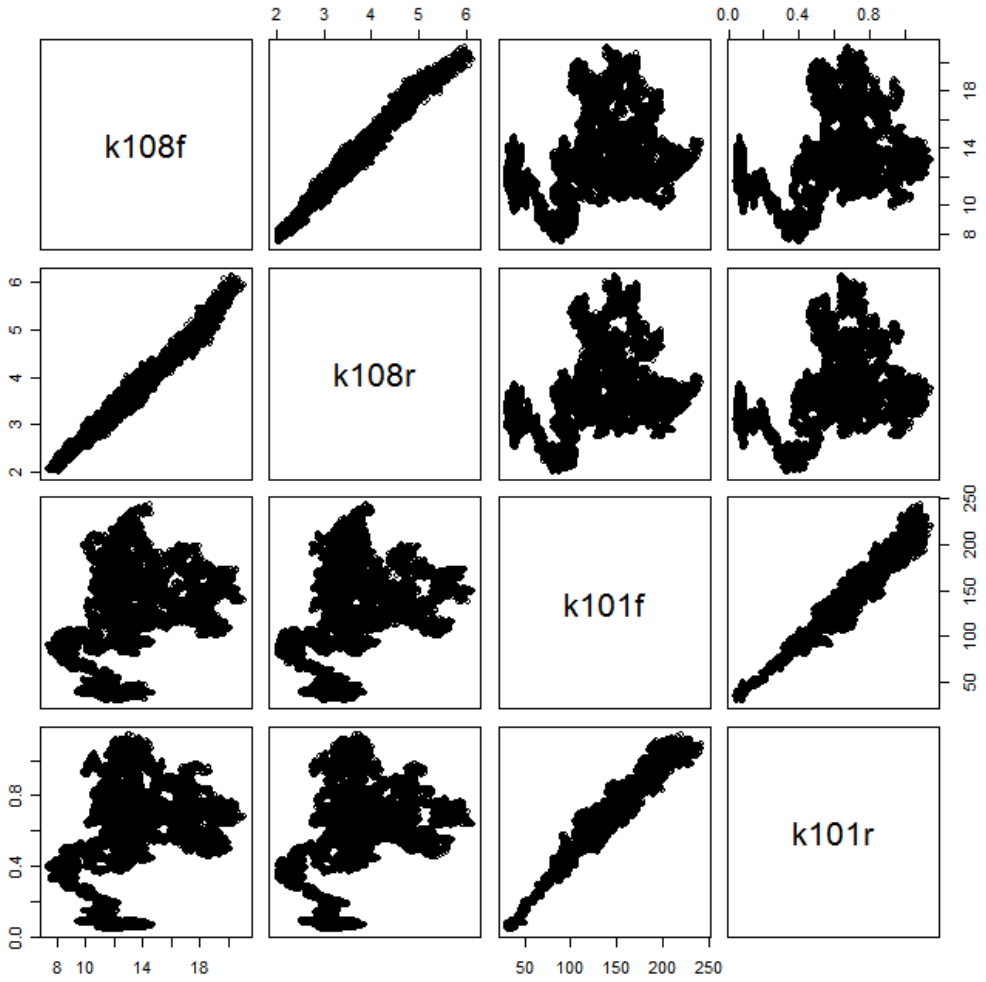

$$\Pr(\theta \mid y) = \frac{f(y \mid \theta)\Pr(\theta)}{\Pr(y)}$$

$$\Pr(y_{\text{new}} \mid y) = \int f(y_{\text{new}} \mid \theta)\Pr(\theta \mid y)\, d\theta$$

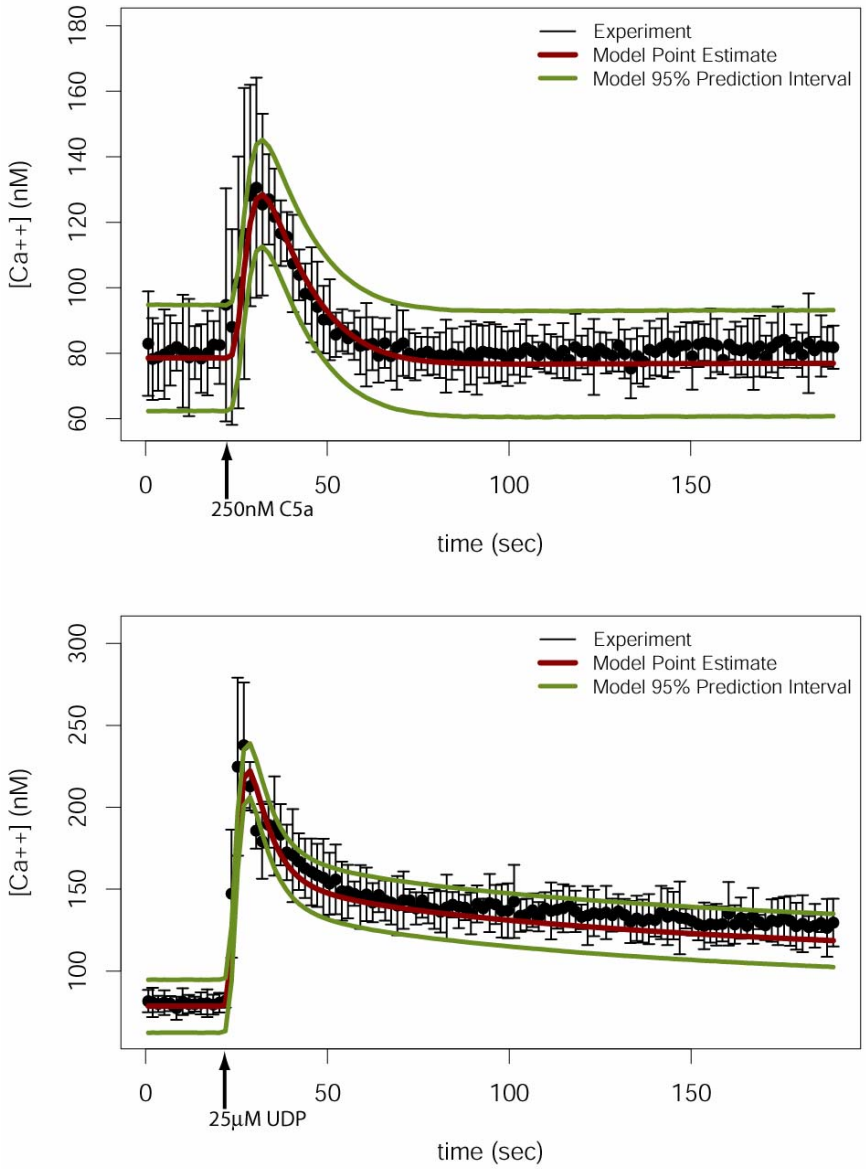



- Prior parameter information is coded as a prior density function.
- Single ligand data is used to obtain a posterior distribution over parameters.

# Posterior Parameter Correlation

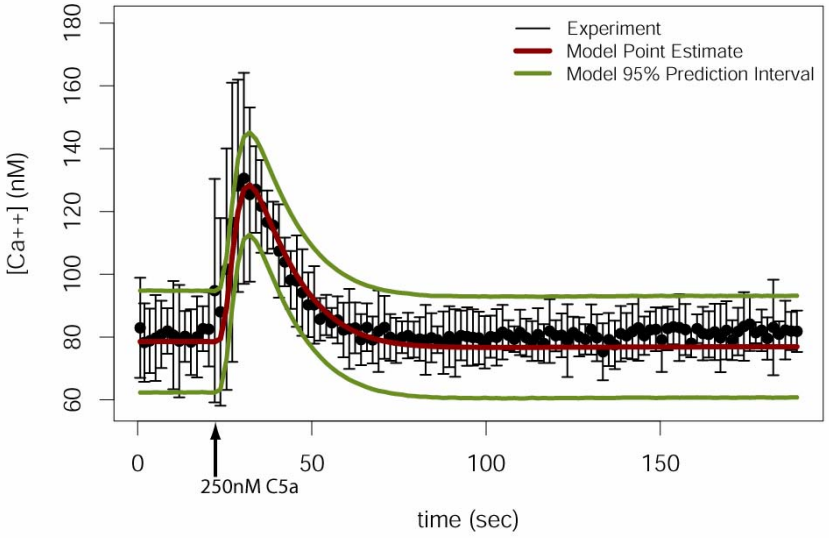


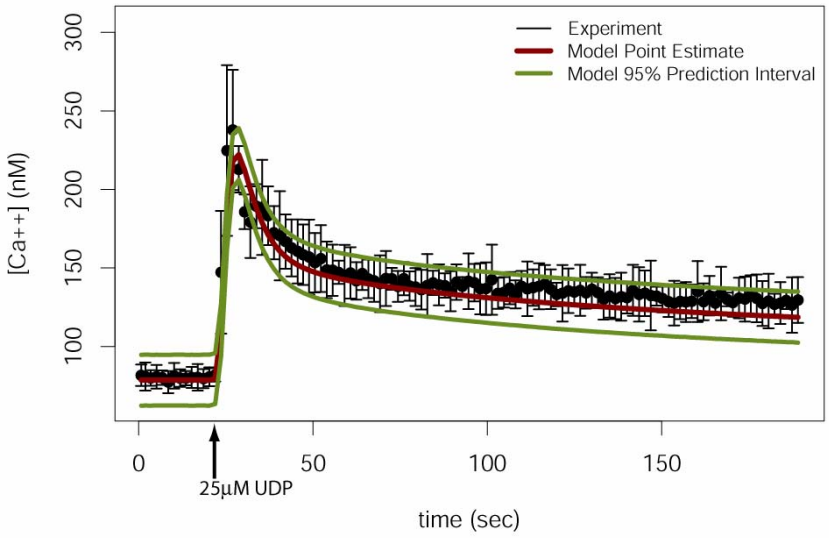
# Model Prediction Intervals

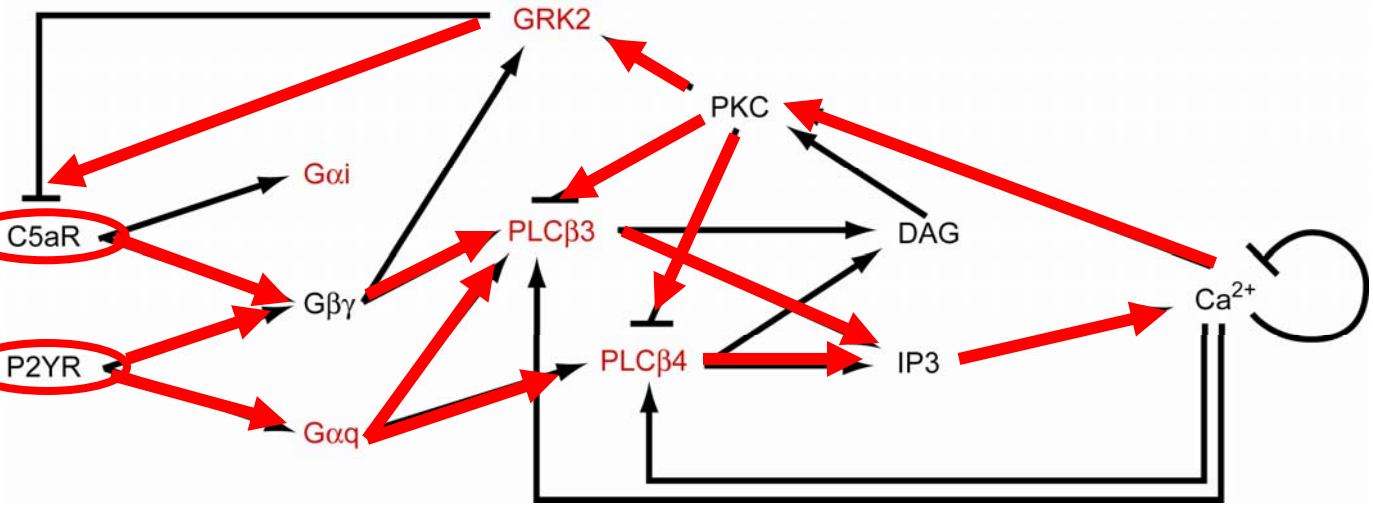



- Posterior parameter uncertainty and measurement uncertainty are combined to give prediction intervals.
- Prediction intervals are computed for complex experiments to quantify how surprising or consistent the data is with the model.

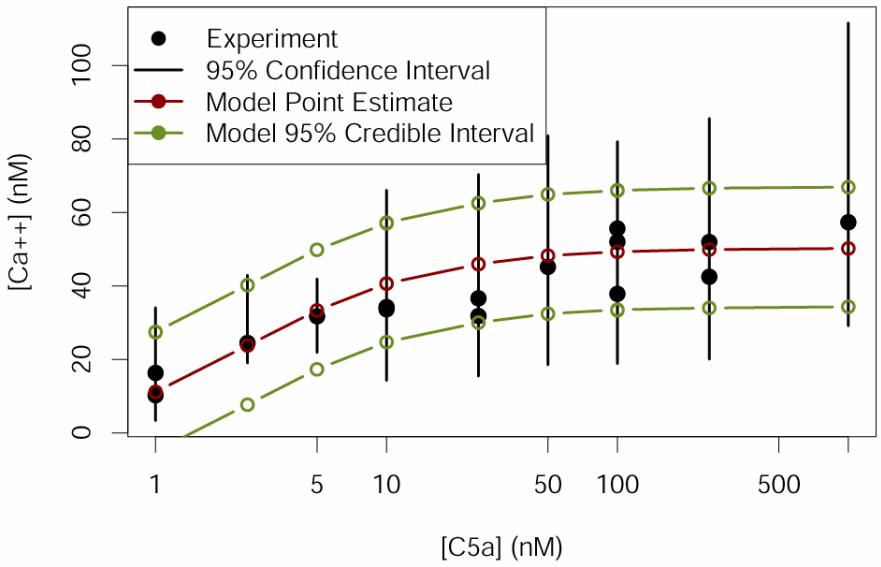
# Single Ligand - Wild-type cells

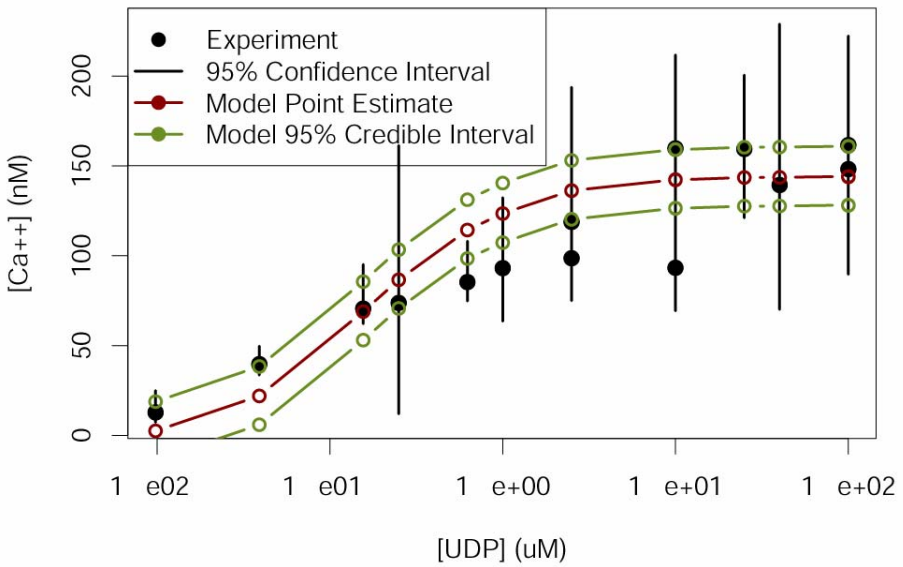


# Single Ligand Dose Response

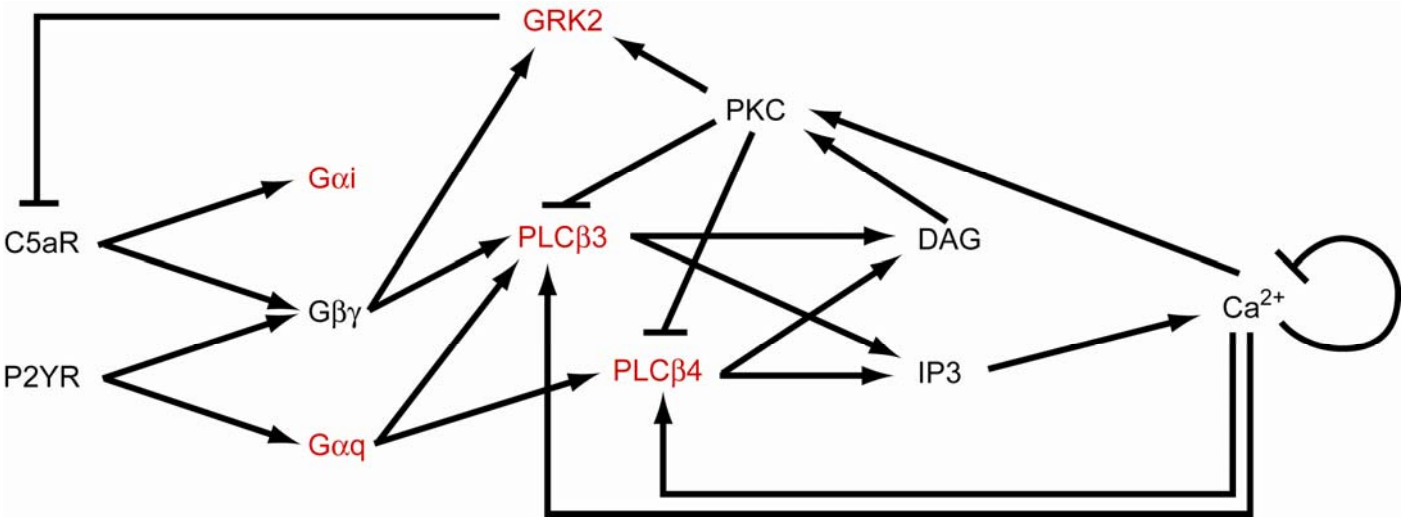



- Model fit to peak height data is within measurement error for both C5a and UDP.
- As expected, the calcium response saturates at a lower dose for C5a than for UDP

# Single Ligand - Knockdown Cells Data Set

| | | Fraction Knockdown | | | | |
|---|---|---|---|---|---|---|
| | Cell Line | qRT-PCR | Western | Nominal | Lower | Upper |
| GRK2 | 2 | 90% ± 7%, n=5 | 40% ± 6%, n=6 | 40.0% | 22.0% | 58.0% |
| Gαi2 | 3 | 83% ± 5%, n=4 | 73% ± 6%, n=5 | 73.0% | 55.0% | 91.0% |
| Gαq | 3 | 70% ± 8%, n=7 | 66% ± 23%, n=2 | 66.0% | 0.0% | 95.0% |
| PLCβ3 | 1 | - | 83% ± 15%, n=3 | 83.0% | 38.0% | 100.0% |
| PLCβ4 | 1 | 87% ± 6%, n=5 | - | 87.0% | 69.0% | 100.0% |



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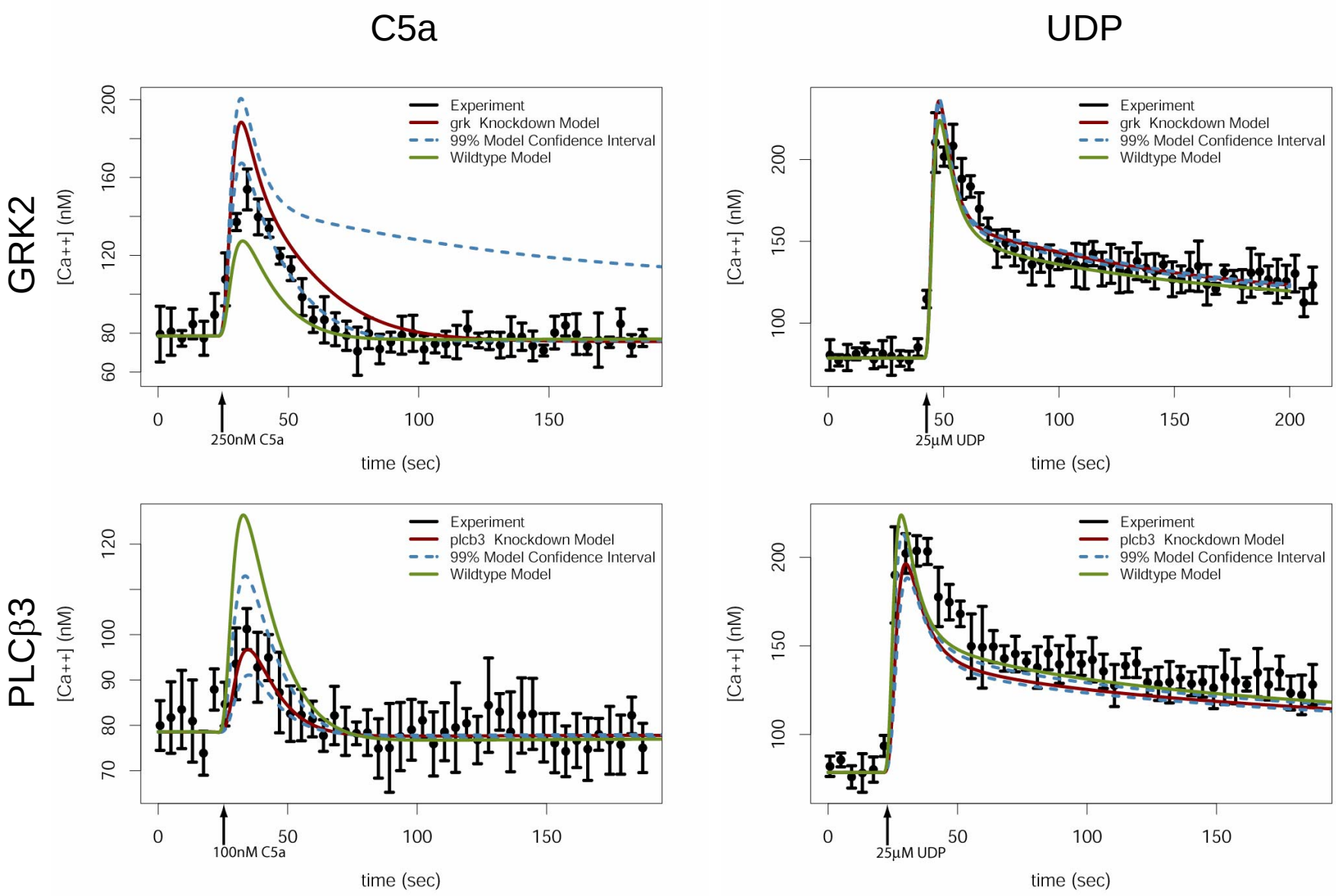

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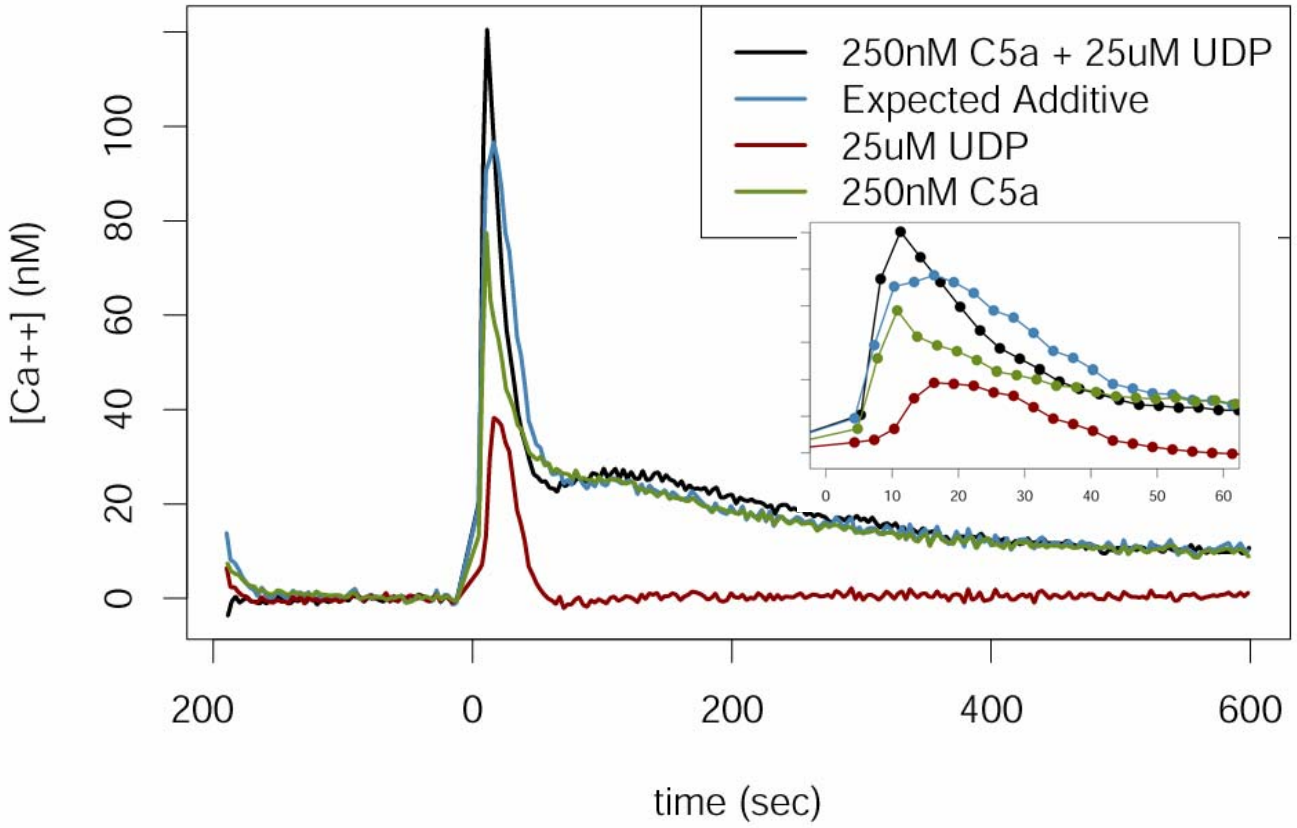
- Model Structure & Statistical Methods
  - system components & structure
  - parameter inference
  - posterior prediction intervals
- **Double Ligand Experiments**
  - **synergistic $Ca^{2+}$ release dose response to C5a+UDP**
- **Signal Transduction Mechanism**
  - **isoform specificity**
  - **delayed feedback**

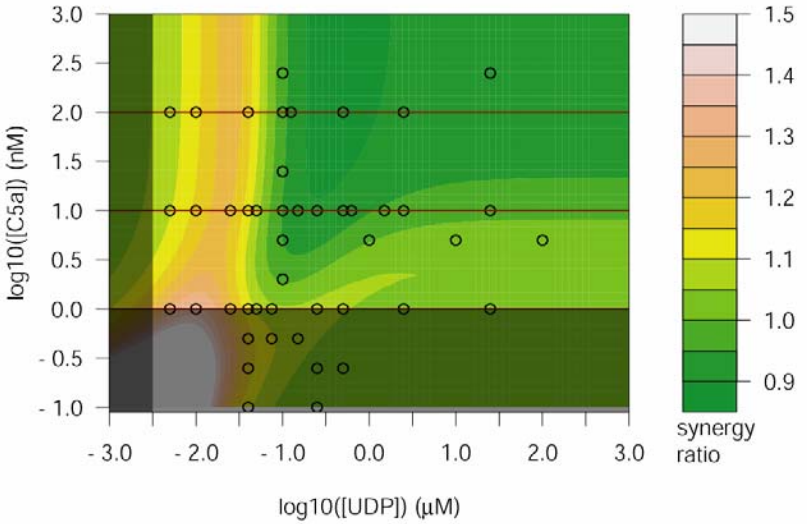
# Double Ligand Experiments

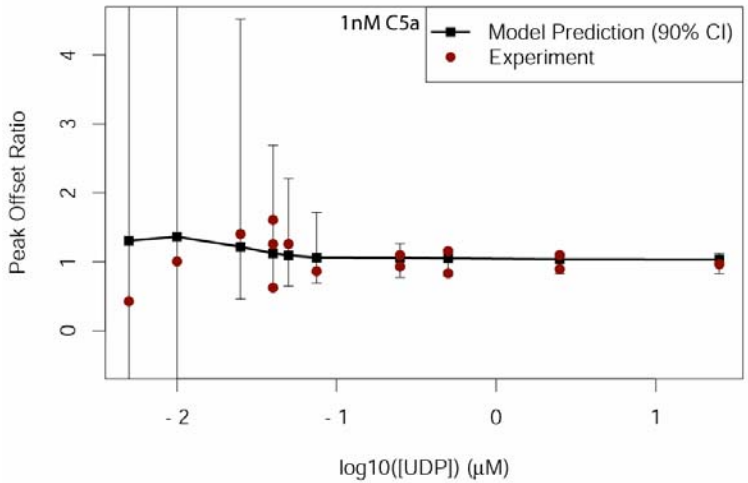
- Stimulating the cell with C5a and UDP simultaneously releases more $Ca^{2+}$ than expected if the effects were additive.

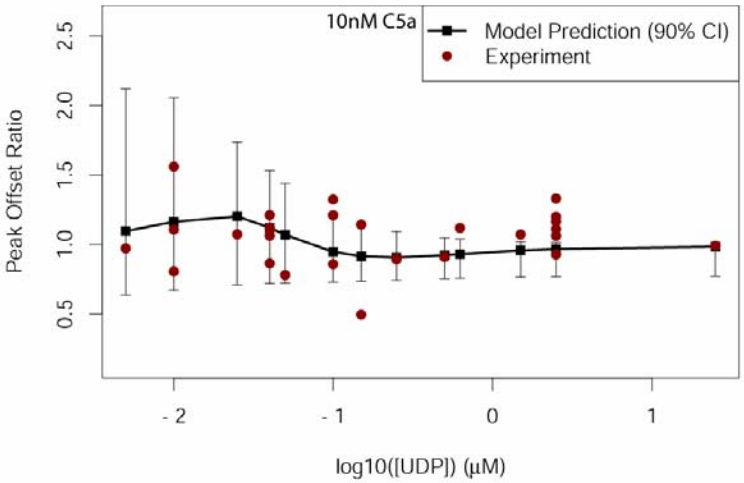


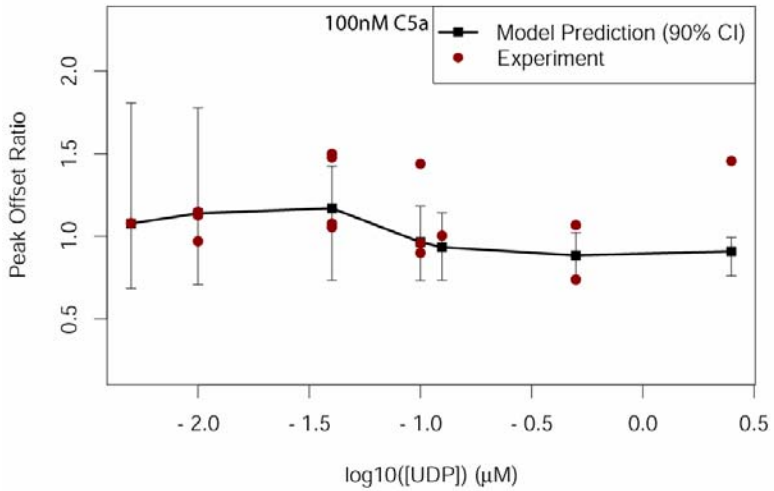

Peak Height Ratio=1.24

# Double Ligand Synergy Data



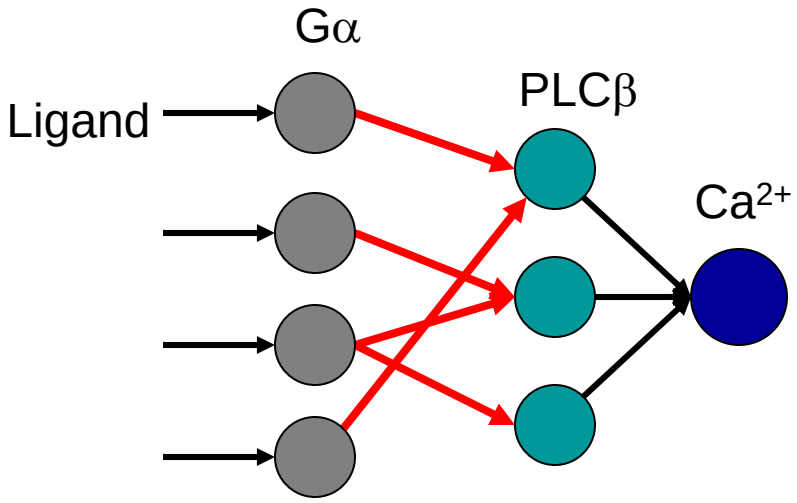




- Ridge in peak height synergy ratio is expected (from the model) for moderate UDP dose
- 90% prediction confidence intervals indicate where we expect 90% of the observation to fall based on the uncertainty in the model parameters and measurement variance

# Single & Double Ligand Summary

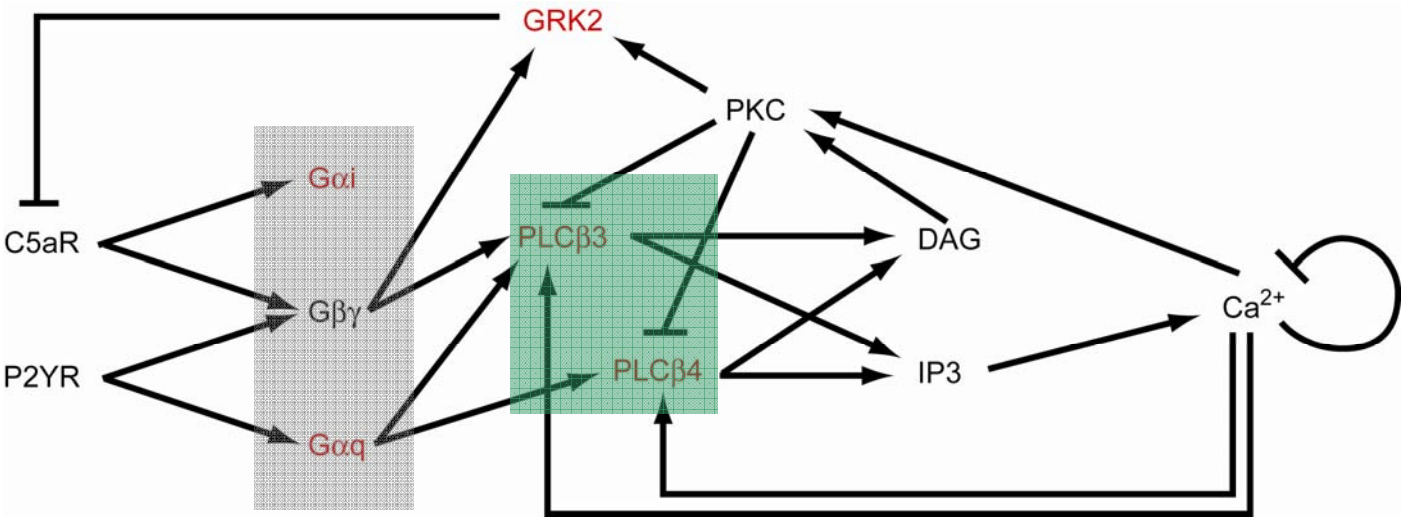
- We estimated the posterior parameter density using 96 experiments
  - wild-type and knockdown cell lines
  - range of doses of C5a and UDP
- The model fits the single ligand data
- We used the model to predict the double-ligand synergy ratio dose response surface
  - experimental data is consistent with our model

# Isoform Specificity

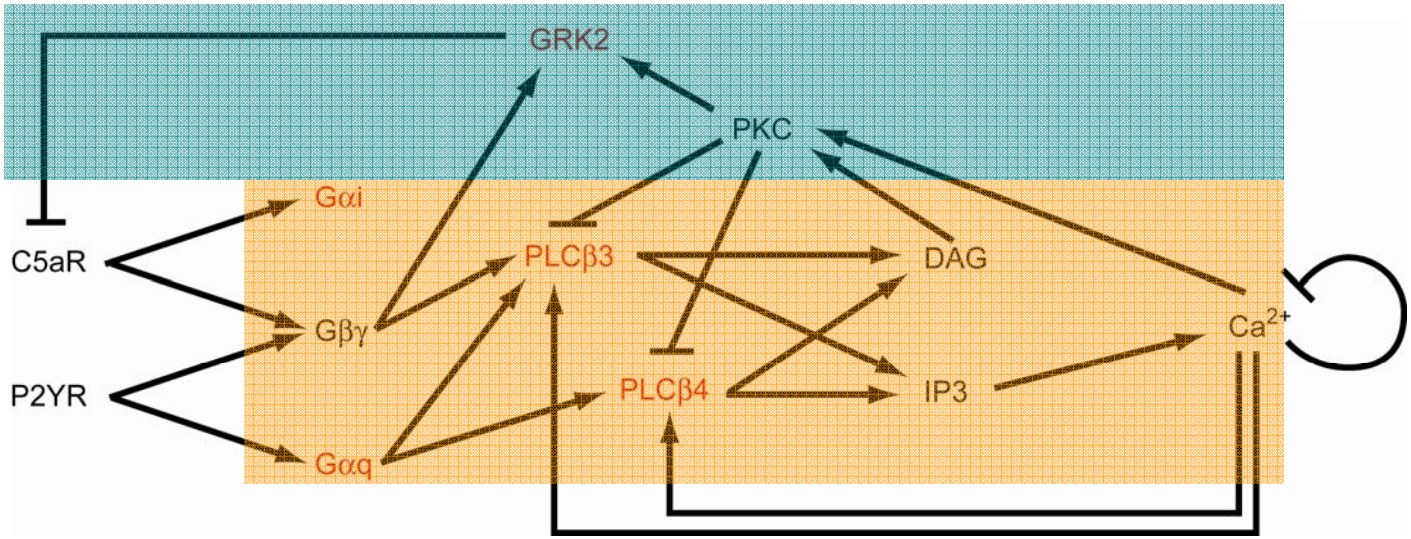



- Ligands feed into specific pattern of Gα/Gβγ subunits
- Gα and Gβγ isoforms are specific for PLCβ isoforms

The pattern of connections between Gα/Gβγ and PLCβ determines the shape of the calcium response.



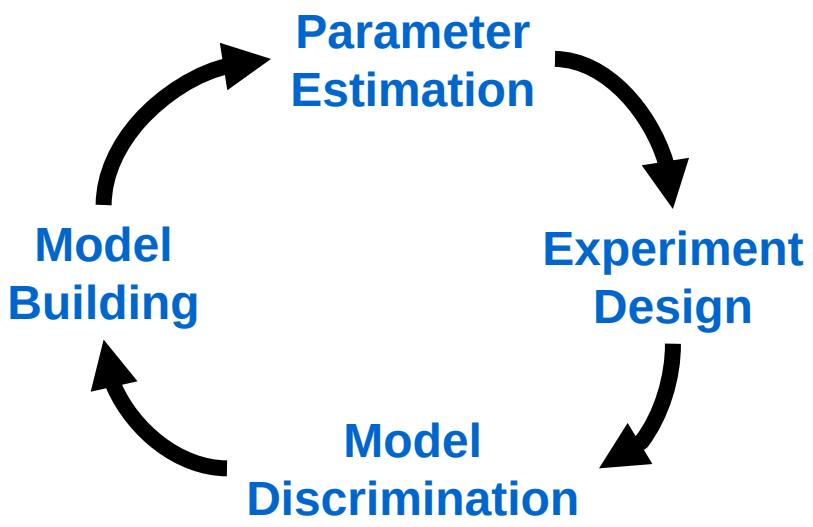
# Delayed Feedback




- When stimulate with C5a & UDP speed of signal propagation through feedforward direction is faster than with C5a or UDP alone.
- Speed of signal through feedback pathway is saturated.

# Summary

- At the outset we asked: How does the GPCR system integrate signals from multiple receptors?
- We layered a statistical model on our kinetic model to inform our parameter estimates.




- The model gave us predictions with confidence intervals for double ligand experiments and those experiment proved to be consistent with the model.
- We analyzed the model to understand how the system might exhibit signal buffering and synergy
  - isoform specificity
  - delayed feedback
- Our model is used as computational tool to aid in the design and analysis of experiments and to understand the interacting components of this complex system

# Recent Observations

**Data**

BMDM data

1. GRK2,5,6 desensitizes C5a receptor
2. PLCβ3 KO affects C5a and UDP signaling
3. PLCβ4 KO affects UDP signaling
4. dominant use of PLCβ3 by C5a, UDP, LPA and PAF
5. some dependence on PLCβ4 by UDP
6. All ligand/receptors tested that can couple with Gαq are able to synergize with Gαi-linked C5aR for an increased $Ca^{2+}$ response
7. sustained UDP response removed by EGTA

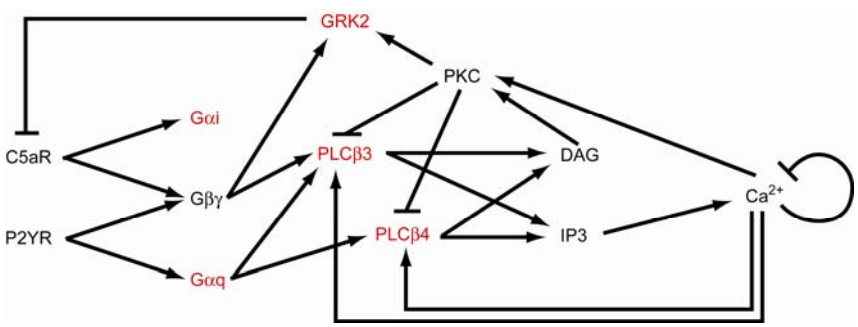
**Model**

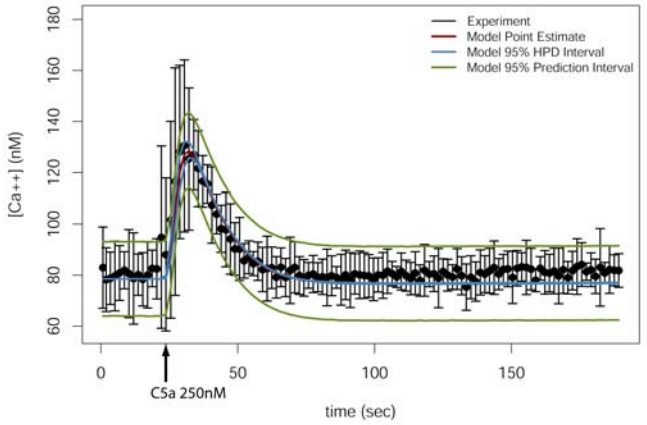
1. GRK2 desensitizes C5a receptor
   - model effect of knockdown is *greater* than observed effect
2. C5a & UDP signal through PLCβ3 by Gβγ
3. UDP signals through Gαq -> PLCβ4
4. PLCβ3 (83%) knockdown diminishes $Ca^{2+}$ response due to C5a + UDP more than PLCβ4 (87%) knockdown
5. PLCβ4 knockdown minimally(5-10nM peak height) affects $Ca^{2+}$ response
6. hypothetical synergy mechanism does not depend on specifics of C5a receptor (may depend on GRK desensitization)
7. sustained $Ca^{2+}$ response to UDP is due to maintained stimulation of IP3 receptor

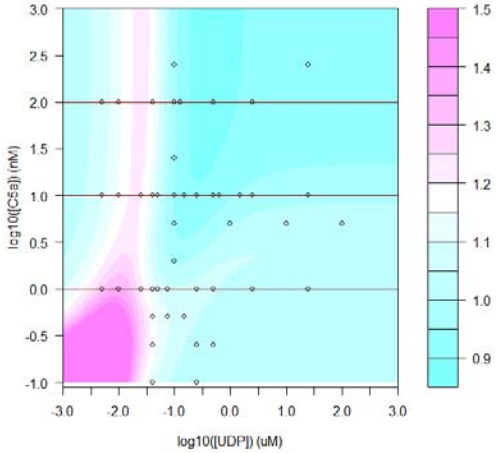
# Future Work

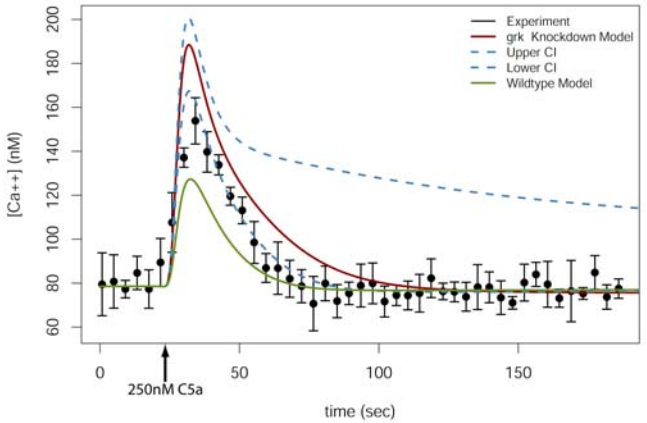
- We find a similar structure in the cAMP pathway – these may be common dynamical motif in GPCR signal transduction systems.
- We can investigate dynamics and single cell stochastic effects with a simpler phenomenological model.
- Does overexpressing GRK and PKC individually and together eliminate UDP-C5a synergy?

# Results Summary



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