Modeling Dynamic Cellular Reaction Networks: Applications to drug discovery and development

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ATOM Summer
Intern Program

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My Experience

- 30+ years at the NIH
- Drug Discovery and Development, both wet and dry lab
- 12 years at Mount St. Mary's University, Emmitsburg, MD

Education

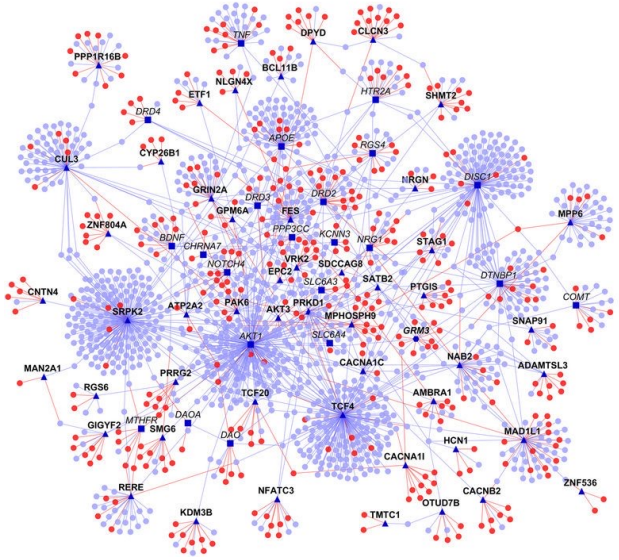
- BS, MS, PhD, George Washington University

Outside Interests

- Quilting, Gardening, Bird watching, Reading
- Dog training
  - My dog Bibbs won two blue ribbons in Conformation

Computational Modeling in Biology

- Since the 1970s, investigators have used mathematics to describe biology
  - Tyson studied the cell cycle and the role of phosphorylation
  - Others studied cell migration and the actin cytoskeleton
  - All accomplished mostly by hand with ODEs
- Historically, called Computational Biology
- Reports on cellular networks were limited
  - Limited by knowledge
  - Limited by computing
    - CPUs
    - Little or no easy access for the biologist w/o math training



Software Development

- In the 2000s, GUIs were developed that improved access for biologists and modelers
  - COPASI (COmplex PAthway SImulator)
  - http://copasi.org/

  - Virtual Cell
  - https://vcell.org/

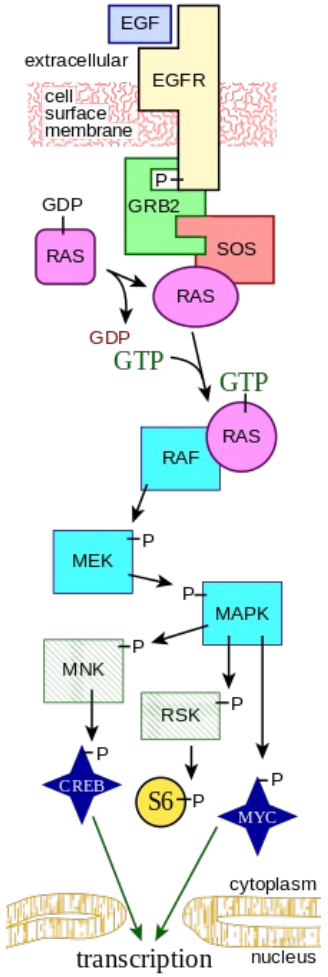
  - RuleBender, the IDE for BioNetGen
  - http://bionetgen.org/

In Common

- All are more accessible to the biologist
- Interesting Aspects
  - COPASI – can do rigorous analysis of model such as stability measures
  - VCell – has a GUI that allows simple drawings of reactions of the model
    - Is also integrated with RuleBender
    - Spatial Modeling available (e.g.. incorporating diffusion)
  - RuleBender – facile integration of biological knowledge and accounting for combinatorial complexity (later slides!)
  - All can be exported to SBML (Systems Biology Markup Language)

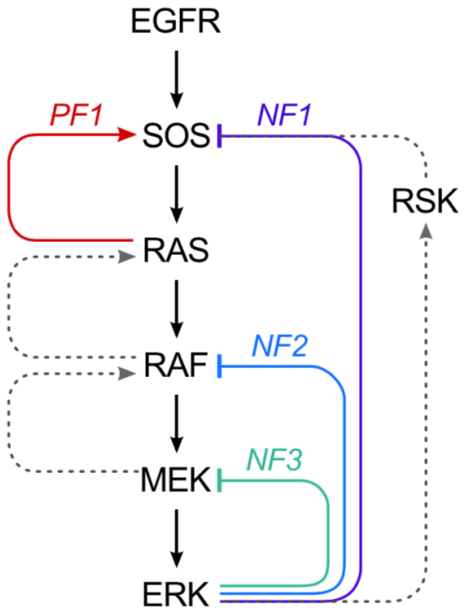
Knowledge Explosion

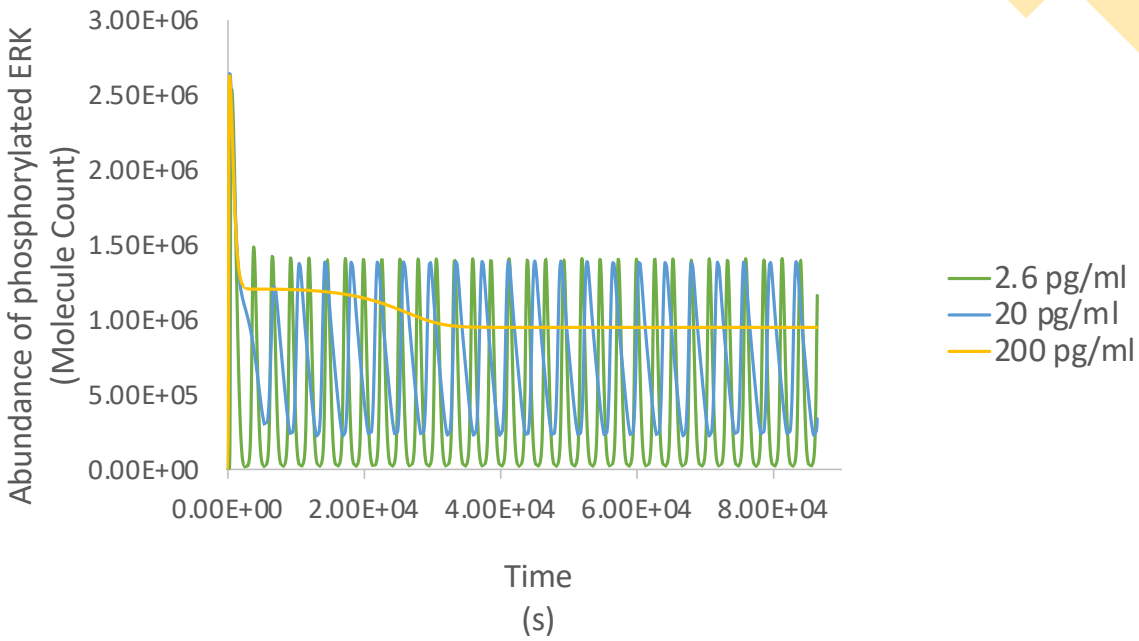
- It is now apparent that early studies which emphasized a “linear” reaction network are **not** “linear”
  - MAPK pathway is classically understood this way
- Typically, final output is translocation to the nucleus by phospho-ERK1/2 to promote transcription of cell cycle genes
- It is nearly impossible to translate outcomes now without the aid of a computational model
  - Accounts for Positive and Negative Feedback Loops
  - In the case of MAPK, at least 2 Positive and 3 Negative



MAPK Signaling with Positive and Negative Feedback Loops

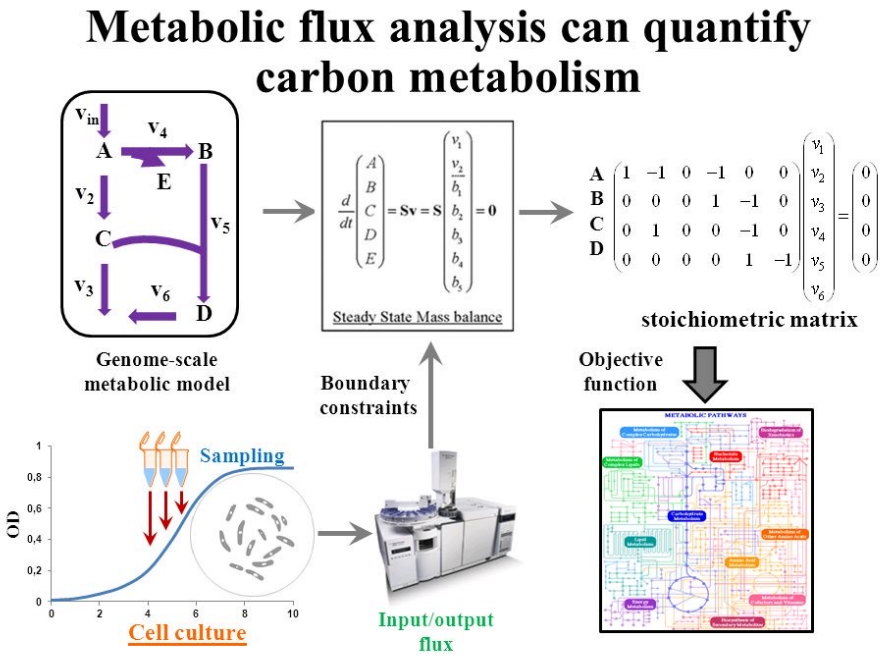
- Output considering complexity:
  - Amount of phospho-ERK1 oscillates
  - Oscillations themselves are dependent of growth factor concentration
  - This has been verified experimentally





Modeling Biology

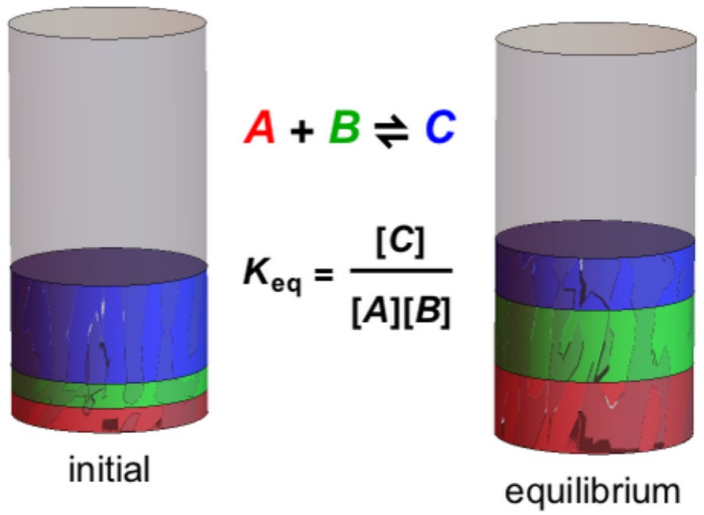
- In the simplest terms, capturing Reaction Networks
- Equations that underpin this are
  - *Ordinary differential equations*
    - Can be analyzed in a deterministic or stochastic fashion
  - *Partial differential equations*
    - A cell is not a well mixed solution
    - There are gradients, diffusion and other processes that require other mathematical analyses



Modeling Biology

- Describing reaction networks mathematically
  - Reactions have rates that can be described in different ways
    - For example: Law of Mass Action
      - Where rate depends on concentration
    - For example: Michaelis Menten kinetics
      - Where rate depends on substrate concentration
      - There is a maximum rate at a point where a change in substrate concentration does not change the rate
    - For example: Hill Equation
      - Where rate depends on a cooperativity factor
    - And others

Law of Mass Action



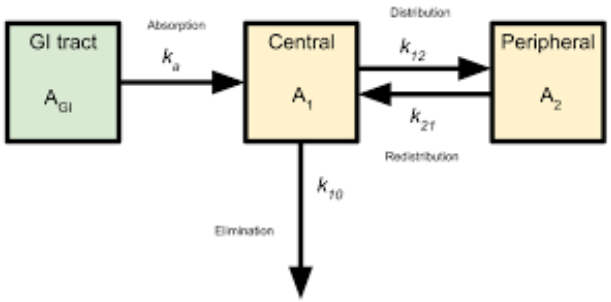
Nomenclature for Mathematical Models in Biology

- Transition to ATOM has required careful definitions of these models

- 1. Computational Biology
- 2. ODE Models
- 3. Mechanistic Models (Reaction Network Models)
- 4. Pharmacodynamic Models*

Modeling in Biology and Drug Discovery and Development

- Traditionally, Pharmacokinetics has utilized mathematical modeling to understand rates of change of drug concentration in body tissues
  - Compartmental Modeling
  - Connection to ODEs mentioned earlier



- One difference between Pharmacokinetic modeling and Pharmacodynamic modeling is a question of scale
- “Quantitative Systems Pharmacology”

Pharmacodynamic Modeling in Drug Discovery and Development

- Pharmacodynamics is the study of drug MOA and desired outcome
  - In vitro assays support and supported these studies
  - In the simplest terms, effect of drug on reaction network
- Opportunity has now arisen to capitalize on PD modeling
  - Create Network of interest, input drug, and analyze output
  - Is dependent on parameters
    - This is to say there is uncertainty
    - BUT there limits! Biology occurs within a range of parameters and the physical and chemical laws
      - For example, rates cannot be too slow, diffusion rates have limits due to size and conditions
- Notably: Models themselves can be studied for emergent system properties
- Notably: Models, ideally, can be confirmed with wet lab experiments

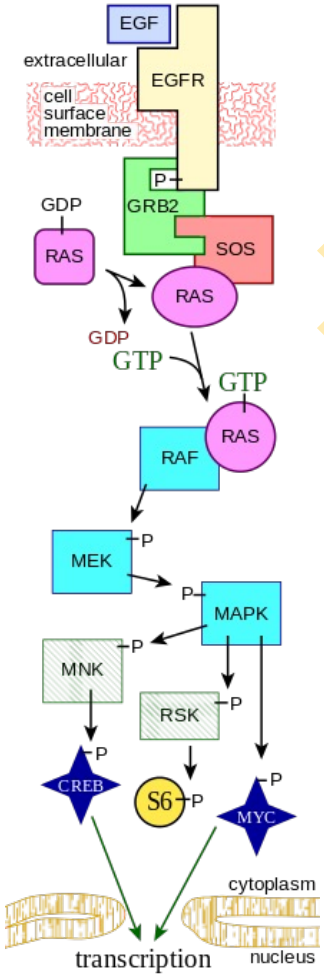
Pharmacodynamic Modeling in Drug Discovery and Development

- Mechanistic and PD Models are of interest

- 1. Mechanistic Models can describe normal and diseased state
  - Evaluate differences in outcome
  - E.g. modeling cancer mutations
- 2. Mechanistic Models can be used to evaluate drug MOA
- 3. Mechanistic Models can be examined for potential molecular targets in *in silico* knockout studies
- 4. Mechanistic Models can be interrogated for biomarkers of drug response

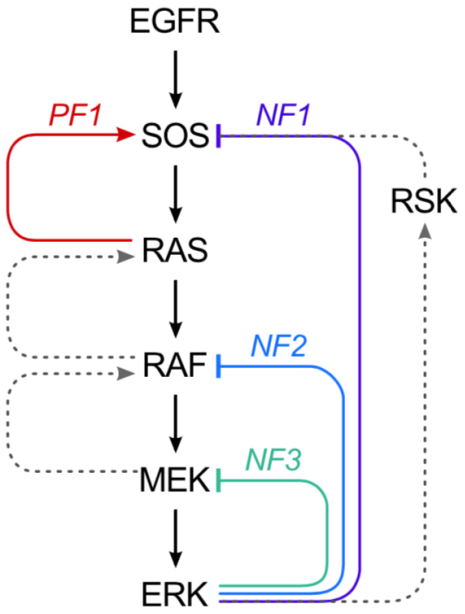
Mechanistic Model: MAPK Signaling and RAS Mutants

- RAS mutations are exceptionally common in cancer
  - 25% of all cancers
  - Considered ”Undruggable”
  - RAS Initiative at FNL
- Role of RAS
  - 3 genes (HRAS, KRAS (two isoforms), NRAS)
  - Of interest - KRAS
  - Multiple mutants that result in MAPK signaling “ON”
  - GTPase
    - In the GTP bound state, have binding of positive effectors such as RAF, a kinase
    - GTPase cycle is complex
      - Both RAS and other effectors can shut off signaling
      - Again, in mutants, signaling remains on

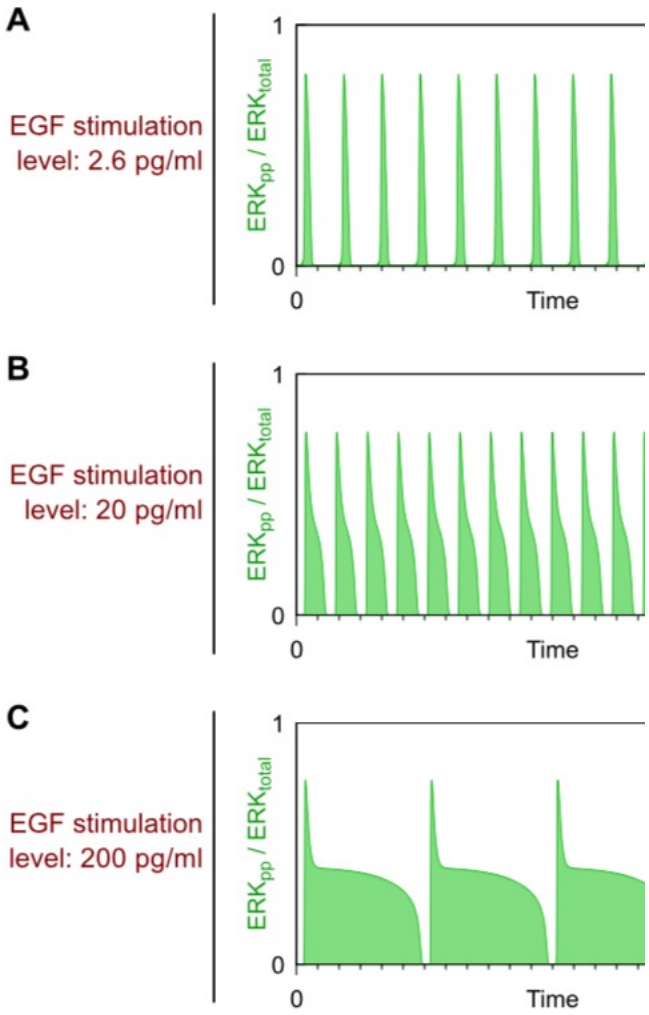


Model – Kochanczyk, et al. 2017

- MAPK signaling with
  - 1 Positive feedback loop
  - 3 Negative feedback loops
  - Goal was to understand the impact of the feedback loops on ERK activation (or, phosphorylated ERK)
  - Model does not necessarily contain parameters that are directly measured in the literature, but would qualify that they fall in the known range of biological measurements
  - In a stepwise fashion, added loops
  - Summary: Found different cellular behaviors (state changes)
    - Monostable Inactive, Monostable Active, and Oscillations



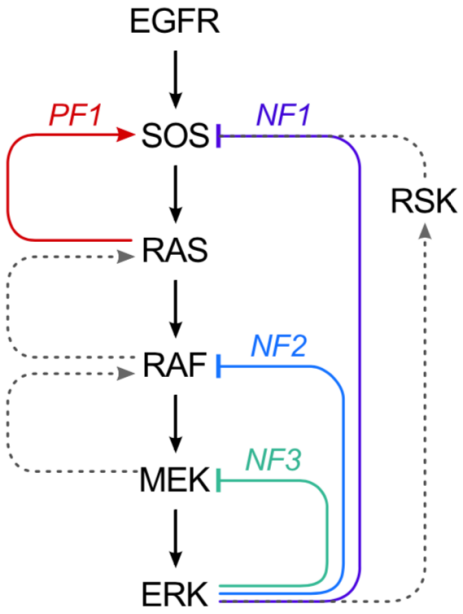
All Feedback Loops Predict Oscillatory Behavior of phosphorylated ERK



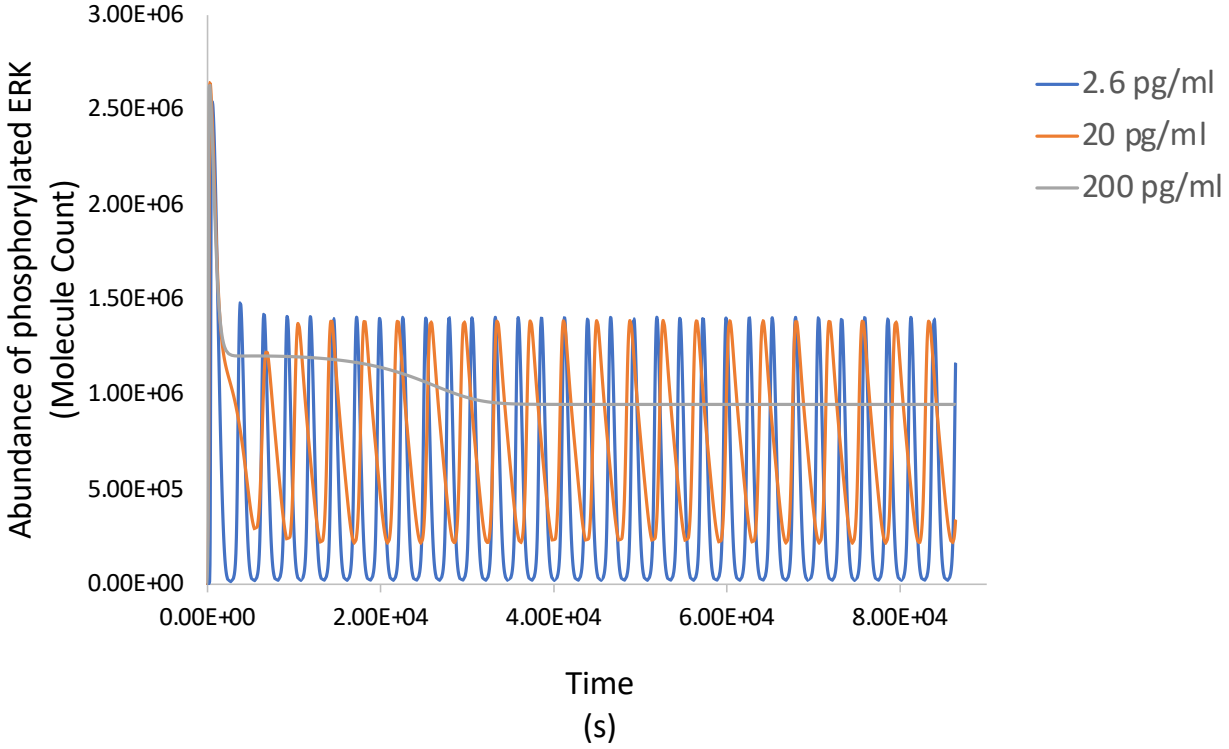
Kochanczyk, 2017

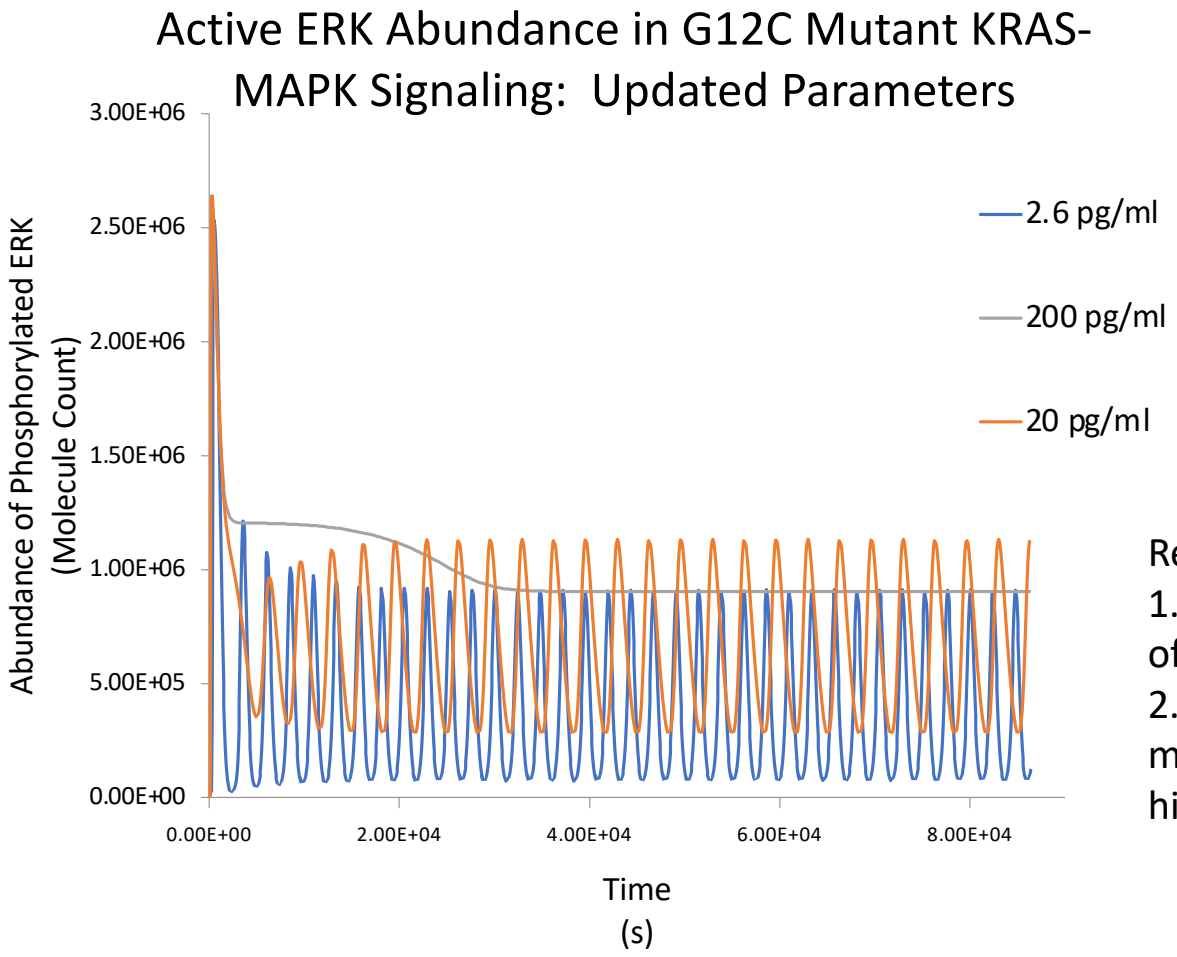
Adapting Model – Kochanczyk, et al. 2017

- Detailed short-term goals of modeling:
  - 1. Incorporate nucleotide exchange (by SOS) and intrinsic hydrolysis rates (of RAS) for wild type and G12C mutant RAS
  - 2. Determine the effect of ARS-853, a covalent inhibitor of G12C mutant RAS using parameters in Patricelli et al. 2016
  - 3. Because it is likely that the covalent inhibitor depletes G12C RAS, synthesis and degradation of RAS were modeled



Active ERK Abundance in Wild Type KRAS-MAPK Signaling: Updated Parameters

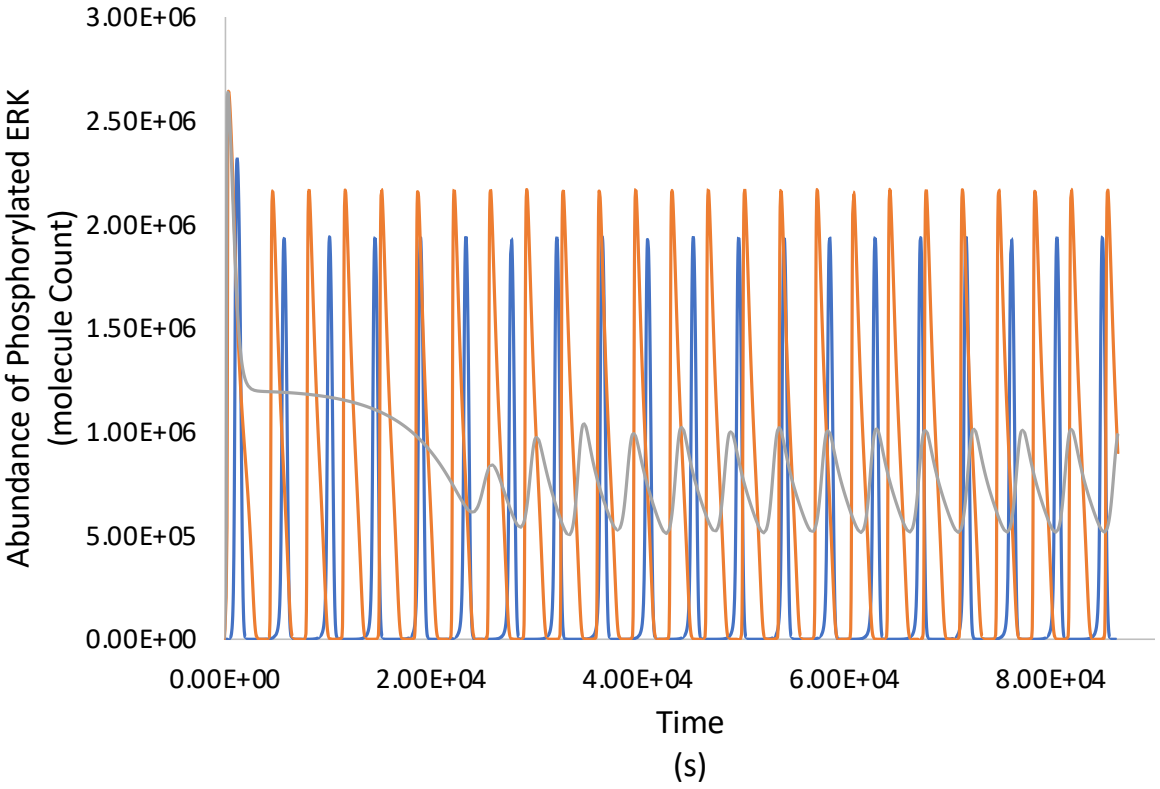




Results:

1. Decreased amplitude of oscillations
2. No change in monostable activation for highest dose

Active ERK Abundance in G12D KRAS Mutant MAPK Signaling

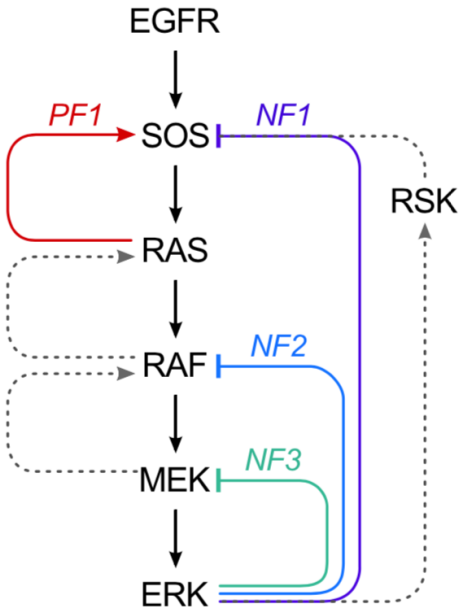


Results:

1. Increased amplitude of oscillations
2. Change to oscillatory behavior for high dose EGF stimulation

Brief Summary

- Updating model with wild type RAS parameters altered outcomes
  - Not surprising
  - Changes can be verified
- Different mutants altered outcomes
  - Path to discovery novel therapeutics for each mutant?



Modeling in Biology and Drug Discovery and Development

- Mechanistic Models are underdeveloped
- PD Models are reaching the forefront
  - Quantitative Systems Pharmacology
  - Example in MAPK Signaling follows

Modeling in Biology

- Information overload is exceeding human thought
- Will be required in Drug Discovery and Development

Acknowledgements

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