THE CENTER FOR CANCER SYSTEMS PHARMACOLOGY

Laboratory of Systems Pharmacology

HARVARD MEDICAL SCHOOL

Building kinetic models with complex drug-protein interactions

Luca Gerosa

Postdoctoral Fellow, Sorger Lab, Harvard Medical School

ROSA Webinar, 2021, 20th January

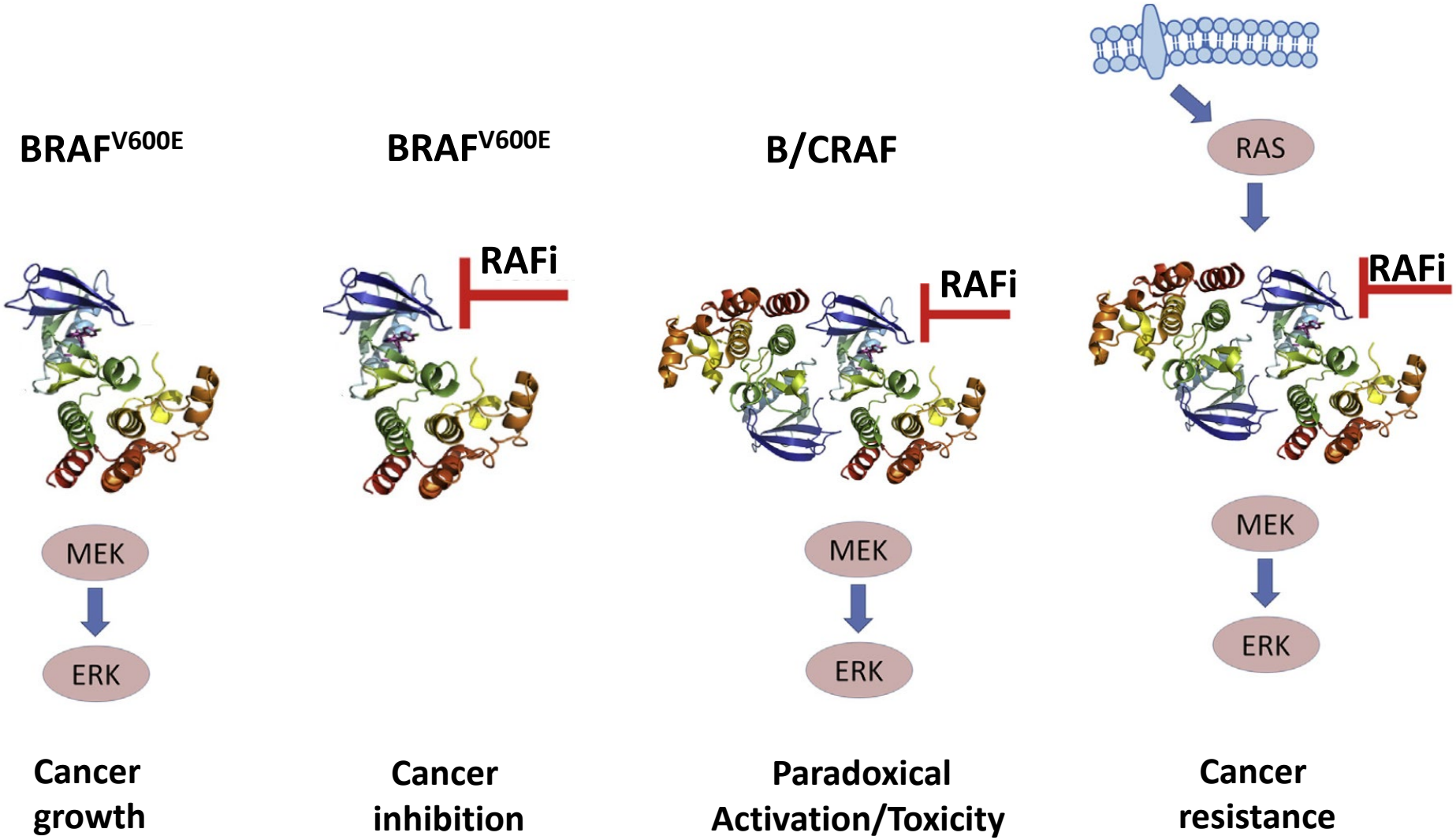
Outline

1. A **modeling framework** to build kinetic models with complex drug-protein interactions

2. A **predictive model** for the targeted inhibition of oncogenic MAPK signaling in cancer

Understanding complex drug-protein interactions is essential for Quantitative Systems Pharmacology

PMID: 26343583

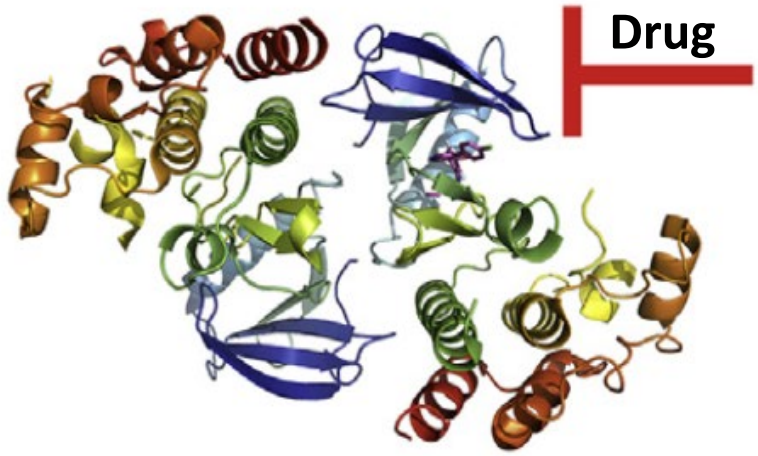


Complex drug-protein interactions are due to positive and negative cooperativity and must be considered in drug studies

Negative cooperativity

Positive Cooperativity

Drug

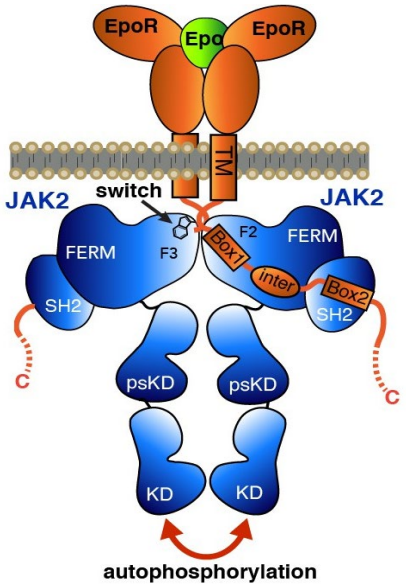


Drug

Modulate biological functions = contribute to drug efficacy

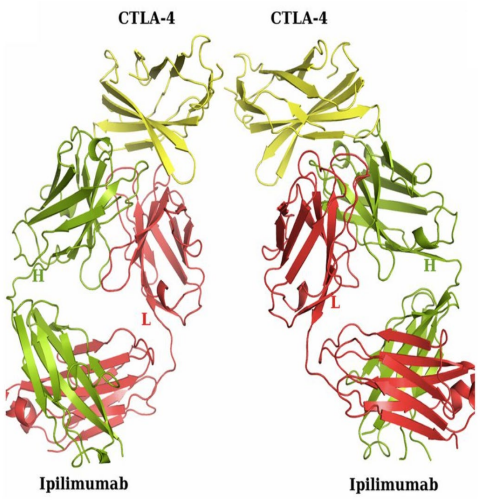
Complex drug-protein interactions appear in multiple scenarios with therapeutic relevance

Inhibitors of dimerizing kinases
(JAK, EGFR, MEK, etc..)



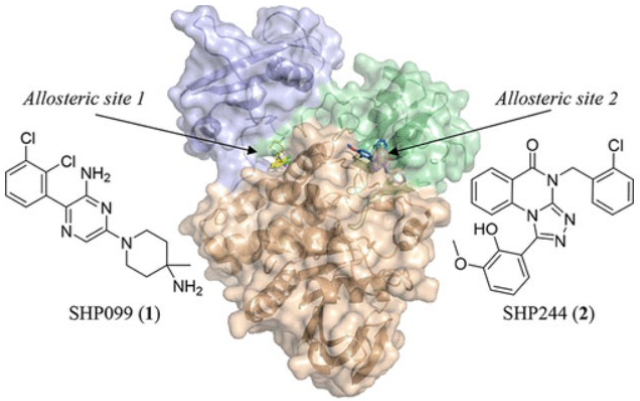
PMID: 30044226

Immune checkpoint inhibitors
(CTLA4, PD1, etc..)



PMID: 28484017

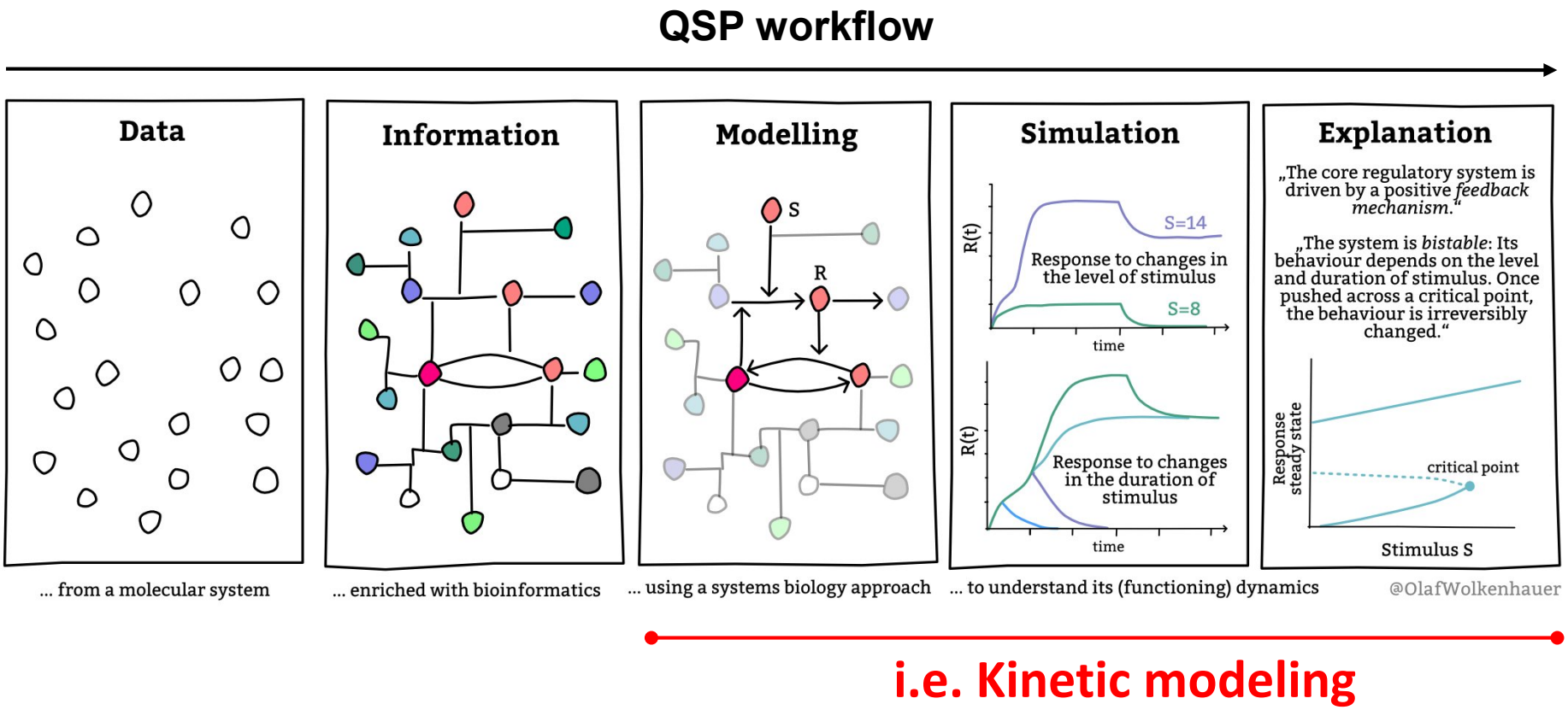
Inhibitors of other signaling components
(SHP2,RAS, etc..)



SHP2 Phosphatase with Allosteric Inhibitors SHP099 (**1**), SHP244 (**2**).

PMID: 29304282

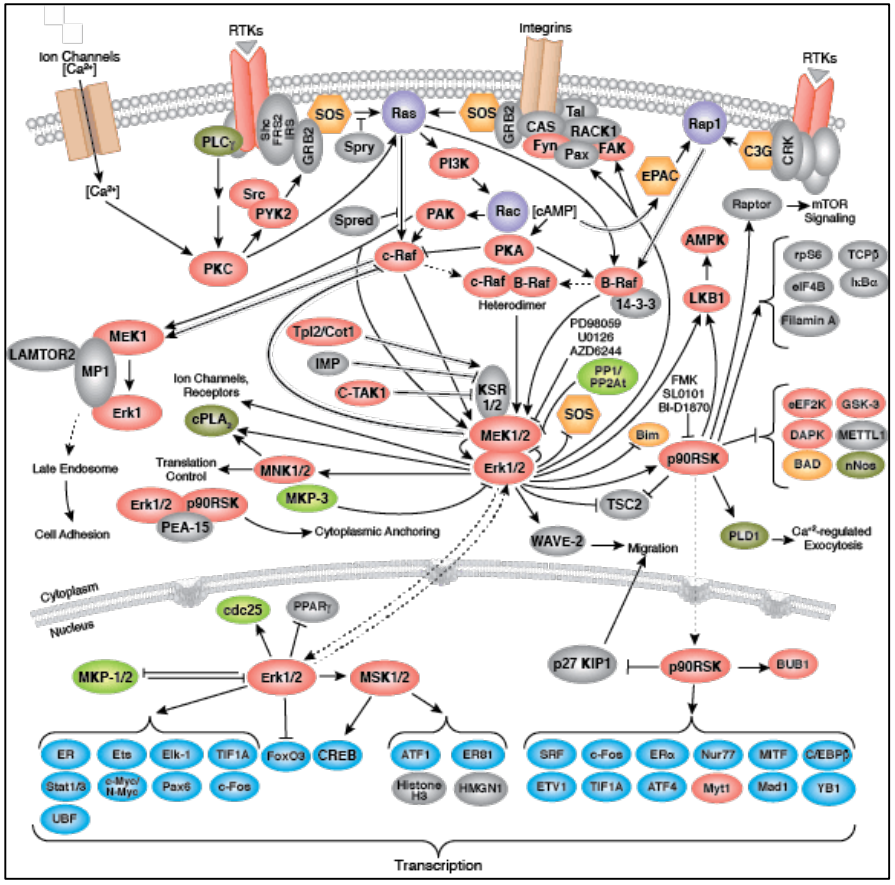
Quantitative Systems Pharmacology relies on kinetic modeling to describe and understand drug action



i.e. Kinetic modeling

Kinetic modelling requires writing ODE systems that properly describe molecular complexity of biological system

Biological system



MAPK signaling pathway – from CST

Ordinary differential equation systems

$$\frac{d[pEGFR]}{dt} = k_{on}*[EGFR]*[EGF] - \ldots$$

$$\frac{d[Ras\text{-}GTP]}{dt} = k_{on}*[Ras]*[GTP] - \ldots$$

$$\frac{d[RAF_2]}{dt} = k_{on}*[RAF]^2 - k_{off}*[RAF_2] + \ldots$$

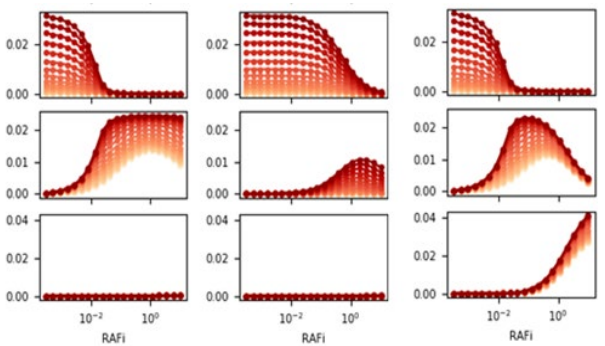
$$\frac{d[BRAF^{V600E}]}{dt} = k_{exp} - k_{deg}*[BRAF^{V600E}] + \ldots$$

$$\frac{d[pMEK]}{dt} = k_{on}*[MEK]*[BRAF^{V600E}] - \ldots$$

$$\frac{d[pERK]}{dt} = k_{on}*[ERK]*[pMEK] - \ldots$$

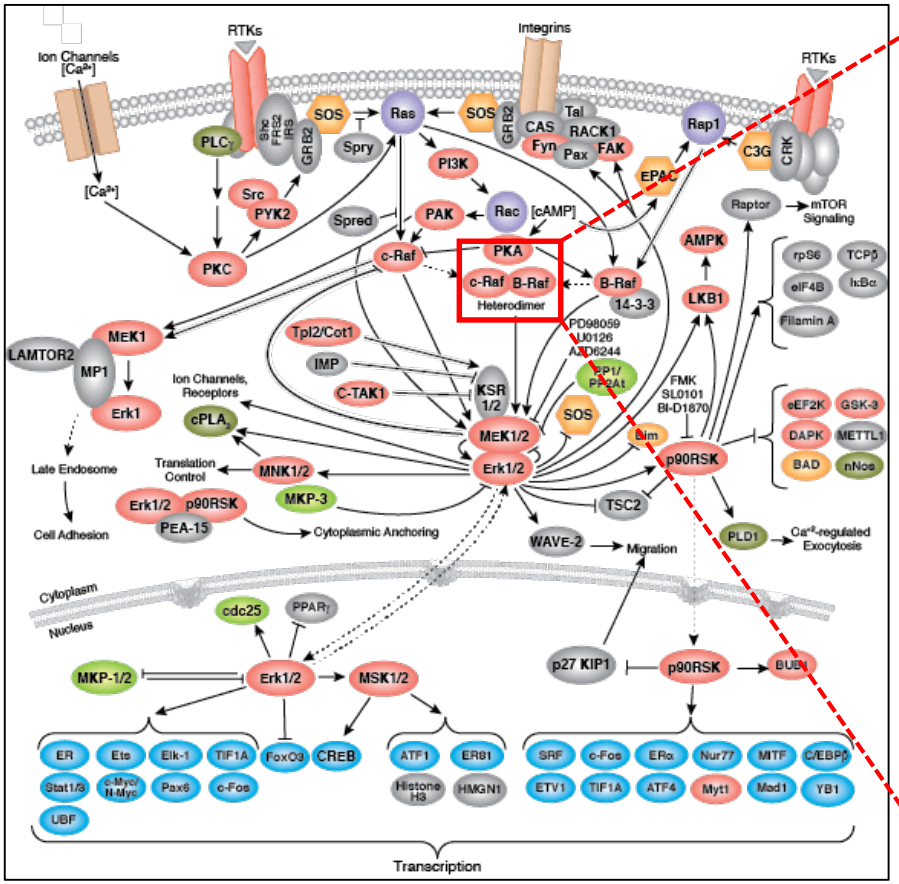
$$\frac{d[CyclinD1]}{dt} = k_{exp} - k_{deg}*[CyclinD1] + \ldots$$

Kinetic model



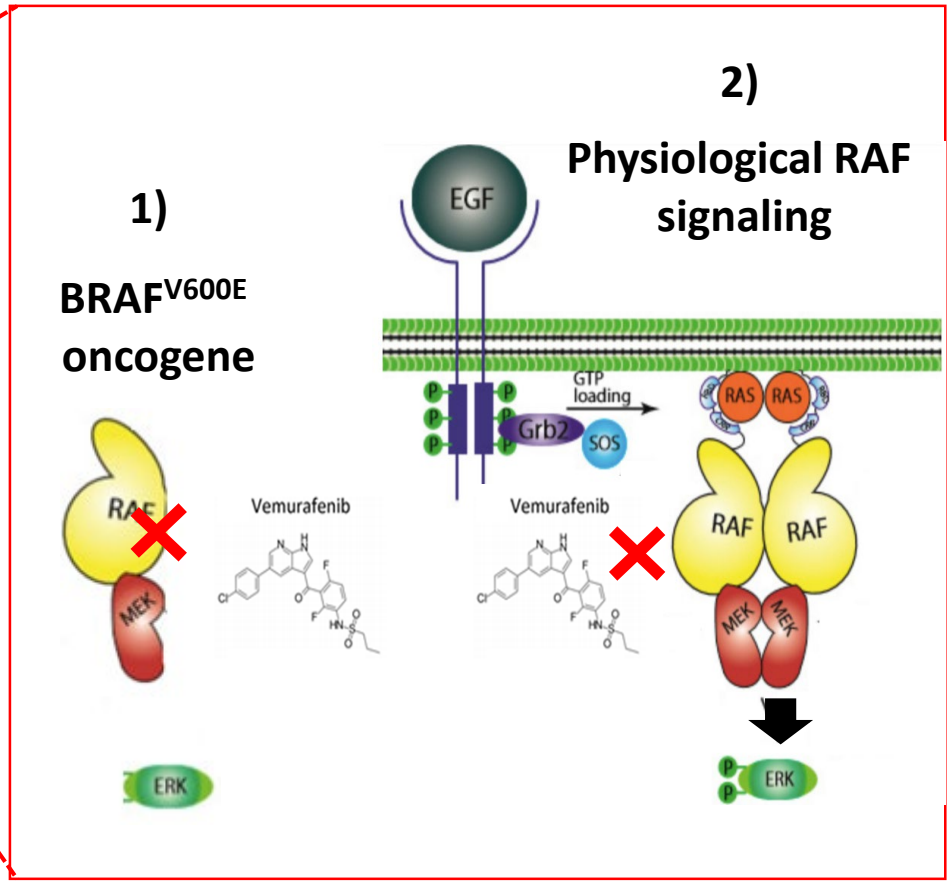
Kinetic modeling for QSP requires describing complex drug-protein interactions

Pathway interactions



MAPK signaling pathway – from CST

Drug-protein and protein-protein interactions



Degirmenci et al, 2020 Cells

A toy example of RAF and RAF inhibitors to introduce a framework for kinetic modeling with energy description

Components:

R: RAF (target)

I: RAF Inhibitor (drug)

Binding schema:

I – R – R – I

Reaction	Dissociation constant
$R + I \leftrightarrow RI$	K_{RI}
$R + R \leftrightarrow RR$	K_{RR}
$RR + I \leftrightarrow RRI$	$f \cdot K_{RI}$
$RRI + I \leftrightarrow IRRI$	$g \cdot K_{IRRI}$

Cooperative reactions

Thermodynamic factors

$f = K_{RRI} / K_{RI}$ fold change in drug affinity to 1st RAF in dimer

$g = K_{IRRI} / K_{RI}$ fold change in drug affinity to 2nd RAF in dimer

Kholodenko, 2015, Cell Reports

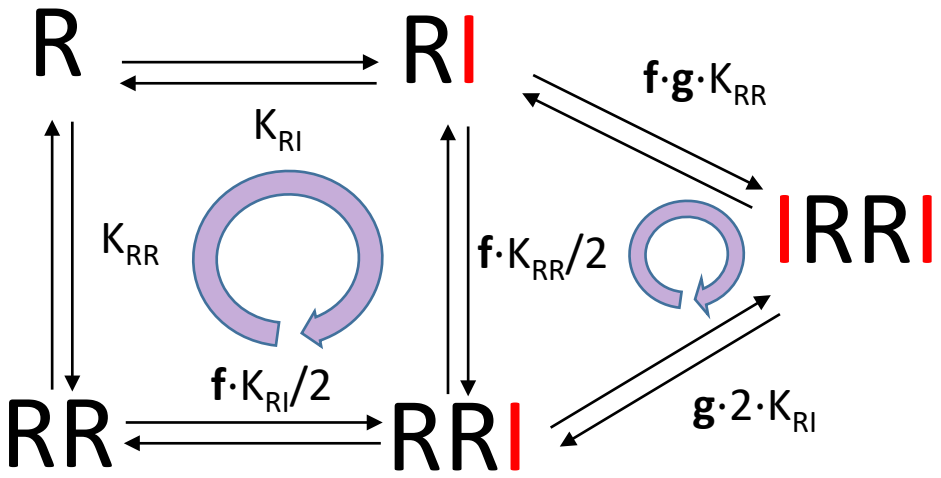
The two problems in properly describing drug-protein interactions: combinatorial and contextual complexity

1. Combinatorial complexity:

the explosion of the reaction network size due to combinations of multi-domain protein and drug interactions

2. Contextual complexity:

the constraints in cooperative reaction rates due to context-dependency of cooperative interactions



Detailed balance must be satisfied!

ODE model

$$d[\boldsymbol{R}]/dt = k_{RR}^{-}[\boldsymbol{RR}] + ..$$

$$d[\boldsymbol{I}]/dt = k_{RI}^{-}[\boldsymbol{RI}] + ..$$

$$d[\mathbf{RI}]/dt = k_{RI}^{+}[\boldsymbol{R}][\boldsymbol{I}] + ..$$

$$d[\mathbf{RRI}]/dt = f\, k_{RI}^{+}[\boldsymbol{RR}][\boldsymbol{I}] + ..$$

$$d[\mathbf{IRRI}]/dt = fg k_{RI}^{+}[\boldsymbol{IRR}][\boldsymbol{I}] + ..$$

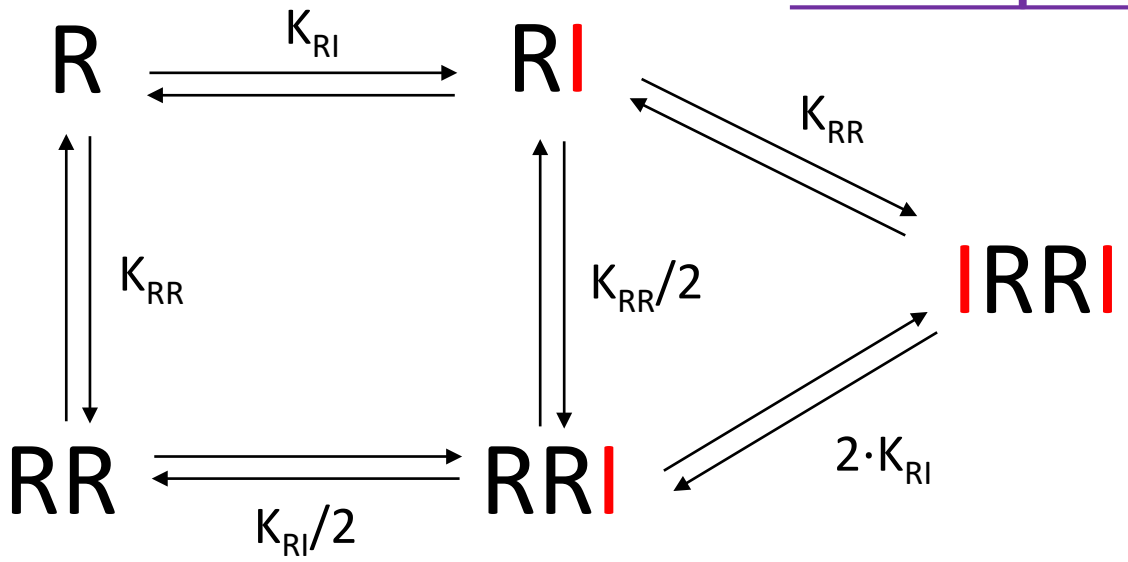
Classic rule-based modeling solves combinatorial but not contextual complexity problem

Reaction rules:

$R+R \leftrightarrow RR : K_{RR}\,(k^{+}_{RR}, k^{-}_{RR})$

$R+I \leftrightarrow RI : K_{RI}\,(k^{+}_{RI}, k^{-}_{RI})$

Rates independent of context = no cooperativity !



How to solve the problem of building kinetic models with combinatorial and contextual complexity?

Solution:

Rule-based modeling with energy descriptions*

**Hogg JS, PhD Thesis, 2013, Faeder lab@UPitt*

Rule-based modeling with energy descriptions solves combinatorial and contextual complexity at once

Reaction rules:

$R+R \leftrightarrow RR : K_{RR}(k^+_{RR}, k^-_{RR})$

$R+I \leftrightarrow RI : K_{RI}(k^+_{RI}, k^-_{RI})$

Baseline energies (kJ/mol):

$E_0^{RR} = \emptyset \cdot \log(K_{RR}) - \log(k^+_{RR})$

$E_0^{RI} = \emptyset \cdot \log(K_{RI}) - \log(k^+_{RI})$

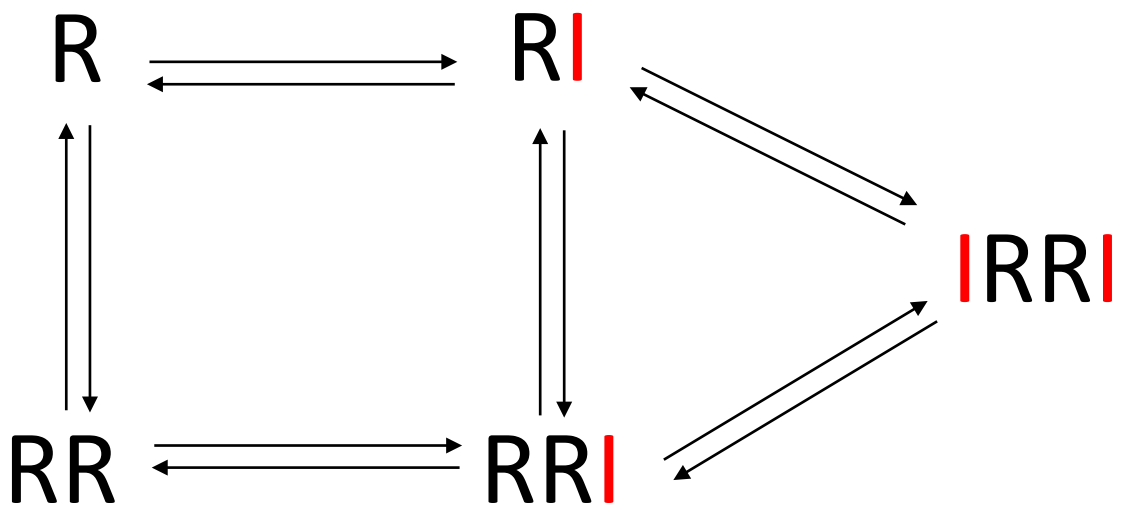
Energy Patterns (kJ/mol):

$ep(RR) = \log(K_{RR})$

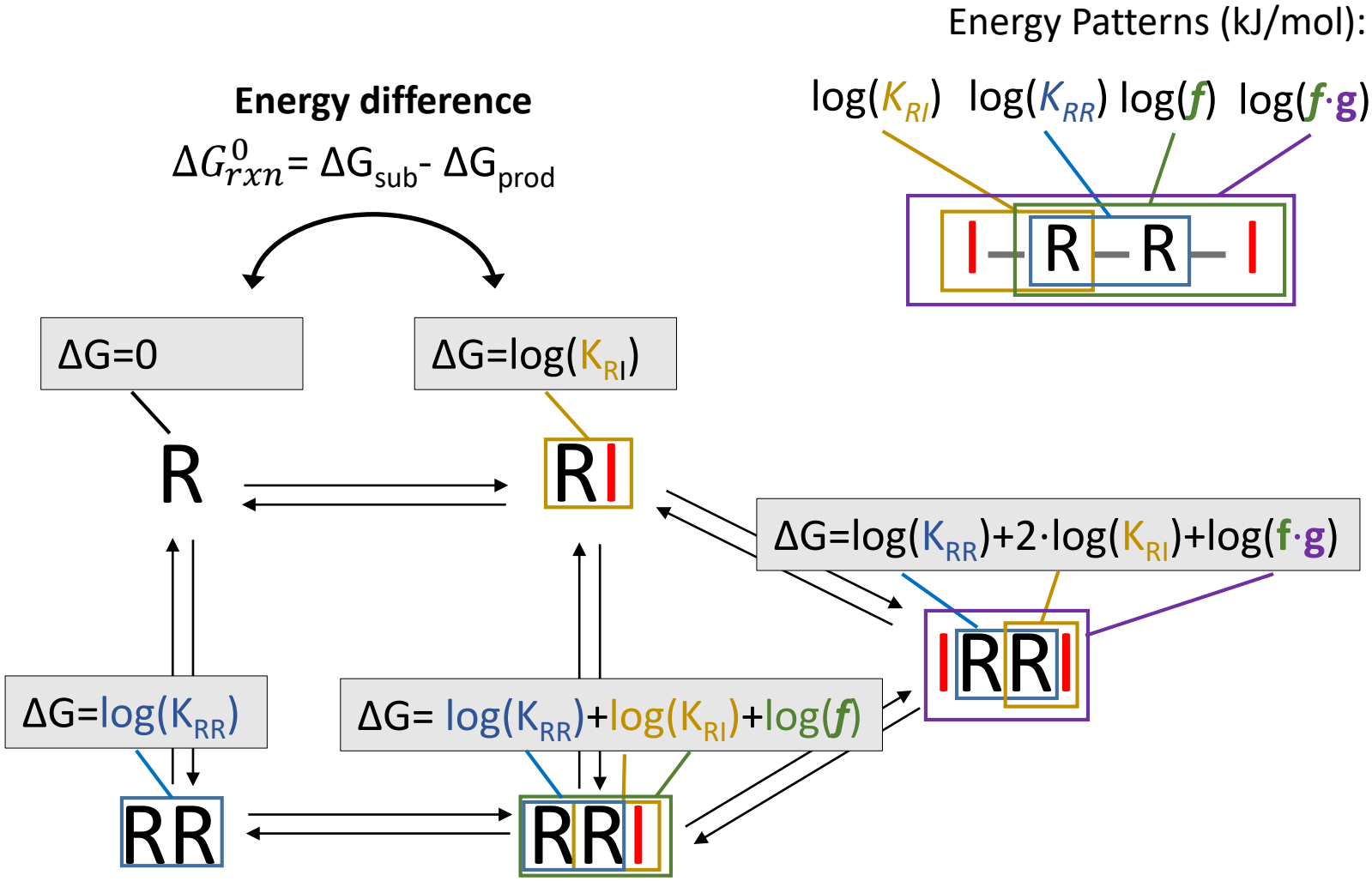
$ep(RI) = \log(K_{RI})$

$ep(RRI) = \log(\mathbf{f})$

$ep(IRRI) = \log(\mathbf{f} \cdot \mathbf{g})$



Rule-based modeling with energy descriptions solves combinatorial and contextual complexity at once

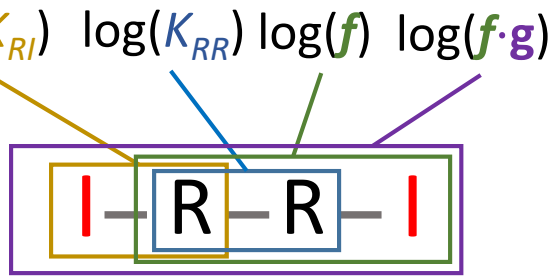


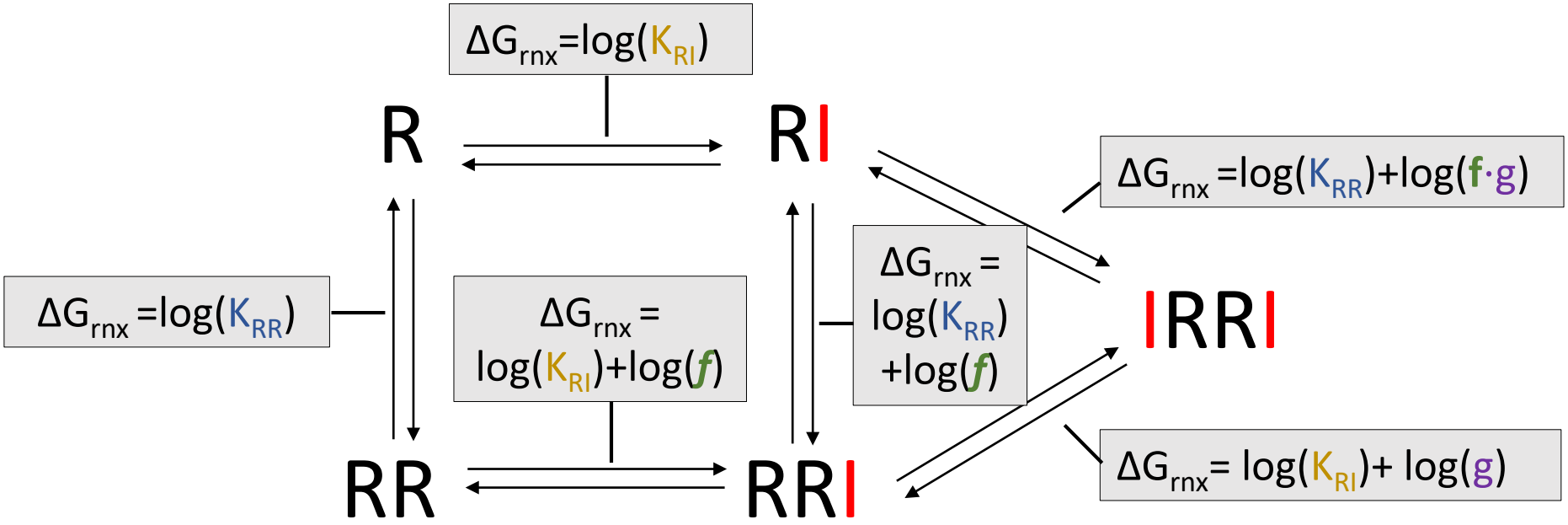
Rule-based modeling with energy descriptions solves combinatorial and contextual complexity at once

Energy Patterns (kJ/mol):

Energy difference

$\Delta G^0_{rxn} = \Delta G_{sub} - \Delta G_{prod}$





The theory behind the energy formulation in BioNetGen

Biological Network Generator

E_0	ΔG^0_{rxn}	$\emptyset$
baseline activation energy	free energy change	rate distribution

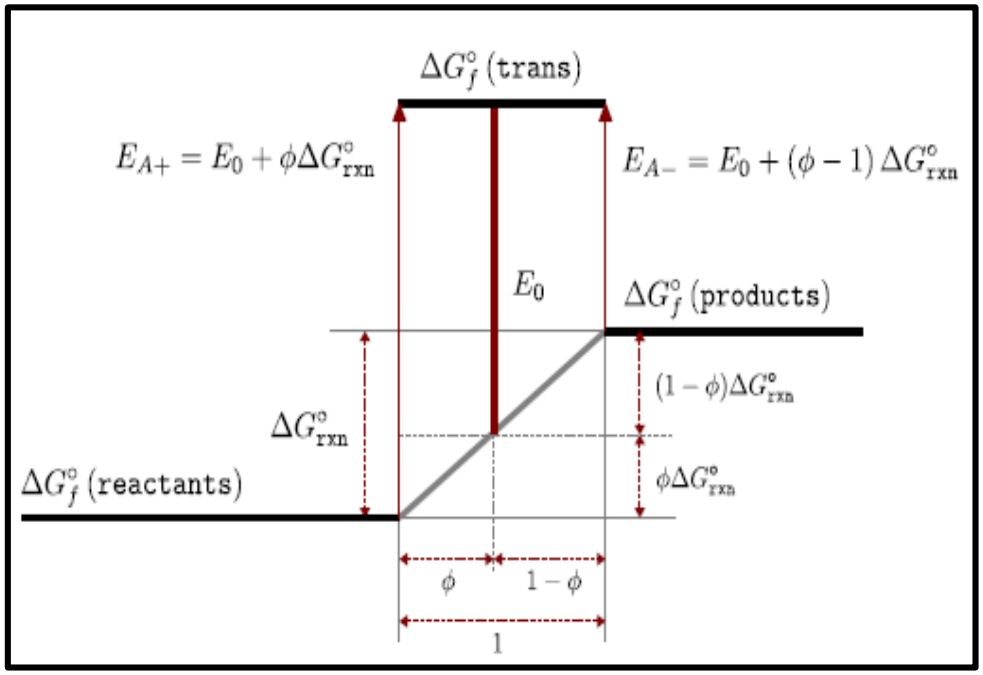
Linear transition state theory. The activation energy is linearly related to the standard change in free energy of a reaction:

$$\mathrm{E_A} = E_0 + \emptyset \cdot \Delta G^0_{rxn}$$

Arrhenius equation. The reaction rate constant of a reaction is determined by the activation energy:

$$\mathrm{k} = \mathrm{C} \cdot \exp \frac{-\mathrm{E_A}}{\mathrm{RT}}$$

From Justin Hogg PhD Thesis 2012 & Sekar et al 2017 BIBM -Faeder Lab@UPitt



Conversion rules from energies to kinetics:

$$\mathrm{k}_{on} = C \cdot \exp\left(\frac{-E_0 + \emptyset \cdot \Delta G^0_{rxn}}{RT}\right)$$

$$\mathrm{k}_{off} = C \cdot \exp\left(\frac{-E_0 + (\emptyset - 1) \cdot \Delta G^0_{rxn}}{RT}\right)$$

Rule-based modeling with energy descriptions solves combinatorial and contextual complexity at once

Baseline energies (kJ/mol):

$E_0^{RR} = \emptyset_{RR} \cdot \log(K_{RR}) - \log(k^+_{RR})$

$E_0^{RI} = \emptyset_{RI} \cdot \log(K_{RI}) - \log(k^+_{RI})$

Rate distributions:

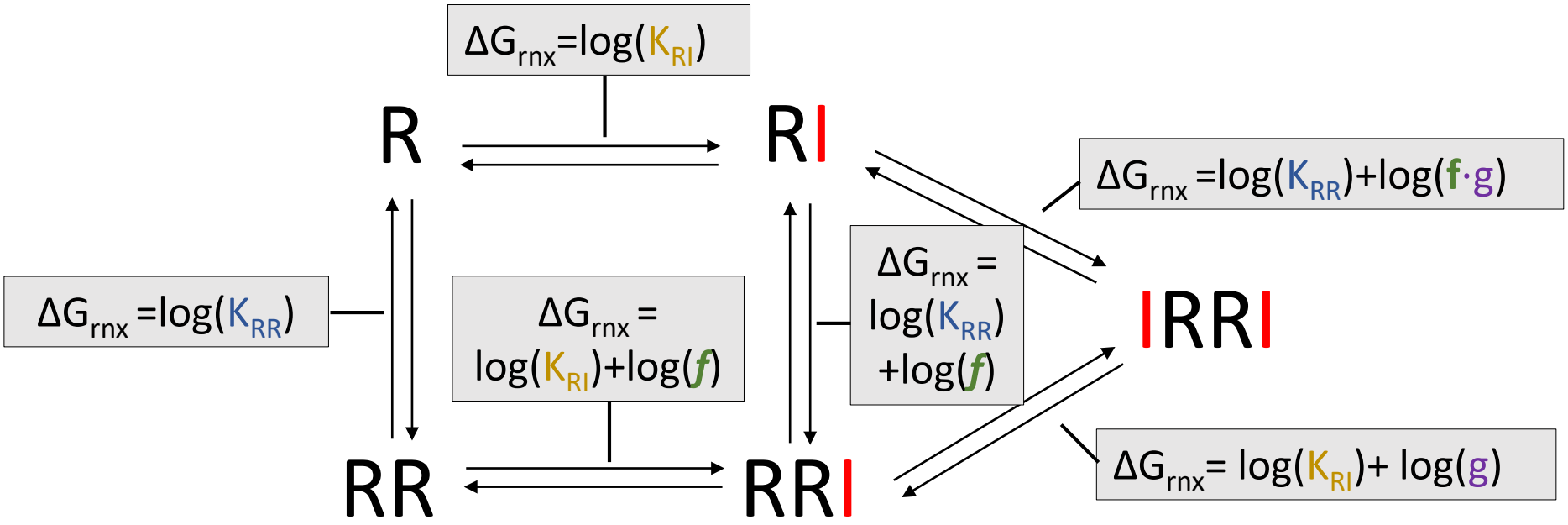
$\emptyset_{RR}$

$\emptyset_{RI}$

Conversion rules:

$$k_{on} = C \cdot \exp\left(\frac{-E_0 + \emptyset \cdot \Delta G^0_{rxn}}{RT}\right)$$

$$k_{off} = C \cdot \exp\left(\frac{-E_0 + (\emptyset - 1) \cdot \Delta G^0_{rxn}}{RT}\right)$$



Rule-based modeling with energy descriptions solves combinatorial and contextual complexity at once

Baseline energies (kJ/mol):

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Rate distributions:

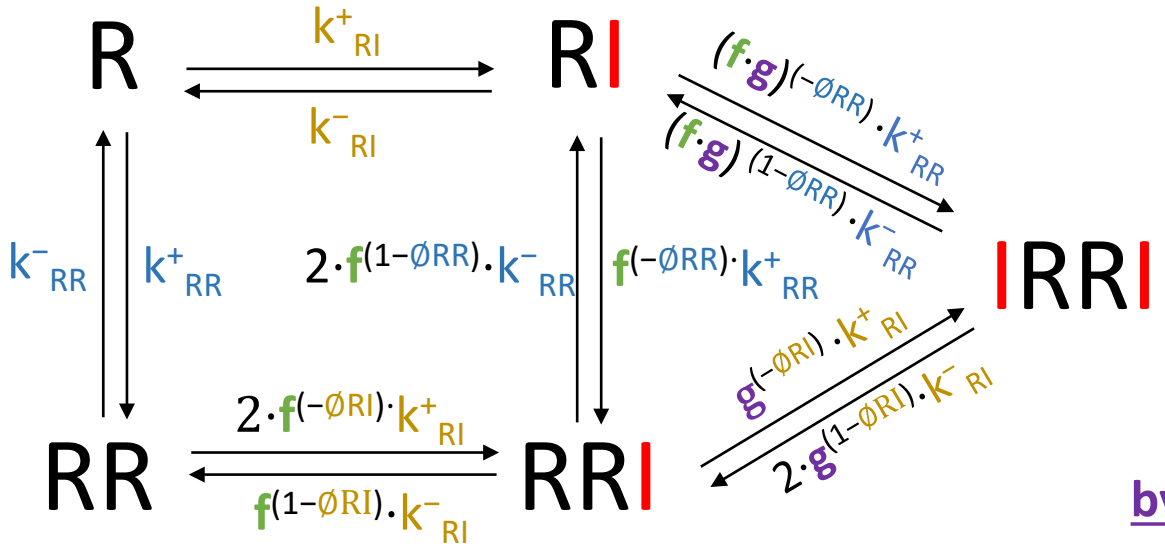
$\emptyset_{RR}$

$\emptyset_{RI}$

Conversion rules:

$$k_{on} = C \cdot \exp\left(\frac{-E_0 + \emptyset \cdot \Delta G^0_{rxn}}{RT}\right)$$

$$k_{off} = C \cdot \exp\left(\frac{-E_0 + (\emptyset - 1) \cdot \Delta G^0_{rxn}}{RT}\right)$$



Detailed balance is SATISFIED by construction!

Rule-based modeling with energy descriptions solves combinatorial and contextual complexity at once

Automatically derived rates:

$$RR \underset{\mathbf{f}^{(1-\emptyset RI)}\cdot k^{-}_{RI}}{\overset{2\cdot\mathbf{f}^{(-\emptyset RI)}\cdot k^{+}_{RI}}{\rightleftharpoons}} RRI$$

Dissociation constant:

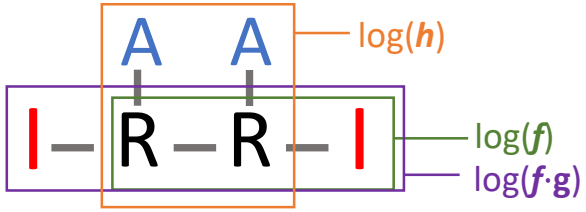
$$\frac{k^{-}}{k+} = \frac{\mathbf{f}^{(1-\emptyset RI)}\cdot k^{-}_{RI}}{2\cdot\mathbf{f}^{(-\emptyset RI)}\cdot k^{+}_{RI}} = \frac{\mathbf{f}^{(1-\emptyset RI+\emptyset R)}\cdot k^{-}_{RI}}{2\cdot k^{+}_{RI}} = \boxed{\frac{\mathbf{f}\cdot K_{RI}}{2}}$$

Thermodynamic factor	Distribution rate	
f=0.01	$\emptyset_{RI}$=1 (on k^{+})	$RR \underset{k^{-}_{RI}}{\overset{2\cdot\mathbf{100}\cdot k^{+}_{RI}}{\rightleftharpoons}} RRI$
	$\emptyset_{RI}$=0 (on k^{-})	$RR \underset{\mathbf{0.01} * k^{-}_{RI}}{\overset{2\cdot k^{+}_{RI}}{\rightleftharpoons}} RRI$
	$\emptyset_{RI}$=0.5 (shared)	$RR \underset{\mathbf{0.1} * k^{-}_{RI}}{\overset{2\cdot\mathbf{10}\cdot k^{+}_{RI}}{\rightleftharpoons}} RRI$

$$\boxed{\frac{\mathbf{0.01}\cdot K_{RI}}{2}}$$

PySB with support for eBNG allows to write energy-balanced kinetic models as compact python programs

R: RAF
I: RAFi
A: Ras^{GTP}



PySB Systems biology modeling in Python

BioNetGen Biological Network Generator

PySB extended with energy BioNetGen
by Jeremy Muhlich

PySB code (Python)

```
#Rules
Rule('RR', R(r=None)+R(r=None)<>R(r=1)%R(r=1), RR_phi, Ea0_RR)
Rule('RI', R(i=None)+I(r=None)<>R(i=1)%I(r=1), RI_phi, Ea0_RI)
Rule('RA', R(a=None)+A(r=None)<>R(a=1)%A(r=1), RA_phi, Ea0_RA)

#Energy Patterns
EnergyPattern('ep_RR', R(r=1)%R(r=1), Gf_RR)
EnergyPattern('ep_RI', R(i=1)%I(r=1), Gf_RI)
EnergyPattern('ep_RA', R(i=1)%I(r=1), Gf_RI)
EnergyPattern('ep_IRR', I(r=1)%R(r=2)%R(r=2,i=None), ep_IRR)
EnergyPattern('ep_IRRI', I(r=1)%R(i=1,r=2)%R(r=2,i=3)%I(r=3), ep_IRR)
EnergyPattern('ep_ARRA', A(r=1)%R(a=1,r=2)%R(r=2,a=3)%A(r=3), ep_ARRA)

#Expressions
Expression('Gf_RR', log(RR_kD))
Expression('Gf_RI', log(RI_kD))
Expression('Gf_RA', log(RA_kD))
Expression('Ea0_RR', -(RR_phi*log(RR_kD)+log(RR_kf)))
Expression('Ea0_RI', -(RI_phi*log(RR_kD)+log(RI_kf)))
Expression('Ea0_RA', -(RA_phi*log(RR_kD)+log(RA_kf)))
Expression('ep_IRR', log(f))
Expression('ep_IRR', log(g)+log(f))
Expression('ep_ARRA', log(h))
```

ODE system

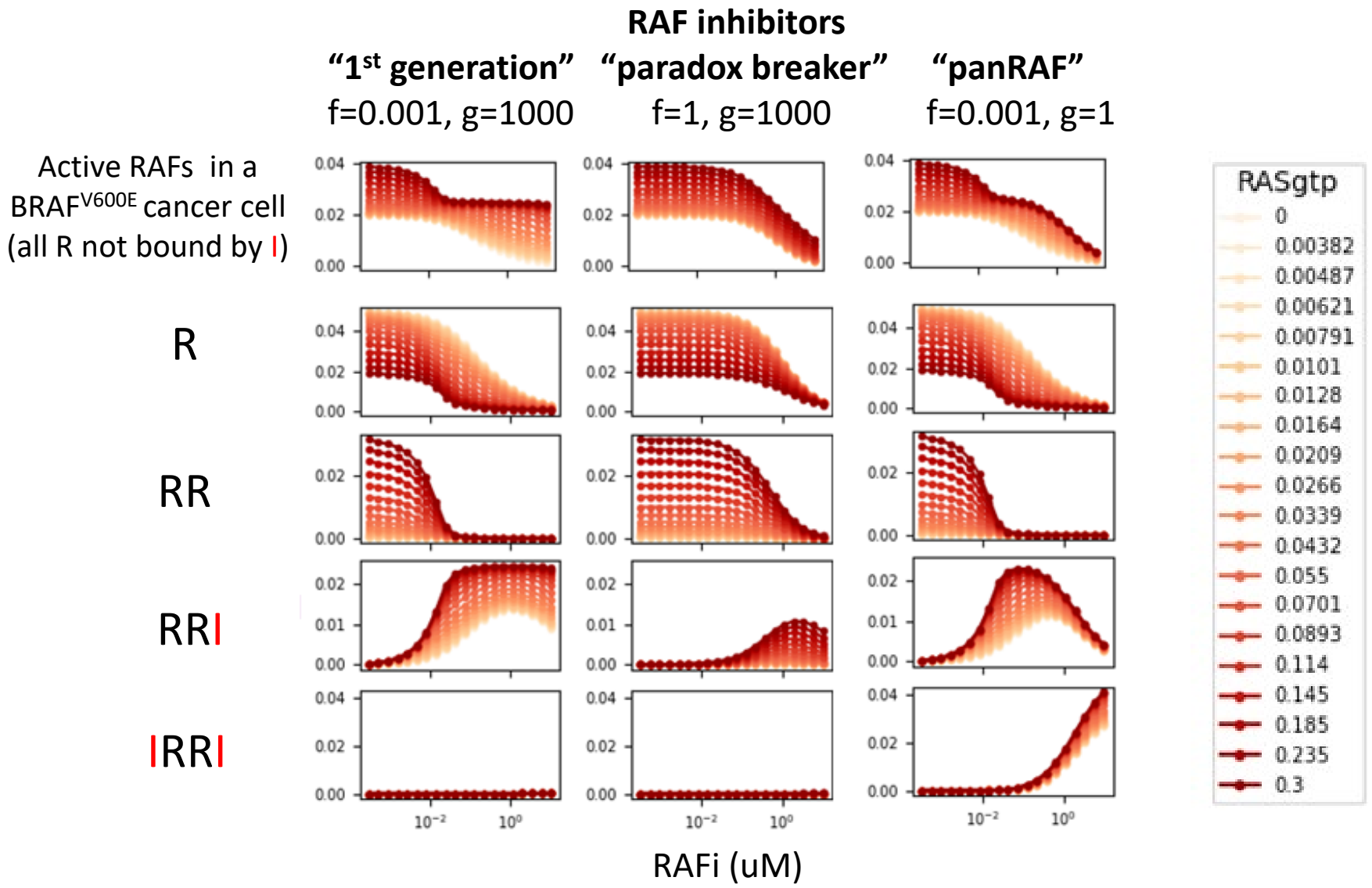
```
In [204]: model
Out[204]: <Model 'pysb_energy.model.example1'
          (monomers: 3, rules: 3,
           parameters: 13,
           expressions: 32)

In [205]: len(model.parameters)
Out[205]: 13

In [206]: len(model.species)
Out[206]: 16

In [207]: len(model.reactions)
Out[207]: 60
```

A toy kinetic model with energies recapitulates the efficacy of different classes of RAF inhibitors

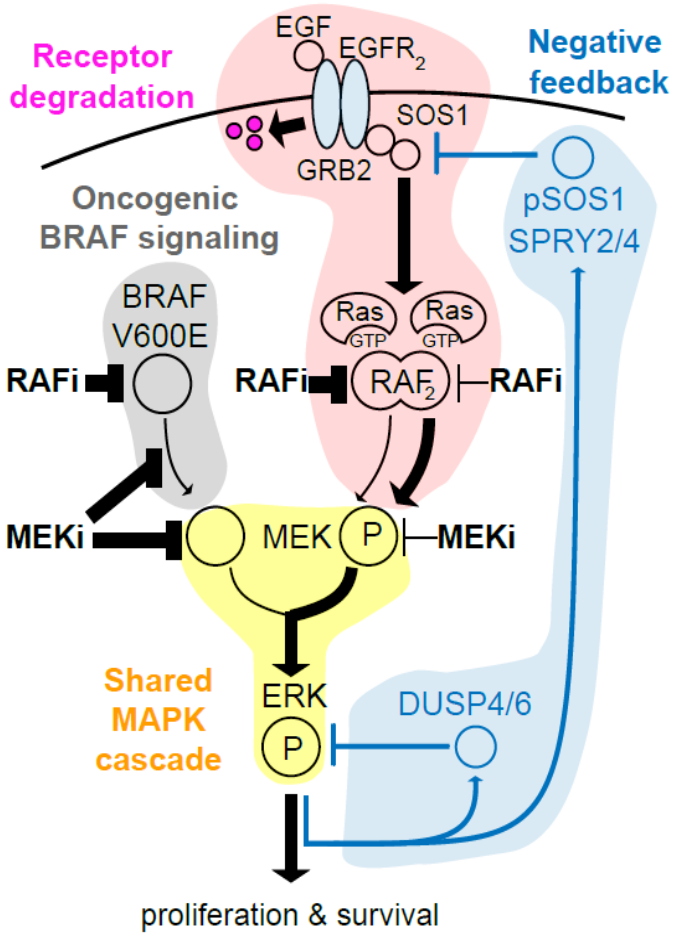


Conclusions on rule-based modelling framework with energies

- Remapping kinetic parameters as energies using energy patterns allows to solve the combinatorial and contextual complexity of kinetic models at once
- Convenient description of high-order cooperativity in drug-protein and protein-protein interactions
- Applicable to much more complex biological descriptions with multiple high-order cooperativities as for example:
  - Mutations, PTMs, asymmetric complexes, localization, etc..
- Newly implemented support for energy-BNG in PySB allows to write kinetic models with energy description as compact Python programs

Construction of a mechanistic model of MAPK signaling with high-order cooperativity in drug-protein interactions

MAPK signaling cascade
in BRAF-mutant cancer



Proteins: **11**

Drugs: **2**

Reaction Rules: **54**

Energy Patterns: **27**

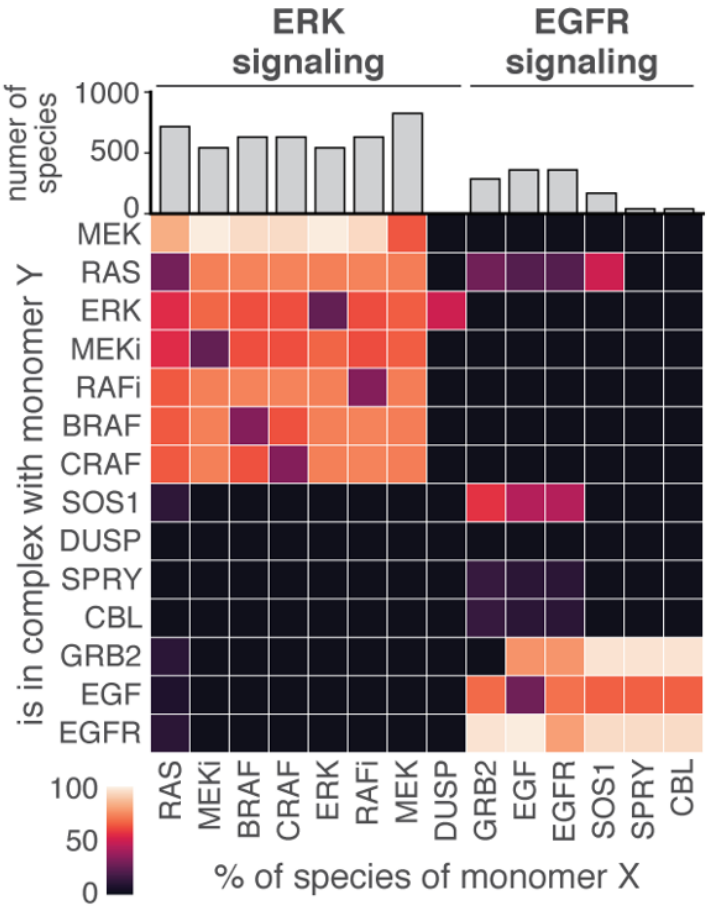
Parameters: **63**

Protein Complexes (ODEs): **1007**

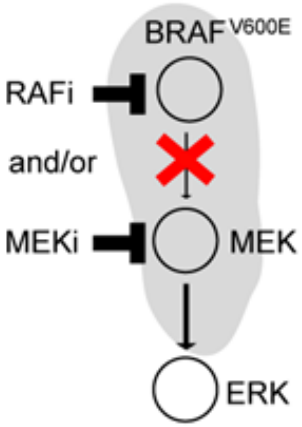
Reactions: **13164**

The MAPK signaling model with energy descriptions properly accounts for complexity of protein and drug-protein assembly

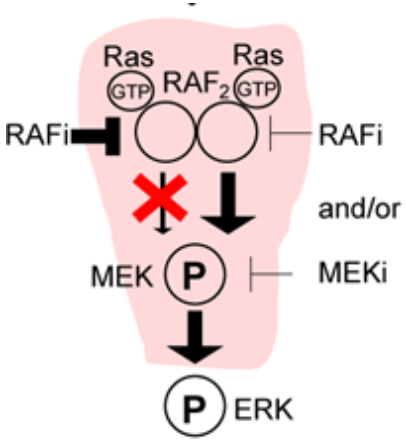
Interaction diagram



Examples of complex drug-protein species



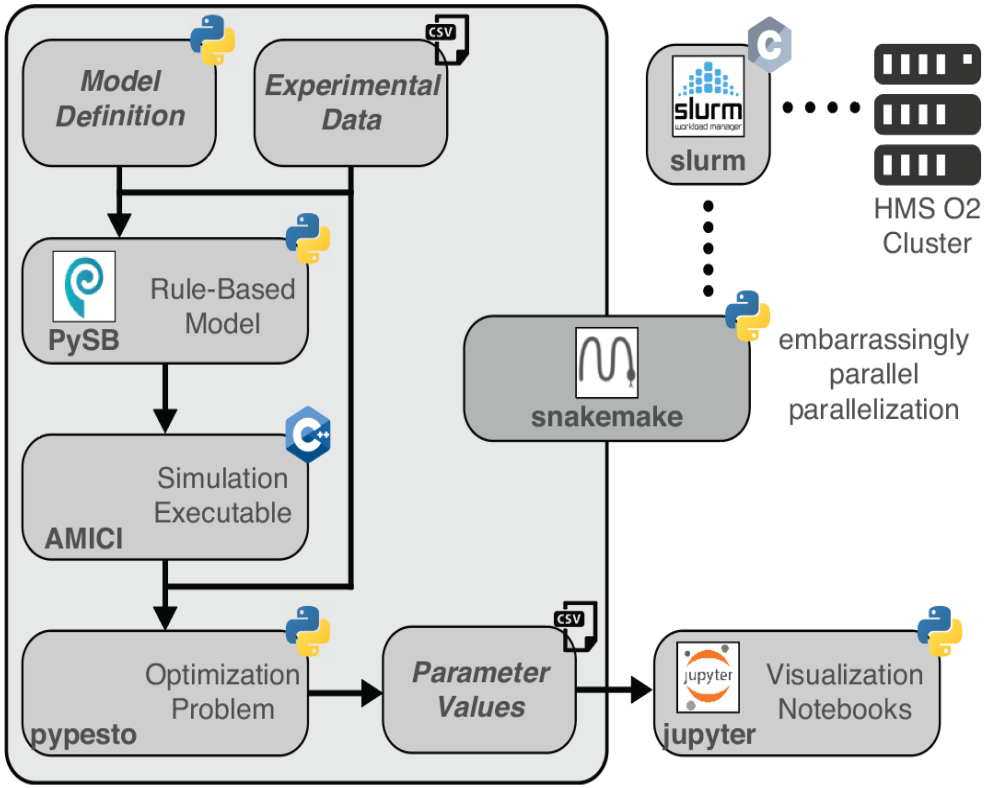
Sensitive to RAFi and/or MEKi



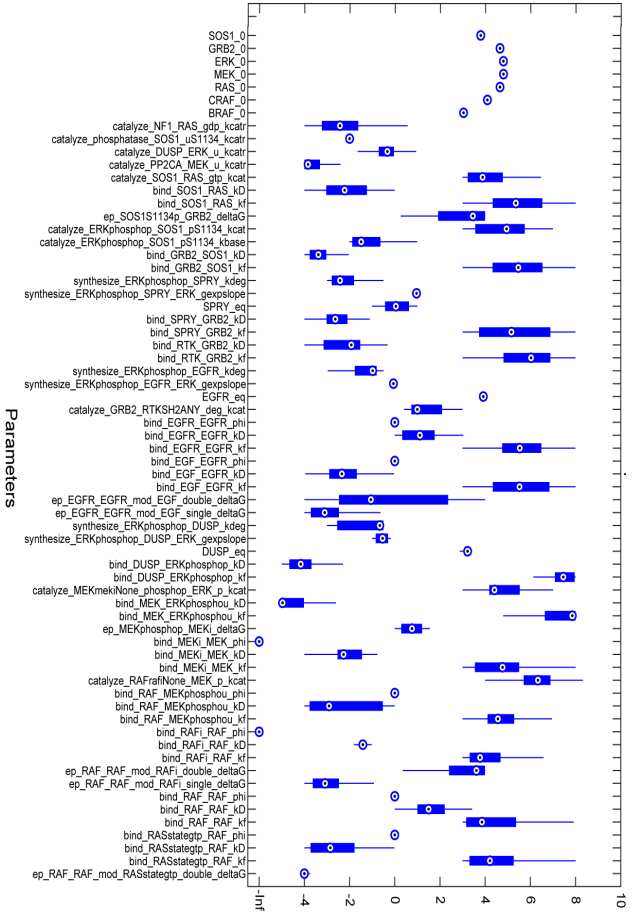
Resistant to RAFi and/or MEKi

Large-scale parameter fitting provides estimations for the 63 kinetic and energy parameters determining MAPK signaling

Parameter fitting pipeline



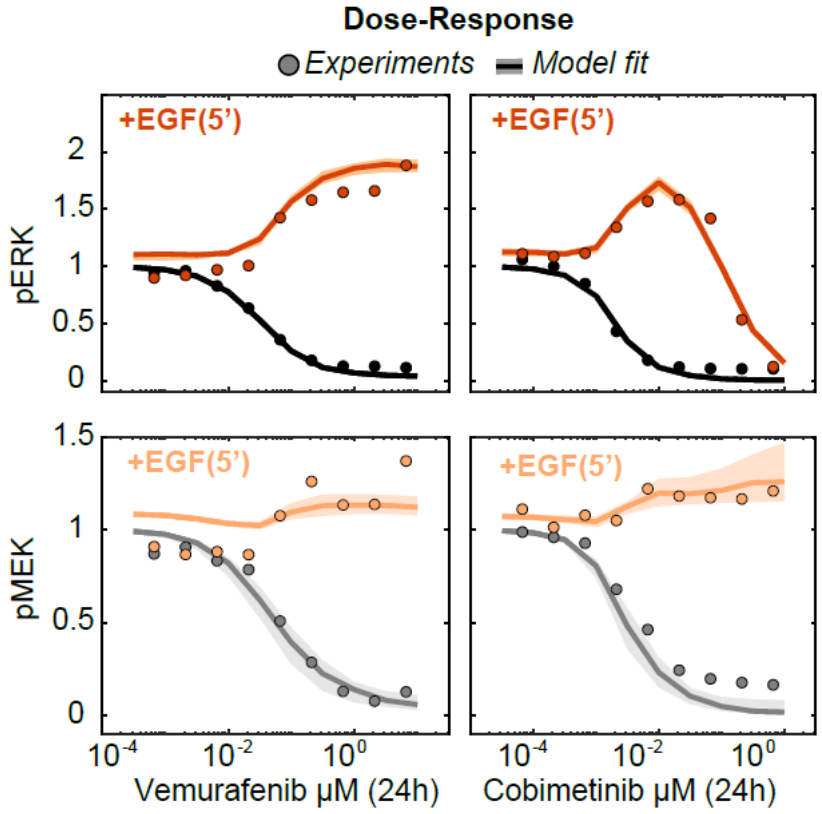
63 estimated parameters



by Fabian Fröhlich

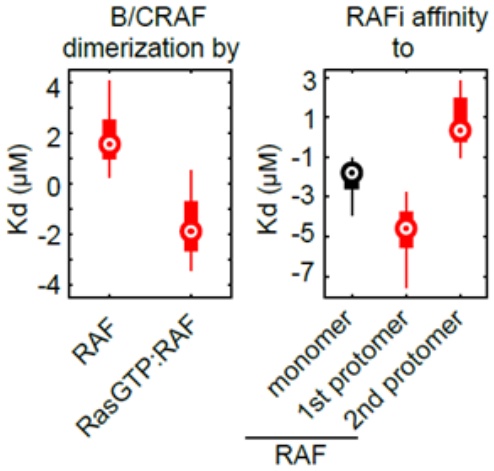
Kinetic parameters show that drug resistance to RAF and MEK inhibitors is due to RAF dimers assembled by receptor activation

Model fit to data

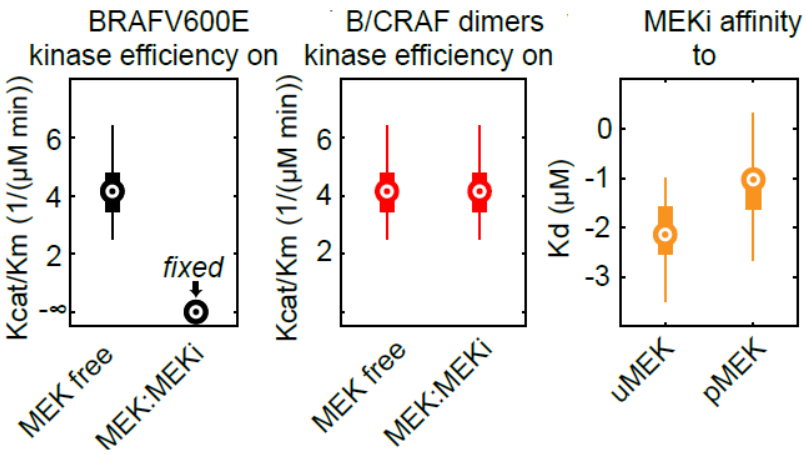


A375 melanoma cell line

Parameters

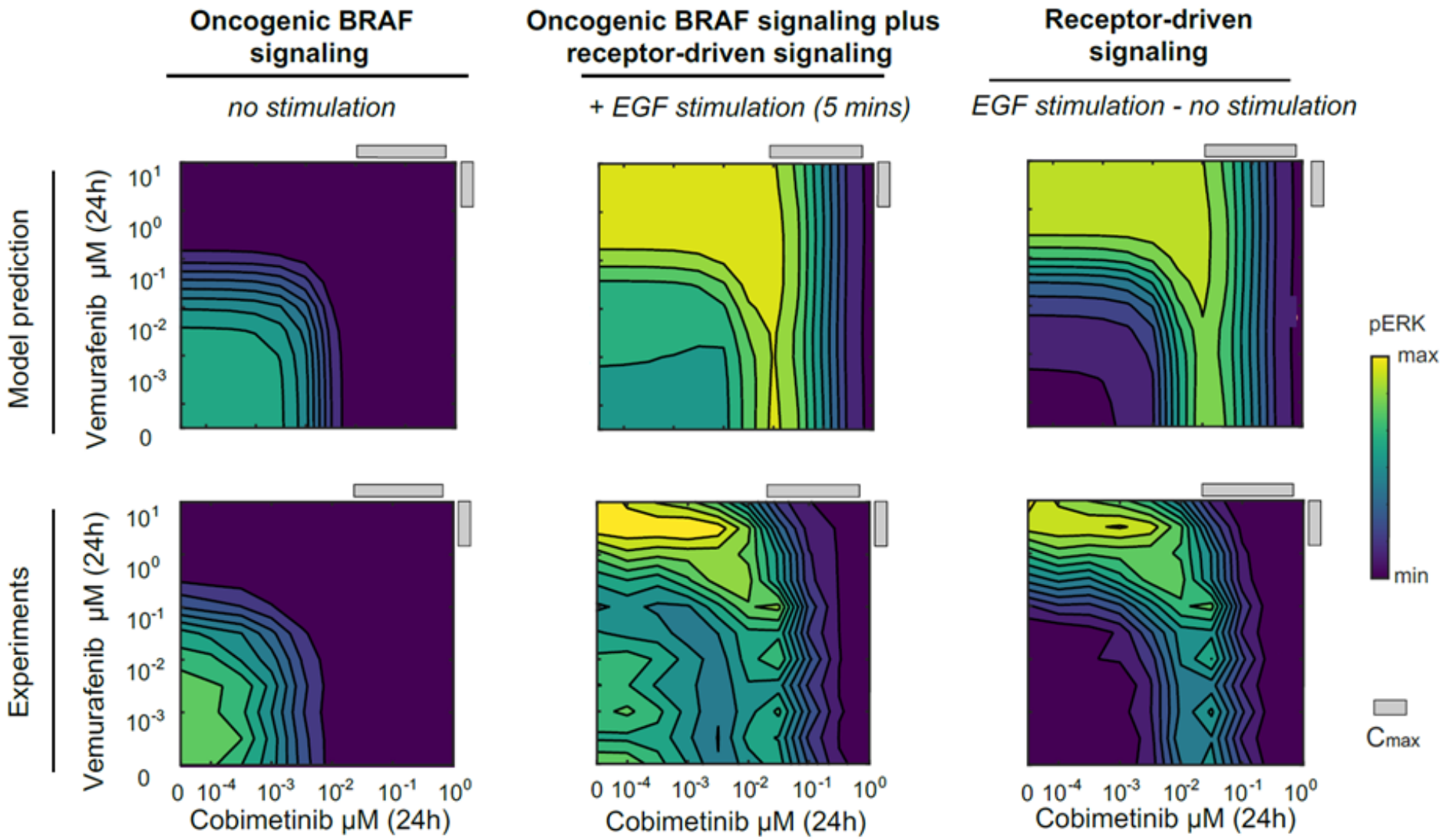


RAS-RAF-RAFi



MEK-MEKi

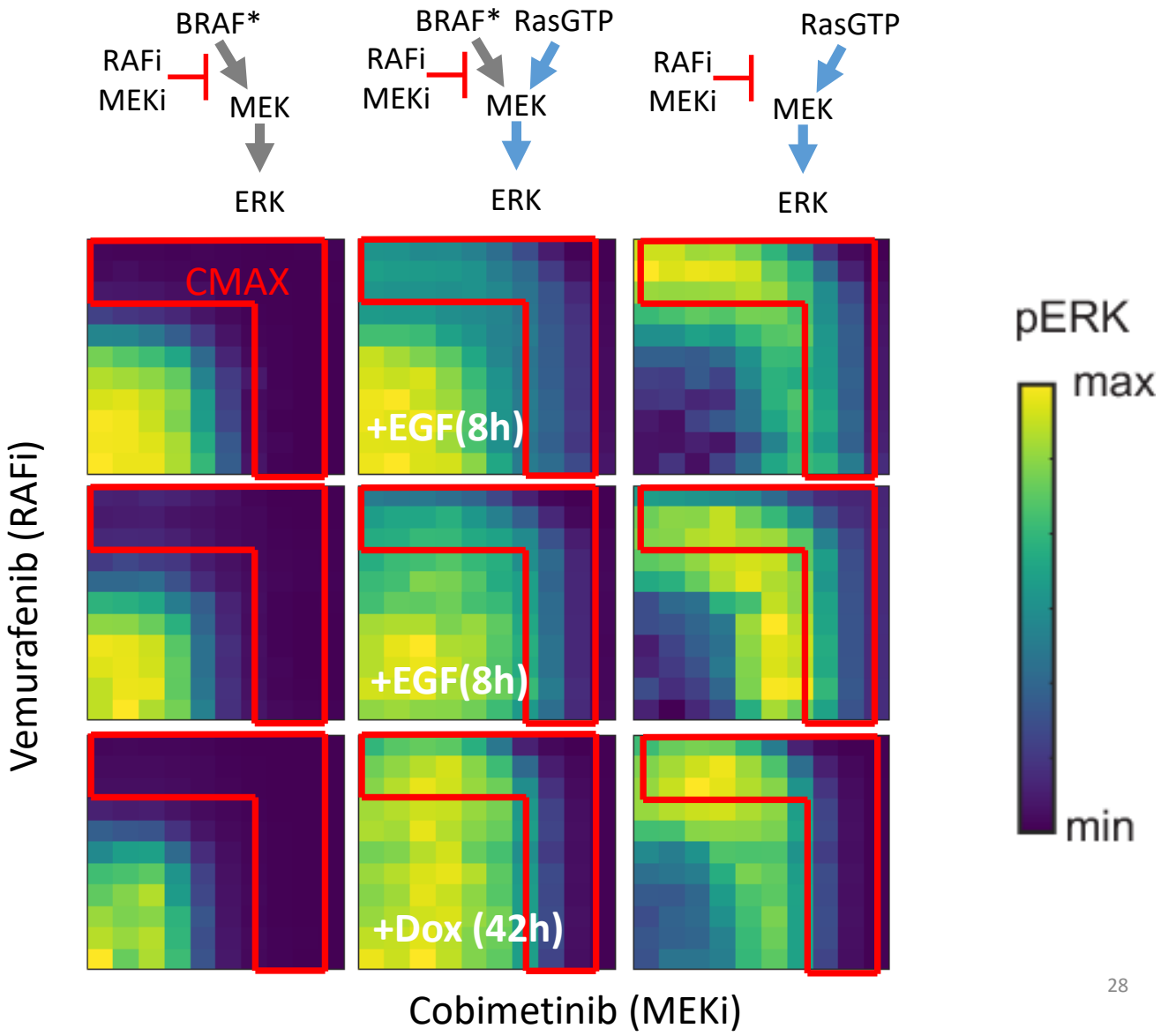
Modelling predicts wide dose range of combined RAF/MEK inhibition in which receptor-driven ERK signaling is possible



A375 melanoma cell line

RasGTP activation by receptors or mutations is resistant combined RAF and MEK inhibitors

Experimental validation of unified model



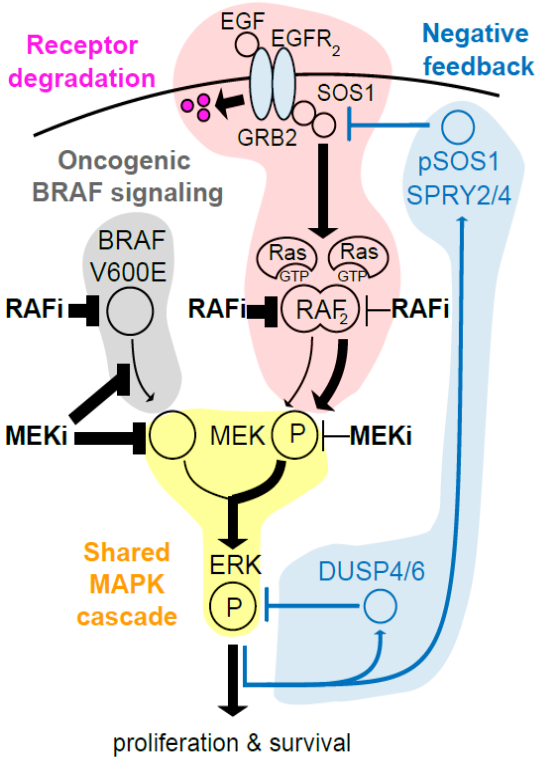
A375 EGFR overexpression (CRISPRa)

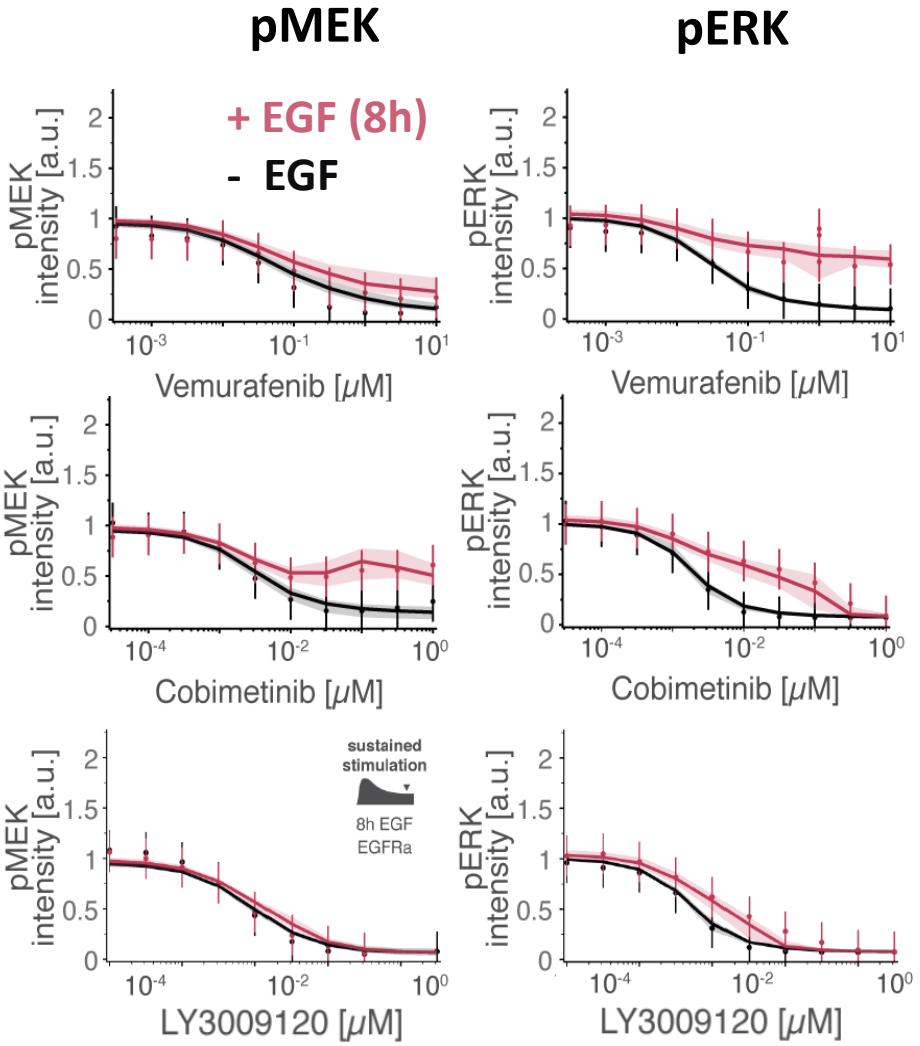
HT29 EGFR expressing (colorectal)

A375 with NRAS Q61K overexpression

The model accurately predicts resistance through MAPK reactivation driven by EGFR overexpression

Simulated and experimental (CRISPRa) overexpression of EGFR by x10 times



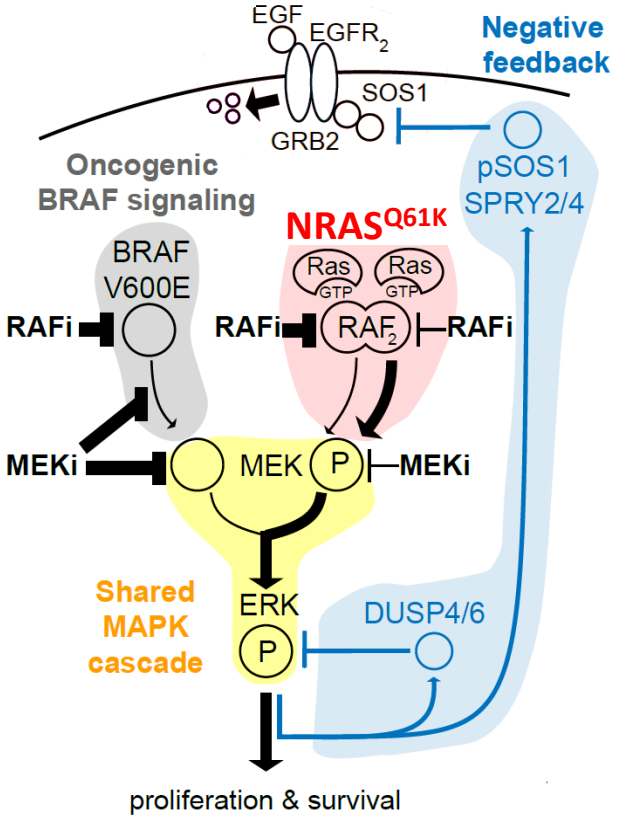


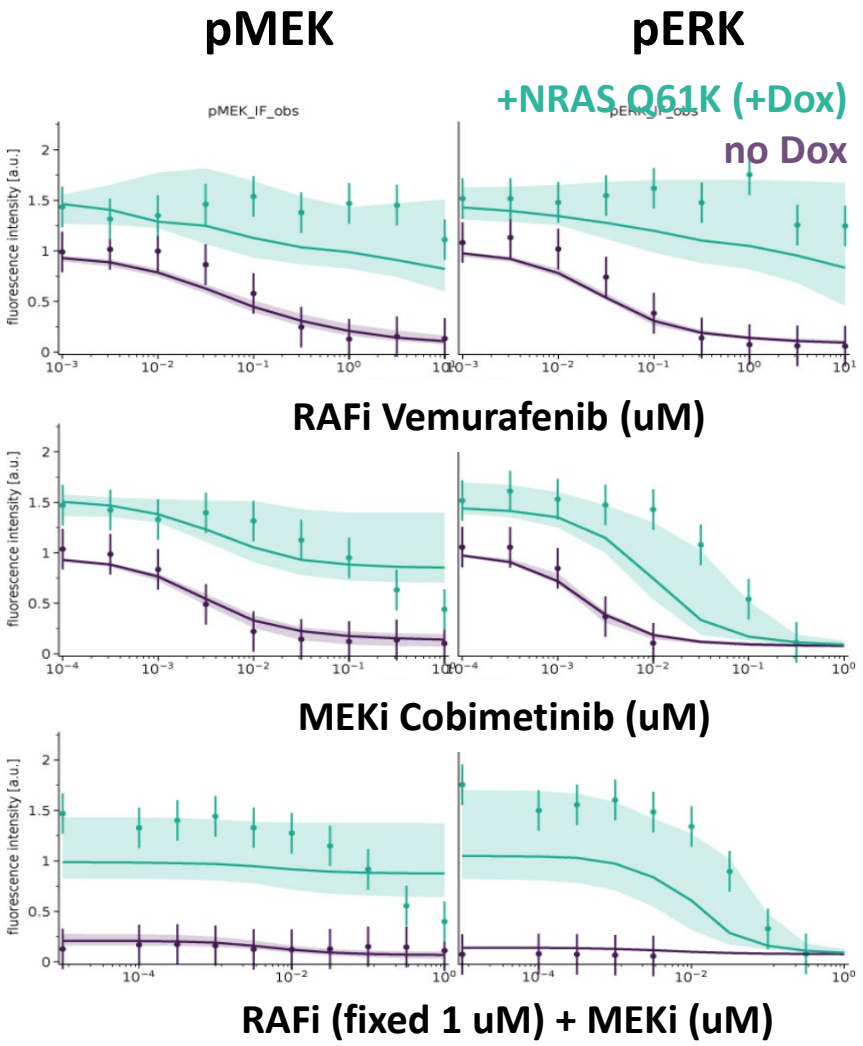
Experiments

Modelling

The model accurately predicts resistance through MAPK reactivation driven by RAS mutations

Simulated and experimental (Dox inducible) expression of NRAS^{Q61K} with x10 lower GTPase activity

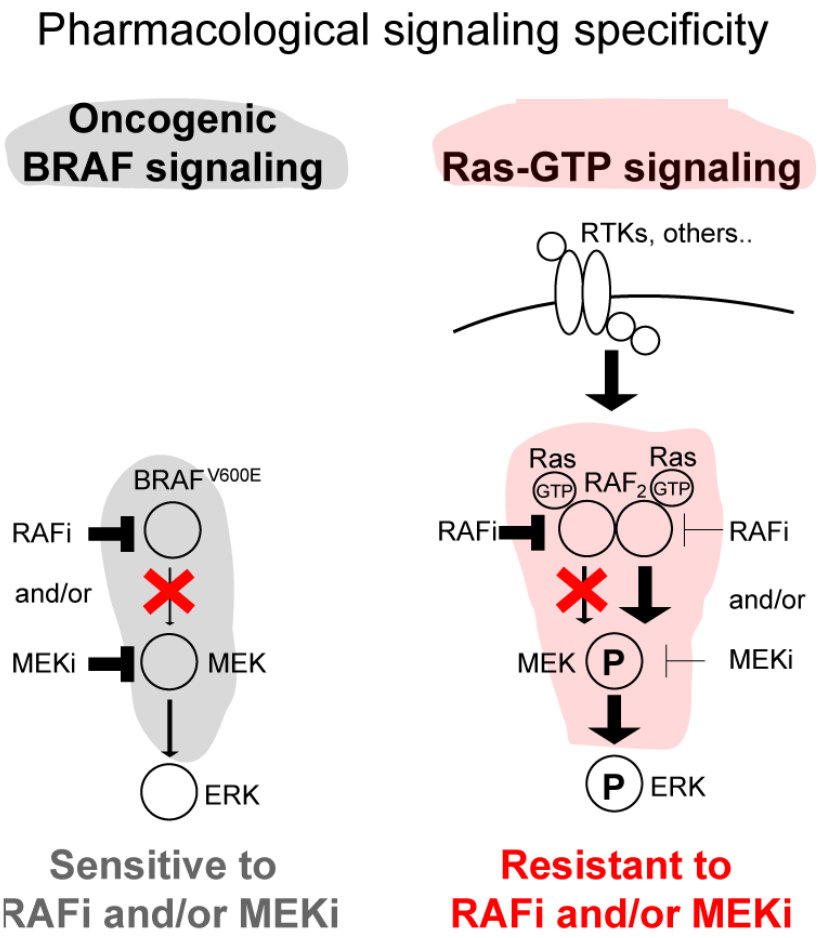




Experiments

Modelling

Conclusions on modeling complex drug-protein interactions in the MAPK signaling of BRAF-mutant cancers



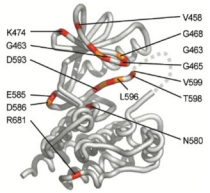
1. MAPK reconfiguration by Ras signaling assemble RAS-RAF-MEK complexes with lower affinity for drugs.

2. Genetic and adaptive resistance to RAF/MEK inhibitors is through MAPK reconfiguration

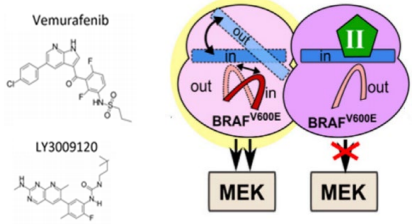
3. Likely both a source of drug resistance and drug tolerability

Outlook: Energy-based kinetic modeling is ideal to integrate structural biology information and omics data into predictive models

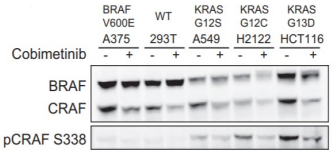
Mutational status



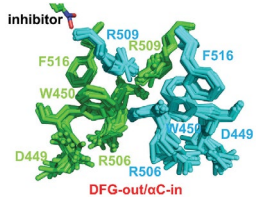
Drug-protein conformations



Protein abundances and PTM



Molecular dynamic simulations



Energy-based kinetic modeling

```
#Rules
Rule('RR', R(r=None)+R(r=None)<>R(r=1)%R(r=1), RR_phi, Ea0_rr)
Rule('RI', R(i=None)+I(r=None)<>R(i=1)%I(r=1), RI_phi, Ea0_ri)
Rule('RA', R(i=None)+A(r=None)<>R(i=1)%I(r=1), RI_phi, Ea0_ri)

#Energy Patterns
EnergyPattern('ep_RR', R(r=1)%R(r=1), Gf_RR)
EnergyPattern('ep_RR', R(i=1)%I(r=1), Gf_RI)
EnergyPattern('ep_IRR', I(r=1)%R(r=2)%R(r=2,i=None), ep_IRR)
EnergyPattern('ep_IRRI', I(r=1)%R(r=2)%R(r=2,i=3)%I(r=3), ep_IRRI)

#Expressions
Expression('Gf_RR', log(RR_kD))
Expression('Gf_RI', log(RI_kD))
Expression('Ea0_RR', -(RR_phi*log(RR_kD) + log(RR_kf)))
```

PySB Systems biology modeling in Python

$$\frac{d[pEGFR]}{dt} = k_{on}*[EGFR]*[EGF] - \ldots$$

$$\frac{d[Ras\text{-}GTP]}{dt} = k_{on}*[Ras]*[GTP] - \ldots$$

$$\frac{d[RAF_2]}{dt} = k_{on}*[RAF]^2 - k_{off}*[RAF_2] + \ldots$$

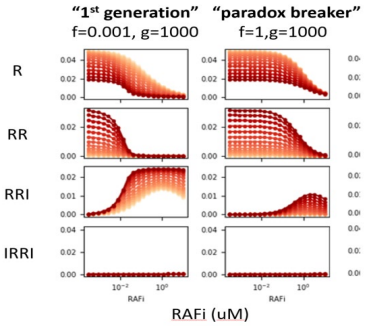
$$\frac{d[BRAF^{V600E}]}{dt} = k_{exp} - k_{deg}*[BRAF^{V600E}] + \ldots$$

$$\frac{d[pMEK]}{dt} = k_{on}*[MEK]*[BRAF^{V600E}] - \ldots$$

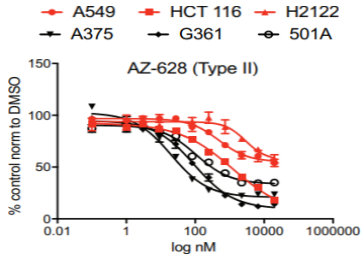
$$\frac{d[pERK]}{dt} = k_{on}*[ERK]*[pMEK] - \ldots$$

$$\frac{d[CyclinD1]}{dt} = k_{exp} - k_{deg}*[CyclinD1] + \ldots$$

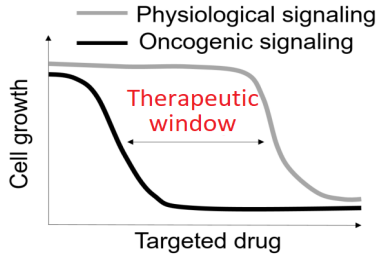
Drug design



Drug combinations



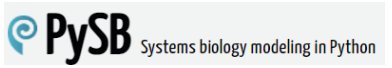
PK/PD analysis



Resources

- **Code for this webinar:** https://github.com/lgerosa/rosa_webinar_20Jan2021
  - Toy examples of thermodynamic model implementations in PySB
- **MAPK model:** https://github.com/labsyspharm/marm1-supplement
  - *Receptor-Driven ERK Pulses Reconfigure MAPK Signaling and Enable Persistence of Drug-Adapted BRAF-Mutant Melanoma Cells*
    Gerosa L, et al. - Cell Systems, 2020

- **PySB:** http://pysb.org/
  - *Programming biological models in Python using PySB*
    Lopez CF, Muhlich JL, Bachman JA, Sorger PK - Molecular systems biology, 2013



- **Foundational work:**
  - Energy-BNG by ***Faeder lab*** @ UPittsburgh:
    - *Advances in Rule-based Modeling: Compartments, Energy, and Hybrid Simulation, with Application to Sepsis and Cell Signaling*, Hogg JS , PhD Thesis , U Pitt - 2013
    - *Energy-based modeling in BioNetGen*, Sekar JAP, Hogg JS , Faeder JR
      IEEE International Conference on Bioinformatics and Biomedicine - 2016
  - Thermodynamic derivations by ***Kholodenko lab*** @ UCDublin
    - *Drug resistance resulting from kinase dimerization is rationalized by thermodynamic factors describing allosteric inhibitor effects*, Kholodenko BN , Cell reports - 2016
    - *Dissecting RAF inhibitor resistance by structure-based modeling reveals ways to overcome oncogenic RAS signaling*, Rukhlenko OS , et al, Cell systems – 2018

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parameter estimation

Peter Sorger
Director@LSP

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Leadership

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Miles Miller@MGH (In-vivo imaging)

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