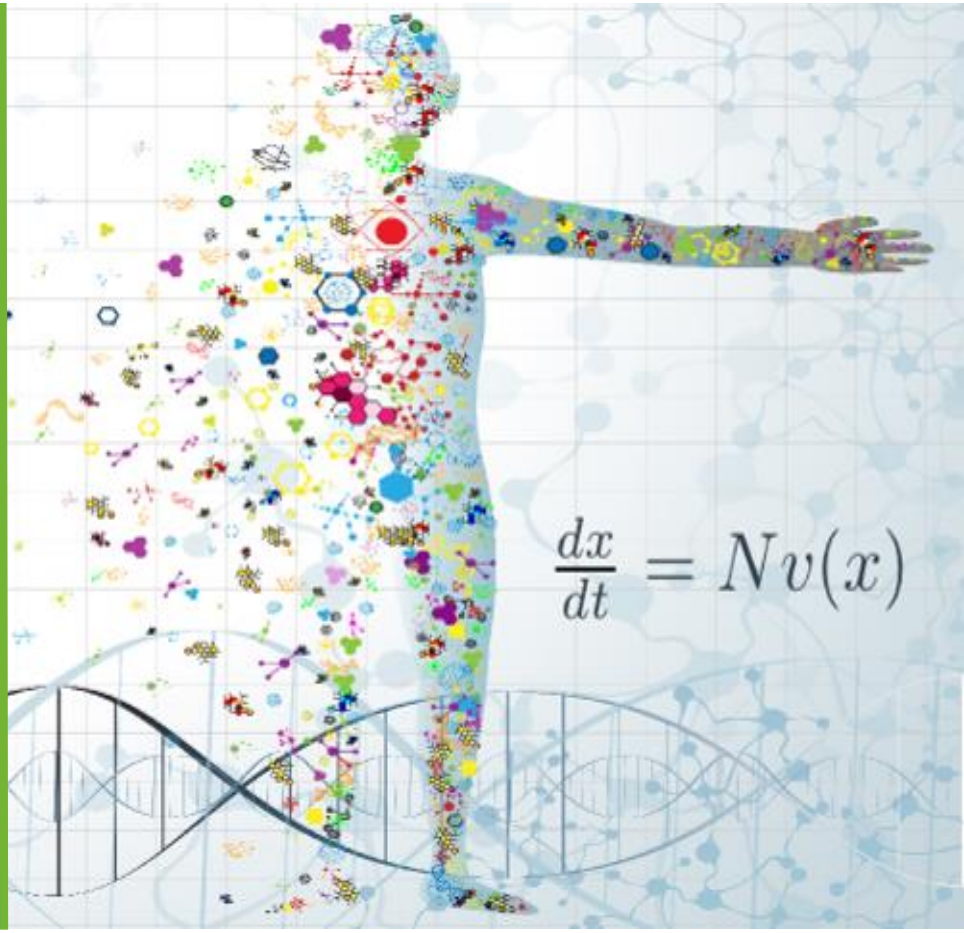
Using network reconstruction and modelling to improve cancer therapies



Walter Kolch

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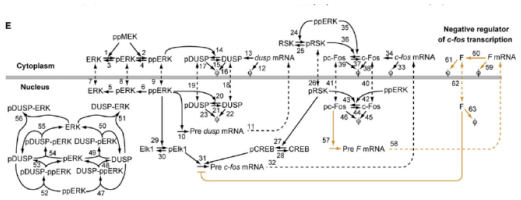
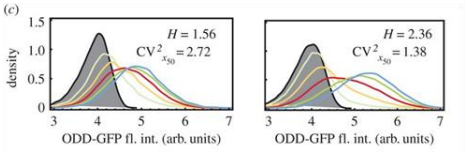
From networks to knowledge

Network ***mapping***

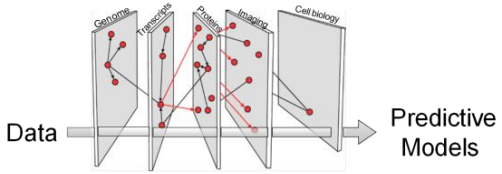
Network ***reconstruction***

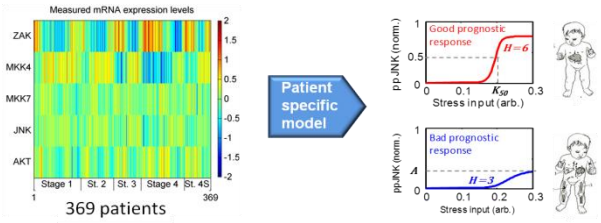




Network ***analysis***

Network ***integration***





Network ***knowledge application***

1) From probabilistic to mechanistic network models

2) Relating pathway models to tissue physiology

3) Network motifs that convey drug resistance

4) Making drugs work based on thermodynamic models

1) From probabilistic to mechanistic network models

2) Relating pathway models to tissue physiology

3) Network motifs that convey drug resistance

4) Making drugs work based on thermodynamic models

SCIENCE SIGNALING | RESEARCH ARTICLE

CANCER

Integrating network reconstruction with mechanistic modeling to predict cancer therapies

Melinda Halasz,[1,2]* Boris N. Kholodenko,[1,2,3] Walter Kolch,[1,2,3]* Tapesh Santra[1]*

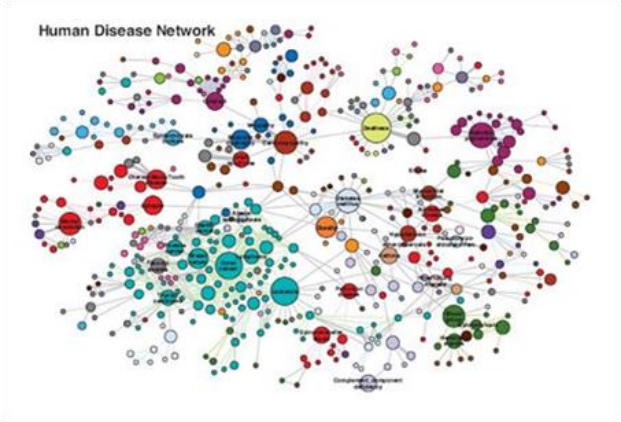
From probabilistic to mechanistic network models

Statistical models:

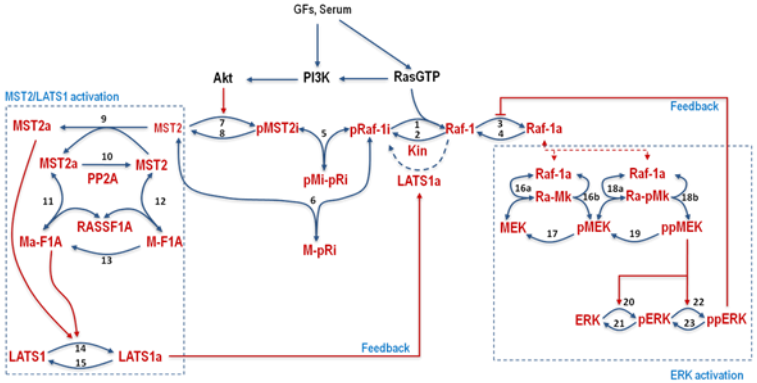
- Networks of correlations
- Limited predictive power
- No dynamic responses
- No mechanisms

Dynamic deterministic models:

- Networks of causal relationships
- Predictive power
- Dynamic responses
- Mechanisms



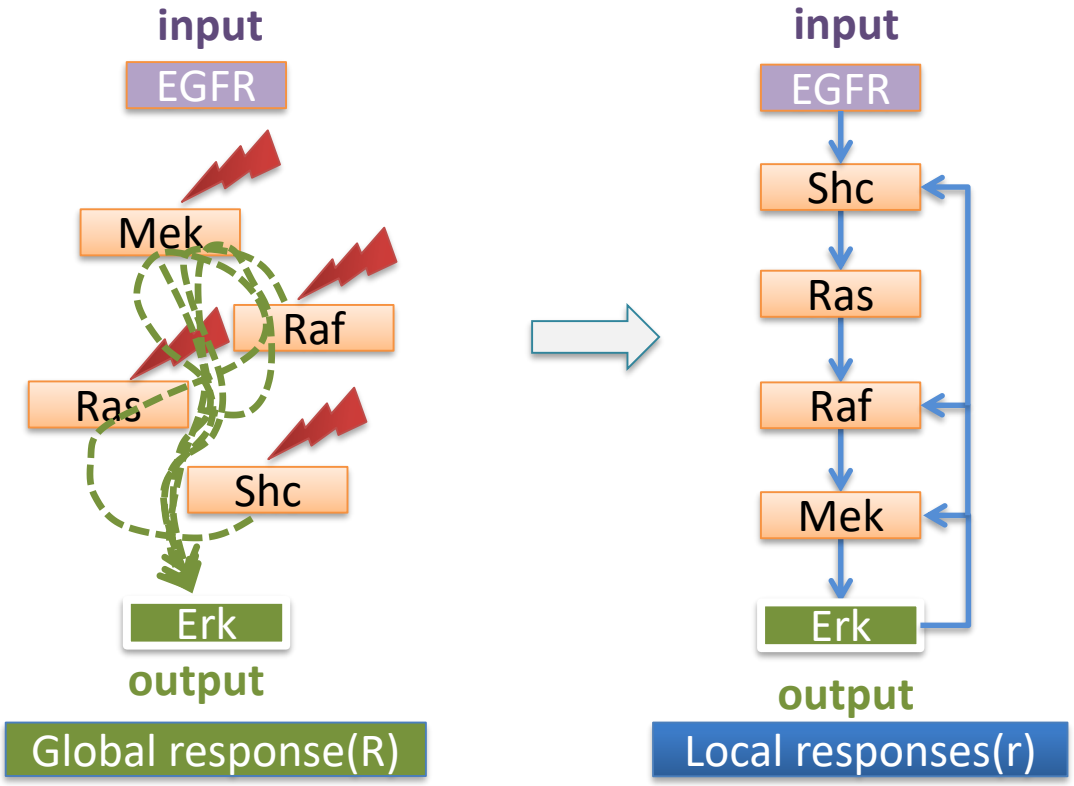
Ideal for -omics data



Needs quantitative data

Modular Response Analysis (MRA)

Reconstructing the local responses, i.e. network connections, from global responses through systematic perturbations



Untangling the wires: A strategy to trace functional interactions in signaling and gene networks, Kholodenko et. al., 2002, PNAS, vol. 99, page- 12841–12846

Modular Response Analysis (MRA)

The **local response coefficients** give the connection and connection strength between nodes, but they cannot be directly measured. However, they can be **recovered** from **experimental perturbation data**:

Local response coefficients

$$\sum_{j=1, j\neq i}^{n} r_{ij} R_{jk} = R_{ik}; i \neq k; i, k = 1 \ldots n$$

Global response coefficients = fractional change in the measured protein due to perturbation of the perturbed protein

$$R_{ik} \approx 2\left(\frac{x_i^k - x_i^0}{x_i^k + x_i^0}\right)$$

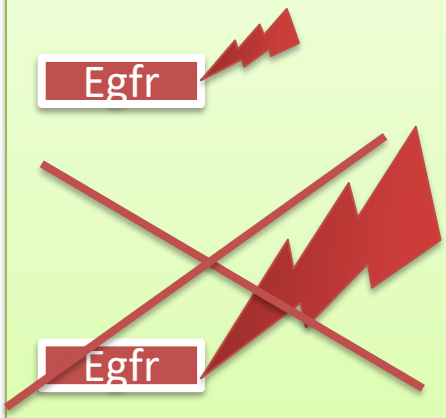
Untangling the wires: A strategy to trace functional interactions in signaling and gene networks, Kholodenko et. al., 2002, PNAS, vol. 99, page- 12841–12846

Modular Response Analysis (MRA)

Conditions and Assumptions

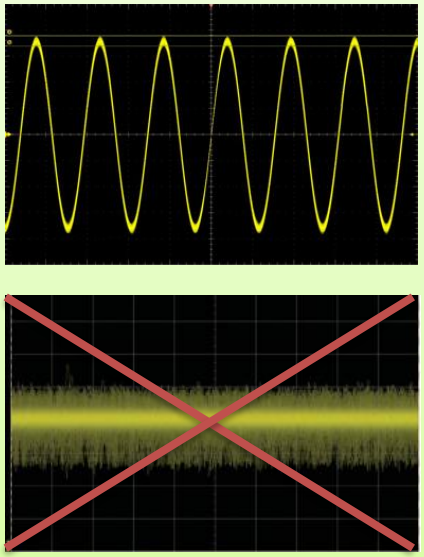
Condition 1

Perturbations are infinitesimally small



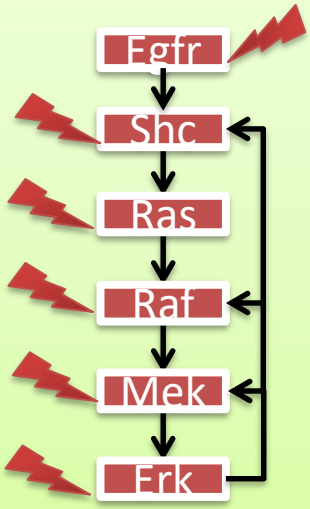
Condition 2

Noise free measurements



Condition 3

Requires as many perturbations as the number of nodes



Untangling the wires: A strategy to trace functional interactions in signaling and gene networks, Kholodenko et. al., 2002, PNAS, vol. 99, page- 12841–12846

Modular Response Analysis (MRA)

Advantages
- Gives direction of connections
- Quantifies strengths of connections

Disadvantages
- Every network component needs to be perturbed
- Very sensitive to measurement & biological noise

Bayesian Modular Response Analysis (BMRA)

$$\sum_{j=1,j\neq i}^{n} r_{ij}R_{jk} = R_{ik}; i \neq k; i,k = 1 \ldots n$$

$$\sum_{j=1,j\neq i}^{n} A_{ij}r_{ij}R_{jk} + \epsilon_{ik} = R_{ik}; i = 1 \ldots n, k = 1 \ldots n_p^i, i \neq k$$

Binary Variable:
Aij=0 if rij =0
Aij=1 if rij≠0

Stochastic variable that accounts for the error caused by noise

Determined by Bayesian Variable Selection Algorithm (BVSA), which is a MCMC approximation to estimate whether a connection exists or not

BVSA can handle missing perturbations and is more resistant to noise

Signal dependent noise →

Signal independent noise ↓

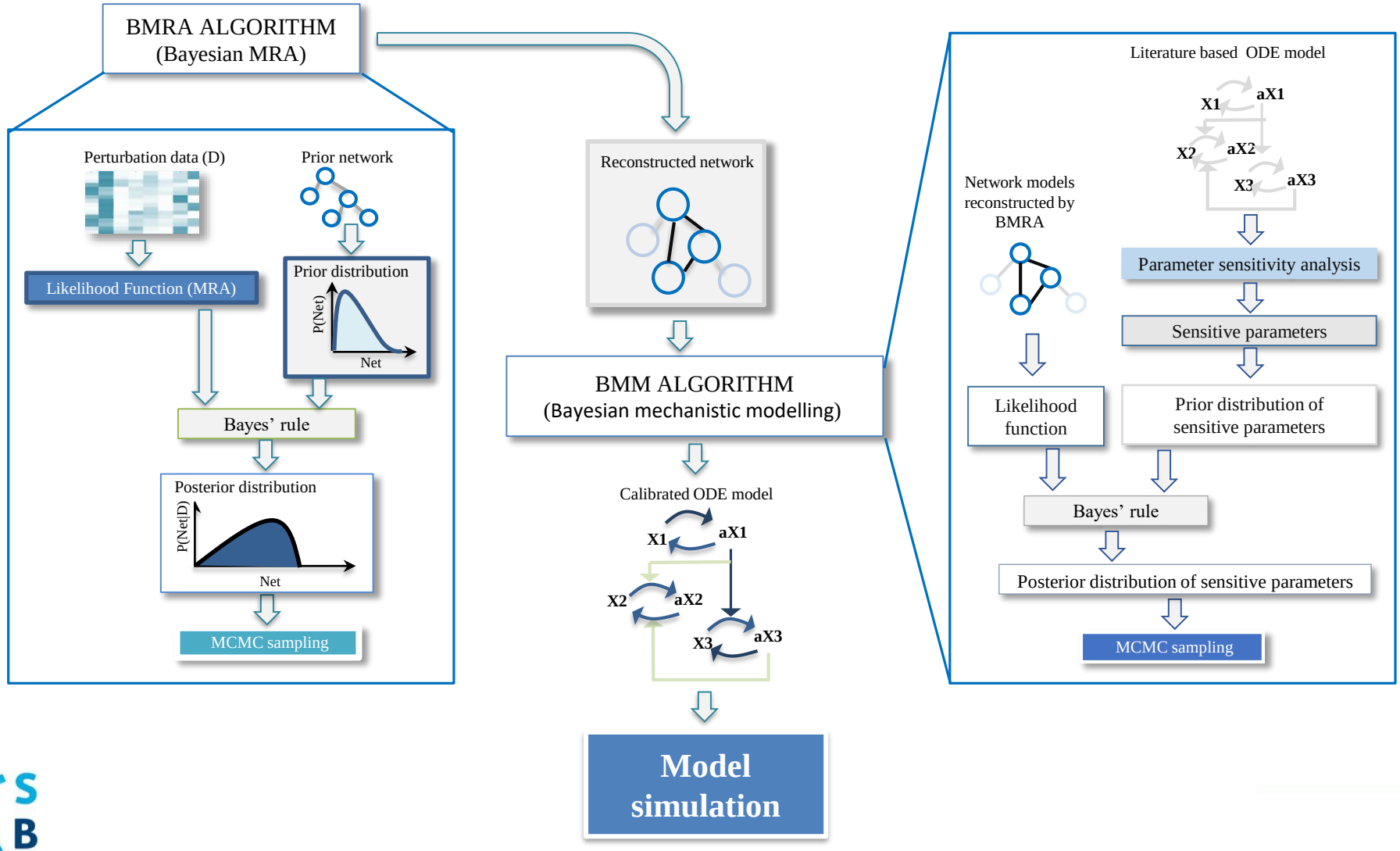
Best performer in terms of AUROC

	β_s=0.1	β_s=0.2	β_s=0.3	β_s=0.4	β_s=0.5	β_s=0.6	β_s=0.7	β_s=0.8	β_s=0.9	β_s=1.0
α_b=0.01	BVSA	BVSA	BVSA	BVSA	BVSA	BVSA	SBRA	SBRA	SBRA	SBRA
α_b=0.02	BVSA	BVSA	BVSA	BVSA	BVSA	SBRA	BVSA	BVSA	BVSA	SBRA
α_b=0.03	BVSA	BVSA	BVSA	SBRA	BVSA	BVSA	BVSA	BVSA	BVSA	SBRA
α_b=0.04	BVSA	BVSA	BVSA	BVSA	BVSA	SBRA	BVSA	BVSA	LMML	BVSA
α_b=0.05	LMML	LMML	BVSA	BVSA	BVSA	BVSA	BVSA	BVSA	BVSA	SBRA
α_b=0.06	BVSA	BVSA	BVSA	BVSA	NONE	BVSA	BVSA	BVSA	BVSA	BVSA
α_b=0.07	SBRA	BVSA	BVSA	BVSA	LMML	BVSA	BVSA	BVSA	BVSA	BVSA
α_b=0.08	LMML	BVSA	BVSA	BVSA	BVSA	BVSA	BVSA	BVSA	BVSA	LMML
α_b=0.09	LMML	BVSA	MRA	BVSA	BVSA	BVSA	BVSA	BVSA	BVSA	BVSA
α_b=0.10	MRA	BVSA	LMML	LMML	BVSA	BVSA	LMML	BVSA	LMML	BVSA

(a)

Santra, T., W. Kolch and B. N. Kholodenko (2013). "Integrating Bayesian variable selection with Modular Response Analysis to infer biochemical network topology." BMC Syst Biol **7: 57.**

From probabilistic to mechanistic network models



Benchmarking BMM

Accuracy of network reconstruction

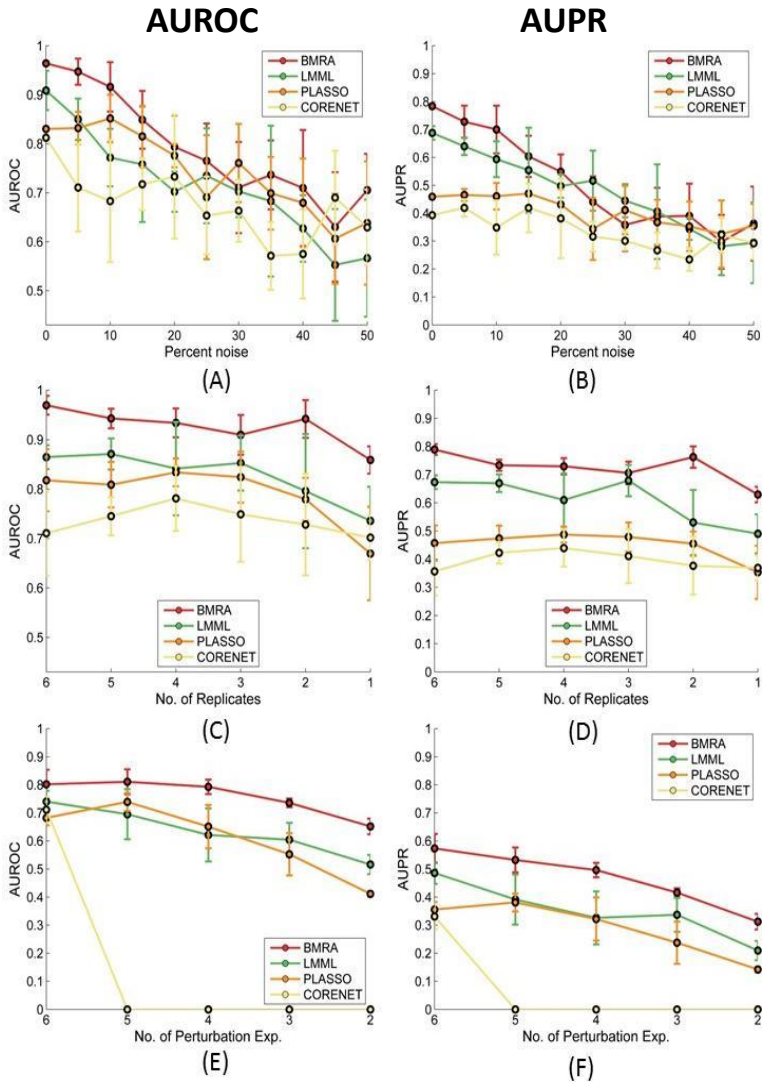
AUROC					AUPR				
Net1	**Net2**	**Net3**	**Net4**	**Net5**	**Net1**	**Net2**	**Net3**	**Net4**	**Net5**
0.985	**0.984**	**0.992**	**0.994**	**0.9594**	**0.930**	**0.937**	**0.968**	**0.9569**	**0.7738**
0.972	0.841	0.99	0.954	0.928	0.916	0.547	0.968	0.852	0.761
0.967	0.796	0.916	**0.936**	0.822	0.881	**0.543**	0.682	**0.774**	0.673
0.934	**0.751**	0.869	0.922	0.776	**0.740**	0.382	0.659	0.698	0.508
0.884	0.657	**0.8675**	0.902	**0.7403**	0.673	0.301	**0.650**	0.693	**0.4996**
0.864	0.655	0.824	0.884	0.723	0.623	0.243	0.646	0.675	0.424
0.844	0.567	0.864	0.82	0.673	0.507	0.288	0.572	0.645	0.385

Performances of BMRA (red and blue) and top five performers of the DREAM 4 challenge (black).

Results of BMRA without prior knowledge are shown in blue.

Results of BMRA with prior knowledge (50% wrong connections) are shown in red.

Benchmarking BMM



Sensitivity of BMM to

Increasing noise (1-50%)

Decreasing number of replicates (6-1)

Decreasing number of perturbation experiments (6-2)

Application to real data

Molecular Systems Biology 9; Article number 673; doi:10.1038/msb.2013.29
Citation: *Molecular Systems Biology* 9:673
www.molecularsystemsbiology.com

OPEN ACCESS SOURCE DATA TRANSPARENT PROCESS molecular systems biology

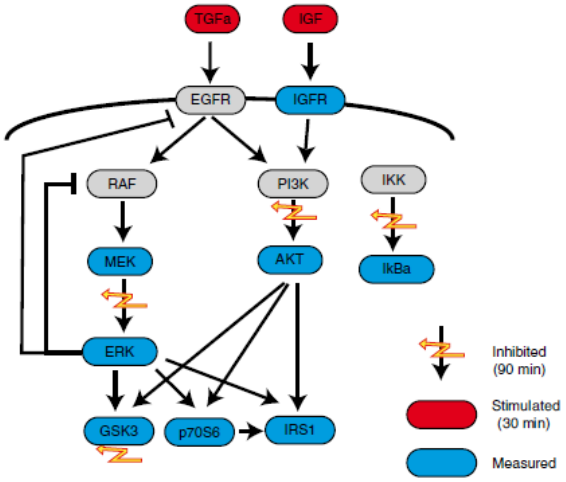
Network quantification of EGFR signaling unveils potential for targeted combination therapy

Bertram Klinger[1,2,6], Anja Sieber[1,6], Raphaela Fritsche-Guenther[1,6], Franziska Witzel[1,2], Leanne Berry[3], Dirk Schumacher[1], Yibing Yan[4], Pawel Durek[1,2,5], Mark Merchant[3], Reinhold Schäfer[1,5], Christine Sers[1] and Nils Blüthgen[1,2,*]

Stimulation with TGFα (a ligand of EGFR) and IGF-1

Measurements of phosphorylation changes in eight key signalling proteins (IGF-1R , IRS-1, AKT, MEK, ERK2 p70S6K, GSK3α/β, IkBα)

Four different inhibitory drugs



Five colorectal cancer cell lines with different mutations

Table 1 Mutation spectrum of the cell line panel

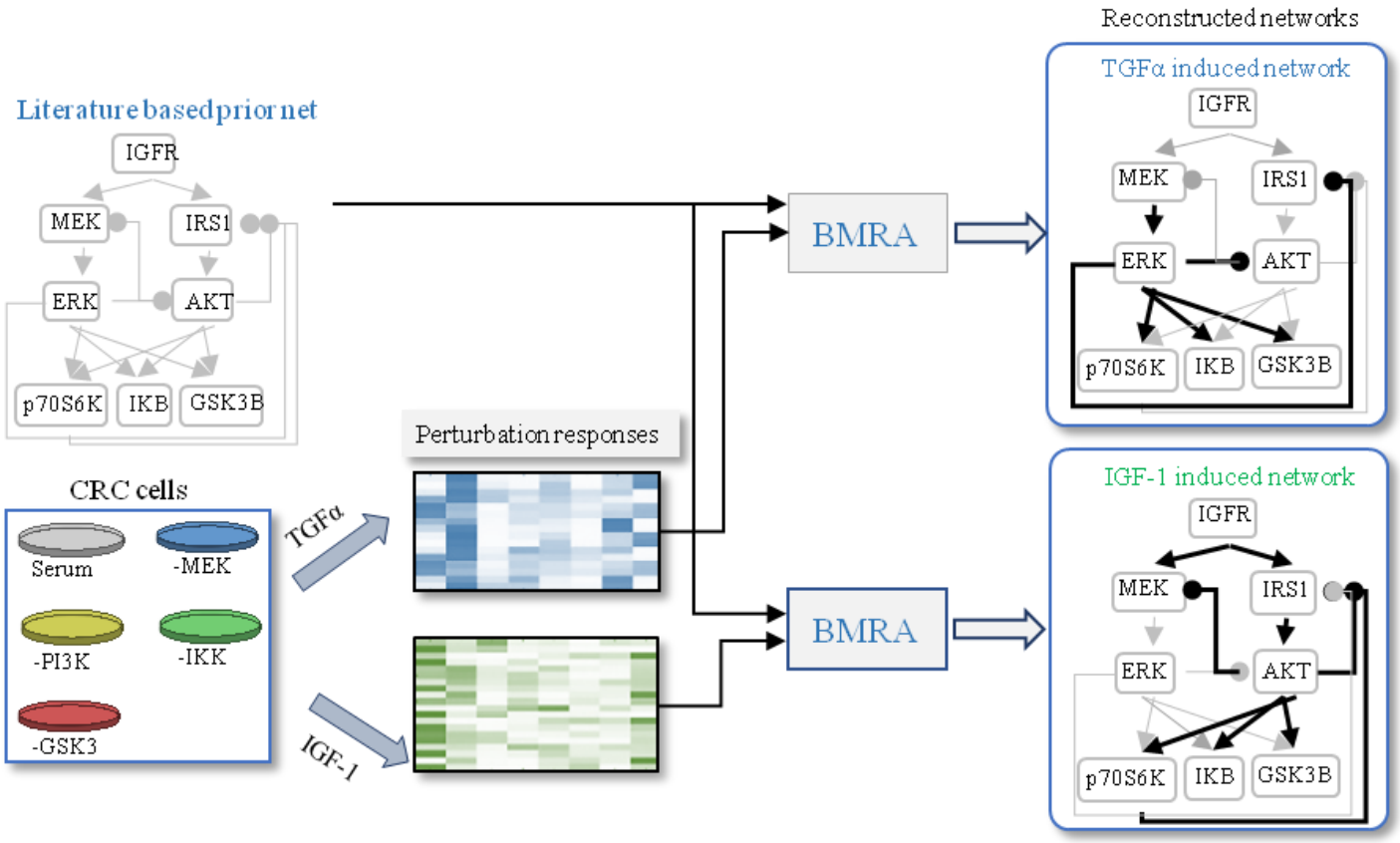
Gene symbol	LIM1215	HCT116	SW403	SW480	HT29
ABL1	P309A	Y257C			
APC		K1462R		A1457T/K1462R	
BRAF					V600E*
CTNNB1	T41A[H]				
EGFR3		S400R		S400R	
KRAS	A146T	G13D*	G12V*,[H]	G12V*,[H]	
PIK3CA		H1047R*			
SMAD4					Q311X*,[H]
SMO		V404M			
STK11		G58S		G58S	
TP53			R273H[H]	R273H[H]	R273H*,[H]

10-15%

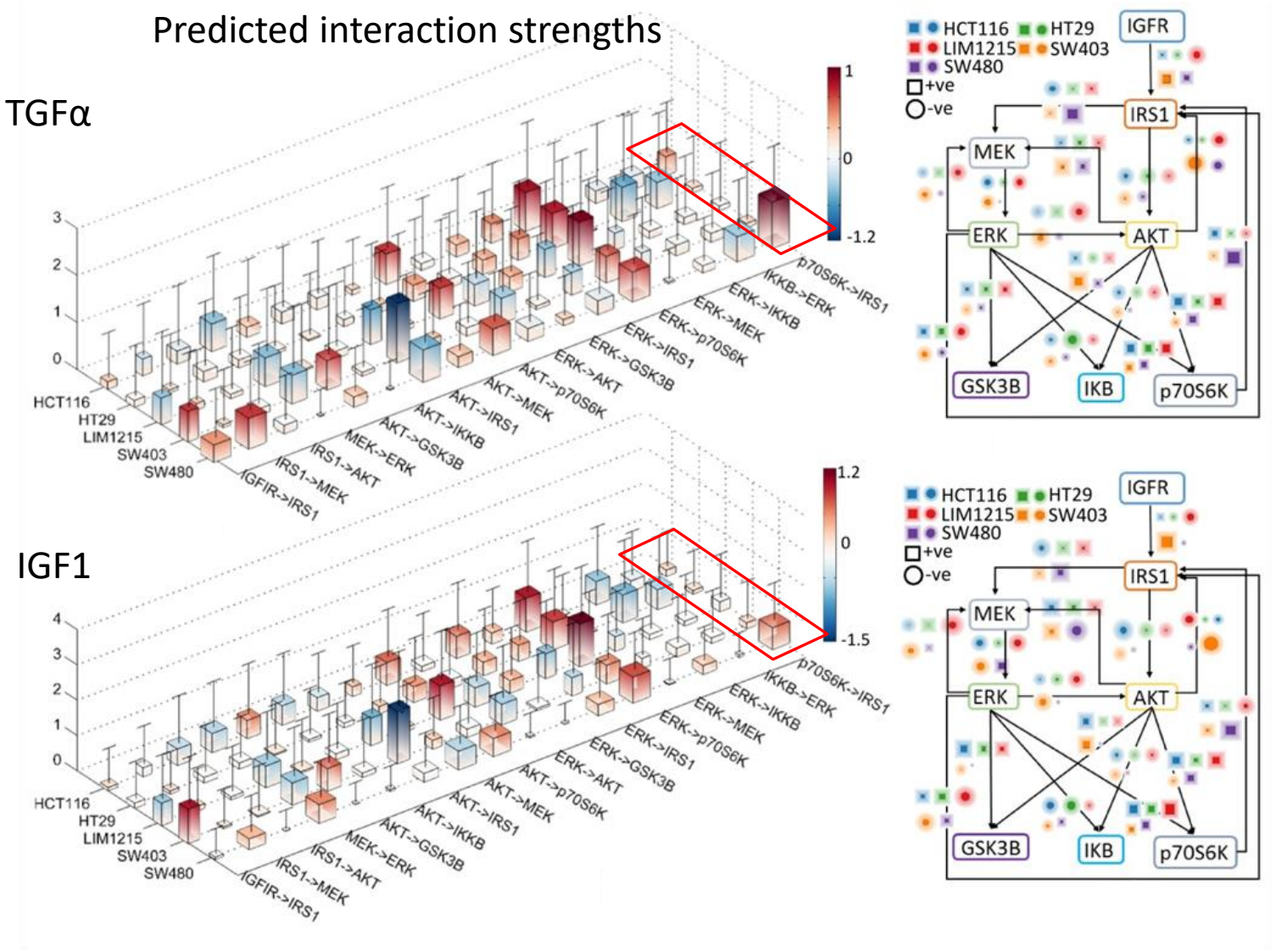
20-30%

No targeted therapy available

Network reconstruction



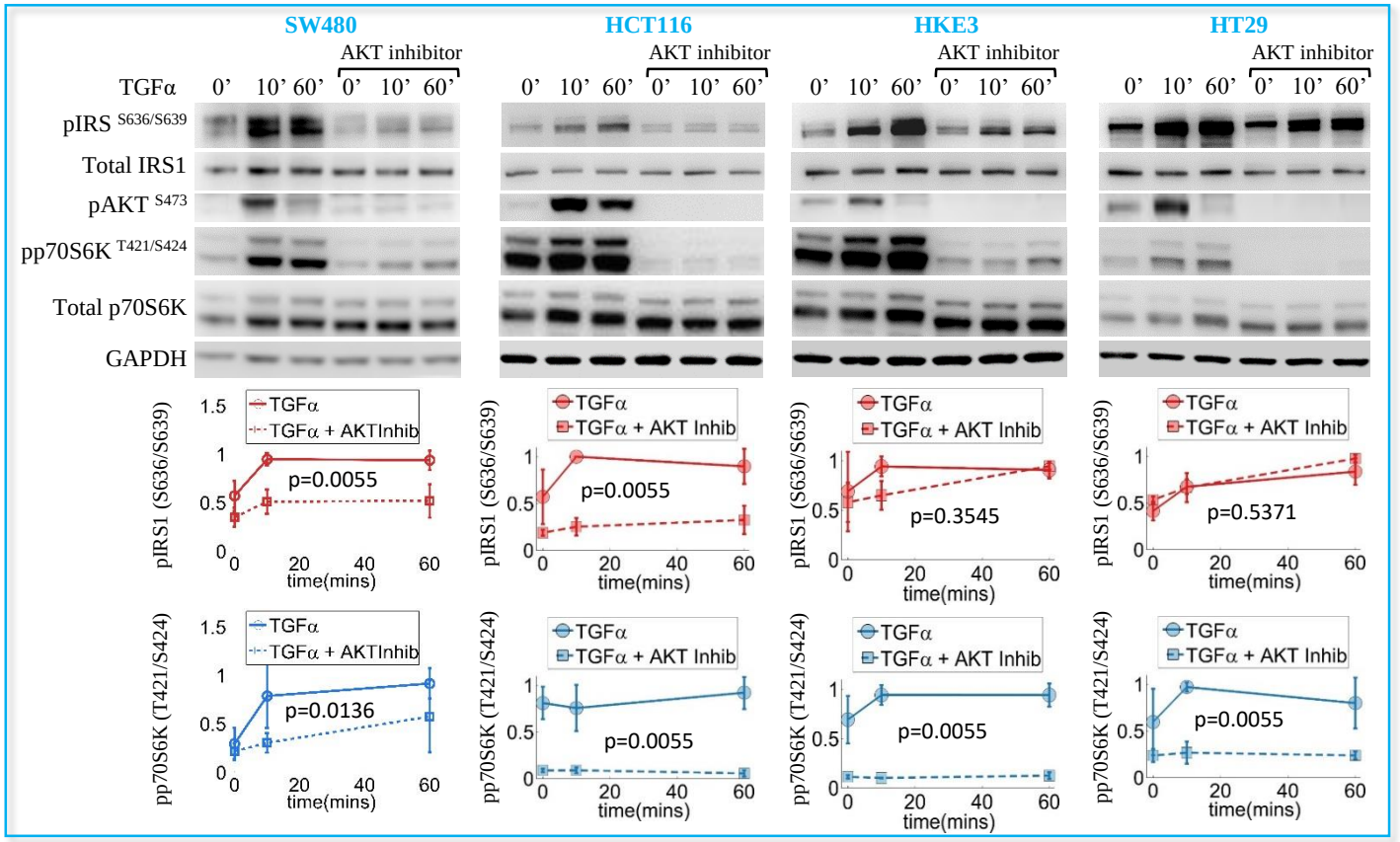
Cell type specific connections



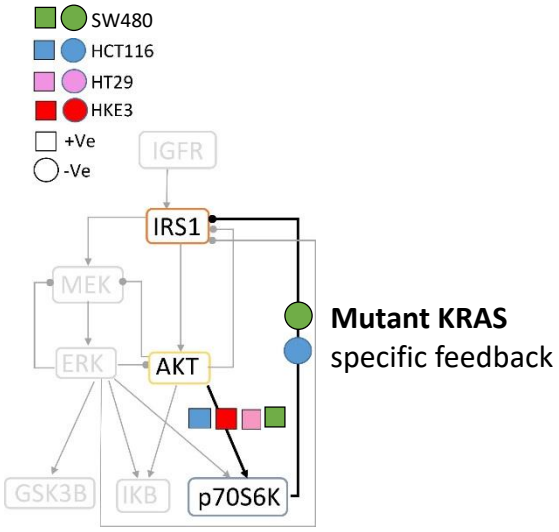
Experimental validation of connectivities

Effect of AKT inhibition on pIRS1 & pp70S6K

Mutant KRAS Mutant KRAS ~~Mutant~~ KRAS Mutant BRAF



Experimental validation summary



Mutant KRAS
specific feedback

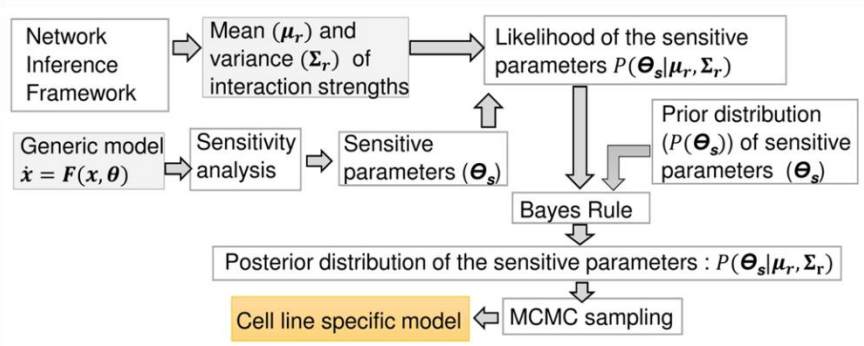
An effective CRC therapy is cetuximab, an antibody that inhibits the EGFR

20-30% of CRC patients have a mutated KRAS gene that renders them resistant to EGFR targeted therapies

Approximately half of the patients with wild type KRAS will develop resistance to EGFR inhibition

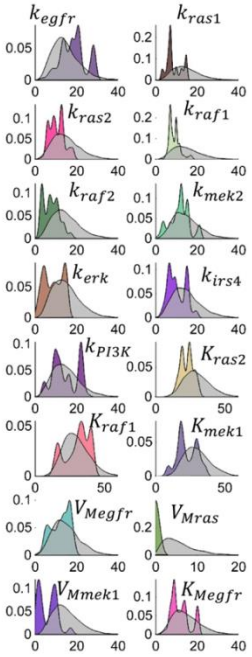
Can the model address these clinical problems?

From Bayesian networks to ODE models

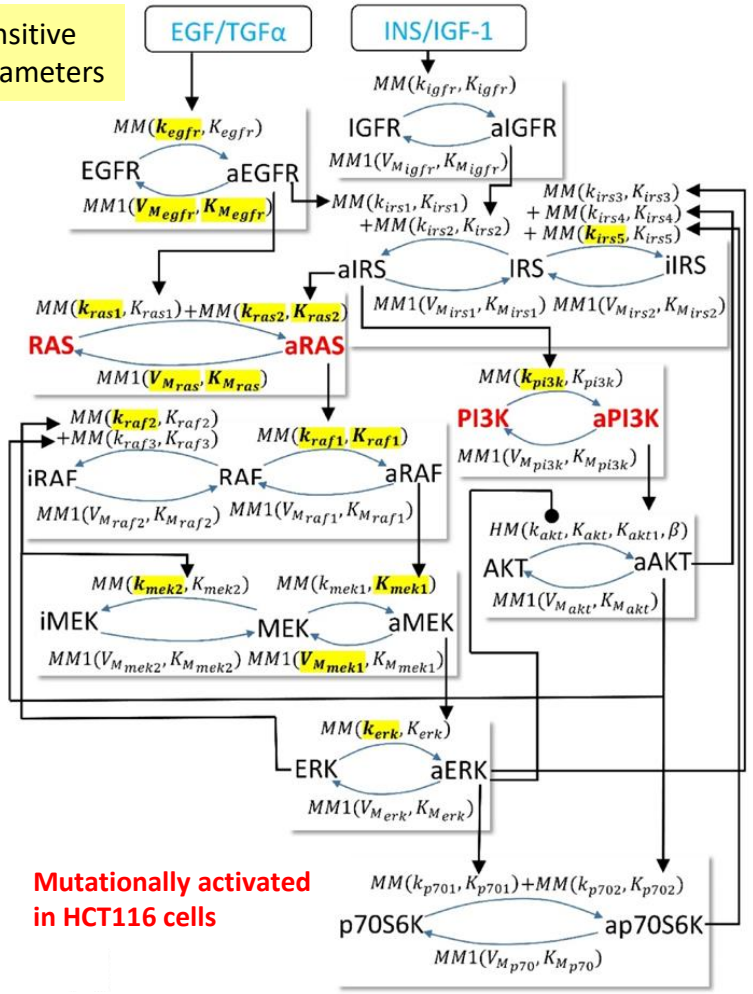


Prior & posterior distribution of sensitive parameters

Posterior parameters are often bimodal reflecting kinetic differences in EGFR and IGFR signalling

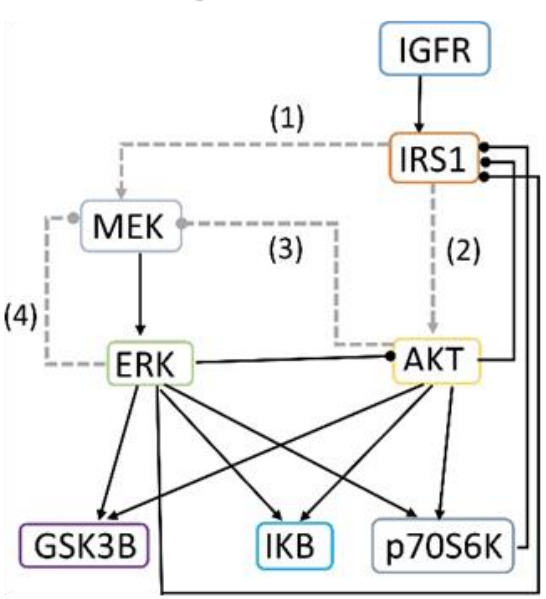


sensitive parameters

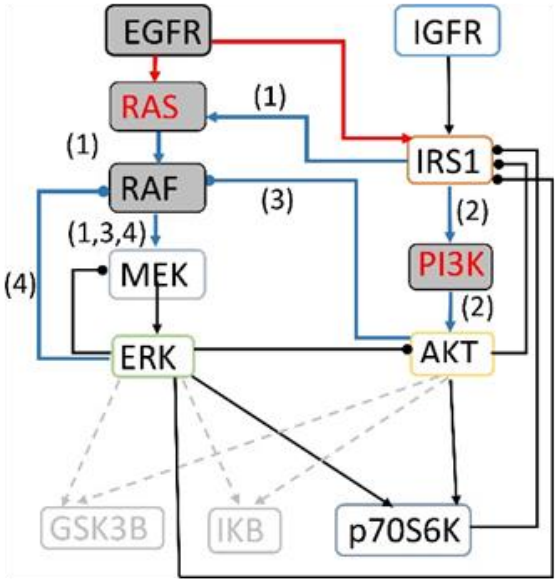


From Bayesian networks to ODE models

Prior generic STN model



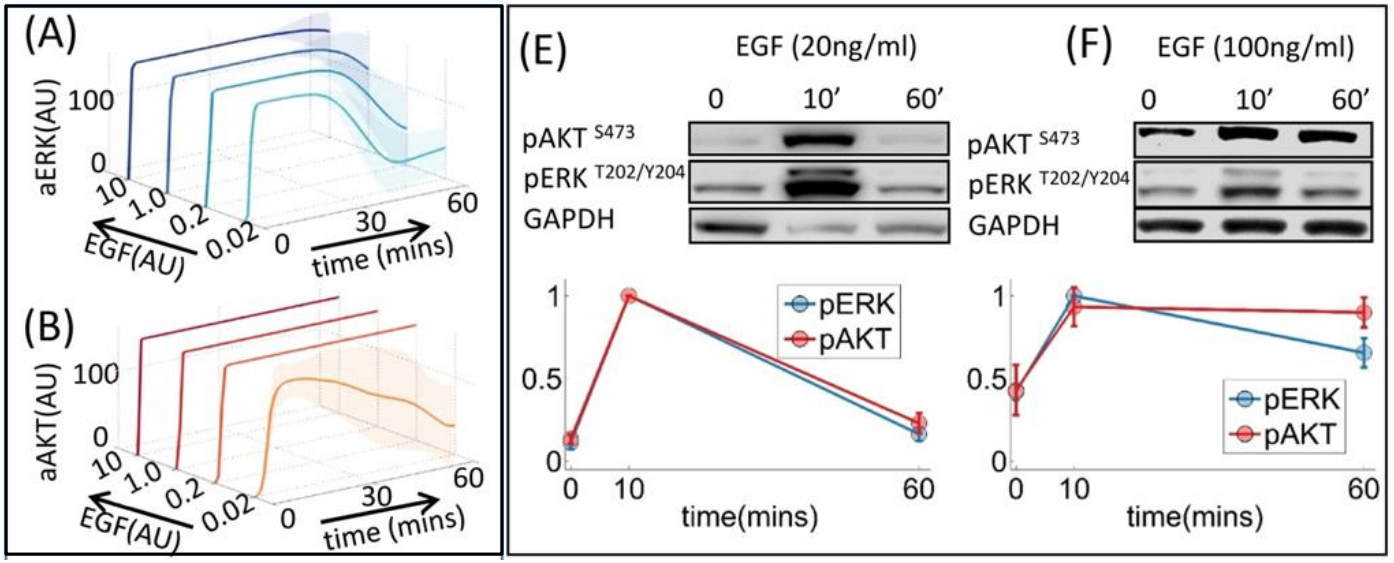
Posterior & calibrated STN model



Experimental validation of ODE model

Model simulation

Experiment (HCT116 cells)

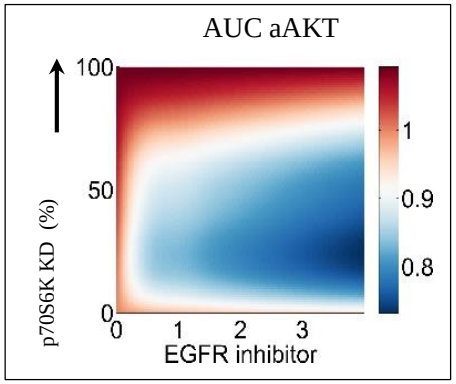


Stimulation with increasing dose of EGF

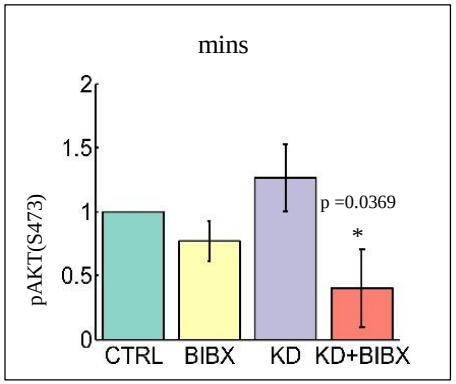
Reducing p70S6K feedback sensitizes mtKRAS cells to EGFR inhibition

Synergy between p70SK KD & EGFR inhibitors to inhibit AKT activation in mutant KRAS cells (HCT116)

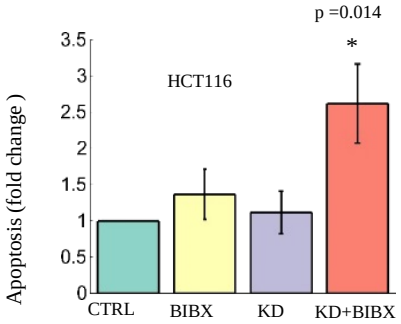
Model

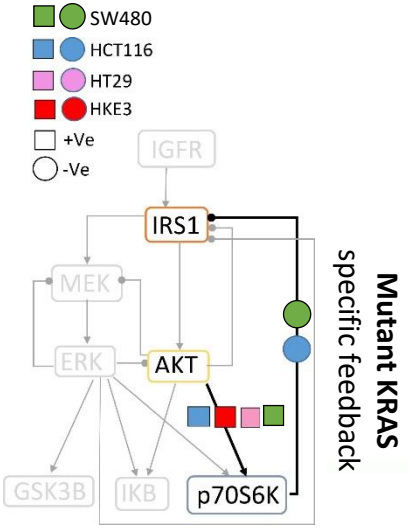


Experiment

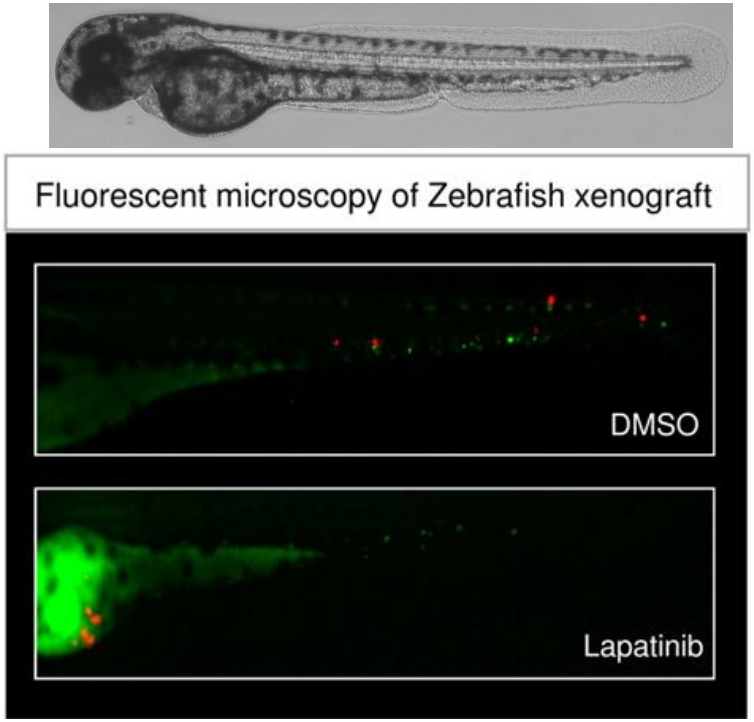


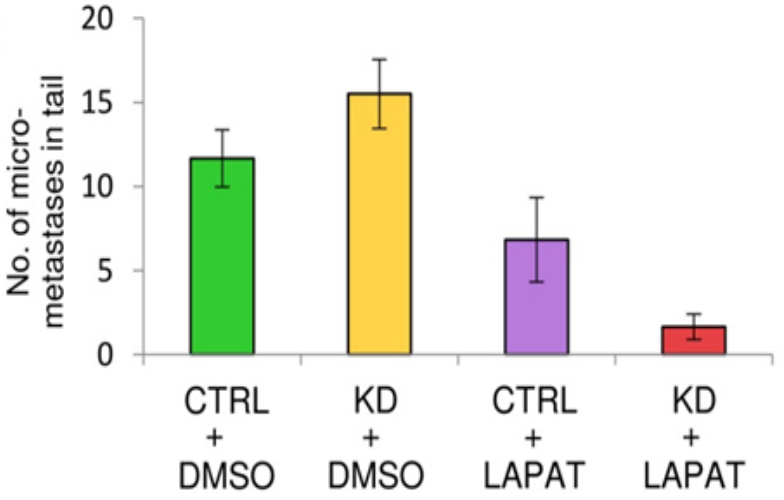
Synergy between p70SK KD & EGFR inhibitors to promote apoptosis of mutant KRAS cells (HCT116)





EGFR inhibition & S6K knockdown synergise to inhibit in vivo invasion





Conclusions

We have developed a two stage modelling framework that can
- infer the structure of STNs using noisy and incomplete perturbation data
- derive mechanistic ODE models to simulate adaptive STN responses

Feedback inhibition of IRS1 by p70S6K correlates with resistance to EGF receptor (EGFR) inhibition

Breaking the p70S6K mediated feedback sensitises mutant KRAS cells to EGFR inhibitors

Model predictions were validated in a zebrafish model of metastasis

1) From probabilistic to mechanistic network models

2) Relating pathway models to tissue physiology

3) Network motifs that convey drug resistance

4) Making drugs work based on thermodynamic models

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Cell Reports
Report

The APC Network Regulates the Removal of Mutated Cells from Colonic Crypts

Je-Hoon Song,[1] David J. Huels,[2] Rachel A. Ridgway,[2] Owen J. Sansom,[2] Boris N. Kholodenko,[3,4,5] Walter Kolch,[3,4,5] and Kwang-Hyun Cho[1,*]

[1]Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea
[2]The Beatson Institute for Cancer Research, Garscube Estate, Glasgow G61 1BD, UK
[3]Systems Biology Ireland, University College Dublin, Belfield, Dublin 4, Ireland
[4]Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland
[5]School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4, Ireland

SBI

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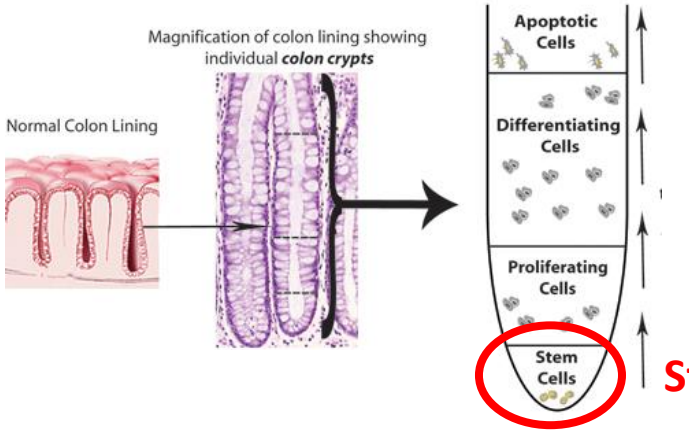
Fondúireacht Eolaíochta Éireann
Science Foundation Ireland

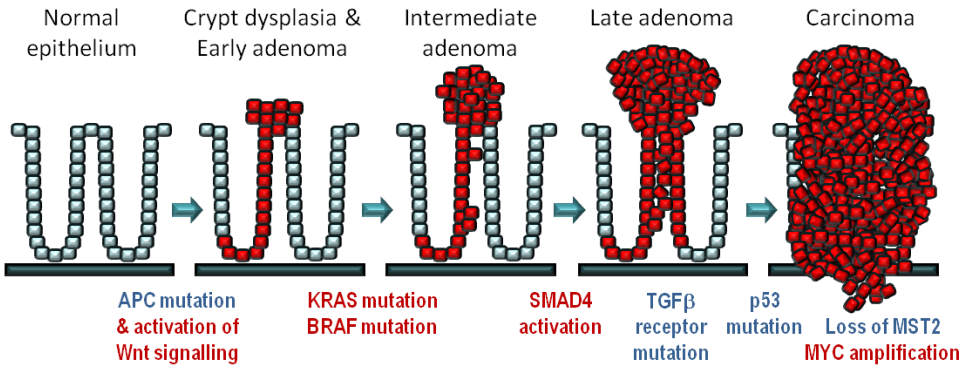
How colorectal cancer develops



The normal regeneration of the colon mucosa

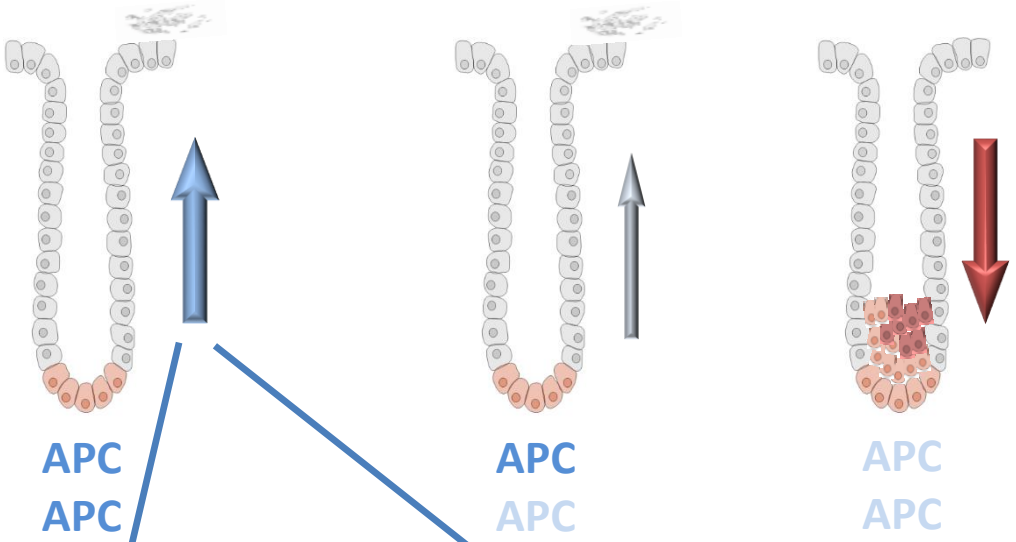
Stem cell mutations!

The pathological sequence of events

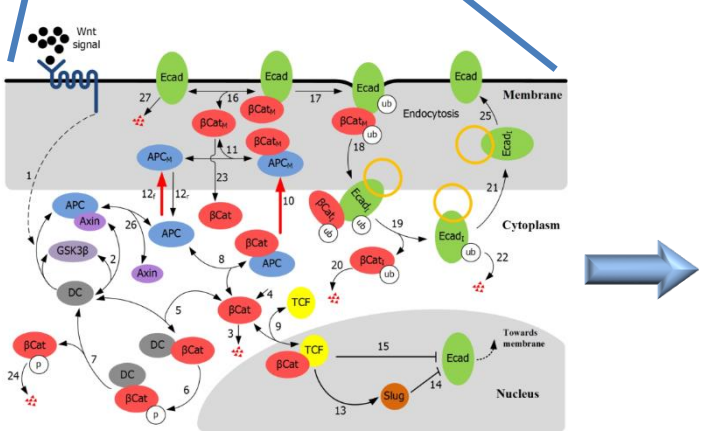


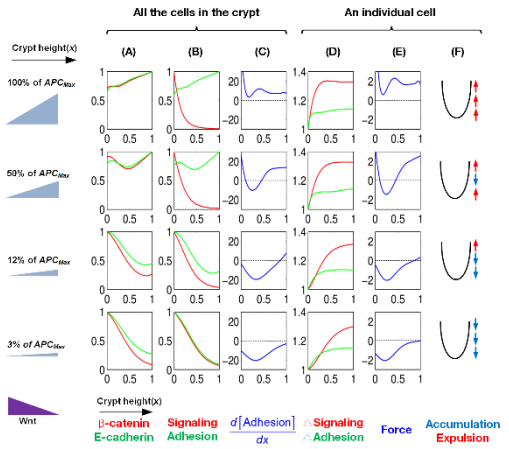
The role of the APC tumour suppressor

The observation

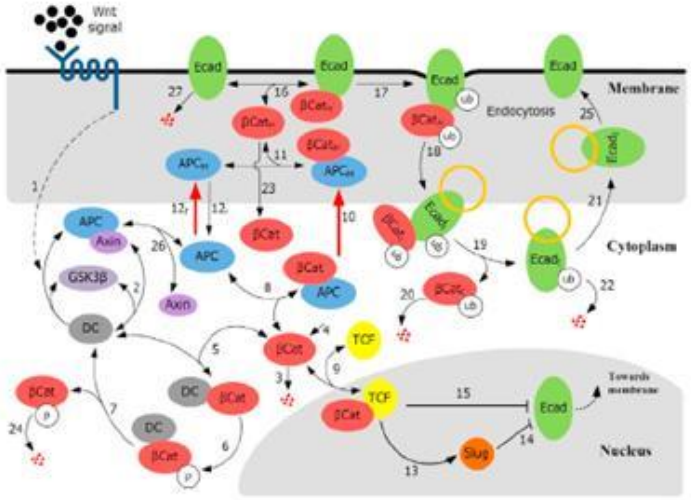


The explanation

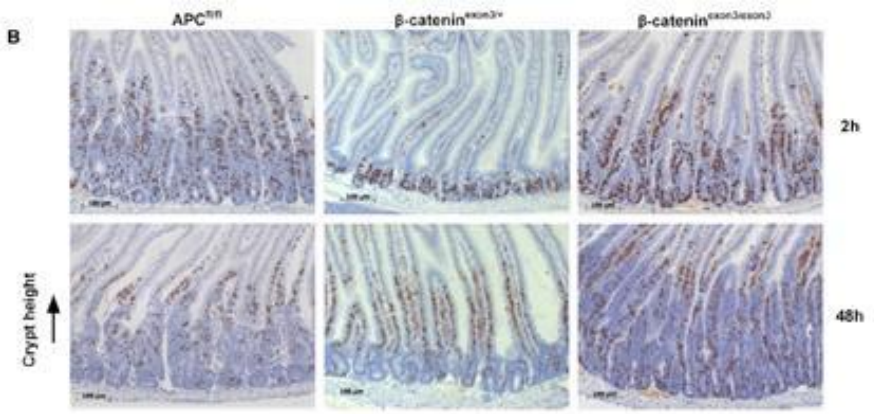




From signalling model to mouse model



The APC tumour suppressor acts as a safeguard that eliminates mutated cells from the colonic crypt.



1) From probabilistic to mechanistic network models

2) Relating pathway models to tissue physiology

3) Network motifs that convey drug resistance

4) Making drugs work based on thermodynamic models

RESEARCH ARTICLE

COMPUTATIONAL BIOLOGY

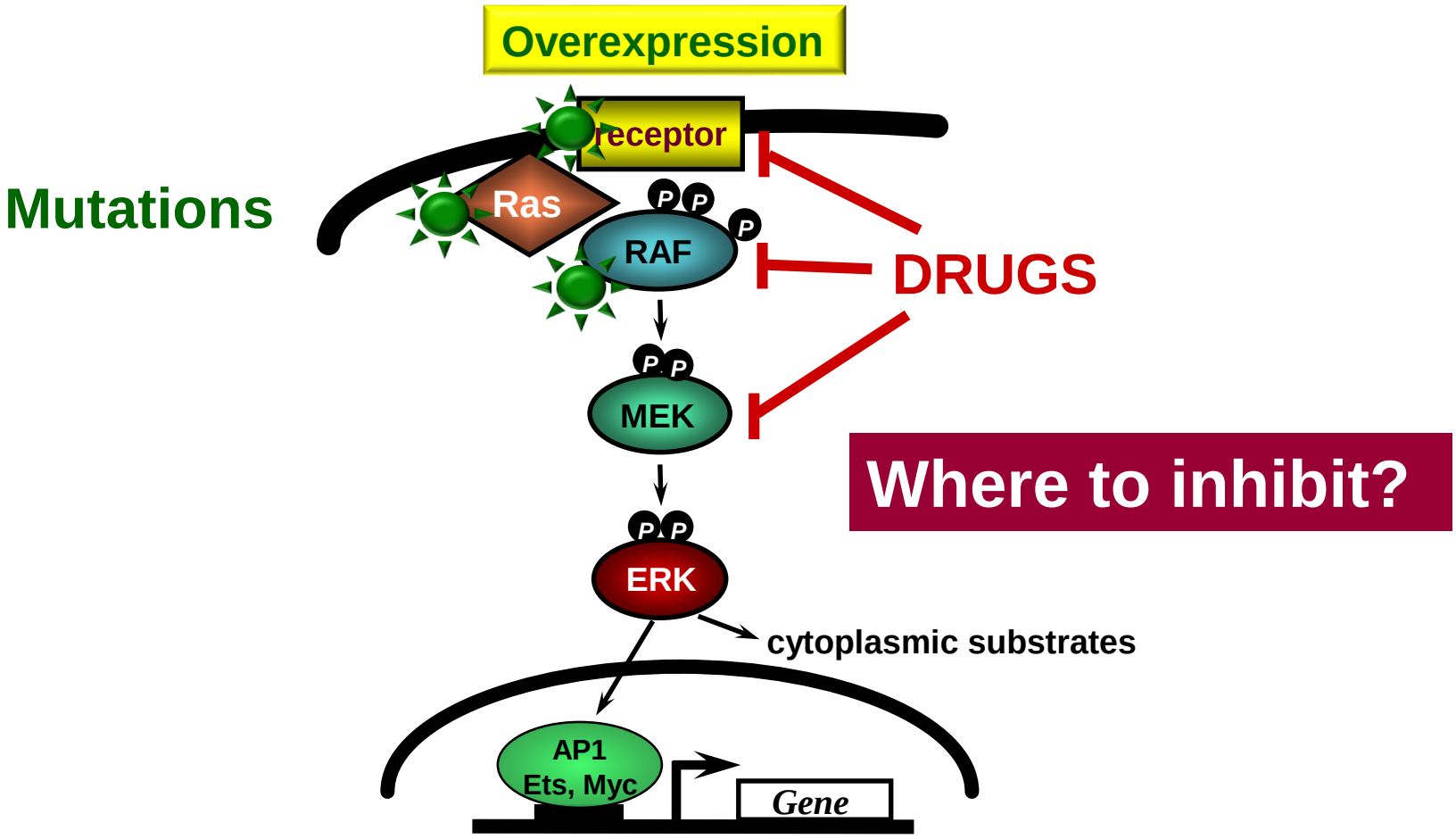
The Mammalian MAPK/ERK Pathway Exhibits Properties of a Negative Feedback Amplifier

Oliver E. Sturm,[1*†] Richard Orton,[1*‡] Joan Grindlay,[2*] Marc Birtwistle,[3] Vladislav Vyshemirsky,[1§] David Gilbert,[1||] Muffy Calder,[1] Andrew Pitt,[4] Boris Kholodenko,[3] Walter Kolch[3,5¶]

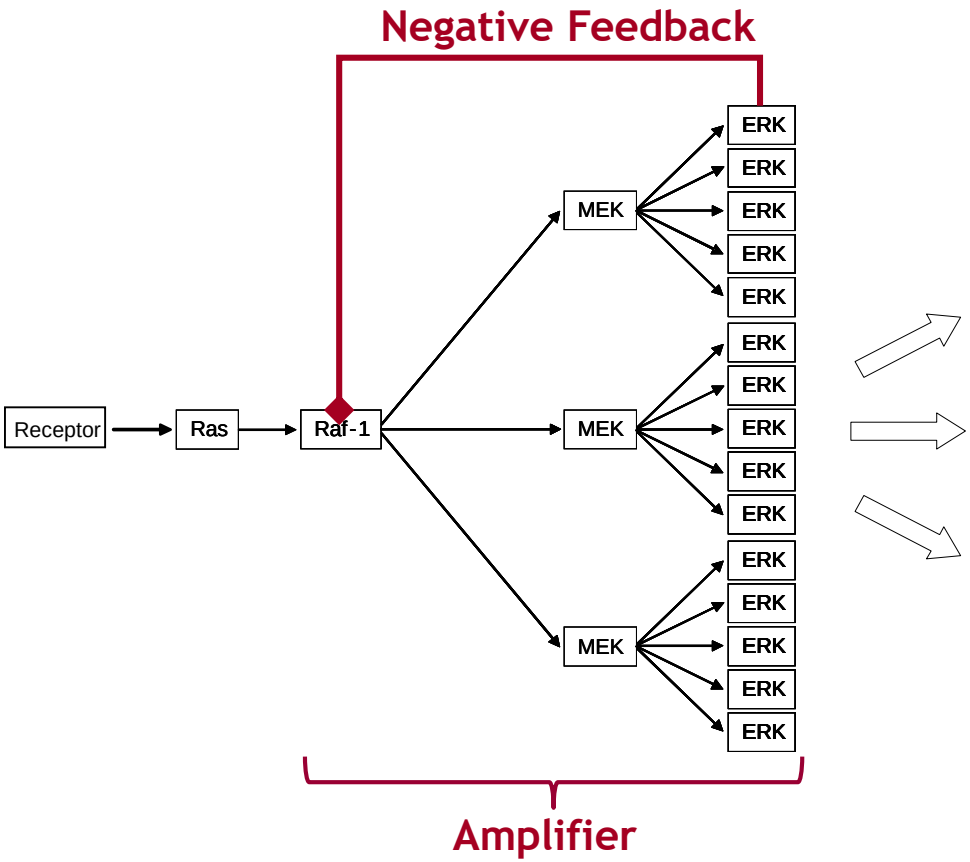
www.SCIENCESIGNALING.org 21 December 2010 Vol 3 Issue 153 ra90

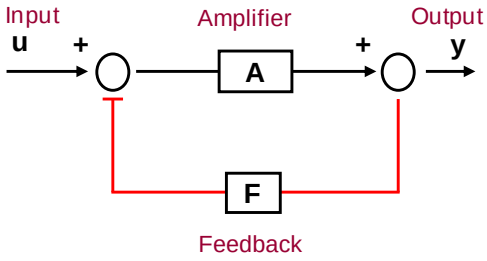
Targeting the Ras-Raf pathway

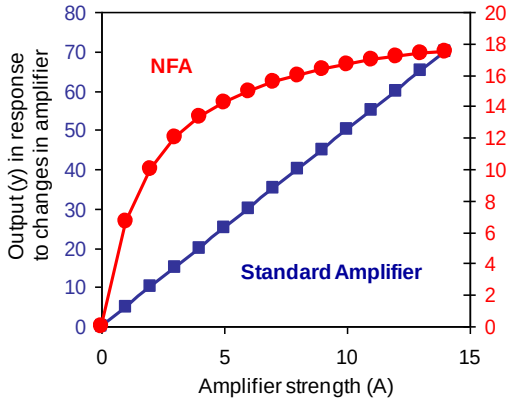
The Ras-Raf pathway is activated in >50% of all human cancers



The Raf pathway as negative feedback amplifier



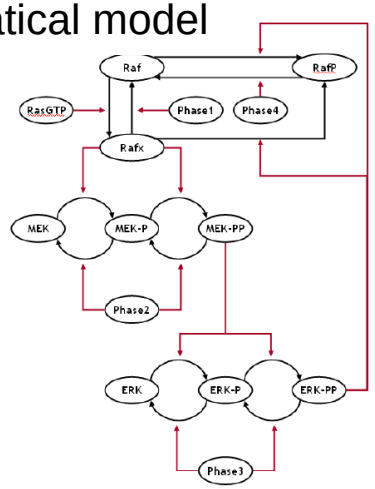




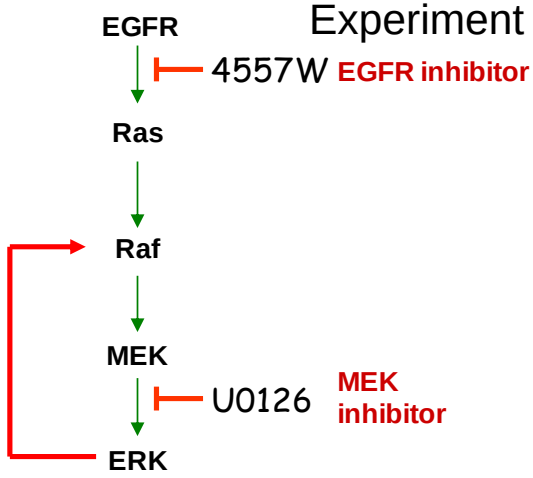
The Negative Feedback Amplifier conveys robustness to perturbations of the amplifier

Where should we inhibit?

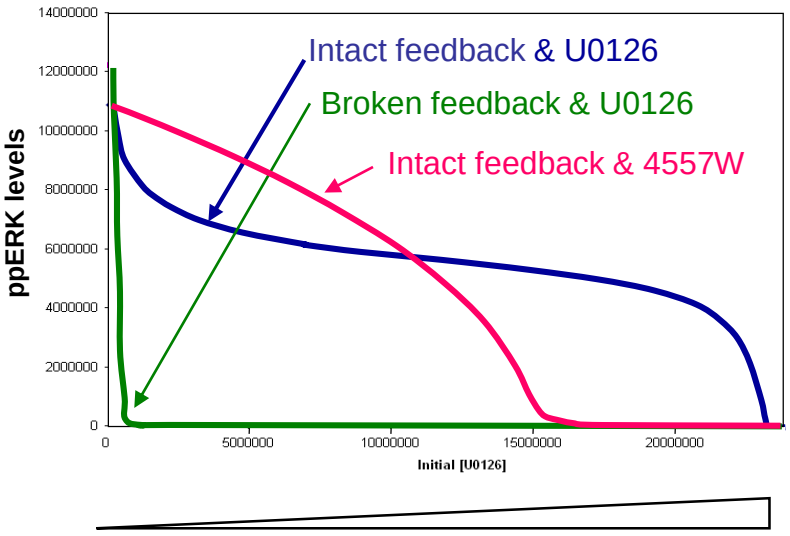
Mathematical model

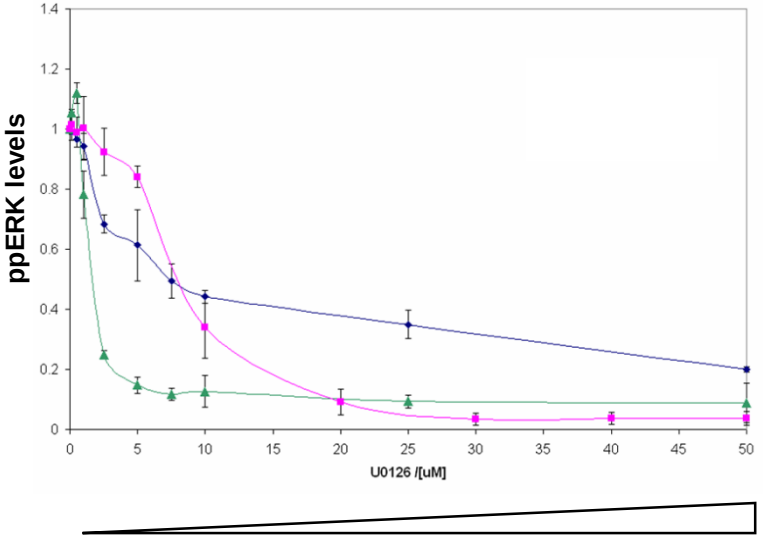


Experiment



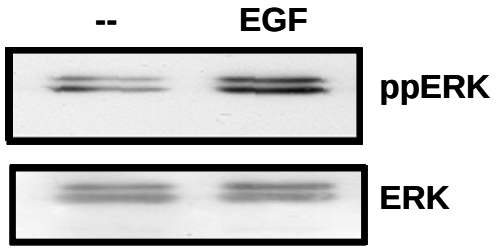
Model Predictions & Comparison to Wet Lab data

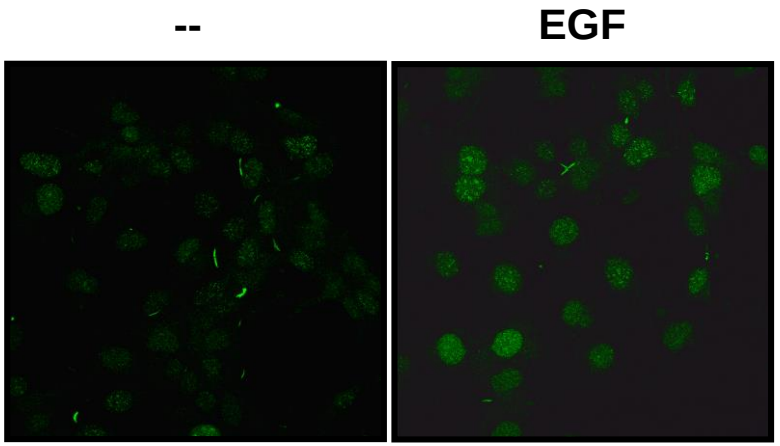




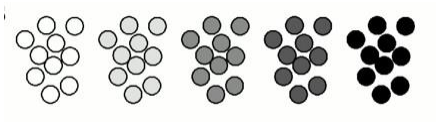
The NFA reduces heterogeneity

Feedback Intact



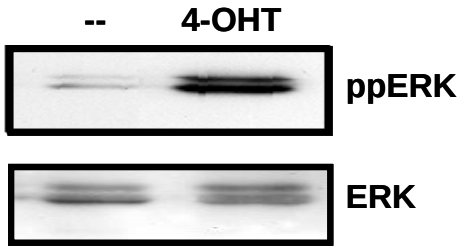


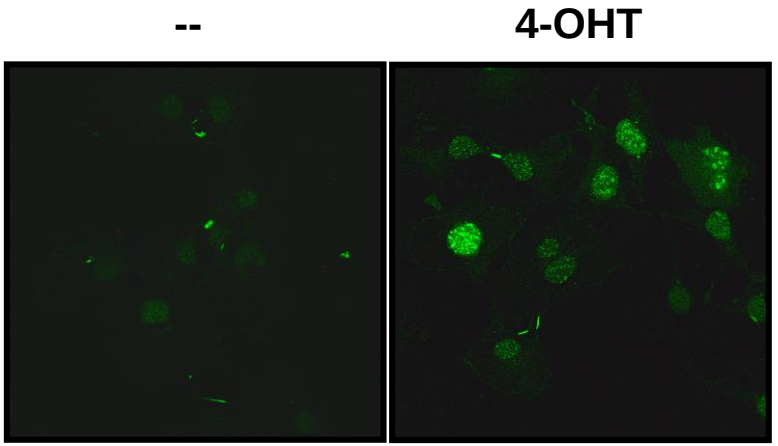
Anti-ppERK



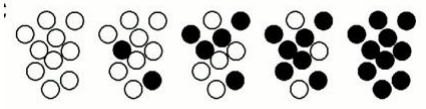
Increasing stimulus ->

Feedback Broken





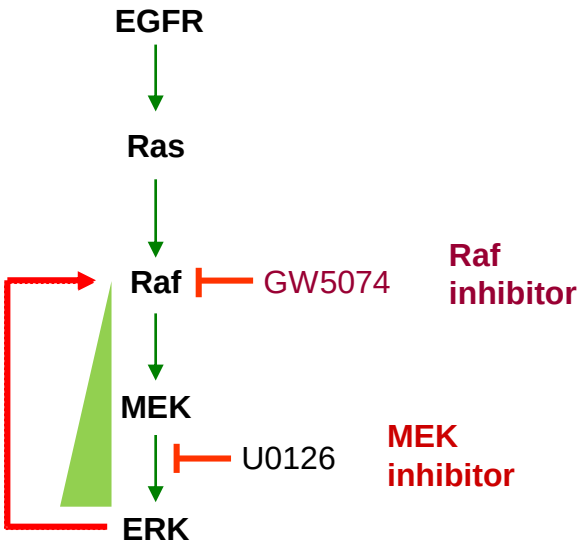
Anti-ppERK



Increasing stimulus ->

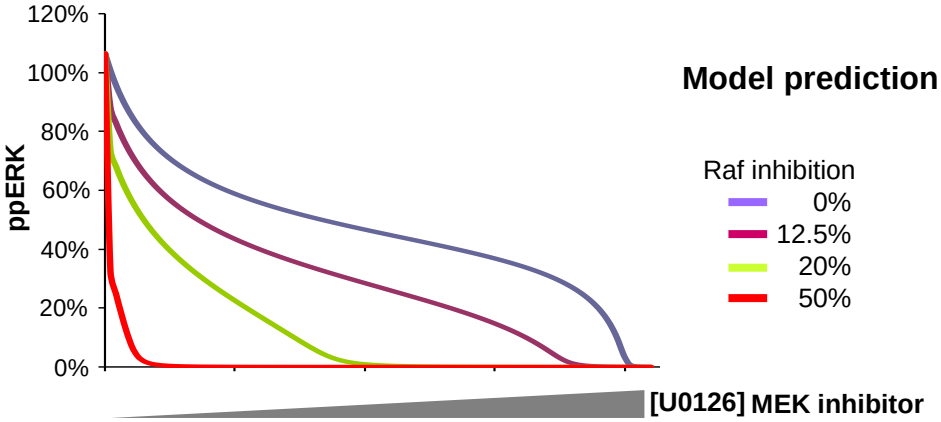
Where should we inhibit?

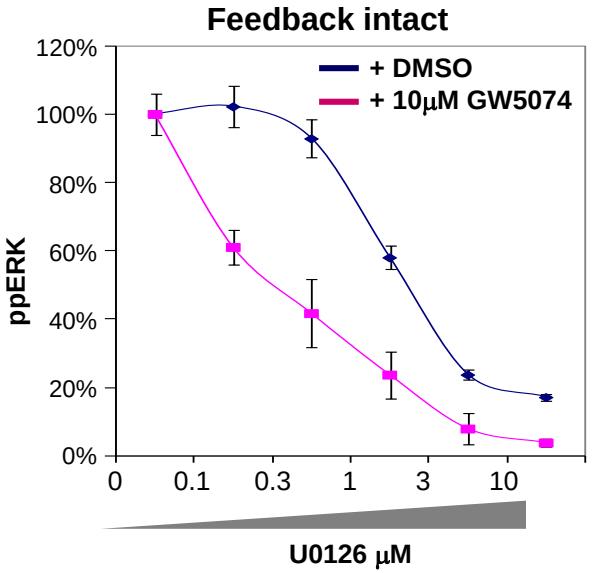
Drug targets embedded in a negative feedback amplifier are difficult to inhibit

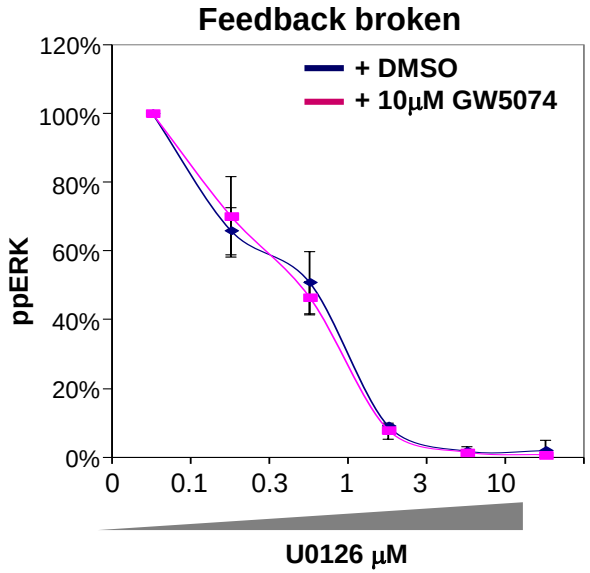


What if we weaken the negative feedback by a Raf inhibitor?

Predicting drug combinations: Synergism between Raf & MEK inhibitors

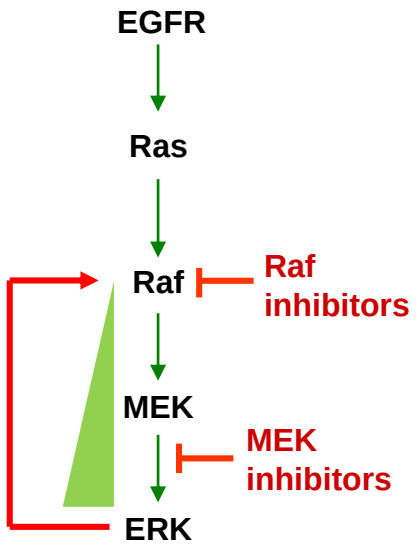






Conclusions

- ❑ **The negative feedback amplifier can cause drug resistance**
- ❑ **Mathematical model analysis suggests ways to break resistance: Raf & MEK inhibitors should synergise**



MEK & Raf inhibitors cooperate in clinical studies in melanoma treatment:

•Flaherty, K. Et al., N Engl J Med. 367:1694-703, 2012

•Kim et al. J Clin Oncol.31: 482-9, 2013

1) From probabilistic to mechanistic network models

2) Relating pathway models to tissue physiology

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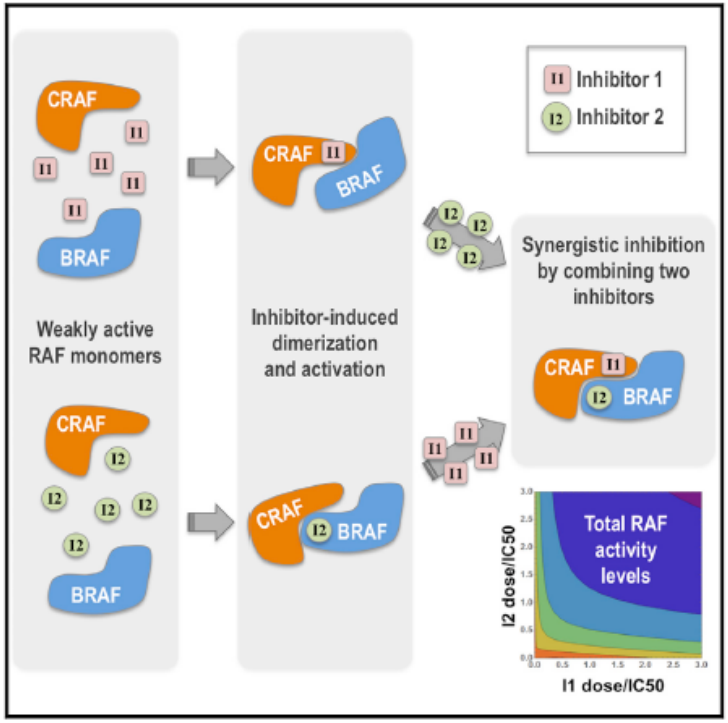
Article

Cell Reports

Drug Resistance Resulting from Kinase Dimerization Is Rationalized by Thermodynamic Factors Describing Allosteric Inhibitor Effects

Graphical Abstract



Authors

Boris N. Kholodenko

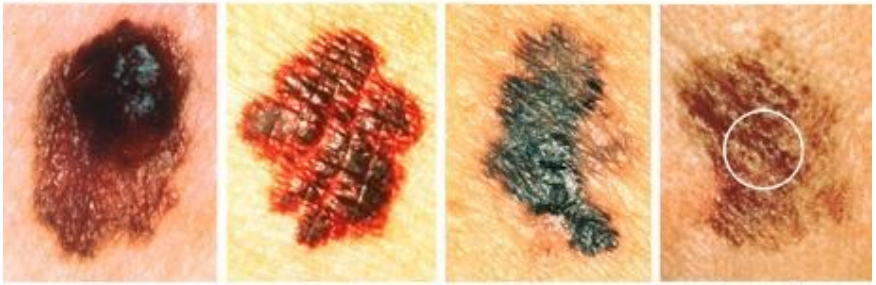
Correspondence

boris.kholodenko@ucd.ie

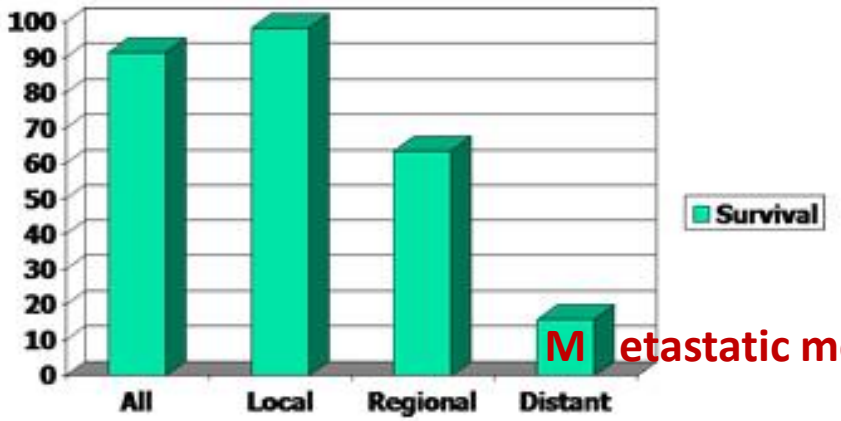
In Brief

Kholodenko has developed a model that describes drug-facilitated dimerization and the emergence of differing drug affinities between free kinase monomers versus dimers. Importantly, the model suggests ways of overcoming drug resistance.

Malignant melanoma

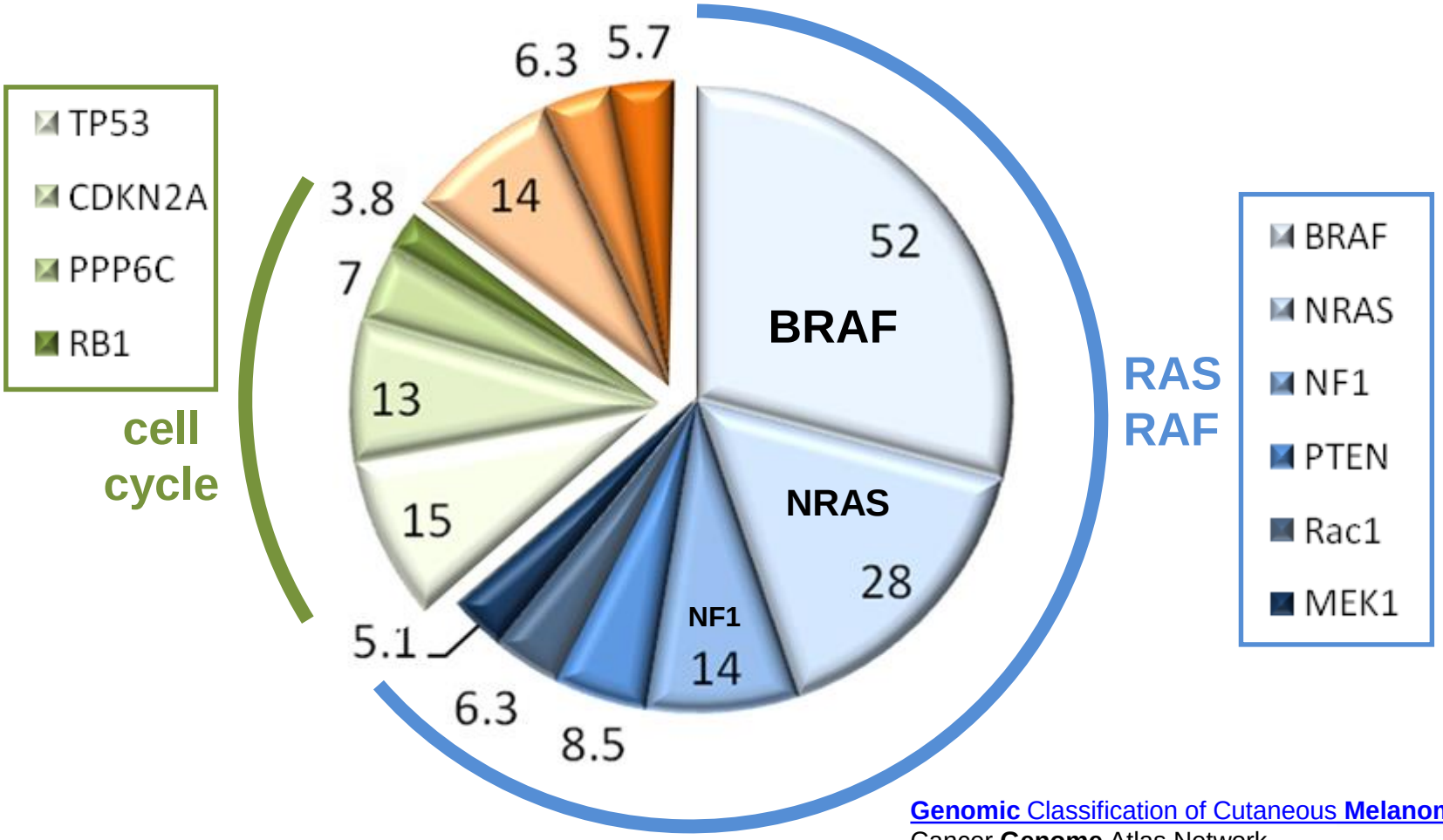


Surgery



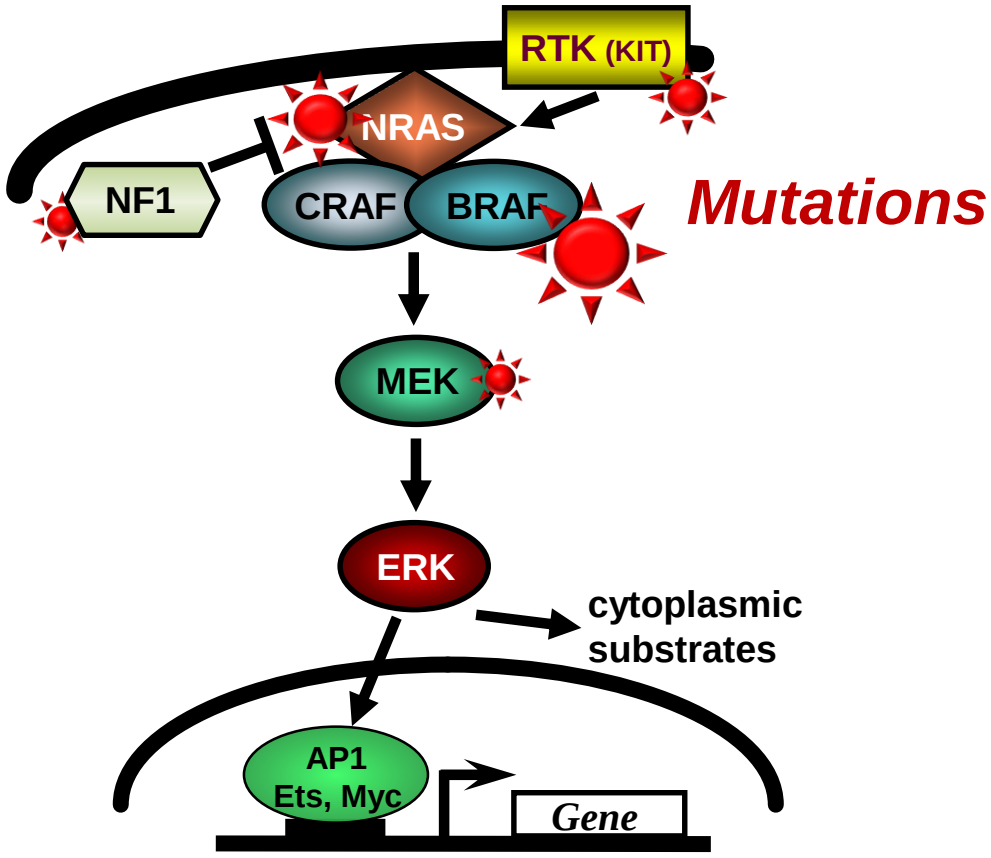
Metastatic melanoma

Common mutations in cutaneous melanoma

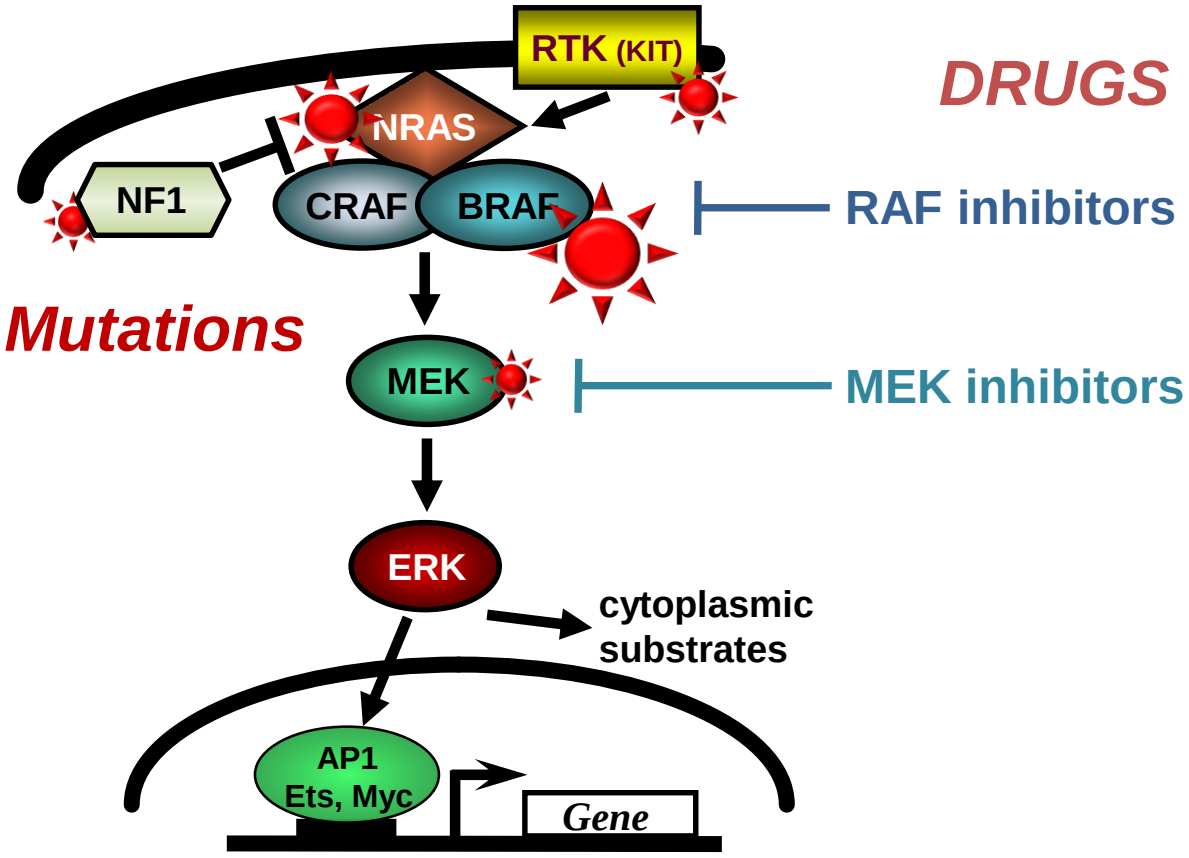


Genomic Classification of Cutaneous **Melanoma**.
Cancer **Genome** Atlas Network.
Cell. 2015 Jun 18;161(7):1681-96.

The central role of the RAF-MEK-ERK pathway

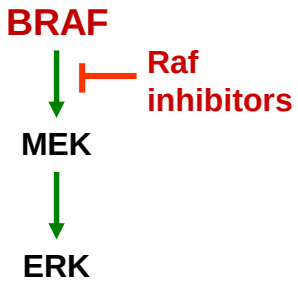


The central role of the RAF-MEK-ERK pathway



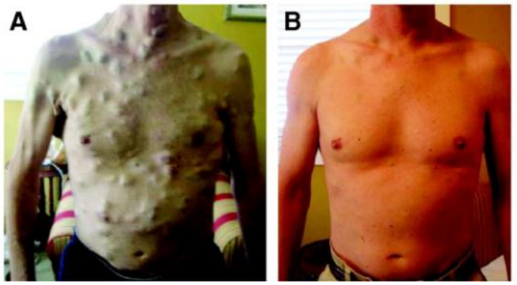
RAF inhibitors in the treatment of metastatic melanoma

50% of melanoma is caused by a mutation in BRAF

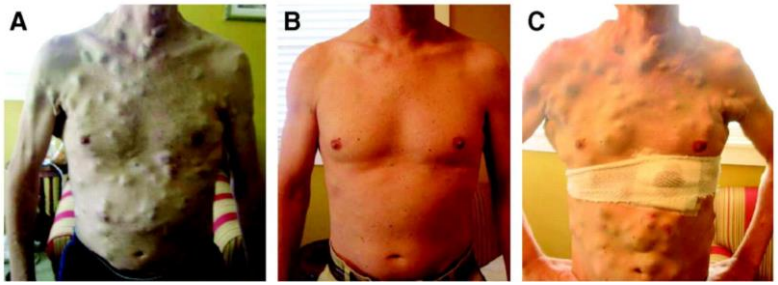


which we can treat with Raf inhibitors

with spectacular success



Before **after 2 weeks**



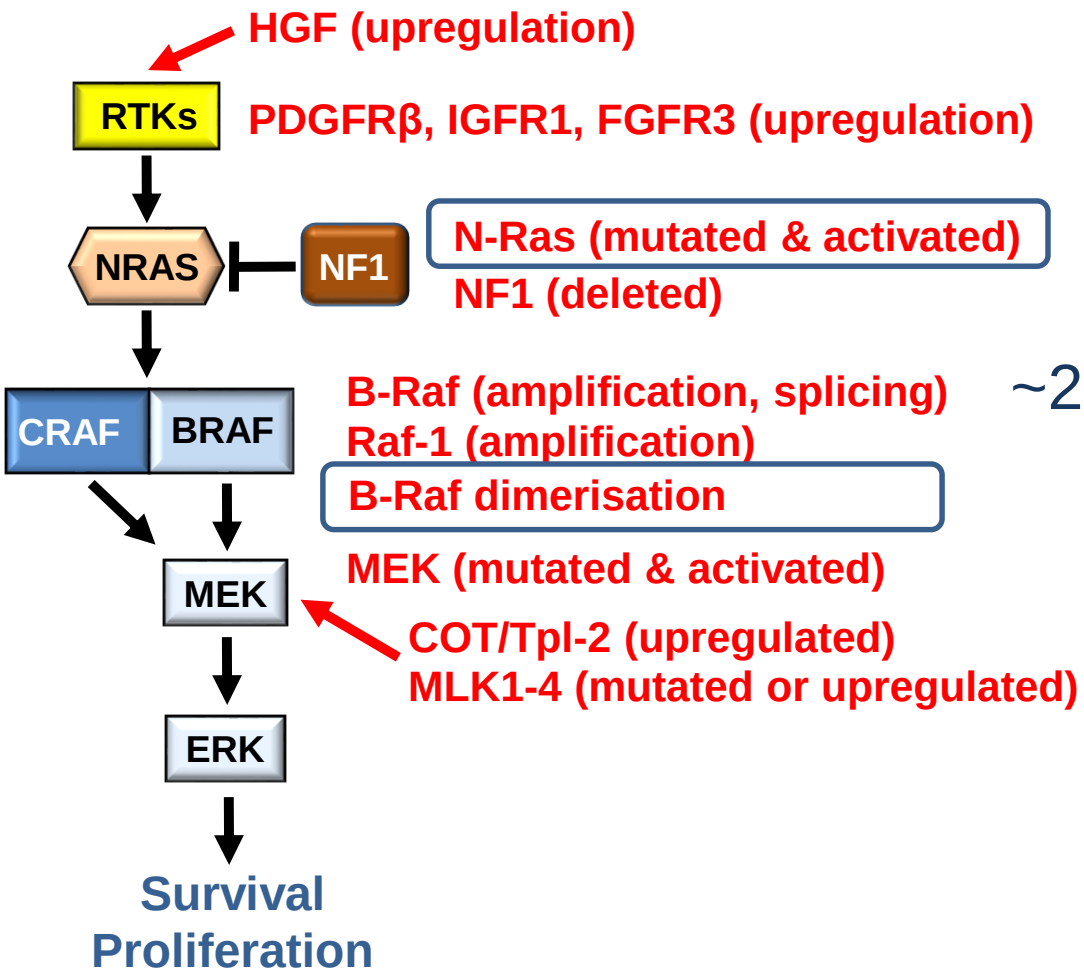
Before **2 weeks** **8 months**

However, resistance develops fast

NRAS mutated melanomas are resistant to RAF inhibitors

Network adaptations that restore ERK signalling

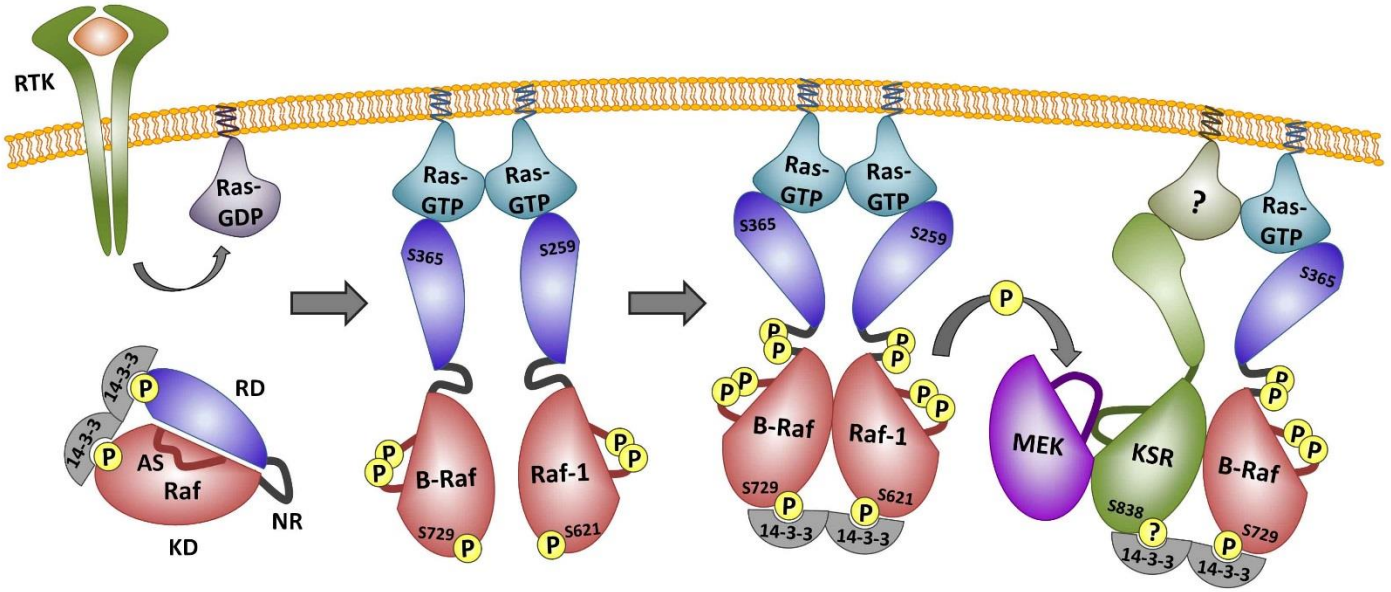
Mechanisms of resistance to Raf inhibitors



Re-activation of ERK

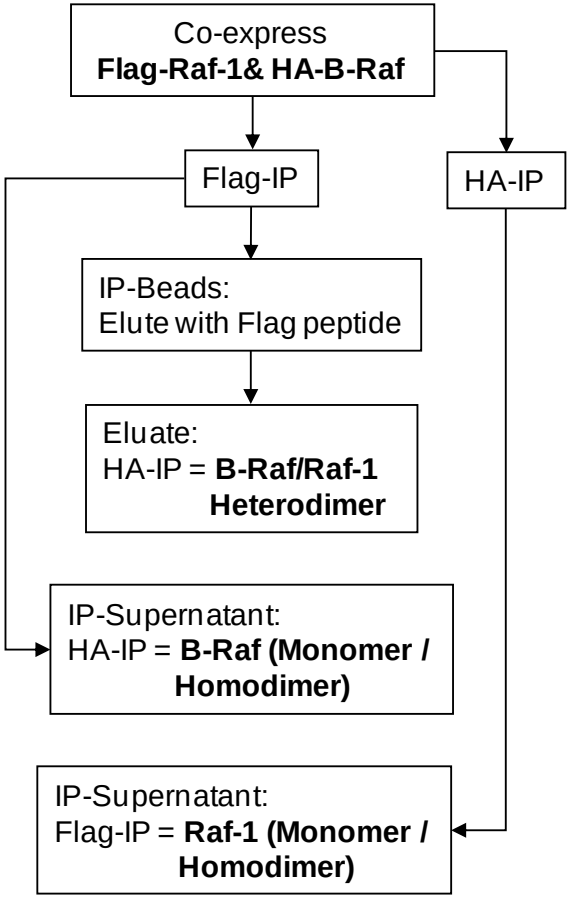
The role of BRAF-CRAF heterodimerisation

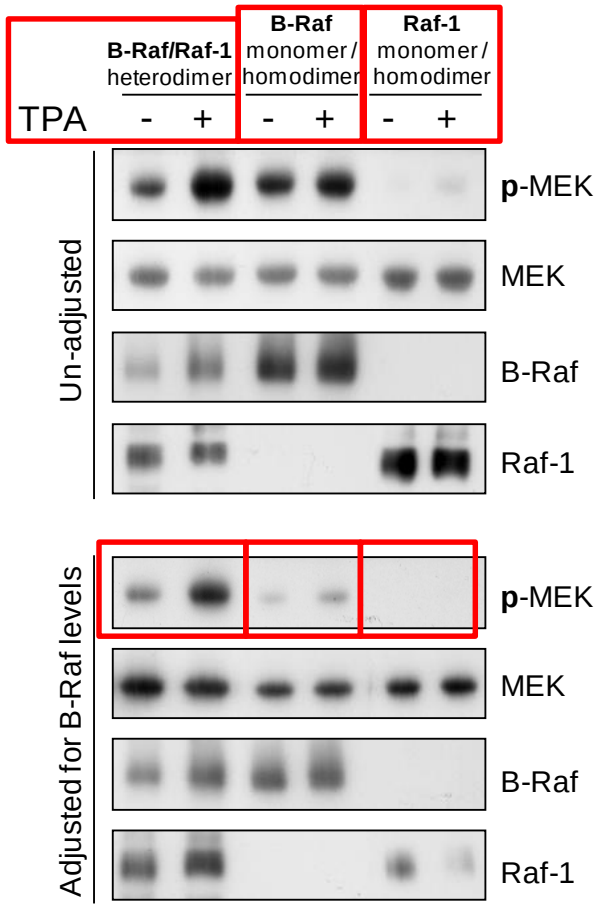
BRAF (B-Raf) – CRAF (Raf-1) heterodimerisation is part of physiological RAF activation



Baljuls A, Kholodenko BN, Kolch W. It takes two to tango--signalling by dimeric Raf kinases. Mol Biosyst. 2013 Apr 5;9(4):551-8.

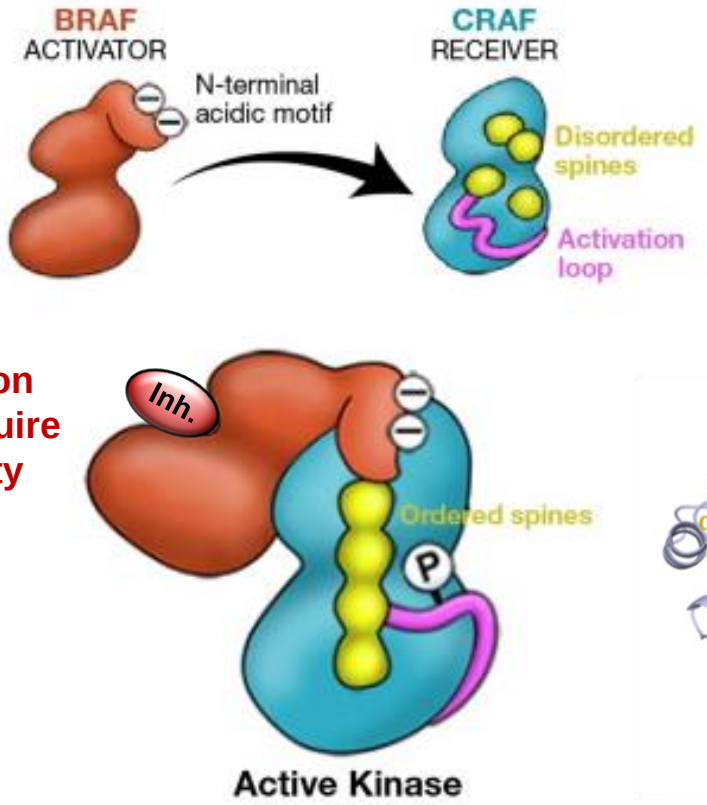
Raf-1 – B-Raf heterodimerisation increases Raf kinase activity >30fold





Rushworth et al. Mol Cell Biol. 26:2262-72, 2006

BRAF-CRAF heterodimerisation causes allosteric transactivation

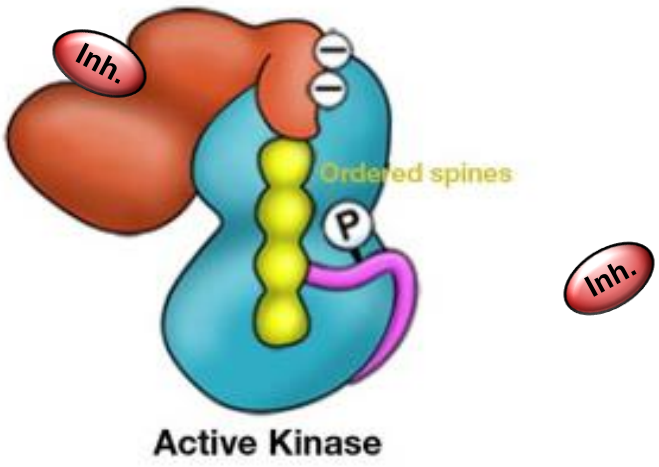


Allosteric transactivation does not require kinase activity

Hu et al. Allosteric activation of functionally asymmetric RAF kinase dimers. Cell. 2013 Aug 29;154(5):1036-46.

BRAF-CRAF heterodimerisation conveys drug resistance

Why is the RAF dimer drug resistant?



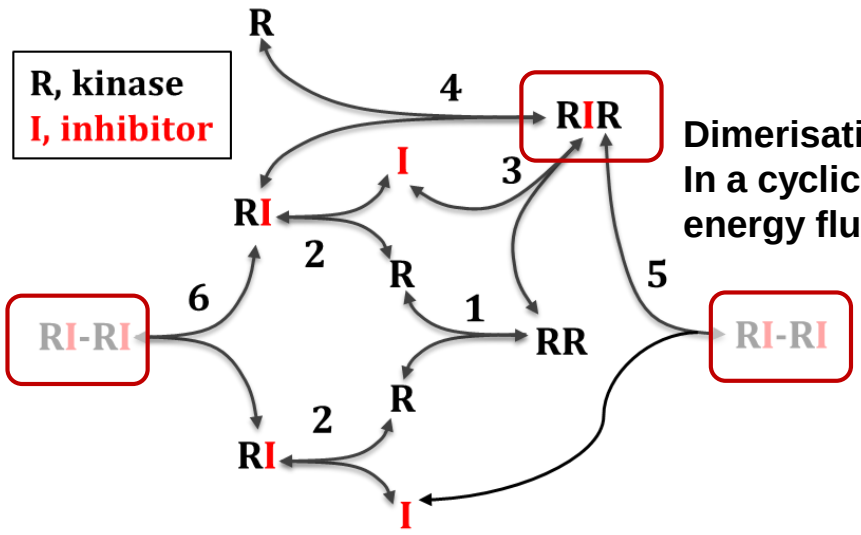
Kholodenko BN.Cell Rep. 2015 Sep 2 Drug Resistance Resulting from Kinase Dimerization Is Rationalized by Thermodynamic Factors Describing Allosteric Inhibitor Effects.

Yao et al. Cancer Cell. 2015 Sep 2. BRAF Mutants Evade ERK-Dependent Feedback by Different Mechanisms that Determine Their Sensitivity to Pharmacologic Inhibition.

Peng et al. Cancer Cell. 2015 Sep 2.Inhibition of RAF Isoforms and Active Dimers by LY3009120 Leads to Anti-tumor Activities in RAS or BRAF Mutant Cancers.

Kinase dimers & drug resistance – a thermodynamic view

Kholodenko BN.Cell Rep. 2015 Sep 2 Drug Resistance Resulting from Kinase Dimerization Is Rationalized by Thermodynamic Factors Describing Allosteric Inhibitor Effects.



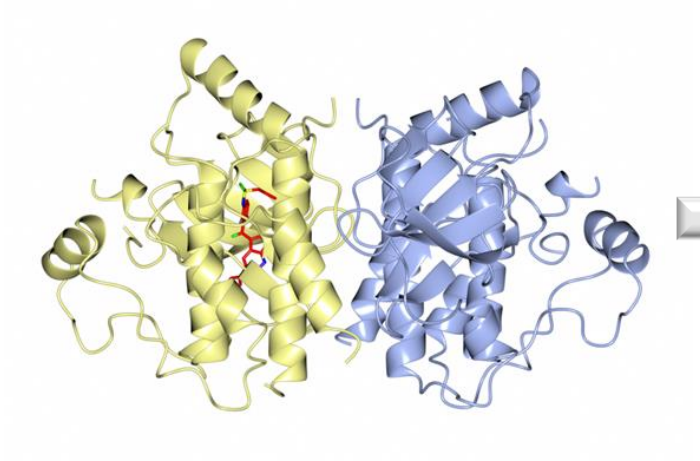
Dimerisation & drug binding is a cycle. In a cyclic process the thermodynamic energy fluxes = 0 at equilibrium

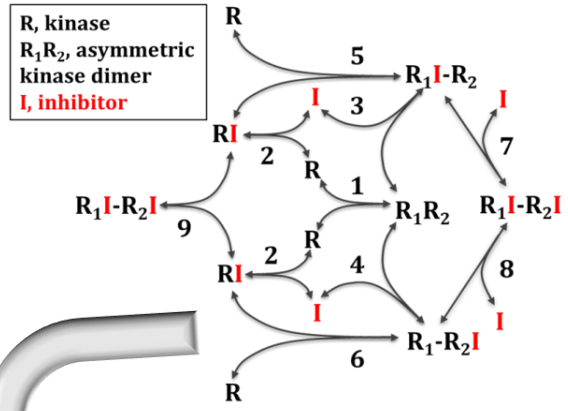
That means

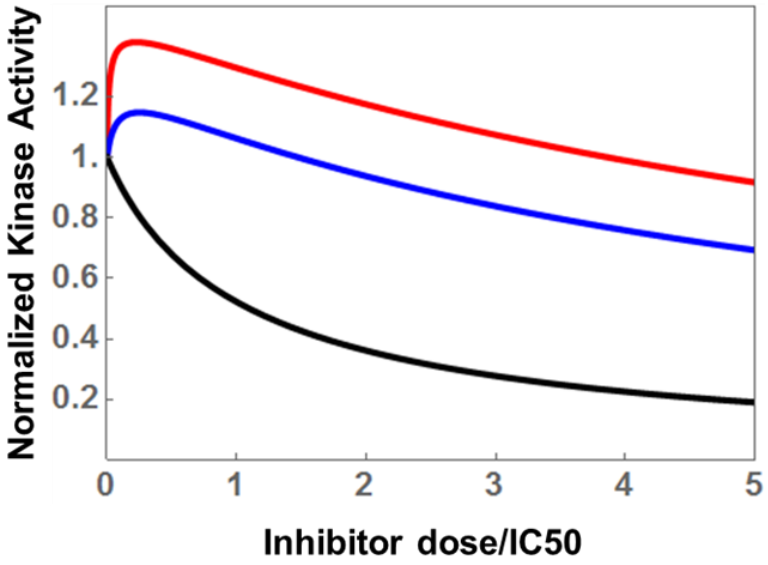
1) Inhibitors that induce dimerisation will preferentially induce RIR

2) The RIR to RI-RI transition is disfavoured as the binding constant for the second inhibitor molecule drops (Yao et al. Cancer Cell. 2015 Sep 2)

BRAF-CRAF heterodimerisation – thermodynamic view





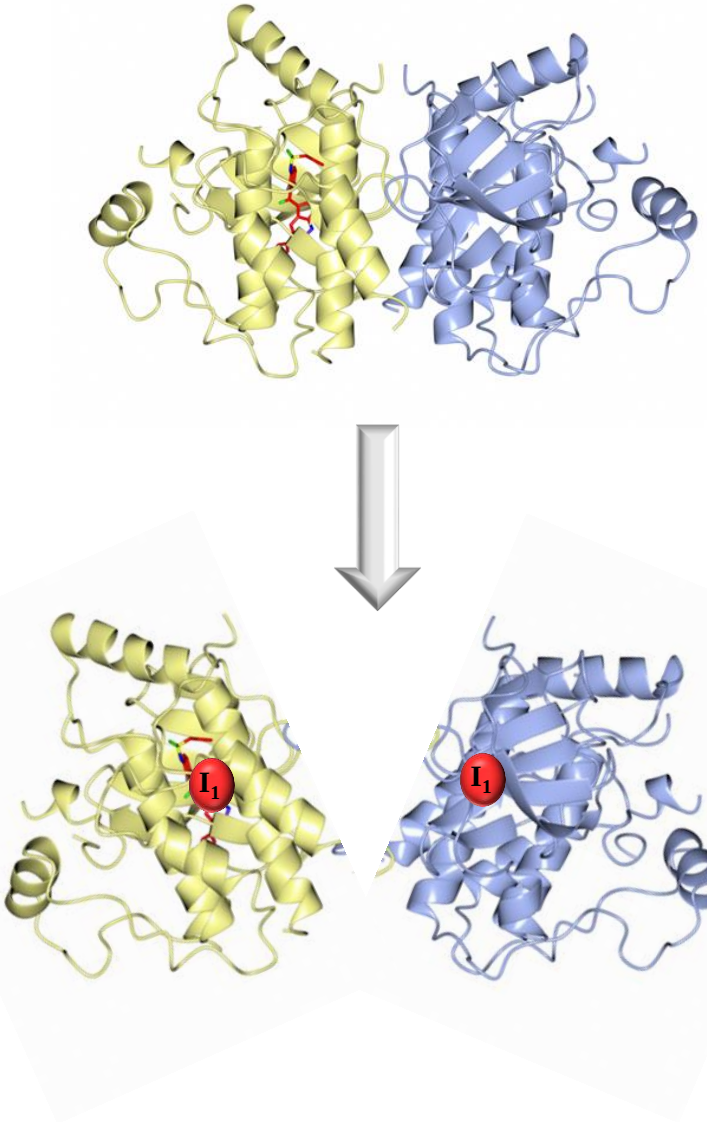


Inhibitor binding & dimerisation are highly cooperative

Inhibitor binding & dimerisation are cooperative

Inhibitor binding & dimerisation are independent events

Breaking dimerisation induced drug resistance



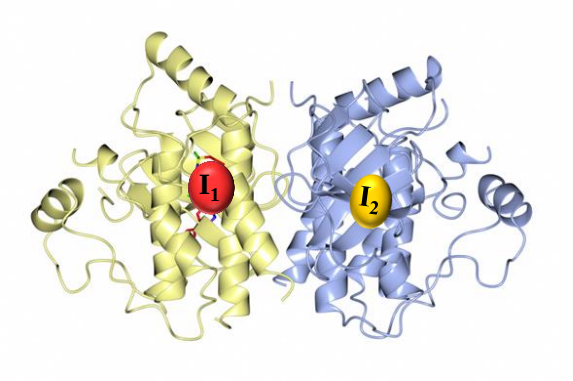
Break dimerisation

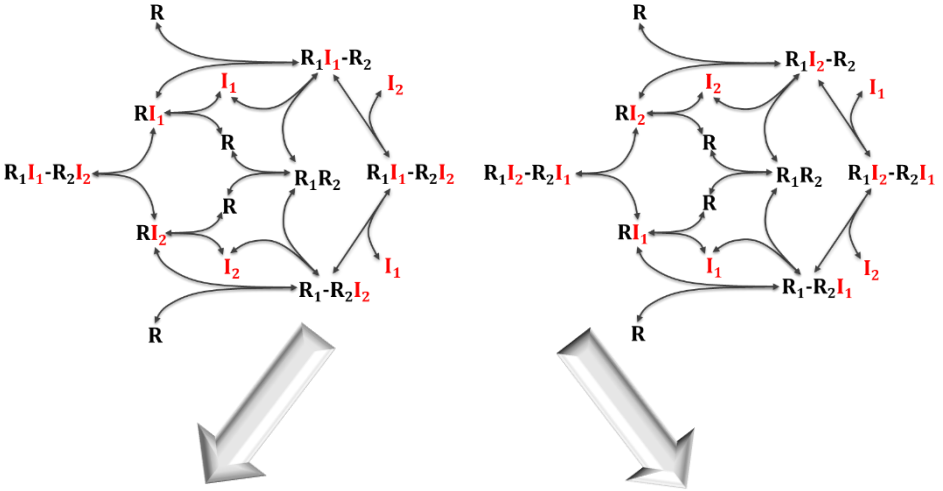
Girotti et al. Cancer Cell. 2015 Jan 12;27(1):85-96.Paradox-breaking RAF inhibitors that also target SRC are effective in drug-resistant BRAF mutant melanoma.

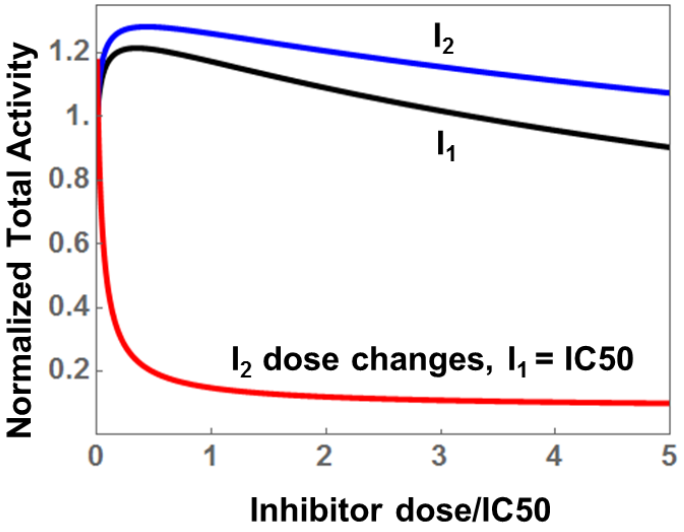
Le et al. Pigment Cell Melanoma Res. 2013 Jul;26(4):509-17. Selective RAF inhibitor impairs ERK1/2 phosphorylation and growth in mutant NRAS, vemurafenib-resistant melanoma cells.

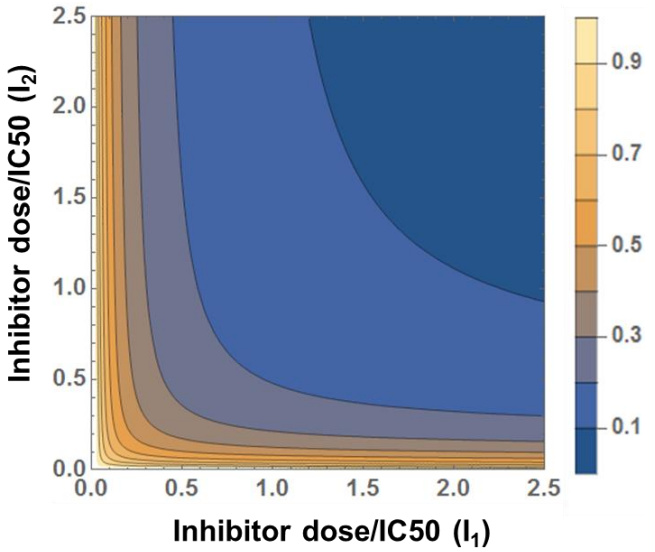
Breaking dimerisation induced drug resistance

Consider thermodynamics

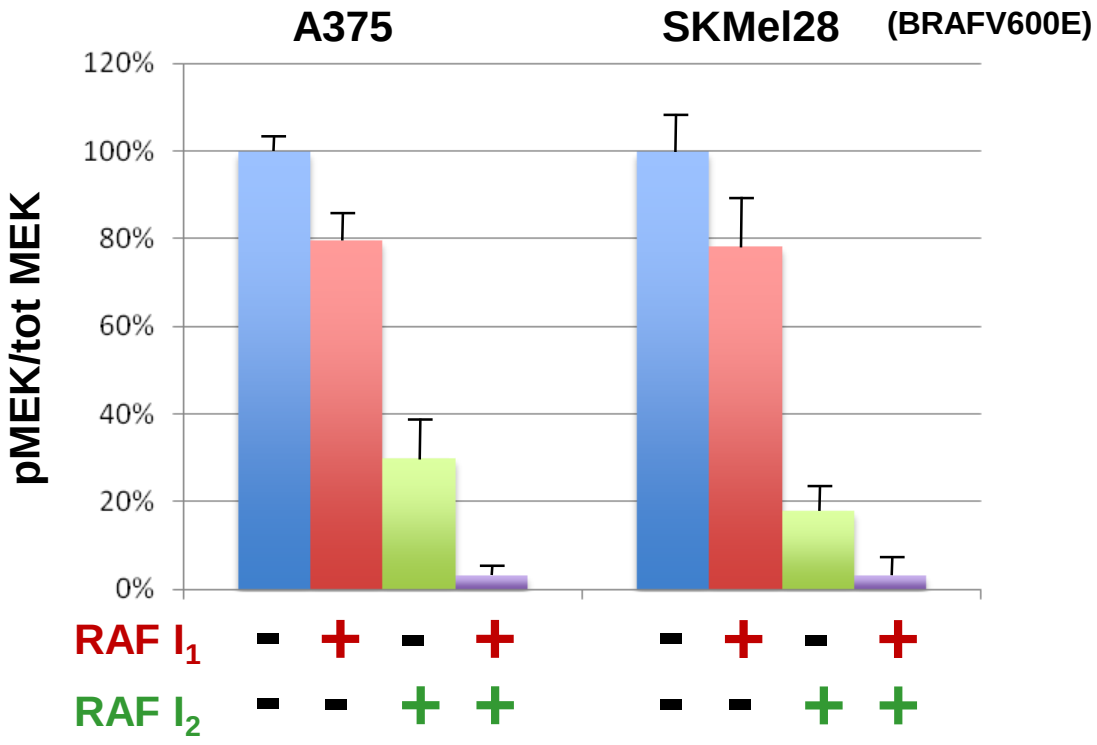








Combining different RAF inhibitors to overcome dimerization induced drug resistance



RAF I_1 + RAF I_2

Expected combined inhibition: 18.9%

Observed combined inhibition: 3.2%

Summary

- Kinase dimerisation causes drug resistance due to thermodynamic principles

- In the case of BRAF-CRAF dimers this resistance can be broken by
  - Designing RAFi that do not dimerise
  - Designing RAFi that can bind to different RAF conformations
  - Combining RAFi that bind to different conformations

Thank you

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http://www.ucd.ie/sbi/
http://www.ucd.ie/conway/

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NDP National Development Plan 2007 - 2013

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