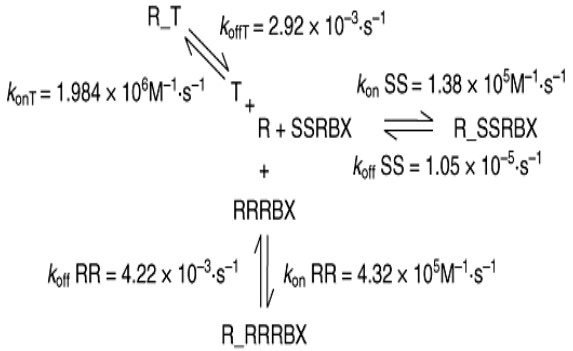
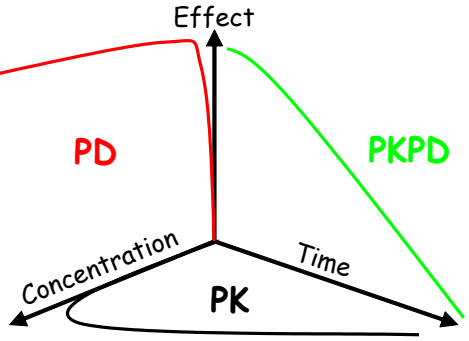
Early estimation of clinically efficacious drug dose using Systems Pharmacology approaches; application to the Nerve Growth Factor pathway

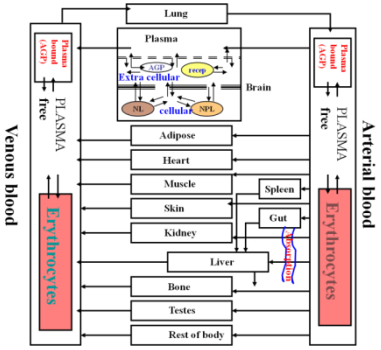
Dr Neil Benson (neil@xenologiq.com)

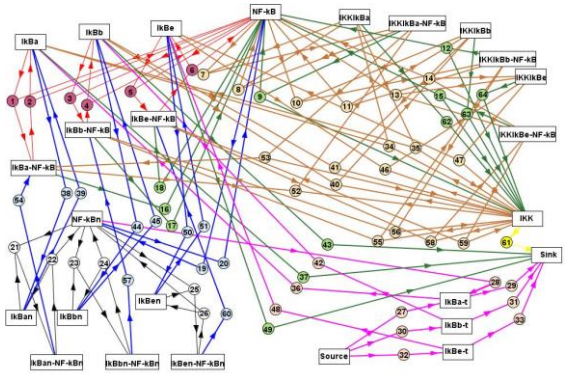
PKUK, Bath, Nov 5th -7th 2014.

What is systems pharmacology?









Requires mathematical modelling approach

Understanding of behaviour of a drug in a disease

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Why do we need Systems Pharmacology (SP)? Attrition & ROI

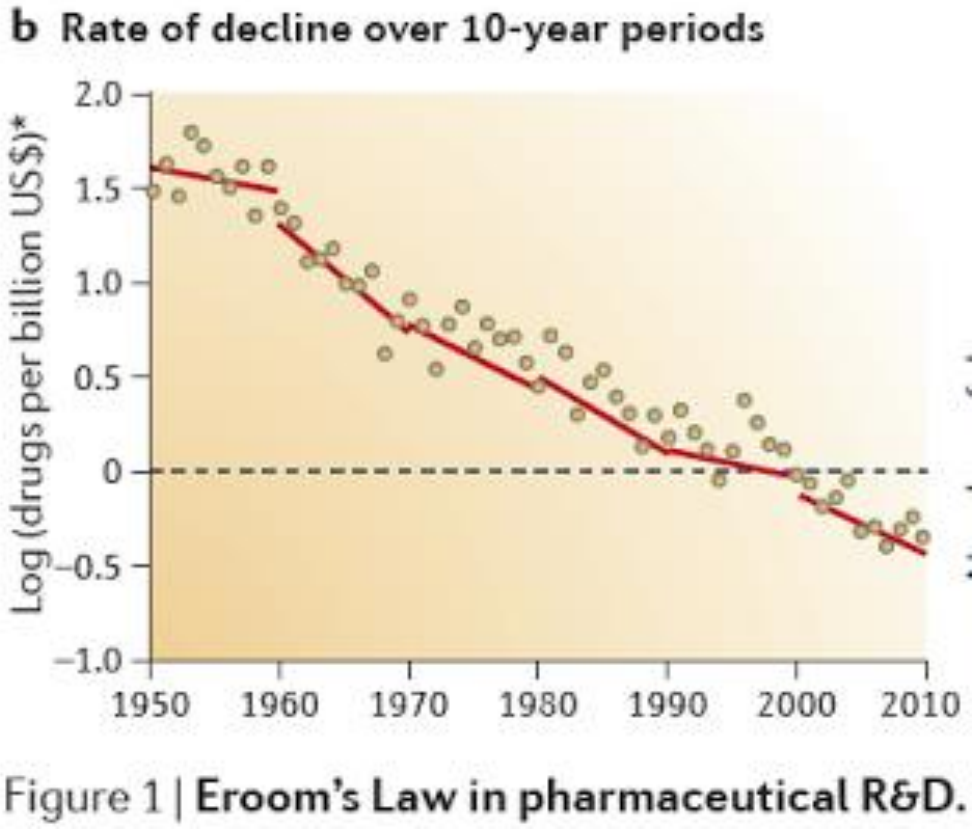
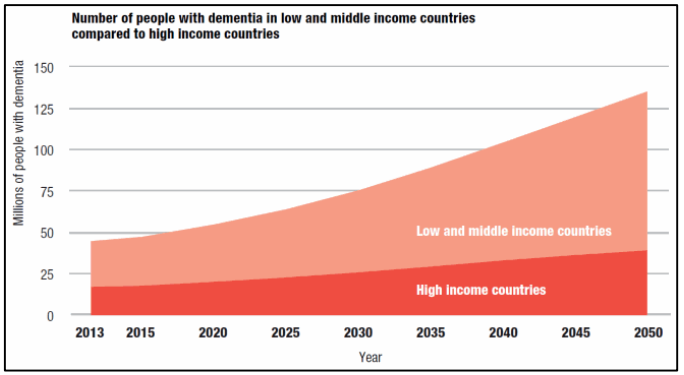


Figure 1 | **Eroom's Law in pharmaceutical R&D.**

Why do we need Systems Pharmacology? The patients

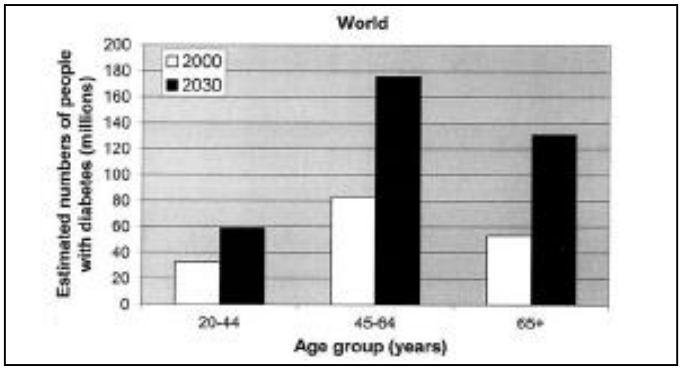
Dementia*

- 2013 there were an estimated 44.4 million dementia sufferers WW
- This number will increase to an estimated 135.5 million in 2050
- Developing nations disproportionately impacted



Diabetes^

- 2000 171M people with diabetes WW
- 2030 projected 366 M
- Developing nations disproportionately impacted

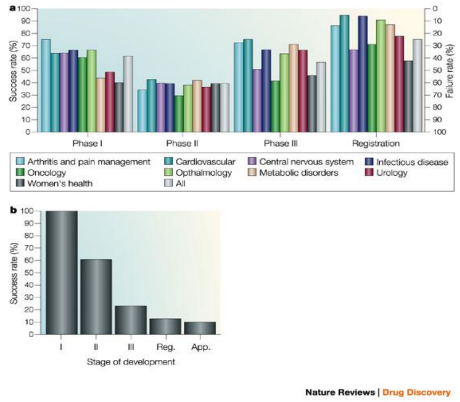


Lots of complex, multi-factorial diseases impacting people worldwide that need treatments

-Will current methods deliver the solution?

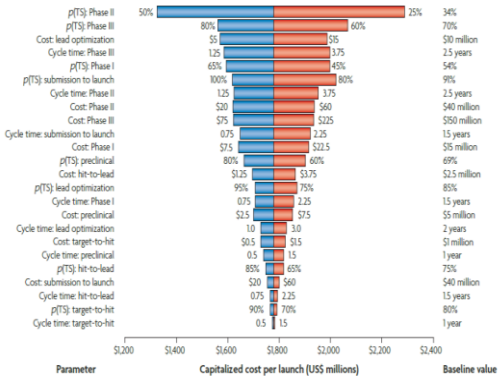
10 years of evaluation & proposals and arguably the main problem remains

2004



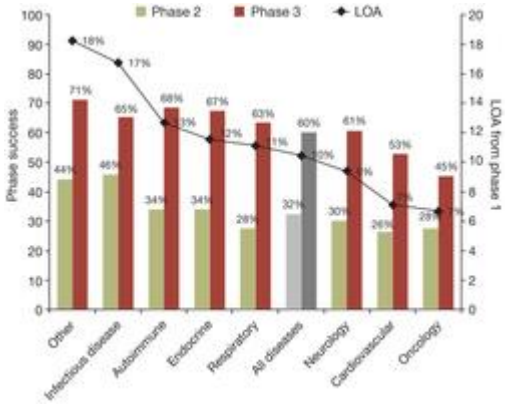
Kola & Landis, Nature Rev DD (2004); PHII attrition due to lack of efficacy or safety key issue

2010



Paul et al, Nature reviews, DDT, (2010) – PHII attrition by far largest contributor to productivity

2014

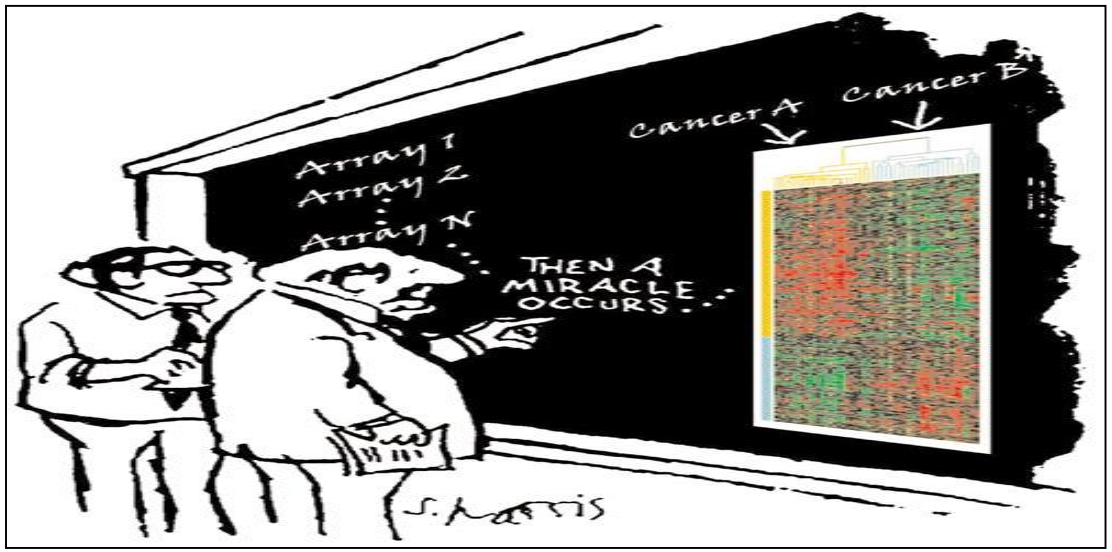


Hay et al (2014); Nature biotech;
The attrition statistics showed a decrease in probability of success compared to previous evaluations

The need to experiment with genuinely novel approaches in drug discovery could not be clearer or more urgent

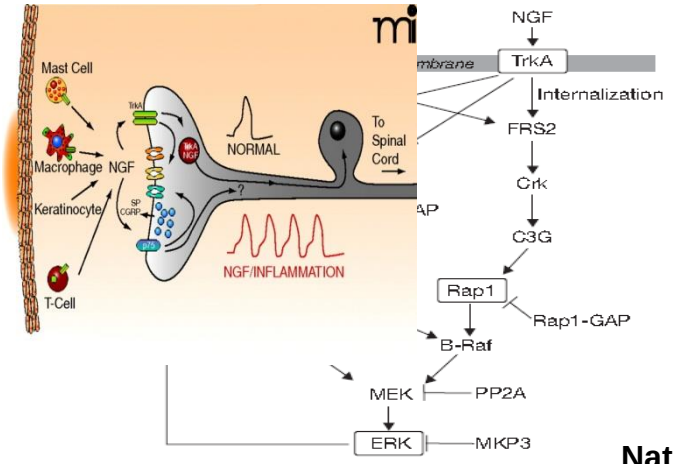
What is the problem?

- All analyses point to Phase II attrition due to lack of efficacy or safety
  - ie we didn't understand the biology -> consequences perturbing a complex system

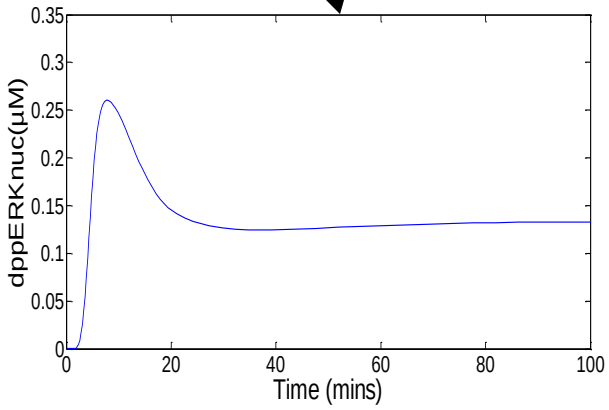


- **Mathematical modelling & simulation proven approach for tackling complexity in many areas of science and engineering**

Systems pharmacology of the Nerve Growth Factor pathway



Nat Cell Biol

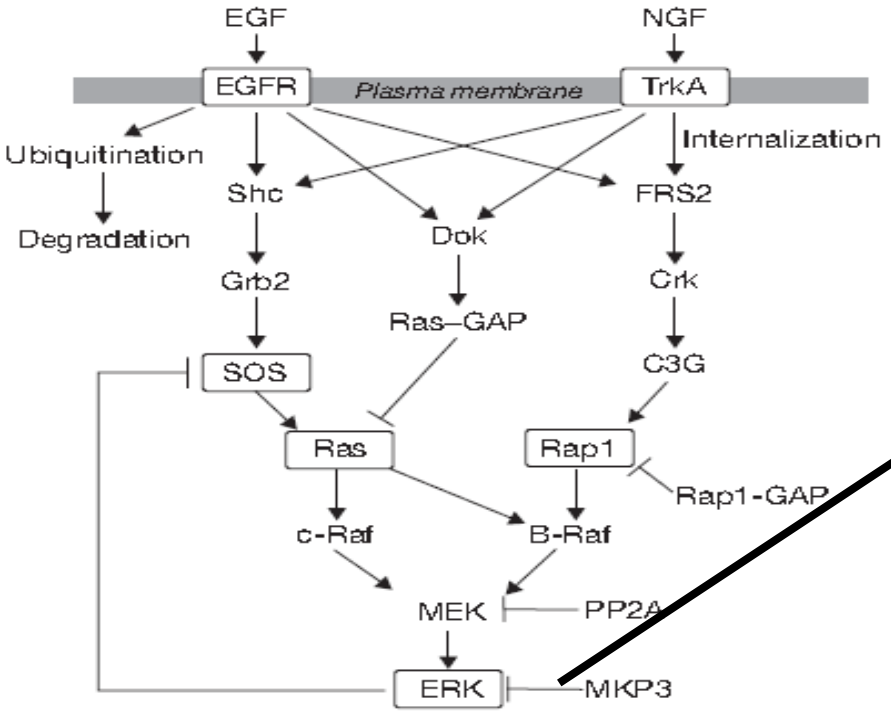


- Extracellular Regulated Kinase (ERK) activation controls pain response (eg trka levels, ion channel activity etc)

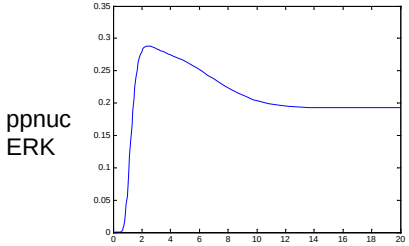
- Team questions;
  - what are the best pain targets ?
  - what is the dose & regimen?

Acknowledgements; Pinky Dua (Pfizer, Neusentis) and Oleg Demin (Institute for Systems Biology). Cesar Pichardo (XenologiQ Ltd), Lambertus Peletier, Dept Mathematics, Leiden University, Netherlands & Piet van der Graaf ;LACDR, Leiden University, Netherlands.

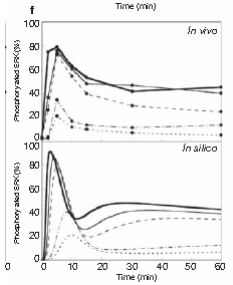
Critical assumptions



Pfizer model



Sasagawa et al

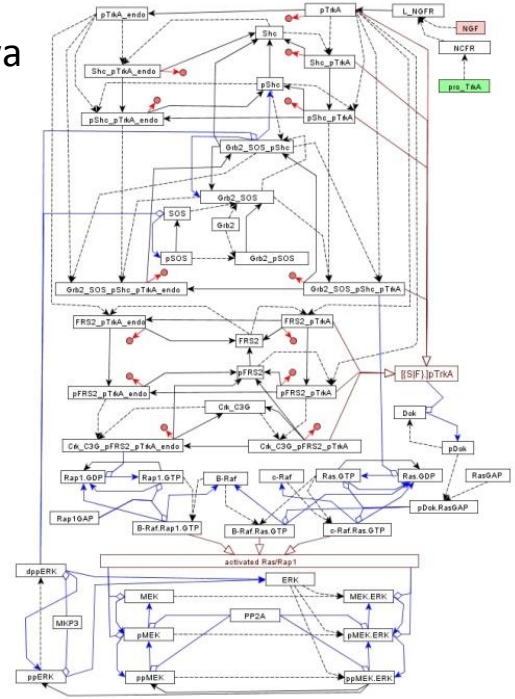


dppERKnuc = pain

As a base case;
success = stop response

Models in literature

Sasagawa et al, Nat Cell Biol, 2005



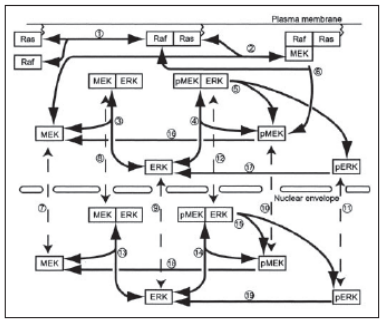


FIGURE 6. Minimum essential model of the Ras/ERK MAPK cascade. The cascade starts from Ras, which is arbitrarily set to reproduce the EGF-induced activation. The arrows represent the reactions specified in the supplemental material. p, phospho-

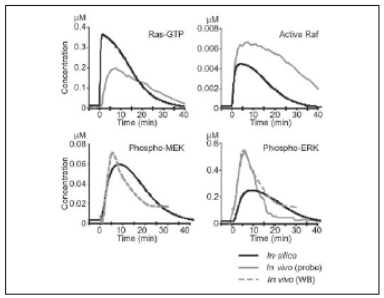
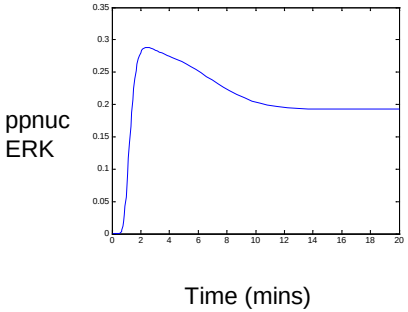


FIGURE 7. Kinetics of the components of the Ras/ERK/MAPK cascade. The activation of Ras, Raf, and ERK was monitored *in vivo* by FRET imaging (probe) and/or Western blotting (WB). The kinetics of MEK and ERK activation were from Fig. 2.

Fujioka et al, J Biol Chem, 2006

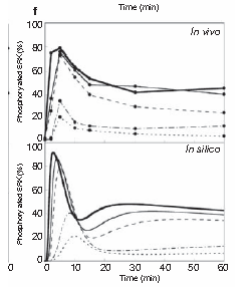
Pfizer model



ppnuc ERK

Time (mins)

Sasagawa et al



- One compartment integrated 'systems biology' model constructed (59 molecular species and 233 parameters)

Benson & Dua et al **Interface Focus,** 2013 **vol. 3 no. 2,** 20120071.

Interface Focus

Systems pharmacology of the nerve growth factor pathway: use of a systems biology model for the identification of key drug targets using sensitivity analysis and the integration of physiology and pharmacology

Neil Benson, Tomomi Matsuura, Sergey Smirnov, Oleg Demin, Hannah M. Jones, Pinky Dua and Piet H. van der Graaf

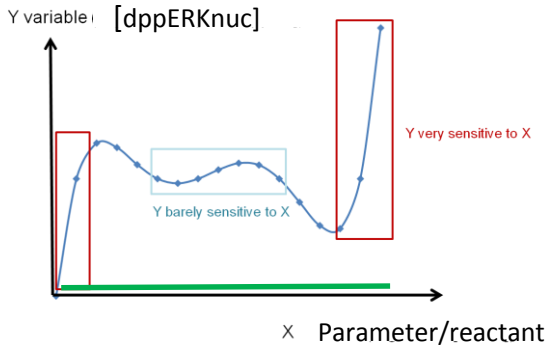
Interface Focus 2013 3, 20120071, published 21 February 2013

Finding optimal targets; sensitivity analysis of the model

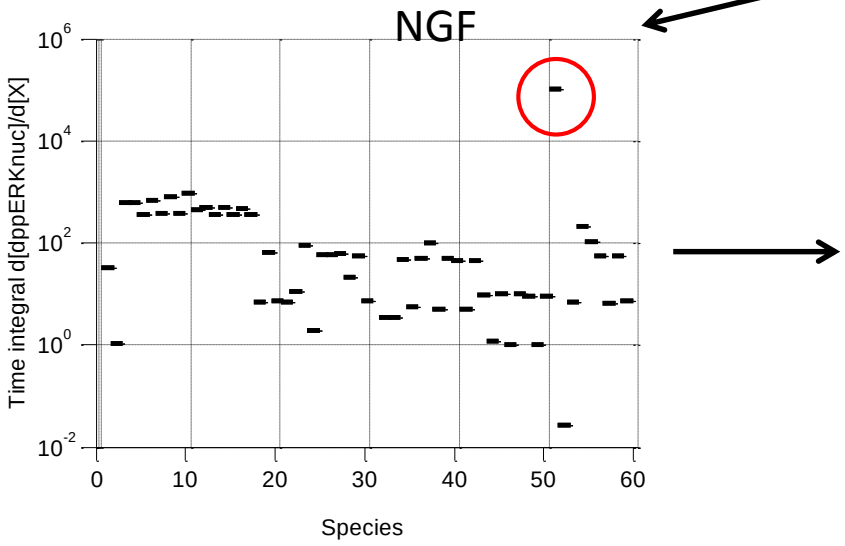
$$\left(\frac{dx(t)}{dk}\right)$$

Change in the species
(eg [dppERKnuc])

Change in variable
(eg parameter or reactant conc)



By calculating integrals -> rank of importance



Rank (dppERKnuc)	Species	Initial value	Unit	Description
1	NGFext	30	pM	NGF concentration in extracellular matrix
2	Grb2_SOS_pShc_pTrkA	0	µM	Downstream multi-protein complex
3	pShc_pTrkA	0	µM	Downstream multi-protein complex
4	Shc_pTrkA	0	µM	Downstream multi-protein complex
5	pTrkA	0	µM	Phosphorylated TrkA concentration
6	L_NGFR	0	µM	NGF NGFR complex
7	FRS2_pTrkA	0	µM	Downstream multi-protein complex
8	pFRS2_pTrkA	0	µM	Downstream multi-protein complex
9	Crk_C3G_pFRS2_pTrkA	0	µM	Downstream multi-protein complex
10	Grb2_SOS_pShc_pTrkA_endo	0	µM	Downstream multi-protein complex

Finding optimal targets; sensitivity analysis (SA) of the model

- SA provides a way to rank targets & further triage by druggability criteria -> interesting targets;

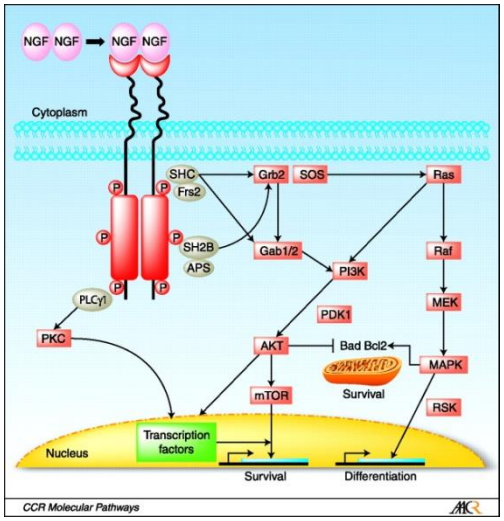
1. NGF (Consistent with NGF mAb efficacy in pain; eg Lane et al, 2010)

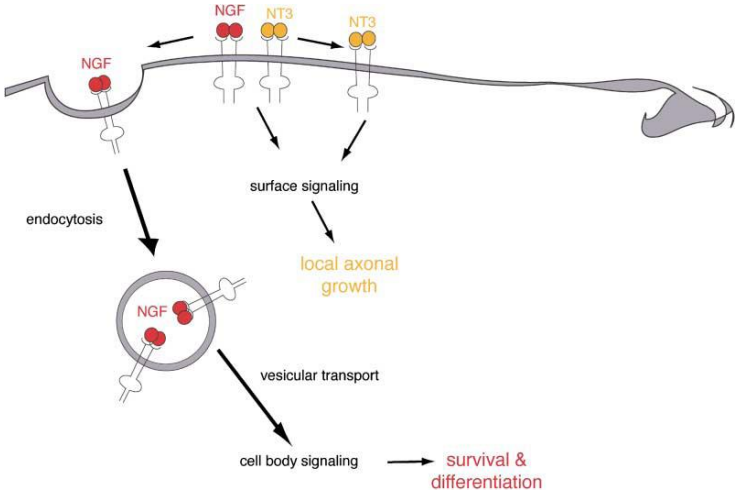
2. Trka kinase activity (eg Expert Opin Ther Pat. 2009 Mar;19(3):305-19. Trk kinase inhibitors as new treatments for cancer and pain).

3. RAS activity (Mutations in NF1 gene associated with a chronic pain phenotype (J Neurophysiol 94: 3659-3660, 2005)

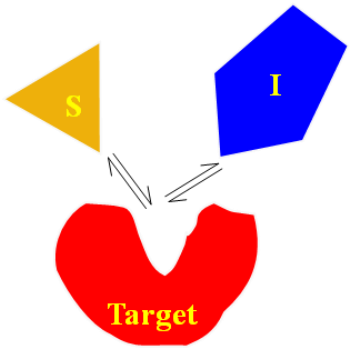
Trka kinase

- On binding NGF trka autophosphorylates and is internalised
- Model suggests phosphorylation could be a key controlling step
- Kinases 'good' targets – viewed as druggable
- ..but what does success look like in terms of dose ?





Inhibitor binding assumptions



$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI$$

M = Molar ie Moles/litre

k_1 2nd order rate constant (units typically $M^{-1}s^{-1}$)

k_{-1} 1st order rate constant (units typically s^{-1})

$$K_i = k_{-1}/k_1 = s^{-1}/M^{-1}s^{-1} = \textbf{Molar}\ (\mu M/nM)$$

Why systems pharmacology matters

For a hypothetical TrkA kinase inhibitor K_i= 0.1 nM…

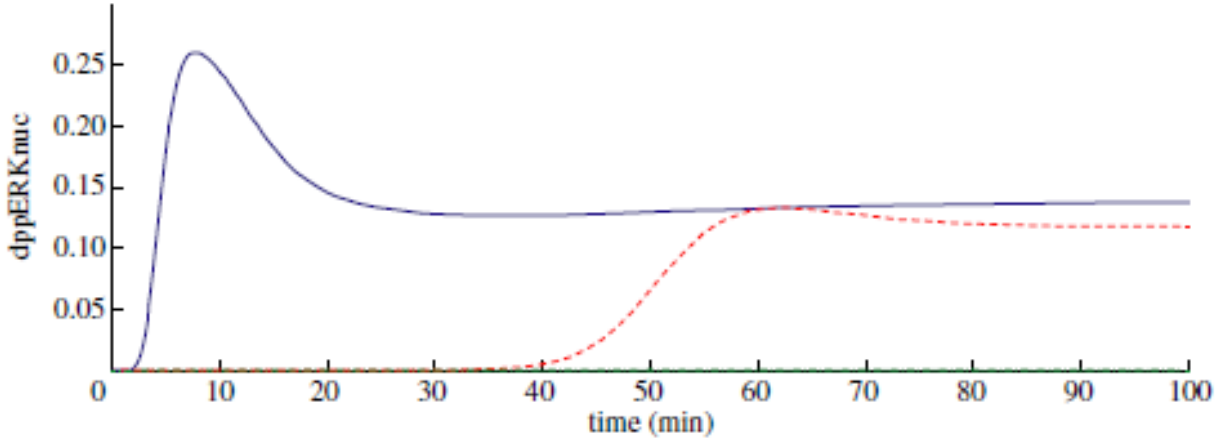


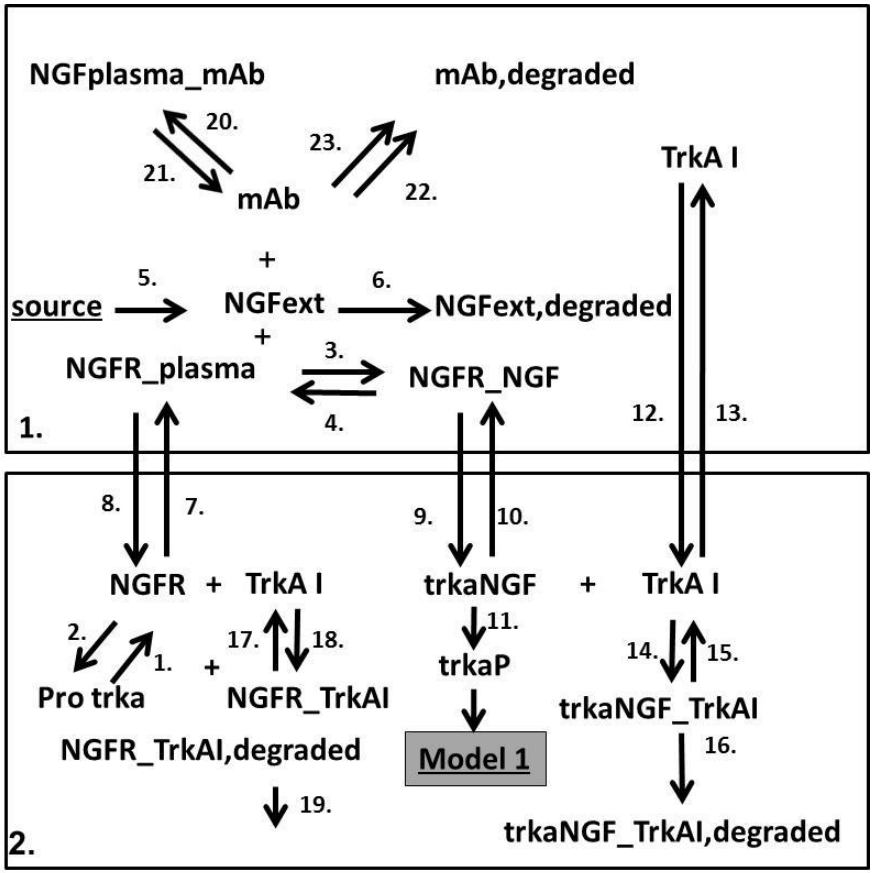
Figure 1. Model 1 simulated time course of dppERKnuc response to 30 pM NGF (solid line). The dashed line shows the response in the presence of a TrkA binding inhibitor given at $t = 0$, $K_i = 0.1$ nM (at $1000 \times K_d$ of the inhibitor) and the dashed-dotted line at $1 \times 10\,000 \times K_i$. (Online version in colour.)

- Model 1; **10,000xKi Trka inhibitor (uM)** needed to block the response

  - High > 1QD dose
  - uM plasma concs -> Is there a therapeutic index ?

The Systems biology model 1 was incorporated in appropriate physiological context; the 'systems pharmacology' model 2

Benson & Dua et al, Systems pharmacology of the NGF pathway; **Interface focus, 2013.**



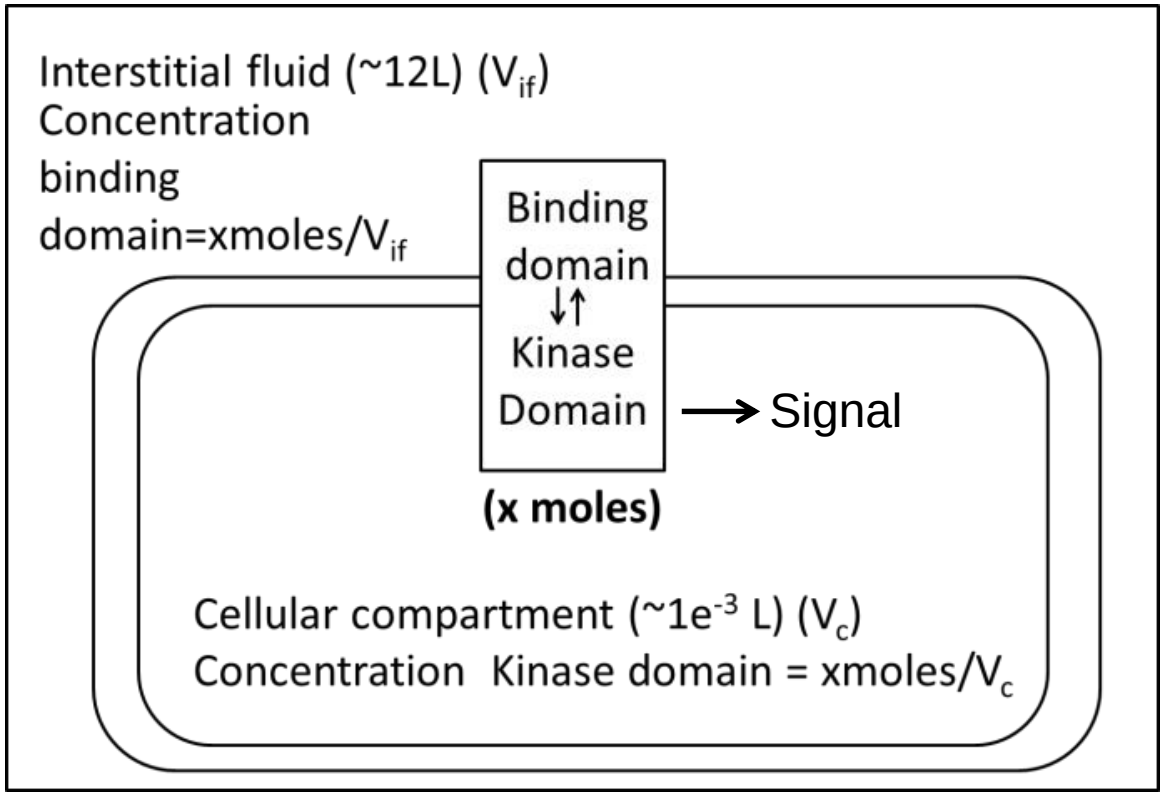
Model 1 = systems biology model

- 2 compartments

1. Extracellular body water (~5-15L)
2. Neuronal compartment (~0.001 L)

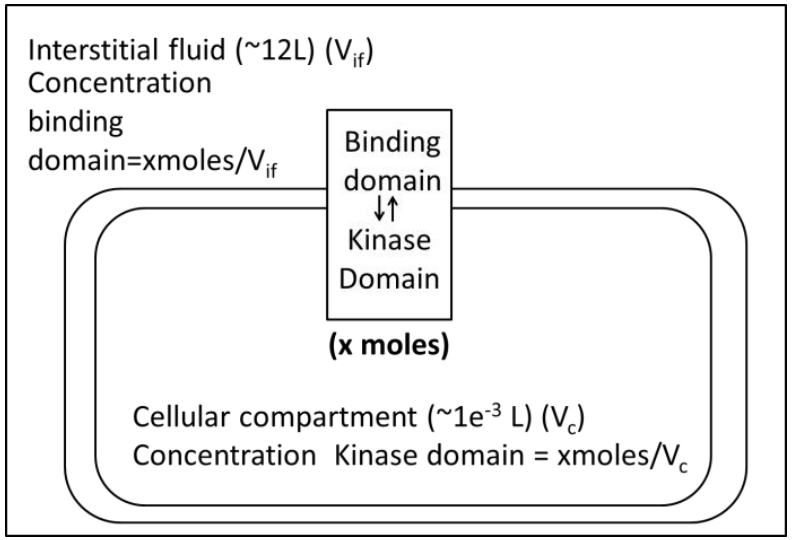
- Signal transduction elements in neuronal compartment
- Cross membrane signal transfer (Benson, Peletier & van der Graaf, J Math Biol. 2012 Dec 2. [Epub ahead of print])
- Enables dose projection simulations

Dealing with cross membrane signalling



How do we represent this mathematically ?

Dealing with cross membrane signalling



$$\begin{cases} \xrightarrow{k_{\mathrm{syn}}} L_1 + R_1 \underset{k_{\mathrm{Lb}}}{\overset{k_{\mathrm{Lf}}}{\rightleftarrows}} RL_1, & L_1 \xrightarrow{k_{\mathrm{deg}}} \\ R_1 \underset{k_{\mathrm{R21}}}{\overset{k_{\mathrm{R12}}}{\rightleftarrows}} K_2, & RL_1 \underset{k_{\mathrm{K21}}}{\overset{k_{\mathrm{K12}}}{\rightleftarrows}} KL_2 \\ K_2 \underset{k_{\mathrm{PKf}}}{\overset{k_{\mathrm{PKb}}}{\rightleftarrows}} PK_2, & KL_2 \xrightarrow{k_{\mathrm{cat}}} P_2 \end{cases} \tag{2.1}$$

J. Math. Biol.
DOI 10.1007/s00285-012-0620-z

Mathematical Biology

Cross-membrane signal transduction of receptor tyrosine kinases (RTKs): from systems biology to systems pharmacology

Neil Benson · Piet H. van der Graaf · Lambertus A. Peletier

- Assume infinitely thin membrane & V_if>>V_c (5-15L vs 0.001L)
- Very rapid exchange (2000 min^{-1}) of mass between extra-cellular facing part of the receptor and the intracellular (s timescale)
- -> equal mass but receptor concentration
  - cell **uM**
  - interstitial fluid **pM**

Why systems pharmacology matters

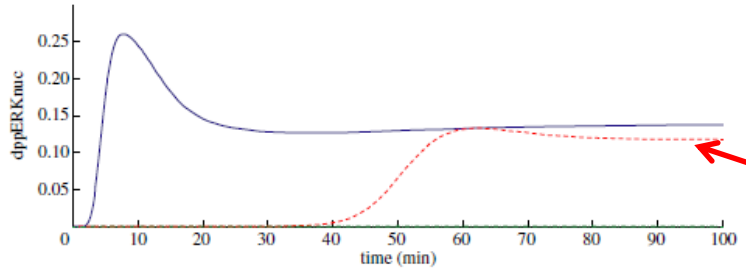


Figure 1. Model 1 simulated time course of dppERKnuc response to 30 pM NGF (solid line). The dashed line shows the response in the presence of a TrkA binding inhibitor given at $t = 0$, $K_i = 0.1$ nM (at $1000 \times K_d$ of the inhibitor) and the dashed-dotted line at $1 \times 10\,000 \times K_i$. (Online version in colour.)

Model 1; **10,000xKi Trka inhibitor** can't block the reaction (red)

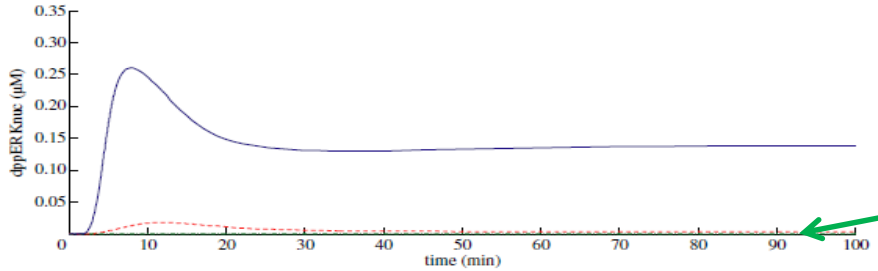


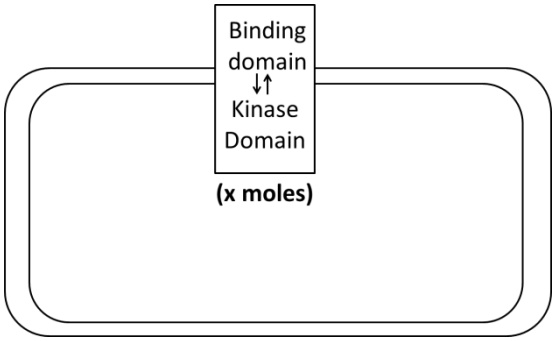
Figure 4. Model 2 simulated time course of dppERKnuc response to NGF (solid line). The dashed line shows the response in the presence of a TrkA binding inhibitor $K_i = 0.1$ nM (at $100 \times K_i$ of the inhibitor) and the dashed-dotted line at $1000 \times K_i$, both given at $t = 0$. (Online version in colour.)

Model 2; **1000xKi Trka inhibitor** blocks the reaction (green)

- Predicting a dose from model 1 is subject to >10x over-estimate
  - Impacts dose & safety margin

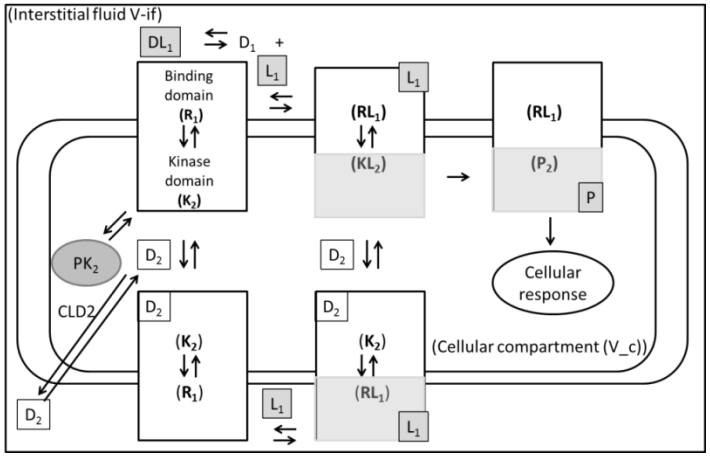
- Related to inappropriate calculation of mass transfer in model 1

Recap; systems biology -> systems pharmacology



Systems biology model;

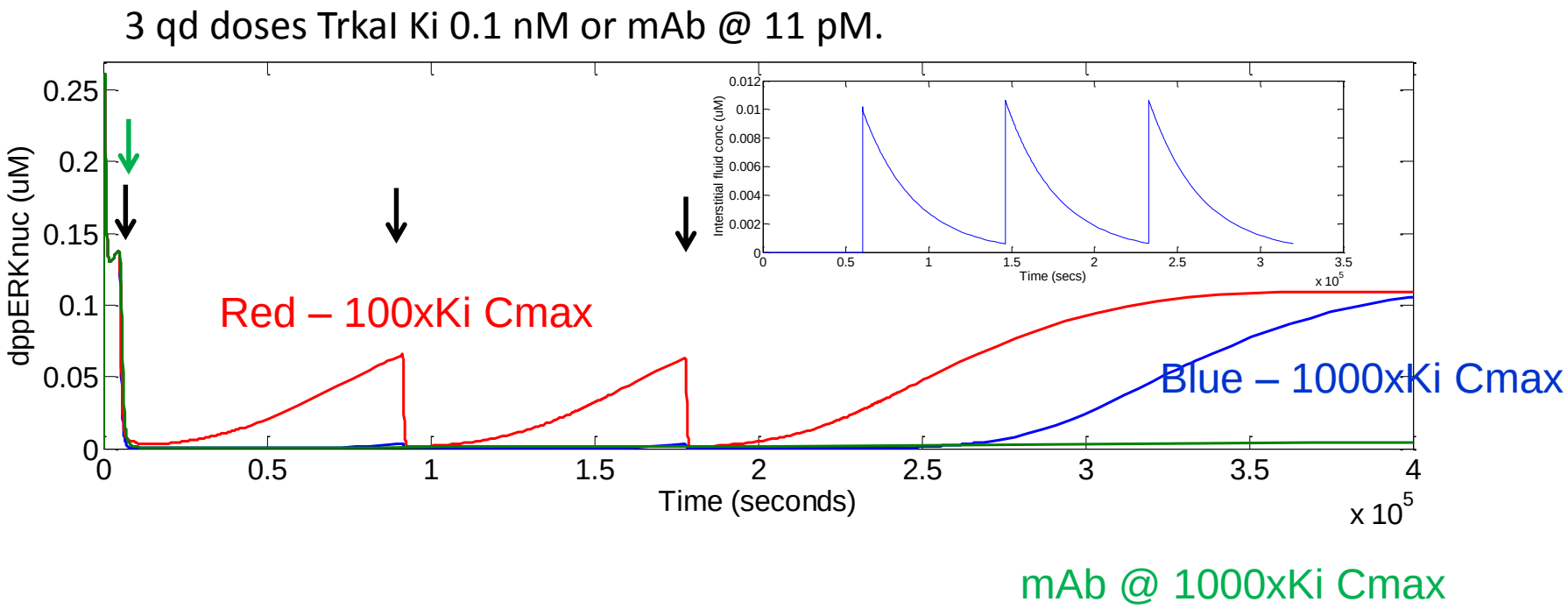
- One compartment
- No physiological data
- No easy way to compare eg mAb's and small molecules
- Gives large dose over-prediction



Systems pharmacology model;

- Two (n) compartments
- Physiological information (eg compartment volume)
- Separate compartments enable comparison of small molecules and mAb's
- Can be used for dose prediction
- Clear to non-expert

Dose simulations enabled; compare small molecules with restricted V_d (eg mAbs)



Require 1000xKi mAb or trka kinase I @ Cmax to stop response

Cross-membrane signal transduction of RTK's: Impact on drug dose and administration, Peletier et al, manuscript in preparation.

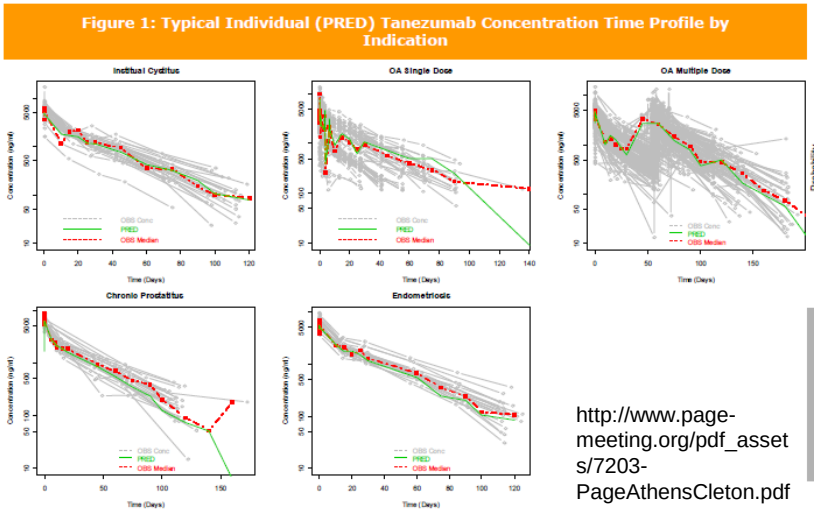
NGF antagonists

- NGF identified as the optimal target in model
- mAbs directed against NGF have clinical efficacy in some pain states (Lane et al, 2010)
- As for TrkA kinase, model predicts higher than expected NGF binding drug concentration (>1000 x K_D) for maximum efficacy
- Dose = (3.5L)*$K_{D,\ NGF}$ (0.01nM^)*1000*1e-9*150,000*1000 = **5mg**
  - **=> Steady state 5-10 mg to maintain effect**

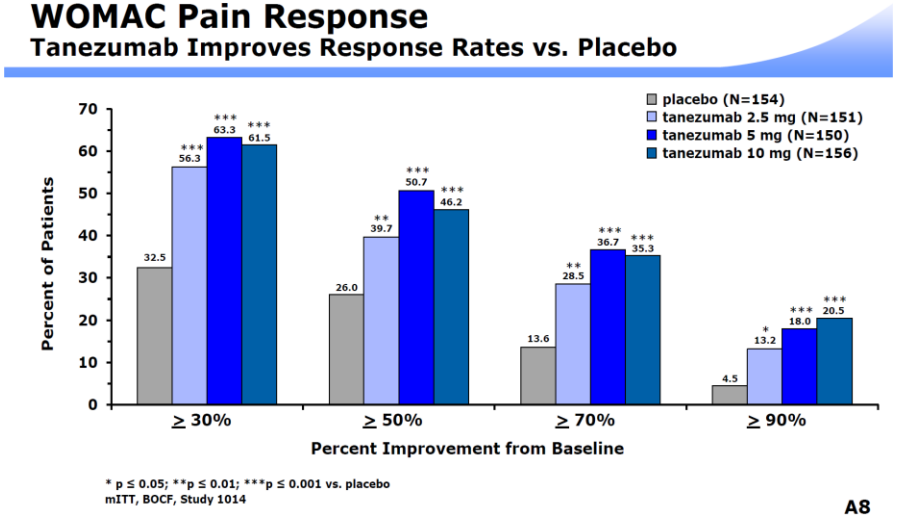
Model predictions quantitatively concordant with data for prototype NGF mAbs eg tanezumab

- **Model prediction consistent with clinical pain data**

- **Clinical optimal dose for efficacy reported ~ <u>5-10 mg</u>**



http://www.page-meeting.org/pdf_assets/7203-PageAthensCleton.pdf

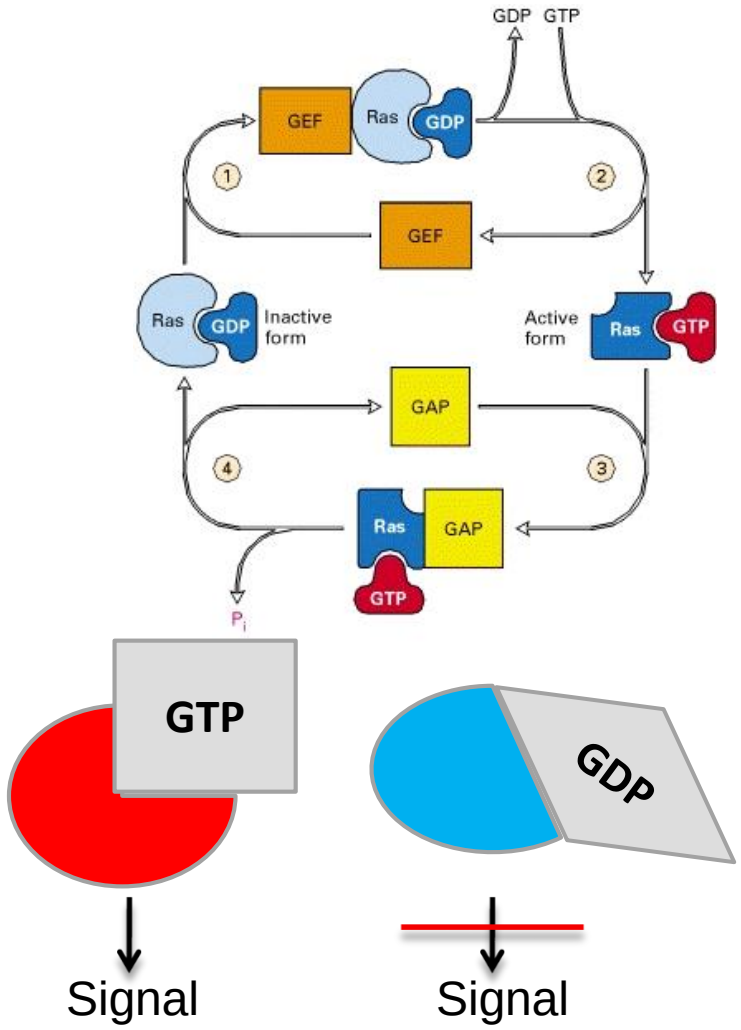


http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ArthritisAdvisoryCommittee/UCM301305.pdf

⇒ <u>Efficacy & dose could have been predicted from '05 model (before any clinical data were published)</u>

Ras biology

- Ras - a switch in many signal transduction pathways
- Switch on with GTP bound and off when GTP is turned over to GDP
- Typically the GTP/GDP turnover is accelerated by binding a GTPase activating protein (a GAP)
- SA identified a GAP as critical in signal transduction to ERK



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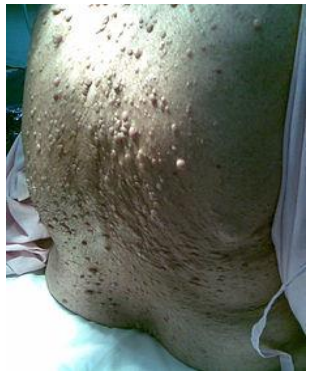
Human Genetic supporting data ?



- Mutations in NF1 gene associated with **'neurofibromatosis' & a chronic pain phenotype** (J Neurophysiol 94: 3659-3660, 2005)
- NF1 has GAP activity & is expressed in neuronal cells
- Mutation causes loss of function – delayed hydrolysis of GTP
- Sensory Neurons from Nf1 haploinsufficient mice Exhibit **increased excitability** (J Neurophysiol, 94, 3670-, 2005)

Summary

- Development of '05 published model predicted efficacy & (non-intuitive) dose for NGF mAb ahead of any PHII data

- Model predicts importance of RAS signalling in pain
  - Supported by genetic evidence

- Systems pharmacology (SP) approach
  - Can incorporate systems biology
  - Deals appropriately with mass transfer between compartments & enables dose prediction
  - Can be presented graphically to non-expert
  - Allows comparison of drugs operating in restricted compartments (eg mAb's versus small molecules)

- SP is a valuable novel tool in drug development

About XQ

Located Canterbury Innovation Centre, UK. ~60 mins from central London

AstraZeneca

BAYER

CREABILIS

astellas
Leading Light for Life

MMV
Medicines for Malaria Venture

Pfizer

Roche

Levicept

modern biosciences
creating value from innovative research

Interested in application of PKPD and systems pharmacology in drug discovery

Backups

Model 3;
impact of the complexity in model 1

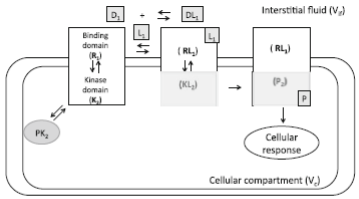
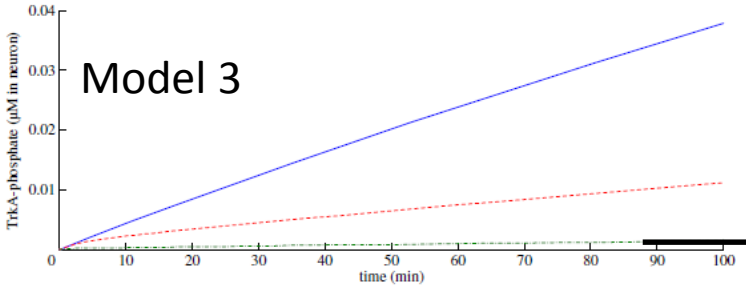


Fig. 2 Schematic picture of the cell and its membrane. On the *left*, the receptor in its free state, with its binding domain R_1 and its kinase domain K_2, the latter in equilibrium with the receptor pool PK_2. In the *middle* the receptor in its bound state with its binding domain RL_1 and its kinase domain KL_2, the latter shaded to highlight a conformational change. On the *right* KL_2 is shown in its phosphorylated state P_2, inducing a cellular response

Model 3; model 2 without model 1 systems biology



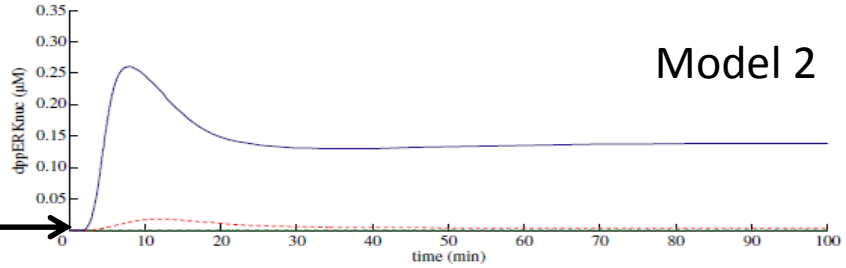
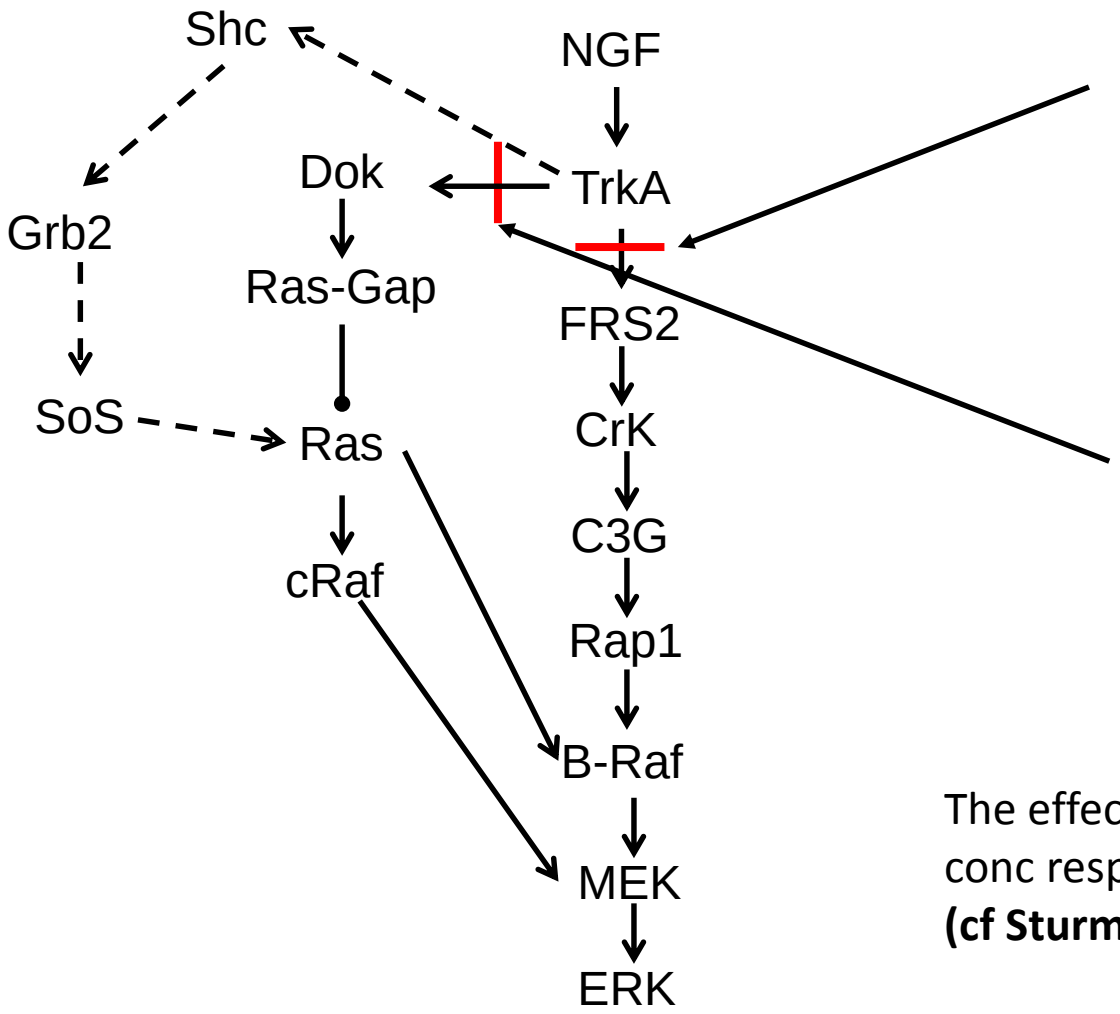


Figure 5. Model 3 simulations with and without hypothetical inhibitor of TrkA kinase, given at $t = 0$, $K_i = 0.1$ nM. Graph shows the accumulation of phosphorylated-NGF–TrkA complex concentration in the neuronal compartment over time with and without TrkA inhibitor. No inhibitor (solid line) $10 \times K_i$ (dashed line) $100 \times K_i$ (dashed-dotted line). (Online version in colour.)

re 4. Model 2 simulated time course of dppERKnuc response to NGF (solid line). The dashed line shows the response in the presence of a TrkA binding inhibitor 0.1 nM (at $100 \times K_i$ of the inhibitor) and the dashed-dotted line at $1000 \times K_i$, both given at $t = 0$. (Online version in colour.)

- 100xKi TrkA inhibitor blocks rate of production of Trka-P 99% (as expected)
- 100xKi TrkA inhibitor – significant response (~5% AUC of non-inhibited signal)
- 1000xKi needed to block response completely (model 2 green)

Hypothesis1 ; impact of negative feedback



Block here **attenuates** dppERKnuc signalling

+

Block here weakens Ras-Gap activation; potentiates signalling through Ras & in turn **potentiates** dppERKnuc signalling

The effects counteract one another -> shifts conc response curve to weaker drug effect **(cf Sturm et al, 2010, Sci signal, 3)**

Hypothesis 2

- Apparent disconnect a lack of clarity around the non-steady state behaviour of dppERKnuc?
  - How do we make an effective enquiry into this?

- How does the dppERKnuc biomarker signal relate to pain?

Other approaches?

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Cell Surface Receptors for Signal Transduction and Ligand Transport: A Design Principles Study

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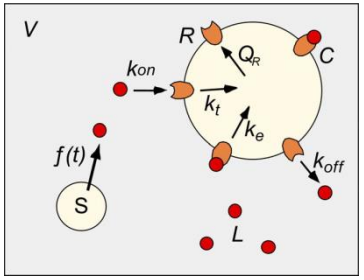
Shankaran et al Plos comp biol



$$dR/dt = -k_{on}RL + k_{off}C - k_tR + Q_R \quad (1a)$$

$$dC/dt = k_{on}RL - k_{off}C - k_eC \quad (1b)$$

$$dL/dt = [-k_{on}RL + k_{off}C]/(N_{av}V) + f(t) \quad (1c)$$

V is volume per cell assuming 2x10^7 cells/ 10 ml
C, R unit molecules, L nM, kon nM-1s-1,

Equivalent to converting neuron to amount?
External concs then corrected for volume ie

d[NGFext]/dt = ksynth-NGFext*kdeg –kon*NGFext*NGFR*(Vcell/(Vcell+Vif)) etc (unit uM-1min-1), Vcell = volume of neurons (1e-3L), Vif interstitial fluid volume = 12L.

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Analysis of Receptor Internalization as a Mechanism for Modulating Signal Transduction

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The past decade has witnessed a profound explosion of knowledge in the field of signal transduction mediated by receptor tyrosine kinases. Upon binding of cognate extracellular ligands, these receptors interact with various enzymes and other signaling molecules

See also Lauffenberger and Haugh, J Theor Biol, 1998 & Krippendorf and Huisinger,