

Simulating the EGF signal transduction pathway to understand drug action

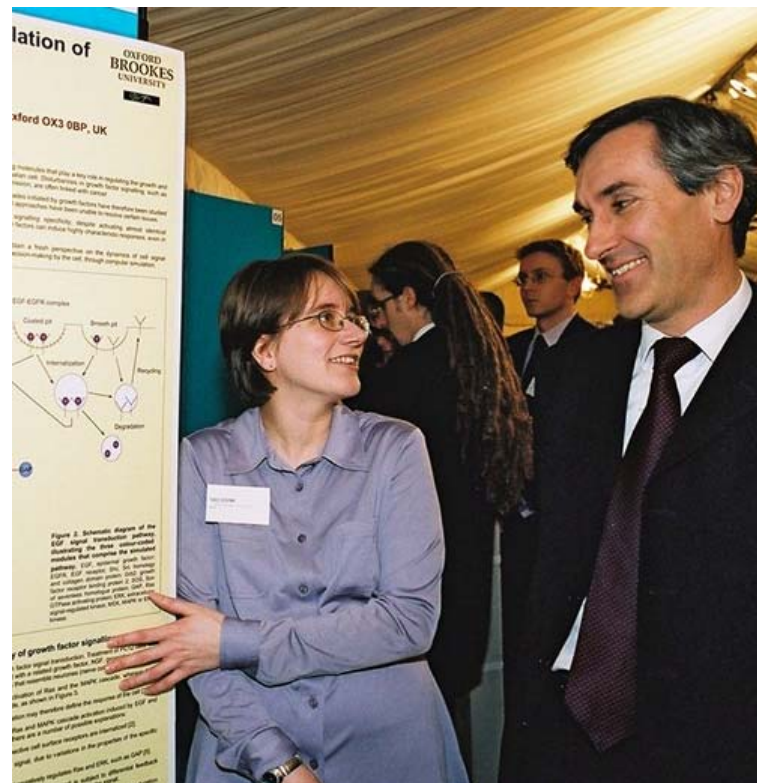
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Acknowledgement

Frances Brightman



The need

- High throughput screening, combinatorial chemistry, structure based design, gene discovery have all increased drug research costs.
- High failure rates at later stages of development (toxicity, inefficacy in clinical trials) where costs are even higher.
- Better selection in the early stages could reduce costs and increase productivity.
- Aberrant signal transduction is involved in many diseases, especially cancer, but understanding of the effects of intervention in signal pathways is poor.

Outline

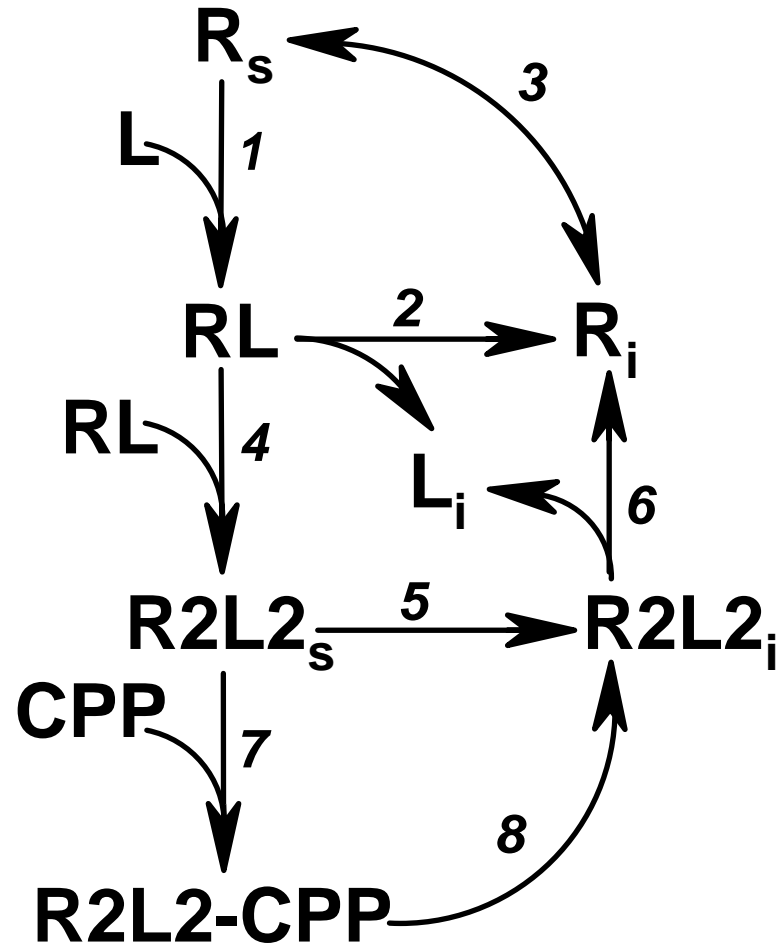
- Description of the model.
- Sensitivity analysis
- Exploration of some drug effects

Features of the EGF-MAPK model

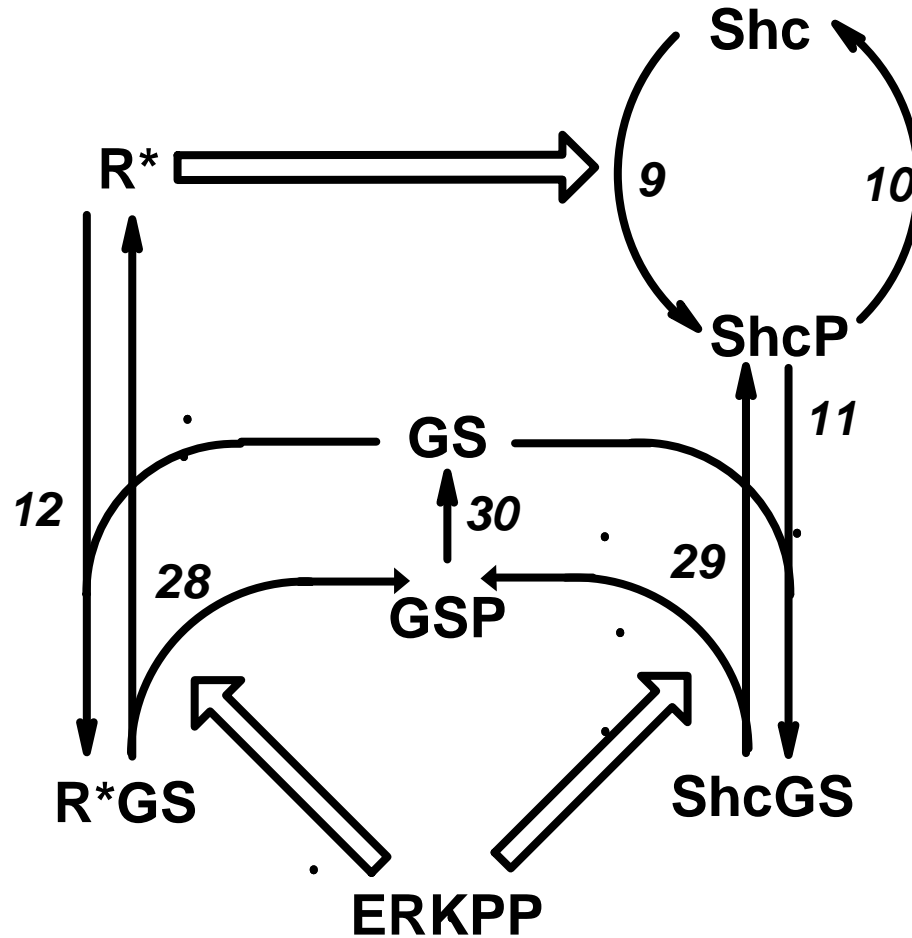
- Spatial distribution not represented (except higher local concentrations for membrane-associated species).
- Based on responses of PC12 cells for 60 min after stimulation with EGF.

Brightman & Fell (2000), FEBS Lett. 482, 169-174.

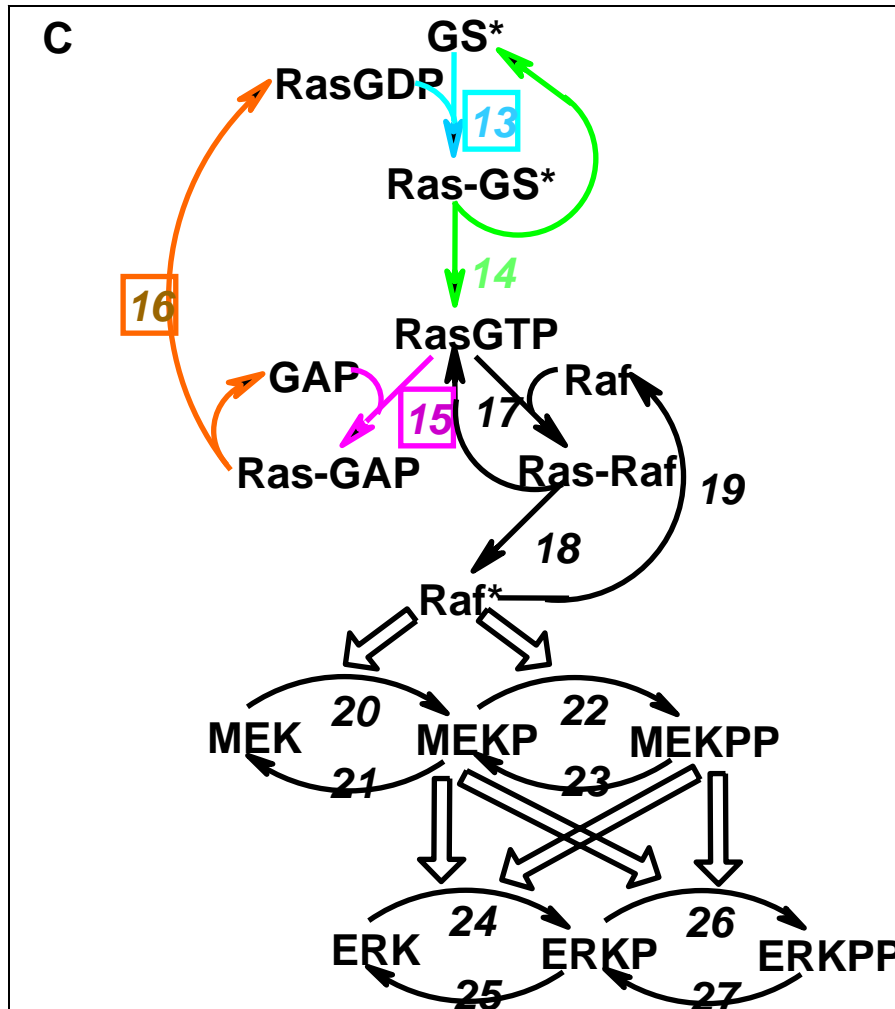
Receptor binding module of EGF model

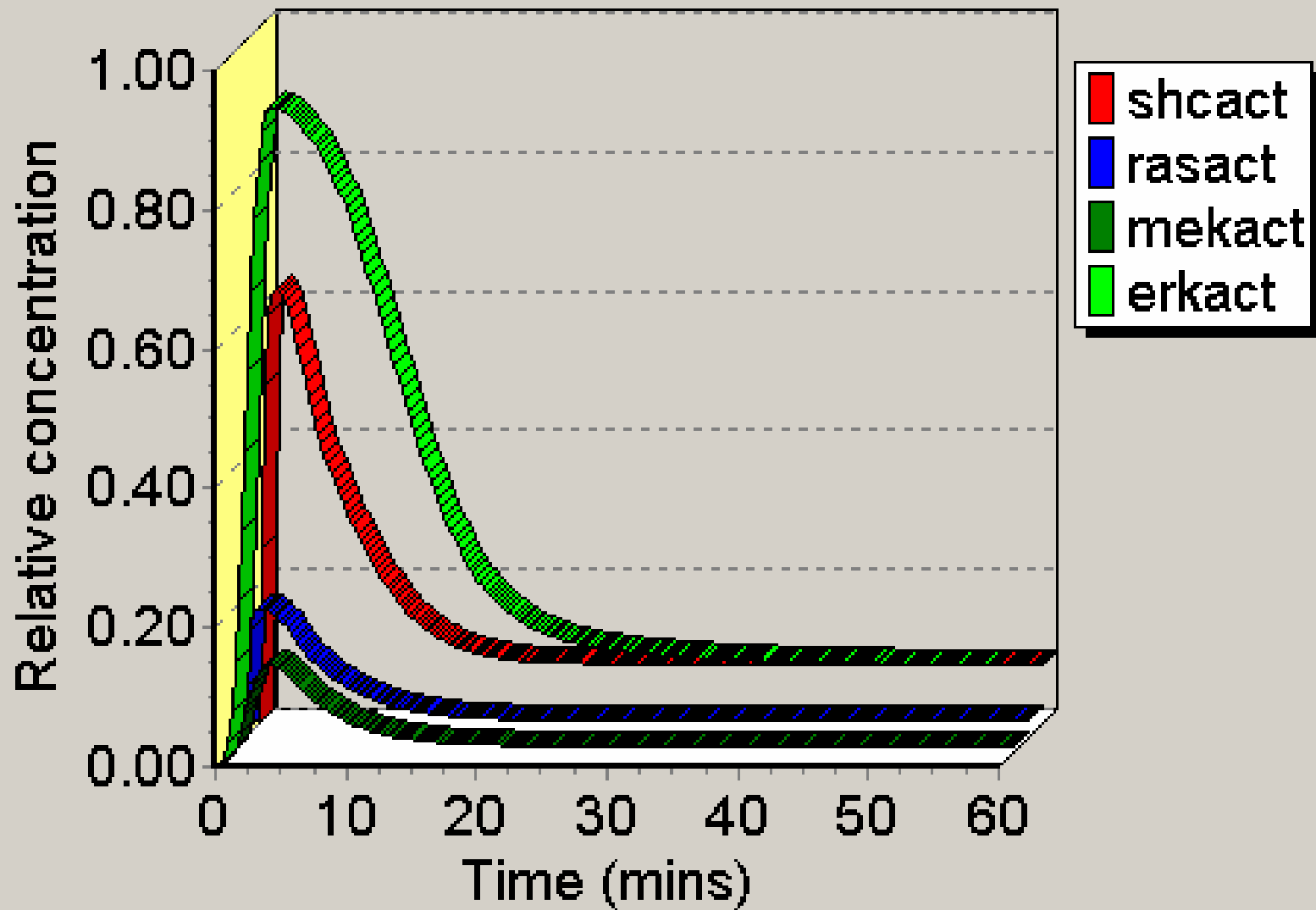


Intermediate module

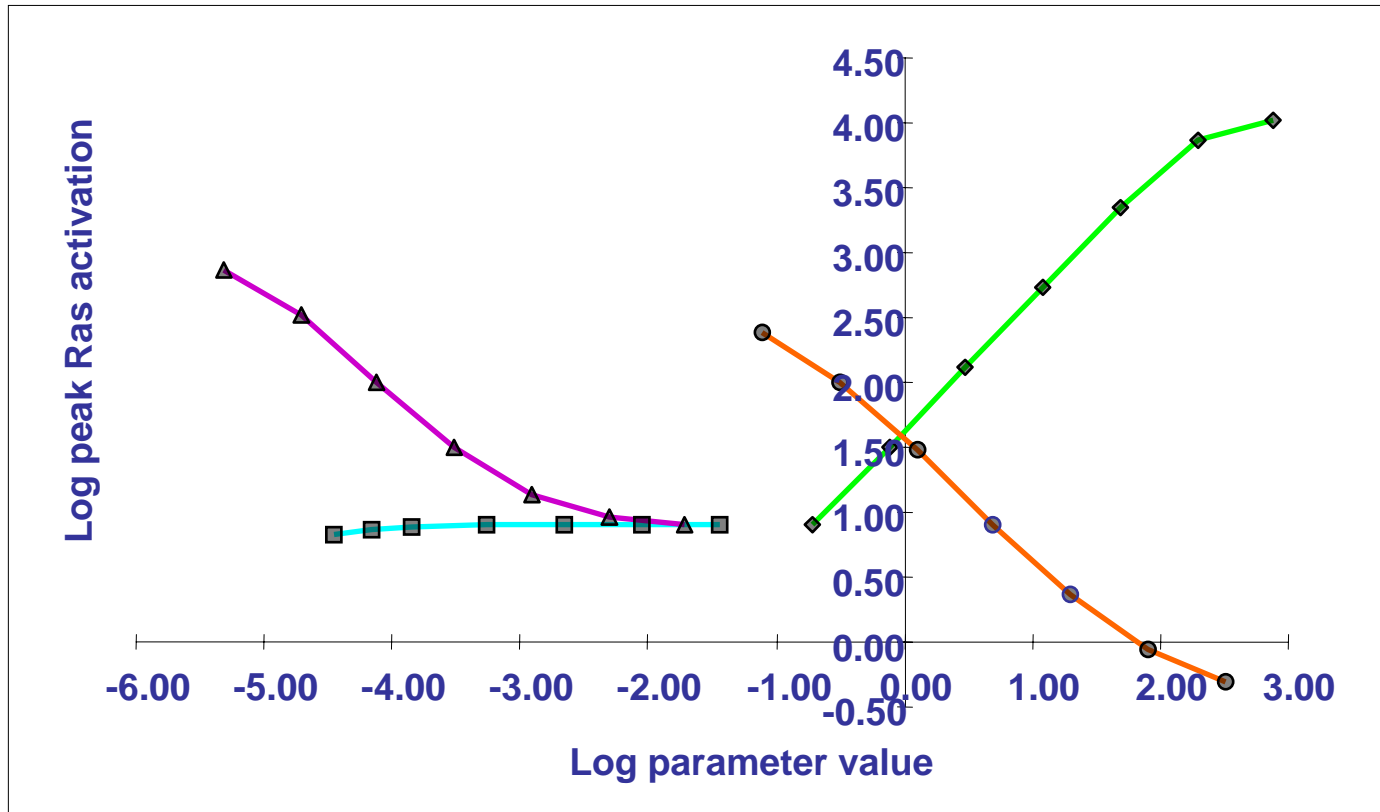


MAPK module of EGF model

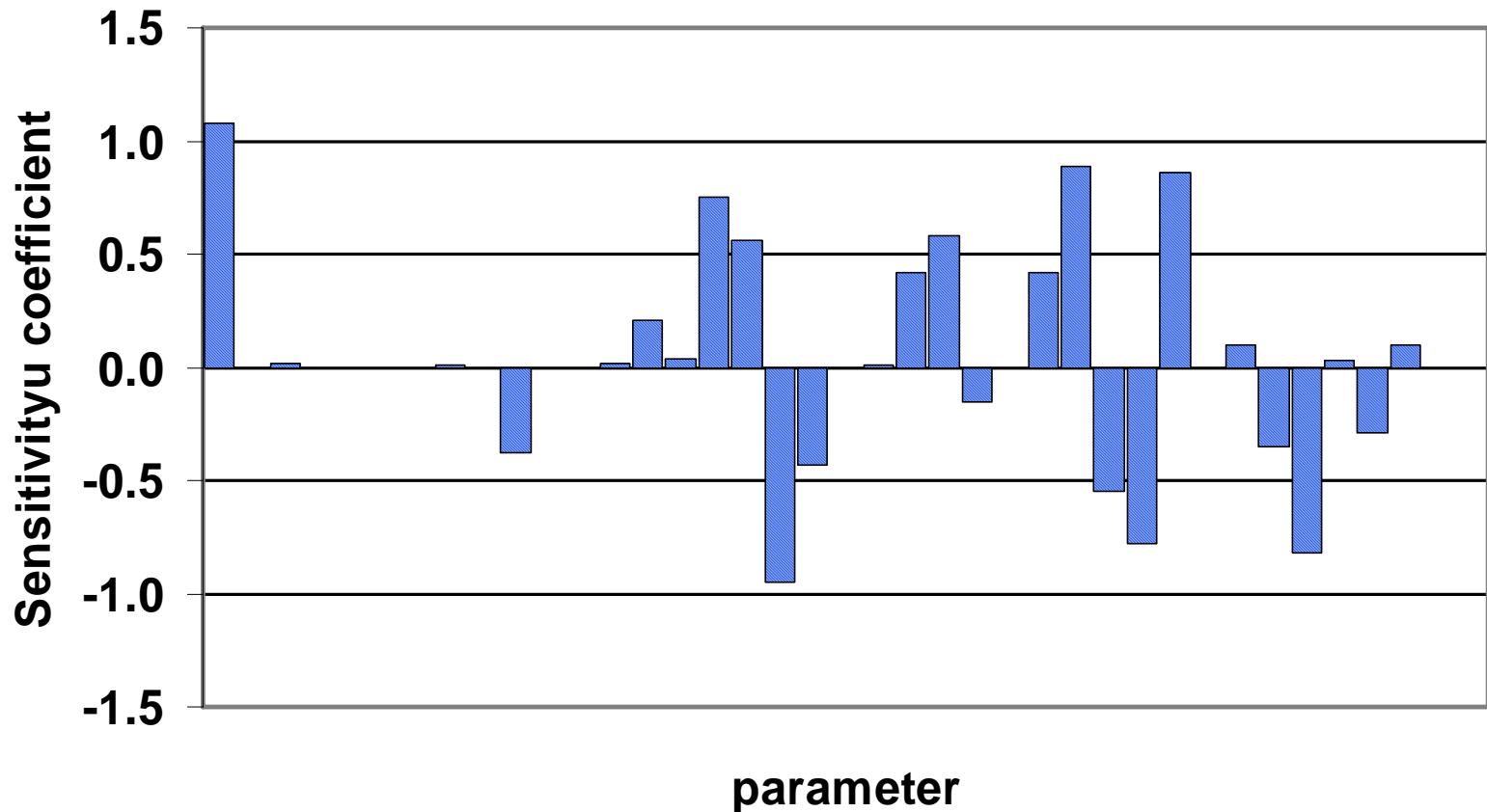




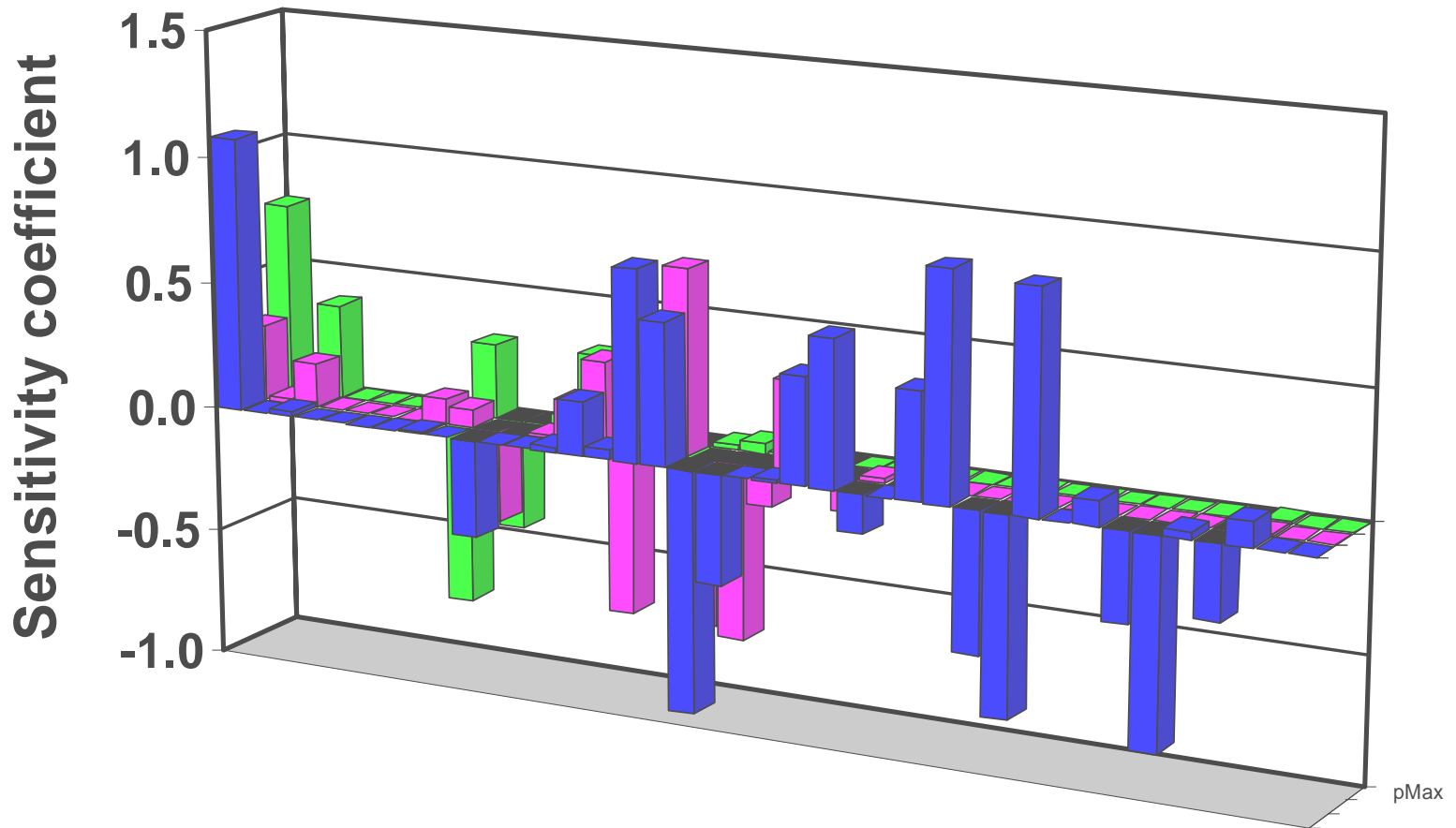
Sensitivity of peak Ras to some model parameters



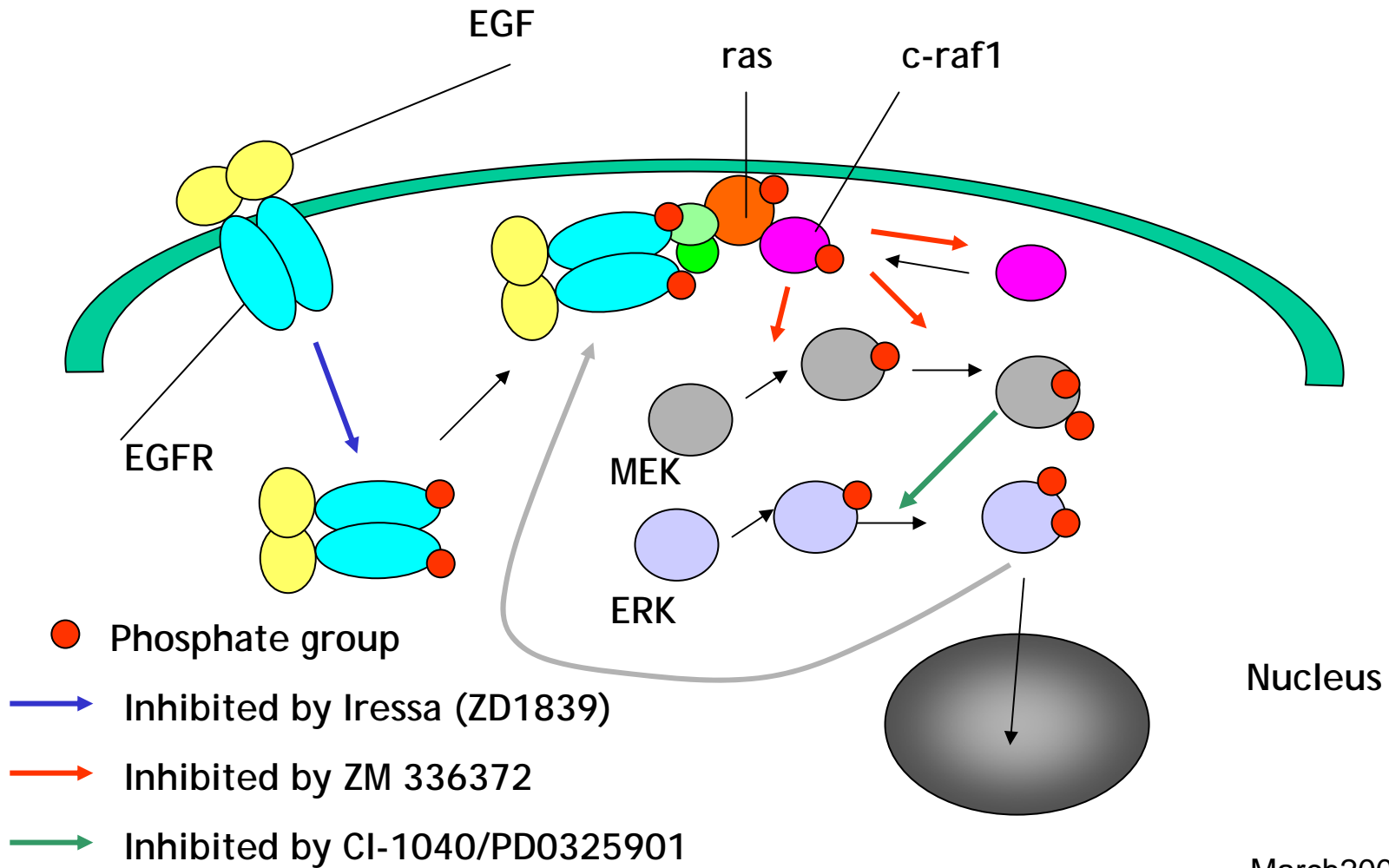
Sensitivity of maximum ERK phosphorylation is variable



Peak Shc, Ras and ERK phosphorylation



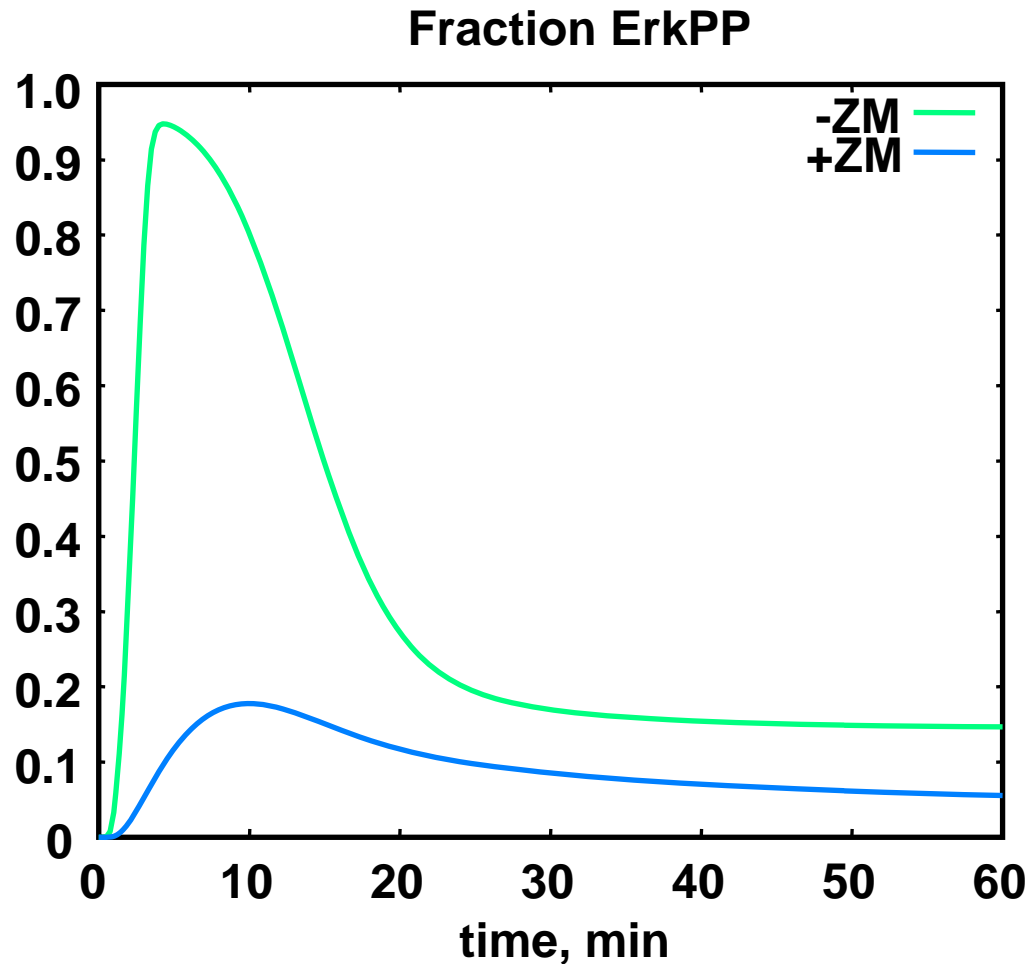
EGF Inhibition



Paradoxical activation of Raf

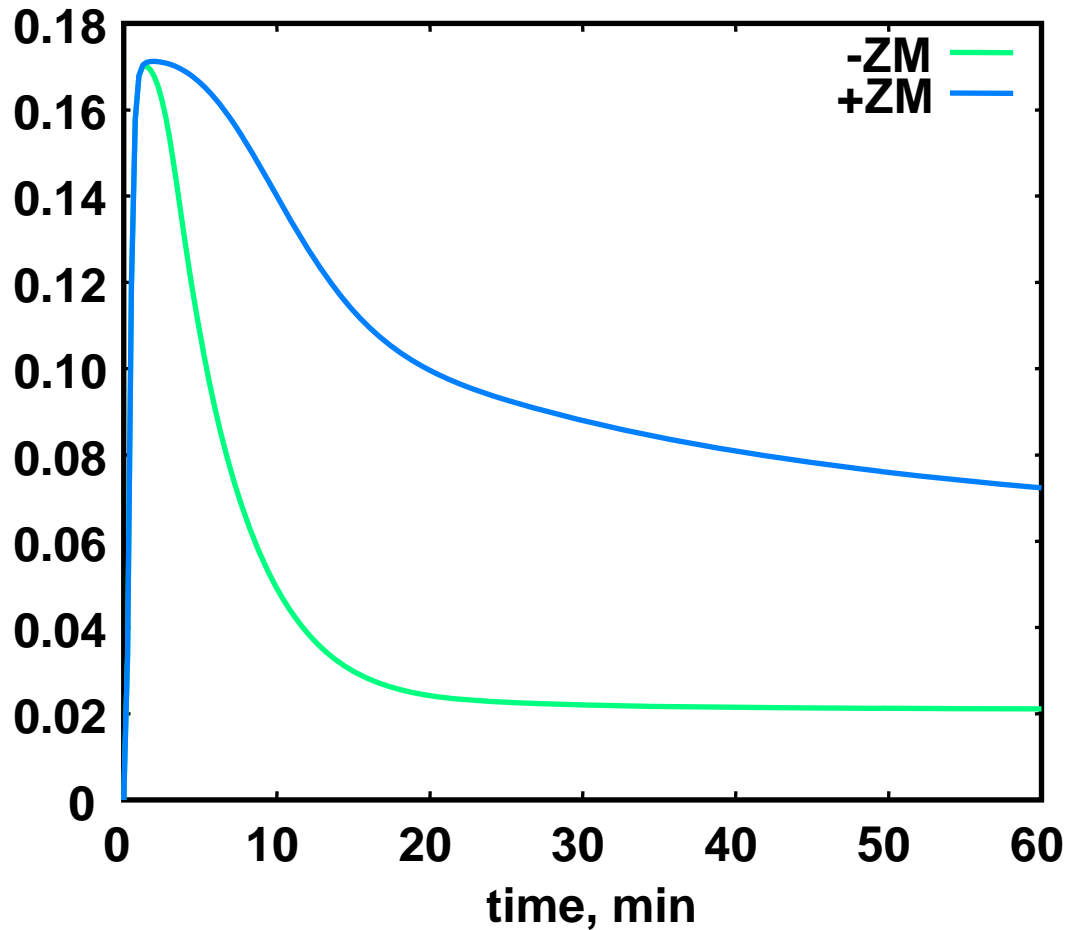
- Zeneca developed ZM 336372 as an inhibitor of Raf.
- Although it inhibits phosphorylation of MAPK cascade components by Raf, it results in higher levels of Raf in treated cells.
- This Raf activation was termed 'paradoxical' by Hall-Jackson et al (*Chem & Biol.* 6, 559-568, 1999).
- The following simulations of PC12 cells are for drug at 5x its IC₅₀.

Simulation of ZM336372: ERK



Simulation of ZM336372: Raf

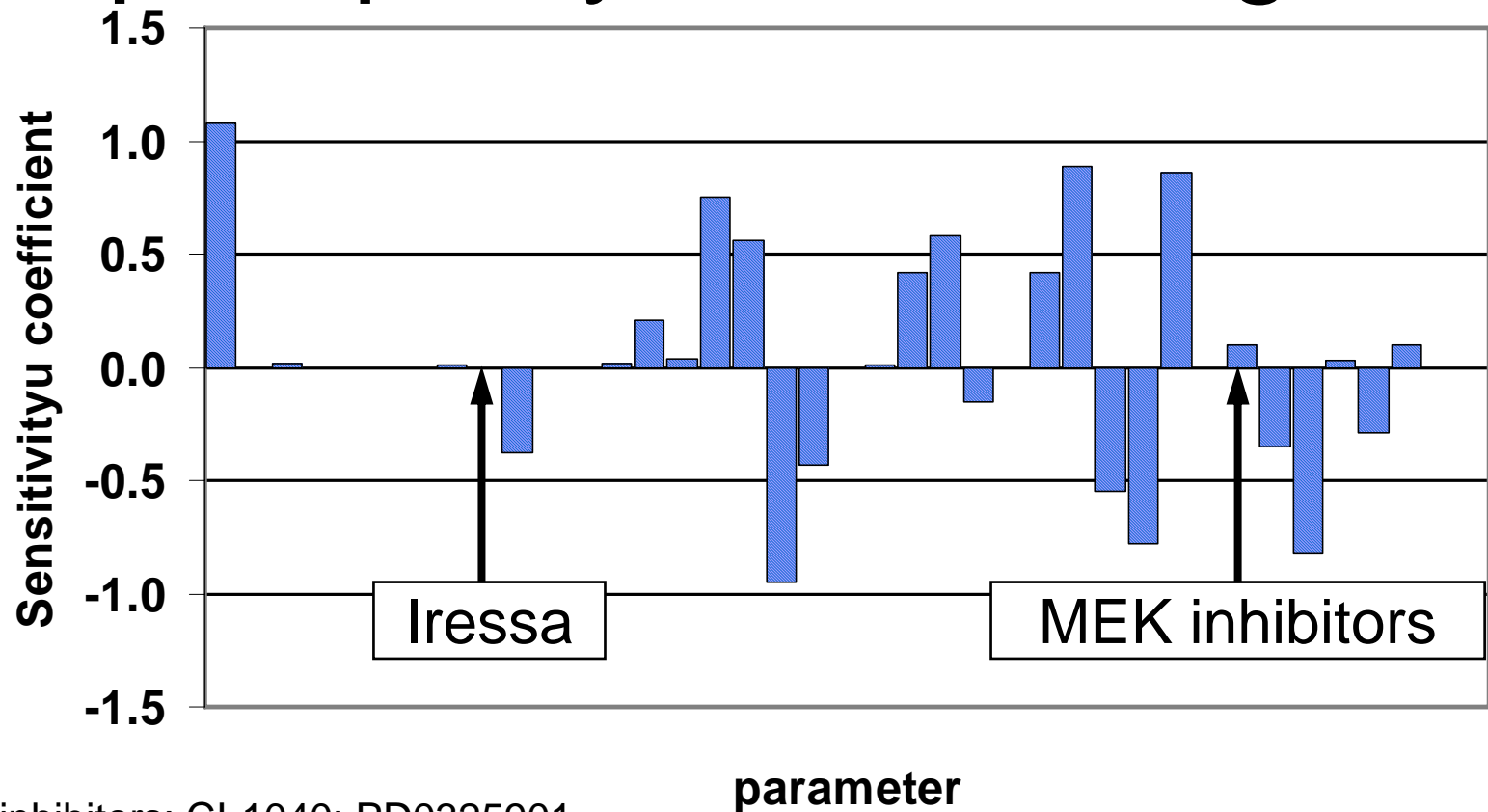
Fraction active Raf



Iressa

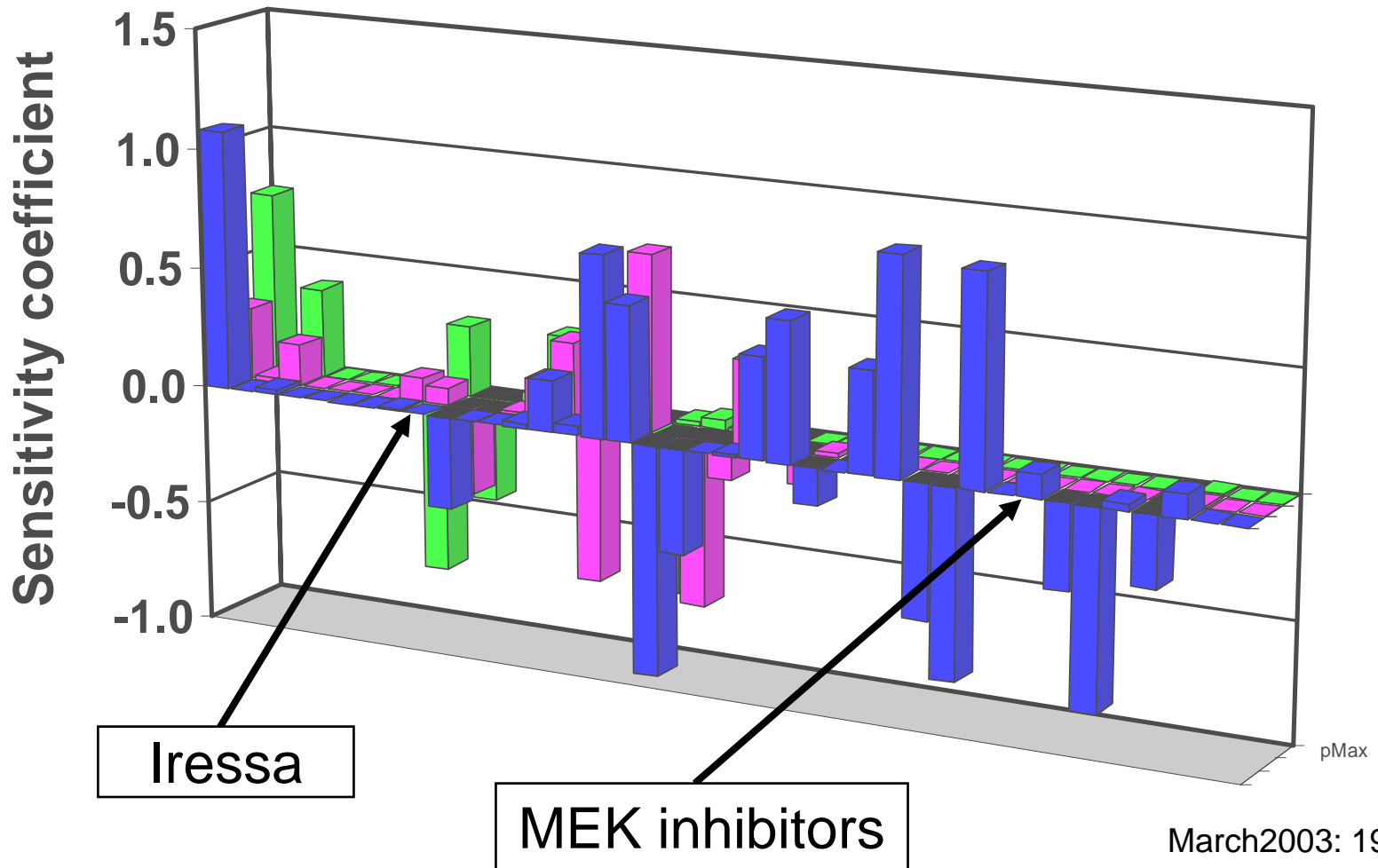
- IC_{50} *in vitro* 0.023 μM ; *in vivo* 0.080 μM
- Blood level at 24 hours 5.7 μM
- Adding drug to cells already EGF stimulated diminishes ERK activity by >95% BUT
- Activity returns to c 50% of normal within 12 hours
- Peak drug levels of 68 μM strongly inhibiting >98%

Relative sensitivity of maximum ERK phosphorylation to drugs



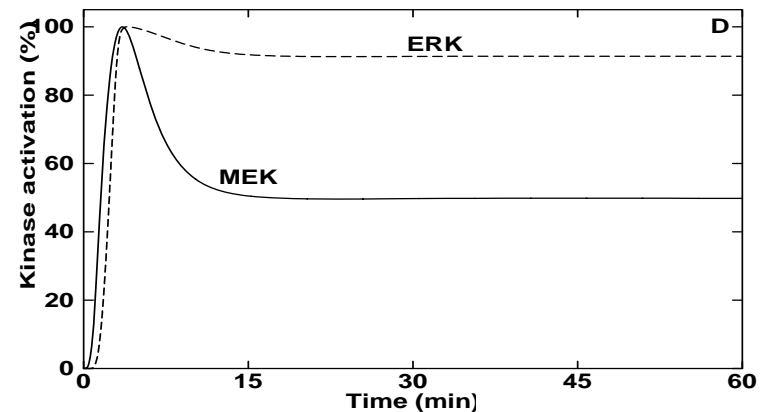
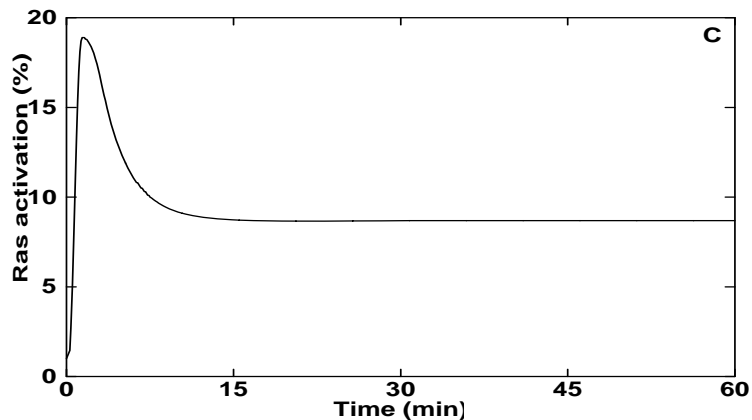
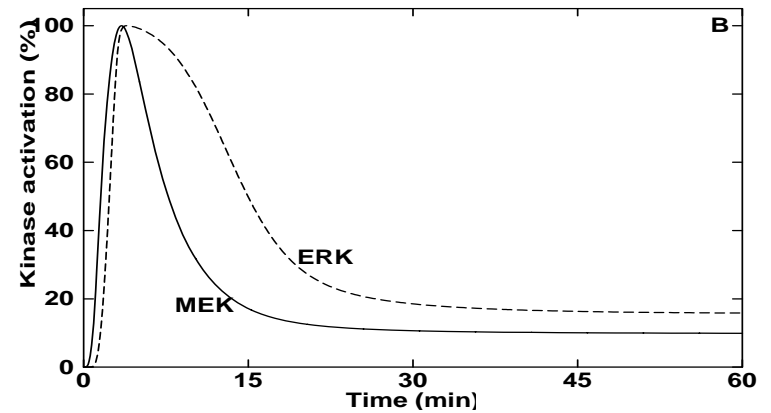
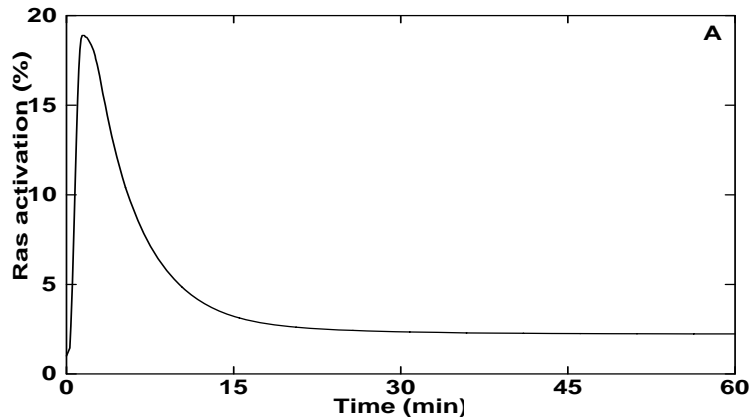
MEK inhibitors: CI-1040; PD0325901

Peak Shc, Ras and ERK phosphorylation



Conclusion

- Simulating signal transduction networks is challenging but feasible.
- Quantitative data on concentrations and kinetics is an issue, and is needed for a range of normal and tumour cells.
- Spatial representation may need to be improved; longer time scales need to be simulated.
- Even so, it is possible now to gain understanding of drug effects.



Simulated time courses of Ras and MAPK cascade activation in PC12 cells over 60 minutes continuous exposure to 100 nM EGF. A & B: transient activation of Ras, MEK and ERK. C & D: sustained activation of the Ras, MEK and ERK resulting from a 40-fold increase in the rate of SOS dephosphorylation. Percentage of total Ras in the active GTP-bound form (A & C). Percentage of maximal MEK and ERK activation (B & D).