Sensitivity and information theoretic analyses of biochemical networks

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mcisb

“Progress in science depends on new techniques, new discoveries, and new ideas, probably in that order”

Sydney Brenner, Nature, June 5, 1980

“But one thing is certain: to understand the whole you must study the whole”

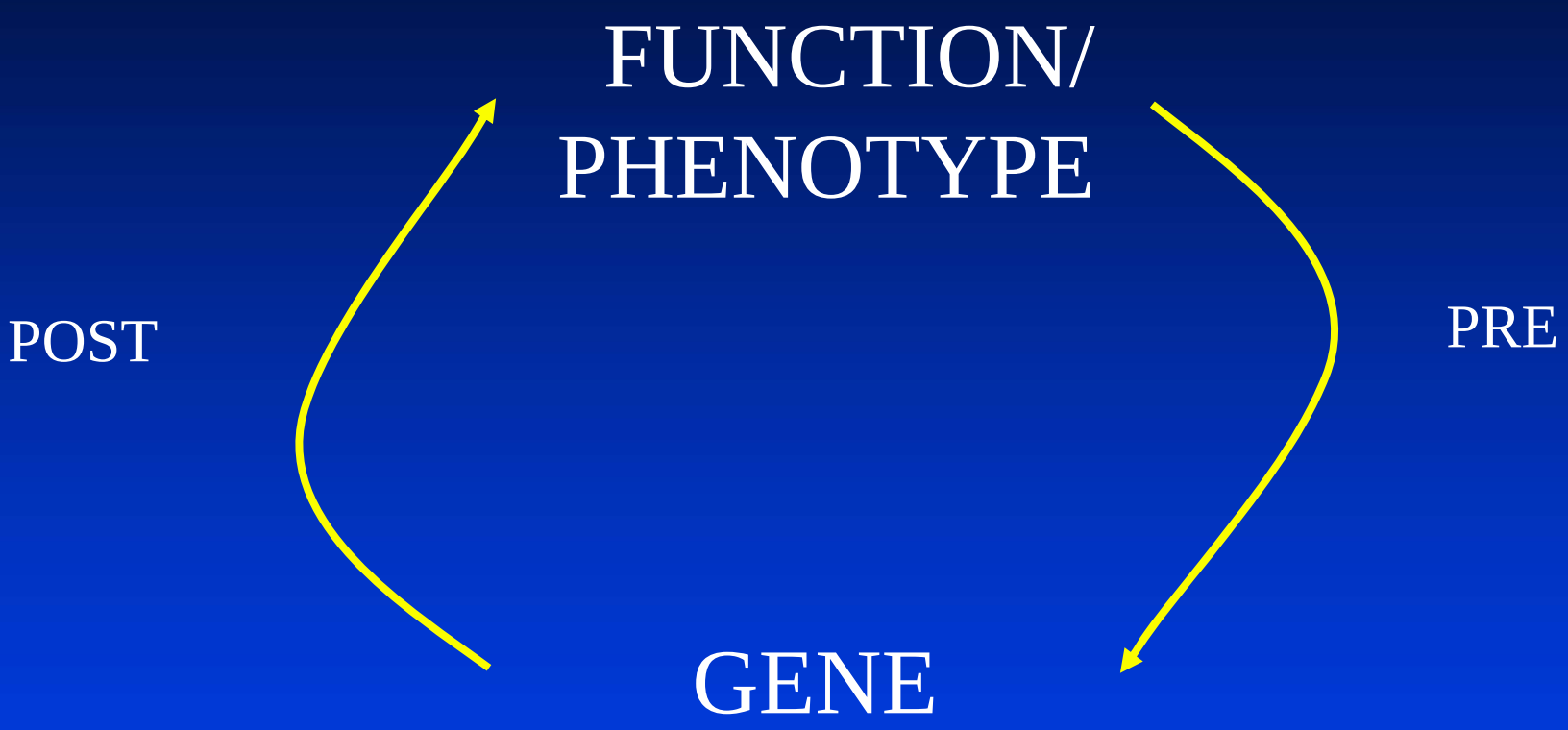
Henrik Kacser, 1986

Synopsis of talk

New techniques, new discoveries, and new ideas

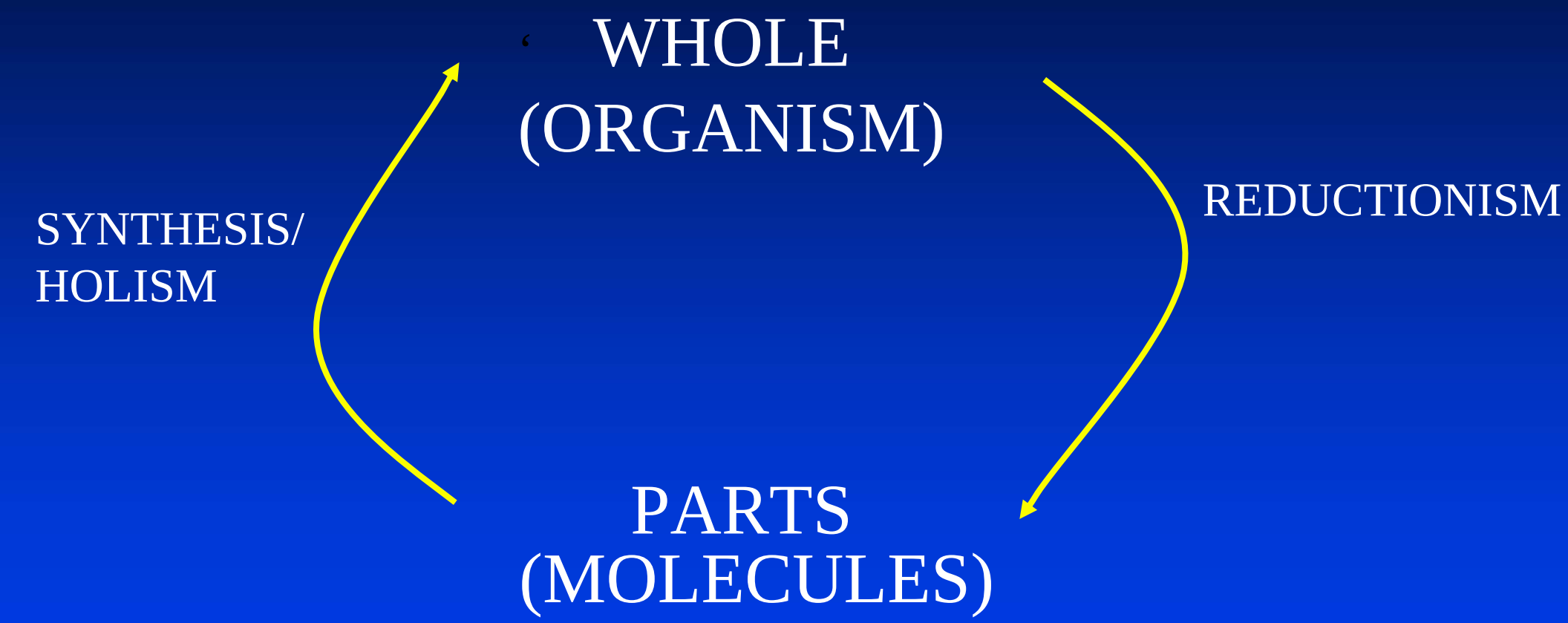
- **Biology is changing – a new philosophy of Systes Biology**
- **SBML, Taverna and modelling in modern systems biology**
- **Sensitivity analyses of the NF-κB signal transduction pathway**
- **New ways of encoding information in biology**
- **Conclusion**

Pre- and post-genomics

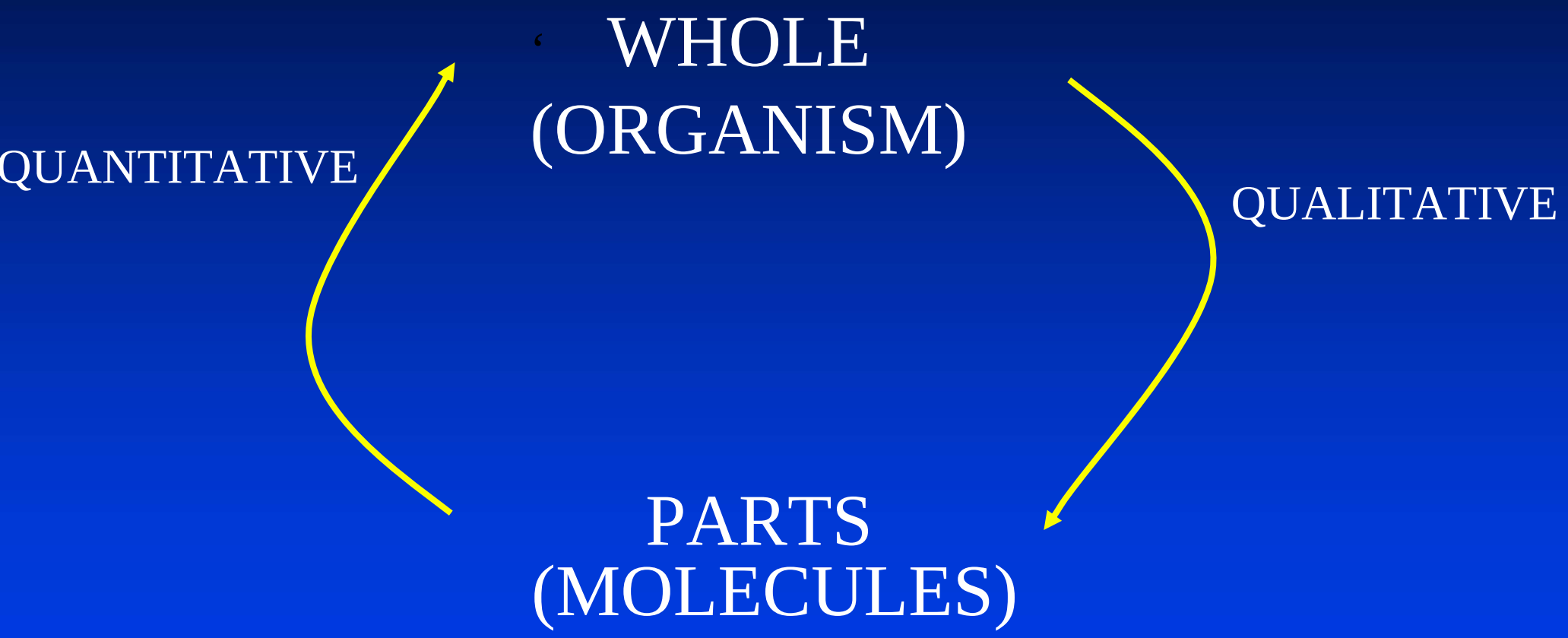


BUT THE SYSTEMATIC GENOME SEQUENCING PROGRAMMES SHOWED WE HAD MISSED ~50% OF THE GENES EVEN IN WELL-STUDIED ORGANISMS

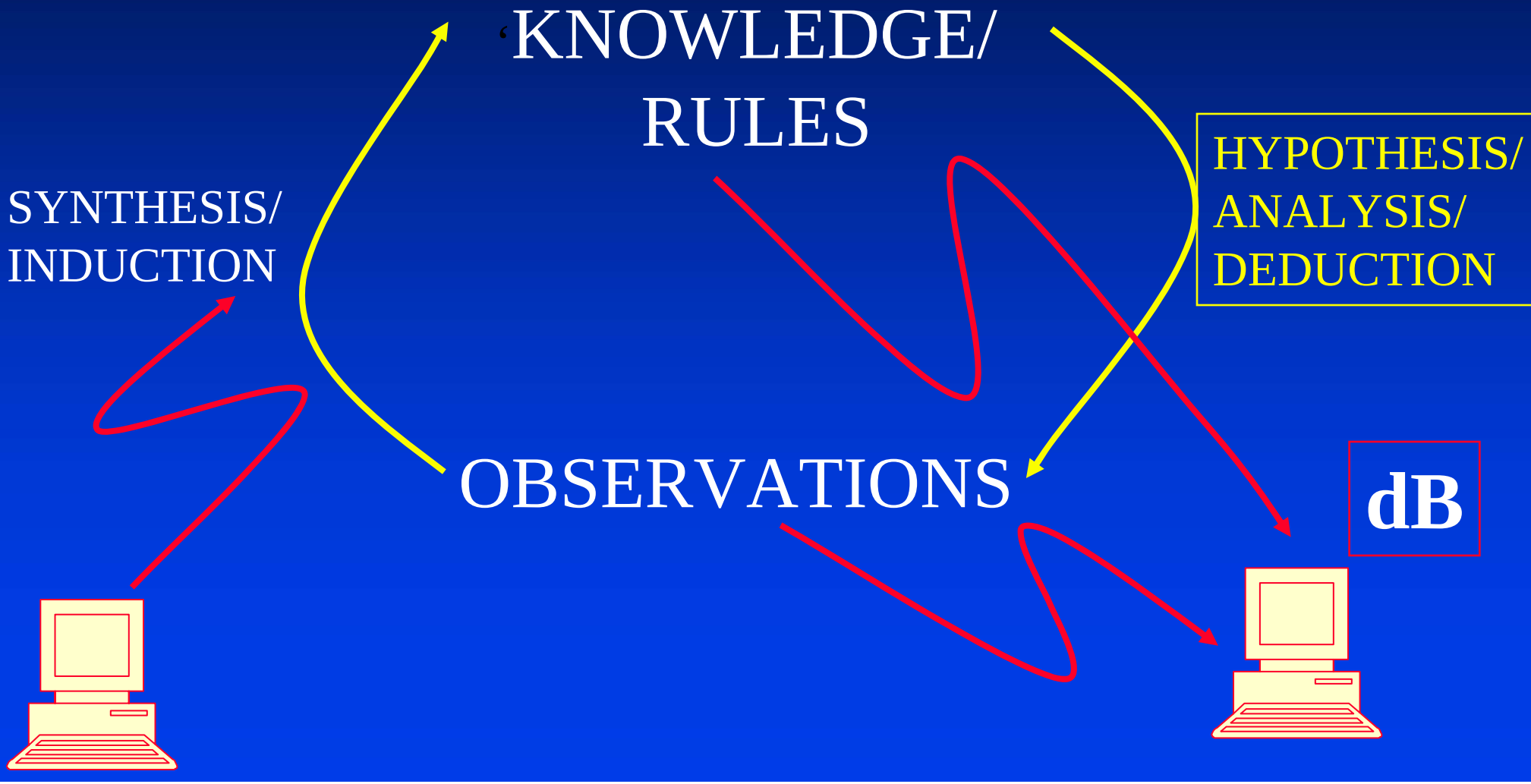
Holism/reductionism



Holism/reductionism



The cycle of knowledge

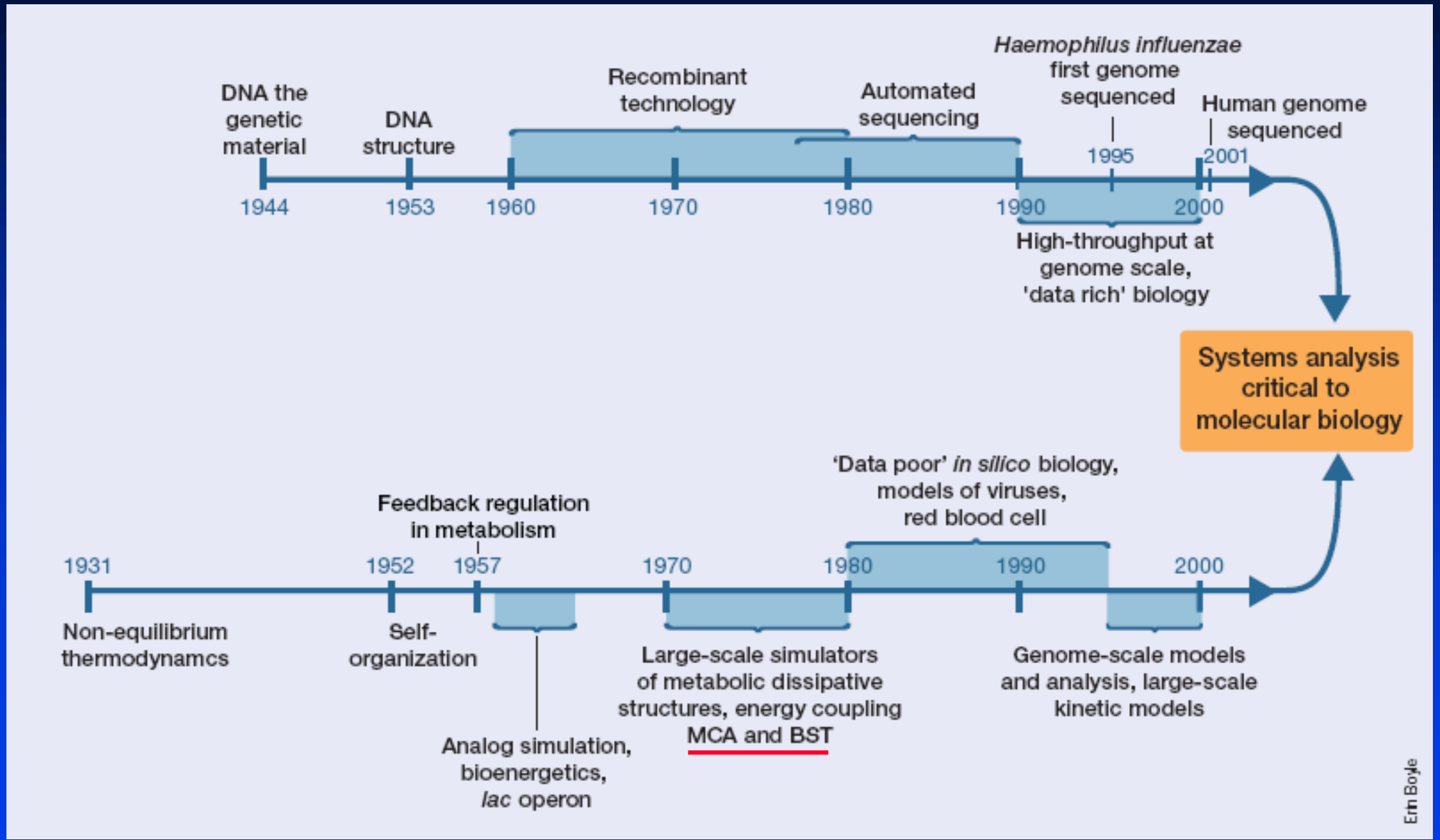


Here is the evidence, now what is the hypothesis? The complementary roles of inductive and hypothesis-driven science in the post-genomic era

Douglas B. Kell[1]* and Stephen G. Oliver[2]

BioEssays 26:99–105, © 2003 Wiley Periodicals, Inc.

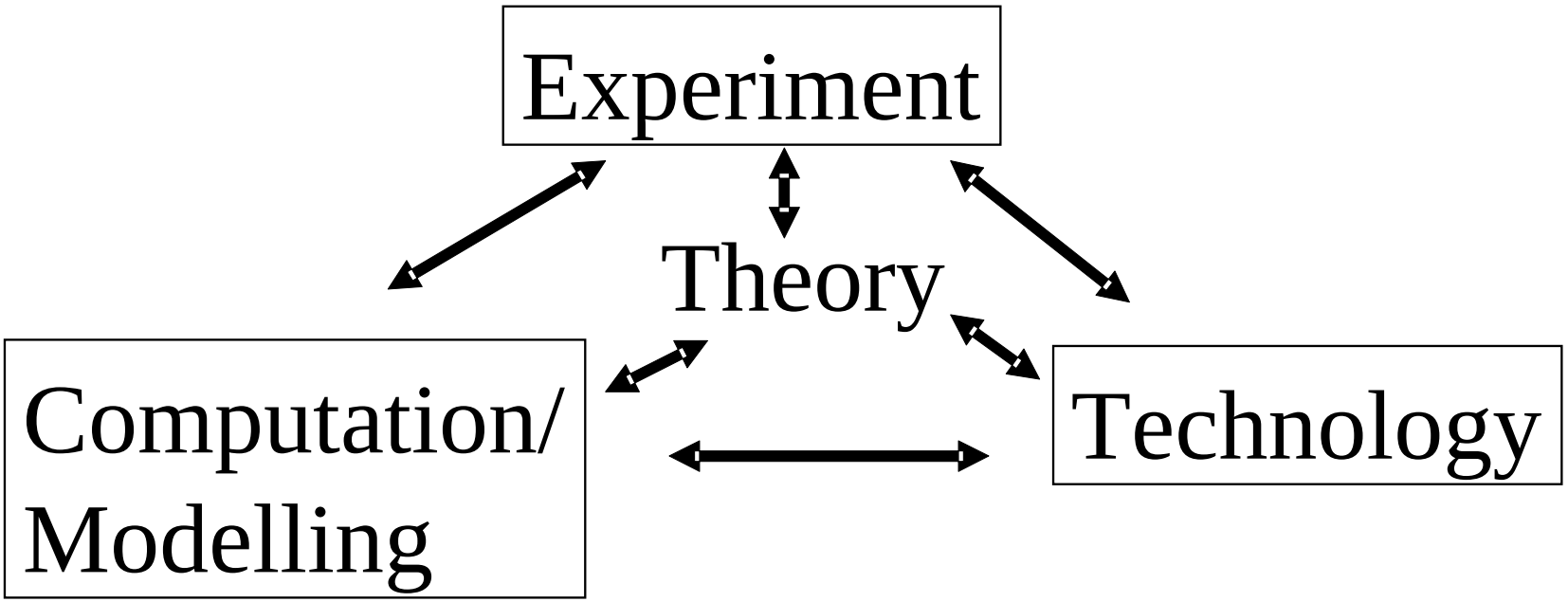
Westerhoff & Palsson NBT 22, 1249-52 (2004)



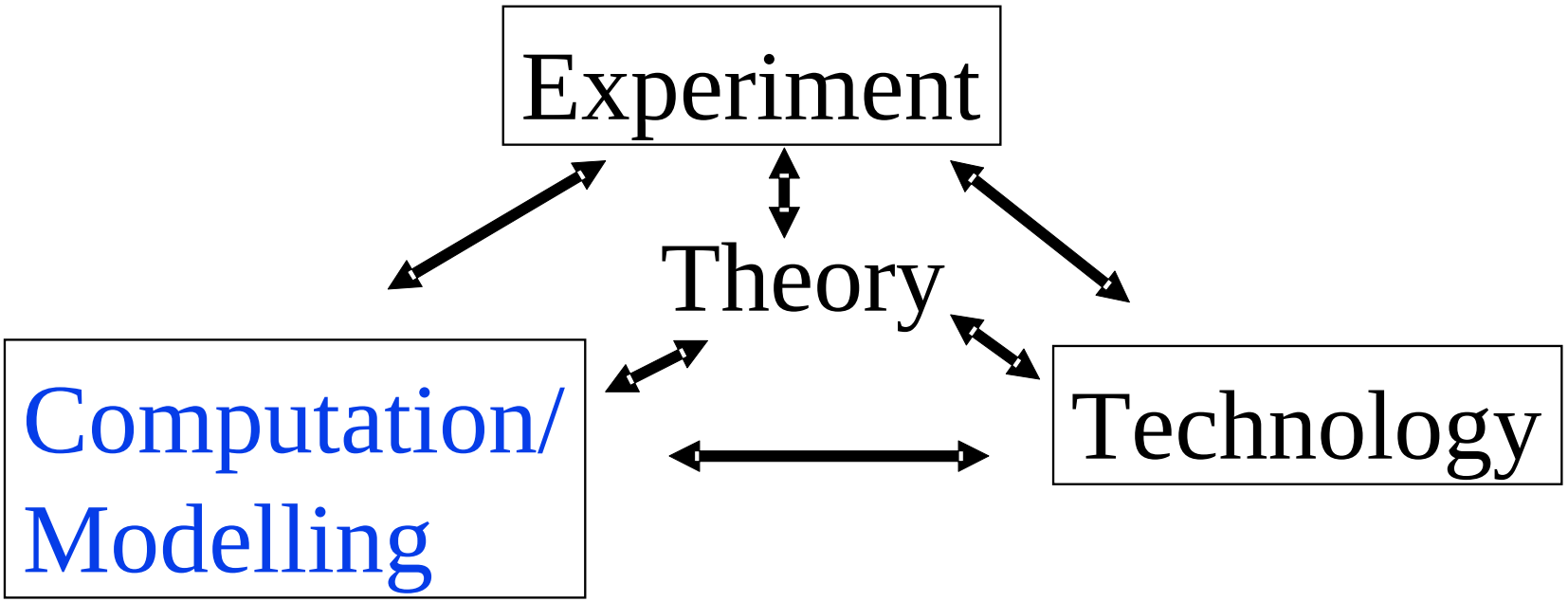
Molecular → Systems Biology

Traditional molecular biology	**The new systems biology**
Study molecules in isolation	Study systems as a whole
Qualitative	Quantitative
Reductionist	Holistic/synthetic
Largely hypothetico-deductive	Largely inductive
Little need for computation	Computation and modelling at the core
The importance of technology development is barely recognised	The importance of technology development is explicit

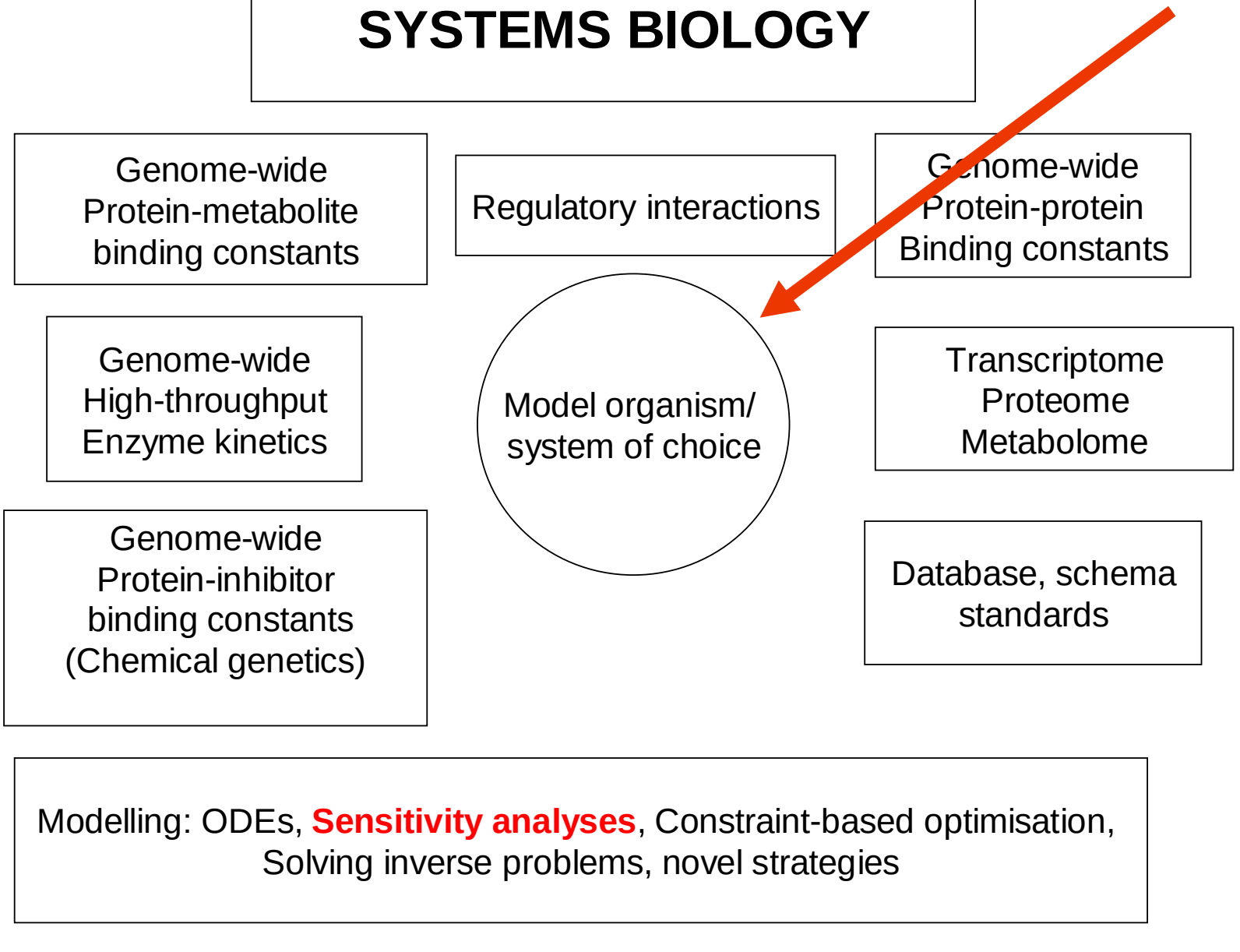
One view of systems biology



One view of systems biology



SYSTEMS BIOLOGY



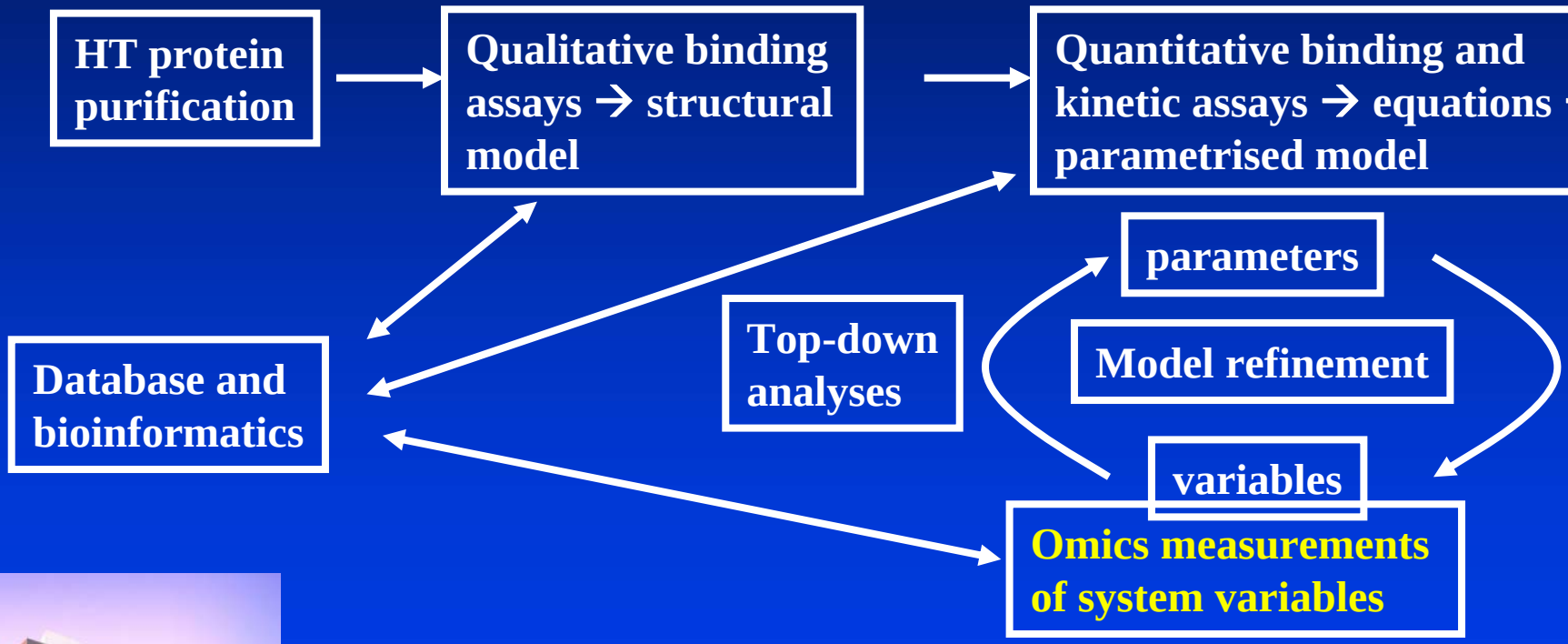
‘Bottom-up’ Systems Biology pipeline (dry)

1. Qualitative (‘structural’) model – who talks to whom as substrate, product or effector →
2. Quantitative model including ‘real’ or approximate equations describing individual steps →
3. Parametrisation of those equations →
4. Run the model and assess its most important parameters
5. Iteratively , with wet data, GOTO 1….

Systems biology experiments (including the wet side) ….

- Set up a well-defined system
- Effect systematic perturbations (genetic, environmental, chemical)
- Measure a time series of as many concentrations of variables, especially RNAs, proteins, metabolites (the ’omes) as possible
- Model the system and compare the experimental time series to those generated by the model
- Repeat iteratively (adjusting in silico parameters as needed – ‘system identification’)

Basic ‘bottom-up’-driven Systems Biology pipeline at MCISB



Bringing together metabolomics and systems biology models

Drug Discovery Today • Volume 11, Numbers 23/24 • December 2006 REVIEWS

Systems biology, metabolic modelling and metabolomics in drug discovery and development

Reviews • INFORMATICS

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[2] The Manchester Centre for Integrative Systems Biology, The Manchester Interdisciplinary Biocentre, 131, Princess St, Manchester, M1 7DN, UK

Drug Discovery Today 11, 1085-1092 (2006)

Systems biology and modelling are all about representation

The main representation for systems biology models is SBML

BIOINFORMATICS

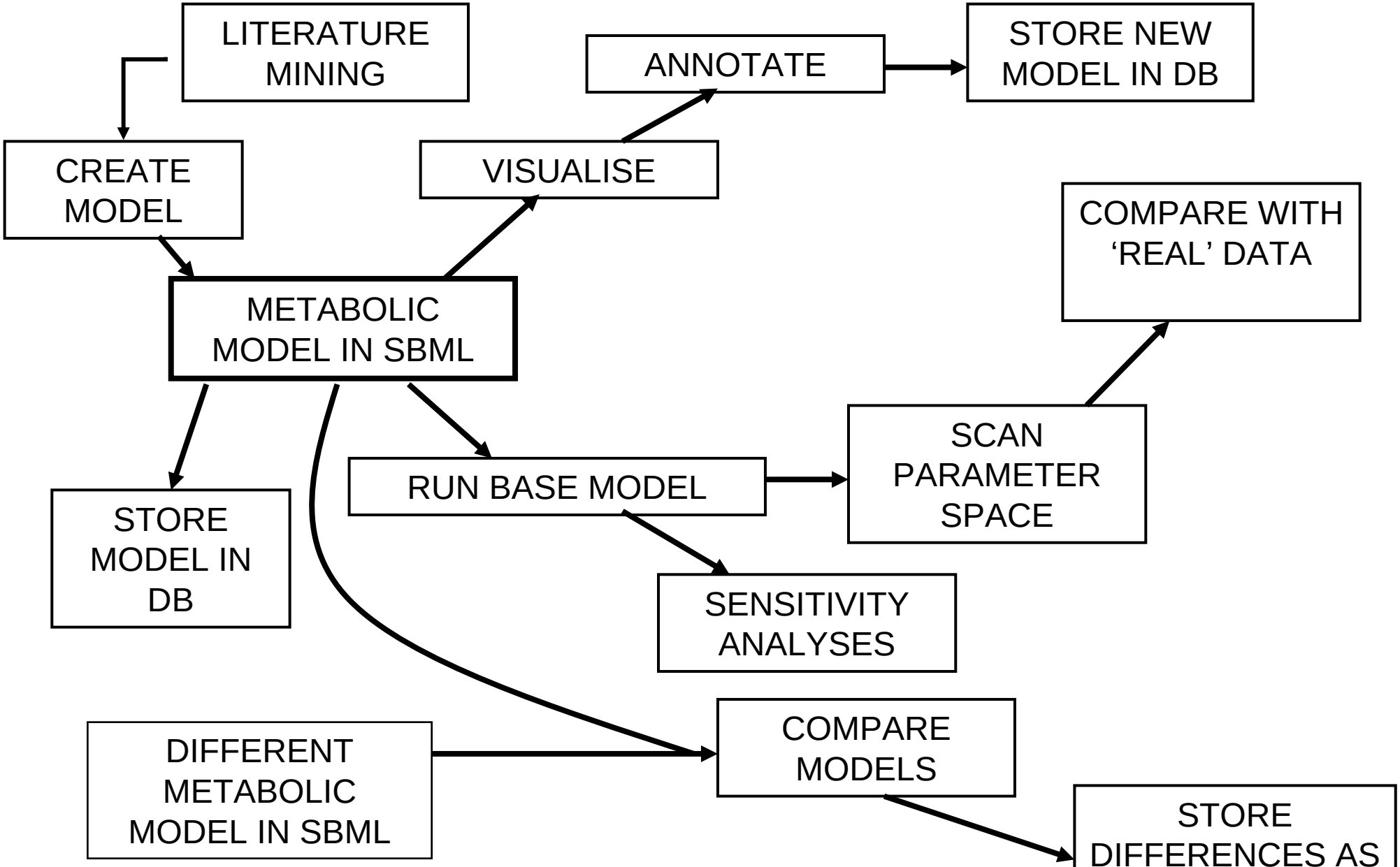
Vol. 19 no. 4 2003, pages 524–531
DOI: 10.1093/bioinformatics/btg015

The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models

M. Hucka[1, 2,], A. Finney[1, 2], H. M. Sauro[1, 2], H. Bolouri[1, 2, 3], J. C. Doyle[1], H. Kitano[1, 2, 4, 16, 18],* and the rest of the SBML Forum: *A. P. Arkin[5], B. J. Bornstein[6], D. Bray[7], A. Cornish-Bowden[8], A. A. Cuellar[9], S. Dronov[10], E. D. Gilles[11], M. Ginkel[11], V. Gor[6], I. I. Goryanin[10], W. J. Hedley[9], T. C. Hodgman[10], J.-H. Hofmeyr[12], P. J. Hunter[9], N. S. Juty[10], J. L. Kasberger[5], A. Kremling[11], U. Kummer[13], N. Le Novère[7], L. M. Loew[14], D. Lucio[14], P. Mendes[15], E. Minch[19], E. D. Mjolsness[20], Y. Nakayama[16], M. R. Nelson[17], P. F. Nielsen[9], T. Sakurada[16], J. C. Schaff[14], B. E. Shapiro[6], T. S. Shimizu[7], H. D. Spence[10], J. Stelling[11], K. Takahashi[16], M. Tomita[16], J. Wagner[14]* and *J. Wang[17]*

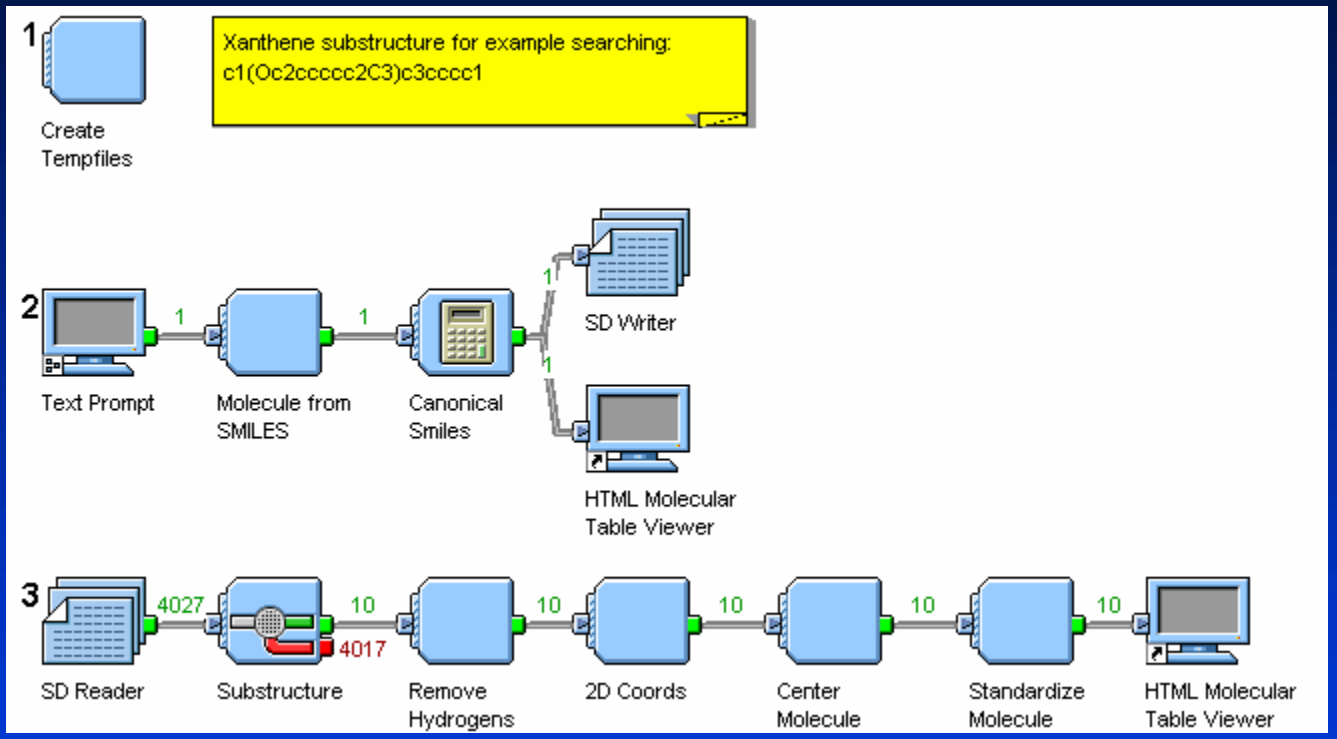
SBML Systems Biology Markup Language

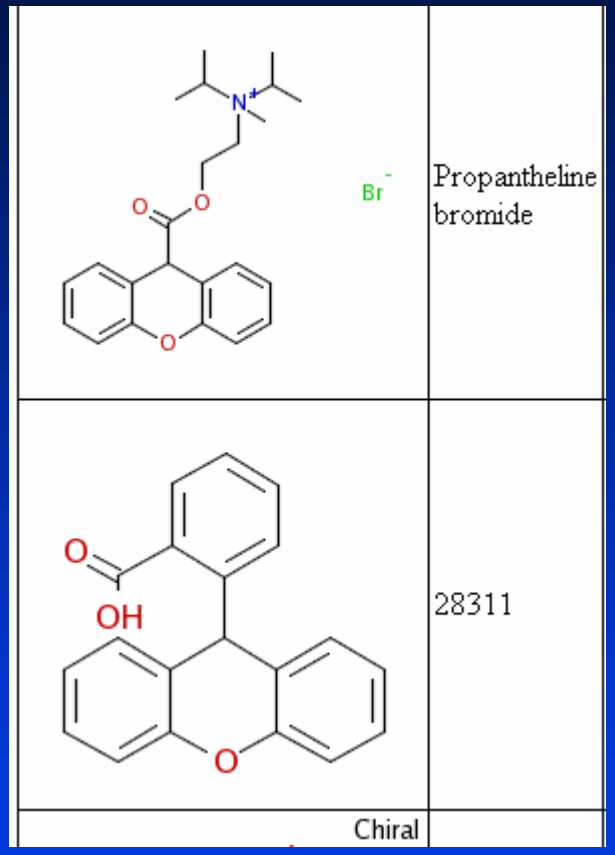
www.sbml.org



SYSTEMS BIOLOGY WORKFLOWS

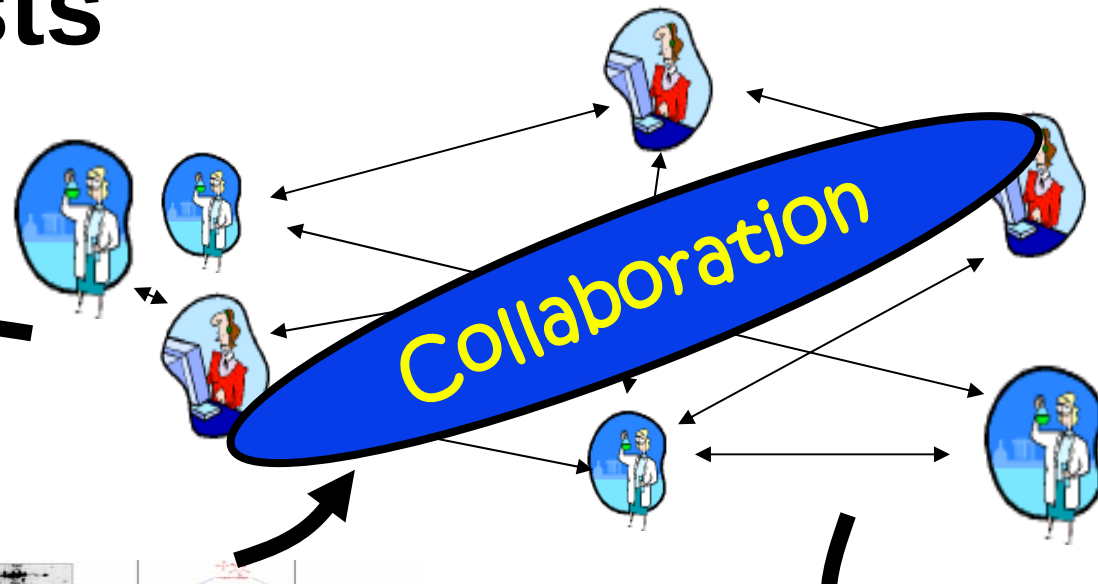
Pipeline Pilot workflow



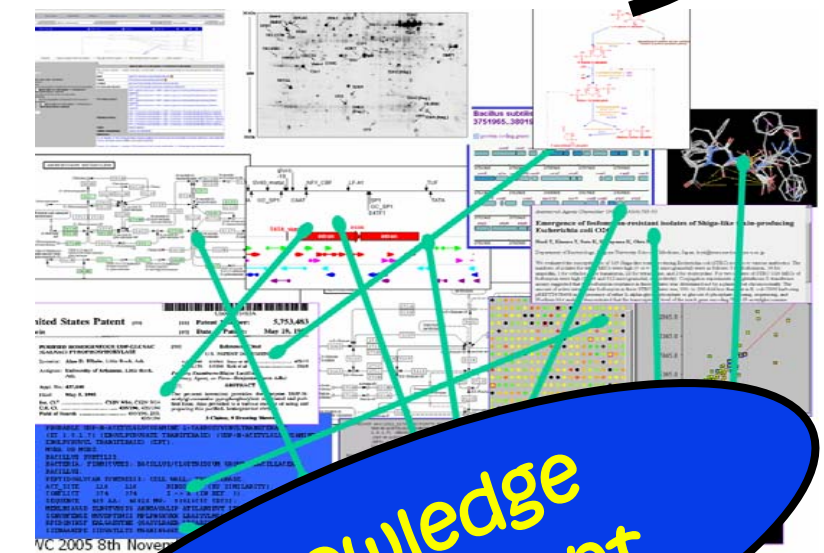


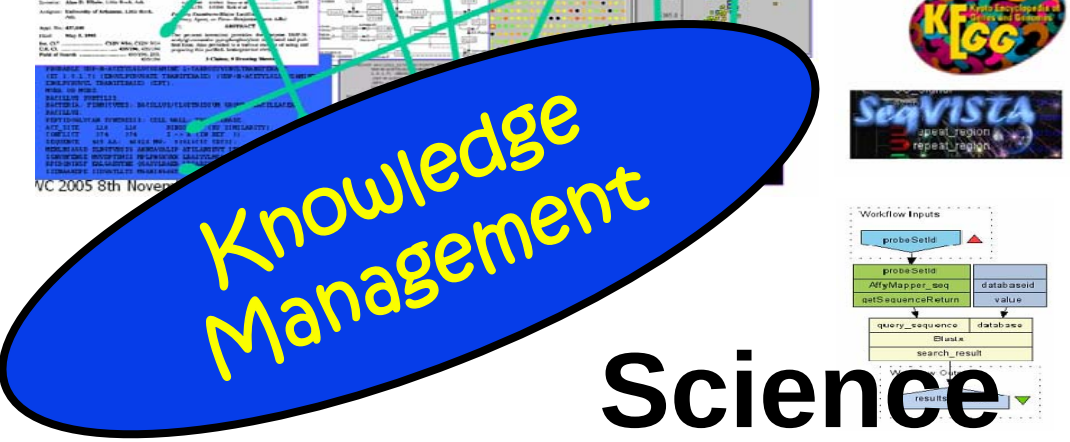
etc…

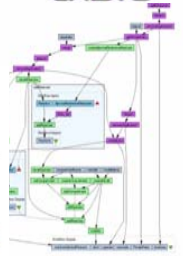
Scientists

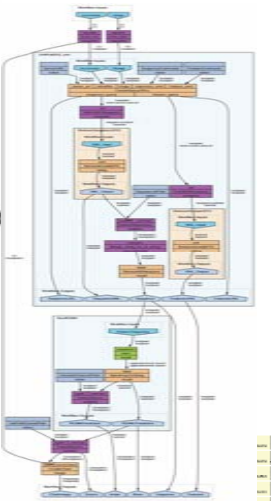


Decoupled suppliers & consumers









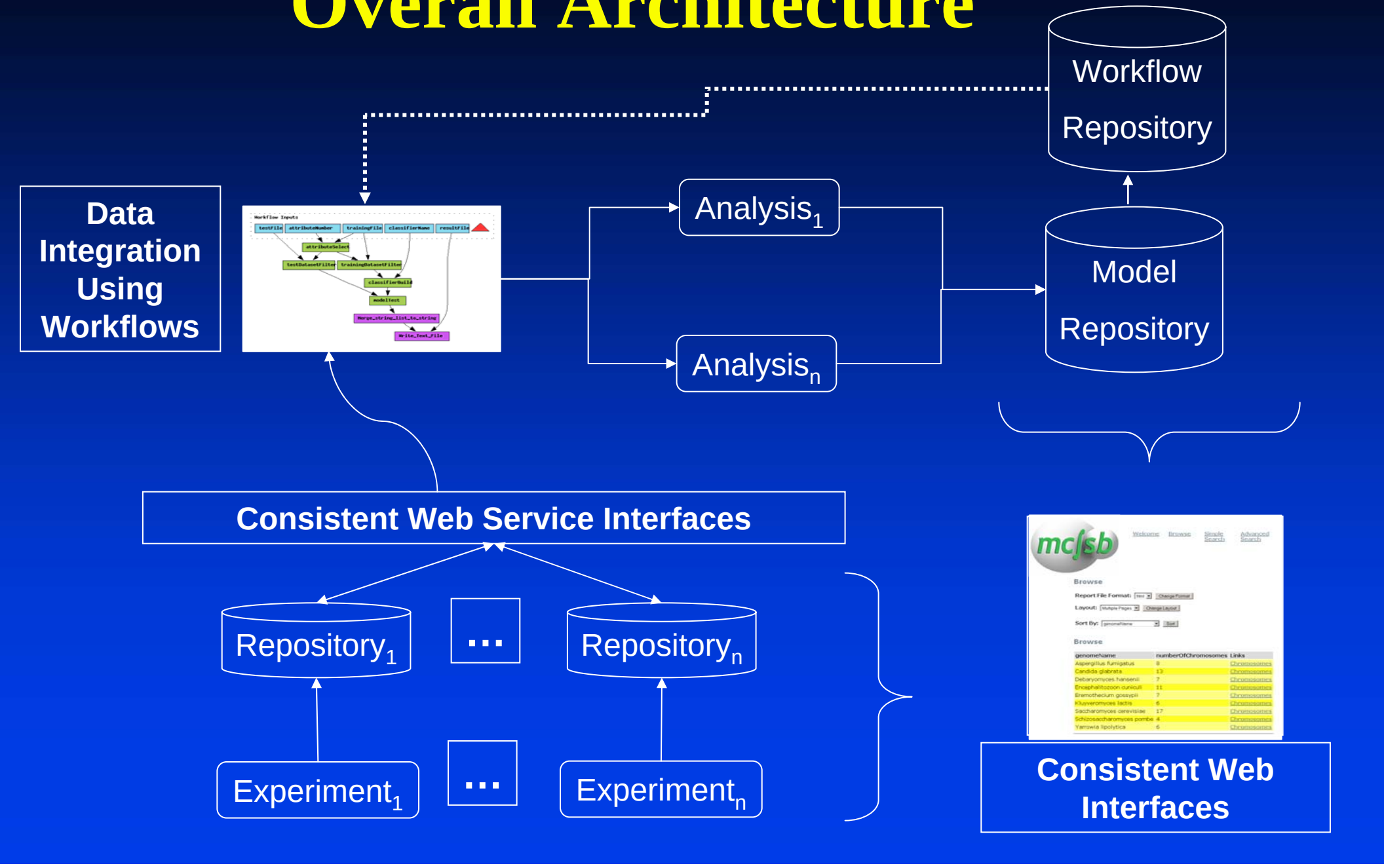
Knowledge Management

Science

‘Warehouse’ vs distributed workflows

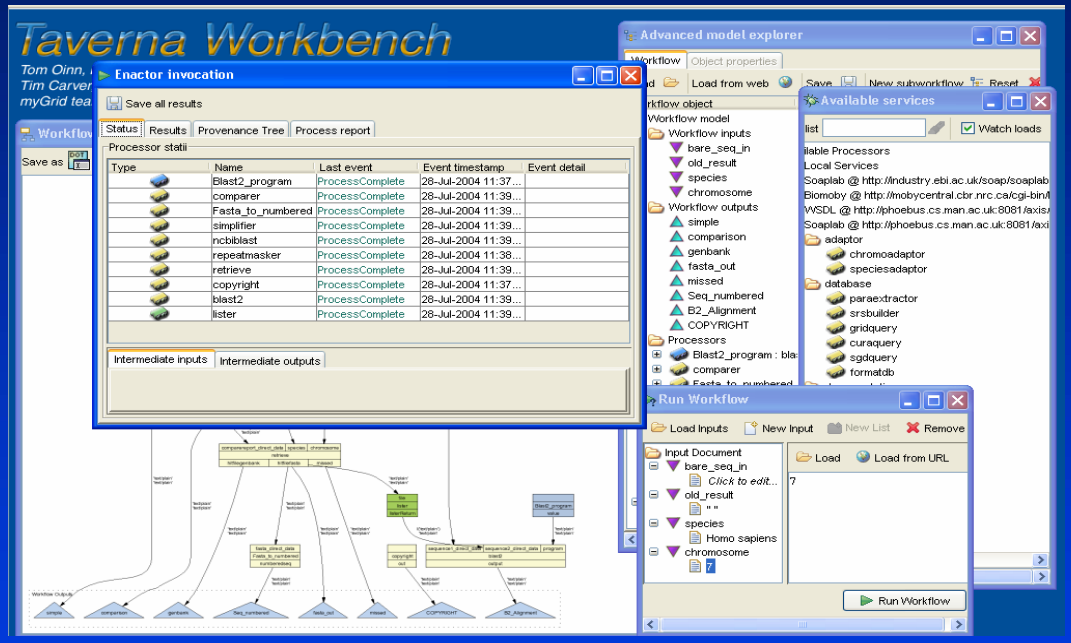
- Different ‘modules’ developed in different labs can reside on different computers anywhere, and expose themselves as Web Services
- Labs can then specialise in what they are best at
- All that is then needed is an environment for enacting bioinformatic workflows by coupling together these service-oriented architectures
- One such is Taverna
- This is arguably the best way to combine metabolomic SBML models with metabolomic data, and is what we plan to do at MCISB

Overall Architecture



Taverna Workflow Environment

- Workflow environment for authoring scientific workflows.
- Developed by myGrid e-Science Pilot project.
- Downloads: over 1000 a month during 2006.



http://taverna.sourceforge.net/

Taverna (sits on myGrid)
www.mygrid.org.uk
www.taverna.sf.net

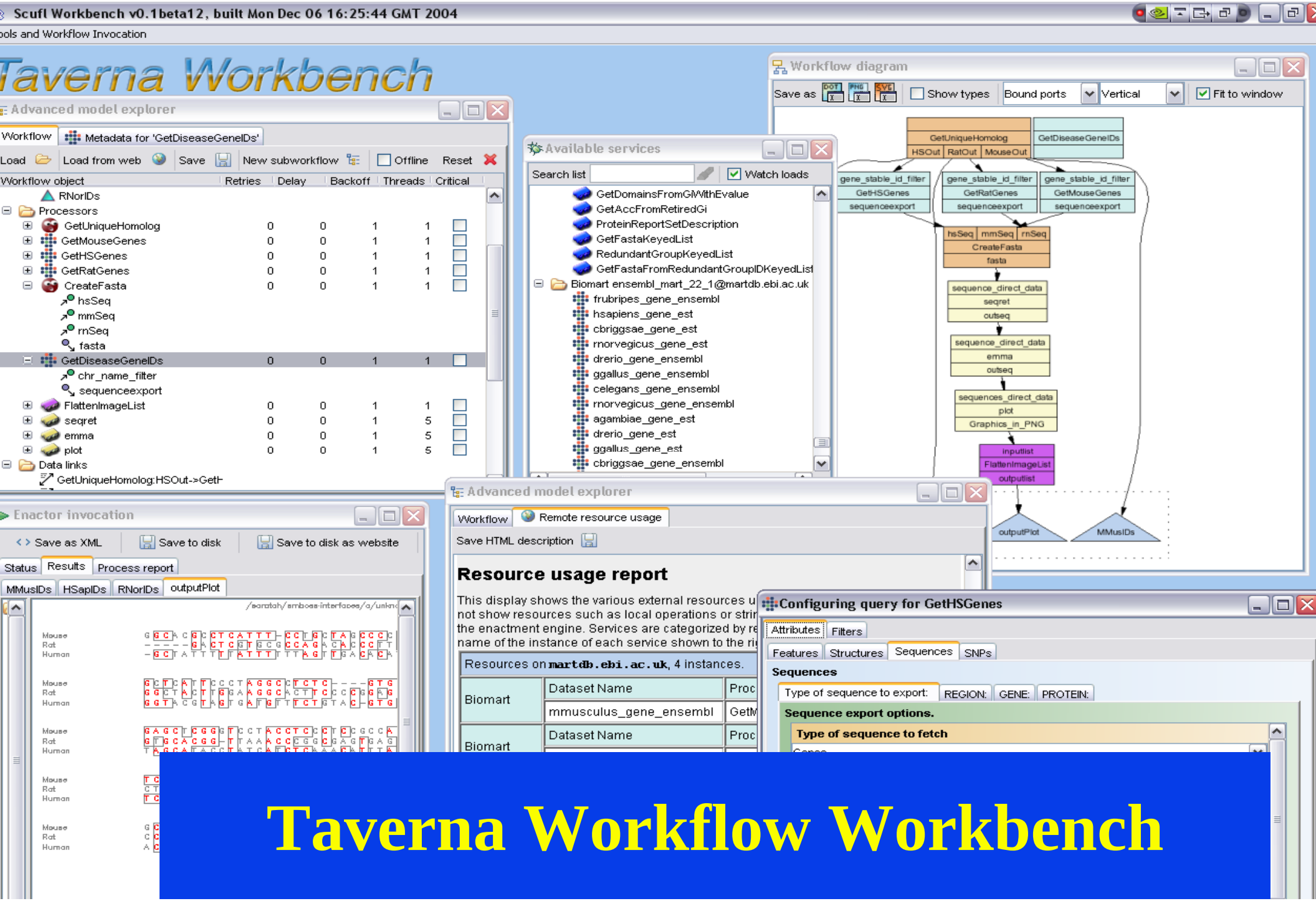
BIOINFORMATICS

Vol. 20 no. 17 2004, pages 3045–3054
doi:10.1093/bioinformatics/bth361

Taverna: a tool for the composition and enactment of bioinformatics workflows

Tom Oinn[1], Matthew Addis[2], Justin Ferris[2], Darren Marvin[2], Martin Senger[1], Mark Greenwood[3], Tim Carver[4], Kevin Glover[5], Matthew R. Pocock[6], Anil Wipat[6] and Peter Li[6,]*

myExperiment.org



Taverna Workflow Workbench

Key issues and strategic benefits

- Easy to find workflows (Feta/Find-o-matic semantic discovery engines)
- Easy to reuse and edit workflows
- Easy to share workflows (myExperiment)
- Talks directly to Utopia data analysis and visualisation engine
- Easy to configure for and extend to systems biology simply by wrapping the tools and data sources as Web Services – preferably with proper semantic annotation in WSDL
- Usability for biologists vs bioinformaticians….

Now for some sensitivity analysis…

- The NFκB system

NFκB (1)

- NF-κB is a nuclear transcription factor that can modify the expression of many (200-300…) other genes
- It is held inactive in the cytoplasm of non-stimulated cell by three IκB isoforms.
- It is widely and diversely implicated in cancer, apoptosis and in diseases such as arthritis

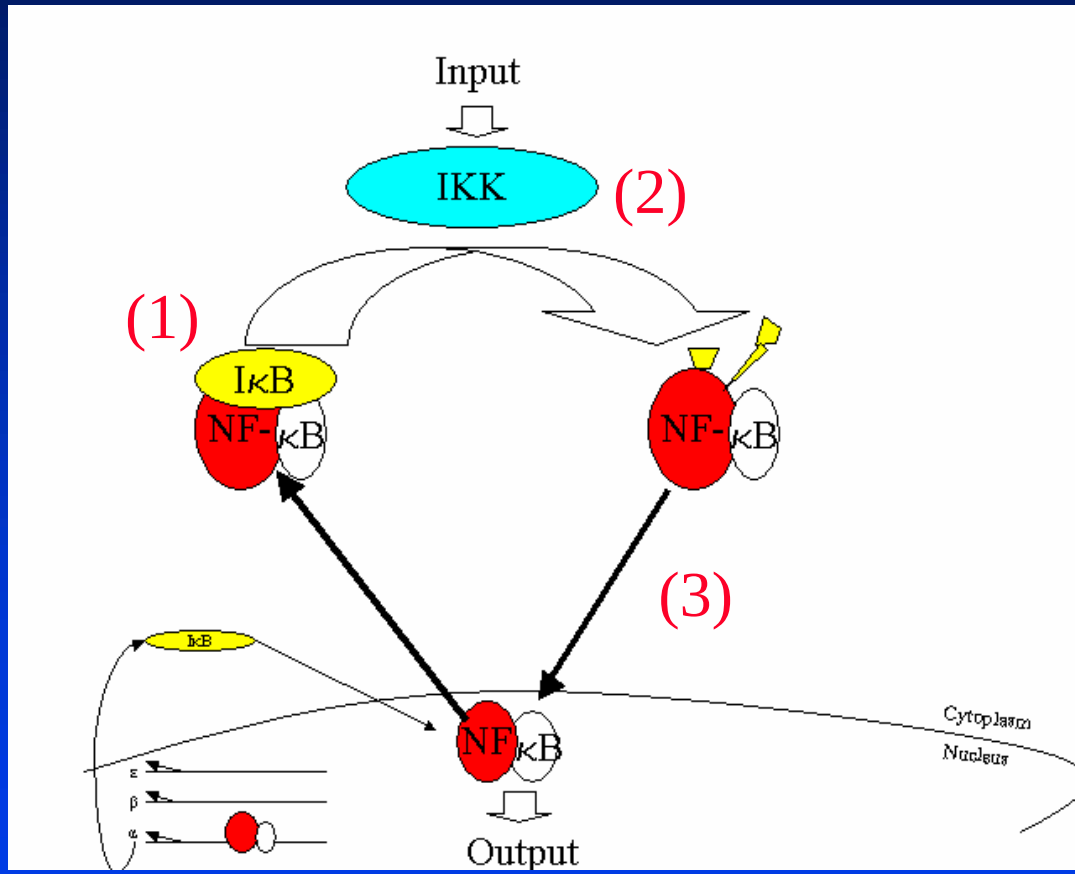
Question 1: so what is a good drug target in the NFκB pathway?
Question 2: and how do we measure that?

The big question…
(aka the ‘crosstalk problem’)

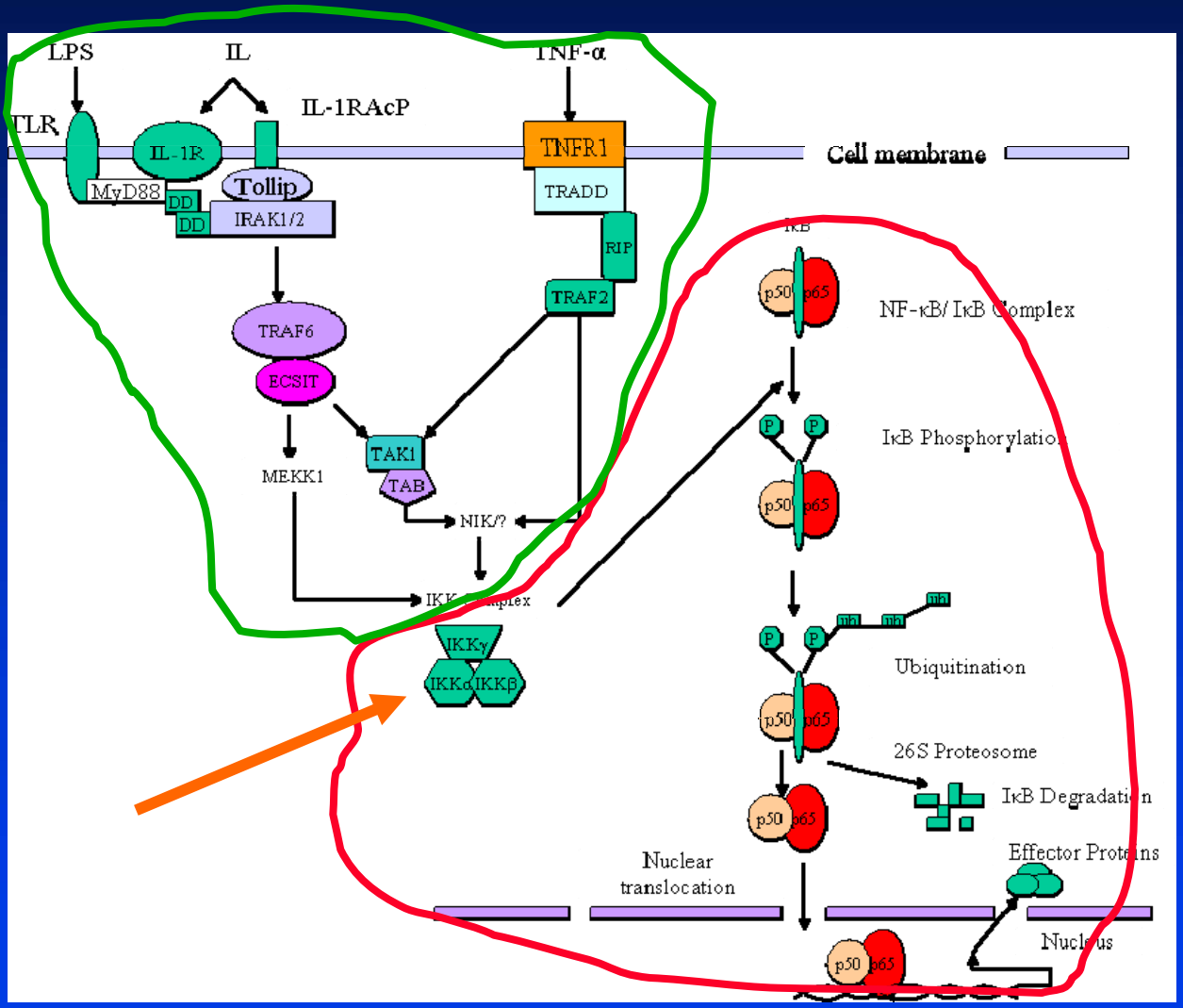
How can the same thing (i.e. NF-κB) – it is assumed by changes in its concentration in the nucleus – be ‘involved’ both in cell proliferation in cancer and in apoptotic cell death (two processes that are pretty well opposite in character)?!

Summary of NF-κB – 3 steps

1. NF-κB is a nuclear transcription factor and is held inactive in the cytoplasm of non-stimulated cell by three IκB isoforms
2. During cell stimulation, the IKK complex is activated, leading to phosphorylation and ubiquitination (and removal) of the IκB proteins.
3. Free NF-κB translocates to the Nucleus, activating genes including IκBα. IκBβ& -ε are synthesised at a steady rate, allowing for complex temporal control of NF-κB activation involving negative feedback



Many effectors (e.g. TNFα) can activate IKK



Hoffman *et al* (2002) produced a reduced model for cells lacking two IκB isoforms (IκBβ and IκBε)

The IκB–NF-κB Signaling Module: Temporal Control and Selective Gene Activation

Alexander Hoffmann,[1]* Andre Levchenko,[2]* Martin L. Scott,[3]† David Baltimore[1]‡

Nuclear localization of the transcriptional activator NF-κB (nuclear factor κB) is controlled in mammalian cells by three isoforms of NF-κB inhibitor protein: IκBα, -β, and -ε. Based on simplifying reductions of the IκB–NF-κB signaling module in knockout cell lines, we present a computational model that describes the temporal control of NF-κB activation by the coordinated degradation and synthesis of IκB proteins. The model demonstrates that IκBα is responsible for strong negative feedback that allows for a fast turn-off of the NF-κB response, whereas IκBβ and -ε function to reduce the system's oscillatory potential and stabilize NF-κB responses during longer stimulations. Bimodal signal-processing characteristics with respect to stimulus duration are revealed by the model and are shown to generate specificity in gene expression.

Hoffman et al used the modelling system *Gepasi* written by Pedro Mendes

BIOINFORMATICS

Vol. 14 no. 10 1998
Pages 869–883

Non-linear optimization of biochemical pathways: applications to metabolic engineering and parameter estimation

Pedro Mendes and Douglas B. Kell

Institute of Biological Sciences, University of Wales Aberystwyth, Aberystwyth, Ceredigion SY23 3DD, UK

Received on July 27, 1998; revised on August 31, 1998; accepted on September 4, 1998

BIOINFORMATICS **APPLICATIONS NOTE**

Vol. 17 no. 3 2001
Pages 288–289

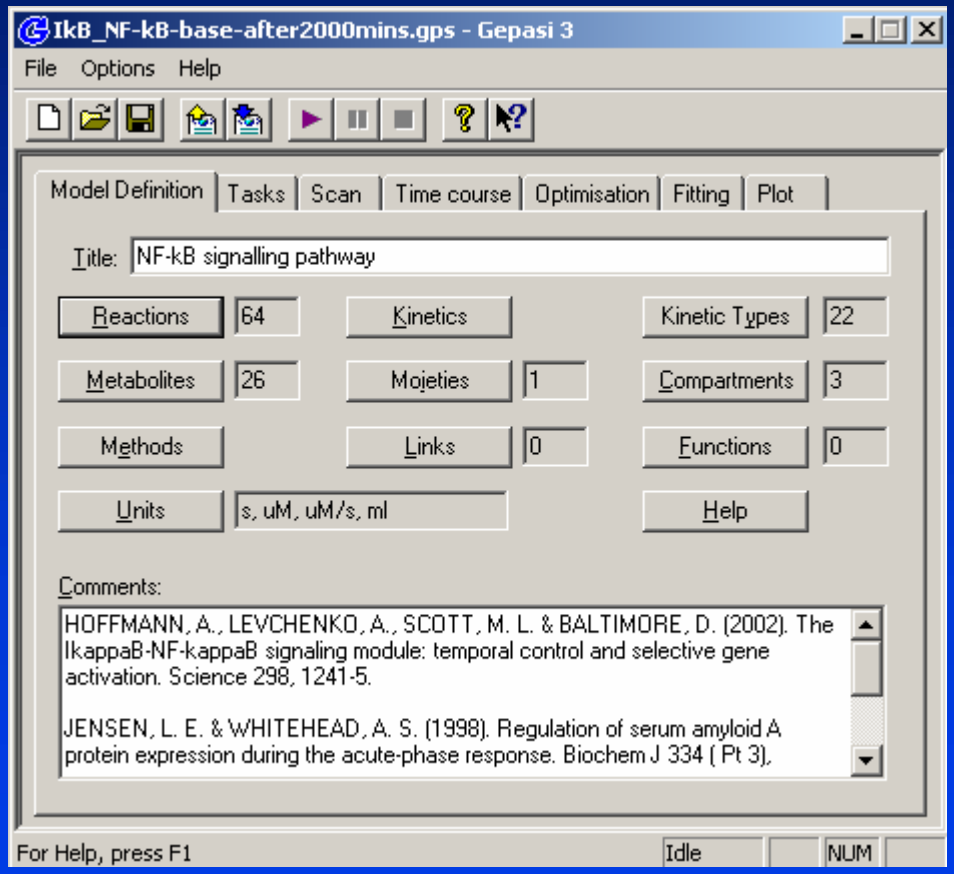
MEG (Model Extender for Gepasi*): a program for the modelling of complex, heterogeneous, cellular systems*

Pedro Mendes[1,*] *and Douglas B. Kell*[1]

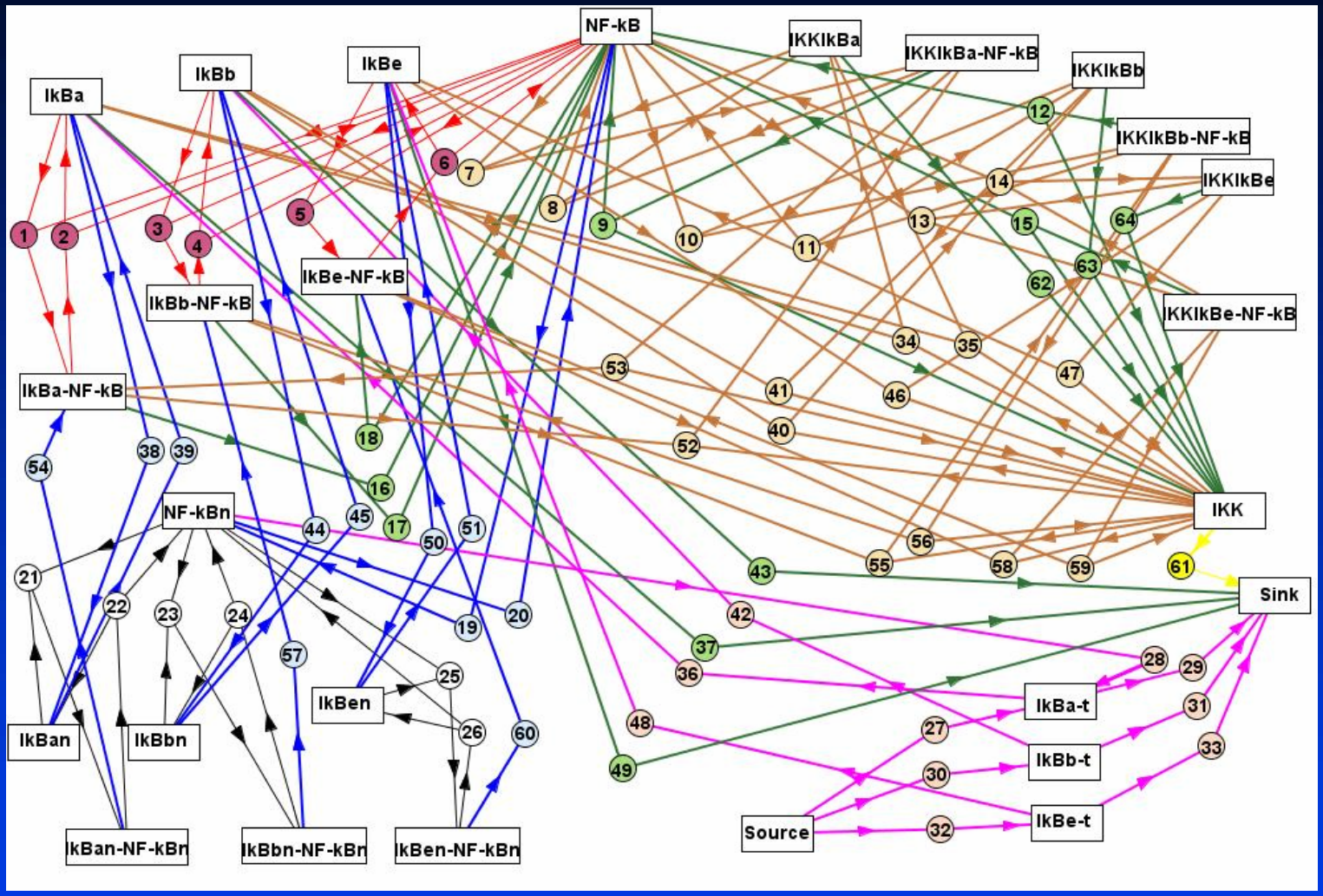
[1]*Institute of Biological Sciences, University of Wales, Aberystwyth SY23 3DD, UK*

Received on July 6, 2000; revised on September 19, 2000; accepted on October 6, 2000

We have reproduced this model (modified to remove mistakes in the original publication, now corrected) using Gepasi

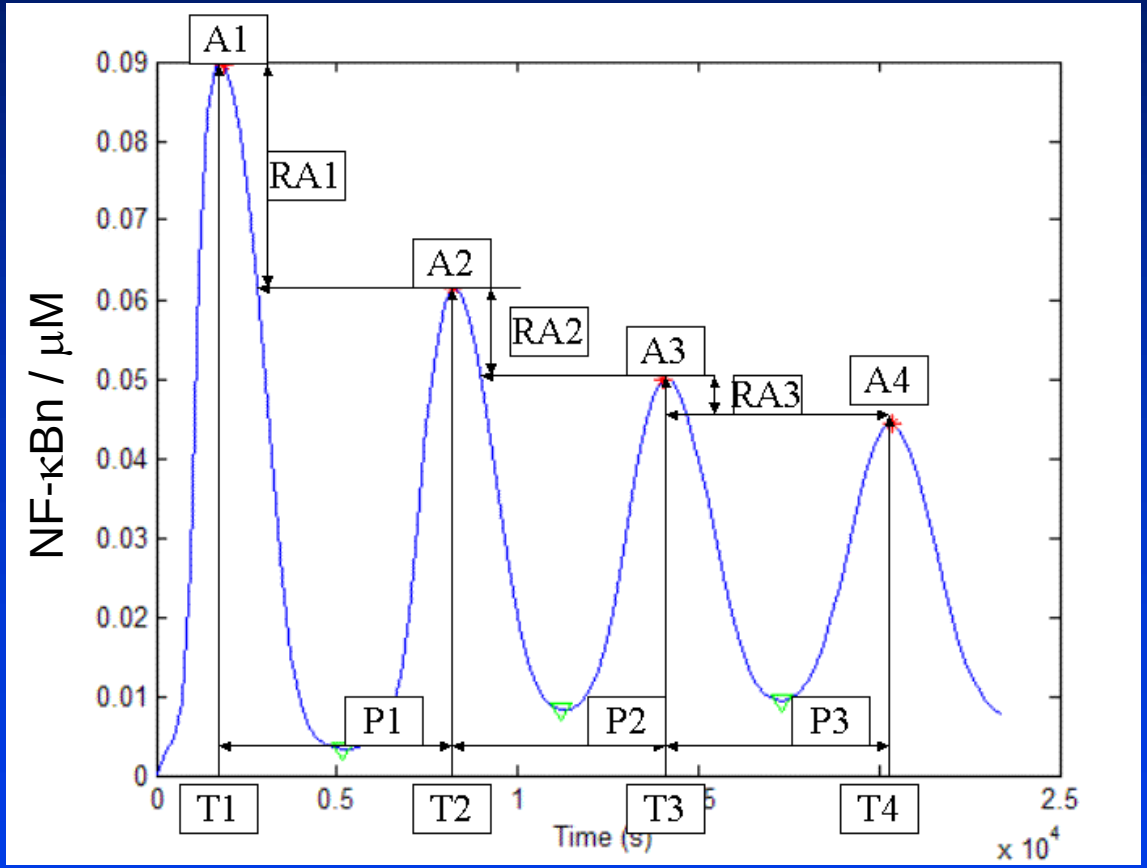


The model has 64 unidirectional reactions & 26 variables

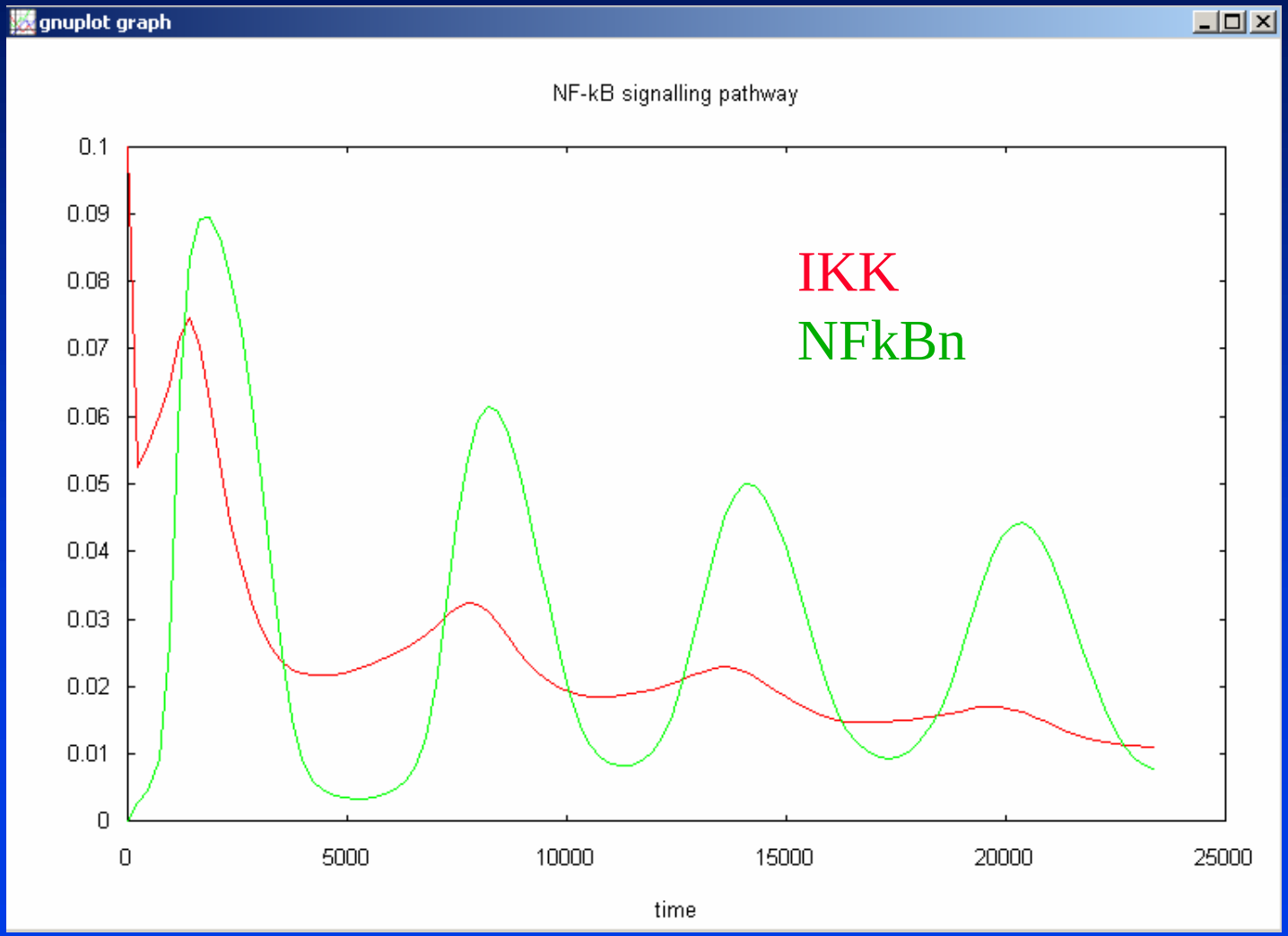


Violet red circles = IκB-NF-κB cytoplasmic reactions; Blue Arrows and circles = Nuclear Transport; Magenta Arrows and Pink circles = IκB mRNA synthesis (including transcription, translation and degradation); Black Arrows and white circles = IκB-NF-κB nuclear reactions; Light Green Arrows and circles = IκB Phosphorylation and Degradation reactions; Brown Arrows and brown circles = Bimolecular IKK- IκB and tri-molecular IKK- IκB-NF-κB; Yellow Arrows and circles = IKK slow adaptation coefficient

Cartoon of nuclear NF-κB after IKK addition



After pre-equilibration for 2000s, IKK is ‘added’ at 0.1 μM



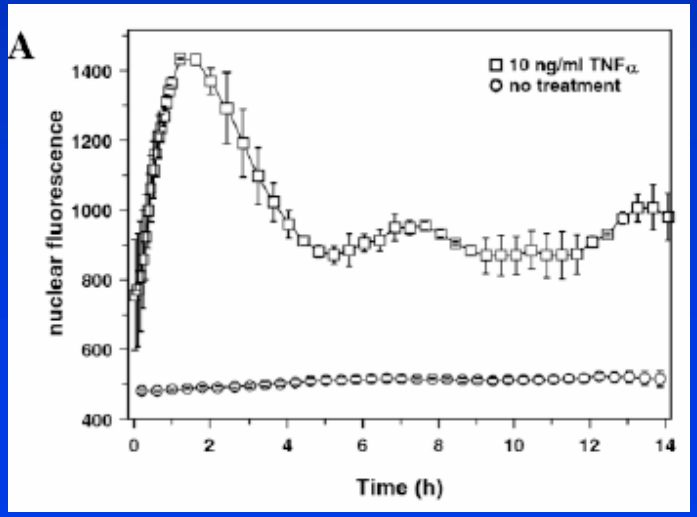
“Real” oscillations of GFP-NFκBn observed microscopically (and averaged)

Multi-parameter analysis of the kinetics of NF-κB signalling and transcription in single living cells

Glyn Nelson[1], Luminita Paraoan[1], David G. Spiller[1], Geraint J. C. Wilde[1], Mark A. Browne[3], Peter K. Djali[1,3], John F. Unitt[2], Elaine Sullivan[2], Eike Floettmann[2] and Michael R. H. White[1,*]

[1]School of Biological Sciences, University of Liverpool, Crown Street, Liverpool, L69 7ZB, UK
[2]AstraZeneca R&D Charnwood, Molecular Biology, Bakewell Road, Loughborough, Leicestershire, LE11 5RH, UK
[3]Kinetic Imaging Ltd, 2 Brunel Road, Wirral, CH62 3NY, UK
*Author for correspondence (e-mail: mwhite@liv.ac.uk)

Accepted 13 December 2001
Journal of Cell Science 115, 1137-1148 (2002) © The Company of Biologists Ltd



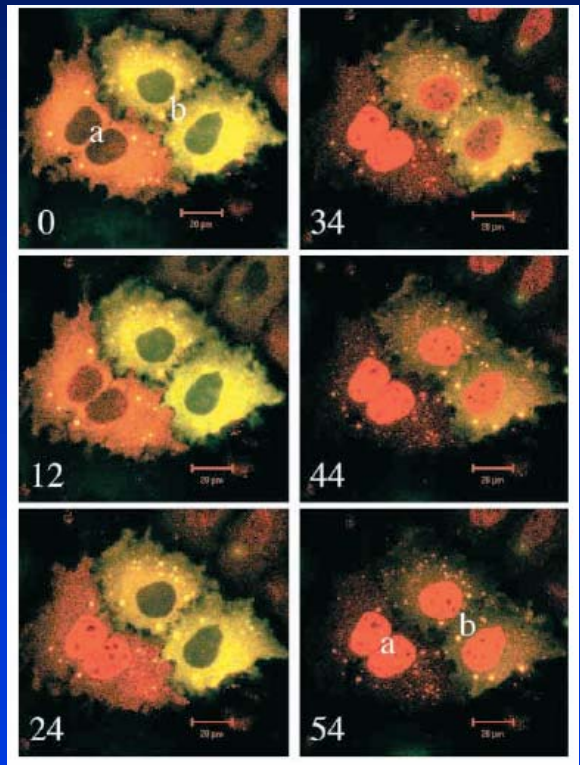
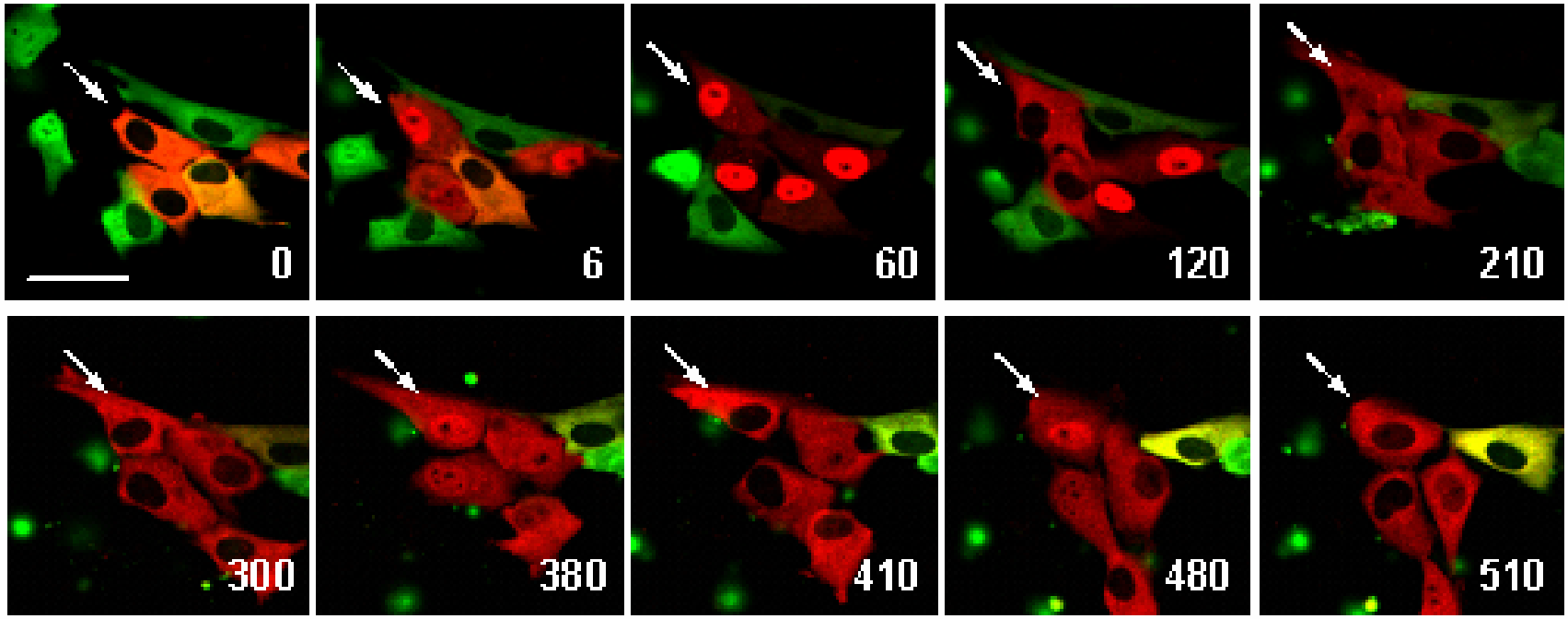


Fig. 4. Confocal microscopy of p65-dsRed and IκBα-EGFP in living cells. Time series images of IκBα-EGFP and p65-dsRed fluorescence at stated times (in minutes) after addition of 10 ng/ml TNFα. Green and red fluorescence were recorded as separate images and then merged for visualisation. Green IκBα-EGFP and red p65-dsRed co-localisation are represented as yellow in the presence of higher IκBα-EGFP:p65-dsRed ratios, and orange in the presence of higher p65-dsRed:IκBα-EGFP ratios.

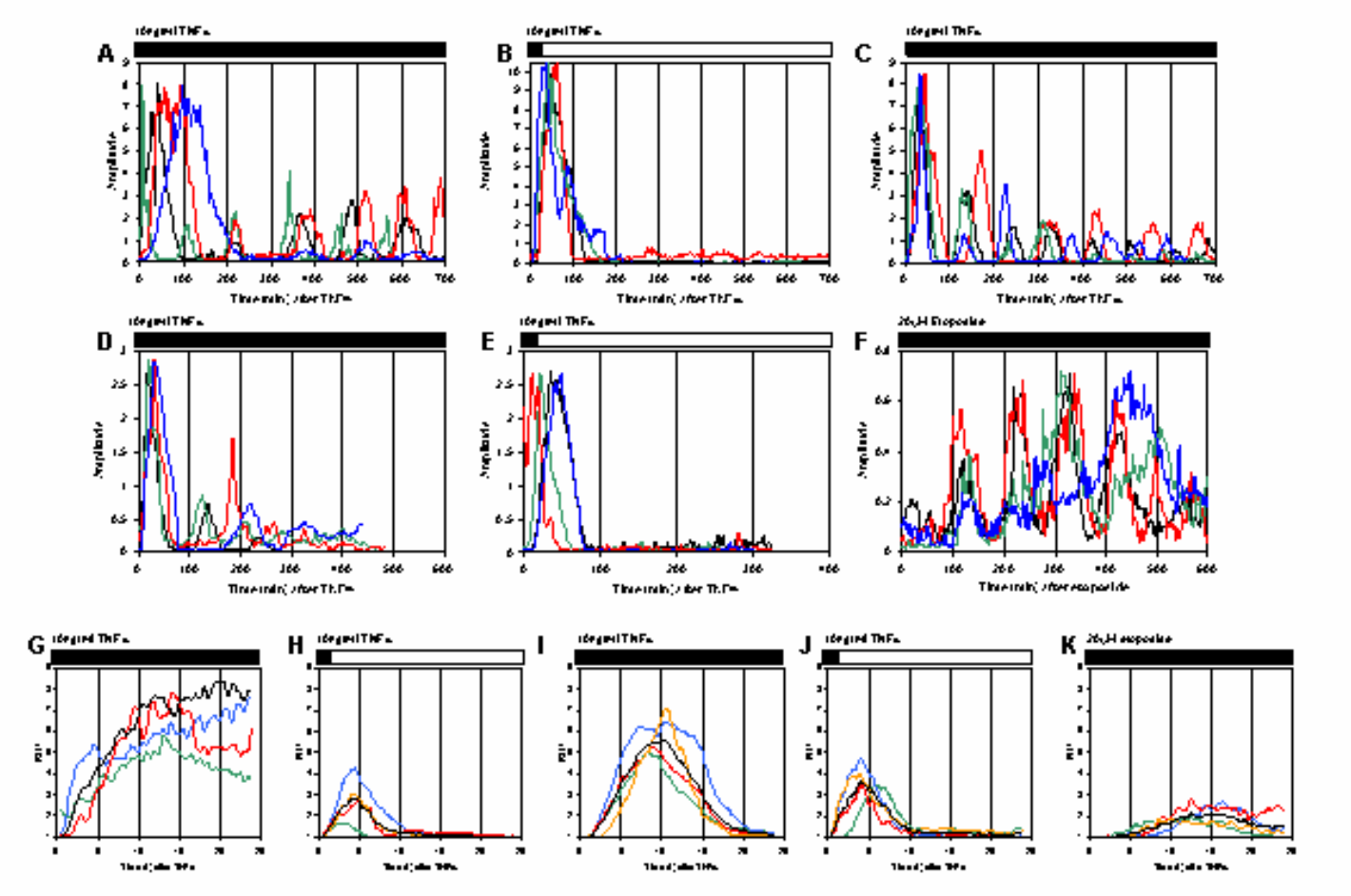
“Real” oscillations of GFP-NFκBn observed microscopically with labelled IκBα and NFκB



Nelson et al, Science 2004

NB we measure individual cells, not ensembles

The timing and amount of oscillations depend strongly on the type of stimulation (various amounts and times of TNFα, different individual cells)



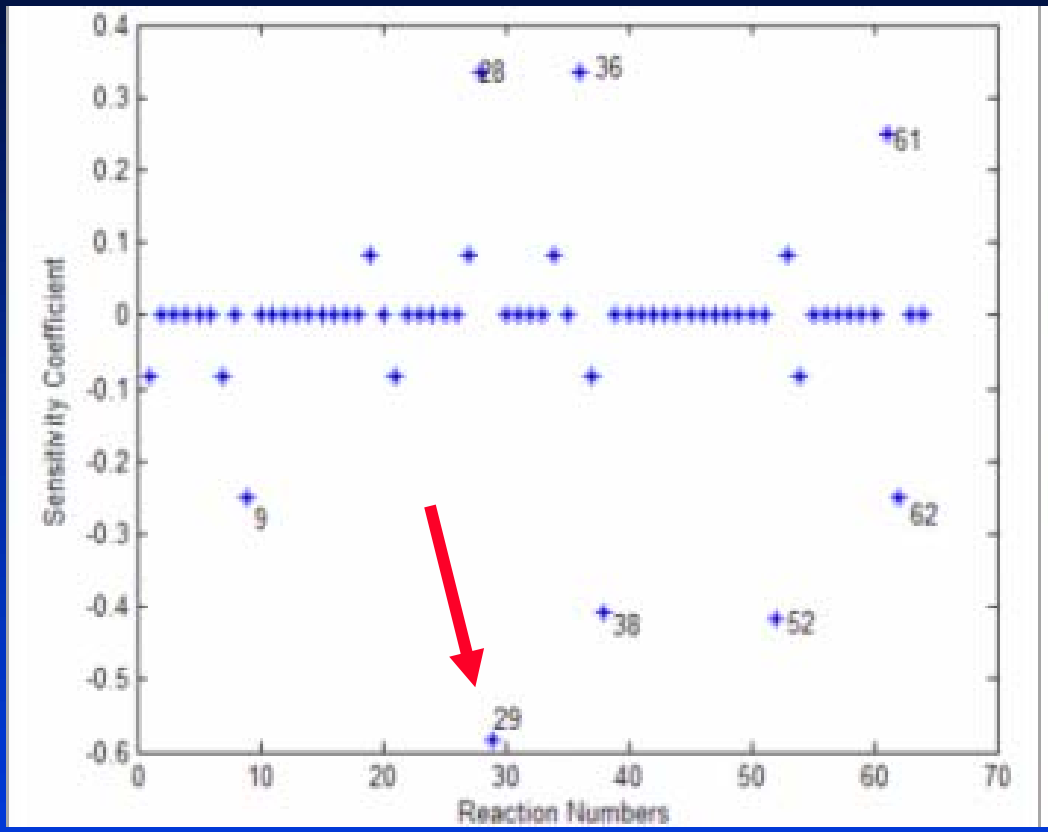
Nelson et al
Science 2004

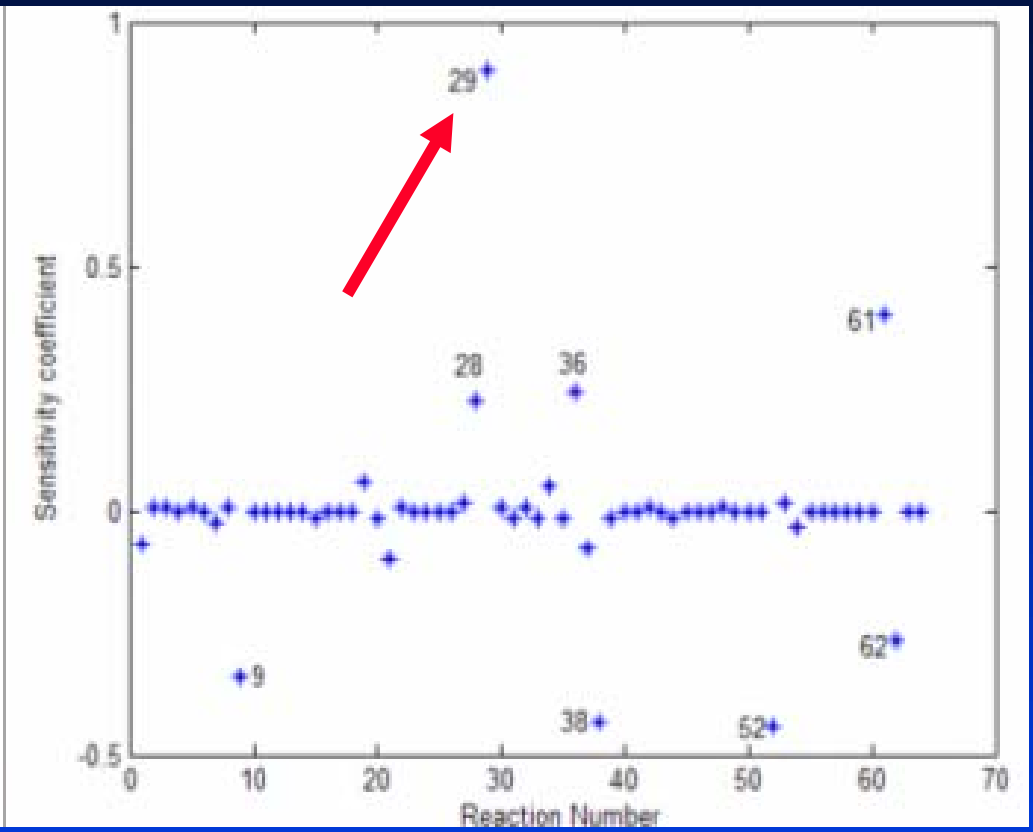
What about the model? Sensitivity analysis

$$S_P^M = \frac{\delta M / M}{\delta P / P}$$

- A generalised form of the control coefficients of MCA
- Dimensionless
- Describe quantitatively which reactions are most ‘important’
- In favourable cases (especially steady states) there are summation theorems
- We here discuss <u>local</u> sensitivity analyses

Sensitivity coefficients of T3 for δP of 10% or 100%





- Only 8 reactions have significant sensitivity coefficients when T3 is measured
- Note the change in sign for reaction 29 – very nonlinear system

9 important reactions

9: IKKIκBα-NF-κB catalytic rate constant
28: IκBα (IκBα-t) Inducible mRNA synthesis rate constant
29: IκBα (IκBα-t) mRNA degradation rate constant
34 : IKKIκBα association rate constant
36: Constitutive IκBα translation rate constant
38: IκBαn nuclear Import Rate constant
52: IKKIκBα-NF-κB association rate constant
61: IKK signal onset slow adaptation coefficient
62: IKKIκBα catalysis rate constant

What do they have in common?

They all involve free IKK and/or IκBα

9: IKKIκBα-NF-κB catalytic rate constant

28: IκBα (IκBα-t) Inducible mRNA synthesis rate constant

29: IκBα (IκBα-t) mRNA degradation rate constant

34 : IKKIκBα association rate constant

36: Constitutive IκBα translation rate constant

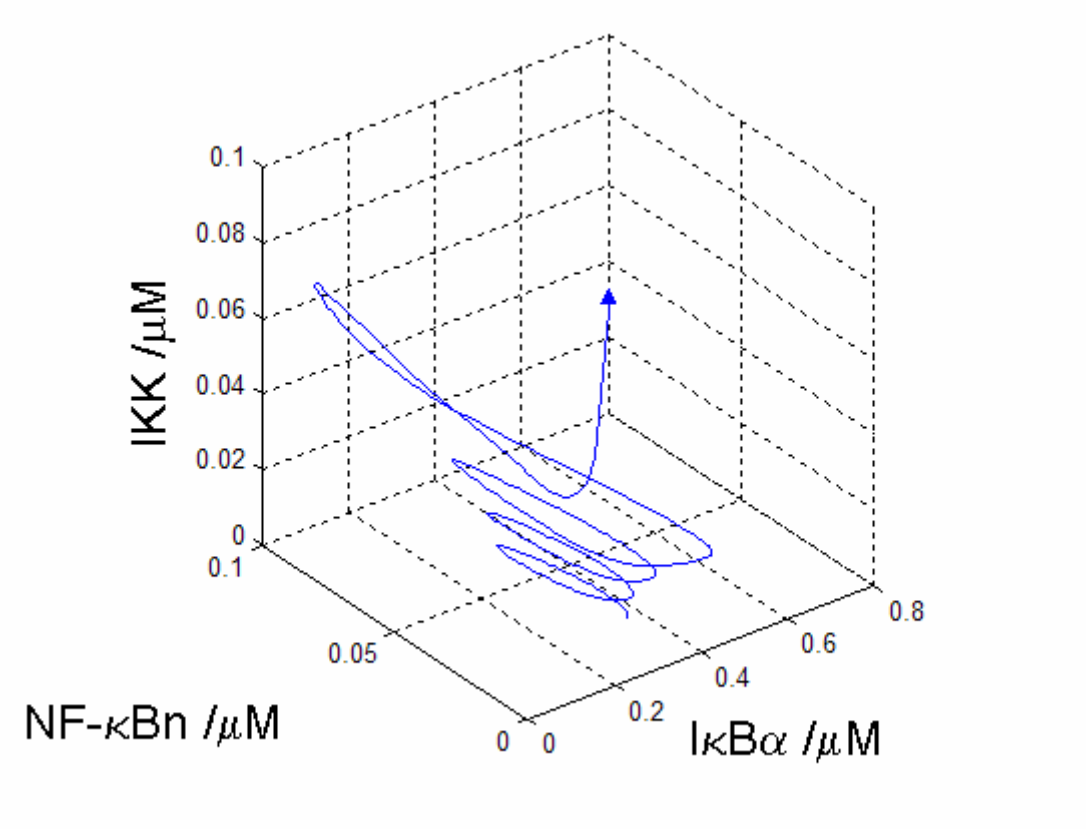
38: IκBαn nuclear Import Rate constant

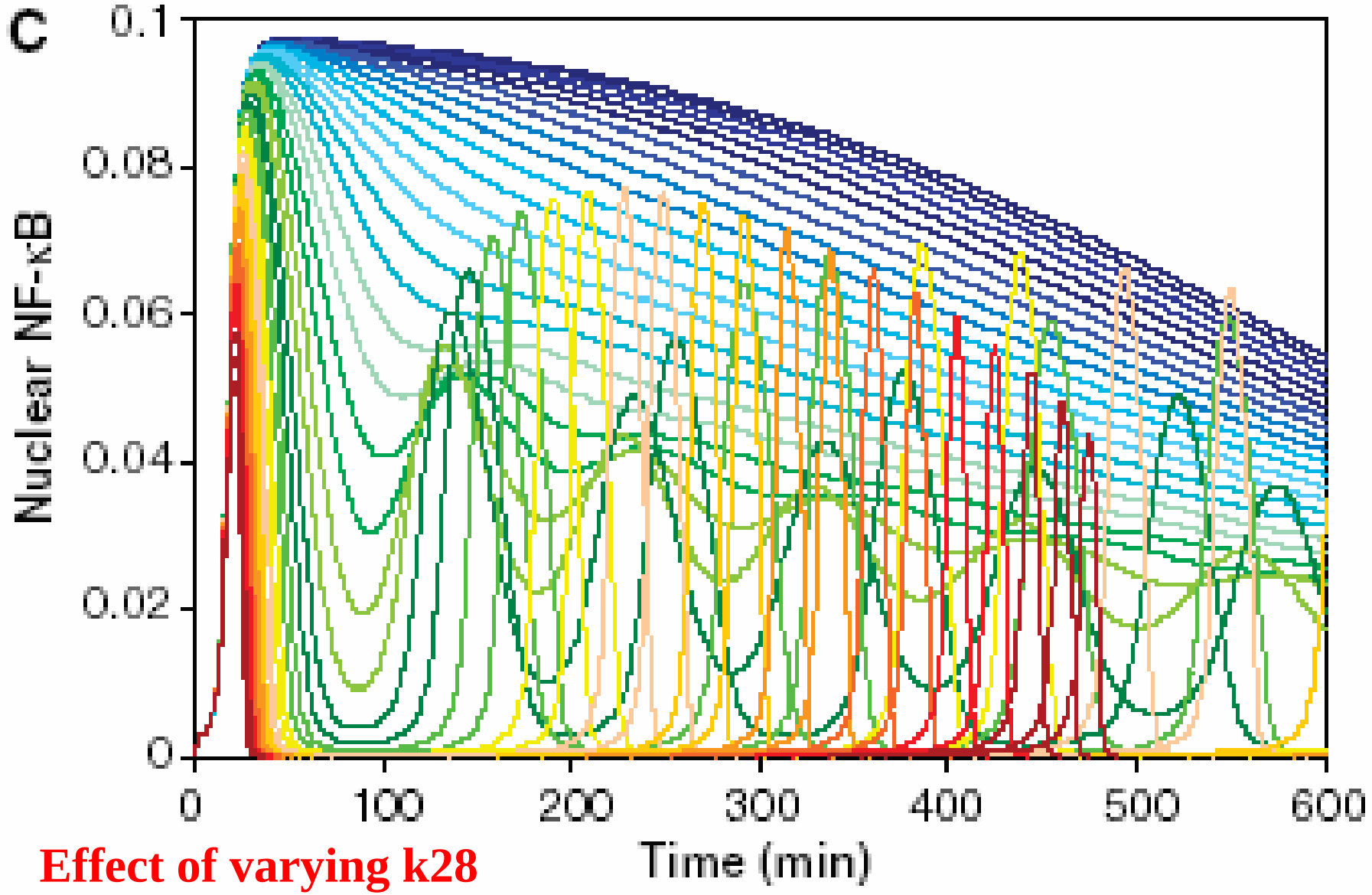
52: IKKIκBα-NF-κB association rate constant

61: IKK signal onset slow adaptation coefficient

62: IKKIκBα catalysis rate constant

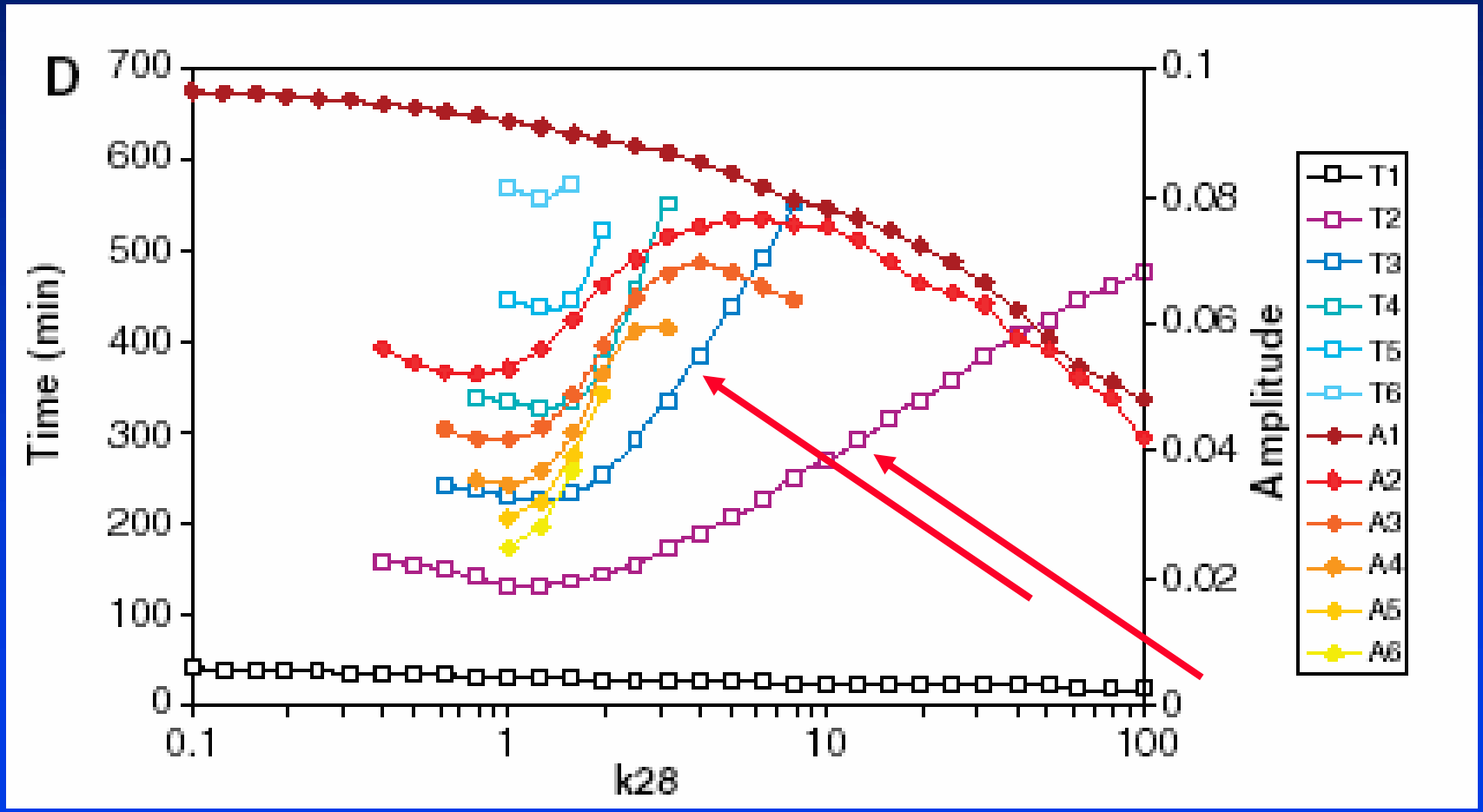
A phase plane plot shows the intimate connection between IKK, IκBα and NFκBn



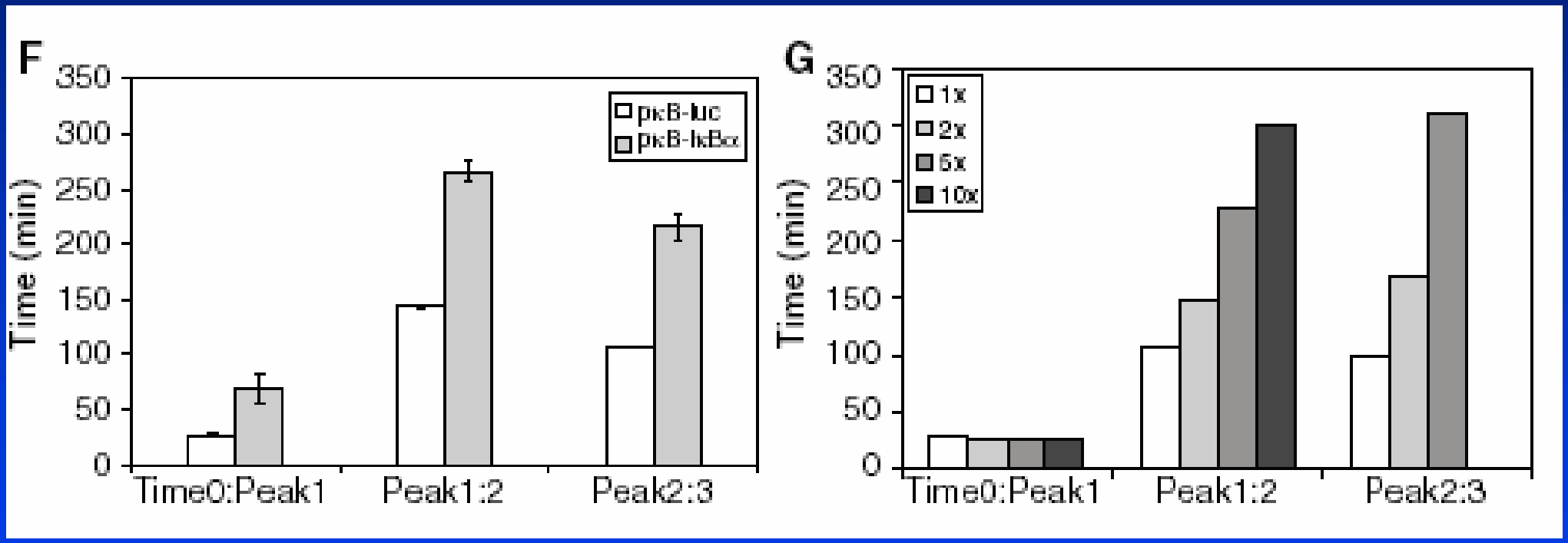


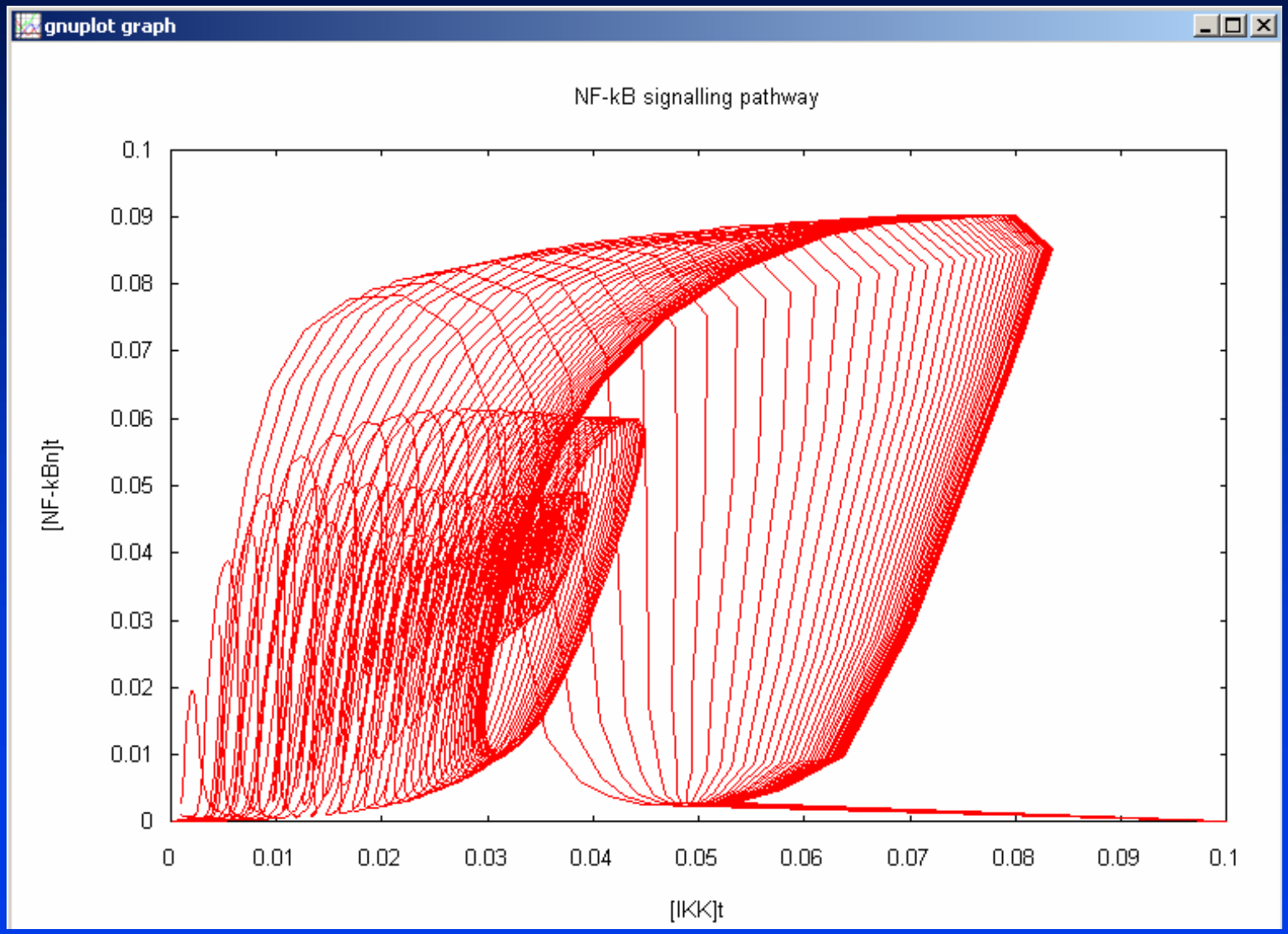
Effect of varying k28

Prediction: increasing k28 will increase the period of the oscillations (e.g. T2 and T3)



Experiment (left) matches simulation (right)



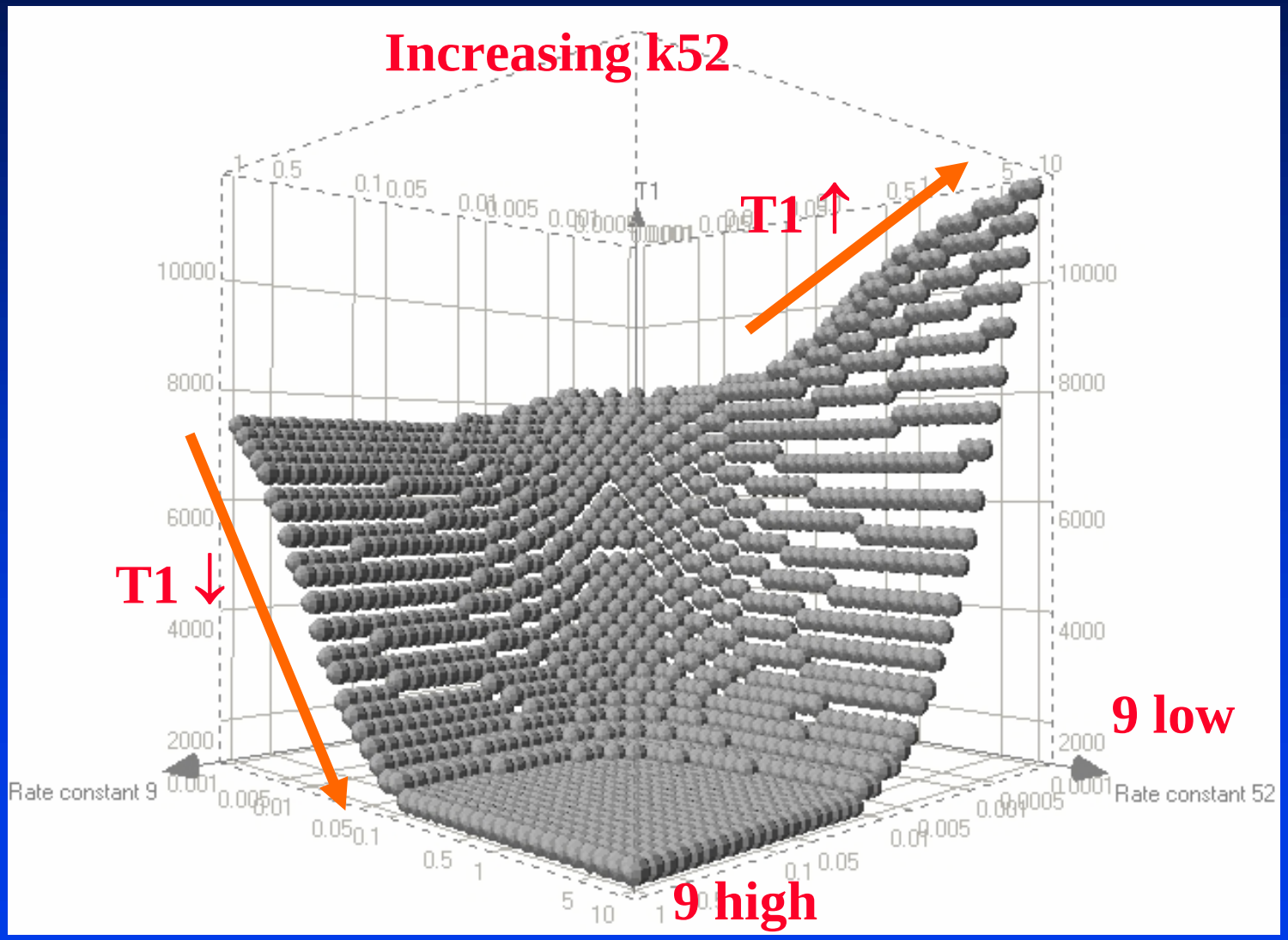

gnuplot graph
NF-kB signalling pathway
[NF-kBn]t
[IKK]t
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1

IEEE Systems Biol 152, 153-160 (2005)

Synergistic control of oscillations in the NF-κB signalling pathway

A.E.C. Ihekwaba, D.S. Broomhead, R. Grimley, N. Benson, M.R.H. White and D.B. Kell

Synergistic effects in the NF-κB pathway – even qualitative differences when the effect of 1 rate constant is observed at different values of another!



Mol Biosyst 2, 640-649 (2006)

PAPER www.rsc.org/molecularbiosystems | Molecular BioSystems

Insights into the behaviour of systems biology models from dynamic sensitivity and identifiability analysis: a case study of an NF-κB signalling pathway†

Hong Yue,*[ab] Martin Brown,[c] Joshua Knowles,[ab] Hong Wang,[c] David S. Broomhead[bd] and Douglas B. Kell[ab]

Similar behaviour is found for nuclear NF-κB using dynamic sensitivity analysis…

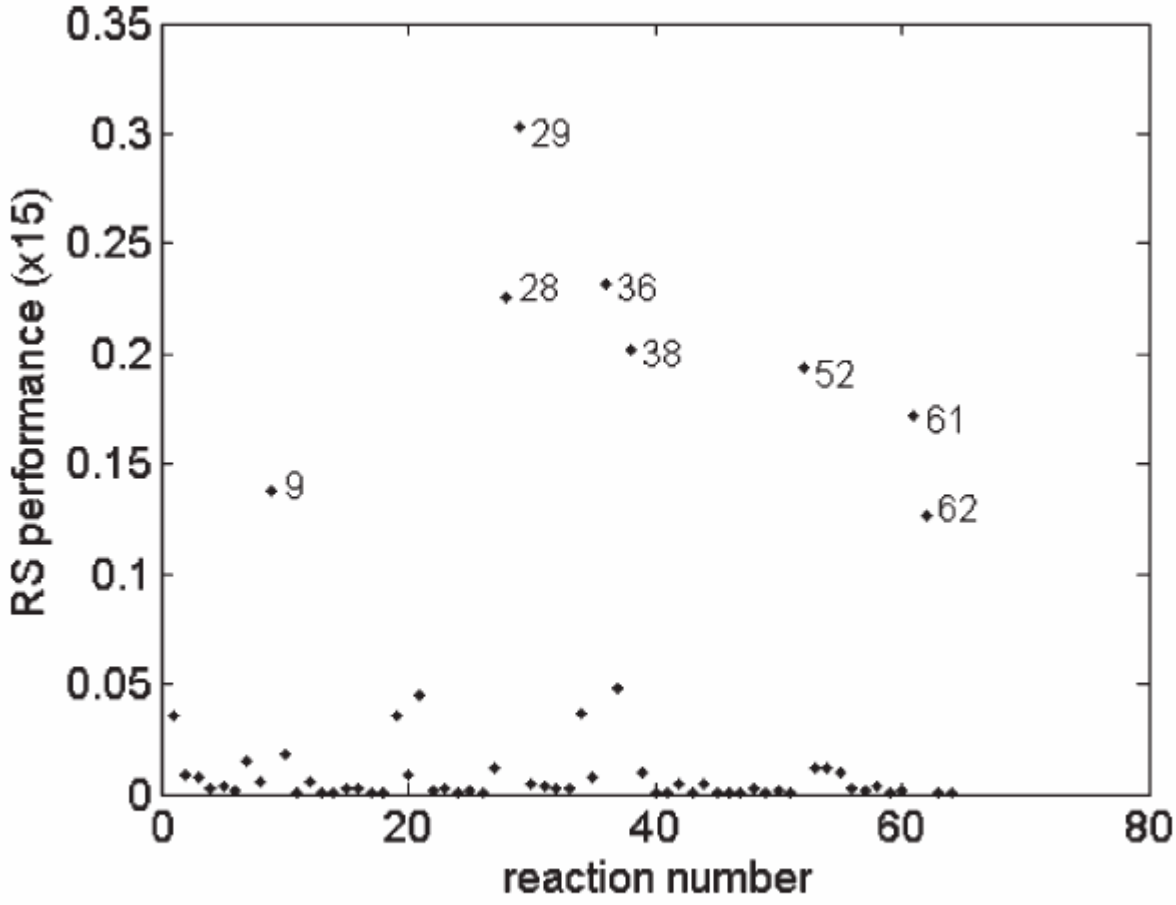


Fig. 4 Performance index $RS_{15,j}$ (variable: NF-κB$_n$).

This was true when all variables were included, since NF-κB_n is dominant

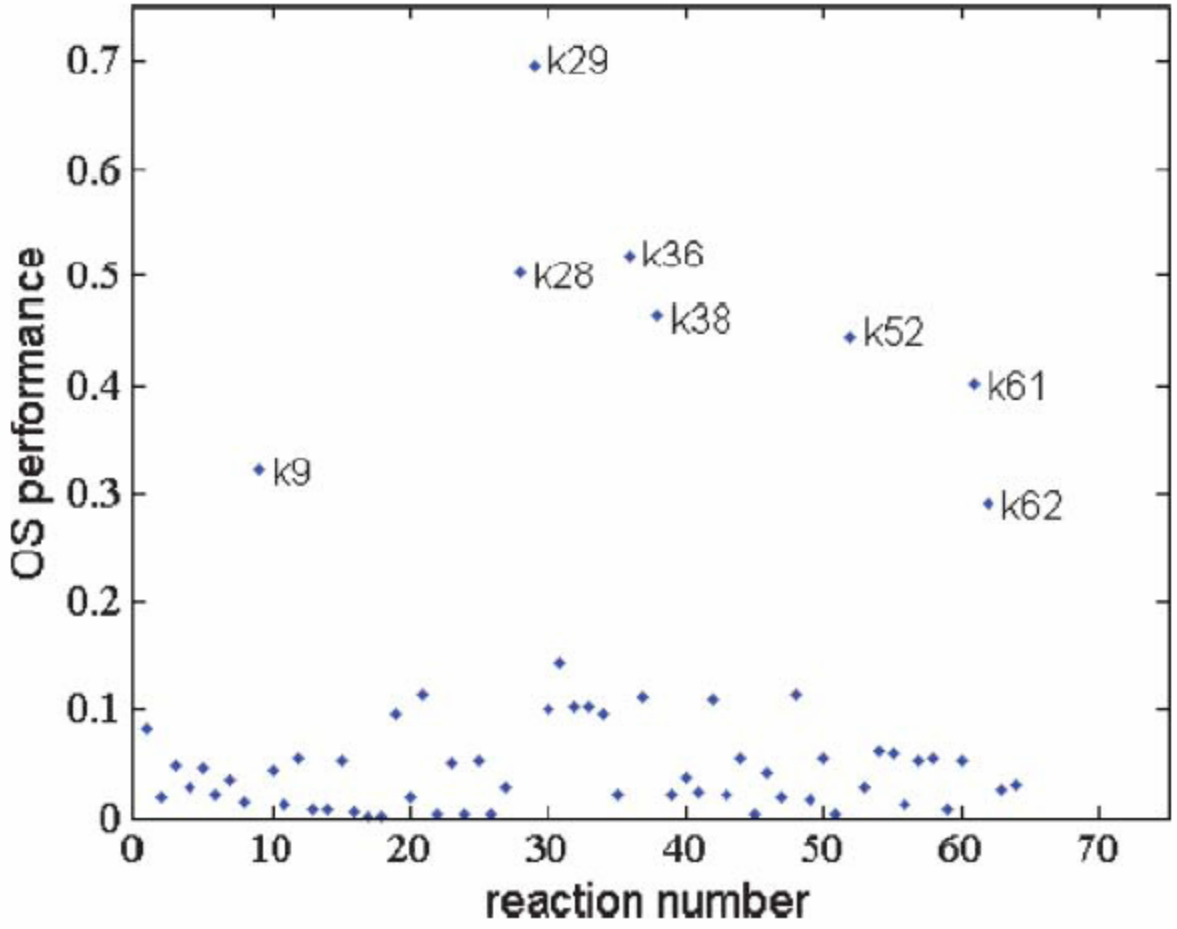


Fig. 8 Overall integral performance OS_j in natural order.

Many of these were the most identifiable parameters

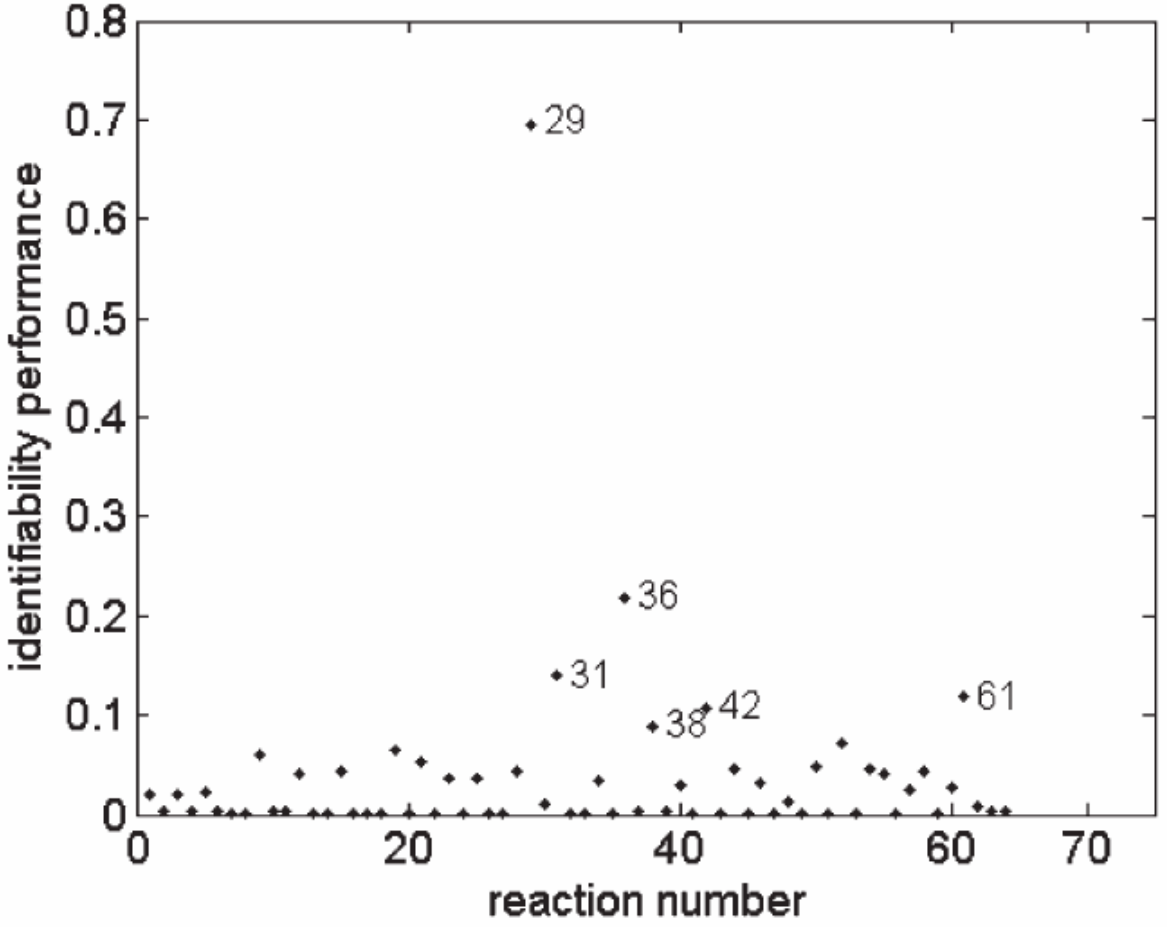


Fig. 11 Parameter identifiablity results by orthorgonal forward selection.

Improving Data Fitting of an Signal Transduction Model By Global Sensitivity Analysis

Yisu Jin, Hong Yue, *Senior Member, IEEE*, Yizeng Liang, and Douglas B. Kell

- **Using sensitivity information assists greatly in parameter fitting**

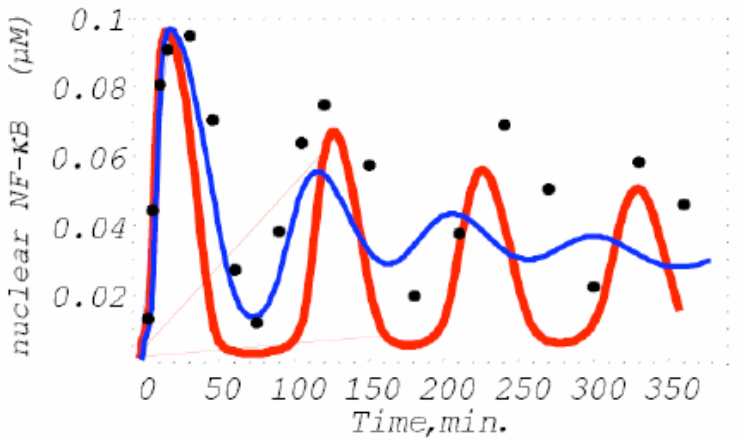


Fig. 1. Comparison of the fitting methods applied to the oscillatory NF-κB activation profile in IκBβ-/- IκBε-/- cells. The experimental data are shown as black filled circles; the "semi-quantitative" fit is shown in red and the result of random search fitting in blue (See Fig. S1 in the supplementary material to [14]).

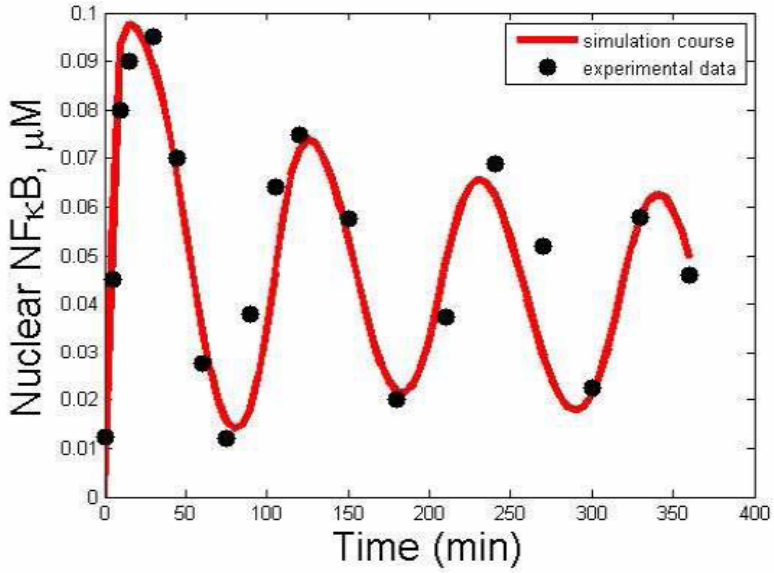


Fig. 3. The fitting result of NF-κB$_n$ in the IκBα-IKK-NF-κB model. The experimental data are shown as black filled circles; the best solution of fitting course is shown in red.

Proximate parameter tuning

Proximate Parameter Tuning for biochemical networks with uncertain kinetic parameters

Stephen J. Wilkinson[a,b], Neil R. Benson[c] & Douglas B. Kell*[a,b]

[a]School of Chemistry and [b]The Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, The University of Manchester, Princess St, Manchester, M1 7DN, UK

[c]Pfizer Central Research, Ramsgate Road, Sandwich, Kent, CT13 9NJ, UK

ITSA – Information Theoretic Sensitivity Analysis

Information-theoretic Sensitivity Analysis:
a general method for credit assignment in complex networks

[1,2]Niklas Lüdtke, [3]Stefano Panzeri, [4]Martin Brown, [5]David S. Broomhead, [1,2,6]Joshua Knowles, [3]Marcelo A. Montemurro and [1,2,*]Douglas B. Kell

[1]School of Chemistry, [2]The Manchester Interdisciplinary Biocentre, [3]Faculty of Life Sciences, [4]School of Electrical and Electronic Engineering, [5]School of Mathematics and [6]School of Computer Science, The University of Manchester, 131 Princess St, Manchester M1 7DN, UK

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- Treats a system as a communication channel
- Decomposes mutual information between inputs and outputs into main and interaction terms, in a principled way
- Unlike variance based schemes this approach can accommodate correlated inputs

We usually consider biological circuit elements such as enzymes as 'responding' solely to amplitudes

e.g. irreversible Michaelis-Menten:

$$v = (V_{max}.S)/ (S + K_m)$$

Thus, *v* depends ONLY on the 'instantaneous' concentration of S

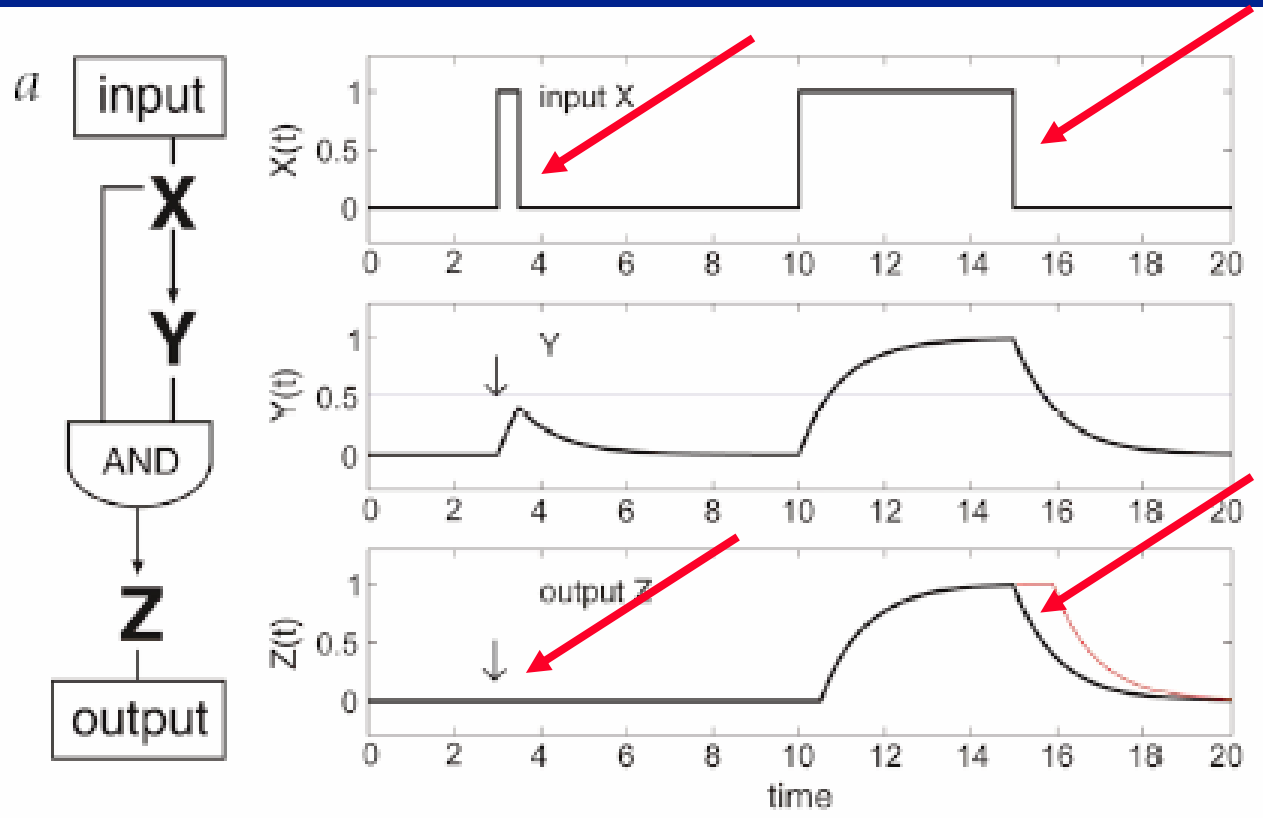
Frequency encoding

- Having the effective signal frequency-encoded allows the same ‘medium’ (NF-kB) to carry different ‘messages’ using changes in the frequency or dynamics rather than the amplitude of oscillatory signals *per se*
- There is thus no ‘crosstalk’ (and no crosstalk problem)
- But this also means that great care must be used if such systems are to be exploited for providing novel drug targets simply by inhibiting particular steps, as the downstream events are not easily related to the activities of the individual steps
- (Additional means of avoiding crosstalk are likely also present, e.g. extra transcription factors providing a logical AND.)
- More generally, we need to recognise signalling systems as signal processing systems

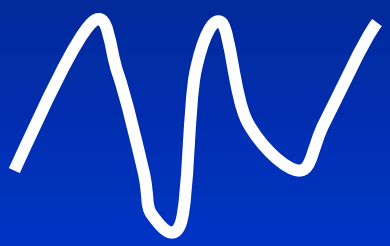
Network motifs such as the coherent feedforward loop respond to frequency, not amplitude *per se*

Network motifs in the transcriptional regulation network of *Escherichia coli*

Shai S. Shen-Orr[1], Ron Milo[2], Shmoolik Mangan[1] & Uri Alon[1,2]



The same signal can lead to two different outputs depending on the filtering/detector



“But one thing is certain: to understand the whole you must study the whole”

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Conclusions

- SBML allows rich and principled representations of biochemical networks, including much useful metadata
- Taverna allows us to construct Systems Biology workflows, including those performing sensitivity analyses
- Sensitivity analyses (local, global, static, dynamic) proved extremely important in understanding the highly nonlinear NFκB system, and thereby uncovered some new biological principles
- Further and better algorithms will allow unique insights into the parameterisation, control and identification of biochemical systems

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- **John McCarthy**
- **Pedro Mendes (from 1/4/07)**
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- **Norman Paton**
- **Hans Westerhoff**

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- ** - BBSRC, EPSRC, RSC, BHF, Pfizer

Sensitivity and information theoretic analyses of biochemical networks

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