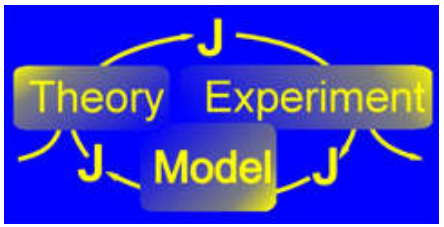
Overview of Research Activities

Prof Johann Rohwer

Laboratory for Molecular Systems Biology
Department of Biochemistry
Stellenbosch University
South Africa



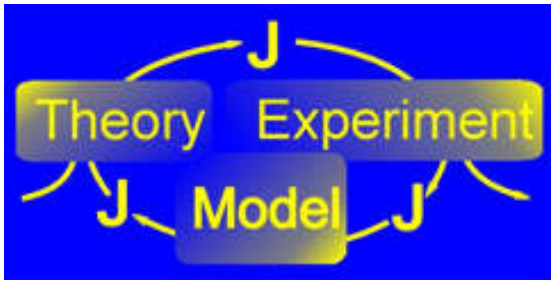
Biochemistry Honours Students
7 April 2017

Theory

- generalised supply-demand analysis
- rate equations for modelling
- symbolic MCA
- *in vitro vs. in vivo* kinetics

Experiment

- NMR “metabolomics”
  - *in vivo, in situ, in vitro* metabolite measurements
- enzyme kinetics for modelling
  - *in vivo* enzyme kinetics
  - pH, macromolecular crowding



Model

- development of software (PySCeS, psctb, SymCA, RateChar)
- kinetic models of cellular systems
  - microbial energy metabolism
  - cellular redoxin networks (with Dr C Pillay, UKZN)
  - plant metabolism
  - glucocorticoid receptor dimerisation (with Prof A Louw)

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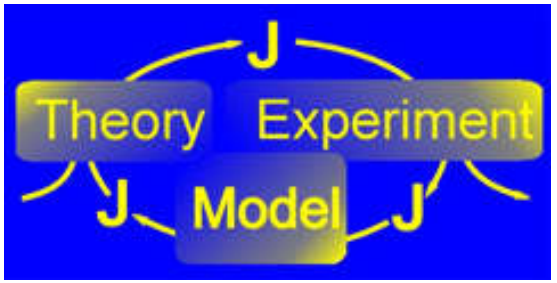

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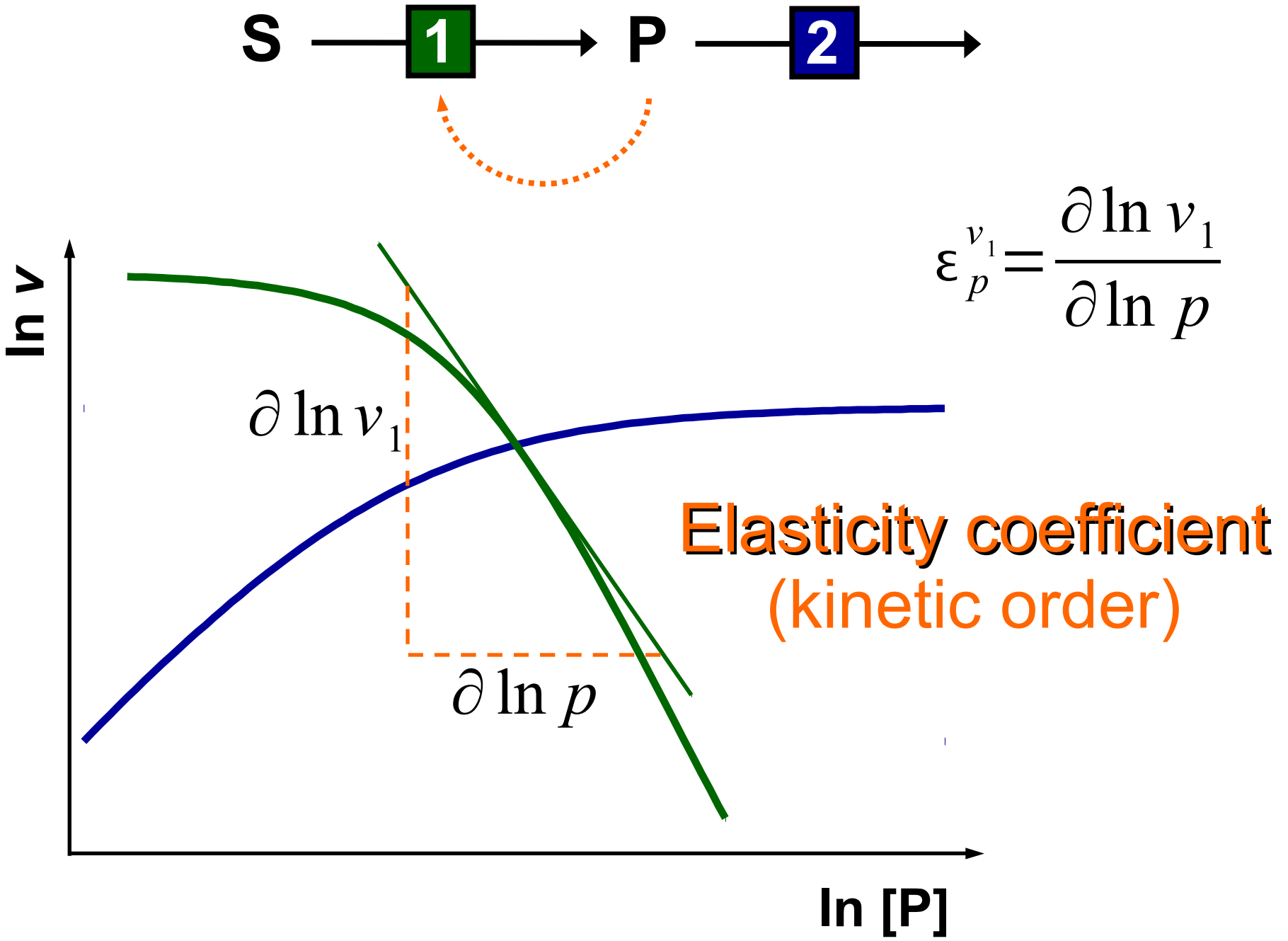
Theory: Example

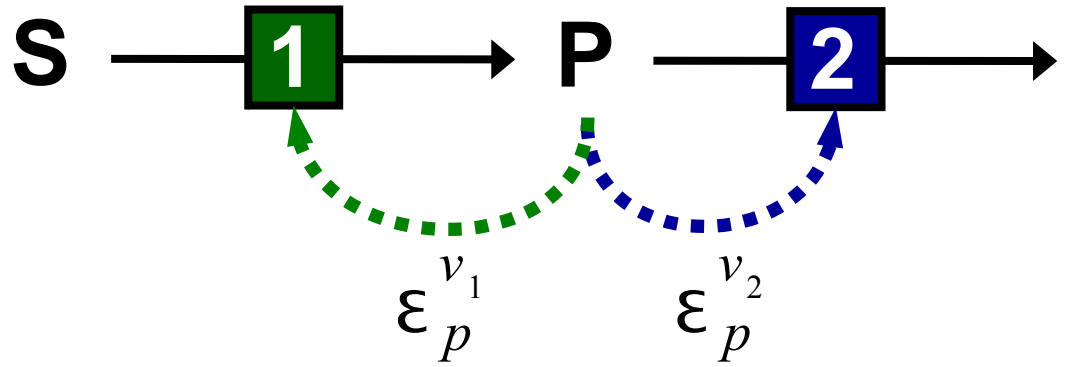
Generalised
supply-demand analysis



$$\varepsilon_p^{v_1} = \frac{\partial \ln v_1}{\partial \ln p}$$

Elasticity coefficient
(kinetic order)

Control analytic expressions



$$C_1^J = \frac{\varepsilon_p^{v_2}}{\varepsilon_p^{v_2} - \varepsilon_p^{v_1}} \qquad C_2^J = \frac{-\varepsilon_p^{v_1}}{\varepsilon_p^{v_2} - \varepsilon_p^{v_1}}$$

$$C_1^p = \frac{1}{\varepsilon_p^{v_2} - \varepsilon_p^{v_1}} \qquad C_2^p = \frac{-1}{\varepsilon_p^{v_2} - \varepsilon_p^{v_1}}$$

Problems with SDA

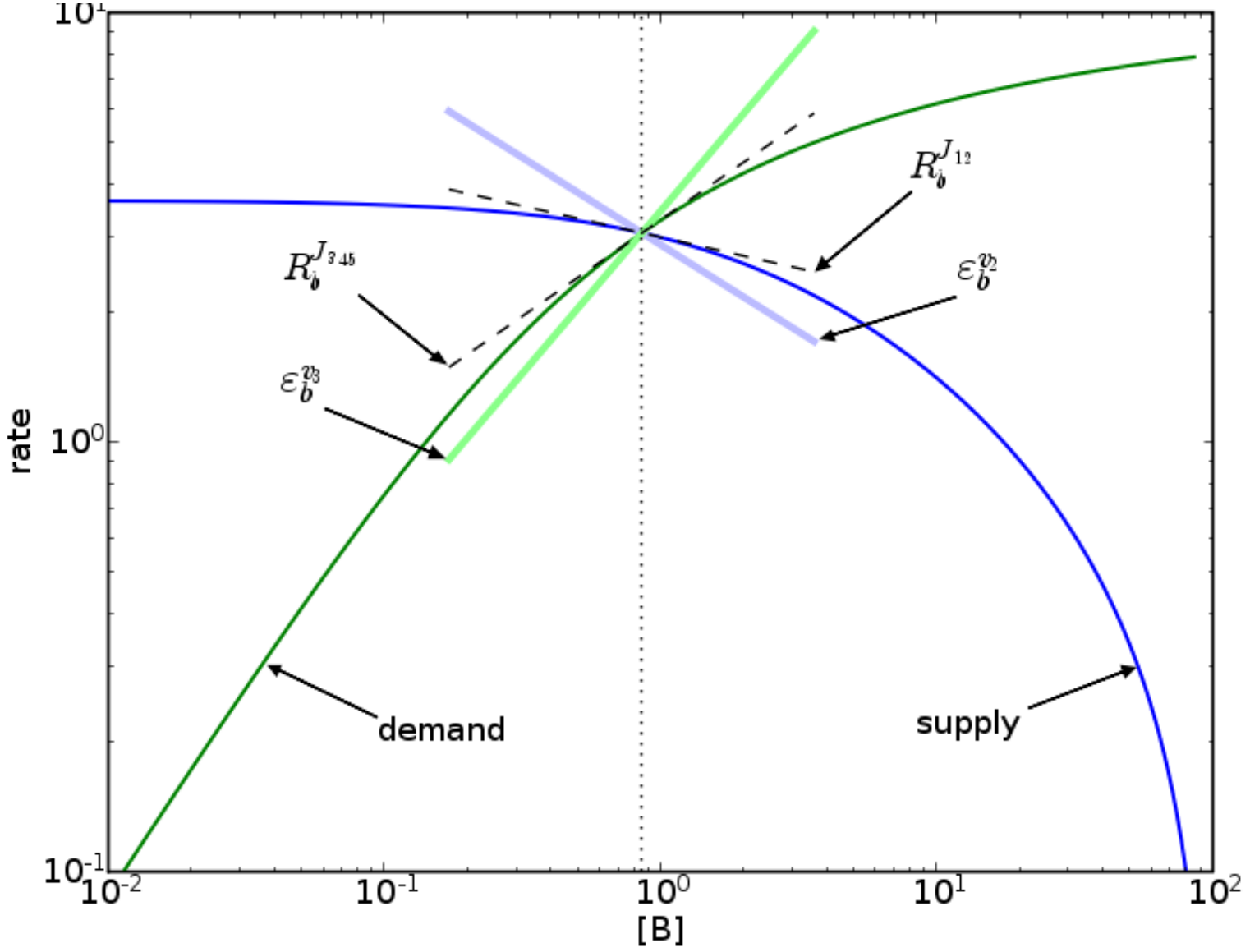
- Where to split system? Choice of intermediate
- Need for a generalised, programmatic, unbiased approach

Generalised supply-demand analysis

- Clamp each variable species of a model in turn and vary above/below steady-state value
- Plot fluxes through supply and demand reactions in log-log rate characteristic
- Compare elasticities and response coefficients

SDA around B


S
1
A
2
B
3
C
4
D
5
P

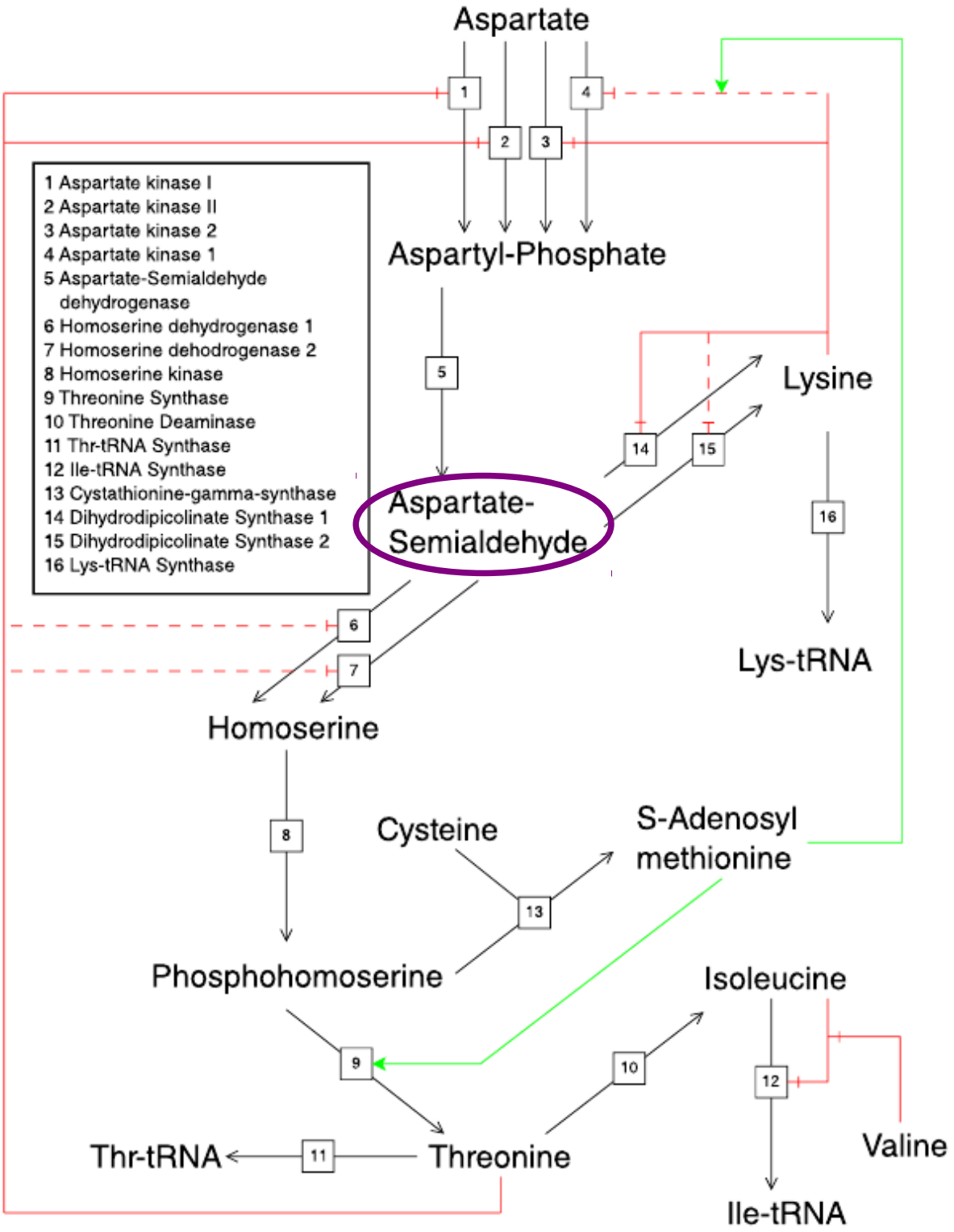

rate
[B]
demand
supply
$R_b^{J_{12}}$
$R_b^{J_{345}}$
$\varepsilon_b^{v_2}$
$\varepsilon_b^{v_3}$

What can GSDA tell us?

- Potential sites of regulation
- $R = \varepsilon \neq 0$ → regulatory metabolite
- $R \to 0, \varepsilon \to 0$ → functional differentiation
- Different routes of communication quantified

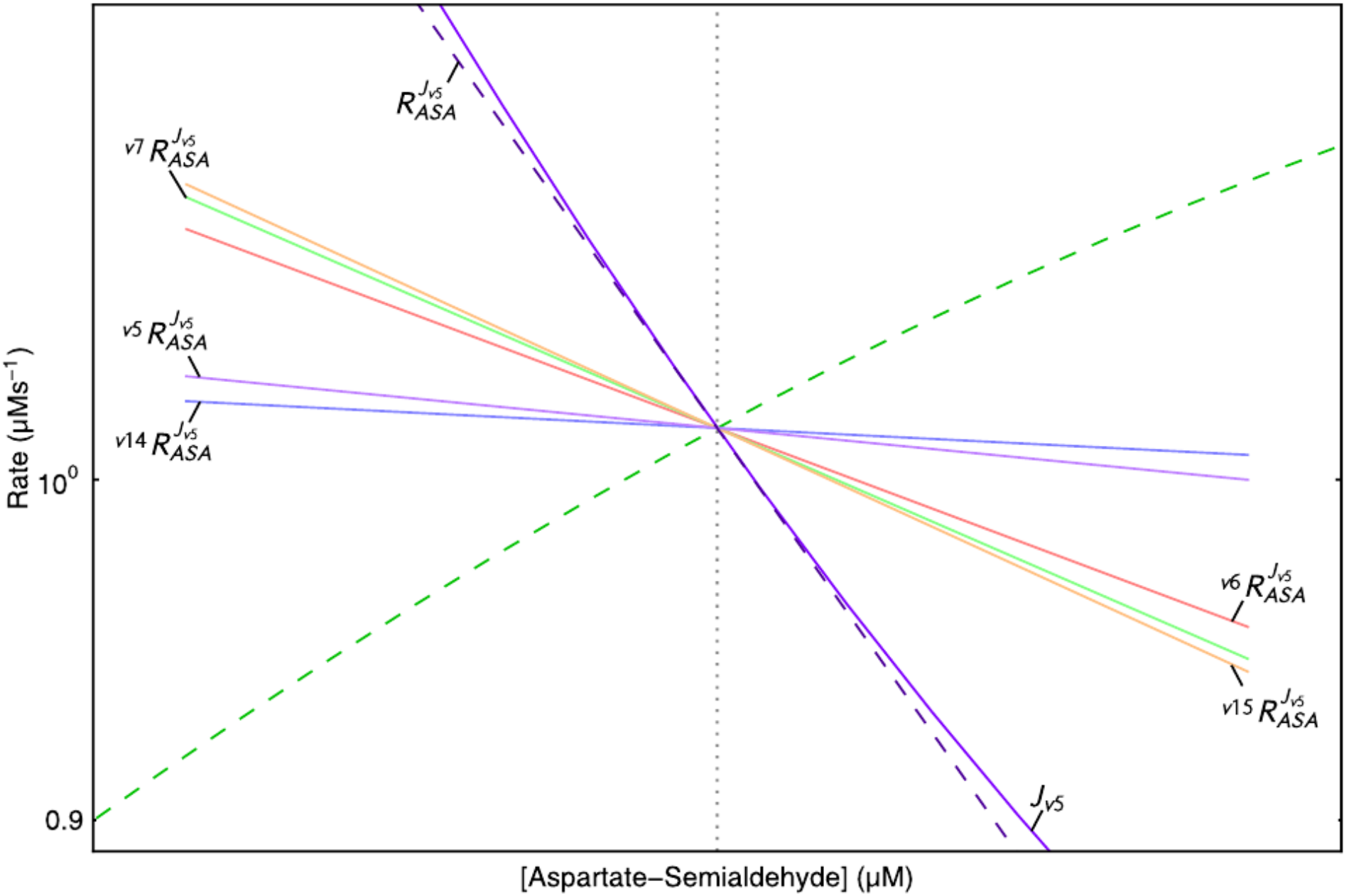
Rohwer & Hofmeyr, J. Theor. Biol. (2008)

“Real” model

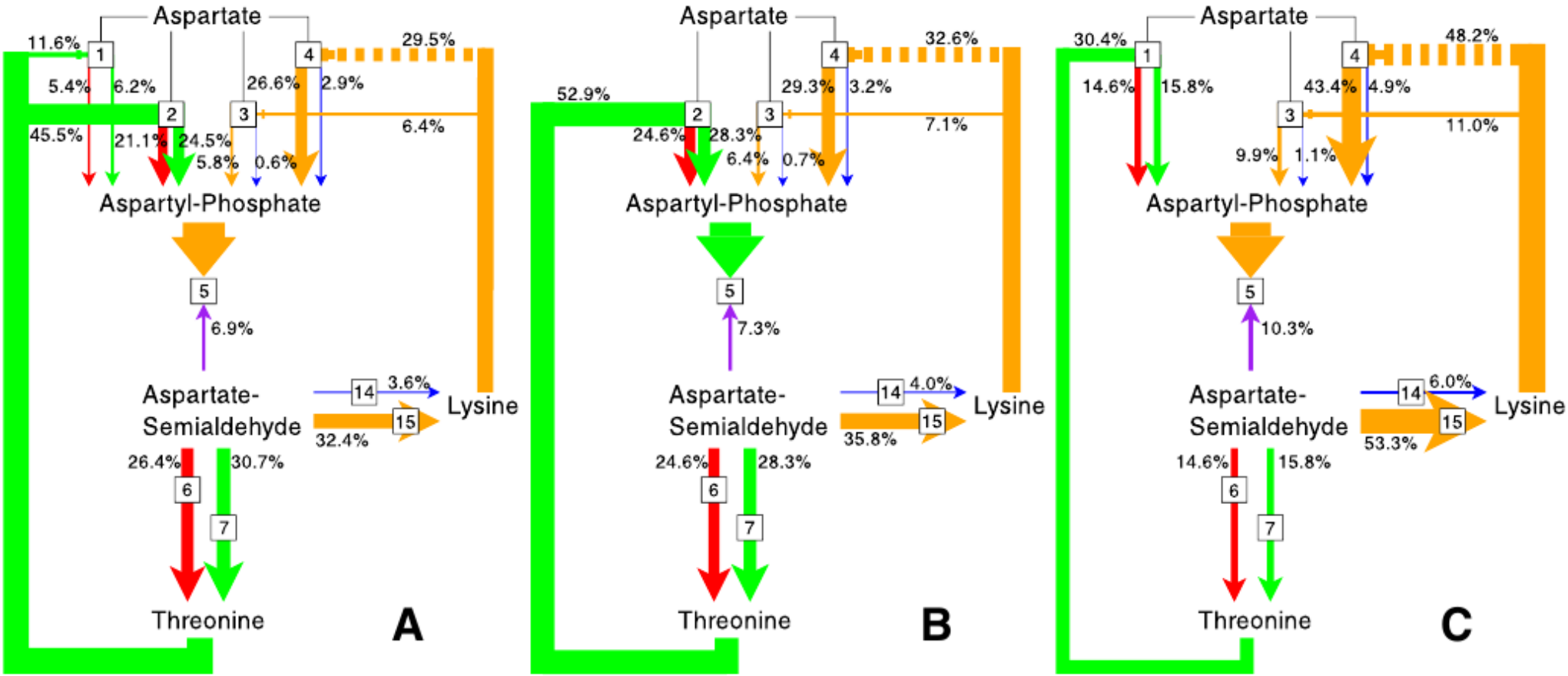


Curien *et al.* (2009, 2010)

GSDA around ASA



Knockout mutants



wild-type

AK I knockout

AK II knockout

Ongoing work

- Application to various “real-life” models
- Integration with symbolic MCA and enzyme kinetics/regulation
- Development of visualisation tools
- Extension of PySCeS
  - PySCeS toolbox (psctb)
    - RateChar
    - SymCA
    - thermokin

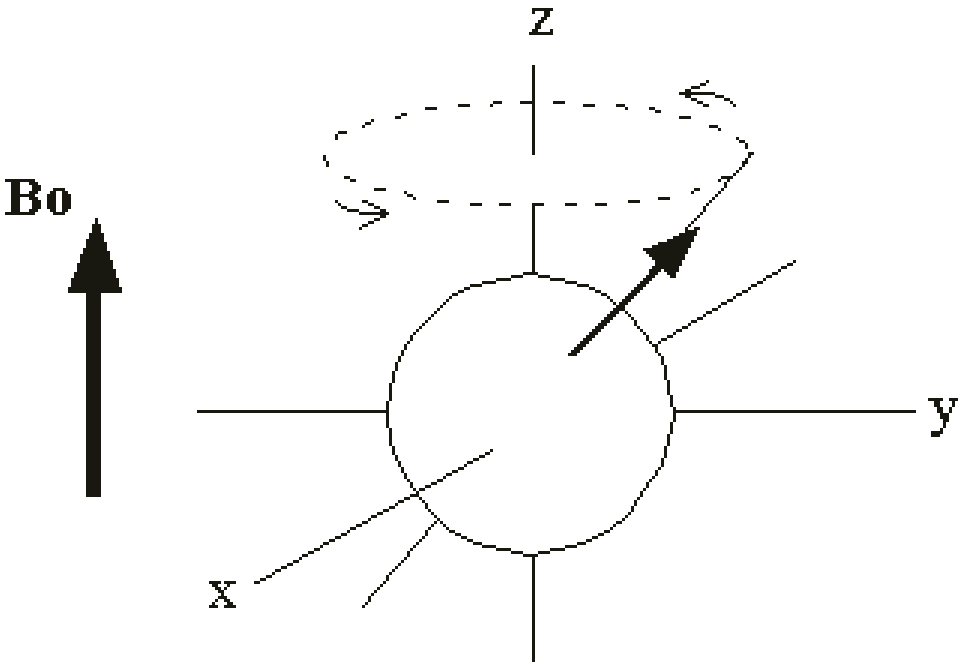
People

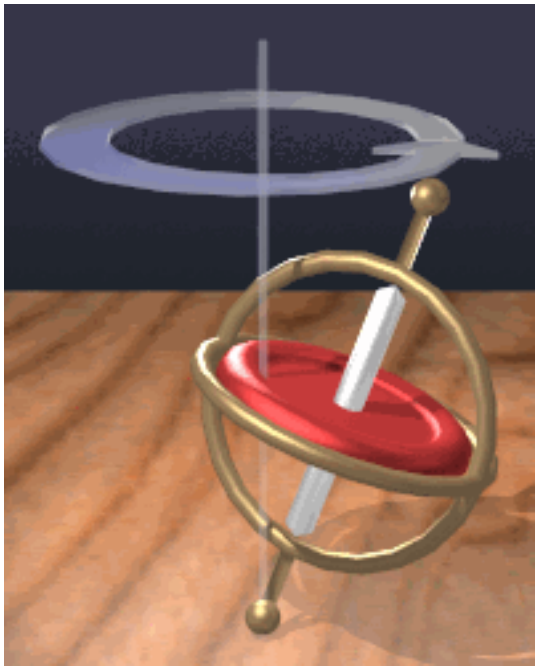
- Academic staff
  - Prof Jannie Hofmeyr
  - Dr Stefan van der Walt
- Students
  - Theo van Staden (Hons 2009)
  - Carl Christensen (Hons 2010, MSc 2011-2012, PhD 2013-2016, post-doc current)

Experiment: Example

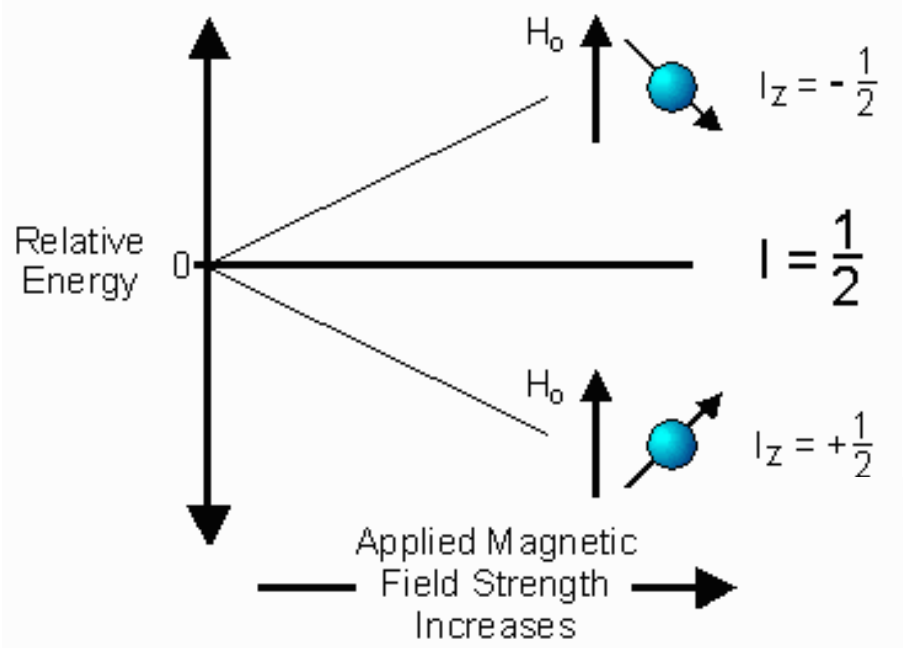
Enzyme kinetics for modelling by NMR spectroscopy
("*In vivo* enzyme kinetics")

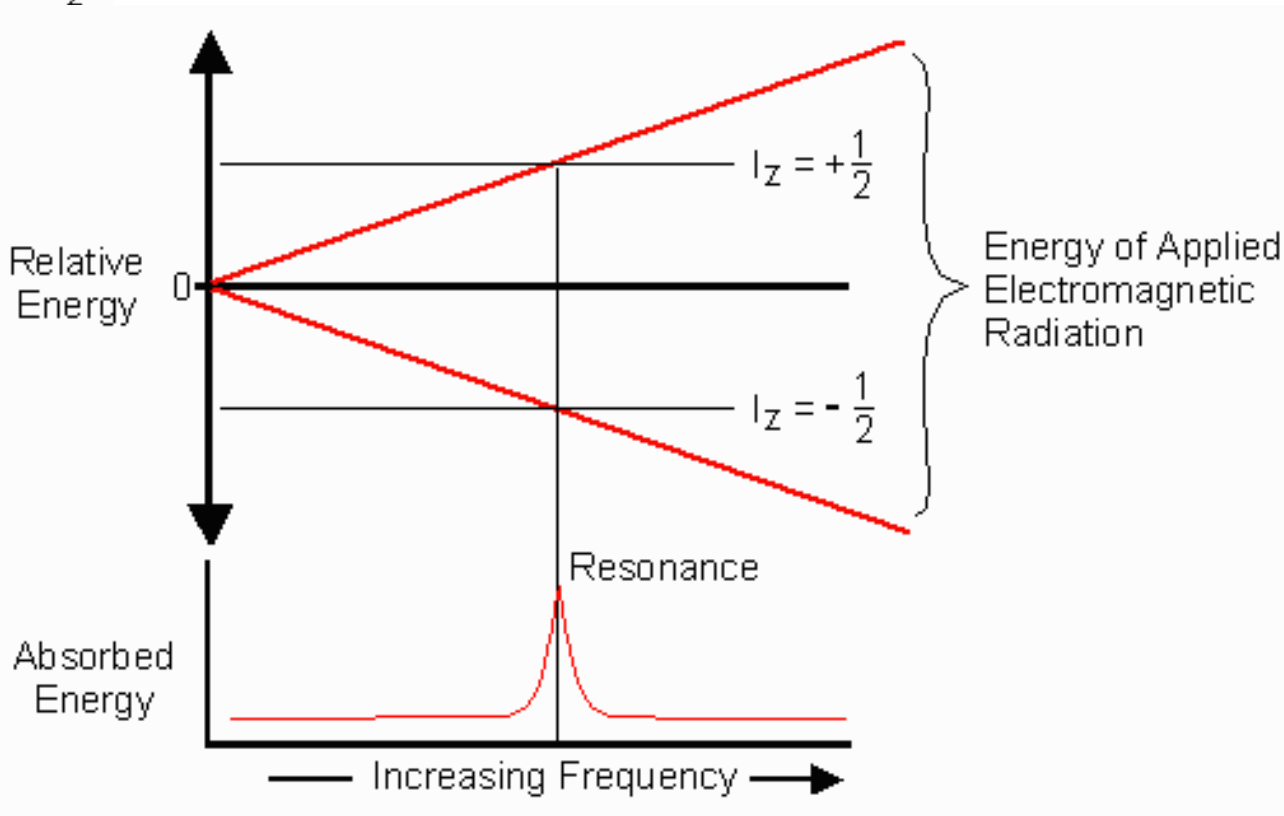
Brief NMR introduction: Nucleus in magnetic field



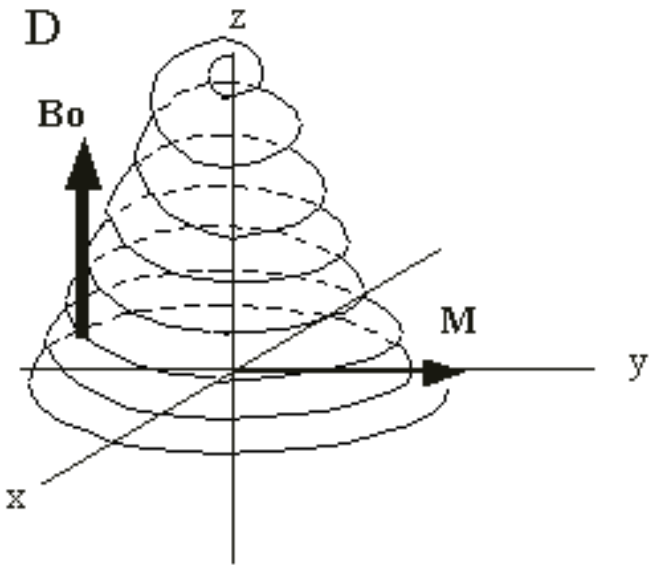


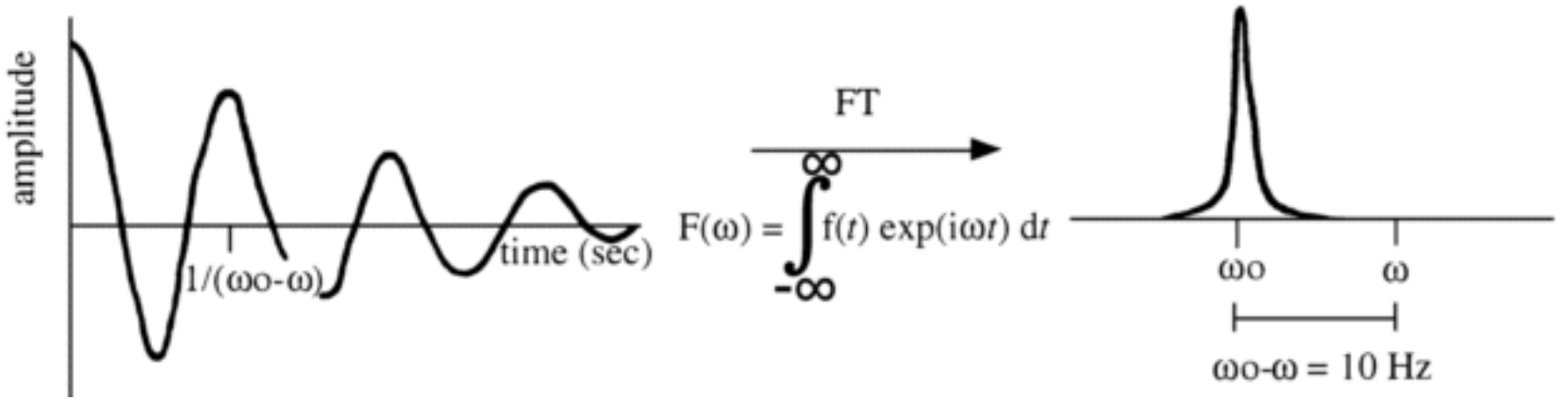
Split in energy levels





Relaxation and Fourier Transform



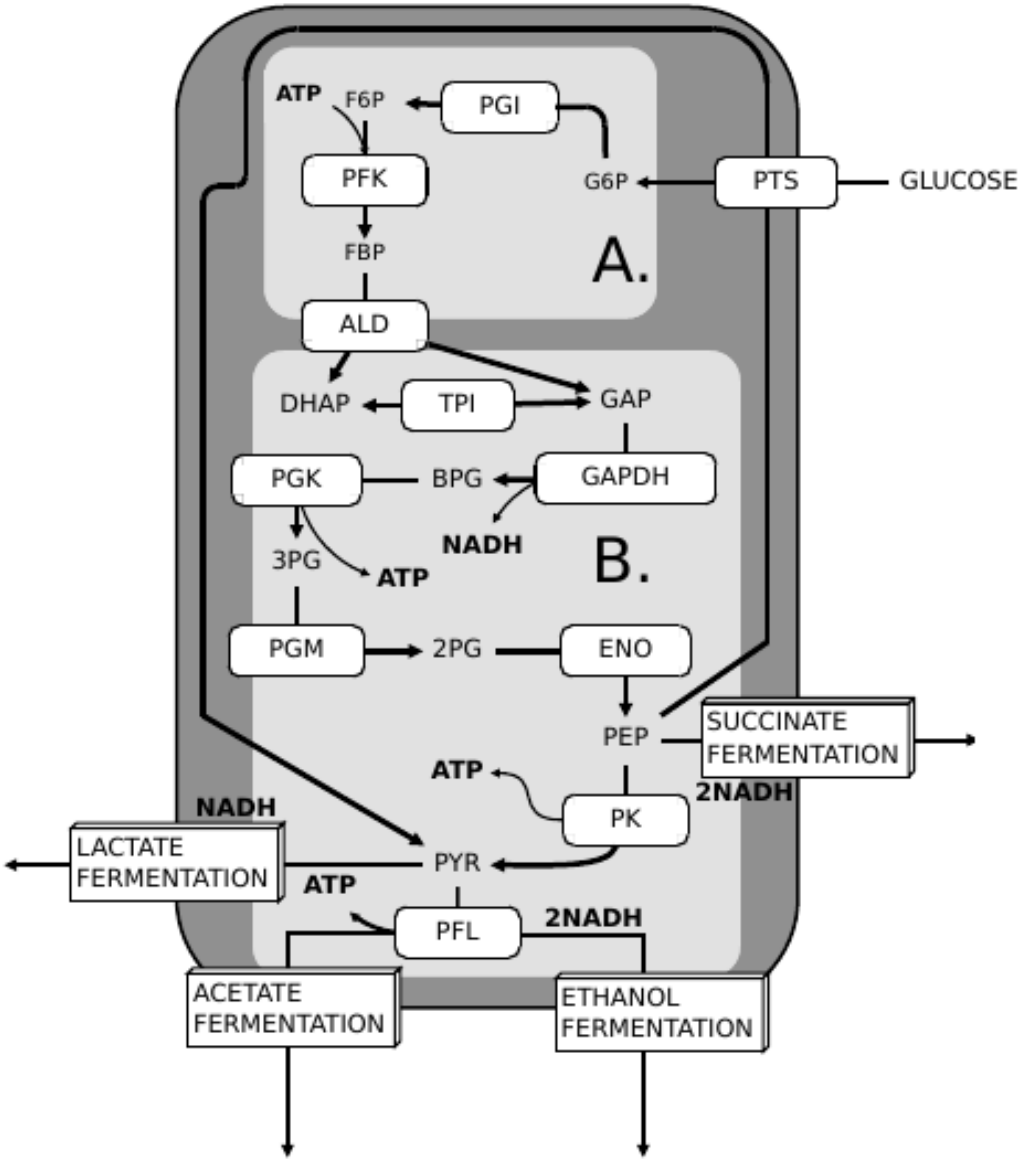


GOAL

- explore central carbon metabolism in *E. coli*
- under microaerobic conditions
- by building a model of the pathway
- using NMR spectroscopy

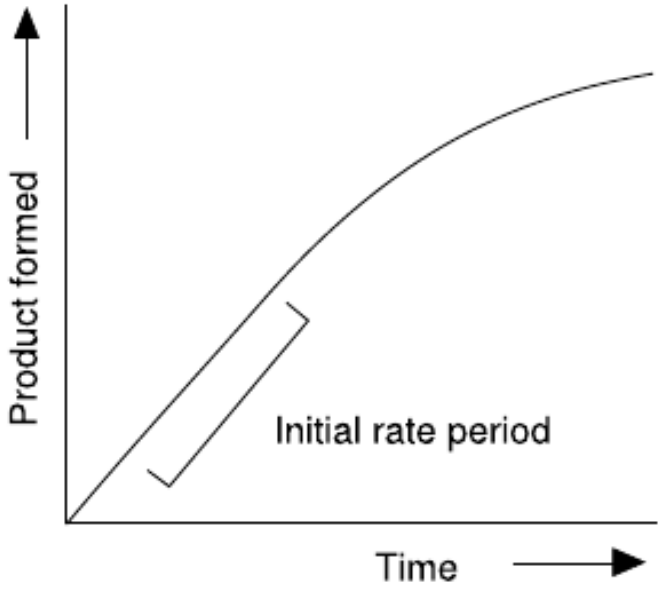
E. coli central carbon metabolism

- Mixed acid fermentation provides substrate and O_2 flexibility
- PhosphoTransferase System
- PFL vs. PDHC

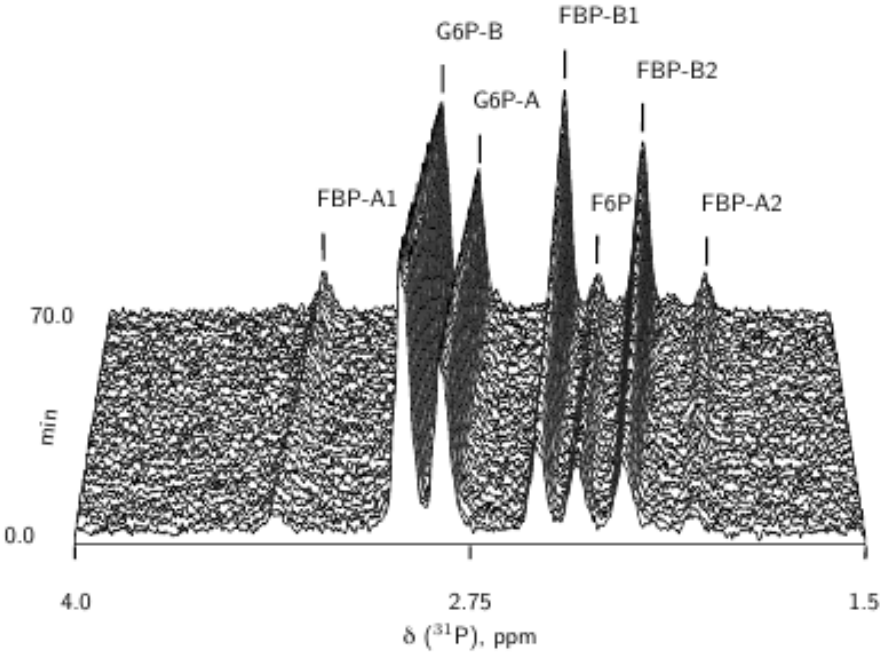


Why use NMR?

Initial rate assays

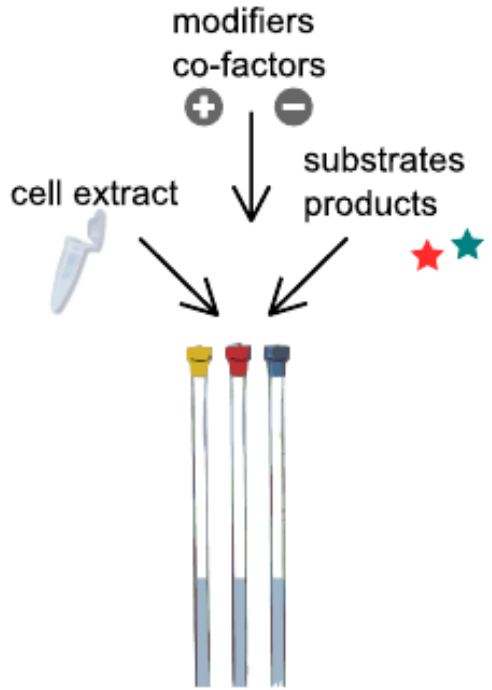


NMR progress curve assays

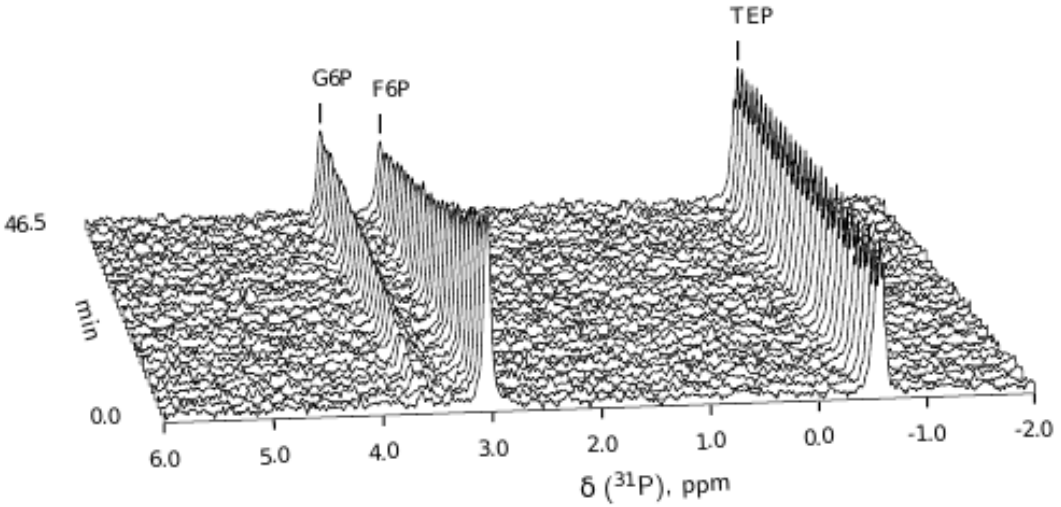


Workflow

1. Incubate, possibly supplementing with ^{13}C-labelled substrate

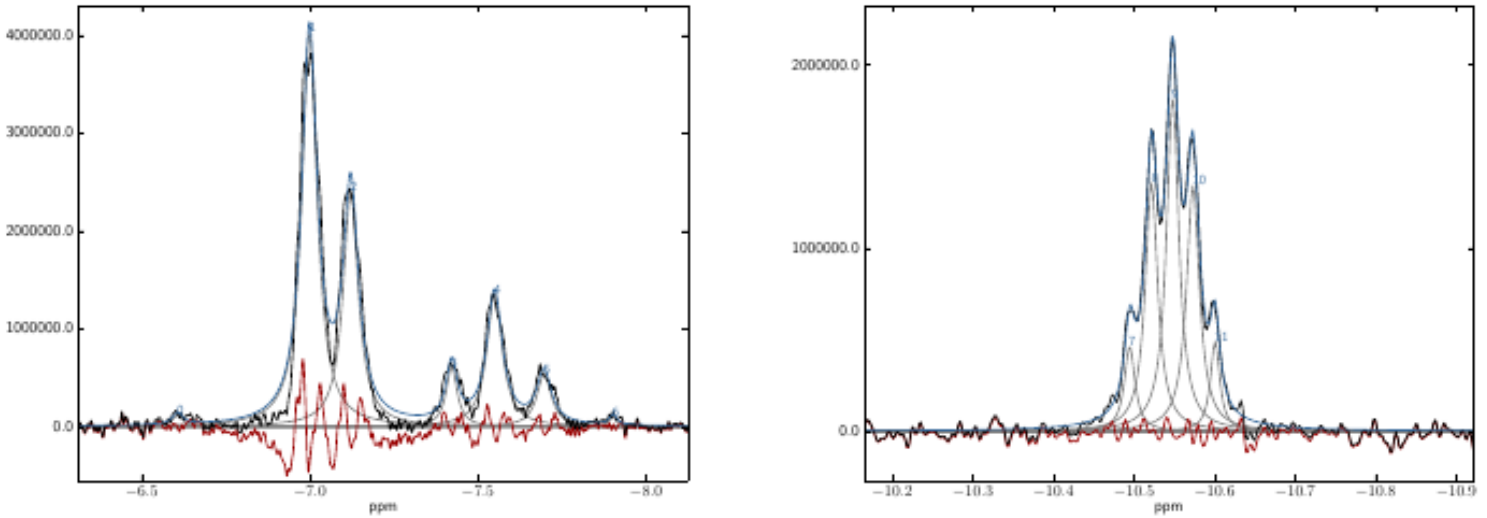


2. Acquire time series of NMR spectra



Workflow (2)

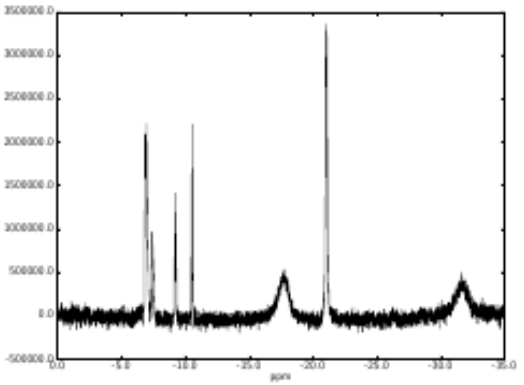
3. Deconvolute spectra to determine peak area

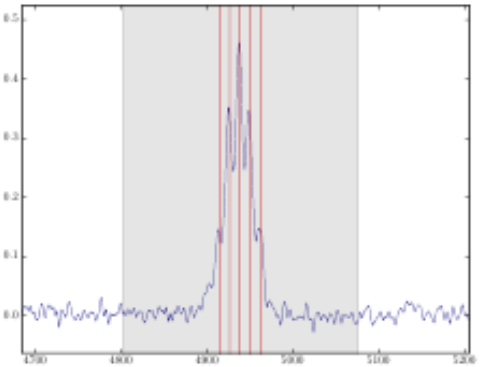


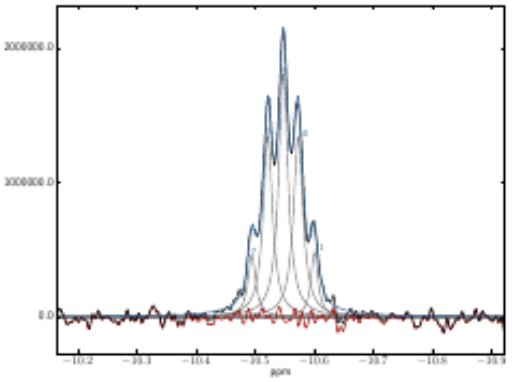
4. Fit splines to concentration time-courses, determine rates
5. Global fit of data to parameterise rate equation

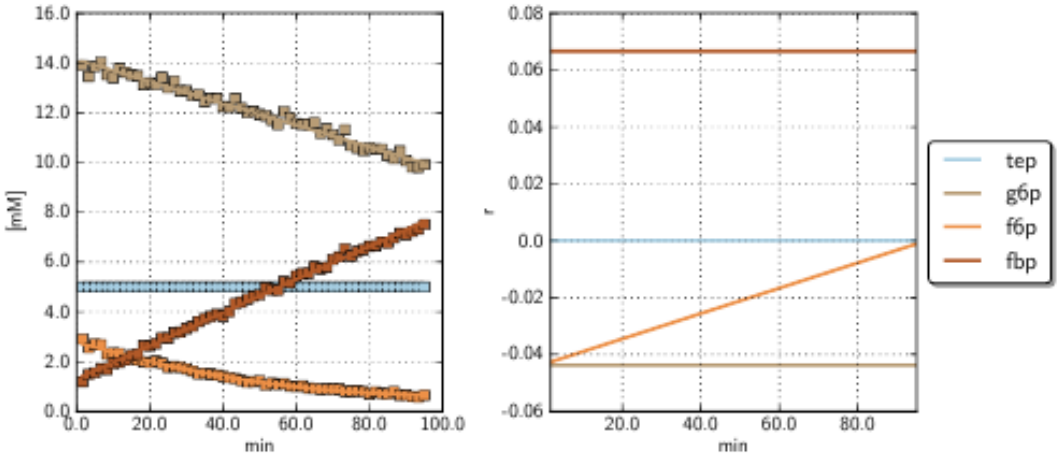
NMRPy

- Python NMR processing software suite
- no adequate software (proprietary or free)
- functionality:
  - processing, integration and deconvolution
  - arrayed spectra
  - interactive or batch processing
  - visualisation
- https://github.com/jeicher/NMRPy

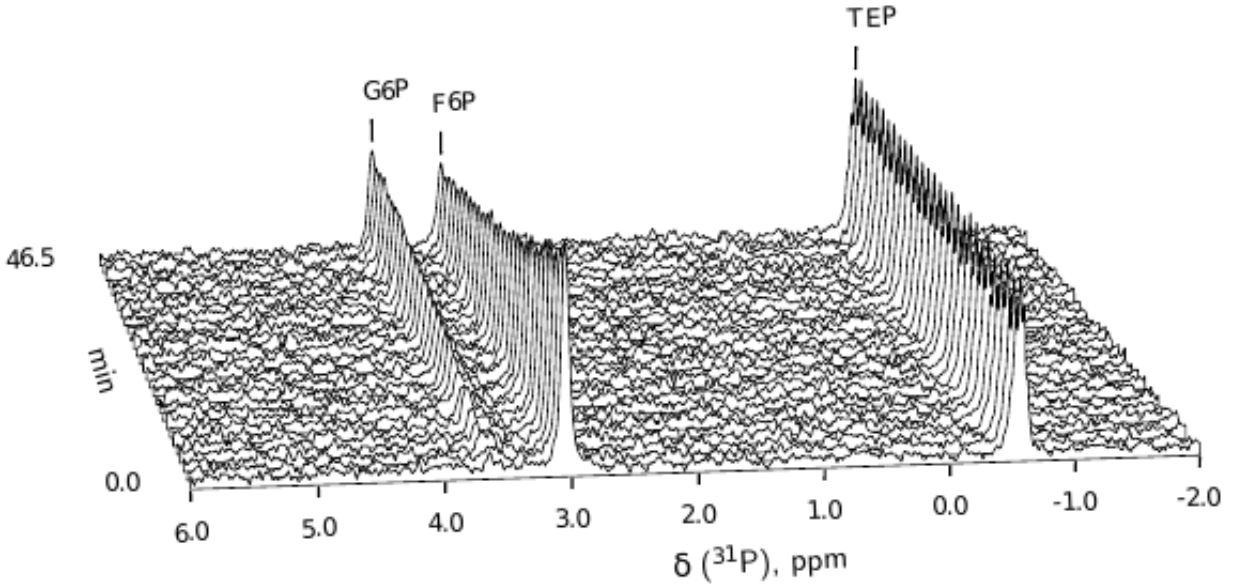


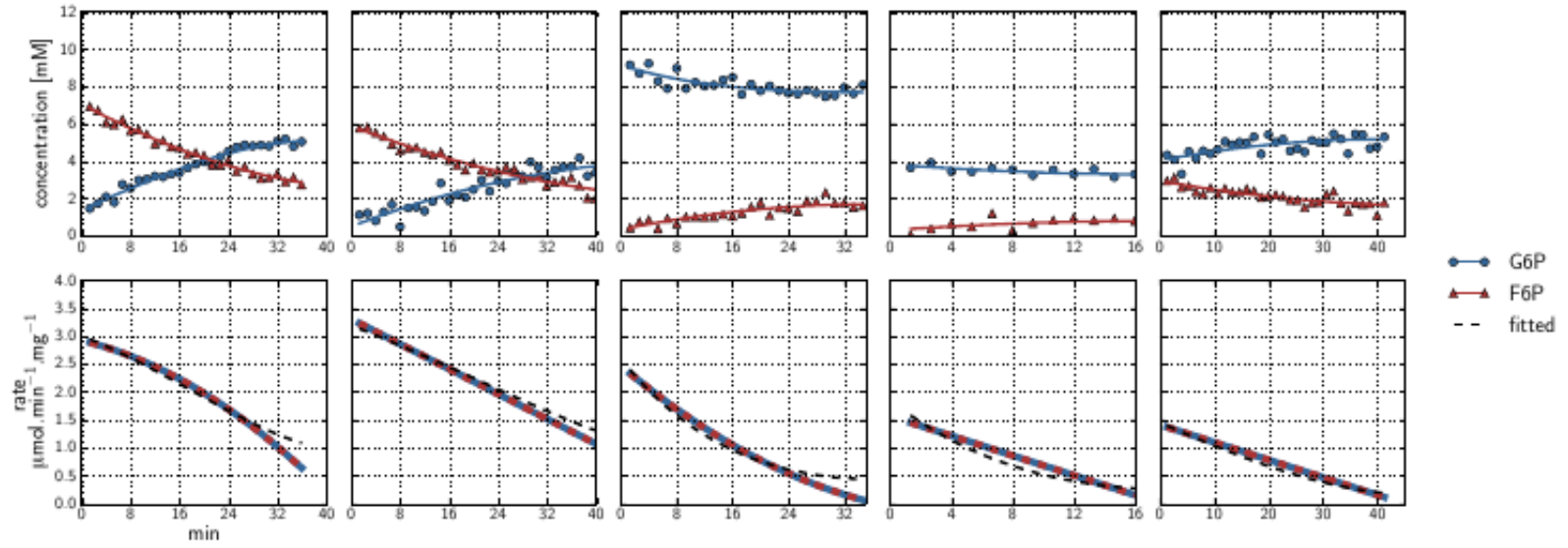




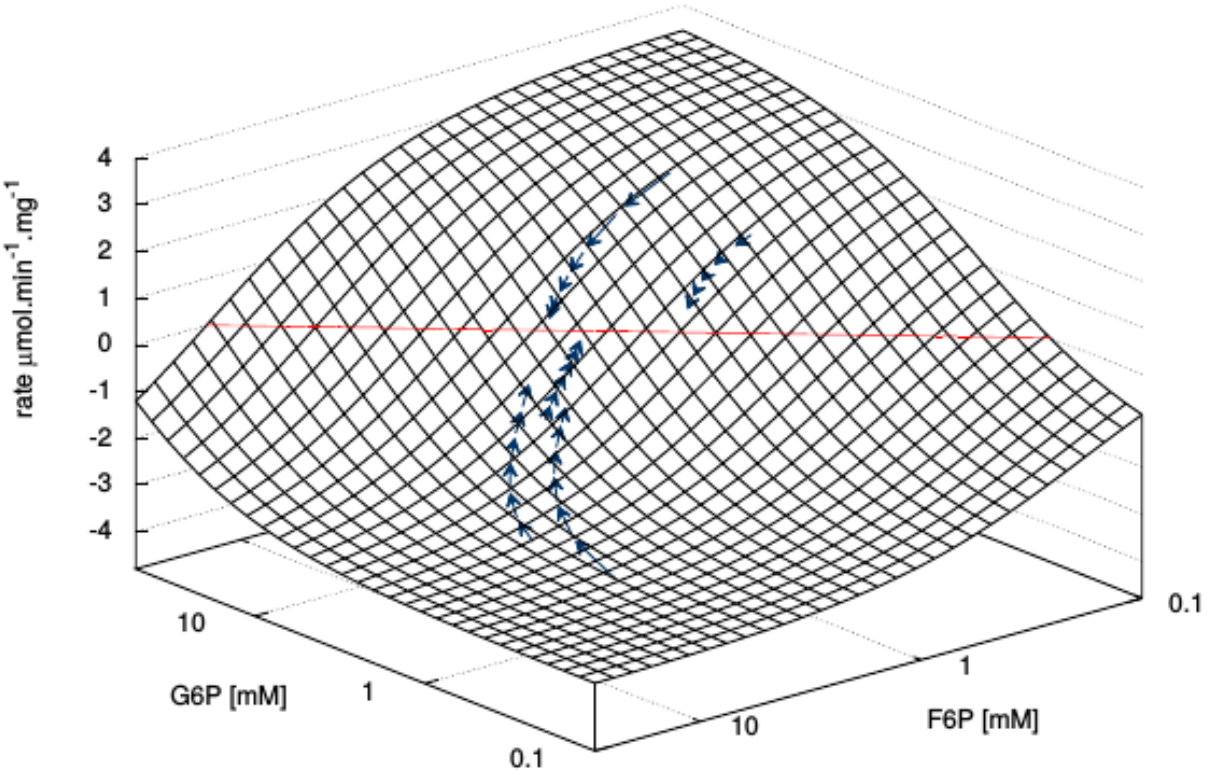


^{31}P-NMR time-course and spline-fitting of PGI





Rate equation fitting to PGI data



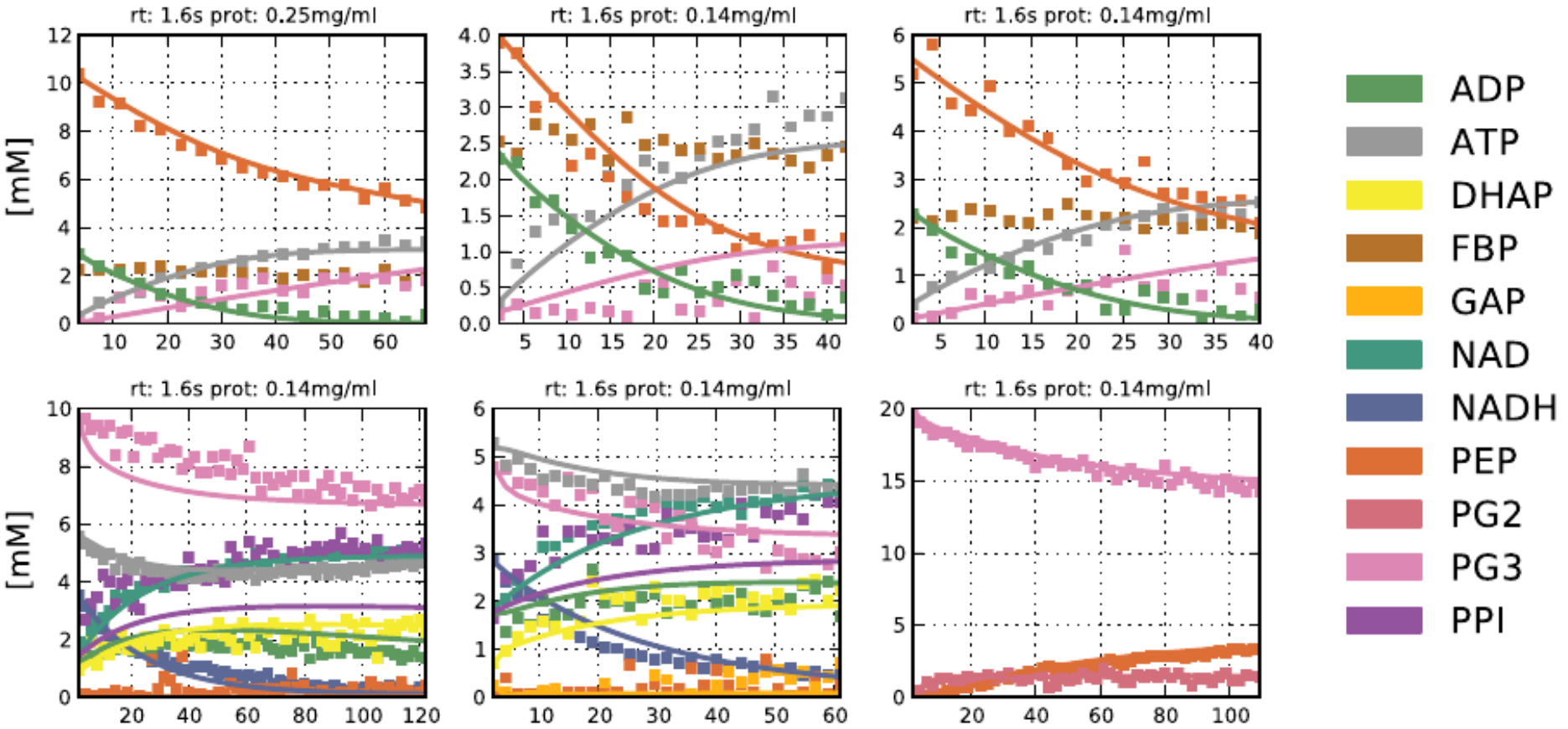
$$v = V_f \frac{g6p\left(1 - \frac{\Gamma}{K_{eq}}\right)}{1 + g6p + f6p}$$

Fitted parameters:

Param.	Value
V_f	3.551 ± 0.050
$G6P_{0.5}$	0.550 ± 0.236
$F6P_{0.5}$	0.152 ± 0.017
K_{eq}	$0.286 \pm 8 \times 10^{-6}$

(rates: $\mu mol.min^{-1}.mg^{-1}$)
(concentrations: mM)

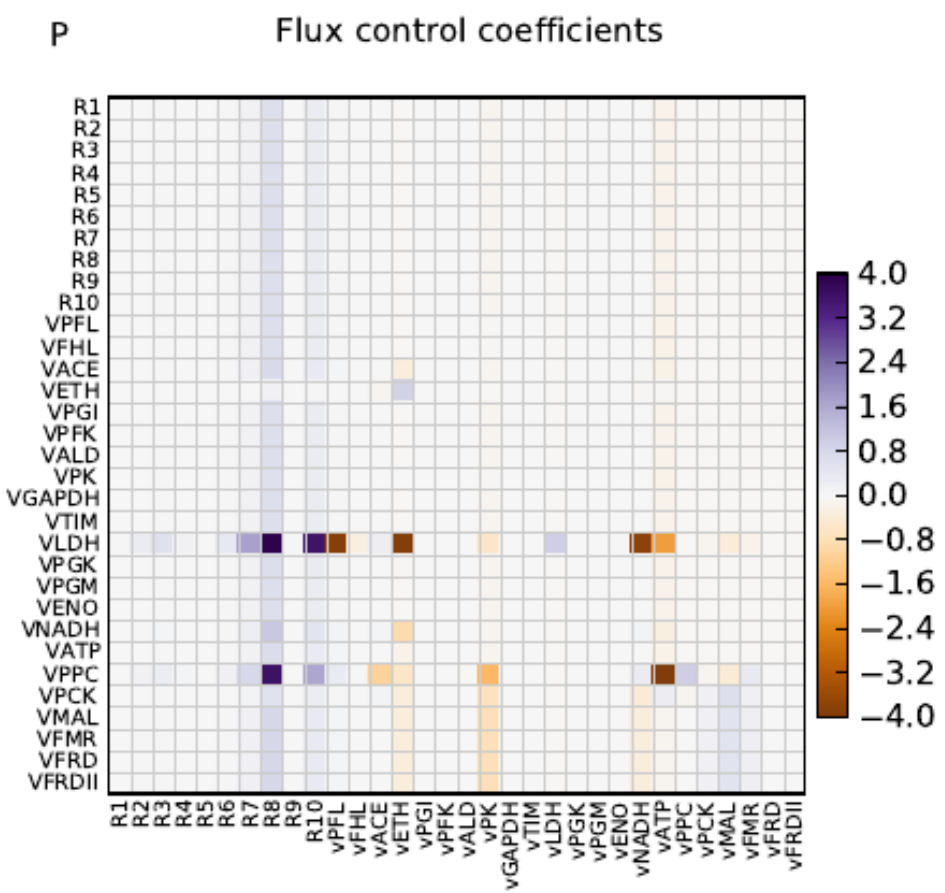
Fitted ^{31}P-NMR time-courses for lower glycolysis

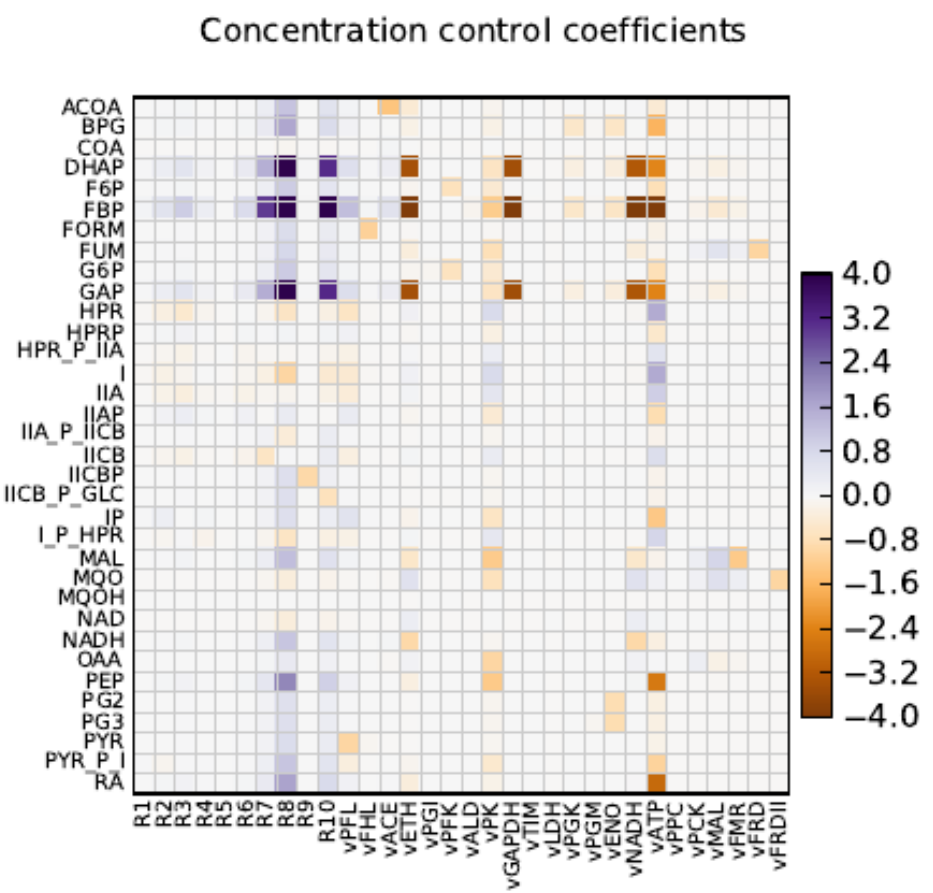


Parameters from global fit of lower glycolysis time-courses

$\alpha_{FBP}PK$	2.2282 ± 0.0649	K_{NADH}	14.6237 ± 0.4825
$hENO$	1.2984 ± 0.0437	$K_{PEP}PK$	5.8857 ± 0.2349
$hPGK$	3.0249 ± 0.1068	$K_{3PG}PGK$	4.4152 ± 0.1291
$hPGM$	3.0952 ± 0.5659	$K_{PPI}GAPDH$	0.3984 ± 0.0252
hPK	1.0659 ± 0.1022	$K_{PYR}PK$	19.8315 ± 0.6827
$hTIM$	2.9615 ± 0.1812	$K_{PEP}ENO$	3.43 ± 0.2353
$K_{ADP}PGK$	0.0048 ± 0.0003	$K_{2PG}ENO$	4.6794 ± 0.284
$K_{ADP}PK$	0.6337 ± 0.0385	$K_{2PG}PGM$	0.6046 ± 0.0124
$K_{ATP}PGK$	1.9728 ± 0.0857	$K_{3PG}PGM$	0.0749 ± 0.0026
$K_{ATP}PK$	0.2343 ± 0.0135	V_fADK	0.0058 ± 0.0001
$K_{BPG}GAPDH$	3.1083 ± 0.3757	V_fENO	2.536 ± 0.1273
$K_{BPG}PGK$	0.0109 ± 0.0002	V_fGAPDH	1.2265 ± 0.0693
$K_{DHAP}TIM$	2.3903 ± 0.0634	V_fNADH	3.388 ± 0.1169
$K_{GAP}GAPDH$	0.0219 ± 0.0011	V_fPGK	4.8766 ± 0.4135
$K_{GAP}TIM$	0.4725 ± 0.0222	V_fPGM	2.82 ± 0.1773
$K_{NAD}GAPDH$	1.9096 ± 0.0646	V_fPK	3.8881 ± 0.1649
$K_{NADH}GAPDH$	0.1697 ± 0.026	V_fTIM	13.8716 ± 0.7641

in silico: Metabolic Control Analysis





$$C_v^y = \frac{\partial y_i / y_i}{\partial v_j / v_j} = \frac{\partial \ln y_i}{\partial \ln v_j}$$

$C_{R8}^{J_{glyc}}$	0.63	C_{vATP}^{RA}	−2.87
$C_{R10}^{J_{glyc}}$	0.29	C_{R8}^{RA}	1.71
$C_{vATP}^{J_{glyc}}$	−0.15	C_{R10}^{RA}	0.78
$C_{R7}^{J_{glyc}}$	0.14	C_{R7}^{RA}	0.37

People

- Students who have completed their degrees
  - Christiaan Crous, MSc (*Z. mobilis* SDA)
  - Sandra Alberts, MSc (*L. lactis* SDA)
  - Justin Smith, MSc (*S. cerevisiae* SDA & kinetics)
  - Johann Eicher, PhD (*E. coli* SDA, kinetics & modelling)
- Academic staff
  - Prof Jacky Snoep

Ongoing work

- Question: are kinetic measurements in the *test tube* a realistic representation of *conditions in the cell*?
- Investigate "*in vivo*" enzyme kinetics
  - effect of macromolecular crowding
    - intracellular environment has very little accessible solvent space, [protein] = 250 mg/ml
  - effect of pH changes
    - intracellular pH not as constant as always thought, signal?
- *E. coli* and *S. cerevisiae* glycolytic enzymes

Current Students

- Z. mobilis kinetics and modelling
  - Theo v. Staden, PhD (*Z. mobilis* kinetics & modelling)
- Macromolecular crowding
  - Chris van Niekerk, PhD
  - Julian Wissing, MSc
- pH effect on kinetics and modelling
  - Tiaan Swanepoel, MSc

Model: Example

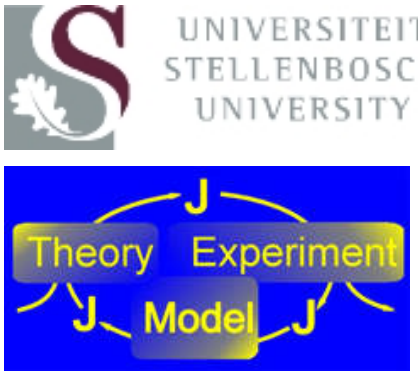
Modelling cellular redoxin networks

Johann Rohwer

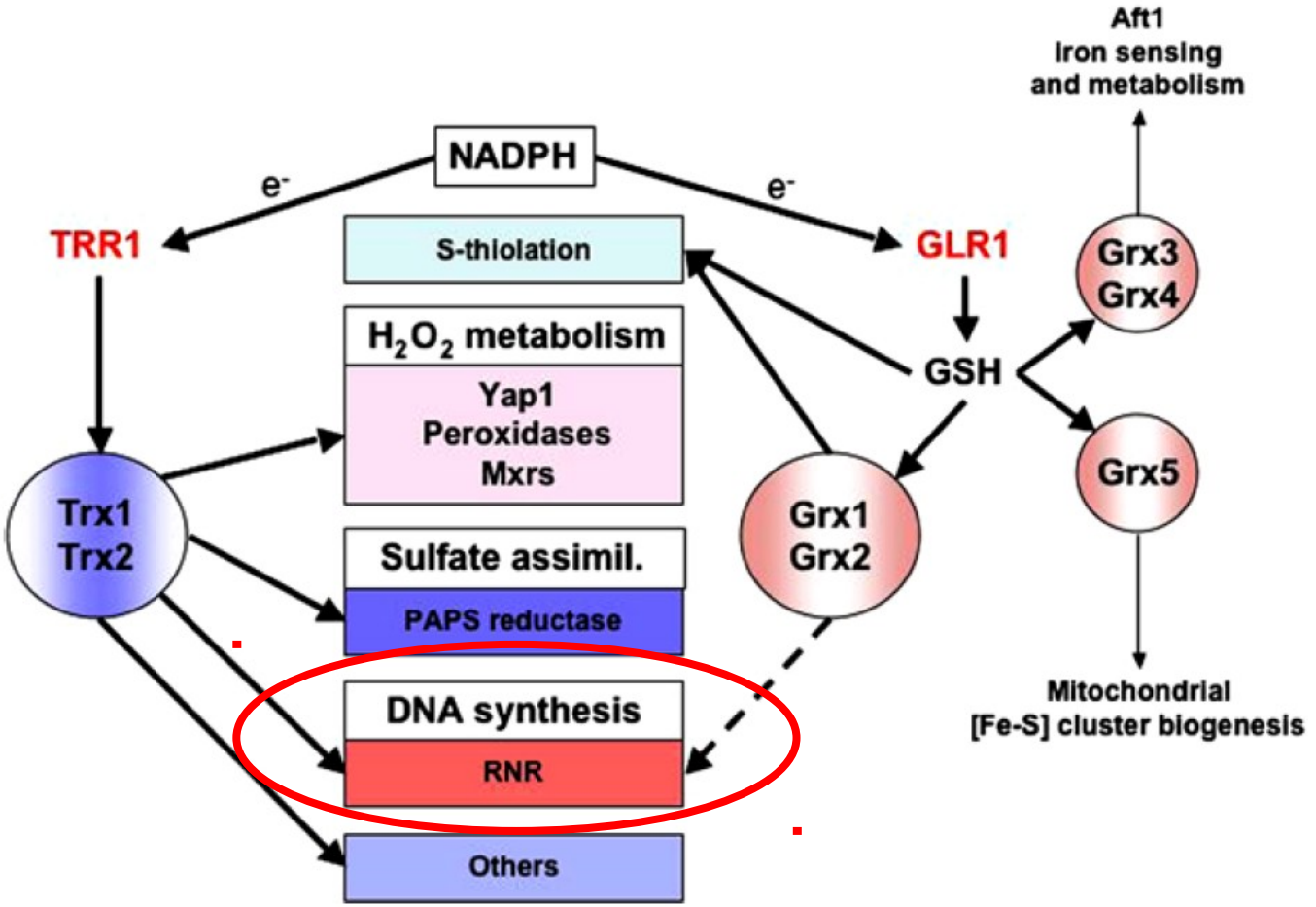
Laboratory for Mol. Systems Biology
Dept. of Biochemistry
Stellenbosch University
South Africa

Ché Pillay

School of Life Sciences
UKZN
Pietermaritzburg
South Africa

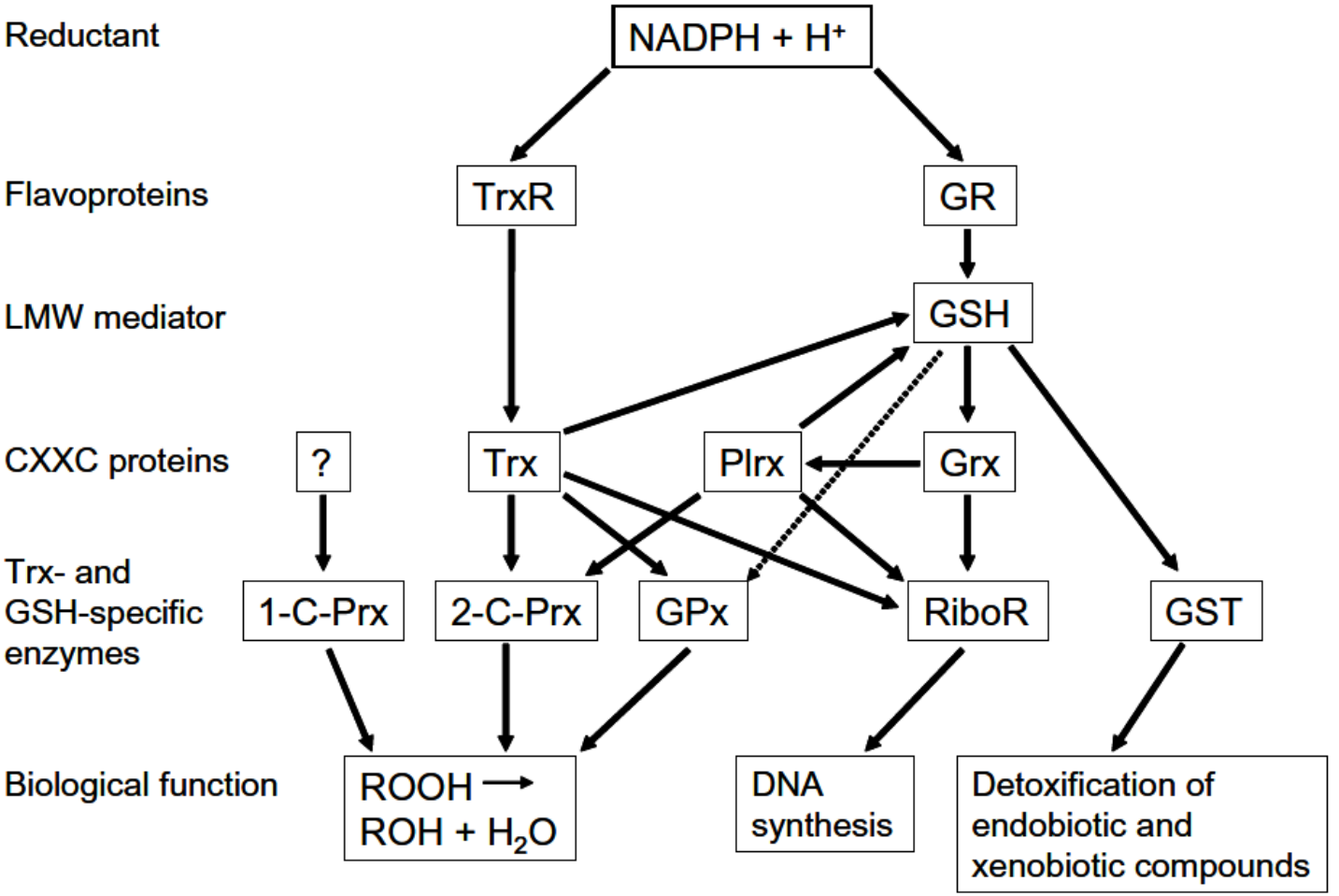


Redoxin networks are essential for all living organisms



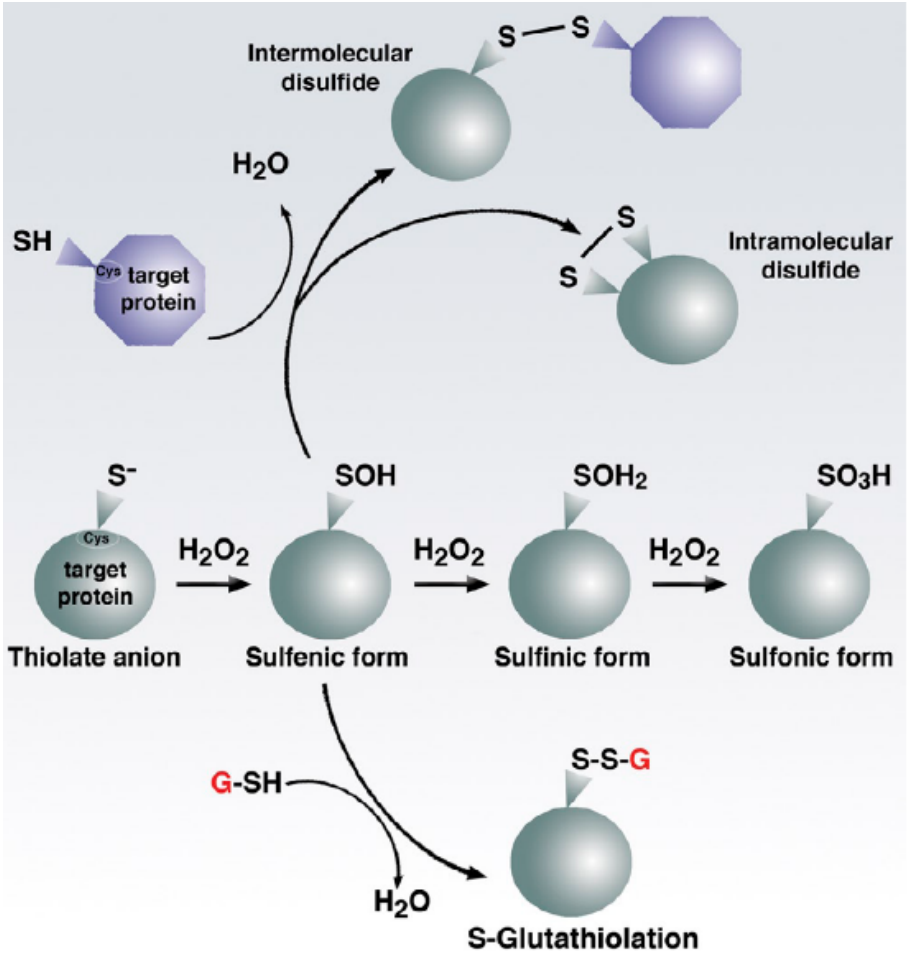
Toledano et al. (2007) FEBS Lett.

Redoxin networks play important roles in health and disease



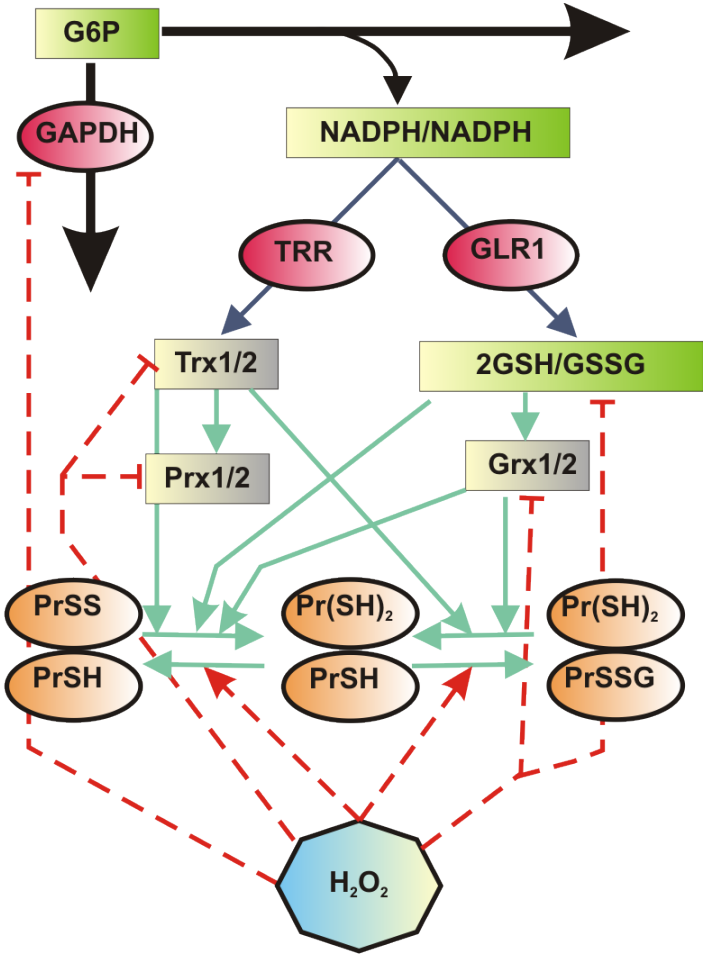
Jaeger, Flohé (2006) BioFactors

Oxidation states of biological thiol groups

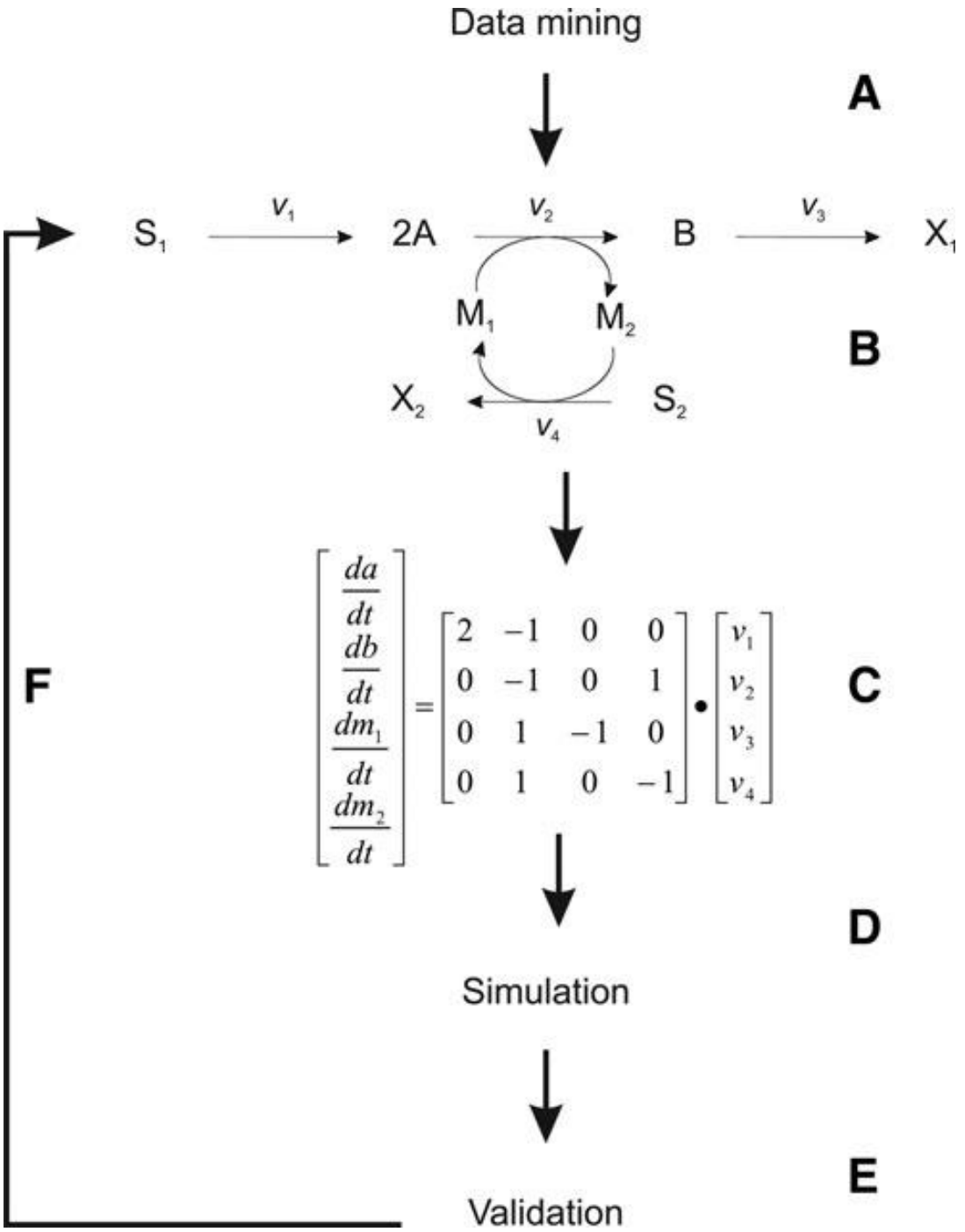


Finkel (2011) J. Cell Biol.

Complex interconnectivity of redoxin networks



Kinetic modelling workflow



Contents

- Enzymes or redox couples?
- The “logic” of the *E. coli* thioredoxin system
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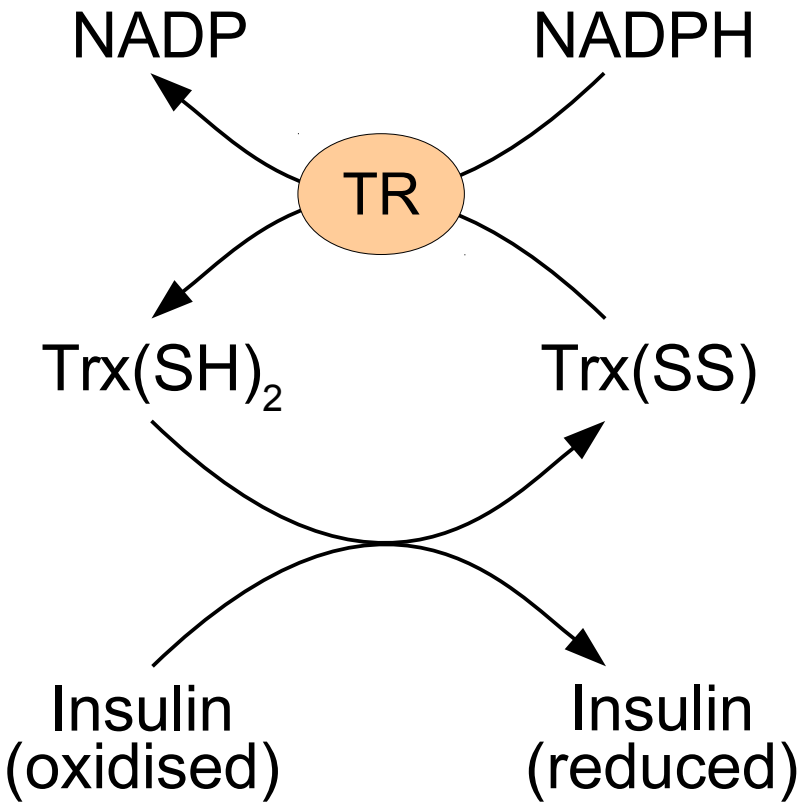
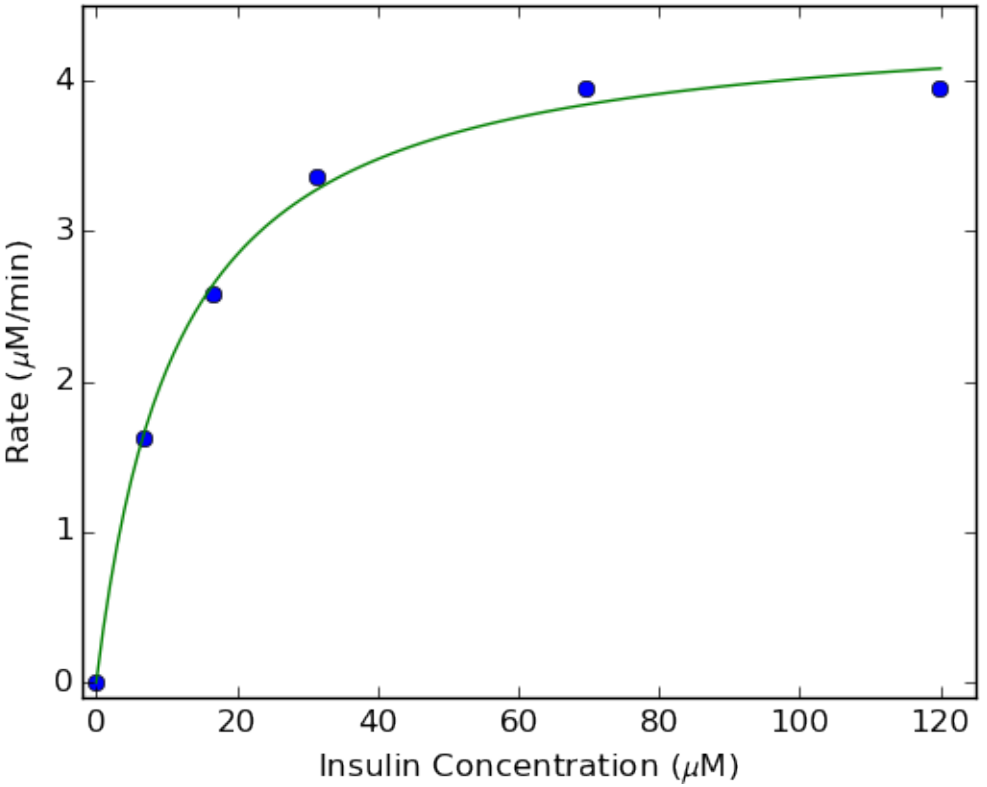
Thioredoxin system

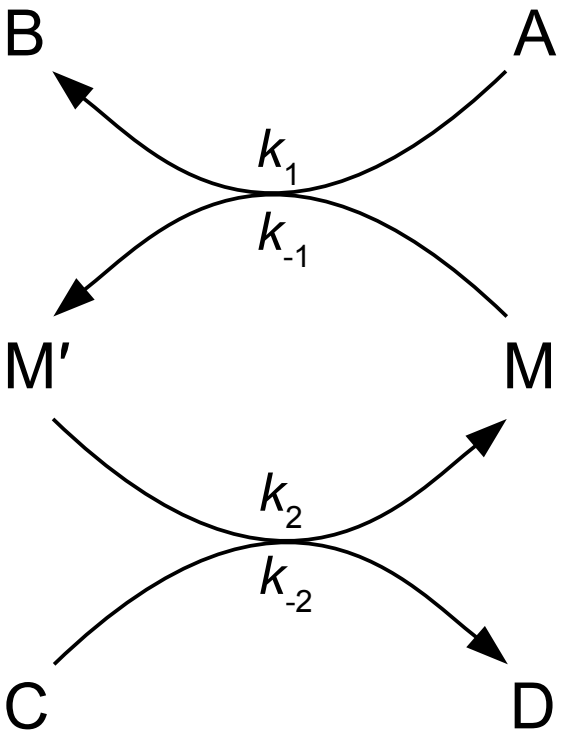




Data: Holmgren 1979 JBC
Model fit: Pillay et al. 2009 Biochem. J.

- classic Michaelis-Menten response
- model with *mass action*

Core model



- differential equation

$$\frac{dm'}{dt} = k_1 am - k_{-1} bm' - k_2 cm' + k_{-2} dm$$

- solve for *m* subject to steady state

$$m = \frac{m_t (k_{-1} b + k_2 c)}{k_1 a + k_{-2} d + k_{-1} b + k_2 c}$$

- calculate v_2

$$v_2 = \frac{k_1 k_2 m_t a c \left(1 - \frac{\Gamma}{K_{eq}}\right)}{k_1 a + k_{-2} d + k_{-1} b + k_2 c}$$

- irreversible case

$$v_2 = \frac{(k_1 a m_t) c}{\frac{k_1}{k_2} a + c}$$

Apparent V_{max}: $k_1 a m_t$

Apparent K_m: $\frac{k_1}{k_2} a$

$$\therefore \quad \frac{k_{cat}}{K_m} = k_2$$

Pillay et al. (2009) Biochem. J.

Changes in apparent Michaelis-Menten parameters

Model Parameters				Apparent M-M Parameters			
V_{TR}	k_2	$[Trx]_t$	NADPH	K_m	V_{max}	k_{cat}	k_{cat}/K_m
1	1	2	1	0.186	0.386	0.193	1.035
10	1	2	1	2.183	4.378	2.189	1.003
100	1	2	1	21.925	43.860	21.930	1.000
1	10	2	1	0.012	0.348	0.174	14.620
1	100	2	1	0.001	0.333	0.167	263.158
1	1	50	1	0.001	0.496	0.010	8.333
1	1	0.1	1	0.462	0.046	0.463	1.002
1	1	2	10	0.356	0.724	0.362	1.018
1	1	2	0.1	0.026	0.065	0.032	1.236

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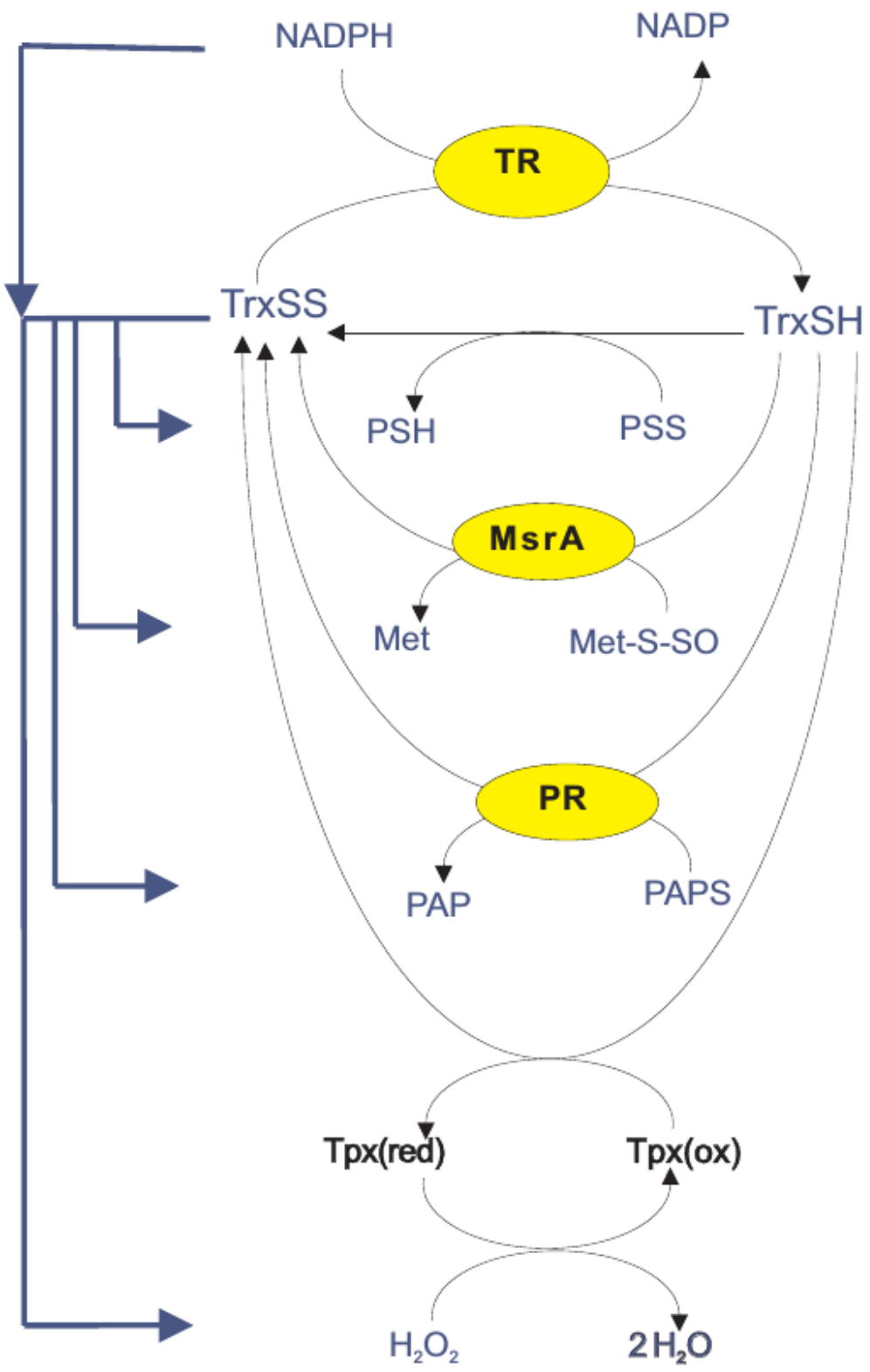
Thioredoxin system of *E. coli*

- numerous molecular targets of TrxSH

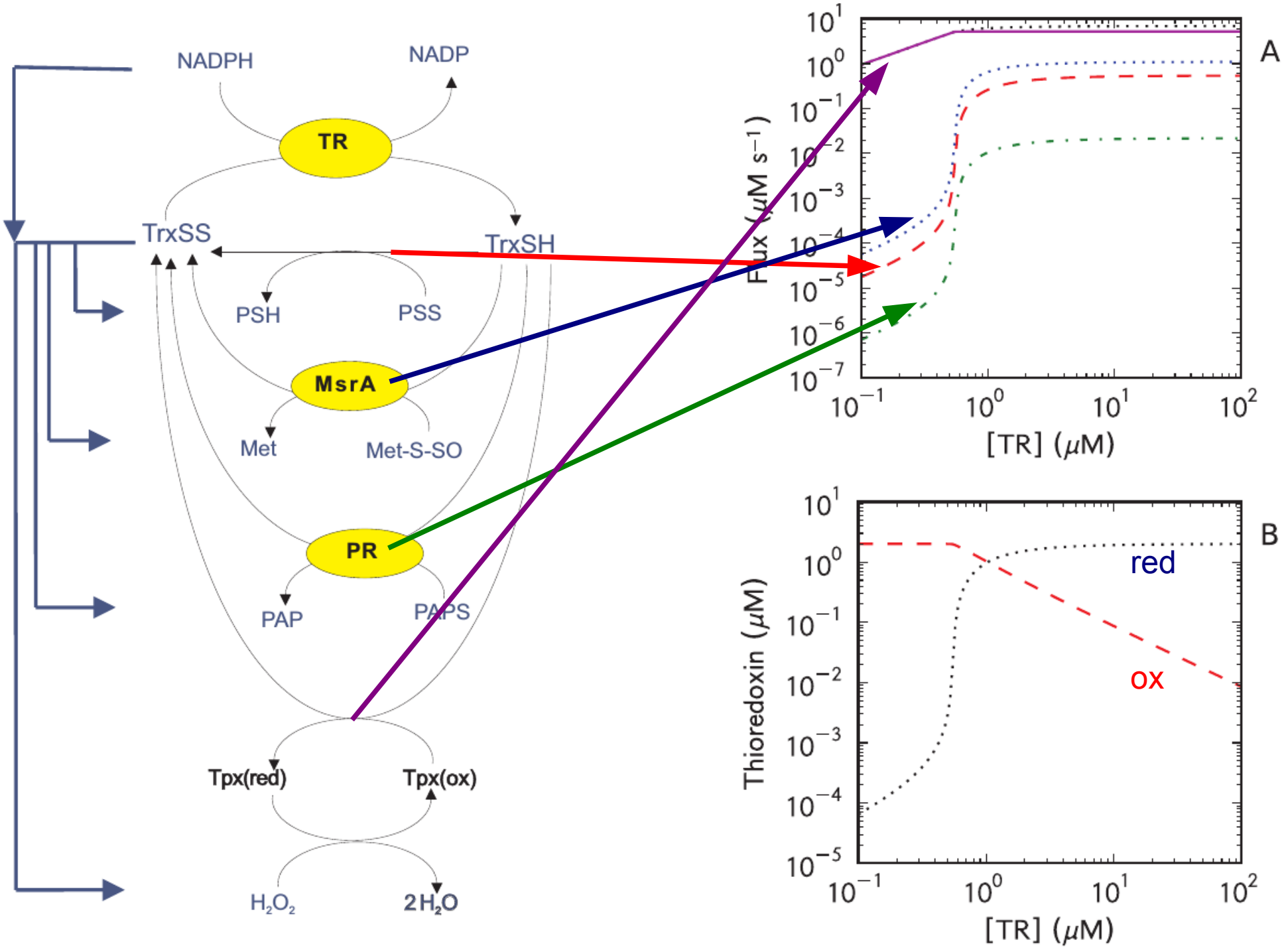


Kinetic motifs

- adaptable systems
- ultrasensitive responses
- interconnectivity
- insulated vs. sensitive subsystems

Pillay, Hofmeyr, Rohwer (2011) BMC Syst. Biol.

Ultrasensitive responses



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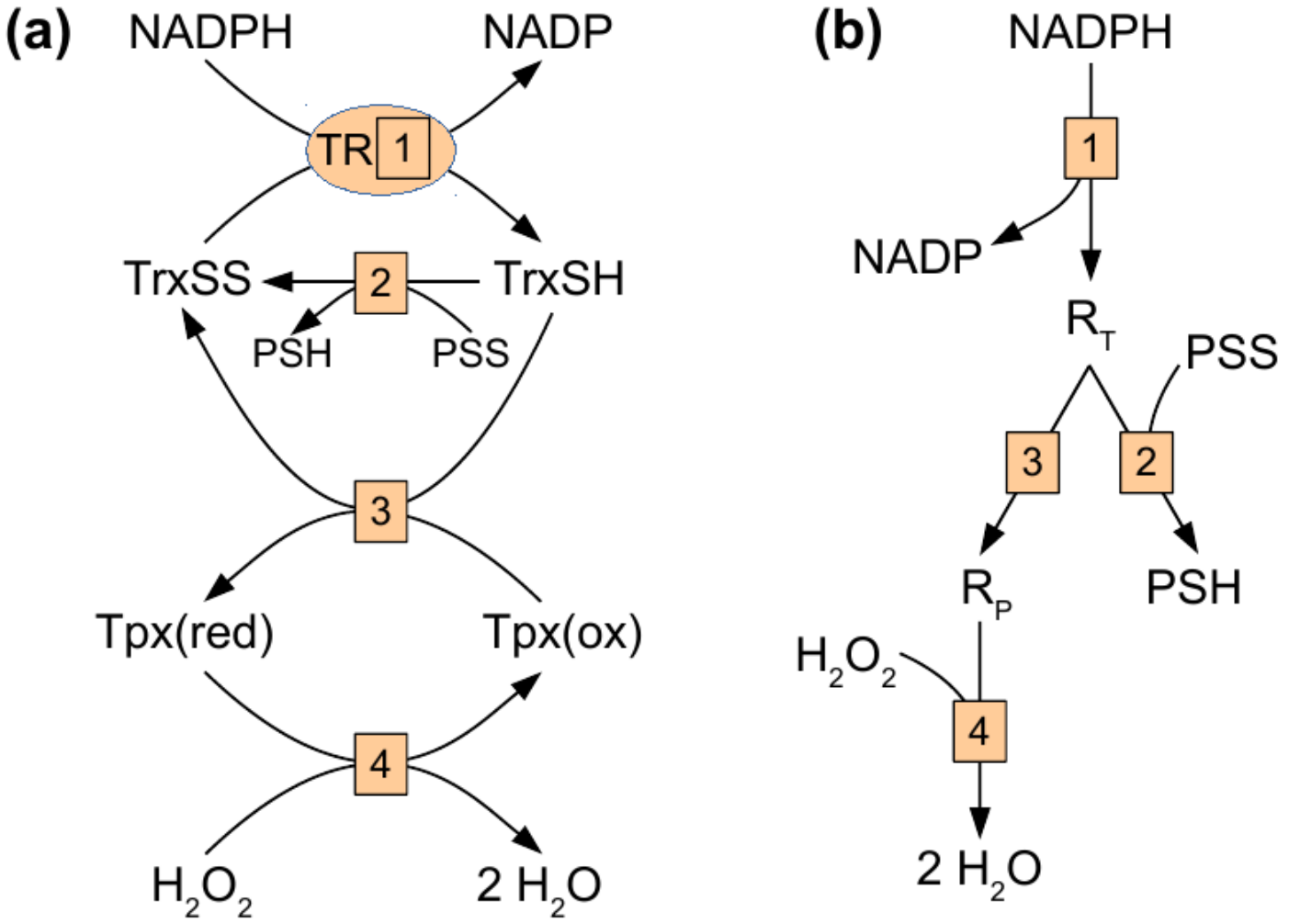
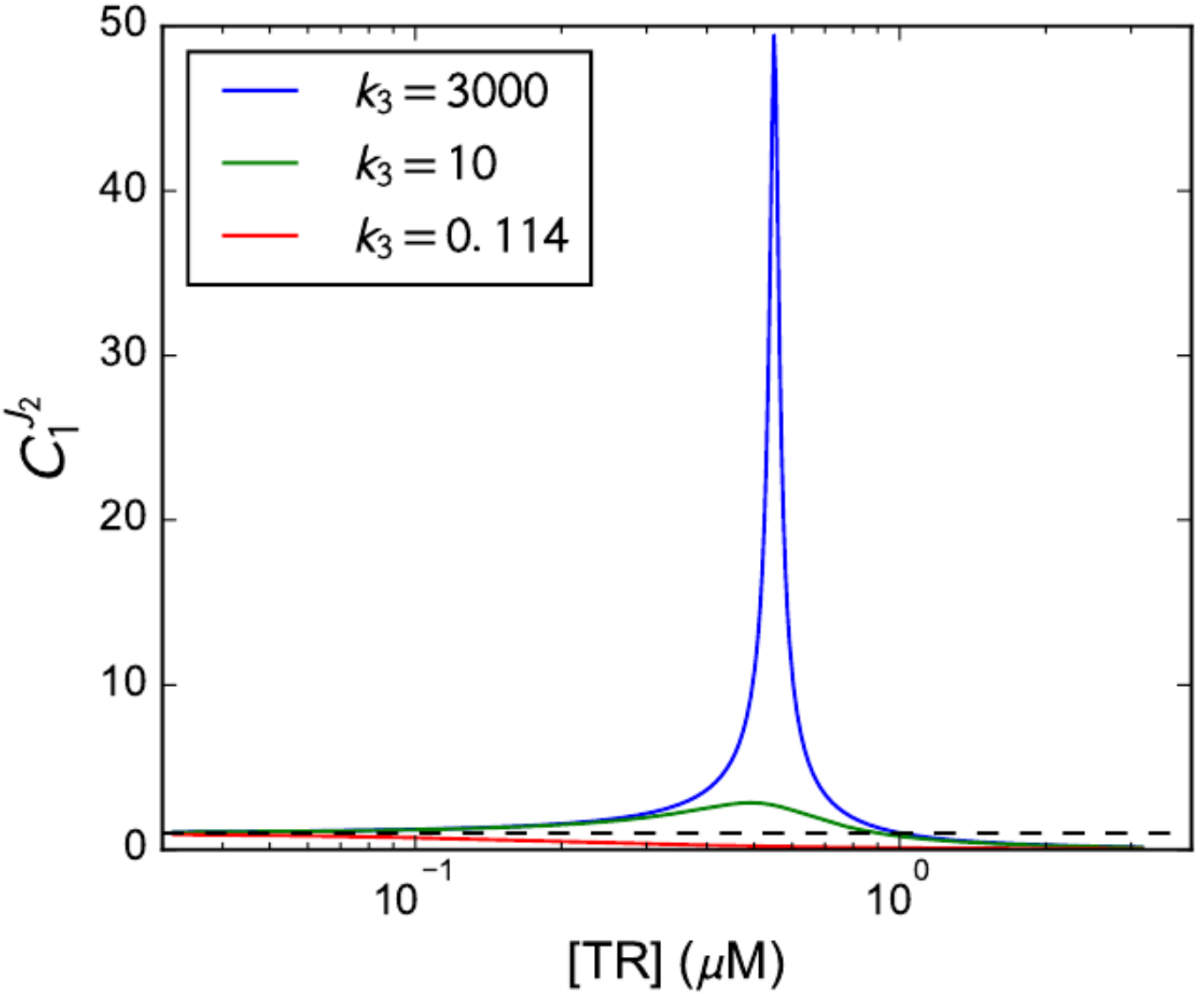
Simplified model



Ultrasensitivity i.t.o. MCA



Conditions for ultrasensitivity

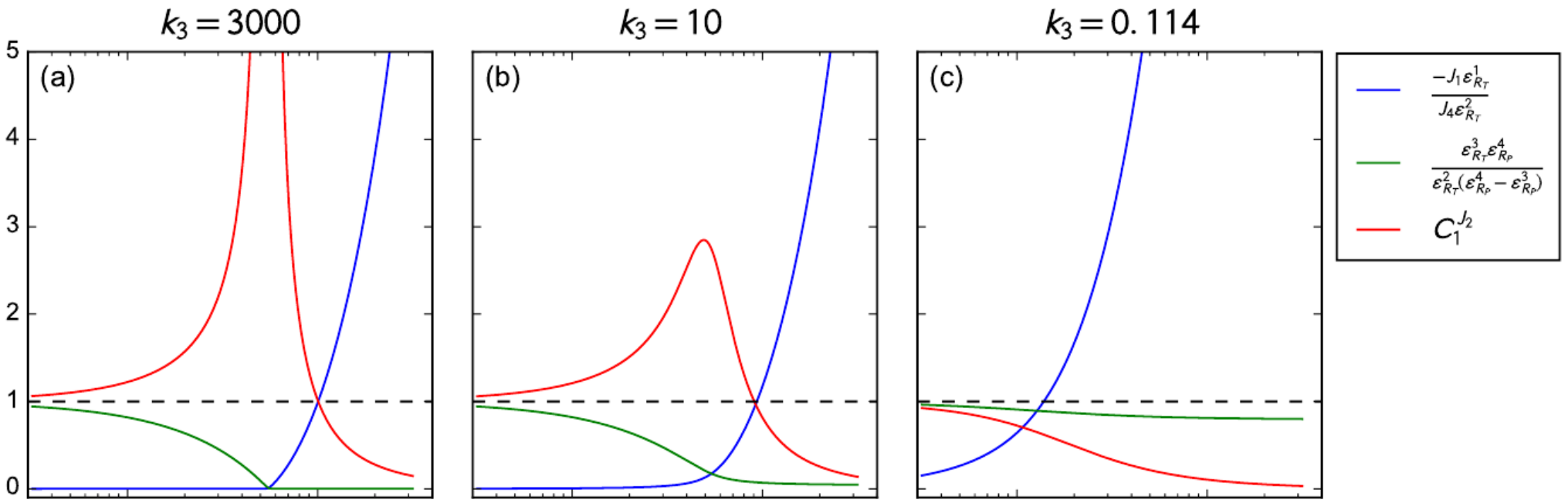
$$C_1^{J_2} = \frac{-J_1\varepsilon_{R_T}^{v_2}\varepsilon_{R_P}^{v_3} + J_1\varepsilon_{R_T}^{v_2}\varepsilon_{R_P}^{v_4}}{J_1\varepsilon_{R_T}^{v_1}\varepsilon_{R_P}^{v_3} - J_1\varepsilon_{R_T}^{v_1}\varepsilon_{R_P}^{v_4} - J_2\varepsilon_{R_T}^{v_2}\varepsilon_{R_P}^{v_3} + J_2\varepsilon_{R_T}^{v_2}\varepsilon_{R_P}^{v_4} + J_4\varepsilon_{R_T}^{v_3}\varepsilon_{R_P}^{v_4}}$$

$$-\frac{J_1\varepsilon_{R_T}^{v_1}}{J_4\varepsilon_{R_T}^{v_2}} + \frac{\varepsilon_{R_T}^{v_3}\varepsilon_{R_P}^{v_4}}{\varepsilon_{R_T}^{v_2}\left(\varepsilon_{R_P}^{v_4} - \varepsilon_{R_P}^{v_3}\right)} < 1$$

Condition 1: $$-\frac{J_1\varepsilon_{R_T}^{v_1}}{J_4\varepsilon_{R_T}^{v_2}} < 1$$

Condition 2: $$\frac{\varepsilon_{R_T}^{v_3}\varepsilon_{R_P}^{v_4}}{\varepsilon_{R_T}^{v_2}\left(\varepsilon_{R_P}^{v_4} - \varepsilon_{R_P}^{v_3}\right)} < 1$$

Plot conditions



Summary

- redoxin systems should be modelled as redox couples with mass-action kinetics
- these systems exhibit kinetic motifs:
  - adaptability
  - ultrasensitivity
  - interconnectivity vs. insulation

Work in progress...

- comparative analysis of redoxin networks
  - Generalised Supply-Demand Analysis
- quantitative analysis of redox signalling
- develop kinetic models of
  - peroxiredoxins
  - *Mycobacterium tuberculosis* redoxin network

Conclusions

- Why should I model redoxin networks?
  - detect kinetic motifs
  - fit parameters from experimental data
  - explore untested scenarios
  - quantify control parameters
  - unravel mechanism

People

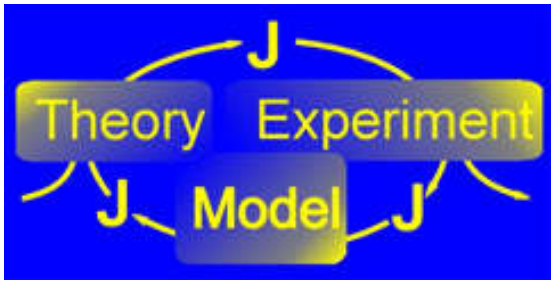
- University of KwaZulu-Natal (Pietermaritzburg)
  - Dr Ché Pillay
  - Lefentse Mashamaite (PhD)
  - Nolyn John (MSc)
- Stellenbosch University
  - Prof Jannie Hofmeyr
  - Charl Viljoen (BSc Hons 2015)
  - Chris Barry (PhD 2017-)

Theory

- generalised supply-demand analysis
- rate equations for modelling
- symbolic MCA
- *in vitro vs. in vivo* kinetics

Experiment

- NMR “metabolomics”
  - *in vivo, in situ, in vitro* metabolite measurements
- enzyme kinetics for modelling
  - *in vivo* enzyme kinetics
  - pH, macromolecular crowding



Model

- development of software (PySCeS, psctb, SymCA, RateChar)
- kinetic models of cellular systems
  - microbial energy metabolism
  - cellular redoxin networks (with Dr C Pillay, UKZN)
  - plant metabolism
  - glucocorticoid receptor dimerisation (with Prof A Louw)

Interested in joining the lab?

- Contact me any time to discuss options!
- jr@sun.ac.za or 021-808-5843