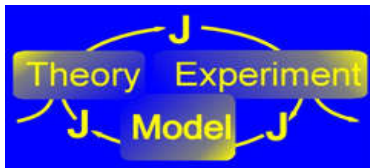


Overview of Research Activities

Biochemistry Honours Students
11 April 2024

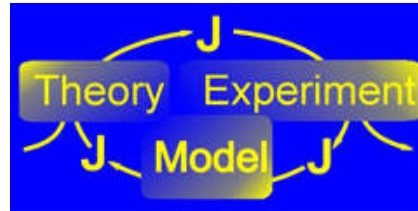
Prof Johann Rohwer

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



Theory

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



Model

- kinetic models of cellular systems
 - microbial energy metabolism
 - cellular redoxin networks (with Dr C Pillay, UKZN)
 - plant metabolism

Experiment

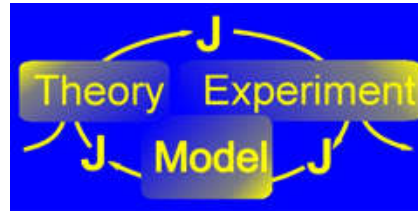
- NMR “metabolomics”
 - *in vivo*, *in situ*, *in vitro* metabolite measurements
- enzyme kinetics for modelling
 - *in vivo* enzyme kinetics
 - pH, macromolecular crowding

Model / Experiment

- software development
 - PyscesToolbox
 - NMRPy
 - PyEnzyme
 - LabNexus
- CoA metabolism

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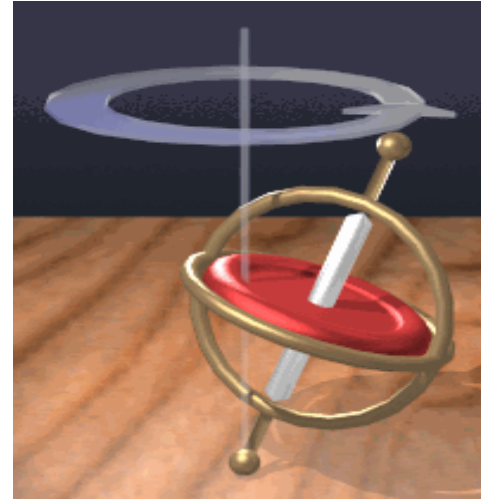
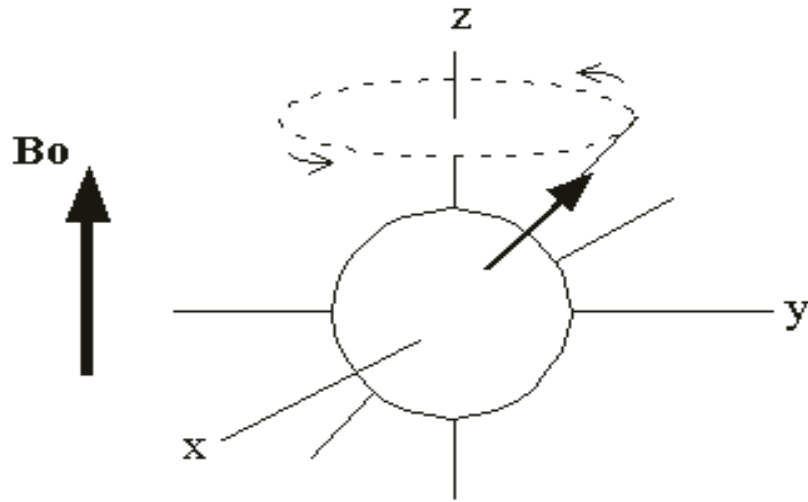
Model / Experiment

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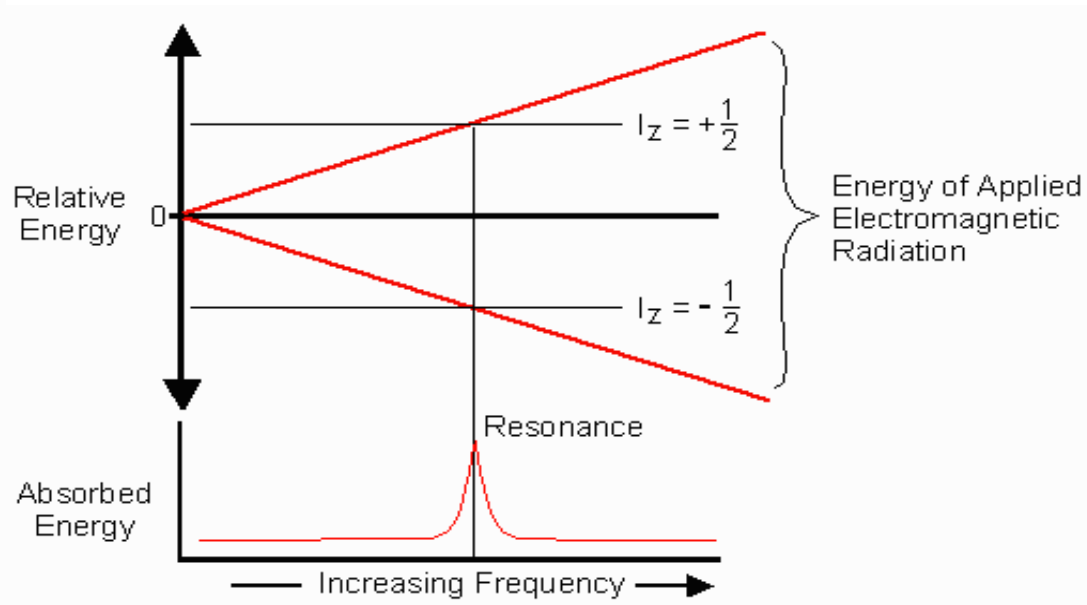
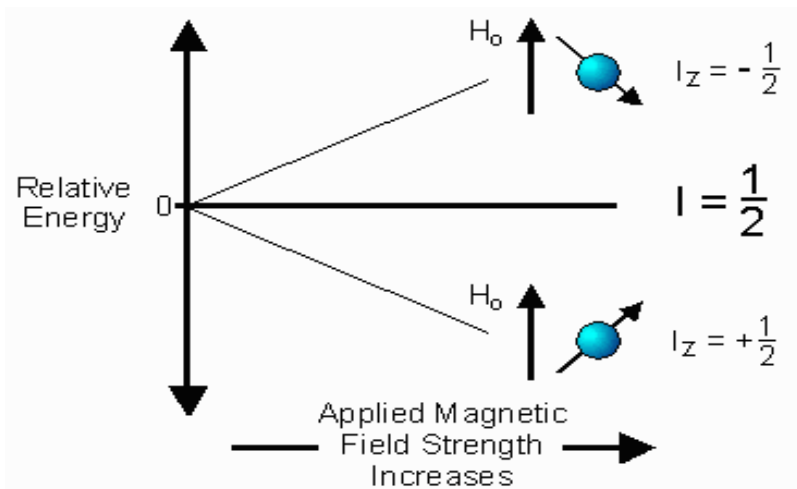
Experiment: Example

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

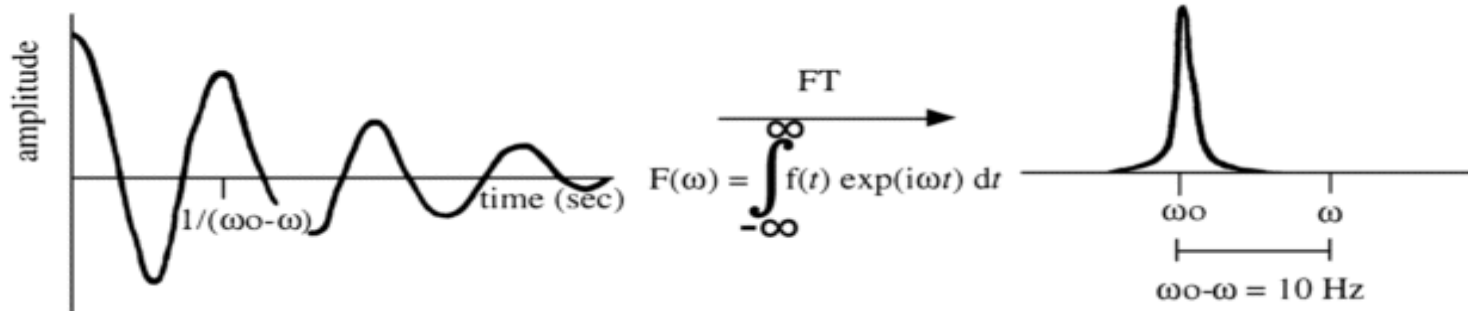
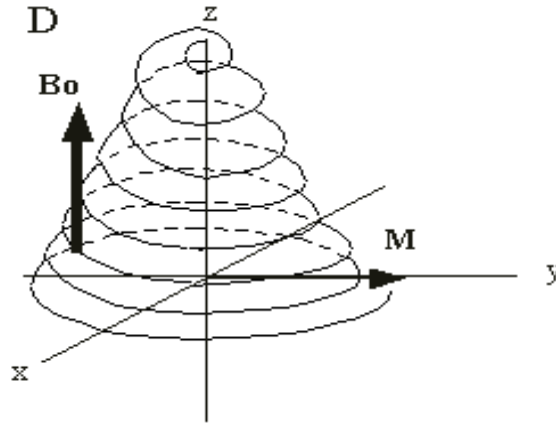
Brief NMR introduction: Nucleus in magnetic field



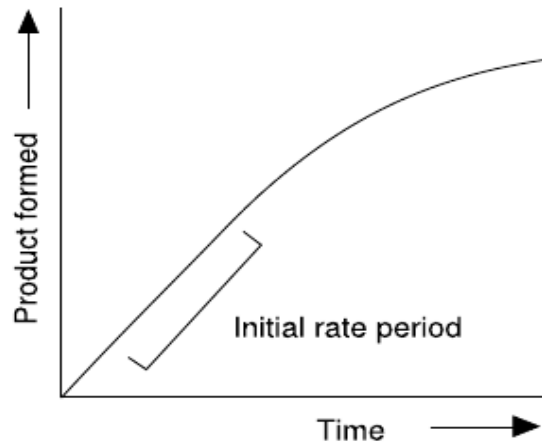
Split in energy levels



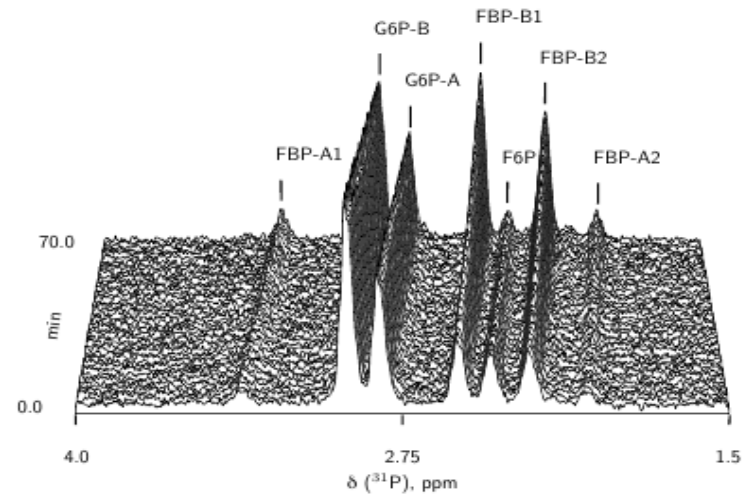
Relaxation and Fourier Transform



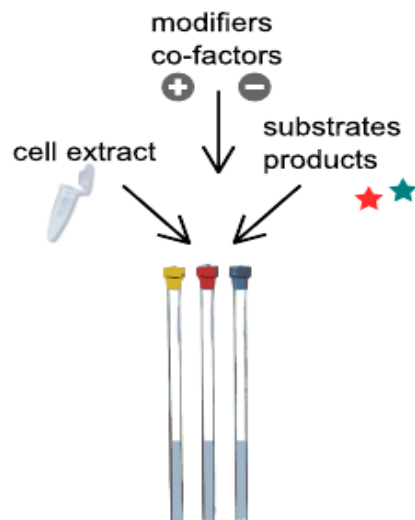
Initial rate assays



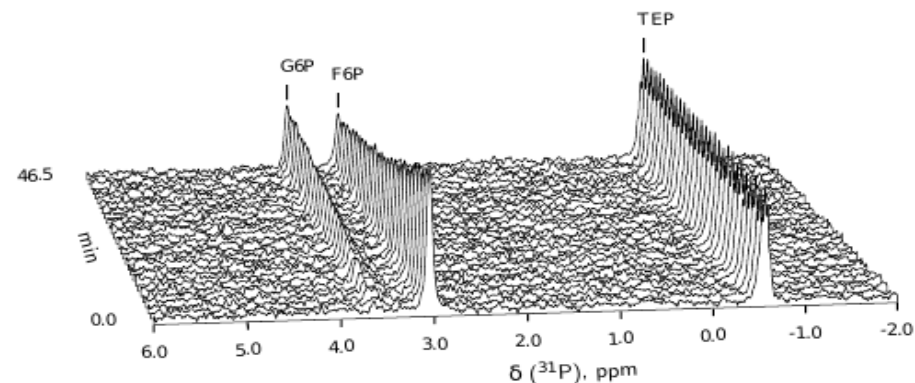
NMR progress curve assays



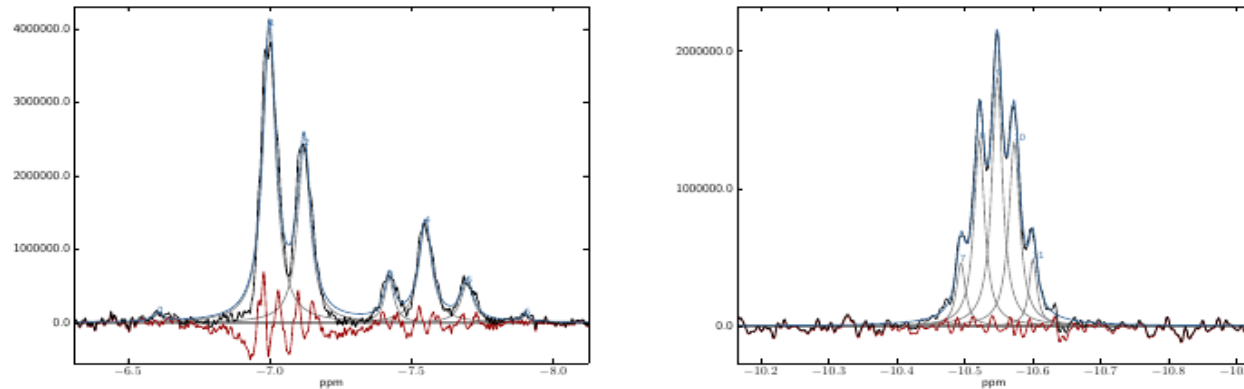
- 1 Incubate, possibly supplementing with ^{13}C -labelled substrate



- 2 Acquire time series of NMR spectra



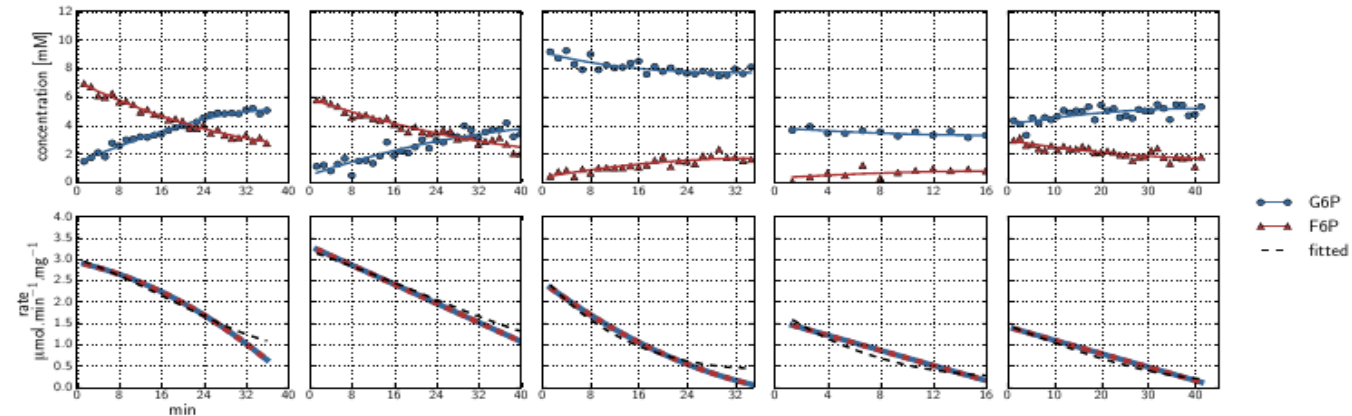
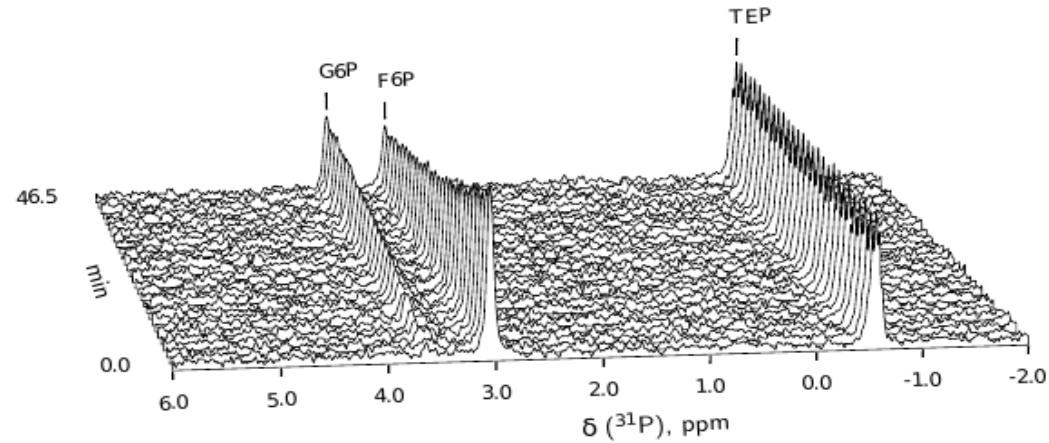
3 Deconvolute spectra to determine peak area



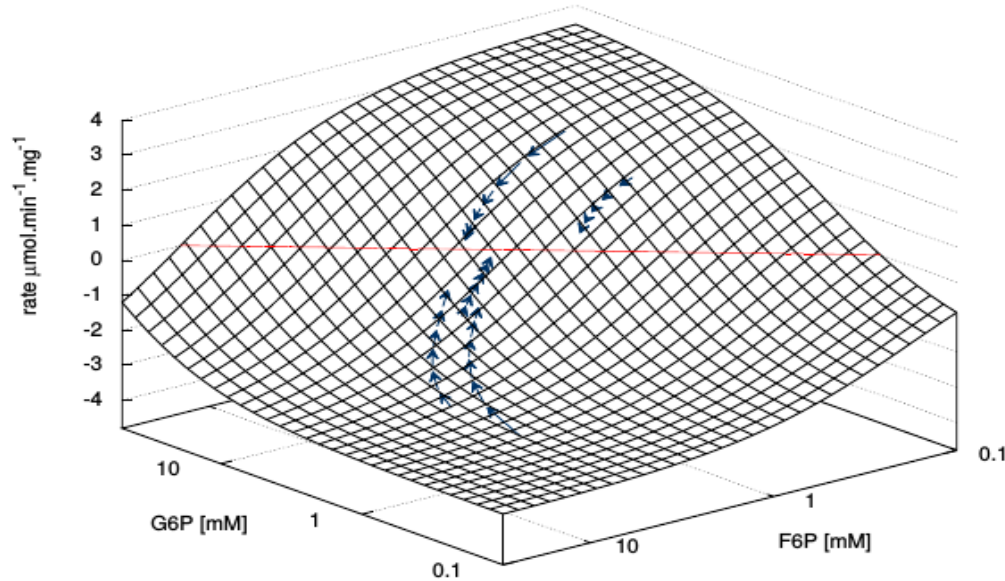
4 Fit splines to concentration time-courses, determine rates

5 Global fit of data to parameterise rate equation

^{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}	$0.286 \pm 8 \times 10^{-6}$

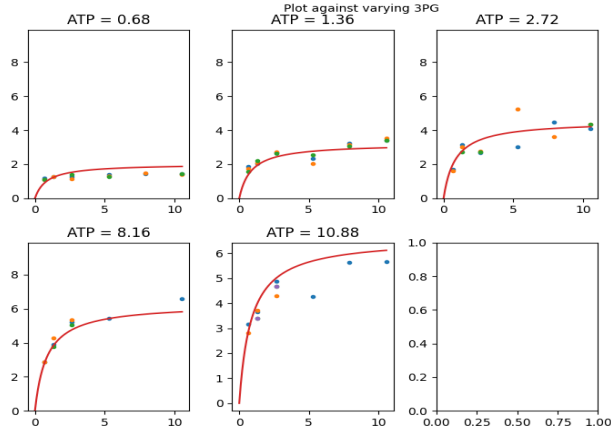
(rates: $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)
(concentrations: mM)

Ongoing work

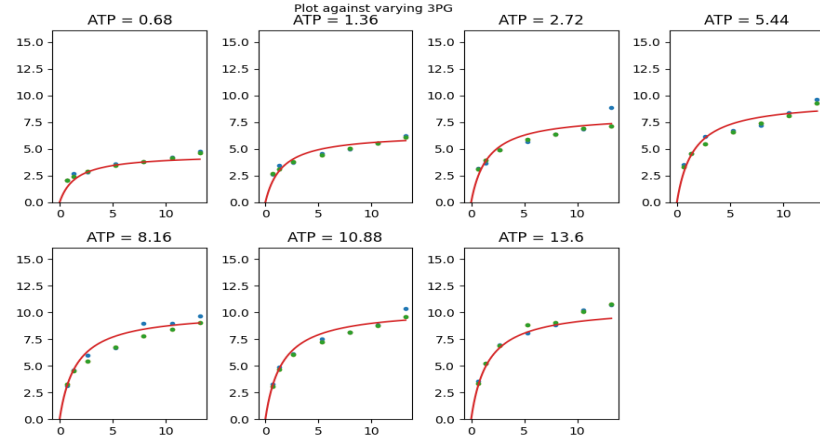
- Question: are kinetic measurements in the *test tube* a realistic representation of *conditions in the cell*?
- Investigate “*in vivo*” enzyme kinetics
 - effect of macromolecular crowding
 - intracellular environment has very little accessible solvent space, [protein] = 250 mg/ml
 - effect of pH changes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

Effect of pH on PGK kinetics

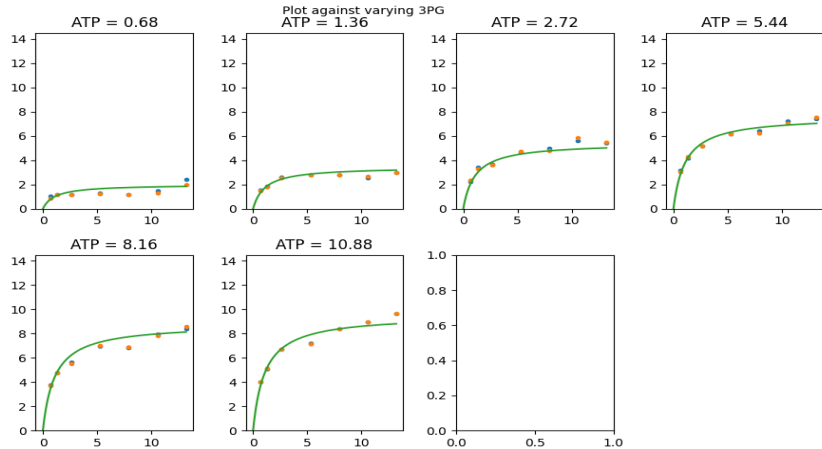
pH 5.5



pH 7



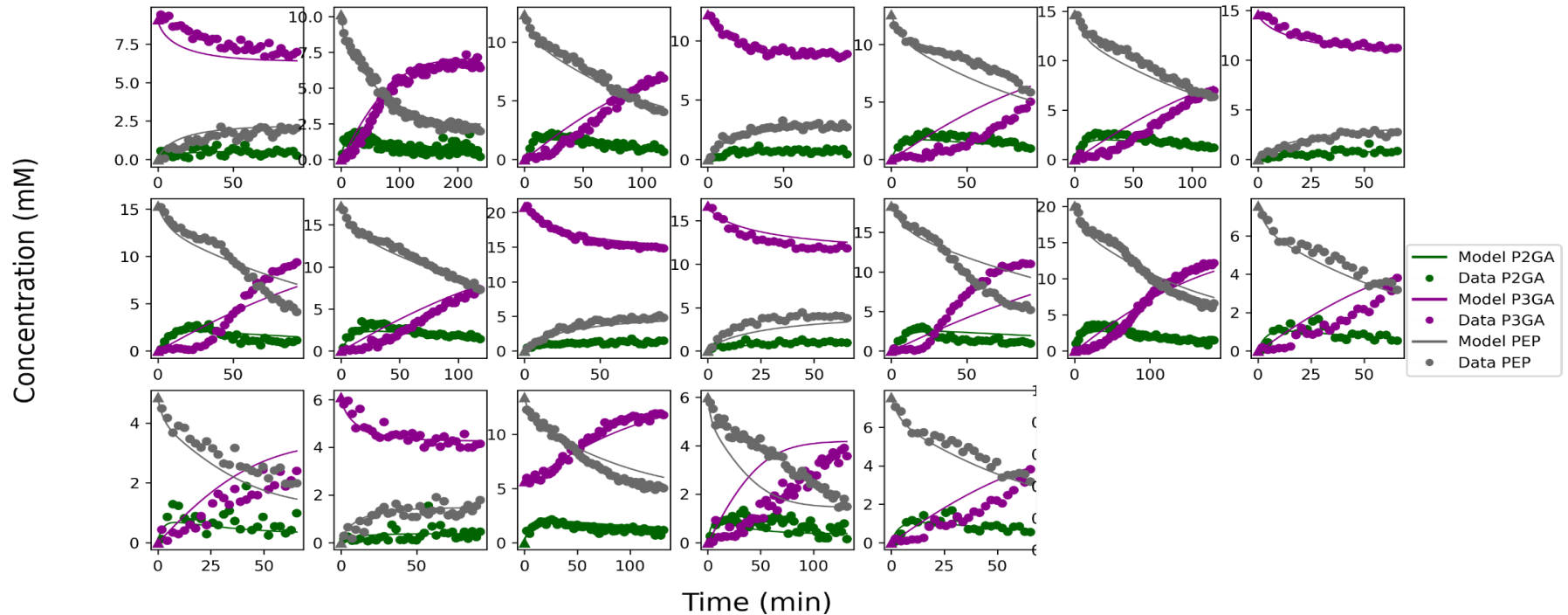
pH 8



Parameter	pH 5.5	pH 7	pH 8
V_f	7.8 ± 0.3	11.4 ± 0.2	12.7 ± 0.3
K_{ATP}	1.9 ± 0.2	1.0 ± 0.1	3.7 ± 0.2
K_{3PG}	0.85 ± 0.08	1.6 ± 0.1	1.1 ± 0.1

Effect of crowding on PGM/ENO kinetics

10% dextran



Effect of crowding on PGM/ENO kinetics



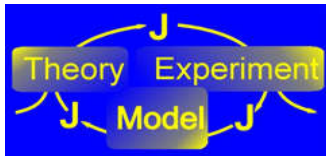
Parameter	No crowder	10% dextran	20% dextran
$V_{f_{\text{eno}}}$	4.97 (4.9 - 31.13)	1.05 (0.31 - 1.87)	6.27
$K_{\text{eq}_{\text{eno}}}$	2.37 (2.01 - 2.46)	2.87 (2.1 - 3.10)	5.34
$K_{\text{p2ga}_{\text{eno}}}$	0.00022	0.00011	1.47e-4
$K_{\text{pep}_{\text{eno}}}$	3.4e-5	5.54e-5	1.94e-5
$V_{f_{\text{pgm}}}$	13.2	2.78 (0.68 - 5.41)	56.9
$K_{\text{eq}_{\text{pgm}}}$	0.11 (0.088 - 0.12)	0.14 (0.13 - 0.15)	0.075
$K_{\text{p2ga}_{\text{pgm}}}$	3.04 (0.80 - 4.61)	4.80 (1.13 - 10.9)	1.47e-4
$K_{\text{p3ga}_{\text{pgm}}}$	657.8	316.81 (79.05 - 645.06)	2.86

Model: Example

Modelling cellular redoxin networks

Johann Rohwer

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

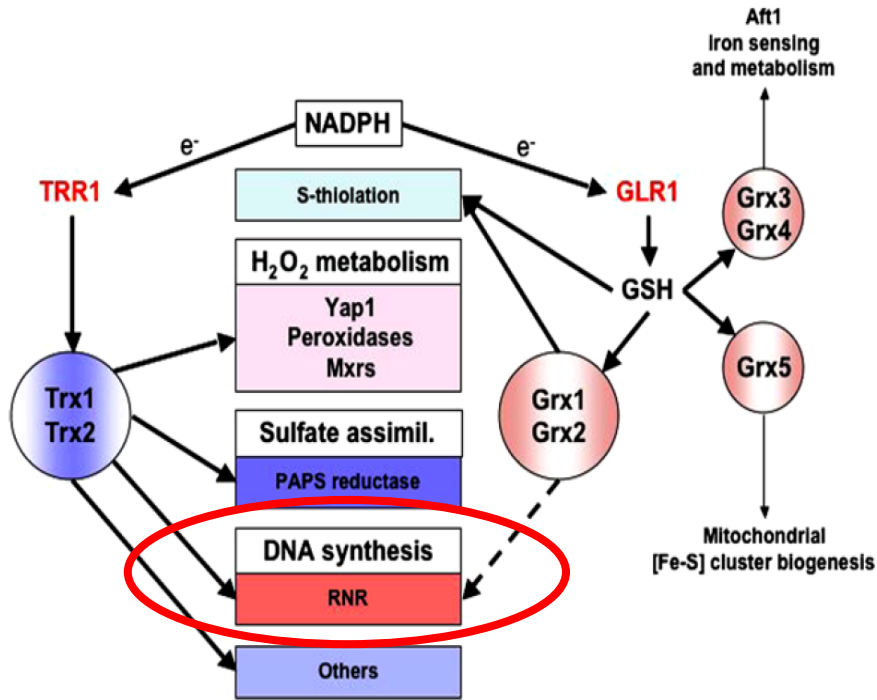


Ché Pillay

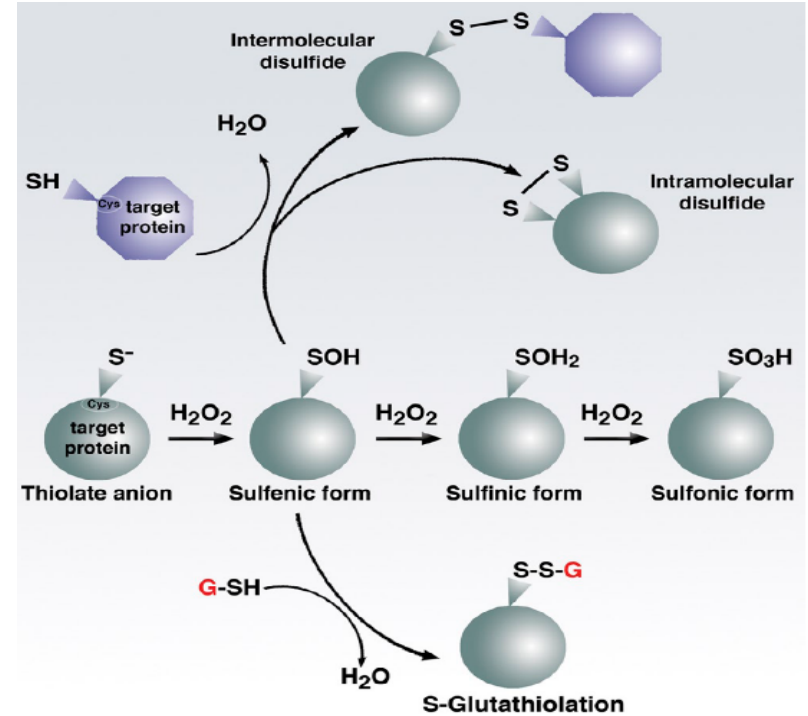
School of Life Sciences
UKZN
Pietermaritzburg
South Africa



Redoxin networks are essential for all living organisms



Toledano et al. (2007) FEBS Lett.

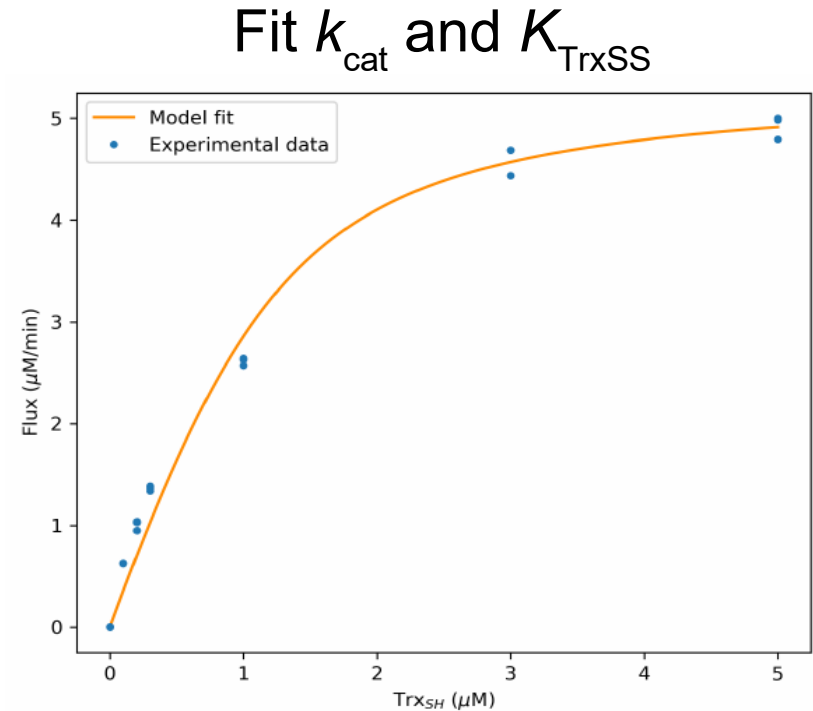
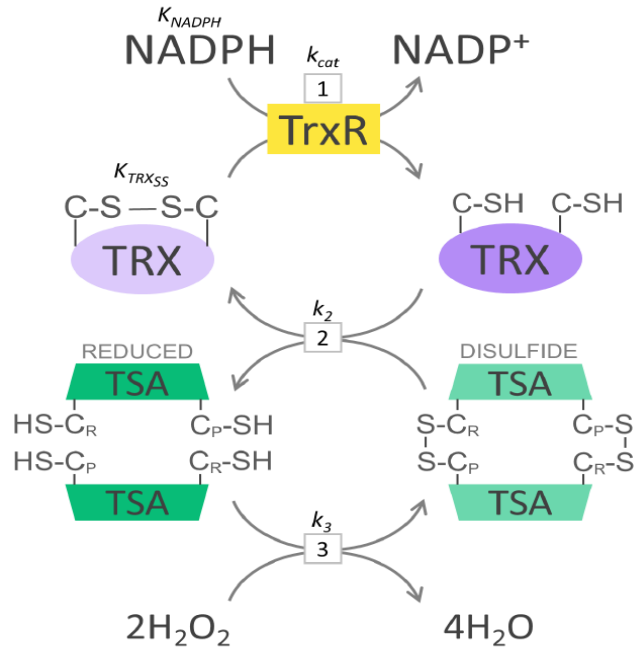


Finkel (2011) J. Cell Biol.

Redoxin networks – Aspects

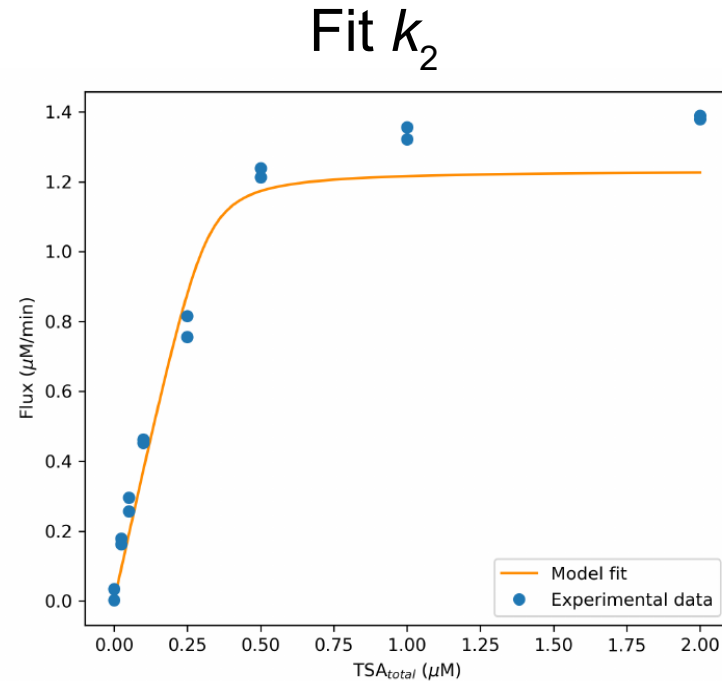
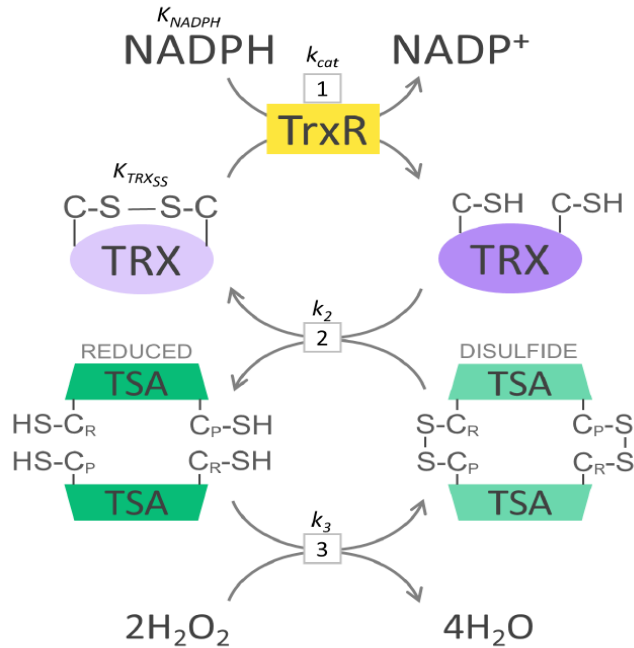
- Kinetic characterisation of peroxiredoxin system in *S. cerevisiae*
- Effect of peroxiredoxin decamerisation

Peroxioredoxin system in *S. cerevisiae*



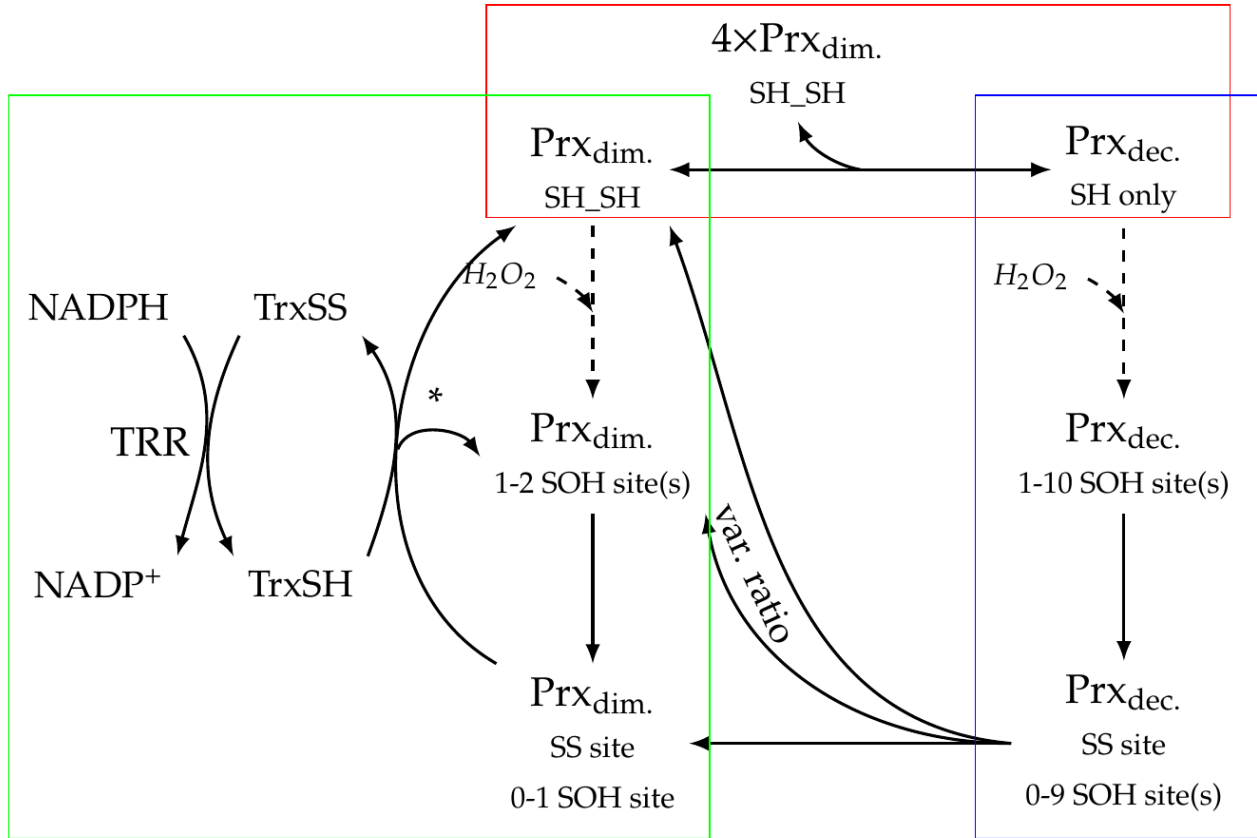
M. Badenhorst, MSc thesis (2020)

Peroxioredoxin system in *S. cerevisiae*



M. Badenhorst, MSc thesis (2020)

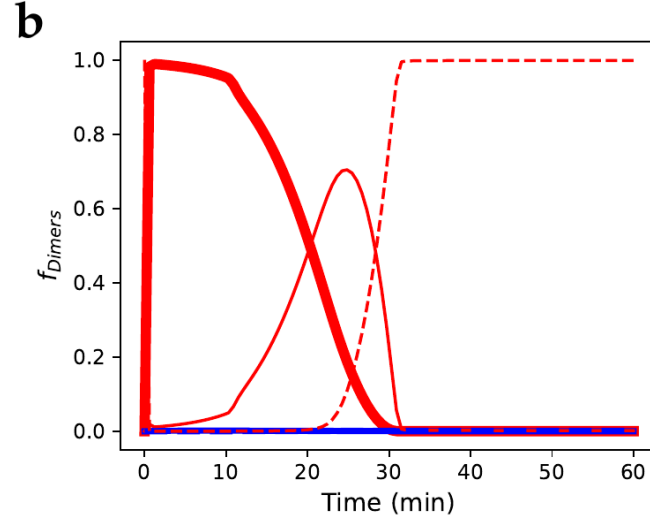
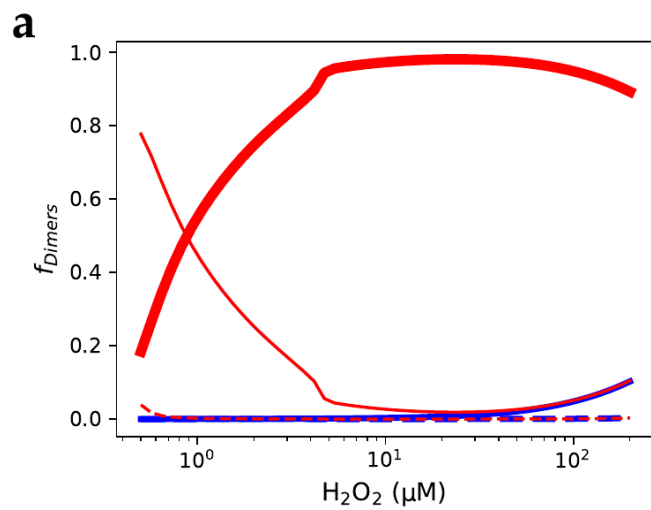
Decamerisation of Prx



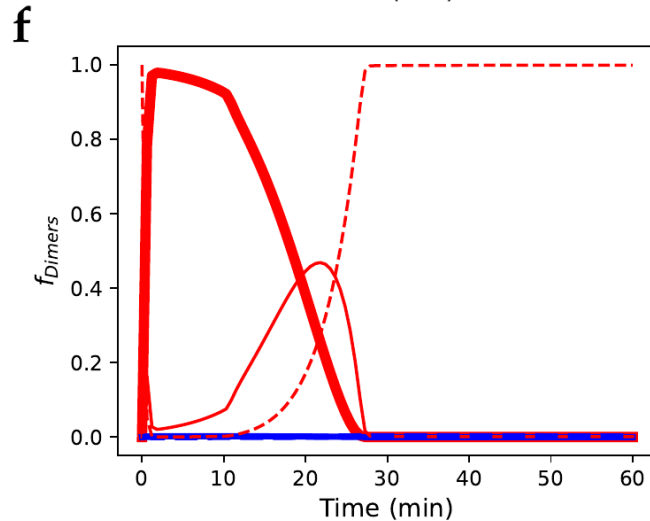
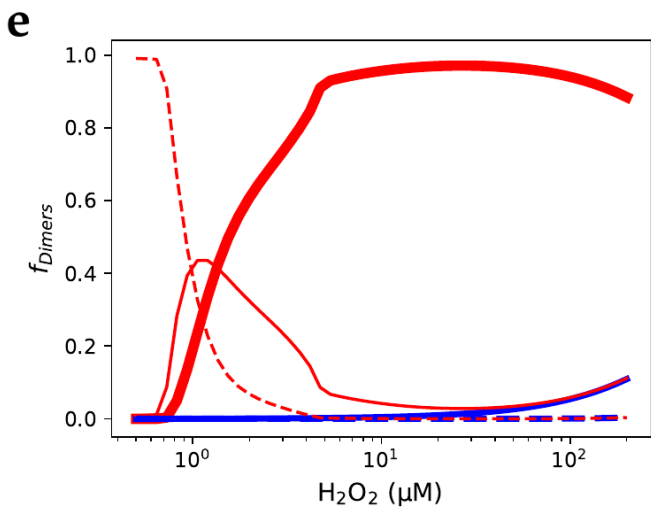
- decamers $\sim 100\times$ more active than dimers
- previously modelled with “inhibited” form

Barry et al. (2023)
Antioxidants, 12:1707

Decamerisation can reconcile models and data



- model with decamerisation



- model with postulated “inhibited form”, no experimental evidence

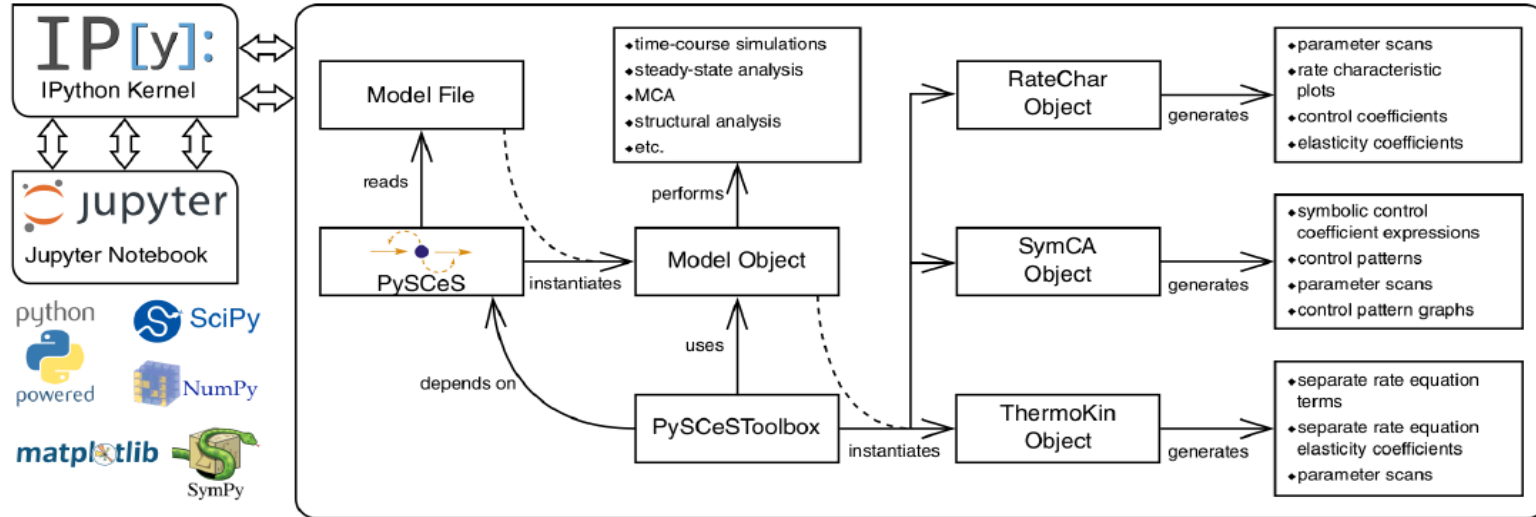
Redoxins: current work

- Detailed kinetic model of redoxin systems in *S. cerevisiae*
- Redox signalling and transcriptional regulation
- Redox probes to monitor intracellular redox state
- Dynamics of oxidative stress and recovery

Interface of Modelling and Experiment

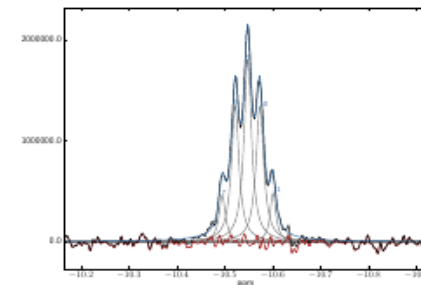
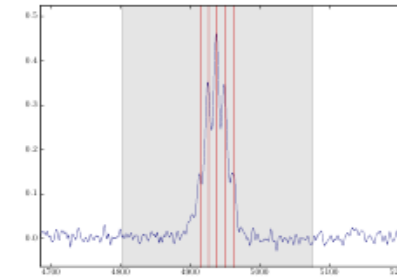
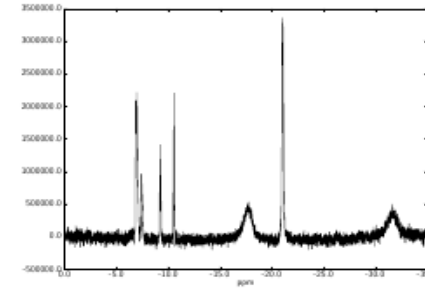
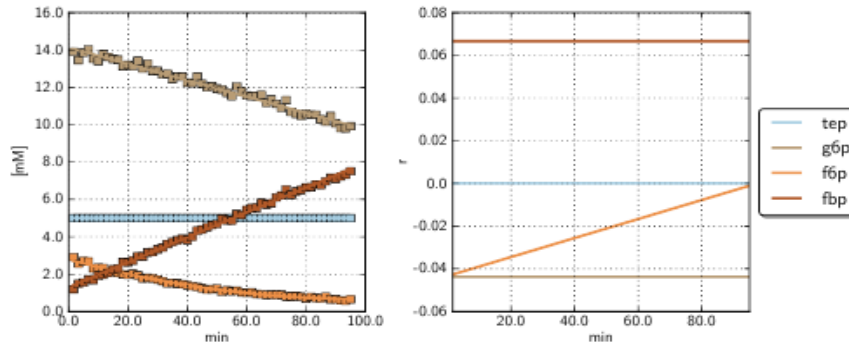
**Software tools
developed and used
(Python programming language)**

PyscesToolbox



Christensen et al. (2018)
Bioinformatics, 34:124-125

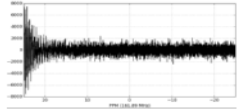
- Python NMR processing software suite
- no adequate software (proprietary or free)
- functionality:
 - processing, integration and deconvolution
 - arrayed spectra
 - interactive or batch processing
 - visualisation
- <https://github.com/NMRPy/nmrpy>



Tools and Techniques

NMR spectroscopy

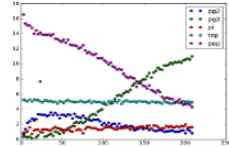
NMR time-courses



Perform enzyme assays and collect NMR time-course data for all reactions in a multi-enzyme system as they progress towards equilibrium.

NMRPy

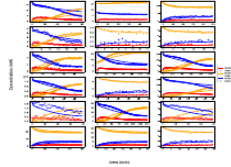
Progress curves



Process NMR data by deconvolution of NMR spectra to obtain progress curves (metabolite concentrations versus time).

NMRPy
Numpy
Scipy
Matplotlib

Parametrisation (multiple enzymes)

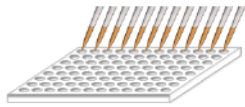


Fit representative rate equations to aggregated progress curves to obtain kinetic parameters for the enzymes in the system.

PySCeS
LmFit

Microtitre plate assays

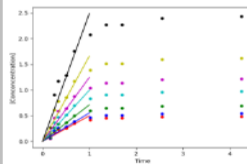
Microtitre plate assays



Perform enzyme assays at various substrate concentrations and monitor the change in light-absorbing species in the assay mixture over time (progress curves).

Pandas

Initial rates



Calculate initial rates of the reactions by linear regression of the first few data points of each progress curve.

Numpy
Scipy
Matplotlib

Parametrisation (single enzyme)

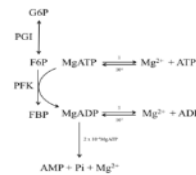
$$v_{PGI} = \frac{V_f \epsilon_i \left(\frac{[G6P]}{K_{m,G6P}} \right) \left(1 - \frac{[G6P]}{K_{eq}} \right)}{1 + \left(\frac{[G6P]}{K_{m,G6P}} + \frac{[F6P]}{K_{m,PFK}} \right)}$$

V_f	3.551 ± 0.050 ($V_f = 3.431$)
$K_{m,G6P}$	0.550 ± 0.236
$K_{m,PFK}$	0.152 ± 0.017
K_{eq}	$0.286 \pm 8 \times 10^{-6}$

Fit the rate-vs.-substrate concentration data to a rate equation (e.g. Michaelis-Menten or Hill) to obtain kinetic parameters.

PySCeS
LmFit

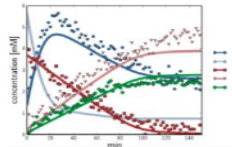
Model construction



After parameterization, combine the kinetic data for all the enzymes in the pathway under study into a kinetic model.

PySCeS

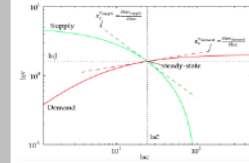
Model validation



Test the validity and accuracy of the model by comparing model output to validation data that were not used in model construction.

PySCeS
LmFit

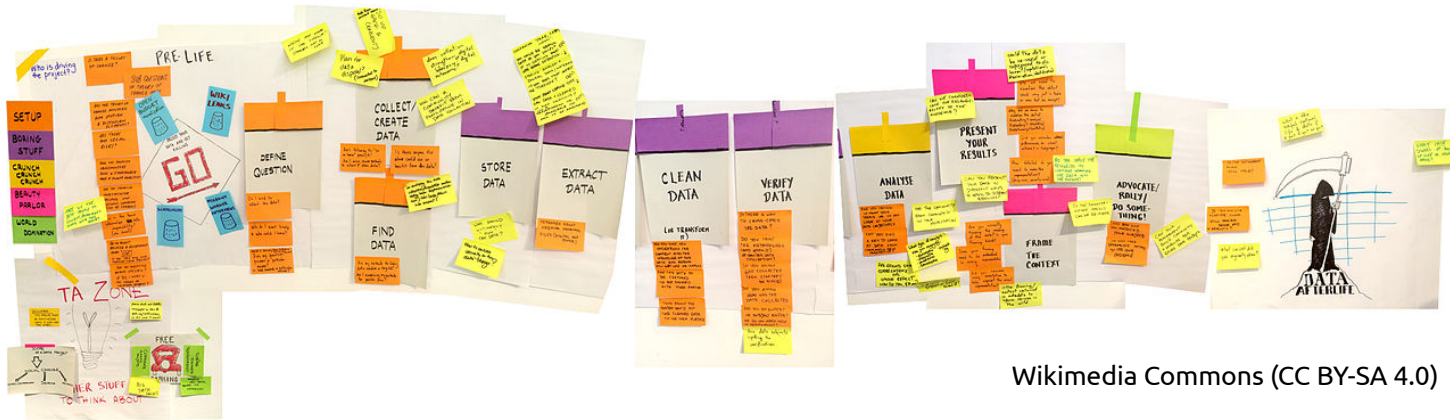
Further analysis



Perform further analyses on the model, e.g. steady-state analysis, numeric and symbolic metabolic control analysis, and general supply-demand analysis.

PySCeS
PyscesToolbox

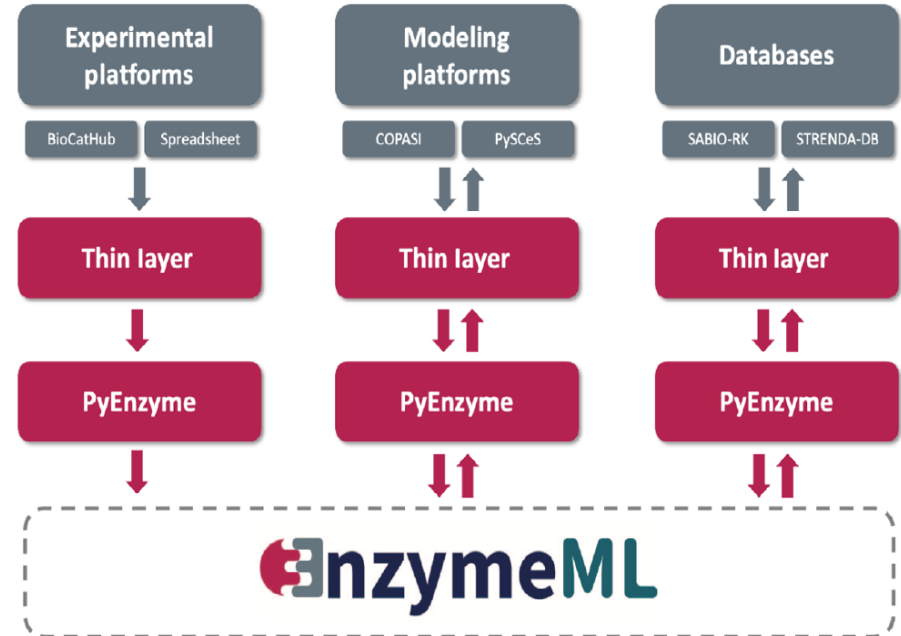
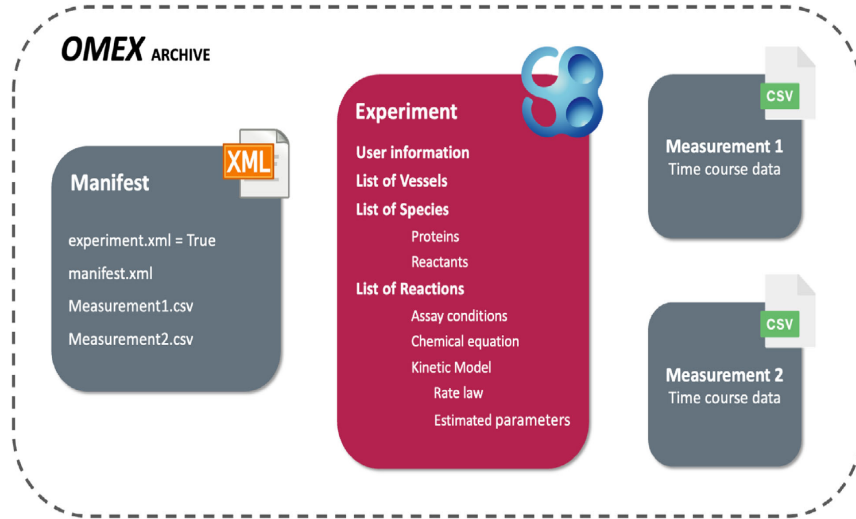
Challenges of data management



Wikimedia Commons (CC BY-SA 4.0)

- Manual copy and paste
- Medium- to high-throughput data acquisition
- Transfer of data and metadata
- Robotics, automation, integration with ELNs/LIMS
- New data analysis and modelling methods
- Quality of data
- New methods of experimental design
- Labour-intensive entry of data into STRENDA DB

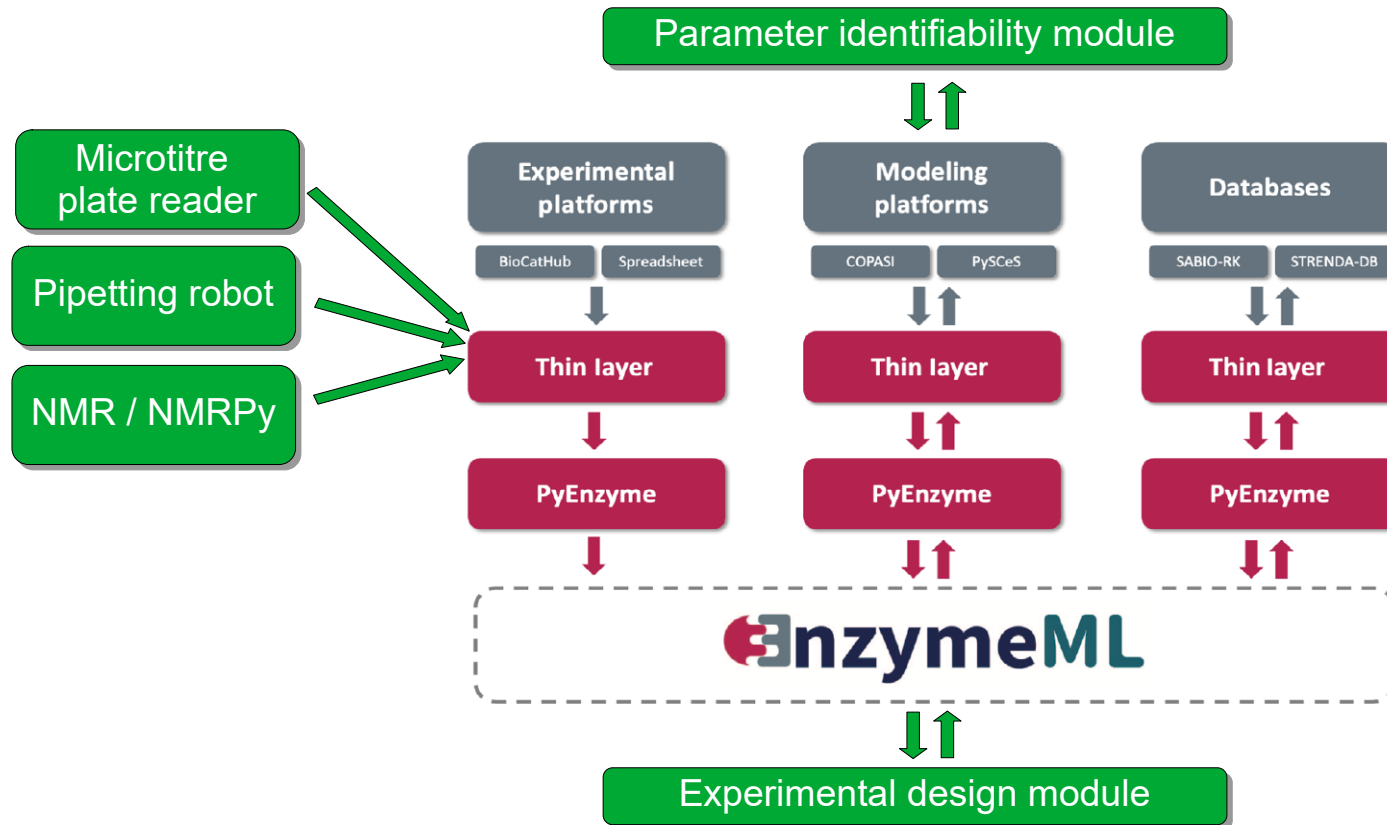
EnzymeML



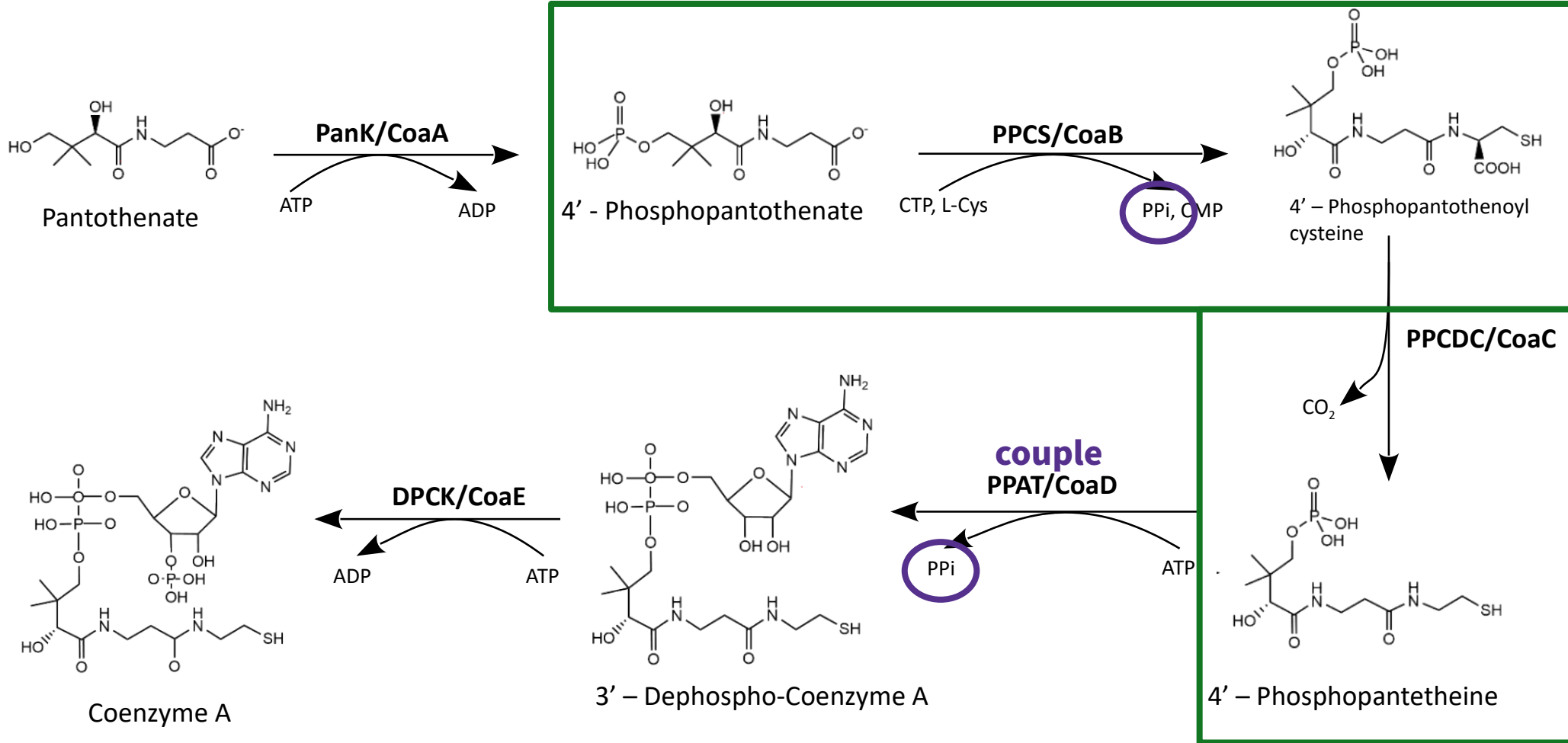
Range et al. (2021) *FEBS J.* doi:10.1111/febs.16318

Lauterbach et al. (2023) *Nat. Meth.* doi:10.1038/s41592-022-01763-1

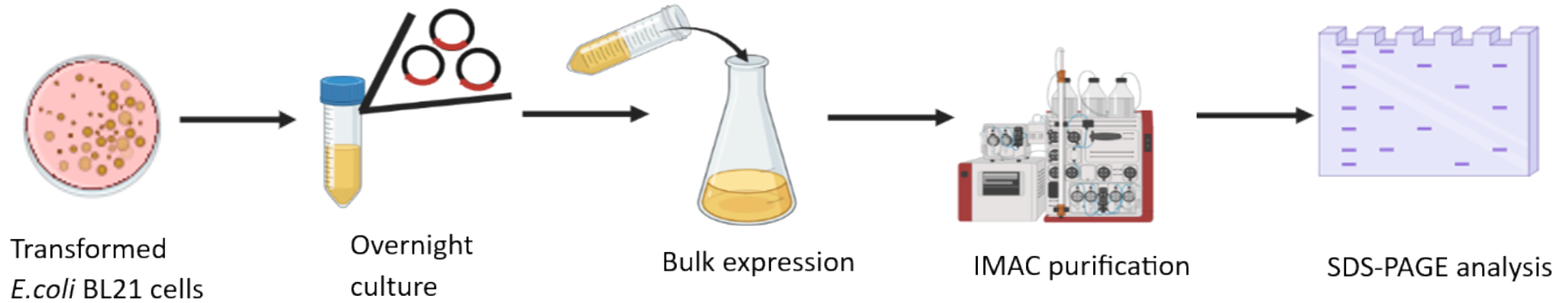
FAIR Enzymology: PyEnzyme extensions



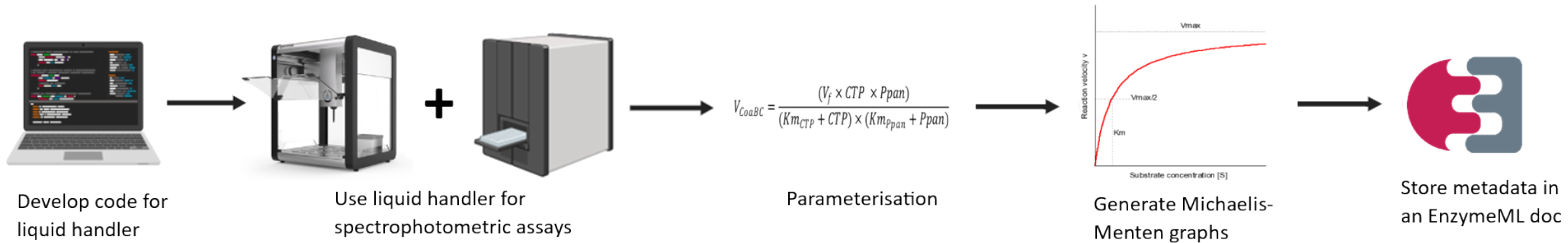
Example: CoaBC from CoA Biosynthesis Pathway



Protein purification workflow



Kinetic characterisation workflow



Automation in data acquisition: Programmed liquid handling

```
from opentrons import protocol_api

metadata = {'protocolName': 'Characterisation of CoaB', 'author': 'Francel',
            'description': 'Preparing mastermix',
            'apiLevel': '2.10'}

def run(protocol: protocol_api.ProtocolContext):
    # loading required labware
    tips2 = protocol.load_labware('opentrons_96_tiprack_300ul', 1)
    p300 = protocol.load_instrument('p300_single_gen2', mount='left', tip_racks=[tips2])

    # loading in custom labware:
    source_TRIS = protocol.load_labware('opentrons_10_tuberack_nest_4x50ml_6x15ml_conical', 7)
    source = protocol.load_labware('opentrons_24_tuberack_ependorf_1.5ml_safelock_snapcap', 5)

    # Preparing the mastermix:
    # MilliQ:
    p300.pick_up_tip(tips2['A1'])
    p300.flow_rate.aspirate = 210
    p300.flow_rate.dispense = 210
    p300.well_bottom_clearance.aspirate = 1
    p300.well_bottom_clearance.dispense = 2
    p300.transfer(176.8, MilliQ, Mastermix, blow_out=True, blowout_location='destination well', new_tip='never')
    p300.flow_rate.blow_out = 210

    # Tris - Buffer:
    p300.flow_rate.aspirate = 160
    p300.flow_rate.dispense = 160
    p300.well_bottom_clearance.aspirate = 60 #falcon tube
    p300.well_bottom_clearance.dispense = 2
    p300.transfer(136, Tris_Buffer, Mastermix, blow_out=True, blowout_location='destination well', new_tip='never')
```

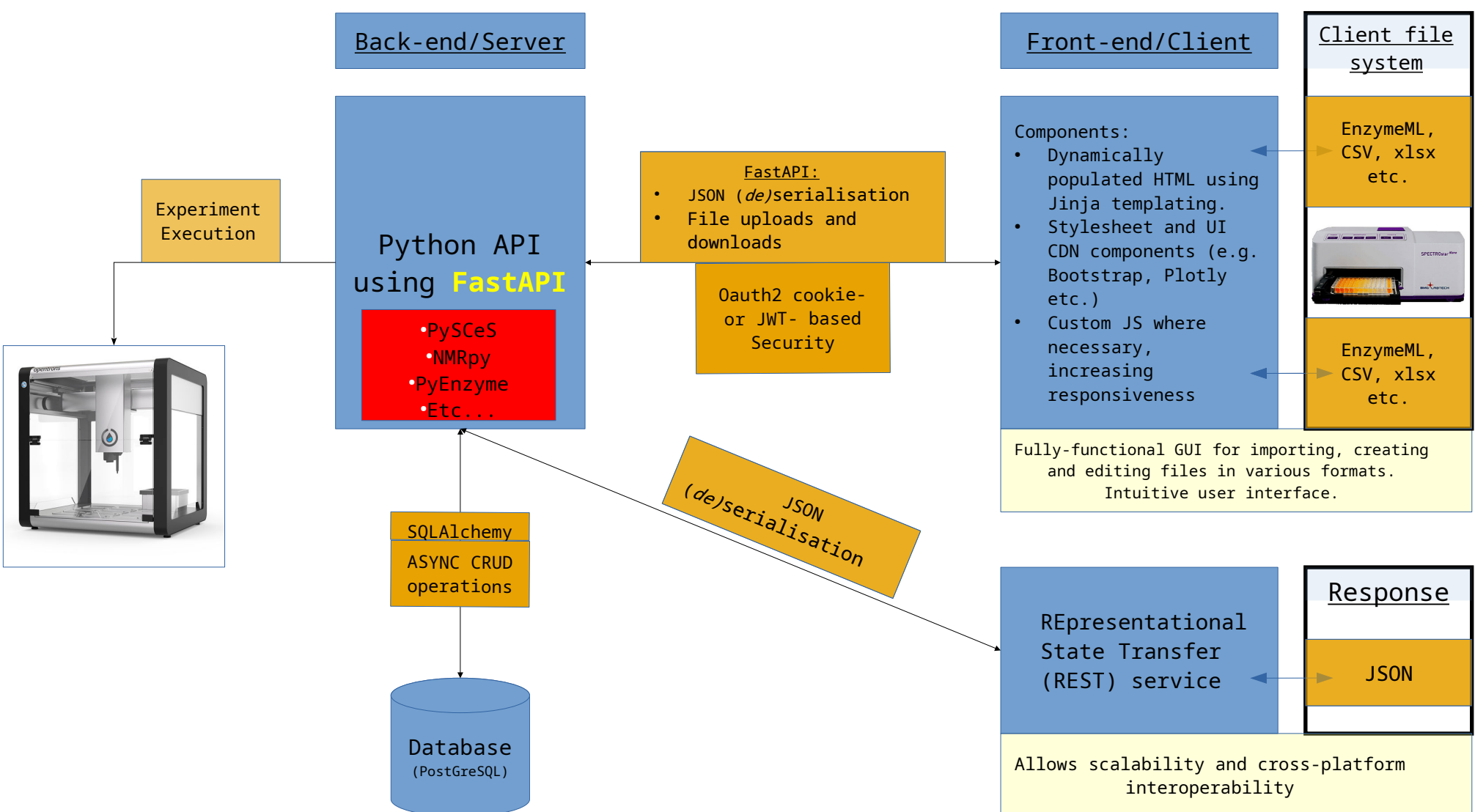


- Opentrons OT-2

Francel Wessels, MSc student

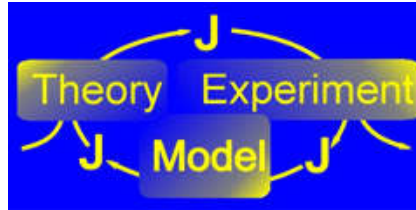
Automation in Data Management: Labnexus

- Server/client architecture
- User-friendly GUI, web-based interface
- https://github.com/CdeBeer7th/labnexus_server
 - currently private, in development
- Standalone installation or docker build



Theory

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



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Model / Experiment

- software development
 - PyscesToolbox
 - NMRPy
 - PyEnzyme
 - LabNexus
- CoA metabolism

Interested in joining the lab?

- Please contact me to discuss options!
- jr@sun.ac.za or 021-808-5843