# Overview of Research Activities

Biochemistry Honours Students
27 March 2025


## Prof Johann Rohwer

Laboratory for Molecular Systems Biology
Department of Biochemistry
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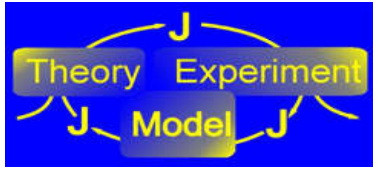
South Africa

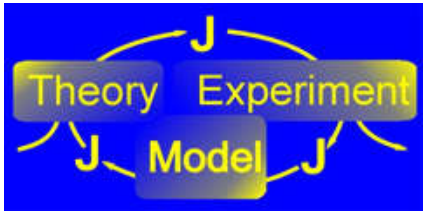




## Theory

- 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

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

## Model / Experiment

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

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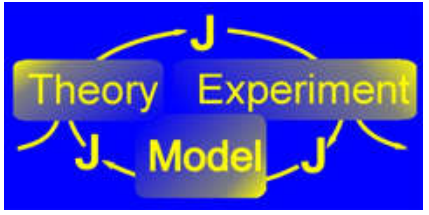


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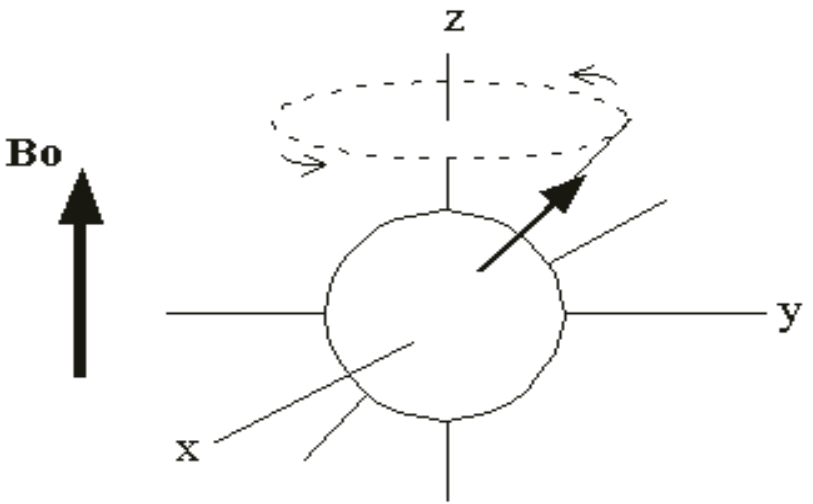
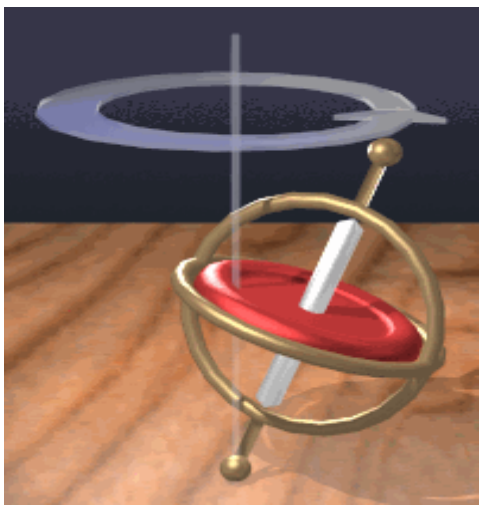
# Experiment: Example

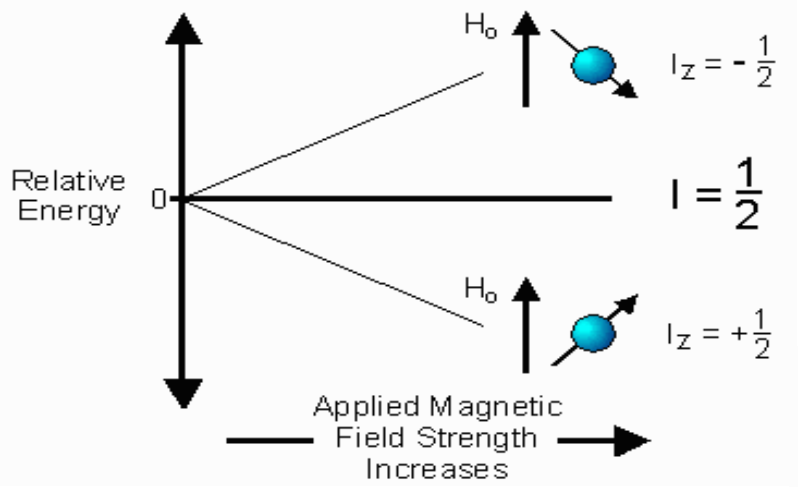
Enzyme kinetics for modelling by NMR spectroscopy
("*In vivo* enzyme kinetics")

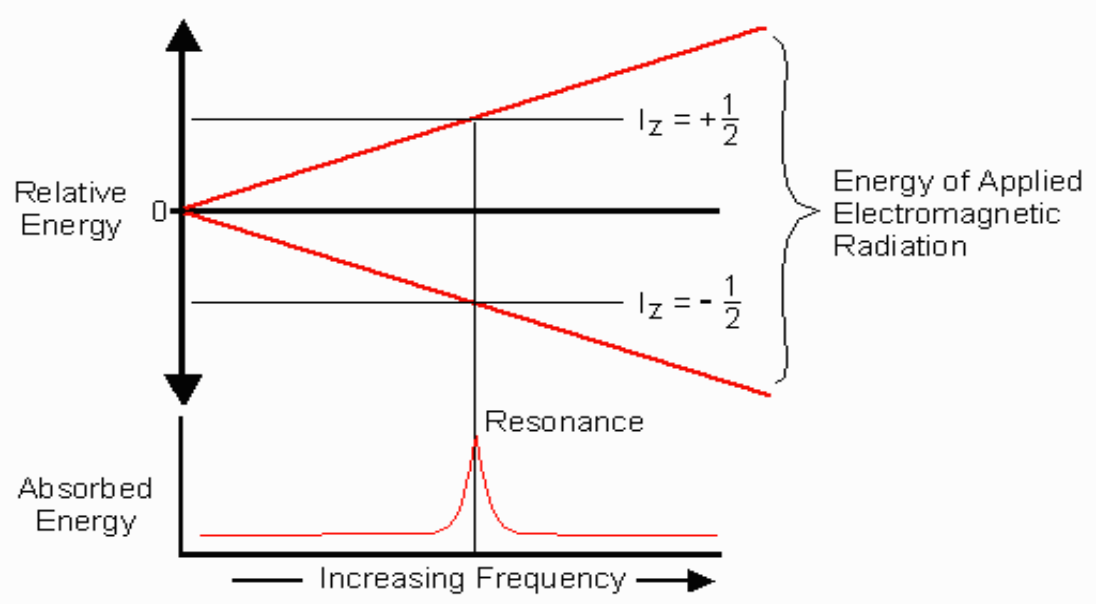
# Brief NMR introduction: Nucleus in magnetic field



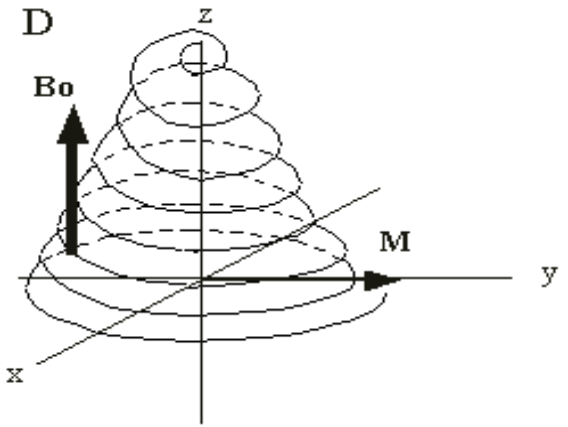



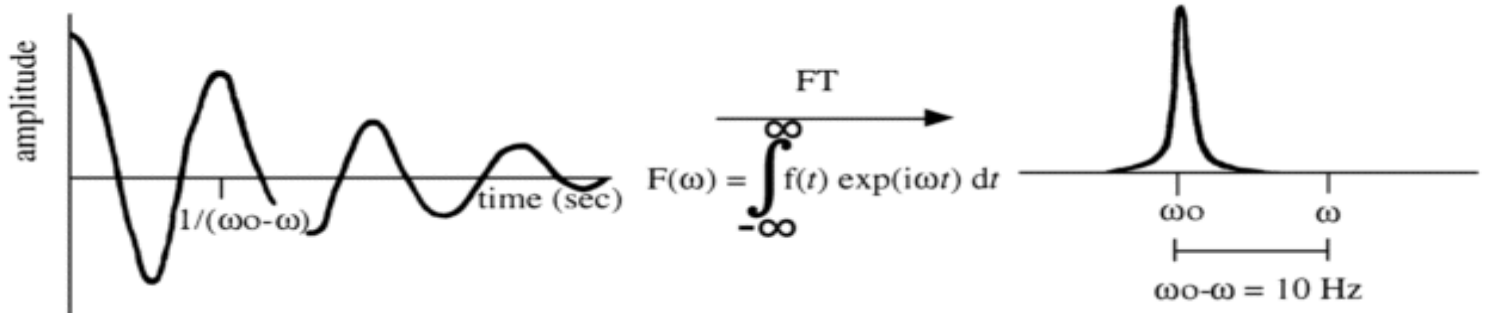
# Split in energy levels



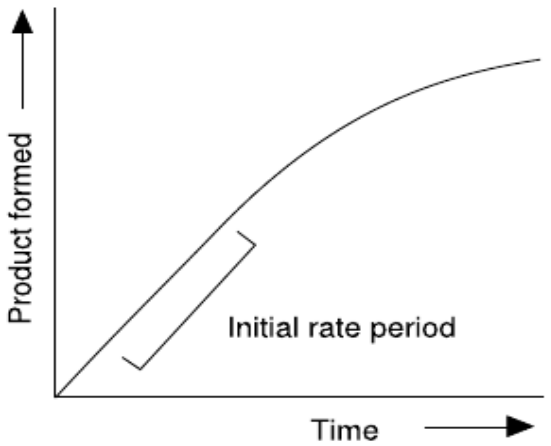
# Relaxation and Fourier Transform



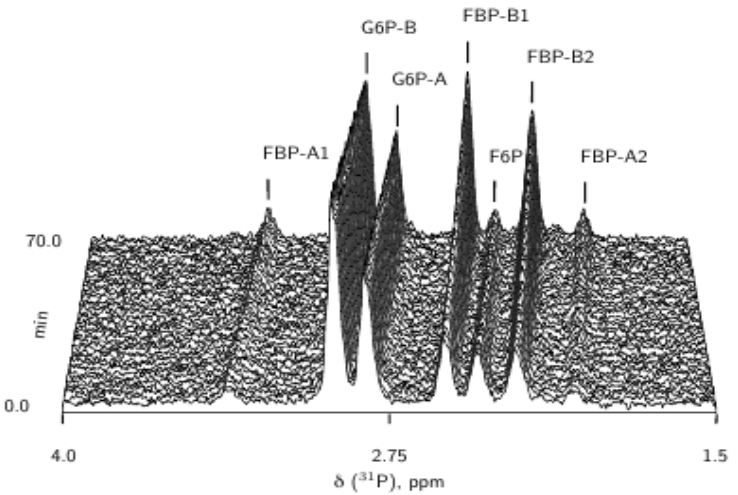



# Why use NMR?

## Initial rate assays

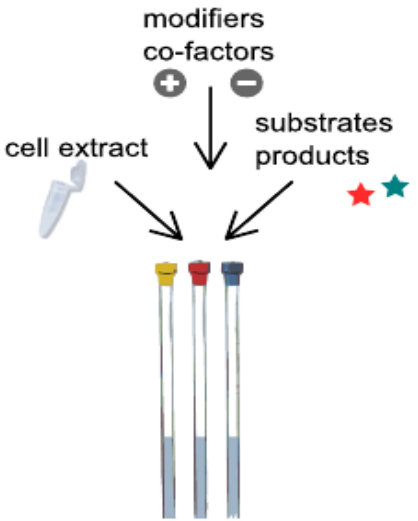



## NMR progress curve assays

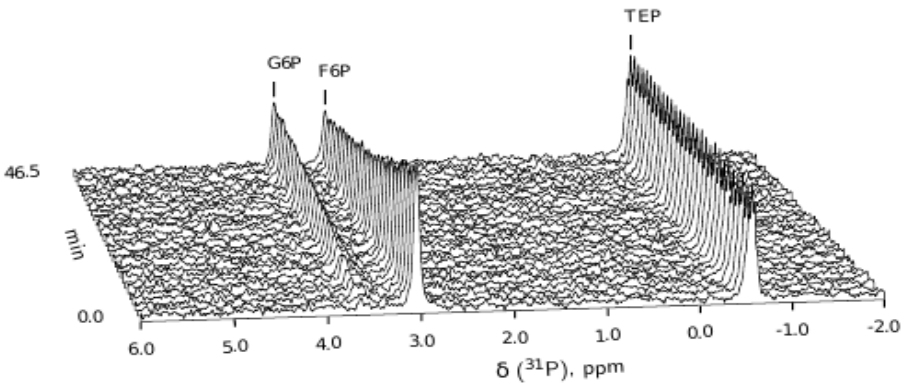


# Workflow

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

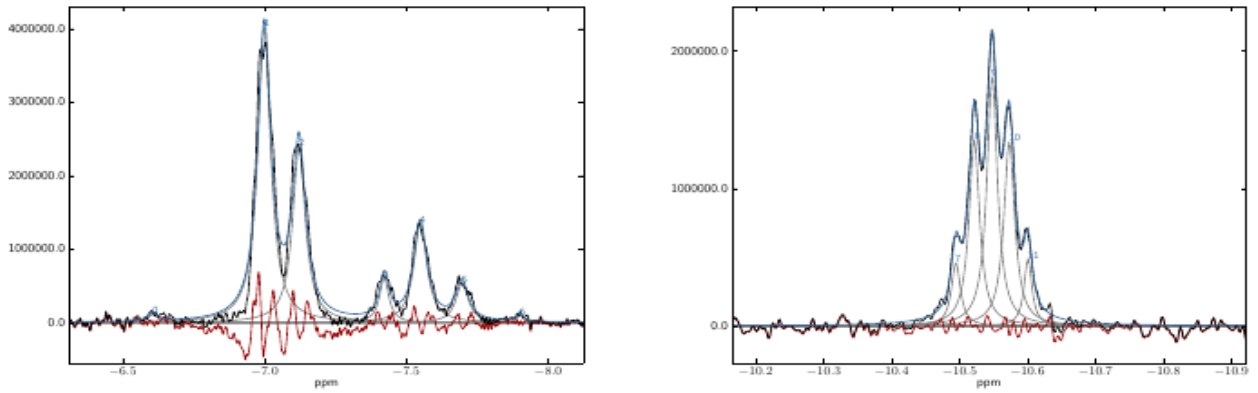



2. Acquire time series of NMR spectra



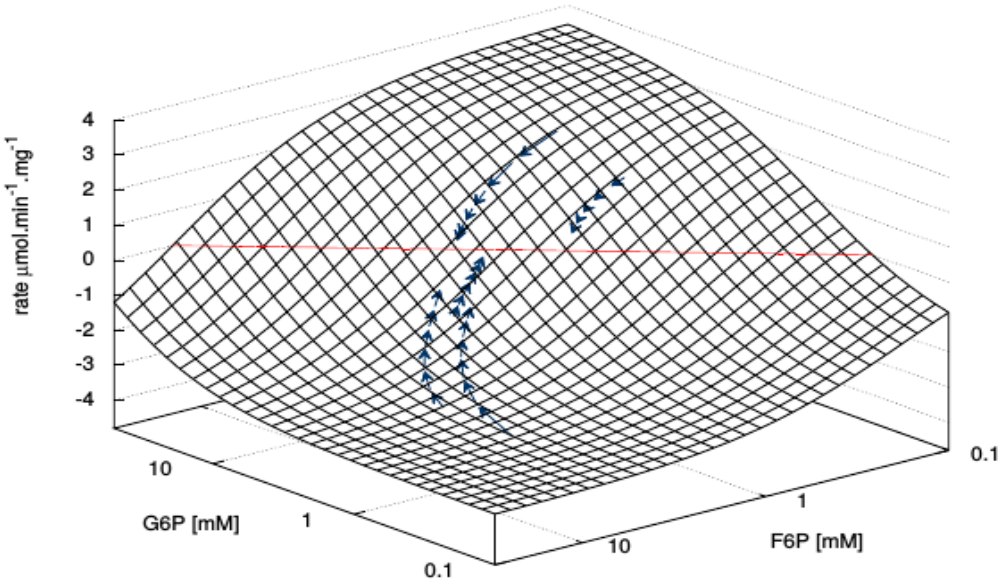
# Workflow (2)

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

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

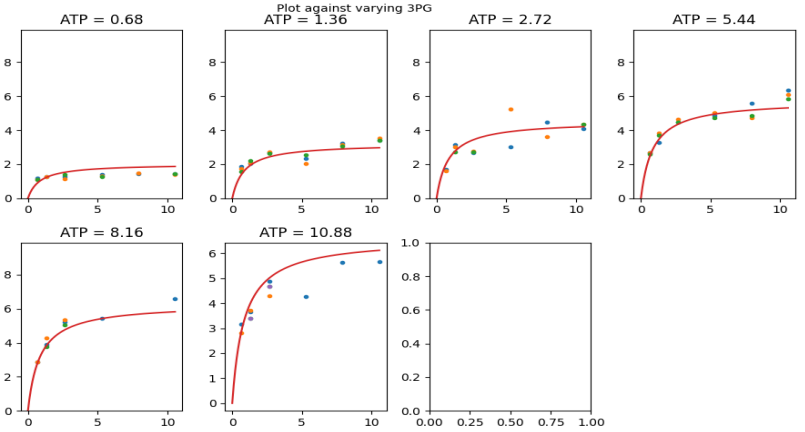
(rates: $\mu mol.min^{-1}.mg^{-1}$)
(concentrations: mM)

# Ongoing work

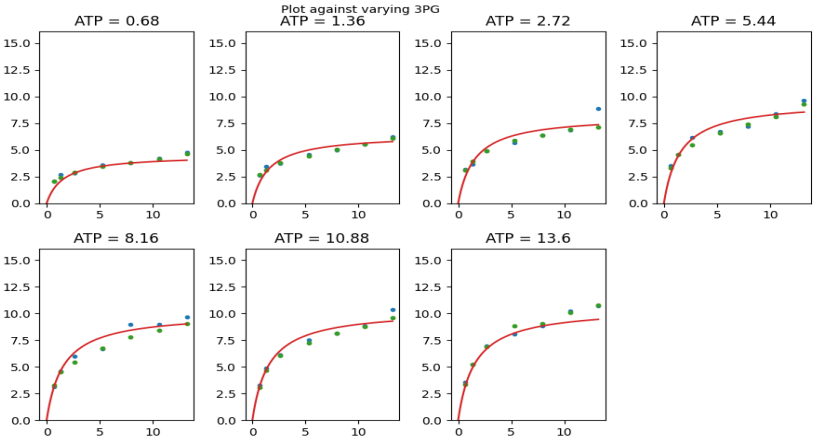
- Question: are kinetic measurements in the *test tube* a realistic representation of *conditions in the cell*?
- Investigate "*in vivo*" enzyme kinetics
  - effect of macromolecular crowding
    - intracellular environment has very little accessible solvent space, [protein] = 250 mg/ml
  - effect of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

# Effect of pH on PGK kinetics

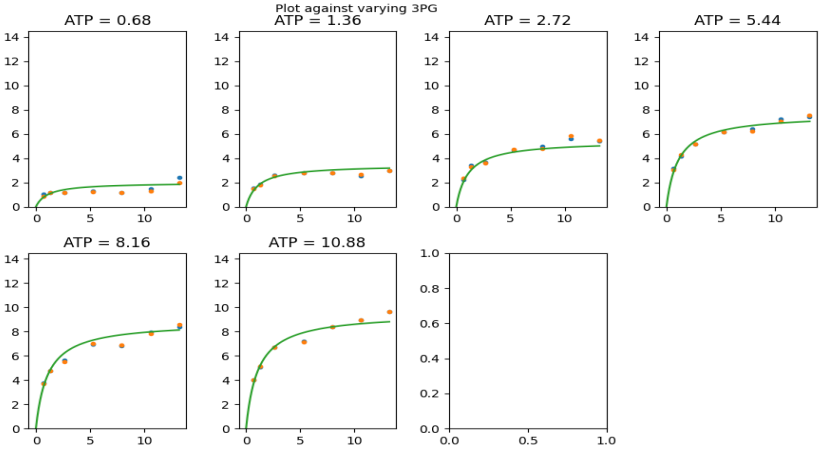
## pH 5.5




## 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 |

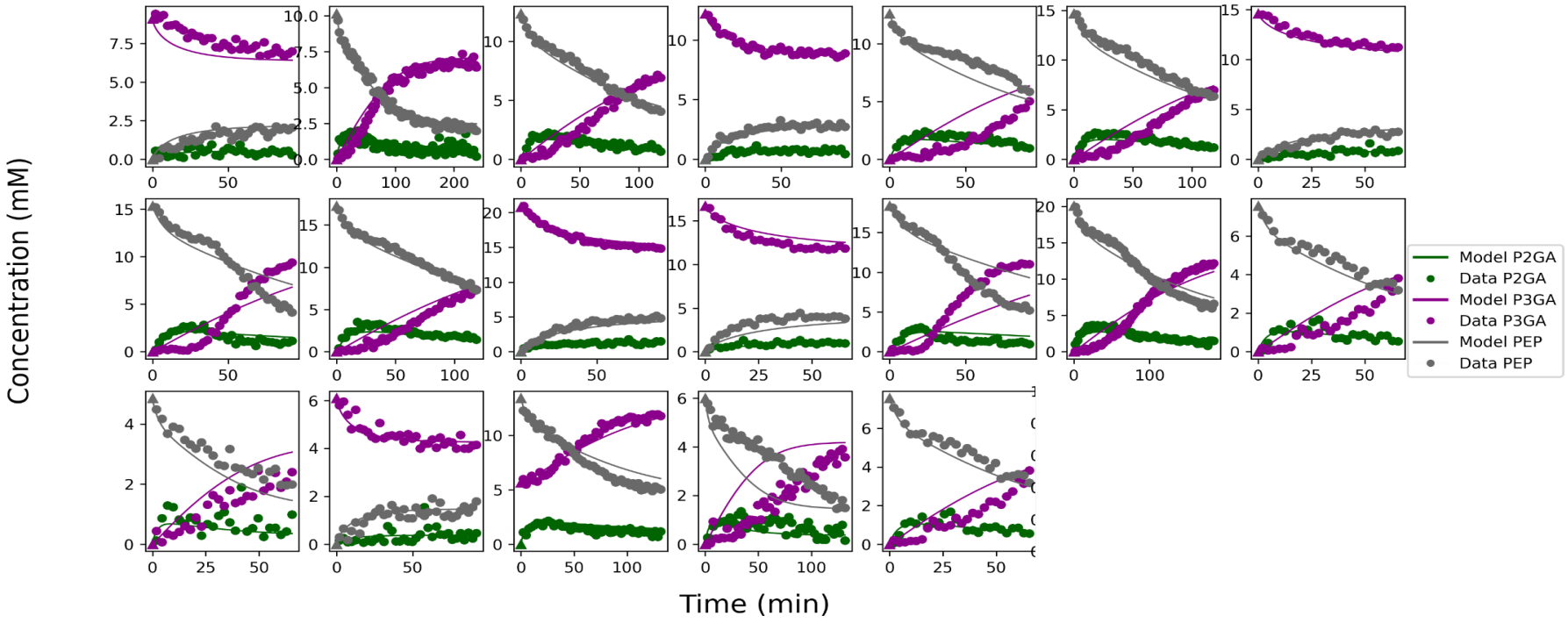
A. van der Spuy, PhD student

# Effect of crowding on PGM/ENO kinetics

10% dextran

**3PG** $\leftrightarrow$ **2PG** $\leftrightarrow$ **PEP**

PGM ENO




K. Matenchi, PhD student

# Effect of crowding on PGM/ENO kinetics

**3PG** $\overset{\text{PGM}}{\leftrightarrow}$ **2PG** $\overset{\text{ENO}}{\leftrightarrow}$ **PEP**

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

K. Matenchi, PhD student

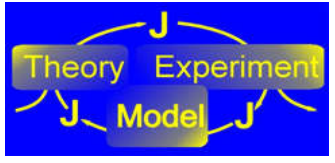
# Modelling cellular redoxin networks

**Johann Rohwer**

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

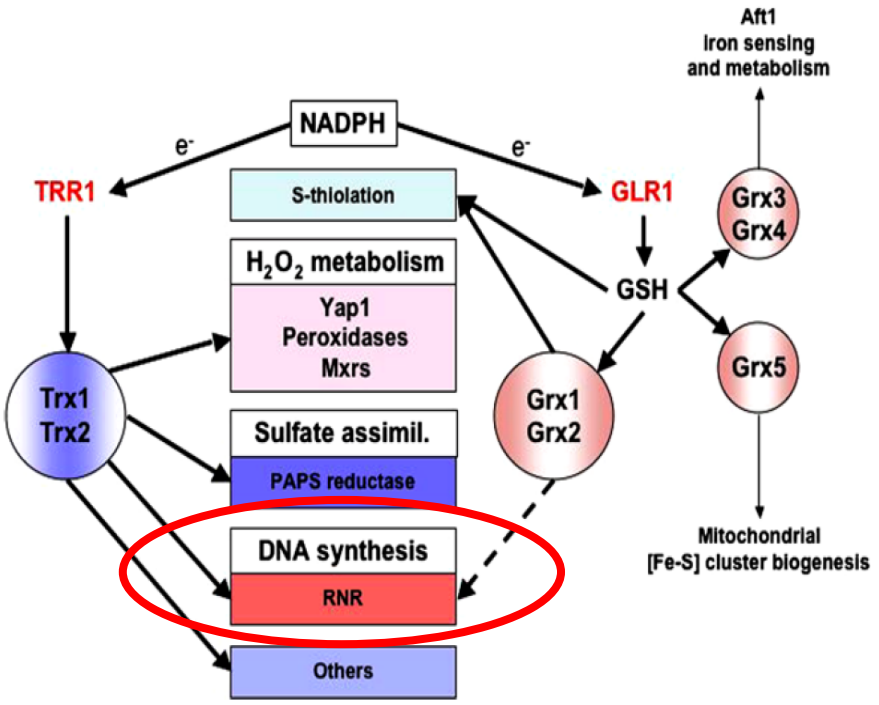
**Ché Pillay**

School of Life Sciences
UKZN
Pietermaritzburg
South Africa

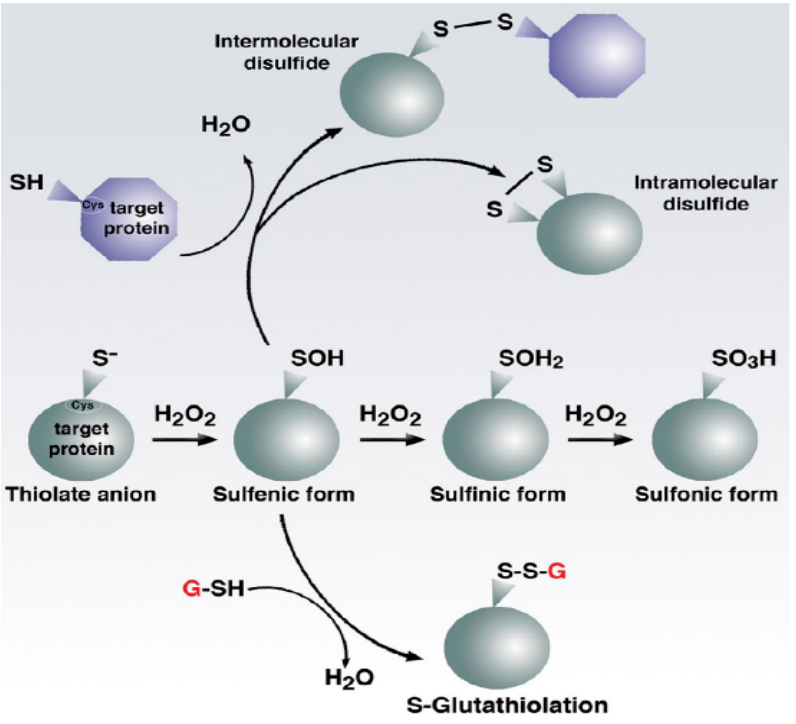




# Redoxin networks are essential for all living organisms




Toledano et al. (2007) FEBS Lett.

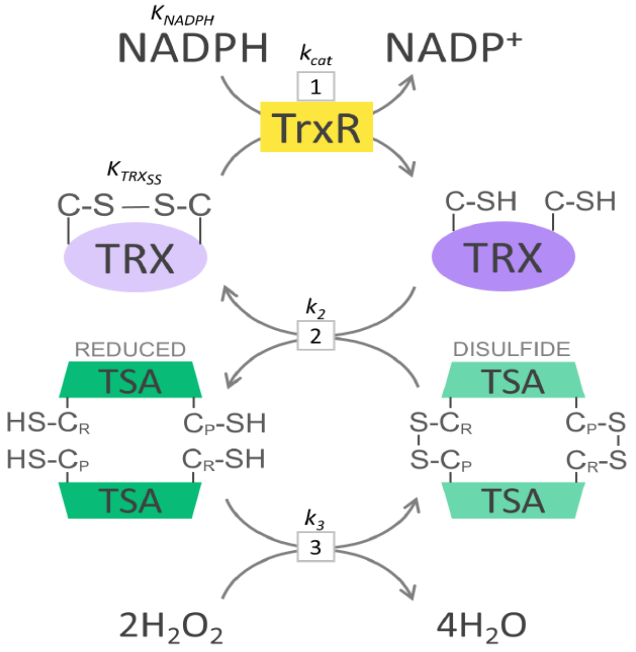



Finkel (2011) J. Cell Biol.

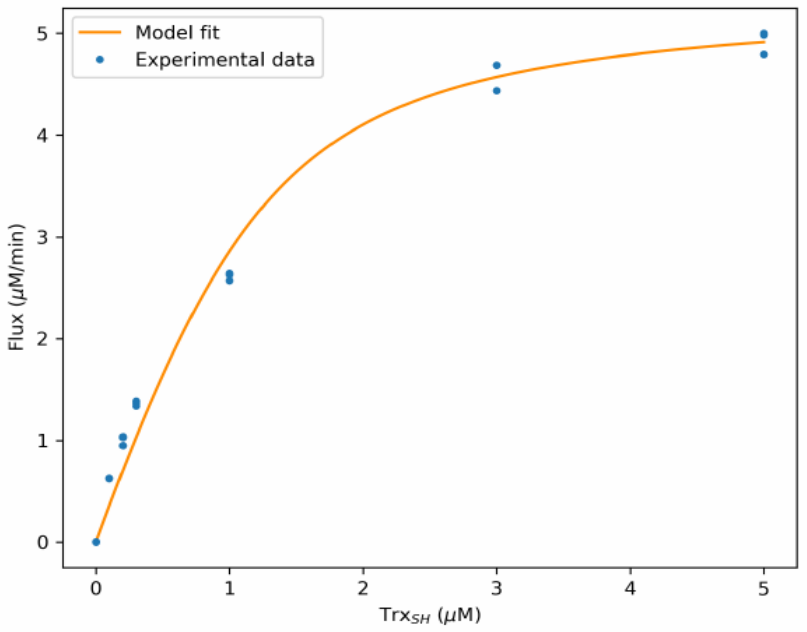
# Redoxin networks – Aspects

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

# Peroxiredoxin system in *S. cerevisiae*

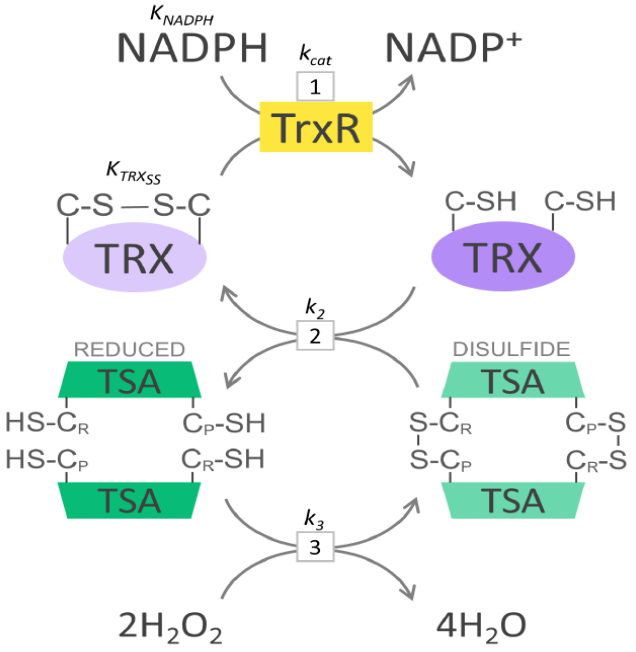



Fit $k_{cat}$ and $K_{TrxSS}$

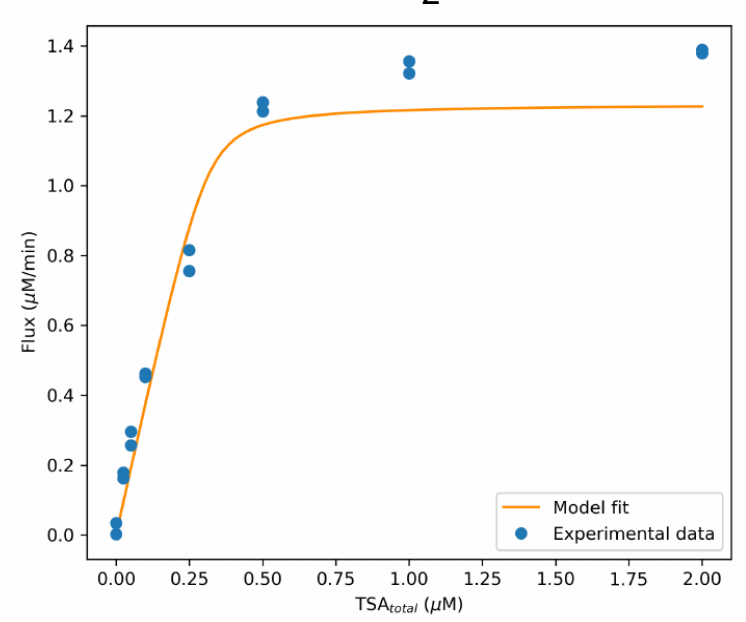



M. Badenhorst, MSc thesis (2020)

# Peroxiredoxin system in *S. cerevisiae*

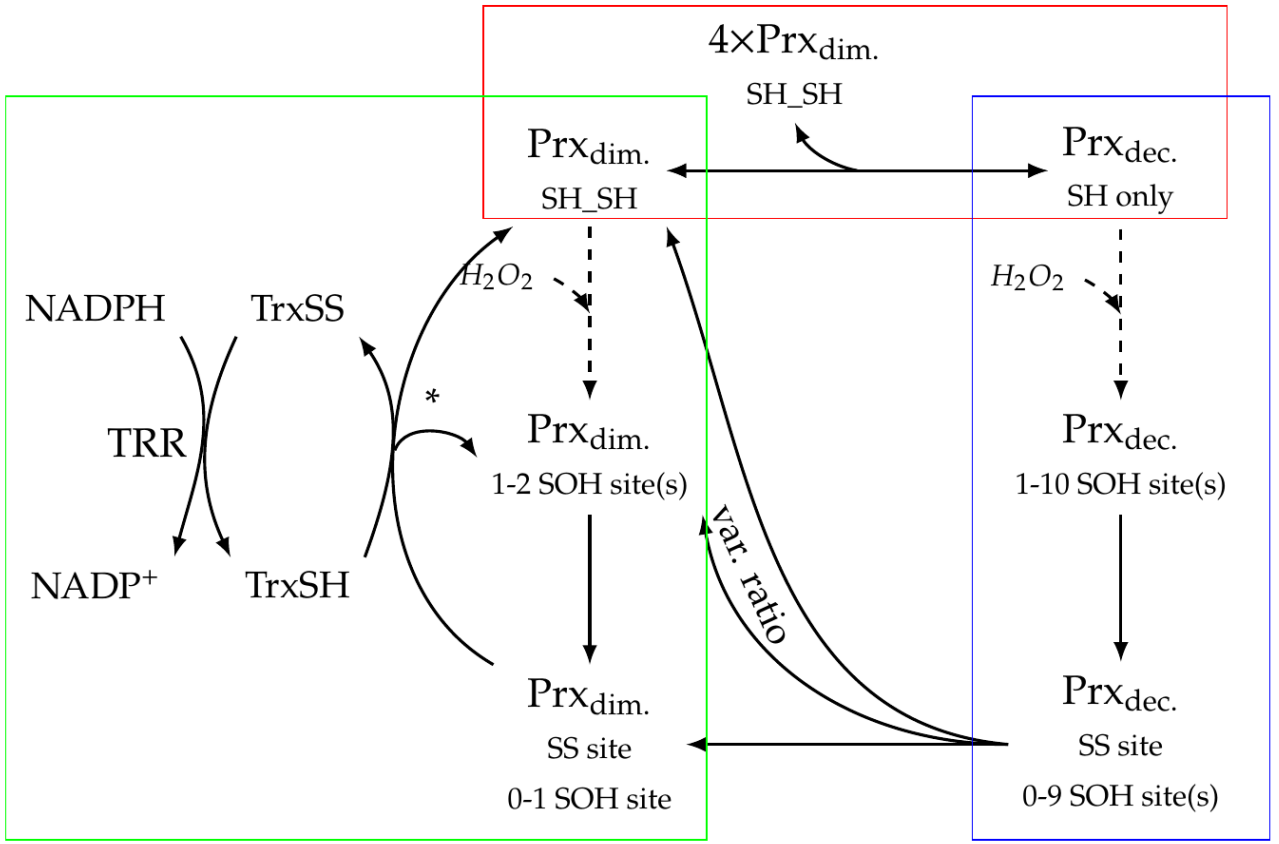



Fit $k_2$




M. Badenhorst, MSc thesis (2020)

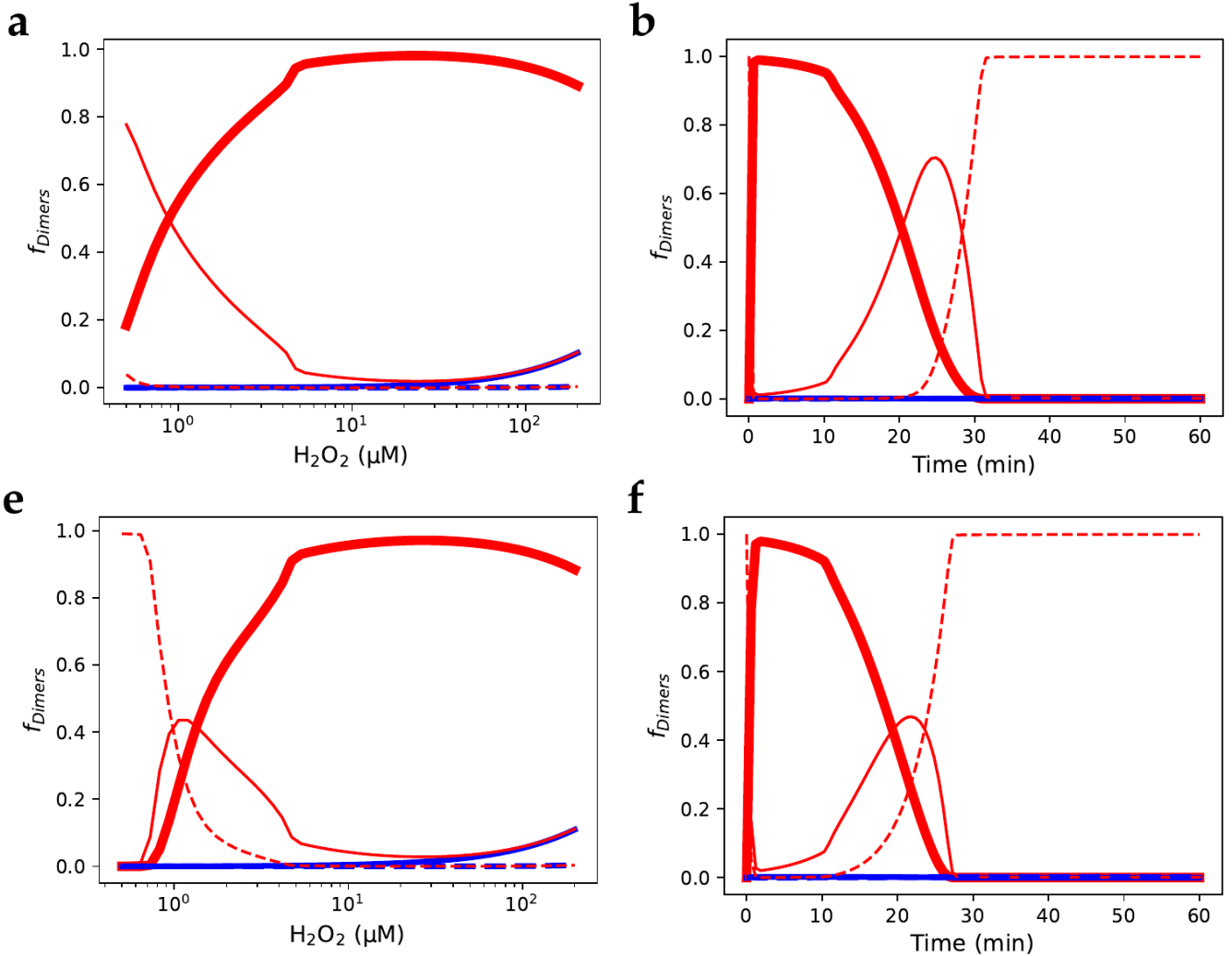
# Decamerisation of Prx




- decamers ~100× more active than dimers
- previously modelled with “inhibited” form

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

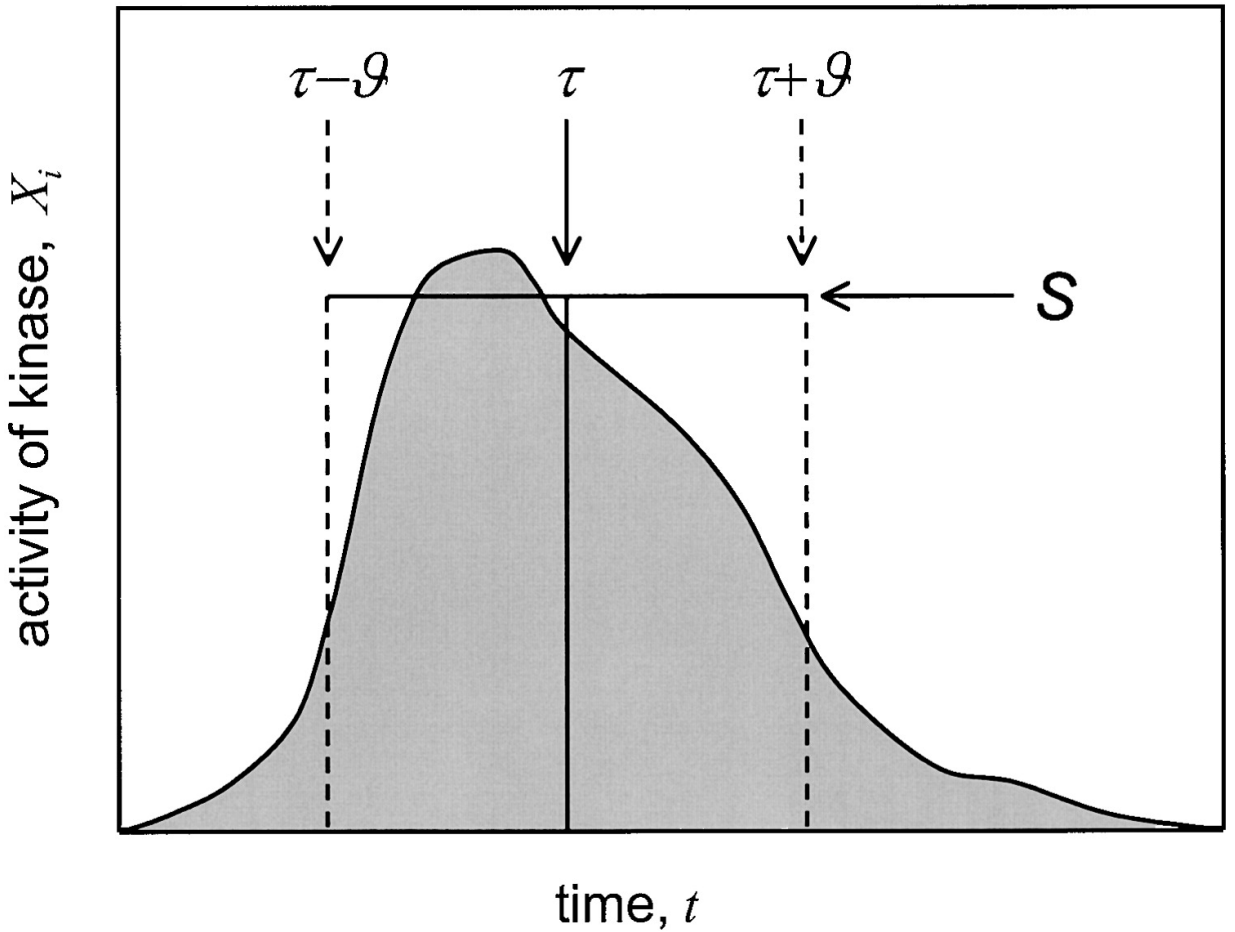
# Decamerisation can reconcile models and data




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

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

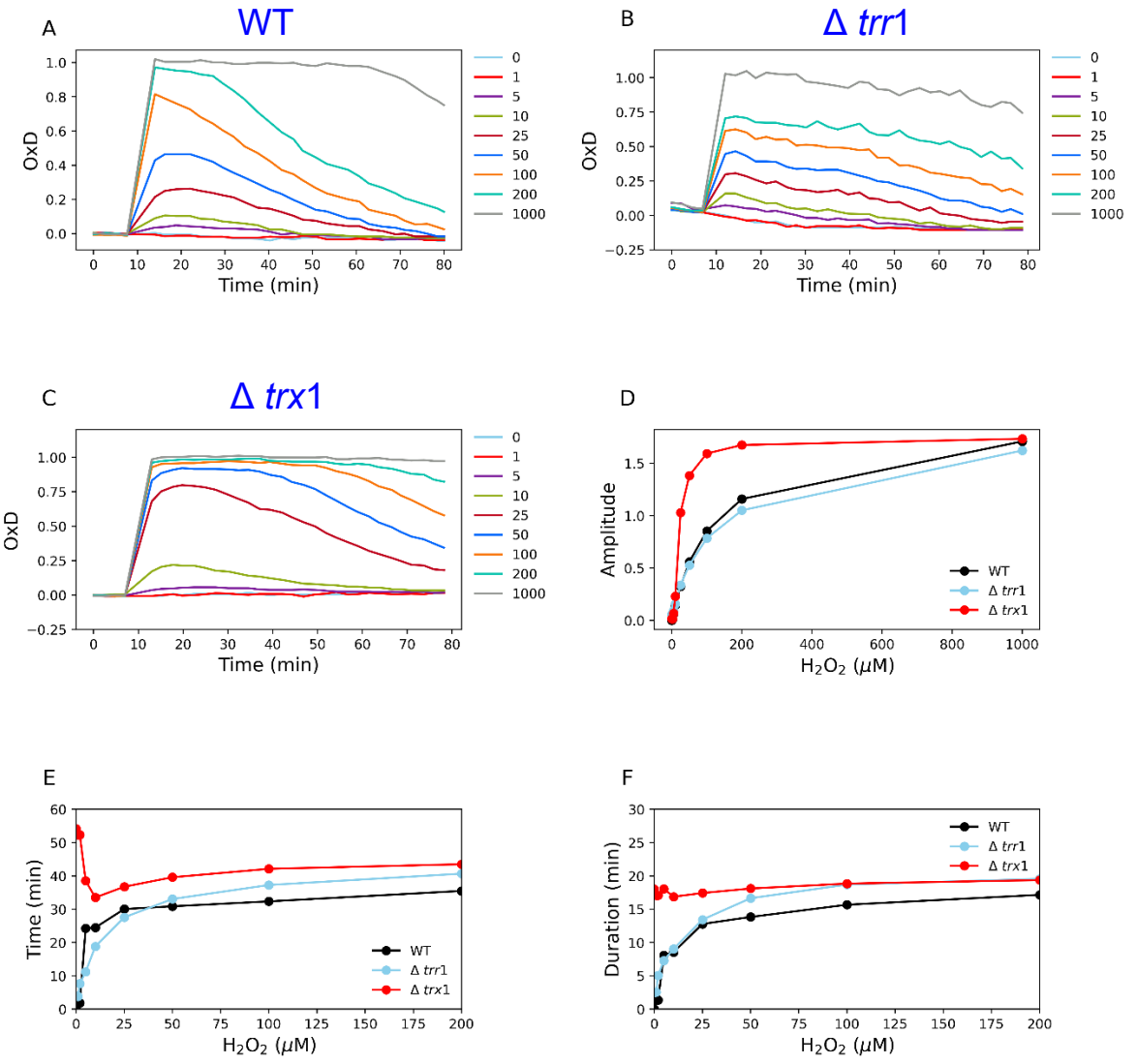
# Signalling parameters




- $\tau$ – signal time
- $\vartheta$ – signal duration
- $S$ – signal amplitude

Heinrich et al. (2002)
*Mol. Cell*, 9:957-970

# Fluorescent redox probes




Lind et al.,
*unpublished*

# Redoxins: current work

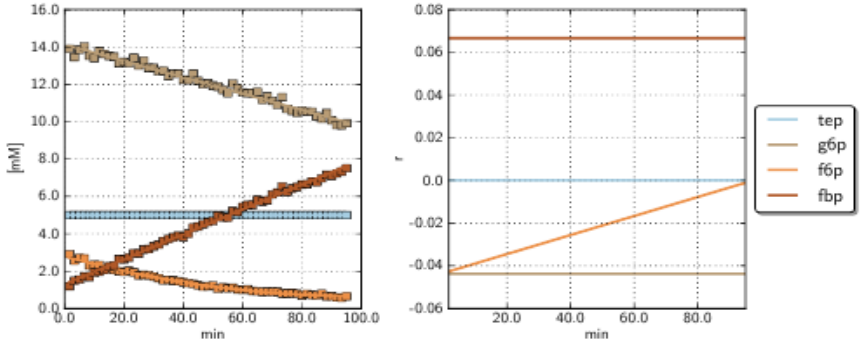
- Detailed kinetic model of redoxin systems in *S. cerevisiae*
- Redox signalling and transcriptional regulation
- Redox probes to monitor intracellular redox state
- Dynamics of oxidative stress and recovery

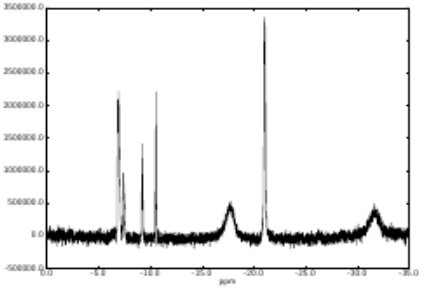
# Interface of Modelling and Experiment

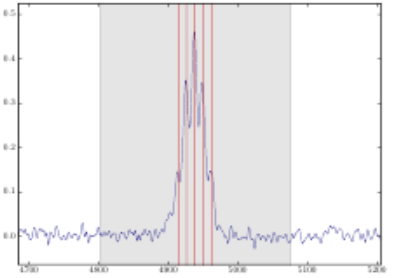
Software tools
developed and used
(Python programming language)

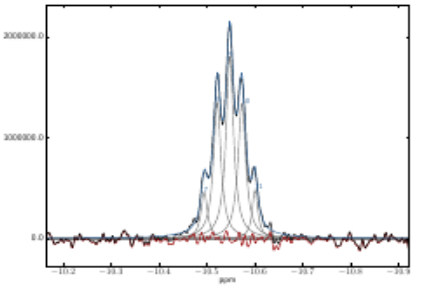
# NMRPy

- 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/NMRPy/nmrpy



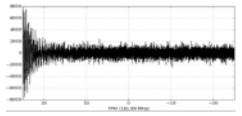




# Tools and Techniques

NMR spectroscopy

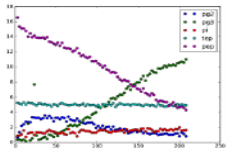
**NMR time-courses**



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

`NMRPy`

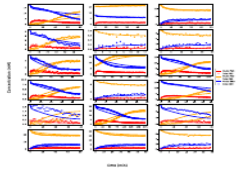
**Progress curves**



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

`NMRPy`
`Numpy`
`Scipy`
`Matplotlib`

**Parametrisation (multiple enzymes)**

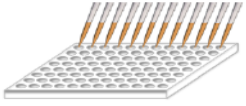


Fit representative rate equations to aggregated progress curves to obtain kinetic parameters for the enzymes in the system.

`PySCeS`
`Lmfit`

Microtitre plate assays

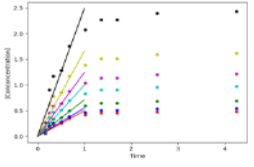
**Microtitre plate assays**



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)**

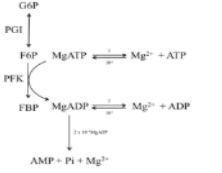
$$v_{PGI} = \frac{V_f \, e_t \left(\frac{g6p}{K_{m,G6P}}\right)\left(1 - \frac{f6p}{g6p \cdot K_{eq}}\right)}{1 + \left(\frac{g6p}{K_{m,G6P}} + \frac{f6p}{K_{m,F6P}}\right)}$$

| | |
|---|---|
| $V_f$ | $3.551 \pm 0.050$ ($V_r = 3.431$) |
| $G6P_{0.5}$ | $0.550 \pm 0.236$ |
| $F6P_{0.5}$ | $0.152 \pm 0.017$ |
| $K_{eq}$ | $0.286 \pm 8 \times 10^{-6}$ |

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

`PySCeS`
`Lmfit`

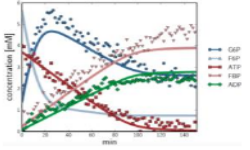
**Model construction**




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

`PySCeS`

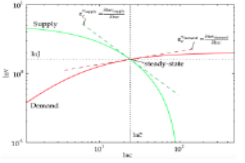
**Model validation**



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`

Badenhorst et al. (2019) *Processes*, 7:460

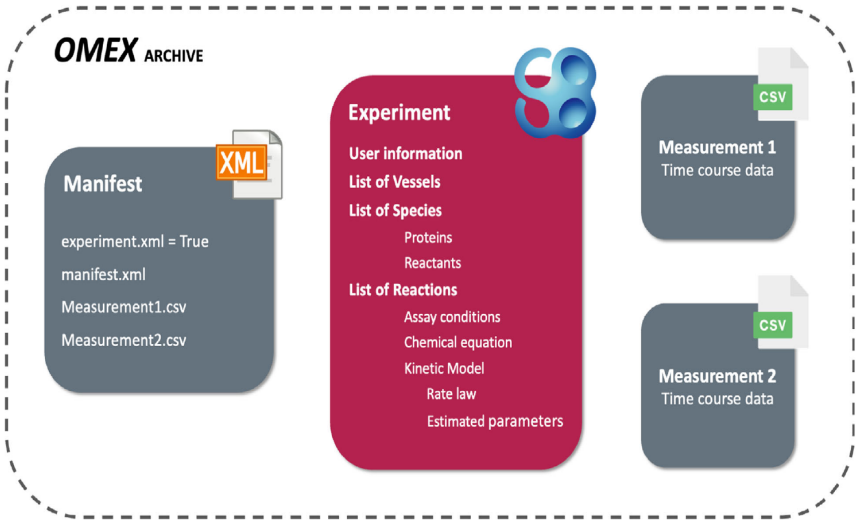
# Challenges of data management

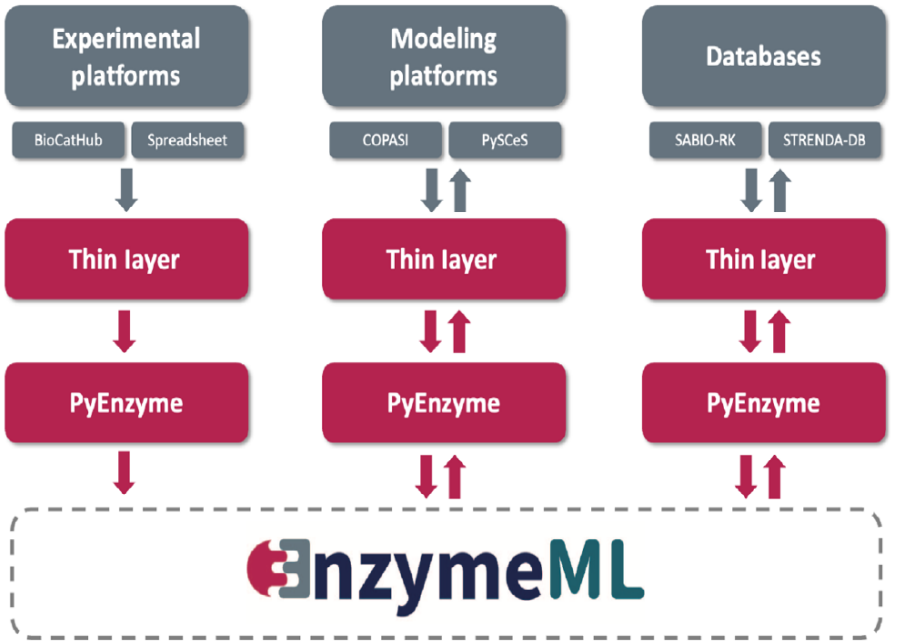



- Manual copy and paste
- Medium- to high-throughput data acquisition
- Transfer of data and metadata
- Robotics, automation, integration with ELNs/LIMS

- New data analysis and modelling methods
- Quality of data
- New methods of experimental design

# EnzymeML

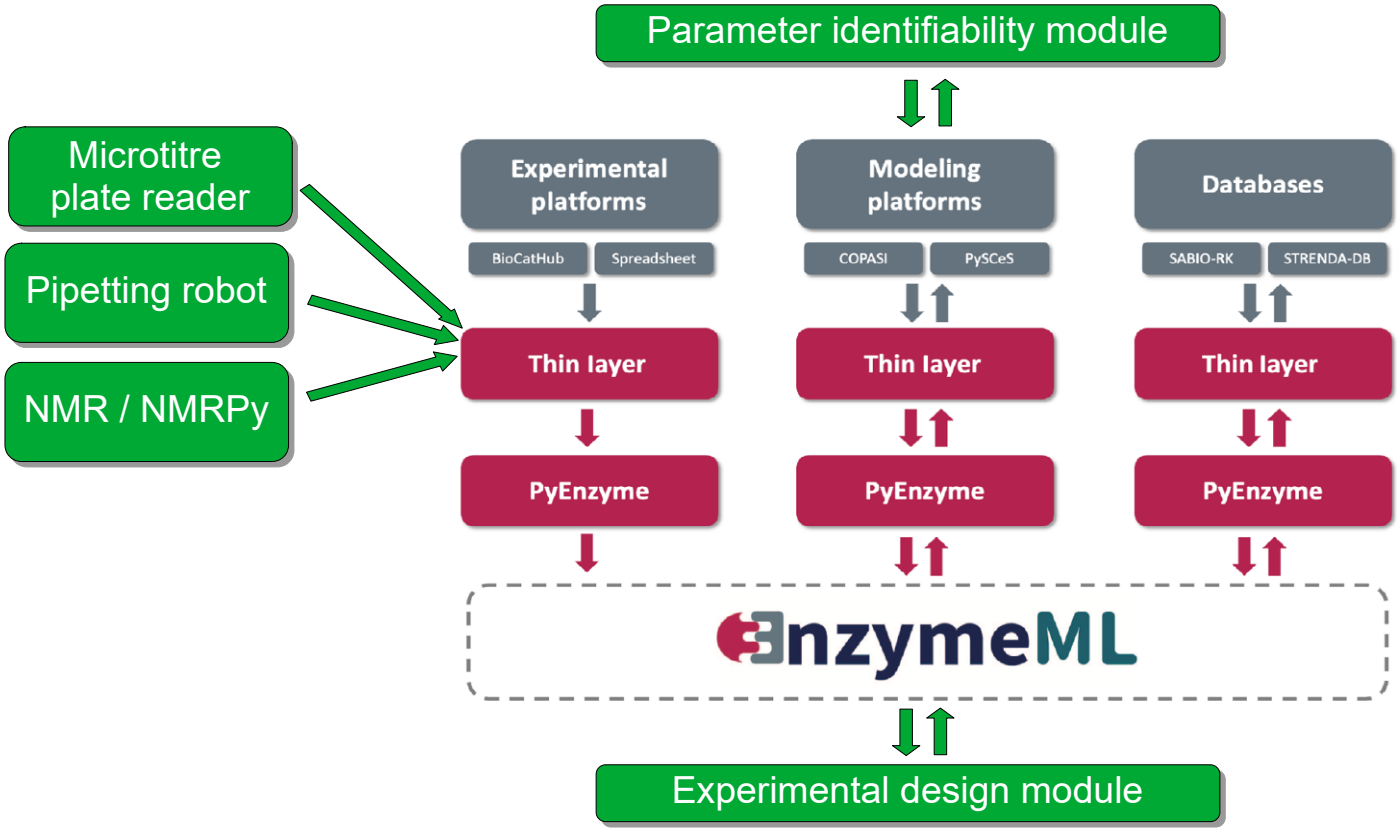


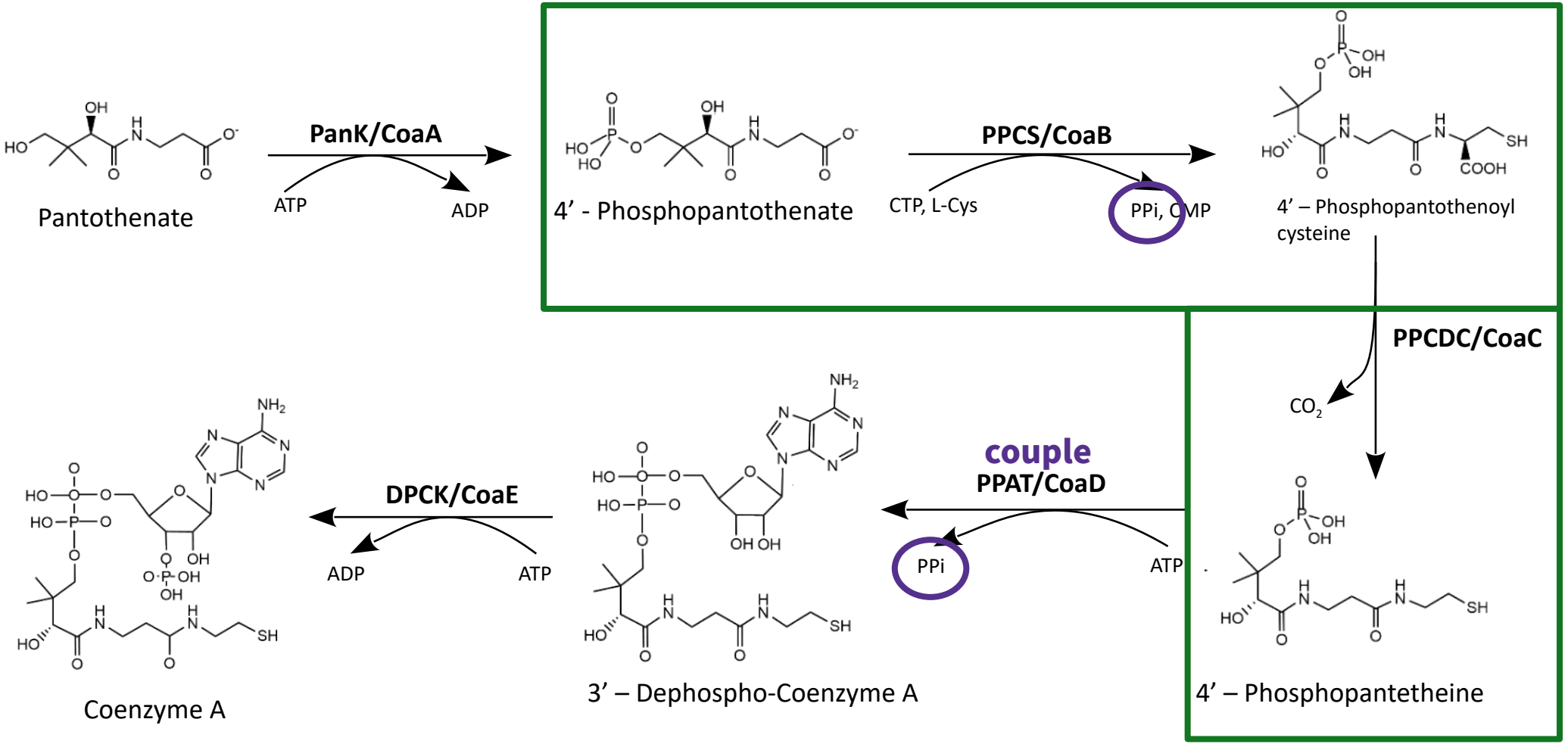


Range et al. (2021) *FEBS J*. doi:10.1111/febs.16318
Lauterbach et al. (2023) *Nat. Meth.* doi:10.1038/s41592-022-01763-1

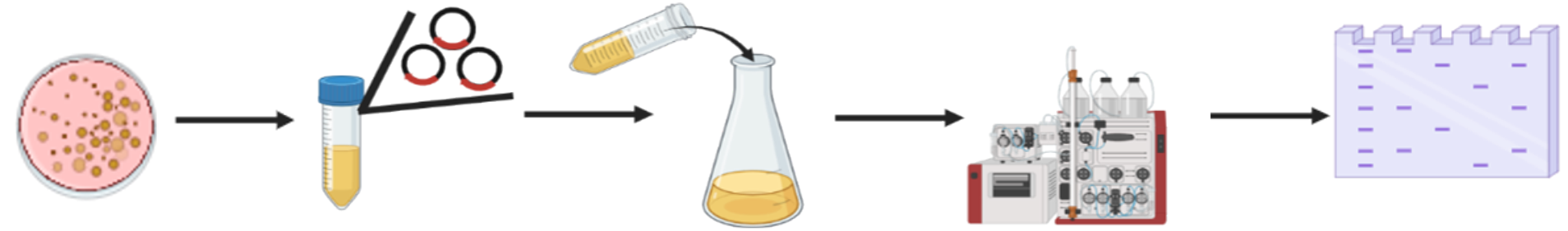
# FAIR Enzymology: PyEnzyme extensions



# Example: CoaBC from CoA Biosynthesis Pathway



# Protein purification workflow



Transformed *E.coli* BL21 cells

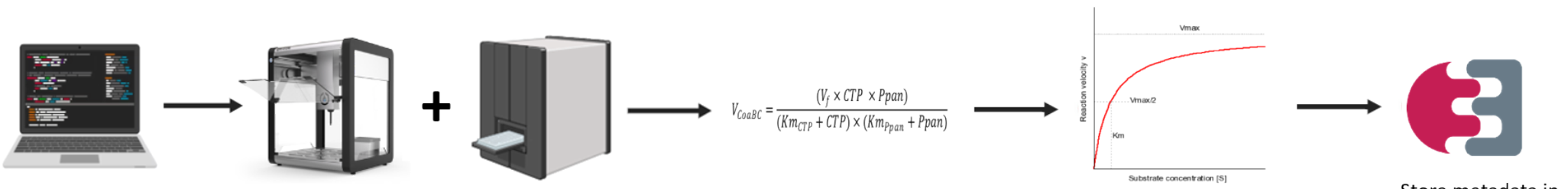
Overnight culture

Bulk expression

IMAC purification

SDS-PAGE analysis

# Kinetic characterisation workflow




Develop code for liquid handler

Use liquid handler for spectrophotometric assays

Parameterisation

Generate Michaelis-Menten graphs

Store metadata in an EnzymeML doc

Francél Wessels, MSc thesis (2025)

# Automation in data acquisition: Programmed liquid handling

```python
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_eppendorf_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

Francél Wessels,
MSc thesis (2025)

# 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

Back-end/Server

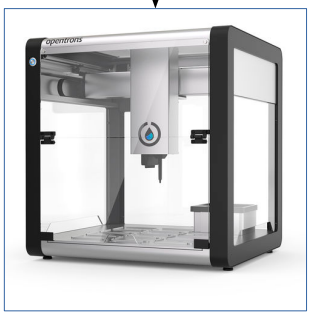
Front-end/Client

Client file system

Python API using FastAPI

- PySCeS
- NMRpy
- PyEnzyme
- Etc...

Experiment Execution



FastAPI:

- JSON (*de*)serialisation
- File uploads and downloads

Oauth2 cookie- or JWT- based Security

Components:

- Dynamically populated HTML using Jinja templating.
- Stylesheet and UI CDN components (e.g. Bootstrap, Plotly etc.)
- Custom JS where necessary, increasing responsiveness

EnzymeML, CSV, xlsx etc.

EnzymeML, CSV, xlsx etc.

Fully-functional GUI for importing, creating and editing files in various formats. Intuitive user interface.

JSON (*de*)serialisation

SQLAlchemy ASYNC CRUD operations

Database (PostGreSQL)

REpresentational State Transfer (REST) service

Response

JSON

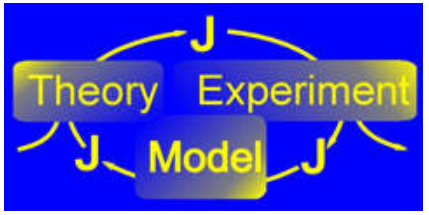
Allows scalability and cross-platform interoperability

## 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

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

## 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