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Extending a dynamic model of trypanosome metabolism

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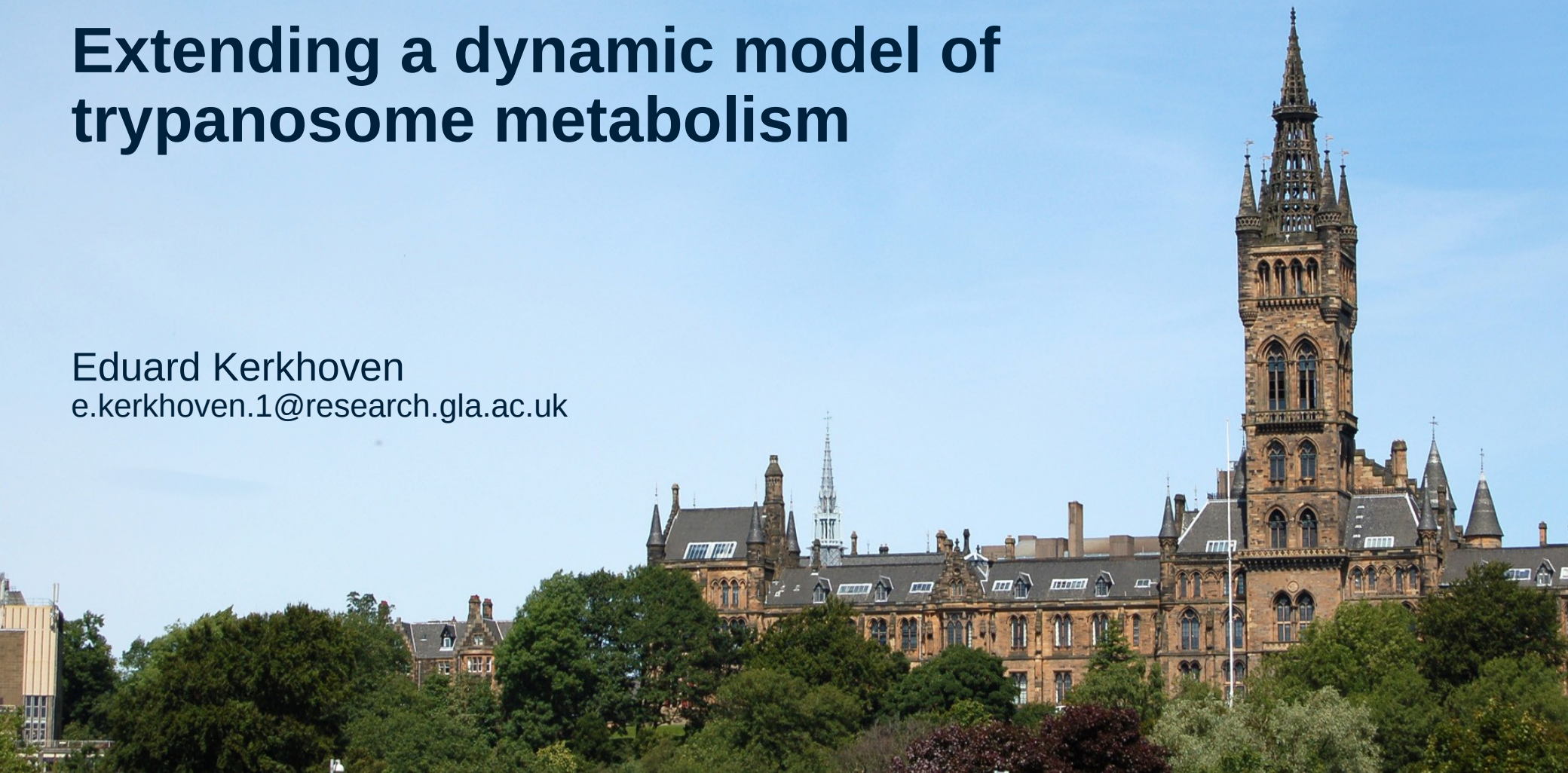

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Overview

- **Introduction to *SilicoTryp***
  - Current model of glycolysis
- **Extension of model**
  - Trypanothione
  - Pentose phosphate pathway (PPP)
  - Wiki-page
- **Cytosolic PPP**
- **Glycosomal PPP**
  - Phosphate leak
  - Two hypotheses
- **Future extension**
- **Conclusions**

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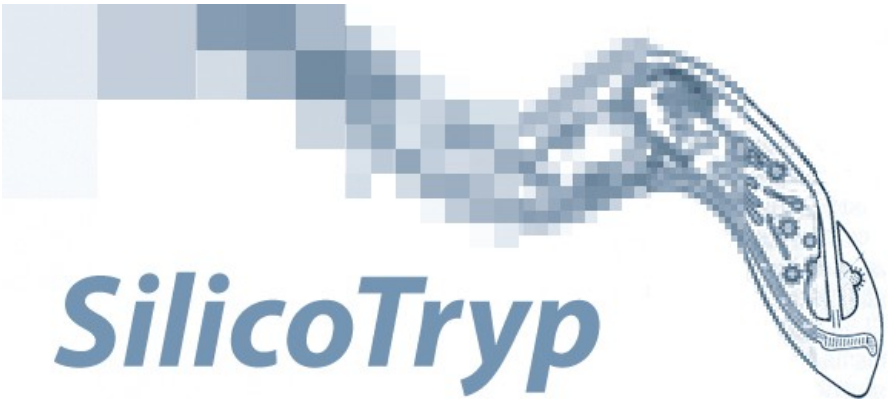
SilicoTryp

To create a ‘Silicon Trypanosome’, a comprehensive, experiment-based, multi-scale mathematical model of trypanosome physiology.



Bakker BM, Krauth-Siegel RL, Clayton C, Matthews K, Girolami M, Westerhoff HV, Michels PAM, Breitling R & Barrett MP (2010) Parasitology 137: 1333-41

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Rijksuniversiteit Groningen, NL

Universitair Medisch Centrum Groningen, NL

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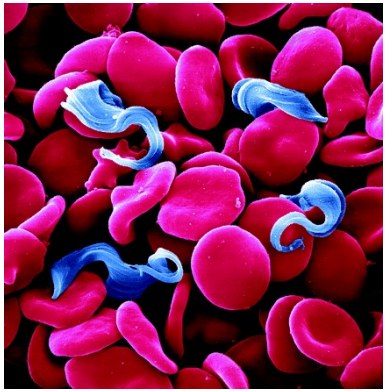
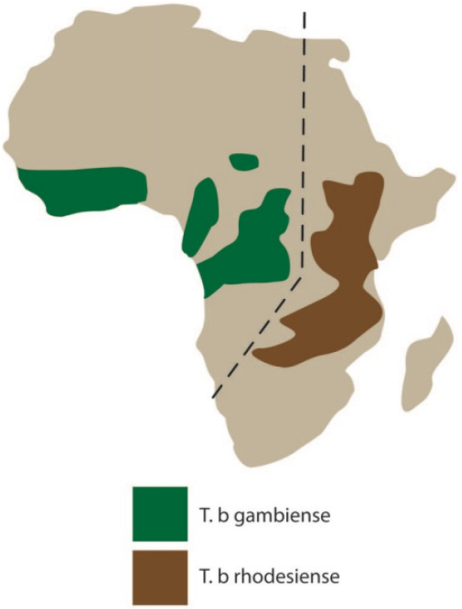
Trypanosoma brucei

- Protozoan parasite
- African sleeping sickness in human
- Nagana in cattle
- Fatal if untreated
- Drugs toxicity, difficulties in administration, emerging resistance





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Dynamic model of bloodstream form *T. brucei* glycolysis

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Glycolysis in Bloodstream Form *Trypanosoma brucei* Can Be Understood in Terms of the Kinetics of the Glycolytic Enzymes*

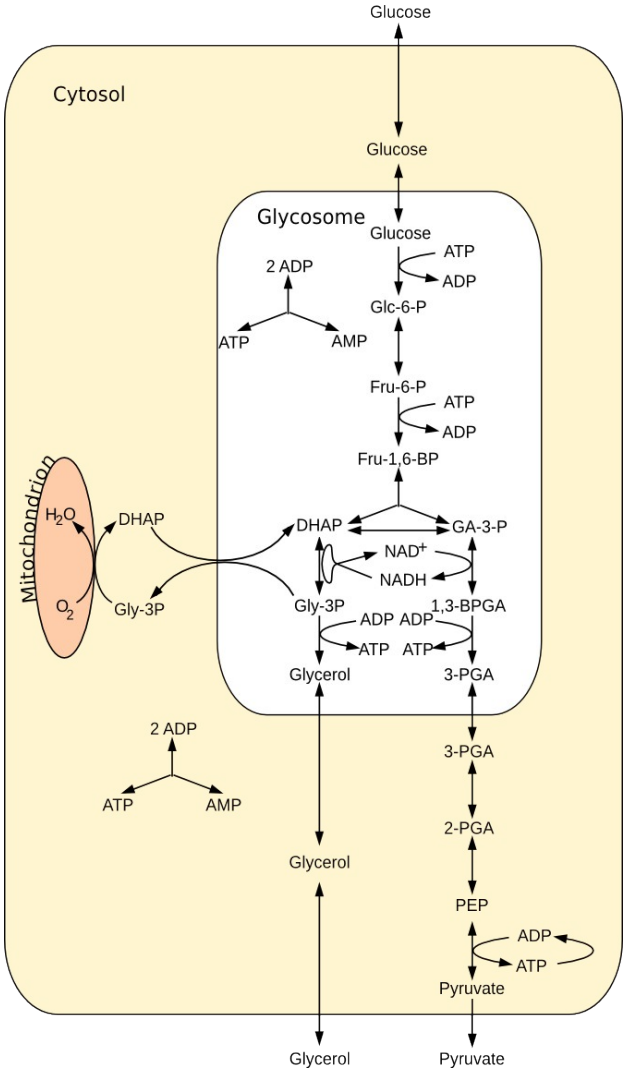
(Received for publication, July 10, 1996, and in revised form, October 9, 1996)

Barbara M. Bakker‡§, Paul A. M. Michels¶, Fred R. Opperdoes¶, and Hans V. Westerhoff‡‖

$$v_{\mathrm{PGI}} = V_{fmax} \cdot \frac{\frac{[\mathrm{Glc6P}]_g}{K_{m,\mathrm{Glc6P}}} \cdot \left(1 - \frac{[\mathrm{Fru6P}]_g}{[\mathrm{Glc6P}]_g \cdot K_{eq,\mathrm{PGI}}}\right)}{1 + \frac{[\mathrm{Glc6P}]_g}{K_{m,\mathrm{Glc6P}}} + \frac{[\mathrm{Fru6P}]_g}{K_{m,\mathrm{Fru6P}}}}$$

$$\frac{d[\mathrm{Fru6P}]}{dt} = v_{\mathrm{PGI}} - v_{\mathrm{PFK}}$$

- Part of glycolysis is localized in an unique peroxisome-like organelle: the glycosome



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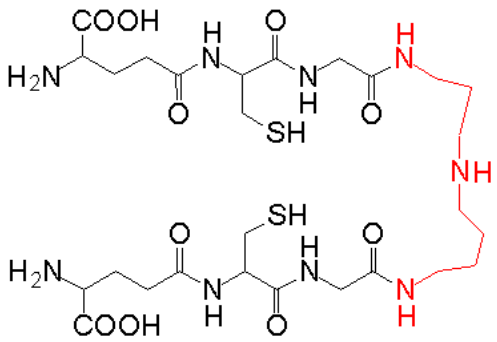
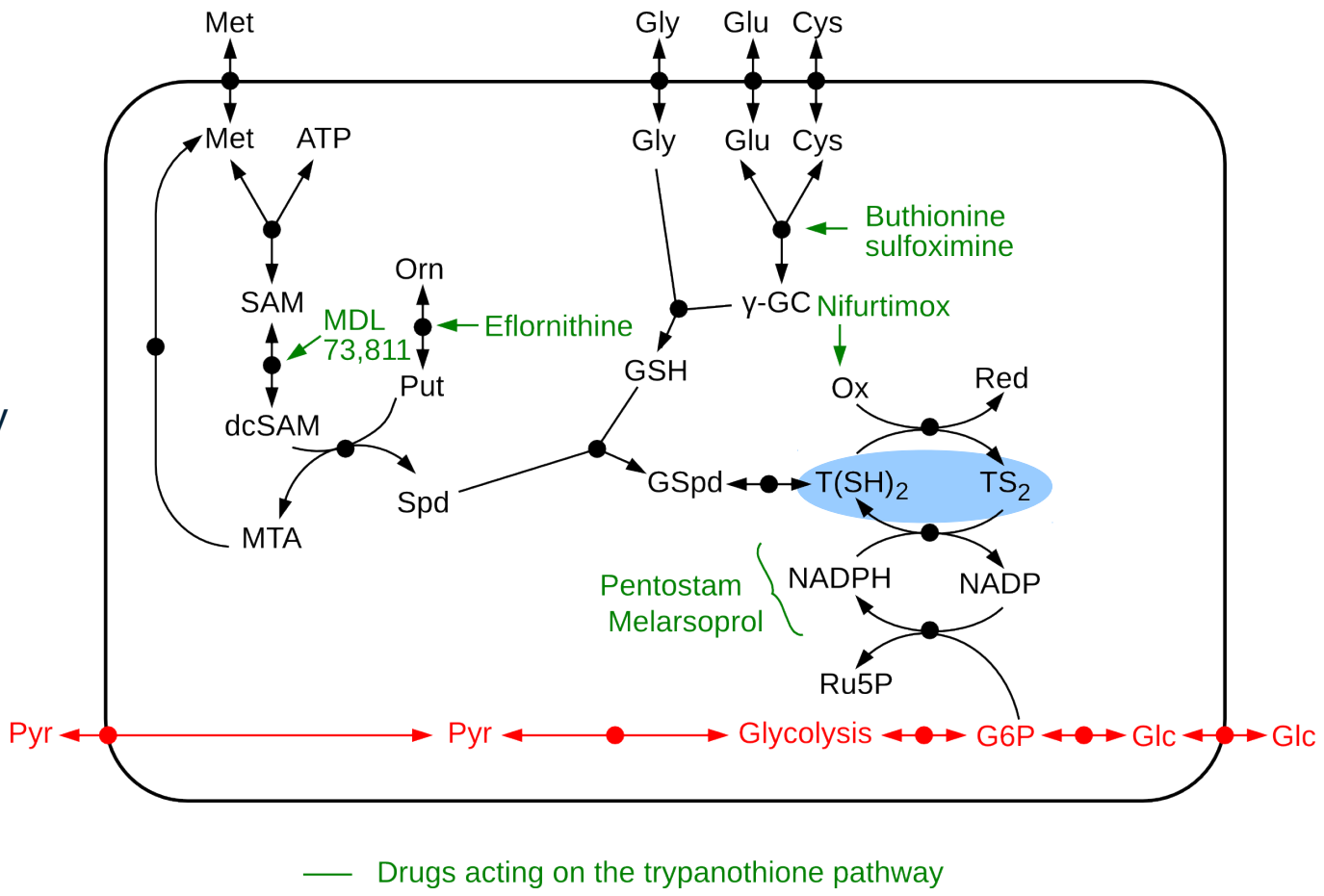
A unique thiol: trypanothione

- Oxidative stress protection
- Trypanothione = 2x glutathione + spermidine
- Eflornithine is suicide inhibitor of ornithine decarboxylase
- Pentose phosphate pathway provides NADPH





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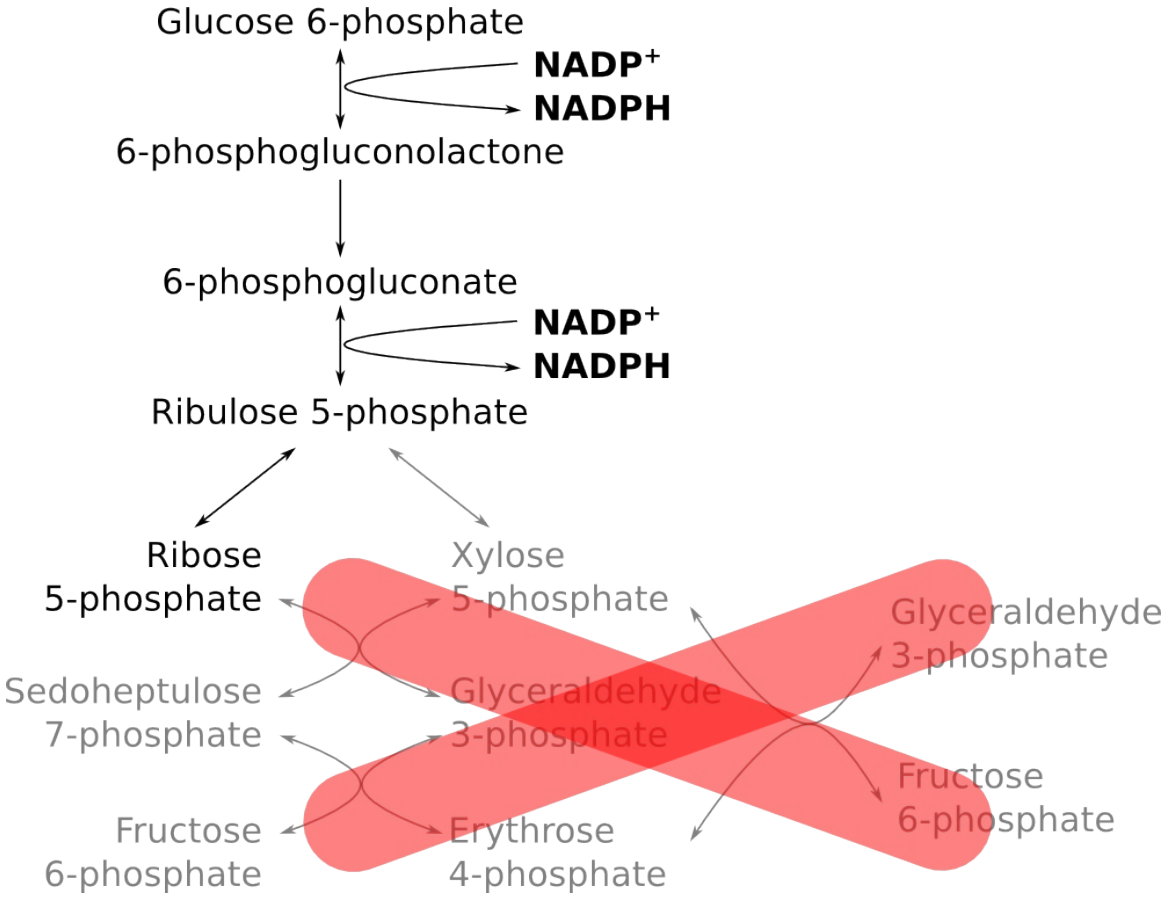
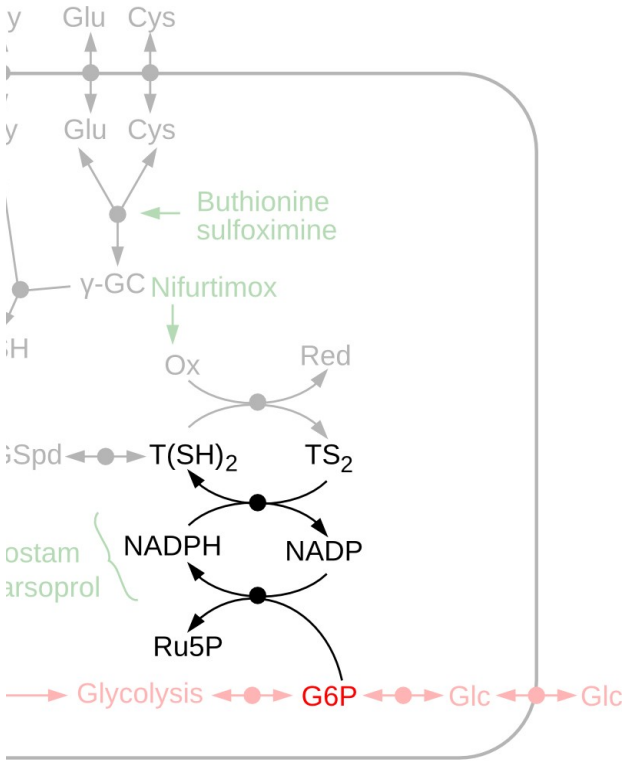
Pentose phosphate pathway in bloodstream form *T. brucei*





This wiki is work in progress and contains unpublished data. Please treat with confidentiality.

Phosphofructokinase

Navigation

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Chemical equation [edit]

$$Fructose\text{-}6\text{-}phosphate_{glycosome} + ATP_{glycosome} \rightarrow Fructose\text{-}1,6\text{-}biphosphate_{glycosome} + ADP_{glycosome}$$

Rate equation [edit]

$$v_{PFK} = V_{fmax} * \frac{K_i^1}{Fru16BP_g + K_i^1} * \frac{\frac{Fru6P_g}{Km_{Fru6P}} * \frac{ATP_g}{Km_{ATP}}}{(1.0 + \frac{Fru6P_g}{Km_{Fru6P}} + \frac{Fru16BP_g}{K_i^2}) * (1.0 + \frac{ATP_g}{Km_{ATP}})}$$

Parameters [edit]

$V_{fmax} = 1708\ nmol.min^{-1}.mg\ of\ cell\ protein^{-1}$	The value was measured for the 2005 version of the model[1](see supplementary information table S1). The assay is described in Misset and Opperdoes (1984)[2] (pH=7.6, T=25°C). The reported value is 1708 +/-299 (3) (average +/- SEM from n experiments).
$Km_{Fru6P} = 0.82\ mM$	From Cronin et al. (1985) [3]. The value was measured at pH=6.7 and reported in table 3:

Table 3. *Effects of enzyme concentration on the kinetics of fructose 6-phosphate phosphorylation by phosphofructokinase from T. brucei*

Enzyme activity was determined by using the coupled assay for fructose 1,6-bisphosphate formation. [Fru-6-*P*]$_{0.5}$ is the concentration of fructose 6-phosphate required to give half-maximum velocity, and *h* is the Hill constant.

Concn. of enzyme (μg/ml)	$V_{max.}$ (units/mg)	[Fru-6-*P*]$_{0.5}$ (mM)	*h*
0.100	267	0.82	1.2
0.010	267	1.00	1.3
0.005	267	1.26	1.3
0.002	267	1.91	1.4

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Dynamic modelling under uncertainty: The case of *Trypanosome brucei* energy metabolism

Fiona Achcar

PS016

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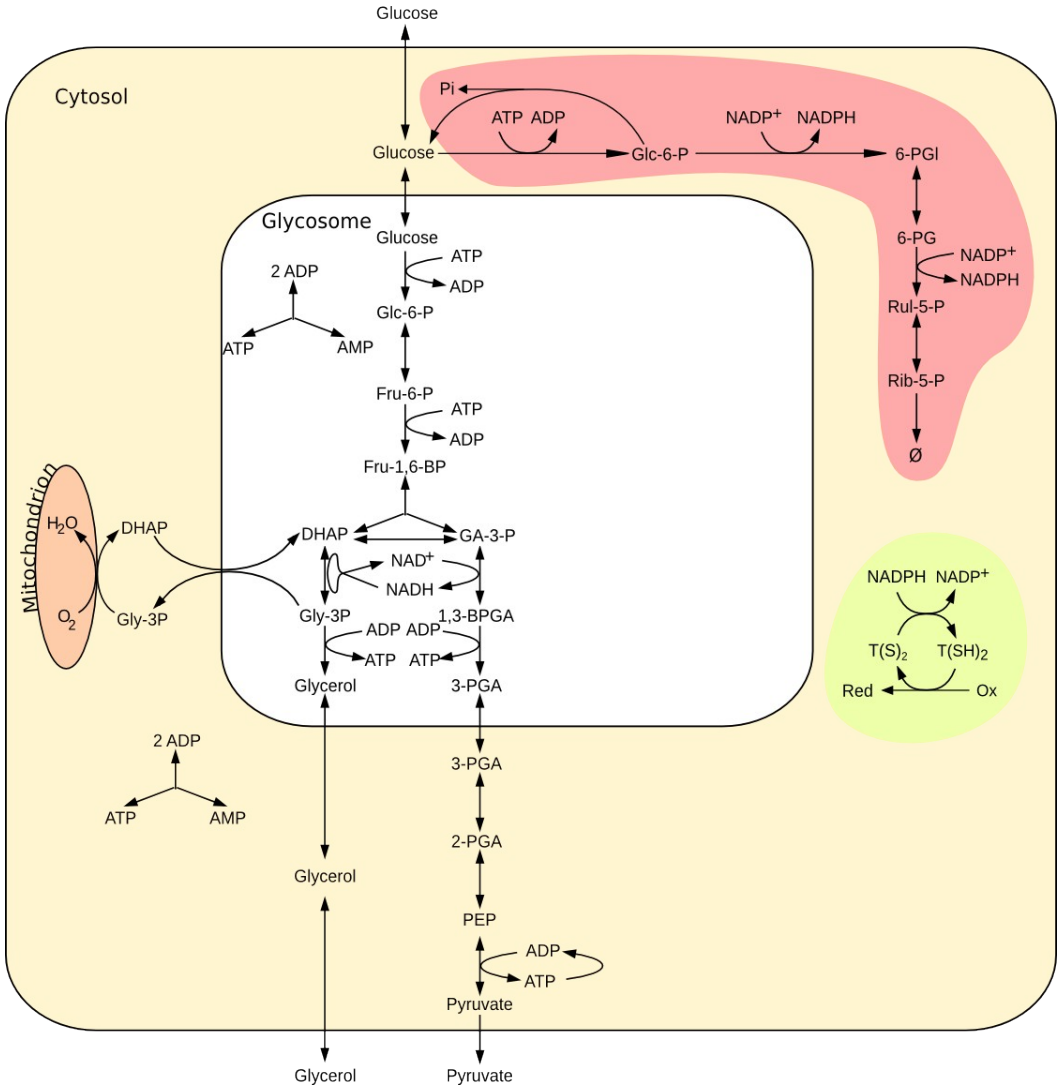
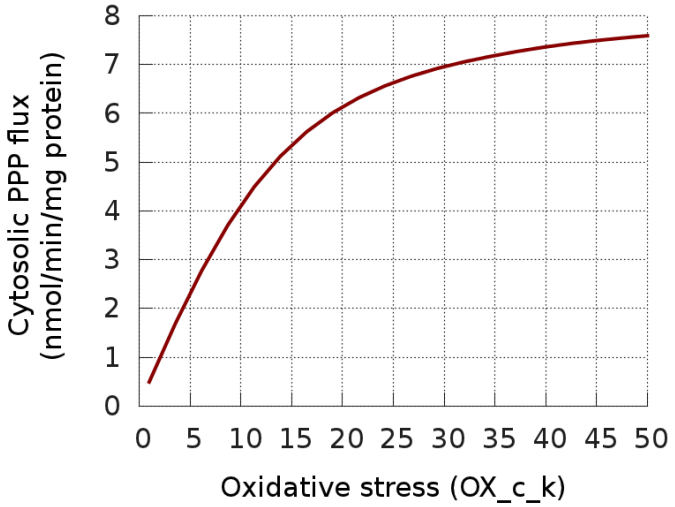
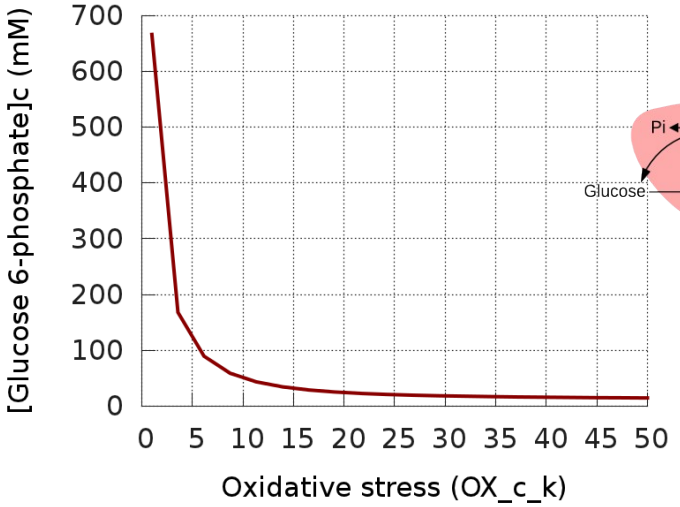
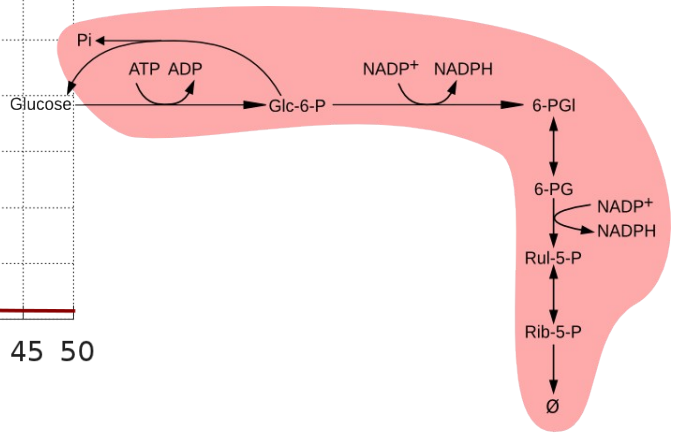
Cytosolic pentose phosphate pathway



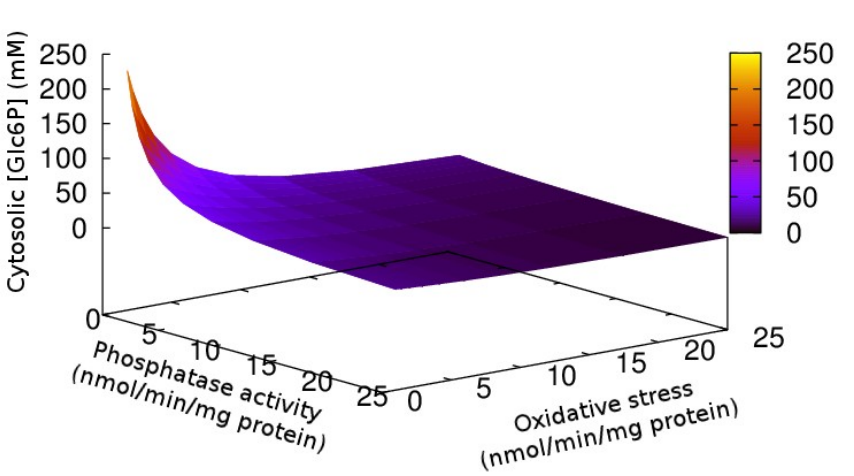
Extension with the cytosolic PPP: an (aspecific) phosphatase?

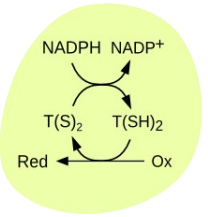






- Level of oxidative stress regulates flux through PPP
- Low oxidative stress leads to Glc6P accumulation
- Phosphatase activity would prevent this accumulation
- Initial experiments have confirmed this activity





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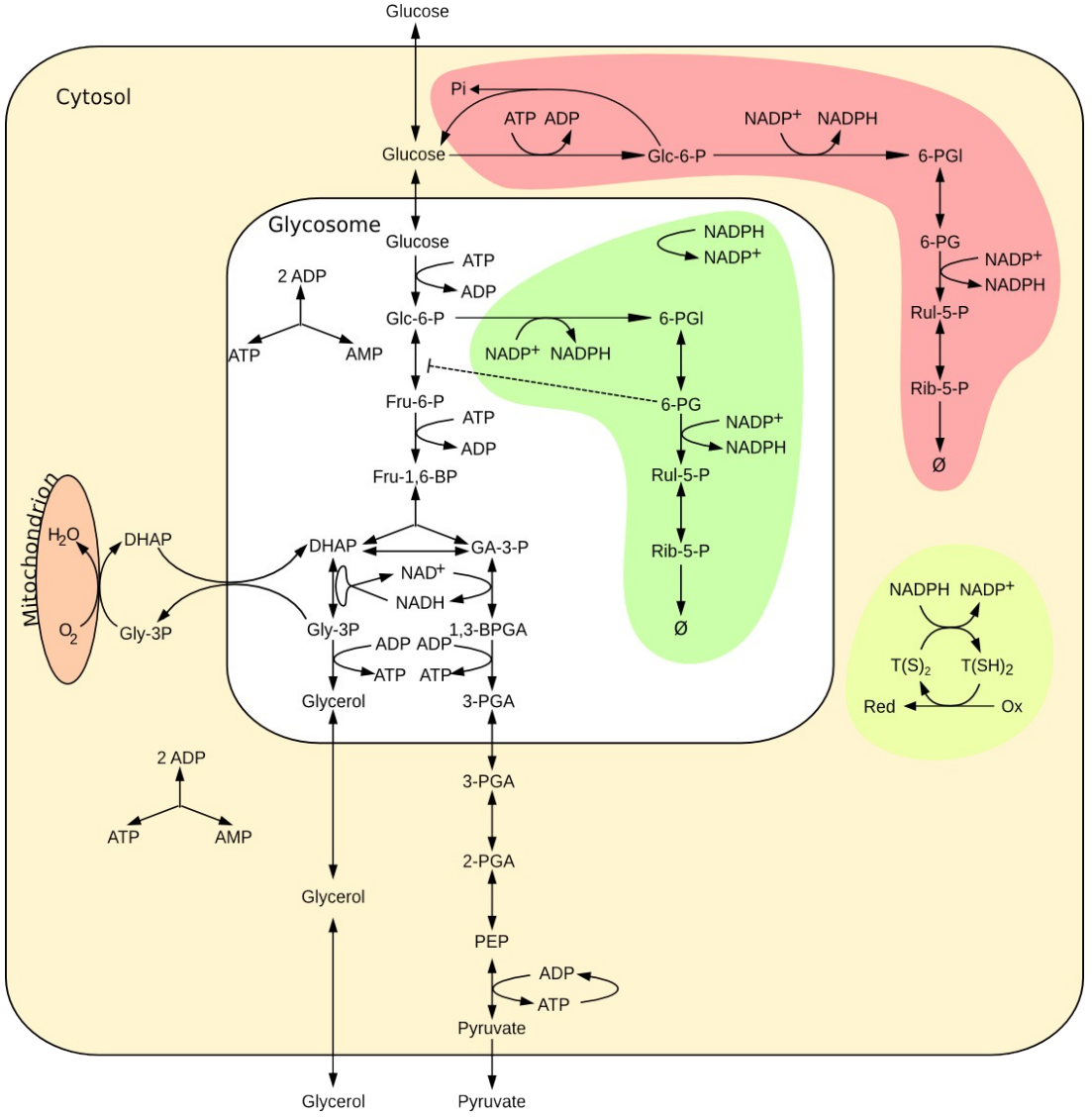
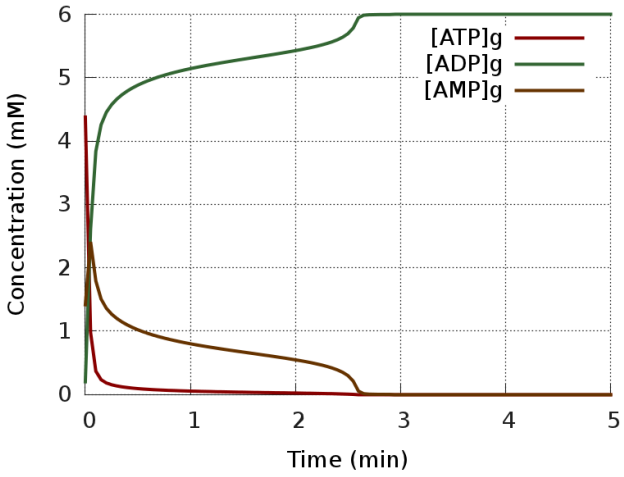
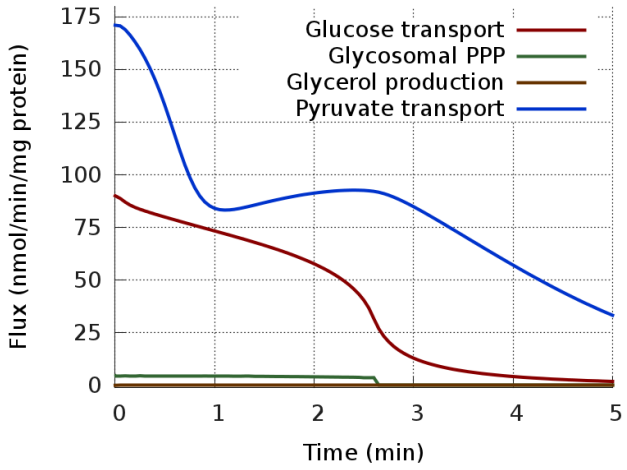
Glycosomal pentose phosphate pathway

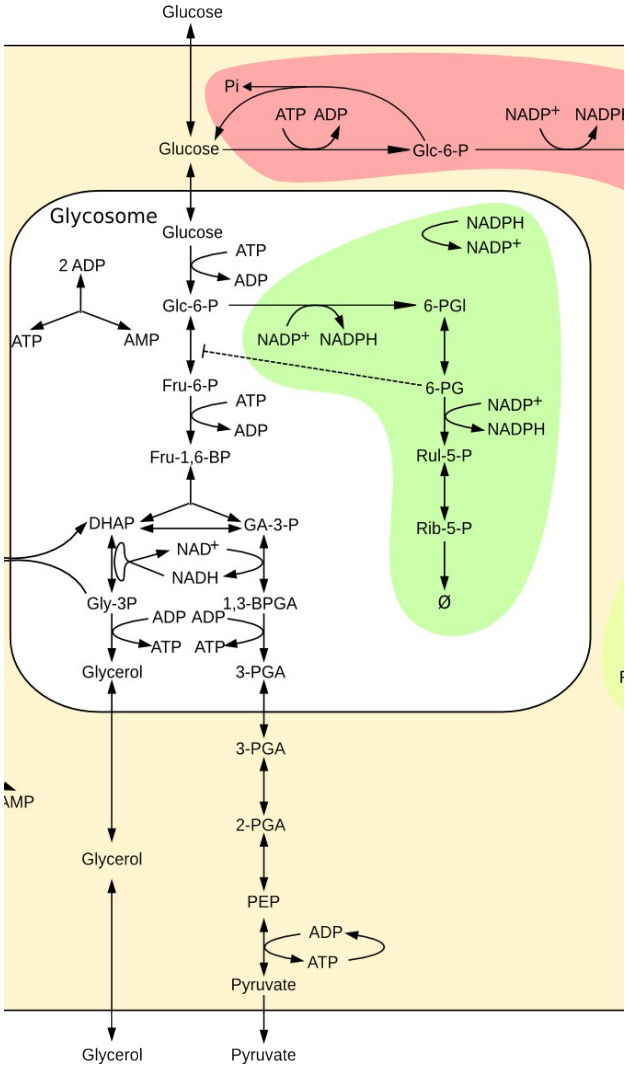


Extension with the glycosomal PPP: a 'phosphate leak'

- Glycosomal PPP introduces a 'phosphate leak'
- ATP/ADP balance in glycosome is perturbed
- Fluxes decline rapidly → non viable state
- Several hypotheses were tested

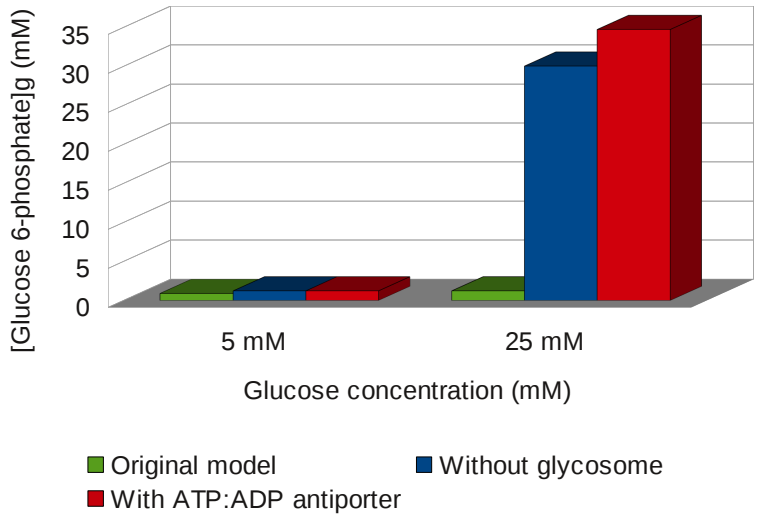


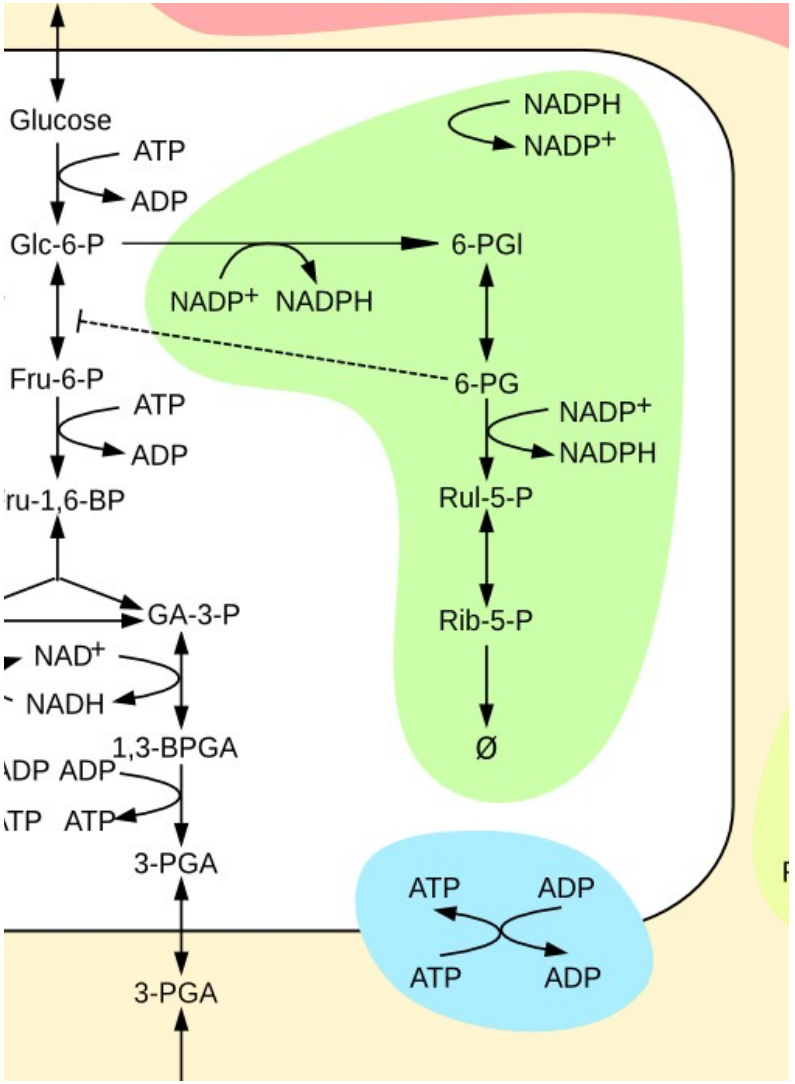




Hypothesis 1: ATP:ADP antiporter

- Works at 5 mM glucose (blood)
- Glycosomal compartmentation of ATP is essential
- Accumulation of sugar phosphates at 25 mM glucose (culture medium)
- ATP:ADP antiporter mimicks absence of glycosome

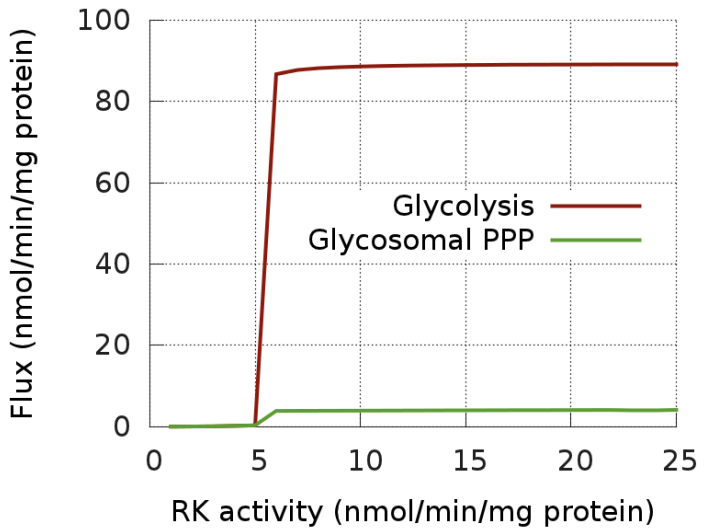


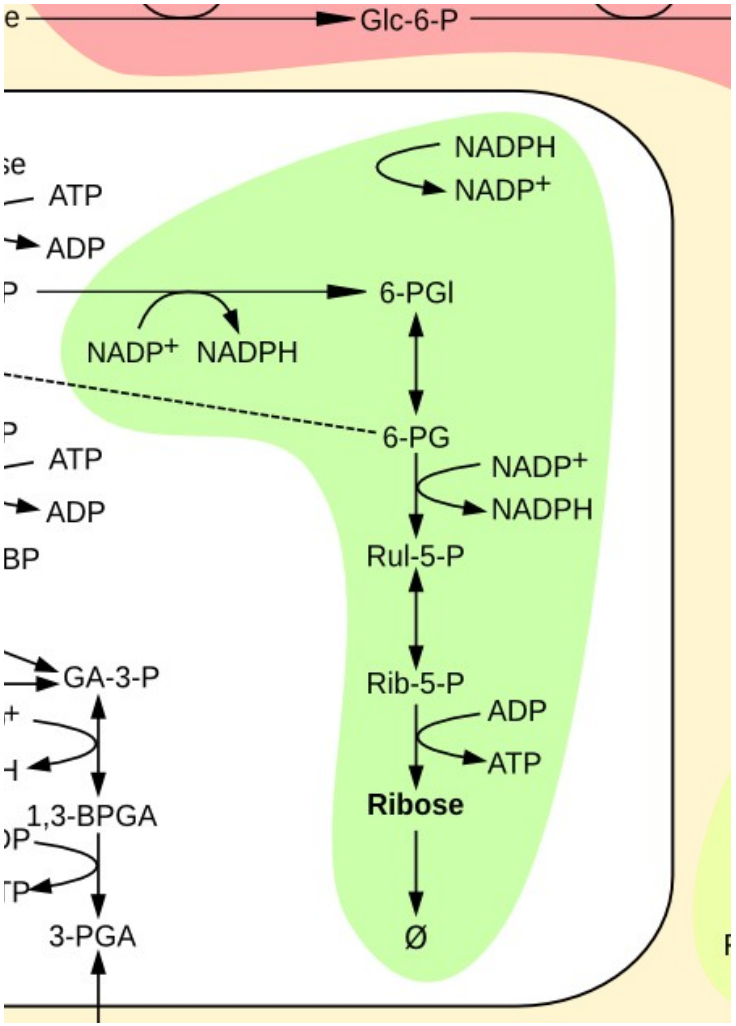


Bakker, B.M., *et al*. PNAS 2000
Haanstra, J.R., *et al.* PNAS 2008

Hypothesis 2: Ribokinase

- Ribokinase in reverse restores the ATP:ADP balance
- Ribose is produced from glucose
- Putative *T. brucei* ribokinase enzyme showed activity in both directions
- Knockdown (RNAi) and knockout experiments hint at essentiality





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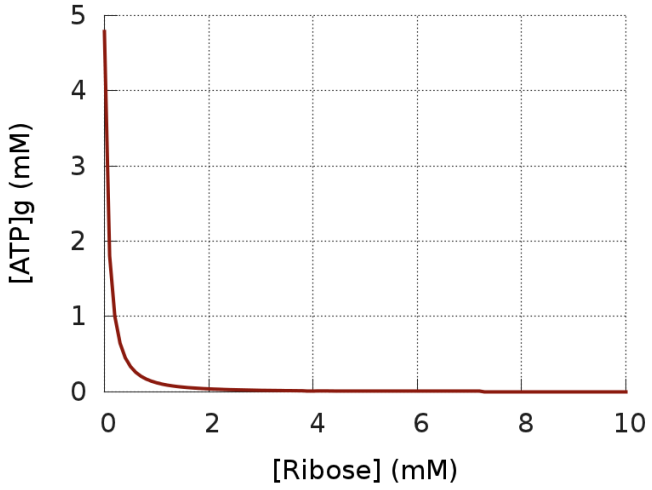
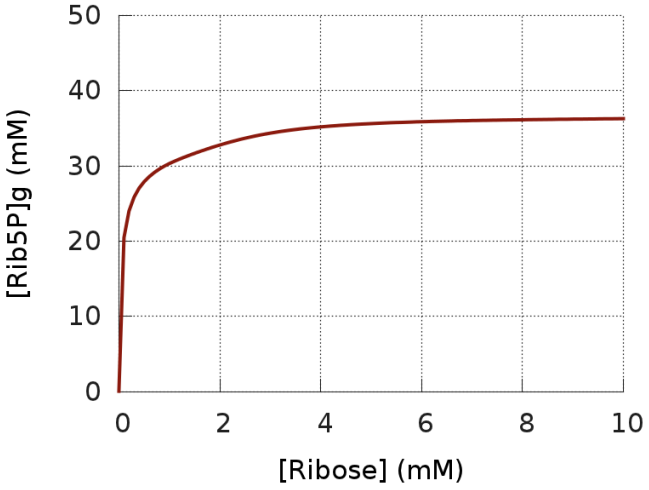
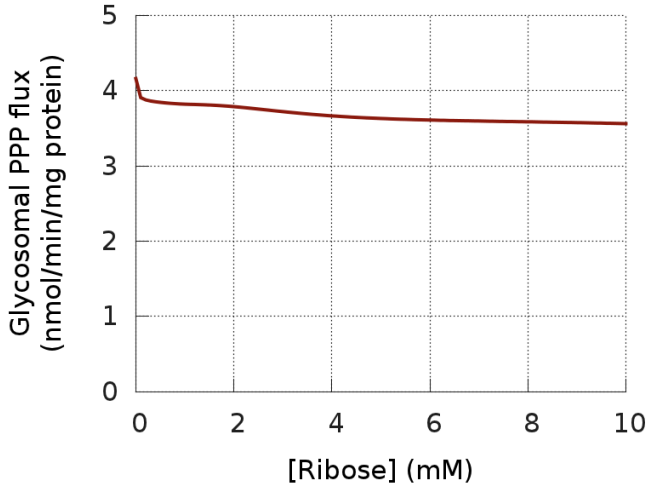
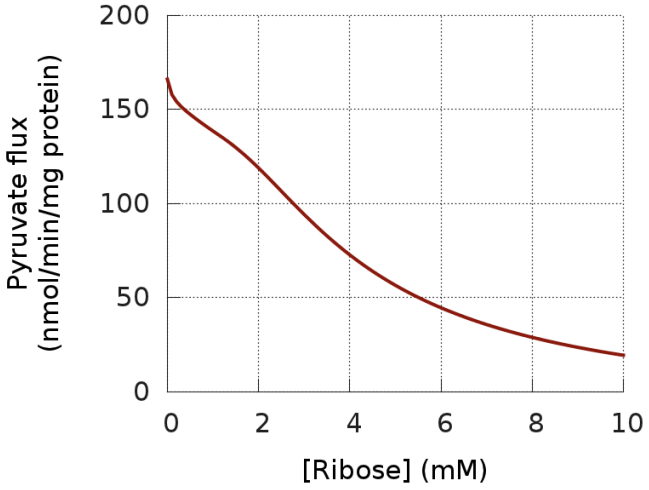
Inhibition by ribose

- Model suggests sensitivity for ribose concentration
- 5 mM should be lethal
- Not reproducible in lab
- Not unexpected, as we know there are more pathways involved in ribose metabolism









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Conclusions

***Trypanosoma brucei* biology:**

- **Phosphatase activity prevents accumulation of cytosolic glucose 6-phosphate**
- **Ribokinase restores phosphate balance in the glycosome**

Systems biology:

- **Even small extension of a well curated model brings up many questions**
- **Iterative cycle of experiments and modelling is necessary (and still continuing)**
- **Stress your model!**

Acknowledgements

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