AMOR·SCIENTIA·QVE·INSERVIANT·CORDI

MEXICO

INSTITVTO·N·DE
CARDIOLOGIA
IGNACIO·CHAVEZ

Departamento de Bioquímica
Instituto Nacional de Cardiología

Kinetic modeling and Metabolic Control Analysis to understand the control and regulation of metabolic pathways

Emma Saavedra

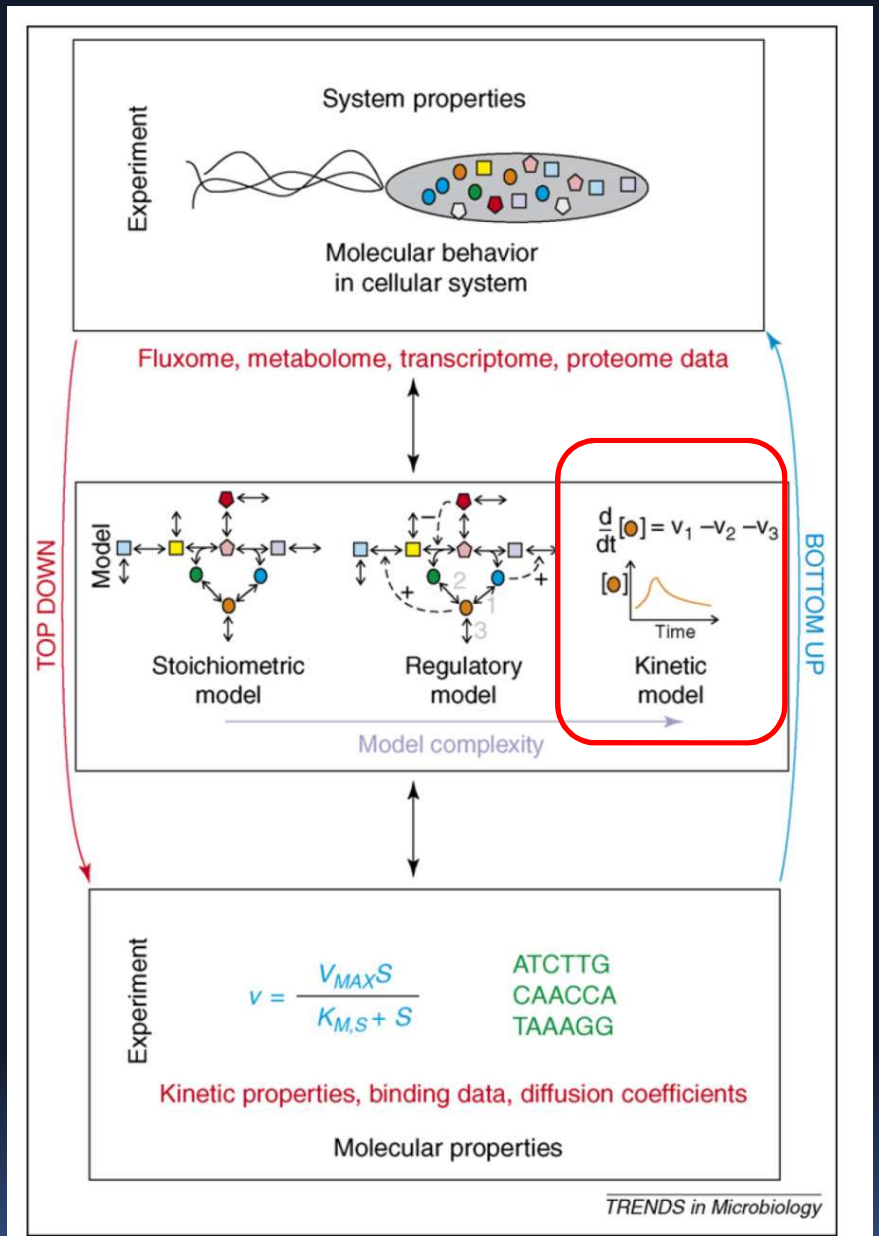
Department of Biochemistry
National Institute of Cardiology
Mexico City, MEXICO
emma_saavedra2002@yahoo.com

Systems Biology aim: to build computational models of complex cellular networks

Top-Down strategy

-Uses high-throughput "omic" data.

-Describes behavior patterns and cause-effect relationships of macromolecules.



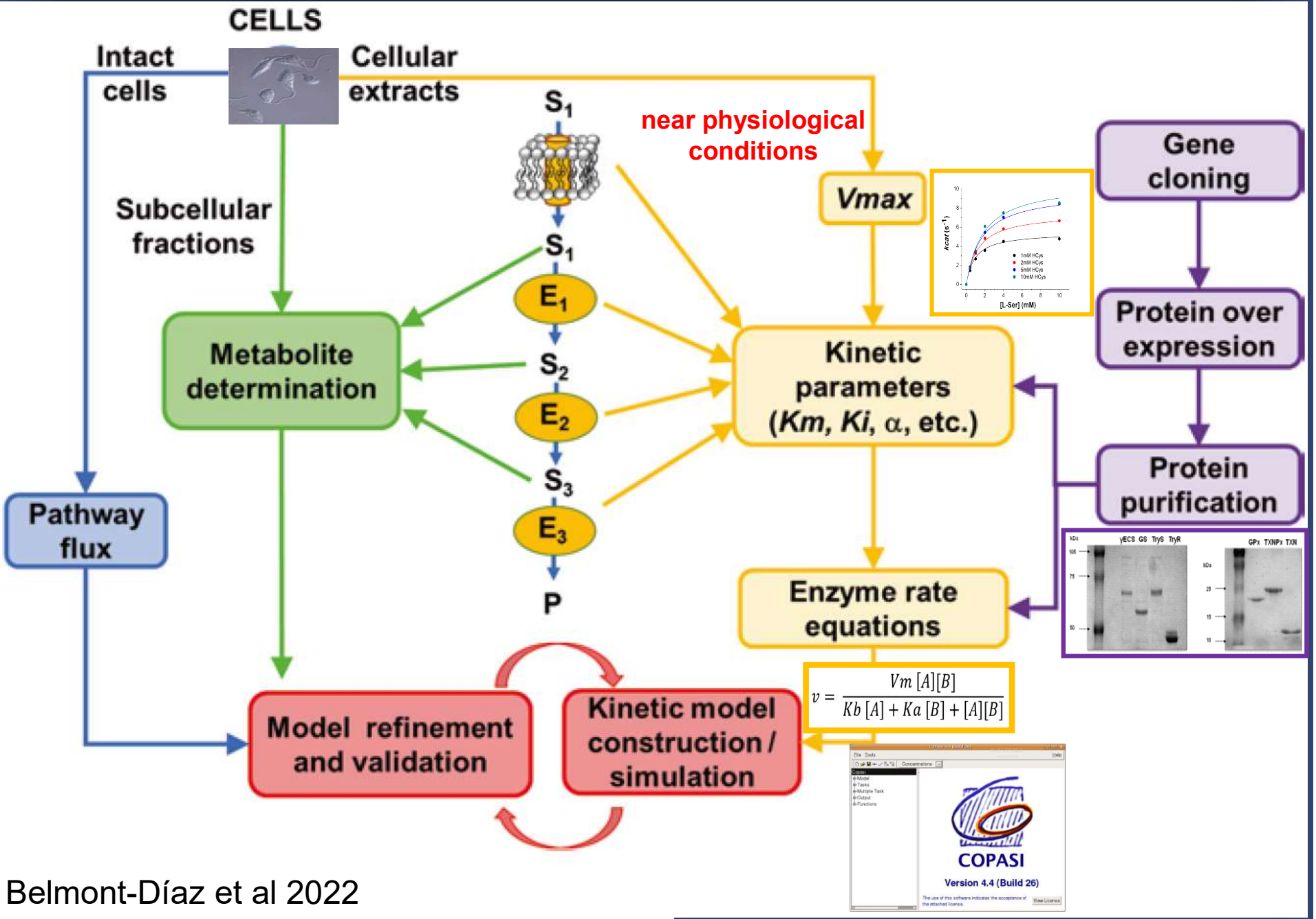
Bottom-Up (kinetic modeling)

-Based on the kinetic properties from the individual enzymes, thermodynamic parameters

- Allow to elucidate the underlying controlling and regulatory mechanisms

Bruggeman & Westerhoff 2006
Trends Microbiol

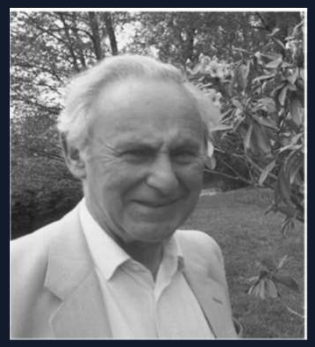
What is needed to build a kinetic model of a metabolic pathway?



Belmont-Díaz et al 2022

What is Metabolic Control Analysis?

- Henrik Kacser and James A Burns (Edinburgh, Scotland) 1973



- Molecular basis of dominance

- Reinhart Heinrich and Tom Rapoport (Berlin, Germany) 1973

- Mathematical modeling of glycolysis in erythrocytes

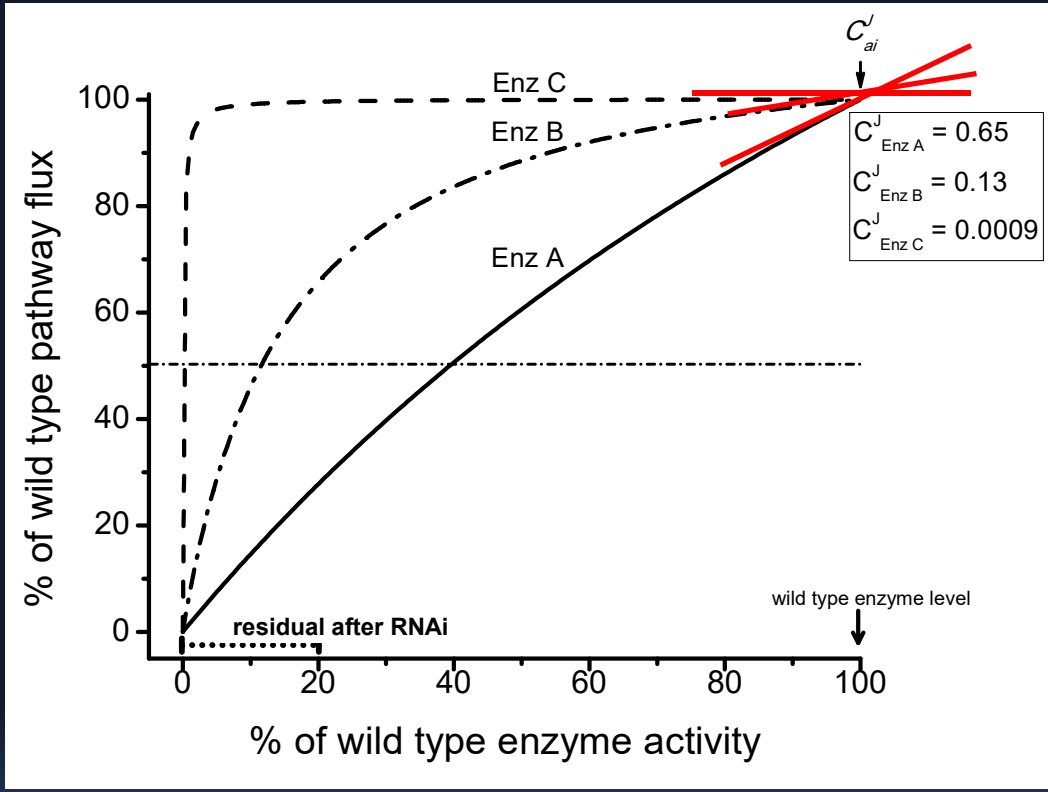
In metabolic pathways there is not a unique rate-limiting step or bottleneck reaction

Metabolic Control Analysis (MCA) *

*Saavedra et al 2019 Curr Med Chem doi: 10.2174/0929867325666180917104242

MCA allows to quantitatively determine the degree of control that each enzyme has on the pathway flux (flux control coefficient; C^{J}_{ai})

- As the C^{J}_{ai} approaches to 1, means the enzyme has the highest control on flux
- The sum of all C^{J}_{ai} have to add up 1
- Usually there are two-three main controlling steps



The enzymes with the highest therapeutic potential are those with the highest C^{J}_{ai}

Kinetic modeling and MCA to understand the controlling mechanisms in metabolic pathways

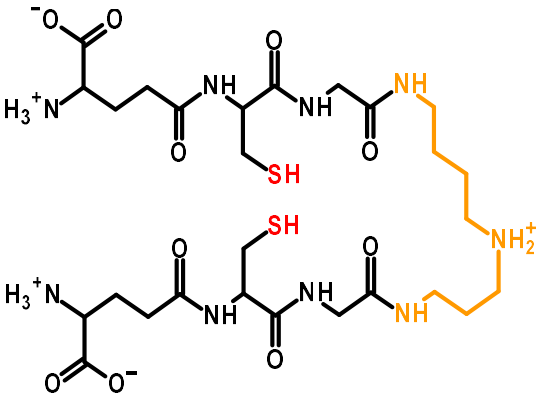
(and drug target prioritization for therapeutic intervention against parasites)

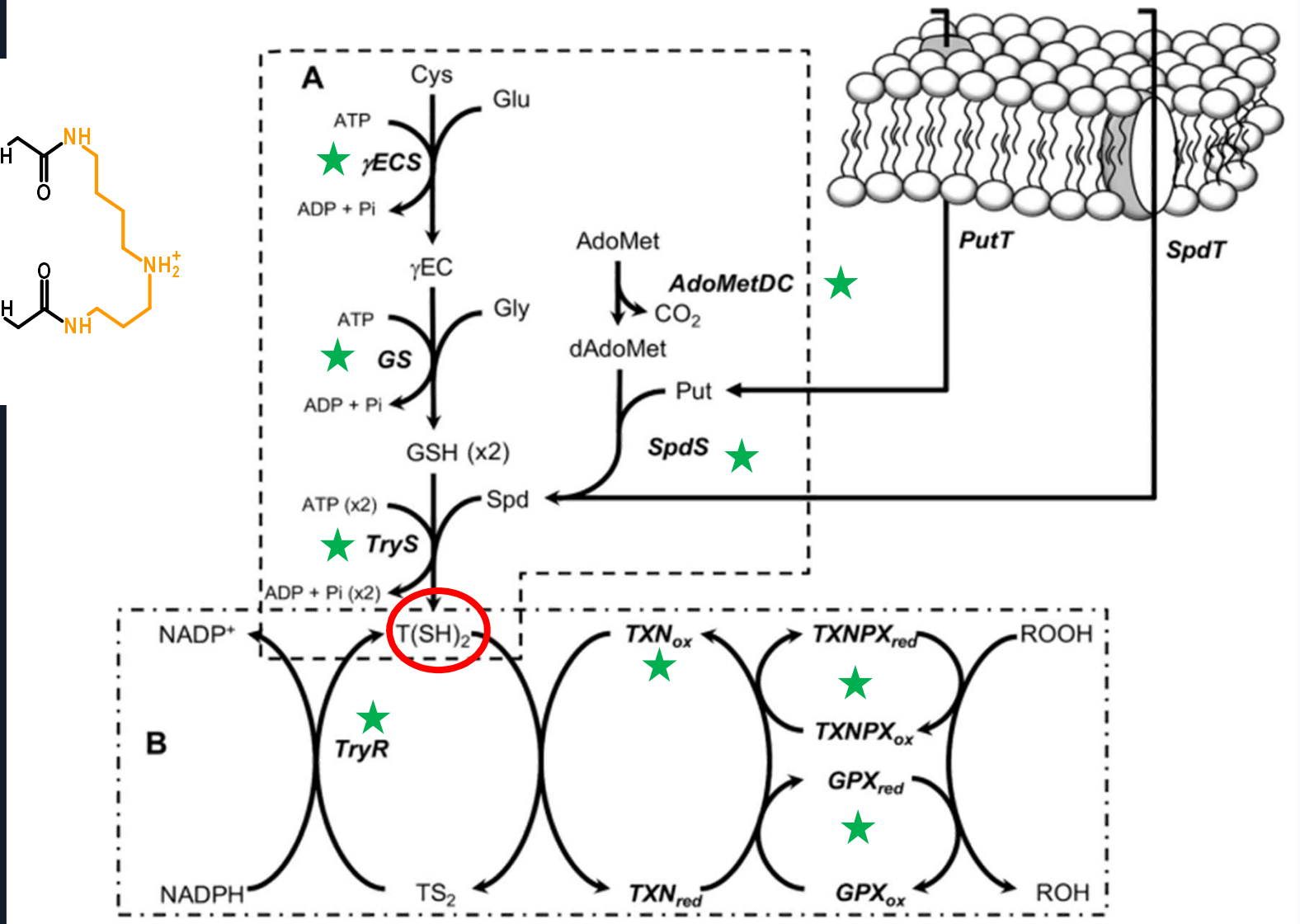
-Trypanothione metabolism in *Trypanosoma cruzi*

-Glycolysis in *Entamoeba histolytica*

Tripanothione $T(SH)_2$

$T(SH)_2$-dependent antioxidant metabolism in *Trypanosoma cruzi*





González-Chávez et al. Redox Biology 26 (2019) 101231

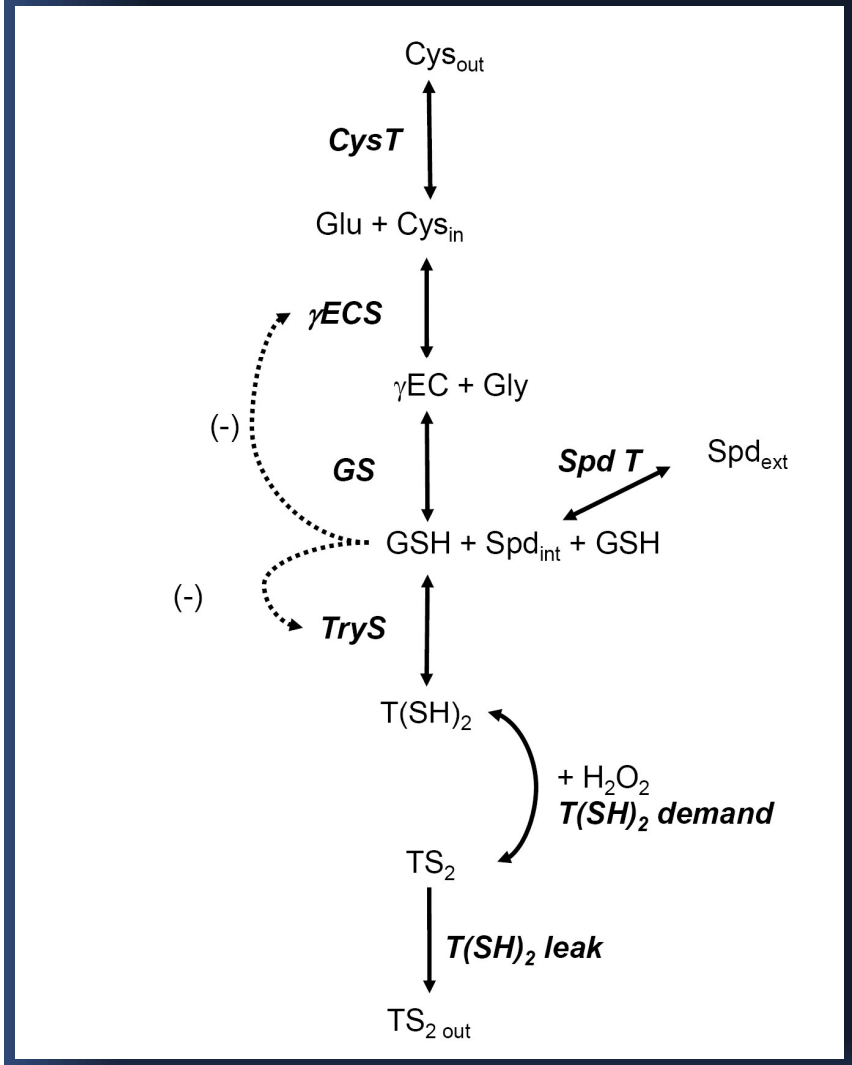
★ **All essential as determined by knockdown or knockout, thus all proposed as putative drug targets**

Additional criteria for drug target prioritization

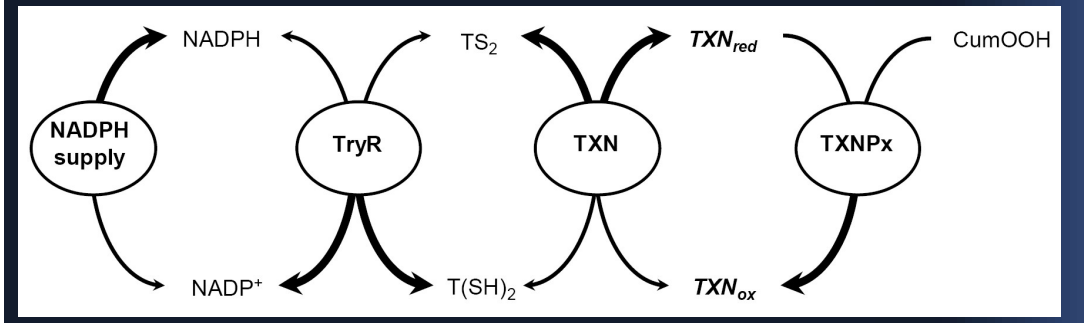
-identification of the main controlling steps by kinetic metabolic modeling and MCA

Reactions included in the kinetic models

Trypanothione synthesis



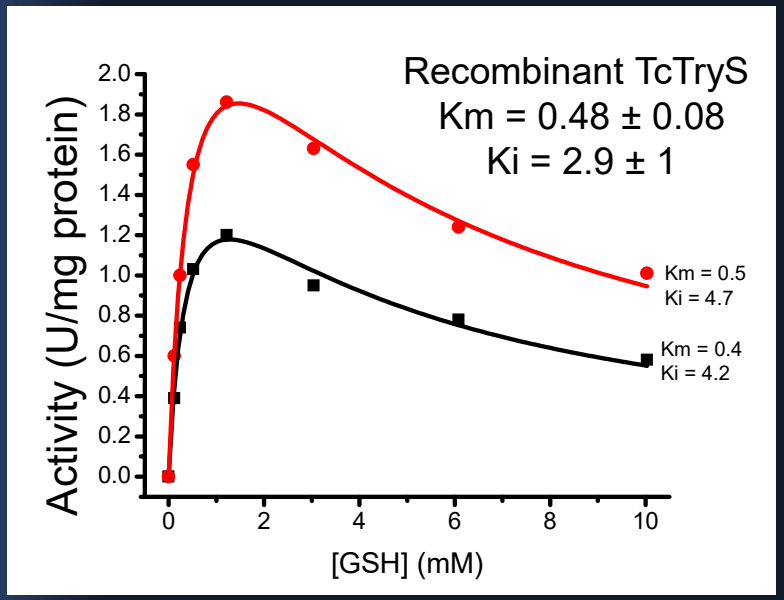
Trypanothione-dependent peroxide reduction pathway

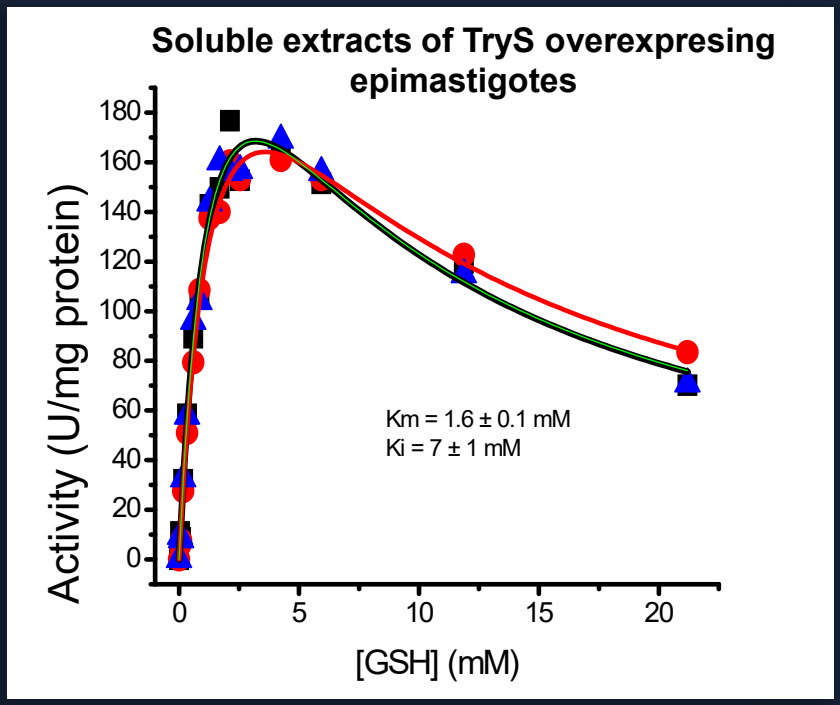


Kinetic mechanisms
- Random bi-bi
- Random tri-uni
- Ordered-bi
- Ping pong
- Haldane
- Mass action

Gonzalez-Chavez *et al*, 2019 Redox Biology 101231

Regulatory loop: TryS inhibition by GSH

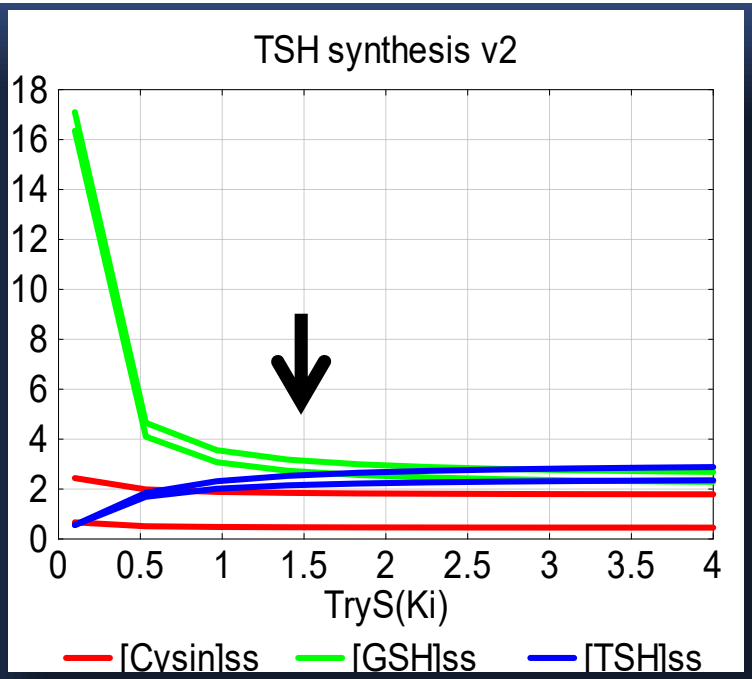


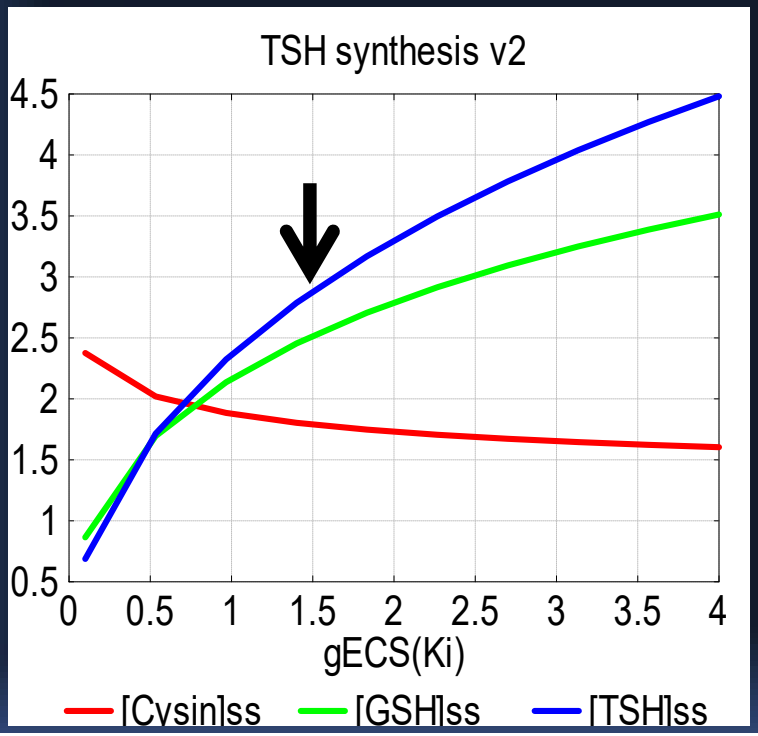


GSH + Spd + GSH = gEC

Random Tri uni with mixed inhibiton by GSH

$$\frac{\frac{Vm}{alfa \cdot Ka \cdot Kb \cdot Kc \cdot \left(1+\frac{I}{Ki}\right)} \cdot \left(A \cdot B \cdot C - \frac{P}{Keq}\right)}{1+\frac{A}{Ka}+\frac{B}{Kb}+\frac{C}{Kc}+\frac{A \cdot B}{alfa \cdot Ka \cdot Kb}+\frac{A \cdot C}{alfa \cdot Ka \cdot Kc}+\frac{B \cdot C}{alfa \cdot Kb \cdot Kc}+\frac{A \cdot B \cdot C}{alfa \cdot Ka \cdot Kb \cdot Kc}+\frac{A \cdot I}{alfa \cdot Ka \cdot Ki}+\frac{B \cdot I}{alfa \cdot Kb \cdot Ki}+\frac{C \cdot I}{alfa \cdot Kc \cdot Ki}+\frac{P}{Kp}}$$





Concentration control coefficients

TryS inhibition by GSH has local homeostatic effects on GSH

	$C^{[GSH]}_{ai}$	$C^{[TSH2]}_{ai}$
CysT	**0.09**	**0.11**
gECS	**0.74**	**0.91**
TryS	**-0.74**	**0.18**
TSH demand	**-0.01**	**-0.22**
TSH leak	**-0.06**	**-0.98**

γECS inhibiton by GSH has higher homeostatic effects on $T(SH)_2$ than on GSH concentrations

Kinetic models simulations

$T(SH)_2$ synthesis

[Metabolite] (mM)	*In vivo*	Model
Cysin	1 ± 0.2	0.5
GSH	1.4±0.4	1.36
Spd	0.8±0.2	1.06
$T(SH)_2$	0.9±0.4	0.75
pathway flux (nmol/min*mg cell protein)	0.6±0.2 (max)	0.12

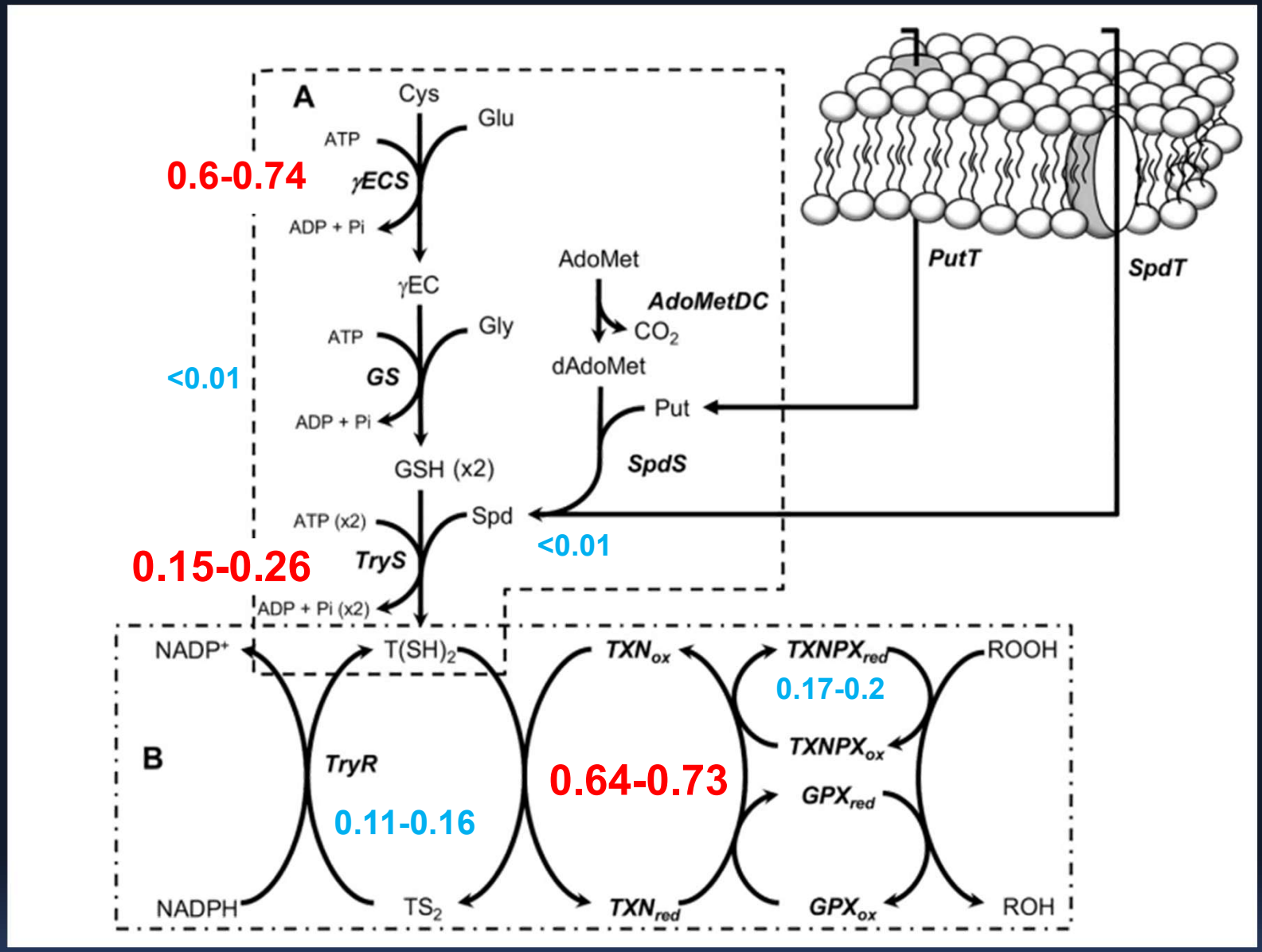
Peroxide reduction

[Metabolite] (mM)	Ex vivo	Model
$T(SH)_2$	0.45±0.4	0.465
TXN ox	0.022-0.078	0.083-0.089
Pathway flux (nmol/min*mg cell protein)	11±5 (max)	6.1-7.8

- The models can simulate the pathways behaviors

***González-Chávez et al Redox Biol. 2019 ;26:101231.*

Flux control coefficients (C^{J}_{ai}) of the antioxidant system in *T. cruzi* obtained by kinetic modeling



González-Chávez et al. Redox Biology 26 (2019) 101231

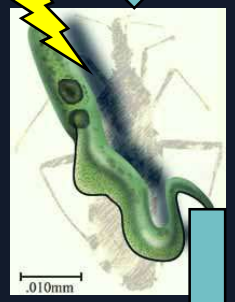
In vivo determination of the flux control coefficients in *T. cruzi* trypanothione metabolism

Experimental procedure

Gene constructions

1. pTREXn
2. γECS
3. TryS
4. TryR
5. TXN

Transfection



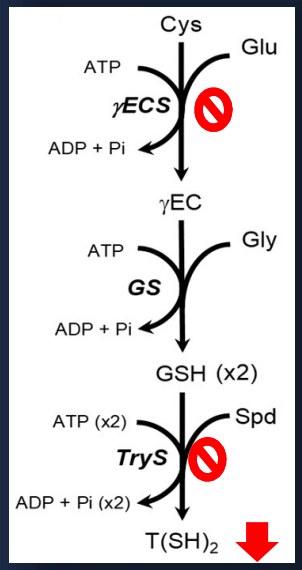
Cloning

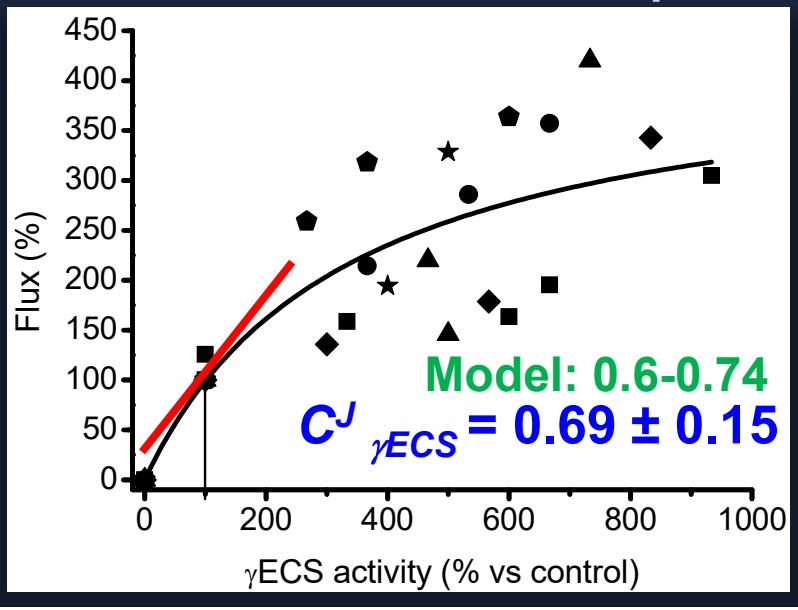
WT
Mock
Overexpresing

- Enzymatic activities
  - Oxidative stress handling
    - Metabolite concentrations
    - Peroxide resistance
  - Metabolic fluxes
    - Flux control coefficient

González-Chávez et al. Redox Biology (2019) 101231

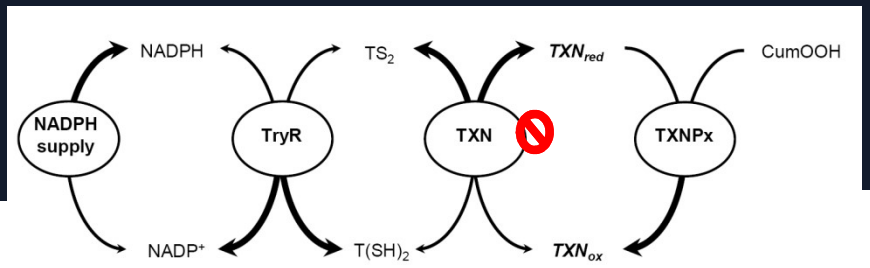
In vivo flux control coefficients are similar to those predicted by kinetic modeling

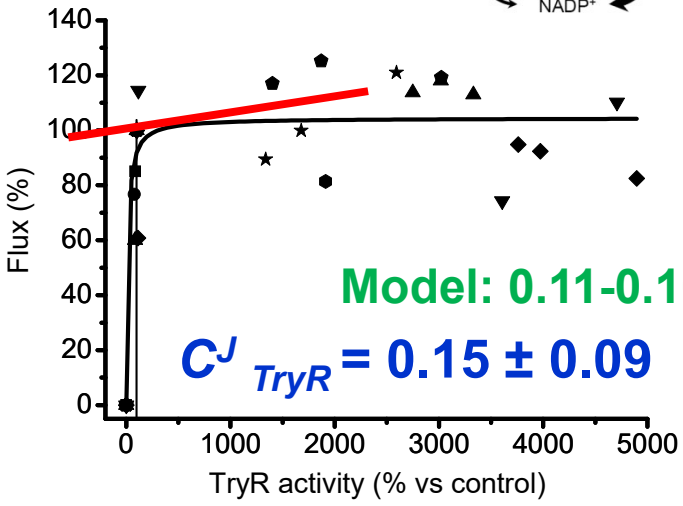


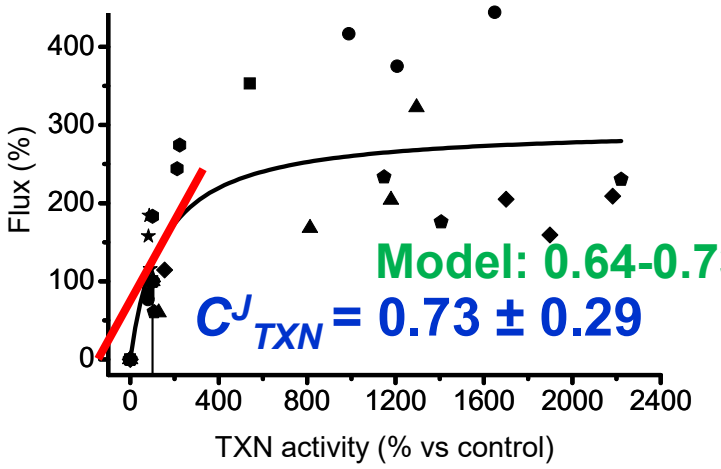


González-Chávez et al.
Redox Biology (2019) 101231

C^J_{TryS} = 0.15-0.26

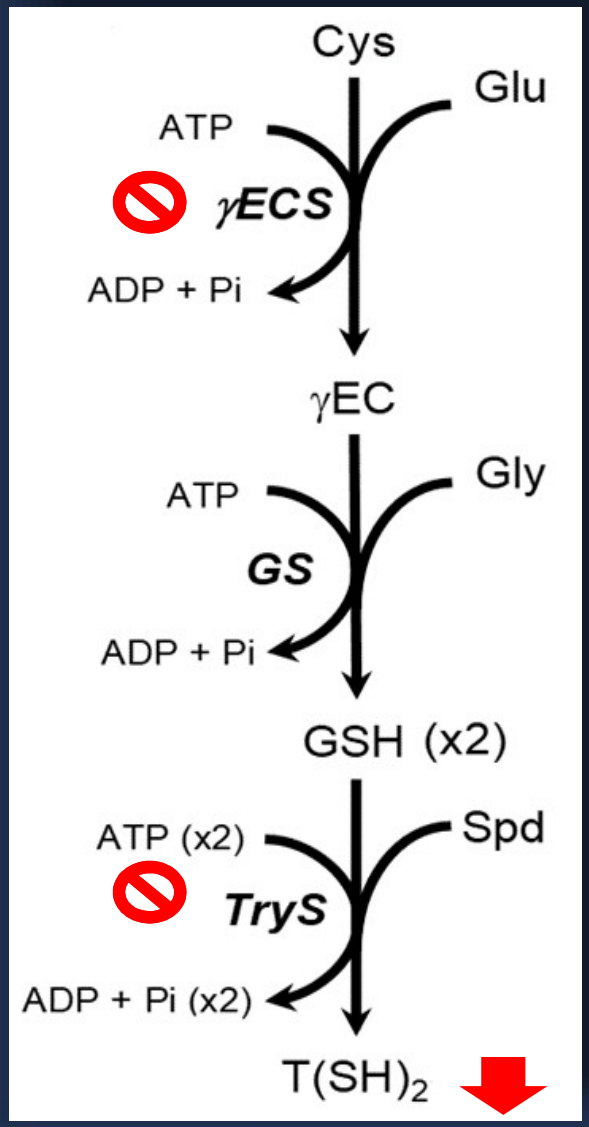


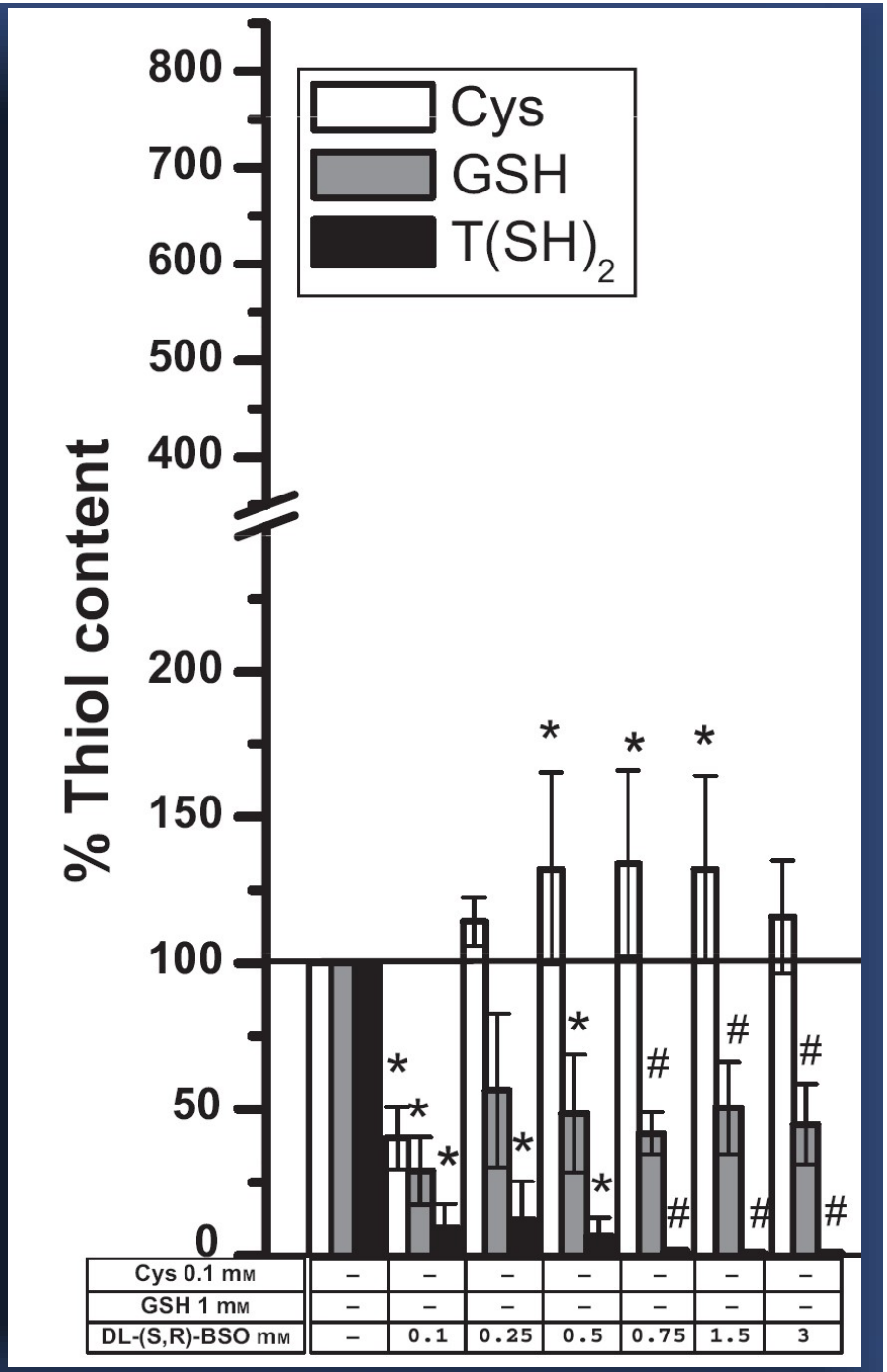




γECS, TRYS AND TXN INHIBITION WILL AFFECT THE ANTIOXIDANT DEFENSE IN *T. CRUZI*

Parasites treated with BSO decreases $T(SH)_2$ because it inhibits γECS and TryS inhibition

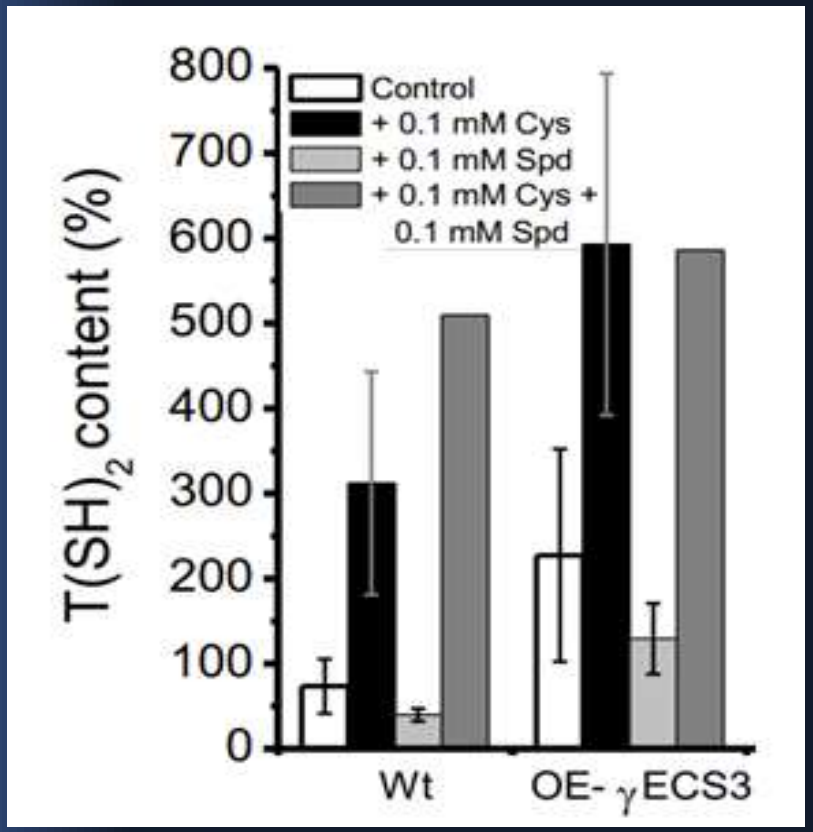




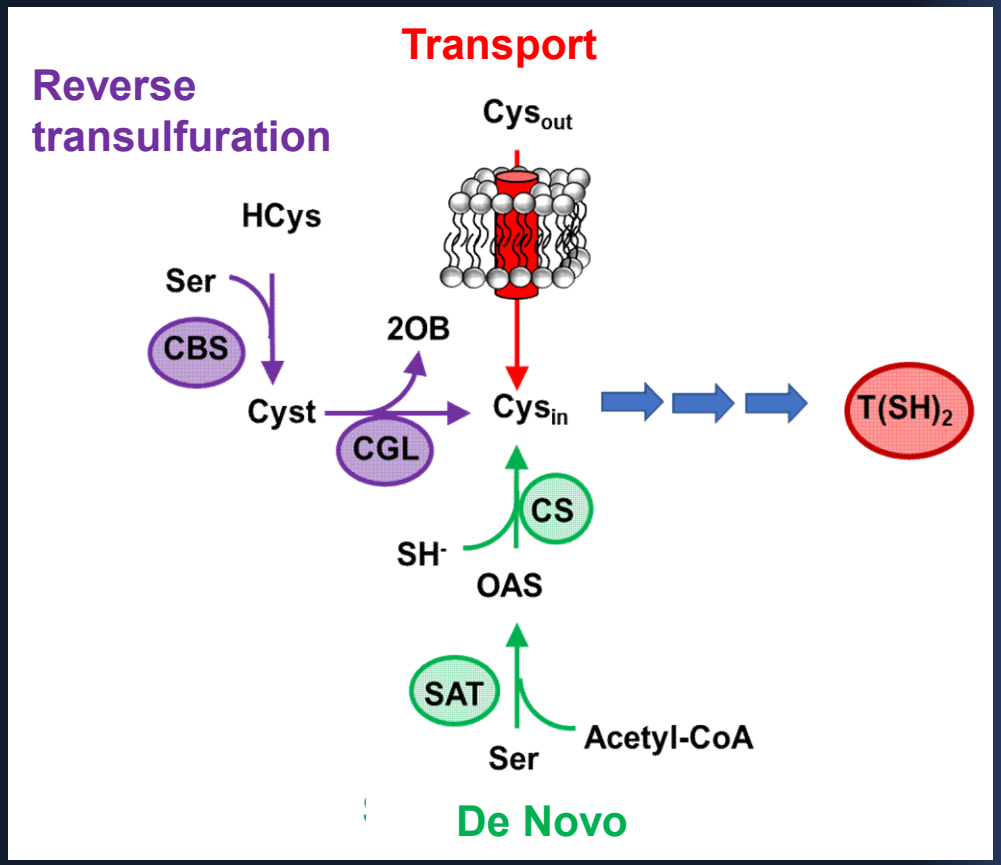
Vazquez et al 2017. *FEBS Lett*

According to the model, cysteine is limiting for $T(SH)_2$ biosynthesis

Epimastigotes supplied with cysteine (Cys) but not with spermidine (Spd), increased $T(SH)_2$ content.



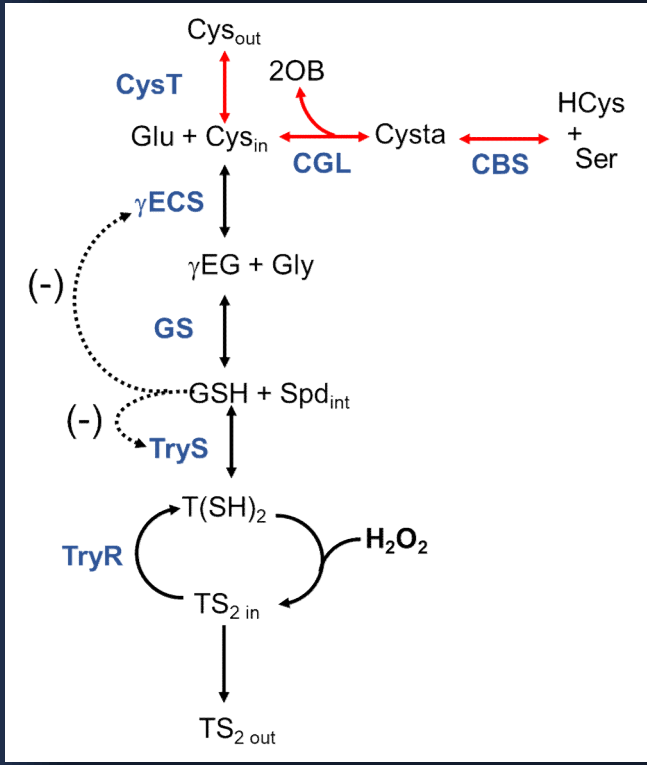
Pathways of Cys supply in T. cruzi



Citlali Vázquez, Javier Belmont, Rusely Encalada

Kinetic model with the reverse transulfuration pathway of Cys syntthesis

Ping pong kinetics



Intermediary (mM)	In vivo	Model
Cys in	1 ± 0.2	2.1
GSH	1.4 ± 0.4	1.8
TSH2	0.9 ± 0.4	1.36
Cysta	0.88± 0.5	2.2
Flux T(SH)$_2$ synthesis	0. 6 ± 0.2 max	0.18
Flux Cys synthesis	1.13 ± 0.3 max	0.4

Flux in nmoles /min*mg cell protein

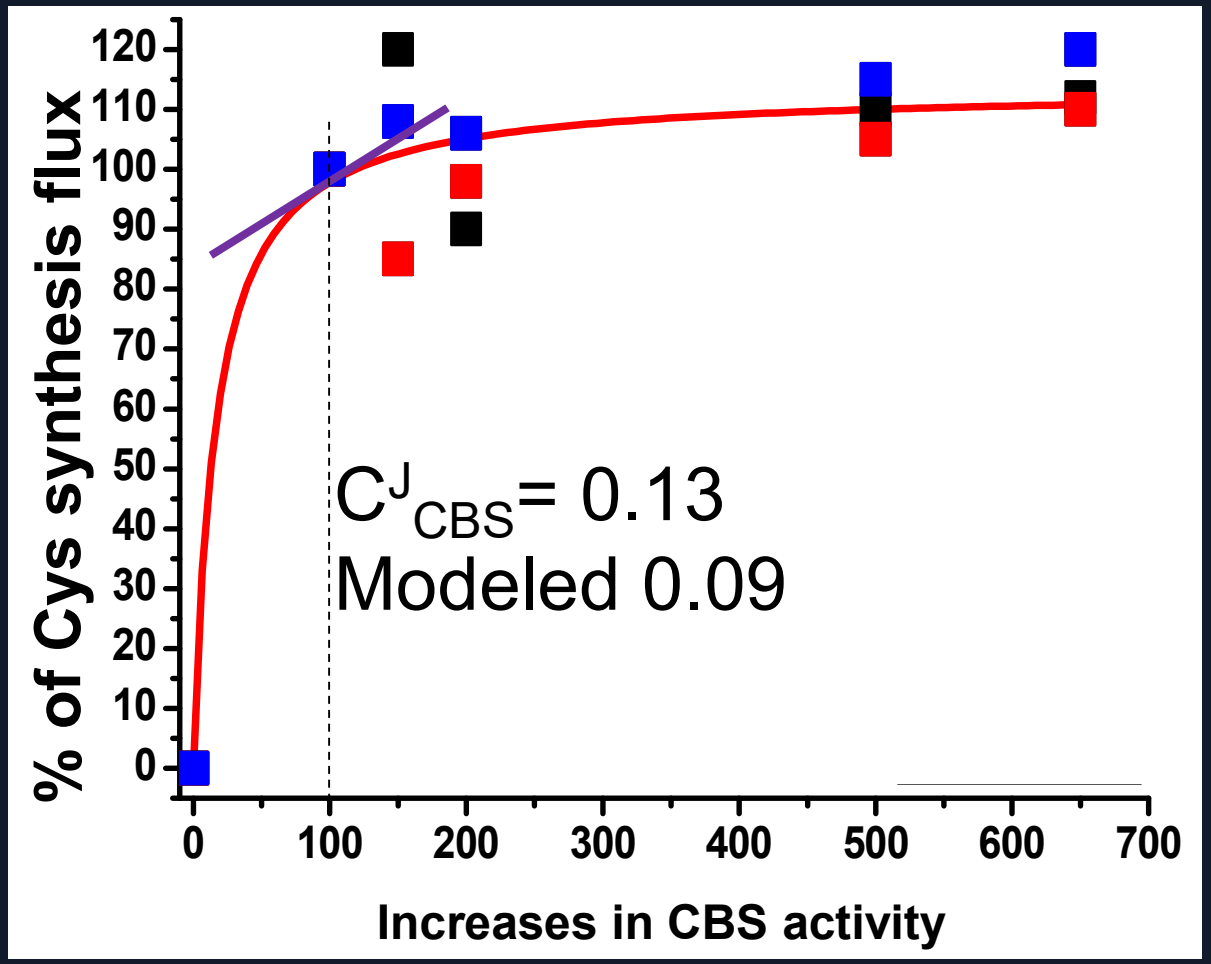
C^J_{ai} on T(SH)$_2$ synthesis

C^J CBS + CGL	0.05
C^J γECS	0.7
C^J TryS	0.21

C^J_{ai} on Cys synthesis

C^J CBS	0.09
C^J CGL	0.36
C^J γECS	0.34
C^J TryS	0.1

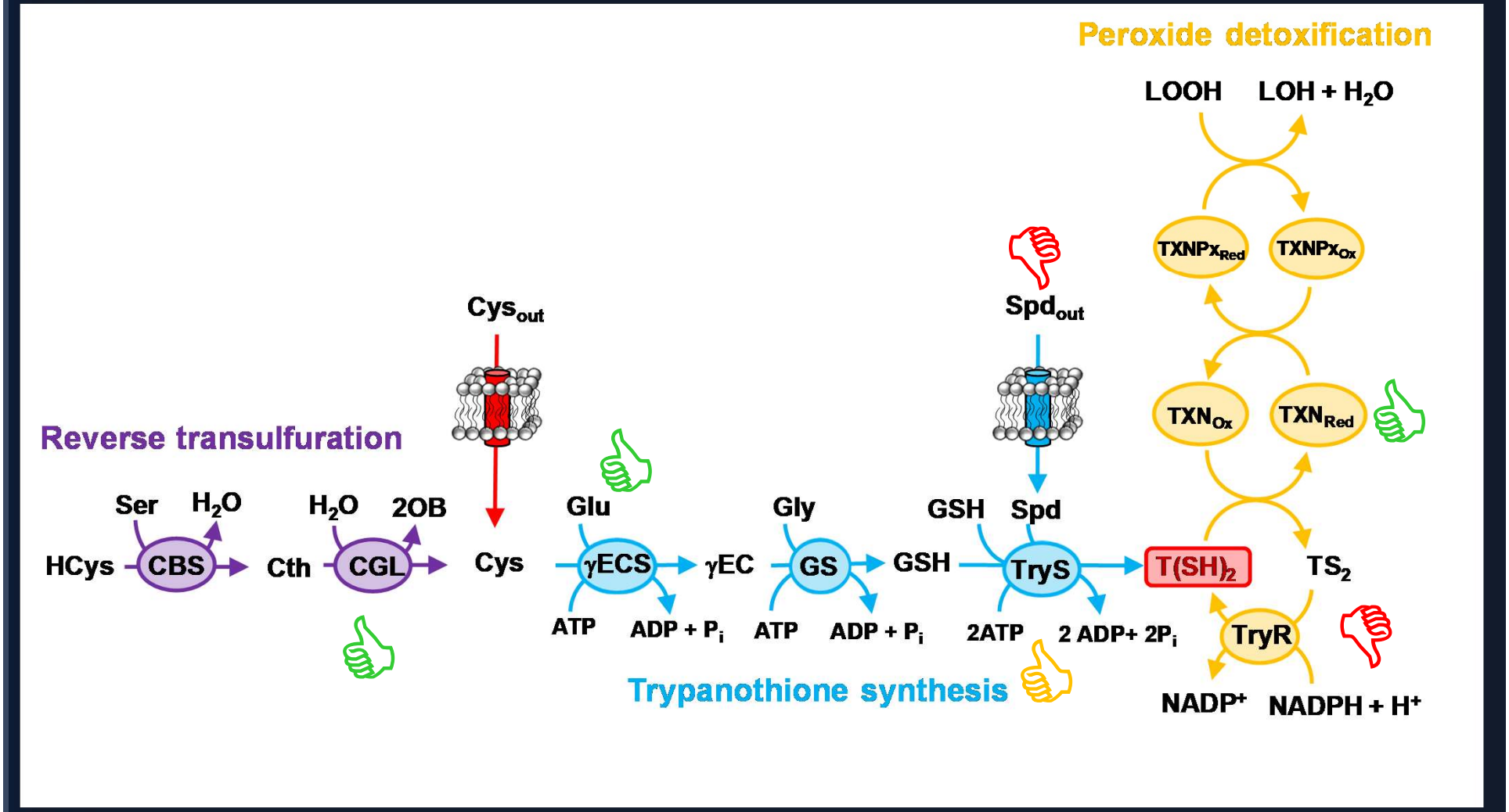
Cystathionine β synthase flux control coefficient on Cys synhtesis



CGL has high control on Cys synthesis flux

Citlali Vazquez, Rusely Encalada

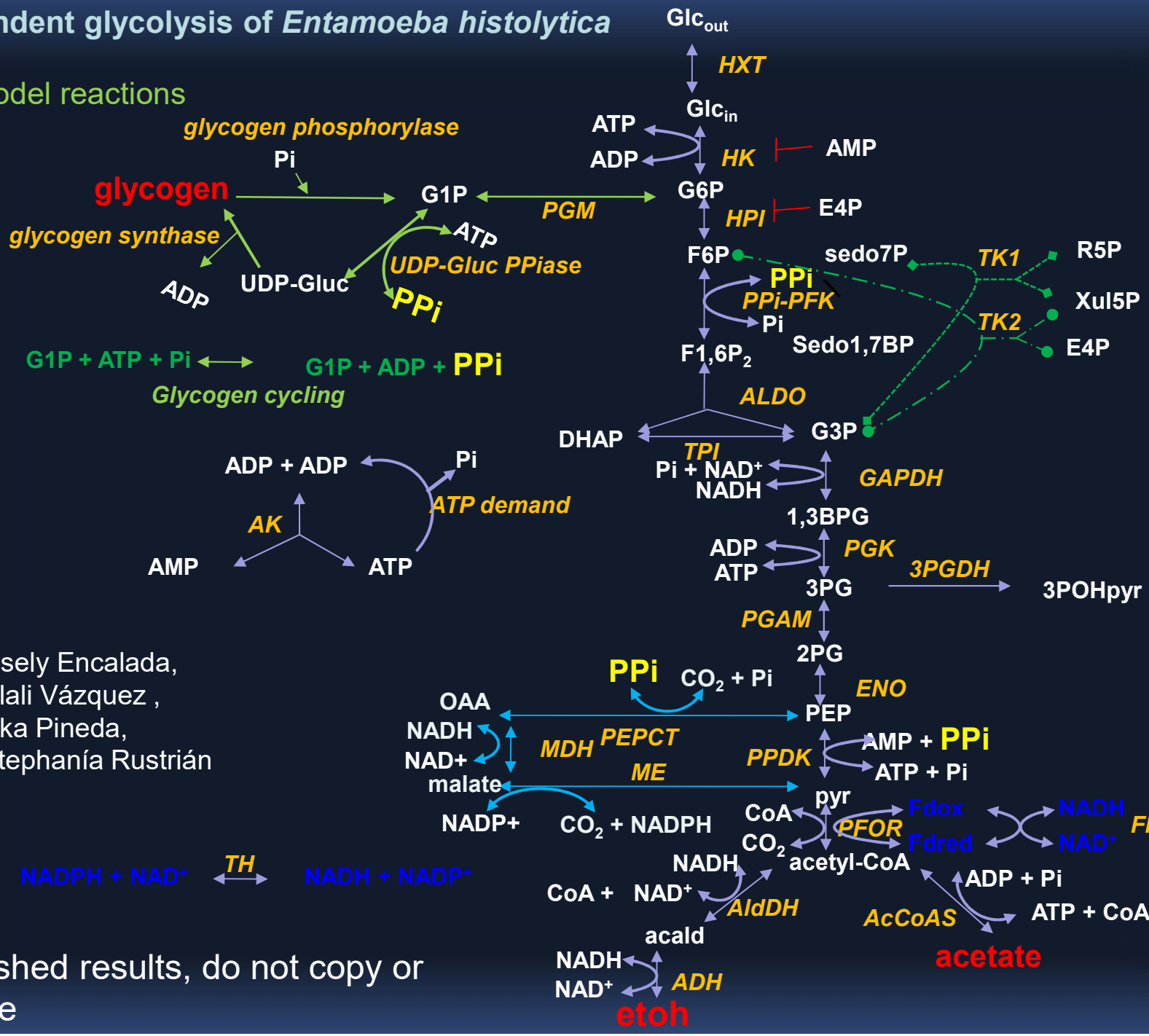
Enzymes with the highest therapeutic potential



Kinetic modeling of *Entamoeba histolytica* glycolysis to understand its controlling mechanisms and identify putative drug targets

PPi-dependent glycolysis of *Entamoeba histolytica*

Kinetic model reactions

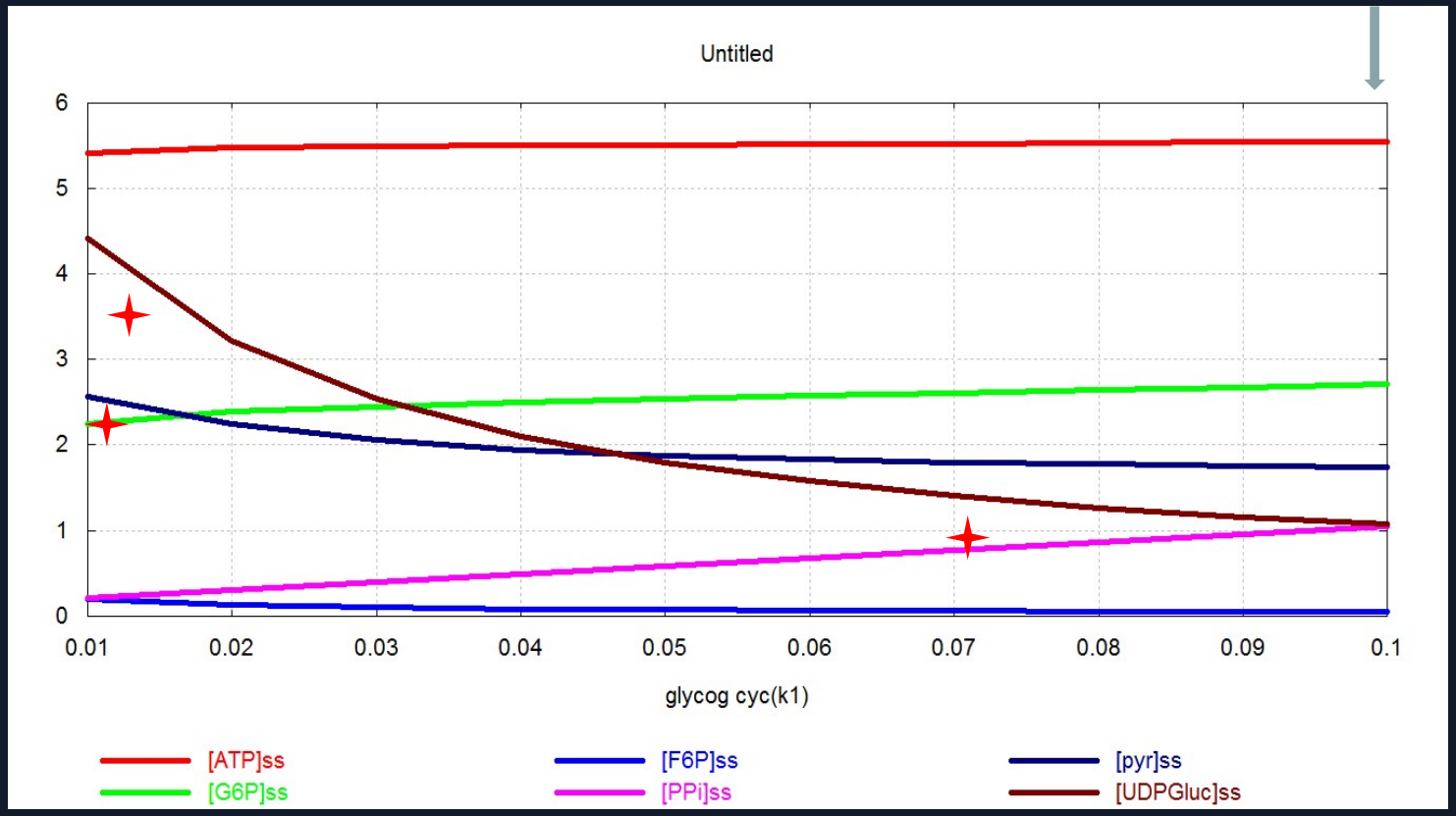


Rusely Encalada,
Citlali Vázquez ,
Erika Pineda,
Estephanía Rustrián

Glycogen cycling (variation in k1)

G1P + ATP + Pi = G1P + ADP + PPi

Conserved moiety ATP + ADP + AMP + UDPGlc



Kinetic modeling suggests that glycogen cycling might be the most important source of PPi

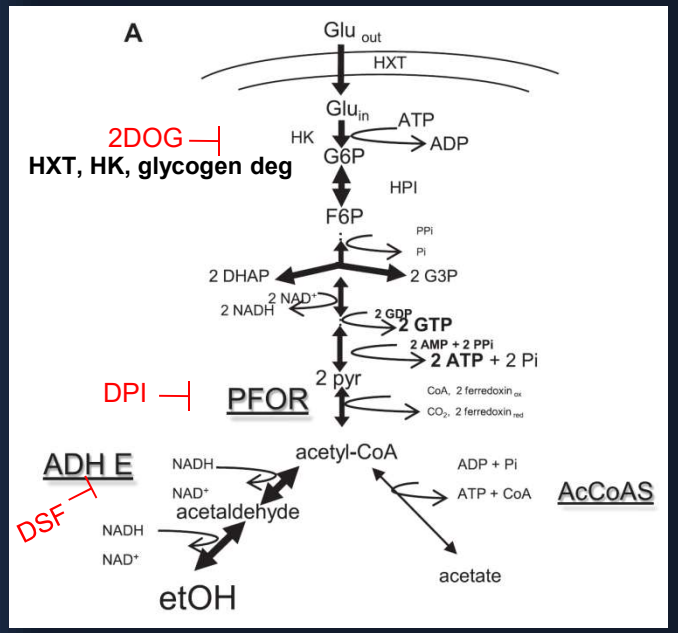
Enzyme/process	*model*	*Titration with inhibitors in live cells**	*Elasticity analysis in live cells *****
HXT	0.34	~0.7 (DOG)	0.72 -0.79
HK	0.02		
Glycogen degradation	0.39		
HPI	0.01		
PGM	0.002		
UDPGlucPPiase Glycog synthase	-0.06 -0.03		-0.13 -> -0.22
PPi-PFK	0.014		
ALDO	0.015		
TPI	0.0005		
GAPDH	0.01		
PGK	0.006		
PGAM	0.28		0.2-0.28
ENO	0.03		
PPDK	0.001		
PFOR	0.0003	0.07 (DPI)	0.13
AldDH + ADH	0.16 + 0.05	0.33 (disulfiram)	0.18
AcCoAS	-0.09	-0.05 knock-down	-0.08

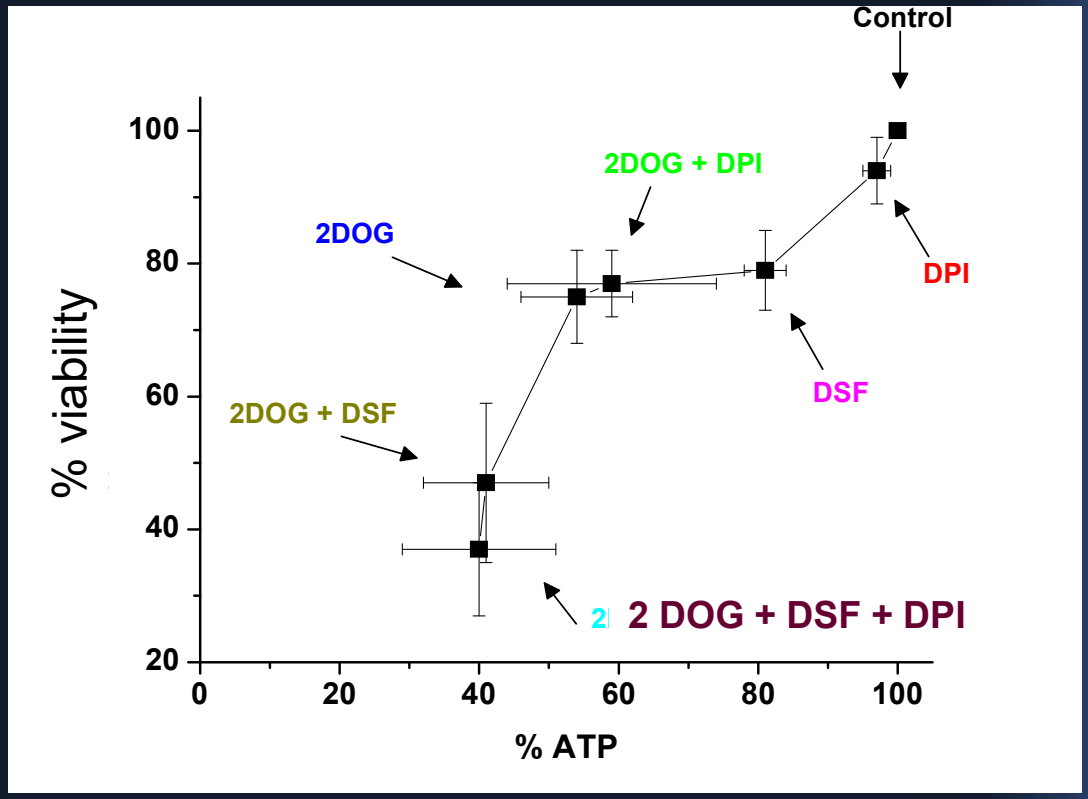
Flux control coefficients ($C^{J\ etoh}_{vi}$) on EtOH flux determined through different MCA strategies

- **Main controlling steps are: HXT, glycogen degradation, PGAM and ADHE**
- **The control of the patwhay flux predicted by pathway modeling is similar to that determined in live cells.**

*Pineda et al 2013 FEBS Lett
** Pineda et al 2015 FEBS J

Effect of the Inhibition of the main controlling steps of amebal glycolysis on ATP content and cell viability





10 mM glucose, 30 mM DOG, 50 nM DSF, 100 nM DPI,2h

Pineda et al 2015 FEBS J

The most controlling enzymes can be proposed as relevant drug targets to decrease the glucose catabolism in amebas.

General conclusions

- Kinetic modeling allows understanding of regulatory and controlling mechanisms of metabolic pathways
- Kinetic modeling and MCA helps to identify the main controlling steps which can be proposed as drug target candidates.

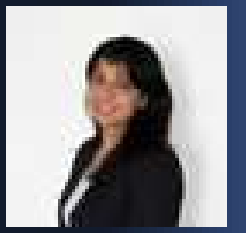
Acknowledgments

- Lab members: Zabdi González, Citlali Vázquez, Rusely Encalada, Javier Belmont, Moisés Rivera, Isabel Jiménez, Aketzalli Silva, Marlen Mejía, Samantha Alvarez, Viridiana Olin, Erika Pineda









- Grants CONACyT-México 178638, 264292, 272941, 282663
- Colaborators

Rafael Moreno-Sánchez (INCar, México) ;

Alvaro Marín Hernández INCar;

Alfonso Olivos † (UNAM), Rebeca Manning (CINVESTAV, México)