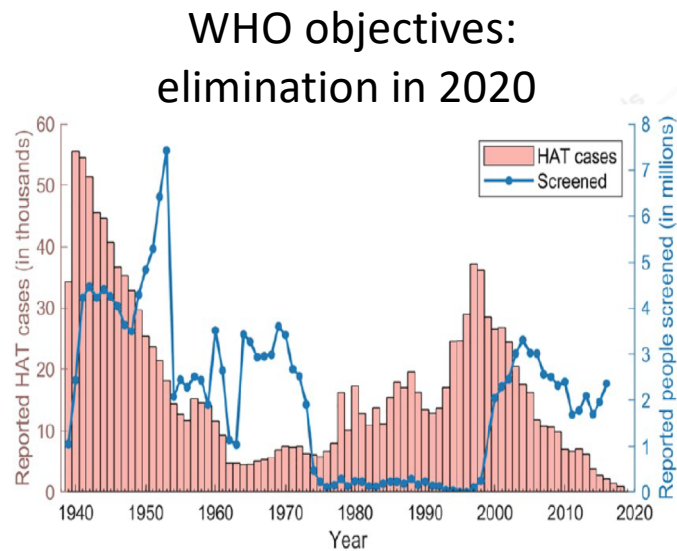


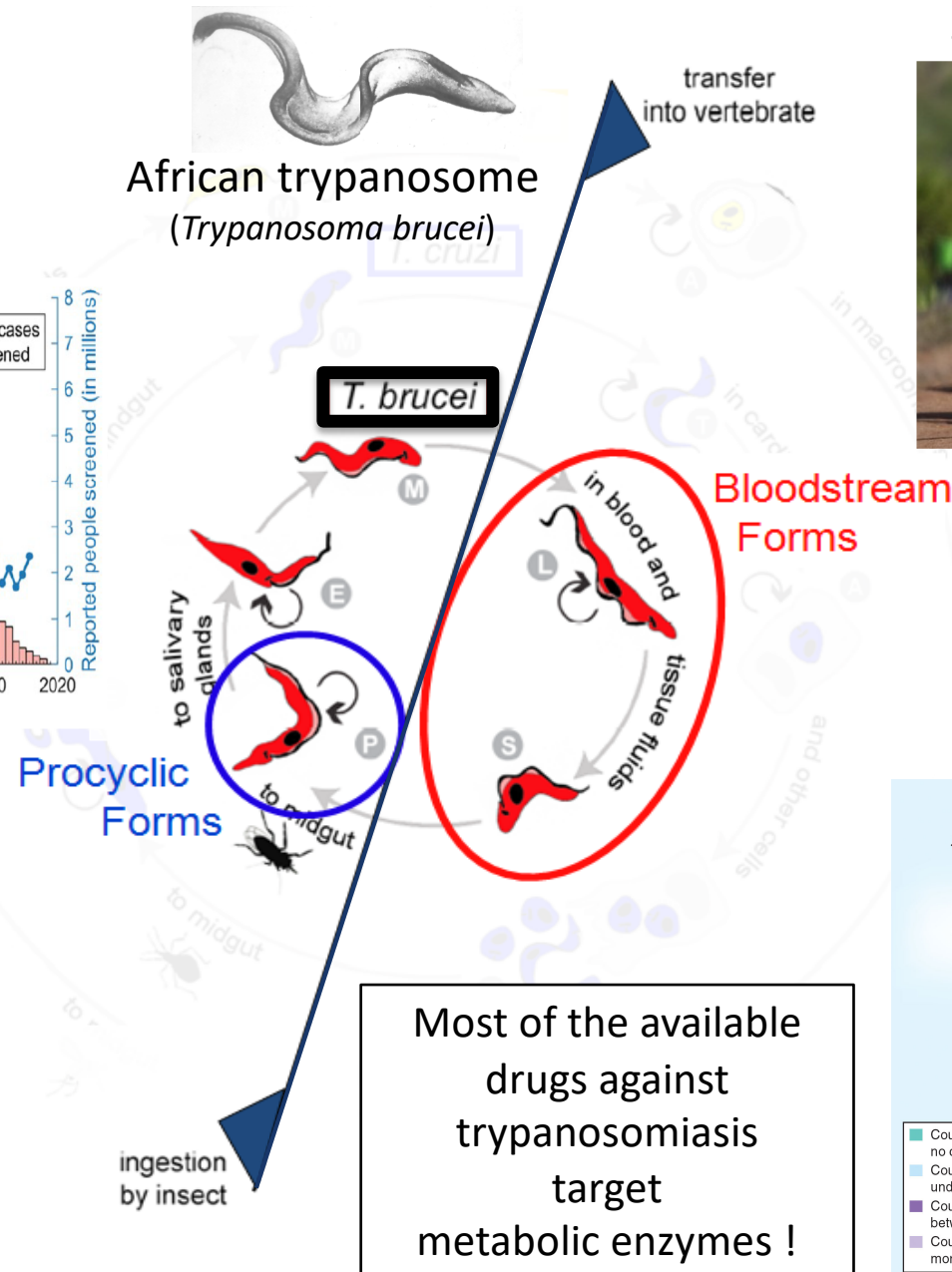
Adaptation of trypanosomes to available carbon sources and carbon source preference

Frédéric Bringaud
on behalf of the iMET team

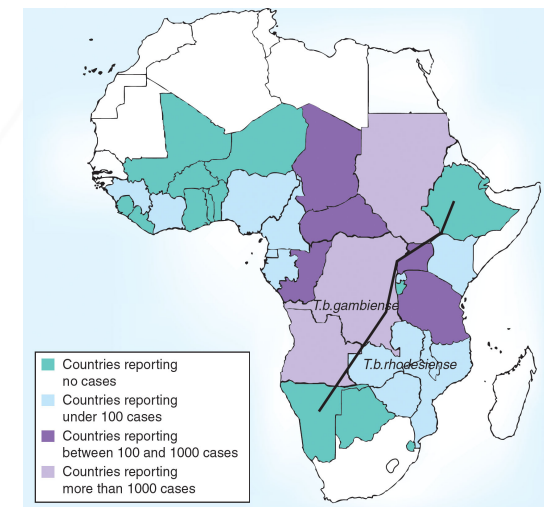




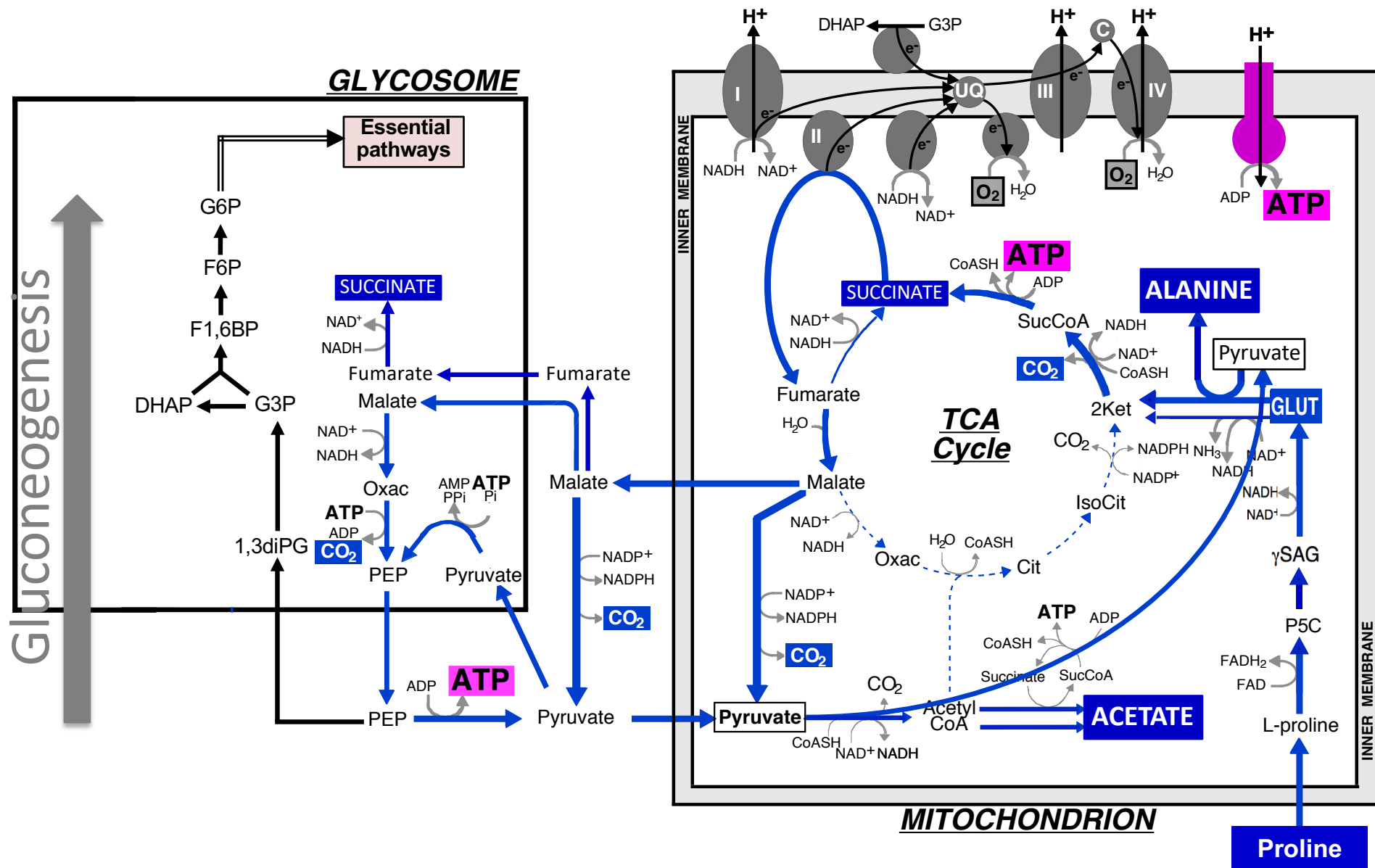
Tsetse fly



Nagana, etc. (AAT)



November 18-19, 2021

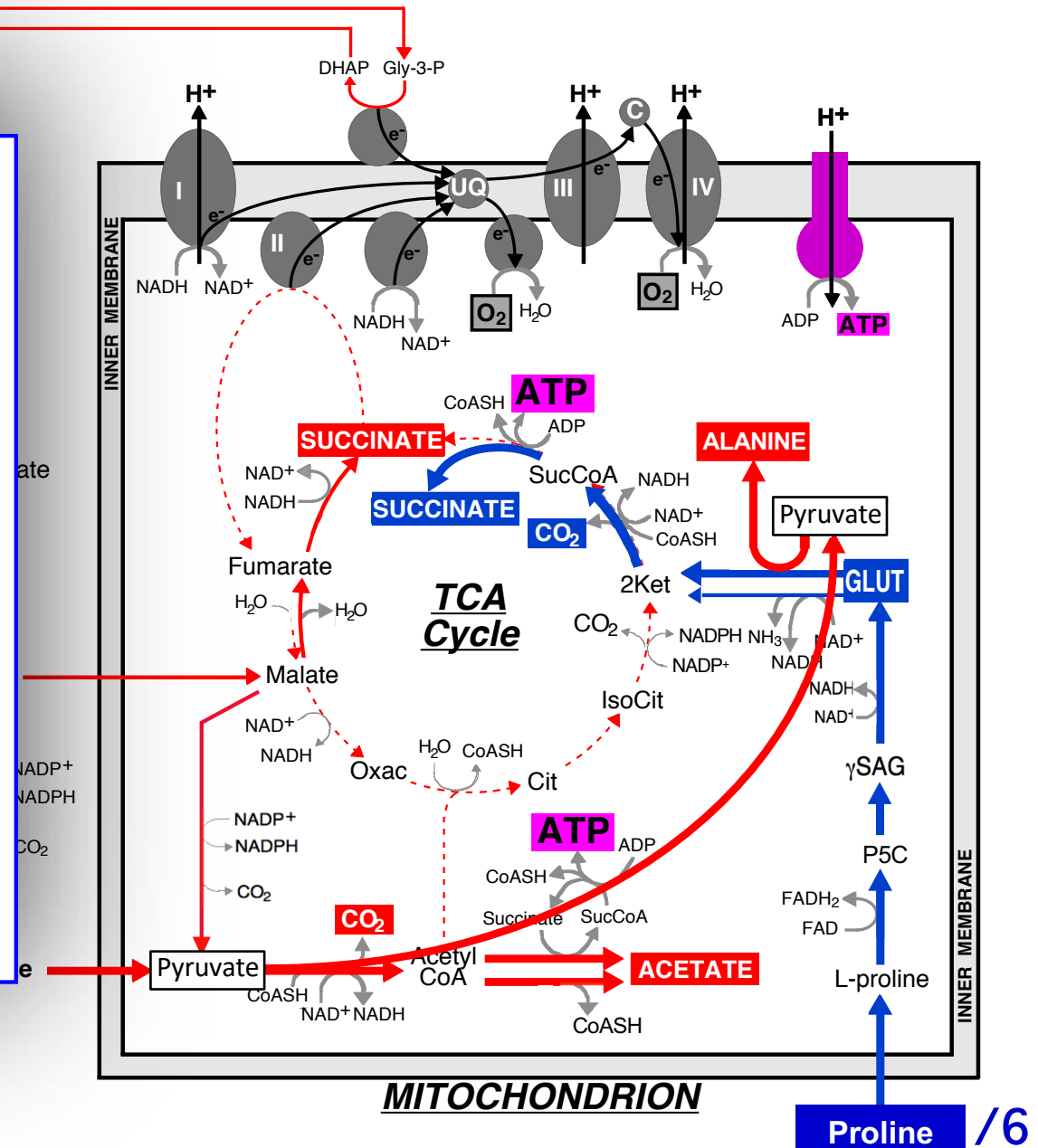
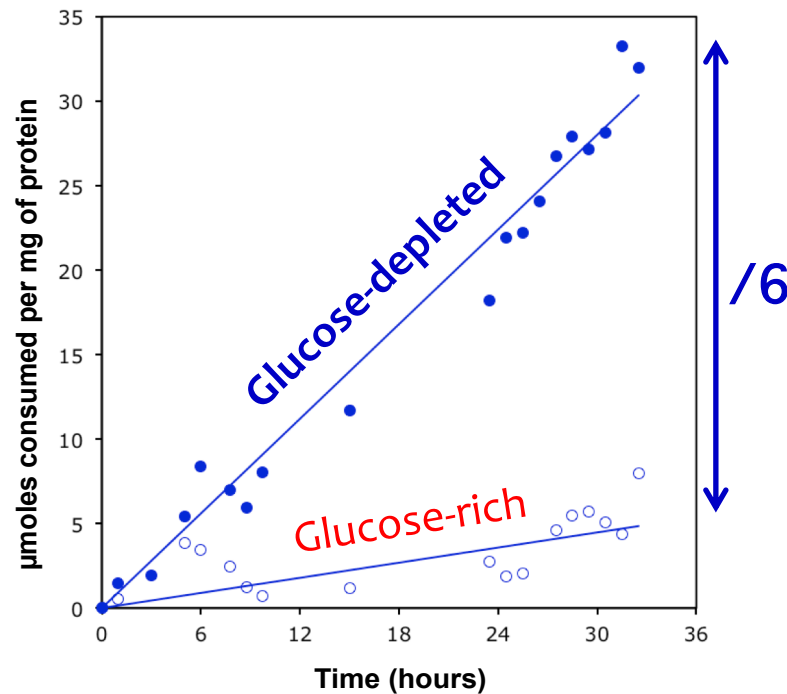


Coustou et al., 2008, JBC; Allmann et al., 2013, JBC; Wargnies et al., 2018, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

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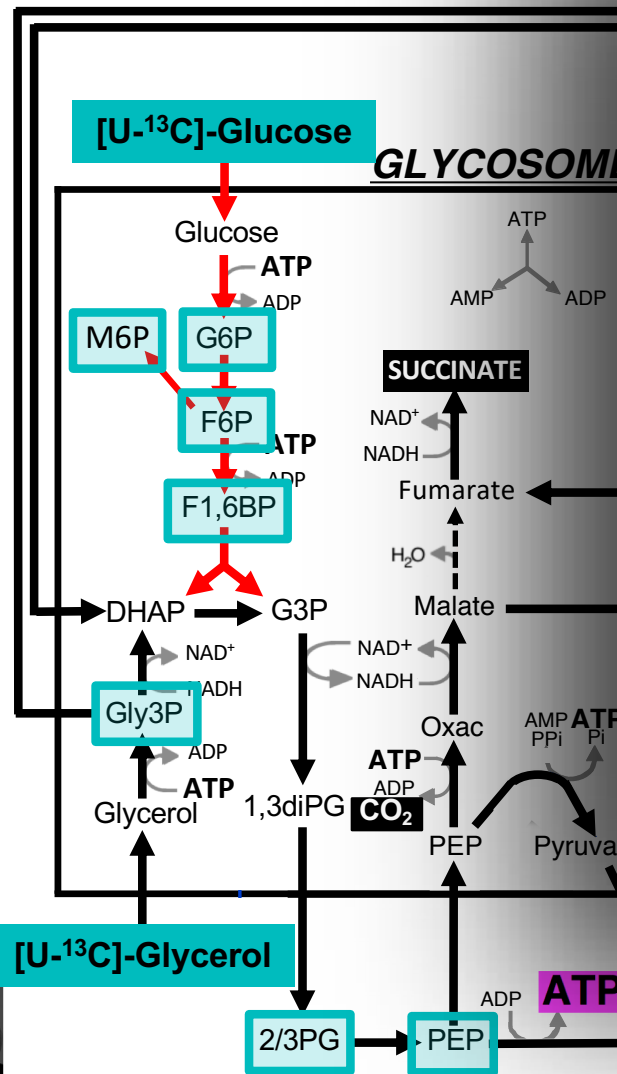
Glucose down-regulates proline metabolism



Lamour et al., 2005, JBC; Coustou et al., 2018, JBC

"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021



2x10⁷ procyclic cells (exponential phase)
↓
Suspension in 10 ml **PBS** with 2 mM **[U-¹³C]-carbon source** and 2 mM of another carbon source

Consumption of **[U-¹³C]-carbon source** and the other carbon source
(**2 hours of incubation** at 27°C)

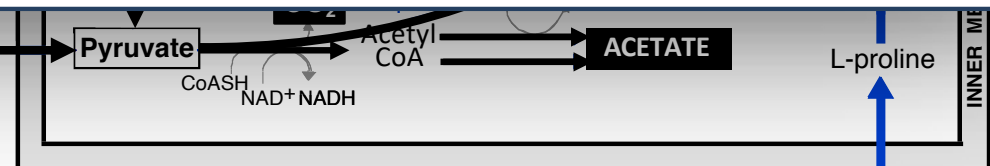
Recovery of the cells by **fast filtration** on membranes

Quenching of metabolic activity in liquid nitrogen

Cell lysis in 50 ml boiling water

IC-MS/MS analysis

Determination of ¹³C-incorporation into glycolytic intermediates

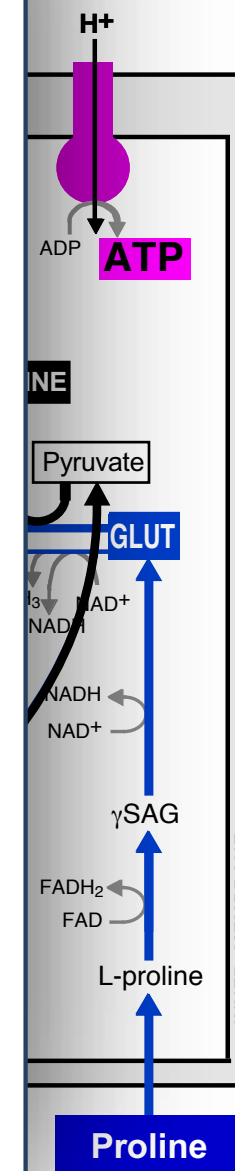
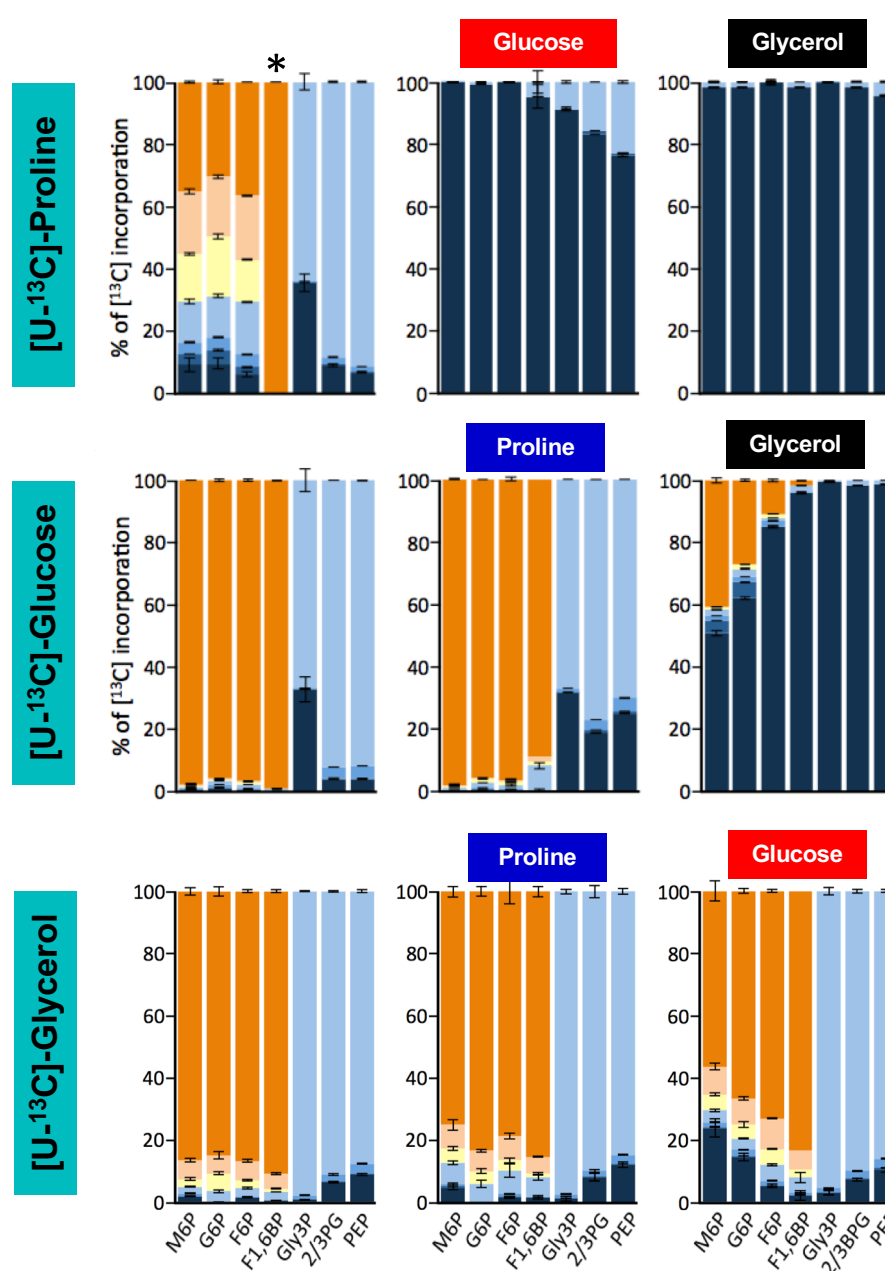
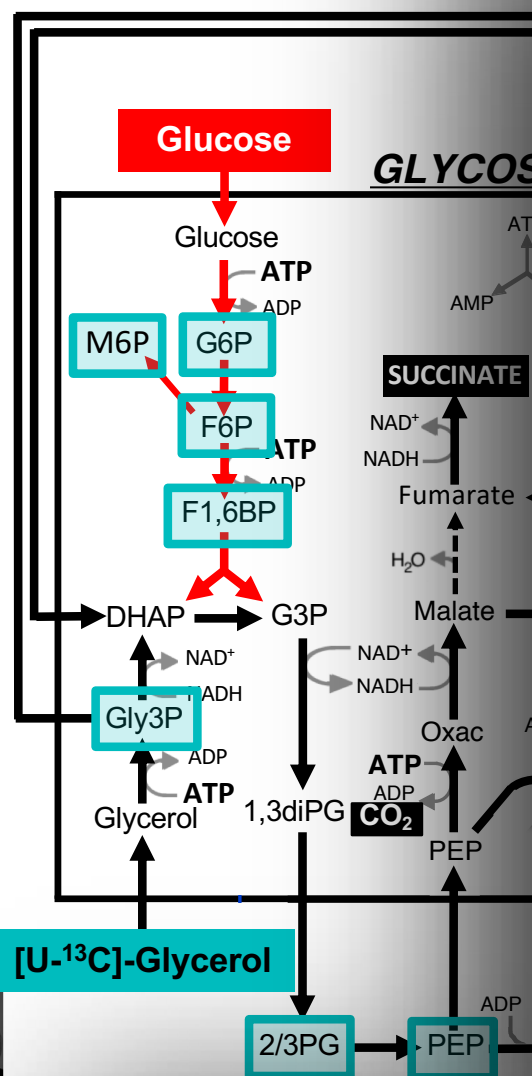


[U-¹³C]-Proline

Allmann, Wargnies, Plazolles *et al.*, 2021, PLoS Biol

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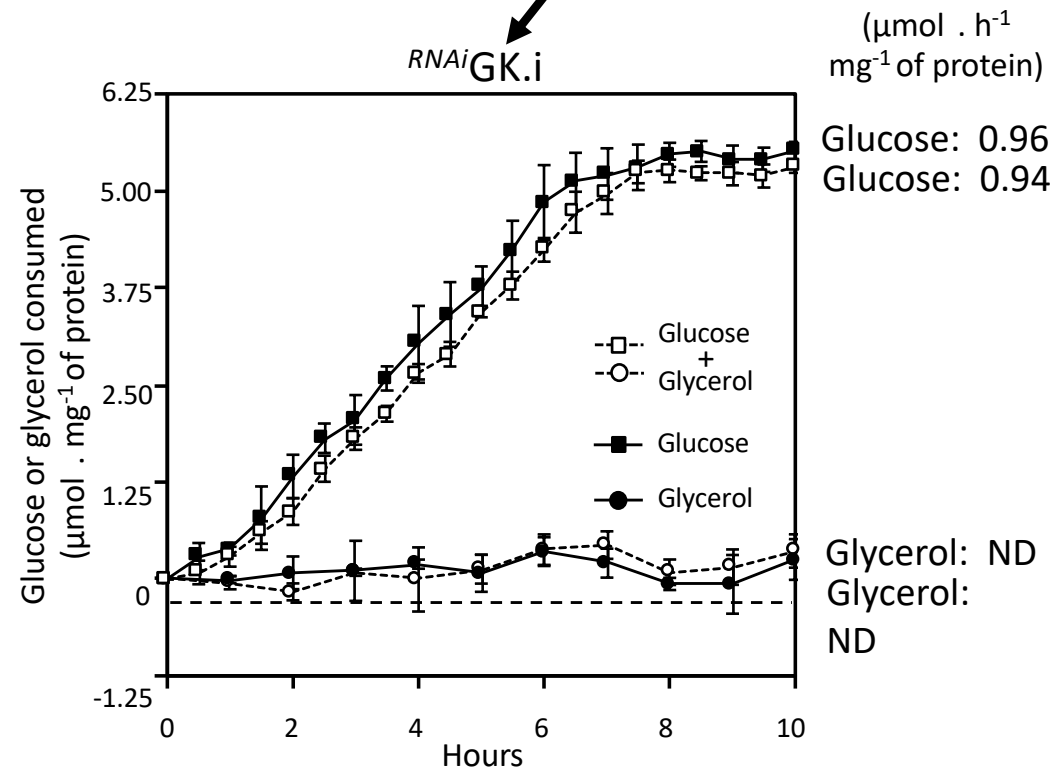
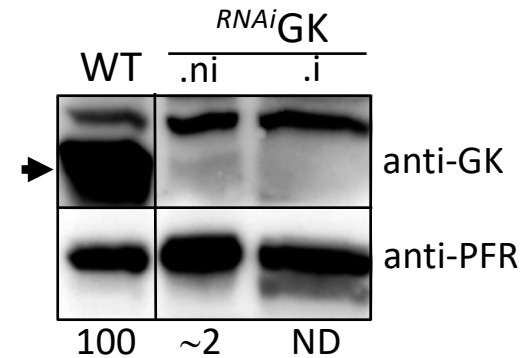
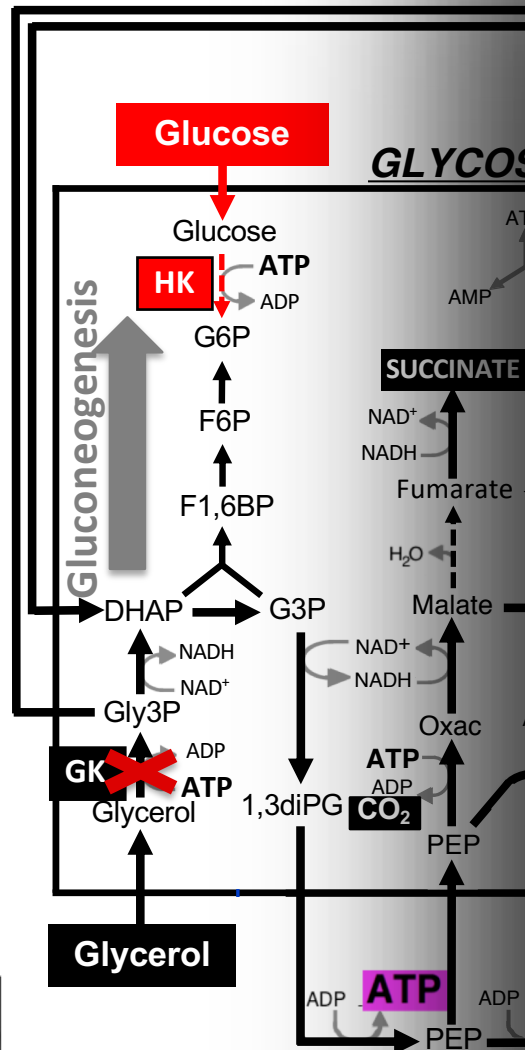


Allmann, Wargnies, Plazolles et al., 2021, PLOS DIET

"Metabolism and mathematical models: Two for a tango"

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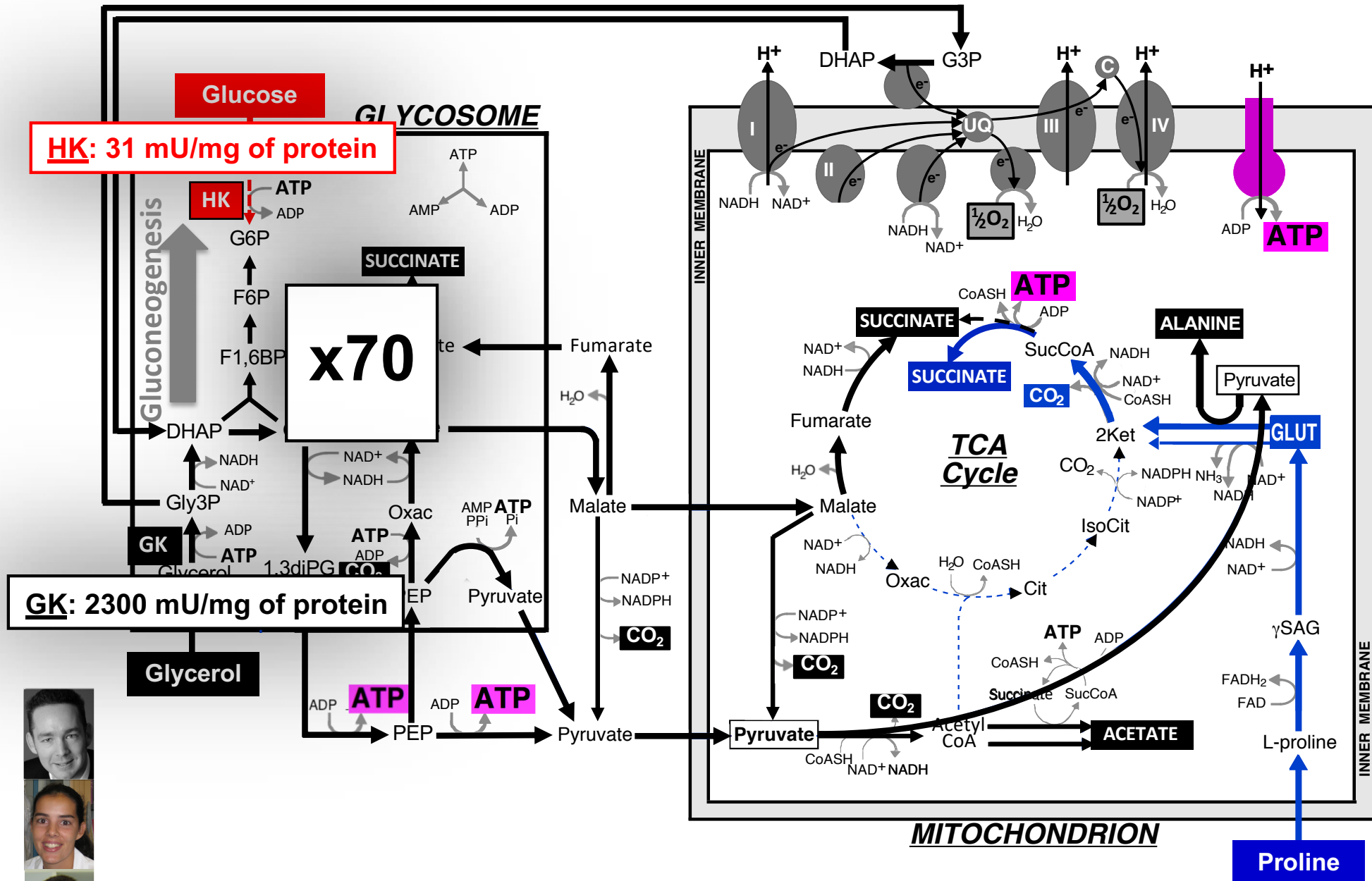




Allmann, Wargnies, Plazolles et al., 2021, PLOS Biol

"Metabolism and mathematical models: Two for a tango"

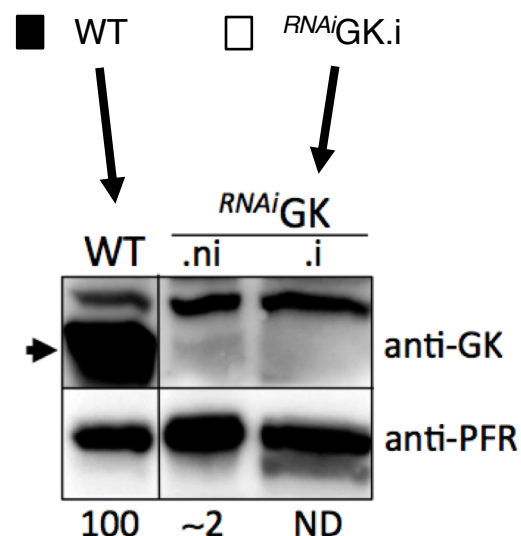
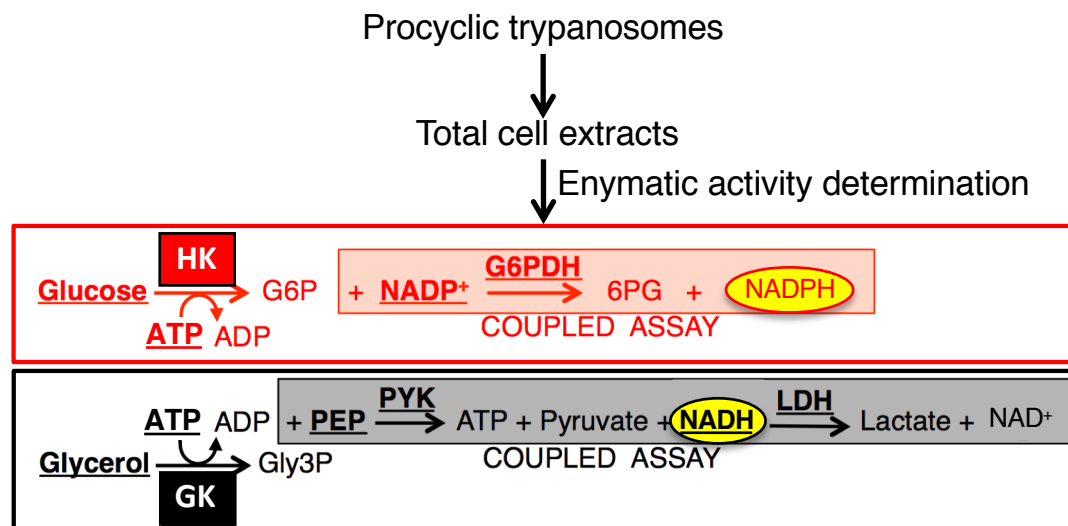
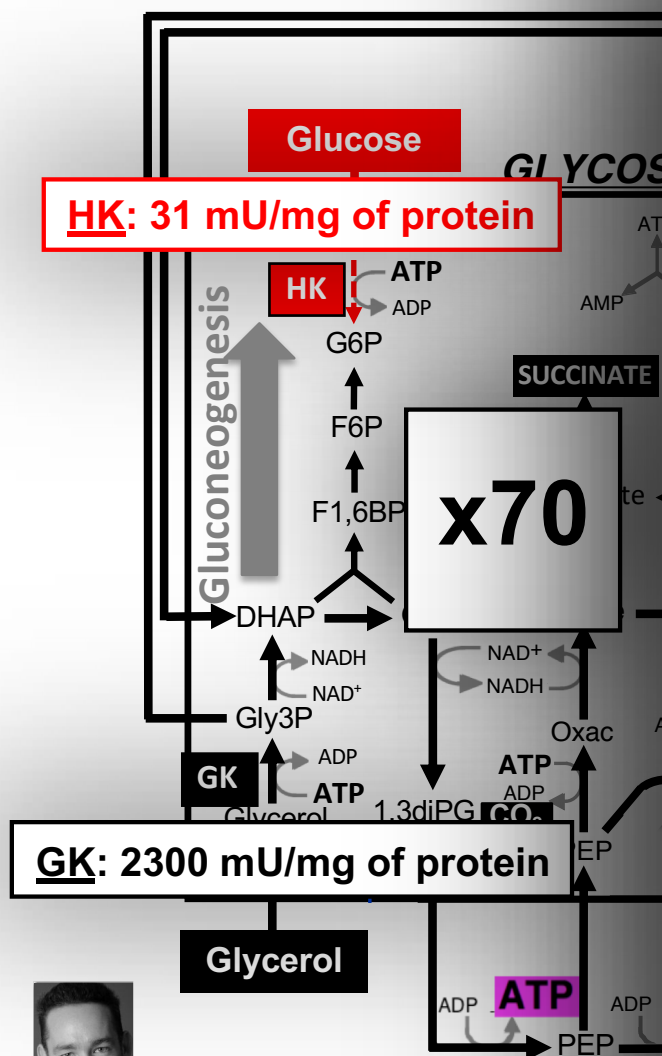
November 18-19, 2021



Allmann, Wargnies, Plazolles et al., 2021, PLoS Biol

"Metabolism and mathematical models: Two for a tango"

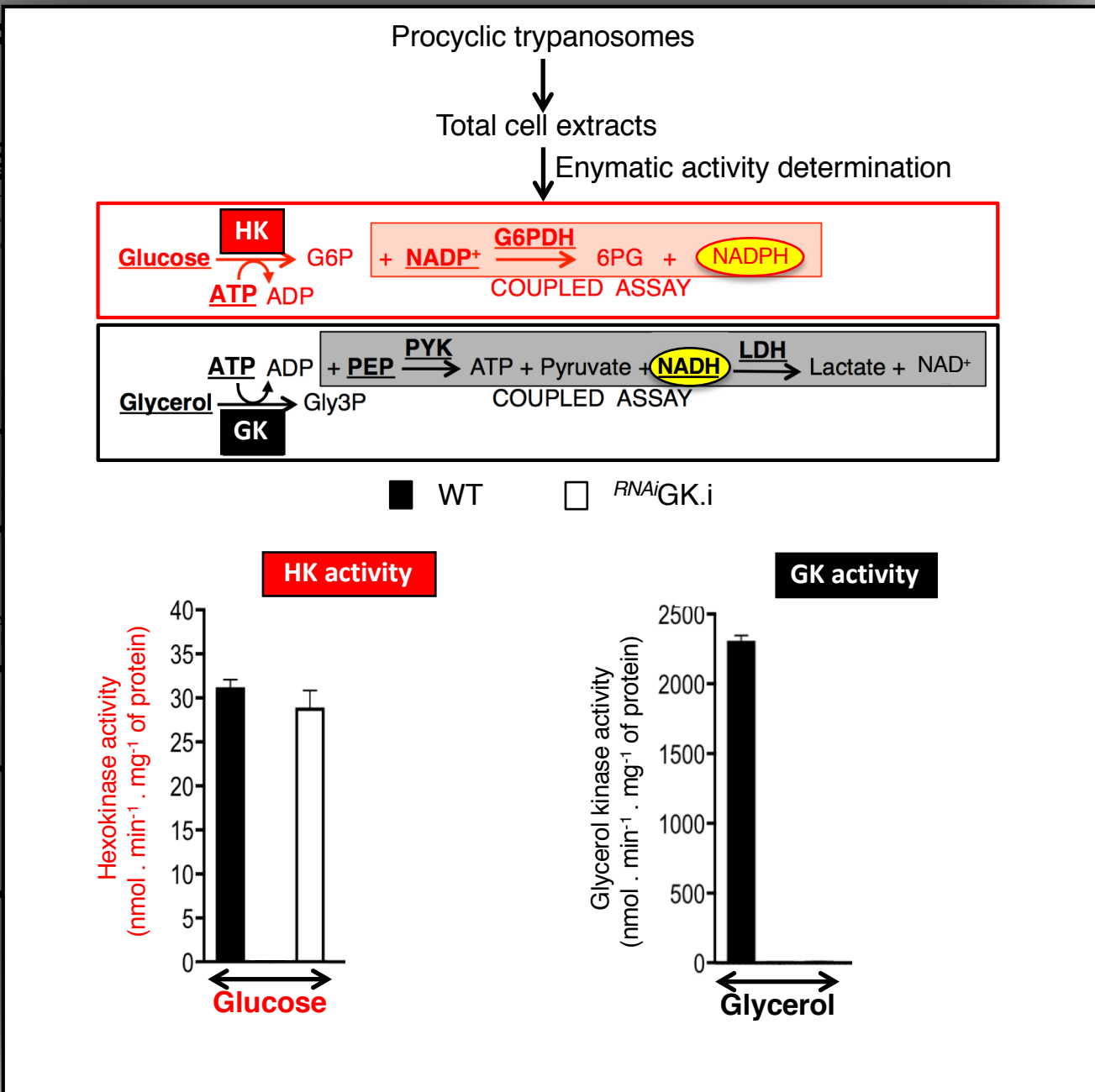
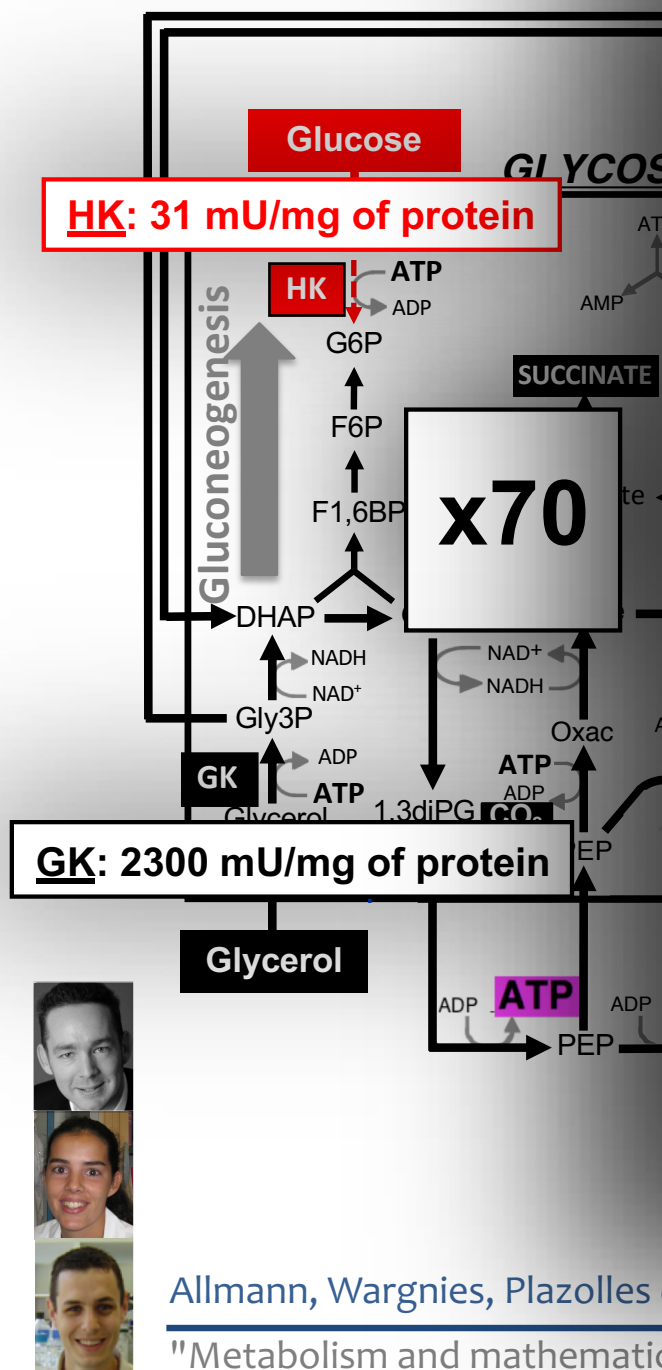
November 18-19, 2021



Allmann, Wargnies, Plazolles et al., 2021, PLOS Biol

"Metabolism and mathematical models: Two for a tango"

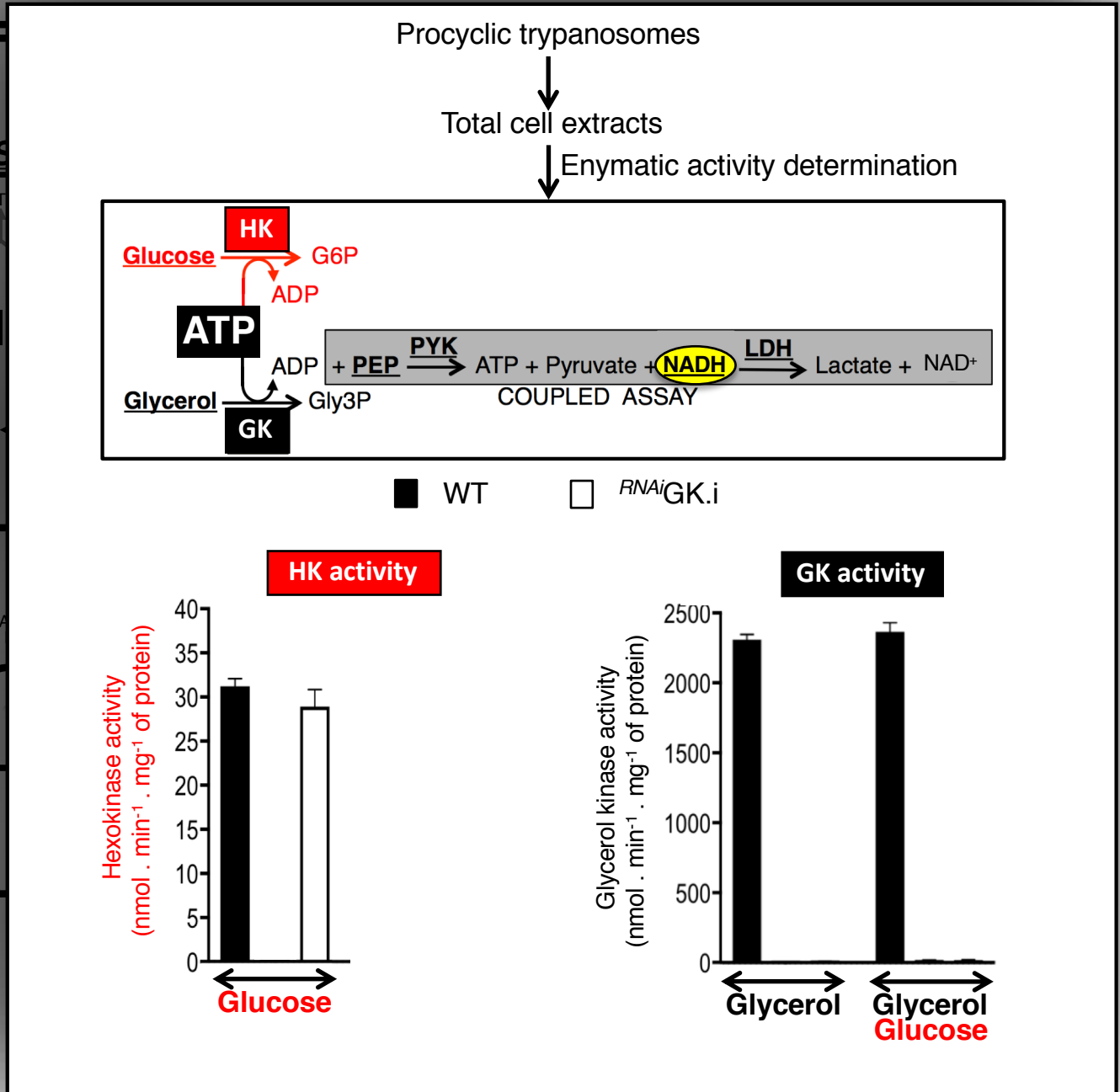
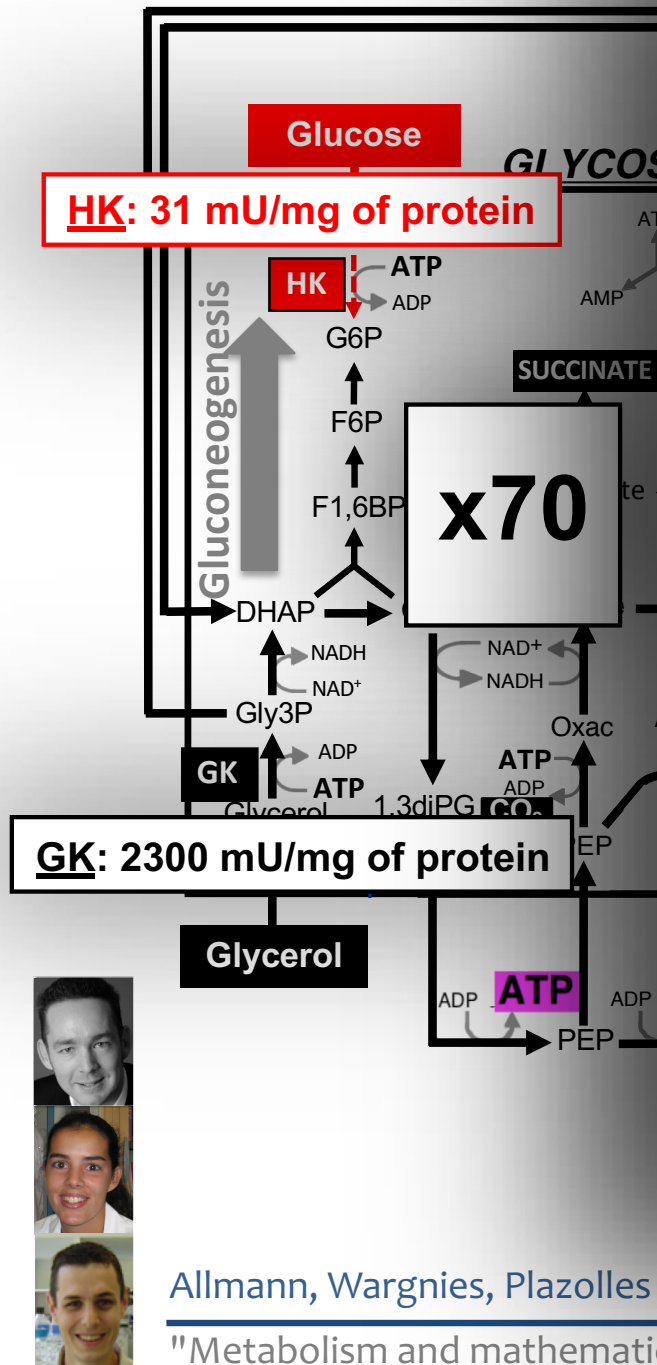
November 18-19, 2021



Allmann, Wargnies, Plazolles et al., 2021, PLOS Biol

"Metabolism and mathematical models: Two for a tango"

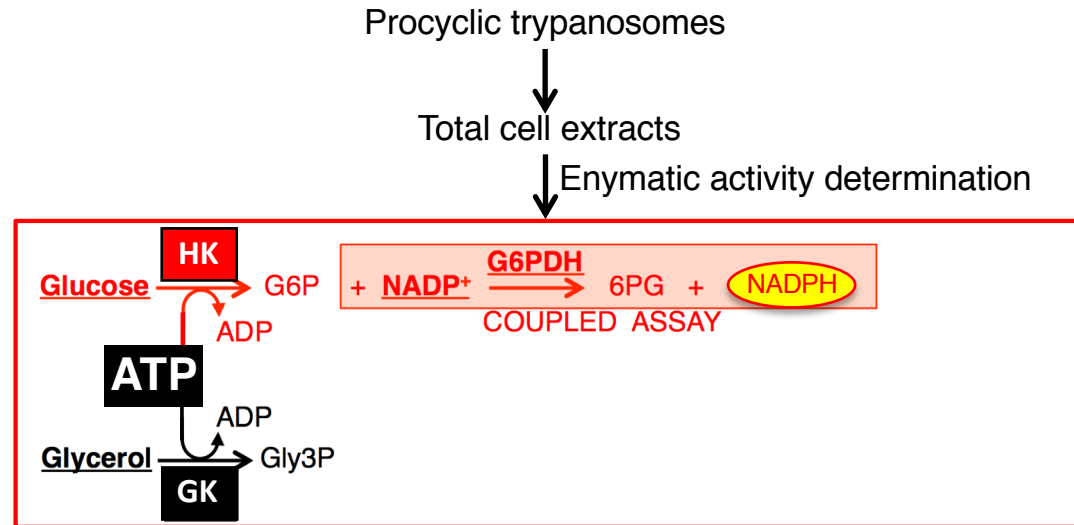
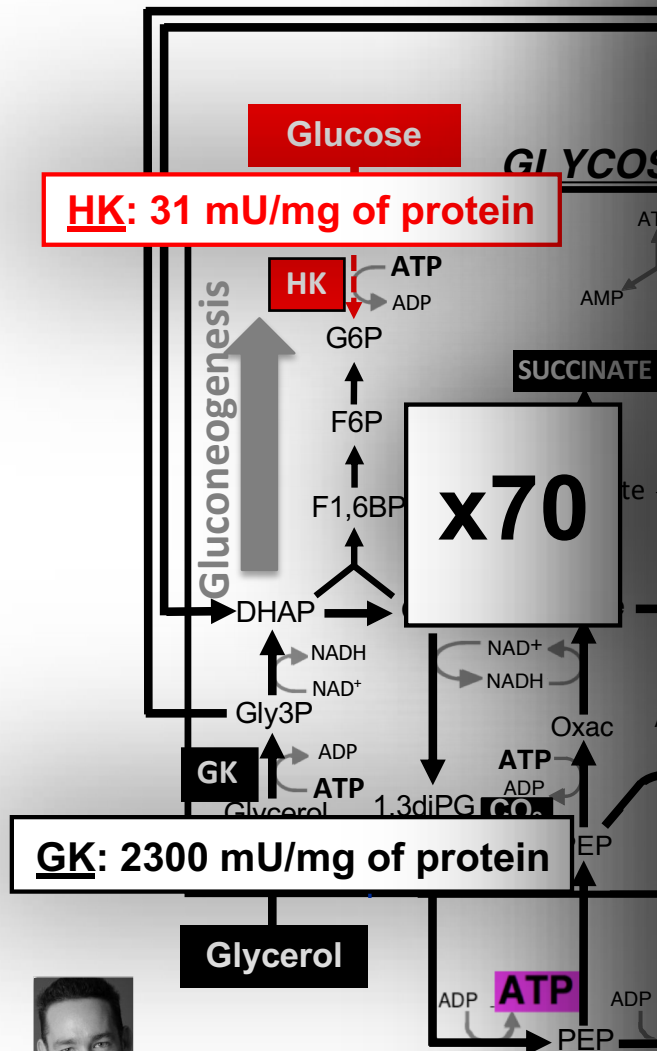
November 18-19, 2021



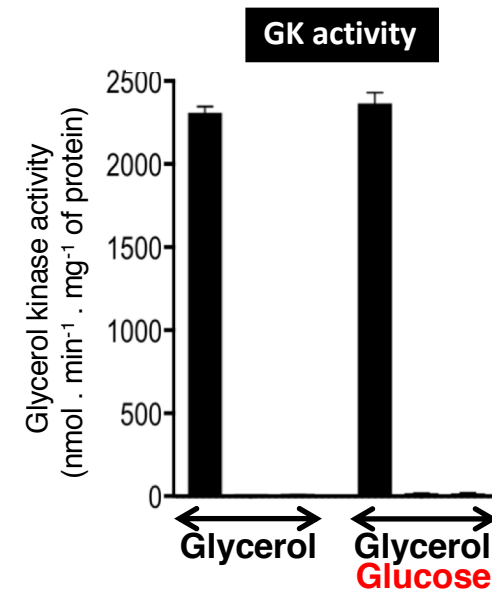
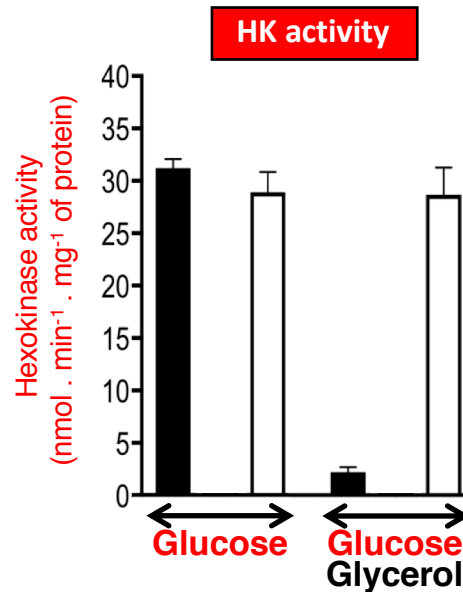
Allmann, Wargnies, Plazolles et al., 2021, PLOS Biol

"Metabolism and mathematical models: Two for a tango"

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■ WT □ *RNAi*GK.i

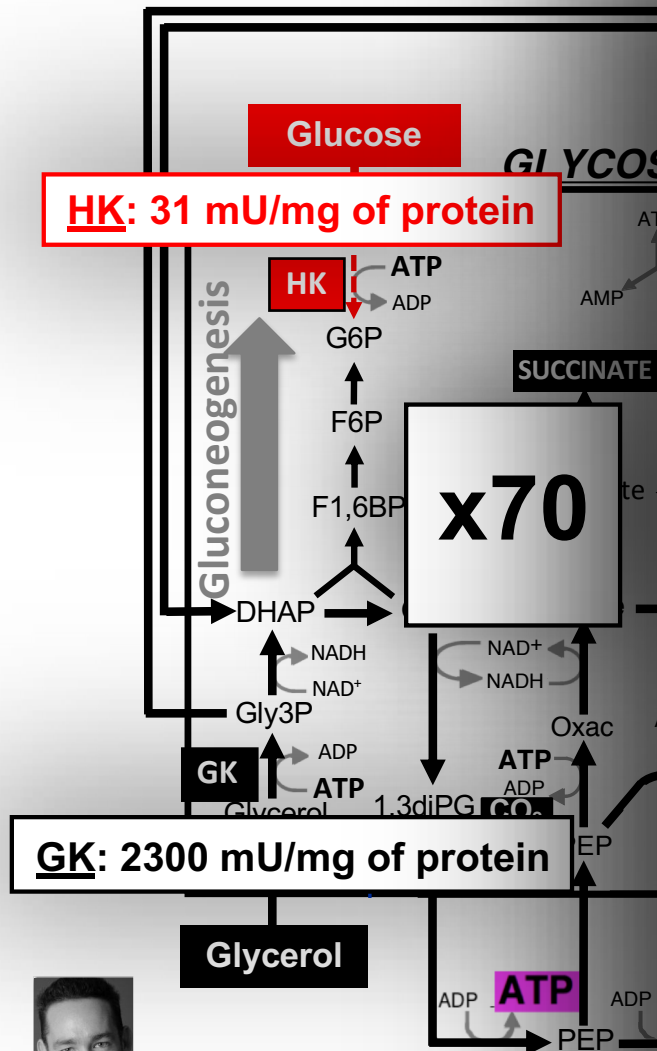


Allmann, Wargnies, Plazolles et al., 2021, PLOS Biol

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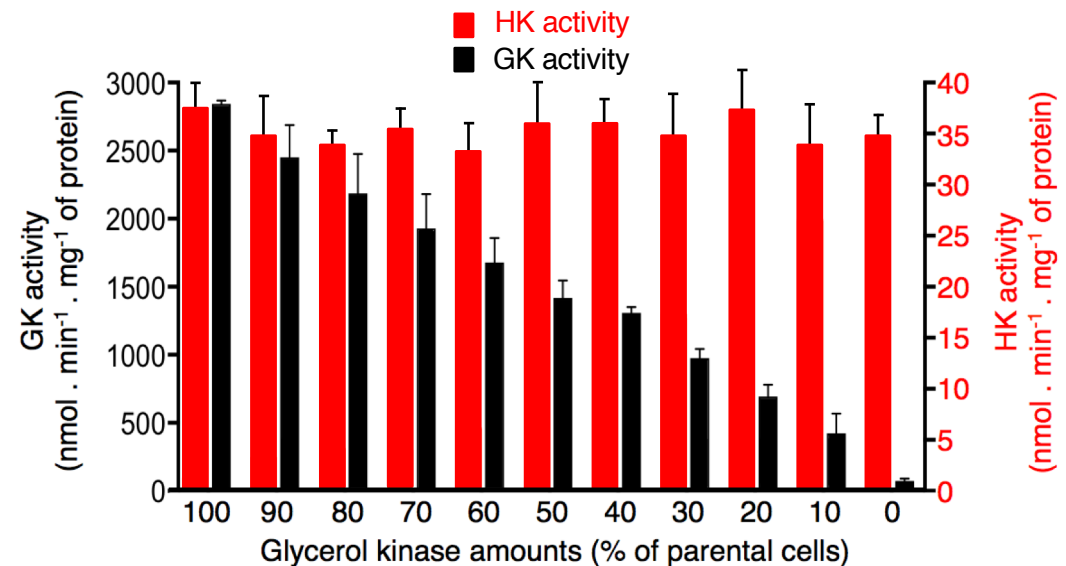
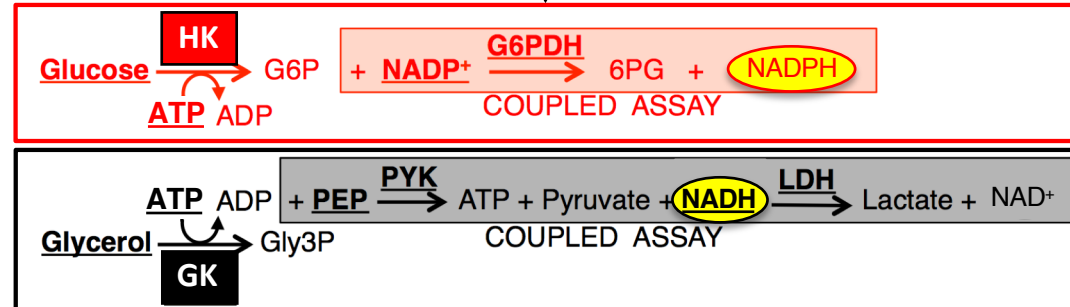




Procyclic trypanosomes

Total cell extracts

Enzymatic activity determination

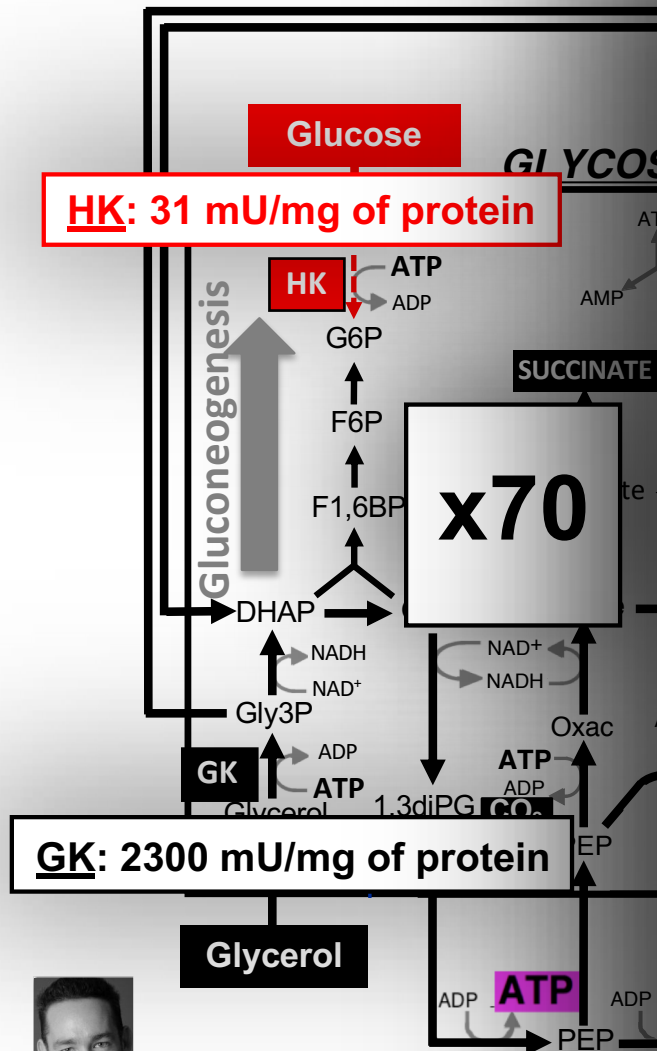


WT (%)	100	90	80	70	60	50	40	30	20	10	0
<i>RNAi</i> GK.i (%)	0	10	20	30	40	50	60	70	80	90	100

Allmann, Wargnies, Plazolles et al., 2021, PLOS Biol

"Metabolism and mathematical models: Two for a tango"

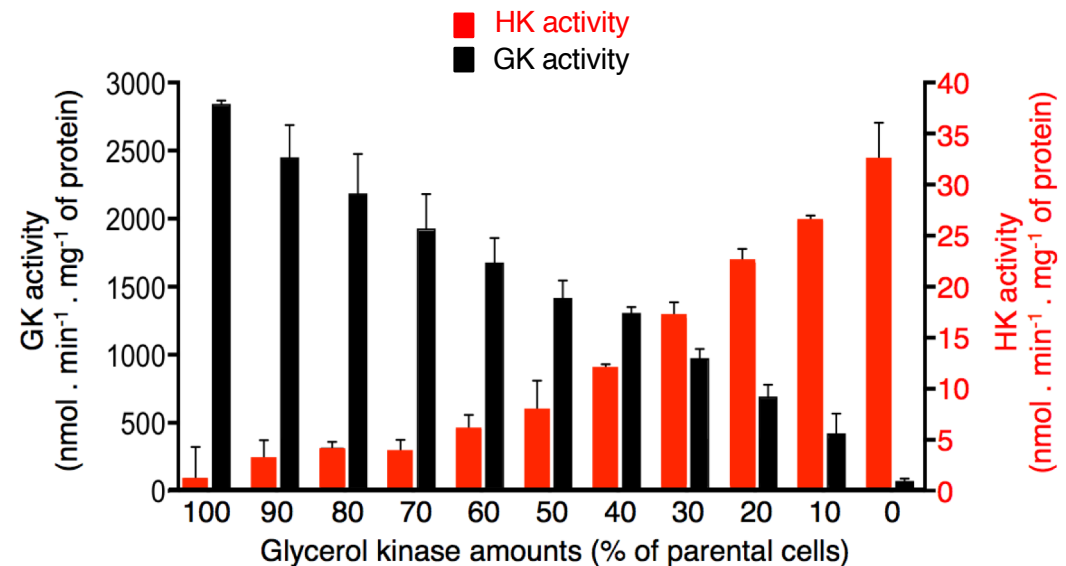
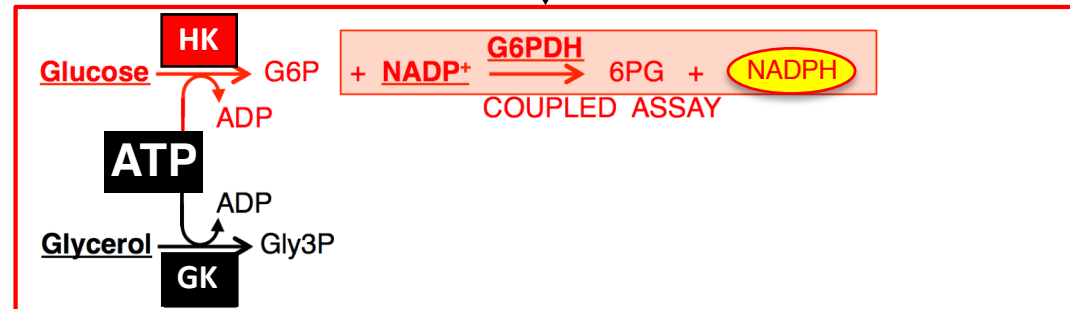
November 18-19, 2021



Procyclic trypanosomes

Total cell extracts

Enzymatic activity determination

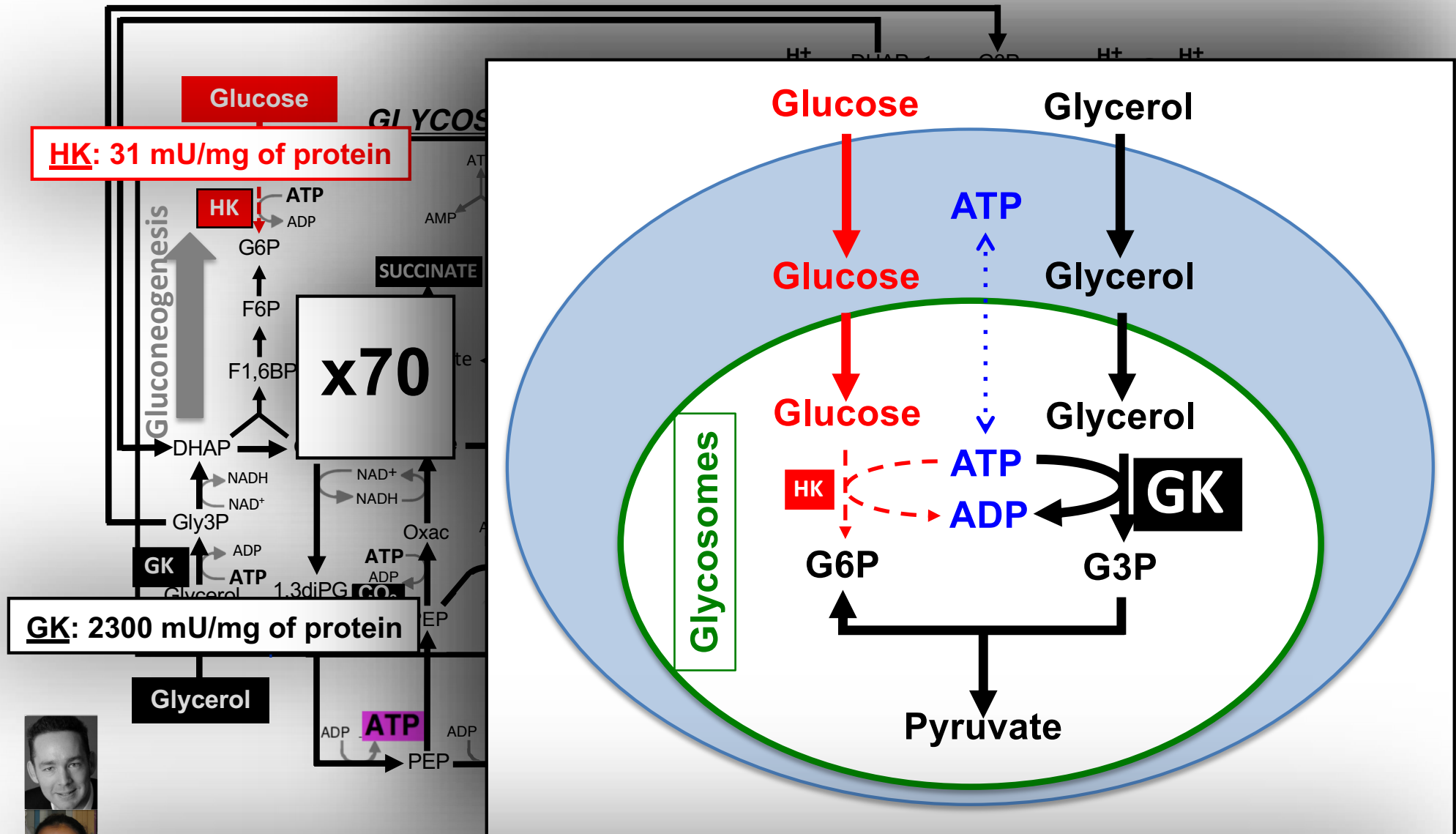


WT (%)	100	90	80	70	60	50	40	30	20	10	0
<i>RNAi</i> GK.i (%)	0	10	20	30	40	50	60	70	80	90	100

Allmann, Wargnies, Plazolles et al., 2021, PLOS Biol

"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021



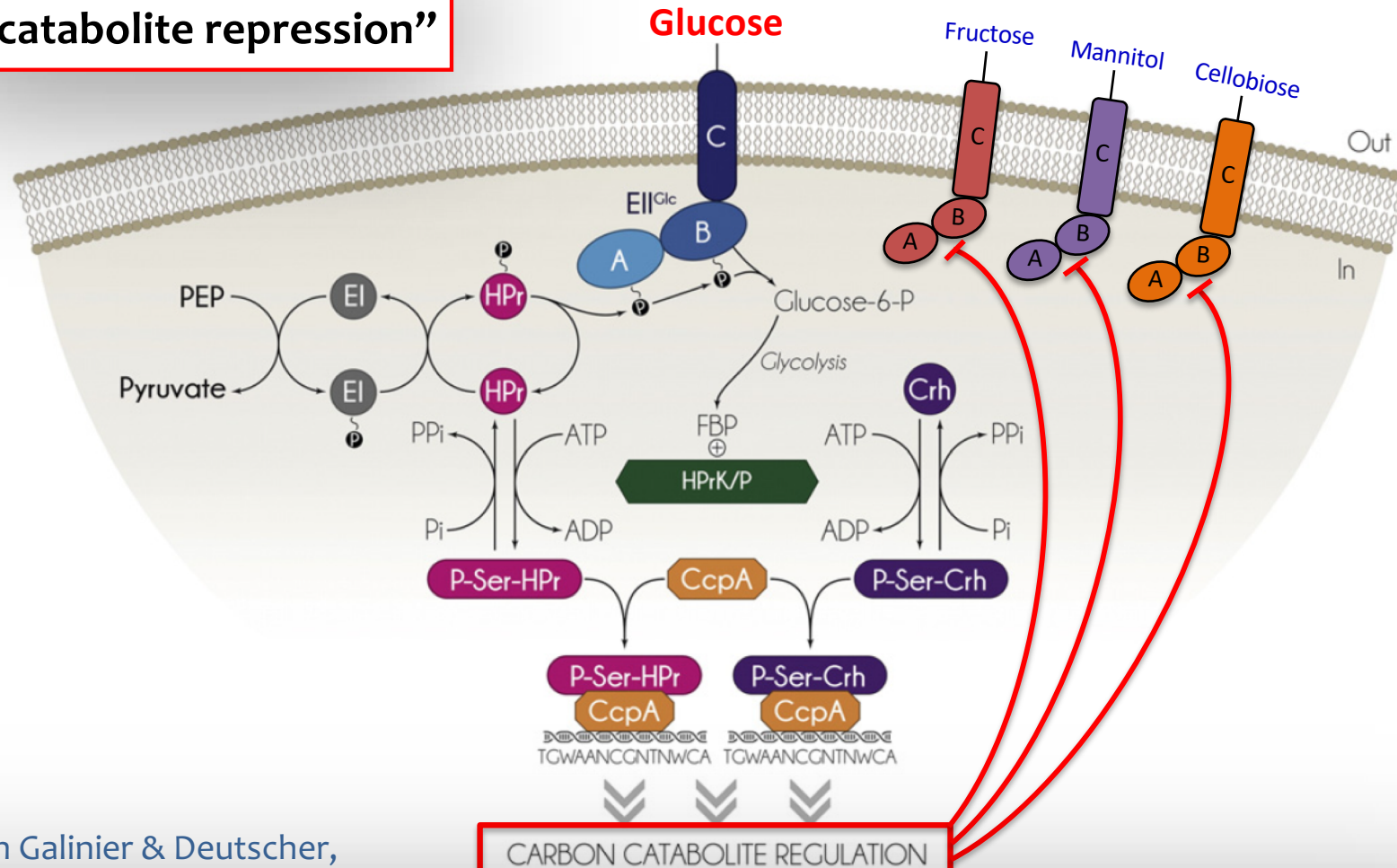
"Metabolic contest" depends on the combination of 3 conditions

Allmann, Wargnies, Plazolles *et al.*, 2021, PLoS Biol

"Metabolism and mathematical models: Two for a tango"

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"catabolite repression"

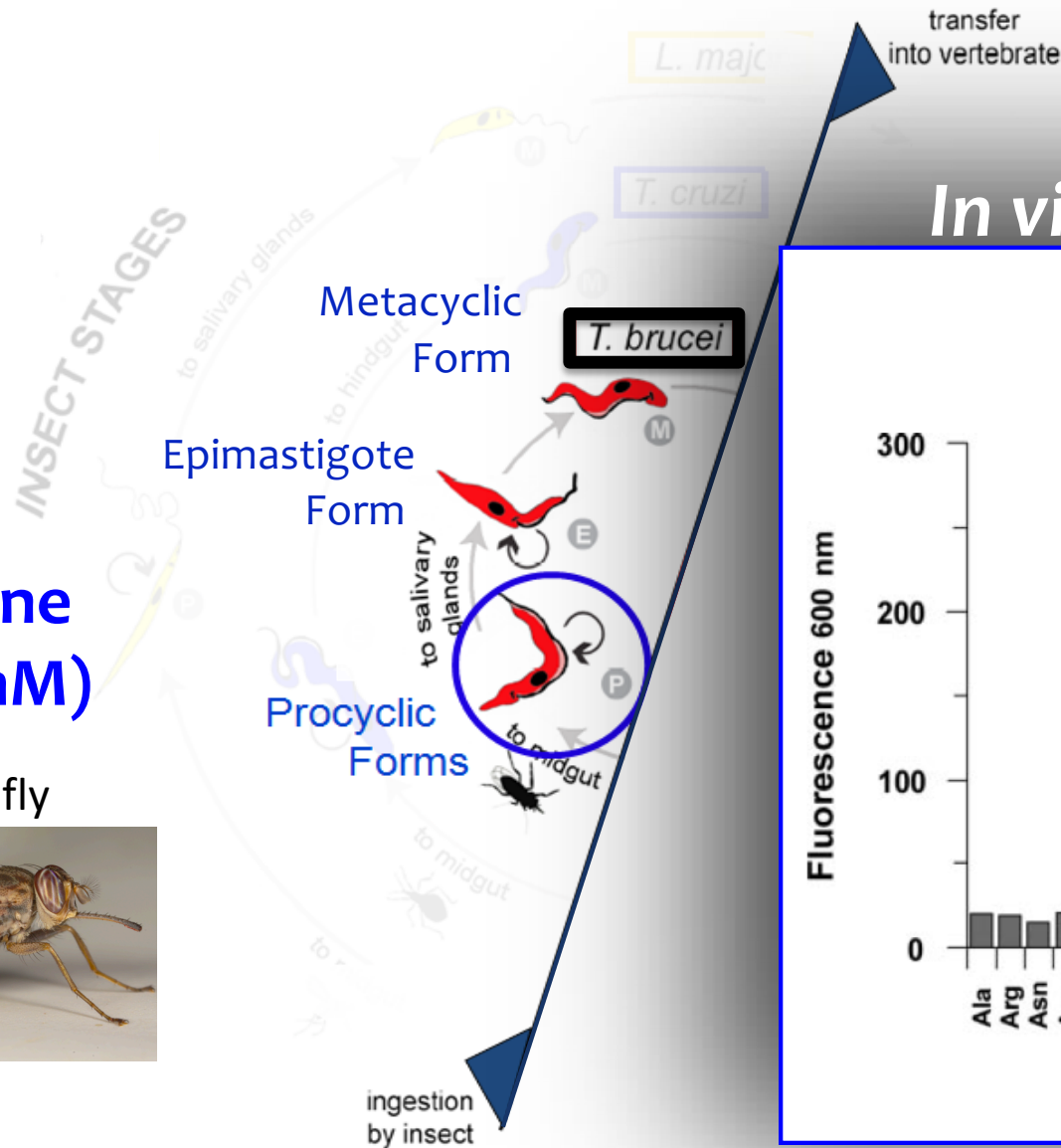


From Galinier & Deutscher,
2017, J Mol Biol

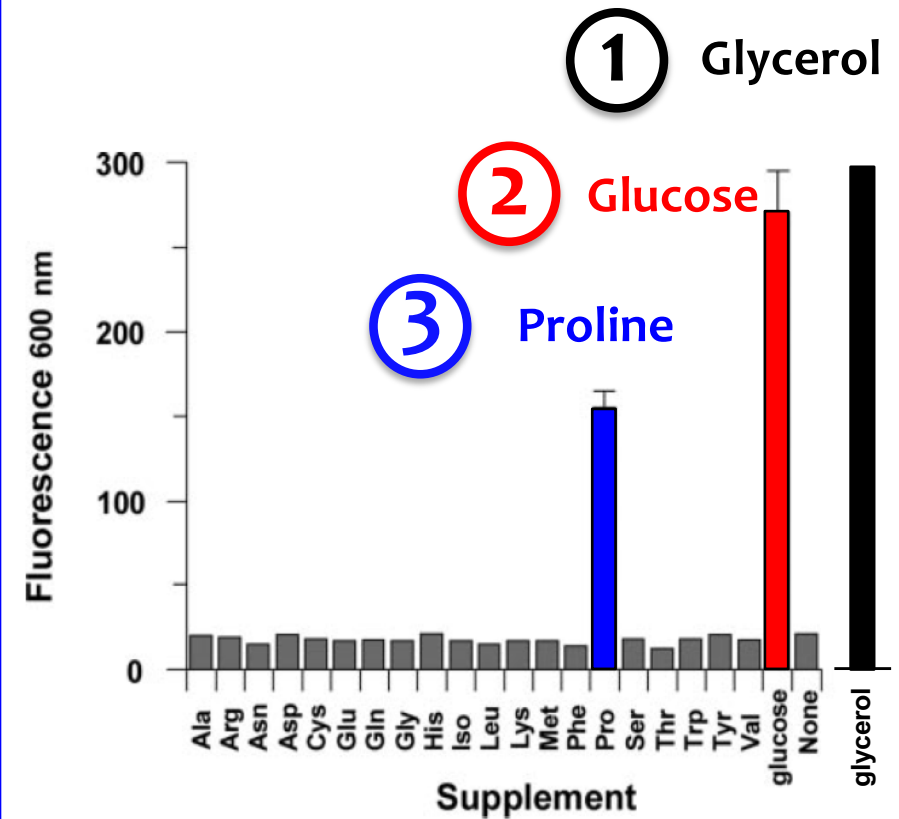
"Metabolic contest" differs greatly from "catabolite repression"

**Proline
(1-2 mM)**

Tsetse fly



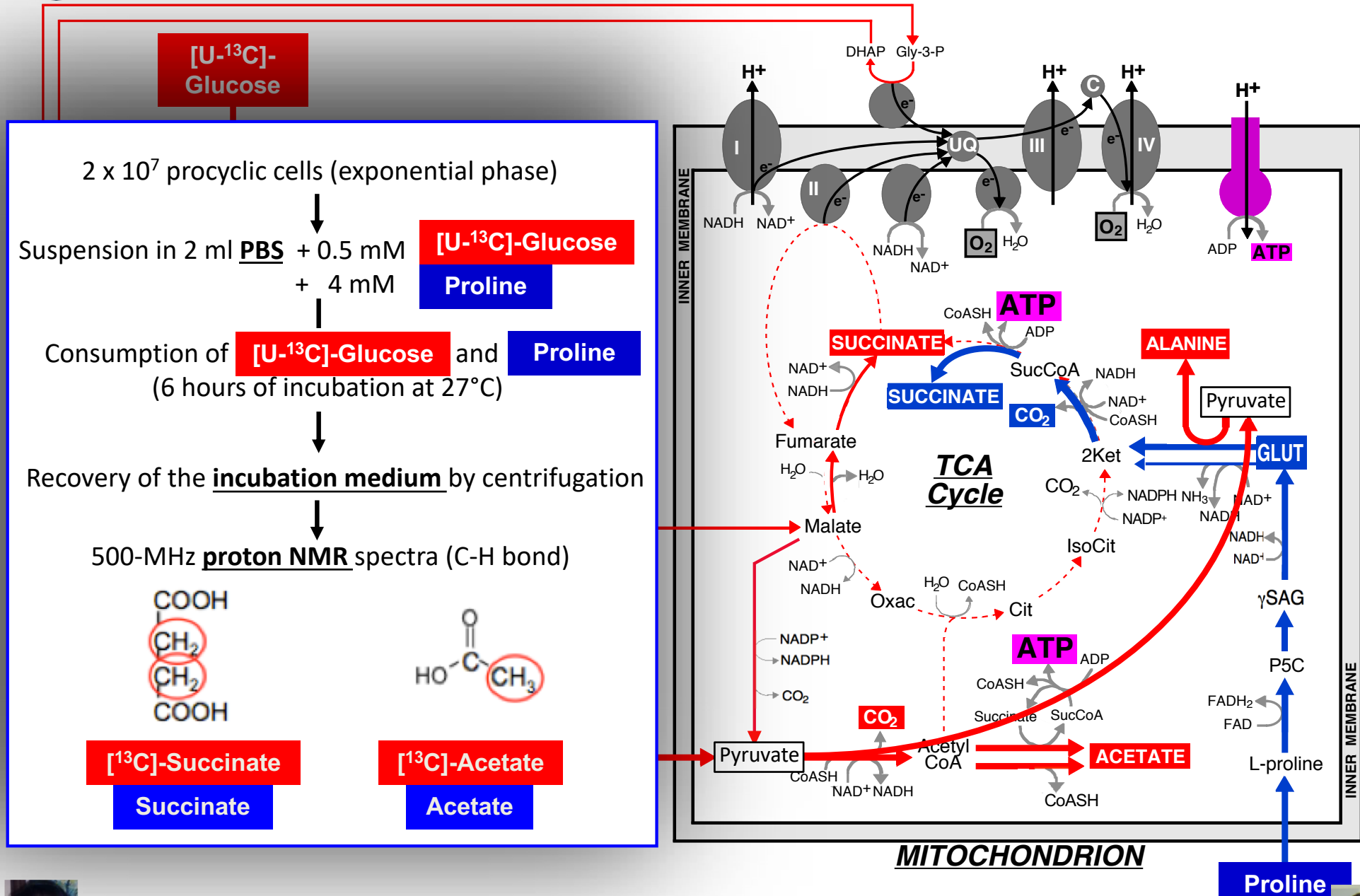
In vitro preference



Villafranz *et al.*, 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021



Villafranz et al., 2021, PLoS Pathog

N. Plazolles

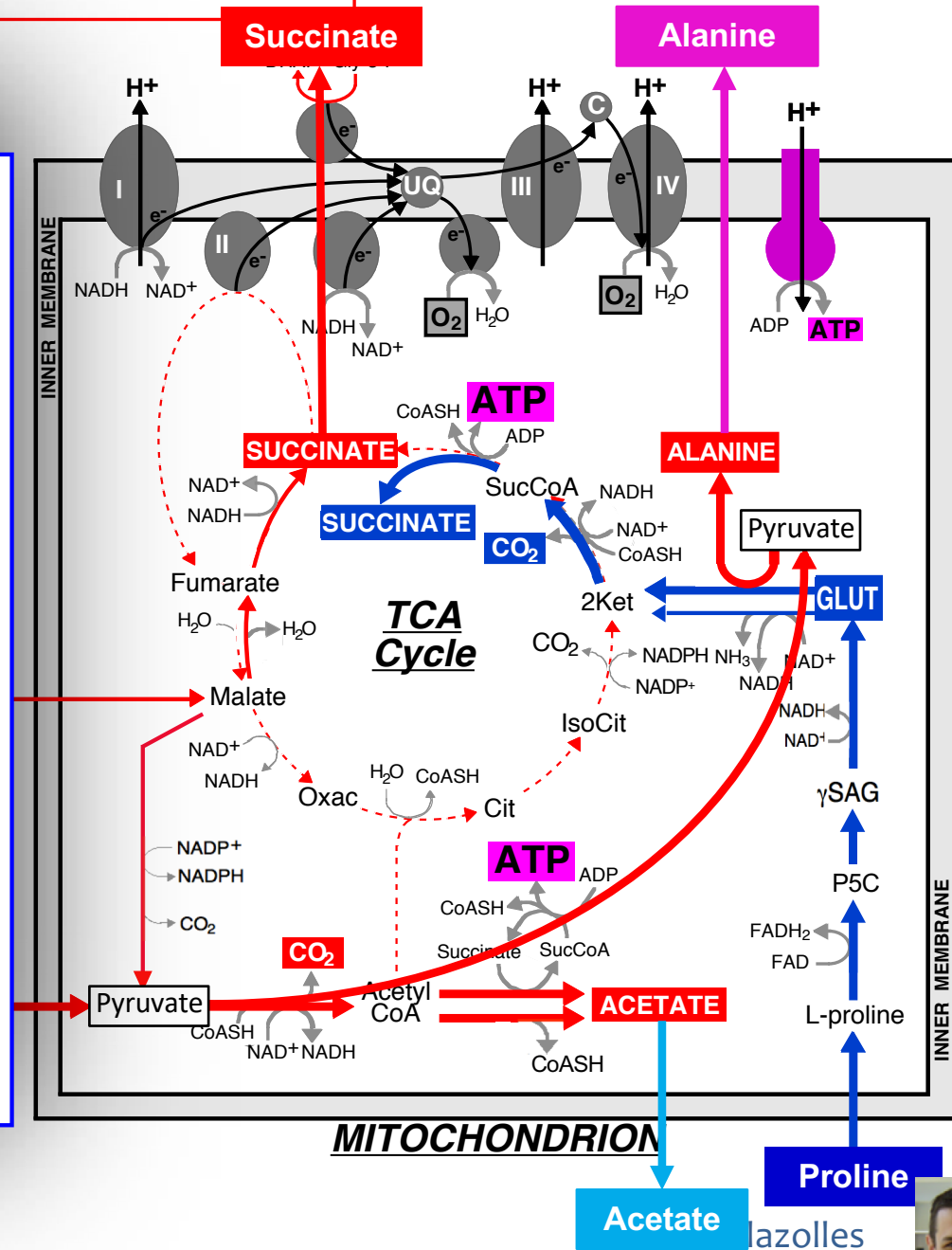
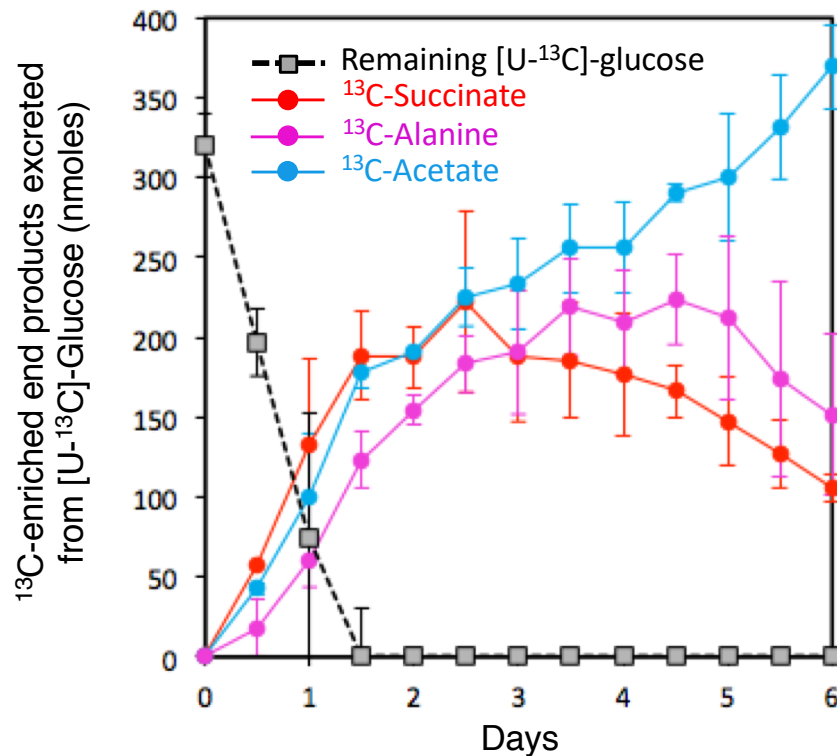


"Metabolism and mathematical models: Two for a tango"

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[U-¹³C]-
Glucose

[U-¹³C]-Glucose (0.5 mM) + Proline (4 mM)



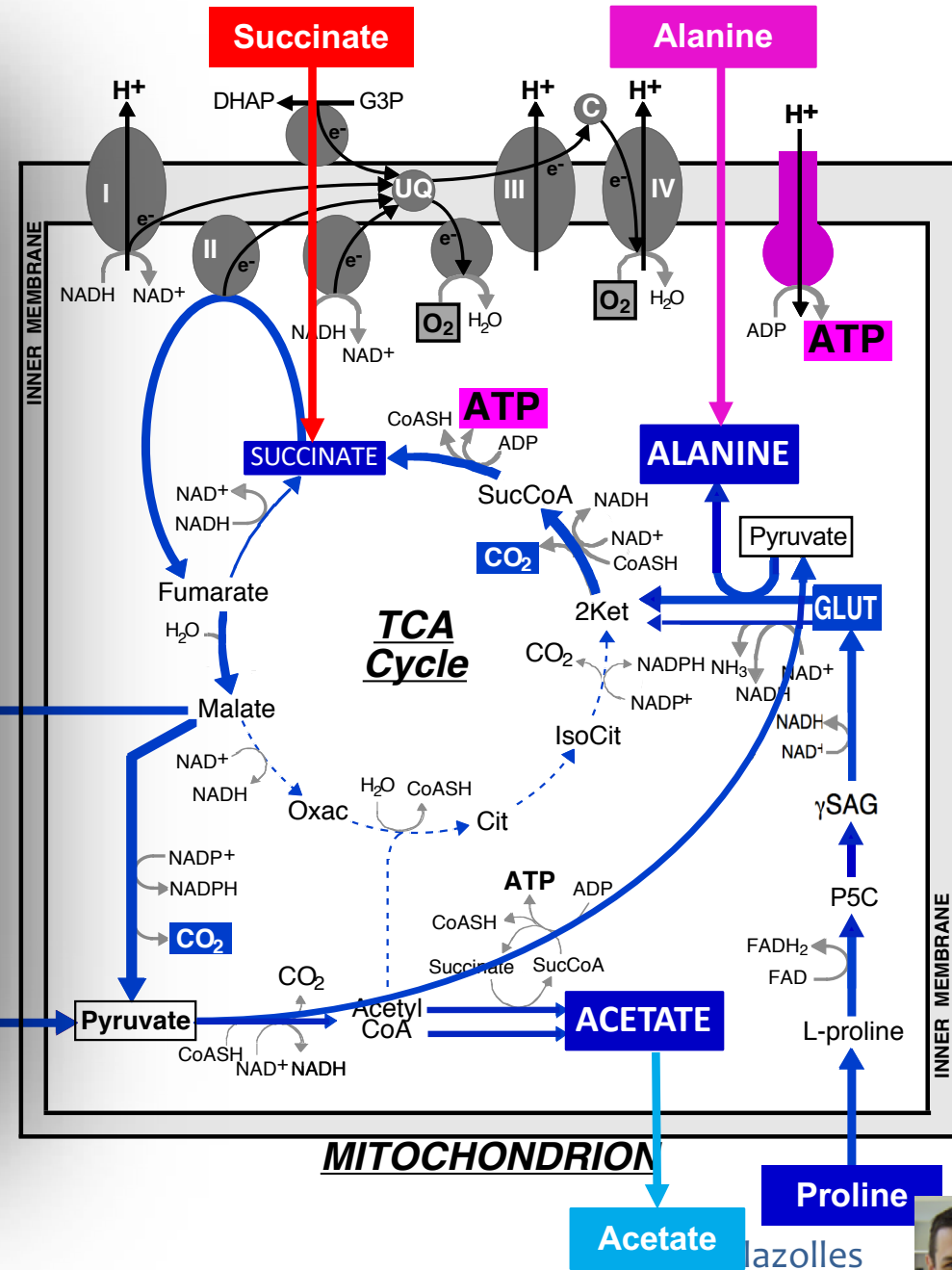
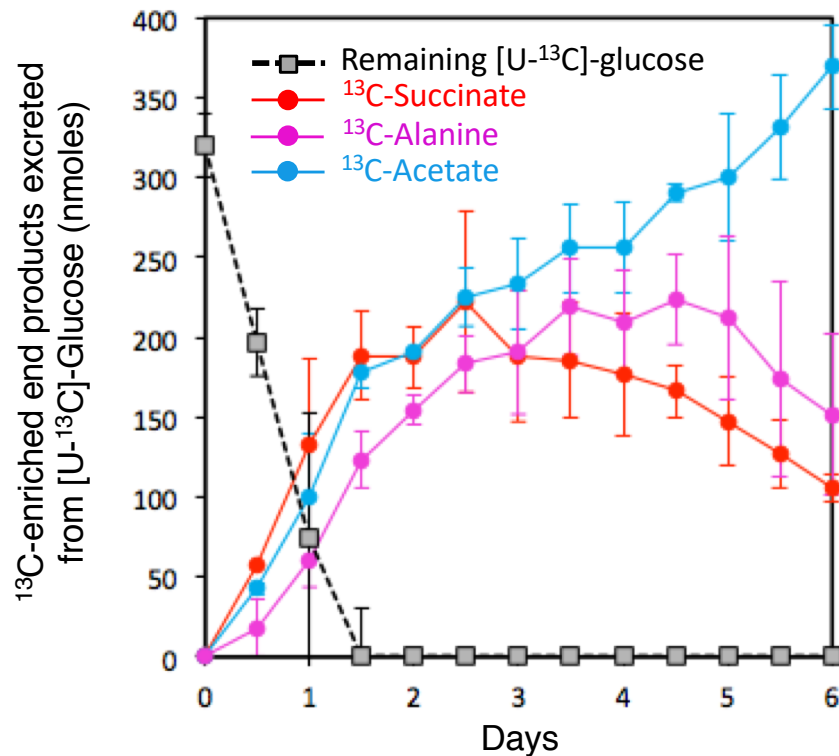
Villafranz et al., 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021



[U-¹³C]-Glucose (0.5 mM) + Proline (4 mM)



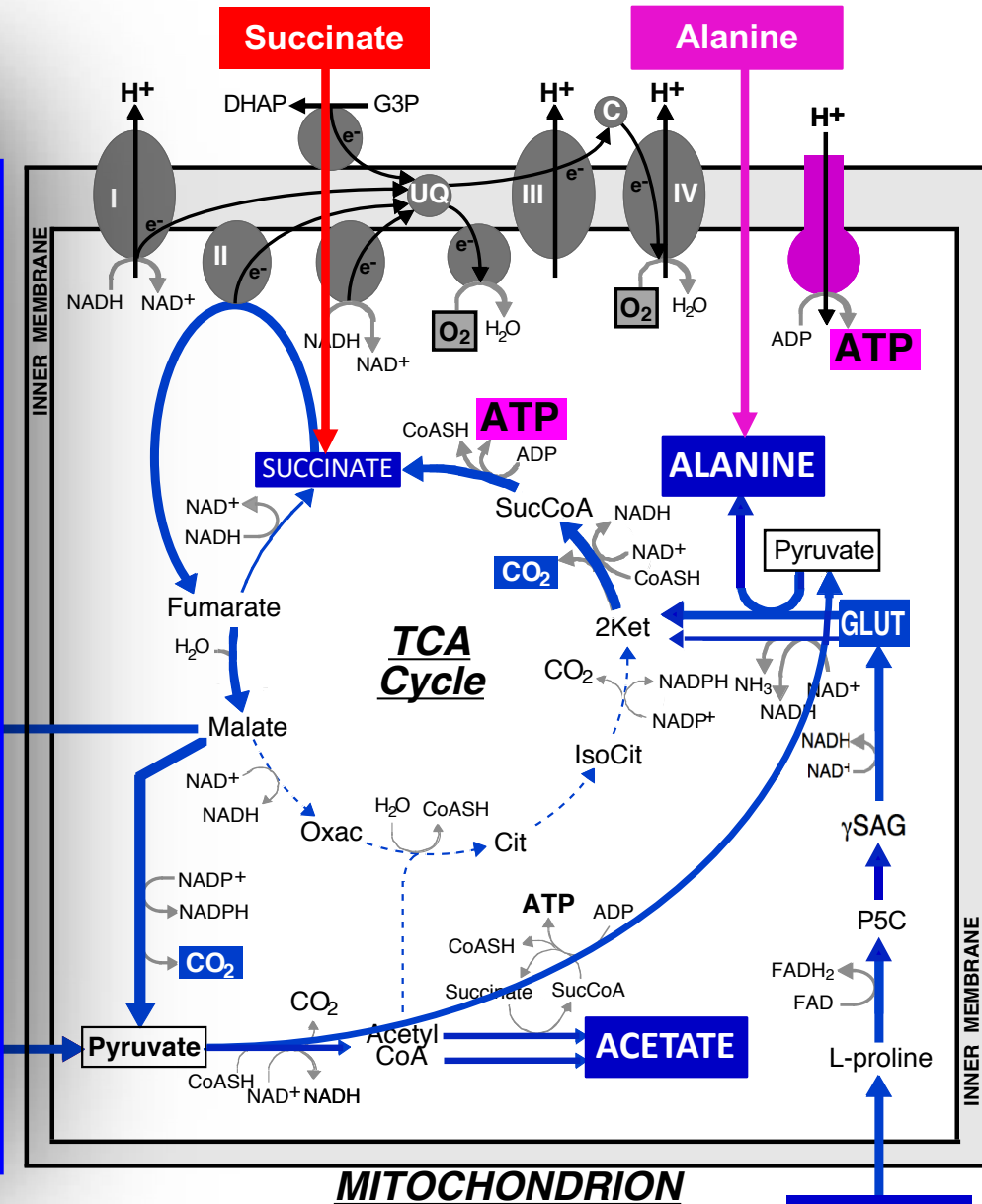
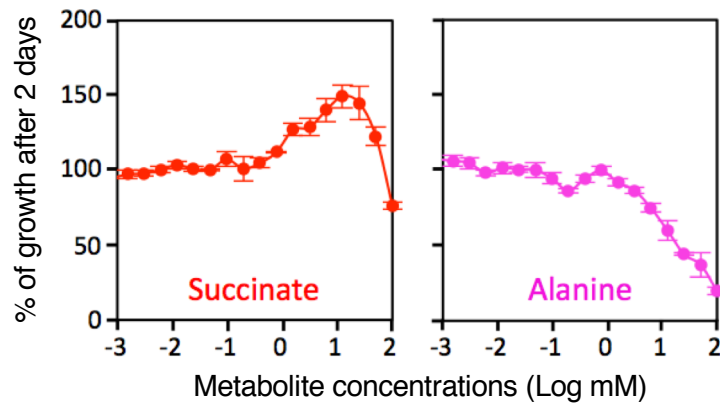
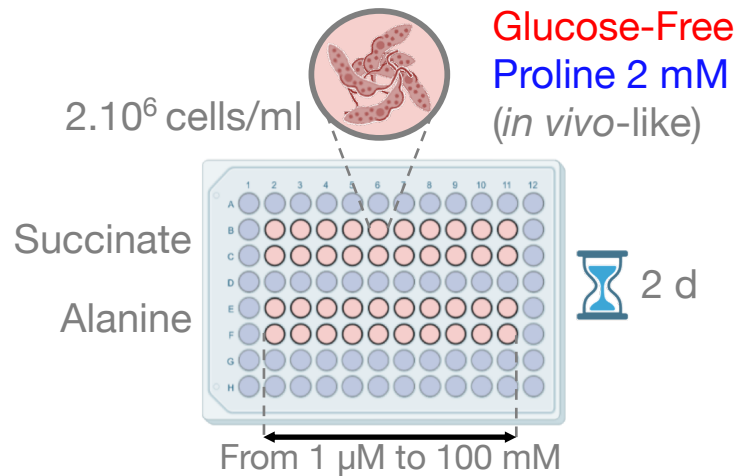
Villafranz et al., 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

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Alamar Blue assay



Villafranz et al., 2021, PLoS Pathog

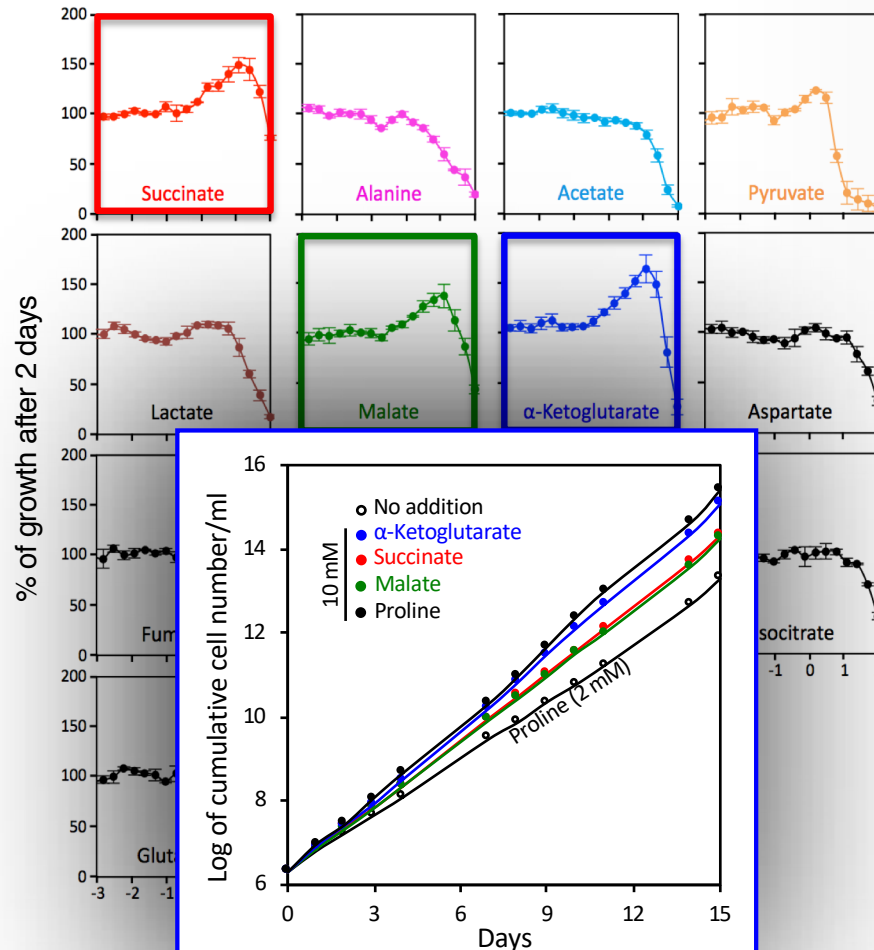
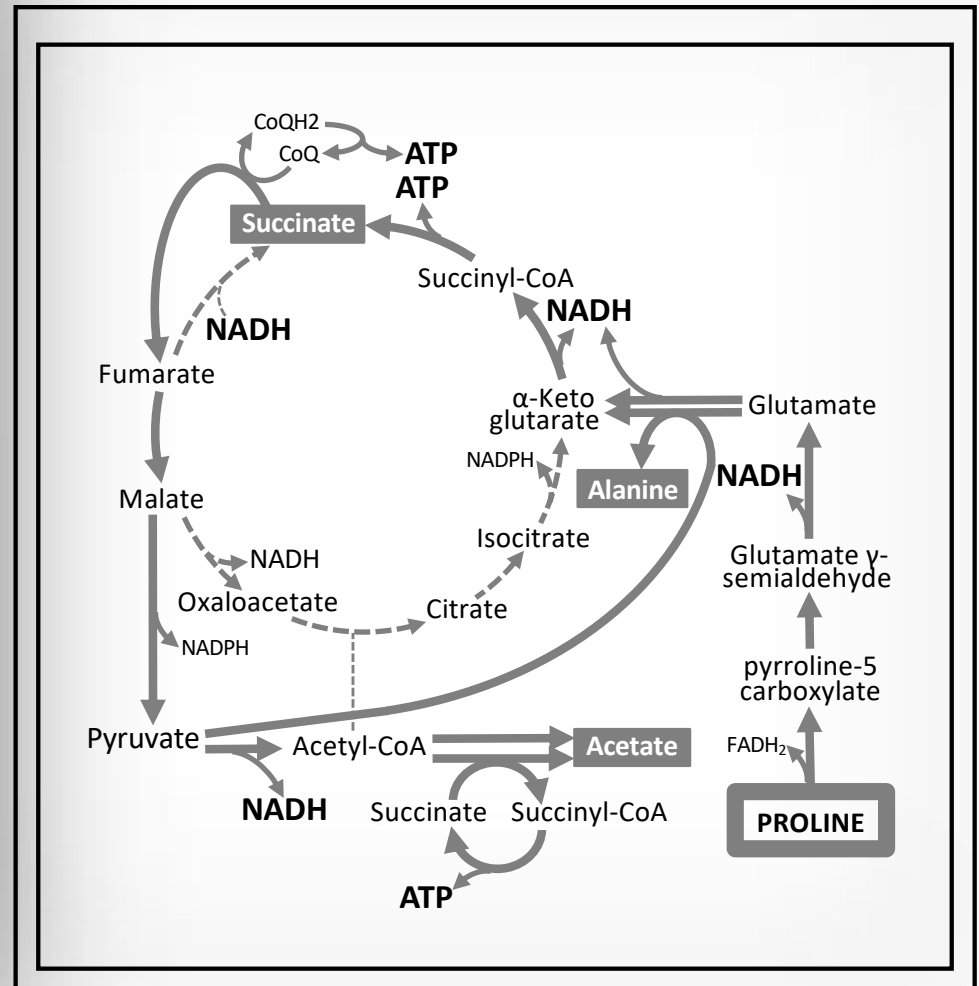
N. Plazolles



"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

Mitochondrion



Villafranz *et al.*, 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

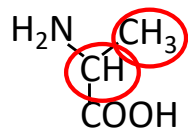
2×10^7 procyclic cells (exponential phase)

Suspension in 2 ml **PBS** + 4 mM **[U-¹³C]-Proline**
+ 4 mM **Succinate**

Consumption of **[U-¹³C]-Proline** and **Succinate**
(6 hours of incubation at 27°C)

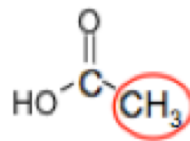
Recovery of the incubation medium by centrifugation

500-MHz proton NMR spectra (C-H bond)



[¹³C]-Alanine

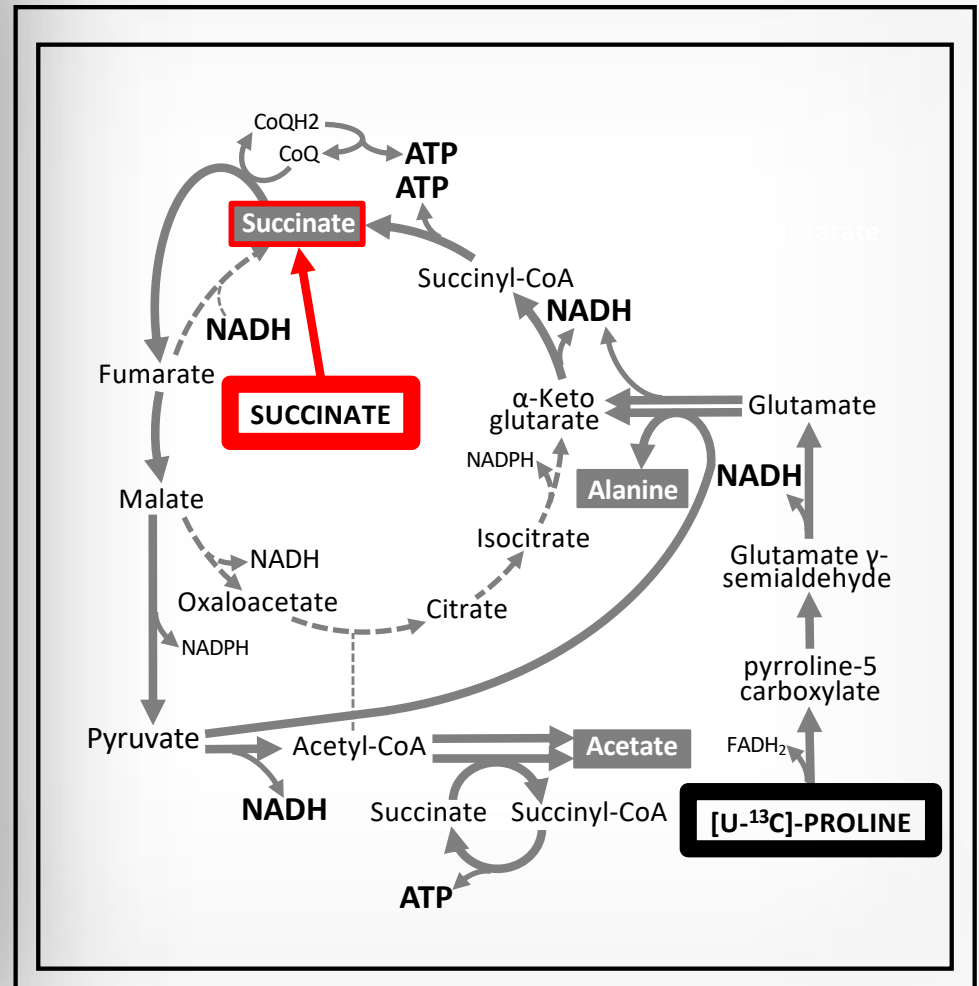
Alanine



[¹³C]-Acetate

Acetate

Mitochondrion

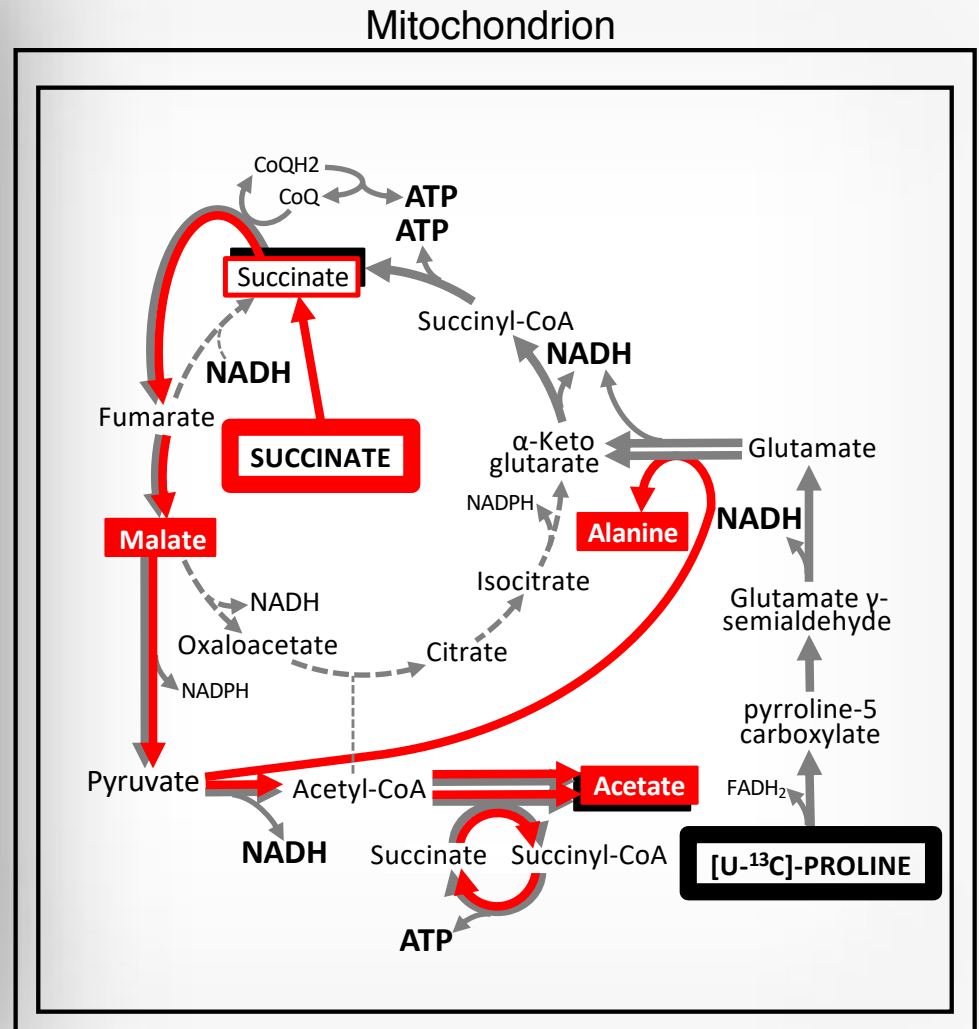
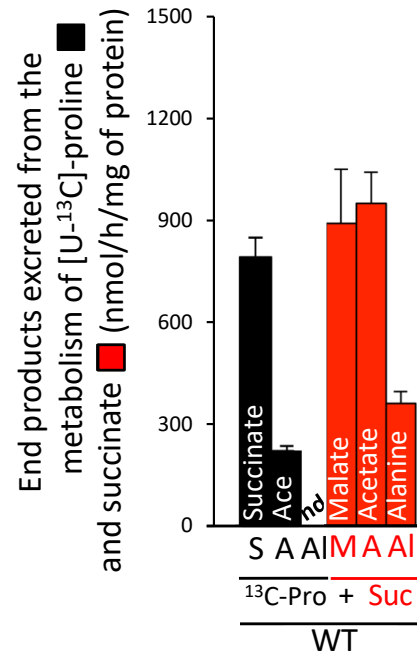


Villafranz *et al.*, 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

4 mM [U-¹³C]-proline + 4 mM succinate

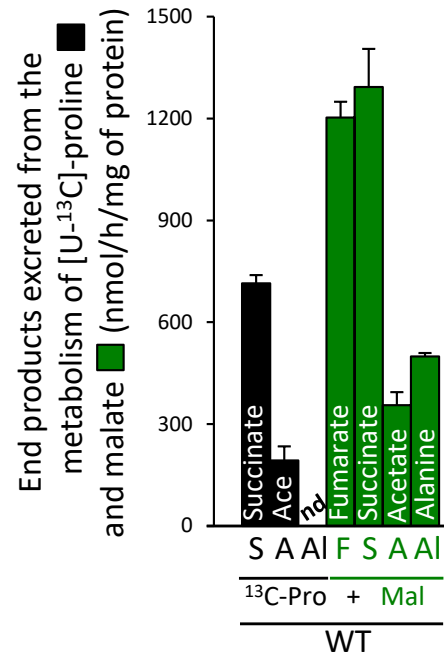


Villafranz et al., 2021, PLoS Pathog

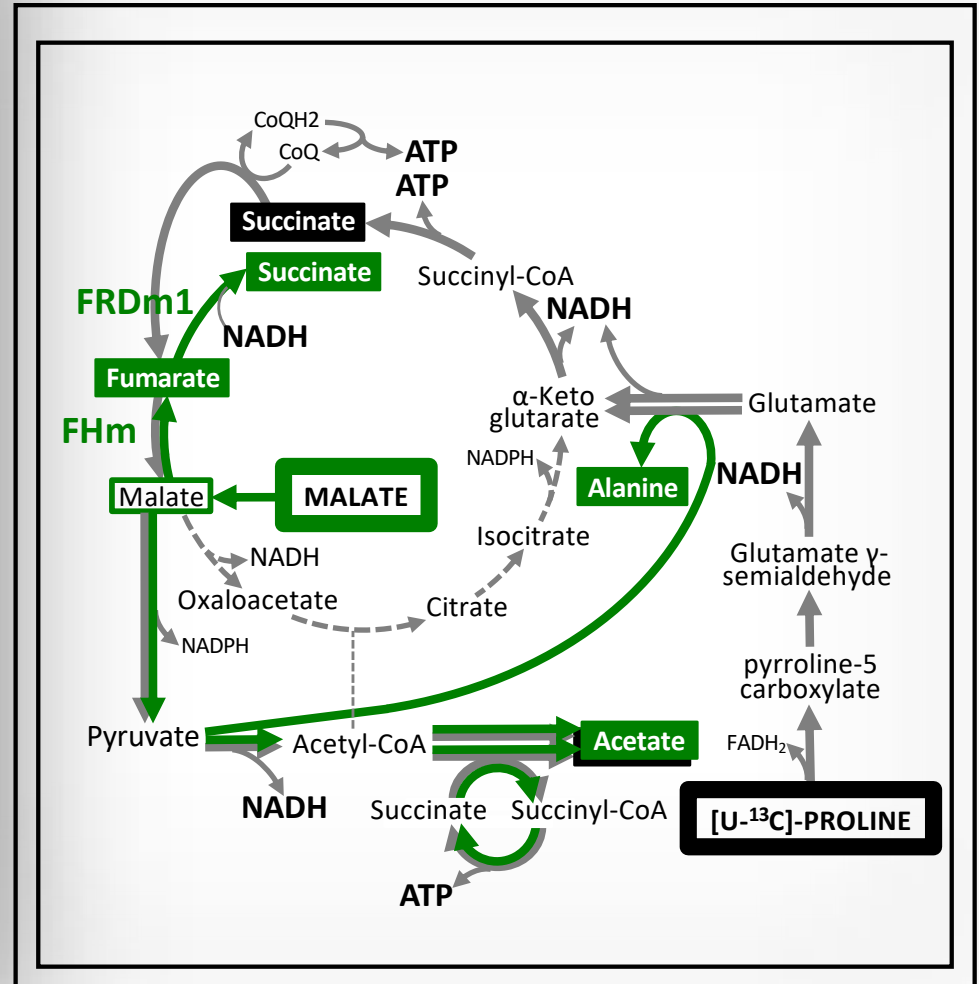
"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

4 mM [U-¹³C]-proline + 4 mM malate



Mitochondrion

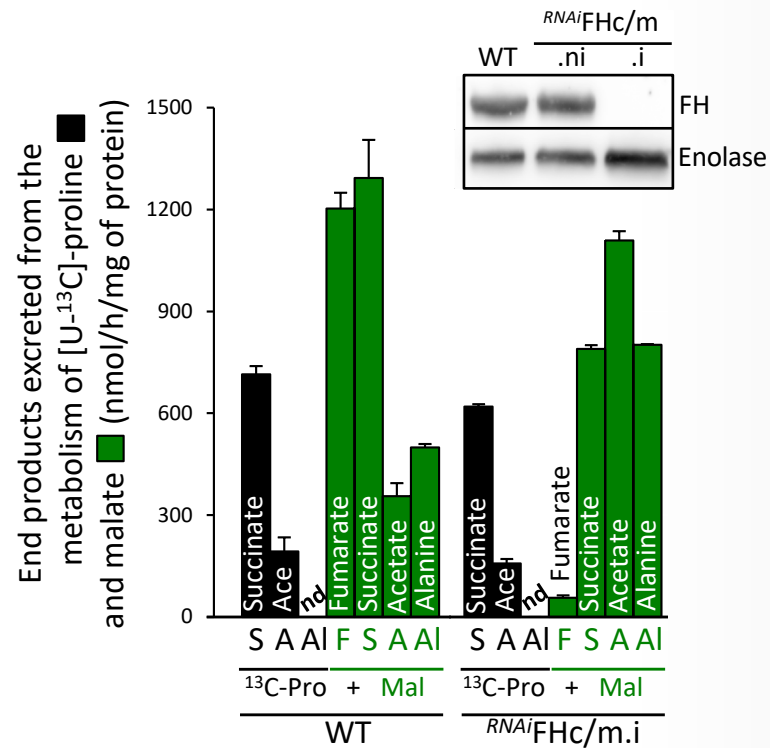


Villafranz *et al.*, 2021, PLoS Pathog

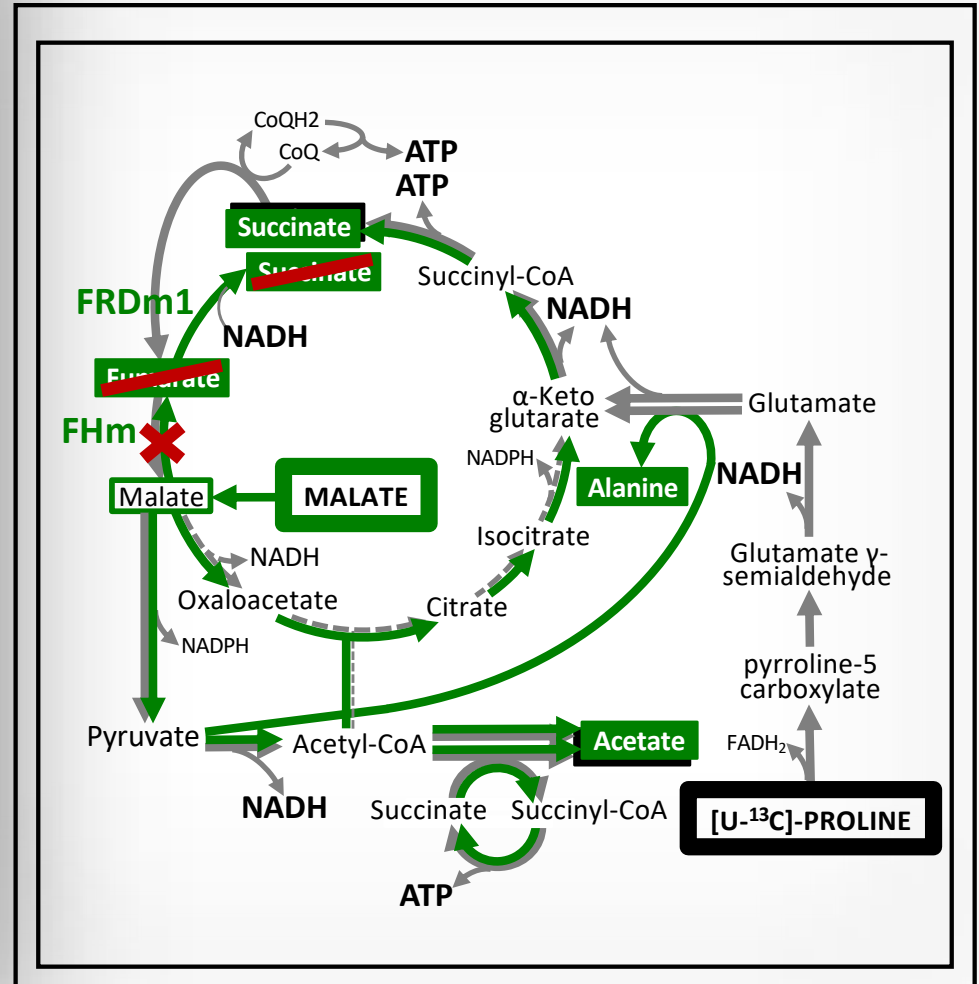
"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

4 mM [U-¹³C]-proline + 4 mM malate



Mitochondrion

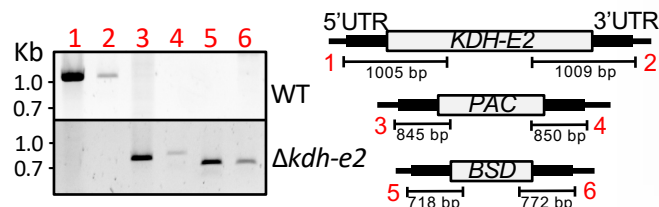
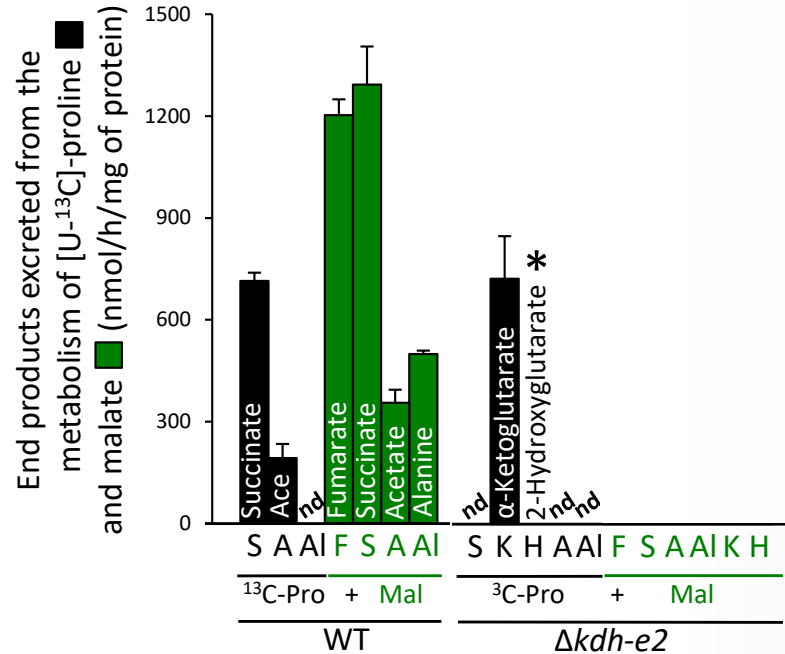


Villafranz et al., 2021, PLoS Pathog

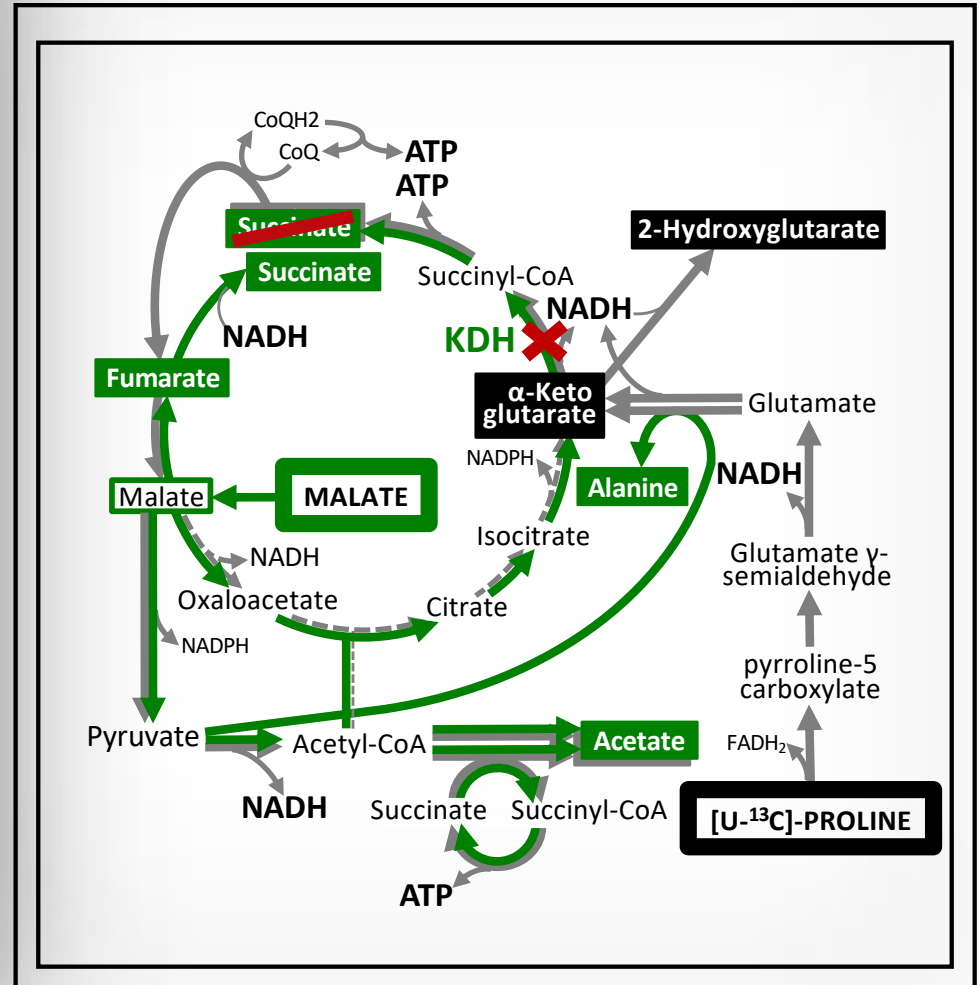
"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

4 mM [U-¹³C]-proline + 4 mM malate



Mitochondrion

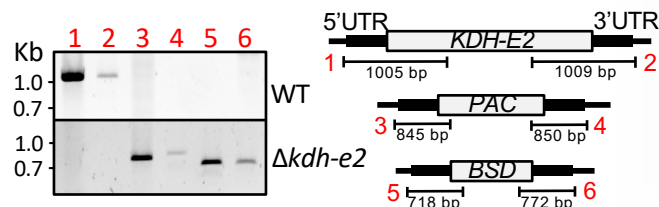
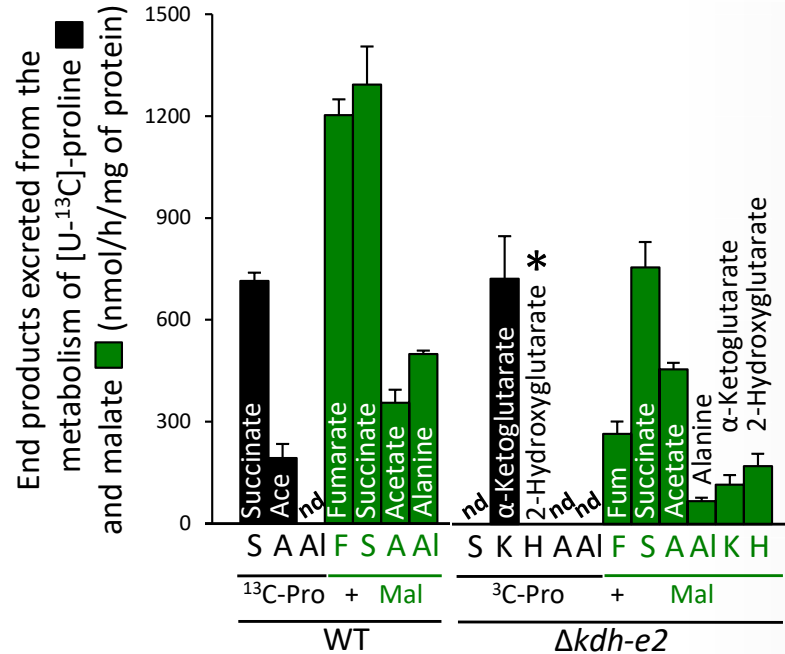


Villafranz et al., 2021, PLoS Pathog

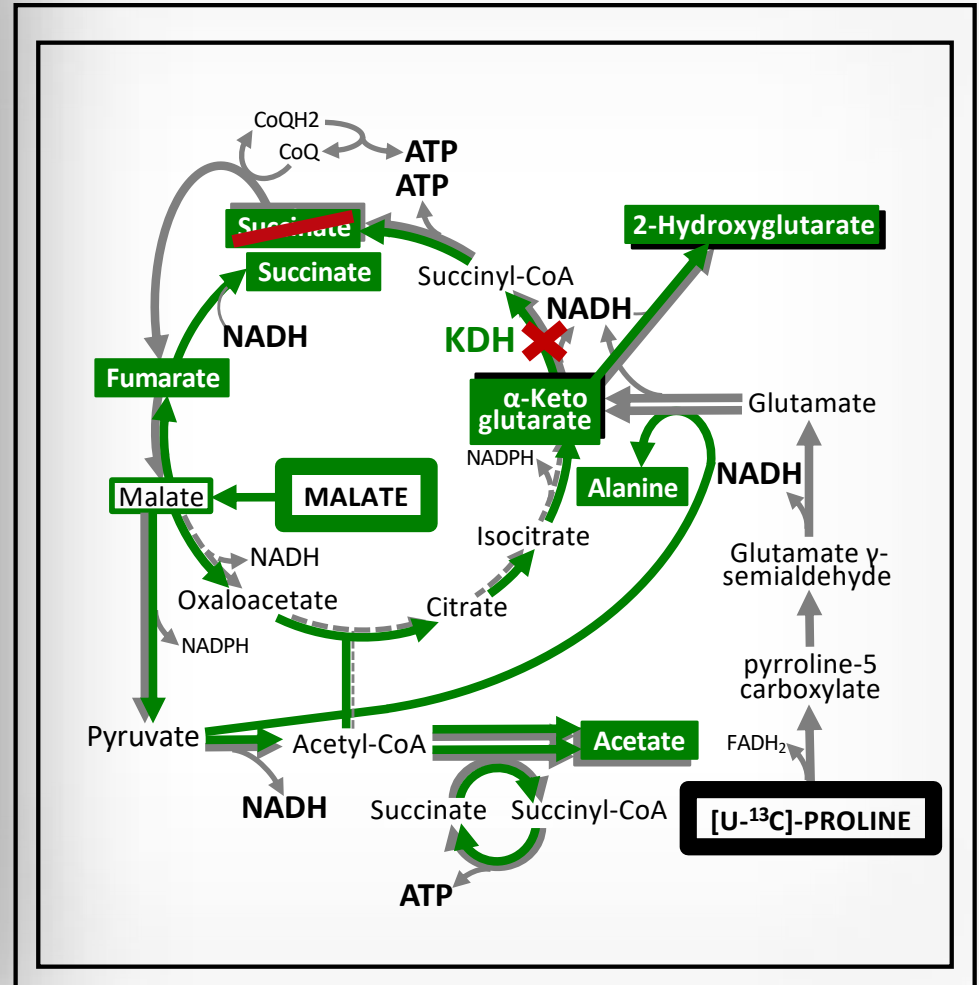
"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

4 mM [U-¹³C]-proline + 4 mM malate



Mitochondrion

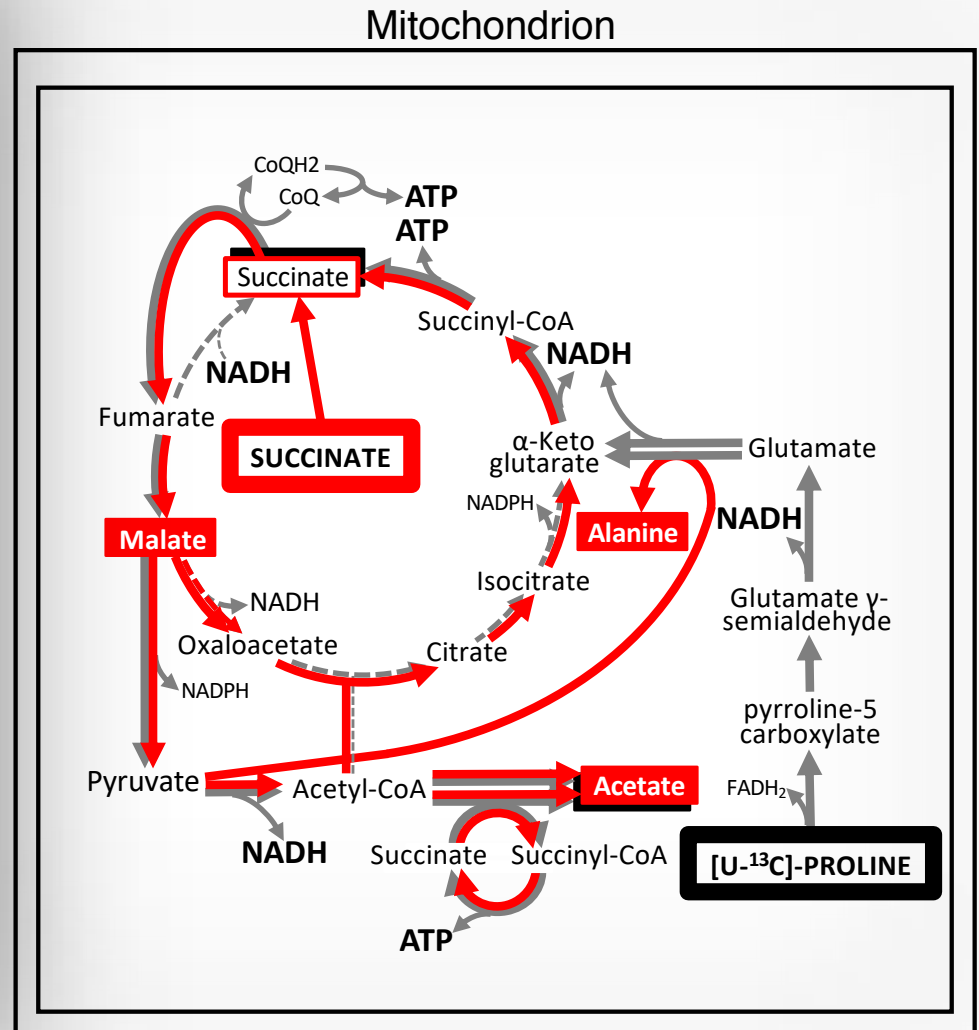
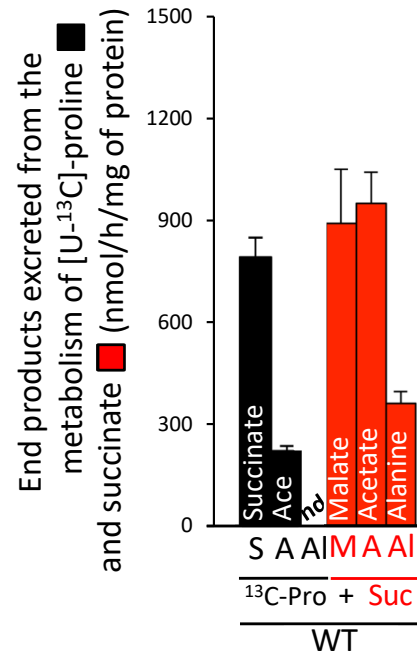


Villafranz et al., 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

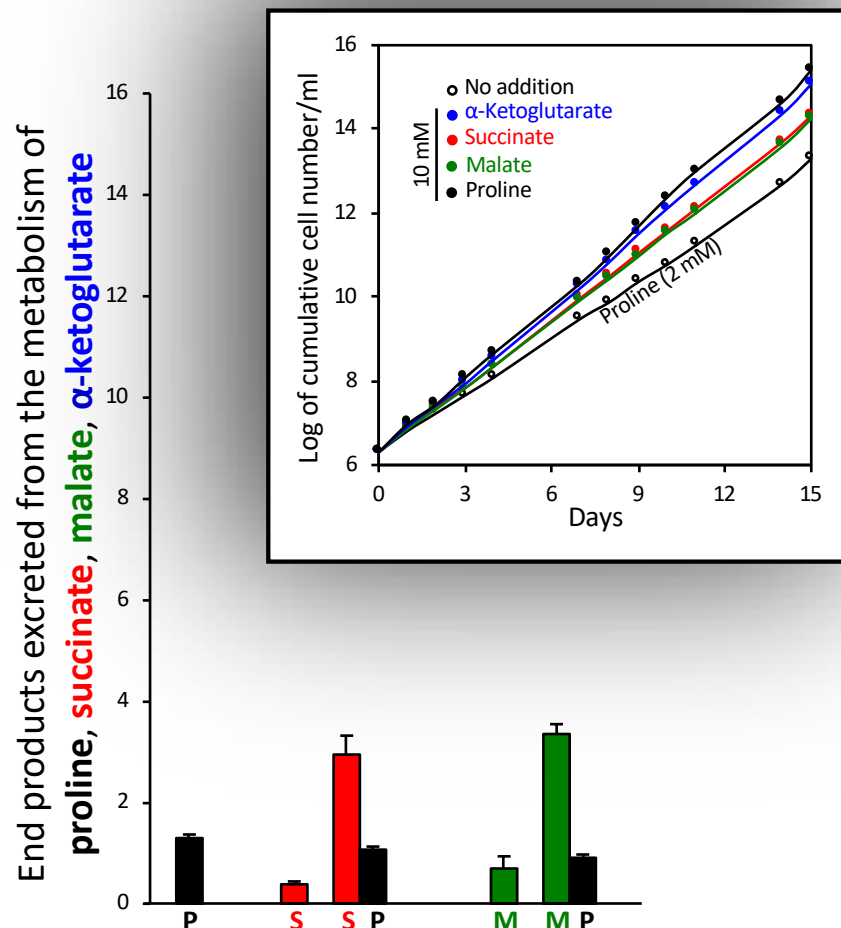
4 mM [U-¹³C]-proline + 4 mM succinate



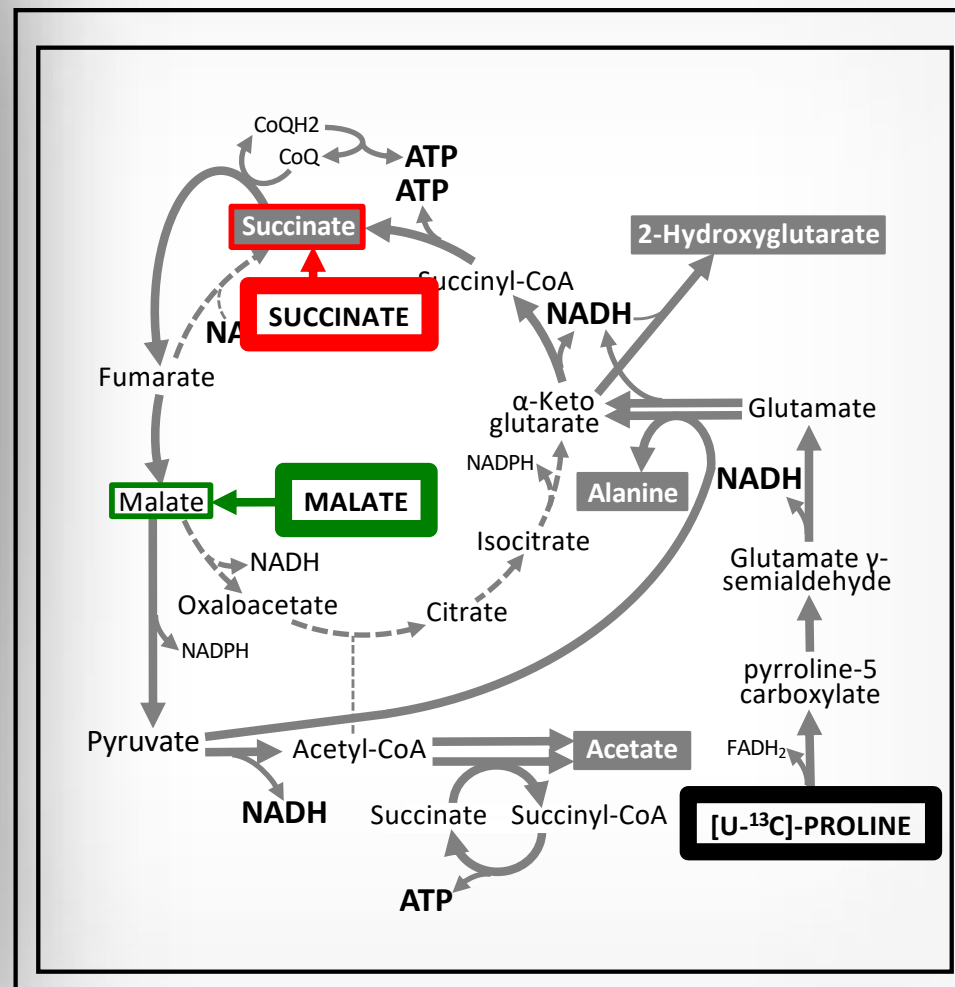
Villafranz *et al.*, 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

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Mitochondrion

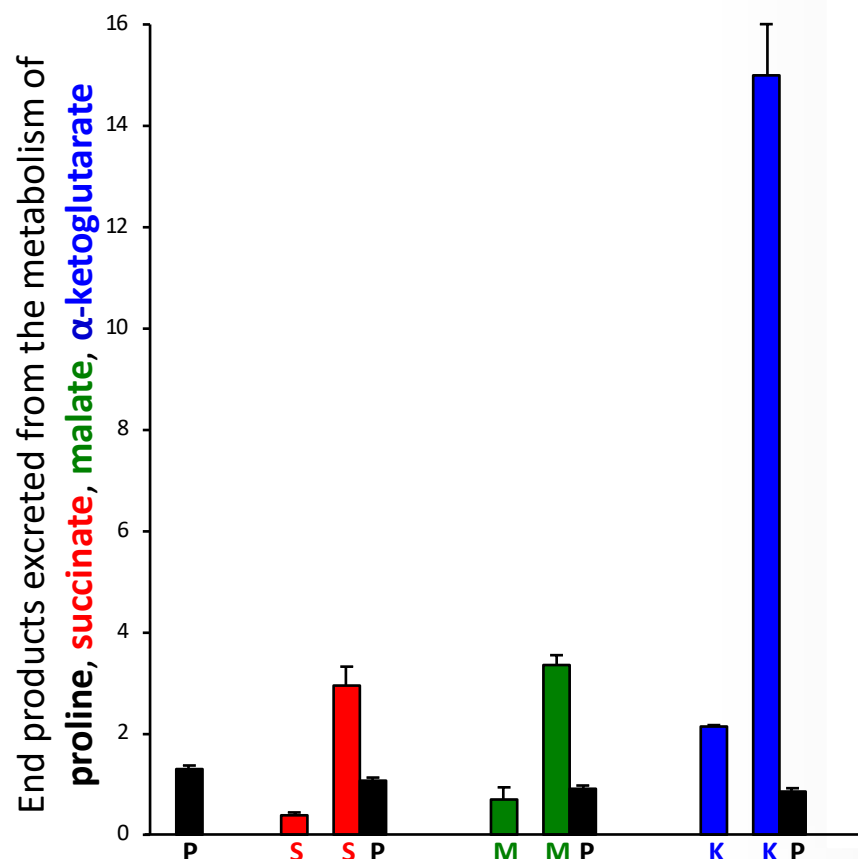


Villafranz et al., 2021, PLoS Pathog

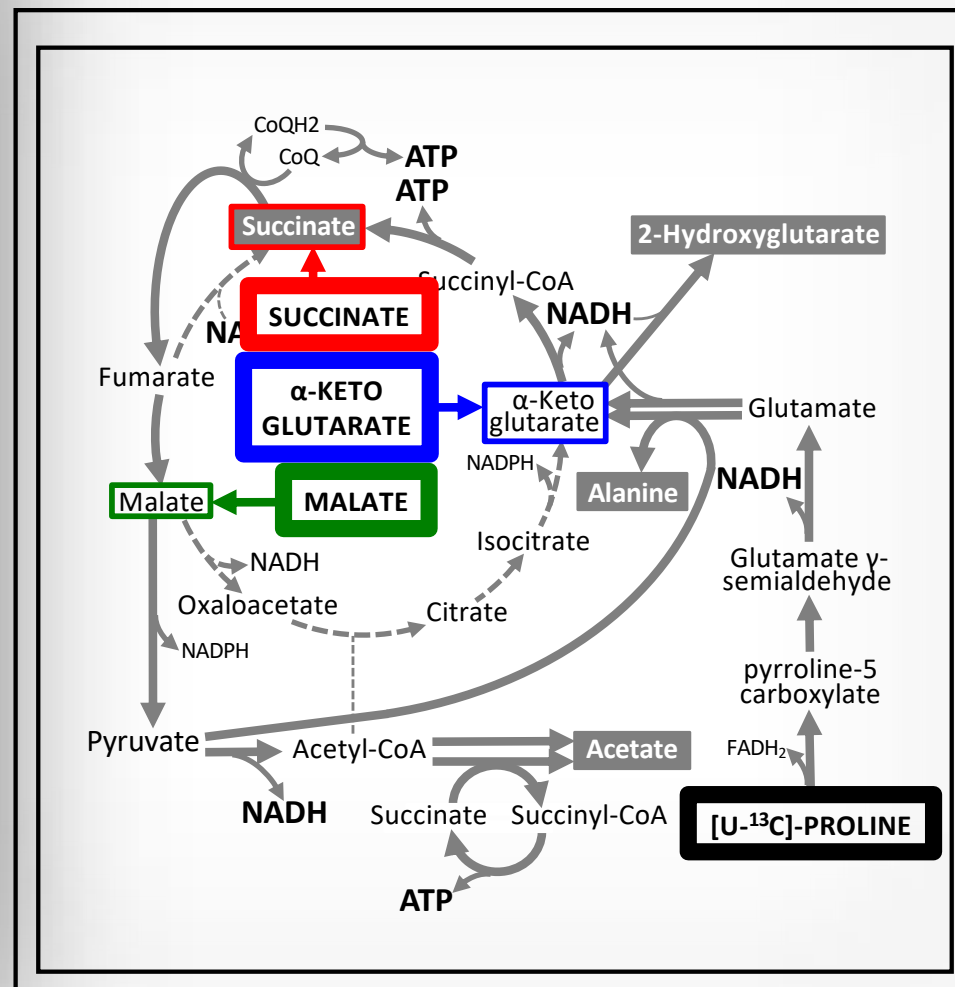
"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

α -ketoglutarate is consumed
20 times faster than proline



Mitochondrion

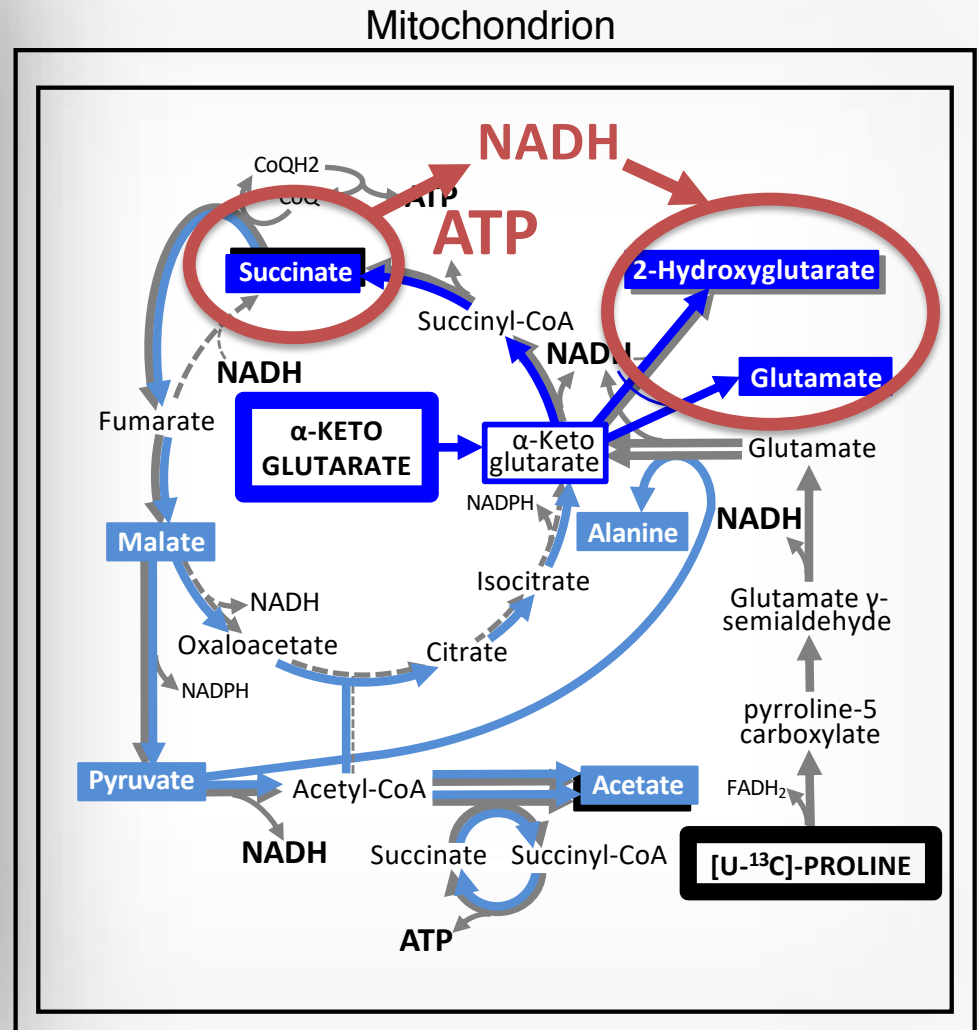
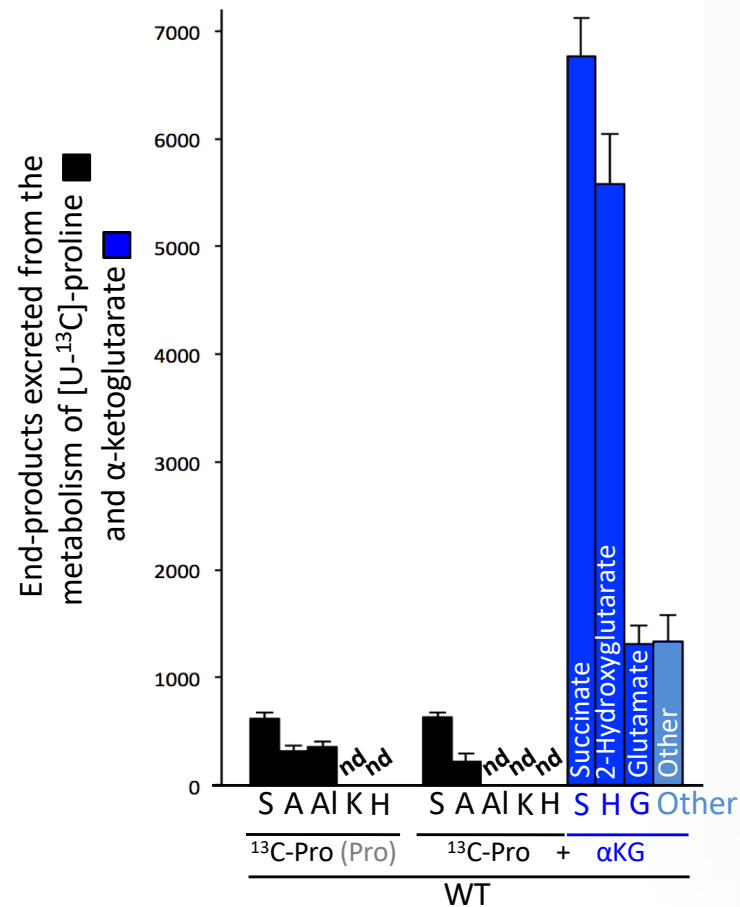


Villafranz et al., 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021

4 mM [U-¹³C]-proline + 4 mM α -Ketoglutarate



Villafranz *et al.*, 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

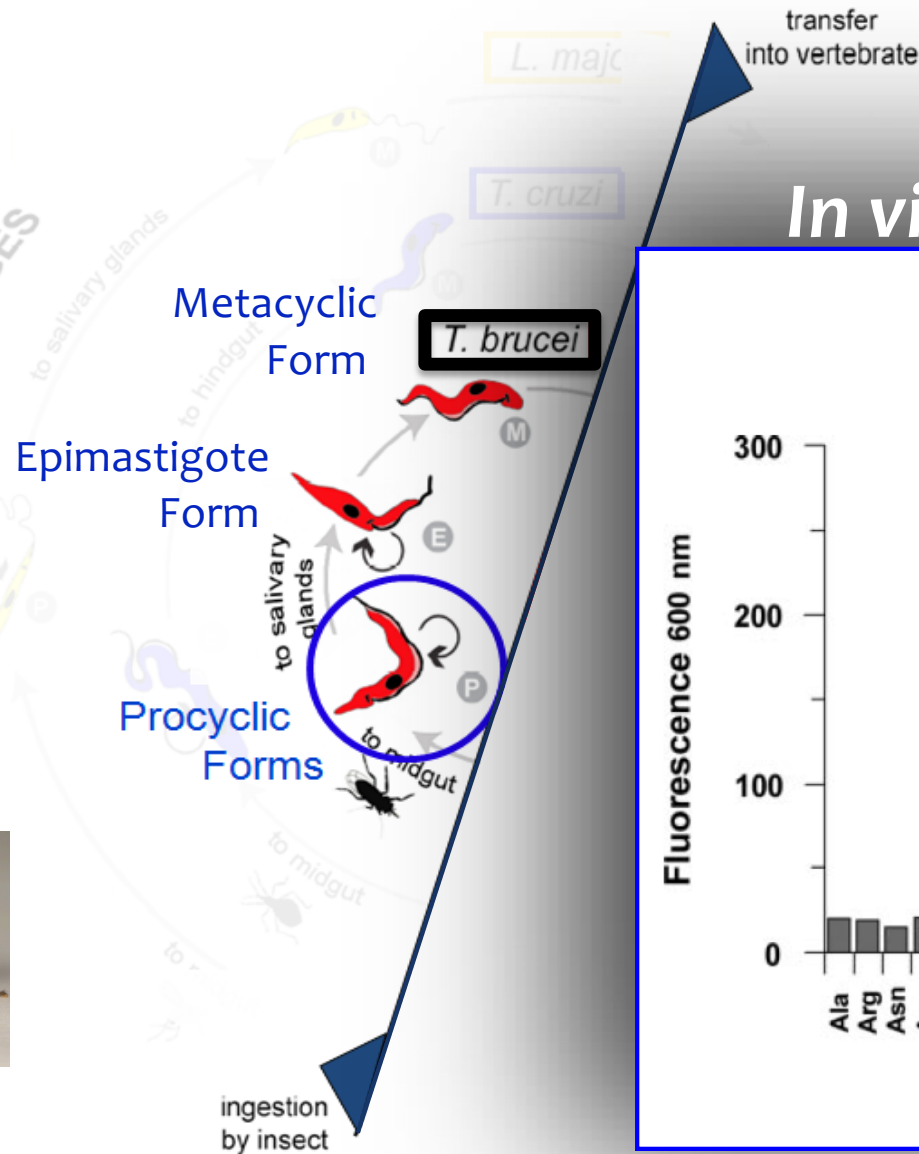
November 18-19, 2021

Other
carbon
Sources ?

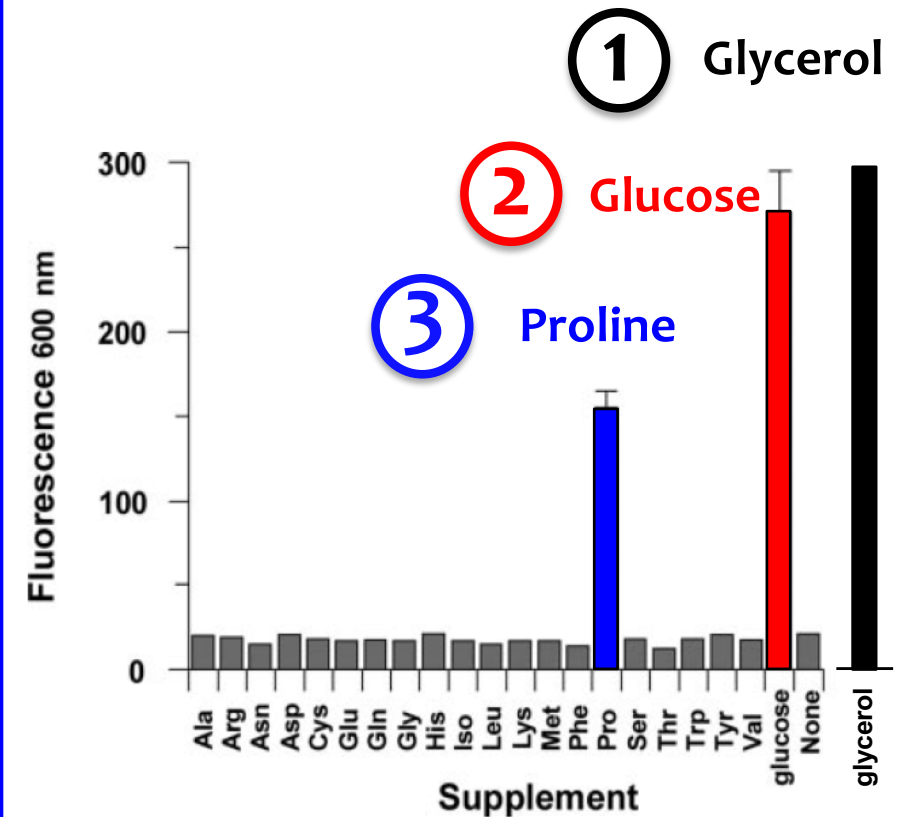
+

Proline
(1-2 mM)

Tsetse fly



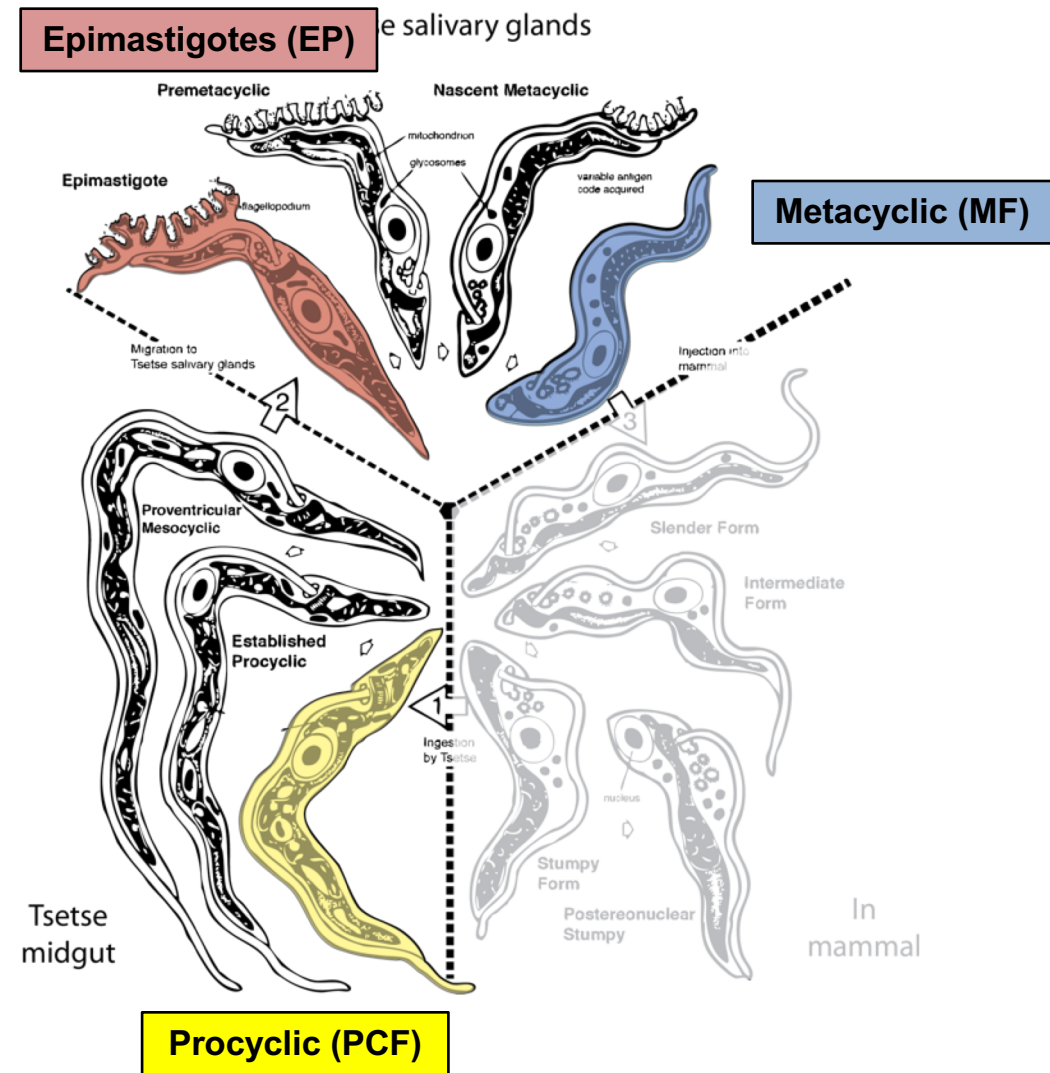
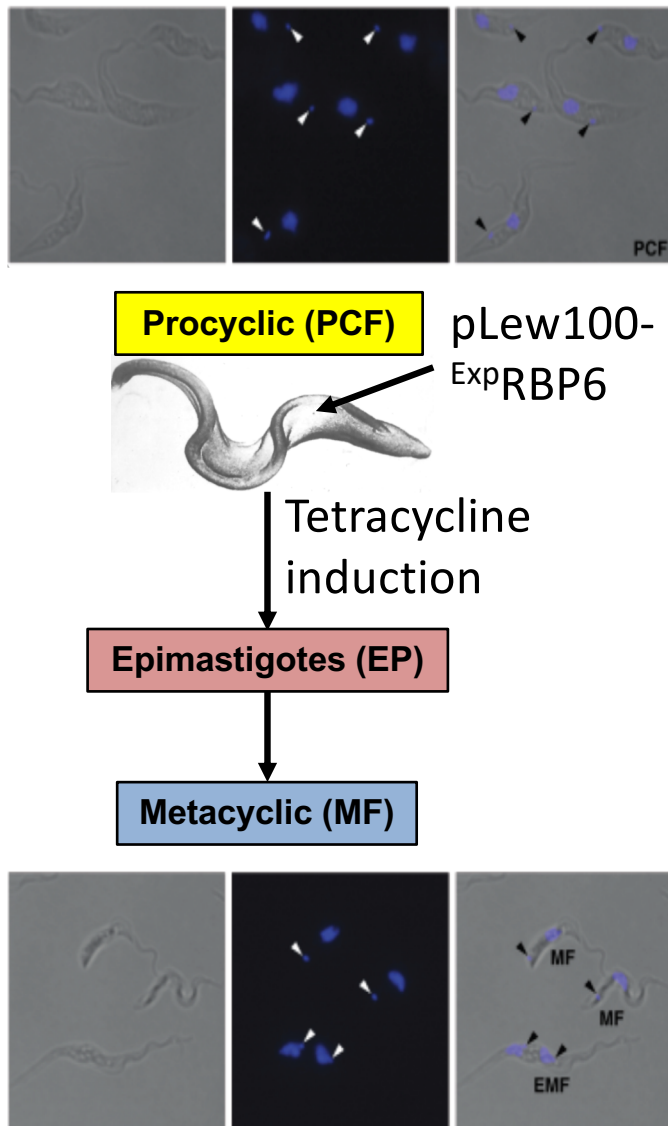
In vitro preference



Villafranz *et al.*, 2021, PLoS Pathog

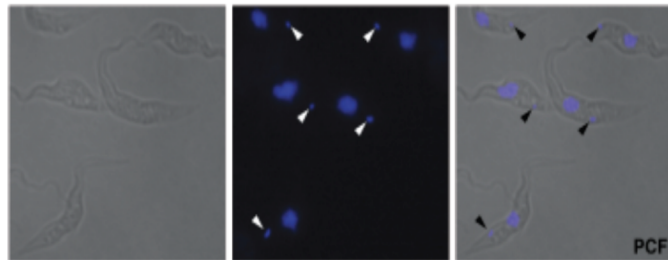
"Metabolism and mathematical models: Two for a tango"

November 18-19, 2021



Kolev et al., 2012, Science

S. Allmann and M. Boshart (Wargnies et al., 2018, PLoS Pathog); Dolezelova et al., 2020, PLoS Biol



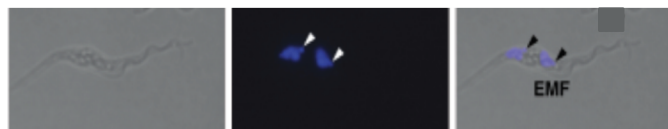
Procyclic (PCF)

pLew100-
ExpRBP6

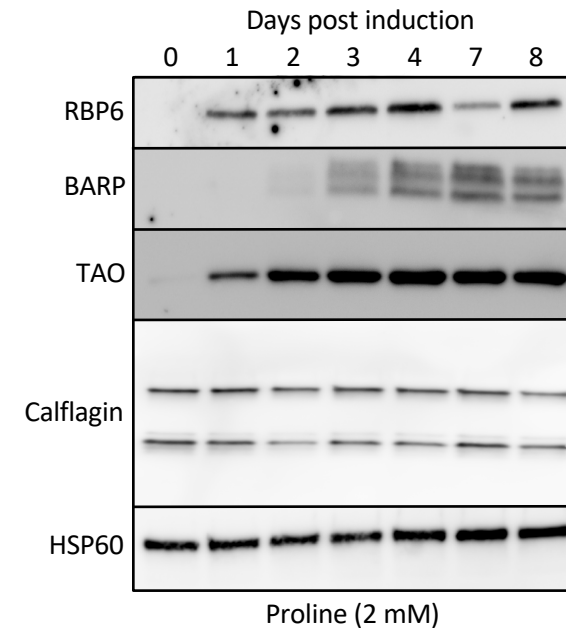
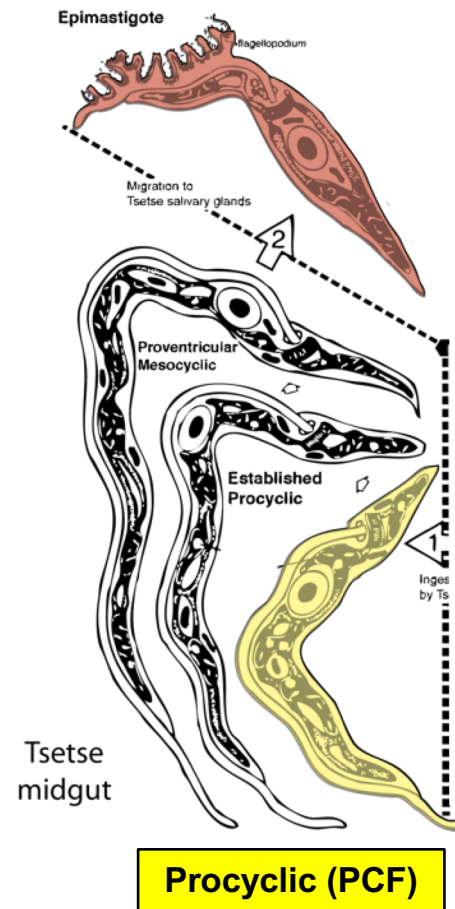


Tetracycline
induction

Epimastigotes (EP)



Epimastigotes (EP)



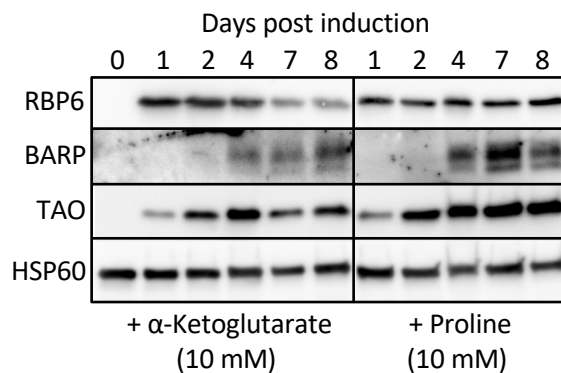
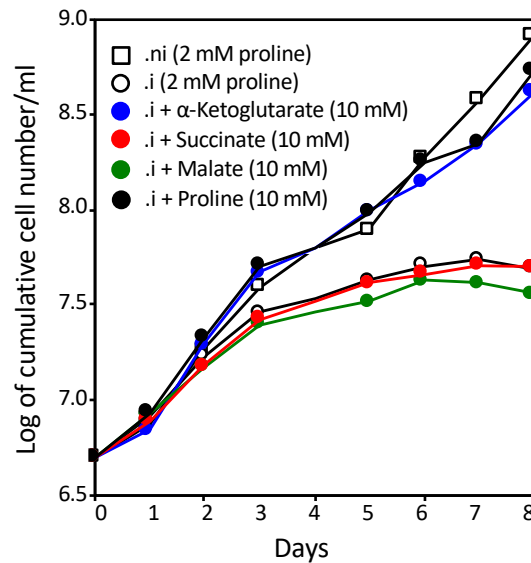
Villafranz et al., 2021, PLoS Pathog

"Metabolism and mathematical models: Two for a tango"

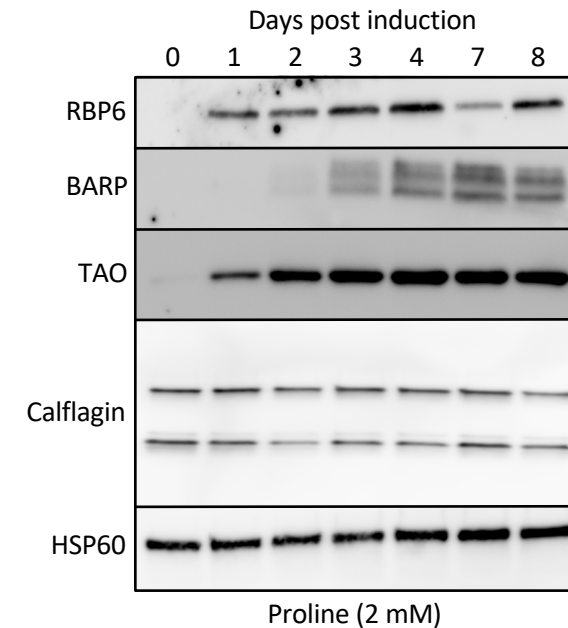
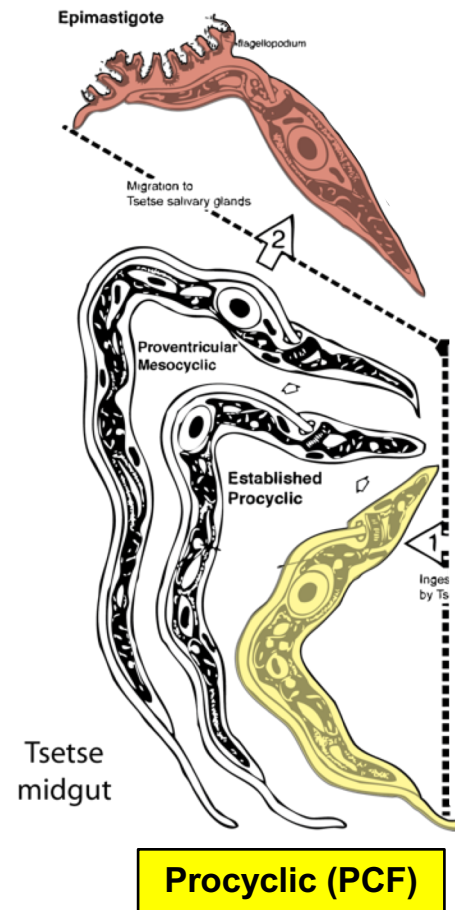
E. Pineda

November 18-19, 2021



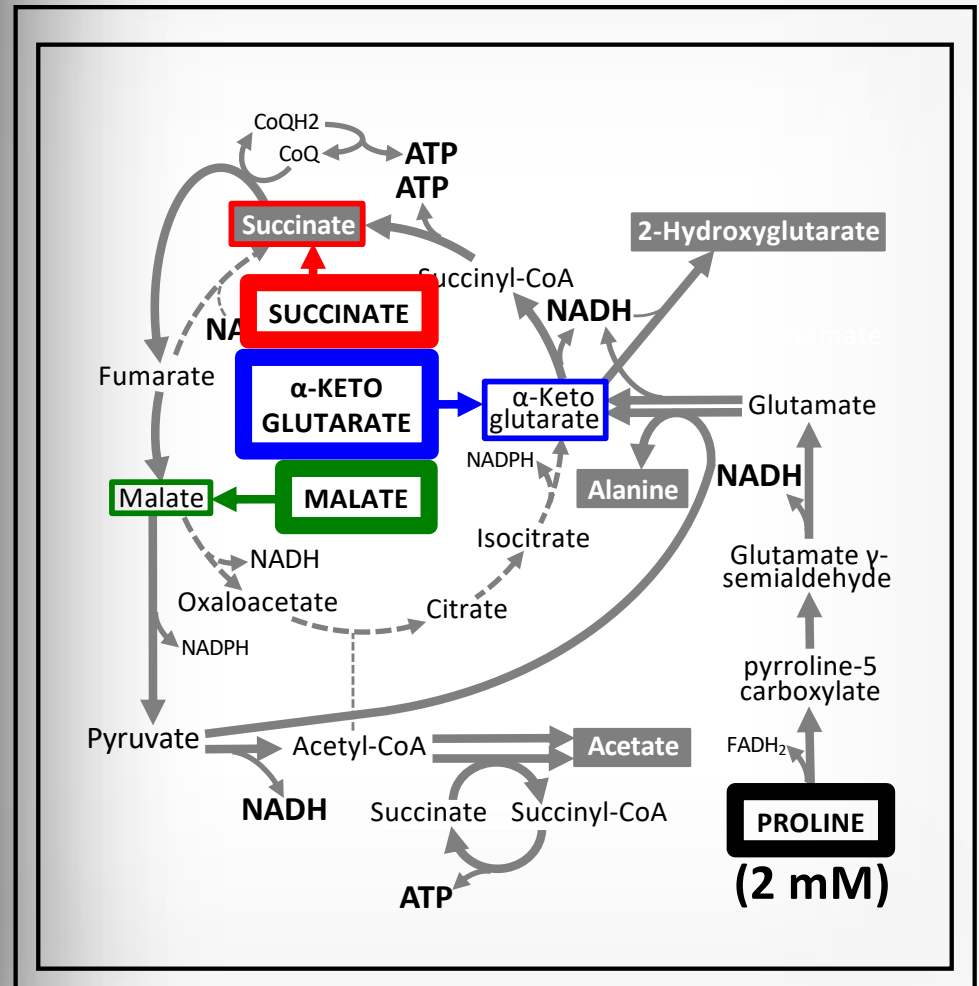


Epimastigotes (EP)



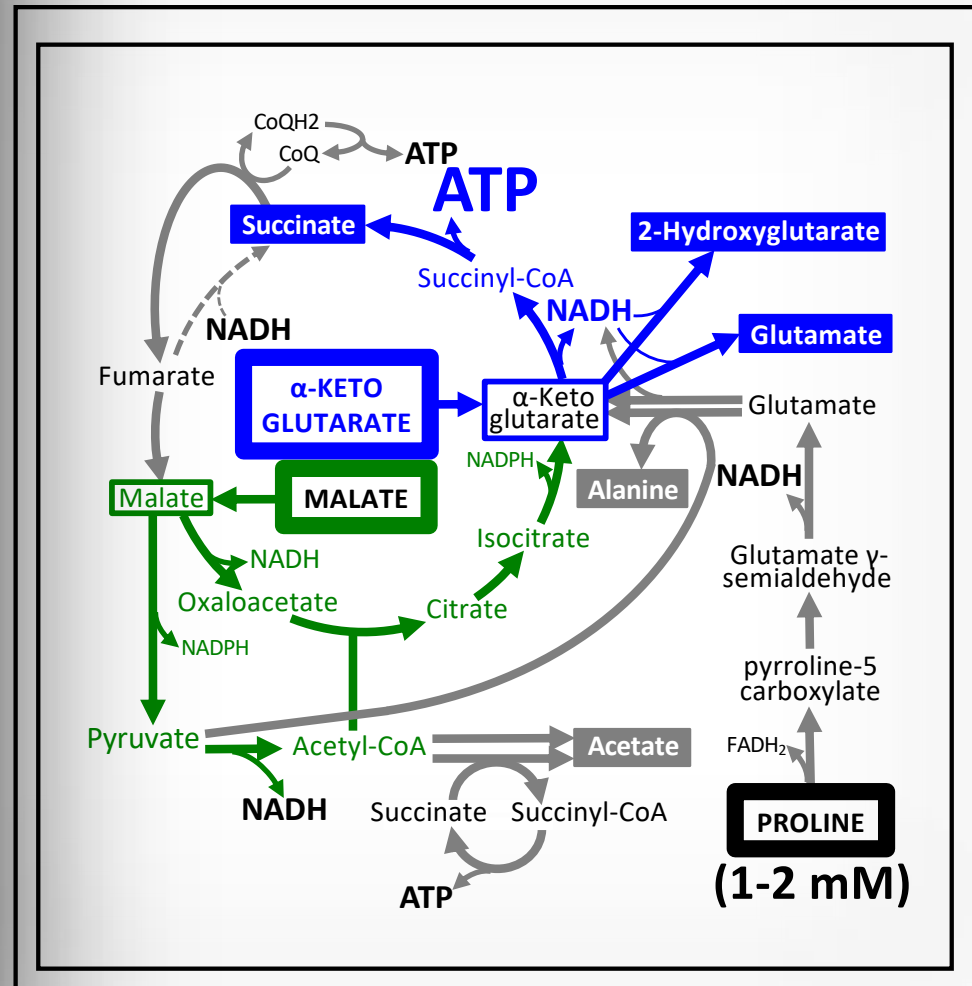
- Growth of procyclics and epimastigotes is stimulated by TCA cycle intermediates, in the presence of physiological amounts of proline (1-2 mM)
- ➔ **Proline is probably not the only carbon source used**

Mitochondrion



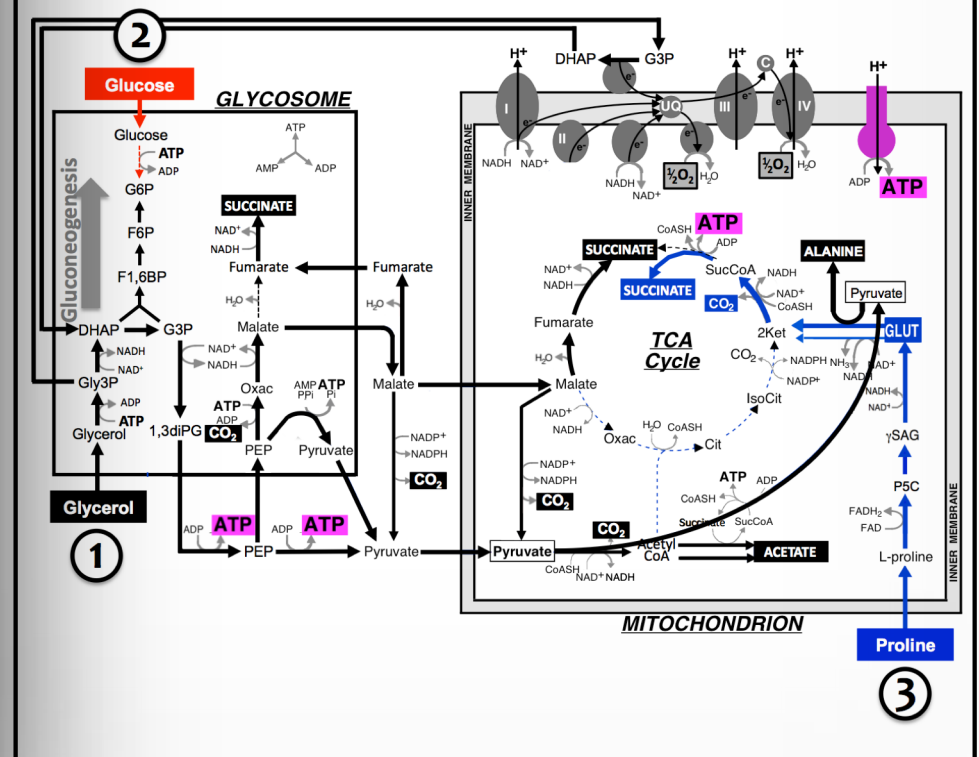
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- The TCA cycle is fully functional and has a high metabolic capacity
- ➔ **PCF have the ability to play with ATP production depending on the availability of carbon source**

Mitochondrion



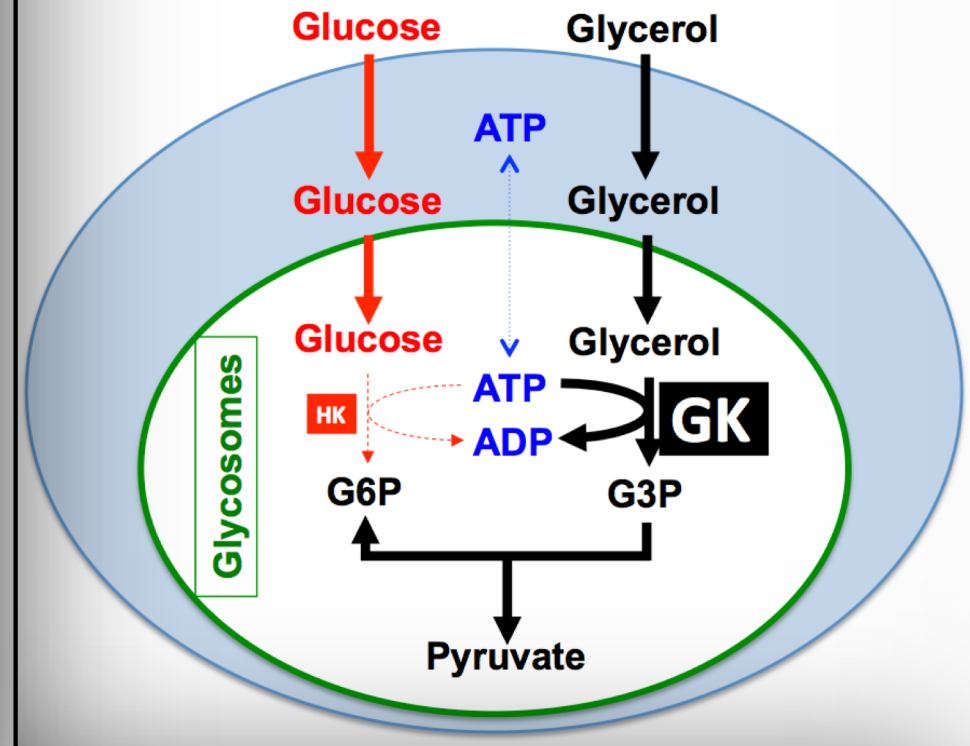
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- PCF prefers glycerol over glucose and glucose over proline, although proline is the main carbon source used *in vivo*
 - ➔ **What is the role of glucose and glycerol metabolism for the parasite *in vivo* ?**

Carbon source preference: Glyc >> Glc >> Pro



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 - ➔ **What is the role of glucose and glycerol metabolism for the parasite *in vivo* ?**
- *Trypanosoma brucei* is the only known microorganism performing gluconeogenesis in the presence of glucose
 - ➔ **Metabolic contest could be performed by any organism provided that ...**
 - ➔ **What is the role of glycerol preference for the parasite *in vivo* ?**

Metabolic contest: Glyc >> Glc





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