Systems Biology to Investigate Biochemical Pathways for Metabolic Functions *in vivo*

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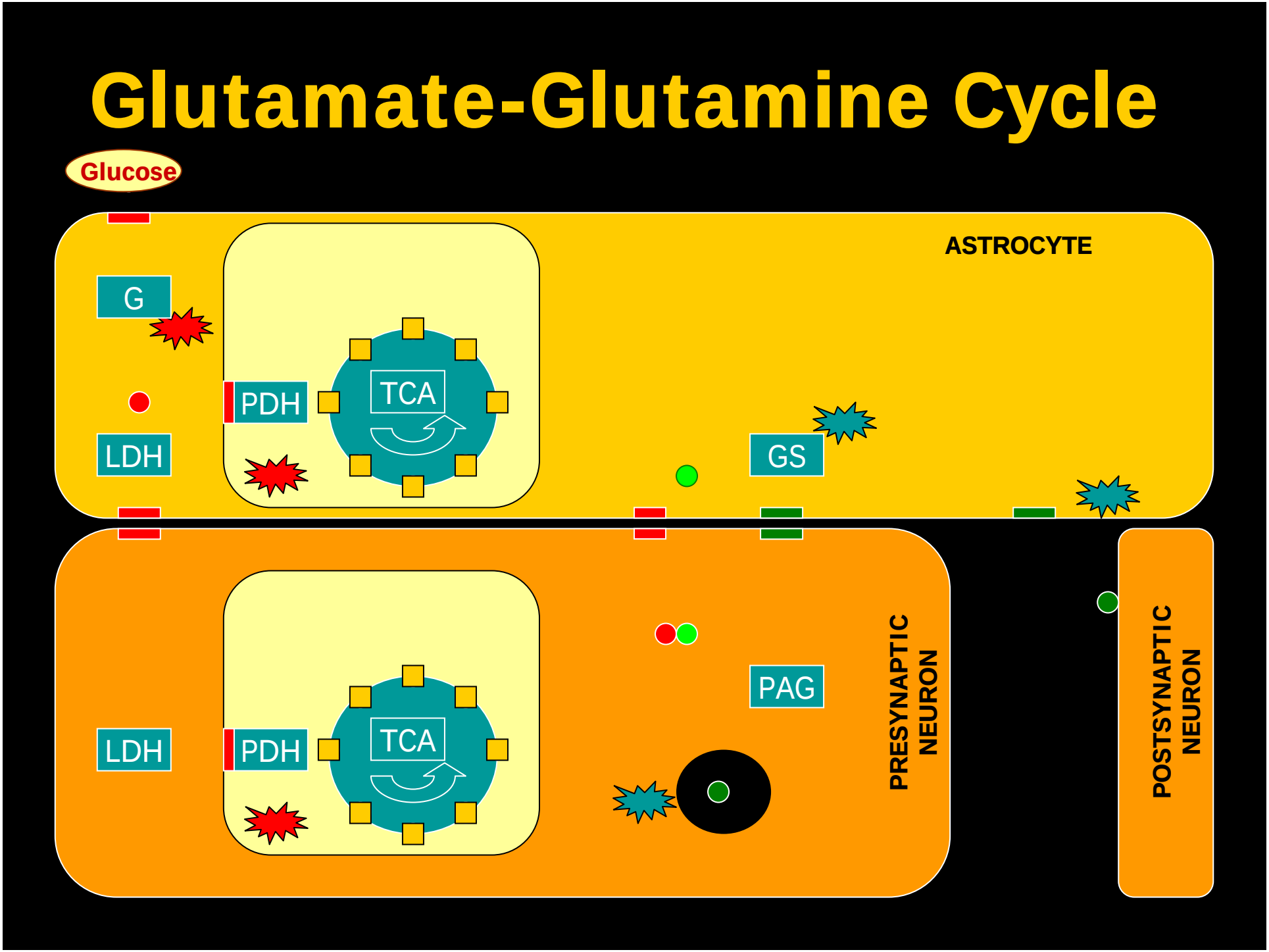
Motivations

- Broad Aim
  - To understand the biochemical processes that sustain metabolic/physiological functions
- Narrow Aim
  - To understand the biochemical processes that sustain neurotransmitter trafficking in brain

Glutamate-Glutamine Cycle



Approach

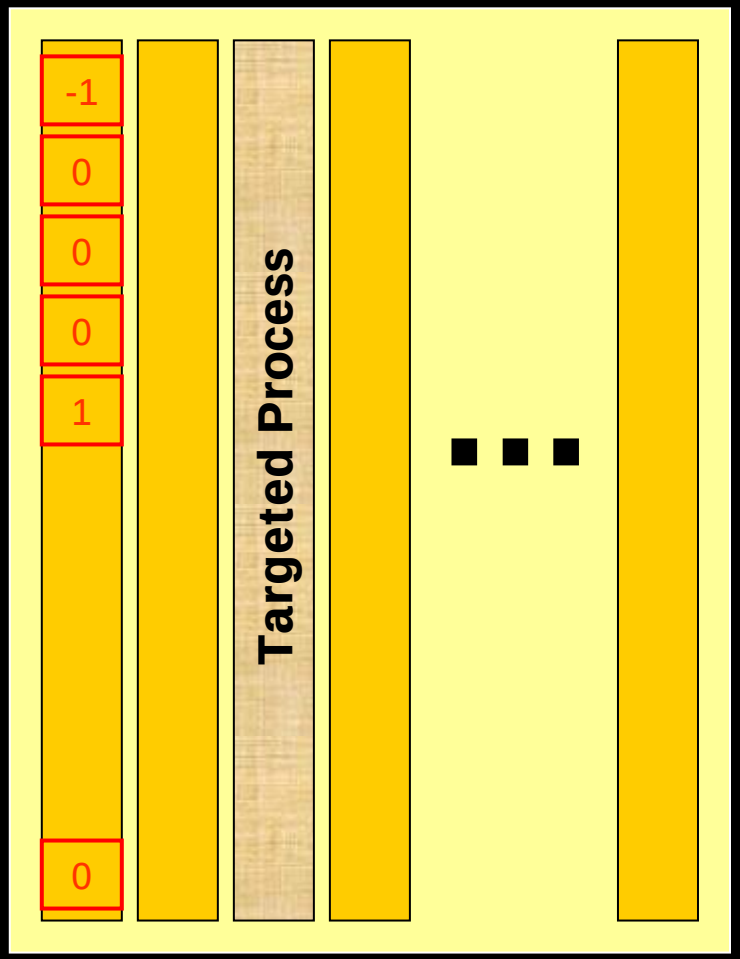
- Use a variant of metabolic flux analysis to construct possible metabolic cycles that sustain a specific physiological function

- Properties of metabolic cycles
  - Operate at steady state
  - Consume a basic energy substrate
  - Execute a metabolic process that corresponds to a physiological function

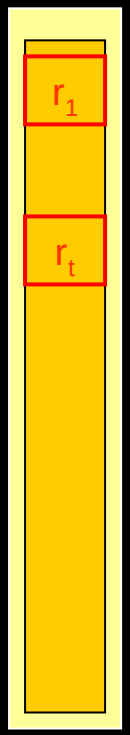
Basic Ideas

- Metabolic enzymes and metabolite carrier proteins represent metabolic processes
- Metabolic processes can be connected by flows of metabolites
- Some combinations of connected metabolic processes constitute metabolic cycles that execute a specific metabolic process corresponding to a specific physiological function

Mathematical Formulation



Process Stoichiometry Matrix (P)



Process Rate Vector (r)

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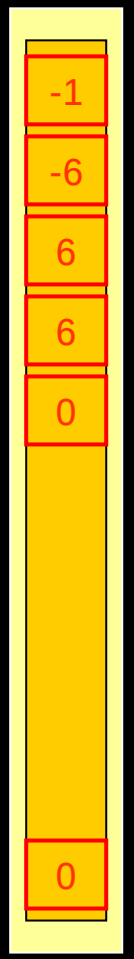
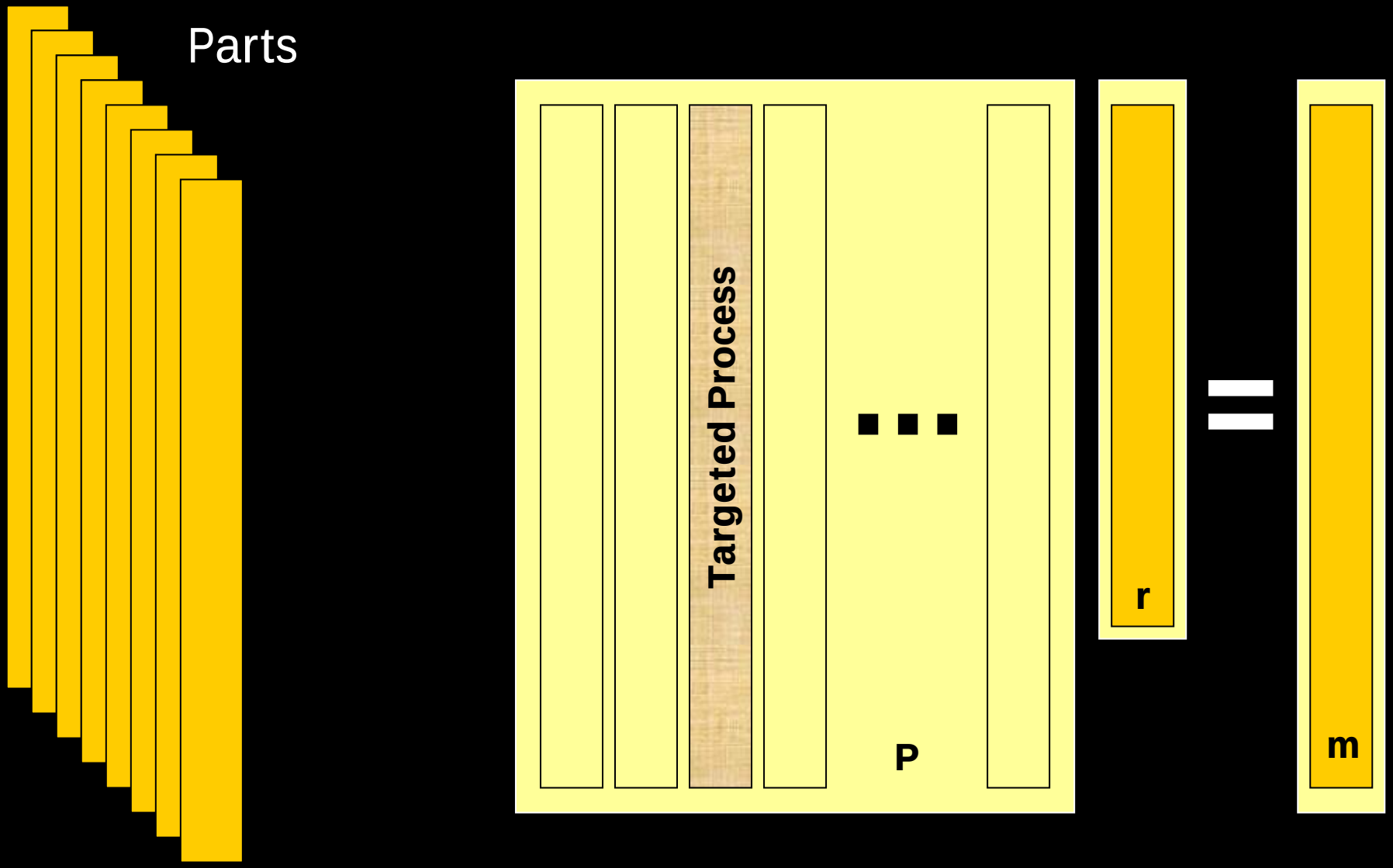


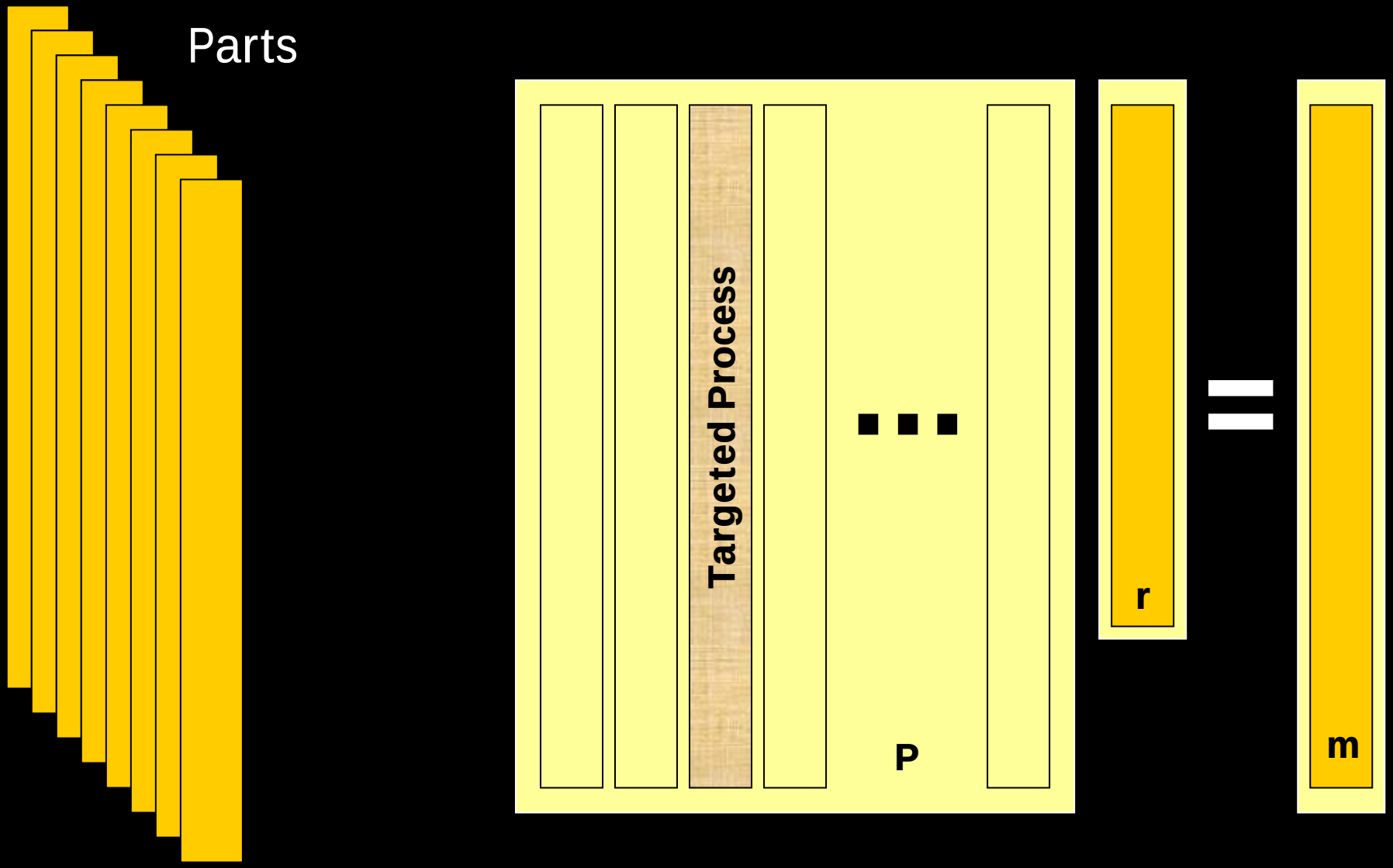
Metabolite Production Vector (m)

xGluc
O_2
CO_2
H_2O
cGluc

Procedure



Procedure



Issues

- There are many possible sets of processes that include the targeted process of interest
- Typically, sets of processes contain more metabolites (rows) than processes (columns) – existence of a solution is not guaranteed
- Even if there is a mathematical solution, that solution may not be physiologically meaningful (e.g., if it includes irreversible processes operating in the wrong direction)

Glutamate Trafficking in Synaptic Neurotransmission

- *in vivo* MRS carbon labeling experiments for estimating neurotransmitter trafficking depend on a priori specifications of metabolic pathways
- Metabolic pathways for neurotransmitter trafficking *in vivo* are not known a priori
- Neurotransmitter trafficking *in vivo* may be accomplished by multiple, functionally redundant pathways

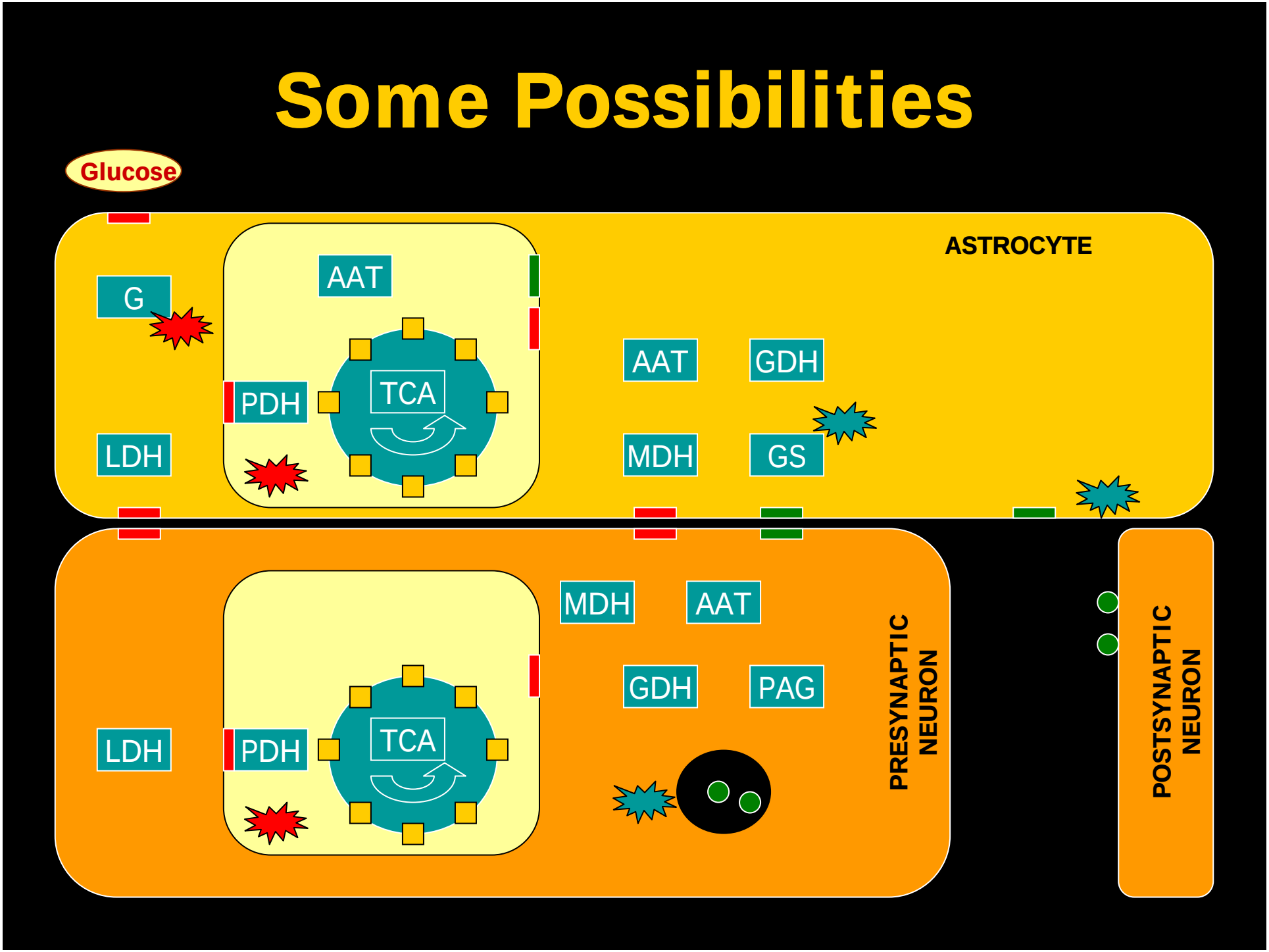
Parts Lists

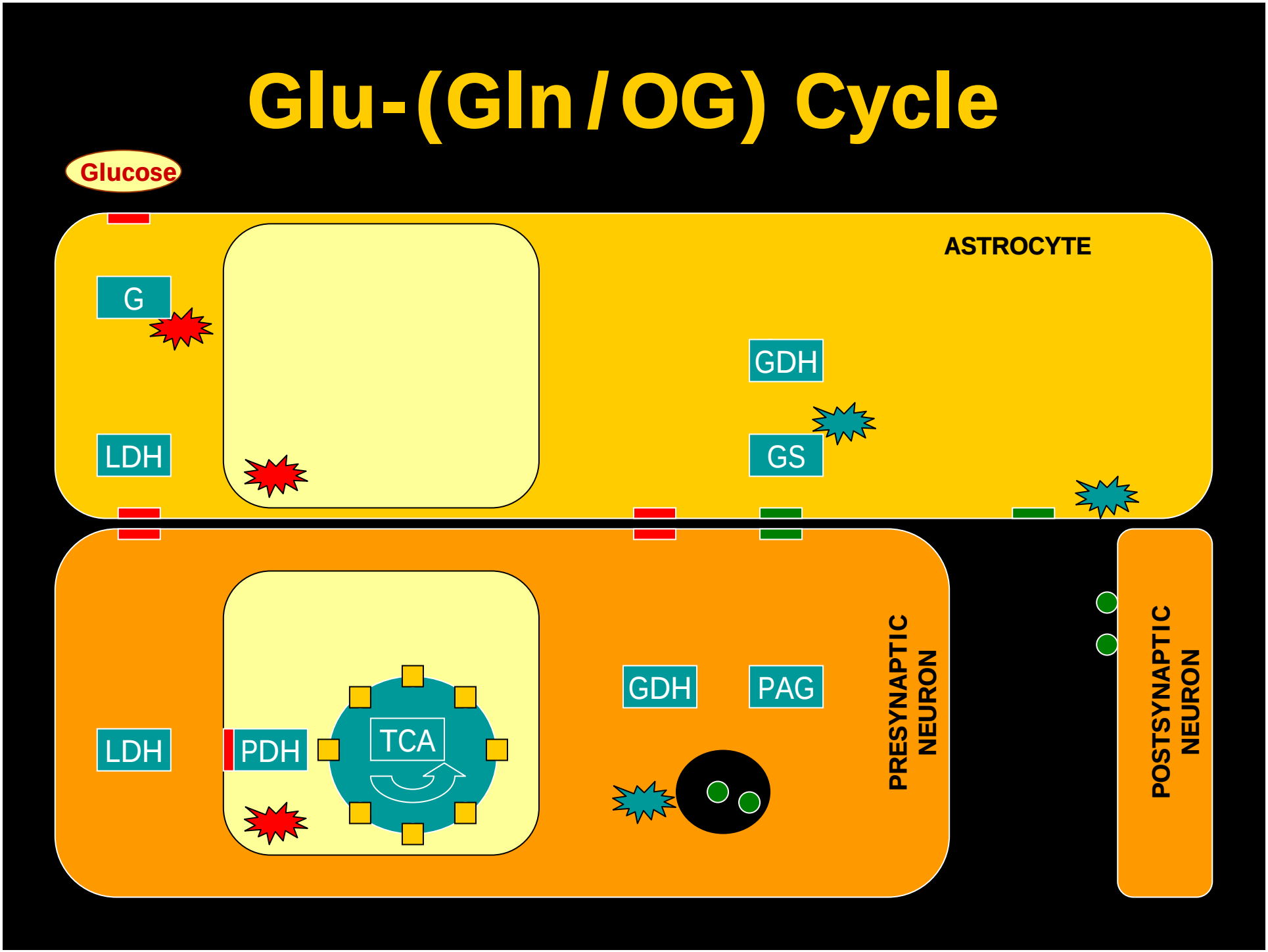
Reaction	PN	A
cLDH	√	√
cGS		√
cPAG	√	
cME		√
cGDH	√	√
cAAT	√	√
cALAT	√	√
cBCAT	√	√
cN	√	√
mPDH	√	√
mCS	√	√
mAH	√	√
mIDH	√	√
mOGDH	√	√
mSCS	√	√
mSDH	√	√
mFH	√	√
mMDH	√	√
mNDK	√	√
mAAT	√	√

Transport	PN	A
pmGLUT(Gluc)	√	√
pmGLAST(Glu)		√
pmSNAT3(Gln)		√
pmSNAT1(Gln)	√	
pmSNAT1(Asp)	√	√
pmSNAT1(Ala)	√	√
pmSBAT1(BCAA)	√	√
pmMCT(BCKA)	√	√
pmMCT(Lac)	√	√
pmNaCT(OG)	√	√
pmNaCT(Mal)	√	√
pmNaCT(Cit)	√	√
pmKCNK	√	√
pmNHE	√	√
mmMCT(Pyr)	√	√
mmP_iC	√	√
mmAAC	√	√
mmOGC	√	√
mmCIC	√	√
mmAGC	√	√

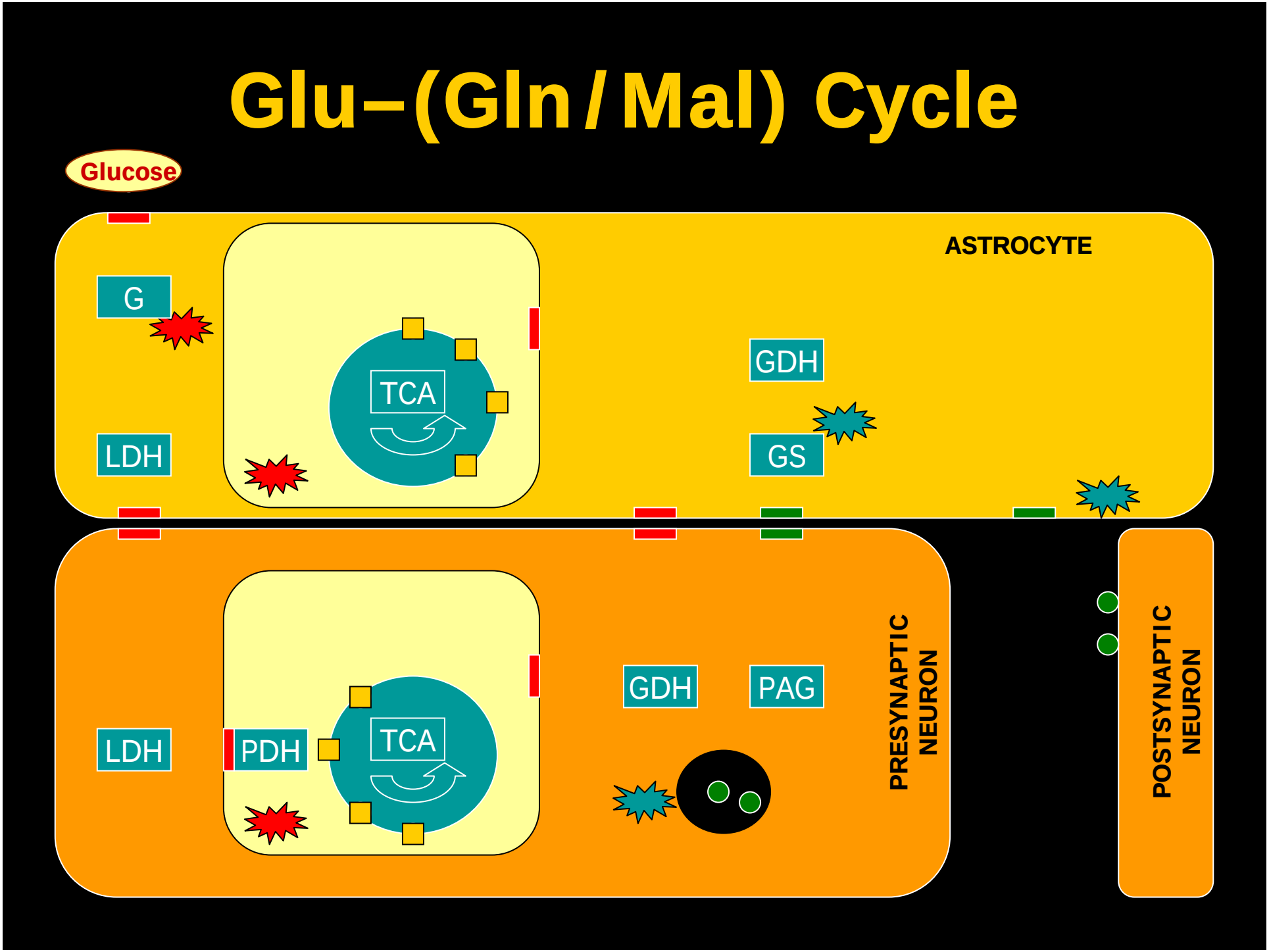
Composite	PN	A
cG	√	√
MAS	√	√
ET1	√	√
ET2	√	√
mmATPase	√	√
cATPase	√	√
pmNaKATPase	√	√
pmExoATPase	√	

Some Possibilities

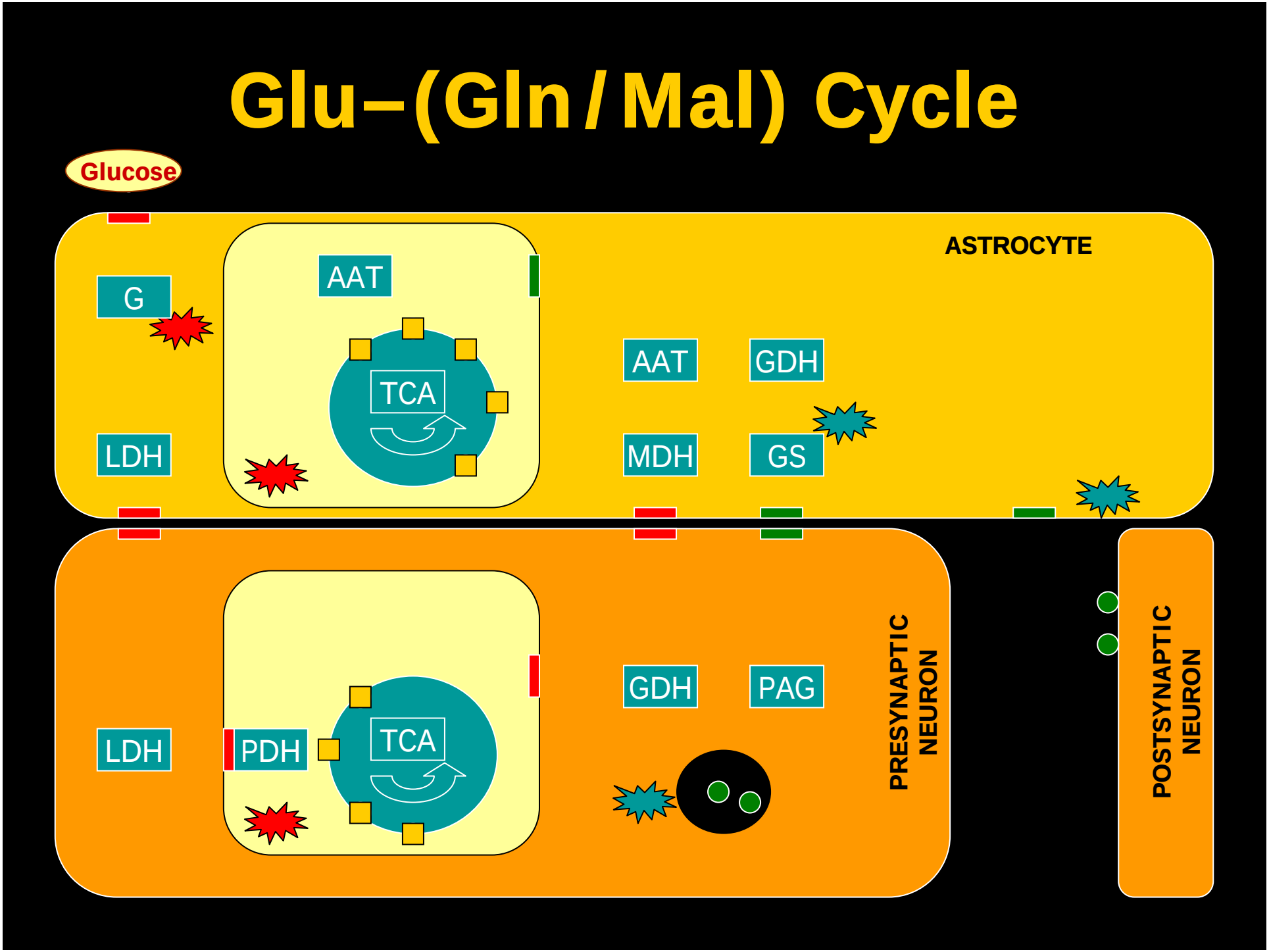



Glu-(Gln/OG) Cycle
Glucose
ASTROCYTE
G
LDH
GDH
GS
LDH
PDH
TCA
GDH
PAG
PRESYNAPTIC NEURON
POSTSYNAPTIC NEURON

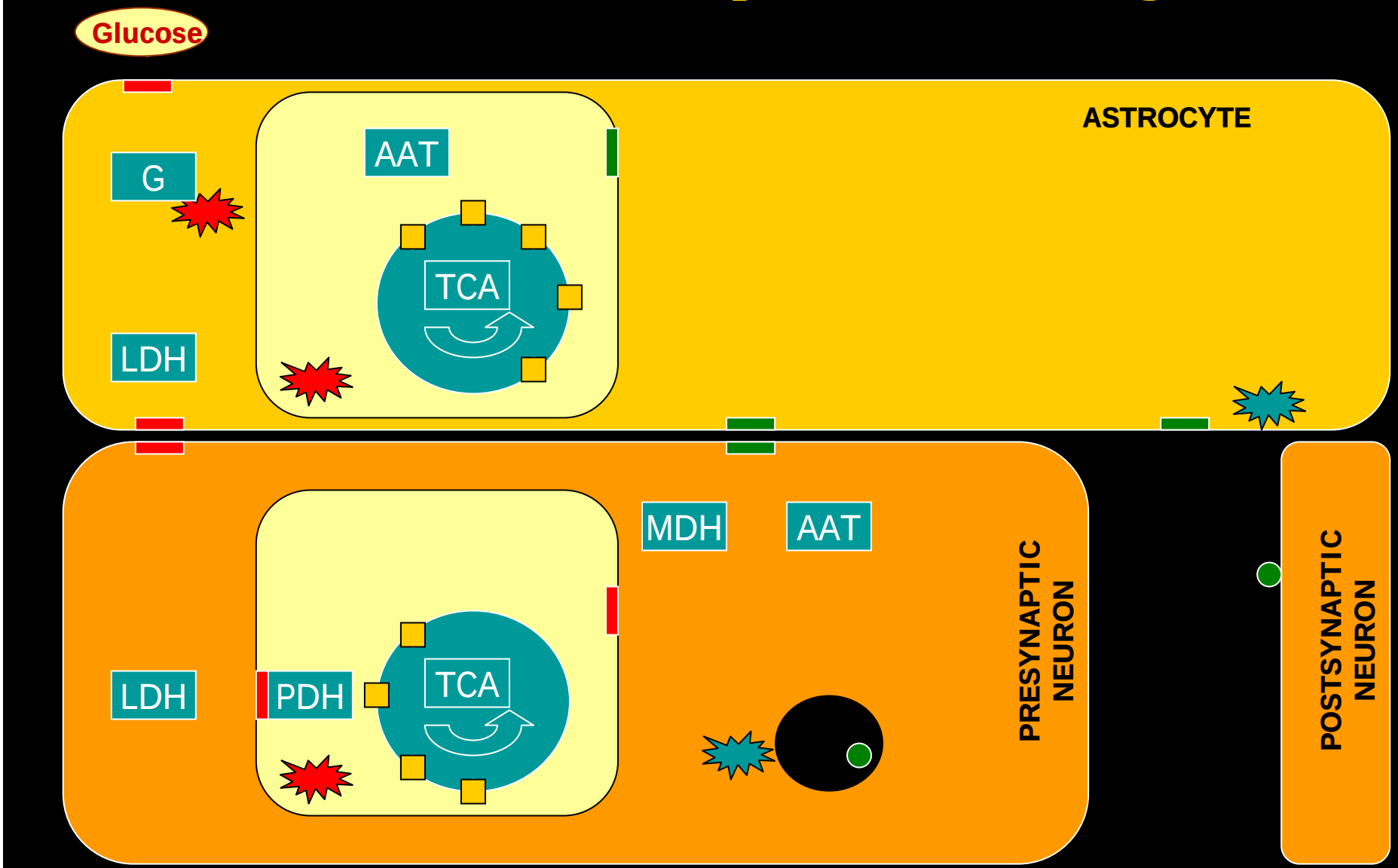
Glu–(Gln / Mal) Cycle



Glu–(Gln / Mal) Cycle



Glutamate-Aspartate Cycle



Relative Process Rates
Glu-(Gln/Mal) Cycle

Reaction	PN	A
cLDH	1/2	-1/2
cGS	N/A	1/2
cPAG	1/2	N/A
cGDH	-1/2	1/2
mPDH	1/2	0
mCS	1/2	0
mAH	1/2	0
mIDH	1/2	0
mOGDH	0	1/2
mSCS	0	1/2
mSDH	0	1/2
mFH	0	1/2
mMDH	1/2	0
mNDK	0	1/2

Transport	PN	A
pmGLUT(Gluc)	0	1/4
pmGLAST(Glu)	N/A	1
pmSNAT3(Gln)	N/A	1/2
pmSNAT1(Gln)	-1/2	N/A
pmMCT(Lac)	-1/2	1/2
pmNaCT(Mal)	-1/2	1/2
pmKCNK	1	1/3
pmNHE	-1	1 1/2
mmMCT(Pyr)	1/2	0
mmPiC	3 3/4	3 5/8
mmAAC	3 3/4	3 5/8
mmOGC	1/2	-1/2

Composite	PN	A
cG	0	1/4
MAS	0	1/2
ET1	1 1/2	1
ET2	0	1/2
mmATPase	3 3/4	3 1/8
cATPase	1 1/4	2 23/24
pmNaKATPase	1/2	2/3
pmExoATPase	1	N/A

Relative Production Rates Glu-(Gln/Mal) Cycle

Molecule	PN	A	Net
xGlucose	0	$-1/4$	$-1/4$
dO_2	$-3/4$	$-3/4$	$-1\,1/2$
dCO_2	1	$1/2$	$1\,1/2$
dH_2O	1	$1/2$	$1\,1/2$
xLactate	$-1/2$	$1/2$	0
xMalate	$-1/2$	$1/2$	0
xGlutamate	1	-1	0
xGlutamine	$-1/2$	$1/2$	0
xH^+	$-1/2$	$1/2$	0

Intended Applications

- Design and interpretation of carbon labeling experiments
  - *in vivo* MRS of brain energy metabolism and neurotransmitter trafficking
- Further systems-theoretical investigations of alternative cycles
  - Metabolic Control Analysis


CK THIS OUT... THE
ROWAVE'S TURNING
FOOD ONE WAY,
T? WELL, NOW I
SE IT, AND RUN
AGAIN AND...
...NOW THE FOOD
IS TURNING THE
OTHER WAY!!
whoa...