The Petri Net Model of the Sucrose-to-Starch Breakdown in the potato tuber

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Outline

- Introduction

- Sucrose-to-starch breakdown in the potato tuber

- The Petri net model

- Qualitative analysis

- Simulation of the net

- Conclusions

Introduction

Cooperation: Björn Junker, Max Planck Institute for Molecular Plant Physiology, Golm

Situation before starting kinetic modelling:

incomplete kinetic data

literature search

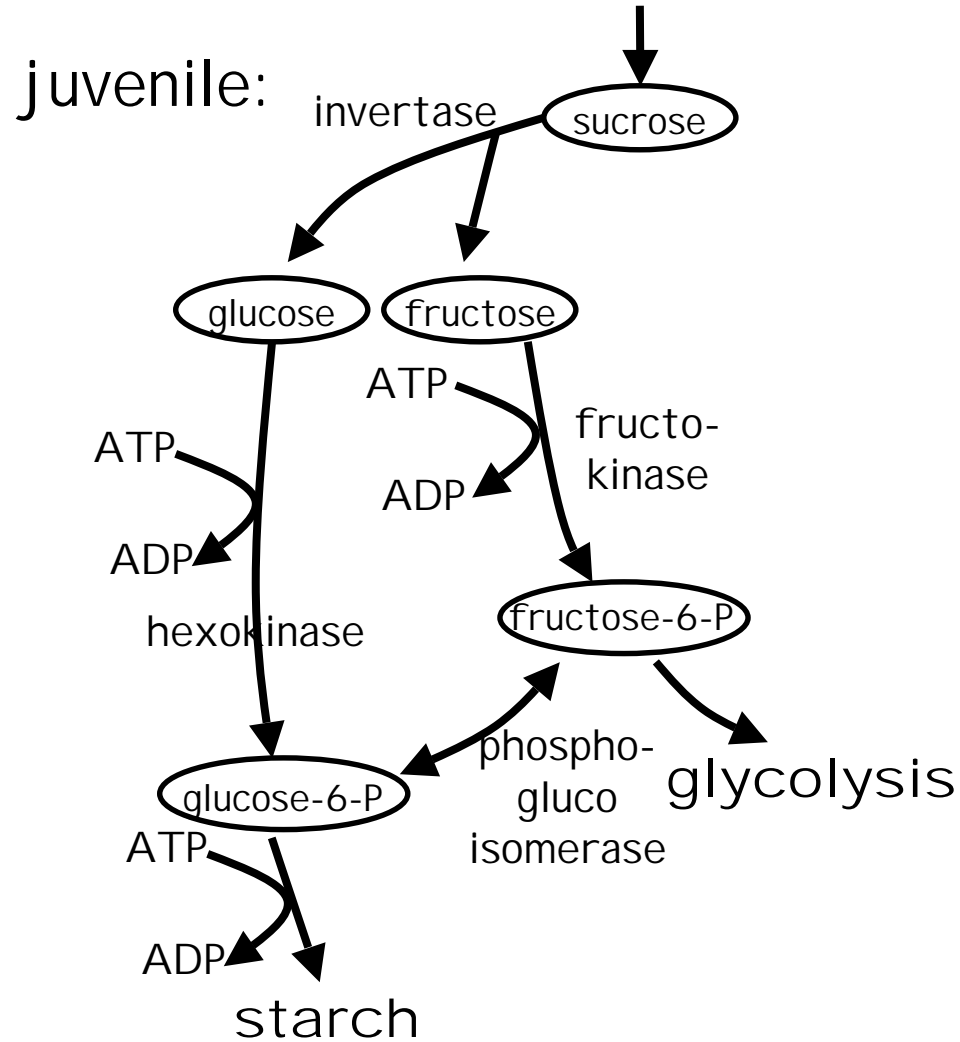try-and-error-technique to find the steady state

using GEPASI   Mendes, *Comp.Appl.Biosci.* (1993)

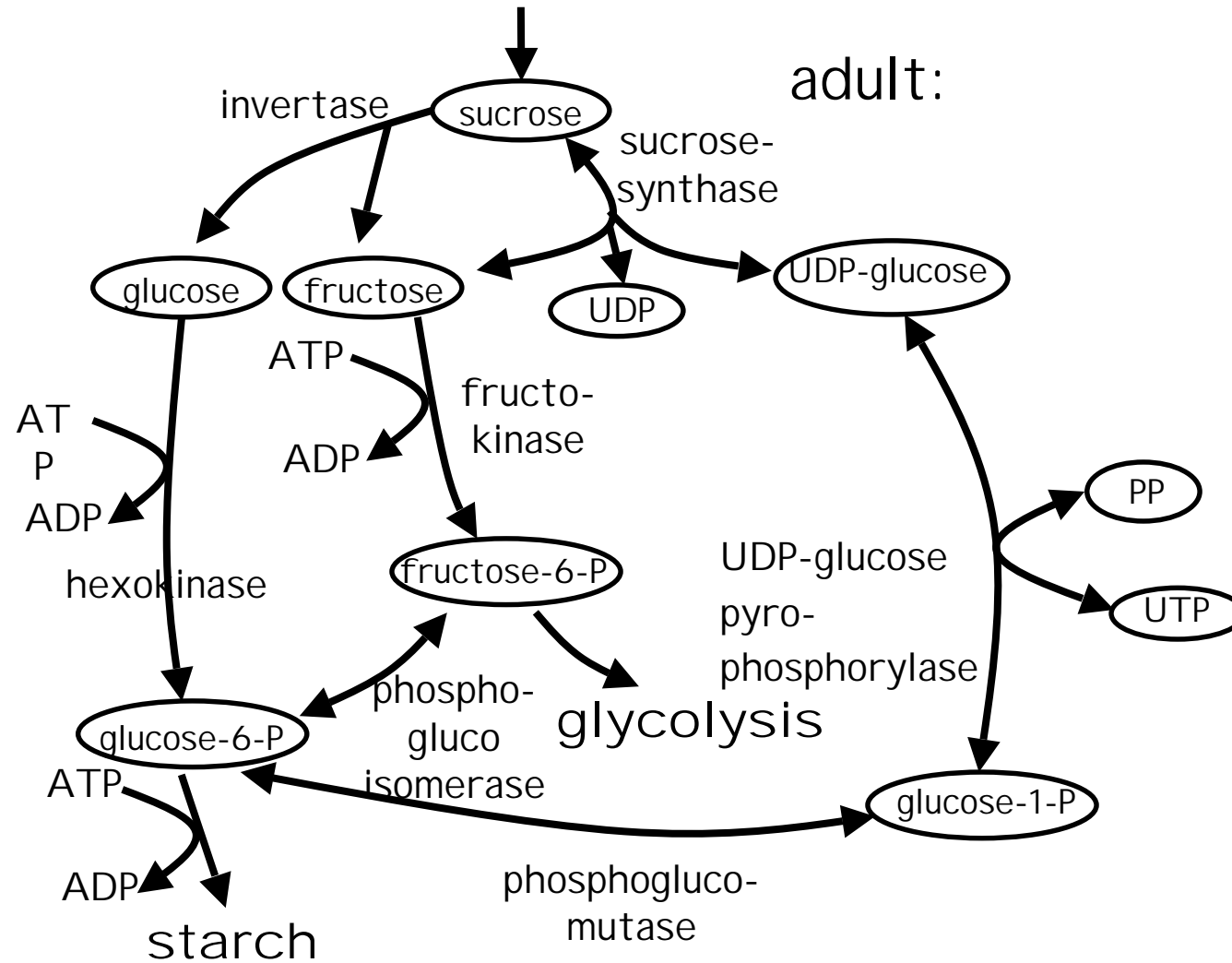Qualitative modelling as the first step

Basic dynamic properties: liveness, reversibility, boundedness,

dead states, deadlocks, traps

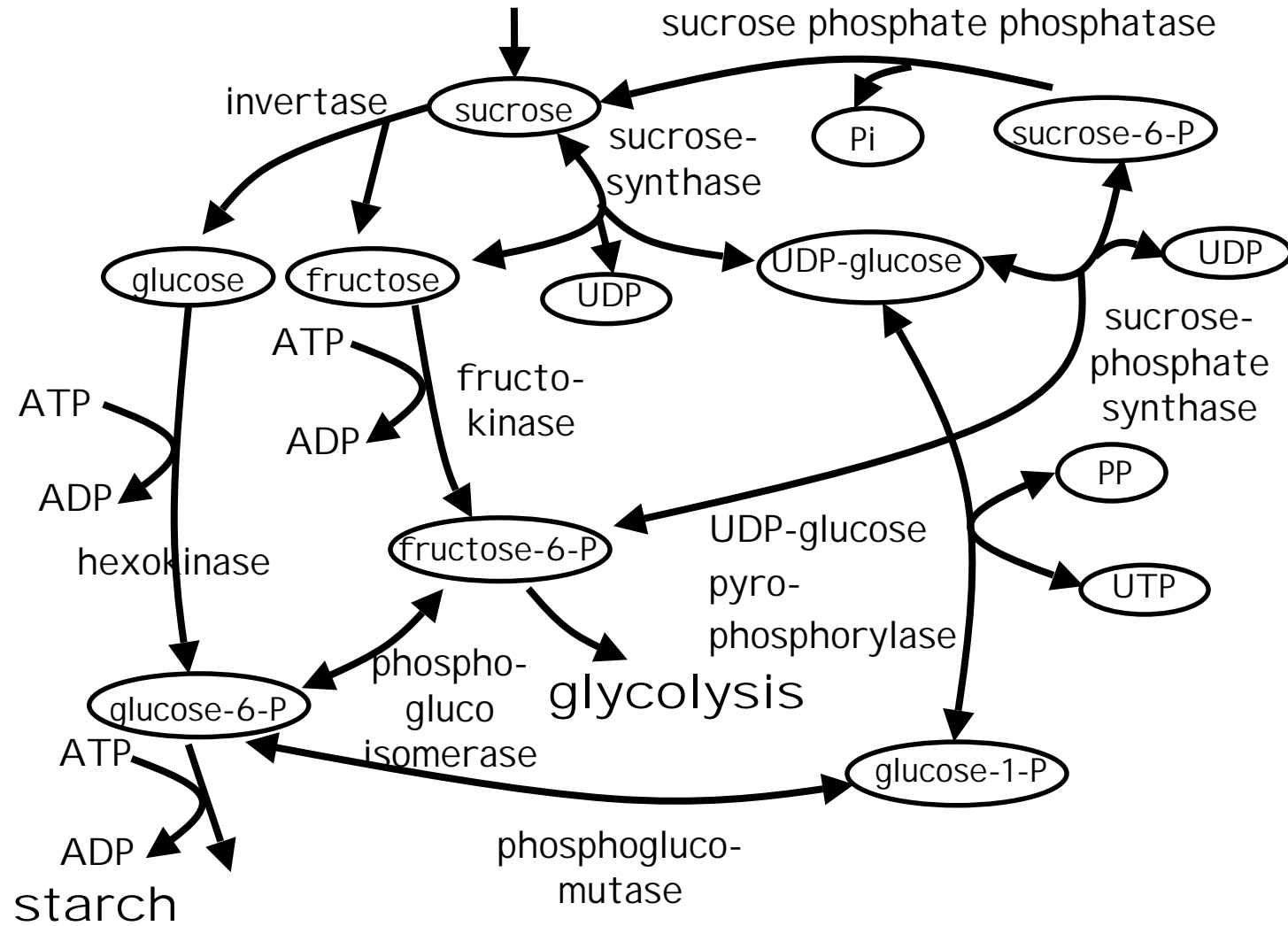Basic structure properties: invariants, robustness, alternative pathways,

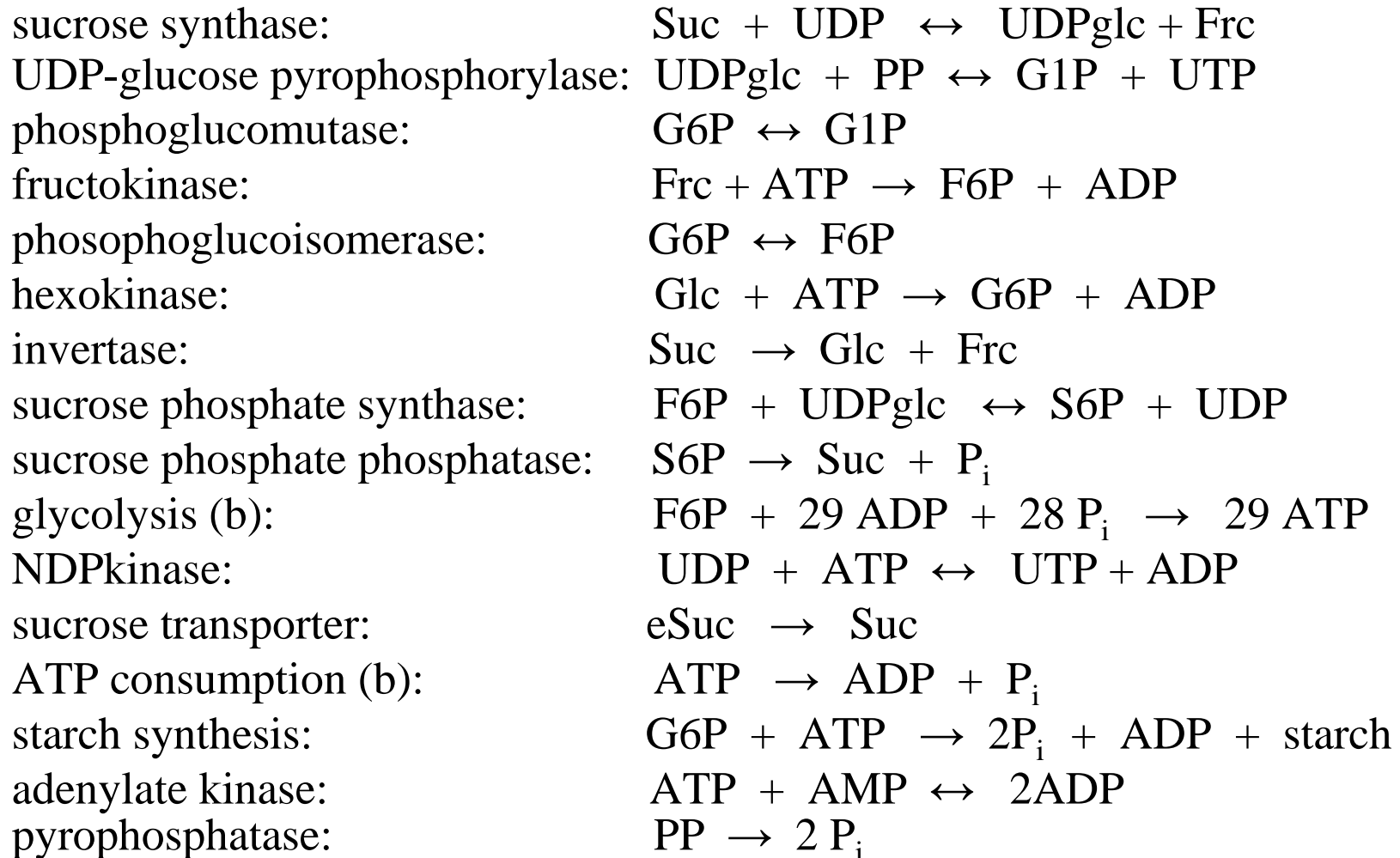# ucrose-to-starch-pathway in potato tuber

ucrose-to-starch-pathway in potato tuber

ucrose-to-starch-pathway in potato tuber

ucrose-to-starch-pathway in potato tuber

sucrose synthase:	$Suc + UDP \leftrightarrow UDPglc + Frc$
UDP-glucose pyrophosphorylase:	$UDPglc + PP \leftrightarrow G1P + UTP$
phosphoglucomutase:	$G6P \leftrightarrow G1P$
fructokinase:	$Frc + ATP \rightarrow F6P + ADP$
phosophoglucoisomerase:	$G6P \leftrightarrow F6P$
hexokinase:	$Glc + ATP \rightarrow G6P + ADP$
invertase:	$Suc \rightarrow Glc + Frc$
sucrose phosphate synthase:	$F6P + UDPglc \leftrightarrow S6P + UDP$
sucrose phosphate phosphatase:	$S6P \rightarrow Suc + P_i$
glycolysis (b):	$F6P + 29\,ADP + 28\,P_i \rightarrow 29\,ATP$
NDPkinase:	$UDP + ATP \leftrightarrow UTP + ADP$
sucrose transporter:	$eSuc \rightarrow Suc$
ATP consumption (b):	$ATP \rightarrow ADP + P_i$
starch synthesis:	$G6P + ATP \rightarrow 2P_i + ADP + starch$
adenylate kinase:	$ATP + AMP \leftrightarrow 2ADP$
pyrophosphatase:	$PP \rightarrow 2\,P_i$

etri net basics

Nodes :   places   transitions
(vertices)

places	transitions
passive elements	active elements
conditions	events
states	actions
chemical compounds	chemical reactions
metabolites	conversions of metabolites catalysed by enzymes

event

etri net basics

Nodes :   places   transitions
(vertices)

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chemical compounds	chemical reactions
metabolites	conversions of metabolites
	catalysed by enzymes

Arcs:
(edges)

event

pre-conditions   post-conditions
pre-places   post-places

etri net basics

Nodes : (vertices)	places	transitions
	passive elements	active elements
	conditions	events
	states	actions
	chemical compounds	chemical reactions
	metabolites	conversions of metabolites catalysed by enzymes

Arcs:
(edges)

Tokens
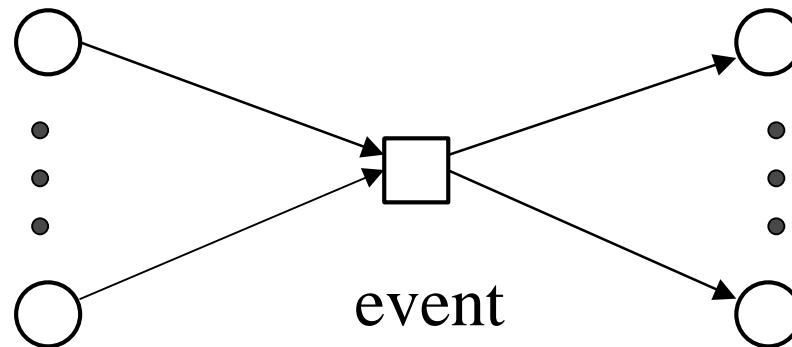
pre-conditions
pre-places

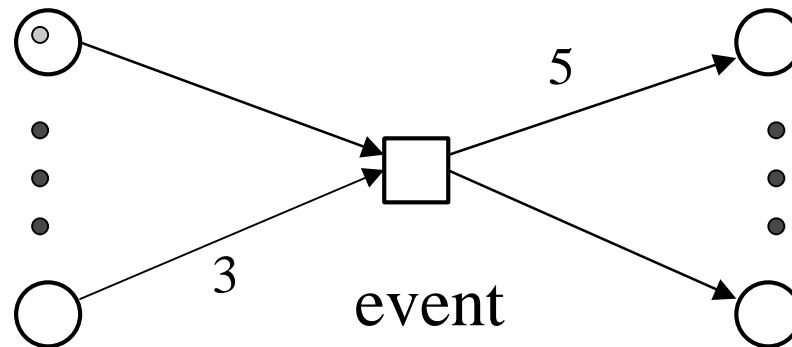post-conditions
post-places

etri net basics

Tokens: movable objects in discrete units,
e.g. units of substances (mole)

◯   condition is not fulfilled

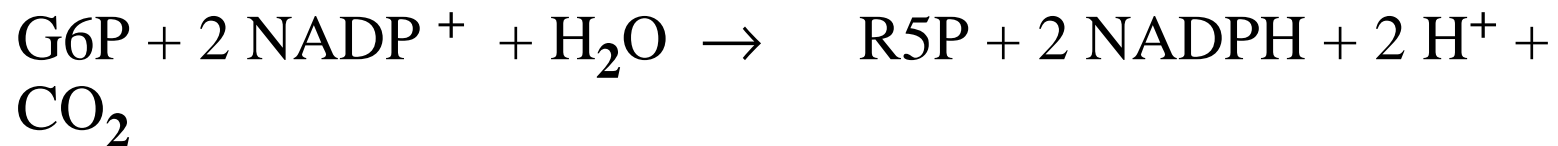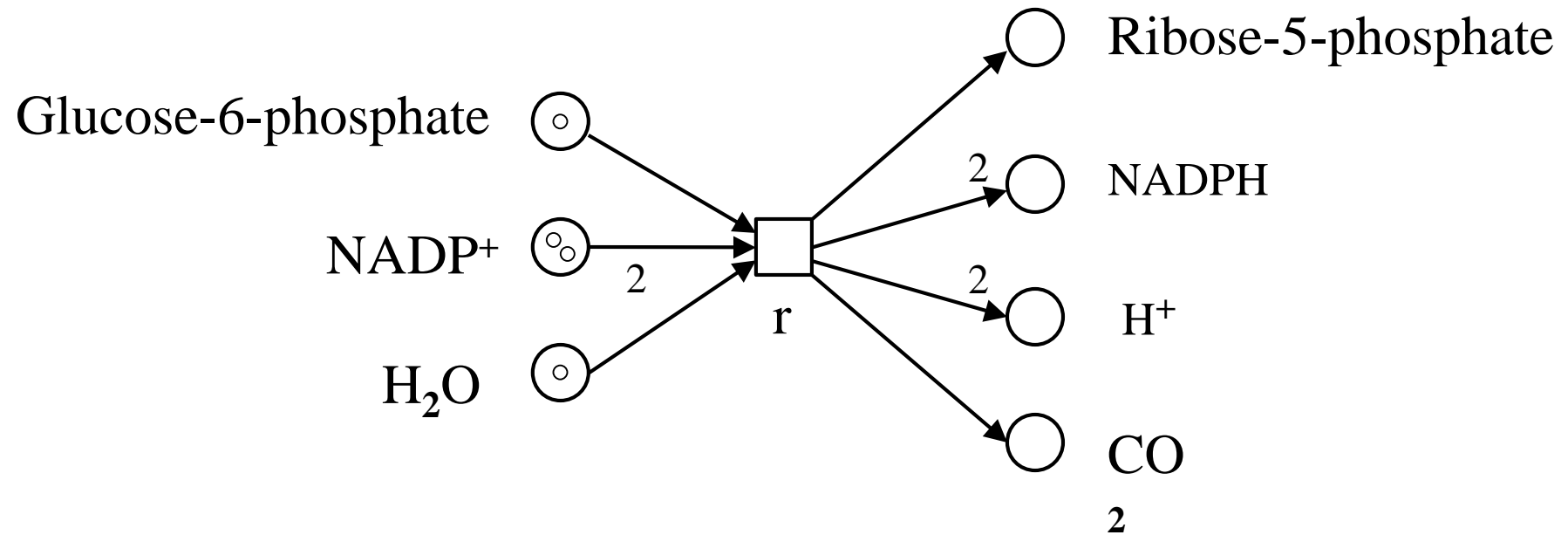◉   condition is (one time) fulfilled

(n)   condition is n times fulfilled

Marking: system state, token distribution, initial marking
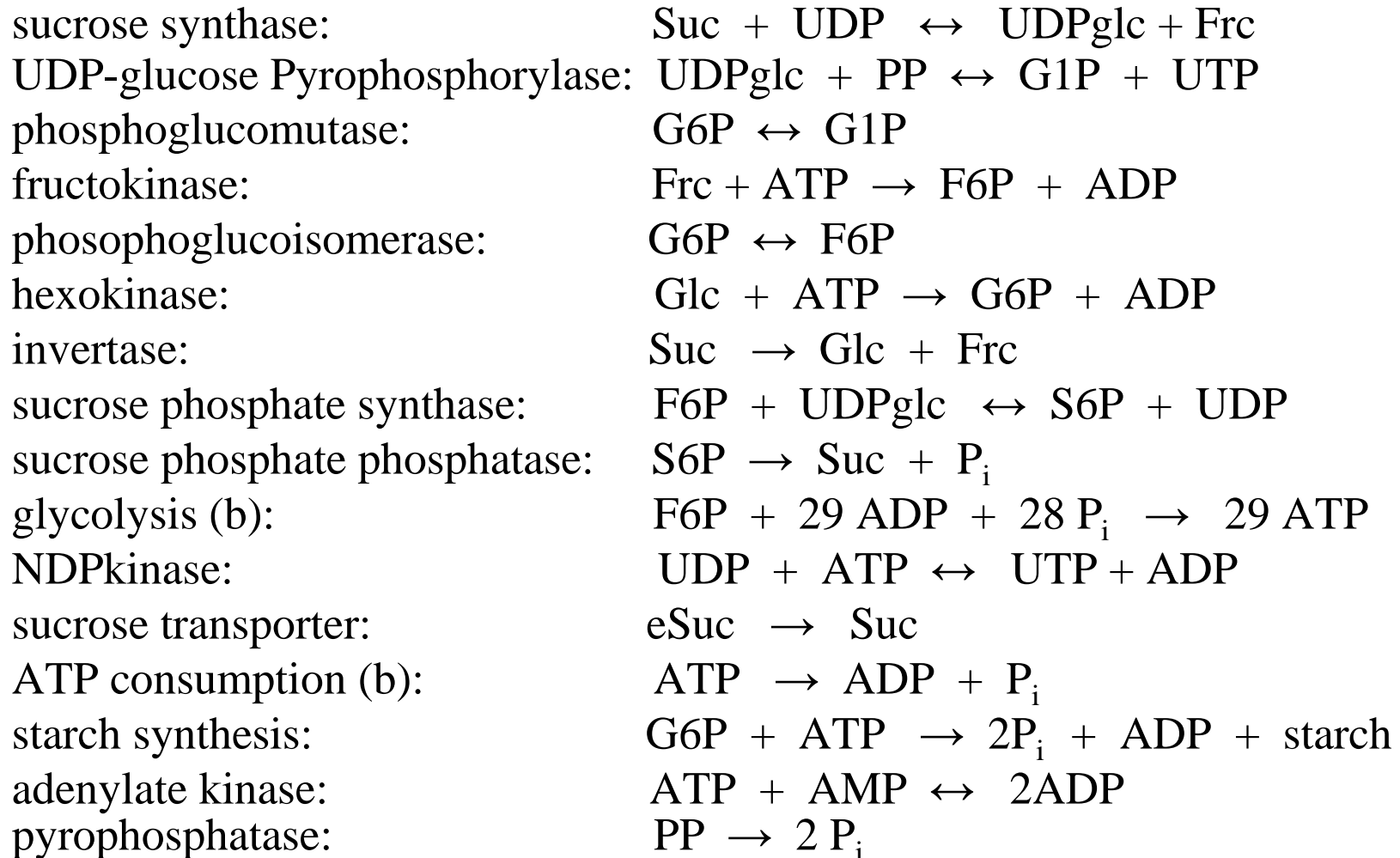
Token flow: occurring of an event (firing of a transition)
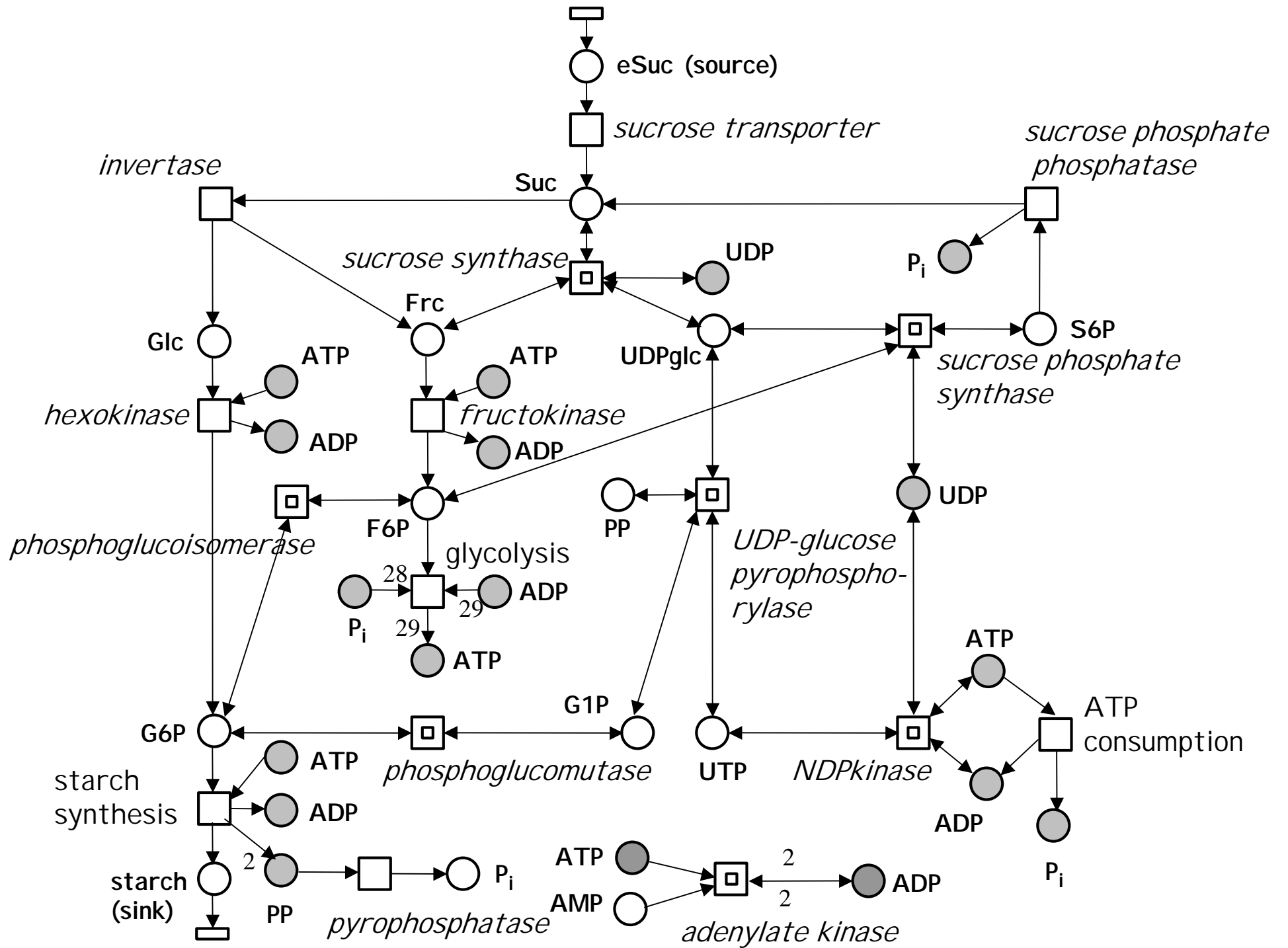
etri net basics

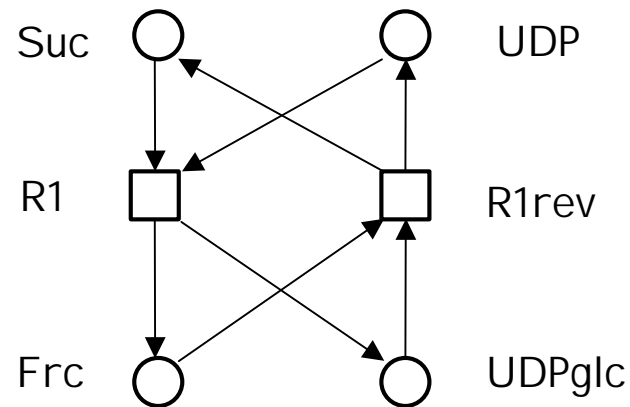Example: Pentose Phosphate Pathway - sum reaction

Glucose-6-phosphate

NADP$^+$

2

H$_2$O

r

Ribose-5-phosphate

2 NADPH

2 H$^+$

CO$_2$

G6P + 2 NADP$^+$ + H$_2$O $\rightarrow$ R5P + 2 NADPH + 2 H$^+$ + CO$_2$

ucrose-to-starch-pathway in potato tuber

|	
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UDP-glucose Pyrophosphorylase:	$UDPglc + PP \leftrightarrow G1P + UTP$
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ucrose-to-starch-pathway in potato tuber

A hierarchical node:

Interface to the environment
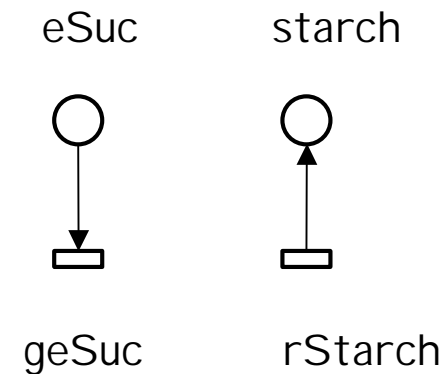
Tools:

Editing: Ped   Heiner BTU Cottbus

Animation: PedVisor   http://www.informatik.tu-cottbus.de/~wwwdssz/

Qualitative analysis: INA   Starke HU Berlin

http://www.informatik.hu-

berlin.de/~starke/ina.html

odel validation

(1)   Dynamical (behavioural) properties

(2)   Reachability analysis

(3)   Structural analysis

(4)   Invariant analysis

(5)   Model checking

ynamic (behavioural) properties

Liveness and Reversibility

- a net is live, if all its transitions are live in the initial marking

- a net is reversible, if the initial marking can be reached from each possible state

- How often can a transition fire? (0-times, n-times, ∞ times)

- infinite systems behaviour, search for dead transitions

- prediction of system deadlocks

ynamic (behavioural) properties

Boundedness

- a net is bounded, if there exists a positive integer number k, which represents a maximal number of tokens on each place in all states

- What is the maximal token number for a place?
      $(0, 1, k, \infty\ )$ boundedness (k-bounded)

- for bounded nets special algorithms exist

eachability analysis

How many and which system states could be reached ?   (0, 1, k, ∞)

• the reachability graph represents all possible states

• computational problem for large and dense biological networks

• for unbounded networks: computation of the coverability graph

• Is a certain system state again and again reachable?
progressiveness
• Is a certain system state never reachable?   safety

tructural analysis

- aims at discovering net structures to derive conclusions on dynamic properties

Elementary properties:

ordinary: the multiplicity of every arc is equal one

homogeneous: for any place all outgoing edges have the same multiplicity

pure: there is no transition, for which a pre-place is also a post-place (loop-free)

conservative: for each place the sum of input arc weights is equal to the sum of output arc weights – a conservative net is bounded
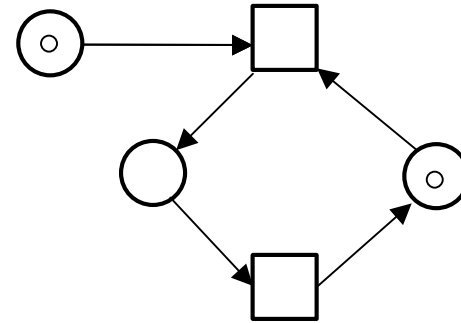
static conflict-free: there are no transitions with a common pre-place

connected, strongly connected: in graph-theoretical sense
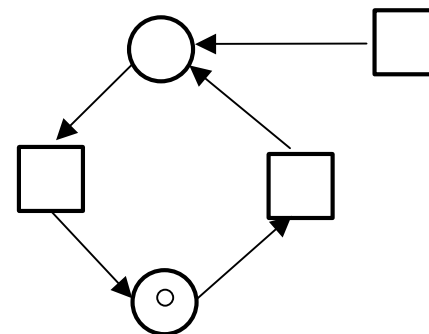
tructural analysis

structural deadlock:

a set of places that delivers its
tokens until a state is reached,
where the place set is empty
and there is no possibility to
get a new token

trap:

the opposite situation that
tokens cannot be removed
from a place set
(accumulation of substances)

nvariant analysis

- properties, which are conserved during the working of the system
- independent of the initial marking
- only the net structure is relevant for their calculation

Are there invariant structures, which are independent from firing of the system?

Place-invariants (P-invariants)
Transition-invariants (T-invariants)

nvariant analysis

Interpretation

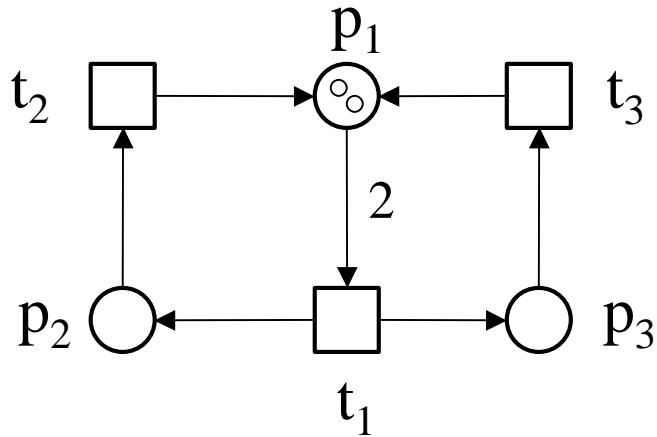| P-invariants	T -invariants
- set of places, whose weighted sum of tokens is constant	- set of transitions, whose firing reproduces a given marking
- covered by P-invariants: sufficient condition for boundedness liveness	- covered by T-invariants: necessary condition for
- set of metabolites, whose total net concentrations remain unchanged ADP, ATP NADP$^+$, NADPH	- minimal set of enzymes which could operate at steady state - indicate the presence of cyclic firing sequences

Elementary modes
Schuster, Hilgetag, Schuster (1993)

nvariant analysis

incidence matrix $C = P \times T$

$$
C = \begin{array}{c} \\ p_1 \\ p_2 \\ p_3 \end{array} \begin{array}{ccc} t_1 & t_2 & t_3 \\ \left(\begin{array}{ccc} -2 & 1 & 1 \\ 1 & -1 & 0 \\ 1 & 0 & -1 \end{array} \right) \end{array}
$$

place (P-) invariant:

$x\, C = 0$

$-2x_1 + x_2 + x_3 = 0$

$x_1 - x_2 \qquad = 0$

$x_1 \qquad - x_3 = 0$

transition (T-) invariant:

$C\, y = 0$

$-2y_1 + y_2 + y_3 = 0$

$y_1 - y_2 \qquad = 0$

$y_1 \qquad - y_3 = 0$

nvariant analysis

Minimal semi-positive solutions are of interest with
- all components of the solution vector are ≥ 0
- basis of the semi-positive solution space such that none solution is contained in another solution, Lautenbach (1973)

The calculation
- of all integer solutions is in P
- of all semi-positive solutions is in P
- of all semi-positive integer solutions is NP-complete, Schrijver (1999)

Extreme Pathways   Schilling et al. (2000)
- minimal basis of semi-positive integer solutions
- subset of T-invariants – biological interpretation?

Qualitative analysis using INA

Elementary properties

The net is not statically conflict-free.
The net has transitions without pre-place.
The net is not covered by semipositive P-invariants.
The net is not structurally bounded.
The net is not safe.
The net has transitions without post-place.
The net is not ordinary.
At least the following transitions are live: 0.SucTrans, 1.Inv, 18.geSuc,
At least the following places are simultaneously unbounded: 0.Suc, 1.eSuc, 2.Glc, 3.Frc,
The net is marked.
The net is not homogenous.
The net has no nonempty clean trap.
The net has no places without post-transition.
The net is connected.

The net is pure.
The net is not strongly connected.
The net is not bounded.
The net is not live and safe.
Transition 18.geSuc has no pre-place.
Transition 21.rStarch has no post-place.
The net is not conservative.

The net is not marked with exactly one toke
The net has not a non-blocking multiplicity
The net has no places without pre-transition
Maximal in/out-degree: 6

ORD HOM NBM PUR CSV SCF CON SC Ft0 tF0 Fp0 pF0 MG SM FC EFC ES
 N   N   N   Y   N   N   N   Y   N   Y   Y   N   N   N   N   N   N

Qualitative analysis using INA

Structural properties

DTP	CPI	CTI	B	SB	REV	DSt	BSt	DTr	DCF	L	LV	L&S
	?	N	Y	N	N	?	?	?	?	?	?	?	N

- liveness could not be decided because the net is unbounded and the reachability graph cannot be calculated
- the coverability graph has more than 4 million states

smaller bounded version: more than 10^{10} states of the reachability graph

Invariant analysis

The net is not covered by P-invariants.

Following P-invariants were calculated:

1. UDPglc, UTP, UDP

2. ATP, AMP, ADP

3. G6P, F6P, G1P, UTP, ATP(2), ADP, S6P, P_i, PP(2)

The net is covered by 19 T-invariants

7 trivials: 1. SPS, SPS_rev,   2. UGPase, UGPASE_rev,
               3. SuSy_SuSy_rev,   4. PGM, PGM_rev,
               5. NDPkin, NDPkin_rev, 6. AdK, AdK_rev,
               7. PGI, PGI_rev

T-invariant 14

geSuc

eSuc

sucrose transporter

sucrose phosphate phosphatase

invertase

Suc

sucrose synthase

UDP

P$_i$

Frc

S6P

Glc

ATP

ATP

UDPglc

sucrose phosphate synthase

hexokinase

ADP

fructokinase

ADP

UDP

phosphoglucoisomerase

F6P

PP

UDP-glucose pyrophosphorylase

glycolysis

28

ADP

ATP

P$_i$

29

29

ATP

ATP

G1P

ATP consumption

G6P

phosphoglucomutase

UTP

NDPkinase

starch synthesis

ATP

ADP

ADP

P$_i$

starch

2

PP

P$_i$

AMP

2

ADP

rStarch

pyrophosphatase

2

adenylate kinase

T-invariant 14

T-invariant 14

geSuc

eSuc

sucrose transporter

sucrose phosphate phosphatase

invertase

Suc

UDP

P_i

sucrose synthase

Frc

Glc

ATP

ATP

UDPglc

S6P

sucrose phosphate synthase

hexokinase

ADP

fructokinase

ADP

phosphoglucoisomerase

F6P

glycolysis

PP

UDP

UDP-glucose pyrophosphorylase

28

ADP

P_i

29

29

ATP

ATP

G1P

G6P

ATP consumption

starch synthase

ATP

phosphoglucomutase

UTP

NDPkinase

ADP

ADP

starch

2

ATP

2

P_i

PP

ADP

pyrophosphatase

AMP

2

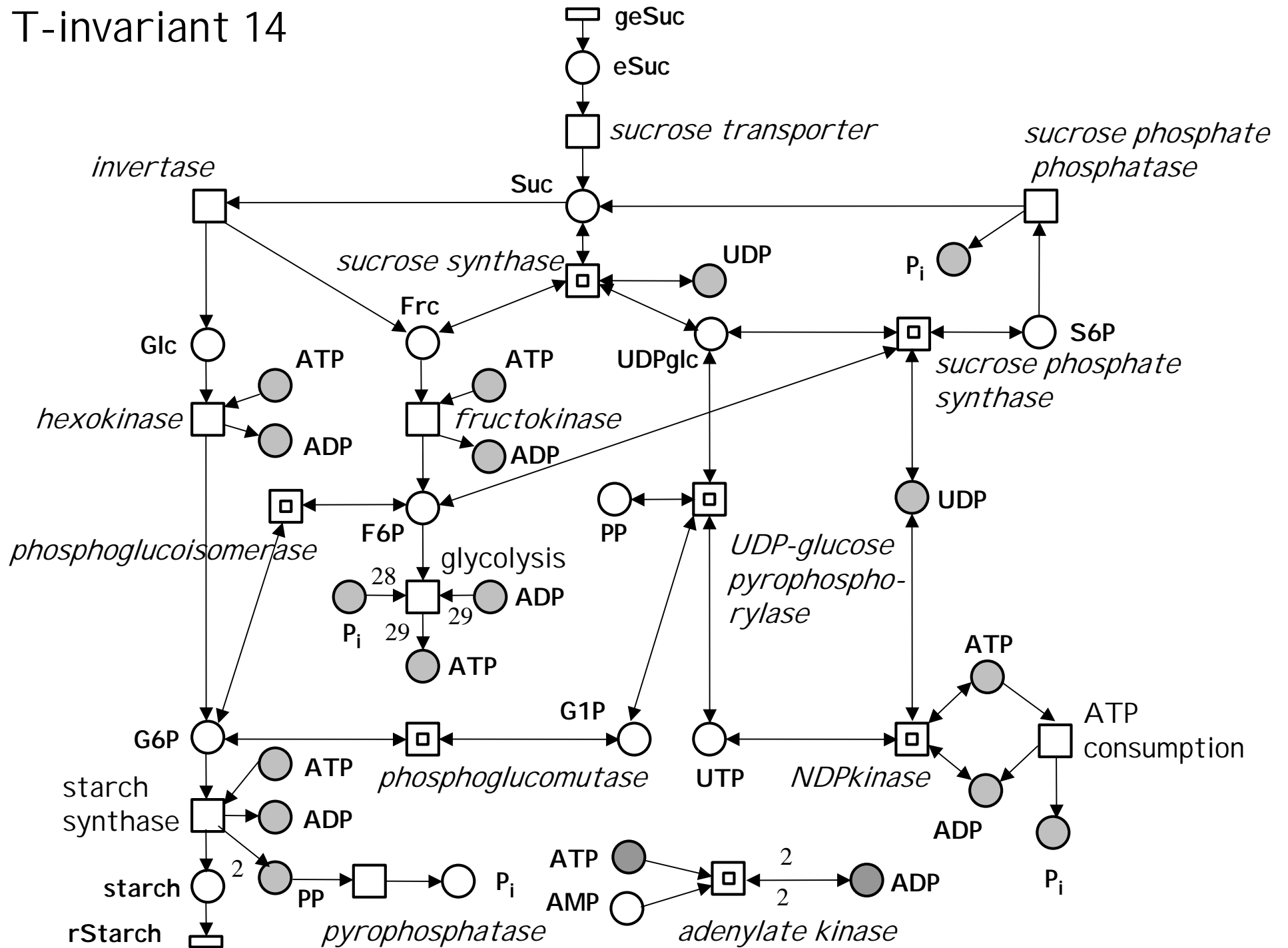*adenylate kinase*

rStarch

P_i

P_i

T-invariant 14
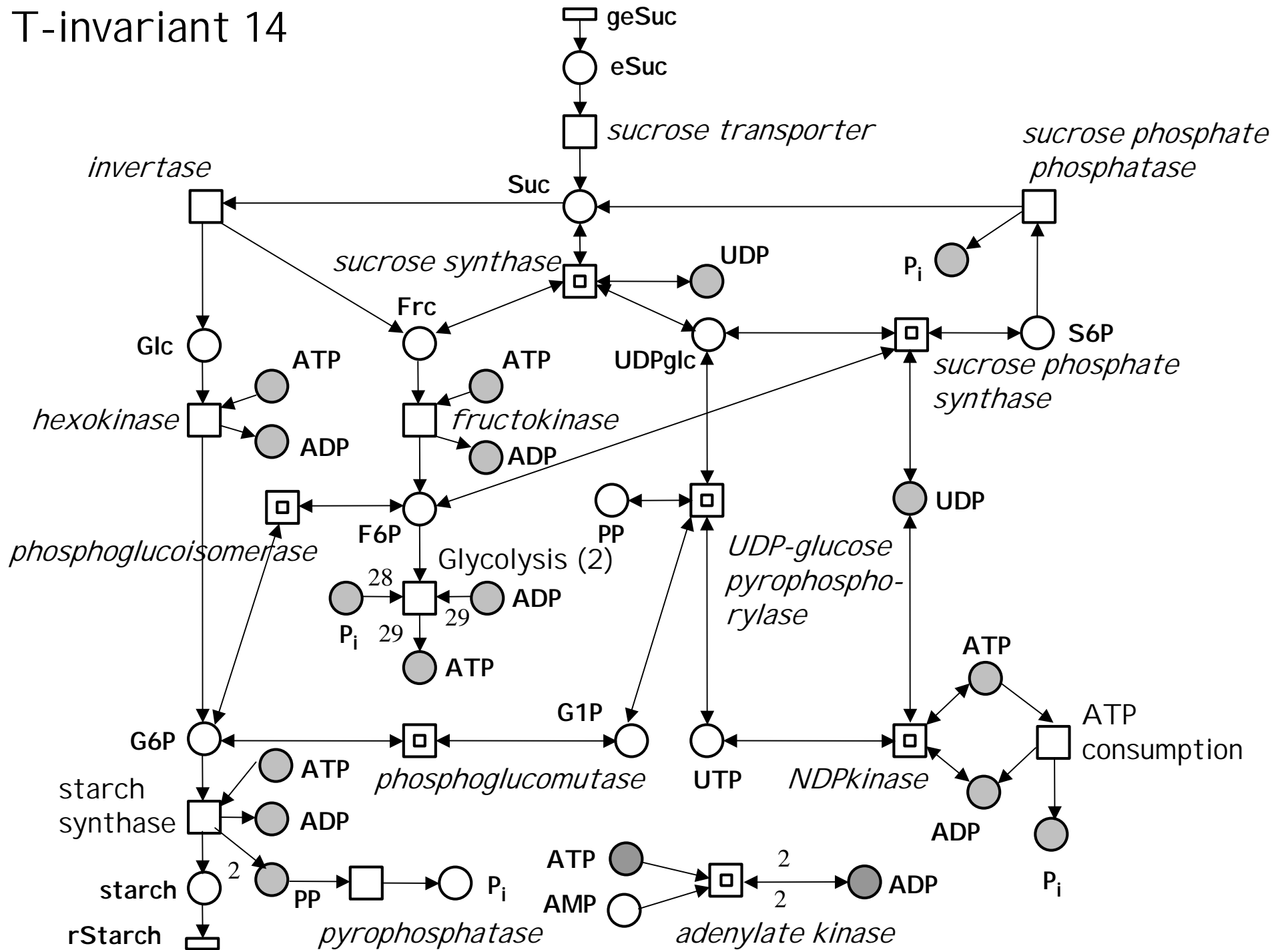
T-invariant 14

T-invariant 14

T-Invariant analysis

```
21  |      0.gGlu        :   1,
    |      1.R1          :   1,
    |      2.gADP        :   2,
    |      3.gPi         :   2,
    |      4.gNAD        :   2,
    |      5.PGIsomerase:   1,
    |      7.Aldolase1  :   1,
    |     10.TPIsomerase:   1,
    |     11.GAPDH       :   2,
    |     13.PGKinase    :   2,
    |     15.PGMutase1   :   2,
    |     17.Enolase     :   2,
    |     19.rNADH       :   2,
    |     26.rATP        :   2,
    |     27.rPyr        :   2,
    |     28.PFKinase    :   1,
    |     29.PyrKinase1 :   1
```
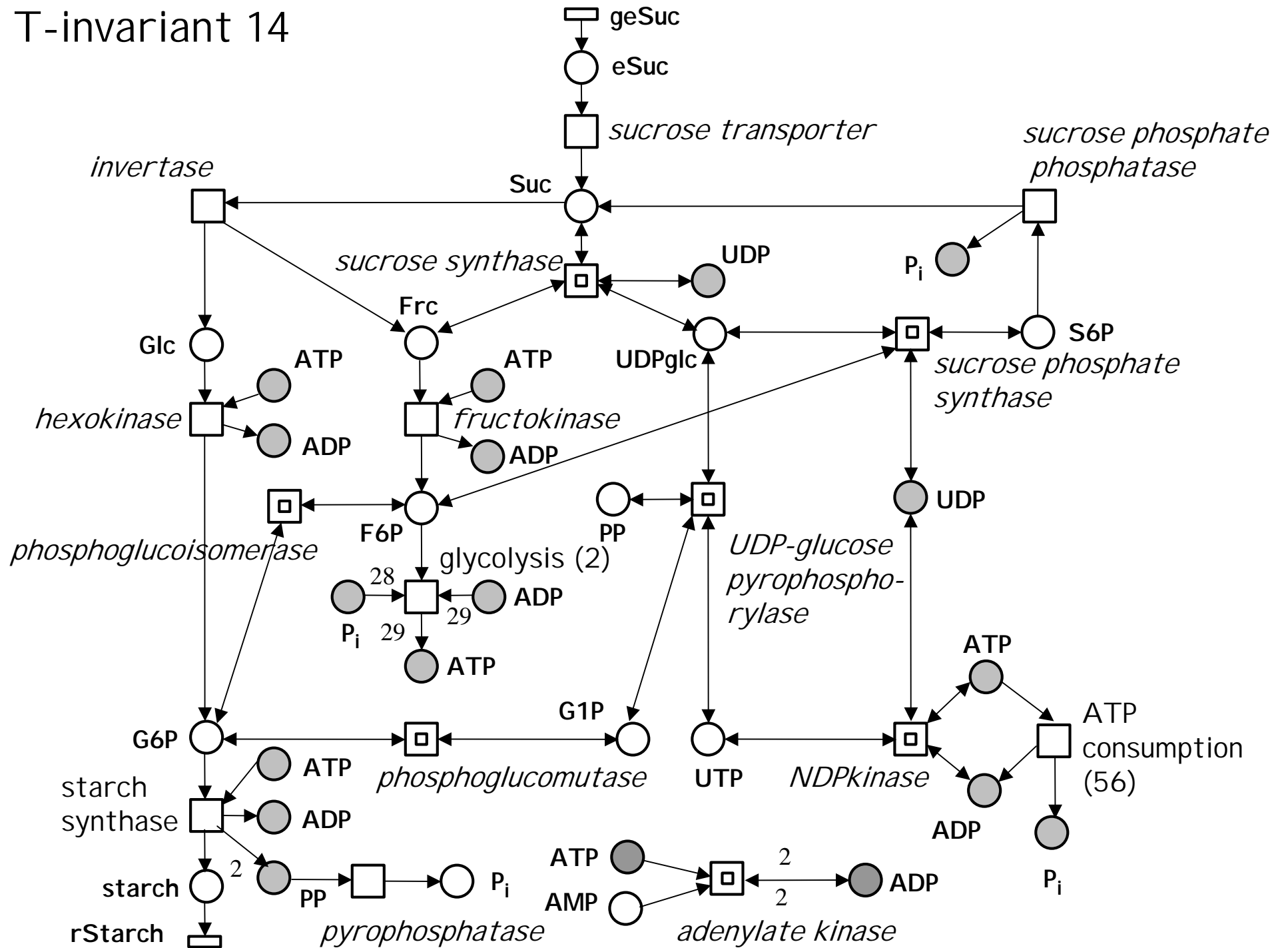
T-Invariant analysis
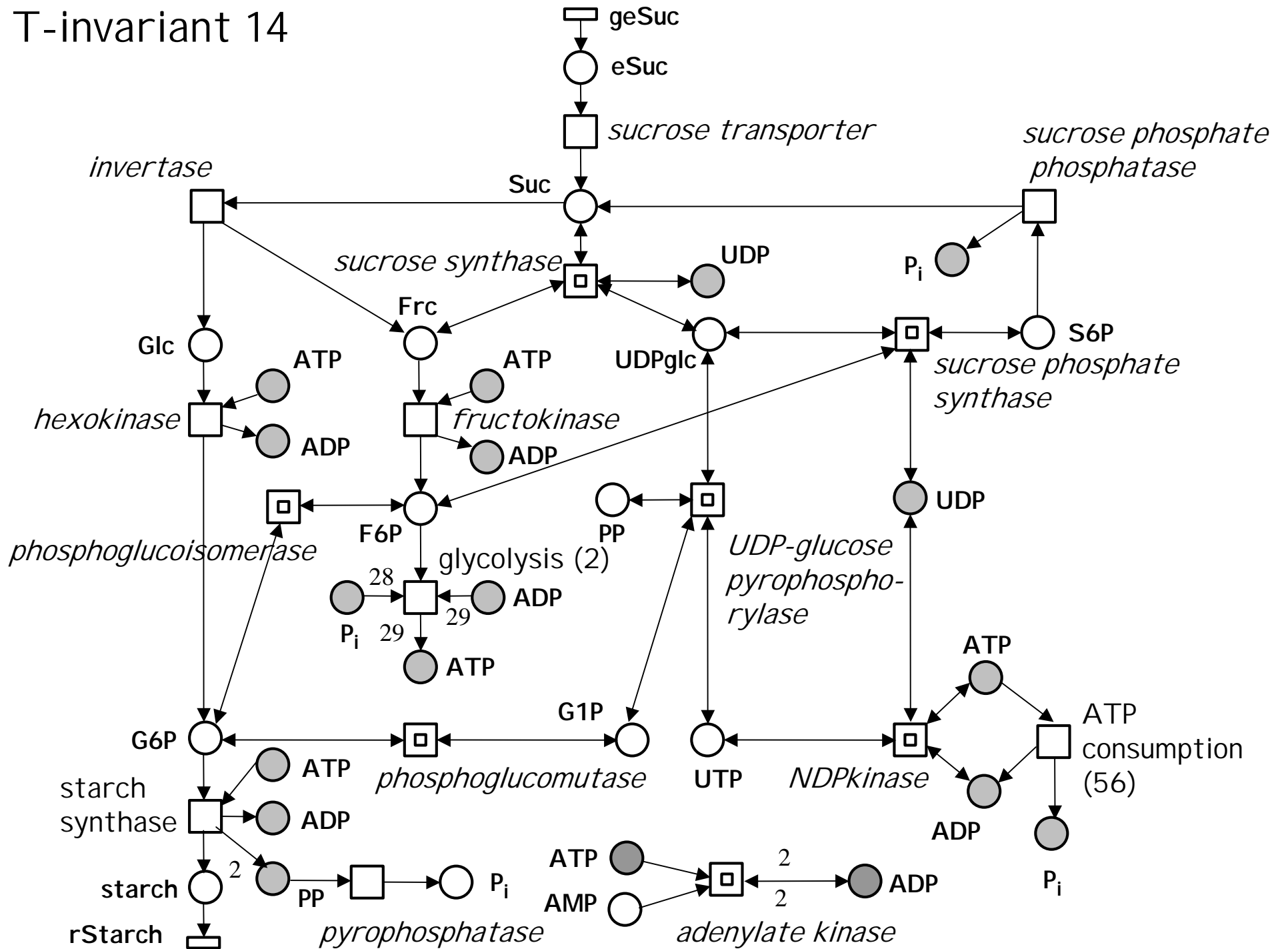
```
21  |      0.gGlu        :  1,
    |      1.R1          :  1,
    |      2.gADP        :  2,
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    |     26.rATP        :  2,
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    |     28.PFKinase    :  1,
    |     29.PyrKinase1 :  1
```

Substrates:

Glucose, 2ADP, $2P_i$, $2NAD^+$

T-Invariant analysis

```
21  |      0.gGlu        :   1,
    |      1.R1          :   1,
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    |     19.rNADH       :   2,
    |     26.rATP        :   2,
    |     27.rPyr        :   2,
    |     28.PFKinase    :   1,
    |     29.PyrKinase1  :   1
```

Substrates:

Glucose, 2ADP, 2P_i, 2NAD^+

Products:

2Pyruvate, 2ATP, 2NADH

T-Invariant analysis

```
21  |      0.gGlu        :  1,
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    |      3.gPi         :  2,
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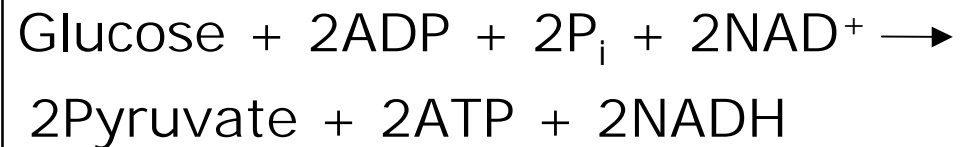
Substrates:

Glucose, 2ADP, 2P$_i$, 2NAD$^+$

Products:

2Pyruvate, 2ATP, 2NADH

Glucose + 2ADP + 2P$_i$ + 2NAD$^+$ $\longrightarrow$

2Pyruvate + 2ATP + 2NADH

T-Invariant analysis

Invariant number	sucrose cleavage		hexoses go into		ATP cons	ATP used for cycling		
	SuSy	Inv	Glyc	StaSy		Inv SuSy_rev	Inv SPS, SPP	SuSy SPS, SPP
8	x		x	x				x
9	x		x	x	x			
10	x		x	x				
11		x	x	x		x		
12		x	x	x			x	
13		x	x	x				x
14		x	x	x	x			
15		x	x	x				
16		x	x			x		
17		x	x				x	
18		x	x					x
19		x	x		x			

Robustness

Robustness: sensitivity of the system against parameter
(fragility)   changes (altered enzyme activity, mutations)
(Voit, 2000)

Stelling et al., Nature (2002): linear correlation between robustness
and the number of elementary modes
(T-invariants)

Our suggestion: - enzyme distribution over T-invariants
- number of alternative paths

Potato net: - fructokinase occurs in all T-invariants
- there is no enzyme that occurs in only one
T-invariant

Conclusions & Outlook

Petri nets provide

(1)   a unique description of biological networks

(2)   methods for qualitative analysis to check models by the calculation of system properties.

(3)   The complexity of biological systems make it necessary to extend Petri net methods.

(4)   Automatic interpretation of T-invariants is necessary.