Applying Petri nets for the analysis of the GSH-ASC cycle in chloroplasts

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- Petri nets are a useful framework for the analysis of biological systems in various complementary ways, integrating both qualitative and quantitative studies.

- Petri nets are a useful framework for the analysis of biological systems in various complementary ways, integrating both qualitative and quantitative studies.
- We apply this formalism to the Glutathione Ascorbate Redox cycle (GSH-ASC) in chloroplasts case study, considering structural Petri net techniques from standard Petri nets to validate the model and to infer new properties, as well as continuous Petri nets in order to have a behaviour prediction.

Goals of this paper

(i) The application of continuous Petri nets to this specific biological process, which provides us with a graphical representation of this biochemical process, which becomes easier to modify and analyze than the corresponding (equivalent) ODEs.

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(i) The application of continuous Petri nets to this specific biological process, which provides us with a graphical representation of this biochemical process, which becomes easier to modify and analyze than the corresponding (equivalent) ODEs.

(ii) The application of the classical theory and tools of Petri nets (in the discrete Petri net), and specifically the structural theory in order to get a better understanding of the biological model and conclude the relationship between the structural elements (invariants) of the underlying discrete Petri net with the biochemical properties of this biological process.

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- The glutathione-ascorbate redox (GSH-ASC) pathway in chloroplasts is a complex network of spontaneous, photochemical, and enzymatic reactions for detoxifying hydrogen peroxide.

E. Valero, M.I. González-Sánchez, H. Macià and F. García-Carmona

Computer Simulation of the Dynamic Behavior of the Glutathione-Ascorbate Redox Cycle in Chloroplasts

Plant Physiology, Vol. 149, pp. 1958-1969, 2009.

Chemical reactions involved in the cycle

Reaction	Notation Reaction
$MDA + MDA \rightarrow ASC + DHA$	k_1
$DHA + 2\ GSH \rightarrow ASC + GSSG$	k_4
$2\ O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$	k_5
$O_2^- + ASC \rightarrow H_2O_2 + MDA$	k_6
$O_2^- + 2\ GSH \rightarrow H_2O_2 + GSSG$	k_7
$H_2O_2 + 2\ ASC \rightarrow 2\ H_2O + 2\ MDA$	k_8

Reactions involved in the APX mechanism.

Reaction	Notation Reaction
$APX + H_2O_2 \rightarrow CoI + H_2O$	k_1^{APX}
$CoI + ASC \rightarrow CoII + MDA$	k_2^{APX}
$CoII + ASC \rightarrow APX + MDA$	k_3^{APX}
$CoI + H_2O_2 \rightarrow APX_i$	k_4^{APX}
synthesis *de novo* of APX	k_5^{APX}

Steady-state rate equations used for the enzymes involved in the model

Enzyme	Rate equation
SOD	$k_{SOD}[SOD]_0[O_2^-]$
DHAR	$\frac{k_{cat}^{DHAR}[DHAR]_0[DHA][GSH]}{K_i^{DHA}K_M^{GSH1} + K_M^{DHA}[GSH] + (K_M^{GSH1} + K_M^{GSH2})[DHA] + [DHA][GSH]}$
GR	$\frac{k_{cat}^{GR}[GR]_0[NADPH][GSSG]}{K_M^{NADPH}[GSSG] + K_M^{GSSG}[NADPH] + [NADPH][GSSG]}$

List of kinetic constants values used to simulate the model under *standard* conditions.

F	640	k_{cat}^{GR}	595	k_{cat}^{DHAR}	142	k_{SOD}	200
k_1^{APX}	12	k_2^{APX}	50	k_3^{APX}	2,1	k_4^{APX}	0,7
k_5^{APX}	0,01	k_1	0,5	k_4	0,1	k_5	0,2
k_6	0,2	k_7	0,7	k_8	$2E-6$	k_{12}	1,3
k_{13}	42,5	k_N	0,5	K_M^{NADPH}	3	K_M^{GSSG}	200
K_M^{GSH}	2500	K	$5E5$				

List of initial concentrations used to simulate the model under *standard* conditions

Enzymes	Initial concentration (μM)	Species	Initial concentration (μM)
GR	1,4	NADPH	150
DHAR	1,7	GSH	4000
SOD	50	ASC	10000
APX	70		

ODEs (I)

$$\frac{d[NADPH]}{dt} = -v_{GR} - k'_N[CO_2][NADPH] + k_{12}[NADP^+] \quad (1)$$

$$\frac{d[NADP^+]}{dt} = v_{GR} + k'_N[CO_2][NADPH] - k_{12}[NADP^+] \quad (2)$$

$$\frac{d[GSH]}{dt} = 2\,(\,v_{GR} - v_{DHAR} - k_7[O2^-][GSH] - k_4[DHA][GSH]\,) \quad (3)$$

$$\frac{d[GSSG]}{dt} = -v_{GR} + v_{DHAR} + k_7[O2^-][GSH] + k_4[DHA][GSH] \quad (4)$$

$$\frac{d[ASC]}{dt} = v_{DHAR} + k_1[MDA]^2 + k_4[DHA][GSH] + k_{13}[MDA] - k_2^{APX}[ASC][CoI] - k_3^{APX}[ASC][CoII] - k_6[O2^-][ASC] - 2\,k_8[H_2O_2][ASC] \quad (5)$$

$$\frac{d[DHA]}{dt} = -v_{DHAR} + k_1[MDA]^2 - k_4[DHA][GSH] \quad (6)$$

$$\frac{d[MDA]}{dt} = k_2^{APX}[ASC][CoI] + k_3^{APX}[ASC][CoII] - 2\,k_1[MDA]^2 + k_6[O_2^-][ASC] + 2\,k_8[H_2O_2][ASC] - k_{13}[MDA] \quad (7)$$

ODEs (II)

$$\frac{d[H_2O_2]}{dt} = v_{SOD} - k_1^{APX}[H_2O_2][APX] - k_4^{APX}[H_2O_2][CoI] + k_5[O_2^-]^2 + k_6[O_2^-][ASC] + k_7[O2^-][GSH] - k_8[H_2O_2][ASC] \quad (8)$$

$$\frac{d[APX]}{dt} = -k_1^{APX}[H_2O_2][APX] + k_3^{APX}[ASC][CoII] + k_5^{APX}([APX]_0 - [APX] - [CoI] - [CoII]) \quad (9)$$

$$\frac{d[CoI]}{dt} = k_1^{APX}[H_2O_2][APX] - k_2^{APX}[ASC][CoI] - k_4^{APX}[H_2O_2][CoI] \quad (10)$$

$$\frac{d[CoII]}{dt} = k_2^{APX}[ASC][CoI] - k_3^{APX}[ASC][CoII] \quad (11)$$

$$\frac{d[APX_i]}{dt} = k_4^{APX}[H_2O_2][CoI] \quad (12)$$

$$\frac{d[O_2^-]}{dt} = -2\,v_{SOD} + F - 2\,k_{12}[NADP^+] - 2\,k_5[O_2^-]^2 - k_6[O_2^-][ASC] - k_7[O2^-][GSH] - k_{13}[MDA] \quad (13)$$

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- We use continuous Petri nets, obtained from Ordinary Differential Equations for a quantitative study.

D. Gilbert, M. Heiner

From Petri Nets to Differential Equations - an Integrative Approach for Biochemical Network Analysis.

Proc. ICATPN 2006, Springer LNCS 4024, pp. 181-200, 2006

Some considerations

(i) There is a constant electron source in the model, F, whose flux is divided among three competitive routes: the photoproduction of O_2^- (transition k5), the photoreduction of $NADP^+$ (transition k12) and the photoreduction of MDA (transition k13).

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$$\begin{aligned}\frac{d[O_2^-]}{dt} = & -2\,v_{SOD} + F - 2\,k_{12}[NADP^+] - 2\,k_5[O_2^-]^2 \\ & - k_6[O_2^-][ASC] - k_7[O2^-][GSH] - k_{13}[MDA] \end{aligned} \quad (13)$$

Some considerations

(ii) The synthesis de novo of APX is considered as

$$k_5{}^{APX}([APX]_0 - [APX] - [CoI] - [CoII])$$

Then, read arcs are used for CoI and $CoII$ places. We have then considered four new transitions in the continuous Petri net model, with the following associated kinetic constants: k51APX with $k_5{}^{APX}[APX_0]$, and k52APX, k53APX and k54APX with $k_5{}^{APX}$.

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$$\frac{d[APX]}{dt} = -k_1^{APX}[H_2O_2][APX] + k_3^{APX}[ASC][CoII] + k_5^{APX}([APX]_0 - [APX] - [CoI] - [CoII]) \quad (9)$$

$$\frac{d[CoI]}{dt} = k_1^{APX}[H_2O_2][APX] - k_2^{APX}[ASC][CoI] - k_4^{APX}[H_2O_2][CoI] \quad (10)$$

$$\frac{d[CoII]}{dt} = k_2^{APX}[ASC][CoI] - k_3^{APX}[ASC][CoII] \quad (11)$$

Some considerations

(iii) $[CO_2]$ is considered constant, so that the flux of *NADPH* consumption by the Calvin cycle (and other electron-consuming reactions) is $kN = k'_N[CO_2]$.

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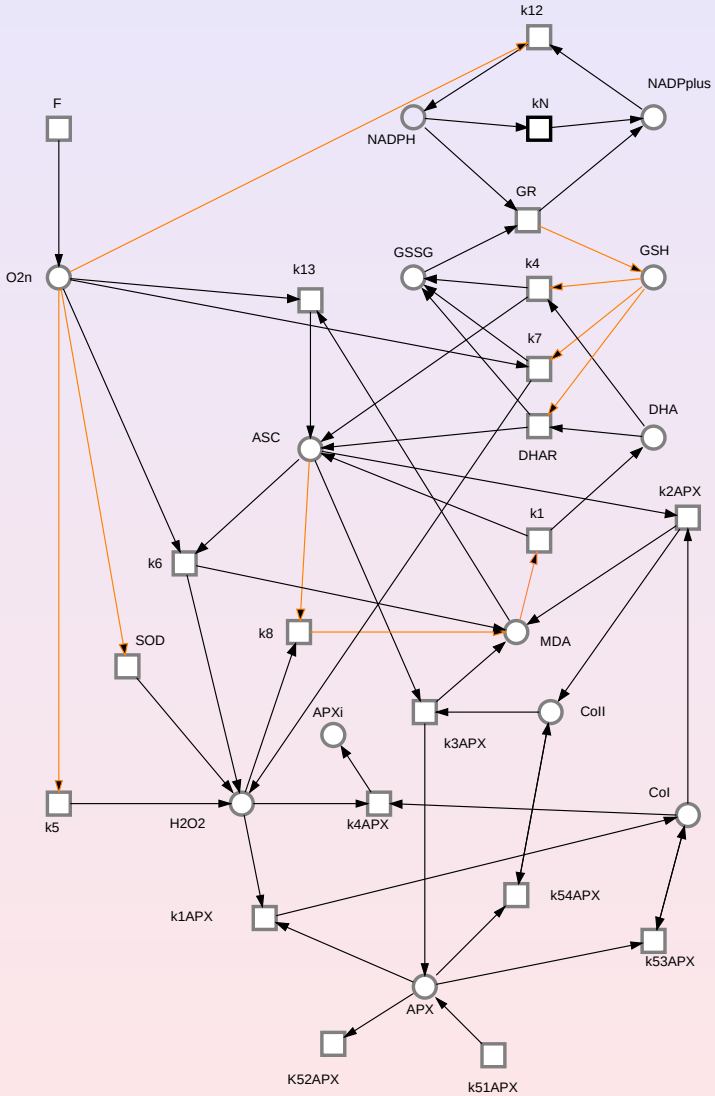
$$\frac{d[NADPH]}{dt} = -v_{GR} - k'_N[CO_2][NADPH] + k_{12}[NADP^+] \quad (1)$$

$$\frac{d[NADP^+]}{dt} = v_{GR} + k'_N[CO_2][NADPH] - k_{12}[NADP^+] \quad (2)$$

- We depict the continuous Petri net for the GSH-ASC cycle, which has been obtained with the Snoopy tool.

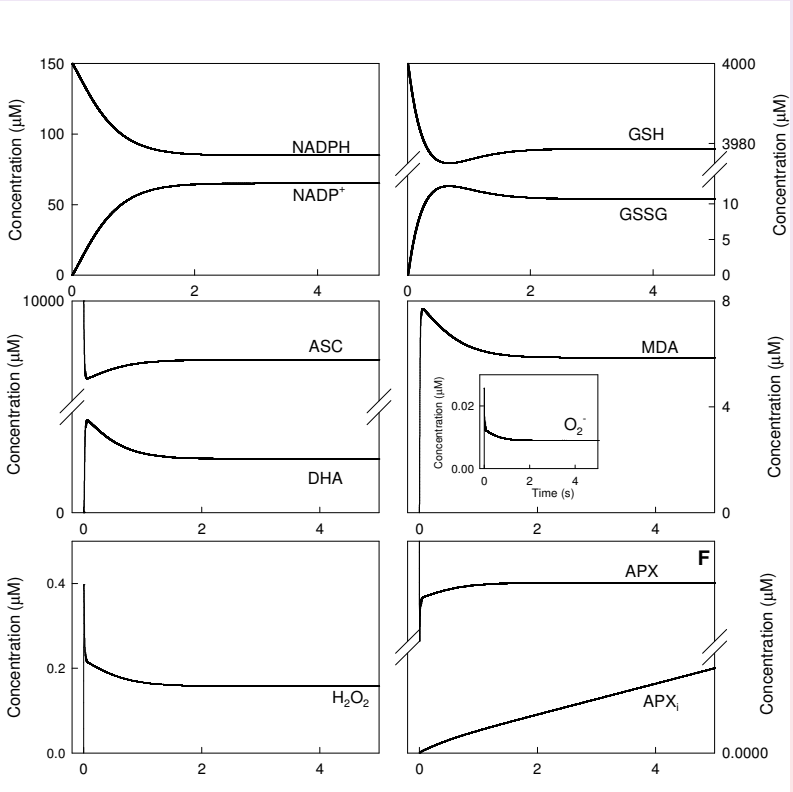
- We depict the continuous Petri net for the GSH-ASC cycle, which has been obtained with the Snoopy tool.
- From this Petri net model, we can obtain the corresponding ODEs, consisting of 13 molecular species and 21 reactions defining the equations.

Continuous Petri net model for the GSH-ASC cycle



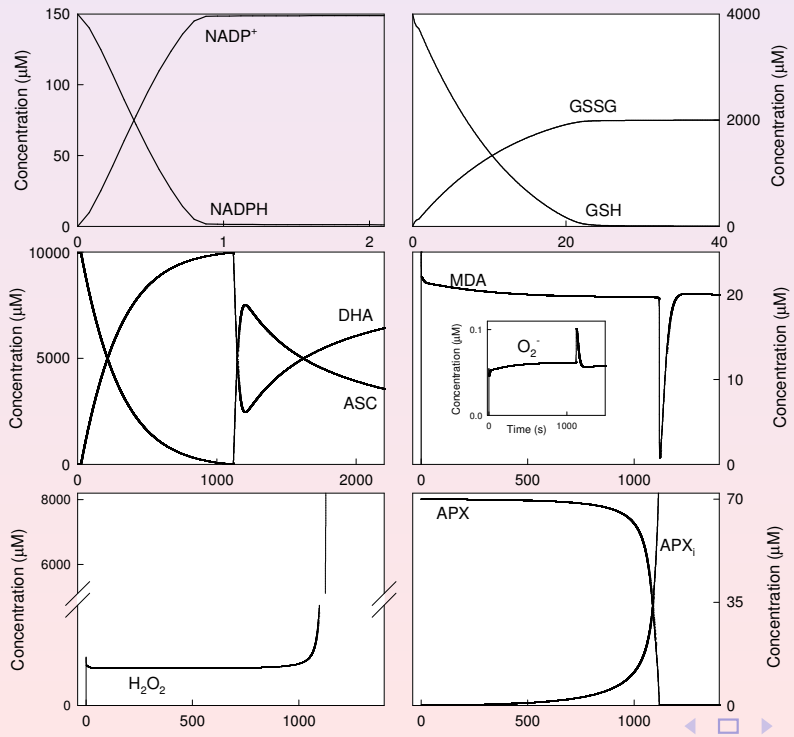
Simulated progress curves corresponding to the species involved in the mechanism with $F = 640$.

Under these conditions, a steady state was rapidly achieved by the system, in which metabolite concentrations remained constant.



Simulated progress curves corresponding to the species involved in the mechanism with $F = 2400$ (intense light exposure).

Under these conditions, the antioxidant concentration in the chloroplast gradually decreased, in the order *NADPH*, *GSH* and *ASC*, so that their respective oxidized species concentrations increased. The disappearance of *ASC* was followed by the rapid inactivation of *APX*, reflecting what occurs in reality, accompanied by a sharp increase in APX_i and H_2O_2.



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- We apply Petri net structural techniques to verify and analyze the metabolic pathway. [Charlie tool]

discrete Petri net

- We remove two edges leaving the place O_2^-, those reaching transitions $k12$ and $k13$, since in the discrete model we do not require any tokens on O_2^- to fire these transitions. These edges are related to electron source.

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- We remove from the continuous Petri net the read arcs, and we join the transitions *k52APX*, *k53APX* and *k54APX* into a single output transition from *APX*, named *k5APXo*, and we also rename the input transition *k51APX* in *k5APXi*.

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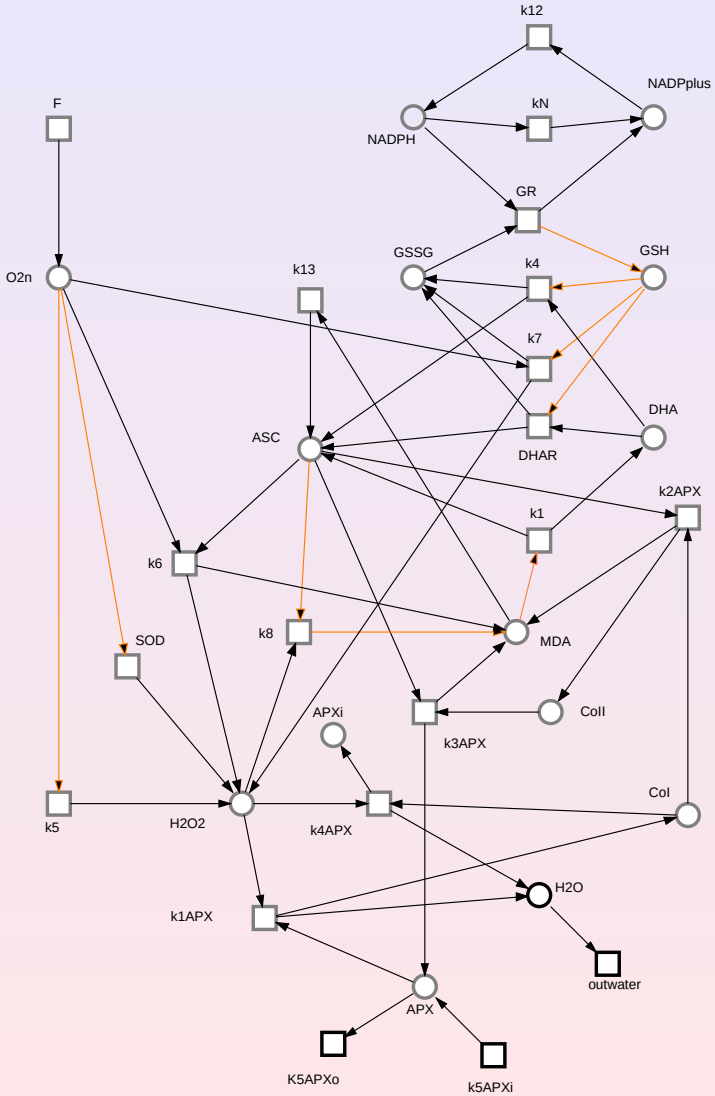
- We remove from the continuous Petri net the read arcs, and we join the transitions *k52APX*, *k53APX* and *k54APX* into a single output transition from *APX*, named *k5APXo*, and we also rename the input transition *k51APX* in *k5APXi*.
- In order to identify the I/O behaviour we add a new place that represents the water that is generated by the reactions (transitions) *k4APX* and *k1APX*, and a new transition (*outwater*) that models the water self control of chloroplasts.

Petri net model for the GSH-ASC cycle



A P-invariant, under the biological point of view, defines a mass conservation law and has associated its corresponding biological interpretation.

$P - inv_1$	$=$	$\{$ NADPH, $NADP^+$ $\}$
$P - inv_2$	$=$	$\{$ 2 GSSG, GSH $\}$
$P - inv_3$	$=$	$\{$ ASC, DHA, MDA $\}$

P-invariants

(i) $P - inv_1 = \{ NADPH, NADP^+ \}$ is associated with the consumption of $NADPH$ by the Calvin cycle and GR, and its corresponding recovery in daylight.

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P-invariants

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(iii) $P - inv_3 = \{ ASC, DHA, MDA \}$ is related to the interconversion of ASC both spontaneously and catalyzed by $DHAR$ and APX.

- A T-invariant, under the biological point of view, defines a state-conserving subnetwork and has associated its corresponding biological interpretation.

- A T-invariant, under the biological point of view, defines a state-conserving subnetwork and has associated its corresponding biological interpretation.
- We have obtained 26 minimal semipositive transition invariants.

T-invariants (I)

T-invariant	Transitions/Reactions (number of fires)
$T - inv_1$	k5APXo (1), k5APXi (1)
$T - inv_2$	kN (1), k12 (1)
$T - inv_3$	k13 (3),k6 (1), k8 (1), F (1)
$T - inv_4$	k13 (2), k8 (1), SOD (1), F (2)
$T - inv_5$	k13 (2), k8 (1), k5 (1), F (2)
$T - inv_6$	GR (1), k12 (1), k7 (1), k13 (2), k8 (1), F (1)
$T - inv_7$	k13 (3), k2APX (1), k3APX (1), k6(1), k1APX (1), F (1), outwater (1)
$T - inv_8$	k13 (2), k2APX (1), k3APX (1), SOD (1), k1APX (1), F (2), outwater (1)
$T - inv_9$	k13 (2), k2APX (1), k3APX (1),k5 (1), k1APX (1),F (2), outwater (1)
$T - inv_{10}$	GR (1), k12 (1), k7 (1), k13 (2), k2APX (1), k3APX(1), k1APX (1), F (1), outwater (1)

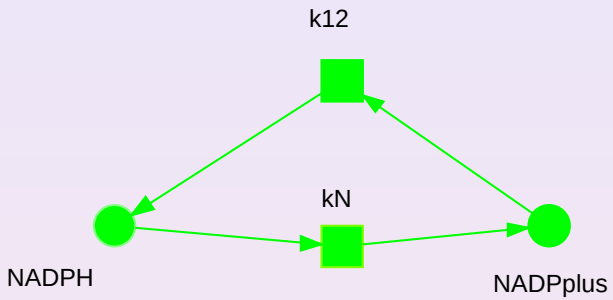
T-invariants (II)

T-invariant	Transitions/Reactions (number of fires)
$T - inv_{11}$	GR (3), k12 (3), k4 (3), k1 (3), k6 (2), k8 (2), F (2)
$T - inv_{12}$	GR (1), k12 (1), k4 (1), k1 (1), k8 (1), SOD (1), F (2)
$T - inv_{13}$	GR (1), k12 (1), k4 (1), k1 (1), k8 (1), k5 (1), F (2)
$T - inv_{14}$	GR (2), k12 (2), k4 (1), k7 (1), k1 (1), k8 (1), F (1)
$T - inv_{15}$	GR (3), k12 (3), k4 (3), k1 (3), k2APX (2), k3APX (2), k6 (2), k1APX (2), F (2), outwater (2)
$T - inv_{16}$	GR (1), k12 (1), k4 (1), k1 (1), k2APX (1), k3APX (1), SOD (1), k1APX (1), F (2), outwater (1)
$T - inv_{17}$	GR (1), k12 (1), k4 (1), k1 (1), k2APX (1), k3APX (1), k5 (1), k1APX (1), F (2), outwater (1)
$T - inv_{18}$	GR (2), k12 (2), k4 (1), k7 (1), k1 (1), k2APX (1), k3APX (1), k1APX (1), F (1), outwater (1)
$T - inv_{19}$	GR (3), k12 (3), DHAR (3), k1 (3), k6 (2), k8 (2), F (2)
$T - inv_{20}$	GR (1), k12 (1), DHAR (1), k1 (1), k8 (1), SOD (1), F (2)

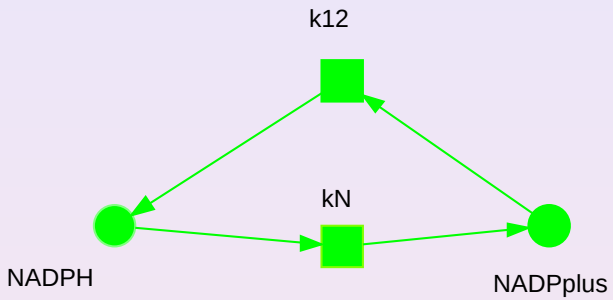
T-invariants (III)

T-invariant	Transitions/Reactions (number of fires)
$T - inv_{21}$	GR (1), k12 (1), DHAR (1), k1 (1), k8 (1), k5 (1), F (2)
$T - inv_{22}$	GR (2), k12 (2), k7 (1), DHAR (1), k1 (1), k8 (1), F (1)
$T - inv_{23}$	GR (3), k12 (3), DHAR (3), k1 (3), k2APX (2), k3APX (2), k6 (2), k1APX (2), F (2), outwater (2)
$T - inv_{24}$	GR (1), k12 (1), DHAR (1), k1 (1), k2APX (1), k3APX (1), SOD (1), k1APX (1), F (2), outwater (1)
$T - inv_{25}$	GR (1), k12 (1), DHAR (1), k1 (1), k2APX (1), k3APX (1), k5 (1), k1APX (1), F (2), outwater (1)
$T - inv_{26}$	GR (2), k12 (2), k7 (1), DHAR (1), k1 (1), k2APX (1), k3APX (1), k1APX (1), F (1), outwater (1)

T-inv2

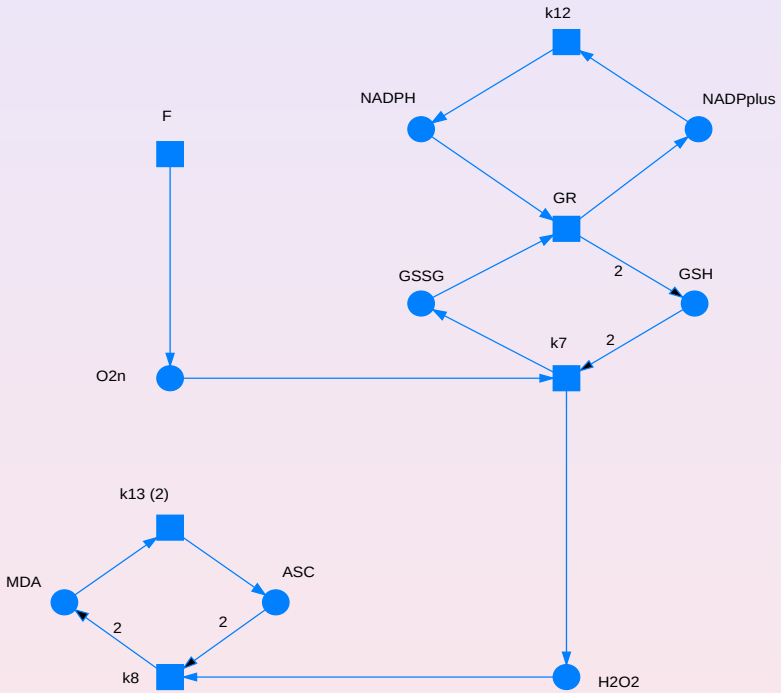


T-inv2

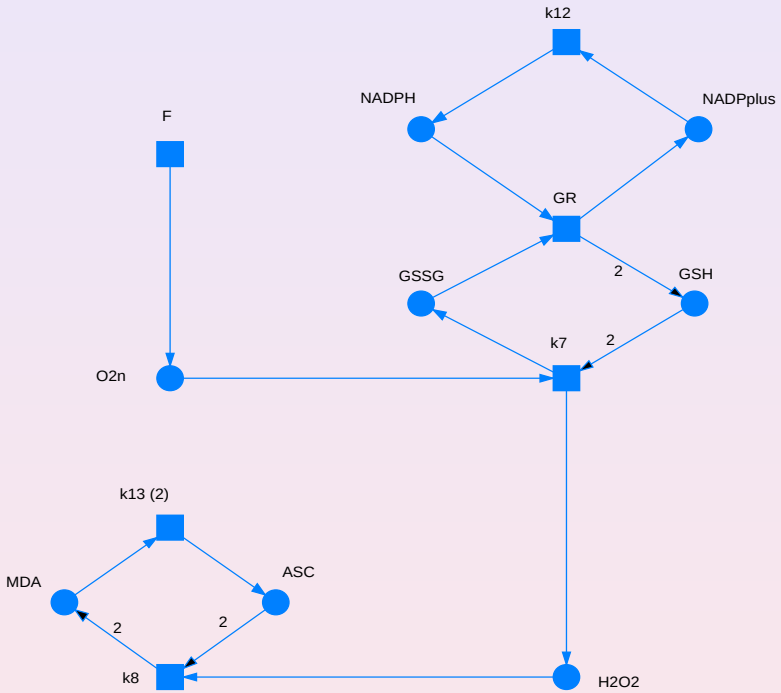


$T - inv_2$ is a trivial T-invariant.
These transitions capture a reversible reaction, each one modeling a direction in this reaction. Biologically speaking, it corresponds to the consumption and regeneration of *NADPH* in the two stages of the photosynthesis.

T-inv6

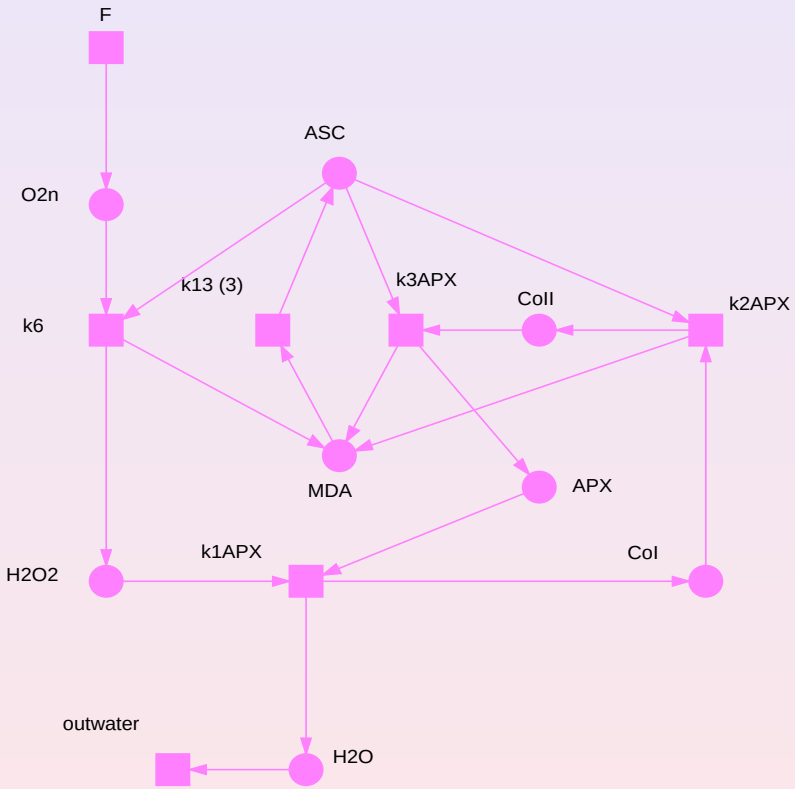

k12
NADPH
NADPplus
F
GR
GSSG
2
GSH
k7
2
O2n
k13 (2)
MDA
ASC
2
2
k8
H2O2

T-inv6

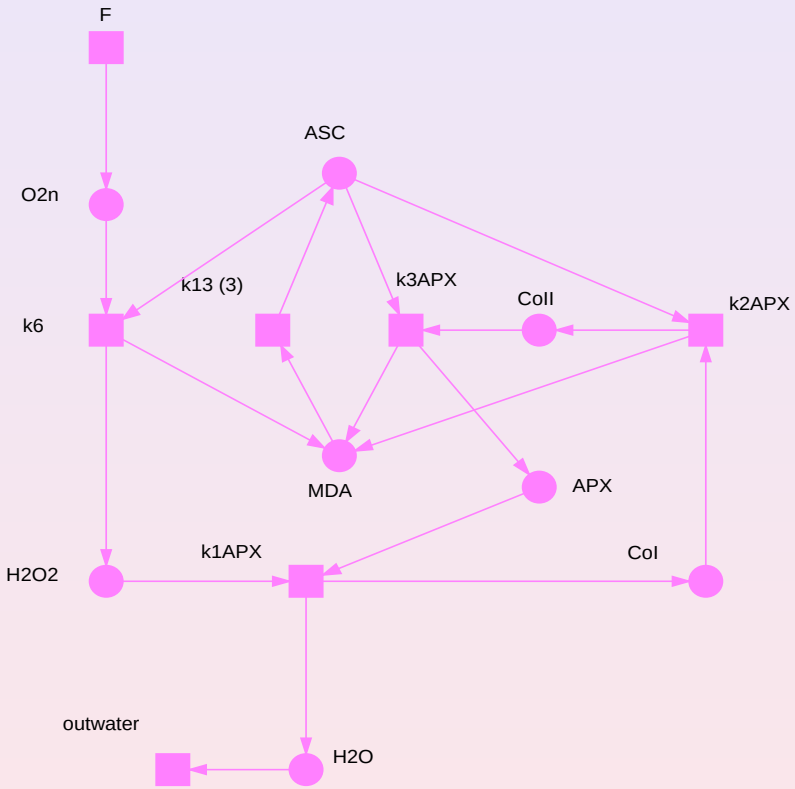


$T - inv_6$ represents in a very clear way the removal of O_2^- and H_2O_2 (reactive oxygen species) by reaction with the reducing agents *GSH* and *ASC*, at the expense of the reducing power of *NADPH*.

T-inv7

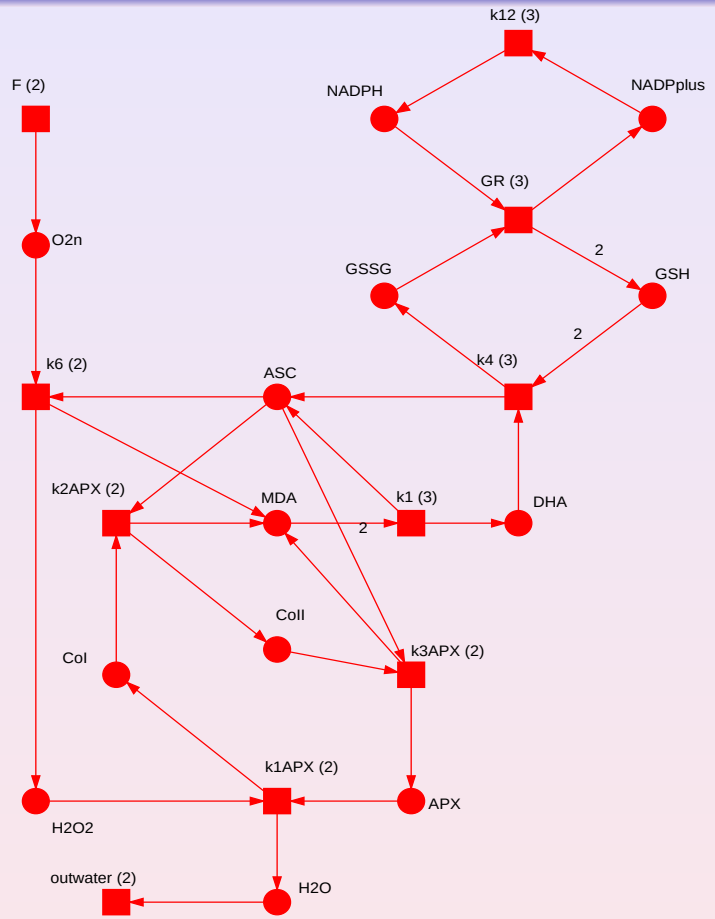

F
ASC
O2n
k13 (3)
k3APX
CoII
k2APX
k6
MDA
APX
k1APX
CoI
H2O2
outwater
H2O

T-inv7

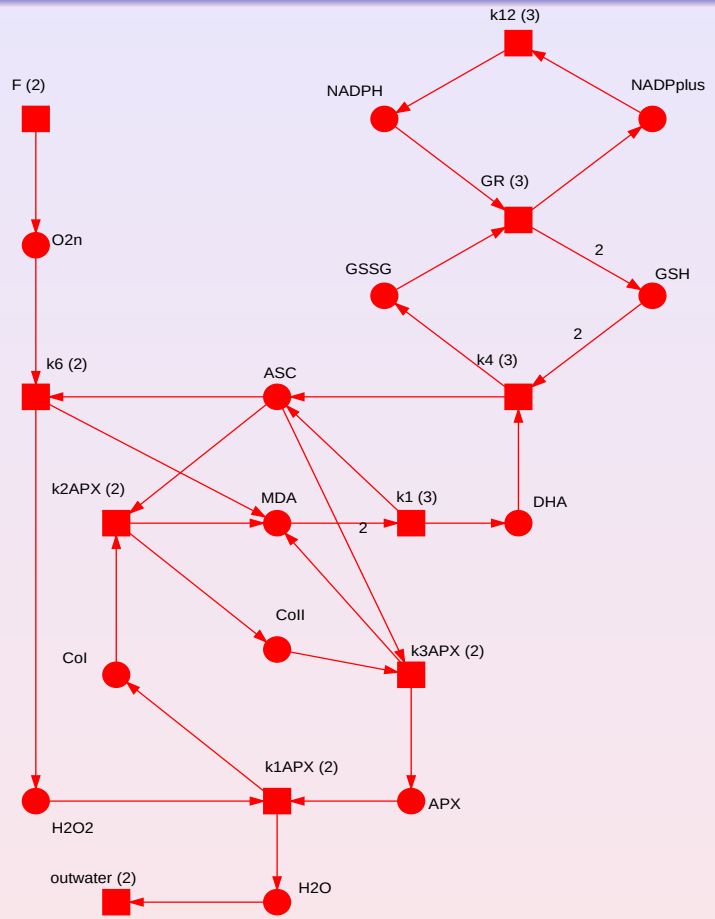


$T - inv_7$ refers to the catalytic cycle of APX.

T-inv15

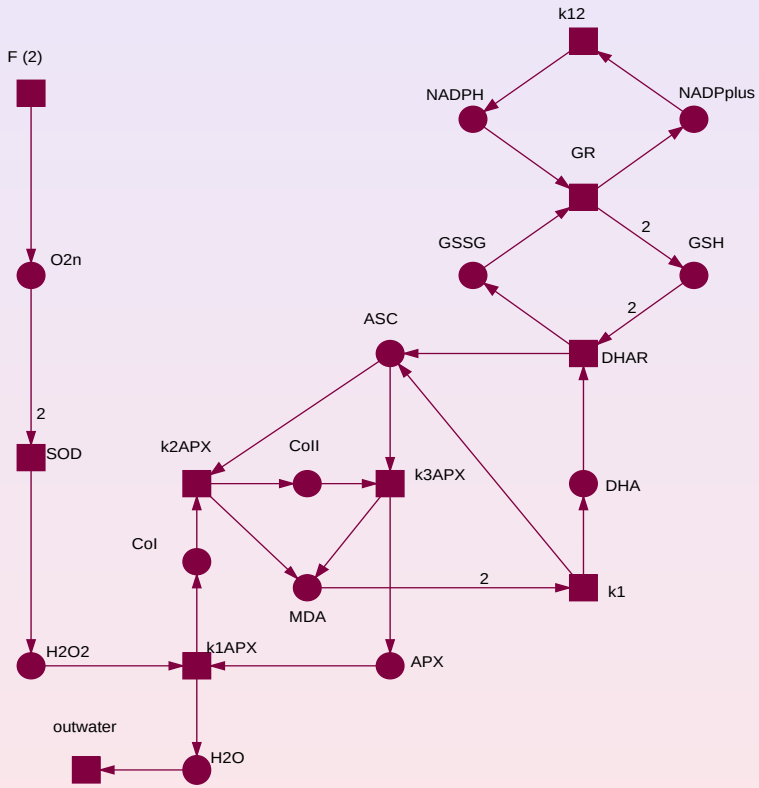

k12 (3)
F (2)
NADPH
NADPplus
GR (3)
O2n
2
GSSG
GSH
2
k4 (3)
k6 (2)
ASC
k2APX (2)
MDA
k1 (3)
DHA
2
CoII
CoI
k3APX (2)
k1APX (2)
APX
H2O2
outwater (2)
H2O

T-inv15

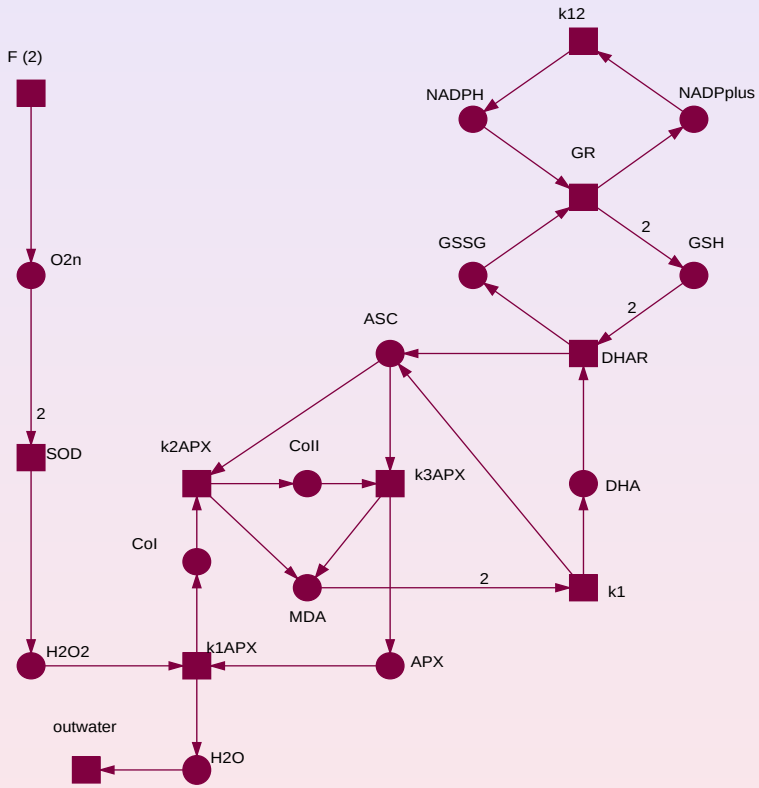


$T - inv_{15}$ means the removal of O_2^- by its spontaneous reduction to H_2O_2 in the presence of *ASC*, the subsequent removal of H_2O_2 by the catalytic cycle of *APX*, and the recovery of *ASC* through the substrate cyclying of *GSH* and *NADPH*.

T-inv24


k12
F (2)
NADPH
NADPplus
GR
2
GSSG
GSH
2
O2n
ASC
DHAR
2
SOD
k2APX
CoII
k3APX
DHA
CoI
2
k1
MDA
H2O2
k1APX
APX
outwater
H2O

T-inv24



$T - inv_{24}$ is a reflection of the enzymatic steps involved in the pathway: *SOD*, *GR*, *DHAR* and *APX*.

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- For instance, in order to consider the same cycle in dark conditions, we only have to remove in the Petri net the transitions F, $k12$ and $k13$.
- Then, if we now apply structural analysis we obtain the same three P-invariants, but we only obtain the first T-invariant, $T - inv_1$, which are the input (synthesis *de novo*) and output (inactive enzyme) of APX.

In order to identify the core net that represents the network's dynamics, we consider:

M. Heiner, K. Sriram,

Structural Analysis to Determie the Core of Hypoxia Response Network
PLoS ONE 5(1): e8600, doi:10.1371/journal.pone.0008600, 2010.

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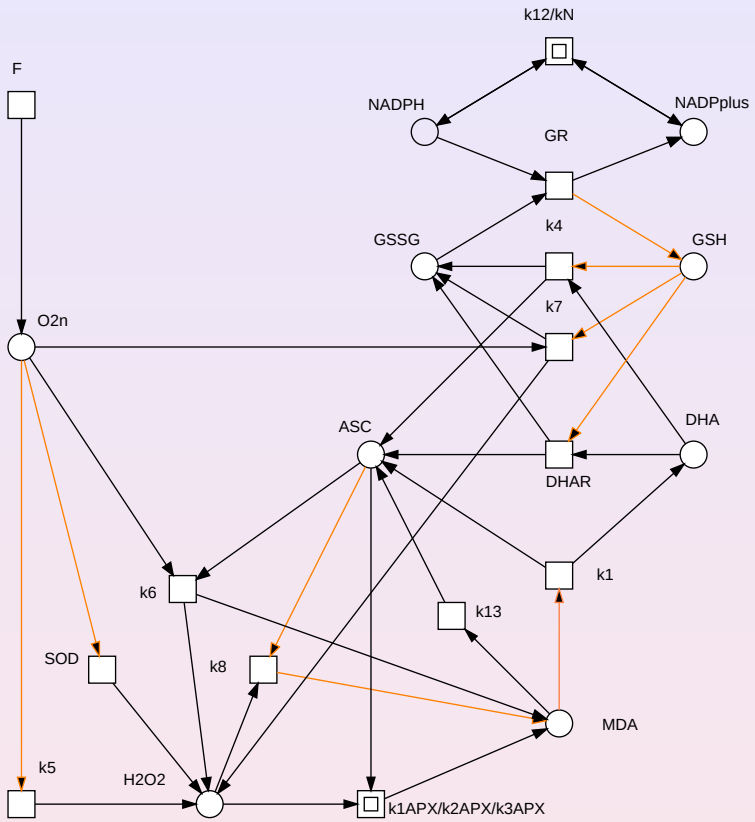
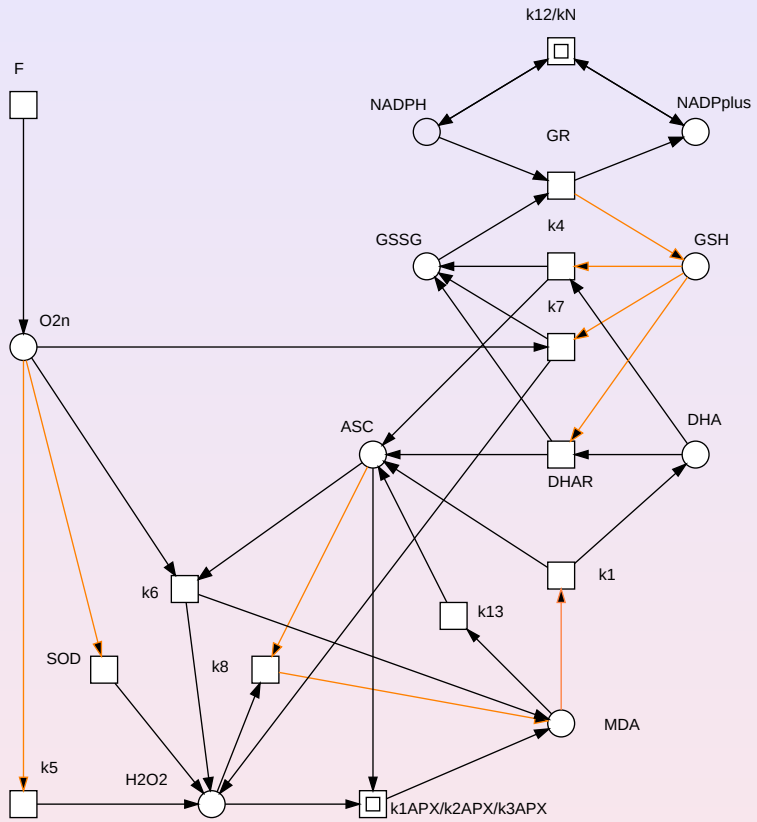
- $T - inv_2$ is a trivial T-invariant we can use a macro-transition to represent these transitions.
- We compute the maximal Abstract Dependent Transition (ADT) sets, considering that two transitions depend on each other if they occur always together in the set of T-invariants, obtaining in this case an only connected ADT set $\{k1APX, k2APX, k3APX\}$, that we can put also in a macro-transition (together with $T - inv_1$).


k12/kN
F
NADPH
NADPplus
GR
k4
GSSG
GSH
k7
O2n
ASC
DHA
DHAR
k1
k6
k13
SOD
k8
MDA
k5
H2O2
k1APX/k2APX/k3APX



This coarse network contributes to a better understanding of the behavior, allowing to test the robustness and the identification of the fragile nodes.

Robustness is defined as the ability of the system to maintain its function against internal and external perturbations.

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In the pathway under study, it has been very clearly shown that robustness is directly related to *APX* activity.

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- In the *GSH-ASC* cycle, if a mutation block *SOD*, there is a parallel spontaneous step for O_2^- dismutation (k_5).
- The same holds for *DHAR* (k_4), in such a way that chloroplasts can recover the reducing power necessary to detoxify reactive oxygen species in the absence of these enzymes.
- Redundancy of the pathway under study is clearly revealed by comparison of $T - inv_{15}$ and $T - inv_{24}$, which represent the chemical and the enzymatic pathways, respectively, to eliminate H_2O_2.

Another information that is teased out from the coarse network is that *NADPH* is the shared node for two pathways: the *Calvin* cycle and the *GSH-ASC* cycle.

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If the recovery of *NADPH* is silenced, it results in a complete loss of function of both pathways in the core network indicating that it is indeed the fragile node in the network.

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Conclusions

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- For that purpose, we have defined the specific continuous Petri net model that corresponds to the network of chemical and enzymatic steps involved in the cycle, and we have studied it in two ways: the quantitative one, which helps us to make a prediction behaviour; and the qualitative one, applying structural techniques, considering the core structure. We have obtained their corresponding biological interpretation that help us to understand this biological system.

Future Work

- We would like to add more steps into the pathway, that would help us to understand the biological behaviour considering new features such as dark-light interactions.

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- We also intend to apply other known formal techniques to the study of the GSH-ASC cycle in chloroplasts, for instance, we may apply model checking techniques in order to conclude whether a certain property is fulfilled or not by the biochemical system.

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- We also intend to apply other known formal techniques to the study of the GSH-ASC cycle in chloroplasts, for instance, we may apply model checking techniques in order to conclude whether a certain property is fulfilled or not by the biochemical system.
- It can also be of interest to derive probabilistic informations from a biochemical system, i.e., we can use a probabilistic framework, like stochastic Petri nets (SPNs), for the modeling of the GSH-ASC cycle in chloroplasts, and derive the relevant stochastic information of the system.

Thank you !

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