

# Qualitative modelling and analysis of Photosystem II<sup>\*</sup>

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**Abstract.** In this paper, a work-in-progress aiming at qualitative modelling of photosynthesis at the mechanistic cellular level by means of Petri nets is described. Presented preliminary results concentrate on modelling and analysis of photosystem II, a crucial component of photosynthesis. By employing qualitative model checking combined with invariant analysis we obtain new insights into electron transfer mechanisms studied in photosystem II.

## 1 Introduction

Photosynthetic reactions of green plants and some bacteria take place on a membrane of specialized organelles called thylakoids where a lot of protein complexes reside. The most important are photosystem I, photosystem II and cytochrome complex  $b_6f$ . These complexes are responsible for absorbing light and they bind the light energy that is further used and transformed in later phases of photosynthesis. Products of these reactions are oxygen and energy bound in ATP and NADPH. The first complex in the whole reaction cascade is photosystem II which is responsible for absorbing light, splitting molecule of water and exciting electrons that further reduce subsequent complexes. [15]. Photosystem II is also considered to have main influence to measured data (especially light-induced fluorescence)[9].

Photosynthesis is often modelled using photochemical and redox reactions reflecting only some of measurable values, e.g., chlorophyll fluorescence, concentration of carbon dioxide or oxygen [15]. In the case of chlorophyll, fluorescence absorbance/emission is driven by femtosecond-scale reactions considered to be transitions of electrons. For these reactions, determination of kinetic rates is very difficult even impossible. Moreover, the reactions are influenced by many other factors such as value of pH or environment temperature.

Recently, some estimating methods have been already used for creation of quantitative models of photosystem II using differential equations [11]. There are also many studies trying to understand precise function of each component of photosystem II. The measured data are moreover commonly explained by very different theories [9]. Some models were analysed qualitatively [10], nevertheless, their development has been still based on incomplete quantitative knowledge.

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The potential of using qualitative modelling in system biology is commonly underrated because of the high level of abstraction. On the other hand, it has been shown many times that qualitative modelling is very powerful and contributive in cases of lack or incompleteness of data [21].

Petri nets are very powerful formalism which has been proved useful for modelling of complex biological systems and for providing some interesting hypothesis about their behaviour only on bases of knowledge of interactions between its components. Notable examples include discovery of various metabolite functions and components with very specific behaviour, e.g., cycling or accumulation [18]. For example, Petri nets have been successfully applied to models of metabolic cascade [6–8]. Though based on a purely qualitative model, the analyses have brought new insights. Other applications show the advantages of using Petri nets for high level modelling of multi-cellular organisms [3].

To the best of our knowledge, there exist only a few applications of Petri nets to modelling of photosynthesis. A general photosynthesis reaction is used as a simple example in [16] while a more serious case study is provided in [14] targeting non-photochemical quenching by means of metabolic P systems.

This paper describes a work-in-progress aiming at qualitative modelling of photosynthesis at the mechanistic cellular level by means of Petri nets. Our first results concentrate on modelling and analysis of photosystem II, a crucial component of photosynthesis. For model development and analysis, we have employed the tools Snoopy [19] and Charlie [5]. We show that qualitative model checking is able to verify some commonly accepted theories about photosystem II.

## 2 Background and Problem Formulation

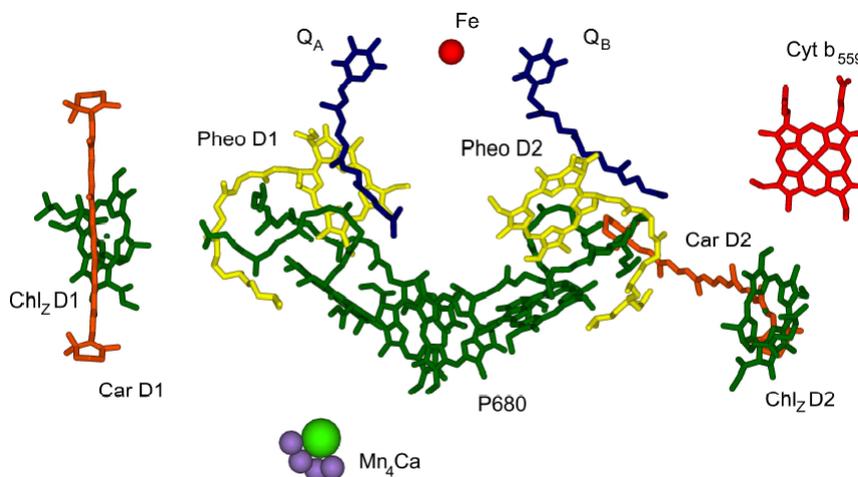


Fig. 1: Structure of photosystem II. Taken from [12].

Photosystem II can be divided into reaction center, oxygen evolving complex (OEC) and light harvesting complex (LHC). Reaction center is composed of *cytochrome b<sub>559</sub>*, *chlorin complex P680* and two other proteins very similar to each other ( $D_1$  and  $D_2$ ). Each of them binds other redox active components: *pheophytin (Pheo)*, *quinone ( $Q_a$  for  $D_1$ ,  $Q_b$  for  $D_2$ )*, *chlorophyll ( $Chl_z$ )*, *carotene (Car)* and *tyrosine ( $Y_Z$  for  $D_1$ ,  $Y_D$  for  $D_1$ )* [17, 20]. Because literature does not describe precise transitions inside complex P680, in this paper we neglect the fact that this complex consists of more than a single molecule. Very important is *oxygen-evolving complex (OEC)* bounded to P680. This complex is responsible for splitting the molecule of water and can be non-functional under some conditions [22].

The photosynthetic process begins with impact of photon to the LHC. Its energy is transited to the reaction center where it causes excitation of electron [13].

## 2.1 Basic electron path

The whole electron path begins with oxidation of complex of chlorins P680. Its electron reduces pheophytin Pheo. Then the electron continues to quinone  $Q_A$  and finally to quinone  $Q_B$  which is capable of carrying two electrons [20]. After receiving a second electron, quinone  $Q_B$  (now  $Q_B^{-2}$ ) is now neutralized by two hydrogen cations from the stroma (the outside of thylakoid) to quinol  $Q_B^{-2}H_2$  and leaves PSII into PQ-pool(space between PSII and complex  $b_6f$  containing 7-10 molecules of quinone/quinol). Into its place there comes a new neutral molecule of quinone  $Q_B$ . After leaving PSII, quinol transports electrons to the next protein complex ( $b_6f$ ) and releases hydrogen into the thylakoid inner space turning again into quinone that is able to recycle back to PSII [9].

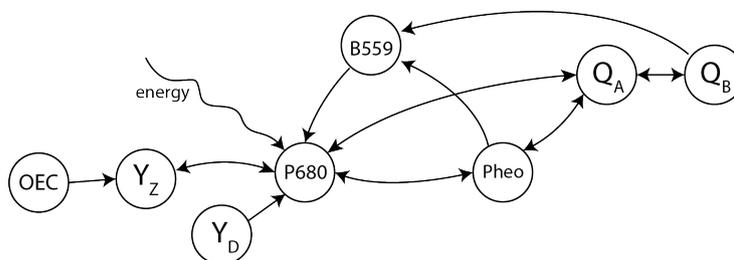


Fig. 2: Possible electron paths in Photosystem II.

When the P680 complex is oxidised, it receives one electron from *tyrosine  $Y_Z$*  (protein is situated between OEC and P680) which is subsequently reduced by one of the four electrons of OEC. After the fourth excitation of the electron from P680, the last electron is taken from OEC which is now capable to split two molecules of water. This reaction provides needed electrons and releases four hydrogen cations and two atoms of oxygen [20].

## 2.2 Alternative electron paths

Literature presents also other possible transitions of electron. In particular, there are possibilities of reverse transitions. Existence of those transitions is known between molecules of quinone  $Q_A$  and  $Q_B$ [11], Pheo and P680[23],  $Q_A$  and Pheo, P680 and  $Y_D$ , and finally  $Y_D$  and OEC[17]. There might be also a reverse reaction of binding (unbinding) of quinone  $Q_B$ (quinol  $Q_B^{-2}H_2$ ) [11].

Except for those reverse transitions, literature describes also another direct transition of electrons. There is a possible transition from reduced  $Q_A^{-1}$  to oxidised P680 [20]. Other path is reduction of oxidised P680 by an electron from cyt b559 which then can receive an electron from a molecule of Qb reduced at least once [20]. Oxidised cyt b559 can receive an electron also from reduced pheophytin Pheo [1]. Such paths through cyt b559 are important during inhibition of OEC function (e.g., by low temperature or damage). As long as P680 is oxidised, it becomes a strong oxidation agent and may cause destruction of the whole system. The alternative path through cyt b559 prevents such events by periodic reduction of P680 [1, 22]. Finally, another possible transitions are oxidation of tyrosine  $Y_D$  (a protein analogous to  $Y_Z$  bound to  $D_2$ ) and reduction of oxidised P680 where  $Y_D$  serves as the electron donor only once and during permanent illumination it stays in the oxidised state [22].

## 2.3 Existing models of photosystem II

It is worth noting that the literature shows only model structures created to better understand its content or those derived from quantitative models. There exist more models (described at structural level) differing in composition and interpretation of components and related transitions. This is the case mainly for OEC, pheophytin, cytochrome b559, and both molecules of tyrosine. OEC is modelled by four transitions between states  $S_i$  where  $i \in \{0, 1, 2, 3\}$  refers to number of missing electrons. In some cases, a free electron state  $S_4$  is used which is connected to  $S_0$  signifying the split of a molecule of water.

Structures are always described by a network of electron transitions, state transitions of a system, or by a reaction network of individual subunits.

Combination of these approaches was presented by Zhu et al. [23]. He divided photosystem into two subunits (P680/Pheo a  $Q_A/Q_B$ ) and presented their state graphs. Moreover, his model contains OEC with  $S_i$  states and a molecule of tyrosine. All mentioned parts are connected by electron transitions.

Nedbal [15] showed very comprehensive structure composed by many state graphs of the subunit  $Y_Z/P680/Chl_D/Pheo/Q_A$ . These graphs were connected by transitions referring to change of four states of  $Q_B$  ( $Q_B$ ,  $Q_B^{-1}$ ,  $Q_B^{-2}$  and  $E$  representing absence of non-presence of  $Q_B$ ). Finally, these graphs were obtained by interconnecting the sub-graphs with reactions representing changes among  $S_i$  states of OEC.

Lazar [10] discussed several structures of quantitative models and the possibility of using first-order and second-order kinetics for modelling reactions of (un)binding  $Q_B$  and reactions between OEC and subunit  $P680/Q_A/Q_B$ . Subsequent analysis of these two approaches was focused mainly on quantitative features.

Other important model structures can be found also in [2, 20].

## 2.4 Problem specification

As mentioned above, existing models differ in the level of detail or in the modelling approach employed. Naturally, there is an inevitable need to unify the notation of models. There are also many biological questions to be solved. For example:

- Does the behaviour and features of models depend on presence/absence of particular photosystem II subunits?
- Are all the current theories about photosystem II valid?
- Can the model reach some final (stable) state?
- Are there any possible electron cycles which have not yet been observed?
- Are the existing models correct?

## 3 Results

For answering these questions we need to create a bunch of models reflecting the absence/presence of particular components. We perform static analysis (discovering invariants and their interpretation) and dynamic analysis using computational tree logic (CTL).

### 3.1 Model development

There exist several approaches to modeling photosystem II using Petri nets depending on interpretation of places and transitions. Some of them can be seen in Fig. 3. In this paper, we employ places to represent states of observed components of photosystem II and transitions to represent electron transitions. Only exception is the case of  $Q_B$  where places refer to states of binding site or bounded molecule of  $Q_B$  and transitions represent electron transfer ( $Q_A \leftrightarrow Q_B^-$ ,  $Q_B^- \leftrightarrow Q_B^{-2}$ ) or (un)binding of  $Q_B$  or  $Q_B^{-2}$ .

We consider a basic model including only irreversible reactions and quinone  $Q_B$  modeled without E state. Refining the model by all possible states and related reactions results in a combinatorial explosion. Number of possible models created on a basis of knowledge mentioned in Sec. 2 is 4320. For this work, we have selected 11 models:

- A is basic model of PSII (see Fig. 4). It does not contain any irreversible reaction while it contains only complex P680,  $Q_A$  and  $Q_B$  without E state. This model reflects only the basic forward path of electron as mentioned in [20].
- B is an extension of the model A. There are considered molecules of pheophytin and tyrosine in the basic path. Moreover,  $Q_B$  is modelled with state E.
- C represents the model B extended by the reverse electron flow from quinone  $Q_A$  to P680.
- D represents model B extended by molecule of tyrosine  $Y_D$ .
- E refers to model C extended by molecule of cytochrome B599 and related transitions.
- F is a model created by the union of models E and D. It makes the complete model without any reversible transitions.

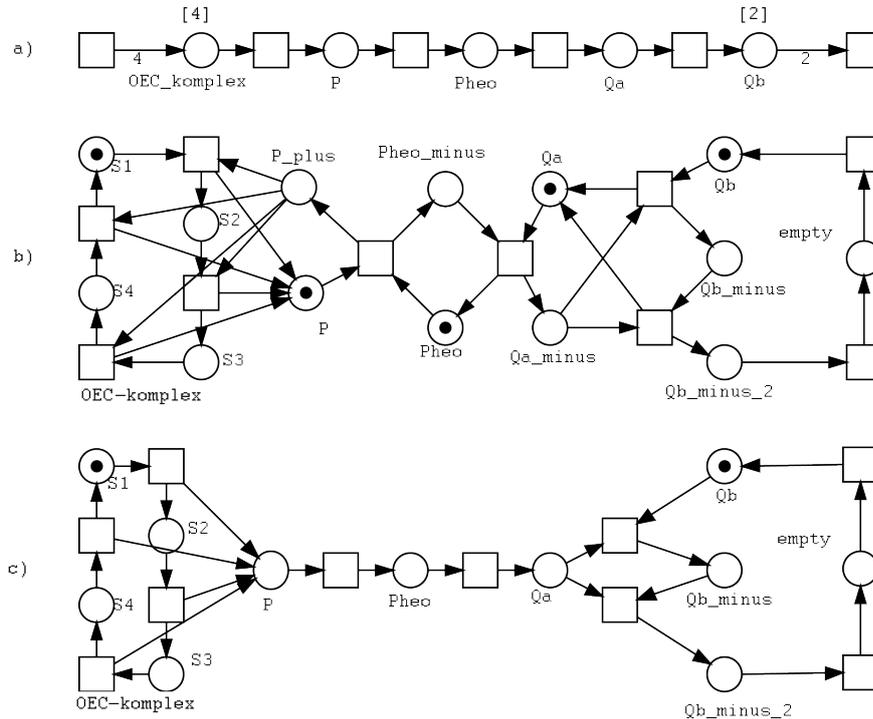


Fig. 3: The figure shows different approaches to modelling of photosystem II using Petri nets. In (a), places represent the component itself. In this case, the maximal number of tokens per place must be specified (normally it is 1 but  $Q_B$  can carry 2 and OEC up to 4 electrons). In (b), places represent states of individual components. Model *c* is created as combination of models *a* and *b*.

G represents model B extended by reverse transitions between  $Y_Z$  and P680, P680 and Pheo, Pheo and  $Q_A$ .

H represents model F extended by reverse transitions between  $Q_A$  and  $Q_B$ .

I is a model created by the union of models D and H.

J refers to model H extended by reverse transitions between states  $Q_B^{-2}$  and E (E and  $Q_B$ ) which represents possible reverse binding of  $Q_B^{-2}$  (reverse unbinding of  $Q_B$ ).

K is the complete and the most complex model containing all components and transitions mentioned in literature (see Fig. 4).

Moreover, we have created all models in two forms. Variant (a) (e.g.,  $K_a$ ) denotes models containing OEC. Models marked (b) refer to the system with a non-functional OEC.

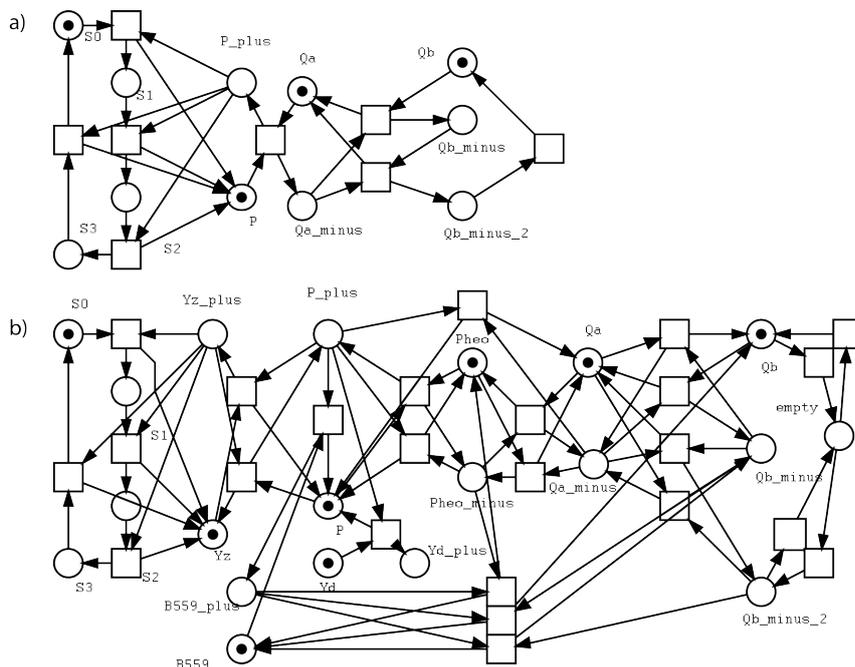


Fig. 4: The figure shows the simplest (a) and the most complex (b) model with the respective initial marking.

### 3.2 Initial marking construction

Before the analysis we need to specify what is the initial state (marking) of our system. It would not be necessary for models with the state space making a single strongly connected component. In this case, all states are reachable regardless of current state of model. Nevertheless, there exist models that do not satisfy that. The connected component in the state space can be interpreted as a permanent outflow/inflow of the electron from/into some system subunit. For the whole analysis we expect all components in neutral state and OEC in the state  $S_0$  (with four electrons present). Employing these constraints, the initial marking  $M_0$  is the following:

$P_{680}$	$P_{680}^+$	$P_{heo}$	$P_{heo}^-$	$Q_A$	$Q_A^-$	$Q_B$	$Q_B^-$	$Q_B^{-2}$	$E$	$Y_Z$	$Y_Z^-$	$S_0$	$S_1$	$S_2$	$S_3$	$Y_D$	$Y_D^-$	$B_{559}$	$B_{559}^-$
1	0	1	0	1	0	1	0	0	0	1	0	1	0	0	0	1	0	1	0

### 3.3 Invariants

*P-invariants* determine sets of places where the total number of tokens is conserved. For our models this can be interpreted as preserving all of redox active components in the model, e.g., P-invariants show that all components were present in the model during the process execution.

	$t_1$	$t_2$	$t_3$	$t_4$	$t_5$	$t_6$	$t_7$	$t_8$	$t_9$	$t_{10}$	$t_{11}$	$t_{12}$
$P \rightarrow Pheo$	1	0	0	0	0	0	0	1	1	1	1	4
$Pheo \rightarrow P$	1	0	0	0	0	0	0	0	0	0	0	0
$Pheo \rightarrow Q_A$	0	1	0	0	0	0	0	0	1	1	1	4
$Q_A \rightarrow Pheo$	0	1	0	0	0	0	0	0	0	0	0	0
$Q_A \rightarrow Q_B$	0	0	1	0	0	0	0	0	1	0	0	2
$Q_A \rightarrow Q_B^-$	0	0	0	1	0	0	0	0	0	1	0	0
$Q_B^- \rightarrow Q_A$	0	0	1	0	0	0	0	0	0	0	0	0
$Q_B^- \rightarrow Q_A$	0	0	0	1	0	0	0	0	0	0	0	0
$Q_B^{-2} \rightarrow E$	0	0	0	0	1	0	0	0	0	0	0	2
$E \rightarrow Q_B^{-2}$	0	0	0	0	1	0	0	0	0	0	0	0
$E \rightarrow Q_B$	0	0	0	0	0	1	0	0	0	0	0	2
$Q_B \rightarrow E$	0	0	0	0	0	1	0	0	0	0	0	0
$Y_Z \rightarrow P$	0	0	0	0	1	0	0	0	0	0	0	4
$P \rightarrow Y_Z$	0	0	0	0	1	0	0	0	0	0	0	0
$S_0 \rightarrow Y_Z$	0	0	0	0	0	0	0	0	0	0	0	1
$S_1 \rightarrow Y_Z$	0	0	0	0	0	0	0	0	0	0	0	1
$S_2 \rightarrow Y_Z$	0	0	0	0	0	0	0	0	0	0	0	1
$S_3 \rightarrow Y_Z$	0	0	0	0	0	0	0	0	0	0	0	1
$Y_D \rightarrow P$	0	0	0	0	0	0	0	0	0	0	0	0
$Q_A \rightarrow P$	0	0	0	0	0	0	0	1	0	0	0	0
$Pheo \rightarrow B559^+$	0	0	0	0	0	0	1	0	0	0	0	0
$Q_B^- \rightarrow B559^+$	0	0	0	0	0	0	0	0	1	0	0	0
$Q_B^{-2} \rightarrow B559^+$	0	0	0	0	0	0	0	0	0	1	0	0
$B559 \rightarrow P$	0	0	0	0	0	0	1	0	1	1	0	0

Table 1: Table shows which T-invariants were (1) or were not (0) found in particular models. All related models of model group  $b$  contain the same invariants with the only exception of  $t_{12}$ .

*T-invariants* refer to multi sets of reactions which can be interpreted as sequences of reactions the triggering of which will not change the system state. All T-invariants found are shown in Tab. 1. Invariants  $t_1 - t_7$  refer respectively to triggering single reactions and their related reverse counterparts. These t-invariants are usually called trivial invariants. Other invariants (except  $t_{12}$ ) describe more complicated cycles of electrons inside photosystem II. The most complex is invariant  $t_{12}$  expressing the whole set of reactions needed for a sequence of two subsequent unbinding events of  $Q_B^{-2}$ , e.g., sending 4

	$t_1$	$t_2$	$t_3$	$t_4$	$t_5$	$t_6$	$t_7$	$t_8$	$t_9$	$t_{10}$	$t_{11}$	$t_{12}$
A	0	0	0	0	0	0	0	0	0	0	0	1
B	0	0	0	0	0	0	0	0	0	0	0	1
C	0	0	0	0	0	0	0	0	1	0	0	1
D	0	0	0	0	0	0	0	0	0	0	0	1
E	0	0	0	0	0	0	0	1	1	1	1	1
F	1	1	1	1	1	0	0	1	1	1	1	1
G	1	1	0	0	1	0	0	0	0	0	0	1
H	1	1	1	1	1	0	0	0	0	0	0	1
I	1	1	1	1	1	0	0	0	0	0	0	1
J	1	1	1	1	1	1	1	0	0	0	0	1
K	1	1	1	1	1	1	1	1	1	1	1	1

Table 2: Rows refer to the models described in the first column. Numbers stand for numbers of occurrences of the transition required to revisit the original state. T-invariants are specified in columns.



*Formula*  $\phi_1$  describes the possibility of quinone  $Q_B$  reduction that allows unbinding of  $Q_B$  from the system thus making other parts of photosynthetic reactions possible.

$$\phi_1 = EG(EF(P \wedge Q_A \wedge Q_B) \wedge !(Q_B^{-2})) \quad (1)$$

Formally,  $\phi_1$  requires there exists a path in the reachability graph such that P/Qa/Qb is always reachable and Qb never reaches the twice reduced state. Validity of  $\phi_1$  means that Qb is never left unbound from PSII.

The formula has the same truth value for all corresponding models from both groups. Thus we can assume that the expressed property is independent of OEC function.

The formula is false only for models where the electron can cycle through reversible transitions without reaching  $Q_B^{-2}$ . In other words, presence of any other T-invariant than  $t_{12}$  violates the formula.

*Formula*  $\phi_2$  verifies if there exists for every state a possibility to reach a state where P680 is neutralized. This can be interpreted as the possibility to avoid the irreversible photosystem damage caused by long time oxidation of P680.

$$\phi_2 = AG(P^+ \rightarrow EF(P)) \quad (2)$$

Formula  $\phi_2$  is true for all models from group *a* where P680 can be always reduced by an electron from OEC. For models  $A_b$ ,  $B_b$  a  $D_b$  the formula is false because there are no reverse transition capable of reducing P680. In terms of T-invariants, the models do not contain any other T-invariant than only  $t_{12}$ .

Despite of the fact that models  $C_b$ ,  $G_b$  and  $H_b$  enable the excited electron to return, a specific state can be reached where the electron cannot be returned back. If is P680 after first excitation reduced by electron from  $Y_Z$  and this electron is also excited and transited to quinone  $Q_B$ , both of these electrons can be taken away and P680 will stay oxidised. Model  $E_b$  ( $I_b$ ) contains in addition cyt b559 (tyrosine  $Y_D$ ) capable of donating one electron to the system. The number of electrons is therefore 3 and although two of them can leave the system, one will be always able to cycle and periodically reduce P680.

Interesting is model  $F_b$  which contains both cyt b559 and tyrosine  $Y_D$ . Thus double oxidation of  $Q_B$  can be performed two times which would lead to exhaustion of system and permanently oxidised P680.

Formula is also true for models  $J_b$  and  $K_b$  where it is always possible to reduce P680 by a sequence of reverse transitions even from the PQ-pool.

*Formula*  $\phi_3$  describes cyclic behaviour of P680. Verifying the assumption that P680 is neutralized or oxidized, it will always get to the state where is oxidised or neutralized, respectively. In other words, the property ensures that the system does not stack with P680 permanently oxidised or neutralized which would lead to its disfunction caused by the absence of electron excitation events.

$$\phi_3 = AG((P \rightarrow AF(P^+) \wedge (P^+ \rightarrow AF(P))) \quad (3)$$

The formula is false for models containing any other reverse reaction than the one related with complex P680, in other words, it is false for all models containing any

t-invariant not related with P680. System in these reaction can cycle. This holds also for group *b*. Moreover this formula is true only in case when  $\phi_2$  holds, i.e., a final state satisfying P680 cannot be never reduced can be reached. Only model  $E_b$  does not have any final state and thus the formula is true. The reason is that the model contain only reverse reactions related to P680 ensuring its periodic reduction.

Formula  $\phi_4$  expresses the condition that if P680 is oxidised and  $Q_A$  and  $Q_B$  are neutralized, there is only one possible action - neutralization of  $P680^+$ .

$$\phi_{4a} = AG((P^+ \wedge Q_A \wedge Q_B) \rightarrow AX((P \wedge Q_A \wedge Q_B))) \quad (4)$$

To make the property clearer we define another formula  $\phi_{4a}$  varying for different models in order to ensure that all oxidizable (reducible) components are oxidized (reduced), respectively. This state displays an absolute lack of electrons in the system. For example, for the model including molecules  $Y_Z$  and  $Y_D$  the formula has the following form:

$$\phi_{4b} = AG((P^+ \wedge Q_A \wedge Q_B \wedge Y_D^+ \wedge Y_Z^+) \rightarrow AX((P^+ \wedge Q_A \wedge Q_B \wedge Y_D^+ \wedge Y_Z^+))) \quad (5)$$

Formula  $\phi_{4a}$  is true only for basic models  $A_a$  and  $A_b$ . In other models, there is included some other component capable of another step than reducing oxidised P680. More specific formula  $\phi_{4b}$  ensures that it will not happen. It is false only for models  $J_a, K_a, J_b$  and  $K_b$  which contains t-invariant  $t_7$  that means unbinding of neutral quinone  $Q_B$  ( $Q_B \rightarrow E$ ).

### 3.5 Discussion

Petri nets showed up as a feasible modelling formalism suitable for our problem. All models, even the most complex ones, are simple and transparent.

Considered binary interpretation of places and transitions seems to be optimal, because other interpretations would not be so explicit about states of components and it would be necessary to define maximal number of tokens present in every place.

T-invariants provided a simple way for representation and specification of non-trivial transitions needed for revisiting the initial state.

Formulas used for expressing observed features were also very transparent and easily understandable. Nevertheless, they proved some behaviour of system is possible despite it is actually very improbable, even impossible. An example is infinite cycling of electron using a forward reaction and its respective reverse direction.

An interesting result is that some formulas have the same truth value for corresponding model variants (*a*) and (*b*). Thus we can assume that expressed features do not dependent on the functionality of OEC. A special attention should be paid to models without OEC because some of them, especially the very comprehensive model  $F_b$ , can reach critical state with permanently oxidised P680. Special attention is needed during formulas formulation, e.g., formula  $\phi_{4a}$ . It is valid only for the simplest models in the hierarchy. An alternative formula  $\phi_{4b}$  has to be employed for other models.

Details on models, formulas and (im)possibility of verifying the requested features should be an objective of further discussions with scientists interested in photosystem II.

## 4 Conclusions and Future Work

In this paper, known facts about photosystem II have been summarized and we have shown preliminary results on its qualitative modelling and analysis. Created models have been compared wrt validity of CTL formulas representing crucial features of the photosystem. It has been shown that some features do not depend on the functionality of OEC. Moreover, it has been shown that some models can reach the final state in which the complex P680 is oxidised. Such a scenario would lead to disfunction and irreversible damage of photosystem II.

For future work we consider addition of formulas reflecting other features of the system. Furthermore, our research aims at targeting other protein complexes in the photosynthesis chain. Their models can be analysed individually and also integrated into a complex photosynthesis model. Obtained information about models can be also validated in a real system (if it is possible) or compared to existing quantitative models. Other direction could be an implementation of software capable of creation and analysis of all 4320 possible models of photosystem II and visualization of validity of specified formulas in similar way as in the graph shown in Fig. 5.

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