New Proposals for the Formalization of Membrane Proteins

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Abstract. This paper presents three new proposals to take advantage, in the framework of P systems, from proteins acting in bacteria. One attempt aims to focus on the transport protein that act as a logic AND gate at the cell membrane. The multiplicity of type of transporters involved in maintaining osmotic pressure within physiological values, both at short and long term level are also presented, as an example of parallelism occurring in living cell. Finally, the change of enzyme activity by reversible aggregation could be important for P systems as a new rule to follow, and process to model.

1 The Regulation of G6P-dehydrogenase

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that catalyzes (that is, it significantly increases the speed of) the reaction that takes away two atoms of hydrogen (thus the name dehydrogenase) from the chemical glucose-6-phosphate. This reaction is one essential step in glucose metabolism, occurring in almost all cells able to metabolize glucose. The activity of G6PD is modulated in cyanobacteria [20, 24, 13] by reversible transition of the enzyme between distinct aggregation states, which differ in catalytic activity. This process was discovered in 1978 by Schaffer and Stanier [20] who showed that the catalytic activity of this enzyme is much lower when it is found as single protein as compared with the catalytic activity of the aggregated protein.

We formalize the functioning of D6PD as follows. We denote by c one occurrence of the enzyme, and by c_n one occurrence of a cluster consisting of n molecules of enzymes linked together. In order to keep the formalization closer to the usual way of writing chemical reactions in Biology, we will denote the occurrence of n enzymes as nc and not as c^n, as it is usual, instead, in P systems.

The isolated enzyme c and the cluster c_n perform the same reaction, but with different speeds, and they both remove two atoms of hydrogen from the substrate G6P. Moreover, the assembly of the enzymes is reversible.

We also denote a molecule of G6P by the symbol a, and the product of the reaction by the symbol b.

The functioning of G6PD can be described by the set of rules:

1. $ca \overset{speed_1}{\longrightarrow} b$,
2. $c_n a \overset{speed_2}{\longrightarrow} b$,
3. $nc \longrightarrow c_n$,
4. $c_n \longrightarrow nc$.

Rule 1 shows that the enzyme, as single protein not aggregated with other identical proteins, catalyzes the dehydrogenation of glucose-6-phosphate, with a relatively low catalytic activity (denoted as the rule condition $speed_1$).

Rule 2 shows that the enzyme, in its aggregated form, catalyzes the same reaction of dehydrogenation of glucose-6-phosphate but with a significantly higher catalytic activity (denoted as the rule condition $speed_2$).

The aggregation (rule 3) and separation (rule 4) occur, e.g., in cyanobacterial cells as a mechanism to integrate G6PD activity in the assembly of the cell metabolism. It is beyond the aim of this article to illustrate the magnificent picture of the interplay between the whole metabolism and the activity of this enzyme, in time and space. However, the change in activity of an enzyme simply by reversible aggregation could be used in P systems as a new type of evolution rule or property for a specific (multi)set of objects, which could be seen either as a cluster or as separate elements with distinct properties. In the near future, following the trend in P systems [17, 18, 4, 7, 9, 25, 11, 6], reversible aggregation could also become a biochemical process to model.

2 Trap Systems as AND Gates at the Cell Membrane

The tripartite ATP-independent periplasmic (TRAP) transporter carriers are secondary uptake carriers requiring a periplasmic solute binding protein. They are active in prokaryotes (*Bacteria* and *Archaea*) and form a distinct family of transporters (for details see [16, 15]). They have been discovered in the anoxygenic phototrophic bacterium *Rhodobacter capsulatus*, its biological function being the unidirectional transport inside the cell of organic solutes such as succinate, malate, fumarate. These substances are needed by the bacterium for photosynthesis, respiration, growth and related biological processes. However, in natural environments, these substances are usually at very low concentrations and TRAP transporters allow their unidirectional passage from the environment into the cell. This uptake accomplished by TRAP transporters use the electrochemical ion gradient as energy. In Microbiology TRAP are important because they have distinct biochemical properties. In short:

- TRAP transporters differ from conventional secondary transporters (e.g., antiporters) with respect to the absolute need of an extracytoplasmic solute receptor, which

is able to bind the solutes (succinate etc.) even at very low concentration. Together with conventional secondary transporters, TRAP use the electrochemical ion gradient as energy source;

- TRAP transporters differ from ABC (from ATP-binding cassette, see [19]) system because ATP is not used as source of energy to perform the uptake against the concentration gradient; however, both systems have in common the occurrence of the extracytoplasmic solute receptor.

Taking into account the real interest of P systems for symport/antiport [18] it could be possible, as already suggested in [2], that TRAP transporters could receive some attention from P systemists. In this paper, we focus our attention on the possibility to describe its biochemical function as an AND logic gate.

To this aim, we denote by the symbol A the intrinsic TRAP membrane protein and by B the extracytoplasmic solute binding protein. Then, we associate to the membrane a set L of labels which corresponds to the proteins involved in the functioning of the TRAP system, that is $L = \{A, B\}$. We also consider the possibility of removing or attaching the protein B to the membrane, at any time. Hence, the cell membrane will be formally described as $[_A, [_B, [_{A,B}$ in the cases only protein A, only protein B or both A and B are present in the membrane, respectively. We also assume that protein B can be found outside the membrane, in any number of occurrences, while protein A always remains within the membrane (when already present, otherwise it cannot be attached to or detached from the membrane).

We denote by the symbol c the chemical which is transported across the membrane by the TRAP system, when both proteins are present.

We use communication rules of the form $c\ [_l \rightarrow [_l\ c$ $(l \in L)$ to describe the passage of the object c from the environment to the cellular compartment, and detaching/attaching rules of the form $B\ [_A \rightarrow [_{A,B}, [_{A,B} \rightarrow B\ [_A$ to describe the inactivation and the activation of the TRAP system. Note that only symbols c can pass through the membrane, and only symbols B can be attached or removed from the membrane (that is, added or deleted from the current label of the membrane).

The following rules describe the functioning of the TRAP system, which actually behaves as and AND gate:

1. $c\ [_{A,B} \rightarrow [_{A,B}\ c$,
2. $c\ [_A \rightarrow c\ [_A$,
3. $c\ [_B \rightarrow c\ [_B$,
4. $c\ [\rightarrow c\ [$,
5. $B\ [_A \rightarrow [_{A,B}$,
6. $[_{A,B} \rightarrow B\ [_A$.

Rule 1 determines the communication of the object c in the case the membrane is labelled with both symbols A and B, while rules 2 and 3 avoid the passage of objects when only one of the two membrane labels is present. This description is consistent with the structure and the functioning of the TRAP symbol.

Note that rules 2, 3 and 4 do not have any effect on the communication of objects nor on the change of membrane labels, though they provide here a complete description of the TRAP system as AND gate.

Proteins acting at the plasma membrane, such as TRAP transporters briefly described in this section (as well as other membrane proteins), could be taken into account for future lab experiments in looking for an implementation of P systems on *bio-ware*. Here, we only stress on the two following expected features of the proposed TRAP-based lab experiments:

- the high speed of the solute transport, thus a detectable output being produced at the time level of seconds;
- the possibility to control this uptake process by electrochemically adjusting the ion gradient across the membrane.

These only features would also make TRAP proteins (together with other protein systems acting as logic gates at the cellular level) valuable candidates for constructing a molecular computing device, a contest already opened by several works in the area of DNA computing. A similar approach has been used, e.g., in [26] where the use of synthesis rate of DNA binding proteins as (input/output) logic signals is proposed for the design and the implementation of digital logic circuits *in vivo*.

3 Short and Long Term Responses to Osmotic Pressure

Mechanosensitive channels of large conductance are involved in the response of *Escherichia coli*, as well as other bacteria, to hypo-osmotic shock [22, 23] and they already received some attention in the framework of P systems [3, 7, 6].

Mechanosensitive channels are not the only structures involved in osmoregulation and water balance in a prokaryotic cell, either on short term basis or on long term basis. Other structures involved in these processes are porins, aquaporins, glycerol facilitators, aquaglyceroporins, K transporters and osmoprotectant transporters, whose structure and functioning we briefly discuss in the following [8, 19, 1, 5].

Porins are proteins found only in the external membrane of Gram negative bacteria; three molecules belonging to the same type of porin form together a pore across the external membrane. This pore is filled with water and allow the passage of some ions and molecules according to the concentration gradient. Bacterial porins are not static, permanently open pores, but these channels can switch between short-lived open states and closed conformations, and can also remain in an inactivated, non-ion conducting state for prolonged periods of time [10]. For instance, in *E. coli* several porins are known, such as OmpC, OmpF (where Omp stays for "outer membrane protein"), and so on. The total amount of OmpC and OmpF are fairly constant but the ratio changes with the osmolarity of the medium. The proportions of these porins can be different for cells grown under given conditions. In *E. coli* the increase in the osmolarity of the growing medium induces an increase in the synthesis of the porin OmpC (and a decrease in the synthesis of the porin OmpF). Thus, at high osmolarity the outer membrane in *E. coli* contains more OmpC and less OmpF [10].

Aquaporins are transmembrane water channel proteins that are tetrameric assemblies of four subunits each containing its own aqueous pore; permeability studies indicate flow

rates in the range of 1 billion water molecules per channel and per second (more details [21, 1, 5]).

Glycerol facilitators are channels permeable to glycerol or small uncharged molecules whereas *aquaglyceroporins* are a new class of water channels which are also permeable to glycerol, but to a lesser degree than glycerol facilitators (for more details see [12, 14]).

K transporters act in this way: when *E. coli* is placed in a medium of high osmolarity (not produced by K ions), the cells respond by synthesizing a specific uptake system for potassium whose specific and controlled entry into the cell will contribute to maintaining the internal osmotic pressure in optimum limits. In *E. coli* there are Trk transporter, Kdp transporter and a sensor kinase (KdpD) that catalyze K uptake by *E. coli* with different kinetic parameters (for more details see [27]) during osmotic up shifts.

Osmoprotectant transporters become active also during osmotic upshifts, thus enabling the cell to accumulate osmoprotectants from the external medium. The best known osmoprotectant transporters are ProP transporter in *E. coli* and Betp transporter in *Corynebacterium glutamicum* (more details in [27]).

So, the interplay between different structures involved in the response to osmotic challenges, either upshifts or downshifts, at short and long term level - which are all hot topics in Microbiology - would be positively affected by the contribution from P system. Moreover, it has to be stressed that during osmotic challenges also other fundamental processes inside the cell are affected, such as respiration [27]. The integration of these changes in a more generalized mathematical model could benefit from the power of P systems to model biological processes occurring at the whole cell.

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