Chapter
Modelling bacterial hyperstructures with cellular automata

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#.1 Introduction

The physiology of prokaryotic and eukaryotic cells has been proposed to be determined at the level of hyperstructures [Norris *et al*. 1999] or modules [Hartwell *et al*. 1999] that would constitute a level intermediate between macromolecules and whole cells. Non-equilibrium hyperstructures include assemblies of genes, mRNA, enzymes and lipids brought together to fulfil a particular function and dismissed when no longer needed [Norris, et al. 1999]. For example, enzymes in the same or related metabolic pathways that are actively engaged in processing their substrates may have an increased probability of co-localization. To determine the values of the parameters governing the formation of hyperstructures in the membrane and cytoplasm of bacteria, we have constructed a program that, in its present version, simulates the dynamics of the formation of hyperstructures comprising enzymes responsible for the transport and metabolism of sugars due to changes in the affinities of its enzymes for one another. These changes result from the binding of enzymes to their substrates and result in increased diffusion coefficients [Norris *et al*. 1999]. In essence, the program uses cellular automata to represent both the cytoplasm in 3-D and the surrounding cytoplasmic membrane in 2-D. Each unit volume of the bacterium corresponds to a cellular automaton that can contain an enzyme (or, according to the size of the unit

volume, another molecule such as a lipid or a stretch of nucleic acid). The diffusion process of each enzyme in either the membrane or the cytoplasm is based on models of the diffusion of gas molecules on lattices. Up to 20 different types of enzymes in the cytoplasm and 1 type of membrane receptor can be studied using this program which we have applied here to the relatively well-understood system of glucose transport and metabolism in *Escherichia coli* [Saier 2000].

#.2 Hyperstructures

A myriad different constituents or elements (genes, proteins, lipids, ions, small molecules *etc*.) participate in numerous physico-chemical processes to create bacteria that can adapt to their environments to survive, grow and, *via* the cell cycle, reproduce. To try to explain how cells steer their way through the hyperastronomical combinations of these elements [Kauffman 1996], we have argued that certain genes, their products and associated lipids interact to structure membranes and cytoplasm into *hyperstructures* [Norris, et al. 1999]. Hyperstructures constitute a level intermediate between macromolecules and cells and correspond to the *modules* recently proposed to occupy a similar level in eukaryotic cells [Hartwell, et al. 1999]. Certain of these hyperstructures would assemble when needed and disassemble when no longer needed [Norris, et al. 1999; Norris, et al. 1999]. Examples of likely non-equilibrium hyperstructures in the bacterium *E. coli* include nucleolar-like hyperstructures for ribosome assembly [Lewis *et al*. 2000; Woldringh *et al*. 1994; Zaritsky *et al*. 2000], complexes of a replication hyperstructure to supply precursors directly to the DNA replication machinery and to prevent multiple initiation events [Norris *et al*. 2000] and a division hyperstructure to bring together cell division proteins and their genes at the cell equator at the time of cell division [Buddelmeijer *et al*. 1998; Norris & Fishov 2000]. Chemoreceptor complexes are clustered in *E. coli* into what may also be a hyperstructure since this clustering probably regulates sensitivity [Bray *et al*. 1998].

#.2.1 Non-equilibrium hyperstructures engaged in metabolism

In chloroplasts, association between glyceraldehyde-3-phosphate (an enzyme in the glycolytic pathway in bacteria) and phosphoribulokinase leads to the latter's activation which persists even after the enzymes separate [Lebreton *et al*. 1997]. In bacteria, evidence that substrate binding can alter enzyme affinities so as to promote assembly of complexes has been shown for protein export [Letoffe *et al*. 1996] and chemotaxis [Li & Weis 2000]. In our hyperstructure approach to the bacterial cell, enzymes in the same or related metabolic pathways that are actively engaged in processing their substrates (*active* enzymes) have an increased probability of colocalization due to altered affinities [Norris, et al. 1999]. Processing of a substrate may promote

association of transport proteins in the plane of the membrane together with metabolons of enzymes in downstream pathways in the adjacent cytoplasm to form a substrate-induced hyperstructure. These hyperstructures would dissipate upon depletion of substrate and hence would provide an efficient and adaptable metabolism. The essence of the idea of a non-equilibrium hyperstructure is that the clustering of integral transport proteins in the membrane and the structuring of related metabolic enzymes in the proximal cytoplasm depend on the *activities* of the relevant transporter and enzyme constituents involved. This might allow rapid reorganization of alternative hyperstructures in response to changing environmental conditions. Rapid adaptation of this type would contrast with slow adaptive mechanisms involving, for example, transcriptional regulation.

#.2.2 The phosphotransferase system and glycolytic pathway

The phosphotransferase system (PTS) of *E. coli* has been intensely studied and is therefore particularly useful for studying the dynamics of hyperstructure formation. The enzymes that constitute the PTS are responsible for the sensing and uptake of a large number of extracellular sugars and for feeding their products, cytoplasmic sugar phosphates, directly to the enzymes that constitute the glycolytic cycle [Saier 2000]. In *E. coli*, for example, there are many sugar-specific PTS permeases or Enzyme II complexes, and each consists of three or four proteins or protein domains, IIA, IIB, IIC and sometimes IID. The IIC and IID components are always integral membrane constituents while the IIA and IIB components are localized to the cytoplasmic surface of the membrane. Glucose transport, for example, depends on a membrane-bound $IICB^{Glc}$ which interacts with a cytoplasmic IIA^{Glc}, and IIA^{Glc}-P is in turn phosphorylated by another cytoplasmic protein, P-HPr. P-HPr derives its phosphoryl group from phosphoenolpyruvate in a reaction catalyzed by EI. Phospho-Enzyme I, HPr, IIA and IIBC are thus intermediates in the transfer of the phosphoryl group derived from phosphoenolpyruvate to glucose. It has been proven that IIC is dimeric and is likely that the Enzymes II form multiprotein complexes with the PTS energy-coupling enzymes, Enzyme I and HPr (for references see [Saier 2000]). However, it is not known whether formation of this complex or *metabolon* is substrate-induced.

Glucose-6-phosphate, released from the Enzyme II complex of the PTS, enters the glycolytic pathway. Evidence also exists for an extensive *glycolytic metabolon* [Srere 1994]. In eukaryotic cells, interactions between sequential pairs of glycolytic enzymes have been demonstrated, with glycolytic enzymes being partitioned

reversibly between cytoplasmic and cytomatrix-bound states depending on physiological conditions (for references see [Welch & Easterby 1994]). In *E. coli*, the glycolytic pathway has been isolated as an equimolar multi-enzyme complex in which compartmentation of substrates can be demonstrated. One such complex was reported to have a molecular mass of 1.65 megadaltons, similar to that calculated for an equimolar complex of the enzymes of glycolysis, and it exhibited a particle diameter of 30-40 nm [Gorringe & Moses 1978; Mowbray & Moses 1976]. Colocalization of Enzymes II actively engaged in sugar transport with a glycolytic metabolon would not only facilitate channeling of substrates but could also provide Enzyme I of the PTS with a high local concentration of the phosphoryl donor for sugar uptake, phosphoenolpyruvate, the product of glycolysis.

To obtain an idea of the numbers of enzymes to model, we used the gene-protein database which allows identification of many *E. coli* proteins *via* radio-labelling and separation according to pI and mass on large 2-dimensional gels (Nyström, unpublished;[VanBogelen *et al*. 1996]). In several cases we were able to confirm our results with literature available on the PTS and glycolysis (for references see [Saier 2000]). This information has been compiled in Table I.

Enzyme/gene	**Molecular Mass kDa**	**Radioactive counts PPM**	**Copies per cell**	**Comments**
$IICB^{glc}$ ptsG	45	802	2361	Membrane-bound, dimeric
IIA^{glc} crr	18.23	2819	20490	Forms oligomers, in operon with ptsH and ptsI
HPr ptsH	109	2710	39420	
E1 ptsI	63.412	3570	7836	dimeric
Phosphoglucose isomerase pgi				
Phosphofructo-kinase pfkA	34.758	2750	10483	
Fructose -1,6-P2 aldolase fbaA	33.4/33.9			
Triose-P isomerase tpi	26.971			
Glyceraldehyde 3-Phosphate dehydrogenase A complex gapA	35.5			

Phosphoglycerate kinase pgk	41.118			
Phosphoglycerate mutaseA gpmA	28.425	1400	6526	Assuming these counts do not apply to GpmB
Enolase eno	47.798	7870	21816	
Pyruvate kinase pykF	50.308	2010	5294	

Table 1. Genes/enzymes in the PTS/glycolytic pathway. The numbers of enzymes were obtained by labelling with radioactive sulphate taking into account that under the conditions and growth rate used 10^9 cells contain 220 micrograms of protein; we allowed for differences in cysteine and methionine content.

#.3 A cellular automaton approach to the PTS

Cellular automata are used to model many physical and biological phenomena [Vichniac 1984]. Once the cells that constitute the automata have been assigned initial states, the evolution of these states can then depend on both the previous history of the state and on the state of neighboring cells. Hence, cellular automata can be particularly suitable for modeling the dynamics of interactions between molecules in 3 dimensions.

To determine the values of the parameters governing the formation of hyperstructures in bacteria, we have constructed a cellular automaton program that simulates the dynamics of the localization of the PTS and glycolytic enzymes in both a 2 dimensional membrane and a 3 dimensional cytoplasm. Each unit volume represents a 10nm*10nm*10nm cube in a cell that can have a maximum volume of 200*200*200 unit volumes or $8\mu m^3$. This is more than sufficient to represent *E. coli* which is modeled here with a realistic volume (for the growth conditions used) of 2 cubic microns. Each cubic unit volume in the membrane is surrounded by 8 other unit volumes and each unit volume in the cytoplasm is surrounded by 26 others.

At each time step, all enzymes are considered in a random order. Each can move into a free neighboring unit volume. Given that the order of magnitude of the coefficient of diffusion in a bacterium is $1\mu m^2 s^{-1}$ [Elowitz *et al*. 1999], we consider that each enzyme diffuses across its 10 nm-sided cube in the equivalent of 0.1 milliseconds. The probability of a enzyme moving from its unit volume (initial state) to a specific unit volume (final state) is proportional to $P=P_0*\exp(\Delta\mu/(k*T))$ where P_0 is a random number, k the Boltzmann constant, T the temperature, and $\Delta\mu$ the difference in chemical potential between the two states or, to put it differently, the difference between the sum of the affinities of the enzyme in question for the enzymes neighboring the free unit volume into which it could move (note that its actual position is one of these free neighboring volumes) and the sum of its affinities for the enzymes neighboring its actual position. At each time step, each enzyme moves into

the free neighboring unit volume for which this probability is the greatest. In practice, the affinity between two enzymes, A and B, can be calculated from the affinity constant *K* for their interaction. The concentrations of enzymes A and B and AB complex can be used to determine *K* where *K*=[AB]/[A][B]. At equilibrium, *K* is equal to $e^{-\Delta G^\circ/RT}$ $mole^{-1}$ where R = N_A * k (N_A is the Avogadro number) and ΔG° is the free energy of binding of the AB complex. Binding energies for simple interactions in biological systems are typically in the range 4-17 kcal/mole. ΔG° is the affinity of 1 mole of A for 1 mole of B, hence the affinity of one enzyme of A for one of B is obtained by dividing ΔG° by N_A.

P_0 represents the complex interactions between an enzyme and the rest of the enzymes in the cell (collision, repulsion forces, etc.) that cause the diffusion of that enzyme. P is equal to P_0 when the enzyme has no neighbors with which it has an affinity; when, however, the enzyme does have neighbors, P_0 allows us to take into account the myriad interactions between intracellular enzymes that lead to them moving in a way that is not determined by affinities alone.

A_0 represents membrane receptors, A_1, the enzymes that catalyze the first cytoplasmic reaction in the PTS/glycolytic pathway, A_2, the enzymes that catalyze the second reaction and so on. The activation of a membrane receptor A_0 is represented by an increase of its affinity for any enzyme A_1 in its neighborhood. The activation of an enzyme A_1 is represented by an increase of its affinity for any enzyme A_0 or A_2 in its neighborhood. More generally, for any enzyme A_n, its activation is represented by an increase of its affinity for any enzyme A_{n-1} or A_{n+1} in its neighborhood.

A membrane receptor is activated by binding to a molecule of sugar. A_1 is activated by contact with an activated receptor (and that can release its substrate to A1). In general, enzyme A_n is activated by contact with an activated enzyme A_{n-1}.

In this model, we have assumed that the characteristic times of changes of affinities are very small compared to the time step (which is the characteristic time for the diffusion of an enzyme across its unit volume). This hypothesis implies that the effective activation of a receptor by a sugar (or of one enzyme by another) lasts the time step. In other words, when there is a concentration of sugar sufficient to saturate the receptors, these receptors are activated and *can activate the next enzyme* throughout the time step. We assume this concentration of sugar is physiologically reasonable.

In the present version of the program, up to 20000 enzymes of 20 different types in the cytoplasm and 5000 enzymes of 1 type of membrane receptor can be studied. The program runs on a PC.

#.3.1 Simulation results

The results that are presented here are preliminary results because they are only qualitative : we actually checked the size and the number of hyperstructures and the times when the entire structure of the cytoplasm appeared to be stationary by visualization of the cellular automata (see the following figures). Another limitation of the program is that the total number of cytoplasmic enzymes it permits in its present version is, for the moment, less than the PTS/glycolytic enzymes present in a real *E. coli* (see Table 1).

We carried out numerical experiments on a bacterium of 200x100x100 unit volumes ($2\mu m^3$) containing 5 types of cytoplasmic enzymes, each present in 3000 copies, and 1 type of membrane receptor, present in 2000 copies.

The bacterium was first modeled in the absence of glucose with an initial random distribution of enzymes in the membrane and in the cytoplasm. Under these conditions, we consider that there are identical, low, affinities between the enzymes. The enzymes diffused rapidly in both membrane and cytoplasm and hyperstructures did not form (see figure 1).

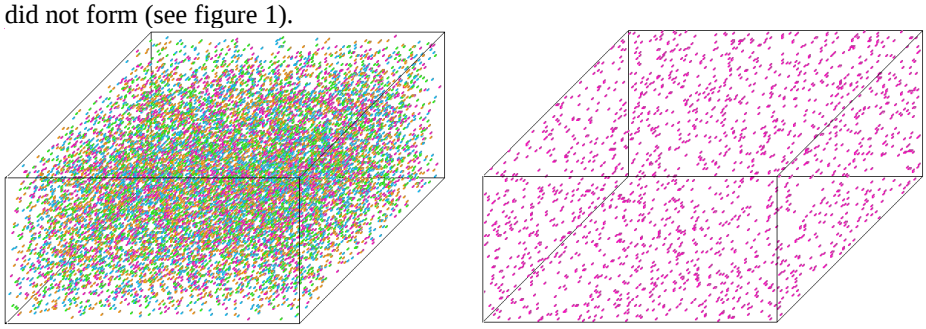

Figure. 1. Distribution of cytoplasmic enzymes (left) and membrane receptors (right) in the absence of glucose.

Subsequently, a series of numerical experiments were performed that modeled the bacterium in the presence of glucose at a concentration sufficient to saturate all receptors. At the start of each experiment, the enzymes in the membrane and in the cytoplasm were distributed randomly similar to that shown in figure 1. In each experiment, the same value for the increase in affinity between enzymes was used for all enzymes. However, in different experiments, values for this parameter ranging from 0.1 kcal/mole up to 50 kcal/mole were used. These showed that hyperstructures formed when the increase in affinity was greater than 0.3 kcal/mole; in these conditions, the bacterium reached a stationary state in which the overall distribution of hyperstructures did not change. The mobility of these hyperstructures was limited in comparison with the individual enzymes in the absence of glucose. The size of the hyperstructures seems to be related to the density of enzymes and to the value of the increase of affinity. The largest hyperstructures were obtained with small increases in affinity and high densities of enzymes. A typical example (Figure 2) shows that, with an increase of affinity equal to 0.5 kcal/mole, the system reaches a stationary state

after about 1.2 second (12000 time steps) where enzymes and receptors assemble into around 200 hyperstructures containing between 50 and 500 enzymes.

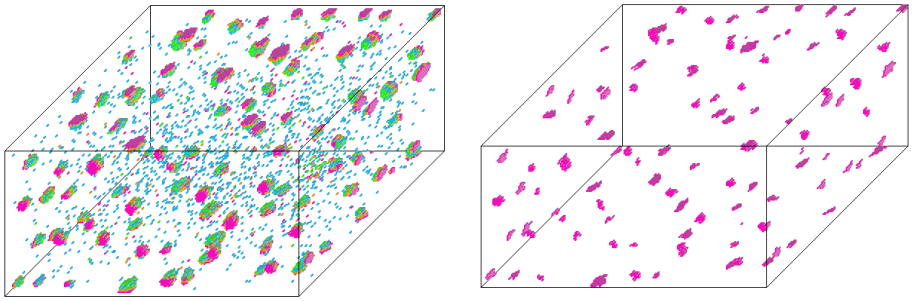

Figure. 2. Stationary distributions of cytoplasmic enzymes (left) and membrane receptors (right) obtained after the equivalent of 1.2 seconds in the presence of glucose, starting from random initial distributions.

#.4 Discussion

To reason in terms of hyperstructures, it is essential to understand the factors responsible for their formation. In the case of non-equilibrium hyperstructures that are assembled when needed and disassembled when no longer needed, our results suggest that a change in the affinities of enzymes for one another in the presence of substrates is a plausible factor. The PTS/glycolytic hyperstructures explored here involve a structuring of both membrane and adjacent cytoplasm and hyperstructures were generated containing up to 500 enzymes. An increase in affinity below the threshold of 0.3 kcal/mole did not allow formation of hyperstructures. At the densities of enzymes studied, large increases in affinity (e.g. above 10 kcal/mole) resulted in smaller hyperstructures than did smaller increases. It should be noted that although the concentrations of membrane receptors used were realistic for the PTS, the concentrations of cytoplasmic enzymes were considerably lower. This remains an important parameter to be studied with a more advanced version of our program.

In vivo, many factors must play an important role in creating large hyperstructures. For example, membrane domains and associated cytoplasmic structures are probably created by *transertion*, the coupled transcription, translation and insertion of proteins into and through membranes [Binenbaum *et al*. 1999]. We are therefore developing the program to model the effect of tethering the proportion of the receptors corresponding to nascent proteins to a patch of membrane. We are also developing it to give quantitative information on the sizes of hyperstructures. This should allow a quantitative evaluation of the effects of transertion.

In principle, our model (and related ones) could help interpret several metabolic or signaling pathways given details of affinities, interactions and concentrations. This possibility may encourage biochemists to obtain them.

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