Biosimulation and disease: the identification of new drug targets

Finding drug targets traditionally: high financial cost and ethical problems
Many diseases are caused by the activity of unwanted cells in the human body. These may be cancer cells, or parasitic microorganisms such as certain bacteria and fungi. With respect to the bacteria mankind has been quite successful in finding so-called antibiotics. These are substances that inhibit the growth of microorganisms. However, more and more bacteria are becoming resistant to the common antibiotics and therefore new and better antibiotics need to be developed. Moreover, a substantial number of parasitic microorganisms, such as the fungi, are chemically very similar to human cells. Consequently it is difficult to find good antibiotics against these that do not harm the patient. Indeed, most humans have experienced the difficulty of getting rid of fungal infections.

Traditionally much of the search for antibiotics was empirical. Multitudes of compounds were screened for their effects on microorganisms or cancer cells. Subsequently, the compounds that worked were tested on animals, and on cells in the test tube. The ones that showed much promise were ultimately tested at harmless dosages in humans, before the most successful of them were actually used as medication against parasitic infections or cancer. This involved an enormous amount of work at tremendous cost, and in the later stages also animal and human experimentation, which came close to the borderlines of ethics. For every new potential drug molecule, the entire procedure was repeated. Information of the work with earlier drugs was not always used intensively, nor was the wealth of information stemming from years of intensive research at many universities and other research institutions worldwide.

Biosimulation coming to the rescue
This may seem highly surprising, as the walls of many university laboratories show extensive pictures and maps that describe the functioning of living organisms. Indeed, much is known about that functioning. However, the situation is much like that of knowing where all the stones and bits of asphalt are of the streets of New York city: This information would not tell one where the traffic would flow, and where the traffic jams would be, as this would still depend on where the people are and where they want to go to. To understand the flow of traffic one needs to study the city in action. That action depends on all streets and all human activities at the same time and is therewith highly complex to understand. This is an aspect of living systems that is studied by Systems Biology (www.systembiology.net).

Biosimulation runs copies of processes that happen in real Life, in the computer. In the past biosimulations needed to simplify what happens in real living organisms. This was because too little was known of those organisms. Now, with the tremendous advance of knowledge about the building blocks of living organisms (i.e. about the stones and asphalt and about he destinations of the human being in the New York analogy), it is becoming more and more possible to run more precise simulations of the actual processes in living organisms.

For this all the knowledge about the parts of the living organism that are important for a certain function (such as its growth rate), is put into a computer program, which then serves as a computer replica of the pathways in the organism that deliver that

function. This replica is called a 'silicon pathway' and collections of such silicon pathways that are close to describing important aspects of the functioning of the organism, are called 'silicon cells'.

When the computer program is started, the computer calculates the functioning of the organism. It is important that scientists check that the functioning calculated by the computer indeed corresponds to the actual functioning of the organism, but after that has been done, the computer replica can be used more extensively for biosimulation.

Over the entire world thousands of scientists are collecting the molecular information that is important for the functioning of pathways in living cells. Some scientists put this information together and make computer replicas of pathways. Many of these silicon pathways are collected in libraries of models. The silicon cell initiative (SiC!; www.siliconcell.net) is one such library with the special feature that one can do experiments with these silicon pathways though the world wide web (www.jjj.bio.vu.nl). This silicon cell initiative participates in the BioSim network of excellence (http://chaos.fys.dtu.dk/biosim), and promotes biosimulation in this way.

Example of the use of biosimulation in finding a drug target
A tremendous asset of BioSim and SiC! is that anyone in the world with access to the world-wide web can now begin to engage in simple cases of drug design. Members of and contributors to BioSim can be similarly involved in the identification of actual drug targets by the used of silicon versions of the pathways worked on in the consortium. There is a training course for the use of the siliconcell live modelbase/library on the web:
http://www.bio.vu.nl/hwconf/teaching/Mathbiochemie/playsic8.htm .

We shall illustrate this here for the search for new drugs against sleeping sickness, the disease causing havoc in much of Africa by killing cattle and humans. The causative agent is a eukaryotic microorganism, called Trypanosome. After infection, this organism lives in the blood of cattle and humans, thriving on their abundant sugar. It grows by extracting energy from the sugar in the form of ATP. Thanks to a collaboration between the groups of Michels and Opperdoes in Brussels and Bakker and Westerhoff in Amsterdam, a silicon version of the pathway that leads from the blood sugar glucose to ATP in this organism, has been constructed and made accessible to the international community through the silicon cell library of live models.

This silicon pathway of trypanosomes is found by clicking www.jjj.bio.vu.nl. This will exhibit the opening page of the live model library. By clicking on 'Model Database' one is led to a list of silicon pathways. In this list one finds 'Glycolysis in *Trypanosoma brucei'*. Clocking on the adjacent 'model', one is led to the web pages where this model is live. On the right-hand side one sees a diagram describing the pathway. The pathway starts from glucose (which is the sugar and is written as 'GlcE' for 'Glucose external to the Trypanosome, i.e. in the patient's blood'), to ATP which is produced in reaction 12 almost at the bottom of the diagram. In the left-hand panel one finds the Values of all the relevant properties ('Parameters') of the molecules in the pathway. These properties have been determined experimentally and reflect a multitude of man-years of work in the two above groups and in many other scientific laboratories: here use is made of all the accumulated knowledge.

If one clicks on 'Evaluate model' the computer simulates what happens for the first 5 minutes after Trypanosomes are given the sugar (one may need to keep the Ctrl key pressed when clicking in order to enable the pop up to appear; one may need to continue to do this also below). The results of this simulation are shown in a new window. The concentrations of the compounds ('meatbolites') in the cell are given as a function of time.

We will here focus on finding a target for a drug against the organism. To this aim one now clicks on 'MCA' and then on 'Evaluate Model'. Now a complex matrix of numbers comes up. These numbers are so-called' control coefficients, which indicate the importance ('C') of all the molecules for the functions of the trypanosome. We are interested in the energy the organism can use for growth and this function is called 'JATPase'. Therefore we scroll down to the row in this matrix where one finds C's with as right-hand superscript 'JATPase'. Their magnitudes given as numbers below them, denote the importances of the molecules ('enzymes') indicated as the right-hand subscript, for the provision of ATP, hence for the possible growth rate of the organism. The C on this line (in this row) with the largest magnitude should be the process that is most important (most critical) for the growth of the organism. One finds that vGlcTr has an importance of 0.728413, and is much more critical (much larger in magnitude) than any of the others. vGlcTr refers to the transport of the sugar into the trypanosome. The implication is the glucose transport system is the preferred drug target.

We may now wish to simulate what the effect would be of a 50 % inhibition of the sugar transport system with a drug. To do this we click the matrix away, we click 'State', then on the circle in front of 'Steady state', and then again on 'Evaluate model' (sometimes one needs to click twice; also, do not forget holding the Ctrl key if the pop up is blocked by your computer). The computer replica responds with a window with a Table describing all the concentrations and rates at steady state. Again]we are interested in the energy, hence we look at the v[ATPase]. We write down its value on a piece of paper: 141.789.
We then move or click away this window and look up Vm1 in the Parameter list in the Table on the left. This is the maximum rate of the glucose uptake system; its value reads 106.2. We click on this value, use the backspace to remove it, and then type 53.1, which is 50 % of the original value. This will simulate an inhibitor at a dose that inhibits this transporter for 50 %. Then we hit the 'Return' key on our keyboard. Subsequently we again click on 'Evaluate model' and read the new v[ATPase]. This has reduced to 74.9961, which is indeed a strong reduction (from 141.789): the biosimulation has shown that a drug inhibiting the glucose uptake system should significantly affect the growth rate of trypanosomes.

Is the drug target specific enough?
We now found that a drug acting against glucose transport in trypanosomes should work against the growth of the organism. A problem could be that the same inhibition of glucose transport might also strongly inhibit the energy metabolism of the patient. This cannot be quite checked at the moment, because not for all human cells silicon cells are available. However, a silicon cell for human red blood cells ('erythrocytes'). *is* available on the siliconcell website thanks to the work of Holzhütter and colleagues.

We will now check that a drug inhibiting glucose transport in human red blood cells does not have a strong effect on the energetics of those cells.
To this purpose we click on Model Database. Then we seach in the list for 'Kinetic model of human erythrocytes' and we click on 'model' adjacent to this. We click on 'State' then on the circle in front of 'Steady state', then on 'Evaluate model' and then note that the rate of ATPase is 0.0258318. In the parameter Table on the left we note that Vmaxvo is 33.6. We click on this number, remove it with the back space and type in 16.8 instead (which is 50 %). We hit the 'Return' key of our keyboard and then click on 'Evaluate model' and read the v[ATPase] as: 0.0257983. We note that 50 % inhibition of the glucose transport system has hardly any effect on the ATP flux of 'silicon' erythrocytes (only reducing this from 0.0258318 to 0.0257983: a drug hitting the glucose transport system in both organism should kill the trypanosomes without significantly affecting the red blood cells of the patient.

The future
Biosimulation should now produce more silicon cell versions of other cells of the human body and ensure that also there inhibition of glucose transport has much less effect than the same inhibition of glucose transport in Trypanosomes. And, in parallel, silicon cells of other parasites and of tumor cells should be made, in order to discover new drug targets there.

Of course the drug targets calculated by this biosimulation approach should be tested experimentally and this may still involve some research with cell lines, experimental animals and eventually humans. However, because there is now a better selection of drug targets, the amount of this experimentation required per newly developed drug, should be reduced quite considerably, ultimately perhaps by a factor of more than 10.