Modeling of protein secretion by cells

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1 Abstract

Protein secretion by cells can be measured with surface plasmon resonance. However, based on the fact that the protein immobilized on the sensor surface and the produced protein are specific to each other, this method only measures the produced protein that binds to the surface. It was unknown beforehand how much protein doesn't reach the SPR sensor and stays in the bulk of the cell medium. To find out about this, a model was devised that sufficiently reflected the practical experiment and led to a simulation. From this simulation, it can be concluded that, under given circumstances, 99.1% of the total cell product binds to the surface. Therefore, single cell SPR measurements are an accurate way to determine cell production rates.

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2 Introduction

The experiments are performed with hybridomas which are specially engineered cells for laboratory applications. They are a fusion of antibody-producing cells and cancer cells. The result is a cell that can be cultured without dying after a few passages and which additionally produces antibodies. So, the cells can live indefinitely when supplied with sufficient nutrition. In this case, the amount of produced antibodies is measured in order to be able to select the hybridomas with the highest production rate, which would be beneficial for the pharmaceutical industry as they would be interested in cloning only the highest producer.[1–3]

The used method to measure production rates of single cells is surface plasmon resonance (SPR). There is a cell on a sensor made of gold coated with hydrogel containing preactivated ester groups. The ester groups of the hydrogel are able to bind proteins/antigens which is achieved in a process called immobilization. If the immobilized antigen gets recognized by the antibodies that are produced by the hybridomas a binding will occur. Polarized light shines from one side of the sensor, gets reflected and detected by a detector. The detector measures the intensity of the reflected light at different angles. At a certain angle there is a minimum in reflection intensity. With the binding of antibodies to the sensor surface the angle with minimum intensity changes. From this angle shift the amount of bound material on the sensor surface can be deduced.[4;5] This is also a disadvantage of the SPR measurement. Antibodies that are not bound to the surface, cannot be measured.

In order to support experimental results, a computer simulation is desired. Simulations have several advantages over experiments. They are not linked to a certain environment or circumstances and can be made as simple or complicated as necessary. It is also possible to only change one input and see the influence of certain parts of the experiment without the need to change the whole setup. Therefore, simulations are more flexible and require less effort when they are not too complicated. Additionally, all produced proteins can be seen. This simulation is made to quantify the amount of produced antibodies that are not bound to the surface of the sensor. In other words, how accurate is the production rate of a single cell measured with SPR?

In this special case, the typical SPR measurement can take up to several hours. Cells have to be cultured, the sensor has to be prepared, the experiment needs thorough preparation. The simulation should be as easy as possible and at the same time accurate enough. So, first a mathematical model needs to be developed that describes the experiment. This description needs to be imported in a simulation to get a result that agrees with experimental values within a certain standard deviation.

3 Theory

3.1 Model

The experiment that needs to be simulated, is a measurement with a single hybridoma cell on a sensor. The sensor is the bottom of a chamber which is filled with cell culture medium to keep the cell alive and producing. By diffusion the antibodies spread in all directions through the chamber and the ones that reach the surface of the sensor, bind to the antigen. This gets registered by the sensor which gives a signal.

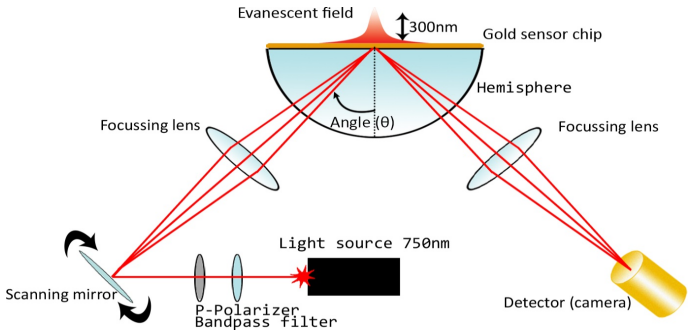

Figure 1: Schematics of an SPR measurement; antibodies binding to antigen which is immobilized on the sensor [6]

There is no flow in the chamber, that means the antibodies get transported only by diffusion which can by described by Fick's second law of diffusion:

$$\frac{\partial c}{\partial t} = D\nabla^2 c \tag{1}$$

Here, c is the bulk concentration of the antibodies in the medium and D is the diffusion constant. In this case it can be described with the Stokes-Einstein relation:

$$D = \frac{k_B T}{6\pi\eta r} \tag{2}$$

It shows that the diffusion constant depends on the Boltzmann constant k_B, the temperature T, the viscosity η and the radius of the spherical particle r. [7] The cell produces antibodies which means there is an inflow of concentration P at the cell boundary which depends on the production rate of the cell.

$$\frac{\partial c}{\partial t} = P \tag{3}$$

At the surface of the sensor, the antibodies bind to the antigen.

$$\frac{\partial c}{\partial t} = -k_{ads}(c_{max} - c_{ads})c + k_{des}c_{ads} \tag{4}$$

The change of surface concentration per time unit depends on k_{ads}, the adsorption rate, c_{max}, the highest possible surface concentration adsorbed on the sensor which is due to

a certain antigen density, c_{ads}, the surface concentration adsorbed on the sensor, and k_{des}, the desorption rate. Thus, specificity of the antigen to the antibody but also the amount of immobilized antigen on the sensor and the bulk concentration in the medium determine the rate at which the surface concentration changes. This change in adsorbed concentration on the sensor surface is described by the following equation:

$$\frac{\partial c_{ads}}{\partial t} = k_{ads}(c_{max} - c_{ads})c - k_{des}c_{ads} \tag{5}$$

There is no lateral interaction on the boundary, that means the antibodies aren't able to move once they are adsorbed. Matter can only enter or leave the system through the cell surface or the sensor surface. The other boundaries of the model are impermeable. [4;6;8]

3.2 Setting up the simulation

To make a simulation of this model, the software Comsol multiphysics (COMSOL, Inc, version 4.3b, released june 2013) was chosen. It is able to solve models numerically with the finite element method. It offers a graphical user interface which makes it easy to work with. Comsol offers a suitable predefined module called "Transport of diluted species" which is used for volume diffusion. On the boundary, the mathematical interface was used because it offers more control and transparency. The equations 1 to 4 serve as input and are at first implemented on a side view of the cell on the sensor.

From this view, the simulation is symmetric with respect to the y-axis. This can be used to keep the computation time low. That means Comsol computes only half of a 2D solution. Comsol also offers to rotate that 2D solution around the symmetry axis and predict the solution in 3D which is more practical because of the lower computation time. At the same time, it doesn't offer less information than solving for the 3D case in the first place.

The system (equations, domain, boundary conditions) is invariant for rotations around an axis through the center of the cell, and oriented along the z-direction. Therefore, the diffusion equation only needs to be solved on a half plane through this axis. Then the solution needs to be revolved around this axis to get the 3D concentration profile. This axisymmetric model is equivalent to a 3D approach, but much less demanding in terms of memory and computational time.

When implementing a new simulation with Comsol multiphysics, first one has to specify the number of spatial dimensions. In this case, it is an axisymmetric 2D simulation. In the next step, the physics have to be added that means the Transport of diluted species" and the "Coefficient Form Boundary PDE". The following step means choosing for a time-dependent study and that completes the setup of the simulation framework.

For further implementation, a geometry is needed. The rectangle in figure 2 represents the domain in which the experiment takes place. The bottom is the surface of the sensor with the cell as a cut out circle on top of it. The red line is the symmetry axis. The remaining sides are impermeable borders.

For a better overview, all important constants are inserted in the parameters node under global definitions. When a value is needed in another part of the simulation it

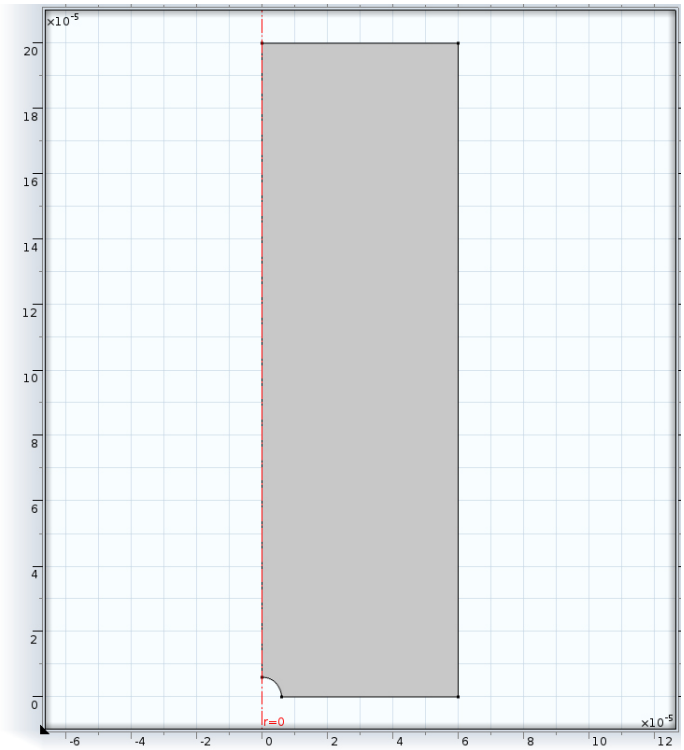

Figure 2: Geometry in Comsol: Side view of a cell on a sensor, cut in half because of symmetry; cell in the lower left corner, sensor surface at the bottom, the measurements along the horizontal and vertical axis are in meter

can be called upon with the constant name. Under the definitions node in model 1 a new variable node can be added for the change in bulk and surface concentration(see equation (4)).

The mesh divides the geometry in smaller subdomains. That enables the solver to find smaller subsolutions which form part of the complete solution of the simulation. For this purpose, the mesh should be very fine close to the cell and the sensor surface for an accurate, continuous solution. Further away from the sensor the mesh can be quite coarse to save computation time. Figure 3 shows the used mesh. In order to optimize the mesh size a couple of simulation runs were done, each with a finer mesh. At some point, the changes due to refining of the mesh don't lead to significant changes in the solution and this procedure gives a reasonable mesh size, that means the mesh is as fine as necessary and as coarse as possible.

Under the study node, the run time of the simulation can be entered. This results in solutions that could be measured in the experiment after the specified run time.

Comsol automatically creates a series of plots under the results node to see the dependent variables from the physics modules. For more information on how to work with Comsol multiphysics, see "Introduction to COMSOL Multiphysics" or the "Comsol

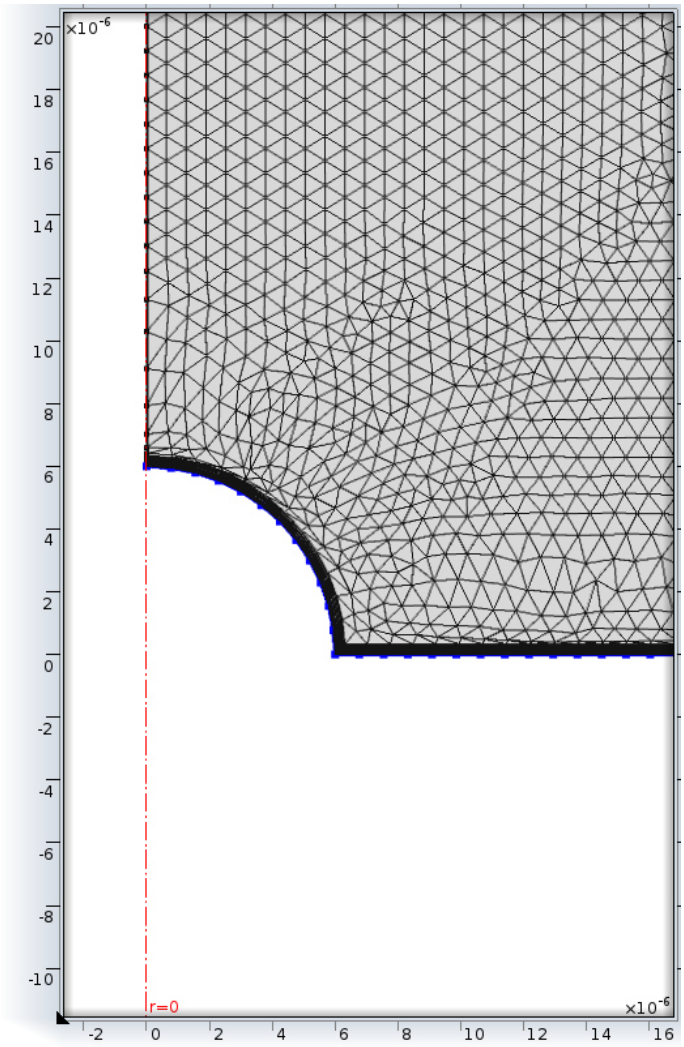

Figure 3: Mesh in Comsol: Division of the problem at hand in subdomains as preparation for the Finite Elements Method, only the part around the cell is shown; the whole mesh can be seen in figure 11

Multiphysics Reference Manual". [9;10]

It is important to note that this is a numerical solution. That means it is not exact but easier and faster to compute than an analytical solution. In this case, the solution gets approximated with the finite elements method.

The first step in this method is to divide the problem in several parts - subproblems - and to assume that every part has a certain shape. The smaller the parts are, the smoother is the solution but it also takes more time to compute. The specified mesh controls how the solution is divided. Then, Comsol looks for a function in every subproblem in such a way that the solution is continuous on the boundaries of the mesh

elements. Also, the boundary conditions and the initial values have to be fulfilled. The outcome of this is a smooth solution over the whole domain. [11]

As internal validation method, a full 3D version of the model was made to see if there are missing conversion factors due to the extra dimension.

4 Results

The following values were used to run the simulation:

physical quantity	value in SI-units
radius of the cell	$6 \times 10^{-6}\,\mathrm{m}$
production rate of a single cell	$0.3\,\mathrm{pg/h}$ ($1.86 \times 10^{-18}\,\mathrm{mol/h}$)
adsorption rate constant	$2.6 \times 10^{8}\,\mathrm{m^3/(mol * s)}$
desorption rate constant	$3.3 \times 10^{-5}\,\mathrm{1/s}$
capacity of the sensor surface	$1.0589 \times 10^{-8}\,\mathrm{mol/m^2}$
temperature	$310.15\,\mathrm{K}$ ($37\,^{\circ}\mathrm{C}$)
viscosity	$0.692\,\mathrm{mPa * s}$
radius of antibodies	$12\,\mathrm{nm}$
height of flow cell	200 um
width of flow cell	60 um
maximum amount of captured antibodies	$1.699 \times 10^{-6}\,\mathrm{kg/m^2}$
weight of antibodies	$160\,000\,\mathrm{Da}$

The production rate, the adsorption and desorption rate constants, the capacity of the sensor surface, the temperature and the size of the flow cell are directly measured in the laboratory. The other values are taken from literature. [12;13]

Figure 4 shows the surface concentration on the sensor after one hour. In Figure 5 the bulk concentration after one hour is visible. The total amount of antibodies on the surface is $1.859\,12 \times 10^{-18}$ mol while the antibodies not bound to the surface amount to $1.670\,59 \times 10^{-20}$ mol.

The full 3D simulation can be seen in figures 9 and 10. Here, the amount of antibodies on the surface is $1.698\,42 \times 10^{-18}$ mol with $1.617\,98 \times 10^{-20}$ mol of antibodies in the bulk of the medium.

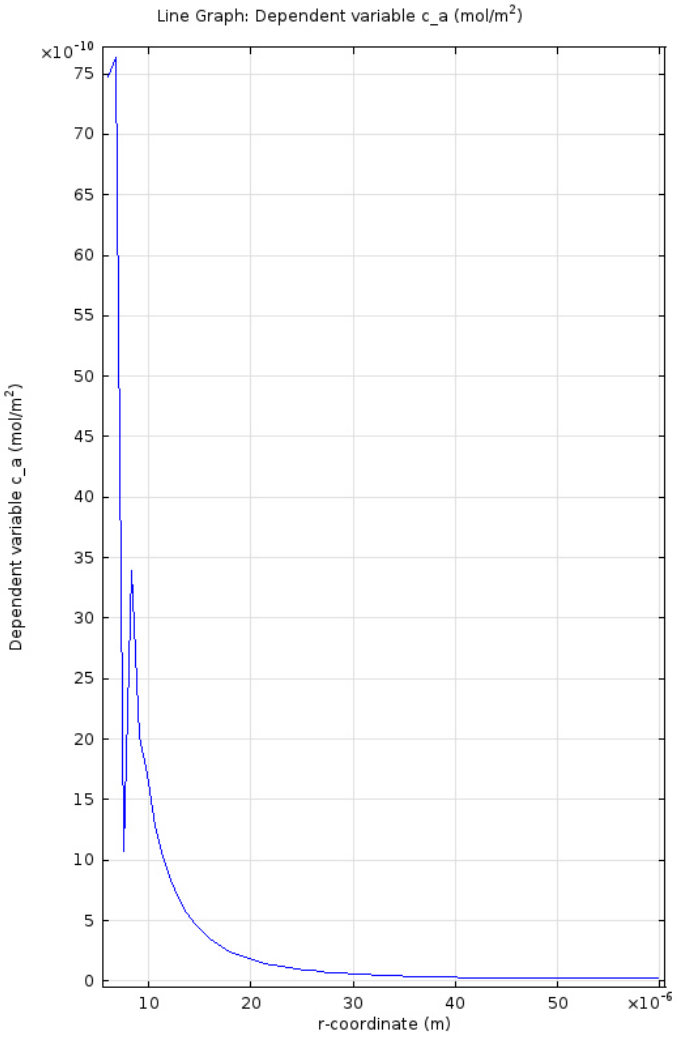

Figure 4: surface concentration along the sensor after one hour, with r the distance from the center of the cell

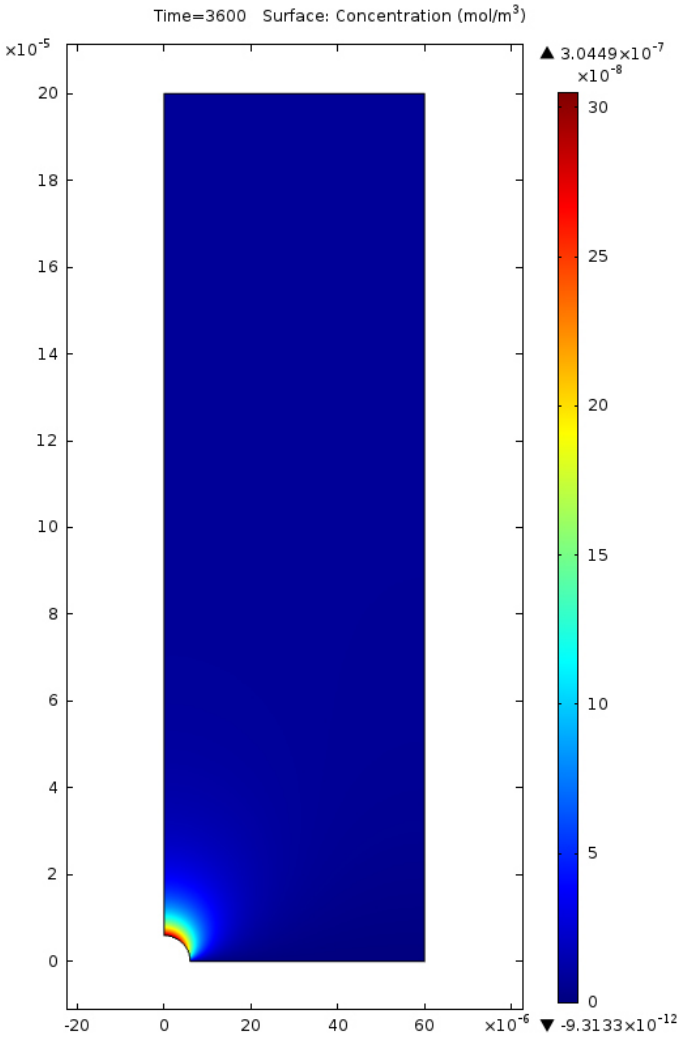

Figure 5: bulk concentration after one hour; concentration (see legend) in mol/m^3, with the distance from the center of the cell along the sensor r on the horizontal axis in m, the height z on the vertical axis in m

5 Discussion

First of all, these results were obtained with the model described in chapter 2.1. The model sufficiently describes the practical experiment but it also has some shortcomings that are introduced into the simulation.

The diffusion constant D is described by formula (2) but that is just an approximation that assumes antibodies are spherical. Antibodies are not spherical but they are relatively small compared to the size of the cell or the size of the domain. Measuring D was not possible in the accessible laboratory. Also, the surface of the sensor is not a flat, smooth surface the antibodies attach themselves to. There is a hydrogel on top of it that contains the antigen. The antibodies can enter the hydrogel layer and somewhere in that layer can bind to the antigen. However, the adsorption coefficients were measured and the result should not be affected by the different structure of the surface.

One big difference between model and reality is that cells are living organisms. In the model a cell has a constant production. In reality, cells not only try to produce antibodies but also divide under certain circumstances, they get older and react to their environment. These factors influence the production rate of antibodies by the cells. Therefore, the production rate used as input is a single cell production value obtained from several measurements of the same cell line.

As mentioned under 2.2, the result is a numerical solution to the problem at hand, hence an approximation computed with the finite element method. From that follows that the solution depends on the mesh. In general, the finer the mesh is the smoother and more accurate is the solution. In practice, available computation power also dictates how fine the mesh can be. This also explains the peak in figure 4 at about 6 µm. That is at the cell boundary. On the left of the cell boundary the surface concentration is zero and to the right of it the surface concentration can be quite high. To calculate a smooth solution at that point, Comsol requires a very dense mesh that was too taxing for the used computer. However, figure 4 gives an idea about the distribution of surface concentration. Also, the total amount of antibodies integrated over the whole sensor surface is just as expected.

For the full 3D version, the problem was similar. The mesh here is quite coarse. There are much more elements because of the added dimension. It could be seen that a finer mesh brings the amount of antibodies on the surface closer to the value of the solution computed with a 2D cut. The mesh size not only affects the accuracy of the solution but also the integration over the surface of the sensor.

The implementation of the model was also a challenge as there was not much experience prior to this project. Therefore, the first implementation was with Matlab. The used model only contained diffusion on a surface with discrete time and space (see figure 6 - 8). Introducing other important aspects of the model in Matlab seemed quite hard. Matlab doesn't appear to be suitable as a solver for partial differential equations. After that, the choice to proceed with Comsol was made because it has a detailed user interface and is specifically made for physics simulations. At the beginning, there was a steep learning curve and it was decided to use a purely equation based implementation.

However, Comsols documentation isn't clear about how exactly it converts the modeled 2D side cut to a 3D solution. This led to using the "Transport of diluted species" physics Comsol already comes with. The conversion is automatically made there while it sticks to the model described here.

Unfortunately, the measured value to control the result of the simulation is also the value used as input for it. There is no other method known that can measure the production rate of single cells under similar circumstances. It was tried to measure the production rate of cells by measuring the production of several million cells with another method. However, the environment the cells were measured in, was very different to the SPR measurement. Several samples had to be taken of the producing cells in medium and every sample meant disturbing the cells which in turn meant that the cells lower their production. Also, the cells were floating in cell medium while they stick to the SPR sensor. This has an influence on the cell production rate. Therefore, these production rates could not be compared.

6 Conclusion

The amount of antibodies on the surface after one hour in the simulation $1.859\,12 \times 10^{-18}$ mol is the same as the amount this specific cell produces within an hour according to the SPR measurement. That suggests that the earlier in this report explained experiment can be described by the simulated model. Furthermore, the amount of unbound antibodies is 0.9

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7 Appendix

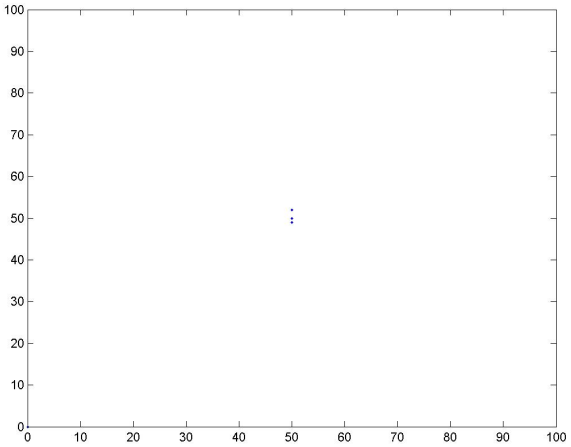

Figure 6: particles on a surface after three time steps, axis don't indicate a certain space unit

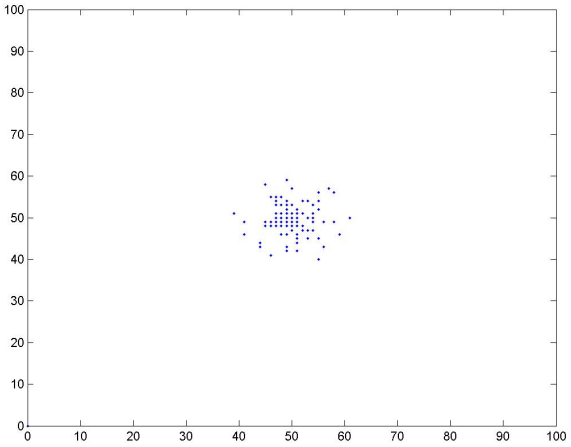

Figure 7: particles on a surface at a later time, axis don't indicate a certain space unit

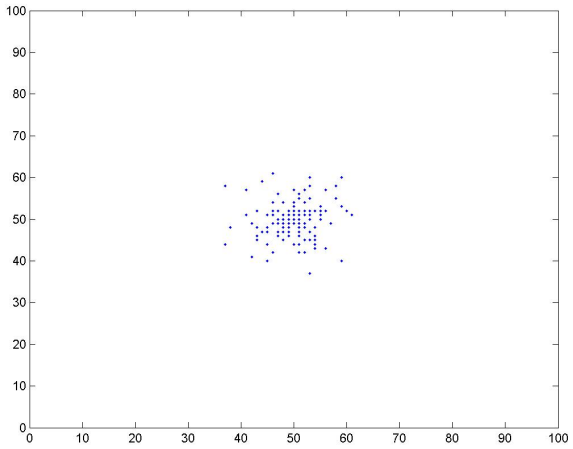

Figure 8: particles on a surface at the end of the simulation, axis don't indicate a certain space unit

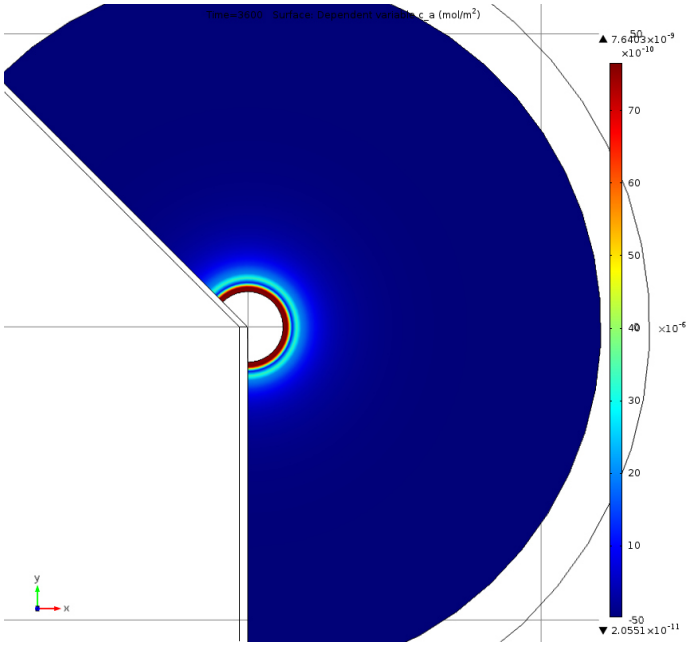

Figure 9: surface concentration after one hour, 3D case; concentration (see legend) in mol/m^2, with the distance from the center of the cell along the sensor r in m

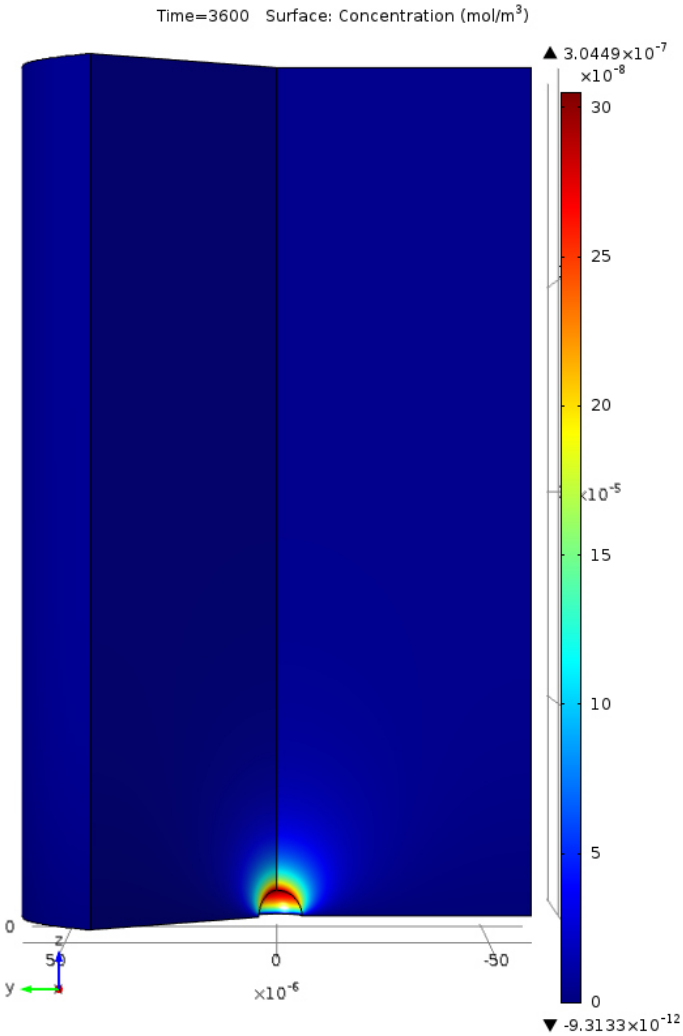

Figure 10: bulk concentration after one hour; concentration (see legend) in mol/m^3, with the distance from the center of the cell along the sensor r on the horizontal axis in m, the height z on the vertical axis in m

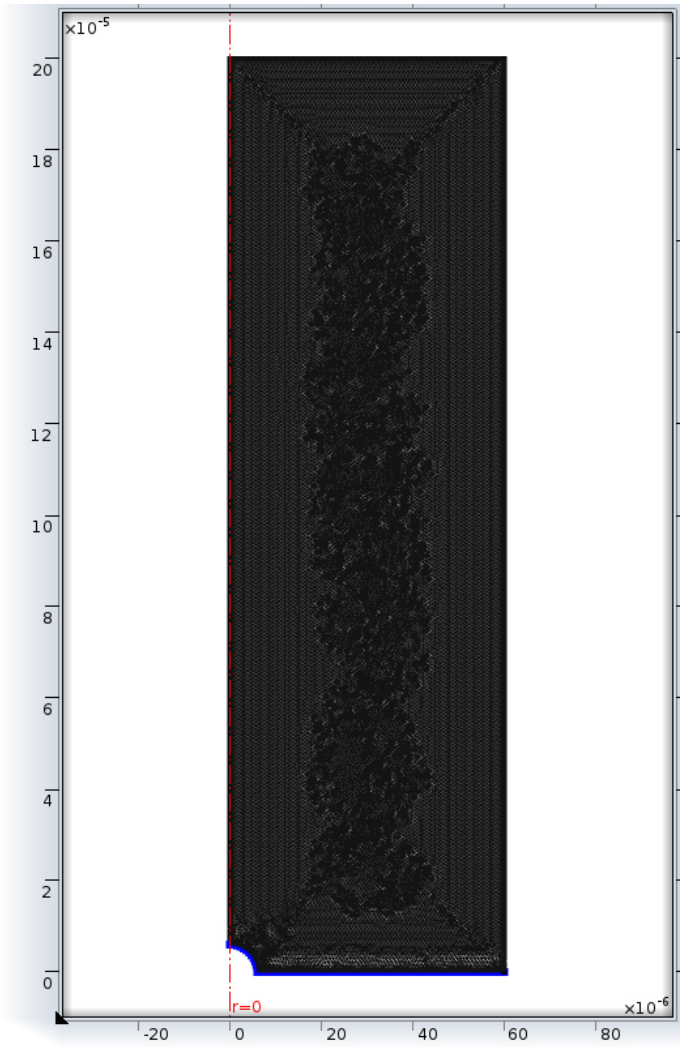

Figure 11: complete mesh