Short Visit Scientific Report
Modelling of competitive protein adsorption: The Vroman effect

Pol Vilaseca Mainar

July 15, 2010

Abstract

We study the process of competitive adsorption onto a surface, also known as the Vroman Effect. Using molecular dynamics we simulate a system composed by three families of proteins interacing with an attractive surface. The proteins are schematically modeled as soft spheres of different radius and masses. The interaction potential between each class of protein and the surface is an effective atraction resulting from implicit factors such as hydrophobicity or electrical attraction. We find that the surface is occupied in a sequential way, first by the light proteins that diffuse quickly, and later by massive and slowly diffusing proteins. The surface concentration of each protein shows a peak and later decreases, being displaced by higher affinity proteins. Whe plan to extend this study to higher number of families of proteins and to curved surfaces, replicating the surface of a nanoparticle.

Purpose of the visit

Nanoparticle-protein complexes are the result of the interactions among nanoparticles, with characteristic diameter between 50 and 250 nanometers, and the proteins dispersed in a complex aqueous solution. A typical solution used in the experiments is the human plasma, with more than 3700 different species of proteins with characteristic lengths, in the folded state, between 2 and 10 nanometers. Once the nanoparticles are introduced in the physiological environment, the charge interactions and the hydrophobic/hydrophilic interactions are responsible for an effective attraction between the nanoparticles and the proteins. This interaction leads to the formation of a layer (corona) of proteins adsorbed on the nanoparticles surface. The corona can constantly evolve, due to the interchange of proteins with the environment, or can be stable (hard caorona). The resulting complex, formed by the inorganic nanoparticle and the proteins, has different biological features and potential applications depending on the different nature of the proteins forming the corona.

In order to understand the mechanisms that lead to the formation of the corona around the nanoparticles it is necessary to characterize the physics of the interaction between proteins and the surfaces of the nanoparticles. The study of such interactions is important for the formulation of a model able to reproduce the experimental results and with predictive power. The analysis of such a model will make possible the investigation of new research directions for further experimental exploration.

During this visit we planned to start a strong collaboration with the experimentalists at the CBNI to design experiments suitable to elucidate the main contributions to the biomolecules-nanopaticles interactions. In such a way we want to establish the starting point for the theory of these bio-nano-systems, in the context of molecular and statistical physics. This theoretical work, in turn, will allow performing computer simulations, a powerful tool that can lead to a deeper understanding of how each single component of the system affects the global behaviour of the protein corona.

Description of the work carried out during the visit

Starting from the experimental information, we developed a schematical model for understanding the process of competitive adsorption of proteins to a surface, also known as the Vroman effect.

Whenever a solution containing dissolved proteins comes into contact with a surface the proteins diffuse towards it and form an adsorbed layer. The nature and composition of the protein layer depends on a large number of factors such as the concentration of proteins in the complex or the properties of the surface material. The different diffusion rates of different proteins and their surface binding strengths makes the protein adsorption a dynamic process. The initial adsorption is quickly followed by a turnover of the adsorbed protein and the replacement by another protein. In this way, the concentration of a protein in the surface increases in time reaching a peak before being replaced by a slower diffusing protein with higher binding strength. This process is sequentially repeated until the surface is occupied by the proteins with a higher adsorption affinity.

In this study we develop an explanation of the competitive adsorption process based only in the different diffusivities of the proteins and the interaction energy with the surface. The purpose of the study is not to reproduce quantitatively the experimental results but to show that the Vroman effect can be understood in a simple way through the menctioned terms.

The model

We design a simple model with three families of proteins, where each protein is represented schematically as a soft ball with mass M_i and hydrodynamic radius Rh_i. The attraction with a surface is represented by an effective interaction described by a Lennard-Jones potential given by

$$V(r) = 4\epsilon_i \left(\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6} \right) \qquad (1)$$

with different depths ϵ_i of the attractive well for each familly, and where r is the distance to the attractive wall. The parameters M_i and Rh_i will determine the different diffusivities in the bulk for each familly of proteins, and the depth of the attractive well ϵ_i determines the affinity of each protein to adsorb to the surface.

In order to compare qualitatively to experimental results we choose the values of the parameters to be those of Albumin, Inmunoglobulin$-\gamma$ and Fibrinogen (Table 1). The concentration of this proteins in the model is fixed to be the concentration found in human plasma. Time and temperature are given in Molecular Dynamics internal units. Temperature is chosen to be $T = 0.5$, value low enough so that the proteins with less affinity remain attached to the surface in absence of the higher affinity proteins.

	$Albu$	$Inmu-\gamma$	$Fibri$
$M_i(KDa)$	67	150	340
$Rh_i(nm)$	3.55	5.51	11.00
$\epsilon_i(M.D.units)$	1.00	2.79	6.08

Figure 1: Parameters for the mass, the radius and the binding energy for each familly of proteins used in the simulations. The values for the radius correspond to those of the hydrodynamic radius obtained by measuring the diffusion coeficient of each specific protein..

Description of the main results obtained

We perform Molecular Dynamics simulations in a rectangular volume with periodic boundary conditions in the x and y axis, and with the attractive surface placed in the lower side of the z-axis. The upper side of the z-axis is closed with a rigid wall. In order to keep the volume concentration of each protein constant we do not keep the number of proteins fixed. Whenever a protein adsorbs to the surface a new protein of the same class is created in the bulk. If a protein in the surface is displaced back to the bulk, a protein of the same class is annihilated. The annihilated protein is chosen to be the one located at the farest distance from the attractive surface.

We keep track of the number of proteins of each class attached to the surface as a function of time. Measures are taken for different plasma concentrations $C = 100\%$, 50% and 25%. We average over 14

independent simulation runs for each concentration, starting from different initial condition.

The behaviour of the system is qualitatively the same for all the concentrations simulated, finding a sequential competitive adsorption for all the cases (Fig. 2). At very short times the albumin, with a higher abundance and diffusivity, moves fast and adsorbs to the surface. The surface concentration of albumin reaches a maximum value and decreases when inmunoglobulin proteins arrive to the surface and displace albumin. This process is repeated at intermediate times, where inmunoglobulin surface concentration increases until it reaches a peak and decrease when the massive slow moving and high affinity fibrinogen proteins arrive to the surface. For long times the fibrinogen proteins slowly occupy the surface displacing the remaining albumin and inmunoglobulin until equilibrium values are reached. As the protein complex is diluted, that is at lower volume concentrations, the full process happens in the same sequence but dilated in a longer time scale.

Conclusions

Using a schematical model we have reproduced the sequence in which proteins occupy a surface. Hence, our simulations prove that the competitive adsorption of proteins to a surface, also known as the Vroman effect, can be understood in a simple way through the different diffusivities of the proteins, caused by the different masses and sizes, and the different affinities, caused by the different binding energies. We have seen that this ingredients are enough to generate the competition for assorption to the surface.

Future prespectives

As the next steps that naturaly follow our work we plan to

- Study the process of competition for a higher number of classes.
- Study the process of competition in a spherical surface of different radius to study the effect of curvature in the sequence of adsorption.
- Study the process of competition adding a more complex modelization of single proteins, allowing shape deformations or more detailed interactions between proteins.

We believe that by completing the previous tasks we will gain a deeper understanding of the mechanisms controlling the formation of a layer of proteins when a surface enters in contact with a protein complex.

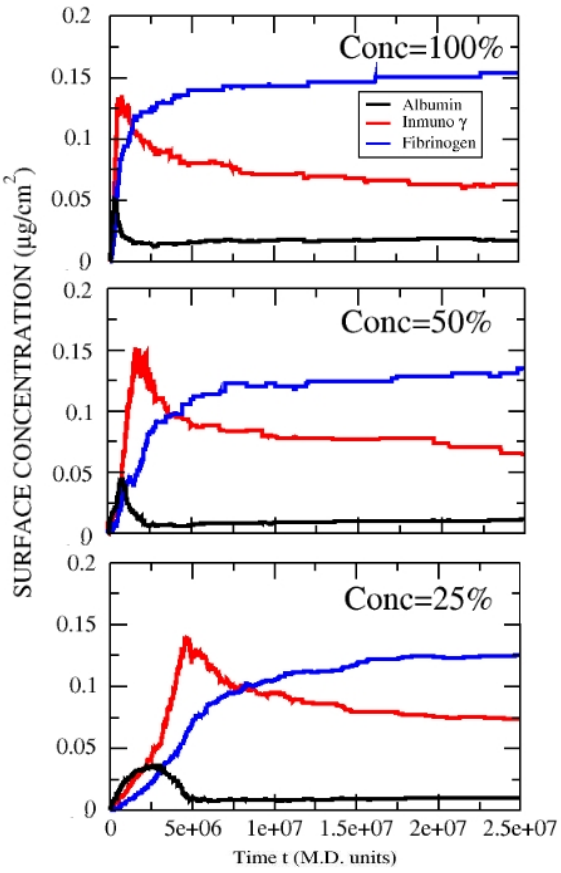

Figure 2: Surface concentration of Albumin, Inmunoglobulin$-\gamma$ and Fibrinogen as a function of time for plasma concentrations $C = 100\%$, 50% and 25%.

Projected publication

The current results are planned to be published together with the results of the study with a higher number of classes of proteins once this last are completed. The two steps of the study will conform a

detailed study of the competitive adsorption for flat surfaces.