

Molecular Dynamics Simulations of the Nanoparticle-Protein Corona

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Abstract: When a nanoparticle (NP) is introduced in a biological fluid, which is composed of several types of proteins, the system formed by the NP and proteins corresponds to a transitory regime characterised by complex behaviours such as competition between proteins and cooperative adsorption. The NP surface rapidly gets covered by some of these biomolecules, whose combination forms a corona around the NP called “protein corona” (PC), which can be categorised into two principal layers with different features. Here, we consider a silica NP in a model plasma made of three blood proteins: Human serum albumin (HSA), Transferrin (Transf) and Fibrinogen (Fibr). We study the adsorption process of these proteins that eventually leads to the formation of the “Hard Corona” (HC) using molecular dynamics simulations of a coarse-grained model. In particular, we notice that for a given biologically relevant time, the corona composition is different depending on the NP radius. This “size-effect” can prove to be of great importance when dealing with biomedical applications of nanotechnology. Furthermore, we study how the composition and structure of the corona changes with the introduction of a 3-body interaction between two proteins and the NP, which accounts for the formation of the more dynamic outer layer of the corona referred to as the “Soft Corona” (SC).

I. INTRODUCTION

Nanoparticles (NPs) in contact with a biological solution are very rapidly coated by a “corona” made of adsorbed biomolecules. In case of a protein solution, understanding how the protein corona (PC) evolves is relevant in fields such as medicine, nanotoxicology, nanotechnology, etc. These entities, which effectively give the NP its biological identity, are recognised by cells and organs and are capable of crossing biological barriers, such as the blood-brain barrier, and possibly reaching almost all cellular compartments [1].

Thanks to experiments, it has been hypothesized that the corona is made up of various layers of proteins. The present view is that initially, small and fast-diffusing proteins adsorb on the NP surface and are gradually substituted by larger, slow-diffusing proteins with a higher binding affinity. These larger proteins are those that eventually form the so called *hard corona* (HC). Meanwhile the smaller proteins, with a lower surface affinity, continuously exchange with the biological media forming a more dynamic corona called the *soft corona* (SC).

However, it is still debated if HC and SC have the same biological relevance. One might assume that the SC, if it is exchanged with the solution over seconds or less, might be less relevant than the HC, that is possibly adsorbed for longer times. Therefore, recently experimental and numerical studies of the PC kinetics have been combined to clarify the short-time behavior, inaccessible by experiments, and to match it with the long-time kinetics observed in the experiments [2]. Furthermore, these studies have revealed non-equilibrium effects in the long-time PC composition, depending on the protein incubation order that leads to a *memory effect* possibly relevant in biological processes [2]. The experimental memory effect has been reproduced by the simulations by modeling the

NP-proteins adsorption via a 3-body interaction [2]. Another puzzling behavior related to the PC kinetics that has been proposed based on experiments is the dependence of the PC composition on the NP size.

Here we investigate this dependency by performing molecular dynamics (MD) simulations of the coarse-grain model of a NP in a protein solution that has been capable of revealing the PC kinetics for fixed NP size [2]. We will follow the same protocol that has been adopted in experiments and simulations in the previous investigation [2]: We will introduce in solution three proteins, human serum albumin (HSA), transferrin (Transf) and fibrinogen (Fibr), at three different times and at a prefixed concentrations, all competing for the NP surface. We will study how the surface coverage of the NP changes with time as a function of the NP radius and test the dependency of our results on the presence of 3-body protein interaction.

II. THE COMPUTATIONAL MODEL

In our model [2], we consider three types of interactions with implicit solvent: a NP-protein interaction, a 2-body contribution of the protein-protein interaction and a 3-body contribution of the protein-protein interaction. The NP-protein interaction depends on the binding affinities of the proteins to the NP surface and is responsible for the formation of the HC. The protein-protein 2-body contribution is repulsive for proteins that do not aggregate in solutions. The 3-body interaction is attractive between *adsorbed* proteins and *free* proteins, mimicking the effect of conformational changes of adsorbed proteins. The latter interaction enables the creation of the SC [2, 3].

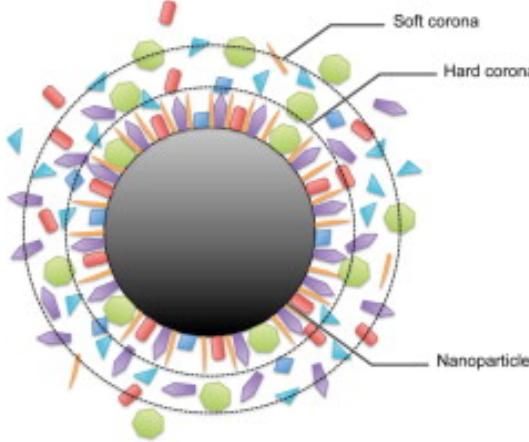


FIG. 1: Schematic representation of the different layers forming the protein corona.

A. NP-PROTEIN INTERACTION

Following the theory for colloids, we can describe the balance between two forces, electrostatic repulsion and van der Waals attraction, adopting DLVO potentials. The electrostatic part of the DLVO interaction is computed in the mean field approximation in the limit of low surface potentials, that is when the potential energy of an elementary charge on the surface is much smaller than the thermal energy scale, $k_B T$. The van der Waals contribution is described by Lennard-Jones potential. Hence, we have:

$$U_{DLVO} = U_{elec.} + U_{VdW} \quad (1)$$

with

$$U_{elec.}(r) = \frac{\pi k_B \gamma_{prot.} \gamma_{NP} \rho_\infty}{\kappa^2} \frac{R_h R_{NP}}{R_h + R_{NP}} e^{-\kappa r} \quad (2)$$

$$U_{VdW}(r) = \frac{A_H}{2520} \frac{2R_h R_{NP}}{R_h + R_{NP}} \left(\frac{\sigma}{d} \right)^6 \frac{1}{r} - \frac{A_H}{12} \frac{2R_h R_{NP}}{R_h + R_{NP}} \frac{1}{r^3} \quad (3)$$

Here A_H , related to the experimental Hamaker constants, sets the energy interaction between the proteins and the NP, R_{NP} is the NP radius, R_h is the shortest characteristic length scale of the protein, σ is the minimum-approach distance between the NP and the protein, $d \geq \sigma$ is the distance between the NP surface and the center of the protein, $k_B T$ is the thermal energy, $\gamma_i = \tanh[ze\phi_i/4k_B T]$ is the reduced surface potential, κ^{-1} is the Debye-Hückel screening length, ze are the valence in electron charges e and ρ_∞ is the ions concentration in solution (Fig. 2).

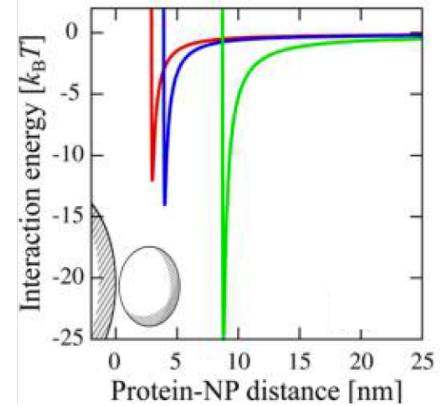


FIG. 2: The NP-Protein interaction for each type of protein: HSA - red, Transf - blue and Fibr - green. The schematic representation in the lower left corner depicts part of the NP on the left in contact with a HSA protein.

B. PROTEIN-PROTEIN INTERACTION (2-BODY CONTRIBUTION)

This contribution is short-range and describes the protein-protein interaction in solution. Because we are considering proteins that do not aggregate in solution, this interaction is repulsive. In our coarse-grain, we consider the proteins globular. However, they cannot be approximated by spheres because of their shapes characterized by at least two principal axes. We therefore describe them as ellipsoids and adopt a potential [4] that accounts for these two principal axes, a large diameter ($2R_s$) and a short diameter ($2R_h$), and associate to the short diameter a higher interaction energy. As a consequence, depending on the surface concentration, each protein can be adsorbed on the NP surface in two different conformations: laying down (lower energy) and standing up (higher energy). The adopted effective potential is [4]:

$$U_2(r_{ij}) = U_2^h(r_{ij}) + U_2^s(r_{ij}), \quad (4)$$

with the following contributions:

$$U_2^h(r_{ij}) = \epsilon \left[\frac{R_{h,i} + R_{h,j}}{r_{ij}} \right]^{24} \quad (5)$$

$$U_2^s(r_{ij}) = \epsilon \frac{1}{1 + \exp \left[\frac{r_{ij} - (R_{s,i} + R_{s,j})}{R_{h,i} + R_{h,j}} \right]} \quad (6)$$

where $r_{ij} = |r_i - r_j|$ is the protein-protein distance and ϵ is the energy cost for the standing-up conformation (Fig. 3).

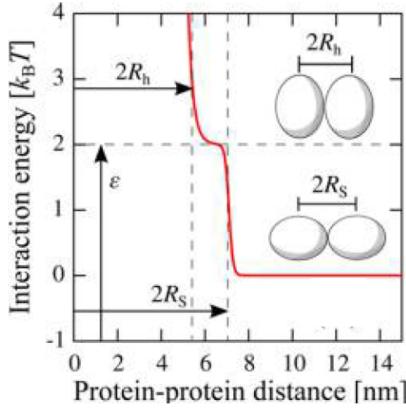


FIG. 3: The 2-body contribution of the protein-protein interaction. The dashed horizontal line marks the characteristic energy ϵ corresponding to the standing-up proteins conformation ($2R_h$) as depicted in the inset.

C. PROTEIN-PROTEIN INTERACTION (3-BODY CONTRIBUTION)

The 3-body contribution allows to account for the memory effect that is observed in experiments for high protein concentration. This interaction could be associated to conformational changes of the adsorbed proteins and is defined based on the distances between the NP and two proteins (i and j). It is define as [2, 3]:

$$U_3(r_i, r_j, r_{NP}) = \epsilon_{i,j} \left[\frac{-\sqrt{d_i d_j}}{d_3} \right] \exp \left[\frac{-(r_{ij} - \delta_{ij})^2}{2\omega_{i,j}^2} \right] \quad (7)$$

where d_3 is the 3-body interaction range, r_i is the i -protein position, r_{NP} is the NP position, $d_i = |r_i - r_{NP}| - R_{h,i} - R_{NP}$ is the distance between the protein centres and the NP surface, $\delta_{ij} = R_{h,i} + R_{h,j}$ is the shortest distance between protein-protein and $\omega_{ij} = \delta_{ij}/4$ is the cut-off range.

We fix the range of the 3-body interaction to $d_3 = 60$ nm, other protein parameters as indicated in Table 1 and NP and simulation parameters as in Table 2. In particular, we consider SiO_2 NP as in [2]. Finally, in order to study any size effects of the NP on the protein corona (i.e. surface coverage), we perform simulations for NP radii ranging from 10 to 50 nm.

III. NUMERICAL METHOD

The numerical method is based on simulating the coarse-grained model for a NP with a concentration of 0.001 mg/ml, and three types of proteins: HSA with a concentration of 0.35 mg/ml or 1.00 mg/ml, Transf with a concentration of 0.35 mg/ml and Fibr with a concentration of 0.50 mg/ml. In order to carry out the simulations, we use a box with lateral size of 375 nm, immersed

	HSA	TRANSF	FIBR
Mass (kDa)	67	80	340
R_h (nm)	2.70	3.72	8.50
R_s (nm)	3.60	3.72	11.0
ϵ_3 ($k_B T$)	0.20	2.50	0.00
A_H ($k_B T$)	9.75	8.40	7.00

TABLE I: Proteins parameters for the coarse-grained model. R_h and R_s are the shortest and largest protein semi-axes, respectively, ϵ_3 is the 3-body interaction energy between proteins of the same type and A_H is the Hamaker constant [2].

Temperature ($k_B T$)	1.00
Number of particles	256*4
Lateral size of the box (nm)	375
SiO_2 NP density ($\frac{g}{cm^3}$)	2.65
t_{sim}	1 i.u = 1.510^{-7} s

TABLE II: General parameters for the coarse-grained model [2].

in a buffer whose function is to keep the concentrations of proteins constant in the solution, releasing or trapping proteins to compensate those exchanged with the PC (Fig. 4).

We simulate the system with the following protocol: We generate an initial configuration with the proteins randomly placed throughout the box and buffer at concentrations high enough to compensate the adsorption of any type. In a first equilibration time we allow each protein to diffuse *without* adsorbing on the NP surface in such a way to achieve the desired protein concentration in solution, thanks to the action of the buffer. Next, we incubate the NP *only* with HSA proteins, allowing them to adsorb on the NP surface. After a fixed incubation time, we add Transf from the buffer and allow it to adsorb on the NP surface for a fixed incubation time. Finally we add Fibr following the same steps.

IV. RESULTS AND DISCUSSION

In order to calculate the NP surface coverage of each proteins, we first calculate for each type of proteins the maximum number of adsorbed proteins in a monolayer on the NP by performing simulations in mono-component solutions at concentration high enough to saturate the NP surface. The estimate of this maximum number is then used for calculating the surface coverage of each protein in our simulations with competing proteins. This process is repeated for every value of the NP radius.

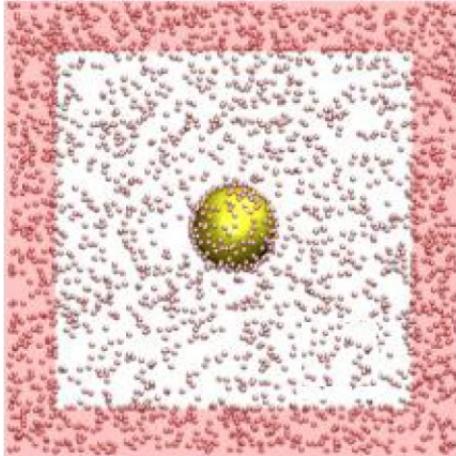


FIG. 4: Snapshot of the simulation box, with the NP (golden sphere in the centre), the protein solution (small spheres) and the external buffer that keeps constant the protein concentration in solution.

A. SURFACE COVERAGE OF THE NP WITHOUT THE 3-BODY INTERACTION POTENTIAL

We perform simulations for different sizes of NPs and calculate the surface coverage in each case to study any size effects in the formation of the corona. Additionally, we carry out the simulations for two values of HSA concentration: 0.35 mg/ml and 1.00 mg/ml, in order to study the effect of concentration. We first consider the model without the 3-body contribution, for which only HC is formed, fixing our observation time as 10 seconds and varying the NP radius (Fig. 5).

We find that the composition of the corona after 10s depends on both the NP size and the HSA incubation concentration. In particular, we find a dramatic effect of the NP size: For $R_{NP} = 50$ nm the Transf has the larger surface coverage and Fibr the smaller after 10s. For $30 < R_{NP} < 50$ nm the Fibr overcomes the HSA and for $R_{NP} < 30$ nm Fibr overcomes also the Transf. Therefore, the greater the curvature of the surface the easier is for the Fibr proteins to displace the smaller HSA and Transf proteins. We interpret this effect as the consequence of the larger affinity of Fibr for the surface and the reduction of possible contact points between the proteins and the strongly curved NP.

Furthermore, for higher HSA concentration (Fig. 7 bottom panel), Fibr is able to displace the smaller proteins only for $R_{NP} < 15$ nm within the first 10s. Although the effect could be easily interpreted as a consequence of steric hindrance between proteins, is surprising how the HSA concentration during the pre-incubation can strongly affect the protein competition over a time scale of biological relevance. To investigate how this effect depends on incubation time-scales goes beyond the scope of the present work and would require a more detailed analysis.

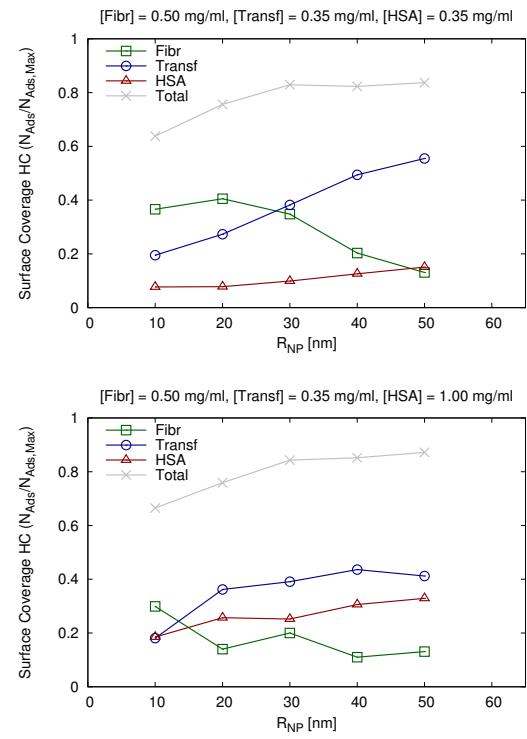


FIG. 5: Surface coverage as a function of the NP radius R_{NP} after 10 seconds. With protein concentrations of [HSA]=0.35 mg/ml, [Transf]=0.35 mg/ml and [Fibr]=0.50 mg/ml for the top panel and [HSA]=1.00 mg/ml, [Transf]=0.35 mg/ml and [Fibr]=0.50 mg/ml for the bottom panel.

B. SURFACE COVERAGE OF THE NP WITH THE 3-BODY INTERACTION POTENTIAL

We repeat the analysis described in the previous subsection with the 3-body interaction potential between two proteins of the same type. This interaction generates a SC, beyond the HC, leading to a surface coverage > 1 due to the second adsorption layer (Fig. 6). Here we consider a limited range of radii due to the high adsorption of proteins on the surface. For larger radii we would need to use a larger buffer and generate much more proteins at the beginning of the simulation which would prove to be much more costly while possibly leading to the same qualitative results.

We find (Fig. 6) that in the case of the HC the surface is always saturated with Transf, which has the strongest 3-body interaction (see table 1). Therefore, there is only a limited change in the surface coverage for different NP sizes. On the other hand, we find a non monotonic behavior of Transf surface coverage within the SC, with a dramatic decrease for the larger $R_{NP} = 30$ nm. We find that this decrease in surface coverage, however, is a result mainly due to the lack of Transf proteins in the buffer for compensating the adsorbed ones (Fig. 7). Hence, the simulation should be repeated with a higher initial number of Transf proteins, a goal beyond the scope of the

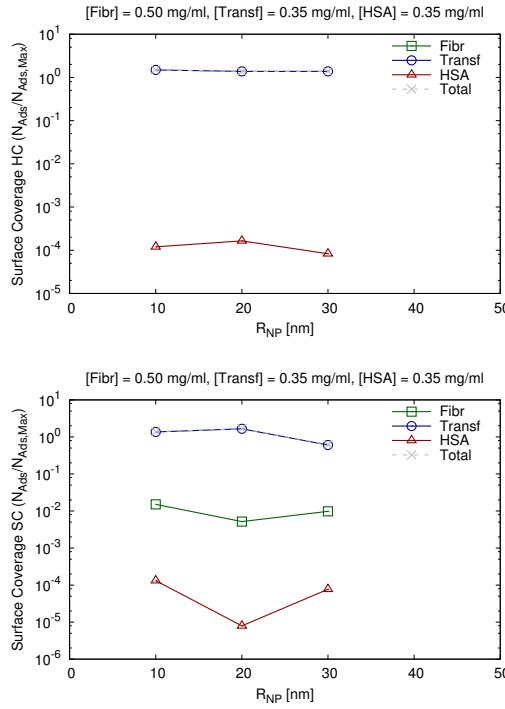


FIG. 6: Surface coverage as a function of the NP radius for the HC (top) and SC (bottom) with protein concentrations of $[HSA]=0.35 \text{ mg/ml}$, $[Transf]=0.35 \text{ mg/ml}$ and $[Fibr]=0.50 \text{ mg/ml}$. Fibr is not represented in the surface coverage for the HC because no Fibr proteins adsorb on the surface throughout the simulation.

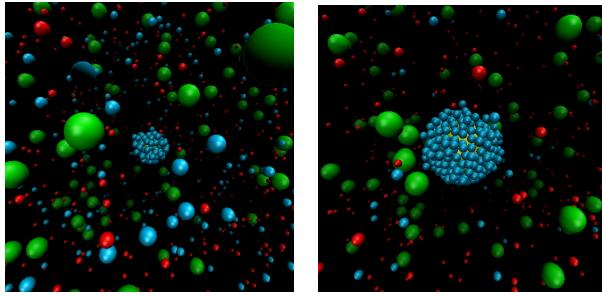


FIG. 7: Final snapshots of the simulations involving a NP with a radius of 10 nm (left) and 30 nm (right). HSA is red, Fibr is green and Transf is blue. For a radius of 30 nm we notice a lack of free Transf proteins in the simulation box.

present work.

V. CONCLUSIONS

- The spontaneous formation of the protein corona in biological systems is an essential and new concept that is drastically changing our view about how NPs interact with living organisms. In the last ten years it has become increasingly clear that it is the protein corona that provides the biological identity of the NP and not just the NP chemistry.

- In this framework, we have studied the adsorption process of three competing proteins (HSA, Transf, Fibr) on a SiO_2 NP surface through simulations of a coarse-grained model that has enabled us to gain insight into the mechanisms behind the formation of the protein corona. According to our results, for a given time of incubation, the composition of the corona depends on i) the protein concentrations, and ii) the NP size. The NP size, therefore, is a fundamental control parameter for designing NPs for specific application in biological systems.
- Finally, we have analyzed the PC composition after a fixed time, considering the case only with HC and the case including also the SC. We find a significant change in the corona composition with the NP size. Given our selection of parameters for the protein interactions, we find that Transf is the largest component of the PC for larger NPs. Comparison with experiments are needed to establish if our parameter choice is reasonable, or if it needs to be tuned for better agreement with experiments.

Acknowledgments

I wish to express my sincere thanks to Sotiris Samatas and Oriol Vilanova for helping me in setting up the simulations and for discussions about the results. I would also like to thank my parents and my friends for their support during these last years.

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