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Competition of opsonins and dysopsonins on the Nanoparticle surface

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ABSTRACT: The biological behavior and fate of nanoparticles are dependent on their retention time in the blood circulation system. The protein corona components, especially opsonins, and dysopsonins, adsorbed on the nanoparticle surface determine their blood circulation time. The protein corona formation is a dynamic process that involves the competition between different proteins to be adsorbed on the nanoparticles. Therefore, studying how proteins compete and are oriented on the nanoparticle surface is essential. We hypothesized that the presence of opsonins (Immunoglobulin (IgG)) might affect the adsorption of dysopsonins (Human Serum Albumin (HSA)) and vice versa. Using the molecular dynamics simulations, we showed that the adsorption of HSA on the GO surface after the IgG adsorption is more probable than the opposite order of adsorption. It was also observed that the higher lateral diffusion of the HSA compared to the IgG helped the system reach a more stable configuration while the initial adsorption of the HSA limits the lateral diffusion of IgG. Therefore, replacing IgG adsorbed on the GO surface with HSA is plausible while the reverse process is less likely to occur. This study revealed that albumin might extend the blood circulation time of GO by replacing opsonins (IgG).

Keywords: protein corona, Human Serum Albumin, dysopsonin, Immunoglobulin, opsonin

Introduction

Nanotechnology provides new and innovative approaches to the diagnosis and treatment of diseases^{1, 2}. Current therapeutic and diagnostic approaches often have limitations, such as low efficacy, high toxicity, and low specificity. Nanotechnology can overcome these challenges by enabling the development of targeted drug delivery and imaging techniques, and highly sensitive diagnostic tools^{1, 3, 4}. One of the main challenges in the application of nanoparticles (NPs) in medicine is the formation of a biomolecular/protein corona on the surface of their surfaces. When NPs are introduced into the body, biomolecules/proteins of the biological fluids are adsorbed on their surfaces forming a biomolecular/protein corona that can alter the physicochemical properties of the NPs and influence their biological behavior and fate in the body^{5, 6}. The therapeutic and diagnostic efficacies of NPs are dependent on the type and amount/number of proteins adsorbed on their surfaces⁷⁻⁹. The protein corona decoration on the NPs is affected by NP physicochemical properties (e.g., size, charge, composition, and surface chemistry), time of circulation (*in vivo*)/incubation (*in vitro*), and the plasma changes mediated by different diseases¹⁰⁻¹⁴. The protein corona consisted of hard corona and soft corona layers that refer respectively to tightly bound layers of proteins that directly interact with the surface of the NPs and the more loosely bound layer of proteins that surround the hard corona¹⁵. Therefore, depending on the time of adsorption and location of the protein in the corona layers, corona proteins are adsorbed on the NP surface through NP-protein interaction and/or protein-protein interactions. The soft corona is a dynamic layer that changes over time through competitive adsorption/desorption of proteins on the NP surface¹⁵. Tenzer *et al.*,¹² demonstrated that the protein content of coronas changes over time not their protein composition in *in vitro* experiments. We showed that both protein content and composition change over time in the animal body (*in vivo* experiments)¹⁶. The protein exchange

on the NPs affects their retention time in the blood circulation system. Any changes in the content of proteins extending (dysopsonins) and/or shortening (opsonins) the NP circulation time can affect the plasma half-life of NPs¹⁶. Opsonins are a group of proteins that enhance the phagocytosis of foreign particles by immune cells, such as macrophages and neutrophils. Opsonization of nanoparticles can lead to their rapid clearance from circulation and accumulation in the liver and spleen, which can limit their therapeutic efficacy. Dysopsonins, on the other hand, are proteins that inhibit the phagocytosis of foreign particles by immune cells. Dysopsonization of nanoparticles can prolong their circulation time and enhance their accumulation in the target tissues, which can improve their therapeutic efficacy¹⁷. Therefore, the balance between opsonins and dysopsonins adsorbed on the nanoparticle surfaces is critical for determining their biological behavior and fate *in vivo*. We identified new proteins acting as opsonins and/or dysopsonins, and change over time¹⁶. The protein replacement on the NP surface could be occurred through a process called the Vroman effect in which some proteins initially adsorbed on the NP surface are subsequently exchanged by other proteins having higher affinity to NP surface over time¹⁸. In some cases, proteins adsorbed on the NPs prevent the adsorption of some proteins through a competitive protein adsorption process. Due to differences in the affinities of proteins to NP surfaces, some proteins could be easily replaced by those having higher affinities¹⁹. Albumin (dysopsonin) and IgG (opsonin) are known as dominant components of protein corona adsorbed on most NPs and affect their circulation time in the animal body¹⁷. To study how these proteins compete to be adsorbed on the NP surface and understand the mechanism of protein replacement on the nanoparticle surface, we performed molecular simulation studies to assess the affinity and orientation of proteins on the NP surface. We revealed that IgG is easily replaced by albumin while albumin restricts the adsorption of IgG on the graphene.

Results and Discussions

One protein adsorption on the GO

Both HSA and IgG are known as abundant proteins in the protein corona adsorbed on the GO. In some cases, it was shown that HAS and immunoglobulins, especially IgG, are the primary constituents of graphene regardless of the surface functionalization²⁰. Not only for the protein corona of GO, but these molecules are also the prominent components of protein corona adsorbed on other types of NPs²¹. In addition, HSA and IgG are abundant proteins present in human blood plasma that respectively act as dysopsonins and opsonins when they are absorbed on the NPs^{17, 22, 23}. On this basis, we selected HSA and IgG as focal points of this study to evaluate the competition of opsonins and dysopsonins on GO. To obtain the preferable orientation for each of the IgG and HSA proteins on the GO surface, the interaction of one protein with the nanoparticle is investigated at the first stage of the current study. As the initial minimum distance of 5 Å is large enough to sample the protein nanoparticle interaction space, the protein is placed in that distance from the GO surface²⁴. Different orientations of the protein with respect to the GO surface is considered and for each orientation a simulation of at least 40 ns of real time is done. As the simulation box size is the same for each protein in different initial orientations, the potential energy parameter can be used to compare the favorability of different configurations.

In figures 1.a and 1.b the orientation of 0 degree is depicted for IgG and HAS, respectively. Different orientations are obtained through rotating the protein around X and Y axes of the Cartesian coordinate system. Since the potential energy noise is usually high in molecular dynamics simulations, 2 ns averaging is used to smoothen the energy values²⁵. Changes in the

potential energy during the simulations are shown in figures 2.a and 2.b for IgG and HSA, respectively. It is apparent from the figures that in the course of the simulation the system has reached an equilibrium status. The minimum potential energy for each system during the simulation is utilized to compare different orientations of the proteins. Its value for each orientation is illustrated in figures 1.d and 1.f for IgG and HSA, respectively. For the IgG protein, the orientation of 35 degrees with respect to the X axis gives the highest absolute value for the potential energy. In this orientation the protein not only has the maximum contact area with GO, but also its heavy chains touch the nanoparticle surface. While for the HSA protein the 90-degree rotation with respect to the Y axis bears the highest potential energy as it leads to the highest contact area between the protein and the GO surface.

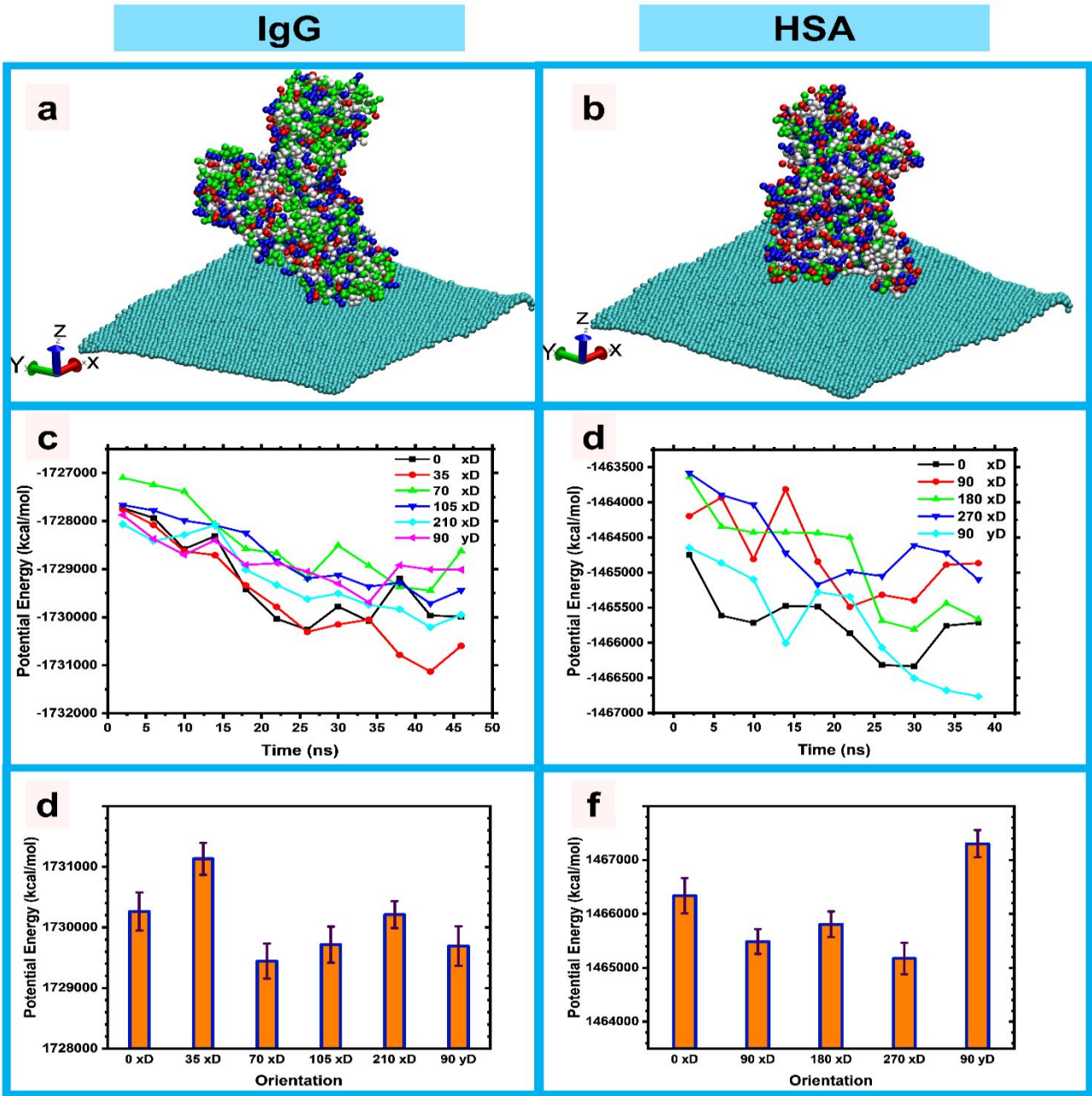


Figure 1. The preferable orientation of IgG and HSA for the adsorption on the GO surface. The snapshot shows the protein in zero-degree orientation for a) IgG and b) HSA. The potential energy curves during the simulation for c) IgG and d) HSA showed that the systems had reached an equilibrium. The values of the minimum potential energy during the simulation for a) IgG and f) HSA showed the preferred orientation of 35 degrees with respect to the X axis and 90 degrees with respect to the Y axis for IgG and HSA, respectively.

The adsorption of two proteins on GO surface

Referring to the outcome of the previous section, the favorable orientation of each protein with respect to the GO surface is known. In this section, the proteins are introduced to the simulation box in two different orders: a) first HSA is absorbed onto the surface (HSA->IgG) and b) HSA is put into the simulation box after the IgG protein adsorption (IgG->HSA). The main aim of the simulations in this section is to compare the potential energy of the system for these two strategies and see if the sequence in which the proteins absorb onto the surface affect their competition for the adsorption on the GO surface. To this end, HSA and IgG proteins are placed in the simulation box with the vertical orientation obtained from the previous section. Because the GO system considered here might have directional properties, two different lateral orientations of 0 and 90 degree with respect to z axis are taken into account (Orientations 1 and 2 in figures 2.a-2.d).

Eight different sets of simulations are considered in this part. In simulations sets of figures 2.e – 2.h, the HSA is absorbed onto the GO surface first, while in the figures 2.i – 2.l IgG was the first protein adsorbed on the GO surface. After the first protein reaches the equilibrium (52 ns), the second protein is placed inside the simulation box. The minimum distance of the second protein from the first one and also from the GO surface is equal to 5 Å and this stage of the simulation lasts for 50 ns.

As it can be seen in figure 2, the IgG protein usually moves in the vertical direction towards the GO surface and it does not have any lateral movement or diffusion on the GO surface. However, the HSA protein has some lateral diffusion on the GO surface and rotates to find a more favorable lateral orientation with respect to the GO surface, regardless of order of HAS and IgG adsorption on the GO surface (figures 2.e and 2.k). Furthermore, when the IgG is the first protein adsorbed on the GO surface, it does not need lateral movement to reach a favorable position on

the GO surface since there is no other protein on the GO surface and the whole surface is almost the same. As a result, in the simulations related to the IgG->HSA (figure 2.i – 2.l), both the HSA and IgG has reached their preferred orientations on the surface, while for the HSA->IgG set just the HSA has a favorable orientation on the surface.

For instance, for both the figure 2.k and 2.f, HSA has reached the preferred orientation of HSA1 (figure 2.a), even though the initial orientation for HSA in figure 2.k is HSA2. In figure 2.k, IgG has also reached a favorable orientation of IgG1, while IgG in figure 2.f, due to its inability to have lateral movement, has not reached a lower energy orientation on the surface. As another example, in both the simulation sets in figures 2.h and 2.i the initial distance of the HSA and IgG from each other is equal to 5 Å. For the IgG->HSA case (figure 2.l), HSA moves vertically to avoid unfavorable contacts with IgG. However, for the HSA->IgG set (figure 2.h) the HSA has already adsorbed strongly on the surface, it does not move laterally and also IgG adsorbs without any horizontal movement. Thus, it seems that if IgG protein is the first protein to be absorbed on the GO surface, the system has a more favorable potential energy rather than the case with IgG approaching the surface after HSA.

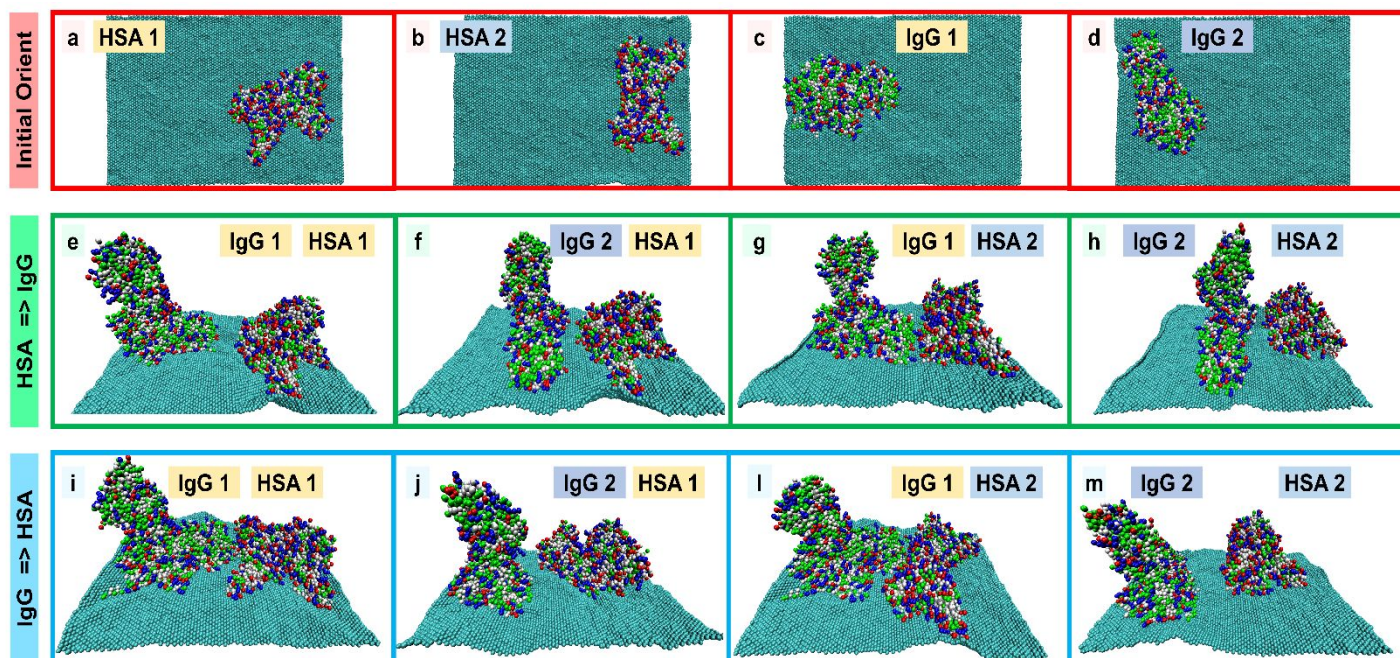


Figure 2. a-d) The initial lateral orientations of the proteins for the simulation box containing two proteins. a) HSA orientation 1 b) HSA orientation 2 c) IgG orientation 1 d) IgG orientation 2. Final configuration of the system for the adsorption of e-h) IGG after HAS and i-l) IgG before HSA for different initial orientations. For all the simulations the vertical orientation is chosen as the orientation with minimum energy obtained in figure 1.

The potential energy of the systems with these two different orders of HSA and IgG adsorption for different initial lateral orientations is summarized in figure 3.a. In order to obtain each potential energy value, at least three simulations were done and the standard error of mean is utilized as a measure of error. For the HSA1-IgG1 and HSA1-IgG2, the difference between potential energies of HSA->IgG and IgG->HSA is the highest. The reason for this high difference is that the orientation of HSA for this orientation is different between these two order (figure 2.e-2.f and 2.i-2.j). For other lateral orientations the difference between the sequences of protein adsorption is lower. However, for all the lateral orientations the IgG->HSA sequence has a higher amount of absolute potential energy than the HSA->IgG and the average value of

potential energy for different lateral orientations for the former (IgGT->HSAT) is higher than the latter (HSAT->IgGT). This huge difference is due to the fact that if the HSA is absorbed first, the IgG has less opportunities to find its minimum potential energy configuration on the GO surface and avoids unfavorable interactions with the already absorbed HSA since it has lower lateral movement on the surface. On the other hand, for IgG->HSA sequence, the IgG has found its preferred adsorption mode on the GO surface before the HSA reaches the surface. Since HSA has higher lateral movement on the surface it is very easy for this protein to absorb on a more favorable position on the surface. For IgG->HSA sequence subsequently, both the proteins have the opportunity to be adsorbed on an energetically favorable mode.

Moreover, the simulation results showed that when the first protein adsorbs on the GO surface, it is so stable on the surface that it will not move laterally on the surface in the time scale accessible to the simulation. Consequently, the only means by which the proteins can avoid unfavorable contacts and reach more stable potential energies, is the lateral moving of the second protein before its adsorption. As a result, higher lateral diffusion of the second protein is equivalent to having higher absolute value of potential energy.

Besides, the MSD values of IgG and HSA proteins during adsorption on the GO surface is measured to calculate their lateral diffusion constants (figure 3.b). The value of the lateral diffusion constant on the GO surface for the HSA and IgG proteins are equal to $5.61 \pm 1.17 \times 10^{-3}$ and $0.95 \pm 0.22 \times 10^{-3} \text{ \AA}^2/\text{ps}$, respectively. Higher lateral diffusion constant of HSA on the GO surface corroborates the idea that higher lateral movement of HSA favors the IgG->HSA adsorption sequence.

To shed more light on the effect of the lateral movement of the proteins and the diffusion on the surface, two extra simulation sets similar to HSA1->IgG2 and IgG1->HSA2 are done in which

the distance between the proteins are kept fixed. The results of the two simulation sets (shown as HSA1f->IgG2f and IgG1f->HSA2f) alongside with the original sets are depicted in figure 3.c.

Considering the error bars, the difference between IgG1f->HSA2f and IgG1->HSA2 simulations is meaningful. As in the former simulation set, the distance between the proteins kept fixed, the HSA protein is not able to have surface diffusion and reach a higher amount of absolute potential energy value. Thus, its potential energy value decreases with respect to its unconstrained counterpart. However, as the lateral movement is not so significant for the other case, the decrease in the potential energy value is not meaningful for that case considering the error bars limit.

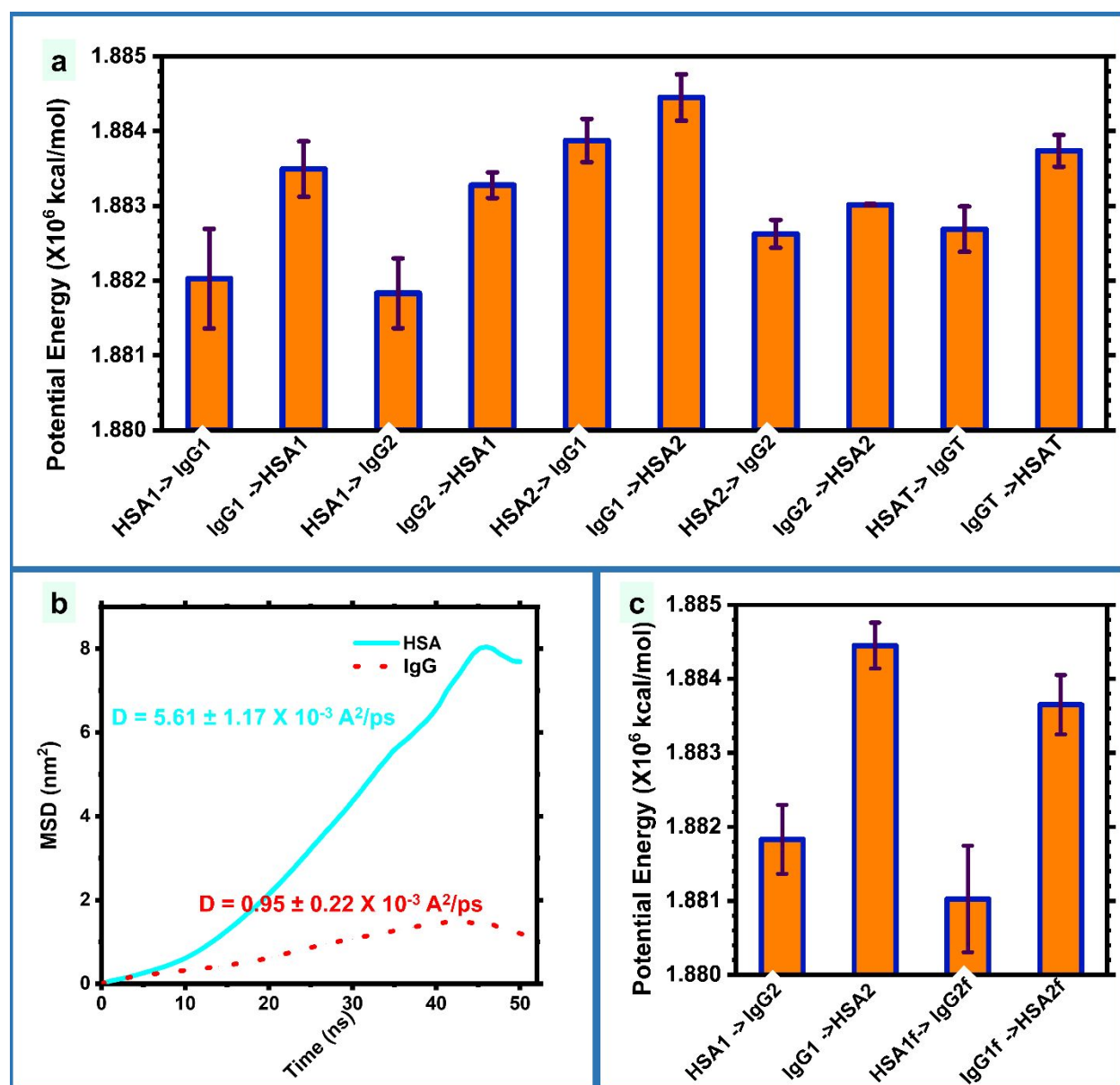


Figure 3. a) The minimum of potential energy for the different sequences and orientations of the protein adsorption for the two protein system containing HSA and IgG. HSA- $\rightarrow$ IgG and IgG- $\rightarrow$ HSA refer to the adsorption of IgG after and before HSA respectively. The lateral orientations are the same as those depicted in figure 2. The IgGT- $\rightarrow$ HSAT and HSAT- $\rightarrow$ IgGT corresponds to the average of all the initial orientations for IgG- $\rightarrow$ HSA and HSA- $\rightarrow$ IgG sequences. b) The variations in the MSD values during the adsorption of HSA and IgG on the GO surface calculated for one protein adsorption simulations. c) The minimum of potential energy for HSA1- $\rightarrow$ IgG2 and IgG1- $\rightarrow$ HSA2 alongside with their counterparts having a fixed HSA – IgG distance (HSA1f- $\rightarrow$ IgG2f and IgG1f- $\rightarrow$ HSA2f).

The free energy change is composed of two components of the enthalpy and entropy change. According to the two box method²⁵, the enthalpy can be calculated as a difference between the potential energy of the system and another reference box containing the same elements with higher protein nanoparticle distance. If all the simulation boxes are the same in the size and the number of particles, as it is the case here, the reference box will be the same for all of them and the enthalpy is calculated as an offset to the potential energy. Thus, if the free energy and enthalpy changes are considered the same, the change in the potential energy can be considered as a synonym to the free energy. Besides, the residence time for different contact residues in the simulations done in the current study are roughly the same (figure 4). As a result, the entropy change is almost the same for different systems considered here and the change in the free energy can be regarded as an equivalent to potential energy.

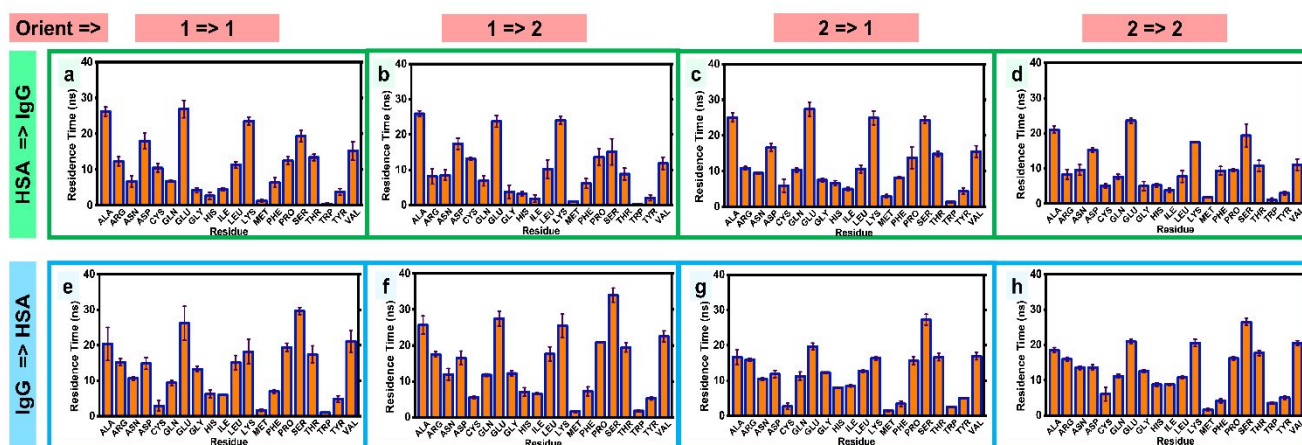


Figure 4. Residence time for different contact residues for two protein adsorption simulations. This figure implies that the contact residues of the protein is almost the same for different orientations and sequences of two protein adsorptions. As a result, the entropy change is the same for different cases and enthalpy change can be used as a measure of the free energy change.

Three simulations were run in which both the proteins were put into the simulation box at the same time. To provide the proteins with enough time to possibly switch places, the new simulations were done for 880 ns instead of the original 100 ns simulations. Interestingly, the proteins showed a propensity towards aggregation during the adsorption which was not happen in the previous simulations in which the proteins sequentially adsorbed on the surface. Also, the potential energy of the system in this case was lower. It is possible that the free energy barrier between this case and the original ones is so high that a much longer simulation times is needed to switch between these cases. Also, a lateral diffusion coefficient of $1.45 \times 10^{-3} \text{ A}^2/\text{ps}$ in this case was observed which is between the values of 0.95×10^{-3} and 5.61×10^{-3} computed for IgG and HSA, respectively and closer to the corresponding value for the IgG protein. Thus, in the case that both proteins adsorb on the GO at the same time the lower surface diffusion ability of IgG alongside with the HSA-IgG aggregation prevents structural changes that minimizes the system energy. Some simulation snapshots for this simulation are shown in figure 5.

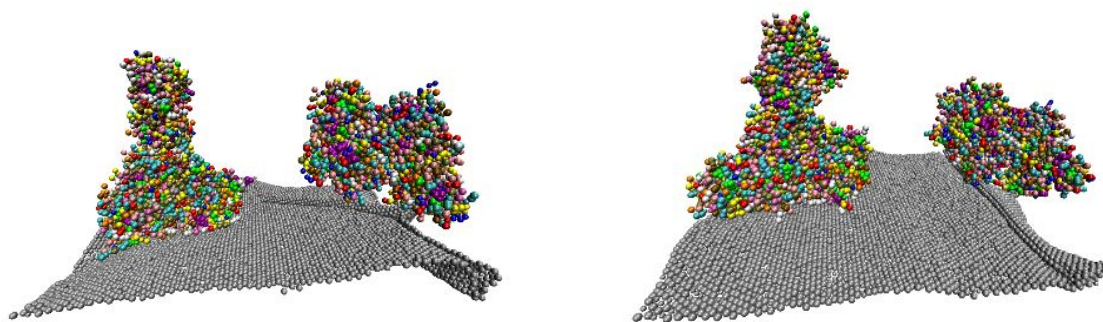


Figure 5. Results of two simulations in which both the proteins are adsorbed on adsorbed at the same time. Please note that the protein contacts each other through periodic boundary conditions.

Plasma contains many proteins that compete to be adsorbed on the NP surface²¹. In addition, corona decoration on the NPs is a dynamic and competitive process affected by the presence/absence of proteins^{7, 10}. Any changes in plasma protein content mediated by different diseases may affect the corona decoration on the NP surface. For example, we showed that identical NPs incubated with the plasma of patients with hypofibrinogenemia (patients with low fibrinogen) and diabetes (patients with glycosylated proteins) have different plasma protein patterns¹⁰. It's noteworthy to mention that the presence/absence of other plasma proteins may affect the adsorption or desorption of HSA and IgG on the GO surface. Due to simulation limitations, it's not possible to simulate the competition of all plasma proteins for accessing the NP surface, their interaction with the NP surface, and the protein-protein interactions. Therefore, we chose HSA and IgG as opsonins and dysopsonins adsorbed on the Go surface and most NPs.

Plasma proteins compete for the available surface area of NPs and they are adsorbed on the NPs through NP-protein and protein-protein interactions¹⁵. In the beginning, Plasma proteins such as HSA and immunoglobulins may occupy the particle surface, but over time, proteins with higher affinity and slower kinetics, such as apolipoprotein A-I, will take their place, despite being less abundant^{18, 26}. Conversely, in cases where the total protein concentration is lower than the available surface area of nanoparticles, lower affinity proteins are absorbed on the NP surfaces. However, the HSA and IgG are the main corona components adsorbed on the NPs.

In our previous study, we showed that fluorescence resonance energy transfer-labeled fibronectin can access the NP surface and directly attach to the NP surface through protein replacement when it faces the corona-coated NPs¹⁹. Addition, deletion, or modification of plasma proteins mediated by different diseases or health conditions affect the corona composition on the NPs surface¹⁰. We

showed that identical NPs may have different corona decorations in different patients and introduced the concepts of personalized protein corona and disease-specific protein corona^{7, 10}. Various proteins show different affinities to identical NPs. For example, HSA has a higher affinity to single-walled carbon nanotubes compared to IgG²⁷. Some plasma proteins undergo structural changes after adsorption on the NPs²⁸. Proteins may easily detach or strongly attach to the NP surfaces after conformational changes. We showed that some proteins located in the outermost layers (soft corona) or those involved within the corona through protein-protein interaction undergo negligible conformational change while those directly attached to NPs suffer severe structural change and denaturation¹⁹. We showed that albumin undergoes insignificant structural changes when it adsorbs on the GO surface⁷. IgG aggregated and denatured after adsorption on the polystyrene²³. The NP-induced protein structural change is a hidden issue at the nano-bio interface that can affect the affinity of proteins to the NPs. Therefore, the dynamic decoration of protein corona on the NPs is a multifactorial process.

Conclusion

Over time, the protein corona can undergo changes as proteins continue to compete for adsorption or replace proteins that are already bound to the NPs. Our study revealed that for all the initial lateral orientations, the HSA->IgG sequence has a lower absolute value of potential energy change compared to the IgG->HSA. This finding implies that adsorption of HSA on the GO surface after the IgG adsorption is more probable than the opposite sequence. The values of diffusion constant for the proteins calculated through the MSD values shows higher lateral diffusion of HSA on the GO surface. Thus, higher diffusion constant of HSA causes higher favorability of the adsorption sequence of IgG->HSA than the other sequence. Therefore, the

IgG adsorbed on the GO could be replaced by HSA while the reverse does not occur. We suggest one of the ways in which albumin can prolong the retention time of GOs in bloodstream might be replacing opsonins. As in the biological protein corona is made of variety of other proteins that can compete or affect the competition of these proteins for the adsorption on the GO surface, in following studies the effect of other proteins on this effect will be investigated.

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