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Ultra-small Spherical Gold Nanoparticle Interaction with Fibrinogen: A computational Approach

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Abstract
Nanotechnology has an outstanding contribution in numerous fields, and thus it has led to considerable progress. Particularly, in biomedicine, nanoparticles are manipulated in diagnosis, and treatment, specifically in drug delivery. However, novel nanoparticles are recently manipulated widely without estimating their possible risks on health. Especially in intravenous administration of the nanoparticle, where their first contact is with blood components, which can produce potentially harmful effects, especially for plasma proteins. So, the development of more hemo-compatible nanoparticles is necessary. Gold nanoparticles (GNPs) possess outstanding properties, such as simple synthesis, manageable size and shape, and excellent biocompatibility. Consequently, GNPs have extensively been used in biomedicine, as cancer treatment, bio-catalysis, bio-imaging, and in drug delivery systems. So, the wide range of their applications may lead to possible risks environmentally, as well as on public health. Therefore, it is important to assess their expected toxicity, which in turn has an impact on their therapeutic efficiency. So, in this study we have conducted atomistic level molecular dynamics simulation (MD), to study the effect of an ultra-small (1.5 nm in radius) spherical gold nanoparticle on C-terminal domain of fibrinogen protein's gamma chain, which is crucial in the blood clotting process and is accomplished via calcium ions located in fibrinogen protein surface at distinct binding sites. So, the calcium ion present in this domain was included in the MD system. Structural analysis represented by Root-Mean-Square of Deviations (RMSDs), Radius- of-Gyration (Rg), Root-Mean-Square of Fluctuations (RMSFs), Solvent-Accessible Surface-Area (SASA), and the total secondary structure content, indicate that there is no substantial change in conformation of the protein. To probe GNP effect on calcium ion's binding to the protein, the free energy of their binding was calculated, and represented in terms of energy components and the energy contribution of individual protein's amino acids located within active-site region. Based on the free energy results, there is no detectable change in the binding upon the introduction of GNP. Structural stability is reflected on the stability of the distribution of the charge on the protein surface and was expressed in terms of electrostatic potential surface. So, Ultra-small GNP of radius 1.5 nm is suitable candidate for biomedical applications, since it has no effect on the protein functionality.
Keywords: Fibrinogen; blood clotting; molecular dynamics simulation; electrostatic potential surface; Gold nanosphere.

1. Introduction

Nanoparticles are manipulated across a wide range of applications, especially in biomedicine, where they are spreading across fields such as imaging, therapy, and diagnostics [1][2]. However, knowledge about their toxic effects on human beings is short of appropriate regulatory measures [3][4]. Especially, novel nanoparticles that are widely used currently lacking a thorough measurement of their expected risks on health. Therefore, studies have to be performed, to help to recognize, understand the mechanism of their action and predict their effects on molecular, cellular, or tissue levels and quantify their effects. Once nanoparticles encounter tissues and cells, they can initiate interactions of nanomaterial with the tissue, that can cause harm, cause severe, and even long-lasting inflammations. So far, biomaterials that are applied to human beings can induce tissue response when they encounter healthy or sick tissue. These effects can occur at nano, molecular or even cellular levels [5][6][7].

Nanoparticles that are involved in diagnosis by imaging, or treatment as nano-transporters, they often administered via intravascular route. Once a nanoparticle enters blood, it interacts with its cells, complement and plasma proteins. The binding of Nanoparticles to the plasma proteins, and their subsequent possible interactions, affects significantly their fate in the blood stream. Consequently it influences their functionality and affects plasma proteins as well [8]. Nanoparticle-protein interaction is complex, and relays on its biological environment, as well as nanoparticle surface properties, such as size, shape, and composition [9].

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According to the purpose of nanoparticles usage, they are designed in a specific size, shape, charge, etc. Their size is determined based on the nature of required interaction with the protein of interest. The size of various proteins ranges from 5-20 nm. So, for ultra-small (1-2 nm) nanoparticles, it acts as protein's epitopes and is located on its surface as active site, for instance to trigger a specific immune response. Nanoparticles of size up to 5 nm are medium sized particles, that can interact with proteins on a one-to-one basis. Larger nanoparticles up to 20 nm adsorb the proteins on their surface, where the nanoparticle itself is usually bigger than the majority of proteins, this resulted in protein corona formation [9]. Consequently, we have selected ultra-small spherical nanoparticles for our study, to avoid corona formation on the surface of the nanoparticle, when it is introduced to the blood. Because it will hinder their passage to the required site of action and affect their functionality. The ultra-small nanoparticles were successfully synthesized, such as pare (colloidal) gold nanoparticles[10].

Gold nanoparticles are characterized by distinct and outstanding properties, that give them an remarkable reactivity and wide range of applications such as nano sensors [11]. Moreover, their surface area is large and surplus active sites, gold nanoparticles of small size show high reactivity, which qualify them as an suitable candidates for a variety of applications in biomedicine [12]. Owing to GNPs interesting medicinal, and optical, and electrical properties. Moreover, due to their antimicrobial activity, they have been employed extensively in biomedical application, such as drug-delivery, orthopedic supplements, bandages, and in antimicrobial materials [13]. Although they have expressed great contribution to the growing nanobiotechnology field, some side effects have been stated [14], [15].

Recently, intensive research was performed on the interaction of nanoparticle with the protein, that aids in illustrating the mechanism of NPs adsorption on proteins [16]. Apart from the pattern of adsorption resulted in the so called "protein's corona", experimental studies have focused on NPs effect on protein three-dimensional conformation, aiming to understand how to employ their ability to alter protein structure and its flexibility [17], [18], [19]. For example, Jonsson et al. found that nanoparticles of smaller size (NPs), didn't have enough strength to denature proteins, compared to larger ones [20]. Nanomaterials which are coated with anti-biofouling agents as polyethylene glycol, and zwitterions are found to help in maintaining proteins structure, possibly by moving them away from nanoparticles [21], [22].

Ubiquitin protein and silver NPs interaction was investigated through the use of molecular simulations and by experiments [23], [24]. The obtained results showed that ubiquitin binds to the NP across successive routes and the presence of the NP cause destabilization of the helical structure. Moreover, fibrinogen expressed significant structural changes in comparison to albumin in the presence of graphene oxide [25] and silica NPs [26]. Transferrin encounters structural changes, which are irreversible and hence lose its main function, which is the transport of iron among living cells, as a consequence of their adsorption to the iron oxide NPs [27]. In contrary, uncoated spherical gold nanoparticle has no effect on human serum albumin's secondary and tertiary structures [28]. The structural changes in some proteins may stimulate inflammatory responses. For example, C–terminus of γ-chain (γ377–395) of fibrinogen after interaction with poly (acrylic acid)-coated gold NPs, caused structural changes, which in turn triggered inflammation response and caused undesirable successive pathways. According to this study nanoparticle coating was the main cause for this response [29].

It can be concluded from the previously mentioned experimental and theoretical studies, more studies are needed to test NPs effects against different plasma proteins to investigate their limit of toxicity. As mentioned previously, it is possible to manufacture pare/colloidal ultra-small gold nanoparticles [30], aiming to enhance their uses in different applications as well as reducing their toxicity. For our case we have modelled the effect of ultra-small spherical gold nano particle of 3nm diameter on fibrinogen protein's C-terminal domain of γ -chain.

Fibrinogen (Fg) is a blood protein, which is abundantly found and has a vital role in immunity system activation and in blood clotting process. Fg composed of 6 polypeptides (α; β; γ)2 chains, that are connected by di-sulfide bridges [31]. We have selected C-terminal part of γ-chain domain of fibrinogen protein to (simply Fg) study, since the γ- chain contains the polymerization domain, which vary mostly among fibrinogen-related proteins and characterized by containing acidic residues. The charged/acidic residues make a major contribution to the total dipole moment of fibrinogen; hence it may allow electrostatic directing, which in turn, helps to align fibrin monomers throughout the polymerization process. Fibrinogen protein has distinct binding sites for calcium ions, with high affinity such as that of γ chain, and are associated with four organizing amino acids, and are γAsp318, γAsp320, γGly324, and γPhe322. At physiological Ca^{+2} concentrations, their dissociation constant is high enough to maintain these ions in their binding sites. These ions protect γ chain from enzymatic degradation, and control fibrin polymerization through the enhancement lateral aggregation to make thick fibers [31], [32].

This work aims to probe the influence of using uncoated ultra-small spherical nanoparticle of 3nm in diameter, on the structural flexibility and dynamics of C-terminal part of γ-chain fibrinogen (Fg) protein as representative of plasma proteins. This particular size of the spherical gold nanoparticle was selected to check their

effect on 1:1 basis with respect to the protein dimensions, and at the same time small enough to have a controllable size of the system, thus allowing atomistic level molecular dynamics to be performed in a convenient time frame.

2. Computational Details

The MD simulation box is constructed as follows; the GNP was created as a sphere of a radius 1.5 nm, using Nanomaterial Builder implemented in CHARMM-GUI [33]. The 30 kDa C-terminus γ-chain of Fibrinogen crystalline structure (Fg) with (PDB ID:1FID), was obtained from the Protein Data Bank. It has −3 e net charge. A calcium ion present in the experimental structure in the active site was kept in position, because it is crucial to the protein functionality. The Fg-NP complex is inserted at octahedron periodic box center, maintaining a water layer of 2 nm thickness around it. The solvation process was achieved through the use of solution builder scheme of CHARMM-GUI portal [34]. Hence, this explicitly accounts for solvation effects on the protein, while keeping the number of atoms in periodic box to be convenient, which in turn helps to conduct MD simulation for 200 ns. The gold NP was inserted in the periodic box, using GROMACS program "gmx insert-molecules". To avoid any close contact between the atoms of the protein on the surface, and GNP was avoided by slightly shifting the NP away from the protein. To neutralize the system, while maintaining the NaCl salt concentration at 0.15 M, some water molecules were replaced by Na^+ and Cl^- ions. The final solvated systems have 66,000 atoms. Fig. 1 shows the initial configuration of Fg-GNP complex. A reference simulation of the GNP-free protein was performed for a 200 ns. The Fg protein, gold atoms and ions potential energy was described by the CHARMM36m force field, that is developed and modified by Mackrell eta.al, group, where the force field describes the gold NP atoms interfacial properties. The solvent/water molecules are modeled using the TIP3P water model, that is recommended to be used with CHARMM36m force field [35]. Atomistic level MD simulation was used to investigate the Fg protein structural properties, and its flexibility in the complex and in the bulk solution. The simulation was performed after energy minimization and equilibration steps. The temperature is adjusted at 300 K by Nose-Hoover thermostat [36]. The gold atoms were treated as a rigid body, so their atomic positions were held fixed in both minimization stage, and in production run. The Non bonded term in the forcefield was calculated as the summation of short-ranged Van der Waals and long-ranged electrostatic interactions. Van der Waals was expressed by Lennard-Jones 6–12 function with a cut-off distance (1.0 nm) , while long-ranged electrostatics was expressed by Coulombic function using particle mesh Ewald summation [37], while, for gold atoms within the NP, the non-bonded interaction energy is neglected. Hydrogen bond length is constrained to the equilibrated length by applying LINCS-algorithm [38]. The bonded interactions were expressed using CHARMM36m force field. The data for the Fg-GNP complex and its reference structure were collected over the 200 ns. Atomistic MD simulations were conducted Gromacs-2023 version [39].

We have calculated the change in the complex the Root-Mean-Square of Deviations (RMSDs), the protein's average Radius-of-Gyration (Rg), Root-Mean-Square of Fluctuations (RMSFs), The Solvent-Accessible-Surface-Area (SASA), were calculated over the simulation course. The total secondary structure content compared to the reference across 200 ns was calculated using DSSP program implemented in GROMACS [40]. Visual Molecular Dynamics (VMD) program [41], was also used for visualizing different protein configurations. The free energy of calcium ion binding to Fibrinogen protein was calculated in the presence of GNP and in reference structure using gmx-MMPBSA tool implemented in GROMACS[42]. The electrostatic potential surface for the protein in the free and bound states after 200ns was calculated by Adaptive-Poisson–Boltzmann-Solver (APBS) software [43].

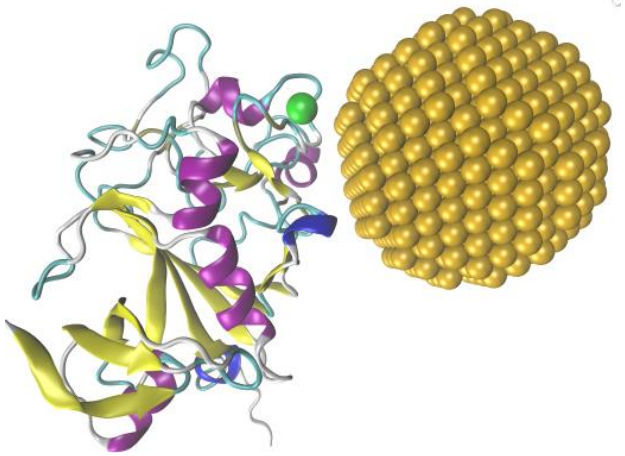

Figure 1 γ-chain fibrinogen (Fg) in complex with spherical gold nanoparticle (GNP) of 3 nm diameter (yellow-colored beads) and calcium ion is located in its active site within the protein (light green sphere).

3. Results and Discussion

3.1. Fg protein remains folded when bound to Ultra-small gold nanoparticle

C-terminal domain of fibrinogen protein γ-chain (simply Fg) in presence of GNP remains folded during the simulation and this was confirmed through performing structural analysis. Starting with the measurement of Root-Mean-Square of Deviations (RMSD) of protein atomic positions relative to its starting structure for free and GNP bound states for the 200ns, shown in figure 2A. For the first 50 ns, in the presence of GNP, the protein experiences an increase in RMSD from 0.25 to 0.55 nm, then it returns to 0.25 nm for the next 50 ns. At the rest of the simulation (i.e. last 100 ns) the reference and the protein in complex exhibits similar behavior. The protein's average Radius-of-Gyration (Rg) was also plotted for both systems as shown in figure 2B. Rg of the protein in the presence of GNP fluctuates from 1.8 to 1.86 nm, while it changes from 1.8 to 1.88 nm, which is considered insignificant difference. The protein flexibility is averaged over 200 ns for each amino acid and expressed as root mean square of flexibility (RMSF) as shown in figure 2C. According to RMSF graph for the protein with GNP and in free state, there is no detectable change in the protein flexibility, apart from residues 352 to 362, and this region is away from the calcium ion binding site, where calcium ion binding site corresponds to ASP318, ASP320, GLY324, and PHE322. The Solvent-Accessible-Surface-Area (SASA) of the protein in free and bound states, was also calculated, as shown in figure 2D. As illustrated both systems exhibit the same pattern with water solvation. The total number of residues forming secondary structure motifs (alpha helices, beta sheets, and turns) of protein's structure compared to the starting structure (i.e. secondary structure percentage) was calculated as a function of time for both systems as illustrated in figure 2E. There is no change in protein's secondary structure in complex with GNP compared to reference system. Based on the protein structural analysis, there is no substantial change in the conformation of the protein, particularly in the active site region as indicated from the RMSF analysis.

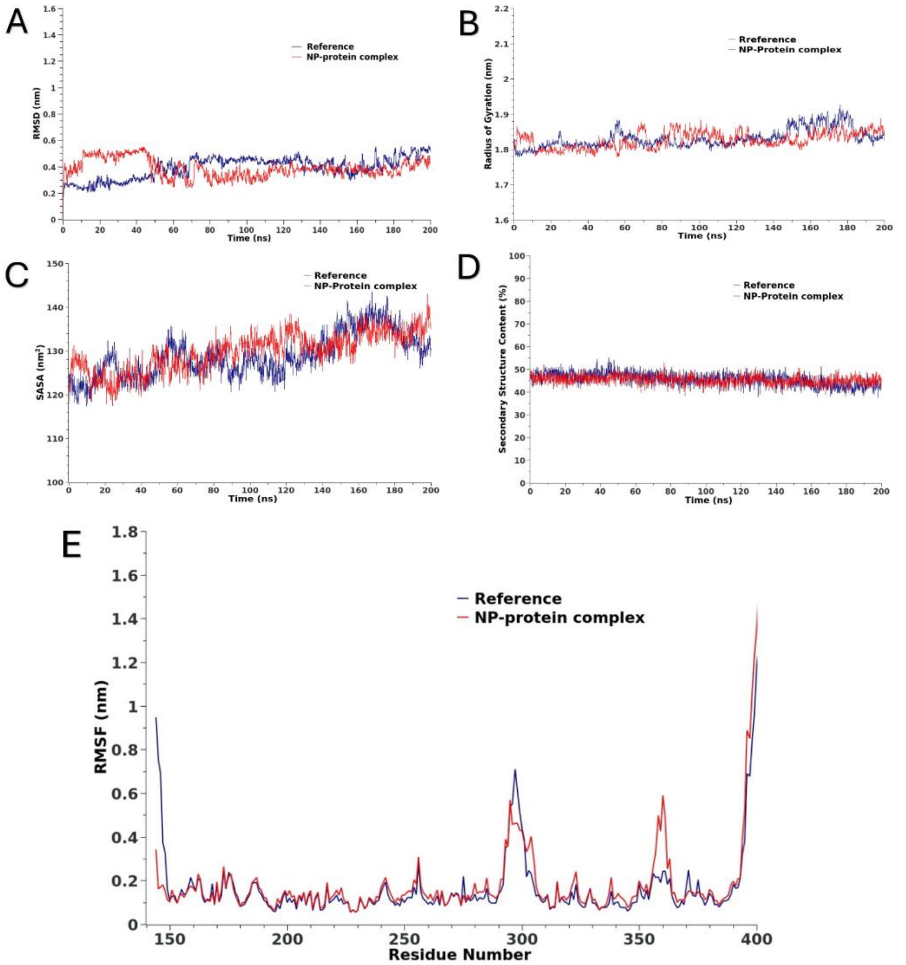

Figure 2 Molecular dynamics simulation structural analysis of both Fg protein (blue) and Fg-GNP complex (red). (A) The root-mean-square of deviation (RMSD), (B) The average radius of gyration (Rg), (C) Solvent accessible surface area (SASA), (D) The percentage of secondary structure content with respect to the starting structure, versus the simulation time. (E) The root mean square of fluctuation (RMSF) of both Fg-GNP complex (red) and Fg protein in bulk solution (blue).

3.2. Molecular Mechanics-Generalized Born Surface Area (MM-GBSA)

3.2.1. The Decomposition of Binding Free energy of Calcium ion to fibrinogen

Components of free energy of calcium ion binding to the fibrinogen protein in the presence of GNP. and for the reference system was calculated by molecular mechanics-generalized born surface area (MM-GBSA) method, and is expressed in figure 3A. Solvation energy contribution to binding was 791.11 Kcal/mol for protein in the presence of GNP compared to 749.82 Kcal/mol for the reference. The electrostatic energy contribution was -749.4 Kcal/mol in comparison to -753.14 Kcal/mol for reference system. the Van Der Waals energy contribution was -24.15 Kcal/mol in comparison to -23.64 Kcal/mol. This gives an overall binding energy of 20.86 Kcal/mol of protein in the presence of GNP compared to 20.32 Kcal/mol of the reference. So, there is not any significant effect on the calcium ion binding to the protein in the presence of GNP.

3.2.2. Per-residue contribution to the binding free energy

The total free energy of binding for residues within 10 A˚ of calcium ion for the protein in GNP complex and in the free states, was illustrated in Figure 3-B. The amino acid residues, which are responsible for calcium ion binding mentioned previously in the literature, were also have significant contribution to the binding energy. Namely, ASP318 with -25.23 Kcal/mol of the protein in complex with GNP, and -26.55 Kcal/mol of the reference, ASP320 with -23.49 Kcal/mol compared to -25.18 of the reference, PHE322 -9.39 Kcal/mol, and -8.36 Kcal/mol of reference system, and GLY324 with -12.21 Kcal/mol compared to -12.4 Kcal/mol. This indicates that there is no effect on calcium binding in terms of binding free energy.

Moreover, the simulation (with its own) shows that the Ca^{+2} is still intact to the protein in its binding pocket, even in the presence of GNP and the reference for 200ns. Figure 4 shows the corresponding final structures of the protein with Ca^{+2} for (A) reference, (B) protein extracted from protein-GNP complex, and (C) the two structures overlapping to show any changes in the conformation. The first two structures are colored by electrostatic potential surface within the range of -1 (red) to +1 (blue) K_bT/e. The stability of the protein structure upon the introduction of GNP is reflected in distribution of the charge on the protein's surface and hence on its electrostatic potential as illustrated in Figure 4.

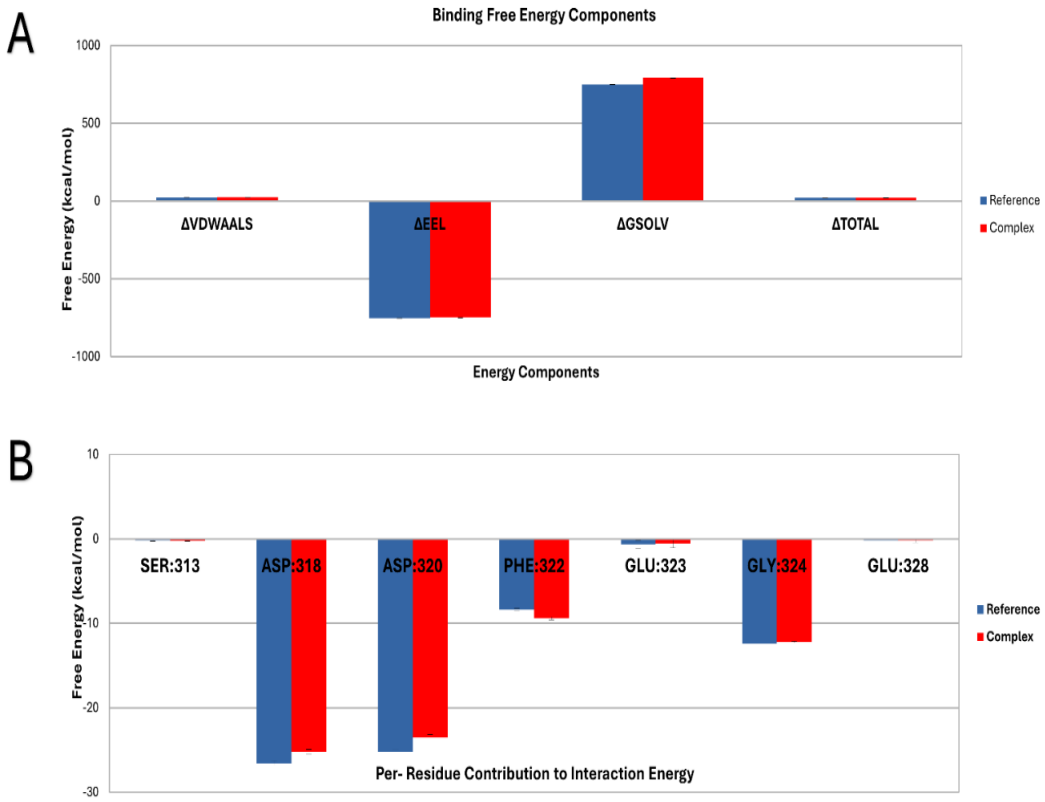

Figure 3 (A) free energy decomposition analysis for Fg protein (blue) and Fg-GNP complex (red). (B) Per-residue interaction energy (kcal/mol) of residues within 5 A˚ from calcium ion (Binding site) in reference (blue) and complex (red).

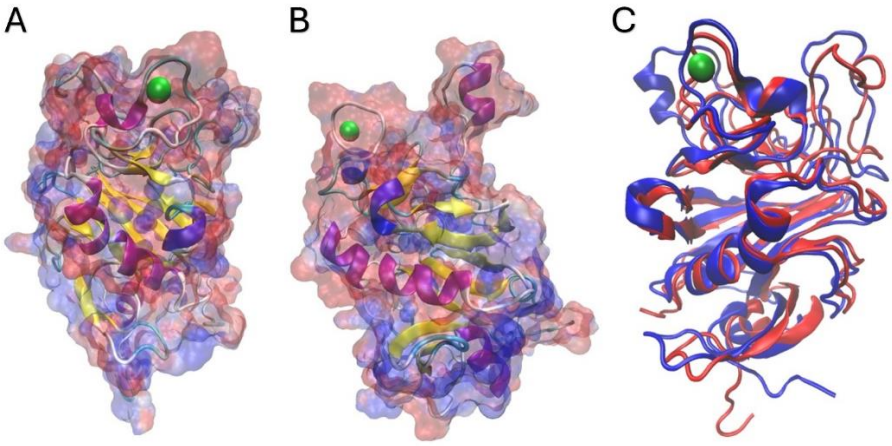

Figure 4 Representative final structures of (A) reference, (B) NP-protein complex after 200 ns, and (C) comparison of both structures in blue and red respectively. The first two structures are represented by a secondary structure (cartoon representation), the protein surface is colored by electrostatic potential ranging from -1 (red) to +1 (blue) K_bT/e

4. Conclusion

We have tested the effect of using ultra small Gold Nanoparticle (GNP) of radius 1.5 nm on C-terminal of fibrinogen's γ-chain of (Fg), using atomistic level molecular dynamics (MD) simulation for 200 ns to protein-GNP and GNP-free (reference) systems, while maintaining the calcium ion in its binding pocket for both systems, due to the fact that Calcium ion is crucial to protein biological function. The structural analysis of the protein indicates that GNP presence, has an insignificant effect on the protein conformation, which was indicated via the measurements of RMSD, RMSF, Rg, and protein's secondary structure content in the free and GNP bound states.

To have an insight into binding free energy of fibrinogen protein to calcium ion, analysis using MM-PBSA was performed. Free energy decomposition and amino acids contributions to the binding show minor differences between the two systems. Moreover, the presence of the calcium ion through the whole simulation course in the presence of GNP, is strong evidence of the persistent binding of calcium ion to the protein and their stability. Consequently, structural stability is reflected on the distribution of the charge on the protein surface, which is maintained and well-illustrated via the measured electrostatic potential surface.

So, calcium binding to the protein is structurally and energetically stable. According to our study the use of ultra small GNP of 1.5 nm is safe in biomedical applications, where it has no effect on gamma chain of fibrinogen protein, which is principal component of plasma proteins in the human body. Thus, Probing the effect of ultra-small spherical GNP is of great importance, as it enables researchers in the biomedical field to optimize the therapeutic efficacy of gold nanoparticles particles, while keeping their toxicity at a minimal level as much as possible.

This study paves the way for more in-depth studies, aiming in the future to be considered; The development of forcefields that account for the polarizability effects of gold atoms, even if it is chemically inert. Moreover, investigating the effect of Gold-nanoparticles with different shapes, that will pave the way for their manipulation in different applications.

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