**In silico** modeling and experimental evidence of coagulant protein interaction with precursors for nanoparticle functionalization

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The design of novel protein–nanoparticle hybrid systems has applications in many fields of science ranging from biomedicine, catalysis, water treatment, etc. The main barrier in devising such tool is lack of adequate information or poor understanding of protein–ligand chemistry. Here, we establish a new strategy based on computational modeling for protein and precursor linkers that can decorate the nanoparticles. *Moringa oleifera* (MO2.1) seed protein that has coagulation and antimicrobial properties was used. Superparamagnetic nanoparticles (SPION) with precursor ligands were used for the protein–ligand interaction studies. The molecular docking studies reveal that there are two binding sites, one is located at the core binding site; tetraethoxysilane (TEOS) or 3-aminopropyl trimethoxysilane (APTES) binds to this site while the other one is located at the side chain residues where trisodium citrate (TSC) or Si60 binds to this site. The protein–ligand distance profile analysis explains the differences in functional activity of the decorated SPION. Experimentally, TSC-coated nanoparticles showed higher coagulation activity as compared to TEOS- and APTES-coated SPION. To our knowledge, this is the first report on in vitro experimental data, which endorses the computational modeling studies as a powerful tool to design novel precursors for functionalization of nanomaterials; and develop interface hybrid systems for various applications.

**Keywords:** molecular docking; magnetic nanoparticle; *Moringa oleifera*; surface coating; coagulation activity; binding free energy

**Introduction**

Over the last 50 years, nanomaterials have been successfully synthesized for diverse applications in many disciplines (De, Ghosh, & Rotello, 2008), thereby creating opportunities to bridge and integrate biology and synthetic materials science. A wide range of core materials have been investigated in the synthesis of nanoparticles such as metals (Fe, Ni, and Co); metal oxides (Fe3O4 and γFe2O3); metal alloys (FePt and CoPt); and ferrites (MFe2O4, where M = Cu, Mn, Ni, and Co) (Burdah, Chen, Narayanan, & El-Sayed, 2005; Faraji, Yamini, & Rezaee, 2010). Their physicochemical properties including surface area, optical, and magnetic behaviors make nanoparticles an appealing tool for numerous applications (De et al., 2008). For instance, magnetic nanoparticles functionalized with antibodies are used for specific capturing of analyte molecules while, it can also serve as a detection method or used as separating technique for a particular molecule from complex substances.

The interaction of nanoparticles with biological systems can be mediated through proteins that are covalently attached or physically adsorbed in order to carry out their significant function. However, it is essential to understand the protein binding, structures, and functions that are influenced by nanoparticle characteristics such as size, curvature, aspect ratio, morphology, crystal structure, and surface chemistry (Gagner, Lopez, Dordick, & Siegel, 2011). Vertegel, Siegel, and Dordick (2004) reported that lysozyme retains its secondary structure and enzymatic activity on smaller and highly curved hydrophilic silica nanospheres than on larger particles. The dispersibility and stability of the nanoparticles are determined by the surface properties. Thus, organic monolayers used to passivate nanoparticles play a key role in controlling the surface properties of the nanoparticles.
role in their biological functions (Rana, Yeh, & Rotello, 2010). The topology and chemical nature of the nanoparticles are considered to be vital for biointerface interactions.

To develop a functional hybrid system of nanoparticle for various applications, including biotechnology, medicine, and catalysis, the proteins need to be conjugated either covalently or noncovalently for e.g. via some other physical means such as electrostatic interaction, van der Waals interaction, or hydrogen bonding. To create such systems without affecting the protein structure and function is a challenge (Rana et al., 2010). The protein surface interactions may not be universal for all proteins and it greatly depends on the protein affinity and nanoparticle surface (Roach, Farrar, & Perry, 2006). The charge on the ligand and chemical characteristics of the nanoparticle has a strong influence on protein immobilization, stability, and functionality (Aubin-Tam & Hamad-Schifferli 2005, 2008). According to Hung et al. (2011), both ligand chemistry and nanoparticle surface determine the orientation and conformation, as described using adsorbed proteins of cytochrome c by computational molecular dynamic study.

Studying the interactions between functionalized nanoparticles and proteins at the molecular level has many applications in different fields ranging from photodynamic therapy, wastewater treatment, biomedical imaging, and drug delivery (De et al., 2008; Satishkumar & Vertegel, 2008). The theoretical modeling tools can be used in a cost-effective manner to understand and design novel linkers to mediate protein–nanoparticle interactions. Thanks to technological advancement in developing fast computers and progress in finding efficient algorithms for solving numerical equations and compute interactions (such as particle Ewald mesh) (Darden, York, & Pedersen, 1993), it is possible to study or model any complex structure and event. It can be used in biology to study the structure and function of biostructures such as proteins, membranes, and various processes including protein folding, conformational changes, and segmental dynamics. In addition, the protein–protein interactions, DNA–protein interactions, and protein–drug interactions can be modeled. The theoretical techniques can analyze biological systems at the microscopic level with a time scale ranging from femtosecond to microsecond and a length scale ranging from few tens of nanometers; hence the information obtained regarding protein substrate interaction can potentially be used to design novel nanoparticle linkers for functional hybrid structures.

The present study aims to prepare protein–functionalized nanoparticle hybrid system for Moringa oleifera (MO2.1) coagulant protein, possessing coagulation and antimicrobial properties (Broin et al., 2002; Ghebremichael, Gunaratna, Henriksson, Bruner, & Dalhammar, 2005; Suarez et al., 2005). The binding sites of MO2.1 protein are poorly understood; hence, it is vital to understand the molecular interactions of protein–linker that lead to develop a biointerface system for wide-range applications. The computational modeling study as well as experimental evidence for the interaction between the protein and organic linkers (precursors) that can decorate the surface of magnetic nanoparticles is reported. The prepared magnetic nanoparticles consist of iron oxide core functionalized with inorganic shell that has either carboxyl, amino, silanol, or hydroxyl functional groups. The aforementioned nanoparticles were analyzed for protein binding. Theoretical modeling tools were employed to understand the interaction of protein with precursor ligands on magnetic nanoparticles; while the binding energy required were also calculated and compared. Usually, the linkers or precursors have long tails that may establish a direct interaction with proteins while the nanoparticles themselves are immersed in the media. Hence, the modeling study was focused on the interaction of these organic precursors with the protein. The functionality of the protein attached core-shell nanoparticles was analyzed. Experimental data were correlated with theoretical modeling and the results are discussed.

**Materials and methods**

**In silico studies**

Theoretical modeling tools were used to understand the structure of a flocculating protein, MO2.1, from natural seed extract of Moringa oleifera coagulant protein (MOCP) and its ability to bind to various precursors as well as hydroxyl groups functionalized nanoclusters. Different theoretical tools have been employed in the following sequence: (1) Molecular docking studies were carried out to identify different binding sites of this protein and to investigate the binding strength and mode of binding of different organic molecules and hydroxyl groups functionalized silicon clusters with this protein. (2) Since the docking studies mimic the zero temperature situations, we have also carried out molecular dynamics (MD) simulations for the docked protein–ligand structures in aqueous solution to study finite temperature effect. (3) In addition, based on the trajectory obtained from the MD simulations, the binding free energy calculations have been carried out to estimate the interaction strength between the organic precursors and protein.

**Docking studies**

The 3D structure of the protein, MO2.1, has been adapted from our previous manuscript which was based on homology modeling of polypeptide sequence of MO2.1 (P24303) through superimposition with the mabinlin II sequence (Li et al., 2008). This has the structural identity of 97% with MO2.1 protein. The sequence of the protein that has been used in this study is:
Many possible minimum energy protein–ligand structures are found and reported based on scoring function that includes various contributions such as electrostatic, van der Waals, and hydrogen-bonding interactions between protein–ligand complexes. Only the global (lowest) minimum energy protein–ligand structures were analyzed.

**MD calculations**

Starting with the three docked protein–ligand structures, MD simulations were carried out. Firstly, the protein–ligand complex was solvated with aqueous solution followed by subsequent addition of Na\(^+\) and Cl\(^-\) ions to neutralize the system. In this study, generalized AMBER force field was used for all three ligands namely TSC, APTES, and TEOS; and PARM99 and TIP3P force fields have been used for protein and water, respectively. The atomic charges for the ligand molecules were obtained by fitting to molecular electrostatic potential using the charges from electrostatic potential using a grid (CHelpG) based method as implemented in Gaussian09 software (Frisch, 2009). The interaction between the protein and ligands include van der Waals and electrostatic energies. The simulations were performed in isothermal–isobaric ensemble. The calculations were carried out in room temperature and at 1 atmosphere pressure. Different structural and energetic parameters were looked for convergence. The calculations were carried out to check the stability of the protein–ligand complexes at ambient condition. In addition, the trajectories obtained in the simulation for the protein–ligand complexes were used for the binding free energy calculations.

**Binding free energy calculations**

The molecular docking studies provide quick solutions for the possible minimum energy structures for protein–ligand complexes. However, in these calculations the aqueous solution is not included explicitly therefore, it lacks the conformational sampling over the protein and ligand degrees of freedom (Brandsdal et al., 2003). Nonetheless, the contributions from conformational sampling and solvents to the binding free energy can be obtained from molecular mechanics Poisson–Boltzmann surface area technique (MM-PBSA) employed on the trajectory that is obtained from the MD simulations (Kollman et al., 2000). In the MM-PBSA calculations, the coordinates of only protein and ligands are used excluding all the solvents and ions. In these calculations, the binding free energy is computed using the following equation:

\[ \nabla G = G_{\text{complex}} - G_{\text{protein}} - G_{\text{ligand}} \]

The free energies for the protein–ligand complex, protein, and ligands are computed separately as an average over many configurations. Each free energy term includes the average molecular mechanical energy and solvation free energy. The binding free energy calculations were carried out using MMPBSA.PY module as implemented in Ambertools1.5. We have carried out the binding free energy calculations using both MM/PBSA and MM/generalized born surface area (GBSA) models (Brandsdal et al., 2003; Kollman et al., 2000). These two models differ with respect to how they describe electrostatics between solute (i.e. protein or ligand or its complex) and solvents. However, both belong to a continuum description for solvents. A negative value for binding free energy implies that the protein–ligand complex formation is favorable. The computed binding free energies from both MM/PBSA and MM/GBSA models for MO\(_{2.1}\)–TSC, MO\(_{2.1}\)–APTES, and MO\(_{2.1}\)–TEOS are given in Table 1 (Supporting information). The results are discussed in the following section.

**In vitro studies**

**Synthesis of magnetic nanoparticles**

Superparamagnetic iron oxide nanoparticles (SPION) were synthesized by controlled coprecipitation method as described (Okoli et al., 2011). Briefly, the iron source was prepared by dissolving FeCl\(_3\) and FeCl\(_2\) in Milli-Q water in the mole ratio of 2:1 followed by precipitation of nanoparticles with ammonia solution at 70°C under vigorous mechanical stirring. The reaction was allowed to proceed for 45 min under N\(_2\) atmosphere in a closed system. The black precipitated nanoparticles were collected with an external magnetic field and then washed several times with Milli-Q water. The obtained magnetic...
nanoparticles was resuspended in water and stored at 4°C for further use and characterizations.

**Trisodium citrate (TSC) coating**

Surface coating of the SPION with TSC was carried out according to the method described by Young-Hui et al. (2005) (Deng, Wang, Hu, Yang, & Fu, 2005) with some modifications. Magnetic nanoparticles were subjected to vigorous stirring at 90°C upon the addition of TSC solution. The TSC-modified SPION obtained was resuspended in water after several washing steps. The surface coating of SPION was evaluated by Fourier transform infrared spectroscopy measurement (Okoli et al., 2011).

**Tetraethoxysilane (TEOS) coating**

Stöber method with some modifications (Stöber, Fink, & Bohn, 1968) was used in order to obtain silica surface coating using TEOS as precursor. Magnetic nanoparticles suspension was diluted with water, alcohol, and aqueous ammonia. The dispersion was homogenized by ultrasonic vibration in water bath. Upon continuous mechanical stirring, TEOS was slowly added drop wise to the reaction mixture. After stirring for 12 h, silica was formed on the surface of the magnetite nanoparticles through hydrolysis and condensation reaction.

**3-Aminopropyltriethoxysilane (APTES) coating**

Amino-silane coupling agent (APTES) was used for the surface modification of SPION. The amino-silane agent of this precursor is well suited to obtain a high density of –NH2 surface functional group. A 4 mL magnetic nanoparticles suspension (18 mg/mL) was dispersed in 35 mL of Milli-Q water. APTES was added drop wise into the mixture under oxygen free environment at 40°C for 1 h (Shen, Fang, Zhou, & Liang, 2004). The solution was cooled to room temperature. The prepared APTES-modified SPION was collected with an external magnet and washed with ethanol followed by Milli-Q water three times. Finally, APTES-modified SPION was resuspended in 20% ethanol and kept at 4°C prior to use.

**Protein-attached SPION and coagulation activity test**

In order to study the interaction/binding mechanism of surface modified SPIONs with MOCP, a protein–nanoparticle composite was developed. Binding of MOCP onto the surface modified SPIONs was performed to prepare protein–SPION complex. In a typical experiment procedure, TSC–SPION, SiO2–SPION, and APTES–SPION were equilibrated with 10 mM ammonium acetate buffer, pH 6.7. Volume of each solution was adjusted such that total weight of magnetic particles was having the same concentration (~2.5 mg) in the resultant solution. Binding of MOCP onto the nanoparticles was carried out in 10 mM ammonium acetate buffer, pH 6.7. After room temperature incubation for 1 h, the protein-attached SPIONs were washed with ammonium acetate buffer and then separated with external magnet. The separated protein-attached SPIONs were suspended in the same buffer or Milli-Q water and kept at 4°C prior to use. The functionality of the prepared protein-attached SPIONs was tested in clay suspension according to the protocol reported in our previous work (Ghebremichael et al., 2005).

**Results and discussion**

The information about the binding sites of MO2.1 protein and its mode of interaction with charged and neutral organic precursors and the nature of molecular interactions stabilizing protein–linker conjugates is useful for wide-range of applications (De et al., 2008; Satishkumar & Vertegel, 2008). An extensive study was carried out to find different binding sites for charged and neutral organic precursors; also the mode of binding and nature of interaction between precursor and proteins. The aforementioned precursors, i.e. TSC, APTES, and TEOS, interact with the surface of MO2.1 protein via carboxyl, amino, and silanol functional groups, respectively, while the magnetic core (SPION) does not influence the binding capacity of the ligands. The functionality of protein-attached SPION was analyzed and the results were discussed.

**In silico studies**

**Interactions of organic linkers with MO2.1 protein**

The 3D structure for coagulant protein MO2.1 from *M. oleifera* has been obtained from homology modeling studies based on the reference structure of Mabinlin II protein (Li et al., 2008) and since the structure of the protein is not yet resolved. Overall sequence identity of the model protein was around 97% with the original MO2.1 protein. The earlier predicted structure for MO2.1 protein from homology modeling with napin protein had 71% of structural identity (Suarez et al., 2005). The approximate size of the *M. oleifera* (MO2.1) protein is around 3.1 nm which has been obtained as an average over the size of the three helices. Docking studies provide first-hand information like the binding mode, orientation of ligand to protein, and also provide details of various contributions to the total interaction energy. Since, it is difficult to use iron oxide core molecule for computational studies due to the unavailability of force fields and system size, the surface coated precursors were used as a ligand for analyzing the interaction with protein residues. Moreover, the linkers have usually long
tails; only the polar groups of the surface coated linkers are involved in the direct interaction with the proteins thereby neglecting the nanoparticles in the modeling study was a valuable approximation. Blind docking study was performed where there is no need to specify the active site or binding site of the protein and this becomes handy when there is no such information available for MO$_{2.1}$ protein. From the docking studies, it was found that all three organic linker molecules studied were bound to the protein even though their relative binding energies and sites were different. The docking views of these three ligands with _M. oleifera_ (MO$_{2.1}$) protein were shown in Figure 1(a)–(c).

Overall, docking studies reveal that there were at least two binding sites and the size and charge of the ligand decide the binding site. Among the two binding sites, one was located at the core of the protein surrounded by Cys12, Ala28, and Ala32 residues while the other one was located at the side chain of the 3rd helix around Arg48 and Arg52 residues. In the case of APTES and TEOS, Cys12 residues of protein were involved in hydrogen bonding interaction and appear to be a binding site. However, the TSC ligand binds to Arg48 and Arg52 where the two COO$^-$ groups of TSC interact like a clip. The MD studies performed for these protein–ligand structures show that even at ambient conditions, the ligands were localized in the binding site. The root mean square displacement (results not shown) computed for the ligands shows localized dynamics of ligands in the protein binding sites and suggests that all three ligands form stable protein–ligand complexes in ambient temperature and pressure suitable for practical applications. Functionalized nanoclusters may serve as a suitable linker for proteins. These have advantages when compared to organic linkers due to relatively larger surface area and size dependent optical properties of these clusters. The interaction of Si$_{60}$ nanoclusters functionalized with hydroxy groups was studied and it was observed that the Si$_{60}$ cluster binds to Arg48 and Arg52 residues of the protein (Figure 1(d)). The position of binding site in protein was decided by the size and charge of the ligands. The negatively charged ligands such as TSC or hydroxy groups functionalized Si$_{60}$ bind to the positively charged Arg48 and Arg52 residues. The ligands such as silanol group from TEOS and amino group from APTES bind to core binding site. The relative strength of these protein–ligand complexes is discussed below from the binding free energy calculations (Table 1, Supporting information).

The negative values for the total binding free energy suggest that there was a formation of favorable protein–ligand complexes. The comparable values for the binding free energies (ranging between $-15$ and $-17$ kJ/mol) for all three protein–ligand complexes suggest that these

![Docking study of protein (MO$_{2.1}$) interaction with ligand molecules. The following ligand interactions are shown (a) TSC ligand; (b) TEOS; (c) APTES; and (d) Si$_{60}$ cluster. Notes: The important protein residues are highlighted, arginine (green); cysteine (yellow); alanine (white); and Si$_{60}$ nanoclusters with OH group (red and white).]
complexes have comparable stabilities. In all three cases, the van der Waals and electrostatic interactions between protein and ligand favor the formation of protein–ligand complexes. Particularly, in the case of protein–TSC complex, the electrostatic interactions are highly dominant and the value was many folds larger than the other two cases. The electrostatic contribution in the case of protein–TSC complex stabilizes the formation of such complex. Interestingly, differences in the total free energies of the protein extracted from the three protein–ligand complexes were observed. As discussed in the later section, this has to be attributed to the ligand-induced structural changes in the protein.

Protein residues–ligand distance profile analysis

There have been detailed reports about the effect of protein on the structure and dynamics of hydration layer (Pal, Peon, Bagchi, & Zewail, 2002; Pal, Peon & Zewail, 2002; Pal & Zewail, 2004) and on the molecules (Murugan & Ågren, 2009; Murugan, Jha, & Ågren, 2009; Murugan, Kongsted, Rinkevicius, & Ågren, 2012) that were bound to the cavity of protein or on its surface. The reports suggest that the structure, dynamics, and electronic structure of these molecules are dramatically affected in the vicinity of protein. Interestingly, there were reports on the ligand-induced structural changes in proteins even though the size of the ligand subsystem was much smaller when compared to protein (Mobley & Dill, 2009; Najmanovich, Kuttner, Sobolev, & Edelman, 2000; Zidek, Novotny, & Stone, 1999). It was shown that there was an increased conformational flexibility of protein backbone when it binds to a hydrophobic ligand (Mobley & Dill, 2009). Some proteins show increased side chain motion upon binding. Furthermore, there were reports of ligand binding induced structural ordering in some proteins (Hilser & Thompson, 2007; Radivojac et al., 2007). Here, our focus was to study the linker-induced changes in structure and dynamics of proteins. If the linker molecules alter the structure of the proteins drastically, it would also affect the functionality of the proteins. Basically, it would be of interest to look for the linkers that would bind to the proteins but still would not change the antimicrobial and flocculating functionality of the protein. Hence, it was focused to carry out protein residues–ligand distance profile analysis to study the structural changes in the protein due to the binding of linkers.

The instantaneous distance was computed (defined as \( R_{N\text{-ligand}} \) where \( N \) refers to the residue number and ligand refers to the precursors namely TSC, APTES, and TEOS) between each of the 60 residues of protein and the ligand center of mass. In addition to that, the average of \( R_{N\text{-ligand}} \) was also computed. This calculation has been done for 1000 configurations obtained from the MD study corresponding to the three protein–ligand complexes.

The results are shown in Figure 2 for all the three linkers such as protein–TSC, protein–TEOS, and protein–APTES. It shows the average distance value for each residue and its distribution with respect to the average value. The width was directly related to the amplitude of the residue motion (with respect to the ligand). A larger \( r \)-value for the width means a large amplitude motion for the residue, while the smaller \( r \)-value for the width means a localized motion for the particular residue. As can be seen in Figure 2(a) for the distance profile of protein–TSC complex, the residues Arg48 and Arg52 are in closer contact with the TSC ligand. Interestingly, we observe smaller width computed for these two residues (among all 60 residues). This clearly shows that the binding of the ligand makes the dynamics of these two residues sluggish. When compared to other two cases, we observed relatively smaller distance for widths of other residues in the 3rd helix. So,
the effect of ligand binding was in some sense can be seen over the whole helix. Larger width for the residues that form 1 and 2 helices means increased conformational flexibility for these two helices when compared to 3rd helix that was involved in binding.

For the ligands of TEOS and APTES, the 1st and 2nd helices have restricted dynamics over the 3rd helix since these two helices are involved in binding. Particularly, Cys12 of helix 1, Ala28, and Ala 32 of the protein appear at smaller r-value in Figure 2(b) and (c), hence involved in binding. As discussed above, in these two cases, a smaller width for the residues namely Cys12, Ala28, and Ala32 was also observed. The features seen for protein–TEOS and protein–APTES were almost the same except in the region corresponding to the residues 39-50. A relatively larger width for the residues in the case of protein–APTES was observed, which implies increased conformational freedom/flexibility for the 3rd helix; the phenomenon, explains the increased activity for this case when compared to protein–TEOS. Overall, our study shows that the ligand binding brings some sort of structural ordering to the residues that were involved in ligand binding.

Since MO2.1 protein has the possibility of forming homodimers (Broin et al., 2002), it is of interest to study the interaction of homodimeric protein and ligand molecule (Figure 3). The structure for the dimeric protein has been taken from our previous study. The binding free energy calculations were performed for one and two TSC with dimer of MO2.1 protein in order to verify the change in binding ability of this protein due to dimerization. Interestingly, the results show that the binding was stronger for TSC with dimer than with the monomer since the binding free energies for TSC with monomer and dimer are, respectively, around −15 and −32 kcal/mole (Table 2, Supporting information). However, the addition of second TSC molecule to the dimer does not yield such strong binding, which was seen from the lowering in the value of binding free energy to around −8 kcal/mole. Moreover, either monomer or dimer, TSC still binds to Arg48 and Arg52 residues implying the specificity in binding irrespective of the oligomerization (or dimerization). This study gives firsthand information evidencing that TSC molecule was interacting with one protein molecule of the homodimer thus suggesting one protein–one ligand interaction. This makes it suitable for functionalizing the nanoparticle with specific interactions for MO2.1. The coagulation activity of the dimer protein was reported. Altogether, calculations suggest that the linker binding ability of the protein was retained in its dimeric form and in addition the binding site remains unaltered.

Based on the in silico studies, it is evident that the interaction of protein with TSC-coated nanoparticles implies the importance of C terminal region in binding, while the N-terminal region is more likely for interacting with other molecules. It is of interest to ascertain the in silico investigation with in vitro experiments.

In vitro analysis

Synthesis and surface modification of magnetic nanoparticles

Superparamagnetic iron oxide nanoparticles (SPION) were synthesized by coprecipitation approach and then modified with COO−, SiO2, and −NH2 functional groups to study their interaction/binding mechanism with MOC2. The surface modified SPIONs of the present study have been characterized using different techniques (Okoli et al., 2011), and their efficiency and stability was enhanced after surface coating with precursors.

Protein-attached SPION and coagulation activity test

Data from the experimental results (Figure 4) demonstrate that there is a significant interaction between the surface modified SPION and protein from M. oleifera; these results support the data earlier published (Okoli, Boutonnet, Mariey, Jaras & Rajarao, 2011; Okoli et al., 2011). Moreover, it also implies that the adsorbed

Figure 3. Homodimer formation of coagulant protein (MO2.1) and its interaction with TSC precursors. Homodimer interaction with (a) one TSC precursor and (b) two TSC precursors.
Notes: Important protein residues are highlighted (arginine – green; cysteine – yellow; and alanine – white).
protein from MOCP is still retaining its functionality even after binding to the nanoparticles. The protein-attached surface modified SPION exhibited an enhanced efficiency in the reduction of turbid clay suspension compared to the MOCP alone due to the effect of large surface-to-volume ratio of the nanoparticles that initiated increase in protein-attached particle density. Among the different surface coated SPION, TSC-coated nanoparticles showed highest coagulation activity of ~80% compared to TEOS-coated nanoparticles (~60%). APTES-coated nanoparticles depict 65% activity, which falls in between TSC- and TEOS-coated nanoparticles.

The protein–ligand distance profile analysis confirms the difference in functional activity of the surface modified SPION. As can be seen in Figure 4, TSC-coated nanoparticle attached with protein showed highest coagulation activity demonstrating that there are free Arginine residues in helix 1 and 2 available for interaction. This has been confirmed with protein–ligand distance profile analysis where helices 1 and 2 are having increased conformational flexibility for interaction with other molecules (Figures 1 and 2). The least interacting surface was TEOS-coated nanoparticle attached with protein, given that the structure is rigid with very restricted dynamics. The APTES-coated nanoparticle showed better activity even though the binding residues were similar to that of TEOS. As seen in Figure 2, the residues are relatively flexible in helix 3 making the protein interaction efficiently than TEOS. Altogether, the binding energy, the binding site, and distance in motion are favorable for TSC interacting with protein compared to the other two ligand molecules as evidenced by in vitro results. Suarez et al. (2005) pointed out that the glutamine residues and positive charges in the protein molecule were responsible for functional activity. This could be the possible region for protein functionality even though protein binds with ligand molecule does not interfere with its functionality.

To our knowledge, this is the first report on coagulant protein and its interaction study with different precursors used for functionalizing nanomaterials. This study endorses that the protein interaction with ligand was correlating with the experimental results; thus implying that in silico study could be a useful tool to understand complex molecular structures while developing interface hybrid systems for various biological applications.

**Conclusions**

Molecular docking studies have been carried out to identify the precursor ligand binding sites with the *M. oleifera* (MO₂₋₁) coagulant protein and also, their mode of interactions. It showed that there were at least two binding sites one at Cys12 (a core binding site) and other located closer to Arg48 and Arg52 residues (a surface binding site). Binding to these sites was decided by the size and charge of the ligands. The binding free energy calculations suggest that all three protein–ligand complex formation was favorable. Particularly, a negatively charged TSC binds to the surface binding site of protein where two positively charged Arginine (Arg) residues are located. The binding site and binding ability remain similar even in dimer form of the protein. TSC-coated nanoparticles showed higher coagulation activity compared to TEOS and APTES. The in vitro experimental data confirmed the computational modeling studies; thus, provide a tool in designing and validating different precursors for functionalization of nanomaterials and to develop interface hybrid structures for various applications.

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**Supplementary material**

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