

# Molecular dynamics simulations on constraint metal binding peptides

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## Abstract

Molecular dynamics (MD) simulations are performed using constraint polypeptides that were combinatorially selected to have binding affinities for the noble metal platinum (Pt). We analyzed the effects of the threonine-serine-threonine (T-S-T) amino acid sequence because this domain is common among strong binders. Using pair correlation functions, intermolecular interactions are evaluated between peptide residues and the metal surface in the presence of solvent water. In explicit simulations in the absence of metal surface, we find that among the experimentally verified strong binders the side chain groups within the T-S-T region make hydrogen bonding with water molecules, i.e. being more solvent exposed. In MD simulations including the metal, the T-S-T region interacts with the substrate to an extent greater than those with the non-polar residues. However, it is also observed that carbonyl and amide groups on the backbone and certain residues, such as Arg and Pro, also exhibit close interactions with the surface. Backbone torsional angle auto-correlation functions indicate that threonine and serine residues impart the highest flexibility to the backbone of the chains in solvent simulations in the absence of the surface. This flexibility of the peptides and their interactions with the metal surface are major players in binding. The simulations also reveal that the flexibility of the whole chain is considerably hindered upon binding. These results have significant implications in understanding of how constraint peptides selectively bind to a metal surface and may provide insight into the design of new sequences.

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## 1. Introduction

Proteins interact with other macromolecules and inorganics to control the structures and functions of all biological tissues in organisms [1]. Biological hard tissues such as bones, dentin, enamel, and spines contain proteins together with inorganic minerals [2]. Design and engineering of proteins with desired properties such as specific binding affinity to selected inorganics, would open avenues to the engineering of materials with novel properties and broaden the knowledge about protein physics [3–5]. Thus, binding of polypeptides to metal surfaces is of great interest in the field of nanotechnology, biomaterials and biomimetics. In biomaterials, protein adsorption is critical for the integration of an implant with tissue [6,7]. In the nanotechnology aspect, protein–surface interactions

constitute the basis for functional biological/electronic constructs such as sensors, activators, etc. [4]. Biomimetics, i.e., gaining inspiration and guidance from nature have also gained considerable attention in recent years for the design with novel properties [1]. In view of this approach, combinatorial and evolutionary techniques have been adopted for the creation and isolation of peptide sequences that bind specifically to solid surfaces, similar to the natural proteins that regulate crystal growth [4].

Recent studies with protein–surface interactions basically aim to combine inspirations adopted from nature and engineering to develop systems to operate at the nanoscale with the advantage of the functional properties of these novel protein [4]. Whaley et al. [8] have investigated peptide adsorption on several semiconductor surfaces by using phage-display techniques. 12-mer peptide chains is found to have high affinity and specificity to bind on semiconductor surfaces, and the adsorption affinity is found to be enhanced with the presence of serine (Ser, S) and threonine (Thr, T) rich regions. Studies on the adsorption of peptides on stainless steel surfaces suggest that the adsorption behavior of a peptide depends not only on the amino acid residue content but also on their sequence [9,10].

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Table 1  
Simulation details

Chains	No surface		With surface	
	No. of runs	Simulation time (ns)	No. of runs	Simulation time (ns)
1 (strong binder)	6	2	2	2
2 (strong binder)	4	2.5	–	–
3 (weaker binder)	3	2	2	2
4 (weaker binder)	2	2	2	2

Braun et al. [3] have presented a study based on structure predictions for three gold-binding protein sequences selected by combinatorial techniques and have further utilized molecular dynamics to assess the interactions responsible for the binding process. The results indicate that the main contribution to the adsorption energy comes from the polar residues, i.e. serine and threonine, in the presence of equal number of polar side chains and hydrophobic residues. Sarikaya et al. [1] and Brown et al. [11] have reported 28 experimentally determined polypeptide sequences exhibiting affinity to bind on various solid surfaces including Au, Pt, Pd, Ag, SiO<sub>2</sub>, zeolites and some Zn, Fe compounds. Specific antibody development to bind to crystal surfaces for controlling the crystal growth [12,13] and nanoparticle–protein integration studies [14–16] are extensively reported.

To gain a better insight on the mechanism of peptide recognition and binding we carried out molecular dynamic simulations on cyclic polypeptide chains, shown experimentally to have binding affinity to noble metal platinum

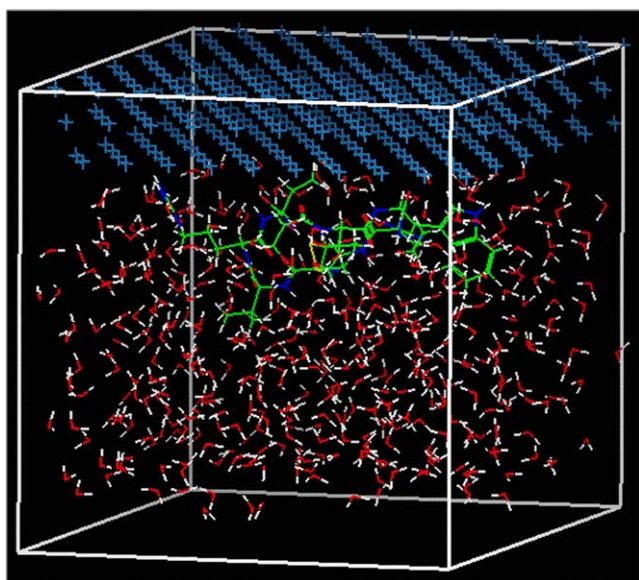


Fig. 1. Water box (28 Å × 28 Å × 28 Å) including the Pt {1,1,1} surface and the cyclic peptide.

(unpublished). Explicit solvent simulations are carried out in the absence and presence of the metal surface with four different peptide sequences, three of which share a common motif of Thr–Ser–Thr (T–S–T) sequence with binding affinity. The results provide insight on the common characteristics in equilibrium and dynamic properties of these sequences and an analysis of their interactions with the Pt surface.

## 2. Material and method

The cyclic peptides investigated here, each having different binding affinity for Pt surface, consist of nine amino acid residues including two cysteine residues at the ends for ring closure. Four peptide chains investigated in the present project have the following sequences: (1) Cys–Pro–Thr–Ser–Thr–Gly–Gln–Ala–Cys; (2) Cys–Gln–Ser–Val–Thr–Ser–Thr–Lys–Cys; (3) Cys–Val–Arg–Thr–Ser–Thr–Trp–Arg–Cys; (4) Cys–Ile–Met–Arg–Asp–Gly–Pro–Met–Cys. According to peptide binding experiments carried out using immunolabelling fluorescence microscopy, the first two sequences are known to be strong metal binders, whereas the latter two are relatively weaker binders (S. Dincer et al., to be published).

MD simulations were performed using the commercial software, Accelrys [17]. Single chains were initially generated by assigning random torsion angles to the backbone with the Builder module of Accelrys [17]. The two cysteine residues were then connected by S–S bridge for cyclization, and energy minimization was performed by the Discover\_3 module [17]. As a result, different conformations were obtained for each peptide sequence. Then each cyclic chain was soaked in a periodic box filled with water molecules assuring a sufficiently thick water layer around the chain in all directions. The cubic box lengths varied between 25–30 Å depending on the sequences. Several independent simulations were performed for each chain listed in Table 1. As a result, multiple trajectories were considered in the analysis so that common characteristics among the chains, if any, can be observed in dilute solution.

The final peptide conformation equilibrated in water at the end of each run was then manually placed next to a Pt {1,1,1} surface, composed of three layers, generated by the Accelrys Solids Builder module [17]. The TST sequence known to have binding affinity was specifically located close to the surface. Then the peptide was soaked in water together with the surface (Fig. 1). For each periodic box containing surface, peptide, and water molecules, independent MD simulations were performed following brief energy minimization to resolve undesirable close contacts among the different components in the system. The resulting non-orthogonal box dimensions including the surface, peptide, and water molecules are 28 Å × 28 Å × 28 Å.

The group-based and atom-based summation methods

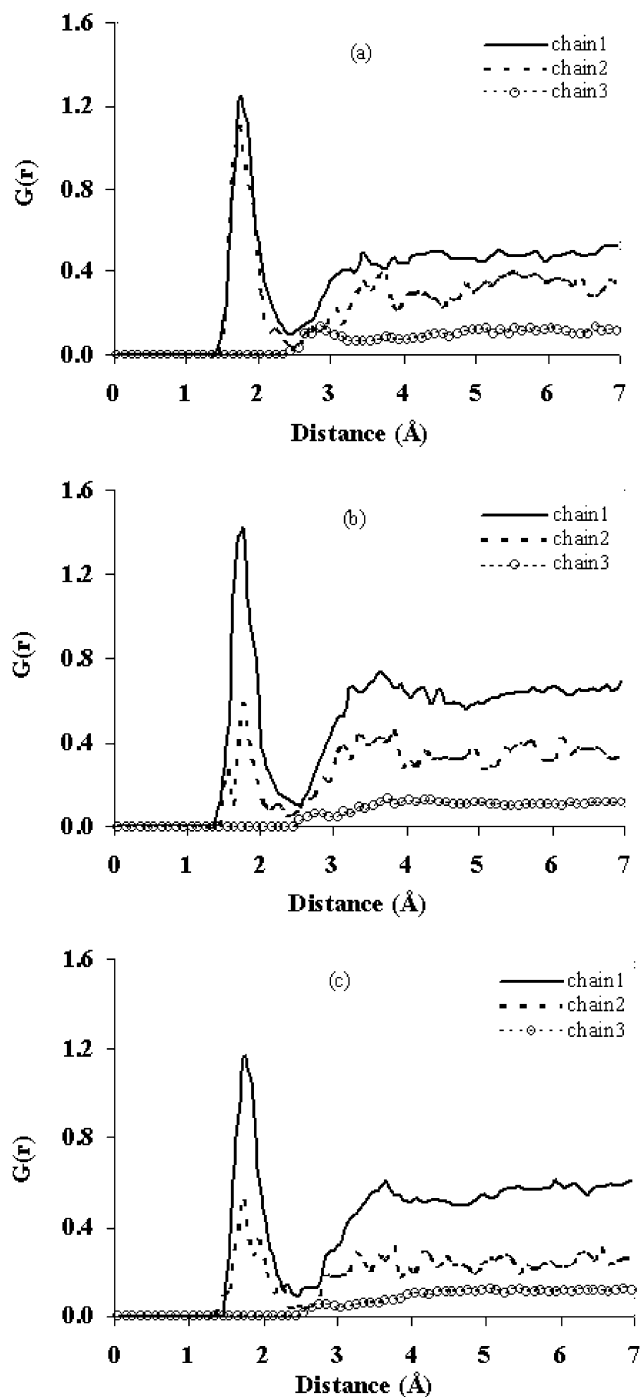


Fig. 2. Pair correlation function between water molecules and side chain OH groups of (a) Thr1, (b) Ser, (c) Thr2 in chains 1, 2 and 3, in explicit water simulations with no surface.

were applied in solvent simulations (no surface) and in surface simulations, respectively. In all simulations (Discover\_3 module), the cutoff distance for the non-bonded interactions was set to 10 Å, using a buffer width of 1.5 Å. The number of residues in the chains varied between 432 and 443 with total number of atoms including water and metal surface varying between 1723 and 1744. Quasi Newton–Raphson minimization method; Velocity Scaling

temperature control methods (at 300 K), Verlet integration algorithm (with 1 fs time step) were used in the simulations. The initial 500 ps period of each run was discarded in the analysis as the equilibration period.

Consistent valence force field (CVFF) [18] was used in all simulations. CVFF is a generalized forcefield for which the parameters are provided for amino acids, water and a variety of other functional groups including metals. The potential energy expression of CVFF includes the bonded interaction terms representing the energy of deformation of bond lengths, bond angles, torsional angles, and out-of-plane interactions; the bonded off-diagonal (cross terms) representing the couplings between deformations of internal coordinates; and the non-bonded terms, namely the Lennard–Jones potential for van der Waals interactions and Coulombic form for electrostatic interactions. The cross-terms were not included in our simulations for efficiency and the dielectric constant was taken as 1.0.

Analysis of the trajectories mainly concentrated on the equilibrium properties, i.e. intermolecular interactions (with surrounding water and the metal surface) were investigated via pair correlation functions and dynamic properties, i.e. time-dependent conformational behavior of the peptide bonds, were investigated by backbone torsional-angle relaxations.

### 3. Results and discussion

#### 3.1. Equilibrium properties (intermolecular interactions of the peptide chains with water and surface)

Intermolecular interactions of the chains are analyzed by using the pair correlation function,  $G(r)$ , in the absence and in the presence of metal surface. The pair correlation function can be expressed as [19]:

$$G(r) = \frac{\langle \rho_A(r) \rangle}{\langle \rho_A \rangle_{\text{local}}} = \frac{1}{\langle \rho_A \rangle_{\text{local}}} \frac{1}{N_B} \sum_{i \in A} \sum_{j \in B} \frac{\delta(r_{ij} - r)}{V(r)} \quad (1)$$

where,  $\rho_A(r)$  is the density of type A particles at distance  $r$  from particle B. The summation term shows the number of  $ij$  pairs that are separated by a distance  $r$ . These pair are determined by taking spherical shells with volume  $V(r)$  and normalized with the average density  $\langle \rho_A \rangle_{\text{local}}$  in the whole volume and the total number of B particles ( $N_B$ ).

Special attention is devoted to the polar threonine–serine–threonine (T–S–T) sequence, which is common in strong binders and known to contribute to the metal binding affinity as reported in literature [4,9]. The intermolecular interactions of the peptide chains with surrounding water molecules (in the absence of Pt surface) are presented in Fig. 2. Panels (a)–(c) demonstrate the pair correlation functions between water molecules and the side chain –OH groups of Thr1, Ser and Thr2 residues, respectively. The distribution of water molecules around Thr1 and Thr2, which refer to the

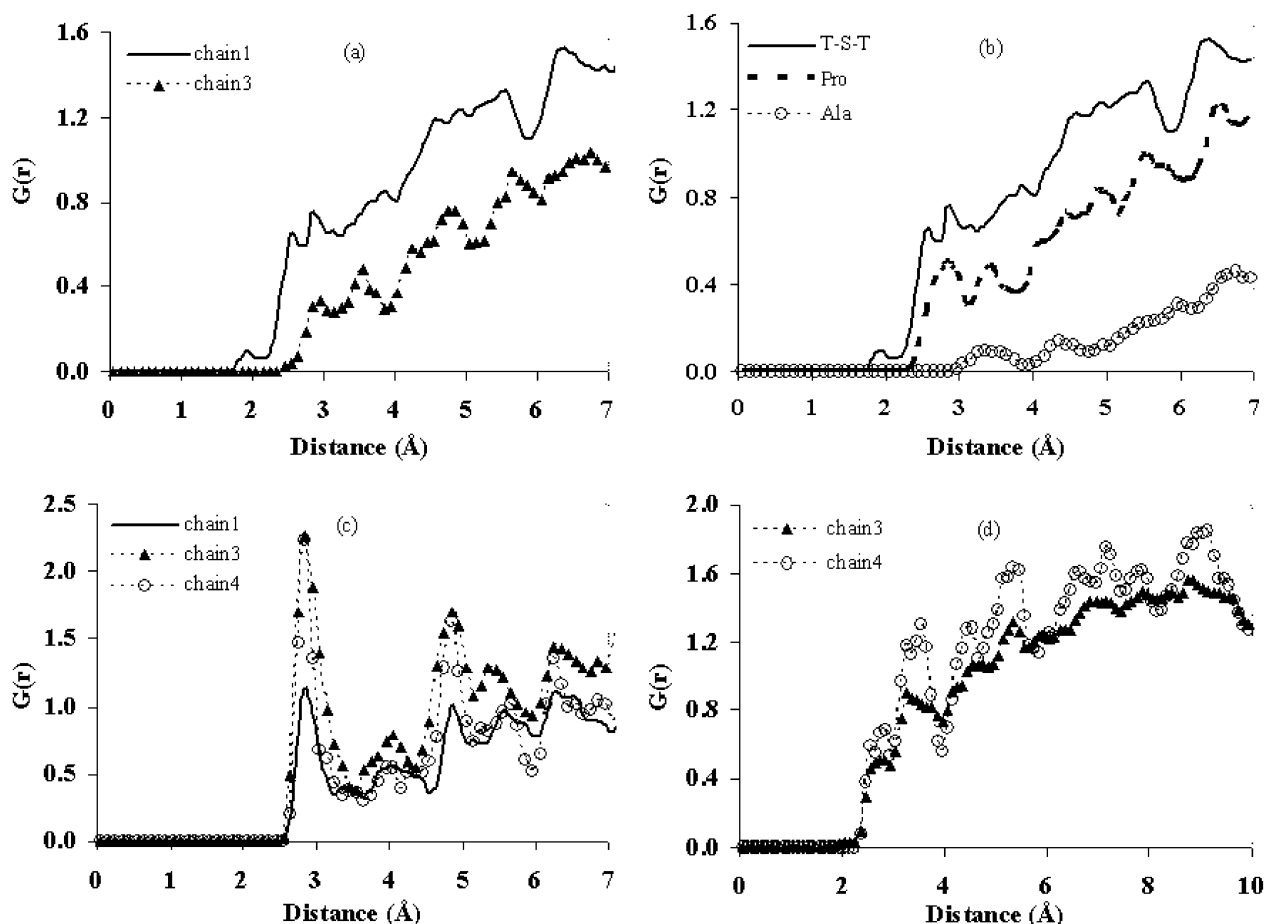


Fig. 3. Pair correlation function between Pt surface and: (a) Side groups of TST residues in chains 1, 3, (b) Polar and non-polar residues in chain 1, (c) Backbone oxygens in chains 1, 3, 4, (d) Arg residues in chains 3, 4 (chain 3 contains two Arg's, combined effect shown).

first and second Thr's in the sequences listed above, and Ser residues indicate quite similar behavior for the different chains. Hydrogen bonding observed around 2 Å is prominent for the relatively strong binders (chains 1, 2). Thus, the T-S-T sequence has more affinity to make hydrogen bonds (H-bonds) with surrounding water molecules as compared to the weaker binder (chain 3), which means that the side chains of these residues are more exposed to solvent in stronger binders. In chain 3, the intramolecular H-bonds may be responsible for the hindrance of solvent exposure of T-S-T. In order to see the stability of these H-bonds, annealing simulations are carried out at higher temperature (400 K) for chains 1, 2, and 3, which indicate that these pair correlations are still persistent at higher temperature.

The intermolecular interactions of the peptide chains with the Pt surface are presented in Fig. 3. The difference between the binding affinities of the T-S-T region in stronger (chain 1) and weaker binder (chain 3) can be observed in Fig. 3(a), where the -OH groups in the strong binder lie relatively closer to the surface. Polar and non-polar residue interactions with the metal surface for chain 1 are shown in Fig. 3(b). It is noticed that the T-S-T sequence (polar) interacts with the metal surface to a greater extent

than the non-polar residues (Ala, Pro). Although Ala and Pro are classified as non-polar, Pro exhibits unexpectedly high binding affinity as compared to Ala. Fig. 3(c) and (d) significantly indicate that the T-S-T region may not be the only factor that governs metal binding, since backbone oxygen (panel c), and nitrogen groups (similar but not shown), and residues like Arg (panel d) also show significant binding affinity.

### 3.2. Dynamic properties (torsional angle relaxations)

The time-dependent conformation behavior of the sequences are analyzed by torsional angle auto-correlation function expressed as [20]

$$G(\tau) = \langle \cos[\Phi(t + \tau) - \Phi(t)] \rangle \quad (2)$$

Here,  $\Phi$  refers to the torsional angle of a virtual bond connecting successive  $\alpha$ -C atoms.  $\Phi(t + \tau)$  and  $\Phi(t)$  are the torsional angles of a specific virtual bond at respective times  $t + \tau$  and  $t$ .

The backbone torsional angle relaxations evaluated according to Eq. (2) are presented in Fig. 4. Panels (a)–(c) show the torsional angle relaxations in the absence of metal

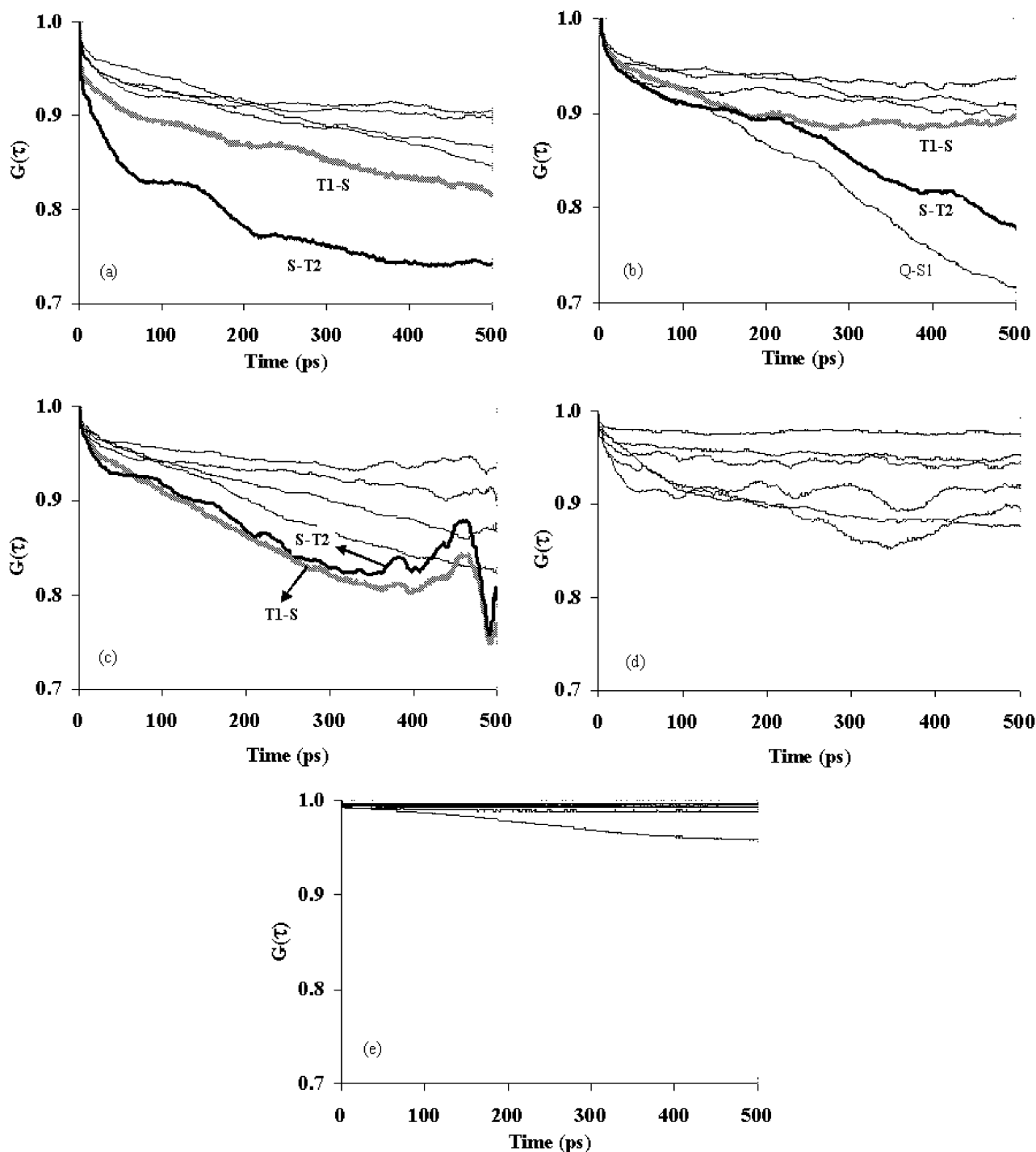


Fig. 4. Torsional angle auto-correlation function of backbone virtual bonds of (a) chain 1, (b) chain 2, (c) chain 3, (d) chain 4 without surface and (e) chain 1 with surface. The highly flexible bonds T1-S (gray), S-T2 (black) and Q-S1 are indicated on the figures (see text).

surface for chains 1, 2, and 3, respectively. Specifically, the torsion angles for T1-S (gray) and S-T2 (black) bonds are distinguished by thick solid lines on these figures. The T-S-T sequence is observed to constitute the most flexible region in all three chains. Moreover, the torsion angle of the additional Ser residue in chain 2 indicated by Q-S2 also

imparts significant flexibility. As a result, the flexibility of Ser and Thr residues in general is thought to play an important role in their binding to surface. For comparison, the relaxation of backbone torsional angles in chain 4, which does not include T-S-T sequence, is shown in panel (d). As compared to the TST regions of the first three chains, the



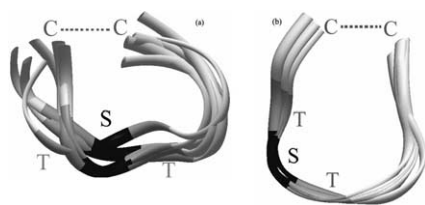


Fig. 5. Several superimposed snapshots of chain 1 during a run (a) without surface, and (b) with surface. The RMSD values between the conformations superimposed are given in Tables 2 and 3, respectively.

backbone of chain 4 exhibits relatively less flexibility. Fig. 4(e) represents the change in angle relaxations for chain 1 in the presence of metal surface. It is observed that all backbone torsional angle relaxations are considerably inhibited (reduction is valid for all chains; plot is given for chain 1 only).

### 3.3. Root-mean-square displacement/deviation (RMSD)

Mean-square displacement also provides a means to follow the quantitative conformational changes upon binding. Root-mean-square displacement is given by [21]:

$$\Delta r(t) = \left[ \frac{1}{N} \sum_{i=1}^N (r_i(t) - r_i(0))^2 \right]^{1/2} \quad (3)$$

where,  $N$  is the number of atoms over which the RMSD is measured,  $r_i(t)$  and  $r_i(0)$  are the coordinates of atom  $i$  at time  $t$  and at a reference time.

Snapshots taken from simulations carried out in the absence and presence of Pt surface are superimposed for comparison of RMSD values. For chain 1, the superimposed conformations are demonstrated in Fig. 5. The RMSD values presented in Tables 2 and 3 indicate that maximum RMSD values attained are 2.23 and 0.70 Å in the absence and presence of Pt surface, respectively, and this reduction is observed in all sequences. This clearly shows that the conformational flexibility and possible rearrangements are significantly dampened due to binding. In other words, the Pt surface limits the peptide chain rearrangement.

Table 2

RMSD values between conformations of chain 1 in a single run without surface

Frames (ps)	RMSD (Å)
1–400	1.674
400–800	0.746
800–1200	1.779
1200–1600	0.419
1600–2000	1.020
1–2000	2.235

Table 3

RMSD values between conformations of chain 1 in a single run with surface

Frames (ps)	RMSD (Å)
1–500	0.257
500–1000	0.236
1000–1500	0.274
1500–2000	0.701
1–2000	0.162

## 4. Conclusions

In the present study, the analysis of MD simulations of four cyclic (constraint) peptides in the presence and absence of the surface suggest some equilibrium and dynamic properties of chains that might possibly be important for surface binding: The T–S–T region in stronger binders (chains 1,2) in the absence of metal surface makes more intermolecular hydrogen bonds with the surrounding water molecules as compared to the relatively weaker binder (chain 3). However, the T–S–T sequence may not be the only factor leading to effective binding, but other residues such as Arg where present and backbone –C=O and N–H groups also show significant binding affinity in all the chains studied. On the other hand, the analysis of the torsional angle auto-correlation functions displays that Thr and Ser residues in the absence of surface, if present, correspond to the highly flexible regions in all chains, which implies the importance of the flexibility in surface binding. All torsional angle rotational mobilities are reduced in the presence of the surface. Similarly, the RMSD values demonstrate that in the presence of Pt surface the conformational rearrangements are significantly limited. It appears that the entropy loss due to the binding with reduced flexibility is an important aspect of the binding, which thus should be compensated with the affinity of the binding residues for the binding stability.

The present study presents an initial analysis of the relationship between sequence, structure and binding process for metal binding peptides. For an extensive analysis, the development of hierarchical computational tools combining atomistic and coarse-grained simulations seem to be necessary to efficiently screen the conformational and dynamic properties of a larger set of sequences that are known to bind to specific inorganic surfaces [22,23].

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