Role of Wybutosine and Mg\(^{2+}\) Ions in Modulating the Structure and Function of tRNA\(^{\text{Phe}}\): A Molecular Dynamics Study

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ABSTRACT: Transfer RNA remains to be a mysterious molecule of the cell repertoire. With its modified bases and selectivity of codon recognition, it remains to be flexible inside the ribosomal machinery for smooth and hassle-free protein biosynthesis. Structural changes occurring in tRNA due to the presence or absence of wybutosine, with and without Mg\(^{2+}\) ions, have remained a point of interest for structural biologists. Very few studies have come to a conclusion correlating the changes either with the structure and flexibility or with the codon recognition. Considering the above facts, we have implemented molecular modeling methods to address these problems using multiple molecular dynamics (MD) simulations of tRNA\(^{\text{Phe}}\) along with codons. Our results highlight some of the earlier findings and also shed light on some novel structural and functional aspects. Changes in the stability of tRNA\(^{\text{Phe}}\) in native or codon-bound states result from the conformations of constituent nucleotides with respect to each other. A smaller change in their conformations leads to structural distortions in the base-pairing geometry and eventually in the ribose-phosphate backbone. MD simulation studies highlight the preference of UUC codons over UUU by tRNA\(^{\text{Phe}}\) in the presence of wybutosine and Mg\(^{2+}\) ions. This study also suggests that magnesium ions are required by tRNA\(^{\text{Phe}}\) for proper recognition of UUC/UUU codons during ribosomal interactions with tRNA.

1. INTRODUCTION

Protein biosynthesis is an essential process crucial to the survival of every living cell. Various biological macromolecules contribute critically to run a smooth and in-phase functioning of this process.\(^1\) Transfer RNA is a key molecule that acts as an adapter to decode the genetic code into a functional array of a variety of proteins.\(^7\) It is well known that tRNA has an inverted-L-like three-dimensional (3D) structure. This 3D orientation of the molecule is one of the key features that govern its stability and interactivity.\(^3\) Similar to other biomolecules, tRNA also shows structure-dependant functional properties, which can be seen through its stability and interactions with codons.\(^1\) Also, its proper 3D fold is essential for its interactions with various proteins and the decoding center itself.\(^2\) tRNA is unique in its base composition as it encompasses a variety of modified nucleic acid bases in addition to the usual RNA bases, A, U, G, and C.\(^6\) Various studies have reported that these modified bases are crucial elements contributing to various properties of the molecule.\(^1-14\) Some studies also show that the absence of a specific modified nucleoside in tRNA causes structural deformities, which in turn cause considerable damage to native functions of the molecule.\(^14-17\)

The presence of the hypermodified nucleoside, wybutosine, at the 37\(^{\text{th}}\) position in tRNA and its structural and functional effects have remained a topic of discussion over the years.\(^18-24\) It has been well documented that wybutosine exhibits multiple conformations in the anticodon stem loop.\(^22,24\) It has also been proved that in the absence of wybutosine HIV replication progresses faster.\(^25\)

This study was undertaken to understand the role of modified nucleoside wybutosine present at the 37\(^{\text{th}}\) position in tRNA\(^{\text{Phe}}\). Wybutosine and its derivatives are known to play a crucial role during codon recognition. They are reported to support the codon–anticodon base pairing by maintaining a proper translational reading frame\(^26\) and also by preventing the formation of extended Watson–Crick base pairing.\(^14\) Various other bases present at the 37\(^{\text{th}}\) position in tRNA are also known to support the codon recognition process by some cooperative interactions with the base present at the 34\(^{\text{th}}\) (wobble) position.\(^27\) The structural properties of hypermodified base wybutosine have been documented by various research groups over decades.\(^18,24,28-33\) In the present study, we have tried to explore its structural and functional role during the codon–anticodon recognition process. The results of this study will be helpful in understanding the pattern of UUC/UUU codon recognition by yeast phenylalanine tRNA. Also, the preference in codon recognition under various physiological conditions can be understood. Prevention of ribosomal slippage during codon recognition due to the presence of wybutosine can probably be seen in the presence and absence of wybutosine with the two
cognate codons (UUC/UUU). This phenomenon is of critical importance to study the initial steps of retroviral replication.

2. RESULTS AND DISCUSSION

2.1. Stability of the Whole tRNAPhe Molecule. RMS deviations of the ribose-phosphate backbone were used to evaluate the stability of all model systems (Figure 1). We noticed that the RMSD for the whole tRNA molecule shows occasional fluctuations for all models. Comparatively, the RMSD for model 1 (Figure 2) shows stability as compared to that for model 2.

Similar stability is also observed in the case of models 3 and 5 as compared to the lower stability of models 4 and 6, respectively (Figures 3 and 4). These changes in the RMSD of multiple models are correlated with the structure of the tRNA molecule, and it is found that the molecule shows minute conformational changes within its component domains, which are reflected in such RMS deviations in systems. RMSD observations for the whole tRNA systems seem to be a cumulative effect of the dynamics of its component domains on the whole tRNA structure. Overall, the systems show a higher level of dynamism in the structures with respect to time, which can be accorded to changes occurring in various loop regions of the tRNA molecule (Figure 5a). From this RMSD analysis, it can be inferred that model 3 (tRNA without Mg2+) shows a smaller RMSD than model 4, which contains Mg2+. Similarly, among the codon-
bound models, model 7 and model 5 show a smaller RMSD as compared to models 1, 2 and models 6, 8, respectively (Figures 2−4).

2.2. Stability of the ACL, ASL, T-Loop, and D-Loop Domains of tRNA. Transfer RNA comprises various component domains, such as the D-loop, ACL, ASL, and T-Loop.
loop. All of these loops coordinate with each other structurally and make the tRNA molecule ready for further functions. Primarily, these loops are crucial to maintain the 3D fold of tRNA. During maturation of tRNA, the D- and T-loops fold over each other such that the T-loop region folds back over the D-loop. Thus, the D-loop is involved in stabilization of the 3D structure of the tRNA molecule. The D-loop structure is also essential for recognition of the tRNA molecule by aminoacyl tRNA synthetases.42,43 Similarly, the importance of the anticodon loop and anticodon stem loop has been well documented in previous reports.13,17 Hence, the stability of these component domains was individually assessed to check for local structural changes with the help of parameters such as the RMSD or structural visualization.

From RMSD results, it was observed that the whole tRNA\textsuperscript{Phe} shows some significant structural changes. However, RMSD analysis of the ACL, ASL, T-loop, and D-loop shows stability, which could be useful to determine the structure and function of tRNA\textsuperscript{Phe}. Four constituent loops, viz., di-hydro-uridine (D-loop), anticodon stem loop (ASL), anticodon loop (ACL), and TyC or T-loop of the molecule, were considered for detailed RMSD analysis.

The RMSD of the D-loop (residue no. 10–25) shows smaller values in the case of models 1, 4, and 6 as compared to those of their respective control models 2, 3, and 5. From the figure (Figure 6), it can be easily seen that the RMSD of model 1 (green) is considerably lower as compared to that of model 2 (red). In the case of model 3, the RMSD is larger as compared to model 4. Also, in the case of model 5, the RMSD is seen to be larger as compared to model 6. This behavior of the molecule from models 2 and 6 (both in the absence of WBG) suggests the occurrence of local conformational changes in the backbone residues of the D-loop. This change can also be correlated with the changes observed in Figure 5, wherein a structural change in models 2 and 6 can be seen as compared to their control models 1 and 5. From these observations, it can be seen that the presence and absence of wybutosine has a distinguishable effect on the stability of the D-loop (Figure 6).

RMSD analysis for the anticodon step loop (ASL) region (residue no. 27–43) is depicted in Figure 7, in which models 1 and 2 show similar behavior with occasional variations. ASL regions of models 3 and 4 show a stable RMSD of model 3 (green) as compared to the fluctuating RMSD of model 4 (yellow). In models 5 and 6 (Figure 8), the effect of the presence and absence of wybutosine on the binding of UUU codons can
be seen. In spite of the presence of wybutosine at the 37th position, the RMS deviation observed in model 5 (Figure 8) is an indication of the codon preference for UUC over UUU of tRNAPhe.28 Comparing the RMSD of models 1, 2 and 5, 6 for codon preference (Figures 2 and 8), one can observe that tRNA\(^{\text{Phe}}\) in the presence of wybutosine can recognize UUC codons more preferably over UUU codons. It can also recognize UUU codons but with some structural adjustments in the tRNA. The anticodon loop (ACL) region (residue no. 32−38) is crucial to the function of each tRNA as it actually influences the codon−anticodon interactions. The RMSD for this region of tRNA for all models is depicted in Figure 9. This domain of the tRNA molecule shows more dynamics as compared to other domains. This might occur due to the presence of various modifications and also because of the open-loop structure itself.44 Hence, the RMSD for model 1 shows more deviations as compared to that for model 2, while model 7 shows the least deviations as compared to models 1 and 2. In the case of model 1, the presence of WBG and Mg\(^{2+}\) might be a strong reason for occasional RMS deviations, which might, otherwise, get compensated for by the absence of WBG in the case of model 2. This presence and absence of WBG show changes in the conformation of the ACL, which is evident from Figure 5. The ACL can also be seen affected by the presence or absence of Mg\(^{2+}\) ions. From the RMSD of the ACL (Figure 9), it can be seen that the absence of Mg\(^{2+}\) in models 3, 7, and 8 imparts more stability to the ACL as compared to models 4, 1, and 5, correspondingly. The presence of Mg\(^{2+}\) highly destabilizes the ACL in the case of model 4. The ACL RMSDs of models 1 and 2 show occasional fluctuations. Similar fluctuations are also observed in the case of models 5 and 6, where model 5 shows higher deviations as compared to model 6. This behavior of model 5 in the presence of wybutosine can be correlated with a previous study,28 wherein it has been experimentally demonstrated that tRNA\(^{\text{Phe}}\) prefers UUC over UUU codons.

The RMSD for the TΨC loop (residue no. 49−65) is depicted in Figure 10. Model 1 shows a stable RMSD as compared to model 2, while models 3 and 4 both show similar RMSDs with minute fluctuations. Contrary to this, the RMSD for model 5 is less stable as compared to that for model 6. This suggests that interactions with the UUU codon influence the structure of tRNA\(^{\text{Phe}}\). Overall, the structure of the TΨC loop is highly affected by either the presence or absence of wybutosine and of the UUC/UUU codons. The presence of wybutosine lowers the RMSD in the case of model 1 as compared to that of model 2 (Figure 10), while its absence in model 6 lowers the deviations in the backbone of the T-loop in comparison with model 5, favoring recognition of UUU codons. Hence, it can be easily concluded that the presence or absence of WBG in models 5 and 6 has a minor effect on the structure of the TΨC loop. The RMSDs of models 3 and 4 (Figure 10) show that the absence or presence of Mg\(^{2+}\) does not hamper the TΨC loop structure.

2.3. RMSF. RMS fluctuations of all eight models along with RMSFs of their constituent loops have been calculated and are depicted in Figure 11. RMSFs for whole tRNA with UUC/UUU codons indicate that residues from models 1 and 5 show smaller atomic fluctuations as compared to those from models 2 and 6, respectively. In the case of models 3 and 4, RMS fluctuations can be seen to increase for model 4, while RMS fluctuations in the case of model 3 are comparatively lower. From the RMSF of the ASL (Figure 12), it can be clearly seen that models 1 and 5 show a reduced level of RMSF as compared to models 2 and 6. Overall, the RMSF results indicate that the presence of wybutosine is essential for codon recognition. Similarly, it is also evident that binding of codons induces structural changes in
the tRNA molecule; essentially in the D-loop, ASL, ACL, and TψC loop domains. The RMSF of the ASL suggests that codon binding residues from 34 to 36 along with wybutosine at the 37th position show elevated fluctuations.

Hence, correlating the RMSD and RMSF results, we can observe that model 1 represents the exact native state of the molecule describing comparatively lower RMSD and RMSF values, especially in the ASL region of the molecule. The
presence of wybutosine is helpful in recognizing UUC codons, while in its absence, UUU codons can be read. The function of Mg$^{2+}$ ions can be better understood by considering the ribosomal environment in simulation. Aufter in 1995 also reported similar structural stability of tRNA$^{Asp}$ in the absence of Mg$^{2+}$. We would like to highlight at this point that though Mg$^{2+}$ is essential for in vivo activity of the molecule, it has lower contribution toward governing the structure of the molecule.

2.4. Analysis of Base Stacking. Base stacking interactions within tRNA$^{Phe}$ of all eight models were analyzed for their stable behavior. For this purpose, representative base pairs of each of the stem loops were monitored. Out of these, the interaction between A$_{31}$····PSU$_{39}$ (Figure 13) is indicative of the level of base stacking within the ASL, while terminal base stacking interactions between G$_{1}$····C$_{72}$ (Figure 14a−c) show the effect of structural conservation or distortion occurring in the overall structure of all models. The base stacking interaction between N(1)A$_{31}$····H(3)PSU$_{39}$, which is indicative of the typical U-turn feature of tRNA, is seen to be well maintained only in the case of models 1 and 5, whereas it fluctuates for models 2 and 6 (Figure 13). This indicates that base stacking within the ASL of models 2 and 6 is disturbed, while that of models 1 and 5 is well conserved. Similarly, the terminal base stacking interaction between G$_{1}$····C$_{72}$ is assessed.
with the help of three interactions, viz., H(1)G₁···N(2)C₇₂, H(21)G₁···O(2)C₇₂, and O(6)G₁···H(41)C₇₂ (Figure 14a–c). Overall, these interactions show a high level of distortions in the last base stacking pair, i.e., G₁···C₇₂ only for models 2 and 6. This shows that the interactions are well maintained in the presence of wybutosine, i.e., for models 1 and 5.
Table 1. Binding Energy Analysis of the Whole tRNA<sup>Phe</sup> with UUC/UUU Codons in the Presence/Absence of WBG and Mg<sup>2+</sup>

| model | \(\Delta E_{\text{MM}}\) | \(\Delta E_{\text{elec}}\) | \(\Delta G_{\text{ps}}\) | \(\Delta G_{\text{nps}}\) | \(\Delta G_{\text{psolv}}\) | \(\Delta G_{\text{bind}}\) |
|-------|----------------|----------------|-------------|--------------|----------------|----------------|
| M1    | -22.68 ± 2.67 | -1296.7 ± 38.29 | -1303.41 ± 40.51 | -1296.71 ± 38.29 | -3.034 ± 0.14 | -1299.74 ± 38.35 | -18.597 ± 2.04 |
| M2    | -16.076 ± 2.07 | -1320.71 ± 52.72 | -1328.83 ± 54.61 | -1320.71 ± 52.72 | -2.583 ± 0.14 | -1323.29 ± 52.76 | -10.53 ± 2.84 |
| M5    | -21.94 ± 2.21 | -1286.60 ± 35.47 | -1297.59 ± 37.27 | -1286.60 ± 35.47 | -2.856 ± 0.19 | -1289.46 ± 35.53 | -13.80 ± 3.26 |
| M6    | -19.20 ± 2.71 | -1320.46 ± 28.74 | -1333.09 ± 30.12 | -1320.46 ± 28.74 | -2.53 ± 0.09 | -1323.00 ± 28.68 | -9.11 ± 5.35 |
| M7    | -23.93 ± 3.32 | -1240.22 ± 53.20 | -1247.44 ± 54.16 | -1240.22 ± 53.20 | -3.083 ± 0.10 | -1243.30 ± 53.20 | -19.78 ± 2.86 |
| M8    | -22.27 ± 4.14 | -1239.28 ± 44.22 | -1250.90 ± 45.27 | -1239.27 ± 44.23 | -2.98 ± 0.29 | -1242.25 ± 44.14 | -13.63 ± 3.62 |

\[\Delta G_{\text{binding}} = \Delta E_{\text{MM}} + \Delta G_{\text{nps}}\text{ where, } (\Delta E_{\text{MM}} = \Delta E_{\text{elec}} + \Delta E_{\text{nps}} \text{ and } \Delta G_{\text{nps}} = \Delta G_{\text{psolv}} + \Delta G_{\text{ps}})\].

2.5. Binding Free Energy Analysis. Binding free energy analysis was performed to determine the strength of bonds formed between the codons and anticodons for each model. MM-PBSA results (Figure 15a–c and Table 1) suggest that binding of codons in the case of models 1 and 5 is more energetically favorable than that seen in the case of models 2 and 6.

Models 1 and 5, containing wybutosine at the 37th position, show average binding energies of -18.597 and -13.808 kcal/mol, respectively, while for models 2 and 6, the average binding energy values recorded are -10.532 and -9.11 kcal/mol. This analysis suggests that model 1 having wybutosine at the 37th position and UUC codon is energetically more favorable than model 2 lacking wybutosine at the 37th position and with UUC codon. Similarly, in the presence of wybutosine, model 5 with UUU codon is energetically more favorable than model 6 lacking wybutosine and with UUU codon. The energy difference of models 1 and 5, for recognition of UUC codons over UUU, is 4.789 kcal/mol. This also supports the observation of RMSD, RMSF, and base stacking analysis that tRNA Phe prefers +4.789 kcal/mol. This also supports the observation of RMSD, RMSF, and base stacking analysis that tRNA Phe prefers +4.789 kcal/mol.

Instead, from RMSD, RMSF, and binding energy calculations, it can be seen that the absence of Mg<sup>2+</sup> has been found to be more crucial to the energetics of the whole system. In the present study, this effect was investigated using a control system (model 3), which consisted of a system similar to models 1, 2, 5, 6, 7, and 8 except for the codons and Mg<sup>2+</sup> ions. Comparative RMSD and RMSF plots, along with structural comparisons (Figures 1, 5, and 12), show that codon binding definitely induces structural

2.6. Effect of Mg<sup>2+</sup> Ions on the Structure of tRNA. In this study, we have observed that the presence of Mg<sup>2+</sup> ions in models 1, 2, 4, 5, and 6 does not contribute to tRNA stability. Instead, from RMSD, RMSF, and binding energy calculations, it can be seen that the absence of Mg<sup>2+</sup> has been found to be more favorable for the stability of the system. A previous study reported that Mg<sup>2+</sup> ions are the stabilizing factors for the tRNA structure, whereas another study reported stability of the tRNA structure even in the absence of Mg<sup>2+</sup> ions in codon recognition. They found that the concentration of Mg<sup>2+</sup> ions influenced the structure and thereby the function of tRNA<sup>Phe</sup> in recognizing UUC/UUU codons. Hence, we have simulated another two systems (models 7 and 8) in the absence of Mg<sup>2+</sup> ions to understand the preference of codon recognition. Here, models 1, 2, 7 and 5, 6, 8 can be compared for UUC and UUU codons, respectively. In both the groups, we can see that the absence of Mg<sup>2+</sup> in model 7 induces smaller RMS deviations as compared to models 1 and 2 (Figure 2). However, in the case of model 8, the RMSD is higher than that of models 5 and 6 (Figure 4). This behavior can be the result of changes in the composition of the respective systems. It has been reported that Mg<sup>2+</sup> ions are key players during attachment of tRNA to A/P sites of the ribosome.

2.7. Effect of Codon Binding on the Structure of tRNA. It has been documented previously that binding of codon to a tRNA induces certain conformational changes, which are crucial to the energetics of the whole system. In the present study, this effect was investigated using a control system (model 3), which consisted of a system similar to models 1, 2, 5, 6, 7, and 8 except for the codons and Mg<sup>2+</sup> ions. Comparative RMSD and RMSF plots, along with structural comparisons (Figures 1, 5, and 11), show that codon binding definitely induces structural
and conformational changes in the core domains of the tRNA molecule. Structural comparisons show some notable changes near the wybutosine base at the 37th position and also near the acceptor arm region. According to the visualization, we can say that the native structure of tRNA is conserved only in the case of models 1 and 5, while models 2 and 6 show some deformities at the loop structures and acceptor arms (Figure 5). These structural changes will be of great interest in the context of peptidyl transferase enzyme, which helps in recognizing the tRNA molecule in the P-site and transferring the respective amino acid to the growing peptide chain.

2.8. Effect of the Presence and Absence of Wybutosine on the tRNA Structure and Codon–Anticodon Interactions. In this study, the contribution of wybutosine to the structural changes in tRNA was investigated. Structural comparisons (Figure 5) show that in the absence of wybutosine, as in the case of models 2 and 6, tRNA adopts some structural changes in its ribose-phosphate backbone, especially near the anticodon stem loop, D-loop, and near the acceptor arm.

Figure 17. Codon–anticodon interactions of various base pairs.
(Figures 1 and 5). Remarkably, such distortions are not observed in the presence of wybutosine. These minute conformational changes cause other bases to change their native base-pairing geometry, as can be clearly seen from Figure 16a,b. Similarly, a change in the backbone of the base stacking pair along with its interactions near the acceptor arm in the absence of WBG is very crucial, as it might hamper the polymerization of the protein molecule being synthesized.

3. CONCLUSIONS

Structural aspects of the whole tRNA were investigated using fully solvated multiple molecular dynamics (MD) simulations on eight model systems of yeast tRNA^Phe. The effect of Mg$^{2+}$ on the tRNA tertiary structure is worth considering in the context of ribosomes, as there are few reports that shed some light on its interactions with ribosomal residues. Otherwise, the presence of Mg$^{2+}$ in the native state of tRNA may result in some structural deformities. Transfer RNA phenylalanine prefers the UUC codon over UUU because of its energetics in the presence of wybutosine. The highly hydrophobic WBG restricts the anticodon loop structure and maintains it in the open-loop conformation, which allows easy access to the UUC codon for Watson–Crick pairing during the codon recognition process. In contrast, UUU codon recognition is less favored in the presence of wybutosine at the 37th position (Figure 17). The presence of a hydrophobic wyosine side chain does not allow formation of Crick pairing during the codon recognition process. In every model, UUU codon recognition is less favored in the presence of wybutosine at the 37th position (Figure 17). These model systems were built using commercially available SYBYL ver 7.3 [SYBYL] and UCSF Chimera.35 Codon trinucleotide segments were manually docked to tRNA by holding proper alignment and distance for each hydrogen bond.18,23 Each of these systems was then subjected to MD simulations.

4. COMPUTATIONAL METHODS

4.1. System Nomenclature. Referring to 3D coordinates from the crystal structure of the whole tRNA$^{\text{Phe}}$ from yeast (Saccharomyces cerevisiae) (RCSB PDB ID: 1EHZ), totally eight tRNA$^{\text{Phe}}$ model systems were constructed (Table 2) for the study as follows.

Table 2. Nomenclature of the tRNA Model Systems under Study

| no. | model code | tRNA model | 37th position residue | codon | Mg$^{2+}$ |
|-----|------------|------------|----------------------|-------|----------|
| 1   | M4         | whole tRNA$^{\text{Phe}}$ | WBG | no codon | + |
| 2   | M3         | whole tRNA$^{\text{Phe}}$ | WBG | no codon | − |
| 3   | M1         | whole tRNA$^{\text{Phe}}$ | WBG | UUC | + |
| 4   | M2         | whole tRNA$^{\text{Phe}}$ | WBG | UUC | + |
| 5   | M7         | whole tRNA$^{\text{Phe}}$ | WBG | UUC | + |
| 6   | M5         | whole tRNA$^{\text{Phe}}$ | WBG | UUU | + |
| 7   | M6         | whole tRNA$^{\text{Phe}}$ | WBG | UUU | + |
| 8   | M8         | whole tRNA$^{\text{Phe}}$ | WBG | UUU | − |

These model systems were built using commercially available SYBYL ver 7.3 [SYBYL] and UCSF Chimera.35 Codon trinucleotide segments were manually docked to tRNA by holding proper alignment and distance for each hydrogen bond.18,23 Each of these systems was then subjected to MD simulations.

4.2. Molecular Dynamics Simulations. MD simulations were performed by taking the initial coordinates from model systems as mentioned above. Parameters for the modified nucleosides occurring in tRNA$^{\text{Phe}}$ were retrieved from the AMBER parameter database maintained by the Bryce group (http://research.bmh.manchester.ac.uk/bryce/amber). The solvated system was neutralized by 74 Na$^+$ counterions. The water density of the rectilinear box was maintained at 1.0. All simulations were run under periodic boundary conditions with the particle-mesh Ewald method for calculating long-range interactions.36 MD trajectories were recorded at a 2 fs time step using the SHAKE algorithm37 for hydrogen atoms with a nonbonded cutoff of 9 Å. The Berendsen coupling algorithm38 with 2 fs was used to calculate the trajectories by maintaining constant pressure (1 atm) and temperature (300 K).

The equilibration protocol consisted of 4 steps of MD and 2 steps of energy minimization. Initially, 10 000 steps of the steepest descent method were used to minimize steric clashes between atoms. This system was then subjected to four steps of equilibration MDs as per our earlier studies.13,17 A final minimization with 10 000 steps of the steepest descent method was then used to remove any steric clashes between the atoms. These equilibrated model systems were further used for production MD simulations of 50 ns using constant temperature and pressure. All MD simulation experiments were performed on IBM-HS22 and HP-Proliant DL 180-G6 rack servers using the AMBER10 software suite.39

4.3. Data Analysis. The data generated from MD simulations were analyzed using the CPPTRAJ module of AmberTools16.41 Snapshots and average structures were generated by clustering the data over a selected time period using CPPTRAJ and UCSF Chimera.35 Postprocessing of trajectories was done to evaluate the binding energies of the codons and anticodons using the MM-PBSA method42 for calculating binding energies between the codon–anticodon complexes. For graphical and statistical representations of the results, SigmaPlot ver 10 was used.

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Notes
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