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Insight, Innovation and Integration:

Codon recognition is crucial biochemical process featured by proper codon-anticodon interactions. This study focuses on the analysis of codon anticodon interactions promoted by the modified nucleosides L and t⁶A present in anticodon loop of tRNA^{Ile}. Theoretical insights of this critical process are important to understand biophysical view of codon recognition. This molecular dynamic simulation analysis provides comprehensive biophysical scenario of codon anticodon interactions. This study also emphasizes the importance of modified nucleosides to provide structural stability to anticodon loop of tRNA. Here, we have analyzed codon anticodon interactions in detail at the atomic level and studied the role of modified bases L and t⁶A to recognize AUA codon instead of AUG.

Influence of hypermodified nucleosides lysidine and t⁶A to recognize AUA codon instead of AUG: A molecular dynamics simulation study

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Abstract

Hypermodified nucleosides lysidine (L) and *N*6-threonylcarbamoyladenine (t^6A) influence codon-anticodon interactions during protein biosynthesis process. Lysidine prevents misrecognition of AUG codon as isoleucine and that of AUA as methionine. The structural significance of these modified bases has not been studied in detail at the atomic level. Hence, in the present study we performed multiple molecular dynamics (MD) simulations of anticodon stem loop (ASL) of tRNA^{Ile} in presence and absence of modified bases 'L' and ' t^6A ' at 34th and 37th positions respectively along with trinucleotide 'AUA' and 'AUG' codons. Hydrogen bonding interactions formed by tautomer form of lysidine may assist to read third base adenine of 'AUA' codon, unlike guanine of 'AUG' codon. Such interactions might be useful to restrict codon specificity to recognize isoleucine tRNA instead of methionine tRNA. The t^6A side chain interacts with purine ring of the first codon nucleotide adenine which might provide base stacking interactions and could be responsible to restrict extended codon-anticodon recognition. We found that ASL tRNA^{Ile} in absence of modifications at 34th and 37th positions cannot establish proper hydrogen bonding interactions to recognize isoleucine codon 'AUA' and subsequently disturbs the anticodon loop structure. The binding free energy calculations revealed that tRNA^{Ile} ASL with modified nucleosides prefers codon AUA over AUG. Thus, these findings might be useful to understand role of modified bases L and t^6A to recognize AUA codon instead of AUG.

Keywords: Anticodon, codon recognition, modified nucleosides, tRNA, MD simulations

Introduction

Codon recognition during translation is a very convoluted process which depends on different components of ribosome.¹ Codon-anticodon interactions play a crucial role in mRNA decoding on ribosome. The canonical structure of the anticodon loop is important for decoding codons at ribosomal sites and is evolutionary conserved.² Modified bases in tRNA increases stability by providing additional hydrogen-bonding and base stacking interactions.³ Transfer RNA molecules undergoes posttranscriptional modifications, which is one of the processing events that resulted in functional tRNA. Modifications present at wobble (34th) position along with conserved purines at 3'-adjacent (37th) position to the anticodon loop are involved in recognition of specific codons.⁴ It has also been known that the wobble modifications coincidentally act as determinants to recognize cognate aminoacyl tRNA synthetases.⁵ However, base modifications occur at position 37 stabilizes tRNA-mRNA complex by improving codon-anticodon stacking interactions.⁶ Recently, it has been indicated that tRNA modifications enhance decoding⁷ and restore ribosomal binding.⁸ Previous experimental studies concluded that, modification defect in anticodon first letter of mutant tRNA^{Lys} molecule leads to disturb codon-anticodon interactions, severely harming mitochondrial translation that finally result in mitochondrial diseases such as MELAS and MERRF.⁹

The hypermodified nucleoside lysidine (L), 4-amino-2-(N⁶-lysino)-1-(ribofuranosyl) pyrimidinum, naturally occur at wobble position of anticodon loop of tRNA^{Ile} in varied range of organisms as well as plant mitochondria to decode AUA codon.¹⁰ Conjugation of lysine or agmatine to the C(2) carbon of the cytosine induces a tautomeric conversion of the base from enamine to imine, enabling the base to pair with adenosine instead of guanosine at the third position of codons.¹¹ In most archaea, 'AUA' codon has deciphered by tRNA^{Ile} bearing 2-agmatidyl cytidine (agm²C or agmatidine) at the wobble position.^{12,13} The enzymes, Tils and Tias use ATP as a substrate and synthesize structurally similar cytidine derivatives by distinct catalytic mechanism in which Tils activates the C2 carbon of C34 by adenylation, while Tias activates the target site by phosphorylation.¹⁴ In a previous experimental study, it has been shown that modified nucleoside agmatidine side chain interacts with downstream ribose 4' oxygen or phosphate oxygen.¹⁵ Agmatidine and lysidine are structurally similar cytosine modifications differing in terminal groups.¹³ A crystal structure of ribosome complex containing agmatidine with its specific codon AUA has been solved to understand molecular interactions.¹⁵

Previously, conformational preferences of different forms of isolated lysidine have been studied.¹⁰ It has been proved that only tautomer form of lysidine may provide compatible hydrogen bond donor and acceptor sites to enable base pairing with third codon 'A' and thus may recognize the codon 'AUA' instead of 'AUG'.^{10,13} The modification N⁴-acetylcytidine (ac⁴C), prevents tRNA^{Met}_{CAU} to read AUA through wobble geometry^{16,17} while lysidine (L)^{10,18} and agmatidine (agm²C)^{12,15} prevents tRNA^{Ile}_{CAU} from reading codon AUG in bacteria and archaea. It has been stated that decoding system for the AUA codon relies strictly on the wobble modification of tRNA.¹⁹ Molecular dynamic simulation study of anticodon domain containing hypermodified base wybutosine at 37th position has been made to understand the interactions with codon trinucleotide to observe the alterations in the structure and dynamics of anticodon domain tRNA^{Phe}.²⁰ Similarly, structural significance of modified nucleosides present at specific sites in tRNA have been investigated by computational methods previously.²¹⁻²⁵

However, the recognition of 'AUA' minor isoleucine codon by L and t⁶A containing tRNA has not been studied in detail at the atomic level. Hence, we performed multiple molecular dynamic simulations of tRNA^{Ile} anticodon stem loop with 'AUA' and 'AUG' codons in presence and absence of modifications at 34th and 37th positions. The MD simulation revealed that tRNA^{Ile} anticodon stem loop having modified bases L and t⁶A at 34th and 37th positions respectively show proper stacking interactions with AUA as compared to AUG codons. So this study might be helpful to understand role of modified bases L and t⁶A to provide structural stability to codon-anticodon complex during protein biosynthesis process.

Material and methods:

Computational details:

Molecular dynamics simulation:

In this study the anticodon stem loop (ASL) of tRNA^{Ile} from position 27-43 has been considered as shown in figure 1. A model of tRNA^{Ile} anticodon stem and loop (ASL) was generated by using yeast phenylalanine tRNA crystal structure at 1.9 Å resolution (PDB ID-1EHZ).²⁶ Then nucleoside bases were substituted according to tRNA^{Ile} sequence²⁷ by using 'Sybyl' software.²⁸ Three-dimensional models of 'AUA' and 'AUG' codons were generated using Sybyl and then manually docked to ASL of tRNA^{Ile} model by maintaining proper orientation and Watson-Crick hydrogen bonding interactions with the help of Chimera.²⁹ The ASL model of tRNA^{Ile} contains hypermodified nucleosides lysidine (L) at 34th 'wobble' position,

N6-threonylcarbonyl adenosine (t^6A) at 3'-adjacent to anticodon (37^{th}) position and Pseudouridine at 39^{th} position.¹⁸ Similar type of unmodified ASL model having Cytosine at 34^{th} , Adenine at 37^{th} and Pseudouridine at 39^{th} positions were constructed and then docked with 'AUA' codons. In further discussions nucleotide bases of codons are subscripted in hydrogen bonding description.

Molecular dynamic simulations were performed over two models of ASL (with and without modifications) docked with 'AUA' and 'AUG' codons. Each model was solvated by 3633 SPC/E water molecules and neutralized by 19 Na^+ ions in a rectilinear box having 55.36 x 66.73 x 54.97 dimensions.³⁰ MD trajectories were propagated at 2.0 fs time step utilizing shake algorithm³¹ to all hydrogen atoms with non-bonded cut off 9.0 Å. The non-bonded pair list was updated by every 10 steps. The trajectories were calculated by maintaining a constant temperature (300 K) and constant pressure (1atm) at 2.0 fs time step according to Berendsen coupling algorithm.³² Simulations were performed under periodic boundary conditions by employing Particle Mesh Ewald method³³ to calculate long range interactions.

An equilibrium protocol similar to earlier molecular dynamic simulation studies of nucleic acids were applied.^{34,35} The equilibration protocol consisted of 5000 steps of steepest descent minimization followed by 50 ps of MD at 300K applied to relaxation of initial strain present between water molecules and model ASL segment docked with codon. Next the model segment was fixed while water molecules and Na^+ counter ions were allowed to relax 100 K (10 ps), 200 K (10 ps), and finally to 300 K for 930 ps, thus equilibration protocol was completed at 1000 ps. Equilibrated system was further subjected to 5000 steps of steepest descent minimization in order to remove bad contacts between water molecules and model segment of tRNA^{lle}. In further steps of MD simulation no positional constraints were applied to the system and the temperature was progressively increased to 300 K in steps of 50 K with 1ps at each step. Finally, system was subjected to production MD run up to 10 ns at 300 K temperature and constant pressure (1 atm) with fully solvated and neutralized system using ffbsc0 force field in Amber 10 software.³⁶ Modified nucleoside parameters were taken from 'Modified Parameters Database server'.³⁷ PTRAJ module of Amber 10 and VMD software were used for analysis of average and snapshot structures.³⁸ Molecular dynamics simulation was performed with Amber 10 simulation suit on (HP ProLiant-ML150G6) server.

Molecular Electrostatic Potential and binding free energy Calculations:

Molecular Electrostatic Potential (MEPs) of base pair models $L_{(34)}:A_{(3)}$, $L_{(34)}:G_{(3)}$, $C_{(34)}:A_{(3)}$, and $C_{(34)}:G_{(3)}$ were calculated using *Spartan ver 6.0.1*.⁴¹ The color-coded surface gives information of size of MEP surface and to trace positive (deepest blue color, i.e., repulsion of positive charges) and negative (deepest red color, i.e., attraction of positive charges) electrostatic potentials.

The Molecular Mechanics-Poisson Boltzmann Surface Area (MM-PBSA) method was used to calculate binding free energies as described in earlier studies for biomacromolecules.^{39,40} The binding energy calculations were carried out on 10 snap shot structures evenly chosen from 0 to 10 ns MD simulation trajectory.

Results:

MD Simulation of ASL with AUA codon:

Molecular dynamics simulation was performed to address conformational elasticity of hypermodified nucleosides lysidine (L) and threonylcarbonyl adenine (t^6A) side chains in presence of AUA codon. Figure 2 illustrates root mean square deviation of ASL-codon backbone during molecular dynamic simulation, in presence and absence of modified nucleosides at 34th and 37th positions. The RMSD of ASL with modified nucleosides at 34th and 37th positions in presence of codon shows deviation around 1-3.5 Å, whereas unmodified ASL-codon RMSD increases quickly with deviation around 6 Å (Fig. 2). The modified ASL model has less divergence than unmodified ASL from the initial structure. These results show that anticodon stem loop structure with modified bases provides more hydrogen bonding and stacking interactions as compared to unmodified bases.

Hydrogen bonding interactions of L:

Torsion angle α_1 prefers value 180° (Fig. 5a) similar to earlier studies¹⁰ which help to maintain 'trans' orientation of the lysine substituent. Hydrogen bonding interaction between 2'-oxygen atom of ribose ring and HN(2) of lysine side chain contribute to retain 'trans' orientation of lysine substituent (Fig. 5a). Figure 4 depicts fluctuations of hydrogen bonding throughout MD simulation period. Here, we found that O(12a) and HN(11) of lysidine side chain interacts respectively with hydrogen and oxygen of 2'-OH group of third base of codon 'AUA' (Fig. 3c, 3d, 5a, 5b). This hydrogen bonding interaction might play an important role to read adenine at 3rd position of codon to decode the 'AUA' codon by 'LUA' anticodon of tRNA^{Ile}.

Tautomer form of lysidine has hydrogen bond donor –HN(3) and acceptor –N(4) groups which interacts with N(1) and HN(6) of third codon (Adenine) respectively (Fig. 5a). This would allow tautomer form of lysidine to recognize third codon of isoleucine by forming Watson-Crick base pairing interactions as described in earlier studies.^{10,18} Such type of double hydrogen bonding interaction has also been reported in case of imino form of agmatidine.⁴² We have also found single hydrogen bonding interaction between N(4) of lysidine ‘L’ side chain and N(1) of A₍₃₎ codon [Fig. 5c] similar to crystal structure of 70S ribosome complex of archaeal tRNA^{Ile} with agm²CAU.¹⁵ Hydrogen bonding interaction between O(1)P₍₃₆₎ and HN(3)₍₃₃₎ commonly known as U-turn feature⁴³ has been preserved during 10 ns simulation studies of ASL tRNA^{Ile} with modifications continuously as compared to without modifications at 34th and 37th positions in presence of codon ‘AUA’ (Fig. 4a). Another hydrogen bonding interaction N(7)₍₃₅₎...HO2’₍₃₃₎ (Fig. 4b) may help to maintain U-turn feature in anticodon loop.⁴³ This interaction was found stable at last 1 ns simulation period of tRNA^{Ile} ASL containing modified nucleosides whereas it was disturbed at the end in case of tRNA ASL without modifications.

Hydrogen bonding interactions of t⁶A:

The interaction of HN(11) with N(1) (Fig. 3a) preserves distal orientation of t⁶A side chain throughout the simulation period similarly as found in previous studies.^{44,45} The hydrogen atom of O(14) of t⁶A was found interacting with N(7) of the first codon ‘adenine’ during MD simulation (Fig. 3b, 5c). Similarly, atom O(13b) of t⁶A interacts with HN(6) of first codon adenine (Fig. 5c). These interactions would be helpful to maintain proper base stacking interactions of t⁶A₍₃₇₎ with first codon ‘A₍₃₎’ which would concurrently prevent extended Watson-Crick base pairing during codon reading process.

Hydrogen bonding interactions of pseudouridine:

Apart from modifications at 34th and 37th positions, pseudouridine is present at 39th place in anticodon loop of tRNA^{Ile}. Pseudouridine forms hydrogen bond with A₍₃₁₎ which has been found distorted during MD simulation of tRNA^{Ile} ASL without modifications unlike MD simulation results of tRNA^{Ile} ASL with modifications which maintains throughout the simulation time period. There may be some contribution of 37th modification to assist hydrogen bonding between bases 31st and 39th in stem loop of tRNA^{Ile} as can be seen in figure 4c and 4d. The root mean square fluctuations (RMSF) of every residue in ASL with codon (Fig. 6) show

significantly greater fluctuations of ASL without modifications at 34th and 37th residues as compared to ASL with modifications.

Codon recognition process is facilitated by Watson and Crick hydrogen bonding between first and second codon with 36th and 35th nucleosides of ASL respectively while hydrogen bonding between 34th wobble base and 3rd codon was not that much stringent. These results revealed that normal base pair hydrogen bonding interactions found disturbed in unmodified ASL during MD simulation period. On the other hand ASL having modified bases at 34th and 37th positions preserves Watson-Crick base pairing till end of simulation.

MD simulation of ASL with AUG codon:

The results obtained from MD simulation of ASL with modifications at 34th and 37th positions along with isoleucine codon 'AUA' then compared with ASL with methionine codon 'AUG'. The RMSD of ASL with 'AUA' as well as 'AUG' shows deviation around 1 to 3.5 Å (Fig. 2). The base stacking in codon-anticodon mini helix is destabilized in the MD simulation of ASL with AUG codon (Fig. 7). The wobble base pairing between lysidine and guanine G₍₃₎ of 'AUG' codon found distorted during MD simulation (Fig. 7b). Such type of destabilization of wobble pairing consequently hampered stability of base pairing at 1st and 2nd position in codon-anticodon mini helix. During MD simulation, tautomer form of lysidine interacts properly with 'AUA' codon instead of 'AUG' codon (Fig. 7a).

Molecular Electrostatics Potential and binding free energy calculations

Molecular Electrostatic Potential (MEPs) calculations have been performed over the base pair model of L₍₃₄₎:A₍₃₎, L₍₃₄₎:G₍₃₎, C₍₃₄₎:A₍₃₎, and C₍₃₄₎:G₍₃₎ using *Spartan ver 6.0.1* software⁴¹ as shown in (Fig. 8 a-d) similar to earlier studies^{16,46}. The L:A model shows two electrostatic potential tunnels between N(4)₍₃₄₎...HN(6)₍₃₎, N(1)₍₃₎...HN(3)₍₃₄₎ (Fig. 8a). The MEPs color scale range for the model L₍₃₄₎:A₍₃₎ is in between -1.1 and -4.7 eV. The L₍₃₄₎:G₍₃₎ model shows only hydrogen bonding interaction between HN(4)₍₃₄₎...O(6)₍₃₎ (Fig. 8b). The MEPs color scale range for the model L₍₃₄₎:A₍₃₎ is in between -1.2 and -4.9 eV. The model C₍₃₄₎:G₍₃₎ depicts standard Watson and Crick base pairing with hydrogen bonding interaction between O(2)₍₃₄₎...NH(6)₍₃₎, N(1)₍₃₄₎...HN(1)₍₃₎, and O(2)₍₃₎...HN(4)₍₃₄₎ (Fig. 8d) while, model C₍₃₄₎:A₍₃₎ does not show any hydrogen bonding interaction (Fig. 8c). The MEPs color scale range for the model C₍₃₄₎:G₍₃₎ is in between -9.2 and -4.3 eV. Likewise, the MEPs color scale range for the model C:A has been found in between -8.3 and -4.5 eV.

The binding free energy calculations have been performed using MM-PBSA method implemented in Amber software. The average binding free energy of ASL with codon AUA and AUG are -44.24 Kcal/mol and -24.17 Kcal/mol respectively as shown in figure 9.

Discussion:-

Various attempts have been made to find out accurate mechanism of translation of mRNA on ribosome. Perhaps it is difficult to understand dynamic nature of the modified nucleosides in the translation process. Here, we have tried to investigate structural dynamics of modified nucleosides lysidine, (L) and *N*6-threonylcarbamoyladenosine, t⁶A in ASL tRNA^{lle} in presence and absence of modifications along with trinucleotide codons.

The RMSF values calculated for ASL with AUA/AUG codons show deviation per residue (Fig. 6). This RMSF graph showed less fluctuation of residues in ASL with modification than ASL without modification (Fig. 6). As per the earlier report third (wobble) position of the codon-anticodon base pair is usually less stringently monitored than the first and second positions in the ribosome.⁴⁷ Wobble position modification may restrict or enlarge the scope of codon recognition. Lysidine restricts such scope of codon recognition to 'AUA' for isoleucine and discriminate it from methionine codon 'AUG'. As we know that normal Watson and Crick base pairing is not possible between cytosine and adenine. Hydrogen bonding interaction might be possible within the modified cytosine and adenine if cytosine is in imino form where N(4) works as a hydrogen bond acceptor and N(3) acts as a hydrogen donor as per earlier report.¹⁰ Though it is not perfect Watson-Crick hydrogen bonding interaction, it can be compensated by hydrogen bonds formed by the modified part of cytosine. In case of lysidine modification, a long lysine side chain may form hydrogen bond to stabilize codon-anticodon interactions at the wobble position.

Molecular dynamic simulation of ASL along with codon analysis shows that lysidine, a cytosine modification at C(2) position, in its tautomer form can make Watson-Crick like base pairing interaction with adenine at the third codon position. Likewise, single hydrogen bonding interaction between N(4) of lysidine 'L' side chain and N(1) of A₃ codon was also found in our MD simulation study (Fig. 5b). Recently, it has been showed that single hydrogen bonding interaction between agmatidine and third codon adenine might be involved to recognize AUA codon¹⁵. The amino and carboxyl groups of long lysine substituent of lysidine nucleoside also interacts with 2' oxygen atom of ribose ring of third codon to compensate another hydrogen

bond similarly as described in crystal structure.¹⁵ The possibility of two different hydrogen bonding strategies shows dynamic behaviour of modified nucleoside lysidine during recognition of isoleucine codon AUA.

Hydrogen bonding between $N(4)_{(34)} \dots HN(6)_{(3)}$ and $N(1)_{(3)} \dots HN(3)_{(34)}$ (Fig. 5) are well maintained during MD simulation in presence of modified bases in ASL. The hydrogen bonding interactions between lysidine and third base of codon AUA might be responsible to recognize 'AUA' codon whereas, ASL containing unmodified bases dose not form such type of hydrogen bonding interactions with 'AUA' codon at wobble '34th' position. Lysidine side chain interacts with 2' oxygen atom of ribose ring of third base of codon 'AUA' to support codon recognition process (Fig. 5). However, these results are observed in absence of rRNA because various bases from rRNA, interacts with mRNA which influence codon-anticodon interactions.^{47,48} Molecular dynamic simulation shows that lysidine at the wobble position can interact with Adenine, $A_{(3)}$ at third codon position, with two hydrogen bonds within bases and one hydrogen bond between lysine side chain and 2' oxygen atom of ribose. The $L_{(34)}:A_{(3)}$ pair might maintain wobble base pairing geometry. Hydrogen bonding maintained during MD simulation held Adenine in case of modified cytosine, but could not hold Adenine properly in absence of modification at wobble position. Similarly, tautomer form of lysidine could not accommodate guanine, $G_{(3)}$ properly due to steric hindrance between lysine substituent of lysidine and $-NH_2$ group of guanine base (Fig. 7). The base stacking interactions were found disturbed in $L_{(34)}:G_{(3)}$ pair, which subsequently could damage remaining normal base pairing in codon-anticodon mini helix.

Watson and Crick hydrogen bonding between $U_{(36)}:A_{(3)}$ and $A_{(35)}:U_{(2)}$ found disturbed during simulation period in absence of modifications in ASL at 34th and 37th positions. The general purpose of $t^6A_{(37)}$ modification appears to strengthen codon binding by positioning residue 37 for stacking over the first anticodon-codon base pair as explained in earlier report.⁴¹ Adenine modification $t^6A_{(37)}$ interacts with first codon adenine which might establish stacking interactions with codon anticodon duplex similarly as described by Durant and co-workers in 2005.⁴⁹ Pseudourine increases base stacking and thermodynamic stability of the anticodon arm.⁵⁰ Interestingly, adenine present at 31st position and pseudouridine at 39th position shows stable hydrogen bonding interactions in presence of modified bases at wobble and 37th positions as compared to ASL in absence of modifications (Fig. 4c, d). Several structural studies predicted an important role of t^6A in translational fidelity by allowing cross-strand stacking of A38 and

t^6A_{37} with the first position of the codon.^{49, 51-54} Such cross strand stacking interactions might get destabilized in absence of t^6A at 37th position and subsequently there would be strain created on base pairing interactions of stem region in between 39-31 nucleotides.

The MEPs calculations revealed the structural significance of models $L_{(34):A_{(3)}}$ over $L_{(34):G_{(3)}}$. MEPs of model $L_{(34):A_{(3)}}$ (Fig. 8) shows two positive potential tunnels between hydrogen bond donor and acceptor groups while the $L_{(34):G_{(3)}}$ (Fig. 8) model only one positive potential tunnel could observe. The MEPs calculations revealed the potential of the tautomeric form of lysidine to recognize Adenine as third codon to identify isoleucine tRNA instead of methionine tRNA. Binding free energy of ASL with codon AUA and AUG results show that ASL with modified nucleosides prefers codon AUA over AUG.

Conclusion:

The present MD simulation results revealed that tautomer form of lysidine recognizes 'AUA' codon for isoleucine by forming hydrogen bonds with $A_{(3)}$ of 'AUA' codon and avoids misrecognition of 'AUG' as a methionine codon. Long lysine moiety of lysidine may form hydrogen bond with ribose ring oxygen to support wobble interactions during codon recognition process. Theronylcarbonyl moiety of t^6A interacts with first codon ' $A_{(1)}$ ' of AUA which might be useful to maintain base stacking interactions during codon-anticodon interactions. Thus, this study would be useful to understand role of modified bases L and t^6A to recognize proper codons in detail at the atomic level.

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Conflict of interest

All authors have no conflict of interest.

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Figure 1 Model anticodon stem and loop (ASL) with codon 'AUA' in [a] Clover leaf model and [b] Three dimensional structure of tRNA^{Ile} considered for MD simulation.

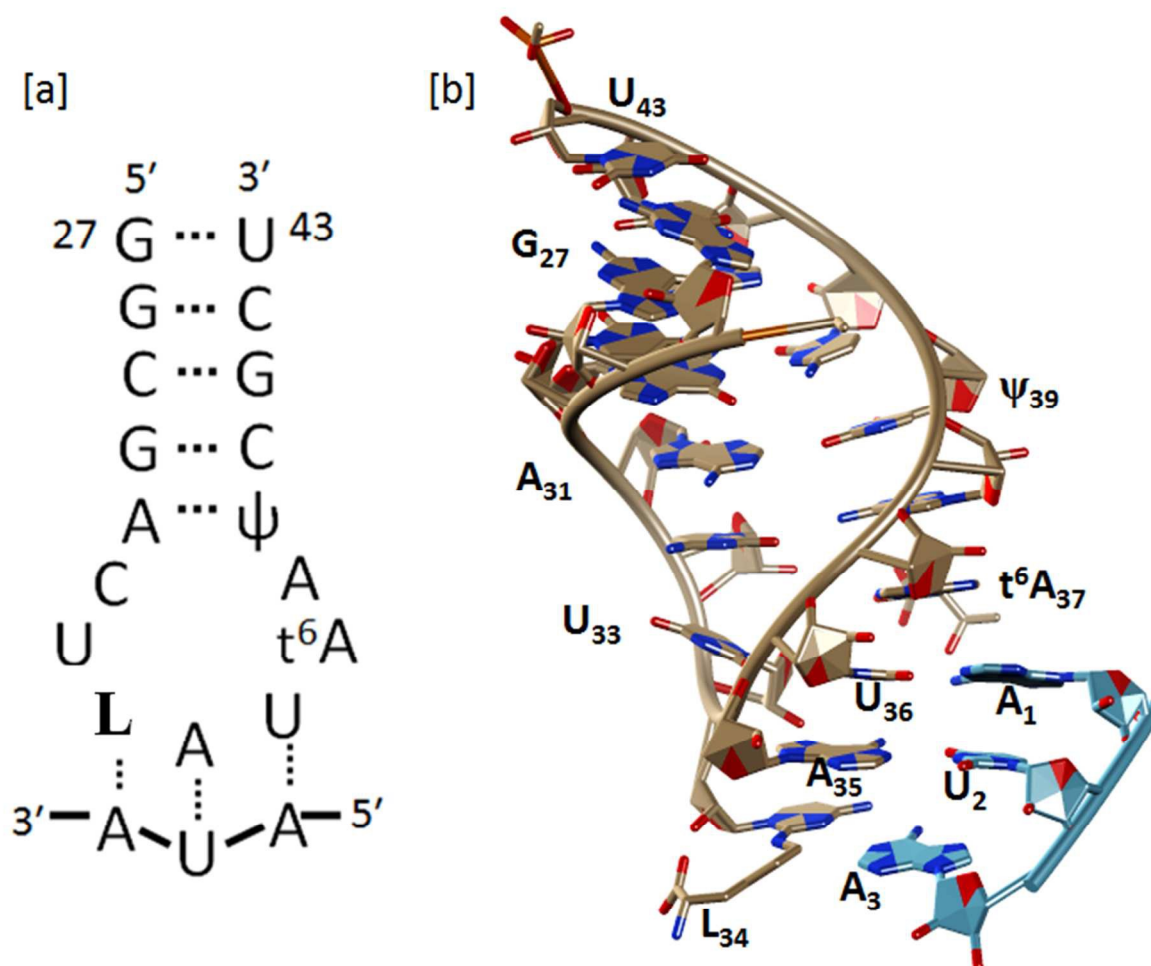


Figure 2 RMS Deviation of ASL with codon model sugar phosphate backbone during MD simulation in presence (blue line) and absence (red line) of modifications at 34th and 37th positions.

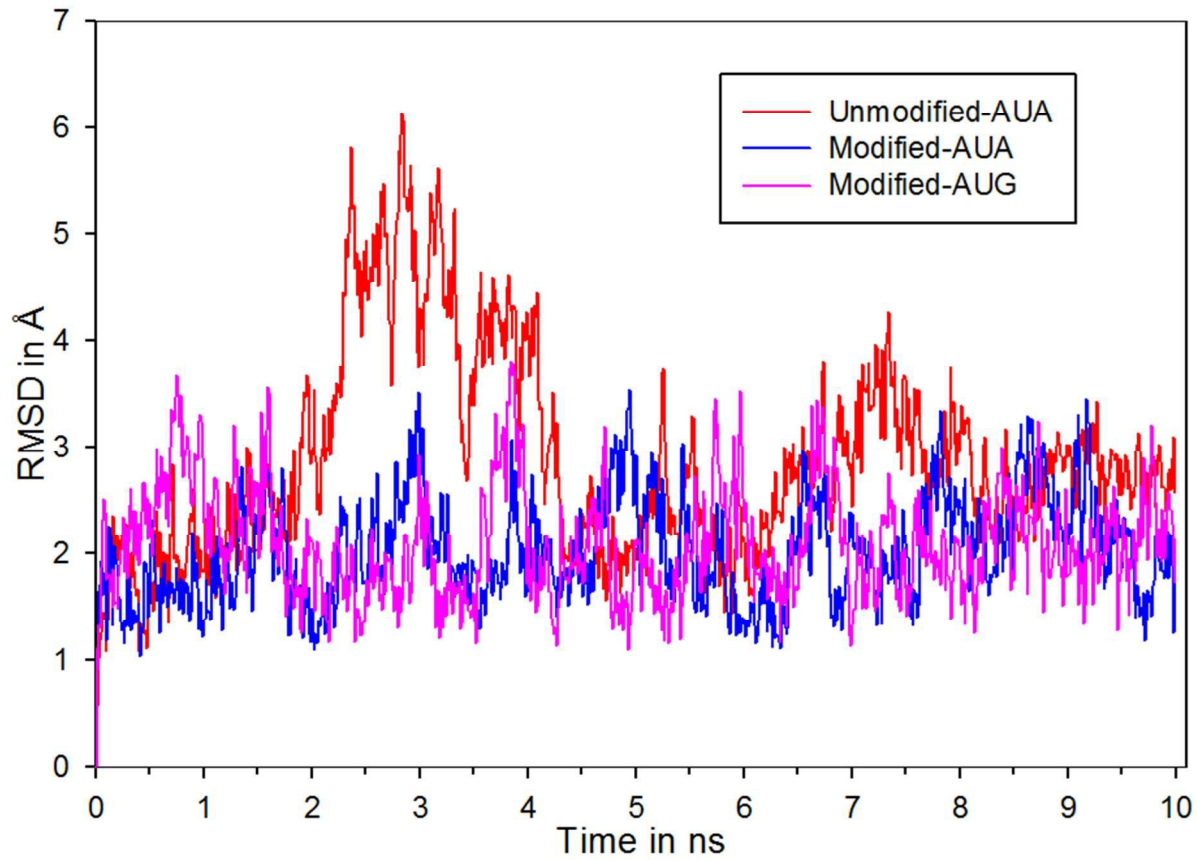


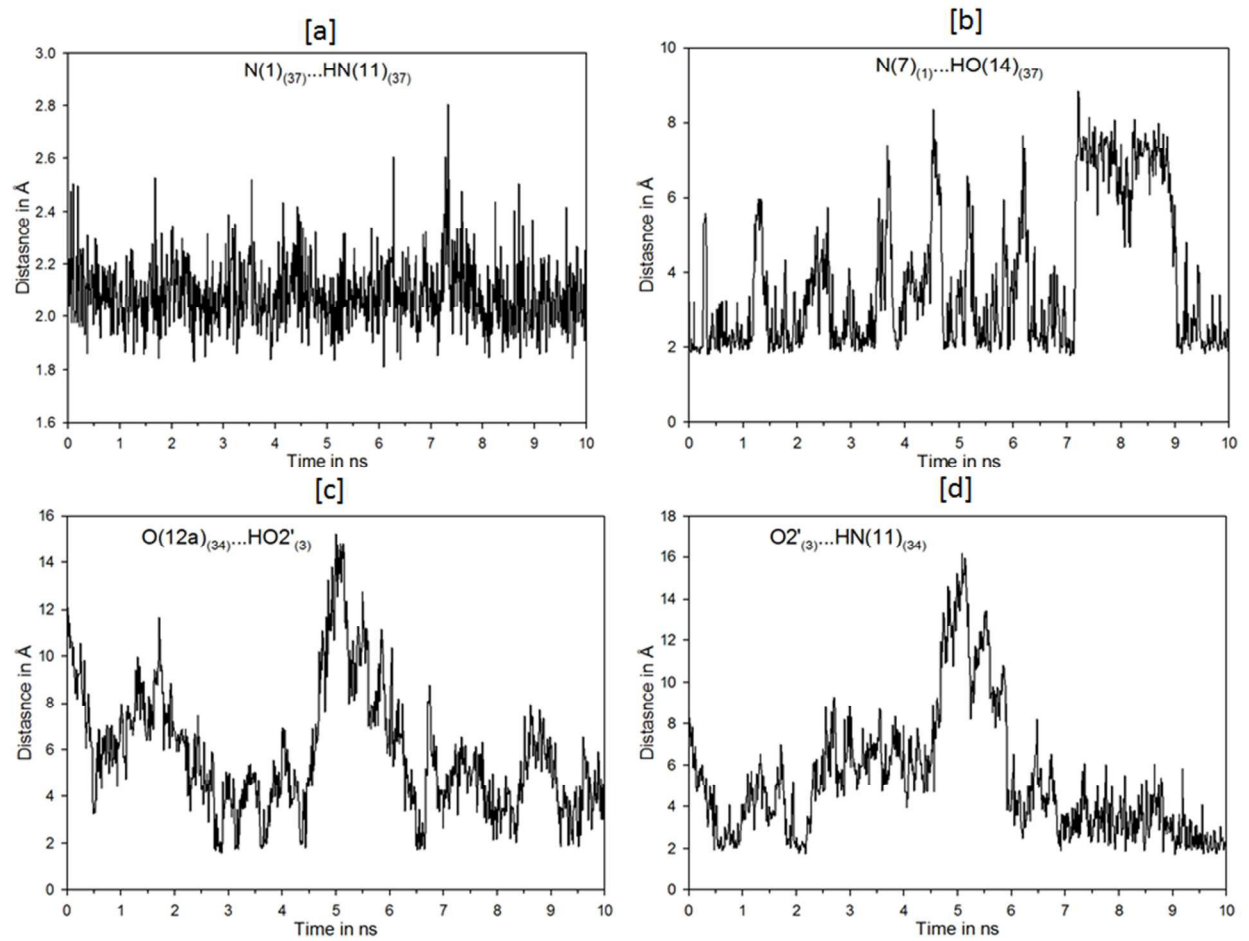
Figure 3 Analysis of hydrogen bonding during MD simulation period.

Figure 4 Comparative analysis of hydrogen bonding during MD simulation period in presence (blue) and absence (red) of modifications at 34th and 37th position of ASL.

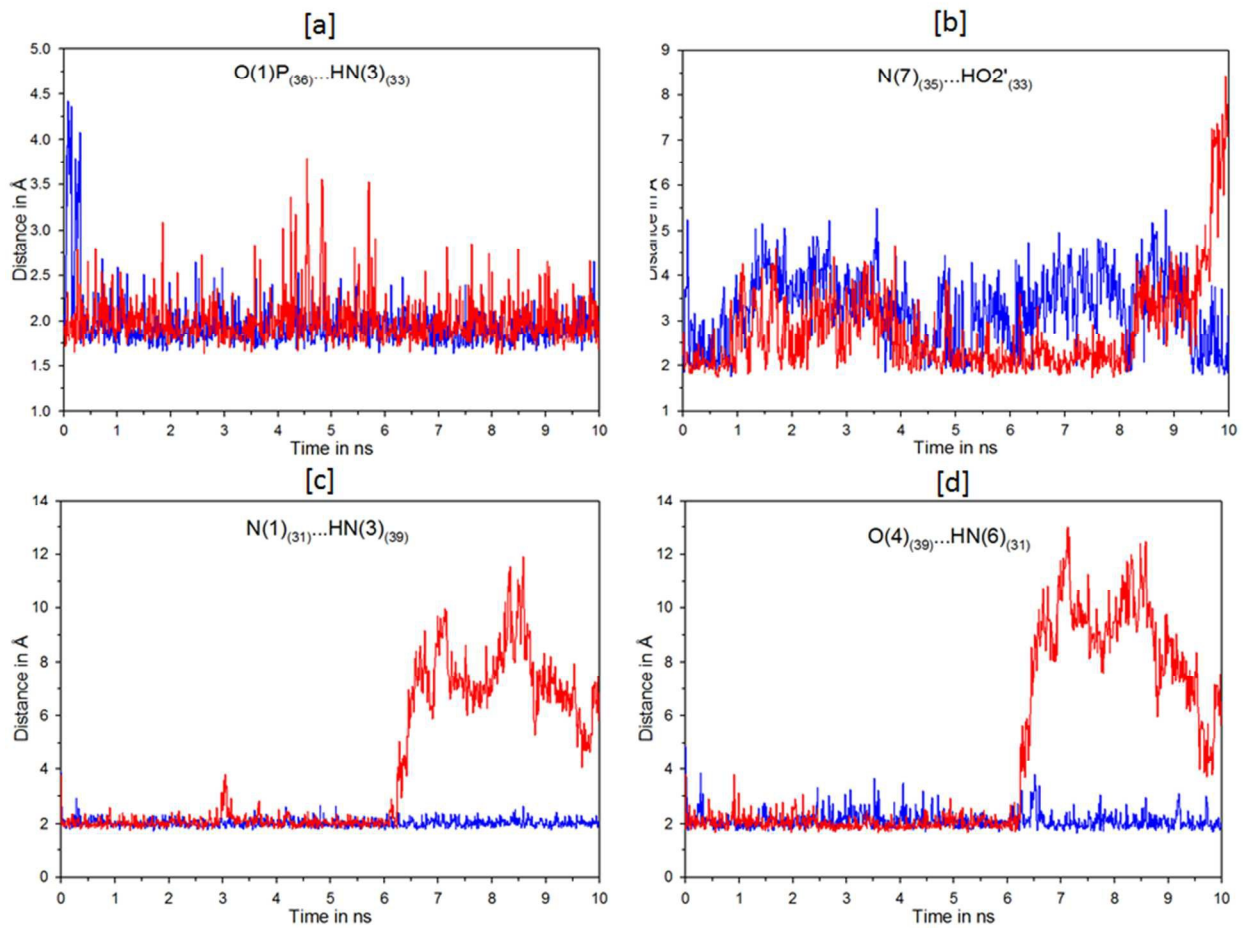


Figure 5 Hydrogen bonding Interactions of [a] $L_{(34)}$ with third codon adenine double hydrogen bond geometry, [b] single hydrogen bond geometry and [c] $t^6A_{(37)}$ and $U_{(36)}$ with first codon adenine during MD simulation.

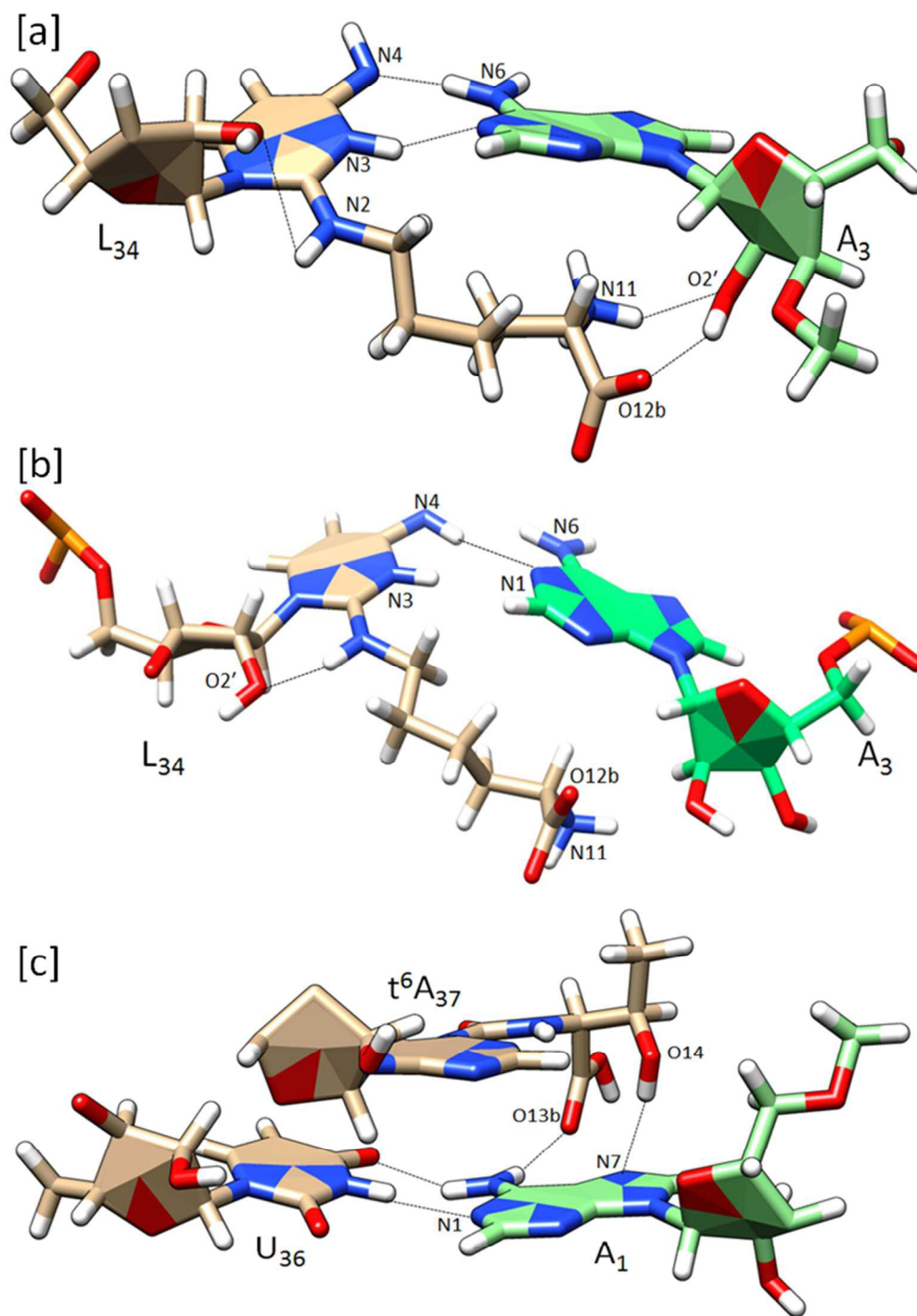


Figure 6 Root mean square fluctuations (RMSF) of ASL residues with codons over 10 ns time scale. Residue no. 44, 45, and 46 represents codon A₁, U₂, and A₃ respectively.

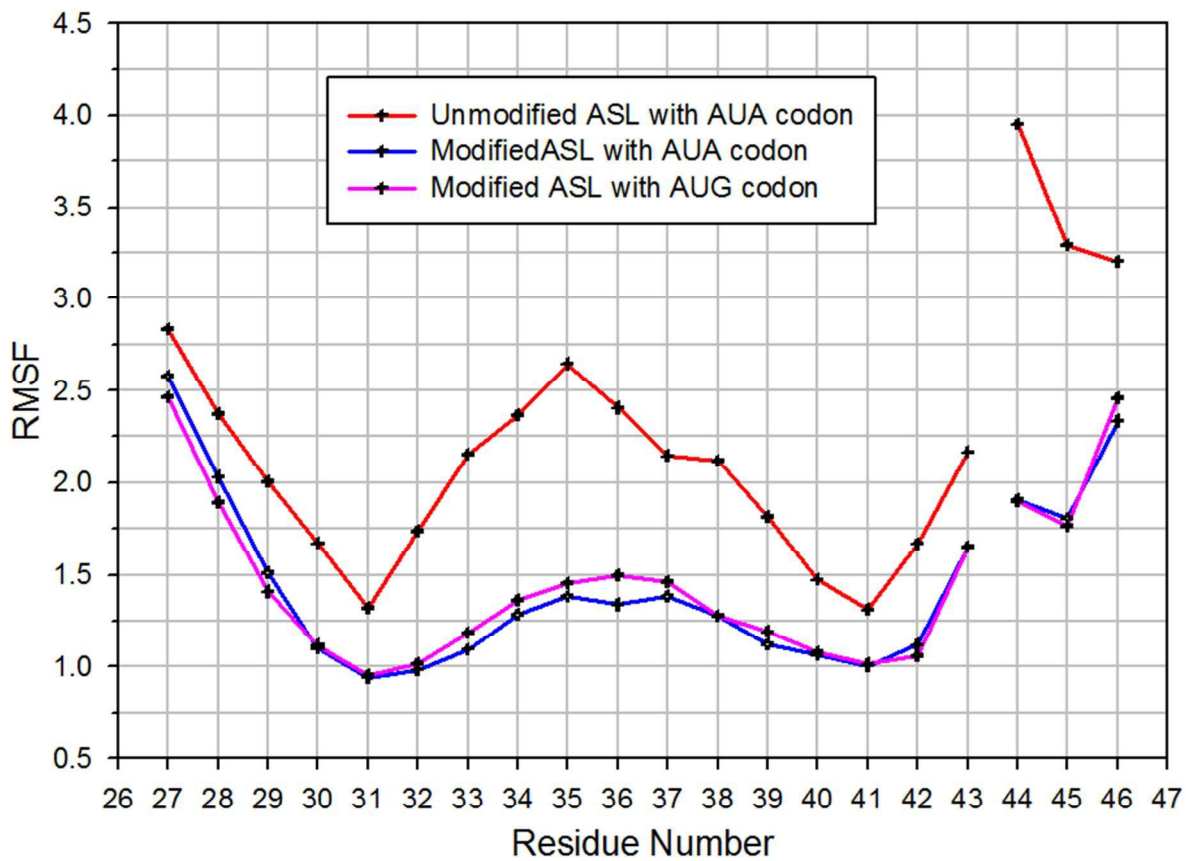


Figure 7 The 10ns snap shot structure showing hydrogen bonding and base stacking interactions between ASL (tan color) and [a] 'AUA' codon (light blue), [b] 'AUG' codon (light green)

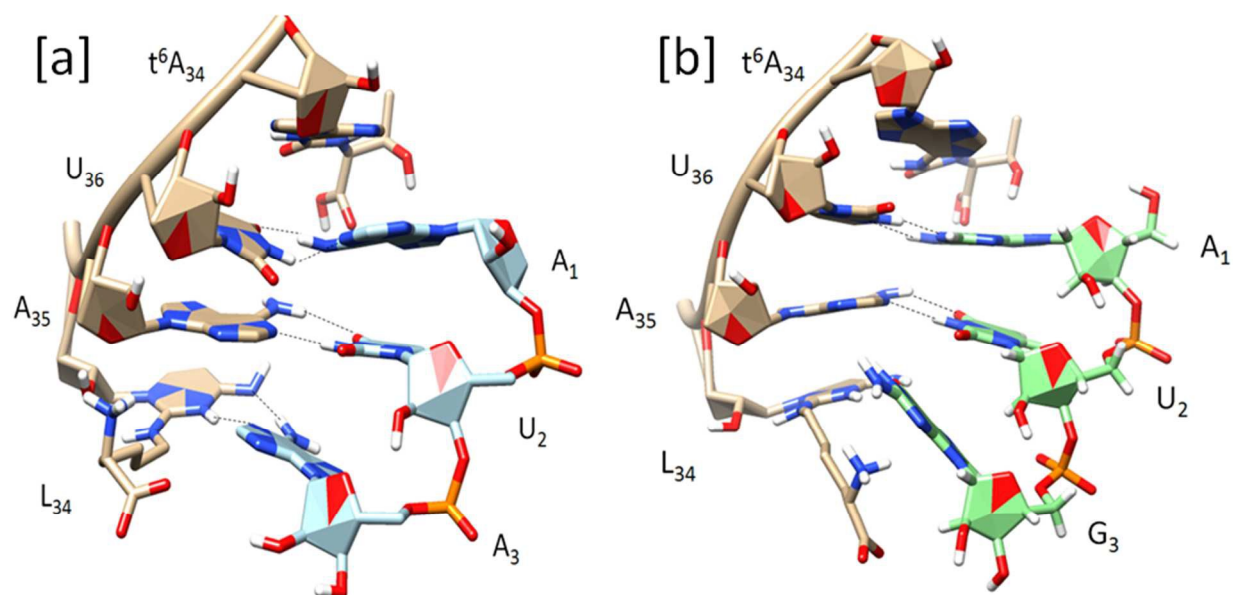


Figure 8 Molecular Electrostatics Potential surface calculations of base pair of models [a] $L_{(34)}:A_{(3)}$ [b] $L_{(34)}:G_{(3)}$ [c] $C_{(34)}:A_{(3)}$ [d] $C_{(34)}:G_{(3)}$

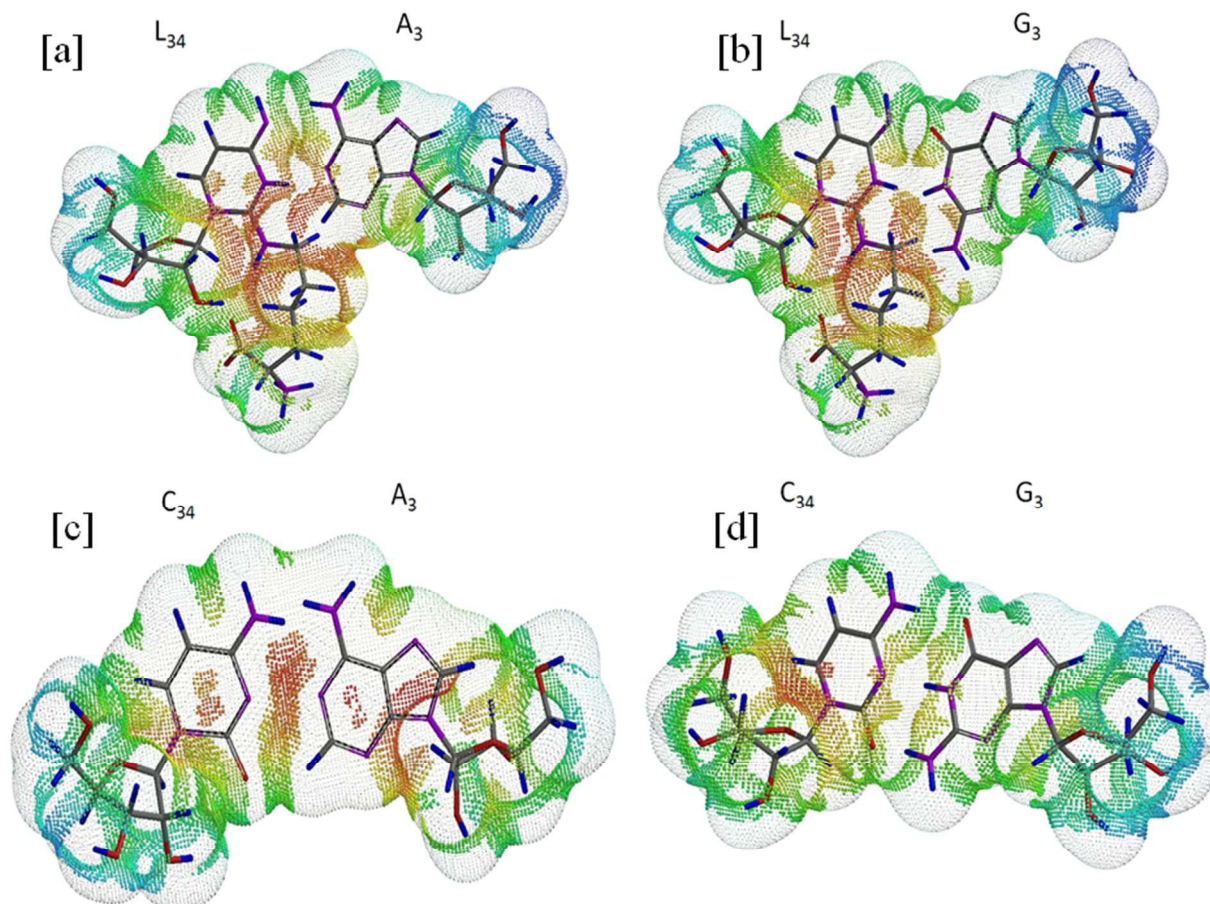


Figure 9: Binding free energy of snapshots taken over 10 ns trajectory of MD simulation