Supplementary Information

To

Structural landscape of base pairs containing post-transcriptional base modifications in RNA

PREETHI S. P,[1] PURSHOTAM SHARMA[2,*] and ABHIJIT MITRA[1,*]

[1]Center for Computational Natural Sciences and Bioinformatics, International Institute of Information Technology (IIIT), Gachibowli, Hyderabad, Telangana, 500032, India.

[2]Computational Biochemistry Laboratory, Department of Chemistry and Centre for Advanced Studies in Chemistry, Panjab University, Chandigarh, 160014, India.

Corresponding authors: psharma@pu.ac.in, abi_chem@iiit.ac.in

Table of contents

Section S1. Details of geometrical and energetic analysis of modified base pairs ……1
Section S2. Effect of type and position of modification on base pair geometry and stability……..…..……..……...8
Figure S1. Distribution of modified base pairs in the RNA crystal structure dataset...……10
Figure S2. Structural alignment of crystal occurrences of modified base pairs (with occurrence frequency ≥ 10) ……..……..……..11
Figure S3. Structural alignment of crystal structures of modified base pairs (with occurrence frequency ≤ 10) ……..……..12
Figure S4. RMSD graph of modified base pairs of crystal and optimized geometries……..13
Figure S5. E-values graph of modified base pairs of crystal and optimized geometries…..14
Figure S6. Local intra base pair parameters graphs of modified base pairs of crystal and optimized geometries……..……15
Figure S7. Structural context analysis of s^4U8:A modified base pair in tRNA ……..……16
Figure S8. Structural context of the m^5U:A modified base pair……..…...17
Figure S9. Structural context of the Ψ:G W:WC base pair ……..…...18
Figure S10. Structural context of the D:U W:WC base pair ……..…19
Figure S11. Structural representation of hinge regions in tRNA ……..……...20
Figure S12. Illustrating effect of type and position of modification on base pair geometry and stability ……21
Table S1. List of various type of RNAs searched in the dataset ……..……...21
Table S2. Distribution of crystal structures within the dataset ……..……...23
Table S3. List of PDBs with at least one modified base pair ……..……24
Table S4. List of unpaired modified bases in the crystal structure ……..……...25
Table S5. List of modified base pairs involving W:WC and W:WT pairing geometry ……..…26
Table S6. List of modified base pairs involving W:HT and W:HC pairing geometry 28
Table S7. List of modified base pairs involving S:WC, S:WT, W:ST, H:SC, S:HT, S:SC and S:ST pairing geometry ……..……..29
Table S8. Structural context of modified base pairs in various types of RNA ……..….30
Table S9. Presence of modified base pairs in structural elements of RNA ……..……32
Table S10. Modified base pairs from tRNA sequence dataset ……..……..34
Table S11. List of 36 unique modified base pairs identified in the dataset ……..……....35
Table S12. Structural deviation of modified base pairs within all crystal occurrences, with reference to optimized structure and with unmodified counterpart ……..……...36
Table S13. Deviation of E-values of modified base pairs ……..……...37
Table S14. Deviation of rotational base pair parameters of modifies base pair ….....……38
Table S15. Deviation of translational base pair parameters of modifies base pair ...……..…39
Table S16. Interaction energies of modified and unmodified base pairs ……..……40
Table S17. List of Triples and Quartets formed by modified base pairs ……..…..41
Table S18. Cartesian coordinates of the 36 modified base pair ……..……...42

SECTION S1

Details of geometrical and energetic analysis of modified base pairs

S1.1. Modified base pairs involving B–B interactions observe higher RMSD and better interbase hydrogen bonding within crystal occurrences, compared to those involving B–S and S–S interactions

Analysis of RNA crystal structures reveals that 18 of the total 24 base pairs combinations involving B–B interactions have more than one occurrences. 15 of these 18 base pairs show little deviation among their respective crystal occurrences (average root mean square deviation ($rmsd_{av}$) < 0.1 Å, Supplemental Table 9, Supplemental Figure S4). However, three modified base pairs, *viz.* $m^2{}_2G$:A W:WC, Um:A W:WC and D:U W:WT, show significantly high average rmsd values of 0.4, 0.2 and 0.2 respectively. On the other hand, 3 of the 6 modified pairs involving B–S interactions have multiple occurrences in RNA crystal structures. Within these base pairs, s^4U:A W:ST shows the highest deviation, with $rmsd_{av}$ of 0.2 Å, whereas m^7G:A S:WT and Ψ:C S:WC base pairs showed the lowest $rmsd_{av}$ (0.1 Å). Similarly, among 3 base pairs showing multiple occurrences among the 6 base pairs involving S–S interactions, Gm:G S:SC pair showed greatest deviation with $rmsd_{av}$ (0.1 Å), whereas the $m^6{}_2A$:G and Am:G S:ST pairs showed the least deviation with an $rmsd_{av}$ less than 0.04 Å.

The absolute values of average buckle ($|\kappa_{av}|$) within the crystal occurrences of modified base pairs is greater for base pairs involving S–S interactions ($12° \leq |\kappa_{av}| \leq 40°$), compared to those involving B–S interactions ($3° \leq |\kappa_{av}| \leq 35°$) and B–B interactions ($0° \leq |\kappa_{av}| \leq 23°$). This indicates that the involvement of ribose sugar provides greater flexibility to base pairs and results in deviation from ideal base pairing geometries. Although 8 of the 24 modified base pairs that involve B–B interactions show relatively high buckle ($|\kappa_{av}| > 10°$, Supplemental Table S11), the deviation in buckle across all crystal occurrences of each base pair is highest for the D:U W:WT (standard deviation (κ_{SD}) = 27.0) and Um:A W:WC (κ_{SD} = 21.4) pairs. However, the deviation in buckle parameter is relatively small among B–S interactions, and even smaller among S–S interactions.

In contrast to buckle, the absolute values of average propeller twist ($|\pi_{av}|$) are higher for B–S interactions ($4° \leq |\pi_{av}| \leq 40°$), compared to B–B ($0° \leq |\pi_{av}| \leq 22°$) and S–S($3° \leq \pi_{av} \leq 12°$) interactions. Within the B–B interactions, $|\pi_{av}|$ is relatively higher ($> 13°$) for two W:WC ($m^2{}_2G$:A and Gm:A) and one W:HT (A:m^1A) base pairs. However, 13 of the 24 B–B

interactions observe significantly small π_{av} ($0° \leq |\pi_{av}| \leq 5°$, Supplemental Table S11), albeit with a relatively high standard deviation (π_{SD}), ($0 \leq \pi_{SD} \leq 17$). On the other hand, highest $|\pi_{av}|$ is observed in D:G W:ST base pair among B–S interactions and s^4U:A S:SC base pair among S–S interactions.

The absolute values of average open angle ($|\sigma_{av}|$) are higher for B–S ($1° \leq |\sigma_{av}| \leq 59°$), compared to B–B interactions ($4° \leq |\sigma_{av}| \leq 14°$) and S–S ($4° \leq |\sigma_{av}| \leq 39°$) interactions. Among the B–B interactions, three W:WC base pairs (m^2G:C+, m^5U:G and Gm:A) showed the high $|\sigma_{av}|$ ($> 10°$), whereas 6 modified base pairs possess $|\sigma_{av}| < 1.0°$. Within the B–S interactions, base pairs containing dihydrouridine showed low $|\sigma_{av}|$ ($< \pm 0.1°$), whereas lowest $|\sigma_{av}|$ ($4°$) among the S–S interactions is observed in the D:U S:ST pair. Further, the trend in $|\sigma_{av}|$ correlates with the observed trend in stretch (S_z, Supplemental Table S12), which is smaller for B–B interactions (2.5 Å < S_z < 3.0 Å) compared to B–S (2.8 Å < Sz < 4.5 Å) and S–S interactions (2.7 Å < S_z < 3.6 Å).

The absolute values of average stagger ($|S_{x(av)}|$) are up to 1.4 Å for B–B interactions, 0.7 Å for B–S interactions, and 1.8 Å for S–S interactions. Except for two (m^2G:C+, $m^2{}_2$G:A) W:WC base pairs with $|S_{x(av)}|$ 0.7 Å and 1.4 Å respectively, the B–B interactions observe an $|S_{x(av)}|$ value of only up to 0.3 Å (Supplemental Table S12). In B–S interactions, three base pairs Ψ:C S:WC, A:m7G W:ST and D:G H:SC showed high $|S_{x(av)}|$ (>0.5 Å), whereas the D:G W:ST showed the lowest $|S_{x(av)}|$ (0.12 Å). Among the S–S interactions, the smallest $|S_{x(av)}|$ (0.3 Å) is observed for the Am:G S:ST pair.

The absolute values of the average shear parameter ($|S_{y(av)}|$) range up to 2.8 Å for B–B and B–S interactions, and up to 3.7 Å for S–S interactions. Within the B–B interactions, 7 out of 24 base pairs showed high $|S_{y(av)}|$ (~2.0 Å), whereas 5 base pairs showed low $|S_{y(av)}|$ (<0.1 Å). In the B–S interactions, the highest $|S_{y(av)}|$ is observed for the A:m^7G W:ST pair (2.8 Å), whereas D:G H:SC showed the lowest $|S_{y(av)}|$ (0.2 Å) . Similarly, in the S–S interactions, D:U S:ST showed the highest $|S_{y(av)}|$ (3.7 Å), whereas s^4U:A S:ST showed the lowest $|S_{y(av)}|$ (1.2 Å, Supplemental Table S13).

In addition to the analysis of geometrical variations of each modified base pair in different macromolecular crystal contexts, it is important to analyse the deviation in inter base hydrogen bonding interactions within different crystal occurrences (Supplemental Table S10). For this, we measured the E–value (E_{av}) of base pair occurrences in crystal contexts (See Materials and Methods section). E_{av} is a quantitative parameter which measures the quality

of hydrogen bonds between the pairing bases, where smaller E_{av} indicates better base pairing. Our analysis reveals that the average E_{av} of base pairs involving B–B interactions (0.1 and 1.0) are smaller than those involving B–S (0.4 and 1.3) and S–S (0.7 and 1.2) interactions. Among the 24 B–B interactions, 7 base pairs possess E_{av} < 0.3, whereas three modified base pairs m^2G:C+ W:WC, m^5U:G W:HT and m^1A:m^5U H:WT possess high E_{av} (between 0.8 and 1.0). Within the B–S interactions, 2 W:ST (A:m^7G and s^4U:A) and Ψ:C S:WC base pairs possess high E_{av} (~ 1.0), whereas the other three B–S interactions have E_{av} ranging between 0.4 and 0.7. Except Am:G S:ST (E_{av} = 0.7), all S–S interactions possess high E_{av} (>1.0).

S1.2. Crystal geometries of some modified base pairs deviate significantly compared to isolated optimized geometries

Geometry optimizations were carried out starting from crystal geometry to understand deviation in base pair geometries in RNA crystal structures from their respective isolated geometries optimized using quantum chemical methods reveals that the $rmsd_{av}$ ranges between 0.0 Å and 1.2 Å for B–B interactions, 0.1 Å and 0.3 Å for B–S interactions and 0.2 Å and 0.7 Å for S–S interactions (Supplemental Table 9, Supplemental Figures S2, S3 ad S4). Within the B–B interactions, two W:WC pairs ($m^2{}_2$G:A and Um:A) pairs exhibit the highest $rmsd_{av}$ (0.8 Å and 1.2 Å respectively). However, although 7 pairs observe moderate conformational changes (rmsdav between 0.3 and 0.1), 15 pairs deviate insignificantly (rmsdav < 0.1 Å) on optimization. Within the B–S interactions, Ψ:C S:WC showed relatively low $rmsd_{av}$ (0.15 Å), whereas s4U:A W:ST and H:SC base pairs showed high $rmsd_{av}$ (~ 0.3 Å). Within the S–S interactions, D:U S:ST show the highest deviation ($rmsd_{av}$ = 0.7 Å), whereas Gm:G S:SC and m^7G:A S:ST showed the lowest deviation ($rmsd_{av}$ = 0.2 Å).

19 out of 24 pairs involving B–B interactions show decrease in buckle, whereas 5 pairs show slight increase in buckle after optimization. However, within the B–S interactions, although three base pairs ((s^4U:A, D:G) W:ST and Um:G H:SC) exhibit decrease in buckle ($|\kappa_{av}|$ = 8.4°,17.6° and 0.5° respectively) on optimization, there is significant increase in buckle in Ψ:C S:WC, A:m^7G S:ST and D:G H:SC pairs ($|\kappa_{av}|$ = 38.8°, 53.2° and 32.7° respectively) on optimization. On the other hand, in the S–S interactions, all the pairs showed large increase in buckle after optimization (Supplemental Table 11).

In contrast, 15 out of 24 pairs show decrease in propeller after optimizations (0° ≤ $|\pi_{av}|$ ≤ 1° on optimization). Although 7 out of 24 modified base pairs showed moderate increase in

propeller, two W:WT modified base pairs (D:U and m5C:G) showed very high increase in propeller after optimization ($|\pi_{av}|$ of 23.4° and 39.6° respectively). Within B–S interactions, two pairs (Ψ:C S:WC and A:m7G S:ST) showed decreased $|\pi_{av}|$ (upto 12°) on optimization, whereas the other pairs showed increase in $|\pi_{av}|$. Among these, two pairs D:G W:ST and Um:G H:SC exhibited high increase in π_{av} on optimization (upto 20°). Within S–S interactions, m62A S:ST showed no change in $|\pi_{av}|$, two pairs (Gm:G S:SC and D:U S:ST) observe decrease in $|\pi_{av}|$, whereas other pairs exhibit increase in $|\pi_{av}|$ on optimization (Supplemental Table 11).

13 out of 24 pairs of B–B interactions showed decrease in $|\sigma_{av}|$, in contrast with other base pairs on optimization. Within B–S interactions, three pairs (A:m7G W:ST, s4U:A W:ST and Um:G H:SC) exhibited decrease in $|\sigma_{av}|$, whereas three pairs (D:G W:ST, D:G H:SC and Ψ:C S:WC) exhibited high increase in $|\sigma_{av}|$ on optimization. In S–S interactions, two S:ST pairs (m62A:G and m7G:A) showed decrease in $|\sigma_{av}|$ (upto 15°), whereas two pairs (Gm:G S:SC and D:U S:ST) showed tremendous increase in $|\sigma_{av}|$ (>14°) on optimization (Supplemental Figure S5).

7 out of 24 pairs observes decrease in $S_{x(av)}$ and 16 out of 24 pairs in B–B interactions showed insignificant change in $S_{x(av)}$ (< 0.2 Å) on optimization, however D:U W:WT showed slight increase in $S_{x(av)}$ (~ 0.3 Å). Within B–S interactions, 4 out of 6 pairs showed increase in $S_{x(av)}$ on optimization and in contrast, D:G H:SC showed decrease in $S_{x(av)}$ on optimization. Within S–S interactions, four pairs showed decrease in $S_{x(av)}$ and two pairs showed increase in $S_{x(av)}$ on optimization (Supplemental Table 12).

5 out of 24 base pair showed increase in $S_{y(av)}$ and 9 out of 24 pairs showed decrease in $S_{y(av)}$ and others showed no change on optimization. Within B–S interactions, 4 out of 6 pairs exhibited decrease in $S_{y(av)}$ and 2 pairs (s^4U:A W:ST and D:G H:SC) showed increase in $S_{y(av)}$ on optimization. Within S–S interactions, three pairs exhibited decrease in $S_{y(av)}$ and two pairs exhibited no change in $S_{y(av)}$, however D:U S:ST showed increase in $S_{y(av)}$ on optimization. With the exception of s^4U:A S:SC where $S_{z(av)}$ increased, all the base pairs showed insignificant change in $S_{z(av)}$ on optimization.

The E_{av} ranges between 0.1 and 0.6 for B–B interactions, 0.3 and 1.5 for B–S interactions and 0.9 and 1.2 for S–S interactions. With the exception of Ψ:G W:WC where E_{av} increased,19 base pairs observe low E_{av} and four pairs showed insignificant change in

E_{av} on optimization. Within B–S interactions, with the exception of D:G H:SC where E_{av} increased, all the base pairs observe low E_{av} on optimization. All the pairs in S–S interactions showed low E_{av} on optimization (Supplemental Figure S4).

S1.3. Comparison of geometries of modified and unmodified base pairs highlight the effect of base modification.

The $rmsd_{av}$ between optimized geometries of modified and unmodified base pairs ranges between 0.0 Å and 1.5 Å for B–B interactions, 0.0 Å and 0.4 Å for B–S interactions and 0.3 Å and 2.1 Å for S–S interactions. Among B–B interactions, two W:WC base pairs Ψ:G and m^2G:C+ showed highest deviation ($rmsd_{av}$= 1.5 Å and 1.4 Å respectively). However, the modified and unmodified geometries of 13 out of 24 base pairs are very similar ($rmsd_{av}$ < 0.1 Å). Among B–S interactions, two W:ST (s^4U:A and D:G) pairs, D:G H:SC and Ψ:C S:WC showed significant deviation ($rmsd_{av}$ > 0.1 Å). However, A:m^7G W:ST and Um:G H:SC are very similar to their unmodified counterparts ($rmsd_{av}$ ~ 0.03 Å). Among S–S interactions, Gm:G S:SC and $m^6{}_2$A:G S:ST showed relatively greater variation ($rmsd_{av}$ = 2.1 Å and 1.7 Å respectively), whereas s^4U:A S:SC and two S:ST (Am:G and m^7G:A) pairs showed the lowest deviation ($rmsd_{av}$ < 0.6 Å, Supplemental Table 09).

In B–B interactions, 19 out of 24 modified base pairs showed E_{av} similar to unmodified base pairs with minute difference ($\Delta E_{av} \leq 0.1$, Supplemental Table 10). However, 4 out of 24 pairs exhibited minute increase in E (upto 0.4) and m^7G:G W:HT showed low E, with respect to their counterparts. In B–S interactions, three pairs (s4U:A W:ST, D:G W:ST, and Um:G H:SC) were similar to unmodified structures ($\Delta E_{av} \leq 0.2$), whereas the other pairs showed high E than unmodified counterparts (ΔE_{av} ~ 1.0). Within S–S interactions, all the pairs of S:ST family showed similar E_{av} to unmodified counterparts, whereas S:SC pairs showed high E than the unmodified counterpart ($\Delta E_{av} \leq 0.6$, Supplemental Table 10)

17 out of 24 B–B pairs have high κ compared to their unmodified counterparts. On the other hand, although $m^2{}_2$G:A W:WC as well as three W:HT (m^5Um:A, Um:A and D:U) pairs showed high κ, both in their crystal and optimized structures, where their respective unmodified structures are close to planar (κ ~ 0°). Although most of the B–B and S–S interactions exhibited high κ than their unmodified counterparts, κ is low in modified B–S base pairs.

In 22 of the 24 B–B pairs, propeller twist (π) remains same as their unmodified counterpart. However, 2 pairs (A:m^1A W:HT and D:U W:WT) showed significant increase in

π (upto 23.0°) upon modification. In B–S interactions, although π decreases slightly (up to 7°) in two base pairs (Ψ:C S:WC and A:m^7G W:ST), remaining four pairs (s^4U:A W:ST, D:G W:ST, D:G H:SC and Um:G H:SC) observe (up to 54°) increase in propeller on modification. However, within the S–S interactions, all pairs observe increase in π (upto 10°) on modification.

8 out of 24 B–B pairs showed increase in open on modification. Among these, m^2G:C+ W:WC pair showed high σ on modification. However, 9 B–B pairs observed similar σ to unmodified pairs. On the other hand, 13 pairs showed decrease in open on modification. In B–S interactions, three modified base pairs showed significant increase in open on modification. However the other three base pairs (Ψ:C S:WC, D:G W:ST and Um:G H:SC), showed high open on modification (σ_{av} –32.9°, –31.0° and –2.1° respectively). In S–S interactions, 5 out of 6 modified base pairs showed increase in open on modification. However, s^4U:A S:SC showed decrease in open on modification (Supplemental Table 11).

S1.4. Interaction energies of modified base pairs and their comparison to unmodified base pairs.

Interaction energies of modified base pairs range between –43.4 and –9.8 kcal/mol. In B–B interactions, with the exception of protonated base pair m^2G:C+ W:WC, the interaction energy range between –38.5 and –9.8 kcal/mol. Six W:WC (m^7G:C, m^7G:G, m^5C:G, Gm:C, m^2G:C and Cm:Gm) and m^7G:G W:HT modified base pairs have high interaction energy and identified to be greater than –27.0 kcal/mol. In six W:WC (m^2G:U, $m^2{}_2$G:A, Ψ:G, Um:A, m^5U:G and Gm:A) and five W:HT (m^5U:A, Ψ:A, m^5Um:m^1A, A:m^1A, s^4U:A and Um:A) modified base pairs, the interaction energies were moderate and range between –23.30 and –15.4 kcal/mol. In Ψ:A W:WC, m^5U:G W:HT and W:WT (D:U and m^5C:G) modified base pairs the interaction energy is relatively low and range between –14.8 and –9.8kcal/mol. In B–S interactions, It is observed that, W:ST modified base pairs A:m7G and D:G, showed relatively high interaction energy of –22.5 and –15.4 kcal/mol respectively. Rest of the modified base pairs, Ψ:C S:WC, s^4U:A W:ST and H:SC (D:G and Um:G) showed moderate interaction energies that range between –14.6 and –12.6 kcal/mol. In S–S interactions, m^7G:A S:ST showed highest interaction energy of –34.9 kcal/mol, subsequently S:SC (Gm:C and s^4U:A) and three S:ST ($m^6{}_2$A:G, Am:G and D:U) modified base pairs showed interaction energies within range of –21.5 and –15.9 kcal/mol (Supplemental Table S13).

Interaction energy difference between modified and unmodified base pairs range between –15.3 and 6.3 kcal/mol. In B–B interactions, the interaction energy difference range between –15.30 and 0.86 kcal/mol. In base pair interactions involving positive charge bases such as m^7G:C W:WC, four W:HT (m^7G:G, m^1A:m^5U, m^1A:m^5Um and m^1A:A), m^7G:A S:WT and m^7G:A S:ST the difference in modified and unmodified base pairs range between –6.0 and –15.30 kcal/mol. In W:WC and W:WT base pairs, the change in interaction energy in most of the base pairs is less than $\pm$ 1.0 kcal/mol, however slight increase in interaction energy is observed in four W:WC base pairs m^2G:C+, $m^2{}_2$G:A, Ψ:G and Cm:Gm. In W:HT base pairs, there is significant increase in interaction energy upon modification in base pairs m^5U:A, Ψ:A and s^4U:A. In B–S interactions, the change in interaction energy range between –6.00 and 6.00 kcal/mol, upon modification there is decrease in interaction energy in Ψ:C S:WC and D:G W:ST base pairs. In S–S interactions, the change in interaction energy range between –13.91 and 1.1 kcal/mol, Gm:G S:SC showed decrease interaction energy.

Overall, not only the protonated base pair m^2G:C(+) W:WC showed high interaction energy of –43.37 kcal/mol but also the base pair interactions that involve positively charged bases such as m^1A and m^7G. In these interactions, the interaction energy is relatively high and the energy difference between the modified and unmodified base pairs range between –6.0 and –15.3 kcal/mol. In case of sugar methylations, 2'–O–ribose methyl modifications slightly effected the base pair stability and the change in interaction energy range between –2.2 and 1.1 kcal/mol (Supplemental Table 13).

SECTION S2. Effect of type and position of modification on base pair geometry and stability.

S2.1. Uridine modifications reveal the effect of type of modification on the base pair geometry and stability

As mentioned earlier, our study reveals that a significant fraction of modified base pairs in RNA involve the modification of either uridine (rU) nucleoside. This natural variety in base pair structures containing uridine modifications can be utilized to analyze the effect of the type of base modification on the geometry and stability of base pairs. Specifically, our crystal structure analysis revealed six distinct modified forms of the U:A W:HT base pair, *viz.* s^4U:A, Ψ:A, m^5U:A, m^5U:m^1A, m^5Um:m^1A and Um:A, each of which contain a different uridine modification (Supplemental Figure 10). Whereas pseudouridylation leads to increase in N1–N9 distance, sugar methylation increases the RMSD between the crystal occurrences upto 0.3 Å. Similarly, buckle parameter is also relatively high in m^5Um:A (7.9°) and Um:A (3.8°) base pairs.

Upon modification, U:A modified base pairs that are composed of charged bases showed high increase in interaction energies. For example, m^5U:m^1A base pair possess stronger interaction (by 7.4 kcal/mol) compared to the U:A pair. However, on addition of extra methyl substituent at 2'-O-ribose of m^5U (in m^5Um:m^1A), the interaction energy is 5.9 kcal/mol lower compared to the unmodified counterpart. This might be attributed to change in structure with sugar methylation. In Um:A, slight decrease in energy was observed due to changes in structure upon sugar methylation.

S2.2. Guanosine, cytidine and adenosine modifications illustrate the effect of position of modification on the base pair geometry and stability

Our database analysis reveals five examples of modified base pairs corresponding to the native G:C W:W cis pair, viz. m^2G:C, m^2G:C+, m^7G:C, G:m^5C, Gm:C and Gm:Cm, and three modified base pairs corresponding to the A:G S:ST base pair, viz. $m^6{}_2$A:G, Am:G and A:m^7G which differ in the position of modification on the interacting nucleobase.

All five modifications of the G:C W:WC pairs (Supplemental Figure 10, Supplemental Table S14) possess very similar geometries to that of unmodified pair with $rmsd_{av}$ within 0.01 Å, and similar N9-N1 distances. Moreover, the binding energy of the m^2G:C, m^5C:G and Gm:C pairs are very similar to unmodified base pair, with interaction energy change of $<$1.0 kcal/mol. However, the presence of (positively charged) m^7G base in the m^7G:C pair likely

increases the electrostatic interactions between the pairing nucleobases, which enhances the interaction energy by 15 kcal/mol, compared to the unmodified base pair.

In context of the modifications at the A:G S:ST base pair, three modified variants that include single methyl substituted at positions N7 of Guanine (m^7G) or 2'–O–ribose of Adenine (Am) or dimethyl substituent at N6 of exocyclic amino group of adenine ($m^6{}_2A$) were identified. Crystal structures showed low $rmsd_{av}$ of 0.02 Å and deviation in geometry was observed upon optimization (0.2 Å < $rmsd_{av}$ < 0.4 Å). However, $m^6{}_2A$:G S:ST showed much deviation in geometry from unmodified structure with $rmsd_{av}$ of 1.7 Å and the other two Am:G and A:m^7G showed less deviation from unmodified structure with $rmsd_{av}$ 0.3 Å and 0.5 Å respectively. The observed base pair parameters buckle (κ), open (σ) and propeller (π) for these modified base pair are very high. These structures showed high E-values (~1.0). Sugar methylated base pair, Am:G S:ST showed slight decrease in interaction energy (~ 1 kcal/mol), whereas the charged base pair m^7G:A S:ST showed high increase in interaction energy (~14 kcal/mol). Dimethyl modification in $m^6{}_2A$:G had not affected the stability of the base pair as the interaction energy is similar to unmodified base pair.

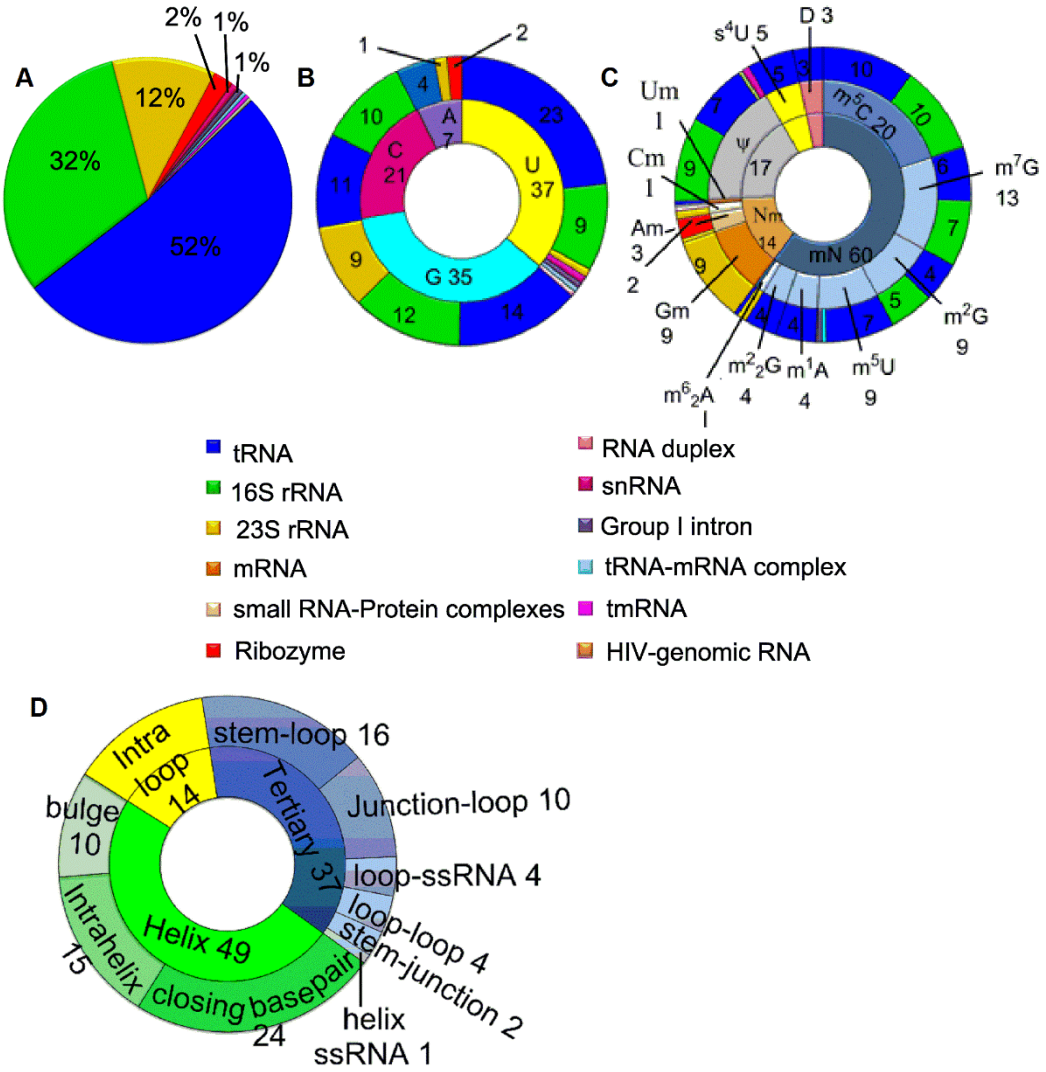

FIGURE S1. (A) Distribution of 453 modified base pairs as a function of type of RNA. (B) Distribution of modified base pairs as function of nucleotide that undergoes modification (first layer), and their occurrence in various types of RNA (second layer). (C) Distribution of modified base pairs based on type of modification (first and second layers) and their occurrence in various types of RNA (third layer). (D) Percentage distribution of 453 modified base pairs as function of secondary structural elements.

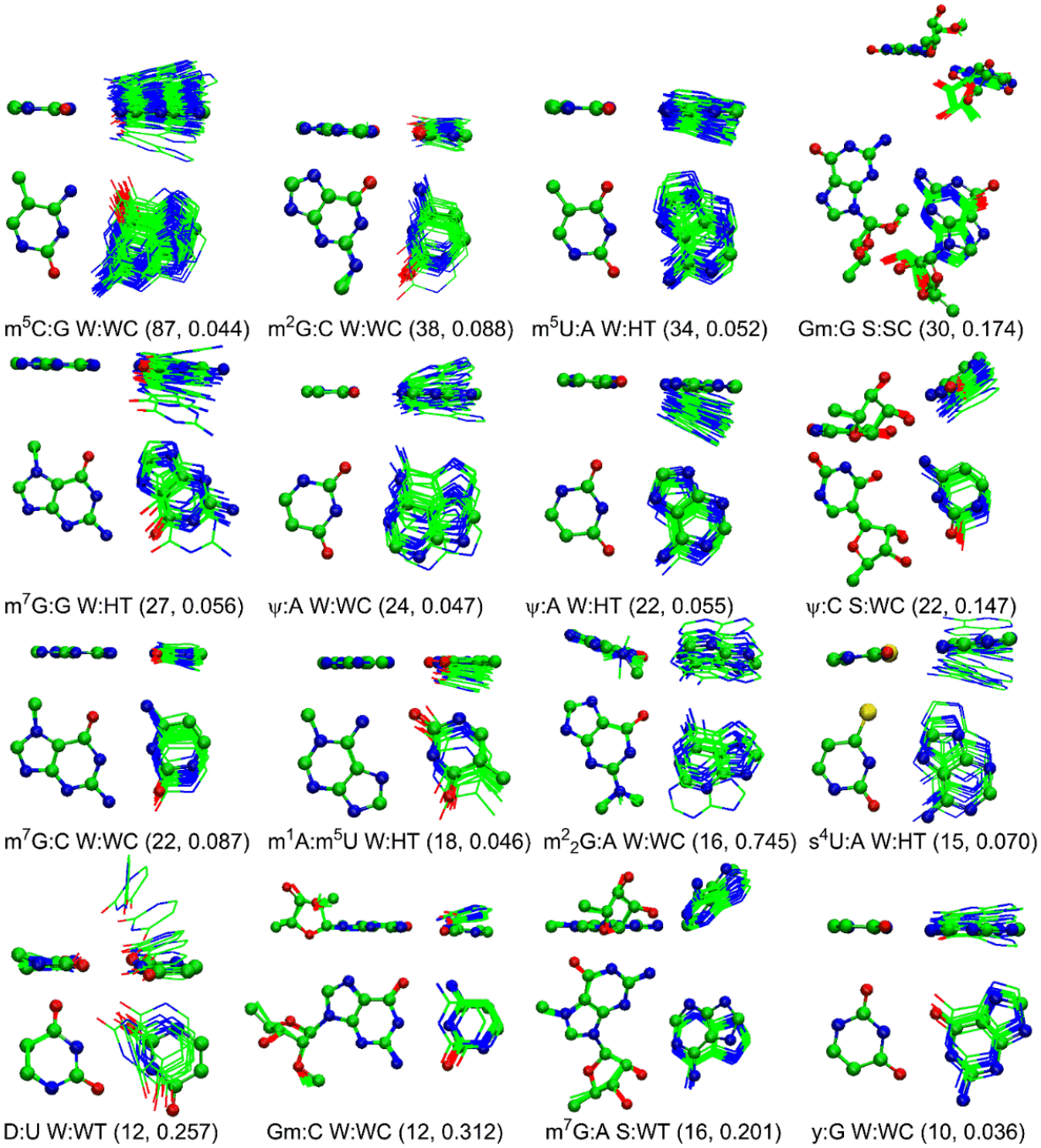

FIGURE S2. Structural alignment of crystal occurrences of modified base pairs (with occurrence frequency ≥ 10) with their corresponding optimized structures. Occurrence frequency and average RMSD with respect to the optimized structure (in Å) given in parenthesis.

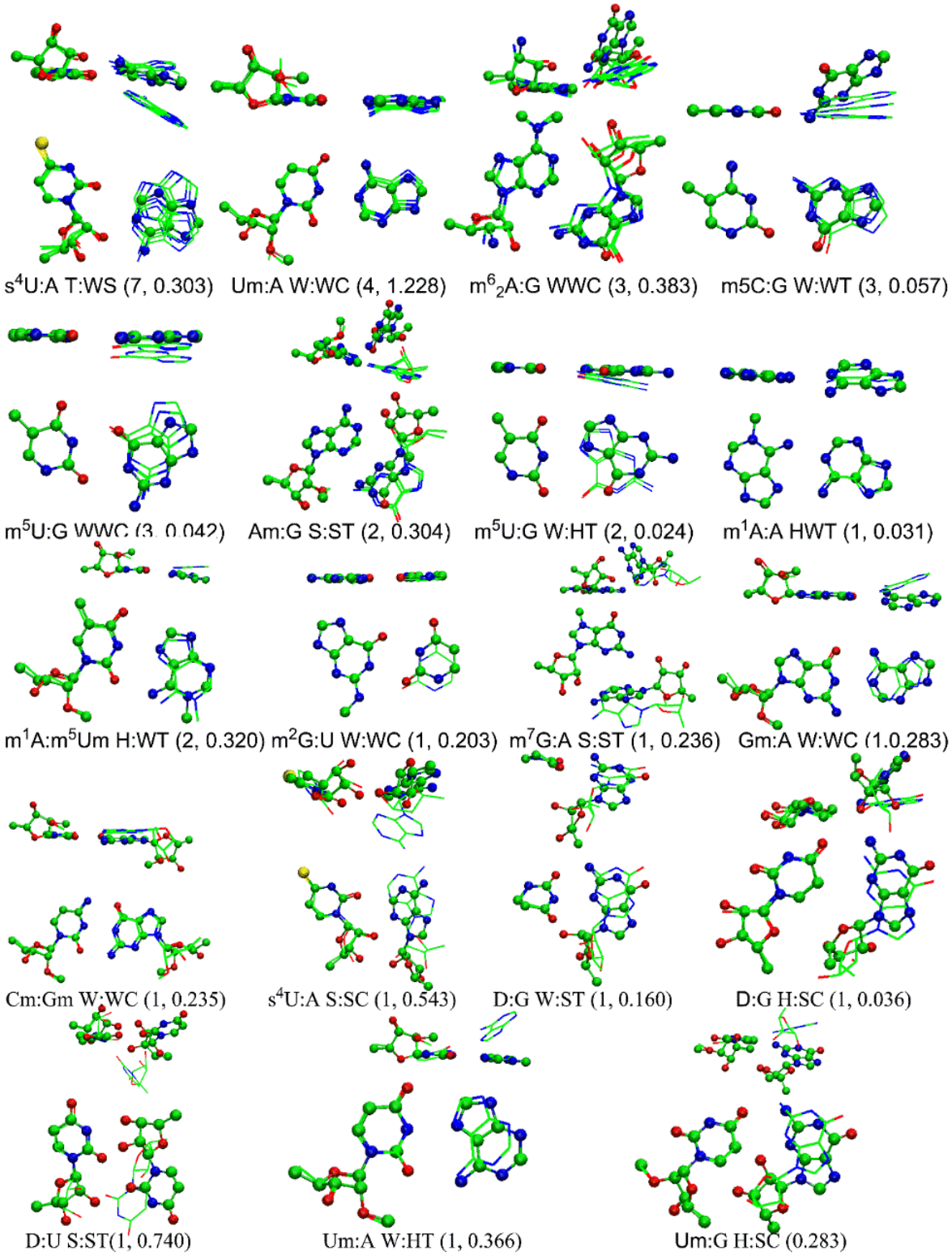

FIGURE S3. Structural alignment of crystal structures of modified base pairs (with occurrence frequency ≤ 10) with their corresponding optimized structures. Occurrence frequency and RMSD (in Å) given in parenthesis.

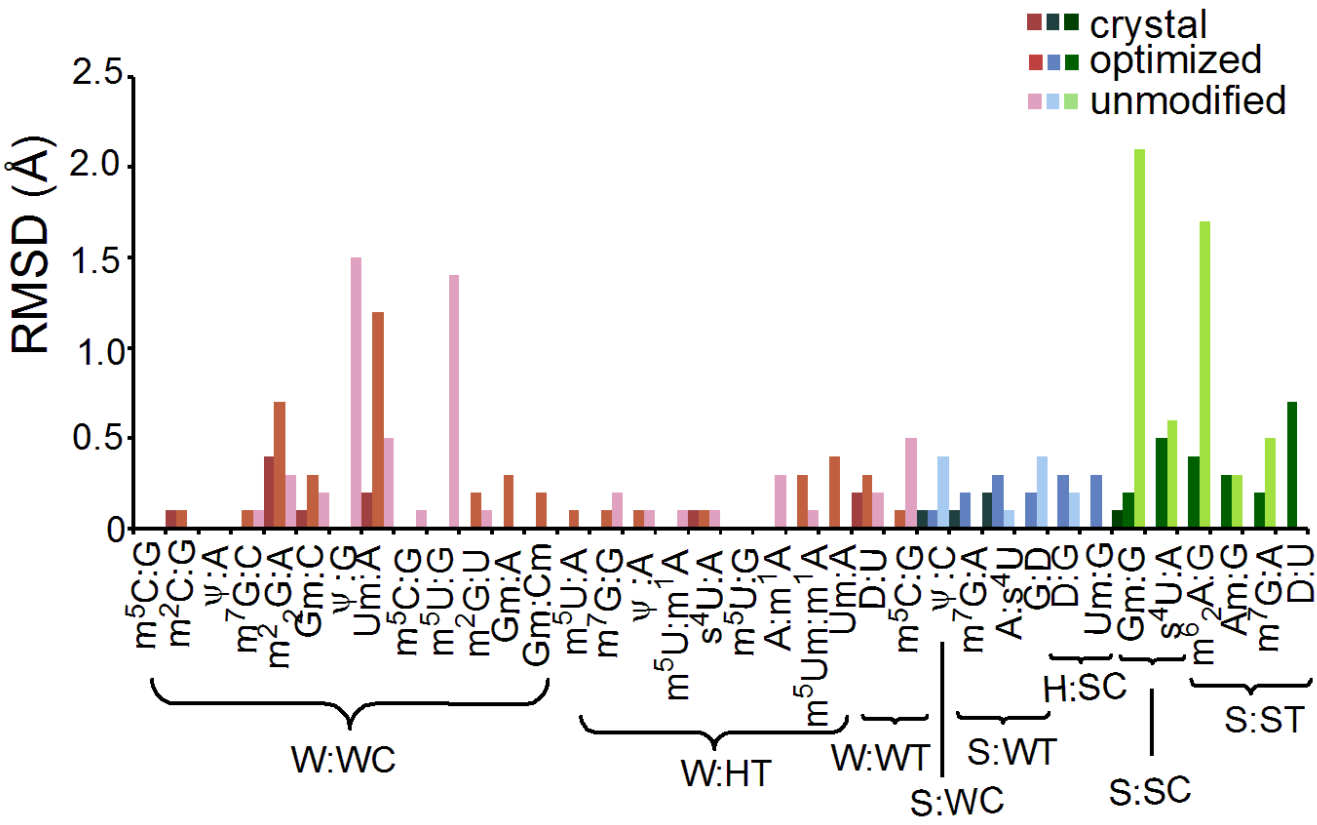

FIGURE S4. Average RMSD within the crystal occurrences of each base pair.

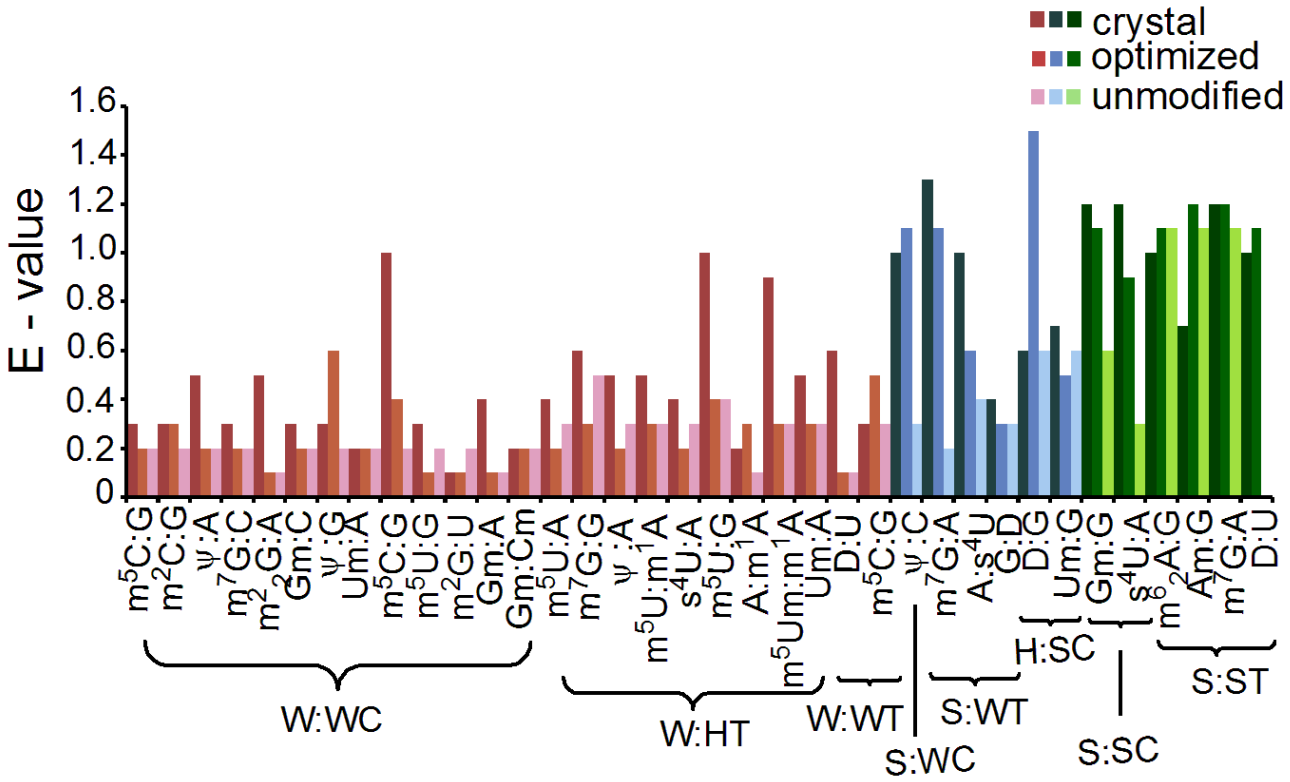

FIGURE S5. Average E-value of the crystal geometries of each base pair with respect to the optimized structure.

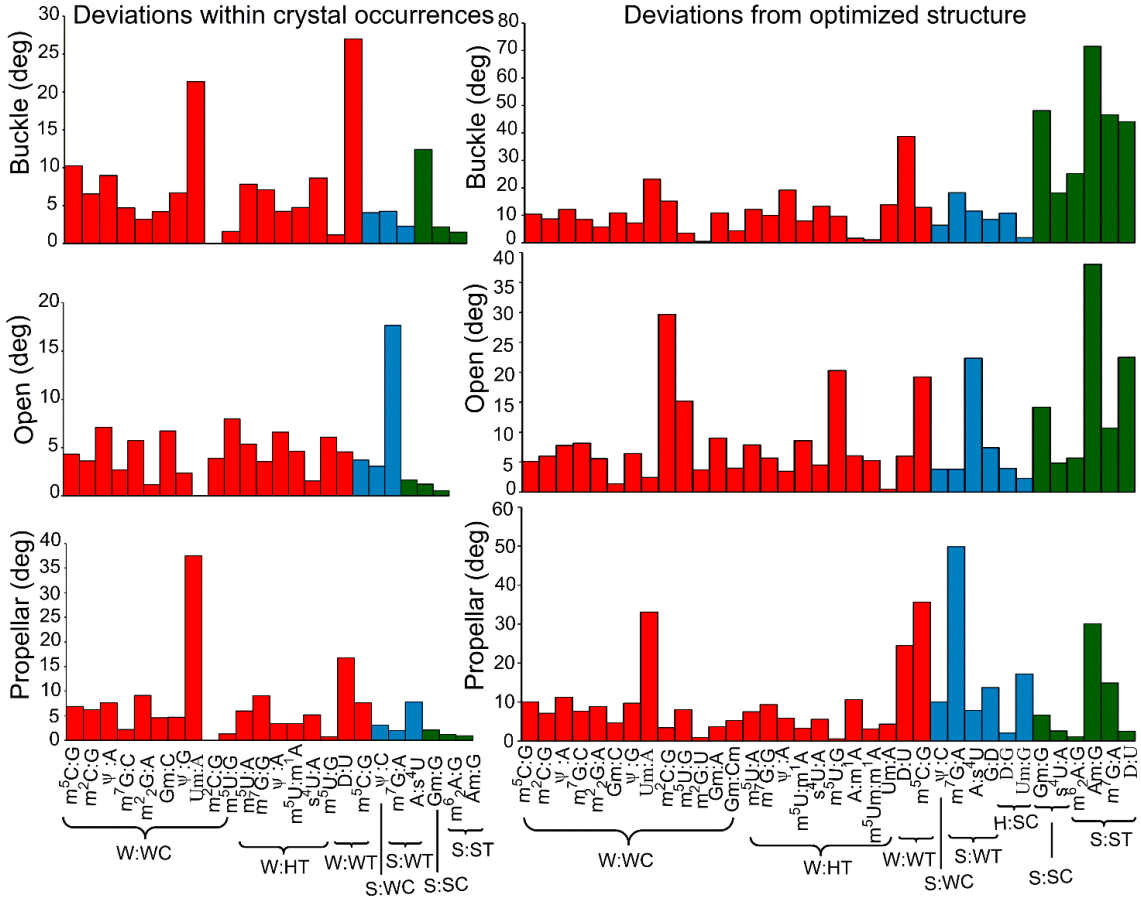

FIGURE S6. Graphs representing average RMS deviation and standard deviation of local intra base pair parameters buckle (κ), open (σ) and propeller (π). Colour coding according to the base pair interactions – B–B interactions (red), B–S interactions (blue) and S–S interactions (green).

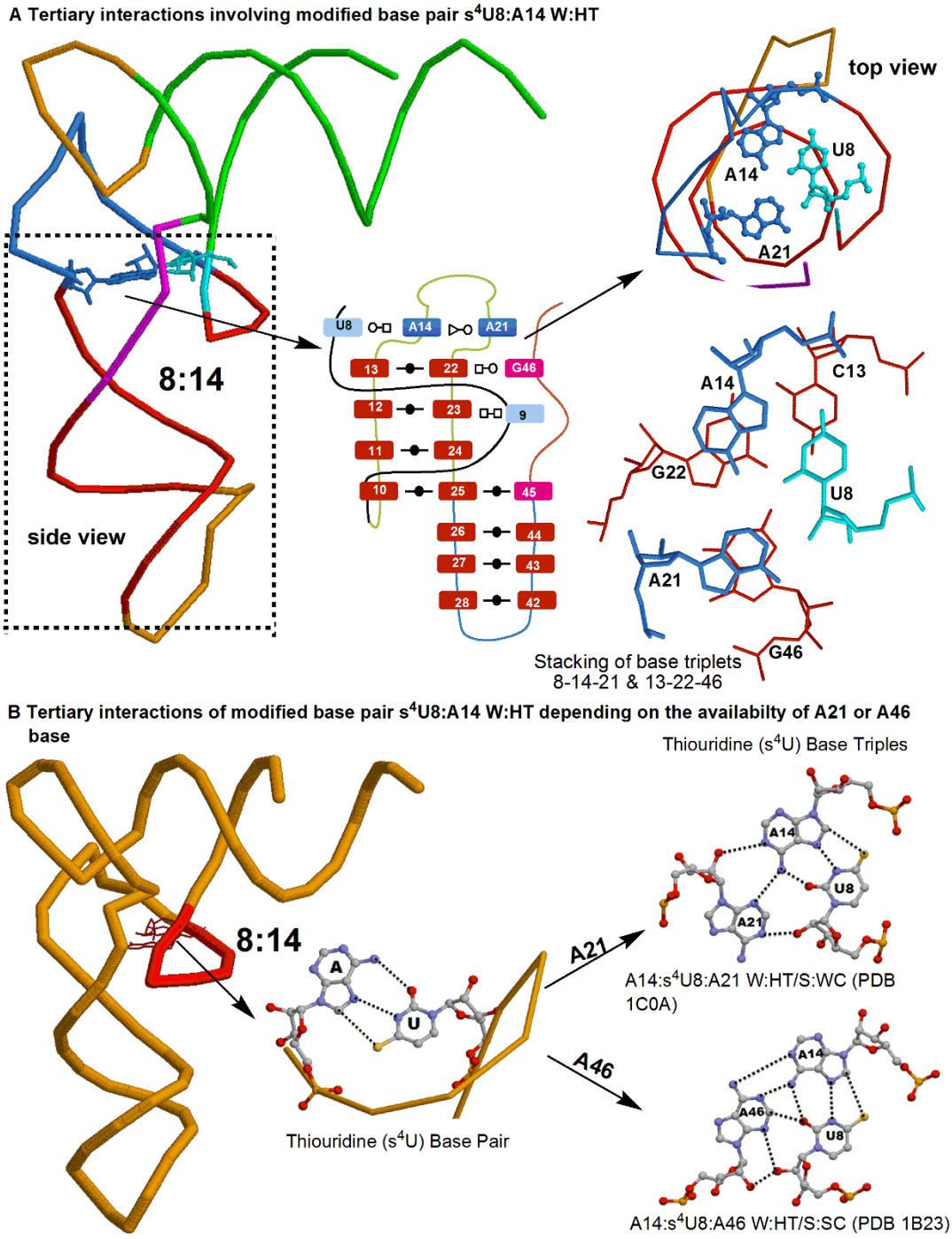

FIGURE S7. Structural context analysis of s^{4}U8:A14 W:HT modified base pair in tRNA. (A) 4–thiouridine base pair positioned at 8:14 of tRNA, interacts with A21 nucleobase and forms a 8:14:21 base triple. The base triple is stacked upon other triplet 13:22:46 (PDB 1C0A). (B) Alternate tertiary interactions, where the modified base pair interacts with base 46, forming the 8:14:46 base triple (PDB 1B23).

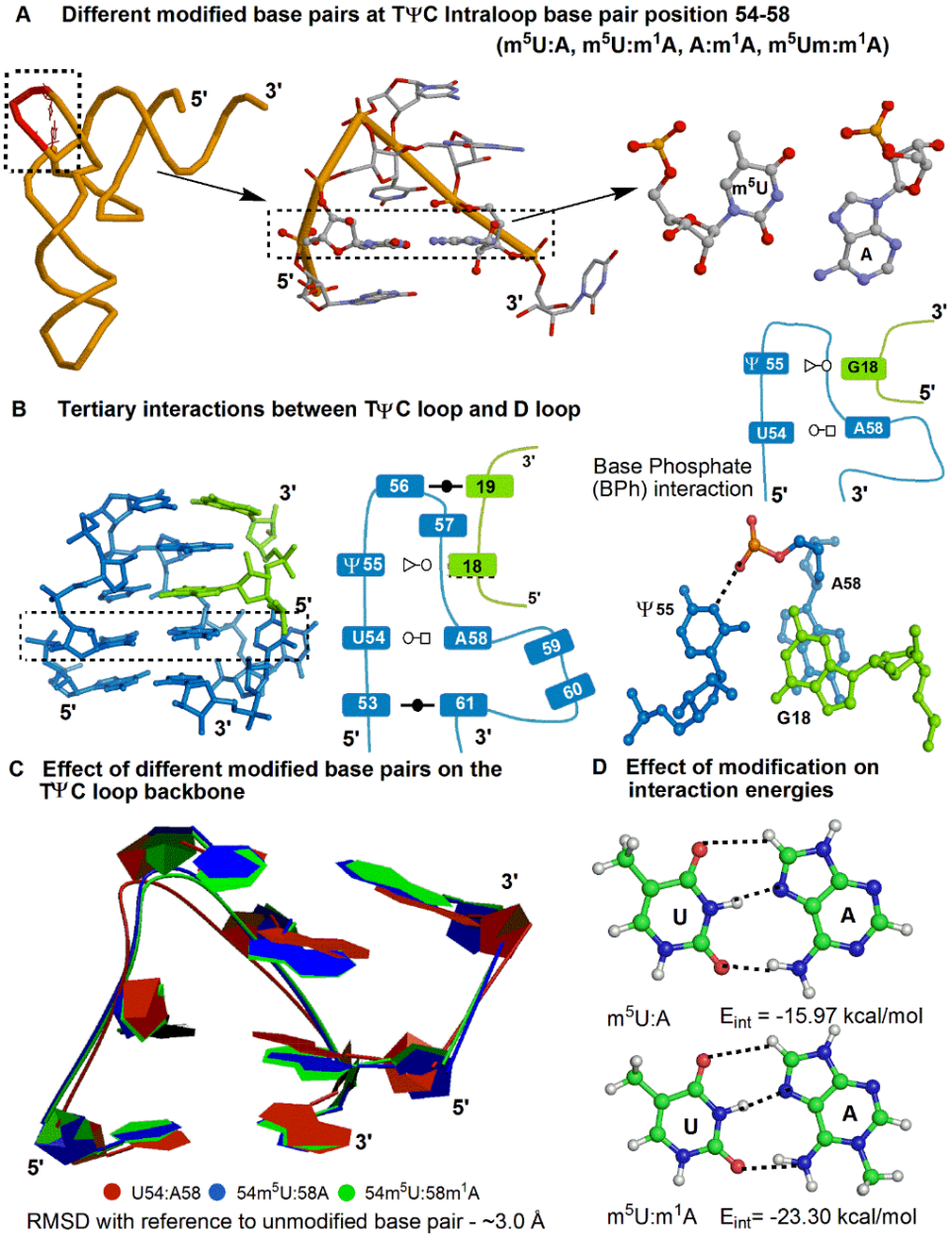

FIGURE S8. Structural context of the m^{5}U modified base pair in the T–loop of tRNA. (A) tRNA TΨC loop showing the 54:58 base pair. (B) Kissing loop interaction between the TΨC loop and the D–loop, stabilized by two base pairs, 18:55 and 19:56, along with a base phosphate (BPh) interaction between Ψ55:A58 (PDB 1EFW). (C) Backbone alignment of the TΨC loop in presence of U:A, m^{5}U:A and m^{5}U:m^{1}A at 54:58 position. (D) Geometry and interaction energies of m^{5}U:A and m^{5}U:m^{1}A base pairs.

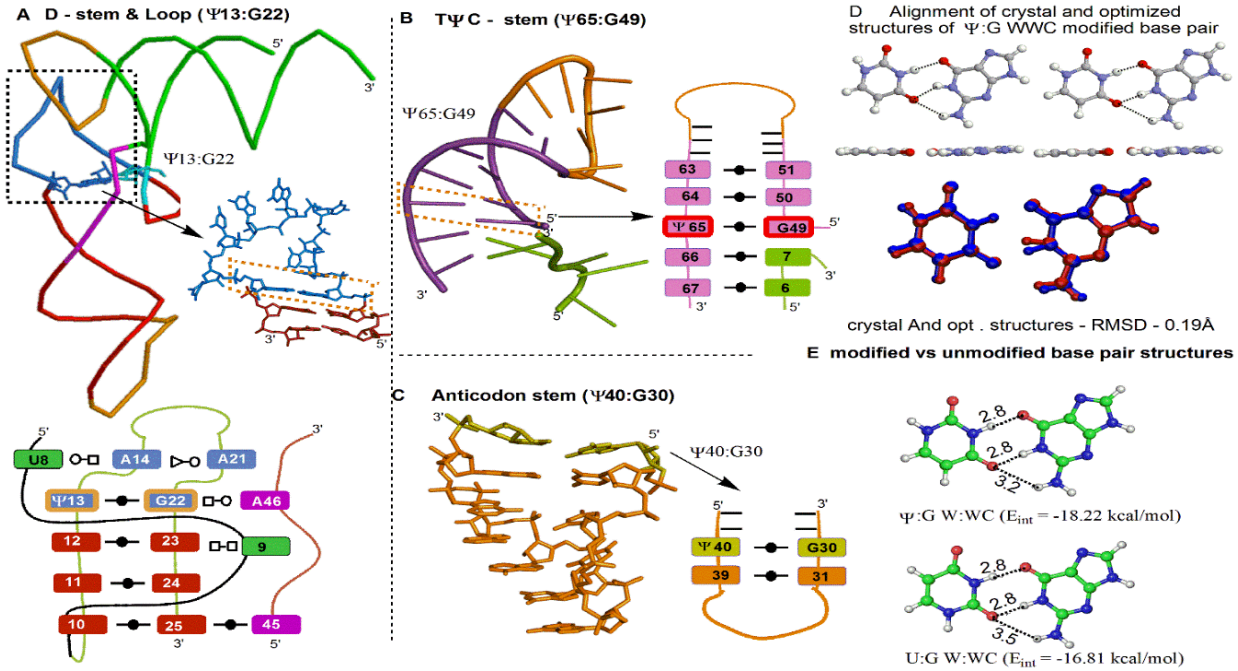

FIGURE S9. Structural context of the Ψ:G W:WC base pair as the closing base pair (A) at position 13:22 of D–stem of tRNA. (B) at position 65:49 of the TΨC stem of tRNA. (C) at position 40:30 of the anticodon stem in tRNA. (D) Comparison of crystal and optimized structures of the Ψ:G W:WC base pair. (E) Geometry and interaction energies of the Ψ:G W:WC base pair and its unmodified counterpart U:G W:WC base pair.

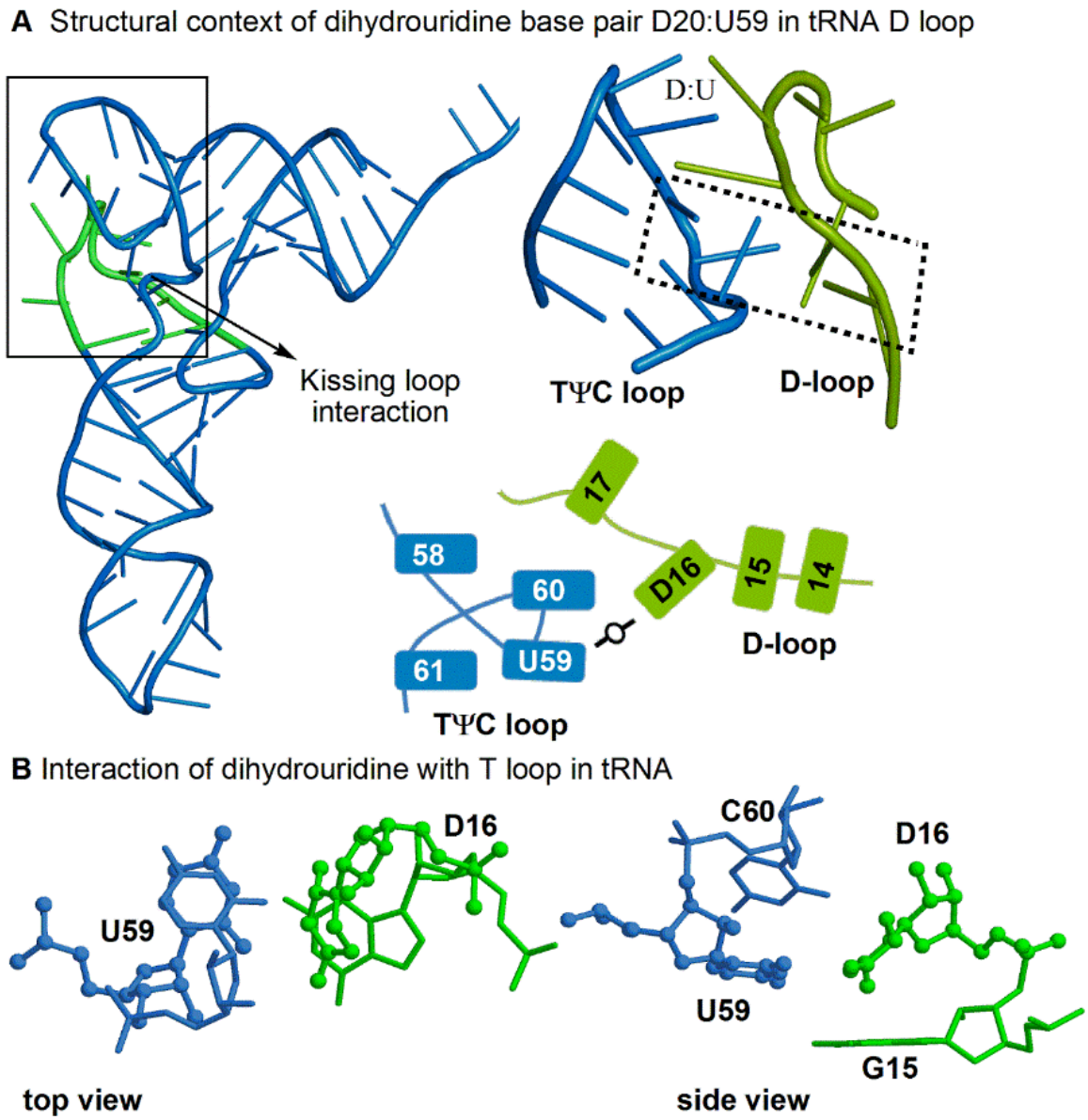

FIGURE S10. Structural context of tertiary interactions of dihydrouridine modified base at position 16:59. (A) D16:U59 W:WT base pair at the interface of D–loop and T–loop of tRNA. (B) Tertiary interactions of D16 and U59 in tRNA (PDB 1ASY).

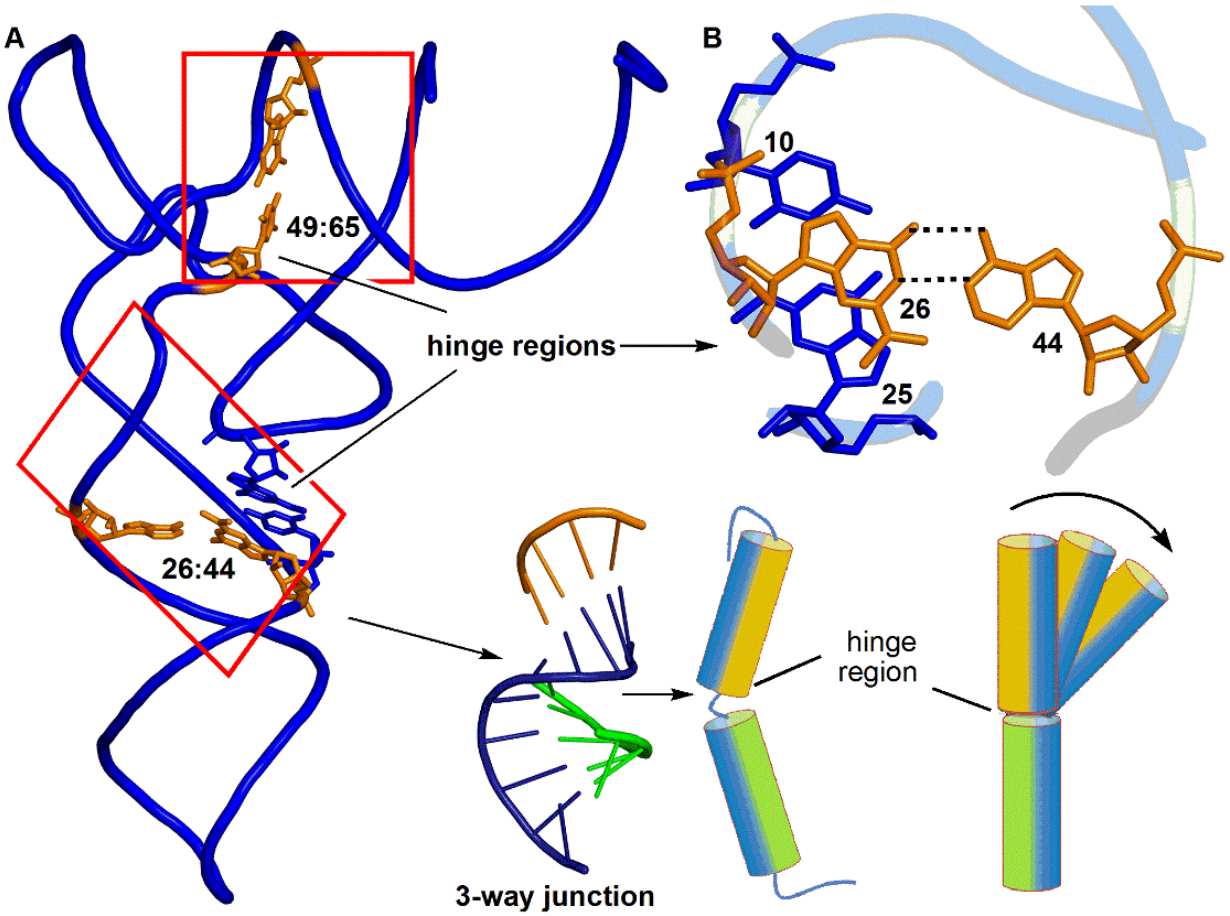

FIGURE S11. (A) Cartoon representation of tRNA showing the presence of modified base pairs (m^2G:C, $m^2{}_2G$:A amd m^5C:G) at 10:25, 26:44 and 49:65 positions respectively in the two hinge regions (in red boxes). (B) Representation of the flexibility of the hinge region. (C) The 10:25 and 26:44 pairs zoomed for clarity.

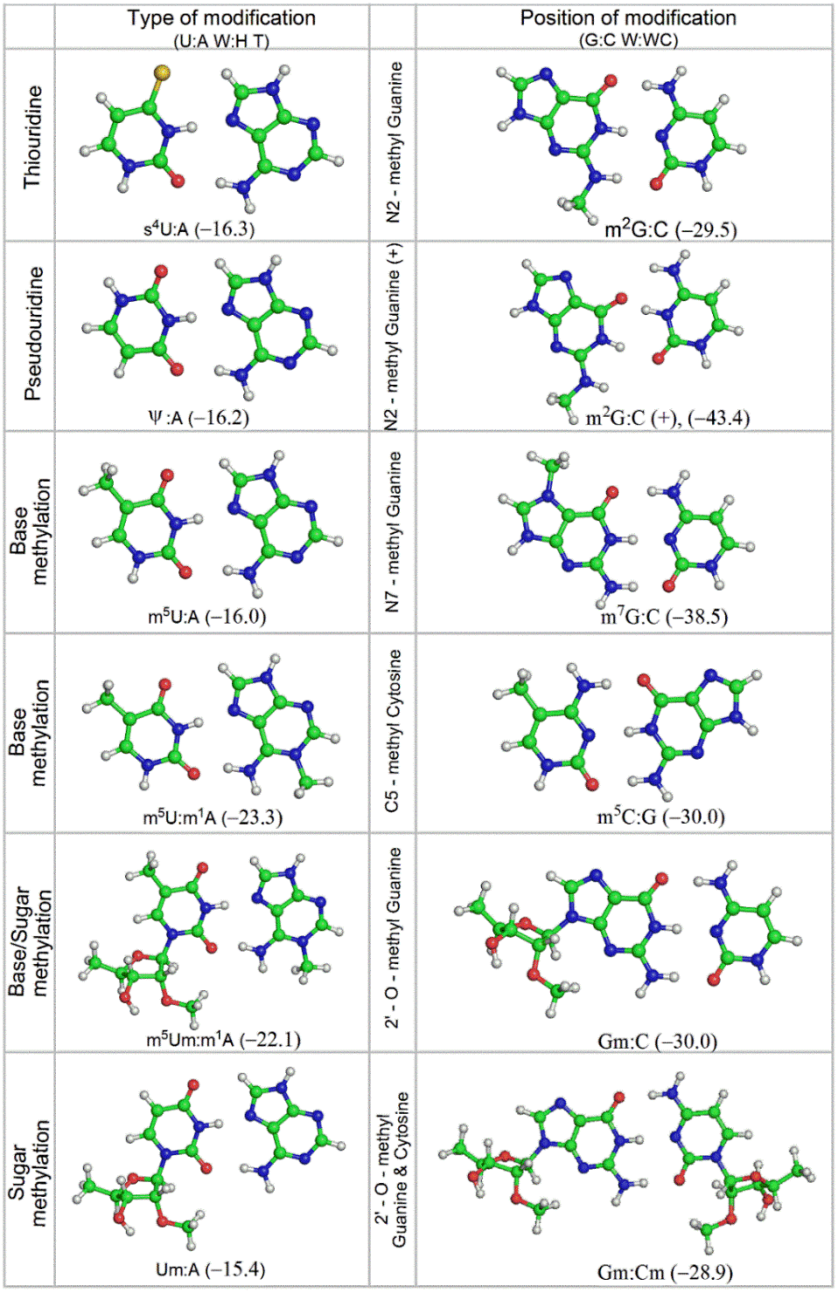

FIGURE S12. Schematic representation of some modified base pairs illustrating effect of type (e.g. U:A W:HT) and position (e.g. G:C W:WC) of modification on the stability of the base pair. Interaction energy is represented in parenthesis (in kcal/mol).

TABLE S1. List of various RNA types searched in the dataset containing 207 crystal structures

RNA Types	PDB Accession IDs
tRNA	1asy, 1asz, 1b23, 1c0a, 1efw, 1ehz, 1evv, 1f7u, 1f7v, 1fir, 1gsg, 1h3e, 1h4q, 1h4s, 1i9v, 1il2, 1n32, 1n33, 1ob2, 1ob5, 1qf6, 1ser, 1tn1, 1tn2, 1tra, 1ttt, 1vtq, 1yfg, 2czj, 2dlc, 2fmt, 2j00, 2j02, 2tra, 2wdg, 2wdh, 2xqd, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 3b0u, 3bt7, 3cw5, 3cw6, 3kfu, 3kiq, 3kis, 3t1h, 3t1y, 3tra, 4byb, 4byd, 4dr5, 4dr6, 4gcw, 4jv5, 4jya, 4jyz, 4k0l, 4qcm, 4qco, 4qcq, 4qcs, 4qcu, 4qcw, 4qcy, 4qd0, 4qjt, 4rb5, 4rb7, 4rb9, 4rbb, 4rbd, 4rbf, 4rbh, 4rbj, 4tna, 4tra, 5ccx, 6tna
16S rRNA	1n32, 1n33, 2j00, 2j02, 2wdg, 2wdh, 2xqd, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 3t1h, 3t1y, 4byb, 4byd, 4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4jv5, 4jya, 4k0l, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4qcm, 4qco, 4qcq, 4qcs, 4qcu, 4qcw, 4qcy, 4qd0, 4qjt, 4rb5, 4rb7, 4rb9, 4rbb, 4rbd, 4rbf, 4rbh, 4rbj, 4x62, 4x64, 4x65, 4x66, 5br8
23S rRNA	1jbs, 1s72, 1vq4, 1vq5, 1vq6, 1vq7, 1vq8, 1vq9, 1vqk, 1vql, 1vqm, 1vqn, 1vqo, 1vqp, 1yhq, 1yi2, 1yij, 1yit, 1yj9, 1yjn, 1yjw, 2otj, 2otl, 2qa4, 2qex, 3cc2, 3cc4, 3cc7, 3cce, 3ccj, 3ccl, 3ccm, 3ccq, 3ccr, 3ccs, 3ccu, 3ccv, 3cd6, 3cma, 3cme, 3dw4, 3dw5, 3g4s, 3g6e, 3g71, 3i55, 3i56, 3kiq, 3kis, 4hub, 4y27, 5d99
mRNA	1n32, 1n33, 2czj, 2j00, 2j02, 2wdg, 2wdh, 2xqd, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 3t1h, 3t1y, 4dr5, 4dr6, 4jv5, 4jya, 4k0l, 4qcm, 4qco, 4qcq, 4qcs, 4qcu, 4qcw, 4qcy, 4qd0, 4qjt, 4rb5, 4rb7, 4rb9, 4rbb, 4rbd, 4rbf, 4rbh, 4rbj
Small RNA–protein complexes	1eqq, 1h2t, 1D, 1n1h, 1xmm, 2v0j, 3b0u, 3d45, 3g1h, 3mj0, 3o6e, 3o7v, 3t3n, 3t3o, 4gim, 4jnp, 4o8r, 5in3
Ribozyme	1m5k, 2d2k, 2goz, 2oeu, 2oue, 3b5a, 3b5s, 3bbi, 3bbm, 3cr1, 3i2q, 3l3c, 3zd3, 3zd4, 3zd5, 3zp8, 5dun
RNA fragments	1i7j, 2dqq, 310d, 4pcj, 4rbz, 4rc0, 4wcp, 4wcq, 4wcr, 4wcs
snRNA	3cgp, 3cgq, 3cgr, 3cgs, 3l3c
Group I Introns	1t42, 1u6b, 1zzn
tmRNA	2czj
HIV genomic RNA	3dvv

TABLE S2. Distribution of crystal structures within the dataset as a function of the type of RNA.

Structure category	Structures with at least one modified base	Structures with at least one modified base pair	Structures with only unpaired modified bases
tRNA	50	49	1
16S rRNA	32	22	10
23S rRNA	48	46	2
RBP	32	8	24
Ribosomes	21	2	19
ribozyme	16	6	10
Others	8	2	6
Total	207	135	72

TABLE S3. List of PDBs with at least one modified base pair, distributed with respect to RNA types.

RNA Types	Occurrence	PDB Accession IDs
tRNA	49	1asy, 1asz, 1b23, 1c0a, 1efw, 1ehz, 1evv, 1f7u, 1f7v, 1fir, 1h3e, 1h4q, 1h4s, 1i9v, 1il2, 1n33, 1ob2, 1ob5, 1qf6, 1ser, 1tn1, 1tn2, 1tra, 1ttt, 1vtq, 1yfg, 2dlc, 2fmt, 2j00, 2j02, 2tra, 2wdh, 2xqd, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 3cw5, 3cw6, 3kfu, 3tra, 4jyz, 4tna, 4tra, 6tna
16S rRNA	22	4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8
23S rRNA	47	1jbs, 1s72, 1vq4, 1vq5, 1vq6, 1vq8, 1vq9, 1vqk, 1vql, 1vqm, 1vqn, 1vqo, 1vqp, 1yhq, 1yi2, 1yij, 1yit, 1yj9, 1yjn, 1yjw, 2otj, 2otl, 2qa4, 2qex, 3cc2, 3cc4, 3cc7, 3cce, 3ccj, 3ccl, 3ccm, 3ccq, 3ccr, 3ccs, 3ccu, 3ccv, 3cd6, 3cma, 3cme, 3dw4, 3dw5, 3g4s, 3g6e, 3g71, 3i55, 3i56, 5d99
Ribozyme	7	1m5k, 2oue, 3b5a, 3b5s, 3bbi, 3cr1, 3l3c
snRNA	3	3cgq, 3cgr, 3cgs
Group I Intron	3	1t42, 1u6b, 1zzn
tRNA–mRNA	2	4jv5, 4jya
HIV genomic RNA	1	3dvv
tmRNA	1	2czj

Table S4. List of unpaired modified bases in the crystal structure (207 RNA crystal structures)

Modified Bases	Occur. Freq	No. of PDBs	Base Position	Occur. Freq	No. of PDBs	PDB Accession Codes
m^1A	53	48	1MA:8	5	4	4wcp, 4wcq,4wcr,4wcs
			1MA:31	4	4	4wcp, 4wcq,4wcr,4wcs
			1MA:58	1	1	5ccx
			1MA:628	43	43	1s72, 1vq4, 1vq5, 1vq6, 1vq8, 1vq9, 1vqk, 1vql, 1vqm, 1vqn, 1vqo, 1vqp, 1yhq, 1yi2, 1yij, 1yit, 1yj9, 1yjn, 1yjw, 2otj, 2otl, 2qex, 3cc2, 3cc4, 3cc7, 3cce, 3ccj, 3ccl, 3ccm, 3ccq, 3ccr, 3ccs, 3ccu, 3ccv, 3cd6, 3cma, 3cme, 3g4s, 3g6e, 3g71, 3i55, 3i56, 4hub,
$m^6{}_2A$	0	–	–	–	–	–
Am	12	10	Am	12	10	2d2k, 3bbm, 3i2q, 3kiq, 3kis, 3l3c, 4byb, 4byd, 4rbz, 4rc0,
m^2G	1	1	2MG:10	1	1	1ttt
$m^2{}_2G$	25	25	M2G:966	25	25	1f7u, 1f7v, 1ttt, 4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8,
m^7G	14	11	7MG:1152, 651	3	2	1h2t, 3d45
			7MG:1153	2	1	1h2u
			7MG:46	6	6	2xqd, 2y0y, 2y12, 4qco, 4qcs, 4rbj,
			G7M:1424	1	1	1n1h
			G7M:503, 1502,	2	1	1xmm
Gm	23	18	OMG:34	15	11	1ehz, 1evv, 1ob2, 1ob5, 1tn1, 1tn2, 1tra, 1ttt, 4tna, 4tra, 6tna,
			OMG:21	2	2	3kiq, 3kis
			OMG:2661	1	1	4y27
			OMG:903, 904	2	1	3t3o
			OMG:918	1	1	4jyz
			OMG:905	1	1	3t3n
			OMG:518	1	1	2dlc
m^5C	61	39	5MC:32	16	16	4qcm, 4qco, 4qcq, 4qcs, 4qcu, 4qcw, 4qcy, 4qd0, 4rb5, 4rb7, 4rb9, 4rbb, 4rbd, 4rbf, 4rbh, 4rbj,
			5MC:501	1	1	4jnp
			5MC:967	22	22	4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8,
			5MC:1400	22	22	4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8,
Cm	35	29	OMC:6	6	6	2goz, 2oeu, 3zd3, 3zd4, 3zd5, 3zp8,
			OMC:32	26	21	1ehz, 1evv, 1ob2, 1ob5, 1tn1, 1tn2, 1tra, 1ttt, 2fmt, 2xqd, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 4tna, 4tra, 6tna,
			OMC:901	1	1	3t3o
			OMC:903, 904	2	1	3t3n
Ψ	231	182	PSU:55	93	59	1asy, 1asz, 1b23, 1c0a, 1efw, 1ehz, 1evv, 1f7u, 1f7v, 1fir, 1h3e, 1h4q, 1h4s, 1il2, 1ob2, 1ob5, 1qf6, 1ser, 1tn1, 1tn2, 1tra, 1ttt, 2czj, 2dlc, 2fmt, 2xqd, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 3cw5, 3cw6, 3kfu, 4jyz, 4qcm, 4qco, 4qcq, 4qcs, 4qcu, 4qcw, 4qcy, 4qd0, 4qjt, 4rb5, 4rb7, 4rb9, 4rbb, 4rbd, 4rbf, 4rbh, 4rbj, 4tna, 4tra, 6tna,
			PSU:2621	44	44	1s72, 1vq4, 1vq5, 1vq6, 1vq8, 1vq9, 1vqk, 1vql, 1vqm, 1vqn, 1vqo, 1vqp, 1yhq, 1yi2, 1yij, 1yit, 1yj9, 1yjn, 1yjw, 2otj, 2otl, 2qa4, 2qex, 3cc2, 3cc4, 3cc7, 3cce, 3ccj, 3ccl, 3ccm, 3ccq, 3ccr, 3ccs, 3ccu, 3ccv, 3cd6, 3cma, 3cme, 3g4s, 3g6e, 3g71, 3i55, 3i56, 4hub
			PSU:1541	21	21	4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8,
			PSU:1540	19	19	4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8,

			PSU:32, 1932	32	18	1asy, 1asz, 1b23, 1il2, 1vtq, 2tra, 3tra, 4qcm, 4qco, 4qcq, 4qcs, 4qjt, 4rb5, 4rb7, 4rb9, 4rbb, 4rbh, 4rbj,
			PSU:39	11	11	1ehz, 1ob2, 2dlc, 3t1h, 3t1y, 4qco, 4qcs, 4x62, 4x64, 4x65, 4x66,
			PSU:54	3	3	1vtq, 2tra, 3tra
			PSU:38	3	2	4dr6, 4jyz
			PSU:35	2	2	1h3e, 2dlc
			PSU:1	1	1	4jya
			PSU:400	1	1	4gim
			PSU:5	1	1	4pcj
s^4U	1	1	4SU:8	1	1	4qd0
m^5U	15	10	5MU:54	11	9	2czj, 2wdg, 2xqd, 2y0y, 2y12, 2y14, 2y16, 3bt7, 4rb9,
			5MU:801, 802, 803, 900	4	1	1eqq
D	101	44	H2U:16	28	27	1asy, 1c0a, 1efw, 1ehz, 1evv, 1f7u, 1f7v, 1fir, 1qf6, 1tn1, 1tn2, 1tra, 1vtq, 1yfg, 2tra, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 3tra, 4tna, 4tra, 6tna,
			H2U:17	25	21	1ehz, 1evv, 1ob2, 1ob5, 1qf6, 1tn1, 1tn2, 1tra, 1ttt, 2xqd, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 4tna, 4tra, 6tna,
			H2U:20	28	20	1b23, 1c0a, 1efw, 1fir, 1qf6, 2fmt, 2xqd, 2y0u, 2y0w, 2y0y, 2y10, 2y12, 2y14, 2y16, 2y18, 3b0u, 3cw5, 3cw6, 3kfu, 4jyz,
			H2U:19	11	8	1asy, 1asz, 1f7u, 1f7v, 1il2, 1vtq, 2tra, 3tra,
			H2U:47	4	4	1f7u, 1f7v, 1fir, 1yfg
			H2U:21	1	1	1b23
			H2U:229	1	1	3g1h
			H2U:301	1	1	4o8r
			H2U:402	1	1	5in3
			H2U:1453	1	1	2v0j
Um	59	56	OMU:2587	44	44	1s72, 1vq4, 1vq5, 1vq6, 1vq8, 1vq9, 1vqk, 1vql, 1vqm, 1vqn, 1vqo, 1vqp, 1yhq, 1yi2, 1yij, 1yit, 1yj9, 1yjn, 1yjw, 2otj, 2otl, 2qa4, 2qex, 3cc2, 3cc4, 3cc7, 3cce, 3ccj, 3ccl, 3ccm, 3ccq, 3ccr, 3ccs, 3ccu, 3ccv, 3cd6, 3cma, 3cme, 3g4s, 3g6e, 3g71, 3i55, 3i56, 4hub,
			OMU:19	4	4	3kiq, 3kis, 4byb, 4byd
			OMU:14	2	2	3o6e, 3o7v
			OMU:902	2	2	3t3n, 3t3o
			OMU:73, 74	2	1	4gcw
			OMU:901, 906	2	1	3t3n
			OMU:9	1	1	3mj0
			OMU:932	1	1	4jyz
			OMU:5	1	1	5dun

TABLE S5. List of modified base pairs involving W:WC and W:WT pairing geometry.

S.No	Base Pair	Position	PDB Accession IDS
W:WC			
		m^5C1404:G1497 (22)	4dr2, 4dr3, 4dr5, 4dr6, 4duy, ,4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8
		m^5C1407:G1494 (22)	4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8
		m^5C40:G30 (15)	1ehz, 1evv, 1ob2, 1ob5, 1tn1, 1tn2, 1tra, 1ttt, 4tna, 4tra, 6tna
		m^5C48:G64 (3)	1ttt, 1vtq, 2tra
		m^5C49:G65 (25)	1asy, 1asz, 1ehz, 1evv, 1fir, 1f7u, 1f7v, 1il2, 1ob2, 1ob5, 1tn1, 1tn2, 1tra, 1ttt, 1yfg, 4tna, 4tra, 6tna
		m^2G10:C25 (15)	1ehz , 1evv , 1fir , 1f7u, 1f7v, 1ob2 , 1tn1 , 1tn2 , 1tra , 1ttt , 1yfg , 4tna , 4tra , 6tna
		m^2G1207:C1051 (22)	4dr2 , 4dr3 , 4dr5 , 4dr6 , 4duy , 4dv6 , 4dv7 , 4ji0 , 4ji1 , 4ji3 , 4ji7 , 4lf4 , 4lf6 , 4lf7 , 4lf8 , 4lf9 , 4lfb , 4x62 , 4x64 , 4x65 , 4x66 , 5br8
3	m^2G:U	m^2G6:U67	1fir
4	$m^2{}_2$G:A (16)	$m^2{}_2$G Ψ26:A44 (16)	1ehz , 1evv , 1ob2 , 1ob5 , 1tn1 , 1tn2 , 1tra , 1ttt , 1yfg , 2dlc , 4tna , 4tra , 6tna
5	m^7G:A (22)	m^7G527:C522	4dr2 , 4dr3 , 4dr5 , 4dr6 , 4duy , 4dv6 , 4dv7 , 4ji0 , 4ji1 , 4ji3 , 4ji7 , 4lf4 , 4lf6 , 4lf7 , 4lf8 , 4lf9 , 4lfb , 4x62 , 4x64 , 4x65 , 4x66, 5br8
6	m^5U:G (3)	m^5U1:G10	1t42 , 1u6b , 1zzn,
		Ψ39:A31 (14)	1b23 , 1evv , 1fir , 1ob5 , 1tn1 , 1tn2 , 1tra , 1ttt , 4tna , 4tra , 6tna
		Ψ6:A21 (2)	3cgp, 3cgq
		Ψ7:A19 (1)	3cgr
		Ψ4:A36 (2)	4jv5, 4jya

		Ψ27:A43 (3)	1fir, 1f7u, 1f7v
		Ψ1:A72 (2)	1f7u, 1f7v
		Ψ13:G22 (5)	1asy, 1asz, 1vtq , 2tra , 3tra
		Ψ65:G49 (3)	1c0a, 1efw
		Ψ6:G20 (1)	3cgs
		Ψ30–G40(1)	1n33
9	Gm:C (12)	Gm2588:C75 (12)	1vq4 , 1vq5 , 1vq6 , 1vqk , 1vql , 1vqm , 1vqn , 1vqo , 1vqp , 3cd6 , 3cma , 3cme , 3i55
10	Gm:A (1)	Gm2588:A76	3cme
11	Am:Um (2)	Am26:Um4	1jbs
		Um2650:A2670	3dw4, 5d99
		Um3:A21	3dvv
W:WT			
1	D:U (12)	D16:U59	1asy , 1asz , 1il2 , 1ob2 , 1ob5 , 1ttt
2	m^5C:G(3)	m^5C48:G15	1fir, 1yfg, 2dlc

TABLE S6. List of modified base pairs involving W:HT and W:HC pairing geometry.

S.No	Base Pair	Position	PDB Accession IDS
W:HT			
1	m^7G:G (27)	m^7G46:G22	1c0a, 1efw, 1ehz, 1evv, 1fir, 1ob2, 1ob5, 1qf6, 1tn1, 1tn2, 1tra, 1ttt, 1yfg, 2y0u, 2y0w, 2y10, 2y14, 2y16, 2y18, 4tna, 4tra, 6tna
2	m^5U:A (34)	m^5U54:A58	1asy, 1asz, 1b23, 1c0a, 1efw, 1i9v, 1il2, 1qf6, 1ser, 1vtq, 2czj, 2fmt, 2j00, 2j02, 2tra, 2wdh, 2y0u, 2y0w, 2y10, 2y18, 3cw5, 3cw6, 3kfu, 3tra, 4jyz
3	m^5U:G (2)	m^5U54:G58	1h4q, 1h4s
3	m^1A:m^5U (18)	m^1A58:m^5U54	1ehz, 1evv, 1f7u, 1f7v, 1h3e, 1ob5, 1tn1, 1tn2, 1tra, 1ttt, 2dlc, 4tna, 4tra, 6tna
	m^1A:U (1)	m^1A58:U54	1ob2
	m^1A:A (1)	m^1A58:A54	1yfg
4	s^4U:A (12)	s^4U8:A14	1efw, 2y10, 2y18, 1b23, 1c0a, 2fmt, 2y0u, 2y0w, 2y0y, 2y12, 2y14, 2y16, 3cw5, 3cw6
5	m^5Um:m^1A (1)	m^5Um54:m^1A58	1fir
6	Um:A (1)	Um2656:A2665	3dw5
7	Ψ:A (22)	Ψ516:A533	4dr2, 4dr3, 4dr5, 4dr6, 4duy,4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8
W:HC			
1	Ψ:A	Ψ39:A31	1ttt

TABLE S7. List of modified base pairs involving S:WC, S:WT, W:ST, H:SC, S:HT, S:SC and S:ST pairing geometry.

S.No	Base Pair	Position	PDB Accession IDs
S:WC			
1	Ψ:C (22)	Ψ516:C519	4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8
S:WT			
1	m^7G:A (10)	m^7G527:A535	4dr2, 4dr5, 4dv6, 4dv7, 4ji1, 4ji3, 4ji7, 4lf6, 4lf7, 4lf8
2	s^4U:A (7)	s4U8:A	1c0a, 2y0y, 2y12, 2y14, 2y16, 3cw5, 3cw6
W:ST			
1	D:G (1)	D20:G15	1ser
2	$m^2{}_6$A:G (1)	$m^6{}_2$A76:G2618	1vq6
3	Am:G (1)	Am0:G57	3l3c
H:SC			
1	Um:G (1)	Um2656:G2655	3dw5
2	D:G (1)	D620:G619	1c0a
S:HT			
1	Am:A (7)	Am5:A9	1m5k , 2oue , 3b5a , 3b5s , 3bbi , 3cr1
S:SC			
1	Gm:G (30)	Gm2588:G2617	1s72 , 1vq8 , 1vq9 , 1yhq , 1yi2 , 1yij , 1yit , 1yj9 , 1yjn , 1yjw , 2otj , 2otl , 2qa4 , 2qex , 3cc2 , 3cc4 , 3cc7 , 3cce , 3ccj , 3ccl , 3ccm, 3ccq , 3ccr , 3ccs , 3ccu , 3ccv , 3g4s , 3g6e , 3g71 , 3i56
2	s^4U:A (1)	s^4U8:A46	1b23
S:ST			
1	$m^2{}_6$A:G (3)	$m^6{}_2$A76:G2618	1vq4, 1vq5, 3i55
2	Am:G (2)	Am0:G57	3l3c
3	m^7G:A (1)	m7G527:A535	4ji0
4	D:U (1)	D16:U60	2xqd

TABLE S8. Categorization of modified base pairs with respect to their occurrence in various RNA types.

RNA Type	Base pair position	Modified Base Pair	Occurrence	Context
tRNA	54–58	A:m^{1}A H:WT	01	tRNA 58:54; Intra loop interaction in TΨC loop of tRNA
		m^{5}U:m^{1}A W:HT	18	
		m^{5}Um:m^{1}A W:HT	01	
		m^{5}U:A W:HT	34	
		m^{5}U:A W:HT	34	
		m^{5}U:G W:HT	02	
	10:25	m^{2}G:U W:WC	03	closing base pair(CB) of D–stem
	6:67	m^{2}G:U W:WC	01	Next to CB of amino acceptor arm
	26:44	m$^2_2$G:A W:WC	16	closing base pair of anticodon stem–loop
	22:46	m^{7}G:G W:HT	27	D–loop–Variable loop interaction
	48:15	m^{5}C:G W:WT	04	D–loop–Variable loop interaction
	8:14	s^{4}U:A W:HT	15	junction loop–D–loop interaction
	8:21	s^{4}U:A W:ST	07	junction loop–D–loop interaction
	8:46	s^{4}U:A S:SC	01	junction loop–Variable arm interaction
	31:39	Ψ:A W:WC	15	tRNA 31:39; Anti codon stem
	1:72		2	CB of amino acceptor arm
	13:22	Ψ:G W:WC	3	tRNA 13:22; CB of D–loop
	65:49		3	tRNA 65:49; CB of TΨC loop
	40:30		1	tRNA 40:30; Anti codon stem
	20:15	D:G W:ST	01	Intraloop [D–loop] interaction; base platform
	20:19	D:G H:SC	01	tRNA 20:19; Intraloop [D–loop] interaction; base platform
	16:59	D:U W:WT	12	tRNA 16:59; Interloop interaction [D and TΨC loop]
	16:60	D:U S:ST	01	tRNA 16:60; Interloop interaction [D and TΨC loop]
16S rRNA	527:522	m^{7}G:C W:WC	22	next to CB; H18 (5′ Domain)
	527:535	m^{7}G:A S:ST	01	H18 (5′ Domain), A minor interaction
	527:535	m^{7}G:A S:WT	10	H18 (5′ Domain), A minor interaction
	516:533	Ψ:A W:HT	22	CB of H18 (5′ Domain)
	516:519	Ψ:C S:WC	22	H18 (5′ Domain)
23S rRNA	2588:2617	Gm:G S:SC	30	H92 (V Domain)
	2650:2670	Um:A W:WC	04	23S rRNA 2650:2670; H95 (VI Domain)

	2656:2665	Um:A W:HT	01	23S rRNA 2656:2665; sarcin–ricin domain
	2655:2656	Um:G H:SC	01	23S rRNA; Sarcin–ricin domain
		Cm:Gm W:WC		sarcin/ricin domain RNA
Both tRNA and 16S rRNA	10:25 (tRNA)	m^2G:C W:WC		closing base pair(CB) of D–stem
	1207:1051 (16S rRNA)			Next to CB of H34 (3′M domain).
	49:65 (tRNA)	m^5C:G W:WC		
	40:30 (tRNA)			
	1407:1494 (16S rRNA)			in helix 44 of 3m domain of 16S rRNA
	1404:1497 (16S rRNA)			in helix 44 of 3m domain of 16S rRNA
tRNA–rRNA complex	tRNA (76):rRNA (G2618)	$m^6{}_2A$:G S:ST	01	23S rRNA (2618):tRNA analogue (76); H91 (V Domain);
	tRNA (76):rRNA (G2588)	Gm:A W:WC	01	23S rRNA H92 (V Domain):tRNA acceptor arm
	tRNA (76):rRNA (2588)	Gm:C W:WC	12	23S rRNA–H92 (V Domain)
Ribozyme	0:57	Am:G S:ST	02	*Homo sapiens* glmS ribozyme
snRNA	1:10	m^5U:G W:WC	03	Exon bound to U1 SNRNP

TABLE S9. Structural context of modified base pairs in RNA crystal structures.

Secondary structure element	Modified base pair	Category	Occurrence frequency	Type of RNA	Context
Helix					
(i) within helix	m^2G1207:C1051 W:WC	Base methylation	22	Tth 16S rRNA	helix 34 of 3′M domain of 16S rRNA
	m^2G6:U67 W:WC	Base methylation	1	HIV primer tRNA	Amino acid acceptor arm of tRNA
	Ψ27:A43 W:WC	Pseudouridylation	3	Eco tRNA	Anticodon stem of tRNA
	m^5C:40:G30 W:WC	Base methylation	15	Sce tRNA	Anticodon stem of tRNA
	Um2656:A2670 W:WC	Sugar methylation	2	Eco 23S rRNA	Helix 95 of domain VI of 23S rRNA
	m5U1:G10 W:WC	Base methylation	4	Hsp Group I Intron	P1 helix of Group I Intron
	Um3:A21 W:WC	Sugar methylation	2	HIV–1	HIV–1 Genomic RNA
	Ψ6:A21 W:WC	Pseudouridylation	2	snRNA	snRNA
	Ψ27:A43 W:WC	Pseudouridylation	2	HIV primer	HIV transcription primer tRNA
	Um4:Am26 W:WC	Sugar methylation	2	Aspergillus 23S rRNA	Sarcin–ricin domain RNA analog
	Am5:A9 S:HT	Sugar methylation	7	Ribozyme	Ribozyme
	Ψ6:G20 W:WC	Pseudouridylation	1	snRNA	snRNA
	Um2656:G2655 H:SC	Sugar methylation	1	E.coli 23S rRNA	Sarcin–ricin domain RNA analog
	Gm24:Cm6 W:WC	Sugar methylation	1	Aspergillus 23S rRNA	Sarcin–ricin domain RNA analog
	Um2656:A2665 W:HT	Sugar methylation	1	E.coli 23S rRNA	Sarcin–ricin domain RNA analog
	Ψ7:A19 W:WC	Pseudouridylation	1	snRNA	snRNA
(ii) closing base pair	Ψ13:G22 W:WC	Pseudouridylation	5	Sce tRNA	D–stem of tRNA
	m^2G10:C25 W:WC	Base methylation	19		D–Stem of tRNA
	Ψ65:G49 W:WC	Pseudouridylation	3	Eco tRNA	TΨC stem of tRNA
	Ψ1:A72 W:WC	Pseudouridylation	2	Eco tRNA	Amino acid acceptor arm of tRNA
	Ψ39:A31 W:WC	Pseudouridylation	15	Sce tRNA	Anticodon stem of tRNA
	G65:m^5C49 W:WC	Base methylation	25	Sce tRNA	TΨC stem of tRNA
	$m^2{}_2$G26:A44 W:WC	Base methylation	16	Sce tRNA	Anticodon stem of tRNA
	m^7G527:G522 W:WC	Base methylation	22	Tth 16S rRNA	helix 18 of 5M domain of 16S rRNA
(iii) Internal Bulges	m^5C1407:G1494 W:WC	Base methylation	22	Tth 16S rRNA	helix 44 of 3m domain of 16S rRNA
	m^5C1404:G1497 W:WC	Base methylation	22	Tth 16S rRNA	helix 44 of 3m domain of 16S rRNA
	Um2650:A2670 W:WC	Sugar methylation	2	Eco 23S rRNA	Bulge in Helix 95 of domain VI of 23S rRNA
Intra loop Interactions	m^5U54:m^1A58 W:HT	Base methylation	18	Sce tRNA	TΨC loop of tRNA
	m^5U54:A58 W:HT	Base methylation	34	Eco tRNA, Tth tRNA	TΨC loop of tRNA

	m^5U54:G58 W:HT	Base methylation	2	Tth tRNA	TΨC loop of tRNA
	m^1A58:A54 H:WT	Base methylation	1	Sce tRNA	TΨC loop of tRNA
	m^1A58:U54 H:WT	Base methylation	1	Sce tRNA	TΨC loop of tRNA
	D20:G19 H:SC	Reduction	2	Eco tRNA	D–loop tRNA
	D20:G15 W:ST	Reduction	1	Eco tRNA	D–loop tRNA
	Cm32:A38 W:W(+)C	Sugar methylation	2	Eco tRNA	Anti–codon loop of tRNA
Tertiary interactions					
Helix–single strand	$m^6{}_2A76$:G2618 S:ST	Base methylation	4	Hma 23S rRNA	between H89–H91 domain V and anti–codon stem loop of tRNA
Loop–single strand	Gm2588:C75 W:WC	Sugar methylation	12	Hma 23S rRNA	between H89–H91 domain V and anti–codon stem loop of tRNA
Loop–single strand	Gm2588:A76 W:WC	Sugar methylation	1	Hma 23S rRNA	between H89–H91 domain V and anti–codon stem loop of tRNA
Loop–single strand	Am0:G57 S:ST	Sugar methylation	2	Bacillus Ribozyme	glms ribozyme
Loop–single strand	Ψ4:A36 W:WC	Pseudouridylation	2	Tth tRNA–mRNA	Anti–codon loop of tRNA and mRNA complex
Stem–junction	m^7G527:A535 S:WT	Base methylation	10	Tth 16S rRNA	helix 18 of 5M domain of 16S rRNA
Stem–junction	m^7G527:A535 S:ST	Base methylation	1	Tth 16S rRNA	helix 18 of 5M domain of 16S rRNA
Loop–stem	Gm2588:G2617 S:SC	Sugar methylation	30	Hma 23S rRNA	Stem between H89–H91 & H92 of domain V
Loop–stem	Ψ516:A533W:HT	Pseudouridylation	22	Tth 16S rRNA	helix 18 of 5M domain and 530 loop of 16S rRNA
Loop–stem	Ψ516:C519 S:WC	Pseudouridylation	22	Tth 16S rRNA	helix 18 of 5M domain
Junction–loop	m^7G46:G22 W:HT	Base methylation	23	Sce tRNA	Anti–codon stem loop of tRNA
Junction–loop	s^4U8:A14 W:HT	Thiolation	15	tRNA	Acceptor–D stem junction and D–loop
Junction–loop	s^4U8:A21 S:WT	Thiolation	7	tRNA	Acceptor–D stem junction and D–loop
Junction–loop	s^4U8:A46 S:SC	Thiolation	1	tRNA	Acceptor–D stem junction and D–loop
loop–loop	m^5C48:G15 W:WT	Base methylation	03	tRNA	D–loop and variable arm
loop–loop	D16:U59 W:WT	Reduction	12	Sce tRNA	D–loop and TΨC loop interaction
loop–loop	D16:U60 S:ST	Reduction	1	Tth tRNA	D–loop and TΨC loop interaction

TABLE S10. Modified base pairs ranked according to their occurrence in all the tRNA sequences.

Base pair	Rank[a]	Frequency	Occurrence in aminoacyl tRNAs	Other combinations observed
54m^{5}U:58m^{1}A H:WT	02	124	L, F, S, R, Y, V, M, I, K	m^{5}U:A(126); U:A(60); m$^1\Psi$:A(49); U:m^{1}A(30); A:m^{1}A(22); Ψ:m^{1}A(15); m^{5}Um:m^{1}A(10)
10m^{2}G:25C W:WC	02	128	L, K, F, Y, R, M, N, W, V, G, I, M^{Ini}	G:C(246); G:U(37); C:G(19); m^{2}G:U(13); m$^2{}_2$G:U(12);
26m$^2{}_2$G:44A W:WC	01	90	F, Y, I, A, R, M, M^{Ini}, N, T, W	A:G(62); G:A(49); A:A(38); m$^2{}_2$G:Um(33); A:C(25); G:U(22); A:U(20); m^{2}G:A(19); m$^2{}_2$G:U(19); U:A(18); U:C(17); G:C(15)
22G:46m^{7}G H:WT	01	168	F, K, M^{Ini}, M, I, Y, A, W, P, R, N, T, V	G:G(61); A:G(50);G:A(47); U:A(27); A:A(25); A:U(19); U:m^{7}G(18); U:G(10)
49m^{5}C:65G W:WC	02	88	G, E, F, Q, V, A, R, P, D, H	G:C(177); A:U(67); C:G(57); G:U(54); U:A(10)
8s^{4}U:14A W:HT	02	53	S, M^{Ini}, P, Y	U:A(382); U:m^{1}A(9), U:G(4)
54m^{5}U:58A W:HT	01	126	L, S, G, F, I, R, E, P, Y, M, D, W, T, V, A, Q, K, H	m^{1}A:m^{5}U(124); U:A(60); m$^1\Psi$:A(49); U:m^{1}A(30); A:m^{1}A(22); Ψ:m^{1}A(15); m^{5}Um:m^{1}A(10)
31A:39Ψ W:WC	01	148	F, Y, L, C, K, Q	C:G(130); G:C(122); U:A(22); A:U(14)
49G:65Ψ W:WC	07	09	W, I, D, R, P	G:C(177); m^{5}C:G(88); A:U(67); C:G(57); G:U(54); U:A(10)
13Ψ:22G W:WC	04	36	Q, E, D, H, G, F, N	C:G(229); G:A(64); Ψ:U(45); A:A(15); U:G(14); A:m^{1}A(12); U:A(10)
30G:40Ψ W:WC	03	13	R, A, Q, M^{Ini}, P, S	G:C(359); C:G(83); U:G(5)
16D:59U W:WT	08	38	Y, S, R, Y, V, I, Q, G, H	D:C(72); D:G(68); U:C(60); U:U(41); U:G(40); C:C(40); C:G(39);

[a] represents the populated modified base pair combinations at a given base pair combination in the hierarchy.

TABLE S11. List of 36 unique modified base pairs identified in the dataset and used for quantum chemical calculations.

Modified base pairs	Occur. Freq.	RNA type	Position	Organism	PDB id
m^5C:G W:WC	87	tRNA	49:65	*S. cerevisiae*	1EHZ
m^2G:C W:WC	38	tRNA	10:25	*S. cerevisiae*	1EVV
Ψ:A W:WC	24	tRNA	39:31	*E. coli*	1B23
m^7G:C W:WC	22	16S rRNA	527:522	*T. thermophilus*	4DR1
$m^2{}_2G$:A W:WC	16	tRNA	26:44	*S. cerevisiae*	1YFG
Gm:C W:WC	12	23S rRNA	2588:75	*H. marismortui*	1VQ0
Ψ:G W:WC	10	tRNA	13:22	*S. cerevisiae*	1ASY
Um:A W:WC	4	23S rRNA	2650:2670	*E. coli*	3DW4
m^5U:G W:WC	3	snRNA	1:10	*Azoarcus*	1U6B
m^2G: C W:W(+)C	3	tRNA	10:25	*S. cerevisiae*	1Q5S
m^2G:U W:WC	1	tRNA primer	6:67	Bos taurus	1FIR
Gm:A W:WC	1	23S rRNA	2588:76	*H. marismortui*	3CME
Cm:Gm W:WC	1	23S rRNA	29:1	Aspergillus sp.	1JBS
m^5U:A W:HT	34	tRNA	54:58	*T. thermophilus*	1SER
m^7G:G W:HT	27	tRNA	46:22	*S. cerevisiae*	1YFG
Ψ:A W:HT	22	tRNA	533:516	*T. thermophilus*	4DR1
m^1A: m^5U H:WT	18	tRNA	54:58	*S. cerevisiae*	1EHZ
s^4U:A W:HT	15	tRNA	8:14	*E. coli*	1B23
m^5U:G W:HT	2	tRNA	54:58	*T. thermophilus*	1H4Q
m^1A:A H:WT	1	tRNA	54:58	*S. cerevisiae*	1YFG
m^1A:m^5Um H:WT	1	tRNA primer	58:54	*Bos taurus*	1FIR
Um:A W:HT	1	23S rRNA	2656:2665	*E. coli*	3DW5
D:U W:WT	12	tRNA	16:59	*S. cerevisiae*	1IL2
m^5C:G W:WT	3	tRNA primer	48:15	*Bos taurus*	1FIR
Ψ:C S:WC	22	16S rRNA	519:516	*T. thermophilus*	4DR1
m^7G:A S:WT	10	16S rRNA	527:535	*T. thermophilus*	4DR1
s^4U:A W:ST	7	tRNA	8:21	*E. coli*	1C0A
D:G W:ST	1	tRNA	20:15	*T. thermophilus*	1SER
D:G H:SC	1	tRNA	20:19	*E. coli*	1C0A
Um:G H:SC	1	23S rRNA	2656:2655	*E. coli*	3DW6
Gm:G S:SC	30	23S rRNA	2588:2617	*H. marismortui*	3CME
s^4U:A S:SC	1	tRNA	8:46	*E. coli*	1B23
$m^6{}_2A$:G S:ST	3	23S rRNA	2618:76	*H. marismortui*	3I55
Am:G S:ST	2	ribozyme	0:57	*H. sapien*	3L3C
m^7G:A S:ST	1	16S rRNA	527:522	*T. thermophilus*	2OW8
D:U S:ST	1	tRNA	16:60	*E. coli*	2XQD

TABLE S12. Average RMSD of all crystal occurrences, average RMSD of crystal structures with reference to optimized geometry and deviation between optimized geometries of modified and unmodified base pairs.

Modified base pairs	Occurrence Frequency	Average RMSD (Å)				
		All crystal structure	Standard deviation	w.r.t Fopt geometry	Standard deviation	Modified vs Unmodified
m^5C:G W:WC	87	0.0	0.0	0.0	0.0	0.0
m^2G:C W:WC	38	0.1	0.0	0.1	0.0	0.0
Ψ:A W:WC	24	0.0	0.0	0.0	0.0	0.0
m^7G:C W:WC	22	0.0	0.0	0.1	0.0	0.1
$m^2{}_2G$:A W:WC	16	0.4	0.2	0.7	0.3	0.3
Gm:C W:WC	12	0.1	0.0	0.3	0.0	0.2
Ψ:G W:WC	10	0.0	0.0	0.0	0.0	1.5
Um:A W:WC	4	0.2	0.1	1.2	0.1	0.5
m^2G: C W:W(+)C	3	0.0	0.0	0.0	0.0	0.1
m^5U:G W:WC	3	0.0	0.0	0.0	0.0	1.4
m^2G:U W:WC	1	–	–	0.2	–	0.1
Gm:A W:WC	1	–	–	0.3	–	0.0
Cm:Gm W:WC	1	–	–	0.2	–	0.0
m^5U:A W:HT	34	0.0	0.1	0.1	0.1	0.0
m^7G:G W:HT	27	0.0	0.0	0.1	0.0	0.2
Ψ:A W:HT	22	0.0	0.0	0.1	0.0	0.1
m^1A: m^5U H:WT	18	0.0	0.0	0.0	0.0	0.1
s^4U:A W:HT	15	0.1	0.0	0.1	0.0	0.1
m^5U:G W:HT	2	0.0	0.0	0.0	0.0	0.0
m^1A:A H:WT	1	–	–	0.0	–	0.3
m^1A: m^5Um H:WT	1	–	–	0.3	–	0.1
Um:A W:HT	1	–	–	0.4	–	0.0
D:U W:WT	12	0.2	0.1	0.3	0.1	0.2
m^5C:G W:WT	3	0.0	0.0	0.1	0.0	0.5
Ψ:C S:WC	22	0.1	0.0	0.1	0.0	0.4
m^7G:A S:WT	10	0.1	0.0	0.2	0.0	0.0
s^4U:A W:ST	7	0.2	0.1	0.3	0.1	0.1
D:G W:ST	1	–	–	0.2	–	0.4
D:G H:SC	1	–	–	0.3	–	0.2
Um:G H:SC	1	–	–	0.3	–	0.0
Gm:G S:SC	30	0.1	0.1	0.2	0.0	2.1
s^4U:A S:SC	1	–	–	0.5	–	0.6
$m^6{}_2A$:G S:ST	3	0.0	0.0	0.4	0.0	1.7
Am:G S:ST	2	0.0	0.0	0.3	0.0	0.3
m^7G:A S:ST	1	–	–	0.2	–	0.5
D:U S:ST	1	–	–	0.7	–	–

TABLE S13. Average (E_{av}) and standard deviation (SD) of E–values of all Crystal occurrences, E_{av} and SD of crystal occurrences with respect to optimized geometry, E_{av} between optimized geometries of modified and unmodified base pairs.

Modified Base pair	Occu. Freq.	Crystal structures		optimized geometry	SD w.r.t opt	Unmod. opt. geometry	Mod – Unmod (E_{diff})
		Avg.	Stdev				
m^5C:G W:WC	87	0.3	0.2	0.2	0.3	0.2	0.0
m^2G:C W:WC	38	0.3	0.2	0.3	0.2	0.2	0.1
Ψ:A W:WC	24	0.5	0.3	0.2	0.4	0.2	0.0
m^7G:C W:WC	22	0.3	0.1	0.2	0.2	0.2	0.0
$m^2{}_2$G:A W:WC	16	0.5	0.2	0.1	0.5	0.1	0.0
Gm:C W:WC	12	0.3	0.2	0.2	0.2	0.2	0.0
Ψ:G W:WC	10	0.3	0.3	0.6	0.4	0.2	0.4
Um:A W:WC	4	0.2	0.1	0.2	0.1	0.2	0.0
m^2G: C W:W(+)C	3	1.0	0.0	0.4	0.6	0.2	0.2
m^5U:G W:WC	3	0.3	0.1	0.1	0.3	0.2	–0.1
m^2G:U W:WC	1	0.1	0.0	0.1	0.0	0.2	–0.1
Gm:A W:WC	1	0.4	0.0	0.1	0.1	0.1	0.0
Cm:Gm W:WC	1	0.2	0.0	0.2	0.0	0.2	0.0
m^5U:A W:HT	34	0.4	0.2	0.2	0.3	0.3	0.0
m^7G:G W:HT	27	0.6	0.4	0.3	0.6	0.5	–0.3
Ψ:A W:HT	22	0.5	0.2	0.2	0.4	0.3	–0.1
m^1A: m^5U H:WT	18	0.5	0.2	0.3	0.3	0.3	0.0
s^4U:A W:HT	15	0.4	0.3	0.2	0.4	0.3	0.0
m^5U:G W:HT	2	1.0	0.0	0.4	0.7	0.4	0.0
m^1A:A H:WT	1	0.2	0.0	0.3	0.0	0.1	0.2
m^1A: m^5Um H:WT	1	0.9	0.0	0.3	0.3	0.3	0.1
Um:A W:HT	1	0.5	0.0	0.3	0.0	0.3	0.0
D:U W:WT	12	0.6	0.5	0.1	0.7	0.1	0.0
m^5C:G W:WT	3	0.3	0.1	0.5	0.2	0.3	0.2
Ψ:C S:WC	22	1.0	0.2	1.1	0.2	0.3	0.8
m^7G:A S:WT	10	1.3	0.3	1.1	0.3	0.2	0.9
s^4U:A W:ST	7	1.0	0.5	0.6	0.6	0.4	0.2
D:G W:ST	1	0.4	0.0	0.3	0.0	0.3	0.1
D:G H:SC	1	0.6	0.0	1.5	0.8	0.6	0.9
Um:G H:SC	1	0.7	0.0	0.5	0.0	0.6	–0.1
Gm:G S:SC	30	1.2	0.2	1.1	0.2	0.6	0.6
s^4U:A S:SC	1	1.2	0.0	0.9	0.1	0.3	0.6
$m^6{}_2$A:G S:ST	3	1.0	0.5	1.1	0.4	1.1	0.1
Am:G S:ST	1	0.7	0.0	1.2	0.3	1.1	0.2
m^7G:A S:ST	1	1.2	0.0	1.2	0.0	1.1	0.2
D:U S:ST	1	1.0	0.0	1.1	0.0	–	–

TABLE S14. Local intra base pair rotational parameters (Buckle (κ), Open (σ) and Propeller (π)) calculated for crystal, optimized and unmodified structures (values in deg(°))

Modified Base Pair	Frequency	Crystal structures						Optimized structures						Un modiifed structures					
		Buckle (deg)		Open (deg)		Propeller (deg)		Buckle (deg)		Open (deg)		Propeller (deg)		Buckle (deg)		Open (deg)		Propeller (deg)	
		Avg	SD	Avg	SD	Avg	SD	Opt. value	SD	Opt. value	SD	Opt. value	SD	Opt. value	Δ(M–U)	Opt. value	Δ(M–U)	Opt. value	Δ(M–U)
m^{5}C:G W:WC	87	2.3	10.3	–0.2	4.4	–7.3	6.9	0.0	10.5	–2.9	5.1	0.1	10.1	0.0	0.0	–2.9	–0.1	–0.1	0.1
m^{2}G:C W:WC	38	–5.8	6.5	1.2	3.6	–3.6	6.3	0.1	8.7	–3.7	6.0	0.1	7.2	–1.0	1.0	–3.5	–0.2	0.0	0.1
Ψ:A W:WC	24	7.9	9.0	–0.3	7.1	–8.4	7.6	–0.4	12.1	–3.8	7.8	0.0	11.2	0.1	–0.5	2.3	–6.1	0.1	0.0
m^{7}G:C W:WC	22	7.2	4.7	9.5	2.7	7.3	2.3	0.1	8.5	1.8	8.2	0.0	7.6	–1.0	1.0	–3.5	5.3	0.0	0.0
m$^2{}_2$G:A W:WC	16	23.4	3.2	–3.0	5.7	–22.3	9.2	18.6	5.8	–3.6	5.6	–22.4	8.9	–0.6	19.2	–2.4	–1.2	–19.3	–3.1
Gm:C W:WC	12	8.0	4.2	–4.2	1.2	0.5	4.6	–2.0	10.8	–3.4	1.4	1.9	4.7	–1.0	–1.1	–3.5	0.1	0.0	1.9
Ψ:G W:WC	10	4.1	6.7	1.8	6.7	–6.9	4.7	0.7	7.2	1.1	6.4	1.7	9.7	–2.9	3.6	1.1	–0.1	–0.6	2.3
Um:A W:WC	4	–10.1	21.4	3.4	2.3	7.0	37.5	0.6	23.1	1.9	2.4	0.0	33.1	0.1	0.6	2.3	–0.5	0.1	–0.1
m^{2}G: C W:W(+)C	3	–15.3	0.0	12.2	0.0	–3.6	0.0	–0.1	15.2	–17.4	29.6	–0.2	3.4	–1.0	0.8	–3.5	–13.9	0.0	–0.2
m^{5}U:G W:WC	3	0.0	1.6	–13.4	3.9	–7.5	1.4	3.3	3.5	1.5	15.2	0.5	8.1	–2.9	6.1	1.1	0.4	–0.7	1.2
m^{2}G:U W:WC	1	1.7	–	6.2	–	1.6	–	2.4	0.5	1.0	3.7	0.3	0.9	2.9	–0.4	1.1	–0.1	–0.7	1.0
Gm:A W:WC	1	16.6	–	10.3	–	15.0	–	1.3	10.9	–2.4	9.0	20.2	3.7	–0.6	1.9	–2.4	0.0	–19.3	39.5
Cm:Gm W:WC	1	0.1	–	0.6	–	0.1	–	1.0	4.3	–3.5	4.0	4.9	5.2	0.0	1.0	–3.5	0.0	0.0	4.9
m^{5}U:A W:HT	34	9.3	7.8	3.4	8.0	4.7	6.0	–0.1	12.2	3.1	7.9	0.0	7.6	0.0	–0.1	3.4	–0.3	0.0	–0.1
m^{7}G:G W:HT	27	7.4	7.1	0.6	5.4	3.0	9.1	0.3	9.9	2.7	5.7	0.2	9.4	–7.4	7.7	10.4	–7.7	–3.5	3.6
Ψ:A W:HT	22	18.7	4.2	–0.5	3.6	4.8	3.4	–0.1	19.2	–0.4	3.5	–0.1	5.9	0.0	–0.1	–3.4	3.1	0.0	–0.1
m^{1}A: m^{5}U H:WT	18	6.4	4.8	3.4	6.6	0.4	3.4	0.0	7.9	–2.3	8.6	0.0	3.3	0.0	0.0	–3.4	1.2	0.0	0.0
s^{4}U:A W:HT	15	10.7	8.6	3.0	4.6	3.5	5.2	0.4	13.3	2.8	4.5	1.0	5.6	0.0	0.4	–3.4	6.2	0.0	1.0
m^{5}U:G W:HT	2	8.6	1.1	2.6	1.6	1.2	0.7	–1.0	9.7	22.9	20.3	1.5	0.6	–0.8	–0.2	22.8	0.1	1.4	0.1
m^{1}A:A H:WT	1	–4.0	–	0.5	–	13.1	–	–1.7	1.6	–8.1	6.1	28.1	10.6	0.0	–1.8	–0.6	–7.5	1.6	26.5
m^{1}A: m^{5}Um H:WT	1	6.4	–	7.7	–	–2.0	–	7.9	1.1	0.3	5.2	2.4	3.1	0.0	7.9	–3.4	3.8	0.0	2.4
Um:A W:HT	1	–18.9	–	2.5	–	–6.2	–	3.8	13.8	4.8	0.5	0.4	4.4	0.0	3.7	–3.4	8.2	0.0	0.4
D:U W:WT	12	–22.0	27.0	–2.0	6.1	4.9	16.8	6.8	38.8	–0.6	6.0	23.4	24.5	0.0	6.8	0.0	–0.6	0.0	23.4
m^{5}C:G W:WT	3	–5.9	4.0	7.4	4.6	–4.5	7.6	–18.3	12.9	–11.4	19.2	–39.6	35.7	17.6	–35.9	–11.6	0.2	39.1	–78.7
Ψ:C S:WC	22	33.8	4.3	16.4	3.7	–21.9	3.1	38.8	6.5	17.6	3.8	–12.4	10.0	47.1	–8.3	–32.9	50.5	–19.4	7.0
m^{7}G:A S:WT	10	–35.1	2.3	41.2	3.1	–31.0	2.0	–53.2	18.2	38.7	3.8	18.9	49.8	–5.1	–48.2	7.6	31.1	34.2	–15.3
s^{4}U:A W:ST	7	9.9	12.4	59.3	17.7	–10.8	7.8	8.4	11.6	44.0	22.4	–13.9	7.9	22.0	–13.6	–4.3	48.3	8.9	–22.7
D:G W:ST	1	29.6	–	–0.7	–	39.8	–	17.6	8.5	–11.2	7.4	59.2	13.7	–32.2	49.8	–31.0	19.8	5.4	53.7
D:G H:SC	1	–17.5	–	0.9	–	–7.6	–	–32.7	10.7	6.5	4.0	–10.5	2.0	–52.6	20.0	–2.1	8.6	1.9	–12.3
Um:G H:SC	1	–3.2	–	–5.2	–	3.5	–	–0.5	1.9	–2.0	2.3	27.9	17.2	–52.6	52.1	–2.1	0.2	1.9	26.0
Gm:G S:SC	30	–11.6	2.2	8.7	1.7	11.2	2.1	–59.7	48.1	22.8	14.1	4.9	6.7	5.3	–65.0	–10.8	33.5	–61.1	66.0
s^{4}U:A S:SC	1	19.8	–	–29.1	–	–12.2	–	45.4	18.1	–35.9	4.8	–15.9	2.6	2.6	42.8	–38.5	2.6	–43.3	27.4
m$^6{}_2$A:G S:ST	3	–37.9	1.5	–21.3	1.2	11.1	1.2	–67.0	25.2	–14.8	5.7	11.9	1.1	–5.1	–61.9	7.6	–22.5	34.2	–22.3
Am:G S:ST	2	–19.8	1.0	17.3	0.5	–10.3	0.9	–91.3	71.5	–20.8	38.0	19.8	30.1	–5.1	–86.3	7.6	–28.4	34.2	–14.4
m^{7}G:A S:ST	1	–40.8	–	–38.7	–	2.7	–	–106.6	46.5	–23.6	10.7	23.8	14.9	–5.1	–101.5	7.6	–31.2	34.2	–10.4
D:U S:ST	1	20.4	–	–3.9	–	–11.3	–	–41.9	44.1	27.8	22.5	–7.6	2.6	–	–41.9	–	27.8	–	–7.6

TABLE S15. Local intra base pair translational parameters (Stagger (Sx), Shear (Sy) and Stretch (Sz)) calculated for crystal, optimized and unmodified structures (values in Å)

Modified Base Pair	Frequency	Crystal structures						Optimized structures						Unmodified structures					
		Stagger (Å)		Shear (Å)		Stretch (Å)		Stagger (Å)		Shear (Å)		Stretch (Å)		Stagger (Å)		Shear (Å)		Stretch (Å)	
		Avg	SD	Avg	SD	Avg	SD	Opt.	SD	Opt.	SD	Opt.	SD	Opt.	Δ(M–U)	Opt.	Δ(M–U)	Opt.	Δ(M–U)
m^5C:G W:WC	87	–0.1	0.4	–0.1	0.5	2.8	0.2	0.0	0.4	2.4	2.5	2.9	4.3	0.0	0.0	2.4	0.1	2.9	0.1
m^2G:C W:WC	38	–0.2	0.3	–0.3	0.5	2.8	0.2	0.0	0.3	–0.1	0.5	2.9	0.2	0.0	1.0	–0.1	0.2	2.9	0.1
Ψ:A W:WC	24	–0.2	0.5	–0.6	0.6	2.8	0.2	0.0	0.5	–0.1	0.8	2.8	0.2	0.0	0.5	0.1	6.1	2.8	0.0
m^7G:C W:WC	22	–0.2	0.2	–0.1	0.3	2.8	0.1	0.0	0.3	–0.2	0.3	2.9	0.1	0.0	1.0	–0.1	5.3	2.9	0.0
$m^2{}_2$G:A W:WC	16	–0.4	0.6	–0.5	0.7	2.8	0.2	–0.4	0.6	–0.1	0.8	3.0	0.2	–0.2	19.2	0.0	1.2	2.9	3.1
Gm:C W:WC	12	0.2	0.1	0.0	0.1	3.0	0.2	0.0	0.2	–0.1	0.2	2.9	0.2	0.0	1.1	–0.1	0.1	2.9	1.9
Ψ:G W:WC	10	0.1	0.5	1.9	0.5	2.8	0.1	0.1	0.4	2.4	0.6	2.8	0.1	–0.1	3.6	2.4	0.1	2.8	2.3
Um:A W:WC	4	–0.2	0.4	0.0	0.3	2.7	0.2	0.0	0.4	–0.1	0.3	2.9	0.2	0.0	0.6	–0.1	0.5	2.9	–0.1
m^2G: C W:W(+)C	3	–1.4	0.0	–1.9	0.0	3.0	0.0	0.0	1.2	–2.2	0.3	2.9	0.1	–0.6	0.8	–3.4	13.9	2.6	–0.2
m^5U:G W:WC	3	0.7	0.3	–2.3	0.1	2.8	0.1	0.1	0.6	–2.4	0.1	2.8	0.1	–0.1	6.1	2.4	0.4	2.8	1.2
m^2G:U W:WC	1	0.3	–	–1.9	–	3.0	–	–0.1	0.2	–2.4	0.3	2.8	0.1	–0.1	0.4	2.4	0.1	2.8	1.0
Gm:A W:WC	1	0.2	–	–0.3	–	2.5	–	–0.2	0.3	0.0	0.2	2.9	0.3	–0.2	1.9	0.0	0.0	2.9	39.5
Cm:Gm W:WC	1	–0.2	–	0.2	–	2.8	–	0.1	0.1	0.1	0.1	2.9	0.2	0.0	1.0	–0.1	0.0	2.9	4.9
m^5U:A W:HT	34	0.2	0.5	0.4	0.5	2.8	0.2	0.0	0.5	0.1	0.6	2.8	0.2	0.0	0.1	0.1	0.3	2.8	0.1
m^7G:G W:HT	27	0.4	0.6	0.3	0.5	2.9	0.3	0.0	0.7	0.3	0.5	2.8	0.3	0.1	7.7	0.2	7.7	2.9	3.6
Ψ:A W:HT	22	–0.3	0.3	–0.1	0.2	2.9	0.1	0.0	0.4	–0.1	0.2	2.9	0.1	0.0	0.1	0.1	3.1	2.8	–0.1
m^1A: m^5U H:WT	18	0.1	0.3	0.0	0.4	2.9	0.1	0.0	0.3	0.2	0.4	2.9	0.1	0.0	0.0	0.1	1.2	2.8	0.0
s^4U:A W:HT	15	0.1	0.4	0.7	0.6	2.8	0.2	0.0	0.4	0.1	0.9	2.9	0.2	0.0	0.4	0.1	6.2	2.8	1.0
m^5U:G W:HT	2	–0.1	0.2	0.4	0.1	2.5	0.1	0.0	0.1	0.0	0.4	3.0	0.5	0.0	0.2	0.0	0.1	3.0	0.1
m^1A:A H:WT	1	–0.1	–	2.8	–	2.7	–	0.2	0.2	2.4	0.2	2.8	0.1	0.0	1.8	2.6	7.5	3.0	26.5
m^1A: m^5Um H:WT	1	–0.2	–	–0.7	–	2.9	–	0.0	0.2	0.2	0.6	2.9	0.0	0.0	7.9	0.1	3.8	2.8	2.4
Um:A W:HT	1	–0.1	–	0.0	–	2.6	–	0.0	0.1	0.1	0.0	2.8	0.2	0.0	3.7	0.1	8.2	2.8	0.4
D:U W:WT	12	0.3	0.6	–1.8	0.3	2.8	0.3	–0.6	1.0	–2.6	0.8	2.9	0.3	0.0	6.8	–2.6	0.6	2.9	23.4
m^5C:G W:WT	3	0.0	0.2	–2.4	0.3	2.9	0.1	–0.1	0.2	–2.5	0.2	3.0	0.1	0.1	35.9	–2.5	0.2	3.0	78.7
Ψ:C S:WC	22	–0.5	0.3	1.3	0.2	4.5	0.1	–0.7	0.3	0.4	0.9	4.4	0.1	–1.2	8.3	–0.7	50.5	4.3	7.0
m^7G:A S:WT	10	–0.6	0.3	–2.8	0.2	2.8	0.1	1.4	2.0	2.0	4.8	2.7	0.2	0.9	48.2	1.5	31.1	3.2	15.3
s^4U:A W:ST	7	0.2	0.3	–0.4	0.6	4.4	0.4	0.8	0.6	0.7	1.2	4.1	0.5	0.8	13.6	–0.2	48.3	3.0	22.7
D:G W:ST	1	–0.1	—	–0.7	–	3.1	–	–0.1	0.0	–0.4	0.2	3.0	0.1	–0.1	49.8	–0.8	19.8	4.7	53.7
D:G H:SC	1	–0.7	–	0.2	–	3.1	–	0.2	0.6	0.9	0.5	3.3	0.1	0.0	20.0	1.0	8.6	3.7	12.3
Um:G H:SC	1	–0.2	–	1.2	–	3.3	–	0.6	0.5	0.4	0.5	3.4	0.1	0.0	52.1	1.0	0.2	3.7	26.0
Gm:G S:SC	30	1.6	0.1	2.5	0.1	3.0	0.1	1.1	0.6	1.6	0.8	3.1	0.1	–0.1	–65.0	1.8	33.5	3.8	66.0
s^4U:A S:SC	1	–1.8	–	–1.2	–	3.2	–	–0.9	0.6	–0.9	0.2	4.3	0.7	–0.8	42.8	–0.6	2.6	4.2	27.4
$m^6{}_2$A:G S:ST	3	–0.7	0.1	1.3	0.2	2.7	0.4	–0.4	0.3	1.5	0.2	3.3	0.6	0.9	61.9	1.5	22.5	3.2	22.3
Am:G S:ST	2	–0.3	0.3	1.4	0.2	3.4	0.1	1.3	1.5	1.4	0.2	3.0	0.4	0.9	86.3	1.5	–28.4	3.2	14.4
m^7G:A S:ST	1	–0.7	–	2.3	–	3.6	–	–1.5	0.6	1.1	0.8	2.8	0.5	0.9	101.5	1.5	–31.2	3.2	10.4
D:U S:ST	1	–0.8	–	3.7	–	3.3	–	–0.2	0.5	5.0	0.9	3.0	0.2	–	41.9	–	27.8	–	7.6

++ SD for single occurrence are not applicable in crystal structures.

TABLE S16. Interaction energies (kcal/mol) of modified and unmodified base pairs.

Modified base pairs	Occurrence Frequency	Interaction Energy of Modified base pairs	Interaction Energy of Unmodified base pairs	Difference between Modified and Unmodified base pairs
m^5C:G W:WC	87	–30.00	–29.43	–0.57
m^2G:C W:WC	38	–29.47	–29.43	–0.04
Ψ:A W:WC	24	–14.79	–15.26	0.47
m^7G:C W:WC	22	–38.45	–29.43	–9.02
$m^2{}_2$G:A W:WC	16	–17.69	–17.84	0.15
Gm:C W:WC	12	–29.99	–29.20	–0.79
Ψ:G W:WC	10	–18.22	–16.80	–1.42
Um:A W:WC	4	–15.78	–15.40	–0.38
m^2G: C W:W(+)C	3	–43.37	–39.97	–3.40
m^5U:G W:WC	3	–17.18	–16.81	–0.37
m^2G:U W:WC	1	–16.90	–16.81	–0.09
Gm:A W:WC	1	–18.08	–18.37	0.29
Cm:Gm W:WC	1	–28.94	–27.44	–1.50
m^5U:A W:HT	34	–15.97	–15.90	–0.07
m^7G:G W:HT	27	–35.10	–19.85	–15.25
Ψ:A W:HT	22	–16.18	–15.90	–0.28
m^1A: m^5U H:WT	18	–23.30	–15.90	–7.40
s^4U:A W:HT	15	–16.34	–15.90	–0.44
m^5U:G W:HT	2	–9.76	–10.02	0.26
m^1A:A H:WT	1	–22.99	–13.36	–9.63
m^1A: m^5Um H:WT	1	–22.12	–16.15	–5.97
Um:A W:HT	1	–15.41	–16.15	0.74
D:U W:WT	12	–11.94	–11.82	–0.12
m^5C:G W:WT	3	–14.11	–13.72	–0.39
Ψ:C S:WC	22	–13.79	–20.09	6.30
m^7G:A S:WT	10	–22.50	–16.51	–5.99
s^4U:A W:ST	7	–12.58	–12.37	–0.21
D:G W:ST	1	–15.46	–18.29	2.83
D:G H:SC	1	–12.88	–12.40	–0.48
Um:G H:SC	1	–14.58	–12.40	–2.18
Gm:G S:SC	30	–15.89	–15.39	–0.50
s^4U:A S:SC	1	–20.96	–19.63	–1.33
$m^6{}_2$A:G S:ST	3	–21.49	–21.00	–0.49
Am:G S:ST	2	–19.91	–21.00	1.09
m^7G:A S:ST	1	–34.91	–21.00	–13.91
D:U S:ST	1	–16.29	–	–

TABLE S17. List of Triples and Quartets formed by modified base pairs along with crystal structure PDBs.

Modified base	Modified base triple	Freq.	PDB accession no.	Base Pair I	Base Pair II	Quartet
Gm (56)	G2617_C2542_Gm2588_WWC–SSC	43	1s72, 1vq4, 1vq5, 1vq6, 1vq8, 1vq9, 1vqk, 1vql, 1vqm, 1vqn, 1vqo, 1vqp, 1yhq, 1yi2, 1yij, 1yit, 1yj9, 1yjn, 1yjw, 2otj, 2otl, 2qa4, 2qex, 3cc2, 3cc4, 3cc7, 3cce, 3ccj, 3ccl, 3ccm, 3ccq, 3ccr, 3ccs, 3ccu, 3ccv, 3cd6, 3cma, 3cme, 3g4s, 3g6e, 3g71, 3i55, 3i56,	Gm2588:G2617 S:SC	–	–
	Gm2588_C75_G2617_WWC–SSC	12	1vq4, 1vq6, 1vqk, 1vql, 1vqm, 1vqn, 1vqo, 3cd6, 3cma, 3i55, 1vq5, 1vqp,	Gm2588:G2617 S:SC	Gm2588:C75 WWC	C2542
	Gm2588_A76_G2617_WWC–SSC	1	3cme	Gm2588:G2617 SSC	Gm2588:A76 WWC	–
m^7G (31)	G22_C13_ m^7G46_WWC–HWT	20	1c0a, 1ob2, 1ob5, 1yfg, 1ehz, 1evv, 1fir, 1tn1, 1tn2, 1tra, 1ttt, 2y0u, 2y0w, 2y10, 2y18, 4tna, 4tra, 6tna, 1efw, 1qf6,	m^7G46:G22 WHT	–	–
	m^7G 527_C522_A535_WWC–swT	11	4ji0, 4dr2, 4dr5, 4dv6, 4dv7, 4ji1, 4ji3, 4ji7, 4lf6, 4lf7, 4lf8,	m^7G527:C522 WWC	m^7G527:A535 SWT	–
Ψ (28)	Ψ516_A533_C519_WHT–SWC	22	4dr2, 4dr3, 4dr5, 4dr6, 4duy, 4dv6, 4dv7, 4ji0, 4ji1, 4ji3, 4ji7, 4lf4, 4lf6, 4lf7, 4lf8, 4lf9, 4lfb, 4x62, 4x64, 4x65, 4x66, 5br8,	Ψ516:A533 WHT	Ψ516_C519 SWC	A520
	G22_Ψ13_A46_WWC–H+T	4	1il2, 1asy, 1asz, 1il2,	Ψ13:G22 WWC	–	–
	Ψ4_A36_A1493_WWC_SSC	2	4jv5, 4jya	Ψ4:A36 WWC	–	–
s^4U (13)	s^4U8_A14_A21_WHT–swT	12	1c0a, 1efw, 2xqd, 2y0y, 2y12, 2y14, 2y16, 3cw5, 3cw6, 4jyz, 2y10, 2y18,	s^4U8:A14 WHT	s^4U8_A21 SWT	
	s^4U8_A14_A46_WHT–ssC	1	1b23	s^4U8:A14 WHT	s^4U8_A46 SSC	
m^5U (3)	G10_m^5U1_A58_WWC–ssC	3	1t42, 1u6b, 1zzn,	m5U1:G10 WWC	–	–
D (2)	G15_C48_D20_WWT–SWT	1	1ser	D:G SWT	–	–
	G619_C656_D620_WWC–shC	1	1c0a	D:G HSC	–	–
m^5C (2)	G1497_m^5C1404_A1518_WWC–swT	2	4lf7, 4lf8	m^5C:G WWC	–	–
$m^6{}_2A$ (1)	G2618_U2541_ $m^6{}_2$A76_WWC–swT	1	1vq6	$m^6{}_2$A:G WST	–	–
Um (1)	Um2656_G2655_A2665_hsC–WHT	1	3dw5	Um:G HSC	Um2656:A2665 WHT	–

TABLE S18. Cartesian coordinates of the 36 modified base pair

Cartesian coordinates of the modified base pair m^5C:G W:WC

Calculated energy (in Hartrees) = –976.876111

ATOM	X	Y	Z
N	–0.044066	–0.235488	0.035823
C	0.049193	–0.275313	1.437433
O	1.163484	–0.429038	1.949913
N	–1.109867	–0.139816	2.133369
C	–2.282743	0.023000	1.509601
N	–3.372981	0.147837	2.273291
C	–2.396564	0.066394	0.060258
C	–1.228300	–0.070300	–0.619151
C	–3.721039	0.250079	–0.627087
N	–0.875016	–0.298189	9.007076
C	–2.230746	–0.134498	9.250370
N	–2.935548	–0.025101	8.156172
C	–2.004211	–0.120493	7.134986
C	–2.161275	–0.073125	5.713632
O	–3.202585	0.068950	5.054104
N	–0.923598	–0.213889	5.057051
C	0.298171	–0.377128	5.667897
N	1.369683	–0.491076	4.856096
N	0.456389	–0.423272	6.984335
C	–0.711970	–0.291263	7.644711
H	–3.298430	0.115790	3.308387
H	–1.180561	–0.054969	–1.703131
H	–4.422815	–0.556837	–0.380987
H	–4.197840	1.197529	–0.345715
H	–3.599245	0.256291	–1.713368
H	–2.623099	–0.105780	10.257839
H	2.261337	–0.619471	5.303782
H	0.827218	–0.337779	–0.465129
H	–0.133904	–0.403582	9.682875
H	1.301719	–0.474007	3.835718
H	–0.963597	–0.190141	4.023923
H	–4.277698	0.271381	1.851009

TABLE S19: Cartesian coordinates of the modified base pair m^2G:C W:WC

Calculated energy (in Hartrees) = –976.86511642

ATOM	X	Y	Z
N	–0.060887	–0.092608	0.034176
C	–0.052398	–0.031656	1.419587
N	1.153296	0.019736	1.920050
C	1.987004	–0.008383	0.814428
C	3.415138	0.022157	0.713579
O	4.252380	0.082705	1.627748
N	3.834640	–0.026371	–0.627309
C	3.009253	–0.095058	–1.731048
N	3.617262	–0.133371	–2.935083
C	2.855389	–0.205192	–4.166303
N	1.685096	–0.123793	–1.648600
C	1.249696	–0.078485	–0.372398
N	8.634629	0.011569	–2.387821
C	7.233017	–0.014464	–2.231551
O	6.533402	–0.075238	–3.247478
N	6.749055	0.029642	–0.962904
C	7.571102	0.095026	0.092489
N	7.019474	0.135397	1.308267
C	9.007013	0.121676	–0.064253
C	9.489346	0.077656	–1.330635
H	–0.971601	–0.029070	1.989516
H	2.193240	0.660109	–4.280881
H	2.233466	–1.106377	–4.202280
H	3.561620	–0.226124	–4.998168
H	5.987190	0.116300	1.422418
H	9.669907	0.174456	0.789507
H	10.548640	0.092294	–1.562225
H	8.968677	–0.022396	–3.340691
H	–0.861144	–0.139080	–0.577816
H	7.605723	0.184841	2.125398
H	4.857734	–0.007416	–0.770969
H	4.637457	–0.109466	–2.992329

TABLE S20: Cartesian coordinates of the modified base pair Ψ:A W:WC

Calculated energy (in Hartrees) = –882.182649

ATOM	X	Y	Z
N	0.339967	–0.037448	–0.099052
C	0.253650	0.023039	1.277640
N	1.420666	0.110258	1.868101
C	2.325576	0.106981	0.820507
C	3.737680	0.177094	0.785590
N	4.496172	0.267877	1.890646
N	4.331290	0.149939	–0.429417
C	3.575611	0.059061	–1.541839
N	2.248530	–0.012522	–1.630151
C	1.678965	0.015808	–0.415591
N	9.267164	0.402395	0.112229
C	7.894321	0.353107	0.331045
N	7.142757	0.265569	–0.808888
C	7.613275	0.223019	–2.133518
C	9.069457	0.281279	–2.254122
C	9.830430	0.367424	–1.143451
O	7.434335	0.387943	1.472332
O	6.829921	0.143783	–3.069803
H	–0.701561	–0.001711	1.784550
H	5.512541	0.314515	1.820098
H	4.139474	0.043779	–2.471901
H	10.913465	0.413625	–1.169731
H	–0.418956	–0.107182	–0.760063
H	9.503256	0.253217	–3.243841
H	4.042680	0.285312	2.789375
H	6.103065	0.225392	–0.674437
H	9.833608	0.467253	0.945048

TABLE S21: Cartesian coordinates of the modified base pair m^7G:C W:WC

Calculated energy (in Hartrees) = –977.279020

ATOM	X	Y	Z
N	4.588670	–0.749589	0.000493
C	3.204366	–0.959407	–0.000349
O	2.787861	–2.124885	–0.000601
N	2.398983	0.139644	–0.000780
C	2.927447	1.373061	0.000033
N	2.084526	2.413571	–0.000585
C	4.348886	1.592413	0.001188
C	5.143351	0.492311	0.001218
N	–4.338760	–1.097529	0.000501
C	–4.809890	0.169472	0.001019
N	–3.788603	1.020184	0.000895
C	–2.611688	0.281977	0.000182
C	–1.228441	0.668820	–0.001011
O	–0.790360	1.822180	–0.001747
N	–0.404116	–0.456326	–0.001246
C	–0.838308	–1.770053	–0.000321
N	0.090789	–2.718057	0.000272
N	–2.138000	–2.115375	–0.000053
C	–2.948961	–1.067045	0.000109
C	–3.879478	2.486540	0.000303
H	1.074641	2.260816	–0.000663
H	4.768424	2.589574	0.001525
H	6.226115	0.541636	0.001950
H	–0.233218	–3.673503	0.001124
H	–4.930999	2.772585	0.000822
H	–3.380066	2.876127	0.887388
H	–3.381290	2.874929	–0.887990
H	–4.903113	–1.937771	0.000875
H	0.629354	–0.255972	–0.001664
H	1.106478	–2.509289	0.000743
H	2.438827	3.356120	0.002056
H	5.158948	–1.585135	0.000506
H	–5.852230	0.447333	0.001412

TABLE S22: Cartesian coordinates of the modified base pair $m^2{}_2$G:A W:WC

Calculated energy (in Hartrees) = –1088.539726

ATOM	X	Y	Z
N	–0.140740	0.012901	–0.005843
C	–0.301172	0.173502	1.360439
N	0.835749	0.248638	2.002281
C	1.796044	0.133033	1.014197
C	3.228790	0.114662	1.094651
O	3.940760	0.208391	2.097403
N	3.813853	–0.055265	–0.184523
C	3.124855	–0.136737	–1.373779
N	3.872062	–0.272778	–2.528742
N	1.807776	–0.120149	–1.450947
C	1.209928	–0.014893	–0.242114
C	3.122810	–0.482850	–3.764494
C	5.081925	0.534241	–2.709338
N	10.458868	–2.178816	0.516977
C	10.698766	–1.606368	1.750948
N	9.668284	–0.952569	2.228617
C	8.696748	–1.100837	1.254211
C	7.358144	–0.645148	1.176726
N	6.779556	0.087007	2.139032
N	6.652609	–0.965602	0.061412
C	7.241081	–1.717080	–0.889620
N	8.477946	–2.208175	–0.922240
C	9.162687	–1.861719	0.179856
H	–1.282742	0.226838	1.811221
H	3.817690	–0.842753	–4.528040
H	2.644165	0.438808	–4.124926
H	2.344465	–1.227154	–3.603287
H	4.838416	1.546935	–3.065427
H	5.650686	0.614956	–1.785397
H	5.721087	0.053353	–3.454630
H	11.655972	–1.709248	2.243983
H	5.759092	0.207764	2.139187
H	6.609930	–1.951180	–1.744385
H	–0.861380	–0.060716	–0.707884
H	11.095021	–2.731032	–0.038075
H	7.295476	0.222594	2.994728
H	4.819429	–0.298742	–0.158987

TABLE S23: Cartesian coordinates of the modified base pair Gm:C W:WC

Calculated energy (in Hartrees) = –1397.875810

ATOM	X	Y	Z
C	6.618932	–1.405668	–0.812750
C	5.623942	–0.277774	–0.633936
O	4.360627	–0.660366	–1.210700
C	5.266543	0.071357	0.822259
O	6.226539	0.843473	1.497570
C	3.907578	0.778802	0.647590
O	4.200470	2.148157	0.409087
C	3.105969	3.028031	0.659396
C	3.317035	0.098855	–0.620988
N	2.202102	–0.795681	–0.353524
C	2.229961	–2.156029	–0.061814
N	1.045235	–2.658626	0.166787
C	0.180743	–1.587110	0.026814
C	–1.242915	–1.503775	0.146232
O	–2.052332	–2.402791	0.418328
N	–1.700799	–0.192111	–0.094715
C	–0.915927	0.893188	–0.401264
N	–1.552701	2.070925	–0.577103
N	0.405121	0.827488	–0.512414
C	0.879725	–0.420931	–0.297212
N	–6.531332	1.453008	–0.196725
C	–5.128520	1.318534	–0.248249
O	–4.457540	2.313708	–0.540529
N	–4.611451	0.093475	0.031070
C	–5.404699	–0.941556	0.338028
N	–4.823317	–2.116899	0.591753
C	–6.841418	–0.805476	0.396354
C	–7.356428	0.417900	0.120110
H	7.569582	–1.126155	–0.349074
H	6.794185	–1.612881	–1.871373
H	5.990951	0.635897	–1.126883
H	5.124265	–0.858879	1.385467
H	6.019093	1.763714	1.265396
H	3.244872	0.669512	1.514564
H	2.221038	2.745368	0.076959
H	2.840241	3.025635	1.725848
H	3.436454	4.028580	0.373139
H	2.949242	0.871526	–1.305935
H	3.159961	–2.703699	–0.071694
H	–0.989099	2.848282	–0.877413
H	–5.387873	–2.919225	0.818572
H	–7.480415	–1.640904	0.650812

H	–8.419685	0.630064	0.132892
H	–6.889837	2.373658	–0.408230
H	–3.792966	–2.222480	0.526871
H	–2.572061	2.156141	–0.564248
H	–2.726065	–0.063609	–0.048064
H	6.254714	–2.321378	–0.335576

TABLE S24: Cartesian coordinates of the modified base pair Ψ:G W:WC

Calculated energy (in Hartrees) = –957.421341

ATOM	X	Y	Z
N	–0.114040	0.022505	0.058581
C	–0.061686	–0.016001	1.458741
N	1.232018	–0.030783	1.962609
C	2.406747	–0.010796	1.220669
C	2.239547	0.028354	–0.221936
C	0.992760	0.043791	–0.744688
O	–1.069124	–0.034190	2.135047
O	3.516478	–0.025519	1.774111
N	4.324143	–0.172596	8.461269
C	2.995748	–0.224812	8.853802
N	2.165308	–0.207889	7.845249
C	2.979214	–0.140128	6.728661
C	2.657771	–0.101000	5.332000
O	1.553088	–0.121842	4.777349
N	3.829553	–0.032365	4.548506
C	5.112755	–0.011684	5.022557
N	6.097459	0.103619	4.082520
N	5.428054	–0.058363	6.302089
C	4.330084	–0.117295	7.091831
H	0.801808	0.073880	–1.811847
H	2.717470	–0.273550	9.897687
H	7.032376	–0.092701	4.403081
H	3.124477	0.045519	–0.841562
H	5.142875	–0.169712	9.050471
H	5.878544	–0.105936	3.119849
H	1.309301	–0.061410	3.001289
H	–1.048011	0.033884	–0.325251
H	3.675409	–0.021847	3.527840

TABLE S25: Cartesian coordinates of the modified base pair Um:A W:WC

Calculated energy (in Hartrees) = –1342.504906

ATOM	X	Y	Z
N	4.620099	17.721264	5.685428
C	3.395825	17.114434	5.377956
N	3.147769	16.946672	4.037493
C	3.975014	17.317055	2.977218
C	5.214495	17.952721	3.381555
C	5.481013	18.132370	4.695997
C	4.899954	17.927248	7.129827
C	5.333884	16.624264	7.850104
O	4.846896	16.586954	9.181805
C	3.573006	15.957345	9.335292
C	6.861924	16.797269	7.967014
C	6.988210	18.321959	8.129171
O	7.438398	16.049726	9.005576
O	5.960413	18.850175	7.257281
C	8.329286	18.915543	7.752123
N	–2.800568	13.866185	2.570743
C	–2.705490	13.862117	1.193091
N	–1.601262	14.409657	0.747298
C	–0.932506	14.795853	1.896024
C	0.311441	15.439401	2.098246
N	1.117495	15.812490	1.093260
N	0.685044	15.679801	3.377507
C	–0.121992	15.306447	4.389498
N	–1.304960	14.698437	4.320273
C	–1.657422	14.469133	3.045416
H	3.981124	18.318059	7.575297
H	5.032750	15.727884	7.297598
H	3.309809	16.046302	10.391051
H	3.629123	14.894570	9.063365
H	2.811351	16.436179	8.713749
H	7.341250	16.492858	7.028146
H	6.737784	18.580243	9.167954
H	6.758934	16.020896	9.699965
H	9.099841	18.509635	8.414225
H	8.321875	20.004216	7.853044
H	–3.486020	13.439329	0.575041
H	2.010844	16.272362	1.284559
H	0.258168	15.539747	5.381479
O	2.617915	16.758494	6.259004
O	3.638178	17.105563	1.808709
H	5.900425	18.284263	2.614560
H	6.373282	18.624872	5.058109

H	2.236044	16.483974	3.800829
H	8.593642	18.663369	6.719598
H	0.827098	15.626772	0.147075
H	–3.553527	13.503433	3.135681

TABLE S26: Cartesian coordinates of the modified base pair m^5U:G W:WC

Calculated energy (in Hartrees) = –996.742093

ATOM	X	Y	Z
N	–0.274496	–0.122337	–0.031376
C	1.090861	–0.317364	0.105609
N	1.757112	0.801578	0.212498
C	0.789971	1.787849	0.141654
C	0.898685	3.216752	0.206101
O	1.900744	3.924261	0.347919
N	–0.372528	3.821699	0.083448
C	–1.564058	3.165109	–0.061635
N	–2.672709	3.954717	–0.201502
N	–1.683328	1.853719	–0.110067
C	–0.484433	1.231730	–0.010247
N	0.496318	8.660775	0.595878
C	0.562181	7.287010	0.484106
N	1.824801	6.767583	0.554381
C	3.021240	7.499033	0.723789
C	2.854532	8.958205	0.832288
C	4.091928	9.785508	1.013594
C	1.607216	9.467503	0.763450
O	–0.470343	6.619782	0.332560
O	4.098279	6.930567	0.772734
H	1.520678	–1.309623	0.117937
H	–2.623491	4.911973	0.114948
H	4.783404	9.633832	0.178550
H	3.853651	10.850120	1.084513
H	4.629033	9.483242	1.918333
H	1.399790	10.529713	0.834670
H	–0.431588	9.052914	0.539206
H	–0.991736	–0.824387	–0.132468
H	–3.554629	3.478900	–0.091013
H	1.913961	5.734701	0.478448
H	–0.373822	4.850727	0.129732

TABLE S27: Cartesian coordinates of the modified base pair m^2G: C W:W(+)C
Calculated energy (in Hartrees) = –977.265178

ATOM	X	Y	Z
N	59.148545	48.015478	16.084783
C	60.167773	48.958139	16.049635
N	61.360161	48.428551	16.094487
C	61.121900	47.067495	16.162453
C	62.007338	45.972222	16.232819
O	63.270792	46.004629	16.249153
N	61.350906	44.741143	16.287465
C	59.972949	44.578990	16.276763
N	59.513436	43.309577	16.319100
C	58.087506	43.004942	16.363387
N	59.130889	45.593683	16.215463
C	59.744808	46.789458	16.157401
H	59.952509	50.016151	15.990804
H	61.943944	43.909972	16.329975
H	60.172851	42.559342	16.455953
H	57.634175	43.336282	17.303017
H	57.571727	43.499465	15.537995
H	57.966849	41.925716	16.265893
N	65.580195	41.871373	16.483223
C	64.508186	42.760215	16.428030
O	63.341927	42.394114	16.437223
N	64.877399	44.085884	16.362206
C	66.149059	44.538753	16.348149
N	66.323159	45.855931	16.280604
C	67.218107	43.593724	16.405824
C	66.881484	42.275780	16.472107
H	67.247251	46.260045	16.266258
H	65.516490	46.473933	16.241606
H	68.252014	43.909780	16.397250
H	67.625635	41.489080	16.518967
H	58.153189	48.186518	16.062117
H	65.337241	40.889032	16.531802
H	64.062542	44.847579	16.316249

TABLE S28: Cartesian coordinates of the modified base pair m^2G:U W:WC
Calculated energy (in Hartrees) = –996.728780

ATOM	X	Y	Z
N	0.085151	0.035719	0.083342
C	0.174404	–0.115489	1.458291
N	1.408103	–0.162664	1.886203
C	2.175650	–0.035812	0.742611
C	3.599035	–0.021164	0.562331
O	4.498614	–0.123162	1.402981
N	3.930409	0.134965	–0.800084
C	3.041769	0.249424	–1.840432
N	3.592777	0.418026	–3.074912
C	2.784161	0.399614	–4.280633
N	1.730864	0.225337	–1.691081
C	1.369298	0.088992	–0.393146
N	8.880883	–0.033702	–0.894416
C	7.536817	–0.017114	–0.558099
O	6.679948	0.107506	–1.442235
N	7.273697	–0.144973	0.775727
C	8.231364	–0.287219	1.812522
O	7.881565	–0.393118	2.972807
C	9.619261	–0.291561	1.346653
C	9.891691	–0.166834	0.033773
H	–0.709793	–0.183145	2.077235
H	1.941320	1.084111	–4.168541
H	3.402062	0.730680	–5.117734
H	2.383167	–0.597532	–4.503599
H	10.400633	–0.396963	2.086157
H	10.898062	–0.163064	–0.369333
H	–0.750474	0.097716	–0.478202
H	4.938646	0.152661	–1.002626
H	6.272773	–0.138290	1.060172
H	9.077565	0.063331	–1.879344
H	4.586481	0.261342	–3.157281

TABLE 29: Cartesian coordinates of the modified base pair Gm:A W:WC
Calculated energy (in Hartrees) = –1470.245784

ATOM	X	Y	Z
C	0.185767	–0.725044	–0.078515
C	0.205560	–0.335728	1.384757
O	1.514893	0.158089	1.732008
C	–0.732445	0.819460	1.778366
O	–2.084366	0.465188	1.913848
C	–0.082531	1.314509	3.085949
O	–0.624813	0.513029	4.125945
C	–0.492446	1.078966	5.426476
C	1.425342	1.004389	2.865250
N	2.240712	2.185268	2.612957
C	2.524937	2.792880	1.395725
N	3.261951	3.867217	1.514459
C	3.482233	3.987159	2.872750
C	4.222168	4.962692	3.621360
O	4.828441	5.958176	3.217359
N	4.201239	4.661172	5.005449
C	3.525502	3.621821	5.580471
N	3.641643	3.506298	6.945920
N	2.820542	2.732594	4.910953
C	2.857412	2.952556	3.571158
N	7.803160	9.001055	9.048138
C	7.873476	10.040839	8.141796
N	7.315280	9.762567	6.989025
C	6.851152	8.468879	7.145585
C	6.167648	7.597551	6.262451
N	5.832461	7.945080	5.013623
N	5.842905	6.364525	6.732376
C	6.206890	6.024782	7.984606
N	6.849469	6.750780	8.895231
C	7.143655	7.971343	8.417270
H	–0.816223	–1.073672	–0.345585
H	0.900834	–1.526443	–0.282650
H	–0.035916	–1.206577	2.013368
H	–0.666139	1.605920	1.016765
H	–2.168229	0.119235	2.817495
H	–0.256236	2.378599	3.286292
H	0.554821	1.299674	5.665678
H	–1.074969	2.007313	5.511027
H	–0.884264	0.343099	6.131623
H	1.834841	0.520390	3.759577
H	2.179228	2.361635	0.468808
H	2.997279	2.845503	7.354835
H	8.350146	10.978259	8.394453

H	6.141708	8.843446	4.676507
H	5.939876	5.011890	8.282500
H	5.467760	7.250701	4.349268
H	8.167694	8.980653	9.988674
H	3.768608	4.361598	7.468115
H	4.796026	5.262798	5.609221
H	0.437154	0.132547	–0.711027

TABLE S30: Cartesian coordinates of the modified base pair Cm:Gm W:WC
Calculated energy (in Hartrees) = –1858.197781

ATOM	X	Y	Z
N	–4.483947	0.552236	–0.573894
C	–3.091601	0.359235	–0.411559
N	–2.292873	1.448577	–0.328800
C	–2.799061	2.689146	–0.397084
C	–4.207062	2.905977	–0.582470
C	–5.002083	1.808857	–0.670482
O	–2.669000	–0.808643	–0.345640
N	–1.949704	3.715433	–0.292969
C	–5.328041	–0.665733	–0.671948
C	–5.569888	–1.335135	0.705261
O	–5.647880	–2.746619	0.584376
C	–4.403189	–3.428956	0.740353
C	–6.995730	–0.867724	1.058962
C	–7.650085	–0.797500	–0.331567
O	–6.592195	–0.302010	–1.187211
O	–7.655198	–1.701837	1.974675
C	–8.861138	0.104761	–0.443555
C	8.838891	–0.079906	1.487186
C	7.663504	–0.801823	0.862174
O	6.439446	–0.317186	1.445296
C	7.462743	–0.564360	–0.645285
O	8.327899	–1.291046	–1.479748
C	5.973598	–0.927152	–0.822452
O	5.931484	–2.325317	–1.069356
C	4.718230	–2.773385	–1.669397
C	5.358016	–0.579651	0.564637
N	4.488004	0.584325	0.556631
C	4.833120	1.924486	0.702245
N	3.812677	2.734231	0.594937
C	2.732698	1.901638	0.360893
C	1.345493	2.192681	0.162467
O	0.778954	3.294433	0.145364
N	0.597218	1.012142	–0.031221
C	1.099487	–0.265810	–0.045562

N	0.215648	–1.262448	–0.281311
N	2.383432	–0.546741	0.132212
C	3.131243	0.562489	0.334260
H	–4.622897	3.902322	–0.661849
H	–6.069862	1.856003	–0.836725
H	–0.937609	3.555165	–0.138544
H	–4.809084	–1.352208	–1.345593
H	–4.813847	–1.046428	1.442860
H	–4.607151	–4.487841	0.569763
H	–4.009582	–3.294221	1.757085
H	–3.657431	–3.067354	0.026811
H	–6.957895	0.140302	1.490583
H	–7.907813	–1.819131	–0.644515
H	–7.327547	–2.596086	1.781300
H	–9.654455	–0.277579	0.205551
H	–9.236630	0.137200	–1.469883
H	9.765595	–0.425185	1.019403
H	8.752559	1.001056	1.336206
H	7.749293	–1.886571	1.031765
H	7.606316	0.502211	–0.857417
H	7.895339	–2.151828	–1.605286
H	5.483059	–0.376814	–1.634350
H	4.789974	–3.860075	–1.749567
H	3.844776	–2.502210	–1.064511
H	4.596611	–2.344060	–2.673935
H	4.753007	–1.425368	0.912275
H	5.850837	2.211868	0.918533
H	–0.420821	1.145870	–0.156546
H	0.566558	–2.198920	–0.167565
H	8.895149	–0.275041	2.561500
H	–8.620377	1.125721	–0.128001
H	–0.794309	–1.101552	–0.282977
H	–2.297900	4.659475	–0.329967

TABLE S31: Cartesian coordinates of the modified base pair m^5U:A W:HT
Calculated energy (in Hartrees) = –921.506889

ATOM	X	Y	Z
N	0.119475	–0.051615	0.030122
C	0.220051	–0.213682	1.403298
N	1.508164	–0.213350	1.869587
C	2.679539	–0.070875	1.118814
C	2.481864	0.095676	–0.327591
C	3.697580	0.253895	–1.191816
C	1.214192	0.096852	–0.797776
O	–0.776812	–0.343743	2.112598
O	3.777845	–0.089051	1.667191

N	3.350981	–0.677725	6.378809
C	3.229544	–0.519924	5.020366
N	1.976843	–0.527170	4.626741
C	1.232387	–0.698116	5.781771
C	–0.158184	–0.788607	6.033570
N	–1.093541	–0.713438	5.069419
N	–0.556584	–0.959468	7.312858
C	0.373908	–1.034642	8.274990
N	1.706333	–0.964078	8.168895
C	2.073549	–0.795017	6.893409
H	3.426156	0.369537	–2.244604
H	4.282418	1.126519	–0.883094
H	4.358970	–0.613167	–1.093924
H	0.983515	0.214345	–1.851293
H	4.073549	–0.403087	4.353893
H	–0.872765	–0.584805	4.084525
H	–0.012600	–1.172430	9.282904
H	–0.820261	–0.048625	–0.337917
H	4.205044	–0.705034	6.914912
H	–2.056383	–0.789059	5.356698
H	1.629337	–0.332826	2.901302

TABLE S32: Cartesian coordinates of the modified base pair m^7G:G W:HT
Calculated energy (in Hartrees) = –1124.896396

ATOM	X	Y	Z
N	0.099128	0.036437	–0.035682
C	0.073833	0.115904	1.338458
N	1.281638	0.133868	1.850597
C	2.137065	0.063346	0.769395
C	3.562380	0.039630	0.722275
O	4.380890	0.076077	1.645099
N	4.013711	–0.044682	–0.615442
C	3.207552	–0.096328	–1.735157
N	3.825234	–0.221026	–2.938074
N	1.890996	–0.070035	–1.681525
C	1.416831	0.000684	–0.421260
N	1.745881	0.460007	8.599610
C	0.425037	0.492170	8.887290
N	–0.271409	0.446740	7.755572
C	0.624276	0.382914	6.697226
C	0.429138	0.316255	5.270989
O	–0.637450	0.301368	4.666528
N	1.671958	0.267731	4.620832
C	2.903957	0.280021	5.234075
N	3.987677	0.226926	4.459693
N	3.065791	0.342789	6.567409

C	1.911133	0.390667	7.220975
C	–1.735089	0.460540	7.632729
H	–0.839682	0.157677	1.914438
H	4.788640	0.049663	–3.058105
H	4.878952	0.236181	4.931856
H	–2.170416	0.517629	8.630067
H	–2.060021	–0.449079	7.127611
H	–2.035556	1.322326	7.036812
H	3.225544	–0.144433	–3.746198
H	5.020842	–0.110114	–0.709551
H	–0.696691	0.007065	–0.657366
H	1.603171	0.217497	3.571746
H	3.976927	0.174664	3.433642
H	2.498340	0.482467	9.275776
H	0.002891	0.546136	9.878259

TABLE S33: Cartesian coordinates of the modified base pair Ψ:A W:HT
Calculated energy (in Hartrees) = –882.216500

ATOM	X	Y	Z
N	4.225970	0.855955	–0.000712
C	2.876883	1.170653	–0.001263
N	2.044222	0.071652	–0.001180
C	2.427919	–1.266051	–0.000321
C	3.858121	–1.493576	0.000520
C	4.697372	–0.429901	0.000480
O	2.466420	2.328496	–0.002081
O	1.569264	–2.161495	0.000031
N	–2.429907	2.236634	0.000661
C	–1.075659	2.040423	0.000426
N	–0.745606	0.765415	–0.000253
C	–1.954173	0.087423	–0.000154
C	–2.288009	–1.289201	–0.000437
N	–1.372080	–2.271486	–0.000939
N	–3.602323	–1.619233	–0.000232
C	–4.516291	–0.636061	0.000279
N	–4.328641	0.688261	0.000638
C	–3.019746	0.992906	0.000399
H	5.776520	–0.527754	0.001404
H	–0.371709	2.860681	0.000574
H	–0.368462	–2.091759	–0.000531
H	–5.553263	–0.967684	0.000306
H	4.229430	–2.508982	0.001413
H	4.870416	1.636542	0.000126
H	1.024102	0.278947	–0.000895
H	–1.697097	–3.227223	–0.000398
H	–2.904167	3.130282	0.001363

TABLE S34: Cartesian coordinates of the modified base pair m^1A: m^5U H:WT
Calculated energy (in Hartrees) = –961.213955

ATOM	X	Y	Z
N	–0.027749	0.101952	0.249615
C	0.090506	0.011609	1.613752
N	1.376184	–0.074178	2.057404
C	2.554388	–0.080224	1.289754
C	2.340660	0.019341	–0.157633
C	3.544651	0.020153	–1.052292
C	1.065806	0.104985	–0.603074
O	–0.897616	0.009700	2.366103
O	3.638768	–0.164514	1.852001
N	2.975850	–0.439911	6.760762
C	2.966084	–0.354743	5.388348
N	1.747389	–0.269308	4.903672
C	0.924335	–0.299425	6.000693
C	–0.486700	–0.240902	6.095858
N	–1.300029	–0.139727	5.057700
N	–0.964412	–0.299307	7.394024
C	–2.417846	–0.247375	7.632817
C	–0.110436	–0.405566	8.481997
N	1.186356	–0.461494	8.430757
C	1.671826	–0.405847	7.172760
H	3.259959	0.098037	–2.103981
H	4.128704	–0.895176	–0.914180
H	4.207930	0.855285	–0.806022
H	0.821548	0.181563	–1.656490
H	3.863418	–0.358293	4.783117
H	–2.301033	–0.101002	5.176559
H	–2.910028	–1.095012	7.148571
H	–2.602012	–0.304699	8.704056
H	–2.827902	0.694490	7.258615
H	–0.607431	–0.442615	9.445192
H	3.787451	–0.513954	7.361093
H	–0.968316	0.166445	–0.113405
H	1.503153	–0.143372	3.075047
H	–0.959006	–0.093585	4.068533

TABLE S35: Cartesian coordinates of the modified base pair s^4U:A W:HT
Calculated energy (in Hartrees) = –1205.140661

ATOM	X	Y	Z
N	0.108459	0.299979	0.140505
C	0.162715	0.419471	1.522832
N	1.416825	0.236385	2.064801
C	2.579822	–0.045745	1.366891
C	2.425780	–0.151276	–0.064758

C	1.209497	0.022233	–0.631610
O	–0.844239	0.669004	2.176388
S	4.048609	–0.246467	2.137188
N	2.671516	0.450668	6.865818
C	2.715052	0.325472	5.501000
N	1.535064	0.475926	4.940890
C	0.665489	0.713424	5.998530
C	–0.728340	0.953562	6.083493
N	–1.541580	0.993343	5.012673
N	–1.266130	1.149043	7.306893
C	–0.464848	1.107005	8.380020
N	0.853976	0.891163	8.436903
C	1.359183	0.701364	7.212607
H	3.298376	–0.369871	–0.662802
H	1.035389	–0.045474	–1.699291
H	3.624203	0.123409	4.950902
H	–1.204394	0.880429	4.061214
H	–0.959383	1.272229	9.335297
H	–2.515829	1.186165	5.181771
H	3.445495	0.375834	7.508404
H	–0.806738	0.432914	–0.265322
H	1.459665	0.323167	3.106799

TABLE S36: Cartesian coordinates of the modified base pair m^5U:G W:HT
Calculated energy (in Hartrees) = –996.730150

ATOM	X	Y	Z
N	–4.668439	66.914772	124.834116
C	–4.542709	66.147363	123.674359
N	–3.574544	66.626363	122.806360
C	–2.763536	67.747640	122.993277
C	–2.972809	68.487348	124.243665
C	–2.127459	69.701594	124.493116
C	–3.914810	68.033413	125.100840
O	–5.228692	65.162677	123.475664
O	–1.929363	68.081033	122.151258
N	–1.386325	65.771852	118.494997
C	–1.849733	66.210865	119.716147
N	–2.815035	65.452055	120.179465
C	–2.995879	64.470460	119.222019
C	–3.921041	63.367205	119.170495
O	–4.786211	63.006130	119.946137
N	–3.715083	62.630513	117.950325
C	–2.793636	62.908004	116.974007
N	–2.805591	62.102170	115.857886
N	–1.953356	63.914187	117.035073
C	–2.110950	64.655975	118.161625

H	–2.373547	70.165706	125.452310
H	–1.063287	69.443425	124.493015
H	–2.266431	70.444004	123.700249
H	–4.127883	68.525039	126.044623
H	–1.463118	67.079343	120.232615
H	–3.045480	61.129497	115.989015
H	–5.368604	66.585616	125.481928
H	–0.654248	66.184179	117.936661
H	–2.006181	62.253434	115.258286
H	–4.382728	61.880064	117.820339
H	–3.444112	66.092237	121.929828

TABLE S37: Cartesian coordinates of the modified base pair m^1A:A H:WT
Calculated energy (in Hartrees) = –974.392266

ATOM	X	Y	Z
N	0.007861	–0.102049	–0.140508
C	–0.118448	0.158223	1.210050
N	1.033168	0.291372	1.823572
C	1.964701	0.112082	0.821102
C	3.374966	0.123180	0.841159
N	4.080594	0.354454	1.963220
N	4.020102	–0.094634	–0.332000
C	3.288196	–0.329182	–1.448593
N	1.972514	–0.368849	–1.580225
C	1.352588	–0.136779	–0.410671
N	9.145877	–0.843908	2.546486
C	7.804591	–0.823168	2.844884
N	7.058562	–0.470505	1.821212
C	7.957528	–0.252975	0.800713
C	7.757898	0.145400	–0.546980
N	6.580825	0.359751	–1.101150
N	8.937145	0.300275	–1.265982
C	8.871196	0.725516	–2.674827
C	10.178609	0.054451	–0.703713
N	10.396645	–0.333601	0.515849
C	9.264058	–0.474085	1.236773
H	–1.088705	0.236880	1.681115
H	3.566323	0.426230	2.828289
H	3.868062	–0.510636	–2.351810
H	7.434764	–1.073177	3.829467
H	5.626536	0.154744	–0.627369
H	8.404788	1.711559	–2.750835
H	9.882574	0.789987	–3.072125
H	8.311160	–0.005381	–3.264545
H	11.018938	0.209171	–1.371726
H	9.908870	–1.078634	3.168485

H	–0.736529	–0.245172	–0.808107
H	6.528512	0.675014	–2.058694
H	5.071442	0.129887	1.985915

TABLE S38: Cartesian coordinates of the modified base pair m1A: m^5Um H:WT
Calculated energy (in Hartrees) = –1421.531287

ATOM	X	Y	Z
N	–1.890965	0.559467	–0.336997
C	–0.541988	0.337989	–0.178073
N	0.202819	1.449030	0.074495
C	–0.231351	2.780513	0.180605
C	–1.668697	2.950047	–0.007722
C	–2.238003	4.336663	0.066429
C	–2.411491	1.845256	–0.259869
O	–0.032276	–0.799952	–0.254604
O	0.586721	3.665915	0.405380
C	–2.767266	–0.614000	–0.601104
C	–3.319289	–1.281319	0.689907
O	–3.342146	–2.692686	0.557397
C	–4.794828	–0.827633	0.704249
C	–5.100375	–0.718940	–0.800328
O	–5.645975	–1.676160	1.422043
O	–3.873414	–0.177973	–1.354760
C	–6.263517	0.173614	–1.174539
C	–2.168051	–3.352219	1.018961
N	5.130456	1.938039	0.429763
C	3.784700	2.221837	0.466396
N	3.046858	1.154542	0.258053
C	3.940767	0.132301	0.073273
C	3.724215	–1.236941	–0.205551
N	2.528360	–1.789436	–0.338707
N	4.888331	–1.974396	–0.333920
C	4.803390	–3.416635	–0.624420
C	6.140164	–1.390405	–0.200783
N	6.374232	–0.136947	0.047844
C	5.250507	0.598933	0.177314
H	–3.316514	4.333922	–0.106410
H	–1.766557	4.988474	–0.675922
H	–2.041727	4.783841	1.046183
H	–3.474112	1.894458	–0.454843
H	–2.154758	–1.326608	–1.159268
H	–2.761793	–0.981564	1.585679
H	–4.867332	0.169643	1.156179
H	–5.252349	–1.731331	–1.200509
H	–5.310164	–2.575418	1.276742
H	–6.385565	0.229177	–2.259184

H	–7.180915	–0.235278	–0.741626
H	–2.307293	–4.416431	0.820149
H	–1.274417	–2.991371	0.495811
H	–2.031463	–3.201847	2.098903
H	3.384143	3.212378	0.640548
H	4.301836	–3.579572	–1.582187
H	5.810623	–3.824554	–0.686889
H	4.267860	–3.931500	0.177675
H	6.973112	–2.074088	–0.323023
H	–6.122560	1.188205	–0.787226
H	5.894034	2.589689	0.559839
H	1.214385	1.303411	0.179338
H	2.425251	–2.769279	–0.554293
H	1.639027	–1.243968	–0.261482

TABLE S39: Cartesian coordinates of the modified base pair Um:A W:HT
Calculated energy (in Hartrees) =–1342.503811

ATOM	X	Y	Z
N	0.081125	0.369898	0.144847
C	0.055813	0.413612	1.539320
N	1.268289	0.574288	2.144403
C	2.523125	0.698749	1.525046
C	2.460715	0.634633	0.073269
C	1.270655	0.469946	–0.545723
O	–1.007833	0.317361	2.163026
O	3.537100	0.841145	2.199861
C	–1.222755	0.193430	–0.539968
C	–2.020700	1.517466	–0.684536
O	–3.413978	1.297359	–0.533982
C	–3.895973	1.426669	0.803694
C	–1.810745	1.878865	–2.169245
C	–1.719845	0.485087	–2.814098
O	–2.812631	2.705952	–2.699326
O	–0.983710	–0.295202	–1.843486
C	–1.017796	0.424562	–4.154234
N	2.658514	0.716310	6.820743
C	2.654635	0.753436	5.448200
N	1.446862	0.623744	4.948851
C	0.611469	0.492695	6.044722
C	–0.785452	0.314206	6.179898
N	–1.627044	0.243783	5.130127
N	–1.291823	0.214485	7.426939
C	–0.453536	0.288869	8.471417
N	0.874623	0.453052	8.477364
C	1.349217	0.547712	7.230061

H	1.263399	0.599759	3.190087
H	–1.791496	–0.525508	0.055297
H	–1.669279	2.288885	0.010133
H	–4.960451	1.186261	0.774970
H	–3.766330	2.455384	1.166961
H	–3.374683	0.746256	1.483704
H	–0.850471	2.395470	–2.291469
H	–2.737359	0.077468	–2.899756
H	–3.625591	2.448685	–2.233289
H	–1.578985	1.020818	–4.879722
H	–0.955577	–0.603313	–4.521425
H	3.546970	0.872551	4.848245
H	–2.600350	0.079812	5.332159
H	–0.923259	0.202061	9.449094
H	–0.003176	0.831128	–4.084795
H	3.459442	0.792953	7.429305
H	–1.310048	0.272909	4.164895
H	3.382186	0.704944	–0.488141
H	1.168666	0.380865	–1.618606

TABLE S40: Cartesian coordinates of the modified base pair D:U W:WT
Calculated energy (in Hartrees) = –830.888059

ATOM	X	Y	Z
N	–0.163983	0.204865	–0.043949
C	–0.102043	–0.161163	1.271593
O	0.937282	–0.077855	1.926399
N	–1.292269	–0.618649	1.829561
C	–2.549442	–0.594443	1.235117
O	–3.540314	–0.991492	1.815700
C	–2.579642	0.003988	–0.164621
C	–1.262869	–0.213736	–0.906932
N	0.258565	–1.610112	6.543899
C	0.174246	–1.247324	5.205736
O	–0.863402	–1.429657	4.571105
N	1.318327	–0.691134	4.695851
C	2.532403	–0.463438	5.383273
O	3.485814	0.039675	4.814386
C	2.507469	–0.882394	6.784769
C	1.391489	–1.431128	7.305391
H	–2.773357	1.078609	–0.056672
H	–3.423789	–0.435438	–0.699046
H	–1.233024	0.383041	–1.822066
H	–1.161996	–1.270591	–1.196354
H	3.402170	–0.737736	7.373847
H	1.316823	–1.759604	8.336008
H	–0.580717	–2.017705	6.929034

H	0.742729	0.394041	–0.446877
H	–1.230419	–0.932166	2.807591
H	1.276438	–0.422275	3.701255

TABLE S41: Cartesian coordinates of the modified base pair m^5C:G W:WT
Calculated energy (in Hartrees) = –976.851285

ATOM	X	Y	Z
N	0.091675	–0.138782	–0.120374
C	0.080397	–0.640131	1.171176
N	1.280040	–0.775647	1.673020
C	2.128557	–0.342272	0.668970
C	3.568327	–0.250827	0.635787
O	4.396381	–0.540993	1.485281
N	3.993402	0.273737	–0.625718
C	3.179580	0.622420	–1.670212
N	3.789018	1.127828	–2.785439
N	1.861883	0.529114	–1.643199
C	1.408205	0.058997	–0.456174
N	8.735633	0.058957	–2.250031
C	7.352340	0.320942	–2.282080
O	6.816833	0.793605	–1.282988
N	6.699450	0.022623	–3.447552
C	7.358826	–0.497967	–4.472944
N	6.642728	–0.756634	–5.594486
C	8.779163	–0.779683	–4.464333
C	9.414488	–0.471397	–3.300503
C	9.490827	–1.363365	–5.653886
H	–0.845421	–0.881614	1.675526
H	3.164812	1.223894	–3.571865
H	5.641246	–0.650383	–5.545570
H	10.477988	–0.632944	–3.157869
H	9.101136	–2.354523	–5.920352
H	10.557484	–1.482831	–5.447982
H	9.398520	–0.721917	–6.539054
H	–0.699113	0.059631	–0.713679
H	4.746309	0.852319	–2.990288
H	9.196420	0.274076	–1.375935
H	7.034090	–1.280169	–6.358517
H	5.001244	0.424782	–0.721446

TABLE S42: Cartesian coordinates of the modified base pair Ψ:C S:WC
Calculated energy (in Hartrees) =–1230.802926

ATOM	X	Y	Z
N	187.162514	95.929078	22.327165
C	186.581000	96.631666	21.282166
N	186.199213	95.797799	20.241219
C	186.339255	94.403776	20.138102
C	186.959491	93.769543	21.298519
C	187.325505	94.563754	22.334072
O	186.424961	97.839155	21.283179
O	185.927125	93.838798	19.126754
C	187.102954	92.259320	21.377975
C	188.065881	91.616740	20.353823
O	187.607526	90.312126	20.112684
C	189.373056	91.595826	21.167075
C	188.842051	91.169535	22.543278
O	190.316539	90.719617	20.585941
O	187.618435	91.919801	22.682099
C	189.742712	91.458271	23.730270
N	188.737369	89.834935	15.473336
C	188.331151	90.101607	16.804038
O	188.595983	89.265414	17.669389
N	187.673575	91.269814	17.030337
C	187.485322	92.139525	16.047304
N	186.858095	93.291604	16.359417
C	187.917038	91.897858	14.690682
C	188.540125	90.714642	14.456505
H	187.756071	94.130918	23.227794
H	186.120034	91.794118	21.239562
H	188.144006	92.195819	19.428246
H	188.040842	89.957228	19.307823
H	189.773217	92.622654	21.238899
H	188.609582	90.098109	22.487796
H	191.130378	90.772025	21.102836
H	189.249216	91.194657	24.670310
H	190.661626	90.864605	23.660412
H	186.498647	93.417568	17.303712
H	187.752904	92.615401	13.897080
H	188.902633	90.418643	13.477991
H	190.018160	92.518461	23.770074
H	187.441514	96.490141	23.118955
H	185.758370	96.258878	19.454856
H	186.599379	93.946672	15.641361
H	189.215372	88.956114	15.331343

TABLE S43: Cartesian coordinates of the modified base pair m^7G:A S:WT
Calculated energy (in Hartrees) = –1470.653620

ATOM	X	Y	Z
C	–3.440453	–4.190354	0.283791
C	–2.191467	–3.520744	–0.245454
O	–2.562428	–2.353568	–1.047035
C	–1.236076	–2.944132	0.802814
O	–0.470068	–3.980135	1.359798
C	–0.455645	–1.900919	–0.031437
O	0.481853	–2.569431	–0.825582
C	–1.589982	–1.355505	–0.939539
N	–2.280627	–0.134579	–0.387085
C	–3.594401	–0.091665	–0.081459
N	–3.947311	1.142267	0.274417
C	–2.818166	1.942319	0.187417
C	–2.633632	3.348581	0.420336
O	–3.447982	4.178607	0.781638
N	–1.280556	3.687770	0.157265
C	–0.281643	2.826848	–0.259923
N	0.937824	3.350567	–0.450734
N	–0.496103	1.528990	–0.469750
C	–1.762622	1.148805	–0.236736
C	–5.286149	1.583348	0.688385
N	5.212696	1.774230	0.413875
C	5.941086	0.886115	1.185447
N	5.481522	–0.339191	1.135657
C	4.405253	–0.258787	0.275226
C	3.470745	–1.224610	–0.157804
N	3.466528	–2.496243	0.272343
N	2.522520	–0.827183	–1.040381
C	2.468440	0.456178	–1.419162
N	3.244169	1.467258	–1.024071
C	4.214881	1.048370	–0.186702
H	–3.151788	–5.068079	0.868591
H	–4.010275	–3.517656	0.934690
H	–1.623285	–4.204198	–0.885155
H	–1.825802	–2.416054	1.570957
H	–0.007381	–3.647872	2.139391
H	–0.004607	–1.102699	0.573127
H	1.170984	–1.912226	–1.113865
H	–1.202673	–1.061471	–1.919692
H	–5.586374	2.435040	0.078455
H	–5.262408	1.887343	1.735707
H	–5.981882	0.755938	0.552181
H	6.798406	1.210131	1.759446

H	4.170118	–2.805462	0.924449
H	1.670932	0.702329	–2.113291
H	5.410219	2.753865	0.270048
H	2.711282	–3.112732	0.002634
H	1.104705	4.336315	–0.327446
H	1.734283	2.757849	–0.705417
H	–4.086348	–4.520134	–0.533892
H	–1.075129	4.669846	0.305854
H	–4.249613	–0.944140	–0.149273

TABLE S44: Cartesian coordinates of the modified base pair s^4U:A W:ST
Calculated energy (in Hartrees) = –1626.151569

ATOM	X	Y	Z
N	63.080614	13.734277	18.858436
C	64.288253	13.301401	19.400356
N	65.404870	13.641771	18.658064
C	65.456107	14.346207	17.460547
C	64.162561	14.735258	16.973290
C	63.039849	14.418311	17.667801
O	64.354963	12.677800	20.451503
S	66.914695	14.664153	16.722564
C	61.852106	13.345362	19.612504
C	61.689351	14.136441	20.934492
O	61.095450	13.335587	21.932746
C	60.646347	15.204198	20.551852
C	59.777787	14.438806	19.538627
O	59.937889	15.711753	21.653236
O	60.728256	13.618953	18.810289
C	58.992206	15.291278	18.564573
N	63.764080	8.838648	26.072000
C	62.590139	8.725055	26.789892
N	61.627090	9.491233	26.338237
C	62.201329	10.146075	25.263083
C	61.699032	11.099202	24.353072
N	60.436770	11.574166	24.416326
N	62.529194	11.552322	23.389836
C	63.793650	11.081729	23.324283
N	64.372846	10.188704	24.123935
C	63.531463	9.757246	25.074075
H	64.101210	15.275948	16.039832
H	62.042582	14.665800	17.330457
H	61.940560	12.275311	19.821922
H	62.640264	14.560136	21.272299
H	61.776380	12.739820	22.360083
H	61.136873	16.045705	20.045763
H	59.102843	13.776279	20.097300

H	59.939770	14.986463	22.300454
H	58.276355	15.903512	19.121113
H	58.440107	14.672480	17.851845
H	62.509812	8.057775	27.637334
H	59.798278	11.185601	25.090758
H	64.390909	11.486373	22.512729
H	60.099345	12.194759	23.695452
H	64.631943	8.349496	26.232715
H	59.655304	15.960006	18.005266
H	66.287055	13.334090	19.051445

TABLE S45: Cartesian coordinates of the modified base pair D:G W:ST
Calculated energy (in Hartrees) = –1379.642970

ATOM	X	Y	Z
C	72.648727	61.556358	35.225777
C	72.084038	60.181582	35.519633
O	72.860897	59.181641	34.825300
C	72.139570	59.743637	36.994342
O	71.124679	60.281069	37.805103
C	72.088694	58.211346	36.865808
O	70.707921	57.872975	36.757862
C	72.841516	57.958216	35.536904
N	74.232202	57.542589	35.768142
C	75.388747	58.241408	35.447865
N	76.483590	57.602799	35.773116
C	76.041468	56.423308	36.333741
C	76.789645	55.330897	36.894926
O	77.988127	55.144500	37.006307
N	75.879731	54.333953	37.394490
C	74.510794	54.364760	37.352835
N	73.844897	53.290441	37.857690
N	73.836089	55.368717	36.812584
C	74.647156	56.361200	36.333054
N	69.065170	53.567379	37.011195
C	70.362168	53.918234	37.223950
O	70.992729	53.585392	38.225957
N	70.947043	54.701161	36.215528
C	70.452511	54.845904	34.914857
O	71.088747	55.426139	34.057814
C	69.093503	54.212474	34.670414
C	68.257071	54.154061	35.947761
H	72.064282	62.309398	35.762994
H	72.605477	61.779308	34.156216
H	71.039080	60.120078	35.181244
H	73.103157	60.042682	37.423295
H	70.354845	59.705337	37.671090

H	72.557011	57.689219	37.705364
H	70.628530	56.911725	36.831924
H	72.348861	57.181055	34.942747
H	75.331268	59.203442	34.964332
H	74.341301	52.675829	38.484029
H	69.269815	53.197975	34.291519
H	67.910570	55.160894	36.223040
H	67.372504	53.530458	35.800304
H	68.594571	54.780358	33.883126
H	71.944251	54.946426	36.366353
H	72.848741	53.412588	38.052690
H	76.352973	53.532237	37.792861
H	73.691227	61.625442	35.553131
H	68.626037	53.102172	37.792283

TABLE S46: Cartesian coordinates of the modified base pair D:G H:SC
Calculated energy (in Hartrees) = –958.61992759

ATOM	X	Y	Z
N	0.000000	0.000000	0.000000
C	0.000000	0.000000	1.385860
N	1.204583	0.000000	1.892621
C	2.043331	0.005086	0.793548
C	3.481039	0.011044	0.719161
O	4.333214	0.002207	1.590171
N	3.888349	0.030170	–0.659935
C	3.072276	0.030498	–1.765106
N	3.676219	–0.003108	–2.987989
N	1.754877	0.014954	–1.679268
C	1.313115	0.005451	–0.395514
N	–1.720787	–1.203879	–5.141076
C	–1.650363	0.088202	–5.589374
O	–2.609915	0.784235	–5.866226
N	–0.334924	0.594813	–5.685258
C	0.823318	0.018395	–5.209182
O	1.886555	0.626521	–5.239568
C	0.657593	–1.368574	–4.625677
C	–0.564346	–2.095825	–5.185707
H	–0.922115	–0.000970	1.950691
H	3.084574	0.222156	–3.789211
H	1.575704	–1.927969	–4.818592
H	0.587111	–1.230530	–3.538984
H	–0.365948	–2.437243	–6.212609
H	–0.785823	–2.979718	–4.582855
H	–2.646986	–1.598953	–5.225571
H	–0.799460	0.026454	–0.614556

H	4.629697	0.320135	–3.052188
H	–0.274631	1.556753	–5.997899
H	4.894216	0.007218	–0.774779

TABLE S47: Cartesian coordinates of the modified base pair Um:G H:SC
Calculated energy (in Hartrees) = –1878.046729

ATOM	X	Y	Z
C	0.410266	–4.541402	0.624287
C	–0.055426	–3.238381	–0.019417
O	0.644524	–2.111415	0.572217
C	0.223675	–3.146557	–1.526520
O	–0.653283	–2.246411	–2.197250
C	1.606293	–2.475638	–1.570963
O	1.917120	–1.876527	–2.796760
C	1.512140	–1.490782	–0.377109
N	2.785586	–1.190053	0.243116
C	3.634504	–2.064188	0.914764
N	4.764146	–1.515775	1.274904
C	4.677661	–0.213252	0.819617
C	5.626957	0.866346	0.896913
O	6.746481	0.920715	1.376857
N	5.076894	2.037421	0.268903
C	3.834909	2.158197	–0.307003
N	3.508149	3.361794	–0.854471
N	2.980410	1.154594	–0.369214
C	3.457845	0.012352	0.183631
N	–2.363976	2.060024	0.518257
C	–2.681133	3.039715	–0.421508
N	–1.581506	3.691487	–0.953175
C	–0.222916	3.436751	–0.701213
C	–0.004149	2.407476	0.291430
C	–1.059738	1.774305	0.849793
O	–3.832172	3.291009	–0.752395
O	0.639399	4.073707	–1.305372
C	–3.505604	1.282879	1.073481
C	–3.971759	0.178506	0.094325
O	–5.357817	–0.085972	0.228287
C	–6.193014	0.727885	–0.596755
C	–3.243436	–1.060988	0.642202
C	–3.265608	–0.794082	2.156977
O	–3.828025	–2.282428	0.254240
O	–3.088626	0.642704	2.256161
C	–2.195376	–1.499542	2.965862
H	–0.215767	–5.377735	0.295785
H	1.449884	–4.772875	0.369978
H	–1.123392	–3.098029	0.177466

H	0.210302	–4.127957	–2.019831
H	2.386527	–3.214467	–1.360790
H	1.081899	–1.503337	–3.121887
H	1.106324	–0.532337	–0.717480
H	3.335522	–3.080443	1.127708
H	2.528380	3.510770	–1.101772
H	–4.304233	2.000359	1.274621
H	–3.718179	0.415343	–0.945300
H	–7.223873	0.479502	–0.338202
H	–6.021823	0.510584	–1.659863
H	–6.012078	1.794056	–0.426706
H	–2.206893	–1.066224	0.287461
H	–4.263090	–1.047116	2.542234
H	–4.784003	–2.110452	0.219292
H	–2.392333	–2.576122	2.956740
H	–2.206064	–1.159285	4.004832
H	1.010963	2.123154	0.529742
H	–1.791824	4.400563	–1.645684
H	–0.948013	1.008110	1.604413
H	–1.197943	–1.332089	2.546336
H	4.003875	4.181642	–0.540187
H	5.721749	2.817989	0.252000
H	0.337322	–4.465142	1.712105
C	–1.888016	–2.815983	–2.620908
H	–2.543933	–3.049422	–1.774000
H	–2.377671	–2.067358	–3.248105
H	–1.718792	–3.722881	–3.218348

TABLE S48: Cartesian coordinates of the modified base pair Gm:G S:SC
Calculated energy (in Hartrees) = –1966.483286

ATOM	X	Y	Z
C	–0.397878	–4.659359	–0.047189
C	–0.356863	–3.287213	0.590260
O	–0.730688	–2.276254	–0.397824
C	–1.331430	–3.050555	1.756040
O	–0.886152	–3.549553	2.988781
C	–1.490642	–1.517832	1.723791
O	–0.353097	–0.995440	2.391943
C	–0.515607	0.326571	2.905028
C	–1.444118	–1.204709	0.210699
N	–2.774909	–1.130970	–0.394197
C	–3.534349	–2.161479	–0.938024
N	–4.739409	–1.787248	–1.282947
C	–4.796851	–0.450202	–0.950003
C	–5.876679	0.495929	–1.072608
O	–7.005606	0.380341	–1.508360

N	–5.452142	1.777965	–0.558613
C	–4.220244	2.089510	–0.054338
N	–3.992094	3.396006	0.304066
N	–3.240946	1.213881	0.046722
C	–3.587322	–0.023696	–0.405859
C	6.025152	–2.185737	–0.285538
C	4.521489	–2.107754	–0.457413
O	3.938969	–1.317983	0.612620
C	4.032103	–1.431421	–1.753661
O	4.041627	–2.269794	–2.880682
C	2.622643	–0.974004	–1.334422
O	1.822817	–2.139644	–1.433569
C	2.853322	–0.552783	0.134369
N	3.205487	0.874866	0.243693
C	4.475388	1.410227	0.406067
N	4.493209	2.719402	0.393926
C	3.175998	3.078233	0.213899
C	2.583081	4.386436	0.108012
O	3.072616	5.500317	0.157312
N	1.163819	4.250054	–0.077286
C	0.450666	3.081200	–0.129012
N	–0.921833	3.213593	–0.215931
N	1.002036	1.891439	–0.043805
C	2.356711	1.950560	0.129286
H	–0.123449	–5.406866	0.703199
H	0.311842	–4.724018	–0.875910
H	0.660829	–3.063566	0.931780
H	–2.294554	–3.525342	1.536665
H	–0.251597	–2.893583	3.320754
H	–2.417500	–1.162008	2.188419
H	–0.687030	1.051875	2.103146
H	–1.348624	0.365822	3.619591
H	0.414035	0.576875	3.418535
H	–0.934309	–0.255501	0.020337
H	–3.115898	–3.146219	–1.077194
H	–3.138042	3.534190	0.830541
H	6.288826	–2.635211	0.675775
H	6.444820	–2.804467	–1.084650
H	4.087264	–3.115674	–0.416188
H	4.659701	–0.562381	–1.981508
H	3.227726	–2.795550	–2.813063
H	2.223965	–0.150590	–1.937431
H	0.882908	–1.949207	–1.255573
H	1.964382	–0.721407	0.752042
H	5.328487	0.767869	0.554648
H	–1.423511	2.333296	–0.321505

H	6.485355	–1.193938	–0.342837
H	–1.399091	–4.896376	–0.421108
H	0.672786	5.135792	–0.084019
H	–1.254819	3.930624	–0.848793
H	–4.779511	3.947769	0.612404
H	–6.158838	2.497425	–0.655510

TABLE S49: Cartesian coordinates of the modified base pair s^4U:A S:SC
Calculated energy (in Hartrees) = –2047.169603

ATOM	X	Y	Z
N	–3.655872	–0.616955	–0.436072
C	–3.729568	0.756181	–0.666144
N	–4.902116	1.347758	–0.230477
C	–5.989004	0.742610	0.389855
C	–5.830135	–0.674916	0.562007
C	–4.697760	–1.297367	0.147024
O	–2.827828	1.383633	–1.203711
S	–7.312636	1.638433	0.855288
C	–2.414950	–1.287120	–0.917264
C	–1.176556	–0.989650	–0.028996
O	–0.028432	–0.885240	–0.837968
C	–1.111890	–2.258114	0.837955
C	–1.568658	–3.337606	–0.156121
O	0.186611	–2.431884	1.360104
O	–2.624168	–2.682855	–0.898778
C	–2.106131	–4.623562	0.440946
C	6.419810	–1.775032	1.008456
C	4.958573	–1.823989	0.613407
O	4.222692	–0.824410	1.358988
C	4.656541	–1.495219	–0.860079
O	4.874664	–2.562734	–1.746796
C	3.182994	–1.054977	–0.782217
O	2.419967	–2.240997	–0.823941
C	3.106510	–0.384597	0.612642
N	3.166712	1.086063	0.536253
C	4.201483	1.912270	0.941096
N	3.956229	3.190969	0.772330
C	2.686580	3.216161	0.229711
C	1.847240	4.274539	–0.177486
N	2.224827	5.565874	–0.086339
N	0.626153	3.988976	–0.673096
C	0.256183	2.708589	–0.767279
N	0.961086	1.616836	–0.422125
C	2.175174	1.924145	0.077403
H	–6.633921	–1.234705	1.018132

H	–4.544016	–2.364608	0.229153
H	–2.228931	–0.922946	–1.930363
H	–1.315848	–0.082753	0.565431
H	0.327612	0.047013	–0.751369
H	–1.861873	–2.175953	1.641401
H	–0.735429	–3.555101	–0.837974
H	0.173427	–3.161359	1.992577
H	–2.492528	–5.282730	–0.340794
H	–1.304952	–5.158834	0.962023
H	6.849182	–0.791239	0.791290
H	6.549918	–1.983148	2.074122
H	4.534568	–2.813177	0.835079
H	5.284691	–0.654960	–1.180888
H	4.049320	–3.076024	–1.729797
H	2.897951	–0.357566	–1.579810
H	1.475481	–1.984899	–0.775797
H	2.170774	–0.655653	1.113640
H	5.099062	1.492091	1.367026
H	1.587395	6.284914	–0.387151
H	–0.738429	2.521405	–1.162572
H	3.130502	5.802505	0.283686
H	–2.911939	–4.422335	1.154946
H	6.971535	–2.527293	0.436734
H	–4.957195	2.347593	–0.389332

TABLE S50: Cartesian coordinates of the modified base pair $m^6{}_2$A:G S:ST
Calculated energy (in Hartrees) = –1910.701748

ATOM	X	Y	Z
C	7.096692	0.770065	0.580267
C	5.775000	0.944670	–0.141211
O	5.372395	–0.313330	–0.721500
C	4.584622	1.366874	0.738791
O	4.518749	2.754954	0.979614
C	3.387445	0.855552	–0.093630
O	3.096659	1.844729	–1.070798
C	3.960044	–0.412281	–0.770483
N	3.578697	–1.660017	–0.099743
C	4.380695	–2.484578	0.680329
N	3.771642	–3.571863	1.079176
C	2.505466	–3.477406	0.536829
C	1.391892	–4.385694	0.609284
O	1.263575	–5.462472	1.168811
N	0.287043	–3.849815	–0.136579
C	0.249445	–2.657340	–0.817803
N	–0.906021	–2.353664	–1.481426
N	1.280756	–1.836239	–0.877897

C	2.364902	–2.300190	–0.200610
C	–7.425712	–0.014532	0.733766
C	–6.237717	–0.680905	0.068454
O	–5.583514	0.274819	–0.803697
C	–4.193214	0.020303	–0.865519
N	–3.470085	1.219184	–0.436797
C	–2.136187	1.463146	–0.677430
N	–1.296373	0.695944	–1.391846
C	–0.067542	1.207688	–1.407217
N	0.365129	2.329292	–0.811920
C	–0.492065	3.113054	–0.102289
N	0.004335	4.238621	0.456044
C	–1.845661	2.666533	–0.021607
N	–2.986250	3.157202	0.596396
C	–3.919252	2.276048	0.330876
C	–0.760322	5.088632	1.365949
C	1.417065	4.577253	0.286331
C	–3.917078	–1.202548	0.041252
O	–3.867063	–2.419451	–0.707778
C	–5.112240	–1.167115	1.009250
N	–5.272008	–2.411729	1.725650
H	7.878868	0.435501	–0.106731
H	7.401070	1.726876	1.015492
H	5.881912	1.688190	–0.945620
H	4.633532	0.860005	1.708808
H	4.175362	3.109257	0.140140
H	2.505764	0.622034	0.509512
H	2.160436	2.135415	–0.957414
H	3.605221	–0.466364	–1.805364
H	5.405992	–2.220772	0.885132
H	–1.016420	–1.365858	–1.703117
H	–8.127076	0.369420	–0.011499
H	–7.959252	–0.737899	1.359146
H	–6.592084	–1.529672	–0.541502
H	–3.875726	–0.186124	–1.894852
H	0.688987	0.633946	–1.933517
H	–4.951846	2.344158	0.636437
H	–0.715787	6.127585	1.021428
H	–1.799458	4.770182	1.404084
H	–0.332111	5.042386	2.375050
H	1.707828	4.492393	–0.762438
H	1.555590	5.610479	0.608819
H	2.072996	3.932715	0.882812
H	–2.954471	–1.133060	0.547770
H	–4.566141	–2.420731	–1.376154
H	–4.925760	–0.365307	1.737698

H	–6.118778	–2.421023	2.285052
H	–5.292706	–3.195650	1.080571
H	–7.101842	0.817293	1.367572
H	7.006170	0.034679	1.386427
H	–1.769460	–2.788373	–1.179175
H	–0.522940	–4.457455	–0.140645

TABLE S51: Cartesian coordinates of the modified base pair Am:G S:ST
Calculated energy (in Hartrees) = –1891.250622

ATOM	X	Y	Z
C	7.872976	–0.226560	–1.042719
C	6.624312	0.640860	–1.102449
O	5.477948	–0.213081	–1.369392
C	6.296023	1.401433	0.191879
O	5.791582	2.678437	–0.155077
C	5.181608	0.551922	0.827510
O	4.427945	1.378727	1.681067
C	4.449252	0.028816	–0.434195
C	3.576783	0.719971	2.614952
N	3.715184	–1.216900	–0.265923
C	4.244434	–2.498553	–0.239451
N	3.342991	–3.439953	–0.100541
C	2.148089	–2.749284	–0.035170
C	0.813406	–3.182766	0.108369
N	0.483694	–4.483656	0.231662
N	–0.158796	–2.246374	0.139931
C	0.176424	–0.949387	0.021797
N	1.396631	–0.423074	–0.116220
C	2.351017	–1.369847	–0.144506
C	–7.254411	–1.928655	–0.534775
C	–5.740668	–2.002137	–0.531636
O	–5.197364	–0.916402	–1.319173
C	–5.069673	–1.852746	0.846903
O	–5.056577	–3.030636	1.613431
C	–3.660226	–1.379013	0.439894
O	–2.963426	–2.553977	0.065643
C	–3.965018	–0.477119	–0.782062
N	–4.102442	0.946677	–0.434795
C	–5.279626	1.658096	–0.236684
N	–5.084005	2.927029	0.016648
C	–3.712926	3.077798	–0.018620
C	–2.901323	4.253382	0.156547
O	–3.198057	5.411390	0.394521
N	–1.512492	3.908217	–0.002767
C	–0.997931	2.663898	–0.267240
N	0.359755	2.570076	–0.412960

N	–1.756916	1.597670	–0.417085
C	–3.086520	1.863518	–0.299983
H	8.768607	0.398199	–0.970102
H	7.951010	–0.833936	–1.948125
H	6.701497	1.369307	–1.916781
H	7.169788	1.483647	0.854073
H	5.283784	2.986142	0.611604
H	5.605420	–0.303313	1.375972
H	3.728408	0.781031	–0.777681
H	3.259623	1.478150	3.333844
H	4.114720	–0.073771	3.150637
H	2.690787	0.296521	2.129663
H	5.305390	–2.659076	–0.359582
H	–0.487508	–4.751098	0.250993
H	–0.648977	–0.241417	0.018394
H	–7.604468	–0.992601	–0.087061
H	–7.656222	–2.761599	0.050308
H	–5.407932	–2.953896	–0.968654
H	–5.582010	–1.076525	1.427481
H	–4.288043	–3.522138	1.275496
H	–3.139902	–0.825374	1.229839
H	–1.987067	–2.425299	0.122865
H	–3.165363	–0.553580	–1.526669
H	–6.238835	1.173373	–0.326080
H	0.929465	3.270392	0.036907
H	–7.650301	–1.994629	–1.551940
H	7.851280	–0.900592	–0.179459
H	1.196562	–5.187895	0.129758
H	0.759760	1.633988	–0.421720
H	–0.897115	4.710559	0.052028

TABLE S52: Cartesian coordinates of the modified base pair m^7G:A S:ST
Calculated energy (in Hartrees) = –1892.438401

ATOM	X	Y	Z
C	–5.805286	–3.464739	1.183389
C	–4.717594	–3.026643	0.213814
O	–4.928554	–1.622876	–0.103002
C	–3.275408	–3.133034	0.750239
O	–2.437673	–3.593682	–0.294020
C	–2.951164	–1.675327	1.112798
O	–1.560224	–1.489910	1.203952
C	–3.697666	–0.934176	–0.013064
N	–3.982565	0.462819	0.291878
C	–5.251659	1.083001	–0.141308
N	–4.995962	2.524524	0.022270
C	–3.586644	2.652788	–0.083478

C	–2.774189	3.806960	–0.218109
O	–3.073624	4.993358	–0.358849
N	–1.380445	3.438986	–0.210043
C	–0.878385	2.181309	–0.059081
N	0.468869	2.007625	–0.051705
N	–1.674705	1.133337	0.116042
C	–3.001804	1.418184	0.098774
C	–5.828841	3.388712	–0.801631
C	5.579639	0.297882	2.319275
C	5.887527	1.285445	1.197666
O	5.959828	0.664086	–0.116378
C	4.828426	2.361269	0.976658
O	5.363170	3.487883	0.276819
C	3.784487	1.645771	0.068947
O	3.185632	2.554352	–0.829430
C	4.650211	0.567674	–0.654258
N	4.174083	–0.818705	–0.488852
C	5.022132	–1.919883	–0.433915
N	4.405383	–3.072463	–0.491405
C	3.073768	–2.728379	–0.620501
C	1.911319	–3.518072	–0.744565
N	1.957268	–4.865892	–0.724812
N	0.717160	–2.911788	–0.888289
C	0.694621	–1.570769	–0.899156
N	1.715624	–0.711716	–0.765880
C	2.896352	–1.342161	–0.634176
H	–5.754568	–4.545286	1.352298
H	–5.703589	–2.955893	2.147200
H	–4.776842	–3.603593	–0.716652
H	–3.216227	–3.793983	1.628341
H	–1.530539	–3.290402	–0.110601
H	–3.457608	–1.404683	2.052562
H	–1.379607	–0.557348	0.954240
H	–3.132406	–1.016033	–0.958260
H	–5.472109	0.827407	–1.200825
H	1.076206	2.711804	–0.450136
H	–6.869908	3.316160	–0.470862
H	–5.785681	3.123392	–1.873745
H	–5.486684	4.417471	–0.683259
H	6.306994	–0.517531	2.310665
H	5.647350	0.805313	3.286879
H	6.870706	1.737392	1.361687
H	4.387669	2.749841	1.896775
H	6.036732	3.154236	–0.336244
H	2.999289	1.174236	0.663152
H	3.782268	3.326002	–0.841156

H	4.703708	0.792171	–1.724127
H	6.088181	–1.780014	–0.342067
H	1.102029	–5.386330	–0.835677
H	–0.286525	–1.123153	–1.010448
H	–6.791738	–3.226624	0.775781
H	4.580640	–0.136463	2.230623
H	2.839386	–5.340965	–0.629591
H	0.806341	1.062149	–0.237831
H	–0.757093	4.228720	–0.321568
H	–6.086824	0.744358	0.475783

TABLE S53: Cartesian coordinates of the modified base pair D:U S:ST
Calculated energy (in Hartrees) = –1672.908753

ATOM	X	Y	Z
C	6.145000	2.263000	–0.552000
C	4.647000	2.106000	–0.694000
O	4.338000	0.736000	–1.031000
C	3.823000	2.351000	0.581000
O	3.617000	3.706000	0.888000
C	2.985000	0.478000	–0.711000
C	2.515000	1.586000	0.276000
O	1.654000	2.509000	–0.358000
N	2.907000	–0.892000	–0.149000
C	1.646000	–1.341000	0.090000
O	0.657000	–0.597000	0.032000
N	1.490000	–2.686000	0.411000
C	2.499000	–3.605000	0.672000
O	2.252000	–4.767000	0.932000
C	3.889000	–2.998000	0.631000
C	3.983000	–1.863000	–0.388000
C	–5.056000	–3.068000	–0.735000
C	–3.637000	–2.579000	–0.992000
O	–3.675000	–1.156000	–1.273000
C	–2.659000	–2.771000	0.191000
O	–1.349000	–3.131000	–0.253000
C	–2.588000	–1.352000	0.796000
O	–1.509000	–1.062000	1.619000
C	–2.719000	–0.481000	–0.473000
N	–3.202000	0.880000	–0.217000
C	–2.287000	1.934000	–0.273000
O	–1.114000	1.785000	–0.595000
N	–2.818000	3.161000	0.060000
C	–4.145000	3.478000	0.433000
O	–4.451000	4.627000	0.700000
C	–5.016000	2.313000	0.440000
C	–4.522000	1.097000	0.124000

H	6.660000	2.044000	–1.492000
H	6.532000	1.590000	0.221000
H	4.264000	2.770000	–1.484000
H	4.338000	1.880000	1.428000
H	2.358000	0.526000	–1.612000
H	2.058000	1.159000	1.172000
H	0.744000	2.161000	–0.379000
H	4.602000	–3.793000	0.406000
H	4.116000	–2.620000	1.636000
H	3.917000	–2.262000	–1.410000
H	4.931000	–1.335000	–0.303000
H	–5.456000	–2.658000	0.198000
H	–5.081000	–4.161000	–0.667000
H	–3.226000	–3.080000	–1.880000
H	–3.029000	–3.505000	0.918000
H	–3.502000	–1.213000	1.389000
H	–1.753000	–0.373000	–0.974000
H	–6.058000	2.453000	0.692000
H	–5.139000	0.209000	0.086000
H	–2.167000	3.937000	0.028000
H	–5.712000	–2.761000	–1.554000
H	6.372000	3.293000	–0.262000
H	–1.393000	–4.014000	–0.644000
H	–0.714000	–0.919000	1.059000
H	0.517000	–2.990000	0.476000
H	2.785000	3.935000	0.440000