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Supporting information

Predicting nearest neighbor free energies of modified RNA with LIE: Results for pseudouridine and N1-methylpseudouridine within RNA duplexes

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Methods

Molecular dynamics simulations

In the present work for each of the duplexes 15-18 (Table S1), the centroid structure from the most populated cluster obtained from the last 400 ns of the 500 ns simulations at 300K (detailed protocol in Dutta et al., 2023 [1]), was further subjected to an additional set of minimization, heating, equilibration and production run steps.

The conditions were the same as mentioned in Dutta et al., 2023 [1] except that the heating was carried out from 0K to 310K and the equilibration and production runs were performed at 310K. The production run was carried out for 50 ns and the trajectory corresponding to the last 40 ns simulation was utilized for the analysis of stacking and base pairing energies and calculation of a set of nearest neighbor thermodynamic parameters (at 310K). The MD simulations were performed using the AMBER18 software package using the GPU version of the *pmemd* module.

Analyses

The hydrogen bonding energies and the base stacking energies were calculated following the same method as mentioned in the main text

Table S1. List of the RNA duplexes considered in this work.

Description	**Sl No.**	**Sequences**
unmodified duplexes (containing U(5)-X(14) base pair where X=>A/G/U/C)	1	5′-UCA**GU**CAGU-3′ 3′-AGU**CAG**UCA-5′
	2	5′-UCA**CUG**AGU-3′ 3′-AGU**GAC**UCA-5′
	3	5′-UCA**UUA**AGU-3′ 3′-AGU**AAU**UCA-5′

	4	5′-UCA**AUU**AGU-3′ 3′-AGU**UAA**UCA-5′
	5	5′-UCA**GUC**AGU-3′ 3′-AGU**CGG**UCA-5′
	6	5′-UCA**GUC**AGU-3′ 3′-AGU**CUG**UCA-5′
	7	5′-UCA**GUC**AGU-3′ 3′-AGU**CCG**UCA-5′
Ψ-modified duplexes (containing Ψ(5)-X(14) base pair where X=>A/G/U/C)	8	5′-UCA**GΨC**AGU-3′ 3′-AGU**CAG**UCA-5′
	9	5′-UCA**CΨG**AGU-3′ 3′-AGU**GAC**UCA-5′
	10	5′-UCA**UΨA**AGU-3′ 3′-AGU**AAU**UCA-5′
	11	5′-UCA**AΨU**AGU-3′ 3′-AGU**UAA**UCA-5′
	12	5′-UCA**GΨC**AGU-3′ 3′-AGU**CGG**UCA-5′
	13	5′-UCA**GΨC**AGU-3′ 3′-AGU**CUG**UCA-5′
	14	5′-UCA**GΨC**AGU-3′ 3′-AGU**CCG**UCA-5′
$m^1Ψ$-modified duplexes (containing $m^1Ψ$(5)-X(14)	15	5′-UCA**Gm^1ΨA**GU-3′ 3′-AGU**CAG**UCA-5′

base pair where X=>A/G/U/C)	16	5′-UCA**Cm^{1}ΨG**AGU-3′ 3′-AGU**GAC**UCA-5′
	17	5′-UCA**Um^{1}ΨA**AGU-3′ 3′-AGU**AAU**UCA-5′
	18	5′-UCA**Am^{1}ΨU**AGU-3′ 3′-AGU**UAA**UCA-5′
	19	5′-UCA**Gm^{1}ΨC**AGU-3′ 3′-AGU**CGG**UCA-5′
	20	5′-UCA**Gm^{1}ΨC**AGU-3′ 3′-AGU**CUG**UCA-5′
	21	5′-UCA**Gm^{1}ΨC**AGU-3′ 3′-AGU**CCG**UCA-5′

Table S2. Interaction energies (kcal/mol) of the base-pair steps containing the m^{1}Ψ-A base pair obtained from the last 400 ns of MD simulations at 300K for (A) duplex-Gm^{1}ΨC, (B) duplex-Cm^{1}ΨG, (C) duplex-Am^{1}ΨU (D) duplex-Um^{1}ΨA respectively.

(A)	**Y=m^{1}Ψ** [a]	**Y=U** [b]	**Y=Ψ** [c]
Stacking energies			
Step 1 **4 5** **5'- G Y -3'** **3'- C A -5'** **15 14**	-15.98 (1.84) -15.95 (1.81)	-9.57 (2.6) -12.00 -9.56 -9.59	-10.58 (2.51) -12.48 -10.55 -10.58
Step 2 **5 6** **5'- Y C -3'** **3'- A G -5'** **14 13**	-10.33 (3.21) -10.3 (1.71)	-9.45 (2.33) -12.66 -9.44 -9.42	-9.84 (2.3) -11.99 -9.82 -9.85

Base pairing energies			
4 15 **G - C**	-26.09 (3.04) -26.05 (3.08)	-26.0 (3.10) -23.31 -26.0 -26.01	-25.93 (3.10) -23.18 -25.92 -25.95
5 14 **Y - A**	-10.22 (2.76) -10.53 (2.33)	-9.24 (2.48) -12.66 -9.27 -9.22	-8.99 (2.45) -12.33 -8.96 -8.99
6 13 **C - G**	-26.06 (3.08) -26.06 (2.59)	-26.08 (3.03) -24.02 -26.07 -26.07	-26.10 (3.0) -23.93 -26.08 -26.12

[a] The values reported in Dutta et al., 2023 [1] from the last 400 ns of MD simulations (values from the second set of simulations are shown in purple).
[b] The values reported in Deb et al., 2019 [2] for U-A pair.
[c] The values reported in Deb et al., 2019 [2] for Ψ-A pair.
The values corresponding to the QM energies reported in Deb et al., 2019 [2] are shown in blue.
The values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown.
The values obtained from the last 200 ns (of the 400 ns trajectory) are shown in green.

(B)	**Y=m^{1}Ψ** [a]	**Y=U** [b]	**Y=Ψ** [c]
Stacking energies			
Step 1 **4 5** **5'- C Y -3'** **3'- G A -5'** **15 14**	-11.78 (1.54) -11.74 (1.54)	-8.34 (2.18) -12.31 -8.32 -8.35	-7.9 (2.03) -12.23 -7.9 -7.91
Step 2 **5 6** **5'- Y G -3'** **3'- A C -5'** **14 13**	-13.31 (1.81) -13.42 (1.79)	-8.64 (2.35) -12.71 -8.67 -8.60	-10.24 (2.16) -14.32 -10.24 -10.24

Base pairing energies			
4 15 **C - G**	-26.21 (3.00) -26.23 (2.99)	-25.99 (3.08) -23.42 -25.95 -26.03	-25.94 (3.08) -23.57 -25.97 -25.91
5 14 **Y - A**	-10.80 (2.23) -10.81 (2.22)	-9.65 (2.37) -12.77 -9.64 -9.66	-9.46 (2.24) -12.38 -9.45 -9.47
6 13 **G - C**	-26.06 (3.03) -26.04 (3.01)	-26.05 (3.01) -24.06 -26.07 -26.04	-26.03 (3.00) [d] -24.06 -26.05 -26.02

[a] The values reported in Dutta et al., 2023 [1] from the last 400 ns of MD simulations (values from the second set of simulations are shown in purple).
[b] The values reported in Deb et al., 2019 [2] for U-A pair.
[c] The values reported in Deb et al., 2019 [2] for Ψ-A pair.
[d] This value was recalculated during the present study from the Coulomb and VDW components reported in Deb et al., 2019 [2]
The values corresponding to the QM energies reported in Deb et al., 2019 [2] are shown in blue.
The values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown.
The values obtained from the last 200 ns (of the 400 ns trajectory) are shown in green.

(C)	**Y=m^{1}Ψ** [a]	**Y=U** [b]	**Y=Ψ** [c]
Stacking energies			
Step 1 **4 5** **5'- A Y -3'** **3'- U A -5'** **15 14**	-14.00 (1.82) -14.02 (1.79)	-8.12 (2.16) -11.44 -8.05 -8.18	-8.39 (2.12) -11.14 -8.35 -8.43
Step 2 **5 6** **5'- Y U -3'** **3'- A A -5'** **14 13**	-9.4 (1.39) -9.41 (1.38)	-6.59 (1.92) -9.64 -6.57 -6.61	-7.68 (1.83) -10.69 -7.69 -7.67
Base pairing energies			

4 15 **A - U**	-9.46 (2.39) –9.48 (2.37)	-9.31 (2.49) -12.39 -9.39 -9.22	-9.38 (2.40) -12.47 -9.4 -9.36
5 14 **Y - A**	-10.65 (2.23) -10.62 (2.26)	-9.41 (2.39) -12.19 -9.41 -9.42	-9.15 (2.32) -12.02 -9.17 -9.13
6 13 **U - A**	-9.61 (2.30) -9.62 (2.29)	-9.67 (2.27) -12.65 -9.67 -9.66	-9.71 (2.26) -12.77 -9.70 -9.71
[a] The values reported in Dutta et al., 2023 [1] from the last 400 ns of MD simulations (values from the second set of simulations are shown in purple). [b] The values reported in Deb et al., 2019 [2] for U-A pair. [c] The values reported in Deb et al., 2019 [2] for Ψ-A pair. The values corresponding to the QM energies reported in Deb et al., [2] are shown in blue. The values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown. The values obtained from the last 200 ns (of the 400 ns trajectory) are shown in green.			

(D)	**Y=m^{1}Ψ** [a]	**Y=U** [b]	**Y=Ψ** [c]
Stacking energies			
Step 1 **4 5** **5'- U Y-3'** **3'- A A -5'** **15 14**	-11.17 (1.55) -11.11 (1.59)	-6.5 (1.93) -9.62 -6.55 -6.43	-6.13 (1.88) -9.54 -6.13 -6.13
Step 2 **5 6** **5'- Y A -3'** **3'- A U -5'** **14 13**	-13.74 (1.93) -13.86 (1.92)	-10.42 (2.37) -12.66 -10.34 -10.51	-11.29 (3.16) -13.62 -11.32 -11.26
Base pairing energies			
4 15 **U - A**	-9.55 (2.34)	-9.38 (2.37) -12.19	-9.36 (2.37) -12.23

	-9.56 (2.29)	-9.40 -9.37	-9.35 -9.35
5 14 **Y - A**	-10.74 (2.19) -10.73 (2.17)	-9.63 (2.29) -12.63 -9.65 -9.61	-9.32 (2.25) -12.17 -9.32 -9.33
6 13 **A - U**	-9.70 (2.26) -9.66 (2.28)	-9.70 (2.27) -12.69 -9.69 -9.68	-9.68 (2.29) -12.65 -9.66 -9.68

[a] The values reported in Dutta et al, 2023 [1] from the last 400 ns of MD simulations (values from the second set of simulations are shown in purple).
[b] The values reported in Deb et al, 2019 [2] for U-A pair.
[c] The values reported in Deb et al, 2019 [2] for Ψ-A pair.
The values corresponding to the QM energies reported in Deb et al, 2019 [2] are shown in blue.
The values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown.
The values obtained from the last 200 ns (of the 400 ns trajectory) are shown in green.

Table S3. Interaction energies (kcal/mol) of the base-pair steps containing the $m^1\Psi$-A base pair obtained from the last 40 ns of MD simulations at 310K for (A) duplex-Gm$^1\Psi$C, (B) duplex-Cm$^1\Psi$G, (C) duplex-Am$^1\Psi$U and (D) duplex-Um$^1\Psi$A respectively.

(A)		**(B)**		**(C)**		**(D)**	
Stacking energies							
Step 1 **4 5** **5'- G m$^1\Psi$ -3'** **3'- C A -5'** **15 14**	-15.96 (1.82) -15.97 (1.81)	**Step 1** **4 5** **5'- C m$^1\Psi$ -3'** **3'- G A -5'** **15 14**	-11.67 (1.59) -11.75 (1.52)	**Step 1** **4 5** **5'- A m$^1\Psi$ -3'** **3'- U A -5'** **15 14**	-13.95 (1.82) -14.05 (1.74)	**Step 1** **4 5** **5'- U m$^1\Psi$ -3'** **3'- A A -5'** **15 14**	-11.13 (1.66) -11.11 (1.52)
Step 2 **5 6** **5'- m$^1\Psi$ C -3'** **3'- A G -5'** **14 13**	-10.32 (1.73) -10.26 (1.65)	**Step 2** **5 6** **5'- m$^1\Psi$ G -3'** **3'- A C -5'** **14 13**	-13.30 (1.84) -13.31 (1.80)	**Step 2** **5 6** **5'- m$^1\Psi$ U -3'** **3'- A A -5'** **14 13**	-9.26 (1.43) -9.49 (1.37)	**Step 2** **5 6** **5'- m$^1\Psi$ A -3'** **3'- A U -5'** **14 13**	-13.55 (1.94) -13.84 (1.95)
Base pairing energies							

4 15 **G - C**	-26.00 (3.18) -26.06 (3.07)	**4 15** **C - G**	-26.17 (3.00) -26.2 (3.04)	**4 15** **A - U**	-9.37 (2.46) -9.49 (2.36)	**4 15** **U - A**	-9.51 (2.34) -9.59 (2.29)
5 14 **m^{1}Ψ - A**	-10.47 (2.36) -10.55 (2.30)	**5 14** **m^{1}Ψ - A**	-10.78 (2.22) -10.74 (2.21)	**5 14** **m^{1}Ψ - A**	-10.59 (2.30) -10.66 (2.23)	**5 14** **m^{1}Ψ - A**	-10.66 (2.19) -10.72 (2.15)
6 13 **C - G**	-26.04 (3.07) -26.11 (2.98)	**6 13** **G - C**	-25.99 (2.96) -26.08 (2.94)	**6 13** **U - A**	-9.58 (2.33) -9.60 (2.30)	**6 13** **A - U**	-9.62 (2.26) -9.68 (2.25)
[a]The values obtained in this study from the last 40 ns of MD simulations at 310K (values for the first set of simulations are shown in black and those for the second set of simulations are shown in purple)							

Table S4. Interaction energies (kcal/mol) of the base-pair steps containing the m^{1}Ψ-G mismatch for duplex-Gm^{1}ΨC and those containing the U-G mismatch for duplex-GUC and the Ψ-G mismatch for duplex-GΨC

	Y=m^{1}Ψ [a]	**Y=U** [b]	**Y=Ψ** [b]
Stacking energies			
Step 1 **4 5** **5'- G Y -3'** **3'- C G -5'** **15 14**	-16.76 (3.14) -16.01 (2.86)	-9.7 (2.57) -9.61 -9.78	-10.47 (2.81) -10.42 -10.52
Step 2 **5 6** **5'- Y C -3'** **3'- G G -5'** **14 13**	-6.71 (2.16) -7.07 (2.02)	-6.29 (2.21) -6.29 -6.28	-6.71 (2.08) -6.66 -6.76
Base pairing energies			
4 15 **G - C**	-26.00 (3.05) -25.96 (3.12)	-26.04 (3.01)	-26.08 (2.98)

		-25.99 -26.08	-26.07 -26.06
5 14 **Y - G**	-12.69 (2.64) -13.16 (2.46)	-12.97 (2.33) -12.96 -12.97	-13.56 (2.32) -13.56 -13.59
6 13 **C - G**	-26.08 (3.23) -26.15 (2.99)	-26.13 (2.99) -26.13 -26.14	-26.16 (2.96) -26.15 -26.11

[a] The values reported in Dutta et al. 2023[1] from the last 400 ns of MD simulations (values from the second set of simulations are shown in purple).
[b] The values reported in Dutta et al. 2023 [1] from the last 400 ns of MD simulations (values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown and those obtained from the last 200 ns (of the 400 ns trajectory) are shown in green).

Table S5. Interaction energies (kcal/mol) of the base-pair steps containing the m^{1}Ψ-U mismatch for duplex-Gm^{1}ΨC and those containing the U-U mismatch for duplex-GUC and those containing the Ψ-U mismatch for duplex-GΨC

	Y=m^{1}Ψ [a]	**Y=U** [b]	**Y=Ψ** [b]
Stacking energies			
Step 1 **4 5** **5'- G m^{1}Ψ -3'** **3'- C U -5'** **15 14**	-11.89 (3.22) -12.13 (3.32)	-4.98 (3.18) -4.64 -5.33	-5.65 (2.98) -5.95 -5.35
Step 2 **5 6** **5'- m^{1}Ψ C -3'** **3'- U G -5'** **14 13**	-6.62 (2.65) -6.74 (2.63)	-4.48 (3.10) -4.90 -4.06	-6.14 (2.81) -6.05 -6.23
Base pairing energies			
4 15 **G - C**	-25.81 (3.18) -25.80 (3.16)	-25.56 (3.29) -25.56 -25.55	-25.80 (3.18) -25.82 -25.78

5 14 **m^{1}Ψ - U**	-7.86 (2.97) -7.09 (2.97)	-4.59 (2.91) -4.52 -4.68	-5.66 (2.74) -5.74 -5.58
6 13 **C - G**	-25.43 (3.42) -25.38 (3.62)	-25.63 (3.14) -25.63 -25.61	-25.59 (3.24) -25.61 -25.58

[a] The values reported in Dutta et al. 2023 [1] from the last 400 ns of MD simulations (values from the second set of simulations are shown in purple).
[b] The values reported in Dutta et al. 2023 [1] from the last 400 ns of MD simulations (values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown and those obtained from the last 200 ns (of the 400 ns trajectory) are shown in green).

Table S6. Interaction energies (kcal/mol) of the base-pair steps containing the m^{1}Ψ-C mismatch for duplex-Gm^{1}ΨC and those containing the U-C mismatch for duplex-GUC and the Ψ-C mismatch for duplex-GΨC

	Y=m^{1}Ψ [a]	**Y=U [b]**	**Y=Ψ [b]**
Stacking energies			
Step 1 **4 5** **5'- G m^{1}Ψ -3'** **3'- C U -5'** **15 14**	-14.46 (2.26) -14.52 (2.41)	-8.67 (4.21) -8.46 -8.89	-9.27 (2.53) -9.05 -9.49
Step 2 **5 6** **5'- m^{1}Ψ C -3'** **3'- U G -5'** **14 13**	-9.14 (2.65) -9.31 (2.72)	-7.14 (3.36) -6.71 -7.58	-8.15 (3.33) -7.63 -8.64
Base pairing energies			
4 15 **G - C**	-25.86 (3.12) -25.90 (3.12)	-25.57 (3.28) -25.56 -25.58	-25.71 (3.23) -25.72 -25.69

5 14 **$m^1\Psi$ - U**	-3.48 (2.87) -3.72 (3.04)	-2.53 (2.75) -2.45 -2.62	-2.31 (2.89) -2.09 -2.52
6 13 **C - G**	-25.72 (3.46) -25.44 (3.03)	-25.85 (3.09) -25.83 -25.86	-25.86 (3.12) -25.83 -25.89
[a] The values reported in Dutta et al. 2023 [1] from the last 400 ns of MD simulations (values from the second set of simulations are shown in purple). [b] The values reported in Dutta et al. 2023 [1] from the last 400 ns of MD simulations (values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown and those obtained from the last 200 ns (of the 400 ns trajectory) are shown in green).			

Table S7. Nearest neighbor parameters (free energies in kcal/mol) for the U-A pair calculated using the energy values (A) obtained from MD using the LIE analysis and (B) obtained from QM calculations reported in Deb et al, 2019 [2].

(A)				
Base pair step 1	**GU** **CA**	**CU** **GA**	**AU** **UA**	**UU** **AA**
$E^{MD}_{NN,binding}$	-27.19 -27.195 -27.205	-26.16 -26.115 -26.195	-17.48 -17.45 -17.50	-16.00 -16.075 -15.920
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.24 -2.246 -2.248 **-2.247** **(0.0014)**	-2.12 -2.116 -2.126 **-2.121** **(0.0071)**	-1.08 -1.075 -1.082 **-1.0785** **(0.0049)**	-0.90 -0.911 -0.892 **-0.902** **(0.0134)**
$\Delta G^{\circ}_{37,Experiment}$ (Xia et al., 1998)[3]	-2.24 (0.06)	-2.08 (0.06)	-1.1 (0.08)	-0.93 (0.03)
$\Delta G^{\circ}_{37,Predicted}$ (Chou et al, 2016) [9]	−1.95 ± 0.14	−2.19 ± 0.11	−0.91 ± 0.21	−1.13 ± 0.17

$\Delta G^{\circ}_{37,Predicted}$ (Hopfinger et al, 2020)[11]	−2.03 ± 0.07	−2.16 ± 0.08	−1.11 ± 0.13	-0.94 (0.04)
$\Delta G^{\circ}_{37,Predicted}$ (Zuber et al., 2022)[4]	-2.25 (0.06)	-2.01 (0.07)	-1.09 (0.07)	-0.94 (0.04)
Base pair step 2	**UC** **AG**	**UG** **AC**	**UU** **AA**	**UA** **AU**
$E^{MD}_{NN,binding}$	-27.11 -27.11 -27.065	-26.49 -26.525 -26.450	-16.13 -16.11 -16.15	-20.08 -20.010 -20.155
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.24 -2.236 -2.231 **-2.234** **(0.0035)**	-2.16 -2.166 -2.156 **-2.161** **(0.0071)**	-0.92 -0.915 -0.919 **-0.917** **(0.0028)**	-1.39 -1.383 -1.401 **-1.392** **(0.0127)**
$\Delta G^{\circ}_{37,Experiment}$ (Xia et al., 1998)[3]	-2.35 (0.06)	-2.11 (0.07)	-0.93 (0.03)	-1.33 (0.09)
$\Delta G^{\circ}_{37,Predicted}$ (Chou et al, 2016) [9]	−2.13 ± 0.09	−2.09 ± 0.10	−1.13 ± 0.17	−1.26 ± 0.20
$\Delta G^{\circ}_{37,Predicted}$ (Hopfinger et al, 2020)[11]	−2.18 ± 0.07	−2.19 ± 0.04	−1.02 ± 0.12	−1.34 ± 0.09
$\Delta G^{\circ}_{37,Predicted}$ (Zuber et al., 2022)[4]	-2.42 (0.05)	-2.07 (0.07)	-0.94 (0.04)	-1.29 (0.08)

Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas E (NN,binding) = E_{stack} + 0.5 (E_{HB}) and $\Delta G^{\circ}_{37,MD,Predicted}$ = 0.1201 ∗ $E^{MD}_{NN,binding}$ + 1.0198. E_{stack} represents the stacking energy corresponding to a base pair step and E_{HB} represents the sum of hydrogen bonding (i.e.) base pairing energies of the two base pairs of the base pair step. The average values of the stacking and base pairing energies were considered during this calculation.

The values obtained from the last 400 ns of MD simulations[1]. The values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown and those obtained from the last 200 ns (of the 400 ns trajectory) are shown in green). The mean and average values (of the two 200 ns segments) are shown in black and bold font.

(B)				
Base pair step 1	**GU** **CA**	**CU** **GA**	**AU** **UA**	**UU** **AA**
$E^{QM}_{NN,binding}$	-29.98	-30.4	-23.73	-22.03
$\Delta G^{\circ}_{37,QM,Predicted}$	**-2.08**	**-2.09**	**-1.14**	**-0.89**
$\Delta G^{\circ}_{37,Experiment}$ (Xia et al., 1998)[3]	-2.24 (0.06)	-2.08 (0.06)	-1.1 (0.08)	-0.93 (0.03)
$\Delta G^{\circ}_{37,Predicted}$ (Chou et al, 2016) [9]	−1.95 ± 0.14	−2.19 ± 0.11	−0.91 ± 0.21	−1.13 ± 0.17
$\Delta G^{\circ}_{37,Predicted}$ (Hopfinger et al, 2020)[11]	−2.03 ± 0.07	−2.16 ± 0.08	−1.11 ± 0.13	-0.94 (0.04)
$\Delta G^{\circ}_{37,Predicted}$ (Zuber et al., 2022)[4]	-2.25 (0.06)	-2.01 (0.07)	-1.09 (0.07)	-0.94 (0.04)
Base pair step 2	**UC** **AG**	**UG** **AC**	**UU** **AA**	**UA** **AU**
$E^{QM}_{NN,binding}$	-31	-31.125	-22.06	-25.32
$\Delta G^{\circ}_{37,QM,Predicted}$	**-2.24**	**-2.26**	**-0.89**	**-1.37**
$\Delta G^{\circ}_{37,Experiment}$ (Xia et al., 1998)[3]	-2.35 (0.06)	-2.11 (0.07)	-0.93 (0.03)	-1.33 (0.09)
$\Delta G^{\circ}_{37,Predicted}$ (Chou et al, 2016) [9]	−2.13 ± 0.09	−2.09 ± 0.10	−1.13 ± 0.17	−1.26 ± 0.20
$\Delta G^{\circ}_{37,Predicted}$ (Hopfinger et al, 2020)[11]	−2.18 ± 0.07	−2.19 ± 0.04	−1.02 ± 0.12	−1.34 ± 0.09
$\Delta G^{\circ}_{37,Predicted}$ (Zuber et al., 2022)[4]	-2.42 (0.05)	-2.07 (0.07)	-0.94 (0.04)	-1.29 (0.08)
Here $E^{QM}_{NN,binding}$ and $\Delta G^{\circ}_{37,QM,Predicted}$ were calculated using the formulas E (NN,binding) = E_{stack} + 0.5 (E_{HB}) and $\Delta G^{\circ}_{37,QM,Predicted}$ = 0.1516 * $E^{QM}_{NN,binding}$ + 2.4593. E_{stack} represents the stacking energy corresponding to a base pair step and represents the sum of				

hydrogen bonding (i.e.) base pairing energies of the two base pairs of the base pair step. The average values of the stacking and base pairing energies were considered during this calculation.

Table S8. Nearest neighbor parameters (free energies in kcal/mol) for the Ψ-A pair calculated using the energy values (A) obtained from MD using the LIE analysis and (B) obtained from QM calculations reported in Deb et al, 2019 [2].

(A)				
Base pair step 1	**GΨ** **CA**	**CΨ** **GA**	**AΨ** **UA**	**UΨ** **AA**
$E^{MD}_{NN,binding}$	-28.04 -27.990 -28.050	-25.6 -25.610 -25.600	-17.66 -17.635 -17.675	-15.47 -15.465 -15.470
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.35 -2.342 -2.349 **-2.346 (0.0049)**	-2.05 -2.056 -2.055 **-2.056 (0.0007)**	-1.10 -1.098 -1.103 **-1.1005 (0.0035)**	-0.84 -0.838 -0.838 **-0.838 (0)**
$\Delta G^{\circ}_{37,Experiment}$ (Hudson et al, 2013) [5]	-3.29	-2.77	-2.80	-1.62
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019) [6]	-2.50 (0.08)	-2.10 (0.10)	NA	NA
$\Delta G^{\circ}_{37,Predicted}$ (Hopfinger et al, 2020)[11]	-1.46 (0.03)	-1.56 (0.02)	-0.61 (0.14)	-0.58 (0.08)
Base pair step 2	**ΨC** **AG**	**ΨG** **AC**	**ΨU** **AA**	**ΨA** **AU**
$E^{MD}_{NN,binding}$	-27.38 -27.340 -27.405	-27.98 -27.990 -27.985	-17.11 -17.125 -17.090	-20.79 -20.810 -20.765
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.27 -2.264	-2.34 -2.342	-1.04 -1.036	-1.48 -1.479

	-2.272 **-2.268 (0.0056)**	-2.341 **-2.342 (0.0007)**	-1.033 **-1.0345 (0.0021)**	-1.474 **-1.4765 (0.0035)**
$\Delta G^{\circ}_{37,Experiment}$ (Hudson et al, 2013)[5]	-2.49	-2.20	-2.74	-2.10
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.51 (0.10)	-2.35 (0.08)	NA	NA
$\Delta G^{\circ}_{37,Predicted}$ (Hopfinger et al, 2020)[11]	-1.68 (0.14)	-1.67 (0.02)	-0.66 (0.20)	-0.94 (0.22)
Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas E (NN,binding) = E_{stack} + 0.5 (E_{HB}) and $\Delta G^{\circ}_{37,MD,Predicted}$ = 0.1201 ∗ $E^{MD}_{NN,binding}$ + 1.0198. E_{stack} represents the stacking energy corresponding to a base pair step and represents the sum of hydrogen bonding (i.e.) base pairing energies of the two base pairs of the base pair step. The average values of the stacking and base pairing energies were considered during this calculation. The values obtained from the last 400 ns of MD simulations [1]. The values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown and those obtained from the last 200 ns (of the 400 ns trajectory) are shown in green). The mean and average values (of the two 200 ns segments) are shown in black and bold font.				

(B)				
Base pair step 1	**GΨ CA**	**CΨ GA**	**AΨ UA**	**UΨ AA**
$E^{QM}_{NN,binding}$	-30.24	-30.205	-23.38	-21.74
$\Delta G^{\circ}_{37,QM,Predicted}$	**-2.13**	**-2.12**	**-1.08**	**-0.84**
$\Delta G^{\circ}_{37,Experiment}$ (Hudson et al, 2013)[5]	-3.29	-2.77	-2.80	-1.62
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.50 (0.08)	-2.10 (0.10)	NA	NA
$\Delta G^{\circ}_{37,Predicted}$ (Hopfinger et al, 2020)[11]	-1.46 (0.03)	-1.56 (0.02)	-0.61 (0.14)	-0.58 (0.08)
Base pair step 2	**ΨC**	**ΨG**	**ΨU**	**ΨA**

	AG	**AC**	**AA**	**AU**
$E^{QM}_{NN,binding}$	-30.12	-32.54	-23.08	-26.03
$\Delta G^{\circ}_{37,QM,Predicted}$	**-2.11**	**-2.47**	**-1.04**	**-1.49**
$\Delta G^{\circ}_{37,Experiment}$ (Hudson et al, 2013)[5]	-2.49	-2.20	-2.74	-2.10
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.51 (0.10)	-2.35 (0.08)	NA	NA
$\Delta G^{\circ}_{37,Predicted}$ (Hopfinger et al, 2020)[11]	-1.68 (0.14)	-1.67 (0.02)	-0.66 (0.20)	-0.94 (0.22)
Here $E^{QM}_{NN,binding}$ and $\Delta G^{\circ}_{37,QM,Predicted}$ were calculated using the formulas E (NN,binding) = E_{stack} + 0.5 (E_{HB}) and $\Delta G^{\circ}_{37,QM,Predicted}$ = 0.1516 * $E^{QM}_{NN,binding}$ + 2.4593. E_{stack} represents the stacking energy corresponding to a base pair step and represents the sum of hydrogen bonding (i.e.) base pairing energies of the two base pairs of the base pair step. The average values of the stacking and base pairing energies were considered during this calculation.				

Table S9. Nearest neighbor parameters (free energies in kcal/mol) for the m$^1\Psi$-A pair calculated using the energy values obtained from MD using the LIE analysis.

Base pair step 1	**Gm$^1\Psi$ CA**	**Cm$^1\Psi$ GA**	**Am$^1\Psi$ UA**	**Um$^1\Psi$ AA**
$E^{MD}_{NN,binding}$ (From 300K simulation)	-34.14 -34.24	-30.28 -30.26	-24.05 -24.07	-21.32 -21.26
$\Delta G^{\circ}_{37,MD,Predicted}$ (From 300K simulation)	-3.080 -3.092 **-3.086 (0.0085)**	-2.617 -2.614 **-2.6155 (0.0021)**	-1.869 -1.871 **-1.870 (0.0014)**	-1.541 -1.534 **-1.538 (0.0049)**
$E^{MD}_{NN,binding}$ (From 310K simulation)	-34.22 -34.24	-30.14 -30.22	-23.93 -24.18	-21.22 -21.26
$\Delta G^{\circ}_{37,MD,Predicted}$ (From 310K simulation)	-3.090 -3.072	-2.600 -2.609	-1.854 -1.884	-1.529 -1.534

	-3.081 (0.0127)	**-2.604 (0.0064)**	**-1.869 (0.0212)**	**-1.532 (0.0035)**
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.43 (0.08)	-1.83 (0.09)	NA	NA
Base pair step 2	**m^{1}ΨC AG**	**m^{1}ΨG AC**	**m^{1}ΨU AA**	**m^{1}ΨA AU**
$E^{MD}_{NN,binding}$ (From 300K simulation)	-28.47 -28.56	-31.74 -31.84	-19.53 -19.53	-23.96 -24.06
$\Delta G^{\circ}_{37,MD,Predicted}$ (From 300K simulation)	-2.399 -2.410 **-2.405 (0.0078)**	-2.792 -2.804 **-2.798 (0.0085)**	-1.326 -1.326 **-1.326 (0)**	-1.858 -1.869 **-1.864 (0.0077)**
$E^{MD}_{NN,binding}$ (From 310K simulation)	-28.58 -28.59	-31.68 -31.72	-19.34 -19.62	-23.69 -24.04
$\Delta G^{\circ}_{37,MD,Predicted}$ (From 310K simulation)	-2.413 -2.414 **-2.414 (0.0007)**	-2.785 -2.789 **-2.719 (0.0092)**	-1.303 -1.336 **-1.319 (0.0233)**	-1.825 -1.867 **-1.846 (0.0296)**
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.67 (0.08)	-2.26 (0.07)		

Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas E (NN,binding) = E_{stack} + 0.5 (E_{HB}) and $\Delta G^{\circ}_{37,MD,Predicted}$ = 0.1201 ∗ $E^{MD}_{NN,binding}$ + 1.0198. E_{stack} represents the stacking energy corresponding to a base pair step and represents the sum of hydrogen bonding (i.e.) base pairing energies of the two base pairs of the base pair step. The average values of the stacking and base pairing energies were considered during this calculation.

The values obtained from the last 400 ns of MD simulations[1] (values from the second set of simulations are shown in purple). The mean and average values (of the two 400 ns replicates) are shown in black and bold font.

Table S10. Nearest neighbor parameters (free energies in kcal/mol) for the (A) m^{1}Ψ-G, m^{1}Ψ-U and m^{1}Ψ-C mismatches and (B) the U-G, U-U and U-C mismatches and (C) the Ψ-G, Ψ-U and Ψ-C mismatches, calculated using the energy values obtained from MD using the LIE analysis

(A)			
Base pair step 1	**Gm^{1}Ψ CG**	**Gm^{1}Ψ CU**	**Gm^{1}Ψ CC**
$E^{MD}_{NN,binding}$	-35.62 -35.57	-28.72 -28.58	-29.13 -29.33
$\Delta G^{\circ}_{37,MD,Predicted}$	-3.258 -3.252 **-3.255 (0.0042)**	-2.429 -2.412 **-2.421 (0.0120)**	-2.478 -2.503 **-2.491 (0.0176)**
Base pair step 2	**m^{1}ΨC GG**	**m^{1}ΨC UG**	**m^{1}ΨC CG**
$E^{MD}_{NN,binding}$	-25.62 -26.72	-23.26 -22.98	-23.47 -23.89
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.057 -2.189 **-2.123 (0.0933)**	-1.774 -1.740 **-1.757 (0.0240)**	-1.798 -1.849 **-1.824 (0.0360)**

Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas $E(NN,binding) = E_{stack} + 0.5\ (E_{HB})$ and $\Delta G^{\circ}_{37,MD,Predicted} = 0.1201 * E^{MD}_{NN,binding} + 1.0198$. E_{stack} represents the stacking energy corresponding to a base pair step and represents the sum of hydrogen bonding (i.e.) base pairing energies of the two base pairs of the base pair step. The average values of the stacking and base pairing energies were considered during this calculation.

The values obtained from the last 400 ns of MD simulations[1] (values from the second set of simulations are shown in purple). The mean and average values (of the two 400 ns replicates) are shown in black and bold font.

(B)			
Base pair step 1	**GU CG**	**GU CU**	**GU CC**
$E_{NN,binding}$	-29.21 -29.08 -29.31	-20.06 -19.68 -20.44	-22.72 -22.46 -22.99
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.49	-1.39	-1.71

	-2.473 -2.500 **-2.486** **(0.0191)**	-1.344 -1.435 **-1.389** **(0.0643)**	-1.678 -1.741 **-1.709** **(0.0445)**
$\Delta G^{\circ}_{37,Experiment}$ (Mathews et al, 1999) [7]	-2.51 (0.25)	NA	NA
$\Delta G^{\circ}_{37,Experiment}$ (Chen et al, 2012) [8]	−2.15 (0.10)		
$\Delta G^{\circ}_{37,Predicted}$ (Chou et al, 2016) [9]	-1.96 (0.19)		
$\Delta G^{\circ}_{37,Predicted}$ (Zuber et al, 2022) [4]	-2.23 (0.07)		
Base pair step 2	**UC** **GG**	**UC** **UG**	**UC** **CG**
$E_{NN,binding}$	-25.84 -25.84 -25.84	-19.59 -19.98 -19.21	-21.33 -20.85 -21.82
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.08 -2.08 -2.08 **-2.08 (0)**	-1.33 -1.379 -1.287 **-1.333** **(0.0650)**	-1.54 -1.484 -1.600 **-1.542** **(0.0820)**
$\Delta G^{\circ}_{37,Experiment}$ (Mathews et al, 1999) [7]	-1.53 (0.27)	NA	NA
$\Delta G^{\circ}_{37,Experiment}$ (Chen et al, 2012) [8]	−1.80 (0.09)		
$\Delta G^{\circ}_{37,Predicted}$ (Chou et al, 2016) [9]	-1.50 (0.23)		
$\Delta G^{\circ}_{37,Predicted}$ (Zuber et al, 2022) [4]	-1.80 (0.07)		

Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas E (NN,binding) = E_{stack} + 0.5 (E_{HB}) and $\Delta G^{\circ}_{37,MD,Predicted}$ = 0.1201 * $E^{MD}_{NN,binding}$ + 1.0198. E_{stack} represents the stacking energy corresponding to a base pair step and represents the sum of hydrogen bonding (i.e.) base pairing energies of the two base pairs of the base pair step. The average values of the stacking and base pairing energies were considered during this calculation.

The values obtained from the last 400 ns of MD simulations[1]. The values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown and those obtained from the last 200 ns (of the 400 ns trajectory) are shown in green). The mean and average values (of the two 200 ns segments) are shown in black and bold font.

(C)			
Base pair step 1	**GΨ** **CG**	**GΨ** **CU**	**GΨ** **CC**
$E^{MD}_{NN,binding}$	-30.29 -30.24 -30.34	-21.38 -21.73 -21.03	-23.28 -22.96 -23.59
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.62 -2.612 -2.624 **-2.618** **(0.0085)**	-1.55 -1.589 -1.506 **-1.548** **(0.0586)**	-1.78 -1.738 -1.813 **-1.776** **(0.0530)**
Base pair step 2	**ΨC** **GG**	**ΨC** **UG**	**ΨC** **CG**
$E^{MD}_{NN,binding}$	-26.53 -26.52 -26.61	-21.76 -21.72 -21.81	-22.24 -21.59 -22.84
$\Delta G^{\circ}_{37,MD,Predicted}$	-2.17 -2.165 -2.176 **-2.171** **(0.0077)**	-1.59 -1.588 -1.599 **-1.594** **(0.0077)**	-1.65 -1.573 -1.723 **-1.648** **(0.1060)**
Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas E			

(NN,binding) = E_{stack} + 0.5 (E_{HB}) and $\Delta G^{\circ}_{37,MD,Predicted}$ = 0.1201 ∗ $E^{MD}_{NN,binding}$ + 1.0198. E_{stack} represents the stacking energy corresponding to a base pair step and represents the sum of hydrogen bonding (i.e.) base pairing energies of the two base pairs of the base pair step. The average values of the stacking and base pairing energies were considered during this calculation.

The values obtained from the last 400 ns of MD simulations[1]. The values obtained from the first 200 ns (of the 400 ns trajectory) are shown in brown and those obtained from the last 200 ns (of the 400 ns trajectory) are shown in green). The mean and average values (of the two 200 ns segments) are shown in black and bold font.

Table S11. Comparison of the free energies (kcal/mol) of duplexes containing internal Ψ-A pair studied in this work.

Duplex	$-\Delta G^{\circ}_{37,Expt}$ (Kierzek et al, 2014)[10]	$-\Delta G^{\circ}_{37,MD,Pred}$ (This work)	$-\Delta G^{\circ}_{37,QM,Pred}$ (This work)	$-\Delta G^{\circ}_{37,Pred}$ (Hudson et al, 2013)[5]	$-\Delta G^{\circ}_{37,Pred}$ (Hopfinger et al, 2020)[11]	$\Delta G^{\circ}_{37,Pred}$ (Mauger et al. 2019)[6]
UCAGΨCAGU AGUCAGUCA	12.85	12.594	12.22	13.69	11.12	12.99
UCACΨGAGU AGUGACUCA	14.44	12.378	12.57	13.28	11.21	12.43
UCAAΨUAGU AGUUAAUCA	8.36	8.185	8.17	11.41	7.32	NA
UCAUΨAAGU AGUAAUUCA	8.42	8.134	8.15	9.37	7.34	NA
MSE		1.107	1.0007	3.06	3.92	2.03
RMSE		1.05	1.00035	1.75	1.98	1.42
The predicted values of the free energies of the duplexes were calculated using the Xia et al., 1998 [3] parameters for the WC pairs and the NN free energy parameters for the NN pairs containing Ψ reported in the present study and also those reported in earlier studies as indicated.						

Table S12. Comparison of the free energies (kcal/mol) of duplexes (from Hudson et al, [5]) containing internal Ψ-A pair.

Duplex	$-\Delta G^{\circ}_{37,Expt}$ (Hudson et al,	$-\Delta G^{\circ}_{37,MD,Pred}$ (This work)	$-\Delta G^{\circ}_{37,QM,Pred}$ (This work)	$-\Delta G^{\circ}_{37,Pred}$ (Hudson et	$-\Delta G^{\circ}_{37,Pred}$ (Hopfinger	$-\Delta G^{\circ}_{37,Pred}$ (Mauger et al.

	2013)[5]			al, 2013)[5]	et al, 2020)[11]	2019)[6]
CGAΨACG GCUAUGC	9.93	7.79	7.79	10.12	6.77	NA
CGCΨACG GCGAUGC	10.96	9.82	9.9	11.16	8.79	NA
CGGΨACG GCCAUGC	11.71	9.95	9.75	11.52	8.53	NA
CGUΨACG GCAAUGC	9.10	7.42	7.44	8.83	6.63	NA
CGAΨCCG GCUAGGC	11.92	9.61	9.43	11.53	8.53	NA
CGCΨCCG GCGAGGC	12.93	11.63	11.54	12.57	10.55	11.92
CGGΨCCG GCCAGGC	12.76	11.76	11.39	12.93	10.29	12.16
CGUΨCCG GCAAGGC	9.76	9.24	9.08	10.24	8.39	NA
CGAΨGCG GCUACGC	11.45	9.84	9.95	11.40	8.68	NA
CGCΨGCG GCGACGC	12.35	11.87	12.06	12.44	10.70	11.92
CGGΨGCG GCCACGC	12.59	11.99	11.91	12.80	10.44	12.16
CGUΨGCG GCAACGC	10.34	9.47	9.6	10.11	8.54	NA
CGAΨUCG GCUAAGC	10.42	7.46	7.45	10.87	6.60	NA
CGCΨUCG GCGAAGC	12.06	9.49	9.56	11.91	8.62	NA
CGGΨUCG GCCAAGC	12.51	9.62	9.41	12.27	8.36	NA
CGUΨUCG GCAAAGC	9.51	7.09	7.1	9.58	6.46	NA

MSE		3.35 (number of duplexes =16)	3.54	0.07 (number of duplexes =16)	7.95 (number of duplexes =16)	0.44 (number of duplexes =4)
RMSE		1.83	1.88	0.26	2.82	0.66

Table S13. Comparison of the free energies (kcal/mol) of duplexes containing internal $m^1\Psi$-A pair studied in this work.

Duplex	$-\Delta G^\circ_{37,MD,Pred}$ (This work)	$-\Delta G^\circ_{37,Pred}$ (Mauger et al. 2019)[6]
UCAG$m^1\Psi$CAGU AGUCAGUCA	13.471	13.08
UCAC$m^1\Psi$GAGU AGUGACUCA	13.39	12.07
UCAA$m^1\Psi$UAGU AGUUAAUCA	9.25	NA
UCAU$m^1\Psi$AAGU AGUAAUUCA	9.22	
The predicted values of the free energies of the duplexes were calculated using the Xia et al., [3] parameters for the WC pairs and the NN free energy parameters for the NN pairs containing $m^1\Psi$ reported in the present study and those reported in Mauger et al. [6].		

Table S14. Comparison of the free energies (kcal/mol) of duplexes containing mismatches studied in this work.

Duplex	**Y=U**		**Y=Ψ**		**Y=$m^1\Psi$**
	$-\Delta G^\circ_{37,Expt}$ (Kierzek et al, 2014)[10]	$-\Delta G^\circ_{37,MD,Pred}$ (This work)	$-\Delta G^\circ_{37,Expt}$ (Kierzek et al, 2014)[10]	$-\Delta G^\circ_{37,MD,Pred}$ (This work)	$\Delta G^\circ_{37,MD,Pred}$ (This work)
UCAGYCAGU AGUCGGUCA	12.01 (0.18)	12.55	13.41 (0.27)	12.77	13.36
UCAGYCAGU AGUCUGUCA	8.58 (0.05)	10.70	9.35 (0.13)	11.12	12.16

UCAGYCAGU AGUCCGUCA	8.07 (0.05)	11.23	8.34 (0.02)	11.40	12.29
The predicted values of the free energies of the duplexes were calculated using the Xia et al., 1998 [3] parameters for the WC pairs and the NN free energy parameters for the mismatches reported in the present study.					

Table S15. Interaction energies (kcal/mol) of the base-pair steps containing Y-A, Y-G, Y-U and Y-C pairs (where Y=> U, Ψ, $m^1Ψ$) obtained from MD simulations at 300K using the CHARMM36 parameters.

	Duplex-1 Context: GUC(U-A)	**Duplex-2 Context: CUG(U-A)**	**Duplex-3 Context: UUA(U-A)**	**Duplex-4 Context: AUU(U-A)**
Stacking energies				
Step 1	ELEC 0.60 (1.33) VDW -14.06 (1.23) TOTAL -13.46 (1.81)	ELEC 0.23 (1.32) VDW -14.15 (1.08) TOTAL -13.92 (1.71)	ELEC 1.27 (1.13) VDW -13.50 (1.12) TOTAL -12.23 (1.59)	ELEC 2.01 (1.19) VDW -12.93 (1.52) -10.92 (1.93)
Step 2	ELEC 0.69 (1.38) VDW -14.44 (1.09) TOTAL -13.75 (1.76)	ELEC -0.52 (1.20) VDW -14.50 (0.93) TOTAL -15.02 (1.52)	ELEC -3.19 (1.41) VDW -13.45 (1.01) TOTAL -16.64 (1.73)	ELEC 1.07 (1.25) VDW -13.42 (1.23) TOTAL -12.35 (1.75)
Base pairing energies				
4 15	ELEC -22.02 (2.92) VDW -0.29 (1.51) TOTAL -22.31 (3.28)	ELEC -22.56 (3.48) VDW -0.18 (1.52) TOTAL -23.74 (3.79)	ELEC -10.42 (2.75) VDW -0.29 (1.40) TOTAL -10.71 (3.08)	ELEC -9.95 (3.57) VDW -0.26 (1.39) TOTAL -10.21 (3.83)
5 14	ELEC -10.64 (2.17) VDW -0.38 (1.38) TOTAL -11.02 (2.57)	ELEC -11.27 (1.98) VDW -0.15 (1.44) TOTAL -11.42 (2.45)	ELEC -11.06 (2.11) VDW -0.23 (1.43) TOTAL -11.29 (2.55)	ELEC -9.98 (3.44) VDW -0.28 (1.38) TOTAL -10.26 (3.71)
6 13	ELEC -23.18 (2.47) VDW -0.07 (1.56) TOTAL -23.25 (2.92)	ELEC -22.87 (2.45) VDW -0.21 (1.54) TOTAL -23.08 (2.89)	ELEC -11.09 (1.95) VDW -0.29 (1.39) TOTAL -11.38 (2.39)	ELEC -11.10 (2.05) VDW -0.26 (1.40) TOTAL -11.36 (2.48)
The values reported in this table were obtained from the last 100 ns of 150 ns MD simulations of the duplexes at 300K.				

	Duplex-5 Context: GUC(U-G)	**Duplex-6 Context: GUC(U-U)**	**Duplex-7 Context: GUC(U-C)**

Stacking energies			
Step 1	ELEC -2.88 (2.06) VDW -14.17 (1.34) TOTAL -17.05 (2.46)	ELEC 0.72 (2.25) VDW -9.99 (1.93) TOTAL -9.27 (2.96)	ELEC -0.49 (2.24) VDW -10.58 (1.77) TOTAL -11.07 (2.85)
Step 2	ELEC 1.75 (2.08) VDW -14.10 (1.07) TOTAL -12.35 (2.34)	ELEC 0.48 (2.13) VDW -10.08 (1.84) TOTAL -9.6 (2.81)	ELEC -0.11 (2.36) VDW -10.93 (1.75) TOTAL -11.04 (2.94)
Base pairing energies			
4 15	ELEC -21.48 (2.63) VDW -0.60 (1.43) TOTAL -22.08 (2.99)	ELEC -21.91 (4.89) VDW -0.26 (1.49) TOTAL -22.17 (5.11)	ELEC -21.02 (5.51) VDW 0.35 (1.44) TOTAL -20.67 (5.69)
5 14	ELEC -11.17 (3.28) VDW -0.43 (1.15) TOTAL -11.6 (3.48)	ELEC -1.82 (2.82) VDW -0.29 (0.54) TOTAL -2.11 (2.87)	-2.22 (2.95) VDW -0.36 (0.67) TOTAL -2.58 (3.02)
6 13	ELEC -24.12 (2.29) VDW 0.09 (1.62) TOTAL -24.03 (2.81)	ELEC -22.34 (4.22) VDW 0.14 (1.56) TOTAL -22.2 (4.49)	ELEC -22.80 (2.59) VDW -0.21 (1.55) TOTAL-23.01 (3.02)
The values reported in this table were obtained from the last 250 ns of 300 ns MD simulations of the duplexes at 300K.			

	Duplex-8 **Context: GΨC(Ψ-A)**	**Duplex-9** **Context: CΨG(Ψ-A)**	**Duplex-10** **Context: UΨA(Ψ-A)**	**Duplex-11** **Context: AΨU(Ψ-A)**
Stacking energies				
Step 1	ELEC 0.21 (1.23) VDW -14.38 (1.05) TOTAL -14.17 (1.62)	ELEC 1.96 (1.30) VDW -14.54 (0.90) TOTAL -12.58 (2.5)	ELEC 1.95 (1.10) VDW -13.59 (1.04) TOTAL -11.64 (1.51)	ELEC 2.51 (1.08) VDW -13.39 (1.16) TOTAL -10.88 (1.58)
Step 2	ELEC 0.49 (1.46) VDW -14.73 (1.04) TOTAL -14.24 (1.79)	ELEC -0.46 (1.29) VDW -14.52 (0.90) TOTAL -14.98 (1.57)	ELEC -2.48 (1.42) VDW -13.39 (1.12) TOTAL -15.87 (1.81)	ELEC 0.88 (1.01) VDW -13.78 (0.93) TOTAL-12.9 (1.37)
Base pairing energies				
4 15	ELEC -22.73 (2.68) VDW -0.26 (1.51)	ELEC -23.39 (2.50) VDW -0.19 (1.55)	ELEC -10.87 (2.09) VDW -0.32 (1.39)	ELEC -10.32 (3.01)

	TOTAL -22.99 (3.08)	TOTAL -23.58 (2.94)	TOTAL -11.19 (2.51)	VDW -0.29 (1.39) TOTAL -10.61 (3.32)
5 14	ELEC -10.43 (1.94) VDW -0.16 (1.45) TOTAL -10.59 (2.42)	ELEC -10.73 (1.85) VDW -0.11 (1.44) TOTAL -10.84 (2.34)	ELEC -10.37 (2.36) VDW -0.16 (1.41) TOTAL -10.53 (2.75)	ELEC -10.45 (1.93) VDW -0.13 (1.43) TOTAL -10.58 (2.40)
6 13	ELEC-23.32 (3.26) VDW 0.01 (1.56) TOTAL -23.31 (3.61)	ELEC -23.20 (2.48) VDW -0.19 (0.56) TOTAL -23.39 (2.54)	ELEC -11.11 (1.99) VDW -0.29 (1.38) TOTAL -11.40 (2.42)	ELEC -11.02 (1.99) VDW -0.33 (1.38) TOTAL -11.35 (2.42)
The values reported in this table were obtained from the last 100 ns of 150 ns MD simulations of the duplexes at 300K.				

	Duplex-12 **Context: GΨC(Ψ-G)**	**Duplex-13** **Context: GΨC(Ψ-U)**	**Duplex-14** **Context: GΨC(Ψ-C)**
Stacking energies			
Step 1	ELEC -1.71 (1.93) VDW -14.49 (1.34) TOTAL -16.20 (2.35)	ELEC 1.28 (2.08) VDW -10.82 (1.85) TOTAL -9.54 (2.78)	ELEC -0.24 (1.64) VDW -10.13 (2.19) TOTAL -10.37 (2.74)
Step 2	ELEC 1.32 (1.99) VDW -14.16 (1.06) TOTAL -12.84 (2.25)	ELEC 2.06 (2.38) VDW -11.28 (1.79) TOTAL -9.22 (2.98)	ELEC 1.15 (1.96) VDW -11.28 (1.55) TOTAL -10.13 (2.49)
Base pairing energies			
4 15	ELEC -21.50 (2.78) VDW -0.58 (1.41) TOTAL -22.08 (3.12)	ELEC -21.66 (4.20) VDW -0.23 (1.49) TOTAL -21.89 (4.46)	ELEC -22.31 (3.74) VDW -0.36 (1.47) TOTAL -22.67 (4.02)
5 14	ELEC -10.77 (2.68) VDW -0.36 (1.18) TOTAL -11.13 (2.93)	ELEC -3.77 (3.09) VDW -0.37 (0.77) -4.14 (3.18)	ELEC -2.65 (2.16) VDW -0.29 (0.80) -2..94 (2.30)
6 13	ELEC -24.02 (2.36) VDW 0.07 (1.61) TOTAL -23.95 (2.86)	ELEC -22.31 (4.01) VDW -0.13 (1.54) TOTAL -22.44 (4.29)	ELEC -21.88 (2.68) VDW -0.43 (1.46) TOTAL -22.31 (3.05)

	Duplex-15	**Duplex-16**	**Duplex-17**	**Duplex-18**

	Context: Gm^{1}ΨC(m^{1}Ψ-A)	**Context: Cm^{1}ΨG(m^{1}Ψ-A)**	**Context: Um^{1}ΨA(m^{1}Ψ-A)**	**Context: Am^{1}ΨU(m^{1}Ψ-A)**
Stacking energies				
Step 1	ELEC 0.01 (1.22) VDW -15.44 (1.10) TOTAL -15.43 (1.64)	ELEC 0.05 (1.46) VDW -14.30 (1.06) TOTAL -14.25 (1.80)	ELEC 0.95 (1.19) VDW -13.42 (1.18) TOTAL -12.47 (1.68)	ELEC 2.59 (1.04) VDW -14.58 (1.15) TOTAL -11.99 (1.55)
Step 2	ELEC 0.62 (1.43) VDW -15.15 (1.01) TOTAL -14.53 (1.75)	ELEC 1.17 (1.22) VDW -14.58 (0.90) TOTAL -13.41 (1.52)	ELEC -2.51 (1.49) VDW -13.49 (1.05) TOTAL -16.00 (1.82)	ELEC 0.94 (0.96) VDW -14.28 (0.94) TOTAL -13.34 (1.34)
Base pairing energies				
4 15	ELEC -23.01 (3.21) VDW -0.19 (1.51) TOTAL -23.2 (3.54)	ELEC -23.06 (3.28) VDW -0.21 (1.48) TOTAL -23.27 (3.59)	ELEC -11.01 (2.14) VDW -0.27 (1.43) TOTAL -11.28 (2.57)	ELEC -10.45 (2.92) VDW -0.32 (1.39) TOTAL -10.77 (3.23)
5 14	ELEC -9.73 (1.85) VDW -0.36 (1.36) TOTAL -9.37 (2.29)	ELEC -10.13 (1.89) VDW -0.24 (1.39) TOTAL -10.37 (2.35)	ELEC -9.77 (2.31) VDW -0.34 (1.34) TOTAL -10.11 (2.67)	ELEC -9.87 (1.81) VDW -0.25 (1.39) TOTAL -10.12 (2.28)
6 13	ELEC -23.37 (2.85) VDW -0.002 (1.57) TOTAL -23.37 (3.25)	ELEC -23.70 (2.46) VDW -0.09 (1.58) TOTAL -23.79 (2.92)	ELEC -11.16 (1.95) VDW -0.26 (1.40) TOTAL -11.42 (2.40)	ELEC -10.89 (2.09) VDW -0.37 (1.39) TOTAL -11.26 (2.51)
The values reported in this table were obtained from the last 100 ns of 150 ns MD simulations of the duplexes at 300K.				

	Duplex-12 Context: Gm^{1}ΨC(m^{1}Ψ-G)	**Duplex-13 Context: Gm^{1}ΨC(m^{1}Ψ-U)**	**Duplex-14 Context: Gm^{1}ΨC(m^{1}Ψ-C)**
Stacking energies			
Step 1	ELEC -1.73 (1.86) VDW -15.17 (1.45) TOTAL -16.9 (2.36)	ELEC 0.95 (2.25) VDW -12.17 (1.88) TOTAL -11.22 (2.93)	ELEC -0.37 (1.59) VDW -12.48 (2.19) TOTAL -12.85 (2.71)
Step 2	ELEC 2.08 (2.26) VDW -14.29 (1.15) TOTAL -12.21 (2.54)	ELEC 1.46 (2.04) VDW -11.58 (1.70) TOTAL -10.12 (2.65)	ELEC 0.90 (1.78) VDW -12.48 (1.58) TOTAL -11.58 (2.38)
Base pairing energies			

4 15	ELEC -21.84 (2.75) VDW -0.53 (1.45) TOTAL -22.37 (3.11)	ELEC -22.84 (3.23) VDW -0.16 (1.54) TOTAL -23.00 (3.58)	ELEC -22.34 (4.20) VDW -0.25 (1.51) TOTAL -22.59 (4.46)
5 14	ELEC -10.52 (2.71) VDW -0.44 (1.13) TOTAL -10.96 (2.94)	ELEC -2.95 (2.97) VDW -0.49 (0.69) TOTAL -3.44 (3.05)	ELEC -3.59 (2.09) VDW -0.49 (0.81) TOTAL -4.08 (2.24)
6 13	ELEC -23.79 (2.48) VDW 0.05 (1.62) TOTAL -23.84 (2.96)	ELEC -22.37 (4.68) VDW -0.11 (1.55) TOTAL -22.48 (4.93)	ELEC -23.44 (2.60) VDW -0.18 (1.55) TOTAL -23.62 (3.03)
The values reported in this table were obtained from the last 250 ns of 300 ns MD simulations of the duplexes at 300K.			

Table S16. Nearest neighbor parameters (free energies in kcal/mol) for the nearest neighbor pairs containing Y-A, Y-G, Y-U and Y-C pairs (where Y=> U, Ψ, $m^1\Psi$) calculated using the energy values obtained from MD (with CHARMM36 parameters) using the LIE analysis.

NN pairs	**GU CA**	**CU GA**	**AU UA**	**UU AA**	**GU CG**	**CU GU**	**UU AC**
$E^{MD}_{NN,binding}$	-30.125	-31.50	-21.16	-23.23	-33.89	-21.41	-22.69
$\Delta G^{\circ}_{37,MD,Predicted}$	**-1.97**	**-2.14**	**-0.87**	**-1.12**	**-2.44**	**-0.90**	**-1.06**
$\Delta G^{\circ}_{37,Experiment}$ (Xia et al., 1998)[3]	-2.24 (0.06)	-2.08 (0.06)	-1.1 (0.08)	-0.93 (0.03)	N.A.		
$\Delta G^{\circ}_{37,Experiment}$ (Mathews et al, 1999) [7]	N.A.				-2.51 (0.25)	N.A.	
$\Delta G^{\circ}_{37,Experiment}$ (Chen et al, 2012) [8]	N.A.				−2.15 (0.10)	N.A.	
NN pairs	**UC AG**	**UG AC**	**UA AU**	**UU AA**	**UC GG**	**UG UC**	**UA CU**

$E^{MD}_{NN,binding}$	-30.885	-32.27	-27.98	-23.16	-30.16	-21.76	-23.84
$\Delta G^{\circ}_{37,MD,Predicted}$	**-2.07**	**-2.24**	**-1.71**	**-1.12**	**-1.98**	**-0.94**	**-1.20**
$\Delta G^{\circ}_{37,Experiment}$ (Xia et al., 1998)[3]	-2.35 (0.06)	-2.11 (0.07)	-1.33 (0.09)	-0.93 (0.03)	N.A.		
$\Delta G^{\circ}_{37,Experiment}$ (Mathews et al, 1999) [7]	N.A.				-1.53 (0.27)	N.A.	
$\Delta G^{\circ}_{37,Experiment}$ (Chen et al, 2012) [8]	N.A.				−1.80 (0.09)	N.A.	
Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas $E^{MD}_{NN,binding} = E_{stack} + 0.5\ (E_{HB})$ and $\Delta G^{\circ}_{37,MD,Predicted} = 0.1229 * E^{MD}_{NN,binding} + 1.7277$ (This equation was obtained from linear regression of the $E^{MD}_{NN,binding}$ values with the available experimental nearest neighbor free energies ($\Delta G^{\circ}_{37,Experiment}$) (Xia et al., 1998)[3] for the canonical nearest neighbor pairs).							

NN pairs	**GΨ CA**	**CΨ GA**	**AΨ UA**	**UΨ AA**	**GΨ CG**	**CΨ GU**	**UΨ AC**
$E^{MD}_{NN,binding}$	-30.96	29.79	-21.48	-22.50	-32.81	-22.56	-23.18
$\Delta G^{\circ}_{37,MD,Predicted}$	**-2.08**	**-1.93**	**-0.91**	**-1.04**	**-2.30**	**-1.04**	**-1.12**
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.50 (0.08)	-2.10 (0.10)	N.A.				
$\Delta G^{\circ}_{37,Experiment}$ (Hudson et al, 2013)[5]	-3.29	-2.77	-2.80	-1.62	N.A.		
NN pairs	**ΨC AG**	**ΨG AC**	**ΨU AA**	**ΨA AU**	**ΨC GG**	**ΨG UC**	**ΨA CU**
$E^{MD}_{NN,binding}$	-31.19	-32.09	-23.86	-26.84	-30.38	-22.51	-22.76

$\Delta G^{\circ}_{37,MD,Predicted}$	**-2.11**	**-2.22**	**-1.20**	**-1.57**	**-2.01**	**-1.04**	**-1.07**
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.51 (0.10)	-2.35 (0.08)	N.A.				
$\Delta G^{\circ}_{37,Experiment}$ (Hudson et al, 2013)[5]	-2.49	-2.20	-2.74	-2.10	N.A.		
Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas $E^{MD}_{NN,binding} = E_{stack} + 0.5\ (E_{HB})$ and $\Delta G^{\circ}_{37,MD,Predicted} = 0.1229 * E^{MD}_{NN,binding} + 1.7277$.							

NN pairs	**Gm$^1\Psi$CA**	**Cm$^1\Psi$GA**	**Am$^1\Psi$UA**	**Um$^1\Psi$AA**	**Gm$^1\Psi$CG**	**Cm$^1\Psi$GU**	**Um$^1\Psi$AC**
$E^{MD}_{NN,binding}$	-31.72	-31.07	-22.44	-23.16	-33.56	-24.44	-26.18
$\Delta G^{\circ}_{37,MD,Predicted}$	**-2.17**	**-2.09**	**-1.03**	**-1.12**	**-2.39**	**-1.28**	**-1.49**
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.43 (0.08)	-1.83 (0.09)	N.A.				
NN pairs	**m$^1\Psi$CAG**	**m$^1\Psi$GAC**	**m$^1\Psi$UAA**	**m$^1\Psi$AAU**	**m$^1\Psi$CGG**	**m$^1\Psi$GUC**	**m$^1\Psi$ACU**
$E^{MD}_{NN,binding}$	-30.90	-30.49	-24.03	-26.76	-29.61	-23.08	-25.43
$\Delta G^{\circ}_{37,MD,Predicted}$	**-2.07**	**-2.02**	**-1.39**	**-1.75**	**-1.91**	**-1.11**	**-1.39**
$\Delta G^{\circ}_{37,Experiment}$ (Mauger et al, 2019)[6]	-2.67 (0.08)	-2.26 (0.07)	N.A.				
Here $E^{MD}_{NN,binding}$ and $\Delta G^{\circ}_{37,MD,Predicted}$ were calculated using the formulas $E^{MD}_{NN,binding} = E_{stack} + 0.5\ (E_{HB})$ and $\Delta G^{\circ}_{37,MD,Predicted} = 0.1229 * E^{MD}_{NN,binding} + 1.7277$.							

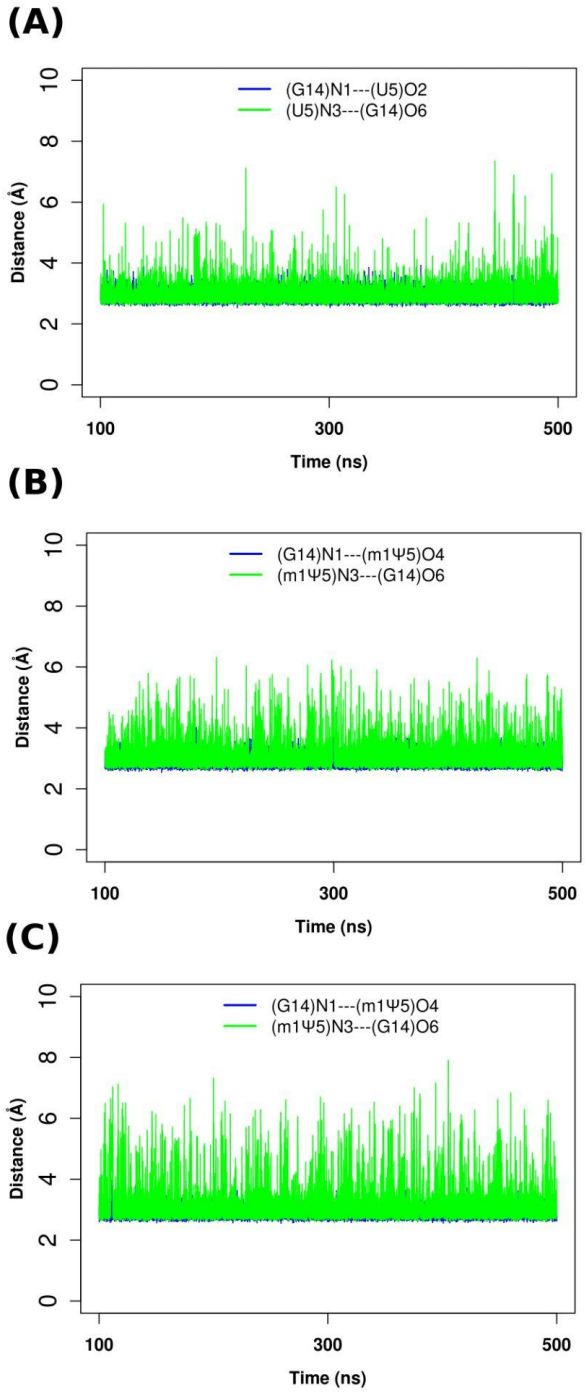

Figure S1. Time series of hydrogen bonding distance (d, i.e. the distance between the hydrogen bond donor and acceptor atoms) for the (A) U-G (B) Ψ-G and (C) m^1Ψ-G pairs. For m^1Ψ-G, the values obtained from simulation replicate 2 are plotted.

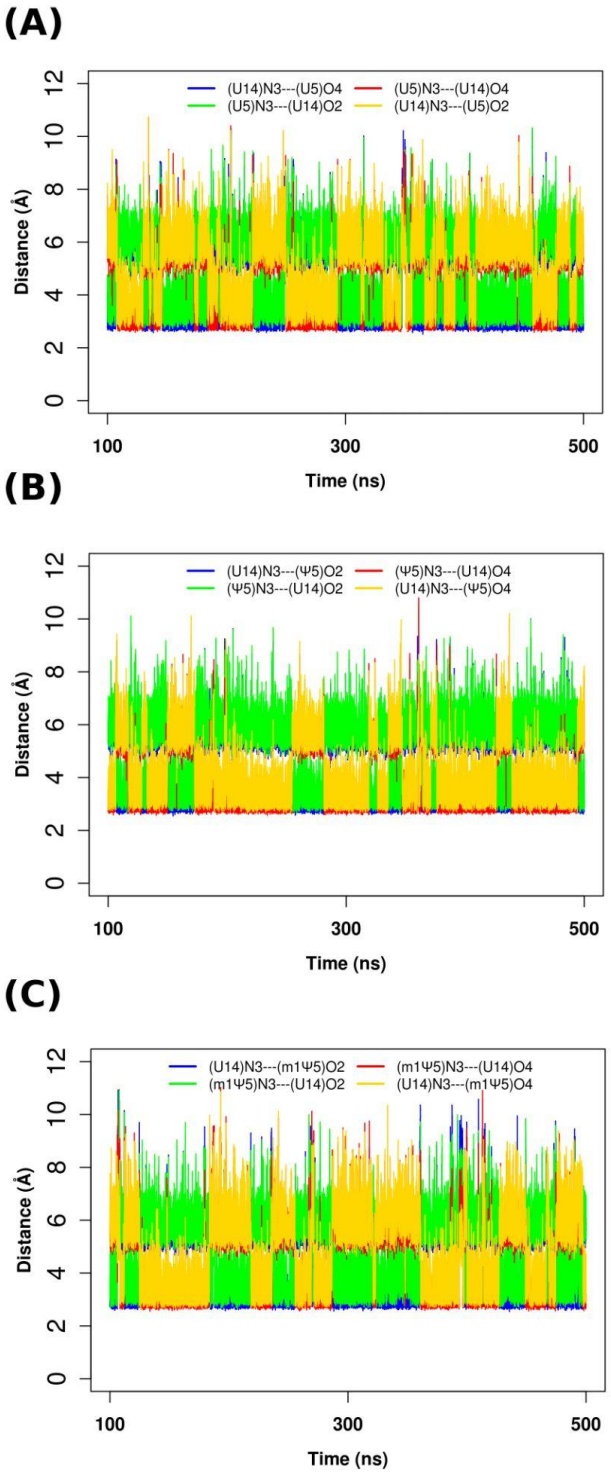

Figure S2. Time series of hydrogen bonding distance (d, i.e. the distance between the hydrogen bond donor and acceptor atoms) for the (A) U-U (B) Ψ-U and (C) m^1Ψ-U pairs. For m^1Ψ-U, the values obtained from simulation replicate 2 are plotted.

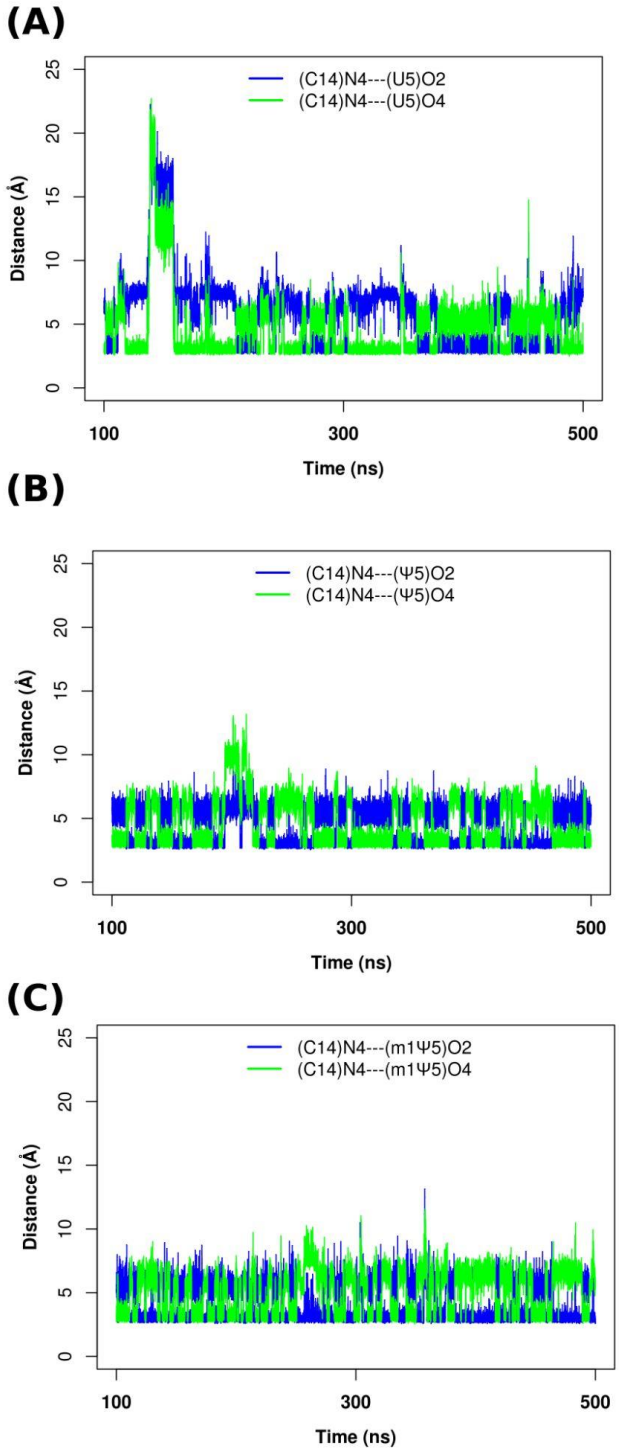

Figure S3. Time series of hydrogen bonding distance (d, i.e. the distance between the hydrogen bond donor and acceptor atoms) for the (A) U-C (B) Ψ-C and (C) $m^1Ψ$-C pairs. For $m^1Ψ$-C, the values obtained from simulation replicate 2 are plotted.

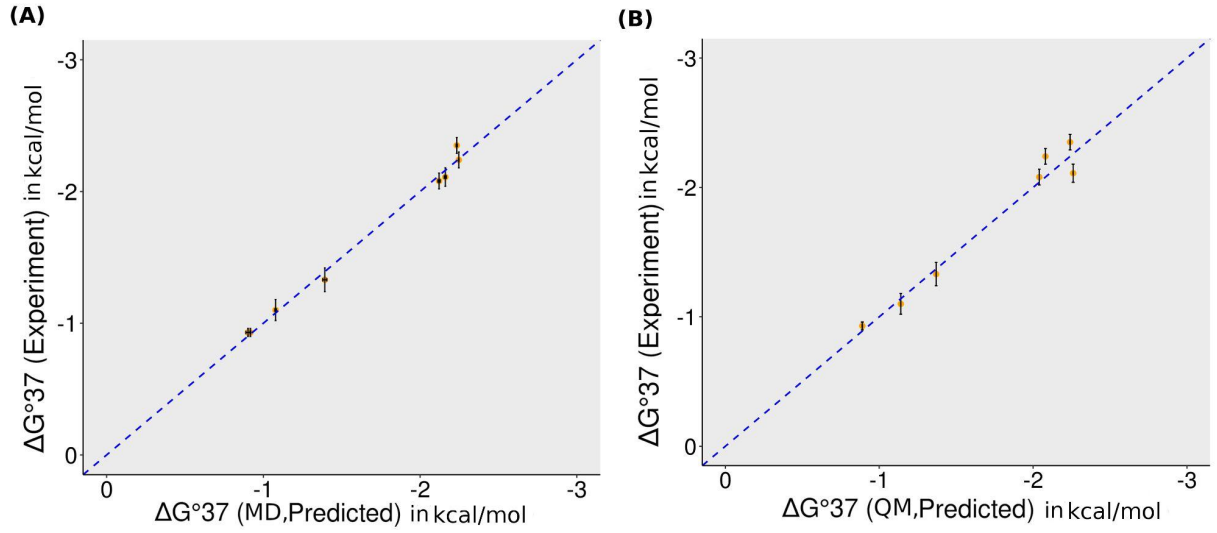

Figure S4. Plots of predicted nearest neighbor free energies (kcal/mol) (A) $\Delta G^{\circ}_{37,MD,Predicted}$ (B) $\Delta G^{\circ}_{37,QM,Predicted}$ vs experimental nearest neighbor free energies ($\Delta G^{\circ}_{37,Experiment}$) for the U-A pair reported by Xia et al., 1998 [3]. The blue dashed line represents the ideal line of regression (with slope =1, and intercept =0).

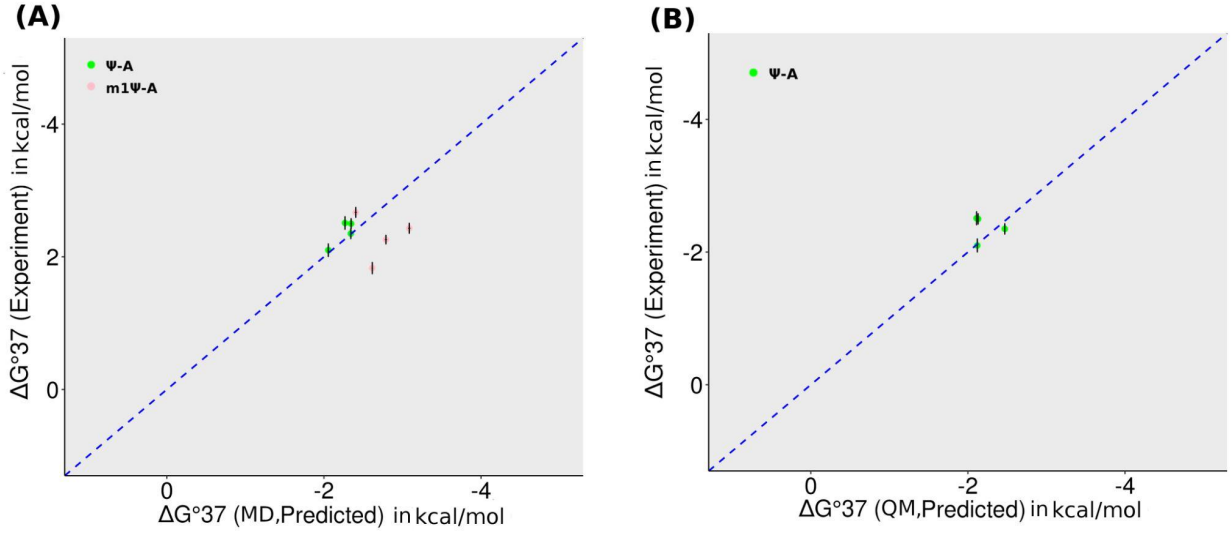

Figure S5. Plots of predicted nearest neighbor free energies (kcal/mol) (A) $\Delta G^{\circ}_{37,MD,Predicted}$ (B) $\Delta G^{\circ}_{37,QM,Predicted}$ vs experimental nearest neighbor free energies ($\Delta G^{\circ}_{37,Experiment}$) for the Ψ-A (panels (A) and (B)) and $m^1\Psi$-A (only panel (A)) pairs reported by Mauger et al., [6]. The blue dashed line represents the ideal line of regression (with slope =1, and intercept =0).

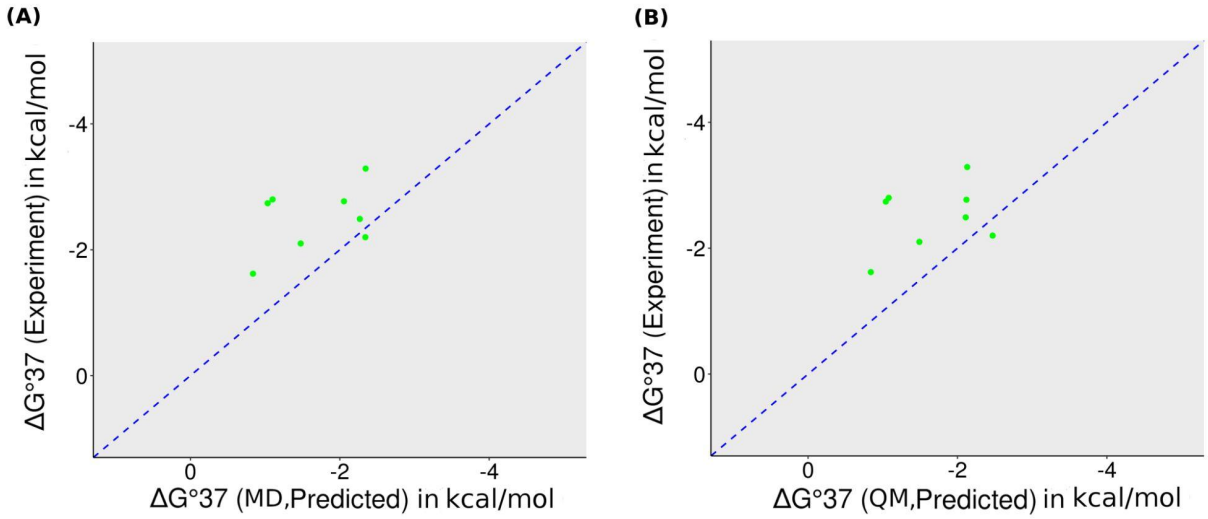

Figure S6. Plots of predicted nearest neighbor free energies (kcal/mol) (A) $\Delta G^{\circ}_{37,MD,Predicted}$ (B) $\Delta G^{\circ}_{37,QM,Predicted}$ vs experimental nearest neighbor free energies ($\Delta G^{\circ}_{37,Experiment}$) for the Ψ-A pair reported by Hudson et al. [5]. The blue dashed line represents the ideal line of regression (with slope =1, and intercept =0).

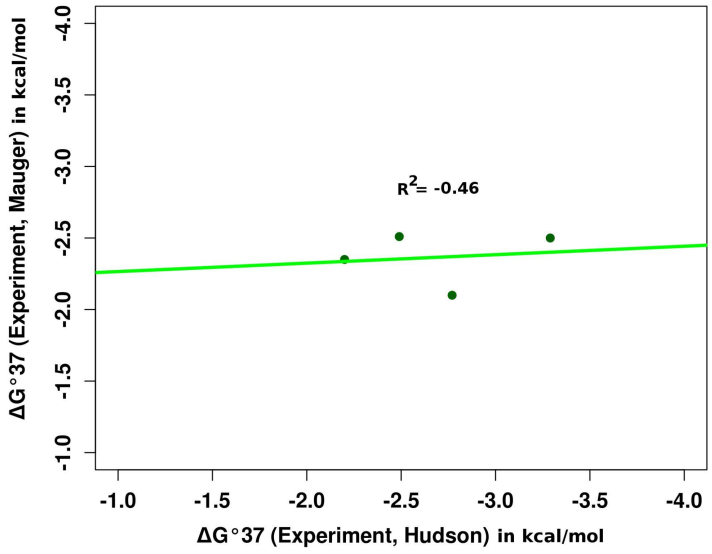

Figure S7. Linear regression plot of the experimental NN free energy values for the Ψ-A pair with the values reported by Hudson et al., 2013 [5] along the x-axis and those reported by Mauger

et al, [6] along the y-axis. The adjusted R-squared value is indicated above the regression line (green line).

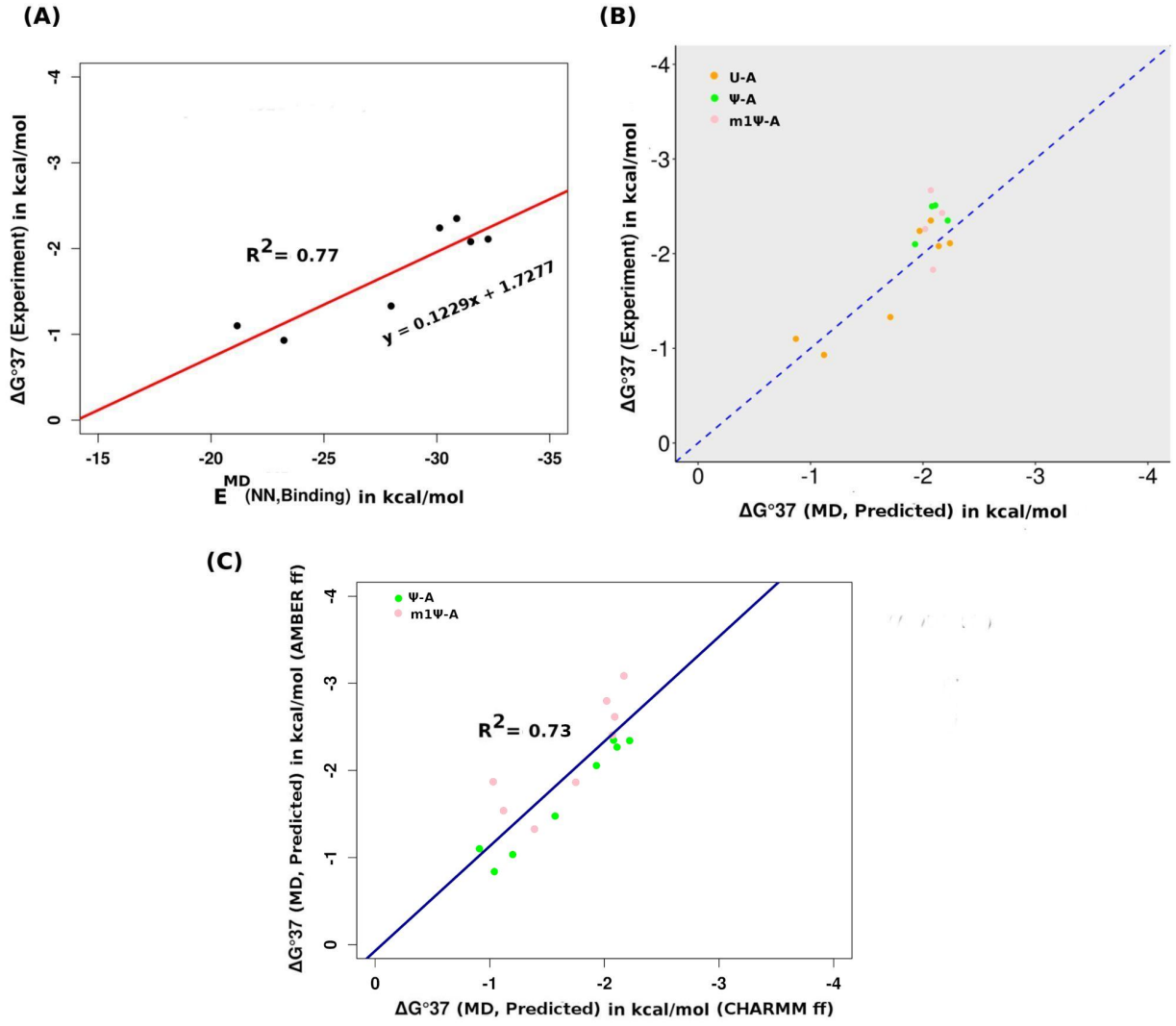

Figure S8. (A) Linear regression between experimental nearest neighbor free energies ($\Delta G^{\circ}_{37,Experiment}$) (kcal/mol) obtained from Xia et al, [3] and the $E_{NN,binding}$ (kcal/mol) values obtained from LIE calculations ($E^{MD}_{NN,binding}$) corresponding to MD simulations carried out with CHARMM36 force field for the NN pairs containing the U-A pair. R^2 value is indicated above the regression line (solid red line) and the equation of the regression line is also shown; (B) Nearest neighbor free energies (kcal/mol) predicted from MD (with CHARMM36 force field) using LIE analysis ($\Delta G^{\circ}_{37,MD,Predicted}$) vs experimental nearest neighbor free energies ($\Delta G^{\circ}_{37,Experiment}$)

for the U-A pair obtained from Xia et al, [3] and Ψ-A and m[1]Ψ-A pairs reported by Mauger et al, [6]. The blue dashed line represents the ideal line of regression (with slope =1, and intercept =0). The R^2 value for the actual regression is 0.776; (C) Linear regression between the predicted NN free energy parameters ($\Delta G^{\circ}_{37,MD,Predicted}$) for the Ψ-A and m[1]Ψ-A pairs in this study using CHARMM (CHARMM36) force field and those predicted using AMBER (FF99 variants) force field. The adjusted R^2 value is indicated above the regression line (solid blue line).

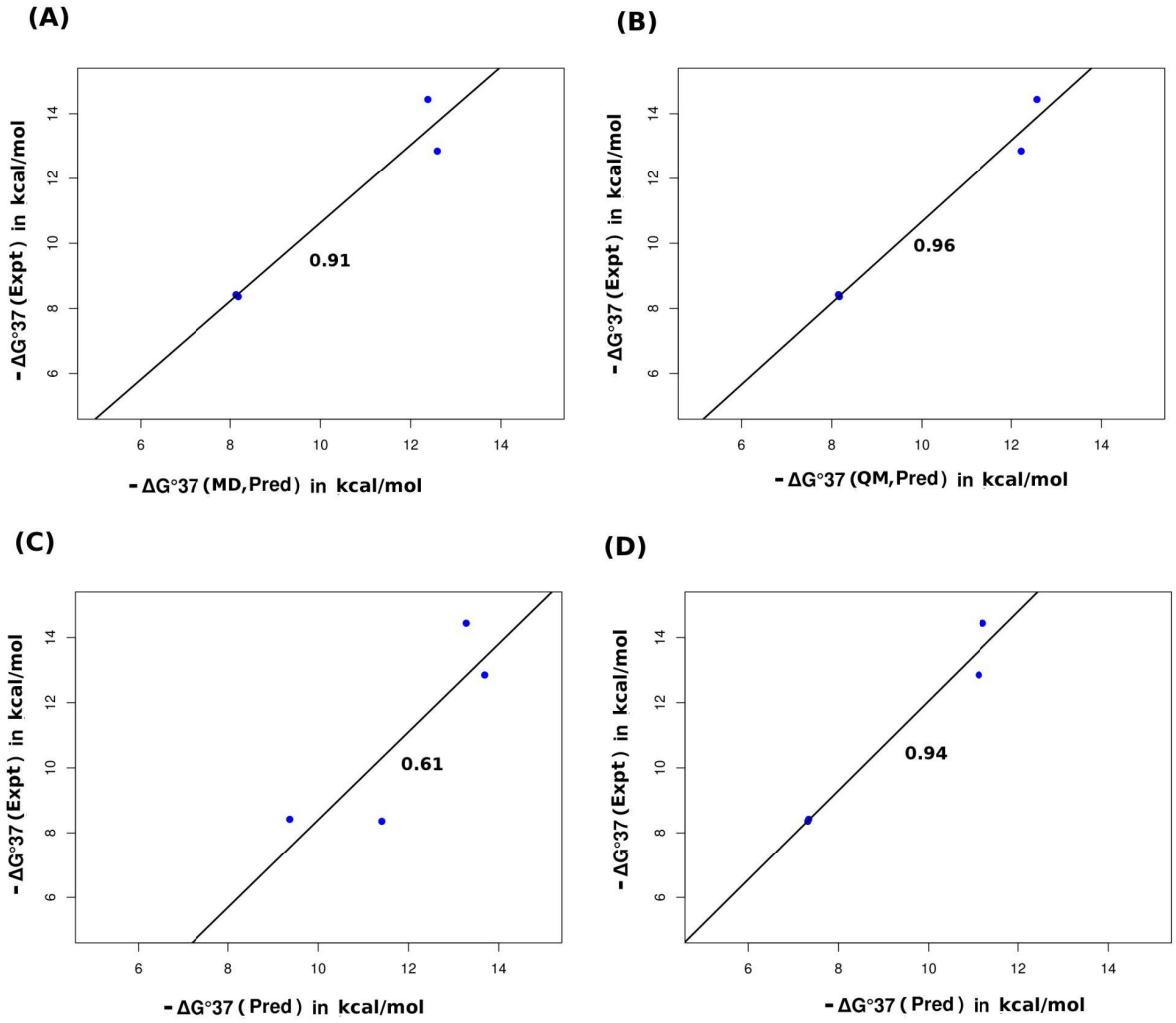

Figure S9. Linear regression plots of predicted folding free energies (kcal/mol) vs experimental folding free energies (reported by Kierzek et al., 2014 [10]) for the duplexes containing internal Ψ-A pair studied in this work. (A) Predicted folding free energies using the NN free energy parameters for Ψ-A predicted from MD (using LIE approach) in this work; (B) predicted folding free energies using the NN free energy parameters for Ψ-A predicted from QM in this work; (C)

predicted folding free energies using the NN free energy parameters for Ψ-A reported by Hudson et al., 2013 [5] and (D) predicted folding free energies using the NN free energy parameters for Ψ-A reported by Hopfinger et al., 2020 [11]. The adjusted R^2 values are indicated below the regression line (black line).

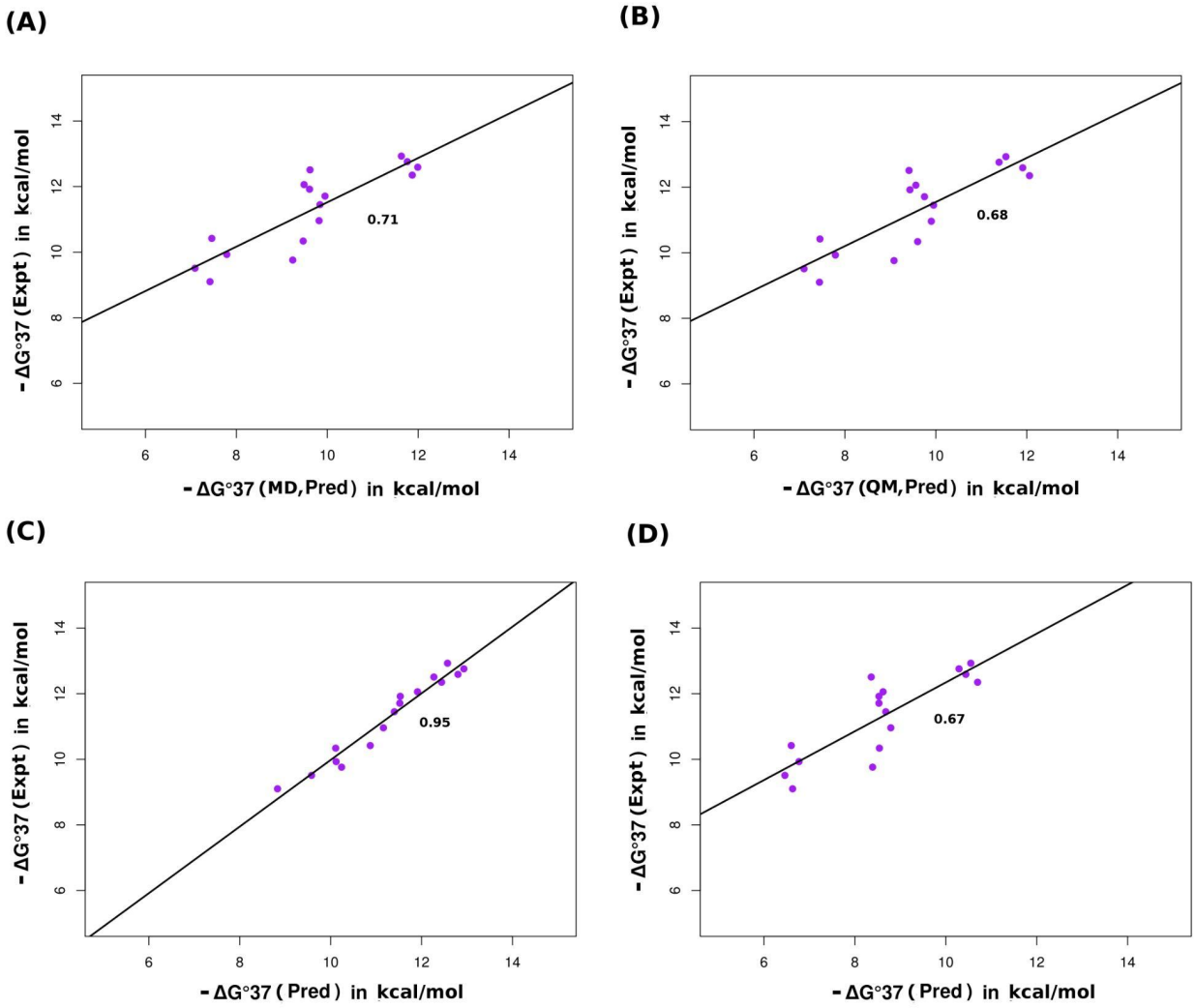

Figure S10. Linear regression plots of predicted folding free energies (kcal/mol) vs experimental folding free energies (reported by Hudson et al., 2013 [5]) for the duplexes containing internal Ψ-A pair in their study[5]. (A) Predicted folding free energies using the NN free energy parameters for Ψ-A predicted from MD (using LIE approach) in this work; (B) predicted folding free energies using the NN free energy parameters for Ψ-A predicted from QM in this work; (C) predicted folding free energies using the NN free energy parameters for Ψ-A reported by Hudson et al.,

2013 [5] and (D) predicted folding free energies using the NN free energy parameters for Ψ-A reported by Hopfinger et al., 2020 [11]. The adjusted R^2 values are indicated below the regression line (black line).

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