Principles of $tRNA^{Ala}$ selection by alanyl-tRNA synthetase based on the critical G3•U70 base pair

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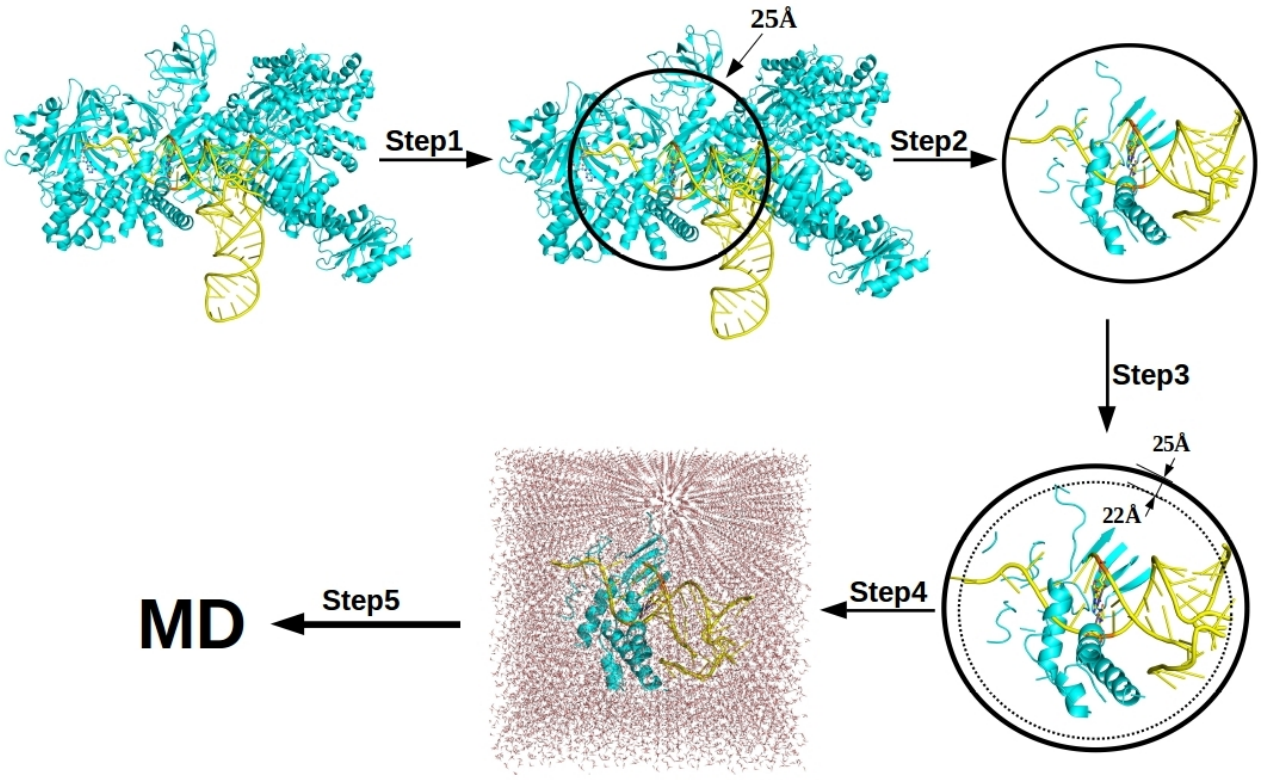

Figure S1: MD setup. Step1: Selection of 25Å radii sphere centred at N9 of G3/A3 (resid 1603) nucelotide of $tRNA^{Ala}$ bound Ala-Synthetase complex (PDB:3wqy, 3wqz). Step 2: Truncated model (25Å sphere) for molecular dynamics simulations. Step 3: Heavy atoms of the "buffer region" (22Å-25Å) are harmonically restrained to their experimentally determined positions. Sept4: Overlay water box of edge length 80Å, equilibrate and proceed for MD.

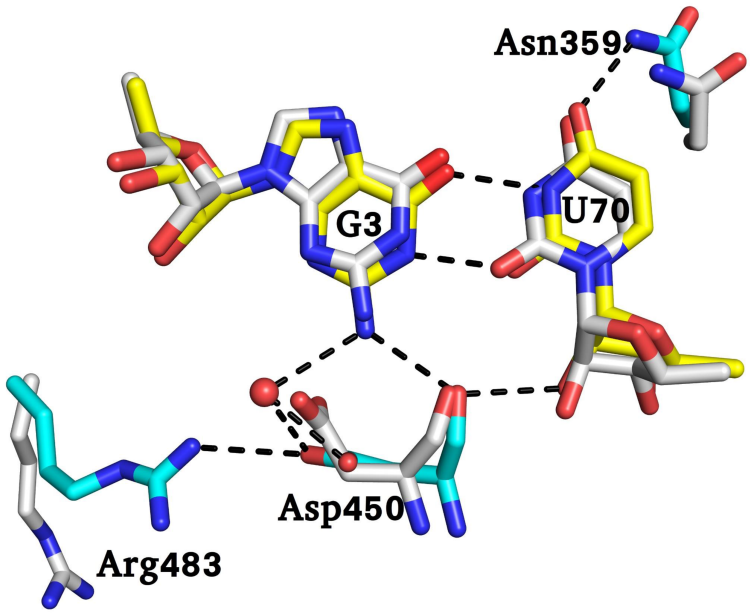

Figure S2. MD structure of reactive AlaRS:$tRNA^{Ala}$/G3.U70 complex (Gray sticks) and after reverse alchemical transformation, i.e, A3.U70 → G3.U70 (yellow sticks for G3.U70 and protein residues in cyan sticks). Arg483-Asp450 salt bridge is present at the end point of the reverse alchemical transformation.

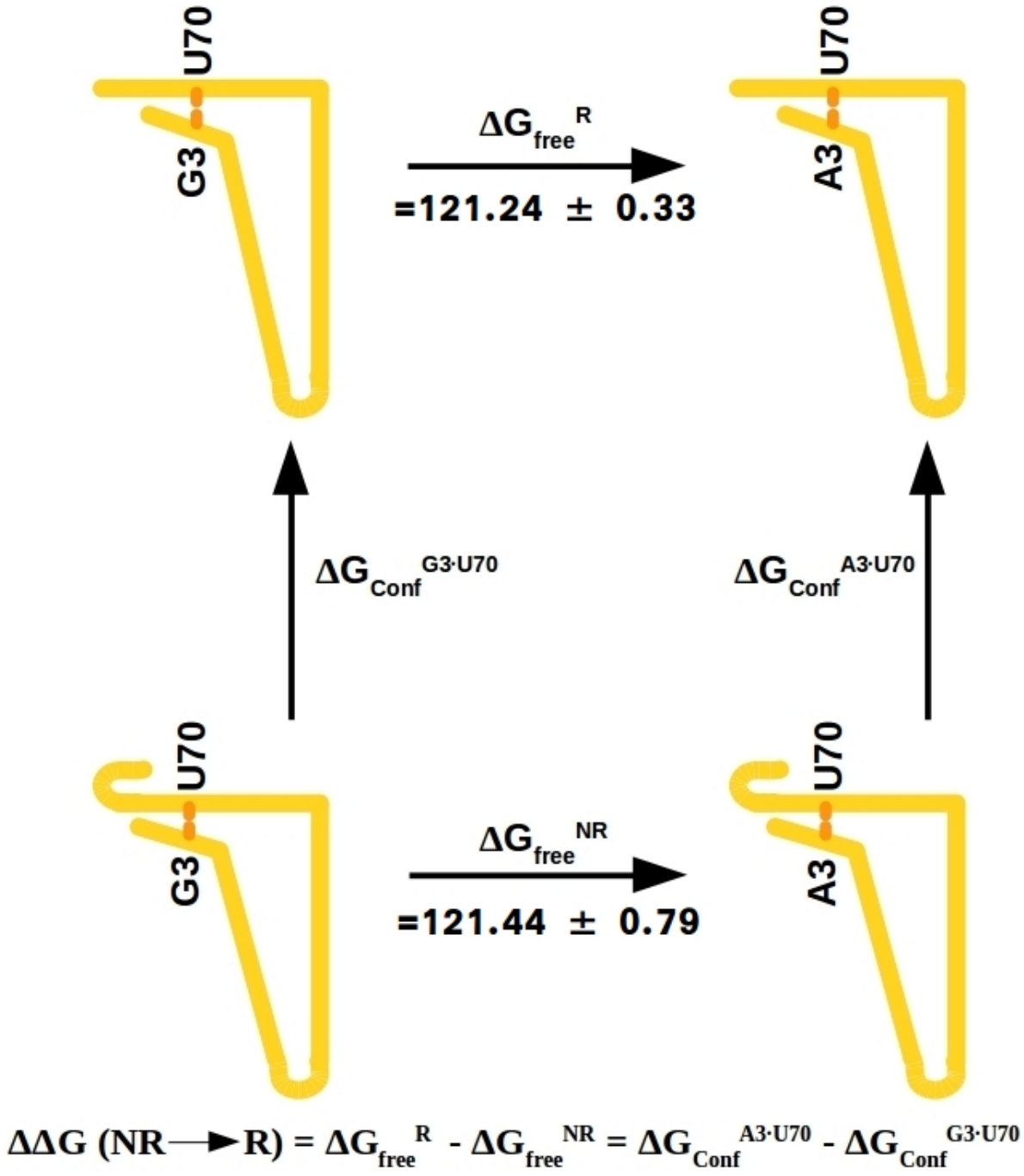

Figure S3. Thermodynamic cycle for alchemical transformation of G3•U70 into A3•U70 in free tRNA in water. Vertical legs correspond to the conformation transformation from non-reactive to reactive; horizontal legs correspond to the alchemical transformation of the identity base pair, either in the reactive conformation (upper leg) or in non-reactive conformation (bottom leg).

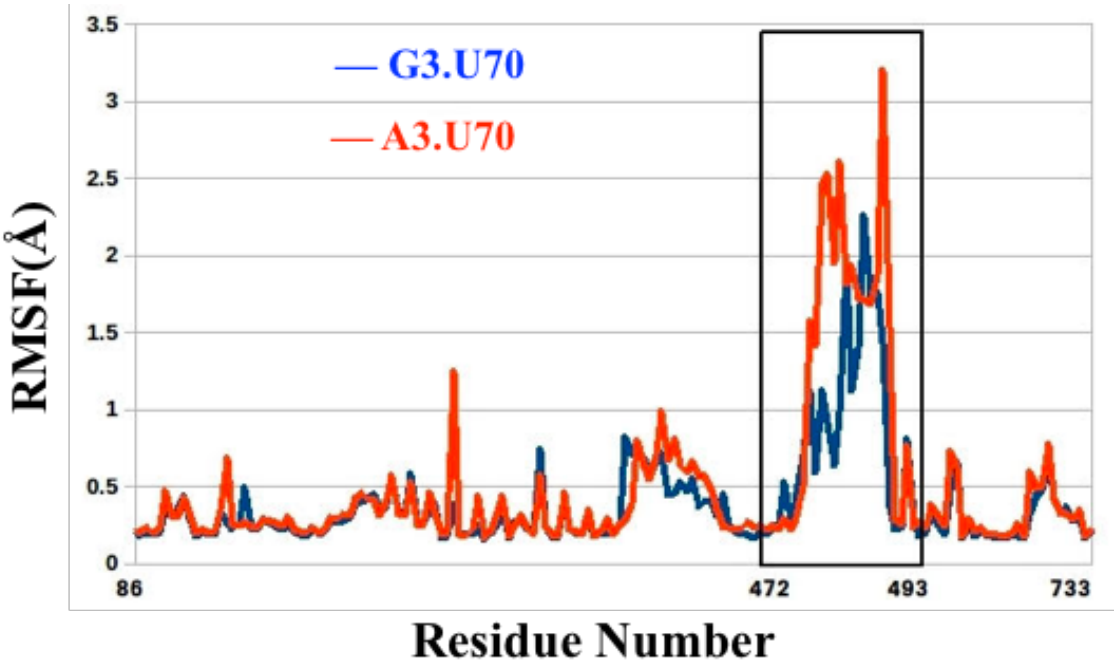

Figure S4: Root-mean-square fluctuation of the heavy atoms of the loop region is highlighted with rectangular box. RMSF plotted was averaged over 7ns MD trajectory with 1 ps interval.

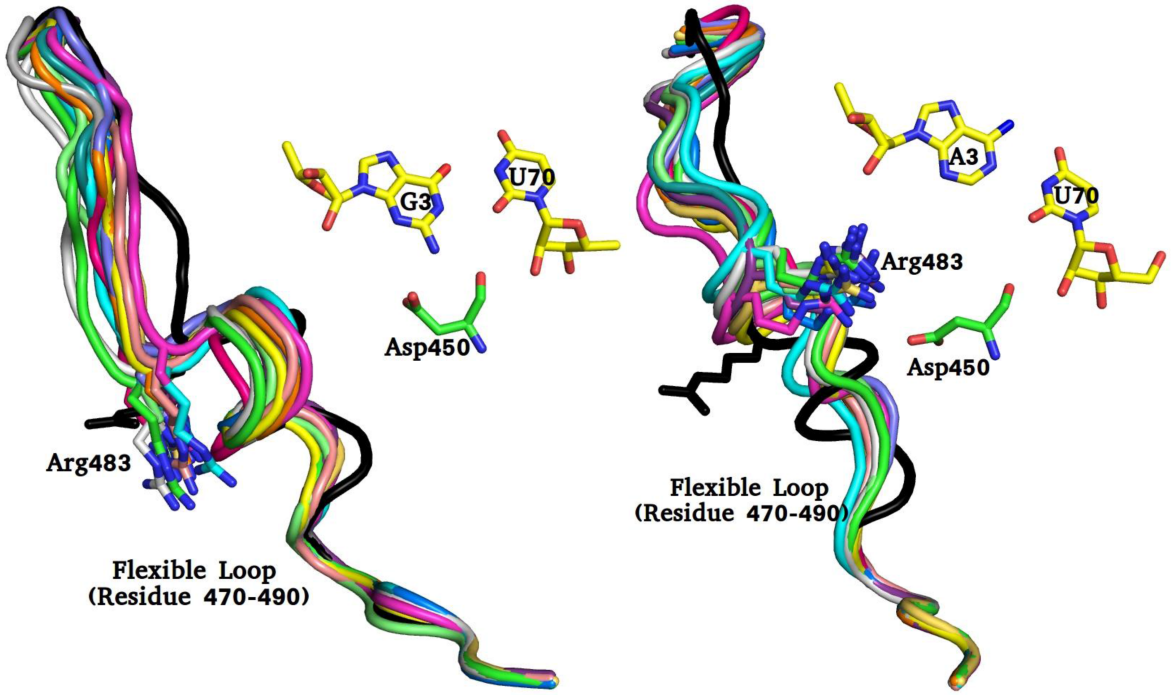

Figure S5. Loop comparison between X-ray (black) and MD structures of AlaRS.tRNA complex with respect to G3.U70 (left) and A3.U70 (right). High flexibility of the loop region (residue 470−490) is visible: overlaid 15 snapshots with a 125 ps spacing from a 2 ns MD trajectory. The distantly different orientation of Arg483 is visible. 3.70 base pair, Asp450 and Arg483 in sticks.

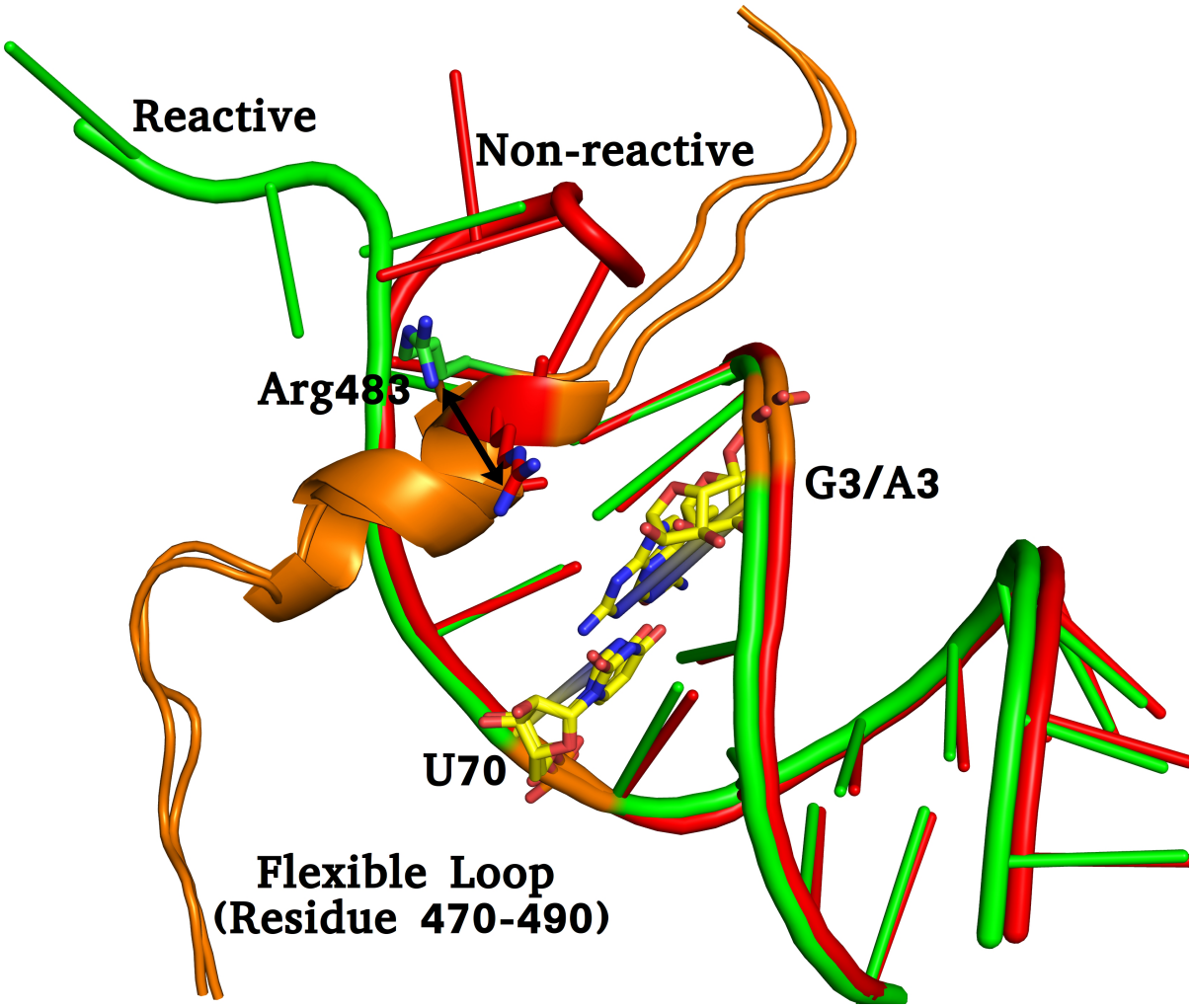

Figure S6. X-ray structure of the reactive (green) and non-reactive (red) complex revealed that Arg483 of highly flexible loop region (orange) is in different orientation (represented by double headed arrow) with respect to 3.70 base pair (yellow sticks).

Table S1: Partial changes of RNA phosphates.

Atom Name	Charges in CHARMM36 FF	Scaling down partial charges
P	1.50	1.75
O1P	-0.78	-0.53
O2P	-0.78	-0.53
O5'	-0.57	-0.32

Table S2:

(a) Free energy change for the alchemical transformation of G3·U70→A3·U70 in reactive complex of AlaRS:tRNA^{Ala}. MD trajectories were divided into two equal halves and the difference between the computed ΔG's from the two-halves is reported as uncertainty in the parenthesis for individual replicas. Uncertainty in the average result is reported as standard error (ΔG's from different replicas). Hysteresis of > 5 kcal/mol observed.

System	**No of runs**	**Simulation Length (Fwd+Rev)**	**Forward ΔG^{comp} (Figure 2a)** (G3·U70) to (A3·U70)	**Reverse ΔG^{comp} (Figure 2a)** (A3·U70) to (G3·U70)
Reactive State (R): AlaRS:tRNA^{Ala} /(G3·U70) Forward G-->A Reverse A-->G	Run1	34 ns	125.23 (1.91)	-121.06 (1.84)
	Run2	34 ns	126.39 (1.33)	-122.37 (1.27)
	Run3	34 ns	128.55 (1.52)	-121.46 (2.01)
	Run4	34 ns	127.90 (1.56)	-120.65 (1.43)
	Run5	34 ns	126.39 (1.91)	-121.33 (2.37)
Average			**126.89 (0.59)**	**-121.37 (0.28)**

(b) Free energy change for the alchemical transformation of G3/A3 → A3/G3 in reactive/non-reactive conformation of tRNA^{Ala} free in water. G3 in non-reactive conformation of free tRNA^{Ala} was modelled by replacing A3 by G3. Uncertainties are calculated in the same way described in Table S1(a).

System	**No of runs**	**Simulation Length (Fwd+Rev)**	**Forward ΔG_{free}^{R} (Figure 2a)** (G3·U70) to (A3·U70)	**Reverse ΔG_{free}^{R} (Figure 2a)** (A3·U70) to (G3·U70)
tRNA free in water (in Reactive conformation): tRNA^{Ala} /(G3·U70) Forward G-->A Reverse A-->G	Run1	34 ns	120.97 (1.49)	-121.63 (0.35)
	Run2	34 ns	121.25 (1.14)	-121.13 (2.13)
	Run3	34 ns	120.14 (1.49)	-122.46 (1.58)
	Run4	34 ns	122.20 (1.45)	-119.39 (1.76)
	Run5	34 ns	121.40 (1.03)	-121.85 (1.10)
Average			121.19 (0.33)	-121.29 (0.52)
Forward+Reverse Average: ΔG_{free}^{R}				**121.24 (0.43)**

System	**No of runs**	**Simulation Length (Fwd+Rev)**	**Forward ΔG_{free}^{NR} (Figure 2a)** (A3·U70) to (G3·U70)	**Reverse ΔG_{free}^{NR}(Figure 2a)**

				(G3·U70) to (A3·U70)
tRNA free in water (Non-reactive conformation): tRNA^{Ala} /(A·U) Forward A-->G Reverse G-->A	Run1	34 ns	-121.65 (1.57)	121.21 (1.57)
	Run2	34 ns	-121.72 (1.76)	122.03 (1.02)
	Run3	34 ns	-121.67 (1.20)	121.71 (1.38)
	Run4	34 ns	-121.57 (0.99)	121.33 (0.93)
	Run5	34 ns	-120.57 (1.17)	121.43 (0.91)
Average			-121.43 (0.22)	121.54 (0.15)
	No of runs	**Simulation Length (Fwd+Rev)**	**Forward ΔG_{free}^{NR}** (G3·U70) to (A3·U70)	**Reverse ΔG_{free}^{NR}** (A3·U70) to (G3·U70)
tRNA free in water (Non-reactive conformation): tRNA^{Ala} /(G·U) - MODELEED Forward G-->A Reverse A-->G	Run1	34 ns	121.41 (1.15)	-121.36 (1.62)
Average			121.41 (1.15)	-121.36 (1.62)
Forward + Reverse Average of G3→ A3, ΔG_{free}^{NR}				**121.44 (0.79)**
$\Delta\Delta G_{free}^{NR\rightarrow R}$ (G3.U70 vs A3.U70) = ΔG_{free}^{R} - ΔG_{free}^{NR}				**-0.2 (0.9)**

(c) Free energy change for the alchemical transformation of G3/A3·U→ A3/G3·U in AlaRS:tRNA^{Ala} non-reactive complex.

System	**No of runs**	**Simulation Length (F+R)**	**Forward ΔG (A·U) to (G·U)**	**Reverse ΔG (G·U) to (A·U)**
Non-reactive State: AlaRS:tRNA^{Ala} /(A3·U70) Forward A-->G Reverse G-->A	Run1	34 ns	-122.54 (1.62)	122.37 (1.63)
	Run2	34 ns	-119.96 (1.59)	118.35 (1.81)
	Run3	34 ns	-121.25 (1.64)	121.85 (2.14)
	Run4	34 ns	-123.98 (1.43)	118.48 (2.57)
	Run5	34 ns	-120.60 (1.98)	120.84 (1.08)
		Average	**-121.67 (0.72)**	**120.38 (0.84)**

System	No of runs	Simulation Length (F+R)	Forward ΔG (G·U) to (A·U)	Reverse ΔG (A·U) to (G·U)
Non-reactive State: AlaRS:tRNA^{Ala} /(G3·U70) - [A3 replaced by reactive state G3.] Forward G-->A Reverse A-->G	Run1	34 ns	123.78 (1.84)	-119.11 (0.66)
	Run2	34 ns	121.73 (1.70)	-119.10 (2.32)
	Run3	34 ns	123.66 (1.37)	-119.40 (1.34)
	Run4	34 ns	122.82 (1.80)	-120.64 (2.58)
	Run5	34 ns	122.17 (1.53)	-122.32 (1.27)
	Average		**122.83 (0.40)**	**-120.11 (0.62)**

(d) Free energy change for the alchemical transformation of G3·U70 → G3·C70 in AlaRS:tRNA^{Ala} reactive complex

System	No of runs	Simulation Length (F+R)	Forward ΔG (G·U) to (G·C)	Reverse ΔG (G·C) to (G·U)
Reactive State: AlaRS:tRNA^{Ala} /(G·U) Forward U-->C Reverse C-->U	Run1	22 ns	14.55 (0.19)	-12.96 (1.00)
	Run2	22 ns	12.70 (1.33)	-12.02 (1.00)
	Run3	22 ns	14.01 (1.08)	-14.50 (0.99)
	Run4	22 ns	13.87 (0.54)	-14.40 (0.78)
	Run5	22 ns	13.06 (0.93)	-11.04 (0.98)
Average			**13.64 (0.33)**	**-12.98 (0.67)**

(e) Free energy change for the alchemical transformation of G3·U70→G3·C70 in AlaRS:tRNA^{Ala} non-reactive complex

System	No of runs	Simulation Length (F+R)	Forward ΔG (G·U) to (G·C)	Reverse ΔG (G·C) to (G·U)
Non-reactive State: AlaRS:tRNA^{Ala} /(G·U) Forward U-->C Reverse C-->U	Run1	22 ns	14.03 (1.14)	-13.23 (1.10)
	Run2	22 ns	15.95 (0.89)	-12.82 (0.96)
	Run3	22 ns	13.36 (0.66)	-11.38 (0.88)
	Run4	22 ns	13.82 (0.94)	-11.71 (0.75)
	Run5	22 ns	13.27 (0.51)	-11.93 (0.75)
Average			**14.09 (0.49)**	**-12.21 (0.35)**

(f) Free energy change for the alchemical transformation of G3·U70→G3·C70 in reactive and non-reactive $tRNA^{Ala}$ free in water

System	No of runs	Simulation Length (F+R)	Forward ΔG (G·U) to (G·C)	Reverse ΔG (G·C) to (G·U)
tRNA free in water (Reactive conformation): $tRNA^{Ala}$ /(G·U) Forward U-->C Reverse C-->U	Run1	22 ns	7.65 (0.11)	-7.37 (0.22)
	Run2	22 ns	7.86 (0.78)	-8.01 (0.51)
	Run3	22 ns	7.64 (0.76)	-8.07 (0.72)
	Run4	22 ns	7.74 (0.65)	-7.97 (0.93)
	Run5	22 ns	6.62 (0.94)	-8.64 (0.84)
Average			**7.50 (0.22)**	**-8.01 (0.20)**

System	No of runs	Simulation Length (F+R)	Forward ΔG (G·U) to (G·C)	Reverse ΔG (G·C) to (G·U)
tRNA free in water (Non-reactive conformation): $tRNA^{Ala}$ /(G·U) Forward U-->C Reverse C-->U	Run1	22 ns	8.02 (0.71)	-8.01 (0.66)
	Run2	22 ns	7.92 (0.45)	-7.74 (0.75)
	Run3	22 ns	7.68 (0.69)	-7.59 (0.64)
	Run4	22 ns	7.70 (0.72)	-8.14 (0.51)
	Run5	22 ns	7.78 (0.72)	-8.08 (0.64)
Average			**7.82 (0.06)**	**-7.91 (0.11)**

(g) Free energy change for the alchemical transformation of A3·U70→A3·C70 in AlaRS:$tRNA^{Ala}$ reactive complex where A3 taken from non-reactive complex

System	No of runs	Simulation Length (F+R)	Forward ΔG (A·U) to (A·C)	Reverse ΔG (A·C) to (A·U)
Reactive State: AlaRS:$tRNA^{Ala}$ /(A3·U70)-A3 modelled from Non-Reactive state Forward U-->C Reverse C-->U	Run1	22 ns	18.35 (1.23)	-18.12 (0.60)
	Run2	22 ns	18.55 (1.44)	-17.69 (0.70)
	Run3	22 ns	17.25 (0.66)	-17.45 (1.59)
	Run4	22 ns	18.60 (0.91)	-17.49 (0.87)
	Run5	22 ns	17.74 (1.39)	-18.00 (1.42)
Average			**18.10 (0.26)**	**-17.75 (0.13)**

(h) Free energy change for the alchemical transformation of A3·U70→A3·C70 in AlaRS:tRNA^{Ala} non-reactive complex

System	**No of runs**	**Simulation Length (F+R)**	**Forward ΔG** (A·U) to (A·C)	**Reverse ΔG** (A·C) to (A·U)
Non-reactive State: AlaRS:tRNA^{Ala} /(A·U) Forward U-->C Reverse C-->U	Run1	22 ns	17.68 (0.38)	-18.59 (1.16)
	Run2	22 ns	17.72 (1.16)	-15.56 (0.55)
	Run3	22 ns	18.23 (1.52)	-16.6 (1.00)
	Run4	22 ns	18.25 (1.35)	-17.77 (1.27)
	Run5	22 ns	18.11 (1.44)	-18.71 (1.60)
		Average	**18.00 (0.12)**	**-17.45 (0.60)**

(**i)** tRNA free in water Free energy change for the alchemical transformation of A3·U70→A3·C70 in reactive and non-reactive tRNA^{Ala} free in water where A in reactive tRNA^{Ala} taken from non-reactive complex

System	**No of runs**	**Simulation Length (F+R)**	**Forward ΔG** (A·U) to (A·C)	**Reverse ΔG** (A·C) to (A·U)
tRNA free in water (Reactive conformation): tRNA^{Ala} /(A·U)-"A" Modelled from Non-reactive state Forward U-->C Reverse C-->U	Run1	22 ns	15.20 (0.87)	-14.88 (0.47)
	Run2	22 ns	14.24 (1.09)	-14.25 (0.74)
	Run3	22 ns	14.43 (1.04)	-14.02 (0.72)
	Run4	22 ns	13.41 (0.59)	-14.87 (0.78)
	Run5	22 ns	14.18 (0.97)	-13.95 (0.87)
		Average	**14.29 (0.29)**	**-14.39 (0.20)**
System	**No of runs**	**Simulation Length (F+R)**	**Forward ΔG** (A·U) to (A·C)	**Reverse ΔG** (A·C) to (A·U)
Non-reactive conformation of tRNA free in water: tRNA^{Ala} /(A·U) Forward U-->C Reverse C-->U	Run1	22 ns	14.60 (0.38)	-13.26 (0.45)
	Run2	22 ns	14.02 (0.91)	-11.50 (0.97)
	Run3	22 ns	14.45 (0.84)	-14.68 (0.89)
	Run4	22 ns	14.92 (0.76)	-14.11 (1.15)
	Run5	22 ns	14.82 (1.14)	-14.41 (0.73)
		Average	**14.56 (0.16)**	**-13.59 (0.57)**