Supplementary Material for:

Mechanistic features of the atypical tRNA m^1G_9 SPOUT methyltransferase, Trm10

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Supplementary Figure Legends

Figure S1: Purified Trm10 proteins. SDS-PAGE of purified yeast and human Trm10 wild type and mutant proteins. Expected molecular weights for the N-terminal His_6-tagged enzymes: ScTrm10, 36.8 kDa; hTRMT10A, 42.0 kDa. MW – Molecular weight standards; WT – wild-type; DM – D210A + D100N ScTrm10 double mutant; TM – D210A + D100N + E111A ScTrm10 triple mutant.

Figure S2: SAM-dependence of Trm10 enzymes. $tRNA^{Gly}$ m^1G_9 methylation activity of ScTrm10 **(A)** and hTRMT10A **(B)** with varying concentrations of SAM (10 – 500 µM for WT, D210N and G206A G207A enzymes and 0.005 – 500 µM for all other variants). Nucleotides corresponding to reaction product (p*m^1G) and unreacted substrate (p*G) are indicated to the left of each assay panel.

Figure S3: SAM binding by human TRMT10A. Isothermal titration calorimetry (ITC) was used to measure the $K_{D,SAM}$ exhibited by hTRMT10A (WT and variants, as indicated). The top panels show raw injection traces (with heat expressed as differential power) and the bottom panels show the corresponding binding isotherms calculated from the integrated injection heats normalized per mole of injectant SAM.

Figure S4: Double mutant cycle analysis. A calculation of the theoretical k_{obs} for the D210A+D100N double mutant (DM) was performed using an additive assumption in which the change in free energy of the transition state associated with the double mutation is equal to the sum of the changes in free energy associated with the single mutations ($\Delta\Delta G^{\ddagger}_{int} = 0$). The measured rate constants (k_{obs}) for wild-

type and each of the single variants (Table 1) was used calculate the k_{obs} for the DM (shown in italics) according to the equations relating activation energy to the ratio of the relevant rate constants, yielding a theoretical value of 0.11 min^{-1}.

Figure S5: Binding to $tRNA^{Gly}$ by Trm10 single residue variants. (A) Binding assay with ScTrm10 enzymes measured by FA to calculate $K_{D,tRNA}$ for D210K (red), D100N (green) and E111A (blue) variant enzymes. Black triangles correspond to BSA control used in the same FA assay. Fits shown correspond to the best fit to the binding isotherm (eq. 4). **(B)** Binding assay with hTRMT10A enzymes measured by FA to calculate $K_{D,tRNA}$ for WT (brown) and D210N (purple) enzymes. Fits shown correspond to the best fit to the binding isotherm (eq. 4).

Figure S1

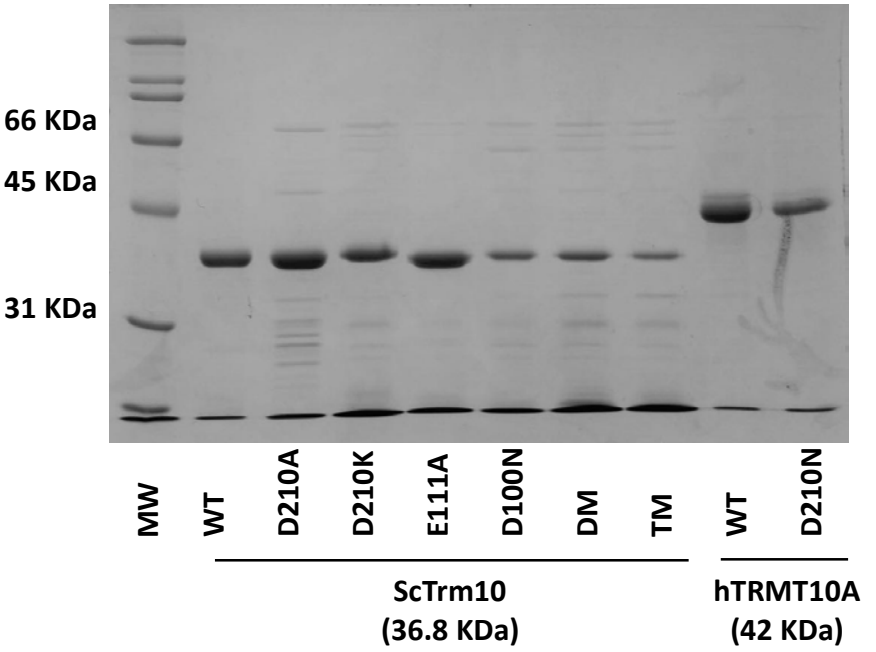

66 KDa
45 KDa
31 KDa
MW
WT
D210A
D210K
E111A
D100N
DM
TM
WT
D210N
ScTrm10
(36.8 KDa)
hTRMT10A
(42 KDa)

Figure S2

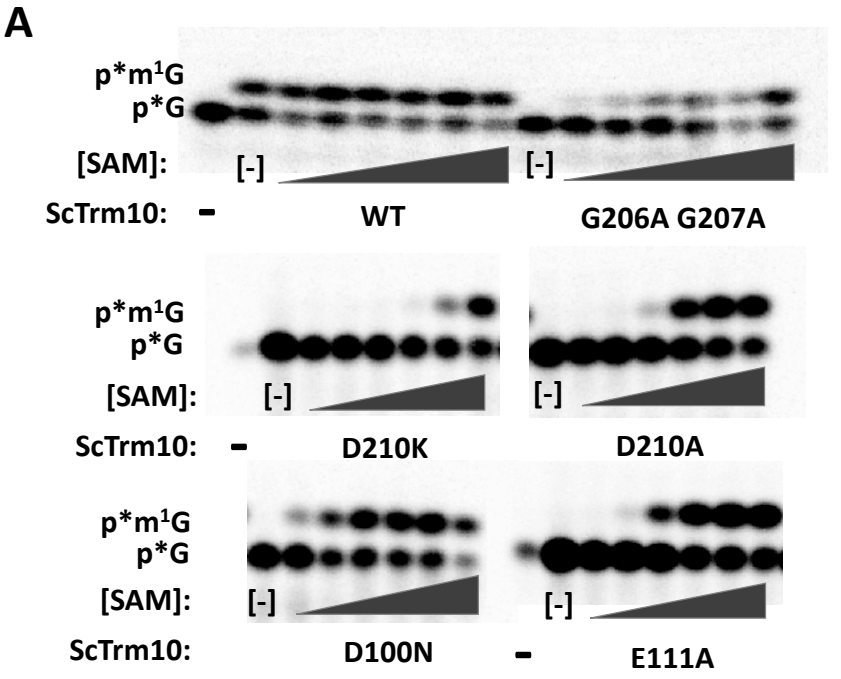

A
p*m1G
p*G
[SAM]:
[-]
[-]
ScTrm10:
-
WT
G206A G207A
p*m1G
p*G
[SAM]:
[-]
[-]
ScTrm10:
-
D210K
D210A
p*m1G
p*G
[SAM]:
[-]
[-]
ScTrm10:
D100N
-
E111A
B
p*m1G
p*G
SAM:
[-]
[-]
hTRMT10A:
-
WT
D210N

Figure S3

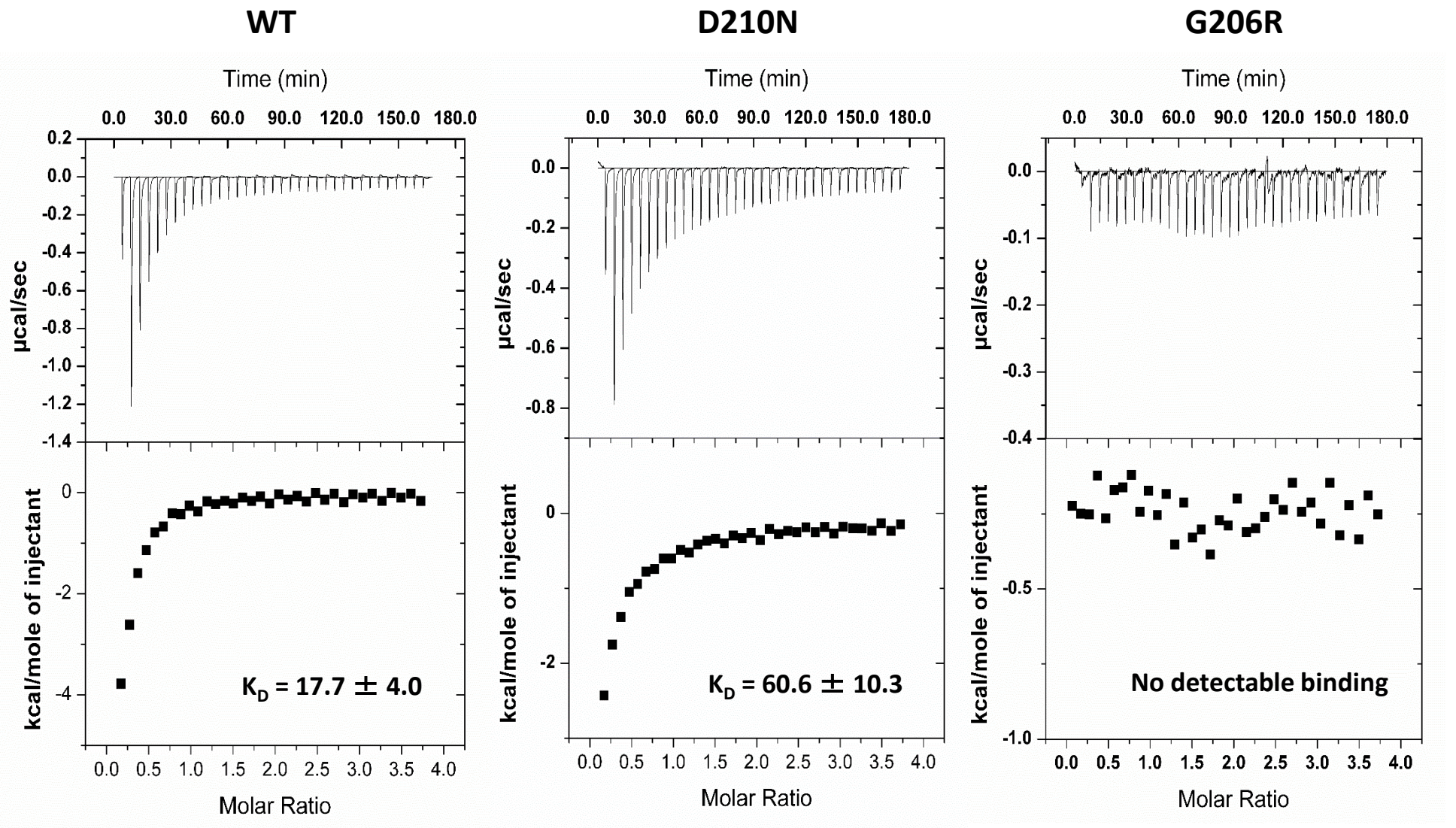
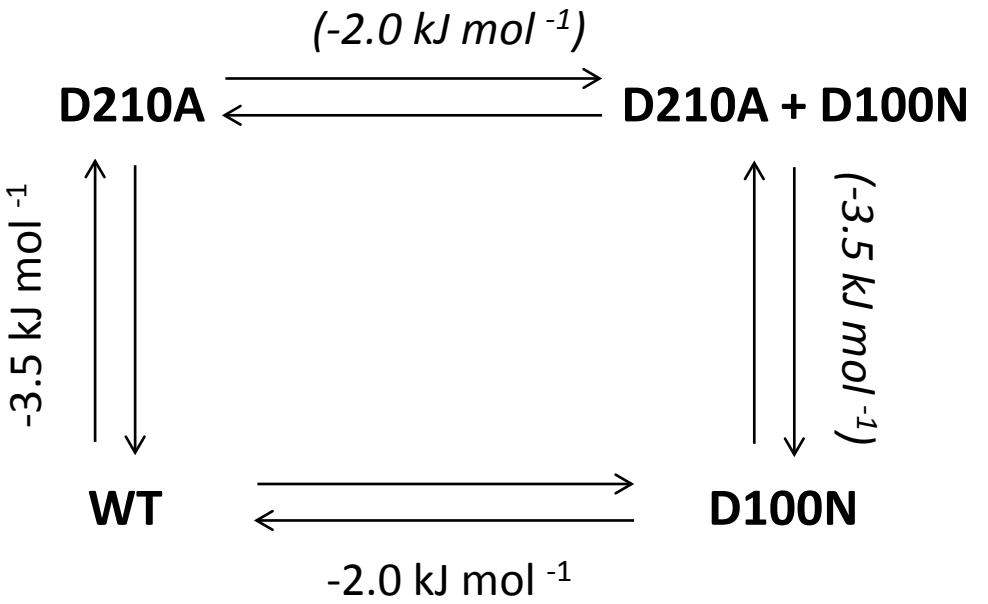

WT
D210N
G206R
Time (min)
0.0 30.0 60.0 90.0 120.0 150.0 180.0
µcal/sec
kcal/mole of injectant
Molar Ratio
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
K_D = 17.7 ± 4.0
K_D = 60.6 ± 10.3
No detectable binding

Figure S4



$\Delta\Delta G^{\ddagger}_{int}$ = 0 (if independent effects on transition state)

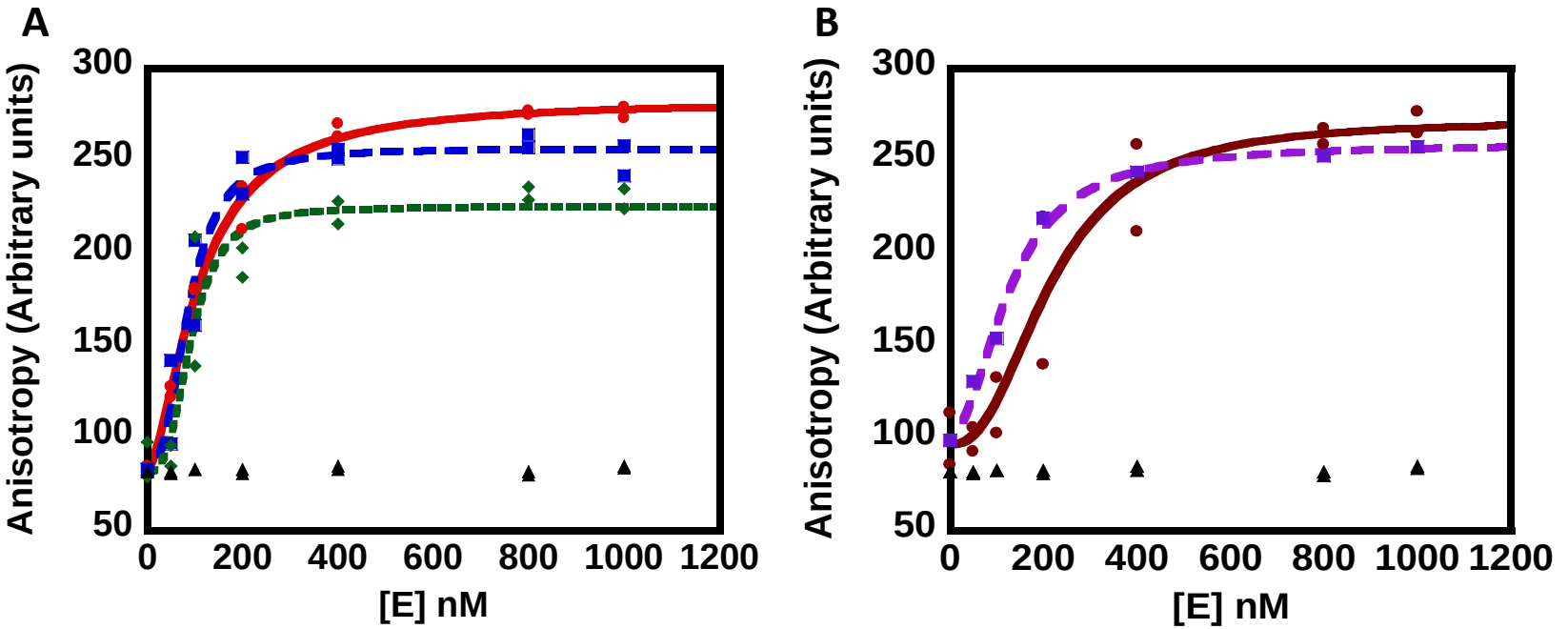
Therefore: $\Delta G^{\ddagger}_{WT \rightarrow D210A} = \Delta G^{\ddagger}_{D100N \rightarrow D210A+D100N}$

$\Delta G^{\ddagger}_{WT \rightarrow D210A} = -RT \ln(k_{obs\ D210A}/k_{obs\ WT})$

$\Delta G^{\ddagger}_{D100 \rightarrow D210A+D100N} = -RT \ln(k_{obs\ D210A+D100N}/k_{obs\ D100N})$

Theoretical $k_{obs\ D210A+D100N} = 0.24*0.44/1.00 = 0.11\ min^{-1}$

Figure S5


A
Anisotropy (Arbitrary units)
300
250
200
150
100
50
0
200
400
600
800
1000
1200
[E] nM
B
Anisotropy (Arbitrary units)
300
250
200
150
100
50
0
200
400
600
800
1000
1200
[E] nM