Supporting Information for:

Comparison of Global Structure and Dynamics of Native and Unmodified tRNA^{val}

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5 pages with 4 tables and figures.

Table S1 Detailed Parameters for NMR experiments on tRNAs

2D II- II NOEST parameters and experimental conditi	UIIS							
Sample	np	ni	nt	SW	sw1	Т	Instru	nent t _{mix}
Native <i>E. coli</i> tRNA ^{val}								
2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM	2048	400	64	13522	13500	25°C	C 600 N	1Hz 200
MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O								
тт 1•0•1 г. <i>1•</i> 4 тэжт 4 Va]								
Unmodified <i>E. coll</i> tRNA ^{TT}	4006	400	64	12500	12500	2500	- 600 N	1Hz 200
0.7 m/r tkinA, 10 m/r Sodium Prosphate pH7.0, 80 m/r NaCi, 5 m/r MgCl 0.1 m/r EDTA 10% D O	4090	400	04	15522	15500	25 0	. 000 N	IHZ 200
MgC12, 0.1 IIIM ED1A, 1070 D20								
¹ H- ¹³ N HSQC parameters and experimental conditions								
Sample		np	ni	nt	SW	sw1	Т	Instrument
Native <i>E. coli</i> tRNA ^{vai}	CI	20.40	1.00	100	10001	2000	2500	500) (11
2.2 mM tKNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCi, 5 mM M	gCI_2 ,	2048	160	0 100	12001	3000	25°C	500 MHZ
$0.1 \text{ mW} \text{ ED1A}, 10\% \text{ D}_2 \text{ O}$								
Unmodified <i>E_coli</i> tRNA ^{val}								
0.7 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM M	gCl ₂	1024	200) 48	14006	3000	25°C	600 MHz
0.1 mM EDTA, 10% D ₂ O	0 - 2)							
,								
¹ H- ¹⁵ N DSSE-HSOC parameters and experimental cond	itions							
Sample	nn	ni	nt	SW	sw1	т		
Jampie	чP	m	m	311	5 W I		Instrumen	t D.O
						-	Instrumen	t D ₂ O split
Native <i>E. coli</i> tRNA ^{val}						-	Instrumen	t D ₂ O split
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM	4096	256	64	14006	3650	25°C	600 MHz	t D ₂ O split
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O	4096	256	64	14006	3650	25°C	600 MHz	t D ₂ O split
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM	4096 4096	256 256	64 8	14006 14006	3650 ±	25°C 15°C	600 MHz 600 MHz	t D ₂ O split - 9.3
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O	4096 4096	256 256	64 8	14006 14006	3650 3650	25°C 15°C	600 MHz 600 MHz	t D ₂ O split - 9.3
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O + C8E5/1-octanol	4096 4096	256 256	64 8	14006 14006	3650 3650	25°C 15°C	600 MHz 600 MHz	t D ₂ O split - 9.3
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O + <i>C8E5/1-octanol</i>	4096 4096	256 256	64 8	14006 14006	3650 : 3650	25°C 15°C	600 MHz 600 MHz	t D ₂ O split 9.3
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O + C8E5/1-octanol	4096 4096	256 256	64 8	14006 14006	3650 : 3650	25°C 15°C	600 MHz 600 MHz	t D ₂ O split - 9.3
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O + C8E5/1-octanol Unmodified E. coli tRNA ^{val} 0.7 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM	4096 4096 4096	256 256 256	64 8 64	14006 14006 14006	3650 3650	25°C 15°C	600 MHz 600 MHz 600 MHz 600 MHz	t D ₂ O split 9.3
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O + C8E5/1-octanol Unmodified E. coli tRNA ^{val} 0.7 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O	4096 4096 4096	256 256 256	64 8 64	14006 14006 14006	3650 3650 3650	25°C 15°C 25°C	600 MHz 600 MHz 600 MHz 600 MHz	t D ₂ O split - 9.3
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O + C8E5/1-octanol Unmodified E. coli tRNA ^{val} 0.7 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM MgCl ₂ , 0.1 mM EDTA, 10% D ₂ O 0.3 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mM	4096 4096 4096 4096	256 256 256 256	64 8 64 96	14006 14006 14006 14006	3650 : 3650 : 3650 : 3650 :	25°C 15°C 25°C 25°C	600 MHz 600 MHz 600 MHz 600 MHz 600 MHz	t D ₂ O split - 9.3 - 8.1
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O+ C8E5/1-octanolUnmodified E. coli tRNA ^{val} 0.7 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O0.3 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O0.3 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O	4096 4096 4096 4096	256 256 256 256	64 8 64 96	14006 14006 14006 14006	3650 : 3650 : 3650 :	25°C 15°C 25°C 25°C	600 MHz 600 MHz 600 MHz 600 MHz 600 MHz	t D ₂ O split - 9.3 - 8.1
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O+ C8E5/1-octanolUnmodified E. coli tRNA ^{val} 0.7 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O0.3 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O0.3 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O+ 15mg/ml Pf1	4096 4096 4096 4096	256 256 256 256	64 8 64 96	14006 14006 14006 14006	3650 3650 3650 3650	25°C 15°C 25°C 25°C	600 MHz 600 MHz 600 MHz 600 MHz 600 MHz	t D ₂ O split - 9.3 - 8.1
Native E. coli tRNA ^{val} 2.2 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O1.0 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O+ C8E5/1-octanolUnmodified E. coli tRNA ^{val} 0.7 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O0.3 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O0.3 mM tRNA, 10 mM Sodium Phosphate pH7.0, 80 mM NaCl, 5 mMMgCl ₂ , 0.1 mM EDTA, 10% D ₂ O+ 15mg/ml Pf1np - the number of complex points in the t ₂ domain	4096 4096 4096 4096	256 256 256 256	64 8 64 96	14006 14006 14006 14006	3650 3650 3650 3650	25°C 15°C 25°C 25°C	600 MHz 600 MHz 600 MHz 600 MHz 600 MHz	t D ₂ O split - 9.3 - 8.1

nt - number of scans

sw - sweep width in Hz in t_2 sw1 - sweep width in Hz in t_1 t_{mix} - mixing time in milliseconds T - temperature

D₂O split - the split of the deuterium of D₂O signal due to anisotropic averaging of the deuterium quadrupole moment

Alignment Tensor Parameters		Arm Or		
D_a (Hz)	R	Bend (°)	Twist (°)	RDC rmsd
15.0	0.55	101	178	5.89
16.0	0.55	102	179	5.64
17.0	0.55	103	179	5.56
18.0	0.55	105	179	5.62
19.0	0.55	106	179	5.85
17.0	0.40	99	181	5.81
17.0	0.45	100	181	5.67
17.0	0.50	102	180	5.58
17.0	0.60	105	178	5.59
17.0	0.65	107	177	5.69

Table S2 Effect of varying magnitudes alignment tensor on the results of thedomain orientation calculations for from *E. coli* native tRNA^{val^a}

^a The magnitudes of the alignment tensor used as input for the domain orientation calculations.







Figure S1 A) Diagram of an imino bond. Structural error was generated by moving only the proton of the imino bond within a cone where the angle of the cone is σ of a Gaussian distribution. B) Fifty structures with 10° structural error (σ =10°) in blue are superimposed on the A-form model tRNA (red). Inset shows close up of residues 2 and 3 to demonstrate that only the imino protons in the structure are moved to simulate structural error.



Figure S2. Normalized RDCs measured for native *E. coli* tRNA^{val} in 5 mM Mg²⁺ at 15°C (blue squares) and unmodified *E. coli* tRNA^{val} at 25°C (red circles) plotted as a function of position in (A) the acceptor arm and (B) the anticodon arm.