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Supporting information for article:

Protein–RNA affinity of ribosomal protein L1 mutants does not correlate with the number of intermolecular interactions

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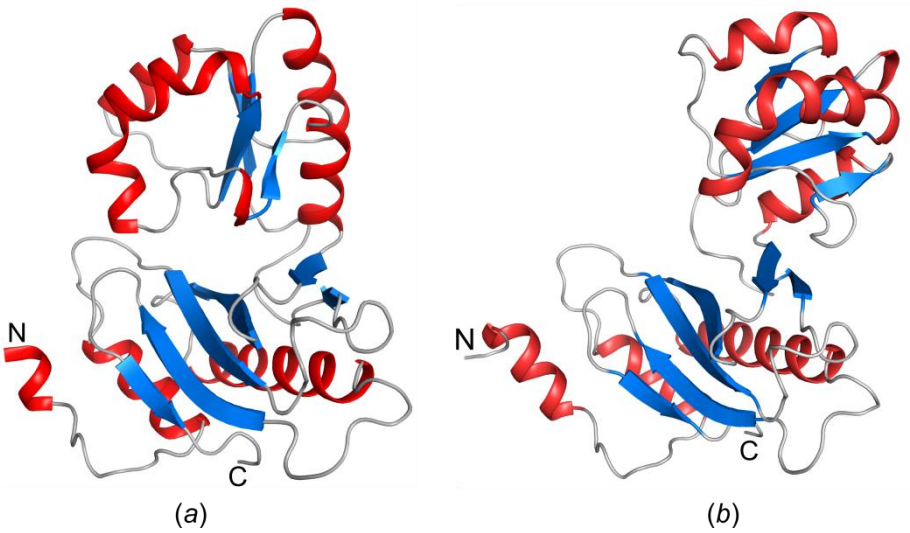

Figure S1 Two conformations of TthL1. (a) TthL1 in the closed conformation (isolated protein). (b) TthL1 in the open conformation (RNA-bound protein).

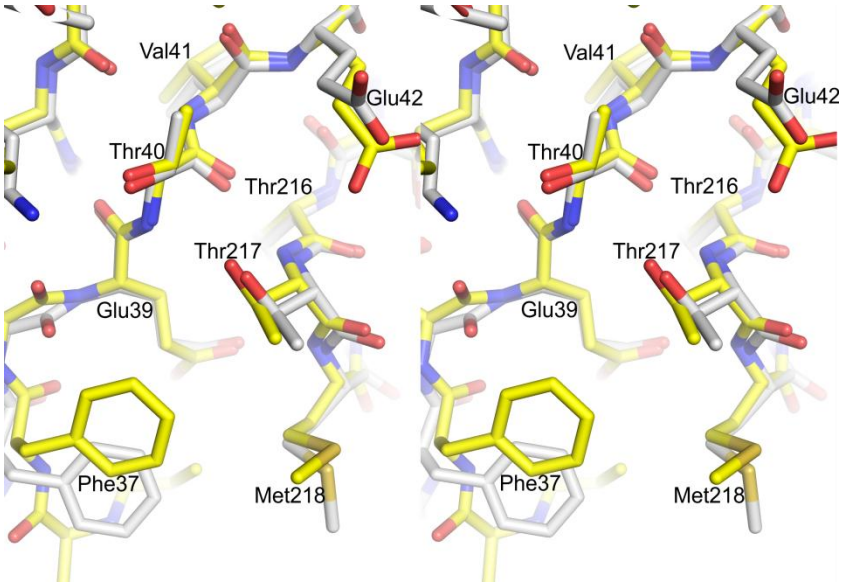

Figure S2 Superposition of the protein-RNA contact regions in the unbound wild-type TthL1 (C-atoms yellow) and RNA-bound TthL1 (C-atoms gray) structures.

Table S1 Intramolecular hydrogen bonds in the complexes formed by wild-type TthL1 and G219V mutant.

Protein				distance (A)	
				wt	G219V
Glu39	OE1 – Thr34	N		----	2.68
Glu39	OE1 – Ala35	N		2.85	2.61
Glu39	OE2 – Thr216	OG1		----	2.67
Glu39	OE2 – Met218	N		2.87	2.95
Thr216	OG1 – Leu32	O		2.71	----
Thr216	OG1 – Gly219	N		3.10	----

Table S2 Superposition of different structure elements of complexes formed by wild-type TthL1 and its mutants with a 80 nt fragment of 23S rRNA.

Component of the complex	r.m.s.d, Å			
	T217A	T217V	M218L	G219V
Domain I (residues 1-67 and 160-228)	0.49	0.32	0.36	0.40
Domain II (residues 68-159)	1.20	1.02	1.18	0.75
rRNA (nucleotides 2105-2184)	1.04	1.27	0.94	1.19
Domain I+ H77 of 23S rRNA	0.48	0.35	0.36	0.45

Table S3 . Average B-factors for the region highlighted in figures 2 through 5.

TthL1	Unbound protein	Bound protein	Bound RNA
WT	21.36	9.67	10.88
T217A	21.69	10.13	10.61
T217V	26.95	12.85	14.11
M218L	31.99	14.13	14.24
G219V	31.71	8.81	9.86

Table S4 Parameters of the interaction of wild type and mutant TthL1 with 80-nt fragment of 23S rRNA

Protein	Reaction model	k_{a1}, $M^{-1}s^{-1}$	k_{d1}, s^{-1}	k_{a2}, s^{-1}	k_{d2}, s^{-1}	K_D, M	K_M, M	χ^2
Wild-type	one-stage	5.64×10^{5}	1.21×10^{-6}			2.14×10^{-12}		2.07
T217A TthL1	one-stage	2.64×10^{5}	3.80×10^{-5}			1.44×10^{-10}		2.11
T217A TthL1	two-stage	1.68×10^{5}	7.57×10^{-6}	2.78×10^{-5}	1.21×10^{-5}		2.10×10^{-10}	2.29
T217V TthL1	one-stage	2.86×10^{5}	2.80×10^{-4}			9.79×10^{-10}		2.30
T217V TthL1	two-stage	1.66×10^{5}	5.27×10^{-6}	2.57×10^{-4}	4.42×10^{-5}		1.58×10^{-9}	1.91
G219V TthL1	one-stage	3.75×10^{6}	9.34×10^{-3}			2.49×10^{-9}		5.59
G219V TthL1	two-stage	1.76×10^{5}	1.38×10^{-6}	3.30×10^{-3}	2.46×10^{-4}		1.87×10^{-8}	1.91
M218L TthL1	one stage	6.57×10^{4}	2.04×10^{-3}			3.10×10^{-8}		4.50
M218L TthL1	two-stage	4.28×10^{3}	8.35×10^{-7}	6.34×10^{-4}	3.10×10^{-5}		1.48×10^{-7}	1.78

$K_D = k_{d1}/k_{a1}$; $K_M = (k_{d1}+k_{a2})/k_{a1}$;

Table S5 Protein-RNA hydrogen bonds in the complexes formed by wild-type TthL1 and its mutants.

Protein			rRNA		distance (A)				
					wt	T217A	T217V	M218L	G219V
Lys	2	NZ	A2108	O1P	-----	2.683	3.077	-----	-----
HIS	3	ND1	C2175	O1P	2.659	2.630	2.730	2.598	2.572
LYS	5	N	U2130	O1P	2.857	2.941	2.883	3.000	2.533
LYS	5	NZ	G2131	O1P	2.769	-----	-----	-----	-----
ARG	6	NE	C2129	O1P	2.895	2.895	2.975	2.974	2.521
TYR	7	OH	A2176	O1P	2.456	2.479	2.546	2.459	2.587
ARG	8	NH2	U2132	O2	2.852	2.884	-----	-----	-----
LYS	36	N	C2128	O1P	2.718	2.726	2.628	2.779	2.651
THR	40	OG1	G2125	O1P	2.690	2.573	2.614	2.684	2.876
GLU	42	OE1	G2123	N2	3.092	3.007	2.952	3.137	3.082
GLU	42	OE2	G2124	O2'	2.679	2.751	2.579	2.699	2.558
LYS	46	NZ	C2178	O2'	3.021	3.100	2.976	3.063	2.713
ARG	134	NE	G2123	O1P	-----	3.216	----	----	-----
ARG	134	NH1	A2170	O1P	2.841	-----	2.813	3.188	2.545
ARG	134	NH2	A2170	O1P	2.628	-----	2.841	2.933	-----
ARG	134	NH2	A2171	O2P	-----	-----	2.956	2.836	2.611
ASP	166	OD2	G2121	N2	2.922	2.747	2.852	2.893	2.951
THR	168	OG1	C2108	O2'	2.622	2.576	2.471	2.572	3.218
SER	211	OG	C2108	O1P	2.656	2.722	2.585	2.709	2.812
THR	217	OG1	G2124	O2'	2.771	-----	-----	2.859	3.116
THR	217	O	G2124	N2	3.131	3.017	3.048	2.978	2.835
GLY	219	O	C2175	O2'	2.638	2.779	2.610	2.674	2.770
SER	221	OG	C2177	O1P	2.610	2.668	2.627	2.793	2.849