Supplementary Material

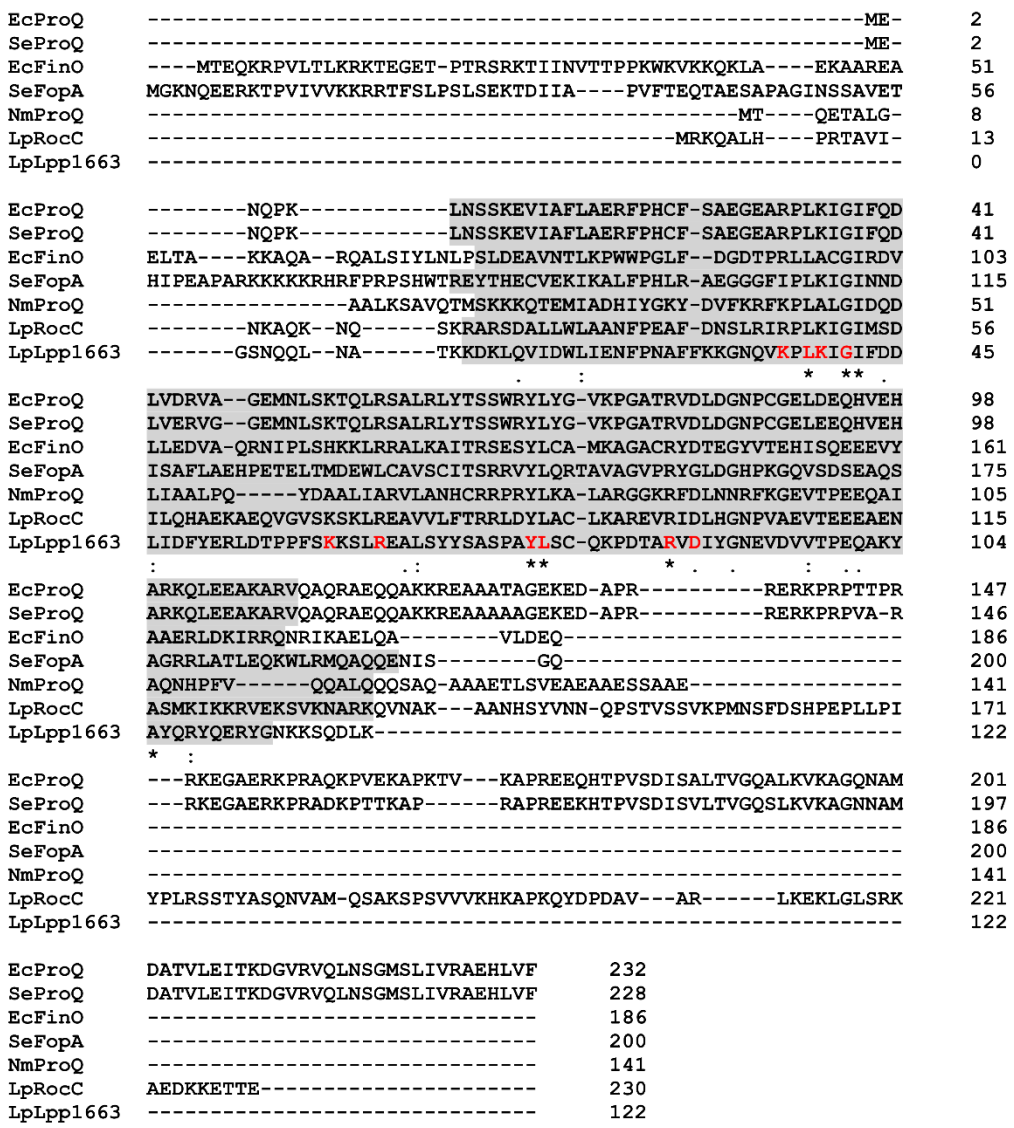
Solution structure and RNA-binding of a minimal ProQ-homolog from *Legionella pneumophila* (Lpp1663)

Carina Immer, Carolin Hacker and Jens Wöhnert*

Institute of Molecular Biosciences and Center for Biomolecular Magnetic Resonance (BMRZ), Johann-Wolfgang-Goethe-University Frankfurt/Germany.

Supplemental Figure S1

A



Supplemental Figure S1

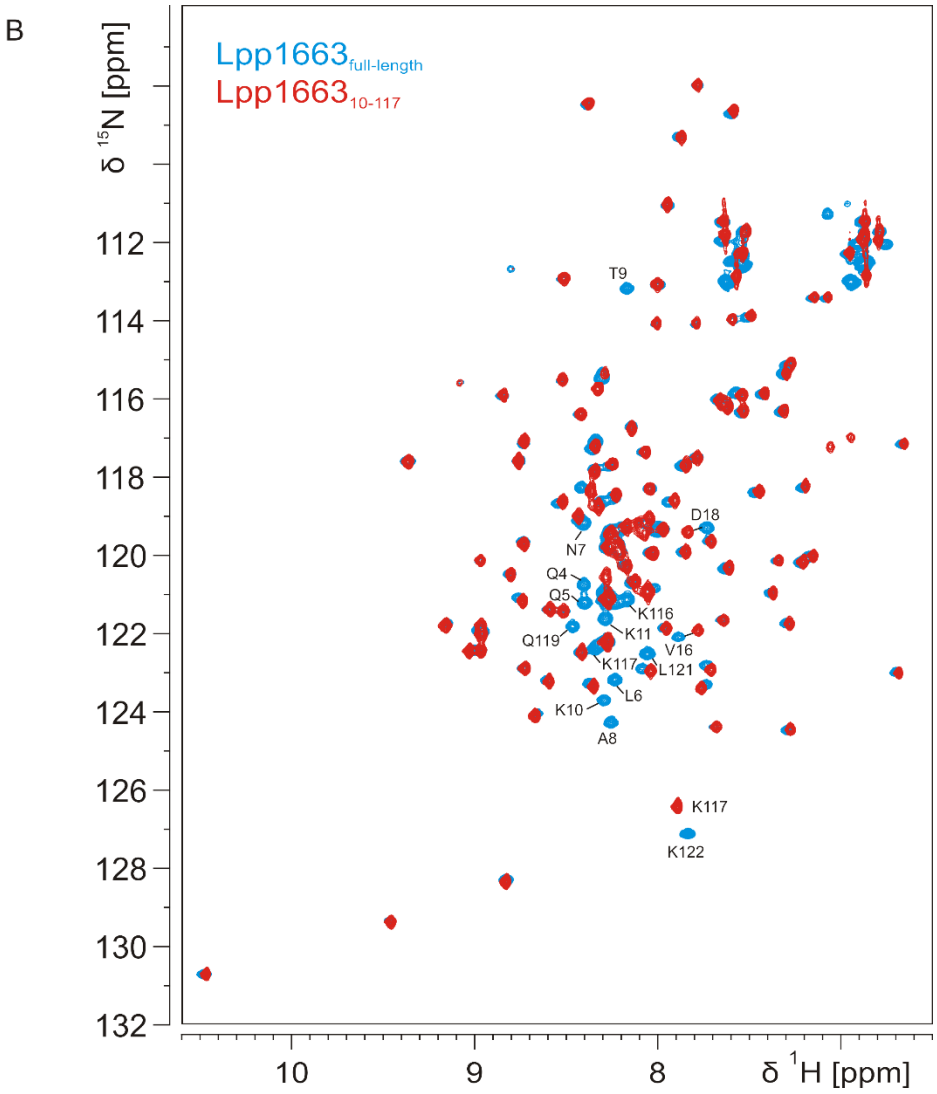


Figure S1: Sequence conservation and stability of the Lpp1663 ProQ domain. A Sequence alignment of the ProQ/FinO homologs used in Figure 1. The central domain that harbours several conserved residues is highlighted in grey. Lpp1663 is numbered according to the construct used in this study. Residues important for RNA-binding are indicated in red. **B** The overlay of ^{1}H, ^{15}N HSQCs of ^{15}N-labelled Lpp1663 (blue) and a truncated version of the protein lacking the flexible N- and C-termini (Lpp1663tr, residues 10-117, red) show that the truncated protein is correctly folded. Residues that disappeared due to the truncation or changed their position are indicated by their residue number.

Supplemental Figure S2

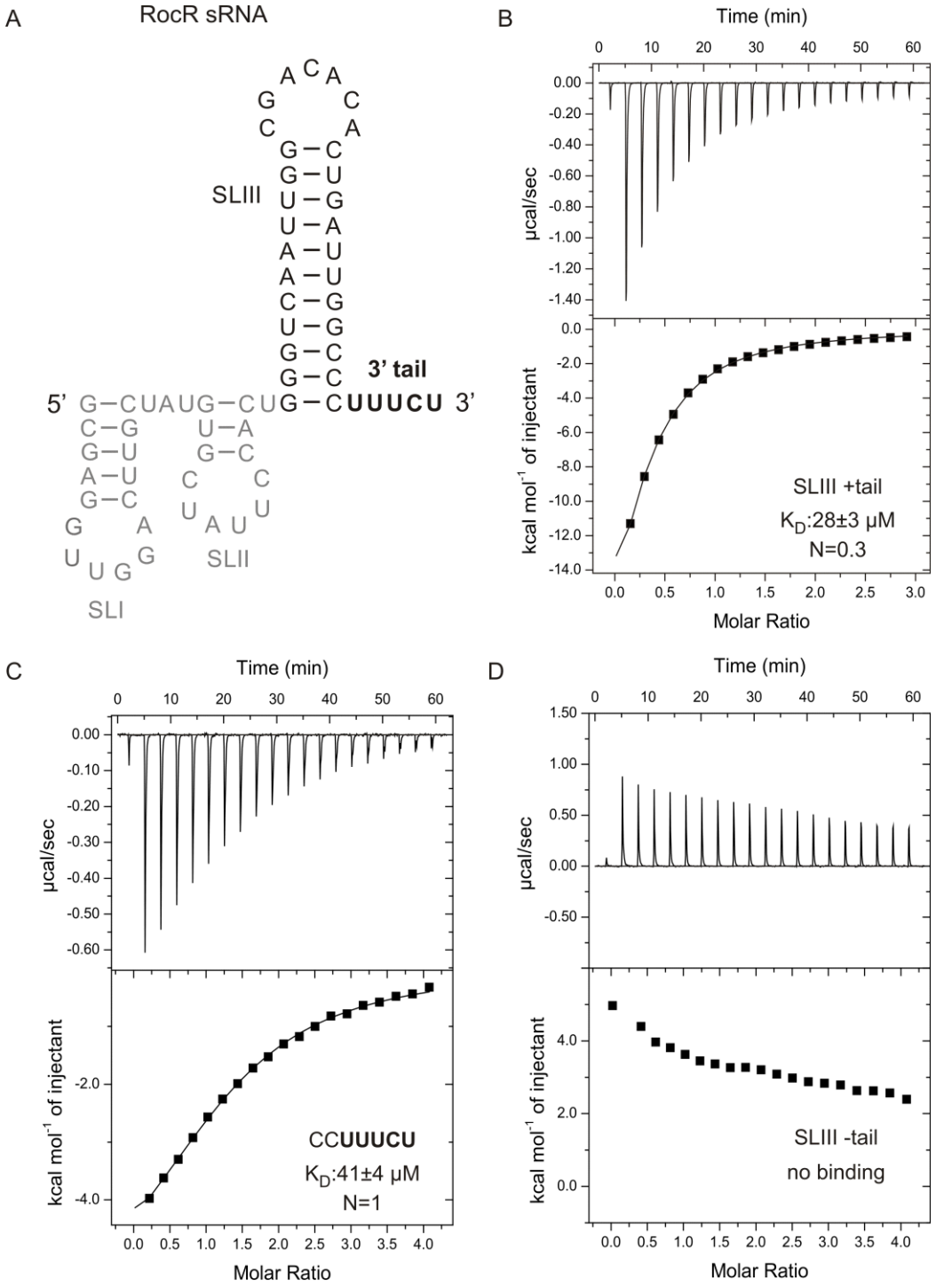
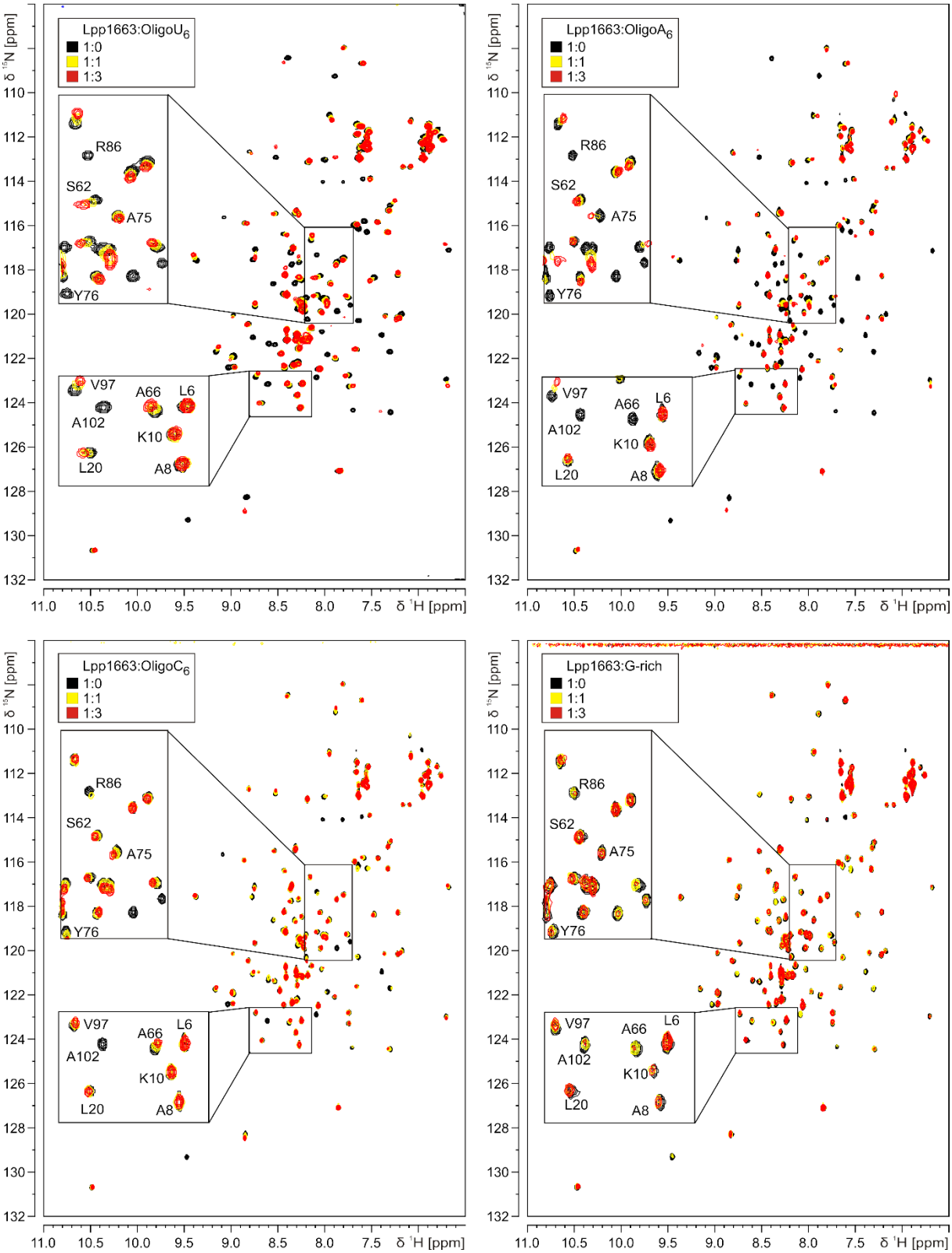


Figure S2: Binding of Lpp1663 to RocR sRNA. A Secondary structure of RocR sRNA according to Attaiech *et al.*, 2016**.** SLIII indicated in black, the 3' extension depicted in bold. **B) C) D)** ITC thermograms of Lpp1663 titrated with RocR SLIII with or without its 3'-single stranded extension or the isolated extension.

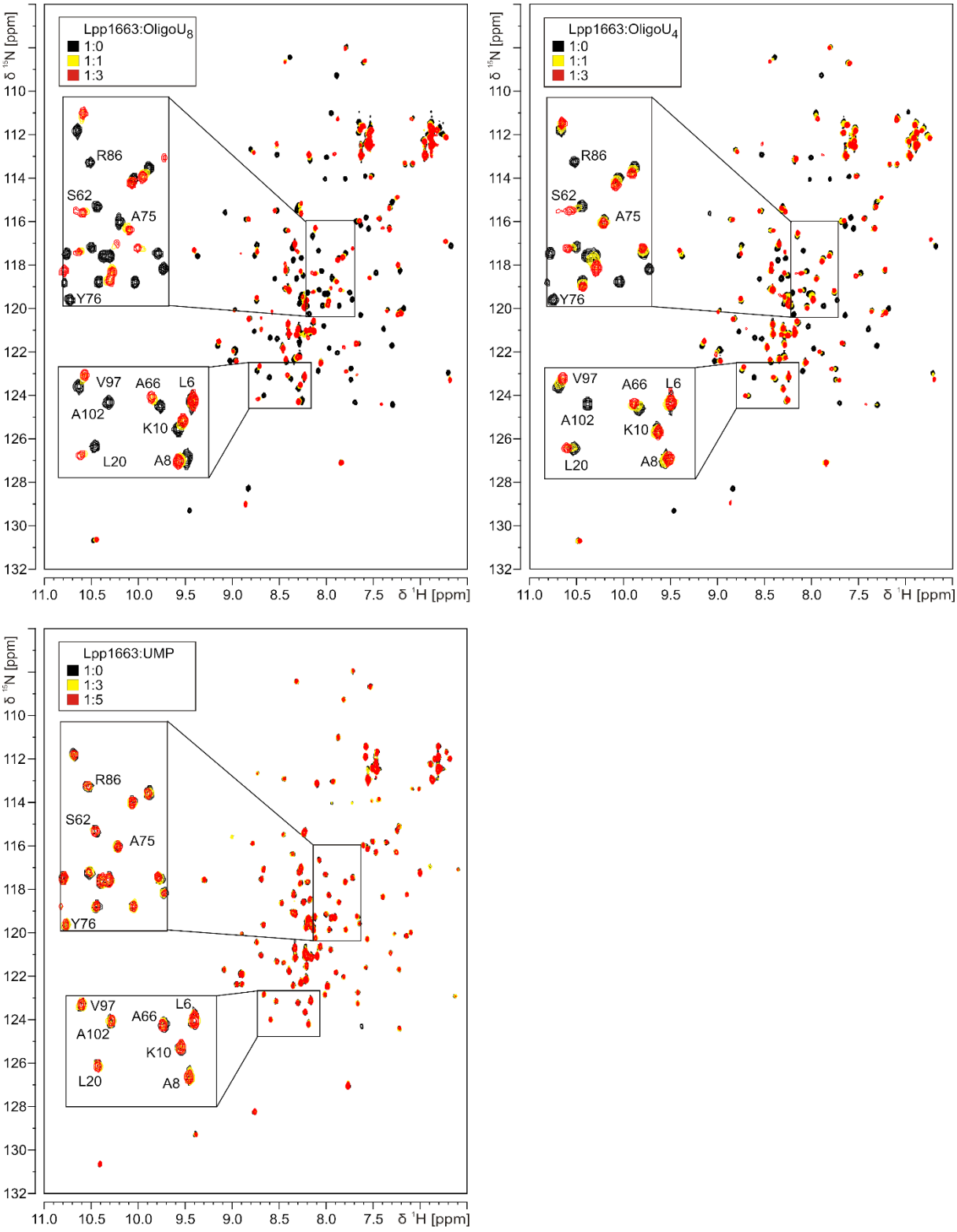
Supplemental Figure S3

S3 A



Supplemental Figure S3

S3 B



Supplemental Figure S3

S3 C

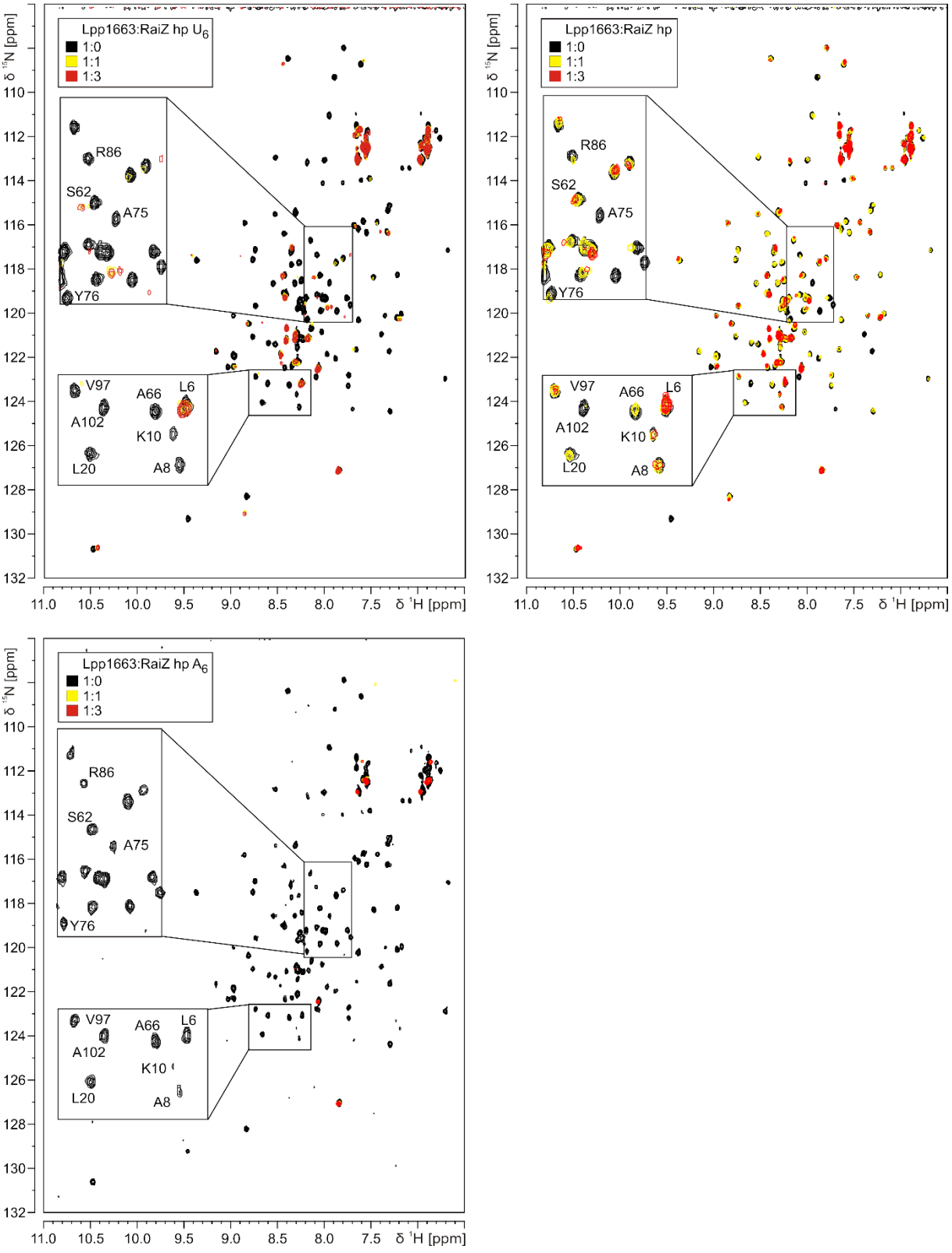


Figure S3 A-C: Overlays of the ^{1}H, ^{15}N HSQC spectra of ^{15}N-labelled Lpp1663 titrated with RNA. 80 µM Lpp1663 was titrated with equivalents of RNA at 298K as indicated by the coloured boxes. Changes in the chemical shift of the corresponding residues were categorized and plotted on the structure or surface of Lpp1663. For all ligands spectra with a 3fold or a 5fold excess are virtually identical. Thus, in most cases only the spectrum at a 3:1 ligand-protein ratio is shown.

Supplemental Figure S4

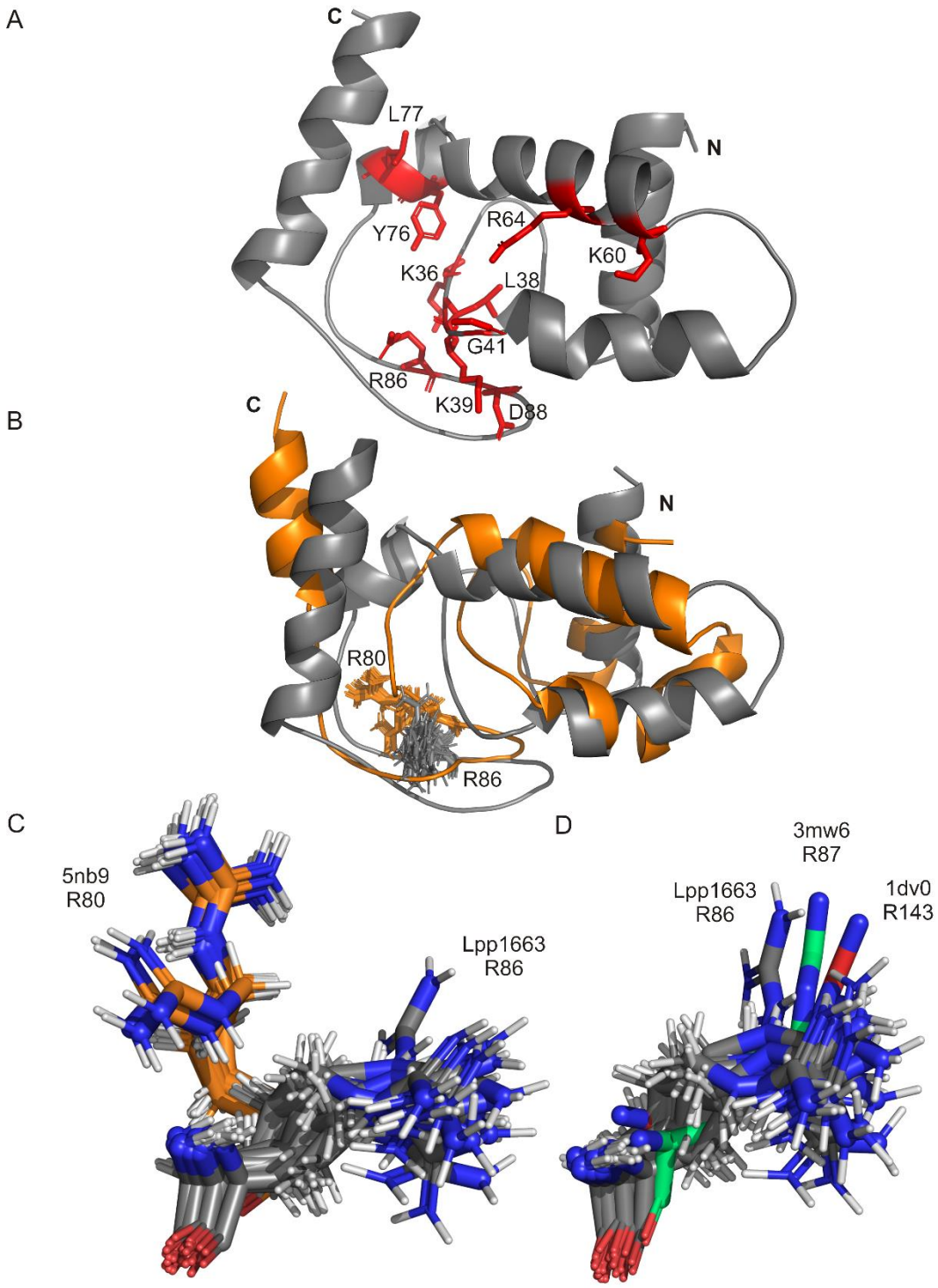


Figure S4: Conserved residues of Lpp1663 involved in RNA-binding and position of R86 in different ProQ/FinO structures. A Conserved residues on the concave site of the Lpp1663 structure involved in RNA binding, shown as red sticks. N- and C- termini indicated as N and C, respectively. **B** Superposition of the Lpp1663 NMR bundle (grey) and *E. coli* ProQ NMR bundle (5nb9, orange), R86 shown as sticks. N- and C-terminus are indicated as N and C, respectively.**C** Overlays of R86 of the Lpp1663 NMR bundle (grey) and corresponding R80 of the 17 lowest energy structures of *E. coli* ProQ (5nb9, orange). **B** Overlay of the arginine residues corresponding to R86 of the twenty final structures of the Lpp1663 NMR bundle (grey) and the crystal structures of NMB1681 (3mw6, green) and FinO (1dvo, red).

Supplemental Figure S5

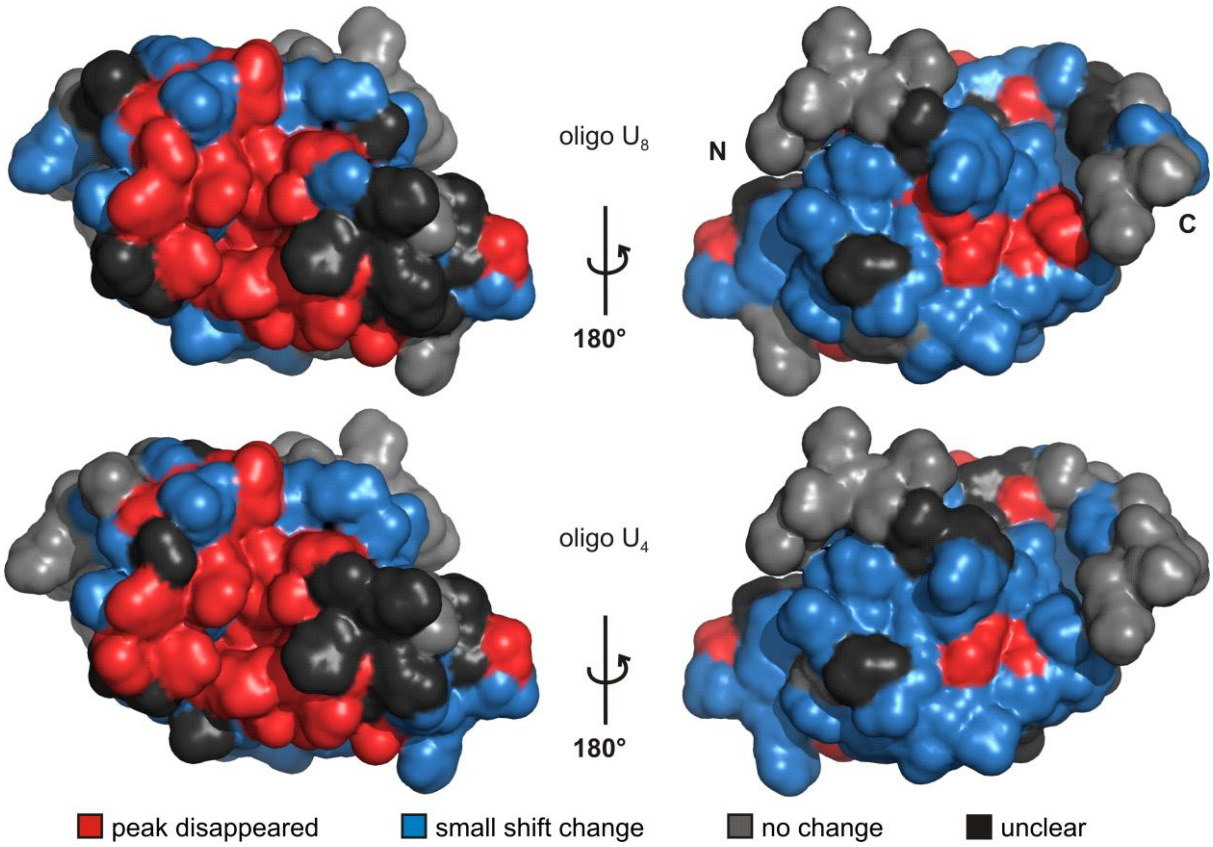


Figure S5, supplementary to Figure 6. In NMR experiments, the chemical shift changes upon oligo U_8 or oligo U_4 RNA titration have very similar patterns as for oligo U_6 (color scheme as described in Figure 5). The most dramatic changes occur on the concave surface patch including the N-terminal half of helix α2, helices α3, α4 and α5, as well as loop L1. N-and C-terminus are indicated as N and C, respectively.

Supplemental Figure S6

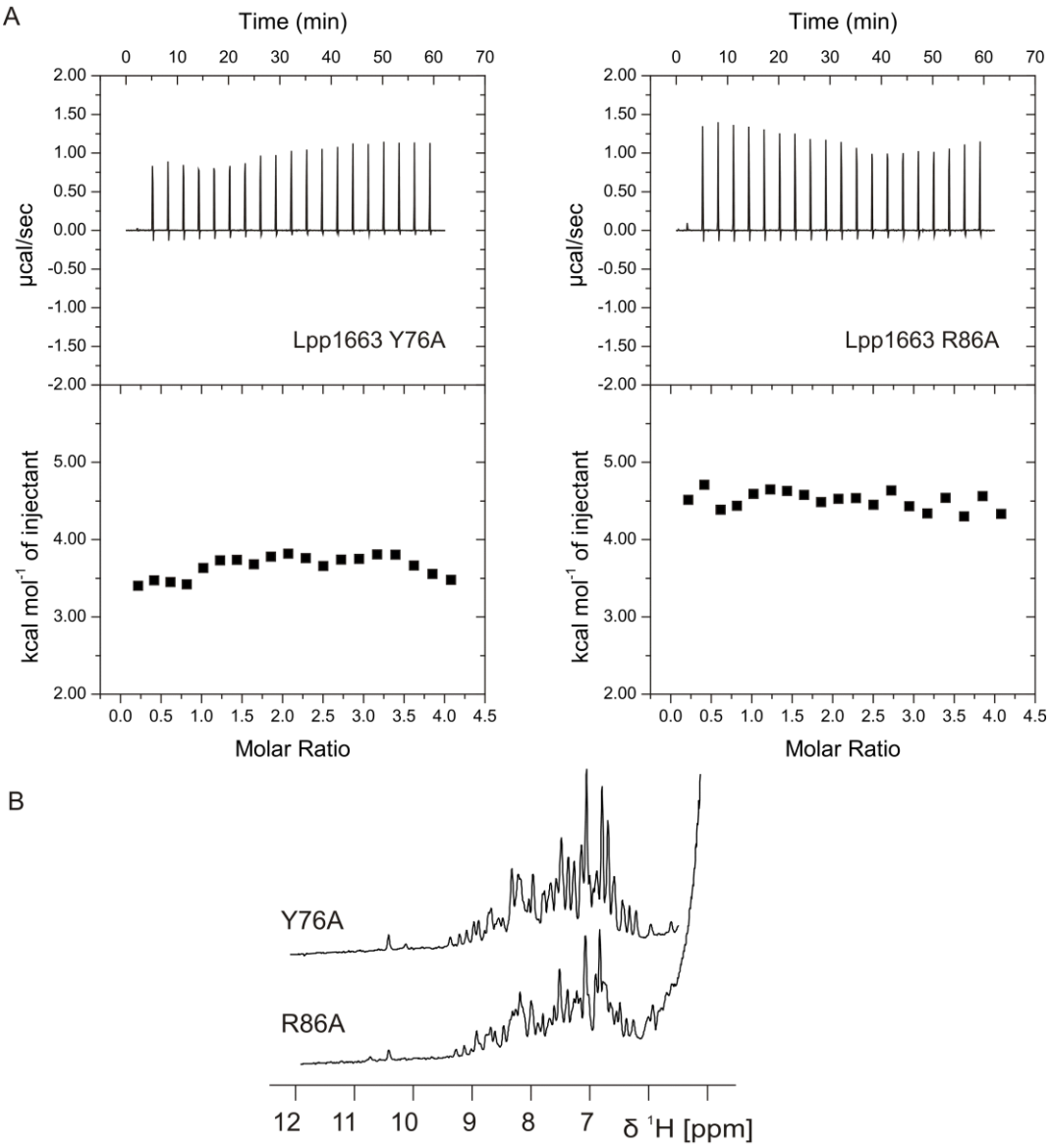


Figure S6: Mutation of the conserved residues Y76 and R86 abolishes RNA binding. A 1D ^{1}H NMR spectra of the amide region of Lpp1663 Y76A and R86A indicate well-folded proteins. The spectra were recorded at 298 K in NMR buffer with ~100 µM protein. **B** ITC thermograms of Y76A and R86A titrated with Oligo U_8 in NMR buffer. No binding could be observed.

Supplemental Figure S7

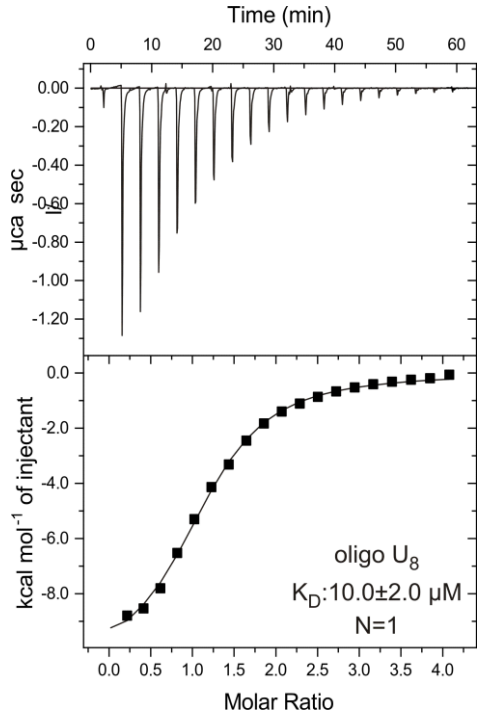


Figure S7: Removal of the flexible N-and C-termini of Lpp1663 does not affect its RNA binding properties. ITC thermograms of Lpp1663tr titrated with Oligo U_8. The truncated protein has nearly the same affinity to the RNA as the full-length protein (~8 µM), indicating that the N-and C-terminus are not required for RNA-binding.

Supplemental Figure S8

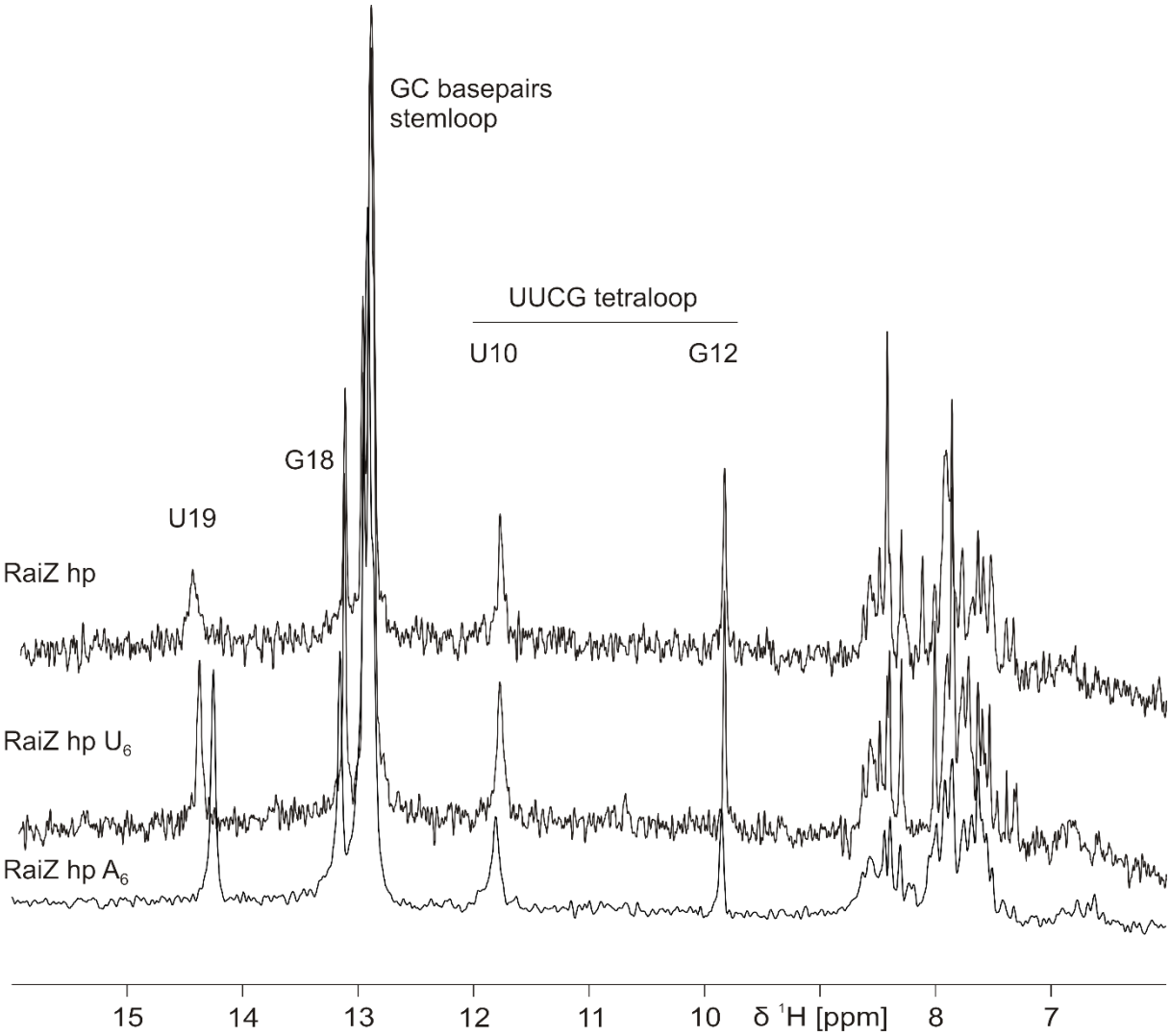


Figure S8: The RaiZ constructs are folded according to the predicted secondary structure. A 1D ^{1}H NMR spectra of 40-50 µM RaiZ hp, RaiZ hp U_6 and RaiZ hp A_6 were recorded at 283 K in NMR buffer. Signals were assigned according to standard chemical shifts for RNA (Fürtig *et al.*, 2003).

Supplemental Figure S9

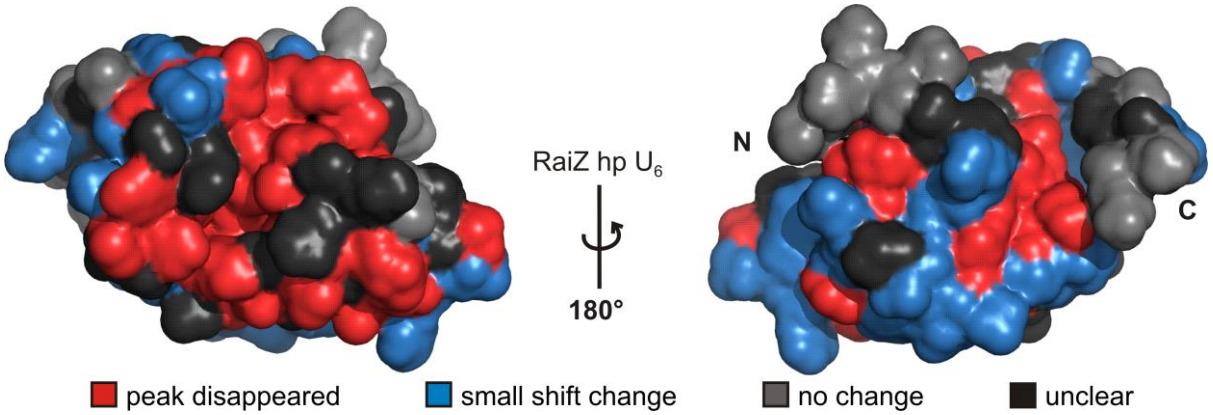


Figure S9, supplementary to Figure 7: Surface view of the chemical shift changes upon titration with RaiZ hp U_6. The main RNA-binding region includes the same surface area on the concave face of Lpp1663 as for the oligo U-RNAs. However, the number of affected residues increases. N-and C-terminus are indicated as N and C, respectively.

Supplemental Figure S10

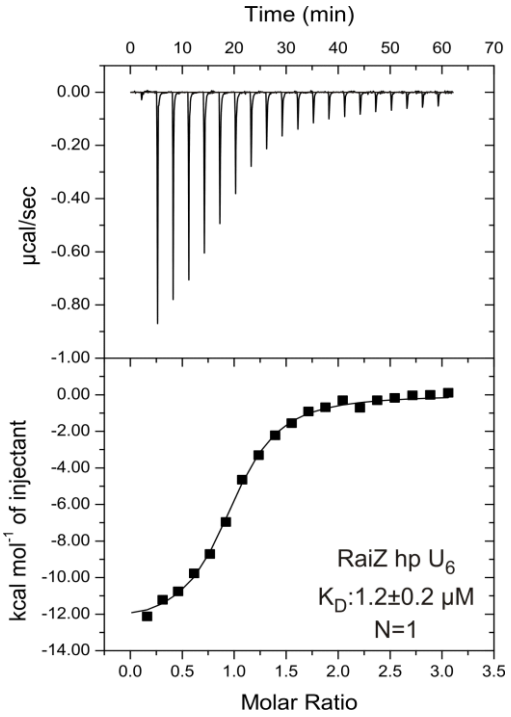


Figure S10: Removal of the flexible N-and C-termini of Lpp1663 does not affect its RNA binding to RaiZ hp U_6. ITC thermogram of Lpp1663tr titrated with RaiZhp U6. The truncated protein has nearly the same affinity to the RNA as the full-length protein (~800 nM), indicating that the N-and C-terminus are not required for RNA-binding.

Table S1: Results of the ITC measurements

Protein	RNA	K_D (µM)	Molar ratio / stoichiometry (N)	ΔH (cal/mol)	ΔS (cal/mol/deg)
Lpp1663	**RocR SLIII -tail**	No Binding			
Lpp1663	**RocR SLIII +tail (1)**	31.0	0.22	-36400	-101
Lpp1663	**RocR SLIII +tail (2)**	26.0	0.29	-28570	-74.8
Lpp1663	**RocR SLIII +tail (3)**	26.0	0.26	-23650	-58.3
	mean	**28.0±3.0**		**-29540±5250**	**-78±18**
Lpp1663	**CCUUUCU (1)**	46.0	1	-7917	-6.76
Lpp1663	**CCUUUCU (2)**	42.0	1	-7360	-4.66
Lpp1663	**CCUUUCU (3)**	36.2	1	-6422	-1.22
	mean	**41.0±5.0**		**-7233±617**	**-4±2**
Lpp1663	**Oligo U_6 (1)**	14.0	1	-4663	-6.56
Lpp1663	**Oligo U_6 (2)**	33.0	1	-8725	-8.76
Lpp1663	**Oligo U_6 (3)**	40.0	1	-10051	-15.2
	mean	**30.0±14.0**		**-7813±2292**	**-10±4**
Lpp1663	**Oligo A_6 (1)**	75.2	1	-1816	12.8
Lpp1663	**Oligo A_6 (2)**	70.4	1	-2028	12.2
Lpp1663	**Oligo A_6 (3)**	61.4	1	-1758	13.4
	mean	**70.0±7.0**		**-1867±116**	**12.8±0.5**
Lpp1663	**Oligo C_6**	No Binding			
Lpp1663	**G-rich**	No Binding			
Lpp1663	**Oligo U_8 (1)**	7.0	1	-9703	-8.94
Lpp1663	**Oligo U_8 (2)**	8.8	1	-7882	-3.31
Lpp1663	**Oligo U_8 (3)**	7.2	1	-7697	-2.3
	mean	**8.0±1.0**		**-8427±905**	**-5±3**
Lpp1663	**Oligo U_4 (1)**	34.8	1	-10340	-14.2
Lpp1663	**Oligo U_4 (2)**	27.6	1	-8863	-8.87
Lpp1663	**Oligo U_4 (3)**	32.9	1	-10130	-13.5
	mean	**31.0±3.0**		**-9778±652**	**-12±2**
Lpp1663	**RaiZ hp**	No Binding			
Lpp1663	**RaiZ hp U_6 (1)**	0.6	1	-11290	-9.71
Lpp1663	**RaiZ hp U_6 (2)**	0.7	1	-11310	-9.85
Lpp1663	**RaiZ hp U_6 (3)**	1.0	1	-10780	-8.77
	mean	**0.82±0.18**		**-11127±245**	**-9.4±0.5**
Lpp1663	**RaiZ hp A6**	No Binding			
Lpp1663 Y76A	**Oligo U_8**	No Binding			
Lpp1663 R86A	**Oligo U_8**	No Binding			
Lpp1663tr	**Oligo U_8 (1)**	9.2	1	-10760	-13
Lpp1663tr	**Oligo U_8 (2)**	8.5	1	-8993	-6.97
Lpp1663tr	**Oligo U_8 (3)**	12.2	1	-13340	-22.3
	mean	**10.0±2.0**		**-11031±1785**	**-14±6**
Lpp1663tr	**RaiZ hp U_6 (1)**	1.4	1	-12480	-15.1
Lpp1663tr	**RaiZ hp U_6 (2)**	1.0	1	-12140	-13.3
Lpp1663tr	**RaiZ hp U_6 (3)**	1.2	1	-12680	-15.4
	mean	**1.2±0.2**		**-12433±223**	**-15±1**