NMR Localization of Divalent Cations at the Active Site of the *Neurospora* VS Ribozyme Provides Insights Into RNA-Metal Ion Interactions

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Supporting Information

Supporting Figures

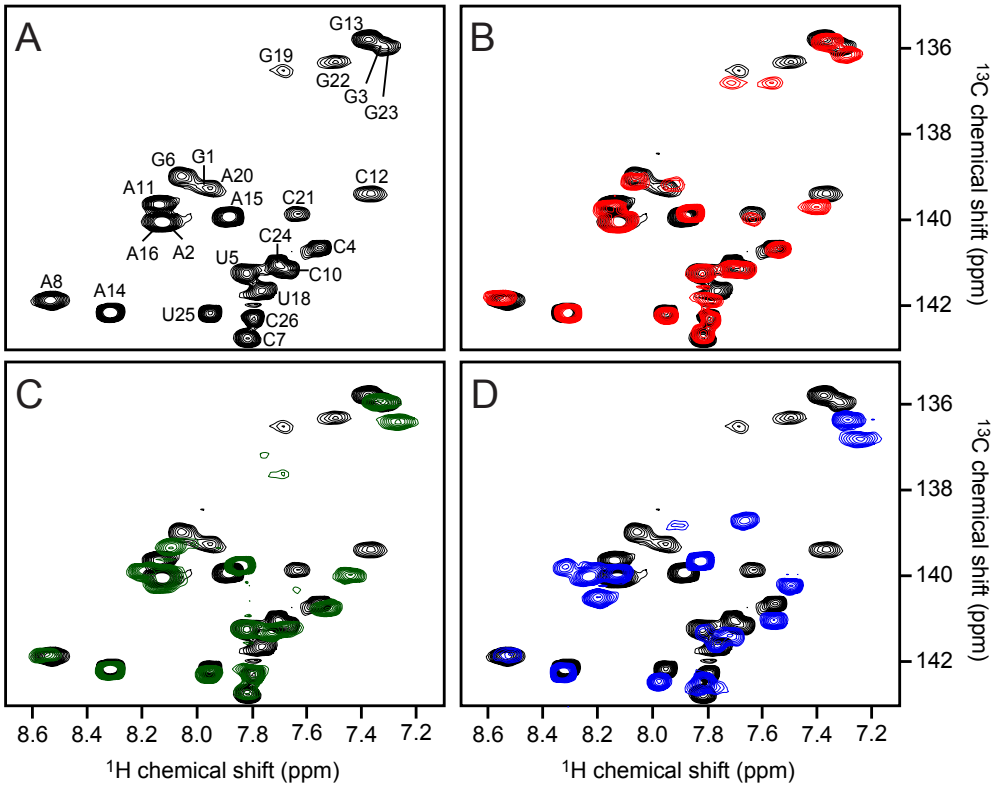

Figure S1. Effect of Cd^{2+} ions on SLVI RNA. Comparison of the H6/H8-C6/C8 region of 2D ^{1}H-^{13}C CT-HSQC spectra of SLVI at selected points of the Cd^{2+} titration. (A)-(D) Spectra are shown for $CdCl_2$:$MgCl_2$ concentrations of (A)-(D) 0.0:5.0 mM (black), (B) 0.25 mM:4.75 mM (red), (C) 1 mM:4 mM (green), and (D) 4 mM:1mM (purple).

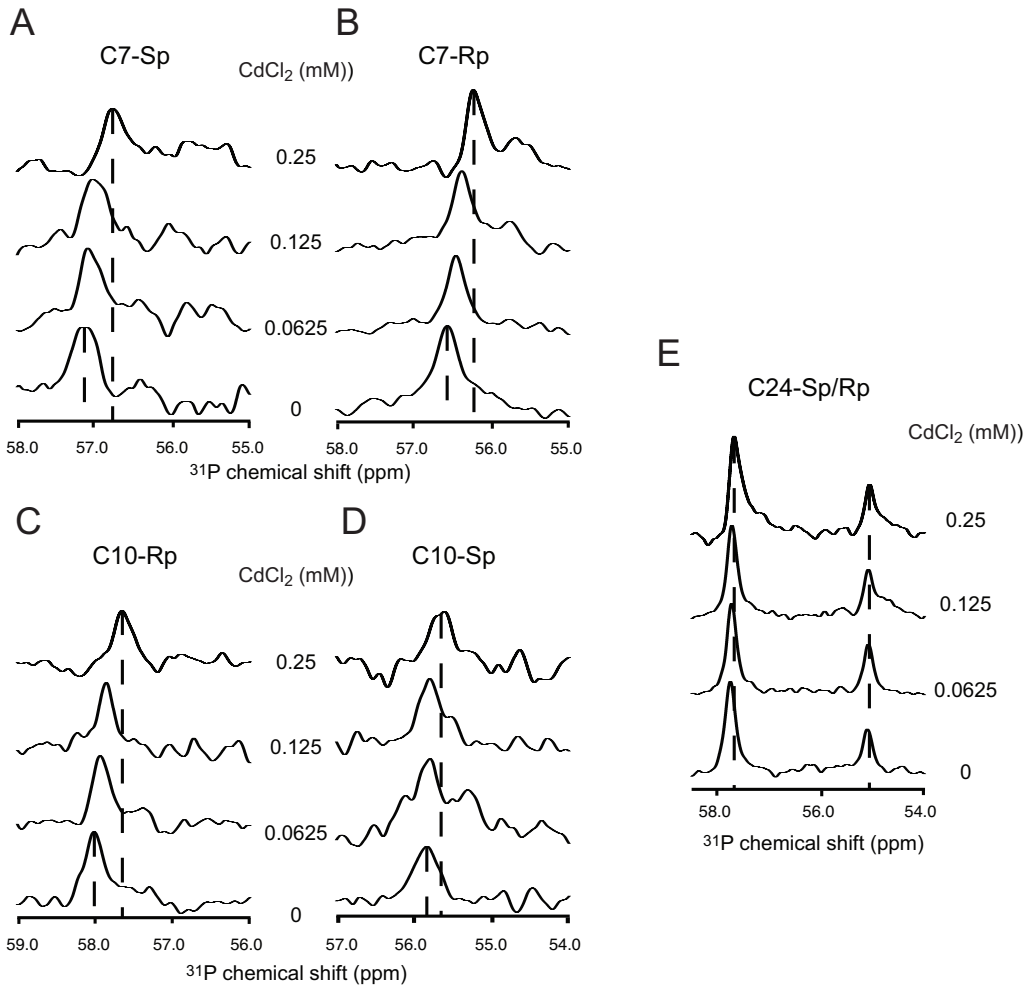

Figure S2. Effect of the addition of Cd^{2+} ions on the ^{31}P NMR spectrum of selected SLVI RNAs containing single phosphorothioate modifications. The 1D ^{31}P NMR spectra of (A) SLVI-C7-S_p, (B) SLVI-C7-R_p, (C) SLVI-C10-R_p, (D) SLVI-C10-S_p and (E) SLVI-C24-S_p/R_p were collected in buffer containing $CdCl_2$:$MgCl_2$ concentrations of 0.0:5.0, 0.0625:4.9375, 0.125:4.875 and 0.25:4.75 mM. Only the most downfield region of the spectra containing the signal from the phosphorothioate is shown.

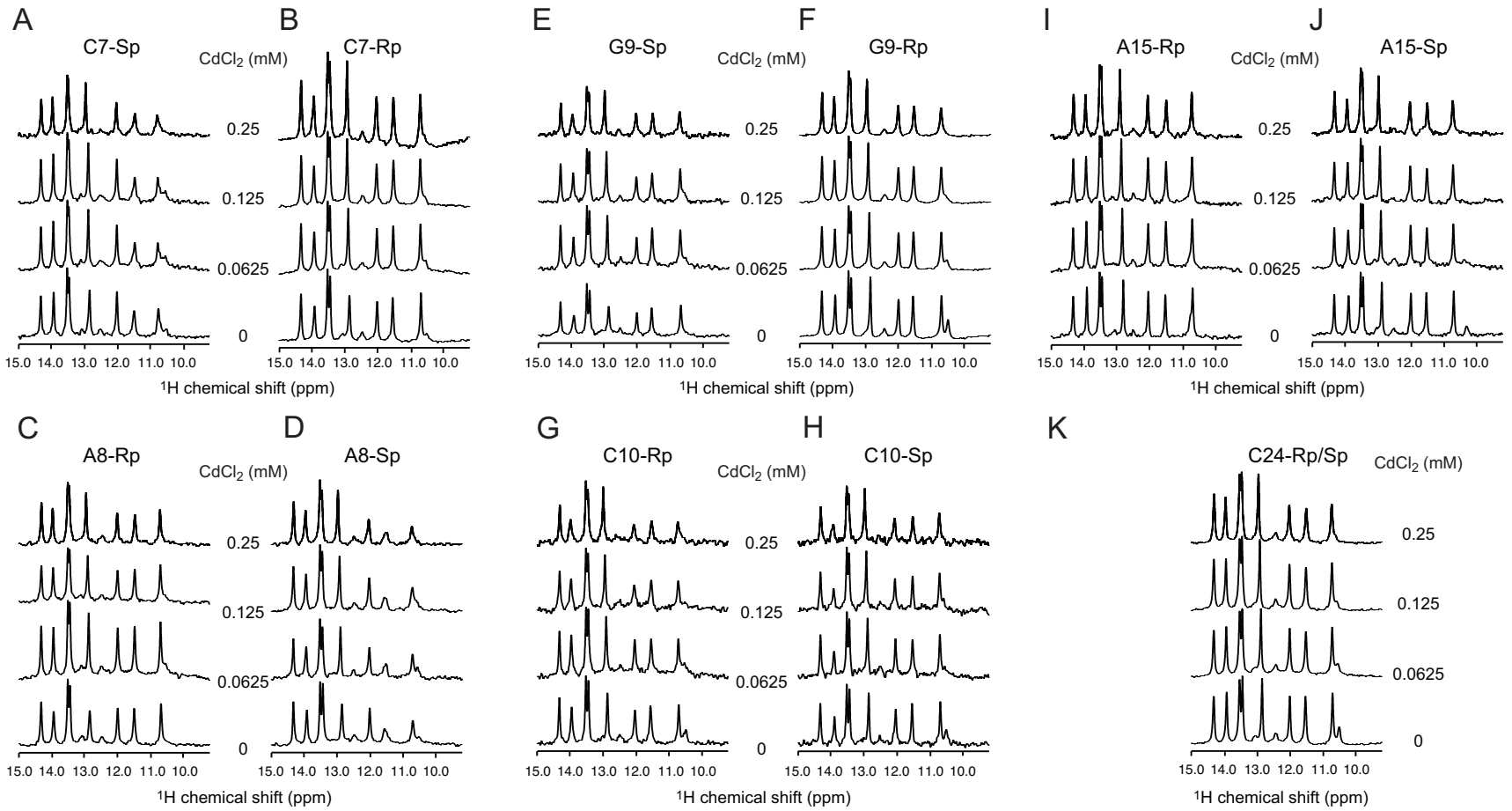

Figure S3. Effect of the addition of Cd^{2+} on the imino region of the ^{1}H NMR spectra of SLVI RNAs containing single phosphorothioate modifications. The 1D ^{1}H spectra of (A) SLVI-C7-S_p, (B) SLVI-C7-R_p, (C) SLVI-A8-R_p, (D) SLVI-A8-S_p, (E) SLVI-G9-S_p, (F) SLVI-G9-R_p, (G) SLVI-C10-S_p, (H) SLVI-C10-R_p (I) SLVI-A15-R_p, (J) SLVI-A15-S_p, and (K) SLVI-C24-S_p/R_p were collected in buffer containing $CdCl_2$:$MgCl_2$ concentrations of 0.0:5.0, 0.0625:4.9375, 0.125:4.875 and 0.25:4.75 mM.

Supporting Tables

Table S1. Distance restraints derived from Mn^{2+}-induced PRE.

Site	Atoms	I_0/I_{Mn}	Restraint
Site 1	G13 N1-H1	51	1.8-7.0 Å
	G13 C8-H8	20	1.8-7.0 Å
	G13 C3'-H3'	8	1.8-7.0 Å
	A16 N7	9	1.8-7.0 Å
	A14 H3'	6	1.8-8.0 Å
	A14 H5' or H5"	5	1.8-8.0 Å
	A15 N7	6	1.8-8.0 Å
	A16 H3'	5	1.8-8.0 Å
	A16 N9	4	1.8-8.0 Å
	A15 N9	7	1.8-8.0 Å
Site 1 or 2	C12 H6	7.5	1.8-10.0 Å
	C12 H5	6	1.8-10.0 Å
	G17 H8	6	1.8-10.0 Å
	G17 H1	5	1.8-10.0 Å
	C12 H41	5	1.8-10.0 Å
	C12 H42	5	1.8-10.0 Å
	G17 H5' or H5"	5	1.8-10.0 Å
Site 2	A11 N7	14	1.8-7.0 Å
	U18 C5-H5	8.5	1.8-7.0 Å
	C10 N4-H42	8	1.8-7.0 Å
	C10 N4-H41	6	1.8-8.0 Å
	G19 H8	7.5	1.8-8.0 Å
	C10 H41	6	1.8-8.0 Å
	A11 N9	5	1.8-8.0 Å
	A11 H8	4	1.8-8.0 Å
	G17 H3'	4	1.8-8.0 Å
	U18 H3	4	1.8-8.0 Å
Site 2 or 3	G9 H8	4	1.8-10.0 Å
Site 3	G6 H2'	6	1.8-8.0 Å
	A8 H8	5	1.8-8.0 Å
	A8 H3'	4	1.8-8.0 Å
	A8 H4'	4	1.8-8.0 Å
	A8 N9	4	1.8-8.0 Å
Site 3 or 4	G6 H8	5	1.8-10.0 Å
Site 4	G23 N7	15	1.8-7.0 Å
	G23 C8-H8	12	1.8-7.0 Å
	G22 C8-H8	9	1.8-7.0 Å
	U5 N3-H3	9	1.8-7.0 Å
	G6 N1-H1	8	1.8-7.0 Å
	A20 N7	5	1.8-8.0 Å
	U5 H5	4	1.8-8.0 Å
Site 4 or 5	G3 N9	6	1.8-10.0 Å
	C24 H42	5	1.8-10.0 Å
	C21 H5	5	1.8-10.0 Å
	C4 H41	4	1.8-10.0 Å
Site 5	G3 N7	15	1.8-7.0 Å
	G1 C8-H8	13	1.8-7.0 Å
	C26 H41	6	1.8-8.0 Å
	A2 N7	5	1.8-8.0 Å
	A2 H8	4	1.8-8.0 Å

Table S2. Results from the WebFR3D motif search.

Site	Searched motif[a]	Number of raw hits[b]	Number of hits with a cation[b]	Number of positive hits[b]	PDB entry (positive hits)	Residues (positive hits)	Metal ion[c] (positive hits)
Site 1	NR G — A C — G 5' 3'	111	13	3	**3U5F** **1FJG** **1JTW**	1121-1126 897-902 6-11	Mg 2162 Mg 1586 Na 25
Site 2	3' 5' C — G A — U C — G 5' 3'	173	18	8	**3U5H** **3U5H** **2ZNI** **3V2F** **3V2F** **3V2F** **3V7E** **3BNL**	1183-1185/1321-1323 1497-1499/1517-1519 26-28/42-44 589-591/666-668 414-416/2407-2409 2678-2680/2727-2729 48-50/85-87 8-10/13-15	Mg 4027 Mg 3997 Ca 82 Mg 3471 Mg 3288 Mg 3626 NCO 910 NCO 026
Site 3 (search 1)	3' 5' C — G G N A N G — C 5' 3'	1	0	0	-	-	
Site 3 (search 2)	3' 5' N — N G N A N N — N 5' 3'	1	0	0	-	-	

Site 4	3' 5' G — C U — G C — G 5' 3'	92	4	3	**1FJG** **3V2F** **3U5H**	1509-1511/1524-1526 1767-1769/1983-1985 1671-1673/1774-1776	Mg 1592 Mg 3524 Mg 4578
Site 5	3' 5' G — C A — U G — C 5' 3'	297	26	16	**3U5H** **3U5H** **3U5H** **3U5H** **3U5H** **1S72** **3U5F** **3IVK** **3V2F** **3T4B** **3CUN** **397D** **2XZM** **2ZJR** **4ENB** **1FJG**	272- 274/291-293 1379-1381/1424-1426 333-335/28-30 2251-2253/2263-2265 2670-2672/2682-2684 116-118/122-124 1478-1480/1527-1529 72-74/83-85 1277-1279/1291-1293 309-311/325-327 66-68/78-80 26-28/37-39 266-268/275-277 1666-1668/1989-1991 8-10/45-47 407-409/433-435	Mg 4333 Mg 3948 Mg 4491 Mg 4458 Mg4118 Mg 8039 Mg 2087 Mg 137 Mg 3500 Ni 1015 Mg 506 Ca 49 Mg 43 Mg 2901 Iri 104 Mg 1609

[a] N are undefined nucleotides and R are purines

[b] See Experimental Procedures

[c] Refers to metal ion residue in the pdb files, where Mg is a magnesium ion, Na is a sodium ion, Ca is a calcium ion, NCO is a cobalt hexamine ion, Ni is a nickel ion and Iri is a iridum hexamine ion.