Structure-function analysis of the ribozymes of chrysanthemum chlorotic mottle viroid: a loop-loop interaction motif conserved in most natural hammerheads

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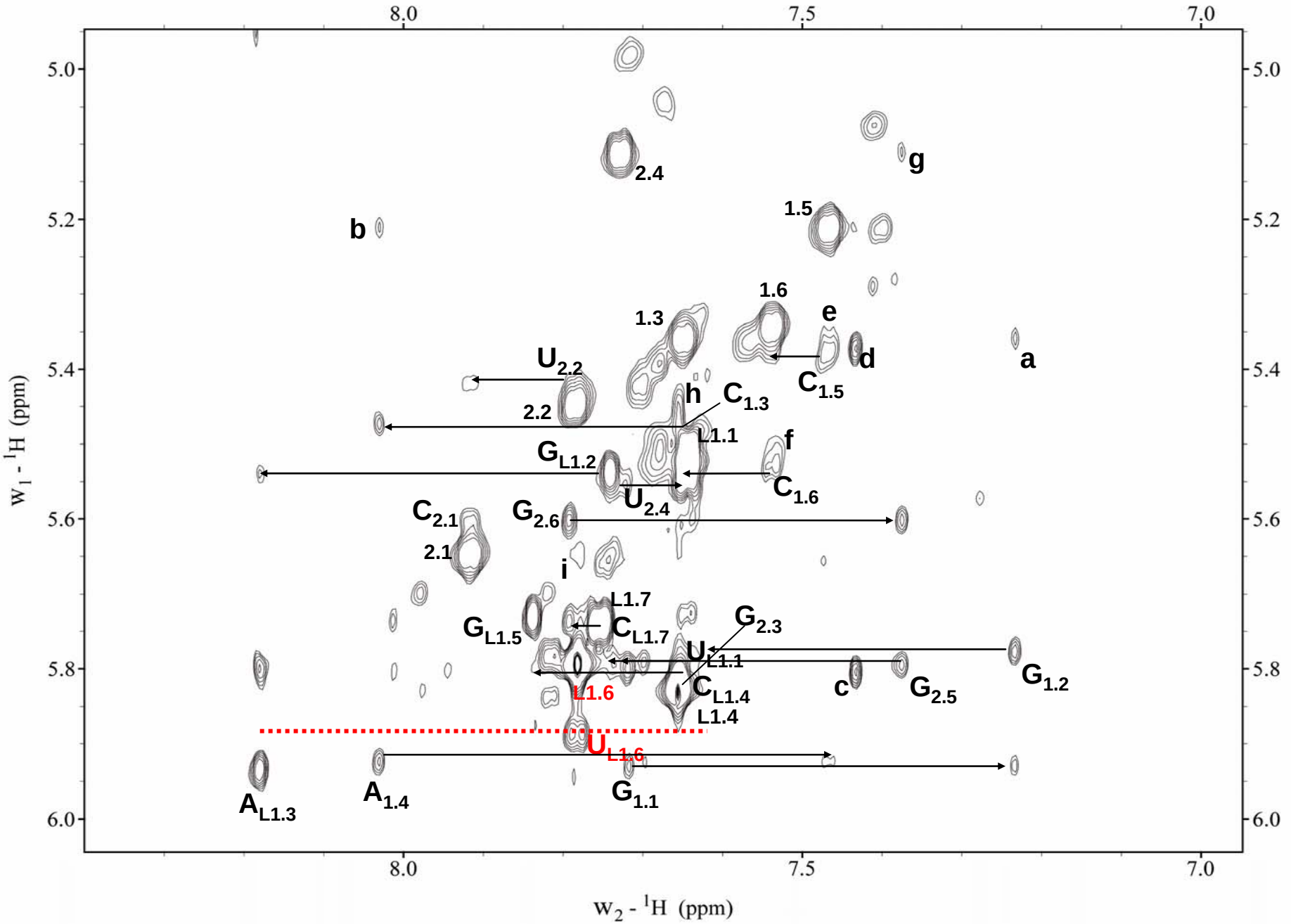
Supplementary Information

Figure S1. Assignments of the H2/H6/H8–H1'/H5 region of the NOESY spectrum (250 ms mixing time) of CChMVd domain I+ at 0.5 mM sodium phosphate and 36^{o}C. Intraresidue H1´-H6/H8 cross-peaks are labeled with residue name and number, intraresidue H5-H6 crosspeaks are labeled with residue number, and sequential NOE connectivities are indicated with horizontal arrows. Cross-peaks (a) to (i) are assigned as follows: a, $G_{1.2}$ H8-$C_{1.3}$ H5; b, $A_{1.4}$ H8-$C_{1.5}$ H5; c, $A_{1.4}$ H2-$G_{2.3}$ H1'; d, $A_{1.4}$ H2-$C_{1.5}$ H1'; e, $C_{1.5}$ H6-$C_{1.6}$ H5; f, $C_{1.6}$ H6-L1.U1 H5; g, $G_{2.5}$ H8-$U_{2.4}$ H5; h, $G_{2.3}$ H8-$U_{2.2}$ H5; i, $U_{2.2}$ H6-$C_{2.1}$ H5. The assignments of the extrahelical L1.U6 residue are shown in red, and the discontinuous line indicates the absence of any significant sequential interactions. The assignments of the terminal base pair (green-colored in Fig. 1B) have been omitted for clarity. The lower intensity, unlabeled cross-peaks are due to a competing duplex. These signals could be identified and minimized by decreasing the ionic strength of the buffer (see Material and Methods) and increasing the temperature of the measurements.

Figure S2. Assignments of the H2/H6/H8–H1'/H5 region of the NOESY spectrum (250 ms mixing time) of CChMVd domain II- at 32^{o}C and 5 mM $MgCl_2$. Intraresidue H1´-H6/H8 cross-peaks are labeled with residue name and number, intraresidue H5-H6 crosspeaks are labeled with residue number, and sequential NOE connectivities are indicated with horizontal arrows. Cross-peaks (a) to (g) are assigned as follows: a, $A_{10.7}$ H8-$C_{10.8}$ H5; b, $A_{10.7}$ H2-$C_{11.6}$ H1'; c, $A_{10.7}$ H2-$C_{10.8}$ H1'; d, $A_{TL.2}$ H2-$A_{TL.3}$ H1'; e, $A_{TL.3}$ H2-$A_{TL.4}$ H1'; f, $A_{TL.4}$ H2-$A_{TL.3}$ H1'; g, $G_{11.8}$ H8-$U_{11.7}$ H5. The assignments of the first base (green-colored in Fig. 1B) have been omitted for clarity. This and other non-exchangeable and exchangeable proton spectra reveal that domain II- forms the hairpin depicted in Fig. 1B, capped by a GAAA tetraloop and containing an asymmetric 2:6 GU:CGCACA internal loop. The broadening and doubling of the resonances of the internal loop and adjacent residues indicates that this internal loop exchanges between two or more conformations in solution.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5.6
5.8
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w2 - 1H (ppm)
2.4
g
1.5
b
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d
a
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h
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f
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C1.6
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C2.1
G2.6
2.1
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L1.7
G2.3
GL1.5
CL1.7
UL1.1
G1.2
c
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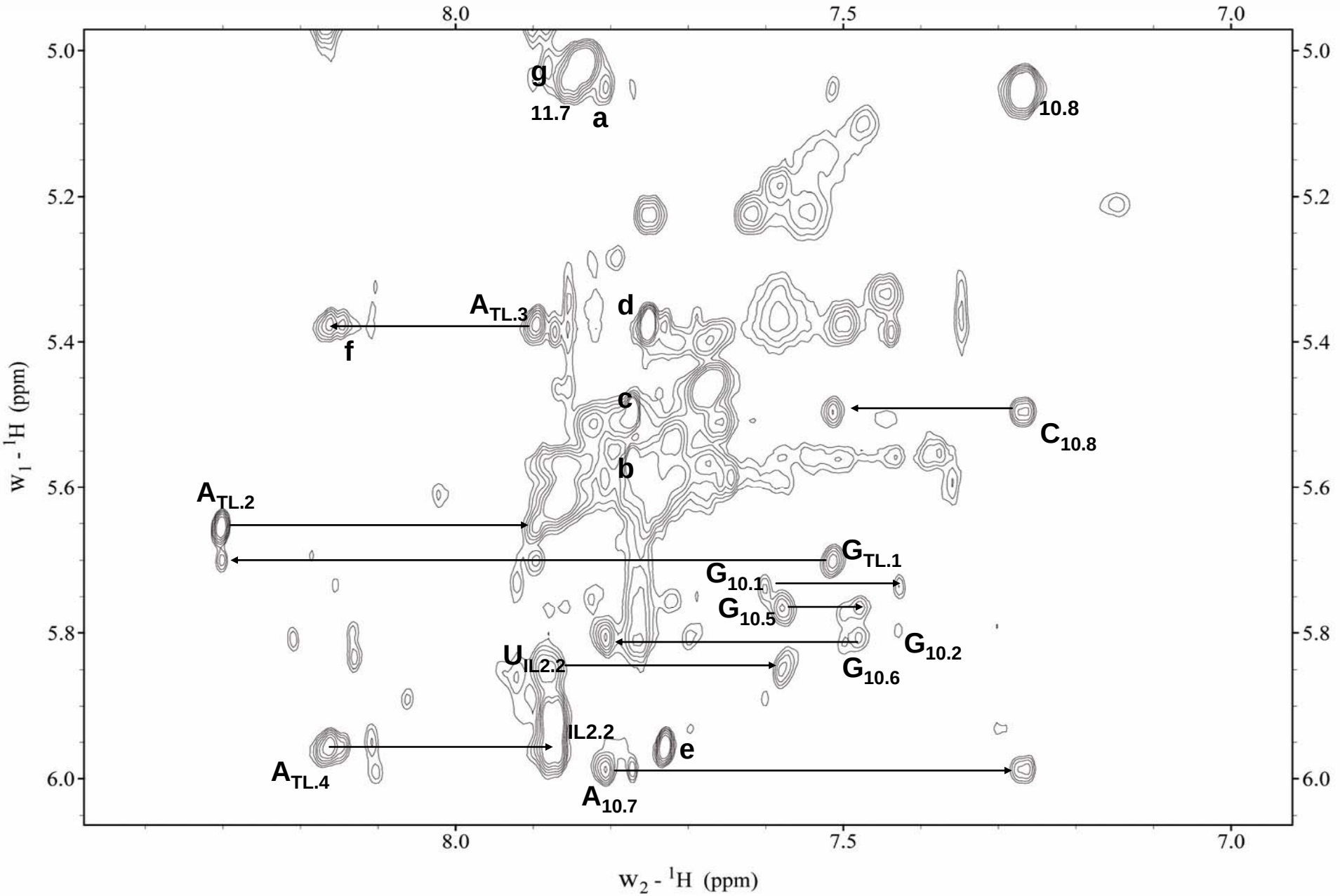
L1.6
CL1.4
L1.4
UL1.6
AL1.3
A1.4
G1.1

Figure S1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7.5
7.0
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5.8
6.0
w1 - 1H (ppm)
w2 - 1H (ppm)
g
11.7
a
10.8
f
ATL.3
d
c
C10.8
b
ATL.2
GTL.1
G10.1
G10.5
G10.2
G10.6
UIL2.2
IL2.2
e
ATL.4
A10.7

Figure S2