Supplemental data

Supplemental figures S1-S5, as well as input and output files from SAFA and NORNALIZE used in this study are freely available for download at https://simtk.org/home/nornalize.

Supplemental figure legends

FIGURE S1. Secondary structure diagram of the P4P6ΔC209 molecule displaying long range intramolecular interactions and crystal contacts. Base pairs are shown according to the nomenclature for base-pairing and base-stacking (Leontis & Westhof 2001; Adams et al. 2004) as output from the software S2S (Jossinet & Westhof 2005). Crystals contacts are shown in black. The inset on the right shows a different local structure adopted by molecule B. The inset at the bottom left shows a view of the overall lattice organization.

FIGURE S2. Comparison of the SHAPE reactivity in crystal and the temperature B-factors of the 2'-hydroxyl groups. Values for the SHAPE reactivity shown in Fig. 3A were used. The horizontal bars correspond to the mean average B-factors for molecules A (purple) and B (pink).

FIGURE S3. A178 and U168 are trapped in reactive conformations at the surface of the molecule. (A) Extensive tertiary interactions (direct and water-mediated H-bonds, metal coordination, and stacking) involving A178. (B) U168 (shown here belonging to molecule B) lies at the surface of the molecule, at the junction between molecules A and B. The 2'-hydroxyl group is indicated by a cyan sphere.

FIGURE S4. Crystal contacts involving the shallow groove of helical domains. (A) Overall stereoview of the packing of the P4 and P5 helices belonging to four different molecules. (B)

Overall stereoview of the local packing around the helical domains P6a/P6b and P5/P5a, centered around U130 from molecule A. Lighter orange and gray shades refer to neighboring molecules. (C) Stereoview showing the local helical packing around U130 from molecule B (orientation as in (B).

FIGURE S5. RNA and NMIA titrations. (A) SHAPE performed using three RNA concentrations in the crystallization solution (78 μM corresponds to the RNA concentration in the crystallization drop). The RNA was refolded either by heating for 10 min at 65°C followed by slow-cooling to room temperature ("65") or by incubating for 15 min at room temperature ("RT"). (B) NMIA titration in the native solution for an RNA concentration of 0.15 μM. Representative nucleotides are indicated on both panels as in Fig. 1B.

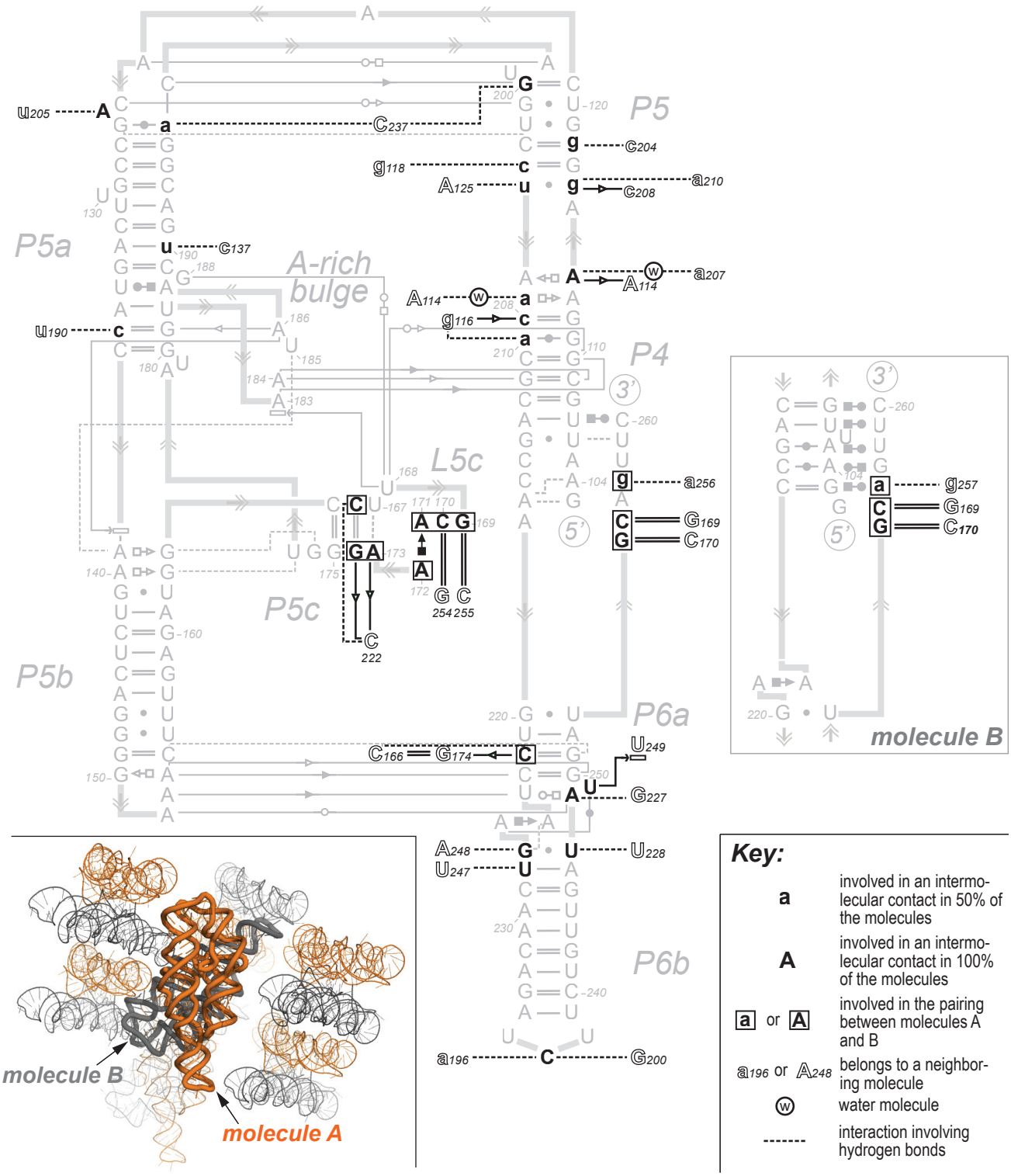
P5
P5a
A-rich bulge
P4
L5c
P5c
P5b
P6a
P6b
molecule B
molecule A
Key:
a involved in an intermolecular contact in 50% of the molecules
A involved in an intermolecular contact in 100% of the molecules
a or A involved in the pairing between molecules A and B
a196 or A248 belongs to a neighboring molecule
w water molecule
------- interaction involving hydrogen bonds

Figure S1

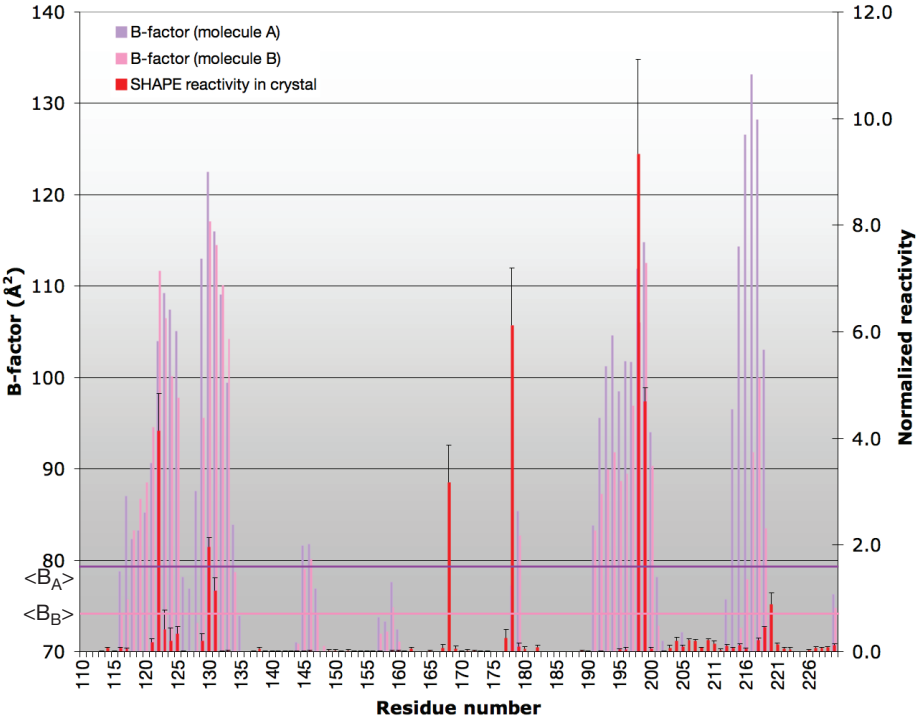
B-factor (molecule A)
B-factor (molecule B)
SHAPE reactivity in crystal
B-factor (Å²)
Normalized reactivity
Residue number
<B_A>
<B_B>
140
130
120
110
100
90
80
70
12.0
10.0
8.0
6.0
4.0
2.0
0.0
110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 211 216 221 226

Figure S2

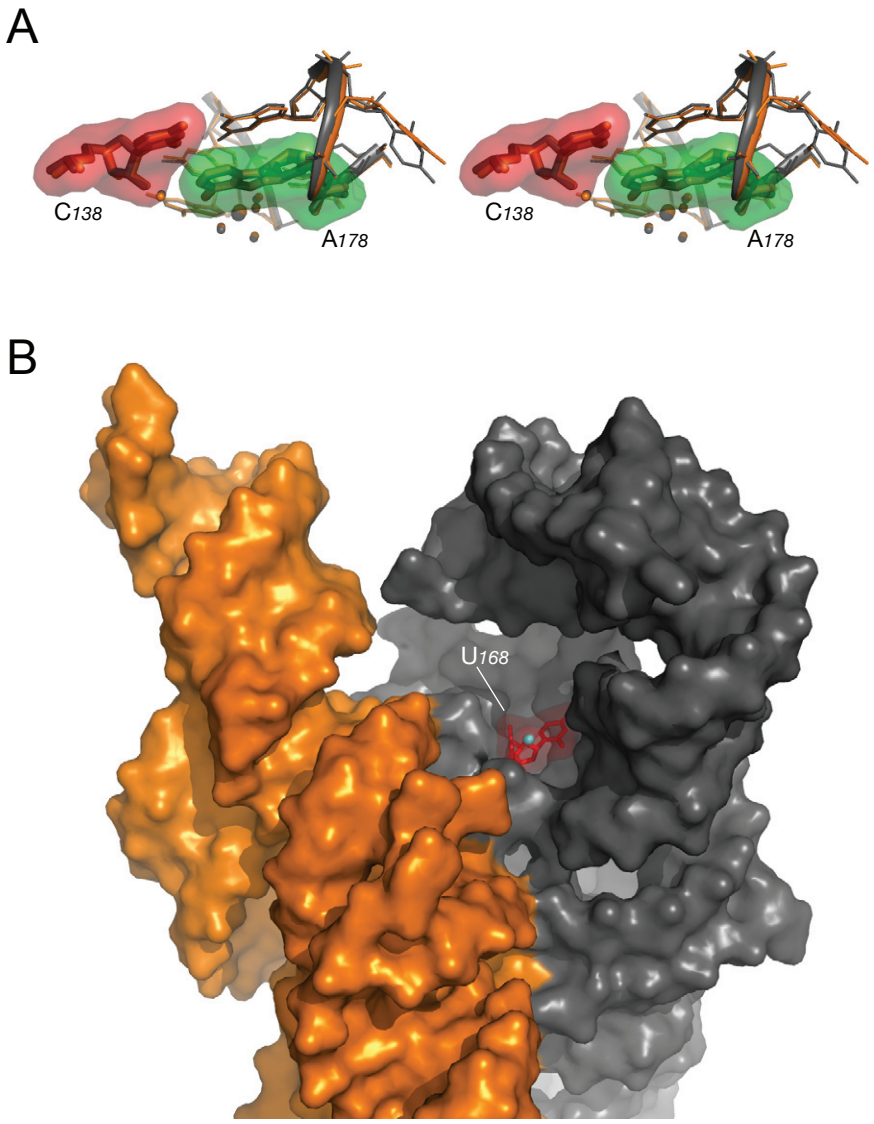

Figure S3

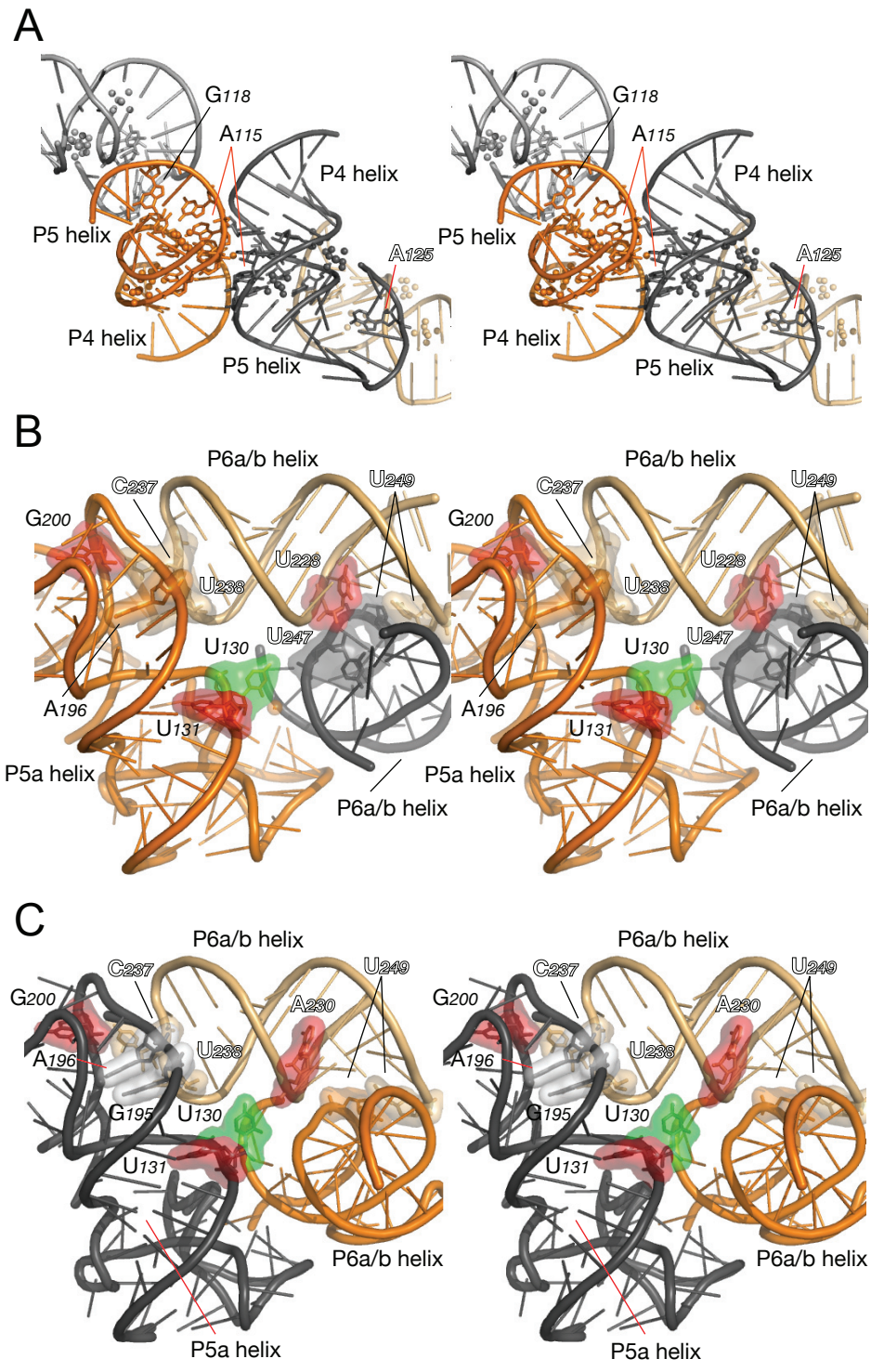
A
G118
A115
P4 helix
P5 helix
A125
P4 helix
P5 helix
G118
A115
P4 helix
P5 helix
A125
P4 helix
P5 helix
B
P6a/b helix
C237
U249
G200
U228
U238
U130
U247
A196
U131
P5a helix
P6a/b helix
P6a/b helix
C237
U249
G200
U228
U238
U130
U247
A196
U131
P5a helix
P6a/b helix
C
P6a/b helix
C237
U249
G200
A230
A196
U238
G195
U130
U131
P6a/b helix
P5a helix
P6a/b helix
C237
U249
G200
A230
A196
U238
G195
U130
U131
P6a/b helix
P5a helix

Figure S4

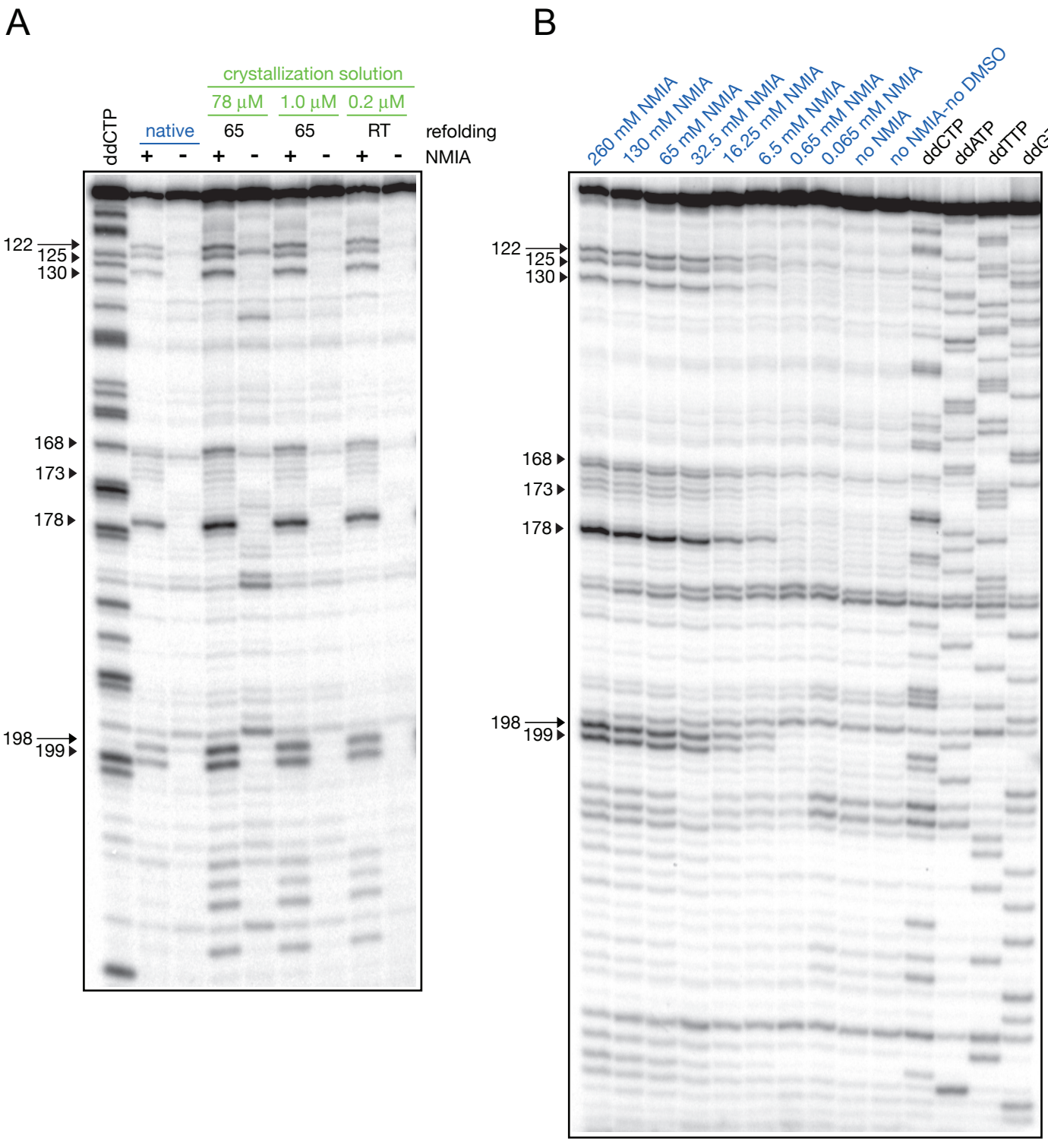
A
crystallization solution
78 µM
1.0 µM
0.2 µM
ddCTP
native
65
65
RT
refolding
+ - + - + - + -
NMIA
122
125
130
168
173
178
198
199
B
260 mM NMIA
130 mM NMIA
65 mM NMIA
32.5 mM NMIA
16.25 mM NMIA
6.5 mM NMIA
0.65 mM NMIA
0.065 mM NMIA
no NMIA
no NMIA-no DMSO
ddCTP
ddATP
ddTTP
ddGTP
122
125
130
168
173
178
198
199

Figure S5