Biophysical Journal, Volume 96

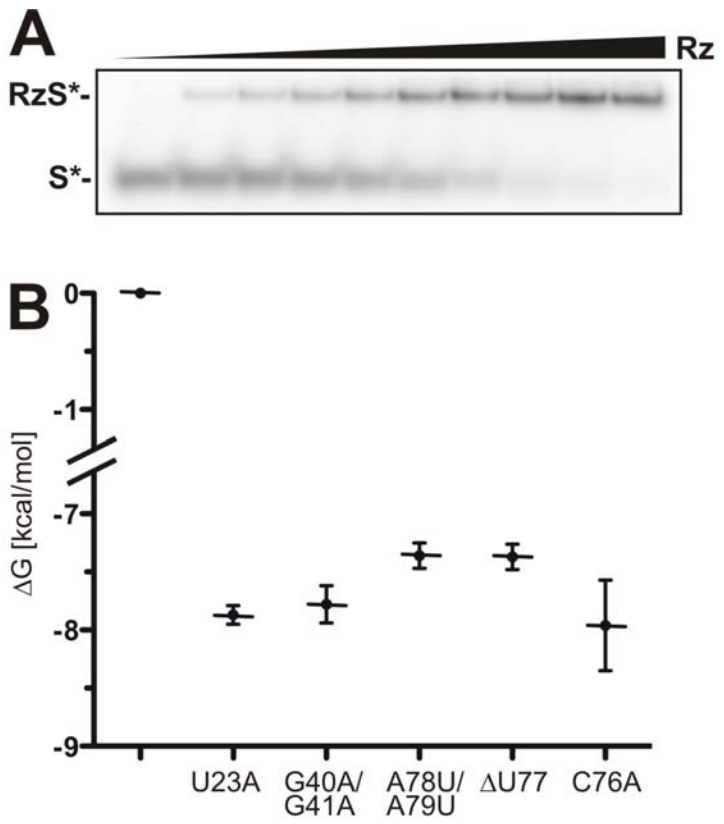
Supplementary Material

Monitoring of an RNA Multistep Folding Pathway by Isothermal Titration Calorimetry

Cédric Reymond, Martin Bisaillon, and Jean-Pierre Perreault

Electrophoretic mobility shift assay (EMSA)

EMSA analyses were performed with the collection of mutated ribozymes. Briefly, trace amounts of 5'-end-^{32}P-labeled substrates were incubated with 0.2 nM - 50 nM of mutated ribozyme in a final volume 10 µL containing 50 mM Tris-borate (pH 7.5) and 10 mM $MgCl_2$. The samples were incubated at 25˚C for 90 min, and then loading dye (50% glycerol, 0.025% bromophenol blue and 0.025% xylene cyanol) added. The samples were then fractionated on native 20% polyacrylamide gels (29:1 ratio of acrylamide to bisacrylamide) using 50 mM Tris-borate (pH 7.5) and 10 mM $MgCl_2$ solution for both gel preparation and running buffer. The gels were run at 4˚C for 18 h at 90 V, exposed to phosphor screens, scanned and analyzed using a PhosphorImager program (Molecular Dynamics). The fractions of bound substrate were determined, and both the K_D and the Gibbs free energy were then calculated (i.e. $\Delta G = -RT \ln K_D$, where ΔG is the change in Gibbs free energy, T the absolute temperature and R the universal gas constant). All of the measurements were performed on a minimum of two independent experiments.



Results of electrophoretic mobility shift assays experiments. (A) Typical autoradiogram of an EMSA assay performed for the Rz-C76A mutant. (B) ΔG values obtained with the various mutants.

TABLE S1: Data from binding shift assays performed with the HDV ribozymes.

Mutant	ĘH [kcal / mol]	TĘS [kcal / mol]	ĘG [kcal / mol]
Rz-U23A	-2.73 ± 0.19	3.10 ± 0.11	-5.83 ± 0.05
Rz-G40A/G41A	-3.95 ± 0.21	1.68 ± 0.24	-5.62 ± 0.06
Rz-A78U/A79U	-3.15 ± 0.54	2.06 ± 0.78	-5.18 ± 0.13
Rz-ĘU77	-5.04 ± 1.29	0.17 ± 0.10	-5.21 ± 0.06
Rz-C76A	-5.30 ± 0.78	0.87 ± 0.14	-6.17 ± 0.13