

Abstract No. Chan0071

Novel Analysis Methods for the RNA Folding

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Beamline(s): X28C

The formation of individual tertiary contacts of the *Tetrahymena* L-21 Sca I ribozyme has been followed by hydroxyl radical ($\cdot\text{OH}$) footprinting and the global conformation has been followed by analytical ultracentrifugation as a function of monovalent ion concentration in the absence of divalent ions. Advanced methods of data analysis, which allow the hydroxyl radical reactivity of every nucleotide to be quantitated, permit monitoring of each and every structural element of the RNA with great details. Monovalent ion mediated global compaction of the ribozyme is accompanied by the formation of native tertiary contacts even without divalent cation. In the absence of magnesium and the presence of 1.5 M NaCl, most native tertiary contacts are evident in hydroxyl radical footprinting data except several sites implicated in direct magnesium coordination by structural studies. Na^+ folding isotherms derived from these analyses indicate non-native tertiary contacts that are present at low but not high concentrations of Na^+ . In addition, some isotherms illustrate complex patterns having biphasic or transient transitions. In light of recent studies that have shown that the presence of monovalent ions greatly accelerates the Mg^{2+} -dependent folding of the *Tetrahymena* ribozyme, the present studies suggest that Na^+ concentration changes not only the starting position of the RNA on its folding funnel but also pushes it deep into the well by forming native tertiary contacts and thus favoring fast and correct folding pathways.