Biophysical Journal, Volume *114*

Supplemental Information

Synergistic SHAPE/Single-Molecule Deconvolution of RNA Conformation under Physiological Conditions

Mario Vieweger and David J. Nesbitt

S1: SHAPE reactivity

The SHAPE reagent (*N*-Methylisatoic anhydride (NMIA)) is an electrophile that preferentially reacts with nucleotides that display an electron density at a 2'OH position that is favorable for oxyanion formation. Nucleotides constrained in hybridized domains present a proximal 3' phosphodiester due to the RNA C3'-endo conformation. The proximity of the 3'-phosphodiester anion to the 2'-hydroxyl destabilize the oxyanion, thus, rendering such conformations unreactive to the SHAPE reagent. [MerinoWeeks2005]

In contrast, single-stranded nucleotides are flexible and can sample micro-conformations that promote oxyanion formation and thus reactivity toward the NMIA electrophile. Based on comparison of SHAPE reactivities with X-ray chrystallography data, reactive conformations are rare conformations that are sampled by single-stranded, non-hybridized nucleotides.[54] As demonstrated by McGinnis et. al., the most reactive nucleotides, so called hyper-reactive nucleotides, exhibit conformations that present the 3' phosphodiester far from the 2' OH. These reactive conformations are sampled during dynamics of flexible nucleotides.

During data analysis, SHAPE reactivities are normalized based on benchmarking results onto a 0-2 scale. On this scale hybridized nucleotides that are inert to electrophilic attack are observed with low reactivities ($R < 0.45$), while highly flexible nucleotides sampling reactive conformations exhibit high reactivities ($R > 0.8$). Intermediate reactivities indicate structurally dynamic RNA domains that result in nucleotides sampling flexible and constrained micro-conformations ($0.45 < R < 0.8$). In addition, hyper-reactive nucleotides which are locked in a highly reactive micro-conformation are observed outside the regular scale with reactivities above 2 ($R > 2$).

To extract [M^{n+}]-induced structural changes to the overall RNA conformation, one thus focusses on nucleotides in the $0 < R < 2$ range that show significant changes in SHAPE reactivity. Small changes in this range, especially between 0 and 1, are most informative as they indicate a change in the hybridization between conformations, i.e. single-stranded to double-stranded and *vice versa*. Hyper-reactive nucleotides, on the other hand, may exhibit a large change in reactivity but due to the origin of the SHAPE reactivity, this change is merely indicative of a change in nucleotide micro-conformation rather than an insignificant structural rearrangement.

S2: SHAPE data analysis

Data processing, analysis and sequence alignment are performed in QuSHAPE [46]. SHAPE reactivities are normalized using a model-free boxplot analysis. To this end, the interquartile range (IQR) is determined for all SHAPE reactivities of the construct and outliers are determined that are larger than 1.5 x IQR (limited to 9%). The reactivities are then normalized to the average of the 10% most reactive nucleotides after exclusion of the outliers. This procedure typically normalizes reactivities onto a 0-2 scale with a few hyperreactive nucleotides showing reactivities above 2. Normalized SHAPE reactivities are represented using a ternary color code (black: $R < 0.4$; orange: $0.4 < R < 0.85$; red: $R > 0.85$) that is based on benchmarking results and comparison with x-ray chrystallography data [54] [41, 42]. This color

code displays non-reactive nucleotides in black, moderately reactive nucleotides in orange, and highly reactive nucleotides in red.

S3: MaxExpect and Probknot structure predictions of 226nt TLS construct

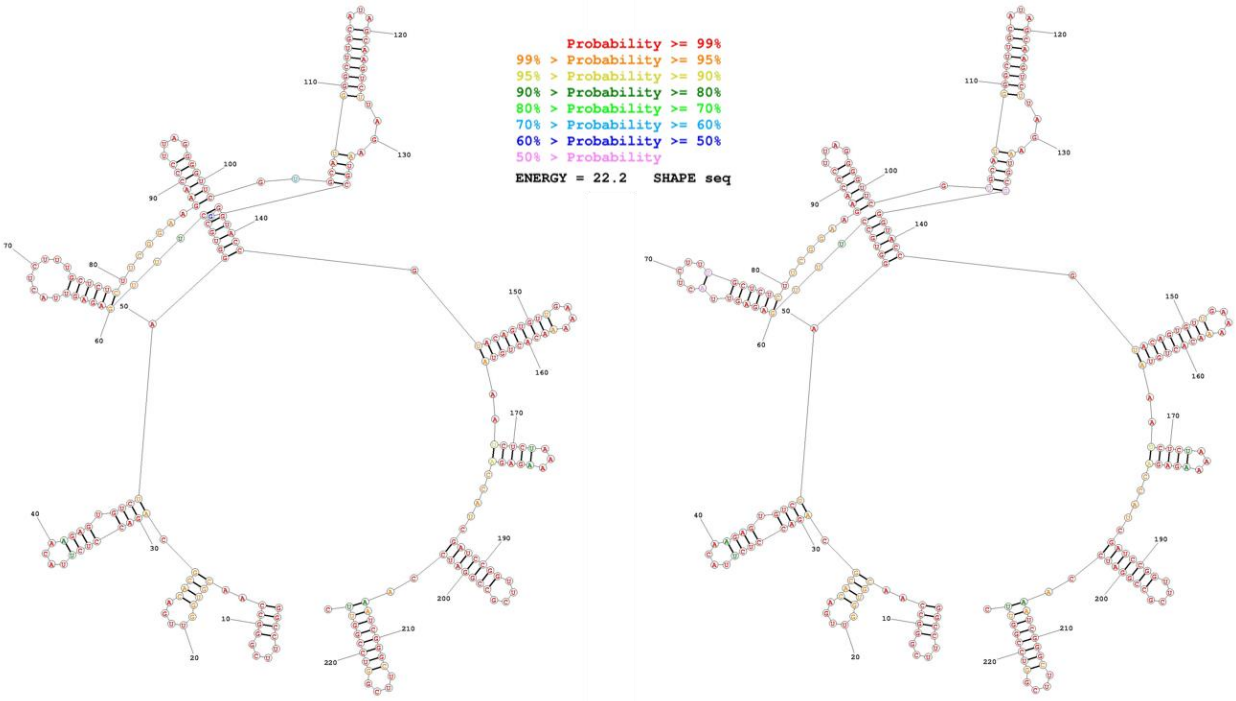
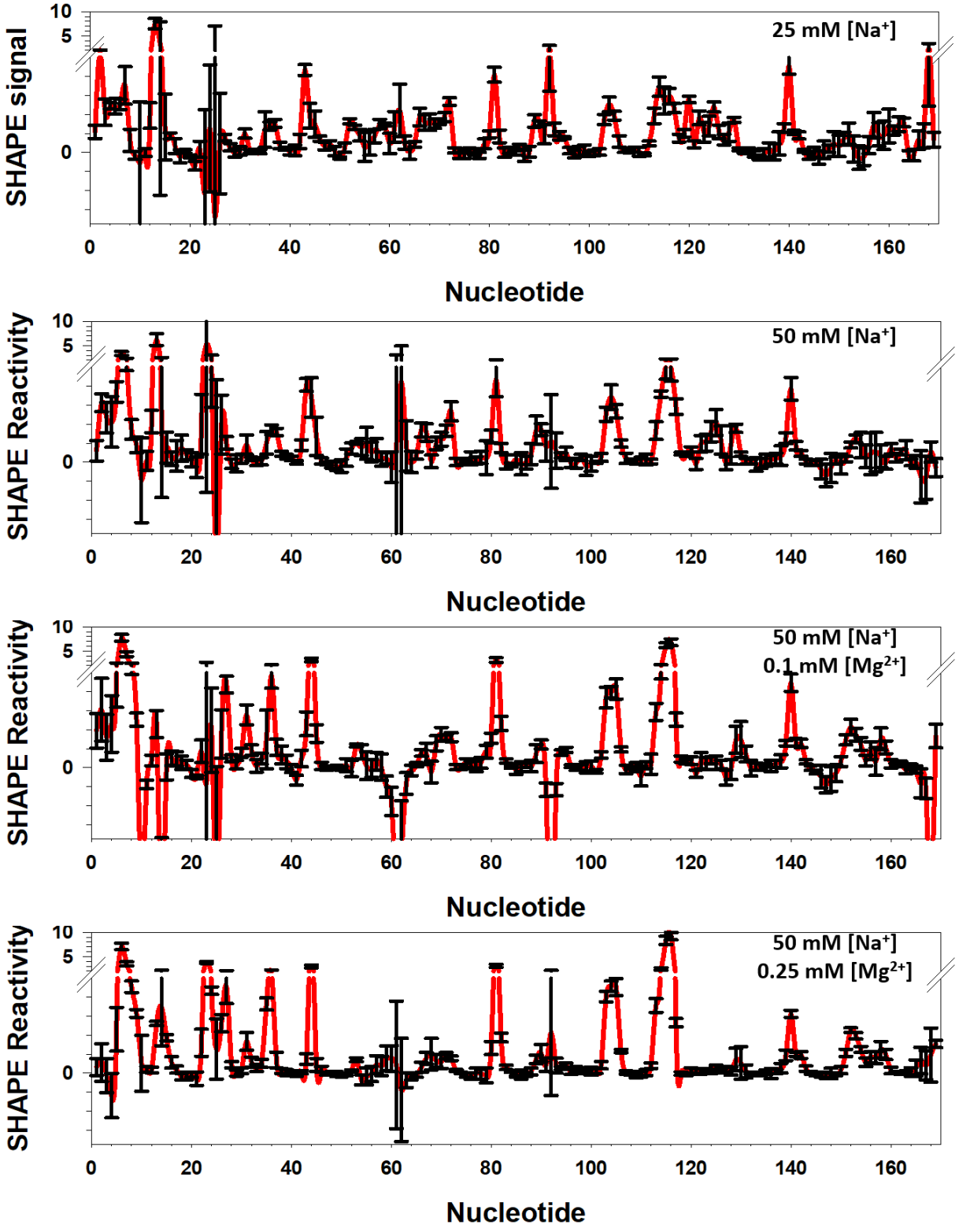


Figure S1: MaxExpect and ProbKnot 2° structure prediction of 226nt TLS construct for SHAPE probing. The 5' nucleotides 1-14 and the two 3' nucleotides 184-226 fold into hairpins that form the structure cassette for SHAPE probing. Prediction from RNAStructure suggests that these additional regions will not interfere with the folding of the TLS construct. (Left: MaxExpect, Right: ProbKnot, Insert: Prediction Probability)

S4: Averages and Standard Deviations of SHAPE probing results



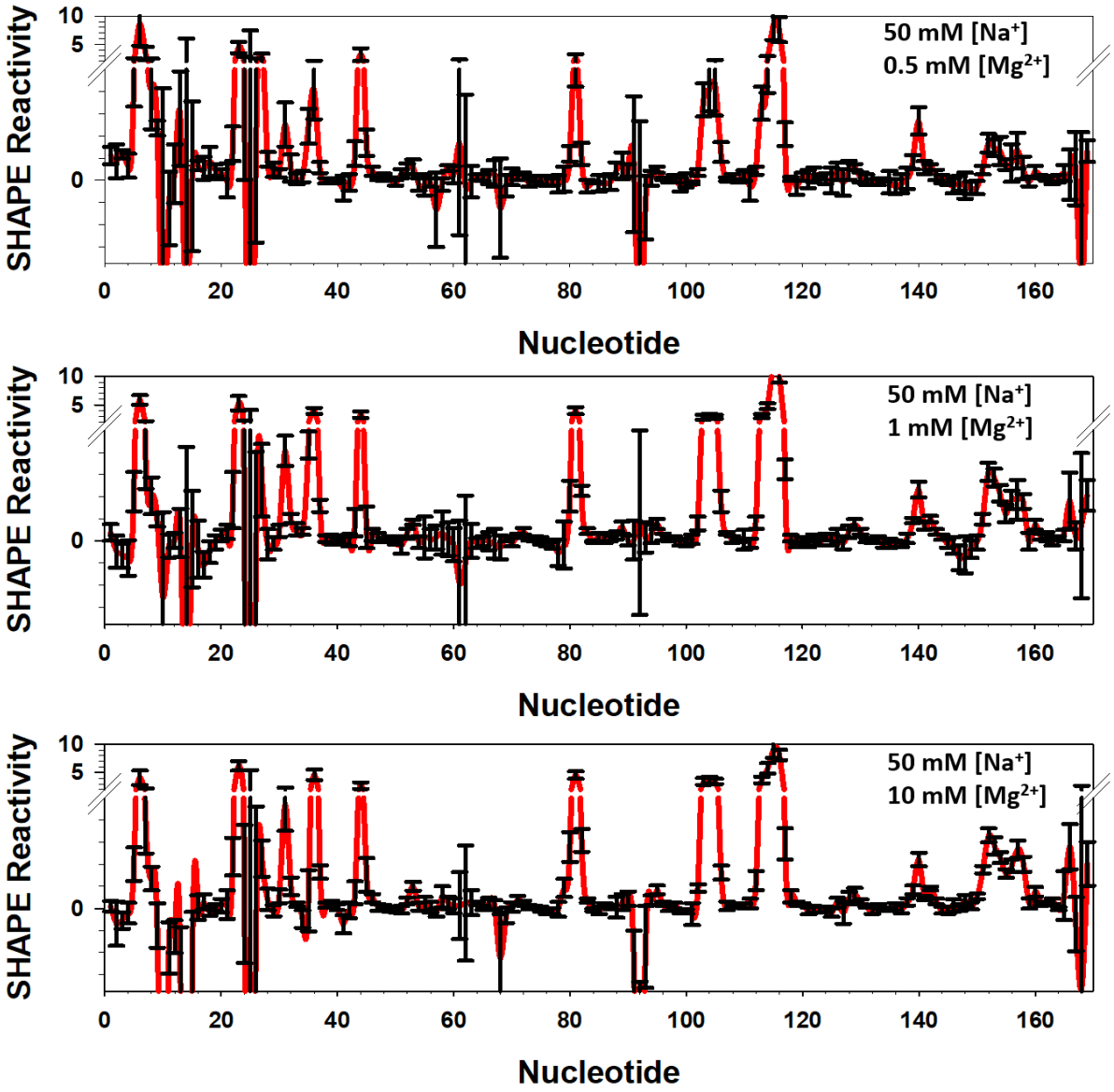


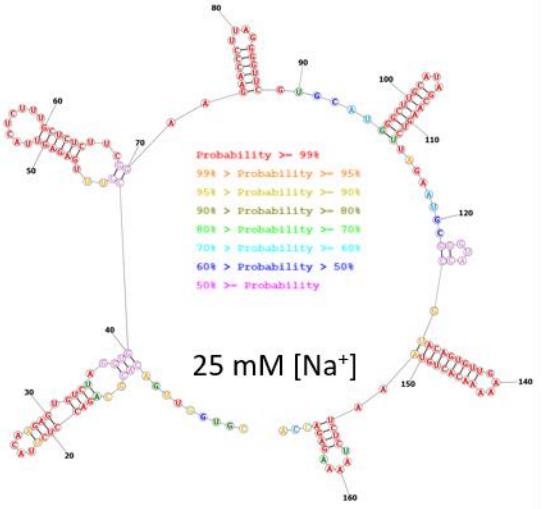
Figure S2: TLS SHAPE probing statistics. Three biological repeats of both positive and negative SHAPE probing experiments were analyzed using QuSHAPE. Individual QuSHAPE results were averaged for each probing condition. Standard deviations were determined at each nucleotide position to determine statistical significance of SHAPE signal intensity and changes thereof with varying SHAPE reactivities.

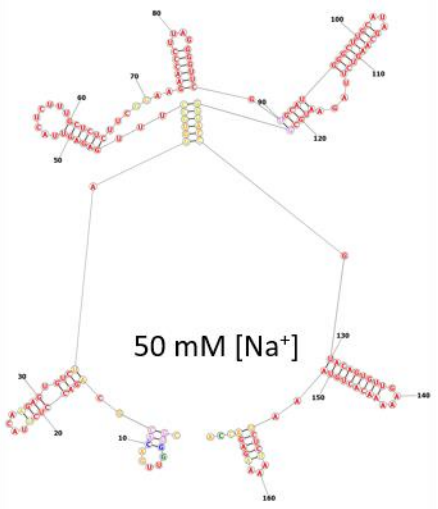
S5: Rejected nucleotides

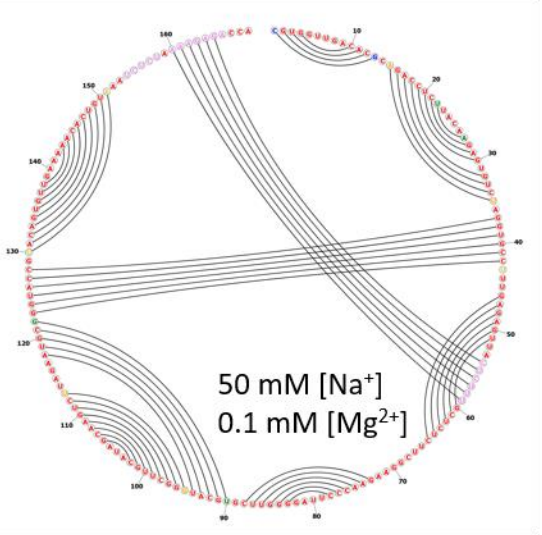
Condition	*[Na^+](mM)*	*[Mg^{2+}](mM)*	*Outliers*	*Rejected Nucleotide positions*
1	25	0	9	10 | 13 | 14 | 23 | 24 | 25 | 26 | 92 | 168
2	50	0	7	10 | 14 | 23 | 25 | 61 | 62 | 92
3	50	0.1	8	14 | 23 | 24 | 25 | 61 | 62 | 92 | 168
4	50	0.25	9	4 | 5 | 10 | 14 | 25 | 61 | 62 | 92 | 168
5	50	0.5	12	10 | 14 | 15 | 23 | 24 | 25 | 26 | 61 | 62 | 91 | 92 | 168
6	50	1	10	10 | 14 | 23 | 24 | 25 | 26 | 61 | 62 | 92 | 168
7	50	10	10	10 | 14 | 15 | 23 | 24 | 25 | 26 | 62 | 92 | 168

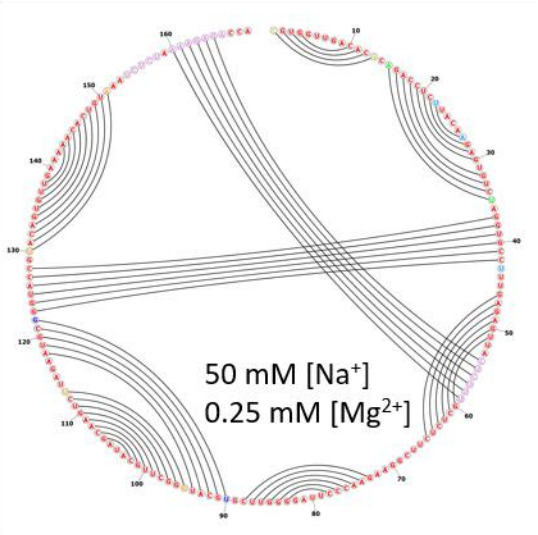
Table S1: Nucleotides that were rejected based on statistical considerations.

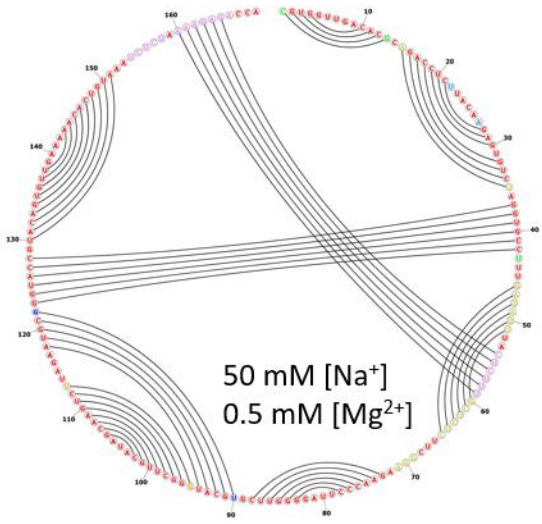
S6: Base-pairing probabilities of SHAPE predicted 2° structures

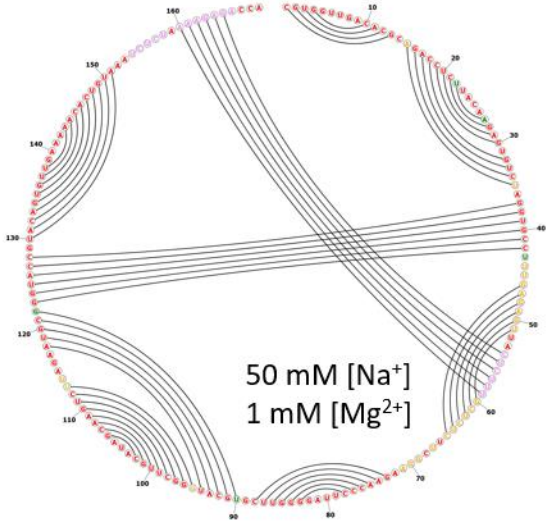












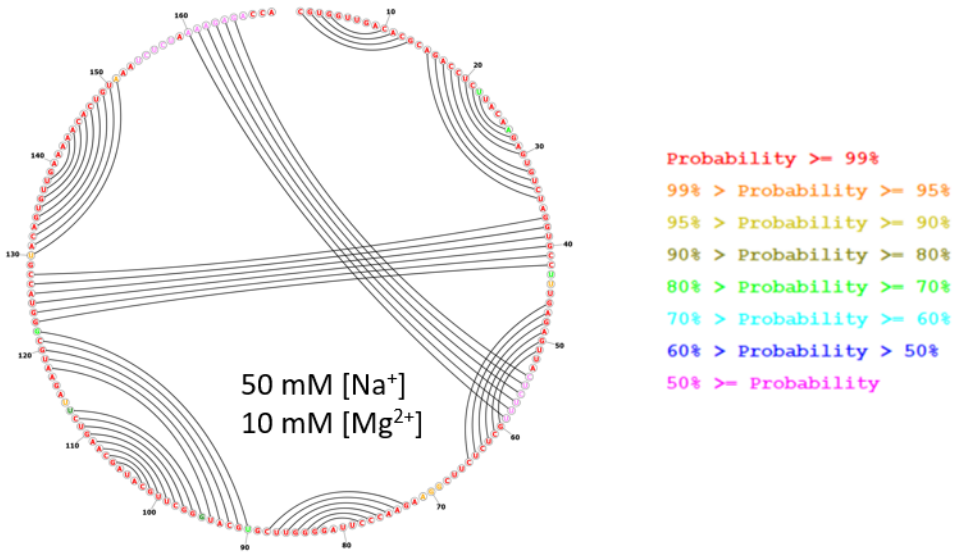


Figure S3: Probability functions of SHAPE-directed structure predictions.

S7: Structure prediction of deconvoluted SHAPE traces

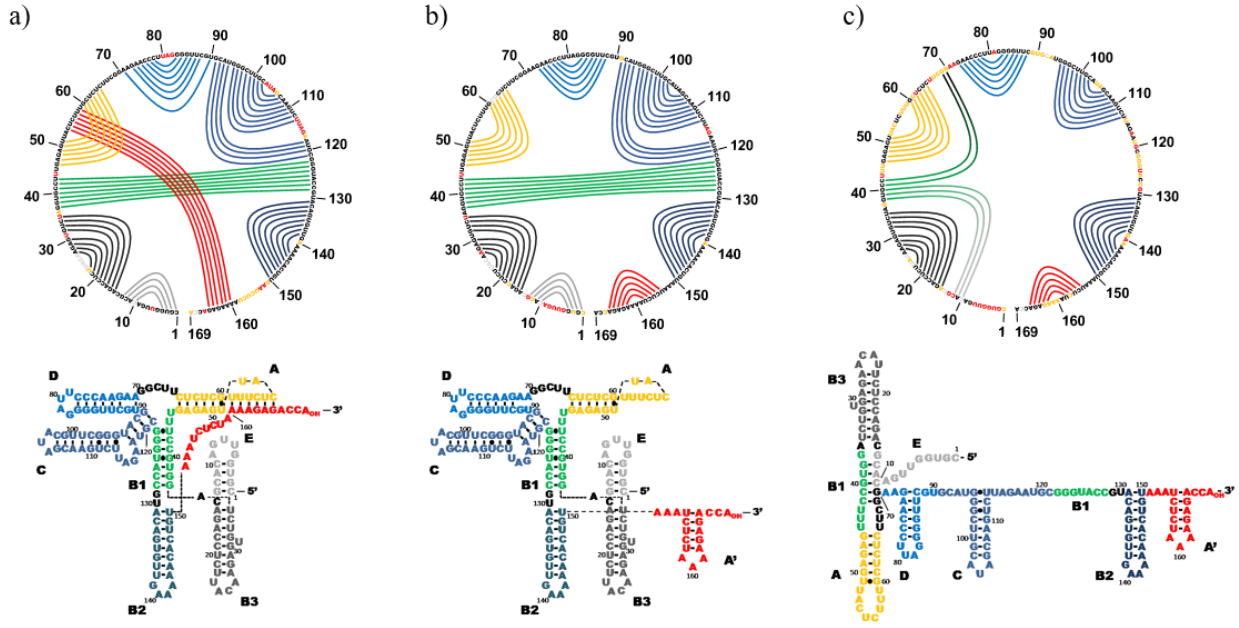


Figure S4: Overlap of deconvoluted SHAPE traces onto 2° structure predictions.