SUPPLEMENTAL MATERIAL

Template switching enables chemical probing of native RNA structures

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Table S1. Switch-MaP oligonucleotides

Oligo Name	Sequence 5′-3′
SHAPE 3′ Adapter[a,b]	rAppNNNNNNNCTGTCTCTTATACACATCTCCGAGCCCACGAGAddC
SHAPE TSO[c]	TiMe-isodCGTCGGCAGCGTCAGATGTGTATAAGAGACAG NNNNNNNrGrGrG

[a]rApp denotes an adenylate group.
[b]ddC denotes a dideoxy cytosine.
[c]iMe-isodC denotes a 5-methyl isodeoxycytosine.

Table S2. Riboswitch gene block sequence

Construct Name	Sequence 5′-3′
SreA gene block	AGTTACGAATTCTAATACGACTCACTATAGGGATCGACGTTTCTT GCTGATGAGTCCGTGAGGACGAAACGGTACCCGGTACCGTCCAA GAAACGTCACTATAGTCACAAATGAAGTGATTTAATAATATGTAGA AATCTTATCCAGAGTGGTGGAGGGAAATGCCCTATGAAGCCCAG CAACCTAAACAATAATTCATTATGTGTTTAAGGTGCTAAGTCATGC AGAACAACTAATTGTTCTGAAAGATGAGAAGGAAGTTAGTCCATT TGAAAAAATGCTGCCTTTCTGCTCATCAGCAGAAAGGCTTTTTTTG GATCCCTAGATA

Table S3. Construct sequences

Construct	Sequence 5′-3′[a,b]
SreA Riboswitch	CAAGAAACGUCACUAUAGUCACAAAUGAAGUGAUUUAAUAAUA UGUAGAAAUCUUAUCCAGAGUGGUGGAGGGAAAUGCCCUAUG AAGCCCAGCAACCUAAACAAUAAUUCAUUAUGUGUUUAAGGUG CUAAGUCAUGCAGAACAACUAAUUGUUCUGAAAGAUGAGAAGG AAGUUAGUCCAUUUGAAAAAAUGCUGCCUUUCUGCUCAUCAGC AGAAAGGCUUUUUUU
SreA Riboswitch Cassette	**GUGGGCACUUCGGUGUCCACAC**CAAGAAACGUCACUAUAGUC ACAAAUGAAGUGAUUUAAUAAUAUGUAGAAAUCUUAUCCAGA GUGGUGGAGGGAAAUGCCCUAUGAAGCCCAGCAACCUAAACA AUAAUUCAUUAUGUGUUUAAGGUGCUAAGUCAUGCAGAACAA CUAAUUGUUCUGAAAGAUGAGAAGGAAGUUAGUCCAUUUGAA AAAAUGCUGCCUUUCUGCUCAUCAGCAGAAAGGCUUUUUUU**U C**GAUCCGGUUCGCCGGAUC**CA**AAUCGGGCUUCGGUCCGGUUC
SreA Riboswitch Switch-MaP	**GG**CAAGAAACGUCACUAUAGUCACAAAUGAAGUGAUUUAAUAA UAUGUAGAAAUCUUAUCCAGAGUGGUGGAGGGAAAUGCCCUA UGAAGCCCAGCAACCUAAACAAUAAUUCAUUAUGUGUUUAAGG UGCUAAGUCAUGCAGAACAACUAAUUGUUCUGAAAGAUGAGAA GGAAGUUAGUCCAUUUGAAAAAAUGCUGCCUUUCUGCUCAUCA GCAGAAAGGCUUUUUUU
SreA Aptamer	**GG**UGUAGAAAUCUUAUCCAGAGUGGUGGAGGGAAAUGCCCUA UGAAGCCCAGCAACCUAAACAAUAAUUCAUUAUGUGUUUAAGG UGCUAAGUCAUGCAGAACAACUAAUUGUUCUGAAAGAUGAGA**C C**
SreA Aptamer Cassette	**GUGGGCACUUCGGUGUCCACACGG**UCUUAUCCAGAGUGGUGG AGGGAAAUGCCCUAUGAAGCCCAGCAACCUAAACAAUAAUUCAU UAUGUGUUUAAGGUGCUAAGUCAUGCAGAACAACUAAUUGUUC UGAAAGAUGAGA**CCUCGAUCCGGUUCGCCGGAUCCAAAUCGGG CUUCGGUCCGGUUC**

[a]Non-Native nucleotides are indicated by **bold**.
[b]Cassette stem-loop sequences are indicated by underline.

Table S4. Primer sequences

Primer Name	Sequence 5′-3′
pUC57-80 Fwd	GGAATTGTGAGCGGATAACAATTTCACACAGGAAAC
SreA FL Rev[a]	mAmAAAAAAGCCTTTCTGCTGATGAGCAGAAAGGCAG
SreA FL RT	AAAAAAAGCCTTTCTGCTGATG
SreA FL cDNA Fwd	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNNCAAGAA ACGTCACTATAGTCA
SreA FL cDNA Rev	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNAAAAAAA GCCTTTCTGCTG
SreA FL-GG Fwd	TAATACGACTCACTATAGGCAAGAAACGTCACTATAGTCACAAATGA AGTGATTTAATAA
SreA FL-Cas-Fwd1	GCACTTCGGTGTCCACACCAAGAAACGTCACTATAGTCACAAATGA AGTGATTTAATAA
SreA Fl-Cas-Fwd2	TAATACGACTCACTATAGTGGGCACTTCGGTGTCCACACCAAG
SreA Fl-Cas-Rev[a]	mGmAACCGGACCGAAGCCCGATTTGGATCCGGCGAACCGGATCG AAAAAAAAGCCTTTCTGCTGATGAGCAG
SreA AD Fwd	AATAGTGAATTCTAATACGACTCACTATAGGTCTTATCCAGAGTGG TGGAGGGAAATGCC
SreA AD Rev[a]	mGmGTCTCATCTTTCAGAACAATTAGTTGTTCTGCATGACTTAGCAC CTT
SreA AD RT	GTCTCGTGGGCTCGG
i5 multiplex[b]	AATGATACGGCGACCACCGAGATCTACAC-X_8-TCGTCGGCAGCGTC
i7 multiplex[b]	CAAGCAGAAGACGGCATACGAGAT-X_9-GTCTCGTGGGCTCGG
OEPCR Primer 1F	TAATACGACTCACTATAGTGGGCACTTCGGTGTCCACACGGTCTTATC CAGAG
OEPCR Primer 2R	GGCTTCATAGGGCATTTCCCTCCACCACTCTGGATAAGACCGTGTGG ACA
OEPCR Primer 3F	GAGGGAAATGCCCTATGAAGCCCAGCAACCTAAACAATAATTCATTAT GT
OEPCR Primer 4R	GTTCTGCATGACTTAGCACCTTAAACACATAATGAATTATTGTTTAGGT TGCTG
OEPCR Primer 5F	GGTGCTAAGTCATGCAGAACAACTAATTGTTCTGAAAGATGAGACCTC GATCCGGTTCG
OEPCR Primer 6R[a]	mGmAACCGGACCGAAGCCCGATTTGGATCCGGCGAACCGGATCGA GGTCTCATCTTT
Cassette RT	GAACCGGACCGAAGCCCG
Cas i7 Fwd Multiplex[b]	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-X_5-GTGGGCACTTCGGTGTCC
Cas i5 Rev Multiplex[b]	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-X_5-GAACCGGACCGAAGCCCG

[a]m denotes a 2′-O-methyl modification.

[b]X denotes variable nucleotides that serve as unique molecular identifiers.

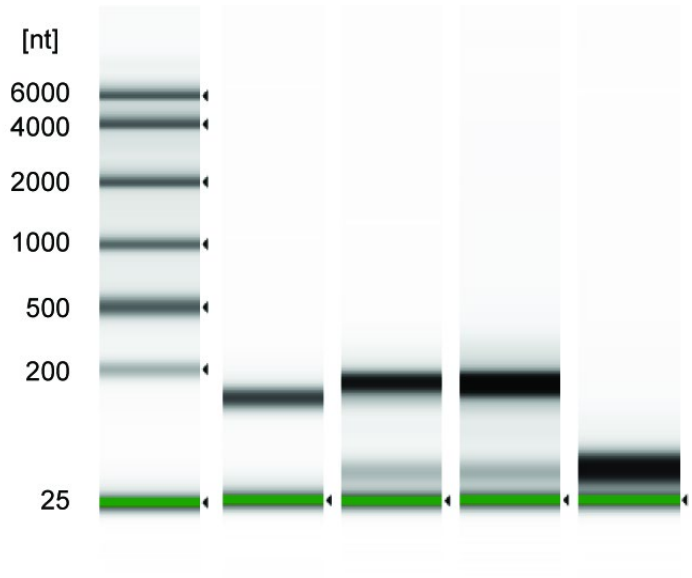

Figure S1. Validation of ligation of pre-adenylated DNA adapter.

Lane 1 contains SreA aptamer domain RNA (121 nt). Lane 2 contains 1M7 treated SreA aptamer domain RNA ligated to the pre-adenylated DNA adapter (162 nt). Lane 3 contains DMSO treated SreA aptamer domain RNA ligated to the pre-adenylated DNA adapter (162 nt). Lane 4 contains the pre-adenylated DNA adapter (41 nt).

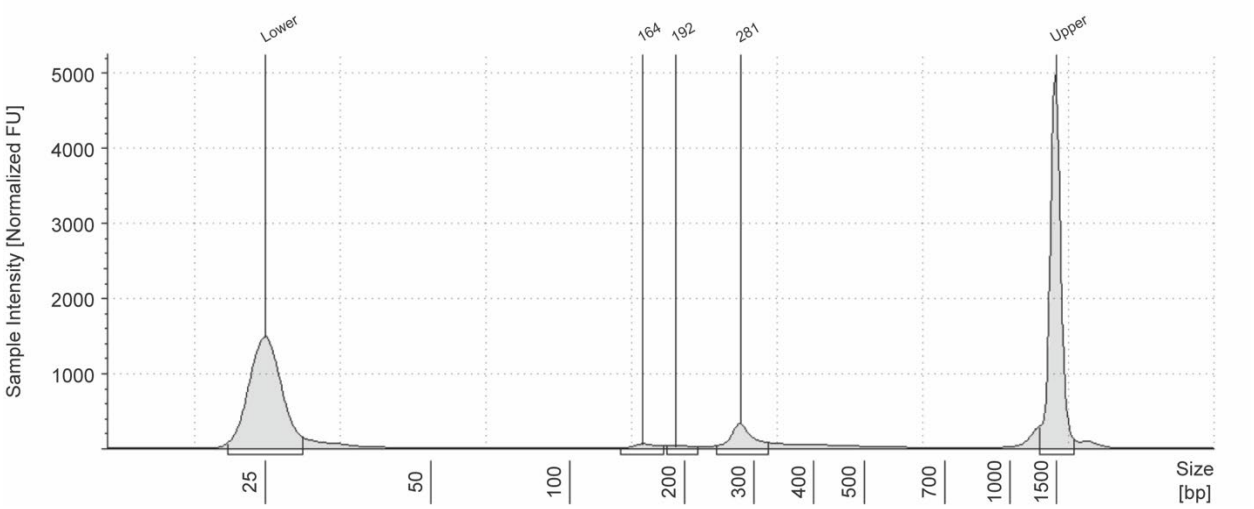

Figure S2. Validation of template switching and cDNA amplification.

SreA aptamer domain cDNA from Switch-MaP-RT reactions was PCR amplified with i5/i7 primers. The presented lower and upper markers are included in the D1000 high sensitivity TapeScreen as size standards. The observed 164 bp peak corresponds to the expected SreA aptamer domain RNA ligated to the pre-adenylated DNA adapter (162 nt). The 192 bp product represents the Switch-MaP cDNA product (202 nt) and the 281 bp product represents to the i5/i7 PCR product (272 bp).

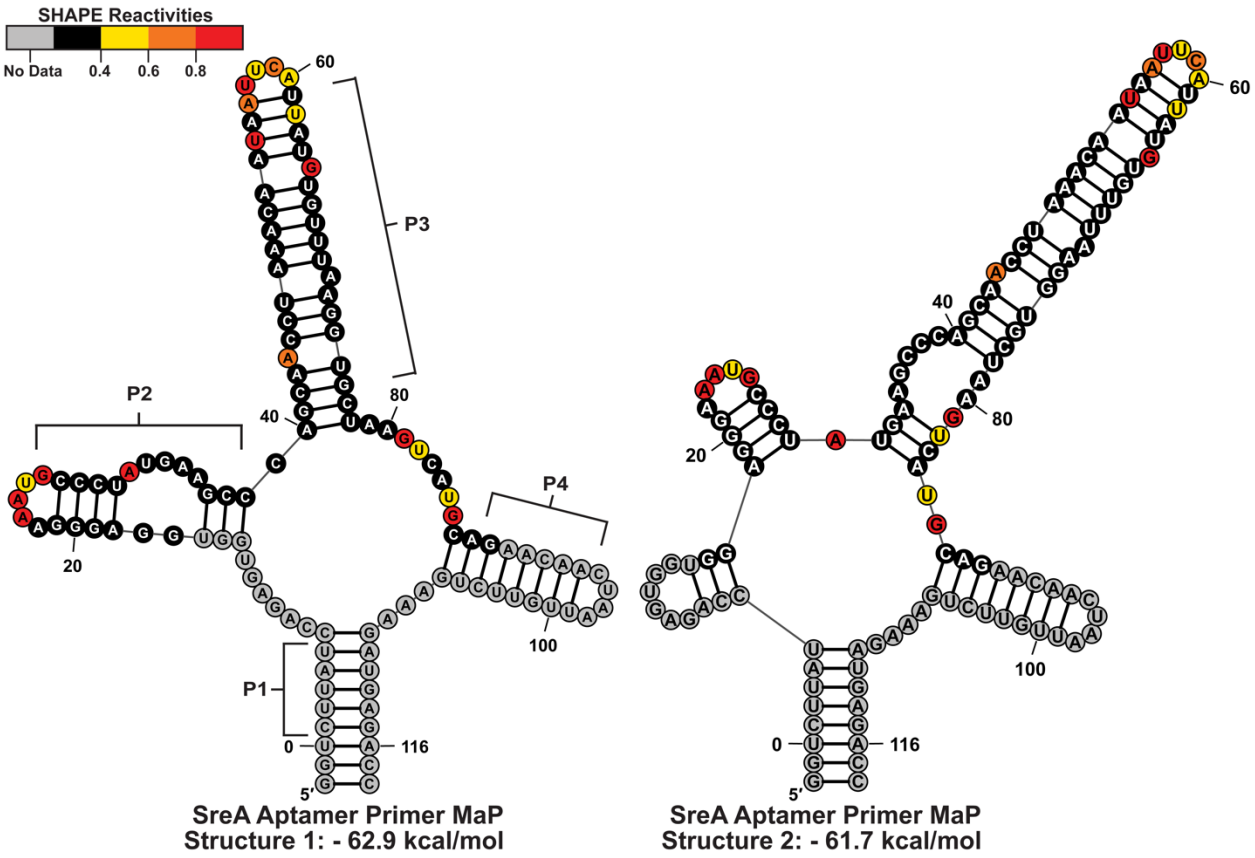

Figure S3. Replicate of SreA aptamer of Primer-Map.

Structure models generated from a replicate of the SreA aptamer primer-MaP experiments in Figure 2. Model structure 1 **(left)** presents the expected aptamer domain fold with P1- 4 helices. Model structure 2 **(right)** possesses the extended P3 helix and bifurcated P2 helix.

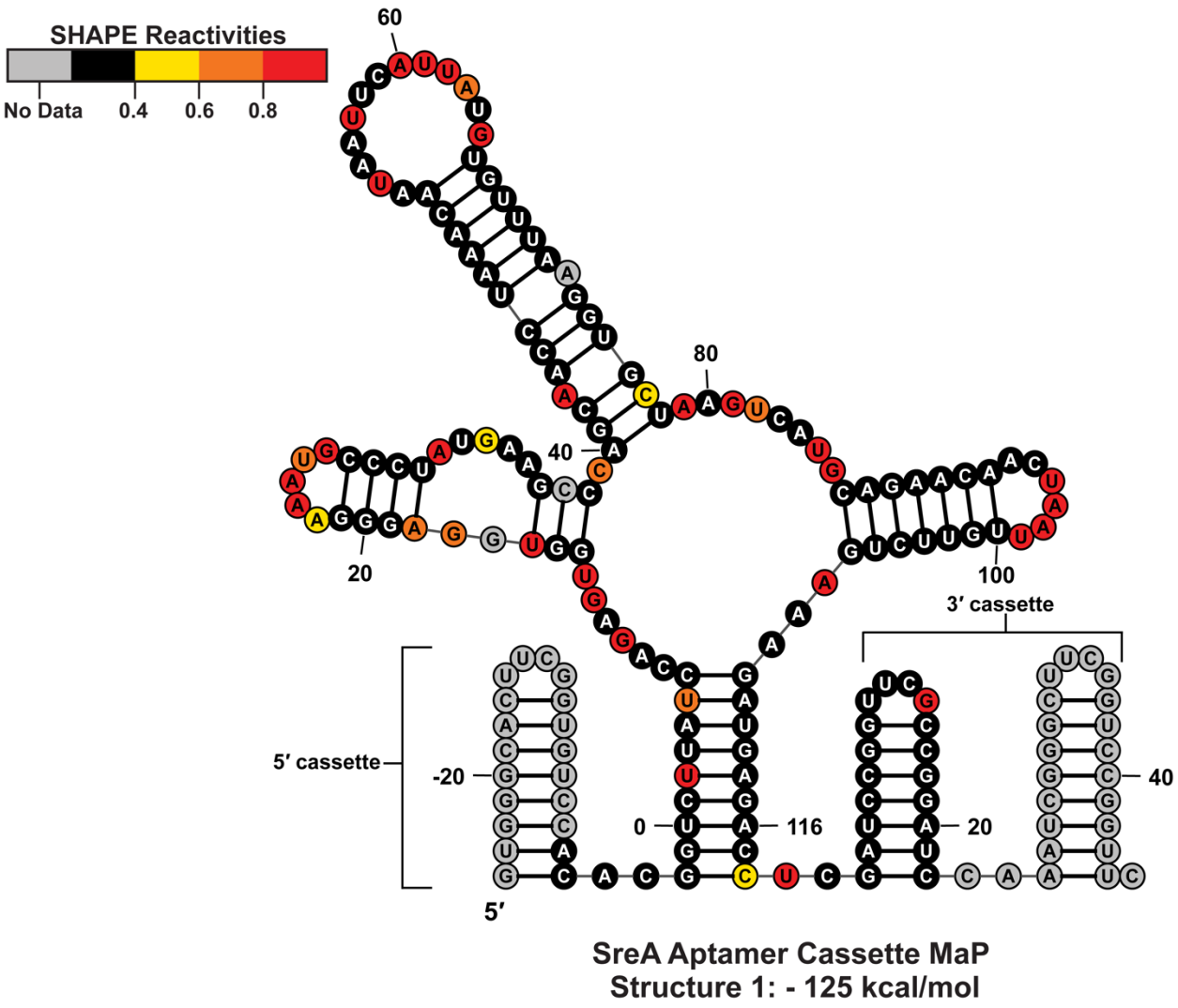

Figure S4. Structural model of SreA aptamer of cassette-MaP replicate experiment.

The structure model generated from a replicate of SreA aptamer cassette-MaP experiment in Figure 4. Small reactivity differences, nucleotides 53-65, produce a more open apical loop conformation in P3.

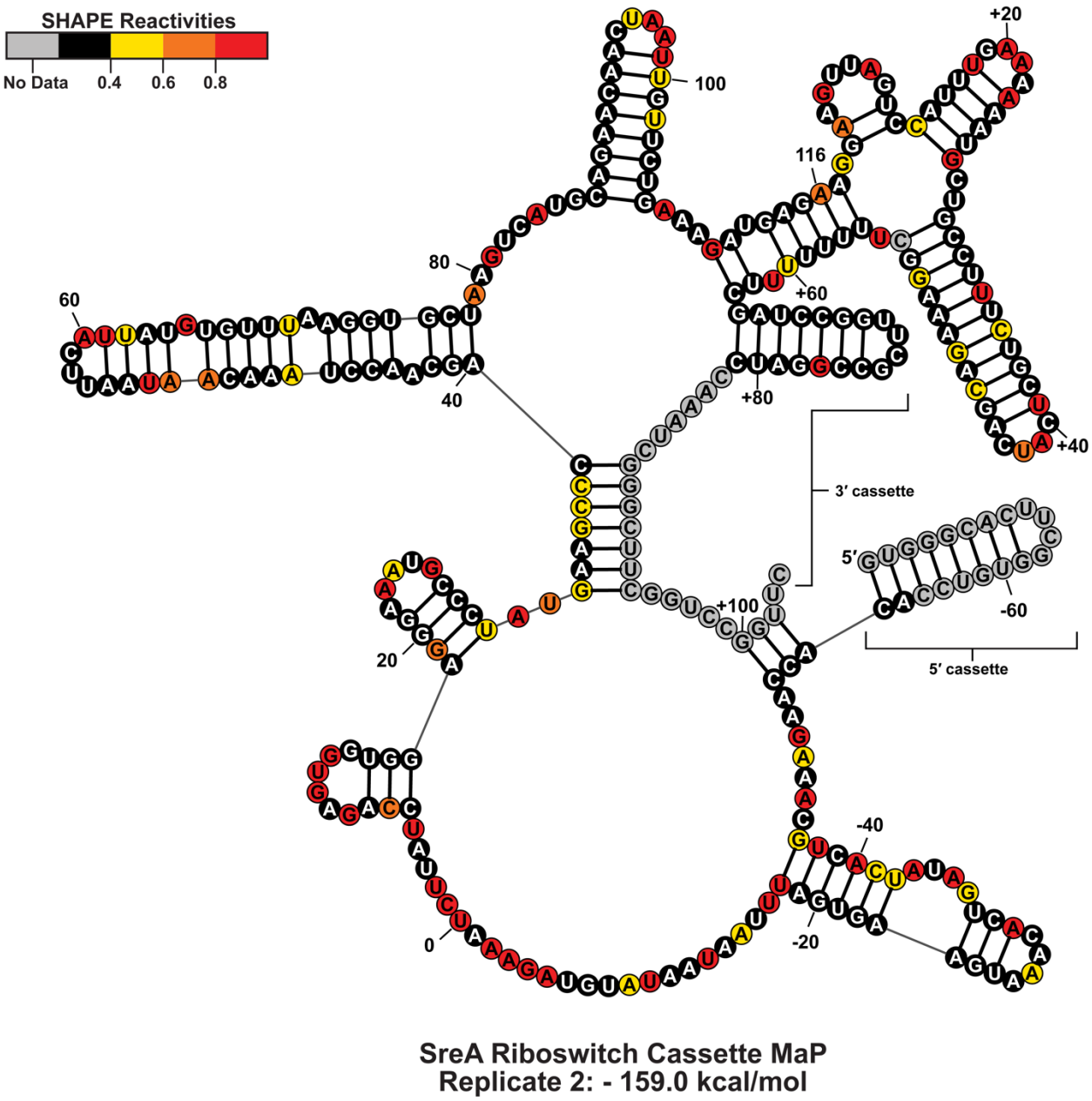

Figure S5. Structural model of SreA Riboswitch cassette-MaP replicate experiment.

The structure model generated from a replicate of SreA Riboswitch cassette-MaP experiment in Figure 4. Slight reactivity differences alter the arrangement of structural motifs around the major junction in the model (nts 87 to +81), however interference of the 3′ structure cassette is retained in this replicate/

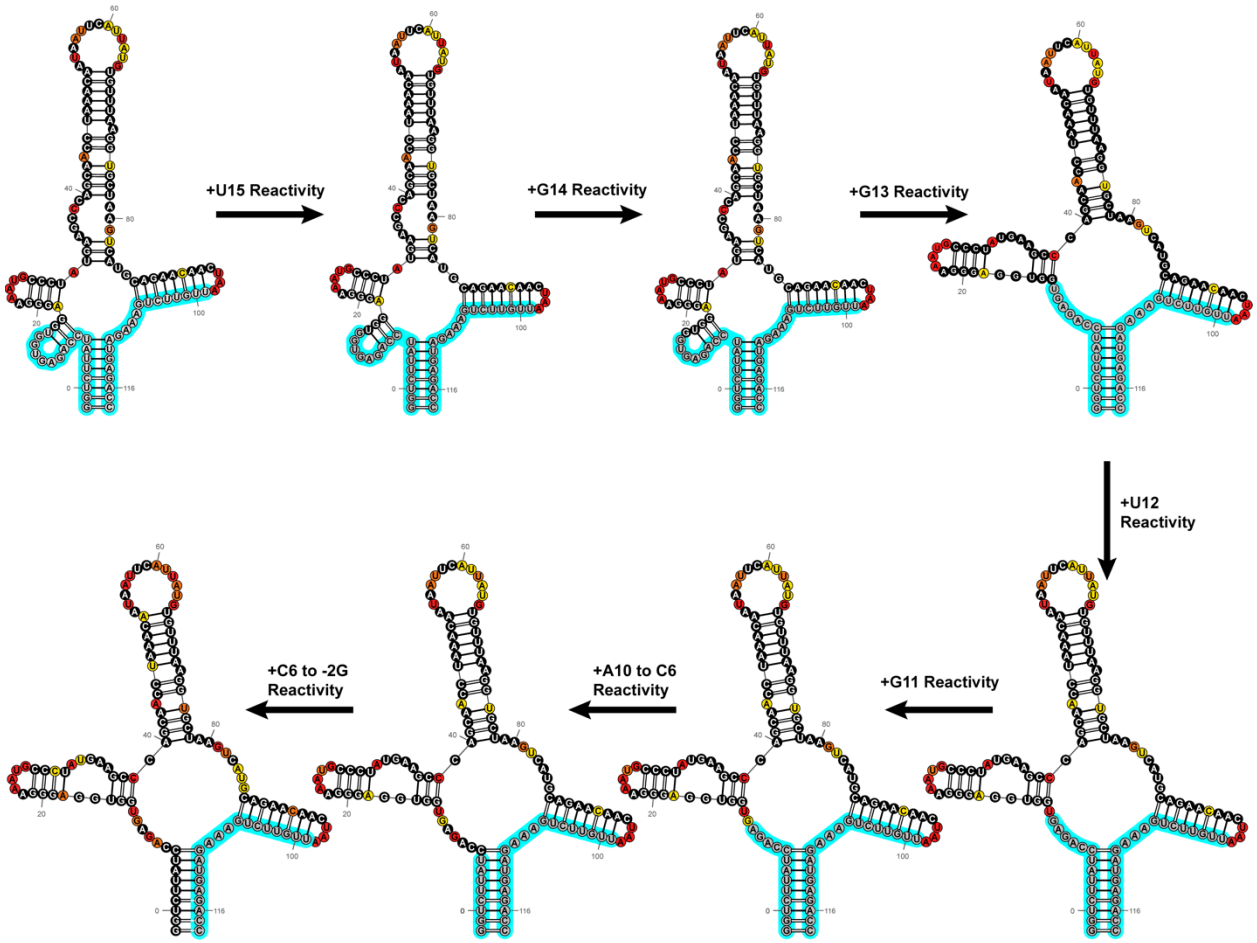

Figure S6. Full 5′ primer mask simulations of SreA aptamer Switch-MaP model.

Nucleotide level resolution of the simulated mask experiment for the SreA aptamer Switch-MaP data from Figure 6. The key determinant to produce the expected 4-way junction fold is the reactivity of G13. Further reduction in the simulated mask produces equivalent structures to the full unmasked.