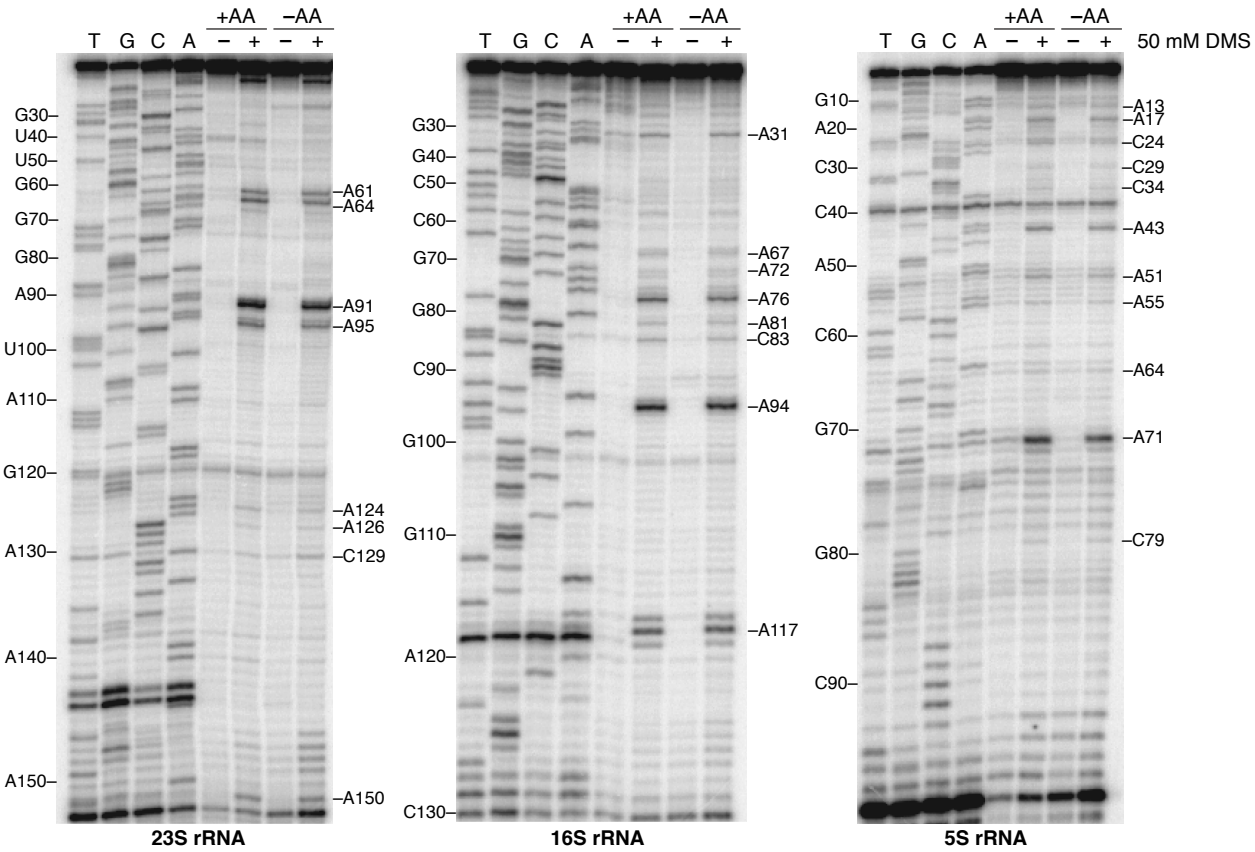
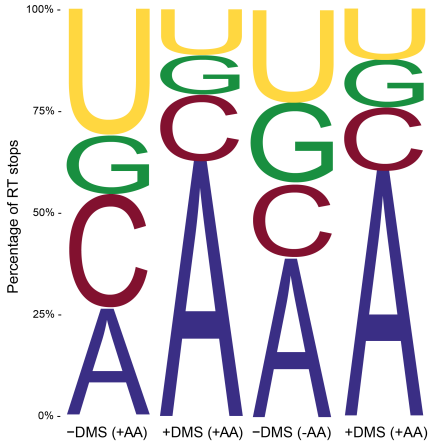
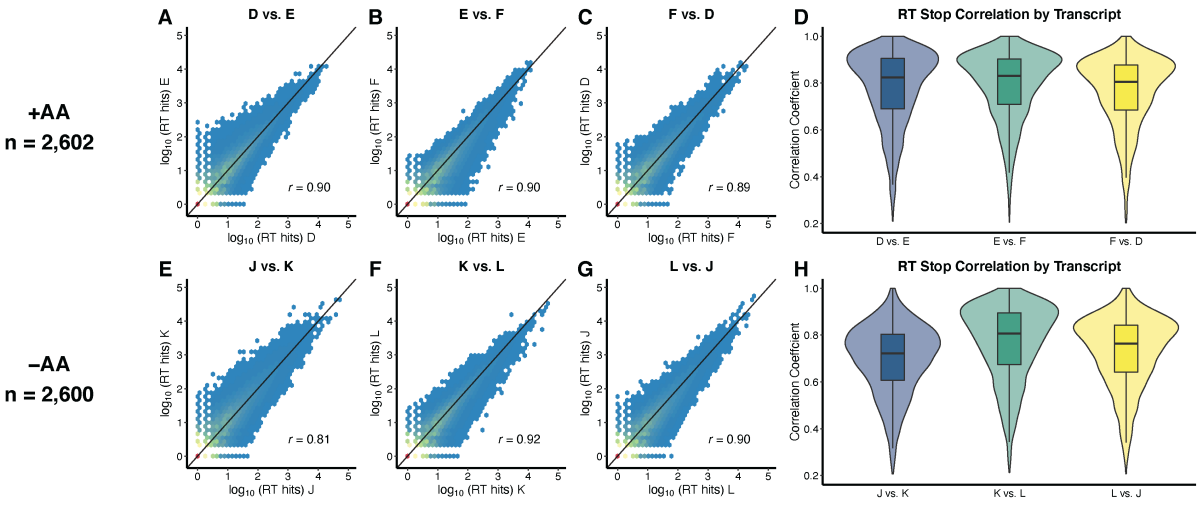


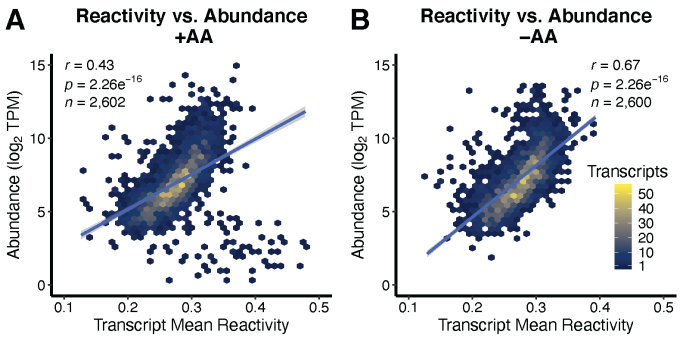
Supplemental Fig. S1. Structure-seq2 in *B. subtilis* was performed with a single-hit kinetics regime. A 5 min treatment with 50 mM DMS produced DMS reactivity within the single-hit kinetics regime, allowing proper analysis of DMS-modified transcripts. The sites of modification within 23S, 16S, and 5S rRNA correspond to A and C residues, as expected given the chemical properties of DMS. Every fifth nucleotide is numbered, and primers were selected to be ~200 nucleotides from the 5′ end of the given transcript to allow visualization of the full-length band.



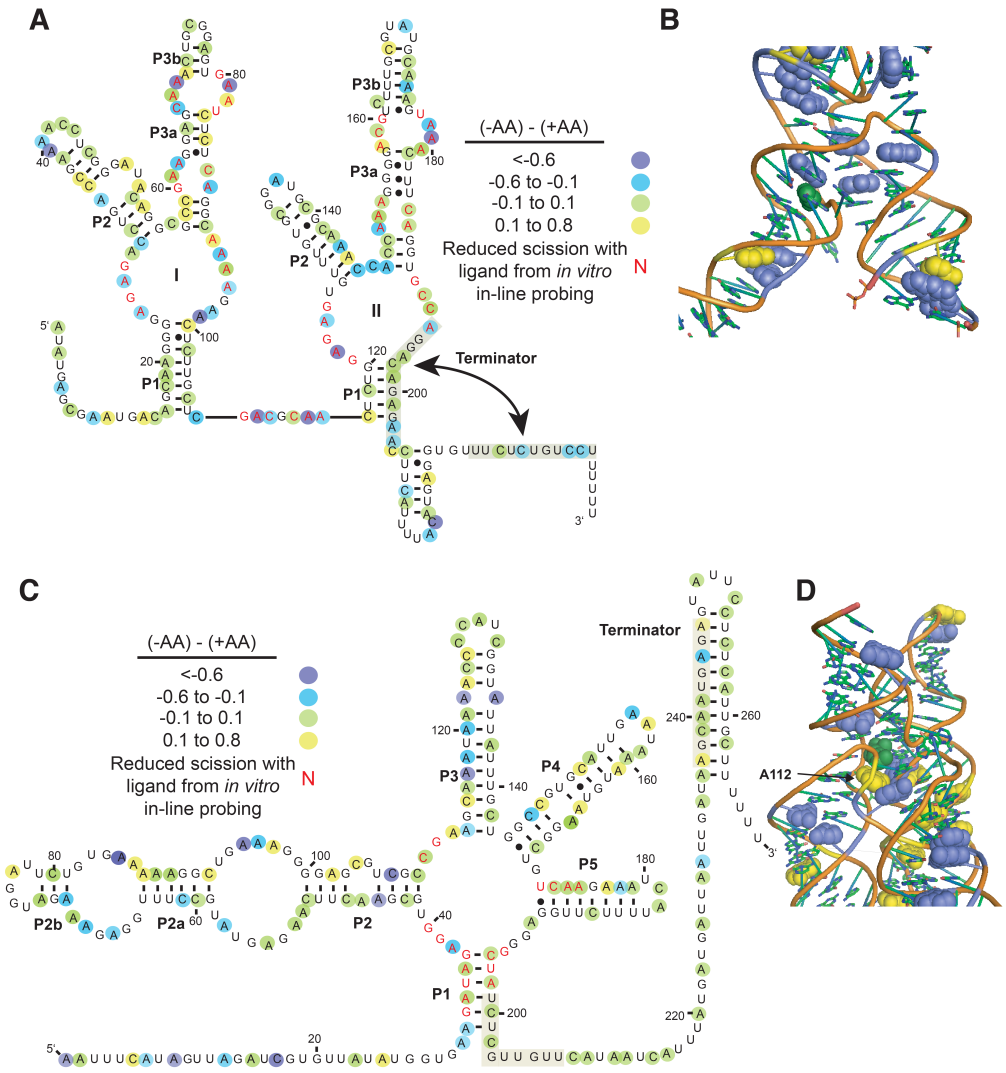
Supplemental Fig. S2. DMS treatment modifies A and C residues. DMS is specific for A and C nucleobases as demonstrated by the percentage of reverse transcriptase stops in the combined data of the specified libraries. The raw data for this figure is contained within Supplemental Table W.4.



Supplemental Fig. S3. Excellent correlation of Structure-seq2 libraries from independent biological replicates. Reverse transcriptase stops from the three replicates of +DMS libraries within each condition (+AA, –AA) were correlated against each other in both a structurome-wide (panels *A*-*C* and *E*-*G*) and per transcript (panels *D, H*) manner, showing high repeatability of DMS modification. All numbers and tests are provided in Supplemental Table T.1



Supplemental Fig. S4. Correlation of reactivity and coverage for each structurome. The reactivity and coverage for each structurome (+AA, -AA) was calculated separately (*A, B*). This shows that in each structurome, more reactive transcripts are typically more abundant. This trend is slightly stronger in -AA conditions (r=0.43 vs. r=0.67, Fisher Z-Test p = <0.0001), pointing to more overall modulation of structure in stress conditions. Additional details are provided in Supplemental Table T.4.



Supplemental Fig. S5. Structure-seq2 data align well with known riboswitches and reveal functionally significant regions. (*A*) Change in DMS reactivity displayed on the secondary structure of the *gcvT* glycine riboswitch from *B. subtilis.* Blue shows nucleotides that had decreased reactivity without amino acids (darker shade shows more extensive change of DMS reactivity), green shows nucleotides that did not change in reactivity, and yellow shows nucleotides that had increased reactivity without amino acids. Nucleotides that had decreased reactivity with bound glycine in previous in vitro studies are in red font (Mandal et al., 2004). (*B*) Change in DMS reactivity displayed on the crystal structure of Domain II of the *VC1422* glycine riboswitch from *Vibrio cholerae* (Huang et al., 2010). Blue and yellow have the same meaning as in (*A*), and glycine is displayed in forest green. (*C*) Change in DMS reactivity displayed on the secondary structures of the *lysC* lysine riboswitch from *B. subtilis.* Blue, yellow and green have the same meaning as in (*A*). Nucleotides that had decreased reactivity with bound lysine in previous in vitro studies are in red font (Sudarsan et al., 2003). (*D*) Change in DMS reactivity displayed on the crystal structure of the *ads* lysine riboswitch from *Thermatoga maritima*. Blue and yellow are the same as in (*C*), and lysine is

displayed in forest green. The glycine riboswitch of *gcvT*, which contains two glycine binding pockets, directly binds glycine and thereby prevents the formation of a transcription terminator (Mandal et al., 2004). The lysine riboswitch from *lysC,* binds lysine and promotes transcription termination (Grundy et al., 2003). (*A, C*) Both of these riboswitches showed similarities between Structure-seq2 and in vitro probing data (Mandal et al., 2004; Sudarsan et al., 2003). (*D*) In the lysine riboswitch, nucleotide A112 shown to assist in lysine recognition within the crystal structure of the *ads* lysine riboswitch from *T. maritima* (Garst et al., 2008), was exposed (i.e. DMS reactive) in the absence of amino acids.

SUPPLEMENTAL REFERENCES

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