Supplemental Information

Glyoxals as *In Vivo* RNA Structural Probes of Guanine Base Pairing

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Supplemental Figure S1. Modification of rice 5.8S rRNA in vitro by glyoxal. All nucleotides within the examined range (G53-G124) are shown. A red dot indicates glyoxalated cytidines at buffer pH 8 and 5 min reaction time, whereas two red dots indicate glyoxalated guanosines. While uridines are shown, glyoxal cannot react with uridines (see Fig. 2 in main text). Therefore, no uridine residues are given red dots. Normalized intensities for each nucleotide are grouped by reaction time, with 5 min reaction times given a lighter color and 15 min reaction times given a darker color. G79, G101, and G120, which are the residues that give natural reverse transcriptase stops, are colored grey.
**Supplemental Figure S2.** *In vitro* modification of guanosine residues with rice 5.8S rRNA by glyoxal. Normalized band intensities of all guanosine nucleotides within the examined range (G53-G124) are shown except for the three guanosines that give natural reverse transcriptase stops (G79, G101, and G120). The baseline value above which glyoxalation is considered significant, 1806, is shown as a red line.
Supplemental Figure S3. Modification of rice 5.8S rRNA in vitro by methylglyoxal. All nucleotides within the examined range (G53-G124) are shown. Two red dots indicate significantly glyoxalated guanosines. Uridines, while shown, cannot be glyoxalated (see Fig. 2 in main text). G79, G101, and G120, which are the residues that give natural reverse transcriptase stops, are colored grey.
Supplemental Figure S4. Modification of guanosine residues with rice 5.8S rRNA *in vitro* by methylglyoxal. Normalized band intensities of all guanosine nucleotides within the examined range (G53-G124) are shown except for the three guanosines that give natural reverse transcriptase stops (G79, G101, and G120). The baseline value above which glyoxalation is considered significant, 3268, is shown as a red line.
Supplemental Figure S5. Modification of rice 5.8S rRNA \textit{in vitro} by phenylglyoxal. All nucleotides within the examined range (G53-G124) are shown. Two red dots indicate significantly glyoxalated guanosines. Uridines, while shown, cannot be glyoxalated (see Fig. 2 in main text). G79, G101, and G120, which are the residues that give natural reverse transcriptase stops, are colored grey.
Supplemental Figure S6. Modification of guanosine residues with rice 5.8S rRNA *in vitro* by phenylglyoxal. Normalized band intensities of all guanosine nucleotides within the examined range (G53-G124) are shown except for the three guanosines that give natural reverse transcriptase stops (G79, G101, and G120). The baseline value above which glyoxalation is considered significant, 3910, is shown as a red line.
Supplemental Figure S7. Modification of rice 5.8S rRNA in vitro by dimethylglyoxal. All nucleotides within the examined range (G53-G124) are shown. Two red dots indicate significantly glyoxalated guanosines. Uridines, while shown, cannot be glyoxalated (see Fig. 2 in main text). G79, G101, and G120, which are the residues that give natural reverse transcriptase stops, are colored grey.
Supplemental Figure S8. Modification of guanosine residues with rice 5.8S rRNA in vitro by dimethylglyoxal. Normalized band intensities of all guanosine nucleotides within the examined range (G53-G124) are shown except for the three guanosines that give natural reverse transcriptase stops (G79, G101, and G120). The baseline value above which glyoxalation is considered significant, 3605, is shown as a red line.
Supplemental Figure S9. Glyoxal modification of *B. subtilis* 5S *in vivo* analyzed by denaturing PAGE after reverse transcription to generate cDNAs. Lane 1 is the control (0 mM glyoxal) while lanes 2-6 show reactions from 5 mM to 100 mM glyoxal. Lanes 7-10 show dideoxy sequencing of U, G, C, and A.