Capillary Electropherograms of Mg^{2+} Titration in the Presence of 100μM S-adenosylmethionine

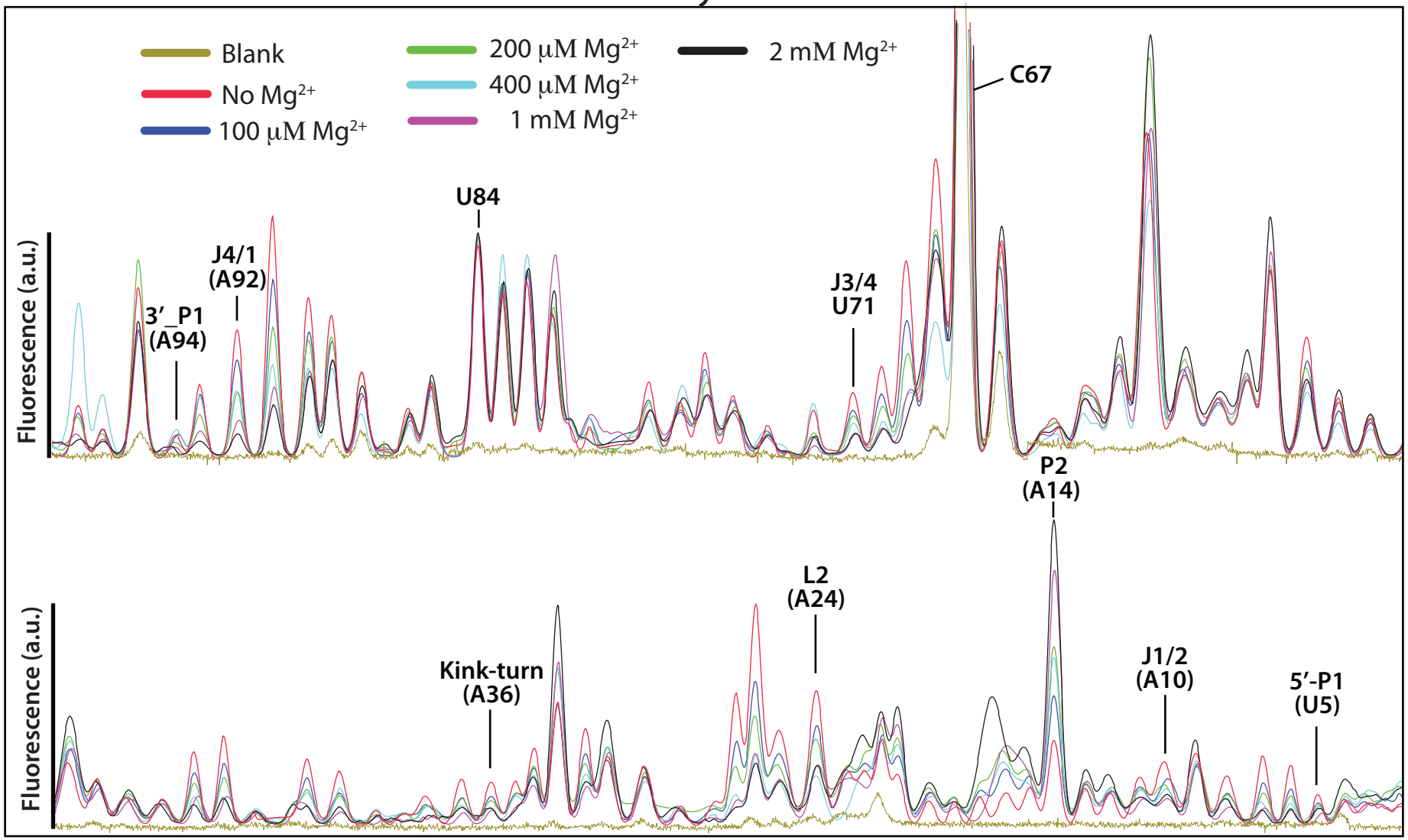

Figure S1. Example Mg^{2+} titration SHAPE probing data set of the SAM I aptamer domain at 100 μM SAM. After reaction with 1M7, primer extension of RNA samples was carried out with an Fluorescently labeled primer using AMV-reverse transcriptase(AMV-RT produces a strong extension stop at the base of P3 at C67). Analysis was performed on an Applied Biosystems 3100 Avant capillary electrophoresis instrument. Peak integration utilized multiple gaussians fits to determine peak areas. The Blank trace is a raw fluorescences electrophergram of primer extension performed on RNA not reacted with 1M7 without integration. Positions of nucleotides used in the analysis of collapse are indicated along with the region they lie within. Nucleotide U84 was used for normalization of data from each experiment.

Capillary Electropherograms of Mg^{2+} Titration without S-adenosylmethionine.

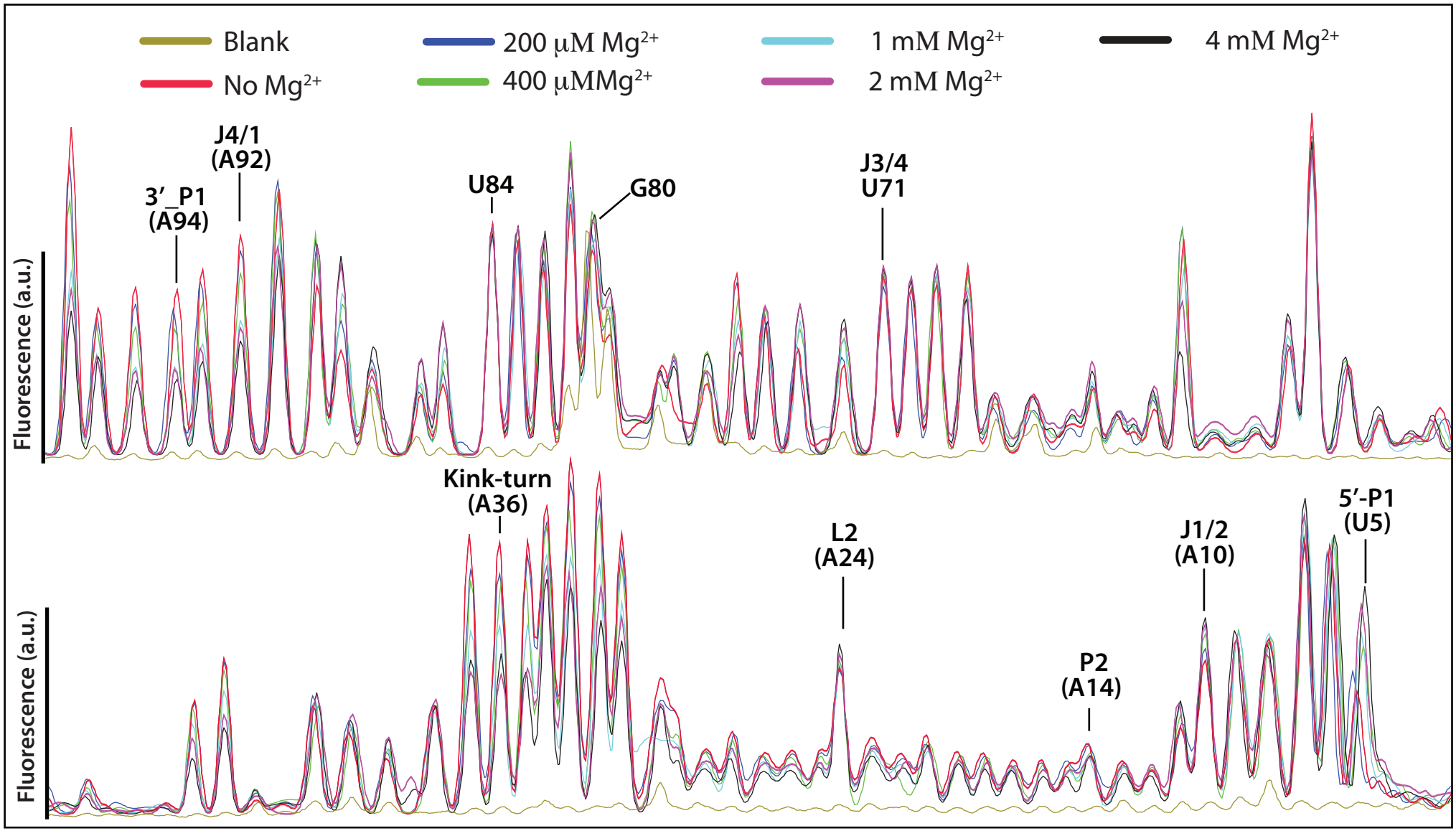

Figure S2. Example Mg^{2+} titration SHAPE probing data set of the SAM I aptamer domain without SAM. For ease of visualization not all Mg^{2+} concentrations used in the titration are included. After reaction with 1M7, primer extension of RNA samples was carried out with an fluorescently labeled primer using Superscript MMLV-reverse transcriptase(MMLV-RT produces anextension stops around G80) . Analysis was performed on an Applied Biosystems 3100 Avant capillary electrophoresis instrument. Peak integration utilized multiple gaussians fits to determine areas for peaks. The Blank trace is a raw fluorescences electrophergram of primer extension performed on RNA not reacted with1-methyl-7-nitroso-isatoic anhydride without integration. Positions of nucleotides used in the analysis of collapse are indicated along with the region they lie within. Nucleotide U84 was used for normalization of data.

SHAPE probing Capillary Electropherograms of S-adenosylmethionine Titration at 1mM Mg^{2+}.

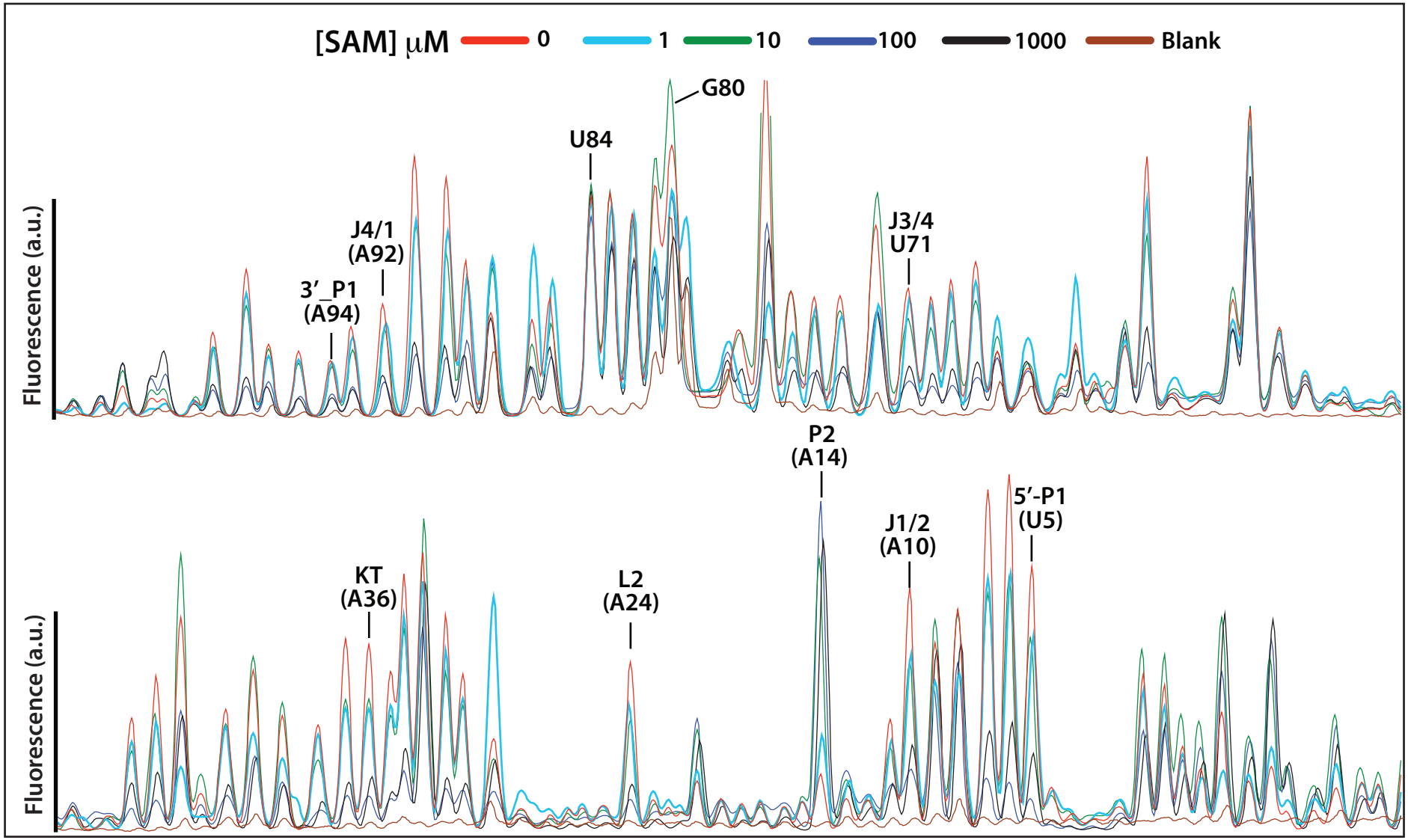

Figure S3. Example SAM titration SHAPE probing data set of the SAM I aptamer domain at 1mM Mg^{2+}. After reaction with 1M7, primer extension of RNA samples was carried out with an Fluorescently labeled primer using Superscript MMLV-reverse transcriptase(MMLV-RT produces extension stops around G80). Analysis was performed on an Applied Biosystems 3100 Avant capillary electrophoresis instrument. Peak integration utilized multiple gaussians fits to determine peak areas. The Blank trace is a primer extension of unmodified RNA showing a raw fluorescences electrophergram without integration. Positions of nucleotides used in the analysis of collapse are indicated along with the region they lie within. Nucleotide U84 was used for normalization of data.

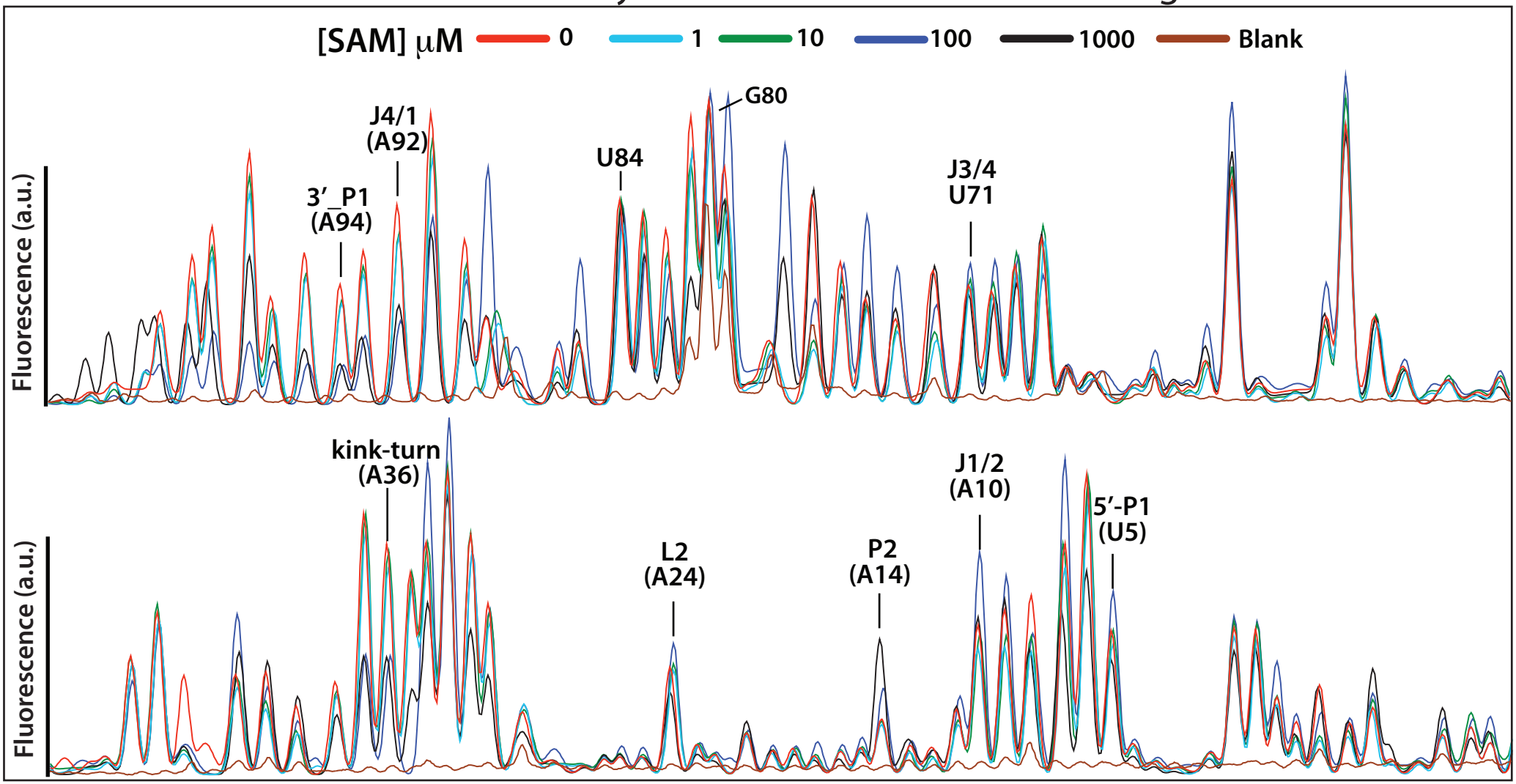

Figure S4. Example SAM titration SHAPE probing data set of the SAM I aptamer domain without Mg^{2+}. After reaction with 1M7, primer extension of RNA samples was carried out with a fluorescently labeled primer using Superscript MMLV-reverse transcriptase (MMLV-RT produces extension stops around G80). Analysis was performed on an Applied Biosystems 3100 Avant capillary electrophoresis instrument. Peak integration utilized multiple gaussians fits to determine peak areas. The Blank trace is a raw fluorescences electrophergram without integration on unmodified RNA. Positions of nucleotides used in the analysis of collapse are indicated along with the region they lie within. Nucleotide U84 was used for normalization of data.

Conformational switching selection scheme for phosphorothioate interference.

1. Aptamer RNA is randomly incorporated with a phosphorthioate nucleotide, 3′- fluorescently labeled and purified.

2. Aptamer RNA is folded with various concentrations of SAM or in a buffer containing $MnCl_2$.

3. RNA is mixed with RNase-H and the RNA-DNA chimeric analog of the anti-terminator sequence and allowed to equilibrate for 1 hour at 37° C.

4. Samples are then denatured and phosphorothioate linkages are cleaved with molecular iodine.

5. Analysis is performed by capillary electrophoresis. All aptamer RNA cleaved by RNase H migrates as a sinlge peak early in the trace leading to a decrease in the intensity of peaks at PSO_3 interfence sites.

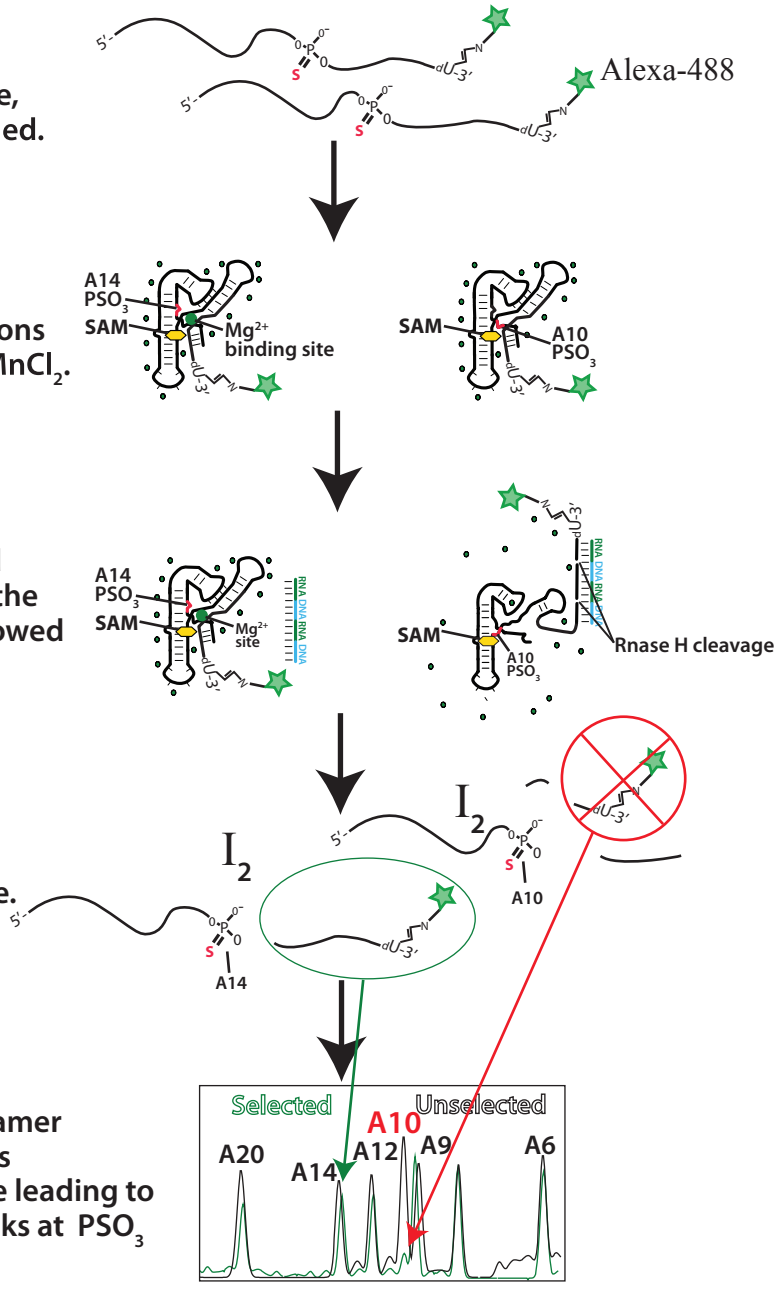

Figure S5. Schematic of the conformational switching phosphorothioate selection method.

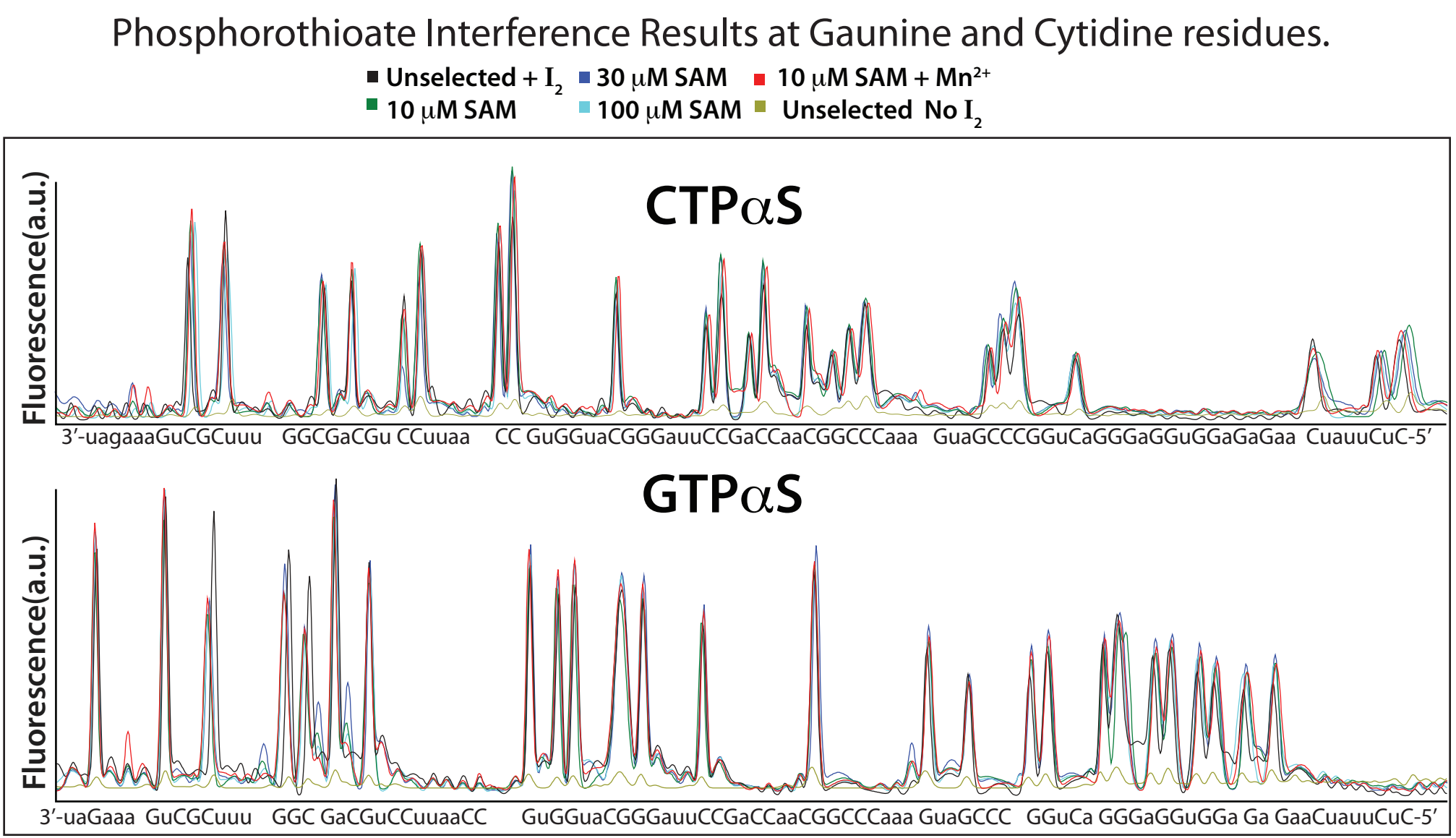

Figure S6. Capillary electrophoresis traces from phosphorothioate interference assay using riboswitch secondary structure switching as selection. Phosphorothioate containing nucleotides are randomly incorporated at C and G positions. The resulting aptamer RNAs were then selected for the ability to maintain the aptamer domain fold when equilibrated with an expression platform oligmer. The RNAs are labeled on their 3′ terminus with an Alexa-488 flurophor prior to selection. Destabilized domains are removed by association with expression platform analog oligomer(see article materials and methods). After selection the PSO_3 containing linkages are cleaved with iodine and the RNA analyzed by capillary electrophoresis(ABI 3100). Peaks are integrated by fitting multiple gaussians to raw fluorescence traces. Plotted above are the gaussian fits of the data. The results show no interference arising from non-bridging sulfur atoms incorporated at C or G positions for any of the SAM concentrations used. The identity of the overlayed traces are indicated above. They include various concentrations of SAM as well as a Mn^{2+} rescue experiment.