Detailed procedure used for chemical interference experiments.

Modification reactions of the simplified chemical sequencing procedure were used in TE buffer containing 80 mM KCl so that the aptamers had adopted their folded structure. 4 Eppendorf tubes were prepared as follows: To 1µl radiolabeled aptamer (about 5 10^5 cpm) was added 8 µl TE +0.1M KCL. The solution was heated 2 min at 98°C, cooled in ice 2 min then incubated 2 hours at 25°C and stored at 4°C until the modification reaction was initiated the following day. For modification of G residues, 1 µldimethylsulfate 2% solution freshly made in water was added, and the resulting solution was incubated for 10 min at room temperature. For depurination (G+A) 1 µl piperidinium formate pH2 was added and the resulting solution was incubated 5 min at 65°C. For modification of T residues, 1 µl of $KMnO_4$ (0.5 mg/ml, freshly made), was added and the resulting solution was incubated 3 min at room temperature followed by addition of 1 µl allyl alcool. In each case, reaction was stopped by addition of 200 µl Stop buffer pH 7.5 (Sodium Acetate 0.3M, EDTA 1 mM) followed by addition of 10 µl tRNA (5mg/ml) and 600 µl ethanol. As a control 1 µl H_2O was added to the 4^{th} tube which was incubated 10 min at 23°C before adding stop buffer, tRNA and ethanol. After 1 hour at –80°C, the tubes were centrifuged 30 min at 14000g at 4°C, pellets were dried, redissolved in 200µl Sodium acetate / 0.3M EDTA 1 mM pH7 and precipitated again by addition of 600 µl ethanol. After 1 hour at –80°C, tubes were centrifuged, the pellets were dried and redissolved in 4 µl buffer A 2X containing 40 µg/ml denatured salmon sperm DNA. After 10 min at room temperature, 4 µl of RNase H in dilution buffer was added. The dilution was choosen to give about 50% retardation in EMSA. After 10 min incubation at room temperature samples were loaded on a native gel in 0.5 X TB buffer and run as described for EMSA experiments. At the end of the run, the gel was autoradiographied and radioactive bands corresponding to bound (retarded) and free aptamers were located, excised, cut in small pieces and eluted overnight with gentle agitation in 500 µl of a solution containing ammonium acetate 0.5M, EDTA 1mM and 1/6 volume of phenol. The eluates were recovered and extracted with one volume of phenol/chloroforme/isoamyl alcohol (50:49:1), After centrifugation the upper aqueous phases were saved and nucleic acids precipitated by addition of 1 ml ethanol. After 1 hour at –80°C the tubes were centrifuged 30 min at 14000 g at 4°C and the pellets were dried in a Speed-Vac. Pellets were redissolved in 10 µl H_2O. Then, 100 µl of pyrrolidine 1.1M (freshly made) was added and tubes were incubated 15 min at 90°C for cleavage at modified sites. Samples were then reduced to dryness in a Speed-vac, resuspended in 20 µl H2O and redried two times. After final drying samples were dissolved in 10 µl and Cerenkôv counted. Identical amounts of radioactivity (usually about 2 10^4 cpm) were mixed with formamide containing tracking dyes, heated 1 min at 98°C and loaded onto a 20% acrylamide 7M urea sequencing gel. For each modification reaction, bound (B) and free (F) aptamers were loaded side by side. After electrophoresis, the gel was wrapped in Saran and autoradiographied.