Supporting Information

Refolding through a linear transition state enables fast temperature adaptation of a translational riboswitch

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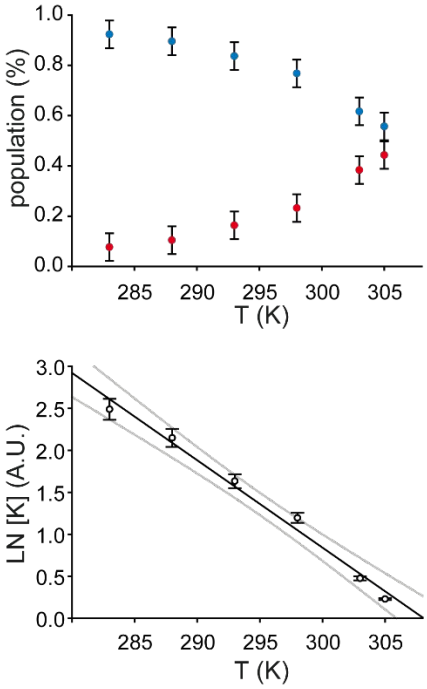

Figure S1: Van't Hoff Analysis of the conformational equilibrium between apoA and apoB.

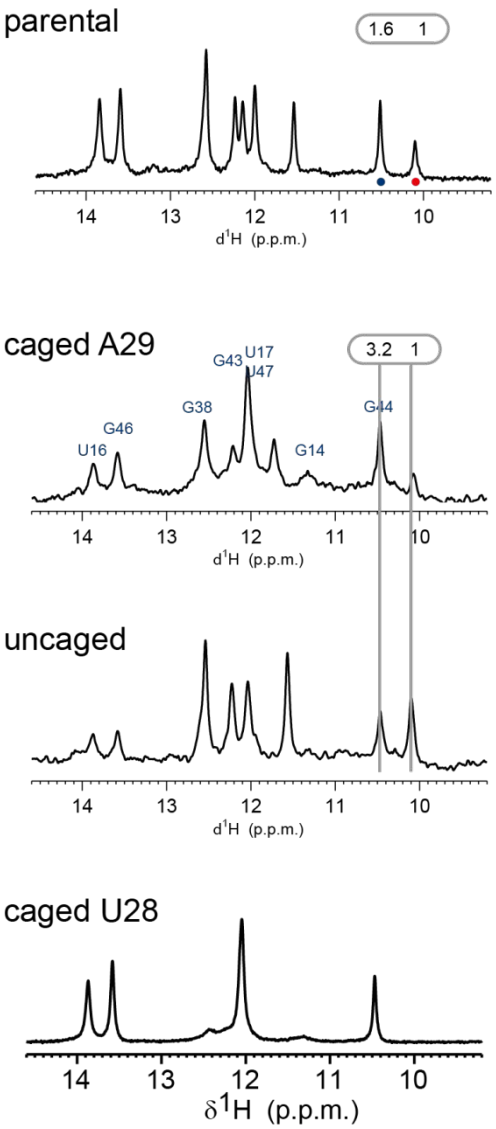

Figure S2: Comparison of imino-proton region of 1D ^{1}H spectra (800MHz, jump return echo for water suppression, 512 scans) of unmodified 35nt long TRM (parental RNA) with the same sequence that contains a NPE-caged A at position 29; although after deprotection (1s laser pulse at 4.5W, 355nm) equilibrium could be restored, incorporation does not yield exclusive stabilization of a single conformation as compared to the TRM caged at position U28 that leads to a exclusive stabilization of conformation apoB.

Introduction of a NPE-caged adenosine at position A29 was initially assumed to destabilize apoA-conformation and consequently shift the equilibrium to an exclusive population of conformation apoB. As evident from the above described experiments, however, the nucleotide at position 29 is not involved in a stable base pair with U41. Consequently, the destabilization effect of the introduced photo-caged adenosine at this position was smaller as the energy difference between the two states and did not result in the preferential population of the apoB conformational state

Experimental Procedures

NMR spectroscopy:
All NMR spectroscopic experiments were carried out on a BrukerAVII 800 MHz spectrometer, equipped with a 5-mm TXI- HCN cryogenic probe with z-axis gradients; RNA assignment experiments[1], laser assisted in situ deprotection experiments[2] and exchange experiments[3,4] were carried out as described in the respective literature.

Biophysical Analysis:

Van't Hoff Analysis
For the determination of the equilibrium constant K the ratio between the populations of conformation apoA and conformation apoB as derived from the relative intensities of the respective imino-proton signals was build. The temperature dependent data were fitted against $\Delta G = -R*T*LN[K]$.

ZZ-Exchange
The ZZ-Exchange curves were analyzed according to the formulae derived in Farrow et al. 1994[4]

Arrhenius/Eyring Analysis

The Gibb energies of activation were extracted from the temperature dependence of exchange rates determined by the ZZ-exchange experiment. It is derived from fitting the Eyring equation
$kex = \frac{k_b T}{h} e^{-\frac{\Delta G}{RT}}$ to the experimental data.

Biochemical Synthesis of isotope labelled RNA:
A uniformly ^{13}C,^{15}N-labelled bistable 35mer RNA derived from the adenine-dependent riboswitch of *Vibrio vulnificus* (5´-pppGCUUCAUAUAAUCCUAAUGAUAUGGUUUGGGAGUU-3´) was prepared by *in vitro* transcription from a *SmaI* linearized DNA template (modified pUC57-plasmid). 100 mM Tris-glutamic acid (pH 8.1), 2 mM spermidine, 45 mM $Mg(OAc)_2$, 4 mM ^{13}C,^{15}N-labelled rNTPs and 300 ng/µL DNA template were incubated for 30 min at 37°C before 20 mM DTT and 70 µg/mL T7-polymerase were added. Incubation was continued for 4 hours with addition of 1 unit/mL pyrophosphatase after 2 hours. The RNA product was purified by anion exchange chromatography using a DEAE sepharose resin with an elution gradient of 0.6 to 3 M NaOAc followed by a reversed phase HPLC. After $LiClO_4$ precipitation and desalting the RNA was folded by heating up to 90°C and then rapidly diluted to 4°C. The final sample concentration was 200 µM RNA in 25 mM potassium phosphate, 50 mM KCl at pH 6.5 in 90% H_2O and 10% D_2O.

RNA Solid-phase synthesis:
The NPE-photocaged phosphoramidites were synthesized according to the literature.[5] The solid-phase syntheses of the RNAs were performed either by Axolabs GmbH (Kulmbach, Germany) or on an Expedite synthesizer from Perseptive Biosystems using 2'-TBDMS RNA SynBaseTM 1000 Å CPG columns and 2'-TBDMS RNA phosphoramidites from SAFC Proligo. The oligonucleotides were synthesized in DMTr-off mode using modified synthesis protocols with 12 minutes coupling time for standard 2'-TBDMS amidites and 15 minutes coupling time for caged amidites. ETT was used as activator in a 0.35 M concentration. Phosphoramidites were used at 0.1 M concentration.
When U-NPE was incorporated G-*i*PrPac-phosphoramidite or fast deprotection amidites, with *tert*-phenoxyacetyl (tac) as exocyclic protection group were used. Fast deprotection Cap A solution, containing 5% *tert*-butylphenoxyacetyl acetic anhydride (TAC_2O) in tetrahydrofuran, was used in place of Cap A solution to ensure that the displacement of *tert*-butylphenoxyacetyl (tac) on guanine bases of the TAC-protected RNA phosphoramidites did not occur.

For RNA that contained an NPE-adenosine, deprotection was performed as follows: The CPG was treated with a mixture of NH_3/EtOH 3:1 at 40°C for 24 h, the supernatant was collected, the CPG washed with DEPC-treated water, vortexed, and the combined supernatants were dried in a vacuum concentrator. The dried RNA was dissolved in a mixture of NMP/TEA/TEA·3HF (300 µL NMP/150 µl TEA/200 µL TEA·3HF) and incubated for 90 minutes at 60 °C. 1.4 mL *n*-Butanol were added and precipitation was performed overnight at -20 °C or for a minimum of 1 h at -80 °C. Centrifugation was performed for 30 minutes at 0 °C and 12500 rpm, the supernatant was discarded, and the RNA was dissolved in DEPC-treated water for further purification via HPLC.
For RNA that incorporated an NPE-uridine and G-*i*PrPac-phosphoramidite, deprotection was performed as follows: The CPG was treated with a mixture of NH_3/EtOH 3:1 at 25°C for 4 h, the supernatant was collected, the CPG was washed with DEPC-treated water, vortexed and the combined supernatants were dried in a vacuum concentrator. The residue was dissolved in a mixture of NMP/TEA/TEA·3HF (300 µL NMP/150 µL TEA/200 µL TEA·3HF) and incubated for 12 h at 25 °C. 1.4 mL *n*-Butanol were added and precipitation was performed over night at -20 °C or for a minimum of 1 h at -80 °C. Centrifugation was performed for 30 minutes at 0 °C and 12500 rpm, the supernatant discarded and the RNA was dissolved in DEPC-treated water for further purification via HPLC.
For RNA that incorporates an NPE-uridine and tac-protection groups, deprotection was performed as follows: The CPG was treated with a mixture of NH_3/EtOH 3:1 at 25 °C for 2.5 h, the supernatant was kept, the CPG washed again with NH_3/EtOH 3:1, vortexed and the combined supernatants were dried in a vacuum concentrator. The residue was dissolved in a mixture of AMA/DMSO (0.5 mL/1.5 mL) and stored for 10 minutes at -20 °C. Then 0.75 mL TEA·3HF were added and the mixture was incubated at 45 °C for 1 h. The pH was adjusted to 5 by adding 0.1 M TEAA (pH 7).

HPLC-Purification

Separation of the RNAs by length was performed via ion exchange-HPLC:
Column: DNA-Pac PA-100, Dionex, 9 x 250 mm
Temperature: 80 °C
Buffer A: DEPC-water
Buffer B: 1 M LiCl in DEPC-water
Flow: 5 mL/min
Gradient: 0% Buffer B → 80% Buffer B in 50 minutes

Separation of the caged and uncaged products were performed via RP-HPLC:
Column: Nucleosil C12, Phenomenex, 9 x 250 mm
Buffer A: 0.1 M TEAA-Puffer, pH 7, in DEPC-water
Buffer B: acetonitrile
Flow: 6 mL/min
Gradient: 0% buffer B → 43% buffer B in 35 minutes

Purification of the tac-protected RNAs were performed on Äkta Purifier from GE Healthcare
Column: Waters XBridge BEH C18 ODB Prep Column, 130 Å, 10 x 50 mm
Temperature: 60 °C
Buffer A: 0.1M TEAA
Buffer B: 0.1M TEAA in 95% MeCN
Flow: 4 mL/min
Gradient: 2%B → 20%B in 35 minutes

Table S 1: Sequences of the synthesized oligonucleotides

	Sequence
1	5‘-GCUUCAUAUAAUCCUA^{NPE}AUGAUAUGGUUUGGGAGUU-3‘
2	5‘-GCU^{NPE}UCAUAUAAUCCUAAUGAUAUGGUUUGGGAGUU-3‘

3	5‘-GCUUCAUAUAAUCCU^{NPE}AAUGAUAUGGUUUGGGAGUU-3‘

Table S 2: ESI-MS data of synthesized oligonucleotides

Sequence	Mass calc. [Da]	Mass found [Da]
1	11312	11317
2	11312	11358 [M+2Na$^+$]
3	11312	11319

References

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