Electronic Supplementary Information

Dehydration from conserved stem regions is fundamental for ligand-dependent conformational transition of adenine-specific riboswitch

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Preparation of *add*-A riboswitch

The riboswitch was transcribed from a DNA template using ScriptMAX Thermo T7 Transcription Kit (Toyobo). The crude transcripts were purified in a denaturing polyacrylamide gel.

For preparation of DNA template, the sense primer containing T7 promoter had the sequence 5'−GCTAATACGACTCACTATAGGCTTCATATAATCCTAATGATATGGTTTGGGAGTTTCT ACCAAGAGCC−3' and the antisense primer sequence was 5'−GACTTCATAATCAAGAGTTTAAG GCTCTTGGTAGAAACTCCC−3'.

Fluorescence measurement of 2-AP

Various concentrations (0−2 μM) of *add*-A riboswitch were mixed with 200 nM 2-AP in total volume of 100 μL buffer containing 50 mM sodium MOPS (pH 7.3) and 100 mM KCl in the presence or absence of PEG200 (5-30 wt%). Reaction mixtures were incubated at 70 °C for 10 min and cooled to 25°C at 1°C min^{-1}. Aliquots of the reaction mixtures (90 μL) were transferred to wells of a 96-well, black plate (Nunc). The fluorescence signal due to 2-AP was measured at 25°C in an Infinite M200 PRO fluorescence micro plate reader (TECAN) with 300 nm excitation and 380 nm emission.

Partial digestion of RNA by RNase T1

A 5'-fluorescein-labeled *add*-A riboswitch was prepared using 5' end tag nucleic acid labeling system (Vector Laboratories) and was purified in a denaturing polyacrylamide gel. A total of 25 pmol labeled riboswitch in buffer containing 50 mM sodium MOPS (pH 7.3) and 100 mM KCl in the presence or absence of 30 wt% PEG200 and with or without 300 μM 2-AP was incubated at 70°C for 10 min and cooled to 25°C at 1°C min^{-1}. The samples were incubated with 0.1 U of RNase T1 (Roche) at 25°C for 10 min and electrophoresed on a 10 % denaturing polyacrylamide gel at 45°C. The fluorescein signal in the gel was imaged using a fluorescence image scanner (FLA-7000, Fuji Film) with 473 nm excitation and 520 nm emission.

Water activity measurement

The water activities were determined via vapor phase osmometry using a model 5520XR pressure osmometer (Wescor).

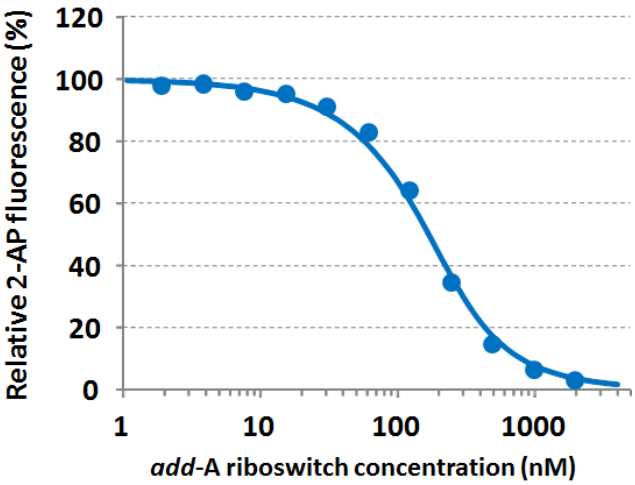

Fig. S1. Relative fluorescence intensities of 2-AP (200 nM) mixed with *add*-A riboswitch in the presence of 20 mM $MgCl_2$ in a buffer solution containing 50mM sodium MOPS (pH 7.3) and 100 mM KCl at 25°C; λex 300 nm and λem 380 nm.

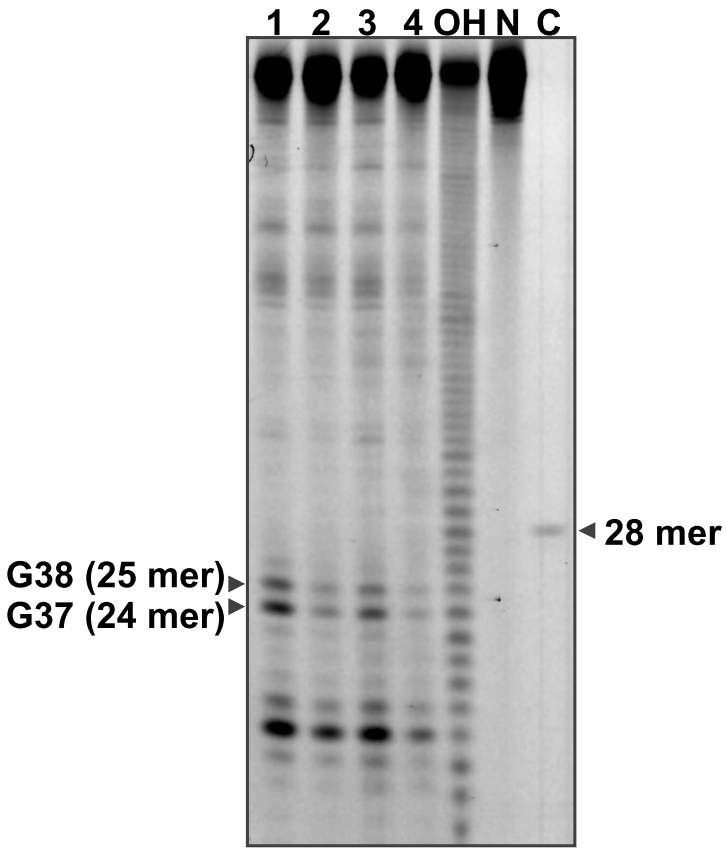

Fig. S2. Partial digestion of the *add*-A riboswitch using RNase T1. The fluorescein-labeled riboswitch was digested in buffer containing 50 mM sodium MOPS (pH 7.3), 100 mM KCl in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of 30 wt% PEG200 and with (lanes 2 and 4) or without (lanes 1 and 3) 300 μM 2-AP. Positions of monitored signals due to digestion at G37 and G38 are shown on the left. Alkaline digested riboswitch (lane OH), intact riboswitch (lane N), and 28-mer control RNA (lane C) were also evaluated.

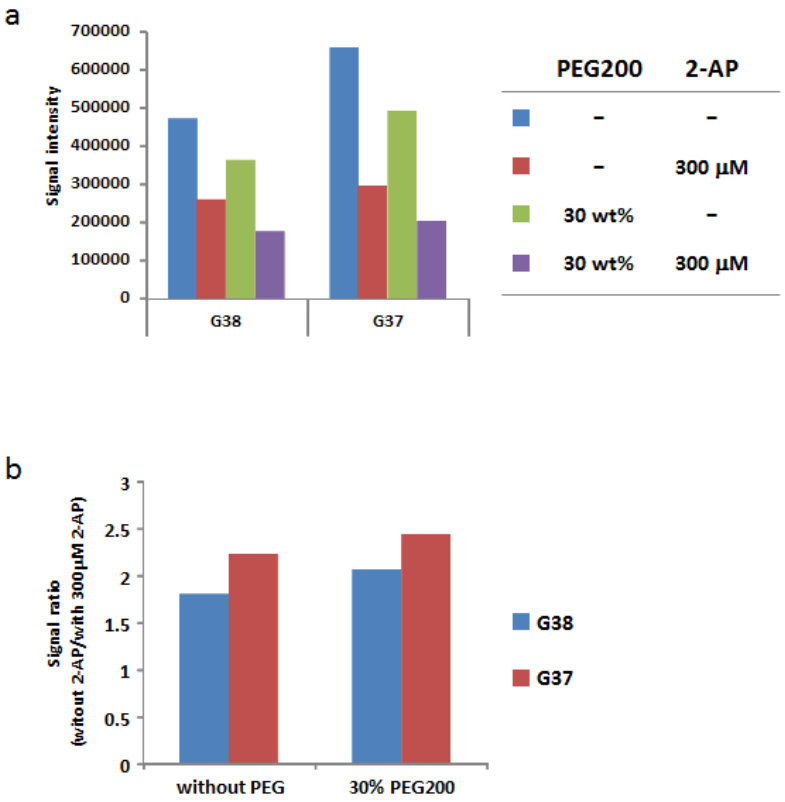

Fig. S3. (a) Signal intensities of *add*-A riboswitch digested by RNase T1 at G37 and G38 positions. (b) Ratios of signal intensities due to digestion at G37 and G38 in the presence and absence of 300 µM 2-AP with and without 30wt% PEG200.

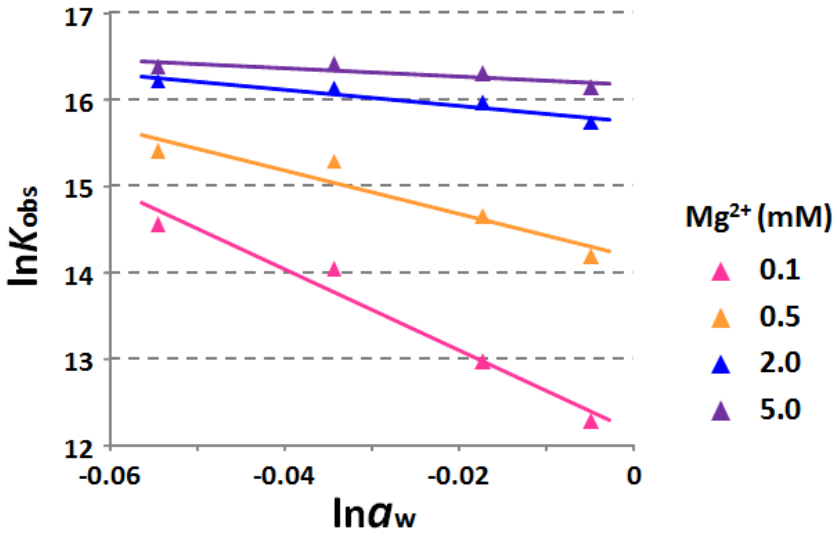

Fig. S4. Plots of ln K_{obs} *vs.* ln a_w for *add*-A riboswitch at different concentrations of $MgCl_2$ in a buffer containing 50 mM sodium MOPS (pH 7.3), 100 mM KCl, and various concentrations of PEG200.

Table S1: Water activity in the presence of different amounts of PEG200 [a]

PEG200 (wt%)	ln a_w
0	- 0.0048
5	- 0.0108
10	- 0.0172
15	- 0.0271
20	- 0.0342
25	- 0.0440
30	- 0.0544

a) All values were measured in the buffer containing 50 mM sodium MOPS (pH 7.3) and 100 mM KCl at 25°C.