Supplementary material

Direct observation of the temperature induced melting process of the *Salmonella* fourU RNA thermometer at base-pair resolution

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MATERIALS AND METHODS

Determination of mononucleotide imino proton exchange rates

In addition, imino exchange rates were measured on UTP and GTP. The composition of the UTP NMR-sample was 10 mM UTP, 15 mM $K_xH_yPO_4$ (pH 6.5), 25 mM KCl, 90% H_2O and 10% D_2O. The GTP NMR-sample contained 10 mM GTP, 15 mM $K_xH_yPO_4$ (pH 6.5), 25 mM KCl, 90% H_2O and 10% D_2O. Exchange rates were determined from the line widths of the imino signals in the ^{1}H-1D spectra which were recorded at temperatures between 1°C-11°C. The line widths $\Delta\nu$ were determined by deconvolution of the imino signal using the software Topspin 2.1 (Bruker). The exchange rates k_{ex} were calculated by $k_{ex} = \Delta\nu*\pi.-R_2-R_{2(B0)}$. R_2 represents the spin-spin relaxation rate constant of the imino resonance whereas $R_{2(B0)}$ corresponds to line broadening caused by B_0 field inhomogeneities. The contribution of R_2 relaxation and $R_{2(B0)}$ were considered to be negligibly small in comparison to the contribution of k_{ex} to the imino line width.

Theoretically derived temperature dependence of the NOE contributions

If one assumes no large structural changes of the RNA molecule upon heating, the temperature dependence of the factor ‘d’ in equation (14) (main manuscript) which reflects the NOE contributions of the magnetization transfer can be calculated. Structural changes of the RNA upon heating would be accompanied by large chemical shift changes upon changes in temperature. In addition, structural changes would also cause changes in the thermodynamic stabilities ΔH_{diss}, ΔS_{diss} and ΔG_{diss}. As a consequence, the three parameter equation (14) would not be sufficient to describe the temperature dependence of the imino proton exchange rates $k_{ex}(T)$ anymore.

The NOE of a large biomacromolecule is proportional to the spectral density at zero frequency J(0). J(0) is linearly proportional to the global rotational correlation time τ_c. τ_c is dependent on the temperature itself, the viscosity of the solvent and the global shape of the biomacromolecule. The temperature dependence of τ_c can be described by equation (1)

$$\tau_c(T) = \frac{\kappa\eta}{k_B T} \quad (1)$$

T is the temperature, η is the viscosity, κ is the shape factor of the molecule and τ_c is the rotational correlation time. In case of a spherical overall shape of the biomacromolecule the shape factor κ calculates to

$$\kappa = \frac{4}{3}\pi r^3 \qquad (2)$$

The temperature dependence of τ_c can be calculated by equation (3)

$$\frac{\tau_c(T_1)}{\tau_c(T_2)} = \frac{\eta(T_1) * T_2}{\eta(T_2) * T_1} \qquad (3)$$

Notably, the shape factor κ is cancelled in equation (3). Thus, equation (3) is valid for arbitrary global shapes as long as the structure is insensitive to changes in temperature. We calculated the temperature dependence of 'd' according to equation (4) by using the η(T) values of water (taken from Handbook of Chemisty and Physics, CRC Press) (1). The resulting d(T)/d(283K) plot is given in Figure S1.

$$\frac{d(T)}{d(283K)} = \frac{\tau_c(T)}{\tau_c(283K)} = \frac{\eta(T) * 283K}{\eta(283K) * T} \qquad (4)$$

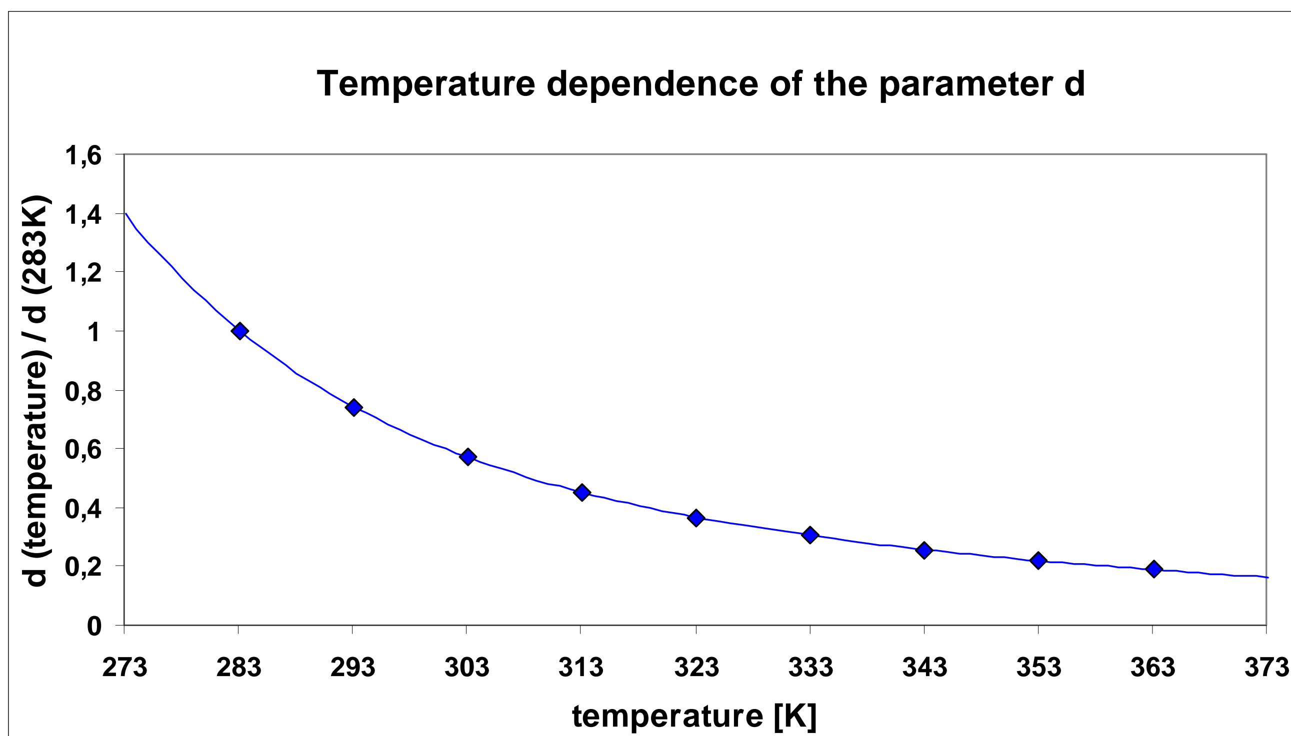

Figure S1: Temperature dependence of the NOE contributions d(T)/d(283K) to the k_{ex} rates determined by the inversion recovery method in aqueous solutions.

The d(T)/d(283K) function was fitted to a double exponential. Thus, d(T) can be described empirically by equation (5)

$$\boldsymbol{d}(\boldsymbol{T}) = \boldsymbol{d}(283\boldsymbol{K}) * \left[\boldsymbol{a} * \exp(-\boldsymbol{b} * (\boldsymbol{T} - 273.1\boldsymbol{K})) + \boldsymbol{c} * \exp(-\boldsymbol{f} * (\boldsymbol{T} - 273.1\boldsymbol{K}))\right] \qquad (5)$$

Parameters have to be set as follows:

a=0.8230; b=0.0505 c=0.5725 f=0.0129

In most cases, it will be sufficient to use d as a temperature invariant parameter in equation (14) (main manuscript). However, there might be cases (other RNAs) in which the temperature dependence of d

may become significant e.g. for larger RNA molecules that show stronger NOEs as a consequence of a longer rotational correlation time τ_c or if the melting is less cooperative so that the observed temperature range becomes larger. In these cases equation (5) (main manuscript: equation (15)) can be used to incorporate the temperature dependence of d in equation (14) (main manuscript).

RESULTS

NMR assignments

The imino proton chemical shifts of the 4U-hp2-wt RNA and the 4U-hp2-A8C-mutant RNA are given in Table S1.

Table S1: Imino proton (N1:guanine; N3: uracil) chemical shifts of the 4U-hp2-wt and the 4U-hp2-A8C-mut RNA in NMR buffer recorded at 10°C. Chemical shifts were referenced to TSP

Chemical shifts	^{1}H 4U-hp2-wt	^{15}N 4U-hp2-wt	^{1}H 4U-hp2-A8C-mut	^{15}N 4U-hp2-A8C-mut
G3	-	-	10.074	141.757
U4	14.139	162.441	14.121	162.454
U5	13.258	162.855	13.243	162.723
G6	9.781	141.354	9.853	141.714
U10	14.070	161.981	13.641	161.568
U11	11.749	157.634	11.698	157.774
U12	12.086	158.092	11.998	158.101
U13	13.431	162.029	13.398	162.026
G14	11.626	145.834	11.616	145.900
U17	-	-	13.352	161.144
U23	13.909	162.313	13.861	162.280
U24	13.845	162.931	13.799	162.920
G27/G28	10.706	143.050	10.668	143.077
G30	13.182	148.356	12.808	147.408
G31	-	-	13.279	148.534
U32	-	-	14.230	162.128
U33	11.625	158.181	11.641	158.401
U36	-	-	11.902	158.997

Temperature dependent exchange rates of the open conformation

In order to determine the thermodynamic parameters ΔG_{TR}, ΔH_{TR} and ΔS_{TR}, the temperature dependences of the imino proton exchange rates of UTP (Figure S2A) and GTP NMR-samples were determined and analyzed using the Eyring formalism (Figure S2B) to extract the thermodynamic parameters of the transition state (TR) for the proton transition from the imino group to H_2O according to the equations (3) and (4) (main article: Materials and Methods, Imino proton exchange rate analysis. Extracted values are given in the main article (Materials and Methods, Imino proton exchange rate analysis). Finally, the extracted ΔG_{TR}, ΔH_{TR} and ΔS_{TR} parameters were assumed to resemble the transition states of guanine and uracil nucleobases in the open conformation of an RNA molecule according to the energy diagram in Figure 1A (main article). Thus, these ΔG_{TR}, ΔH_{TR} and ΔS_{TR} values were used to calculate ΔG_{diss}, ΔH_{diss} and ΔS_{diss} for the individual base pair opening events according to equation (14) (main article: Materials and Methods, Imino proton exchange rate analysis).

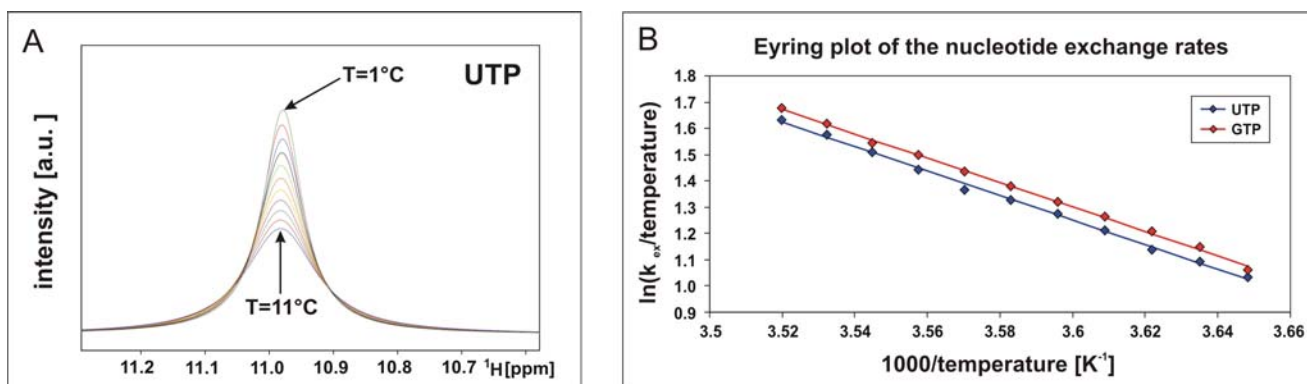

Figure S2: **(A)** Imino proton region of several ^1H-^1D-spectra recorded on a UTP sample at temperatures between 1-11°C. sample composition: 10 mM UTP, 15 mM $K_xH_yPO_4$ (pH 6.5), 25 mM KCl, 90% H_2O and 10% D_2O **(B)** Eyring plot describing the temperature dependence of the imino proton exchange rates k_{ex} for UTP (blue) and GTP (red) in NMR-buffer (10 mM UTP, 15 mM $K_xH_yPO_4$, pH 6.5, 25 mM KCl, 90% H_2O and 10% D_2O).

RNA preparation: native PAA gel electrophoresis

Native gel electrophoresis was used to verify that the purified RNA samples were folded into monomeric homogeneous conformations (Figure S3).

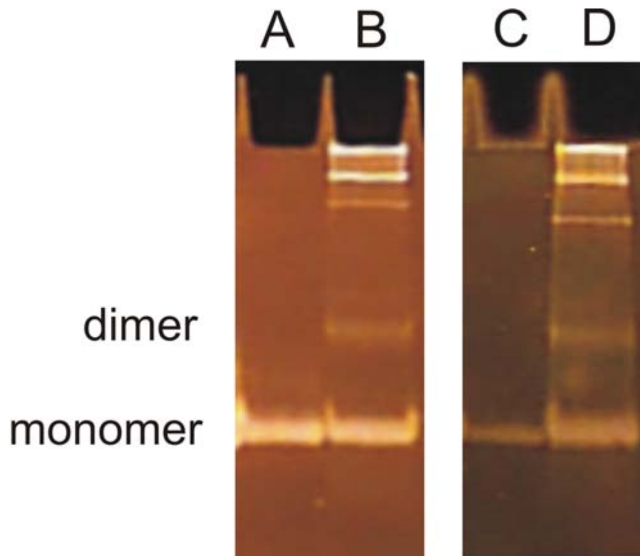

Figure S3: native 15% PAA gel electrophoresis **(A)** 4U-hp2-wt RNA refolded **(B)** 4U-hp2-wt RNA directly after T7 polymerase in vitro transcription **(C)** 4U-hp2-A8C-mutant RNA refolded **(D)** 4U-hp2-A8C-mutant RNA directly after T7 polymerase in vitro transcription

Enthalpy-Entropy correlations (EEC)

The statistical significance of the EEC can be tested by the following inequality (2)

$$\boldsymbol{T_{hm}} - 2\boldsymbol{\sigma} < \boldsymbol{m} < \boldsymbol{T_{hm}} + 2\boldsymbol{\sigma} \qquad (1)$$

Where T_{hm} is the harmonic mean of the temperatures of the imino exchange rate measurements, m is the slope of the $\Delta H_{diss}(\Delta S_{diss})$ correlation plot and σ is the standard deviation of m from the linear regression of the $\Delta H_{diss}(\Delta S_{diss})$ correlation plot. If inequality (1) is fulfilled, the observed $\Delta H_{diss}(\Delta S_{diss})$ correlation is not significant. For the wildtype RNA T_{hm}=295.06 K and for the mutant RNA T_{hm}=297.44 K. The slope is m=316 K for the wildtype RNA and m=329.6 K for the mutant RNA. The standard deviation σ is 2.1 K for the wildtype RNA and 1.5 K for the mutant RNA. Thus, we can wright

290.86 K < 299.26 K < 316.0 K = m (4U-hp2-wt RNA)

294.44 K < 300.44 K < 329.6 K = m (4U-hp2-A8C-mutant RNA)

Since inequality (15) is not fulfilled in both cases, the observed ΔH(ΔS) correlations are clearly statistically significant.

REFERENCES

1. (1974) *Handbook of Chemistry and Physics*. 55th ed. CRC PRESS.
2. Sharp, K. (2001) Entropy-enthalpy compensation: fact or artifact? *Protein Sci*, **10**, 661-667.