Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Aminophthalimide as mimetic of purines and fluorescent RNA base surrogate for RNA imaging

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Supporting Information

Content

1. Materials and methods

RNA synthesis. The oligonucleotides were synthesized under an argon atmosphere on an H-6 DNA/RNA synthesizer from K&A LABORGERÄTE. Controlled pore glass (CPG) with 1 μmol (500 Å) was used as the solid phase. All reagents, phosphoramidites, solvents and CPG columns were obtained from GlenResearch and Sigma Aldrich. To ensure a more effective clutch, the more reactive Activator 42® was used. To ensure RNase-free working, all phosphoramidite tubes were rinsed with ethanol and acetonitrile. The RNA building blocks for the **APht1**, **APht2** and **Dap** modifications were identical to the published DNA building blocks.[1,2] The coupling times were increased due to the sterically demanding 2'-O-TBDMS protecting group. The CPG columns of **RNA1** and **RNA2** were dried under high vacuum, opened and the CPG transferred to an Eppendorf vial equipped with a safe-lock lid. 1 mL 0.5 M DBU and 40 mM thymine in pyridine were added and the mixture was incubated overnight at room temperature. The suspension was centrifuged (3 min, 13000 rpm), the supernatant was pipetted off, the CPG was washed three times with dry pyridine and dried under high vacuum. The solid phase was transferred to an Eppendorf vial with a safe-lock lid. 1 mL K_2CO_3 solution (0.05 M in MeOH) was added and kept overnight at room temperature. 6 µL glacial acetic acid was added for neutralization, the solution was pipetted off and the CPG was washed three times with 300 µL RNase-free water. The solvent was removed using a Christ Alpha RVC vacuum concentrator and the samples were lyophilized using a Christ Alpha 1-2 LD Plus lyophilizer. **RNAD** was cleaved from the resin using conventional methods. To cleave the 2'-O-TBDMS protecting groups lyophilized **RNA1**, **RNA2** and **RNAD** were suspended in 300 µL DMSO. 300 µL $NEt_3 \cdot 3HF$ was added and the samples incubated for 2.5 hours at 60 °C. After cooling to room temperature 600 µL isopropyl trimethylsilyl ether and 10 mL diethyl ether were added and the solution stored over night at -32 °C. The suspension was centrifuged (3 min, 13000 rpm), the supernatant was pipetted off, the precipitated RNA washed with 80% ethanol (3 x 1 mL) and lyophilized. Unmodified RNA strands (**RNAA**, **RNAC**, **RNAU**, **RNAG**) and modified **RNAS** were purchased from Metabion. The RNA samples were dissolved in a defined amount of RNase-free water and the concentration was then determined via the absorption at λ = 260 nm using ε_{260}=22.7 $mM^{-1}cm^{-1}$ for **APht1**, 12.4 $mM^{-1}cm^{-1}$ for **APht2** and 2.24 $mM^{-1}cm^{-1}$ for **Dap**.

Table S1. Standard RNA coupling protocol.

	Time [0.1 s]	Reagent 1	Reagent 2	Target	S.Col.Ptr.	Time [s]	Branch
COUPLING							
1	15	GAS		COL		2	
2	4	TET		COL	ON		
3					ON		
4						2	1
BRANCH							
1	4	TET		COL	ON		
2	6	AMD	TET	COL			
3					ON		
4						99	
5						99	
6	6	AMD	TET	COL	ON		
7					ON		
8						99	
9						99	
10	6	AMD	TET	COL	ON		
11					ON		
12						99	

13						99	
14	5	TET		COL	ON		
15					ON		
16	20	ACN		M_W			
17	20	GAS		M_W			
Coupling							
1						90	
2	10	ACN		M_W		90	
3	10	ACN		COL	ON		
4					ON		
5	35	GAS		COL			
6	20	GAS		M_W			

Table S1. RNA protocol for coupling of the modified RNA building blocks for **APht1**, **APht2** and **Dp**.

	Time [0.1 s]	Reagent 1	Reagent 2	Target	S.Col.Ptr.	Time [s]	Branch
BRANCH							
1	4	TET		COL	ON		
2	4	AMD	TET	COL			
3					ON		
4						99	
5						99	
6						99	
7						99	
8						99	
9						99	
10						99	
11						99	
12	4	AMD	TET	COL	ON		
13					ON		
14						99	
15						99	
16						99	
17						99	
18						99	
19						99	
20						99	
21						99	
22	5	TET		COL	ON		
23					ON		
24	20	ACN		M_W			
25	20	GAS		M_W			

RNase-free working. Certified RNase-free consumables (Eppendorf tubes, pipette tips, falcons) were used for working with RNA. Glassware was heated overnight in a drying cabinet at 200 °C. Gloves and surfaces were previously disinfected with ethanol. To produce RNase-free water, deionized water with 0.1% diethyl pyrocarbonate (DEPC) was stirred at room temperature for about 2 h and then heated to the boiling point for 2 h to destroy excess DEPC.

High Performance Liquid Chromatography (HPLC). The synthesized oligonucleotides were purified on a ThermoFisher system (Dionex UltiMate3000 with autosampler, pump module, column oven, multidiode array, RS fluorescence detector, fraction collector) using the

Chromeleon 7 software). For the semi-preparative separation, a reversed-phase Supelco Discovery® BIO Wide Pore C18 column (250 x 10 mm, 5 μm) was used with a flow rate of 2.5 mL/min. A VDSpher OptiBio PUR 300 S18-SE column (250 x 4.6 mm, 5 μm) with a flow rate of 1.0 mL/min was used for analytical chromatography. A 100 mM ammonium bicarbonate buffer (component A, RNase-free, DEPC-treated) was used as the mobile phase for the purification of RNA. The respective sample could be eluted with an increasing acetonitrile gradient (0-15% B in A over 50 min, 60 °C). Detection was at a wavelength of λ = 260 nm.

MALDI-TOF MS. Mass spectrometry was performed using a Shimadzu Axima Confidence Spectrometer using the following matrix: 3-hydroxypicolinic acid (saturated in MeCN:H_2O=1:1) / diammoniumhydrogencitrat (0.44 M in H_2O) = 9:1.

Optical Spectroscopy. The optical-spectroscopic characterization of the synthesized RNA was carried out in Starna quartz glass cuvettes (path length 1 cm, sample volume 1 mL) at a temperature of 20 °C. The spectra were baseline corrected using the respective solvent. UV/Vis absorption was measured on a Varian Cary 100 Bio spectrometer with a temperature-controlled cell holder (Cary 100 temperature controller) using the Cary WinUV Scan Application software. The following parameters were selected: averaging time 0.1 s, data interval 1.0 nm, scanning speed 600 nm/min, lamp change 350 nm. The melting temperatures of oligonucleotide duplexes were measured on the same device using the Thermal program as software. The change in absorption of the oligonucleotides was detected at 260 nm in a temperature range of 10-90 °C (heating or cooling rate 0.7 °C/min, recording of the measurement data per 0.5 °C). The temperature at the start and end point was maintained for 10 min each. Fluorescence was measured on a Horiba Fluoromax-4. The spectra were corrected for the Raman scattering of the pure solvent. The following parameters were used for the measurements: step size 1.0 nm, increment time 0.2 s, integration time 0.1 s, slit widths 2 nm, 3 nm or 9 nm.

Table S3. Extinction coefficients ε_{260} and MALDI-TOF MS analyses **RNA1**, **RNA2** and **RNAD**. The small differences between calcd. and found m/z data is due to the inaccuracy of the MALDI-TOF-MS instrument.

RNA	ε_{260} [$mM^{-1}cm^{-1}$]	m/z calcd. [$gmol^{-1}$]	m/z found [$gmol^{-1}$]
RNA1	135.5	4148.8	4145.2
RNA2	125.2	4176.8	4172.5
RNAD	115.1	4096.8	4097.9

2. Images of MS analyses

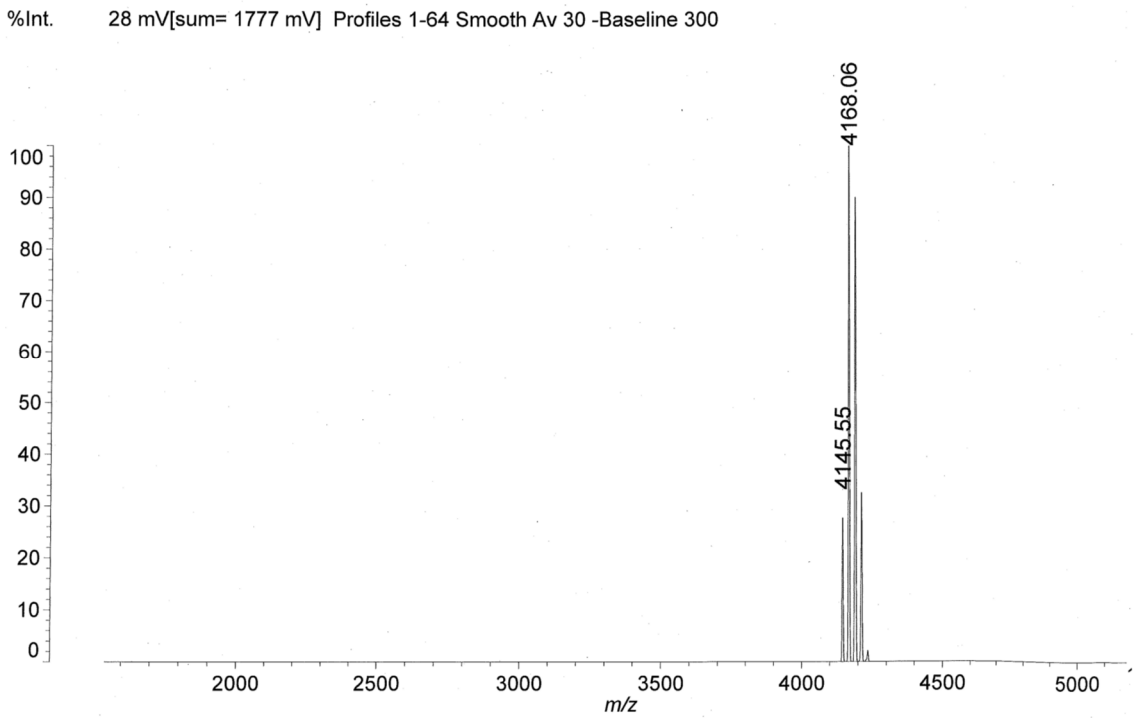

Figure S1. MALDI-TOF MS analysis of **RNA1**.

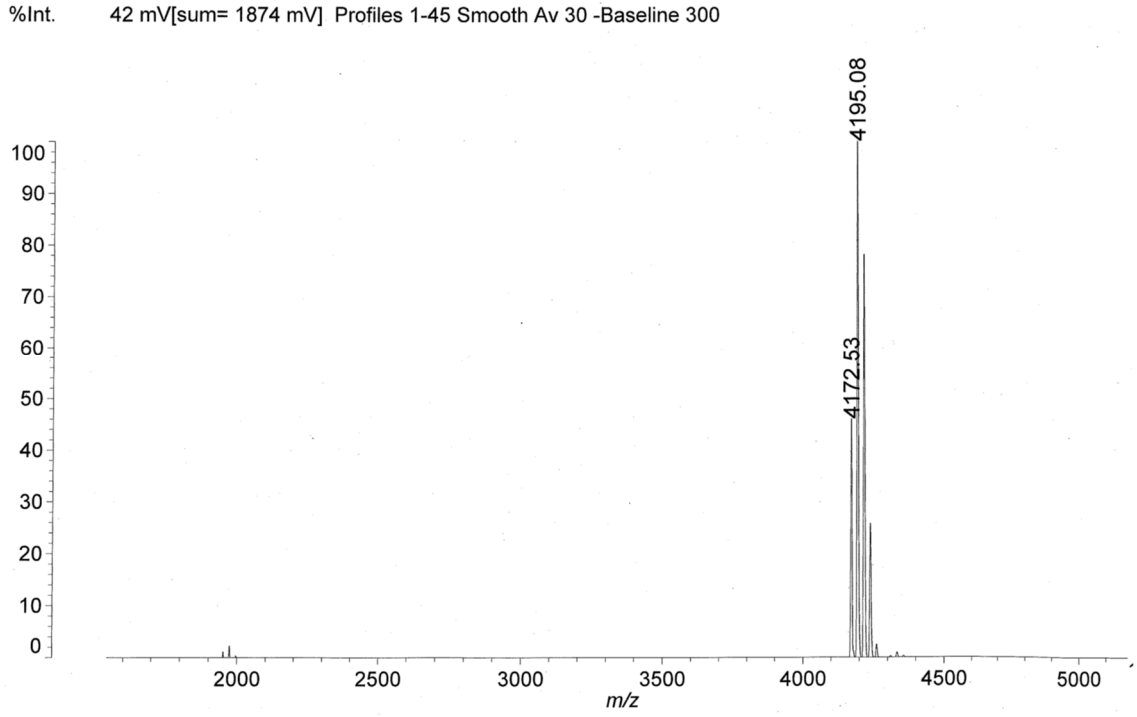

Figure S2. MALDI-TOF MS analysis of **RNA2**.

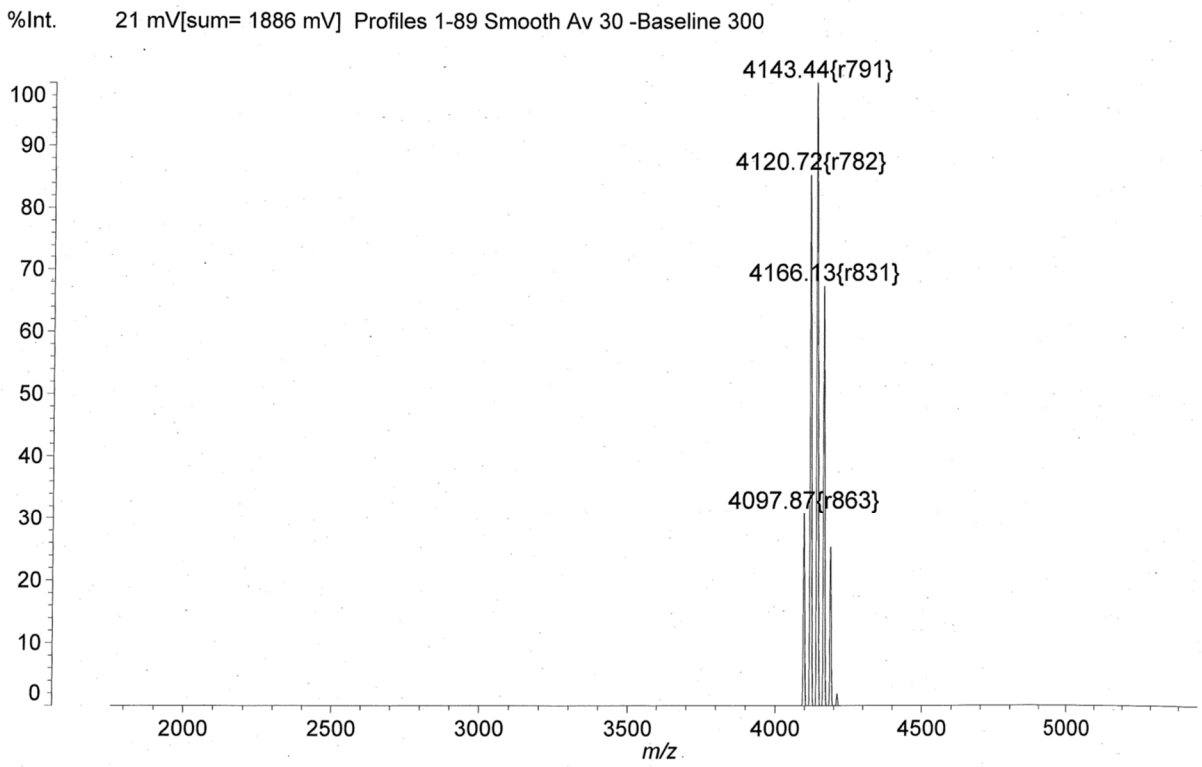

Figure S3. MALDI-TOF MS analysis of **RNAD**.

3. UV/Vis absorbances

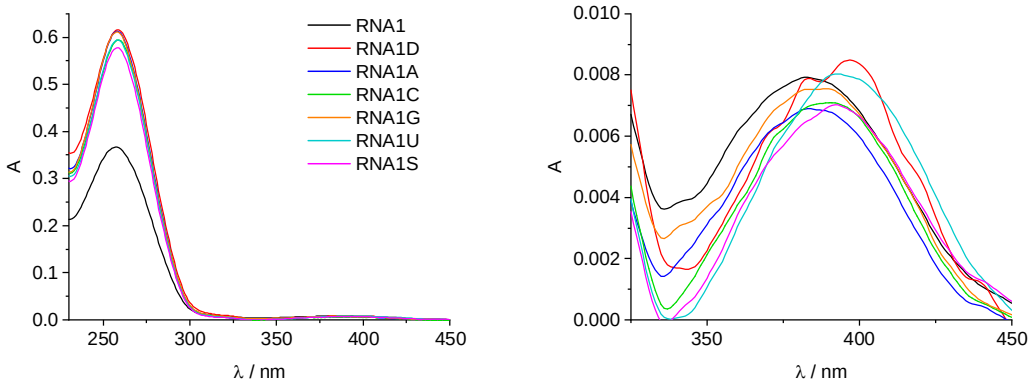

Figure S4. UV/Vis absorbance of **RNA1-RNAX** (**X**=A, C, G, T, D, S); 2.5 µM RNA, 10 mM Na-P_i buffer, 250 mM NaCl, pH 7, 20 °C.

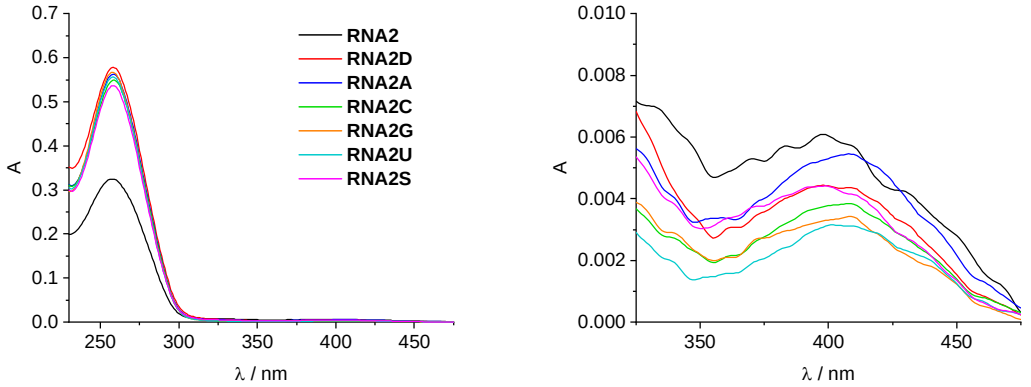

Figure S5. UV/Vis absorbance of **RNA2-RNAX** (**X**=A, C, G, T, D, S); 2.5 µM RNA, 10 mM Na-P_i buffer, 250 mM NaCl, pH 7, 20 °C.

4. Melting temperatures

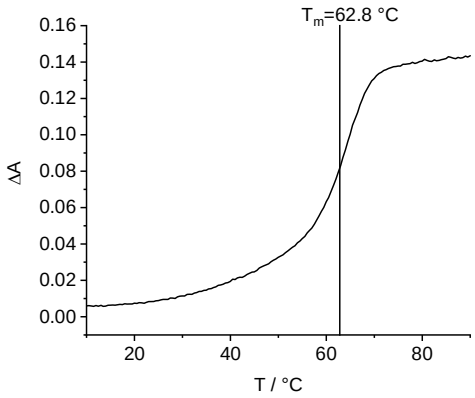

Figure S6. Melting profile of **RNA1-RNAD**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

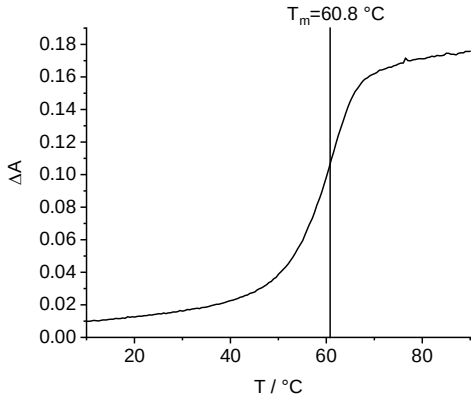

Figure S7. Melting profile of **RNA1-RNAA**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

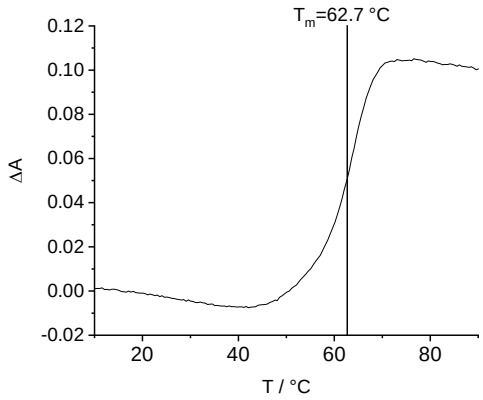

Figure S8. Melting profile of **RNA1-RNAC**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

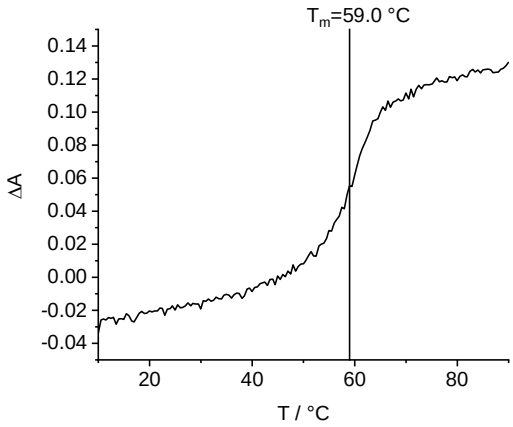

Figure S9. Melting profile of **RNA1-RNAG**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

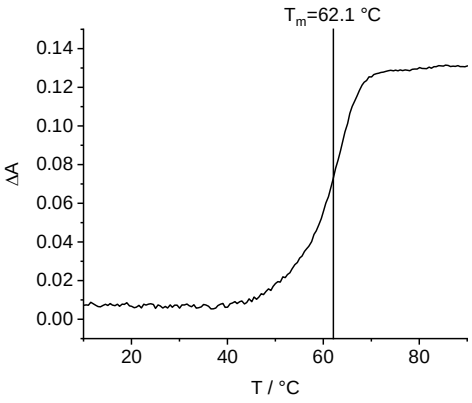

Figure S10. Melting profile of **RNA1-RNAU**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

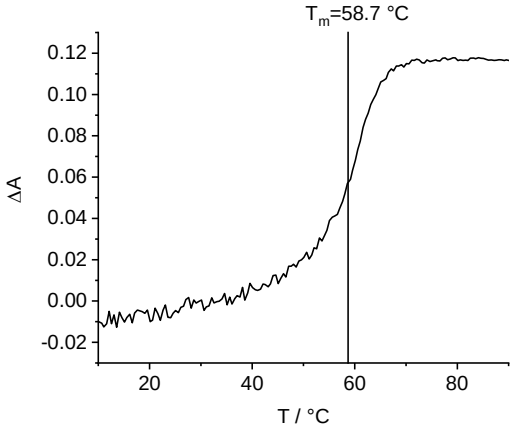

Figure S11. Melting profile of **RNA1-RNAS**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

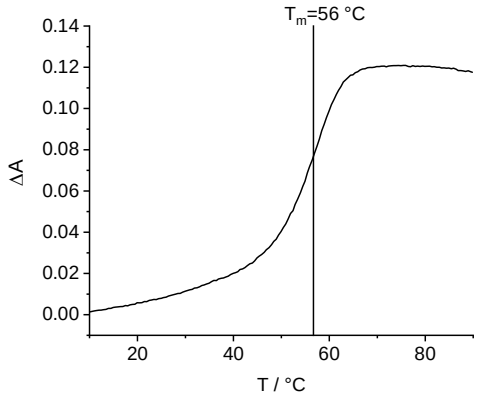

Figure S12. Melting profile of **RNA2D**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

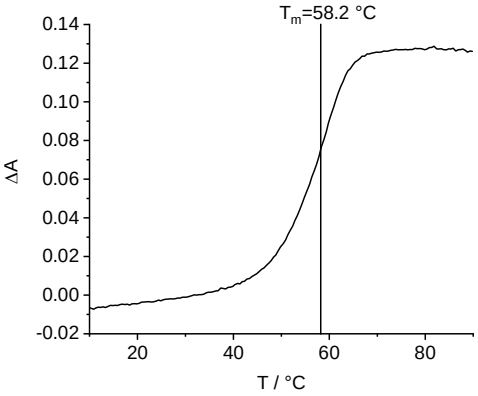

Figure S13. Melting profile of **RNA2-RNAA**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

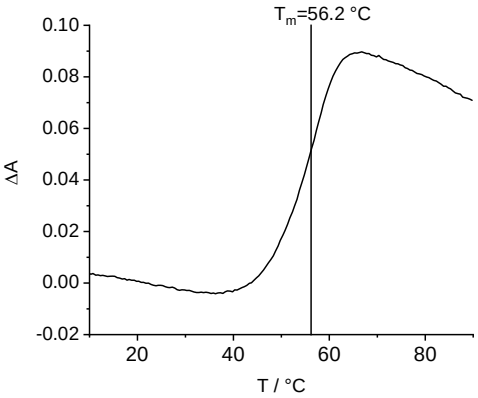

Figure S14. Melting profile of **RNA2-RNAC**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

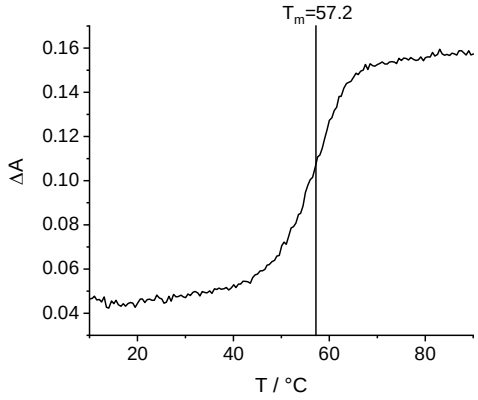

Figure S15. Melting profile of **RNA2-RNAG**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

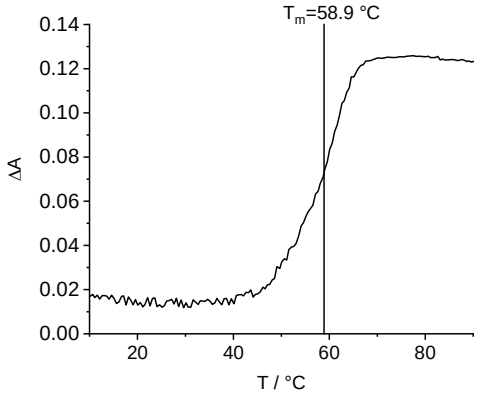

Figure S16. Melting profile of **RNA2-RNAU**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

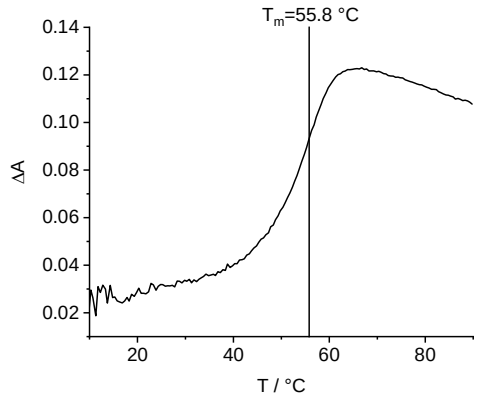

Figure S17. Melting profile of **RNA2-RNAS**; 2.5 µM RNA, 10 mM Na-Pi buffer, 250 mM NaCl, pH 7, 20 °C, 0.7 °C/min.

5. References

1 M. Merkel, L. Dehmel, N. P. Ernsting, H.-A. Wagenknecht, *Angew. Chem. Int. Ed.* **2017**, *56*, 384-388.

2 M. Weinberger, F. Berndt, R. Mahrwald, N. P. Ernsting, H.-A. Wagenknecht, *J. Org. Chem.* **2013**, *78*, 2589-2599.