SUPPLEMENTARY INFORMATION

Time dependence of cisplatin-induced duplex dissociation of 15-mer RNAs and mature miR-146a

Christopher Polonyi and Sofi K. C. Elmroth

EXPERIMENTAL DETAILS

Chemicals and solutions

All oligonucleotides (5′-CUUCUUGGUUCUCUU-3′ and 5′-AAGAGAACCAAGAAG-3′ (RNA-1), 5′-CUUCUUGUGUCUCUU-3′ and 5′-AAGAGACACAAGAAG-3′ (RNA-2), 5′-CCUCUGAAAUUCAGUUCUUCAG-3′ and 5′-UGAGAACUGAAUUCCAUGGGUU-3′ (miR-146a)) were purchased from IBA GmbH (IBA Nucleic Acids Synthesis, Göttingen, Germany) of HPLC grade quality. Cisplatin (*cis*-$[Pt(NH_3)_2(Cl)_2]$, (**1**)) and dimethylformamide (DMF) was obtained from Sigma-Aldrich. Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \bullet H_2O$), disodium hydrogen phosphate dehydrate ($Na_2HPO_4 \bullet 2H_2O$), sodium perchlorate monohydrate ($NaClO_4 \bullet H_2O$) and silver nitrate ($AgNO_3$) was obtained from Merck. Ammonium acetate ($CH_3CO_2NH_4$) was purchased from Scharlau and acetonitrile (CH_3CN, HPLC grade) from LAB-Scan. All water used was of Millipore quality and autoclaved before use.

Kinetic studies

Monoaquated cisplatin (*cis*-$[Pt(NH_3)_2Cl(OH_2)^+]$, (**1a**)) was prepared by dissolving 3.9 mg of **1** in 1.30 ml DMF, by vortexing (1400 rpm) over night at room temperature. $AgNO_3$ solution was prepared by dissolving 2.3 mg $AgNO_3$ in 1.38 ml DMF, by vortexing (2200 rpm) during 5 min at room temperature. 0.98 mole equivalents of $AgNO_3$ were added to the **1** solution. The mixture was incubated at 37 °C during shaking (170 rpm) for 24 h in the dark. Precipitated silver chloride was removed by two consecutive centrifugations (during 3h, and 2 h and 30 min) at room temperature. The supernatant was transferred to new eppendorf tubes after each centrifugation step. Stock solutions of **1a** were stored in a desiccator together with silica gel (the particles (1 – 3 mm) contained a moisture indicator) and were kept in the dark at room temperature.

Kinetic studies were performed on a Varian Cary 4000 spectrophotometer, equipped with a thermal control unit. The oligonucleotide strands for RNA-1, RNA-2 and miR-146a were mixed separately and the measurements were conducted with a total strand concentration (C_T) of 3.0 μM at two different P_i-buffer conditions (the 5-fold concentrated stock solution was prepared according to *Current protocols in molecular biology*[1]); C_{Pi} = 20 mM (pH 5.8 and C_{Na+} = 29 mM) and C_{Pi} = 20 mM, supplemented with $NaClO_4$ (pH 5.7 and C_{Na+} = 129 mM). The complementary oligonucleotides were heated to 90 °C and allowed to hybridize by slow cooling (0.5 °C/min) to 20 °C. The hybridized samples were heated to 38 °C and **1a** was added (the final concentrations were 7.5, 15.0, 22.5, 30.0 and 45.0 μM). The change in absorbance was measured at λ = 260 nm during 19 h after addition of **1a**.

Thermal melting studies

Thermal melting studies were performed on a Varian Cary 4000 spectrophotometer, equipped with a thermal control unit. The oligonucleotide strands for RNA-1, RNA-2 and miR-146a were mixed separately and the measurements were conducted with C_T = 3.0 μM at two different P_i-buffer conditions (the 5-fold concentrated stock solution was prepared according to *Current protocols in molecular biology*[1]); C_{Pi} = 20 mM (pH 5.8 and C_{Na+} = 29 mM) and C_{Pi} = 20 mM, supplemented with $NaClO_4$ (pH 5.7 and C_{Na+} = 129 mM). The complementary oligonucleotides were heated to 90 °C and allowed to hybridize by slow cooling (0.5 °C/min) to 20 °C. The thermal melting points were evaluated by the first derivative method, using the Cary WinUV software. Data points were collected every 0.20 °C and the equidistant data was used for the Savitzky-Golay calculations[2]. These calculations give rise to a minimized signal noise, by obtaining a new derivative point out of a determined number of surrounding data points. The standard deviation of independent measurements of T_m, was estimated to 0.3 °C based on 8 consecutive measurements.

1 *Current Protocols in Molecular Biology*, 1996, A.2.1-A.2.8.
2 A. Savitzky, M. J. E. Golay, *Anal. Chem.*, 1964, **36**, 1627-1639.

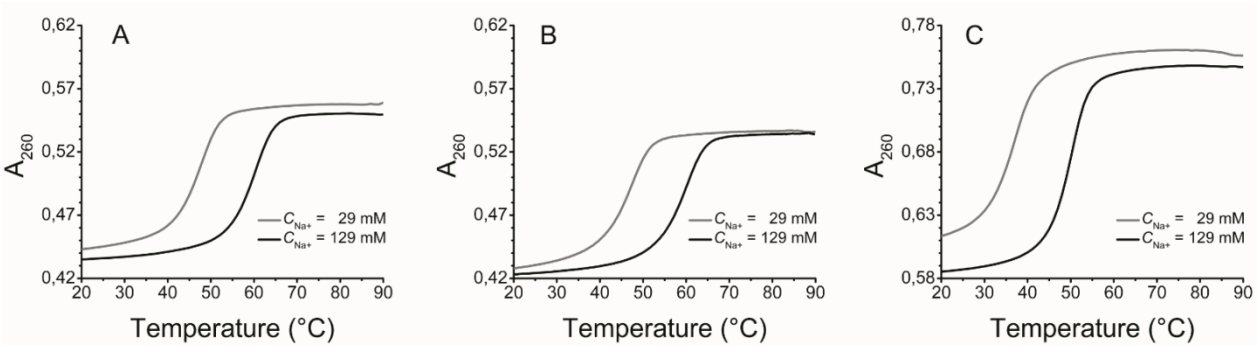

Figure S1. Thermal melting curves obtained for the native RNA duplexes (A) RNA-1, (B) RNA-2 and (C) miR-146a. All measurements were conducted with C_T = 3.0 µM at two different buffer conditions; C_{Na+} = 29 mM (▬; 20 mM Na_2HPO_4/NaH_2PO_4 pH 5.8) and C_{Na+} = 129 mM (▬; 20 mM Na_2HPO_4/NaH_2PO_4 pH 5.7, supplemented with 100 mM $NaClO_4$).

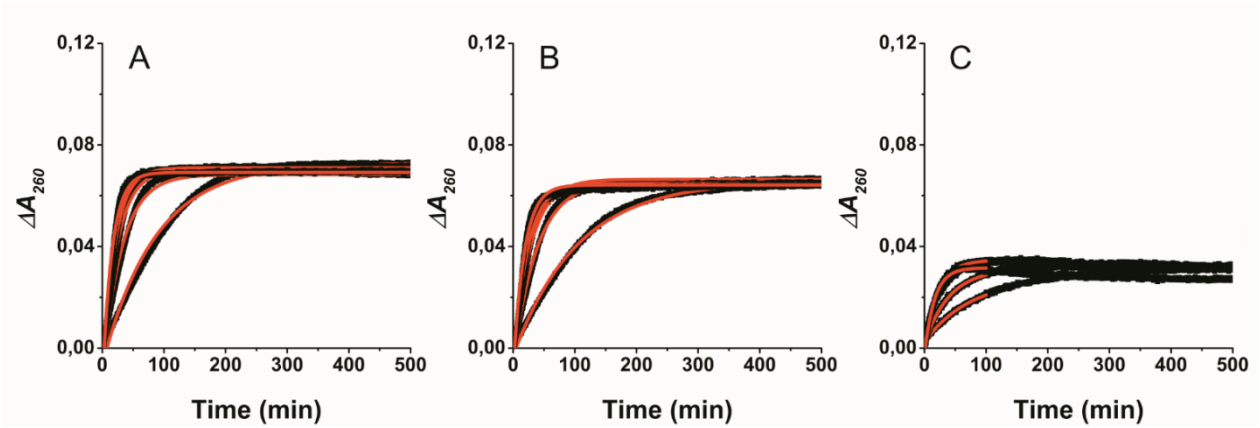

Figure S2. Absorbance change as a function of time after addition of **1a** (C_{1a} = 7.5 - 45.0 μM) to (A) RNA-1, (B) RNA-2 and (C) miR-146a. All measurements were conducted with C_T = 3.0 μM at 38 °C in buffered solution; C_{Na+} = 29 mM (20 mM Na_2HPO_4/NaH_2PO_4 pH 5.8).

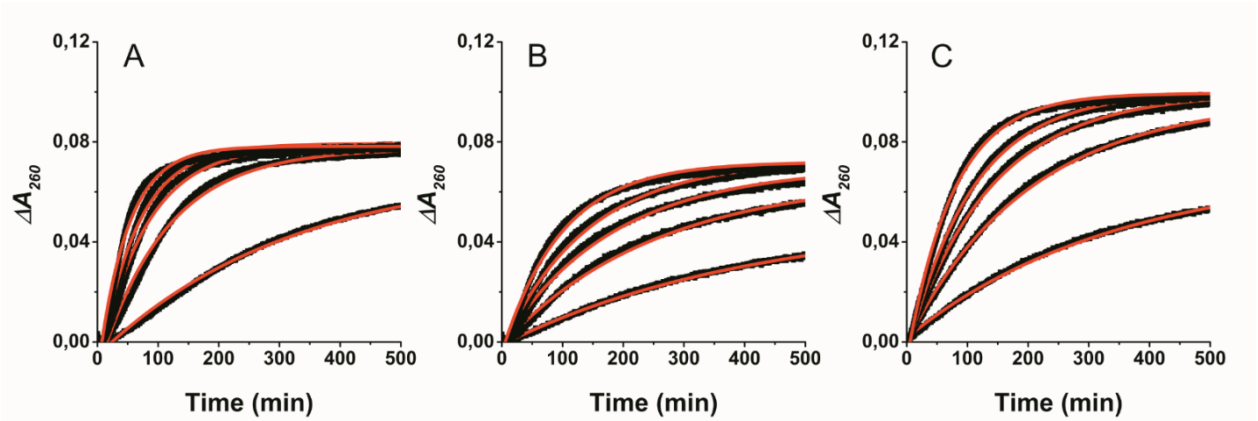

Figure S3. Absorbance change as a function of time after addition of **1a** (C_{1a} = 7.5 - 45.0 μM) to (A) RNA-1, (B) RNA-2 and (C) miR-146a. All measurements were conducted with C_T = 3.0 μM at 38 °C in buffered solution; C_{Na+} = 129 mM (20 mM Na_2HPO_4/NaH_2PO_4 pH 5.7, supplemented with $NaClO_4$).

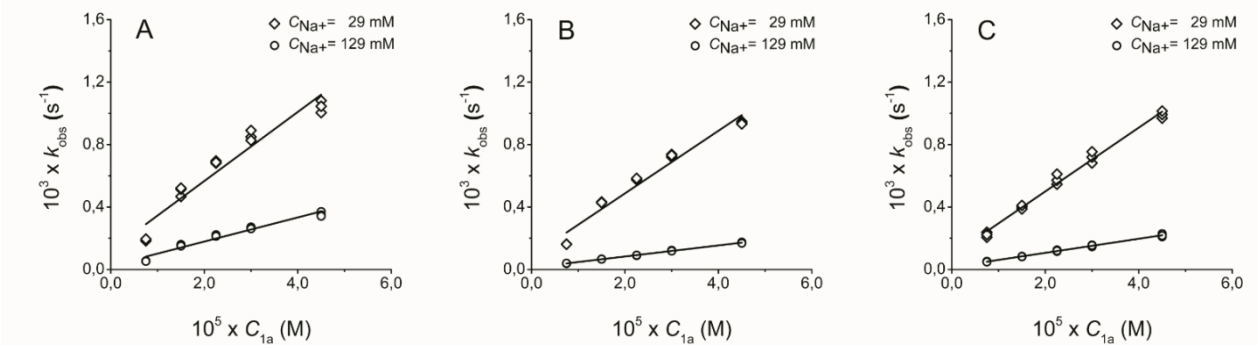

Figure S4. Observed pseudo-first-order rate constants (k_{obs}) plotted as a function of [**1a**] (C_{1a} = 7.5 - 45.0 μM) for (A) RNA-1, (B) RNA-2 and (C) miR-146a. All measurements were conducted with C_T = 3.0 μM and regression lines were fitted to data obtained at two different buffer conditions; C_{Na+} = 29 mM (■; 20 mM Na_2HPO_4/NaH_2PO_4 pH 5.8) and C_{Na+} = 129 mM (○; 20 mM Na_2HPO_4/NaH_2PO_4 pH 5.7, supplemented with 100 mM $NaClO_4$).