

Electronic Supplementary Information to:

Kinetics of cisplatin binding to short r(GG) containing miRNA mimics – influence of Na⁺ versus K⁺, temperature and hydrophobicity on reactivity

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Table S1. Summary of melting temperatures (T_m) and reaction rate constants (k_{obs} and $k_{2,app}$) for RNA-1-1, RNA-1-1-S, RNA-1-3, RNA-1-3-X.^a

| Duplex | T_m^b (°C) | $10^4 \times k_{obs}^c$ (s ⁻¹) | | | | | $k_{2,app}^d$ (M ⁻¹ s ⁻¹) | Reference |
|------------------------|--------------|--|-----------------------|-----------------------|-----------------------|-----------------------|--|--------------------------|
| | | C_{Pt} (μM) 7.5 | C_{Pt} (μM) 15.0 | C_{Pt} (μM) 22.5 | C_{Pt} (μM) 30.0 | C_{Pt} (μM) 45.0 | | |
| $C_{Na^+} = 122$ mM | | | | | | | | |
| RNA-1-1 | 61.6 ± 0.2 | 0.54 | 1.6 | 2.2 | 2.7 | 3.5 | 7.7 ± 0.5 | 2l |
| RNA-1-1-S | 56.7 ± 0.1 | 1.6 ± 0.3 | 2.7 ± 0.2 | 3.3 ± 0.3 | 4.1 ± 0.2 | 5.6 ± 0.5 | 10.5 ± 0.6 | 2n |
| RNA-1-3 | 39.2 ± 0.1 | 2.5 | 5.4 | 7.5 | 9.4 | 14 | 29.7 ± 1.1 | 2n |
| RNA-1-3 ($T = 28$ °C) | | 0.44 ± 0.01 | 0.9 ± 0.2 | 1.5 ± 0.4 | 2.0 ± 0.4 | 2.7 ± 0.1 | 6.0 ± 0.5 | <i>This work</i> |
| RNA-1-3-X | 53.1 ± 0.2 | 1.1 ± 0.3 | 2.3 ± 0.1 | 3.5 ^f | 4.7 ± 0.2 | 7.0 ± 0.2 | 15.8 ± 0.3 | <i>This work</i> |
| $C_{K^+} = 122$ mM | | | | | | | | |
| RNA-1-1 | 59.5 ± 0.3 | 1.3 ± 0.3 | 2.2 ± 0.3 | 3.0 ± 0.1 | 3.8 ± 0.3 | 4.7 ± 0.2 | 9.2 ± 0.6 | <i>This work</i> |
| RNA-1-1-S | 55.0 ± 0.2 | 1.8 ± 0.4 | 2.7 ± 0.3 | 4.0 ± 0.5 | 4.7 ± 0.7 | 6.5 ± 1 | 12.6 ± 1.1 | <i>This work</i> |
| RNA-1-3 | 36.5 ± 0.3 | <i>n.d.</i> | <i>n.d.</i> | <i>n.d.</i> | <i>n.d.</i> | <i>n.d.</i> | <i>n.d.</i> | <i>n.d.</i> ^e |
| RNA-1-3 ($T = 28$ °C) | | 0.7 ± 0.4 | 1.2 ± 0.2 | 1.9 ± 0.4 | 2.6 ± 0.3 | 3.7 ± 0.5 | 8.0 ± 0.6 | <i>This work</i> |
| RNA-1-3-X | 50.9 ± 0.1 | 1.9 ± 0.9 | 3.3 ± 0.7 | 4.9 ± 0.6 | 6.1 ± 0.5 | 9.2 ± 0.7 | 19.4 ± 1.2 | <i>This work</i> |

^a $C_T = 3.0$ μM and $T = 38$ °C if nothing else stated. ^b Measurements performed in triplicates. Indicated errors correspond to the standard deviation. ^c Indicated errors for k_{obs} correspond to the standard deviation. ^d Indicated errors for $k_{2,app}$ correspond to the standard error of the linear curve fit based on data points from five separate measurements; 7.5 μM < C_{Pt} < 45 μM. ^e Reaction over within mixing time. ^f Only measured once.

Table S2. Summary of reaction rate constants (k_{obs} and $k_{2,\text{app}}$) in buffer with $C_{\text{Na}^+} = 50 \text{ mM}$, with and without 10% EtOH for RNA-1-1, RNA-1-3-X and RNA-1-3.^a

| Duplex | T_m ($^{\circ}\text{C}$) | $10^4 \times k_{\text{obs}}^b$ (s^{-1}) | | $k_{2,\text{app}}^c$ ($\text{M}^{-1}\text{s}^{-1}$) |
|---|------------------------------|--|------|---|
| | | C_{1a} (μM) | 15.0 | |
| $C_{\text{Na}^+} = 50 \text{ mM at } 38 \text{ }^{\circ}\text{C}$ | | | | |
| RNA-1-1 | 54.9 ± 0.1 | 3.1 ± 0.7 | | 20.9 ± 5 |
| RNA-1-1-S | 49.8 ± 0.1 | 3.9 ± 0.1 | | 26.1 ± 0.2 |
| RNA-1-3-X | 47.3 ± 0.3 | 5.9 ± 0.7 | | 39.3 ± 4.7 |
| $C_{\text{Na}^+} = 50 \text{ mM} + 10\% \text{ EtOH at } 38 \text{ }^{\circ}\text{C}$ | | | | |
| RNA-1 | 53.7 ± 0.5 | 1.9 ± 0.2 | | 12.9 ± 1.4 |
| RNA-1-1-S | 46.7 ± 0.8 | 2.4 ± 0.4 | | 16 ± 2.5 |
| RNA-1-3-X | 46.3 ± 0.1 | 3.6 ± 0.2 | | 24.3 ± 1.4 |
| $C_{\text{Na}^+} = 50 \text{ mM at } 28 \text{ }^{\circ}\text{C}$ | | | | |
| RNA-1-3 | 32.5 ± 0.2 | 2.4 ± 0.3 | | 15.7 ± 1.8 |
| $C_{\text{Na}^+} = 50 \text{ mM} + 10\% \text{ EtOH at } 28 \text{ }^{\circ}\text{C}$ | | | | |
| RNA-1-3 | 33.0 ± 0.2 | 1.6 ± 0.1 | | 10.7 ± 0.5 |

^a $C_T = 3.0 \mu\text{M}$. ^b Measurements performed in triplicates. Indicated errors correspond to the standard deviation. ^c Indicated errors for the $k_{2,\text{app}}$ correspond to the standard deviation of triplicate data points.

Table S3. Summary of second-order rate constants as a function of temperature and activation parameters for the reaction of **1a** with RNA-1-1, RNA-1-1-S, RNA-1-3 and RNA-1-3-X.

| Duplex | T_m^b (°C) | T (°C) | $k_{2,app}^c$ (M ⁻¹ s ⁻¹) | ΔH^\ddagger (kcal mol ⁻¹) | ΔS^\ddagger (cal K ⁻¹ mol ⁻¹) |
|-----------|-----------------|-------------|---|--|---|
| RNA-1-3 | 39.2 ± 0.1 | 23 | 2.8 ± 0.2 | 31 ± 3 | 49 ± 5 |
| | | 28 | 7.9 ± 0.7 | | |
| | | 33 | 21.6 ± 0.5 | | |
| | | 38 | 36 ^f | | |
| RNA-1-3-X | 53.1 ± 0.2 | 33 | 7.6 ± 0.3 | 34 ± 3 | 57 ± 5 |
| | | 38 | 15.4 ± 0.3 | | |
| | | 43 | 37.0 ± 0.3 | | |
| | | 48 | 111 ± 3 | | |
| RNA-1-1-S | 56.7 ± 0.1 | 33 | 10.3 ± 0.5 | 23 ± 1 | 22 ± 1 |
| | | 38 | 18 ± 1.3 ^f | | |
| | | 43 | 35 ± 1.0 | | |
| | | 48 | 63.3 ± 0.6 | | |
| RNA-1-1 | 61.6 ± 0.2 | 33 | 6.9 ± 0.2 | 28 ± 2 | 36 ± 3 |
| | | 38 | 10.7 ± 0.3 ^f | | |
| | | 48 | 50 ± 15 | | |
| | | 58 | 222 ± 35 | | |

^a $C_T = 3.0 \mu\text{M}$, $C_{\text{Na}^+} = 122 \text{ mM}$. ^b Measurements performed in triplicates. ^c $k_{2,app}$ was calculated as $k_{\text{obs}}/C_{\text{Pt}}$ (15 μM). Indicated error for $k_{2,app}$ correspond to the standard deviation of triplicate data points with $C_{\text{Pt}} = 15 \mu\text{M}$. ^fData from Ref# 3n.

Table S4. Comparison of the products kK_{ass} for the reactions of **1a** with RNA-1-1, RNA-1-1-S, RNA-1-3-X, and RNA-1-3 after adjustment with respect to salt concentration in agreement with mechanism outlined in Eqns (2) – (5).

| Duplex | C_{Na^+} (mM) | $k_{2,\text{app}}$ ($\text{M}^{-1}\text{s}^{-1}$) ^a | $k K_{\text{ass}}$ ($= k_{2,\text{app}} \cdot C_{\text{Na}^+}$ (s^{-1})) ^a |
|-----------|------------------------|--|--|
| RNA-1-1 | 50 | 21 | 1.05 |
| | 122 | 7.7 | 0.939 |
| RNA-1-1-S | 50 | 26.1 | 1.30 |
| | 122 | 10.5 | 1.28 |
| RNA-1-3-X | 50 | 39 | 1.95 |
| | 122 | 15.8 | 1.93 |
| RNA-1-3 | 50 | 15.7 ^b | 0.78 ^b |
| | 122 | 6.0 ^b | 0.73 ^b |
| | 122 | 29.7 | 3.62 |

^a $k_{2,\text{app}}$ determined at 38 °C if nothing else stated, ^b $k_{2,\text{app}}$ determined at 28 °C.

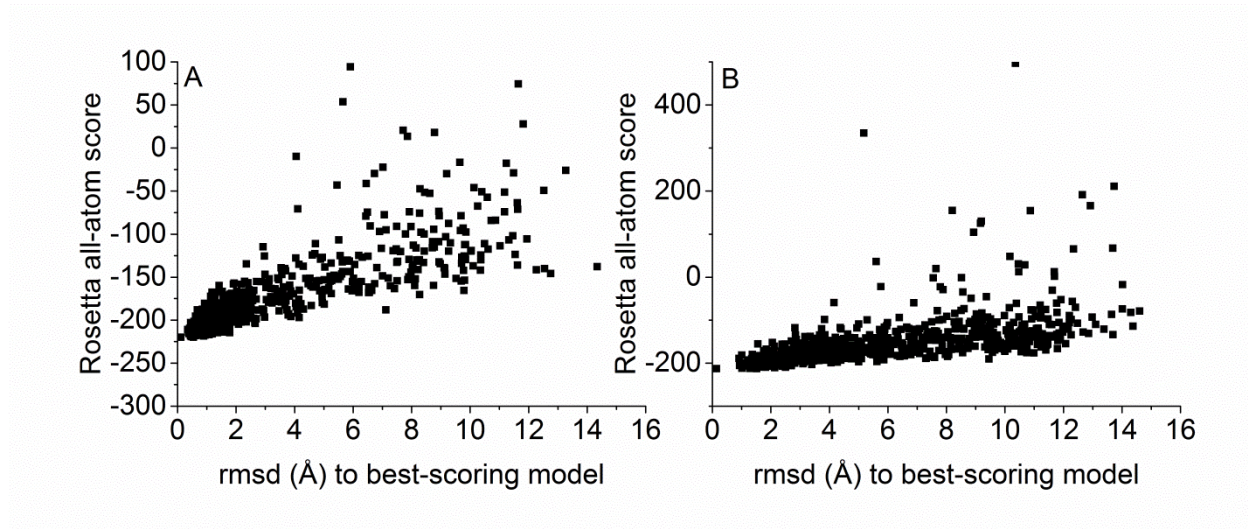


Figure S1. Rosetta all-atom score vs rmsd for (A) RNA-1-1 and (B) RNA-1-3, showing convergence of the structure simulations.

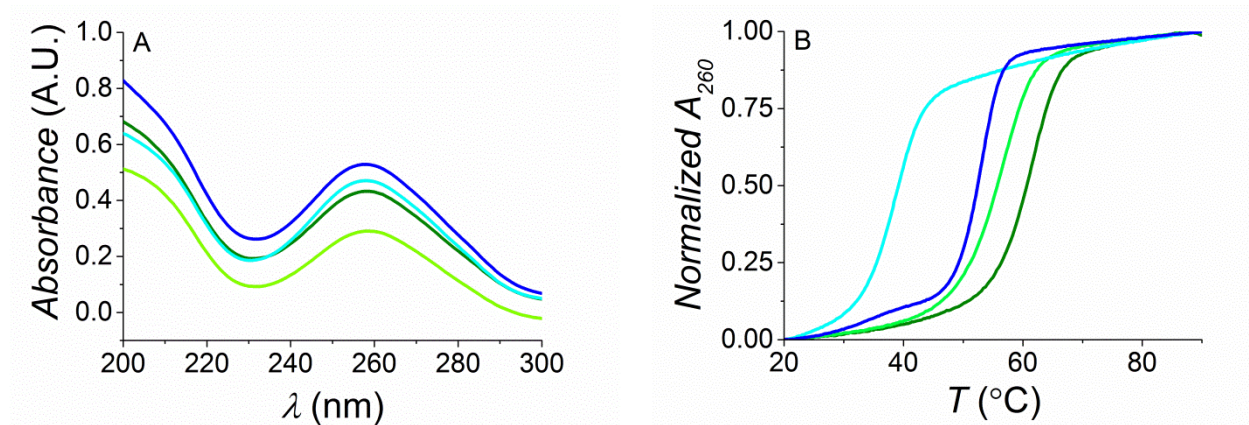


Figure S2. (A) Spectra and (B) melting curves for RNA-1-3-X (dark blue), RNA-1-3 (light blue), RNA-1-1 (dark green), and RNA-1-1-S (light green). The spectra were recorded in Buffer A with $C_{\text{Na}^+} = 122$ mM and $C_{\text{T}} = 3.0$ μM .

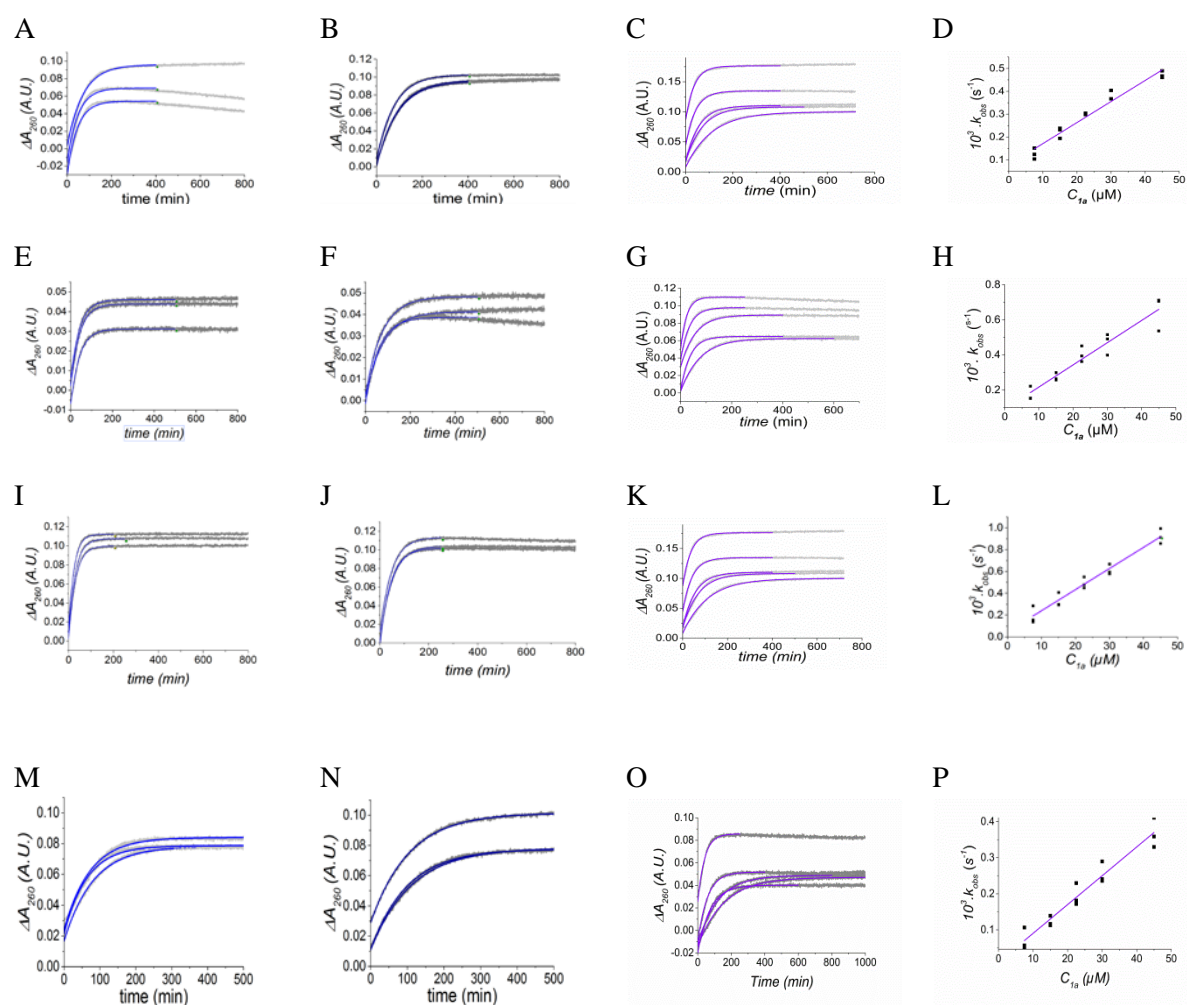


Figure S3. Absorbance change as a function of time (gray), together with fits of a single-exponential function to experimental data (solid lines, blue or purple), in buffered solution; $C_{Na^+} = 50$ mM (Buffer C) after addition of **1a** to (A) RNA-1-1, (E) RNA-1-1-S, (I) RNA-1-3-X and (M) RNA-1-3. All measurements were conducted with $C_{1a} = 15.0$ μM , $C_T = 3.0$ μM . Absorbance change as a function of time, together with fits of a single-exponential function to experimental data (blue lines), in buffered solution; $C_{Na^+} = 50$ mM (Buffer D) after addition of **1a** to (B) RNA-1-1, (F) RNA-1-1-S, (J) RNA-1-3-X and (N) RNA-1-3. All measurements were conducted with $C_{1a} = 15.0$ μM , $C_T = 3.0$ μM . Absorbance change as a function of time, together with fits of a single-exponential function to experimental data (purple lines) in buffered solution; $C_{K^+} = 122$ mM (Buffer B) after addition of **1a** to (C) RNA-1-1, (G) RNA-1-1-S, (K) RNA-1-3-X and (O) RNA-1-3. All measurements were conducted with $C_{1a} = 7.5$ – 45.0 μM , $C_T = 3.0$ μM . Observed pseudo-first-order rate constants (k_{obs}) plotted as a function of C_{1a} in the interval 7.5 – 45.0 μM , together with linear regression lines allowing for determination of $k_{2,app}$ from the slope; (D) RNA-1-1, (H) RNA-1-1-S, (L) RNA-1-3-X, and (P) RNA-1-3. All measurements were conducted in triplicates with $C_T = 3.0$ μM and $C_{K^+} = 122$ mM (Buffer B).

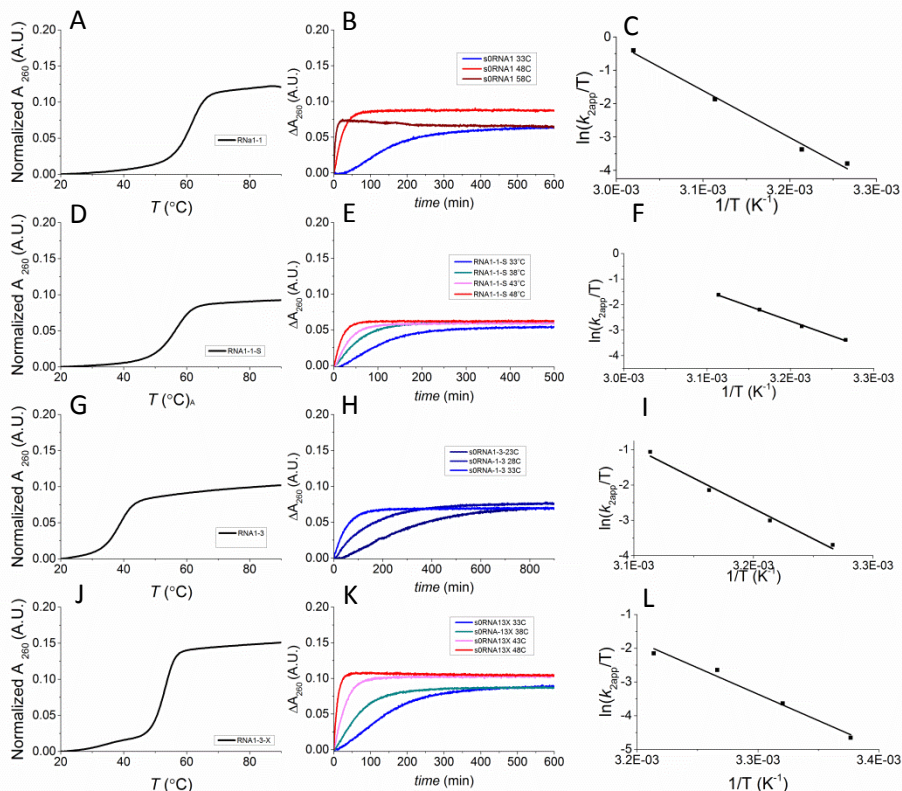


Figure S4. Absorbance change as a function of temperature and time. Melting curve of (A) RNA-1-1, (D) RNA-1-1-S, (G) RNA-1-3, and (J) RNA-1-3-X. Absorbance change as a function of time after addition of **1a** to (B) RNA-1-1, (E) RNA-1-1-S, (H) RNA-1-3, and (K) RNA-1-3-X. All reactions were followed at $\lambda = 260$ nm, and conducted with $C_{1a} = 15.0$ μ M, $C_T = 3.0$ μ M and $C_{K^+} = 122$ mM (Buffer B). Eyring plot of $\ln(k_{2,app}/T)$ as a function of $1/T$ for the reaction of **1a** with (C) RNA-1-1 (F) RNA-1-1-S, (I) RNA-1-3, and (L) RNA-1-3-X. Figures (C) and (I) contain one datapoint each retrieved from Ref#2n (compare Table S3).