Supporting Information

Fluorescence-based Binding Characterization of Small Molecule Ligands Targeting CUG RNA Repeats

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Part I. Aminoglycoside comparison data and RNA sequences

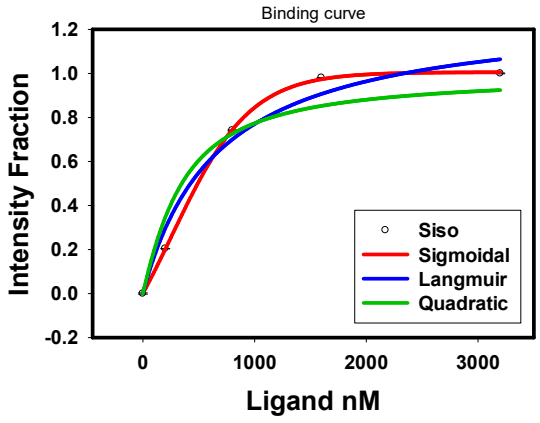

Figure S1. Comparison of different binding models with fluorometer data obtained at room temperature. Sigmoidal fitting (in red): K_d = 945 nM, R^2 = 0.9999. Modified Langmuir isothermal model regression (in blue): K_d = 671 nM, R^2 = 0.9766. Quadratic model fitting (in green): K_d = 247 nM, R^2 = 0.9493.

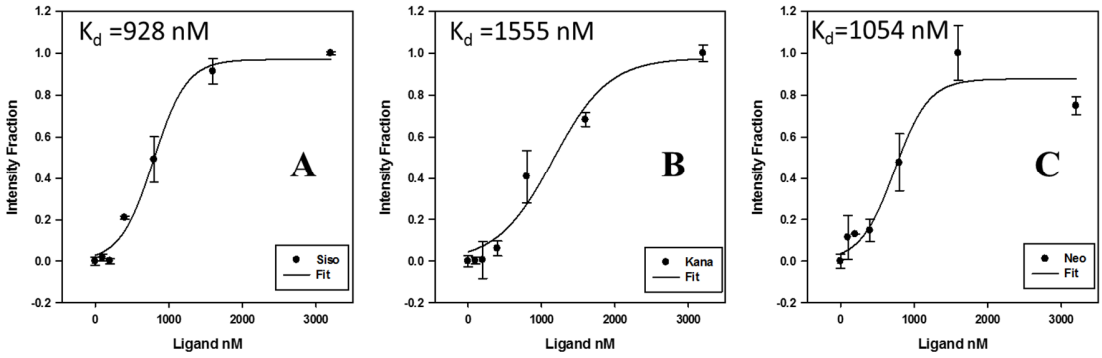

Figure S2. Sigmoidal fitting of aminoglycoside binding data and calculated K_d values. (A) Sisomicin: K_d = 928 nM, R^2 = 0.9898; (B) Kanamycin: K_d = 1555 nM, R^2 = 0.9639; (C) Neomycin: K_d = 1054 nM, R^2 = 0.9471.

Table S1. CUG repeats sequences.

Entry	Sequence
Native r7	5'-CUGCUGCUGCUGCUGCUGCUG-3'
Native r10	5'-CUGCUGCUGCUGCUGCUGCUGCUGCUGCUG-3'
FM1	5'-CUGCUGCUGCUGCfamUGCUGCUGCUGCUGCUG-3'

Part II. UV-melting temperature (T_m) study of RNA CUG repeats

Solutions of RNAs (1.5 μM) were prepared in sodium phosphate buffer (10 mM, pH 7.0) containing 100 mM NaCl. The samples were heated to 95 °C for 5 min, then cooled down slowly to room temperature, and incubated at 4 °C for at least 2 h before T_m measurements. Thermal denaturation was performed on a Cary 300 UV-Visible Spectrophotometer utilizing a temperature controller. The block temperature was the temperature reported. Denaturing curves were acquired at 260 nm with 4 ramps by heating and cooling samples from 5 to 85 °C at a rate of 0.5 °C/min. UV melting of binding studies was conducted with RNA samples subsequently titrated by ligands generating ratios of ligand to RNA from 0 to 16. The thermodynamic parameters of samples were obtained by fitting their melting curves in the MeltWin 3.5 software.

Table S2. Calculated T_m for Siso-CUG binding with Meltwin 3.5.

CUG Repeats	LM0	LM1	LM4	LM4_2	LM8	LM16
r7	59.8 ± 0.5°C	64.0 ± 1.3°C	61.9 ± 0.5°C	60.0 ± 0.3°C	62.4 ± 1.4°C	62.8 ± 1.0°C
r10	57.5 ± 0.5°C	58.7 ± 0.5°C	58.8 ± 0.2°C	59.4 ± 0.3°C	60.4 ± 0.5°C	60.7 ± 0.5°C
r10FAM	55.8 ± 0.6°C	57.4 ± 0.2°C	57.2 ± 0.4°C	57.2 ± 0.6°C	58.7 ± 0.5°C	58.6 ± 0.5°C

r10FAM5µM	57.1 ± 0.5°C	57.3 ± 0.7°C	59.0 ± 1.1°C	59.0 ± 0.7°C	59.6 ± 0.6°C	61.2 ± 1.0°C

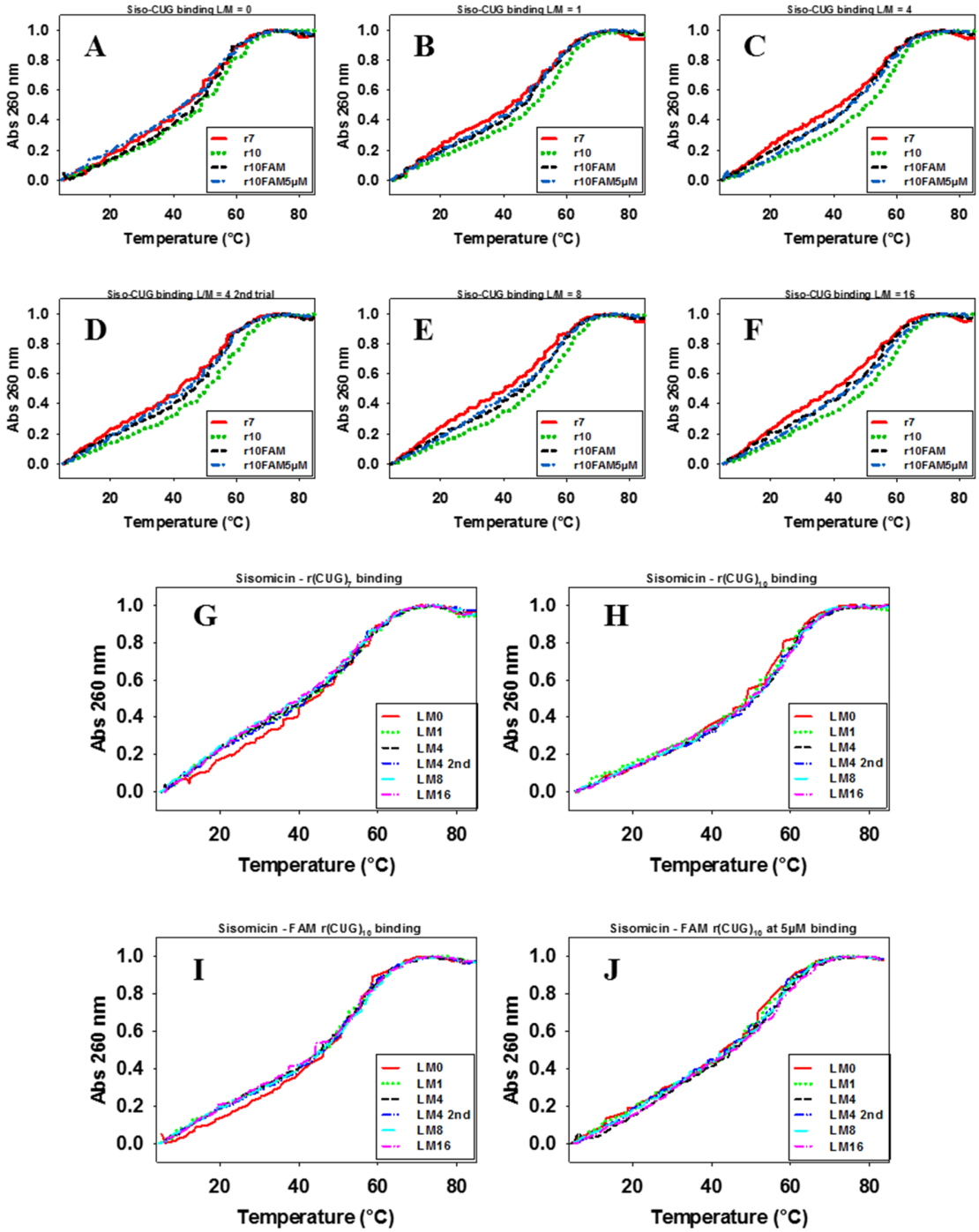

Figure S3. Normalized UV-melting curves of RNA-ligand complexes using sisomicin as titrant against different RNA repeat templates: r7 – native $r(CUG)_{10}$, r10 – native $r(CUG)_{10}$, r10FAM – FM1 $r(CUG)_{10}$, all at 1.5 µM, and r10FAM5µM – FM1 $r(CUG)_{10}$ at 5 µM. (A – F) T_m curves at equivalent ligand/RNA molar ratios of 0, 1, 4, 4, 8, and

16 respectively; LM4 2nd: a repeat of LM4 after volume loss (~10%) compensated by adding DI water. (G – J) Ligand concentration effect on different RNA repeat templates: r7, r10, r10FAM, and r10FAM5μM respectively. UV-melting running conditions: 600 μL annealed sample in sodium phosphate buffer (10 mM, pH 7.0) containing 100 mM NaCl; Absorbance recorded at 260 nm with 4 ramps in 5 – 85 °C (heating/cooling, 0.5 °C/min); T_m calculated with Meltwin 3.5 software.

Part III. Fluorescence characterization on annealed CUG RNA repeats

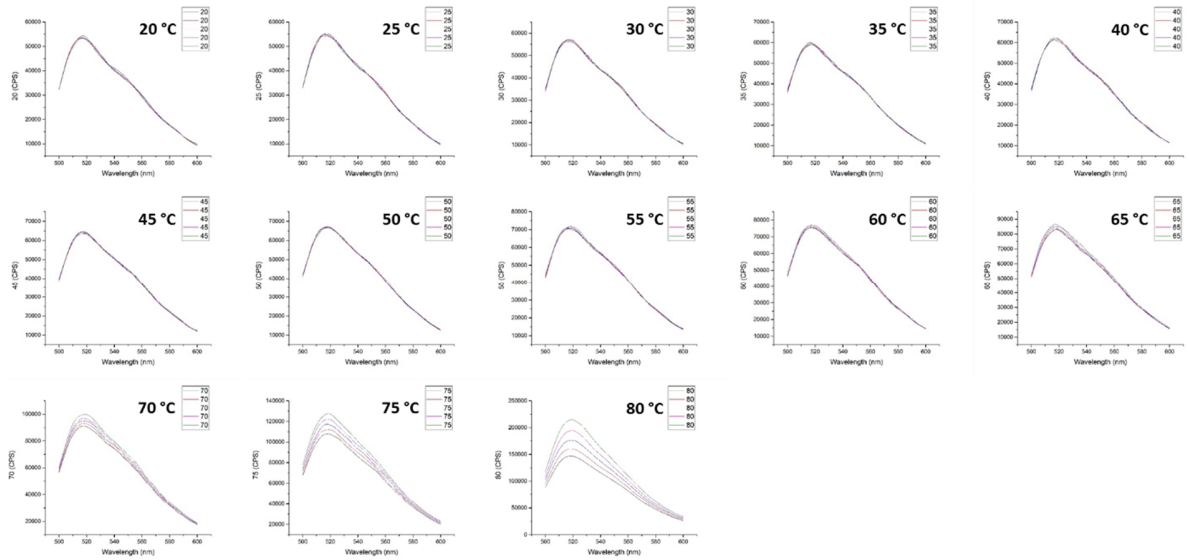

Figure S4. Quintuplicate spectra at each temperature of 20 – 80 °C for annealed RNA repeats. Hold time: 2 min for each scan, totally 10 min at each temperature; 150 μL of 200 nM annealed FM1 r(CUG)$_{10}$ sample with no sisomicin addition; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm.

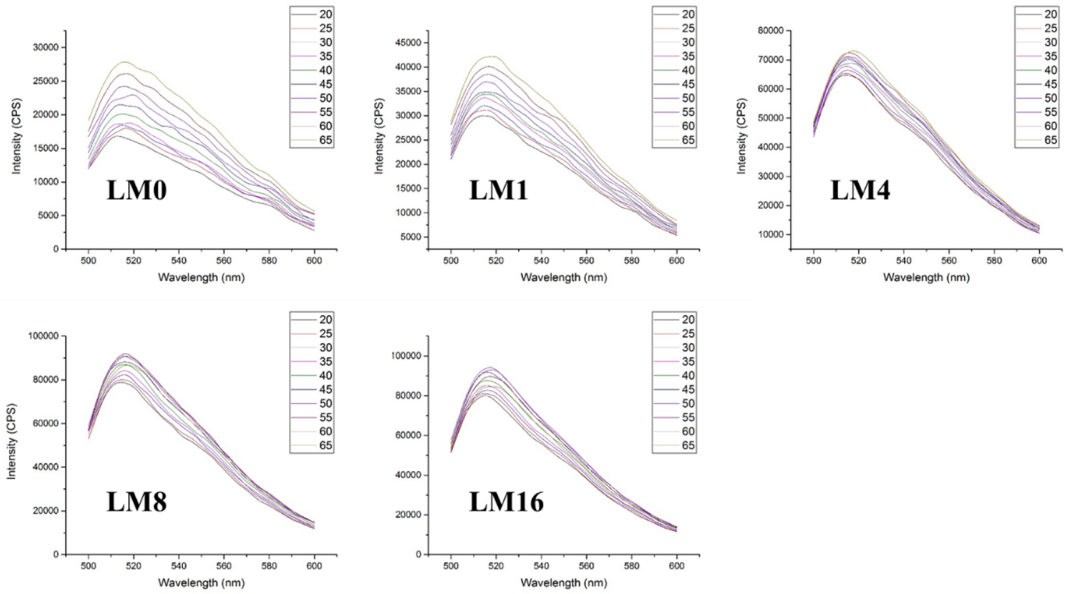

Figure S5. Fluorescence spectra of temperature effect on sisomicin binding towards annealed RNA repeats with different L/M. Annealed FM1 r(CUG)$_{10}$ RNA sample concentration 200 nM and volume size 150 µL; Sisomicin addition volume size 1.5 µL with ligand to RNA molar ratios – L/M at 0, 1, 4, 8, and 16; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm at 20 – 65 °C.

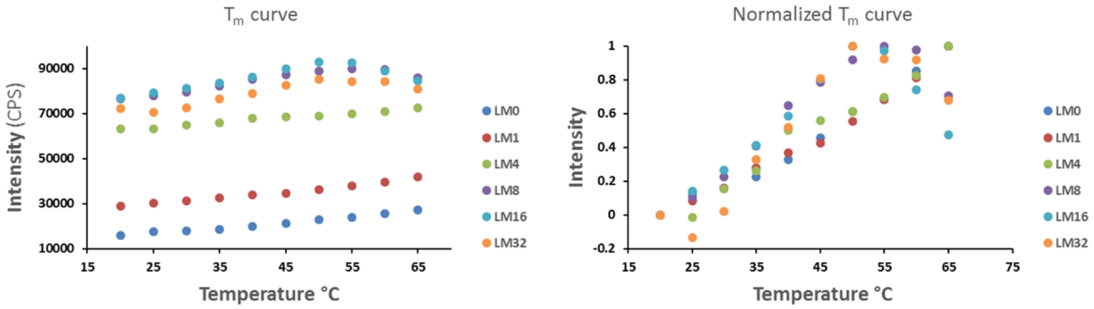

Figure S6. *T_m* curves for sisomicin binding towards annealed RNA with different L/M. Annealed FM1 r(CUG)$_{10}$ RNA sample concentration 200 nM and volume size 150 µL; Sisomicin addition volume size 1.5 µL with ligand to RNA molar ratios – L/M at 0, 1, 4, 8, and 16; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm at 20 – 65 °C.

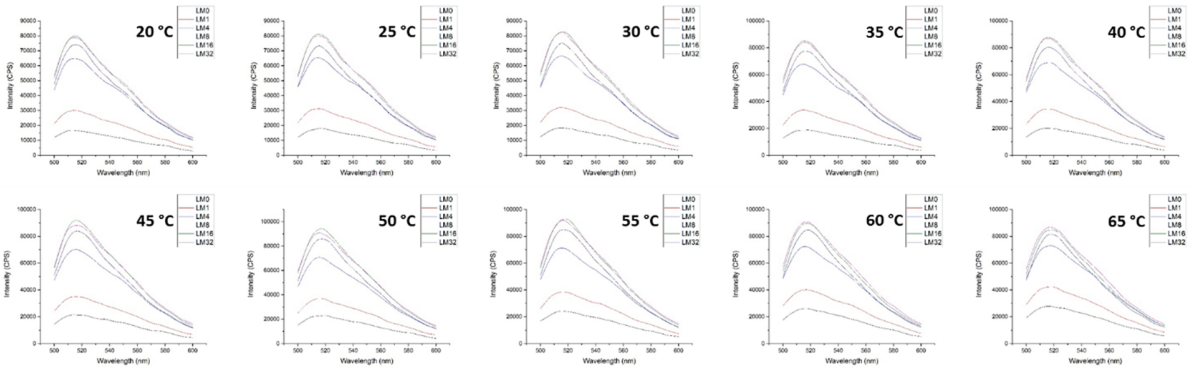

Figure S7. Fluorescence spectra of ligand concentration effect on sisomicin binding towards annealed RNA repeats at 20 – 65 °C. Annealed FM1 r(CUG)$_{10}$ RNA sample concentration 200 nM and volume size 150 µL; Sisomicin addition volume size 1.5 µL with ligand to RNA molar ratios of 0, 1, 4, 8, and 16; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm.

Part IV. Fluorescence characterization on denatured CUG RNA repeats

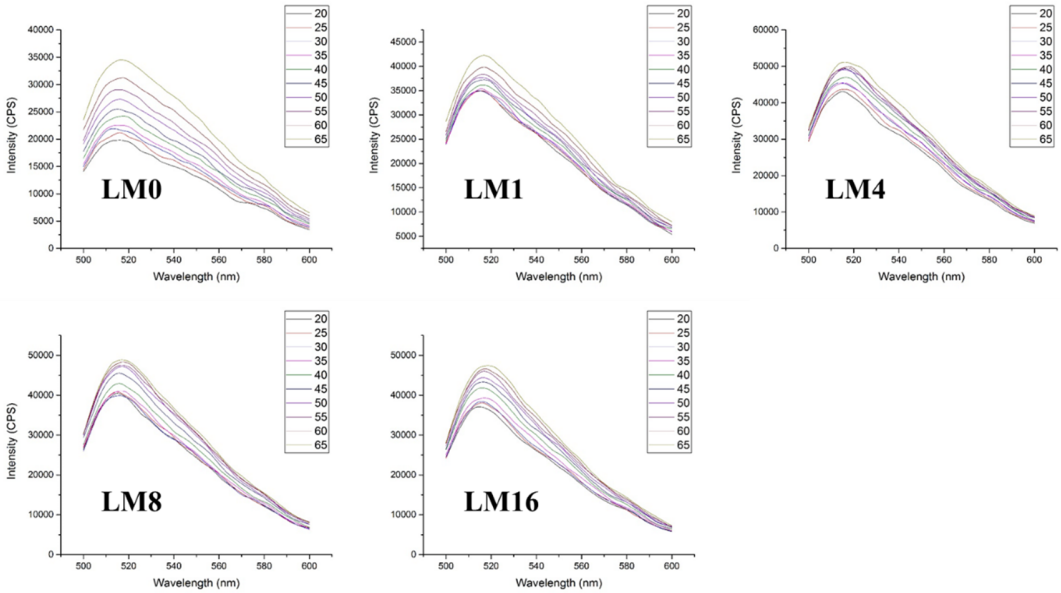

Figure S8. Fluorescence spectra of temperature effect on sisomicin binding towards denatured RNA repeats with different L/M. Denatured FM1 r(CUG)$_{10}$ RNA sample concentration 200 nM and volume size 150 µL; Sisomicin addition volume size 1.5 µL with ligand to RNA molar ratios – L/M at 0, 1, 4, 8, and 16; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm at 20 – 65 °C.

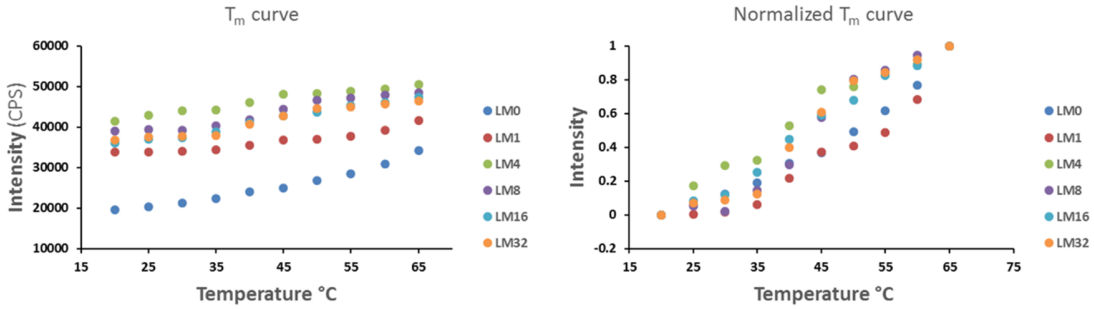

Figure S9. T_m curves for sisomicin binding towards denatured RNA with different L/M. Denatured FM1 r(CUG)$_{10}$ RNA sample concentration 200 nM and volume size 150 µL; Sisomicin addition volume size 1.5 µL with ligand to RNA molar ratios – L/M at 0, 1, 4, 8, and 16; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm at 20 – 65 °C.

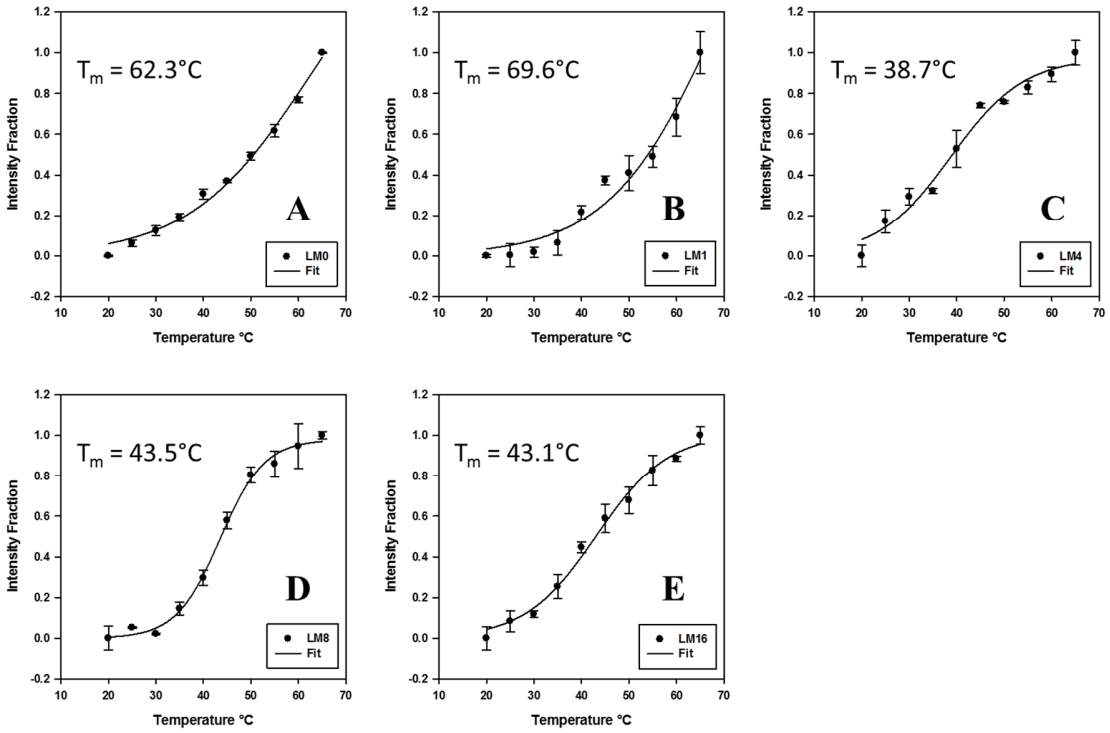

Figure S10. Sigmoidal fittings of T_m curves for titrating sisomicin against denatured FM1 r(CUG)$_{10}$: (A – E) T_m at ligand to RNA molar ratios of 0, 1, 4, 8, and 16. Denatured FM1 r(CUG)$_{10}$ RNA sample concentration 200 nM and volume size 150 µL; Ligand addition volume size 1.5 µL; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm in a range of 20 – 65 °C.

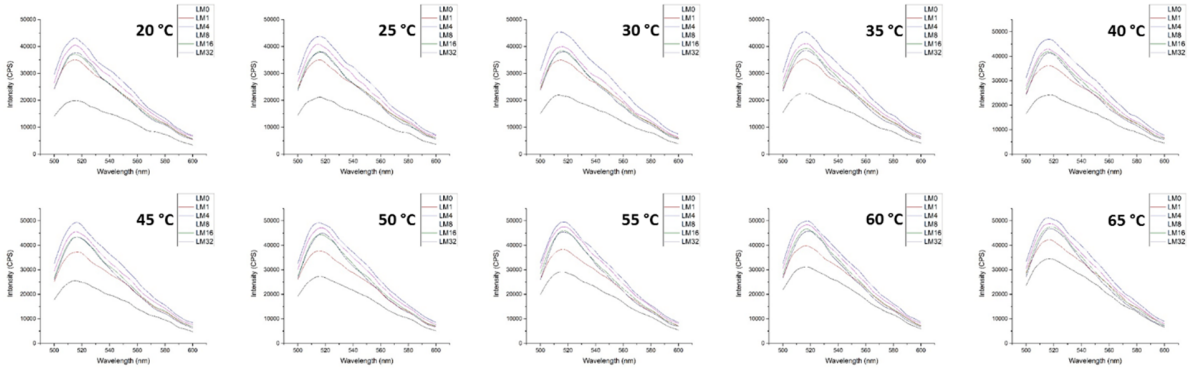

Figure S11. Fluorescence spectra of ligand concentration effect on sisomicin binding towards denatured RNA repeats at 20 – 65 °C. Denatured FM1 r(CUG)$_{10}$ RNA sample concentration 200 nM and volume size 150 µL; Sisomicin addition volume size 1.5 µL with ligand to RNA molar ratios of 0, 1, 4, 8, and 16; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm.

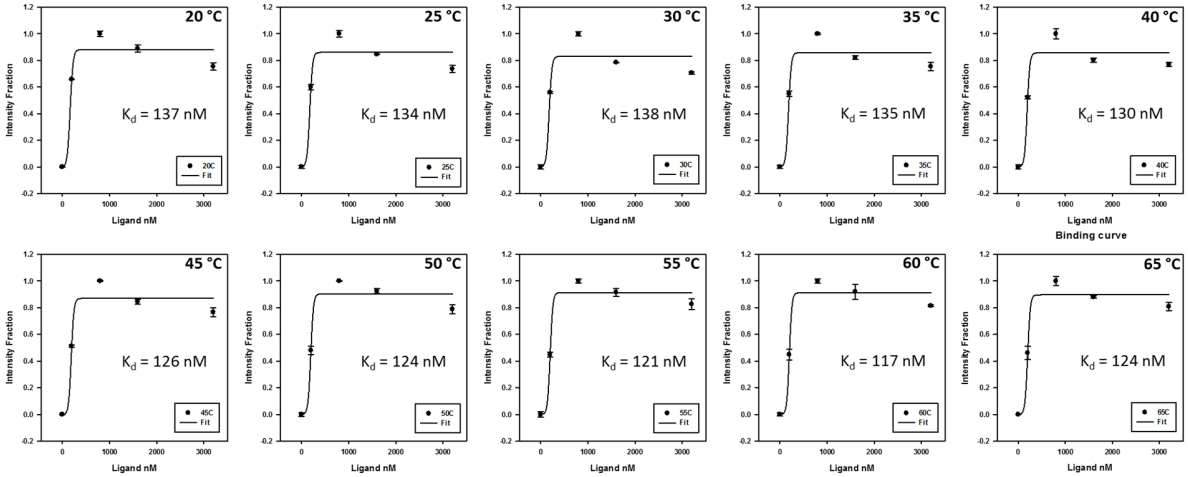

Figure S12. Sigmoidal data fitting of sisomicin binding towards denatured RNA repeats and calculated K_d values at 20 – 65 °C. Denatured FM1 r(CUG)$_{10}$ RNA sample concentration 200 nM and volume size 150 µL; Sisomicin addition volume size 1.5 µL with ligand to RNA molar ratios of 0, 1, 4, 8, and 16; Fluorescence setting: excitation at 485 nm, emission at 500 – 600 nm, and slit width of 3 nm.