Supplementary Information

Measuring RNA-Ligand Interactions with Microscale Thermophoresis

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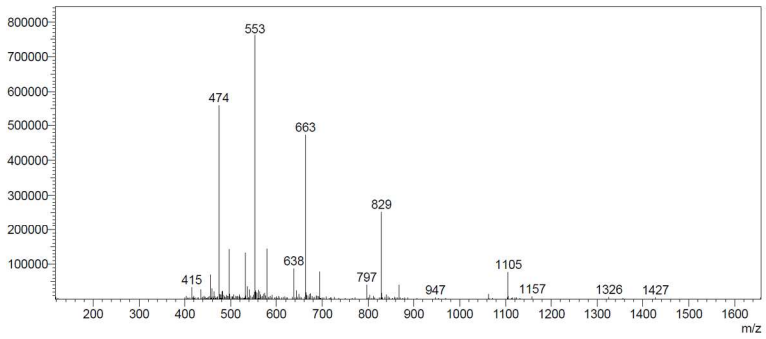
Peptide Synthesis

The fluorescein-labeled Rev peptide was synthesized through Fmoc-based solid-phase peptide chemistry with DIC activation under microwave conditions on Rink amide resin using an automated CEM Liberty Blue peptide synthesizer (Reference for peptide synthesis: Luedtke, N.W.; Tor, Y. *Biopolymers* 2003, 70, 103-119). The following amino acids were used for the synthesis: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Trp(Boc)-OH. N-terminal succinylation was performed by adding 10 eq. of succinic anhydride to the resin-bound peptide in the presence of 20 eq. DIEA in DMF for 3 hrs at room temperature. The resin was then washed three times with DMF, methanol, and DCM and dried. The succinylated peptide was cleaved from the resin using a TFA/thioanisole/1,2-ethanedithiol/anisole (90:5:3:2) cocktail for 2 hrs under an argon atmosphere. The resin was removed from the cleavage cocktail and concentrated. The crude peptide was precipitated with cold diethyl ether and lyophilized. The peptide was purified by reverse-phase HPLC equipped with a Vydac C18 column using a linear gradient of 0-100% of solvent B (0.1% TFA in 9:1 acetonitrile/water) in solvent A (0.1% TFA in water). Following purification and lyophilization, the peptide was dissolved in HEPES buffer (100 mM, pH 7.5) at approx. 0.5 mM and 3 eq. of 5-iodoacetimidofluorescein (from a 10 mg/mL stock in DMF) was added. The mixture was stirred in the dark at room temperature for 3 hrs and lyophilized. The crude mixture was again purified by reverse-phase HPLC as previously described and the purity of the fluorescein-tagged Rev peptide was verified by analytical HPLC and ESI mass spectrometry. MS (ESI) m/z 1104.85 (M+3H)/3 (calc. 1104.22), 828.95 (M+4H)/4 (calc. 828.67), m/z 663.40 (M+5H)/5 (calc. 663.14), m/z 553.05 (M+6H)/6 (calc. 552.78), m/z 474.20 (M+7H)/7 (calc. 473.96).

Fl-Rev Peptide: $_{suc}$TRQARRNRRRRWRERQRAAAAC$_{am}$-Fluorescein

Mass Spec:



HPLC:

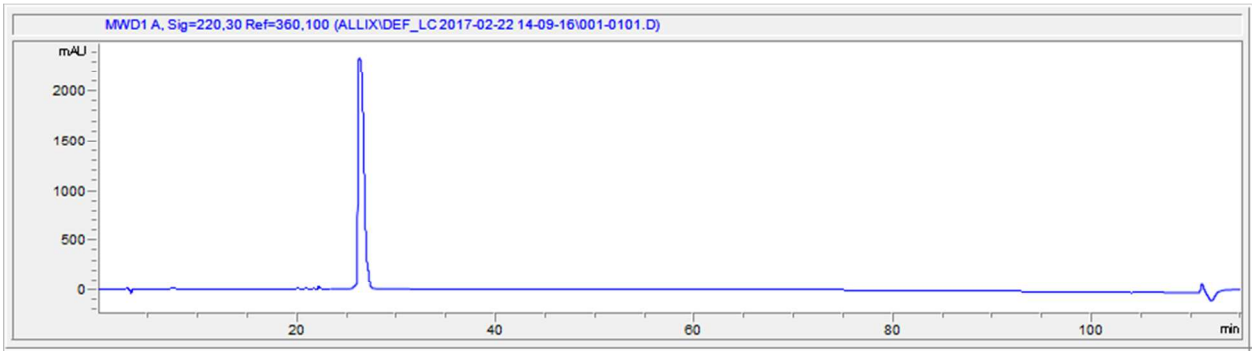
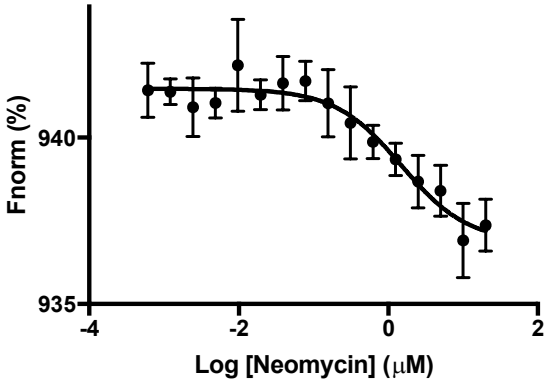
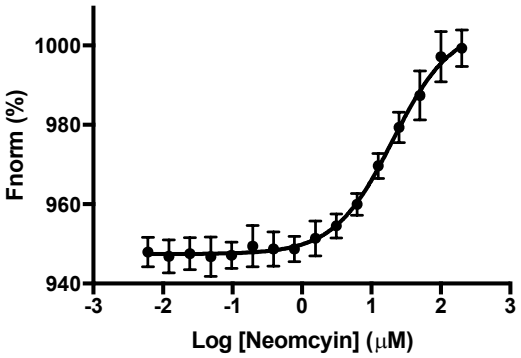


Figure S1. MST curves for RRE/ligand interactions (direct/competitive binding)

RRE Neomycin



Rev-RRE Neomcyin displacement



RRE Rev Neomycin competitive binding