Supporting Information for

Design of a β-Hairpin Peptide-Intercalator Conjugate for Simultaneous Recognition of Single Stranded and Double Stranded Regions of RNA

Lauren L. Cline and Marcey L. Waters*

Department of Chemistry, The University of North Carolina at Chapel Hill, CB# 3290, Chapel Hill, NC 27599. Email: mlwaters@email.unc.edu; Fax: +1 919 962 2388; Tel: +1 919 843 6522

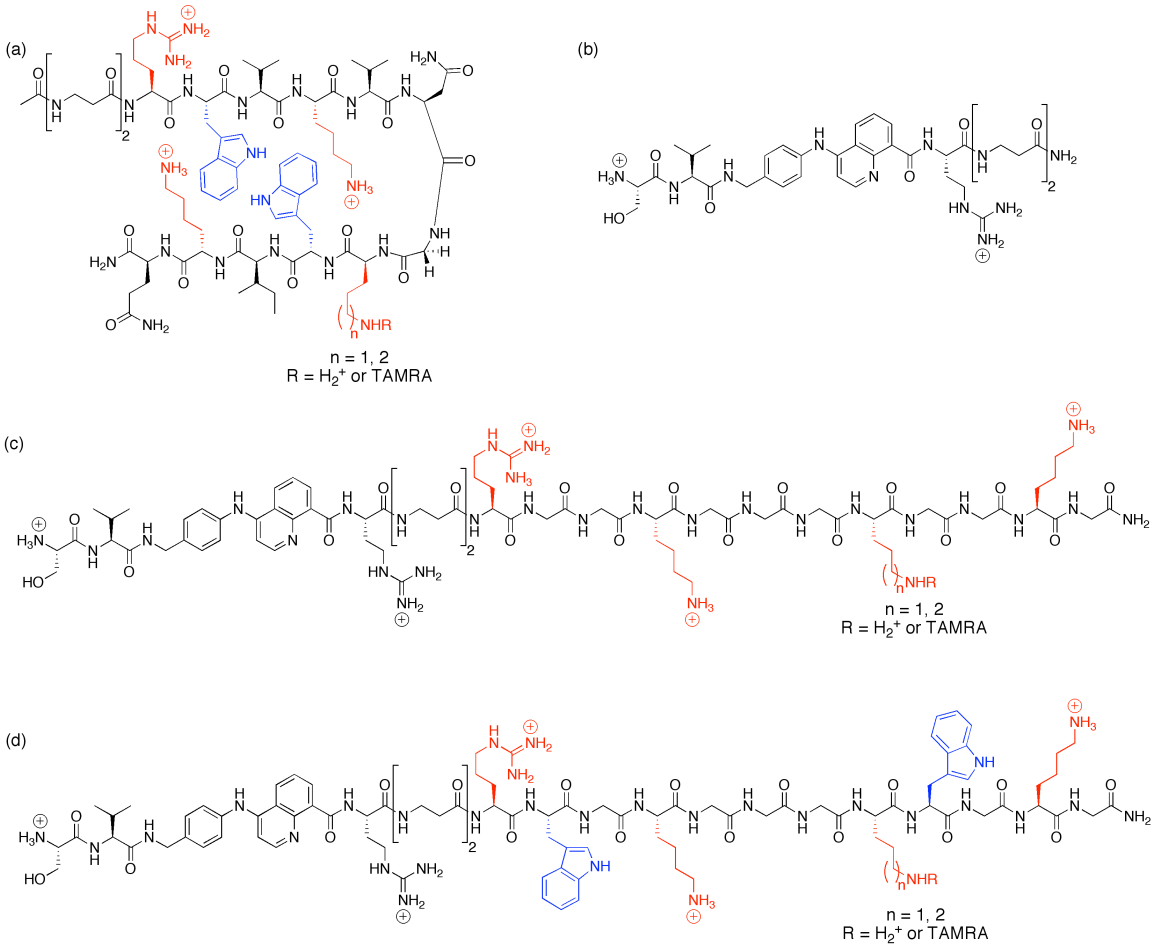

Fig. S1. Structure and sequences of the control peptides. The cationic residues from the WKWK peptide portion of the probes are highlighted in red and the aromatic residues are highlighted in blue. a) **WKWK-control**. b) **Int-control**. c) **GKGK-Int**. d) **GWKWKG-Int**.

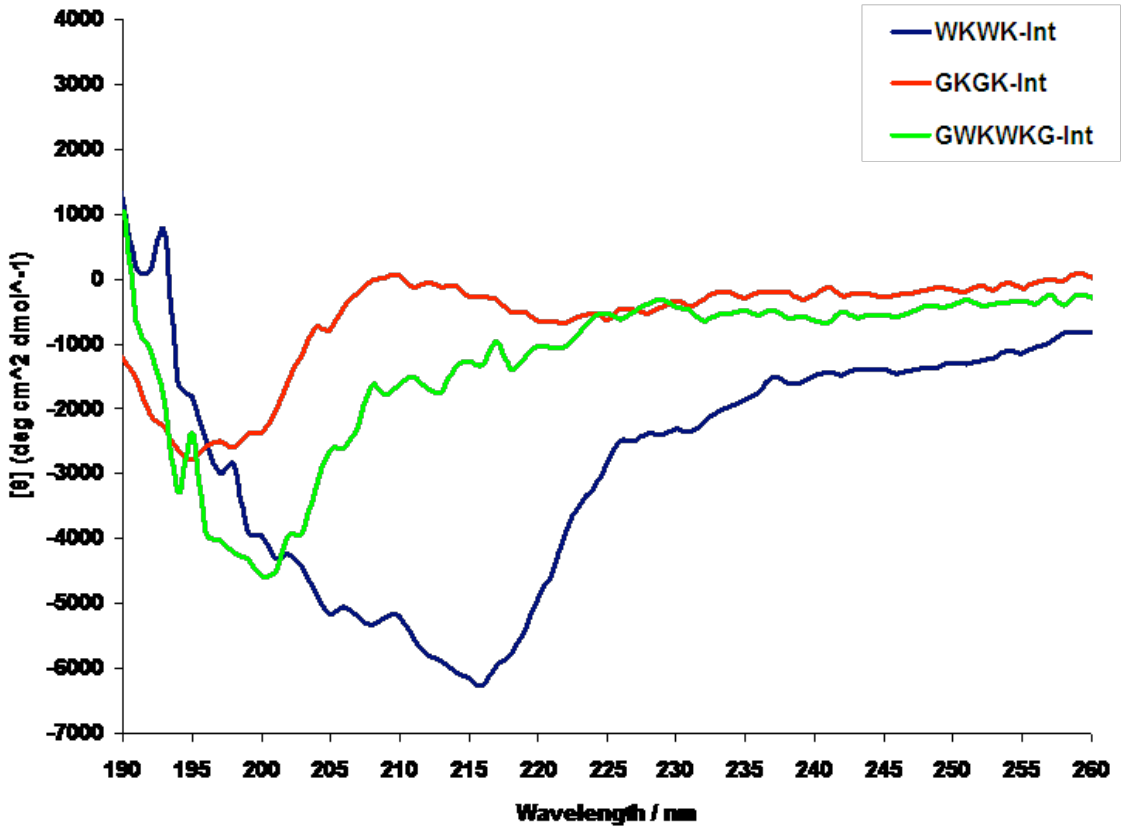

Fig. S2. Circular dichroism spectrum for the **WKWK-Int** probe (blue) and the unstructured controls, **GKGK-Int** (red) and **GWKWKG-Int** (green). The CD experiments were run using 100 μM peptide in 10 mM sodium phosphate buffer (pH 7.6) at 25°C in a 0.1 cm cell. The spectra that are shown are an average of three scans.

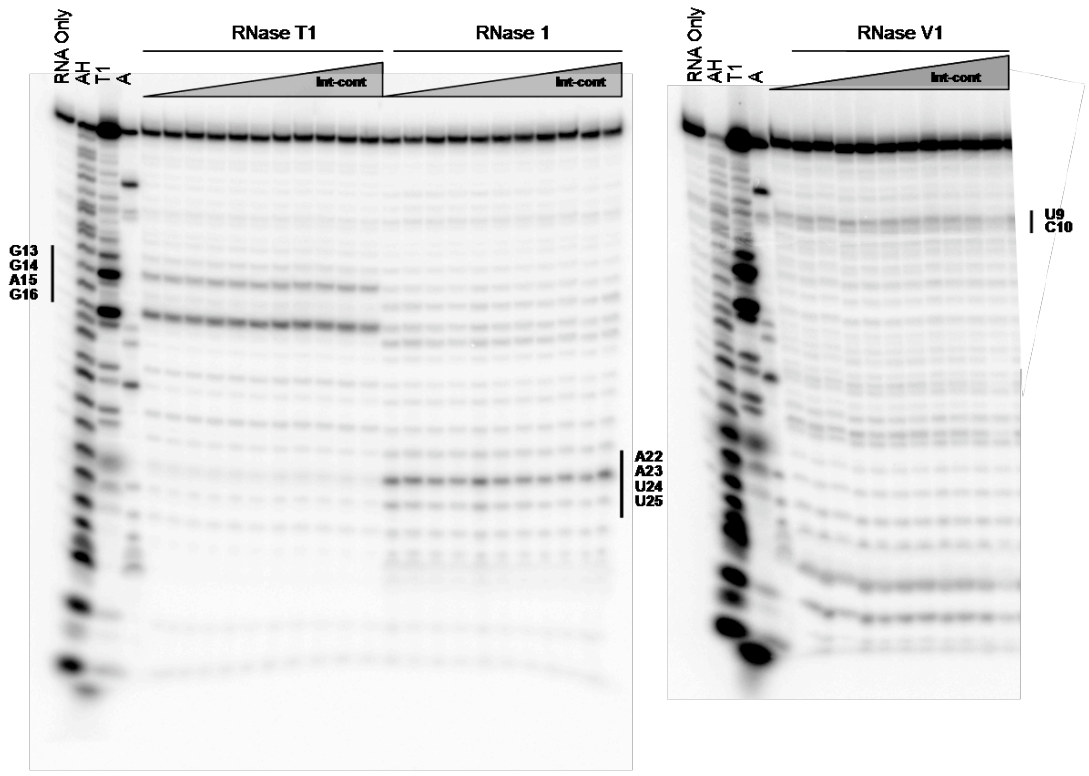

Fig. S3. RNase footprinting experiments with **GGAGloop RNA** and the **Int-cont** peptide. Shown is the phosphorimage of a 20% denaturing polyacrylamide gel. Structure lanes: RNA only, folded RNA in buffer; AH, alkaline hydrolysis; T1, RNase T1 (G-lane); A, RNase A (C and U lane). Increasing concentrations (0 to 100 μM) of **Int-cont** was added to **GGAGloop RNA** (0.18 μM) in 10 mM PBS buffer (140 mM Na^+/K^+Cl^-, 1 mM $MgCl_2$, pH 7.4) and 5 ng/μL yeast tRNA. RNase T1 and RNase 1 were used to visualize the binding in the single-stranded regions of RNA (left plot) and RNase V1 was used to visualize binding in the double-stranded regions of the RNA (right plot). Minimal protection was detected even at the highest concentration of **Int-cont** added.

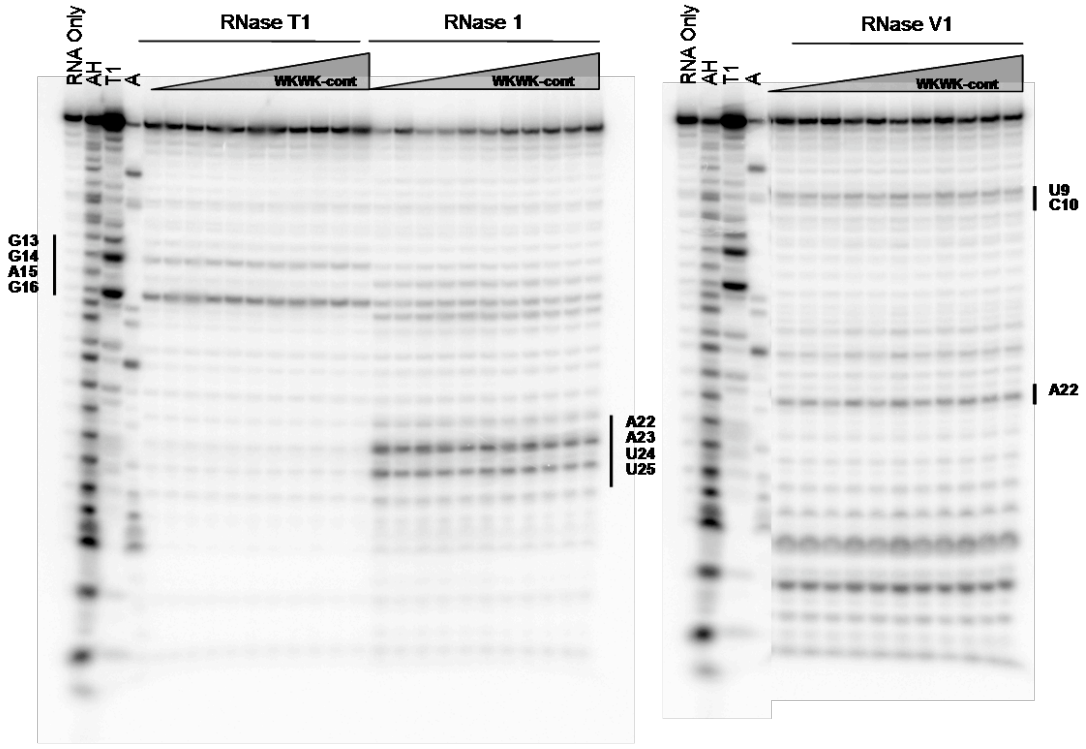

Fig. S4. RNase footprinting experiments with **GGAGloop RNA** and the **WKWK-cont** peptide. Shown is the phosphorimage of a 20% denaturing polyacrylamide gel. Structure lanes: RNA only, folded RNA in buffer; AH, alkaline hydrolysis; T1, RNase T1 (G-lane); A, RNase A (C and U lane). Increasing concentrations (0 to 100 μM) of **WKWK-cont** was added to **GGAGloop RNA** (0.18 μM) in 10 mM PBS buffer (140 mM Na^+/K^+Cl^-, 1 mM $MgCl_2$, pH 7.4) and 5 ng/μL yeast tRNA. RNase T1 and RNase 1 were used to visualize the binding in the single-stranded regions of RNA (left plot) and RNase V1 was used to visualize binding in the double-stranded regions of the RNA (right plot). Minimal protection was detected even at the highest concentration of **WKWK-cont** added.

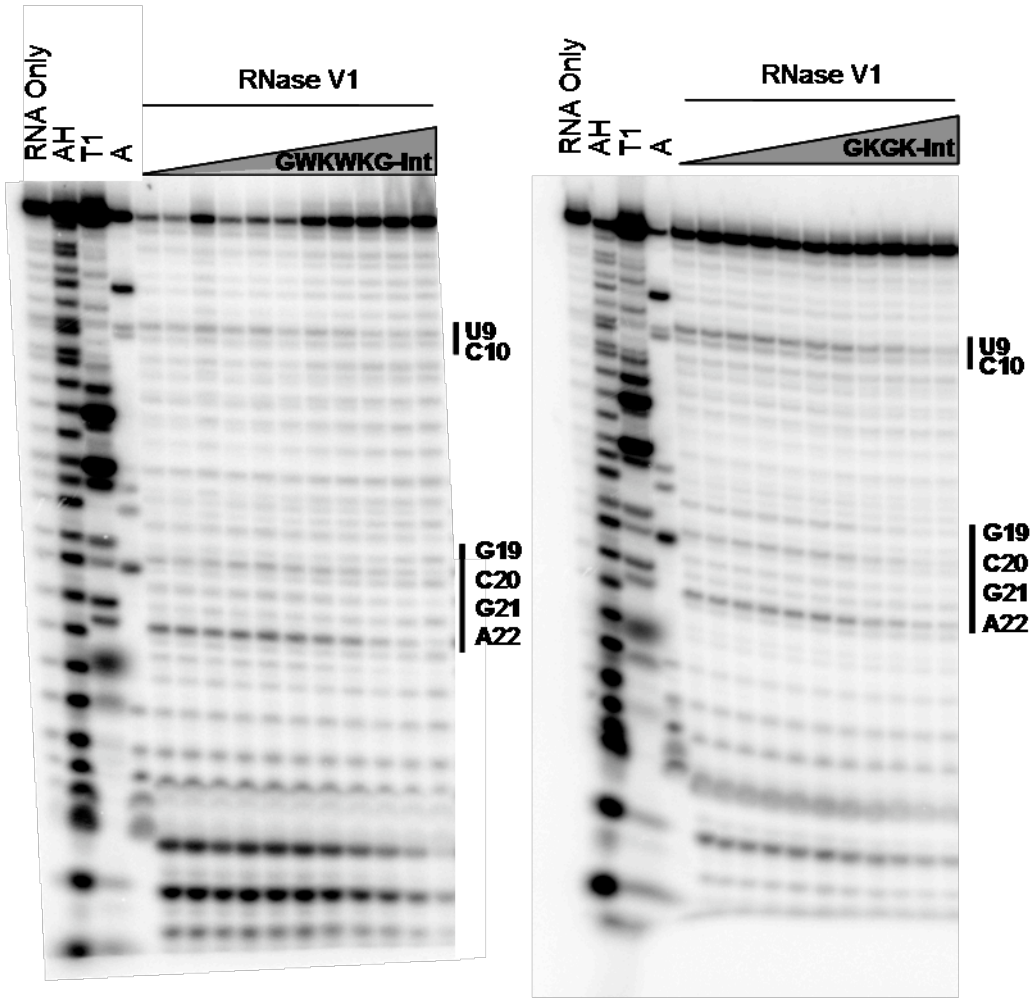

Fig. S5. RNase V1 footprinting experiments of **GGAGloop RNA with GWKWKG-Int and GKGK-Int**. Shown is the phosphorimage of a 20% denaturing polyacrylamide gel. Structure lanes: RNA only, folded RNA in buffer; AH, alkaline hydrolysis; T1, RNase T1 (G-lane); A, RNase A (C and U lane). Increasing concentrations of **GWKWKG-Int** (left plot) and **GKGK-Int** (right plot) were added to **GGAGloop RNA** (0.18 μM) in 10 mM PBS buffer (140 mM Na^+/K^+Cl^-, 1 mM $MgCl_2$, pH 7.4) and 5 ng/μL yeast tRNA. RNase V1 (cleaves double stranded regions) was used to visualize binding in the stem regions of the RNA.

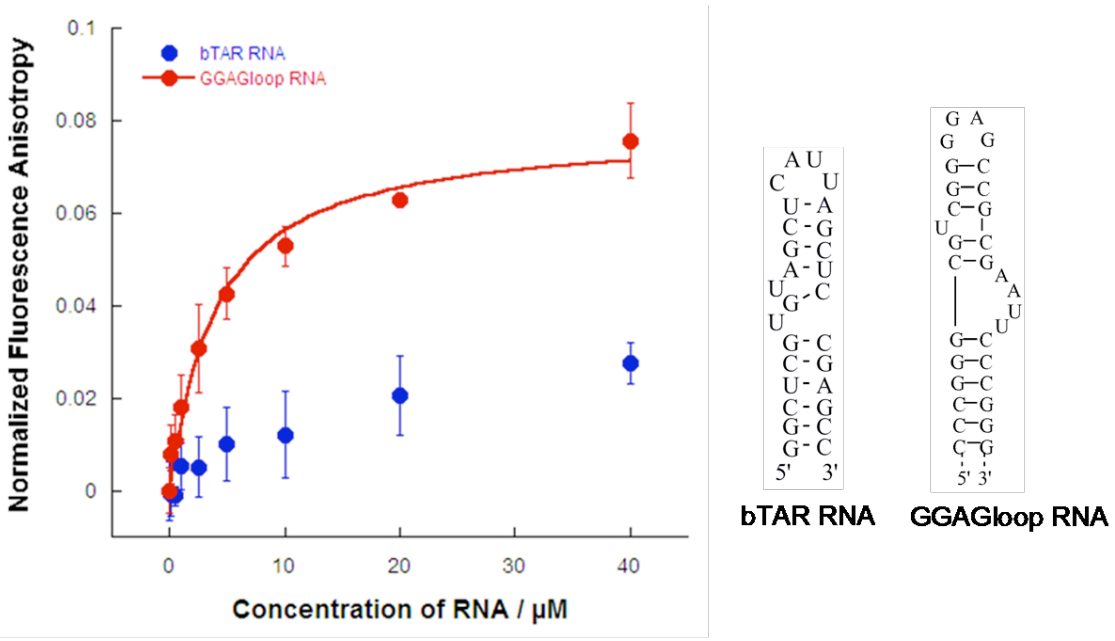

Fig. S6. Fluorescence anisotropy binding experiments of TAMRA-labeled **WKWK-Int** (0.5 μM) binding to **BIV TAR RNA** (K_d > 100 μM) or **GGAGloop RNA** (K_d = 3.7± 0.7 mM). Experiments were done with 0.5 μM **WKWK-Int** in 10 mM PBS buffer (140 mM Na^+/K^+Cl^-, 1 mM $MgCl_2$, pH 7.4) at 25°.

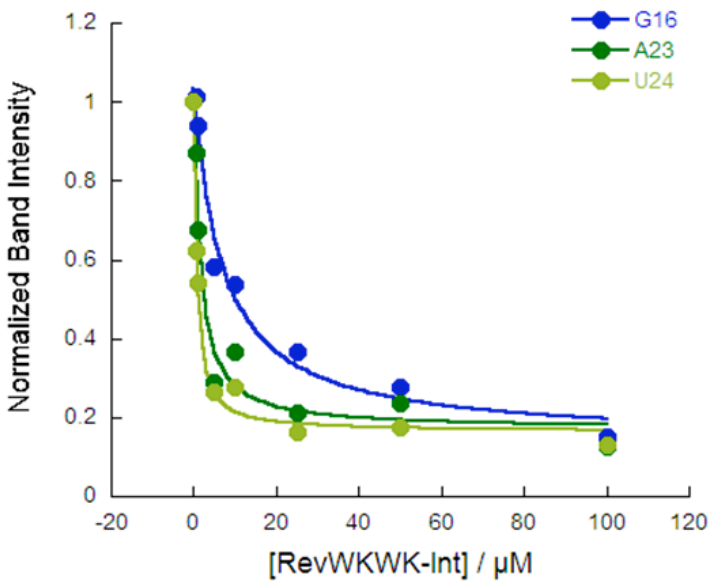

Fig. S7. ImageQuant analysis of RNase footprinting assay of **RevWKWK-Int** binding to **GGAG RNA**. **RevWKWK-Int** binds to bases G16 (light blue), A23 (dark green), and U24 (light green). The local dissociation constants were determined to be 1.5 (± 0.5) µM for base A23, 0.7 (± 0.1) µM for U24, and 6.8 (± 1.8) µM for G16. It is hypothesized that because this peptide has nearly the same local binding affinities as WKWK-Int, it binds with a similar orientation.

Table S1. Mass Spectrometry Data for Peptides Studied in this Work.

Peptide	Expected Mass (Da)	Observed Mass (Da)
WKWKcontrol	1710.0	1710.4
WKWKcontrol(TAMRA)	2136.1	2136.2
Intcontrol	776.4	776.6
Intcontrol(TAMRA)	1316.6	1316.8
WKWK-Int	2285.3	2285.0
WKWK(TAMRA)-Int	2711.5	2711.5
GKGK-Int	1759.0	1758.9
GKGK(TAMRA)-Int	2187.2	2186.1
GWKWKG-Int	2017.1	2017.1
GWKWKG(TAMRA)-Int	2445.3	2444.9
RevWKWK-Int	2285.3	2286.0
RevWKWK(TAMRA)-Int	2710.5	2711.1