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for

Effects of Asymmetric Arginine Dimethylation on RNA Binding Peptides: Methylation of the Rev Peptide Either Reduces or Increases Binding Affinity with RRE RNA

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Experimental details of the following:

I. General

Fmoc-protected α-amino acids and Rink Amide MBHA resin were purchased from Novabiochem (San Diego, CA). N^{α}-Fmoc-N^{ω},N^{ω}-asymmetric dimethylarginine Hydrochloride salt was purchased from *Bachem*. Dimethylformamide (DMF), N,N-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), 1,2-dichloromethane (DCM), and piperidine were purchased from Sigma-Aldrich and used as supplied.

For all RNA-related applications, water was autoclaved and treated with 0.1% diethylpyrocarbonate (DEPC).

II. Syntheses of Peptides

The peptides were synthesized manually using Fmoc solid phase peptide chemistry on Rink amide MBHA resin with loading levels of 0.4-0.6 mmol/g resin. All peptides were synthesized in 0.38 μmol scale using a protocol for the solid phase synthesis. The detail of the syntheses of peptides has been described elsewhere[1]. All peptides were succinylated at the N-terminus except for Rev_{50-34} peptide. All peptides were confirmed by an Auto Flex II MALDI-TOF/TOF mass spectro-meter (Bruker Daltonics, Germany) equipped with a 337 nm nitrogen laser and 1.2 m flight tube. All peptides were HPLC-purified using Agilent 1100 instrument. HPLC traces and MALDI-TOF data are shown in Figure S1 and Table S1, respectively.

III. Circular Dichroism (CD)

CD measurements were performed at 20°C on a *JASCO* model J715 spectropolarimeter equipped with Peltier temperature controller and running *JASCO* Spectra Manager™ software. Spectra were acquired from 190 – 250 nm at a scan speed 20 nm/ min, data pitch 0.5 nm, response time 16 sec, band width 1.0 nm, and sensitivity 100 mdeg using 0.1 cm pathlength cuvette, and the signals were averaged of 2 accumulations. The α-helical content of each peptide was calculated as reported previously[1]. Representative CD spectra of rev peptides in the absence and presence of RRE RNA are shown in Figure S2.

IV. Electrophoretic Gel Mobility Shift Assay

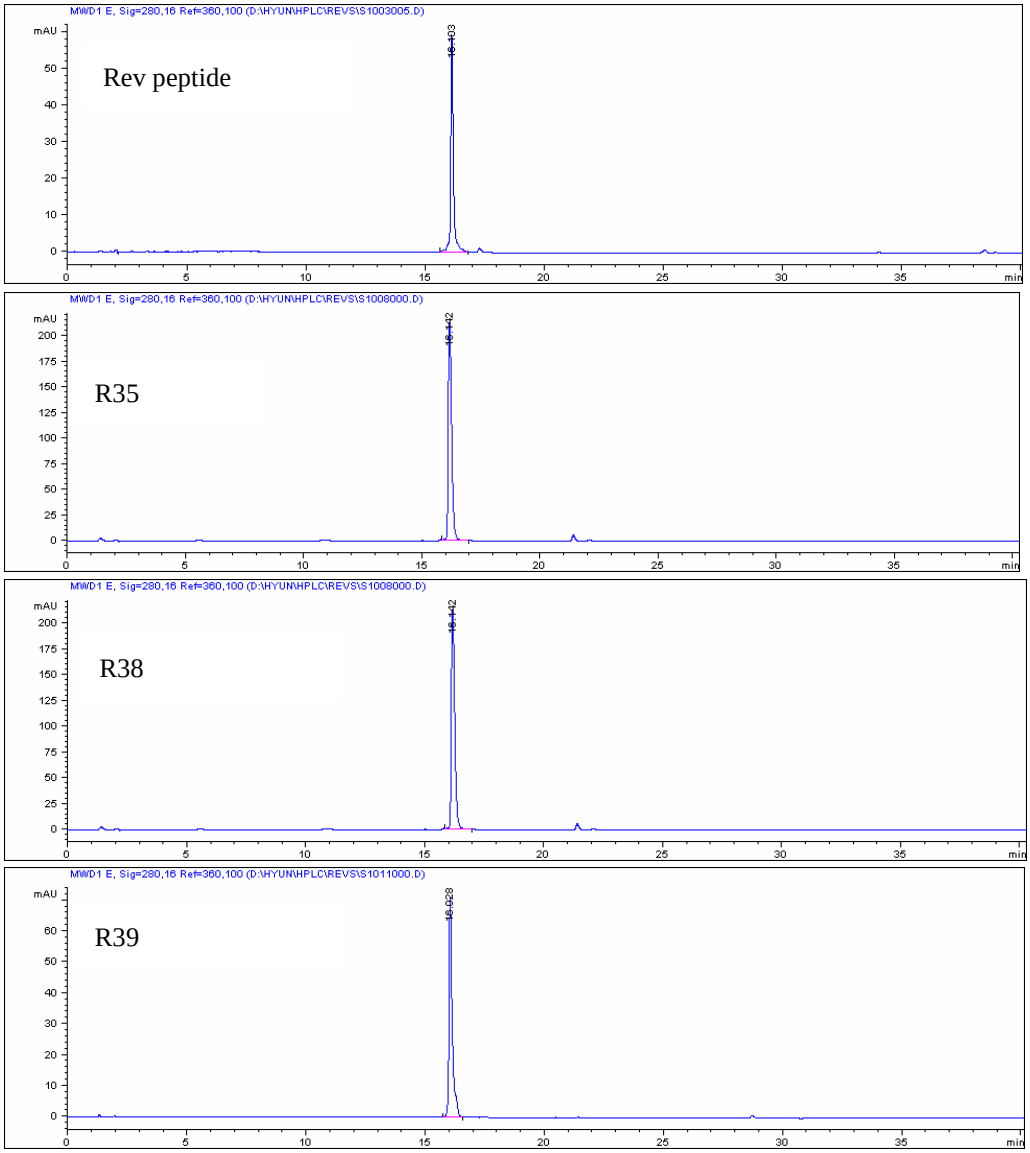
Labeling of RRE RNA. RRE probe (~ 30 pmoles) was radioactively labeled at the 5'-end by using 100 μCi of [γ-^{32} P] ATP (New England Biolabs) and 50 units of polynucleotide kinase (New England Biolabs), after the hydrolysis of 5'-phosphate by 20 units of alkaline phosphatase (CIP, New England biolabs). Labeled probe was separated from small nucleotides by passage through G-25 Sephadex beads (Sigma).

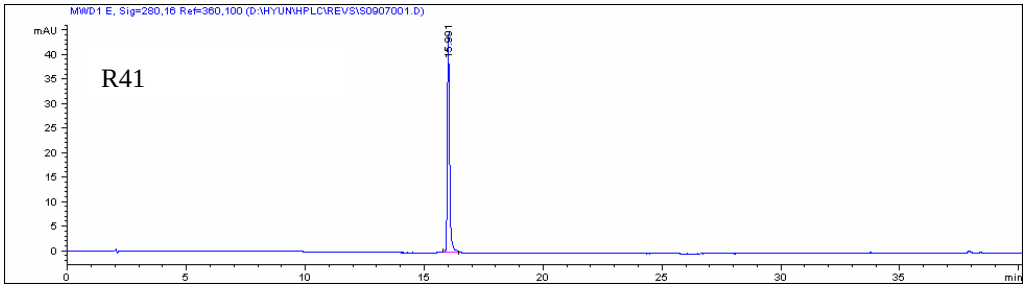
Electrophoretic Gel Mobility Shift Assay. A labeled solution of 10 nM RRE RNA was heated to 65^{o}C for 5 min and slowly cooled to room temperature over over 1 h in a buffer containing 20 mM 4-(2-hydroxyethyl) piperazine-1-ethansulfonic acid (HEPES), 1 mM $MgCl_2$, 5 mM KCl, and 140 mM NaCl at pH 7.4. All binding reaction samples were performed on a 10 μL scale with final concentrations of 1 nM RRE mixed with the indicated amount of peptide in binding buffer (90 mM Tris-borate, 2 mM EDTA, 50 mM $MgCl_2$, 100 mM NaCl, and 0.01% Tween20). The binding mixtures were incubated on ice for 1 hr. To the resulting sample solution, loading buffer (2.6 μL, 50 % glycerol in 1.25 × Tris-Borate and EDTA (TBE) buffer) were added. A 10% native polyacrylamide gel (1 mM $MgCl_2$ with 4% glycerol) that had been pre-run for 30 min was used. Each sample (12 μL) was loaded on the 10% native polyacrylamide gel and run at 150 V, 4^{o}C in 0.5 × TBE running buffer (45 mM Tris-Borate/1 mM EDTA) for 2 h 30 min to 3 h. The gel was exposed to a phosphorimager screen and individual bands were quantified on a FLA-3000 and analyzed with Multi Gauge Ver. 3.0 software (Fuji Photo). Representative autoradiograms of each peptide from EMSA are shown in Figure S3.

References and Notes

(1) Hyun, S.; Kim, H. J.; Lee, N. J.; Lee, K. H.; Lee, Y.; Ahn, D. R.; Kim, K.; Jeong, S.; Yu, J. *J. Am. Chem. Soc*. **2007**, *129*, 4514-4515.

Figure S1. Chromatograms of Rev peptides. A Zorbax C_{18} (3.5 µm, 4.6 x 150 mm) column was used as the stationary phase. For the mobile phase, buffer A (water with 0.1% v/v TFA) and buffer B (acetonitrile with 0.1% v/v TFA) were used as a gradient. The gradient conditions are as follows: 5 min, 5% B followed by linear gradient 5-60% B over 35 min. Each peptide was labeled on a respective chromatogram.

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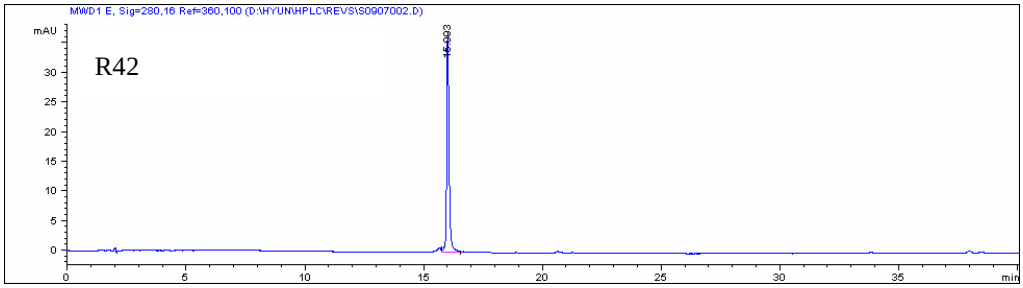
R41
min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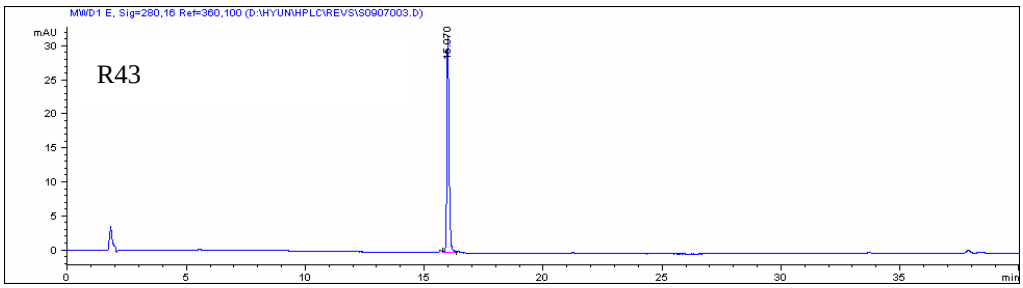
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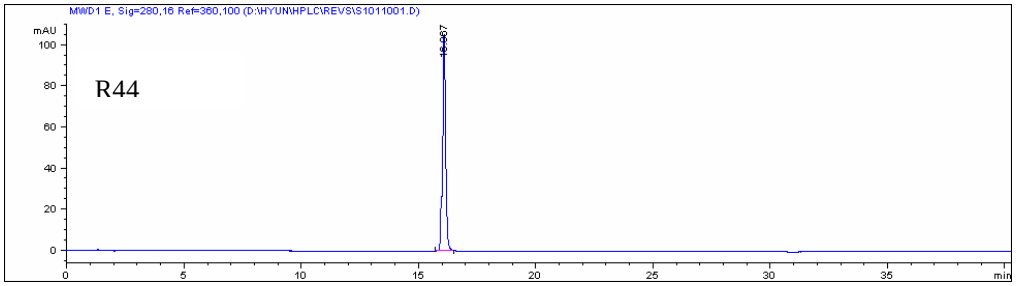
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R44
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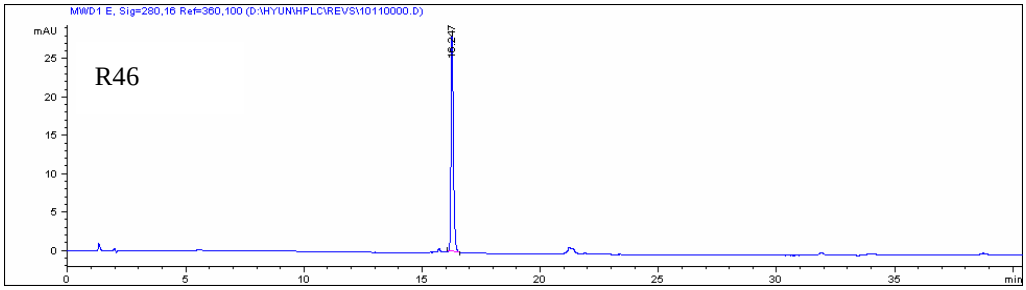
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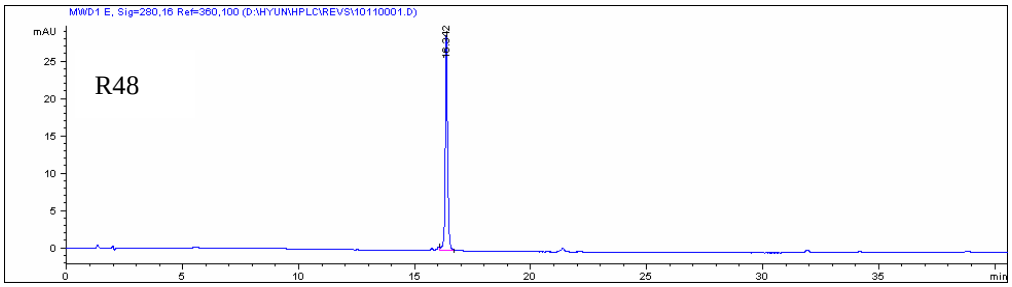
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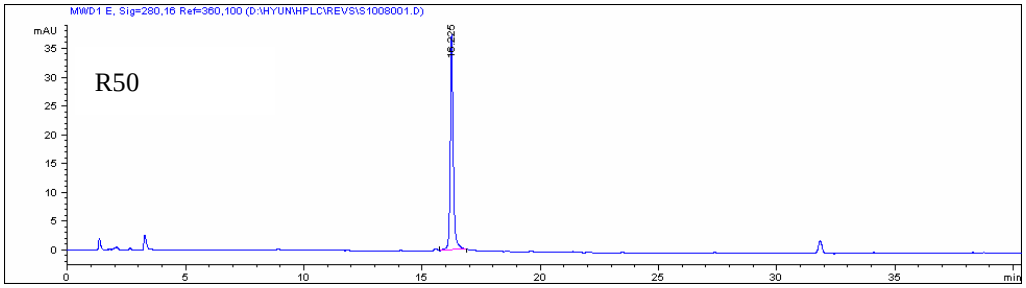
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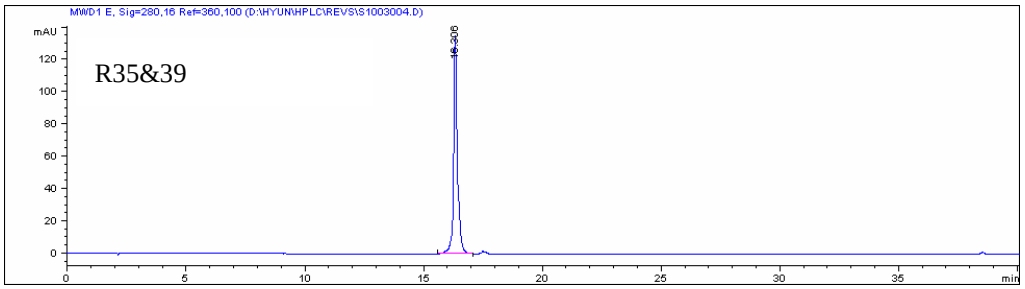
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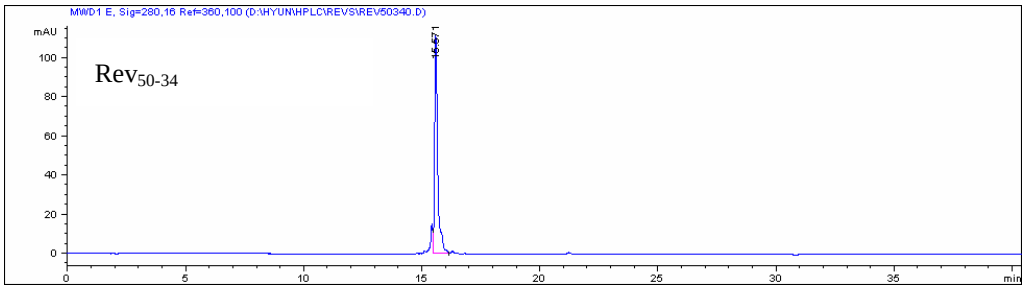
R35&39
min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mAU
16.671
Rev50-34
min

Figure S2. CD spectra of 10 μM Rev peptide, R50, and Rev_{50-34} in the absence and presence of same concentration of RRE RNA, as a representative example. Spectra of peptide and RRE were measured in a buffer solution containing 10 mM potassium phosphate and 150 mM NaF (pH 7.4) at 20°C. Red, 10 μM RRE only; blue, 10 μM of Rev peptide; green, Rev-RRE complex (1:1 stoichiometry); black, 10 μM of R50; pink, R50-RRE complex (1:1 stoichiometry); cyan, 10 μM of Rev_{50-34}; orange, Rev_{50-34}-RRE complex (1:1 stoichiometry).

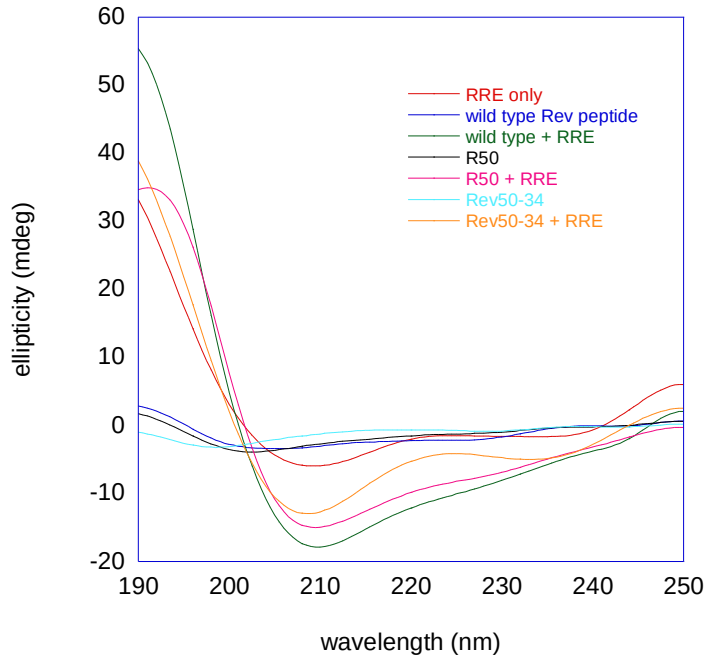
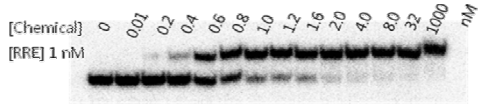


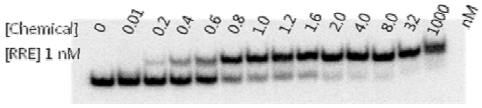
Figure S3. EMSA analysis of Rev peptide and asymmetric dimethylarginine mutants binding to RRE. Indicated increasing amounts of peptides were mixed with radioactively labeled RRE (~1 nM). Values indicate average and one standard deviation of at least three experiments.

wt



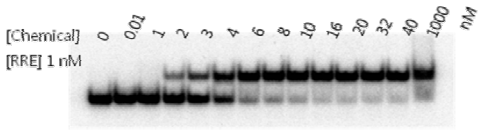
K_d (nM) 0.77 ± 0.12

R35



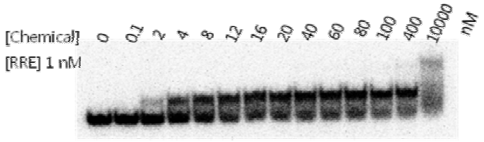
K_d (nM) 0.63 ± 0.062

R38



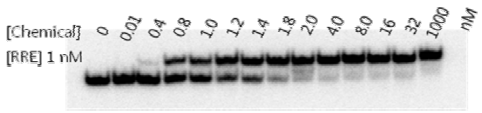
K_d (nM) 3.5 ± 0.12

R39



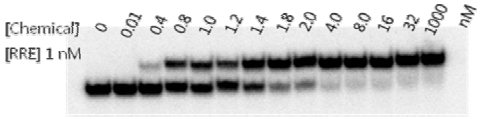
K_d (nM) 19 ± 4.2

R41



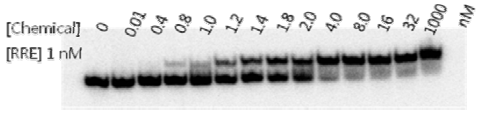
K_d (nM) 1.2 ± 0.23

R42



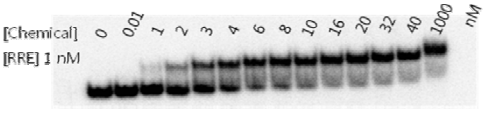
K_d (nM) 1.3 ± 0.19

R43



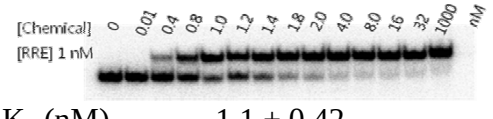
K_d (nM) 1.7 ± 0.11

R44



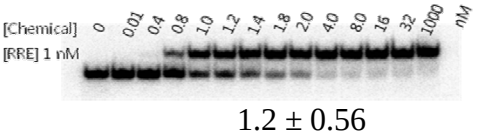
K_d (nM) 4.3 ± 0.44

R46



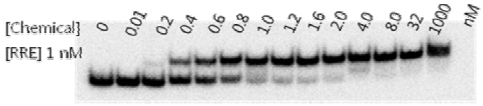
K_d (nM) 1.1 ± 0.42

R48



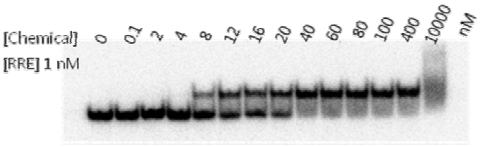
K_d (nM) 1.2 ± 0.56

R50



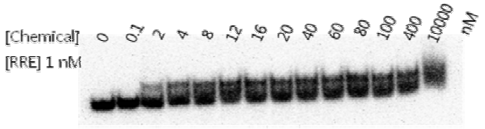
K_d (nM) 0.58 ± 0.073

R35&39



20 ± 9.4

$Rev_{50\text{-}34}$



K_d (nM) 48 ± 7.4

Figure S4. Selectivity analysis of Rev peptide and R50. A, a representative EMSA assay in which 1 nM RRE was mixed with increasing amounts of wild type Rev peptide (0–1000 nM) in the absence of $tRNA^{mix}$, B, a representative EMSA assay of wild type Rev peptide (0–1000 nM) in the presence of 10–fold molar excess of $tRNA^{mix}$, C and D, representative EMSA assays of R50 in the absence and presence of 10–fold molar excess of $tRNA^{mix}$, respectively.

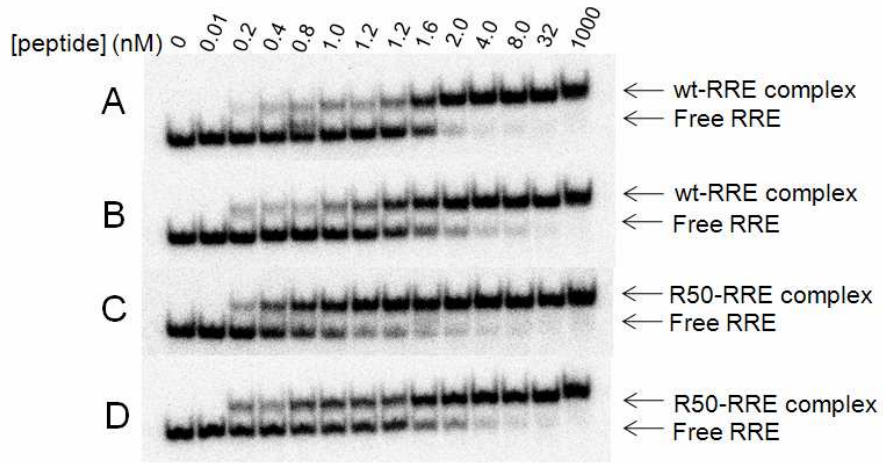


Table S1. Mass spectrometry data for the peptide described in this study.[a]

peptide	Calculated mass	Found mass
wt[b]	2976.670	2976.256
R35	3004.701	3004.828
R38	3004.701	3005.585
R39	3004.701	3004.783
R41	3004.701	3004.327
R42	3004.701	3004.680
R43	3004.701	3004.498
R44	3004.701	3004.742
R46	3004.701	3004.430
R48	3004.701	3004.638
R50	3004.701	3005.490
R35&39	3032.733	3033.868
Rev_{50-34}[c]	2478.415	2477.907

[a]Each number represents the asymmetric dimethylarginine mutant of Rev peptide at the designated position. [b]Amino acid sequence of Rev peptide is Suc-TR(35)QARRN(40)RRRRW(45)RERQR(50)RAAAAR-NH_2. [c]The sequence of Rev_{50-34} is AcRQRERWRRRRNRRAQRT-NH_2.

Table S2. Specificity assay for selected mutants.

Rev peptide	K_d (nM)	RRE specificity ratio[a]
wt	0.77 ± 0.12	1.3
R35	0.63 ± 0.062	2.5
R38	3.5 ± 0.12	2.1
R39	19 ± 4.2	2.3
R44	4.3 ± 0.44	3.6
R50	0.58 ± 0.073	1.4

[a]specificity ratio = K_d in the presence of 10-fold molar excess $tRNA^{mix}$ /K_d in the absence of $tRNA^{mix}$.