Electronic Supplementary Information for RSC Advances

Discovery of a novel small molecular peptide that disrupts helix 34 of bacterial ribosomal RNA

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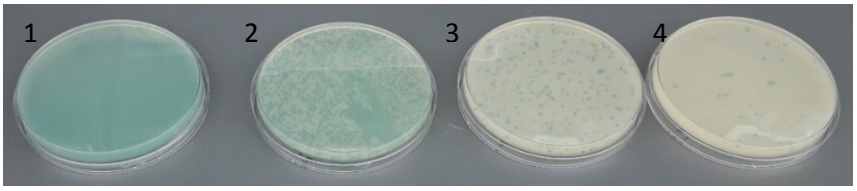

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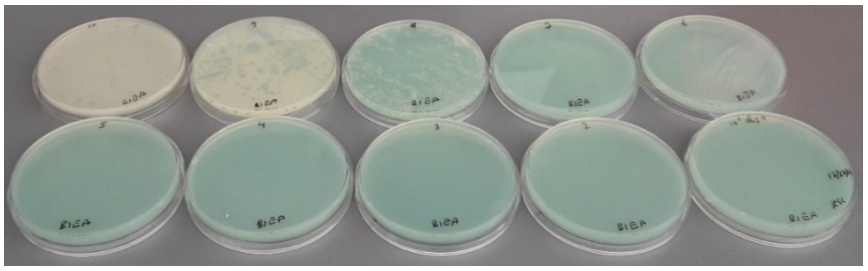

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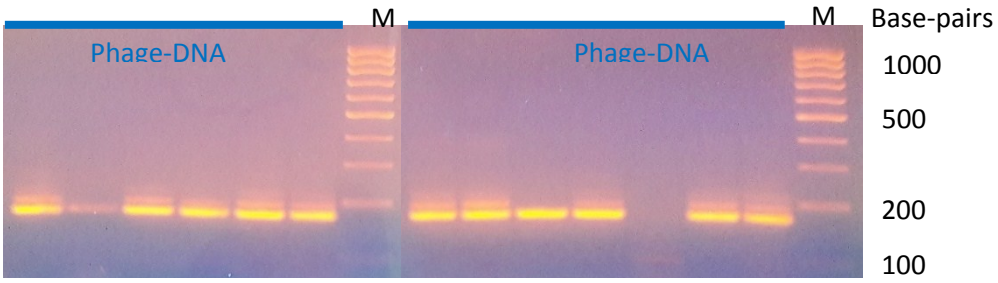

Fig. S1. Screening of phage libraries to discover peptides that bind to target the second strand of helix 34 RNA (3h34). a) Steps of biopanning cycles are shown. b) Representative phage titer plates after biopanning before amplification that shows up to 4th dilution plate {10^4 plaque-forming units (pfu)}. c) Representative phage titer plates after amplification of the eluate that shows up to the 10th dilution plate (10^{10} pfu). d) One percent agarose gel image shows DNA bands just below 200 base-pairs confirm the amplification of the extracted DNA from the isolated phage. M represents the 100 bp DNA ladder (New England BioLabs).

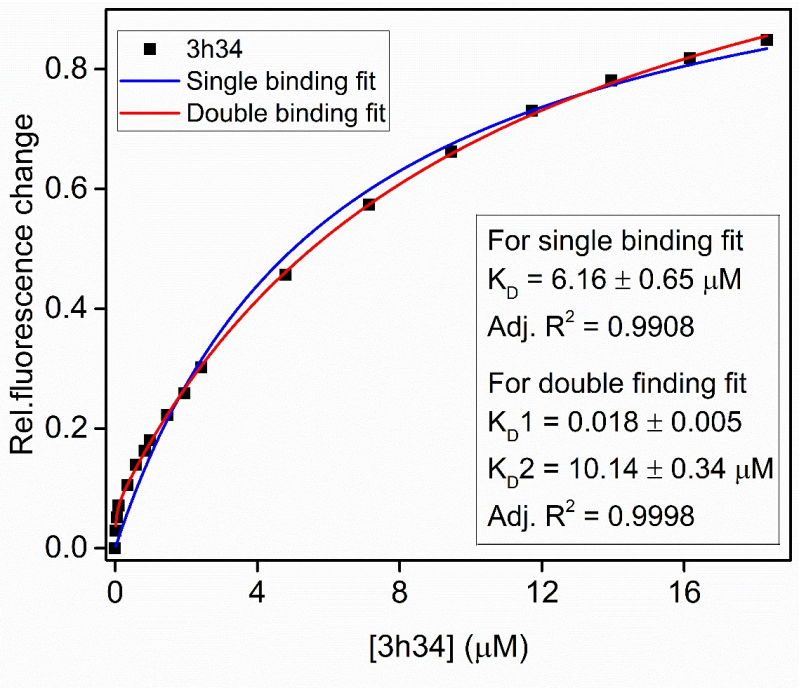

Fig. S2. The relative change in fluorescence of the peptide P1 plotted against the increasing concentration of 3h34 target RNA was fitted to both single binding isotherm (blue fitted line) and double binding isotherm (red fitted line).

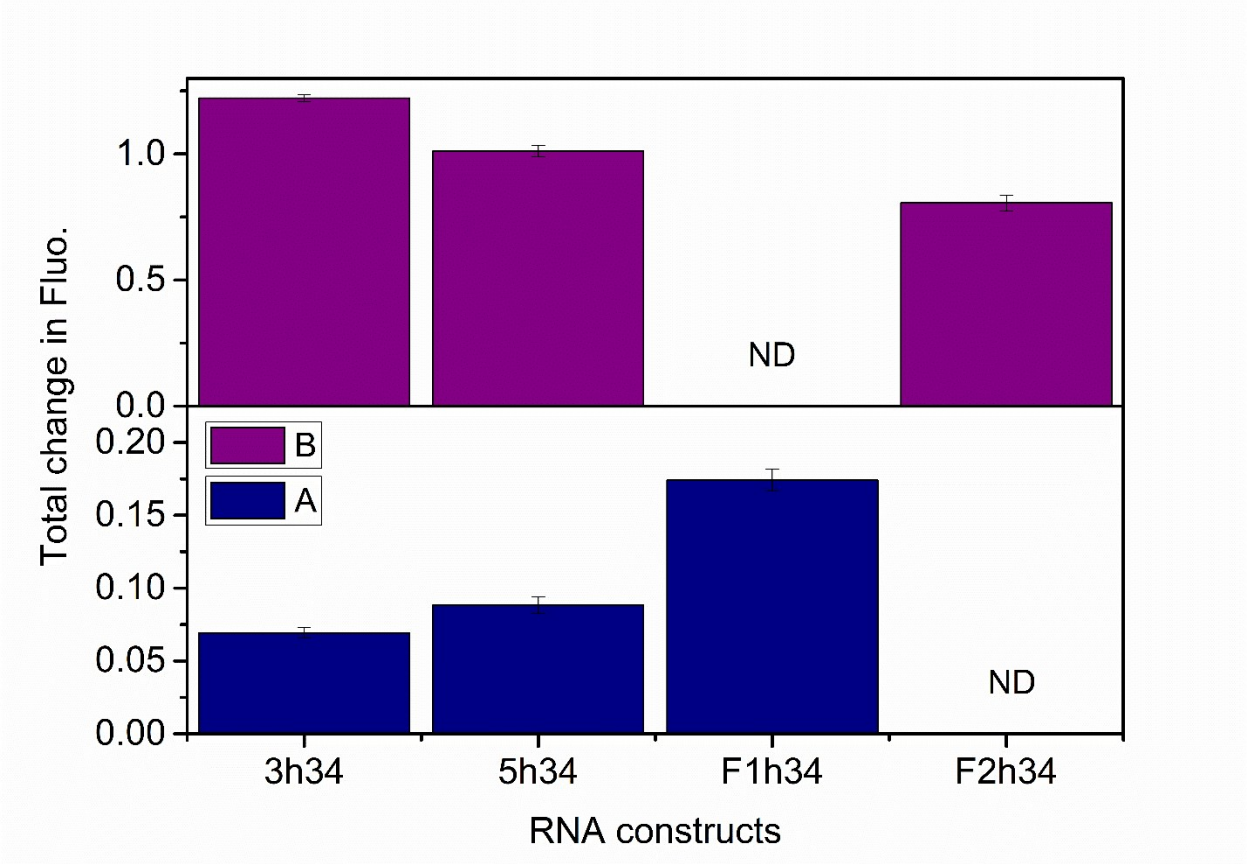

Fig. S3. The change in fluorescence at complete saturation of high-affinity (A) and low-affinity (B) peptide binding sites. These data were obtained from non-linear curve fittings of fluorescence-based titration curves using Equations 2 and 3.

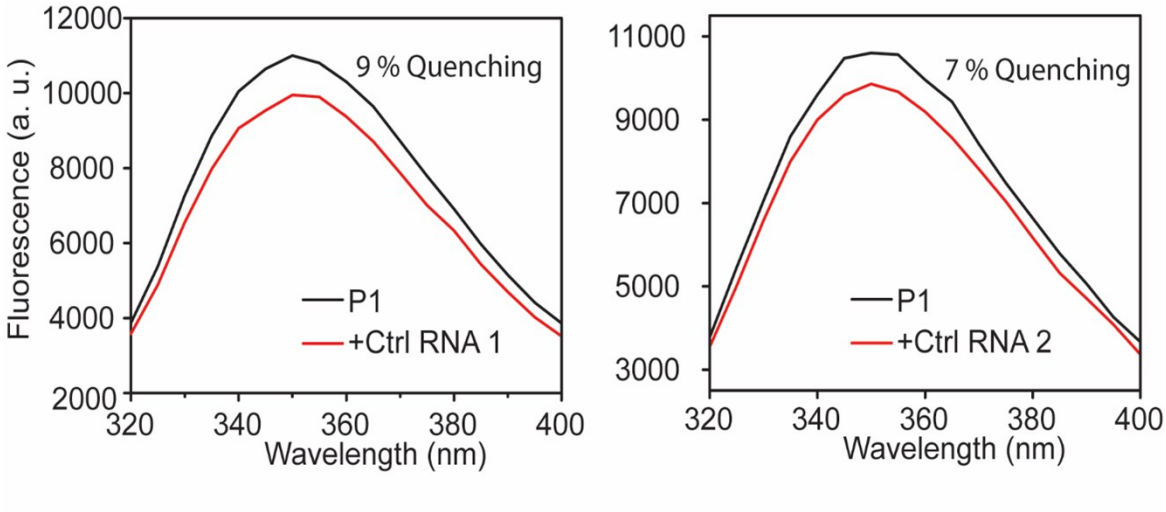

Fig. S4. The decrease in tryptophan fluorescence with the addition of control RNAs are shown. Less than 10% change of fluorescence is observed when two control RNAs (scrambled sequences) were added, compared to 80% change for 3h34 RNA (Fig. 2c).

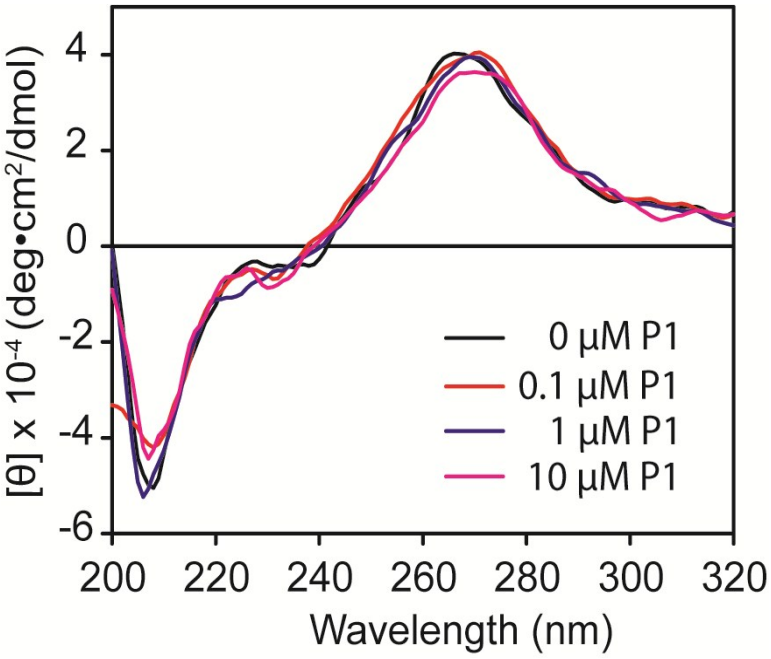

Fig. S5. CD spectra of 5h34 RNA in the presence of various P1 concentrations are shown. The change of CD at 266 nm wavelength is less significant for 5h34 compared to that for 3h34 RNA (Fig. 3a, b).

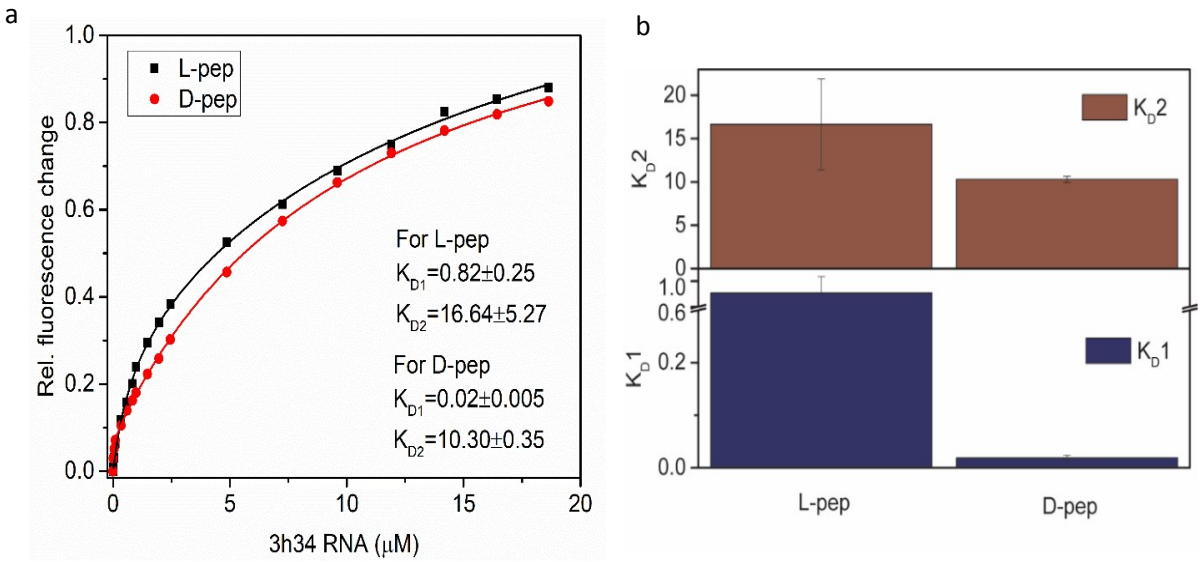

Fig. S6. a) Titration curves obtained by measuring the relative fluorescence changes of 3h34P1- L-peptide (black square) and D-peptide (red circles) in the presence of 3h34 target RNA are shown. b) Equilibrium dissociation constants for peptide-RNA complexes. All different titrations were performed in duplicates.

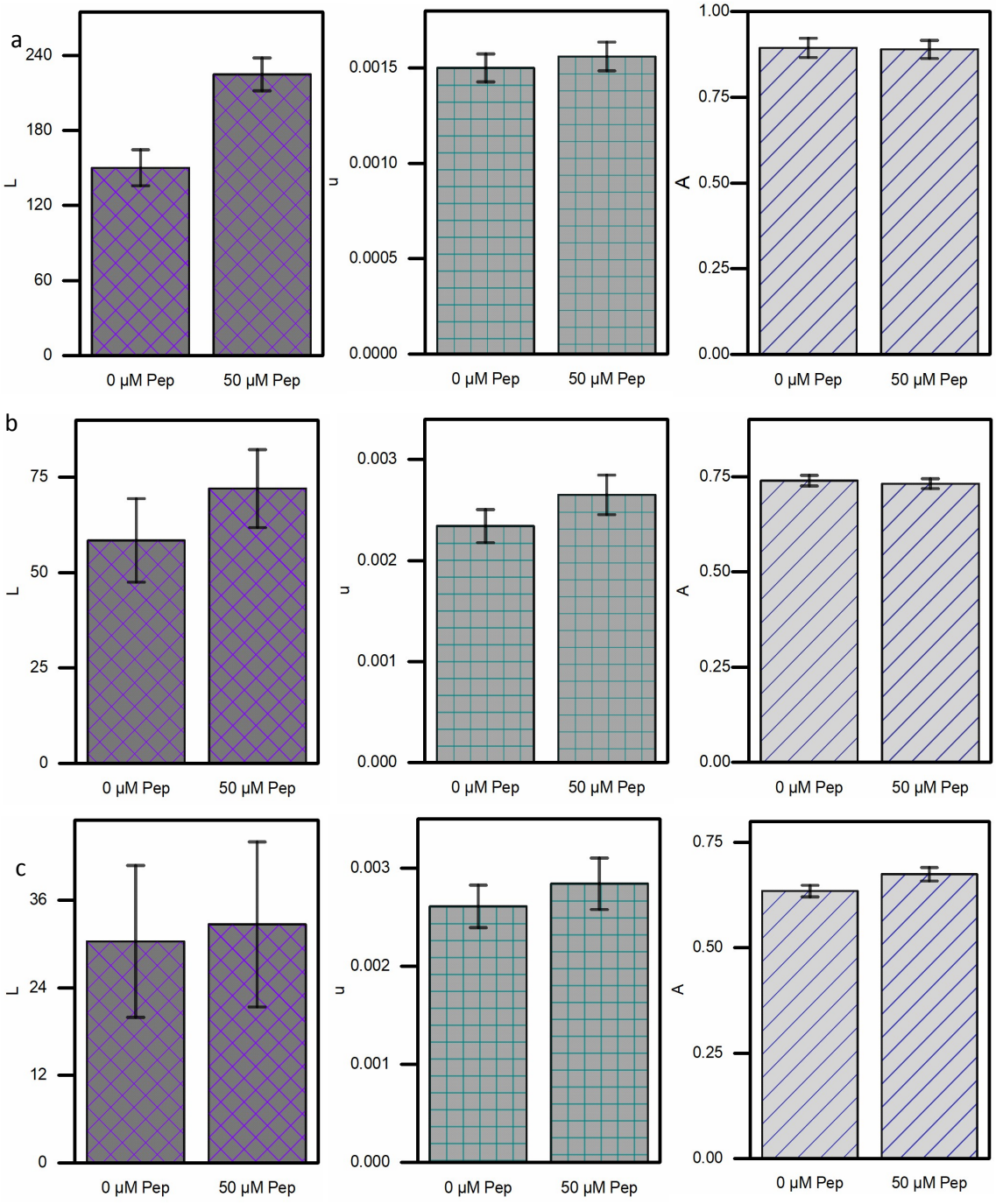

Fig. S7. Growth parameters for E. coli are shown. (a) Growth parameters obtained at 30 °C show mild defects in the growth. The growth lag phase (L) increased in the presence of the 3h34P1 peptide (P1). Growth parameters at (b) 37 °C and (c) 42 °C show no significant growth defects in the presence of 3h34P1. There is almost no change in the growth rates (u) and the maximum growth (A) at any temperatures tested. All the experiments were performed in triplicates.

Table S1. Sequences of RNA oligonucleotides with extinction coefficients used in the study are shown.

#	Oligonucleotides	Sequences (5′ to 3′)	Extinction coefficient L/(mole.cm)
1	3h34	CCGUCAAGUCAUCAUGGCCC	186,400
2	5h34	GGUGCUGCAUGGCUGUCGG	177,600
3	3h34-Cy3	/5Cy3/CCGUCAAGUCAUCAUGGCCC	191,300
4	3h34F1	CCGUCAAGUC	94,200
5	3h34F2	AUCAUGGCCC	93,800
6	Control RNA 1	GCCCUGAAAAGGGC	135,900
7	Control RNA 2	GAGGCCUUCGGGCCUC	143,800

Table S2. Biopanning conditions used in each cycle is shown. The stringency for the peptide selection was increased by changing washing conditions.

Mode of Stringency	BP 1st[a]	BP 2nd[a]	BP 3rd[a]
Tween-20	0.1%	0.2%	0.3%
Target RNA (3h34)	50 nM	50 nM	50 nM
# of washes (1 mL/wash)	2	4	6
Competitor tRNA	N/A[b]	5 µg/ml	N/A[b]
Competitor RNA (5h34)	N/A[b]	N/A[b]	50 nM

[a] Biopanning cycles [b] Not Assigned

Table S3. Phages titers before and after amplification during each biopanning cycle are shown.

Biopanning cycles	Titers (before amplification) pfu/µL	Titers (after amiplificatin) pfu/µL
First	7.6×10^{4}	8×10^{11}
Second	2.9×10^{4}	1.6×10^{11}
Third	3.3×10^{2}	N/A[a]

[a] Not Assigned