Supplementary material

for

A modified fluorescent intercalator displacement assay for RNA ligand discovery

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Figures

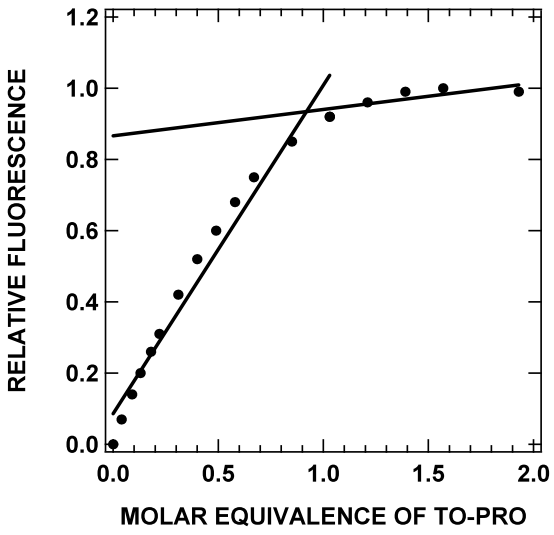

Figure S1: Fluorescence titration data of the A-site RNA (3 μM) with TO-PRO is shown (buffer conditions were 100 mM KCl, 20 mM Tris, pH 7).

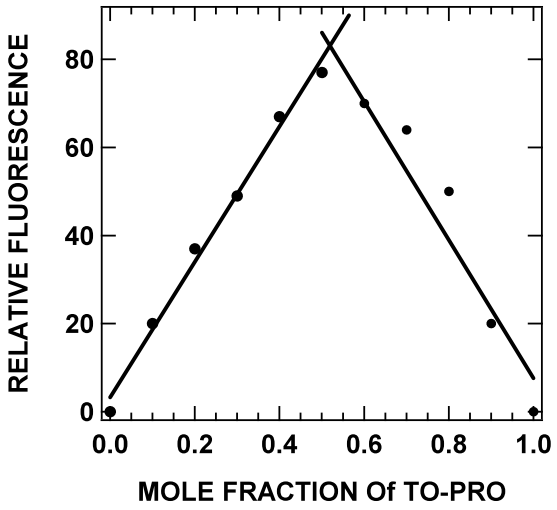

Figure S2: A Job plot of TO-PRO binding to the A-site RNA (3 μM) is given (buffer conditions were 100 mM KCl, 20 mM Tris, pH 7).

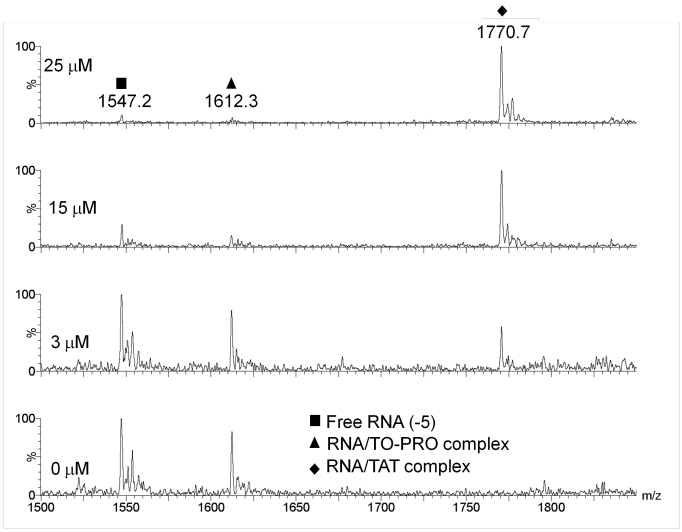

Figure S3: ESI-MS spectra showing a titration of Tat peptide into equimolar concentrations of TAR RNA and TO-PRO complex (1 μM). Buffer conditions are 150 mM ammonium acetate, pH 7.

Tables

Table S1: Quantification of the ESI-MS peak areas of the TO-PRO:A-site RNA complex after titration with 0–15 μM of paromomycin.

[paromomycin] μM	fraction bound (TO-PRO)[a]
0	0.29
1	0.25
3	0.20
6	0.19
10	0.13
15	0.04

[a]The fraction bound of TO-PRO was calculated by dividing the peak area corresponding to A-site RNA/TO-PRO complex by the peak area corresponding to total RNA (peak area of total RNA is equal to the summation of the peak area for free RNA, RNA/TO-PRO complex, and RNA/paromomycin complex).

Table S2: Quantification of the ESI-MS peak areas of TO-PRO:A-site RNA complex after titration with 0–100 μM of chloramphenicol.

[chloramphenicol] μM	fraction bound (TO-PRO)[a]
0	0.23
5	0.26
40	0.29
100	0.26

[a]The fraction bound of TO-PRO was calculated by dividing the peak area corresponding to A-site RNA/TO-PRO complex by the peak area corresponding to total RNA (peak area of total RNA is equal to the summation of the peak area for free RNA, RNA/TO-PRO complex, and RNA/chloramphenicol complex).

Table S3: FID assay of Tat peptide utilizing pre-bound equimolar concentrations of TAR RNA and TO-PRO (1 μM each). Buffer conditions were 150 mM ammonium acetate, pH 7. The data shown is an average of three separate experiments.

dye (μM)	TAR RNA (μM)	Tat (μM)	relative fluorescence (%)
1	0	0	0.3
1	1	0	100
1	1	2	86
1	1	10	49
1	1	19	35
1	1	31	23

Table S4: Quantification of the ESI-MS peak areas of TO-PRO:TAR RNA complex after titration with 0–25 μM Tat peptide.

[Tat], μM	fraction bound (TO-PRO)[a]
0	0.42
3	0.35
15	0.13
25	0.05

[a]The fraction bound of TO-PRO was calculated by dividing the peak area corresponding to TAR RNA/TO-PRO complex by the peak area corresponding to total RNA (peak area of total RNA is equal to the summation of the peak area for free RNA, RNA/TO-PRO complex, and RNA/Tat complex).

Table S5: Comparison of the FID results and dissociation constants for DHR23 and paromomycin binding to H69 and A-site RNA.

	DHR23		paromomycin	
	fluorescence (%)	K_d (μM)[a]	fluorescence (%)	K_d (μM)[a]
A-site RNA	54	19	14	1.3
H69	83	43	36	21

[a] Dissociation constants were obtained from ESI-MS experiments as described previously [25] and in the Materials and Methods section.