Construction of Ratiometric Fluorescent Sensors by Ribonucleopeptides

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Supporting Information

Materials

Tetracycline, dopamine, norepinephrine and tyrosine were purchased from Sigma Aldrich. Chloramphenicol, gel electrophoresis grade acrylamide and bisacrylamide was provided by Wako Chemicals GmbH. Streptomycin was obtained from Nacalai Tesque. N-Fmoc-protected amino acids, Fmoc-NH-SAL-PEG resin (0.23mmol/g), HBTU (2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), HOBt (1-hydroxybenzotriazole), DIEA (N,N-diisopropylethylamine), TFA (trifluoroacetic acid) and distilled DMF (N,N-dimethylformamide) were purchased from Watanabe Chemical Industry. All other chemicals were reagent grade and used without further purification.

Methods

Nucleic acids preparation

The nucleic acids used in this study were prepared according to the procedure previously reported.[6, 8, 9, 14, S1] Concentration of RNA was determined by UV spectroscopy.

SNARF-Rev and SNARF-Abu-Rev preparation

The procedure for the synthesis of fluorophore-labeled Rev peptides were previously reported.[3] Briefly, the two Rev peptides were synthesized on a Shimadzu PSSM-8 peptide synthesizer according to the Fmoc chemistry protocols. In SNARF-Abu-Rev two residues of γ-aminobutyric acid were manually attached to the N-terminus of the peptide applying Fmoc-SPPS strategies (HOBt, HBTU as activators, DIEA as base). SNARF, which was synthesized according to previous report[7], was directly coupled, after activating the carboxyl function, to the N-terminal deprotected residue of Rev peptide on the resin. SNARF-Rev and SNARF-Abu-Rev peptides were then deprotected, cleaved from the resin and purified by using RP-HPLC. Characterization was achieved by MALDI-TOF MS spectrometry (AXIMA-LNR, Shimadzu Biotech) [6-carboxy-SNARF-Rev+H]$^+$ calc. m/z 3314.12 obs.m/z 3313.35; [6-carboxy-SNARF-abu-Rev+H]$^+$ calc. m/z 3729.38 obs. m/z 3728.23.

Fluorescent measurements on the microplate

The 96-well fluorescence measurements were performed on an Infinite M200PRO instrument (TECAN co. ltd.). For tetracycline titration, a binding solution (100 µl) containing 1µM of fluorescent RNP in 10mM Tris HCl (pH 7.0), 250 mM NaCl, 5 mM $MgCl_2$, 0.005% Tween 20 was prepared. For dopamine and other catecholamine derivatives, a binding solution (100 µl) containing 1 µM fluorescent RNP in 10mM Tris HCl (pH 7.60), 300 mM NaCl, 5 mM $MgCl_2$, 0.005% Tween 20 was prepared. For streptomycin, a binding solution (100 µl) of 1 µM concentration of fluorescent RNP was prepared in 50mM Tris-HCl (pH 7.6), 250 mM NaCl, 10 mM $MgCl_2$, 0.005% Tween 20 buffer conditions. Well-mixed samples with different concentrations of substrates were incubated at 25ºC for 30 min followed by the measurement of emission spectra (measured emission spectrum range: 570-700 nm; excitation wavelength utilized: 534 nm).

Determination of dissociation constants

The dissociation constant were achieved applying the following equation:

$$F_{obs} = A(([RNP]_T + [substrate]_T + K_D)-(([RNP]_T + [substrate]_T + K_D)^2 - (4[RNP]_T [substrate]_{T)}^{1/2})/2[RNP]_T)$$

where A is the increase in fluorescence at saturating substrate concentrations (R_{sat}-R_0), K_D is the equilibrium dissociation constant, $[RNP]_T$ and $[substrate]_T$ are the total concentrations of RNP and the substrate, respectively. DHc65-RRE/SNARF-Rev provided an I/I_0 value of 0.85 and a K_D of 1.1 µM. While a norepinephrine titration provide a K_D value of 2.0 µM. Comparable results were achieved from by the standard binding isotherm obtained from the titration curve of DH05-RRE/SNARF-Rev with dopamine and norepinephrine (K_D values respectively of 1.1 µM for dopamine and 1.9 µM for norepinephrine). These values are consistent with the results previously reported [12] showing just a slightly higher affinity.

Fluorescent pH titration

The 96-well fluorescence measurements were performed on an Infinite M200PRO instrument (TECAN co. ltd.). Samples were prepared in a 10mM of a wide-range pH buffer using Tris HCl, Acetate buffer and Mes buffer, in order to achieve a 6.00 to 8.00 interval of pH values. To the binding solution containing 1 µM of fluorescent RNP in 250 mM NaCl, 5 mM $MgCl_2$, 0.005% Tween 20, 3µM tetracycline was added. Well-mixed samples, with different buffer pH values (6.00, 6.25, 6.50, 6.75, 7.00, 7.25, 7.50, 7.75, 8.00) in the presence and in the absence of tetracycline, were incubated at 25ºC for 30 min followed by the measurement of emission spectra (measured emission spectrum range: 570-700 nm; excitation wavelength utilized: 534 nm).

Fluorescent image evaluation

Fluorescent image evaluation was accomplished in the presence and in the absence of tetracycline using Pharos FX Imager. A wide range pH buffer was utilized (Tris HCl, Acetate buffer and Mes buffer). 10 µl of the binding solution containing 1 µM RNP in 250 mM NaCl, 5 mM $MgCl_2$, 0.005% Tween 20 were added to a glass plate (S117814, Matsunami glass int. ltd.) and, after 30 min incubation at room temperature, were scanned (excitation wavelength 534 nm; filters at 580 ± 25 nm and 640 ± 20 nm).

Table S1. Equilibrium dissociation constants K_D (μM) of dopamine-binding RNPs.

Ligand	K_D [μM]			
	DHc65		DH05	
	SNARF-Rev	SNARF-abu-Rev	SNARF-Rev	SNARF-abu-Rev
Dopamine	1.1	1.2	1.1	1.5
Norepinephrine	1.9	1.3	2.0	1.5
Tyrosine	>100	>100	>100	>100

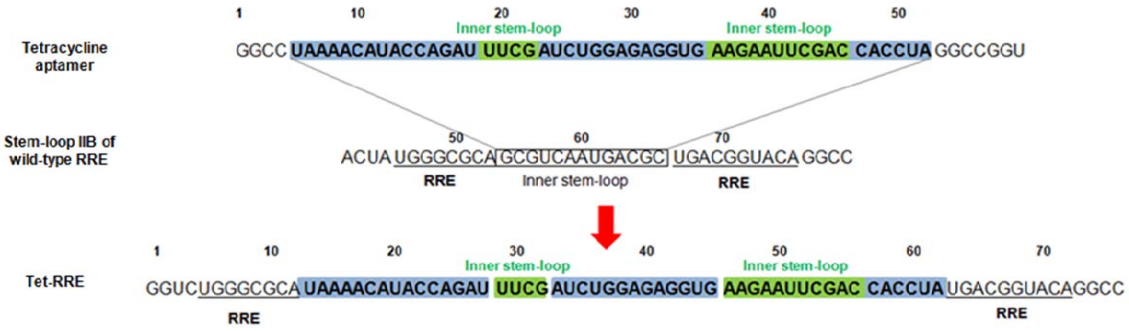

Figure S1. Nucleotide sequences of the tetracycline aptamer, RRE RNA, and modular tetracycline-binding RNP (tet-RRE RNA). The tetracycline aptamer fused to the RRE sequence (tet-RRE) was complexed with 6-carboxy-SNARF-modified Rev (SNARF-Rev and SNARF-abu-Rev).

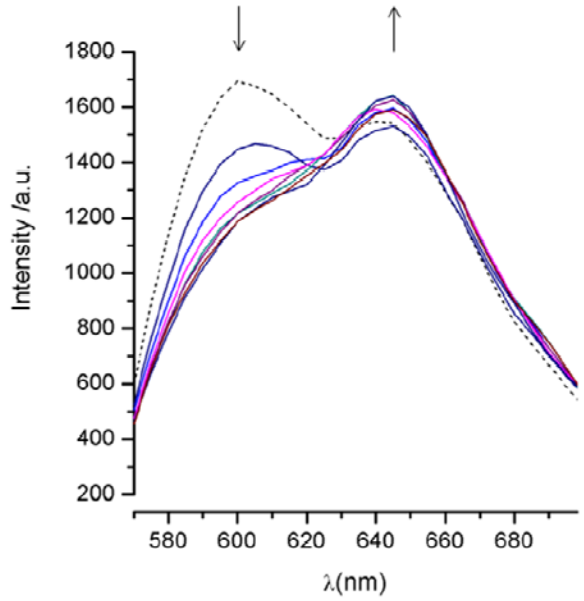

Figure S2. Fluorescence spectral change of tet-RRE/ SNARF-Rev upon the addition of tetracycline (0-30 μM). The arrows show the direction of change of the spectrum at that position as tetracycline concentration is increased.

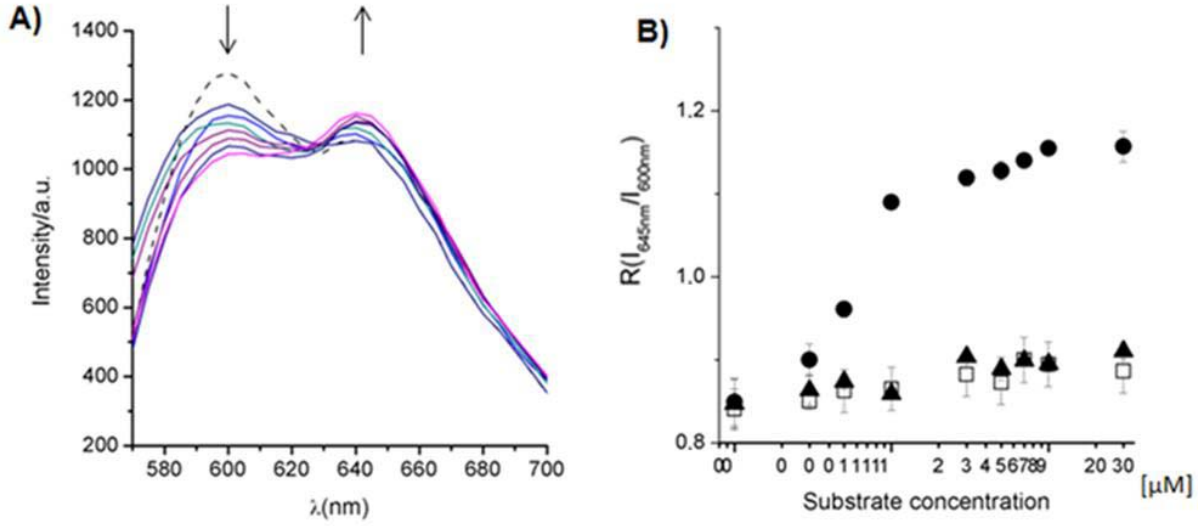

Figure S3. A) Fluorescence spectral change of tet-RRE/ SNARF-abu-Rev upon the addition of tetracycline (0-30 μM). B) Fluorescence titration plot of the emission intensity ratio (645 nm / 600 nm) of tet-RRE/ SNARF-abu-Rev vs. the antibiotic compounds concentration. Tetracycline (black filled circle), streptomycin (black filled triangle), chloramphenicol (black opened square).

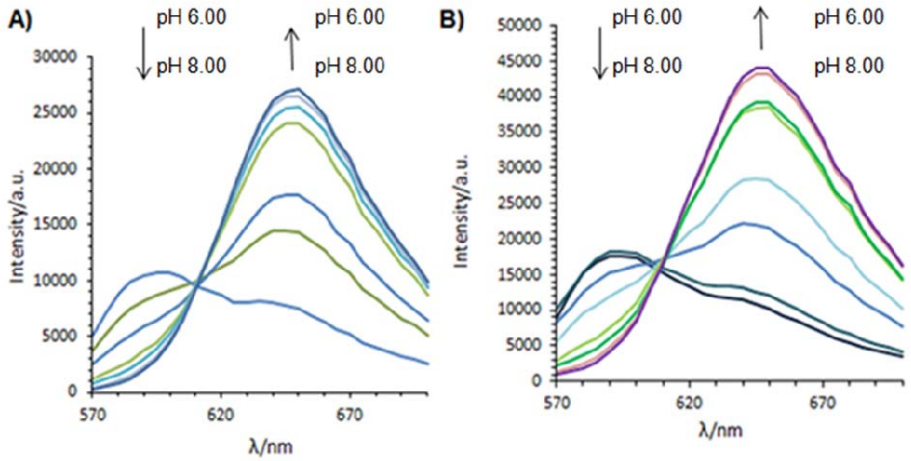

Figure S4. A) Fluorescence spectral change of tet-RRE/ SNARF-Rev at different pH values (6.00 to 8.00). B) Fluorescence spectral change of tet-RRE/ SNARF-abu-Rev at different pH values (pH 6.00 to 8.00).

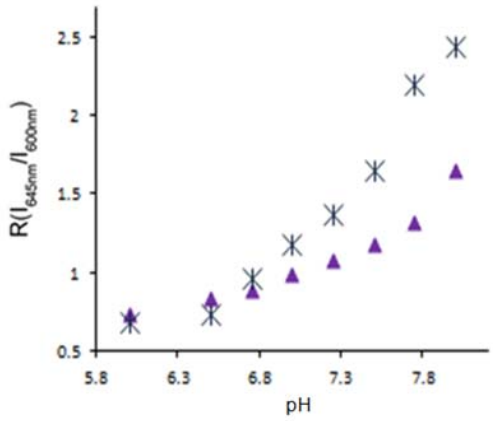

Figure S5. A pH titration (pH 6.00 to 8.00) of tet-RRE/SNARF-Rev RNP (filled purple triangle) and tet-RRE/SNARF-Rev upon 3 μM tetracycline addition (dark snow construction line).

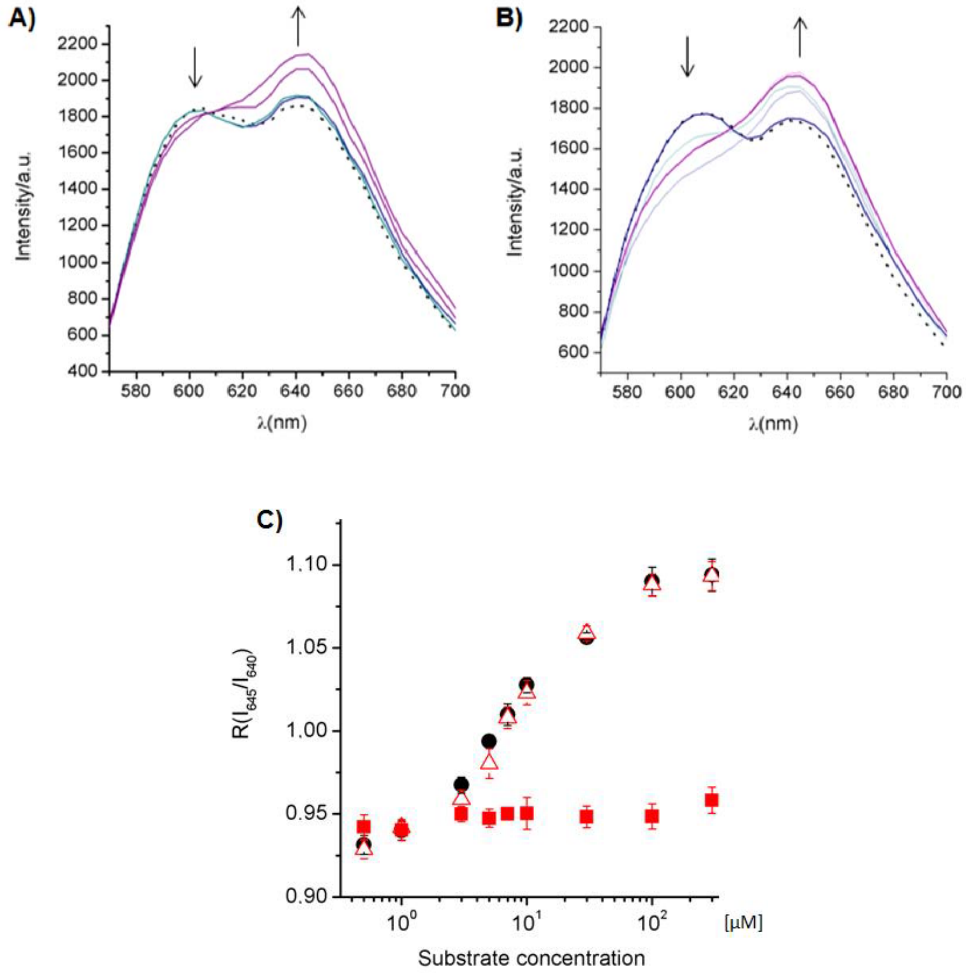

Figure S6. A) Fluorescence spectral change of DHc65-RRE/SNARF-Rev upon the addition of dopamine (0-300 μM). B) Fluorescence spectral change of DHc65-RRE/SNARF-abu-Rev upon the addition of dopamine (0-300 μM). C) Fluorescence titration plot of the emission intensity ratio (645 nm / 600 nm) of DHc65-RRE/SNARF-abu-Rev with dopamine (filled black circle), norepinephrine (open red triangle), L-tyrosine (filled red square).

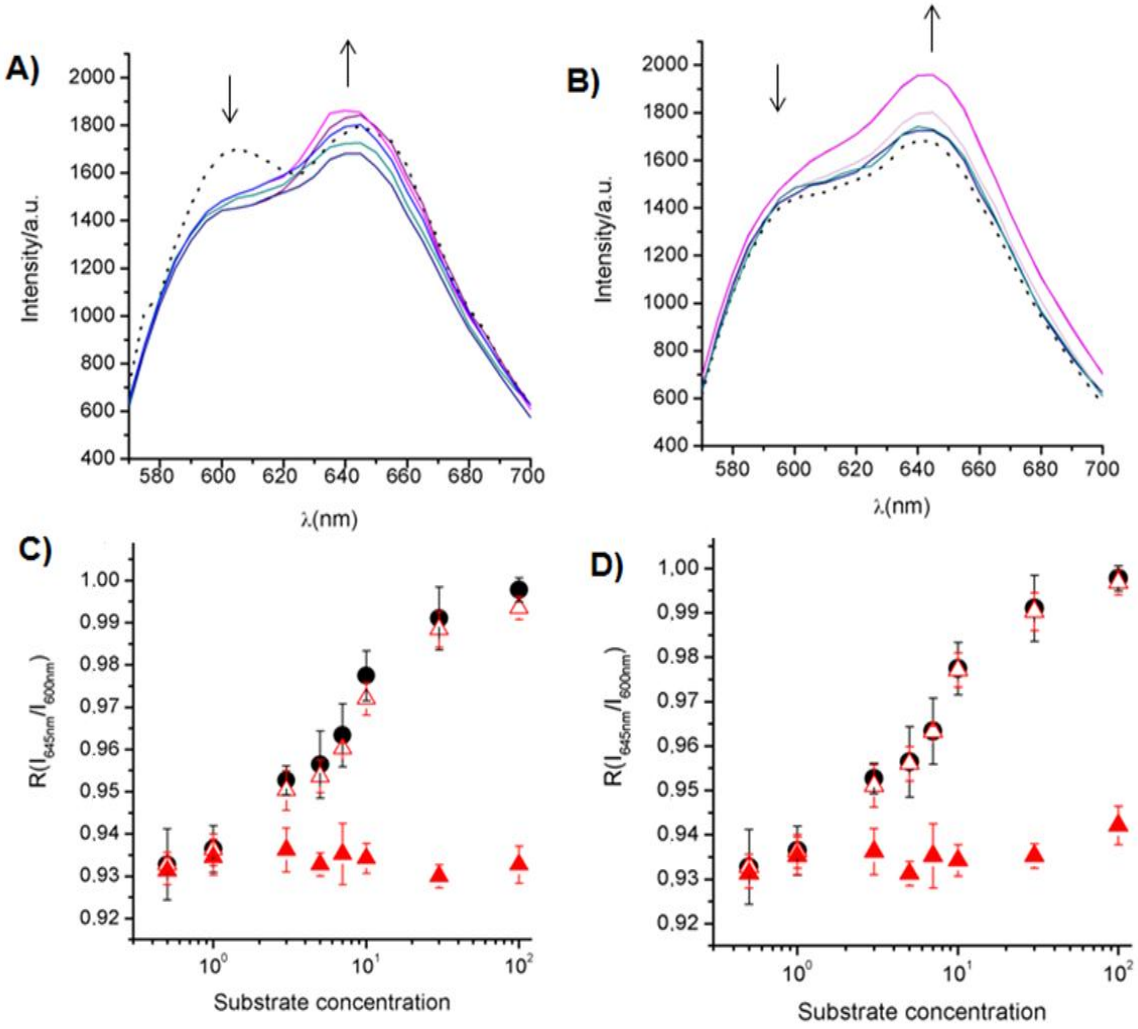

Figure S7. A) Fluorescence spectral change of DH05-RRE/SNARF-Rev upon dopamine addition (0-300 μM). B) Fluorescence spectral change of DH05-RRE/SNARF-abu-Rev upon dopamine addition (0-300 μM). C) Fluorescence titration plot of the emission intensity ratio (645 nm / 600 nm) of DHc65-RRE/SNARF-abu-Rev with dopamine (filled black circle), norepinephrine (open red triangle), L-tyrosine (filled red square).

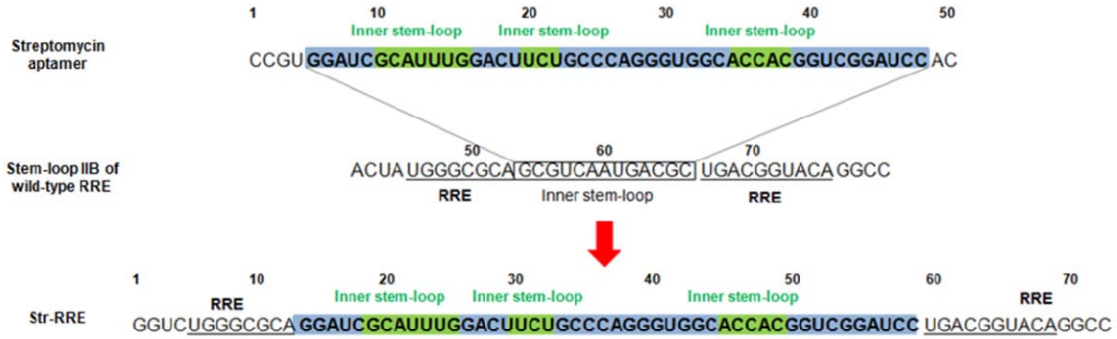

Figure S8. Nucleotide sequences of the streptomycin aptamer, RRE RNA, and modular streptomycin-binding RNP (str-RRE RNA). The streptomycin aptamer fused to the RRE sequence (str-RRE) was complexed with 6-carboxy-SNARF-modified Rev (SNARF-Rev and SNARF-abu-Rev).

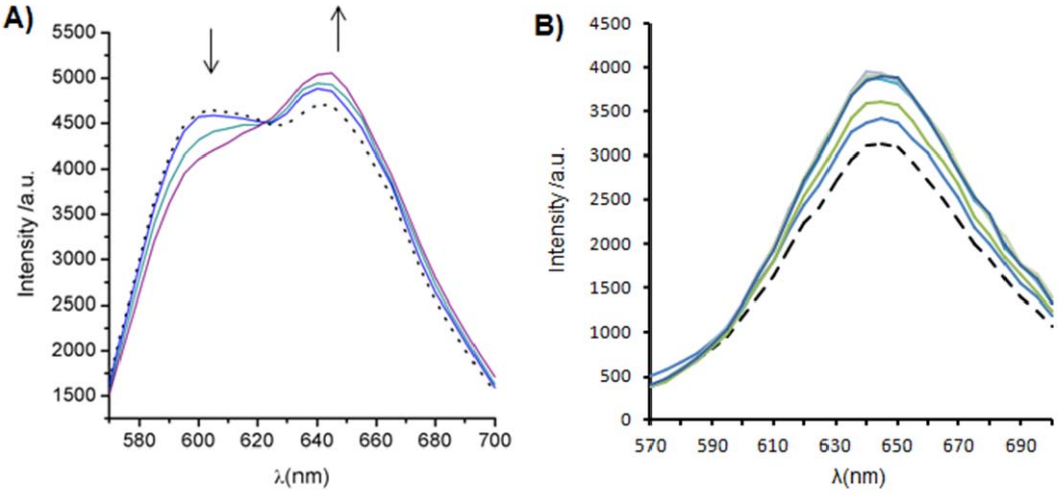

Figure S9. A) Fluorescence spectral change of str-RRE/SNARF- Rev upon the addition of streptomycin (0-300 μM). B) Fluorescence spectral change of str-RRE/SNARF-abu-Rev upon the addition of streptomycin (0-300 μM).

Reference

S1) A. Kertsgurg, G. A. Soukup, *Nucleic Acids Res.* **2002**, *30,* 4599.