Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

for

Modular Blue Fluorescent RNA Sensors for Label-Free Detection of Target Molecules

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1. Preparation of RNA sensors

The dsDNA templates for RNA probes were prepared by PCR amplification using the forward primers (5'–TAA TAC GAC TCA CTA TAG GCC AAG CAG GTT CGT TTT CGA AGC-3' for **RNA1** , 5'-TAA TAC GAC TCA CTA TAG GCC AAG CTT CGT TTT CGA AGC-3' for **RNA2**, 5'-TAA TAC GAC TCA CTA TAG GCC AAG CAG GTT CGG-3' for **RNA3**, 5'-GTA ATA CGA CTC ACT ATA GGT CAT CCA AGC AGG TTC GTT TTC GAA G-3' for **RNA4**, 5'-GTA ATA CGA CTC ACT ATA GCT GGC CAA GCA GGT TCG TTT TCG for **RNA5**), reverse primers (5'-TCC AAG CTT CGA AAA CGA ACC-3' for **RNA1**, 5'-TCC AAG CTT CGA AAA CGA AG-3' for **RNA2**, 5'-TCC AAG CTT CGG ACG TC-3' for **RNA3**, 5'-TTG GAT GAC CGT GTC TGA TTG TCC GGG GTG TTC-3' for **RNA4**, 5'-TTG ACC AGC CTT GCG TAT ACG TGC TCT TCT GGC-3' for **RNA5**), and templates (5'-GGC CAA GCA GGT TCG GAC GTC TTT TGA CGT CCG AAG CTT GGA-3' for **RNA3**, 5'-GCA GGT TCG TTT TCG AAG CTT GGT TGG ATG ACG AGG GGA ATG AAC ACC CCG GAC AAT C-3' for **RNA4**, 5'-CCA AGC AGG TTC GTT TTC GAA GCT TGG TTG GCC AGC CAG AAG AGC ACG TAT AC-3' for **RNA5**). RNA sensors were transcribed from the PCR-generated dsDNA templates using T7 MEGAshortscript kit (Ambion), and purified by 8% PAGE containing 7 M urea.

2. Surface plasmon resonance analysis

SPR analyses were performed with a BIAcore2000 system (GE Healthcare, USA). Chromophore **1** was immobilized on a sensor chip SA (streptavidin) through biotin-avidin interaction accoring to the method reported previously.[1] Binding experiments of immobilized **1** with various concentrations of **RNA4** (10 ~ 600 nM in running buffer) were performed at 25 °C in a continuous flow of running buffer [1× PBS pH 7.4 (10 mM phosphate buffer containing 138 mM NaCl and 2.7 mM KCl) containing 2.5 mM Mg^{2+} and 0 or 3 mM ADP] at a flow rate of 10 μL min^{-1}. Kinetic analyses were performed with BIAevaluation software.

3. Fluorescence measurement

RNA sensors (final conc. 200 nM) were dissolved in the binding buffer [1× PBS pH 7.4 (10 mM phosphate buffer containing 138 mM NaCl and 2.7 mM KCl) containing 2.5 mM $MgCl_2$] in the presence or absence of analytes. The solutions were incubated for 3 min after addition of chromophore **1** (final conc. 200 nM) and subjected to fluorescence measurements. Fluorescence spectra (excitation at 345 nm and emission in the range of 360-650 nm) were measured at 25 °C using a FP-6500 fluorescence spectrometer (JASCO Corp., Japan).

4. Figure S1

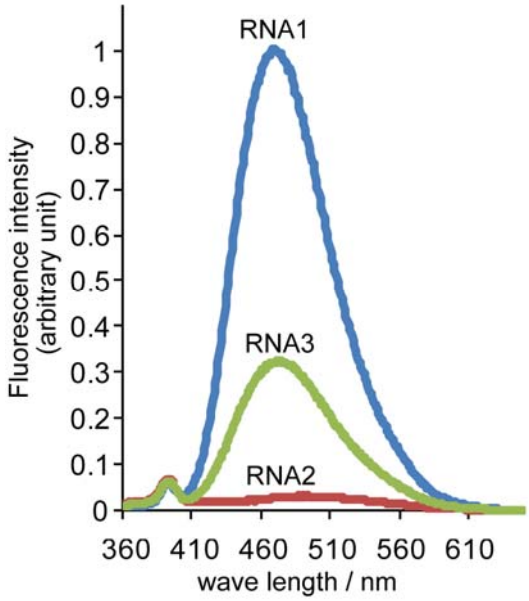

Fig. S1 Fluorescence spectra of chromophore **1** (200 nM) in the presence of **RNA1-3** (200 nM). Fluorescence spectra (excitation at 345 nm) were measured in the binding buffer (1× PBS containing 2.5 mM $MgCl_2$) at 25 °C.

5. Reference

1. S. Sando, A. Narita, M. Hayami and Y. Aoyama *Chem. Commun*., 2008, 3858–3860.