Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Enzyme-Assisted Binary Probe for Sensitive Detection of RNA and DNA

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Experimental Details

DNAse/RNAse-free water was purchased from Fisher Scientific, Inc. (Pittsburgh, PA) and used for all buffers and for the stock solutions of oligonucleotides. RNase HII from E. coli was purchased from New England Biolabs (Ipswich, MA). RO was custom-made by TriLink BioTechnologies, Inc. (San Diego, CA) and additionally purified by HPLC using JASCO X-LC HPLC system (Easton, MD). All other oligonucleotides were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) and used without purification (desalted). The oligonucleotides were dissolved in water and stored at -20 ^{o}C until needed. Stock concentrations of oligonucleotides were calculated by measuring the absorption of the solutions at 260 nm using a Perkin-Elmer Lambda 35 UV/VIS spectrometer (San Jose, CA). Fluorescent spectra were recorded on a Perkin-Elmer (San Jose, CA) LS-55 Luminescence Spectrometer equipped with a Hamamatsu Xenon lamp. Experiments were performed at excitation wavelength of 485 nm. Emission of FAM was monitored at 517 nm. Excitation and emission slits were 5 nm and 20 nm, respectively. The data were processed using Microsoft Excel. RNase HII-assisted binary probe assay was performed for 1 h at 30 ^{o}C in a solution containing 1×ThermoPol buffer (20 mM Tris-HCl, pH 8.8; 10 mM $(NH_4)_2SO_4$,10 mM KCl, 25 mM $MgCl_2$, 0.1% Triton X-100). The concentration of RO was 10 nM. The concentrations of both strands α and β were 100 nM. RNase HII was added in the amount of 10U per 130 μL reaction mixture. The concentration of the analyte was 2 nM for the time-course experiments, and 0.1-3 nM for the sensitivity experiments. To determine the limit of detection (LOD) of the assay, seven independent measurements of the probe's fluorescence in the absence of the analyte (blank) were performed. The fluorescence correspondent to the LOD was calculated as the average fluorescence of the blank plus three standard deviations.[1]

Table S1 Oligonucleotides used in the study

Oligonucleotide	Nucleotide sequence*
RO	5'-FAM-*CGCG*TTAaCATACAATaGAT*CGCG*-BQ1-3'
HCV-DNA	5'-TTCATCGTCTCGCCGCAGTACCACTGGTTT GTGCAAGAAT-3'
HCV-RNA	5'-uucaucgucucgccgcaguaccacugguuugugcaag aau-3'
HCV-strand α	5'-GATCTATTGGCGG CGAGACGAT-3'
HCV-strand β	5'-AACCAGTGGTACT TATGTTAAC-3'
rs 717302-G	5'-GAAAGGCATATCGTATTAACTGTGT**G**GTGA ACG TCTGTCATTAGGTTTAGC-3'
rs 717302-A	5'-GAAAGGCATATCGTATTAACTGTGT**A**GTGA ACG TCTGTCATTAGGTTTAGC-3'
rs717302-strand α	5'-GATCTATTG TCACCACAC-3'
rs717302-strand β	5'-ACCTAATGACAGACGT TATGTTAAC-3'

* The uppercase letters denote deoxyribonucleotides; ribonucleotides are shown in lowercase letters. Stem-forming nucleotides of the reporter are in italic; SNP nucleotides are shown in bold. The analyte-binding arms of stands α and β are underlined.

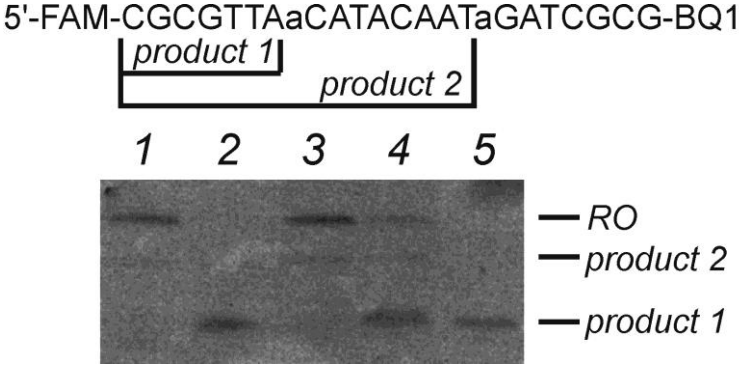

Fig. S1. Polyacrylamide gel analysis of the enzyme-assisted binary probe. Cleavage of the reporter of RNase HII-assisted binary probe in the presence of an analyte. Gel electrophoresis was performed using 20 % polyacrylamide gel in the presence of 8 M urea. The bands are visualized by intrinsic fluorescence of FAM group. OR (100 nM) was incubated at 30 ^{o}C in 1×ThermoPol buffer containing 10U RNase HII alone (lane 1), in the presence of complimentary deoxyribonucleotide 5'-GATCTATTGTATGTTAAC-3' (500 nM) (lane 2), in the presence of HCV-probe strands α and β (1000 nM) (lane 3) or in the presence of strands α and β (1000 nM) and HCV-DNA (100 nM) (lane 4). Lane 5 – RO (100 nM) incubated with 0.2 M NaOH at 37 ^{o}C overnight for complete cleavage.

In lane 1, there is a single band, which corresponds to the intact RO. These data indicate that RO is stable to RNase HII degradation under the assay condition. The same band is observed when the probe is incubated in the absence of the analyte (lane 3). Addition of the HCV-DNA analyte, which is complementary to analyte-binding arms of strands α and β, triggers degradation of OR into product 1, the shortest fluorescent oligonucleotide fragment (lane 4). The same FAM-containing fragment is observed when RO is incubated with RNase HII and the complementary oligonucleotide (lane 2), or when RO was subjected to alkaline cleavage (lane 5). These results prove the suggested scheme for the enzyme-assisted binary probe operation (Scheme 1B). Specifically, the quadripartite complex of RO, strands α and β and the analyte is processed by RNase HII.

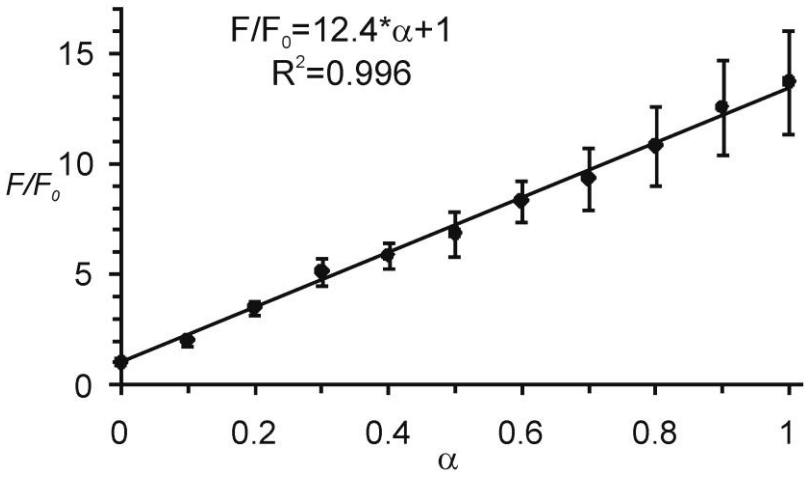

Fig. S2. The dependence of fluorescent signal enhancement from the cleavage degree for RO. The data are shown as average of three independent experiments. The errors are given as a standard deviation of average.

The completely cleaved (0.2 M NaOH at 37 ^{o}C overnight) RO was mixed with the intact RO in definite proportions to obtain cleavage degree (α) of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 and incubated in 1×ThermoPol buffer (20 mM Tris-HCl, pH 8.8; 10 mM $(NH_4)_2SO_4$,10 mM KCl, 25 mM $MgCl_2$, 0.1% Triton X-100) in the presence of HCV strands α and β (100 nM) and HCV-DNA (2 nM) for 20 min. The fluorescence intensities of the mixtures at 517 nm were used to calculate F/F_0 ratio, where F_0 is the fluorescence of intact RO and F is the fluorescence of a sample containing definite amount of the completely cleaved OR. F/F_0 values were plotted against α. The linear correlation of these values was found. The data are average of three independent experiments. The error bars are given as standard deviations.

1 D. MacDouglas, W. B. Crummett, *Anal. Chem.* 1980**, 52**, 2242.