

Electric supplementary information

Liquid–liquid extraction of enzymatically synthesized functional RNA oligonucleotides using reverse micelles with DNA-surfactant

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Experimental Section

Materials

Synthesized DNA oligonucleotides were from Tsukuba Oligo Service Co., Ltd (Ibaraki, Japan).

Synthesized RNA oligonucleotides were from Japan Bio Services Co., Ltd. (Saitama, Japan). The

nucleobase sequences are listed in Table 1. Tris-HCl buffer (10 mM, pH 8) containing 1 mM EDTA

was from Fluka. Thrombin from bovine plasma was from Sigma-Aldrich (St Louis, MO). DLPC and

other chemicals were from Wako Pure Chemicals (Osaka, Japan).

Reverse micellar extraction of ribonuclease A

Sodium bicarbonate buffer (pH 9.0, 0.1 M, 1 ml) containing 1 mg/ml RNase A was mixed with

dimethyl sulfoxide solution (0.1 ml) containing 10 mg/ml fluorescein isothiocyanate and stirred at 4

°C for 8 h. Fluorescein-labeled RNase A was purified twice over a PD-10 column. Fluorescein-labeled RNase A was eluted with Tris-HCl buffer (10 mM, pH 8) containing 1 mM EDTA.

Reverse micellar extraction was performed as follows. The aqueous phase was Tris-HCl buffer (pH 8, 10 mM) containing 1 mM EDTA, 10 mM MgCl₂ and fluorescein-labeled RNase A (0.4 µg/ml). The organic phase was isooctane containing DLPC (10 mM) and 1-hexanol (240 mM). The organic phase (1 mL) was mixed with the aqueous phase (1 mL) in a glass vial. The two phases were gently stirred with a magnetic stir bar at 25 °C for 3 h. The fluorescence of each phase was measured with a luminescence spectrometer, model LS 50B (PerkinElmer, Waltham, MA) at 25 °C to determine the concentration of fluorescein-labeled RNase A in each phase. Excitation and emission wavelengths were 494 and 520 nm, respectively.

Synthesis of DNA-surfactants

The DNA-surfactant was synthesized as previously reported.^{1,2} Briefly, 5'-aminated DNA oligonucleotide (0.5 mM) in phosphate buffer (50 mM, 10 µl) was mixed with a dimethylsulfoxide solution (50 µl) containing oleic acid *N*-hydroxysuccinimide ester (1 mM) at 40 °C for 24 h. The synthesized 5'-oleoyl DNA oligonucleotides (DNA-surfactants) were purified using a high performance liquid chromatograph (Shimadzu LC20 system, Kyoto, Japan) equipped with a C18 column (4 × 250 mm, GL Sciences, Tokyo), initially eluted with 10% acetonitrile in 0.1 M triethylamine acetate buffer for 5 min, then with a linear gradient of 10 to 90% acetonitrile over 30

min and isocratic 90% acetonitrile for 10 min at a flow rate of 1.0 ml/min.

Extraction of target RNA using reverse micelles and a DNA-surfactant

The aqueous phase was a Tris-HCl buffer (pH 8, 10 mM) containing 1 mM EDTA, 10 mM MgCl₂, a fluorophore-labeled target RNA (25 nM) and the DNA-surfactant (25 nM). The organic phase was isooctane containing DLPC (10 mM) and 1-hexanol (240 mM). The organic phase (1 mL) was mixed with the aqueous phase (1 mL) in a glass vial. The two phases were gently stirred using a magnetic stir bar at 25 °C for 3 h, taking care not to disrupt the interface between the two phases. The fluorescence of each phase was measured in a luminescence spectrometer, model LS 50B (PerkinElmer) at 25 °C to determine the concentration of the target RNA in each phase. Excitation and emission wavelengths used to detect the carboxyfluorescein (FAM) fluorophore were 494 and 520 nm, respectively. All experiments were performed in triplicate and error bars in the figures represent standard deviations.

The diameter of reverse micelles was measured using a Zetasizer Nano ZS (Malvern, Worcestershire, UK).

Back-extraction of extracted RNA to an aqueous phase

A DNA-surfactant conjugated with FITC at its 3' end and an unlabeled target RNA were used for back-extraction to monitor the concentration of the DNA-surfactant. To monitor the back-extraction of target RNA, a FAM-labeled target RNA and a DNA-surfactant without FITC were used. After the

reverse micellar extraction, as described above, the organic phase (1 mL) containing the target RNA was mixed with Tris-HCl buffer (1 mL) containing 1 mM EDTA, followed by addition of 0.5 mL 1-butanol at 75 °C to destroy the reverse micelles. The mixture was stirred and then centrifuged to collect the aqueous phase. The aqueous phase contained duplexes of the target RNA and the DNA-surfactant. To remove the DNA-surfactant, the aqueous phase was mixed with isooctane containing DLPC (10 mM) and 1-hexanol (240 mM) at 80 °C. The reverse micelles formed in the organic phase extracted the DNA-surfactant, resulting in single-stranded target RNA separated from DNA-surfactant. At each step, fluorescence of the two phases was measured to determine concentrations of each component.

Enzymatic synthesis of an RNA aptamer

Oligo DNAs A and B (Table S1) were dissolved in Tris-HCl buffer (pH 8, 10 mM) containing 1 mM EDTA and 50 mM potassium acetate to prepare a DNA template solution containing each Oligo DNA at 10 µM. Using the DNA template solution (4 µl) and T7 RiboMAX™ Express Large Scale RNA Production System (Promega, Fitchburg, WI), target 3 was enzymatically synthesized according to the manufacturer's instructions. The synthesized target 3 was used for the following experiments without removal of the DNA template or enzymes. The concentration of synthesized target 3 was determined using SYBR Green II. Excitation and emission wavelengths were 497 and 520 nm, respectively. The amount of synthesized target 3 was determined using a Qubit RNA assay kit (Thermo Fisher Scientific, Waltham, MA).

Assay of thrombin and RNA-aptamer binding

N-Hydroxysuccinimidyl (NHS)-activated Sepharose (700 μ L, GE Healthcare, Little Chalfont, UK) was centrifuged and the supernatant removed. A phosphate buffer solution (pH 8, 100 mM, 700 μ L) containing 0.2 mg/mL thrombin was mixed with the NHS-activated Sepharose at 25 $^{\circ}$ C for 3 h. The reaction mixture was centrifuged and the supernatant removed. The collected thrombin-immobilized beads were then mixed with a Tris-HCl buffer solution (700 μ L) containing 15 nM thrombin-binding RNA aptamer (synthesized enzymatically and purified by reverse micellar extraction), 100 mM NaCl and 100 mM KCl for 3 h. After centrifugation, the supernatant was labeled with SYBR Green II and its fluorescence measured to determine the concentration of the unbound RNA aptamer. The binding ratio of RNA aptamer to thrombin was calculated from the concentration of the unbound RNA aptamer. Because the non-thrombin aptamer was labeled with carboxyfluorescein (FAM), the concentration of the unbound non-thrombin aptamer was determined by measuring FAM fluorescence. Excitation and emission wavelengths used to detect the FAM fluorophore were 494 and 520 nm, respectively.

References

1. J. Shimada, T. Maruyama, M. Kitaoka, H. Yoshinaga, K. Nakano, N. Kamiya and M. Goto, *Chem. Commun.*, 2012, **48**, 6226-6228.

2. T. Maruyama, H. Yamamura, M. Hiraki, Y. Kemori, H. Takata and M. Goto, *Colloids Surf B Biointerfaces*, 2008, **66**, 119-124.

Table S1. Sequences of template DNAs for enzymatic RNA synthesis

Name	Sequence
Oligo DNA A	5'-TAATACGACTCACTATATTTGGAAGATAGCTGGAGAACTAACCAAA-3'
Oligo DNA B	3'-ATTATGCTGAGTGATATAAACCTTCTATCGACCTCTTGATTGGTTT-5'