Supporting information

Oligonucleotides

A 63mer oligonucleotide probe (circularizing probe, padlock probe, 5'-TGTCTTCGCCTTCTTGTTTCCTTTCCTTGAAACTTCTTCCTTTCTTTCTTTCGACTAAGCACC-3') containing 5'-Iodo and 3'-Phosphorothioate was synthesized in our laboratory on an Expedite 8909DNA synthesizer (PE Biosystems, Foster City, CA) via standard phosphoramidite chemistry. All synthesis reagents were purchased from Glen Research (Sterling, Virginia). The 3' phosphorothioates were synthesized on 3' phosphate-CPG by replacing the normal oxidizing reagent with the sulfurizing reagent thiosulfonate. 5' I-dT were deprotected in NH_4OH at room temperature for 24h, purified by denaturing polyacrylamide gel in the presence of 7M urea prior to use.

Aptazyme, biotin-aptazyme, biotin-dT-aptazyme, and biotin-T_{10}-aptazyme were purchased from IDT (Coralville, IA). All aptazymes have the same sequence (5'-CGAAGACAGGTTCCTGGGGGAGTATTGCGGAGGAAGGTGCTTAGTC) except for 5' end modification with various linkers (biotin, biotin-dT, and biotin-T_{10}, respectively) for chip immobilization. Complementary, fluorescent oligonucleotide probe labeled with Cy3 at 5' end (Cy3-GTTTTCGCCTTCTTGTTTCC-3') was from IDT.

General method for Effector-dependent ligation and RCA in solution

Ligation reactions were performed in at 25°C in ligation buffer (50 mM Tris-HCl , pH 7.4 and 50 mM $MgCl_2$) with 0.5 μM aptazyme and 1.0 μM padlock probe in a volume of 20 μL. The aptazymes were heat denatured at 70°C for 3 min and cooled to room temperature in ligation buffer prior to the addition of DTT-treated padlock probe and an effector (ATP). The final

concentration of DTT was 500 μM. Reaction was initiated by the addition of effector (10 mM ATP, pH 7 except for the dose-dependent experiment) and padlock probe. Ligation reaction was terminated by addition of 95% formamide gel-loading buffer. Ligated and unligated species were separated on denaturing 8-12% acrylamide gel containing 7M urea, stained with SybrGold (Molecular probe, Eugene, OR), and quantitated on a Fluoroimager and ImageQuant 5.2 software (Molecular Dynamics, Sunnyvale, CA).

Aliquots of 4μL of ligation reaction mixture were used to template RCA reactions in a final volume of 20 μL of 1x RCA buffer (40 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 5 mM $(NH_4)_2SO_4$ and 4 mM DTT), 0.25mM dNTPs, and 2 ng/μL ϕ29 DNA polymerase (Epicentre, Madison, WI). Reactions were incubated at 37°C period of ranging from 5-120 min. and then terminated by addition of 95% formamide gel-loading buffer. Amplified products were separated on denaturing 8% polyacrylamide gel containing 7M urea, stained with SybrGold, and quantitated on a Fluoroimager.

General method for Effector-dependent ligation and RCA on a chip

Biotin-labled aptazymes were immobilized on the surface of strepavidin coated glass slides (Pierce Biotechnology, Rockford, IL) via strepavidin conjugation. In detail, heat denatured 100 nM aptazyme in ligation buffer containing 5% glycerol was printed using manual arrayer (V&P Scientific, Inc., San Diego, CA) at 60-80% humidity.

After printing, the slides were incubated within the humidity chamber for 1h to allow time for biotinylated moieties of the aptazymes to be bound to the streptavidin coating on the slides. The printed area on each slide was then enclosed within a CoverWell incubation chamber (Schleicher and Schuell, Keene, NH) comprised of a water-tight 4 wells with clear plastic cover. 1 mL of

ligation buffer containing 0.05% Tween 20 was pipetted into each chamber through access ports that were sealed to remove unbound aptazymes. Each chamber was then incubated with 70 μL of ligation mixture (50 mM Tris-HCl , pH 7.4 and 50 mM $MgCl_2$, and 1.0 μM padlock probe) containing effector at 25°C for period of ranging from 5-120 min. The ligation reaction was terminated by washing the chamber with 1 mL of ligation buffer containing 0.05% Tween 20. 70 μL of RCA mixture [1x RCA buffer (40 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM $MgCl_2$, 5 mM $(NH_4)_2SO_4$ and 4 mM DTT), 0.25mM dNTPs, 0.2 mg/μL BSA, and 2 ng/μL ϕ29 DNA polymerase] was applied to each chamber and incubated at 37°C up to 2h. The RCA reaction was terminated by washing the chamber with 1 mL of 2x SSC buffer (300 mM sodium chloride and 30 mM sodium citrate) containing 0.05% Tween 20.

70 μL of 0.5 μM complementary, fluorescent oligonucleotide probe in 2x SSC/0.05% Tween 20 solution was added to each chamber and allowed to hybridize to the RCA product for 30 min at 37°C. Slides were washed with 1 mL 2x SSC/0.05% Tween 20 solution and then the SureSeal hybridization chamber was then peeled off from the surface of the slide, followed by washing twice with 2x SSC/0.05% Tween 20 solution, twice with 1x SSC/0.05% Tween 20 solution, and once with 0.5x SSC. Slides were withdrawn from the washing station and then dried by immediate centrifugation for 5 min at 600rpm at 25°C. Slide were scanned on an Axon Instruments (Union City, CA) 4000B confocal microarray scanner and the resulting images were analyzed using GenePix 4.1 software.

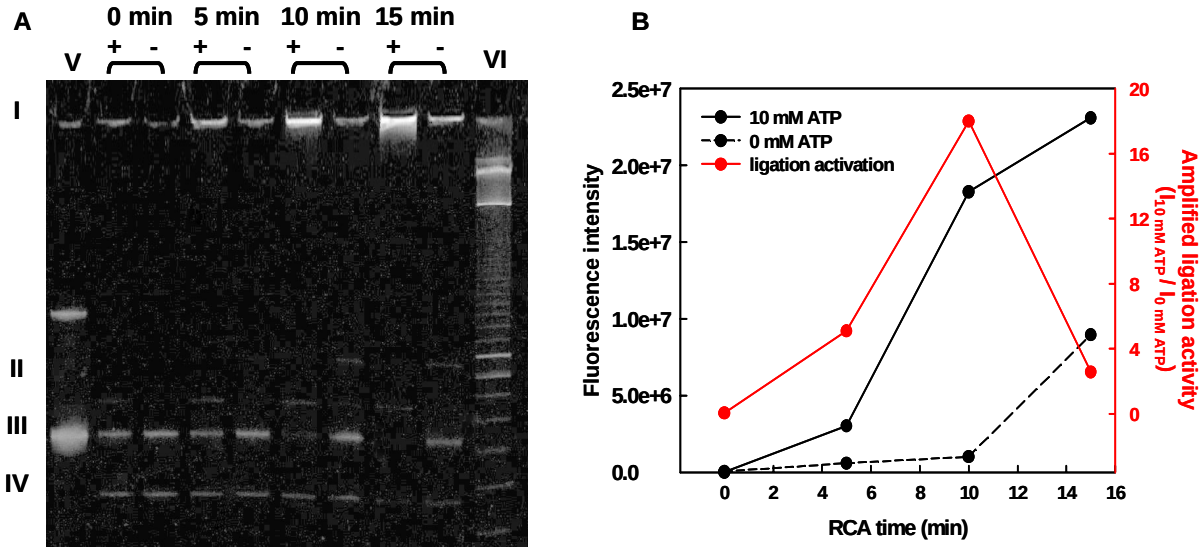

Figure 1. ATP-dependent ligation followed by RCA in solution. (A) Analysis of RCA product by denaturing 8% polyacrylamide gel electrophoresis. (+) and (-) represent ligation in the presence of 10 mM ATP and ligation in the absence of ATP, respectively. Time represents RCA reaction time. I, II, III, and IV correspond to RCA product, circularized padlock probe, original padlock probe and aptazyme, respectively. V and VI are reference padlock probe and 10bp DNA ladder, respectively. (B) Quantitative analysis of RCA product as a function of RCA time. Black line represents % ligation in the presence of ATP, black dotted line represents % ligation in the absence of ATP, and red line represents amplified ligation activity (ratio of % ligation in the presence of ATP versus % ligation in the absence of ATP).

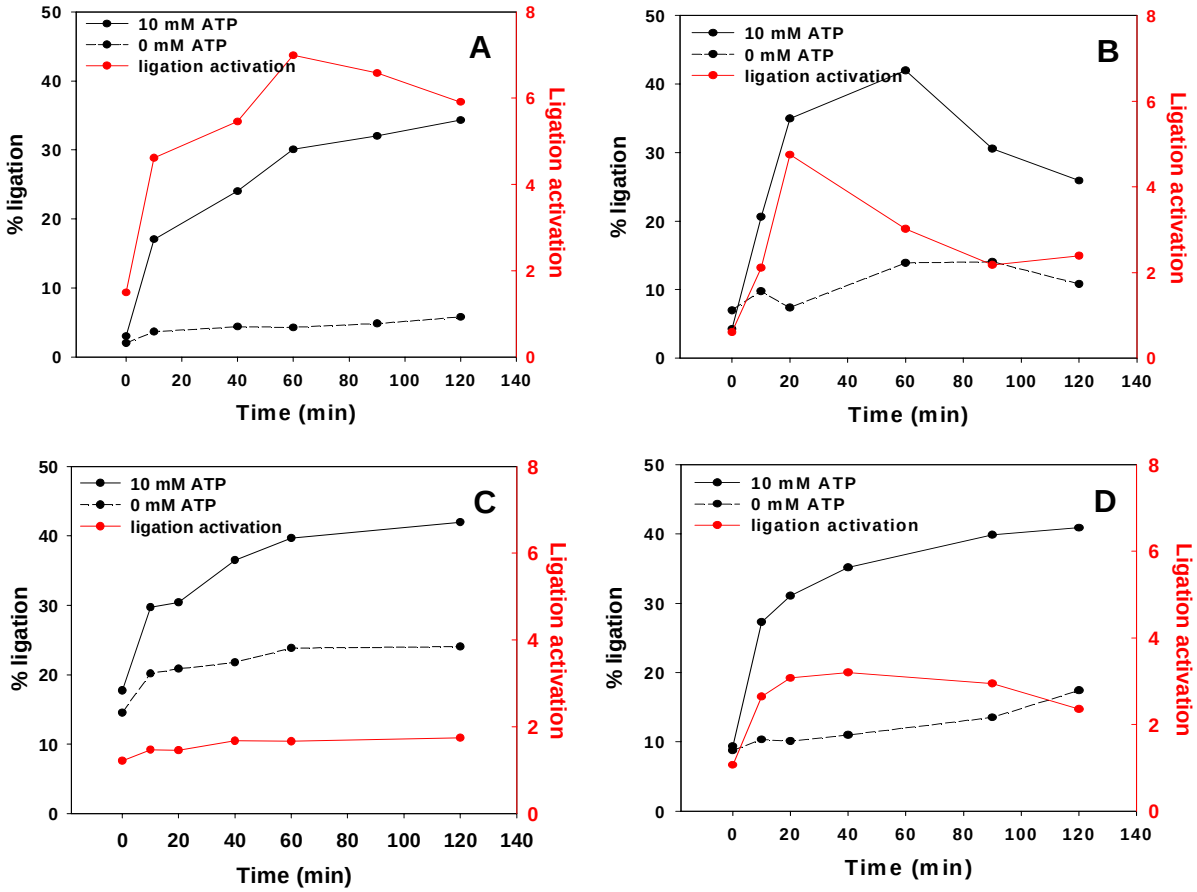

Figure 2. Time course monitoring of ligation activation for biotin-labeled aptazymes with various linkers, no linker, dT, and T_{10} and for unlabeled aptazyme in solution. Each graphs correspond to kinetic analysis of aptazyme (A), biotin-labeled aptazyme (B), biotin-dT-labeled aptazyme (C), and biotin-T_{10}-labeled aptazyme (D), respectively. Black line represents % ligation in the presence of ATP, black dotted line represents % ligation in the absence of ATP, and red line represents ligation activity (ratio of % ligation in the presence of ATP versus % ligation in the absence of ATP).