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Supporting Information for

Target-induced transcription of light-up RNA aptamer to construct a novel method for alkaline phosphatase assay

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Experimental section

Materials

All DNA oligonucleotides used in this work were synthesized and PAGE-purified by Bioneer (Daejeon, Korea). The sequences of oligonucleotides studied in this work are listed in Table S1. Klenow (exo-) DNA polymerase, NEBuffer 2, and lambda protein phosphatase (λ PP) were purchased from New England Biolabs Inc. (Beverly, MA, USA). EZ™ T7 High Yield In Vitro Transcription kit including transcription buffer, $MgCl_2$, rNTP, T7 RNA Polymerase, DTT, and enhancer solution was purchased from Enzynomics (Daejeon, Korea). Alkaline phosphatase (ALP), human serum albumin (HSA), trypsin, lysozyme, thrombin, phosphorylase, acid phosphatase (AP), protein phosphatase 1 (PP1), protein tyrosine phosphatase 1B (PTP1B), acetate kinase (AcK), protein kinase A (PKA), human serum from human male AB plasma, and malachite green chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hexokinase (HK) was purchased from Roche (Basel, Switzerland). All other chemicals were of analytical grade and used without further purification.

Experimental procedures for ALP assay

The reaction solutions for the ALP assay were separately prepared as part A, part B, and part C solutions. Part A solution contains ALP analyte at varying concentrations, 600 nM phosphorylated probe, and 1X NEBuffer 2 (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9). Part B solution contains 600 nM template probe, 1X NEBuffer 2, 750 μM dNTP, and 1 U Klenow (exo-) DNA polymerase. Part C solution contains 1.25X

Transcription buffer, 1.25X $MgCl_2$, 12.5 mM DTT, 1.25X Enhancer solution, 25 mM rNTP, 120 U T7 RNA Polymerase, and 125 μM malachite green chloride. 2 μL of Part A solution was incubated at 37 °C for 10 min, followed by the inactivation of ALP at 90 °C for 5 min. 2 μL of Part B solution was next added to Part A solution and incubated at 37 °C for 10 min. Finally, 16 μL of Part C solution was added to the mixed solution and incubated at 37 °C for 45 min. After the reaction, the fluorescence emission spectra were scanned from 635 nm to 735 nm at an excitation wavelength of 605 nm using Tecan Infinite M200 pro microplate reader (Mannedorf, Switzerland).

For practical applicability test, ALP solution at varying concentrations was spiked into human serum, which was then analyzed according to the same experimental procedures for ALP assay as describe above. The final concentration of human serum in the analyte solution was 1 %.

Polyacrylamide gel electrophoresis

For polyacrylamide gel electrophoresis (PAGE), a 10 μL aliquot of the reaction solution was resolved on 15% polyacrylamide gel at 120 V for 100 min using 1X TBE (0.13 M tris, 45 mM boric acid, 2.5 mM EDTA, pH 7.6) as the running buffer. After ethidium bromide (EtBr) (3 μg/mL) staining, gel was scanned using ChemiDoc™ (Bio-rad, CA, USA).

Optimization of the developed strategy for ALP assay

To maximize the reaction efficiency, we optimized several reaction conditions by examining the signal-to-background ratios (F/F_0, where F and F_0 are the fluorescence intensities at 654 nm from the samples with and without ALP, respectively) under various conditions.

Optimization of reaction times

As shown in Figure S1 (a), as the reaction time for T7 *in vitro* transcription increased, the F/F_0 value increased up to 45 min but rather decreased after 45 min because the increasing rate of F_0 was higher than that of F after 45 min. As shown in Figure S1 (b) and (c), the highest values of F/F_0 were obtained from the reaction samples when ALP-promoted dephosphorylation and PP extension were performed for 10 min.

As a result, 10 min reaction time for both ALP-promoted dephosphorylation and PP extension, and 45 min reaction time for T7 *in vitro* transcription were selected as the optimal reaction times for the proposed strategy.

Optimization of probe concentrations

As shown in Figure S2 (a), the highest value of F/F_0 was obtained when the ratio of TP to PP was 1:1. We next optimized the concentration of both TP and PP. As shown in Figure S2 (b), as we increased the concentration, F/F_0 first increased up to 60 nM but rather decreased over 60 nM. As a result, 60 nM PP and TP was selected as the optimal concentration for the proposed strategy.

Optimization of amounts of enzymes

As shown in Figure S3 (a), as the amount of T7 RNA polymerase increased, the F/F_0 value increased up to 120 U but rather decreased over 120 U because the increasing rate of F_0 was higher than that of F over 120 U. As shown in Figure S3 (b), the highest value of F/F_0 was obtained from the reaction samples when the amount of Klenow (exo-) DNA polymerase was 1 U. As a result, 120 U T7 RNA polymerase and 1 U Klenow (exo-) DNA polymerase were selected as the optimal amounts for the proposed strategy.

Table S1. Oligonucleotide sequences used in this study.

Name	Sequence (5' → 3')
Phosphorylated probe (PP)	TGA GGT AGT AGG TTG TAT AGT T-PO_4
Non-phosphorylated probe (NP)	TGA GGT AGT AGG TTG TAT AGT T
Template probe (TP)	*GGA TCC ATT CGT TAC CTG GCT CTC GCC AGT CGG GAT CC*T CTC CCT ATA GTG AGT CGT ATT A**AA CTA TAC AAC CTA CTA CCT CA**-PO_4
Template probe complementary sequence (Complementary TP)	TGA GGT AGT AGG TTG TAT AGT TTA ATA CGA CTC ACT ATA GGG AGA GGA TCC CGA CTG GCG AGA GCC AGG TAA CGA ATG GAT CC

* The italic letters represent the complementary sequence of malachite green aptamer (MGA). The underlined bases are the T7 promoter sequence. The bold characters indicate the complementary sequence of PP and NP.

Table S2. Comparison of this strategy with alternative strategies for ALP assay.

Key strategy (Signaling method)	Assay time	Detection limit	Limitations	Reference
Iodine-mediated etching of gold nanorods (Colorimetry)	35 min	0.01 U/L	- Synthesis of nanomaterials	[1]
Digital Single Molecule Electrochemistry (Electrochemistry)	30 min	1 aM	- Synthesis of nanomaterials - Requirement of washing step - Sophisticated technique	[2]
Well-controlled assembly on single nanoparticle (SERS)	10 min	0.01 U/L	- Synthesis of nanomaterials	[3]
DNA–silver nanocluster probe (Fluorometry)	~ 3 h	1 U/L	- Low sensitivity - Long reaction time - Synthesis of nanomaterials	[4]
Target-promoted exponential strand displacement amplification (Fluorometry)	80 min	0.47 U/L	- Low sensitivity	[5]
Allosteric-Probe (Fluorometry)	90 min	12 U/L	- Low sensitivity - Labeling with fluorophore	[6]

Dephosphorylation-initiated transcription reaction-mediated dual signal amplification (Fluorometry)	~ 3 h	0.02 U/L	- Long reaction time - Labeling with fluorophore	[7]
rGO and λ exo cleavage (Fluorometry)	50 min	0.01 U/L	- Labeling with fluorophore	[8]
Carbon nitride nanosheets (Fluorometry)	~ 4 h	0.05 U/L	- Long reaction time - Synthesis of nanomaterials	[9]
An enzyme-triggered conformational switch of G-quadruplex (Fluorometry)	70 min	0.503 U/L	- Low sensitivity	[10]
DNAzyme-Au nanoprobe coupled with graphene-oxide–loaded hybridization chain reaction signal amplification (Fluorometry)	~ 3 h	0.14 U/L	- Low sensitivity - Long reaction time - Labeling with fluorophore	[11]
Transcription of light-up RNA aptamer (Fluorometry)	**70 min**	**0.018 U/L**	**-**	**This strategy**

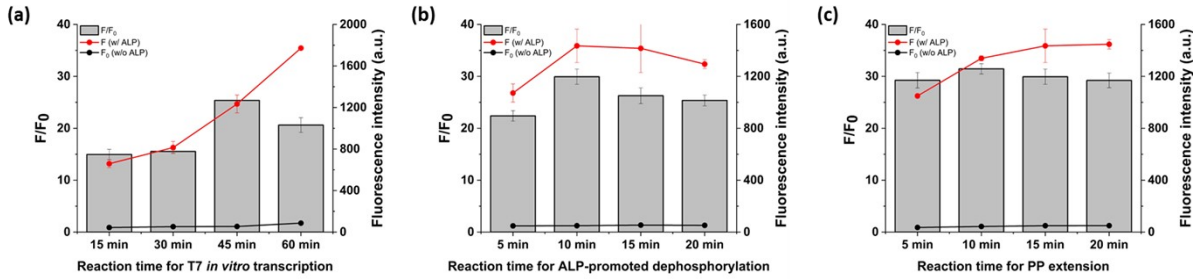

Figure S1. Optimization of reaction times by examining the signal-to-background ratios (F/F_0, where F and F_0 are the fluorescence intensities at 654 nm from the samples with and without ALP, respectively) at different conditions. (a) The signal-to-background ratios (F/F_0) for varying reaction times for T7 *in vitro* transcription. The reaction times for ALP-promoted dephosphorylation and PP extension are 20 min and 15 min, respectively. (b) The signal-to-background ratios (F/F_0) for varying reaction times for ALP-promoted dephosphorylation. The reaction times for PP extension and T7 *in vitro* transcription are 15 min and 45 min, respectively. (c) The signal-to-background ratios (F/F_0) for varying reaction times for PP extension. The reaction times for ALP-promoted dephosphorylation and T7 *in vitro* transcription are 10 min and 45 min, respectively. Through all the experiments, the final concentrations of ALP, PP, and TP are 1000 U/L, 100 nM, and 100 nM, respectively and the amounts of Klenow (exo-) DNA polymerase and T7 RNA polymerase are 2 U and 160 U, respectively. The error bars were determined from triplicate measurements.

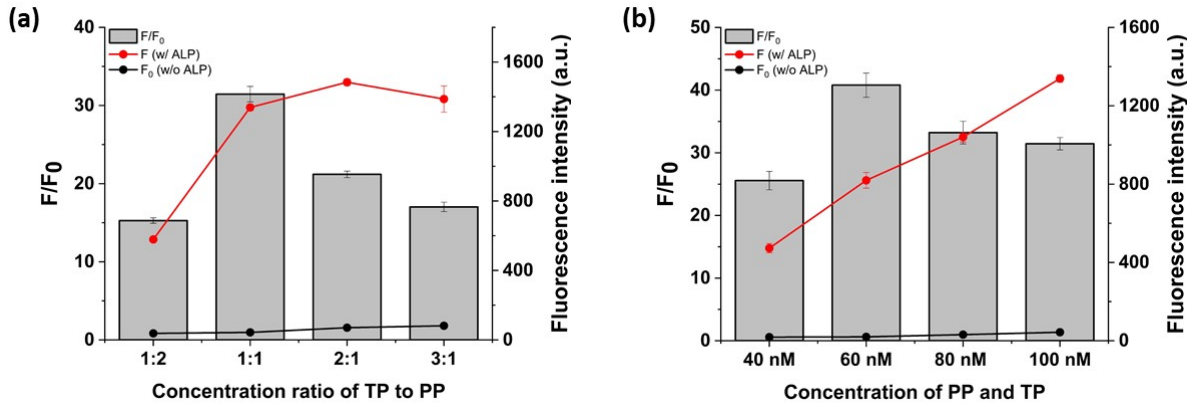

Figure S2. Optimization of probe concentrations by examining the signal-to-background ratios (F/F_0) at different conditions. (a) The signal-to-background ratios (F/F_0) for varying concentration ratios of TP to PP. The final concentration of PP is 100 nM. (b) The signal-to-background ratios (F/F_0) for varying concentrations of PP and TP. PP and TP are employed at the same concentration. Through all the experiments, the final concentration of ALP is 1000 U/L and the amounts of Klenow (exo-) DNA polymerase and T7 RNA polymerase are 2 U and 160 U, respectively. The reaction times for ALP-promoted dephosphorylation, PP extension, and T7 *in vitro* transcription are 10 min, 10 min, and 45 min, respectively. The error bars were determined from triplicate measurements.

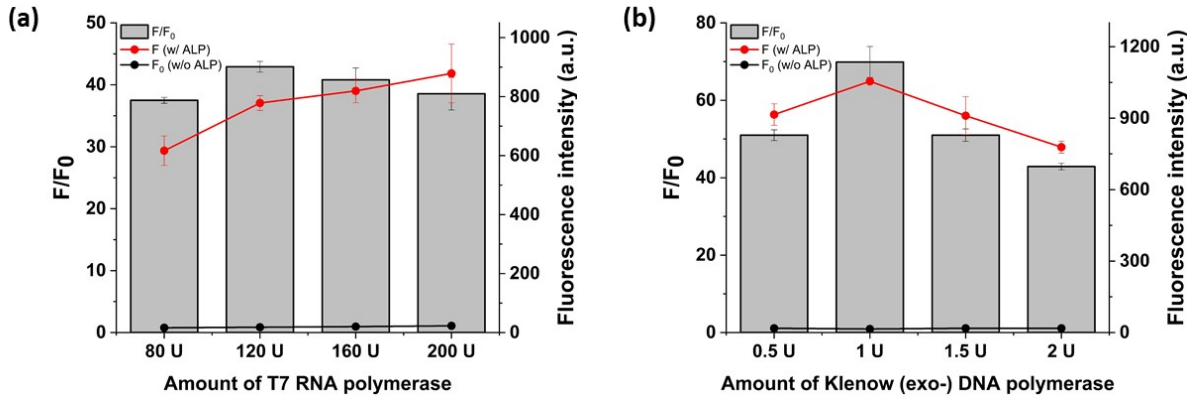

Figure S3. Optimization of amounts of enzymes by examining the signal-to-background ratios (F/F_0) at different conditions. (a) The signal-to-background ratios (F/F_0) for varying amounts of T7 RNA polymerase. The amount of Klenow (exo-) DNA polymerase is 2 U. (b) The signal-to-background ratios (F/F_0) for varying amounts of Klenow (exo-) DNA polymerase. The amount of T7 RNA polymerase is 120 U. Through all the experiments, the final concentrations of ALP, PP, and TP are 1000 U/L, 60 nM, and 60 nM, respectively. The reaction times for ALP-promoted dephosphorylation, PP extension, and T7 *in vitro* transcription are 10 min, 10 min, and 45 min, respectively. The error bars were determined from triplicate measurements.

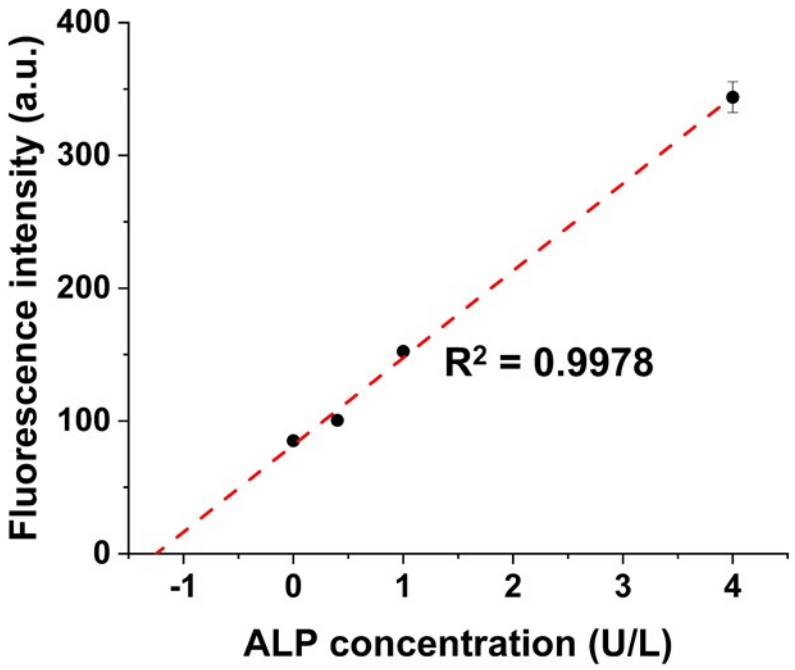

Figure S4. Linear relationship between the fluorescence intensity at 654 nm and the concentration of ALP spiked in 1 % human serum. The final concentrations of PP and TP are 60 nM. The amounts of Klenow (exo-) DNA polymerase and T7 RNA polymerase are 1 U and 120 U, respectively. The reaction times for ALP-promoted dephosphorylation, PP extension, and T7 *in vitro* transcription are 10 min, 10 min, and 45 min, respectively. The error bars were determined from triplicate measurements.

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