Magnetic Field-Activated Fluorescent Sensors for Nucleic Acids

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Supporting Information

1. Chemicals and Reagents

Custom made DNA were purchased from Integrated DNA technologies (IDT, Coralville, IA USA); see the sequences in Table 1 in the paper and applied concentrations are specified in sections below. Trypsin (E.C. 3.4.21.4), fetal bovine serum (FBS), fetal calf serum (FCS), Trypan Blue (stain dye), (1-ethyl-3-[3-(dimethylamino)-propyl] carbodiimide (EDC), N-hydroxysuccinimide (NHS), Triton-X-100, Tween-20, 2-(N-morpholino)ethanesulfonic acid (MES-buffer), 2-amino-2-hydroxyethyl-propane-1,3-diol (Tris-buffer), (4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES-buffer), silicon tetraethoxide (TEOS), (3-aminopropyl)triethoxysilane (APS), α-bromoisobutyril bromide (BIB), ethyl α-bromoisobutyrate (EBIB), N,N,N′,N″,N‴-pentamethyldiethylenetriamine (PMDTA), methane sulfonic acid and other standard organic and inorganic materials and reactants were obtained from Sigma-Aldrich or J.T. Baker and used without further purification. tert-Butyl acrylate (TBA) and poly(ethylene glycol) methyl ether
acrylate (PEGMA) (average molecular mass 480 g/mol) were purchased from Sigma-Aldrich and purified using a flash-chromatography column containing inhibitor removers (Sigma #311340 and Sigma #311332). All solutions for the experiments were prepared using ultrapure water (18.2 MΩ·cm; Barnstead NANOpure Diamond).

2. Detailed Experimental Procedures

*Isolation and culturing of ectocervical cell line HCX from human tissue.* All cell culture preparations were performed following published procedure. Ectocervical cells were cultured in Keratinocyte Serum Free Medium (KSFM) (Gibco).

*Preparation and culturing of MCF-7 cells.* MCF-7 cells were ordered from American Type Culture Collection (ATCC), Manassas, VA, USA. MCF-7 (breast adenocarcinoma) cells were cultured in Eagle’s Minimum Essential Medium (EMEM) from ATCC supplemented with 100 U/mL penicillin, 0.05% (w/v), amphotericin B, 0.02% (w/v), gentamicin and 0.1% (w/v) streptomycin, 0.01 mg/ml human recombinant insulin and fetal bovine serum to a final concentration of 10%.

*Synthesis of Fe₃O₄ magnetic beads (MaB).* Superparamagnetic nanoparticles were synthesized by a co-precipitation methods as described elsewhere. Iron chloride salts, FeCl₃·6H₂O (4.43 g) and FeCl₂·4H₂O (1.63 g) were dissolved in 190 mL of water with a stoichiometric ratio 2:1 using magnetic stirring at room temperature. Then, 10 mL of 25% (w/w) ammonium hydroxide was added to the solution to yield a black precipitate. The supernatant solution was stirred for additional 10 minutes, then the precipitate was separated with a magnet and rinsed 3 times with water using magnetic separation. The colloidal dispersion of magnetic nanoparticles was stabilized with citrate ions by rapid rinsing of the precipitate with a 2 M nitric acid solution (two consecutive rinsing) followed by addition of 5 mL of 0.5 M aqueous solution of trisodium citrate while maintaining pH 2.5 with added sodium hydroxide. After stirring for 1.5 h, the magnetic nanoparticles were magnetically separated, rinsed with water and diluted to 100 mL (pH 6.0) of the volume of the nanoparticle dispersion. The concentration of the magnetic nanoparticles (15 nm in diameter) in the final stock solution was 2% (w/w).

A modified Stöber method was used to coat the synthesized Fe₃O₄ magnetic nanoparticles with a silica shell. The nanoparticle stock solution (2 mL) was diluted with a mixture of 160 mL of ethanol and 40 mL of water. Then, ammonium hydroxide (25% w/w, 5 mL) was added to the nanoparticle dispersion. After 10 minutes of ultrasonic bath treatment, 1 mL of TEOS was added dropwise to the solution. The synthesis was carried out at 0°C under sonication for 3 h. The reaction was stopped by the addition of several droplets of 10% HCl resulting in precipitation of the silica-coated nanoparticles. The precipitate was collected with a magnet, rinsed 3 times with water using centrifugation and re-suspended in a 50 mL centrifuge tube using ultra-sonication. The resulting product represented a stable dispersion of the core-shell nanoparticles (2 mg/mL). The produced nanoparticles (TEM image) are shown in Figure SI1a. The powder was easily redispersable in water and formed a stable colloidal dispersion with a nanoparticle size of 45 nm and, Figure SI1, and zeta potential ξ = -30 mv (pH 7.4).
**PAA-b-PEGMA block copolymer grafted from nanoparticles surface.** Grafting of PAA-b-PEGMA block copolymer from the surface of the nanoparticles was conducted using activator generated by electron transfer (AGET)–atom transfer radical polymerization (ATRP). The polymerization was conducted in two steps. First, poly-tert-butyl acrylate (PTBA) was grafted by polymerization of TBA. The polymerization was followed by grafting of PEGMA blocks. Finally, the post-polymerization treatment was applied to hydrolyze the PTBA blocks and convert them to polyacrylic acid (PAA) blocks. The process was performed according to the steps specified below.

**Immobilization of initiator.** Silica-coated magnetic nanoparticles were transferred to ethanol medium: the stock nanoparticle solution was mixed with ethanol and the particles were extracted using a magnetic separation. This was repeated several times to decrease concentration of water in the ethanol medium. Finally, the nanoparticles were added to 2% (w/w) (3-aminopropyl)triethoxysilane (APS) solution in ethanol and stirred for 2 h. After the APS immobilization, the particles were rinsed 3 times with ethanol and incubated for 1 h in 100 mL of dry dichloromethane with added 2 mL trimethylamine and 1 mL α-bromoisobutyryl (BIB) bromide. The initiator-functionalized particles were rinsed 3 times with chloroform and ethanol.

**Grafting of the copolymer.** A TBA monomer solution was purified using a flash-chromatography column containing the inhibitor removers. Then, 320 µL of 0.1 M CuBr₂, 320 µL of 0.5 M PMDTA and 10 µL of 0.68 M EBIB ethanol solutions were added to a 30% (w/w) monomer solution in ethanol and loaded with the initiator-functionalized magnetic nanoparticles. EBIB was added to the solution for the synthesis of the polymer in the solution for molecular mass analysis. The reaction mixture was deoxygenated by nitrogen purging for 20 min and then heated to 70° C in an oil bath. Then, 500 µL of 1 M ascorbic acid solution was added to the solution and the reactor was sealed. The polymerization reaction was terminated in 15-30 min by opening vial to air. The non-grafted polymer from the solution was separated from the nanoparticles by centrifugation, re-precipitated 3 times with 30% (v/v) aqueous ethanol and analyzed with gel permeation chromatography (GPC). Grafting of the second PEGMA block was carried out by a similar procedure: a 10% (w/w) PEGMA solution in ethanol was polymerized for 1 h at room temperature. PTBA-b-PEGMA was converted to PAA-b-PEGMA by adding methane sulfonic acid. After hydrolysis, the particles were rinsed 3 times with chloroform, ethanol, and water and dried at 50° C in an oven.

**Characterization of the nanoparticles.** The nanoparticle size analysis was carried out using a combination of dynamic light scattering (Malvern Zetasizer Nano) and AFM (Icon, Bruker) methods. The summary of the nanoparticle dimensions and molecular characteristics of the grafted brush are presented in Table SI1, Figures SI1 and SI2.
Table S11. Structure and features of the polymer brush-decorated magnetic nanoparticles

| Schematics | Iron oxide core | Iron oxide core coated with a silica shell | Core-shell particle with grafted PAA-b-PEGMA |
|------------|----------------|----------------------------------------|------------------------------------------|
| Diameter (DLS) at pH 7.4, ± 5 nm | 16 | 50 | 115 |
| ζ-potential, ± 10 mv | -10 | -40 | -5 |
| Brush thickness swollen/dry, ± 5 nm | - | - | 32.5/9.3 |
| Number average molecular mass Mn of the block-copolymer, g/mol, ± 5% | - | - | 17000 |
| Grafting density, ±0.06 nm⁻² | - | - | 0.36 |

Preparation of MaBiDZ. To conjugate NH₂-modified DZa and Hook oligos to the polymer-functionalized Fe₃O₄ magnetic nanoparticles, EDC/NHS carbodiimide coupling was employed. Carboxyl groups on the MaB polymeric brush surface were activated using 20 mM EDC and 50 mM NHS for 25 minutes in a mixture containing 0.05% Tween-20 and pH 4.5 MES-buffer, 50 mM, on a slow tilt shaker. Unreacted EDC, NHS and their reaction low-molecular products were removed through centrifugation for 10 min at 14,000 r.p.m. and the pellet was re-suspended in pH 5.5 HEPES-buffer, 50 mM, containing 50 mM MgCl₂. DZa and Hook, both modified respectively with amino groups at the 5'-ends were then separately incubated with the magnetic beads containing activated carboxylic groups for 1.5 h. Unbound DNA was removed through centrifugation for 10 min at 14,000 r.p.m. and MaB were re-suspended in pH 7.4 HEPES buffer, 50 mM, containing 50 mM MgCl₂. Specific analyte strand (Twist), DZb and the prepared DZa-bound MaB conjugates were pre-incubated in a thermostated water bath at 30°C for 20 min. F-sub was incubated with the prepared Hook-MaB conjugates for 1 h. The F-sub-Hook-MaB conjugates were then centrifuged at 14,000 r.p.m. for 10 min, the supernatant containing unbound F-sub was discarded, and the pellet was re-suspended in pH 7.4 HEPES-buffer, 50 mM.

Attachment of BiDZ was confirmed using Diamond Nucleic Acid dye (Promega). A calibration line was developed by measuring fluorescence of known concentrations of DZa bound to the dye. Fluorescence of the dye bound to a known quantity of MaB was then measured in order to find the average fluorescence per MaB. Lastly the calibration plot was used to relate the fluorescence per MaB to DZa strands per MaB. The measurements of fluorescence per MaB were repeated three times and a value of 120 DZa strands ± 11/MaB was obtained.

Fluorescence measurements. The final MaB concentration was 25 µg particles per well (12.5 µg each of DZa- and Hook-modified nanoparticles) or 83 µg/mL. Neodymium N-52 magnets (K & J Magnetics, Inc., Pipersville, PA, USA) measuring 3/8” × 3/16” × 1/16” were glued to 48-well plates (Nunc™ Cell-Culture Treated Multidishes, ThermoFisher Scientific). The pre-incubated
Twist, DZb and DZa-bound MaB conjugates were added to respective wells followed by the F-sub-Hook-MaB conjugates. Fluorescence measurements were taken using the SpectraMax i3x Microplate Reader (Molecular Devices, LLC., CA, USA).

**Confocal laser scanning microscopy.** For intracellular monitoring we used a Leica TCS SP5 II Tandem Scanning Confocal and Multiphoton Microscope. For magnet-controls, we placed NdFeB, grade 52, magnets measuring 5/16" diameter × 1/8" thick (K & J Magnetics, Inc.) under each cell dish. Cells were seeded at 60-70% confluence 3 days prior to measurements in 35 mm glass bottom dishes (MatTek Corporation, Ashland, MA), USA. Hoechst Stain (Santa Cruz Biotechnology, Dallas, TX, USA) and EpCAM surface antibody stain (BioLegend, San Diego, CA, USA) were added at 0.5% and 1%, respectively, and allowed to incubate for 45 minutes. LysoTracker Green DND-26 (ThermoFisher Scientific) was used to stain endosomal compartments according to package instructions. MaBiDZ probe was then added at a 90 pM concentration (40 µg/mL). Prior to addition, DZb, and the prepared DZa-bound and F-Sub-Hook-bound MaB conjugates were pre-incubated in a thermostated water bath at 30°C for 20 min. Confocal images were taken every hour for four hours, using the 405 nm, 488 nm, and 635 nm lasers to visualize Hoechst staining; Lysotracker staining and MaBiDZ probe fluorescence; and EpCAM staining, respectively.

**Flow cytometry** Cells were seeded in 35 mm glass bottom dishes at 80% confluence. Cells were incubated with MaBiDZ for 2 hours. For magnet controls, a NdFeB, grade 52, magnet measuring 5/16" diameter × 1/8" thick (K & J Magnetics, Inc.) was placed under each dish for 4 hours. Cells were then rinsed with Dulbecco’s phosphate buffered saline (Gibco) and trypsinized with 500 µL trypsin for 20 minutes. Trypsin was neutralized with 500 µL media containing 10% FBS. Cells were centrifuged at 2000 r.p.m. for 3 min. Supernatant was removed and cells were re-suspended in a 1% solution of Alexa Fluor 647-conjugated EpCAM antibody (clone 9C4, Biolegend, San Diego, CA) in 2% FBS and allowed to incubate in the dark at 4°C. Cells were centrifuged at 2000 r.p.m. for 3 min and re-suspended in 500 mL 4% paraformaldehyde in PBS, pH 7, for 20 min. Cells were centrifuged at 2000 r.p.m. for 3 min. Supernatant was removed and replaced with 300 µL of 2% FBS.

**Trypan Blue staining for membrane damage assessment.** Cell membrane damage is examined by using Trypan Blue staining method. MCF-7 cells were incubated with 100 pM of MaBiDZ for 2 h. Cells without any additions, cells with the sensing components but without the magnet, and cells with the sensing components and activated with a magnet were detached from 60 mm culture dishes by using trypsin/EDTA (Gibco™ media) and re-suspended with complete EMEM medium. Cell suspension was added to an equal volume of 0.4% Trypan Blue. Note that Trypan Blue is specifically coloring dead cells. Then, cells were counted by using a Bright-Line™ hemocytometer (Hauser Scientific, PA, USA). Under optical microscope, Trypan Blue-stained cells, which lost the integrity of cell membrane, were counted. Finally, normal cell membrane percentage of each sample was calculated.

**Non-complementary analyte response assay** M.smeg BiDZ probes were ordered from Integrated DNA Technologies (Coralville, IA, USA) with binding arms complementary to the M.smegmatis mRNA analyte not found in mammalian cells. To test response of MCF-7 cells to the M.smeg BiDZ probe, and to the Twist mRNA BiDZ probe, respectively, MCF-7 cells were grown in 60
mm culture dishes to 60% confluence, trypsinized, and lysed using NET-buffer (0.1% Triton-X-100, 100 mM NaCl, 1 mM EDTA, 20 mM Tris-buffer). RNAsecure RNAse inactivation reagent (ThermoFisher Scientific) was added and used according to package instructions. Cell count was quantified before lysis using a Bright-Line™ hemocytometer (Hausser Scientific, PA, USA). To the lysate we added pH 7.4 HEPES-buffer, 50 mM, containing 50 mM MgCl₂, 100 nM DZa, 100 nM DZb and 200 nM F-sub. Fluorescence measurements were taken using a Cary Eclipse spectrophotometer every hour for twelve hours.

**Twist mRNA Detection in cell lysates.** MCF-7 breast cancer cells and HCX primary cervical epithelial cells were grown in culture to 80% confluence, trypsinized, and lysed using NET buffer (0.1% Triton-X-100, 100 mM NaCl, 1 mM EDTA, 20 mM Tris). Prior to lysis, cell count was confirmed using a Bright-Line hemacytometer (Hausser Scientific). RNAsecure RNAse inactivation reagent (ThermoFisher Scientific) was added and used according to package instructions. The solution-based probe (100 nM Dzb, 100 nM Dza, 200 nM Fsub) was added to lysates and fluorescence measurements were taken using a Cary Eclipse spectrophotometer. Measurements were repeated for each cell type three times.
3. Additional Figures and Short Comments.

**Figure S11.** Transmission electron microscopy (TEM) images of magnetic nanoparticles: a) 50 ±5 nm silica-coated magnetic nanoparticles reveal a 15 nm iron oxide magnetic core and a 20 nm thick silica shell; b) magnetic nanoparticles with 12.5±5.0 nm grafted poly(acrylic acid)–poly(ethylene glycol) block-copolymer (PAA-b-PEGMA); the latter is observed as polymer structures bridging between two adjustment nanoparticles. Note that the aggregated state of the nanoparticles was obtained during the TEM imaging and it does not mean the nanoparticle aggregation in the solution state. The TEM images in this figure are shown to present size, shape and structure of individual particles. The particles appear as 2D agglomerated structures. TEM images are obtained for the samples prepared by deposition of a droplet of an aqueous particle suspension on the TEM-grid. Evaporation of water results in receding of the contact line when the capillary forces translocate particles that are “shoveled” by the contact line. The beads form 2D-agglomerates with loosely assembled individual particles. This is very typical for TEM-imaging of nanoparticles. If particles were aggregated in solution, they would appear in TEM images as dense 3D-aggregates.
Figure SI2. Dynamic light scattering (DLS) analysis of the nanoparticles in aqueous dispersions at pH 7.5: 1) Fe₃O₄ magnetic nanoparticles, 2) silica-coated magnetic nanoparticles, 3) magnetic nanoparticles with the grafted PAA-b-PEGMA polymer layer. Distributions by size of the magnetic beads in aqueous environment are shown in this figure when the measurements followed each step of the surface modification. The distribution functions demonstrate that a fraction of particles that exceeds the average diameter by 2-fold is less than 5%. This is a solid evidence that in the absence of the magnetic field the nanoparticle either not aggregated in aqueous solutions or a number of aggregates is negligible.

Figure SI3. Photos of the experimental setups: (A) *ex vivo* experiments and (B) *in vitro* experiments. Aggregate of MaB is indicated by arrow in (A).
Figure S14. BiDZ-Twist sensor in complex with the complimentary Twist analyte. A fragment of Twist is shown. See Table 1 of the main text for the full sequence.

Figure S15. A probe complementary to the M.smeag analyte (not found in MCF-7 cells) was incubated in MCF-7 lysates. Binding arm sequences were as follows: DZa (M.smeag): 5' - CCA TCC CAC ACC GCA AAA GCT TTCC A CAA CGA GAGAACCTT and Dzb(M.smeag) 5' - TGC CCA GGG A GG CTA GCT CCT ACC AGG CC A TGC GAC CAG CAG G. In (A) and (B) we show the responses of the M.smeag and BiDZ sensors respectively in MCF-7 lysate over the course of 12 hours. MCF-7 lysates consist of 600,000 cells each. After 12 hours, the Twist BiDZ probe produced a 5-fold response compared to the M.smeag probe in MCF-7 lysates. This confirms that the fluorescent intensity produced by the sensor is indeed in response to hybridization with analyte present in the cell. Increase in fluorescence observed over the 12 h period in response to the M.smeag probe can be attributed to nuclease-induced non-specific cleavage of F-sub produced by active nucleases.
Figure SI6. Confocal laser scanning microscopy (CLSM) images and flow cytometry data for a series of negative controls to the experiment shown in Figure 2 of the main text. Controls are as follows: (A) Non-sense control consisting of binding arms complementary to a random oligo sequence, (B) MaBiDZ probe without DZb binding arm, (C) cells incubated with only the Hook-F-sub-MaBiDZ species, and (D) cell only control.
Figure S17. Confocal laser scanning microscopy (CLSM) images showing co-localization between MaB and endosomes with and without the presence of a magnetic field. Previous studies\(^4\) show that nanoparticles enter cells by endocytosis, and are subsequently either stored in endosomes or lysosomes, or undergo endosomal escape. If the MaB do not undergo endosomal escape, they are not available for intracellular sensing. To examine a small window of events that occur upon cellular entry of MaB, MCF-7 cells were incubated with MaB conjugated with a 22-mer oligos tagged with the Quasar-670 fluorophore to enable intracellular tracking of the MaB. Cells and MaB were then visualized with CLSM at 30, 60, and 120 min. MCF-7 nuclei are stained with Hoechst nuclear stain and visualized with 408 nm laser, endosomes with Lysotracker green and 488 nm laser, and Quasar 670-labelled oligo-modified MaB with 635 nm laser. Arrows indicate sites of co-localization (yellow) between MaB (red) and endosomes (green). Scale bar is 20 µm. As shown, sites of colocalization are much more numerous in the “No Magnet” controls than in the “Magnet” controls. Likewise, free particles (visible in red in the images) are much more evident in the samples when the magnetic field is applied. Thus, the data clearly shows that application of a magnetic field is consistent with decreased endosomal sequestering and a greater degree of free particles in the cytoplasm.
Figure SI9. MaBiDZ cytotoxicity presented as a fraction of live cell counts vs. magnetic beads concentration after incubation for 12 h. The iron oxide magnetic beads affect cell viability at concentration greater than 50 µg/mL in good agreement with literature. The concentration of MaBiDZ used in cell culture samples was 40 µg/mL (for a ca. 300 mm² surface area), which is within the non-cytotoxic concentration limit. In addition, since the cells were incubated with MaBiDZ for up to four hours only, negligible cytotoxicity was observed.

Figure SI8. (A) Quantification of co-localization between quasar 670-modified MaB (Q670-MaB) fluorescence with MCF-7 endosomes (labeled with Lysotracker Green DND-26 (ThermoFisher Scientific)) after 0.5 h, 1 h, and 2 h of treatment, for samples with (a) and without (b) a magnetic field. To quantify co-localization we used several CLSM images for the two controls to calculate Pearson coefficients using the ImageJ co-localization threshold plugin. (B) Cell membrane damage is examined by using Trypan Blue staining method. Results show that the force acting on the MaB in a magnetic field does not induce significant membrane damage.
Figure SI20. To validate the MaBiDZ-generated fluorescence signal in response to Twist levels shown in CLSM and flow cytometry measurements, we tested the response to our probe in MCF-7 and non-cancerous cervical epithelial cell lysates. Cells were grown in culture to 80% confluence, trypsinized, and lysed using NET buffer (0.1% Triton-X-100, 100 mM NaCl, 1 mM EDTA, 20 mM Tris). Cell count was confirmed using a Bright-Line hemocytometer (Hausser Scientific). The solution-based probe was added to lysates and fluorescence measurements were taken using a Cary Eclipse spectrophotometer every hour for 12 hours. In order to determine if the signal difference between cancer and normal cells was statistically significant, we did a two sample T-test and found that the difference between the two populations is statistically different after three hours. The fluorescent response produced by the two populations was distinct, validating the CLSM and flow cytometry results.
To calculate the internal cellular concentration of the DNAzyme probe, a calibration line was developed based on the fluorescence of Quasar670-labeled DNA bound to MaBs (Q670-MaB). MCF-7 cells were seeded into a 96-well plate (Greiner Bio), incubated with Q670-MaBs, and fluorescence was quantified (640 nm excitation, 670 nm emission) using a Spectramax i3x microplate reader. The fluorescence of cells alone was subtracted from the fluorescence of Q670-MaB-incubated cells, and this value was divided by the number of cells (quantified using a hemocytometer (Bright-Line)). This value was related to concentration from the developed calibration line and used to calculate the average number of particles inside the cells, which was determined to be ca. $1 \times 10^6$ Q670-MaBs/cell. This value is in good agreement with the literature. Using the value of DNA/MaB calculated earlier (see SI, “Preparation of MaBiDZ”), a value for the MaBiDZ/cell was obtained. Using the volume of the cell, the value obtained for concentration of MaBiDZ/cell was 5.8 µM. It is important to note that the amount of correctly configured DNAzyme arms that allow for formation of the core is much lower. Further lower is the number of catalytically active DNAzyme cores/cell, as is the active DNAzyme that is able to hybridize with the target mRNA. Thus, the calculated internal cellular concentration of MaBiDZ is not related to the sensitivity of this method.

Figure SI21. To calculate the internal cellular concentration of the DNAzyme probe, a calibration line was developed based on the fluorescence of Quasar670-labeled DNA bound to MaBs (Q670-MaB). MCF-7 cells were seeded into a 96-well plate (Greiner Bio), incubated with Q670-MaBs, and fluorescence was quantified (640 nm excitation, 670 nm emission) using a Spectramax i3x microplate reader. The fluorescence of cells alone was subtracted from the fluorescence of Q670-MaB-incubated cells, and this value was divided by the number of cells (quantified using a hemocytometer (Bright-Line)). This value was related to concentration from the developed calibration line and used to calculate the average number of particles inside the cells, which was determined to be ca. $1 \times 10^6$ Q670-MaBs/cell. This value is in good agreement with the literature. Using the value of DNA/MaB calculated earlier (see SI, “Preparation of MaBiDZ”), a value for the MaBiDZ/cell was obtained. Using the volume of the cell, the value obtained for concentration of MaBiDZ/cell was 5.8 µM. It is important to note that the amount of correctly configured DNAzyme arms that allow for formation of the core is much lower. Further lower is the number of catalytically active DNAzyme cores/cell, as is the active DNAzyme that is able to hybridize with the target mRNA. Thus, the calculated internal cellular concentration of MaBiDZ is not related to the sensitivity of this method.
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