(Supporting Information)

Aptamer Nano-Flares for Molecular Detection in Living Cells

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Materials and Methods

Oligonucleotide Synthesis

Oligonucleotides were synthesized on an Expedite 8909 Nucleotide Synthesis System (ABI) using standard solid-phase phosphoramidite methodology. Bases and reagents were purchased from Glen Research. The oligonucleotides were purified using reverse-phase high performance liquid chromatography (RP-HPLC) using a Varian Microsorb C18 column (10 μm, 300 × 10 mm) with 0.03 M triethylammonium acetate (TEAA), pH 7 and a 1%/min gradient of 100% CH3CN at a flow rate of 3 mL/min, while monitoring the UV signal of DNA at 254 nm. Sequences used to prepare and test ATP aptamer nano-flares are given in Fig. 1b.

Preparation of Aptamer Nano-flares

Citrate-stabilized gold nanoparticles (13 ± 1 nm) were prepared using published procedures.[1] Thiolated aptamer or control oligonucleotides were mixed with reporter strands (1:1.2) in phosphate buffered saline (PBS; 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, pH 7.4, Hyclone), heated to 70 ^{o}C, slowly cooled to room temperature, and stored in the dark for at least 12 hours to allow hybridization. These pre-formed DNA duplexes were then added to gold nanoparticles at a concentration of 3 nmol duplexes per 1 mL of 10 nM colloid and shaken overnight. After 12 hours, sodium dodecylsulfate (SDS) solution (10%) was added to the mixture to achieve a 0.1 % SDS concentration, phosphate buffer (0.1 M; pH = 7.4) was added to the mixture to achieve a 0.01 M phosphate concentration, and the salt concentration of the mixture was slowly increased to 0.3 M NaCl. The solution containing the functionalized particles was centrifuged (15,700 g, 20 min) and resuspended in phosphate buffered saline three times to produce the purified Au NPs used in all subsequent experiments. The concentration of the particles was determined by measuring their extinction at 524 nm (ε = 2.7 x 10^{8} L mol^{-1} cm^{-1}). Purified oligonucleotide functionalized Au NPs were suspended at a concentration of 25 nM in PBS.

Fluorescence Experiments

Aptamer nano-flare probes or control particles were diluted to a concentration of 1 nM in PBS containing 2 mM $MgCl_2$ (Sigma). All of the fluorescence spectra were recorded on a Jobin Yvon

Fluorolog FL3-22 exciting at 633 nm and measuring emission from 655 to 755 nm in 1 nm increments. To determine DNA duplex loading, the conjugate was treated with 2 mM KCN to oxidize the gold nanoparticles and the concentration of DNA duplexes was determined by fluorescence measurements and comparing to a standard curve.

Cell Culture

HeLa cells (ATCC) were grown in Eagle's Minimal Essential Medium (EMEM), with 10 % heat inactivated fetal bovine serum and maintained at 37 ^{o}C in 5 % CO_2. Cells were seeded in 12 or 24 well plates and were grown for 24 hours to reach 80% confluence prior to treatment with particles. After 24 hours, the cells were washed with PBS and fresh opti-MEM (Invitrogen) was added. The particles (25 nM) were filtered (0.20 μm acetate syringe filter) and added to the media of the freshly washed cells at a final concentration of 1nM. After 2 hours, the cells were washed in PBS, trypsinized, and collected for analysis. Cells were counted and measured for viability using a Guava EasyCyte Mini (Guava Technologies). Viability after incubation was > 98%.

Imaging

Cells were grown on Lab-Tek®II Chamber #1.5 German Coverglass System (Nalge Nunc International). After 1 day, the media was replaced with opti-MEM containing filtered aptamer nano-flares and control particles (particle concentration, 1 nM). After 2 hours of treatment, the cells were washed with PBS and fresh EMEM was added. Live cells were stained with TubulinTracker™ Green reagent, and Hoechst 33342 (Invitrogen) following the manufacturer's instructions. All images were obtained with a Zeiss 510 LSM at 40x magnification using a Mai Tai 3308 laser (Spectra-Physics). Fluorescence emission was collected at 390 – 465 nm, 500 – 550 nm, and 650 – 710 nm, exciting at 729, 488, and 633 nm respectively. In all experiments, the pinhole and gain settings of each individual collection channel were determined using control nanoparticle treated cells. The settings were held constant throughout the experiment.

Flow Cytometry

Cells were incubated with 5 mM 2-deoxy-D-glucose (Sigma) and oligomycin (Sigma) (0, 2, 5, 10g/mL) for 1 hour, followed by co-incubation with aptamer nano-flares or control particles as

described above for 2 hours (particle concentration, 1 nM). After treatment, cells were detached from culture flasks using trypsin. The solution containing treated cells was centrifuged (2,300 g, 5 min) and resuspended in PBS three times to remove the cell debris. Flow cytometry was performed using BD LSR II System, exciting at 633 nm. All flow cytometry experiments were preformed in triplicate and averaged, and each measurement used 10,000 cells.

Nanoparticle Cellular Uptake Quantification

Cells were treated with 5 mM 2-deoxy-D-glucose and oligomycin (0, 2, 5, 10g/mL) for 1 hour, followed by co-incubation with 1 nM aptamer nano-flares or control particles as described above for 2 hours. After treatment, cells were washed 3 times in PBS buffer, collected, and counted. To prepare samples for inductively coupled plasma mass spectrometry (ICP-MS) (Thermo-Fisher), the cells were dissolved with neat nitric acid at 60°C overnight. The Au content of the cell digest was determined by ICP-MS.[2, 3] Each cell sample was prepared in a matrix consisting of 3% HNO_3, 5 ppb Indium (internal standard), and Nanopure™ water. The number of nanoparticles taken up by each cell was calculated based on the concentration of Au found in the sample. This was done using the molecular weight of Au and the diameter of the nanoparticle to calculate Au atoms per particle (6.78×10^4 atoms/particle). Once the number of particles was calculated, this particle number was divided by the cell count to determine the number of particles per cell. All ICP-MS experiments were preformed in triplicate and averaged.

Cellular ATP Determination

Cells were incubated with 5mM 2-deoxy-D-glucose and oligomycin (0, 2, 5, 10μg/mL) for 3 hours. After treatment, cells were detached using trypsin and counted as described above. The ATP amounts were determined by ATP bioluminescent somatic cell assay kit (Sigma) following the manufacturer's instructions. Luminescence was measured using a GloMax® 96 microplate luminometer. The cellular ATP level is calculated as: $[ATP_{(cellular)}]=ATP_{(total)}/(\text{Total cell population} \times \text{Cell volume})$. Cell volume was 2.6×10^{-12} L based on literature values. [4] All luminescence measurements were preformed in triplicate and averaged.

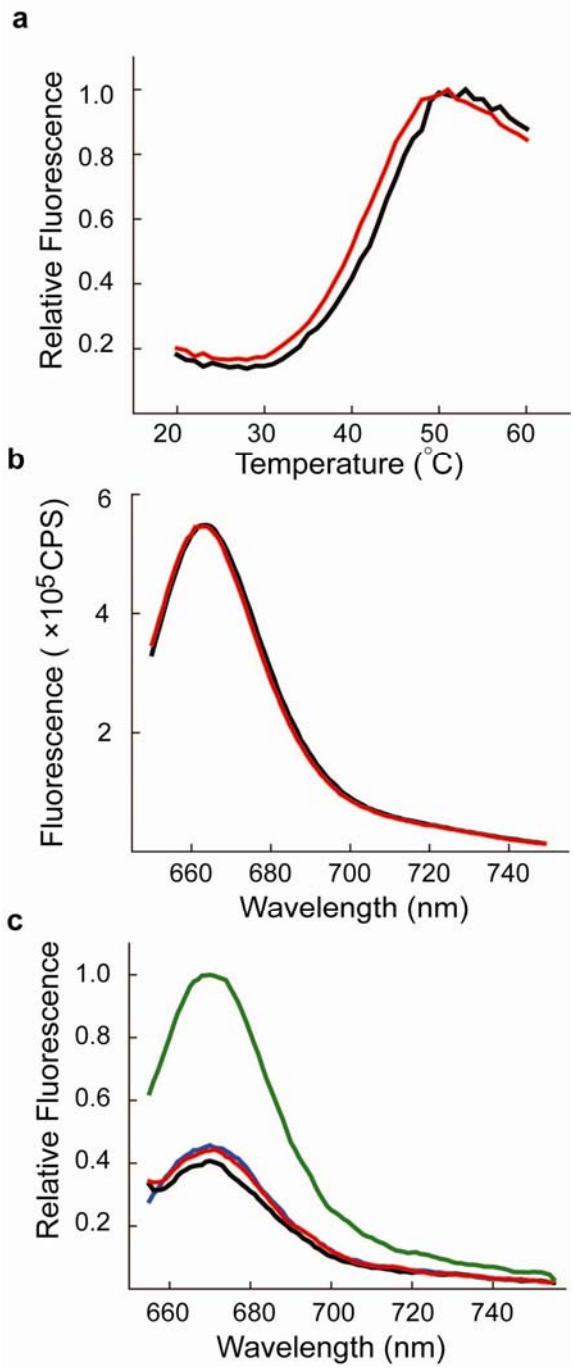

Figure S1. (a) Fluorescence melting curves of aptamer nano-flares (red) and control nanoparticles (black). (b) Total fluorescence signal of aptamer nano-flares (red) and control particles (black) after dissolving the gold. (c) Fluorescence signal of aptamer nano-flares (blue) and control nanoparticles (black) alone. Fluorescence signal of aptamer nano-flares (green) and control nanoparticles (red) in the presence of 2 mM ATP. These measurements demonstrate that both the aptamer nano-flares and control particles have very similar signaling and thermal stability properties.

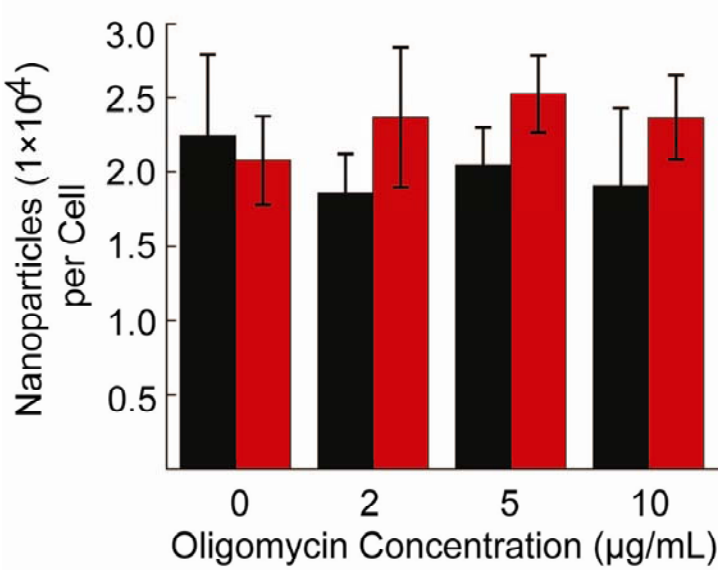

Fig S2. Cellular uptake of aptamer nano-flares (red) and control particles (black).

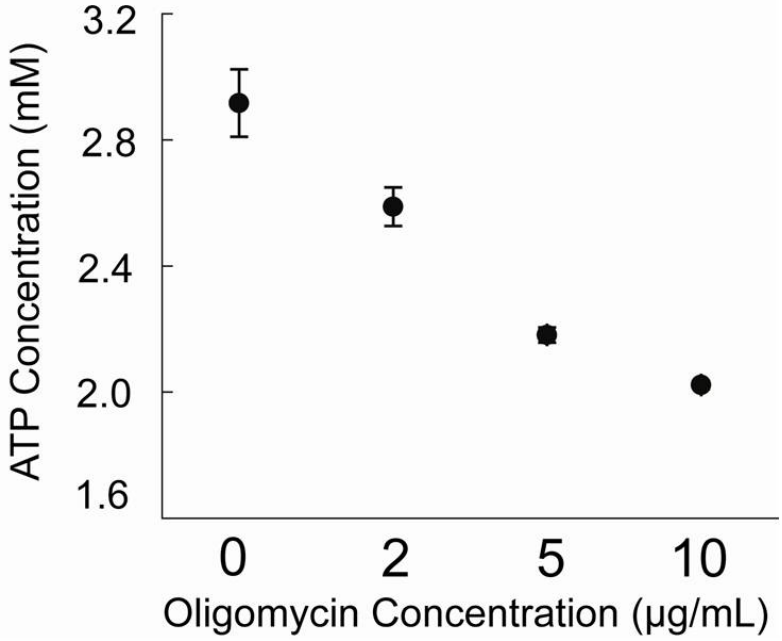

Fig S3. Calculated cellular ATP concentration after treatment with doses of oligomycin.

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