Published in final edited form as:

Small. 2011 September 5; 7(17): 2517–2525. doi:10.1002/sml.201100591.

Long Range Nanoparticle Surface Energy Transfer Ruler for Monitoring Photothermal Therapy Response

Anant K. Singh, Wentong Lu, Dulal Senapati, Sadia Afrin Khan, Zhen Fan, Tapas Senapati, Teresa Demeritte, Lule Beqa, and Paresh Chandra Ray*

Department of Chemistry, Jackson State University, Jackson, MS, USA

Abstract

Gold nanotechnology driven recent approach opens up a new possibility for the destruction of cancer cells through photothermal therapy. Ultimately, photothermal therapy may enter into clinical therapy and as a result, there is an urgent need for techniques to monitor on time tumor response to therapy. Driven by the need, in this article we report nanoparticle surface energy transfer (NSET) approach to monitor photothermal therapy process by measuring the simple fluorescence intensity change. Fluorescence intensity change is due to the light-controlled photothermal release of ssDNA/RNA via dehybridization during therapy process. Our time dependent results show that just by monitoring fluorescence intensity change, one can monitor photothermal therapy response during therapy process. Possible mechanism and operating principle of our NSET assay have been discussed. Ultimately, this NSET assay could have enormous potential applications in rapid, on-site monitoring of photothermal therapy process, which is critical to providing effective treatment of cancer and MDRB infections.

Introduction

Presence of cancer remains as the greatest challenge in public health care in today's world.¹⁻¹⁰ Recently, gold nanoparticles of different sizes and shapes with optical properties tunable in the near-infrared (NIR) region have been exploited for the hyperthermic destruction of cancer cells and upon successful trial, they can be used as drugs in photothermal therapy⁴⁻¹⁶. Due to biocompatibility, lack of toxicity and ability to generate high temperatures at a desired site, perhaps the greatest promise of impact of gold nanotechnology for society will be the therapeutic challenges of cancer disease¹⁰⁻³⁶. Ultimately, photothermal therapy may enter into clinical therapy and as a result, currently there is an urgent need for an assay to monitor photothermal therapy response during the therapy process, so that the physician can find out whether therapy is successful during therapy process. When light of appropriate wavelength is absorbed by gold nanoparticles, it is converted into heat by rapid electron-phonon relaxation followed by phonon-phonon relaxation⁴⁻³⁰. This highly localized heat generated by gold nanoparticle kills cells selectively and also help to optically elicit the controlled release of desired oligonucleotides or small molecules, which are anchored to the nanoparticle¹⁷⁻²⁶. In this paper, we have designed long range nanoparticle surface energy transfer (NSET) ruler³⁷⁻⁵⁰ based on light-controlled photothermal release of ssDNA/RNA via dehybridization of double-stranded DNA/RNA, to monitor photothermal therapy response during the photothermal process. For our study, a well-characterized human prostate cancer cell line LNCaP which expresses a high level of prostate-specific membrane antigen (PSMA)^{6,27-28} relative to normal cells of the prostate has been used. For designing long range optical ruler, we have modified

*paresh.c.ray@jsums.edu, Fax: 601-979-3674 .

popcorn shape gold nanoparticle with extended A9 RNA aptamer, which is specific to human prostate cancer cell, PSMA. We have used extension to the A9 aptamer to serve as a hybridization site for complementary Cy3 coated RNA, which will be released during photothermal process due to thermal de-hybridization. As a result, during therapy process, fluorescence intensity increases and by monitoring simple fluorescence intensity change one will be able to monitor in-situ photothermal process.

Optical ruler based distance measurements are essential for tracking biomolecular conformational changes, drug discovery, and cell biology³²⁻⁵⁷. Förster Resonance Energy transfer (FRET), in which excited fluorophores transfer energy to neighboring chromophores, is well characterized in photochemistry and have been used in a wide range of applications in biology^{51,56-57}. This process results from dipole-dipole interactions and is thus strongly dependent on the center-to-center separation distance. It also requires a nonzero integral of the spectral overlap between donor emission and acceptor absorption^{51, 56-57}. Although FRET technology is applied routinely at the single molecule detection limit, the length scale is limited by the nature of the dipole-dipole mechanism, which is on the order of maximum 10 nm. Recently, several groups including ours³³⁻⁵⁰, have experimentally demonstrated that nanomaterial based surface energy transfer (NSET) ruler is capable of measuring distances more than twice the FRET distance. Though NSET is a through space mechanism like FRET, it is geometrically different from FRET because it works through a dipole-surface resonance mechanism³²⁻⁵⁰. This mechanism effectively breaks the distance barriers of FRET, thereby increasing the probability of energy transfer³²⁻⁵⁰. Using those above advantages, in this article, we have demonstrated that popcorn shape gold nanomaterial based long range NSET ruler can be used for on-site monitoring of photothermal therapy response during therapy process of different human cancer cell lines.

Results and Discussions

For selective therapy and monitoring of therapy process, in our present study we have used three different cell lines and these are 1) The human prostate cancer cell line LNCaP which over expresses a high level of prostate-specific membrane antigen (PSMA), 2) PSMA negative human prostate cancer cell line (PC-3), and 3) human skin HaCaT keratinocytes, a normal skin cell line. We have used an enzyme-linked immunosorbent assay kit to quantify PSMA in different tested cells. Our experimental results indicated that amount of PSMA in LNCaP cell was 7.8×10^6 / cells, whereas PSMA amount was only 1.9×10^3 / cells in case of PC3 cell, which is comparable to the reported concentration of PSMA in different cancer cell lines²⁷⁻²⁸. Our result also shows that there is no PSMA in HaCaT cell line, which is the normal skin cell. The sequence of the A9 RNA aptamer with the extended sequence used was 5' GGG AGG ACG AUG CGG ACC GAA AAA GAC CUG ACU UCU AUA CUA AGU CUA CGU UCC CAG ACG ACU CGC CCG AGA AUU AAA UGC CCG CCA UGA CCA G-SH, where underlined sequence was the extended sequence. For covalent attachment of -SH modified A9 RNA with popcorn shape gold nanoparticle, the CTAB surfactant on the nano-popcorn surface was replaced by mercaptohexanoic acid (MHA) by reported method¹⁷. As shown in Figure 1, after that popcorn shape gold nanoparticle was functionalized with -SH modified A9 RNA extended aptamers using reported protocol as we described in experimental section^{17,22-25,37-43}. In the next step, we hybridized extended part of A9 aptamer with capture Cy3 modified RNA (CTG GTC ATG GCG GGC ATT TAA TTC) which is complementary to the aptamer extension sequence. In this situation, since the acceptor gold nanoparticle and donor organic dyes are close together, there is dipole-surface type energy transfer from dye molecular dipole to nanometal surface, which is known as NSET³⁷⁻⁵⁰. As a result, the fluorescence from Cy3-labelled nucleic acid is mostly quenched by gold nanoparticles as shown in Figure 2A. Duplex RNA lengths less

than 100 base pairs are typically assumed to be adequately modeled by a rigid rod approximation with only high-frequency oscillations along the backbone, as described by Hagerman⁵⁶. Since after hybridization, Cy3 modified duplex RNA is attached with gold nanoparticle, the separation distance between the nanoparticle and the donor dye can be evaluated using the model reported by Clegg et al⁵⁷. The distances are estimated by taking into account size of the fluorescent dye, 0.32 nm for each base pair, 1.8 nm for Au-S distance + base pair to dye distance. Our experimental results show that though after hybridization the distance between gold nanoparticle and Cy3 dye is around 10 nm, we see huge amount of fluorescence quenching, which is not possible by general FRET mechanism. To understand whether our assumption of rigid rod approximation is valid in our case, we have used dynamic light scattering (DLS) measurement, using Malvern Zetasizer Nano instrument. Popcorn shape gold nanoparticles have average size is about 30 nm, which can be seen from our TEM data. The addition of duplex RNA aptamer to the gold nanoparticle, changes the diameter to about 49 nm, which indicates that the distance between gold nanoparticle and Cy3 dye is around 10 nm.

In case of FRET approximation, one assumes that there is no perturbation between the donor and acceptor dye molecules. Since the metal nanoparticle has a strong electric field when a single-point dipole donor is placed close to a metal nanoparticle, experimental data have shown that there are notable changes in the radiative and nonradiative rates of decay due to coupling of the donor to the metal's local electric field^{46,49,52}. As a result, in case of NSET, the metal oscillators are considered to be strongly coupled, rather than a single dipole, as assumed in case of FRET model. The above coupling of the donor dipole to the metal surface, leads to huge quenching enhancement, depending on the projection of the electric field from the metal nanoparticle surface.

To understand what is the distance limit for the energy transfer in the case of gold nanopopcorn based NSET, we have used complimentary strand of extended A9 RNA of different lengths, as shown in Figure 2B. After hybridization, by varying the RNA lengths, the separation distance between popcorn shape gold nanoparticle and Cy3 dye can be systematically varied between 8 nm and 33 nm, by varying the number of base pairs. The quenching efficiency for each sample was measured by comparison against control ds RNA–dye in the absence of NP, using the following equation³⁹:

$$Q_{eff} = 1 - \frac{I_{sample} A_{control}}{I_{control} A_{sample}} \quad (1)$$

Where, Q_{eff} is the quenching efficiency due to the energy transfer, I_{sample} and $I_{control}$ are the integrated intensity under the curve for the fluorescence peak due to the sample and control. A_{sample} and $A_{control}$ are the absorption of the sample and control at the peak of the dye. For each set of experiment, initially we have measured total amount of ds RNA–dye attached to the gold nanoparticle using 10 μ M potassium cyanide which oxidizes the gold nanoparticle as we discussed in the experimental section and then we have used same amount ds RNA–dye in the absence of NP, as a control. Also for distance dependent experiment, we have used the same amount of dye attached RNA for each experiment. Figure 2C shows how the quenching efficiency varies with the increase in the distance between gold nanoparticle and Cy3 dye for popcorn shape gold nanoparticles of 30 nm particle size. Our result shows that 30 nm size popcorn shape gold nanoparticle based NSET ruler is highly sensitive to small changes in the dye-particle distance even if they are separated more than 40 nm, which is 4 times larger than general FRET distance. To understand the distant dependent quenching process, we tried to fit our data (as shown in Figure 2C) with theoretical modeling using general FRET and NSET used by Jeening et. al.^{39-41,47}. The quantum efficiency of energy transfer can be written as^{37-41,47},

$$\Phi_{EnT} = \frac{1}{1 + \left(\frac{R}{R_0}\right)^n} \quad (2)$$

where R is the distance between donor and acceptor, R_0 is the distance between donor and acceptor at which the energy transfer efficiency is 50%. In the case of Förster or dipole-dipole energy transfer, $n = 6$ and in case of NSET, $n = 4$ as reported by Jeening et. al.^{39-41,47}. For our theoretical fitting we have used reported R_0 data^{37,39-41}.

As shown in Figure 2C, our results indicate that the long distance quenching rate is better described with a slower distance-dependent quenching rate than the classical $1/R^6$ characteristic of Förster energy transfer and also our data show poor agreement with NSET model which provides a better description for gold nanoparticle of size below 2 nm⁴¹. In general, the interactions between nanoparticle and dye are quite complex due to the involvement of several parameters like illumination polarizations and wavelength, plasmonic overlap, distance ranges, and particle sizes. Since FRET physically originates from the weak electromagnetic coupling of two dipoles, one can imagine that providing more coupling interactions can circumvent the FRET limit. Light induces oscillating dipole moments in each gold particle, and their instantaneous $(1/r)^3$ coupling results in a repulsive or attractive interaction, modifying the plasmon resonance of the system³⁷⁻⁵⁰. Due to that, the softer dependence of the interaction strength on particle separation distance r results in a much longer interaction range compared to general FRET. Quenching efficiency is also highly dependent on the excited-state donor lifetime. At small distances, the large fluorescence quenching efficiency of 99.8% is due to the two facts and these are, 1) gold nanoparticles increase the nonradiative rate R_{nonrad} of the molecules due to energy transfer and the radiative rate R_{rad} of the molecules decreases because the molecular dipole³⁷⁻⁵⁰ and 2) the dipole induced on the gold nanoparticles radiates out of phase if the molecules are oriented tangentially to the gold nanoparticle's surface. At higher distance, the distance dependent quantum efficiency is almost exclusively governed by the radiative rate as reported recently³⁷⁻⁵⁰. Recently Seelig et. al.⁴⁶ reported that nanoparticle-induced lifetime modification can serve as a nanoscopic ruler for the distance range well beyond 10 nm.

Their data indicate that for bigger size nanoparticle (20-40 nm in diameters), radiative life time (τ) is highly sensitive to small changes in the dye-particle distance even if they are separated by up to 40 nm, which explains our observation of long range optical ruler. Now, one has to remember that there is a possibility for RNA to interact with the Au NP surface. Due to the effect of surface charge, surface coverage, mutual strand interaction and interaction with gold nanoparticle, the apparent length of the RNA can be little smaller than their expected molecular length which may change the actual experimental distance. As shown in Figure 2C, the quenching rate become slower after 20 nm and it may be due to the fact that as reported by Lakowicz et. al.¹⁴ after 20nm, there are possibility of small amount of surface enhanced fluorescence. As a result, after 20 nm, both the process, fluorescence enhancement and quenching will compete with each other, which decrease the quenching rate.

Next we have used this long range fluorescence ruler to monitor photothermal therapy process of prostate cancer cell line by just monitoring fluorescence intensity change. For this purpose, we have used Cy3 modified extended A9 RNA aptamer ruler attached propcorn shape gold nanoparticle which can selectively bind with human prostate cancer cell line LNCaP which over expresses a high level of prostate-specific membrane antigen (PSMA). To demonstrate selectivity, we have also used PSMA negative human prostate cancer PC-3 cell line and human skin HaCaT keratinocytes, a normal skin cell line. As we have discussed before⁶, our enzyme-linked immunosorbent assay indicated that the amount of PSMA in

LNCaP cell is 7.8×10^6 /cells, whereas PSMA amount was only 1.9×10^3 /cells in case of PC3 cell and we do not find any measurable PSMA in our HaCaT cell line. As shown in Figure 3B, our TEM image shows that when aptamer modified popcorn shape gold nanoparticle were mixed with LNCaP cancer cell, several nanoparticles can bind to PSMA receptors in one cancer cell. On the other hand, as shown in Figure 3C, our TEM image also shows that the PSMA negative human prostate cancerous PC-3 cells are poorly labeled by the A9 RNA aptamer attached nanoparticles even after 4 hours of incubation. We observed the same phenomena for HaCaT normal cell. In our photothermal therapy experiment, we have used 80-120 mW 785 nm NIR light for 40 min using a 785 nm OEM laser. Since the irradiation wavelength matches with the plasmon bands of the cancer cell conjugated popcorn shape gold nanoparticles, during photothermal therapy, the light will be absorbed by the gold nanoparticles and it will generate heat. This heat will lead to irreversible cell destruction through protein denaturation and coagulation as well as cell membrane destruction. Our bright field inverted microscope image as shown in Figure 3D, shows that cancer cells are deformed during photothermal therapy process. This cell death following nanoparticle exposure to NIR radiation could be due to numerous factors, including nanoparticle explosion, shock waves, bubble formation, and thermal disintegration. As shown in Figure 4C, our time interval MTT test indicates that within 30 minutes most of the cancer cells died. We have also performed same tests with PSMA negative prostate cancerous (PC-3) and non-cancerous (HaCa T) cells (as shown in Figure 4C). Our result shows that PC-3 and HaCaT cells are mostly alive even after 40 minutes of irradiation and it is mainly due to the fact that PC-3 and HaCaT cells are poorly labeled by the A9 RNA aptamer attached nanoparticles. Our experimental data also indicate that the LNCaP cancer cells required less than half the laser energy (8 W/cm²) for photothermal lysis in comparison to the normal cells (20 W/cm²).

Now the lattice heat content can be sufficient enough to lead to raise the local temperature above the melting temperature (T_m) of the RNA duplex and as a result the Cy-3 modified nonthiolated RNA strand will dissociate into the surrounding medium, while its complement A9 RNA will remain attached to the nanoparticle, as shown in Figure 1. The temperature at which melting or dehybridization of RNA duplex in solution occurs is dependent on many factors including composition and orientation of neighboring base pairs, sequence length, and salt concentration¹⁶⁻²⁵. First, we have measured the RNA melting curves by monitoring the fluorescence in the gel column as a function of solution-ambient temperature. We have found out that the melting temperature of the duplex RNA (extended part of A9 RNA aptamer) sequence in solution without gold surface was 39 °C at 40 mM salt concentration. Recently, several articles have reported¹⁶⁻²⁵ that there is a possibility of significant decrease in the DNA/RNA duplex melting temperature when DNA/RNA is bound to the gold nanoparticle surface relative to the anticipated solution-phase T_m . As a result, we can easily anticipate that irradiation with 100 mW 785 nm continuous-wave (CW) lasers raised the local temperature above the melting temperature (T_m) of the RNA duplex, which will trigger the Cy3 modified non-thiolated strand to dissociate into the surrounding medium while its complement remained attached to the nanoparticle, as shown in Figure 1. In this condition, since the distance between gold nanoparticle and Cy3 dye changes abruptly, the quenching efficiency decreases and as a result, fluorescence signal increases. Now to monitor dye release process, we have performed time dependent photothermal therapy process as well as fluorescence intensity measurement experiment. For this purpose, we have used 785 nm continuum laser for photothermal therapy and 532 nm continuum laser (5 mw) for fluorescence intensity measurement. At first the NIR laser at 785 nm was exposed to popcorn shape gold conjugated with cancer cell for different time intervals and then the conjugated solution was centrifuged. After that, we have used fluorescence spectroscopy to quantify the released Cy3 attached RNA in supernatants. To quantify how much percentage of dye attached RNA has been released during therapy process, we have used ds RNA–dye

in the absence of nanoparticle as control. Before performing the photothermal experiment in each case, we have measured the amount of ds-RNA-dye attached with the gold nanoparticle. For this purpose, we have treated the Cy3 modified aptamer conjugated gold nanoparticle with 10 μ M potassium cyanide to oxidize the gold nanoparticle. After that, the solution containing the released Cy3-labeled aptamers were collected for the fluorescence analyses. The amount of Cy3-labeled aptamer was measured by fluorescence. We have used the same amount of ds RNA-dye in the absence of nanoparticle as the control. We have also performed 10 μ M potassium cyanide treatment after the therapy and we have found out that the measured fluorescence intensity before and after therapy are the same, after KCN treatment. This indicates that dye photo-bleaching is minimal during therapy process. As shown in Figure 4A, we observed a very distinct emission intensity change during photothermal therapy. Our time dependent fluorescence measurement shows that as the photothermal therapy progresses, the fluorescence intensity increases. It is due to release of dye attached oligonucleotides during therapy process. It is also interesting to see that, as shown in Figure 4B and 4C, there is not any significant photothermal killing or any significant fluorescence intensity change for first 10 minutes of photothermal therapy process. Now, since during therapy process the temperature of the solution changes, it can also change the fluorescence intensity. To understand that, we adsorbed Cy3 dyes on the gold nanoparticle first and then Cy3 fluorescence intensity was monitored as a function of solution-ambient temperature. We have noted negligible fluorescence change even at the temperature of 45⁰ C, which confirms that the fluorescence intensity change during therapy process is mainly due to the RNA dehybridization.

Figure 4B shows time dependent fluorescence intensity change due to the Cy-3 modified non-thiolated RNA melting, where $F(t)$ is the fluorescence intensity at time t , during photothermal therapy and $F(0)$ is the fluorescence intensity from the control. As shown in Figure 4B, our result shows that after therapy for 40 minutes a nanoparticle has only around 15% RNA duplex attached. To understand whether there is any correlation between % of cell death and fluorescence intensity recovered, we have plotted % of cell viability and fluorescence intensity change. As shown in Figure 4D, we see a nice linear plot between % of cell viability and fluorescence intensity change where $F(t)$ is the fluorescence intensity at time t , during photothermal therapy and $F(0)$ is the initial fluorescence intensity, from ds-RNA-Cy3 aptamer as a standard. Our result shows that simple fluorescence intensity change due to duplex RNA melting can be used to monitor in-situ photothermal-therapy response during therapy process. Although, MTT assay always used to find the amount of cell death after photothermal therapy, but for MTT assay it needs 24h incubation before the treatment, as a result, it is time consuming and also it is less sensitive than our NSET assay.

To understand whether the fluorescence intensity change is dependent on the formation of conjugation between A9 aptamer attached gold nanoparticle and cancer cell line, we have performed time dependent fluorescence intensity change during photo-thermal process for PSMA negative PC-3 and normal HAcAT cell line in the presence of A9 aptamer coated popcorn shape gold nanoparticle. As we discussed before, due to the lack of enough PSMA, A9 aptamer attached gold nanoparticle did not conjugate well with PC-3 and HAcAT cell line and as a result, we did not expect much nano-therapy activity for these cell lines when they were exposed to 100 mW, 785 nm laser light for 30 minutes. As shown in Figure 4C, fluorescence intensity changes as well as cell death are very little in the case of PC-3 and HAcAT cell line, whereas significant cell death and fluorescence intensity changes have been observed for LNCAP cell line. This may be due to the fact that aggregation of gold nanoparticles on cell membranes or in intracellular environments led to the high enhancement of photothermal performance as per many studies⁵⁸⁻⁶⁰. Since in our case, aggregates were formed only in the presence of LnCaP cancer cell, which has strong absorption at 785 nm, photothermal therapy effect should be highly efficient in the presence

of aggregates. Also A9 RNA aptamer attached popcorn shape gold nanoparticle with PC-3 or HaCaT cells exhibit very little absorption at 785 nm. Our data show that our NSET assay for photothermal-therapy monitoring is highly selective and nanoparticle conjugation with cancer cell line is necessary to monitor therapy process.

Conclusion

In conclusion, in this article, we have reported development of long range nanoruler to monitor photothermal therapy response during photothermal therapy process of human prostate cancer cells. Our experimental data shows that popcorn shape gold nanoparticle based NSET ruler is highly sensitive to small changes in the dye-particle distance even if they are separated more than 30 nm, which is 3 times larger than general FRET distance. Our result indicates that the long distance quenching rate is better described with a slower distance-dependent quenching rate than the classical $1/R^6$ characteristic of Forster energy transfer. Our time dependent experimental results demonstrate that as the photothermal therapy progresses, the fluorescence intensity increases due to duplex RNA melting, which changes the distance between gold nanoparticle and dyes. We have shown that just by monitoring fluorescence intensity change during therapy process, one can monitor the photothermal therapy response in time. Our experimental data indicate a nice linear plot between % of cancer cell death and fluorescence intensity change, which shows that it is highly feasible to use long range fluorescence assay for the measurement of in-situ photothermal therapy response during therapy process, which is critical to providing effective treatment of cancer. Although we have shown promising advances in popcorn shape gold nanoparticle based NSET assay for monitoring photothermal therapy response, we still need a much greater understanding of how to control surface architecture and clinical sample environment in order to stabilize and maximize the assay response for clinical study.

Materials and Experiments

All the chemicals were purchased from Sigma-Aldrich. Aptamers were purchased from Midland Certified Reagent. The human prostate cancer cell lines were purchased from ATCC. Human skin HaCaT keratinocytes, a transformed human epidermal cell line, was obtained from Dr. Norbert Fusenig of the Germany Cancer Research Center.

Synthesis of Popcorn Shape Gold Nanoparticle

Our gold nano-popcorn synthesis was achieved through a two-step process, as we have reported recently⁶. In the first step, very small, reasonably uniform, spherical seed particles are generated. In the second step, we have used ascorbic acid and CTAB to grow into larger particles of popcorn shape morphology we desired. JEM-2100F transmission electron microscope (TEM) and UV-visible absorption spectrum were used to characterize the nanoparticles (as shown in Figure 3A).

Synthesis of Aptamer attached Popcorn Shape Gold Nanoparticle

As we discussed popcorn shape gold nanoparticles were synthesized using seed-mediated growth procedure in the presence of CTAB. Since CTAB is known to be cytotoxic, it will not be ideal for in vivo diagnosis. Since CTAB is positively charged at physiological pH, it will be able to attract negatively charged proteins easily. To overcome this problem, the CTAB surfactant on the nano surface was replaced by mercaptohexanoic acid using round-trip phase transfer ligand exchange method, as reported recently by Wijaya et. al¹⁷. After that -SH RNA aptamers were gradually exposed to gold nanoparticle in presence of Sodium dodecyl sulfate (SDS) NaCl and PBS buffer over a 16-hour period¹⁷⁻²⁵. To remove the unbound RNA, we centrifuged the solution at 8,000 rpm for 20 minutes and the precipitate was redispersed in 2 mL of the buffer solution. To measure the number of aptamer

molecules in each gold nanoparticle, after conjugation, we have treated the Cy3 modified aptamer conjugated gold nanoparticle with 10 μ M potassium cyanide to oxidize the gold nanoparticle. After that, the solutions containing the released Cy3-labeled aptamers were collected for the fluorescence analyses. The amount of Cy3-labeled aptamers was measured by fluorescence. By dividing the total number of Cy3-labeled aptamers by the total number of nanoparticles, we estimated that there were about 350-430 aptamers per popcorn shape gold nanoparticles. This experiment has been performed 5-6 times and average values are reported in this manuscript

Design of different length RNA optical ruler

To design different length RNA based optical ruler, we have used complimentary strand of extended A9 RNA of different lengths, as shown in Figure 2B and discussed before. After hybridization, by varying the RNA lengths, the separation distance between popcorn shape gold nanoparticle and Cy3 dye can be systematically varied between 8 nm and 33 nm, by varying the number of base pairs. Some of the capture sequences are shown below.

6mer 5 -Cy3- CTG GTC -3

12mer 5 -Cy3- CTG GTC ATG GCG -3

18mer 5 -Cy3- CTG GTC ATG GCG GGC ATT -3

24mer 5 -Cy3- CTG GTC ATG GCG GGC ATT TAA TTC-3

30mer 5 -Cy3- CTG GTC ATG GCG GGC ATT TAA TTC -3

36mer 5 -Cy3- CTG GTC ATG GCG GGC ATT TAA TTC TCG GGC -3

45mer 5 -Cy3- CTG TTC GCG CTG GTC ATG GCG GGC ATT TAA TTC TCG GGC -3

63mer 5 -Cy3- CTG GCG GGC TAA TTC ATG ATT TTC GCG CTG GTC ATG GCG GGC ATT TAA TTC TCG GGC -3

Cell Culture and Cellular Incubation with Aptamer Conjugated Nanoparticle

Cancer cells were grown in a 5% CO₂ incubator at 37 °C using RPMI-1640 medium (ATCC, Rockville, MD) supplemented with 10% premium fetal bovine serum (FBS) (Lonza, Walkersville, MD) and antibiotics (10 IU/mL penicillin G and streptomycin) in 75-cm² tissue culture flasks. An enzyme-linked immunosorbent assay kit was used to quantify PSMA in different tested cells, as we discussed before. Different numbers of cells were then immersed into the multifunctional popcorn shape gold nanoparticle solution for 30 min at room temperature before performing the experiment.

Photothermal Therapy and % of live cell determination

For photothermal therapy experiment, we have used a continuous wavelength portable OEM laser operating at 785 nm, as an excitation light source for 40 minutes. After that we have used MTT test to find the amount of live cell during nanotherapy process. For this purpose, prostate cancer cells were seeded in 96-well plates (well diameter 6.4 mm) with a density of 10,000 cells/well and allowed to attach for 24 h at 37 °C in a 5% CO₂ incubator, before the treatment. Cell viability was determined 1 h after photothermal treatment, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay kit (ATCC CA# 30-1010k). This experiment has been performed 5-6 times and average values are reported in this manuscript.

NSET Study

For NSET experiment, we have used a continuous wavelength 532 nm OEM laser, as an excitation light source. Excitation light source was first attenuated using appropriate neutral density (ND) filter and coupled to the excitation arm of the Y-shaped reflection probe through a plano-convex lens (f :4.5mm). We have used miniaturized QE65000 scientific-grade Spectrometer from Ocean Optics as a NSET detector, which has remarkable sensitivity for low-light level applications.

Fluorescence Study to quantify amount of Cy3 labeled RNA release during therapy process

To quantify how much of dye attached RNA release during photothermal process, we have performed time dependent photothermal therapy process as well as fluorescence intensity measurement experiment. For this purpose, NIR laser at 785 nm was exposed to popcorn shape gold conjugated with cancer cell for different time intervals and then the conjugated solution was centrifuged. After that, we have used 532 nm continuum laser (5 mw) for fluorescence spectroscopy to quantify the released Cy3 attached RNA in supernatants. In all cases, we have used ds RNA–dye in the absence of nanoparticle as control.

Acknowledgments

Dr. Ray thanks NIH-SCORE grant # S06GM 008047 and NSF-PREM grant # DMR-0611539 for their generous funding. We also thank reviewers whose valuable suggestions improved the quality of this manuscript.

References

1. Bray F, Møller B. Nature Reviews Cancer. 2006; 6:63–74.
2. <http://www.who.int/cancer/en>
3. Deutscher SL. Chem. Rev. 2010; 110:196–3211.
4. Louie A. Chem. Rev. 2010; 110:3146–3195. [PubMed: 20225900]
5. Ray PC. Chem. Rev. 2010; 110:5332–5365. [PubMed: 20469927]
6. Lu W, Singh AK, Khan SA, Senapati D, Yu H, Ray PC. J. Am. Chem. Soc. 2010; 132:18103–18104. [PubMed: 21128627]
7. Bunz UHF, Rotello VM. Angew. Chem. Int. Ed. 2010; 49:3268.
8. Jain PK, Huang X, El-Sayed IH, El-Sayed MA. Acc. Chem. Res. 2008; 41:1578–1586. [PubMed: 18447366]
9. Jang B, Kim YS, Choi Y. Small. 2011; 7:265–270. [PubMed: 21213392]
10. Chen H, Shao L, Ming T, Sun Z, Zhao C, Yang B, Wang J. Small. 2010; 6:2272–2280. [PubMed: 20827680]
11. Kennedy LC, Bickford LR, Lewinski NA, Coughlin AJ, Hu Y, Day ES, West JL, Drezek RA. Small. 2011; 7:169–183. [PubMed: 21213377]
12. Yang L, Mao H, Wang YA, Cao Z, Peng X, Wang X, Duan H, Ni C, Yuan Q, Adams G, Smith MQ, Wood WC, Gao X, Nie S. Small. 2009; 5:235–243. [PubMed: 19089838]
13. Agasti SS, Chompoosor A, Chang-Cheng Y, Ghosh P, Kim CK, Rotello VM. J. Am. Chem. Soc. 2009; 131:5728–5729. [PubMed: 19351115]
14. Zhang J, Lakowicz JR. Opt. Express. 2007; 15:2598–2606. [PubMed: 19532498]
15. Lu W, Arumugam SA, Senapati D, Singh AK, Arbneshi T, Khan SA, Yu H, Ray PC. ACS Nano. 2010; 4:1739–1749. P C. [PubMed: 20155973]
16. Yu H, Javier D, Yaseen MA, Nitin N, Richards-Kortum R, Anvari B, Wong MS. J. Am. Chem. Soc. 2010; 132:1929–1938. [PubMed: 20092330]
17. Wijaya A, Schaffer SB, Pallares IG, Hamad-Schifferli K. ACS Nano. 2009; 3:80–86. [PubMed: 19206252]
18. Giljohann DA, Mirkin CA. Nature. 2009; 462:461–464. [PubMed: 19940916]

19. Barhoumi, Aoune; Huschka, Ryan; Bardhan, Rizia; Knight, Mark W.; Halas, Naomi J. Chem. Phys. Lett. 2009; 482:171–179.
20. Bretschneider JC, von Plesse G, Simon U. Small. 2008; 4:607–610. [PubMed: 18454511]
21. Richardson HH, Carlson MT, Tandler PJ, Hernandez P, Govorov AO. Nano Letters. 2009; 9:1139–1146. [PubMed: 19193041]
22. Huschka R, Neumann O, Barhoumi A, Halas NJ. Nano Letters. 2010; 10:4117–4122. [PubMed: 20857946]
23. Bakhtiari AB, Hsiao D, Jin G, Gates BD, Branda NR. Angew Chem Int Ed Eng. 2009; 48:4166–9.
24. Poon L, Zandberg W, Hsiao D, Erno Z, Sen D, Gates BD, Branda NR. ACS Nano. 2010; 4:6395–6403. [PubMed: 20958080]
25. Stoeva SI, Lee J-S, Smith JE, Rosen ST, Mirkin CA. J. Am. Chem. Soc. 2006; 128:8378–8379. [PubMed: 16802785]
26. Huang X, El-Sayed IH, Qian W, El-Sayed MA. J. Am. Chem. Soc. 2006; 128:2115–2120. [PubMed: 16464114]
27. Javier DJ, Nitin N, Levy M, Ellington A, Richards-Kortum R. Bioconjugate Chem. 2008; 19:1309–1312.
28. Sardana G, Jung K, Stephan C, Diamandis EP. J. Proteome Res. 2008; 7:3329–3338. [PubMed: 18578523]
29. Dasary SSR, Singh AK, Senapati D, Yu K, Ray PC. J. Am. Chem. Soc. 2009; 131:13806–13812. [PubMed: 19736926]
30. Wang S, Singh AK, Senapati D, Neely A, Yu H, Ray PC. Chem. A Eur. J. 2010; 16:5600–5606.
31. Darbha GK, Singh AK, Rai US, Yu E, Yu H, Ray PC. J. Am. Chem. Soc. 2008; 130:8038. [PubMed: 18517205]
32. Dubertret B, Calame M, Libchaber AJ. Nat. Biotechnol. 2001; 19:365–370. [PubMed: 11283596]
33. Griffin J, Singh AK, Senapati D, Lee E, Gaylor K, Boone JJ, Ray PC. Small. 2009; 5:839–845. [PubMed: 19219836]
34. Tabor, Christopher; Murali, Raghunath; Mahmoud, Mahmoud; El-Sayed, Mostafa A. J. Phys. Chem. A. 2009; 113(10):1946–1953. [PubMed: 19090688]
35. Fan C, Wang S, Hong JW, Bazan GC, Plaxco KW, Heeger AJ. Proc. Natl. Acad. Sci. U.S.A. 2003; 100:6297–6301. [PubMed: 12750470]
36. Spriet C, Meehan TD, Battersby BJ, Lawrie GA, Héliot L, Trau M. Small. 2009; 5:839–845. [PubMed: 19219836]
37. Griffin J, Singh AK, Senapati D, Rhodes P, Mitchell K, Robinson B, Yu E, Ray PC. Chem. Eur. J. 2009; 15:342–351. [PubMed: 19035615]
38. Griffin J, Ray PC. J. Phys. Chem. B. 2008; 112:11198–11201. [PubMed: 18702540]
39. Jennings TL, Singh MP, Strouse GF. J. Am. Chem. Soc. 2006; 128:5462–5467. [PubMed: 16620118]
40. Singh MP, Strouse GF. J. Am. Chem. Soc. 2010; 132:9383–9391. [PubMed: 20560666]
41. Jennings TL, Schlatterer JC, Singh MP, Greenbaum NL, Strouse GF. Nano Lett. 2006; 6:1318–1324. [PubMed: 16834403]
42. Skewis LR, Reinhard BM. Nano Lett. 2008; 8:214–220. [PubMed: 18052230]
43. Darbha GK, Ray A, Ray PC. ACS Nano. 2007; 3:208–214. [PubMed: 19206651]
44. Neely A, Perry C, Varisli B, Singh AK, Arbneshi T, Senapati D, Kalluri JK, Ray PC. ACS Nano. 2009; 3:2834–2840. [PubMed: 19691350]
45. Singh AK, Senapati D, Wang S, Griffin J, Neely A, Candice P, Naylor KM, Varisli B, Kalluri JR, Ray PC. ACS Nano. 2009; 3:1906–1912. [PubMed: 19572619]
46. Seelig J, Leslie K, Renn A, Kuhn S, Jacobsen V, van de Corput M, Wyman C, Sandoghdar V. Nano Letter. 2007; 7:685–689.
47. Singh MP, Jennings TL, Strouse GF. J. Phys. Chem. B. 2009; 113:552–558. [PubMed: 19099435]
48. Chen Y, O'Donoghue MB, Huang Y-F, Kang H, Phillips JA, Chen X, Estevez M-C, Yang CJ, Tan W. J. Am. Chem. Soc. 2010; 132:16559–16570. [PubMed: 21038856]

49. Pons T, Medintz IL, Sapsford KE, Higashiya S, Grimes AF, English DS, Mattoussi H. Nano Lett. 2007; 7:3157–3164. [PubMed: 17845066]
50. Sen T, Jana S, Koner S, Patra A. J. Phys. Chem. C. 2010; 114:19667–19672.
51. Stryer L, Haugland RP. Proc. Natl. Acad. Sci. 1967; 58:719–725. [PubMed: 5233469]
52. Dulkeith E, Ringler M, Klar TA, Feldman J, Javier AM, Parak WJ. Nano Lett. 2005; 5:585–589. [PubMed: 15826091]
53. Johnson PB, Christy RW. Phys. Rev. B. 1972; 6:4370–4379.
54. Gersten J, Nitzan A. J. Chem. Phys. 1981; 75:1139–1152.
55. Kuhn H. J. Chem. Phys. 1970; 53:101–108.
56. Hagerman PJ. Annu. Rev. Biophys. Biophys. Chem. 1988; 17:265–286. [PubMed: 3293588]
57. Clegg RM, Murchie AIH, Zechel A, Lilley DMJ. Proc. Natl. Acad. Sci. 1993; 90:2994–2998. [PubMed: 8464916]
58. Govorov AO, Richardson HH. Nano Today. 2007; 2:30.
59. Richardson HH, Carlson MT, Tandler PJ, Hernandez P, Govorov AO. Nano Lett. 2009; 9:1139. [PubMed: 19193041]
60. Wang S, Chen KJ, Wu TH, Wang H, Lin WY, Ohashi M, Chiou PY, Tseng HR. Angew. Chem. Int. Ed. 2010; 49:3777–3781.

Figure 1. Schematic representation shows the development of NSET optical ruler and its working principle to monitor photothermal therapy process.

Figure 2.

A) Plot showing fluorescence intensity from Cy3 modified RNA (CTG GTC ATG GCG GGC ATT TAA TTC) mostly quenched after the addition of popcorn shape gold nanoparticle. B) Schematic illustration of 5 Cy3 and 3 -SH modified ds-RNA of different lengths. C) Plot shows how quenching efficiency varies with the distance between gold nanoparticle and Cy3 dye for popcorn shape gold nanoparticles of 30 nm particle size. It also shows theoretical fitting data for the variation of the quenching efficiency with distance using FRET, NSET formula as described in Equation 2.

Figure 3.

TEM image showing A) aptamer conjugated popcorn shape gold nanoparticles before the addition of cancer cell line, B) after the addition of 10^5 human prostate cancer LNCaP cells/ml for 180 minutes and C) after the addition of 10^5 human prostate cancer PC-3 cells/ml for 30 minutes. D) Bright field inverted microscope images of aptamer conjugated popcorn shape gold nanoparticle conjugated LNCaP prostate cancer cells stained with Typan Blue, before irradiation. E) Bright field inverted microscope images after irradiation with 100 mW, 785 nm NIR radiation and then staining with Typan Blue

Figure 4.

A) Plot demonstrating time dependent fluorescence intensity increases (two minutes interval) during nanotherapy progress of LNCaP prostate cancer cell. B) Plot demonstrating fluorescence intensity change when R9 extended attached conjugated popcorn shape gold nanoparticle with LNCaP, PC-3 and HaCaT cells were exposed to 785 nm NIR continuous-wave radiation of different powers for 30 minutes. C) Plot showing cell viability measured by MTT test after popcorn shape gold nanoparticle conjugated LNCaP cells, PC-3 and HaCaT cells were exposed to 100 mw 785 nm NIR continuous-wave radiation. D) Plot showing linear relationship between % of LNCaP cell viability and NSET intensity change.