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Multifunctional Oval Shape Gold Nanoparticle Based Selective Detection of Breast Cancer Cells Using Simple Colorimetric and Highly Sensitive Two-Photon Scattering Assay

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Abstract

Breast cancer is the most common cancer among women and it is the second leading cause of cancer deaths in women today. The key to the effective and ultimately successful treatment of diseases such as cancer is an early and accurate diagnosis. Driven by the need, in this article, we report for the first time a simple colorimetric and highly sensitive two-photon scattering assay for highly selective and sensitive detection of breast cancer SK-BR-3 cell lines in 100-cells/ml level using multifunctional (monoclonal anti-HER2/c-erb-2 antibody and S6 RNA aptamers conjugated) oval shape gold nanoparticle based nanoconjugate. When multifunctional oval shape gold nanoparticles were mixed with breast cancer SK-BR-3 cell line, a distinct color change occurs and two-photon scattering intensity increases by about 13 times. Experimental data with HaCaT non-cancerous cell line, as well as with MDA-MB-231 breast cancer cell line clearly demonstrated that our assay was highly sensitive to SK-BR-3 and it was able to distinguish from other breast cancer cell line which expresses low levels of HER-2. The mechanism of selectivity and assay's response change, have been discussed. Our experimental results reported here open up a new possibility of rapid, easy and reliable diagnosis of cancer cell lines by monitoring the colorimetric change and measuring TPS intensity from multifunctional gold nanosystems.

Keywords

Breast Cancer; Oval Shape Gold Nanoparticle; Two-Photon Scattering; Plasmonics; Colorimetric

Introduction

Cancer has been described in early medical texts from antiquity, but till now it remains the second leading cause of death in our world^{1–5}. Breast cancer is the most common cancer among women and it is the second leading cause of cancer deaths in women today, after lung cancer^{3–5}. Rapid selective detection of cancer cells is an important challenge for the diagnosis and treatment of tumors^{1–9}. The key to the effective and ultimately successful treatment of diseases such as cancer is an early and accurate diagnosis. Oncogene like Human Epidermal growth factor Receptor 2 (HER2)/neu, overexpression is found in about 30 percent of different breast cancer cases and 20 percent of ovarian cancer cases^{1–24}. Also there are mounting evidences for the role of HER2 overexpression in patients with gastric cancer^{1–18}. As a result, immunophenotypic analyses of cancer cells using antibody probes for specific surface antigens has limitation due to the fact that antigens used for cell recognition are normally not exclusively

expressed on any single cell type, dramatically influencing selectivity, and resulting in false positive signals^{13,16,17}. Target cell-specific aptamers have the potential to serve as molecular probes for specific recognition of the cancerous cells from complex mixtures including whole blood samples^{5,13–14,16,17}. Most aptamers reported for breast cancer cell lines have weak binding affinity and thus low signal in molecular imaging, limiting their ability for highly sensitive detection of cancer cells^{5,16,17}. In addition, during the early stages of cancer development, cancer cells will have a very low density of target membrane proteins for recognition of specific cancer cell. In this case, single-aptamer/antibody binding will not be enough to detect early stage cancer development^{1–17}. Given the complexity and diversity of cancers, in order to increase sensitivity and selectivity, multivalent binding is usually considered to be essential for early stage disease diagnostics^{1–30}. To address this, in this article, we report the use of oval shape gold nanoparticle based multifunctional (antibody and aptamer) nanoconjugate platform for highly selective and sensitive detection of breast cancer cell line (as shown in Scheme 1). Since nanoparticle provides large surface area, it will be easier to incorporate several recognition elements in same surface. A well-characterized breast cancer cell line, SK-BR-3, which over expresses the epidermal growth factor receptor HER2/c-erb-2/Neu (HER-2) on the cell surface, has been used to demonstrate the ultra-sensitive detection capability. For selective and sensitive detection of SK-BR-3 cell line, we have conjugated oval shape gold nanoparticles by multiple HER-2 specific targets and these are anti HER-2 antibody and S6 RNA aptamer ($K_d = 94.6 \text{ nM}$ ⁵). Both of them are known to exhibit highly specific targeting for SK-BR-3 cell line^{4–7}.

Due to the unique optical properties of nanomaterials, since last 5 years several groups have been developing suitable nanomaterials for cancer imaging and therapy^{5–20}. In noble metal gold nanoparticles, the coherent collective oscillation of electrons in the conduction band induces large surface electric fields, which greatly enhances the radiative properties of these nanoparticles^{5–42}. As a result, the light scattering cross-section of metal nanoparticles are several orders of magnitude more intense than that of organic dyes, which makes it as an excellent candidate for sensors development and novel contrast agents for optical detection^{15–57}. In the last couple of years, several publications have shown that^{27–28,50–57} two-photon scattering (TPS) properties can be greatly enhanced ($\sim 10^4$ – 10^6) for molecules on a roughened versus an unroughened metal surface, which is comparable to very large enhancements (10^4 – 10^{14}) observed in surface enhanced Raman scattering (SERS) from organic dyes on colloidal solution^{29–41}. Average SERS enhancement is in the order of 10^4 – 10^8 have been reported^{35–39, 41} and in case single molecule SERS experiment, enhancement as high as 10^{14} have been reported^{29–32,40}. Using the above unique two-photon optical property of gold nanoparticle, we report here that the TPS properties of oval shape gold nanoparticles can be used for rapid, highly sensitive and selective detection of human breast cancer cell line SK-BR-3. The TPS or hyper Rayleigh scattering technique^{27–28,50–62} is based on light scattering. TPS can be observed from fluctuations in symmetry, caused by rotational fluctuations, where scattering by a fundamental laser beam can be detected at the second harmonic wavelength^{50–62}. Our group and other groups have demonstrated that this technique can easily be applied to study a very wide range of materials, because electrostatic fields and phase matching are not required^{27–28,50–62}.

Results and Discussion

Our oval shape gold nanoparticle based colorimetric and two-photon scattering approach for the detection of selective SK-BR-3 cell line is based on the fact that multifunctional conjugated oval shaped gold nanoparticle can readily and specifically identify breast cancer cell line, through HER2/c-erb-2/Neu receptor recognition (as shown in Scheme 1). For a SK-BR-3 cell line, there are many surface epidermal growth factor receptor HER2/c-erb-2/Neu available for specific recognition with monoclonal anti-HER2/c-erb-2 antibody and S6 aptamers

conjugated oval shaped gold nanoparticle. Therefore, in the presence of SK-BR-3 cell line, several nanoparticles can bind to HER2 receptors in the cancer cell, thereby producing nanoparticle aggregates (as shown in Scheme 1). Our TEM image (as shown in Figure 1B) shows clearly that multifunctional nanoparticle and SK-BR-3 cancer cell interaction is highly specific and nanoparticles are strong aggregates on the surface of cancer cell. As a result, a colorimetric change has been observed from pink to bluish color (as shown in Figure 2B) and a new broad band appears around 150 nm far from their plasmon absorption band, as shown in Figure 2A. This red shift might be due to two factors. One is the change in the local refractive index on the nanoparticle surface caused by the specific binding of the multifunctional nanoparticle (NP), which binds to HER2/c-erb-2 on the SK-BR-3 cell surface. The other is the interparticle interaction resulting from the assembly of nanoparticles on the cell surface^{6–50}. As shown in Figure 2B, the colorimetric pattern of oval shaped gold nanoparticles remains unchanged when we added anti-HER2 antibody/S6 RNA aptamer coated NP to the HaCaT noncancerous cells as well as MDA-MB-231 breast cancer cell line. As shown in Figure 1D, our TEM image also clearly demonstrated that the HaCaT noncancerous cells are poorly labeled by the nanoparticles and as a result, we have not observed clear colorimetric change. As noted from Figure 1D, some oval shaped gold nanoparticles are also found on the HaCaT noncancerous cells and it is mostly due to the nonspecific interactions between the antibodies and the proteins on the cell surface, and thus the nanoparticles are randomly distributed on the whole cells. As a result, we have not observed the colorimetric change as we observed for SK-BR-3 cell line.

Figure 1C, shows TEM image, which clearly demonstrated that the MDA-MB-231 breast cancerous cells are also quite poorly labeled by the nanoparticles and as a result, we have not observed clear colorimetric change. MDA-MB-231 is the breast cancer cell line, which expresses low levels of HER-2, and as a result, there is weak interaction between monoclonal anti-HER2/c-erb-2 antibody and S6 aptamers conjugated oval shaped gold nanoparticle and MDA-MB-231 cancer cell line. Due to the lack of strong interaction, nanoparticles do not produce larger aggregates and as a result, no color change has been observed. This contrast difference clearly demonstrates that our multifunctional oval shaped gold nanoparticles based colorimetric assay is highly specific for SK-BR-3 cancer cell lines and even it can distinguish between different breast cancer cell lines. To evaluate the sensitivity of our multifunctional oval shape gold nanoparticle based colorimetric assay, different concentration of SK-BR-3 cells from one stock solution were evaluated. As shown in Figure 2C, our colorimetric assay is highly sensitive to the concentration of SK-BR-3 cancer cells. Our result clearly shows that the sensitivity of colorimetric assay is 10^4 cells/ml.

To improve the assay sensitivity towards SK-BR-3 cancer cell detection, we have employed TPS technique^{27–28,50–62}. TPS is a powerful method to determine the small change in the size of the particles and therefore, even it has capability to separate dimer from the monomer^{27–28,50–57}. As shown in Figure 3A, when monoclonal anti-HER2/c-erb-2 antibody and S6 aptamers conjugated oval shaped gold nanoparticle were mixed with various concentrations of SK-BR-3 cells, two-photon scattering intensity increases by about 13 times (as shown in Figure 3A). Our experimental results demonstrated a very distinct two-photon scattering intensity change even upon the addition of 100 SK-BR-3 cancer cells/ml, whereas, we observed colorimetric change only after addition of 10^4 cells/ml (shown in Figure 2C). So our result clearly shows that TPS assay is two orders of magnitude more sensitive than the normal colorimetric assay.

Two- photon scattering signal from multifunctional gold nanoparticles can be expressed as^{27–28, 50–63},

$$I_{TPRS} = G \langle N_w \beta_w^2 + N_{nano} \beta_{nano}^2 \rangle I_\omega^2 e^{-N_{nano} \epsilon_{2\omega}} \quad (1)$$

where G is a geometric factor, N_w and N_{nano} the number of water molecules and monoclonal anti-tau antibody-conjugated gold nanoparticle per unit volume, β_ω and β_{nano} are the quadratic hyperpolarizabilities of a single water molecule and a single monoclonal anti-tau antibody-conjugated gold nanoparticle, $\epsilon_{2\omega}$ is the molar extinction coefficient of the gold nanoparticle at 2ω , l is the path length and I_ω the fundamental intensity. The exponential factor accounts for the losses through absorption at the harmonic frequency. Considering the size of nanoparticle, the approximation that assumes that the electromagnetic fields are spatially constant over the volume of the particle may not be suitable anymore. As a result, the total nonlinear polarization consists of different contributions such as multipolar radiation of the harmonic energy of the excited dipole and possibly of higher multipoles, as we discussed in our previous publication or reported by others^{27–28,50–57}.

The TPS intensity change upon aggregation of multifunctional oval shape gold nanoparticles in the presence of SK-BR-3 cell line consists of several contributions and these are: 1) The first one is the electric dipole approximation, which may arise due to the defects in nanoparticle. This contribution is actually identical to the one observed for any non-centrosymmetrical point-like objects^{57–63}. After the aggregation in the presence of SK-BR-3 cells, multifunctional oval shape gold nanoparticles loses the center of symmetry and as a result, one can expect significant amount of electric dipole contribution to the two-photon scattering intensity. Since electric dipole contributes several times higher than that of multipolar moments, we expect two-photon scattering intensity to increase upon the addition of SK-BR-3 cells. 2) The second contribution is multipolar contribution like electric quadrupole contribution^{27–28,50–57}. This contribution is very important when aggregation occurs due to the addition of SK-BR-3 cells. Since after aggregation, the size of the particle is no longer negligible compared to the wavelength, one cannot neglect multipolar contribution, as we and others reported before^{27–28,50–57}. As result, after aggregation one can expect very high multipolar contribution. To probe the multipolar contribution, we performed angle resolved HRS measurement. For this purpose, the fundamental input beam was linearly polarized, and the input angle of polarization was selected with a rotating half-wave plate. The configuration of the experimental setup was such that the fundamental beam was propagating in the Z direction with the electric field polarized in the $\{X, Y\}$ plane with the polarization angle and the harmonic light was collected along the Y direction, at right angle from the fundamental beam propagation direction.

As shown in Figure 4A, our experimental plot shows two lobes for multifunctional oval shape gold nanoparticles, which are similar to the one for pure electric dipole response from noncentrosymmetric organic molecules, as reported before by several groups^{60–63}. As shown in Figure 4B in presence of cancer cell line, the nature of the plot changes significantly. Polar plot contribution pattern shows clearly four lobes. The asymmetric four-lobe pattern is no longer predominantly dipolar in origin. So our experimental observation shows that due to the aggregation in presence of cancer cell, one cannot neglect multipolar contribution, and the origin of the very high nonlinearity after the addition of SK-BR-3 cell is due to the presence of both dipolar and multipolar contributions. 3) The third contribution is the resonance contribution. As shown in Figure 1F, a clear colorimetric change is observed when SK-BR-3 cells were added to multifunctional oval shape gold nanoparticles and as a result absorption spectra shifted 150 nm far, as shown in Figure 1E. Now this new absorption band appearing at 700 nm upon the addition of SK-BR-3 cell line can influence the TPRS intensity very highly due to single photon resonance. According to the two-state model,⁶³

$$\beta^{\text{two state}} = \frac{3\mu_{eg}^2 \Delta\mu_{eg}}{E_{eg}^2} \frac{\omega_{eg}^4}{(\omega_{eg}^2 - 4\omega^2)(\omega_{eg}^2 - \omega^2)} \quad (2)$$

static factor dispersion factor

where ω is the fundamental energy of the incident light, μ_{eg} is the transition dipole moment and ω_{eg} is the transition energy between the ground state $|g\rangle$ and the charge-excited state $|e\rangle$, $\Delta\mu_{eg}$ is the difference in dipole moment between $|e\rangle$ and $|g\rangle$ states. Since $\omega_{eg} \propto 1/\lambda_{\text{max}}$, β should change tremendously upon the addition of SKBR3 cell and as a result, the two-photon scattering intensity should change tremendously with the addition of SKBR3 cell. 4) The fourth contribution is due to the size dependent scattering properties. Since size increases tremendously with aggregation, the two-photon scattering intensity should increase with the increase in particle size.

To evaluate whether our assay is highly selective, we have also performed how two-photon scattering intensity changes upon addition of HaCaT non-cancerous cell and MDA-MB-231 breast cancer cell line. As shown in Figure 3B, two-photon scattering intensity changes only 1.3 times in the presence of 10^5 HaCaT cells/ml and 2.2 times when we added 10^5 MDA-MB-231 breast cancer cells/ml to multifunctional oval shape gold nanoparticles. So our experiments clearly demonstrate that our multifunctional oval shape gold nanoparticles based two-photon scattering assay is highly specific for SK-BR-3 cancer cell lines and even it can distinguish between different breast cancer cell lines.

To understand how the colorimetric and TPS assay responses with single bio-functional nanoparticle, we have also performed experiments on the addition of 10^5 SK-BR-3 cancer cells/ml, with only S6-RNA-aptamer conjugated oval shape gold nanoparticle or anti-HER2/c-erb-2 antibody conjugated nanoparticles, as well as multifunctional nanoparticles. Figure 5 clearly demonstrated that colorimetric change was only observed when we used multifunctional gold nanoparticle. No colorimetric change observed for single bio-functional nanoparticle. As shown in Figure 3B, TPS intensity changes only 4.1 times when SK-BR-3 cancer cell was added to anti-HER2/c-erb-2 antibody conjugated nanoparticles and TPS intensity changes around 6.1 times in case of S6-RNA-aptamer conjugated oval shape gold nanoparticle, whereas, TPS intensity changes 13 times when we used multifunctional nanoparticles. So by using multifunctional gold nanoparticles, we increased our TPS assay signal more than twice as much.

To understand whether our TPS assay sensitivity vary with the aspect ratio (length/diameter) of gold nanoparticle, we have performed our experiment using 1–2.4 aspect ratio (σ) of different gold nanoparticles, where $\sigma = 1$ denote spherical gold nanoparticles. Our experimental results (as shown in Figure 6) show that TPS intensity change is highly dependent on the aspect ratio of gold nanoparticles. As the particle aspect ratio increases, TPS intensity change becomes higher and higher which indicates that sensitivity becomes better. This variation of sensitivity efficiency with particle aspect ratio can be due to the increase in surface area as well as higher contribution from multipolar moments.

In the last 15 years, surface enhanced Raman scattering technique has been shown to be unique for ultrasensitive biological and chemical analysis and cancer cell imaging^{29–41}. To compare the sensitivity of our TPS technique with established SERS technique^{29–41} we have also performed Raman tagged Rhodamine 6G (Rh-6G)-labeled multifunctional gold nanoparticle based SERS for SKBR3 cell detection. We have used a continuous wavelength DPSS laser from laser glow technology (LUD-670) operating at 670 nm, as an excitation light source and miniaturized QE65000 Scientific-grade spectrometer from Ocean Optics as a Raman detector

⁴¹. The spectral response range of this mini Raman spectrometer is 220–3600 cm^{-1} . Detailed experimental set up has been reported recently by our group ⁴¹. Raman spectrum was collected with Ocean Optics data acquisition SpectraSuite spectroscopy software. As we discussed before, since in the presence of SKBR3 cell line multifunctional gold nanoparticles undergo aggregation, it formed several hot spots and provided a significant enhancement of the Raman signal intensity by several orders of magnitude through electromagnetic field enhancements (as shown in Figure 7). The Raman modes at 236, 252, 273 and 376 cm^{-1} are N-C-C bending modes of ethylamine group of the Rh6G ring and the Raman modes at 615, 778, 1181, 1349, 1366, 1511, 1570, 1603 and 1650 cm^{-1} are due to C-C-C ring in-plan bending, C-H out-of-plan bending, C-N stretching and C-C stretching, as we reported before ^{41,64}.

To evaluate the sensitivity of SERS probe, different concentrations of SKBR3 from one stock solution were evaluated. As shown in Figure 7, the SERS intensity is highly sensitive to the concentration of SKBR3. Our experimental results show that the detection capability of SERS probe is as low as 40 SKBR3 cells, which is 2.5 times more sensitive than our TPS probe. Though our experimental results shows that SERS is a highly promising technology to detect SKBR3 cells at very low concentration, the necessity of Raman dye tagging makes it difficult to use it as a biosensor for real life application.

Conclusion

In conclusion, in this article, we have demonstrated a label-free, fast and highly sensitive multifunctional (monoclonal anti-HER2/c-erb-2 antibody and S6 RNA aptamers conjugated) oval shape gold nanoparticle based simple colorimetric and highly sensitive two-photon scattering assay for the selective detection of breast cancer SK-BR-3 cell line in 100-cells level/ml. We have shown that when multifunctional oval shape gold nanoparticle were mixed with breast cancer SK-BR-3 cell line, a distinct color change occurs and two-photon scattering intensity increases by about 13 times. Our experimental data with HaCaT non-cancerous cell line, as well as with MDA-MB-231 breast cancer cell line clearly demonstrated that our colorimetric and TPS assay is highly sensitive to SK-BR-3 and it can distinguish from other breast cancer cell line which expresses low levels of HER-2. Our experiment indicates that this bioassay is quite rapid and it can be two orders of magnitude more sensitive than the usual colorimetric technique. We have also shown that using multifunctional gold nanoparticles, we are able to see colorimetric change directly upon the addition of cancer cell line and also using multifunctional nanoparticle, TPS assay signal increases more than twice as much. Although we have shown promising advances in multifunctional oval shape gold nanoparticle based colorimetric and TPS assay, we still need a much greater understanding of how to control surface architecture in order to stabilize and maximize the assay response. Continued optimization of different parameters is necessary to monitor cancer cell line in point of care complex environments. We believe that assay has enormous potential for application of cancer cell detection from clinical sample.

Materials and Experiments

Hydrogen tetrachloroaurate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), NaBH_4 , sodium citrate, CTAB and silver nitrate were purchased from Sigma-Aldrich and used without further purification. Monoclonal anti-HER2/c-erb-2 antibody were purchased from Thermo Fisher Scientific, 3'-SH modified S6 RNA aptamer were purchased from Midland Certified Reagent. The human adenocarcinoma breast cell lines SK-BR-3, which over expresses HER2/c-erb-2 gene product, were obtained from the American Type Culture Collection (ATCC, Rockville, MD). MDA-MB-231 breast cancer cell line was also 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, Heidelberg, Germany.

Synthesis of Oval Shape Gold Nanoparticle

Oval shape gold nanoparticles (aspect ratio, 1.3, as shown in Figure 7A) were synthesized using seed-mediated growth procedure in presence of CTAB. At first, we made gold nano-seed (~3nm diameter particles) using NaBH_4 reduction method as reported before. For preparing oval shape gold nanoparticle, we have used 4.75ml of 0.0085M CTAB solution in a small vial and then we added 0.2ml 0.01M $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ under constant stirring. After that, we used 0.03ml 0.01M AgNO_3 drop-wise to allow the solution mix properly. Once the solution mixed properly, we added 0.032 ml of 0.1M ascorbic acid slowly, as a reducing agent. The solution turned colorless. To this colorless solution, we added 0.01ml gold-seed at a time and gently mixed the solution for 30sec. Color changed immediately and became dark blue within 2 minutes. TEM image shows (as shown in Figure 8) that aspect ratio of these oval shape nanoparticle is 1.3.

This oval shape gold nanoparticle has only one plasmon bond like spherical gold nanoparticle but their λ_{max} shifted about 35 nm, in comparison to the spherical gold nanoparticle of same size.

Preparation of Multifunctional Oval Shape Nano-conjugates

Oval shape gold nanoparticles (aspect ratio, 1.3, as shown in Figure 8A) were synthesized using seed-mediated growth procedure in the presence of CTAB (details have been discussed in the experimental section). The above procedure produced oval shaped gold nanoparticle with CTAB coating. CTAB is known to be cytotoxic and as a result, it will not be ideal for in vivo diagnosis. Furthermore, since CTAB is positively charged at physiological pH and will be able to attract negatively charged proteins easily. As a result, CTAB coated oval shaped gold nanoparticle can face severe nonspecific binding problems. To overcome this problem, we have modified the oval shape gold nanoparticle surface by -3'-SH-S6 RNA aptamers and cystamine dihydrochloride (as shown in Scheme 1) using reported method⁵⁵⁻⁵⁶. -SH labeled RNA aptamers were gradually exposed to gold nanomaterial in presence of 0.1 M NaCl in a PBS buffer over a 16-hour period according to a procedure we reported before⁵⁵⁻⁵⁶. To remove the unbound RNA, we centrifuged the solution at 13,000 rpm for 20 minutes and the precipitate was redispersed in 2 mL of the buffer solution. We have continued this process three times. To measure the number of aptamer molecules in each gold nanoparticle, we have performed the above process with Cy3-labeled RNA aptamers. After conjugation, we have treated the aptamer conjugated gold nanoparticle with 10 μM 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 600–700 aptamers per oval shape gold nanoparticles.

To modify the gold nanoparticle surface by amine groups (as shown in Scheme 1), we have added 30 mM cystamine dihydrochloride to 50 mL of gold nanoparticle and the solution was kept at 50° C for several hours under constant sonication. After that, the excess cystamine dihydrochloride was removed by centrifugation at 8000 rpm for several minutes. For covalent immobilization of the monoclonal anti- HER2/c-erb-2 antibody onto the amine, we have used highly established glutaraldehyde spacer method^{30,43}. To remove the excess antibody, we have washed aptamers and anti- HER2/c-erb-2 antibody conjugated nanoparticles several times with PBS. To measure the number of anti- HER2/c-erb-2 antibody molecules in each gold nanoparticles, we have performed the above process with Rh6G-labeled anti- HER2/c-erb-2 antibody. After conjugation, we have performed exactly same process what we did for Cy3 labeled aptamers. The amount of Rh6G-labeled anti- HER2/c-erb-2 antibody was measured by fluorescence. By dividing the total number of Rh6G-labeled anti-HER2/c-erb-2 antibody

by the total number of nanoparticles, we estimated that there were about 70–80 anti-HER2/c-erb-2 antibody per oval shape gold nanoparticle. During aptamer conjugation and immobilization of the antibody, we have not noted any aggregation of gold nanoparticles as examined by TEM (as shown in Figure 8A) and UV-visible absorption spectroscopy (as shown in Figure 8B).

Cell Culture and Cellular Incubation with multifunctional nanoparticle

Cancer cells were grown in McCoy's 5a medium (ATCC, Rockville, MD) supplemented with 10% premium fetal bovine serum (FBS) (Lonza, Walkersville, MD) and antibiotics (10 IU/mL penicillin) in 75-cm² tissue culture flasks (Falcon; Becton Dickinson Labware Europe, Meylan, France) at 37°C under 5% CO₂/95% O₂, humidified incubator. Before the experiments, the cells were resuspended at a concentration of 1×10^6 cells/mL in PBS buffer medium. An enzyme-linked immunosorbent assay kit was used to quantify HER-2 in different tested cells. Our experimental results indicate that amount of HER2 in SK-BR-3 Cell was 480 ng/ml, where as HER2 amount was only 80 pg/ml in case of HaCaT non-cancerous cell. In case of MDA-MB-231 cell, we found HER2 level was 590 pg/ml. Different numbers of cells were then immersed into the multifunctional oval shape gold nanoparticle solution for 30 min at room temperature before performing the experiment.

Two-photon Rayleigh scattering spectroscopy

Detailed experimental set up for TPS experiment has been reported before^{27–28,43,55–56}. For the TPS or hyper Rayleigh Scattering (HRS) experiment, we have used a mode-locked Ti:sapphire laser delivering at fundamental wavelength of 860 nm with a pulse duration of about 150 fs at a repetition rate of 80 MHz. We performed TEM data before and after exposure of about 5–10 minutes to the laser and we have not noted any photo-thermal damage of multifunctional oval shape gold nanoparticles within our TPS data collecting time. The TPS light was separated from its linear counterpart by a high-pass filter, 3 nm bandwidth interference filter and a monochromator and then detected with a cooled photomultiplier tube. The pulses were counted with a photon counter.

The fundamental input beam was linearly polarized, and the input angle of polarization was selected with a rotating half-wave plate. Since λ_{max} for multifunctional oval shape gold nanoparticle (550 nm) and aggregates (700 nm) are very far from excitation source (860 nm) or second harmonic generated frequency (430 nm), we can eliminate the two-photon luminescence (TPL) contributions in our HRS experiment. To make sure that only second harmonic signal is collected by PMT, we have used 3 nm interference filter and a monochromator in front of PMT. To understand whether the two-photon scattering intensity at 430 nm light is due to second harmonic generation, we performed power dependent as well as concentration dependent studies (as shown in Figure 9). A linear nature of the plot implies that the doubled light is indeed due to the two-photon Rayleigh scattering signal.

Aptamer Concentration Dependent Sensitivity of TPS Probe

To understand how the sensitivity of our TPS probe varies with the concentration of the aptamer conjugated to each oval shape gold nanoparticle, we performed TPS experiment with different numbers of conjugated aptamers in the presence and absence of SKBR3 cell lines. Figure 10 shows how the TPS intensity change varies after addition of SKBR3 cells when number of aptamers/gold nanoparticle changes from 200 to 1000 aptamers/oval shape gold nanoparticle. Our result clearly shows that TPS intensity changes till 500–600 aptamers/gold nanoparticle and then remains unchanged. As a result, we have used 600 aptamers/oval shape gold nanoparticle for our experiments.

Anti- HER2/c-erb-2 antibody Concentration Dependent Sensitivity of TPS probe

To understand how the sensitivity of our TPS probe varies with the concentration of the anti-HER2/c-erb-2 antibody conjugated in each oval shape gold nanoparticles, we also performed TPS experiment with different numbers of conjugated anti- HER2/c-erb-2 antibody in the presence and absence of SKBR3 cell lines. Figure 11 shows how the TPS intensity change varies after addition of SKBR3 cells when number of anti- HER2/c-erb-2 antibody/gold nanoparticle changes from 20 to 100 antibody/oval shape gold nanoparticle. Our result indicates that TPS intensity changes till 70–80 anti- HER2/c-erb-2 antibody/gold nanoparticle and then remains unchanged. To get the maximum sensitivity, we have used 80 antibody/oval shape gold nanoparticle for our experiments.

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Figure 1.

A) TEM image showing anti HER-2 antibody and S6 RNA aptamer conjugated oval shape gold nanoparticles before addition of cell line. B) TEM image demonstrating aggregation of multifunctional oval shape gold nanoparticles after the addition of 10^4 SK-BR-3 cells/ml. C) TEM image showing very little aggregation after the addition of 10^4 MDA-MB-231 cell/ml on multifunctional oval shape gold nanoparticles. D) TEM image demonstrating about no aggregation after the addition of 10^4 HaCaT cells/ml, where nanoparticles are randomly distributed on the whole cells.

Figure 2.

A) Absorption profile variation of multifunctional oval shape gold nanoparticles due to the addition of different cancerous and non-cancerous cells. B) Photograph showing colorimetric change upon addition of different cancer cells (10^4 cells/ml). C) Photograph demonstrating colorimetric change upon the addition of different numbers of SK-BR-3 cells.

A

B

Figure 3.

A) Plot demonstrating two-photon scattering intensity changes upon the addition of different concentrations (number of cells/ml) of SK-BR-3 breast cancerous cells on multifunctional oval shape gold nanoparticle. B) Plot demonstrating selectivity of our TPS assay over other cancerous and non-cancerous cell line. Two-photon scattering intensity changes only two times upon the addition of 10^5 MDA-MB-231 breast cancerous cells, whereas TPS intensity changes 13 times upon the addition of same number of Sk-BR-3 cells.

Figure 4. Polar plot of the HRS intensity as a function of the incoming fundamental beam polarization angle (ϕ) from multifunctional oval shape gold nanoparticle A) before addition of cancer cells and B) after the addition of cancer cells.

Figure 5.
Photograph showing colorimetric change only when multifunctional oval shape gold nanoparticle has been used.

Figure 6.
Demonstrating aspect ratio dependent TPS intensity change upon the addition of SKBR3 cancer cell to multifunctional gold nanoparticle

Figure 7.
SERS spectra from Rh-6G modified multifunctional gold nanoparticle in the presence of the different concentrations of SKBR3 cells (cells/ml)

Figure 8. A) TEM image showing oval shape gold nanoparticles (14 nm length and 18 nm width), B) Absorption profile of oval shape gold nanoparticles.

Figure 9.
Power dependence of scattering intensity for different concentrations of multifunctional gold nanoparticle

Figure 10.
Demonstrating aptamer concentration dependence TPS intensity change after addition of SKBR3 Cells.

Figure 11. Showing anti- HER2/c-erb-2 antibody concentration dependence TPS intensity change after addition of SKBR3 Cells.

Scheme 1.

First two steps show schematic representation of the synthesis of monoclonal anti-HER2 antibody and S6 RNA aptamers-conjugated oval shape gold nanoparticles. Third step shows schematic representation of multifunctional oval shape gold nanoparticle based sensing of SK-BR-3 breast cancer cell line.