Analyses of protein corona on bare and silica-coated gold nanorods against four mammalian cells

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Introduction

Nanoparticles are comparable in size with many organic entities and subcellular compounds; thus, nanoparticles may interact with a range of biological systems, depending on composition and specific application. Such interactions may be helpful for treating various diseases; however, the use of these nanoparticles could also have adverse effects leading to toxicity and cellular death. Numerous studies have described the interactions of gold nanorods (AuNRs) with various mammalian cells.\(^1\)\(^-\)\(^3\) AuNRs have been described as not entering the nucleus but remaining entrapped in the vesicles. However, the exact pathway for AuNRs is not known. In general, larger particles move through cells via phagocytosis, whereas receptor-mediated endocytosis is considered the most important working mechanism.\(^4\)

While it is essential to understand the potential benefits of AuNRs on cellular behavior, it is equally important to address any toxicity concerns. Thus, another key challenge is determining the exact mechanism responsible for cellular toxicity. Several biochemical tests have been applied to determine the levels of nanomaterials that were toxic to cell lines, using viability, reactive oxygen species (ROS), and...
genotoxicity assays. Two major components were deemed to be responsible for the toxic effects of AuNRs covered with surface ligands, which were identified as cetyltrimethylammonium bromide (CTAB) bilayers and residual or desorbed reagent-free CTAB molecules. This surfactant was shown to be poisonous to cells, even at low concentrations. It was reported that oxidative stress by nanoparticles was correlated with increased ROS. Furthermore, the interaction of nanomaterials with the biological fluids creating protein layers on the surface of the nanomaterials, termed the “protein corona”, has garnered much attention. Therefore, in the present study, we investigated the interaction of nanomaterials and mammalian cells to determine the cytotoxicity mechanism induced by AuNRs and AuNRs functionalized with a silica coating (SiO₂-AuNRs) in four different cell lines: cervical cancer cells (HeLa), fibroblast cells (FY-11), human umbilical vein endothelial cells (HUVECs), and neuroblastoma cells (SH-SY5Y). Cytotoxicity was analyzed using several cell viability assays, including the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay and a CellTiter-Glo® assay. For this evaluation, we focused on the oxidative effects induced by nanomaterials, which resulted in decreased cellular viability and increased cellular death. In addition, mass spectroscopy (MS) analysis indicated involvement of the protein corona layer formed on the AuNRs and SiO₂-AuNRs in inducing free-radical production inside the cells.

Materials and methods

Materials

Gold (III)chloride trihydrate (HAuCl₄), sodium borohydride (NaBH₄), CTAB, ascorbic acid (AA), and silver nitrate (AgNO₃) were purchased from Sigma-Aldrich (St Louis, MO, USA). 3-Mercaptopropyltrimethoxy silane (MPS) and ammonium hydroxide (NH₄OH) were purchased from Aldrich (Milwaukee, WI, USA). Ultrapure deionized water was used for preparing all solutions and subsequent experiments.

Phosphate-buffered saline (PBS, pH 7.4), MTT assay reagent, and dihydrorhodamine-123 (DHR) were purchased from Sigma-Aldrich. Dulbecco’s Modified Eagle’s Medium (DMEM) and DMEM/F12 were purchased from Gibco-Invitrogen (Grand Island, NY, USA). Endothelial Cell Basal Medium-2 (EBM-2) was purchased from Lonza (Walkersville, MD, USA). Heat-inactivated fetal bovine serum (FBS), penicillin and streptomycin, and other tissue culture reagents were purchased from Thermo Scientific (Waltham, MA, USA). The CellTiter-Glo® assay kit was purchased from Promega (Madison, WI, USA).

Synthesis of AuNRs

AuNRs were synthesized according to previously described methods. Briefly, 0.25 mL of aqueous 0.01 M HAuCl₄·3H₂O solution and 7.5 mL of 0.10 M CTAB were mixed in a conical flask, after which 0.6 mL of 0.01 M ice-cold NaBH₄ solution was added to the flask. Following the addition of NaBH₄, the clear white solution turned to a brick-brown color, indicating the formation of Au nanoparticles. This solution was aged for 2.5 hours at 25°C–28°C to form the seed solution.

Meanwhile, in another beaker, 9.5 mL of 0.1 M CTAB, 0.4 mL of 0.01 M HAuCl₄·3H₂O, and 0.03 mL of 0.01 M AgNO₃ were mixed. A volume of 0.064 mL of 0.1 M ascorbic acid was added to the mixture, which immediately turned the solution from orange–yellow to colorless. Finally, 0.010 mL of the seed solution was added, the solution was gently mixed for 10 seconds, and then it was left undisturbed for 24 hours.

Synthesis of SiO₂-AuNRs

SiO₂-AuNRs were synthesized according to a previously published method. A volume of 3 mL of the AuNRs solution was centrifuged to remove excess CTAB and was redispersed in distilled water. A solution containing 5.58 μL of MPS in 20 mL of ethanol was prepared, and 80 μL of the solution was added to the AuNRs solution under vigorous magnetic stirring for 30 minutes to achieve a silica coating of intermediate thickness (around 3 nm). A volume of 20 μL of NH₄OH (pH 9) was then added, and the solution was vigorously stirred for 1 hour. The solution was washed three times with ethanol by centrifugation and was treated with 70% ethanol to eliminate any bacteria. A schematic representation of the AuNRs and SiO₂-AuNR synthesis process is shown in Figure 1.

Characterizations

Transmission electron microscopy (TEM) was performed using a JEM-3010 microscope (JEOL, Tokyo, Japan) operating at 300 kV. TEM samples were prepared by depositing 10 μL of the SiO₂-AuNRs suspension on carbon-coated copper grids, which was followed by the removal of excess solution and vacuum drying in an oven for 24 hours. Ultraviolet-visible (UV-Vis) spectra of the SiO₂-AuNRs were measured using an Optizen 2120 UV spectrophotometer.
analyses of protein corona on bare and silica-coated AuNRs

Figure 1 Schematic representation of AuNRs and SiO$_2$-AuNRs synthesis along with protein corona formation.

Abbreviations: CTAB, cetyltrimethylammonium bromide; AA, ascorbic acid; AuNRs, gold nanorods; MPS, mercaptopropyltrimethoxy silane; SiO$_2$-AuNRs, gold nanorods functionalized with silica.

(Figs & Tables)

Cell culture and treatment with AuNRs/SiO$_2$-AuNRs

FY-11 cells were cultured in DMEM containing 10% (v/v) FBS and 1% (w/v) penicillin/streptomycin at 37°C in a humidified atmosphere of 5% CO$_2$ for 24 hours. Then, cells were seeded at a density of 2×10$^4$ cells/well in flat-bottom 96-well plates (SPL Life Sciences, Seoul, Korea) and maintained at the same temperature and atmospheric conditions for up to 24 hours to allow cells to attach to the bottom of the plate. The cells were then washed with PBS and treated with AuNRs and SiO$_2$-AuNRs in a serum-free medium at concentrations ranging from 0.7 μg/mL to 12 μg/mL for 24 hours. After 24 hours, the medium was removed and the cells were washed twice with PBS to remove excess NRs. Cells cultured with NR-free medium served as control samples.

Cell viability analysis using MTT assay

The effect of AuNRs and SiO$_2$-AuNRs on FY-11 cell viability was evaluated using an MTT colorimetric assay, as described in previous studies. MTT solution (approximately 0.5 mg/mL) was added to wells containing fresh medium and previously cultured cells. The cultures were incubated at 37°C for 2 hours. Formazan crystals were then dissolved in dimethyl sulfoxide by discarding the medium. UV absorbance was measured using a microplate reader, and the data were interpreted as the percentage of viable cells relative to the control. The same procedure was repeated for HeLa cells, but for the SH-SY5Y and HUVEC cells, DMEM/F12 and EBM-2 medium, respectively, was used for culturing the cells.

CellTiter-Glo® assay of cell viability

The CellTiter-Glo® assay was performed according to the manufacturer’s protocol. The previously described culture procedures were repeated for the HeLa cells. DMEM/F12 and EBM-2 medium was used for culturing the SH-SY5Y and HUVEC cells, respectively.

Cell mortality assay

Cell mortality was assessed as previously described using a Trypan blue assay (Sigma). The FY-11 cells were plated in 12-well plates, with each well containing 2×10$^4$ cells/well. The cells were treated with various concentrations of AuNRs and SiO$_2$-AuNRs (0.7, 1.5, 3, 6, and 12 μg/mL) that were added to the culture medium. Cells cultured in
nanoparticle-free medium were used as the control. After 24 hours, the supernatant was collected, and the cells were detached with 300 μL of trypsin–ethylenediaminetetraacetic acid solution. The mixture, consisting of the supernatant and detached cells, was centrifuged at 1,200 rpm for 5 minutes. The obtained pellet was then dispersed in 500 μL of Trypan blue. After staining for 5 minutes, the cells were counted using a Countess Automated Cell Counter (Invitrogen, Grand Island, NY, USA). Cell mortality (%) was expressed in terms of dead cell number/total cell number. This procedure was repeated for the HeLa, SH-SY5Y, and HUVEC cell lines.

**Measurement of intracellular ROS**

ROS generation was determined using DHR-123, as described previously. Cells were plated into 96-well plates. After 24 hours of incubation, the medium was discarded, and the cells were preincubated with 100 μL of 10 μM DHR-123 solution and the growth medium at 5% CO₂, 95% air at 37°C for 30 minutes. Following the incubation period, the medium was removed and cells were washed three times with PBS. The cells were then exposed to AuNRs and SiO₂-AuNRs at concentrations of 0.75, 1.5, 3, 6, and 12 μg/mL for 24 hours. The fluorescence intensity of each well was analyzed using a microplate reader (Victor 3; Perkin-Elmer, Waltham, MA, USA) with an excitation filter of 485 nm and an emission filter of 535 nm. This procedure was repeated for the HeLa, SH-SY5Y, and HUVEC cell lines.

**Identification of the protein corona using MS**

AuNRs and SiO₂-AuNRs were incubated in DMEM and Roswell Park Memorial Institute (RPMI) medium for 1 hour at 37°C with rotation. After 1 hour, the samples were centrifuged at 18,000×g for 30 minutes, and the supernatant was discarded. PBS was then added to resuspend the AuNRs and SiO₂-AuNRs. This washing procedure was repeated three times, and the samples were then sent for MS determination at Diatech (Korea) to confirm the formation of the protein corona.

**Statistical analysis**

Statistical analysis performed was based on three replicates of each experiment. The significant differences were examined using Student’s t-test. Significance was analyzed at P<0.05.

**Results**

**Characterization of AuNRs and SiO₂-AuNRs**

The CTAB-stabilized AuNRs were encapsulated with a CTAB bilayer on their surface. For typical SiO₂-AuNRs synthesis, removal of the unbound CTAB is essential; therefore, the washing step must be performed very carefully. Here, with the use of a silane-coupling agent, uniform layers of SiO₂ were formed, with an aspect ratio of 3.0±0.2. A uniform silica coating over AuNRs can be seen in Figure 2.

**Characterization UV-Vis spectra**

The UV-Vis spectra of the AuNRs before and after coating with SiO₂ showed that the physiochemical properties of the AuNRs are altered (Figure 3). The prepared AuNRs have a weak transverse plasmon band at 522 nm and a strong longitudinal plasmon band at 630 nm, whereas for the SiO₂-AuNRs, the longitudinal surface plasmon band was red-shifted by 5 nm. This shift is attributed to an increase in the local refractive index of the medium surrounding the AuNRs after the formation of SiO₂ shell.

**Characterization of zeta potential**

The AuNR surface is positively charged due to the presence of polycations; thus, the zeta potential value was observed to be

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**Figure 2** Transmission electron microscope images of AuNRs (A) and intermediate SiO₂-AuNRs, showing a silica shell thickness of around 3 nm (B and C).
analyses of protein corona on bare and silica-coated AuNRs

Figure 3 UV-Vis spectra of AuNRs and SiO$_2$-AuNRs.

Note: AuNRs show a weak transverse plasmon band at 522 nm and a strong longitudinal plasmon band at 630 nm, whereas for the SiO$_2$-AuNRs, the longitudinal surface plasmon band was red shifted by 5 nm.

Abbreviations: AuNRs, gold nanorods; SiO$_2$-AuNRs, gold nanorods functionalized with silica; UV-Vis, ultraviolet-visible spectroscopy.

66.2 mV, whereas after coating with SiO$_2$, the surface becomes negatively charged with a value of $-25.7$ mV, as shown in Figure 4A and B. These zeta potential values confirm the stability and decreased aggregation of the AuNRs and SiO$_2$-AuNRs, and therefore the zeta potential results confirm the coating of the AuNR surfaces with SiO$_2$.

Cellular viability based on the CellTiter-Glo® assay

The mitochondrial function and cellular viability of the HeLa, FY-11, SH-SY5Y, and HUVEC cells, in the presence of AuNRs and SiO$_2$-AuNRs, are shown in Figure 5A–D. AuNRs induced toxicity even at the lowest concentration, whereas SiO$_2$-AuNRs maintained more than 80% of cellular viability for all concentrations. Similar viability was observed in the case of all four cell types.

Cellular mortality

In this study, cellular mortality was monitored using the Trypan blue assay, where dead cells were stained blue, while live cells remained unchanged. Mortality was expressed as the ratio of dead cells to total cells. Here, greater cell mortality (%) was observed in the presence of AuNRs, whereas cellular mortality was relatively low with the SiO$_2$-AuNRs. As shown in Figure 6, the toxic effect of the AuNRs on mitochondrial activity increased with increasing concentrations.

NR-induced ROS generation

The formation of intracellular free-radical levels could induce oxidative damage to cellular components, ultimately resulting in necrosis. The potential of AuNRs and SiO$_2$-AuNRs to induce oxidative stress was determined by measuring the ROS levels. Significant ROS elevation was observed for the HeLa, FY-11, SH-SY5Y, and HUVEC cells after 24 hours of exposure to the AuNRs and SiO$_2$-AuNRs at concentrations including 0.7, 1.5, 3, 6, and 12 μg/mL.
Figure 5 shows AuNRs and SiO₂-AuNRs impact on cellular viability of HeLa (A), FY-11 (B), SH-SYSY (C) and HUVEC (D) cells as determined by CellTiter-Glo® assay. Notes: AuNRs and SiO₂-AuNRs were incubated with cells for 24 h at 0.7–12 μg/ml. Control group was treated with media.
Abbreviations: AuNRs, gold nanorods; SiO₂-AuNRs, gold nanorods functionalized with silica; FY-11, fibroblast cells; HeLa, cervical cancer cells; SH-SYSY, neuroblastoma cells; HUVEC, human umbilical vein endothelial cell.

These results demonstrate that the formation of free radicals is significantly induced by AuNRs and SiO₂-AuNRs in different cell lines. Based on these obtained results, for 12 hours, a negligible increase in the production of hydrogen peroxide (H₂O₂), hydroxyl radical (•OH), and superoxide anion (O₂•⁻) was observed from the AuNRs, whereas the production from the SiO₂-AuNRs was almost similar to the control. After an 18-hour incubation period, the AuNRs increased ROS production by an average of 20%. Cells containing SiO₂-AuNRs exhibited a slight increase in ROS by around 5%, which was similar to the control. Finally, after 24 hours of incubation, it was observed that cells treated with AuNRs produced a higher...

Figure 6 Cell viability of HeLa (A), FY-11 (B), SH-SYSY (C) and HUVEC (D) cells after exposure to increasing doses of AuNRs and SiO₂-AuNRs for 24 h, as determined by MTT assay.
Note: The data represents AuNRs to be significantly toxic than SiO₂-AuNRs.
Abbreviations: AuNRs, gold nanorods; SiO₂-AuNRs, gold nanorods functionalized with silica; FY-11, fibroblast cells; HeLa, cervical cancer cells; SH-SYSY, neuroblastoma cells; HUVEC, human umbilical vein endothelial cell; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide.
Analyses of protein corona on bare and silica-coated AuNRs

percentage of $\text{H}_2\text{O}_2$, $\cdot$OH, and $\text{O}_2^\cdot$ than those treated with SiO$_2$-AuNRs did, as shown in Figure 8, which led to cellular death. AuNRs produced almost 60% of free radicals in all cell lines, whereas the production induced by the SiO$_2$-AuNRs remained around 20%, resulting in greater cellular viability.

Effects of the protein corona on NRs
To present a comprehensive characterization, MS analysis was performed to determine the biomolecular entities formed by dispersing AuNRs and SiO$_2$-AuNRs into the cell culture medium. Table 1 reports the number of proteins attached to AuNRs and SiO$_2$-AuNRs incubated in DMEM and RPMI.

This analysis showed that in both types of cell culture medium (DMEM and RPMI), the total number of proteins attached to the AuNR surface was less than that of the SiO$_2$-AuNR surface. As previously shown, this nano–bio interface is due to three dynamically interacting components: 1) the surface of NRs whose characteristics are determined...
by their physiochemical composition, 2) the changes that occur following the solid–liquid interface when the particle interacts with components in the surrounding medium, and 3) the contact area of the solid–liquid interface with its biological substrates. Specific NR properties have been shown to greatly contribute to the NR interactions with medium. For example, certain NR properties may result in increased adsorption of ions, proteins, organic materials, and detergents, which permits double-layer formation and minimizes the free surface energy by surface modification. There are several factors affecting the protein corona formation, including protein–AuNRs interactions, protein–protein interactions, and protein–medium interactions.

As shown in Figure 9, the DMEM and RPMI mediums encouraged the attachment of a comparable number of proteins. The major difference observed was with the surface

| Medium | Sample      | Total proteins | Within criteria | Without criteria |
|--------|-------------|----------------|-----------------|------------------|
| DMEM   | AuNRs       | 146            | 102             | 44               |
|        |             | 100            | 66              | 34               |
| RPMI   | SiO₂-AuNRs  | 153            | 106             | 47               |
|        |             | 122            | 84              | 42               |

Abbreviations: DMEM, Dulbecco’s Modified Eagle’s Medium; RPMI, Roswell Park Memorial Institute; AuNRs, gold nanorods; SiO₂-AuNRs, gold nanorods functionalized with silica.

Figure 9 Proteins attachment on AuNRs and SiO₂-AuNRs surfaces incubated in DMEM and RPMI medium for 1 h.
Notes: (A) Represents the attachment of larger numbers of unique proteins (50%) on DMEM incubated AuNRs, whereas on DMEM incubated SiO₂-AuNRs a lesser number of unique proteins are attached. (B) Represents the attachment of a large number of unique proteins (50%) on RPMI medium incubated AuNRs, whereas on RPMI medium incubated SiO₂-AuNRs a lesser number of unique proteins are attached. (C) and (D) Represents the number of unique proteins attached on DMEM and RPMI medium incubated AuNRs and SiO₂-AuNRs, which are almost similar. Hence, it is apparent that the attachment solely depends on the surface properties and charges, rather than differences in the medium.
Abbreviations: DMEM, Dulbecco’s Modified Eagle’s Medium; RPMI, Roswell Park Memorial Institute; B, bare; Si, silica.
Analyses of protein corona on bare and silica-coated AuNRs

Figure 10 Unique proteins attached on the surface of DMEM (A, B) and RPMI medium (C, D) incubated AuNRs and SiO₂-AuNRs.

Notes: (A) Represents the presence of cell matrix adhesion proteins on AuNRs; (B) represents the absence of cell matrix adhesion proteins on SiO₂-AuNRs; (C) represents the presence of cell matrix adhesion proteins on AuNRs; (D) represents the absence of cell matrix adhesion proteins on SiO₂-AuNRs.

Abbreviations: DMEM, Dulbecco’s Modified Eagle’s Medium; RPMI, Roswell Park Memorial Institute; AuNRs, gold nanorods; SiO₂-AuNRs, gold nanorods functionalized with silica.

Discussion

Interest in the use of AuNRs for biomedical applications has grown due to their unique physiochemical properties; however, their current use has been limited because of major concerns over their toxicity. AuNRs are toxic to cells due to the presence of CTAB, which is required for AuNR stabilization. Therefore, surface functionalization, such as with SiO₂, has been used in an effort to reduce AuNR toxicity.

For AuNRs, the intensity of the longitudinal plasmon band corresponding to the long axis of the NRs has been shown to be much higher than that of the transverse plasmon band corresponding to the short axis of the NRs, because of the enhanced surface electric field due to surface plasmon excitation.¹⁵
Therefore, in the case of SiO\textsubscript{2}-AuNRs, the shift in the longitudinal plasmon band is larger than that of the transverse band, which is at a wavelength close to the characteristic band of Au nanoparticles of similar diameter.

Every particle in a mixture carries some charge, which is typically negative rather than positive. This is attributed to the presence of chemical groups on the surface of the particle, which are ionized to form a charged surface. Sometimes, ions with an opposite charge may be adsorbed to the surface, and at other times chemical compounds may be intentionally added to yield a specific charge. In this study, CTAB acts as a chemical compound that generates the charge for the AuNRs. Since the CTAB-stabilized AuNRs possess a highly positive surface charge, the wrapping of negative ions around the metal is strongly favored. Hence, a SiO\textsubscript{2} coating over the AuNRs was formed, and zeta potential analysis was performed to confirm the coating of SiO\textsubscript{2}-AuNRs.

In the present study, a visible difference in cell viability was observed based on the results of the viability assays used, as different mechanisms were involved. For the MTT assay, water-soluble MTT was converted to an insoluble formazan crystal. The formazan was then solubilized by an inorganic solvent, and the concentration was determined by determining the optical density at 570 nm. Alternatively, for the CellTiter-Glo\textsuperscript{®} assay, a homogeneous method based on the amount of adenosine triphosphate (ATP) present, which indicates the presence of metabolically active cells, was used to determine the number of viable cells.

Typically, polycationic materials exhibit higher cytotoxicity. AuNRs stabilized with CTAB and washed once with water by centrifugation showed strong cytotoxicity due to free CTAB remaining in the solution. However, in the case of the SiO\textsubscript{2}-AuNRs, more than 70% cell viability was observed even at the highest concentrations, indicating that the removal of excess CTAB and modification with SiO\textsubscript{2} contributed to a significant decrease in cytotoxicity. The SiO\textsubscript{2}-AuNRs exhibited lower toxicity, which is essential for biomedical applications using AuNRs. In addition, the absence of CTAB on the AuNR surfaces has been shown to affect biological processes inside the cells, while binding to the cell membrane.\textsuperscript{19}

Since material properties affect the kinetics of cell death, the mechanisms of nanomaterial-mediated cell toxicity may vary depending on the composition of the material each cell type interacts with. ROS generation has been suggested as an initial cellular response to nanomaterial internalization and subsequent cell death. In this study, the nanomaterial-mediated cell responses prior to cell death, specifically the production of intracellular ROS, were measured at 6 hours via the DHR assay. Our results showed that AuNRs increased the production of ROS in all cells by 60%, depending on the time, whereas ROS production for the SiO\textsubscript{2}-AuNRs was negligible. These results indicated that SiO\textsubscript{2} scavenged the production of the ROS. The mechanism for the production of free radicals and its relation to toxicity are described as follows.

Typically, ROS generated by cells within an enclosed environment may easily turn into a source of cell and tissue injury. O\textsubscript{2} is essential for human survival, and aerobic energy metabolism depends upon oxidative phosphorylation, which plays a vital role through which the oxidoreduction energy of mitochondrial electron transport is eventually converted to the high-energy phosphate bond of ATP. Aerobic organisms use O\textsubscript{2} as the final electron acceptor for mitochondrial cytochrome c oxidase, which is able to catalyze the four electron reduction of O\textsubscript{2}, leading to H\textsubscript{2}O formation (Equation 1). During mitochondrial oxidative phosphorylation, and other electron transfer reactions, including those of the superoxide anion (O\textsuperscript{2-}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and hydroxyl radicals (\textbulletOH) can be formed within cells (Equation 2). These reactive O\textsubscript{2} metabolites are usually collectively referred to as ROS, and their generation in a biological environment exposes most living organisms to the so-called “oxygen paradox”. O\textsubscript{2} is essential for life, but it is also potentially hazardous, since ROS may become a source of cell and tissue injury.

\textbf{Equation 1}

In a human body, four electron reduction reactions occur, leading from O\textsubscript{2} to H\textsubscript{2}O production:

| Protein          | DMEM AuNRs | DMEM SiO\textsubscript{2}-AuNRs | RPMI AuNRs | RPMI SiO\textsubscript{2}-AuNRs |
|------------------|------------|----------------------------------|------------|----------------------------------|
| Alpha-actinin-1  | 10.21      | Absent                           | 10.18      | Absent                           |
| Fibronectin      | 460.32     | 20.16                            | 530.35     | 240.32                           |
| Angiotensin      | 140.33     | 60.26                            | 190.32     | 80.29                            |
| Nidogen          | 10.15      | Absent                           | 20.22      | Absent                           |
| Vitronectin      | 90.33      | 70.29                            | 110.33     | 80.33                            |

\textbf{Abbreviations:} DMEM, Dulbecco’s Modified Eagle’s Medium; RPMI, Roswell Park Memorial Institute; AuNRs, gold nanorods; SiO\textsubscript{2}-AuNRs, gold nanorods functionalized with silica.
formation completely depended on the material surface on the AuNRs and SiO₂ MS that two different groups of protein corona were formed. Based on our hypothesis, we confirmed with experiments and to identify the cause of toxicity observed on the two types of NRs. In addition, different reports have shown that the corona could force the toxicity of materials. The presence of such proteins could be the reason for excessive free-radical production in cells leading to cell death. The list of proteins involved in corona formation are mentioned in the supplementary information. Hence, we concluded that the biological impact of the AuNRs was not exactly associated with their properties but associated with the attributes of the corona NR complexes. Further studies of the protein corona and its behavior could provide a clearer picture of this relationship.

**Equation 2**

When the human body is exposed to a foreign material (metal nanoparticles), then carbon-centered free radicals are generated by interaction with ROS:

1. \( R–H + *\text{OH} \rightarrow R + \text{H}_2\text{O} \) (organic radical)
2. \( R^* + O_2 \rightarrow \text{ROO}^* \) (peroxyl radical)
3. \( \text{ROO}^* + R–H \rightarrow \text{ROOH} + R^* \) (organic peroxide)
4. \( \text{ROOH} \rightarrow \text{RO}^* + *\text{OH} + \text{nanoparticle} \) (alkoxy radical).

Thus, the free radicals produced may be very dangerous, leading to cell death. In this study, the results obtained were different for each cell type; however, the production of intracellular ROS increased due to the presence of AuNRs, while SiO₂–AuNRs showed scavenging properties.

Based on the principles involved in ROS production, the question arises of what induces increased free-radical production within cells. Recently, many researchers have addressed the relationship between nanomaterials and biological fluid interaction. A layer formed over the nanomaterial has been defined as the “corona”. When NRs enter a biological fluid (medium), they are coated with proteins that may undergo conformational changes, leading to the exposure of new epitopes, altered function, and/or avidity effects. The concept of the NR–protein corona is important for tuning the surface properties, charges, resistance to aggregation, and toxicity of NRs. Notably, in this study, we showed that the interactive NR surface may be prebound to chemical substances that reflect its prior history and could subsequently influence its protein adsorption kinetics.

**Conclusion**

The results of this study demonstrate that the AuNRs due to the presence of CTAB molecules are the source of toxicity. Providing a coating over the CTAB-coated AuNRs with alkoxysilane is one way to prevent toxicity, which explains why the overcoated rods examined in this work were far more biocompatible.

In addition, we analyzed the protein corona formed over AuNRs and SiO₂–AuNRs to determine any significant differences and to identify the cause of toxicity observed on the two types of NRs. Based on our hypothesis, we confirmed with MS that two different groups of protein corona were formed on the AuNRs and SiO₂–AuNRs, respectively. The corona formation completely depended on the material surface properties. The MS data suggested the presence of cell matrix adhesion proteins on the AuNRs, and the absence of those proteins on the SiO₂–AuNRs. Cell matrix adhesion proteins such as immunoglobulin are associated with the recognition and phagocytosis of NRs into the cells. The adsorbed proteins regulate the NR–cellular interactions, thus making them toxic or nontoxic to cells. In addition, different reports have shown that the corona could force the toxicity of materials. The presence of such proteins could be the reason for excessive free-radical production in cells leading to cell death. The list of proteins involved in corona formation are mentioned in the supplementary information. Hence, we concluded that the biological impact of the AuNRs was not exactly associated with their properties but associated with the attributes of the corona NR complexes. Further studies of the protein corona and its behavior could provide a clearer picture of this relationship.

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**Disclosure**

The authors report no conflicts of interest in this work.

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Supplementary materials

As shown in this section, a list of proteins involved in the protein corona layer due to the incubation of gold nanorods (AuNRs) (bare) and gold nanorods functionalized with silica (SiO$_2$-AuNRs) (Si) in Dulbecco’s Modified Eagle’s Medium (DMEM) or Roswell Park Memorial Institute (RPMI) medium is provided. As shown in the tables, “common proteins” indicates proteins involved in both DMEM-bare and DMEM-Si (Tables S3, S4, S7 and S8). On the other hand, “uncommon proteins” indicates unique proteins involved in either DMEM-bare or DMEM-Si. Similar designations are specified for the RPMI samples (Tables S5, S6, S9 and S10).

Here, we compared DMEM-bare with DMEM-Si (Table S1) (to show the difference between “bare” and “Si” in DMEM), RPMI-bare with RPMI-Si (Table S2) (to show the difference between “bare” and “Si” in RPMI), DMEM-bare with RPMI-bare (Table S9) (to show the difference between “DMEM” and “RPMI” in bare), and DMEM-Si with RPMI-Si (to show the difference between “DMEM” and “RPMI” in Si) (Table S10).

Table S1 Protein corona list of DMEM-bare and DMEM-Si

| DMEM-bare                                                                 | DMEM-Si                                                                 |
|--------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Alpha-1-antiproteinase precursor                                         | PREDICTED: apolipoprotein B                                             |
| Protein AMBP precursor                                                   | Complement C3 preproprotein                                             |
| Talin-1                                                                  | Serum albumin precursor                                                 |
| Thyroglobulin precursor                                                  | Alpha-2-macroglobulin                                                   |
| Apolipoprotein E precursor                                               | Antithrombin-III precursor                                              |
| Alpha-2-antiplasmin precursor                                            | Apolipoprotein E precursor                                              |
| Apolipoprotein A-I preproprotein                                         | Inter-alpha-trypsin inhibitor heavy chain H2                            |
| Hemoglobin fetal subunit beta                                            | Apolipoprotein A-I preproprotein                                        |
| Coagulation factor V precursor                                           | Fibulin-1                                                               |
| Hyaluronan-binding protein 2                                              | Alpha-1-antiproteinase precursor                                        |
| C4b-binding protein alpha chain precursor                                | Alpha-2-HS-glycoprotein precursor                                       |
| Kininogen-2 isoform II                                                   | Complement C4                                                           |
| Complement component C9 precursor                                        | Periostin                                                               |
| PREDICTED: apolipoprotein B                                              | Inter-alpha-trypsin inhibitor heavy chain H3                            |
| Tubulin, beta 1                                                          | Hemoglobin subunit alpha                                                |
| PREDICTED: similar to complement component 4A                            | Vitronectin                                                             |
| PREDICTED: heparan sulfate proteoglycan 2                                | Heparin cofactor 2                                                      |
| Hemoglobin subunit beta                                                  | Prothrombin                                                             |
| Hypothetical protein LOC510860                                           | Inter-alpha-trypsin inhibitor heavy chain H4                            |
| Heat shock protein HSP 90-alpha                                          | Hemoglobin fetal subunit beta                                           |
| Serpin A3-1 precursor                                                    | Angiotensinogen                                                        |
| Factor Xlla inhibitor precursor                                          | PREDICTED: complement component 4 binding protein, alpha chain-like    |
| Actin, aortic smooth muscle                                              | Gelosin isoform a                                                       |
| Tubulin alpha-4A chain                                                   | Protein AMBP precursor                                                  |
| Kininogen-2 isoform I                                                    | Fetuin-B precursor                                                      |
| Inter-alpha-trypsin inhibitor heavy chain H4 precursor                   | Lipopolysaccharide-binding protein precursor                            |
| Mannan-binding lectin serine protease I                                  | Plasma serine protease inhibitor precursor                              |
| Apolipoprotein M                                                          | Complement component C9 precursor                                       |
| Fibrinogen beta chain                                                    | Actin, aortic smooth muscle                                             |
| Tenascin-X                                                                | Coagulation factor XIII A chain precursor                               |
| PREDICTED: complement component 4 binding protein, alpha chain-like     | Talin-1                                                                |
| Collagen alpha-1 (XI) chain                                              | PREDICTED: apolipoprotein B                                             |
| Heat shock protein HSP 90-beta                                           | Serotransferrin precursor                                               |
| Tubulin beta-5 chain                                                     | Hemoglobin subunit beta                                                |
| Fibromodulin                                                             | Alpha-2-antiplasmin precursor                                           |
| Fetuin-B precursor                                                       | Phosphoglycerate kinase I                                               |
| Lumican precursor                                                        | Inter-alpha-trypsin inhibitor heavy chain H1                            |
| Alpha-actinin-1                                                          | Actin, cytoplasmic                                                      |
|                                                                             | L-lactate dehydrogenase B chain                                         |
|                                                                             | Apolipoprotein A-II precursor                                           |

(Continued)
Table S1 (Continued)

| DMEM-bare | DMEM-Si |
|-----------|---------|
| Vitamin D-binding protein precursor | Fibronectin |
| Neurpin-1 | Hypothetical protein LOC510860 |
| Echinoderm microtubule-associated protein-like 5 | Heat shock protein HSP 90-beta |
| PREDICTED: alpha-2-macroglobulin-like, partial | Fermitin family homolog 3 |
| Aggrecan core protein | Phospholipid transfer protein |
| PREDICTED: GLIS family zinc finger 3, partial | Pyruvate kinase isozymes M1/M2 |
| Plasma serine protease inhibitor precursor | Adenosylhomocysteinase |
| PREDICTED: calcium channel, voltage-dependent, L type, alpha 1S subunit-like | Complement component 8, beta polypeptide |
| Myosin, heavy chain 9, nonmuscle | Kininogen-2 isoform II |
| Glyceraldehyde-3-phosphate dehydrogenase | Kelch-like ECH-associated protein 1 |
| Caspase-14 | Alpha-enolase |
| tRNA-dihydouridine synthase I-like | PREDICTED: similar to complement component 4A |
| Myosin-11 | Apolipoprotein M |
| PREDICTED: KRAB-A domain containing 2 | Glyceraldehyde-3-phosphate dehydrogenase |
| Thrombospondin-3 | Transmembrane and coiled-coil domain-containing protein 2 |
| Neurpin-2 | Glyceraldehyde-3-phosphate dehydrogenase, testis-specific |
| Beta-casein precursor | Probable arginyl-tRNA synthetase, mitochondrial precursor |
| PREDICTED: collagen, type VI, alpha 3-like isoform 4 | Serpin A3-I precursor |
| Sucrase-isomaltase, intestinal | Vitamin D-binding protein precursor |
| Transmembrane and coiled-coil domain-containing protein 2 | L-lactate dehydrogenase C isomorph 1 |
| Complement factor B precursor | Nucleolar GTP-binding protein 2 |
| Interleukin-1 beta precursor | Thyroxine-binding globulin precursor |
| PREDICTED: NIMA (never in mitosis gene a)-related kinase 11 | Heat shock protein HSP 90-alpha |
| Coagulation factor X | C4b-binding protein alpha chain precursor |
| SPARC-like protein 1 | Neutrophil cytosol factor 1 |
| PREDICTED: FYVE and coiled-coil domain-containing protein 1-like | Apolipoprotein D precursor |
| PREDICTED: thiolester containing protein II-like | Thrombospondin-1 precursor |
| Carbohydrate sulfotransferase 3 | Zinc finger protein 668 |
| PREDICTED: zinc finger protein 347-like | PREDICTED: mcg144546-like |
| C-type lectin domain family 11 member A | Dynamin heavy chain 2, axonemal |
| Terminal uridylyltransferase 4 | Coagulation factor V precursor |
| Nucleolar GTP-binding protein 2 | Leucine-rich repeat-containing protein 49 |
| Alpha-S1-casein precursor | Tyrosine-protein kinase BAZ1B |
| Collectin-11 precursor | Alpha-S1-casein precursor |
| Mannan-binding lectin serine protease 2 | Coiled-coil domain-containing protein 71 |
| N-acetylated-alpha-linked acidic dipeptidase 2 | Pigment epithelium-derived factor precursor |
| Pentraxin-related protein PTX3 precursor | Clusterin preproprotein |
| 6-Phosphogluconate dehydrogenase, decarboxylating | PREDICTED: PDSS, regulator of cohesion maintenance, homolog B (Saccharomyces cerevisiae) |
| Nidogen-1 | Splicing factor 3B subunit 2 |
| Mitochondrial-processing peptidase subunit alpha precursor | Collagen alpha-1 (XII) chain |
| Coiled-coil alpha-helical rod protein 1 | PREDICTED: SYF2 homolog, RNA splicing factor-like, partial |
| Ankyrin repeat domain-containing protein 32 | Carboxypeptidase B2 precursor |
| Complement C5a anaphylatoxin | PREDICTED: titin |
| von Willebrand factor | Seryl-tRNA synthetase, mitochondrial precursor |
| PREDICTED: hemolytic complement-like | Probable phospholipid-transporting ATPase IA |
| PREDICTED: ZAR1-like protein-like | Coagulation factor XIII B chain |
| Homeobox protein Hox-A4 | 26S proteasome non-ATPase regulatory subunit 1 |
| PREDICTED: recombination activating gene 1 | Filamin-C |
| PREDICTED: FLJ00002 protein-like | Glycine N-acetyltransferase |
| Heat-stable enterotoxin receptor | PREDICTED: hypothetical protein |
| Reelin | PREDICTED: phospholipase D family, member 3-like |
| Hypothetical protein LOC100124506 | von Willebrand factor C domain-containing protein 2-like |
| Kelch repeat and BTB domain-containing protein 12 | Probable ATP-dependent RNA helicase DDX49 |
| Tenasin C | PREDICTED: preferentially expressed antigen in melanoma-like |
| Nucleoporin p58/p45 | Protein Shroom1 |

(Continued)
Table S1 (Continued)

| DMEM-bare                                                                 | DMEM-Si                                                                 |
|--------------------------------------------------------------------------|-------------------------------------------------------------------------|
| PREDICTED: transcriptional regulating factor 1-like                      | Heat shock 70 kDa protein 1A                                            |
| Endoplasmic precursor                                                    | Hypothetical protein LOC614478                                           |
| PREDICTED: chloride channel protein CIC-Ka-like                         | Insulin-like growth factor-binding protein complex acid labile subunit  |
| AP-1 complex subunit mu-2                                                | PREDICTED: collagen, type VI, alpha 3-like isoform 4                    |
| TAF6-like RNA polymerase II p300/CPB associated factor 65 kDa subunit 6L| Actin-related protein 2                                                  |
| Cyclin-G-associated kinase                                               |                                                                        |
| ARF GTPase-activating protein GIT2                                       |                                                                        |
| Hexokinase-I                                                             |                                                                        |
| PREDICTED: histone cluster 1, H2bd-like                                  |                                                                        |
| Desmoplakin                                                             |                                                                        |
| Ras-related protein Rap-1b precursor                                     |                                                                        |
| PREDICTED: transforming growth factor, beta receptor III                 |                                                                        |
| Acetyl-CoA carboxylase I                                                 |                                                                        |
| PREDICTED: microtubule-associated protein 7-like                         |                                                                        |
| PREDICTED: centrosomal protein 110 kDa                                   |                                                                        |
| Serpin A3-7                                                              |                                                                        |
| Voltage-dependent N-type calcium channel subunit alpha-1B               |                                                                        |
| Ribonucleoside-diphosphate reductase M1 chain                            |                                                                        |
| Probable phospholipid-transporting ATPase IB                             |                                                                        |
| PREDICTED: hypothetical protein                                          |                                                                        |
| Tubulin alpha-1D chain                                                  |                                                                        |
| Kinesin family member C2                                                |                                                                        |
| Selenoprotein S                                                          |                                                                        |
| PREDICTED: mcg14546-like                                                 |                                                                        |
| Nucleoporin NUP188 homolog                                              |                                                                        |
| Actin, cytoplasmic I                                                    |                                                                        |
| PREDICTED: centromere protein E, 312kda                                  |                                                                        |
| PREDICTED: zinc finger, DHHC-type containing 18-like                     |                                                                        |
| Serylprophilin-like protein                                              |                                                                        |
| PREDICTED: sodium channel protein type 11 subunit alpha-like            |                                                                        |

**Abbreviations:** DMEM, Dulbecco’s Modified Eagle’s Medium; Si, silica; tRNA, transfer ribonucleic acid; ATP, adenosine triphosphate.
Table S2 Protein corona list of RPMI-bare and RPMI-Si

| RPMI-bare                                      | RPMI-Si                                      |
|------------------------------------------------|----------------------------------------------|
| **PREDICTED**: apolipoprotein B                 | **PREDICTED**: apolipoprotein B               |
| Fibronectin                                    | Complement C3 preproprotein                  |
| Complement C3 preproprotein                    | Alpha-2-macroglobulin                        |
| Inter-alpha-trypsin inhibitor heavy chain H2   | Serum albumin precursor                      |
| Alpha-2-macroglobulin                          | Inter-alpha-trypsin inhibitor heavy chain H2 |
| Serum albumin precursor                        | Fibronectin                                  |
| Inter-alpha-trypsin inhibitor heavy chain H3 precursor | Inter-alpha-trypsin inhibitor heavy chain H3 precursor |
| Inter-alpha-trypsin inhibitor heavy chain H1 precursor | Inter-alpha-trypsin inhibitor heavy chain H1 precursor |
| Gelsolin isoform a                             | Antithrombin-III precursor                   |
| Talin-1                                        | Apolipoprotein E precursor                   |
| Complement C4                                  | Heparin cofactor 2                           |
| Fibulin-1                                      | Gelsolin isoform a                           |
| **PREDICTED**: pregnancy-zone protein-like     | **PREDICTED**: pregnancy-zone protein-like   |
| Angiotensinogen                                | Alpha-2-HS-glycoprotein precursor            |
| Thrombospondin-4 precursor                     | Apolipoprotein A-I preproprotein             |
| Antithrombin-III precursor                     | Heparin cofactor 2                           |
| Heparin cofactor 2                             | Gelsolin isoform a                           |
| Prothrombin                                    | Vitronectin                                  |
| Vitronectin                                    | Periostin                                    |
| Inter-alpha-trypsin inhibitor heavy chain H4 precursor | Talin-1                                      |
| Periostin                                      | **PREDICTED**: pregnancy-zone protein-like   |
| Alpha-2-HS-glycoprotein precursor              | Alpha-2-HS-glycoprotein precursor            |
| Alpha-1-antiproteasease precursor              | Apolipoprotein A-I preproprotein             |
| Protein AMBP precursor                         | Heparin cofactor 2                           |
| Hyaluronan-binding protein 2                   | Gelsolin isoform a                           |
| Alpha-2-antiplasmin precursor                  | Vitronectin                                  |
| Coagulation factor V precursor                 | Angiotensinogen                              |
| Tubulin, beta 1                                | Complement factor B precursor                |
| Tubulin beta-5 chain                           | Serotransferrin precursor                    |
| **PREDICTED**: apolipoprotein B                 | Inter-alpha-trypsin inhibitor heavy chain H4 precursor |
| Fibulin-1                                      | Alpha-1-antiproteasease precursor            |
| Tubulin alpha-4A chain                         | Hemoglobin fetal subunit beta               |
| Pigment epithelium-derived factor precursor    | Prothrombin                                  |
| Cartilage oligomeric matrix protein            | Hemoglobin subunit alpha                     |
| Hypothetical protein LOC510860                 | Protein AMBP precursor                       |
| Heat shock protein HSP 90-beta                 | Fettin-B precursor                           |
| C4b-binding protein alpha chain precursor      | Tubulin, beta 1                              |
| Mannan-binding lectin serine protease I        | Complement C5a anaphylatoxin                 |
| Hemoglobin subunit beta                        | Plasma serine protease inhibitor precursor   |
| Kininogen-2 isoform I                          | Lipopolysaccharide-binding protein precursor  |
| Kininogen-2 isoform II                         | Kininogen-2 isoform II                       |
| Apolipoprotein E precursor                     | Fermitin family homolog 3                    |
| Tubulin alpha-4A chain                         | Complement component C9 precursor           |
| Coagulation factor X                           | Actin, aortic smooth muscle                  |
| PREDICTED: complement component 4 binding protein, alpha chain-like | Thrombospondin-1 precursor                   |
| Fibrinogen alpha chain precursor               | Alpha-2-antiplasmin precursor                |
| Coagulation factor X                           | Hemoglobin subunit beta                     |
| PREDICTED: complement component 4 binding protein, alpha chain-like | Phospholipid transfer protein                |
| Complement component C9 precursor              | Complement component 8, beta polypeptide     |
| C-type lectin domain family 11 member A        | Fibrinogen beta chain                        |
| Filamin-A                                      | Alpha-fetoprotein precursor                  |
| Fettin-B precursor                             | Thrombospondin-4 precursor                   |
| Serpin A3-1 precursor                          | **PREDICTED**: apolipoprotein B               |
| Hemoglobin subunit alpha                       | Properdin                                    |
|                                                | Hypothetical protein LOC510860               |
|                                                | C4b-binding protein alpha chain precursor    |
|                                                | Coagulation factor V precursor               |
|                                                | **PREDICTED**: collagen, type VI, alpha 3-like isoform 4 |
|                                                | Heat shock protein HSP 90-alpha              |
|                                                | **PREDICTED**: complement component 4 binding protein, alpha chain-like |

(Continued)
Table S2 (Continued)

| RPMI-bare                                         | RPMI-Si                                           |
|---------------------------------------------------|---------------------------------------------------|
| Alpha-fetoprotein precursor                       | Apolipoprotein A-II precursor                     |
| PREDICTED: similar to complement component 4A     | Coagulation factor X                              |
| Factor Xla inhibitor precursor                    | Elongation factor I-alpha 2                       |
| Neuropilin-2                                      | Pyruvate kinase isoenzymes M1/M2                  |
| Fibrinogen gamma-B chain precursor                | Kininogen-2 isofrom I                             |
| Nidogen-1                                         | PREDICTED: similar to complement component 4A     |
| Collectin-11 precursor                            | Heat shock protein HSP 90-beta                   |
| Serpin A3-7                                       | Apolipoprotein M                                  |
| PREDICTED: sushi, von Willebrand factor type A, EGF, and pentraxin domain containing I, partial | PREDICTED: GLIS family zinc finger 3, partial     |
| Thyroglobulin precursor                           | Glyceraldehyde-3-phosphate dehydrogenase         |
| Lumican precursor                                 | PREDICTED: putative zinc finger protein ENSP00000330994-like, partial |
| Complement C5a anaphylatoxin                      | Vitamin D-binding protein precursor               |
| Collagen alpha-1 (XII) chain                      | Glyceraldehyde-3-phosphate dehydrogenase, testis-specific |
| Vitamin D-binding protein precursor               | Tubulin alpha-4A chain                            |
| Fibromodulin                                      | Hyaluronan-binding protein 2                      |
| Pentraxin-related protein PTX3 precursor          | Actin, cytoplasmic I                              |
| 6-Phosphogluconate dehydrogenase, decarboxylating | Adenosylhomocysteinease                           |
| PREDICTED: GLIS family zinc finger 3, partial     | Actin, alpha cardiac muscle I                     |
| Neuropilin-1                                      | PREDICTED: hCG1647286-like                        |
| PREDICTED: heparan sulfate proteoglycan 2         | Transhurten precursor                            |
| Reelin                                            | PREDICTED: calcineurin binding protein I          |
| Lipopolysaccharide-binding protein precursor       | Carboxypeptidase b2 precursor                    |
| CTAGE family, member 5                            | Leucine-rich repeat-containing protein 49         |
| Rab GDP dissociation inhibitor beta               | Neural cell adhesion molecule 1 precursor         |
| PREDICTED: VGF nerve growth factor inducible-like | Biglycan precursor                               |
| Actin, aortic smooth muscle                       | Tubulin alpha-1D chain                            |
| Eukaryotic elongation factor 2 kinase             | PREDICTED: phosphorylase kinase, beta             |
| Thrombospondin-3                                  | C-type lectin domain family 11 member A          |
| von Willebrand factor                             | Alpha-1-acid glycoprotein precursor               |
| SPARC-like protein 1                              | Kelch-like ECH-associated protein I               |
| Alpha-actinin-1                                   | Coagulation factor XIII A chain precursor         |
| Apolipoprotein A-I preproprotein                  | PREDICTED: cadherin 4, type I preprote in-like    |
| Carbohydrate sulfotransferase 3                   | PREDICTED: neurofilament, medium polypeptide      |
| Serine/threonine-protein phosphatase 2A 65 kda    | ATP synthase subunit D, mitochondrial             |
| regulatory subunit A alpha isoform                | PREDICTED: mcg144546-like                         |
| Actin, cytoplasmic I                              | Neutrophil cytosol factor 1                       |
| tRNA-dihydrouridine synthase 1-like               | Alpha-S1-casein precursor                        |
| Retinoic acid receptor responder (tazarotene induced) | Serpin A3-1 precursor                           |
| Interleukin-1 beta precursor                      | 6-Phosphogluconate dehydrogenase, decarboxylating |
| NACHT, LRR, and PYD domains-containing protein 5  | Pigment epithelium-derived factor precursor       |
| Hypothetical protein LOC781988                    | Serpin A3-7                                       |
| Tenascin-X                                        | PREDICTED: collagen, type IV, alpha 2, partial    |
| Heat shock protein HSP 90-alpha                   | Tyrosine-protein kinase BAZ1B                     |
| Mannan-binding lectin serine protease 2           | Inner nuclear membrane protein MANI              |
| Galectin-3-binding protein precursor              | PREDICTED: NIMA (never in mitosis gene a)-related kinase 11 |
| Adenosine deaminase-like protein                  | Nucleolar GTP-binding protein 2                   |
| Ankyrin repeat domain-containing protein 32       | Annexin A2                                       |
| PREDICTED: hCG1647286-like                        | SPARC-like protein 1                              |
| Fatty acid synthase                               | L-lactate dehydrogenase C isoform 1              |
| Tenascin C                                        | Casein kinase II subunit alpha                    |
| Apolipoprotein M                                  | Glycine N-acyltransferase                        |
| Ras-related protein Rap-1b precursor              | Fibrinogen alpha chain precursor                 |
| Zinc finger protein 366                           | Peroxisomal biogenesis factor 3                   |
| PREDICTED: multiple C2 domains, transmembrane 1-like |                                  |

(Continued)
Table S2 (Continued)

| RPMI-bare                                                                 | RPMI-Si                                                                 |
|---------------------------------------------------------------------------|------------------------------------------------------------------------|
| DnaJ homolog subfamily C member 27                                        | PREDICTED: kinesin family member 13A                                    |
| Nucleosome assembly protein 1-like                                         | Filamin-C                                                               |
| Chromodomain helicase DNA binding protein 6                               | Heat shock 70 kDa protein 1A                                             |
| PREDICTED: tudor domain-containing protein 12-like                        | MKL/myocardin-like protein 2                                             |
| Plasma serine protease inhibitor precursor                                | Sulphydryl oxidase 1                                                    |
| Transforming growth factor-beta-induced protein (g-h3)                    | Alpha-1b-glycoprotein precursor                                          |
| Reversion-inducing cysteine-rich protein with Kazal motifs               | PREDICTED: KIAA1747 protein-like                                         |
| Transcription elongation factor B polypeptide 1                           | PREDICTED: mkiaa4091 protein-like                                        |
| PREDICTED: mcg144546-like                                                 | Thiamin pyrophosphokinase 1                                             |
| Dnaj homolog subfamily C member 27 (zinc transporter), member 1-like     | Noll/nop2/sun domain family, member 5                                    |
| Pregnancy-associated glycoprotein                                         | L-lactate dehydrogenase B chain                                          |
| Leucine-rich repeat-containing protein 49                                  | Coiled-coil domain-containing protein 50                                 |
| PREDICTED: tyrosine-protein phosphatase non-receptor type 21-like         |                                                                        |
| PREDICTED: olfactory receptor, family 5, subfamily 1, member 1-like      |                                                                        |
| Histamine N-methyltransferase                                             |                                                                        |
| PREDICTED: ZAR1-like protein-like                                         |                                                                        |
| Regulator of G-protein signaling like 1                                   |                                                                        |
| ELKS/Rab6-interacting/CAST family member 1                               |                                                                        |
| Elongation factor Ts, mitochondrial precursor                             |                                                                        |
| Neural cell adhesion molecule 1 precursor                                |                                                                        |
| Homeobox protein Hox-A4                                                  |                                                                        |
| PREDICTED: dystroclin-like                                                |                                                                        |
| PREDICTED: centromere protein 32 kDa                                     |                                                                        |
| Importin subunit beta-1                                                  |                                                                        |
| PREDICTED: apobe-1 complementation factor-like isofrom 2                 |                                                                        |
| Choline transporter-like protein 4                                        |                                                                        |
| Sperm associated antigen 7                                                |                                                                        |
| PREDICTED: similar to uncharacterized protein c10or90                     |                                                                        |
| Probable ATP-dependent RNA helicase DDX17                                 |                                                                        |
| DnaJ homolog subfamily C member 21                                        |                                                                        |
| Leucine-rich repeat-containing protein 48                                 |                                                                        |
| UTP – glucose-1-phosphate uridyltransferase                              |                                                                        |
| PREDICTED: FLJ00002 protein-like                                          |                                                                        |
| Hephaestin-like protein 1                                                 |                                                                        |
| Serine/threonine-protein phosphatase PPI-alpha catalytic subunit          |                                                                        |
| PREDICTED: hypothetical protein                                           |                                                                        |
| Acetyl-CoA carboxylase 1                                                 |                                                                        |
| PREDICTED: zinc finger protein 107-like                                   |                                                                        |
| Zinc finger protein 180                                                   |                                                                        |
| Ribonucleoside-diphosphate reductase M1 chain                             |                                                                        |
| AT-rich interactive domain-containing protein 5A                         |                                                                        |
| Protein farnesyltransferase/gerany1geranyltransferase type-1 subunit alpha|                                                                        |

**Abbreviations:** Si, silica; ATP, adenosine triphosphate; tRNA, transfer ribonucleic acid; RPMI, Roswell Park Memorial Institute.
### Table S3 List of DMEM common proteins involved in both AuNrs and SiO₂-AuNrs

| DMEM common                  |
|------------------------------|
| PREDICTED: apolipoprotein B   |
| Alpha-2-macroglobulin        |
| Fibronectin                  |
| Complement C3 preproprotein  |
| Inter-alpha-trypsin inhibitor heavy chain H2 |
| Inter-alpha-trypsin inhibitor heavy chain H3 precursor |
| Serum albumin precursor      |
| Complement C4                |
| Inter-alpha-trypsin inhibitor heavy chain H1 precursor |
| Fibulin-I                    |
| Gelsolin isoform a           |
| Angiotensinogen              |
| Heparin cofactor 2           |
| Alpha-2-HS-glycoprotein precursor |
| Vimentin                     |
| Prothrombin                  |
| Periostin                    |
| Antithrombin-III precursor   |
| Alpha-1-antiproteasone precursor |
| Protein AMBP precursor       |
| Talin-I                      |
| Apolipoprotein E precursor   |
| Alpha-2-antiplasmin precursor |
| Apolipoprotein A-I preproprotein |
| Hemoglobin fetal subunit beta |
| C4b-binding protein alpha chain precursor |
| Kininogen-2 isof orm II      |
| Complement component C9 precursor |
| PREDICTED: apolipoprotein B   |
| PREDICTED: similar to complement component 4A |
| Hemoglobin subunit beta      |
| Hemoglobin subunit alpha     |
| Hypothetical protein LOC510860 |
| Heat shock protein HSP 90-alpha |
| Serpin A3-I precursor        |
| Actin, aortic smooth muscle  |
| Inter-alpha-trypsin inhibitor heavy chain H4 precursor |
| Apolipoprotein M PREDICTED: complement component 4 binding protein, alpha chain-like |
| Heat shock protein HSP 90-beta |
| Fetuin-B precursor           |
| Vitamin D-binding protein precursor |
| Plasma serine protease inhibitor precursor |
| Glycerophosphate-3-phosphate dehydrogenase |
| Transmembrane and coiled-coil domain-containing protein 2 |
| Nucleolar GTP-binding protein 2 |

**Abbreviations:** DMEM, Dulbecco’s Modified Eagle’s Medium; AuNrs, gold nanorods; SiO₂-AuNrs, gold nanorods functionalized with silica.

### Table S4 List of RPMI common proteins involved in both AuNrs and SiO₂-AuNrs

| RPMI common                  |
|------------------------------|
| PREDICTED: apolipoprotein B   |
| Fibronectin                  |
| Complement C3 preproprotein  |
| Inter-alpha-trypsin inhibitor heavy chain H2 |
| Alpha-2-macroglobulin        |
| Serum albumin precursor      |
| Complement C4                |
| Inter-alpha-trypsin inhibitor heavy chain H3 precursor |
| Thrombospondin-1 precursor   |
| Inter-alpha-trypsin inhibitor heavy chain H1 precursor |
| Gelsolin isoform a           |
| Talin-I                      |
| Complement C4                |
| Fibulin-I                    |
| PREDICTED: pregnancy-zone protein-like |
| Angiotensinogen              |
| Thrombospondin-4 precursor   |
| Antithrombin-III precursor   |
| Heparin cofactor 2           |
| Prothrombin                  |
| Vimentin                     |
| Inter-alpha-trypsin inhibitor heavy chain H4 precursor |
| Periostin                    |
| Alpha-2-HS-glycoprotein precursor |
| Alpha-1-antiproteasone precursor |
| Protein AMBP precursor       |
| Tubulin, beta I              |
| Kininogen-2 isof orm II      |
| PREDICTED: apolipoprotein B   |
| PREDICTED: collagen, type VI, alpha 3-like isoform 4 |
| Hemoglobin fetal subunit beta |
| Kininogen-2 isof orm I       |
| Apolipoprotein E precursor   |
| Tubulin alpha-4A chain       |
| Hypothetical protein LOC510860 |
| Heat shock protein HSP 90-beta |
| C4b-binding protein alpha chain precursor |
| Hemoglobin subunit beta      |
| Coagulation factor X         |
| PREDICTED: complement component 4 binding protein, alpha chain-like |
| Fibrinogen beta chain        |
| Complement component C9 precursor |
| C-type lectin domain family 11 member A |
| Fetuin-B precursor           |
| Hemoglobin subunit alpha     |
| Alpha-fetoprotein precursor  |
| Apolipoprotein A-I preproprotein |
| Actin, cytoplasmic I         |
| Heat shock protein HSP 90-alpha |
| PREDICTED: hCG1647286-like   |
| Apolipoprotein M              |

**Abbreviations:** AuNrs, gold nanorods; SiO₂-AuNrs, gold nanorods functionalized with silica; RPMI, Roswell Park Memorial Institute.
### Table S5 List of DMEM unique proteins involved in either AuNRs or SiO$_2$-AuNRs

| DMEM uncommon | DMEM-Si |
|---------------|---------|
| **DMEM-bare** | **DMEM-Si** |
| PREDICTED: pregnancy-zone protein-like | Lipopolysaccharide-binding protein precursor |
| Thrombospondin-1 precursor | Coagulation factor XIII A chain precursor |
| Thrombospondin-4 precursor | Serotransferrin precursor |
| Thyroglobulin precursor | Phosphoglycerate kinase 1 |
| Coagulation factor V precursor | Actin, cytoplasmic 1 |
| Hyaluronan-binding protein 2 | L-lactate dehydrogenase B chain |
| Tubulin, beta 1 | Apolipoprotein A-II precursor |
| PREDICTED: heparan sulfate proteoglycan 2 | Fermitin family homolog 3 |
| Fibrinogen alpha chain precursor | Phospholipid transfer protein |
| Factor XIIa inhibitor precursor | Pyruvate kinase isozymes M1/M2 |
| Tubulin alpha-4A chain | Adenosylhomocysteinase |
| Kininogen-2 isoform I | Complement component 8, beta polypeptide |
| Mannan-binding lectin serine protease 1 | Kelch-like ECH-associated protein 1 |
| Fibrinogen beta chain | Alpha-1-endorse |
| Tenascin-X | Glyceraldehyde-3-phosphate dehydrogenase, testis-specific |
| Collagen alpha-1 (XII) chain | Probable arginyl-tRNA synthetase, mitochondrial precursor |
| Tubulin beta-5 chain | L-lactate dehydrogenase C isoform 1 |
| Fibromodulin | Thyroxine-binding globulin precursor |
| Lumican precursor | Neutrophil cytosol factor 1 |
| Alpha-actinin-1 | Apolipoprotein D precursor |
| Neuruplin-1 | |
| Echinoderm microtubule-associated protein-like 5 | |
| PREDICTED: alpha-2-macroglobulin-like, partial | |
| Aggrecan core protein | |
| PREDICTED: GLIS family zinc finger 3, partial | |
| PREDICTED: calcium channel, voltage-dependent, L type, alpha 1S subunit-like | |
| Myosin, heavy chain 9, nonmuscle | |
| Caspase-14 | |
| tRNA-dihydouridine synthase 1-like | |
| Myosin-1 | |
| PREDICTED: KRAB-A domain containing 2 | |
| Thrombospondin-3 | |
| Neuruplin-2 | |
| Beta-casein precursor | |
| PREDICTED: collagen, type VI, alpha 3-like isoform 4 | |
| Sucrase-isomaltase, intestinal | |
| Complement factor B precursor | |
| Interleukin-1 beta precursor | |
| PREDICTED: NIMA (never in mitosis gene a)-related kinase 11 | |
| Coagulation factor X | |
| SPARC-like protein 1 | |
| PREDICTED: FYVE and coiled-coil domain-containing protein 1-like | |
| PREDICTED: thiolester containing protein II-like | |
| Carbohydrate sulfotransferase 3 | |
| PREDICTED: zinc finger protein 347-like | |
| C-type lectin domain family 11 member A | |
| Terminal uridylyltransferase 4 | |
| Alpha-S1-casein precursor | |
| Collectin-11 precursor | |
| Mannan-binding lectin serine protease 2 | |
| N-acetylated-alpha-linked acidic dipeptidase 2 | |
| Pentraxin-related protein PTX3 precursor | |
| 6-phosphogluconate dehydrogenase, decarboxylating | |
| Nidogen-1 | |
| Mitochondrial-processing peptidase subunit alpha precursor | |
| Coiled-coil alpha-helical rod protein 1 | |

**Abbreviations:** DMEM, Dulbecco’s Modified Eagle’s Medium; AuNRs, gold nanorods; SiO$_2$-AuNRs, gold nanorods functionalized with silica; tRNA, transfer ribonucleic acid.
Table S6 List of RPMI unique proteins involved in either AuNRs or SiO$_2$-AuNRs

| RPMI-uncommon          | RPMI-Si                                      |
|------------------------|---------------------------------------------|
| Tubulin beta-5 chain   | Complement factor B precursor               |
| Pigment epithelium-derived factor precursor | Serotransferrin precursor                   |
| Cartilage oligomeric matrix protein | Plasma serine protease inhibitor precursor |
| Mannan-binding lectin serine protease I | FERMitin family homolog 3                   |
| Fibrinogen alpha chain precursor | Phospholipid transfer protein               |
| Filamin-A              | Complement component 8, beta polypeptide   |
| Serpin A3-1 precursor  | Properdin                                   |
| Factor Xlla inhibitor precursor | Apolipoprotein A-II precursor               |
| Neuropilin-2           | Elongation factor 1-alpha 2                 |
| Fibrinogen gamma-B chain precursor | Pyruvate kinase isozymes M1/M2              |
| Nidogen-I              | Glycerolaldehyde-3-phosphate dehydrogenase |
| Collectin-I precursor  | PREDICTED: Putative zinc finger protein     |
| Serpin A3-7            | ENSP00000330994-like, partial               |
| PREDICTED: sushi, von Willebrand factor type A, EGF, and pentraxin domain containing 1, partial | Glycerolaldehyde-3-phosphate dehydrogenase, testis-specific |
| Thyroglobulin precursor | Adenosylhomocysteinase                      |
| Collagen-alpha-I (XII) chain | Actin, alpha cardiac muscle 1               |
| Fibromodulin           | Transthyretin precursor                     |
| Pentraxin-related protein PTX3 precursor | PREDICTED: calcineurin binding protein I    |
| 6-phosphogluconate dehydrogenase, decarboxylating | Carboxypeptidase B2 precursor               |
| Neuropilin-1           | Leucine-rich repeat-containing protein 49   |
| PREDICTED: heparan sulfate proteoglycan 2 | Neural cell adhesion molecule 1 precursor  |
| Reelin                 | Biglycan precursor                          |
| CTAGE family, member 5 | Tubulin alpha-1D chain                     |
| Rab GDP dissociation inhibitor beta | PREDICTED: phosphorylase kinase, beta       |
| PREDICTED: VGF nerve growth factor inducible-like | Alpha-1-acid glycoprotein precursor         |
| Eukaryotic elongation factor 2 kinase | Kelch-like ECH-associated protein 1         |
| Thrombospondin-3       |                                             |
| von Willebrand factor  |                                             |
| SPARC-like protein 1   |                                             |
| Alpha-actinin-1        |                                             |
| Carbohydrate sulfotransferase 3 |                                             |
| Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform |                                             |
| tRNA-dihydouridine synthase 1-like |                                             |
| Retinoic acid receptor responser (tazarotene induced) 1 |                                             |
| Interleukin-1 beta precursor |                                             |
| NACHT, LRR, and PYD domains-containing protein 5 |                                             |
| Hypothetical protein LOC781988 |                                             |
| Tenascin-X             |                                             |
| Mannan-binding lectin serine protease 2 |                                             |
| Galectin-3-binding protein precursor |                                             |
| Adenosine deaminase-like protein |                                             |
| Ankyrin repeat domain-containing protein 32 |                                             |
| Fatty acid synthase    |                                             |
| Tenascin C             |                                             |
| Ras-related protein Rap-I b precursor |                                             |
| Zinc finger protein 366 |                                             |

**Abbreviations:** AuNRs, gold nanorods; SiO$_2$-AuNRs, gold nanorods functionalized with silica; Si, silica; tRNA, transfer ribonucleic acid; RPMI, Roswell Park Memorial Institute.
### Table S7 List of AuNRs attached common proteins involved in both DMEM and RPMI

| BARE common                  |                  |
|------------------------------|------------------|
| PREDICTED: apolipoprotein B   |                  |
| Alpha-2-macroglobulin        |                  |
| Fibronectin                  |                  |
| Complement C3 preproprotein  |                  |
| Inter-alpha-trypsin inhibitor heavy chain H2 | |
| Inter-alpha-trypsin inhibitor heavy chain H3 precursor | |
| Serum albumin precursor      |                  |
| Complement C4                |                  |
| Inter-alpha-trypsin inhibitor heavy chain H1 precursor | |
| Fibulin-1                    |                  |
| PREDICTED: pregnancy-zone protein-like | |
| Gelsolin isoform a            |                  |
| Thrombospondin-1 precursor   |                  |
| Angiotensigen                |                  |
| Heparin cofactor 2           |                  |
| Alpha-2-HS-glycoprotein precursor | |
| Vitronectin                  |                  |
| Prothrombin                  |                  |
| Periostin                    |                  |
| Thrombospondin-4 precursor   |                  |
| Antithrombin-III precursor   |                  |
| Alpha-1-antitrypsinase precursor | |
| Protein AMBP precursor       |                  |
| Talin-1                      |                  |
| Thyroglobulin precursor      |                  |
| Apolipoprotein E precursor   |                  |
| Alpha-2-antiplasmin precursor | |
| Apolipoprotein A-I preproprotein | |
| Hemoglobin fetal subunit beta | |
| Coagulation factor V precursor | |
| Hyaluronan-binding protein 2 |                  |
| C4b-binding protein alpha chain precursor | |
| Kininogen-2 isoform II       |                  |
| Complement component C9 precursor | |
| PREDICTED: apolipoprotein B   |                  |
| Tubulin, beta 1              |                  |
| PREDICTED: similar to complement component 4A | |
| PREDICTED: heparan sulfate proteoglycan 2 | |
| Hemoglobin subunit beta      |                  |
| Fibrinogen alpha chain precursor | |
| Hemoglobin subunit alpha     |                  |
| Hypothetical protein LOC510860 |                  |

### Table S7 (Continued)

| BARE common                  |                  |
|------------------------------|------------------|
| Heat shock protein HSP 90-alpha |                  |
| Serpin A3-1 precursor         |                  |
| Factor XIIa inhibitor precursor |                |
| Actin, aortic smooth muscle   |                  |
| Tubulin alpha-4A chain        |                  |
| Kininogen-2 isoform I         |                  |
| Inter-alpha-trypsin inhibitor heavy chain H4 precursor | |
| Mannan-binding lectin serine protease I | |
| Apolipoprotein M              |                  |
| Fibrinogen beta chain         |                  |
| Tenascin-X                    |                  |
| PREDICTED: complement component 4 binding protein, alpha chain-like Collagen alpha-I (XII) chain | |
| Heat shock protein HSP 90-beta |                  |
| Tubulin beta-5 chain          |                  |
| Fibromodulin                  |                  |
| Fetuin-B precursor            |                  |
| Lumican precursor             |                  |
| Alpha-actinin-I               |                  |
| Vitamin D-binding protein precursor |             |
| Neuruplin-I                   |                  |
| PREDICTED: GLIS family zinc finger 3, partial tRNA-dihydouridine synthase 1-like Thrombospondin-3 | |
| Neuruplin-2                   |                  |
| PREDICTED: collagen, type VI alpha 3-like isoform 4 Interleukin-1 beta precursor | |
| Coagulation factor X          |                  |
| SPARC-like protein 1          |                  |
| Carbohydrate sulfotransferase 3 |               |
| C-type lectin domain family 11 member A Collectin-11 precursor | |
| Mannan-binding lectin serine protease 2 Pentraxin-related protein PTX3 precursor | |
| 6-phosphogluconate dehydrogenase, decarboxylating Nidogen-1 | |

**Abbreviations:** AuNRs, gold nanorods; DMEM, Dulbecco’s Modified Eagle’s Medium; tRNA, transfer ribonucleic acid; RPMI, Roswell Park Memorial Institute.
**Table S8** List of SiO$_2$-AuNRs attached common proteins involved in both DMEM and RPMI

| Si common |
|-----------|
| PREDICTED: apolipoprotein B |
| Complement C3 preproprotein |
| Serum albumin precursor |
| Alpha-2-macroglobulin |
| Antithrombin-III precursor |
| Apolipoprotein E precursor |
| Inter-alpha-trypsin inhibitor heavy chain H2 |
| Apolipoprotein A-I preproprotein |
| Fibulin-1 |
| Alpha-1-antiproteinase precursor |
| Alpha-2-HS-glycoprotein precursor |
| Complement C4 |
| Periostin |
| Inter-alpha-trypsin inhibitor heavy chain H3 precursor |
| Hemoglobin subunit alpha |
| Vitronectin |
| Heparin cofactor 2 |
| Prothrombin |
| Inter-alpha-trypsin inhibitor heavy chain H4 precursor |
| Hemoglobin fetal subunit beta |
| Angiotensinogen |
| PREDICTED: complement component 4 binding protein, alpha chain-like |
| Gelsolin isoform a |
| Protein AMBP precursor |
| Fetuin-B precursor |
| Lipopolysaccharide-binding protein precursor |
| Plasma serine protease inhibitor precursor |
| Complement component C9 precursor |
| Actin, aortic smooth muscle |

**Table S8 (Continued)**

| Si common |
|-----------|
| Talin-1 |
| PREDICTED: apolipoprotein B |
| Serotransferrin precursor |
| Hemoglobin subunit beta |
| Alpha-2-antiplasmin precursor |
| Inter-alpha-trypsin inhibitor heavy chain H1 precursor |
| Actin, cytoplasmic 1 |
| Apolipoprotein A-II precursor |
| Fibronectin |
| Hypothetical protein LOC510860 |
| Heat shock protein HSP 90-beta |
| Fermitin family homolog 3 |
| Phospholipid transfer protein |
| Pyruvate kinase isozymes M1/M2 |
| Adenosylhomocysteinase |
| Complement component B, beta polypeptide |
| Kininogen-2 isoform II |
| Kelch-like ECH-associated protein 1 |
| Apolipoprotein M |
| Glyceraldehyde-3-phosphate dehydrogenase |
| Glyceraldehyde-3-phosphate dehydrogenase, testis-specific |
| Vitamin D-binding protein precursor |
| Heat shock protein HSP 90-alpha |
| C4b-binding protein alpha chain precursor |

**Abbreviations:** SiO$_2$-AuNRs, gold nanorods functionalized with silica; Si, silica; DMEM, Dulbecco’s Modified Eagle's Medium; RPMI, Roswell Park Memorial Institute.
Table S9 List of AuNRs attached unique proteins involved in either DMEM or RPMI

| Bare uncommon | DMEM-bare                                                                 | RPMI-bare                                                                 |
|---------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
|               | Echinoderm microtubule-associated protein-like 5                          | Pigment epithelium-derived factor precursor                                |
|               | PREDICTED: alpha-2-macroglobulin-like, partial                            | Cartilage oligomeric matrix protein                                         |
|               | Aggreccan core protein                                                    | Filamin-A                                                                  |
|               | Plasma serine protease inhibitor precursor                                | Alpha-fetoprotein precursor                                                |
|               | PREDICTED: calcium channel, voltage-dependent, L type, alpha 1S subunit-like | Fibrinogen gamma-B chain precursor                                         |
|               | Myosin, heavy chain 9, nonmuscle                                           | Serpin A3-7                                                                |
|               | Glyceraldehyde-3-phosphate dehydrogenase                                  | PREDICTED: sushi, von Willebrand factor type A, EGF, and pentraxin domain containing 1, partial |
|               | Caspase-14                                                                | Complement C5a anaphylatoxin                                                |
|               | Myosin-I1                                                                 | Reelin                                                                    |
|               | PREDICTED: KRAB-A domain containing 2                                     | Lipopolysaccharide-binding protein precursor                               |
|               | Beta-casein precursor                                                     | CTAGE family, member 5                                                     |
|               | Sucrase-isomaltase, intestinal                                             | Rab GDP dissociation inhibitor beta                                         |
|               | Transmembrane and coiled-coil domain-containing protein 2                 | PREDICTED: VGF nerve growth factor inducible-like                          |
|               | Complement factor B precursor                                             | Eukaryotic elongation factor 2 kinase                                      |
|               | PREDICTED: NIMA (never in mitosis gene a)-related kinase II               | von Willebrand factor                                                      |
|               | PREDICTED: FYVE and coiled-coil domain-containing protein 1-like          | Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform |
|               | PREDICTED: thiolester containing protein II-like                          | Actin, cytoplasmic 1                                                       |
|               | PREDICTED: zinc finger protein 347-like                                    | Retinoic acid receptor responder (tazarotene induced) 1                    |
|               | Terminal uridylyltransferase 4                                             | NACHT, LRR, and PYD domains-containing protein 5                           |
|               | Nucleolar GTP-binding protein 2                                            | Hypothetical protein LOC781988                                            |
|               | Alpha-S1-casein precursor                                                 | Galexin-3-binding protein precursor                                        |
|               | N-acetylated-alpha-linked acidic dipeptidase 2                             | Adenosine deaminase-like protein                                           |
|               | Mitochondrial-processing peptidase subunit alpha precursor                | Ankyrin repeat domain-containing protein 32                               |
|               | Coiled-coil alpha-helical rod protein 1                                   | PREDICTED: hCG1647286-like                                                 |
|               |                                                                          | Fatty acid synthase                                                        |
|               |                                                                          | Tenascin C                                                                 |
|               |                                                                          | Ras-related protein Rap-1b precursor                                       |
|               |                                                                          | Zinc finger protein 366                                                    |

Abbreviations: AuNRs, gold nanorods; DMEM, Dulbecco’s Modified Eagle’s Medium; RPMI, Roswell Park Memorial Institute.
## Table S10 List of SiO₂-AuNRs attached unique proteins involved in either DMEM or RPMI

| DMEM-Si | RPMI-Si |
|---------|---------|
| Coagulation factor XIII A chain precursor | PREDICTED: pregnancy-zone protein-like |
| Phosphoglycerate kinase 1 | Complement factor B precursor |
| L-lactate dehydrogenase B chain | Tubulin, beta 1 |
| Alpha-enolase | Complement C5a anaphylatoxin |
| Transmembrane and coiled-coil domain-containing protein 2 | Thrombospondin-1 precursor |
| Probable arginyl-tRNA synthetase, mitochondrial precursor | Fibrinogen beta chain |
| Serpin A3-1 precursor | Alpha-fetoprotein precursor |
| L-lactate dehydrogenase C isoform 1 | Thrombospondin-4 precursor |
| Nucleolar GTP-binding protein 2 | Properdin |
| Thyroxine-binding globulin precursor | Coagulation factor V precursor |
| Neutrophil cytosol factor 1 | PREDICTED: collagen, type VI, alpha 3-like isoform 4 |
| Apolipoprotein D precursor | Coagulation factor X |
| | Elongation factor 1-alpha 2 |
| | Kininogen-2 isoform 1 |
| | PREDICTED: GLIS family zinc finger 3, partial |
| | PREDICTED: Putative zinc finger protein |
| | ENSP00000330994-like, partial |
| | Tubulin alpha-4A chain |
| | Hyaluronan-binding protein 2 |
| | Actin, alpha cardiac muscle 1 |
| | PREDICTED: hCG1647286-like |
| | Transhyretin precursor |
| | PREDICTED: calcineurin binding protein 1 |
| | Carboxypeptidase B2 precursor |
| | Leucine-rich repeat-containing protein 49 |
| | Neural cell adhesion molecule 1 precursor |
| | Biglycan precursor |
| | Tubulin alpha-1D chain |
| | PREDICTED: phosphorylase kinase, beta |
| | C-type lectin domain family 11 member A |
| | Alpha-1-acid glycoprotein precursor |

**Abbreviations:** SiO₂-AuNRs, gold nanorods functionalized with silica; DMEM, Dulbecco's Modified Eagle's Medium; Si, silica; tRNA, transfer ribonucleic acid; RPMI, Roswell Park Memorial Institute.