Interactions of variously coated gold and silver nanoparticles with a bis(triarylborane) photodynamic therapy (PDT)-dye; their cellular uptake, cytotoxicity and photo-activity

Abstract

**Background and purpose:** Diethynylarene-linked bis(triarylborane) tetracations can be used as probes for fluorimetric and Raman sensing of biomacromolecules, as well as promising theragnostic agents. Among them, bis(triarylborane) fluorophore (TAB3), when bonded to Ag nanoparticles (NP), stood out with specific properties such as Raman signal enhancement of the TAB3 dye in a cuvette. However, TAB3 dye - nanoparticle composites have not been studied in biological systems. For this reason, questions arose as to whether different types of metal nanoparticles (Au or Ag-based) with different coatings (negatively charged citrate or neutral PVP) could be efficiently stained with the TAB3 dye in a cuvette. The aim of this research was to examine Au and Ag nanoparticles of similar size (20-25 nm) with different stabilizers for their cellular uptake, cytotoxicity in the dark and under visible light radiation, to characterize the interactions of nanoparticles with the TAB3 fluorophore, and to study NP-TAB3 composites in cells, evaluate their intracellular staining, as well as possible photoinduced release and biological activity.

**Materials and methods:** The binding constants of Au- and Ag-based nanoparticles with TAB3 were determined by fluorimetric titrations. The cytotoxic effect of NPs was determined by the survival of A549 cells (MTT assay). Cellular uptake of both NP and NP-TAB3 composites were performed by live cell imaging experiments.

**Results:** The Au- or Ag-based NPs with different coatings bind to the TAB3 with high affinity. These NPs, as well as TAB3-NP complexes, efficiently enter living human cells, accumulating in cytoplasm with no apparent selectivity for a particular organelle. Even prolonged 3-day treatment with the NPs studied did not show any toxic effect on the cells. Bioimaging studies in cells revealed that the TAB3-NP complex does not intracellularly dissociate; the previously reported photo-bioactivity of TAB3 is completely inhibited by binding to NPs.

**Conclusion:** Au- and Ag NPs were non-covalently stained by TAB3, irrespective of the different coatings, with similar binding affinities. Emission from TAB3 is strongly quenched by the NPs, but not completely. Experiments on living human cells revealed that neither free NPs, nor their composites with TAB3, were toxic. Bioimaging studies by confocal microscopy revealed that all NPs efficiently enter living cells within 90 min. Co-localization experiment with simultaneous collection of data in the reflection
and fluorescence modes demonstrated that the TAB3 dye remained bound to NPs inside cells. Strong irradiation of TAB3-NP inside cells with a 457 nm laser did not yield any damage to the cells, at variance with our previously shown very strong photo-bioactivity of the TAB3 dye alone. Thus, binding of a chromophore to a nanoparticle can inhibit the chromophore’s ability to undergo photo-induced singlet oxygen production, consequently blocking its photo-bioactivity.

INTRODUCTION

The potential application of metal nanoparticles (NPs) is indeed broad, including fields such as catalysis, photonics, optoelectronics, surface-enhanced Raman scattering (SERS) (1) to applications in biomedicine, such as anticancer therapy (2), drug delivery (3), cell labeling, non-invasive diagnostics (bioimaging and MR imaging), (5–8) and cosmetics (9). The most commonly researched nanoparticles for biomedical purposes are silver nanoparticles (AgNP) due to their previously known antibacterial efficacy (10), and gold nanoparticles (AuNP). Their biological activity, cellular uptake, as well as their toxicity highly depends on their physicochemical characteristics, i.e., their size (11), shape (12), surface charge/coating (13) as well as on the interaction of various nanoparticles (including gold NPs) with physiological fluids (14).

Enea et al. (15) investigated the influence of size, capping agent, and the shape of AuNP on cellular uptake and cytotoxicity in two different hepatic cell models. They showed that, in general, AuNPs have a low toxicity towards hepatic cells. However, they demonstrated that the cellular uptake depended significantly on the type of hepatic cells, with smaller AuNPs being more toxic than the larger ones (~15 nm vs 60 nm) and their cellular uptake was higher. Furthermore, citrate-coated NPs showed higher toxicity than 11-mercaptoundecanoic acid-coated ones, and cellular uptake for 60 nm nanostars was higher than that of nanospheres of the same size. Many experimental data showed a similar trend for AgNPs (16,17).

The increased cellular uptake and cytotoxicity of smaller NPs could be explained by their higher probability of interaction with the biological medium due to their larger specific surface area, i.e., more atoms are on the surface of the NPs (18). The charge, solubility, and biocompatibility of NPs is defined by their coating. In general, positively charged NPs have a longer lifetime in the blood stream compared to negatively charged ones, making them more suitable for drug-delivery purposes (19). Also, their tendency to agglomerate in interactions with a biological medium is well known to affect cellular uptake and cytotoxicity (20). Some nanoparticles also show outer stimuli-induced drug-release (21) or, in particular, photo-induced bioactivity (photo-thermal induced therapy) (22).

We recently reported diethynylarene-linked bis(triarylborane) tetracations for dual fluorimetric and Raman-SERS (SERS = surface-enhanced Raman scattering) sensing of biomacromolecules (23) and promising photodynamic therapy (PDT)-based biological activity on human cell lines and adenovirus type 5 (HAdV5), thus acting as theranostic agents (24). Among these, TAB3 dye (Figure 1A and 1B) excelled in intracellular properties: fluorimetric staining of several organelles and consequent photo-induced bioactivity, as well as in Raman (SERS) signal amplification of the TAB3 dye in a cuvette caused by binding to silver nanoparticles (23). However, these silver-nanoparticle/TAB3 dye composites were not studied in biological systems.

For that reason, questions arose as to whether different types of metal nanoparticles (Au- or Ag-based) with different coatings (negatively charged citrate or neutral PVP) could be efficiently stained by our TAB3 dye in a cuvette. If yes, such novel nanoparticle/TAB3 dye composites could show intriguing cellular activity, both in the dark and under visible light irradiation.

The aim of this research was to screen several Au- and Ag-based nanoparticles of similar size (20–25 nm), differing in their coating (citrate vs PVP), for their cellular uptake, cytotoxicity in the dark and under visible light irradiation. Furthermore, interactions of these Au- and Ag-based nanoparticles with our very recently developed photo-active fluorophore TAB3 were characterized, and

![Figure 1](image_url)

Figure 1. A) Au or Ag nanoparticles studied with various coatings; B) schematic presentation of TAB3 dye binding to a nanoparticle surface (23); C) study of intracellular uptake of the free nanoparticles (A) and the same nanoparticles stained with the TAB3 dye (B).
novel nanoparticle-dye composites were also studied in cells assessing their intracellular staining, eventual photoinduced release, and biological action.

**MATERIALS AND METHODS**

**Synthesis of NPs**

**Materials**

Tetrachloroauric(III) acid trihydrate (HAuCl$_4$·3H$_2$O, ≥99.9% trace metal basis), trisodium citrate dihydrate (C$_6$H$_5$Na$_3$O$_7$·2H$_2$O, puriss, p.a., ASC reagent, ≥99.0%) and polyvinylpyrrolidone (PVP-50, (C$_6$H$_9$NO), powder, M$_w$ ≈ 50000) were purchased from Sigma-Aldrich. Nitric acid (HNO$_3$, 65%, M$_w$ ≥ 99,9% trace metal basis), trisodium citrate dihydrate (Na(CH$_3$)$_2$AsO·3H$_2$O, 98%) for buffer preparation was purchased from Fluka. Sodium cacodylate trihydrate (sodium cacodylate, Na(CH$_3$)$_2$CO$_3$·3H$_2$O, 98%) was purchased from Fluka. Sodium cacodylate buffer solution (Na$_2$CO$_3$ buffer, pH = 7.0) was prepared using Na$_2$CO$_3$·3H$_2$O, pH 7.0).

UV-Vis measurements were performed on a Cary 100 Bio spectrophotometer (Agilent Technologies) and fluorescence measurements were performed on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies). Resulting titration data were fitted with an exponential function obtaining $R^2 > 0.99$ in all cases. All measurements were performed in 10 mm x 10 mm cuvettes at room temperature (25 °C).

Scanning electron microscopy (SEM) measurements were carried out in order to determine the morphology and size of the nanoparticles. Plain view images were taken using a field-emission microscope Jeol JSM 7000F at an acceleration voltage of 1 kV. SEM samples were prepared by depositing a drop of a diluted nanoparticle suspension onto a silicon wafer and air-drying at room temperature. SEM images were analyzed using ImageJ software (25) and the average diameter of the nanoparticles was obtained from the analysis.

**Synthesis of gold nanoparticles**

Citrate-coated gold nanoparticles (AuNP/cit) were synthesized following the previously published two-step procedure (synthesis of Au seeds and then immediate growth of the seeds) (26) with minor modification. In a three-neck round bottomed flask, 50 mL of an aqueous 2.2 mM sodium citrate solution was heated to a boil. A condenser, attached to the flask, was used to prevent the evaporation of the solvent. After 5 min of boiling, 400 µL of 25 mM HAuCl$_4$ was injected into the solution. The color of the solution changed quickly from yellow to bluesh-grey and then to pink within 10 min, when the Au seeds were formed. After seed formation, the reaction was cooled to 85 °C. The growth of AuNPs occurred immediately after the seed formation, in the same flask. After cooling to 85 °C, 400 µL of 25 mM HAuCl$_4$ was injected into the reaction. After 25 min, after the first growing step was finished, an additional 400 µL of 25 mM HAuCl$_4$ was injected into the reaction mixture. When the second growing step was complete (after 25 min), the reaction was cooled to room temperature. To remove excess trisodium citrate salt, the AuNPs obtained were centrifuged at 12 000 x g for 10 min and then dispersed in ultrapure water. The molar concentration of the AuNPs was calculated from a UV-Vis spectrum using extinction coefficients obtained from Mie theory calculations for citrate-coated spherical Au nanoparticles (27).

**Synthesis of silver nanoparticles**

Silver nanoparticles were synthesized using both trisodium citrate and tannic acid as reducing agents according to a published protocol (28). Briefly, in a three-neck round bottomed flask, 50 mL of a solution containing 5 mM trisodium citrate and 0.1 mM tannic acid was heated to a boil. A condenser, attached to the flask, was used to prevent the evaporation of the solvent. After 3 min of boiling, 500 µL of 25 mM AgNO$_3$ was injected into the solution. The solution immediately changed color to bright yellow. After 25 min, the reaction was complete and was cooled to room temperature. To remove tannic acid and excess trisodium citrate salt, the AgNPs obtained were centrifuged at 12 000 x g for 10 min and then dispersed in ultrapure water. The molar concentration of citrate stabilized Ag nanoparticles was calculated from UV-Vis spectrum using experimentally obtained extinction coefficient data for citrate stabilized spherical silver nanoparticles (29).

**Preparation of PVP-coated NPs**

PVP-coated gold and silver nanoparticles were prepared by adding 500 µL of 1 mM PVP-50 into 1 mL of a suspension of the nanoparticles. After 12 h of incubation, the solution was centrifuged once at 12 000 x g for 10 min and the nanoparticles were dispersed in sodium cacodylate buffer solution (I = 0.05 M; pH = 7.0).

The concentration of the stock solutions for both citrate- and PVP-50-coated AuNPs (AuNP/cit and AuNP/ PVP-50, respectively, in further text) suspensions were adjusted to be 5 x 10$^{-10}$ M and for AgNP suspensions...
Determination of binding constants of TAB3 with nanoparticles

The binding constant of TAB3 with nanoparticles was determined by fluorimetric titration of TAB3 ($c = 2.1 \times 10^{-7}$ M) in water or sodium cacodylate buffer ($pH = 7.0, I = 0.05$ M) by adding aliquots of NPs' suspensions. For titration purposes, the concentration of the stock solution of AuNP (PVP-50 and citrate coated) was $5 \times 10^{-10}$ M and that of AgNP was $5 \times 10^{-11}$ M. The quenching of emission of TAB3 was recorded at 531 nm (in experiments with AgNPs) and 522 nm (for AuNPs) after excitation at 413 nm. Titration data were fitted in Origin 7.0 software to the first exponential equation, giving apparent binding constants ($K_{app}$).

**Biology**

**Cells**

A549 cells (human lung carcinoma; ATCC CCL-185) were obtained from the ATCC Cell Biology Collection and were cultured according to the manufacturer's instructions. Cells were grown in Dulbecco Modified Eagle's Medium (DMEM, Sigma Aldrich, USA) supplemented with 10% of fetal bovine serum (FBS, Sigma Aldrich, USA) at 37 °C and 5% CO$_2$ in a humidified atmosphere.

**Cytotoxicity assay – MTT**

Cells were seeded on 96 well plates at concentrations of $7 \times 10^3$ cells/well in 100 µL of DMEM (10% FBS) and left in the incubator overnight (37 °C, 5% CO$_2$). The next day, 100 µL of the working solution of the samples was added to the wells, thus the final volume was 200 µL/well. The stock solutions used for antiproliferative screening were $3.5 \times 10^{-2}$ M for AuNPs and $4 \times 10^{-10}$ M for AgNPs. The concentrations tested were $3.5 \times 10^{-10} - 3.5 \times 10^{-12}$ for AuNPs and $4 \times 10^{-11} - 4 \times 10^{-13}$ M for AgNPs. All measurements were made in quadruplicate. The plates were then incubated for the next 72 h (37 °C, 5% CO$_2$). After incubation, the medium was removed, and 40 µL of an MTT solution was added to each well. The plates were incubated in the cell incubator for $3 h$, allowing formazan crystals to form. After 4 h, 170 µL of DMSO was added to each well and the plates were placed on a shaker for 20 min, allowing the crystals to dissolve. The absorbance of the MTT-formazan product was measured with a microplate reader at 600 nm, and the absorbance value correlates directly with cell survival. For irradiation experiments, cell culture plates, prepared as above, were treated with the compounds studied, incubated for 90 min (37 °C, 5% CO$_2$) in order to allow the compounds to enter the cells, and irradiated in a Luzchem reactor with UV light, 350 nm, in total 8 W, dose 50.6 mw*m$^{-2}$, 18 cm lamp to cell-plate. Irradiation for 10 min and 30 min per day was performed on three consecutive days at the same times each day.

**Live cell imaging**

Live imaging of the cells treated with the compounds was performed on the A549 cell line. Cells were seeded in Ibidi imaging cell chambers (Ibidi®, Germany) in 500 µL of medium, with a concentration of $5 \times 10^4$ cells/well and left in the cell incubator for 48 h (37 °C, 5% CO$_2$). After two days, cells were treated with a $1 \times 10^{-11}$ M solution of the NP system to be tested and left in the cell incubator for 90 min or overnight to allow the compound to enter the cells. Then, the cells were rinsed and observed using a Leica SP8 X confocal microscope (Leica Microsystems, Germany) with a 63 x/1.40 oil-immersion objective for imaging, using the 457 nm laser and operating in the reflection mode to collect Reflection Interference Contrast Microscopy (RICM) images, or in the fluorescence mode to collect emission images (only in the presence of the TAB3 dye). The images were analyzed using LAS X (Leica Microsystems, Germany) and ImageJ (NIH, USA) software and they showed maximum projections of confocal stacks, unless otherwise indicated.

**RESULTS AND DISCUSSION**

**Studies in aqueous solutions (cuvette)**

**Characterization of gold and silver nanoparticles**

As the cellular uptake depends on the size and shape of the nanoparticles, citrate-coated gold and silver nanoparticles were characterized by UV-Vis spectroscopy and SEM.

Figure 2A shows typical UV-Vis spectra of AgNP/cit and AuNP/cit in ultrapure water with maximum absorbances of 410 nm and 519 nm, respectively. SEM microscopy (Figure 2B and 2C) showed that both AgNP/cit and AuNP/cit were homogeneous and quasi-spherical in shape, with average diameters of 24.5 nm ± 2.8 nm and 20.7 nm ± 2.6 nm (insets in Figure 2B and 2C), respectively. The shape and size of both AgNP/cit and AuNP/cit are in good agreement with results of published protocols (26, 28).

**Interactions of the TAB3 dye with various NP**

Stock solutions of the well-defined AgNPs and AuNPs were then titrated into buffered aqueous solutions of the TAB3 dye, monitoring the change of TAB3 emission (Figures 3 and 4). In general, emission from TAB3 was hypsochromically shifted and strongly quenched, although the extent of quenching was dependent on the type of NP (Table 1; $\text{III}_i$). This result suggests that bind-
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Calculation of binding constants which could be attributed to the TAB3-NP complex is quite challenging as it is not possible to determine the exact number of binding sites available on the surface of a particular NP. However, non-covalent equilibrium of the TAB3-NP interaction was very fast (as shown by almost immediate equilibration of emission upon every addition of an NP aliquot during titration), strongly supporting non-covalent interactions.

**Figure 2.** A) UV Vis spectra of AgNP/cit (full blue line) and AuNP/cit (dashed red line); B) SEM image of AgNP/cit with INSET: AgNP/cit diameter distribution; C) SEM image of AuNP/cit with INSET: AuNP/cit diameter distribution.

**Figure 3.** Change in the fluorescence spectrum of TAB3 (c = 2.1 \times 10^{-7}, \lambda_{ex} = 413 nm) at 298 K upon titration with: A) AgNP/cit in water; B) AgNP/cit in sodium cacodylate buffer; C) AgNP/PVP–50 in sodium cacodylate buffer; D) emission dependence at \lambda_{max} = 531 nm of TAB3 on c(AgNP), red line-fit to the 1:1 stoichiometry complex formation.
Figure 4. Change in the fluorescence spectrum of TAB3 (c = 2.1 × 10⁻⁷ M, λmax = 413 nm) at 298 K upon titration with: A) AuNP/cit in water; B) AuNP/cit in sodium cacodylate buffer; C) AuNP/PVP–50 in sodium cacodylate buffer; D) emission dependence at λmax = 522 nm of TAB3 on c(AuNP), red line-fit to the 1:1 stoichiometry complex formation.

Thus, presuming that all TAB3 molecules bind non-co-operatively in the same way, we obtained an excellent fit of the experimental data to the first exponential equation, giving us apparent binding constants (Kapp; Table 1). Such Kapp values do not represent exact binding constants of 1:1 stoichiometry (K1:1), but an estimated Kapp value which is useful for the determination of the TAB3-NP ratio necessary to have all dye bound to the particular NP, which is essential information for the biological experiments described below. More detailed analysis of Kapp values revealed that the affinity of TAB3 toward AgNPs is an order of magnitude higher than toward AuNPs. However, coating of particular NP had only a negligible impact on the Kapp values. It should be stressed that after partial quenching by NPs, TAB3 still emits fluorescence sufficient for imaging studies in cells.

### Biology

#### Antiproliferative screening

In order to study the cytotoxic effect of NPs we determined the survival of A549 cells after exposing them to different concentrations of NPs. The cells were treated with 3.5 × 10⁻¹⁰ M, 3.5 × 10⁻¹¹ M or 3.5 × 10⁻¹² M solutions of AuNP/cit or AuNP/PVP-50, or 4 × 10⁻¹⁰ M, 4 × 10⁻¹¹ M or 4 × 10⁻¹² M of AgNP/cit or AgNP/PVP-50, and cell survival was assessed by the MTT assay (Figure 5). Both AuNP/cit and AuNP/PVP-50 showed cytotoxic effects at higher concentrations, namely 3.5 × 10⁻¹⁰ M and 3.5 × 10⁻¹¹ M. At the lowest concentration, 3.5 × 10⁻¹² M, AuNP/cit or AuNP/PVP-50 did not interfere with cell survival. Ag nanoparticles were toxic only at the highest concentration studied, 4 × 10⁻¹⁰ M. When applied at the...
lower concentration, neither AgNP/cit nor AgNP/PVP-50 were toxic to the A549 cells. The effect of nanoparticles on cell survival was tested also after exposure to UV light. Regardless of the exposure to UV light, both Au- and Ag-based nanoparticles remained non-toxic at the picomolar concentration range (3.5 × 10⁻¹² M for Au nanoparticles and 4 × 10⁻¹² M for Ag nanoparticles) (Figure 6).

**Live cell imaging**

To determine whether non-toxicity is a result of non-efficient cellular uptake, we performed live cell imaging experiments on A549 cells treated with AuNP/cit, AgNP/cit or AgNP/PVP-50, all at a concentration of 1 × 10⁻¹¹ M, using a Leica TCS SP8 X confocal laser scanning microscope (Leica Microsystems) equipped with an HC PL APO CS2 63x/1.40 oil objective, using the 457 nm laser and operating in reflection mode.

We used two different incubation times upon addition of nanoparticles to cells (90 min and overnight – 16 h) and, under both conditions, all nanoparticles studied efficiently entered cells, as clearly demonstrated in reflection mode images (Figure 7) in comparison to reference experiments performed on cells not treated with NPs (Supporting Information Fig S1).

To confirm the presence of nanoparticles in the cells, we stained AgNP/cit nanoparticles with TAB3 according to the above determined binding constants (Table 1), i.e., with c(TAB3) = 1µM, and then incubated the cells with NP/TAB3 for 90 min.

Confocal microscopy experiments in fluorescence mode using the excitation wavelength of the TAB3 dye (457 nm), revealed efficient cellular uptake and significant co-localization (Pearson’s coefficient \( r = 0.808 \)) of TAB3 emission (Figure 8B, green) with simultaneous microscopy analysis of the same sample in reflection mode (Figure 8A and 8C). It should be noted that while monitoring the sample in Figure 8 in real time, we noticed that dots attributed to the NPs move, the intensity of movement being proportional to the intensity of laser irradiation. This effect can be attributed to photo-induced heating of the metal NPs. This movement, even at low laser power, interfered to a minor extent with the collection of subsequent images in reflection and fluorescence modes, thus resulting in a bit less than 100% co-localization (Figure 8D).

We recently reported that the TAB3 dye, under intense irradiation at 457 nm for only 1 min, causes severe cellular damage due to singlet oxygen production (24), demonstrated by the confocal microscope movie in the Supp. Info. to ref. 24. Thus, we irradiated TAB3-AuNP/cit composites in cells under the same conditions, but no visible toxicity or cellular damage was observed (data not shown,
movie available on request). Also, Figure 6 showed that irradiation of cells treated with TAB3-NP in a photochemical reactor for even 30 min did not produce a measurable cytotoxic effect, at variance to the results for TAB3-treated cells (24), where, under the same conditions, all cells were killed.

This result could be correlated with the TAB3 emission quenching upon binding to NPs (Figures 3, 4 and Table 1), attributed to the enhanced non-radiative decay of the fluorophore caused by the metal nanoparticle surface. We showed above, in co-localization experiment in reflection and fluorescence modes, that TAB3 remained bound to

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**Figure 7.** Uptake and localization of AuNP/cit, AgNP/cit and AgNP/PVP-50 in live A549 cells. Cells were incubated with studied compounds overnight (16h) in concentration of $1 \times 10^{-11} \text{ M}$ (shown in red; $\lambda_{\text{exc}} = 405 \text{ nm}$). Reflection Interference Contrast Microscopy (RICM) is shown in grey.

**Figure 8.** Uptake and localization of AgNP/cit coupled to TAB3 in live A549 cells. Cells were incubated with AgNP/cit in concentration of $1 \times 10^{-11} \text{ M}$ (A) shown in red) and TAB3 at a concentration of 1 µM (B) shown in green) for 90 min. Reflection Interference Contrast Microscopy (RICM) is shown in grey (C) and overlay of a,b,c given in (D).
NP s in a cellular environment. As singlet oxygen production by TAB3 is directly related to intersystem crossing (ISC) from singlet to triplet excited state (24), which takes place on a timescale roughly similar to that of fluorescence, NP-caused fluorescence quenching by enhanced non-radiative decay rates will also likely interfere with ISC by providing another competing pathway for deactivation of the S state, while those molecules which did undergo ISC to the triplet state might also undergo enhanced non-radiative decay, both of which would result in diminishing the photo-induced bioactivity.

CONCLUSIONS

Au and Ag nanoparticles (NPs) were non-covalently stained by the fluorescent dye TAB3 irrespective of the different coatings (negatively charged citrate or neutral PVP) with similar binding affinities. Emission from TAB3 is strongly quenched by the NPs, but not completely; thus, the remaining emission allowed monitoring of stained TAB3-NP in biological systems.

Experiments on living human cells (A549) revealed that neither free NPs, nor their composites with TAB3, were toxic. Bioimaging studies by confocal microscopy, performed in the reflection mode and also in the fluorescence mode with TAB3-stained NPs, revealed that all NPs efficiently enter living cells within 90 min. Co-localization experiment with simultaneous collection of data in the reflection and fluorescence modes demonstrated that the TAB3 dye remained bound to NPs inside cells.

However, strong irradiation of TAB3-NP inside cells with a 457 nm laser did not yield any damage to the cells, at variance with our previously shown very strong photobiologic activity. This finding can be applied in two ways: either for suppressing harmful effects of photo-active chromophores or for the design of novel photodynamic therapy (PDT) systems. The later PDT-composites would be based on non-active PDT-drug/NP composites which efficiently enter living cell, and, inside the cells, the PDT drug would be released by internal or external stimuli, thus becoming photo-bioactive.

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