Peptide Functionalized Gold Nanoparticles as a Stimuli Responsive Contrast Medium in Multiphoton Microscopy

Johan Borglin,† Robert Selegård,‡ Daniel Aili,*‡ and Marica B. Ericson*†

† Biomedical Photonics Group, Department of Chemistry and Molecular biology, University of Gothenburg, 412 96 Gothenburg, Sweden
‡ Division of Molecular Physics, Department of Physics, Chemistry, and Biology (IFM), Linköping University, 581 83 Linköping, Sweden

Supporting Information

ABSTRACT: There is a need for biochemical contrast mediators with high signal-to-noise ratios enabling noninvasive biomedical sensing, for example, for neural sensing and protein–protein interactions, in addition to cancer diagnostics. The translational challenge is to develop a biocompatible approach ensuring high biochemical contrast while avoiding a raise of the background signal. We here present a concept where gold nanoparticles (AuNPs) can be utilized as a stimuli responsive contrast medium by chemically triggering their ability to exhibit multiphoton-induced luminescence (MIL) when performing multiphoton laser scanning microscopy (MPM). Proof-of-principle is demonstrated using peptide-functionalized AuNPs sensitive to zinc ions (Zn²⁺). Dispersed particles are invisible in the MPM until addition of millimolar concentrations of Zn²⁺ upon which MIL is enabled through particle aggregation caused by specific peptide interactions and folding. The process can be reversed by removal of the Zn²⁺ using a chelator, thereby resuspending the AuNPs. In addition, the concept was demonstrated by exposing the particles to matrix metalloproteinase-7 (MMP-7) causing peptide digestion resulting in AuNP aggregation, significantly elevating the MIL signal from the background. The approach is based on the principle that aggregation shifts the plasmon resonance, elevating the absorption cross section in the near-infrared wavelength region enabling onset of MIL. This Letter demonstrates how biochemical sensing can be obtained in far-field MPM and should be further exploited as a future tool for noninvasive optical biosensing.

KEYWORDS: Gold nanoparticles, multiphoton microscopy, contrast media, optical sensing, peptide

One of the major translational challenges for the development of biosensing contrast mediators is to develop biocompatible sensors with high biochemical contrast while avoiding a raise of the background signal. Gold nanoparticles (AuNPs) have been extensively studied as optical sensors, primarily based on their biocompatibility and optical properties in the linear regime.¹ The optical properties of the particles varies with size and shape.¹−³ Furthermore, the particles can be surface modified with a wide range of biofunctional molecules to tailor them for specific applications.⁴ In the nonlinear optical regime, AuNPs can be visualized by MIL,⁵−¹⁰ which makes them suitable contrast mediators in combination with multiphoton-laser scanning microscopy (MPM).¹¹,¹² MPM enables noninvasive visualization of intact biological tissue with cellular resolution and has become an important and versatile tool allowing for in vivo imaging in preclinical¹³,¹⁴ and clinical applications, for example, cancer diagnostics.¹⁵ Focused near-infrared (NIR) light from femtosecond-pulsed lasers, operating in the tissue transparent wavelength region, give rise to confined nonlinear excitation processes of biomolecules. When employing MPM in biomedical research, exogenous fluorophores are generally used to provide contrast, both in vitro as well as preclinically;⁶,¹⁷ however, for clinical applications signal generation is so far predominantly restricted to autofluorescence.¹⁵,¹⁸,¹⁹ In addition, other applications such as neural²⁰ and immune cell dynamics²¹ and monitoring protein–protein interactions²² are potential applications where in vivo optical sensing is expected to play an important role. To facilitate noninvasive biosensing, biocompatible contrast mediators with high signal-to-noise ratios are desired. Targeted gold nanorods have been proposed to facilitate, for example, tumor diagnostics.¹¹,¹² The problem with this strategy is that the presence of unbound particles not specifically targeted to the cells of interests will increase background signal and thereby decrease contrast and signal-to-noise ratio.

Received: February 13, 2017
Revised: February 18, 2017
Published: February 20, 2017
In this paper, we demonstrate a concept for acquiring a stimuli responsive contrast medium with high biochemical contrast in connection to far field MPM. The concept is based on locally tuning the MIL signal from spherical AuNPs through aggregation. Figure 1 illustrates the concept of sequential two photon absorption of two NIR photons (a,b) leading to MIL (c). The energy levels represent the sp- and d-band structures of Au. F indicates the Fermi surface. The absorption of the first photon (a) leads to an intraband indirect transition within the sp-band from a conduction electron, leaving behind a hole below the Fermi-level. The bent arrow imply that electronic transitions are coupled to phonon excitations. After the second absorption step, a hole in the d-band is created (b). This step is dependent on the time delay of the photons and will primarily be determined by the pulse duration and photon flux of the excitation NIR laser. When particles aggregate, the LSPR of the first absorption is red-shifted (a), which enhances the absorption in the NIR range, and thereby elevates probability for absorption of this subsequent photon. The luminescence (c) is represented as an interband transition occurring when an sp-band electron refills the hole in the d-band. The dashed arrow indicates that thermalization and scattering might precede transition. The emission has been described as a continuum as a signature of the recombination of d-band electron holes. As concluded from our previous paper, the first sp−sp transition (a) is related to the LSPR of the particle. Because the absorption cross section in this range is negligible for small spherical AuNPs but enhanced by formation of clusters, the MIL can be tuned based on the aggregation state of the particles, as will be utilized in this paper.

Figure 2. (a) Schematic representation of JR2EC functionalized spherical AuNPs (20 nm) in their dispersed and aggregated state before and after addition of Zn2+, respectively. (b) Extinction spectrum of JR2EC-AuNPs in buffer before (green) and following addition of Zn2+ after 5 min (orange), 60 min (red), and subsequent addition of EDTA (dashed green). (c) Evolution of the extinction at 800 nm as a function of time after addition of Zn2+. (d,e) JR2EC-AuNPs visualized in far-field MPM (d) in dispersed state before addition of Zn2+ and (e) in aggregated state after addition of Zn2+. Imaging conditions were kept constant for both experiments. Each field of view corresponds to 424 × 424 × 151 μm. (f) Intensity of MIL from JR2EC-AuNPs in suspension during three cycles of sequential additions of 5 mM Zn2+ followed by 10 mM EDTA. Signal is measured as the average of a ~ 500 × 500 μm region. Error bars show standard error of mean. According to theoretical understanding of the concept of MIL, emission only occurs when spherical particles in this size range are aggregated, which is here clearly demonstrated by switching the aggregation state of the JR2EC-AuNPs.
controlled aggregation. The rationale behind is based on the underlying physical principles of MIL. In this process, two or more photons are sequentially absorbed generating a hole in the $d$-band, illustrated by Figure 1. During the first absorption step, an intraband transition in the $sp$-band is generated leaving a hole in the $sp$-band below the Fermi level. During the second absorption in which probability will depend on the lifetime of the $sp$-hole, an interband transition takes place leaving behind a hole in the $d$-band. A subsequent recombination of the electron–hole pair leads to emission of a photon with higher energy than the separate excitation photons corresponding to generation of MIL. We have recently demonstrated that dispersed small spherical AuNPs ($<40$ nm) will not exhibit MIL when excited with NIR.\textsuperscript{20} This is understood from the fact that the probability of the first intraband $sp$→$sp$ transition is related to the localized surface plasmon resonance (LSPR) of the particle, which is known to depend on the shape and size of the particle. Because the absorption cross section, $\sigma_{sp-sp}$ in this range is negligible for small spherical AuNPs, these will not exhibit MIL when excited with NIR; however, when particles aggregate, the LSPR is red-shifted, which enhances the absorption in the NIR range, and thereby enables MIL as illustrated by the figure. In this setting, the MIL signal can be switched on and off based on the chemical environment. By triggering aggregation of the AuNPs, the distance between the particles can be tuned so that a significant red shift of the plasmon band is obtained and MIL can hence be enabled.

Proof-of-principle is here demonstrated for spherical AuNPs (20 nm) modified with a zinc ($Zn^{2+}$) and protease (MMP-7) responsive polypeptide. The peptide (JR2EC) is de novo designed to fold into a helix–loop–helix motif and homodimerize into four-helix bundles in the presence of millimolar concentrations of $Zn^{2+}$. It is well-known that $Zn^{2+}$ plays an important role in biomedical process, for example, carcinogenesis\textsuperscript{26} and neural signaling,\textsuperscript{27} which is why this $Zn^{2+}$-responsive system is interesting to explore in combination with MPM. The presence of $Zn^{2+}$ causes peptides immobilized on separate AuNPs to homodimerize, resulting in extensive but specific and reversible aggregation of the particles.\textsuperscript{28–30} In addition, JR2EC contains two recognition sites for the matrix metalloproteinase MMP-7 with important roles in cancer progression\textsuperscript{31,32} and inflammation.\textsuperscript{33} This system has previously been investigated for phosphatase and protease activity detection and protein sensing\textsuperscript{34–36} but is here for the first time explored with MPM, providing a novel concept for design of stimuli responsive contrast mediators which should be further exploited for biochemical in vivo sensing.

Results and Discussion. Data supporting proof-of-principle for a switchable AuNP-system using far-field MPM are presented in Figure 2, together with an illustration of the concept. Aggregation of the JR2EC functionalized AuNPs (JR2EC-AuNPs), triggered by the addition of $Zn^{2+}$, causes a red shift of the LSPR band of the AuNPs from 525 to around 630 nm as visualized in the one-photon extinction spectrum (Figure 2b). This shift has earlier been demonstrated to be the result of a specific and folding mediated bridging aggregation of the particles, caused by the zinc-triggered folding and homodimerization of the immobilized peptides.\textsuperscript{28–30,36–38} Both absorption and scattering contributes to the extinction spectrum; however, as the scattering component for small particles is small, the predominant contribution to the spectra will be due to particle absorption, which also agrees with the acquisition of MIL. The maximum peak shift was obtained about 60 min after addition of $Zn^{2+}$. In addition to the shift of the LSPR band, a spectral broadening was observed, as expected. This shift leads to a more than 5-fold increase in absorption in the NIR spectral region, as shown in Figure 2c. As previously demonstrated for spherical AuNPs deposited on glass substrates,\textsuperscript{25} this enhanced absorption in the NIR region significantly elevates the possibility for generating MIL. The hypothesis is confirmed (Figure 2d,e), by investigating the system in the MPM. The figure shows three-dimensional (3D) volume images of particles in dispersed (Figure 2d) and aggregated (Figure 2e) states, that is, before and after addition of $Zn^{2+}$, respectively (see also Supplementary Video S1). After addition of $Zn^{2+}$ solution, bright particle clusters appear in the field of view. No signal distinguishable from noise could be seen for the dispersed JR2EC-AuNPs nor for a solution containing only 30 mM Bis-Tris (pH 7.0) buffer and 5 mM $Zn^{2+}$. The addition of NaOH to the $Zn^{2+}$ buffer, causing precipitation of $Zn(OH)_{2}$, did not elevate the signal at experimental conditions supporting the observation is purely due to aggregation of AuNPs.

The observed effect could effectively be reversed by complexation of $Zn^{2+}$ by EDTA (Figure 2f). By consecutive additions of $Zn^{2+}$ and EDTA in excess, the MIL signal could be repeatedly switched on and off, indicating that appropriately functionalized AuNPs can be used to probe the biochemical environment noninvasively in far-field MPM. This clearly demonstrates the relation between proximity effect and MIL. The effect is understood based on the fact that particle aggregation causes plasmonic coupling and shift of the LSPR band, which enhances the absorption cross-section in the NIR range, thereby elevating the probability of the sequential two-photon absorption required to generate MIL, as previously demonstrated.\textsuperscript{25} This agrees with the theory of MIL being based on a sequential two-photon absorption where the cross-section of the first intraband transition, $\sigma_{sp-sp}$, is driven by LSPR. It further confirms the conclusion that spherical AuNPs in this size range have to be clustered in order to be visualized in far-field MPM.

In addition to the responsiveness to $Zn^{2+}$, the MIL signal was also significantly elevated after addition of matrix metalloproteinase matrilysin-7 (MMP-7), as demonstrated by Figure 3. Matrix metalloproteinases are known to play important roles in cancer progression\textsuperscript{31,32} and for inflammatory processes.\textsuperscript{33} The JR2EC-AuNP system has been introduced as a concept for colorimetric detection of MMP-7 based on the fact that the enzyme digests the immobilized peptide, reducing the electrostatic stabilization of the nanoparticles, which result in aggregation.\textsuperscript{36} As shown by the figure, the presence of MMP-7 generates a 2-fold increase in MIL signal compared to the control buffer. Even though the elevated MIL signal is not as pronounced as after the addition of $Zn^{2+}$, the experiment demonstrates proof-of-principle that MIL can be applied as a sensitive readout of enzyme activity and that particle clustering can provide a novel approach for noninvasive biomolecular recognition using MPM.

It should also be noted that background signal in Figure 3 acquired from the particles in the PBS buffer was higher, compared to the Bis-Tri buffer used for the $Zn^{2+}$-experiments. This effect is most likely attributed to the higher ionic strength of PBS, which causes the particles JR2EC-AuNPs to aggregate to a small extent. Interestingly, this effect has not previously been observed in absorption measurements,\textsuperscript{36} thus further supporting the high sensitivity of MIL to detect presence of
aggregating particles. This effect is also important to consider in future work, moving in the direction of biomedical experiments as physiological conditions are required.

The addition of Zn²⁺ to JR2EC-AuNPs causes the formation of larger aggregates that eventually precipitates. This effect is observed in Figure 2e as the brighter area of the bottom of the 3D volume, corresponding to a layer of deposited particle clusters. When investigating the particles that had precipitated further, an increase in signal was observed during imaging, as shown by Figure 4 and Supplemental Video S2. The figure demonstrates a series of MPM images acquired from sedimented particles after sequential exposures. The MIL signal from a pre-exposed region of the surface is significantly elevated as compared to the unexposed region. After repeated exposures using a 2.5 mW average laser power and a 500 ms exposure time over the scan area, the MIL from the particles increases until reaching a saturated level. This effect was most predominant for the sedimented particle aggregates but could also be observed for the clusters in suspension (data not shown). Because the signal reaches a saturation level, it cannot simply be explained by increased deposition of particles. A more plausible explanation to the signal increase is the possibility that the femtosecond-laser NIR pulses causes irreversible changes to the particles after repeated exposures, which are further investigated below.

To investigate the elevated MIL signal from repeated exposure of sedimented clusters of AuNPs, the particles were deposited on carbon-coated TEM grids and imaged in TEM after exposure in the MPM. As shown in Figure 5, the pre-exposed particles are separated corresponding to the thickness of the peptide layer (Figure 5a,d). After low exposure by femtosecond-pulsed NIR laser light in the MIL, the particle spacing is reduced (Figure 5b,c). The small interparticle separation of the repeatedly exposed aggregates indicates that the peptide may have unfolded due to local plasmonic heating or desorbed during exposure, further increasing the red shift of the LSPR-band. The particles were seen to coalesce, forming larger particles of a size of approximately 100 nm in diameter, likely as a result of Ostwald ripening. Eventually, after repeated laser-light exposure the AuNPs are subject to irreversible morphological changes (Figure 5c,d). Indeed particle reshaping due to femtosecond exposure has recently been reported for gold nanorods. The average energy deposited to each particle cluster can be estimated to 1 pJ. The approximated energy required to melt a gold nanorod with an average diameter of 210 nm is 1.6 fJ, based on earlier reports. Thus, the deposited energy after repeated exposure is likely to induce irreversible changes due to melting. The larger particles formed exhibit an increased absorption in the NIR region due to the reduced interparticle separation, which in turn give rise to higher levels of MIL, ultimately reaching a saturation level as observed in Figure 4. This effect was opposite to the “photobleaching” observed in our previous work. The major difference from the previous study was that here the particles were dispersed, while previously the AuNPs were immobilized on a substrate. The mobility of the AuNPs therefore seems to be of importance for how the particulate systems respond to repeated exposure. Further studies should be done in order to elucidate what importance this effect has in a biological environment, for example, to study particle reorganization on the cellular membrane. Coalescence of AuNPs could potentially be both a benefit and a drawback for in vivo sensing applications. It is beneficial in the respect that an increasing particle size elevates the MIL signal further but is a disadvantage as the reversible switching feature potentially will be lost due to the desorption of the responsive surface coating. In addition, the elevated absorption should be an advantage for treatment purposes, as highly confined particle heating could be used as a tool to stimulate biochemical reactions or, more drastically, to kill cells with high precision. Thus, these observations are of importance for the future development.

Taken together, this Letter demonstrates proof-of-concept for using functionalized AuNPs as a stimuli responsive contrast media sensitive to changes in the local environment, here demonstrated using a Zn²⁺ and MMP-7 responsive peptide, in combination with MPM. The nanoparticulate system consists of AuNPs functionalized with a synthetic helix–loop–helix

Figure 3. Intensity of MIL from JR2EC-AuNPs before and after addition of Zn²⁺ or MMP7. Buffers were 30 mM Bis-Tris (pH 7.0) and 10 mM PBS (pH 7.4) for the two experiments, respectively. MIL signal was measured after 2 h for the different experiments and here represented as the mean over the full acquired z-stack. Error bars show standard error of mean.

Figure 4. MPM images of deposited JR2EC-AuNPs in aggregated state after addition of Zn²⁺ after repeated exposure. A pre-exposed region is visible in the right corner. Images show evolution of MIL after repeated exposure (from left to right). Field of view: 424 × 424 μm.
peptide designed to coordinate Zn$^{2+}$ and dimerize and fold into four-helix bundles. It was demonstrated that the MIL signal is strongly related to the aggregation state of the particles, mediated by the dimerization and folding of the immobilized peptides modulated by Zn$^{2+}$ concentration or MMP-7 activity. The onset of MIL could be reversed by complexation of Zn$^{2+}$, demonstrating proof-of-principle of a metal-ion responsive sensor allowing for biochemically tuning MIL. In addition, the MIL signal was found to be elevated after repeated exposure, taking place in two stages of structural change, that is, (i) decreased interparticle distance because of unfolding or desorption of the immobilized peptides, and (ii) thermally induced particle ripening. Both of these effects will contribute to a shift of the LSPR band toward longer wavelengths. Because of the biocompatibility of AuNPs and the fact that MPM operates in the NIR optical window of biological tissue, this type of system holds promise for future applications for noninvasive biosensing. Important fields where the concept could be applied are neural sensing, protein-protein interactions, and tumor diagnostics. By tuning the particle aggregation, the MIL can be utilized as a highly sensitive readout. Because of the sensitivity of the approach, careful design of the functionalization is required in order to target the desired purposes. The concept can be further explored as contrast mediator providing high sensitivity and specificity as well as high signal-to-noise ratios, for example, allowing for detection of local biochemical changes in subcellular environments, opening up a new window toward noninvasive optical biosensing in combination with MPM.

**Methods.** All chemicals were obtained from Sigma-Aldrich (Stockholm, Sweden) if not otherwise stated. The polypeptide JR2EC (H2N-NAADLEKAIEALEKHLEAKGPCDAAQLEK-QLEQAFEAFERAG-COOH) was synthesized on an automated peptide synthesizer (Protein Technologies, Inc.) using standard fluorenylmethoxy carbonyl (Fmoc) chemistry as described earlier. Briefly, the synthesis was carried out in a 0.1 mmol scale using Fmoc-Gly-Wang (Iris biotech Gmbh) as solid support. Each coupling was performed using a 4-fold excess of amino acid (Iris biotech Gmbh) with O-(7-benzotriazole-1-yl)-1,1,3,3-tetramethyuronium tetrafluoroborate (Iris biotech Gmbh) as activator and diisopropylamine (Iris biotech Gmbh) as base. Deprotection of the coupled amino acids was accomplished by treatment with piperidine in dimethylformamide (20%, Applied Biosystems). The resulting resin-bound peptide was side chain deprotection and cleavage using a solution containing trifluoroacetic acid (TFA)/ethandithiol/water/triisopropylsilane (94:2.5:2.5:1) for 2 h followed by filtration and evaporation of the solvent. The crude peptide was precipitated twice in cold diethyl ether and then lyophilized. Purification of the crude peptide was carried out using a 30 min gradient of 30–41% aqueous isopropanol with 0.1% TFA on an ACE 5 C-8 column (250 × 21.2 mm) attached to a semipreparative HPLC system (Dionex). The identity of the purified peptide was confirmed by MALDI-TOF MS.

Spherical AuNPs (20 nm, 0.06 mg/mL, BB International) were functionalized with the thiol-containing JR2EC peptide by incubating the AuNPs with 10 μM JR2EC (10 mM sodium citrate, pH 6) overnight at 4 °C. The AuNPs were then repeatedly centrifuged and the supernatant was exchanged for Bis-Tris buffer (2,2-Bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol, 30 mM, pH 7.0) and resuspended to a final concentration of 25 nM, calculated using the absorbance at 526 nm and an extinction coefficient of 8.78 × 10^3. The particles were diluted to 0.25 nM in 30 mM Bis-Tris buffer for analysis with UV−vis and MPM.

Extinction spectra were acquired using a Cary 50 UV−vis spectrometer (Agilent Technologies). AuNPs (0.25 nM) were dispersed in 30 mM Bis-Tris buffer (pH 7.0) containing 5 mM Zn$^{2+}$. Spectra were recorded every minute for 60 min over the range 400–800 nm.
MMP was performed using two different microscope systems. For particle imaging, a commercial upright LSM 710 NLO set up (Carl Zeiss MicroImaging GmbH, Germany) was utilized. The system was equipped with a Mai Tai DeepSee tunable NIR Ti:Sapphire femtosecond-laser (Spectra-Physics, Newport Corporation, U.S.A.) with a repetition rate of 80 MHz and a W Plan-ApoChromat 20×/1.0 DIC M27 75 mm objective lens. The laser wavelength was set to 800 nm, and the average power as measured at the back aperture of the objective lens was 2.5 mW. The detection was obtained between 415 and 735 nm. The suspensions with JR2EC-AuNPs (0.25 nM) or JR2EC-AuNPs (0.25 nM) with Zn2+ (5 mM) added were placed in custom-built imaging chambers, consisting of two cover glasses mounted on a metal microscopy slide. An additional experiment was performed where JR2EC-AuNPs dissolved in 10 mM PBS (pH 7.4) were exposed to MMP7 (2 uM). In four samples, where particles were aggregated by addition of Zn2+, the imaging chambers also contained a transmission electron microscopy (TEM)-grid onto which the aggregated particles were allowed to sediment. Image acquisition was done with ZEN 2011 (Carl Zeiss) using line scanning over an area of 424 μm × 424 μm. The samples were imaged at several locations, keeping imaging conditions constant for all samples. Both time series and volume stacks were acquired.

For signal switching using Zn2+ and EDTA, an experimental inverted MPM setup was applied, facilitating access to sample during acquisition. In brief, this system consisted of a Tsunami tunable NIR Ti:sapphire femtosecond-laser operating at 800 nm, 80 MHz repetition rate and at approximately 15 mW average power, measured at the back aperture, and a scan area of 424 μm × 424 μm. The sample was scanned after the addition of Zn2+ (5 mM), and then repeatedly after alternating addition of EDTA (10 mM) and Zn2+ (5 mM), choosing the volumes to ensure 1:1 ratio of Zn2+ and EDTA after each Zn2+-EDTA cycle. Detection was made using two channels with band-pass filters (525/50 and 580/150) by photomultiplier tubes (H7422P-40 MOD, Hamamatsu). Data were acquired by time-correlated single photon counting using a SPCI50 card (Becker-Hickl, Germany).

For the TEM-grid samples, MPM exposure was obtained using the LSM 710 NLO setup, operating at 2.5 mW measured at the back aperture, and a scan area of 424 μm × 424 μm for 500 ms. Each exposure corresponded to a deposited light dose of 685 mJ/cm². Exposure was repeated up to 10 times. After MPM, the TEM-grams were dismounted from the imaging chambers and examined using TEM (Technai G2 F20U Twin microscope, running at 200 kV) at 9500×. Both exposed and unexposed particles were imaged.

ASSOCIATED CONTENT
2 Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.7b00611.

Full descriptions of Videos S-1 and S-2 (PDF)
Rendering of z-stacks of JR2EC-AuNPs after and before addition of Zn2+ (MOV)

AUTHOR INFORMATION
Corresponding Authors
*E-mail: marica.ericson@chem.gu.se.
*E-mail: daniel.aili@liu.se.

ORCID
Marica B. Ericson: 0000-0002-5987-5915

Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding
Financial support was obtained from the Swedish Research Council (621-2011-5189), the Foundation for Strategic Research (ICA10-0002), and Swedish Government Strategic Research Area in Materials Science on Functional Materials at Linköping University (Faculty Grant SFO Mat LiU No 20090971).

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
The authors would like to acknowledge the Centre for Cellular Imaging, University of Gothenburg, for use of imaging equipment and the kind support from the staff. We also thank the Centre for Skin Research (SkinResQU), Gothenburg, for use of infrastructure.

ABBREVIATIONS
AuNPs, spherical gold nanoparticles; EDTA, ethylenediaminetetraacetic acid, JR2EC, de novo synthesized peptide; LSPR, localized surface plasmon resonance; MIL, multiphoton-induced luminescence; MPM, multiphoton laser scanning microscopy; NIR, near-infrared; TEM, transmission electron microscopy

REFERENCES