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Rationally designed Raman dyes for multiplexing with SERRS nanotags

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Surface-enhanced resonance Raman scattering (SERRS) is widely used in nanodiagnostics for the selective detection of biomolecules with nanotags conjugated to target-specific ligands. SERRS nanotags comprise Raman dyes adsorbed on the surface of noble metal nanoparticles (NPs). The brightness of SERRS nanotags/labels depends on the scattering cross section of both the plasmonic nanoparticle and the Raman reporter molecule. Optimum Raman dyes exhibit high scattering cross sections and possess surface-seeking groups such as amines or thiols for chemisorption onto the NP surface.

For ultrabright SERRS labels we use Raman dyes with large scattering cross sections like Rhodamine. Bright and photostable fluorescent dyes are modified by the introduction of thiol groups on one side for attachment on the NP surface and on the other side by the introduction of carboxylic acid groups for bioconjugation (Fig.1 A).

We have investigated the SERRS signal of our rationally designed Raman dyes adsorbed on gold nanostars in colloidal suspension (Fig.1 B).

The introduction of deuterium atoms at selected carbon atoms of the chromophore offers exciting opportunities for sensitive and multiplexed biomedical nanodiagnostics with SERRS.

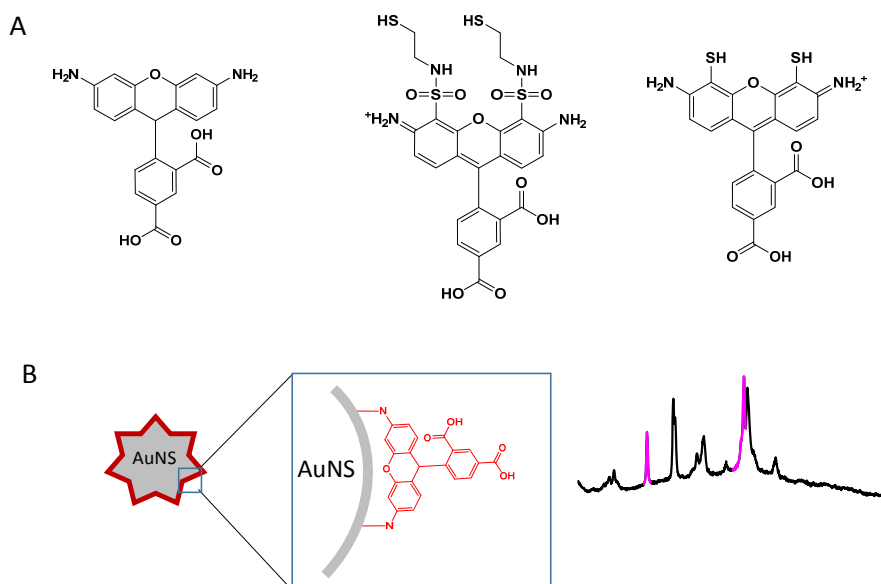


Figure 1: A: Rationally designed Raman dyes. B: Detection of SERRS from Rhodamine dye adsorbed on the surface of gold nanostar

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Nanoparticle-peptide conjugates are used as model system for in vitro bioapplications considering precise and quantitative charge balancing

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Bioconjugated gold nanoparticles (AuNPs) are used in medicine, e.g. for bioimaging^[1]. In cell selective imaging, nanoparticles have to be translocated across the plasma membrane and furthermore should be spectroscopically detectable inside the cell. This detection based on scattering is only possible for species >60 nm^[2]. This can be managed by a design based on the bioconjugation of small particles with cell penetrating peptides (CPPs)^[3] that induce the formation of agglomerates with high extinction cross sections.

This work focuses on charge effects between nanoparticles and oppositely charged ligands. Small monodisperse AuNPs were generated by pulsed laser ablation in liquid providing a bare surface, free from any surfactants. Particles were *ex situ* conjugated with different types of CPPs and subsequently investigated concerning their aggregation tendencies and long-term stabilities. This model system enables the examination of effects like charge compensation depending on the peptide's charge and length. Due to charge balancing effects, defined agglomerates may be synthesized. For future bioapplications, the time and concentration dependent endosomal uptake of these agglomerates was monitored in cell culture experiments. Furthermore, the controlled laser-induced rupture of endosomes loaded with plasmonic AuNPs could be used as an efficient drug release system.

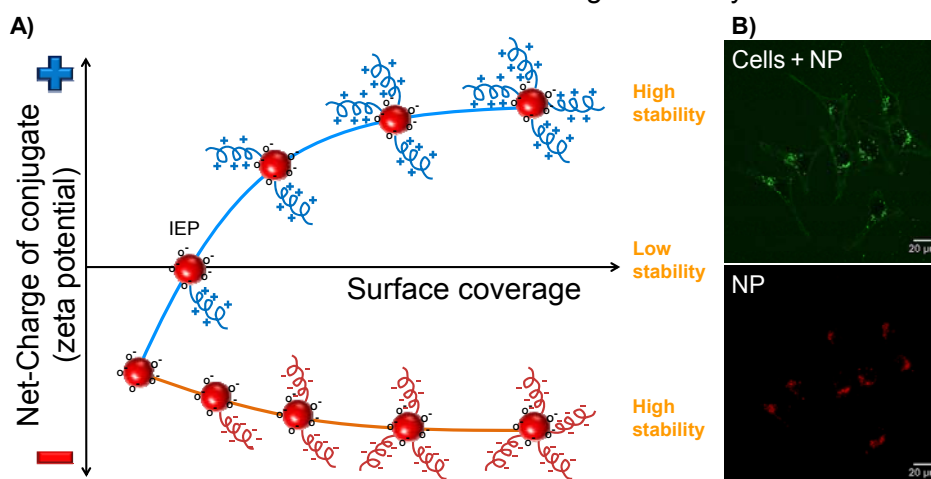


Figure 1: A) Scheme of dependence of resulting net-charge and stability of conjugates due to the charge of utilized peptides. B) Multiphoton-microscopy images of cells treated with AuNP-CWR₁₀-conjugates.^[3]

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A photochromic bacterial photoreceptor for superresolution microscopy

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Biological photoreceptors serve as converters of information obtained from incident light into a biological signal, allowing the organism to adapt to environmental conditions. Many blue light sensing photoreceptors are based on flavins as chromophores. Besides their role in biological signaling, flavins also provide a strong fluorescence. The photoreceptor protein YtvA from *Bacillus subtilis* has a flavin-based photochemistry and regulates the stress response of this bacterium. Whereas the parental state is strongly fluorescent, the signaling state shows practically no fluorescence, but can be re-converted into the parental state by selective irradiation. This property allows using YtvA as a switchable fluorescence marker even in living cells. Its application in FPALM yields a resolution of ca. 33 nm. As flavin-binding photoreceptors are generated independent of oxygen (in contrast to GFP derivatives in which chromophore formation is oxygen dependent) they provide an alternative to the frequently used GFP derived fluorescent proteins.

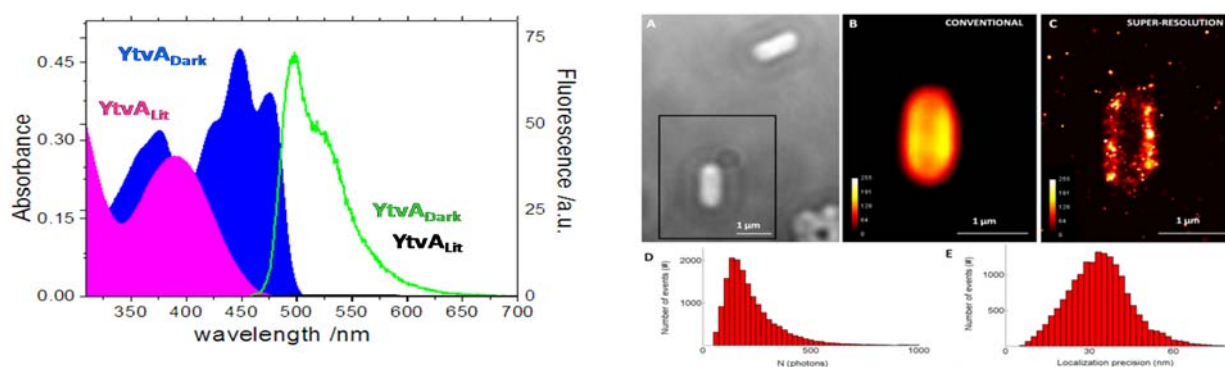


Fig. 1: Left, Photocycle of YtvA; blue, green: absorbance and fluorescence emission spectrum of the parental state (YtvA_{Dark}), magenta: absorbance of the signaling photoproduct state (YtvA_{Lit}). Right, microscopy of *E. coli* cells expressing YtvA; A, light microscopy, B, normal fluorescence microscopy, C, FPALM microscopy, D, E, relative photon count and histogram for optical resolution determination.

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Laser-generated alloy nanoparticles for biological and biomedical applications

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The versatile use of the alloys in biology and medicine has arisen many years ago as alloying allows a combination of desirable properties in one material and the alloy may significantly differ from its components. While this is common knowledge in bulk materials, similar tendencies can be observed, when alloy materials are reduced to a nanoscopic scale. The most prominent feature in this context are optical properties, which are relevant e.g. for bioimaging. The prominent example is the system AgAu, where the plasmonic properties are directly linked to the material's composition.

Synthesis of alloy nanoparticles by pulsed laser ablation in liquids (PLAL) is a promising method to obtain alloy nanoparticles with controlled homogeneous elemental distributions (solid solution), which are currently very difficult to obtain via chemical synthesis (Figure 1A)¹. These materials can be useful in imaging applications, e.g. as detection components in lateral-flow assays where their enhanced extinction cross sections may significantly boost sensitivity in comparison to pure gold standards. Furthermore, risk assessment of alloy nanoparticles is a topic of great public concern. In this context we could recently show that nanotoxicity of AgAu alloy nanoparticles is ruled by the particle's composition, which was verified for gametes (Figure 1B)², as well as for bacteria and mammalian cell lines³.

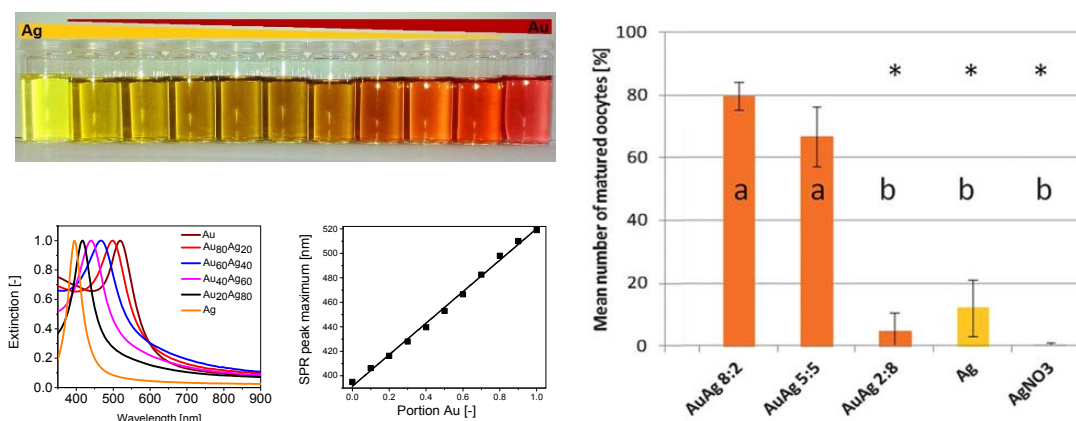


Fig. 1: A) Optical properties of laser-generated Ag-Au solid solution alloy nanoparticles B) Composition dependent toxicity of AgAu alloy nanoparticles in oocyte maturation

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Using functionalized core-shell nanoparticles for optical passivation

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Plasmonic nanoparticles (p-NPs) have various applications in sensing, molecular diagnostic imaging, Raman spectroscopy, and photovoltaics through both scattering and plasmonic effects. Such p-NPs are chemically active or introduce non-radiative decay channels [1] for neighbouring molecules as well as a temperature rise in the host medium; hence their presence is capable to alter the performance of the overall system [2]. Adding a shell with an optimized thickness can «passivate» the p-NPs without deteriorating scattering and plasmonic effects, while enabling both a reduced absorption and the inhibition of non-radiative decay channels [3]. The performance of functionalized core-shell p-NPs is studied in the context of a thin-film solar cell where we introduced Ag-SiO₂ core-shells p-NPs (core \square : 50nm; shell: 10nm) into a 200nm thick c-Si active layer with a 55nm Si₃N₄ layer on top acting as an anti-reflection coating.

For the proper analysis we used the finite-element-method (FEM) based simulation platform COMSOL MultiphysicsTM together with revised Mie calculations to compute the spectral response of the p-NP's respective the active layer's absorption yielding reliable data to validate the impact of the functionalized core-shell p-NPs on the surrounding medium. Fig.1(a) depicts the plasmon resonance at 435nm that is properly confined to the p-NP's 10nm SiO₂ shell, leaving the host medium virtually unaffected. The influence of the shell on the absorption inside the p-NP is shown in Fig.1(b), which results in a 58% reduction of the spectrally integrated absorption for the core-shell NP (with higher absorption in the c-Si host). Fig.1(c) displays the absorption data for the surrounding c-Si material yielding lower absorption at short wavelengths (due to field localization in the shell) and an increased aggregate absorption for the 650–800nm range. Hence, optical passivation of p-NPs offers control over location and strength of the absorption as well as the preservation of the plasmonic effect.

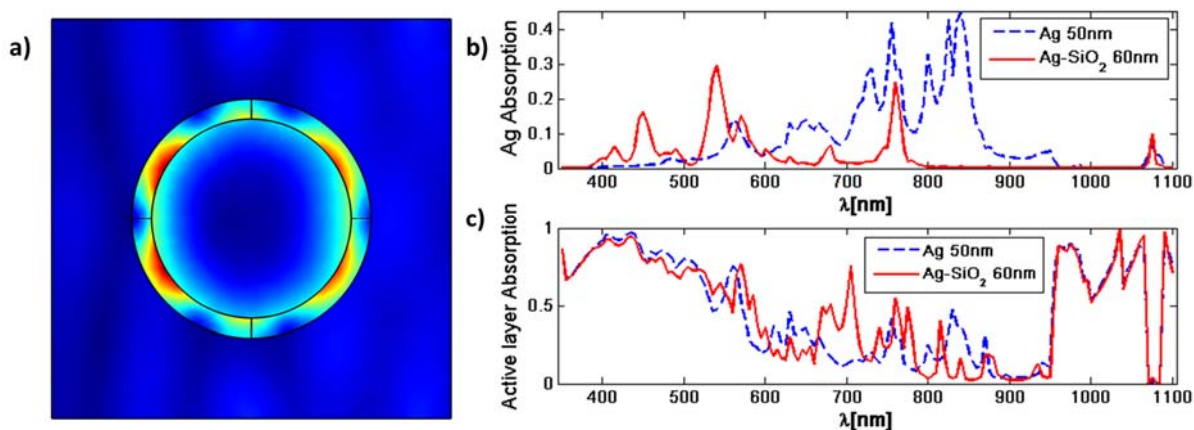


Fig. 1: (a) Distribution of the electric field strength at 435nm; (b) absorption inside the Ag core without and with 10nm SiO₂ shell; (c) absorption in the surrounding c-Si active layer of the solar cell without and with 10nm SiO₂ shell.

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Integrated approach to design fluorescent calcium phosphate nanoparticles for *in vivo* imaging and photodynamic therapy

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Application of nanotechnology for the biomedical diagnosis and treatment has the potential to enhance conventional methods as well as to develop multiplex approaches for simultaneous detection and therapy. Among the inorganic nanoparticles calcium phosphate has been frequently utilized as carrier of active biological molecules or fluorescent compounds for cellular delivery and treatment (1). The modification of nanoparticles with a silica shell enables the covalent attachment of targeting moieties (2). The targeting ability of nanoparticles can be analyzed *in vitro* and *in vivo*, typically by making them fluorescent (**Fig. 1**). Near infrared fluorescence (NIRF) imaging has attracted much interest due to its noninvasive, highly sensitive and real-time imaging features. Silica-modified calcium phosphate nanoparticles were labelled with the NIR dye DY-682, loaded with the photosensitizer mTHPC and finally conjugated with the targeting peptide RGDfK. The behavior of multifunctional nanoparticles was evaluated in a tumor-bearing mouse using NIRF imaging. The therapeutic efficacy of the particles after PTD treatment was analyzed *in vivo* by detecting apoptosis and tumor vascularization (3).

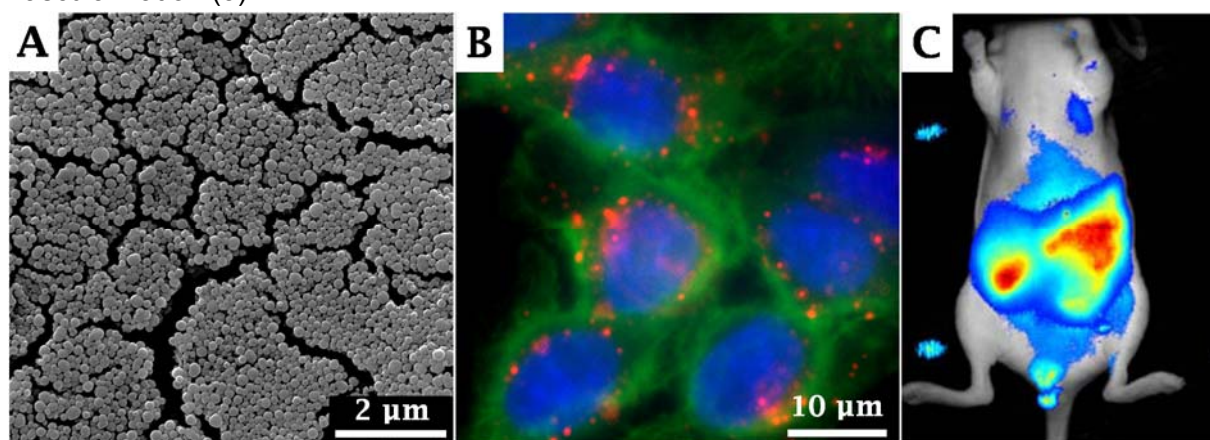


Fig. 1: SEM-image of calcium phosphate nanoparticles (**A**). Detection of fluorescent nanoparticles *in vitro* (**B**) and *in vivo* (**C**).

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Autofluorescent ultra-small silver-gold nanoparticles for cellular uptake studies

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Metal nanoparticles have received considerable attention due to their attractive chemical and physical properties and their potential applications in the biomedical sciences and engineering. Especially gold and silver nanoparticles have led to their utilization in a number of very important applications including diagnostic imaging, cancer diagnosis and therapy, and as antibacterial agents (silver) (1). Recently, ultra-small noble metal nanoparticles (diameters of two nanometers and below), also denoted as clusters, have gained special interest. These ultra-small nanoparticles show molecular-like properties, for example autofluorescence, making them highly useful in analytical science, cellular and biological imaging (2, 3). Functionalized silver-gold nanoparticles were obtained in a one-pot synthesis from aqueous medium. Carboxylic acid-functionalized silver-gold nanoparticles were prepared with nine different metal compositions with a diameter up to 2 nm (**Fig. 1A**). Ultra-small bimetallic nanoparticles with a silver content up to 60 : 40 showed autofluorescence with a large Stokes shift of about 250–300 nm. The intracellular localization of fluorescent bimetallic silver–gold nanoparticles was studied in HeLa cells by confocal laser scanning microscopy (CLSM) (**Fig. 1B**). Toxicity studies showed that the silver–gold nanoparticles are not cytotoxic up to a metal concentration of 5 $\mu\text{g mL}^{-1}$ after 24 h of incubation.

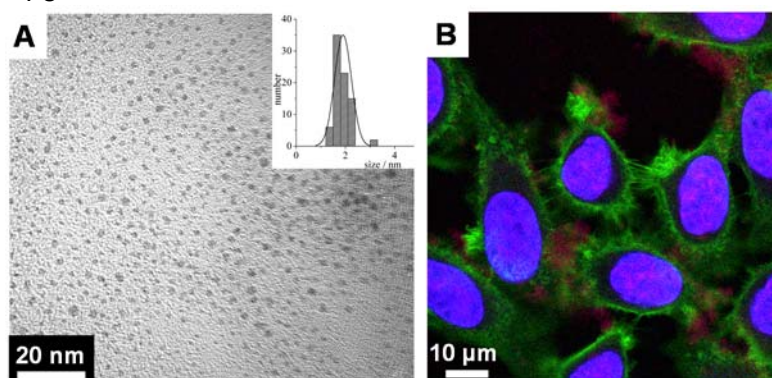


Fig. 1: TEM-image of silver-gold (30:70) nanoparticles (**A**) and CLSM-image of HeLa cells after incubation with fluorescent ultra-small silver-gold nanoparticles (**B**).

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Change of Particle Properties by Laser Irradiation for Catalytic Applications

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The growing interest in nanomaterials has raised many syntheses routes for the controlled generation of nanostructures and particles. The technique of laser-generated nanoparticles bares significant advantages as no strong oxidation and reduction agents are necessary (as for classical bottom-up synthesis) and no abrasion is generated (like in physical grinding processes). The laser generation of nanoparticles in liquids can be distinguished in two approaches. When an intense laser beam is focused onto a bulk material plate the material is vaporized and captured in the surrounding liquid. This pulsed laser ablation in liquids (PLAL) differs from the technique of pulsed laser fragmentation in liquids (PLFL) where suspended microparticles are formed into nanoparticles. Beside the simple change of particle size PLFL enables to change particle properties like e.g. the bandgap energy [1] or chemical composition [2]. Further, stable and ultra-small gold nanoparticles can be obtained when PLFL is carried out in presence of an oxidative reagent stabilizing the particles [3]. These ultra-small nanoparticles can be transferred to graphene nano sheets and form a potential catalyst. Another method to change particle size in an opposite way to PLFL can be achieved by laser irradiation below the fragmentation threshold [3, 4]. This pulsed laser melting in liquid (PLML) leads to sub-micrometer spheres formed by aggregates of joint primary particles. We will present how the different laser irradiation techniques of particle suspensions can lead to micro/nano hybrid materials [4] and how they can be used in heterogeneous catalysis.

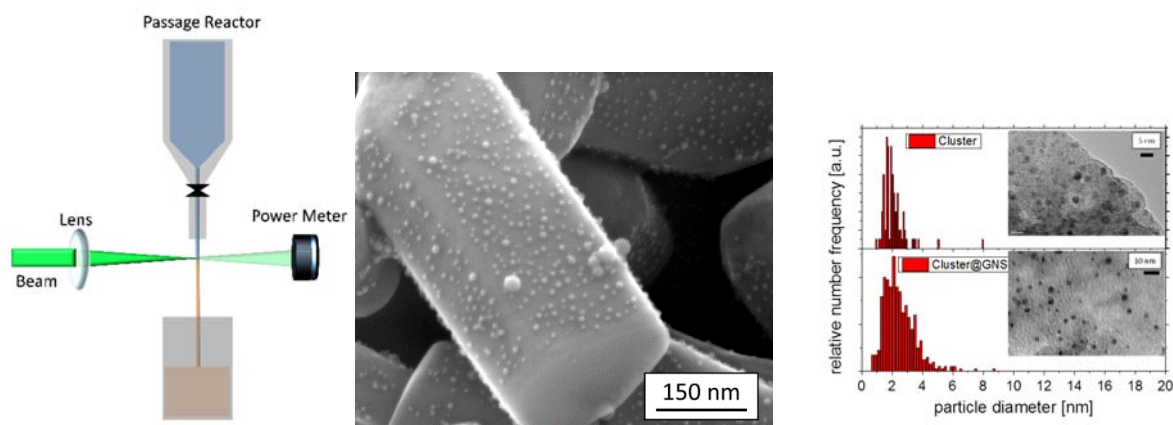


Figure: Schematic illustration of experimental design for laser fragmentation and pulsed laser melting (left) [1], micro/nano hybrid material (middle) [4] and ultra-small gold nanoparticles transferred to graphene nano sheets [3]

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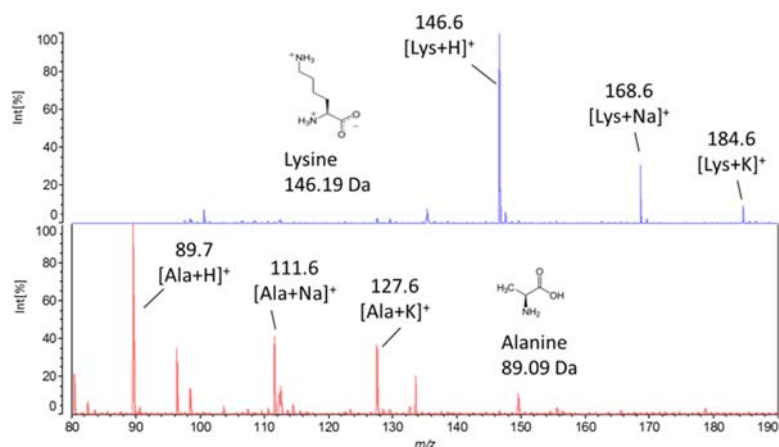
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LDI-MS with Gold Nanoparticles

Duxin Li, Ti Hau Bui, Susanne Stephan, Stephan Barcikowski, Oliver J. Schmitz

Laser desorption ionization mass spectrometry (LDI) is a powerful technique for the analysis of a variety of substances like organic polymers, proteins or carbohydrates. The use of organic matrices in matrix assisted laser desorption ionization (MALDI) allows the ionization of molecules up to 1,000,000 Da. Problems may occur in a mass range below 500 m/z because common MALDI matrices form fragment ions which result in intensive background signals in this lower mass range. A lower background and therefore better results for analytes below 500 m/z can be received using gold nanoparticles (AuNPs) instead of a conventional organic matrix. In this project the applicability of laser generated gold nanoparticles, ligand-free or stabilized with different ligands, for the AuNPs-LDI analysis of a variety of compounds like carbohydrates, amino acids and mycotoxins was tested.

A simple sample preparation technique spotting a layer of sample solution onto a dried layer of gold nanoparticles emerged to produce the best results. The ligand-free gold nanoparticles agglomerate faster than the ones stabilized with ligands like lipoic acid, but show the lowest background and result in the best mass spectra for most of the compounds.



▲ Fig.1: AuNPs-LDI mass spectrum of the amino acids lysine and alanine.

Different monosaccharides with a molar mass of 180.16 Da like glucose and mannose could be detected as [M+Na]⁺ and [M+K]⁺. Amino acids like lysine and alanine even showed peaks for [M+H]⁺, [M+Na]⁺ and [M+K]⁺.

Furthermore it was possible to detect the mycotoxins Aflatoxin B1 and Ochratoxin A and steroid structure cortisol, which are all in the mass range of 300 to 400 Da.

Further steps will be to produce complete salt free laser generated gold nanoparticles to determine the applicability of this technique for peptides and proteins.

The Effect of CLPFFD-functionalized Hollow Gold Nanospheres on β -Amyloid Fibril Formation

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Amyloidogenesis has a devastating role in neurodegenerative diseases such as Alzheimer. Therefore, the development of new methods to inhibit the aggregation process of peptides into β -amyloid fibrils, the deposition of such fibrils in the extracellular matrix of the brain and the dissolution of already formed β -amyloid aggregates would be highly desirable. One promising approach to inhibit the β -amyloid fibril formation is the use of specific peptides containing the LPFFD sequence that attaches selectively to the amyloidogenic $A\beta_{1-42}$ structures of the fibrils and thereby affects their aggregation behavior.

Recently, inhibition of β -amyloid fibril aggregation and dissolution of formed β -amyloid aggregates has been demonstrated by means CLPFFD functionalized 15 nm solid gold nanoparticles upon weak microwave irradiation. However, with respect to *in vivo* application near infrared irradiation in the optical window of biological tissues would be favorable. Therefore, hollow gold nanospheres (HAuNS) exhibiting optical absorbance maximum between 600 and 900 nm are considered promising.

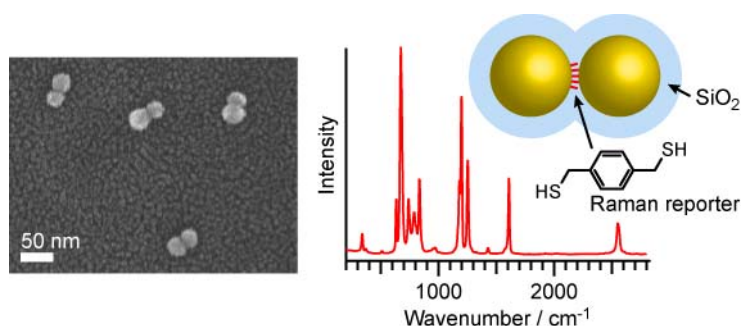
In this work we report the synthesis of HAuNS having a diameter of 36 nm, a shell thickness of 5 nm and an absorbance maximum at 855 nm. HAuNS were functionalized with the CLPFFD peptide in two different ways: a direct functionalization (HAuNS-CLPFFD) and a functionalization on previously PEGylated particles (HAuNS-PEG-CLPFFD). The binding affinity to β -amyloid fibrils and the positive influence on the selective fibril dissolution will be discussed.

Molecularly Bridged Noble Metal Nanoparticle Dimers for Nanodiagnostics by SERS

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The excitation of localised surface plasmons in metallic nanoparticles (NPs) leads to high local electric fields on the surface of the NP. Extremely high electric fields enhancement, so called hot spots, occur in the gap between two closely spaced NPs (dimer). This can be exploited for surface-enhanced Raman scattering (SERS) of molecules which are present in the hot spot. Raman reporter molecules with two surface-seeking groups such as aromatic dithiols can induce the formation of NP dimers/clusters. After bioconjugation to target-specific ligands such molecularly bridged noble metal NP dimers and NP clusters can be employed for highly sensitive nanodiagnostics. We have developed an efficient and selective route to prepare SERS NPs dimers in high yield using a solid support-assisted approach (figure).^[1] The physico-chemical characterization of these metal NP dimers at the single-particle level is correlated with results obtained by transmission electron microscopy, elastic light scattering, and SERS spectroscopy.^[2]



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A Home-Made FRET Sensor Composed of Novel Biological Photoreceptors: Characterization of a LOV(C62S)-1393GAF3 Fusion-Protein and Determination of Intramolecular Fluorescence Resonance Energy Transfer (FRET)

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This artificial fusion protein is composed of two naturally photosensor domains, i.e., the LOV-domain from the bacterial flavo-protein YtvA (*Bacillus subtilis*) and the GAF3-domain from a cyanobacteriochrome *Synechocystis* sp PCC 6803. We used the C62S-mutation in YtvA to convert this protein as constitutively fluorescent. Both domains have been characterized individually^[1,2] and here we demonstrate that these domains from biological photoreceptors show promising potential for fluorescence resonance energy transfer (FRET).

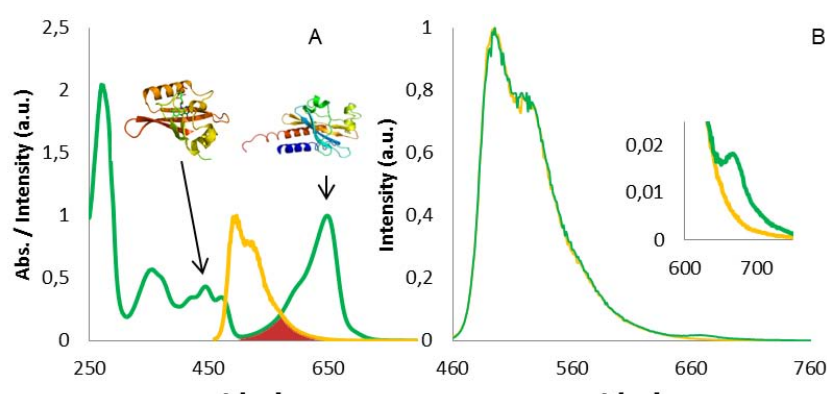


Fig. 1: A: Absorption (green) and fluorescence emission spectrum (yellow) of LOV-GAF_C62S with the GAF domain of slr1393 (GAF domain carries PCB as chromophore), λ_{ex} = 450 nm; red area: Spectral overlap between emission (donor) and absorption spectra (acceptor). B: Emission spectra of LOV-GAF_C62S both with (green) and without (yellow) PCB chromophore in the GAF domain. Three dimensional crystal structures are presented for each of the protein domains

Fehler! Ungültiger Eigenverweis auf Textmarke. A shows the spectral overlap (in red) of the emission spectra of the LOV-domain and the absorption spectrum of the GAF-domain. Fluorescence measurements yielded an increase of 1393GAF specific emission at 670 nm in the fusion protein compared to the emission of the 1393GAF-domain alone (**Fehler! Ungültiger Eigenverweis auf Textmarke.** B, compare green vs. yellow traces).

Calculations^[3] showed a FRET-radius of 17 Å and an efficiency of 78 %. Ongoing experiments will focus on a variation of chromophores in the GAF-domain of the fusion protein. Furthermore, time-resolved fluorescence measurements as well as ongoing attempts to crystallize the fusion protein will reveal further molecular details. We here demonstrate in a test case the capability of both photoreceptor domains for modern fluorescence microscopy applications.

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Model System: Hybrid Fusion Proteins Comprising Natural Phycobilin- and Artificial Chlorophyll-Binding Domains. *J. Am. Chem. Soc.* **135**, 13479-13487.

Title: Analysis of “Neutrophil Extracellular Trap” – associated proteins and structures using super resolution fluorescence microscopy.

Authors: Manuel Stecher, Mike Hasenberg, Anthony Squire, Alexandra Breuer and Matthias Gunzer

Beside phagocytosis the synthesis of Neutrophil Extracellular Traps (NETs) provides Neutrophil Granulocytes with another effective counter measure to oppose various invasive pathogens. These NETs appear as web like structures formed by highly decondensed nucleosomal DNA which is associated with various cytotoxic proteins. They are usually visualized through either electron microscopy or fluorescence microscopy. Until now, neither of them was able to resolve on a sub-diffraction level while marking all observable proteins effectively.

Using the recently developed superresolution microscopy methods (SIM, STED and STORM), which allow resolution way beyond the Abbe-limit of 200 nm, our work aims to clarify the structure of neutrophil extracellular traps on a molecular level. More precisely we take a look at part of the nucleosome complex (histone H1) and the NET associated proteins Myeloperoxidase and Neutrophil Elastase. Therefore we generate either murine and human NETs and mark them with specific monoclonal antibodies coupled with (for each microscopy method) suitable fluorophores to identify and even quantify those protein complexes and their links.

Sample figure:

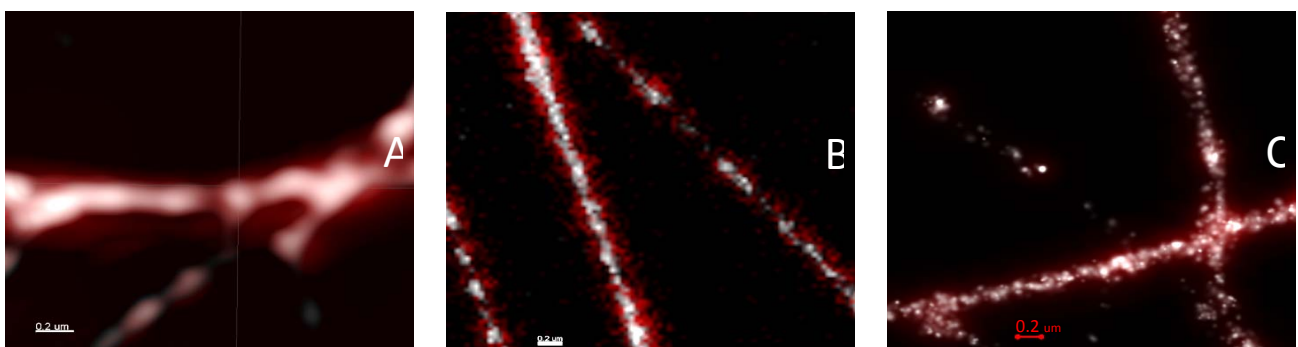


Fig. 1: Resolution comparison of human NET fibers using SI-Microscopy (A), STED-Microscopy (B) and STOR-Microscopy (C).

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Plasmonic gold nanoparticles as platform for biomolecules counteracting protein misfolding diseases

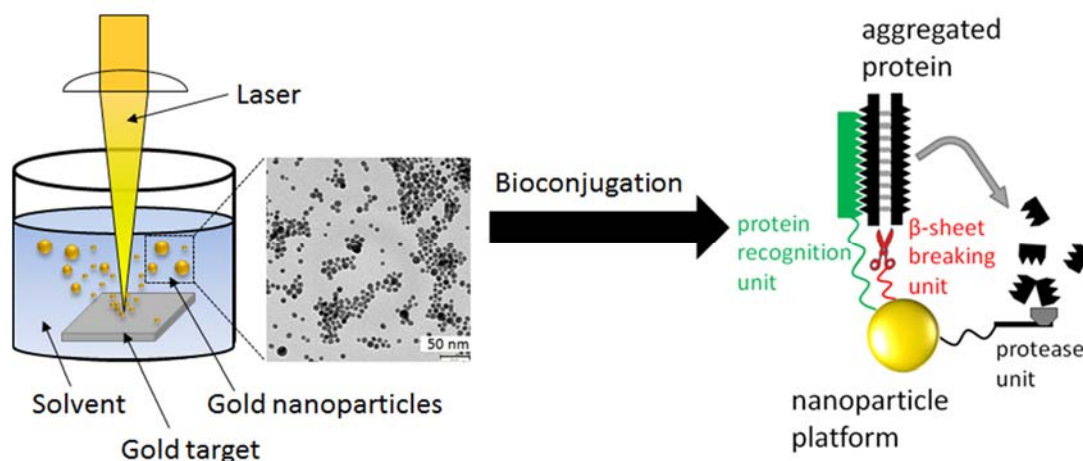
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Many neurodegenerative disorders are associated with protein misfolding. Therefore, the development of new methods to detect, understand and prevent pathological protein aggregation processes is essential. In our approach, tailored nanoparticles are used as organizational platforms and transport vehicles to combine different functional units. These are intended to cooperate synergistically in order to simultaneously perform peptide recognition, beta-sheet breakage and cleavage of the Alzheimer peptide A β [1].

Laser-generated gold nanoparticles were systematically conjugated with A β -specific ligands. Laser ablation in liquid was chosen as fabrication method because it yields completely ligand-free nanoparticles [2], which feature a high purity and surface accessibility for subsequent ligand functionalization. The adsorption of cationic ligands on negatively charged gold nanoparticles resulted in a reversal of the conjugate's zeta potential as a function of ligand charge and ligand concentration.[3] Consecutively, the conjugates were incubated with A β and changes in the surface plasmon resonance peak of the nanogold cores served as indicator for protein binding. Overall, the results show that A β binds to both, ligand-free and ligand-functionalized nanoparticles. However, only functionalized nanoparticles seem to inhibit A β aggregation, whereas non-functionalized nanoparticles do not have an effect. In future, even more effective bi- and trifunctional nanoparticles will be synthesized, which are expected to counteract A β aggregation even in substoichiometric doses.



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Orange fluorescent proteins constructed from cyanobacteriochromes chromophorylated with phycoerythrobilin

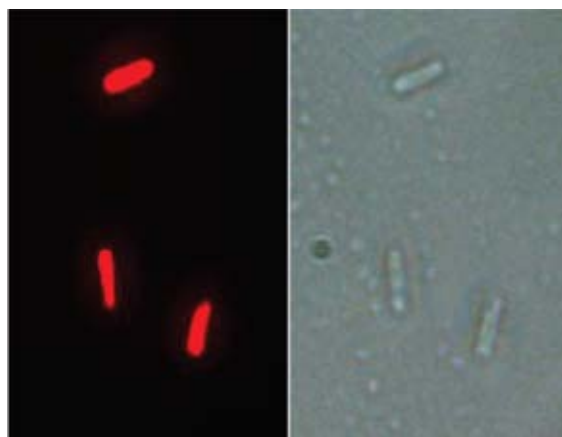
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Cyanobacteriochromes are a structurally and spectrally highly diverse class of phytochrome-related photosensory biliproteins¹. They contain one or more GAF domains that bind phycocyanobilin (PCB) autocatalytically. We tested the chromophorylation with the non-photochromic phycoerythrobilin (PEB) of 16 cyanobacteriochrome GAFs from *Nostoc* sp. PCC 7120, of Slr1393 from *Synechocystis* sp. PCC 6803^{2,3}, and of Tlr0911 from *Thermosynechococcus elongatus* BP-1. Nine GAFs could be autocatalytically chromophorylated in vivo/in *E. coli* with PEB, resulting in highly fluorescent biliproteins with brightness comparable to that of fluorescent proteins like GFP. In several GAFs, PEB was concomitantly converted to phycourobilin (PUB) during binding. This not only shifted the spectra, but also increased the Stokes shift. The chromophorylated GAFs could be oligomerized further by attaching a GCN4 leucine zipper domain, thereby enhancing the absorbance and fluorescence of the complexes. The presence of both PEB and PUB makes these oligomeric GAF-“bundles” interesting models for energy transfer akin to the antenna complexes found in cyanobacterial phycobilisomes. The thermal and photochemical stability and their strong brightness make these constructs promising orange fluorescent biomarkers.

Fig. Fluorescence (left panel) and bright-field (right panel) micrographs of *E. coli* cells expressing PEB-All2699 GAF1. *E. coli* cells expressing only PEB did not fluoresce (data not shown). Excitation was through a band-pass filter (540–555 nm), and emission was collected through a lowpass filter (>590 nm).



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Evaluating the signal brightness of SERS nanoparticle labels using single nanoparticle tracking analysis

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Surface-enhanced Raman scattering (SERS) is widely used for the sensitive detection of biomolecules. SERS is observed for Raman-active molecules on or nearby the surface of plasmonic nanostructures. SERS nanoparticle labels for nanodiagnostics typically consist of metal nanoparticles (NPs) (20-100 nm) coated with Raman reporters (Ra) for identification [fig. 1]. In contrast to fluorescent tags, SERS NP labels have a higher capability for multiplexed detection due to the narrow line width of Raman bands of the corresponding Ra. ^[1-2]

Spectrally distinct SERS signals are generated by different Ra on the NP surface. For the same Ra, the SERS signal intensity depends on the type of plasmonic NP. Therefore, SERS activities of Ag/Au nanoshells, Au nanostars, and Au/Au superstructures coated with the Raman reporters 4-NTB and MBA are determined and compared with one another.

The direct comparison of the different plasmonic NPs requires knowledge on their concentration. However, the concentration of Ag/Au nanoshells, Au nanostars, and Au/Au superstructures are not directly accessible by UV/Vis extinction spectroscopy. For determining the NP concentration we employ single nanoparticle tracking analysis. By normalizing the SERS signal intensity of the different nanoparticles it allows us to compare the different SERS signal brightnesses.

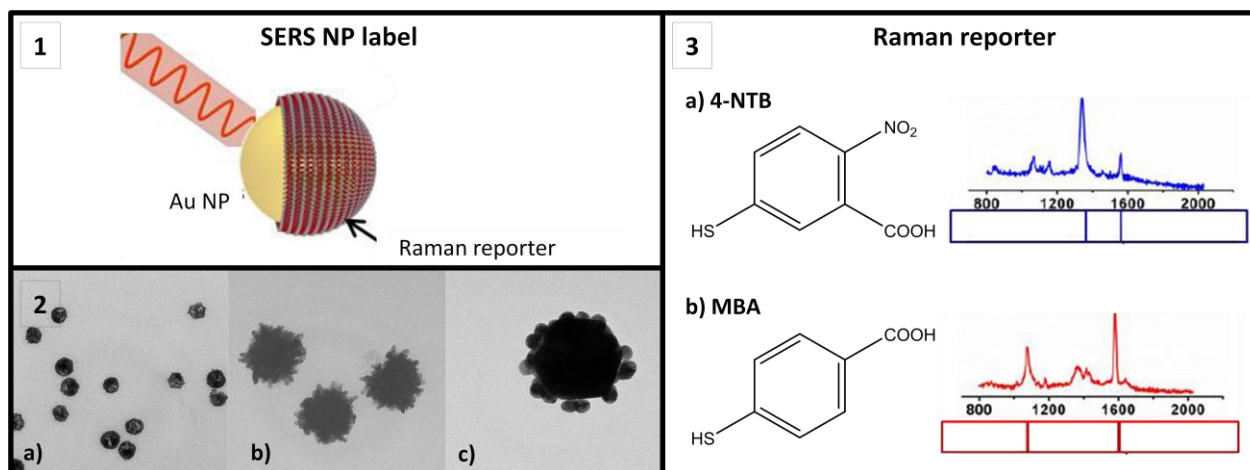


Fig. 1 – SERS NP label (1), TEM images (2) of a) Ag/Au nanoshells, b) Au nanostars and c) Au/Au superstructures, Raman reporter (3) a) 4-NTB and b) MBA with corresponding SERS spectra and barcode.

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Biocompatibility of Perfluorodecalin-filled Albumin-nanocapsules as potential artificial oxygen carriers

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In the field of artificial blood substitutes, Perfluorodecalin-filled nanocapsules are already discussed as an artificial oxygen carrier^{1, 2}. This dose escalation study analyzes the preclinical safety and biocompatibility of different amounts of Perfluorodecalin-filled nanocapsules with an albumin shell. The nanocapsules were prepared using the power of ultrasonic amplitudes³. After intravenous infusion to anesthetized rats, effects on systemic parameters, acid base/metabolic status and organ damage were evaluated. Generally all animals tolerated intravenous infusion the nanocapsules well. Nevertheless some slight increase of plasma enzymes such as lactate dehydrogenase, creatine kinase, alanine aminotransferase and aspartate aminotransferase could be observed. Furthermore, significant injury of the spleen was detected. Encouraged by these overall positive results of this first *in vivo* application, we are now seeking for more detailed information on the chemical and physical properties of Perfluorodecalin-filled Albumin-nanocapsules.

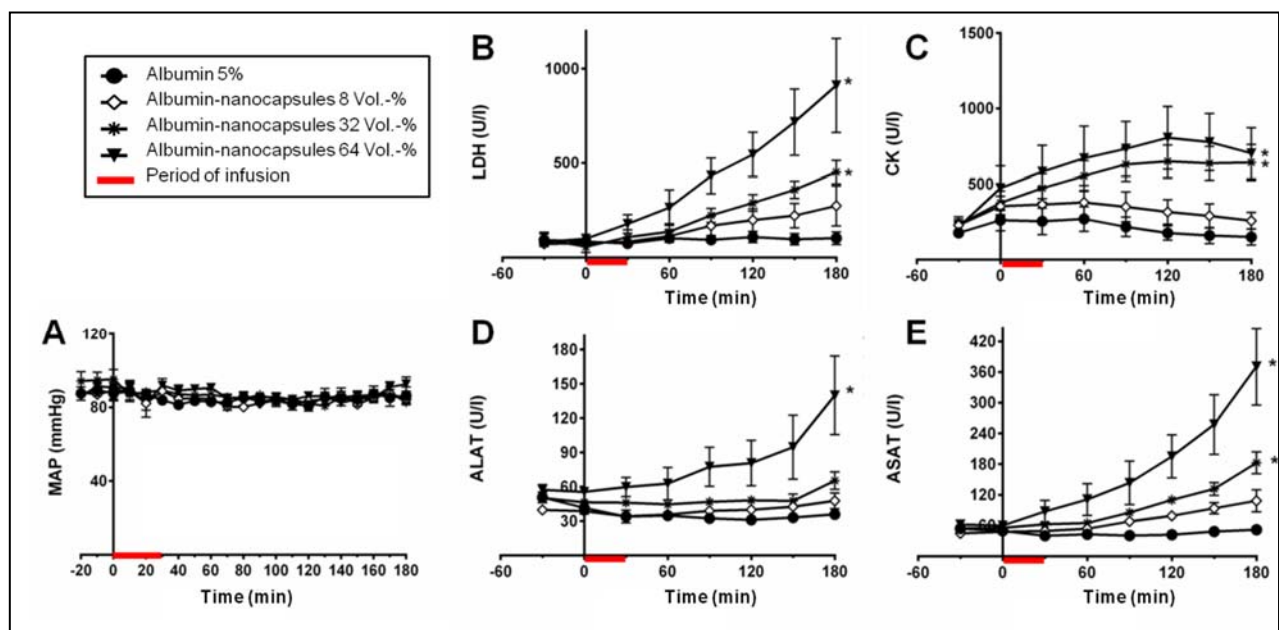


Figure 1: Effects of Albumin-nanocapsule infusion on mean arterial blood pressure (A) and plasma enzyme activity of lactate dehydrogenase (B), creatine kinase (C), alanine aminotransferase (D) and aspartate aminotransferase (E). Albumin-nanocapsules or Albumin 5% were infused over a period of 30 min (10 ml/kg body weight). The values plotted are mean \pm SEM of 6 individual experiments per group, *p < 0,05 compared to Albumin 5% group.

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Biological Photoreceptors with Red-/Green Photochromic Conversion and Switchable Fluorescence

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Cyanobacteriochromes (CBCRs) are phytochrome-related photoreceptors found in cyanobacteria, which are capable of autocatalytically binding the chromophore by a single GAF domain. CBCRs show reversible photoconversions between two photostates (similar to canonical phytochromes), however, they extend the photosensory range to shorter wavelengths of visible light and near-UV. Their primary photochemical process is a selective double bond isomerization (from the 15-*Z* to the state 15-*E* state). Considering their photo-switchable properties and relatively small size, these proteins are promising candidates for biomarkers and optogenetic studies. Representing these proteins, we studied the GAF3 domain of a red/green-switching cyanobacteriochrome, encoded by *slr1393* from *Synechocystis* sp. PCC 6803. This GAF3 domain shows a green-red photochromism between its 15-*E* state ($\lambda_{\text{max}} = 539$ nm) and 15-*Z* state ($\lambda_{\text{max}} = 650$ nm). Both states revealed noticeable fluorescence (quantum yield ≈ 0.03 and 0.08 with emission maxima at 618 and 670 nm, respectively). A three-dimensional structure and the micro- to milliseconds time-resolved absorption spectroscopy have been determined. Besides the *in vitro* analysis, the photoswitching dynamics were demonstrated in two preparations: GAF3 protein immobilized on glass surface and GAF3 expressed in living *E. coli* cells.

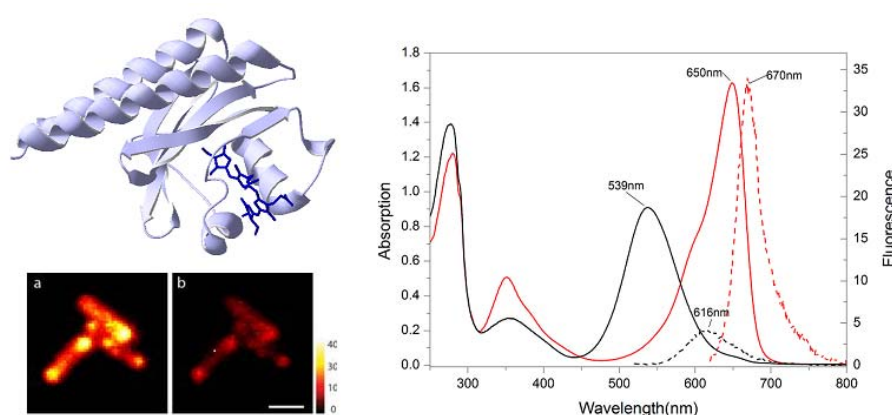


Figure 1: (top left) Three dimensional structure of GAF3 of *slr1393* from *Synechocystis*; (bottom left) Fluorescence picture of living *E. coli* cells expressing *slr1393g3* for both red- and green

absorbing states (taken from an inverted fluorescence microscope); (right) Absorbance and fluorescence spectra for both photo-switchable states of slr1393g3.

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Immuno-SERS microscopy for protein localization in tissue biopsies

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Immuno-SERS (surface-enhanced Raman scattering) microscopy is a novel imaging method in tissue diagnostics, which is based on antibodies labeled with molecularly functionalized noble metal colloids. Compared with existing approaches, immuno-SERS microscopy offers the advantages of multiplexing, photo stability, quantification, and improved image contrast by red or near-infrared laser excitation¹. Traditional bioconjugation of antibodies to colloids involves chemical modifications of the antibodies that can be difficult and time consuming to optimize; for example, the antibodies attach on the nanoparticle surface in random orientations, which may lead to a decreased binding capacity. In contrast, using chimeric protein G as an adaptor protein avoids this problem². In our study, Au/Ag nanoshells (Fig.1 a) were covalently coupled to protein G, which binds to the Fc region of the antibody, therefore, presenting the antibody in an optimal orientation for target binding³ (Fig.1 b).

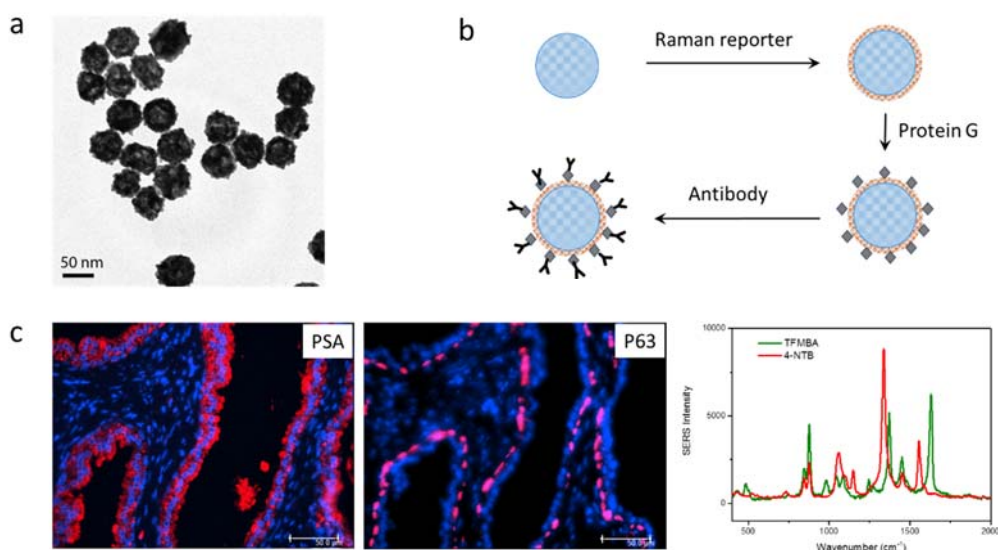


Fig.1 a, TEM image of Au/Ag nanoshells; b, scheme of fabricating immuno-SERS tags; c, example of immuno-fluorescence and SERS results.

Non-neoplastic prostatic tissue was chosen for establishing this methodology using SERS NP-protein G-antibody conjugates. Localization of prostate-specific antigen (PSA) in the epithelium of the prostate gland and P63 in the nuclei of the basal cells is a clear test that immuno-SERS microscopy provides the necessary specificity for selective staining (Fig.1 c, left). Spectrally distinct SERS signals were obtained by simply using two different Raman reporters on the surface of the metal colloid (Fig.1 c, right). Overall, we have demonstrated multi-color immuno-SERS microscopy for tissue-based cancer diagnosis using prostate biopsies as an example.

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