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**Entwicklung neuer Sonden für bioanalytische Anwendungen der oberflächenverstärkten Raman-Streuung**

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Surface-enhanced Raman scattering (SERS) has been established as a versatile tool for probing and labeling in analytical applications, based on the vibrational spectra of samples as well as label molecules in the proximity of noble metal nanostructures. The aim of this work was the construction of novel SERS hybrid probes. The hybrid probes consisted of Au and Ag nanoparticles and reporter molecules, as well as a targeting unit. The concept for the SERS hybrid probe design was followed by experiments comprising characterization techniques such as UV/Vis-spectroscopy (UV/Vis), Transmission electron microscopy (TEM) and Dynamic Light Scattering (DLS), respectively. SERS experiments were performed for studying and optimizing the plasmonic properties of nanoparticles with respect to their enhancement capabilities.

The SERS-probes had to meet following requirements: biocompatibility, stability in physiological media, and enhancement of Raman-signals from Raman reporter molecules enabling the identification of different probes even in a complex biological environment. Au and Ag nanoaggregates were found to be the most appropriate SERS substrates for the hybrid probe design.

The utilization of Raman reporters enabled the identification of different SERS probes in multiplexing experiments. In particular, the multiplexing capability of ten various reporter molecules *para*-aminobenzenethiol, 2-naphthalenethiol, crystal violet, rhodamine (B) isothiocyanate, fluorescein isothiocyanate, 5,5'-dithiobis(2-nitrobenzoic acid), *para*-mercaptobenzoic acid, acridine orange, safranin O und Nile blue was studied using NIR-SERS excitation. As demonstrated by the results the reporters could be identified through their specific Raman signature even in the case of high structural similarity. Chemical separation analysis of the reporter signatures was performed in a trivariate approach, enabling the discrimination through an automated calculation of specific band ratios. The trivariate identification could be a promising method for SERS-multiplexing in analytical applications.

Multivariate methods such as Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were as well applied for discrimination and imaging of the reporter signatures. With the help of multivariate imaging methods based on cluster analysis, it could be for the first time demonstrated that such methods provided a fast identification of various SERS hybrid probes inside the biological matrix, this was demonstrated using living 3T3 cells. Further, in a duplex imaging approach, the probes fulfill the requirements for the sensitive detection of both the specific reporter signatures and intrinsic information coming from eukaryotic cells. The results of cluster methods and principal components approaches for discrimination indicate that fast, multivariate evaluation of whole sets of multiple probes is feasible, beyond the visual inspection of individual spectra that has been practiced so far. This suggests multiplexing applications with SERS hybrid nanoproboscopes and SERS tags in very high density sensing and biological imaging applications, where fast read-out is required.

The pH-sensitivity of SERS-Tags that consisted of different reporter molecules attached to aggregated Au and Ag nanoparticles in the range between pH~3-10 was studied. It could be demonstrated that the reporter molecules provided pH-dependent SERS signatures and could therefore be suitable for the sensitive pH-detection, e.g., inside cellular compartments.

The construction of targeted SERS probes was based on the integration of a goat-anti-mouse antibody as targeting element. Antibodies were coupled to Au and Ag nanoproboscopes surrounded by a Bovine Serum Albumin (BSA) coating which served as a carrier for the covalent linkage of a Raman reporter molecule and the targeting units at the same time. In experiments with BSA and a conjugated reporter the spectra of the BSA-coupled reporters provided an indication of the secondary structure of BSA which is related to the BSA-reporter coupling.

procedure In *in vitro*-experiments with BSA-coupled nanoprobe inside 3T3 cells reporter signatures and intrinsic information from the cellular matrix could be delivered BSA enabled the coupling of reporter molecules as well as the targeting of antibodies and served as stabilizer of the gold nanoaggregates The functionality of the coupled antibodies after their integration into the SERS probe was retained This was verified by the results of a direct Enzyme-Linked Immunosorbent Assay (ELISA) Conjugates with implemented reporter molecules could be characterized using SERS The application of the complete probes suggested a use of these novel biocompatible stable and targeted SERS probes that can be excited out-of-resonance also for other bioanalytical applications.

On the basis of the constructed SERS hybrid probes comprising a large number of BSA-coupled reporters could e.g. be implemented for automated high-throughput immuno-assays where they are arranged on a microstructured device for the simultaneous and multilevel SERS-readout in one step .