A new generation of Raman spectroscopists:
Thinking diversity in the analytical sciences

Janina Kneipp[1,2*], Ulrich Panne[1,3], Dorota Bartkowiak[1,2], Dimitra Gkogkou[1,4,7], Christian Heck[1,3,5], Wan-Ing Lin[1,8,11], Tilmann Neubert[1,2], Elena Pavlenko[1,6], Christine Joy Uy Querebillo[1,7], Bita Rezania[1,8], Maximilian Ries[1,4,12], Victor M. Rodriguez Zancajo[1,2,9], Radwan Mohammed Sarhan[1,6], Gergo Péter Szekeres[1,2,10], Yanlong Xing [1,4,13], Anur Yadav[1,2], Zhiyang Zhang[1,2,3], and Vesna Zivanovic[1,2]

[1]*Humboldt-Universität zu Berlin, School of Analytical Sciences Adlershof (SALSA),*
Albert-Einstein-Str. 5-9, 12489 Berlin, Germany

[2]*Humboldt-Universität zu Berlin, Department of Chemistry, Brook-Taylor-Str. 2, 12489 Berlin, Germany*

[3]*BAM Federal Institute for Materials Research and Testing, Richard-Willstätter-Str. 11, 12489 Berlin, Germany*

[4]*Leibniz-Institut für Analytische Wissenschaften-ISAS-e.V., ISAS Berlin, Schwarzschildstr. 8, 12489 Berlin, Germany*

[5]*University of Potsdam, Institute of Chemistry, Karl-Liebknecht-Str. 24–25, 14476 Potsdam, Germany*

[6]*University of Potsdam, Institute of Physics and Astronomy, Karl-Liebknecht-Str. 24–25, 14476 Potsdam, Germany*

[7]*Technische Universität Berlin, Department of Chemistry, Straße des 17. Juni 135, 10623 Berlin, Germany*

[8]*Humboldt-Universität zu Berlin, Department of Physics, Newtonstr. 15, 12489 Berlin, Germany*

[9]*The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture,*
The Hebrew University of Jerusalem, Rehovot 7610001, Israel

[10]*Department of Physical and Analytical Chemistry, University of Oviedo, 33006 Oviedo, Spain*

[11]*Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland*

[12]*Technische Universität Berlin, Institute of Solid State Physics, Straße des 17. Juni 135, 10623 Berlin, Germany*

[13]*Department of Biosystems Science and Engineering, ETH Zurich, 4058 Basel, Switzerland*

Analytical Raman spectroscopy finds itself at the interface of physics, chemistry, biology, modeling, and data science, and is evolving at a high pace due to the interaction of scientists trained in different fields. To address the major challenges regarding sensitivity and selectivity in the applications of Raman scattering, to push its physical limits, and to connect different levels of understanding of complex systems in the natural sciences, future generations of spectroscopists will need a diverse background. In this paper, we report on the activities of junior scientists of all genders in the field of Raman spectroscopy in a multidisciplinary, multi-institution graduate school. We demonstrate that bias-free recruitment, yielding a high percentage of female researchers, infrastructural support, and an environment promoting exchange leads to high-level research in basic and applied Raman spectroscopy. The examples illustrate our research activities in surface-enhanced Raman scattering (SERS) and molecular plasmonics, advances in the characterization and utilization of low-dimensional materials, and the application of Raman scattering for the characterization of complex biological systems. © Anita Publications. All rights reserved.

Corresponding author
e mail:janina.kneipp@chemie.hu-berlin.de (Janina Kneipp)

1 Introduction

Raman scattering, revealing the structure of matter based on vibrational and rotational states of molecules and materials has diversified over almost a century to serve in almost all fields of the natural sciences, from solid-state physics over astronomy and biochemistry, to forensics, diagnostics, and archeology. In order to develop the field of analytical Raman spectroscopy, the scientific exchange must take place across disciplines, so that spectroscopists become aware of the problems that are addressed in the specific fields and of the relevance of particular solutions they may find. One of the most prominent examples are probably Raman applications in disease diagnostics, where Raman spectroscopists, pathologists, microbiologists, engineers, data scientists, and members of other communities have been working hand in hand to generate immense progress over the past three decades. The same is true for approaches to elucidate the structure and composition of a chemical system by other types of measurements, e.g., in mass spectrometry or electrochemistry. Raman-based methods have been an integral part in addressing the important challenges in the broader field of Analytical Sciences. Summarizing these challenges by the terms 'Sensitivity and Selectivity' and 'Limits and Scales', with implications in many walks of chemistry and physics, we identified an important theme as a general approach towards tackling them: 'Make and Measure'. In the context of Raman experiments, the term 'Make' stands for the synthesis of new vibrational probes and new materials that will enable higher detection sensitivity, spatial confinement, faster probing, as well as the modification of molecules and materials so that their optical properties can be better understood.

With this article, we would like to stimulate discussions on the role of future generations of scientists in promoting exchange between disciplines by enhancing diversity in science. Such diversity is not necessarily restricted to gender, but in our experience crucially relies on scientists from diverse scientific backgrounds. At the same time, we will illustrate that progress in the applications and basic new findings in Raman spectroscopy and related fields can be pushed in an environment that embraces the diversity of people, regarding gender, personality, and cultural background, and regarding their future aspirations. We would like to illustrate this here by showcasing the scientific achievements of a graduate program that has been running for almost ten years and successfully graduated ~60 young scientists in the past five years. Aiming to overcome the fragmentation of the Analytical Sciences and prove wrong those who view them as a retrospective discipline with a 'service' character, a group of ~50 faculty have founded a multidisciplinary school of Analytical Sciences. They come from the three Berlin universities, the University of Potsdam, several non-university institutes including the Federal Institute for Materials Research and Testing (BAM), Helmholtz Center for Materials and Energy with BESSY synchrotron, all in Germany, and the international partner universities ETH Zürich, University of Oviedo in Spain, and the Hebrew University of Jerusalem, Israel. Its research projects are anchored in groups from physics, analytical and physical chemistry, synthetic organic and inorganic chemistry, biology, modeling, and even didactics. The research comprises the development of new probes for Raman-based analytics in biological environments, with a specific focus on making use of plasmonic enhancement, as well as Raman microscopy and imaging. Moreover, research in the project deals with a comprehensive analysis of materials that has a strong connection to materials synthesis and to other areas of materials characterization. So far, more than one-third of the graduates have actively contributed to progress in the field of Raman spectroscopy, with many collaborative publications. These achievements were made by people of all genders, through their research, teaching, and scientific discussion. As will be explained here, a rigorous selection of the best junior scientists, innovative projects, a climate that embraces discussion and exchange, and a self-determined educational program, together with a professionalized supporting structure for any non-scientific affairs, including families, ensures a high percentage (of over 60%) of women Raman spectroscopists in the program. We demonstrate the success of the program by some examples from a range of research results obtained in the field of Raman spectroscopy in our former doctoral work.

2 Recruiting the best worldwide leads to gender balance

In 2012, the graduate School of Analytical Sciences Adlershof (SALSA), focused on a structured doctoral education, was founded at Humboldt Universität zu Berlin in the framework of the German 'excellence initiative'. From the beginning and through the past decade, research in Raman spectroscopy has been an important direction of the school, both in research and in teaching. A total of 92 doctoral students from ~30 nations, selected over the years from ~870 applicants have entered SALSA since 2013. Currently, the program has 27 fellows. The average time-to-degree has been four years, less than the average at all participating university departments. The average output in peer-reviewed articles per finished thesis project amounts to 4-5. The scientific achievements made by SALSA doctoral fellows and postdoctoral researchers were recognized by many awards, including poster or presentation prizes, prestigious postdoctoral fellowships, and competitive awards for young researchers at the international level (e.g., Chinese Academy of Sciences, etc.). Compared with other graduate schools or similar structured doctoral programs, SALSA probably stands out because it does not focus on a single research area. Instead, we focus on analytical problems that can be found virtually everywhere in science – and anybody who is willing to think about their research from a problem-solving perspective is welcome. We have therefore attracted extremely diverse cohorts of graduate students over the years. In a competitive application process only genuinely motivated students can succeed. Several students – particularly female fellows – also explicitly state that they want to achieve a degree in the Analytical Sciences because they want the chance to shape policy and decision-making in their home countries.

Doctoral researchers in SALSA perform their research projects in co-supervision by two PIs, who together provide a framework for a project, described in a short proposal by the two faculty. Starting from this proposal, they ideally develop the topic together with their co-supervisors, sharpening the project goals themselves as the project evolves. In line with this, international candidates are selected first regarding their scientific excellence only and in a highly competitive process, and after being accepted, an existing project with the best fit is identified for/by them. As we found in the course of the recruitment rounds in the recent few years, a very large number of fellows has a strong interest in materials-related topics, which led to the very successful research in this direction and matches the expertise of many faculties. As examples, the possibilities that are opened by an advanced characterization of molecules at surfaces and interfaces incorporate a wide range of research topics, from bio-interfaces to novel organic-inorganic hybrid materials. Other groups study nanostructures and low dimensional materials, both from the fundamental perspective and for new applications in analytical sensing or catalysis.

Our recruitment strategy has become best practice for several graduate schools throughout Germany. An important step is that after submission, the applications are anonymized, i.e. cleared of any details in terms of gender, origin, ethnicity, and age by the school's Equal Opportunity Officer. Only after this, the admission board reviews suitable applications in this anonymized form and ranks the applications. After this first stage, video conferences are conducted with the most promising applicants. Based on their scientific qualifications, personal fit/talent, motivation, and the impression gained from the remote interview, the applicants are also invited to continue with a presentation about their previous scientific work (Master thesis or similar) in English and discuss it with the members of the admission board. This is followed by an invitation to Berlin for personal interviews and discussions with the potential thesis supervisors of several particular research projects that are pre-selected by the applicants based on the short proposals of the faculty. There, the candidates present previous work and justify the selection of particular projects in a discussion. The main aim of this workshop format is the matching process, as well as an exchange with other fellows. Based on these results, the admission board decides on admission to the program and on matching applicants to projects. In the first four years of the program, seven cohorts of doctoral fellows were recruited using this three-stage process, with 10-16 members per cohort. After a break due to an unresolved financial situation

in 2018, two more cohorts have been recruited in the past two years. An average percentage of ~60% female researchers has been maintained. Table 1 provides details on the first five application rounds.

Table 1. Percentage of male and female applicants and accepted doctoral fellows in the first three years (five calls for applications) of the program.

| | Applicants | | | Accepted | |
	Total number	Male (%)	Female (%)	Male (%)	Female (%)
2013 (1)	101	65	35	33	67
2013 (2)	164	60	40	8	92
2014 (3)	88	69	31	50	50
2014 (4)	120	62	38	57	43
2015 (5)	83	70	30	50	50

3 Case-based learning and teaching in the analytical sciences

As part of the SALSA project, we focus strongly on collaboration and development of a common 'language' at the interface between chemistry, physics, and the life sciences. The main motivation is to educate excellent, highly specialized young researchers who think outside the box to collaborate, as many different problems must be solved – today and in the future, using many different toolboxes, including optical spectroscopies. One of the hallmarks of Analytical Sciences is their focus on the individual sample – its composition, structure, and dynamics. The process underpins the strong focus on real-world samples. Not surprisingly, the inductive nature of Analytical Sciences is reflected in many of the well-known analytical concepts, including the limit of detection, selectivity, uncertainty, validation, or traceability. Therefore, a case- and inquiry-based education has been the central pillar of the development of a new curriculum in Analytical Sciences in the graduate school. Learning and teaching patterns are not traditional, and in the first years of the collaborative project, scientists engaged in didactics became involved in the development, including quantitative assessment, of a curriculum for the multidisciplinary graduate school. At this point, it should be mentioned that a structured 'classroom type' scientific education at the graduate level is unusual in the German system and many others. For an effective design of our approach to education and research, we acknowledged that students entering the graduate school did not arrive with a uniform level of education but with broad variation in the depth, direction, and level of specialization of their undergraduate experiences. The curriculum connects doctoral researchers in the school across all research groups through learning and teaching and has been stimulating the research by disseminating 'analytical thinking' to students and their supervisors.

While a structural framework (for curriculum and supervision) is provided by the program, the actual scientific contents are determined by the doctoral fellows. As examples, they contribute to the topics and focus of the multidisciplinary discussions based on their own interests, propose the structure of particular seminars, render decisions on which guests and teachers to invite, and identify their own knowledge gaps, to be bridged by particular lectures or lab courses. The role of the faculty, apart from serving as co-advisers of the thesis work, is to offer accompaniment of seminars and consultation according to their respective expertise and help in 'vertical' networking (e.g., with renowned senior researchers in a particular field). We have established several unique but successful teaching and learning formats. For example, we host sessions prepared by the more advanced doctoral fellows, who share the approaches and methods they are most familiar with, and explain them to their fellow students. Such sessions, in particular, see the 'teachers' and 'students' working in groups to solve analytical problems. They also invite senior scientists from the school to give short lectures on specific aspects of the topic. Of course, all our faculty involved in the different applications of Raman spectroscopy have been engaging in corresponding discussions, including, e.g., Nobert Esser, Peter Fratzl,

Peter Hildebrandt, Inez Weidinger, Renato Zenobi, and many others. Not to our surprise, we found that the fellows of the graduate school really benefit from working in groups and taking on an active role in mutual teaching. We still teach face-to-face a lot, and student-to-student teaching is invaluable in graduate education. Blended formats and the online exchange of data, video teaching, and discussions are crucial and have been normal to us long before pandemic times, due to common classes with the different partners, e.g., an excellent class by Renato Zenobi taught at ETH Zurich involving students in the Berlin area.

A yearly conference /Summer University, where renowned international guests are interviewed by doctoral students in public discussions has become a success story [1]. The format of this Make and Measure conference series has been recognized also by students at the ~25 universities of (former) international conference guests. The preparation sessions for the conference are an important example of peer-to-peer teaching, the interviews with the guests give evidence of the active role taken by the doctoral fellows in shaping the academic profile of the school. Before the conference, for a few months of regular meetings, a group of ~5 or so Ph D students prepare an in-depth discussion ('challenge session') with an international guest, by reading their papers, trying to understand the background of their research, and their experimental approach. During the conference, they use half a day to conduct the discussion session that is attended by the public. They present to the guest and the public their perspective on the guest's research, with the guest commenting. In the past, this has included the re-creation of a Raman setup in a seminar room, presentations of the students regarding simulation results that they obtained for molecular systems from the guests' work etc. On the same day, in the afternoon, the guest gives a plenary lecture, as in a conventional conference, but with a well-prepared audience.

Observing gender and diversity equality has been an important element in the graduate school. Equality at both the structural and personal levels resulted in a personal diversity among female and male researchers. An important objective is to convince female students to continue their academic careers after completing their degrees, so providing role models is key. Many renowned scientists of all genders who perform work in the area of vibrational spectroscopy visited the program for short lectures, teaching, the summer universities, and discussions, they include Maria Paula Marques, Giulietta Smulevich, Lia Addadi, Lutgarde Buydens, Janina Maultzsch, Achim Kohler, Gerwin Puppels, Sebastian Schlücker, Laura Lechuga, Roy Goodacre, Christy Haynes, Luis Liz-Márzan, Cees Otto, and many others. The graduate school has hosted two outstanding international female senior researchers from the field of Analytical Sciences as a guest professors, Rivka Elbaum and Maria Montes-Bayón who actively engaged in teaching and started collaborative research projects in the area of analytical Raman spectroscopy as well and eventually joined as faculty.

4 Support through infrastructure: Raman in the application labs

SALSA runs two so-called Application Labs that provide facilities for graduate projects (in addition to the labs of the hosting faculty) and are home to a junior research group in Photonics, as well as to a new full professorship for Micro- and Nanoanalytics. The functions of the labs are diverse, in addition to advanced hands-on teaching they can be used as Raman spectroscopy facility for all researchers in the project, and they are home to postdoctoral researchers working on applied aspects of Raman spectroscopy as well. As an example, interesting new results on the Raman spectroscopy of minerals have been obtained there in the past years which have applications in practical issues, e.g. related to restoration [2-5]. The Application Lab 'Applied Microspectroscopy' is equipped with a Raman spectrometer and a Raman microscope with several excitation lasers and possibilities for light microscopy, environmental scanning-electron microscopy and other instrumentation. During lab courses taught there, everyone has the possibility to familiarize with Raman scattering by characterizing own samples, and to apply it to problems that they may encounter during work on their respective project. This led to new cooperations within the graduate school and to new results in the research in very diverse topics [6-7].

5 Research results

From the beginning, two major research areas have been in the focus of the program. Both rely on methodological advancements in Raman spectroscopy (i) biomolecule detection and characterization in complex environments, specifically emphasizing the challenge of 'sensitivity and selectivity', and (ii) regarding the analytics of structures and networks, where many materials-related topics have been discussed that connect different scales in hierarchical structures, that investigate surfaces, composite materials, and low-dimensional systems. The following examples will illustrate the need in both areas for input from research on basic spectroscopy and adaptation to complex systems, as well as the connection of Raman spectroscopy with approaches in materials synthesis and characterization.

5.1 Surface-enhanced Raman scattering and molecular plasmonics

MgF$_2$-coated gold nanostructures as a plasmonic substrate for analytical applications [8]

Plasmonic substrates can be a powerful tool for analytical applications. In order to broaden the spectrum of their applications and to push the detection limits of analytical spectroscopy, new plasmonic substrates are developed. The motivation of this work was to coat plasmonic nanostructures with magnesium fluoride. Coatings of magnesium fluoride are porous but exhibit high mechanical stability and extraordinary optical properties including a low refractive index and a wide optical window. Combining these properties with the beneficial properties of plasmonic nanostructures is of interest for manufacturing advanced plasmonic substrates for analytical applications. The approach based on immobilization of gold nanoparticles on glass and coating them with magnesium fluoride using a dip-coating method provides plasmonic substrates that are characterized by a high nanoscopic homogeneity of the gold nanoparticles distribution, a high mechanical stability, interesting optical properties and enhancement factors of optical signals that allow for real analytical applications (Fig 1) [9]. The coating of gold nanoparticles immobilized on the glass with magnesium fluoride results in very promising substrate that can be used for sensing and other applications in the future.
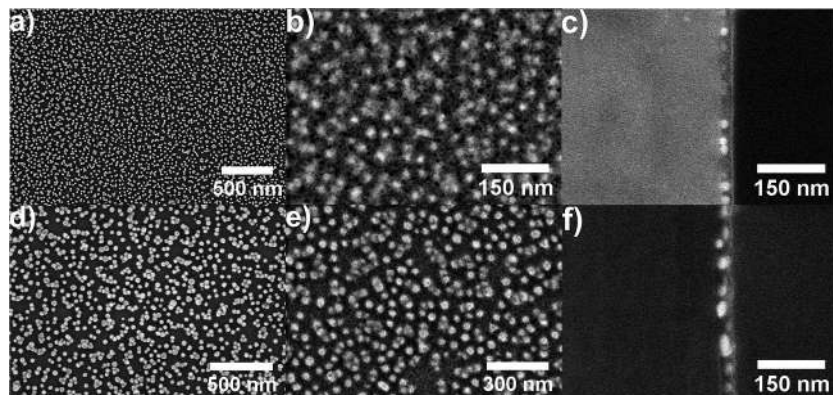

Fig 1. Scanning electron micrographs of 26 nm (a–c) and 36 nm (d–f) sized gold nanoparticles: (a and d) uncoated particles immobilized on glass, (b and e) immobilized particles coated with magnesium fluoride, (c and f) cross sectional images of immobilized and coated gold nanoparticles. Reproduced with permission from ref. [9] Copyright 2016, the authors of ref. [9].

Anisotropic plasmonic nanoparticle arrays for surface-enhanced biosensors [10]

Plasmonic structures exhibiting surface enhancement properties can lead to hypersensitive, label-free and analyte-specific detection of organic molecules. The motivation of this work is the realization and study of optically anisotropic plasmonic substrates for combining surface enhanced Raman scattering (SERS) and surface enhanced infrared absorption spectroscopy (SEIRA). These properties can be ultimately used for chemical- and bio-sensing purposes. Silver was deposited on pre-patterned rippled silicon surface,

with ripple periodicity in the range of 40-50 nm. The silver nanoparticles grow preferentially in the direction along the ripples forming arrays. The structural anisotropy introduces optical anisotropy, investigated with ellipsometry. A smaller, narrower resonance appears in the direction across the ripples and a red-shifted, stronger and broader resonance in the direction along the ripples (Fig 2). Chemical functionalization was achieved by the adsorption of the 4-mercaptobenzonitrile (4-MBN) self-assembled monolayer (SAM) to the silver surface. The position of the plasmonic resonances can be tuned by controlling the size and aspect ratio of the nanoparticles. One combination involves both of the resonances in the visible range, employed for SERS [11]. SERS measurements at varying excitation wavelengths and polarization angles, exhibited a sinusoidal profile of the 4-MBN peak intensity. The signal modulates according to the polarization angle and laser wavelength, exciting the respective resonances. The SERS polarization dependency has been confirmed up to the NIR range [11]. It was applied for the signal differentiation between surface-attached molecules (4-MBN) and solution molecules (cyclohexane), demonstrating potential sensing uses. The SERS enhancement factors were estimated to be in the range of 10^4. A second combination involves both resonances red-shifted to the MIR region demonstrating considerable SEIRA properties. The enhancement factor was estimated to be 170, with the peaks exhibiting a Fano-shaped profile. IR ellipsometry and IR polarized microscopy demonstrated the polarization dependence, according to the excitation direction, of the SEIRA activity [12]. The combination of this type of substrate and characterization techniques introduces new possibilities in the structural characterization of molecules with high sensitivity. The third combination involves the resonance in the direction across the ripples peaking in the visible range and the resonance in the direction along the ripples peaking in the NIR. Due to broadening, the longitudinal resonance extends to the MIR enhancing the vibrational signal and potentially allowing the dual detection of SERS and SEIRA on a single spot [13]. An additional method for the combination of SERS and SEIRA properties on a single substrate is proposed by the evaporation of a silver layer of gradient thickness on silicon. The variation of the surface morphology and therefore optical activity along the sample, results in SERS and SEIRA active areas. The proximity of these sites can be controlled by the deposition parameters. The gradient thickness substrate is an alternative solution for combined SERS and SEIRA detection by performing a line scan. The results demonstrate the controlled design and characterization of plasmonic nanostructures with specific surface enhancement properties and investigate their potential bio-sensing applications. The combined SERS and SEIRA properties allow for acquisition of the maximum vibrational information of an analyte in high sensitivity and selectivity.
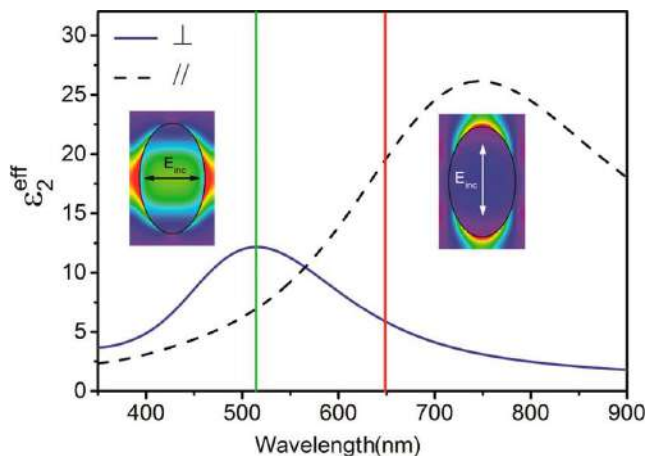

Fig 2. Optical resonances for the directions ⊥ (blue), with dipole excitation on the short axis, and ‖ (dashed black), with dipole excitation on the long axis. The vertical lines indicate the positions of the 514 and 647 nm excitation wavelengths used in the SERS experiments. Reproduced with permission from ref. [11].© 2016, American Chemical Society.

Gold and silver nanolenses self-assembled by DNA origami [14]

Nanolenses are linear chains of differently-sized metal nanoparticles, which can theoretically provide extremely high field enhancements. The complex structure renders their synthesis challenging and has hampered closer analyses so far. Here, the technique of DNA origami was used to self-assemble DNA-coated 10 nm, 20 nm, and 60 nm gold or silver nanoparticles into gold or silver nanolenses. Three different geometrical arrangements of gold nanolenses were assembled, and for each of the three, sets of single gold nanolenses were investigated in detail by atomic force microscopy, scanning electron microscopy, dark-field scattering and Raman spectroscopy. The SERS capabilities of the single nanolenses were assessed by labelling the 10 nm gold nanoparticle selectively with dye molecules. The experimental data were complemented by finite-difference time-domain simulations [15-16]. For those gold nanolenses which showed the strongest field enhancement, SERS signals from the two different internal gaps were compared by selectively placing probe dyes on the 20 nm or 60 nm gold particles. The highest enhancement was found for the gap between the 20 nm and 10 nm nanoparticles, which is indicative of a cascaded field enhancement. The protein streptavidin was labelled with alkyne groups and served as a biological model analyte, bound between the 20 nm and 10 nm particles of silver nanolenses [17]. Thereby, a SERS signal from a single streptavidin could be detected. Background peaks observed in SERS measurements on single silver nanolenses could be attributed to amorphous carbon. It was shown that the amorphous carbon is generated *in situ* [18].

Hybrid nanolayer architectures for ultrafast acousto-plasmonics in soft matter [19]

The goal of this project was to explore the interaction between gold nanorods (GNRs) and hyper-sound waves. For the generation of the hyper-sound Azobenzene-containing polymer transducers were used. Multilayer polymer structures with well-defined thicknesses and smooth interfaces were built via layer-by-layer deposition. Anionic polyelectrolytes with Azobenzene side groups (PAzo) were alternated with cationic polymer PAH, for the creation of transducer films. PSS/PAH multilayer were built for spacer layers, which do not absorb in the visible wavelength range [20]. The properties of the PAzo/PAH film as a transducer were carefully characterized by static and transient optical spectroscopy. The optical and mechanical properties of the transducer were studied on the picosecond time scale. In particular the relative change of the refractive index of the photo-excited and expanded PAH/PAzo is $\Delta n/n = 2.6*10^{-4}$. Calibration of the generated strain was performed by ultrafast X-ray diffraction calibrated the strain in a mica substrate, into which the hyper-sound is transduced. By simulating the X-ray data with a linear-chain-model the strain in the transducer under the excitation was derived to be $\Delta d/d \sim 5*10^{-4}$. Additional to the investigation of the properties of the transducer itself, a series of experiments to study the penetration of the generated strain into various adjacent materials was performed. By depositing the PAzo/PAH film onto a PAH/PSS structure with gold nanorods incorporated in it, it was shown that nanoscale impurities can be detected via the scattering of hyper-sound. Prior to the investigation of complex structures containing GNRs and the transducer, several sets of experiments on GNRs deposited on a small buffer of PSS/PAH were performed. The static and transient response of GNRs is investigated for different fluence of the pump beam and for different dielectric environments (GNRs covered by PSS/PAH). A systematic analysis of sample architectures was performed in order to construct a sample with the desired effect of GNRs responding to the hyper-sound strain wave. The observed shift of a feature related to the longitudinal plasmon resonance in the transient reflection spectra is interpreted as the event of GNRs sensing the strain wave [21]. We argue that the shift of the longitudinal plasmon resonance is caused by the viscoelastic deformation of the polymer around the nanoparticle. The deformation is induced by the out of plane difference in strain in the area directly under a particle and next to it. Simulations based on the linear chain model support this assumption. Experimentally this assumption was proven by investigating the same structure, with GNRs embedded in a PSS/PAH polymer layer. The response of GNRs to the hyper-sound wave was also observed for the sample structure with GNRs embedded in PAzo/PAH films. The response of GNRs in this case is explained to be driven by the change of the refractive index of PAzo during the strain propagation.

5.2 Towards the utilization of low-dimensional systems

Small molecules intercalated within graphene and graphene oxide interfaces [22]

The behavior of molecules confined by graphene or graphene oxide (GO) has proven to be a promising area of research owing to the remarkable structural and optical properties of these quasi two-dimensional materials. One of the projects focused on the hydration behavior of GO and the behavior of small molecules confined by graphene. In this work, scanning force microscopy (SFM) has been employed to investigate the hydration of GO. The results show a gradual and a step-like increase of the average interlayer distance for relative humidities below 80% and in liquid water, respectively. These experimental observations are consistent with XRD results on multilayered graphite oxide as reported in the literature [22]. However, the results exclude the postulated interstratification effect, for hydration of GO at low relative humidities. Instead, the gradual expansion is attributed to the continuous incorporation of water molecules into single GO layers, while the step-like expansion when completely immersed in water, is attributed to the insertion of a full monolayer of water. On the other hand, the interface between graphene and its substrate may exhibit a confined electric field, a common phenomenon due to charge transfer at interfaces. This subject was addressed using Rhodamine 6G (R6G) as a probe molecule confined between graphene and mica. A red shift of the RG6 peaks at low relative humidities is argued to be due to both, electric fields acting on the molecules and mechanical deformation of the R6G structure at the interface. The strength of the field is estimated from the graphene Raman spectra to be on the order of 1 V/nm [22].

Metal-organic nanowires [23]

Metal-organic nanowires exhibit not only the properties of one-dimensional structures including ultra-small scale, large surface-to-volume ratio etc., but also obvious advantages in their tunable properties and label-free sensing ability by optical or electrical readout. Thus, the evaluation of metal-organic materials by the use of transition metal ions and organic ligands including tetrathiafulvalene (TTF) and tetracyanoquinodimethane (TCNQ) were studied. Concerning the synthesis of metal-organic nanowires, microfluidics offers various benefits, e.g., laminar flow, reduced sample/reagent consumption and control of self-assembly of nanostructures. Therefore, microfluidic techniques have been mainly applied to the synthesis and application of nano-/microstructures. Label-free biosensors based on *in situ* formed and functionalized gold-tetrathiafulvalene (Au-TTF) wires were developed using an integrated microfluidic system [24-26]. Au-TTF microwires were formed and immobilized inside the microchip. Then, different surface modification protocols were applied to modify Au-TTF wires which were used for sensitive label-free detection of catecholamines and human IgG by Raman spectroscopy. Following, a study of molecular self-organization in individual Au-TTF nano-/microwire by polarized confocal Raman spectroscopy was performed to understand the growth mechanism of Au-TTF [27]. Single nanowires were analyzed using non-destructive polarized Raman spectroscopy. Angular polarization Raman measurement of a single TTF crystal and single nanowire showed the periodic variations in typical Raman bands, indicating preferential ordering of molecules in both crystal and Au-TTF wire (Fig 3). Based on the density functional theory (DFT) calculation and simulation of depolarization ratio, the molecular assembly in a single TTF crystal was confirmed [25]. The tilted stacking of TTF units in single Au-TTF nanowire along the long axis was also proved. Afterwards, the formation of fibres and particles made of metal salts and TTF derivatives on a microfluidic device and in a conventional reaction flask was investigated. Their morphologies, optical properties and electrical conductivities were characterized. This study provided a comprehensive overview of the morphologies of the products obtained from reactions between metals and different commercially available TTF derivatives. Finally, a microfluidic-assisted synthesis of copper-tetracyanoquinodimethane (Cu-TCNQ) nanostructures based on TCNQ was performed [26]. A two-layer microfluidic device comprising parallel actuated microchambers was used for the synthesis, and enabled the excellent fluid handling for the continuous and multiple chemical reactions in confined ultra-small chambers. The as-prepared Cu-TCNQ

wire bundles showed good conductivity and hysteresis reversing memory effect, which proved the possibility in using them to build advanced nanoelectronics.
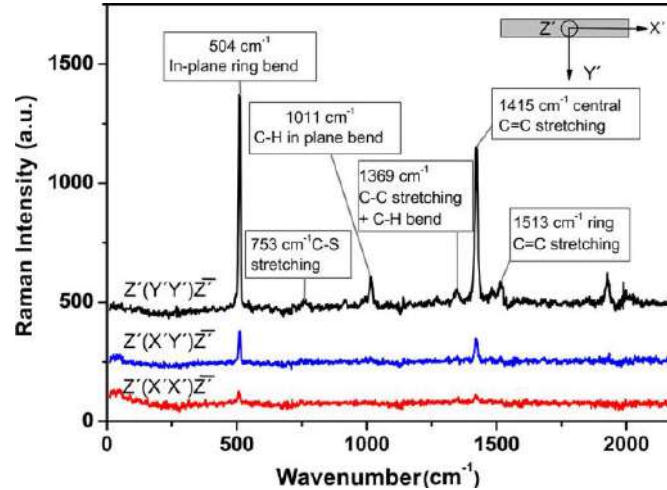

Fig 3. Raman spectra of a single Au–TTF wire under parallel (black and red spectra) and crossed (blue spectrum) polarization configurations. The full spectrum under Z′(Y′Y′) configuration shows the typical Raman active vibrations of TTF^{n+} ($0 < n < 1$). The figure inset indicates the definition of X′Y′Z′ directions for a single wire. refers to the direction of backscattered light. Reproduced with permission from ref. [27]. © 2017, American Chemical Society.

InN and InGaN nanostructures [28].

Nanostructures of group-III-V materials, such as quantum wells (QWs) or nanowires (NWs), are widely deployed in modern solid-state technology. The applicability of the III-nitride system originates in the direct fundamental bandgap covering the whole visible range and expanding even into the near-infrared and the ultraviolet (UV). Optical applications based on these materials exhibit extraordinary efficiency over most emission wavelengths. However, the green spectral region is governed by an efficiency drop. Miscibility problems leading to strong compositional fluctuations and disorder, combined with a large quantum-confined Stark effect, are the main problems in devices relying on InGaN QWs. NWs can potentially solve some of these problems, but the technology requires further advancement. The nanoscopic dimensions necessitate experimental techniques able to resolve subwavelength features. InN surfaces and InGaN single quantum well (SQW) samples were investigated to explore the reason for the occurrence of the additional modes in tip-enhanced Raman spectroscopy (TERS) [29-30]. A connection with charge carrier accumulations close to the surface is found. Multi-wavelength Raman spectroscopic studies were employed to resolve the interrelationship between charge carriers and vibrational states. The observation of a similar near-field effect in InGaN SQW samples suggests the existence of locally degenerate regions [30]. Supported by $\boldsymbol{k} \cdot \boldsymbol{p}^-$ -calculations, photoluminescence (PL), and UV-Raman spectroscopy, as well as transmission electron microscopy (TEM) with nano-cathodoluminescence, the sensitivity of the SQW towards compositional and thickness fluctuations, defects, strain, and surface distance were analyzed and highlighted. The interplay between structural and optical properties in InGaN nanostructures was continued with the characterization of self-assembled InGaN-GaN NWs. The origin of the emission redshift observed in the room temperature PL was examined in PL series dependent on temperature, intensity, and polarization. Calculations of the surface band bending emphasize a bulk carrier concentration beyond 10^{18} cm^3 to enable a Stark effect comparable to the observed redshift. A negative depolarization ratio, UV-Raman, and TEM results demonstrate the presence of structural disorder associated with InGaN and the spontaneous formation of inclusions during the growth.

Combined vibrational spectroscopy and electrochemistry for studying titania nanotubes (*TiO₂-NTs*) [31].

In the materials system, anodized titania nanotubes (TiO₂-NTs) with inherently different localized electric field (EF) enhancement properties (TiO₂-NT|low and TiO₂-NT| high for low and high EF enhancements, respectively; TiO₂-NT|high|BD-PATP or TiO₂-NT|low| BD-PATP for the electrodes deposited with the dye, benzidine-*p*-aminothiophenolate (BD-PATP)) were used as substrate for the laser-induced photocatalytic degradation of BD-PATP, which was resolved using time-dependent SERR spectroscopy. [32,33]. The degradation rate constant for TiO₂-NT |high| BD-PATP, 5.1 (1.4) s^{-1}, is ~70 % higher than the one measured for TiO₂-NT |low| BD-PATP, 3.0 (0.6) s^{-1}. Applying an external potential of +0.4 V, resulted in a significantly higher (~2.5 times) rate constant for the decay of TiO₂-NT |high| BD-PATP, 25.5 (5) s^{-1}, vs. of TiO₂-NT|low|BD-PATP, 10.1 (3) s^{-1} indicating an improved performance of TiO₂-NT|high vs. TiO₂-NT|low. Degradation was found to depend on the excitation at 413 nm, indicating a degradation pathway involving the excited state of the dye and no contributions from the charges of the TiO₂-NTs. Low-wavenumber Raman measurements at 488 nm show that the E$_g$ mode of the TiO₂-NT|high is downshifted compared to that of TiO₂-NT|low (145.5 cm^{-1} vs. 147.3 cm^{-1}) indicative of possible size-dependent charge-transfer surface enhancement of the Raman signals. The high capacitance values from electrochemical impedance spectroscopy data obtained at negative potentials reveal the poor electron-acceptor character of TiO₂-NTs at these potentials possibly due to proton/cation intercalation, which increases the injected electron-oxidized dye recombination rate. On the other hand, the lower capacities of TiO₂-NTs at positive potentials and of TiO₂-NT|high relative to TiO₂-NT|low, reveal their improved electron-accepting capabilities at higher potentials, necessary for efficient surface-to-bulk electron transport. Based on this, the higher decay rate for TiO₂-NT|high at +0.4 V can be justified by a combined effect of better electron-accepting property and photon-capturing ability of the material.

Enhanced Raman scattering of molecular monolayers [34].

The quest to achieve ultrahigh sensitivity, surface specificity and high spatial resolution has led to the development of plasmon- and chemically- enhanced Raman spectroscopy, including techniques such as SERS and TERS. In one of the projects, gap-mode TERS was used, which allows studying even molecularly thin layers of very weakly scattering molecules. With the nanoscale spatial resolution provided by TERS, the spontaneous segregation in a mixed thiol system on a gold surface could be resolved, while scanning tunneling microscopy (STM) could not discern the nanodomains via their apparent height difference [36]. Furthermore, since graphene and a flat gold surface both are known to provide some Raman enhancement through mainly a chemical mechanism, sandwiching copper phthalocyanine (CuPc) molecules between graphene and a flat gold surface allowed electrons to be transferred in both directions, and thereby to address the question whether chemical enhancements with different origins in SERS can add to each other. The results suggest that the chemical enhancements were influenced by the two individual surfaces, and a 68-fold enhancement of sandwiched CuPc between graphene and gold was observed, as compared to CuPc on mica. TERS was applied to study this sandwiched structure [36]. Molecules self-assembled on a gold surface and covered by transferred graphene acted as optical probes. Such an arrangement has interesting properties in the sense that molecules are protected and encapsulated by graphene. Also, a possible ultrahigh Raman enhancement together with localized spatial resolution may be achieved due to the combined effects from SERS and TERS. The results showed that a tip can improve graphene-enhanced Raman scattering (GERS) further by 4 orders of magnitude, but graphene exerts some shielding effect to gap-mode TERS.

Isolated Graphene Edge Nanoelectrodes: Fabrication, Selective Functionalization, and Electrochemical Sensing [37,38].

A simple photolithography-based method was used to realize the isolated monolayer graphene edge nanoelectrode on an insulating substrate. The millimeter-long and a nanometer-wide graphene edge is found to behave like a nanowire with a high aspect ratio of 1000000-to-1. Further, the use of electrochemical

modification (ECM) is demonstrated to selectively functionalize the graphene edge with metal nanoparticles and organic moieties in a non-covalent/ covalent manner to tune the chemistry of the edge. The attachment of metal nanoparticles was used to exploit SERS to characterize the chemistry of both the pristine and the functionalized graphene edge. The graphene edge electrodes were found to exhibit very high mass transport rates, characteristic of nanoelectrodes. Accordingly, the voltammetric response is found to be dictated by the kinetics of heterogeneous electron transfer (HET), attributed to the nanoscale geometry and a unique diffusional profile at such electrodes. At the graphene edge electrode, high HET rates are observed: at least 14 cm/s for outer-sphere probe, ferrocenemethanol (FcMeOH) with a quasi-Nernstian behavior; and 0.06 cm/s or higher for inner-sphere probe, ferricyanide ($[Fe(CN)_6]^{3-}$) with a kinetically controlled response. Upon selective modification of the edge with gold nanoparticles, the HET is found to be reversible, with a mass-transport-limited Nernstian response for both probes. Furthermore, the fast HET kinetics enables the sensing of the reduced form of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) with low onset potentials and down to low micromolar concentrations. In this work, isolated graphene edge as a new class of nanoelectrode was realized which forms an important basis within the fields of fundamental electrochemistry and analytical sciences.

Interfacial phenomena at the graphene-liquid-interface in nanostructure devices: Faradaic effect, edge-gating and van der Waals heterostructures [37,39,40].

Graphene has been studied broadly as the transducing element in electrochemical and field-effect sensors. Such devices operated in aqueous solution show great potential as label-free sensors for the detection of analytes, e.g. with medical, biological or environmental relevance. At the graphene-liquid-interface, an electrical double-layer (EDL) forms. In graphene field-effect devices, the EDL is reminiscent of a double-plate capacitor. Changes in charge distribution at the EDL can cause a sensor response via electrostatic interactions. In electrochemical measurements, on the other hand, electron transfer processes at the EDL are studied at graphene as a working electrode. Both approaches share similar set-ups, as in both cases the potential at graphene is modulated by a reference electrode. Several aspects of the design and function of sensors based on graphene operated in liquid have been investigated, providing new insight into fundamental processes at the graphene-liquid-interface. Using photolithographic methods, field-effect transistors (FET) based on graphene have been fabricated. The pH sensitivity of graphene was revisited and established as a reference characterization tool for the devices. First, the effect of the presence of redox active molecules in the analyte solution during the operation of electrochemically gated graphene FETs was explored. During operation, heterogeneous electron transfer may occur at relevant potentials leading to Faradaic currents at the graphene channel. These lead to doping-like shifts of the transfer curve of graphene, as the graphene-liquid-interface represents a partially polarizable electrode. The occurrence of these shifts depends mainly on the relation between the Dirac point of graphene and the standard potential of the redox species. This relation determines whether cathodic or anodic currents are present dictating the direction of the Dirac point shift. Due to the origin of the shifts, this observation is termed Faradaic effect. It is fundamentally different from typically discussed transduction mechanisms like electrostatic interactions. Parameters influencing the direction and magnitude of the Faradaic effect are discussed in detail, e.g., the shifts are the stronger, the larger the area of the graphene channel. The edge of graphene represents a nanoscopic one-dimensional defect of the two-dimensional material. It attracts great attention due to its special properties [38,39]. A new type of graphene FET was introduced based on electrochemical gating of graphene exclusively via the EDL at its edge. To achieve edge-gating, the basal part of graphene is passivated by a photoresist and shielded entirely from interaction with the solution. Devices with narrow graphene widths (< 1 μm) below the resist showed ON/OFF ratios of up to 10. Furthermore, it was demonstrated that the edge of graphene can be functionalized covalently via electrografting. This changes the charge density at the graphene edge-liquid-interface effectively, while maintaining the favorable transfer characteristics of the devices. This

strategy may pave the way for new graphene based one-dimensional sensors with high selectivity. Finally, a novel approach towards graphene edge devices was pursued in the form of hexagonal boron nitride (hBN) encapsulated graphene. The graphene edge is exposed at the edge of such van der Waals heterostructures. Electrochemical gating and charge transfer were probed on the edge of these heterostructures. Especially for electroanalytical applications, these devices are promising due to low interfacial capacitance at the EDL leading to small capacitive currents, while high Faradaic currents could be observed for two analytes, ferrocene methanol and the neurotransmitter dopamine. The detection of both species at scan rates of up to 1000 V/s was demonstrated in fast-scan cyclic voltammetry, which is promising for the detection of dopamine *in vivo*, where high temporal resolution is required.

5.3 SERS for studies of plasmon-driven chemistry

Plasmonic metal nanostructures can be tuned to efficiently interact with light, converting the photons into energetic charge carriers and heat. Therefore, the plasmonic nanoparticles such as gold and silver nanoparticles act as nano-reactors, where the molecules attached to their surfaces benefit from the enhanced electromagnetic field along with the generated energetic charge carriers and heat for possible chemical transformations. Hence, plasmonic chemistry presents metal nanoparticles as a unique playground for chemical reactions on the nanoscale remotely controlled by light. However, defining the elementary concepts behind these reactions represents the main challenge for understanding their mechanism in the context of the plasmonically assisted chemistry. In two projects [41,42], SERS was used to probe the dimerization reaction of 4-nitrothiophenol (4-NTP, PNTP) as a model example of plasmonic chemistry (Fig 4) [6,44]. It was demonstrated that plasmonic nanostructures such as gold nanotriangles and nanoflowers have a high SERS efficiency, as evidenced by probing the vibrations of R6G and 4-NTP. The high signal enhancement enabled the measurements of SERS spectra with a short acquisition time, which allows monitoring the kinetics of chemical reactions in real time. To get insight into the reaction mechanism, several time-dependent SERS measurements of the 4-NTP were performed under different laser and temperature conditions. Analysis of the results within a mechanistic framework showed that the plasmonic heating significantly enhances the reaction rate, while the reaction is probably initiated by the energetic electrons [6,43]. The reaction was shown to be intensity-dependent. First attempts to scale up the plasmonic catalysis have been performed showing the necessity to achieve the reaction threshold intensity. Meanwhile, the induced heat needs to quickly dissipate from the reaction substrate, since otherwise the reactants and the reaction platform melt. The results might open the way for further work seeking the possibilities to quickly dissipate the plasmonic heat generated during the reaction and therefore, scaling up the plasmonic catalysis.
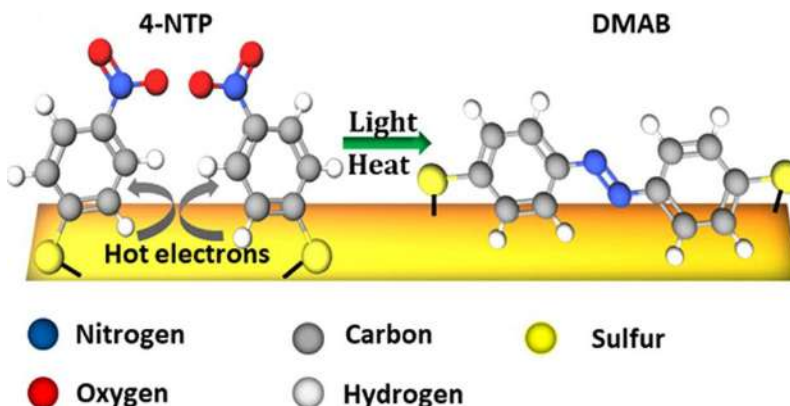

Fig 4. Schematic of the formation of 4,4′-dimercaptoazobenzene (DMAB) from 4-aminothiophenol (4-ATP, PATP) on a plasmonic surface. Reproduced with permission from ref. [6]. © 2019, the authors of ref. [6].

One of the central concepts in plasmon-assisted chemistry is the utilization of hot carriers for the reaction. Hot carriers, generated from the non-radiative decay of the localized surface plasmons, can induce redox reactions of molecules adsorbed on metal nanoparticles. Determining catalytic properties and understanding the underlying mechanisms of a catalytic reaction helps to utilize heterogeneous catalysis in new applications, and to screen catalyst materials. It is challenging for conventional analytical methods to study heterogeneous catalysis in situ, mostly due to the insufficient sensitivity and selectivity. In another project, SERS was used to study other heterogeneous reactions as well, plasmonic and non-plasmonic [42]. Plasmon-catalyzed redox reactions of the model SERS probes, *p*-nitrothiophenol (PNTP, 4-NTP) and *p*-aminothiophenol (PATP, 4-ATP), were investigated under different conditions. Specifically, the role of metal ions in the plasmon-catalyzed oxidation reaction of PATP on metal nanoparticles [43] and the enhancement by ligands in the hot electron reduction of PNTP on metal nanoparticles was demonstrated [44]. Based on the observation of ligand-supported light harvesting in this and other model reactions, the role of Cl$^-$ ions in the pH reversible response of the PATP SERS spectrum could be elucidated [46] and a mechanism for the hot electron reduction on silver nanoparticles was proposed. Moreover, new optofluidic approaches including a SERS mapping and a new reusable SERS microfluidic platform were proposed [47]. They are specifically used for the characterization of nanoparticle-catalyzed reactions and enable new detailed findings on the inhomogeneity of plasmonic catalysis on supported gold nanostructures, the role of O_2 in PATP dimerization, and the formation of an anion radical intermediate in the reduction of PNTP by $NaBH_4$ on gold. As application, a method for surface molecular patterning on supported metal nanoparticles using plasmonic catalysis was shown (Fig 5) [48]. The results have important implications for future studies of heterogeneous catalytic reactions by SERS.
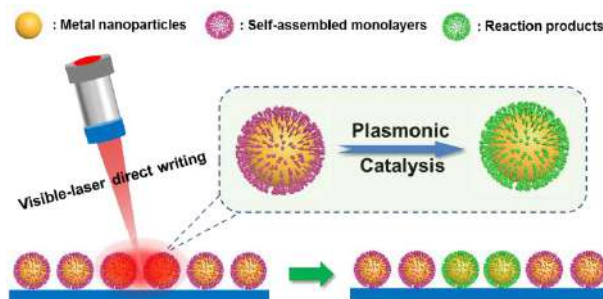

Fig 5. Schematic illustration of surface molecular patterning of self-assembled monolayers on a plasmonic substrate based on direct writing with a laser operating in the visible wavelength range. The self assembled monolayers can be formed by different molecules, proof-of-principle was reported for several plasmon-catalyzed reactions: the formation of DMAB from PNTP (4-NTP), of DMAB from PATP (4-ATP), and the reduction of PNTP to PATP. The obtained molecular patterns can be erased by other reactions, enabling different strategies for rewriting, encryption, or stepwise functionalization. Reproduced with permission from ref. [48]. © 2021, American Chemical Society.

5.4 SERS in bioenvironments: From molecules and materials to whole cells

Combined vibrational spectroscopy and electrochemistry for structural studies of a hemoprotein sensor [31].

In the putative hemoprotein redox sensor MsmS(-sGAF2) from *M. acetivorans*, resonance Raman (RR) spectroscopy revealed that a six-coordinated low spin (6cLS) and a five-coordinated high spin (5cHS) state is the prevailing configuration in the oxidized and reduced state, respectively [49]. Comparative studies on engineered variants showed that H702 is most likely the proximal axial ligand which serves as a potent hydrogen-bond donor to a nearby amino acid residue. This conclusion is based on the relatively low wavenumber and the H/D sensitivity of the ν_4 mode of the 5cHS ferrous heme, as well as the upshift of the ν_4 mode of the 5cHS form in the reduced state of the H702 mutant variants (H702G, K661G/H702G,

and H702/708G). The findings are consistent to those for the analogous protein MA0863 for which similar effects on the ν_4 mode have been observed. An increased amount of heme groups in the 5cHS configuration was noted when polar amino acid residues of MsmS-sGAF2 in the distal region were mutated (Y665F and H646A). Temperature-dependent RR spectroscopy revealed that the 6th ligand acts as a strong ligand as reflected by the gradual decrease in the 5cHS/6cLS ratio with increasing temperatures. An energy-minimized structural model confirmed that the distal pocket region with its polar residues can accommodate a small polar molecule. These studies suggest that the second-coordination sphere plays an important role for MsmS's ligand-binding function, which could influence the heme redox state. Using surface-enhanced resonance Raman (SERR) spectroscopy and infrared absorption spectroscopic techniques, MsmS-sGAF2 was found to display redox reversibility accompanied by reversible conformational changes. SERR spectroscopic redox titration of MsmS-sGAF2 immobilized on 8-aminooctanethiolate|roughened silver yielded midpoint potentials (E_{midpt}) for the $Fe^{3+/2+}$ transition of –0.28 V (vs. Ag/AgCl, 3 M KCl) and –0.29 V for the redox transitions of 5cHS and 6cLS species for the aerobic BL21(DE3) *E. coli* preparation, respectively, as well as –0.32 V and –0.20 V for the 5cHS and 6cLS species for the anaerobic Nissle 1917 *E. coli* preparation, respectively. The average values (5cHS+6cLS) are similar to that obtained from cyclic voltammetry of the anaerobic preparation, E_{midpt} = –0.297 V.

Structure and composition of the protein corona in animal cells

The characterization of the protein-nanoparticle interactions in complex biomolecular systems such as a living cell is vital for pharmaceutical, medical, and environmental research fields. In such biomolecular systems, proteins readily adsorb on the surface of nanoparticles forming the protein corona. As was shown, SERS, together with other analytical approaches, can be used to characterize the protein corona in living cells combining different analytical approaches. SERS experiments on pure protein solutions revealed the concentration dependence of the protein-gold nanoparticle interactions resulting in different SERS spectra, and allowed for the determination of protein segments binding to citrate-stabilized gold nanoparticles [50,51]. In live cell SERS experiments, the presence of protein fragments in the innermost layer of the protein corona, called the hard protein corona, was revealed [52]. An analytical method combining sodium dodecyl sulfate-polyacrylamide gel electrophoresis and high-performance liquid chromatography-coupled electrospray ionization mass spectrometry was developed to identify the constituents of the hard protein corona [53]. The proteomics, SERS, and cryo soft X-ray nanotomography data, the latter providing information of the three dimensional ultrastructure of the cell, reveal the uptake mechanism, processing, accumulation site, molecular environment, and the induced cellular responses of internalized gold nanoparticles [54]. This work validates the use of SERS in the analysis of the protein corona in the solution of model proteins and in living cells, and presents a suitable method for the analysis of the unaltered hard protein corona formed in living cells.

SERS as an approach to monitor lysosomal function.

Lysosomes are cellular compartments that play a crucial role in cellular homeostasis. Monitoring lysosomes that accumulate lipids represents a considerable challenge. SERS approaches were adapted to monitor intact lysosomes, in particular regarding the influence of drugs that interfere with lipid metabolism. To evaluate the potential of SERS for studying lysosomes in live cells, the interactions between tricyclic antidepressants and acid sphingomyelinase were studied. First, model systems were investigated. The interactions between the antidepressants and gold nanoparticles were characterized by SERS [55]. The data showed that molecules interact with the nanoparticles. As a model system of the lipid-rich environment, composites of liposome and gold nanoparticles were studied by SERS and cryo-EM [56]. The SERS spectra are characteristic of the vesicles' lipid composition. The interactions between the antidepressants and the lysosomes were studied in the fibroblast cell line 3T3 by SERS and complementary methods [57]. In agreement with the SERS spectra of model systems, the SERS spectra of live cells show signatures of both, the antidepressants and the lipids. To reveal the differences in the lysosomes between treated and non-treated cells, a random forest approach was used [57]. Moreover, SERS was used to study the lipid distribution in

Leishmania-infected macrophages known to accumulate lipids (Fig 6) [58]. The results show that SERS can be used to investigate lipid composition in live cells of different cell types. As a new methodological development, the random forest analysis of SERS data shows that machine learning approaches can be useful for a better understanding of data from biological systems.
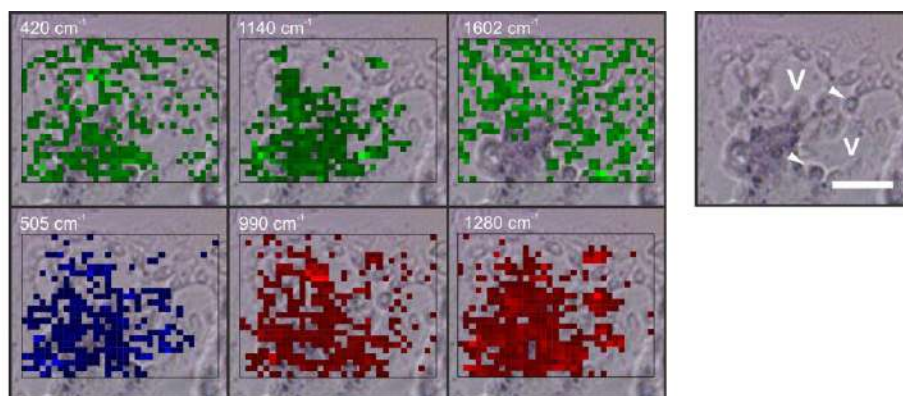

Fig 6. Distribution of SERS signals that are related to molecular species characteristic of the infection by *Leishmania mexicana*, representing lipids (green), proteins (blue), and phosphate (red) species together with bright field images of the investigated cell. The chemical images are generated by mapping the intensity of the bands at 420 cm^{-1} assigned to cholesterol, 505 cm^{-1} of disulfide in proteins, 990 cm^{-1} mainly assigned to phosphate in phosphoproteoglycans, 1140 cm^{-1} of phospholipid alkyl chains, 1278 cm^{-1} of phosphate in proteins and phopholipids, and 1602 cm^{-1} of ergosterol, which is specific for the parasite. The cells that contain the parasites in vacuoles (V) were incubated with gold nanoparticles that serve as plasmonic substrate. Arrowheads in the brightfield image (right most) indicate individual parasites; Scale bar: 10 μm. Reproduced with permission from ref. [58]. © 2018, American Chemical Society.

5.5 Raman in multimodal approaches to characterize plant silicification

The topic of bio-silicification is part of a series of research projects dealing with biomineralization and interfaces in materials. Apart from discussions and seminars several doctoral research projects were co-supervised by SALSA PIs on silicification, and promising new results by SALSA fellows were obtained. The approach to study the composition of the silicifying plant tissues combines plant physiology with state-of-the-art materials analysis. The main objective of this research is to unravel the chemical and molecular mechanisms involved in plant bio-silicification [59]. This can open new ways to improve crop plants. Silica deposition is a common phenomenon that correlates with plant tolerance to various stresses. Plants accumulate amorphous silica in microscopic particles termed phytoliths, through yet unclear mechanisms. With the aim to gain better understanding of the processes that govern silica deposition, different vibrational techniques were used on sorghum leaves and molecular models to obtain chemical and structural information addressing different length scales. Solid-state Nuclear Magnetic Resonance and thermogravimetric analysis showed that phytolith extraction methods affect silica structure. Nevertheless, Raman and IR analysis of individual phytoliths revealed differences in the structure and composition between phytolith types (Fig 7), suggesting the existence of different biological pathways for silica deposition [7]. The environment of sorghum tissues where silica is deposited was assessed using a multimodal approach consisting of fluorescence, brightfield and scanning electron microscopies, while chemical composition was mapped using Raman and Fourier transform infrared microspectroscopy. Scattering-type near-field optical microscopy in the mid-infrared region was used to characterize the plant tissues, in both fixed and native plant samples. The nano-IR images and the mechanical phase image enabled a combined probing of mechanical material properties together with the chemical composition and structure of both the cell walls and the phytolith structures [60]. *In vitro* reactions simulating lignin-silica co-precipitation and silica polymerization with peptides revealed strong

interaction between these compounds and silica, and their possible involvement in silica deposition in the plant. This project provided a better understanding of the chemical process that control plant silicification, suggests new methodologies to characterize plant samples, and evaluated the current methods used in plant science.
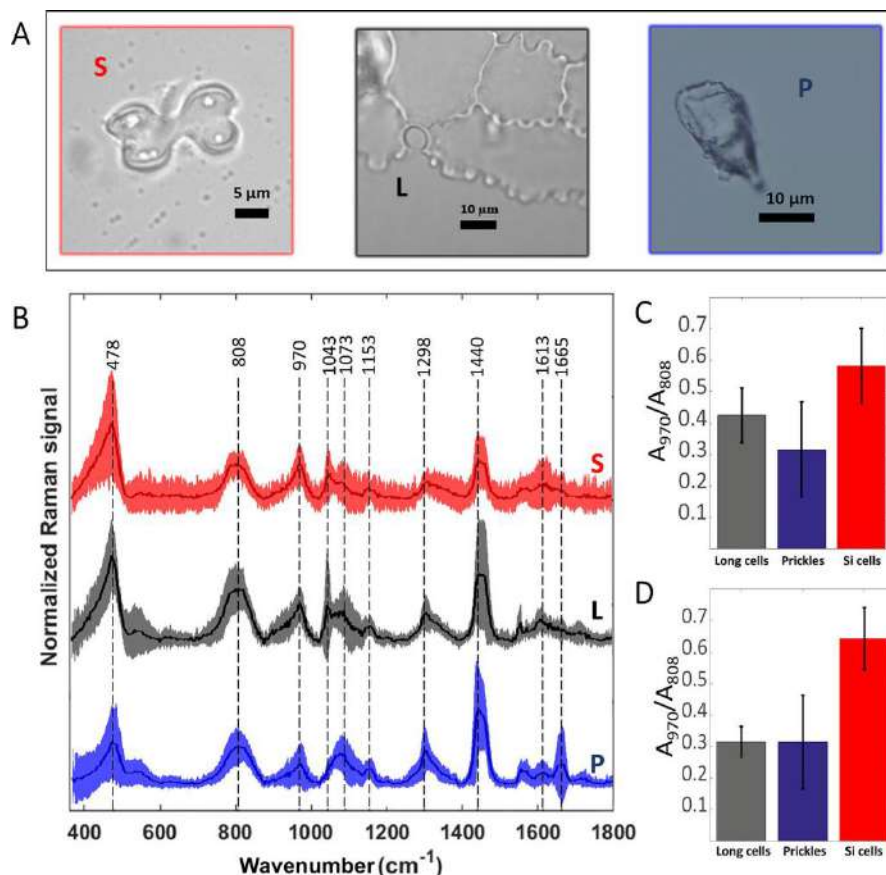

Fig 7. Preprocessed mean Raman spectra of the most abundant sorghum leaf phytoliths and intensity ratio of the Si-OH to Si-O-Si Raman bands. Bilobate silica cells (S, red), long cells or plates (L, black) and prickles (P, blue) are shown in bright-field micrographs **(A)**. Mean spectra ± standard deviation are plotted in the same respective colour and denoted with the same abbreviations **(B)**. Averages of 25 spectra of phytoliths of each type extracted by microwaved-assisted digestion (MAD) are shown. The area of the peak at 970 cm^{-1}, assigned to Si-OH surface groups, was normalized to the area of the 808 cm^{-1} band, assigned to Si-O-Si stretching. Ratios of band areas calculated in spectra of bilobate cells were significantly higher ($p < 0.05$) than both prickles and long cells under MAD **(C)** and sulphuric acid-hydrogen peroxide-nitric acid extraction (SONE) **(D)** methods. Reproduced with permission from ref. [7]. ©2019, the authors of ref. [7].

6 Conclusions

The overview of our research results given here illustrates that new developments in harnessing new materials and molecules for the more sensitive and specific Raman characterization of matter originate in collaboration between spectroscopists of different disciplines, applying new protocols for synthesis and for complementary characterization. It also enables addressing specific analytical problems from the perspective of a particular sample. Moreover, we have described a few of the measures that in our opinion are key to ensure gender equality in any field of science.

Author information

JK and UP are co-founders of the program, all other authors are alumni of the SALSA doctoral program and graduated between 2016 and 2021.

Acknowledgements

References

1. Montes-Bayón M, Kneipp J, Panne U, Report on KOSMOS Summer University at the School of Analytical Sciences Adlershof (Berlin): limits and scales in analytical sciences, *Analytical and Bioanalytical Chemistry*), 407(2015) 4869–4872.

2. Schmid T, Jungnickel R, Dariz P, Insights into the $CaSO_4$–H_2O system: A Raman-spectroscopic study, *Minerals*, 10(2020)115; doi.org/10.3390/min10020115.

3. Schmid T, Jungnickel R, Dariz P, Raman band widths of anhydrite II reveal the burning history of high-fired medieval gypsum mortars, *J Raman Spectrosc*, 50(2019)1154–1168.

4. Dariz P, Schmid T, Phase composition and burning history of high-fired medieval gypsum mortars studied by Raman microspectroscopy, *Mater Charact*, 151(2019)292–301.

5. Dariz P, Schmid T, Ferruginous phases in 19th century lime and cement mortars: A Raman microspectroscopic study, *Mater Charact*, 129(2017)9–17.

6. Sarhan R M, Koopman W, Schuetz R, Schmid T, Liebig F, Koetz J, Bargheer M, The importance of plasmonic heating for the plasmon-driven photodimerization of 4-nitrothiophenol, *Sci Rep*, 9(2019)3060; doi.org/10.1038/s41598-019-38627-2.

7. Zancajo V M R, Diehn, S.; Filiba, N.; Goobes, G.; Kneipp, J.; Elbaum, R., Spectroscopic Discrimination of Sorghum Silica Phytoliths, *Front Plant Sci,* 10(2019); doi.org/10.3389/fpls.2019.0157.

8. Bartkowiak D, MgF_2-coated gold nanostructures as a plasmonic substrate for analytical applications, Doctoral Thesis, (Humboldt-Universität zu Berlin), 2018.

9. Bartkowiak D, Merk V, Reiter-Scherer V, Gernert U, Rabe J P, Kneipp J, Kemnitz E, Porous MgF_2-over-gold nanoparticles (MON) as plasmonic substrate for analytical applications, *RSC Adv*, 6(2016)71557–71566.

10. Gkogkou D. Anisotropic plasmonic nanoparticle arrays for surface-enhanced biosensors, Doctoral Thesis, (Technische Universität, Berlin, Berlin), 2017.

11. Gkogkou D, Schreiber B, Shaykhutdinov T, Ly H K, Kuhlmann U, Gernert U, Facsko S, Hildebrandt P, Esser N, Hinrichs K, Weidinger I M, Oates T W H, Polarization- and Wavelength-Dependent Surface-Enhanced Raman Spectroscopy Using Optically Anisotropic Rippled Substrates for Sensing, *ACS Sensors*, 1(2016)318–323.

12. Gkogkou D, Shaykhutdinov T, Oates T W H, Gernert U, Schreiber B, Facsko S, Hildebrandt P, Weidinger I M, Esser N, Hinrichs K, Characterization of anisotropically shaped silver nanoparticle arrays via spectroscopic ellipsometry supported by numerical optical modeling, *Appl Surf Sci*, 421(2017)460–464.

13. Gkogkou D, Shaykhutdinov T, Kratz C, Oates T W H, Hildebrandt P, Weidinger I M, Ly K H, Esser N, Hinrichs K, Gradient metal nanoislands as a unified surface enhanced Raman scattering and surface enhanced infrared absorption platform for analytics, *Analyst*, 144(2019)5271–5276.

14. Heck C, Gold and silver nanolenses self-assembled by DNA origami, (Doctoral Thesis, University of Potsdam), 2018.

15. Heck C, Prinz J, Dathe A, Merk V, Stranik O; Fritzsche W; Kneipp J, Bald I, Gold Nanolenses Self-Assembled by DNA Origami, *ACS Photonics*, 4(2017)1123–1130.

16. Prinz J, Heck C, Ellerik L, Merk V, Bald I, DNA origami based Au-Ag-core-shell nanoparticle dimers with single-molecule SERS sensitivity, *Nanoscale*, 8(2016)5612–5620.

17. Heck C, Kanehira Y, Kneipp J, Bald I, Placement of Single Proteins within the SERS Hot Spots of Self-Assembled Silver Nanolenses, *Angew Chem Int Ed*, 57(2018)7444–7447.

18. Heck C, Kanehira Y, Kneipp J, Bald I, Amorphous carbon generation as a photocatalytic reaction on DNA-assembled gold and silver nanostructures, *Molecules*, 24(2019)2324; doi.org/10.3390/molecules24122324.

19. Pavlenko E, Hybrid nanolayer architectures for ultrafast acousto-plasmonics in soft matter, Doctoral Thesis, (University of Potsdam), 2016.

20. Pavlenko E S, Sander M, Mitzscherling S, Pudell J, Zamponi F, Rossle M, Bojahr A, Bargheer M, Azobenzene - functionalized polyelectrolyte nanolayers as ultrafast optoacoustic transducers. *Nanoscale*, 8(2016)13297–13302.

21. Pavlenko E S, Sander M, Cui Q, Bargheer M, Gold Nanorods Sense the Ultrafast Viscoelastic Deformation of Polymers upon Molecular Strain Actuation, *J Phys Chem C*, 120(2016)24957–24964.

22. Rezania B, Optical spectroscopy and scanning force microscopy of small molecules intercalated within graphene and graphene oxide interfaces, Doctoral Thesis, (Humboldt-Universität, zu Berlin), 2022.

23. Xing Y, Metal-organic nanowires, Doctoral Thesis, (Technische Universität, Berlin), 2016.

24. Xing Y, Wyss A, Esser N, Dittrich P S, Label-free biosensors based on in situ formed and functionalized microwires in microfluidic devices, *Analyst*, 140(2015)7896–7901.

25. Xing Y, Esser N, Dittrich P S, Conductive single nanowires formed and analysed on microfluidic devices, *J Mater Chem C*, 4(2016)9235–9244.

26. Xing Y, Sun G, Speiser E, Esser N, Dittrich P S, Localized Synthesis of Conductive Copper–Tetracyanoquinodimethane Nanostructures in Ultrasmall Microchambers for Nanoelectronics. *ACS Appl Mater Interface*, 9(2017)17271–17278.

27. Xing Y, Speiser E, Singh D K, Dittrich P S, Esser N, Bi-Axial Growth Mode of Au–TTF Nanowires Induced by Tilted Molecular Column Stacking, *J Phys Chem C*, 121(2017)23200–23206.

28. Ries M D C, Optical analysis of InN and InGaN nanostructures, Doctoral Thesis, (Technische Universität, Berlin, Berlin), 2021.

29. Poliani E, Seidlitz D, Ries M, Choi S J, Speck J S, Hoffmann A, Wagner M R, Strong Near-Field Light–Matter Interaction in Plasmon-Resonant Tip-Enhanced Raman Scattering in Indium Nitride, *J Phys Chem C*, 124(2020)28178–28185.

30. Seidlitz D, Poliani E, Ries M, Hoffmann A, Wagner M R, Nanoscale InN clusters and compositional inhomogeneities in InGaN epitaxial layers quantified by tip-enhanced Raman scattering, *Appl Phys Lett*, 118(2021)162107; doi.org/10.1063/5.0040760.

31. Querebillo C J U, Combined vibrational spectroscopy and electrochemistry for studying biological and materials systems, Doctoral Thesis, (Technische Universität Berlin, Berlin), 2020.

32. Querebillo C J, Öner I H, Hildebrandt P, Ly K H, Weidinger I M, Accelerated Photo-Induced Degradation of Benzidine-p-Aminothiophenolate Immobilized at Light-Enhancing TiO_2 Nanotube Electrodes, *Chem Eur J*, 25(2019) 16048–16053.

33. Öner I H, Querebillo C J, David C, Gernert U, Walter C, Driess M. Leimkühler S, Ly K H, Weidinger I M, High Electromagnetic Field Enhancement of TiO_2 Nanotube Electrodes, *Angew Chem Int Ed*, 57(2018)7225–7229.

34. Lin W.-I, Enhanced Raman scattering of molecular monolayers, Doctoral Thesis, (Humboldt-Universität zu Berlin), 2017.

35. Lin W I, Shao F, Stephanidis B, Zenobi R, Tip-enhanced Raman spectroscopic imaging shows segregation within binary self-assembled thiol monolayers at ambient conditions, *Anal and Bioanal Chem*, 407(2015)8197–8204.

36. Lin W.-I, Gholami M F, Beyer P, Severin N, Shao F, Zenobi R, Rabe J P, Strongly enhanced Raman scattering of Cu-phthalocyanine sandwiched between graphene and Au(111), *Chem Commun*, 53(2017)724–727.

37. Yadav A, Iost R M; Neubert T J, Baylan S, Schmid T, Balasubramanian K, Selective electrochemical functionalization of the graphene edge, *Chem Sci*, 10(2019)936–942.

38. Yadav A, Wehrhold M, Neubert T J, Iost R M, Balasubramanian K, Fast Electron Transfer Kinetics at an Isolated Graphene Edge Nanoelectrode with and without Nanoparticles: Implications for Sensing Electroactive Species, *ACS Appl Nano Mater*, 3(2020)11725–11735.

39. Neubert T J, Wehrhold M, Kaya N S, Balasubramanian K, Faradaic effects in electrochemically gated graphene sensors in the presence of redox active molecules, *Nanotechnology,* 31(2020); doi.org/10.1088/1361-6528/ab98bc.

40. Wehrhold M, Neubert T J, Yadav A, Vondráček M, Iost R M, Honolka J, Balasubramanian K, pH sensitivity of interfacial electron transfer at a supported graphene monolayer, *Nanoscale*, 11(2019)14742–14756.

41. Sarhan R M, Plasmon-driven photocatalytic reactions monitored by surface-enhanced Raman spectroscopy, Doctoral Thesis, (University of Potsdam), 2019.

42. Zhang Z, *In situ* Characterization of Plasmonic Catalysis Using Surface-enhanced Raman Scattering, Doctoral Thesis, (Humboldt-Universität zu Berlin), 2018.

43. Sarhan R M, Koopman W, Pudell J, Stete F, Rössle M, Herzog M. Schmitt C N Z, Liebig F, Koetz J, Bargheer M, Scaling Up Nanoplasmon Catalysis: The Role of Heat Dissipation, *J Phys Chem C*, 123(2019)9352–9357.

44. Zhang Z, Merk V, Hermanns A, Unger W E S, Kneipp J, Role of Metal Cations in Plasmon-Catalyzed Oxidation: A Case Study of *p*-Aminothiophenol Dimerization, *ACS Catal*, 7(2017)7803–7809.

45. Zhang Z, Li Y, Frisch J, Bär M, Rappich J, Kneipp J, *In situ* surface-enhanced Raman scattering shows ligand-enhanced hot electron harvesting on silver, gold, and copper nanoparticles, *J Catal*, 383(2020)153–159.

46. Zhang Z, Kneipp J, Ligand-supported hot electron harvesting: Revisiting the pH-responsive surface-enhanced Raman scattering spectrum of *p*-aminothiophenol, *J Phys Chem Lett*, 12(2021)1542–1547.

47. Zhang Z, Gernert U, Gerhardt R F, Höhn E M, Belder D, Kneipp J, Catalysis by Metal Nanoparticles in a Plug-In Optofluidic Platform: Redox Reactions of *p*-Nitrobenzenethiol and *p*-Aminothiophenol, *ACS Catal*, 8(2018)2443–2449.

48. Zhang Z, Kneipp J, Surface Molecular Patterning by Plasmon-Catalyzed Reactions, *ACS Appl Mater Interfaces*, 13(2021)43708–43714.

49. Fiege K, Querebillo C J, Hildebrandt P, Frankenberg-Dinkel N, Improved Method for the Incorporation of Heme Cofactors into Recombinant Proteins Using Escherichia coli Nissle 1917, *Biochemistry*, 57(2018)2747–2755.

50. Szekeres G P, Kneipp J, Different binding sites of serum albumins in the protein corona of gold nanoparticles, *Analyst*, 143(2018)6061–6068.

51. Szekeres G P, Kneipp J, SERS probing of proteins in gold nanoparticle agglomerates, *Front Chem*, 7(2019)30; doi.org/10.3389/fchem.2019.00030.

52. Szekeres G P, Montes-Bayón M, Bettmer J, Kneipp J, Fragmentation of Proteins in the Corona of Gold Nanoparticles as Observed in Live Cell Surface-Enhanced Raman Scattering, *Anal Chem*, 92(2020)8553–8560.

53. Szekeres G P, Fernández-Iglesias N, Kneipp J, Montes-Bayón M, Bettmer J, Mass spectrometric approach for the analysis of the hard protein corona of nanoparticles in living cells, *J Proteomics*, 212(2020)103582; doi.org/10.1016/j.jprot.2019.103582.

54. Szekeres P G, Werner S, Guttmann P, Spedalieri C, Drescher D, Živanovic V, Montes-Bayón M, Bettmer J, Kneipp J, Relating the composition and interface interactions in the hard corona of gold nanoparticles to the induced response mechanisms in living cells, *Nanoscale*, 12(2020)17450–17461.

55. Živanović V, Madzharova F, Heiner Z, Arenz C, Kneipp J, Specific Interaction of Tricyclic Antidepressants with Gold and Silver Nanostructures as Revealed by Combined One- and Two-Photon Vibrational Spectroscopy, *J Phys Chem C*, 121(2017)22958–22968.

56. Živanović V, Kochovski Z, Arenz C, Lu Y, Kneipp J, SERS and Cryo-EM Directly Reveal Different Liposome Structures during Interaction with Gold Nanoparticles, *J Phys Chem Lett*, 9(2018)6767–6772.

57. Živanović V, Seifert S, Drescher D, Schrade P, Werner S, Guttmann P, Szekeres G P, Bachmann S, Schneider G, Arenz C, Kneipp J, Optical Nanosensing of Lipid Accumulation due to Enzyme Inhibition in Live Cells, *ACS Nano*, 13(2019)9363–9375.

58. Živanović V, Semini G, Laue M, Drescher D, Aebischer T, Kneipp J, Chemical mapping of Leishmania infection in live cells by SERS microscopy, *Anal Chem*, 90(2018)8154–8161.

59. Rodriguez Zancajo V M, Vibrational spectroscopy as a tool to understand plant silicification, Doctoral Thesis, (Humboldt-Universität, zu Berlin), 2021.

60. Zancajo V M R, Lindtner T, Eisele M, Huber A J, Elbaum R, Kneipp J, FTIR Nanospectroscopy Shows Molecular Structures of Plant Biominerals and Cell Walls, *Anal Chem*, 92(2020)13694–13701.