The structural organization of cellular membranes has an essential influence on their functionality. The membrane surfaces currently are considered to consist of various distinct patches, which play an important role in many processes, however, not all parameters such as size and distribution are fully determined. In this study, purple membrane (PM) patches isolated from *Halobacterium salinarum* were investigated in a first step using TERS (tip-enhanced Raman spectroscopy). The characteristic Raman modes of the resonantly enhanced component of the purple membrane lattice, the retinal moiety of bacteriorhodopsin, were found to be suitable as PM markers. In a subsequent experiment a single *Halobacterium salinarum* was investigated with TERS. By means of the PM marker bands it was feasible to identify and localize PM patches on the bacterial surface. The size of these areas was determined to be a few hundred nanometers.

1. Introduction

Cell membranes are part of every living organism and thus are the structural basis of life as we know it. Mainly consisting of amphiphilic lipids and a variety of proteins, the membrane acts as a selective barrier between the cytoplasm and the surrounding medium. Many important mechanisms take place at this site, ranging from transport of nutrients and waste to cell signalling events [1–3]. For a long time the so-called fluidic mosaic model, proposed by Singer and Nicolson in 1972, as an organisational basis of the membrane remained unchallenged. In this model comparatively few proteins are immersed in lipids and every molecule diffuses randomly in two dimensions [4]. In reality, however, a manifold of...
proteins fill the membrane surface and the distribution is nowhere near random. In fact, it is dominated by structurally and functionally distinct patches consisting of domains with a specific composition. Examples are so called lipid rafts, which are known to be segregated regions in cell surfaces or even in pure lipid vesicles [5]. Consequently, the new model predicts membranes with variable patchiness, thickness and higher protein occurrences than considered before [6]. Since the exact composition, the typical size and variability of such segregated regions are mostly unknown, the need of analytical tools to unravel these parameters is obvious.

An attractive method to elucidate nanoscale surface domains is tip-enhanced Raman spectroscopy (TERS) [7–10]. By combining surface-enhanced Raman spectroscopy (SERS) [11–13] with atomic force microscopy (AFM) or scanning tunnelling microscopy (STM), TERS provides a sensitive, molecule specific and non-destructive approach with a spatial resolution in the range of at least 10 nm [14–16]. The resolution is determined by a single metal nanoparticle attached to the apex of a commercial AFM tip. When a laser beam is focused on such a particle, localized surface plasmon polaritons are excited, which can enhance the Raman signals by several orders of magnitude. Consequently, spectroscopic information from a sample volume well below the optical diffraction limit is gained. Since spectral and topographic information are collected simultaneously, a structurally specific characterization of the sample surface is feasible. Compared to other techniques no additional labels are required, which favours TERS over fluorescence based imaging techniques where an interference due to the label is difficult to rule out [17–19]. TERS has been already applied to several biological samples [20–24]. Of particular interest for the present studies are TERS investigations on human cell surfaces [25], cell organelles [26] and membrane models [27, 28], the application to specific membranes is straightforward.

In this contribution the target is the characterization and localization of specific membrane domains on the bacterial surface of the archean Halobacterium salinarum [29, 30] on the nanometer scale with TERS. Isolated purple membrane (PM) patches were investigated for comparison and as a reference prior to the actual experiments on the more complex bacterial surface. The inner membrane of the organism consists of two parts: the red membrane, the basis of the plasma membrane, and the PM, which forms patches in the plasma membrane. However, a distinctive knowledge regarding size and distribution does not exist yet.

To differentiate between specific membrane constituents and their environment the resonantly enhanced retinal moiety of bacteriorhodopsin (bR) within the purple membrane is utilized. With a fraction of 75% the purple membrane consists of bR surrounded by a phospholipid matrix [31, 32]. The bacteriorhodopsin acts as a light-driven proton pump and the resulting electrochemical gradient is applied for energy storage in the cell [33–37].

2. Experimental

2.1 Materials

Wild-type bacteriorhodopsin isolated from Halobacterium salinarum strain S9 as purple membrane (protein to lipid ratio ~3:1 [32]) was purchased from Sigma Aldrich. Salts for the buffer solution (150 mM KCl, 10 mM Tris, 2 mM CaCl2 in water, pH 7.4) were obtained either from Sigma Aldrich or Merck.

2.2 Sample preparation

2.2.1 Bacteria and isolated purple membrane

Halobacterium salinarum S9 cells were grown according to Oesterheldt and Stoeckenius (1971) [38]. After inoculation in 3 mL medium and growth for 48 h at 42 °C and 110 rpm in a shaker the culture was turbid. With this pre-culture 50 mL medium were inoculated and grown in the dark until a turbidity of OD600 0.9 was reached. This culture was transferred to another shaker and exposed to light for 2 days to express sufficient amounts of bacteriorhodopsin. Small amounts (10–20 μL) of the halobacterial cell culture were dropped on glass plates and dried. The evaporation of water must not happen too fast, since it would stress the cells, yielding a salt covered sample. Slow evaporation leads to areas of varying salt content. Low/no-salt areas alternate with high-salt areas. Large salt crystals were removed mechanically. Suitable bacteria (5–10 μm long, 1–2 μm wide) for the measurements were found between salt dendrites in locations of low salt content.

For the direct Raman and TERS investigation of isolated patches, PM fragments dissolved in aqueous buffer solution (10 μL, 1 μM) were deposited on a glass cover slide and dried under argon.

2.3 Instrumental setups

2.3.1 TERS setup

General features of the setup used for TERS measurements on Halobacterium salinarum and on isolated purple membrane patches have been described.
in detail previously [9, 39]. Particular care was taken to exclude fake signals by tip contamination during the TERS measurement by repeated reference measurements [40]. An inverted micro-Raman system in backscattering mode (HR LabRam, Jobin Yvon Horiba (France), 600 lines/mm grating, N₂ – cooled CCD device (1024×256 pixels)) is combined with an AFM setup (NanoWizard AFM, JPK Instrument AG, Germany). Spectra were recorded using an excitation wavelength of 568.2 nm (Kr⁺ ion laser, Coherent, USA) and a sampling time of 10 s (Halobacteri a) and 20 s (PM patch), respectively (P ~ 1 mW, measured at the sample). For all measurements the laser was focused using an oil immersion microscope objective (60×, NA 1.45, Olympus) through sub- strate and sample onto the silver coated AFM tip. Therefore, the spot was slightly defocused to match the antenna condition of the TERS-probe. The sample is scanned independently using the intermittent contact mode (modulation amplitude ~6 nm), to maintain focusing on the TERS tip all the time. The Raman scattered light is collected in transmission mode through the same microscope objective. The non-contact AFM tips (NSG10, resonant frequency 190–325 Hz, NT-MDT) were coated with 20 nm silver (99,99% pure, Balzers Materials). These TERS tips were used directly after evaporation within two days. The diameter of the silver coated AFM tips is estimated to be approximately 20 nm, as typical SEM pictures of similar TERS probes show [39].

2.3.2 FTIR and Raman measurements

600 μg purified purple membrane sheets were sus- pended in 1 M KCl and 100 mM Tris at pH 7. The protein pellet – after centrifugation at 200,000 g for 2 h – was squeezed between CaF₂ windows, trans- ferred into an airtight cuvette and stabilized at 20 °C during the whole measurement. The data was ac- quired using a modified Bruker IFS66V spectrometer recording with 4 cm⁻¹ spectral resolution and a time resolution of up to 30 ns (41). The photocycle was triggered by an excimer laser (Lambda Physics 305i) driven dye laser containing Coumarin153.

Resonance Raman measurements of isolated PM patches were done using the setup described for TERS measurements, at 530.9 nm (P ~ 1.6 mW, sampling time ~ 30s).

3. Results and discussion

In a first experiment, isolated purple membrane patches were investigated. After deposition of the PM patches on glass cover slides from an aqueous buffer solution, TERS spectra were recorded on these structures. Figure 1 shows four selected spectra [1–4] recorded on a PM patch with a tentative assign- ment given in Table 1 [42–45]. The TERS spectra are reproducible with respect to their spectral po- sitions, but compared to Resonance-Raman or FTIR – difference-spectra the band widths and intensity ratios can differ substantially. Such varying intensities and intensity ratios can be attributed to specific nanoscale effects, which are related to the small number of molecules in the tip-sample interaction region resulting in the detection of specific molecular orienta- tions of the micro ensembles [20]. This behaviour is reflected in the observed variation of band intensity ratios. Each peak can be tentatively assigned to be characteristic for bacteriorhodopsin (br) or lipid (Lipid), respectively. Therefore the

![Figure 1](www.biophotonics-journal.org)  
Selected TERS spectra recorded on an isolated purple membrane patch (λexc = 568.2 nm, tacq = 20 s, P ~ 1 mW). Significant bacteriorhodopsin marker bands are assigned as “bR”. Further spectral information from the lipid ma- trix is assigned as “Lipid”.

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TERS bands are listed in Table 1. Since the excitation wavelength (568.2 nm) is almost at the maximum of the visible absorption profile of the protein, resonantly enhanced Raman modes of bR dominate. The main contribution is related to the retinal moiety, due to the resonant enhancement [42, 43]. In particular the C–C and C–NH stretching modes around 1540 cm⁻¹ and 1635 cm⁻¹ serve as unique Raman markers for the protonated Schiff base Retinal [46]. The N–H and C–H deformation modes of the retinal around 1340–1380 cm⁻¹ cannot directly serve as unique bR Raman marker bands, although their high intensity indicates a resonance enhancement and points towards the presence of bR. The lipid matrix shows typical bands at approximately 1095 cm⁻¹ and 1230 cm⁻¹ in spectrum 1 and 2, which can be tentatively assigned to the phosphate residue. However, these modes are not always detected. This corresponds to the estimated protein – lipid ratio (3:1) [32] and demonstrates the crucial influence of an exact tip positioning with respect to the lateral and vertical resolution capabilities. For example, recent TERS studies on isolated mitochondria revealed a distinct spectral contribution of non-resonant components compared to the resonantly enhanced cytochrome c [26]. This finding could be explained by the different sample-TERS probe distances in z-direction. The same effects must be considered for the present experiments as well. Here, the probe is able to approach the bacteriorhodopsin very closely and the spectral characteristics of purple membrane patches are dominated by the retinal moiety of bR. Nevertheless still surprisingly many features can be found that are related to the lipid environment. These features are not observed in standard resonance Raman scattering and again support the fact that molecules closer to the tip (here lipid and protein) experience a higher electromagnetic enhancement compared to molecules or fragments (Schiff base) further away. Hence, distance effects can match electronic resonance effects and lead to the observed spectral features. To support our findings on the compositional assignment of the purple membrane patches, conventional Raman and Fourier Transform Infrared (FTIR) spectroscopy measurements are performed. Since both techniques have been widely used to study bR and purple membrane [37, 47, 48] we present two selected spectra in Figure 2 for comparison with our TERS results of isolated PM patches. Figure 2 shows a resonance Raman spectrum (black line) of purple membrane and a time resolved inverted M-bR infrared difference spectrum (red line) of purple membrane. The Raman and the FTIR difference spectra show nice agreement regarding the chromophore bands at 1641 cm⁻¹, 1530 cm⁻¹, 1202 cm⁻¹, 1166 cm⁻¹ and 1009 cm⁻¹. In the FTIR difference spectra in addition protein bands as for example protonation of Asp85 at 1762 cm⁻¹ is observed. However, the intensity patterns of the Resonance and FTIR spectra deviate largely from the TERS spectra in Figure 1 of the PM. The resonance Raman spectrum is dominated by the bands of the chromophore, exhibiting the same prominent Raman markers at approx. 1530 cm⁻¹ (C=C) and 1641 cm⁻¹ (C=NH) as the TERS spectra in Figure 1, even though in a different intensity ratio and slightly shifted in frequency, which can be understood by specific surface selection rules [49]. Interestingly, TERS lacks the prominent band at 1200 cm⁻¹. FTIR is equally sensitive for all components, and the additional bands in the FTIR spectra can be assigned to molecular changes during the bR to M transition (with ground state (bR) contributions pointing upwards), like the bands at approx. 1166 cm⁻¹, 1253 cm⁻¹, 1348 cm⁻¹, 1556 cm⁻¹, 1660 cm⁻¹ and 1690 cm⁻¹. Some of these signals can also be identified in the TERS spectra in Figure 1, hence, supporting the distance dependence discussed above. For the subsequent TERS investi-
gation of *Halobacterium salinarum*, the more complex composition of the bacterium compared to the bR patches has to be considered [32]. Here, purple membrane sections are embedded in a so-called red membrane, where they are presumably organized in patch-like domains. The red membrane contains a variety of proteins and \(\beta\)-carotene. This ensemble forms the inner membrane which is covered by a protein envelope, the so-called S-layer (50). This S-layer is not present on isolated PM-patches.

Prior to the actual TERS experiments single isolated *Halobacterium salinarum* cells were localized using the AFM topography. Figure 3(a) shows a typical AFM topography image of two halobacterial organisms (256 \(\times\) 256 pixels). Single points (4 \(\times\) 5) separated by 75 nm are measured on a 300 \(\times\) 375 nm grid. Positions characteristic for purple or red membrane are highlighted in the enlarged colour map in purple or red, respectively. (b) Selected TERS spectra (\(\lambda_{\text{exc}} = 568.2\) nm, \(t_{\text{aqc}} = 10\) s, \(P \approx 1\) mW) were recorded on the positions indicated in the colour map in (a). Intensive bands of the purple membrane in spectra 1 and 2 are labelled with an asterisk. In spectra 3 and 4 characteristic red membrane bands are labelled with a diamond.
were recorded by subsequent positioning of the TERS probe on the preselected grid while oscillating. In addition to the characteristic PM signals, bands of proteins and lipids were detected, reflecting the increased complexity of the real bacterial surface. A compositional assignment of each single spectrum was accomplished depending on the presence of the distinct PM marker bands and is schematically shown in the coloured grid of Figure 3(a). Each pixel represents the assignment of a single spectrum and is either coloured purple (PM) or red (red membrane – no PM features), respectively. The colour map shows a contiguous spectral distribution, which gives a hint to the organisation of the halobacterial surface. Figure 3(b) shows four selected TERS spectra characterizing PM patches [1, 2] and domains without PM [3, 4]. The latter is used to classify the red membrane. A detailed assignment of the spectra is provided in Table 2 [42–45].

The complete spectral data of the entire grid can be found in the supplementary section. Here, the individual positions of the TERS-probe are not shown in the AFM-image for a better visibility of the halobacterial surface. In spectra 1 and 2 the most prominent signals refer to PM marker bands. Considering the aforementioned position sensitive aspects of TERS and focussing mainly on the band positions, the PM marker bands are labelled with an asterisk (†) shown in Figure 3 recorded on Halobacterium salinarum [42–45].

### Table 2 Tentative assignment of TERS spectra (cm$^{-1}$) shown in Figure 3 recorded on Halobacterium salinarum [42–45].

<table>
<thead>
<tr>
<th>Spec 1</th>
<th>Spec 2</th>
<th>Spec 3</th>
<th>Spec 4</th>
<th>assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>620 w</td>
<td>606 w</td>
<td>623 m</td>
<td>620 w</td>
<td>Phe, Trp</td>
</tr>
<tr>
<td>670 w</td>
<td>679 w</td>
<td>δ CH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>746 w</td>
<td>779 w</td>
<td>Trp, v$_l$ O–P–O (L)</td>
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<tr>
<td>797 w</td>
<td>801 w</td>
<td>799 w</td>
<td>v$_l$ O–P–O (L)</td>
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<tr>
<td>849 w</td>
<td>837 s</td>
<td>833 s</td>
<td>v$_{as}$ O–P–O (L)</td>
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</tr>
<tr>
<td>907 w</td>
<td>939 m</td>
<td>945 w</td>
<td>v C–C (P), δ C–H (L)</td>
<td></td>
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<tr>
<td>990 m</td>
<td>v C–C (P)</td>
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<td></td>
<td></td>
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<tr>
<td>1041 w</td>
<td>1051 w</td>
<td>1040 s</td>
<td>1045 w</td>
<td>v C–O–P (L), δ CH$_2$ (bR)</td>
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<tr>
<td>1081 m</td>
<td>1075 w</td>
<td>v$_l$ PO$_2$(L)</td>
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<td></td>
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<tr>
<td>1113 w</td>
<td>1132 s</td>
<td>1132 s</td>
<td>v C–C (L), (P)</td>
<td></td>
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<tr>
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<td>1153 w</td>
<td>1153 w</td>
<td>v C–C (bR)</td>
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<tr>
<td>1199 m</td>
<td>1176 w</td>
<td>1201 w</td>
<td>v C–C (bR), Tyr</td>
<td></td>
</tr>
<tr>
<td>1254 (S)</td>
<td>1247 s</td>
<td>v (C–C)/lysine</td>
<td></td>
<td></td>
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<tr>
<td>1294 s</td>
<td>1289 s</td>
<td>1274 m</td>
<td>1278 m</td>
<td>v (CH)$_2$/amide III (bR), amide III (P)</td>
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<tr>
<td>1300 m</td>
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<td>1314 w</td>
<td>v$_{ip}$–CH$_2$ (L)</td>
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<tr>
<td>1355 vs</td>
<td>1346 w</td>
<td>1332 w</td>
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<td>δ CH$_2$ (L)</td>
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<tr>
<td>1366 vs</td>
<td>1380 m</td>
<td>δ CH$_3$ (bR, L), Trp (bR)</td>
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<tr>
<td>1404 m</td>
<td>1397 w</td>
<td>1409 w</td>
<td>1394 m</td>
<td>δ CH$_2$ (L)</td>
</tr>
<tr>
<td>1452 w</td>
<td>1475 m</td>
<td>δ CH$_3$ (bR), δ CH$_3$(L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1495 w</td>
<td>1484 m</td>
<td>δ CH$_2$ (L), amide II (P)</td>
<td></td>
<td></td>
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<tr>
<td>1513 s</td>
<td>1513 w</td>
<td>v C–C (bR)</td>
<td></td>
<td></td>
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<tr>
<td>1523 m</td>
<td>1542 s</td>
<td>1545 s</td>
<td>v$_{as}$ C–C (bR)</td>
<td></td>
</tr>
<tr>
<td>1542 s</td>
<td>1545 s</td>
<td>1566 m</td>
<td>amide II</td>
<td></td>
</tr>
<tr>
<td>1583 w</td>
<td>1599 m</td>
<td>v C–C (bR, L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1614 w</td>
<td>1611 s</td>
<td>1611 w</td>
<td>v C–C (bR, L)</td>
<td></td>
</tr>
<tr>
<td>1638 s</td>
<td>1622 (S)</td>
<td>v C–NH$_2$(bR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1688 w</td>
<td>1664 s</td>
<td>1672 m</td>
<td>amide I (bR, P)</td>
<td></td>
</tr>
</tbody>
</table>

w: weak, m: middle, s: strong, vs: very strong, L: lipid contribution, bR: bacteriorhodopsin contribution (retinal with protein envelope), P: protein contribution (without bR envelope) v: stretch, δ: deformation, r: rocking, t: torsion, wag: wagging, s: symmetric, as: asymmetric, ip: in plane, Phe: Phenylalanine, Trp: Tryptophan, Tyr: Tyrosine
Spectra 3 and 4 do not show any PM characteristics, based on the missing C=C and the C=N/C=NH+ stretching modes of the retinal moiety. Distinct lipid phosphate modes were detected at approximately 800 cm⁻¹ and 1080 cm⁻¹. A β-carotene contribution is mainly indicated by a shoulder at approximately 1150 cm⁻¹ and the C=C stretching at 1513 cm⁻¹, both are labelled with a diamond. Typical protein Raman modes such as the amide I and amide III bands can be assigned to non-resonant proteins within the red membrane. All spectra assigned to the retinal free domain in the grid do not show the specific bR bands signals, as depicted by 2 examples in Figure 2, right side.

In Figure 4 the results of the TERS measurements on halobacterial surface are summarized schematically. The protein envelope covering the inner membrane (S-layer), is not shown for simplification. Estimating an apex of approximately 20 nm the silver covered TERS tip scans the inner membrane of the surface sequentially. The localized field enhancement is generated by the laser excitation at the nano particle and decays rapidly (after a few nanometers) with increasing distance of the particle surface. The spectral information is based on the molecular composition in the range of this evanescent field. Position 1 shows the tip localized on a purple membrane domain containing bR. Typically the proteins are organized as bR trimers, ordered in a hexagonal lattice [32]. The red circles symbolize components present in the red membrane, like β-carotene and proteins, which are randomly embedded in the lipid matrix. Hence, position 2 in Figure 4 refers to a purple membrane free domain. Compositional differences of the lipid molecules between purple and red membrane may cause a slightly differing matrix environment, which is not the topic of the current investigation.

The results confirm a patch like organization of purple and red membranes on the halobacterial surface. From the experiments the patch size of red and purple membrane can be estimated to be on the order of a few hundred nanometers.

4. Conclusion

In this work purple membrane patches isolated from Halobacterium salinarum cells and whole bacterial organisms were investigated using tip-enhanced Raman spectroscopy. TERS experiments on bacteriorhodopsin containing purple membrane (PM) patches were performed in order to identify specific PM Raman marker bands. These are the very specific C=C and C=N stretching modes as well as intensive C–H and N–H deformation modes of the resonantly enhanced retinal moiety of bacteriorhodopsin. These data, supported by resonance Raman and FTIR spectra, allowed the nanoscale differentiation of distinct membrane domains on the halobacterial surface using TERS. The spectra could be specifically assigned to purple membrane (retinal containing) and retinal free domains, most likely red membrane, the two major components of the membrane surface of Halobacterium salinarum. The established distribution indicates a patch-wise organization of the components with typical length scales of several hundred nanometers. Considering the signal to noise ratio of the actual TERS-spectra, a shorter sampling time is feasible. This allows smaller step sizes and would improve the resolution accordingly.

The present results signify an important step towards a nanoscale structural mapping of specific domains on membrane surfaces. The distribution as well as the arrangement of cell surface compartments are important parameters and will broaden the understanding of cell operations. Specifically nanoscale structural investigations are required to address the still unclear function of the so-called lipid rafts and TERS seems to be ideally suited for this task.

Further progress in the instrumental development will allow also large scale structural studies with nanometer lateral resolution which will enable to investigate the structural and functional composition of an entire membrane system.
Tanja Deckert-Gaudig studied chemistry at the University of Würzburg. In 1992 she received an Erasmus fellowship for a stay at the University College of Swansea (Wales) and in 1994 a DAAD research grant at the University of Tokyo (Japan). She obtained her Ph.D. in Organic Chemistry in 1997 with Prof. S. Hünig at the University of Würzburg. After a baby break she started working with Dr. V. Deckert at the University of Dresden in 2002 and moved to the Institute for Analytical Sciences in Dortmund in the same year. Since 2009 she works at the Institute of Photonic Technology in Jena. Her main research interest is the characterization of protein related biopolymers with TERS.

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Jürgen Popp is Professor for Physical Chemistry at the Friedrich-Schiller University Jena and Scientific Director of the Institute of Photonic Technology Jena, Germany. He received his Ph.D. in chemistry from the University of Würzburg in 1995 and subsequently spent a year in the Department of Applied Physics at the Yale University, USA. After his habilitation in the group of Prof. Dr. W. Kiefer he became a full professor of the University Jena in 2002. In June 2006 he was also appointed scientific director of the Institute of Photonic Technology. His work was awarded by the faculty prize of chemistry (1995), the Bayerische Habilitationsförderpreis (1997), the Förderpreis der Würzburger Korporationen (2001) and the Kirchhoff-Bunsen award (2002). His research interests are focussed on problems being resolved by means of innovative frequency-, time- and spatially resolved laser spectroscopic methods. Thereby, bio- and material photonics are setting up the two main priorities of the research activities.

Klaus Gerwert received his Ph.D. in biophysical chemistry from the University of Freiburg in 1985. From 1986–1989 he conducted research at the Max-Planck-Institute in Dortmund, then as Heisenberg-fellow from 1990–1993 at the Scripps Research Institute, La Jolla, USA and also in Dortmund.
Since 1993 he has been appointed as full professor at the Ruhr-University Bochum, where he holds a chair of biophysics since 2009. He is director at the Max-Planck-CAS partner-institute in Shanghai (dual appointment) and Max-Planck-fellow. His research focuses on proteins using time-resolved FTIR-spectroscopy combined with x-ray structure analysis and computational biology to elucidate molecular reaction mechanisms and interactions of membrane and membrane-bound proteins: microbial rhodopsins; Ras-superfamily; in addition vibrational imaging of cells and tissues in translational clinical research.

Volker Deckert holds a joint position at the Institute of Physical Chemistry and the Institute of Photochemical Technologies, both in Jena, Germany. He obtained his Diploma and Ph.D. from the University of Würzburg, Germany, working on Raman spectroscopy. As a postdoc he worked on non-linear and time-resolved laser spectroscopy at the University of Tokyo and KAST, in Kawasaki. During his habilitation at the ETH Zurich, he came into contact with near-field optical spectroscopy, a subject he still pursues and applies to mainly biological samples in his current position.

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