Raman Microscopy

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A Spectral Window to the Cell

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Drug development—from the synthesis of putative pharmacological compounds through preclinical and clinical studies up to the approval and release of the product on the market—is a time-consuming and expensive process. Implementation of the principles of rational design for the discovery of drugs and for developing drug-delivery procedures may lower the costs and reduce the expenditure of time, at least for preclinical studies. These strategies, however, strongly rely upon the knowledge of the molecular processes of the drug from the transfer into the cell to the interaction with the target biomolecule. While structural biology methods and molecular modeling techniques provide valuable insight into drug–target interactions, there is still an urgent need for appropriate experimental approaches to monitor drugs in cellular systems on molecular and microscopic levels simultaneously.

Twenty years ago it had already been suggested that this gap might be closed by Raman spectroscopy, which can provide molecular structure information and is not limited to specific sample arrangements, an important prerequisite for in situ studies. Thus, with the development of confocal Raman spectroscopy and the possibility of probing molecular species with spatial resolution (Raman microscopy), cellular systems and specifically medical applications became research targets. In fact, the seminal work by Puppels et al.[1] motivated a large number of studies on single cells as well as on tissues, frequently aimed at diagnostic applications of Raman microscopy. However, the initial enthusiasm was soon followed by a certain disenchantment owing to the intrinsic limitations of Raman spectroscopy: its relatively low sensitivity and selectivity. Attempts to overcome these drawbacks by exploiting the preresonance enhancement of drugs or target molecules proved to be problematic since the required higher excitation energies may cause unwanted photoinduced degradation processes in the cellular systems.[2] As a consequence, the application of Raman microscopy in medicine has been restricted to specific niches, despite some recent methodological advances. Coherent anti-Stokes Raman spectroscopy (CARS) and stimulated Raman spectroscopy (SRS) have been developed for operation in the imaging mode.[3,4] Both techniques exhibit a substantially increased sensitivity and display, in principle, potential for in situ studies of cellular systems and thus for medical applications. However, both approaches are technically quite demanding, which would limit routine use. Furthermore, these techniques, like conventional Raman spectroscopy, also suffer from low selectivity.

Improved selectivity can be achieved by utilizing appropriate spectral windows, in which the characteristic vibrational bands of solely the target molecules appear. One approach is to shift the Raman signals of specific compounds from cogent to largely free spectral regions by isotopic labeling, as demonstrated in Raman microscopic studies in which deuteration of lipid components was used to enhance the contrast between the target vibrational bands and the unwanted spectral background.[5] Such studies may provide valuable information about specific processes in cellular systems, but the required isotopic substitutions prohibit more general application. Nevertheless, these studies emphasize the advantage of utilizing spectral windows. Marker bands of natural reporter groups that can be observed in spectral windows are the stretching vibrations of CN and CO ligands at metal centers, like those in enzymes such as hydrogenases.[6] These bands, which appear in the region between 1900 and 2100 cm^{-1}, can be selectively probed by IR spectroscopy without interference from the much more intense vibrational bands of the protein matrix. This approach has been successfully applied to the enzymes in complex biological environments such as whole membranes.[7]

In a recent Raman microscopic approach,[8] the CO stretching mode was used to look inside cells; the uptake and the intracellular distribution of a pharmacologically important compound, a CO-releasing molecule (CORM), was tracked.[9] CORMs are metal carbonyl complexes that act as a CO reservoir; they release CO molecules photochemically upon irradiation with near-UV light. Thus, the intriguing advantage of CORMs is that the harmful action of CO towards biomolecules can be triggered with high selectivity, which may have a strong impact on cancer therapy. Clearly, the potential of such compounds can be fully exploited only if the conditions for cellular uptake and the transfer to the target components of the cells are optimized, a challenge that can be guided by the Raman microscopic approach developed by Meister et al.[8]

The objective of the study was to monitor the uptake and distribution of $[Mn(tpm)(CO)_3]Cl$ (tmp = tris(pyrazol-2-yl)-methane) in human HT29 colon cancer cells. This compound

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displays a Raman-active CO stretch at 1963 cm^{-1} in aqueous solution. Despite its low relative intensity, this band can be detected readily in single cells after $[Mn(tpm)(CO)_3]Cl$ uptake, since the much stronger Raman bands of the cell constituents are more than 250 cm^{-1} away (Figure 1). Spectra

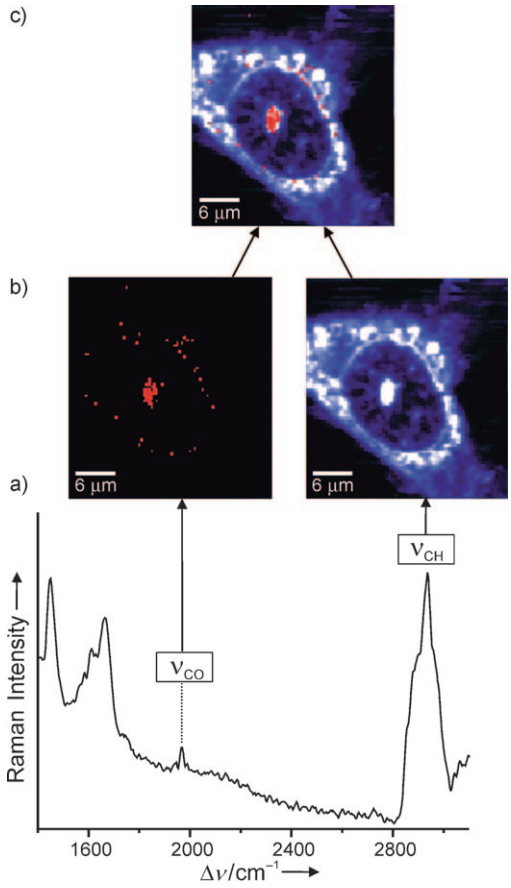

Figure 1. a) Raman spectrum of a HT29 cell after incubation with $[Mn(tpm)(CO)_3]Cl$; b) images of the intensity distribution of the CH stretching (right; white) and CO stretching modes (left, red); c) superposition of the images in (b) (graphics courtesy of K. Meister).

were recorded using a conventional confocal Raman spectrometer and an excitation line of 532 nm, which is too low in energy to induce photodissociation of CO from the metal center. Once detectability was demonstrated, experiments were carried out in the imaging mode using a high-precision motorized microscope table. Imaging was performed by scanning the laser focus over the cell and accumulating complete spectra at each raster point. Thus, it was possible to obtain images based on different Raman bands simultaneously, which hence are unambiguously correlated. In this study the authors used the CO stretching band of $[Mn(tpm)(CO)_3]Cl$ and the envelope of the CH stretching bands of all constituents of the cell. The latter image thus reflects the contour and morphology of the cell, which nicely agrees with the image of the same object obtained by light microscopy. The intensity of the CO stretching band reflects the only the distribution of the metal carbonyl complex inside the cell. Combining both images indicates a particularly high concentration of the metal carbonyl complex at the nucleus. This finding suggests that the photoinducible cytotoxic activity of $[Mn(tpm)(CO)_3]Cl$[10] results from the attack of CO specifically at the nucleus. In addition to the lateral resolution, the technique also provides a profile of the $[Mn(tpm)(CO)_3]Cl$ distribution in the z direction, which may become of particular interest for tissue studies.

The present approach offers exciting perspectives for future studies. One may try to exploit the intrinsic advantages of vibrational spectroscopy more comprehensively by probing the molecular interactions with the cellular target that are reflected by spectral changes of the CO stretching mode. Particularly, attempts should be made to distinguish between $[Mn(tpm)(CO)_3]Cl$ species in the unbound state and complexed to (specific) biopolymers in the cell. Comparative in vitro studies with purified proteins and DNA may then help identify the target components in the cell. In addition, the Raman microscopic approach seems to be suitable to monitor photoinduced CO release with microscopic resolution and to identify the reaction products. Obtaining this information might require appropriate measures to improve the signal-to-ratio; however, such efforts are justified as the outcome may guide the synthesis of further and improved CORMs. More generally, studies one may simply focus on the reporter function of the CO group attached to various compounds. After such labeled compounds are introduced into a cell, their processes can be followed by Raman spectroscopy with a spatial resolution better than that of IR microscopy.[11] This strategy may be applied to elucidate molecular interactions in cells in fundamental and applied research.

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