Abstract:

UV resonance Raman investigations of enzymes in action

Co-supervisor 1: Janina Kneipp, Humboldt-Universität zu Berlin, Dept. of Chemistry

Co-supervisor 2: Norbert Esser, ISAS e.V. and Technical University Berlin, Physics Dept.

In this project, enzyme structure at membranes and of membrane proteins will be studied using resonance Raman spectroscopy with excitation in the UV (UVRR). While several UVRR studies were done with proteins in buffers and solutions, this project aims to extend such research in the direction of studying proteins specifically at and in artificial vesicles, that is, in their native environment, including the discussion of both intra-protein contacts and protein-membrane interactions. Building on existing protein-membrane models, in particular that of an enzyme involved in cellular lipid metabolism, as well as other protein models studied in the UniSysCat context - providing interaction with several other groups in EC2 and UniSysCat-, catalysis by proteins will be better understood. In order to study the protein substrate and other components of the model system, visible and NIR excited non-resonant Raman spectra of the enzyme substrate will be combined in a multiblock statistical analysis.

Extended description version of the project:

UV resonance Raman investigations of enzymes in action

Co-supervisor 1: Janina Kneipp, Humboldt-Universität zu Berlin, Dept. of Chemistry

Co-supervisor 2: Norbert Esser, ISAS e.V. and Technical University Berlin, Physics Dept.

1. Overall goal of the project

UV resonance Raman spectroscopy (UVRR) will be used to probe aromatic amino acid side chains and structural changes in the protein backbone of protein enzymes in lipid environments. It is expected to reveal very sensitively small changes in protein structure during enzyme catalysis. The main advancements compared to previous UVRR work on proteins will be (i) a selective, microscopy-based collection of spectra from model lipid environments, important to study enzymes in membranes, as well as (ii) the combination of the UVRR data with non-resonant Raman microspectra of enzyme substrates and molecules participating in the reactions both in the experiments and in a multiblock analysis approach in order to attain correlation of UVRR and non-resonant signals. The project will contribute to the goals of research area E of UniSysCat (Signal-controlled multicomponent catalysis), specifically by expanding the spectroscopic basis to better understand aspects of biocatalysis and structure in native biological environments.

2. State of the art

UVRR has been used for the characterization of proteins for several decades. While information on the aromatic amino acid side chains can be attained using longer excitation wavelengths of 229 nm and 266 nm,[1] the probing of the peptide backbone requires excitation in the deep UV wavelength range ~200 nm and resulted in work that contributed e.g., to elucidate the folding of proteins into beta-sheet structures. Because of the challenging sampling conditions, especially with respect to the competing photodecomposition when in resonance with an electronic transition in the molecule and when using pulsed lasers, UVRR experiments on proteins embedded in membranes are still rare. [2-3]. Nevertheless, in order to study the structure of membrane bound enzymes, selective probing of the secondary structure in the native environment is crucial. We will shed new light on protein UVRR by employing a unique Raman microspectroscopic setup operating at 220 nm with cw excitation that was adapted in collaboration by the two PI in the past three years to accommodate sensitive UVRR spectroscopy on proteins and other biomolecules [4] (UVRR application lab at ISAS) to measure microspectra of vesicle preparations that contain proteins. At this wavelength, our previous work has shown that the strong signals of tyrosine, tryptophan and phenylalanine are still present but give way to signals of the amide backbone,[5] which in principle then can be selectively probed in pre-resonance, at much lower sample destruction than ~195 nm. An important prerequisite to achieve this is deconvolution and if possible, combination with other spectral data that are obtained ideally at similar spatial resolution from the same system. Such a combination of many different types of spectra from the same sample can be achieved mathematically by consensus principal component analysis (CPCA), with which we have linked structural/chemical data from different analytical methods in the recent past,[6] and that we want to use to add non-resonant Raman spectra excited in the visible and NIR wavelength range to the UVRR spectra.

3. Specific aims and how they may be reached:

The project has the following specific aims:

(i) Enable UVRR probing of proteins in a lipid environment as experimental basis.

(ii) Combine UVRR spectra with non-resonant Raman spectra excited in the visible and NIR wavelength range and other experimental data, e.g., from optical pH measurements

(iii) Study enzymes in their lipid environments

The work program is structured according to these aims:

WP1 UVRR spectra of liposomes containing proteins and molecules involved in enzymatic processes. We will study protein structures in vitro using excitation at 220 nm. There we would like to focus on the possibilities to monitor changes in secondary structure, indicated by the (weak, as pre-resonant) amide III spectral region and interaction via changes in vibrational modes associated with the aromatic amino acid side chains. Excitation conditions must be identified, including adaptation of the microscope sample stage, probing of different locations in the micro-structured sample, control of temperature (e.g. PT cooling) and pH. Initially, well studied proteins (BSA), and model peptides devoid of aromatic side chains (polylysine) will be incorporated in lipid vesicles at varying concentration and pH and spectra will be measured. Molecules that play an important role as energy sources in catalysis, such as GTP and ATP, will be investigated in these environments as well, using excitation at 266 nm and 355 nm to attain high detection sensitivity.

WP2 Multi-block analysis of different UVRR, non-resonant Raman, and other vibrational spectra. These data sets constitute several respective individual data blocks for a CPCA, where changes in the UVRR data and in non-resonant Raman spectra can be correlated. This may be an important tool to help identify characteristic structural changes in the UVRR protein spectra and to observe them under varied conditions, e.g., different pH. We have studied many different lipid-protein environments by non-resonant Raman microscopy, and sample preparation and measurement conditions for the non-resonant (and non-SERS) experiments are established. [7] This enables an investigation of the underlying global information, since complementary chemical data are combined.

WP3 Experiments with enzymes at the membrane. This part of the project will use enzymes in the membrane environment and benefit from another project in EC2, where the protein ASM is prepared in liposomes for studies by SERS. The non-resonant Raman spectra can also report on changes in enzyme substrates, in particular lipids, as recently demonstrated in the case of glycolipids, sphingomyelin, and cholesterol esters.[7]

Resources and collaboration

The UVRR data will be obtained in the UVRR application lab that was recently established at ISAS Berlin. Raman microspectroscopic experiments in the visible and NIR will be done in the Kneipp group. The vesicle-protein composites will be prepared in the Kneipp group. Multiblock analysis will be performed in the Kneipp group. cryo-EM studies of the vesicles will be conducted in the SALSA environment.

References

[1] Kneipp, J. et al. *Journal of Molecular Biology* **2006,** *356* (2), 335-353. [2] Sanchez, K. M.; Neary, T. J.; Kim, J. E., *The Journal of Physical Chemistry B* **2008,** *112* (31), 9507-9511. [3] Wei, X. et al.. *Journal of Raman Spectroscopy* **2021,** DOI 10.1002/jrs.6252. [4] Merk, V. et al. *Appl. Spectrosc.* **2021,** *75* (8), 994-1002. [5] Kneipp, J. et al. *Journal of Physical Chemistry B* **2004,** *108* (40), 15919-15927. [6] Diehn, S. et al., *Frontiers in Plant Science* **2020,** *10*. [7] Živanović, V. et al. *Analytical Chemistry* **2018,** *90* (13), 8154-8161.