Abstract:

Title of Project: Monitoring enzyme function at lipid membranes by SERS

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

Co-supervisor 2: Christoph Arenz, Dept. of Chemistry, Humboldt-Universität zu Berlin

Text: ca. 150 Words

In this project, methodological tools will be developed that enable observations of enzyme function at membranes and of membrane proteins. The first aspect concerns the ability to probe the action of intra-membrane enzymes by SERS, and monitoring and control of this environment during enzyme insertion, interaction, and catalysis. It will lead to versatile models for future studies of different enzymes, and to improved targeting of SERS probes to monitor enzymes in cells. The second aspect, detection and characterization of enzyme metabolites, is crucial for probing enzyme action in complex environments such as cells. Such metabolites include lipids, e.g., from biological membranes, as well as proteins and peptides. In order to address these objectives, probing of lipid molecules in environments of different complexity will be at the center of this project, ranging from vesicle and membrane models to cellular models where the abundance of specific lipids will be controlled, and enzyme action can be monitored.

Title of Project: Monitoring enzyme function at lipid membranes by SERS

Co-supervisor 1: Janina Kneipp, Dept. of Chemistry, HU

Co-supervisor 2: Christoph Arenz, Dept. of Chemistry, HU

1. Overall goal of the project

In this project, methodological tools will be developed that enable observations of enzyme function at membranes and of membrane proteins. In the detection of products from enzyme-catalyzed reactions, it is often not possible to rely on strong molecular resonances to enhance Raman scattering, therefore surface-enhanced Raman scattering (SERS) spectroscopy is ideal to monitor enzyme action. Here, SERS probing of lipids in different environments of varying complexity will be pursued with the following two objectives:

(i) to enable a comprehensive characterization of intra-membrane and membrane-associated enzymes together with their lipid membrane environment and

(ii) to detect lipid species that are substrates and metabolites of such enzymes by SERS in cellular models.

2. State of the art

In Raman experiments, molecules that reside in the close proximity of noble metal nanostructures can be detected and characterized by SERS, an effect that significantly improves the sensitivity of spontaneous Raman scattering. The starting point of the project are developments, by us and others, of gold-nanoparticle based SERS probes that can be applied in eukaryotic cells to study the molecular composition and interaction in different compartments of these complex systems, see e.g.,[1] for an overview. Nevertheless, while many active (drug) molecules produce a high SERS signal, [2] a major challenge is posed by the strong variations in Raman cross-sections of different molecular groups in the biomolecular species: Specifically, reports of in situ and in vivo lipid SERS spectra are rare and restricted to SERS substrates that connect directly to the outer cell membranes [3] or to exosomes. [4] Recently, significant contributions to SERS spectra by lipids in cells where the abundance of lipids is particularly high were found in the Kneipp group.[5] In this project, cells with high abundance of specific lipids will be studied in collaboration with the Arenz group, who has experience with cell models of lipid storage disorders [6,7], and studies important lipases in the physiological context, in cultured cells by mimicking known inborn metabolic disease by generating patient-associated genotypes using CRISPR/Cas technology.

Outside cells, some SERS spectra of pure lipids and lipid vesicles were reported. [8,9] First results show that the structure of lipid vesicle models can be controlled and monitored by SERS. [10] Very recently, both co-PI showed that SERS can be used to characterize lipids in endosomes.[11] In the proposed project, the model will be extended towards membranes interacting with enzyme molecules. First, in vesicle models, the influence of specific lipids that play an important role in lipid storage diseases will be delineated, to later on move to whole cells. These molecules are cholesterol, ceramide and sphingomyelin. The group of Christoph Arenz has shown several possibilities of labeling both lipids as well as intact enzymes. [7,12] Here, the molecules will be labeled by functional groups that have characteristic Raman bands in a spectral window outside the typical vibrations of other biomolecules. Thereby, additional tools for SERS detection are at hand for the interpretation of the spectra in the vesicle models, and for tracing of lipids and enzymes in live cells.

(1) Kneipp, J. *ACS Nano* **2017**, *11*, 1136-1141. (2) Živanović, V. et al. *J. Phys. Chem. C* **2017**, *121*, 22958-22968. (3) Caprettini, V. et al. *Advanced Science* **2018**, *5*. (4) Stremersch, S. et al. *Small* **2016**, *12*, 3292-3301. (5) Živanović, V. et al. *Analytical Chemistry* **2018**, *90*, 8154-8161. (6) Kirkegaard, T. et al. *Nature* **2010**, *463*, 549. (7) Kirkegaard, T. et al. *Science Translational Medicine* **2016**, *8*, 355ra118-355ra118. (8) Suga, K. et al. *Analytical Chemistry* **2015**, *87*, 4772-4780. (9) Taylor, R. W. et al. *Scientific Reports* **2014**, *4*, 5940. (10) Živanović, V. et al. *The Journal of Physical Chemistry Letters* **2018**, *9*, 6767-6772. (11) Živanović, V. et al. *ACS Nano* **2019**, *13*, 9363-9375. (12) Pinkert, T. et al. *Angew.Chem. Int. Ed.* **2017**, *56*, 2790-2794.

3. Specific aims and how they may be reached:

Both main objectives (i) characterization of enzymes at/in the membrane together with their lipid membrane environment and (ii) monitoring of enzyme action using an enzyme metabolite will be addressed by lipid-enzyme models at varying complexity. Starting from lipid-gold nanoparticle composites and vesicles as model environment, cellular models that contain specific lipids in high abundance will be used. They are derived from naturally occurring metabolic disorders that are based on a dysfunction of membrane associated enzymes involved in lipid metabolism. Lipid storage phenotypes will be used to validate specific lipid signatures within their natural cellular environment.

WP1 SERS spectroscopy of vesicle models: influence of vesicle composition

Generation of liposome-gold nanoparticle composite structures for varied composition and labeling in the lipids will build on preliminary work published in ref.[10]. Ceramide and sphingomyelin will labeled by azide, nitrile or alkyne functions in both, their acyl- or sphingosyl parts. In addition, sphingomyelin can be labeled within its polar choline moiety by introduction of an azide or alkyne group. Cholesterol can be labeled in its extracyclic portion by the previously mentioned alterations.

WP2 Inclusion of enzyme molecules in a combined lipids- enzyme vesicle model

Enzyme molecules will be included in an optimum liposome model obtained in WP1, for example acid sphingomyelinase with and without labels and characterized by SERS

WP3 Generation of cell models for Niemann-Pick disease type A/B or C for SERS characterization.

Niemann-Pick disease (NPD) type A/B is a sphingomyelin storage disorder of varying severity, caused by a defect in the gene for acid sphingomyelinase. NPD type C is a cholesterol storage disorder, caused by mutations in either NPC1 or NPC2 gene. Since the lysosomal degradation of sphingomyelin can only proceed in the absence of cholesterol, sphingomyelin is an associated storage compound. Both cellular models will be established by introducing well-known patient-associated mutations using the CRISPR/Cas9 technology, performed in the Arenz lab. Cells of the desired geno-/phenotypes will be enriched using FACS in combination with recently developed live cell probes for enzymatic activity. Since the respective diseases are very well-studied, these cellular models would be valuable tools for validating the signatures of the storage compounds within living cells in correlation with their known sub-cellular distribution.

WP4 SERS characterization of the cellular models

Spectroscopic mapping of the cells will be performed, and identification of the lipid spectral signatures will be achieved by multivariate statistics and application of random forest (machine learning) algorithms, recently shown to be able to enhance mapping of lipid spectral signatures in such systems.[11]

Resources and collaboration

All SERS experiments will be done in the Kneipp group. The vesicle-gold nanoparticle composite structures will be generated in both groups using, e.g. the expertise in labeling of molecules. The cellular models will be generated in the Arenz group. Multivariate analysis will be performed in the Kneipp group. cryo-EM studies and cryo-XRT will be conducted in the SALSA environment.