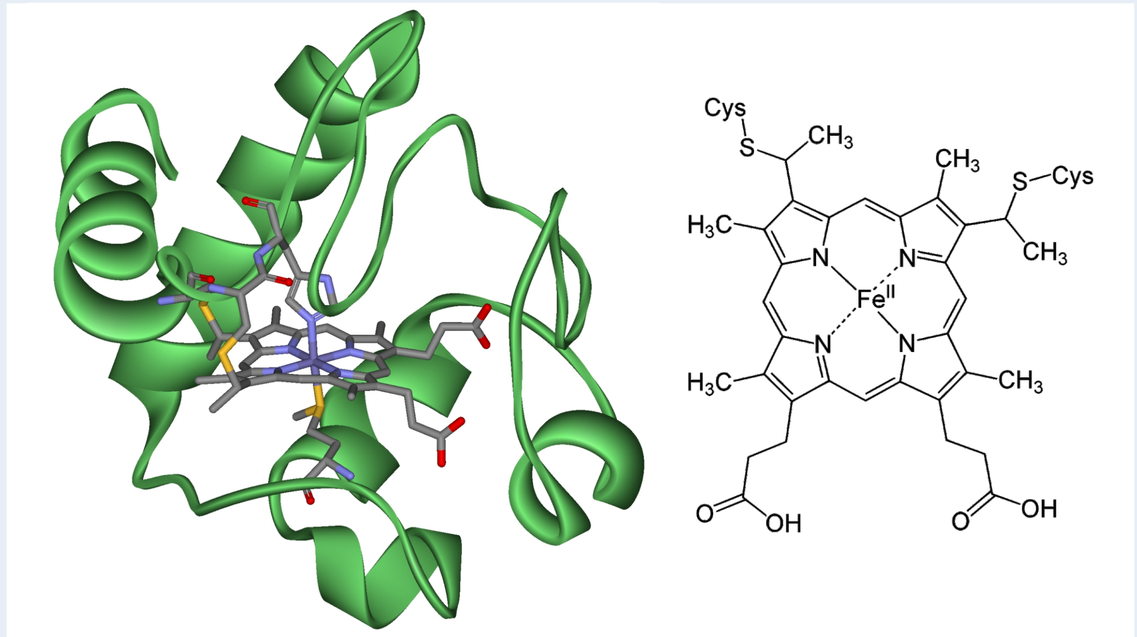
Optical Technologies.

Leibniz Universität Hannover

Bacterial Phylogeny in vivo

cyt-c based resonance Raman microscopy

A. Kniggendorf, B. Roth
Hanover Center for Optical Technologies, Leibniz University Hanover



3D structure of cytochrome-c with the heme embedded in the protein moiety (left) and the basic structure of heme-c (right).

Concept & Challenge

Confocal Resonance Raman Microscopy tuned for the porphyrin ring resonances of heme-c is used to record the Raman spectrum of cytochrome-c (cyt-c) of individual bacterial cells *in vivo*.

Hierarchical Cluster Analysis (HCA) categorizes the analyzed bacteria cells into clusters based on the spectral similarity of their cytochromes, thus determining how closely the analyzed bacteria are related.

Scientific Challenge: most bacteria build multiple types of cyt-c, all of which undergo evolutionary change, thus each obtained spectrum is actually an indivisible superposition of the Raman spectra of many different cyt-c.

Spectral similarity [%]

single spectrum	Reference spectrum DSM 158	DSM 2340	Nm-02	Nm-50	Nm-53
DSM 2340	48.61	96.55	-	56.31	59.65
Nm-02 006 004	0.00	20.85	94.13	58.04	73.62
Nm-02 008 000	0.00	21.64	94.82	60.98	75.51
Nm-02 013 001	0.00	30.21	97.30	69.43	82.51
Nm-50 016 022	10.45	50.23	91.91	92.84	92.10
Nm-50 029 031	16.50	56.06	87.81	94.95	93.98
Nm-50 032 014	14.80	53.90	85.93	95.40	89.82
Nm-50 044 023	10.55	51.01	92.52	92.33	92.87
Nm-53 011 030	17.85	63.41	84.96	78.81	95.72
Nm-53 011 041	16.28	63.84	87.67	81.54	96.03
Nm-53 012 029	12.89	60.63	90.32	80.18	94.73
Nm-53 020 045	19.89	66.55	87.19	86.35	96.95
Nm-53 025 017	17.80	67.18	87.20	83.20	96.38

Color key

identification inconclusive (multiple reference matches)
same chromophore, same genus, same species, same strain
same chromophore, same genus, same species, different strain
same chromophore, same genus, different species
slightly variant chromophore, different genus
different chromophore, different genus
different chromophore, same genus, same species, different strain

Proof of Concept: Spectral similarities of Raman spectra recorded from bacteria *in vivo*:
~ 95 % if the cells are from the same strain (blue)
~ 84 % if different strains of the same species (green)
~ 78 % if closely related different species (yellow)

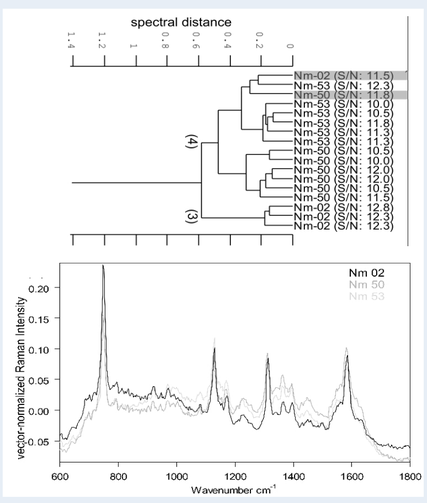
Scientific Background & Techniques

Cytochromes-c (cyt-c) are ubiquitous heme proteins, found in the majority of life forms on Earth. Phylogenetic trees based on extracted and purified cyt-c were published years ago. Bacterial cyt-c are extremely flexible proteins, showing dramatic changes in the protein moiety while the functional heme-c, clamped into the protein matrix, remains unaltered.

Heme-c consists of an iron-ion centered in a ring porphyrin connected with the protein solely via two cystein-bridges. X-ray analyses of cyt-c protein cystals revealed that the normally planar porphyrin is deformed by the surrounding protein.

Resonance Raman spectroscopy on extracted cyt-c confirmed these deformations to be specific for the protein moiety, thus for the respective life form.

Confocal Raman microscopy allows the *in vivo* analysis of microbial samples on the scale of single cells.



HCA of heme-c resonant Raman spectra recorded in vivo from individual bacteria cells of three closely related strains of ammonium oxidizing bacteria. [algorithm: WAL, distance measure: Euclidian]

Raman spectra as used for the HCA recorded in vivo in planktonic AOB cultures.

State of the Project

Optimization of Raman measurement parameters for recording cyt-c resonant Raman spectra from bacteria in planktonic culture and/or environmental samples *in vivo*.

Identification of the critical spectral parameters for successful HCAs.

Performance analysis of existent clustering algorithms for HCA of resonance Raman spectra originating from the same chromophor (high intrinsic similarity) or different chromophors (low intrinsic similarity).

Reliable identification of multiple bacteria strains *in vivo* based on individual cells and discrimination against different strains of the same species and a closely related species (see Table).

Next steps

Mapping a whole bacteria phylum: does cyt-c based RRS provide an optical phylogenetic tree comparable to existing ones?

Determination of the phylogenetic range: What are the closest and the most distant bacteria strains still categorized reliably?

Effects of different culture conditions on bacterial cyt-c resonant Raman spectra: applicability range of the method for analyzing complex environmental samples.

A. Kniggendorf, *Resonanz Raman Mikro-Spektroskopie zur Analyse bakterieller Biofilme*, Dissertation

contact: A. Kniggendorf
phone: +49 511 762-17910
fax: +49 511 762-17909
email: ann.kathrin.kniggendorf@hot.uni-hannover.de

Hanover Center for Optical Technologies
Nienburger Straße 17, 30167 Hanover, Germany
phone: +49 511 762-17908, fax: +49 511 762-17909
web: http://www.hot.uni-hannover.de