Abstract:

Elucidating the mechanistic role of aromatic amino acids in photosensors and enzymes by UV RR spectroscopy

Co-supervisor 1: Norbert Esser, ISAS e.V. and TU Berlin, Physics Department

Co-supervisor 2: Peter Hildebrandt, TU Berlin, Chemistry Department

The overall goal of the project is to establish ultraviolet (UV) resonance Raman (RR) spectroscopy as an instructive tool to analyse structural and mechanistic properties of proteins. UV RR spectroscopy sensitively probes aromatic amino acids, specifically tyrosine (Tyr) and tryptophan (Trp), which may be involved in the reactions of photoreceptors and enzymes. Here, we will employ continuous-wave UV excitation and a spectroscopic set-up that is based on a novel echelle spectrometer. Supported by site-directed mutagenesis, the project first aims at identifying structural changes of individual Tyr and Trp residues in biological photoswitches (phytochromes), such as hydrogen bonding interactions. Introducing nitrile-substituted Tyr or Trp at specific positions in the protein will then be used to monitor local electrostatic changes by exploiting the vibrational Stark effect. In addition, the dynamics of the structural and electrostatic changes will be addressed by extending the UV RR spectroscopy to the time-resolved domain in pump-probe experiments.

Extended description version of the project:

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1. Overall goal of the project

The overall goal of the project is to establish ultraviolet (UV) resonance Raman (RR) spectroscopy as an instructive tool to analyse structural and mechanistic properties of proteins. UV RR spectroscopy sensitively probes aromatic amino acids, specifically Tyr and Trp, which may be involved in the reactions of these biopolymers. In this project, we will employ continuous-wave (cw) UV excitation within a new spectroscopic set-up to minimize the risk of laser-induced protein degradation. The approach will be first developed and optimized on the basis of biological photoswitches that may adopt two distinct states with different conformations or interactions of individual Tyr or Trp residues. Upon extending the technique to the time-resolved domain in pump-probe experiments, we expect to gain insight into the structural and reaction dynamics of the protein.

2. State of the art

Amino acids as the building blocks of proteins do not only define the structure of these biopolymers. In many cases, individual amino acids are part of (biocatalytic) reaction pathways by (i) orientational or positional changes to generate reactive conformational states of the protein, (ii) alteration of their pK_a to assist proton or ion translocation, or (iii) participation in electron transfer chains via transient changes of the redox potential. It is therefore of utmost importance to establish tools that may report on the structural changes of amino acids during the reaction pathways of enzymes and proteins in general. In principle, vibrational spectroscopies are ideal techniques to probe structure and reaction dynamics of proteins but in most cases the target groups that can be monitored are protein cofactors (RR, IR) or carboxylic side chains of Glu and Asp (IR difference). With the development of lasers with emission lines in the deep UV, also aromatic amino acids like Tyr and Trp became accessible for RR spectroscopy [1]. The applicability of this technique, however, is so far largely restricted to a few robust systems since the typically used pulsed-laser excitation with high photon fluxes as well as long acquisition times bear the risk of protein degradation during the measurements.

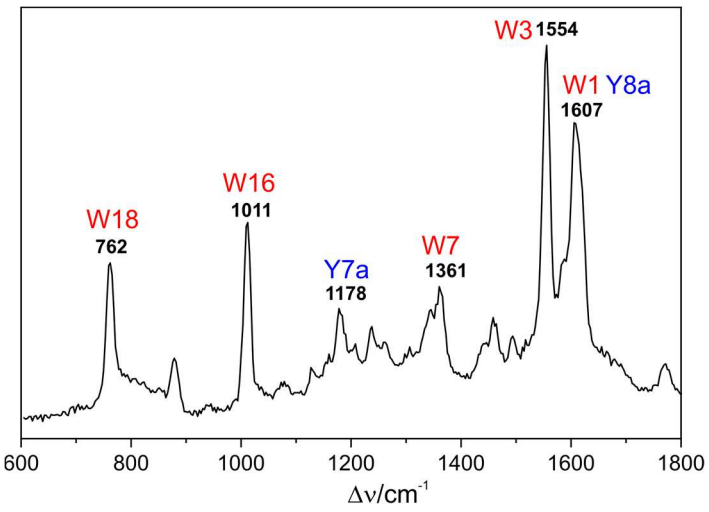

Fig. 1. UVRR spectrum of the parent state of the cyano-bacteriochrome Slr1939-g3 obtained with 220 nm excitation. Raman bands of Trps and Tyrs are labeled by red and blue symbols, respectively.

To overcome this drawback, we have developed a new UV-RR spectroscopic set-up with low photon-flux cw

excitation (e.g., 354, 266, and 220 nm). It is based on an echelle spectrometer that allows for the acquisition of high-quality spectra of proteins within a few minutes. The technique has already successfully been tested with a cyanobacteriochrome, a reversible biological photoswitch (Fig. 1).

3. Specific aims and how they may be reached

The project can rely upon available set-ups for UV-RR spectroscopy (ISAS) and visible RR and IR (difference) spectroscopy (TUB). Biochemical and molecular biology work will be supported by the infrastructure at the TUB and collaboration partners in UniSysCat and the CRC1078.

WP1. Identification of structural differences of Trp and Tyr in bacteriophytochromes.

Starting point of the project will be a bacteriophytochrome variant (Agp1 or Agp2) which exists in two stable states that can be interconverted by visible light [2]. First, conditions for UV RR spectroscopic experiments will be optimized according to the kinetic and photochemical properties of the protein such that spectral changes of the Trps and Tyrs can be safely determined in the parent states. Assignment of these changes to individual residues will be supported by available crystal structures and comparative studies of engineered variants with stepwise substitutions of the ca. five Trps and 10 Tyrs. Integrity of the variants will be checked by conventional RR spectroscopy and IR difference spectroscopy probing the biliverdin cofactor and protein structural changes, respectively [3].

WP2. Probing changes of the local electrostatics in bacteriophytochromes.

Introducing nitrile-substituted Tyr and Trp into bacteriophytochromes allows monitoring changes of the local electrostatics by monitoring the vibrational Stark effect of the nitrile function [4]. While such variants of Agp2 have already been successfully studied using IR spectroscopy, UVRR spectroscopy may provide complementary information. As shown previously, resonance enhancement at 220 nm is sufficient to detect the nitrile stretching [5]. In addition, band shape analysis may allow disentangling hydrogen bonding and non-covalent electric field effects. The evaluation of the data will carried out in conjunction with the results by conventional RR and IR (difference) spectroscopy, supported by theoretical calculations of the Mroginski group.

WP3. Extension of the project to the time-resolved domain.

The conversion between the two parent states of the bacteriophytochrome variants by light occurs on the long millisecond time scale [2]. To probe the key intermediates Meta-F/Meta-R thus requires an additional pump laser (P1) to initiate the photoconversion and a second pump laser (P2) to re-convert the photoreceptor to the original state. Such a pump-probe-pump scheme with millisecond time-resolution can be established by appropriate positioning the cw-laser foci on the flowing sample [1]. The desired time-resolution is then given by the flow rate of the sample and the spatial separation of the probe beam from the P1 pump beam.

4. References:

[1] Buhrke D, Hildebrandt P (2020) Probing structure and reaction dynamics of proteins using time-resolved Resonance Raman spectroscopy. Chem. Rev. 120, 3577-3630.

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[4] Fried SD, Boxer SG (2015) Measuring Electric Fields and Noncovalent Interactions Using the Vibrational Stark Effect. Acc. Chem. Res. 48, 998–1006.

[5] Weeks CL, et al. (2008) Investigation of an unnatural amino acid for use as a resonance Raman probe: detection limits and solvent and temperature dependence of the nC N band of 4-cyanophenylalanine. J. Raman Spectrosc. 39: 1606–1613.