Workshop - Vibrational Spectroscopy of Proteins

Hands-on Laboratory Work

Biochemistry and Vibrational Spectroscopy on [NiFe]-hydrogenase

The laboratory activities have been designed for **master/PhD students** and **young Postdoc researchers**, aiming to familiarize with the practical aspects of the biochemical and vibrational spectroscopic characterization of metalloenzymes. The course is limited to a max of **9-10 participants** due to Covid-19 laboratory safety. Candidates are expected to submit a **motivation letter**. Please include **skills**, **previous experience** and **qualifications** showing why you would be suitable for the course. Explain why you would like to attend and how you think you will benefit from the course.

Free Registration at: workshop.timb3@chemie.tu-berlin.de

If you are a member of a laboratory affiliated with the TIMB³ Consortium you are eligible for support that will cover your expenses. If you wish to apply for this support please submit a letter addressed to the course organizers indicating your current affiliation and outlining how your current research interests match the mission of the TIMB³ project, together with your application.

Experimental activities at TU Berlin on 25th-26th March 2021**

Meeting point: Max-Volmer-Laboratorium für Biophysikalische Chemie (PC building), TU Berlin.

**** The lab activities might be postponed due to Covid-19 restrictions**

9-10 Students divided in three groups (**Groups 1, 2 and 3**)

The lab activities start with a 20 minutes introduction on hydrogenases, catalytic cycle and details about biochemistry, IR and RR spectroscopies.

The aim of the proposed experimental activities is to introduce the students to the biochemical and vibrational spectroscopic methods used to investigate hydrogenase protein samples. The course will cover the purification of the regulatory [NiFe]-hydrogenase from *Ralstonia eutropha* using Strep-tag-II affinity chromatography, the determination of protein concentration and the determination of H_2-oxidation activity. The RH enzyme purified by the students will be characterized by means of IR and Resonance Raman spectroscopy. The metal cofactors of the RH, i.e. the $NiFe(CO)(CN)_2$ active site and [4Fe4S] clusters, will be detected in different redox states and the catalytic mechanism of [NiFe]-hydrogenases will be illustrated.

The proposed activities (A, B, C) are:

Biochemistry

Time scheduled: 6-8 h

A - Purification of the RH [NiFe]-hydrogenase (10 g of wet cell pellet), activity measurements (H_2 oxidation) and protein quantitation via BCA (bicinchoninic acid assay). At the end of the activity **A**, the students will fill a table reporting the protein yield and their specific activity of the RH preparation. The purified protein will be used for activities in **B** and **C. The protein solution will be aliquoted and stored in liquid N_2 by operators.**

Vibrational spectroscopy

Resonance Raman

Time scheduled: 5h

B – Introduction to **Resonance Raman** spectroscopy and measurement of as-isolated RH at three different excitation wavelengths (458, 514 and 648 nm) to monitor **Fe-CO/CN** and **C≡O/C≡N** vibrations of the active site and **Fe-S** bands of the [FeS]-cluster**. Every group will measure at one wavelength** the **low-frequency** (0 - 700 cm^{-1}) and **high-frequency** region (1100 - 2300 cm^{-1}) of the spectrum. At the end of their experiment, the results will be discussed with the operator. Each group will also receive a document with the expected RR results at the used excitation line and two additional RR spectra recorded with the other excitation lines. Students are expected to fill in a table reporting the more prominent RR bands at their specific excitation line and will assign them according to the material received.

Infrared

Time scheduled: 4-5h

C – **IR transmission** of RH. The students will record IR spectra of the protein samples in **A** detecting the protein amide II band as well as the absorptions of the CO and CN^- ligands of the NiFe active site in i) as-isolated and ii) H_2 reduced form (*). The operators will illustrate the difference of the IR spectra, related to redox changes in the hydrogenase samples. Based on literature data, **the students will assign the Ni_a-S, Ni_a-C and Ni_a-SR** catalytic intermediates.

*Reduction will be performed by a qualified operator and students will note the precautions to take when working under anaerobic conditions. Two glove boxes will be available to avoid multiple people in the same room: anaerobic tent in MVL and TC.

Groups	Experimental activity	Day and time schedule
Group 1	A	25th, 9:00 – 17:00
Group 2	A	25th, 11:00 – 19:00
Group 3	A	26th, 9:00 – 17:00
Group 1	B	26th, 9:00 – 13:30
Group 2	B	26th, 14:30 – 19:00
Group 3	B	25th, 9:00 – 13:30
Group 1	C	26th, 14:30 – 19:00
Group 2	C	26th, 9:00 – 13:30
Group 3	C	25th, 14:30 – 19:00