PhD course "Chemistry and biology of regulatory heme: demystification of a multifaceted molecule"

SYLLABUS

1 Lecturer information

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2 Title of the course

Chemistry and biology of regulatory heme: demystification of a multifaceted molecule

3 Course program

Labile heme, released from hemoglobin of ruptured red blood cells, has been considered an „alarmin" for around 30 years because of its cytotoxic, proinflammatory and procoagulant properties. The molecular and structural basis of the heme-protein interactions and their consequences, however, were unknown for a long time. Studies on the binding and functional effects of labile heme on proteins using artificial peptides and numerous native proteins supported the idea of labile heme as a regulator of protein function and thus an effector molecule in biochemical processes, yet also disease development (e.g., thrombosis, inflammation). Heme associates with proteins as an axial ligand, based on a coordinate bond between the central iron ion and an amino acid side chain containing heteroatoms (e.g. C, H, Y) and significant contributions of adjacent amino acids. The use of combinatorial peptide library screening, molecular and structural analysis by different spectroscopic methods (UV/Vis, rRaman, cwEPR, 2D NMR spectroscopy) enabled the establishment of a classification scheme for Cys-, His- and Tyr-based heme-binding motifs (HBMs). The course will teach the interdisciplinary approach (chemistry, biochemistry, biology, pharmacy) to elucidating the molecular basis of heme-protein interactions, including verification of protein function, and the importance of this knowledge for the development of biomedical applications in diagnosis and therapy.

4 Course content detailed per lesson of two hours (possibly with dates and room, real and virtual)

Tentative outline (in person/real):

Lesson 1: Introduction into heme (as a prosthetic group) and hemoprotein chemistry, biochemical background (physiology), heme-related diseases (pathophysiology).

Lesson 2: Heme research with focus on heme as an effector molecule (not as a prosthetic group), analysis methods based on peptides and proteins, elucidation of heme-binding/regulatory motifs in proteins, introdcution into spectroscopic analysis of heme binding.

Lesson 3: Methods for heme binding studies and structural analysis of heme-peptide/protein complexes (UV/Vis, rRaman, cwEPR, 2D NMR), prediction of heme-binding motifs.

Lesson 4: Applications and clinical relevance (from bacteria to humans).

Note: Interaction with students is encouraged and desired through the processing of worksheets and question papers.

5 Suggested reading

1. *Heme Biology: The Secret Life of Heme in Regulating Diverse Biological Processes (Book)*
   Edited By: Zhang Li, World Scientific, https://doi.org/10.1142/7484 | July 2011, Pages: 228
2. *Heme and erythropoieis: more than a structural role. (Review)*
   Chiabrando D, Mercurio S, Tolosano E. Haematologica. 2014 Jun;99(64.):973-83. doi: 10.3324/haematol.2013.091991.
3. *Regulatory Fe(II/III) heme: the reconstruction of a molecule's biography. (Review)*
   Kühl T, Imhof D. Chembiochem. 2014 Sep 22;15(14):2024-35. doi: 10.1002/cbic.201402218.
4. *Red alert: labile heme is an alarmin. (Review)*
   Soares MP, Bozza MT. Curr Opin Immunol. 2016 Feb;38:94-100. doi: 10.1016/j.coi.2015.11.006.
5. Other references (including e.g., Ref 1) will be announced and/or provided during the course.

6 Learning Objectives

- Acquire basic knowledge about porphyrins and their biological relevance with a particular focus on Fe(II/III) protoporphyrin IX
- Understand how combinatorial peptide libraries can help to establish consensus sequences for specific protein motifs
- Understand how instrumental analysis and biophysical methods can be applied to unravel biochemical ligand-protein interactions on a molecular and structural basis
- Interpret MS spectra and UV/Vis binding studies, determination of K_D values with different biophysical methods
- Plan own experiments to solve a relevant question concerning the analysis of protein interactions, including biochemical analysis of the impact of a specific interaction on a proteins' function

7 Knowledge and Skills to be acquired

General knowledge:

- Fundamental aspects of hemoproteins and heme as a prosthetic group
- Heme biosynthesis and heme-related diseases
- Heme as an effector molecule through transient binding to proteins
- Peptides as tools for analysing ligand/protein-protein interactions
- Use of bioanalytical and biophysical methods to solve biochemical questions

Specific skills:

- Interpretation of MS spectra from PED-MALDI-MS applications in the context of combinatorial peptide library screening
- Planning of UV/Vis experiments of ligand-peptide/protein complexes, interpretation of spectra and analysis of binding constants
- Basics in interpretation of spectra from other biophysical methods, critical evaluation of all experimental data and analysis results
- How to prove a hypothesis in a biochemical context using chemical (peptide) models and biophysical methods
- Transfer of knowledge and skills to other applications and scientific fields

8 Prerequisites

- Basic knowledge about amino acids, peptides, and proteins, including the structural levels of proteins
- Basics in organic chemistry, in particular solid-phase peptide synthesis
- Basic knowledge in spectroscopy (instrumental analysis) and biophysical methods (binding studies)

9 Teaching Methods

O MODE 1 – Pre-recorded lessons uploaded on the moodle platform (a meeting must be organized with PhD students in ordert o clarify eventual doubts)

x MODE 2 (preferred) – Lessons delivered in-person and in remote with simultaneous recording by the WEBEX platform

10 Further Information

n.a.

11 Type of Assessment

The final evaluations will have to be validated maximum 1 month after the end oft he course.
Evaluation sheets will be handed out directly after the course.

12 Period

Thursday 22, Friday 23, Monday 26, Tuesday 27th February 2024 - 3 CFU - 12 h including final exam