TECHNIQUES FOR STUDYING IRON IN HEALTH AND DISEASE

EMBL, Heidelberg, May 2-4, 2019

1st International laboratory course dedicated to analytical methods for assessing iron in living entities in physiological and pathological states

Directors: Dorine Swinkels(NL) and Ioav Cabantchik (IL)

https://www.embl.de/training/events/2019/BIR19-02/index.html

***Venue*: ATC premises of EMBL Heidelberg, Germany prior to the EMBL-8th Bioiron International conference May 5-10, 2018:**

https://www.embl.de/training/events/2019/BIR19-01/

The workshop is designed to expose the various experimental tools used for:

a. tracing iron dynamics in biological systems, from solution to cells to organs, with major emphasis on fluorescence techniques available in most laboratories

b. assessing iron status in humans in health and disease and

c. exploring methods to study iron sulphur cluster (ISC) proteins (ISP).

The 3-day workshop is planned for up to 18 students. Morning sessions comprise lectures and experimental planning and afternoon-evening data analysis and discussions. Bursaries covering the course registration fees are available for selected 10 applicants.

TOPICS

I. Thursday May 2 2019. GENERATION AND INTERPRETATION OF ASSAY TEST RESULTS FOR BIOMARKERS OF IRON HOMEOSTASIS AND DYSHOMEOSTASIS

Coordinator: Dorine W. Swinkels

The aim is to reach a level of understanding for adequately generating and interpreting the results of assays for biomarkers of iron homeostasis and dyshomeostasis in both research and clinical setting. Specifically, the students should acquire a level of understanding to be able to:

a. Clinically interpret test results of a broad range of conventional and novel iron biomarkers
b. Apply fundamentals of implementing an assay and generating a reliable test result in both the research and clinical setting.

In the workshop we will:

- Discuss interpretation of test results generated by a fully validated assay for the diagnosis of iron disorders. Students will be challenged to solve case –puzzles in small groups, and present their “diagnosis” for the whole group for discussion.
- Discuss design of a validation plan for an assay of an analyte. In the afternoon, students will apply this knowledge by performing a validation of a commercial hepcidin ELISA kit in the lab in small groups. By the end of the day each group will present their analytical findings for the whole group for discussion.

II. May 3 2019. DYNAMICS OF IRON SULFUR CLUSTER (ISC) PROTEINS (ISP): FROM SOLUTION TO CELLS

Coordinator: Coordinators: Stehling, Lill & Nechushtai

The aim is to get acquainted with the applicartion of spectroscopic methods for assessing mitochondrial ISP maturation within the cellular environment.

I. Biophysical and biochemical analysis of recombinant ISP

a. Determination of iron and sulphide content of holo-ISP: ISCA1 and FDX2 as paradigms.

b. Assessment of ISC cofactor stability under reducing and oxidizing conditions (UV/Vis spectroscopy).

c. Tracing ISC transfer between proteins by native gel chromatography and UV-VIS spectroscopy-CISD2-gene product NAF-1 (wild type and mutants) as paradigm.

II. Assessing the consequences of ISP assembly defects in cells in tissue culture reflected in:

a. morphological and metabolic changes of mitochondria; b. Activity measurements of key mitochondrial ISC enzymes by spectroscopic assays; c. ISC-dependent protein modification (lipoylation of DLAT and DLST).

b. ISC transfer between proteins by native gel chromatography and UV-VIS spectroscopy: CISD2-gene product NAF-1 (wild type and mutants) as paradigm.

d. the roles of amino acid residues in the ISC domain of NAF-1 on cell properties by fluorescence spectroscopy**:** a. mitochondrial membrane potential; mitochondrial labile (chelatable) iron; mitochondrial ROS susceptible to iron chelators.

Techniques: fluorescence plate reader and microscope imaging, UV-VIS spectroscopy, affinity chromatography and native protein gel chromatography

III. Thursday May 4 2019. FLUORESCENCE MONITORING OF LABILE IRON TRAFFICKING IN CELLS AND IRON SPECIATION IN ANIMAL FLUIDS AND ORGANS.

Coordinators: Pourzand, Hider and Cabantchik

We will explore how can fluorescence probes be designed and used for sensing iron dynamically in solution (fluorescence plate reader) or in cell compartments (fluorescence plate reader, FACS and fluorescence microscopy imaging). Theoretical aspects of labile iron sensing with the aid of fluorescence probes will be provided and application in solution and in cells demonstrated by:

I. Monitoring on line iron ingress (influx) into cytosol and mitochondria compartments with organelle targeted probes using both transferrin-iron and non-transferrin iron as iron substrates and. K562 as model cells.

II. Assessing the level of labile iron in biological fluids of clinical relevance: simulants of labile plasma iron as direct target of chelation. Performance of assays and data evaluation.

III. The application of a novel mitochondrial targeted iron sensor as a biochemical and pharmacological tool in iron research- applied to cell model.

Techniques: fluorescence plate reader, fluorescence microscope imaging and FACS.