March 25, 2024

Laboratory Class HS 2024 "Biological Chemistry B: New Enzymes from Directed Evolution Experiments"

General concept: The course deals with a specifically designed and genuine **research project**. We intend to carry out biological-chemical enzyme evolution experiments using molecular genetic mutation technologies and *in vivo* selection in recombinant bacterial strains. By working in parallel, teams of 2 participants each will generate a variety of different variants of a catalyst. Individual proteins will be purified and subsequently characterized using several different spectroscopic methods. The detailed chemical-physical analyses include determination of the enzymes' kinetic parameters and the integrity of the protein structure. The results obtained from the individual evolution experiments will be compared and discussed at the end of the class in a final seminar. We expect that during this lab course we will not only generate novel enzymes, but also gain new insights into the reaction mechanism of the investigated catalyst.

Course format: The program runs over 3.5 weeks at 3.5 days per week (Tue afternoon to Fri evening). The class is a **"block course"** (involving experiments that require **long working days with a tight schedule!**), consisting of the practical course itself and an integrated series of 1-2 hours daily lectures. All technologies used for the experiments will be explained to the students in theory and in practice with the goal that they will be able to independently apply them for the course project and in future research endeavors. After the course, an individual report about the results obtained must be prepared. The teaching language is **English**.

Dates of the course: Fall semester, 3rd quarter: **Nov 5 through Nov 27, 2024**.

Locations: ETH Campus at Hönggerberg, 8093 Zurich, building HCI.
Practical work: **HCI E 392**; *Lectures:* **HCI J 374** (= *meeting place for the introductory session on Nov 5, 2024,* ***exactly at 12:45****).*

Participants: BSc ETH Biochemistry-Chemical Biology & ETH/UZH Biology students in their 3rd year of study; MSc and Ph.D. students.

Performance assessment: Credit points can only be earned if the candidate *(i)* actively takes part in the **entire** program, *(ii)* turns in a final report, and *(iii)* obtains at least the passing grade 4.0 (overall performance).

In charge/main contact: Prof. Peter Kast, Laboratory of Organic Chemistry, ETH Zurich,
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Signing up for HS 2024: Must use the reservation system for the biology block courses offered in the 5th semester (third year of study). The maximum number of participants for this laboratory class is 12, but surplus applicants may contact P. Kast directly to have their names added to a **waiting list**.

A valid registration is considered a **commitment for attendance of the entire course**, as involved material orders and experimental preparations are necessary and, once the class has started, the flow of the experiments must not be interrupted by individual absences. In case of an emergency, please immediately notify P. Kast.

Information on the web: http://www.kast.ethz.ch/teaching.html

(Presumed)
Program for the Laboratory Course "Biological Chemistry B: New Enzymes from Directed Evolution Experiments"

	Experiment
Week 1:	– Construction of gene libraries using PCR – Analytical and preparative agarose gel electrophoresis – Preparative endonuclease digestion of DNA – Extraction and purification of DNA fragments – Ligation of DNA and transformation (by electroporation) – Preparation of growth and selection media (autoclaving, pouring agar plates) – *In vivo* selection experiments for functional library clones
Week 2:	– Purification (streak out) of promising candidate clones – Preparation of transformation-competent bacterial cells – Phenotypic confirmation (Master Plates) – Plasmid extraction and purification – Restriction analysis of DNA – Retransformation into competent cells (by heat shock) – DNA quantitation for external DNA sequencing – Colony PCR of non-selective library clones
Week 3:	– Computer-assisted evaluation of DNA sequences – *In vivo* complementation assay (optional) – DNA sequencing reactions (cycle sequencing) of non-selective library clones – Gene expression of 2 new mutants using a T7 RNA polymerase system – Preparative cultivation of bacteria, harvesting, breaking open cells – Protein purification by metal affinity chromatography – SDS polyacrylamide gel electrophoresis – Dialysis – Determination of protein concentration (BCA assay) – Michaelis-Menten enzyme kinetics (k_{cat}, K_m) by UV spectroscopy – Liquid chromatography-mass spectrometry (LC-MS) of purified proteins
Week 4:	– Michaelis-Menten enzyme kinetics (k_{cat}, K_m) by UV spectroscopy – Circular dichroism (CD) spectroscopy – Native polyacrylamide gel electrophoresis (optional) – ^{1}H, ^{15}N-HSQC (2-D protein NMR) spectroscopy (optional) – Thermal-Shift Assay (TSA) – Comparing features of the newly evolved proteins with the wild-type enzyme (or the starting material) – Collection of samples for ongoing research
Final data evaluation	In a final seminar (Wednesday afternoon/evening of week 4), the results of the course will be presented and discussed. A report will have to be turned in subsequently.