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Minutes

Expression, purification and basic characterization of target protein samples for drug binding studies

Monday 8 September – Wednesday 10 September 2025

Latvian Institute of Organic Synthesis, Riga, Latvia

Training School of the COST Action CA21111
One Health drugs against parasitic vector borne diseases in Europe and beyond
OneHealthdrugs

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Minutes

1. LIST OF ATTENDANTS

Number	Name	Country
1.	Walliyulahi Ajibola	HU
2.	Hanifi Atif Arslan	FI
3.	Hatice Aslı Bedel	TR
4.	Foteini Batsolaki	GR
5.	Shapla Bhattacharya	LV
6.	Cagla Burcin Bicakci	TR
7.	Maria Paola Costi	IT
8.	Francesco Calzaferri	IE
9.	Rossella Castagna	LV
10.	Michael Lyngbæk Christensen	DK
11.	David Cisneros	ES
12.	Johander Inacio Dos Ramos Azuaje	PT
13.	Sheraz Gul	DE
14.	Atis Jekabsons	LV
15.	Mindaugas Lesanavicius	LT
16.	Teodors Pantelejevs	LV
17.	Emilio Parisini	LV
18.	Alessio Perazzoli	LV
19.	Filippo Piazza	IT
20.	Cecilia Pozzi	IT
21.	Anastasija Rudnickiha	LV
22.	Elina Zeltkalne-Ratniece	LV
23.	Şener Çintesun	TR
24.	Leonid Rozanov	LV

2. DESCRIPTION OF THE ACTIVITIES

A competitive selection procedure was implemented by the organizing committee to finalize the list of trainees, ultimately admitting 12 participants. Candidates were chosen primarily on the basis of their academic preparation and professional expertise to ensure a well-matched cohort. Following the selection, official invitations were distributed via the e-COST platform.

The training school was organized by the local committee (Emilio Parisini, Rossella Castagna, Elina Zeltkalne-Ratniece) and included three lectures and multiple hands-on experiences, which were provided and guided by a panel of three speakers (Teodors Pantelejevs, Emilio Parisini, Cecilia Pozzi) and five laboratory trainers (Shapla Bhattacharya, Atis Jekabsons, Alessio Perazzoli, Anastasija Rudnickiha , Leonid Rozanov).

Day 1 – 08/09/2025

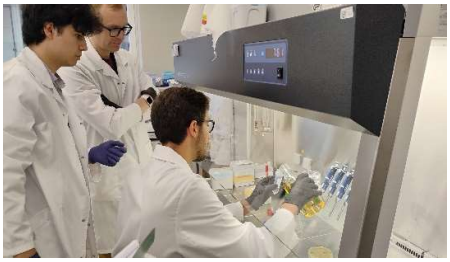
The conference was formally opened by the Coordinator of the COST Action project, Prof. Maria Paola Costi (online), who provided an overview of the initiative. During the opening session, the project's objectives, methodology, and overall framework were presented, offering participants a clear understanding of the context and goals of the training school.

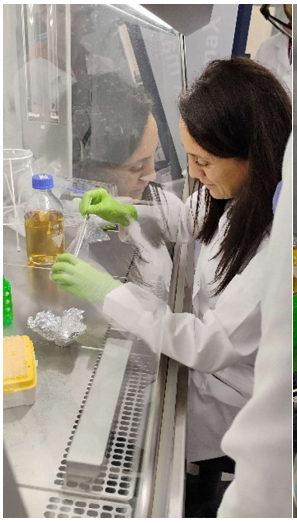
Lecture 1 - Introduction to protein expression in bacteria (Dr. Teodors Pantelejevs, LIOS)

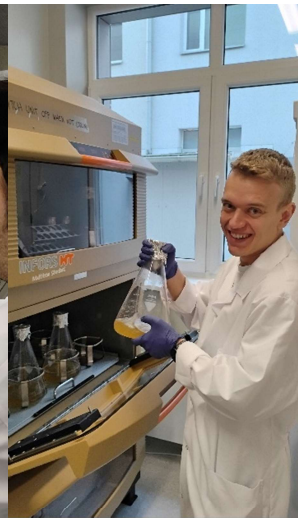
The lecture provided an overview of recombinant protein production in E. coli, discussing the advantages of in-house expression, such as cost-effectiveness, higher yield, and flexibility for experimental needs. It compared natural, synthetic, and recombinant protein sources, emphasizing considerations like post-translational modifications, disulfide bonding, and protein toxicity when choosing an expression host. Key elements of an expression construct were outlined, including plasmid vectors, antibiotic selection markers, promoters (especially the T7/lac system), and affinity or solubility tags. The importance of strain choice was stressed, with examples like BL21(DE3) derivatives designed for toxic proteins, rare codon optimization, or disulfide formation. Practical aspects of expression were covered, including transformation, media composition, carbon source supplementation, and controlled induction with IPTG during mid-log growth. The lecture concluded by emphasizing small-scale expression screening and optimization of temperature, duration, and inducer concentration before scaling up to maximize yield and protein quality.

Laboratory activities – Day 1

The laboratory activities focused on producing recombinant protein using E. coli as the expression system. Students were divided in 4 small groups to allow them to gain hands-on experience. The team began by introducing the gene construct into bacterial cells and allowing colonies to form on selective media. A single colony was then used to start a liquid culture, which was grown to the appropriate density before protein expression was induced. The cells were harvested and collected as a pellet. This pellet was stored for subsequent purification, completing the workflow from transformation to preparation for protein isolation.



Day 2 – 09/09/2025

Lecture 2 - SDS Page and Protein Purification

(Prof. Emilio Parisini, Latvian institute of Organic Synthesis and University of Bologna)

The lecture introduced the principles of protein purification, emphasizing its importance for studying protein structure, function, and applications in research or industry. It outlined the purification workflow starting from recombinant expression, cell lysis (physical, sonication, osmotic shock), centrifugation, chromatography, and final purity assessment by SDS-PAGE. Affinity chromatography, particularly Immobilized Metal Affinity Chromatography (IMAC), was explained in detail, including the use of His-tags and elution strategies with imidazole or pH shifts. Other affinity systems such as GST-, FLAG-, and MBP-tags were presented as alternative strategies for efficient single-step purification. The lecture also covered size-exclusion chromatography for separation by size and ion-exchange chromatography for separation by charge, describing how pH and salt gradients are used to elute proteins. Finally, it stressed the importance of understanding a protein's biochemical properties (stability, charge, cofactors, aggregation behavior) to select the most effective purification strategy and achieve high-quality samples for downstream applications.

Laboratory activities – Day 2

On the second day, the focus shifted to extracting and purifying the expressed protein from the bacterial cells. After breaking open the cells to release the protein into solution, the lysate was clarified and subjected to a series of purification steps. The workflow began with affinity chromatography to selectively capture the target protein, followed by size-exclusion chromatography to separate it based on molecular size, and concluded with ion-exchange chromatography to refine purity based on charge properties. Samples were collected at each stage to monitor progress, and the final purified protein was analyzed to confirm its concentration and quality. This sequential process efficiently progressed from crude lysate to a purified protein ready for further characterization.

On the evening of the second day, all participants chose to meet independently for a group dinner at a restaurant in Riga's city center. This informal gathering provided a relaxed setting for participants to share experiences, deepen personal connections, and engage in conversations beyond the laboratory. The dinner also offered the opportunity to meet other researchers from LIOS, helping participants to establish new professional connections and strengthen the sense of community within the training school.

Day 3 – 10/09/2025

Lecture 3 - Biophysical assessment of protein quality

(Prof. Cecilia Pozzi, University of Siena)

The lecture explored the main biophysical techniques for protein quality assessment, emphasizing their use in structural analysis, stability testing, and interaction studies. Circular Dichroism (CD) spectroscopy was presented as a method to investigate secondary and tertiary structure by analyzing far-UV and near-UV spectra, enabling detection of folding states and conformational changes. Differential Scanning Fluorimetry (DSF) was explained as a thermal unfolding assay that measures changes in fluorescence during protein denaturation, allowing determination of melting temperature (Tm) and the effects of ligands or buffer conditions on stability. The lecture also introduced MALDI-TOF mass spectrometry, describing the process of sample preparation, matrix crystallization, laser ionization, and time-of-flight detection for protein identification and characterization. Applications of these methods were highlighted in protein quality control, formulation development, and drug discovery, where understanding structural integrity and stability is crucial. Finally, practical considerations such as removing salts for MALDI-TOF and optimizing DSF signal quality were discussed to ensure accurate results.

Laboratory activities – Day 3

A series of complementary biophysical and analytical techniques were employed to characterize the reference protein. The investigation began with circular dichroism spectroscopy to assess the protein's secondary structure, which revealed a conformation consistent with the previously determined crystal structure. Protein stability was then evaluated using differential scanning fluorimetry, allowing determination of its thermal melting temperature and providing insight into its folding properties. Finally, mass spectrometry (MALDI-TOF) was used to confirm the protein's identity and purity, revealing the expected molecular weight along with the presence of oligomeric forms. Together, these analyses provided a comprehensive overview of the protein's structural integrity and stability, integrating information from both spectroscopic and mass-based techniques to validate the quality of the purified sample.

Final student's flash-presentation

The student concluded the training school with a flash presentation summarizing the laboratory activities. The presentation retraced each step of the experiments and provided a critical analysis of the results obtained.

CONCLUSIONS

The 6th OHD Training School unfolded seamlessly, guiding students through the complete workflow of protein research in a clear and engaging manner. Beginning with the expression of a reference recombinant protein in E. coli, participants learned essential skills in bacterial transformation, colony selection, and controlled induction of protein expression. Through the purification steps, the participants gained hands-on experience with affinity, size-exclusion, and ion-exchange chromatography, learning how to monitor and optimize protein yield and purity at each stage. On the final day, students explored biophysical and analytical techniques (circular dichroism, differential scanning fluorimetry, and MALDI-TOF mass spectrometry) developing the ability to assess protein structure, stability, and identity. Across all activities, they also strengthened critical laboratory competencies such as experimental planning, data collection, and troubleshooting. The smooth execution of the experiments, combined with the instructors' guidance, allowed students to connect theoretical knowledge with practical application. The experience culminated in a flash presentation, where the student retraced the laboratory journey, critically analyzed the results, and demonstrated a clear understanding of the methods and their interpretation. Overall, the training provided participants with both practical expertise and scientific insight, fostering confidence in executing and evaluating protein production and characterization experiments.