

COURSE LAYOUT

1. GENERAL

SCHOOL	APPLIED BIOLOGY & BIOTECHNOLOGY		
DEPARTMENT	BIOTECHNOLOGY		
STUDY LEVEL	<i>Undergraduate</i>		
COURSE CODE	346	SEMESTER	9 th
COURSE TITLE	Advanced Topics in Structural Biology		
INDEPENDENT TEACHING ACTIVITIES		WEEKLY TEACHING HOURS	ECTS
Lectures		3	1,56
Laboratory exercises		2	0,64
Group and/or individual assignments			0,64
Independent study			2,16
TOTAL			5,00
COURSE TYPE <i>general background, special background, specialised general knowledge, skills development</i>	Specialised		
PREREQUISITES	No, but successful examination in the courses: Biomolecular Biochemistry, Molecular Recognition, Recombinant DNA Technology is recommended.		
LANGUAGE	Greek		
IS THE COURSE OFFERED for ERASMUS STUDENTS?	No		
COURSE WEB PAGE	https://oeclass.aua.gr/eclass/courses/6351/		

2. LEARNING OUTCOMES

Learning Outcomes
Upon successful completion of the course, the student will be able to: - Analyze protein sequences and select appropriate domains for structural studies, assessing the functional relevance of potential truncations or fusions. - Use structural and functional bioinformatics tools (UniProt, Pfam, CATH/SCOP, solubility and intrinsic disorder prediction, crystallizability prediction). - Understand the advantages and limitations of expression systems used in Structural Biology. - Select appropriate expression systems based on the specific requirements of a given project. - Design molecular constructs taking into account the expression system, codon optimization, and mRNA-level stability. - Understand the particular challenges of membrane proteins and the strategies for their isolation, purification, and stabilization. - Apply protein stabilization strategies using ligands, transition-state analogues, Fabs, nanobodies, and protein fusions. - Perform the basic steps of structure determination and refinement (molecular replacement, model building, and refinement). - Understand the information contained in the Protein Data Bank (PDB), from the asymmetric unit to the biologically relevant assembly.

- Evaluate the quality of structural models and understand the structure deposition process in the PDB.

General Competences

1. Search and analysis of data and information using the necessary technologies.
2. Adaptation to new situations.
3. Decision making.
4. Autonomous work.
5. Group work.
6. Generation of new research ideas.
7. Project design and management.
8. Promotion of free, creative and inductive thinking.

3. COURSE CONTENT

I. THEORY

1. Introduction to Structural Biology project design.
2. Workflow concepts and the notion of a “molecular construct”.
3. Domain selection using UniProt, AlphaFold, Pfam, CATH/SCOP.
4. Functional assessment of truncations.
5. Disordered regions and their functional significance. Intrinsically disordered regions (IDRs): when and why they are removed.
6. Solubility of non-membrane proteins and crystallizability (use of CamSol, Aggrescan3D, PsiPred, CrystalPred, etc.).
7. Protein fusion with stable partners (T4 lysozyme, MBP, GST) to enhance expression, folding, and crystallizability.
8. Expression systems in Structural Biology (bacteria, yeast, insect cells, mammalian cells) and post-translational modifications (glycosylation, phosphorylation). Addressing the “glycosylation problem.”
9. Codon optimization and its impact on mRNA structure. Partial or full optimization? Criteria and conditions for implementation.
10. Membrane proteins in Structural Biology (expression, purification strategies, use of detergents and nanodiscs).
11. Stabilization of recombinant proteins using ligands (agonists, antagonists, inhibitors).
12. Nanobodies and stabilization of transient conformational states. Applications in crystallography and cryo-electron microscopy.
13. Protein structure deposition procedures in the PDB. Evaluation of structural quality and interpretation of the information contained in the PDB, using real structure examples.

II. LABORATORY

1. Selection of an initial protein domain and functional assessment

- Sequence retrieval from UniProt
- Structural analysis using AlphaFold DB
- Use of information from CATH and SCOP
- Prediction of intrinsically disordered regions (e.g., IUPred)
- Initial assessment of potential truncations

Objective: A well-documented proposal of a protein domain, including justification of its boundaries and commentary on functional regions that must be preserved.

2. Assessment of solubility and crystallizability

- Analysis using CamSol and Aggrescan3D
- Secondary structure prediction using PsiPred

- Crystallizability prediction using XtalPred
- Discussion of the impact of disordered regions on crystallization

Objective: A concise report correlating solubility, structural order, and crystallization propensity.

3. Truncations and introduction of protein fusions

- Proposal of truncations that improve the properties assessed in Exercise 2
- Evaluation of their functional impact
- Introduction of fusion partners (MBP, GST, T4 lysozyme)
- Discussion on providing interaction surfaces or stabilizing protein structure

Objective: A comparative table of different molecular constructs with respect to stability (and potential expression) and crystallizability.

4. Selection of expression system and construct design

- After selecting an expression system (E. coli, yeast, insect, mammalian), evaluation of the relevance of post-translational modifications (e.g., glycosylation) and purification strategy, followed by selection of an appropriate expression plasmid
- Design of suitable tags and proteolytic cleavage sites
- Implementation of the molecular construct in Benchling

Objective: Selection of an appropriate heterologous expression system and design of the molecular construct.

5. Codon optimization and mRNA analysis

- Codon optimization using IDT
- Analysis of rare codons using SnapGene
- Prediction of mRNA secondary structure using RNAfold
- Identification of potential inhibitory structures affecting translation

Objective: Adaptation of the nucleotide sequence to the chosen expression system and identification of potential translational issues.

6. Molecular replacement and initial electron density map

- Selection of an appropriate model from the PDB
- Execution of molecular replacement (Phaser/Phenix)
- Evaluation of the solution and the initial electron density map

Objective: Introduction to structure determination by molecular replacement.

7. Model building, reciprocal- and real-space refinement, and ligand fitting

- Use of Phenix/refine for refinement and Coot for model correction based on electron density (application of geometric restraints, TLS concepts, etc.)
- Ligand building
- Alternative conformations
- Addition of water molecules and metal ions

Objective: Improvement of the structural model and incorporation of ligands.

8. Structure validation and deposition in the PDB

- Analysis of real structures from the PDB
- Interpretation of validation reports (MolProbity, PDB)
- Temperature factors and structural quality: implications for structural information content
- Identification of problematic regions
- Simulation of the structure deposition process

Objective: Understanding the deposition process of structural data and critical evaluation of the information accompanying each structure based on established quality criteria.

4. TEACHING and LEARNING METHODS - Evaluation

TEACHING METHOD	In suitably equipped teaching rooms	
USE OF INFORMATICS and COMMUNICATION TECHNOLOGIES	Use of powerpoint presentations in lectures, use of e-class website and videos to inform, distribution of educational material, delivery & grading of laboratory exercises, molecular graphics software	
TEACHING ORGANISATION	<i>Activity</i>	<i>Work Load</i>
	Lectures	39 h
	Laboratory exercises	16 h
	Group and/or individual assignments	16 h
	Independent study	54 h
	<i>Course total (25 hours of student work load per ECTS)</i>	<i>125 h (5 ECTS)</i>
STUDENTS EVALUATION	I. Written final exam on theory (50%), which includes: • Multiple choice questions. • Questions of short and/or detailed development. • Problems/exercises based on theoretical knowledge developed in the lectures. II. Written final laboratory examination (40%), including: • Analysis of a new structural biology project, different from those addressed in the laboratory exercises, and responses to multiple-choice and short-answer questions. Individual assignments related to the laboratory exercises (10%)	

5. BIBLIOGRAPHY

1. Introduction to protein structure (Branden & Tooze) (1999). Garland Publishing Inc, ISBN 0-8153-0270-3
2. Biochemistry (Stryer) (2015) Broken Hill Publishers..
3. Biomolecular crystallography, Principles, Practice and Application in Structural Biology (Rupp Bernhard) (2010) Garland Science, ISBN 978-0-8153-4081-2
4. Expression, Purification, and Structural Biology of Membrane Proteins (Camilo Perez, Ana Ramirez) (2026), Humana Press, Springer Protocols