课程大纲
COURSE SYLLABUS

1.	**课程代码/名称** **Course Code/Title**	**蛋白质工程 Protein Engineering and Molecular Targeting（PTME）**
2.	**课程性质** **Compulsory/Elective**	**选修课 Elective**
3.	**课程学分/学时** **Course Credit/Hours**	3 学分/48 学时
4.	**授课语言** **Teaching Language**	英语 English
5.	**授课教师 Instructor(s)**	Peter Pimpl
6.	**先修要求** **Pre-requisites**	普通生物学，生命科学概论 General Biology， Introduction to Life Science

7. 教学目标 Course Objectives

蛋白质是结合当今生命科学各个方面的关键因素。蛋白质是基础研究和技术应用开发的关键参与者。因此，工程改造蛋白质的能力是成功开展项目研究并在该领域建立未来事业的关键。
本课程阐明了蛋白质工程中的基本原理和策略，并提供了在活细胞中使用功能性工程蛋白来为基础项目研究和应用科学开发新的研究工具和实验策略的机会。本课程的内容以实际问题为基础，旨在为学生自己的研究项目或研究兴趣提供解决问题的方法，而不依赖于特定的模式生物。
学生将学习蛋白质工程学的方法、策略和目标（L1-3）；如何工程化蛋白质以控制细胞的形态和功能（L4）；了解真核和原核生物中蛋白质合成，加工和转运的分子机制（L5-6），这是成功分析蛋白质/细胞功能和优化蛋白质表达以最大化重组蛋白质产量的要求（L7）；了解生成特异性抗体以进行蛋白质功能战略分析的优势（L8-9）；如何生成用于快速克隆多域融合蛋白的个性化载体系统（10）；了解在生命科学（L11-12）中使用工程化纳体的新机会，以及如何在真核细胞中设计和利用纳体-表位融合蛋白用于战略研究和生物技术应用（L13-14）。
在与每次主题相对应的研讨课上，学生的口头报告可以加深对主题的理解，每一个主题之后也会着重对实验方法、实验策略和获得的结果进行简短的讨论。
Proteins are the key players that unite all aspects of today’s Life Sciences. Proteins are crucial key players for basic research and development of technical applications. The ability to engineer proteins is therefore the key to successfully perform research projects and to establish a future career in this field.
The course pinpoints fundamental principles and strategies in protein engineering and illustrates the opportunities of using functionally engineered proteins in living cells to develop new research tools and experimental strategies for basic research projects and for applied sciences. The content of the course is illustrated based on real-life problems and aims at offering problem-solving approaches for the students' own research projects or research interests, independent of specific model organism.
The students will learn about methods, strategies and aims in protein engineering (L1-3), how to engineer proteins to control morphology and function of cells (L4), understand the molecular mechanisms of synthesis, processing and trafficking of proteins in eukaryotic and prokaryotic cells (L5-6) as requirement towards successful analysis of protein/cellular functions and the optimization of protein expression to maximize production yields of recombinant proteins (L7), understand the advantages of generating specific antibodies for strategic analysis of protein functions (L8-9), how to generate a personalized vector system for fast cloning of multidomain fusion proteins (10), understand the new opportunities of using engineered nanobodies in Life Science (L11-12), how to design and utilize nanobody-epitope fusion proteins in eukaryotic cells for strategic research and biotechnological applications (L13-14).
The lecture topics are deepened in accompanying seminars by oral presentations by the students, each of which is followed by a short discussion with focus on methods, experimental strategies and results obtained.

8. 教学方法 Teaching Methods

Lectures (L) and seminars (S) with oral presentations by students (individual/group)

9. 教学内容 Course Contents

Section 1	Lecture 1: Protein engineering for research and technological applications: Aims and strategies, rational design, directed evolution & de novo design of proteins, prerequisites and consequences.
Section 2	Lecture 2: Tagging proteins for purification, detection and functional analysis: Epitope tags, enzymatic tags, fluorescent protein tags, interaction tags, Seminar 1 (L1/2)
Section 3	Lecture 3: Engineering proteins to modify function and to improve stability: Kinetic stability, thermodynamic stability and process stability. Considerations for preproduction, production and postproduction pipelines.
Section 4	Lecture 4: Engineering cellular functions in eukaryotic cells: Design of logical gates for information processing: NOT. NOR, S-R latch via promotor/transcription factor engineering to control morphogenetic modules. Seminar 2 (L3/4)
Section 5	Lecture 5: Protein expression in eukaryotic cells: Principles of synthesis, folding and processing of proteins in eukaryotic cells, considerations and prerequisites for establishing protein expression strategies for soluble proteins, type I and type II membrane proteins and for multi-spanning membrane proteins.
Section 6	Lecture 6: Protein sorting and transport in eukaryotic cells. Prerequisites for successful targeting of engineered proteins to intracellular locations. Protein sorting mechanisms of soluble /membrane proteins, receptor transport, vesicle-mediated intracellular protein transport. Selective protein targeting strategies. Seminar 3 (L5/6)
Section 7	Lecture 7: Design and use of quantifiable and fluorescent marker and reporter proteins to analyse protein-protein interactions and protein function in eukaryotic cells. Transient gene expression systems in eukaryotic cells, transfection methods, protein expression and knock-down strategies, expression of dominant-negative deletion/substitution mutants and protein-protein interaction analysis.
Section 8	Lecture 8: Generation of specific polyclonal antibodies. Structure and function of IgG antibodies, cloning, expression and purification of antigens for immunizations, characterization of immune sera and antibody specificity. Seminar 4 (L7/8)
Section 9	Lecture 9: Strategic use of specific antibodies to analyse protein location, function and protein-protein interactions. Antibody-based analyses: Immune fluorescence microscopy, immune electron microscopy, immune precipitation, co-immune precipitation, SDS-PAGE, cell identification and cell sorting.
Section 10	Lecture 10: Designing vector systems for fast and efficient generation of N-/C-terminal fusion proteins for signal-peptide/sorting signal-containing proteins for designing and generating complex multidomain fusion proteins. Seminar 5 (L9/10)
Section 11	Lecture 11: Nanobodies: generation, structure and function of nanobodies. Nanobody-based applications in research, diagnostics and therapeutics. Engineering strategies to enhance nanobody-epitope interactions. Strategies for epitope mapping and nanobody-epitope interaction analysis for in vivo applications.
Section 12	Lecture 12: Design of Nanobody and epitope-tagged fusion proteins for assembly and intracellular targeting of proteins complexes in eukaryotic cells by nanobodies-epitope interaction. Strategies for post-translational protein labelling, intracellular targeting, protein

		tracing and molecular trapping in eukaryotic cells. Seminar 6 (L11/12)
	Section 13	Lecture 13: Design of dual epitope proteins for assembly of differential nanobody-fusion protein populations in eukaryotic cells. In vivo crosslinking of membrane proteins in eukaryotic cells by dual-epitope linker proteins, design and use of dual-epitope linker to analyze and manipulate protein transport and cellular functions.
	Section 14	Lecture 14: Nanobody-based i*n vivo* immunoprecipitation (iVIP) strategies. iVIP systems to analyze composition and stoichiometry of cytosolic protein complexes and to manipulate cellular functions in eukaryotic cells, comparison iVIP vs. FRET-FLIM/IP/Co-IP. Seminar 7 (L13/14)
	Section 15	Review session with questions/answers & conclusions

10.	课程考核 Course Assessment
	出勤 10%+课堂表现 10%+研讨课表现 30%+家庭作业 50% Attendance 10%+Class performance 10%+Seminar performance 30 %+Homework 50%
11.	**教材及其它参考资料 Textbook and Supplementary Readings**
	Textbooks for general reading: 1. Molecular Biology of the Cell, Bruce Alberts, Alexander D. Johnson, Julian Lewis, David Morgan, Martin Raff, 6th edition 2014. 2. Molecular Cell Biology, Harvey Lodish, Arnold Berk, Chris A. Kaiser, Angelika Amon, Hidde Ploegh, 8th edition 2016.