Methods in Biochemistry (KB8002)

AIM

'Methods in Biochemistry' will introduce students to the fundamental principles behind several basic and more advanced techniques commonly used in biochemical research. During the course, the strengths and weaknesses of each technique will be covered to explain which techniques are suitable for particular applications. Upon establishing this foundation, the students will be taught how to combine different methods to overcome problems associated with the individual techniques. The theoretical aspect of the course will be supplemented with several lab practicals and one demostration where the students will apply many of these techniques themselves. In the end, the students should be able to design strategies to address complex biological questions using the techniques they have been exposed to from a theoretical and practical perspective.

Upon completion of the course you should be able to:

- Describe the principles behind a number of common biochemical techniques.
- Explain the strengths and weaknesses of a technique for particular applications.
- Combine different biochemical methods to address a complex biological question.
- Troubleshoot biochemical methods based on their scientific principles.
- Analyze generated data and communicate them in writing and orally.
- Read, communicate and critically evaluate course-related scientific literature.

You will be expected to:

- Attend and actively participate in the lectures and tutorials.
- Participate in discussions with other students and the different faculty.
- Read the assigned literature and complete the assignments on time.
- Present the lab practical results in writing and make the appropriate corrections.

COURSE CONTENT

-Cell homogenization and fractionation The organization and chemical composition of pro- and eukaryotic cells will be discussed in relationship to different homogenization methods, which are used to enrich for particular fractions from these cells.
-Materials on how to make buffers and solutions (incl. sterilization), pH, the metric system, statistics and making lab. notes are provided at the beginning of the course.

-'History of molecular biology' Students will be taught the history behind the main organisms used for molecular biology and how these are handled, genotyped, cultured and stored.

-Centrifugation The theoretical basis for multiple centrifugation techniques will be explained as well as their applications. These include: differential centrifugation, density gradient centrifugation and analytical ultracentrifugation.

-Recombinant DNA techniques Several modern DNA analysis methods will be covered ranging from DNA isolation and sequencing to PCR and a variety of molecular biology techniques for DNA manipulation. Significant time will be spent on: restriction enzyme digests and other reactions that modify DNA, PCR, primer design, sequencing methods, homology cloning, Gibson cloning, recombineering, Cas9-based genome editing, forwarded evolution etc.

-Protein production Several different eukaryotic and prokaryotic protein production platforms are available. These will be thoroughly explained from both a theoretical and need perspective based on the current protein production bottlenecks. Specifically, we will address how to choose an organism, the expression system, and the design of the target gene.

-Protein isolation using physiochemical properties The biochemical principles for a number of basic protein purification techniques will be covered. This will include the buffer systems and resins that support: affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography, and gel-filtration. We will also go over which of these are suitable for HPLC and why.

-Protein-protein interactions Bacterial and yeast two hybrid systems, co-affinity purification, cross-linking, 2D BN-PAGE etc.

-Antibodies and their applications We will discuss the factors to consider for generating a polyclonal/monoclonal antibody, alternative binding approaches that can replace antibodies, and several applications such as: immuno-blotting, immuno-precipitations, and ELISAs.

-Mass spectrometry Basics of mass spectrometry, MALDI-TOF, MS/MS (incl. sequencing proteins), quantitative proteomics (*e.g.*, iTRAQ, iCAT, spectral counting), characterization of post-translational modifications using MS etc.

-Gel-electrophoresis and detection methods Different gel-electrophoresis techniques for DNA and protein will be explained as well as a number of methods for staining and detection.

-Microarrays DNA + protein arrays, RNA-seq, ribosome profiling etc.

-Spectroscopy Introduction: what is spectroscopy, light – frequencies, lambert-Beer's law, electron spectroscopy, chromophores in biology, applications: CD spectroscopy, infrared spectroscopy emission - fluorescence

-Methods in Enzyme Kinetics Deducing enzyme mechanisms, basics: rates and rate constants, enzyme kinetics- transient *versus* steady-state, transient kinetics: upper limits on rates of biological/chemical reactions and methods for studying rapid enzyme reactions.

-Calorimetric methods for the study of protein-ligand interactions and protein stability Energetics of binding interactions, followed by the principle of isothermal titration calorimetry (ITC), and then practical considerations when running ITC experiments. Advantages, and shortcomings. Linked protonation effects. This will be followed by numerous examples of applications in the life sciences and drug discovery. The lecture will then continue with a brief introduction to protein stability, and then the theory behind the differential scanning calorimetry (DSC) technique, followed by its applications in biochemistry. A demonstration of ITC will also be given on a different occasion.

Lectures and tutorials All the lectures are linked to tutorials, which are a significant part of the course. For the tutorials the students get 'homework', *i.e.*, they have to answer questions based on the lectures and articles they have to read. The homework is handed in and checked (feedback) before the tutorial. There are two special tutorials: a cloning and a paper tutorial. In the cloning tutorial the students design constructs (primer design, plasmid design, they learn how to use different computer programs to facilitate this). In the paper tutorial (using studies in which a plethora of different methods/techniques have been used to address a biological question), the students have to figure out why certain techniques have been used, identify the strengths/weaknesses of the techniques to address a particular question and propose alternative strategies.

Practicals

-Protein production practical Primer design, PCR, cloning, overexpression screening (incl. flow cytometry), monitoring, protein production, cell breakage, centrifugation, protein purification, protein characterization (SDS-PAGE, immuno-blotting, CD). All this is integrated in one project.

-CD practical Using CD conformational changes of peptides are studied and the thermostability of the protein the students produced in the protein production practical is also characterized using CD.

-Laser practical Cytochrome oxidase is used to study enzyme kinetics (flash photolysis approach).

-ITC demonstration

The students write lab. reports (not for the demonstration) that are corrected (until the level of the reports is acceptable).

Student presentations
The students get an assignment; they have to design a strategy to produce a specified protein for a particular purpose. Based on the course content and literature searches the students design a detailed strategy to do so. The students will present their strategy. The presentation will take 10-15 minutes. Thereafter, there is time for questions and to discuss the presentation. Presentations have to be send the first day before the presentations are given to degier@dbb.su.se as a pdf-file (put in the subject lane KB8002 and your name, and label the file with your name).

Assessment
Written exam, and the student have to complete all tutorials and lab. reports on time.

Final mark:
-Exam 75%
-Tutorials + lab reports 15% (given that they have been handed in on time)
-Oral presentation 10%

Practical matters

There will be no more than 12 students.

Seminars:
For a seminar you get 3 hours (plus breaks). Maybe you can already think about a nice question for the tutorials and test. Do not forget to make handouts for the students.

Room:
K241

The teachers:
-JWdG: Jan-Willem de Gier (degier@dbb.su.se)
-PÄ: Pia Ädelroth (piaa@dbb.su.se)
-AN: Agneta Noren (agneta.noren@dbb.su.se)
-JD: Jakob Dogan (jakob.dogan@dbb.su.se)
-DD: Dan Daley (ddaley@dbb.su.se)
-RD: Rob Daniels (robertd@dbb.su.se)

Tutorials:
-Do not forget to prepare some questions for the tutorials and give them to the students (you get 60 minutes/lecture). **The students appreciate this part of the course a lot!** The students have to hand in the answers before the tutorials and they have to attend the tutorials. The teachers have a look at the answers before the tutorial. **Please, give a mark; - (not ok),+ (ok),++ (good). This year the tutorials are part of the final grade. The students have to hand in the answers two days (48 hrs) before the tutorial** (put in the subject lane of your Email KB8002 and your name, and label the file with your name and the name of the tutorial)**. Answers that are handed in too late won't be considered.**

PRACTICALS:

This is a list of all the practicals (we have to prepare ourselves for 12 students. Pia has contacted (will contact?) the assistants, so they know about the course and that they are on this list. It is best if each person responsible for a practical contacts his/her lab. assistant(s). If you have to buy things for the practical, let me know. Not all students will have all the practicals at the same time (therefore we have group A, B, C and D with max 3 persons per group).

When the practicals start you can pick the students up at room K241.

Assistants:
Alexandros Karyolaimos (alexandros.karyolaimos@dbb.su.se)
Zhe Zhang (zhe.zhang@dbb.su.se)
Maximilian Kahle (maximilian.kahle@dbb.su.se)
Pontus Pettersson (Pontus.Pettersson@dbb.su.se)

Practicals (Groups A, B, C; D: 3 persons per group max):
Overexpression practical (JWdG): Alexander + Zhe (groups of 3 max) A,B,C,D
Laser practical (MK): Max (groups of 3 max) A,B,C, D
CD practical (PP): Pontus (groups of 3 max) A,B,C, D
ITC demo (JD): Jakob (groups of 6 people max) A, B

The students have to make short lab reports of around 3-4 pages. **They should hand in the first version of the reports to the lab assistants no longer than two weeks after the last day of the practical. Lab reports that are handed in too late won't be considered.**

Book + course materials:

Principles and Techniques of Biochemistry and Molecular Biology (7th edtion). Edited by Keith Wilson and John Walker (Cambridge University Press). Hand-outs, articles etc. will be provided.

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Some golden rules:
-Be on time (9 o'clock we start).
-Seminars, tutorials and practicals are compulsory. If there is a problem you contact me.
-No lab reports is no final mark (there are lab report deadlines). Also the deadlines for handing in tutorials and lab reports are strict.

Schedule

Morning (9:00-12:00)	Afternoon (13:00 ->)
WEEK 12	
20/3 Start/cell disruption (JWdG)	Molecular biology I (JWdG)
21/3 Molecular biology II (JWdG)	study time
22/3 Molecular biology III (JWdG)	study time
23/3 Molecular biology IV (JWdG)	study time
24/3 Protein overexpression I (JWdG)	study time
WEEK 13	
27/3 Protein overexpression II (JWdG)	study time/ Hand in Tut. Mol. biol. (JWdG)
28/3 Gelelectrophoresis (AN)	study time
29/3 Centrifugation (JWdG)	study time
30/3 Antibody based techniques (JWdG)	study time
31/3 Tut. Mol. Biol./Intro cloning Tut. (JWdG)	Intro Overexpression practical, A, B
WEEK 14	
3/4 Overexpression pract. A, B	Overexpression pract. A, B
4/4 Overexpression pract. A, B	Overexpression pract. A, B
5/4 Overexpression pract. A, B	Overexpression pract. A, B Hand in tut. cloning CD (JWdG)
6/4 Overexpression pract. A, B	Overexpression pract. A, B
7/4 Cloning tutorial CD (JWdG)/Overexpression pract. A, B	Intro Overexpression pract., C, D
WEEK 15	
10/4 Overexpression pract. C, D	Overexpression pract. C, D
11/4 Overexpression pract. C, D	Overexpression pract. C, D
12/4 Overexpression pract. C, D	Overexpression pract. C, D Hand in tut. cloning A, B (JWdG)
13/4 Overexpression pract. C, D	Overexpression pract. C, D Hand in tuts. gel el.(AN)/ab techn. (JWdG)/Overexpression (JWdG)
14/4 Red day	Red day
Week 16	
17/4 Red day	Red day
18/4 Cloning tutorial A, B (JWdG)/Overexpression pract. C, D	Study time
19/4 Microarrays (JWdG)	Study time
20/4 Tut Prot Overexpress (JWdG)/ Intro assigments/paper tutorials	Study time
21/4 Tuts. gel el.(AN)/ab techn. (JWdG)	Study time
	Hand in Tut Prot – Microaarrays (JWdG)
	Hand in overexpr. report A, B v 1
WEEK 17	
24/4 Overview spectroscopic techniques (JD)	Tut Microarrays (JWdG)
25/4 Calometric-based techniques (JD)	study time
26/4 Methods in enzyme kinetics (PÄ)	CD A
27/4 Mass spectrometry (JWdG)	CD B/Laser D
28/4 ITC demos (A, 9-10:30; B, 10:30-12)	CD C/Laser D/
	Hand in tut. Spectr. + Calom. techn (JD)/Enz. Kinetics (PÄ)
WEEK 18	
1/5 Red day	Red day
2/5 Tuts Spectr. + Calom. techn (JD) 9-11/Enz. Kinetics (PÄ) 11->	CD D, Hand in overexpr. report C, D v 1
3/5 Laser C	LaserC/ hand in tuts MS (JWdG)/Microarrays (JWdG)
4/5 Laser B	Laser B
5/5 Tuts JWdG 9-10:30/Tut. JWdG	study time
WEEK 19	
8/5 Protein isolation I (RD)	Laser A
9/5 Protein isolation II (RD)	Laser A
10/5 Protein-protein interactions (DD)	study time/ Hand in paper tut (JWdG)
11/5 study time	study time/Hand in tut. Prot. Isolation (RD)/ Hand in tut. Prot-prot interactions (DD)
12/5 study time	study time Hand in laser/CD reports v1/
WEEK 20	
15/5 Tuts Prot. Isol (RD)/Microarray (JWdG)	paper tutorial (JWdG)
16/5 study time	study time
17/5 study time	study time
18/5 study time	study time
19/5 Project presentations (JWdG/DD)	Project presentations (JWdG/DD)
Week 21	
22/5 study time	study time
23/5 study time	study time
24/5 Test JW (tenta vakt)	Test JW (tenta vakt)
25/5 Red day	Red day
26/5 Red day	Red day
Week 22	
29/5 v1 of lab. reports back	lab. reports
30/5 lab. reports	lab. reports
31/6 lab. reports	lab. reports
1/6 lab. reports	lab. reports
2/6 lab. reports	Hand in v2 of labreport