UNSW
THE UNIVERSITY OF NEW SOUTH WALES

FACULTY OF SCIENCE

SCHOOL OF BIOTECHNOLOGY

AND

BIOMOLECULAR SCIENCES

BIOC3271 Molecular Cell Biology

and

BIOC3671 Molecular Cell Biology (Advanced)

TERM 2, 2019

Table of Contents

1. Information about the Course

NB: Some of this information is available on the UNSW Handbook[1]

Year of Delivery	2019
Course Code	BIOC3271 and BIOC3671
Course Name	Molecular Cell Biology 2 and Molecular Cell Biology 2 (Advanced)
Academic Unit	School of Biotechnology and Biomolecular Science
Level of Course	3rd UG
Units of Credit	6 UOC
Term(s) Offered	T2
Assumed Knowledge, Prerequisites or Co-requisites	BIOC2101, BIOC2201
Hours per Week	7 HPW
Number of Weeks	10 weeks
Commencement Date	03.06.19

Summary of Course Structure (for details see 'Course Schedule')

Component	HPW	Time	Day	Location
Lectures	3			
Lecture 1		10 - 11 am	Monday	Central Lecture Block 2
Lecture 2		12 - 13 pm	Tuesday	Central Lecture Block 8
Lecture 3		13 - 14 pm	Wednesday	Central Lecture Block 1
Laboratory/Discussion classes	4	2 - 6 pm	Friday	E26, Lab 12
TOTAL	7			
Special Details	The lecture programs for BIOC3271 Molecular Cell Biology 2 and BIOC3671 Molecular Cell Biology 2 (Advanced) are 'integrated' such that lectures are common to the two courses. The program of the practical work is different. BIOC3271 students have practical classes and discussion classes, while BIOC3671 students perform a short project with the help of self-identified BABS faculty members. BIOC3671 students are expected to write a review article / report about their project and present it at the final practical classes to BIOC3271 students.			

[1] UNSW Online Handbook: http://www.handbook.unsw.edu.au

2. Staff Involved in the Course

Staff	Role	Name	Contact Details	Consultation Times
Course Convenor		A/Prof. Vladimir Sytnyk	v.sytnyk@unsw.edu.au tel.: 9385 1108	*via email*
Additional Teaching Staff	Lecturers & Facilitators	Prof. Andrew J. Brown	aj.brown@unsw.edu.au	*via email*
		A/Prof. Antony Cooper	a.cooper@garvan.org.au	
		Prof. Sally L. Dunwoodie	s.dunwoodie@victorchang.edu.au	
		A/Prof. Kyle Hoehn	k.hoehn@unsw.edu.au	
		Dr. Michal Janitz	m.janitz@unsw.edu.au	
		A/Prof. Louise Lutze-Mann	l.lutze-mann@unsw.edu.au	
		Dr. Joshua McCarroll	jmccarroll@ccia.org.au	
		Prof. H. Rob Yang	h.rob.yang@unsw.edu.au	
	Tutors & Demonstrators	Dr. Iryna Leshchyns'ka	iryna.leshchynska@unsw.edu.au	
		Ryan Keable	r.keable@student.unsw.edu.au	
		Yvette Aw	y.aw@student.unsw.edu.au	
	Technical & Laboratory Staff	Dr. Elessa Marendy	e.marendy@unsw.edu.au	
		Tim Nguyen	thinh.nguyen@unsw.edu.au	
	Other Support Staff			

3. Course Details

Course Description	The discipline known as Molecular Cell Biology investigates how cells develop, operate, communicate, construct multicellular organisms, control their activities, and (on occasion) go awry. To study the properties of the molecules that contribute to all these activities, modern researchers employ concepts and experimental techniques drawn from biochemistry, molecular biology, genetics and cell biology. The courses will present an overview of our current understanding of the myriad processes that control cellular processes and the techniques that are used to arrive at that understanding.	
Course Aims	The overall aim of the course is to provide a solid foundation in eukaryotic molecular cell biology, to demonstrate techniques used to study cell biology, and to show how this knowledge is applied to solve problems involving eukaryotic cells, for example to improve understanding and treatment of the human diseases	
Course Learning Outcomes (CLO)		
CLOs	**CLO statement**	**Related Tasks & Assessment**
CLO 1	Describe complexity of eukaryotic cells and cellular processes in healthy and diseased cells and tissues using knowledge acquired from facts, concepts, principles and procedures employed in the field of biochemistry, molecular biology, genetics and cell biology.	Mid-term and Final exams (BIOC3271/BIOC3671)
CLO 2	Perform effective and efficient experimental analysis of cells and their functional components. This includes adequate planning of work in the laboratory, recording accurate observations, analysis and interpretation of the results and developing skills in using laboratory equipment safely.	Protein-protein interaction assignment (BIOC3271) Project article (BIOC3671)
CLO 3	Apply acquired knowledge of theory and practical methods for understanding cell biological problems, analysing current strategies and designing new approaches for solving cell biological problems. This includes writing, presenting and discussing the research ideas, projects and outcomes of the research work.	Protein-protein interaction assignment (BIOC3271) Project article (BIOC3671) Discussion classes (BIOC3271) / Project presentation (BIOC3671)
CLO 4	Engage with a specific research project in a selected area of cell biology within a scope of work in an individual laboratory, and work on this project as a team member of the laboratory.	Project article (BIOC3671)

Major Topics (Syllabus Outline)	**CELL ADHESION AND RECOGNITION (3 lectures)** **A/Prof. Vladimir Sytnyk, School of Biotechnology and Biomolecular Sciences, room 3101, L3 West, Bioscience South E26** Cell adhesion and recognition molecules play an important role in the development of individual cells and structuring of the tissue. These lectures will provide an overview of the major classes of cell adhesion molecules and their structure. Using nervous system as an example, we will discuss the mechanisms of cell-to-cell adhesion and recognition and consider how specific types of cell-to-cell contacts are established and maintained. We will discuss the functions of cell adhesion molecules at contacts between cells by considering highly specialized contacts between neurons, which are called synapses. We will also talk about how cell adhesion molecules convert extracellular signals into intracellular signaling cascades, which induce gene expression and cytoskeleton remodeling required for cell survival and morphogenesis. **MAMMALIAN GENOME (1 lecture)** **Dr. Michael Janitz, School of Biotechnology and Biomolecular Sciences, room 3106, L3 West, Bioscience South E26** Mammalian genomes, and human genome in particular, are characterized by highly complex structure and mode of action. This complexity reflects the need of the mammalian cell to control thousands of metabolic processes in a highly coordinated manner. Recent advances in genome research powered by massive parallel sequencing and DNA microarrays technologies revealed a number of common functional and structural features of the genomes in higher vertebrates. The lecture will cover topics of genome replication, structure and function of regulatory sequences, haplotypic variations, the role of non-coding sequences, including small RNAs and RNA interference. **TRANSCRIPTOME REGULATION (1 lecture)** **Dr. Michael Janitz, School of Biotechnology and Biomolecular Sciences, room 3106, L3 West, Bioscience South E26** The term transcriptome refers to the total number of mRNA molecules expressed in a particular cell type, tissue or an organism. The concept of transcriptome emerged as a result of studies of gene expression on the genome-wide level using modern techniques of molecular biology such as DNA microarrays, high-throughput RNA interference and, very recently, next-generation DNA sequencing. In higher vertebrates, including human, there are several common functional and structural features of the transcriptome which allow the genes to be expressed in tightly coordinated manner during development and adult life. Furthermore, changes in transcriptome profile may also indicate the onset of the disease and might be observed long before any clinical symptoms emerge. The lecture will address regulation of gene expression, the role of transcriptional factors, structure of promoter regions, coordination of transcription on the level of the whole genome, molecular technologies to study the transcriptome. **CYTOSKELETON AND HUMAN DISEASE (2 lectures)** **Dr. Joshua McCarroll, Children's Cancer Institute Australia.** The cytoskeleton is a network of fibres that fills the cytosol of all cells. It can be considered to be composed of three fibre systems (microfilaments, microtubules and intermediate filaments). The cytoskeleton is responsible for: giving a cell its shape; supplying a framework for vesicular transport; and

providing an essential role in mitosis.

We shall be studying the monomeric and polymerised forms of these structural molecules and relating them to their function. The interactions among the major filament systems will be considered to illustrate the three points above. The final section of this series of lectures will address the cytoskeleton as a target for anticancer therapy.

CELLULAR STRESS (3 lectures)
A/Prof. Antony Cooper, The Garvan Institute of Medical Research, St Vincent's Hospital
Cells are constantly subjected to various stresses that can lead to cell dysfunction or death. Such stresses originate through either extracellular or intracellular means and include oxidative stress, stress from misfolded proteins (ER stress), DNA damage, hypoxia (a shortage of oxygen). Cell stress is implicated in many diseases including diabetes, neurodegenerative diseases (Parkinson's, Huntington's, Alzheimer's) and heart disease.
The three lectures will focus on types of stresses including ER and oxidative stress, cellular stress sensors, signal transduction and stress responses. These stress responses act initially at reversing or compensating for damage but can eventually trigger cell death/apoptosis if the cellular dysfunction is severe or prolonged.

LIPID TRANSPORT (1 lecture)
Prof. H. Robert Yang, School of Biotechnology and Biomolecular Sciences, room 3104, L3 West, Bioscience South E26
Little is known about the molecular mechanisms of intracellular lipid transport. In this lecture, we will discuss the roles of membrane lipid sensors, flippases and cytoplasmic lipid carriers in the transport of sterols and other lipid species.

LIPID DROPLETS (1 lecture)
Prof. H. Robert Yang, School of Biotechnology and Biomolecular Sciences, room 3104, L3 West, Bioscience South E26
Lipid droplets have long been viewed as an inert storage form of neutral lipids and energy. However, recent studies suggested that lipids droplets may represent a novel cellular organelle. In this lecture, we will discuss the biogenesis and dynamics of lipid droplets including their role in human obesity and diabetes.

CELL BIOLOGY OF ATHEROSCLEROSIS (1 lecture)
Prof. H. Robert Yang, School of Biotechnology and Biomolecular Sciences, room 3104, L3 West, Bioscience South E26
Heart attacks and stroke arise from atherosclerosis. In this lecture, I will provide an overview of the cellular mechanisms leading to atherosclerosis. Oxidized LDL (low density lipoprotein), inflammation, macrophages and form cells will be the focus.

CELLULAR MEMBRANES / LIPID RAFTS (1 lecture)
Prof. A.J. Brown, School of Biotechnology and Biomolecular Sciences, Room 3103, L3 West, Bioscience South E26
The lecture will focus on aspects of cell membranes particularly functions of the plasma membrane that go beyond that of a simple barrier. Topics will include: Properties and types of membrane lipids; Role of cholesterol; Membrane

asymmetry; Membrane phospholipids as a source of signalling molecules; Membrane proteins; Beyond the Fluid-Mosaic Model - Lipid rafts and lipid anchors.

ER-TO-GOLGI TRANSPORT (1 lecture)
Prof. A.J. Brown, School of Biotechnology and Biomolecular Sciences, Room 3103, L3 West, Bioscience South E26
Proteins are made in the endoplasmic reticulum (ER). If destined for other organelles or secretion from the cell, proteins must first be transported to the Golgi. The fidelity of this early protein secretory pathway within cells is essential for the maintenance of intracellular organisation and correct cellular function. I will firstly discuss the assembly of COPII vesicles which mediate this trafficking. We will then explore how this transport may be regulated by the actions of particular kinases and how this then impacts on metabolic processes, with a particular focus on lipid metabolism.

MOLECULAR APPROACHES TO CANCER THERAPY (3 lectures)
A/Prof. L.H. Lutze-Mann, School of Biotechnology and Biomolecular Sciences, room 3108, L3 West, Bioscience South E26
Advances in molecular and cellular techniques have provided a greater understanding of changes that occur in cells as they become tumour cells. It is now possible to exploit this understanding to induce the selective death of cancer cells while leaving normal cells intact. These lectures will provide an overview of these strategies and will focus on some specific examples that have been successfully employed in the clinic.

CELL BIOLOGY OF DEVELOPMENT (2 lectures)
Prof. Sally L. Dunwoodie, Developmental Biology Division, Victor Chang Cardiac Research Institute.
1) Notch signalling, Somitogenesis and Abnormal Vertebral Segmentation.
Somites form during embryonic development and give rise to the axial skeleton and associated muscles and tendons. The Notch signalling pathway is critical for normal somite formation. This lecture describes the process of somite formation in the mouse embryo and describes Notch signalling and the function of various pathway components. Genetically modified mouse models and Notch signalling assays in cultured cells are described. Severe disruption of human vertebral development is seen in the abnormal vertebral segmentation syndrome spondylocostal dysostosis (SCD). That SCD is caused by mutation of Notch pathway components is discussed.

2) Nodal Signalling and Establishing the Left-Right Body Axis of the Embryo
The vertebrate body is bilaterally asymmetric. The left-right body axis is established early in embryonic development and the developing visceral organs take cues from the left-right axis for normal development. The heart is particularly susceptible to alterations in left-right patterning. Nodal (TGFbeta) signalling (on the left of the embryo) determines the left side of the embryo. This lecture describes how bilateral symmetry is broken in the mouse embryo and how the left-right body axis is established though the action of Nodal.

CELL BIOLOGY OF ALZHEIMER'S DISEASE (1 lecture)
A/Prof. Vladimir Sytnyk, School of Biotechnology and Biomolecular Sciences, room 3101, L3 West, Bioscience South E26
Alzheimer's disease is the leading cause of dementia among the elderly. This disease arises from accumulation of a small peptide, the β amyloid, which is

	produced from a precursor protein through sequential proteolysis and which blocks neuronal function and induces neuronal death in the brain. In this lecture, we will discuss methods to study the factors that regulate the generation of β amyloid and cell biological mechanisms of β amyloid toxicity. **METHODOLOGICAL APPROACHES FOR CANCER CELL BIOLOGY (1 lecture)** **A/Prof. K.L. Hoehn, School of Biotechnology and Biomolecular Sciences, 3102, L3 West, Bioscience South E26** This lecture will discuss common techniques that researchers use to investigate cancer cell phenotypes including proliferation, migration, invasion, and anchorage-independent growth. **METHODOLOGICAL APPROACHES FOR MOUSE TUMOUR BIOLOGY (1 lecture)** **A/Prof. K.L. Hoehn, School of Biotechnology and Biomolecular Sciences, 3102, L3 West, Bioscience South E26** Mice are frequently used as pre-clinical models to test anti-cancer therapeutics and to study how genetics and environment (carcinogens, ultraviolet light, etc) affect tumour initiation and progression. This lecture will detail the differences between xenograft, syngeneic, orthotopic, and endogenous models of cancer. Rationale will be provided concerning why specific models should be used for specific experiments including metastasis, tumourigenesis, and anti-cancer therapeutics testing.
Relationship to Other Courses within the Program	BIOC3271 and BIOC3671 are mutually exclusive 6 uoc courses in Term 2. The two courses share the same lecture program but have some different practical components, with BIOC3671 having an increased focus on research experience. They build on concepts and knowledge of biochemistry, cell biology and molecular biology developed in the two pre-requisite courses BIOC2101 and BABS2201. Both courses are core components in Molecular and Cell Biology majors and plans, and electives in other related discipline areas.

4. Rationale and Strategies Underpinning the Course

Teaching Strategies	The teaching in Molecular Cell Biology requires the active engagement of students in the learning process and reflects the active enquiry process underlying scientific research and discovery. Face-to-face lectures and practical classes are complemented by online materials provided at the course website to allow efficient revision and self-directed learning.
Rationale for learning and teaching in this course	While lectures and practical classes provide solid theoretical foundation, the protein-protein interaction assignment (3271), discussion classes (3271) and article-writing (3671) encourage student engagement with the latest developments in research methods and equipment, and the current research literature.

5. Course Schedule

Some of this information is available on the Online Handbook[2] and the UNSW Timetable[3].

Week	Lecture 1 Mon, 10-11 am Central lecture block 2	Lecture 2 Tue, 12-13 pm Central lecture block 8	Lecture 3 Wed, 1-2 pm Central lecture block 1	Practical Fri, 2-6 pm E26 Teaching Lab 12
Week 1 starts on 03/06	Introduction (Vladimir Sytnyk)	Cell Adhesion and Recognition (Vladimir Sytnyk)	Cell Adhesion and Recognition (Vladimir Sytnyk)	
Week 2 starts on 10/06	**Public Holiday**	Cell Adhesion and Recognition (Vladimir Sytnyk)	Mammalian Genome (Michael Janitz)	Cell adhesion
Week 3 starts on 17/06	Transcriptome Regulation (Michael Janitz)	Cytoskeleton and human disease (Josh McCarroll)	Cytoskeleton and human disease (Josh McCarroll)	Functional genomics
Week 4 starts on 24/06	Lipid transport (H. Rob Yang)	Lipid Droplets (H. Rob Yang)	Cell Biology of Atherosclerosis (H. Rob Yang)	Protein analysis I
Week 5 starts on 01/07	Cellular Stress (Antony Cooper)	Cellular Stress (Antony Cooper)	Cellular Stress (Antony Cooper)	Protein analysis II
Week 6 starts on 08/07	Cell biology of development (Sally Dunwoodie)	Cell biology of development (Sally Dunwoodie)	Membranes/lipid RAFTs (Andrew J. Brown)	**Mid-term exam**
Week 7 starts on 15/07	ER to Golgi Transport (Andrew J. Brown)	Molecular Approaches to Cancer Therapy (Louise Lutze-Mann)	Molecular Approaches to Cancer Therapy (Louise Lutze-Mann)	Fluorescent microscopy
Week 8 starts on 22/07	Molecular Approaches to Cancer Therapy (Louise Lutze-Mann)	Cancer (Kyle Hoehn)	Cancer (Kyle Hoehn)	Protein interactions
Week 9 starts on 29/07	Cell Biology of Alzheimer's Disease (Vladimir Sytnyk)	Discussion class I (BIOC3271)	Discussion class II (BIOC3271)	Project presentations (BIOC3671)
Week 10 starts on 05/08	Discussion class III (BIOC3271)			Project presentations (BIOC3671)

[2] UNSW Virtual Handbook: http://www.handbook.unsw.edu.au
[3] UNSW Timetable: http://www.timetable.unsw.edu.au/

6. Assessment Tasks and Feedback

Assessment task and methods		Weighting (%)	Submission methods	Mark and feedback style	Due date (normally midnight on due date)
Formative Assessment:					
Quiz (BIOC3271) Project description (BIOC3671)			In class Online	Demonstrators, in class, verbal Convenor, written	Week 2, 14/06 Week 2, 16/06
Summative Assessment Tasks:					
Assessment 1:	Protein-Protein interaction assignment (BIOC3271)	20	Online	Convenor, written	Week 9, 29/07
	Project article (BIOC3671)	30	Online	Convenor, written	Week 9, 2/08
Assessment 2: Mid-term exam		30	In class	Convenor, provide upon request	Week 6, 12/07
Assessment 3:	Discussion classes (BIOC3271)	10 – presentation 10 – participation	In class	Convenor, verbal	Week 9-10
	Project presentation (BIOC3671)	10	Online, In class	Convenor, verbal	Week 9-10
Assessment 4: Final exam		30	In class	Convenor, provide upon request	Final exam period

7. Additional Resources and Support

Text Books	Alberts B., Johnson A., Lewis J., Morgan D., Raff M., Roberts K. and Walter P. Molecular Biology of the Cell (6th Ed.) Garland Science (2014). availability– bookshop, UNSW library
Course Manual	Course manual and, as far as possible, all course notes and information will be available to view or download from the Course Moodle site. This will include the various sections of the "Course Outline", all practical notes, lecture handouts and/or copies of powerpoint presentations, links to lecture recordings, additional information about assessment tasks and course administration as it becomes available, and announcements. Students should consult the course Moodle site on at least a weekly basis and are encouraged to use it for posting inquiries about the course content or administration.
Required Readings	For Discussion classes, each student will receive a scientific paper published in a scientific journal and available online. The details will be posted on the course website.
Additional Readings	Lodish, H., Berk, A., Kaiser, C.A., Krieger, M., Bretscher, A, Ploegh, H., Amon A., and Martin, K. Molecular Cell Biology (8th Ed.) Macmillan learning (2016). availability – bookshop, UNSW library See also references to original research and review articles given by the lecturers and available online
Recommended Internet Sites	The online Bookshelf of the US National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov/books PubMed Central (PMC) - the U.S. National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature. http://www.ncbi.nlm.nih.gov/pmc/ iBiology: http://www.ibiology.org/ Neuronline: http://neuronline.sfn.org
Societies	The Australia and New Zealand Society for Cell and Developmental Biology http://www.anzscdb.org/ The American Society for Cell Biology http://www.ascb.org/
Computer Laboratories or Study Spaces	Computers can be accessed in Room G08, Biological Sciences Building

8. Required Equipment, Training and Enabling Skills

Equipment Required	Lab coat and safety glasses are required for all practical classes.
Enabling Skills Training Required to Complete this Course	The course is built on concepts and knowledge of biochemistry, cell biology and molecular biology developed in the two pre-requisite courses BIOC2101, BIOC2201. Students have to complete "BABS Health and Safety Quiz" on Moodle / Practicals.

9. Course Evaluation and Development

Student feedback is gathered periodically by various means. Such feedback is considered carefully with a view to acting on it constructively wherever possible. This course outline conveys how feedback has helped to shape and develop this course.

Mechanisms of Review	Last Review Date	Comments or Changes Resulting from Reviews
Major Course Review	01.05.2019	There have been minor changes to the lecture and laboratory program in 2019, reflecting the staff's intention to maintain an overall balance in the content while introducing new concepts and techniques in what is a rapidly developing area of scientific research. The current system of examining lecture content arises from student feedback in previous years.
CATEI[4] myExperience	10.12.2015 01.05.2019	Student evaluative feedback from UNSW's Course and Teaching Evaluation and Improvement (CATEI) and myExperience process is gathered each year, and the staff involved in teaching will review all aspects of the course at the end of the session. Student feedback is valued and forms part of the basis for continual improvement.
Other	10.05.2019	Course manual and other supporting documentation including UNSW and BABS WHS documentation available at BABS website (https://www.babs.unsw.edu.au/) are updated each year using recommendations of the Learning and Teaching Unit and WHS unit at UNSW and BABS.

[4] CATEI process

10. Administration Matters

Expectations of Students	Students are encouraged to attend all lectures. BIOC3271 students are expected to attend all practical classes. Attendance records will be kept in practical classes. Attendance at less than 80% of classes may result in the grade of UF. BIOC3671 students are expected to spend equivalent time working on the project in one of the BABS laboratories. The student's supervisor will be asked to confirm the attendance. Students are expected to consult the course Moodle site on at least a weekly basis.
Assignment Submissions	All written assignments must be submitted as Word (.doc) files or pdf files via the course Moodle site. Late submission of assignments normally attracts a penalty (10% of the maximum possible marks per day). Extensions for late submission of assignments without penalty will only be granted by staff before the submission deadline, not retrospectively.
Occupational Health and Safety[5]	OHS issues in School of BABS are covered at the school website: http://www.babs.unsw.edu.au/ohs/school-babs-workplace-health-and-safety OHS issues for individual practicals are covered in the relevant notes provided for each practical class in this course outline. It is compulsory for all students to study both sources and read "Laboratory Safety" information (see page 16). All students must complete the BABS Health and Safety Quiz and BABS UG Risk Assessment before each practical class using templates provided in this course outline. BIOC3671 students have to discuss Health and Safety requirements with the project supervisors and complete training required for their projects.
Assessment Procedures **UNSW Assessment Policy**[6]	**SPECIAL CONSIDERATION AND FURTHER ASSESSMENT** Students who believe that their performance, either during the session or in the end of session exams, may have been affected by illness or other circumstances may apply for special consideration. Applications can be made for compulsory class absences such as (laboratories and tutorials), in-session assessments tasks, and final examinations. You must submit the application prior to the start of the relevant exam, or before a piece of assessment is due, except where illness or misadventure prevent you from doing so. If you become unwell on the day of the exam or fall sick during an exam, you must provide evidence dated within 24 hours of the exam, with your application. You must obtain and attach Third Party documentation before submitting the application. Failure to do so may result in the application being rejected. UNSW has a fit to sit/submit rule which means that if you sit an exam or submit a piece of assessment, you are declaring yourself fit to do so. Further information on special consideration can be found at https://student.unsw.edu.au/specialconsideration. HOW TO APPLY FOR SPECIAL CONSIDERATION The application must be made through Online Services in myUNSW (My Student Profile tab > My Student Services > Online Services > Special

[5] UNSW OHS Home page
[6] UNSW Assessment Policy

	Consideration). Students will be contacted via their official university email as to the outcome of their application. It is the responsibility of all students to regularly consult their official student email accounts and myUNSW in order to ascertain whether or not they have been granted further assessment. **SUPPLEMENTARY EXAMINATIONS:** The University does not give deferred examinations. However, further assessment exams may be given to those students who were absent from the final exams through illness or misadventure. Special Consideration applications for final examinations and in-session tests will only be considered after the final examination period when lists of students sitting supplementary exams/tests for each course are determined at School Assessment Review Group Meetings. Students will be notified via the online special consideration system as to the outcome of their application. **It is the responsibility of all students to regularly consult their official student email accounts and myUNSW in order to ascertain whether or not they have been granted further assessment.** **For Semester 2 2019, BABS Supplementary Exams will be scheduled on:** **9 Sep 2019 – 13 Sep 2019** **Further assessment exams will be offered on this day ONLY and failure to sit for the appropriate exam may result in an overall failure for the course. Further assessment will NOT be offered on any alternative dates.**
Equity and Diversity	Those students who have a disability that requires some adjustment in their teaching or learning environment are encouraged to discuss their study needs with the course Convenor prior to, or at the commencement of, their course, or with the Equity Officer (Disability) in the Equity and Diversity Unit (9385 4734 or http://www.studentequity.unsw.edu.au/). Issues to be discussed may include access to materials, signers or note-takers, the provision of services and additional exam and assessment arrangements. Early notification is essential to enable any necessary adjustments to be made.

Student Complaint Procedure[7]	**School Contact**	**Faculty Contact**	**University Contact**
	BABS Grievance Officer: A/Prof. Louise Lutze-Mann l.lutze-mann@unsw.edu.au Tel: 9385 2024	Dr Gavin Edwards g.edwards@unsw.edu.au Tel: 9385 4652	The Student Integrity Unit studentcomplaints@unsw.edu.au Tel: 02 9385 8515, University Counselling and Psychological Services[8] counselling@unsw.edu.au Tel: 9385 5418

[7] UNSW Student Complaint Procedure
[8] University Counselling and Psychological Services

11. UNSW Academic Honesty and Plagiarism

What is Plagiarism?

Plagiarism is the presentation of the thoughts or work of another as one's own.
*Examples include:

- direct duplication of the thoughts or work of another, including by copying material, ideas or concepts from a book, article, report or other written document (whether published or unpublished), composition, artwork, design, drawing, circuitry, computer program or software, web site, Internet, other electronic resource, or another person's assignment without appropriate acknowledgement;
- paraphrasing another person's work with very minor changes keeping the meaning, form and/or progression of ideas of the original;
- piecing together sections of the work of others into a new whole;
- presenting an assessment item as independent work when it has been produced in whole or part in collusion with other people, for example, another student or a tutor; and
- claiming credit for a proportion of work contributed to a group assessment item that is greater than that actually contributed.†

For the purposes of this policy, submitting an assessment item that has already been submitted for academic credit elsewhere may be considered plagiarism.

Knowingly permitting your work to be copied by another student may also be considered to be plagiarism.

Note that an assessment item produced in oral, not written, form, or involving live presentation, may similarly contain plagiarised material.

The inclusion of the thoughts or work of another with attribution appropriate to the academic discipline does *not* amount to plagiarism.

The Learning Centre website is main repository for resources for staff and students on plagiarism and academic honesty. These resources can be located via:

http://www.lc.unsw.edu.au/resources

The Learning Centre also provides substantial educational written materials, workshops, and tutorials to aid students, for example, in:

- correct referencing practices;
- paraphrasing, summarising, essay writing, and time management;
- appropriate use of, and attribution for, a range of materials including text, images, formulae and concepts.

Individual assistance is available on request from The Learning Centre.

Students are also reminded that careful time management is an important part of study and one of the identified causes of plagiarism is poor time management. Students should allow sufficient time for research, drafting, and the proper referencing of sources in preparing all assessment items.

* Based on that proposed to the University of Newcastle by the St James Ethics Centre. Used with kind permission from the University of Newcastle
† Adapted with kind permission from the University of Melbourne

12. LABORATORY WORK

LABORATORY HOURS

The hours during which the laboratory will be open and teaching staff on duty, are as follow:

Fridays 2 - 6 pm, E26 Lab 12

Students *should* complete their experimental work within these hours**. Most laboratory sessions will commence with a brief talk by the lecturer in charge. It is imperative that you attend these introductory sessions.** Under no circumstances are students allowed to work in the laboratory without the supervision of a member of the teaching or laboratory staff. *Special permission must be obtained on each occasion if it is necessary to work outside laboratory hours or to use equipment outside the undergraduate practical area*.

Note: Students should prepare for an experiment beforehand in their own time, **not** after the practical class begins. **Before** each practical class you must read the practical notes in this manual.

Satisfactory attendance at the practical sessions is *required to pass the course as a whole*. If you are absent from the practical class for a medical reason, please submit a medical certificate to A/Prof. Vladimir Sytnyk via email: v.sytnyk@unsw.edu.au.

LABORATORY RECORDS AND PREPARATION

It is *expected* that students will record **ALL** their experimental details **during the practical classes** (these sections of your practical book do not have to be pristine but you should try to tabulate raw data). You can either keep records in the space provided in this course manual or purchase a note book (say a 96-page exercise book). Before each practical class you *must* read and understand the procedures to be followed in the practical class. The procedures are described in this course manual. After completion of the experiment, pictures, calculations, graphs, etc. and brief conclusions should be recorded. The experimental details and raw data should be annotated such that, with the use of the laboratory manual, you would be able to come back in one or two years time and repeat the experiments ***essentially as you performed them this year.*** This is training in keeping laboratory records in the form that would be expected for Honours and Postgraduate research students, and for graduates involved in laboratory work in research or industry.

The practical note book should be kept up to date at all times. Any member of academic staff may check on your record keeping at any time during the laboratory classes. The practical note books serve as a record of satisfactory attendance at and completion of the practical component. **In all cases where a student requests special consideration for medical or other reasons, the student may be required to submit their practical note book, which will form part of any additional assessment.**

LABORATORY SAFETY

Biochemical laboratories contain apparatus and chemicals that are potentially dangerous when misused or handled carelessly. Consequently, safe experimental procedures and responsible conduct in the laboratory are essential at all times. The regulations governing conduct in the laboratory have been set down by the NSW Occupational Health & Safety (Hazardous Substances) Regulation 1996, NSW Draft OHS Regulation 2000, and the NSW Workcover Publications and Worksafe National Codes of Practice and Guidance Notes. These policies apply to all university staff and students.

Students are responsible for:

- Complying with the requirements of this policy, legislation and Australian Standards
- Following directions given to them by the person supervising their work
- Co-operating in the performance of risk assessments
- Participating in induction and training programs.

Students must complete the BABS Health and Safety Quiz.
At the beginning of each practical class, students must complete and sign the Risk Assessment form.

ALL ACCIDENTS WITH CHEMICALS OR INJURIES MUST BE REPORTED IMMEDIATELY TO YOUR DEMONSTRATOR OR TO A MEMBER OF THE PREPARATION ROOM STAFF.

1. Students must purchase a laboratory coat and wear it when in the laboratory.

2. Safety glasses should be brought to all practical classes and used when indicated, especially for handling corrosive and toxic compounds.

3. Eating, drinking and smoking are forbidden in the laboratory.

4. Suitable foot protection must be worn. Students with bare feet or thongs or strappy sandals will not be allowed into the working area.

5. Students with long hair should ensure it is tied back.

6. Disposable plastic gloves will be provided for certain manipulations and these should be discarded after use or if torn. They should be removed by turning them inside out from the wrist and then placed in one of the “solids waste” containers (bins on top of bench), never in the Paper Only bins.

7. For your own protection and those whom you will be working with, you should read, before each week's experiment is started, the Risk Assessment and instructions and take note of any hazards in the particular procedures.

8. Risk Assessments have been carried out on all practicals to highlight the potential for possible risks to the users. This covers chemical, biological and physical hazards. This is to ensure that the proper precautions are taken during those procedures.

9. The chemical risks have been assessed using MSDs (Material Safety Data Sheets). These are available on file in the Prep Room. A copy of the Hazardous Substances Policy is also on file.

10. Strong acids, alkalis and other toxic substances have to be used in some biochemical analyses and the relevant safety instructions will be included at the appropriate places in the manual. Such dangerous materials must never be pipetted by mouth, they should be manipulated with great care and, if any come into contact with skin or clothing, then the affected areas should be washed with water immediately, while assistance is sought and any antidote applied. Poisonous solutions will be provided in automatic dispensers; these should be operated gently and carefully because careless use can cause a spray of the reagent.

11. Fire Extinguishers: It is of utmost importance that these instruments not be tampered with. It may be your own life that is lost if an extinguisher is found empty during an emergency. You should note the location(s) of fire extinguishers in the laboratory.

12. Broken Glassware and other Sharp Objects. Should any breakage of glassware occur, the fragments must be swept up immediately and placed in the special bins provided. These bins are located at the front of each laboratory and are clearly marked "BROKEN GLASS ONLY". Broken glass or other sharp objects MUST NOT be placed in the waste paper bins that are located under the sinks along each laboratory bench, or in any other bins, UNDER ANY CIRCUMSTANCES.

If in doubt about any aspect of safety please consult a member of staff.

Web references:
School of BABS Workplace Health and Safety
http://www.babs.unsw.edu.au/ohs/school-babs-workplace-health-and-safety

PRACTICAL NOTES

Note: changes to practical notes are possible. Please, check the course website for updated practical notes before each laboratory session.

Practical notes are given for Cell Adhesion, Protein Analysis, Protein Interactions and Fluorescence Microscopy practical classes. Functional Genomics session does not involve practical work in the lab and notes will be distributed by the lecturer involved.

PRACTICAL: Cell Adhesion **DATE...../...../.....**

STUDENT NAME.....SIGNATURE.....

BIOLOGICAL HAZARDS	HAZARDS	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY *SPILLS CLEANUP AND FIRST AID
1			
2			
3			
4			
5			

PROCEDURAL (PLANT & EQUIPMENT)	HAZARDS	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY *SHUTDOWN PROCEDURES AND FIRST AID
1			
2			
3			
4			
5			
6			
7			
8			

CHEMICALS	HAZARDS *YOU MAY USE RISK AND SAFETY PHRASES	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY MANAGMENT *SPILLS CLEANUP AND FIRST AID
1			
2			
3			
4			
5			
6			
7			
8			

EXTRA NOTES

THE UNIVERSITY OF NEW SOUTH WALES
SCHOOL OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

BIOC3271/3671

Cell Adhesion

RISK ASSESSMENT

Biological Hazards

Cultured cells – potentially may be contaminated with infectious microorganisms
Cell culture medium contains serum of animal origin

Procedural Hazards

Microscopes – electrical, high intensity light

INTRODUCTION

Cultured cells are used in all laboratories working in the field of biomedical research. Primary cell cultures are prepared by dissociating tissues obtained directly from animals before each experiment. These cells live in culture only for a limited time and have a limited capacity to proliferate. That is why most of the work is performed using cell lines which consist of cells that acquired the ability to proliferate indefinitely either through random mutation (e.g. cancer cells isolated from tumors) or introduced modification, such as artificial expression of the telomerase gene. These cells can be maintained in culture continuously for very long time. They can be used as a cellular model system for analysis of various cell biological processes using microscopy, and biochemical and cell biological methods.

Different cell lines are currently commercially available through various sources (e.g. see American Type Culture Collection (ATCC) at http://www.atcc.org/). In this practical you will use human neuroblastoma SHEP cells.

SHEP cells and a number of other cell lines and primary cells are adherent cells, i.e. they grow in culture as a monolayer of cells attached to the surface of a plastic or specially treated glass dish or substrate. Often, these cells must be detached from the substrate to be re-plated into another dish or used for biochemical analysis. Detachment of cells is achieved by transient disruption of cellular adhesion to the substrate. In this practical class, you will use a protocol given below to detach cells from the plastic surface of a culture dish. You will then seed the cells into another dish and observe how adhesion interactions are restored. You will also use some of the cells to prepare a sample for protein gel electrophoresis and Western blot analysis, which will be performed in your next practical classes.

AIM

The aims of this practical class are
- to demonstrate conditions for culturing adherent cells in the laboratory
- to show how the interactions between cells and artificial substrates can be manipulated
- to demonstrate how changes in adhesion affect cell morphology
- to demonstrate how cells can be used to prepare a biological sample for further biochemical analysis

Experimental Part

All students will obtain 35 mm culture dishes (1 dish per pair of students) containing human neuroblastoma SHEP cells which grow in the culture medium (DMEM containing serum and antibiotics).

1. Take the dish, place under the microscope and find cells. Use 10X objective. Make a rough sketch of cells.

2. Place the dish on the bench and carefully remove the culture medium using a 1 ml pipette. Do not let the cells become dry! To avoid this, always leave a thin layer of culture medium covering cells.

3. Wash cells 2 times with warm DMEM culture medium by adding each time 1 ml of DMEM to the dish with cells. Then discard the medium.

4. Add 1 ml of Trypsin + EDTA solution to the dish with cells.

5. Place the dish under the microscope and observe using 10X objective at 2 min and 5 min after application of Trypsin + EDTA solution. Note the changes happening with the cells.

6. Carefully collect the medium containing cells detached from the substrate into a 1.5 ml aliquot using a pipette and 1 ml tip.

7. Centrifuge the cell suspension for 5 min at 1000 rpm.

8. Carefully remove the Trypsin-EDTA solution. Do not disturb the pellet containing cells.

9. Add 1 ml DMEM with 10% serum.

10. Re-suspend cells using the 1 ml pipette.

11. Centrifuge cell suspension for 5 min 1000 rpm.

12. Carefully remove the media. Do not disturb the pellet containing cells.

13. Add 1 ml DMEM with 10% serum.

14. Re-suspend cells using the 1 ml pipette

15. Take an empty 35 mm dish. Fill it with 1 ml DMEM containing 10% serum.

16. Add 500 µl of the cell suspension to the culture dish to re-plate cells. Place the aliquot containing the remaining cell suspension on ice because you will need it later.

17. Observe the behaviour of cells under the microscope at 10 min, 30 min and 1 h after the application of the cell suspension into the culture dish. Keep cells in a CO_2 incubator between the observations.

18. Make a rough sketch and description of changes in the cell shape.

Preparation for the next practical class

In this part of the practical class, you will prepare a sample from your cells, which will be used for biochemical analysis in further practical classes.

19. Take the remaining cell suspension (see step 16 in the first part of the practical class). Centrifuge the aliquot for 5 min at 1000 rpm.

20. Carefully remove the supernatant. Do not disturb the pellet containing cells.

21. Add 1 ml of phosphate buffered saline (PBS) and resuspend cells.

22. Centrifuge cell suspension for 5 min at 1000 rpm

23. Carefully remove PBS. Steps 20-23 are needed to remove the serum from cells. Serum contains various proteins which may interfere with the biochemical analysis.

24. Add 70 µl of lysis RIPA buffer to the cells. Resuspend cells and incubate for 15 min at room temperature.

25. Centrifuge for 10 min at 16,000 rpm (the highest setting on the centrifuge)

26. Transfer the supernatant into a clean aliquot, label it, and give it to the demonstrator.

Reagents used in the practical class:

DMEM - Dulbecco's Modified Eagle Medium. To maintain cells this medium is supplemented with
antibiotics - Penicillin-Streptomycin (100 U of penicillin, 100 µg of streptomycin) and
serum - Fetal Bovine Serum (10%).
PBS - phosphate-buffered saline (2.67 mM KCl, 1.5 mM KH_2PO_4, 140 mM NaCl, 8 mM $Na_2HPO_4*7H_2O$)
trypsin-EDTA - phosphate-buffered saline solution containing 0.025% trypsin and 0.01% EDTA.

Questions

1. You have used Trypsin-EDTA solution to disrupt adhesion between cells and the plastic substrate. Can you explain the mechanism? What kind of adhesion molecules are targeted by Trypsin and EDTA?

2. Trypsin is a protease. Can you explain how cells can survive this treatment although proteins, which are targeted by proteases, are essential for the cell survival?

3. How can you explain that cells are able to re-attach to the substrate after removal of the Trypsin-EDTA solution?

Make a rough sketch of adherent cells
before treatment with Trypsin-EDTA

during application of Trypsin-EDTA

after removal of trypsin-EDTA
and seeding of cells into a new culture dish

Demonstrator: Signature:

PRACTICAL: PROTEIN ANALYSIS **DATE...../...../.....**

STUDENT NAME.....SIGNATURE.....

BIOLOGICAL HAZARDS	HAZARDS	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY *SPILLS CLEANUP AND FIRST AID
1			
2			
3			
4			
5			

PROCEDURAL (PLANT & EQUIPMENT)	HAZARDS	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY *SHUTDOWN PROCEDURES AND FIRST AID
1			
2			
3			
4			
5			
6			
7			
8			

CHEMICALS	HAZARDS *YOU MAY USE RISK AND SAFETY PHRASES	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY MANAGMENT *SPILLS CLEANUP AND FIRST AID
1			
2			
3			
4			
5			
6			
7			
8			

EXTRA NOTES

THE UNIVERSITY OF NEW SOUTH WALES
SCHOOL OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

BIOC3271/3671

TECHNIQUES OF PROTEIN ANALYSIS:

GEL ELECTROPHORESIS, WESTERN BLOT, IN VITRO PROTEIN POLYMERISATION ANALYSIS

RISK ASSESSMENT

Chemical Hazards
2-mercaptoethanol
methanol
Polyacrylamide Gels

Biological Hazards
Lysate of human cells
Purified actin from rabbit muscle

Procedural Hazards
High voltage power packs
Needles

INTRODUCTION

Proteins play major roles in cells. Protein composition of cells depends on the cell type and function. In disease, levels of some proteins can change, some proteins may not be present anymore, whereas new proteins can be expressed. Knowledge about the proteins present in cells is useful for our understanding of how cells live and function, and what causes their death, abnormal function, or results in uncontrolled proliferation and cancer. We also need to know how individual protein function to understand their role in cells and why changes in their levels can lead to a disease.

We can learn about the proteins present in cells by isolating proteins from cells, for example by treating cells with detergents and collecting solubilised proteins. However, these samples contain large numbers of proteins. To estimate the numbers of proteins, identify the most abundant proteins or characterise differences in expression of proteins, protein gel electrophoresis can be used. This technique is based on the separation of proteins according to their molecular weight during their migration in the gel where larger proteins migrate slower than smaller proteins.

Immunoblotting or Western blot is used to analyse levels of a specific protein using antibodies developed against this protein. Proteins separated by gel electrophoresis are transferred from the gel onto a membrane support, thus making a replica of the separated proteins. The location of the specific proteins is then determined by using antibodies specific to the protein of interest. Immunoblotting can be used to determine a number of important characteristics of proteins. Essentially any experimental question that requires one to know the presence and quantity of a specific protein or the relative molecular weight of the protein can be answered by immunoblotting. Immunoblotting can also be used to determine posttranslational modifications of the protein, e.g. its tyrosine phosphorylation, if the antibodies against these modifications are available. Immunoblotting is also used for the analysis of the composition of protein complexes. In this case, the protein complex is usually isolated by using another method (e.g. by co-immunoprecipitation), and the composition of the complex is then analysed by Western blot using antibodies against proteins which are expected to be in the complex.

Isolated and purified proteins can also be used to study the role that the proteins play in the cell, and how this role is regulated. For example, purified channels can be used to study their ability to allow flow of different ions across the membrane, purified cell adhesion molecules are used to study their ability to mediate recognition between cells, while purified cytoskeleton proteins are used to study the assembly of the cytoskeleton.

AIM

- To demonstrate how protein gel electrophoresis can be used for analysis of protein diversity and abundance in cells;

- To demonstrate how Western blot can be used for analysis of the levels and molecular weight of a specific protein (actin) in cells;

- To demonstrate how purified actin protein can be used to study the assembly of the cytoskeleton *in vitro*.

In this practical class, you will analyse differences in the presence and abundance of different proteins in the extracts of detergent-soluble proteins obtained from mouse brain cells and human neuroblastoma SHEP cells, which are often used to study cellular mechanisms of brain development and function. Cell extracts contain a huge number of proteins. To compare protein composition in these samples, proteins in the extracts will be separated into discrete bands using SDS polyacrylamide gel electrophoresis (PAGE). For comparison, you will also analyse commercially available purified actin. The gel will be labelled with Colloidal Coomassie stain to visualise and analyse separated proteins. The analysis will be completed in Session 1.

In Session 1, you will also separate proteins in another gel, which will be used for Western blot analysis of a specific protein, actin, a protein component of the cytoskeleton. Proteins in this gel will be transferred onto a membrane, which consist of the material (nitrocellulose or PVDF), which binds proteins non-specifically. After the transfer, this ability of the membrane to bind other proteins will be blocked by incubating the membrane in skim milk solution. In Session 2, the membrane will be incubated with the primary antibody, which in your case binds strongly and specifically only to actin. To visualise the primary antibody, a horseradish peroxidase-conjugated secondary antibody will be added, followed by the addition of TMB (3, 3´, 5, 5´-tetramethylbenzidene) substrate resulting in coloured precipitate which localizes the antigen as a coloured band or spot on the membrane. To estimate the molecular weight of actin, you will compare the position of the protein band detected with the actin antibodies to the position of the so called standard protein bands of known molecular weights. These pre-labelled protein standards will be applied on the gel in parallel with your sample.

In Session 2, you will also analyse polymerisation of the purified actin by observing formation of the actin filaments in solution using light microscopy. You will compare behaviour of actin to the behaviour of a control protein, bovine serum albumin, which does not polymerise.

EXPERIMENTAL

Session 1: Protein analysis by protein gel electrophoresis using SDS PAGE, preparation to Western blot analysis (transfer proteins from the gel to a PVDF membrane and place in blocking solution).
Session 2: Protein analysis by Western blot (incubations with antibodies, washes and development of the blot), analysis of actin polymerisation in vitro.

Session 1
Protein Gel Electrophoresis

1. Students will work in pairs and each pair should prepare a sample of human neuroblastoma SHEP cell proteins. To make the sample, use a separate microfuge tubes to combine 20 µl of Laemmli buffer and 20 µl of the protein extract from SHEP cells, which you have prepared in the practical class on Cell Adhesion.
   Help your demonstrator to prepare samples of mouse brain cell proteins and purified actin.
2. Boil samples for 5 minutes at 95°C.
3. Assemble the electrophoresis chamber with the help of the demonstrator.
4. Load 10 µl of the SHEP cell protein sample onto a first 10% SDS polyacrylamide gel and 10 µl of the protein sample onto the second 10% SDS polyacrylamide gel.
   Help your demonstrator to load molecular weight markers, mouse brain cell protein sample and purified actin sample on each gel.
5. Run the gels at 100 volts for 1.5 hours, or until the dye-front is at the bottom of the gel.
6. Carefully remove the gels from the plastic cassette. Mark the gel to establish the orientation (cut a small section on the bottom right hand corner of the gel).
7. Place the first gel into the fixative solution and incubate for 30 min at room temperature. Discard the fixative to the container for the fixative waste. Apply the Colloidal coomassie stain onto the gel, sign it and give it to your demonstrator. Check the labelling at the end of this class and during the next class.
8. Place the second gel in a container and rinse it for 20 minutes in the transfer buffer. Cut a piece of PVDF membrane with the size corresponding to the size of the gel (6x9 cm, wear gloves and only handle the membrane by the edges). Use a pencil to write the date and descriptions of samples near the top edge of the membrane. Wet the PVDF membrane in methanol for 30 seconds, soak in distilled water for 5 minutes and then soak in transfer buffer for 15 minutes.
9. Immerse gel, membrane, and filter papers pads in transfer buffer in a Pyrex dish to ensure they are thoroughly soaked. Be careful to exclude air bubbles from the support pads.
10. The demonstrator will help you to assemble a sandwich for transfer on a Trans-Blot Semi-Dry transfer system: pre-wet filter paper (3 sheets), pre-wet membrane, equilibrated gel, pre-wet filter paper (3 sheets). Roll out air bubbles between layers.
11. Secure top stainless-steel cathode and safety cover.
12. Transfer at 5 mA / cm^2 of the gel for 30 min.
13. After transfer, disassemble the gel sandwich.
14. Place the PVDF membrane into TBS buffer and wash for 1-2 min.
15. Place the membrane in 5% skim milk powder in TBS. The membrane will be stored in this solution until next week.

If required, steps 13-15 will be done for you by the staff.

Session 2
Western blot analysis

16. Remove the membrane from the blocking solution and wash 3 times 5 minutes each in TBS.
17. Place the blot in a plastic bag.
18. Add the primary antibody (anti-actin) solution. Use 5 ml per membrane. The primary antibody is diluted 1:1000 in 0.3% Tween-20 in TBS (TBST).
19. Seal the bag, taking care to ensure that all air bubbles have been removed from the bag.
20. Incubate the blot with primary antibody for 40 minutes at room temperature with agitation.
21. Wash the blot 3 times 5 minutes each in TBST.
22. Add horseradish peroxidase-labelled secondary antibody, diluted 1:1000 in TBST. Incubate for 1 hour at room temperature with agitation.
23. Wash the membrane 3 times 5 minutes each in TBST.
24. Incubate the membrane with the TMB Substrate until the protein bands become visible.

Actin polymerisation analysis

When your membrane is incubated with primary antibodies (step 20), use this time to start the analysis of actin polymerisation.

1. Obtain an aliquot with solution containing purified actin from your demonstrator. Apply a drop of this solution (10 µl) on a glass slide.
2. Add 20 µl of the polymerisation buffer to the drop with purified actin. Incubate at room temperature.
3. Place the slide on the stage of the microscope. Observe formation of the filaments at 10 min intervals. Record your observations.
4. Repeat steps 1-3 for control protein (BSA).

Reagents:

Laemmli buffer	0.125 M Tris-Cl pH 6.8, 4% SDS, 20% glycerol, 0.05% (reducing) bromophenol blue, 10% 2-mercaptoethanol
Fixative:	40% ethanol, 10% acetic acid
Coomassie stain:	QC colloidal Coomassie stain from Bio-Rad
Transfer buffer	3 g Tris and 11.26 g glycine dissolved in 800 ml water. Add 200 ml methanol before use.
RIPA buffer	0.15M NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.05 M Tris-Cl pH 7.5
TBS	20 mM Tris-Cl pH 8, 150 mM NaCl
TBST	0.05% Tween-20 in TBS
Blocking buffer	5% skim milk powder in TBS
Washing buffer	TBST

5 X SDS PAGE running buffer

Tris base	9.0 g
Glycine	43.2 g
SDS	3.0 g

Make up to 600 ml with distilled water. Store at 4^{o}C; warm to 37^{o}C if precipitation occurs. Dilute 60 ml 5 X stock with 240 ml distilled water for one electrophoresis run.

QUESTIONS:

You have used “blocking solution”. What does it block? Can you use something else instead of milk? Why milk is used?

Compare Colloidal coomassie stain of the gel with Western blot. Discuss the difference with the demonstrator.

What are the applications of the Western blot? Discuss with the demonstrators.

How many protein bands do you expect to see in one lane on the Western blot?

If you see more than one band in one lane of the Western blot, how can you explain the bands which appear above or below the expected molecular weight?

Using the Western blot analysis, can you extract any information about the aggregation and/or degradation of the protein of interest? Can you obtain this information by protein gel electrophoresis and Colloidal coomassie staining?

The molecular weight of tubulin is 55 kDa, and the molecular weight of spectrin is 220 kDa. How the gel electrophoresis bands of these two proteins will be located with respect to the actin protein band?

Experimental observations

Make a rough sketch of the gels and protein bands identified by protein gel electrophoresis and Colloidal coomassie staining. SHEP cells are often used as a model of brain cells, but it is debated whether they are a suitable model. What do you think?

Make a rough sketch of the protein bands identified by Western blot analysis. Determine the molecular weight of actin. Is the solution with purified actin free of contaminants?

Make a rough sketch of the actin filaments. Can you use Western blot to detect them? Explain your answer.

Demonstrator: Signature:

PRACTICAL: PROTEIN INTERACTION **DATE...../...../.....**

STUDENT NAME.....SIGNATURE.....

BIOLOGICAL HAZARDS	HAZARDS	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY *SPILLS CLEANUP AND FIRST AID
1			
2			
3			
4			
5			

PROCEDURAL (PLANT & EQUIPMENT)	HAZARDS	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY *SHUTDOWN PROCEDURES AND FIRST AID
1			
2			
3			
4			
5			
6			
7			
8			

CHEMICALS	HAZARDS *YOU MAY USE RISK AND SAFETY PHRASES	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY MANAGMENT *SPILLS CLEANUP AND FIRST AID
1			
2			
3			
4			
5			
6			
7			
8			

EXTRA NOTES

THE UNIVERSITY OF NEW SOUTH WALES

SCHOOL OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

PROTEIN INTERACTION ANALYSIS BY ELISA

RISK ASSESSMENT

Chemical Hazards

Solutions of antibodies and peptide contain **sodium azide** and **thymol** as preservatives. These preservatives are toxic. Care should be taken in handling, to avoid ingestion or skin contact.

Substrate (**Tetramethylbenzidine or TMB**) is toxic. Avoid any skin contact.

Stop solution is a strong acid. Avoid any skin contact.

Biological Hazards

Antibodies were isolated from animals. They should be handled as if capable of transmitting infectious disease.

Procedural Hazards

Plate reader (electrical hazard, contains moving parts)

AIMS

To demonstrate a technique for analysis of the interaction between two proteins.

INTRODUCTION

Interactions between proteins and other biomolecules, and especially other proteins, play a critically important role in the life of a cell. A number of methods have been developed to analyse these interactions. In this practical class you will use one of such methods, ELISA, which is essentially a protein-ligand binding assay. While in case of ELISA one of the proteins is usually an antibody, a similar assay can also be used to detect interactions between two different proteins, none of which is an antibody. In this practical class, you will use ELISA to analyse the interaction between two proteins – Aβ peptide, which is a portion of Amyloid precursor protein, and a mouse monoclonal antibody against the Aβ peptide.

The enzyme-linked immunosorbent assay (ELISA) was described for the first time in 1971 in two independent publications (Engvall and Perlman, 1971; Van Weemen and Schuurs, 1971). ELISA is a highly sensitive, versatile and quantitative technique that requires little equipment.

Amyloid precursor protein (APP) is a cell adhesion molecule expressed at the cell surface of neurons in the brain. It is important for neuronal development and correct functioning of synapses between neurons. It can be proteolytically cleaved by proteases, which results in production of proteolytic products, peptides of APP. In Alzheimer's disease, accumulation of Aβ peptide derived from APP is observed in brains of patients. These peptides are neurotoxic and contribute to neuronal death observed in brains of patients with Alzheimer's disease. Aβ peptides can also serve as biomarkers of Alzheimer's disease.
Imagine that you work in the laboratory studying Alzheimer's disease, and you have developed a new monoclonal antibody detecting a portion of APP corresponding to Aβ peptide (anti-Aβ peptide mAb). You have to check whether your work was successful, i.e. whether your antibody indeed binds the Aβ peptide. You also want to check whether you can use this antibody to detect levels of Aβ peptide in biological samples containing unknown concentrations of Aβ peptide, i.e. to screen samples from patients to identify samples containing abnormally high levels of Aβ peptide characteristic of Alzheimer's disease. To address these questions, in this practical class, you will use ELISA to analyse binding between your antibody, i.e. a mouse monoclonal antibody developed against Aβ peptide (anti-Aβ peptide mAb), and synthetic Aβ peptide.

To analyse whether anti-Aβ peptide mAb binds Aβ peptide, the antibody is immobilized in wells of a microtitre plate. Wells are then incubated with Aβ peptide to allow formation of the monoclonal antibody / Aβ peptide complex. Wells are then washed to remove unbound proteins.

Since both of your proteins (mouse monoclonal antibody and Aβ peptide) are not labelled, you need a detection reagent to detect formation of a complex between the two proteins. In your case, you will use another antibody, anti-Aβ peptide rabbit polyclonal antibody, to detect Aβ peptide within the monoclonal antibody / Aβ peptide complex. After washing, the rabbit antibody is then detected with anti-rabbit secondary antibodies coupled to peroxidise. This

reaction results in the attachment of peroxidase enzyme to the whole mouse monoclonal antibody/ Aβ peptide / rabbit polyclonal antibody complex. Following a further wash, the substrate for peroxidase enzyme, Tetramethylbenzidine or TMB, is added and the presence and amount of the mouse monoclonal antibody/ Aβ peptide protein complex is determined in a microtitre plate reader by estimating subsequent colour production.

To estimate whether the technique is quantitative, i.e. whether it allows estimation of the amount of protein complexes formed, you will use different concentrations of Aβ peptide and analyse whether an increase in the amount of Aβ peptide added to wells and consequent increase in the amount of monoclonal antibody / Aβ peptide complexes formed will result in increased optical density measured by the spectrophotometer. You will then plot a standard curve (optical density as a function of Aβ peptide concentration) and use it to estimate the concentration of Aβ peptide in samples with unknown levels of Aβ peptide that you will be provided. Note, that in this way ELISA is widely used as a diagnostic tool to estimate levels of different antigens in biological samples.

References:

Engvall E, Perlman P (1971). Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry 8 (9): 871–4.
Van Weemen BK, Schuurs AH (1971). Immunoassay using antigen-enzyme conjugates. FEBS Letters 15 (3): 232–6.

Reagents:

Aβ 42 Mouse ELISA Kit (Cat Nr KMB 3441) from Life Technologies.

EXPERIMENTAL

1. Each pair of students will be supplied one test strip from a microtitre plate consisting of 8 wells. The monoclonal antibody against Aβ peptide was already immobilised in each well of the microtitre plate strip that you are provided.

Be careful not to scratch the bottom of the wells during further steps or you will not have a good optical surface for the plate reader.

2. Wash wells once by adding 100 µl of Standard diluent buffer. Thoroughly aspirate solution from wells and discard the liquid.

3. Pipette 100 µL of 0, 6, 25, 50, 100 and 200 pg/ml Aβ peptide in wells 1-6.

4. Pipette 100 µL of samples with unknown concentration of Aβ peptide (collect from laboratory staff) into wells 7-8.

5. Cover plate with plate cover and incubate for 1 hour at 37°C.

6. Thoroughly aspirate solution from wells and discard the liquid. Wash wells 4 times with 200 µl of Wash buffer.

7. Pipette 100 µL of rabbit polyclonal antibody against Aβ peptide into each well. This solution will have a blue colour. Tap gently on the side of the plate to mix.

8. Cover microwells with lid and incubate for 45 minutes at 37°C.

9. Thoroughly aspirate solution from wells and discard the liquid. Wash wells 4 times with 200 µl of Wash buffer.

10. Add 100 µL of anti-rabbit secondary antibody coupled to HRP (anti-rabbit HRP, yellow) into all wells.

11. Cover microwells with lid and incubate for 30 minutes at 37°C.

12. Thoroughly aspirate solution from wells and discard the liquid. Wash wells 4 times with 200 µl of Wash buffer.

13. Add 100 µL of substrate solution into all wells. The liquid in the wells will begin to turn blue.

14. Cover microwells with lid and incubate for 15 minutes at room temperature (20-25°C). Cover the wells with a piece of paper to keep wells in dark.

15. Pipette 100 µL of stop solution into all wells in the same timed sequence as for substrate solution addition.

16. Carry out an end-point reading at 450 nm.

Experimental observations

17. When you have collected your data from the microplate reader, compare signals from wells with different concentrations of Aβ peptide. Plot the curve describing the relationship between the concentration of the peptide and the optical density in the wells. Does your experiment confirm that the antibody binds to the peptide or not? Why do you think so?

18. Can you use the curve (see step 17) to estimate the concentration of Aβ peptide in your sample with unknown levels of Aβ peptide? Perform this estimation.

19. If you need to analyse the binding of protein A to protein B, you can also use ELISA. Make a rough diagram of how you would perform this experiment.

QUESTIONS:

Why are the secondary antibodies designed to recognise rabbit Ig and why are they peroxidase (HRP) coupled? Is it possible to use another label?

Can you predict the shape of the curve describing the relationship between OD and Aβ peptide concentration if you use even higher concentrations of the Aβ peptide in your binding assay? Can you explain? Can you use this information to estimate the concentration of the mouse monoclonal antibody immobilised in wells of the microtitre plate? Note, that the monoclonal antibody is bivalent, i.e. each antibody molecule can bind two molecules of Aβ peptide.

Demonstrator: Signature:

PRACTICAL: Fluorescence microscopy **DATE...../...../.....**

STUDENT NAME.....SIGNATURE.....

BIOLOGICAL HAZARDS	HAZARDS	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY *SPILLS CLEANUP AND FIRST AID
1			
2			
3			
4			
5			

PROCEDURAL (PLANT & EQUIPMENT)	HAZARDS	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY *SHUTDOWN PROCEDURES AND FIRST AID
1			
2			
3			
4			
5			
6			
7			
8			

CHEMICALS	HAZARDS *YOU MAY USE RISK AND SAFETY PHRASES	RISK CONTROL STRATEGIES *PROCEDURAL / PPE	EMERGENCY MANAGMENT *SPILLS CLEANUP AND FIRST AID
1			
2			
3			
4			
5			
6			
7			
8			

EXTRA NOTES

THE UNIVERSITY OF NEW SOUTH WALES

SCHOOL OF BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES

Fluorescence microscopy

RISK ASSESSMENT

Chemical Hazards

Nile Red is classified as non-hazardous at the given concentration. However, the manufacturer recommends handling of all chemicals with caution. Care should be taken in handling, to avoid ingestion or skin contact.

Biological Hazards

Culture media and all samples should be treated as potentially infectious.

Procedural Hazards

Electrical, high intensity light. Note, fluorescence microscopes contain high intensity light sources for specimen illumination. Avoid any eye or skin exposure.

AIMS

The aim of the practical class is to provide basics of the fluorescence microscopy technique and demonstrate that it can be applied for visualization of subcellular structures / organelles.

INTRODUCTION

Fluorescence microscopy is an important technique for visualising cells, cell morphology, cellular organelles and the localisation of fluorescence labelled molecules including proteins.

In this practical class, you will use a fluorescent dye, Nile Red, which is used to localize and quantitate lipids. When cells are incubated with this dye, it binds to neutral lipids which are accumulated within cells in specialized organelles, called lipid droplets. Lipid droplets are emerging as important organelles. They are responsible for the storage of fat within cells. Your aim is to visualize these organelles and estimate their number and morphology in cells.

In this experiment you will use fluorescence microscopy to compare the lipid droplet morphology in yeasts. You will compare wild type, i.e. normal, yeast cells to a mutant strain (*fld1Δ*) known to have abnormal lipid droplet morphology. You should calculate the mean number of these organelles per cell, and estimate the morphology (round, elongated, etc.) and compare size of the organelles in two strains of yeast you are provided.

Experimental procedure

Students work in pairs and each pair of students analyse 1 sample of wild type and 1 sample of mutant yeasts.

In the laboratory:

1. Mutant and wild-type yeast cultures have been grown overnight in SD (synthetic defined) media to stationary phase. Each pair of students will be given 50 µl of either culture.
2. Add 1 µl of Nile red stock solution (provided by demonstrator) to the culture.

NOTE: Nile red is light sensitive. Light exposure of the stained sample should be kept to a minimum.

3. Gently mix the dye by flicking.
4. Label a glass slide with your pair number and sample type (i.e. mutant or wild-type)
5. Pipette 6 µl of the stained culture onto the glass slide.
6. Cover the sample with a cover slip and put the slide into a dark box (see demonstrator) to protect it from light exposure. The demonstrator will take you to the microscopy room.

In the microscopy room:

1. Add one drop of immersion oil to the top of the cover slip.
2. Examine the slide under the 100X objective using normal light. Adjust the focus until you see the oval shaped yeast cells.
3. Turn off the visible light and switch on the fluorescence with the GFP (blue) filter.
4. The lipid droplets should be visible as bright red bodies within the cells. Note the motility of the lipid droplets – they are attached to the cytoskeletal machinery.
5. For 10 cells count the number of lipid droplets per cell. Use the table provided below.

In the laboratory,
1. Perform statistical analysis of your observation
Average the numbers and compare results between mutant and wild type populations. Discuss results with other students.

Table 1. A comparison of numbers of small and large lipid droplets in wild type and *fld1Δ* yeasts.

wild type **lipid droplet number** **small** **large**	***fld1Δ*** **lipid droplet number** **small** **large**
Mean	**Mean**
Standard error of mean	**Standard error of mean**

2. Document your visual observations. Make a rough sketch of the yeast cells of both strains. Show the morphology of lipid droplets in both strains.

wild type ***fld1Δ***

QUESTIONS:

1. Did you observe any differences in the number and size of lipid droplets in yeasts that you analysed? Are these observations supported by your statistical analysis?

2. In *fld1Δ* strain of yeast, *fld1Δ* gene was deleted. Can you speculate about the role that the protein encoded by this gene plays in cells?

3. You used only one dye in your experiment. Discuss whether it is possible to use 2 or more dyes simultaneously. Are there any limitations? Suggest other structures, organelles and compartments that you would look at in parallel to Nile red if you have this possibility. Draw a sketch of what you would expect to see based on your observations.

Demonstrator: Signature: