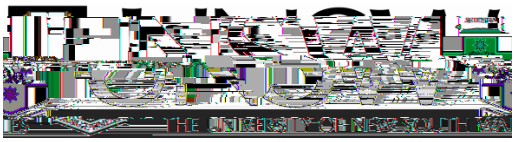

FACULTY OF SCIENCE

SCHOOL OF BIOTECHNOLOGY

AND

BIOMOLECULAR SCIENCES

BIOC3271 Molecular Cell Biology

and

BIOC3671 Molecular Cell Biology (Advanced)

SESSION 2, 2021

Table of Contents

1. Information about the Course

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

Year of Delivery	2021
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
Session(s) Offered	S2
Assumed Knowledge, Prerequisites or Co-requisites	BIOC2101, BIOC2201
Hours per Week	7 HPW
Number of Weeks	10 weeks
Commencement Date	31.05.21 **Number**

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*
		Dr. Frances Byrne	frances.byrne@unsw.edu.au	
		A/Prof. Antony Cooper	a.cooper@garvan.org.au	
		Dr. Gavin Chapman	g.chapman@victorchang.edu.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	
		Prof. H. Rob Yang	h.rob.yang@unsw.edu.au	
	Tutors & Demonstrators	A/Prof. Vladimir Sytnyk	v.sytnyk@unsw.edu.au tel.: 9385 1108	

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)

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, .11 aolt'3(nsoLduectie)5.6().2(R and stresstrepcones.e stresstrepcessesracs inatxing(orcompenslatingfce)5.6rA damag bT ut(cao)5.6(prlmoned.t

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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. Andrew 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. Louise 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

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 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 amyloid and cell biological mechanisms of amyloid toxicity.

METHODOLOGICAL APPROACHES FOR CANCER CELL BIOLOGY (1 lecture)
Dr. Frances Byrne, 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.

5. Course Schedule

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

Week

[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:					
Reports at the end of practical classes (BIOC3271)			Online	Convenor, written / verbal	after practical classes
Project description (BIOC3671)			Online	Convenor, written	Week 3, 14/06
Summative Assessment Tasks:					
Assessment 1:	Protein-Protein interaction assignment (BIOC3271)	20	Online	Convenor, written	Week 10, 02/08
	Project article (BIOC3671)	30	Online		

8. Required Equipment, Training and Enabling Skills

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

10. Administration Matters

Expectations of Students	Students are encouraged to attend all online lectures and participate in Q/A sessions. Students should use online lecture recordings if they cannot attend the lectures. BIOC3271 students are expected to attend practical classes and submit reports by the end of the practical classes. Submissions of reports will be considered as confirmation of attendance at 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 mark 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 BIOC3671 students must 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 practical classes, 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/special-consideration. 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 Consideration). Students will be contacted via their official university email as to the outcome of

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

	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.** **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	In an ideal world, science would be objective. However, the reality is much of science is subjective and is historically built on a small subset of voices. In this course, I will make an effort to expose you to literature from a diverse group of scientists, despite limits still existing on this diversity. I acknowledge that it is possible that there may be some biases in the material due to the lens with which it was written, and please contact me if you have any suggestions to improve the quality (or diversity) of the course materials. There are challenges inherent in communicating between people from other cultures, but I will strive to ensure my passion for science is appreciated through different eyes. I have a genuine desire to experience new cultures, expand my own horizons, and transcend any barriers that interacting with diverse groups could impose. I am acutely aware of the importance of diversity and inclusion in all aspects of life and want to uphold these values as an educator. The School of BABS is dedicated to creating a positive, inclusive educational environment that embraces diversity in all forms and rejects any form of hostile workplace, discrimination, or bullying. We have a clear statement of behavioural expectations (as well as definitions of discrimination, (sexual) harassment and bullying, which can be found here: https://student.unsw.edu.au/harassment. On this website, you can also find resources and contacts for reporting issues. In addition, the Science Equity,

	Beyond the University and Faculty protocols, **it is my goal as course convenor to create a learning environment for my students that supports a diversity of thoughts, perspectives and experiences, and honors your identities** (including race, gender, class, sexuality, religion, ability). To help accomplish this: - If you choose, please let me and the class know your chosen name and pronouns. - Your classmates and demonstrators (like many people) are still in the process of learning about diverse perspectives and identities. If something was said in class (by anyone) that made you feel uncomfortable, please talk to me about it. - As a participant in course discussions, you should also strive to honor the diversity of your classmates (e.g. make sure all voices are being heard, etc.). - If you feel like your performance in the class is being impacted by your experiences outside of class, please do not hesitate to contact me. Finally, the School recognises the added challenges faced by students during the coronavirus outbreak, in particular those related to teaching and learning remotely while public health is managed. Specific details on how this course will be managed are given throughout this manual and will be highlighted further in the first lecture, but please be assured I will strive to minimise stress to students while still endeavoring to deliver a high-quality teaching experience. 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 (+61 2 8374 9201 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: Dr. Megan Lenardon m.lenardon@unsw.edu.au Tel: 9385 8047	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

1. 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.

12. LABORATORY WORK FOR BIOC3271 STUDENTS

LABORATORY HOURS

Online laboratory classes will take place on Fridays 2 - 6 pm.

Students are expected to complete their experimental work within these hours**. Most laboratory sessions will commence with a brief talk by the lecturer in charge and will conclude with the Q/A session. It is imperative that you attend these sessions.**

Students are encouraged to prepare for the class 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*. At the end of the class, you will need to submit via Moodle a short report summarizing your calculations and conclusions. This report will serve as confirmation of your presence at the class.
If you are absent from the practical class for a m6( )5.otheson,sc

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 l 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 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.

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 l 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.

Online Part (to be completed during breaks in the experimental part and submitted as a report) can be accessed on Moodle.

Additional Questions:

1. Trypsin-EDTA solution was used 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?

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BIOC3271/3671

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?

reaction results in the attachment of peroxidase enzyme to the whole mouse monoclonal antibody/ A 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/ A 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 A peptide and analyse whether an increase in the amount of A peptide added to wells and consequent increase in the amount of monoclonal antibody / A 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 A peptide concentration) and use it to estimate the concentration of A peptide in samples with unknown levels of A 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 A 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 l 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 A peptide in wells 1-6.

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

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

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

7. Pipette 100 µL of rabbit polyclonal antibody against A 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 37ºC.

9. Thoroughly aspirate solution from wells and discard the liquid. Wash wells 4 times with 200 l 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 37ºC.

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

Online Part (to be completed during breaks in the experimental part and submitted as a report) can be accessed on Moodle.

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 A peptide concentration if you use even higher concentrations of the A 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 A peptide.

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**

Online Part (to be completed during breaks in the experimental part and submitted as a report) can be accessed on Moodle.

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.