Year and Semester: Semester 1, 2012
Unit convenor: Associate Professor Bridget Mabbutt

Sureshan & Mabbutt (2011, unpublished)
ABOUT THIS UNIT

This unit outlines molecular principles underlying today’s developments in protein science. As well as detailing modern separation technologies, the course addresses structural biology, protein analysis and sequence informatics. Practices common in the biotechnology and pharmaceutical industries to purify macromolecules (including recombinant proteins) are emphasized. Analysis methods are introduced in relation to proteomics, genomics and biomedical research. Molecular properties leading to the 3D shape of proteins are detailed, and contemporary structure methods outlined.

Practical and project segments of the unit involve hands-on protein skills, molecular graphics and use of key protein web resources.

1 - 3 FUNCTIONAL GROUPS IN PROTEINS

4 ISOLATING BIOMOLECULES
- recombinant sources
- quantitation & detection

5 & 6 SEPARATION OF PROTEINS
- separation by precipitation
- gel filtration for separation

7 - 10 CHROMATOGRAPHY FOR PURIFICATION
- ion exchange
- hydrophobic/reversed-phase
- affinity chromatography

11 - 13 PROTEIN ANALYSIS
- 2D gel electrophoresis (MM)
- mass spectrometry (PH)
- sugar/glycoprotein analysis (NP)

14 - 16 PROTEIN FOLDS AND DOMAINS
- all α-structures (globin fold, helix bundles)
- all β structures (antiparallel barrels, the beta helix)
- mixed α/β folds

17 - 19 TERTIARY STRUCTURE DETERMINATION
- x-ray crystallography
- NMR spectroscopy

20 - 21 HOW PROTEINS FOLD IN SOLUTION
- thermodynamics of protein folds
- circular dichroism

22 & 23 BIOINFORMATICS
- structure prediction methods
- the CASP project

24 MEMBRANE PROTEINS (KH)

25 FLUORESCENCE TECHNOLOGIES (LB)
TEACHING STAFF

- Unit convenor and lecturer Bridget Mabbutt (room 338, F7B) can be contacted by email within the online iLearn interface (ilearn.mq.edu.au). You are welcome to arrange personal meeting times by appointment.

- A/Prof. Mabbutt holds a general office consultation for the full hour after each Friday lecture (i.e. 9 – 10 pm).
  
  This is a dedicated time just for 832 and 332 students to raise queries or seek assistance with their assignment work. Please make use of this time to discuss your progress or any concerns.

- The following members of CBMS will deliver individual lectures in their areas of research speciality: Dr Louise Brown, Prof. Paul Haynes, A/Prof. Mark Molloy, Prof. Nicki Packer and Dr Karl Hassan. Each of these lecturers can be contacted via email (mq.edu.au addresses).

CLASSES

- Lectures will be twice weekly: Tuesday (12 pm) and Friday (8 am). Some additional lectures will be delivered in the lab hours of Week3.

- It is essential that you be available to attend all classes. Lectures are NOT made available during session, as the classroom experience is integral to the subject material of this Unit.
  
  Should you not be able to attend a specific class (for reason of illness or misadventure), please contact A/Prof Mabbutt (via iLearn) to organise access to the relevant lecture file.

- The course syllabus is defined by all of the subject material presented in lectures and practicals, much of which is beyond standard textbooks.

- Laboratory Sessions: Wednesday afternoons (1-5 pm). This time will also include seminar and workshop activities. These will generally be held in the office and lab areas of E7B 349/350.

- Attendance is compulsory on the allocated days of class. If you are sick, please consult with A/Prof. Mabbutt to ensure all laboratory and project work is completed.

- Please check the location of each laboratory activity, as classes start promptly at 2pm. Latecomers may be excluded from class.
• My Lab Group is _______________.
You are not permitted to change groups during semester.

<table>
<thead>
<tr>
<th>WEEK 1</th>
<th>Feb29</th>
<th>No practical classes</th>
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<tbody>
<tr>
<td>WEEK 2</td>
<td>Mar7</td>
<td>Literature workshop:</td>
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<tr>
<td></td>
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<td>Searching primary literature for your Pet Protein</td>
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<tr>
<td></td>
<td></td>
<td>1-3pm Group A</td>
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<td></td>
<td></td>
<td>3-5pm Group B</td>
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<tr>
<td>WEEK 3</td>
<td>Mar14</td>
<td>Lectures 6 &amp; 7</td>
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<td></td>
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<td>1-3pm all students (venue tba)</td>
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<td>WEEK 4</td>
<td>Mar21</td>
<td>Purification workshop</td>
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<td>Protein chromatography practical (report 30/3)</td>
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<td>Group A</td>
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<td>WEEK 5</td>
<td>Mar28</td>
<td>Protein chromatography practical (report 5/4)</td>
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<td>Purification workshop</td>
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<td></td>
<td>Mar30</td>
<td>PET PROTEIN PART A DUE</td>
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<td>all students</td>
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<td>WEEK 6</td>
<td>Apr4</td>
<td>Mass spectrometry informatics (report 23/4)</td>
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<td>all students (venue tba)</td>
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<td>mid-term break</td>
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<td>Peer assessment task</td>
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<td>832 students only</td>
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<td>WEEK 7</td>
<td>Apr25</td>
<td>No practical class (Anzac Day)</td>
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<td>Apr27</td>
<td>Peer assessment due</td>
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<td>832 students only</td>
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<tr>
<td>WEEK 8</td>
<td>May2</td>
<td>Molecular graphics (report 11/5)</td>
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<td>Group A (venue tba)</td>
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<td>WEEK 9</td>
<td>May9</td>
<td>Molecular graphics (report 18/5)</td>
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<td>Group B (venue tba)</td>
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<tr>
<td>WEEK 10</td>
<td>May16</td>
<td>Film: “DNA: Life Story”</td>
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<td>all students (venue tba)</td>
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<tr>
<td>WEEK 11</td>
<td>May23</td>
<td>Seminars (PET PROTEIN, PART B)</td>
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<td>Group A</td>
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<td>WEEK 12</td>
<td>May30</td>
<td>Seminars (PET PROTEIN, PART B)</td>
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<td>Group B</td>
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<tr>
<td>WEEK 13</td>
<td>Jun6</td>
<td>No practical class (revision week)</td>
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</tbody>
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schedule as at February 2012

**REQUIRED AND RECOMMENDED TEXTS**

• The textbook of which you are required to purchase a personal copy is: “Physical Biochemistry: Principles and Applications”, David Sheehan, John Wiley (2nd ed, 2002).

• Because of the multidisciplinary nature of this course, you will be expected to read more widely than this, however. The library has an excellent collection of up-to-date reference material to cover the course and laboratory subjects - explore it!!

• Strongly recommended reference texts available in the library (short-term loan only):
  “Protein Structure and Function”, Petsko & Ringe, New Science Press, 2004
  “Introduction to Protein Structure”, Branden & Tooze, Garland, 1999

• Other general references that you may find useful are:
  Garrett & Grisham, “Biochemistry” (esp. Chs 4 – 6), Harcourt Brace, 1999
  T. Creighton, “Proteins: Structures and Molecular Properties”, Freeman, 1993

UNIT WEB PAGE & WEB RESOURCES

• The Unit will run as an online unit within iLearn. You can get to your own login portal at http://learn.mq.edu.au

This subject is very suitable for web-based resources: there are excellent websites, apps and YouTube presentations to show how protein are made and constantly move around.

It is an expectation that you will become familiar with the following sites during the course:
• www.uniprot.org/
  This is a centralised resource for “vital statistics” of known proteins. Try out your Pet Protein here!
• http://au.expasy.org
  “Proteomics” website hosted by the Swiss Intitute for Bioinformatics. Provides many of the tools for doing your own protein analysis & bioinformatics. Good links, so start here.
• www.ncbi.nlm.nih.gov/pubmed (or just type Pubmed)
  Essential for your literature searches-free and easy searching of all journals-anywhere, anytime.
• www.rcsb.org/pdb (or just type PDB)
  the world’s central protein structure database. Use this to locate structures and view them in 3D.
• www.sunsetlakesoftware.com (or AppleStore)
  free downloadable “Molecules” application:
  “an application for the iPhone and iPod touch that allows you to view three-dimensional renderings of molecules and manipulate them using your fingers. You can rotate the molecules by moving your finger across the display, zoom in or out by using two-finger pinch gestures, or pan the molecule by moving two fingers across the screen at once.”

TECHNOLOGY USED AND REQUIRED

Within this Unit, you will be introduced to Web-based tools, search engines and graphics software that are commonly used today in protein science. Our expectation is that you will have ready access to the internet and have a computer available for web browsing, preparation of your reports and case study analysis.
Handwritten reports will not be accepted. Your project and laboratory reports will be submitted and circulated via the online Turnitin program.

Your practical reports will require you to carry out minor computational tasks, for which a calculator and access to basic statistical software will be required. We place a large emphasis on correct referencing style in all your reports, and use of the program EndNote is encouraged, but not essential.

The capacity to download and install a simple molecular graphics program will assist you greatly in the Unit. Your model-building assessment task can be carried out with very simple materials; it is not an expectation that expensive art supplies need be purchased.

## Assessment Tasks

Three modes of assessment are used to determine your progress in CBMS332. Satisfactory (i.e. pass level) performance must separately be achieved in all three of the assessment areas to obtain a P grading for this Unit.

### I: Final examination (3 hr paper) 40%

- This written exam is based on problem-solving exercises.
- The University Examination period in First Half Year 2012 is from **June 12th to June 20th**. All students must ensure that they are available until the final day of the official examination period.

The only exception to not sitting an examination at the designated time is due to documented illness or unavoidable disruption. In these circumstances you may consider applying for Special Consideration (see below).

### II: Laboratory reports (3) 30%

- Laboratory experiments are based on research-based projects underway at Macquarie. Report write-up of these laboratory experiences will develop your scientific report writing skills, and will require background reading and application of the theory you encounter in lectures.
- Laboratory reports must be submitted as a full report of your experimental data and **discussion and analysis** of your findings. Reports are due **9 days** after completion of the laboratory work, i.e. 9 am on Fridays of the week following your practical. Marks will be deducted for reports handed in after the due date (5% / day).
- Laboratory reports must also be submitted in electronic form to the Turnitin program. Login details will be provided on the iLearn site.
- All written work (assignments and lab reports) are to be submitted directly to Ms Thi Huynh during lecture times.
- Submissions must include a completed and signed cover sheet stapled to the front cover. The Assignment Cover Sheet can be downloaded from the at: web.science.mq.edu.au/new_and_current_students/undergrad/assignments_and_coversheets
• Reports with incorrectly formatted bibliography listings will be returned unmarked for correction and re-submission.

• The following sections must be included:

  **Aims & Background** – outline the background science leading to the experiment carried out. Provide primary citations (i.e. the original sources) for all factual background.

  **Experimental Methods/Results** – present your laboratory results obtained on the day, summarise in appropriate tables and/or figures, give example calculations. *Do not copy out methods from the laboratory instructions.* Although experiments may be carried out in collaboration with laboratory partners, the submitted reports are expected to be your own work (see Academic Honesty section, below).

  **Discussion** - interpret your results, and relate them to the system under study. Compare your observations with appropriate reference material.

  **References** – all references used in your report should be fully listed in a relevant scientific format (author list, title, journal, page numbers). Present your author list either alphabetically or numbered according to appearance in text. Web-based sources are not acceptable for citations.

The University library has very useful guidance details on referencing on its website (public.mq.edu.au/on_campus/library/research/referencing/). Marks are generally awarded separately for this section of your reports.

• All marked work will be returned in class, generally within 3 weeks.

III:  “Pet Protein” Project  
• This is assessment of your research and analytical skills, and continues throughout the semester to enhance each topic area.
• Your individual topic for the term “Pet Protein” project will be distributed in Week 2.
• As well as presenting analysis of your own case study in written form, you will be required to transmit your understanding of the individual protein to your fellow students via seminars and presentation of your own constructed three-dimensional protein model:

  written report, Part A: **due Mar 30** 10%
  seminar & questions (Part B) 10%
  model (Part B: due at seminar) 10%

• Your project will be assessed using the following criteria:
  - the quality and extent of your research
  - the depth and **molecular detail** of your analysis
  - appropriate use of internet tools
PET PROTEIN PROJECT

This is a library assignment and will require extensive literature searching to locate prime sources in books and journals.

You will be given the name of a protein of industrial/pharmaceutical importance. You will need to select a specific species variant that is used in a medical or industrial application.

My “Pet Protein” is: ___________________________

PART A: Methods of Purification (Written report due FRIDAY Mar30)

Find out about:

1. The primary structure (amino acid sequence) and physico-chemical properties (pl, MW, GRAVY value) of the protein that are important considerations for a purification protocol.
   - Use the tools in ExPASy to analyse your sequence. Make sure you use the sequence of the mature form of your protein (i.e. remove any signal peptide)
   - If a glycoprotein, detail the full chemical nature of the carbohydrate portion.
   - Present a hydropathy plot (generated in ProtScale: web.expasy.org/protscale/).
   - Discuss (i) the features of this plot with relation to the specific amino acid sequence and (ii) its relationship to the GRAVY (grand average hydropathy) value. Comment on the implications, if any, for purification.

2. How the protein was initially purified from its natural source when it was first discovered.
   - Search the literature to source the original discovery route for this protein.
   - Present a summary for this first purification as a flow chart. This should present an overall view of the chemical partitioning events, not detailed methods & reagents.
   - Explain and give a detailed description of the molecular events and chemistry of each step in the procedure.
   - Where possible, discuss chromatography traces or stained gel images that indicate the purification achieved at particular steps.

   NOTE: YOUR FLOWCHART MUST INCLUDE BRANCH POINTS TO CLEARLY SHOW HOW THE MIXTURE IS SUCCESSIVELY PARTITIONED TO REMOVE IMPURITIES AND ACHIEVE PURIFICATION.

3. How is the same protein now prepared on an industrial scale, or in recombinant form?
   - For what purpose is your protein produced by industry?
   - Present a detailed protocol for its purification as a flow chart.
   - Then explain and give a detailed description of the molecular events and chemistry of each step in the procedure.
   - Where possible, discuss column traces or stained gel images that indicate the purity of samples.

   NOTE: YOUR FLOWCHART MUST INCLUDE BRANCH POINTS TO CLEARLY SHOW HOW THE MIXTURE IS SUCCESSIVELY PARTITIONED.

4. Finish your written submission with a bibliography citing your primary sources, correctly formatted.
PET PROTEIN PROJECT (CONT.)

PART B Bioinformatics and Structural Analysis
(Assessed by seminar- see your laboratory schedule)

1. Find out what is known about the three-dimensional structure of this protein. Find the coordinate file in the Protein data Bank (www.rcsb.org/pdb/).
   - Describe the structural method(s) used to elucidate its structure.
   - Outline the features and spatial organization of the molecule.
   - Detail how these structural features relate to the function of your protein.

2. Use the BLAST tool available through ncbi (/blast.ncbi.nlm.nih.gov) to locate the closest set of sequence homologues to your selected Pet Protein sequence. Align these in CLUSTAL (/www.clustal.org/).
   - Edit your sequence alignment output into a figure that additionally indicates the location of secondary structure elements from the 3D structure of your Pet Protein.
   - Comment on the relationship between sequence variation in this protein family and 2D (and 3D) structure.

3. Construct a model (hint: wire, tape, kitchen utensils...) that shows the three-dimensional shape of this macromolecule. If your protein is particularly large, with two or more distinct domains, just select one of these for your model.

Some past “Pet Protein” models
- the lucidity of your communication (verbal and written) of the facts and molecular analysis
- the extent to which your model successfully shows the shape and form of your protein in three-dimensions
- correct bibliography layout (i.e. alphabetical or numbered listing), with reference to all primary source material (i.e. journal articles, not web-based information).

**Extension and Special Consideration Requests**

Extension requests for assessable material must be made to your lecturer with appropriate evidence to support the request. Each request will be treated on its merits.

The University is committed to equity and fairness in all aspects of its learning and teaching. The University recognises that there may be circumstances where a student is prevented by unavoidable disruption from performing in accordance with their ability. Details concerning the University’s policy on special consideration can be found at


For students who experience serious and unavoidable disruption, the downloadable form required to submit for a request for special consideration is www.registrar.mq.edu.au/Forms/APScons.pdf

**Expected Learning Outcomes**

The learning outcomes of this unit are to develop a molecular understanding of proteins and to become active practitioners in protein methods relevant to biomedical science and biotechnology:

- exhibit a fundamental understanding of technologies used today in protein separation and analysis
- comprehend the chemical and physical behaviour of proteins, both *in vivo* and *in vitro*
- develop a sound knowledge of protein structure and the spatial information encoded in a protein (or gene) sequence
- gain hands-on practical experience in protein analysis, and competency with contemporary web-based analysis tools
- extract information, interpret and analyse information from a variety of scientific sources concerning proteins
- develop presentation skills in both written and oral communication modes
- demonstrate a creative approach to demonstrating biomolecular forms and architectures

The grades you achieve at Macquarie University are descriptive rather than numeric. Your raw marks from assessments are combined into a weighted sum. The numerical value that you will be issued with (the Standardised Numerical Grade, SNG) is determined to match your descriptive grade.
• The SNG gives you an indication of how you have performed within the band for your descriptive grade.

• It is our professional responsibility as your teachers to assign you a descriptive grade that accurately reflects your performance in a unit. Our grading decisions are subject to scrutiny from our academic colleagues at the Department, Faculty and University Senate level.

The Grades range are defined in the Handbook as follows:

<table>
<thead>
<tr>
<th>Grade</th>
<th>SNG</th>
<th>Description</th>
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<tbody>
<tr>
<td>HD</td>
<td>85-100</td>
<td>Work of outstanding quality. This may be demonstrated in areas such as criticism, logical argument, interpretation of materials or use of methodology. This grade may also be awarded to recognise a high order of originality or creativity in student performance</td>
</tr>
<tr>
<td>D</td>
<td>75-84</td>
<td>Work of superior quality in the same areas of performance as above. This grade may also be awarded to recognise particular originality or creativity in student performance</td>
</tr>
<tr>
<td>Cr</td>
<td>65-74</td>
<td>Work of predominantly good quality, demonstrating a sound grasp of content together with efficient organisation, selectivity and use of techniques</td>
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<tr>
<td>P</td>
<td>50-64</td>
<td>Satisfactory achievement of unit objectives</td>
</tr>
<tr>
<td>F</td>
<td>0-49</td>
<td>Failure to achieve unit objectives</td>
</tr>
</tbody>
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**Graduate Capabilities Developed**

In addition to the discipline-based learning objectives, the activities of CBMS332 are organised so as to develop the following generic skills:

• *Critical, analytical and integrative thinking:* to be capable of reasoning, questioning and analyzing, and to integrate and synthesize learning and knowledge from a range of sources and environments; to be able to think independently and systemically in relation to scholarly activity, in the workplace, and in the world.

• *Problem-solving and research capability:* to be capable of researching; of analyzing, and interpreting and assessing data and information in various forms; to be able to relate their knowledge to complex situations at work or in the world, in order to diagnose and solve problems. We want them to have the confidence to take the initiative in doing so, within an awareness of their own limitations.

• *Creative and innovative:* to be capable of creative thinking and of creating knowledge. They will be imaginative and open to experience and capable of innovation at work and in the community.

• *Effective communication, both written and oral, including case study presentations to your peers:* to communicate and convey your views in forms effective with different audiences. We want our graduates to take with them the capability to read, listen, question, gather and evaluate information resources in a variety of formats, assess, write clearly, speak
effectively, and to use visual communication and communication technologies as appropriate.

**ACADEMIC HONESTY**
All members of Macquarie University (students and staff) are bound by the University’s Academic Honesty policy, outlined at [www.mq.edu.au/policy/docs/academic_honesty/policy.html](http://www.mq.edu.au/policy/docs/academic_honesty/policy.html)

This states that:
- all academic work claimed as original is the work of the author making the claim
- all academic collaborations are acknowledged
- academic work is not falsified in any way
- when the ideas of others are used, these ideas are acknowledged appropriately.

Any cases of plagiarism or cheating will be treated as breaches of this Policy, and may result in disciplinary action by the University.

The following explanations have been provided by Paul Haynes to assist you:

“**ALL WRITTEN WORK MUST BE ORIGINAL.** Students are sometimes tempted to use material which is not their own without due acknowledgment. This constitutes cheating, the penalty for which is failure of the course. Direct copying and/or submitting material from your own work done in previous years is also considered cheating.

**Collusion**
Collusion is the secret and fraudulent production of identical or superficially altered work submitted for assessment by two or more students. It is easily detected by the examiner and programs such as Turnitin.

**Plagiarism**
Plagiarism is the verbatim use of someone else's work, as if it were your own. This also constitutes cheating and will be dealt with accordingly. The "someone else" concerned may be an author, critic, lecturer, or even a fellow student. Plagiarism includes copying of material from practical books obtained from other students in the same or previous years. It also includes the direct copying of material from texts, references etc.

If you need to quote another piece of work, do it correctly. You must provide quotation marks around the quotation and this must be referenced. In other words, the only proper way to indicate that the words are not yours is to show clearly that they are a quotation. It is often desirable and may even be necessary to use other people's ideas but you must not pretend that they are your own. In such cases, your text should include a reference to the source of the idea. You may need to use a figure or table from another source. If so, the legend must indicate the source, with the appropriate reference. The list of referees should include acknowledgment of ideas, data and direct quotations from all sources.

**Collaboration**
Students are often required to work cooperatively in groups when performing experiments. Such collaboration is common and is an essential part of scientific endeavour. However, collaboration must always be acknowledged.

When you perform experimental work as part of a group, you must always acknowledge the collaboration by writing the names of the other members of the group at the start of your practical report. Collaboration in performing an experiment does not extend to writing a report on the experiment where that report is assessed for marks. Students must prepare their own report individually.”
**NEW FOR 2012**

Project work has increased in assessment weighting in 2012, with 10% assessment now being allocated to the presented Pet Protein model.

As well as updating of lecture material on the most topical subjects (e.g. bioinformatics, membrane proteins), proteins utilised in the laboratory practicals will be selected according to current research priorities in the Mabbutt Protein Structure group.

I wish you every enjoyment with the 2012 offering of **Protein Discovery & Analysis**, and look forward to meeting you in class.

A/Prof. Bridget Mabbutt  
Co-ordinator, CBMS332/832