Course Announcement for Spring 2022

Purification and Characterization of Protein and Protein Complexes

Oncology 673

Instructors – Yongna Xing and Richard Burgess

Description: This is a 2-credit lecture course with a short-term, intense format that meets from 1:20-3:15 PM on Tues., and Thurs. for eight weeks before and after Spring Recess of 2022. The classroom will be room 125 old McArdle Building. This course will be given in the spring of every other year, 2022, 2024, etc. It is primarily a lecture course consisting of 26 lectures with two lectures per day, two half-semester exams, some take-home problems, and a term paper on a topic relating to the course. For the term paper, the students are asked to read at least twelve articles and prepare an informative summary of the topic chosen with references.

Important new features include 1) protein preparation and assay development for drug discovery, and protein-chemical interactions; 2) Cryo-EM for determining high-resolution structure of protein complexes, particularly oligomeric macromolecular complexes, which has increasing advantage over X-ray crystallography and NMR; 3) development of antibody, nanobody, and Fab for proteins of interest.

Goals of the course are: 1) to introduce the most important and useful concepts of protein purification and handling, 2) to help students to develop an intuition about how to work with proteins-so that they can "think like a protein", 3) to introduce useful, modern tools for characterizing protein structure and function, and 4) to guide students to ongoing sources of information and resources. Students are also encouraged to discuss their research problems and seek solutions from the knowledge learned in the course and from discussion with other course participants and instructors.

Lecture topics include: Introduction-Protein purification overview; Properties of proteins/types of separation methods; Assays - following an enzyme through a purification; Protein characterization; Protein inactivation and stabilization/solution components; Purification strategy/starting materials/preparing cell/tissue extracts; Precipitation methods; Phase partitioning; Dialysis, desalting, concentration, and ultrafiltration; Preparative electrophoresis, chromatofocusing, isoelectric focusing, capillary electrophoresis; Purification of membrane proteins/glycoproteins; Column chromatography - theory and concepts; Sizing - gel filtration chromatography; Ion exchange, Affinity, Immunoaffinity, and DNA affinity chromatography; HPLC: Columns and hardware, theory, methods development, applications; Micropurification by eluting from SDS gels; Overproduction of cloned gene products; Purification and refolding of insoluble overproduced proteins; Engineering proteins for ease of purification and characterization; Recent advances in studying protein-protein interactions; isolation, assembly, and characterization of protein complexes; Proteomics/protein microarrays; Protein characterization methods; Post-translational modifications and characterization with mass spectrometry; Protein structure determination by X-ray crystallography, NMR, and Cryo-EM; Protein engineering and generation of antibody, nanobody and Fab for elucidating protein structure and function; Drug discovery, small molecules and chemical mimics in protein structure and function.

This course is usually taken for credit by about 10-20 graduate students, and occasionally by senior undergraduates. Auditors or those who wish to sit in on part or all of the lectures are welcome (usually as many as a dozen specialists, advanced graduate students, postdocs, and faculty sit in).

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Undergraduate and graduate students in this course will be graded on a separate curve, which is in compliance with the Higher Learning Commission's requirements for graduate courses with undergraduate participants.

Recommended Texts:

It is expected that students in this course have a basic understanding in chemistry, biochemistry, molecular biology, and cell biology. Should you think that your background knowledge in these topics are lacking, we recommend the following sources.

Biochemistry

1. Biochemistry (J. Berg), 5th Edition, W.H. Freeman and Co., New York, 2002.
2. Lehninger Principles of Biochemistry (With Extended Discussion of Oxygen-Binding Proteins) (A. Lehninger, M. Cox, and D. Nelson), 3rd Edition, Worth Publishers, Inc., New York, 2000.
3. G. Zubay, Biochemistry, 4th Edition, W.C Brown, 1999.

Molecular and Cell Biology

1. Molecular Biology of the Cell (B. Alberts), 4th Edition, Garland Publishers, New York, 2002.

In addition to the assigned articles, students are encouraged to view PubMed searches for articles that are related to their research topic interests.

Recommended Readings:

The following reading materials will provide additional details for some of the topics covered in the course, particularly for traditional purification procedures. The students can also use it for additional information for designing their own purification strategies or solving their practical problems. "For the love of enzymes" provides amazing anecdotes on early-day pioneering work on protein purification that led to the birth of biochemistry.

1. Richard J. Simpson, "Purifying Proteins for Proteomics – A Laboratory Manual", Cold Spring Harbor Press, 2004.
2. Methods in Enzymology, vol 463, Guide to Protein Purification 2nd edition Editors: R. Burgess and M. Deutscher. Elsevier, 2009.
3. Arthur Kornberg, "For the love of enzymes – the Odyssey of a Biochemist", Harvard University Press, 1989.

- **Course Description** (will be published in Course Guide), provided as on Page 1.
- **Explain the relationship and importance of the proposed course to existing programs or future programs.**

Proteins execute the majority of biological and physiological processes. Broad disciplines of biological and biomedical research require purification and characterization of proteins and protein complexes. The recent advances of bioinformatic and system biology tools also greatly facilitate proteomic studies in diverse biological research, which require competence skills and knowledge in preparation and handling of protein samples. Protein purification and characterization are also critical for the success of protein biochemistry and structural biology, as well as drug discovery that target specific proteins or protein complexes. In light of these contexts, this course will benefit, and has historically benefited, graduate students and senior undergraduate students from diverse programs on campus, including Biochemistry, Pharmacology, Biophysics, CMB (Cell and Molecular Biology), Cancer Biology,

Toxicology, CBMS (Comparative Biomedical Sciences graduate program), etc. The course addresses diverse practical aspects of protein purification, preparation, handling and characterization that would enhance the success of research projects and develop the right philosophy in experimental design such that the interpretation of the results does not disobey obvious natures of proteins and is consistent with the properties of proteins.

- **Specify which requirement(s) this course meets**, if any (e.g. satisfies third-level language, meets the major's capstone requirement, fulfills PhD minor requirement).

The course is not an essential requirement for obtaining a specific degree. It is often chosen by students from many different departments who anticipate the need for an understanding of protein biochemistry for their research. It is also often recommended by student's mentors or members of the student's thesis committee.

- **Address the relationship of this course to other UW-Madison courses, including possible duplication of content.**

This course is built on basic molecular biology, protein biochemistry and cell biology courses. The development of protein purification procedures considers and reinforces knowledge covered in these courses. For example, based on the biochemistry of specific target protein, the buffer components can be optimized specifically to maintain the function/activity of the protein. Charge distribution, size, and the interactome of the protein can be used for designing purification procedures. The cellular location and tissue distribution of the protein are also crucial for developing the procedures for purifying specific proteins from tissues or cells for proteomic studies. Besides dynamic application of basic knowledge, this course also discusses topics that are not yet covered by specific courses, such as preparation of protein complexes for electron microscopy (EM). It was increasingly realized that the art of biochemistry and protein purification "reign supreme" for high-resolution structural biology by x-ray crystallography and cryo-EM (Protein Science (2017), 26:69-81). On a more expanded term, the art of biochemistry and protein purification "reigns" the chance of success" of many disciplines of biomedical and biological research. Such philosophy is best revealed and stressed in this course, and reflected by Dr. Ying Ge's course on Mass Spectrometry (CRB 630_Proteomics for Biologists).

This course is built on a previous course on "Protein Purification", which was started by Dr. Burgess in 1986 and has been given every other year since then under the experimental course number, Oncology 675. Starting in 2009, it was taught jointly by Drs. Xing and Burgess. It is now being given by Dr. Xing with some contributions from Emeritus Professor Burgess. This should have been given a permanent course number long ago.

- **Learning goals.**

1) To introduce the most important and useful concepts of protein purification and handling.

2) To help students to develop an intuition about how to work with proteins- so that they can "think like a protein".

3) To introduce useful tools for characterizing protein structure and function.

4) To guide students to ongoing sources of information and resources on protein purification and characterization.

5) To encourage students to discuss their research problems and seek solutions from the knowledge learned in the course, and discussions with local experts.

- **Daily representative readings.**

Specific book chapters from Robert Scopes "Protein Purification: Principles and Practice", and Richard J. Simpson, "Purifying Proteins for Proteomics – A Laboratory Manual" will be provided as additional reading materials associated with each lectures. References for each advanced topic will be suggested for the students as well. For example, for preparing samples for EM studies, one of the references provided would be "PROTEIN SCIENCE 2017 VOL 26:69—81", which covers important general strategies and considerations for preparing EM samples to ensure successful high-resolution EM structure determination.

In addition to the assigned book chapters and references, the students are required to search the literature themselves, which will be elaborated in details in the section for "Graduate Course Work Vigor".

- **Graduate Course Work Vigor**

1) Requiring students to demonstrate advanced methodology/application of new skills and information to significant tasks or issues in the discipline. Students are required to identify a paper at the earlier stage of the course, for which they will elaborate how protein purification was accomplished in the paper, including how different purification approaches and strategies were applied and what considerations were made to ensure successful protein preparations. If new approaches were applied, were they derived by modification of currently available methods, and if not, what new principles were applied? This is the first paper assignment for the students to apply their new skills and knowledge to critically examine the information from literature and elaborates the key points outlined above. Another example for the students to demonstrate their application of new skills is to hand in questions and problems that they encountered in their real life research experience and discuss in class how these problems can be approached and solved based on the knowledge and skills that they learned in the class.
2) Requiring students to demonstrate an increased depth of knowledge beyond that normally attained by a typical bachelor degree holder in the discipline. Built on strategies they learned on protein purification, students are required to exercise comprehensive consideration of diverse basic knowledge they learned from cell biology, biochemistry and molecular biology during their undergraduate training to approach a protein purification problem and design the purification schemes that will most likely give rise to the optimum yield and purity and at the same time maintain the biological function and ideal properties suitable for investigating their structure and function using broad characterization methods. Students are also required to exercise comprehensive thinking on potential mistakes and pitfalls that might lead to loss of normal protein function or misinterpretation of the results from investigating protein function using improperly prepared protein samples and how these mistakes can be identified or avoided by tailored protein characterization methods.
3) Requiring students to demonstrate higher-order synthesis and analysis in the discipline. Students will be given advanced literature review assignment, in which they choose one of the topics in a provided list that is of particular relevance to their own research and search for recent literature that cover advanced progress in the selected topic. Based on the literature that they find, which can be optimized after discussion with the lecturer, the students will then write a term paper to elaborate how the new advances in the field could ease the previous specific difficult tasks in protein purification, the advantage of the new development over traditional approaches, how the recent advance allow us to solve new problems or ask new questions that could not be asked before and tackle previously untraceable areas of biological and biomedical research.

4) A strong emphasis on the literature of the discipline and/or active engagement with the latest research and scholarly activity of the discipline. In addition to the assignment of advanced literature review as detailed above, by discussing in class the problems they encountered in their real life research, students will be exposed to diverse latest research questions that can be potentially solved by optimizing protein purification procedures or monitored by well-considered characterization methods to entail reliable and reproducible results/conclusions.

LECTURE SCHEDULE Spring 2022

Old McArdle building Room 125, 1:20-3:15pm, Tuesday and Thursday

Lect#	Lecturer	Date	Topic
Protein Purification – Classical Methods			
1	Y	Feb 8	Introduction to course- Protein "personality", cofactors, partners (thermophile), protein breathing
2	D	Feb 8	Overview of protein purification, properties of proteins for separation, stabilizing proteins
3	D	Feb 10	Assays - following an enzyme through purification Protein purity, quantitation, purification strategy/starting materials/preparing cell extracts
4	D	Feb 10	Column chromatography – HPLC, gel filtration and ion exchange chromatography
			(Choose topic for term paper on modern aspects of protein purification and characterization)
5	D	Feb 15	Affinity and DNA affinity chromatography
6	D	Feb 15	Making antibodies, immuno-affinity chromatography, and gentle immunoaffinity for complexes
7	D	Feb 17	Precipitation methods, Dialysis, desalting, concentration, ultrafiltration.
8	D	Feb 17	Electrophoresis and acquiring of pure materials from electrophoresis (native gel, blue dye native)
			(Term paper bibliographies due)
Protein Purification – Recombinant Approaches			
9	D	Feb 22	Overproduction of cloned genes and tags for purification and improvement of protein behavior
10	D	Feb 22	Purification of insoluble overproduced proteins, Questions/discussion
11	Y	Feb 24	Different expression hosts, insect, and mammalian cells
12	Y	Feb 24	Protein engineering
13	Y	March 1	Purification of membrane proteins/glycoproteins, lectins
q/d	Y	March 1	Questions/discussions
ex		March 8	**EXAM #1 (for lectures Feb 19 to March 2)**
Modern Approaches for Protein Characterization and Use			
14	Y	March 22	Overview of protein characterization methods
15	Y	March 22	Post-translation modifications, characterization with mass spectroscopy (MS) and role in regulation
16	Y	March 24	"Old dog, new use", protein purification from tissue and natural sources for MS
17	Y	March 24	Isolating and studying protein complexes, interactomes and characterization with MS
			(First draft of term paper due)
18	D	March 29	Studying protein-protein interactions
19	D	March 29	Weak interactions (iFAST), concept of avidity
20	J	March 31	Protein purification and pre-characterization for NMR (John Markley)
21	Y	March 31	Proteomics/protein microarrays, characterization of small linear interaction motifs (SLiMs)
22	Y	April 5	Overview of x-ray crystallography and cryo-EM
23	Y	April 5	Art of protein biochemistry and protein engineering for cryo-EM and x-ray crystallography
24	Y	April 7	Phage selection (Fab, Dab, nanobody) for identifying antibodies for single particle cryo-EM, x-ray crystallography, and development of protein therapeutics
25	Y	April 7	Purification of mega-dalton protein assemblies, ultracentrifugation, PEG precipitation
26	Y	April 12	Protein engineering and protein purification for drug discovery, and protein therapeutics (FRET, BRET, LRET sensors, Nanoluc) (REMOVE host proteins, HCPs, endotoxin, host DNA, defined glycosylation, GMP lab, MS, glycoform-specific antibodies, lectins)
27	Y	April 12	Small molecules and chemical mimics for protein structure and function, in silico screen
q/d	Y	April 14	Questions/discussion
ex		April 14	**EXAM #2 (for lectures April 19-May 3)**
		April 21	**(Final draft of term paper due)**

Y=Yongna, D=Dick, J=John Markley, q/d=Questions/discussion, ex=exam