Course BIOL GU4551

A Structural View of Biology

Spring, 2024, Tues and Thurs 10:10-11:25am, 1000 Fairchild

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Course description

Prerequisites: Intro Bio, organic chemistry.

The course covers a general introduction to the theory and experimental techniques of structural biology (protein expression and purification, protein crystallography, cryo-electron microscopy, nuclear magnetic resonance) and then how to use the structural information to understand biochemical and biological processes. The first part of the course will cover the general introduction to structural biology. The second part of the course will involve discussions and explorations of various structures, led by the instructor but with substantial participation from the students, to understand the molecular mechanisms of selected biochemical and biological processes. In the final part of the course, each student will select and lead discussions on a primary structural biology paper. The overall goal of the course is to increase the understanding of how protein structures are determined, what protein structures look like, and how to use the structures to understand biology.

Part I. Theory and experimental techniques of structural biology

1. Overview. Protein expression, purification, and crystallization.

2. Crystals, unit cell, point group and space group symmetry (*What is a crystal*?)

3. X-rays, diffraction of X-rays, structure factors, phase problem, electron density map (*Why is a crystal needed*?)

4. Phasing methods — multiple isomorphous replacement (MIR), multiple-wavelength anomalous diffraction (MAD), single-wavelength anomalous diffraction (SAD), molecular replacement (MR), interpretation of electron density map, crystal structure refinement (*How is a crystal used*?)

The discussions will be linked to an example of solving the crystal structure of lysozyme by sulfur SAD, using diffraction data collected on the instrument in the Department. This will include crystallization, data collection, data processing, locating sulfur positions, phasing, automatic model building, manual examination of the model, structure refinement.

5. Introduction to NMR (special guest lecture by Art Palmer, Feb. 16)

6. Introduction to electron microscopy (special guest lecture by Joachim Frank, March 7)

Part II. A structural view of biology (instructor-led discussions)

This part of the course will explore the structural features and the mechanisms of selected proteins and protein families, as well as the impact of AlphaFold on the field. The discussions will be led by the instructor, but with substantial participation from the students. The structures will be examined in real time in the class, with the programs PyMOL and Coot, and the structural basis of the biochemical mechanisms will be explained.

The discussions will start with a general introduction to protein structures, and then cover a subset of the following topics (depending on time availability): proteases, nucleases, oxidoreductases, protein kinases and phosphatases, hemoglobin and myoglobin (allostery), channels, transporters, ATP synthase, signal transduction, virus-host interactions, ribosome, DNA polymerase, RNA polymerase, glycolysis, TCA cycle, and others, to be selected based on students' interest and coordination with students' presentations in part III.

The materials to be covered in the discussions on proteases are given in more detail here as an example:

Overall structure of chymotrypsin-like serine proteases
The catalytic machinery and reaction mechanism
The molecular basis of substrate selectivity
Regulation of protease activity
Other proteases
Protease inhibitors as drugs to treat human diseases

Part III. A structural view of biology (student-led discussions)

The exploration of protein structure and mechanism will continue in the third part of the course, in discussions/presentations led by each of the students. The student will choose a topic of his/her interest, and present primarily a paper in the literature.

Grading
30%: results from midterm
35%: quality of the discussion/presentation
35%: Write a short report on a paper presented by another student, in the format of a *Nature News&Views* article.

Academic integrity
As students in this course, you are responsible for the full citations of others' ideas in your research papers and projects; you must be scrupulously honest when taking your examinations; you must always submit your own work and not that of another student, scholar, or internet agent. Any breach of this intellectual responsibility is a breach of faith with the rest of our academic community, and will be dealt with appropriately.

Student with disabilities
Appropriate accommodations will be provided to students in this course with disabilities, in consultation with Disabilities Services at Columbia University.

Weekly syllabus

Week 1: Introduction to the course; Protein Expression, purification and crystallization
Week 2: Introduction to symmetry; Introduction to X-rays
Week 3: X-ray diffraction; data collection
Week 4: Crystal structure determination and refinement
Week 5: Structure determination by cryo-EM and NMR
Week 6: Introduction to protein structures; Mid-term
Week 7: Proteases and nucleases
Week 8: Protein kinases and protein phosphatases
Week 9: Spring recess
Week 10: Metabolic enzymes
Week 11: GPCRs and ion channels
Week 12: Viruses; student nominations
Week 13: Student presentations
Week 14: Student presentations

Reading materials

There is no formal textbook for this course. The reading materials will include-

Powerpoint slides for the lectures
Primary research paper(s) (for part II)
PDB entry/entries to examine (for part II)

These materials will be posted to Courseworks one to two weeks before the lecture.

Detailed syllabus:

What is a crystal?

Symmetry (point group and space group)
Crystals
Unit cells
Convolution
The 7 crystal systems
Fractional coordinates, PDB coordinates
Asymmetric Unit
Solvent Content of Protein Crystals (V_m)
Non-crystallographic Symmetry

Why is a crystal needed?

X-rays
  an electromagnetic wave
  amplitude
  phase
Generation of X-rays
  home source (rotating anode)
  synchrotron radiation source
Diffraction of X-rays
  light microscope analogy
  optical interference analogy
Diffraction from a Crystal
  only electrons diffract X-rays
  regular pattern
  reciprocal space
  Bragg equation
  resolution
  reflections
  atomic structure is encoded by the amplitude (and phase)
  structure factors
X-ray Diffraction Data Collection
  X-ray detectors
  Ewald construction
  Laue crystallography
Phase Problem
Electron Density

How is a crystal used?

Phasing Methods
Multiple isomorphous replacement (MIR)
  Heavy atom derivative
  Patterson function
Multiwavelength anomalous diffraction (MAD) method
  Absorption of X-rays
  Breakdown of Friedel's Law
Molecular replacement (MR) method
  Homologous structures
  rotation function
  translation function
Electron density map
  mini map
  Solvent content of a crystal (V_m)
  Solvent flattening (phase improvement)
Model building
Structure refinement
  R factor
  free R factor
  geometric restraint
  temperature factor

List of papers for presentation in class

* You are welcome to choose a paper yourself, but please discuss the paper with me beforehand.
* To sign up for a paper and presentation slot, go to the Pages tab on Courseworks and type in your name and paper number. Please make sure that the paper has not been selected by another student already.

1. Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah ST, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK, Kobilka BK. Nature. 2011 Jul 19;477(7366):549-55. Crystal structure of the β2 adrenergic receptor-Gs protein complex.

2. Kim HM, Park BS, Kim JI, Kim SE, Lee J, Oh SC, Enkhbayar P, Matsushima N, Lee H, Yoo OJ, Lee JO. Cell. 2007 Sep 7;130(5):906-17. Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran.

3. Sreelatha A, Yee SS, Lopez VA, Park BC, Kinch LN, Pilch S, Servage KA, Zhang J, Jiou J, Karasiewicz-Urbańska M, Łobocka M, Grishin NV, Orth K, Kucharczyk R, Pawłowski K, Tomchick DR, Tagliabracci VS. Cell. 2018 Oct 18;175(3):809-821.e19. Protein AMPylation by an Evolutionarily Conserved Pseudokinase.

4. Wang Y, Juranek S, Li H, Sheng G, Wardle GS, Tuschl T, Patel DJ. Nature. 2009 Oct 8;461(7265):754-61. Nucleation, propagation and cleavage of target RNAs in Ago silencing complexes.

5. Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM. Nature 2002 Jan 31;415(6871):545-9. Structural identification of a bacterial quorum-sensing signal containing boron.

6. Yang W, Hendrickson WA, Crouch RJ, Satow Y. Science 1990 Sep 21;249(4975):1398-405. Structure of ribonuclease H phased at 2 A resolution by MAD analysis of the selenomethionyl protein.

7. Worrall LJ, Hong C, Vuckovic M, Deng W, Bergeron JR, Majewski DD, Huang RK, Spreter T, Finlay BB, Yu Z, Strynadka NC. Nature 2016 Dec 22/29; 540:597-601. Near-atomic-resolution cryo-EM analysis of the Salmonella T3S injectisome basal body.

8. Dong C, Beis K, Nesper J, Brunkan-Lamontagne AL, Clarke BR, Whitfield C, Naismith JH. Nature. 2006 Nov 9;444(7116):226-9. Wza the translocon for E. coli capsular polysaccharides defines a new class of membrane protein.

9. Tanaka H, Kato K, Yamashita E, Sumizawa T, Zhou Y, Yao M, Iwasaki K, Yoshimura M, Tsukihara T. Science. 2009 Jan 16;323(5912):384-8. The structure of rat liver vault at 3.5 angstrom resolution.

10. Deng D, Yan C, Pan X, Mahfouz M, Wang J, Zhu JK, Shi Y, Yan N. Science. 2012 Feb 10;335(6069):720-3. Structural basis for sequence-specific recognition of DNA by TAL effectors.

11. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Nature 1997 Sep 18;389(6648):251-60. Crystal structure of the nucleosome core particle at 2.8 A resolution.

12. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Nature 1998 Jun 18;393(6686):648-59. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody.

13. Zeqiraj E, Filippi BM, Deak M, Alessi DR, van Aalten DM. Science. 2009 Dec 18;326(5960):1707-11. Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation.

14. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liau LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, Su SM. Nature. 2009 Dec 10;462(7274):739-44. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate.

15. Schubert AF, Gladkova C, Pardon E, Wagstaff JL, Freund SMV, Steyaert J, Maslen SL, Komander D. Nature. 2017 Dec 7;552(7683):51-56. Structure of PINK1 in complex with its substrate ubiquitin.

16. Nishimura N, Hitomi K, Arvai AS, Rambo RP, Hitomi C, Cutler SR, Schroeder JI, Getzoff ED. Science. 2009 Dec 4;326(5958):1373-9. Structural mechanism of abscisic acid binding and signaling by dimeric PYR1.
(There are 3 related papers in *Nature* on this structure in the Dec. 3, 2009 issue)

17. Chandran V, Fronzes R, Duquerroy S, Cronin N, Navaza J, Waksman G. Nature. 2009 Dec 24;462(7276):1011-5. Structure of the outer membrane complex of a type IV secretion system.

18. Sun T, Lin FH, Campbell RL, Allingham JS, Davies PL. Science. 2014 Feb 14;343(6172):795-8. An antifreeze protein folds with an interior network of more than 400 semi-clathrate waters.

19. Huang CS, Ge P, Zhou ZH, Tong L. Nature. 2011 Dec 11;481(7380):219-23. An unanticipated architecture of the 750-kDa $\alpha_6\beta_6$ holoenzyme of 3-methylcrotonyl-CoA carboxylase.

20. Schwefel D, Groom HC, Boucherit VC, Christodoulou E, Walker PA, Stoye JP, Bishop KN, Taylor IA. Nature. 2014 Jan 9;505(7482):234-8. Structural basis of lentiviral subversion of a cellular protein degradation pathway.

21. Yao R, Ming Z, Yan L, Li S, Wang F, Ma S, Yu C, Yang M, Chen L, Chen L, Li Y, Yan C, Miao D, Sun Z, Yan J, Sun Y, Wang L, Chu J, Fan S, He W, Deng H, Nan F, Li J, Rao Z, Lou Z, Xie D. Nature. 2016 Aug 25;536(7617):469-73. DWARF14 is a non-canonical hormone receptor for strigolactone.

22. Lazarus MB, Jiang J, Kapuria V, Bhuiyan T, Janetzko J, Zandberg WF, Vocadlo DJ, Herr W, Walker S. Science. 2013 Dec 6;342(6163):1235-9. HCF-1 is cleaved in the active site of O-GlcNAc transferase.

23. Manolaridis I, Kulkarni K, Dodd RB, Ogasawara S, Zhang Z, Bineva G, O'Reilly N, Hanrahan SJ, Thompson AJ, Cronin N, Iwata S, Barford D. Nature. 2013 Dec 12;504(7479):301-5. Mechanism of farnesylated CAAX protein processing by the intramembrane protease Rce1.

24. Lee YY, Lee H, Kim H, Kim VN, Roh SH. Nature. 2023 Mar;615(7951):331-338. Structure of the human DICER-pre-miRNA complex in a dicing state.

Questions to be answered at presentations –

What is the protein?
Why is structural information on it interesting (what is currently known structurally)?
How was the protein sample produced?
How was the structure determined?
What does the structure look like (fold, domains, interfaces, etc)?
(examine the structure and make a few figures/movies yourself using PyMOL and/or Coot)
What does the structure tell us about the biology?

(not a full report of the entire paper, be selective of the structural details)