Protein Structure and Computational Biology, 27617

Good morning and welcome!
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The Course – 27617

- Protein Structure and Computational Biology
  - 6th season
  - Master’s level
  - 13 weeks
  - 5 ECTS
Time & Location

- From 8.00-12.00.
- In building 208, room 062 (basement under CBS).
Who’s the Teacher?

- Thomas Holberg Blicher

- Background in protein crystallography and immunology.

- Associate professor in bioinformatics
  - Center for Biological Sequence Analysis
    • DTU, Department of Systems Biology
  - Novo Nordisk Foundation Center for Protein Research
    • University of Copenhagen, Faculty of Health Sciences

- Areas of interest:
  • Protein structure and function relationships
  • Immunology
More Teachers

- During exercises
  - Thomas N. Pedersen

- Guest lectures
  - Birte Svensson (to be confirmed)
  - Others…
Course Topics

- Protein structure analysis
  - Testable, structure-driven hypotheses
- Protein modelling
  - Modelling and model validation (quality control)
- Structure visualisation
  - PyMOL
  - Aesthetics & rules of thumb
- Presentation of structure-based hypotheses and observations
  - Good practice
  - Group work
Learning Objectives

- Reproduce the **20 natural amino acids** and account for their structural and chemical properties.
- Describe the basic **protein structure elements** and their properties.
- Describe the necessary steps for determination of three-dimensional protein structures using **NMR spectroscopy** and **x-ray crystallography**, and account for essential strengths and weaknesses of the two methods.
- Navigate the **PDB** structure database and the corresponding file types.
- Operate the basic functions of the program **PyMOL** for visualisation of protein structure.
- Predict local structural properties of proteins based on their sequences using common, **web-based prediction tools**.
- Evaluate the **quality** of experimental protein structures based on general **validation** criteria.
- Construct a **homology model** of a protein of unknown structure given the sequence and evaluate its quality.
- Analyse and discuss the **structural context** of annotated **protein features** such as epitopes, post-translational modifications and active sites.
- Predict the effect of point mutations on the interaction with ligands, conformational changes and **other structural properties**.
Learning Objectives – I

- Given the sequence of a protein with unknown structure, you should be able to create a model of the structure of the protein and evaluate the quality of the model.

MVKQIESKTAFAQEALDAAGDKLVVVFSA
TWCQGCKMKPFFHSLSEKYVNFLEVD
VDDCQDVALSECEVKCTPTFQFFKKGQKVGEFSGANKEKLEATINELV
Learning Objectives – II

- You should also be able to discuss the structural context of annotated features of the protein such as epitopes, post-translational modifications and active site.
Learning Objectives – III

- Based on the model of the structure of the protein, you should be able to discuss the effect of point mutations on interactions with ligands (small molecules or other proteins), conformational changes or other features related to protein structure.

*Try changing Asp1 to Ala and predict what happens…*
Core Elements

- Elements of protein structure
- Structure databases
- Structural genomics
- Fold recognition and homology modeling
- Structure validation
- Structure analysis
- Protein structure visualization
Case Study: Influenza Virus Proteins

- Influenza A virus
- Bird Flu H5N1
- Hemagglutinin HA (e.g. H5)
  - Undergoes conformational change
- Neuraminidase NA (e.g. N1)
  - Drug target (tamiflu)
Course Plan for 13 Weeks

1. Introduction to the course and to group work. Introduction to influenza.
2. Levels of protein structure, primary – quaternary structure
   Exercise: building a model of a protein structure by hand
3. Protein structure visualization: PDB & PyMol Tutorial
4. Experimental protein structure determination in the genomics era.
5. Structure validation: the closest homologues and pathological cases
6. Prediction of local structural features – secondary structure, accessibility, disorder etc.
7. Homology modeling 1: homology modeling and structural genomics
8. Homology modeling 2: model refinement, force fields, difficult cases, \textit{ab initio}
9. Protein structure analysis 1: case study – analyzing the active site
10. Protein structure analysis 2: post translational modifications, epitopes
11. Protein engineering – how to modify the properties of a protein
12. Group work on poster and oral presentations
13. Poster session
Exam Project

- Will be initiated two weeks before the end of the course.

- Choose between predefined projects
  - Topics to be announced.

- Present as poster on the last day of the course. Poster session.

- Oral exam.
Assessment

- **Pre-exam:**
  - Mini-quizzes (self-assessment).
  - 2 hand-in exercises (must be approved before exam).
  - Poster presentations by groups.
  - Questions for another group.

- **Exam:**
  - *Individual* oral examination.
  - Based on group project but may enter any topic covered during the course.
Reading Material

- **Textbook:**

- **Supplementary reading (optional):**

- **Notes & copies (to be found online on CampusNet)**
  - Exercise instructions
  - PyMOL manual
  - Articles
Other Material

- **Presentations**
  - Will be available on CampusNet as soon as they are ready.
  - Usually after the lectures, in the afternoon.

- **Reading assignments**
  - Will be posted on CampusNet a week ahead of time.
  - Updated reading assignments with supplementary information will be available on the course homepage.

- **Course homepage**
Feedback Persons

- Two persons are asked to give feedback after each teaching session (volunteers) – preferably new feedback persons for each session.

- What worked and what could be improved.

- Takes 5 minutes of your time.
Exam Dates

- Within the first two weeks after the last day of the course.
  - May 9th and 16th (Wednesdays)

- Comments?
Break

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You’ve Got Flu!

A brief introduction to viruses in general and influenza virus in particular.
Contents

- The basics of the virus biology.
- Special case: Influenza virus.
The Cell
Viruses

- Obligate intracellular pathogens
  - No protein-synthesising machinery.
- RNA or DNA genome enclosed in a coat of protein (capsid).
- Some viruses are covered in a lipid membrane (envelope) with further viral proteins.
- Further proteins can be contained in the virus depending on strategy of replication.
Minimal Virus

- Must have
  - Genome
  - Capsid
  - Entry-facilitating protein (could be capsid)

- Most viruses encode 5-10 proteins
Virus Gallery

Human papilloma virus

Dengue virus

Hepatitis B virus

Human rhinovirus

SARS virus
Virus Entry

- Human immunodeficiency virus (HIV)
Influenza Virus

- Neuraminidase
- Hemagglutinin
- RNA
- M₂ protein (only on type A)
Changes in Influenza Virus

- Mutations
  - Seasonal flu
  - Changes in virus in "unnatural hosts"

- "Gene exchange" = reassorting.
  - Pandemics
The Two Mechanisms whereby Pandemic Influenza Originates.

In 1918, an H1N1 virus closely related to avian viruses adapted to replicate efficiently in humans. In 1957 and in 1968, reassortment events led to new viruses that resulted in pandemic influenza. The 1957 influenza virus (Asian influenza, an H2N2 virus) acquired three genetic segments from an avian species (a hemagglutinin, a neuraminidase, and a polymerase gene, PB1), and the 1968 influenza virus (Hong Kong influenza, an H3N2 virus) acquired two genetic segments from an avian species (hemagglutinin and PB1). Future pandemic strains could arise through either mechanism.
H5N1 – Bird Flu

Areas with confirmed human cases of H5N1 avian influenza since 2003 *

- Turkey: Cases: 12, Deaths: 4
- Azerbaijan: Cases: 8, Deaths: 5
- Iraq: Cases: 3, Deaths: 2
- Egypt: Cases: 19, Deaths: 11
- Djibouti: Cases: 1, Death: 0
- Thailand: Cases: 25, Deaths: 17
- Indonesia: Cases: 80, Deaths: 62
- Cambodia: Cases: 6, Deaths: 6
- Vietnam: Cases: 93, Deaths: 42
- China: Cases: 22, Deaths: 14

* All dates refer to onset of illness

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Data Source: WHO / Map Production: Public Health Mapping and GIS Communicable Diseases (CDS) World Health Organization

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# Influenza Virus Variants

<table>
<thead>
<tr>
<th>Year and Country</th>
<th>Virus</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995, United Kingdom</td>
<td>H7N7</td>
<td>A/Eng/268/95</td>
</tr>
<tr>
<td>1997, Hong Kong</td>
<td>H5N1</td>
<td>A/HK/156/97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/HK/148/97</td>
</tr>
<tr>
<td>1999, Hong Kong</td>
<td>H9N2</td>
<td>A/HK/1073/99</td>
</tr>
<tr>
<td>2003, Hong Kong</td>
<td>H5N1</td>
<td>A/HK/213/03</td>
</tr>
<tr>
<td>2003, the Netherlands</td>
<td>H7N7</td>
<td>A/Neth/33/03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/Neth/219/03</td>
</tr>
<tr>
<td>2003, Hong Kong</td>
<td>H9N2</td>
<td>A/HK/2018/03</td>
</tr>
<tr>
<td>2004, Vietnam</td>
<td>H5N1</td>
<td>A/VN/1203/04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/VN/1194/04</td>
</tr>
<tr>
<td>2004, Thailand</td>
<td>H5N1</td>
<td>A/Thai/16/04</td>
</tr>
<tr>
<td>2004, Canada</td>
<td>H7N3</td>
<td>NA</td>
</tr>
<tr>
<td>2004, Egypt</td>
<td>H10N7</td>
<td>NA</td>
</tr>
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* H5 and H9 viruses have generally been associated with respiratory disease, whereas H7 has generally been associated with conjunctivitis. NA denotes not available.
It’s Not Over…

**Fugleinfluenzaen er tilbage**

Døde fugle, der er skyldet i land i Hongkong, kan være tegn på et alvorligt udbrud af fugleinfluenza i Kina, siger ekspert.

**China records its fourth bird flu death this year**

(CNN) — A woman from China's far northwest has died from bird flu, health authorities said Saturday, making her the country's fourth fatality from the deadly avian influenza so far this year.

The 31-year-old woman, who was identified by her first name Zhang, fell ill on January 10. She died Friday morning, according to the health department in Xinjiang Uygur Autonomous Region.

Tests were positive for H5N1, the department said in a statement posted to the Health Ministry's website.

The official Xinhua news agency said a 16-year-old boy, who had fallen ill on January 8, died in the city of Huaxiu in central Henan province.

The country's Ministry of Health had then moved to allay fears over the outbreak, saying that although further human bird flu cases were possible throughout China, there wouldn't be a large-scale outbreak, Xinhua and CCTV said.

One week ago, a 27-year-old woman from eastern China died of bird flu, the Ministry of Health said in a statement.
H1N1

Easily spread
Rarely fatal

H5N1

Spreads slowly
Often fatal
How do We Fight Viruses?
Definitions

- **Antigen**: Substance capable of eliciting an immune response.

- **Epitope**: Small antigenic part of a molecule
  - Most often protein
  - **Linear epitope**: An antigenic peptide.
  - **Discontinuous epitope**: Surface patch on protein consisting of multiple linear epitopes.
Quote of the Day

- Don C. Wiley (1944-2001):
  
  “I’m sorry, but I just don’t understand anything in biology unless I know what it looks like.”

- Has solved the structures of many immunologically important proteins:
  
  - Class I and II Major Histocompatibility Complex proteins.
  - Has studied viruses including HIV, Herpes and Influenza virus and many more.
  - Special focus on molecules involved in viral entry.
How to See a Virus?

- Typical virus size: 100-200 nm
- Typical size of bacterium: 1 \( \mu \)m (\textit{E. coli} diameter)
- Typical size of eukaryotic cell 10-100 \( \mu \)m

Direct molecular recognition!
The Immune System

- **Purpose:** Elimination of invading microorganisms. (Elimination and control of tumours?)

- Innate and adaptive immune system.
  - Innate: fast-acting (immediately), limited specificity, NO memory.
  - Adaptive: slow-acting (3-7 days), very specific, memory (=immunity).

- Adaptive immunity is mediated by B and T cells.

- **T** cells recognize MHC-molecules containing peptides derived from foreign/mutated proteins.

- **B** cells recognize antigens directly, and subsequently start producing antibodies.
Antigen Processing - Intracellular

- Protein
- Proteasome
- Peptides
- TAP
- ER
- MHC class I
- T<sub>C</sub>
- T<sub>CD8+</sub>
- TCR
- MHC-I
- Elimination
- All cells
Cytotoxic T Cells in Action

Cytotoxic T cell

Target cell
B Cells

- Produced in the bone marrow
- Maturation and selection in bone marrow
- Activation
  - $\text{CD4}^+\,\text{helper T cells (T}_H^2\text{)}$
  - Self-activation through polyvalent antigens
- Functions
  - Antibody production
    - Opsonisation
    - Inactivation

Antigen binding
(variable)

Antibody receptor binding
(constant)
Antigen Processing - Extracellular
Viruses are intracellular pathogens.
  – High host specificity.

The immune system can recognise (fragments of) pathogens
  – in MHC-I molecules when found inside cells.
  – in MHC-II molecules or using antibodies when found outside cells.

The part of an antigen (part of pathogen) recognised by the immune system is known as an epitope.
Break

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"Coming together is a beginning, keeping together is progress, working together is success".

Henry Ford
Group Work

- Randomly selected groups.
  - Intended for exercises as well as the final project, but not fixed and final.

- Justification:
  - The ability to work in teams is considered a core competence of engineers.
  - These are often not self-elected.

- Try them out for the first exercise
  - Next week, manual protein building.
  - Then decide whether to change groups.
Start-Up of Group Work

- Find the rest of your group
- Establish a group name and a logo
- Discuss roles within your group based on the profiles of the individual members
- Agree on a written set of rules for:
  - Meetings
  - Conflict resolution
  - Decision making
  - Communication

Feed in potential conflicts
Feed in any good/bad group experiences
During the whole development of the space program, Larry had not contributed with anything whatsoever, and he knew that this was his absolutely last chance.

Stop! Stop! Don’t just push the button! Ehhh… Count down first! Yes! Countdown! That’s a good idea! Otherwise it will never make it to the Moon! Never!
Deliverables

- Present the members of your group and its written set of rules.
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The test will **NOT** influence your grade for this course – its purpose is to help us set the level for the teaching.

In fact, we **don’t expect** you to be able to get it **100% correct** - you may still do very well in this course with an initial 0% in this test, so don’t feel bad if you score poorly.