Protein design and evolution - focus on amylolytic enzymes

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Course description:

The entire course is principally anchored in protein bioinformatics and divided into two parts: (i) a general information (6 hours of lectures) and (ii) practical examples (9 hours of lectures). Basic molecular-biology sequence and structure databases will be described, such as GenBank and UniProt as well as Protein Data Bank, along with presenting how to retrieve the data from those databases and how to work with them (to align the sequences, to calculate an evolutionary tree, to display a protein structure and to superimpose protein structures).

A special method, the so-called Hydrophobic Cluster Analysis (HCA), for comparison of highly diverged protein sequences, will be introduced together with the BLAST (Basic Local Alignment Search Tool) for, e.g., assigning a potential function for a hypothetical protein or genome mining. Proteins as basic building blocks of living matter will be presented in a detail with emphasis on their structure, stability, evolution and design. Practical examples will be focused on amylolytic enzymes, especially the alpha-amylase that is in the sequencebased classification of all Carbohydrate-Active enZymes (http://www.cazy.org/) classified in the families of glycoside hydrolases (GH) GH13, GH57 and GH119. Various aspects of relationships between the sequence and specificity of different, but related amylolytic enzymes covering hydrolases, transferases and isomerases, will be given together with evolutionary scenarios for particular examples with regard to enzyme specificity as well as taxonomy. Starch-binding domains as distinct modules contributing to the ability of an amylolytic enzyme to bind and degrade the raw starch will also be shown with their mutual evolutionary relatedness.

Syllabus of the lecture subjects (enlisted):

1. Molecular-biology sequence and structure databases – GenBank (EMBL-ENA and DDBJ), UniProt (SwissProt and TrEMBL) and Protein Data Bank; genome sequencing projects; amino acid sequence alignment and evolutionary trees (Clustal; consensual length, sequence identity and similarity).
2. Sophisticated comparison of primary structures of proteins: the Hydrophobic Cluster Analysis method (examples) and the BLAST (Basic Local Alignment Search Tool).
3. Proteins – characterization, their roles; 20 amino acids in proteins; peptide bond and planarity of the peptide unit; dihedral angles, rotation at Calpha-atoms.
4. Structure of proteins – primary, secondary, tertiary and quarternary; supersecondary structure and protein domains; alpha-helix and beta-sheet; loops and turns; Ramachandran diagram; protein structure motifs, protein interactions; classification of proteins according to the content of secondary structure.

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5. Prediction of protein structure – overview, significance; homologous proteins; algorithms for protein secondary structure predictions; approaches and methods for protein tertiary structure predictions; web-servers; molecular evolution; sequence and structure-based alignments; background noise; divergent and convergent protein evolution; horizontal gene transfer.
6. Stability and stabilization of proteins; approaches to obtaining stable enzymes; immobilization, chemical modification, protein engineering; protein denaturation; factors of stability of proteins; thermozymes and coldactive enzymes; rigidity and flexibility of protein structure; hydrophobic interactions; hydrogen bonds; salt bridges; disulphide bridges; stabilization of alpha-helices and loops; covalent destruction.
7. CAZy classification system of carbohydrate-active enzymes; glycoside hydrolases; families of alphaamylase, beta-amylase and glucoamylase.
8. Alpha-amylase family GH13 and clan GH-H – more than 20 thousand sequences and 30 different enzyme specificities.
9. Bacterial, archaeal, fungal, plant and animal alphaamylases as GH13 subfamilies – specific sequence structural features and evolution.
10. Oligo-1,6-glucosidase and neopullulanase subfamilies and intermediary alpha-amylases from the family GH13.
11. Family GH77 4-alpha-glucanotransferases and unique amylomaltases from borreliae.
12. Alpha-amylase from Bacillus aquimaris as a representative of a novel GH13 subfamily with specific sequence features and ability to degrade raw starch.
13. Degradation of raw starch using starch-binding domains classified into more than 10 various carbohydrate-binding module (CBM) families and special examples of animal and plant glucan phosphatases laforin and SEX4 protein, respectively.
14. Sequence fingerprints of the individual specificities from the alpha-amylase family GH57 and relatedness to the family GH119.
15. Evolutionary ancestry shared by the non-enzymatic heavy-chains of heteromeric amino acid transporters (rBAT and 4F2hc) and alpha-glucosidases from the alphaamylase family GH13.

TERMINY ZAJĘĆ			
Data	**Dzień tyg.**	**Godz.**	**Sala**
18 maj 2015	poniedziałek	9.00-12.00	Luwr (Chemia A)
19 maj 2015	wtorek	9.00-12.00	Luwr (Chemia A)
20 maj 2015	środa	9.00-12.00	Luwr (Chemia A)
21 maj 2015	czwartek	9.00-12.00	Luwr (Chemia A)
22 maj 2015	piątek	9.00-12.00	Luwr (Chemia A)