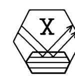

Wednesday, June 28, Session VII

L21

GATHERING RELEVANT INFORMATION FROM WWW-RESOURCES

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The management, organization and validation of biological information is known as Bioinformatics, that is, "*the application of computational techniques to analyse the information associated with biomolecules on a large-scale*" [1-2]. Internet is a tremendous source of information that when approached adequately can facilitate the analysis of a particular problem. In this context, many databases are available through Internet, from genes and genomes to protein sequences, metabolic pathways and protein structures (primary to quaternary); and big efforts are being made to integrate and crosslink different sources of information.

In parallel to the availability of this huge amount of data, many computational web resources and tools have been developed to extract a considerable amount of useful information with just a nucleotide or protein sequence. In this lecture, we will focus on these types of resources and how they can be used to obtain from simple information such as the isoelectric point, molecular weight or the extinction coefficient to more complex data such as flexibility, stability, initial crystallization conditions or structural models.

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L22

IN SILICO STUDY OF PROTEIN STRUCTURE AND FUNCTION

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Understanding the structure and dynamics of protein and interaction of proteins with ligands both in aqueous and non-aqueous solutions can be done by many experimental techniques such as X-ray scattering methods, differential scanning colorimetry, differential scanning fluorimetry, circular dichroism spectroscopy, NMR and many other methods.

One of the complimentary method for experimental techniques is molecular modelling. In order to understand the structure and dynamics of proteins, binding of ligands to enzymes, protein-protein interaction and many other properties of proteins in solution molecular modelling methods are very important because they can give molecular level information.

There are many molecular modelling approaches which are based on stochastic or deterministic phenomenon. In this talk few molecular modelling methods such as quantum chemical calculations (QM), Molecular Mechanics methods, Classical Molecular Dynamics (MD) Simulations, Hybrid QM/MM (quantum mechanics/molecular mechanics) and Monte Carlo simulations of proteins will be discussed briefly and some examples of applications of these methods will be introduced.

L23

HOW DRASTICALLY DIFFERENT FAMILIES OF DUTPASES INTERACT WITH THE SAME INHIBITOR PROTEIN: STRUCTURAL INSIGHTS

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The genetic material encoding all the machinery of a living organism is prone to several sorts of chemical modifications. Removal of the unsought modifications formed by spontaneous processes or induced by damaging agents is of paramount importance. The presence of uracil is one of the common errors, therefore several mechanisms exist to remove this base from DNA. As a part of preventive repair, dUTPase protein encumbers uracil misincorporation into DNA by hydrolysis of dUTP. This important role of dUTPase entitles it as an essential enzyme in most organisms, and also as a target of antiparasite drugs and cancer therapy [1].

It has been shown that Stl, a *Staphylococcus aureus* pathogenicity island regulator protein interacts with several trimeric dUTPases [2,3]. Upon dUTPase-Stl complex formation dUTPase enzymatic activity is inhibited while the repressor function of Stl protein is also perturbed [2,4]. It has been recently revealed that, in spite of its different structure (Fig 1), dimeric dUTPase of NM1 phage also binds to Stl [5,6].

In this study, we attempted to answer how the interaction evolves between the two highly divergent dUTPases and the Stl protein.

We found that the dimeric dUTPase of the NM1 phage is inhibited by Stl with an apparent inhibitory constant of 34 ± 14 nM, which is comparable to that observed in case of trimeric dUTPases [7]. The stoichiometry of the NM1 phage dUTPase-Stl complex was investigated by native gel electrophoresis, chemical crosslinking and native mass spectrometry, both confirming that the interaction exists between Stl and NM1 phage dUTPase monomers. This indicates that in case of this dimeric dUTPase, Stl disrupts the oligomerization of the protein, contrary to the observations for trimeric dUTPases. These results can provide a possible explanation on the mechanism of enzymatic inhibition in case of the phage dimeric dUTPase, where the active centrum is at the dimer interface of the proteins (Fig 1), which is likely affected by Stl binding.

Based on the observed inhibition of the two markedly different dUTPases by Stl it was intriguing to hypothesize that this protein has a substrate mimicking surface which serves as a common interface for complexation with dUTPases. Consonant with this hypothesis it has been shown that the carboxi-terminal segment of Stl has a role in the interaction of the inhibitor protein with both the trimeric and dimeric phage dUTPases [6,8].

In order to investigate the specific interaction surface of the proteins, we analyzed the change in hydrogen deuterium exchange (HDX) rate of those upon complex formation with mass spectrometry (MS) [7].

The disruption of the oligomerization of the dimeric protein should be considered during the interpretation of the HDX-MS results in case of the NM1 phage dUTPase. We found that the isotope exchange rate is close to constant in the putative dimer interface, although it decreases in a segment adjacent to that in the 3D model. Based on this we suggest that the dimer interface of NM1 phage dUTPase is covered by Stl and an additional segment of that is also affected. We found that two different regions of the carboxi-terminal segment of Stl show decrease in isotope exchange velocity in case of the dimer and trimer phage dUTPase complexes. However since Stl also exists in a dimer monomer equilibrium in solution, regions with unchanged exchange rate may also contribute to the complex formation.

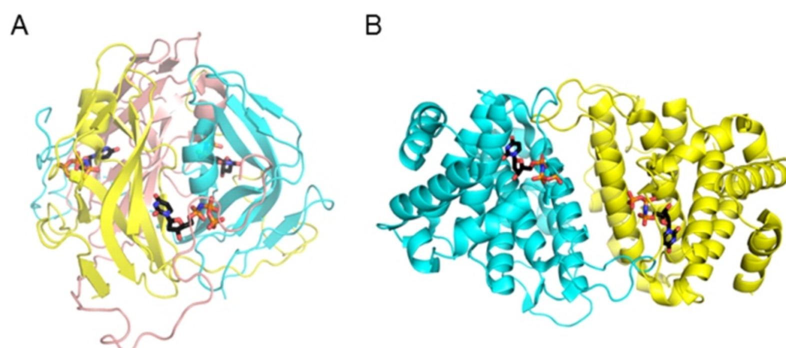
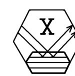


Figure 1. Comparison of the trimeric and dimeric dUTPase structures [3] A) *Mycobacterium tuberculosis* trimeric dUTPase B) *Leishmania major* dimeric dUTPase.



As the structure of Stl either in the dimer or in the monomer state is not yet determined, it is not yet possible to locate these other segments probably involved in complex formation.

In conclusion, we found that two different regions of the Stl carboxi-terminal segment interact with the dimeric and trimeric phage dUTPases, although we cannot exclude that some other parts of the protein may additionally serve as a common interacting surface in different dUTPase-Stl complexes. Based on the differences we found we hypothesize that Stl interact with drastically different families of dUTPases in a different way, which is a hitherto unseen ability of an inhibitor protein.

Based on this knowledge it might be possible to design proteinaceous inhibitor(s) of other dimeric dUTPases essential for parasites as *L. major* or *T. brucei* causatives of leshmainiasis and African sleeping sickness, respectively.

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L24

NOVEL BANGLE LECTIN FROM *PHOTORHABDUS ASYMBIOTICA*: SUGAR-BINDING SPECIFICITY, STRUCTURE AND INTERACTION WITH HOST IMMUNE SYSTEM

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Photorhabdus asymbiotica is gram-negative bioluminescent bacteria living in a symbiotic relationship with nematodes from the genus *Heterorhabditis*. Together with nematode it forms a complex that is highly pathogenic for insects. However, while other three recognized species of the *Photorhabdus* genus are strictly entomopathogenic, *P. asymbiotica* is unique in its ability to act as an emerging human pathogen as well.

Analysis of the *P. asymbiotica* genome identified a novel lectin designated PHL with a sequence similarity to the recently described lectin PLL from *P. luminescens*. Recombinant PHL protein was purified and characterized. It exhibited high affinity for fucosylated carbohydrates and lower affinity to several other mono- and oligosaccharides including saccharides from bacterial cell wall or human blood epitopes. PHL was shown to interact with all types of red blood cells and insect haemocytes. It inhibits the production of reactive oxygen species in human blood and antimicrobial activity both in human blood, serum and insect haemolymph.

In order to further examine its binding abilities, we crystallized the protein and soaked it with methyl- ,L-fucoside, D-galactose and BGH trisaccharide – the human blood cell epitope. The X-ray diffraction data were collected at BESSY synchrotron in Berlin, with resolution of 1.9 – 2.2 Å. The structure analysis of these complexes revealed an unusual organization of binding sites that was not observed in any other lectin so far. Surprisingly, up to twelve binding sites per monomer can be capable of saccharide ligand binding. Additionally, PHL forms a dimer, which is further stabilized by intramonomer disulfide bridge. The presence of high number of binding sites per monomer together with protein dimerization enables high affinity of the lectin towards potential interacting surfaces, e.g. bacteria, immune cells or host epithelia. These results suggest that PHL might play a crucial role in the interaction of *P. asymbiotica* with both human and insect hosts.

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