touchscreens, barcode scanners, digital cameras, and other forms of automation.

Using these data, the *Xtaldb* system organizes experiments and overall target status information into projects and provides tools for data mining and statistical analysis of the crystallization data both on the project and database-wide levels. To test these tools, we used the system in the salvage of a group of ten targets that previously failed to produce a structure in the MCSG pipeline. To date, two structures have been solved and deposited in the PDB, and three others diffract natively: two to 2.7Å, and one to 3.6Å.

Keywords: crystallization of proteins, bioinformatics, structural genomics

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GIXD Investigation of GlnB of *H. seropedicae* Adsorbed on Silicon

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Protein adsorption on solid surfaces has a wide range of applications[1]. The use of Grazing Incidence X-Ray techniques to investigate protein structure adsorbed on interfaces is a promising tool that may lead to the understanding of its function. In diazotroph microorganisms, GlnB of *H. seropedicae* signalizes levels of nitrogen for a series of proteins involved in the regulation of expression and activity of nitrogenase complex. The GlnB-HS structure was already determined by x-ray diffraction revealing a trimmer of (36kDa)[2].

The subject of this investigation is to understand the interaction of protein GlnB-Hs, a globular protein, on Si (111) and Si(100) surfaces under different conditions of deposition. The spin coating technique[3] was used to obtain a uniform thin film. This experiment was conducted on a Huber six-circles diffractometer, at XRD2 beamline(LNLS- Brazil), with energy tuned to approximately 7Kev. The results were used to obtain information on protein layer assembly. The initial scattering profiles of standard θ-2θ obtained in grazing incidence geometries showed signal of protein layers ordering corresponding to a d-spacing of 30Å in Si(111) and 40Å in Si(100) out of plane direction compatible with crystallographic data.

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Keywords: protein assembly, adsorption, grazing incidence diffraction

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Using Multilayer Soft Lithography Formulator Chips to map Precipitations Diagrams of Proteins

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In the post human genome era, the focus has shifted from sequencing genomes to investigate the proteins that are encoded by the genomes. The structural genomics programs have different missions but they all share the fact, that they have put together a high throughput pipeline that make it cheaper, easier and faster to get from gene to the three dimensional structure of the encoded protein. In this pipeline there are several bottlenecks, but it is agreed in general that the most significant bottleneck is to get from the protein solution to protein crystals of diffraction quality.

We have implemented the method of Multilayer Soft Lithography to produce Formulator chips [1] to address the problem of protein crystallization. Using the Formulator chip, the solubility behaviour of a protein can be experimental characterised using minute volumes of sample. The protein is screened against 3000 chemical conditions using less than 10 µL of purified protein sample. Subsequently 30 to 50 chemical conditions from this sparse screen are selected for detailed mapping of the precipitation diagram, in which the concentrations of protein against precipitant are varied. Using the experimental precipitation diagrams tailor made crystallisations experiments are designed, maximizing the probability of producing crystals of the protein.

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Keywords: microfluidics , crystallization, phase diagrams

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Metal Cations Effect on Membrane Crystallized Lysozyme

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Microporous hydrophobic membranes are innovative tools in protein crystallization [1]. In this work, effects of $CoCl_2$ and $CuCl_2$, used as precipitant agents, on membrane-crystallization of hen egg white lysozyme (HEWL), are described. The HEWL*Co^{2+} complex gave rise to a new $P2_12_12_1$ orthorhombic form (a= 36.81 Å, b= 77.56 Å, c= 80.38 Å) beside the ordinary tetragonal one. Literature reports only another similar case: a $P2_12_12_1$ orthorhombic form of an HEWL*Ni^{2+} compound, grown under strong magnetic field [2]. Membrane crystallization of HEWL with $CuCl_2$ allowed to observe new coordination positions of Cu^{2+} to lysozyme (Asp18, Asp87) respect to ones already described (Asp52, Leu129, Arg14) [3]. DSC tests showed cobalt coordination increases lysozyme stability, while copper binding by oxygen atoms is unfavourable and decreases crystals melting point. HEWL specific activity increases after crystallization owing to its further purification and seems to be more affected by copper coordination.

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Mexicain, from the Crystal to the Structure: A Sixty Years Journey

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Mexicain is a cysteine protease from the tropical plant *Pileus Mexicanus* (now, also called *Jacaratia mexicana*) first described by Castañeda and co-workers in 1942 [1]. Previous crystallization trials of mexicain were reported but unfortunately the quality of the crystals was not good enough for accurate X-ray analysis [2, 3].

In this work we present the strategy to find crystallization conditions that produce crystals of the complex protein-inhibitor that are suitable for x-ray diffraction studies. We will present purification protocols and biochemical characterization of mexicain as well as the crystallization of mexicain bound to the inhibitor by vapor diffusion and counter-diffusion techniques. Crystals were cryo-protected with glycerol to a final concentration of 20%. Frozen crystals were analyzed using an X-ray rotating anode source and they diffracted to a resolution of 1.94 Å. The crystal belongs to the monoclinic space group $P2_1$ with unit cell parameters a = 57.36 Å; b = 90.45 Å; c = 80.39 Å; and β = 92.64°. The asymmetric unit contains four molecules of mexicain with a corresponding crystal volume per protein mass (*Vm*) of 2.24 and a solvent content of 45% by volume.

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