amplified by PCR introducing in all of them the same double restriction sites present in a library of expression vectors, which will add different tags (MBP, GB1, His-tag at the N- or C-terminus) to each construct. All constructs are subsequently tested for bacterial overexpression using different strains and at different culture conditions. The last step consists of a solubility screening for each construct tested at a specific strain and expression condition, by using different buffers designed according to a semi-rational approach. The results from different analysis will be presented.

Keywords: methods, expression, solubility

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Community nominated projects at the Joint Center for Structural Genomics

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The Joint Center for Structural Genomics (JCSG), funded through the NIH NIGMS Protein Structure Initiative (PSI), has implemented a high-throughput structural biology pipeline, which has delivered more than 1100 structures over the last 10 years. Numerous technological advances have been incorporated to allow efficient, parallel processing of a large numbers of targets from both bacteria and eukaryotes. Until recently, the primary focus of the PSI was to increase structural coverage of protein sequence space, by determining structures from large protein families that lacked any structural representative. However, in 2010, the PSI entered its third phase (PSI-Biology), where the emphasis shifted to make high-throughput structure determination more broadly available to the biological research community. NIGMS has funded several High-throughput Enabled Structural Biology Centers to focus on specific biological topics, as part of the PSI-Biology Network. In addition, individual researchers are able to propose "community-nominated" targets to the PSI through the PSI Structural Biology Knowledgebase (http://cnt.sbkb.org/CNT/targetlogin.jsp).

Here we will present specific examples of "community-nominated" projects at the JCSG, highlighting the diversity and scope of these biological projects. These projects are sometimes initiated as a follow-up to structures previously solved by the JCSG, where we team up with biologists to perform biochemical studies aimed at elucidating the functional role of these novel proteins. Frequently, the scope of the project is then expanded to target other related proteins. In other cases, biologists come to us with exciting new projects, targeting structures of novel and biologically significant proteins or protein families, proteins involved in interesting pathways, essential proteins but still of unknown function identified through microarray studies, or protein-protein/protein-DNA complexes. Results from some of these projects will be outlined.

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Keywords: high-throughput structural biology, collaborative biological research, community targets

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X-ray fluosescence tool for rapid in-house evaluation of heavy atom derivatives

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The MIR phasing method still plays a crucial role in the structure determination of a novel protein. However, to acquire adequate heavy atom derivatives, one must repeatedly go through the tedious procedure, transferring crystals to various heavy atom solutions with different concentration for different soaking time ranging from minutes to days [1]. Moreover, the result of soaking can only be obtained by checking a difference Patterson map that requires a full data collection. If one can assess the presence of heavy atoms in a protein crystal prior to a data collection, it will greatly reduce the amount of work required to obtain heavy atom derivatives.

To meet this need, we developed an X-ray fluorescence (XRF) tool that can be used to detect heavy atoms introduced by soaking. This XRF tool is composed of an SDD, an XYZ stage, counting circuits and software, and can be added to an existing in-house single crystal system.

We performed some experiments to evaluate this tool. The tool was placed on a Rigaku MicroMax 007HF rotating anode X-ray generator equipped with a VariMax Mo multilayer optic. Some lysozyme crystals were soaked in K_2PtCl_4 solution and subjected to both XRF and X-ray diffraction (XRD) measurements. We found that those crystals showing clear XRF spectra of Pt tend to have discrete peaks corresponding to Pt's on Harker sections and have higher occupancy when refined against ΔF's derived from the XRD measurement.

This XRF tool can be used not only to eliminate unwanted "Native" crystals in-house prior to beam time, but also to estimate amount of heavy atoms introduced in a protein crystal by soaking.

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Keywords: fluorescence, tooling, derivative

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The Joint Center for Structural Genomics

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The Joint Center for Structural Genomics (JCSG; www.jcsg.org) is one of the four PSI:Biology Center for High-Throughput (HT) Structure Determination. Our mission in this 3rd phase of the Protein Structure Initiative (PSI) is to extend HT Structural Biology (HTSB) to a broader biological community. To meet these goals, each of the HT centers has been partnered with Biological Centers that present challenging new opportunities for HTSB, in addition to our internal biomedical-theme projects. The JCSG is in its 12th year of operation and provides a robust and flexible HTSB platform that is applicable to a large variety of targets from both bacteria and eukaryotes. Our main approach involves processing large numbers of targets via an extensive combination of bioinformatics and biophysical analyses to efficiently characterize each target in order to optimize its path through our pipeline. In close collaboration with our PSI:Biology Partnerships and

a more focused approach, the JCSG is leveraging its HTSB platform to address more challenging targets and capitalize on our extensive experience to develop the best strategies to enhance chances of success. In parallel, we process our internal biomedical-theme targets in a HT mode. This project is centered the microbial communities that inhabit specific niches and environments of the human body to investigate the structural basis for host/commensal-microbe interactions. We are initially focusing on secreted proteins from commensal bacteria in the human gut to explore their symbiotic relationship with their human host. The gut microbiota is dominated by poorly characterized bacterial phyla, which contain an unusually high number of uncharacterized proteins and remain largely unstudied. Their influence upon human development, physiology, immunity, and nutrition are only starting to surface and, thus, represents an exciting new frontier for HTSB where we can investigate the contributions of these microorganisms to human health, as well as to disease. Supported by NIGMS: U54-GM094586

Keywords: structural genomics, high-throughput structural biology, human microbiome

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ARCIMBOLDO goes super: *ab Initio* phasing on the supercomputer Calendula FCSCL

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A supercomputer (FCSCL: www.fcsc.es) provides the ideal environment for ARCIMBOLDO, as it opens new dimensions to the incorporation of prior knowledge, allowing to tackle increasingly difficult structures. Extensions in the method and its successes will be reported. It also makes the method accessible to users who do not have a grid.

Ab Initio phasing of macromolecular structures with no heavy atoms has been limited to cases with up to around 1000 atoms in the asymmetric unit, diffracting to atomic resolution [1].

Both the size and resolution barriers have been overcome in the case of several test and previously unknown structures. Thus, cases with a few thousand atoms, diffracting to 2Å have been solved through a combination of location of model fragments such as polyalanine alpha-helices with the program PHASER [2] and density modification with the program SHELXE [3]. Given the difficulties in discriminating correctly positioned fragments, the method has to test many putative groups of fragments in parallel, thus calculations are performed in a grid. The method has been called after the Italian painter Arcimboldo [4], who used to compose portraits out of fruits and vegetables. In the case of our program, most collections of fragments remain a "still-life", but some are correct enough for density modification to reveal the protein's portrait.

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Keywords: ab initio phasing, macromolecule, supercomputing

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Accelerating ab initio phasing with de novo models

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The *ab initio* phasing is one of remaining challenges in protein crystallography. Recent progress in computational structure prediction has enabled the generation of *de novo* models with high enough accuracy to solve the phase problem *ab initio*. This "*ab initio* phasing with *de novo* models" method first generates a huge number of *de novo* models and then selects some lowest energy models to solve the phase problem using molecular replacement. The amount of CPU time required is huge even for small proteins and this has limited the utility of this method. Here, we describe an approach that significantly reduces the computing time required to perform the "*ab initio* phasing with *de novo* models". Instead of performing molecular replacement after the completion of all models, we initiate molecular replacement during the course of each simulation. Our approach principally focuses on avoiding the refinement of the best and the worst models and terminating the entire simulation early once suitable models for phasing have been obtained. In a benchmark dataset of 20 proteins, our method is over two orders of magnitude faster than the conventional approach. We have observed that in most cases molecular replacement solutions were determined soon after the coarse-grained models were turned into full atom representations. We have also found that all-atom refinement could hardly change the models sufficiently to enable successful molecular replacement if the coarse-grained models were not very close to the native structure. Therefore, it remains critical to generate good quality coarse-grained models to enable subsequent all-atom refinement for successful *ab initio* phasing by molecular replacement.

Keywords: phasing, prediction, computation

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Structure analysis of 2D membrane proteins using X-ray powder diffraction data

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The majority of known integral membrane proteins (IMPs) have a natural propensity to form two-dimensional (2D) crystals during the crystallization processes [1]. This limits the possibility that their molecular structures may be obtained using the standard methods of protein crystallography. Powder diffraction methods are, in contrast, not critically sensitive to the quality and dimensions of crystals, which suggests their use in the structure analysis of protein crystals [2]. The application of powder diffraction methods for the structure analysis of proteins, however, is still regarded as intractable because of the large number of unresolved (overlapping) reflections. The development of new methodologies for powder diffraction structure analysis is, therefore, timely and desirable and could significantly expand the list