FA1-MS01-P01

The c-AMP Receptor-Like Protein CLP is a Novel c-di-GMP Receptor Linking Cell-Cell Signaling to Virulence Gene Expression in *Xanthomonas campestris*. Shan-Ho Chou[a], Ko-Hsin Chin[a], Yi-Hsiung Tseng[b], J. Maxwell Dow[c]. [a]*Institute of Biochemistry, National Chung-Hsing University, Taichung, 40227, Taiwan.* [b]*Institute of Microbiology, Immunology, and Molecular Medicine, Tzu Chi University, Hualien 970, Taiwan.* [c]*BIOMERIT Research Center, Department of Micro biology, BioSciences Institute, University College Cork, Cork, Ireland.*
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C-di-GMP controls a wide range of functions in eubacteria, yet little is known about the underlying regulatory mechanisms. In the plant pathogen *Xanthomonas campestris*, expression of sub-set of virulence genes is regulated by c-di-GMP and also by the CAP-like protein *Xc*CLP, a global regulator in the CRP/FNR superfamily. Here, we report structural and functional insights into the interplay between *Xc*CLP and c-di-GMP in regulation of gene expression. *Xc*CLP bound target promoter DNA with sub-μM affinity in the absence of any ligand. This DNA-binding capability was abrogated by c-di-GMP, which bound to *Xc*CLP with μM affinity. The crystal structure of *Xc*CLP showed that the protein adopted an intrinsically active conformation for DNA binding. Alteration of residues of *Xc*CLP implicated in c-di-GMP binding through modeling studies caused a substantial reduction in binding affinity for the nucleotide and rendered DNA binding by these variant proteins insensitive to inhibition by c-di-GMP. Taken together, the current study reveals the structural mechanism behind a novel class of c-di-GMP effector protein in the CRP/FNR superfamily and indicates that *Xc*CLP regulates bacterial virulence gene expression in a manner negatively controlled by the c-di-GMP concentrations.

[1] Chin, K.-H., Lee, Y.-C., Tu, Z.-L., Chen, C.-H., Tseng, Y.-H., Yang, J.-M., Ryan, R. P., McCarthy, Y., Dow, J. M., Wang, A. H.-J. & Chou, S.-H. (2010). J. Mol. Biol. 396, 646-662.

Keywords: c-di-GMP receptor,CLP, quorum sensing

FA1-MS01-P02

EMBL MX1 beamline for Macromolecular crystallography at PETRA III @ Desy, Hamburg Germany. Michele Cianci, Gleb Bourenkov, Stefan Fiedler, Thomas Schneider. *EMBL c/o DESY, Notkestr. 85, 22603 Hamburg, Germany.*
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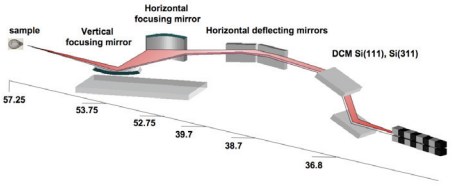
In bio crystallography, new trends and challenges are constantly appearing. At present proteins are increasingly purified directly from natural sources. With no expression possible with recombinant technologies, heavy atom derivatization is the oly method of choice for phasing.
The bacterial ribosomal 30S subunit, a 850-kDa complex, from Thermus Thermophilus, was phased to 5.5 Å resolution with MAD on W and Ta clusters and to higher resolution with Lu and Os compound [1].
The molecular organization of infectious silkworm cypovirus polyhedra, from insect cells, was elucidated to 2Å resolution using crystals of 5–12 microns in diameter with phases from Au, Ag, Se and I derivatives [2].
Working with heavy atom derivatives does not only require tunable beam lines but it is also posing challenges in terms of the number of crystals to be screened, crystal decay, anisotropicity, isomorphism. At synchrotrons such projects require the provision of automation, microbeams, radiation damage issues and new strategies for data collections. Moreover, low-resolution structures may also be further investigated by complementing crystallographic information with *in-crystallo* spectroscopy.
EMBL macromolecular crystallography MX1 beam line is part of the European Molecular Biology Laboratory integrated infrastructure for life science applications at PETRA III, the new 3rd generation synchrotron at DESY in Hamburg.



MX1 double crystal monochromator ('PETRA III design') will be tunable over the energy range from 5(4) to 17 keV to allow crystallographic data acquisition on a broad range of elemental absorption edges for experimental phase determination. In addition to the standard Si(111) it will offer Si (311) for narrow band pass for new developments in phasing and *in-crystallo* spectroscopy. This beam line will also provide a very low beam divergence (0.2 mrad (H) x 0.15 mrad (V)) and variable focus size (20 to 100 microns) to adapt to diverse experimental situations. This is achieved with a bimorph adaptive optics-flexibility Kirk Patrick Baez focusing system. Most notably, the well-collimated beam will allow resolving diffraction patterns from large unit cells. The Maatel/Accel MicroDiffractometer2 combined with a mini kappa goniometer head characterizes the sample stage facilitating the use of small crystals for data collection. We will discuss how ideas and perspectives have been transferred into the design of MX1 beam line.

[1] W.T. Clemons et al, JMB 310 (2001) 827. [2] F. Coulibaly et al., Nature 446 (2007) 97.

Keywords: macromolecular X-ray crystallography, microcrystals, phasing

FA1-MS01-P03

Macromolecular Crystallography Beamlines at Diamond Light Source: Current Capabilities and Future Plans. Ralf Flaig, Macromolecular Crystallography Group, *Diamond Light Source, Harwell Science and Innovation Campus, Chilton, Didcot, Oxfordshire, OX11 0DE, UK.*
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Diamond Light Source [1] is the new UK third generation synchrotron located south of Oxford. Diamond welcomed first users in January 2007.
Currently, there are five operational beamlines (Phase I: I02, I03, I04 (5-25 keV, optimized SAD/MAD); Phase II: I24 (7-25 keV, microfocus), I04-1 (13.53 keV, high throughput MX)) covering the discipline of macromolecular crystallography (MX) and a further one is in the planning stage (Phase III: I23, long wavelength MX) [2].

The three Phase I MX beamlines have been built for state of the art high-throughput data collection on crystals of biological macromolecules and are now well in their fourth year of operation. High quality results have been obtained. Further improvement of the beamlines is ongoing with a focus of automating and streamlining as many components and experimental processes as possible, from beam conditioning to user interaction and data analysis but maintaining experimental flexibility.
Therefore, we are redesigning the end-station table, beam conditioning components and sample environment with the aim to add new and improved features. The system is also designed for ease of maintenance and keeps adaptability to future scientific requirements for the structural biology community. Delivery of the new end-stations is due to begin towards the latter half of 2010.
Further plans include the provision of an improved software user interface, fast sample screening, remote access and category 3 pathogenic sample handling (I03). An update on these developments will also be presented.

[1] http://www.diamond.ac.uk.
[2] http://www.diamond.ac.uk/Home/Beamlines/MX.html.

Keywords: Diamond Light Source, Macromolecular Crystallography, Beamlines

FA1-MS01-P04

Surfaces of Attenuation of Acoustic Waves in Cubic Crystals. Akhmedzhanov F.R.[a], Saidvaliev U.A.[b].
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In contrast to the propagation velocity the anisotropy of attenuation of high-frequency acoustic waves in cubic crystals is investigated insufficiently.
In this work the acoustic attenuation in $Bi_{12}GeO_{20}$ and $Bi_{12}SiO_{20}$ crystals has been investigated on the basis of experimental data on the attenuation of acoustic waves propagating along the main crystallographic directions. Measurements were carried out using Bragg diffraction of light by acoustic waves at room temperature in the frequency range from 0.4 to 1.5 GHz.
According to the known perturbation theory, the attenuation coefficient can be defined in terms of the effective viscosity. Since the viscosity tensor has the same symmetry as the elastic stiffness tensor, three independent constants must be determined for the crystal class 23, to which belong the investigated crystals. All the viscosity components were determined by substituting effective viscosity values obtained from measured attenuation data into the mode viscosity equations.
The obtained viscosity components were used for calculation of the anisotropy of attenuation of three wave modes propagating along any selected direction using equation:

$$\alpha = \omega \cdot \eta_{eff} / 2\rho \cdot V^3$$

where ω is the circular frequency, η_{eff} is the effective viscosity for selected direction, ρ is the density and V is the propagation velocity.
Calculations have been carried out for acoustic waves propagating in (001) and (110) crystallographic planes.
At the same time the contribution of dielectric loss in the total attenuation coefficient of piezoactive waves was assessed for these crystals [1]. It is shown that the dielectric loss can produce a significant influence on the magnitude and anisotropy of the attenuation coefficient for piezoactive longitudinal and transverse waves in $Bi_{12}SiO_{20}$ crystals.

[1] Shaposhnikov I.G., *ZhETF*, 1941, 11, 332.

Keywords: acoustic elastic properties, anisotropic properties, attenuation coefficients

FA1-MS01-P05

Microfocus Beamline for Macromolecular Crystallography MX2@PETRAIII. G. Bourenkov, M. Cianci, S. Fiedler, M. Roessle, T.R. Schneider.
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The undulator beamline MX2 in Sector 9/P14 of the upgraded PETRA III storage ring is a part of the integrated structural biology facility constructed by European Molecular Biology Laboratory at DESY, Hamburg. The purpose of the beamline is to deliver high-quality diffraction data in structural studies of large macromolecular complexes and membrane proteins relying on the usage of small or inherently heterogeneous crystals.
The beamline will provide the focal spot size down to about 4x1 μm^2, matching the dimensions of the smallest macromolecular crystals used for structure determination to date, as well as matching the requirements for spatially resolved sub-crystal diffraction applications on macroscopically disordered crystals. The beam size at the sample position will be adjustable by defocusing in two dimensions, up to ~300x300 μm^2 to permit *in-situ* optimization of signal-to-noise conditions for larger samples. This will be achieved by using an adaptive bimorph focusing mirrors (SESO/BASC/JTEC) in Kirkpatrick-Baez geometry with an ultra-precise surface of the vertical focusing mirror. Microfocusing conditions will be realized at beam divergence <0.5 mrad, sufficient for resolving large unit cells up to ca. 800 Å. For even larger unit cells and for optimal signal-to-noise conditions (e.g. for crystals with very low mosaic spread), further reduction in divergence down to 50 µrad will be achievable. The possibility of using unfocused beam will also be preserved.
The energy range of the beamline will cover most of the absorption edges commonly used for anomalous scattering phasing (Fe-K edge to U-LIII edge), in line with state-of-the-art MAD capabilities. In the high energy range, 17-35 kEV, optimal diffraction signal *versus* sample lifetime conditions are anticipated.
The end station will be equipped with a EMBL-MATTEL microdiffractometer MD2 and a high-efficiency mosaic CCD detector (RAYONIX). The setup was commissioned at DORIS beamline BW7A, and is proven to provide highly accurate data (e.g. for SAD phasing with anomalous signals <0.5%). The MD2 provides repeatability of mechanical positioning better then 0.2 µm, with a sphere of confusion 0.25 µm in a single-axis, and 0.7 µm in a multi-axis mode (r.m.s. values). Later upgrade to a multi-axis diffractometer with even higher precision is planned. Integrated data collection and processing software is being developed on the basis of TINE