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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

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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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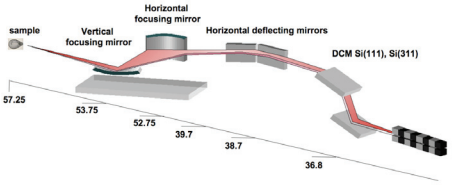
In bio crystallography, new trends and challenges are constantly appearing. At present proteins are increasingly purified directly from natural sources. With no expression possible with recombinant technologies, heavy atom derivatization is the oly method of choice for phasing.
The bacterial ribosomal 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].