Structural basis of thiol-based regulation of formaldehyde detoxification in *H. influenzae* by a MerR regulator with no sensor domain

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Haemophilus influenzae is a major cause of lower respiratory tract diseases. This and other pathogenic bacteria must cope with a range of electrophiles generated in the host or by endogenous metabolism. Formaldehyde is one such compound that can irreversibly damage proteins and DNA through alkylation and cross-linking and interfere with redox homeostasis. HiNmlR is the transcription factor that controls the detoxification of formaldehyde in *H. influenzae*. NmlR belongs to the MerR family of transcription factors, but lacks a specific sensor domain and does not bind metal ions. We found that HiNmlR is a thiol-based genetic switch that modulates *H. influenzae* response to formaldehyde, and its Cys54 residue is critical for its response against a formaldehyde challenge. We obtained crystal structures of HiNmlR in both the DNA-free and DNA-bound forms (Fig. 1). The structures suggest that HiNmlR enhances target gene transcription by twisting of operator DNA sequences in a two-gene operon containing overlapping promoters. The structures provide the first insight into the mechanism of action of MerR regulators that lack sensor domains.

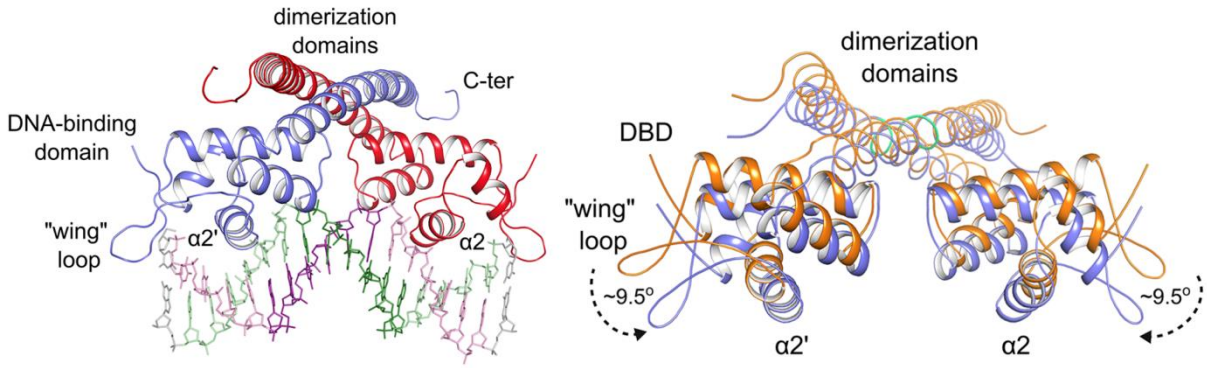

Figure 1. Crystal structures of HiNmlR in DNA-bound and DNA-free forms. Left: crystal structure of HiNmlR dimer bound to the *adhC-estD*/*nmlR* inverted repeat operator DNA. Protein monomers (ribbons) are indicated in different colors. DNA-binding domain (DBD), "wing" (α2-α3) loop and dimerization domain (α5-helix) are indicated; prime denotes the equivalent regions from the other protomer. Operator DNA fragment (stick representation) with template strands for the *adhC-estD* operon and the *nmlR* gene are shown in light pink and green, respectively; the -35 elements are shown in darker shades of the same colors. Right: superposition of DNA-bound (blue) and DNA-free (orange) HiNmlR structures (residues 94'-96' and 95-97 shown in green were used in the superposition)

Lipidic cubic phase injector -sample delivery of protein crystals in a native-like environment

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Over the past decade Lipidic cubic phase (LCP) crystallization has proven extremely beneficial for the high-resolution structure determination of challenging membrane proteins. More recently, sample delivery methods based on LCP technology have begun to yield results for serial crystallography at both the X-ray Free Electron Laser (XFEL) [1] and synchrotron [2].

Here we discuss recent progress in sample delivery techniques that involve extruding gel-like LCP with embedded protein microcrystals, providing a continuously renewed source of material for serial crystallography. Data has been collected at both synchrotron and XFEL facilities from protein crystals ranging in size from sub-micron up to a few tens of microns in size. The results show that a full structural dataset may be produced using less than less than 0.5 mg of purified protein. Here we will also present an update on the current status of the LCP injector project being developed at the Australian synchrotron as part of the ARC Centre of Excellence in Advanced Molecular Imaging.

References

[1] Liu, W., A. Ishchenko, and V. Cherezov, *Preparation of microcrystals in lipidic cubic phase* for serial femtosecond crystallography. Nat. Protocols, 2014. **9**(9): p. 2123-2134.

[2] Nogly, P., D. James, D. Wang, T.A. White, N. Zatsepin, A. Shilova, G. Nelson, H. Liu, L. Johansson, M. Heymann, K. Jaeger, M. Metz, C. Wickstrand, W. Wu, P. Bath, P. Berntsen, D. Oberthuer, V. Panneels, V. Cherezov, H. Chapman, G. Schertler, R. Neutze, J. Spence, I. Moraes, M. Burghammer, J. Standfuss, and U. Weierstall, *Lipidic cubic phase serial millisecond crystallography using synchrotron radiation.* IUCrJ, 2015. **2**(2): p. 168-176.