ESRF

ESRF	**Experiment title:** Structural studies of the rpfF-rpfC complex involved in diffusible signal factor (DSF)-dependent quorum sensing	**Experiment number**: MX-827
Beamline: ID23 1	**Date of experiment**: from: 08/09/2008 to: 09/09/2008	**Date of report**: Feb. 19, 2010
Shifts: 2	**Local contact(s)**: Dr. Sean MCSWEENEY	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Zhihong Cheng* and Haiwei Song
Institute of Molecular and Cell Biology
61 Biopolis Drive
Proteos
Singapore 138673

Report:

RpfF and RpfC are two major components of the diffusible signal factor (DSF)-dependent quorum sensing (QS) system. RpfC integrates signals from the bacterial community and regulates downstream pathogenic activity, while RpfF plays a critical role in DSF production. Here we present the crystal structures of full length RpfF alone and in complex with the REC domain of RpfC. RpfF belongs to the enoyl-coA hydratase/isomerase superfamily and adopts an inactive conformation in the absence of substrate or in the presence of the REC domain. The interaction of RpfF with the REC domain are mediated by the C-terminal helix α-10 and the β-sheet B of RpfF and helices α2 and α3 of REC. Consistently, deletion of α-10 disrupted the interaction with RpfC and abrogated DSF production whereas mutations in the REC domain inhibited its binding to RpfF and consequently reduced substantially the DSF biosynthesis repression. Importantly, the binding of the REC domain to RpfF blocks the substrate entrance to its active site, therefore negatively regulating DSF production.

Table 1 Data collection and refinement statistics

Data collection	RpfF/RpfC-REC
Wavelength (Å)	0.9795
Resolution limit (Å)	2.5
Space group	$P6_5$
Cell parameters	
a/b/c(Å)	130.9/130.9/156.5
α/β/γ (°)	90/90/120
Unique reflections (N)	35459
I/σ	11.5 (2.5)
Completeness (%)	99.4(99.8)
R_{merge}[a]	0.068(0.418)

Refinement Statistics	
Data range (Å)	20-2.5
Used Reflections (N)	56289
Nonhydrogen atoms	8892
R_{work}[b](%)	25.4
R_{free}[c] (%)	30.1
R.m.s deviation	
Bond length (Å)	0.003
Bond angles (°)	1.360
Ramchandran plot	
Allowed (% residues)	96.0%
Generously allowed (% residues)	3.7%
Disallowed (% residues)	0.3%

Values in parentheses indicate the specific values in the highest resolution shell.

[a]$R_{merge} = \sum|I_j - \langle I \rangle| / \sum I_j$, where I_j is the intensity of an individual reflection, and $\langle I \rangle$ is the average intensity of that reflection.

[b]$R_{work} = \sum||F_o| - |F_c|| / \sum|F_c|$, where F_o denotes the observed structure factor amplitude, and F_c denotes the structure factor amplitude calculated from the model.

[c]R_{free} is as for R_{work} but calculated with 5.0% of randomly chosen reflections omitted from the refinement.

Reference:

Zhihong Cheng, Ya-Wen He, Siew Choo Lim, Rohini Qamra, Martin A. Walsh, Lian-Hui Zhang, Haiwei Song (2010). Structural Basis of the Sensor-Synthase Interaction Mediated Autoinduction of Quorum Sensing Signal Biosynthesis. Submittted.