ESRF	**Experiment title:** MAD structure determination of CcbJ, a SAM-dependent methyltransferase which catalyzes the final step of celesticetin biosynthesis	**Experiment number:** MX-973
Beamline: ID14-4	**Date of experiment:** from: 22/06/2009 09:30 to: 23/06/2009 08:00	**Date of report:** 08/02/2012
Shifts: 3	**Local contact(s):** Dr. Joanna TIMMINS	*Received at ESRF:*

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Report:

CcbJ is an S-Adenosylmethionine (SAM) dependent methyltransferase from *S. caelestis* which catalyzes the final step in the biosynthesis of the antibiotic celesticetin. *S. caelestis* has exhibited the ability to synthesize different derivatives of celesticetin depending on the presence of different salicylic acid derivatives in the growth medium. [2] In order to understand how this organism is able to manage this, we have isolated, overexpressed, and purified the individual components of this pathway, including CcbJ. [3]

The crystal structure of free CcbJ was determined by Multiwavelength Anomalous Dispersion and that of the CcbJ–SAM complex was determined by molecular replacement (using the free structure as the search model). In both structures CcbJ crystallized in the $C222_1$ space group with unit cell lengths of $a = 168.02$, $b = 244.55$, and $c = 117.85$. In both crystals, the asymmetric unit contained six monomers arranged as a dimer of trimers.

CcbJ possesses the class I SAM-dependent methyltransferase fold [4], [5]; modifications to the core fold include insertion of a four-stranded β-sheet, which serves as an active site cover, between αE and β5 and a short 3_{10} helix between β4 and αD, which forms part of the SAM binding cleft. There is also an extension to the N-terminus. These insertions match the general pattern seen in other small-molecule methyltransferases. Overall, CcbJ appears to be most similar to glycine N-methyltransferase (GNMT) which also has a similar active site cover and a 3_{10} helix in the SAM binding cleft. Aside from a similar overall shape, the active site of CcbJ is quite different from that of GNMT, having a much larger number of aromatic residues.

One of the most characteristic features of CcbJ is the great degree of flexibility exhibited by the residues in the N-terminal extension preceding αZ. In the free CcbJ structure, these residues were completely disordered in one of the six chains and none of the residues preceding Tyr-17 were visible. In the CcbJ–SAM complex, however, the entire extension was visible in all six chains. The newly ordered residues form an α-helix which passes between the active site cover and αB and forms part of the SAM binding site. Following this helix, the extension passes over part of the active site opening before entering helix αZ. The loop between these two helices contains several proline and glycine residues and is likely to be natively unstructured. This would probably allow it to adopt several different conformations which might allow it to accommodate the several different substrates observed in vivo [2].

[1] This research was supported by VEGA 2/0122/11 in Slovakia and MŠMT 2B08064 in the Czech Republic. This text was originally presented as abstract MS31.P23 at the XXII IUCr Congress and General Assembly held in Madrid, Spain on 22–30 August 2011. It was published in *Acta Cryst* **A67,** C433 (2011). A full manuscript is in preparation.
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