

**High Resolution Data Collection for Crystals of The Proline Dehydrogenase Domain of the Multifunctional PutA Flavoprotein from *Escherichia coli***

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Beamline: X8C

**Introduction:** The PutA flavoprotein from *Escherichia coli* is a multifunctional protein that plays pivotal roles in proline catabolism by functioning as both a membrane-associated bi-functional enzyme and a transcriptional repressor. Peripherally membrane-bound PutA catalyzes the two-step oxidation of proline to glutamate, while cytoplasmic PutA represses the transcription of its own gene and the gene for a proline transporter protein. A recombinant protein (PutA<sub>669</sub>) corresponding to the N-terminal 669 amino acid residues of the 1320 residues of PutA was engineered. Crystals of a PutA<sub>669</sub> have been obtained and the high-resolution data have been collected at NSLS beamline X8C.

**Results:** Native crystals were grown using reservoir solutions of 21-26% PEG 3000, 0.1M citrate buffer, pH 6-7 and cryoprotected by soaking in 15% PEG200-enriched mother liquor for 15 minutes. The crystals occupy space group I222 with a=72.62 Å, b=140.43 Å, c=145.85 Å, and one molecule per asymmetric unit. These crystals diffract to only 2.5 Å resolution using a rotating anode X-ray source, therefore, the high resolution data needed could only be obtained at a synchrotron facility. A KI derivative was prepared by soaking the native crystals in the cryoprotectant for 15 minutes and then soaking in cryoprotectant supplemented with 0.25 M KI for 30 seconds. The native data and the KI data were collected to 2.0 Å at beamline X8C of the National Synchrotron Light Source at Brookhaven National Laboratory. For each crystal, a total of 120° of data were collected using a Quantum 4 CCD detector with an oscillation angle of 1°, an exposure time of 2 minutes per degree of oscillation, and a detector distance of 200 mm. The native data set is 99% complete to 2.0 Å resolution, with an R<sub>Sym</sub> on I of 0.060, <I/σ(I)> of 20.1, and average multiplicity of 5.83. The KI data set is 98% complete to 2.0 Å resolution, with an R<sub>Sym</sub> on I of 0.051, <I/σ(I)> of 15.3, and average multiplicity of 2.58. Structure determination is in progress using multiple isomorphous replacement with anomalous scattering (MIRAS).

**References:** S. Nadaraia, Y.H. Lee, D.F. Becker, and J.J. Tanner, "Crystallization and Preliminary Crystallographic Analysis of the Proline Dehydrogenase Domain of the Multifunctional PutA Flavoprotein from *Escherichia coli*," Acta Cryst. D in press

Table I. Data collection statistics		
	Native	KI
Resolution (Å)	30.0 – 2.0	30.0-2.0
Total reflections	292,513	131,057
Unique reflections	50,208	50,716
Completeness (%) <sup>a</sup>	99.0 (97.9)	98.2 (99.8)
I/σ(I) <sub>b</sub>	20.1 (2.7)	15.3 (2.3)
R <sub>sym</sub>	0.069 (0.61)	0.051 (0.43)
Wilson B (Å)	32.4	29.9
R <sub>iso</sub> <sup>c</sup>		0.152

<sup>a</sup> number in parentheses is statistic for highest resolution shell.  
<sup>b</sup>R<sub>sym</sub> = Σ<sub>h</sub> (Σ<sub>j</sub> | I<sub>j,h</sub> - <I<sub>h</sub>> | / Σ I<sub>j,h</sub>), where h=set of Miller indices and j=set of observations of reflection h.  
<sup>c</sup>R<sub>iso</sub> = Σ<sub>h</sub> | |F<sub>der</sub>| - |F<sub>nat</sub>| | / Σ<sub>h</sub> |F<sub>nat</sub>|, F<sub>der</sub> =observed derivative structure factor amplitude, F<sub>nat</sub> =observed native structure factor amplitude.