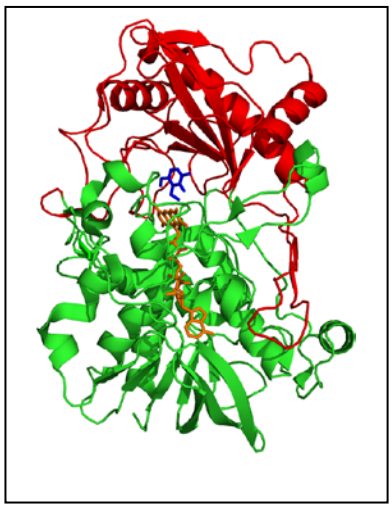
Crystal structure of pyridoxine 4-oxidase from *Mesorhizobium loti*

Andrew Njagi Mugo[a], Jun Kobayashi[b], Taiji Yamasaki[a], Bunzo Mikami[b], Yu Yoshikane[a], Toshiharu Yagi[a] and Kouhei Ohnishi[c].

[a]*Faculty of Agriculture and Agricultural Science Program, Graduate School of Integral Arts and Science, Kochi University, Nankoku, Kochi 783-8502, Japan,* [b]*Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan, and* [c]*Research Institute of Molecular Genetics, Kochi University, Nankoku, Kochi 783-8502, Japan.*

[Aims] To elucidate the complex structure of pyridoxine 4-oxidase (PNOX) with its substrate analogue, pyridoxamine (PM). PNOX, an FAD-dependent enzyme from Mesorhizobium loti MAFF303099, is the first enzyme in the degradation pathway 1 for pyridoxine and belongs to Glucose-methanol-choline (GMC) oxidoreductases family [1].

[Methods] PNOX with a His_6 tag was overexpressed in *E.coli* JM109 cells and purified with a Ni-NTA agarose column and a QA52 column [2]. Crystallization was done by the sitting-drop vapour-diffusion method at 277 K. The structure was solved by molecular replacement method.



[Results] The crystal structures of PNOX and PNOX-PM were determined at 2.2 Å and 2.1 Å resolutions respectively. The overall structure consisted of FAD-binding and substrate-binding domains. The FAD interacts with the PNOX protein through a network of hydrogen bonds which are mainly found in the ribose and pyrophosphate moieties of the FAD molecule. The surface structure of PNOX molecule showed that it had an opening socket for access of substrates. The opening was followed by a bottleneck, formed from mainly hydrophobic residues, and a tunnel. The tunnel was linked to the active site cavity. In the cavity, active site residues were located on the re-face of the isoalloxazine ring of the FAD. A Proline residue, instead of His or Asn in other GMC oxidoreductases family members, was found in the active site [3].

Fig. 1. The Cartoon view of PNOX-PM tertiary structure. The substrate binding-domain is colored red; the FAD-binding domain green; FAD orange and PM blue.

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[2] A.N. Mugo, J. Kobayashi, B. Mikami, K. Ohnishi, T. Yagi, Acta Cryst. Sec. F Struct. Biol. Cryst. Commun. **68,** 66-68 (2012).
[3] A.N. Mugo, J. Kobayashi, T. Yamasaki, B. Mikami, K. Ohnishi and T. Yagi, Biochim Biophys Acta, **1834,** 953-963 (2013).