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# X-Ray Crystal Structure Analysis of Plant Type III Polyketide Synthase

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## 1 Introduction

Type III polyketide synthase (PKS) catalyzes the iterative Claisen-type condensation of the CoA thioesters and cyclization of the poly-*β*-keto intermediates, to produce pharmaceutically and biologically class of compounds, such as chalcone, stilbene, and curcumin. Recently, a type III PKS is implicated to be also involved in the biosynthesis of tropane alkaloids [1], which is a class of compounds that include the pharmaceutically important hyoscyamine and scopolamine. The AbPYKS identified from *Atropha belladonna* was revealed to play a role in the biosynthesis of tropinone, a key tropane intermediate. It is proposed that AbPYKS catalyzes a non-canonical reaction of *N*-methyl-$\Delta^1$-pyrrolium cation with two round of elongation with malonyl-CoA mediated elongation to yield 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoic acid, which is further tailored into tropinone by a cytochrome P450 reductase (Figure 1). However, the detailed enzymatic mechanism employed by AbPYKS remains unknown. Hence, we crystallized the AbPYKS and study its 3-dimensional structure in order to reveal its catalytic mechanism.

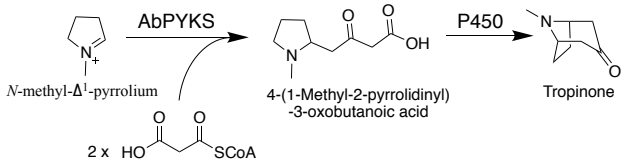


Fig. 1: Proposed biosynthetic pathway for the formation of tropinone catalyzed by AbPYKS.

## 2 Materials and Methods

*Crystallization* – Diffraction-quality crystals of AbPYKS were obtained after one week of incubation at 20 °C, in 100 mM HEPES (pH 7.5) containing 20% PEG4000 with 15 mg/mL of purified AbPYKS solution, by using sitting-drop vapor-diffusion method.

*Data collection* – The crystals were transferred into the soaking solution with 20% (v/v) glycerol for 10 sec for cryoprotection and then flash cooled at -173°C in a nitrogen-gas stream. The X-ray diffractions of crystals were collected at BL1A, processed and scaled with *XDS*. The structure was solved by the molecular replacement method with *Phaser-MR (simple one-component interface)* using curcuminoid synthase (msCHS, PDB ID: 1BI5) as model. The structure was modified manually with *Coot* and refined with *PHENIX*.

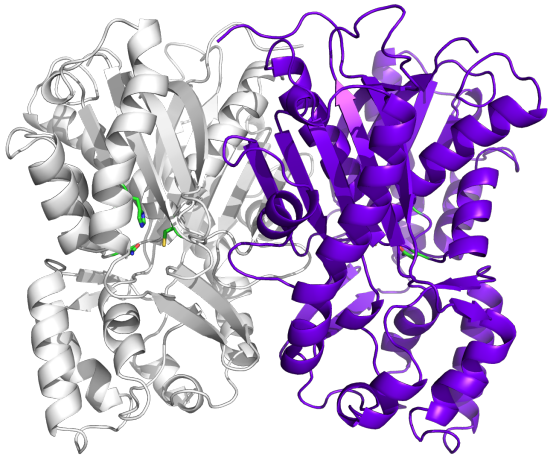

Fig. 2: Overall structure of the AbPYKS. The catalytic triad, Cys167, His306, and Asn339 is highlighted in green.

## 3 Results and Discussion

The crystal structure of AbPYKS was solved by X-ray crystallography at 2.7 Å resolution. The final *R*-value was 17.9% ($R_{free}$ = 25.4%). AbPYKS adopts the typical homodimeric construct and αβαβα-fold architecture that commonly occurs in other type III polyketide synthases (Figure 2). Structural homology search using the Dali Program [2] indicated that AbPYKS is most structurally related to stilbene synthase identified from *Vitis vinifera*, with Cα RMSD at 0.8 Å (PDB ID: 3TSY). Comparisons of the molecular structure of AbPYKS with other type III PKSs and further investigation of the AbPYKS crystal structure complexed with its substrates could highlight the important residues and catalytic mechanism the latter employed to catalyze its non-canonical mechanism.

## Acknowledgement

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## Reference

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