Towards relating the structure of Polyketide Synthases to their metabolic products

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Introduction

Polyketide-synthases (PKS) are a class of multi-domain megasynthases involved in the production of diverse of polyketides. The large class of PKS can be further divided into three different types (I-III) which differ in the way they biosynthesize its products. We have focused on type I PKS that either work iteratively (they use each domain several times) or in a multi-modular way. Modular PKS I are large enzymes composed of several modules, each containing a specific set of catalytic domains. They are similar to fatty-acid-synthases, but they can contain domains for both, total and partial reduction of its substrates.
However, relating the sequence of various catalytic domains present in a PKS biosynthetic gene cluster to the chemical structure of the final product is a challenging task [1,2].
In our studies we have addressed following questions: Can we predict the number of iterative steps catalyzed by an iterative PKS I? Is it possible to predict which substrate is accepted by a given acyl-transferase (AT) domain used for chain elongation?

Can we predict the number of elongation steps that are catalyzed by an iterative PKS I?

Methods

For prediction of the number of iterative steps being catalyzed by an iterative PKS I we measured the correlation between the cavity volume of the keto-synthase (KS) domain and the number of iterations. This methoad was previously proposed by Yadav et al [1]. Therefore homology models of different PKS I were built using the SBSPKS server. Each binding pocket was identified through superpositionig with 1B3N, a homologue β-ketoacyl-carrier-protein synthase that has been co-crystallized with an inhibitor. Cavity volume was measured using Sitemap (Schrödinger Inc.).

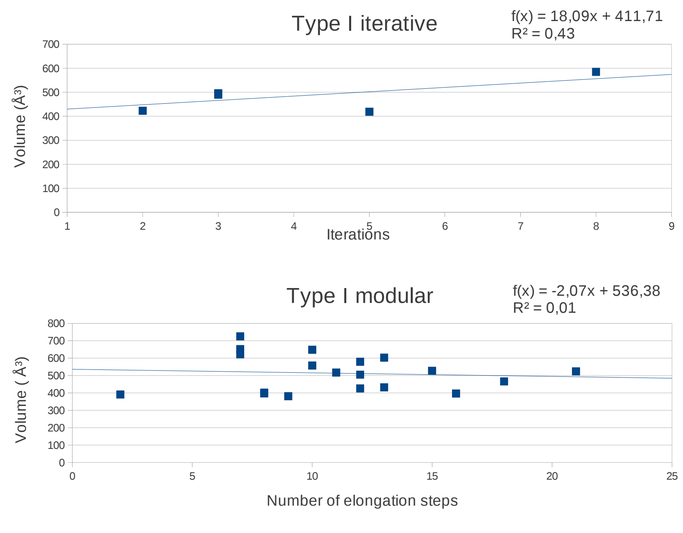


Fig. 1: Correlation between catalytic cavity volume and number of elongation steps for iterative and modular PKS I

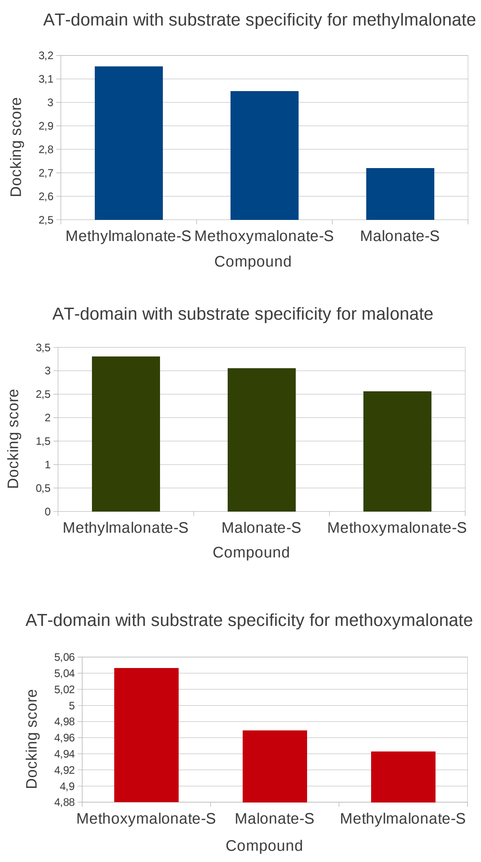
Results

Predicting the number of iterative condensation-steps:
A correlation was found between the cavity volume of the bindingpocket and the number of iterative steps being catalyzed by the corresponding KS domain (Fig.1). The correlation was weaker than previously reported. As expected no significant correlation was observed between the cavity volume of the last KS domain of modular PKS I and the size of its metabolic product.

Prediction of the substrate specificity of an AT domain

Methods

We calculated the affinity of three representative AT domains for three candidate substrates (methylmalonate, malonate and methoxymalonate) using Glide 5.8 (Schrödinger Inc.). The AT domains were first modelled using Prime (Schrödinger Inc.). Templates were obtained from PDB after performing a BLAST search aiming at the identification of homologous proteins with available structural information. Afterwards, an all-atom energy minimization was carried out. To locate the binding pocket we performed a sequence motif search [2]. After docking with the three substrates we compared the corresponding docking scores to identify the favoured substrate. Furthermore, we performed a multiple sequence alignment using Jalview (http://www.jalview.org) to identify amino acids that are important for substrate specificity of AT domains.



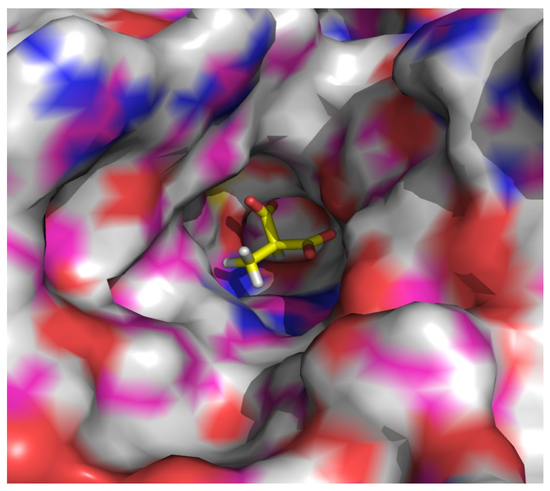


Fig. 2: Representation of the docking scores for each substrate and its specific AT domain and visualization of the binding mode of Methylmalonate in its corresponding binding pocket

Results

Predicting substrate specifity of AT domains: Comparison of the docking scores of different AT domains with possible substrates showed a slight correlation (Fig. 2) with the actual results. However it is to be considered that only the elongation unit (Malonate, Methylmalonate or Methoxymalonate) was used for docking so we cannot exclude that results would differ slightly if their CoA-derivatives were docked. Performing a multiple sequence alignment indicated that specific residues play a key role in substrate specificity for Methylmalonate or Malonate [2]. Moreover, we identified a sequence motif present in AT domains with specificity for Methoxymalonate (Fig. 3).

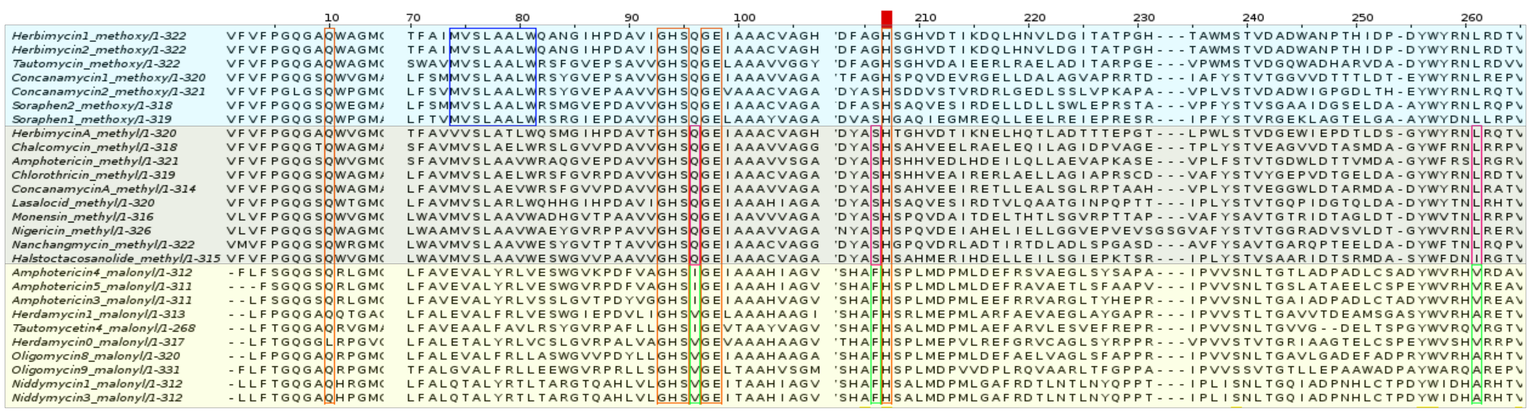


Fig. 3: Residues shared by all AT-domains are marked in orange. Motifs present in AT-domains with substrate specificity for Methoxymalonate are enframed in blue. Residues that are specific for Methylmalonate are highlighted in pink. Green frames indicate substrate specificity for Malonate.

References
[1] Gitanjali Yadav Towards Prediction of Metabolic Products of Polyketide Synthases: An In Silico Analyse, Computational Biology, 2009, Volume 5, Issue 4, S1-1
[2] Gitanjali Yadav Computational Approach for Prediction of Domain Organization and Substrate Specificity of Modular Polyketide Synthase, J. Mol. Biol. 2003,328,335-363
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