

# Predicting Enzyme Function by Docking High-Energy Intermediates of Potential Substrates

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## Introduction

Due to many genome sequencing and structural genomics initiatives, there is an abundance of enzymes of known 3D structure but unknown function. To use *in vitro* experiments to annotate them is clearly not tractable. Moreover, bioinformatics methods are not applicable in all cases, especially when sequence similarity is low. As an alternative and complementary method, docking of high-energy intermediates (HEIs) of substrates is an emerging technology. For the application presented here, we chose the amidohydrolyase (AH) superfamily (Fig. 1), since its members catalyze only few well-characterized reactions (Fig. 2).

## Amidohydrolyase Reactions

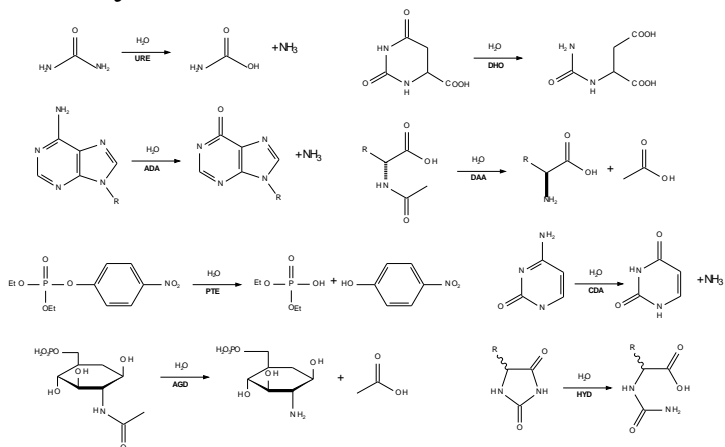


Fig. 2: Examples of characteristic reactions of amidohydrolyases. The attacking nucleophile is  $\text{OH}^-$  and is coordinated by the metal ion(s) in the binding site.

## Method

The basic idea is to use structures mimicking reaction intermediates instead of the ground-states of molecules in the docking calculations, because enzymes are preorganized to recognize and stabilize the intermediate states (Fig. 3). All dockings presented here were done with DOCK [1].

## High-Energy Intermediates

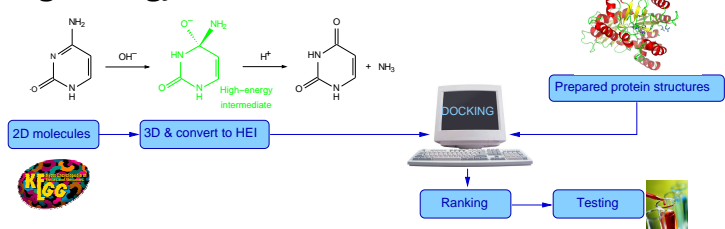
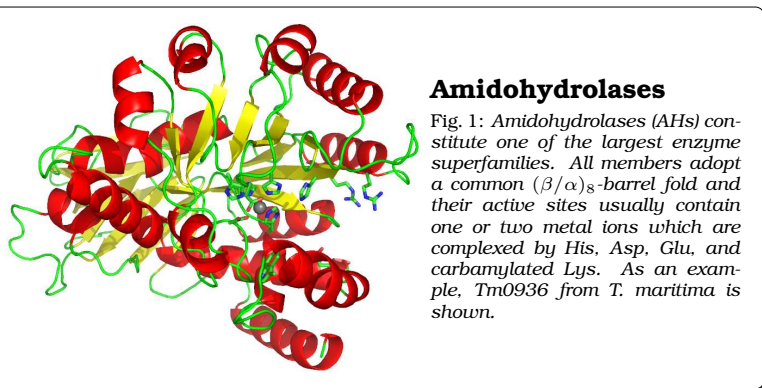
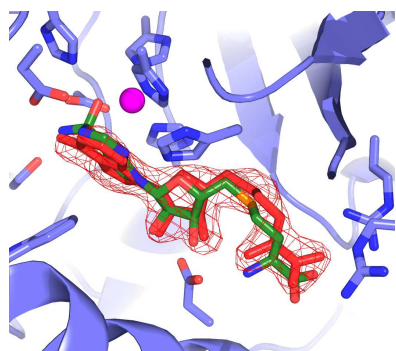


Fig. 3: Docking workflow with high-energy intermediates. An example of a tetrahedral intermediate state is depicted in green. This representation of the molecule is then used in the docking calculations.

## Previous Results

Docking of HEIs has successfully been applied previously [2-4]. Shown is the predicted and crystallographic binding mode of *S*-adenosylhomocysteine (the correctly predicted substrate; green) and its product (red) in Tm0936 from *T. maritima*, an enzyme for which there were no hints as to the function (from [4]).



## Amidohydrolyases

Fig. 1: Amidohydrolyases (AHs) constitute one of the largest enzyme superfamilies. All members adopt a common  $(\beta/\alpha)_8$ -barrel fold and their active sites usually contain one or two metal ions which are complexed by His, Asp, Glu, and carbamylated Lys. As an example, Tm0936 from *T. maritima* is shown.

## Current Predictions

We have now attempted to determine substrates for Dr0930 and Sco3058, two amidohydrolyases from *D. radiodurans* and *S. coelicolor*, respectively. Dr0930 was predicted to catalyze the hydrolysis of lactones (Fig. 4a). The *S. coelicolor* protein is predicted to be a dipeptidase with an Xaa-Glu/Asp specificity (Fig. 4b). Initial experiments have confirmed both functions.

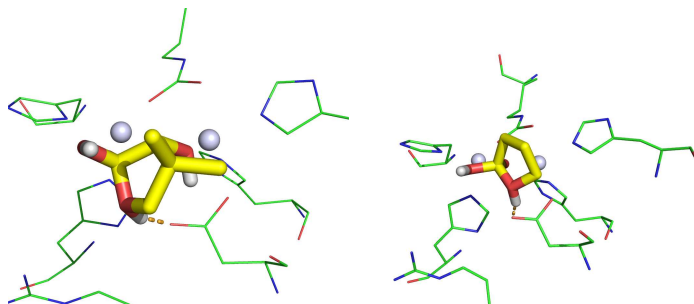


Fig. 4a: Poses of two lactone substrates identified for Dr0930. Metal ions are shown as gray spheres and hydrogen bonds as orange dashed lines.

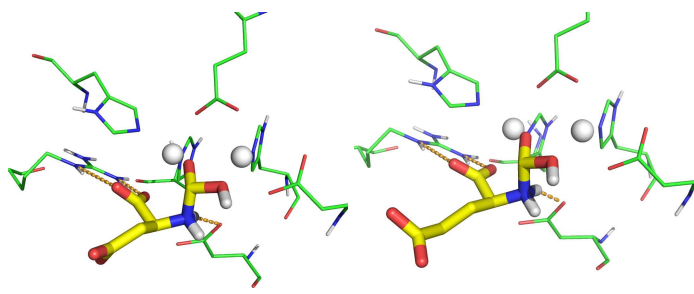


Fig. 4b: Poses of two predicted peptide substrates in Sco3058. Colors as above.

## Conclusions

Structure-based docking of high-energy intermediates should be a useful tool to elucidate the activity of enzymes of unknown function, and will be especially interesting for those targets where bioinformatics methods are not applicable.

## References

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