



# Verification of the $K_{cat}$ Value for AAT via

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## Introduction

Although light activated enzymes such as DNA photolyase do exist, the vast majority of enzymes do not require the absorption of light for catalytic activity. In some cases, however, light can initiate biological activity such as the CO dissociation which can be initiated in hemoglobin and myoglobin by blue light absorption. This brings up the question of whether light excitation can generally affect the catalytic activity of chromophoric enzymes by accelerating cofactor-dependent rate-limiting steps.

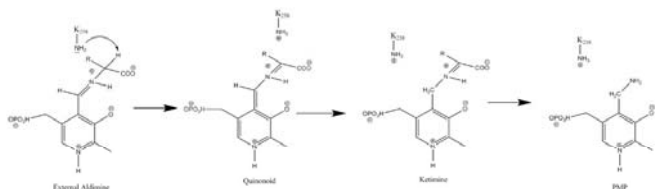
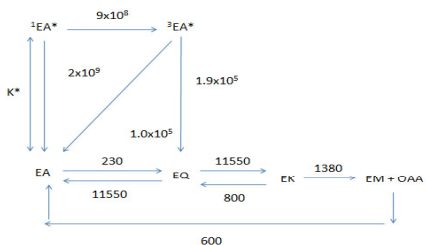


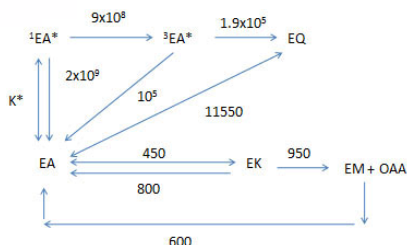
Figure 1: Mechanism of the Transamination Half-Reaction

## Pyridoxal 5'-phosphate (PLP; Vitamin B6) and Aspartate Aminotransferase (AAT)

PLP is a chromophoric cofactor required for catalytic activity by a wide variety of enzymes. While PLP enzymes are thermally activated *in vivo*, it has been reported that some PLP enzymes can be activated by UV light. Previous studies with aspartate aminotransferase (AAT) suggest that the carbanionic quinonoid intermediate is photogenerated by UV laser excitation. AAT is central to nitrogen metabolism in all living systems and has a large body of literature. As such, it is a useful prototype for fundamental studies on this class of enzymes, and was used by Melissa Hill in her paper "Light-Enhanced Catalysis by Pyridoxal Phosphate-Dependent Aspartate Aminotransferase" *J. Am. Chem. Soc.*, 2010, 132 (47), pp 16953-16961



Model 1: The quinonoid is on the productive pathway.

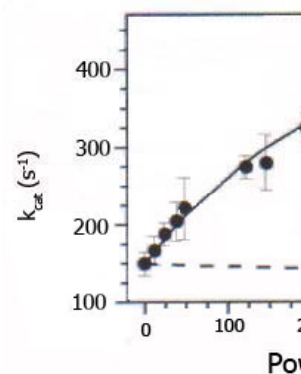


Model 2: The quinonoid is off of the productive pathway

## Verification of Experimental $K_{cat}$ value

In order to verify Melissa Hill's experimental results, the computer program COPASI was used. COPASI stands for Complex Pathway Simulator and is used for solving mathematical models of biological processes. It is a software package that was developed in the early 1990s as a result of an international collaboration between the University of Heidelberg (Germany), the Virginia Bioinformatics Institute (USA). Current development efforts are supported by a grant from the German Ministry of Education.

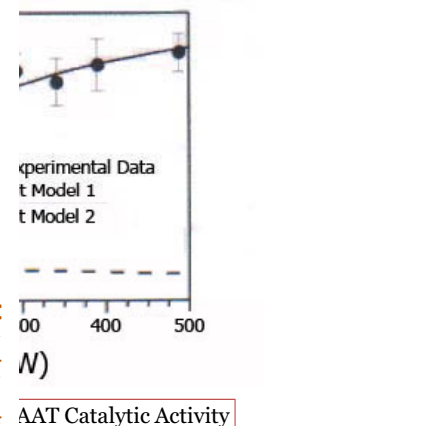
The two models of AAT mechanisms were used to compare the two species and reactions of the mechanism as well as the reactions that would take place. Each reaction was assigned a rate law which was either reversible or irreversible depending on the reaction.



Graph 1: Power Dependence of  $k_{cat}$  (s<sup>-1</sup>)

## via COPASI

the computer program COPASI will be used to verify the experimental data and is an open source application for solving mathematical models of biological processes. It is based on the GEPASI simulation software developed by Pedro Mendes. COPASI is the result of an international collaboration between the University of Manchester (UK), the University of Heidelberg (Germany), and the Virginia Bioinformatics Institute (USA). Current development efforts are supported by a grant from the National Institute of Health and the German Ministry of Education.



Graph 2: Comparison of experimental data and Model 1/2 for AAT catalytic activity

## Conclusions

Unfortunately, the complexity of COPASI and the limited time available prevented the successful verification of the experimental data within the time allotted to this internship. Verification of the  $K_{cat}$  value is planned for the near future.

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