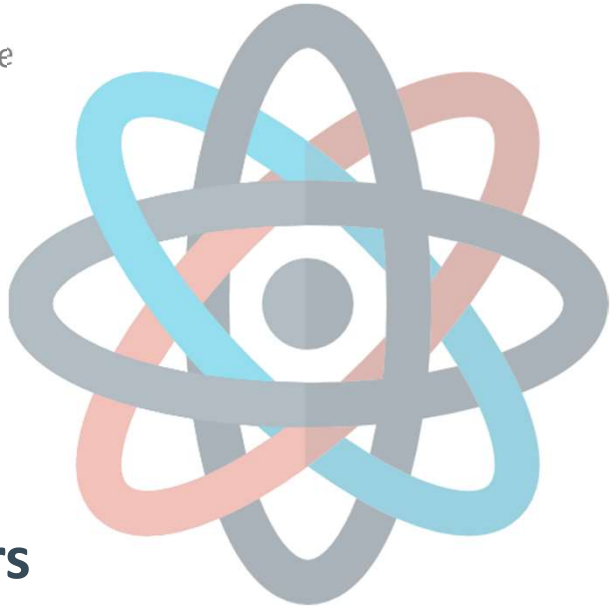

# Quantumzyme

## Catalyze your business

# Overview

01 **About Us**
The context to what we do

02 **Technology**
Who we are
What makes us tick



03 **Collaborators**
Brief about the Qzyme Workbench

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Overview of success stories

# Vision and Mission

## Vision

Clean and Green

## Mission

Simplify complex Chemistry

QM enabled Biotransformation

# QZyme Workbench™

**THE HOME GROWN POWERFUL FRAMEWORK FOR ENZYME ENGINEERING**

While the nature of each project may differ, Quantumzyme executes engagements with a methodology that is tailored to achieve best results and meet Customer expectations

Each project is executed with our powerful QZyme Workbench to make our engagements successful

**QZyme Pilot™**

Initial hypothesis framing from key intelligence and information assimilation based on data, leads from a novel suggestion to an effective proposition

**QZyme Modeller™**

Developing high confidence 3D model in the absence of accurate structural information by applying algorithms best suited for each case

**QZyme CatMec™**

In-depth comparison study of the catalytic mechanism of the enzyme to lay the foundation for a robust enzyme development strategy

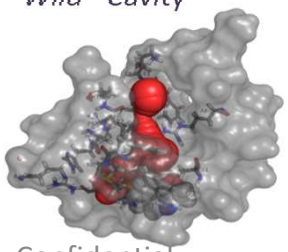
**QZyme Hotspot™**

Discovery of key functional residues that contribute to substrate binding, transition-state stabilization and product release and estimating their

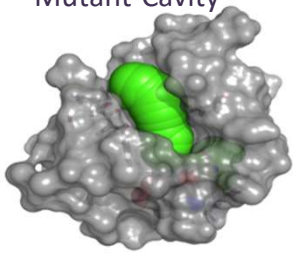
**QZyme Designer™**

Creation of a focused library of variants of optimizing functional hotspots and in depth study of the catalytic mechanism of hundreds of variants designed to

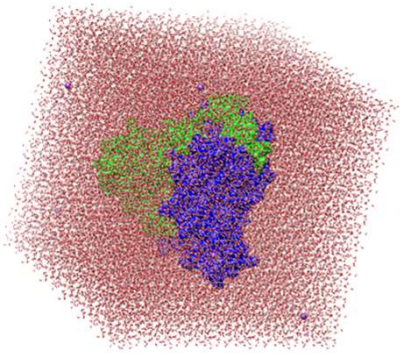
Wild - Cavity



Mutant-Cavity

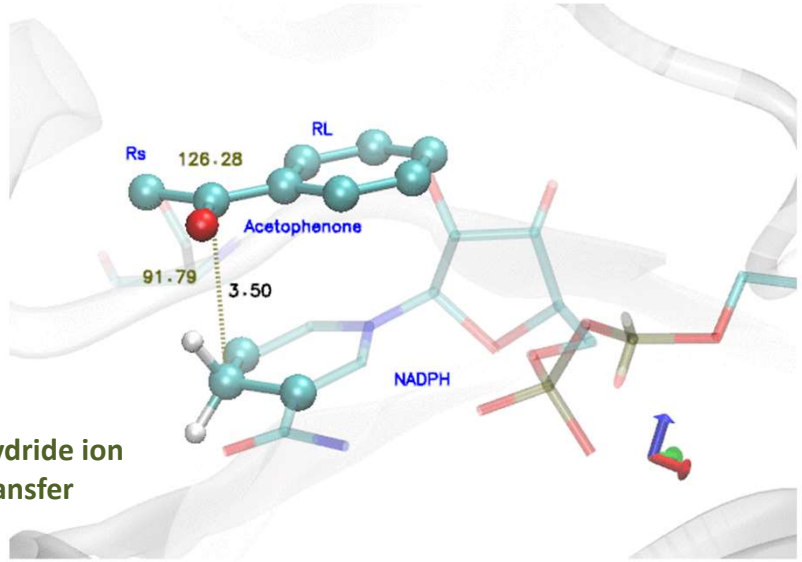


# Execution Methodology

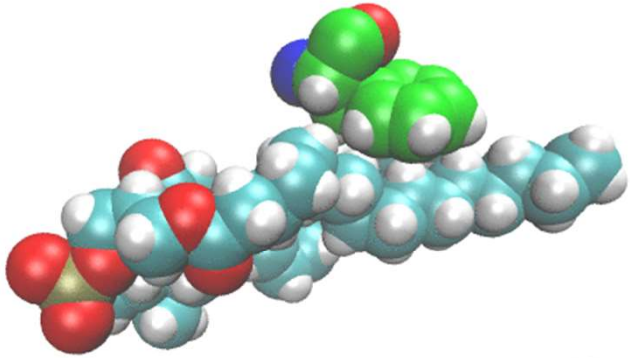


- Identification and Classification of the requirement / problem statement
- Analysis of standard and non-standard use cases within the requirement
- Preparation of parameters / inputs for the QZyme Workbench
- Structured workflow and execution of the project with the QZyme Workbench

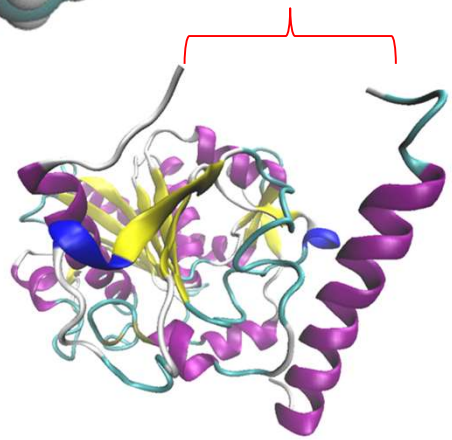
# We see what others don't




Hydride ion transfer

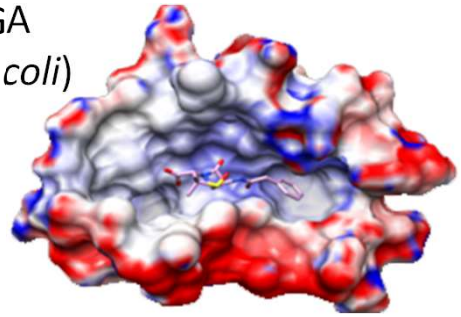


Missing region



PGA (*E.coli*)

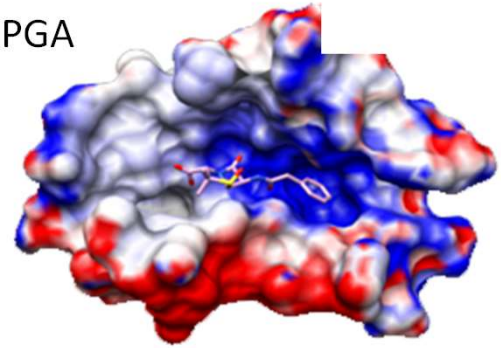
Hydrolysis 100%
Synthesis 25%



Sequence similarity 90%

Active site is 100 % identical

XPGA

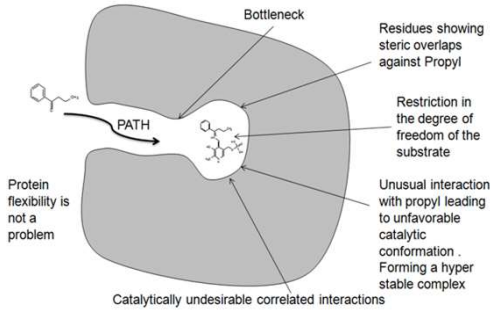


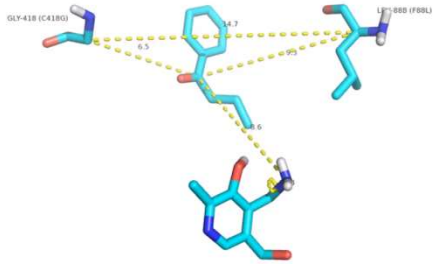
Hydrolysis 100%
Synthesis 50%

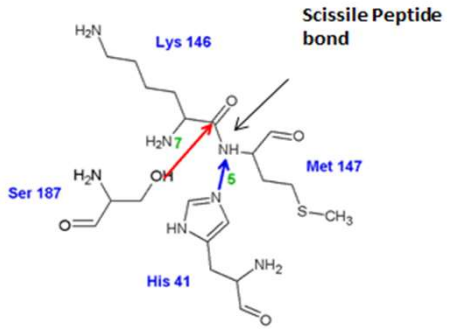
- *Simulation studies show clear change in the electrostatic potential of XPGA compared to PGA*
- *The XPGA having a stronger positively charged active site.*

# Some Benchmark Results

1. 2 log increase in the activity of protease
2. 150 fold increase in the turnover number of transaminase
3. 99% ee : S form of transaminase
4. New chemical synthesis by transaminase
5. Expanding the substrate scope of Penicillin G Acylase



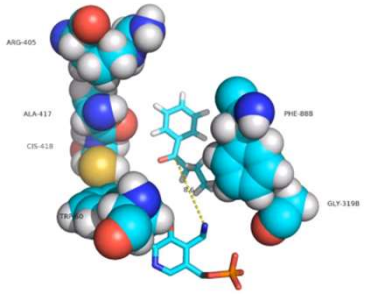





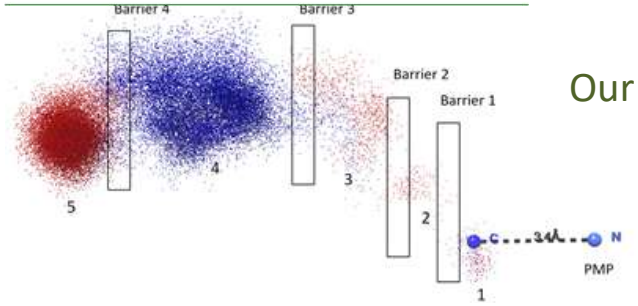

A receptor dependent-4D QSAR approach to predict the activity of mutated enzymes. Scientific Reports, Nature Publication
DOI:10.1038/s41598-017-06625-x

# Why Quantumzyme

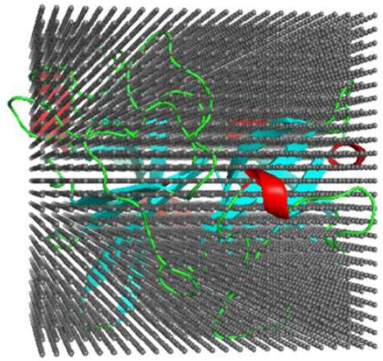
- 4th dimensional view of high resolution details of enzymatic reaction
- MD derived and QM optimised E-S→P reaction coordinates used for alanine scanning
- Unified energy terms to derive activation energy of the enzyme variants



Enzyme limits bulkier substrates




Our findings



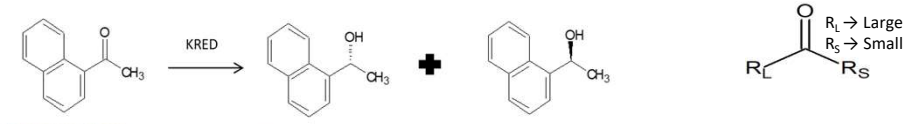
*Grid Based Hotspot prediction*

quantumzyme™

# Case studies

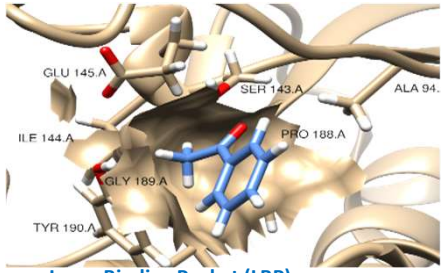
# Producing S-enantiomer variants

**Objective:** Identification of S selective enzymes for a given prochiral substrate from a panel of variant sequences

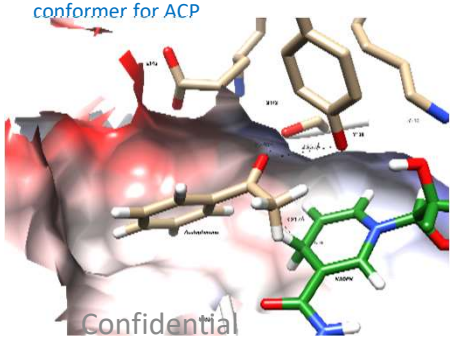



**Results:**

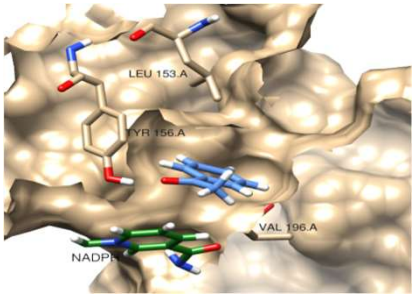
- Total 177 variants of KRED, one being WT and all variants have been modelled
- We have standardized the protocol for Acetophenone and then applied to 1'-Acetonaphthone(ACN)




**Large Binding Pocket (LBP):** Mutations like A94F shrink LBP prevents R-conformer for ACP



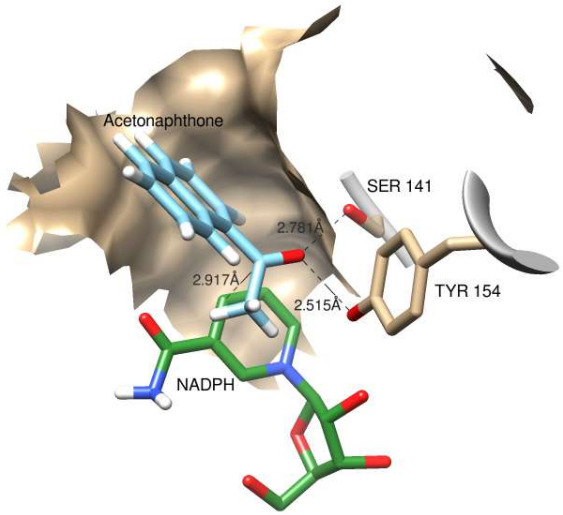
**Small Binding Pocket(SBP):** Native SBP allows R-conformer for Acetophenone(ACP)




**Enlarged Small Binding Pocket (ESBP):** Y190G, E145S allows S-conformer for ACP

**Challenges:**

- Getting Enzyme-Substrate (ES) conformers for each variant
- Understanding the enzyme mechanism by using QM
- Obtaining reaction-specific interaction parameters
- Filtering the reaction-specific conformers and ranking




**S-specific interaction**

**Conclusion:**

- The mutation in the SBP enlarges binding pocket leads to reversal of RL and RS for an S-specific interaction
- QZyme framework successfully identified 60% more S-enantiomer producing variants than the random selection for Acetonaphthone

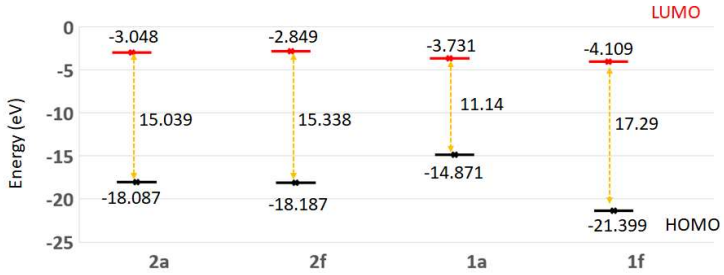
# MD & QC approach to reduce substrate inhibition

**Objective:** To increase enzyme activity by reducing substrate inhibition

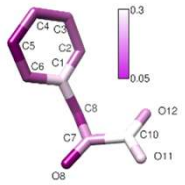
**Challenges:**

- Enzyme-Substrate complex conformation
- Parameterisation of iron for coordination with enzyme
- Multiple transition state for the reaction
- Effect of modification on binding of E-S complex

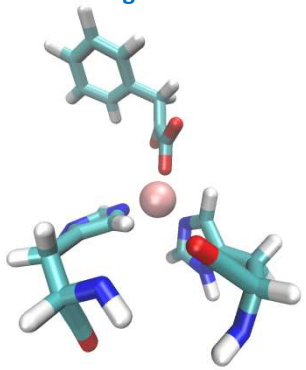
**Results:**

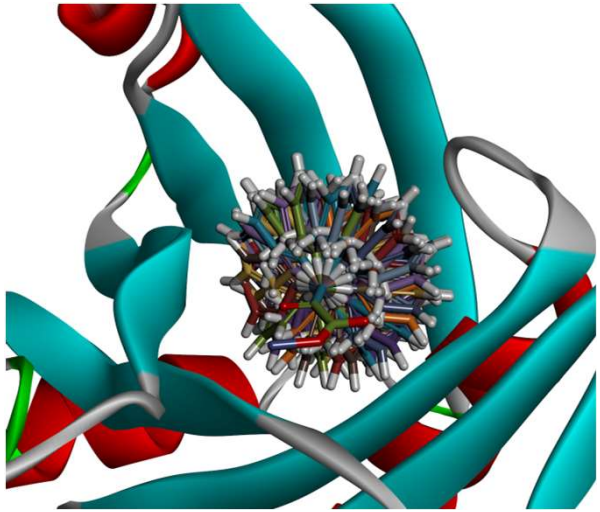



**RMSF heat map of atoms in the substrate**




**Parameterized iron molecule holding the substrate**





**Reference binding modes of substrate generated using QZyme CatMec**

**Conclusion:**

- Obtained optimum michaelis complex
- Parameterisation for iron using QC approach
- Anchoring substrate to reduce instability

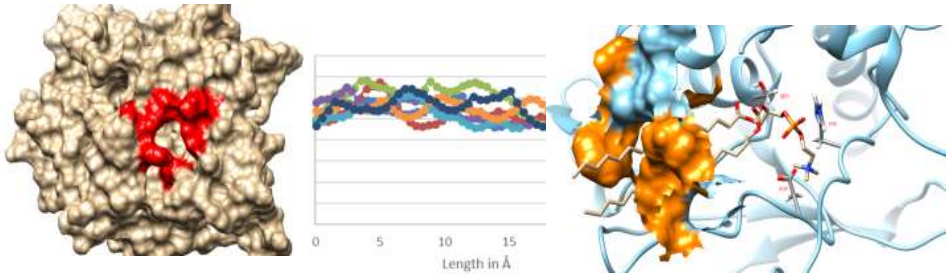
# Engineering Hybrid Phospholipase

**Objective:** To find best possible variants for the enzyme PLA1 to increase its activity by 10 folds

**Challenges:**

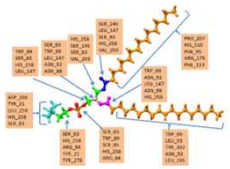
- Missing region in the protein
- Mechanism was not known
- Bigger substrate molecule
- Path for substrate entry

**Path identification for the entry of substrate:**



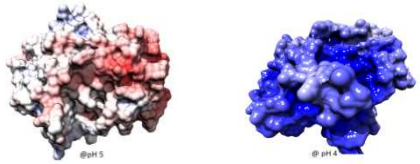

Bottleneck of the substrate entering domain

- The bottleneck radius is 2.38 Å
- The length of the tunnel was 17.5 Å



**Qzyme screener**

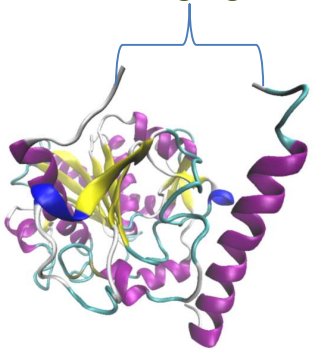
- Enzyme variants screened using Qzyme screener



Normal pH Lower pH

Mutations done based on hotspots of the protein

Missing region

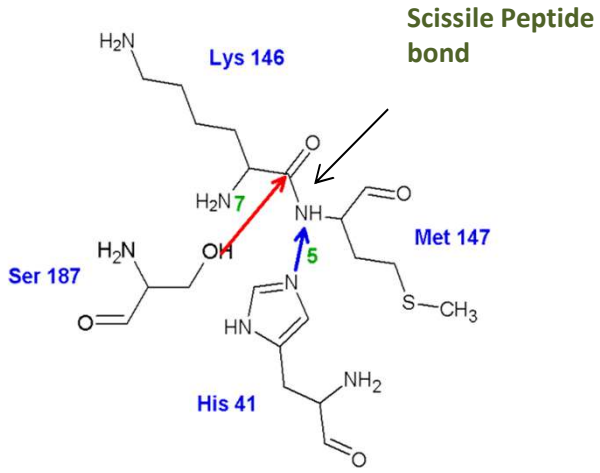


**MD based CAS to find out functional hotspots**

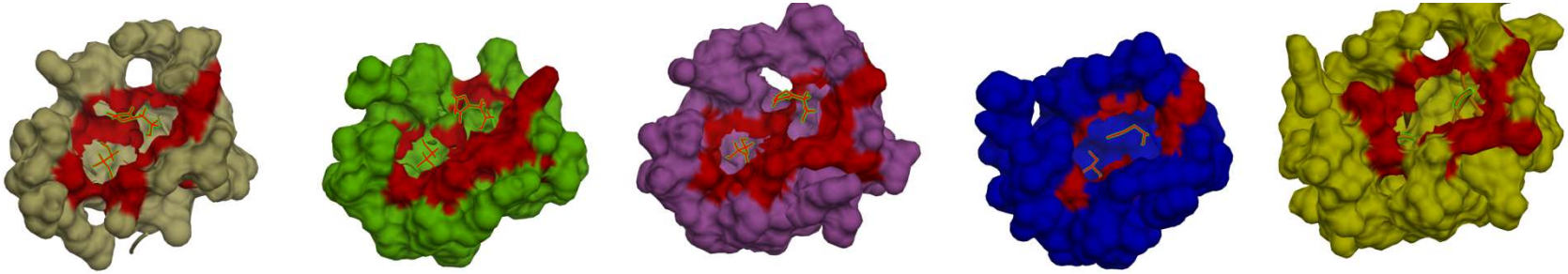
Identified hotspots for normal pH and for lower pH.
CAS was used to find hotspots functions

# Our Experience

- Enetropeptidases – engineered to attain 2 log increase in the activity

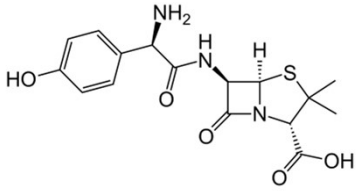



Designed to expose the scissile peptide bond

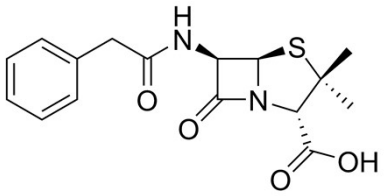


# Our Experience

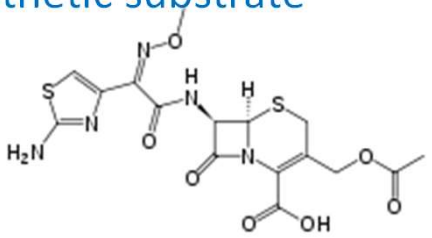
- Penicillin G acylase- engineered to convert semi synthetic substrate




Amoxicillin

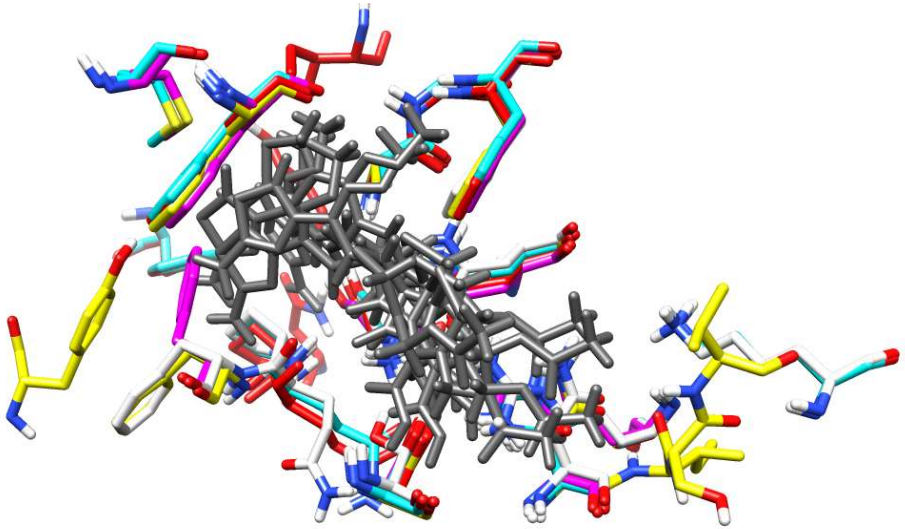



Penicillin G




Cefotaxime

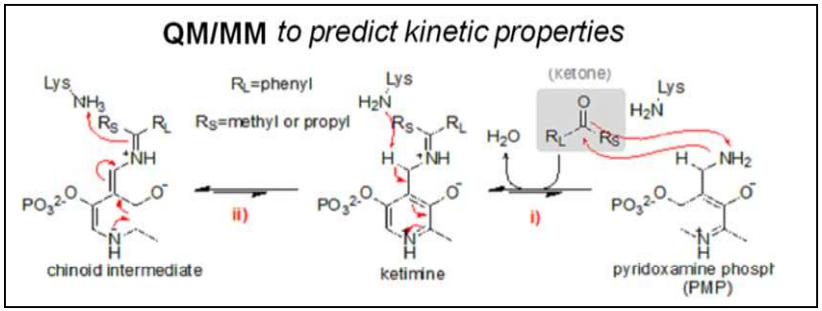
- Penicillin G acylase – engineered to improve the kinetic properties of the enzyme

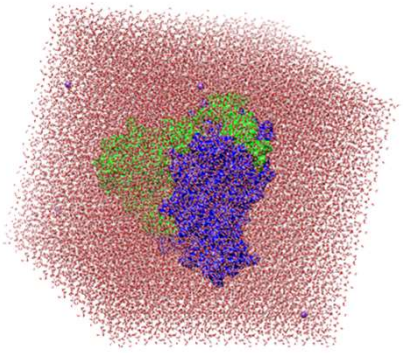


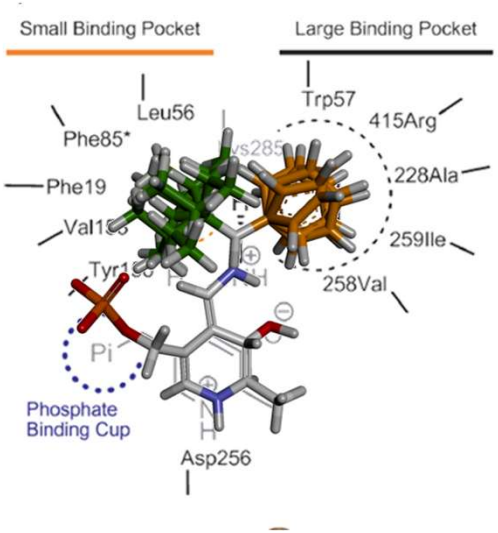
# Our Experience

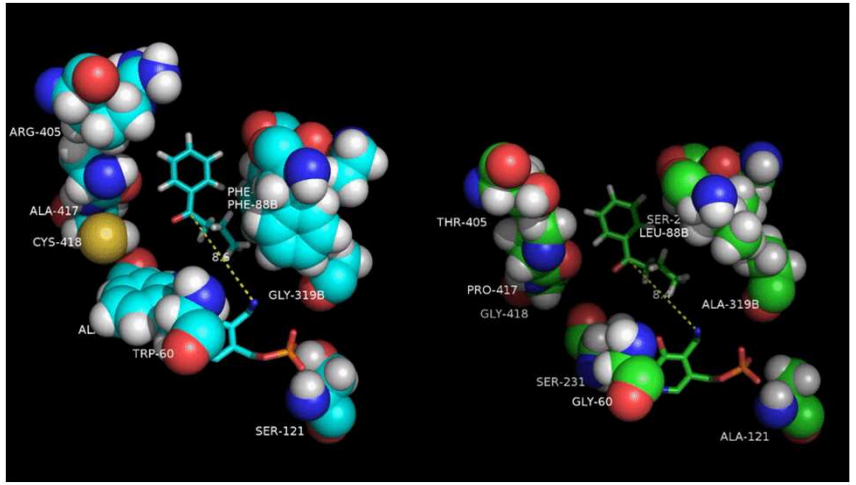
## *Engineered transaminase to expand its substrate scope towards bulky ketones*

The value of drugs containing chiral amines market is estimated to be $88 Billion.
Amine Transaminases (ATAs)are used in the production of chiral amines as an alternative to chemical synthesis to reduce cost incurred by the additional purification steps, inadequate stereo selectivity, and a few other advantages.





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# Collaborations

# Prof. Uwe T. Bornscheuer

**Prof. Uwe T. Bornscheuer**

**Professor at University of Greiswald.** Major research target is the development of tailor-made biocatalysts suitable for industrial applications.

**Enzymes studied :** Transaminases, Baeyer-Villiger Monooxygenases , Esterases/Lipases, Oxidases, Others

**Applications**

1. Synthesis of optically pure compounds, e.g. for pharmaceutical applications
2. Modification of fats and oils, e.g. structured triglycerides
3. Synthesis of detergents, e.g. sugar esters
4. Development of enzyme cascade reactions
5. Investigation of novel reaction systems such as SpinChem

***He has Published 374 articles and written chapters in 34 famous books***

**Major Awards**

- 2015: Stephen S. Chang Award of the American Oil Chemists' Society (AOCS)
- 2014: Wilhelm-Normann-Medal of the German Society of Fat Science e.V. (DGF)
- 2012: Chevreul-Medal from the French Society of Lipid Research (SFEL), Paris, France.

# Prof. Richard A. Gross

**Prof. Richard A. Gross**

**Professor, Constellation Chair, Rensselaer Polytechnic Institute.** His work lies at the interface between Chemistry and Biology; united by the common theme of sustainability and green chemistry. He is developing next-generation bio-based chemicals and materials by processes that are safe and environmentally friendly

**Enzymes studied :** Lipase, cutinase, mono-oxgenase (P450), and metallo-proteins, Others

**Applications**

1. Dielectric structural materials for energy storage
2. Biocomposites
3. Amphiphilic polymers for delivery of fragrants/drugs/flavors
4. bioresorbable polyester implant materials
5. Enzyme catalysts for polymer recycling/modification
6. Tissue engineering matrices for regeneration of articular cartilage and spinal cord repair.

***He has about 600 publications that have been cited over 21,000 times (h-index 76, i10-index 276).***

**Major Awards**

- **2018: ACS Award for Affordable Green Chemistry**
- 2017: Lifetime achievement award by the Bioenvironmental Polymer Society (BEPS)
- 2014: Fellow of the ACS Polymer Division
- 2010: Turner Alfrey Visiting Professor
- 2003: Presidential Green Chemistry Award

# Prof. Lawrence P. Wackett

**Prof. Lawrence P. Wackett**

**Distinguished McKnight University Professor, University of Minnesota.** His work is focused on Biodegradation, Commercial bioremediation, Industrial biocatalysis, Hydrocarbon biosynthesis & Enzyme mechanisms

**Enzymes studied :** Cyanuric acid hydrolase, Others

**Applications**

1. Cyanuric acid hydrolase – Enzyme structure/mechanism and applications
2. Fate of agricultural chemicals and water protection
3. Emerging pollutants - Water remediation of personal care products (PCPs)
4. Aromatic hydrocarbons – Modeling and mechanisms

***He has more than 400 publications that have been cited over 17,000 times (h-index 67, i10-index 172).***

**Biocatalysis**

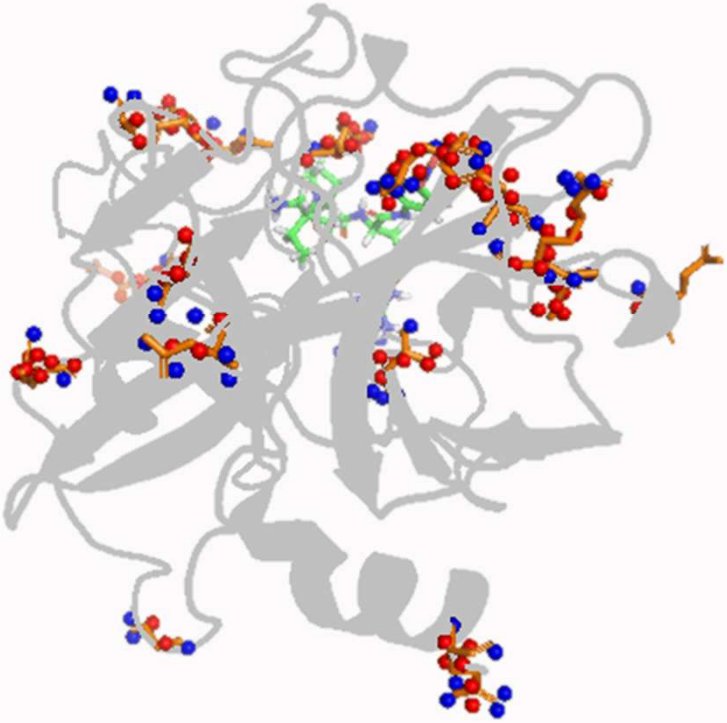
- Biosynthesis of beta-lactone natural products
- Hydrocarbon biosynthesis – Enzyme structure and mechanisms
- Enzyme-based sensors for detecting toxicants
- Predicting biocatalytic potential of enzymes and microbes

# Engagement Model

***Fee For Service – Project to design/engineer the enzyme of interest***
***Milestone based – Risk sharing and payment based on success***

## Requirements from sponsor

- ✓ Clearly define the objectives of the study
- ✓ 3D structure of the enzyme of interest
- ✓ Mode of binding and kinetic properties of the enzyme-substrate reaction
- ✓ Structure of enzyme-substrate complex
- ✓ Reported structure of enzyme complexed with other active substrates OR inhibitor/s
- ✓ Specify process conditions of the reaction
- ✓ Available literature study



# Partial Customer List







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# THANK YOU

**info@quantumzyme.com**