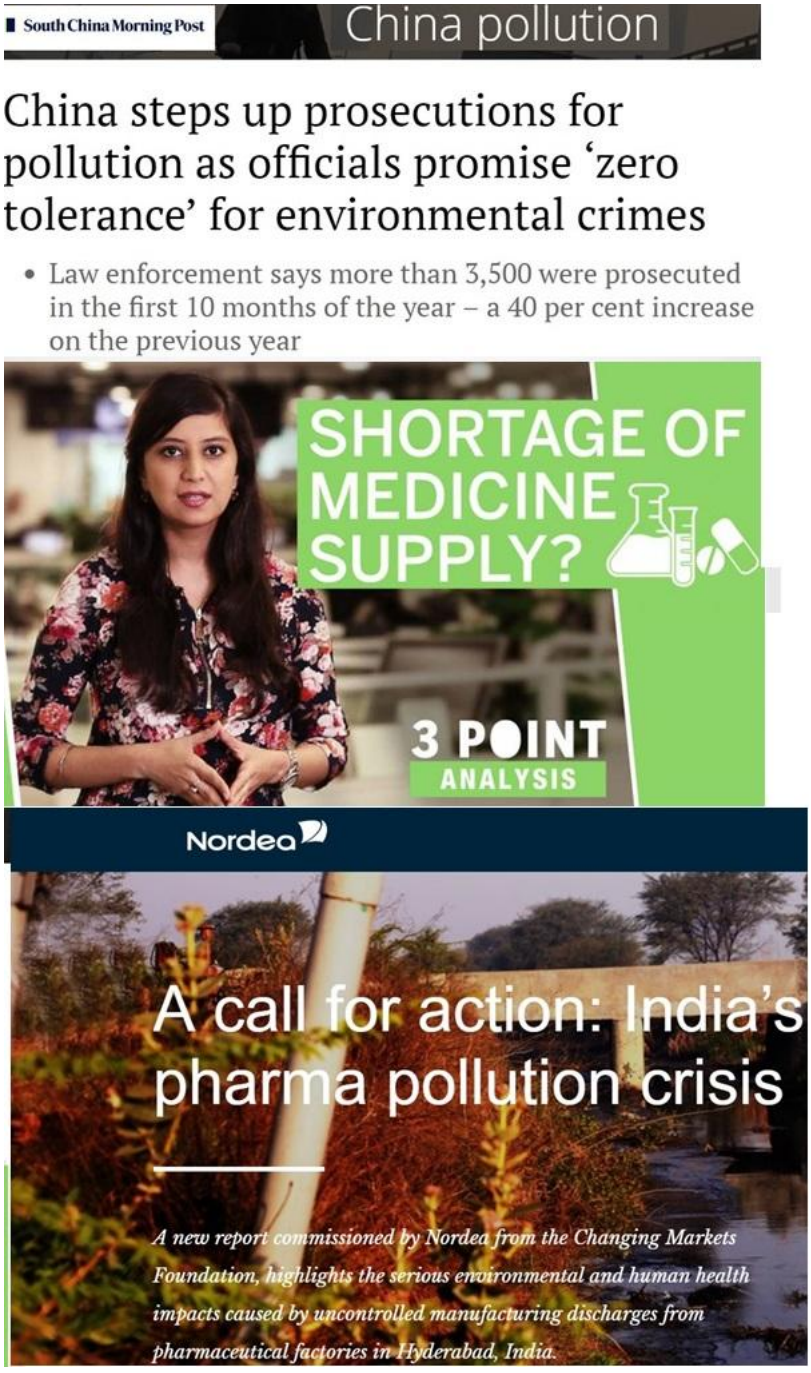
Green and Clean Chemistry – Engineering a sustainable world *In-silico*
Jun 04, 2019



India has become a dump yard for global pharma manufacturing. All the stakeholders, ranging from government to businessmen, profit from the outcomes except the locals who suffer silently.

Green and Clean Chemistry – Engineering a sustainable world *In-silico*

The grand challenge facing the chemical and allied industries in the twenty-first century is the transition to greener, more sustainable manufacturing processes that efficiently use raw materials, eliminate waste and avoid the use of toxic and hazardous materials. It requires a paradigm shift from traditional concepts of process efficiency, focusing on chemical yield, to one that assigns economic value to replacing fossil resources with renewable raw materials, eliminating waste and avoiding the use of toxic and/or hazardous substances. The need for greening of chemicals manufacture is readily apparent from a consideration of the amounts of waste generated per kilogram of product (the E factors) in various segments of the chemical industry.

The following are just a few of the many examples of how enzymes are improving industrial chemical processing. Nitrile hydratases have now supplanted an earlier Cu based method for converting acrylonitrile to acrylamide, the monomer used to prepare polyacrylamide. This process is now conducted on tens of thousands of metric tons per year.

Enzymes can be thought of as renewable catalysts. Unlike metal-based alternatives that rely on mining and harsh, energy intensive processing, biocatalysts are biodegradable and easily replaced through inexpensive and environmentally benign fermentation processes. The minimal environmental and economic burden posed by enzymes is further diminished by the potential to engineer even more active variants than can be found in nature, to optimize expression efficiencies and fermentation yields, and to immobilize and reuse these powerful catalysts.

The widespread adoption of modern protein engineering has led to a number of remarkable applications. Substrates which are dramatically distinct from natural ligands are now able to be transformed with efficiencies suitable for industrial applications.

At Quantumzyme, we have developed an in-house framework - **QZyme Workbench™**, which integrates free and opensource software, as well as our own code to provide a methodology that allows us to engineer enzymes *in-silico.* This has the potential to significantly reduce the time and raw material resources needed to produce enzymes with the desirable properties.

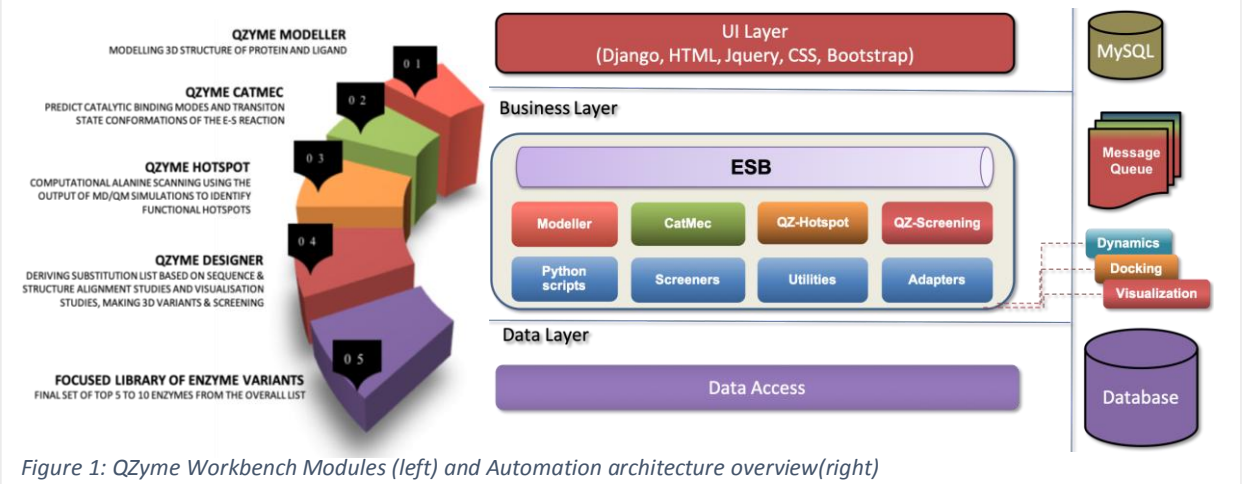


Figure 1: QZyme Workbench Modules (left) and Automation architecture overview(right)

The QZyme Workbench has several components. Some or all components may be used as per requirement.

Quantumzyme works with Chemical and Pharmaceutical companies in India and overseas to solve problems of Biocatalysis and to improve the productivity of the enzymatic reaction.

We present below a recent success story that was published in one of the premier international journals ACS Catalysis.

There are many factors which may affect the activity of the enzyme. One of the most studied and well-known in-silico methods is to obtain the Michaelis-Menten (MM) complex using the methods commonly called Docking, where the binding of the ligand substrate within the active site is engineered. Generally, lower the binding energy, better should be the activity. However, other factors do play a role. How does the substrate enter the active site of the enzyme? How does it attain the MM complex position? Is there a possibility of an alternative position which may not allow the reaction but still bind strongly? The following case study, which is published[2], shows how we have used QZyme Workbench to provide engineered mutants for an enzyme which a lab had struggled to do using in-house methods. In this case, the QZyme Workbench looks solely at the approach tunnel which determines the path for entry of the substrate from outside to inside the active site.

The enzyme amine transaminase from organism *Chromobacterium violaceum* works on small substrates such as acetophenone(1a in Figure 3). However, if the chain length of the substrate is increased, i.e. using propiophenone (2a) or butyrophenone(3a), the activity of the enzyme decreases significantly. What is notable here is that the phenone component of the substrate is the same in all the substrates. Thus the bound substrate in the MM complex are comparably good low-energy bound forms for all of these substrates. The binding of the substrate, which is often the traditional in-silico method was thus not effective here. The QZyme Workbench was thus used here for a different purpose. The entry of the substrate into the active site was looked at in detail. This project was driven by Dr Üwe T. Bornscheuer and his team from Greifswald University in Hamburg, Germany, who is also chairman of the board of directors of an enzyme company: Enzymicals Ag,. The enzyme variants were expressed and tested in their labs. In silico modelling work and enzyme engineering was done by Quantumzyme as follows:

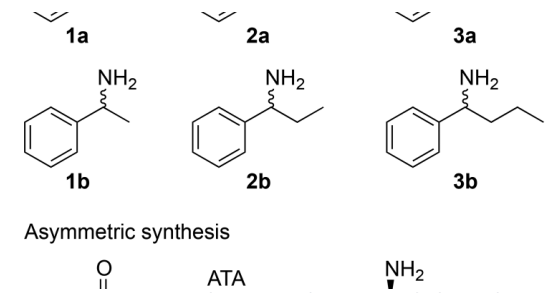


Figure 2: 1a - Acetophenone substrate and 1b Product
2a – Propiophenone and 2b – corresponding product
3a – Butyrophenone and 3b – corresponding product

A thorough molecular dynamics simulation of the substrate within the active site was done. This study showed the substrate moving out of the active site. Clustering of the positions of the substrate show that there are intermediate regions between MM complex position and the position

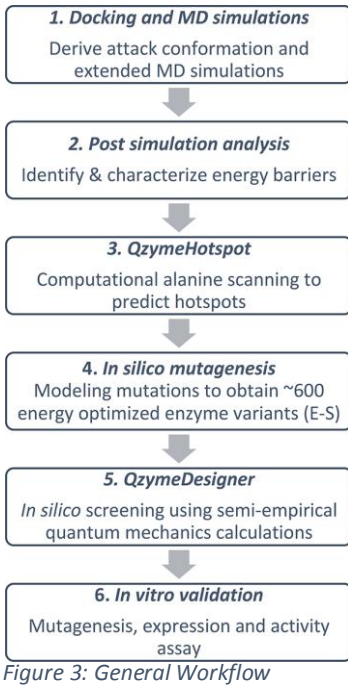


Figure 3: General Workflow

outside the active site where there are large clusters, indicating that the substrate remains comfortably at these cluster positions before eventually coming out. Between these clusters however are regions where the substrate does not remain. These regions represent bottlenecks. Ideally the substrate should move comfortably from point A outside the active site to point B which represents the MM complex position for reaction inside the enzyme. However, that is not happening. There are jumps, where the substrate moves from Point A to a point A1, then a point A2 and then to B. A bottleneck is seen between A and A1, A1 and A2, and A2 and B. This bottleneck may be due to a large residue on the path.

QZyme Workbench was used by innovatively adapting the geometry optimization routine in a semi-empirical Quantum mechanics (QM) software called MOPAC to study the path from A to B. The QZyme workbench can also use free QM software such as CP2K in a similar way. The method has been automated and is iterative. Using the method we were able to detect the bottleneck residues which cause hindrance to the entry path of the substrate. Mutations with smaller residues were then tested using the framework. In the process, two double mutants (F88L/C418(G/L)) were discovered, which when tested in the lab, were shown to give 200-fold increase in the production of 1-phenylbutylamine with high stereo-specificity.

The methodology used here shows one unique application of the QZyme Workbench. The mutations obtained from this framework may then be tested in the lab or used as starting points for further engineering using established evolutionary engineering methods. This can significantly decrease the iterations needed for enzyme engineering and thus the cost of the process.

1. **Nobel Prize 2018 : Frances H. Arnold** *"for the directed evolution of enzymes"* California Institute of Technology, Pasadena, USA
2. **In Silico Based Engineering Approach to Improve Transaminases for the Conversion of Bulky Substrates** Moritz Voss, Devashish Das, Maika Genz, Anurag Kumar, Naveen Kulkarni, Jakub Kustosz, Pravin Kumar, Uwe T. Bornscheuer, and Matthias Höhne *ACS Catalysis* **2018** *8* (12), 11524-11533 DOI: 10.1021/acscatal.8b03900
3. **Introduction: Biocatalysis in Industry**
4. **Engineering a more sustainable world through catalysis and green chemistry**