DTU Chemistry
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# ReaxFF modelling of enzyme catalysis

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**Development of new methods capable of describing chemical reactions fast and accurately is an important research area. Here the development of the ReaxFF method and recent applications within enzyme catalysis is presented.**

## Introduction

**Development of good, reliable and fast methods for *in silico* prediction of reactivity from first principles could potentially revolutionize many disciplines within chemistry and biology, such as: Discovery of new catalysts, catalyst optimization or discovery of new pharmaceuticals. However, for these methods to be a valuable tool they must be several orders of magnitude faster than current state-of-the-art methods (fig. 1), which rely on quantum mechanics (QM) and density functional theory (DFT), and be more accurate than molecular mechanics methods (MM). The reactive force field, ReaxFF [1], is capable of describing chemical reactivity orders of magnitude faster than QM or DFT, and is furthermore very easy and fast to set up.**

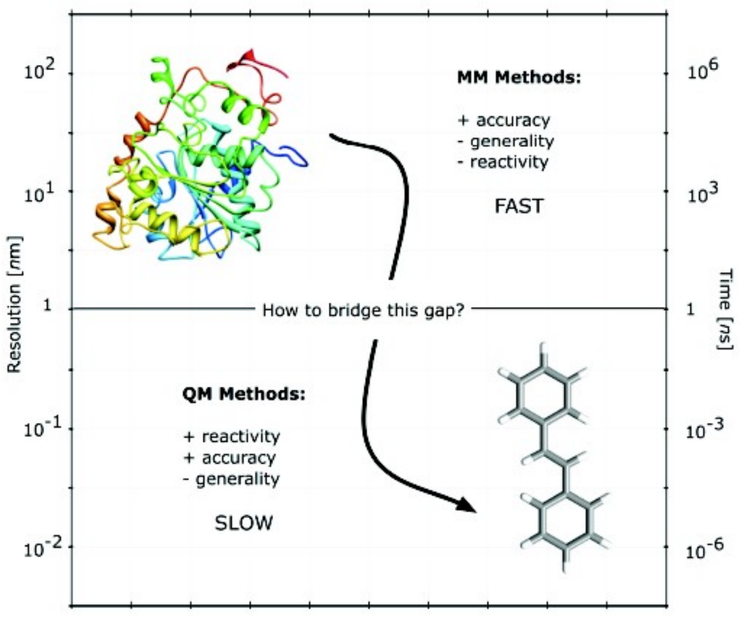


**Figure 1: ReaxFF will have an improved speed/accuracy ratio.**

## Biological model system

***Subtilisin Savinase*® is an enzyme utilised commercially as a component of washing powders, added to remove dirt in the form of proteins. Subtilisins are serine proteases capable of catalyzing hydrolysis of peptide and ester bonds, by binding the substrate in the so-called catalytic triad consisting of a serine, a histidine and an aspartate. The catalytic triad works in a well-understood manner (fig. 2), initiated with a nucleophilic attack by the serine oxygen on the carbonyl carbon of the amide or ester substrate. The serine oxygen is nucleophilic because the histidine acts as a base, thereby removing the proton from the serine during the process [2].**

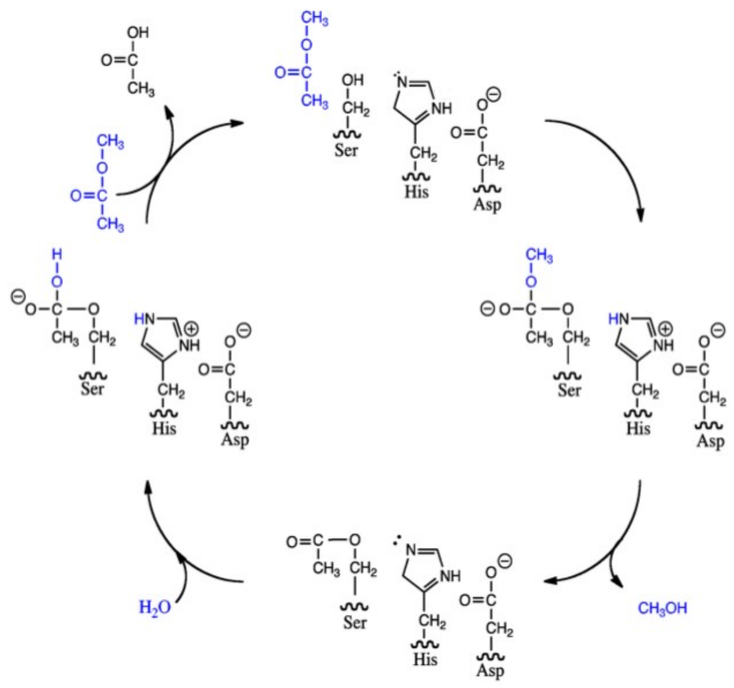


**Figure 2: Subtilisin works in a well-understood manner.**

## Green computer chemistry

**Modelling of chemical systems with the help of a computer has a lot of advantages compared to what people normally think chemistry is (that is synthesis). Computers can be used to model complicated chemical reactions a lot faster, than the time an ordinary experiment would take. This gives one the opportunity to study many different substitutions patterns and mutations, that is possible improvements, of molecules. This can be used as a guideline for selecting the reactions that should be checked in the laboratory – synthesis is still needed to prepare the molecules and furthermore the computational predictions could be inaccurate. In general the ReaxFF method could be used to study every chemical reaction and improve them thereby lowering the amount of experiments needed. In addition to being time-saving, this also reduces the costs and is beneficial to the environment due to lower use of chemicals and reduced waste treatment**

## First results

**To get a better understanding of how *Subtilisin Savinase*® works and can be optimized, the first rate-determining step of the reaction is modelled. The initial studies is carried out using DFT, which is capable of describing the reaction accurately, but very slow - therefore only a small test-system consisting of the catalytic triad and a very simple substrate is used. Because the reaction is relatively complicated two reaction coordinates are used – one for the distance between the carbonyl carbon of the substrate and the serine oxygen (OC) and one for the distance between the nitrogen and hydrogen of the histidine (NH). By varying these two coordinates a ReaxFF energy surface for the reaction can be constructed (fig. 3). The energy surface ”determines” whether the reaction will proceed, what the products will be and how big an activation energy there is needed. The surface obtained by using ReaxFF are similar in shape to the DFT, but calculated a lot faster.**

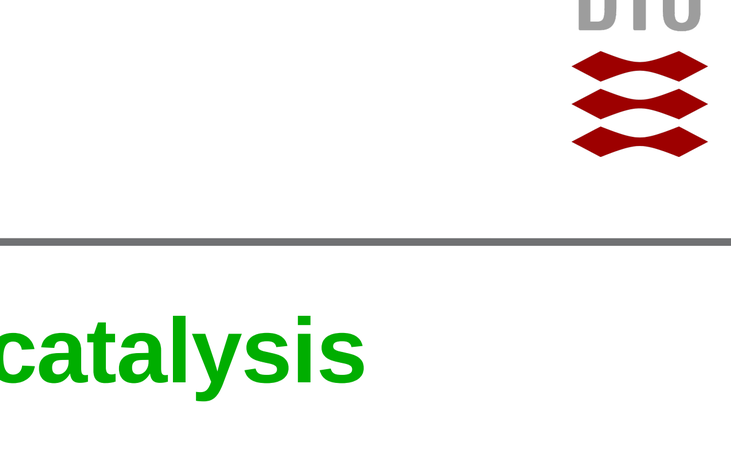


**Figure 3: ReaxFF energy surface for the first step of the hydrolysis. The reactants are in the lower right corner and the tetrahedral intermediate is in the upper left corner.**

## Future studies

***Subtilisin Savinase*® is constantly being engineered to improve its performance (e.g. selectivity, thermal working range and thermo- and pH-stability) – this is generally done by “random” mutations. By understanding the reaction mechanism in more detail one can come up with better “guesses” of what would improve the enzyme's performance. ReaxFF is a very fast method which should be accurately enough to describe the outcome of mutations, and thereby be a valuable tool for the biochemist to select which reactions should be tested in the laboratory.**

**Figure 4: ReaxFF can make washing more environmentally friendly.**

## Acknowledgements

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**References**

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