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Chapter 1

Working title: Calculations

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1.1 Motivation

Let us imagine two companies A and B. Both companies use very similar technical equipment to carry out a biotechnological process where a chemical reaction is catalyzed by an enzyme. Company A uses an enzyme with a rate constant $k_A = 1000s^{-1}$ while company B uses an enzyme with $k_B = 2000s^{-1}$. Letting all other things be equal, the process of company B will therefore only require half the time to produce one Mole of product compared to the time required for company A. Company B therefore can save energy required to heat up the reaction volume, the commercial implications of this are immediate.

The need for efficient catalysts arises from such an outline.¹ Increasing the performance of enzymes however is still far from

¹We use the terms *enzyme* and *bio-/catalyst* interchangeably.

trivial and forms a growing body of research. What is clear though is that the development of such catalysts is costly, in terms of manpower, material and energy – if it is carried out in the laboratory. A number of companies have in fact formed around this demand: Novozymes (DK), Genzyme (US) or DSM (NL) to name but a few[Meyer *et al.* (2013)].

The laboratory costs can however be saved to a large part if the development is carried out *in silico*. The proof that computational results are as reliable as experimental results has been provided not too long ago[Claeysens *et al.* (2006)].

1.2 Introduction

We provide an introduction into the topic of enzyme catalysis modeling for interested people from inside and outside of the field.

1.3 Methods

A variety of methods has been established for the use to model enzyme catalysis. Depending on the properties of interest, the system is treated differently. Molecular mechanics methods allow to study the structural behavior of the enzyme over a significant time period and provide details about rearrangement of loop motives. The description of chemical reactivity however requires the description of the electronic structure of the system (molecular mechanics models do not allow the description of bond formation and braking processes). In this regime, again a number of specialized methods have become available.

Practical applicability of QM based enzyme screening has a couple of problems:

- The structures of the enzyme in the relevant state of the reaction need to be available, ie. an enzyme-substrate complex (ES) and a transition state (TS) analog (usually an inhibited species)

- If the mechanism is not established, the reaction can usually follow various pathways – these would need to be checked in order to find a best guess at the rate determining step
- Identification of the transition state is difficult and depends intimately on the structure, optimizing two structures differing by 0.1 Å in a particular bond length can result in identification or not identification of the TS
- *Every method has it's chemistry.* The results of one QM chemistry need to be verified, usually high-level standard reference methods are computationally too demanding to be applicable to a large system
- Modeling software is available, PyMOL, Schrödinger, Chem?? (Walter Thiel, commercially) but everything is still very much custom work, this is probably the major reason preventing the use of QM as standard method in industry. Compare docking and MD methods have software which is designed towards user friendliness and has become established in industry

1.4 Applications

We have presented recently a number of studies where new functionality has been introduced into an enzyme active site using quantum chemical methods[Hediger *et al.* (2012, 2013a,b)].

Rational arguments alone are not sufficient to design new reactivity in an active site. Put simply, enzymes do not behave in the way the engineer hopes for. Much rather, the only meaningful strategy proves to be to screen a large number of variant candidates for apparent activity of the desired sort. In a second step, identified lead candidates are subjected to higher level computational methods and wet-lab experiments which then allow to further establish or dismiss the nature of the candidate. Based on the vast mutational space available, initial *in silico* screening has to be efficient. Hediger *et al.* therefore chose to use semi-empirical methods for the description of the electronic structure of the enzyme-substrate complex. In their approach, the activities of

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different variants of an enzyme are ordered by comparing the activation energy required for the rate determining step of the overall reaction – low activation energies correspond to high anticipated activity of the variant.

1.4.1 *Candida antarctica* Lipase B Engineering

Introducing amidase functionality into the lipase of *Candida antarctica* (CALB).

CALB is used as a versatile catalyst in a variety of applications among which enzymatic kinetic resolution of enantiomeric mixtures is of high pharmaceutical importance[Gotor-Fernández *et al.* (2006)]. While CALB is also known for its reactive promiscuity[Svedendahl *et al.* (2008)], if the catalyst can be engineered such as to increase its performance for certain of these applications, possible bottle-necks in production could be widened.

1.4.2 *Bacillus circulans* glycoside hydrolase engineering

As a proof of concept and to show general applicability of the approach outlined for CalB, the glycoside hydrolase of *Bacillus circulans* is engineered to be catalytically active towards an artificial substrate.

Bibliography

- Claeyssens, F., Harvey, J., Manby, F., Mata, R., Mulholland, A., Ranaghan, K., Schütz, M., Thiel, S., Thiel, W. and Werner, H. (2006). High-Accuracy Computation of Reaction Barriers in Enzymes, *Angewandte Chemie* **118**, 41, pp. 7010–7013.
- Gotor-Fernández, V., Busto, E. and Gotor, V. (2006). Candida antarctica lipase b: an ideal biocatalyst for the preparation of nitrogenated organic compounds, *Advanced synthesis & catalysis* **348**, 7-8, pp. 797–812.
- Hediger, M. R., De Vico, L., Rannes, J. B., Jäckel, C., Besenmatter, W., Svendsen, A. and Jensen, J. H. (2013a). In silico screening of 393 mutants facilitates enzyme engineering of amidase activity in calb, *PeerJ* **1**, p. e145.
- Hediger, M. R., De Vico, L., Svendsen, A., Besenmatter, W. and Jensen, J. H. (2012). A computational methodology to screen activities of enzyme variants, *PLoS ONE* **7**, 12, p. e49849, doi:10.1371/journal.pone.0049849, URL <http://dx.doi.org/10.1371/journal.pone.0049849>.
- Hediger, M. R., Steinmann, C., De Vico, L. and Jensen, J. H. (2013b). A computational method for the systematic screening of reaction barriers in enzymes: searching for bacillus circulans xylanase mutants with greater activity towards a synthetic substrate, *PeerJ* **1**, p. e111.
- Meyer, H.-P., Eichhorn, E., Hanlon, S., Lütz, S., Schürmann, M., Wohlgemuth, R. and Coppolecchia, R. (2013). The use of enzymes in organic synthesis and the life sciences: perspectives from the swiss industrial biocatalysis consortium (sibc), *Catalysis Science & Technology* **3**, 1, pp. 29–40.
- Svedendahl, M., Carlqvist, P., Branneby, C., Allnr, O., Frise, A., Hult, K., Berglund, P. and Brinck, T. (2008). Direct epoxidation in candida antarctica lipase b studied by experiment and theory, *ChemBioChem* **9**, 15, pp. 2443–2451, doi:10.1002/cbic.200800318, URL <http://dx.doi.org/10.1002/cbic.200800318>.