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PhD Study
Started: August 2014
To be completed: July 2017

# Modification of polymer surfaces to enhance enzyme activity and stability

**Abstract**
The immobilization of enzymes for biocatalytic reactions is an expanding area both in terms of research as well as industrial applications. The essential challenges are to develop catalytic systems with high temperature stability, tolerance to a larger pH range, improved long-term stability and higher activity. For this purpose, the understanding of the interfacial behavior between enzyme carrier surfaces and enzymes is extremely important. Therefore, this project deals with the modification of polymer surfaces to prepare platforms for immobilization of enzymes with increased enzymatic stability and improved activity.

**Introduction**
Enzyme catalyzed processes have been highly studied during the past few decades and are becoming more valuable due to several advantages over convenient metallic organic catalysis, such as mild reaction conditions, energy efficiency, environmentally friendliness and their substrate specificity and selectivity [1,2]. Nevertheless, one of the main challenges is the retention of enzymatic activity of immobilized enzymes. Immobilization is used to provide the necessary stability and tolerance to solvents and pH, however it generally results in a reduction in the activity of the enzyme. Several studies have been performed during recent years, focusing on recent developments and novelties of enzyme immobilization in general [2,3], the activity and selectivity of immobilized enzymes [4], the industrial potential of immobilized enzymes [5] and the interaction between enzyme and material surfaces [6].

**Objectives**
The overall goal of this project is the investigation of interfacial behavior between polymeric surfaces and enzymes in order to determine influencing parameters for the enzymatic activity and stability of immobilized enzymes. For this purpose, polymeric surfaces will be chemically modified, to prepare materials with specific surface properties, such as a given hydrophilicity or hydrophobicity as well as more advanced properties such as pH or temperature responsiveness. In a subsequent step, enzymes will be immobilized and an enzymatic reaction of the immobilized enzyme will provide data, from which various impact factors for the stability and activity of the enzyme will be determined.

**Enzyme Immobilization**
Several different approaches to immobilize enzymes have been developed, which can be divided into a subset of methods. Cross-linking of enzymes is a method requiring no carrier (Figure 1, a), whereas the entrapment of enzymes into a carrier matrix and the binding on a carrier make use of a support material (Figure 1, b and c). For the latter, the immobilization through physical or chemical binding on a support material has gained high importance.

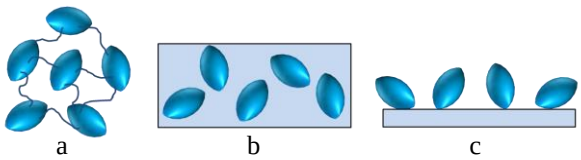


**Figure 1.** Examples of immobilization approaches: a) Cross-linking of enzymes without a carrier; b) entrapment of enzymes in a surface layer and c) direct bonding to a carrier through physical or chemical interaction.

Both methods offer advantages and disadvantages. Physically adsorbed enzymes show higher activity retention compared to chemically bound enzymes, but tend to be more prone to leach out from the support. Covalent bonds between the support and an immobilized enzyme generally lead to a strong binding and a reduced risk of enzyme leaching. However, due to

the fixed conformation of the enzyme, a loss of activity is often observed. In order to identify novel highly efficient immobilization strategies it is necessary to obtain a fundamental understanding of the individual properties of enzyme and support materials as well as the interfacial interaction between both.

**Surface modification for enzyme immobilization**

The surface properties of the support or carrier material can be controlled through introduction of new polymer layers on the surface. Such modifications are generally performed through either "grafting to" or "grafting from" reactions, resulting in the formation of polymer brushes with a specific distribution, density and chemistry [7]. Specifically, it has been found that hydrophobic surfaces and their interaction with enzymes reduce their activity, with lipases being the only exception, which seem to increase their activity when immobilized on hydrophobic surfaces [8,9].

**Covalent Enzyme immobilization**

The immobilization of biomolecules within this new surface layer of polymer brushes can be done by physical interaction or covalent bond formation. For the latter, functional groups from the polymers can be used directly (e.g. epoxide groups, Figure 2, a) or after modification, e.g. the activation of esters with glutaraldehyde or hydroxyl succinimide, maleimide or dithiol pyridyl groups (see Figure 2, b-e, R represents the support material), which enables the polymer structure support to react with different functionalities within the enzymatic structure.

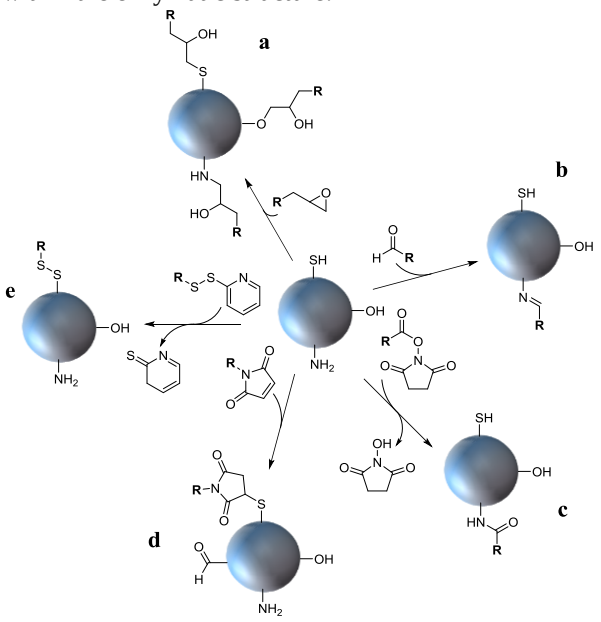


**Figure 2.** Covalent reactions of enzymes suitable for immobilization: a) epoxide groups, b) aldehyde groups (potentially from glutaraldehyde), c) with hydroxyl succinimide, d) with maleimide or e) dithiol pyridyl groups

These are usually residual amino or thiol groups originating from lysine or cysteine in the protein. Site specific binding, such as affinity conjugation can be used for the immobilization of biomolecules in order to immobilize well defined and highly active enzymes. This includes methods such as interaction between polyhistidine (poly-his) and bivalent metal ions [10-14] and the interaction between avidin (or streptavidin) and biotin.

**Conclusion**

For development of novel immobilization strategies of enzymes on surfaces, it is crucial to obtain a broader knowledge of the interfacial interaction between surface and biomolecule. The aim of this project is the investigation of different polymer surface characteristics with regards to enzymatic stability and activity. Ultimately, these findings will be used to prepare a number of novel immobilized enzymes, which will be tested in enzymatic reactor systems.

**Acknowledgements**

The author wants to thank Aage Louis-Hansens Legat for Teknisk Forskning for financial support.

**References**

(1) Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. Nature 2012, 485, 185.

(2) Sheldon, R. A.; van Pelt, S. Chem. Soc. Rev. 2013, 42, 6223.

(3) Cantone, S.; Ferrario, V.; Corici, L.; Ebert, C.; Fattor, D.; Spizzo, P.; Gardossi, L. Chem. Soc. Rev. 2013, 42, 6262.

(4) Rodrigues, R. C.; Ortiz, C.; Berenguer-Murcia, Á.; Torres, R.; Fernández-Lafuente, R. Chem. Soc. Rev. 2013, 42, 6290.

(5) DiCosimo, R.; McAuliffe, J.; Poulose, A. J.; Bohlmann, G. Chem. Soc. Rev. 2013, 42, 6437.

(6) Talbert, J. N.; Goddard, J. M. Colloids Surf. B. Biointerfaces 2012, 93, 8.

(7) Jiang, H.; Xu, F.-J. Chem. Soc. Rev. 2013, 42, 3394.

(8) Shah, S.; Solanki, K.; Gupta, M. N. Chem. Cent. J. 2007, 1, 30.

(9) Petkar, M.; Lali, A.; Caimi, P.; Daminati, M. J. Mol. Catal. B Enzym. 2006, 39, 83.

(10) Kim, Y.-R.; Paik, H.-J.; Ober, C. K.; Coates, G. W.; Batt, C. a. Biomacromolecules 2004, 5, 889.

(11) Balland, V.; Hureau, C.; Cusano, A. M.; Liu, Y.; Tron, T.; Limoges, B. Chemistry 2008, 14, 7186.

(12) Sosna, M.; Boer, H.; Bartlett, P. N. Chemphyschem 2013, 14, 2225.

(13) Cullen, S. P.; Liu, X.; Mandel, I. C.; Himpsel, F. J.; Gopalan, P. Langmuir 2008, 24, 913.

(14) Xu, F.; Geiger, J. H.; Baker, G. L.; Bruening, M. L. Langmuir 2011, 27, 3106.