Enzyme catalysis towards bio-based UV-curable building-blocks

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Akademisk avhandling som med tillstånd av Kungliga Tekniskahögskolan, KTH i Stockholm framlägges till offentlig granskning för avläggande av teknisk doktorsexamen fredagen den 27 september 2019 kl. 10:00 i M3, Brinellvägen 64, Kungliga Tekniska Högskolan, Stockholm. Fakultetsopponent: Professor Katja Loos från The University of Groningen (Nederländerna).

Cover image: *Candida antarctica* lipase B, PDB code: 1LBS

Till Martin och Poppy Matilda

Abstract

Polymeric materials are found in virtually all areas of daily life; they are found in everything from packages keeping our food safe to the buildings where we spend our days, and the production is a worldwide industry. Although polymeric materials play a big part in sustainable solution's, a lot can be done to develop more environmental methods for producing them. Both the process conditions and the resources that go in are important to consider. As more people understand that we need to manage our planet's resources and ecosystem differently the demand for sustainable materials is increasing.

Catalysis is a key for designing chemistry for the environment and an interesting alternative is enzyme catalysis. Enzymes are proteins working as catalysts in biochemical reactions. One of the most prominent features of enzymes' is their selectivity, which means that they have preferences towards forming one product over others. Using enzymes' as catalysts in synthetic chemical reactions the selectivity can be used to produce a wide range of products without side reaction occurring. Further benefits of using enzyme catalysis include high rate acceleration and working under mild reaction conditions.

In the work presented here the selectivity and efficiency of enzymes have been combined with photochemistry in new efficient methods for the synthesis of polymeric materials. The enzymes used were the well-known lipase B form *Candida antarctica* and an esterase/acyltransferase from *Mycobacterium smegmatis*.

The thesis divides into three parts in which three kinds of components were synthesized by enzyme catalysis: (i) unsaturated polyesters; (ii) vinyl ether building-blocks; and (iii) bio-based polyamides. In the first two parts the efficiency and selectivity of enzyme catalysis at low temperatures were utilized to synthesize building-blocks that can be further used for photopolymerization. By using enzyme catalysis structures that can be difficult or even impossible to access with conventional chemistry have been made. In part (iii) photochemistry was used to synthesize a monomer that was polymerized by enzyme catalysis to produce polyamides.

All three parts presented in this thesis show the potential of the combination of enzymes and photochemistry to give access to polymeric materials under benign conditions. The work thus advances the capacity to manufacture building-blocks to create new sustainable polymeric materials.

Sammanfattning

Polymermaterial används till oändligt mycket. Produktion av dem sker i hela världen, men det finns mycket att göra för att tillverka materialen på ett miljövänligare sätt. Det gäller både själva tillverkningsprocessen och vilka råvaror som används i dem. Efterfrågan av förnyelsebara råvaror till denna produktion ökar med medvetenheten om att vi måste hantera vår planets resurser och ekosystemet på ett hållbart sätt.

Katalys är en nyckel för att utforma miljövänliga processer. Till det går det att använda enzymer. De är proteiner som fungerar som katalysatorer i biokemiska reaktioner. En av de mest framträdande egenskaperna hos dem är deras selektivitet. Det vill säga att de har en preferens för att bilda en viss produkt framför andra möjliga. Selektiviteten möjliggör syntes av spännande molekyler, utan sidoreaktioner. Fler fördelar med enzymkatalys inkluderar snabba reaktionshastigheter och möjligheten att utföra reaktioner på ett milt sätt.

I denna avhandling har selektiviteten och effektiviteten hos enzymer kombinerats med fotopolymerisation. Det ger nya effektiva metoder för att syntetisera biobaserade polymermaterial. De använda enzymerna är lipas B från *Candida antarctica* och ett esteras/acyltransferas från *Mycobacterium smegmatis*.

Avhandlingen delas upp i tre delar utifrån vilken typ av komponent som syntetiserats genom enzymkatalys: (i) omättade polyestrar; (ii) vinyleterfunktionella byggstenar; och (iii) biobaserade polyamider.

I de två första delarna kombinerades de selektiva egenskaperna hos enzymer med deras förmåga att utföra effektiv katalys under milda reaktionsbetingelser. Detta för att göra byggstenar som kan reagera vidare i fotopolymerisation och bilda polymera material. Enzymkatalysen möjliggjorde skapandet av byggstenar som kan vara svåra eller rent av omöjliga att producera med konventionell kemi. I del tre användes fotokemin istället i det första steget för att syntetisera en monomer som sedan polymeriserades genom enzymkatalys till polyamider.

Alla delarna som presenteras i denna avhandling visar potentialen i att kombinera enzymkatalys med fotokemi under milda betingelser för att skapa polymermaterial. Arbetet avancerar därmed kapaciteten för att hantera och tillverka byggstenar som kan användas för att tillverka nya polymeramaterial.

List of appended papers

Unsaturated polyesters

Paper I   **Itaconate based polyesters: Selectivity and performance of esterification catalysts.**
S. Brännström*, M. Finnveden*, M. Johansson, M. Martinelle, and E. Malmström.
European Polymer Journal, (2018),103, 370-377.

Paper II   **One-Component Thiol-Alkene Functional Oligoester Resins Utilizing Lipase Catalysis.**
M. Finnveden, S. Nameer, M. Johansson and M. Martinelle.
Macromolecular Chemistry and Physics, (2016), 217, 1335-1341.

Vinyl ether building-blocks

Paper III   **Novel sustainable synthesis of vinyl ether ester building blocks, directly from carboxylic acids and the corresponding hydroxyl vinyl ether, and their photopolymerization.**
M. Finnveden, S. Brännström, M. Johansson, E. Malmström and M. Martinelle.
RSC Advances, (2018), 8, 24716-24723.

Paper IV   **Tailoring Thermo-Mechanical Properties of Cationically UV-Cured Systems by a Rational Design of Vinyl Ether Ester Oligomers using Enzyme Catalysis.**
S. Brännström, M. Finnveden, N. Razza, M. Martinelle, E. Malmström, M. Sangermano and M. Johansson.
Macromolecular Chemistry and Physics, (2018), 219, 1800335.

Paper V   **Mono-substitution of symmetrical diesters: Selectivity of *Mycobacterium smegmatis* Acyltransferase variants.**
M. Finnveden*, S. Semlitsch*, O. He and M. Martinelle.
Catalysis Science & Technology (2019), in press.

Bio-based polyamides

Paper VI   **Lipase Catalyzed Synthesis of renewable plant oil-based polyamides**
M. Finnveden, P. Hendil-Forssell, M. Claudino, M. Johansson and M. Martinelle.
Submitted (2019).

*These authors contributed equally to the work

The author's contributions

Paper I   Major part of planning, catalyst selectivity and efficiency study, CalB-catalyzed synthesis of the polyester, part of the analysis of the polymers, as well as writing a major part of the manuscript.

Paper II   Part of planning and major part of execution and analysis. Enzymatic synthesis and analysis of oligomers, as well as writing major part of the manuscript.

Paper III   Major part of planning, execution and analysis, as well as writing major part of the manuscript.

Paper IV   Part of conceptualization, developed method for synthesis of difunctional vinyl esters. Minor contribution to analysis as well as review and editing manuscript.

Paper V   Part of planning, supervision of master student executing the experimental work, major part of analyses, modeling and manuscript preparation. The construction of the MsAcT-variant was made by Stefan Semlitsch.

Paper VI   All of the experimental work, analyses and manuscript preparation.

Other scientific contributions

Bio Environmental Polymer Society, BEPS, Kansas City, USA, October 2014. Poster presentation.

Biotrans, Vienna, Austria, July 2015. Poster presentation.

PACIFICHEM, Hawaii, USA, December 2015. Poster presentation.

Polymer Networks Group meeting, PNG, Stockholm, Sweden, June 2016. Poster presentation.

Nordic Polymer Days, NPD, Stockholm, Sweden, June 2017. Oral presentation.

Coating Science International, COSI, Noordwijk, Netherlands, June 2017. Oral presentation.

EPF European Polymer Federation, Lyon, France, July 2017. Oral presentation.

Public defence of dissertation

This thesis will be defended on the 27th of September 2019 at 10.00 in M3, Brinellvägen 8 in Stockholm, Sweden.

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Abbreviations

^{1}H-NMR	Proton nuclear magnetic resonance
BD	1,4-butanediol
CalB	*Candida antarctica* lipase B
DP	Degree of polymerization
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DMI	Dimethyl itaconate
DMS	Dimethyl succinate
DSC	Differential scanning calorimetry
DVA	Divinyl adipate
FTIR	Fourier transform infrared spectroscopy
FT-Raman	Fourier transform Raman spectroscopy
GC	Gas chromatography
H_2SO_4	Sulfuric acid
MO-cys	Methyl 10-((2-aminoethyl)thio)octadecenoate
	Methyl 9-((2-aminoethyl)thio)octadecenoate
MsAcT	*Mycobacterium smegmatis* esterase/acyltransferase
PISB	Poly(butylene itaconate succinate)
*p*TSA	*p*-toluenesulfonic acid
SDG	Sustainable development goal
THF	Tetrahydrofuran
T_g	Glass-transition temperature
T	Teterahederal intermediate
TBD	7-Methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene
Ti(OBu)$_4$	Titanium(IV)butoxide
UV	Ultraviolet
wt	Wild type

Note: The word *substrate* has different meanings in biochemistry and material science. In material science, a *substrate* is a material on which a process is conducted. Within the scope of this thesis, the word *substrate* will be used as in biochemistry and refers to a molecule used as a reactant by an enzyme.

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Purpose of the study

The way that we currently live our lives and manage resources is a substantial threat to our planet.(*1, 2*) In order to make a change towards increased sustainability it is important that research activities are focused on the development of new sustainable solutions. Innovation within material science that improves the sustainability of manufacturing processes is a constantly growing field of research.

The main purpose of this study has been to develop sustainable synthesis pathways to produce building-blocks that can be used to synthesize functional polymers and polymer networks by UV-photopolymerization.

Photopolymerization can be used to polymerize and cross-link (cure) smaller molecules into larger polymers or polymeric networks by exposing the system to, for example, ultraviolet (UV) radiation. Photopolymerization reactions are suitable for use in sustainable polymer synthesis since they are generally carried out in solvent-free systems at low temperatures and have low energy requirements.

In this thesis, efficient and benign routes to UV-curable building-blocks have been developed by taking advantage of the capabilities of enzymes to efficiently and selectively catalyze reactions under mild conditions. Throughout the study, the enzyme catalysis was compared to conventional catalysis, unless, as in some cases, the enzyme catalysis was able to produce novel building-blocks that are difficult, or impossible, to synthesize with conventional catalysts.

An additional challenge, addressed in this thesis, has been increasing the use of monomers from renewable resources in polymeric materials. This has been done by focusing on the synthesis of ester and amide building-blocks, since the starting components are easily derived from renewable resources.

Introduction

Nature assembles a variety of complex structures from simple, abundant building-blocks in reactions catalyzed by enzymes. Enzymes enable life and have been used in the oldest chemical transformations performed by humans, in fermentations such as beer brewing or bread making. Although, at that time the mechanism was unknown. The use of enzymes and rapid development of the biocatalysis field followed the famous "lock and key" model. The model explained the mechanism of enzymatic catalysis and its substrate specificity, and was formulated by Emil Hermann Fischer (awarded the Nobel Prize in chemistry 1902 (*3*)). Today, the properties of enzymes (kinetics, stability, etc.) can be matched to a wide range of process conditions, making the field of biocatalysis an interdisciplinary science. Enzymes no longer have to be employed as they are provided by nature, as tools e.g. enzyme engineering and immobilization methods are develop.(*4*) Thanks to their capabilities to be used in many different types of chemical processes, enzymes can be found in various applications both as ingredients (e.g. detergents and pharmaceuticals) and used as catalysts within industries such as food processing, bio-fuel, paper and pulp, detergents, pharmaceutical industries, textiles, and polymer synthesis.(*5*)

Only a century ago the chemical industry was not a frequent user of catalysts.(*6*) Today few chemical companies would be competitive without them. Catalysis offers many benefits including: lower energy requirements and selectivity (decreasing the use of processing and separation agents). Enzymes, with their high selectivity can reduce the number of reaction steps and in some cases offer paths to compounds not possible to make with traditional chemistry. Additionally, immobilizing enzymes and using them as heterogeneous catalysts simplifies their separation from product (eliminating the need for separation through distillation or extraction, which is the case for homogenous catalysts).(*7*)

An area where research on application of enzymes has drastically increased is for the production of polymers, a major building-block for plastic materials. The plastic industry uses catalysts to produce a wide range of materials. Plastics is a word describing a plethora of materials with very different properties, that are used in a huge and growing range of applications.(*8*) As the market of plastics grows so does its ecological footprint and thus, alternative synthetic strategies for polymers have to be considered. Development and applications of enzymes takes part in

attempts to efficiently make use of monomers that can be hard to handle in conventional systems. Designing sustainable systems for the synthesis of polymeric materials there are frameworks that can be used as guidance e.g. *the twelve principals of green chemistry* and *the* United Nations *Sustainable development goals*.

Herein, selective enzyme catalysis was used to synthesize UV-curable building-blocks with predefined structures for coating applications. The synthesized structures are divided into three groups (i) unsaturated polyesters **Papers I** and **II** and (ii) vinyl ether building-blocks **Papers III-V** and (iii) renewabel polyamides **Paper VI**. The core of the thesis is the chapter *Chemo-enzymatic routes towards polymeric materials* which summarizes the research articles, appended at the end. Preceding are chapters providing additional knowledge and context useful for understanding the work.

Sustainability guidelines

In 1962 Rachel Carson published the book Silent Spring. The book has been pointed out as a major catalyst for the modern environmental movement that was shaped during the 1960s and 1970s. The book highlighted that humans are not apart from nature, but rather a part of the large natural network, by bringing light to the effects of pesticides on the natural world. The book raised large debates and led to new policies protecting our environment. Within the chemistry community the field grew to include green chemistry and in 1998 a set of twelve principles, aiming to guide the practice of green chemistry was published by Paul Anastas and John C. Warner.(*9*) Furthermore, with growing concerns for the environment the United Nations formulated the 2030 Agenda for Sustainable Development including the Sustainable Development Goals (SDGs) in 2015. Many of these SDGs concern climate change and the environment. Both *the twelve principals of green chemistry*, and the SDGs act as sustainability guidelines to help recognize, eventual, trade-offs of new technologies.

The twelve principles of green chemistry

The twelve principles of green chemistry were formulated as guidelines to reduce negative chemical related impact on the environment and human health.(*9*) They are "design rules" that can help chemists achieve the United Nations' SDGs.(*10*) When designing the methods presented in this thesis the principles of green chemistry were considered. *The twelve principles of green chemistry* are:

1. Prevent waste
2. Atom economy
3. Less hazardous synthesis
4. Design benign chemicals
5. Benign solvents and auxiliaries
6. Design for energy efficiency
7. Use of renewable feed-stocks
8. Reduce derivatives
9. Catalysis (vs. stoichiometric)
10. Design for degradation
11. Real-time analysis for pollution prevention
12. Inherently benign chemistry for accident prevention

United Nations sustainable development goals

Aiming for a more sustainable future, the United Nations have formulated 17 sustainable development goals (SDGs).(*11*) These are a call for action to address the global challenges we face, promoting prosperity while protecting the planet. All 17 SDGs interconnect and should be reached by 2030. Today, synthesis of fossil-based polymeric materials often utilizes toxic chemicals that can leak out into the environment and upon combustion of polymers they contribute to an increasing amount of greenhouse gases. Decoupling plastics from fossil feedstocks, by using renewable feedstock to produce building-blocks, carbon dioxide is taken up while the plant grows which is later released when the end products are burned. In some cases, bio-based materials can have a closed carbon loop or even act as a carbon sink.(*12*) Thus, development of new sustainable synthetic strategies and decoupling plastics from fossil feedstocks is strongly associated with SDG 12 *responsible consumption and production*. However, several additional SDGs are closely related to the work presented here, for example: SDG 3 *good health and wellbeing*, SDG 13 *climate action*, SDG 15 *protection of life on land* and SDG 14 *life below water*. Also, substituting fossil resources with renewabel resources and developing cleaner synthesis methods can be related to SDG 8 *Decent work and economic growth* as utilizing bio-based feed stock avoids fluctuating oil prices and new job opportunities are expected to arise.

Bio-based building-blocks

Humans have often looked at nature for inspiration in material design. Nature offers a wide-ranging pallet of specific functionalities both in terms of material properties and in composition of chemical groups. The many possibilities for material design combined with: growing awareness of the negative impacts associated with fossil resources, fluctuating oil prices and possible fossil depletion, have sparked a huge interest for bio-based materials.(*12-16*) The 7th principle of green chemistry u*se of renewable feedstocks* highlights the importance of using bio-based building-blocks for the development of sustainable processes.

There are many accessible renewable monomers and polymers. These can, generally speaking, be divided into two groups dependent on their origin: vegetal biomass, e.g. cellulose, terpenes, plant oils or sugars; and animal biomass, such as chitin/chitosan or casein.(*14*) Working towards the SDGs formulated by the United Nations approaches for retrieving renewable starting compounds will vary throughout the world since climate and consumer behavior diverge a lot. In respect to the scope of this thesis, the vegetal biomass used can be divided into two groups (i) sugar derived monomers and (ii) plant-oil based monomers.

Polyesters and polyamides are two large volume groups of synthetic polymers based on: hydroxy acids, diacids, diols, diamines or amino acids.(*8*) Since monomers containing either carboxylic, hydroxy or amine functional groups are abundant in nature polyesters and polyamides are key candidates for replacements with new renewable building-blocks.

Sugar derived chemicals

Integrating manufacture of several products such as: fuels, heat and high-value chemicals, from biomass go hand in hand with *the twelve principles of green chemistry*. In addition, having multiple outputs will likely increase the profitability, making integrated refineries an attractive approach.(*17*)

A wide range of dicarboxylic acids can be reached from fermentation of biomass.(*18*) Dicarboxylic acids are used for the synthesis of polyesters by polymerization with diols. Many diols can be derived by chemical conversion of the dicarboxylic acids. The compounds can then be combined in numerous ways, yielding different macromolecular structures in order to tailor material properties of polymeric material.

Scheme 1 shows two common sugar derived monomers that have been identified by the U.S. Department of Energy as possible economic drivers for the biorefinery.(*19*) Furthermore, some possible derivatives from the two are shown within dotted squares.
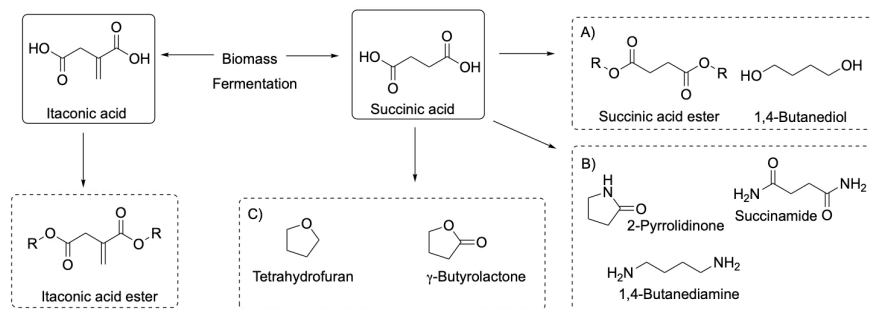
Scheme 1. Two examples of chemicals that can be fermented from biomass: succinic acid and itaconic acid. Dotted squares frame some possible derivatives. Possible products derived from succinic acid: **A)** acylic **B)** nitrogen containing and **C)** cyclic.

Itaconic acid is an unsaturated dicarboxylic acid. Today, no chemical synthesis route to produce itaconic acid can compete with fermentation by fungi, most frequently by *Aspergillus terrus*.(*18*) The annual production of itaconic acid in 2010 was 50 000 Mt.(*20*) Itaconic acid is a trifunctional compound with two different carboxylic acids as well as an unsaturation (Scheme 1). The use of itaconic acid with different diols yielding renewable, unsaturated polyesters for various applications has been extensively researched, as seen in reviews (*21, 22*).

One application where itaconic acid poses as a particularly interesting monomer is for coatings where it is desirable to have a functional group that can be cross-linked after pre-polymerization.(*23*)

Succinic acid is a potential key building-block for both commodity and specialty chemicals.(*24*) In Scheme 1 some compounds that can be derived from succinic acid are shown. As the production of succinic acid was recognized as an important industrial fermentation process, plants to ferment succinic acid were been built. In 2015 the leading producers were Myriant, BioAmber, Reverdia, and Succinity.(*18*) One of the drivers for the projects was the high oil cost raising concern about the future of the chemical industry. However, as oil prices have decreased and the succinic acid production by fermentation has not been able to compete. Consequently, many of the plants closed, with only Succinity remaining out

of the previous leading producers. To get price competitive with oil-based alternatives there are some possible improvements, for example, one of the costliest factors in succinic acid fermentation is the media. An interesting alternative is the use of municipal food waste. It has been reported that, in Sweden, the production of succinic acid from municipal food waste could be feasible from a biotechnical and resource availability perspective.(*25*) Within the work presented in this thesis, compounds derived from succinic acid that can be seen in Scheme 1A were used.

Oils of vegetable origin

Oils from vegetal origin are renewable raw materials, which are versatile and accessible. The fatty acids that constitute the plant oil triglycerides vary in composition depending on the plant and growth conditions.(*26*) The physical and chemical properties of plant oils are affected by the properties of the fatty acids such as: the length of the carbon chain, hydroxyl and epoxy content, their degree of unsaturation as well as the stereochemistry. Providing new properties and renewability, the interest for the use of plant oils as raw material for polymers has grown both in the academic and industrial communities.(*27, 28*) The global production and consumption amounted to 200 Mt June 2018- June 2019.(*29*)

The plant oil based monomers used in the work presented here can be found in Scheme 2. Ricinoleic acid is a characteristic acid for castor oil, forming around 90% of the triglycerides.(*30*) Ricinoleic acid is a building-block from which many molecules can be derived, e.g. 10-undecenoic acid. Another interesting molecule is lipoic acid derived from caprylic acid. Lipoic acid is found in wide variety of foods, commonly at low concentrations. Sources were lipoic acid is most abundant are tissues rich in mitochondria (such as: heart, kidney) or chloroplasts (ex. dark green leafy vegetables such as spinach).(*31*) Oleic acid is a fatty acid occurring in various animal and vegetable oils e.g. in canola oil where it forms around 60% of the triglycerides.
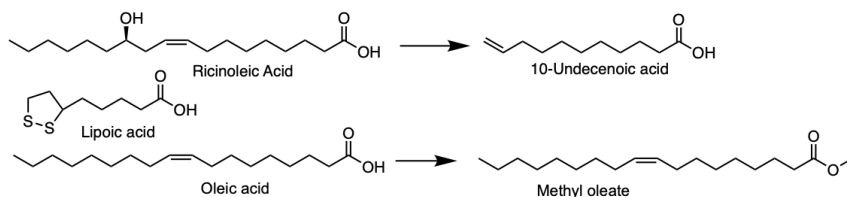
Scheme 2. Some fatty acids found in nature and compounds that can be derived from them.

Selective enzyme catalysis

One of the most important traits of enzymes is their high selectivity. This selectivity enables working life-sustaining metabolism; where enzymes have control over the transformations they perform (e.g. from one form of energy to another, and waste sanitation and synthesis of essential building-blocks). The catalytic power of enzymes arises from their macromolecular structure facilitating the fit of substrates. This is done by rearrangement of structural elements within the surroundings of the active site, the place where catalysis occurs. After binding a substrate, the enzyme selectively stabilizes the species with the highest energy in the transition state. This lowers the activation energy, speeding up the reaction, and determines which reaction coordinate the reaction follows. Enzymes' selective features together with their high activity, often at mild reaction conditions (e.g. room temperature and moderate pH), make enzymes prime candidates as catalysts in sustainable chemistry. An area where enzyme catalysis is of great interest is in material science, especially for the synthesis of bio-based materials. Utilizing the selective properties of enzymes the often great complexity found in bio-based raw materials can be exploited, and the interest within this field is rapidly growing, as seen in reviews (*32-36*).

Specificity, selectivity and promiscuity

To avoid confusion, *specificity* and *selectivity* are within this thesis defined as follows: *specificity* is given by the specificity constant (k_{cat}/K_M), where k_{cat} is the turnover number [time^{-1}] and K_M is the concentration [M] of substrate at which half the active sites are filled. The specificity constant is an absolute number indicating how good an enzyme is at converting a substrate into a product. Enzyme *selectivity* is equal to the ratios of the enzyme specificities towards two substrates A and B (k_{cat}/K_M)$_A$/(k_{cat}/K_M)$_B$.

The selectivity traits displayed by enzymes are generally divided into four different types: 1) Substrate (acting only on one specific substrate); 2) stereo-selectivity [i], (preference towards one of two or more starting compounds with the same chemical formula, but different atom orientations); 3) regio-selectivity, (preference for one direction of bond making or breaking over all other possible directions); and 4) chemo-

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[i] Enantioselectivity if the substrates are optical isomers, i.e. non-superimposable mirror images of each other.

selectivity (preference towards one of two or more different functional groups).(*37*)

Although enzymes are selective, many enzymes diverge from this statement. This behavior is considered to be remaining from ancestral enzymes.(*38*) Enzymes are believed to have diverged from more general ancestral enzymes becoming more specific towards certain type of chemistries. However, if an enzyme has a non-specific trait which does not affect the natural function in the cell, there is no pressure to remove it. Thus, some enzymes can do things they are not expected to do, a behavior which is defined as promiscuous.(*39*) Enzyme promiscuity types have been categorized into three groups: condition promiscuity, substrate promiscuity and catalytic promiscuity[i]. The promiscuous behaviors of enzymes expands their use for numerous synthetic applications, since this behavior allows enzymes to catalyze reactions under, to them, unnatural circumstances.

Carboxylic ester hydrolases

Carboxylic ester hydrolases (E.C 3.1.1) catalyze the hydrolysis of ester bonds into alcohols and carboxylic acids.(*40*) Members of this enzyme sub-subclass are then further divided by their substrate specificities. In this thesis the two carboxylic ester hydrolases used are: *Candida antarctica* lipase B (CalB) and *Mycobacterium smegmatis* esterase/acyltransferase (MsAcT).

Candida antarctica lipase B

Candida antarctica lipase B, (CalB) is often described as a triacylglycerol lipase, but it prefers linear esters (Figure 1).(*41*) Triacylglycerol lipases catalyze the hydrolysis of triacylglycerols, producing free fatty acids and glycerol.(*42*) CalB is made up by 317 amino acids and has a molecular weight of 33 kDa.(*43*) The lipase belongs to the α/β hydrolase family, which is a structural framework common amongst carboxylic ester hydrolases. The α/β hydrolase fold is one of the most versatile and wide spread protein folds known, where a core of predominantly β-stands surrounded by α-helixes provides a stable scaffold for the active site.(*44*)

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[i] Ability of an enzyme to catalyze more than one different chemical transformation, can be divided into accidental or induced.
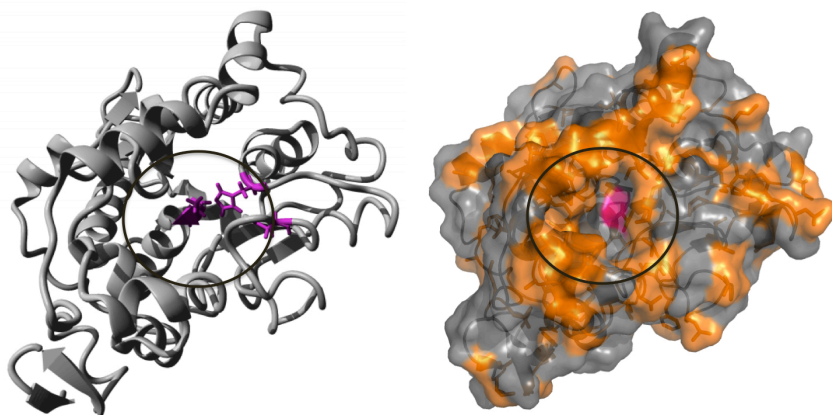
Figure 1. Structure of *Candida antarctica* lipase B shown as ribbon (left structure) and surface representation (right structure). The residues in the catalytic triad (Ser105, His224 and Asp187) are shown in magenta; the substrate binding pocket is circled; and the hydrophobic parts of the surface are shown in orange. PDB code: 1LBS.

In general the backbone of lipases form lids that cover their active sites and are activated when exposed to an interface between water and oil, but no interfacial activation has been observed for CalB and it has no lid on top of the active site.(*43, 45*) CalB has a hydrophobic surface around the entrance to the active site (Figure 1, hydrophobic parts shown in orange) facilitating the diffusion to its natural substrates. The binding site pocket has limited space and is composed of two narrow channels. One hosting the acyl part of the substrate and the other hosts the alcohol-moiety. The alcohol (acyl acceptor) side is narrower, rendering higher selectivity towards accepted alcohols (Figure 2). Primary straight-chain alcohols being the preferred substrates for CalB, additionally secondary alcohols are accepted, while tertiary alcohols are not; CalB is so inactive towards e.g. tert-butanol that it can be used as a solvent.(*46*)

CalB is one of the, if not the, most researched lipase. The interest for CalB ascends from its interesting properties such as: selectivity(*46*), catalytic promiscuity(*39, 47*), and condition promiscuity (for immobilized CalB), e.g. tolerating a wide range of solvents and temperatures (from low temperatures to beyond 100°C).(*32*)
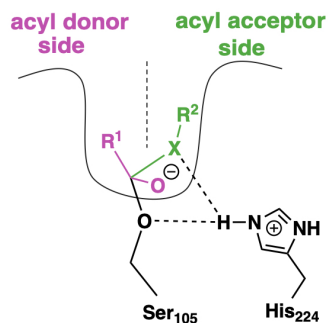
Figure 2. The active site pocket of CalB.(*43*) Where X= O or NH and R^1=C_nH_m. and R^2=H, C_nH_m. The substrate positions itself in the binding pocket with the acyl on one side and the alcohol on one side.

At research level, the use of enzymes as alternatives to the traditional catalysts for polymer synthesis is growing. For polyester synthesis the commercially available Novozyme 435, CalB physically immobilized on an acryl resin, is the most used enzyme formulation and has been proven to be effective for a diverse range of monomers[i], as seen in reviews (*32-36, 46*). Immobilization is a method to increase the stability and condition promiscuity of enzymes. Upon immobilization the enzyme is transformed to an insoluble state by attachment to a carrier or matrix and can thus be used as a heterogeneous catalyst. This transformation has been proven to be a diverse tool that can improve the enzyme's properties, for example: induced stability (both storing and process stability), activity and resistance to chemicals.(*32, 48*) In addition, heterogeneous catalysis simplifies both the initiation of reactions and the separation between product and catalyst and promotes reuse of the catalyst.

In this thesis the Novozyme 435 preparation of CalB, was used (**Papers I-IV** and **VI**). The substrate and condition promiscuity displayed by CalB were utilized, moreover the regio or chemo-selectivity of CalB was exploited in **Papers I, II** and **III**. In **Paper VI** one form of catalytic promiscuity displayed by CalB, by catalyzing the formation of amides, was used.(*49*)

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[i] CalB is capable of catalyzing polyester synthesis both by polycondensation and by ring-opening polymerization. However, ring-opening polymerization will not be covered in this thesis.

Mycobacterium smegmatis esterase/acyltransferase

Mycobacterium smegmatis esterase/acyltransferase (MsAcT) belongs to the SGNH-hydrolase family, having a five-stranded parallel β-sheet structure inserted between α-helices on either side.(*50*) While, SGNH-hydrolases with characterized structures are usually (with one exception) monomeric, MsAcT is suggested to form an octamer in solution. Each subunit consists of 216 amino acids with a molecular weight of 23 kDa.

The interest for MsAcT has grown steadily since its crystal structure was solved a decade ago.(*50*) MsAcT has unique acyltransfer capabilities, in water. This is proposed to arise from its quaternary structure, where three monomers form a hydrophobic channel that restrict the access to the active site (Figure 3). The acyltransfer capabilities of MsAcT have previously been used for ester and amide formation in water.(*51-56*)

In **Paper V** wild type MsAcT and two variants were produced and immobilized. One variant with improved regio-selectivity was used to selectively mono-substitute a symmetric diester.
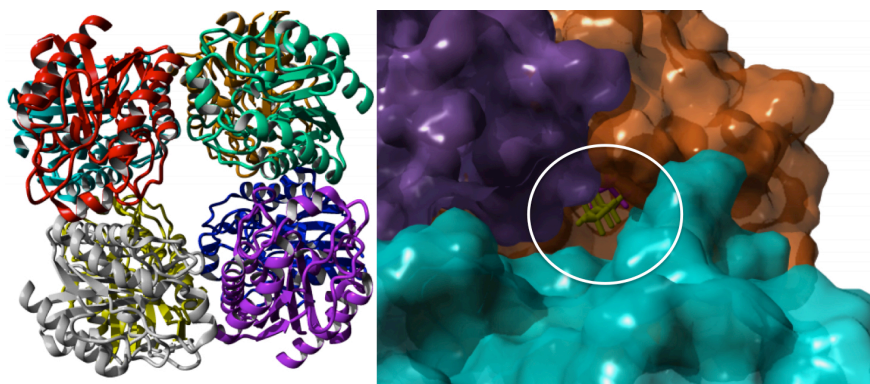

Figure 3. To the left: octameric structure of MsAcT, each subunit is shown in a different color. To the right: a visualization of the hydrophobic channel, restricting the access to the active site, the surface from each subunit is shown in a different color. PDB code 2Q0S.

Kinetics and reaction mechanism

The reaction kinetics of both CalB and MsAcT belong to the serine hydrolase superfamily and follow a ping-pong bi bi mechanism (Scheme 3). The amongst serine hydrolases common catalytic triad composed of Ser-His-Asp can be found in both enzymes. In CalB consisting of Ser105, His224 and Asp187 and in MsAcT Ser11, His195, Asp192. Other important amino acids for the catalysis are the one that make up the oxyanion hole. The oxyanion hole is a pocket in the active site that stabilizes the negative charge formed on the tetrahedral intermediates, during the catalytic cycle (T1 and T2 in Scheme 3). The oxyanion hole in CalB consists of backbone NH and side chain of Thr40 and backbone NH of Gln106(*43*) and in MsAcT from backbone Ala55, Asn94 and backbone NH of Ser11.(*50*)
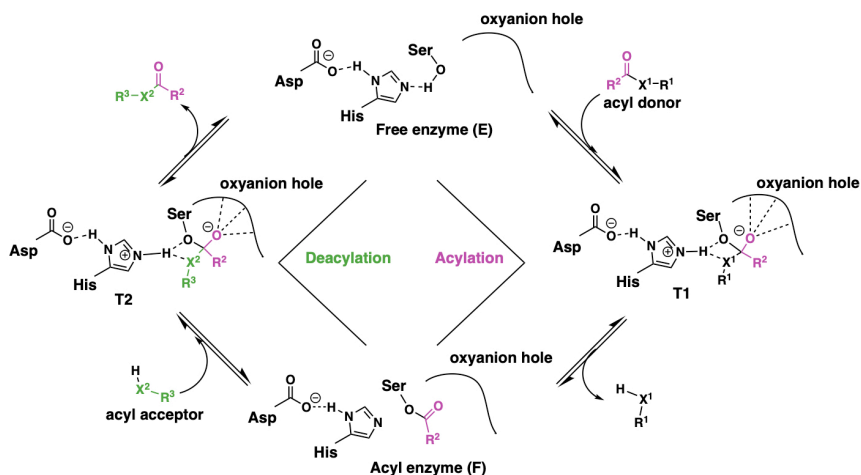
Scheme 3. Schematic representation of chemical mechanism of serine hydrolases for the transacylation of an acyl donor to an acyl acceptor. Where X^1 and X^2= O, NH or S and R^1 and R^3=H, C_nH_m, R^2=C_nH_m.

The catalytic cycle can be divided into two half-reactions, acylation and deacylation. The catalytic triad is arranged so that the pK_a of the catalytic serine is lowered by the histidine acting as a base withdrawing the serine hydroxyl proton. The catalytic aspartate helps by stabilizing the positive charge formed on the histidine through hydrogen bonding. This facilitates a nucleophilic attack from the activated serine on the acyl donor's carbonyl carbon, forming a tetrahedral intermediate (T1). In T1 the oxyanion hole stabilizes the negative charge by hydrogen bonds. The acylation half-reaction is completed by the breakdown of T1 to the acyl-enzyme and the

first product (e.g. water, alcohol, amine or thiol) leaves after accepting a proton from the positively charged catalytic histidine. The acyl-enzyme is formed.

The deacylation half-reaction, a reverse of the acylation, starts with the binding of a nucleophile, the acyl acceptor (e.g. water, alcohol, amine or thiol), to the acyl-enzyme. Thereafter, the acyl acceptor is activated by the catalytic histidine and attacks the carbonyl carbon of the acyl-enzyme forming a new tetrahedral intermediate (T2). The negative charge is stabilized by hydrogen bonding in the oxyanion hole. The deacylation is completed after the breakdown of T2 by the reformation of the carbon-oxygen double bond of the product and the transfer of a proton from the catalytic histidine to the serine. The second product is released, reforming the free enzyme.

Depending on the substrates different products will be formed. For example, during the natural reaction, hydrolysis, the acyl acceptor is water and the final product will be a carboxylic acid. If the acyl acceptor is instead an alcohol, amine or thiol, transacylation reactions take place resulting in a new ester, amide or thioester. Depending on the system: reaction conditions, availability of substrates and the selectivity of the enzyme towards the different substrates the outcome will be different.

The general reaction schemes for enzyme reactions following ping-pong bi bi kinetics can be drawn by Cleland notations (Figure 4). Figure 4A shows a reaction when one acyl donor (denoted A) and one acyl acceptor are present (B). Figure 4B visualizes the competition between two acyl donors (A and A') forming different acyl enzymes (F and F') and depending on which reaction path the enzyme follows; different final products are formed Q or Q'. When the competing substrates are acyl acceptors (B and B'), as shown in Figure 4C, they compete for the same acyl-enzyme (F) yielding product Q or Q'.
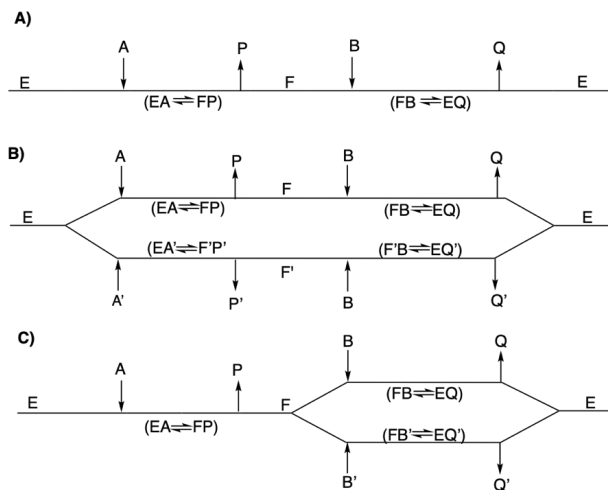
Figure 4. Cleland notations of enzyme reactions following ping-pong bi bi kinetics. During the acylation the acyl donor (A) reacts with the enzyme (E) and releases the first product (P) forming the acyl enzyme (F). During the deacylation the acyl acceptor (B) enters, reacts with F and the final product (Q) is released reforming E: **A)** No competing substrates; **B)** Competing acyl donors (A and A'), competing for E; **C)** Competing acyl acceptors (B and B') competing for the same F.

When multiple substrates are present the selectivity of the enzyme will distinguish the reaction path that takes place. Enzyme selectivity towards different substrates can be determined by different methods. The method used in this thesis is competing substrate systems, where substrates compete with each other and the selectivity is measured by comparing the rates for the different products (Equation 1).

$$\frac{v_A}{v_B} = \frac{\left(\frac{k_{cat}}{K_M}\right)_A [A]}{\left(\frac{k_{cat}}{K_M}\right)_B [B]}$$
(Equation 1)

This method presents some advantages: since the substrates experience the same amount of free enzyme, the enzyme concentration does not have to be known; experimental errors (e.g. setup and measurements) decrease as the reaction rates are measured simultaneously in the same system; and if the enzyme deactivates during the experiment the selectivity is unaffected since the decrease of free enzyme will be the same for all substrates. The disadvantage with competing systems is that less information is obtained, e.g. no kinetic constants (k_{cat} and K_M or absolute k_{cat}/K_M) can be determined. The method of competing substrate systems was used in **Paper I** and **Paper V.**

Polymers

Polymers are present everywhere, both as synthetic polymers and in the natural world (including proteins, carbohydrates, and DNA). The global production of synthetic polymers has increased from 2 Mt in 1950 with an annual growth of 8.4% reaching 380 Mt in 2015.(*57*) They enable life as we know it, for example by insulating our housed protecting us from heat and cold; making cars lighter by using lightweight materials; and providing protection of furniture and houses against its surroundings by coatings. For the synthesis of polymeric materials selecting the right catalyst can greatly improve the efficiency of a reaction by for example: lowering the energy demand; avoiding the use of stoichiometric amount of reagents; and product selectivity.(*10*)

Thermosets

Dependent on their response to heat, polymeric materials can be classified into two groups: materials that flow on heating *thermoplastics* and materials that are cross-linked to avoid flow, *thermosets*.(*23*) Heating thermosets to a high enough temperature will instead lead to their degradation. They are found in various applications (such as adhesives, composites and coatings). Thermosets are prepared from polymers, oligomers and/or multifunctional monomers cross-linked through a process that covalently binds the polymer chains to each other forming networks. There are numerous methods to achieve cross-links including: oxidative, thermal or photo-initiated curing.(*23*)

The wide use of thermosets is due to the possibility of tailoring their properties to fit the application. The material properties depend on factors such as: the constituents of the thermoset (polymers, oligomers and/or multifunctional monomers); type of chemical bonds within the structure (e.g. ester or amide); bulkiness of repeating units or monomers; the arrangement of the constituents; and the cross-linking density.(*58*)

Thermosetting building-blocks

Thermosets are generally based on *thermosetting building-blocks* that participate in cross-linking. The building-blocks can be polymers (often called pre-polymers), oligomers or multifunctional monomers that cross-link through reactive groups, either alone (e.g. acrylates) or by co-polymerization using a cross-linker (e.g. unsaturated polyesters co-polymerized with styrene).(*23*)

The reactive groups can have a single type of chemical function, but by incorporating two or more different chemical groups into the molecule, the potential number of end-use applications can be increased. An example where pre-polymers have more than one type of chemical functionality is the so-called dual cure system, where tunable mechanical properties can be obtained by using different cross-linking chemistries that work in parallel.(*29, 59, 60*) Another method is the off-stoichiometry thiol-ene system, where thermosets with an excess of either thiol or alkene functionalities are synthesized. This concept has been shown useful for the developments of microfluidic devices.(*60*) Functional chemical groups can be introduced into pre-polymers either in the backbone segment or by introducing reactive end-groups. Polymers with functional end-groups are often called telechelics. Telechelic polymers can come in many shapes such as block co-polymers, be used for surface modification, and synthesis of cross-linked materials.(*61*)
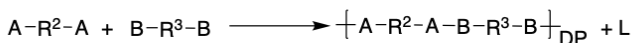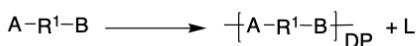
The synthesis of building-blocks with more than one type of chemical functionality can be step intensive and time-consuming. If, for example the chemical groups have a tendency to cross-react or if the functional groups are sensitive to harsh conditions often used in the industry (e.g. high temperatures). However, utilizing selective enzymatic catalysis the synthesis can be performed under mild reaction conditions in one-pot allowing for a large variety of end groups to be incorporated.(*61*)

Methods of polymer synthesis

Polycondensation

Polycondensation refers to a repeated condensation process (i.e. with elimination of simple molecules, most commonly water).(*37*) In polycondensation reactions the polymer chains grow by reacting molecules of all degrees of polymerization (Figure 5). The molecules react via their functional groups, commonly carboxylic acid and alcohol, here denoted as A and B. Starting monomers are either AB-monomers (ex. hydroxy acids), or AA-monomers (ex. dicarboxylic acids or their derivatives[i]) combined with BB-monomers (ex. diols or diamines) in an alternating co-polymer structure (Figure 5). A small low-mass co-product is cleaved off when the functional groups react (e.g. water or methanol). Common polymers produced by polycondensation are polyesters, polyamides and polyurethanes.

Polycondensation

$$A-R^1-B \longrightarrow \left[A-R^1-B\right]_{DP} + L$$

$$A-R^2-A + B-R^3-B \longrightarrow \left[A-R^2-A-B-R^3-B\right]_{DP} + L$$

Growth step

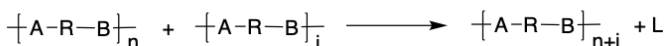$$\left[A-R-B\right]_n + \left[A-R-B\right]_i \longrightarrow \left[A-R-B\right]_{n+i} + L$$

Figure 5. Polycondensation of AB and AA + BB-monomers. In the representation of the growth step the molecules can contain either AB or AA and BB type monomers. Where DP is the degree of polymerization; L is a low-mass co-product; and n and i denote the chains' degree of polymerization.[ii]

To obtain high molecular weights the stoichiometric ratio between A and B should be 1:1. If AB-monomers are used then the ratio between the groups is inherently 1:1. Furthermore, the nature of the polymerization mechanism implies that high conversion is necessary to achieve high molecular weight.(*23*)

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[i] Such as short chain esters or vinyl esters

[ii] In Figure 5, monomers are polymerized into linear structures, but it is also possible to synthesize branched polymers if the number of reactive sites on a monomer is larger than two.

To speed up the reaction a catalyst is often added to the reaction mixture. Common polycondensation catalyst types are: acid catalysts, organometallic catalysts and organobase catalysts. Some common catalysts are shown in Figure 6. Although the catalysts increase the rate of reactions, they are often associated with some trade-offs such as: darker colored products and the need for a separation step to remove the catalyst from the product.(*62*)
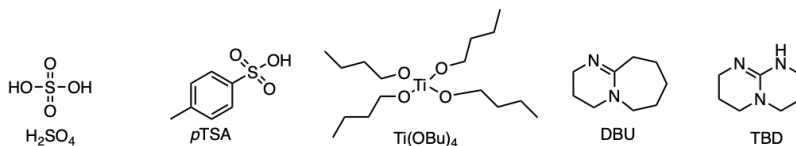

Figure 6. Some common polycondensation catalysts, where sulfuric acid (H_2SO_4) and p-toluenesulfonic acid (*p*TSA) are acid catalysts; titanium(IV)butoxide (Ti(OBu)$_4$) is an organometallic catalyst; 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) are organobase catalysts.

Polycondensation reactions towards polyesters are normally performed at temperatures above 200 °C in the presence of a catalyst. Some catalysts need high temperatures to be active (ex. organometallic). Additionally, high temperatures speed up reactions; the reaction rate per 10°C increase in temperature is reported to increase with a factor between 2-4.(*63*) However, high temperatures equals high energy demand and there may also be implications such as isomerization or thermally initiated reactions between functional groups e.g. gelation of unsaturated polyesters due to radical cross-linking (which can usually be overcome by addition of radical inhibitors such as phenolic compounds(*64*)). Furthermore, when manufacturing unsaturated polyesters (common pre-polymers for thermosets) an inert gas is often used to minimize oxidative degradation, at these elevated temperatures.(*62*) Also at high temperatures it is often necessary to use a stoichiometric excess of the diol, due to evaporation with the co-product that is cleaved of during the reaction, commonly water.

Enzymatic polycondensation

Enzymes are efficient catalysts at comparably lower temperatures and accordingly problems associated with higher temperatures can be circumvented by using enzymatic catalysis. For *in vitro* enzyme-catalyzed polyester synthesis, lipases have been reported as the most efficient catalyst.(*36*) The first lipase-catalyzed polycondensations were reported in the 1980s.(*65, 66*) Since then lipases have proven themselves as powerful

catalysts for the synthesis of polymeric materials. The most widely used catalyst for enzymatic polycondensation is *Candida antarctica* lipase B (CalB). Commonly in the form of the commercially available preparation Novozyme 435, which is CalB that has been immobilized on acrylic resins.(*32*) Polycondensations catalyzed by CalB have been broadly researched and reports span from large scale polycondensation resulting in aliphatic polymers (*67*) to the synthesis of polymers with more rigid structures.(*68*) Moreover, the selectivity of CalB enables synthesis of functional resins without the need of protection chemistry.(*69-72*) In Figure 7A a flow diagram of the polymerization pathway is shown.
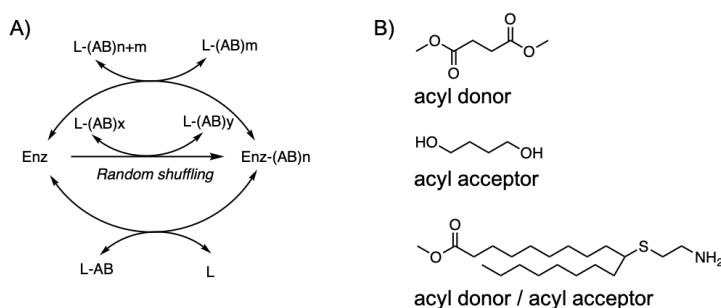

Figure 7. **A)** General flow diagram of the ping-pong mechanism for enzyme catalyzed polycondensation of a AB-type monomer, where A is the acyl donor side and B is the acyl acceptor side; L is a low-mass co-product; and n, m, x and y are degrees of polymerization. **B)** Examples of AA-monomer (acyl donor), BB-monomer (acyl acceptor) and AB-monomer (containing both acyl donor and acceptor in the same molecule).

In the absence of water CalB is *in vitro* capable of utilizing many other different acyl acceptors (nucleophiles). Examples of other acyl acceptors are alcohols and amines leading to esters or amides, respectively. In Figure 7B examples of substrates are shown. When the polycondensation propagates a small co-product, L in Figure 7A, is released (e.g. water or methanol if carboxylic acids or methyl esters are used, respectively). This co-product is usually a substrate for the lipase and needs to be removed for efficient polyesters synthesis. Molecular sieves or reduced pressure can often be used to control removal of co-products. Evaporation of the co-product can be facilitated by combining increased temperature and reduced pressure. Although the use of solvent-free systems provides advantages in terms of chemical use and circumvention of separation, many polymers show an increase in viscosity or crystallization with increasing molecular weight, thus limiting the reaction progression.

Solvents can contribute to reduce these problems. Enzyme catalysis has been shown to be possible with organic solvents as reaction media, in fact organic solvents can enhance the catalytic properties of the enzyme.(*73*)

CalB has been shown to be an efficient catalyst for the production of functional pre-polymers that can be hard to access with other techniques. The pre-polymers can then be further reacted by traditional chemical methods. Both acyl acceptors (**Paper II, Paper IV and VI**) and acyl donors (**Paper I**) can be used as potential end-cappers.

Photopolymerization

Photopolymerization is a technique that uses light (visible or ultraviolet (UV)) to initiate polymerization. Once initiated the polymer grows through chain polymerization, propagating through an active center commonly a radical or a cation (sometimes an anion).(*74-76*)

### Free radical polymerization

Free radical polymerization is the most common UV-initiated commercial chain-polymerization method. One reason being the wide range of photoinitiators, monomers (especially methacrylate and acrylate) and difunctional oligomers available on the market.(*77*) Free-radicals are formed either by cleavage of C-C bonds or by abstraction of a hydrogen atom.(*74*) Common monomers in radical polymerization are acrylates, methacrylates and vinyl monomers such as styrene. This technique can be utilized to form homopolymers of such monomers, but also as a tool for curing and formation of cross-links in a thermoset. One pathway is to use pre-polymers that are either end-capped with unsaturated double bonds or have them in their backbones. In **Paper I** the unsaturation in itaconate is cross-linked by photoinitiated free-radical polymerization. The radical polymerization of itaconate is shown in Scheme 4.
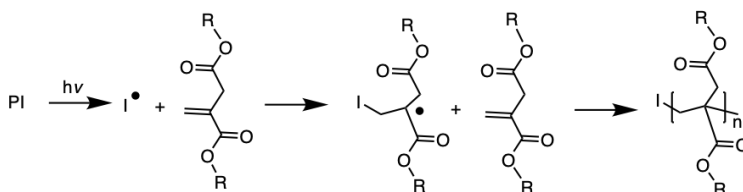
Scheme 4. Photoinitiated radical polymerization of itaconate. Where PI is the photoinitiatior and R=H, C_nH_m or the rest of the polymer.

Thiol-ene chemistry

One type of radical polymerization is the addition of thiol to alkene, thiol-ene chemistry.(*75*) The reaction is initiated and propagated by a thiyl radical and proceeds by step-growth addition to alkenes. The thiyl radical can be generated in different ways: irradiation, thermally or by a photoinitiator. Thiol-ene chemistry was used to cross-link the thiol-ene functional telechelics in **Paper II**, to polymerize the thiol-vinyl ether functional monomer in **Paper III** and to synthesize the monomer used in **Paper VI**. In **Papers II** and **VI** 1,2-disubstituted alkenes were used and thiyl radicals were generated using photoinitiators. The general mechanism for the addition of primary thiols to 1,2-disubstituted alkenes is shown in Scheme 5.
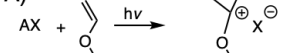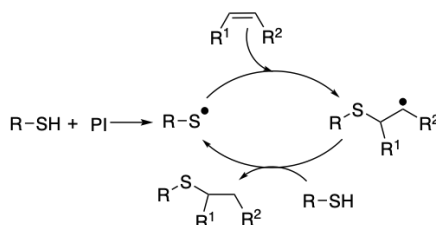

Scheme 5. General mechanism for a thiol-ene reaction between a 1,2 disubstituted alkenes and thiols.

Cationic polymerization

Cationic polymerization is initiated by a photoinitiator complex, excited with UV-light, which dissociates to a strong protic acid or Lewis acid.(*78, 79*) The protic acid protonates the most nucleophilic group on the monomer and then the deprotonated acid acts as a counter ion. While free-radical systems commonly use acrylate and methacrylate, cationic polymerization commonly uses epoxides and vinyl ethers as monomers. The most common monomers for cationic photopolymerization are epoxides. However, vinyl ethers react faster and show low allergenic hazards and low toxicity.(*80*)

Although vinyl ethers exhibit high curing rates the commercial availability of multifunctional vinyl ether monomers is currently limited and consequently few uses in industrial UV-curing applications are found. The synthesis of vinyl ethers has commonly been in super basic conditions and high pressure from alcohols and acetylene. However, there has been a recent development for synthesis routes towards vinyl ethers through more environmentally viable routes.(*81-83*)

Vinyl ethers are sensitive to hydrolysis and may react with acids as well as with alcohols (Scheme 6B), hindering their synthesis, consequently limiting the availability of vinyl ether building-blocks. To expand the availability of vinyl ether monomers methods to synthesize new vinyl ether building-blocks were developed in **Papers III-V**. The general mechanism of a cationic polymerization of vinyl ethers is shown in Scheme 6A.

Scheme 6. **A)** General mechanism for photoinitiated cationic polymerization of vinyl ethers, where AX=photoinitiator. **B)** Some possible side- reactions that may form subsequently when attempting to modify vinyl ethers.

Characterization

Gas chromatography (GC) Compounds elute at different times, called retention time, used to identify analytes. GC can be used both for qualitative and quantitative analysis.

Nuclear magnetic resonance (NMR) is a technique used for chemical structure characterization. The ^{1}H isotope is the most abundant NMR-active nuclei and thus ^{1}H-NMR is the most sensitive. ^{1}H-NMR can be used as a quantitative analytical method. Another common isotope in NMR is [13]C.^{13}C-NMR characterizes the carbon atoms in a material and gives information about the structure of the polymer.

Size exclusion chromatography (SEC) in one of the most commonly used methods for determination of molecular weight and molecular weight distribution. Used to determine the number average molar mass (M_n) weight average molar mass (M_w) and the polydispersity index (PDI= M_n / M_w).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is used for molecular weight analysis of polymers. Quantitative analyses are often hard to obtain, as discrimination of some functionalities and molecules with higher molecular weight, occur.

Fourier transform infrared spectroscopy (FTIR) is used for chemical structure characterization. Used for example to calculate the degree of curing.

Fourier transform Raman spectroscopy (FT-RAMAN) is related to FTIR but, enables analysis of some chemical groups that cannot be separated in FTIR due to light scattering at the same frequencies.

Differential scanning calorimetry (DSC) is used to determine some characteristic properties of a polymer e.g. crystallization, melting temperature (T_m) and glass transition temperatures (T_g).

# Chemo-enzymatic routes towards polymeric materials

Within the scope of this thesis, chemo-enzymatic methods are here defined as methods where conventional synthetic chemistry is combined with enzymatic approaches. The combination of different catalytic systems, each specific for one conversion step, is a versatile strategy to increase the productivity, selectivity, and cost as well as environmental efficiency of synthesis. The chemo-enzymatic routes developed in this thesis are presented in Scheme 7. The chemo-enzymatic procedures developed in (i) and (ii) have been designed so that enzymes are used for their selective properties, synthesizing reactive polymer precursors, which can be or have been further reacted by suitable photopolymerization techniques. In the chemo-enzymatic route developed in the third part of this thesis (iii) the photochemistry was instead used in the first step for the synthesis of a AB-type monomer that was further polymerized by CalB into polyamides. In the enzymatic steps the enzymes used were: *Candida antarctica* lipase B (CalB) as the commercial formulation Novozyme 435 (immobilized on acrylic resin); and the esterase/acyltransferese from *Mycobacterium smegmatis* (MsAcT).
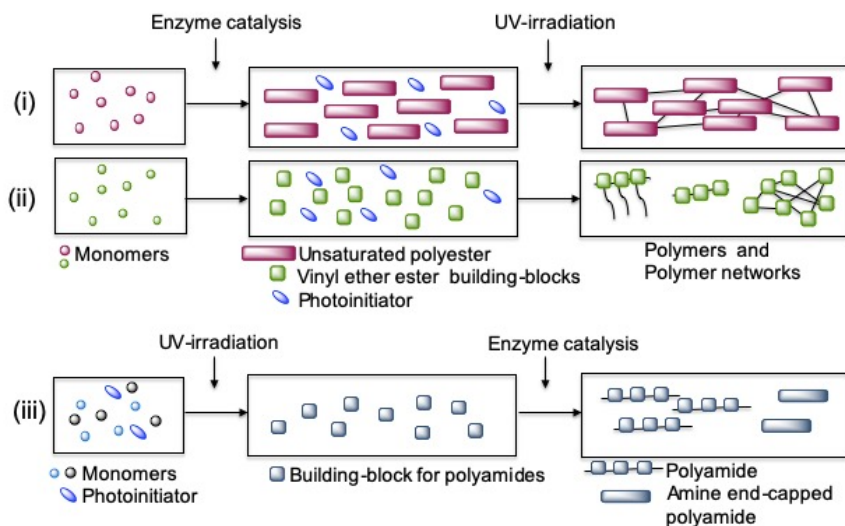
Scheme 7. Summary of chemo-enzymatic routes presented in part **(i)** *unsaturated polyesters*, **(ii)** *vinyl ether building-blocks* and **(iii)** *bio-based polyesters* is not represented.

Unsaturated polyesters

Chemo-enzymatic strategies towards polyester networks were developed in **Papers I** and **II** (Scheme 8). In the enzymatic step, the high selectivity of *Candida antarctica* lipase B (CalB) was used to synthesize unsaturated pre-polymers that were further crosslinked into thermosets. Unsaturated polyester are common cross-linkable polymers for thermosets. These unsaturated polyesters are usually low-molecular-weight prepolymers with common number average molecular weights ranging from 800 to 3000 Da.(*62*)
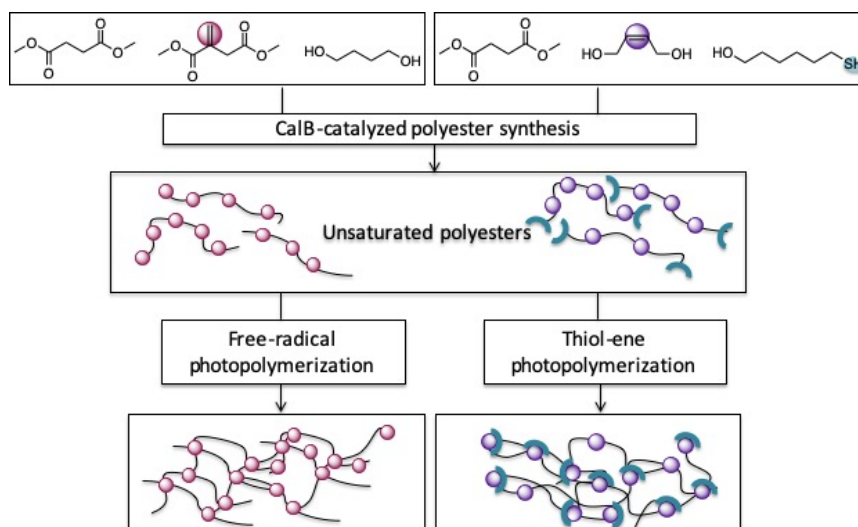

Scheme 8. Summary unsaturated polyesters synthesized by CalB routes presented in this thesis. In **Paper I** dimethyl itaconate was co-polymerized with succinate and butane diol resulting in fully bio-based polyesters. In **Paper II** one component thiol-ene functional polyesters were synthesized.

Today, maleic acid derived from petrochemicals plus a co-monomer, such as styrene is commonly used for cross-linking. The addition of liquid styrene, as a reactive solvent, both enhances the reaction rate and eases the processing. Acrylic monomers can also be used as cross-linkers e.g. for improved outdoor weathering.(*62*) In contrast to maleic acid the unsaturation in DMI, containing a 1,1-substituted double bond, can be radically homopolymerized (Scheme 4 pg. 23). Thus, to cross-link a pre-polyester containing DMI no co-monomer is needed. DMI is a tri-functional monomer, containing a diester and a vinyl group. Therefore, DMI can, in addition to radical polymerization, be incorporated into

polyester backbones by polycondensation with a diol. However, due to its vinyl group ,DMI has one conjugated and one non-conjugated ester with different reactivity's and thus polycondensation of DMI is not as simple as that of conventional diesters, e.g. dimethyl adipate.(*84*)

Furthermore, polycondensation of DMI and diols using traditional catalysts at common reaction conditions (such as high temperatures > 150°C) tend to leads to side-reactions, for exampel: radical cross-linking of the vinyl, isomerization, and attack of the diol on the vinyl group in the Ordelt reaction (Scheme 9).(*85*) To avoid side-reactions of DMI performing the reaction at low temperatures, for example using CalB, has been shown successful.(*86-88*) Aiming to sort out the influence different catalysts have on DMI, during polycondensation with 1,4-butane diol (BD), a comparison of CalB with 5 conventional catalysts: Ti(OBu)$_4$, *p*TSA, H$_2$SO$_4$, DBU and TBD, was conducted in **Paper I** (Table 1).
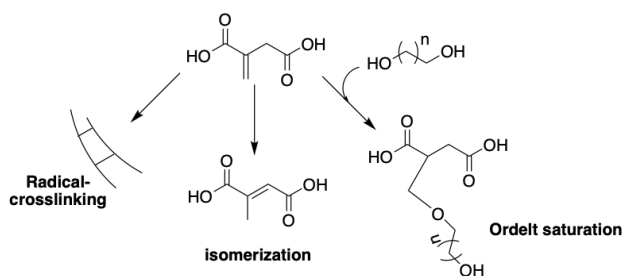
Scheme 9. Some common side-reactions that may occur during the poly-condensation of itaconic acid with diols.(*85*)

**Table 1**. Catalyst initially examined for the polycondensation of DMI and 1,4-butane diol (BD).

Catalyst	Temp [a] [°C]	Suitable catalyst	Comment
CalB	60/100	Yes	No side reactions
Ti(OBu)$_4$	150/160	Yes	Discoloration
*p*TSA	60/160	No [b]	THF formation (from BD)
H$_2$SO$_4$	60/140/160	No	Various side reactions
DBU	100	No	Isomerization
TBD	100	No	No product detected

[a] chosen based on common procedures. [b] Using *p*TSA as a catalyst caused BD to form THF. However, no rearrangements or side-reactions involving DMI were detected.

After the initial screen, in addition to CalB, Ti(OBu)$_4$ and pTSA were considered as interesting catalyst for the synthesis of DMI based polyesters (Table 1). To further deepen the understanding of the catalytic influence on the synthesis of itaconate-based polyester, two different systems were used to study the selectivity towards: I) the different esters of dimethyl itaconate (DMI); and II) the two monomers DMI and dimethyl succinate (DMS), using BD as diol. The results are shown in Table 2.

As can be seen in Table 2, CalB and pTSA favor the formation of the non-conjugated ester (B in Table 2), which agrees with previous results reported for acrylates and DMI.(89, 90) Ti(OBu)$_4$ displayed a 5 times lower selectivity towards the non-conjugated ester compared to CalB. Comparing the total initial reaction rates for CalB presented in Table 2, it can be seen that rate$_{DMI+DMS}$ is 7 times higher compared to rate$_{DMI}$. Thus, it is clear that the vinyl group further influences the reaction rate of transacylation of the non-conjugated side.

Table 2. Catalyst selectivity and efficiency towards: I) the different esters of DMI (grey and green highlight the conjugated and non-conjugated esters, respectively); and II) two monomers DMI and DMS (blue and pink highlight DMI and DMS, respectively), where A and B denotes the new esters non-conjugated over conjugated, respectively.
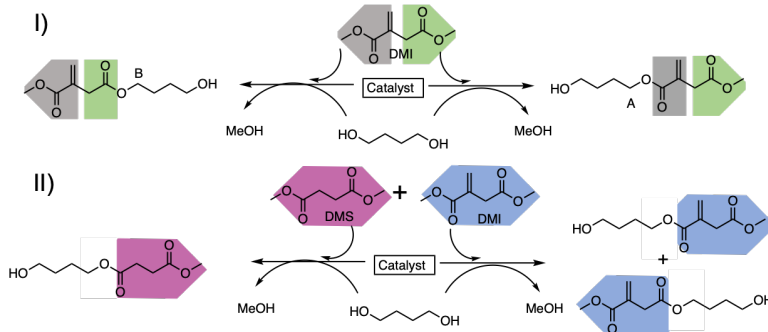

Catalyst	T [°C]	rate$_B$/ rate$_A$	rate$_{DMI}$ [a]	rate$_{DMS}$/ rate$_{DMI}$	rate$_{DMI+DMS}$ [a]
CalB	60	13±3	250±50	NA [b]	1800±100
Ti(OBu)$_4$	160	2.5±0.2	17±3	1.6±0.4	12±1
pTSA	160	7.5±0.4	4.4±0.1	3.5±1	5.1±1

[a] Determined by ^{1}H-NMR, initial reaction rate of total ester formation [μmol substrate * μmol catalyst^{-1}*min^{-1}] and the standard deviation was calculated from the initial rate of a set of three experiments. [b] Not Available, ^{1}H-NMR signal from A was below detection limit.

In the acyl-binding pocket of CalB (Figure 2 pg. 13), acyl chains longer than 5 carbons have to bend at the fourth carbon to fit.(*43, 91*) Due to the conjugation in DMI the acyl chain cannot bend at the fourth carbon when the non-conjugated ester reacts and consequently the activity towards DMI is low. The initial rate of formation of the conjugated ester in DMI (rate$_A$) is 100 times lower than the rate$_{DMI+DMS}$(Table 2). The two esters in DMI resemble methyl propionate and methyl methacrylate respectively, due to the position of the vinyl group. Thus, the difference is selectivity of DMS compares to the conjugated (A) ester in DMI, corroborates with previously reported results showing that CalB is 100 times more selective towards methyl propionate than methyl methacrylate.(*90*) Relating the results to the studies on acrylates compared to methyl propionate, the preference towards the non-conjugated side of DMI by CalB can in addition to the steric effects be attributed both to electronic effects (associated with the conjugated double bond).

To explore the impact of the selective enzyme (CalB) compared to the less selective Ti(OBu)$_4$, the two catalysts were used to synthesize unsaturated polyesters. DMI was co-polymerized with dimethyl succinate (DMS) and BD resulting in poly(butylene itaconate succinate) (PISB) (Scheme 10). Using CalB a high number of conjugated end-groups (from DMI) were obtained, while the less selective Ti(OBu)$_4$ resulted in a more random incorporation of DMI and DMS. The difference can be seen in the ^{1}H-NMR spectra in Figure 8. The formation of the two esters from the conjugated and the non-conjugated esters can be seen at 4.2 ppm (signals c) and 4.1 ppm (signal d), respectively.
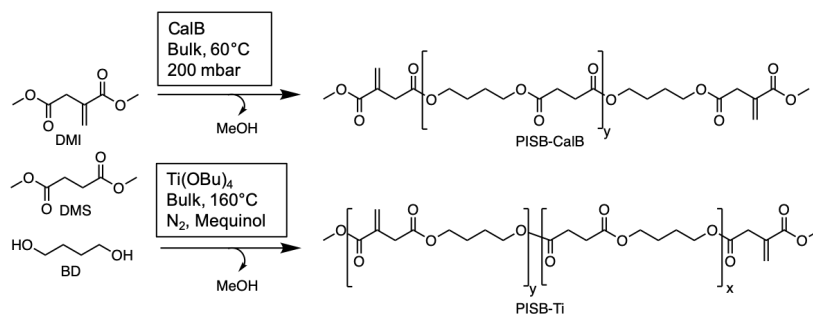
Scheme 10. Two synthesis pathways towards unsaturated polyesters. Top path catalyzed by CalB and the bottom path catalyzed by Ti(OBu)$_4$.
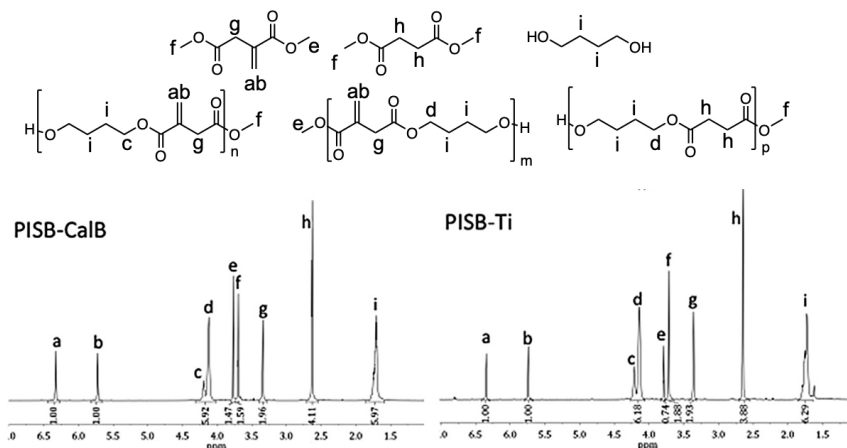
Figure 8. ¹H-NMR spectra of polyesters synthesized from DMI, DMS and BD with CalB and with Ti(OBu)$_4$, where a-i denote signals that have been assigned to hydrogen atoms from the structure shown above; n, m and p denote degree of polymerization; and PISB-CalB and PISB-Ti are the names of the two polyesters. The ¹H-NMR was run in CDCl$_3$.

Analyzing the polyesters with DSC, results displayed that PISB-CalB show a crystallization/melting transition while PISB-Ti is amorphous. It has been shown that when succinic acid and itaconic acid are co-polymerized an increased amount of itaconate decreases the crystallinity.(*92*) Thus, the findings that PISB-CalB has some degree of crystallinity further strengthens that the polyesters synthesized by CalB have a higher number of conjugated end groups (from DMI) compared to PISB-Ti, which allows for longer segments with DMS and BD in the middle of the chain.

The two synthesized unsaturated polyesters PISB-Ti and PISB-CalB were cross-linked by UV-initiated radical polymerization of the 1,1-disubstituted unsaturation in DMI. By following the disappearance of the alkene with FTIR, the degrees of curing were calculated. Relatively high degrees of curing, above 90%, were reached for both polyesters. The thermo-mechanical properties of the cross-linked polyesters (PISB-CalB and PISB-Ti) were studied with DMA and the results showed that the two networks have similar properties.

Comparing the visual appearance of the two films it can be seen that the film synthesized from PISB-Ti was stained by the catalyst; both the polyester and the resulting film were yellow (Figure 9). The film synthesized from PISB-CalB was not stained. Furthermore, CalB was

removed at the end of the polycondensation, while Ti(OBu)$_4$ remains within the cross-linked polyester film. Additionally, by using a lower reaction temperature, 60°C compared to 160°C, the addition of radical inhibitors could be avoided, and the energy demand decreased.

Figure 9. Free-standing films made from PISB-CalB (left) and PISB-Ti (right) showing that the enzymatically synthesized polyester (PISB-CalB) is not stained by the catalyst.

Another interesting alkene is the 1,2-disubstituted double bond, which is commonly found in nature e.g. in fatty acids (Scheme 2 pg. 9). However, this alkene is relatively unreactive and not possible to use in conventional free-radical polymerizations. One way to cure these monomers is to combine 1,2-disubstituted double bonds with other monomers such as thiols.(*93*)

Thiol-ene chemistry is a versatile technique for the coupling of thiols to alkenes and can be used for the formation of thermosets. However, the high reactivity between alkenes and thiols makes the incorporation of both groups within the same resin difficult and often involve protection of the thiol followed by deprotection.(*94, 95*) Therefore, a thiol-ene system commonly consists of two components.(*96*)

In addition, synthesizing polyesters with free pendant thiols is difficult, as many esterification catalysts are not selective towards ester formation over thioester formation. CalB is almost 10^5 times more selective towards alcohols than thiols in transacylation reactions.(*97*) Using CalB Takwa et al. were able to introduce thiol and acrylate end-groups in the same polymer without the need for protection/deprotection chemistry.(*70*)

In **Paper II,** multifunctional aliphatic polyesters (containing varying amounts of alkene and two external thiols) were synthesized. The fraction of alkene within the polyester was altered by varying the degree of polymerization (DP), as the used diol (1,4-butene diol) contained a 1,2-disubstituted alkene. Thiol-ene functional polyesters with DP: 2, 3 and 4, were synthesized and the results are presented in Table 3.

Table 3. Synthesis of unsaturated polyesters using CalB.

| Sample | Ratio A:B:C | DP[a] | Conversion (%)[a] | | | f^{b}_{SH} (%)[a] | T_g (°C)[c] | G' (MPa)[d] |
			A	B	C			
DP2	3:2:2	1.9	98	82	97	82	-15	2.4
DP3	4:3:2	2.9	99	85	98	85	-18	0.4
DP4	5:4:2	3.7	99	82	98	82	-20	0.2

[a] Degree of Polymerization, determined by ^{1}H-NMR. [b] Degree of thiol functionalization. [c] Determined by DSC. [d] Determined by rheology, where T_g is the glass-transition temperature and G' is the shear modulus.

The formation of ester and intact functionalities were confirmed by ^{1}H and ^{13}C-NMR, FTIR and FT-Raman. The two potential side-reactions, disulfide formation and premature thiol-ene coupling, were not detected by NMR. Additionally, the M_n data obtained from SEC agreed with the theoretical values calculated for the three resins and thus the formation of these side-reactions was considered insignificant, since they would increase the molecular weight of the polyesters. A control reaction without enzyme was run at 60°C but no products were observed. However, mixing the monomers at elevated temperature (120°C) resulted in an insoluble network.

The diol, 1,4-butene diol (B in Table 3), was chosen for its 1,2-disubstituted double bond, which displays a relatively low reactivity towards thiols.(*98*) The low reactivity was seen as beneficial for two reasons: the first was that no radical inhibitors had to be added to avoid the premature reaction between the thiol and the alkene; and the second was that the storing stability of the thiol-alkene functional polyester would increase. Both these benefits were proven. The polyesters could be stored for 6 months at ambient conditions, and then dissolved and UV-cured.

The three polyesters synthesized by enzyme catalysis (Table 3), with varying amount of alkene within the structure were cross-linked by thiol-ene coupling. In Figure 10 FT-Raman spectra before and after UV-curing for polyesters with DP 2 and 4 are shown. The polyester with DP 2 has a 1:1 ratio between alkene and thiol resulting in full conversion of both the thiol and the alkene (Figure 10A). When the polyester with DP 4 was UV-cured the thiol was completely consumed while the excess of alkenes was still intact in the network (Figure 10B). The same principle applies when the polyester with DP 3 was UV-cured. Networks based on polyesters with DP3 and DP4, with excess amount of alkene compared to thiol, led to functional networks with intact alkenes meaning that there are opportunities for post-modification. Both T_g and G' decrease when the polyester increases in length (Table 3).
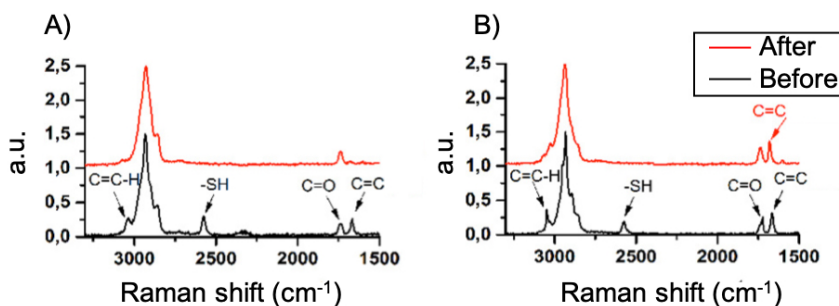

Figure 10. FT-Raman spectra. Before (bottom spectra) and after (top spectra) thiol-ene reaction of polyesters shown in Table 2, where a.u. is arbitrary unit. The spectra are from before and after UV-curing of **A)** DP2 and **B)** DP4.
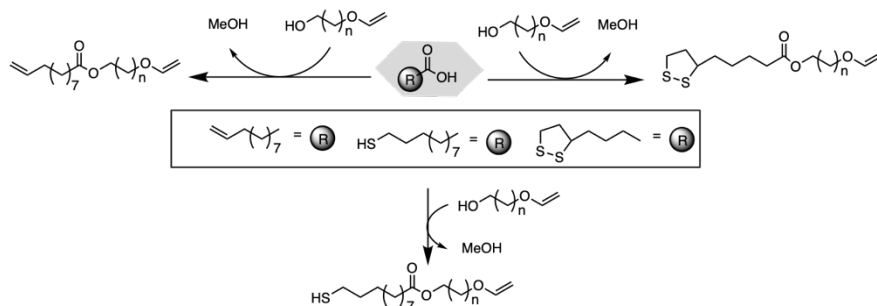
Vinyl ether building-blocks

The synthesis of vinyl ether building-blocks is sometimes difficult since vinyl ethers are prone to react with acids and alcohols and are sensitive to hydrolysis (Scheme 6B pg. 25). Consequently, compared to acrylates, the availability of vinyl ether functional components is considerably lower. Therefore, one of the aims of this thesis was to develop methods for the synthesis of vinyl ether functional ester building-blocks. A key for handling vinyl ethers is operating at mild reaction conditions (e.g. low temperatures and low acidity). Enzyme catalysis working selectively and efficiently at low temperatures was used in **Papers III-V** for the synthesis of a variety of vinyl ether functional components. The two enzymes CalB or MsAcT were used and a summary is shown in Scheme 11.
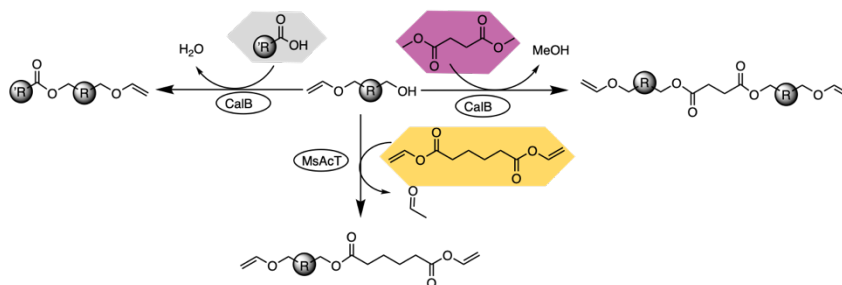
Scheme 11. Summary of enzyme-catalyzed routes towards vinyl ether building-blocks. The acyl donors used in the different routes are highlighted in: grey, generic carboxylic acid (**Paper III**); pink, dimethyl succinate, (used in **Paper IV** as a linker to synthesize difunctional vinyl ethers); and orange divinyl adipate (used in **Paper V** for the synthesis of mixed vinyl ether vinyl ester compounds).

By introducing CalB as catalyst a method for obtaining vinyl ether ester building-blocks from hydroxy vinyl ethers and functional carboxylic acids (Scheme 12) was developed in **Paper III**. Using CalB as catalyst, the acid concentration was rapidly decreased, thus lowering the risk of the carboxylic acid to react with acid-labile vinyl ether. The co-product, water, was removed by molecular sieves (4Å) to push the reactions towards product formation and to avoid hydrolysis of the vinyl ether.

Scheme 12. Schematic overview of the synthesis route towards vinyl ether esters (**Paper III**). Starting directly from carboxylic acids and hydroxy vinyl ether bifunctional building-blocks were synthesized, where n=3 or 5.

The method was evaluated by performing the top left reaction in Scheme 12 under various conditions: under solvent-free conditions at temperatures ranging from 22-90°C; and in different solvents at 22°C. The solvents used were: toluene, methyl *tert*-butyl ether (MTBE), 2-methyl-tetrahydrofuran (Me-THF) and acetonitrile (ACN). The synthesis was shown to work under all tested conditions. Under solvent-free conditions and in toluene at 22°C full conversions were obtained after an hour. At 90°C the lipase-catalyzed reaction reached almost full conversion within 10 minutes, without any observed side products.

Control reactions without any catalyst at the same temperatures as the reactions with CalB were run and in addition the two common esterification catalysts Ti(OBu)$_4$ and TBD were used at 160°C and 80°C, respectively. Specifically, when the reactions were performed at elevated temperatures, the benefits of using CalB were evident. In the control reaction at 90°C no product ester was formed, and the vinyl ether functionality was lost within 80 minutes. Using Ti(OBu)$_4$ at 160°C ester formation was observed, however, at this high temperature the vinyl ether was completely lost within 3 minutes.

The bottom and top right reaction paths in Scheme 12 show the synthesis of thiol vinyl ether functional esters, both these syntheses were run in toluene at 22°C. When the carboxylic acid with a primary thiol was used, a radical scavenger was added to avoid pre-mature reaction by addition of the thiol to the vinyl ether. No radical scavenger was needed when the lipoic acid, containing a di-sulfide was used. High conversions were reached for all synthesized structures. The lowest conversion was reached when lipoic acid was used as carboxylic acid (the top right in Scheme 12),

but was still over 90%. However, the method has since then been further developed by Brännström et al. showing that increasing the temperature to 80°C and running the reaction in bulk gave quantitative yields.(*99*)

To show the versatility of the method, hydroxy vinyl ether was reacted with 3 different carboxylic acids, adding 3 types of additional, to the vinyl ether, functionalities: a thiol, an alkene, and a cyclic disulfide, i.e. bifunctional or trifunctional monomers (Scheme 12). The two bifunctional monomers were cross-linked by UV-initiated radical or cationic polymerization. Depending on which photopolymerization type that was used different architectures could be obtained (Scheme 13). The cationic polymerization of the monomers containing thiol reacted much slower that the monomer containing alkene.
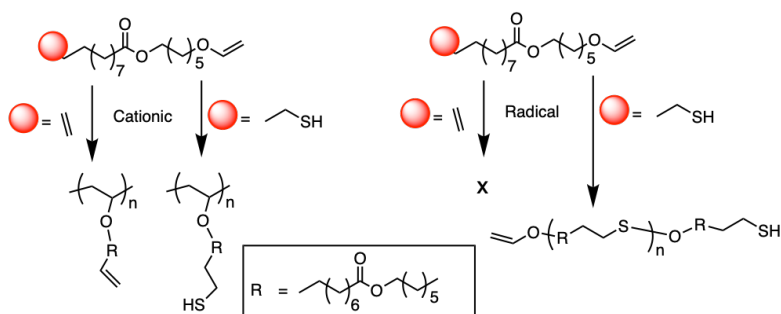
Scheme 13. Proposed structures of the formed polymers after cationic/racical polymerization. After cationic photopolymerization, comb structures with pendant functional groups are synthesized (to the left). After radical photopolymerization a linear polymer is synthesized. The two vinyl groups are both unable to homo-polymerization.

The method is easy to implement and can be extended to other carboxylic acids. It should be noted that the choice of carboxylic is limited by their pK_a. A transition at pK_a 4.8 has been reported, below which the enzyme (CalB) becomes inactive.(*100*) However, there is a plethora of fatty acids with pK_as above 4.8.

The method in **Paper III** was futher developed in **Paper IV**. By using dimethyl succinate and 3 hydroxy vinyl ethers (1,4-butane diol vinyl ether; 1,6-hexanediol vinyl ether; and 1,4-cyclohexanedimethanol vinyl ether) difunctional vinyl ethers were obtained (Scheme 14). The synthesis was performed under solvent-free conditions at 60°C and the reactions were complete within 1 hour. The enzyme was collected after each reaction and reused.
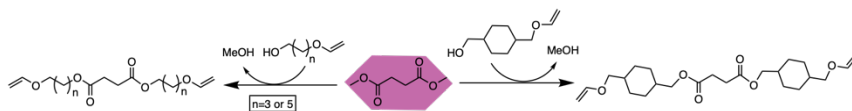
Scheme 14. Enzymatic synthesis of difunctional vinyl ether components. In the left route the two hydroxy vinyl ethers used were, 1,4-butane diol vinyl ether; 1,6-hexanediol vinyl ether.

The series of synthesized vinyl ether functional components were further cross-linked by cationic photopolymerization. By mixing the building-blocks in different ratios the thermo-mechanical properties were evaluated (Figure 11). The homophotopolymerization of the building-blocks yielded cross-linked thermosets with T_g: (1) -10°C; (2) -1°C; and (3) 100°C. Furthermore, by combining (1), (2) and (3) in different ratios it was possible to tailor the T_g within the range -10°C and 100°C.
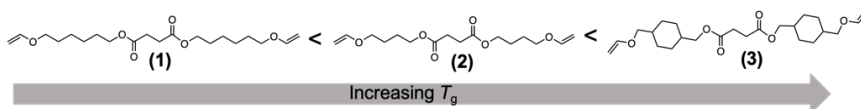

Figure 11. Influence of different vinyl ether difunctional oligomers on T_g. after cationic photopolymerization, where (1), (2) and (3) denote the different oligomers.

Selectively reacting one of two identical chemical groups is a challenge. Hendil-Forssell et al. previously created mutants of the esterase/acyltransferase MsAcT. Looking at one of the mutants, a single point mutant Leu12Ala, we hypothesized that it should be possible to use the mutant to selectively react one, out of two identical, carboxylic esters in the symmetric diester divinyl adipate (DVA) and the method was developed in **Paper V**. The wild type (wt) MsAcT and the mutant MsAcT Leu12Ala were immobilized and explored under solvent free-conditions. The transacylation of divinyl adipate (DVA) catalyzed by MsAcT wt or MsAcT Leu12Ala was performed using 1.5 equivalents of 1,4-butanediol vinyl ether (BVE). By using MsAcT Leu12Ala full conversion of DVA with 95% of the mono-substituted product was observed. The results from two different time points are shown in Table 4 to highlight the differences between the catalysts. The conversion of mono-substituted ester to di-substituted ester from 3% to 5% for the reaction catalyzed by MsAcT Leu12Ala is due to the reaction approaching thermodynamic equilibrium.

Table 4. Enzymatic route for the mono-substitution of divinyl adipate with hydroxyl vinyl ether. Results are shown at two time points for each catalyst.
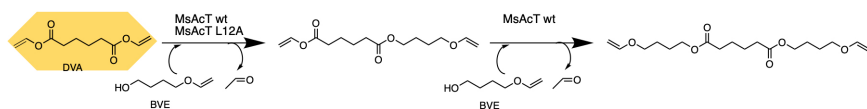
Catalyst	Ratio DVA:BVE	Time [h]	Conversion [a] DVA [%]	Substitution pattern [a] [%]	
				mono	di
MsAcT wt	1:1.5	27	80	80	20
		45	94	66	34
MsAcT Leu12Ala	1:1.5	27	96	97	3
		45	>99	95	5

[a] Analyzed by GC.

By using the limitations displayed by MsAcT Leu12Ala to use long acyl donors, it was possible to react only one of the vinyl esters in DVA. When one side has reacted, the new ester will be too long to fit in the active site. It is important to understand that the amino acids residues around the binding pocket determine the direction of the acyl moiety. In Figure 12, close ups of the active sites of one subunit for MsAcT wt and the variant MsAcT Leu12Ala are shown. The model shows the tetrahedral intermediate of the deacylation step, where both acyl donor (magenta) and acyl acceptor (yellow) are present. The model showed that MsAcT Leu12Ala acquires a deeper binding-site behind the side chain. In the MsAcT wt, acyl moiety (magenta) points toward the entrance of the binding-site in the vicinity of the alcohol binding area in the narrow entrance of the active-site (Figure 12A). When leucine number 12 was mutated into an alanine the new space generated seemingly allows DVA to continue inwards into the enlarged pocket (Figure 12C). However, when the mono-substituted product is released, it is too long to fit into the restricted space generated from the mutation.
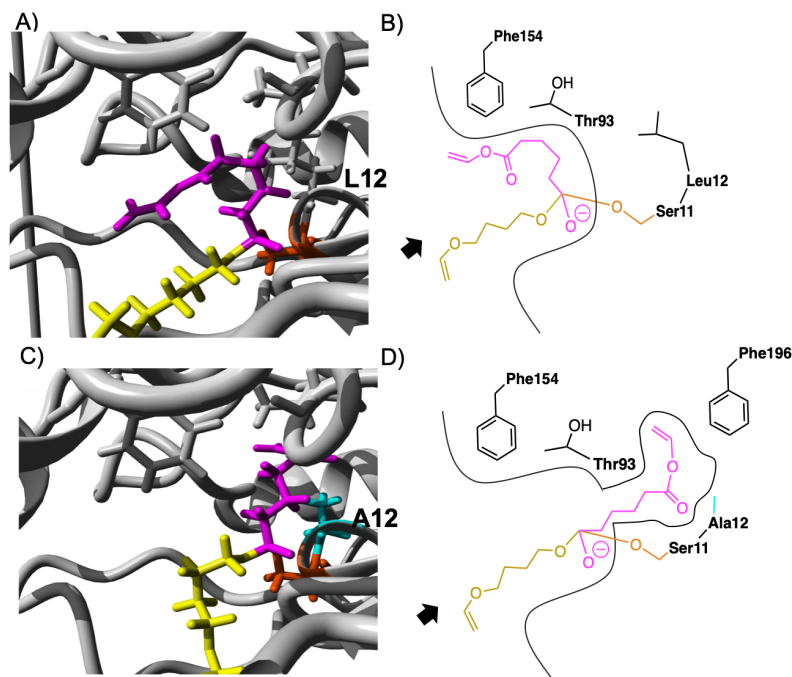
Figure 12. The acyl donor (DVA) orientation in transition state. **A)** and **C)** show close ups of the active sites and **B)** and **D)** schematic representations. Top frames showing MsAcT wt (**A)** and **B)**) and the two bottom frames show MsAcT Leu12Ala (**C)** and **D)**), where: DVA is shown in magenta, the acyl acceptor (BVE) is shown in yellow; the conserved leucine (**A)**) and backbone (shown in ribbon) are shown in grey; the alanine mutation is shown in cyan(**C)**); the catalytic serine is shown in orange and the black arrow indicates the active site entrance. When the leucine is mutated to an alanine the acyl donor can continue inwards into the new space. PDB: 2Q0S.

Additionally, the acyl donor selectivities of MsAcT Leu12Ala were studied for dicarboxylic acids with different lengths: dimethyl succinate (C4); dimethyl adipate (C6); dimethyl suberate (C8); and dimethyl sebacate (C10). The results showed that MsAcT Leu12Ala is 2.5 times more selective towards the 4-carbon dimethyl succinate compared to the 6-carbon dimethyl adipate in mono-substitution of DVA with 1-octanol. No conversion of the 8-carbon dimethyl suberate was observed. The transition for the length of accepted acyl donors thus lies between C6 and C8. This indicates that shorter alcohols than the ones used in **Paper V** (BVE and 1-octanol) can be exploited, and still get the mono-substituted product. However, further experiments have to be made to confirm this claim.

Bio-based polyamides

The third topic of study was renewable polyamides. Although lipases are known to catalyze formation of amide bonds, and polyamides are an important group of polymers, the majority of work reported on lipase-catalyzed polycondensations focuses on polyesters synthesis.(*36*) The high melting temperatures of polyamides (T_m) (130-200°C) have been pointed out as one reason for this.(*101*)

In **Paper VI** a lipase-catalyzed solvent-free route to bio-based polyamides using *Candida antarctica* lipase B (CalB) is presented Scheme 15. A long branched AB-type monomer (MO-cys) was both homopolymerized and co-polymerized with 1,12-diaminododecan to synthesize oligoamides (Scheme 13). The method uses the chemo-enzymatic procedures in a reversed way as compared to the methods presented in **Papers I-V**. The photochemical step is used for the synthesis of the starting monomer by thiol-ene addition of cysteamine to methyl oleate. The resulting monomer, MO-cys, was then polymerized by CalB in the enzymatic part of the reaction.

The method for synthesizing MO-cys was first presented by Türünç et. al.(*102*) Using the organobase TBD Türünç et. al. homopolymerized MO-cys and the product polyamide was still viscous at room temperature. Therefore, we postulated that MO-cys could be a good building-block in lipase-catalyzed synthesis of polyamides, since the product does not solidify at ambient temperatures.

We thus homopolymerized MO-cys under solvent-free conditions using both CalB and TBD as catalysts at 80 and 140°C (Table 5). In addition, MO-cys was co-polymerized with 1,12-diaminododecane at 80°C using CalB as catalyst with two different ratios of the starting monomers (Table 5 entry 5 and 6).

A priori concerns were raised regarding the suitability of the branched MO-cys as a substrate for CalB, which has a narrow entrance pocket to its active site. However, high activities towards MO-cys were observed for CalB in all performed reactions (Table 5, Entries 1-2 and 5-6). The synthesis of amine end-functionalized oligoamides reached high conversion and the reactions were complete within 4 hours.
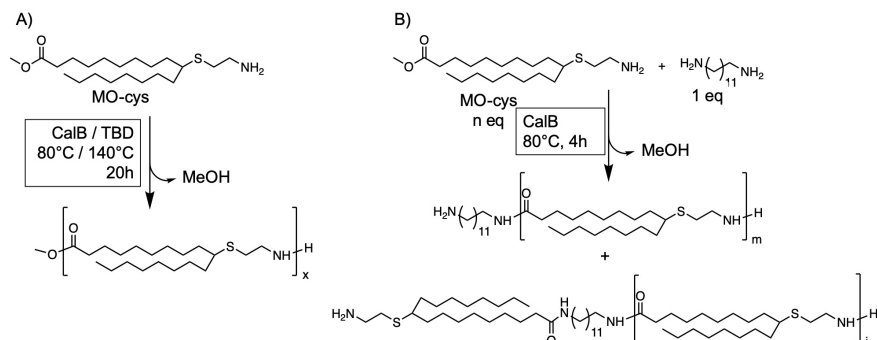
Scheme 15. Schematic overview of lipase-catalyzed solvent-free route to renewable polyamides. **A)** Homopolymerization of MO-cys; and **B)** co-polymerization of MO-cys with 1,12-diaminododecane (n=2 or 4), where m, i and x denote DP. Note: the thiol-ene addition of cysteamine to methyl oleate is neither stereo- nor regio-specific and consequently, MO-cys includes both enantiomers of the 9 and 10-isomers.

**Table 5.** Polycondensation of MO-cys, co-polymerization with 1,12-dodecanediamine (DA) or homopolymerization.

Entry	Catalyst	mol% catalyst[a]	Ratio MO-cys:DA	Temp (°C)	Time (h)	Conv[b] (%)	DP[b]
1	CalB	0.004	1:0	80	20	>85	>6
2	CalB	0.004	1:0	140	20	>95	>20
3	TBD	5	1:0	80	20	>85	>6
4	TBD	5	1:0	140	20	>95	>20
5	CalB	0.004	2:1	80	4	>98	2
6	CalB	0.004	4:1	80	4	>98	4
7[c]	-	-	1:0	80	20	20	-
8[c]	-	-	1:0	140	20	30[d]	-

[a] Assuming 3.3 wt% active lipase on carriers.(*97*) [b] Determined by ^{1}H-NMR. [c] Control reactions without catalyst. [d] The reaction mixture turned black.

For the amounts of catalyst added the two catalysts showed similar catalytic profiles at both 80 and 140°C. It should be noted that 140°C is considered as a high temperature for CalB. Even if the reaction reaches higher conversions at elevated temperatures the possibilities of reusing CalB decreases.(*103*)

The amounts of catalyst (in mol%) added in the two catalytic systems vary by over a factor of 1200, with similar results (Table 4). Organocatalysts are often inspired by the complex nature of enzymes, however the simple, often low molecular weight compounds have a hard time competing with the

complex large protein structure that is responsible for the high catalytic activity and selectivity. In Scheme 16, a comparison between CalB and TBD (proposed mechanism by Pratt et al. (*104*)) in the formation of acyl-catalyst intermediate is shown. Looking at the tetrahedral intermediates in the acylation step the difference in stabilization and orientation of the substrate that can be realized, by the two catalysts, can be visualized.
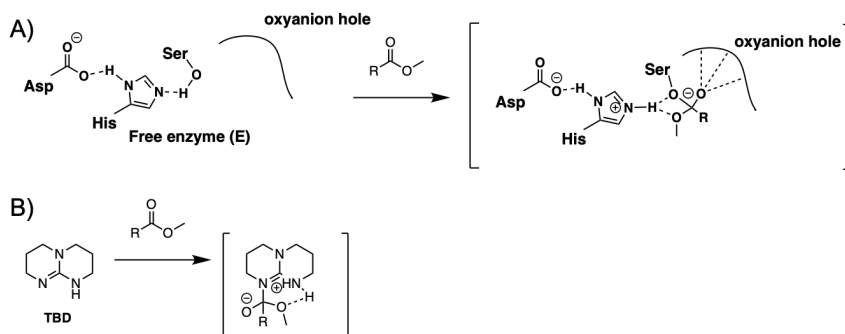
Scheme 16. Comparison of the tetrahedral intermediate in the acylation steps for: **A)** the serine hydrolase CalB; and **B)** the organobase catalyst TBD (*104*), where the transition states are shown within square brackets.

As can be seen in Scheme 16 there are two significant differences in the transition state formed between CalB or TBD (as proposed by Pratt et al. (*104*)) and the acyl substrate. Firstly, in CalB the residues in the catalytic triad (Asp-His-Ser) form a network for charge distribution. Ser is the nucleophilic amino acid and, as can be seen in Scheme 16, it is polarized by the His residue, which is itself stabilized by hydrogen bonding to Asp. In the simpler TBD molecule, there is no such stabilization of the positive charge. Secondly, the negative charge on the tetrahedral intermediate is stabilized in CalB by 3 hydrogen bonds in the oxyanion hole. TBD has no such hydrogen bonds to stabilize the negative charge in the transition state. Both these stabilization systems in CalB help to lowers the activation energy more than for TBD. Thus, explaining the large difference in the amount of catalyst needed for the efficient catalysis by CalB as compared to TBD (mol%, Table 4).

Conclusions

All three parts presented in this thesis advance the knowledge on handling and manufacturing of complex and/or sensitive building-blocks that can be UV-cured to create new polymeric materials.

The influence of different catalysts on the incorporation of dimethyl succinate into unsaturated polyester was studied. The results show the importance of choosing the right catalyst to avoid formation of side-products. The two different catalyst, the enzyme CalB or the organometallic catalyst Ti(OBu)$_4$, were used to synthesize unsaturated co-polyesters from dimethyl itaconate, dimethyl succinate and butanediol. The selectivity of CalB and Ti(OBu)$_4$ were shown to be different and thus, the choice of catalyst effects the macromolecular structure. Using CalB as a selective catalyst, unsaturated co-polyesters with a higher degree of ends constituted of the conjugated ester on dimethyl itaconate, were obtained. While Ti(OBu)$_4$, which is less selective was shown to incorporate itaconate more randomly into the polyester backbone. Although, the macromolecular structures were different comparable results of the final cross-linked network properties were obtained.

Additionally, examples of how the selective properties of enzymes can give access to structures that are difficult to synthesize with traditional catalysts were presented. The first example is the incorporation of thiol- and ene-functional groups in the same molecule. The methods presented used the selectivity of CalB towards formation of ester over thioester, and low reaction temperatures. In this thesis two methods are presented. The first method utilizes a diol with a 1,2-distbstituted alkene. The 1,2-disubstituted alkene is less reactive than other alkenes and thus no radical inhibitors were needed, and the synthesized thiol-alkene functional component could be stored for 6 months.

Furthermore, enzyme catalysis was used to synthesize a series of vinyl ether functional building-blocks. In the first presented method it was possible to use hydroxy vinyl ethers and functional carboxylic acids. Combining a carboxylic acid with a primary thiol at the ω-end with a hydroxy vinyl ether, one-component thiol-vinyl ether monomers were synthesized. A radical inhibitor was added, because of the high reactivity of vinyl ethers for thiol–ene chemistry. Additionally, lipoic acid was used as carboxylic acid. Lipoic acid, containing a ring with a disulfide, was used to provide a stable thiol-containing monomer and thus, radical inhibitors could be avoided.

The second example of how the selective properties of enzymes can be used to gain access to structures, that are difficult to synthesize with traditional catalysts, is the mono-substitution of divinyl adipate. To selectively react one of two identical chemical groups is a challenge. By utilizing the regioselectivity of a MsAcT variant, it was possible to selectively achieve mono-substitution of divinyl adipate. By using a hydroxy vinyl ether as alcohol, molecules with two reactivities, containing both vinyl ether and vinyl ester groups, were synthesized.

Additionally, a method for lipase-catalyzed synthesis of renewable polyamides is presented. The method displays the capability of the lipase CalB to work as an efficient catalyst for polyamide synthesis. Furthermore, the substrate presented to the lipase was a long branched monomer, despite this the lipase accepted the substrate and high conversions were reached.

Connecting back to *the twelve principles of green chemistry*, the synthetic procedures developed, in the work leading to this thesis meet many of the principles. The methods produce a low amount of waste: the co-products generated during synthesis have been water or methanol (which can be distilled and potentially recycled) and in additions most of the syntheses have been performed under solvent-free conditions. Furthermore, it is possible to remove and recycle the immobilized enzymes; the separation of homogeneous catalysts is hard and thus they often remain in the materials. Compared to conventional methods the synthesises were performed at lower temperatures, which reduces the energy consumption. Additionally, the photopolymerizations were performed at ambient temperatures

The thesis presents different strategies to use a wide range of monomers, from both renewable and finite sources. The new synthetic pathways combined enzymatic catalysis and photopolymerization producing polymeric materials in more sustainable ways. I believe that we have just begun to unfold the potential of combining the powers of different catalyst.

Future work

Much research remains for the application of CalB as a catalyst for polymer synthesis. For example, one can continue to investigate possible new building-blocks suitable for UV-curing, with increased bio-based content. Another interesting investigation is the possibility of incorporating monomers that provide more rigid structures into UV-curable molecules or in the backbone of polymers. Monomers could be incorporated by enzymatic polycondensation with monomers such as: isosorbibe, furane or lignin derivatives. Another alternative to incorporate more rigid components is by utilizing thiol-ene chemistry to add rigid groups to backbones containing unsaturation's, as the alkene in the functional networks presented in **Paper II**. Adding rigid groups could led to the possibility of further tuning the material properties, for example to increase T_g.

Another interesting line of research is the use of supercritical carbon dioxide to synthesize polymers with CalB. Super critical carbon dioxide can be used as a potent solvent and is environmentally friendly since the carbon dioxide is able to solubilize compounds that could be hard to dissolve under other conditions. This could, for example, be interesting for enzyme catalyzed synthesis of polyamides.

For MsAcT further investigating the versatility regarding diacids, alcohols and other substrates e.g. amines are interesting. It should for example be possible for the mutant MsAcT Leu12Ala to use amines in the mono-substitution to synthesis amide monomers that can in turn be used to synthesise drugs or as building-blocks in polymeric materials. Additionally, more engineering of MsAcT could be made to for example to create an even deeper site than in Leu12Ala, that could fit larger acyl donors, which would further expand the application scope.

In general, the synthesis routes developed in this thesis can be further developed to suit industry requirements for production volume. Even though we were successful in synthesising vinyl ethers on the 10g scale, much research is needed in order to increase the production volume to industry scale. A possible next step in this direction is to further investigate the immobilization of the catalyst, and to combine this with using a continuous-flow system. The immobilization of catalysts is of great importance to increase the stability of enzymes over several cycles both from an economic perspective and from an environmental point of view.

Further interesting applications of enzymes are not only to build, but also to degrade polymer structures. The work on screening and engineering enzymes for such applications to fight the rising plastic pollution problem, is of increasing importance.

Life cycle assessment (LCA)

Although the methods presented in this thesis are perceived as greener in comparison to traditional chemical routes, it is hard to know without a quantitative measure. One method for quantifying the impact of a product (or a service) is life cycle assessment (LCA). In LCA the environmental effects of products can be calculated by quantifying inputs and outputs of a process.

Although reports comparing the environmental impact of an enzymatic process to its chemical equivalent (with the exception of some pharmaceutical processes) are few; similar results were obtained in two reports that conducted comparative cradle to gate LCAs (i.e. the products life is followed from resource extraction to the factory gate), on the topic. One study looked at different catalytic processes for bio-diesel production, where the immobilized enzyme in the study was a lipase.(*105*) The other study compared the chemical and biochemical synthesis of lactones in Baeyer–Villiger oxidation, where the enzyme used was a monooxygenase.(*106*) The two reports both showed that for the enzymatic synthesis to be of lower environmental impact than the chemical, the recycling of enzyme is a key step. However, no comments were made on if the reactions were performed at the same temperatures. Sufficient environmental assessment tools are crucial in order to make the right choices and additionally, performing LCA based on laboratory-scale data could be used as a tool for improving enzymatic reactions before they are scaled up.

Acknowledgments

Mats Martinelle, my main supervisor. Thank you for this time, for all the guidance, your patience and the possibility to try and fail or succeed and for all the discussion, your open-door policy has been much appreciated.

Mats Johansson, my co-supervisor. Thank you for always sharing your knowledge with such clarity and for being such an engaged co-supervisor. Working with you has always been a lot of fun. *Karl Hult,* you have been an inspiration from start, but foremost I want to thank you for discussions regarding the preparation of this thesis and the last two manuscripts. Your passion for science and nature is contagious. *Eva Malmström*, I'm so happy for the collaboration that started with a discussion on Hawaii and ended up as half of the work in this Thesis. You are a true inspiration to me.

Sara Brännström, my friend and co-author. I think it's rare to find someone where work runs so smoothly. I have really enjoyed all the time we spent together. Now that our shared office, Non-solo, has closed we move on.

To my collaborators: *Samer Nameer*, *Stefan Semlitsch*, *Peter Hendil-Forsell, Mauro Claudino*, *Oscar He*, *Nicoló Razza* and *Marco Sangermano*, thank you for making a lot of this work possible. The *BioPol* group thank you for interesting discussions and a lot of input.

A big thank you to the *Biocats. Peter*, thank you for turning things around and making sure we stay on our toes. Thank you for teaching me the tricks of the trade, you were a great mentor. *Stefan,* thank you for the few hours a day when both of us were awake and in the office. For all the discussions about everything, for always finding my spelling errrs and for Friday magic. *Henrik,* thank you for always being so helpful and for sharing your knowledge about female and male fruits and what not. *Mattias,* for teaching me how to fold origami structure and for always questioning how it is done. *Shan*, *Fabian, Fei, Na, Maria, and Jonatan* for input and discussions and the nice talks in the lunch room. *Per Berglund,* is specially thanked for input under all these years. *Lisa* and *Federica,* thank you for sharing the PhD thesis time with me. It has been great to bounce ideas and navigate through the formal stuff together. *Lisa*, also thank you for always saving me from rain.

There are many others to whom I'm thankful, fellow PhD candidates and co-workers, this time would not have been the same without you! Special thanks to my favorite sceptic *Sarah* and my anatomy teacher *Mathilda*. An extra thank you goes to *Joakim,* for the PhD time, for reading this thesis, for coffee and bun in da sun, for all the conferences and for being a great friend.

Collen Mc Man and the rubber family at the USDA are thanked welcoming me into the group for a short research rotation.

Kära kära *vänner*! Peppen ni har levererat under all denna tid och framför allt nu i slutet har varit ovärderlig! Att få vandra genom livet med er betyder allt. Utan er skulle jag vara noll!

Till min utökade familj, *Maria, Gunnar, Johan, Sara, Anna*, *Rami* och *Mira*, vilket gäng. Tack för att i öppnar mina ögon för naturen och för att ni alltid ställer upp. *Maria* ett extra tack för sommaren då jag fick sitta ostört i ett hörn för att skriva det här.

Till min älskade familj, *Mamma, Pappa, Mårten, Molly, Felix, Sofi, Gustav* och *Sara* Jag har så mycket att vara tacksam för! Tack för att ni lär mig att ta ställning för det som är rätt. Jag har tur som har så mycket kärlek, skratt och briljans runt mig. Ni är alla mina förebilder på olika sätt. *Farmor* jag är så glad över vår relation. Du är så full av kunskap och idéer.

Poppy-Matilda att vara din mamma är det bästa som hänt mig och jag lovar att jag ska hjälpa dig följa dina drömmar, vilka de än må vara

Martin, Bob, min största supporter, kritiker och mitt livs stora kärlek. Hur kan jag ens formulera ett tack till dig, när det här aldrig hade varit möjligt utan dig. Jag viste inte att någon kunde vara som du är och att livet kunde vara som det är med dig. Du slutar aldrig imponera på mig. Tack pga.

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