Summary

Polysaccharides are versatile biopolymers that show their multiple characteristics in nature: cellulose, starch and chitin being examples. The molecular properties of such polysaccharides are excellent in terms of molecular weight and stereoregularity. These special properties are difficult to control with conventional polymer chemistry and hence most glycoscience is based on modifying natural polysaccharides rather than synthesizing them.

In nature, enzymes catalyze the in vivo polymerizations of the above mentioned polysaccharides. In this thesis it is shown that enzymes are also essential laboratory tools for the synthesis of biopolymers with control over macromolecular properties. Conventional polymer synthesis of polysaccharides is in many aspects inferior (or impossible) as compared to the enzyme catalyzed synthesis of polysaccharides investigated in this research. Here we present a method to enzymatically polymerize hyperbranched polysaccharides with control over stereoregularity, degree of branching and molecular weight. Moreover, the possibility to construct hybrid materials consisting of a hyperbranched polyglucan part connected to a synthetic substrate (e.g. polymer, surface, etc) is shown.

Using an enzymatic catalyzed tandem polymerization in which the unique properties of the enzymes potato phosphorylase and glycogen branching enzyme (GBE<sub>DG</sub>; from Deinococcus geothermalis) are combined, a hyperbranched polyglucan was polymerized consisting of (1→4) linked α-D-glucose residues with branches at the glucose C6 hydroxy group. In this tandem polymerization, phosphorylase catalyzes the addition of (1→4) linked α-D-glucose residues from a short oligosaccharide, using glucose-1-phosphate (G-1-P) as donor substrate (monomer). Phosphorylase is the driving force behind the polymerization while GBE<sub>DG</sub> introduces in situ branch points. More specifically, GBE<sub>DG</sub> catalyzes the formation of α(1→6) branch points by the hydrolysis of an α(1→4) linked glycosidic linkage and subsequent inter- or intra-chain transfer of the non reducing terminal fragment to the C6 hydroxyl position of an α-glucan.
A property of phosphorylase, essential for the research as outlined in this thesis, is the donor substrate (primer) dependency. Polymerization is impossible without an oligomeric $\alpha(1\rightarrow4)$ linked D-glucose primer of at least 3 glucose residues.

Chapter 2 shows the fundamentals of the enzyme catalyzed tandem polymerization while chapters 3, 4, and 5 describe the various possible hybrid structures when this essential primer is first coupled to a synthetic substrate.

Chapter 2 describes the isolation and purification of the enzymes, as well as the optimum reaction conditions for a tandem polymerization. Both enzymes showed activity at 37 °C and a pH of 7. The primer used to start the polymerization from, was maltoheptaose, obtained via the acidic catalyzed hydrolysis of $\beta$-cyclodextrin. The enzyme catalyzed tandem polymerization showed characteristics of a living polymerization, including:

- the polymer chains grew linear with time;
- further addition of G-1-P resulted in regrowth of the polymer chain;
- a termination step was absent;
- polymers with a low polydispersity were obtained.

Furthermore, different methods were evaluated to characterize the degree of branching and the side chain distribution profile of the hyperbranched polyglucans. It was shown via $^1$H-NMR that the degree of branching was 11 %. The side chain distribution was measured via MALDI-ToF spectrometry after the enzymatic hydrolysis of the $\alpha(1\rightarrow6)$ linkages. The resulting spectrum showed a rather narrow side chain distribution, ranging from 4 to 15 glucose residues with an optimum length of 7 glucose residues.

Chapter 3 shows the use of the previously described tandem polymerization for the construction of hyperbranched polysaccharide brushes. It is described how brushes were grown from functionalized Si wafers via the grafting from principle. First, a cleaned and oxidized Si wafer was functionalized with an aminosilane agent. This resulted in a surface coverage of 2.8 amino groups nm$^2$. Subsequently, maltoheptaose was coupled to the introduced amino groups via a reductive amination. The conversion of the reaction with the amino groups and the reducing group of the maltoheptaose molecules was determined to be 67 %. These primer functionalized substrates were used to start the enzyme catalyzed tandem polymerization. The thickness of the resulting hyperbranched polysaccharide coating
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was determined by ellipsometry and was in the range of 12-20 nm. Steric hindrance is expected to be the cause of the limited coating thickness.

CHAPTER 4 covers the enzyme catalyzed tandem polymerization started from di- and trivalent primers. Hence, maltoheptaose was coupled to core molecules carrying multiple amino groups. More specifically, di- and trifunctional primers were synthesized by reacting maltoheptaose with, respectively, butanediamine (BDA-(G7)\_3) and tris(2-aminoethyl)amine (TREN-(G7)\_3). This resulted in a mixture of completely and partly functionalized core molecules. It was found that only partly functionalized core molecules adhere to the column material Amberlite IR-120 (H\(^+\)) and hence purification of the mixture was possible with Amberlite beads. Approximately 50% of the product was completely functionalized. Subsequently, the enzyme catalyzed tandem polymerization - started from the purified multivalent primers - was evaluated. An increase in reaction rate was observed as compared with a maltoheptaose primed reaction. However the increase was not as high as expected. The phosphorylase catalyzed reaction may be retarded due to the densely packed multivalent primers which result in a suppression in the reaction rate. By varying the amount of G-1-P, different sized hyperbranched multi arm architectures were polymerized, which was confirmed by DLS.

CHAPTER 5 details the functionalization of poly(ethylene)glycol (PEG) with maltoheptaose as confirmed by MALDI-ToF. This so-called macro-primer was used to start the enzyme catalyzed tandem polymerization, resulting in diblock copolymers consisting of a hyperbranched polysaccharide part and a linear PEG part. By varying the amount of G-1-P, different sized diblock copolymers were polymerized, which was confirmed by DLS measurements.