Oximation optimization and applications in cardiovascular research

Valorisation

Over the past few years an increasing interest is shown in valorization of research performed in an academic setting. Research should have a societal impact and a clear utilization. One of the definitions of valorization is:

"*The process of value-creation out of knowledge, by making this knowledge suitable and available for economic or societal utilization and to translate this into high-potential products, services, processes and industrial activity.*"

This thesis describes the development of a chemical reaction (oximation) and the subsequent (cardiovascular) applications for which it can be used. While it is difficult at first to see a direct benefit to society of this fundamental research, the applications for which it has been used in later chapters already provide a hint towards potential products.

The first chapters (1 and 2) of this thesis discuss the formation and catalysis of the oxime bond. This so-called oximation reaction has long been overlooked in the bioconjugation field, but has received increasing attention over the past few years. With this ever increasing interest, more knowledge is gained about the usefulness of this technique in for example the development of novel medicines. The oxime bond is formed by reaction of a ketone with an aminooxy; both of these functionalities are normally not present in a protein. We have shown that a frequently used keto-acid, levulinic acid, is not suitable for introduction of a ketone in proteins and leads to low yields when performing subsequent oxime ligations. Interestingly, the postulated demise of levulinic acid (Chapter 3) does not hold true for other fields of chemistry. Levulinic acid, a 5-carbon building block, is suggested to be useful for a range of applications and could potentially become the next big biobased chemical. This furthermore shows that although an application might not be immediately apparent, fundamental research is necessary to eventually find these applications.

Optimization of the ketone involved in oxime ligation led to the accidental discovery of reaction rate acceleration by freezing. While we did not test this mechanism with other reactions in aqueous systems, we expect a similar result. Optimization of a chemical reaction reduces possible side reactions, increases yield and with that decreases cost and the environmental strain.

After optimization of the oximation process, the focus changed to applying the acquired knowledge to important questions in cardiovascular research. Atherosclerosis is a major health problem and can lead to serious conditions such as myocardial infarction or stroke. Remarkably, a cause of the disease is not known, and current treatment is mostly based on lifestyle changes (i.e. reducing cholesterol levels) and surgical interventions in late stage disease. Our research focused on the initiation of atherosclerosis. In early stages of disease development leukocytes (predominantly monocytes) play an important role and are attracted to a site of inflammation where

they differentiate into macrophages. This process is governed by small chemotactic cytokines known as chemokines. More than 40 different chemokines exist in the human body, each with its own function and expression pattern. Recent research has shown that these chemokines do not act alone, interactions between chemokines occur to modulate their function. In the process of atherosclerosis an interaction between two chemokines (CCL5 and CXCL4) was hypothesized, because of an increased monocyte arrest when the 2 chemokines were mixed. It was unclear, however, if this effect was a result of the formation of a heterodimer or a result of combined receptor activation. With the synthesis of a covalent heterodimer we could show that the increased monocyte arrest can be attributed to the formation of a heterodimer of chemokines CCL5 and CXCL4. With the synthesis of this heterodimer we have synthesized a molecule that can induce a disease state, the exact opposite of what would be beneficial for the general society. However, this molecule has provided us with insights into its mechanism of action, which can subsequently be used for the development of inhibitors. This study was followed up by an extensive mapping of all possible chemokine interactions. Subsequently, interactions with an atherogenic effect where further investigated and eventually led to the development of inhibitors of these interactions. First results show that this approach can inhibit monocyte arrest in vitro and atherosclerosis in an in vivo mouse model, and thus may be a viable therapeutic approach to inhibit plaque formation in an early stage. In order to develop these inhibitors into approved pharmaceuticals more research is required, but a first step is taken.

A different application of oximation was found in the modification of an existing drug. Tissue plasminogen activator (tPA) is currently being used to dissolve blood clots in stroke patients. The treatment is effective but often leads to bleeding complications as a result of the high required dose. We attempted to overcome this problem by modifying tPA to result in a molecule with a very high affinity for fibrin, which would decrease the required effective dose. We used an oxime bond to link a peptide that is covalently crosslinked to fibrin. Although current constructs show no improvement over unmodified tPA, we are confident that this approach will be beneficial when further improvements are made. Moreover, this method is useful for the modification of other (large) proteins currently being used as treatment for various diseases.