Bioorthogonal antigens as tool for investigation of antigen processing and presentation

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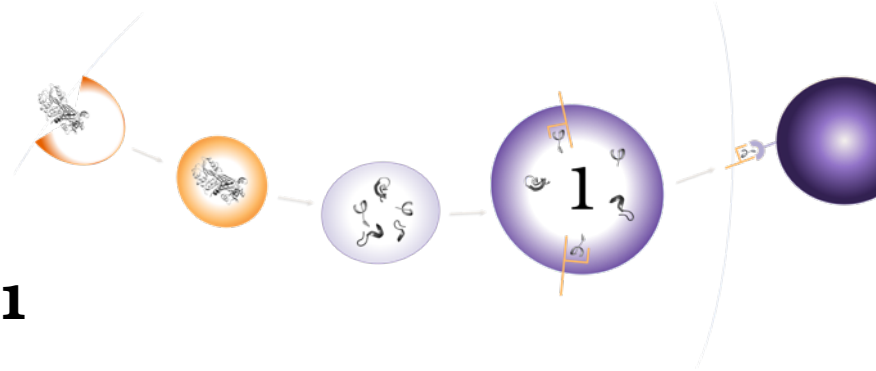

Chapter 1

Research scope

1.1 Studying antigen processing and presentation

In this thesis, the use of bioorthogonal chemistry in the study of the antigen processing and presentation pathway will be discussed. Bioorthogonal chemistry is the type of chemistry that can be performed with a high degree of selectivity in a biological sample - from fixed cell sections [1] to whole live metazoans [2,3]. The advantage regarding antigen processing and presentation - as will become apparent later in this work - is that this approach can be used to make detectable variants of antigenic proteins and peptides that are only minimally altered in their physical properties. As such they behave much more similar to 'real' antigens than all other available reagents.

1.2 Thesis outline

In **Chapter 2** of this thesis, the current mechanistic understanding of the antigen processing and presentation, as a central pathway in adaptive immunity in vertebrates, is outlined, as well as the techniques that are available to study processes involved in these. Furthermore, bioorthogonal chemistry, its scope and limitations and its potential to aid the study of antigen processing and presentation is delineated.

In **Chapter 3**, the expression and characterization of model antigens containing bioorthogonal amino acids is described. Bioorthogonality is achieved by replacing methionine with amino acids carrying chemically reactive groups. These bioorthogonal model antigens are then compared to wildtype antigens, as well as to the other reagents currently used to study antigen processing and presentation. Assessed are *inter alia* similarity of T cell recognition by dendritic cells.

This work is then conducted in more detail in **Chapter 4**, where the fine differences in processing of these bioorthogonal antigens by individual recombinant proteases of the endo-lysosomal system is compared *in vitro*. By assessing the cleavage rates by different recombinant proteases *in vitro*, the effect of the bioorthogonal modification on this degradation is shown. The effect of lysine carbamylation and arginine citrullination on this fine specificity is also described in this chapter. These non-templated post-translational modifications play an important role in i.e., the pathogenesis of rheumatoid arthritis [4] and autophagy [5], but their effects on antigen processing and presentation have not yet

been studied. The application of bioorthogonal antigen chemistry to this field, allows this for the first time.

Chapter 5 elaborates further on the use of bioorthogonal antigens as a method to investigate the influence of citrullination and carbamylation on antigen processing and presentation in an intracellular context. The use of bioorthogonal antigens and bioorthogonal chemistry to study altered degradation of disease-related antigens in live cells is described in this chapter.

Finally, **Chapter 6** summarizes the research objectives of this work in view of the results by summarizing the main findings and limitations of the research described in this thesis. Furthermore it gives impetus for potential future work.
Note that lists with the symbols, figures, tables and abbreviations used in this thesis are summarized at the end of this thesis.