



**Universiteit  
Leiden**  
The Netherlands

## **Bioorthogonal antigens as tool for investigation of antigen processing and presentation**

Pieper Pournara, L.

### **Citation**

Pieper Pournara, L. (2021, November 16). *Bioorthogonal antigens as tool for investigation of antigen processing and presentation*. Retrieved from <https://hdl.handle.net/1887/3239301>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3239301>

**Note:** To cite this publication please use the final published version (if applicable).

# Contents

|   |            |
|---|------------|
| <b>Acknowledgements</b>   | <b>vii</b> |
| <b>Summary</b>  | <b>ix</b>  |
| <b>1 Research scope</b>   | <b>1</b>   |
| 1.1 Studying antigen processing and presentation . . . . .                                      | 1          |
| 1.2 Thesis outline . . . . .  | 1          |
| <b>2 Introduction</b>   | <b>3</b>   |
| 2.1 Mechanisms of antigen processing . . . . .  | 6          |
| 2.1.1 Endo-lysosomal pathway . . . . .  | 6          |
| 2.1.2 The role of endo-lysosomal proteases in antigen presentation . . . . .                    | 7          |
| 2.2 Methods of studying antigen processing . . . . .  | 8          |
| 2.2.1 <i>In vitro</i> and intracellular degradation assays . . . . .                            | 8          |
| 2.2.2 T cell assays . . . . .   | 10         |
| 2.2.3 Mass spectrometry assays . . . . .  | 11         |
| 2.3 Summary of limitations of (above) methods . . . . .   | 11         |
| 2.4 Bioorthogonal chemistry . . . . .   | 12         |
| 2.4.1 Bioorthogonal reactions . . . . .   | 12         |
| 2.4.2 Incorporating bioorthogonal groups into proteins . . . . .                                | 15         |
| 2.4.3 Bioorthogonal chemistry during proteolytic degradation . . . . .                          | 16         |
| <b>3 Bioorthogonal antigens as model for antigen processing and presentation</b>                | <b>17</b>  |
| 3.1 Introduction . . . . .  | 17         |
| 3.2 Results . . . . .   | 18         |
| 3.2.1 Expression of bioorthogonal antigens . . . . .  | 20         |
| 3.2.2 Characterization of bioorthogonal antigens . . . . .                                      | 21         |
| 3.2.3 Visualization of bioorthogonal protein antigens by fluorophore attachment . . . . .       | 22         |
| 3.2.4 Intracellular degradation of bioorthogonal antigens in antigen presenting cells . . . . . | 22         |
| 3.2.5 Antigen presentation of bioorthogonal antigens . . . . .                                  | 24         |
| 3.2.6 Comparison with fluorescently pre-labelled ovalbumin . . . . .                            | 24         |
| 3.2.6.1 Biophysical characterization of fluorescently pre-labelled ovalbumin . . . . .          | 25         |
| 3.2.6.2 T cell activation by fluorescently labelled ovalbumin . . . . .                         | 27         |
| 3.2.6.3 Visualization of fluorescently pre-labelled ovalbumin . . . . .                         | 27         |

|   |            |
|---|------------|
| 3.3 Discussion and conclusion . . . . .   | 28         |
| 3.4 Materials and methods . . . . .   | 31         |
| 3.4.1 Materials . . . . .   | 31         |
| 3.4.2 Methods . . . . .   | 33         |
| <b>4 <i>In vitro</i> protein degradation of bioorthogonal antigens</b>                                      | <b>41</b>  |
| 4.1 Introduction . . . . .  | 41         |
| 4.2 Results . . . . .   | 44         |
| 4.2.1 Post-translational modification of vinculin . . . . .   | 46         |
| 4.2.2 Analysis of the degradation of bioorthogonal variants . . . . .                                       | 46         |
| 4.2.3 Digest of post-translationally modified vinculin variants . . . . .                                   | 49         |
| 4.2.4 Digest of carbamylated vinculin variants . . . . .  | 49         |
| 4.2.5 Digest of citrullinated bioorthogonal vinculin variants . . . . .                                     | 49         |
| 4.2.6 Digest of fluorescently labelled bioorthogonal vinculin variants . . . . .                            | 49         |
| 4.3 Discussion and conclusion . . . . .   | 53         |
| 4.4 Materials and methods . . . . .   | 54         |
| 4.4.1 Materials . . . . .   | 54         |
| 4.4.2 Methods . . . . .   | 55         |
| <b>5 Bioorthogonal antigens to study intracellular processing of post-translationally modified antigens</b> | <b>59</b>  |
| 5.1 Introduction . . . . .  | 59         |
| 5.1.1 Proteolysis in antigen presenting cells . . . . .   | 59         |
| 5.1.2 Structure changes antigenicity . . . . .  | 60         |
| 5.1.3 Post-translational modifications change antigenicity . . . . .  | 60         |
| 5.1.4 Detectable reporter proteins . . . . .  | 60         |
| 5.1.5 Bioorthogonal proteins to study antigen degradation . . . . .   | 61         |
| 5.2 Results . . . . .   | 62         |
| 5.2.1 Chemical analysis of the modified bioorthogonal recombinant proteins                                  | 62         |
| 5.2.2 Intracellular degradation in antigen presenting cells . . . . .                                       | 63         |
| 5.3 Discussion and conclusion . . . . .   | 64         |
| 5.4 Materials and methods . . . . .   | 65         |
| 5.4.1 Materials . . . . .   | 65         |
| 5.4.2 Methods . . . . .   | 66         |
| <b>6 Summary and future perspectives</b>  | <b>69</b>  |
| 6.1 Summary . . . . .   | 69         |
| 6.2 Future perspectives . . . . .   | 70         |
| 6.3 Materials and methods . . . . .   | 73         |
| 6.3.1 Materials . . . . .   | 73         |
| 6.3.2 Methods . . . . .   | 73         |
| <b>Bibliography</b>   | <b>78</b>  |
| <b>List of Figures</b>  | <b>107</b> |
| <b>List of Tables</b>   | <b>109</b> |
| <b>List of Abbreviations</b>  | <b>115</b> |

|                         |            |
|-------------------------|------------|
| <b>Zusammenfassung</b>  | <b>125</b> |
| <b>Περίληψη</b>         | <b>129</b> |
| <b>Samenvatting</b>     | <b>131</b> |
| <b>Curriculum Vitae</b> | <b>133</b> |

