

# **SELF-ASSEMBLY OF BIOMATERIALS AND BIOIMAGING AGENTS USING NOVEL PEPTIDES**

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# TABLE OF CONTENTS

TABLE OF CONTENTS .....	III
ABSTRACT .....	VI
DECLARATION .....	VIII
LIST OF PUBLICATIONS .....	IX
ORAL PRESENTATIONS .....	X
POSTER PRESENTATIONS .....	XI
LIST OF ABBREVIATIONS .....	XII
ACKNOWLEDGEMENTS .....	XVI
CHAPTER 1: SMART PEPTIDES: VERSATILE AND ROBUST TOOLS FOR NANOBIO TECHNOLOGY .....	1
INTRODUCTION .....	2
SMART PEPTIDES: VERSATILE AND ROBUST TOOLS FOR NANOBIO TECHNOLOGY .....	3
ABSTRACT .....	4
PEPTIDES THAT BIND INORGANIC MATERIALS .....	5
GLOSSARY .....	7
INORGANIC-BINDING PEPTIDES AS ‘MOLECULAR VELCRO’ .....	8
INORGANIC PEPTIDES AS MOLECULAR BUILDING BLOCKS: ‘MOLECULAR LEGO’ .....	20
FUTURE PERSPECTIVES .....	26
CONCLUSIONS .....	28
REFERENCES .....	29
MOTIVATION .....	38
AIMS AND SCOPE OF THIS THESIS .....	39

## Table of Contents

---

<b>CHAPTER 2: MATERIALS AND METHODS.....</b>	<b>41</b>
<b>CHAPTER 3: EFFECT OF <i>TRICHODERMA REESEI</i> PROTEINASES ON THE AFFINITY OF AN INORGANIC-BINDING PEPTIDE.....</b>	<b>45</b>
INTRODUCTION .....	46
CONTRIBUTION TO PUBLICATION 1 .....	47
PUBLICATION 1 .....	47
<b>CHAPTER 4: BIOFUNCTIONALISATION OF SILICA-COATED MAGNETIC PARTICLES MEDIATED BY A PEPTIDE .....</b>	<b>65</b>
INTRODUCTION .....	66
CONTRIBUTION TO PUBLICATION 2 .....	67
PUBLICATION 2 .....	67
<b>CHAPTER 5: LUMINESCENCE-ACTIVATING LINKER-PROTEIN G: A NOVEL UNIVERSAL DETECTION REAGENT FOR TIME-GATED LUMINESCENCE BIOIMAGING.....</b>	<b>79</b>
INTRODUCTION .....	80
CONTRIBUTION TO CHAPTER 5 .....	81
LUMINESCENCE-ACTIVATING LINKER-PROTEIN G: A NOVEL UNIVERSAL DETECTION REAGENT FOR TIME-GATED LUMINESCENCE BIOIMAGING .....	82
ABSTRACT .....	83
INTRODUCTION .....	85
MATERIALS AND METHODS .....	89
RESULTS .....	93
DISCUSSION.....	102
ACKNOWLEDGEMENTS.....	108

## Table of Contents

---

REFERENCES.....	109
SUPPLEMENTARY MATERIALS .....	114
<b>CHAPTER 6: SUMMARY AND FUTURE DIRECTIONS.....</b>	<b>117</b>
REFERENCES.....	126

## ABSTRACT

Protein immobilisation on solid matrices commonly relies on non-specific adsorption or the reaction between chemical groups within proteins and those on the matrix surface. In both cases, proteins attach to the surfaces in random orientations that may cause a reduction or loss of biological activity. Some peptides are capable of directing the immobilisation and orientation of proteins on solid surfaces without impeding protein function. They can recognise selectively and bind to a diverse range of inorganic substrates, for example, metals, materials containing carbon and polymers.

In this thesis I present a robust and versatile bioconjugation system that circumvents conventional chemical methods. This technology is based on a unique peptide linker sequence that displays high affinity towards materials that contain silica. The linker sequence can be fused directly to the sequence of a protein of interest using genetic engineering techniques and produced in *Escherichia coli*. The resulting recombinant fusion protein (Linker-Protein) exhibits strong affinity to a range of materials that contain silica, including synthetic silica and zeolites. Several applications of this technology are presented in this thesis.

The filamentous fungus *Trichoderma reesei* was tested as an alternative host to achieve higher yields and extracellular production of a linker-enzyme complex. However, the linker and several derivatives were rapidly degraded by *T. reesei* proteinases and further modification of the sequence is required by a directed evolution technique such as phage display.

In a further application, the linker was incorporated into *Streptococcus* Protein G, an antibody-binding protein. The resulting Linker-Protein G (LPG) was able to direct the attachment of antibodies onto silica-coated magnetic particles. The biofunctionalised particles were used for the antibody-mediated binding and recovery of different cell types for rapid, simple visualisation and identification by microscopy. The application of LPG was extended further by the incorporation of luminescent lanthanide chelates. The Luminescence-Activating LPG (LA-LPG) was shown to impart strong luminescence to antibodies within seconds and represents a novel indirect detection reagent for time-gated luminescence bioimaging.

## DECLARATION

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

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**Andrew Care - December 2014**



## LIST OF PUBLICATIONS

- [1] **Care, A**, P. Bergquist, and A. Sunna (2014) Smart Peptides: Versatile and Robust Tools for Nanobiotechnology. *Invited review for Trends in Biotechnology – submitted.*
  
- [2] **Care, A**, H. Nevalainen, P. Bergquist, and A. Sunna (2014) Effect of *Trichoderma reesei* proteinases on the affinity of an inorganic-binding peptide. *Applied Biochemistry and Biotechnology*, 173:2225-2240.
  
- [3] **Care, A**, F. Chi, P. Bergquist, and A. Sunna (2014) Biofunctionalisation of silica-coated magnetic particles mediated by a peptide. *Journal of Nanoparticle Research*, 16(8):1-9.
  
- [4] **Care A**, N. Sayyadi, R. Connally, A. Try, P. Bergquist and A. Sunna (2014) Luminescence-Activating Linker-Protein G: A novel universal detection reagent for time-gated luminescence bioimaging. *Awaiting provisional patent application to be filed before submission.*

## ORAL PRESENTATIONS

- [1] **Care, A**, P. Bergquist, and A. Sunna 'A robust and versatile bioconjugation method for micro- and nano-particles using an inorganic-binding peptide'. International NanoMedicine Conference 2014 - Sydney, Australia (*Winner for best 3 min presentation and poster*)
  
- [2] **Care, A**, P. Bergquist, and A. Sunna 'Rapid and simple functionalisation of inorganic materials using a peptide linker'. Biosensors 2014 - Melbourne, Australia
  
- [3] **Care, A**, P. Bergquist, and A. Sunna 'Biofunctionalisation of silica-coated magnetic particles using a peptide that binds silica' MQ BioFocus Research Conference 2013 - Sydney, Australia

## POSTER PRESENTATIONS

- [1] **Care, A**, P. Bergquist, and A. Sunna 'A robust and versatile bioconjugation method for micro- and nano-particles using an inorganic-binding peptide'. International NanoMedicine Conference 2014 - Sydney, Australia
  
- [2] **Care, A**, P. Bergquist, and A. Sunna 'Efficient immobilization of enzymes onto a solid matrix for industrial applications' Federation of European Microbiological Societies (FEMS) Congress 2013 – Leipzig, Germany
  
- [3] **Care, A**, P. Bergquist, and A. Sunna 'Expression of a zeolite-binding linker peptide in *Trichoderma reesei*' MQ BioFocus Research Conference 2012 - Sydney, Australia
  
- [4] **Care, A**, P. Bergquist, and A. Sunna 'Efficient immobilization of enzymes onto a solid matrix for industrial applications' BioProcessing Network Conference 2012 – Melbourne, Australia

## LIST OF ABBREVIATIONS

(alphabetical order)

ABP	antibody-binding protein
BHHCT	4,4'-bis(1",1",1",2",2",3",3"-heptafluoro-4",6"-hexanedion-6"-yl)-chlorosulfo-o-terphenyl
BHHST	4,4-bis-(1",1",1",2",2",3",3"-heptafluoro-4",6"-hexanedion-6"-yl)sulfonylaminopropyl-ester-N-succinimide-ester-o-terphenyl
BHTEGS	4,4'-bis (1",1",1",2",2",3",3"-heptafluoro-4",6"-hexanedion-6"-yl) sulfo-o-terphenyl-tetraethylene glycol-N-hydroxysuccinimide
BSA	bovine serum albumin
CE	conjugation efficiency
CEA	carcinoembryonic antigen
CTAB	cetyl trimethylammonium bromide
DC	dendritic cell
DELFI A	dissociation-enhanced lanthanide fluorescent immunoassay
DIC	differential interference contrast
DSSC	dye-sensitized solar cell

DNA	deoxyribonucleic acid
DNS	dinitrosalicylic acid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
FACS	fluorescence-activated cell sorting
FAU	faujasite
Fe-BND	ferrihydrite bionanodots
FITC	fluorescein isothiocyanate
GALD	gated auto-synchronous luminescence detector
GBP1	gold-binding peptide
GEPI	genetically-engineered peptides for inorganics
GRAVY	grand average of hydropathy
IBP	inorganic-binding peptide
IPTG	isopropyl $\beta$ -Dthiogalactoside
LA-PG	luminescence-activating Protein G
LA-LPG	luminescence-activating linker-Protein G
LB	Luria–Bertani medium
LBG	locust bean gum

## List of Abbreviations

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LC	light chain
LC/MS	liquid chromatography–mass spectrometry
LPG	linker-Protein G
ManA	$\beta$ -mannanase
MOPS	3-(N-morpholino)propanesulfonic acid
MRI	magnetic resonance imaging
OPH	organophosphorus hydrolase
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDB	protein database
PEG	polyethylene glycol
PG	Protein G
PONDR	predictor of natural disordered regions
SA	streptavidin
SAM	self-assembled monolayer
SBP	solid-binding peptide
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SiNPs	silica nanoparticles
SNR	signal-to-noise ratio
SP	smart peptide
sulfo-NHS	sulfo-N-hydroxysuccinimide
TGL	time-gated luminescence
TNT	trinitrotoluene
TR-FIA	time-resolved fluoroimmunoassays
SWNT	single-walled carbon-nanotube
UCNC	upconversion nanocrystal
ZBP	zinc oxide-binding peptide

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