Structural modification of proteins and peptides for the creation of nanomaterials

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A dissertation submitted in January 2009 to The University of Adelaide, in collaboration with The University of Canterbury, for the degree of Doctor of Philosophy.

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Abbreviations

AFM	Atomic force microscopy
Cryo-EM	Cryo-electron microscopy
DMSO	Dimethylsulphoxide
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
FTIR	Fourier transform infrared spectroscopy
GdnHCl	Guanidine hydrochloride
HMW	High molecular weight
HOPG	Highly ordered pyrolytic graphite
NaD	*Nicotinia alata* defensin
NMR	Nuclear magnetic resonance
QCM-D	Quartz crystal microgravimetry with dissipation
RsAFP	*Raphanus sativus* antifungal protein/peptide
RCM	Reduced and carboxymethylated
sHSP	Small heat shock protein
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
ThT	Thioflavin T
TEM	Transmission electron microscopy
TFE	Trifluoroethanol
XPS	X-ray photoelectron spectroscopy

Synopsis

Amyloid fibrils are highly ordered β-sheet structures that are formed from a variety of proteins *in vivo*, where they may have biological roles or, more commonly, are found associated with a broad range of diseases (known as amyloidoses). Due to the role of amyloid fibrils in disease and their potential as bionanomaterials, formation of amyloid and amyloid-like fibrils *in vitro* are exciting areas of research activity. This thesis explores the formation and characterisation of nanofibre structures, including amyloid and amyloid-like fibrils, from diverse peptides and proteins.

The growing interest in protein nanofibres in the bionanotechnology industry has led to research into new nanofibre forming target peptides and proteins. In this thesis, *Raphanus sativus* antifungal peptide 19mer (RsAFP-19) and a mutant of β2-microglobulin where the 3rd arginine was replaced with an alanine (R3A β2-microglobulin), were examined for their amyloiogenicity and nanofibre-forming propensity. Generation of nanofibre structures was successful for both, and the characteristics of formation, fibril structure and morphology were explored *via* thioflavin t binding (ThT), transmission electron microscopy (TEM), atomic force microscopy (AFM) and X-ray fibre diffraction. Nanofibre formation was also further characterised for mammalian lens crystallin proteins. Their ability to form protein nanofibres from semi-pure and crude protein mixtures was established, and the resulting fibrils characterised, including for their amyloid-like characteristics. This work demonstrates the ability of crystallin proteins to form inexpensive base materials for use in the bionanotechnology industry.

Inhibition of amyloid fibril formation is an area of current research activity for therapeutic purposes (against amyloidoses). α-Crystallin is a well-known molecular chaperone which traps intermediately structured target proteins, preventing them from both amorphous and ordered (amyloid fibril) aggregation. Like other crystallin proteins, α-crystallin can itself form amyloid fibrils under mildly denaturing conditions. The chaperone activity of αT- and αB-crystallin, in native, amorphously aggregated and fibrillar forms were assessed. Amyloid fibrils

and amorphous aggregates derived from αT and αB-crystallin acted as chaperones, although with modified activity to their native (non-fibrillar) structures. αB-Crystallin fibrils displayed enhanced chaperone activity, compared to native αB-crystallin. The chaperone activity of αB-crystallin was also assessed after its immobilisation onto solid surfaces. Protein immobilisation of the chaperone αB-crystallin was achieved using plasma generated aldehyde polymerisation and Schiff-based covalent bonding of the proteins. Immobilisation was characterised using X-ray photoelectron spectroscopy, AFM and quartz crystal micrography. Immobilised αB-crystallin was shown to act 100-fold to 5000-fold more effectively as a chaperone than native solution αB-crystallin (dependent upon target protein and the type of stress it was exposed to). This research has established that αT- and αB-crystallin can retain chaperone activity, or may even show enhanced activity, under conditions of extreme structural perturbation.

This thesis explores both the induction and inhibition of amyloid fibril, amyloid-like fibril and nanofibre formation, using a range of peptides and proteins, both pure and in crude mixtures. These protein nanofibre structures were characterised, to establish potential future use in bionanotechnology, kinetics and activity of amyloid and amyloid-like fibril formation and assessment of amyloid fibril inhibitors. This work further deliniated aspects of αT- and αB-crystallin chaperone ability, highlighting these proteins' ability to act as effective chaperones under a broad range of conditions.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Adelaide
2009

Acknowledgements

Over the course of this PhD I have had help, advice, assistance and encouragement from many wonderful people. I would primarily like to thank my two supervisors, John Carver and Juliet Gerrard, who have been fantastic in their support, guidance and leadership. John and Juliet provided intresting and successful projects, insight when I was lost, encouragement to follow my own ideas, understanding when things went wrong and good wine with loud laughter at my parties. They have my heartfelt gratitude.

I would also like to thank numerous people who I worked under or with, including: Hans Griesser, Stefani Griesser and Benjamin Thierry, who's assistance and guidance made the immobilised chaperone project very successful and equally fun; Sally Gras who introduced me to XRD and was a joy to work with; Heath Ecroyd and Sarah Meehan, who both taught me so much, especially the glories of α-crystallin and the beauty of amyloid; Grant Pearce and Suzie Meade who provided knowledge and insight into amyloid fibrils and great company for lunch; Matt Nussio, Joe Shapter, Paula Brooksby, Leanne Britcher, Lyn Waterhouse, Daniel Bilusich, Phil Clements and Manfred Ingerfeld, who all provided much needed expertise in a range of highly skilled fields.

I have had the privilege of working as part of two great research groups over the past few years. I'd like to thank everyone who was part of Lab 7 or The Purple Bubble in that time. In particular (other than those already mentioned): Chris Brockwell who kept things real; David Thorn who is my angel; Scott Buckley for all the walks home; Tom Kouldelka for mass spec and tea breaks; Danielle Williams for making me smile so often; Yanqin Liu for so many lovely meals; Francis Dehle and Sean Hudson for being such good fun in very different ways; Shiva Rao for our fibril work; Sean Devenish and Ren Dobson for providing entertainment and advice; and Jackie Healy for looking after me in the lab.

Much of the last few years have been spent thinking and talking about work even when I shouldn't. For their understanding in such matters I'd like to thank all my friends, particularly: Christabel Hough who insipired me; Keina Tiaon for those Melbourne visits; Owen Powell who is always there for me; Thivanka Dedigama, Cat Hyndman, Gajan Tyagarajah and Kim Hynes for drinks and tasty brunches; Mike Maclean and the CMC for scotch and understanding; Kate Langdon for Grace-ful moments; Bruce May for everything except the stalking; Jess 'Sugar' Young for many fanciful tales; Simone Haigh and her ambulances; Steven Poon for all those small joys; Jens Kroeske for all the music and sunny days; Joel Rathus for ukeles and debates; Amy O'Loughlin for the laughter and the puppies; Justin Williams for tea-chats and understanding; Yallana Burgess who makes life shine; Simon Grealy for some champagne moments; and Markus Pietsch with whom each day is brighter and happier than the last.

Finally, I could never have done anything like this if not for my family: my brothers and their wives (Adam, Amor, Nathan and Tara) who are all wonderful and inspirational people; but especially my parents, Ian and Janet Garvey – their love, friendship, advice and support has been strong and constant, through this PhD and for all of my life. I am grateful in so many ways to them all and proud to be part of their family and able to share their lives.