

# The Effects of Macrocyclic Constraints on Electron Transfer in Peptides

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by

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## **Abstract**

Research undertaken in this thesis focuses on electron transfer in peptides constrained into either a  $3_{10}$ -helical or a  $\beta$ -strand conformation in order to progress the field of molecular electronics.

## **Chapter One**

Natural proteins have evolved to promote electron transfer in many biological processes. However, their complex conformational nature inhibits a thorough investigation, so in order to study electron transfer in proteins, simple peptide models containing redox active moieties present as ideal candidates. Chapter One introduces the importance of secondary structure characteristic to proteins/peptides, and its relevance to electron transfer. The proposed mechanisms responsible for such electron transfer are discussed, along with the various approaches used to further constrain the peptides into their geometric conformations. The methods used to characterize the conformation of all peptides synthesized throughout this thesis are outlined, as are details of the electrochemical techniques used to investigate their electronic properties. A literature review describing several factors that have been shown to influence electron transfer in peptides, and a brief summary of molecular electronics follows.

## **Chapter Two**

Two  $3_{10}$ -helical peptides were synthesized, one constrained via a covalent side-chain staple using Huisgencycloaddition, and the other a linear analogue. Both peptides contain a redox active terminal ferrocene moiety, and were separately attached to a single walled carbon nanotube (SWCNT)/gold electrode array for electrochemical analysis. The effect of backbone rigidity imparted by the side-bridge constraint was revealed, which was shown to restrict the necessary torsional motions that lead to facile intramolecular electron transfer along the peptide backbone. High level calculations were used to support the electrochemical observations.

### **Chapter Three**

A series of peptides constrained into either a  $3_{10}$ -helix or  $\beta$ -strand conformation were synthesized, each containing a varied number of electron rich alkene side chains. The ability of the alkene(s) to facilitate electron transfer through the peptides by exploiting a hopping mechanism, and thus act as a “stepping stone” was investigated. Ring closing metathesis was used to further rigidify the backbones of a helical and a  $\beta$ -strand peptide via side chain tethers. The ensuing saturated and unsaturated compounds were electrochemically interrogated in order to explore any possible interplay between the effects of the alkene side-chains and backbone rigidity. High level calculations were conducted to verify the observed electrochemical data.

### **Chapter Four**

Two  $\beta$ -strand peptides were synthesized, one constrained via a covalent side-chain staple using Huisgen cycloaddition, and the other a linear analogue. Both peptides contain a redox active terminal ferrocene moiety, and were separately attached to a SWCNT/gold electrode array for electrochemical analysis. The charge transfer pathway was determined to be intramolecular by measuring the electron transfer rate at various concentrations of the constrained peptidebound to the electrode. This pathway is analogous to charge transfer through a molecular junction involving a single peptide. Theoretical conductance simulations were then undertaken using two peptide analogues in order to establish a link between the electrochemical observations and conductance measurements through a molecular junction.

### **Chapter Five**

Two macrocyclic peptides were synthesized, one constrained into a  $3_{10}$ -helical conformation by linking its  $i$  to  $i+3$  residues to form a lactam bridge, and the other constrained into a  $\beta$ -strand geometry via a lactam-bridge tether, linking its  $i$  to  $i+2$  residues. These peptides were chosen in order to define the role of the amide bond in a lactam bridge constraint. Direct linear analogues of each were used to establish the effect on electron transfer from a terminal amide bond located in an untethered side-chain. High level calculations were also conducted in order to elucidate the mechanism(s) responsible for electron transfer in each of the linear and macrocyclic helical peptides.

## **Declaration and published works**

I certify that 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Date



## **Publications generated from this thesis:**

- (1) Yu, J.; Horsley, J. R.; Abell, A. D. *Australian Journal of Chemistry*, **2013**, *66*, 848.
- (2) Yu, J.; Horsley, J. R.; Moore, K. E.; Shapter, J. G.; Abell, A. D. *Chemical Communications*, **2014**, *50*, 1652.
- (3) Horsley, J. R.; Yu, J.; Moore, K. E.; Shapter, J. G.; Abell, A. D. *Journal of the American Chemical Society*, **2014**, *136*, 12479.
- (4) Horsley, J. R., Yu, J., Abell, A. D. “The Correlation of Electrochemical Measurements and Molecular Junction Conductance Simulations in  $\beta$ -Strand Peptides” *Chemistry A European Journal*, **2015**, *21*, 5926-5933.
- (5) Horsley, J. R., Yu, J., Abell, A. D. “Understanding the Role of the Amide Bond and Electron Transfer Mechanisms in Macrocyclic Peptides” (Prepared in publication format “text in manuscript”).

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## Abbreviations

<u>Abbreviation</u>	<u>Full Name</u>
Å	angstrom
Aib	amino isobutyric acid
Ala	alanine
Boc	<i>tert</i> -butoxycarbonyl
br	broad (spectroscopic)
calcd	calculated
conc	concentrated
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dicloromethane
DIPEA	<i>N,N</i> -diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
EDC.HCl	1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride
ESI	electrospray ionization
equiv	equivalents
eV	electron volt
FTIR	Fourier transform infrared
h	hour
HATU	2-(7-aza-1 <i>H</i> -benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
IR	infrared
Leu	leucine
LRMS	low resolution mass spectroscopy

HRMS	high resolution mass spectroscopy
MeOH	methanol
min	minute
nm	nanometre
NMR	nuclear magnetic resonance
quant	quantitative
RCM	ring closing metathesis
rt	room temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TNBS	trinitrobenzene sulfonic acid
v/v	volume per unit volume
w/w	weight per unit weight

## **Statements of authorship**

Statements of authorship preface each chapter of this thesis where such chapter relates to published work.