

University of Wollongong
Research Online

University of Wollongong Thesis Collection
1954-2016

University of Wollongong Thesis Collections

2006

Mass spectrometric studies of non-covalent biomolecular complexes

Thitima Urathamakul
University of Wollongong, thitima@uow.edu.au

Follow this and additional works at: <https://ro.uow.edu.au/theses>

University of Wollongong

Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material.

Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

Recommended Citation

Urathamakul, Thitima, Mass spectrometric studies of non-covalent biomolecular complexes, PhD thesis, Department of Chemistry, University of Wollongong, 2006. <http://ro.uow.edu.au/theses/663>

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

NOTE

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

UNIVERSITY OF WOLLONGONG

COPYRIGHT WARNING

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

**Mass Spectrometric Studies of Non-Covalent
Biomolecular Complexes**

A thesis submitted in (partial) fulfilment of the requirements
for the award of the degree

Doctor of Philosophy

from

University of Wollongong



by

Thitima Urathamakul

Bachelor of Science (Honours)

Department of Chemistry

October 2006

DECLARATION

I, Thitima Urathamakul, declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The work has not been submitted for qualification at any other academic institution.

Thitima Urathamakul

24th October 2006

ACKNOWLEDGEMENTS

While this thesis is a culmination of three years' worth of work and study, my contribution in the form of its writing is but a small part of the overall process. The following is a list of people who have played an integral part in my life over the past several years – people who have provided me with guidance and support both immeasurable and invaluable. In short, people without whom this thesis would not have been possible.

Firstly, my supervisors Dr Jennifer Beck, Dr Stephen Ralph and Professor Margaret Sheil. Margaret, thank you for giving me the opportunity to complete my postgraduate research here at the University of Wollongong. You have never failed to help and encourage me through difficult times.

Steve, your enthusiasm has been a real driving force that has kept the ruthenium work (and my focus) on track. I have found your energy infectious and your ideas a constant source of inspiration.

Jenny, you have been an amazing supervisor throughout my time working with you. You have always found time to help me both academically and personally, your commitment to your students and your work is tremendous. You are my mentor and my confidant.

The three people mentioned above have been the best supervisors that any student could hope for.

Mr Larry Hick for his knowledgeable advice and assistance with the mass spectrometer. You have always been approachable and helpful to everyone. The lab is a warmer place with your presence. Larry, you are a legend.

Raj Gupta and Stephen Watt for their help in teaching me invaluable skills for the various instruments in the early stages of my degree. David Harman, Karin Maxwell, Stephen Blanksby, Roger Kanitz, Todd Mitchell, Jihan Talib, Karina Gornall, Linda Jessop, Michael Thomas, Jane Deeley, and other past and present members of the Mass Spectrometry group for making this a fun and enjoyable place to work.

Dr Nicholas Dixon and his group (Research School of Chemistry Australian National University) for their kindness in providing the proteins used in this study.

Dr Janice Aldrich-Wright (School of Science, Food and Horticulture, University of Western Sydney, Australia University of Western Sydney) for the ruthenium drugs used for the DNA work.

The Department of Chemistry, around which so much of my life has revolved over the past 8 years. The friendliness, support and guidance I have experienced during my time here have been truly memorable.

My family – my brothers, sister, and most especially my mother for all her support over the years and for giving me the opportunity to study overseas in the first place.

Last but not least, my dearest husband Min for all his endless support and patience, particularly during the tough time of writing up. Min, thank you for believing in me and for always being there for me.

PUBLICATIONS

Beck, J.L., Gupta, R., **Urathamakul, T.**, Williamson, N.L.; Sheil, M.M., Aldrich-Wright, J. R. and Ralph, S.F. (2003) Probing DNA Selectivity of Ruthenium Metallointercalators Using ESI Mass Spectrometry. *Chem. Commun.*, **5**, 626-7.

Urathamakul, T., Beck, J.L., Sheil, M.M., Aldrich-Wright, J.R. and Ralph, S.F. (2004) A Mass Spectrometric Investigation of Non-Covalent Interactions Between Ruthenium Complexes and DNA. *Dalton Trans.*, **17**, 2683-2690.

Beck, J.L., **Urathamakul, T.**, Watt, S.J., Sheil, M.M., Schaeffer, P.M. and Dixon, N.E. (2006) Proteomic Dissection of DNA Polymerisation. *Expert Rev. Proteomics*, **3**, 197-211.

Watt, S.J., **Urathamakul, T.**, Schaeffer, P.M., Sheil, M.M., Dixon, N.E. and Beck, J.L. (2006) Electrospray Ionisation Mass Spectrometry of Oligomers of *E. coli* DnaB Helicase and Mutants. *Rapid Commun. Mass Spectrom.*, **21**, 132-140.

ABSTRACT

Electrospray ionisation mass spectrometry (ESI-MS) was employed to investigate non-covalent associations of macromolecules with ligands, metal ions and other macromolecules. Firstly, ESI-MS was used to examine the interactions of six ruthenium compounds with three different DNA sequences (D1, D2 and D3). The relative binding affinities of these ruthenium compounds towards dsDNA was determined to be: $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+} \geq [\text{Ru}(\text{phen})_2(\text{dpqMe}_2)]^{2+} > [\text{Ru}(\text{phen})_2(\text{dpqC})]^{2+} > [\text{Ru}(\text{phen})_2(\text{dpq})]^{2+} > [\text{Ru}(\text{phen})_2(\text{pda})]^{2+} > [\text{Ru}(\text{phen})_3]^{2+}$. This order was in good agreement with that obtained from DNA melting temperature experiments. Competition experiments involving ruthenium compounds and organic drugs were also conducted to obtain information about the DNA binding modes of the ruthenium compounds. These studies provide strong support for the routine application of ESI-MS as a tool for analysis of non-covalent complexes between metallointercalators and dsDNA.

ESI-MS also proved to be a rapid and efficient tool for investigation of interactions between the N-terminal domain of ϵ ($\epsilon 186$, the exonuclease proofreading subunit of *E. coli* DNA) and three different metal ions (Mn^{2+} , Zn^{2+} and Dy^{3+}). The dissociation constants (K_d) for binding of Mn^{2+} , Zn^{2+} and Dy^{3+} to $\epsilon 186$ were determined from ESI-MS data to be 38.5×10^{-6} , 3.7×10^{-6} and 2.0×10^{-6} M, respectively. Despite binding the least tightly to the protein, incorporation of Mn^{2+} into the enzyme resulted in the highest enzymatic activity as measured by spectrophotometric studies. This suggested that Mn^{2+} is possibly the native metal ion present in $\epsilon 186$. The ability of the metal ions to enhance $\epsilon 186$ enzymatic activity was found to follow the order:

$\text{Mn}^{2+} \gg \text{Zn}^{2+} > \text{Dy}^{3+}$. The results of these experiments also provided evidence that the presence of two divalent metal ions was essential for efficient enzyme-catalysed hydrolysis.

The distribution of different oligomeric forms of wild-type *E. coli* DnaB helicase and DnaB helicase mutants (F102E, F102H, F102W and D82N) was examined using a factory-modified Q-ToF mass spectrometer equipped with a 32,000 m/z quadrupole. Previous experiments showed that the heptameric form of the wild-type protein was favoured in the presence of methanol (30% v/v). In the current work, mixtures of hexamer, heptamer, decamer and dodecamer were observed in solutions containing 1000 mM NH_4OAc , 1 mM Mg^{2+} and 0.1 mM ATP, pH 7.6. When the proteins were prepared in solutions containing a lower concentration of Mg^{2+} (0.1 mM), only the hexameric form was observed for all proteins except D82N, which showed a mixture of hexamer and heptamer. These observations suggest that the higher order structures were stabilised at high concentrations of Mg^{2+} . In addition, the hexamers of DnaB and mutants ($(\text{DnaB})_6$, $(\text{F102W})_6$ and $(\text{D82N})_6$) formed complexes with four to six molecules of the helicase loading partner, DnaC.

ESI-MS was used in conjunction with hydrogen/deuterium exchange studies to probe the unfolding mechanisms of linear and cyclised DnaB-N (the N-terminal domain of DnaB helicase) containing linkers comprised of different numbers of amino acid residues (3, 4, 5 and 9). The unfolding rates for all the cyclised proteins were about ten-fold slower than for the corresponding linear proteins. These observations suggest that enhancement of protein stability against unfolding could be achieved

through cyclisation. Furthermore, the HDX data showed that all the proteins examined exhibited a rare EX1 mechanism at near neutral pH.

ABBREVIATIONS

ϵ 186	N-terminal domain of ϵ
A ₄₂₀	Absorbance at 420 nm wavelength
ADP	Adenosine-5'-diphosphate
AMP-PNP	β , γ -imidoadenosine-5'-triphosphate
ATP	Adenosine 5'-triphosphate
BIRD	Blackbody infrared radiative dissociation
bp	Base pair
bpy	2,2'-Bipyridine
BSA	Bovine serum albumin
CD	Circular dichroism
CI	Chemical ionisation
CID	Collision-induced dissociation
Da	Dalton
DAPI	4',6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
dppz	Dipyrido[3,2- <i>a</i> :2',3'- <i>c</i>]phenazine
dpq	Dipyrido[3,2- <i>d</i> :2',3'- <i>f</i>]quinoxaline
dpqC	Dipyrido[3,2- <i>a</i> :2',3'- <i>c</i>](6,7,8,9-tetrahydro)phenazine
dpqMe ₂	Dipyrido[6,7- <i>d</i> :2',3'- <i>f</i>]2,3-dimethylquinoxaline
DSC	Differential scanning calorimetry
dsDNA	Double-stranded DNA
DTT	D, L-Dithiothreitol
Dy(OAc) ₃	Dysprosium(III) acetate

ECD	Electron-capture dissociation
EDTA	Ethylenediaminetetraacetic acid
EI	Electron ionisation
EM	Electron microscopy
EPR	Electron paramagnetic resonance
ESI	Electrospray ionisation
FAB	Fast atom bombardment
FD	Field desorption
FTICR	Fourier transform ion cyclotron resonance
HDX	Hydrogen/deuterium exchange
HSQC	Heteronuclear single quantum correlation
HMQC	Heteronuclear multiple quantum correlation
HX	Hydrogen exchange
ICP	Inductively coupled plasma
IR	Infrared
ITC	Isothermal titration calorimetry
k_{cat}	Turnover number (Michaelis-Menten kinetics)
K_d	Dissociation constant
kDa	Kilo Dalton
KF	Klenow fragment of Pol I (contains exonuclease domain)
kV	Kilovolts
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption ionisation

Mg(OAc) ₂	Magnesium(II) acetate
MLCT	Metal-to-ligand charge transfer
Mn(OAc) ₂	Manganese(II) acetate
M _r	Molecular mass
MS	Mass spectrometry
MWCO	Molecular weight cut off
NH	Amide hydrogen
NH ₄ OAc	Ammonium acetate
NMR	Nuclear magnetic resonance
NTP	Nucleoside triphosphate
PAGE	Polyacrylamide gel electrophoresis
PAP	Purple acid phosphatase
PD	Plasma desorption
Pda	9,10-diaminophenanthrene
PEG	Polyethylene glycol
phen	1,10-Phenanthroline
pm	Picometres
<i>p</i> NP-TMP	5'- <i>p</i> -nitrophenyl ester of thymidine-5'-monophosphate
Pol I	DNA polymerase I
Pol III	DNA polymerase III
Q-ToF	Quadrupole-time-of-flight
RNA	Ribonucleic acid
SPR	Surface plasmon resonance
SUPREX	Stability of unpurified proteins from rates of H/D exchange
ssDNA	Single-stranded DNA

TMP	Thymidine-5'-monophosphate
Tris-HCl	Tris (hydroxymethyl) amino methane hydrochloride
UV	Ultraviolet
Zn(OAc) ₂	Zinc(II) acetate

TABLE OF CONTENTS

<i>DECLARATION</i>	<i>i</i>
<i>PUBLICATIONS</i>	<i>iv</i>
<i>ACKNOWLEDGEMENTS</i>	<i>ii</i>
<i>ABSTRACT</i>	<i>v</i>
<i>ABBREVIATIONS</i>	<i>viii</i>
<i>TABLE OF CONTENTS</i>	<i>xii</i>
<i>LIST OF FIGURES</i>	<i>xvii</i>
<i>LIST OF TABLES</i>	<i>xx</i>
Chapter 1 Introduction to Biological Mass Spectrometry	1
1.1 Development of Biological Mass Spectrometry	1
1.2 Current Ionisation Techniques Used in Biological Mass Spectrometry	3
1.2.1 Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry.....	3
1.2.2 Electrospray ionisation (ESI) mass spectrometry	5
1.3 Non-Covalent Complexes	8
1.3.1 Brief overview of techniques for studying non-covalent complexes... 10	
1.3.2 ESI-MS studies of non-covalent complexes	14
1.3.2.1 ESI-MS of protein-DNA complexes.....	15
1.3.2.2 ESI-MS of protein-metal and protein-ligand complexes.....	17

1.3.2.3	<i>ESI-MS of dsDNA</i>	18
1.3.2.4	<i>ESI-MS of dsDNA-drug complexes</i>	21
1.3.2.5	<i>ESI-MS of multimeric protein subunits</i>	23
1.4	Scope of the Thesis	25
Chapter 2 Materials & Methods		28
2.1	Materials	28
2.2	Methods	29
2.2.1	Reactions of oligonucleotides with ruthenium compounds	29
	<i>Preparation of oligonucleotides</i>	29
	<i>Preparation of 16-mer double-stranded DNA (dsDNA)</i>	30
	<i>Titration of dsDNA with ruthenium complexes</i>	30
	<i>Competition for dsDNA among ruthenium compounds</i>	31
	<i>Competition between ruthenium compounds and organic drugs</i>	32
	<i>Melting temperatures of drug-DNA complexes determined by UV spectroscopy</i>	33
2.2.2	Preparation of proteins, protein-metal and protein-protein complexes	34
	<i>Determination of protein concentrations</i>	34
	<i>Metal ion binding to ϵ186</i>	35
	<i>Spectrophotometric assay of ϵ186 activity</i>	36
	<i>Oligomerisation of DnaB and DnaB mutants</i>	36
	<i>Formation of (DnaB)₆(DnaC)_x complexes</i>	37
	<i>Hydrogen/deuterium (H/D) exchange of linear and cyclised DnaB-N</i>	39
2.2.3	Mass spectrometry	41
	<i>Conditions for mass spectrometry</i>	41

<i>Processing data</i>	41
Chapter 3 Non-Covalent Interactions between DNA and Metallointercalators	44
3.1 Structure of DNA	44
3.2 DNA-Drug Interactions	49
3.2.1 Covalent (irreversible) binding	50
3.2.2 Non-covalent (reversible) binding	52
3.3 Transition Metal Complexes	57
3.4 Interactions of Ruthenium-Based Intercalators with dsDNA	59
3.5 Applications of Ruthenium and Other Metal-Based Metallointercalators	64
3.6 Scope of This Chapter	66
3.7 Results and Discussion	68
3.7.1 Reactions of ruthenium compounds with individual 16-mer duplexes	68
3.7.1.1 <i>Titration experiments</i>	68
3.7.1.2 <i>Competition experiments between ruthenium compounds</i>	77
3.7.1.3 <i>DNA selectivity</i>	81
3.7.1.4 <i>Saturation experiments</i>	86
3.7.1.5 <i>DNA melting experiments</i>	88
3.7.2 Competition experiments involving ruthenium compounds and organic drugs.....	91
3.7.2.1 <i>Competition between daunomycin and ruthenium compounds</i>	92
3.7.2.2 <i>Competition between distamycin and ruthenium compounds</i>	98

3.8	Conclusions	101
 <i>Chapter 4 Investigation of Interactions of Metal ions with the Exonuclease</i>		
	<i>Subunit of E. coli DNA Polymerase III.....</i>	<i>105</i>
4.1	Introduction	105
4.2	Replication in Escherichia coli	106
4.3	DNA Polymerases	107
4.4	DNA Polymerase III Holoenzyme	109
4.4.1	Epsilon (ϵ).....	110
4.5	Metal Ions in Proteins and Enzymes	112
4.5.1	Metal ion involvement in exonuclease activities of Pol I and Pol III	115
4.6	Scope of This Chapter	118
4.7	Results and Discussion	119
4.7.1	Binding of metal ions (Mn^{2+} , Zn^{2+} and Dy^{3+}) to ϵ 186	119
4.7.2	Spectrophotometric assay of ϵ 186 activity	130
4.8	Conclusions	136
 <i>Chapter 5 Oligomeric Forms of Escherichia coli Replicative Helicase</i>		
	<i>DnaB and Complexes with Its Loading Partner DnaC.....</i>	<i>137</i>
5.1	Helicases	137
5.1.1	DnaB helicase	137
5.1.2	DnaC protein.....	140
5.2	ESI-MS of Large Macromolecular Complexes.....	142
5.3	Scope of This Chapter	144

5.4	Results and Discussion	145
5.4.1	Oligomers of DnaB and DnaB mutants revealed by nanoESI-MS...	145
5.4.2	Effect of Mg ²⁺ concentration on oligomerisation of DnaB and mutants	153
5.4.3	Titration of DnaB, F102W and D82N with DnaC.....	155
5.4.4	Formation of complexes of DnaB and mutants with ADP	158
5.5	Conclusions	160
Chapter 6	<i>Comparison of Unfolding Rates of Linear and Cyclised DnaB-N using Hydrogen/Deuterium Exchange</i>	162
6.1	Introduction	162
6.1.1	Protein splicing	163
6.2	Hydrogen/Deuterium Exchange (HDX)	167
6.3	Techniques for Probing Protein Conformational Dynamics and Interaction Sites of Protein Complexes	170
6.3.1	Hydrogen exchange coupled with mass spectrometry (HX MS).....	171
6.4	Cyclisation of the N-terminal Domain of DnaB (DnaB-N).....	174
6.5	Scope of This Chapter	176
6.6	Results and Discussion	177
6.6.1	Hydrogen/deuterium exchange rates.....	177
6.6.2	Effect of salt concentration on H/D exchange rates.....	187
6.7	Conclusions	192
	<i>REFERENCES.....</i>	194
	<i>APPENDICES</i>	247

LIST OF FIGURES

Figure 1.1 A schematic representation of the matrix-assisted laser desorption ionisation (MALDI) process	4
Figure 1.2 A Schematic representation of droplet formation at atmospheric pressure inside an ESI mass spectrometer source.	6
Figure 3.1 Essential features of the structure of double-stranded (ds) DNA	45
Figure 3.2 The A-, B- and Z-conformations of DNA.	47
Figure 3.3 Examples of small molecules that covalently bind to DNA.....	50
Figure 3.4 Structures of well known minor groove binders.....	54
Figure 3.5 X-ray crystallographic structures of complexes of a minor groove binder and an intercalator with dsDNA.....	55
Figure 3.6 Structures of some intercalators.....	57
Figure 3.7 Enantioselective interactions of a ruthenium compound with B-DNA ...	59
Figure 3.8 Structures of ruthenium metallointercalators used in this study.....	62
Figure 3.9 The “molecular light switch” effect displayed by $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$	63
Figure 3.10 Structure of a synthetic restriction enzyme.....	65
Figure 3.11 Oxidative repair of UV-damaged DNA by a rhodium metallointercalator	66
Figure 3.12 Negative ion ESI mass spectra of reaction mixtures containing different ratios of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and D2.....	69
Figure 3.13 Negative ion ESI mass spectra of reaction mixtures containing a 6:1 ratio of ruthenium compound and duplex D2	71
Figure 3.14 Relative abundances of non-covalent complexes obtained from reaction mixtures containing a 6:1 ratio of ruthenium compound and duplex D2	76

Figure 3.15 Negative ion ESI mass spectra of reaction mixtures containing a 3:3:1 ratio of two ruthenium compounds and D1	79
Figure 3.16 Crystal structure of $\Delta\text{-}\alpha\text{-}[\text{Rh}[(\text{R,R})\text{-Me}_2\text{trien}]\text{phi}]^{3+}$ bound dsDNA	82
Figure 3.17 DNA sequence selectivity of $[\text{Ru}(\text{phen})_2(\text{dpqMe}_2)]^{2+}$	83
Figure 3.18 DNA sequence selectivity of $[\text{Ru}(\text{phen})_3]^{2+}$	84
Figure 3.19 Relative abundances of ions assigned to non-covalent complexes present in ESI mass spectra of reaction mixtures containing $[\text{Ru}(\text{phen})_2(\text{dpqC})]\text{Cl}_2$ and D2.....	87
Figure 3.20 DNA melting curves for D2.....	89
Figure 3.21 Negative ion ESI mass spectra of reaction mixtures containing ruthenium compound, organic drug and D2.....	94
Figure 3.22 Negative ion ESI mass spectra of reaction mixtures containing ruthenium compound, organic drug D3	99
Figure 4.1 Structural model showing the stoichiometry of <i>E. coli</i> DNA polymerase III holoenzyme subunits.....	110
Figure 4.2 Proposed mechanism for hydrolysis of phosphodiester bonds by the ϵ subunit of DNA polymerase III.....	117
Figure 4.3 Positive ion ESI mass spectra (transformed to a mass scale) of ϵ 186 with increasing Mn^{2+} concentrations.....	120
Figure 4.4 Positive ion ESI mass spectra (transformed to a mass scale) of a 1:500 mixture of ϵ 186: Mn^{2+} before and after dialysis	123
Figure 4.5 Relative abundances of ϵ 186, and complexes of ϵ 186 with different numbers of bound Mn^{2+} ions in ESI mass spectra	124
Figure 4.6 Relative abundances of ϵ 186, and complexes of ϵ 186 with different numbers of bound Zn^{2+} ions in ESI mass spectra.	127

Figure 4.7 Relative abundances of $\epsilon 186$ and $\epsilon 186 + 1 \text{ Dy}^{3+}$ in ESI mass spectra of solutions containing different concentrations of Dy^{3+}	129
Figure 4.8 Hydrolysis of <i>p</i> NP-TMP by $\epsilon 186$ in the presence of different metal ions	132
Figure 5.1 Model of the three dimensional structure of DnaB hexamer constructed from cryoelectron micrographs	139
Figure 5.2 Electron micrographs after self-organising map algorithm analysis showing different quaternary structures of the DnaB helicase at different pH.....	140
Figure 5.3 Models of the $(\text{DnaB})_6(\text{DnaC})_6$ complex developed from electron micrographs.....	141
Figure 5.4 A schematic representation of the custom-built Waters Q-ToF Ultima™	143
Figure 5.5 X-ray crystal structure of the dimeric DnaB-N	145
Figure 5.6 Positive ion nanoESI mass spectra of full length DnaB and mutants... 147	
Figure 5.7 Positive ion nanoESI mass spectra of titration experiments of hexameric DnaB and mutants with DnaC hexameric helicase with DnaC.....	157
Figure 5.8 An expansion of the <i>m/z</i> range ~8920-9120 of the 34^+ ion from the nanoESI mass spectrum of F102H.....	159
Figure 6.1 Proposed mechanism of protein splicing	165
Figure 6.2 Kinetic mechanisms of amide hydrogen/deuterium exchange of native proteins.....	169
Figure 6.3 NMR structures of 9-lin- and 9-cz-DnaB-N.....	177
Figure 6.4 ESI-MS analysis of HDX for 3-lin- and 3-cz-DnaB-N	179

Figure 6.5 Relative abundance plots of peaks A and B obtained during HDX experiments for linear and cyclised DnaB-N containing different linker lengths in 10 mM NH ₄ OAc	184
Figure 6.6 First order plots of HDX of linear and cyclised DnaB-N containing different linker lengths in 10 mM NH ₄ OAc.....	185
Figure 6.7 ESI-MS analysis of HDX for 3-lin- and 3-cz-DnaB-N in in 100 mM NH ₄ OAc	188
Figure 6.8 Relative abundance plots of peaks A and B obtained during HDX experiments for linear and cyclised DnaB-N containing different linker lengths in 100 mM NH ₄ OAc.....	190
Figure 6.9 First order plots of HDX of linear and cyclised DnaB-N containing different linker lengths in 100 mM NH ₄ OAc.....	191

LIST OF TABLES

Table 2.1 Compositions of reaction mixtures used for competition experiments among ruthenium compounds and organic drugs	33
Table 2.2 Extinction coefficients (ϵ_{280}) used to determine protein concentrations. .	34
Table 2.3 Examples of compositions of (DnaB) ₆ (DnaC) _x , (F102W) ₆ (DnaC) _x or (D82N) ₆ (DnaC) _x oligomerisation mixtures	39
Table 2.4 ESI-MS conditions used for the analysis of ruthenium-DNA and protein samples	43
Table 3.1 DNA melting temperatures obtained from reaction mixtures containing D2 and different ruthenium compounds.....	90

Table 4.1	Kinetics and equilibrium parameters for ϵ 186 treated with Mn^{2+} , Zn^{2+} or Dy^{3+}	134
Table 5.1	Calculated values of m/z for the 35^+ ion of hexameric DnaB ((DnaB) ₆) and its complexes with ADP and magnesium.....	148
Table 6.1	Peptide sequences of the DnaB-N linkers used in this study.	177
Table 6.2	Average molecular masses of peaks A and B from HDX of DnaB-N with different linker lengths obtained at different salt concentrations.....	182
Table 6.3	Average numbers of amide protons exchanged obtained from solutions containing 10 and 100 mM NH ₄ OAc.....	182
Table 6.4	First order rate constants for unfolding of linear and cyclised DnaB-N with different linker lengths in 99% D ₂ O, 10 mM NH ₄ OAc.	186
Table 6.5	First order rate constants for unfolding of linear and cyclised DnaB-N with different linker lengths in 99% D ₂ O, 100 mM NH ₄ OAc.	192