

**A Study of the Spontaneous Membrane  
Insertion of Chloride Intracellular Ion  
Channel Protein CLIC1 into Model Lipid  
Membranes**

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*A thesis submitted in fulfilment of the requirements for  
the degree of Doctor of Philosophy*



**|U|T|S|**

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## **Certificate of Original Authorship**

I, Khondker Rufaka Hossain, 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 except as fully acknowledged within the text.

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[2] Al Khamici, H., Brown, L., Hossain, K., Hudson, A., Sinclair-Burton, A., Ng, J., Daniel, E., Hare, J., Cornell, B., Curmi, P., Davey, M., and Valenzuela, S. (2015) Members of the Chloride Intracellular Ion Channel Protein Family Demonstrate Glutaredoxin-Like Enzymatic Activity, *PLoS One* 10, e115699.

[3] Yepuri, N., Holt, S., Moraes, G., Holden, P., Hossain, K., Valenzuela, S., James, M., and Darwish, T. (2014) Stereoselective synthesis of perdeuterated phytanic acid, its phospholipid derivatives and their formation into lipid model membranes for neutron reflectivity studies., *Chem Phys Lipids*. 183, 22-33.

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## Table of Contents

Certificate of Original Authorship.....	II
Acknowledgement.....	III
Publications.....	IV
Conference Presentations.....	V
Abbreviations.....	XII
List of Figures.....	XVI
List of Tables.....	XX
Abstract.....	XXI
<b><u>Chapter 1 Chloride Intracellular Ion Channel (CLIC) Proteins</u></b> .....	<b>1</b>
<b>1. Introduction</b> .....	<b>2</b>
<b>1.1 Chloride Ion Channels</b> .....	<b>3</b>
<b>1.2 The CLIC proteins</b> .....	<b>6</b>
<b>1.3 The CLIC1 protein</b> .....	<b>12</b>
1.3.1 Tissue and subcellular distribution.....	12
1.3.2 Physiological function of CLIC1.....	13
<b>1.4 Structure of CLIC1 protein</b> .....	<b>15</b>
1.4.1 The N-terminal and C-terminal domain of CLIC1 protein.....	15
1.4.2 The putative transmembrane regions of CLIC1 protein.....	18
<b>1.5 Structural similarity between GST superfamily and CLIC proteins</b> .....	<b>19</b>
<b>1.6 Conversion of soluble CLIC1 into membrane integral form</b> .....	<b>22</b>
<b>1.7 Factors regulating the membrane insertion of CLIC1</b> .....	<b>25</b>
1.7.1 Redox regulated membrane insertion of CLIC1.....	25
1.7.2 Role of pH on spontaneous membrane insertion of CLICs.....	28
1.7.3 Role of Lipid composition on spontaneous membrane insertion of CLIC1.....	30

1.8 Aims and Objective.....	32
1.9 References.....	34
<b><u>Chapter 2 Theory and Applications of the Langmuir Monolayer and Reflectivity Techniques used in this Study</u></b>	<b>45</b>
<b>2.1 Introduction.....</b>	<b>46</b>
<b>2.2 Langmuir Monolayer Model.....</b>	<b>47</b>
2.2.1 Advantages of the Langmuir Monolayer as a model membrane system...	47
2.2.2 Lipid as amphiphilic molecules for Langmuir Monolayer.....	48
2.2.3 Lipid Monolayer at the Air-Water interface.....	51
2.2.4 Surface pressure – Area isotherm.....	52
2.2.5 Surface pressure- Area isotherms of POPC, POPE and POPS..... monolayers	55
<b>2.3 Lipid-protein interaction at the Air-Water interface.....</b>	<b>57</b>
<b>2.4 X-ray and Neutron Reflectivity studies give structural information about CLIC1 and Lipid Monolayer upon their interaction at the Air-Water interface</b>	<b>59</b>
2.4.1 Fundamental principles for X-ray and Neutron Reflectivity.....	59
2.4.2 Advantages of NR over XR.....	62
2.4.3 Contrast variation of protein and lipids in the monolayer film.....	63
<b>2.5 Conclusion.....</b>	<b>64</b>
<b>2.6 References.....</b>	<b>65</b>
<b><u>Chapter 3 Cholesterol Promotes the Interaction of the protein CLIC1 with Phospholipid Monolayers at the Air-Water Interface</u></b>	<b>70</b>
<b>3.1 Introduction.....</b>	<b>71</b>
<b>3.2 Materials and Method.....</b>	<b>73</b>
3.2.1 CLIC1 heterologous over-expression.....	74

3.2.2 His-CLIC1 fusion protein purification and cleavage.....	75
3.2.2.1 Nickel Affinity Chromatography.....	75
3.2.2.2 Size Exclusion Chromatography.....	77
3.2.3 Protein Concentration Determination.....	78
3.2.4 SDS-PAGE.....	79
3.2.5 Western Blotting.....	79
3.2.6 HEDS Enzyme Assay.....	80
3.3 Langmuir Film Experiments.....	80
3.3.1 Surface activity of CLIC1 protein at the Air-Water interface....	81
3.3.2 Interaction of CLIC1 with Phospholipid or Cholesterol Monolayers.....	81
3.3.3 Interaction of CLIC1 with Mixed Lipid Monolayers .....	82
3.3.4 Pre-incubation of CLIC1 with Cholesterol.....	82
<b>3.4 Results.....</b>	<b>84</b>
3.4.1 Protein Overexpression and Purification.....	84
3.4.1.1 SDS-PAGE analysis of samples collected from Affinity Chromatography.....	84
3.4.1.2 Size Exclusion Chromatography Results.....	86
3.4.2 HEDS Enzyme Assay.....	88
3.4.3 Surface activity of CLIC1 protein.....	89
3.4.4 Interaction of CLIC1 with Lipid Monolayers.....	90
3.4.5 Interaction of CLIC1 with Phospholipid Monolayers containing Cholesterol.....	93
3.4.6 Interaction of CLIC1 with Mixed Lipid Monolayers.....	94
3.4.7 Surface pressure-Area isotherms of Mixed Phospholipid Monolayers.....	96
3.4.7 CLIC1-Cholesterol interaction.....	98
<b>3.5 Discussion.....</b>	<b>101</b>
<b>3.6 Conclusion.....</b>	<b>105</b>

<b>3.7 References</b> .....	106
<b><u>Chapter 4 Elucidating the structure of CLIC1 at the Air-Water Interface: An X-ray and Neutron Reflectivity Study</u></b>	<b>110</b>
<b>4.1 Introduction</b> .....	111
<b>4.2 Materials and Method</b> .....	114
4.2.1 Deuterated-CLIC1 protein expression, purification and activity.....	114
4.2.2 Sample preparation for X-ray and Neutron Reflectivity experiments.....	114
4.2.3 X-ray Reflectometry measurements at the Air-Water interface.....	115
4.2.4 Specular Neutron Reflectometry measurements at the Air-Water interface.....	116
4.2.5 XR and NR data analysis.....	119
<b>4.3 Results</b> .....	123
4.3.1 Functional activity of deuterated CLIC1 (d-CLIC1) protein.....	123
4.3.2 CLIC1 interaction with POPC monolayer in the absence and presence of Cholesterol.....	124
4.3.2.1 Characterisation of CLIC1 insertion into POPC ( $\pm$ cholesterol) monolayer by X-ray Reflectivity.....	125
4.3.2.2 Characterisation of CLIC1 insertion into POPC ( $\pm$ cholesterol) monolayer by Neutron Reflectivity.....	129
4.3.3 CLIC1 interaction with POPE monolayer in the absence and presence of Cholesterol.....	133
4.3.3.1 Characterisation of CLIC1 insertion into POPE monolayer.....	133
4.3.3.2 Characterisation of CLIC1 insertion into POPE:Chol monolayer.	136
4.3.4 CLIC1 interaction with POPS monolayer in the absence and presence of Cholesterol.....	138
<b>4.4 Discussion</b> .....	140
<b>4.5 Conclusion</b> .....	145

4.6 References.....	146
---------------------	-----

**Chapter 5 Sterol structural requirements for interaction of CLIC1 with  
Cholesterol in Phospholipid Monolayers** 151

5.1 Introduction.....	152
-----------------------	-----

5.2 Materials and Method.....	155
-------------------------------	-----

5.2.1 Langmuir Monolayer Experiment.....	155
--	-----

5.2.2 Specular Neutron Reflectivity.....	155
--	-----

5.3 Results .....	157
-------------------	-----

5.3.1 Effects of Sterol Structure on CLIC1 Membrane Interactions.....	157
---	-----

5.3.2 Characterisation of CLIC1 insertion into POPC monolayers containing different natural sterols.....	159
---	-----

5.3.2.1 Characterisation of CLIC1 insertion into POPC:Ergosterol monolayer.....	160
--	-----

5.3.2.2 Characterisation of CLIC1 insertion into POPC:β-Sitosterol monolayer.....	164
--	-----

5.3.2.3 Characterisation of CLIC1 insertion into POPC:Hydroxyecdysone monolayer .....	167
--	-----

5.4 Discussion.....	171
---------------------	-----

5.5 Conclusion.....	177
---------------------	-----

5.6 References.....	178
---------------------	-----

**Chapter 6 A conserved GXXXG motif in the transmembrane domain may  
serve as the Cholesterol-Binding motif for the CLIC1 proteins** 182

6.1 Introduction.....	183
-----------------------	-----

6.2 Material and Method.....	186
------------------------------	-----

6.2.1 Site-directed mutagenesis using polymerase chain reaction (PCR).....	186
--	-----

6.2.1.1 Oligonucleotide primer design.....	189
--	-----

6.2.1.2 Purification of CLIC1-pET-28a plasmid.....	189
6.2.1.3 Polymerase chain reaction (PCR).....	190
6.2.1.4 Transformation into E.coli XL1-Blue super-competent cells.....	191
6.2.1.5 DNA sequencing.....	192
6.2.1.6 Transformation into E.coli BL21 (DE3) pLysS super-competent cells.....	192
6.2.1.7 Preparation of CLIC1-mutant Glycerol Stocks.....	193
6.2.2 Over-expression and Purification of CLIC1 mutants: G18A and G22A....	193
6.2.3 Circular Dichroism Spectroscopy.....	194
6.2.4 Dialysing DTT from CLIC1-wt and mutant proteins in solution.....	196
6.2.5 Functional analysis of G18A and G22A CLIC1 mutants.....	196
6.2.6 Spontaneous membrane insertion of G18A and G22A CLIC1 mutants....	197
<b>6.3 Results.....</b>	<b>198</b>
6.3.1 DNA Sequencing results.....	198
6.3.2 Over-expression and purification of G18A and G22A CLIC1 mutants.....	201
6.3.3 Structural analysis of G18A and G22A CLIC1 mutants using Circular Dichroism Spectroscopy.....	202
6.3.4 Functional activity of G18A and G22A CLIC1 mutants.....	203
6.3.5 Spontaneous membrane insertion of G18A and G22A CLIC1 mutants....	204
6.3.5 Pre-incubation of G18A and G22A CLIC1 mutants with Cholesterol.....	206
<b>6.4 Discussion.....</b>	<b>209</b>
<b>6.5 Conclusion.....</b>	<b>213</b>
<b>6.6 References.....</b>	<b>214</b>
<b><u>Chapter 7 Conclusion and Future Directions</u></b> .....	<b>218</b>
<b>Appendix.....</b>	<b>232</b>

## Abbreviations

2D	Two-dimensional
A	Area per molecule
Å	Angström ( $10^{-10}$ m)
A9C	Anthracene-9-carboxylic acid
ACMW	Air Contrast Matched Water
AEBSF	4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride
AKAP	A Kinase anchor protein
AMP	Adenosine monophosphate
Ano	Anoctamin
ANX	Annexin
APP	Amyloid precursor protein
AQP	Aquaporin
Arg	Arginine amino acid
Asn	Asparagine amino acid
ATP	Adenosine triphosphate
Bcl	B-cell lymphoma
BSA	Bovine serum albumin
C-domain	Carboxyl terminal domain
Ca <sup>2+</sup>	Calcium ion
CaCC	Ca <sup>2+</sup> - activated Cl <sup>-</sup> channel
CaCl <sub>2</sub>	Calcium chloride
CD	Circular Dichroism
CDC	Cholesterol-dependent cytolysins
CFTR	Cystic fibrosis transmembrane conductance regulator
Ch-ane	Cholestane
Ch-one	5-cholesten-3-one
CHO-K1	Chinese hamster ovary cells
Chol	Cholesterol
CIC	Chloride ion channel
Cl <sup>-</sup>	Chloride ion
CLIC	Chloride intracellular ion channel
CV	Column Volume
Cys	Cysteine amino acid

d <sub>31</sub> -POPC	Deuterated 1-palmitoyl-(d31)-2-oleoyl-sn-glycero-3-phosphatidylcholine
<i>DmCLIC</i>	<i>Drosophila melanogaster</i> CLIC protein
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DSC	Differential Scanning Calorimetry
DTT	Dithiothreitol
E-64	Epoxide protease inhibitor
<i>E.coli</i>	<i>Escherichia coli</i>
EDTA	Ethylenediaminetetraacetic acid
EPR	Electron Paramagnetic Resonance
ErbB	Epidermal growth factor receptor
Erg	Ergosterol
ERK	Extracellular signal-regulated kinase
ERM	Ezrin, Radixin and Moesin proteins
EXC	Excretory canal abnormality
EXL	EXC-like
FRET	Fluorescence Resonance Energy Transfer
GABA	gamma-Aminobutyric acid
Gln	Glutamine amino acid
Glu	Glutamic acid amino acid
Gly	Glycine amino acid
GPHR	Golgi pH Regulator
Grx	Glutaredoxin
GSH	Reduced glutathione
GST	Glutathione S-transferase
H <sup>+</sup>	Hydrogen ion
HEDES	2-hydroxyethyl disulphide
Hepes	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His	Histidine amino acid
Hyd	20-hydroxyecdysone
IAA-94	Indanyloxyacetic acid 94
IPTG	Isopropyl-thio-β-D-galactopyranoside
IQGAP	IQ motif containing GTPase activating protein
IR	Infra-Red
IRS	Interfacial Stress Rheometry
Kan	Kanamycin
KCl	Potassium chloride

kDa	kilo-Dalton
KH <sub>2</sub> PO <sub>4</sub>	Potassium dihydrogen phosphate
LC	Liquid condensed phase
LM	Langmuir monolayer
LSM	U6 snRNA-associated Sm-like protein
Lys	Lysine amino acid
M	Moles
MAP	Mitogen activated protein
MC	Monte-Carlo
mCLIC	mouse Chloride intracellular ion channel protein
MgCl <sub>2</sub>	Magnesium chloride
MgSO <sub>4</sub>	Magnesium sulphate
ml	milli-Litre
mM	milli-Molar
N-domain	Amino terminal domain
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NBD	Nucleotide binding domain
NCC27	Nuclear chloride channel protein 27kDa
NEM	N-Ethylmaleimide
Ni-NTA	nickel-nitrilotriacetic acid
NMR	Nuclear Magnetic Resonance
NR	Neutron Reflectivity
p64	Bovine chloride channel protein 64kDa
Panc	Pancreatic cancer cells
PC	Phosphatidylcholine
PCR	Polymerase chain reaction
PE	Phosphatidylethanolamine
PFT	Pore-forming toxin
Phe	Phenylalanine amino acid
PHR	Pam Highwire RPM-1 proteins
pI	Isoelectric point
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine
POPE	1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphatidylethanolamine
POPS	1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphatidylserine
PPI2	Serine/threonine phosphatase (PP1) isoform PP1 gamma 2
Pro	Proline amino acid

PS	Phosphatidylserine
PTMD	Putative Transmembrane Domain
$Q$	Momentum transfer
RyR	Ryanodine receptor
SANS	Small Angle Neutron Scattering
SAXS	Small Angle X-ray Scattering
SDS-PAGE	Sodium Dodecyl Sulfate-PolyAcrylamide
SEC	Size Exclusion Chromatography
Ser	Serine amino acid
Sito	$\beta$ -sitosterol
SLD	Scattering Length Density
SLS	Surface Light Scattering
$t$	Thickness
T84	Human colon cancer cell
tBLM	Tethered lipid bilayers
TCEP	tris-2-carboxyethyl-phosphine
TEMED	NNNN'-tetramethylethylenediamine
TMD	Transmembrane domain
TMR	Transmembrane region
TNF- $\alpha$	Tumour necrosis factor- $\alpha$
Trp	Tryptophan amino acid
Val	Valine amino acid
VGAT	Vesicular GABA transporter
VGLUT	Vesicular glutamate transporter
X	Any amino acid
XR	X-ray Reflectivity
XRD	X-ray Diffraction
$\Gamma$	Surface excess/ Surface coverage
$\Delta A$	Percentage surface area expansion
$\lambda$	Wavelength
$\mu\text{l}$	micro-Litre
$\pi$	Surface pressure
$\sigma$	Roughness
$\Omega$ -GST	Omega class GST

# **List of Figures**

## **Chapter 1**

1.1 Schematic diagrams of the topological structures and mechanism of regulation of Chloride Ion Channels.	5
1.2 Multiple sequence alignment of the six human CLIC proteins.	8
1.3 Multiple sequence alignment of the vertebrate, invertebrate and plant CLIC-like proteins.	11
1.4 Crystal structures of human CLIC family members.	16
1.5 Schematic diagram of reduced CLIC1 in ribbon showing the putative transmembrane region of CLIC1.	17
1.6 The hydrophobic region (PTMD) at the N-terminal domain conserved amongst all human CLIC proteins.	19
1.7 Comparison of A) $\Omega$ -GST and B) CLIC1 structure.	21
1.8 Membrane insertion model of CLIC1 protein.	23
1.9 Oligomerisation model of CLIC1 protein upon membrane interaction.	24
1.10 The oxidised CLIC1 dimer.	26
1.11: The proposed model for the CLIC1 transition from its soluble to membrane-bound form.	29

## **Chapter 2**

2.1 Chemical structures of the phospholipids and cholesterol used in this study.	49
2.2 A Langmuir trough showing the principle assembly of a Lipid monolayer on a water surface: a) expanded, b) partly compressed, and c) close-packed.	51
2.3 The $\pi$ -A isotherm of the KCl/Hepes buffer pH 6.5.	53
2.4 Schematic illustration of a $\pi$ -A isotherm of a lipid monolayer at the air-water interface and descriptors of various phases.	54
2.5 Surface pressure-area ( $\pi$ -A) isotherms of POPC, POPE and POPS phospholipid monolayers.	56
2.6 A schematic diagram of protein insertion into a lipid monolayer at the air-	57

water interface and its subsequent surface area vs. time plots.

2.7 The geometry of specular reflectivity. 60

### **Chapter 3**

3.1 Schematic diagram showing the complex formed between the poly-Histidine tagged protein and a Ni-NTA matrix. 76

3.2 SDS-PAGE gel showing a representative CLIC1 purification. 85

3.3 Eluted fractions of CLIC1-wt protein from Size Exclusion Chromatography Column. 86

3.4 SDS-PAGE, Western blot and Bradford Protein Quantification assay results of the SEC CLIC1-wt fractions. 87

3.5 Oxidoreductase activity of the purified CLIC1 protein. 89

3.6 Adsorption isotherm of CLIC1 to an air-water interface at 25°C at a final concentration of 2 µg/ml. 90

3.7 CLIC1 protein interactions with different phospholipid or cholesterol monolayers. 92

3.8 CLIC1 protein interactions with phospholipid monolayers containing cholesterol. 93

3.9 CLIC1 protein interactions with mixed lipid monolayers. 95

3.10 Surface pressure-Area isotherms of mixed lipid monolayers. 97

3.11 SDS-PAGE gel showing CLIC1- cholesterol pre-incubation. 99

3.12 Percentage area expansion profiles of POPC:Chol monolayer after 3 hours without CLIC1 protein or after injection of recombinant CLIC1-wt and pre-incubated CLIC1 protein. 100

### **Chapter 4**

4.1 Schematic representation of putative structural models of CLIC1 interacting with a lipid monolayer. 112

4.2 Schematic representation of the structural model and contrasts used to fit data from POPC:Chol monolayer after interaction with CLIC1. 118

4.3 Oxidoreductase activity of the purified d-CLIC1 protein. 124

4.4 (A) X-ray reflectivity profile and model data fit and (B) the electron density 125

profile the fit describes for air-water interface containing POPC monolayer held at a constant pressure of 20 mN/m.

4.5 Comparisons of X-ray reflectivity profiles and model data fits and the electron density profile the fits describe for CLIC1-lipid monolayer at the air-water interface in the presence and absence of cholesterol.	127
4.6 Neutron reflectivity profiles and model data fits (A) and the scattering length density profiles these fits describe (B) for CLIC1 interaction with POPC:Chol monolayer in ACMW KCl/Hepes buffer subphase (pH 6.5).	129
4.7 Results from the Monte-Carlo resampling of neutron contrast for CLIC1 layers where the line is a Gaussian fit intended to provide a guide to the eye.	132
4.8 XR and NR profiles and model data fits and the scattering length density profiles these fits describe for (A) POPE monolayer and for (B) CLIC1 interaction with POPE monolayer.	134
4.9 Comparisons of X-ray reflectivity profiles and model data fits and the electron density profile the fits describe for CLIC1-POPE:Chol monolayer at the air-water interface.	137
4.10 Comparisons of X-ray reflectivity profiles and model data fits and the electron density profile the fits describe for CLIC1-lipid monolayer at the air-water interface.	139
4.11 A schematic model summary of the interaction of CLIC1 with phospholipid monolayers in the absence and presence of cholesterol.	143

## Chapter 5

5.1 Chemical structures of the different natural sterols and cholesterol derivatives used in this study.	153
5.2 CLIC1-wt interaction with different POPC:Sterol monolayers.	159
5.3 Monte-Carlo (MC) resampling, Neutron reflectivity profiles and model data fits, and the scattering length density profiles these fits describe for POPC:Ergo monolayer (A) without CLIC1 and (B) with CLIC1.	163
5.4 Monte-Carlo (MC) resampling, Neutron reflectivity profiles and model data fits, and the scattering length density profiles these fits describe for POPC:Sito monolayer (A) without CLIC1 and (B) with CLIC1.	166
5.5 Monte-Carlo (MC) resampling, Neutron reflectivity profiles and model data fits, and the scattering length density profiles these fits describe for POPC:Hyd monolayer (A) without CLIC1 and (B) with CLIC1.	170

## **Chapter 6**

6.1 Schematic diagrams of reduced CLIC proteins in ribbon showing the putative transmembrane region and the GXXXG motif.	185
6.2 Schematic diagram of reduced CLIC1 showing the positions of the different amino acids that were mutated to alanine.	187
6.3 SDS-PAGE gel showing a representative G18A and G22A CLIC1 purification.	201
6.4 Far-UV CD spectra of CLIC1-wt, G18A and G22A CLIC1 proteins.	203
6.5 Oxidoreductase activity of the G18A and G22A CLIC1 proteins.	204
6.6 CLIC1 wild-type and mutant proteins interaction with POPC:Chol monolayer.	206
6.7 Percentage area expansion profiles of POPC:Chol monolayer after 3 hours following injection of non-incubated and pre-incubated CLIC1 wild-type and mutant proteins.	207
6.8 Amino Acid Sequence Alignment of Human CLIC proteins showing the GXXXG motif.	212

## **Chapter 7**

7.1 A schematic representation of a postulated structural model for CLIC1 interacting with A) a lipid monolayer and B) a lipid bilayer.	224
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## List of Tables

Table 1.1: A summary of the molecular characteristics, tissue expression and localization and known functions of human CLIC proteins.	9
Table 4.1 Summary of the Molecular Volumes ( $V_m$ ), theoretical electron densities ( $SLD_e$ ) and neutron scattering length densities ( $SLD_n$ ) of CLIC1 and the different lipids used in this study.	121
Table 4.2 Parameters obtained from fits of XR data from POPC monolayer and POPC:Chol monolayer with and without CLIC1.	128
Table 4.3 Parameters obtained from simultaneous fits of NR data from POPC monolayer without CLIC1 and POPC:Chol monolayer with and without CLIC1.	132
Table 4.4 Parameters obtained from simultaneous fits of XR and NR data from POPE monolayer with and without CLIC1.	135
Table 4.5 Parameters obtained from fits of XR data from POPE:Chol monolayer with and without CLIC1.	137
Table 4.6 Parameters obtained from fits of XR data from POPS monolayer and POPS:Chol monolayer with and without CLIC1.	138
Table 5.1 Summary of the molecular Volumes ( $V_m$ ), theoretical neutron scattering length densities ( $SLD_n$ ) of h/d <sub>31</sub> -POPC, h/d-CLIC1 and the different sterols in ACMW and D <sub>2</sub> O subphase.	156
Table 5.2 Parameters obtained from simultaneous fits of NR data from POPC:Ergo monolayer with and without CLIC1.	162
Table 5.3 Parameters obtained from simultaneous fits of NR data from POPC:Sito monolayer with and without CLIC1.	165
Table 5.4 Parameters obtained from simultaneous fits of NR data from POPC:Hyd monolayer with and without CLIC1.	169
Table 6.1 Sequences of the Oligonucleotides.	188
Table 6.2 PCR reaction mixture and PCR program for site-directed mutagenesis of CLIC1-pET28a plasmid DNA.	191
Table 6.3: CLUSTAL.W alignment of the DNA Sequences of the eleven different CLIC1-mutants with CLIC1-wt.	199

## **Abstract**

Sterols have been reported to modulate conformation and hence the function of several membrane proteins. One such group is the Chloride Intracellular Ion Channel (CLIC) family of proteins. These largely soluble proteins possess the intriguing property of spontaneous insertion into phospholipid bilayers to form integral membrane ion channels. To date, the structure of their membrane-bound form and factors influencing their auto-insertion remains largely unknown. In this thesis, we have performed Langmuir-film, X-ray, and neutron reflectivity experiments to study the interaction of wild-type or mutant versions of the protein CLIC1 with monolayers prepared using various mixtures of different phospholipids and sterol molecules, in order to investigate the regulatory role of the membrane lipid combination on the spontaneous membrane insertion of CLIC1 and to elucidate the structural features of the CLIC1 membrane-bound form within the lipid monolayers.

Our findings have demonstrated that the spontaneous membrane insertion of CLIC1 is dependent on the presence of cholesterol in lipid monolayers. In phospholipid monolayers only, CLIC1 was able to insert within the phospholipid head-group region with no penetration into the acyl chain region of the monolayers. However, in the presence of cholesterol, CLIC1 showed significant interaction with the phospholipid acyl chains thereby, suggesting that cholesterol is required for the penetration of CLIC1 into the hydrophobic tails of the lipid monolayer, which is considered necessary for the formation of functional ion channels. From reflectivity experiments, we were able to show that approximately  $0.8 \text{ mg/m}^2$  of CLIC1 inserted into phospholipid monolayers containing cholesterol such that the protein occupied an area per molecule between  $5 \sim 7 \text{ nm}^2$  with a total CLIC1 thickness ranging from  $\sim 51 \text{ \AA}$  to  $59 \text{ \AA}$  throughout the entire monolayer. We have also demonstrated for the first time that the GXXXG motif in CLIC1 acts as the cholesterol-binding site used by the protein for its initial recognition and binding to membrane cholesterol. Furthermore, Langmuir and reflectivity experiments using different sterols have confirmed that the interaction between CLIC1 and sterols is dependent on an intact  $3\beta\text{-OH}$  group in the sterol ring. Modification of the sterol structure by the introduction of additional hydroxyl

groups and methylation of the sterol alkyl chain was shown to facilitate greater spontaneous membrane insertion of the protein within the phospholipid monolayer. Taken together these findings provide clear evidence for the important role of sterols in the regulation of CLIC1 membrane interactions and a putative mechanism for its initial binding and membrane integration.