Contents

Epigraph iii

Contents v

List of Figures ix

List of Tables xi

Summary xiii

1 Introduction 1

1.1 The architecture of the endoplasmic reticulum 1

1.2 The nuclear envelope 2

1.3 Nuclear Pore Complex 3

1.3.1 Outer rings and Y complex 4

1.3.2 Inner ring 5

1.3.3 Central channel and FG-NUPs 5

1.3.4 Cytoplasmic filaments 5

1.3.5 Nuclear basket 6

1.3.6 Transmembrane NUPs 6

1.4 NE and NPC breakdown and reassembly 7

1.4.1 NEBD and NPC disassembly upon mitosis entry 8

1.4.2 Postmitotic NE formation and NPC reassembly 9

1.4.3 Interphasic NPC *de novo* assembly into the NE 10

1.5 Atlastin 12

1.6 Lamin B Receptor 14

1.7 Transmembrane Protein 209 . . . 15
1.8 Single domain antibodies (Nanobodies) . . . 15
1.8.1 Antibodies and derived fragments . . . 15
1.8.2 Structural characteristics and features . . . 16
1.8.3 Nb library generation . . . 18
1.8.4 Selection of Nbs . . . 19
1.8.5 Research applications . . . 20
1.9 Aim of this study . . . 23

2 Results **25**
2.1 Nanobodies to study Atlastin . . . 25
2.1.1 Generation of nanobodies binding different isoforms, cross-species and multiple nucleotide states . . . 25
2.1.2 Affinity assessment of anti-ATL nanobodies . . . 27
2.1.3 Anti-ATL nanobody LH02H01 produces an ER-specific fluorescent signal 30
2.1.4 Anti-ATL nanobody impairs assembly of import competent nuclei *in vitro* . . . 30
2.2 Nanobodies to study the Lamin B Receptor . . . 31
2.2.1 Generation of nanobodies to target the LBR tudor domain . . . 31
2.2.2 Affinity assessment of anti-LBR nanobodies . . . 32
2.2.3 Anti-LBR nanobodies produce a NE-specific fluorescent signal . . . 35
2.2.4 Anti-LBR nanobodies allow tracking the localization of human LBR during mitosis . . . 36
2.3 Characterization of Transmembrane Protein 209 . . . 38
2.3.1 Generation of nanobodies to target the C-terminal domain of TMEM209 . . . 38
2.3.2 Affinity assessment of anti-TMEM209 nanobodies . . . 39
2.3.3 High-affinity anti-TMEM209 nanobodies specifically label NPCs . . . 41
2.3.4 TMEM209 can be followed during NE assembly in human cells and in reconstituted nuclei *in vitro* . . . 53
2.3.5 TMEM209 incorporates into newly-assembled NPCs in interphase . . . 57
2.3.6 The C-terminal domain of TMEM209 localizes within NPC rings . . . 59

2.3.7 Using anti-TMEM209 Nbs as crystallization chaperones 64

3 Discussion **66**

3.1 Anti-ATL nanobody as a valuable tool to study ATL-mediated membrane fusion 66

3.2 Anti-LBR tudor domain nanobodies for the visualization of the NE and LBR 68

3.3 TMEM209 is associated with the NPC 69

4 Materials and Methods **76**

4.1 Molecular cloning 76

4.1.1 Cloning strategies and validation 76

4.1.2 Restriction enzyme digestion 76

4.1.3 Polymerase chain reaction (PCR) 77

4.1.4 Agarose gel electrophoresis 78

4.1.5 Gibson assembly 79

4.1.6 Ligation using T4 ligase 79

4.1.7 Transformation of bacterial cells 80

4.1.8 Plasmid preparation 82

4.2 Protein expression and purification 82

4.2.1 Expression of recombinant proteins in *E.coli* 82

4.2.2 Immobilized metal ion chromatography 83

4.2.3 Analysis by SDS-PAGE 86

4.2.4 Purification by size exclusion chromatography 87

4.3 Genome editing in human cell lines 87

4.3.1 Cell culture 88

4.3.2 Gene disruption by CRISPR/Cas9 technology 88

4.3.3 Tagging of gene alleles with the open reading frame (ORF) for sfGFP 89

4.4 Nanobody library construction and selection 89

4.4.1 Alpaca immunisation 90

4.4.2 Total RNA preparation and amplification of nanobody sequences . . . 90

4.4.3 Library transformation and phage purification 91

4.4.4 Nanobody selection by phage display 92

4.4.5 Cloning and sequence analysis of enriched nanobody libraries 95

4.5 Nanobody preparation and characterization 95

4.5.1 Nanobody expression and purification 95
4.5.2 Nanobody labeling using maleimide chemistry 96
4.5.3 Kinetic measurements by BioLayer Interferometry (BLI) 97
4.5.4 Assessment of nanobody performance using immunofluorescence microscopy (IFM) 98
4.5.5 Analysis of nuclear membrane fluorescence and perimeter 98
4.6 *Xenopus* egg extract preparation 99
4.6.1 Preparation of interphase high speed egg extract (HSS) 99
4.6.2 Purification of membranes by flotation 100
4.7 Reconstituting postmitotic NE/NPC assembly 101
4.7.1 Nuclei reconstitution from *Xenopus* egg extract 101
4.7.2 Protein composition assessment in fixed reconstituted nuclei 102
4.8 Reconstituting interphasic NPC assembly 103
4.8.1 Assembly of frog NPCs onto permeabilized human cell lines 103
4.9 Characterization of proteins by immunofluorescence microscopy (IFM) 104
4.9.1 Cell cycle synchronization of cultured cells 104
4.9.2 Assessing protein localization during mitosis 105
4.9.3 Preparation of cryostat sections 106
4.9.4 Determine protein localization by super-resolution microscopy 106
4.9.5 Analyses of STED images 107
4.9.6 Assessment of protein topology 108
4.10 Protein crystallography 109
4.10.1 Complex preparation 109
4.10.2 Crystallization 109

References **111**

Abbreviations **133**

Acknowledgements **136**

Curriculum vitae **137**