

# Contents

Table of contents	I
List of figures	V
List of tables	VII
<b>1 Introduction</b>	<b>1</b>
1.1 Human immunodeficiency virus (HIV)	1
1.1.1 Structural and genomic organization of HIV-1	2
1.1.2 The human CD4 receptor and its interactions with HIV-1	3
1.2 Membrane-interacting proteins	5
1.2.1 Integral membrane proteins	6
1.2.2 Peripheral membrane proteins	7
1.2.3 Lipid-anchored membrane proteins	7
1.3 Membrane mimics	8
1.4 Nanodiscs	9
1.4.1 NMR spectroscopy on nanodisc-embedded membrane proteins	10
1.4.2 Nanodiscs in surface plasmon resonance studies	11
<b>2 Aims</b>	<b>13</b>
<b>3 Scientific publications</b>	<b>15</b>
3.1 Integral Membrane Proteins in Nanodiscs Can Be Studied by Solution NMR Spectroscopy	17
3.2 Single Vector System for Efficient N-myristoylation of Recombinant Proteins in <i>E. coli</i>	31
3.3 Nanodiscs Allow The Use of Integral Membrane Proteins as Analytes in Surface Plasmon Resonance Studies	39
<b>Summary</b>	<b>48</b>
<b>Zusammenfassung</b>	<b>51</b>
<b>List of abbreviations</b>	<b>53</b>
<b>Bibliography</b>	<b>57</b>

<b>A</b>	<b>Appendix</b>	<b>63</b>
A.1	Material . . . . .	63
A.1.1	Enzymes and antibodies . . . . .	63
A.1.2	Chemicals and biochemicals . . . . .	63
A.1.3	Bacterial strains . . . . .	64
A.1.4	Kits . . . . .	65
A.1.5	Vectors . . . . .	65
A.1.6	Oligodesoxyribonucleotides . . . . .	66
A.1.7	Lipids . . . . .	67
A.1.8	Gel electrophoresis markers . . . . .	67
A.1.9	Chromatographic equipment . . . . .	68
A.1.10	Laboratory equipment . . . . .	69
A.1.11	Miscellaneous equipment . . . . .	70
A.1.12	Software . . . . .	70
A.1.13	Databases . . . . .	70
A.2	Methods . . . . .	71
A.2.1	DNA techniques . . . . .	71
A.2.1.1	Plasmid DNA purification . . . . .	71
A.2.1.2	DNA gel electrophoresis . . . . .	71
A.2.1.3	DNA extraction from agarose gels . . . . .	71
A.2.1.4	DNA concentration determination . . . . .	72
A.2.1.5	Polymerase chain reaction (PCR) . . . . .	72
A.2.1.6	Restriction digest of DNA . . . . .	73
A.2.1.7	DNA blunting . . . . .	73
A.2.1.8	Plasmid DNA dephosphorylation . . . . .	74
A.2.1.9	DNA ligation . . . . .	74
A.2.1.10	DNA sequencing . . . . .	74
A.2.2	Cloning of vector constructs . . . . .	74
A.2.2.1	Cloning of pTKK19xb/ub_HisUbiquitin_TAA . . . . .	74
A.2.2.2	Cloning of pETDuet-1 $\Delta$ His_hNMT_Nef . . . . .	75
A.2.3	Bacterial cultures . . . . .	75
A.2.3.1	Transformation of <i>E. coli</i> . . . . .	75
A.2.3.2	Growth and storage of <i>E. coli</i> . . . . .	76
A.2.4	Protein techniques . . . . .	76
A.2.4.1	SDS-PAGE according to Laemmli . . . . .	76
A.2.4.2	Coomassie-staining of SDS-PA gels . . . . .	76
A.2.4.3	Protein concentration determination . . . . .	77
A.2.5	Protein expression and purification . . . . .	77
A.2.5.1	Tobacco etch virus protease (S219V) . . . . .	77
A.2.5.2	Membrane scaffold protein (MSP) . . . . .	78
A.2.5.3	CD4mut . . . . .	79
A.2.5.4	HisUbiquitin . . . . .	79
A.2.5.5	HisUbiquitinCD4mut . . . . .	80
A.2.5.6	Nef and N-myristoylated Nef . . . . .	80
A.2.6	Nanodisc assembly . . . . .	82

---

A.2.6.1	Preparation of lipids . . . . .	82
A.2.6.2	Preparation of detergent solubilized membrane proteins . .	82
A.2.6.3	Assembly of empty nanodiscs . . . . .	82
A.2.6.4	Assembly of CD4mut containing nanodiscs . . . . .	83
A.2.6.5	Assembly of HisUbiquitinCD4mut containing nanodiscs . .	83
A.2.6.6	Separation of loaded from unloaded nanodiscs . . . . .	83
A.2.7	NMR spectroscopy . . . . .	84
A.2.7.1	Preparation of NMR samples . . . . .	84
A.2.8	Surface plasmon resonance (SPR) technology . . . . .	84
A.2.8.1	SPR sample preparation . . . . .	84
A.2.8.2	Immobilization of PentaHis monoclonal antibody . . . . .	85
A.2.8.3	Evaluation of SPR data . . . . .	85
A.3	Bibliography . . . . .	86
<b>Danksagung</b>		<b>87</b>
<b>Erklärung zur Promotion</b>		<b>88</b>