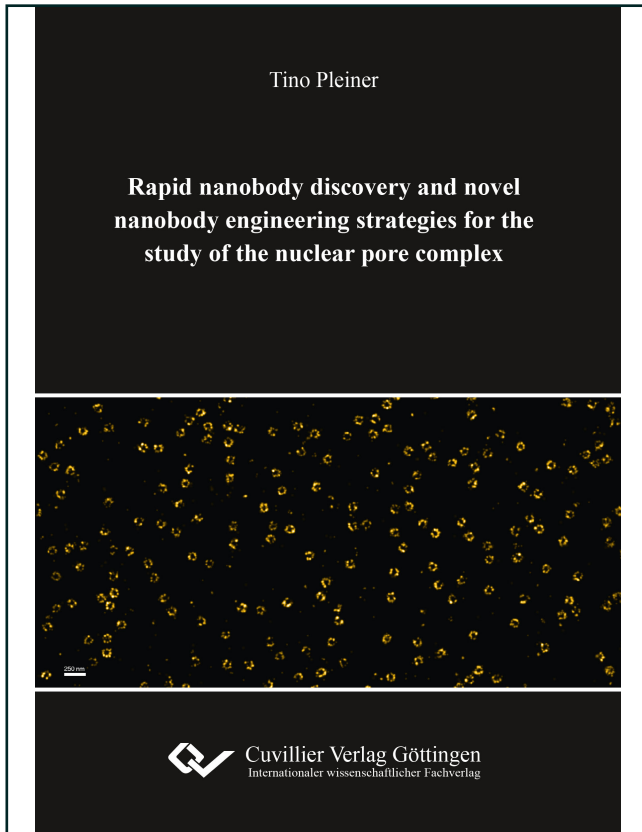




Tino Pleiner (Autor)

**Rapid nanobody discovery and novel nanobody engineering strategies for the study of the nuclear pore complex**



<https://cuvillier.de/de/shop/publications/7508>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen, Germany

Telefon: +49 (0)551 54724-0, E-Mail: [info@cuvillier.de](mailto:info@cuvillier.de), Website: <https://cuvillier.de>



# Table of Contents

<b>1</b>	<b>SUMMARY</b> .....	<b>1</b>
<b>2</b>	<b>INTRODUCTION</b> .....	<b>2</b>
2.1	THE VERTEBRATE NUCLEAR PORE COMPLEX (NPC).....	2
2.1.1	<i>Nucleocytoplasmic transport</i> .....	3
2.1.2	<i>Structure of the nuclear pore complex</i> .....	6
2.1.3	<i>Composition of the NPC</i> .....	7
2.2	ANTIBODY ENGINEERING .....	14
2.2.1	<i>Structure of antibodies</i> .....	15
2.2.2	<i>Monoclonal antibodies</i> .....	17
2.2.3	<i>Recombinant antibody fragments</i> .....	18
2.2.4	<i>Natural single domain antibodies</i> .....	20
2.3	PHAGE DISPLAY.....	23
2.3.1	<i>Filamentous phage life cycle</i> .....	23
2.3.2	<i>Selection of recombinant antibodies via phage display</i> .....	25
2.4	AIMS OF THIS STUDY .....	28
<b>3</b>	<b>RESULTS</b> .....	<b>29</b>
3.1	A RAPID WORKFLOW FOR NANOBODY DISCOVERY .....	29
3.1.1	<i>Total RNA isolation and RNA quality control</i> .....	31
3.1.2	<i>Library construction via Gibson assembly</i> .....	32
3.1.3	<i>Design of the minimal phagemid</i> .....	34
3.1.4	<i>Selective elution of antigen-bound phages</i> .....	37
3.1.5	<i>Rapid quantification of panning by qPCR</i> .....	37
3.1.6	<i>Cloning of enriched sub-libraries and sequence analysis</i> .....	40
3.2	NOVEL NANOBODY ENGINEERING STRATEGIES .....	42
3.2.1	<i>Functional cytoplasmic expression of nanobodies</i> .....	42
3.2.2	<i>Native purification of endogenous protein complexes using nanobodies</i> .....	45
3.2.3	<i>Site-specific fluorescent labeling of nanobodies</i> .....	49
3.2.4	<i>Imaging with site-specifically labeled nanobodies</i> .....	54
3.2.5	<i>Super-resolution imaging with site-specifically labeled nanobodies</i> .....	58
3.2.6	<i>Rapid epitope mapping via crosslinking mass spectrometry</i> .....	60
3.3	ANTI-NUP NANOBODY TOOLBOX APPLIED TO THE STUDY OF THE NPC.....	62
3.3.1	<i>Generation &amp; characterization of the anti-Nup nanobody toolbox</i> .....	62
3.3.2	<i>Selection of nanobodies against natively isolated xlNup107 complex</i> .....	65
3.3.3	<i>NPC assembly investigation with the anti-Nup nanobody toolbox</i> .....	66



3.3.4	<i>Analysis of interphase and mitotic nucleoporins</i> .....	70
<b>4</b>	<b>DISCUSSION</b> .....	<b>72</b>
4.1	A RAPID PHAGE DISPLAY-BASED NANOBODY DISCOVERY WORKFLOW .....	72
4.1.1	<i>Rapid and accurate library construction via Gibson assembly</i> .....	72
4.1.2	<i>A 'minimal phagemid' facilitates construction of large libraries</i> .....	73
4.1.3	<i>qPCR quantification of phage display</i> .....	75
4.1.4	<i>The importance of a characterization strategy</i> .....	76
4.1.5	<i>Comparison to mass spectrometry-based nanobody production workflow</i> .....	77
4.2	CYTOPLASMIC EXPRESSION OF ENGINEERED NANOBODIES.....	78
4.2.1	<i>Native protein complex isolation</i> .....	80
4.2.2	<i>Reliable fluorescent labeling of nanobodies</i> .....	81
4.2.3	<i>Mapping conformational epitopes via crosslinking mass spectrometry</i> .....	84
4.3	EXPLORATION OF THE ANTI-NUP NANOBODY TOOLBOX .....	85
<b>5</b>	<b>MATERIALS</b> .....	<b>87</b>
5.1	REAGENTS.....	87
5.2	BUFFERS AND SOLUTIONS.....	89
5.3	E. COLI STRAINS AND MEDIA .....	90
5.4	LAB EQUIPMENT .....	91
5.5	SOFTWARE & ONLINE TOOLS.....	92
<b>6</b>	<b>METHODS</b> .....	<b>93</b>
6.1	STANDARD METHODS IN MOLECULAR BIOLOGY .....	93
6.2	PROTEIN BIOCHEMISTRY METHODS.....	97
6.3	NANOBODY LIBRARY CONSTRUCTION .....	103
6.4	PHAGE DISPLAY.....	106
6.5	<i>XENOPUS</i> EGG EXTRACT METHODS .....	113
6.6	FLUORESCENCE MICROSCOPY METHODS.....	116
6.7	STRUCTURAL BIOLOGY METHODS.....	119
<b>7</b>	<b>LIST OF ABBREVIATIONS</b> .....	<b>122</b>
<b>8</b>	<b>CONTRIBUTION BY OTHERS</b> .....	<b>124</b>
<b>9</b>	<b>ACKNOWLEDGMENTS</b> .....	<b>125</b>
<b>10</b>	<b>REFERENCES</b> .....	<b>127</b>
<b>11</b>	<b>CURRICULUM VITAE</b> .....	<b>139</b>
<b>12</b>	<b>LIST OF PUBLICATIONS</b> .....	<b>139</b>