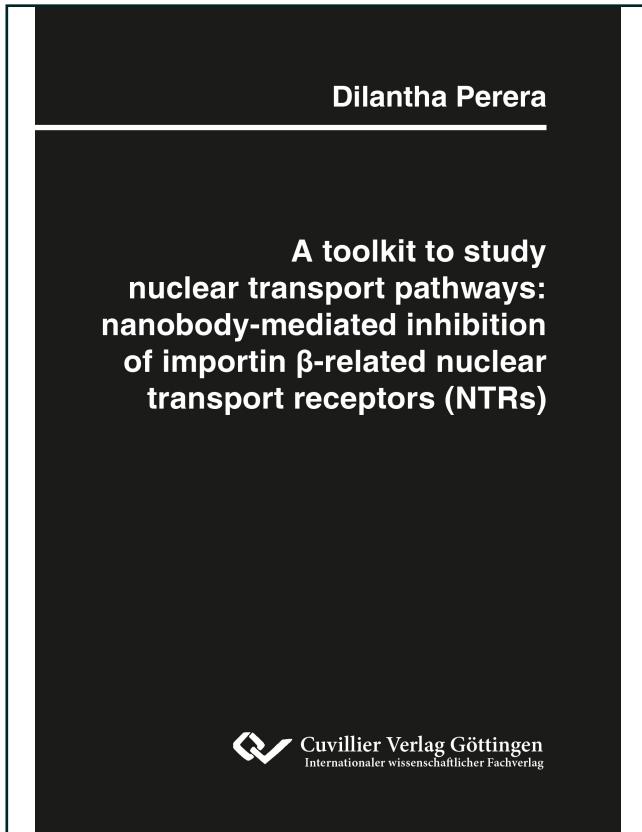




Dilantha Perera (Autor)

**A toolkit to study nuclear transport pathways:
nanobody-mediated inhibition of importin β -related
nuclear transport receptors (NTRs)**



<https://cuvillier.de/de/shop/publications/8870>

Copyright:

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

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

Table of Contents

Thesis Committee and Examination Board Members	i
Epigraph	ii
List of Figures	vi
List of Tables	viii
Summary	1
1 Introduction	2
1.1 Compartmentalization of eukaryotic cells	2
1.2 Nuclear pore complex	2
1.2.1 Architecture of the NPC	3
1.3 Permeability barrier of the NPC	3
1.3.1 FG repeat domains and FG-Nups	4
1.3.2 Models of NPC Transport selectivity	5
1.4 Nuclear transport receptors	7
1.4.1 Characteristics of Importin β -like NTRs	7
1.4.2 RanGTP gradient and NTR transport directionality	8
1.4.3 RanGTP interaction with Importin β -like NTRs	10
1.4.4 Nuclear transport pathways	11
1.4.5 NTR cargo recognition	13
1.5 Overview of selected NTRs	14
1.5.1 Transportin 1	14
1.5.2 Exportin 4 and Exportin 7	16
1.5.3 CAS	18
1.6 Tools to investigate NTR mediated transport pathways	20
1.6.1 Genetic perturbations	20
1.6.2 Targeted protein degradation	21
1.6.3 NTR inhibition using small molecules	21
1.6.4 Nanobodies to investigate NTR pathways	22
1.7 Nanobodies	22
1.7.1 Nanobody library generation	23
1.7.2 Nanobody selection: phage display	24
1.8 Aim of this study	26
2 Results	27
2.1 Biochemical characterization of anti-NTR nanobodies	27
2.1.1 Nanobodies selected against TRN1	28
2.1.2 Nanobodies selected against Xpo7	31
2.1.3 Nanobodies selected against Xpo4	34

2.1.4 Nanobodies selected against CAS	37
2.2 <i>In vivo</i> validation of Nb-mediated NTR pathway inhibition	39
2.2.1 Inhibition of the TRN1 pathway in HeLa cells	39
2.2.2 Inhibition of the CAS pathway in HeLa cells	40
2.3 Inhibition of NTR-facilitated transport through NPCs in permeabilized cells	41
2.3.1 Nb-mediated inhibition of TRN1	43
2.3.2 Nb-mediated inhibition of Xpo7	46
2.3.3 Nb-mediated inhibition of Xpo4	48
2.3.4 Nb-mediated inhibition of CAS	51
2.4 Tag-Nbs to interrupt NTR-facilitated transport through NPCs in permeabilized cells	55
2.4.1 Inhibition of TRN1	55
2.4.2 Inhibition of Xpo7	58
2.4.3 Inhibition of Xpo4	61
2.4.4 Inhibition of CAS	64
2.5 Impeding NTR-facilitated transport into reconstituted FG hydrogels	67
2.5.1 Impeding TRN1-facilitated partitioning into FG particles	68
2.5.2 Impeding Xpo7-facilitated partitioning into FG particles	70
2.5.3 Impeding Xpo4-facilitated partitioning into FG particles	71
2.5.4 Impeding CAS-facilitated partitioning into FG particles	72
2.5.5 Orthogonal tag-Nbs to impede NTR-facilitated partitioning into FG particles	73
3 Discussion	76
3.1 Functional classification of the selected anti-NTR Nbs	76
3.1.1 Semi-inhibitory nanobodies	76
3.1.2 Mildly inhibitory Nbs	78
3.1.3 NTR blockers: red lights on the NTR pathway	79
3.2 Tag-Nbs: the good, the bad, and the redundant	80
3.2.1 The benefits of tag-Nbs	80
3.2.2 Disadvantages and redundancies of tag-Nbs	82
3.2.3 Orthogonal tag-Nbs	84
3.3 Future Outlook	85
3.3.1 Expanding and improving the toolkit	85
3.3.2 Studying the mechanics of Nb-NTR and FG-NTR interactions	86
3.3.3 Unravelling the NTR transport network	86
4 Materials and Methods	88
4.1 Chemicals, proteins, and reagents	88
4.2 Molecular cloning	88
4.2.1 Design and validation	88
4.2.2 Polymerase chain reaction (PCR)	88
4.2.3 Agarose gel electrophoresis	89
4.2.4 Gibson assembly	90

4.2.5 Transformation of bacteria	90
4.2.6 Plasmid DNA preparation	90
4.3 Protein purification	91
4.3.1 Recombinant protein expression	91
4.3.1 Recombinant expression of Nb constructs	92
4.3.2 Immobilized metal ion chromatography	93
4.3.3 Size exclusion chromatography	94
4.3.4 SDS-PAGE	95
4.3.5 Protein labeling using maleimide chemistry	95
4.4 Binding assays	96
4.4.1 Pulldown assays	96
4.4.2 BioLayer Interferometry (BLI)	97
4.5 <i>In vivo</i> experiments	98
4.5.1 Cell culture	98
4.5.2 Transient transfection of HeLa cells	98
4.5.3 Confocal fluorescence microscopy	99
4.6 Permeabilized cell assays	99
4.6.1 Permeabilization of HeLa cells	99
4.6.2 Confocal fluorescence microscopy	100
4.6.3 Data analysis	101
4.7 Experiment using FG phase hydrogels	101
4.7.1 Expression and purification of Nup116 FG domains	101
4.7.2 FG particle assay	103
4.7.3 Confocal fluorescence microscopy	103
4.7.4 Data analysis	104
References	105
Acknowledgements	120
Curriculum vitae	121