



Universiteit
Leiden
The Netherlands

Dynamics and regulation at the tip : a high resolution view on microtubule assembly

Munteanu, L.

Citation

Munteanu, L. (2008, June 24). *Dynamics and regulation at the tip : a high resolution view on microtubule assembly*. Bio-Assembly and Organization / FOM Institute for Atomic and Molecular Physics (AMOLF), Faculty of Science, Leiden University. Retrieved from <https://hdl.handle.net/1887/12979>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/12979>

Note: To cite this publication please use the final published version (if applicable).

DYNAMICS AND REGULATION AT THE TIP
A HIGH RESOLUTION VIEW ON MICROTUBULE ASSEMBLY

Dynamics and regulation at the tip: A high resolution view on microtubule assembly

PROEFSCHRIFT

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus prof. mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 24 juni 2008
klokke 15.00 uur

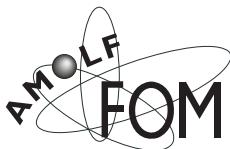
door

EMILIA LAURA MUNTEANU

geboren te Comănești, Romania
in 1978

Promotiecommissie

Promotor: Prof. dr. M. Dogterom
Referent: Prof. dr. K. Visscher (University of Arizona, Tucson, AZ)
Overige leden: Prof. dr. J. P. Abrahams
Dr. A. Akhmanova (Erasmus MC Rotterdam)
Prof. dr. M. E. Janson (Wageningen University)
Dr. ir. J. van Noort
Prof. dr. J. M. van Ruitenbeek



© 2008 by Emilia Laura Munteanu. All rights reserved.

Nederlandse titel: Dynamica en regulatie aan de tip. Een hoge-resolutie-visie op de assemblage van microtubuli.

The work described in this thesis was performed at the FOM Institute for Atomic and Molecular Physics (AMOLF) in Amsterdam, The Netherlands. This work is part of the research programme of the ‘Stichting voor Fundamenteel Onderzoek der Materie’ (FOM), which is financially supported by the ‘Nederlandse Organisatie voor Wetenschappelijk Onderzoek’ (NWO).

Cover by Julien Husson.

ISBN: 978-90-77209-24-0

A digital version of this thesis can be obtained from <http://ub.leidenuniv.nl/>. Printed copies can be obtained by addressing the library at the FOM Institute for Atomic and Molecular Physics (AMOLF): library@amolf.nl; Kruislaan 407, 1098 SJ, Amsterdam, The Netherlands.

Printed in the Netherlands by Ponsen & Looijen BV graphical company, Wageningen.

This is the beginning of a beautiful friendship.

Black Cat, White Cat

This thesis is partly based on the following articles:

Kerssemakers JWJ, Munteanu EL, Laan L, Noetzel TL, Janson ME, Dogterom M (2006) Assembly dynamics of microtubules at molecular resolution. *Nature* **442**: 709-712
(chapter 2 and 3)

Bieling P, Laan L, Schek HT III, Munteanu EL, Sandblad L, Dogterom M, Brunner D, Surrey T (2007) Reconstitution of a microtubule plus-end tracking system *in vitro*. *Nature* **450**: 1100-1105
(chapter 4)

Munteanu EL, Laan L, Brunner D, Surrey T, Dogterom M. Regulation of microtubule dynamics, *in vitro*, by the autonomous microtubule-end tracker Mal3. *to be submitted*
(chapter 5)

Other articles:

Laan L, Husson J, Munteanu EL, Kerssemakers JWJ, Dogterom M. (2008) Force generation and dynamic instability of microtubule bundles. *Proc Natl Acad Sci*: accepted

Dogterom M, Husson J, Laan L, Munteanu EL, Tischer C (2007) Microtubule forces and organization. In: Lenz P, editor, *Cell Motility*, Springer New York. pp. 93-115

Laan L, Munteanu EL, Kerssemakers JWJ, Dogterom M (2006) Meten aan microbuisjes met moleculaire resolutie. *Nederlands Tijdschrift voor Natuurkunde* **72**: 388-391

Tolic-Nørrelykke IM, Munteanu EL, Thon G, Oddershede L, Berg-Sørensen K (2004) Anomalous diffusion in living yeast cells. *Phys Rev Lett* **93**: 078102.1-4

Contents

1	Introduction	11
1.1	Microtubules	13
1.1.1	Structural features	13
1.1.2	Dynamics	15
1.1.3	Force generation	18
1.2	Microtubule plus-end tracking proteins, +TIPs	19
1.2.1	End-tracking mechanisms	21
1.2.2	Regulation of microtubule dynamics	24
1.3	This thesis	24
2	Measuring microtubule dynamics with near molecular resolution	27
2.1	Experimental method	28
2.2	'Keyhole' optical trap	31
2.2.1	Optical tweezers set-up	31
2.2.2	'Keyhole' trap design and features	31
2.2.3	Determining the trap stiffness	32
2.3	Experimental considerations	33
2.3.1	Mechanics of microtubules under load	33
2.3.2	Finite stiffness of the bead-axoneme construct	34
2.4	Dynamic instability of microtubules measured with optical tweezers	35
2.4.1	High-resolution details of microtubule dynamics	35
2.4.2	Dynamics and force generation of multiple microtubules	35
3	Influence of XMAP215 on microtubule dynamics	39
3.1	Microtubule assembly in the presence of XMAP215	40
3.1.1	XMAP215 enhances microtubule growth and catastrophes	40
3.1.2	Assembly dynamics at molecular resolution	41
3.2	Discussion	46
3.3	Methods	47
3.3.1	Measuring microtubule dynamics	47
3.3.2	Step fitting algorithm	50
3.4	XMAP215-tubulin interactions	53

3.4.1	Speckled microtubules	53
3.4.2	FCS measurements on XMAP215-tubulin complex formation . . .	59
3.5	Additional remarks and discussion	63
4	Reconstitution of a microtubule plus-end tracking system <i>in vitro</i>	67
4.1	Results and discussion	68
4.1.1	Mal3 recognizes and autonomously tracks microtubule growing ends	70
4.1.2	Tea2 and Tip1 need each other and Mal3 for efficient plus-end tracking	73
4.1.3	Mal3 acts as a loading factor for the Tea2-Tip1 complex	77
4.1.4	Microtubule dynamics in the presence of Mal3, Tea2 and Tip1	77
4.2	Conclusions	78
4.3	Methods	79
4.3.1	End-tracking assay using TIRF microscopy	79
4.3.2	End-tracking assay using confocal microscopy	80
4.3.3	+TIPs on static microtubules analyzed by confocal microscopy	81
4.3.4	DIC assay to measure microtubule dynamics	81
5	Microtubule dynamics in the presence of Mal3	83
5.1	Introduction	84
5.2	Results	84
5.2.1	Mal3 enhances the dynamic instability of microtubules <i>in vitro</i>	84
5.2.2	Mal3 interacts differentially with the tip and with the lattice of growing microtubules.	87
5.2.3	Mal3 promotes formation of microtubule end-structures.	89
5.3	Discussion	94
5.4	Additional remarks	97
5.5	Experimental procedures and data analysis	98
5.5.1	Measuring the parameters of microtubule dynamic instability by DIC microscopy.	98
5.5.2	Evaluating the amount of Mal3 bound on microtubules by confocal microscopy	100
5.5.3	Microtubule end dynamics measured with optical tweezers	104
6	Microtubule catastrophes at molecular resolution	107
6.1	Results	108
6.1.1	Is there a molecular signature of catastrophes?	109
6.1.2	Microtubule catastrophes in the presence of XMAP215 and Mal3	109
6.2	Discussion on the mechanism of catastrophes	112

7 Discussion and future directions	115
7.1 Discussion	115
7.2 Future directions	117
7.2.1 Regulation of microtubule dynamics by the plus-end tracking complex Mal3-Tea2-Tip1	117
7.2.2 End-tracking of dynamic microtubules by EB proteins	119
7.2.3 Influence of +TIPs on force generating microtubules	120
Bibliography	123
Summary	137
Samenvatting	141
Acknowledgements	145
Curriculum vitae	149

