



Universiteit
Leiden

The Netherlands

Nucleosome dynamics resolved with single-pair fluorescence resonance energy transfer spectroscopy

Koopmans, W.J.A.

Citation

Koopmans, W. J. A. (2009, June 18). *Nucleosome dynamics resolved with single-pair fluorescence resonance energy transfer spectroscopy*. Retrieved from <https://hdl.handle.net/1887/13856>

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/1887/13856>

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

Nucleosome Dynamics Resolved with Single-Pair Fluorescence Resonance Energy Transfer Spectroscopy

Proefschrift

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden,

op gezag van Rector Magnificus prof. mr. P. F. van der Heijden,

volgens besluit van het College voor Promoties

te verdedigen op donderdag 18 juni 2009

klokke 10:00 uur

door

Wiepke Jelle Anthonie Koopmans

geboren te Papendrecht

in 1980

Promotiecommissie

Promotor: prof. dr. T. Schmidt
Co-promotor: dr. ir. J. van Noort
Referent: dr. A. N. Kapanidis (Oxford University, United Kingdom)
Overige leden: prof. dr. J. Brouwer
prof. dr. M. Orrit
dr. ir. E. J. G. Peterman (Vrije Universiteit, Amsterdam)
prof. dr. J. M. van Ruitenbeek
prof. dr. H. Schiessel
prof. dr. C. Wyman (Erasmus Universiteit, Rotterdam)

Cover illustration: nucleosome crystal structure adapted from protein database *1kx5*, Davey *et al.* (2002)

Casimir PhD Series, Delft-Leiden, 2009-03

ISBN 978-90-8593-051-8

An electronic version of this thesis can be found at <https://openaccess.leidenuniv.nl>

Dit werk maakt deel uit van het onderzoekprogramma van de Stichting voor Fundamenteel Onderzoek der Materie (FOM), die financieel wordt gesteund door de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO).

Contents

1	Introduction	1
1.1	The nucleosome	1
1.2	Single-pair Fluorescence Resonance Energy Transfer Spectroscopy	4
1.3	Scope of this thesis	6
	Bibliography	7
2	Engineering Mononucleosomes for Single-Pair FRET Experiments	11
2.1	Introduction	12
2.2	Materials	13
2.2.1	DNA preparation and purification	13
2.2.2	Mononucleosome reconstitution	13
2.2.3	Polyacrylamide Gel Electrophoresis (PAGE)	13
2.2.4	Single-molecule FRET measurements	14
2.3	Single-molecule fluorescence microscopes	16
2.3.1	Widefield TIRF setup	16
2.3.2	Confocal setup	17
2.4	Methods	18
2.4.1	Choice of label positions and primer design	19
2.4.2	DNA preparation and purification	21
2.4.3	Mononucleosome reconstitution	21
2.4.4	Polyacrylamide Gel Electrophoresis	22
2.4.5	Single-molecule FRET measurements	23
2.5	Notes	27
2.6	Conclusion	28
	Bibliography	29

3	Single-pair FRET Microscopy reveals Mononucleosome Dynamics	31
3.1	Introduction	32
3.2	Material and Methods	33
3.3	Experimental Results	36
3.3.1	Bulk fluorescence spectra reveal proper reconstitution of mononucleosomes	36
3.3.2	spFRET microscopy reveals individual nucleosomes together with a large population of dissociated nucleosomes	37
3.3.3	Single-molecule fluorescence footprint of individual nucleosomes	39
3.3.4	Acceptor blinking is the dominant source of spFRET dynamics	40
3.3.5	Suppression of blinking	41
3.3.6	A fraction of the immobilized nucleosomes shows dynamics clearly distinct from blinking	43
3.4	Discussion and conclusion	45
	Bibliography	47
4	Nucleosome Immobilization Strategies for Single-Pair FRET Microscopy	51
4.1	Introduction	52
4.2	Results and Discussion	53
4.2.1	Immobilization through confinement in gels	54
4.2.2	Surface immobilization: binding specificity	58
4.2.3	Surface immobilization: nucleosome integrity	59
4.2.4	Nucleosome breathing dynamics	62
4.3	Conclusion	64
4.4	Experimental Section	64
	Bibliography	66
5	PAGE-ALEX-spFRET-FCS reveals Progressive DNA Breathing	71
5.1	Introduction	72
5.2	Materials and methods	73
5.3	Results	77
5.3.1	ALEX-spFRET resolves nucleosome sample heterogeneity	77
5.3.2	ALEX selection resolves DNA breathing in nucleosomes	79
5.3.3	Monovalent salt promotes DNA unwrapping and nucleosome disassembly	80
5.3.4	Fluorescence correlation analysis of selected nucleosome populations shows unwrapping at low FRET	82

5.3.5	Gel separated nucleosomes are transiently unwrapped in a progressive way from both nucleosome ends	83
5.4	Discussion and Conclusion	87
	Bibliography	90
	Summary	93
	Samenvatting	95
	List of Publications	99
	Curriculum Vitae	101
	Nawoord	103

