

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/29602> holds various files of this Leiden University dissertation

Author: Groeneweg, Fenke Lokke

Title: Corticosteroid receptor dynamics : analysis by advanced fluorescence microscopy

Issue Date: 2014-11-06

Corticosteroid receptor dynamics

analysis by advanced fluorescence microscopy

Femke Lokke Groeneweg

Corticosteroid receptor dynamics, analysis by advanced fluorescence microscopy

Femke Lokke Groeneweg

Thesis, Leiden University

November 2014

ISBN: 978-90-8891-978-7

Cover image: "determination", permitted adaptation of original painting "Mad A." by BYZ (Montreal, Canada).

Cover design: F.L. Groeneweg

Layout: M.A. Groeneweg

Printing: Proefschriftdrukken.nl || uitgeverij BOXPress

© F.L. Groeneweg

No parts of this thesis may be reproduced or transmitted in any form or by any means without written permission of the author.

Corticosteroid receptor dynamics

analysis by advanced fluorescence microscopy

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 6 november 2014
klokke 10:00 uur

door

Femke Lokke Groeneweg
geboren te Leiden
in 1982

Promotiecommissie

<i>Promotor</i>	Prof. Dr. E.R. de Kloet
<i>Co-promotor</i>	Dr. M.J.M. Schaaf
<i>Overige leden</i>	Prof. Dr. A.B. Houtsmuller (Erasmus MC, Rotterdam) Prof. Dr. M. Joëls (UMC, Utrecht) Dr. O.C. Meijer Prof. Dr. T. Schmidt Prof. Dr. H.P. Spaink Prof. Dr. H. Tanke

The studies described in this thesis have been performed at the department of Medical Pharmacology of the Leiden/Amsterdam Center for Drug Research (LACDR) and Leiden University Medical Center (LUMC), The Netherlands. Parts of this research were performed in collaborations with the department of Molecular Cell Biology of the Institute of Biology at Leiden University, the department of Physics of Life Processes of the Institute of Physics at Leiden University, the department of Neuroscience and Pharmacology at the University Medical Center (UMC) Utrecht and the department of Pathology at the Erasmus Medical Center, all in The Netherlands. This research was financially supported by the Royal Dutch Academy of Sciences (KNAW).

List of Abbreviations

AF-1/-2	Activating Function domain (1 and 2)
AR	Androgen Receptor
BLA	Basolateral Amygdala
DBD	DNA Binding Domain
ER	Estrogen Receptor
FRAP	Fluorescence Recovery After Photobleaching
FCS	Fluorescence Correlation Spectroscopy
GFP	Green Fluorescent Protein
GR	Glucocorticoid Receptor
GRE	Glucocorticoid Response Element
HPA	Hypothalamic-Pituitary-Adrenal
LBD	Ligand Binding Domain
LBP	Ligand Binding Pocket
MR	Mineralocorticoid Receptor
PFC	Prefrontal Cortex
PICS	Particle Image Correlation spectroscopy
PR	Progesteron Receptor
PVN	Paraventricular Nucleus
SMM	Single-Molecule Microscopy
TIRF	Total Internal Reflection Fluorescence
YFP	Yellow Fluorescent Protein

Contents

List of Abbreviations	v
1 General introduction	1
1.1 The stress response	2
1.2 Non-genomic corticosteroid signaling	6
1.3 Genomic corticosteroid signaling	8
1.4 The analysis of protein dynamics using advanced fluorescence microscopy techniques	13
1.5 Objective and outline	17
2 Rapid non-genomic effects of corticosteroids through the membrane-associated MR and GR and their role in the central stress response	21
2.1 Rapid effects of corticosterone in the brain	23
2.2 Functional implications of rapid corticosteroid effects in the brain	31
2.3 Molecular aspects of non-genomic corticosterone actions	37
2.4 Concluding remarks	44
3 Potassium currents in neuronal-like cell lines, models to study non-genomic Mineralocorticoid Receptor functionality	47
3.1 Introduction	49
3.2 Methods	50
3.3 Results	54
3.4 Discussion	64
4 A combination of wide-field and TIRF single-molecule microscopy as method to visualize the membrane-associated population of the Mineralocorticoid Receptor	69
4.1 Introduction	71
4.2 Methods	72
4.3 Results	76
4.4 Discussion	83
5 Quantitation of Glucocorticoid Receptor DNA-binding dynamics by Single-Molecule Microscopy and FRAP	87
5.1 Introduction	89
5.2 Methods	90
5.3 Results	94
5.4 Discussion	102

1	
2	
3	
4	
5	
6	
7	
R	

6 Quantitative analysis of the nuclear dynamics of the Mineralocorticoid Receptor reveals ligand-specific modulation of chromatin binding **107**

6.1 Introduction **109**

6.2 Methods **110**

6.3 Results **114**

6.4 Discussion **120**

7 General Discussion **127**

7.1 Summary of main conclusions **128**

7.2 The presence of the MR at the plasma membrane **129**

7.3 *In vitro* MR expression **131**

7.4 Advanced imaging methods to examine protein function and localization **133**

7.5 Towards a unifying model of steroid receptor DNA-binding dynamics **135**

References **141**

Addendum **165**

English summary **166**

Nederlandse samenvatting **170**

Curriculum Vitae **178**

Publication list **179**

