



Universiteit
Leiden
The Netherlands

Spin-label EPR on Disordered and Amyloid Proteins

Hashemi Shabestari, M.

Citation

Hashemi Shabestari, M. (2013, April 16). *Spin-label EPR on Disordered and Amyloid Proteins*. Retrieved from <https://hdl.handle.net/1887/20749>

Version: Not Applicable (or Unknown)

License: [Leiden University Non-exclusive license](#)

Downloaded from: <https://hdl.handle.net/1887/20749>

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

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/20749> holds various files of this Leiden University dissertation.

Author: Hashemi Shabestari, Maryam

Title: Spin-label EPR on disordered and amyloid proteins

Issue Date: 2013-04-16

SPIN-LABEL EPR ON DISORDERED AND AMYLOID PROTEINS

Maryam Hashemi Shabestari

The work reported in this thesis was carried out at the “Leids Instituut voor Onderzoek in de Natuurkunde (LION)” and is part of the research program of the “Stichting voor Fundamenteel Onderzoek der Materie (FOM)”.

An electronic version of this dissertation is available at the Leiden University Repository (<https://openaccess.leidenuniv.nl>).

Casimir PhD series, Delft-Leiden, 2013-6

ISBN: 978-90-8593-150-8

Cover design: M. M. Motazacker

Printed by: CPI Wöhrman Print Service

SPIN-LABEL EPR ON DISORDERED AND AMYLOID PROTEINS

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 dinsdag 16 april 2013
klokke 15.00 uur

door

Maryam Hashemi Shabestari

geboren te Teheran, Iran

in 1979

Promotiecommissie

Promotor:	Prof. dr. E.J.J. Groenen	Universiteit Leiden
Co-promotor:	Dr. M. Huber	Universiteit Leiden
Overige leden:	Prof. dr. E.R. Eliel	Universiteit Leiden
	Dr. C.W.M. Kay	University College London
	Prof. dr. M. Dogterom	FOM Instituut AMOLF, Universiteit Leiden
	Prof. dr. M. Ubbink	Universiteit Leiden
	Dr. A. Alia	Universiteit Leiden

*To my beloved mother and father,
Mitra, Mehran, Masoud,
and – Mahdi of course*

CONTENTS

1 Introduction	1
1.1 Introduction to EPR spectroscopy	2
1.1.1 Zeeman interaction and hyperfine interaction	2
1.1.2 Spin labels for protein EPR.....	4
1.1.3 Dynamics by EPR: the rotation-correlation time	5
1.1.4 Spin-spin interaction.....	6
1.2 The proteins and their properties	10
1.2.1 Structure of proteins.....	10
1.2.2 Misfolding and aggregation of proteins	11
1.2.3 Diseases that involve protein misfolding and aggregation	12
1.2.3.1 Alzheimer's disease: the amyloid β peptide.....	12
1.2.3.2 Parkinson's disease: the α -synuclein protein.....	13
1.2.4 Proteins with disordered regions: the light-harvesting protein CP29	14
1.3 Thesis outline.....	15
2 The effect of a membrane mimicking detergent on amyloid β aggregation	19
<i>Overview and the regime of high detergent concentration</i>	
2.1 Introduction.....	20
2.2 Materials and methods.....	21
2.2.1 Sample preparation protocol	21
2.2.2 EPR experiments.....	22
2.2.3 The amount of spin label in different samples.....	22
2.2.4 Simulations of EPR spectra, interpretation of the rotation-correlation time	22
2.3 Results.....	23
2.3.1 Effect of SDS on the amount of different components.....	26
2.3.2 The size of aggregates at different concentrations of SDS	27
2.4 Discussion.....	28
2.4.1 The high SDS concentration species of A β	28
2.4.2 A β at intermediate SDS concentrations	29
3 Interaction of the amyloid β peptide with a membrane mimicking detergent	33
<i>The regime of sub-micellar detergent concentration</i>	
3.1 Introduction.....	34
3.2 Materials and methods.....	34
3.2.1 Sample preparation protocol	34

3.2.2 EPR experiments.....	35
3.2.3 Simulations of EPR spectra	35
3.3 Results	35
3.3.1 Effect of SDS on the amount of different components	39
3.3.2 Effect of SDS on the rotation-correlation time.....	39
3.4 Discussion.....	40
4 The aggregation potential of 1-15 and 1-16 fragments of the amyloid β peptide and their influence on the aggregation of Aβ40	47
4.1 Introduction	48
4.2 Materials and methods.....	49
4.2.1 Sample preparation protocol	49
4.2.2 EPR experiments.....	50
4.2.3 Simulations of EPR spectra	50
4.2.4 Ratio of the intensity of the “fast” and “slow” components in each spectrum	50
4.2.5 Thioflavin T fluorescence assay.....	51
4.3 Results	51
4.4 Discussion.....	56
5 Elucidating the α-synuclein fibril fold with pulsed EPR.....	61
5.1 Introduction	62
5.2 Materials and methods.....	62
5.2.1 Expression and purification of cysteine variants of α S	62
5.2.2 Preparation and harvesting of fibrillar α S.....	63
5.2.3 Atomic force microscopy (AFM).....	63
5.2.4 Continuous-wave EPR at 80 K and at room temperature.....	64
5.2.5 DEER measurements.....	64
5.2.6 General structure parameters of fibrils.....	65
5.3 Results	65
5.3.1 Continuous-wave EPR.....	66
5.3.2 Pulsed EPR.....	67
5.4 Fibril fold model	71
5.5 Discussion.....	76
6 Exploring the structure of the N-terminus of the plant antenna protein CP29	79
6.1 Introduction	80
6.2 Materials and methods.....	81
6.2.1 Mutagenesis, labeling, and pigment reconstitution.....	81
6.2.2 Continuous-wave EPR measurements.....	82

6.2.3 Simulation of the cw EPR spectra	82
6.2.4. Assessment of the cw EPR spectra	83
6.2.5 Parameters to estimate the length of protein regions	83
6.2.6 Pulsed EPR measurements.....	84
6.3 Results	84
6.3.1 Continuous-wave EPR.....	84
6.3.2 Pulsed EPR	90
6.4 Localization of residue 4 of the N-terminus	93
6.5 Discussion.....	95
7 Structure and first EPR characterization of helical peptides with TOAC spin labels: models for short distances.....	99
7.1 Introduction.....	100
7.2 Materials and methods	101
7.2.1 Synthesis and characterization of peptides	101
7.2.2 Fourier transform infrared absorption spectroscopy	102
7.2.3 EPR spectroscopy.....	102
7.2.4 Preparation of the samples	102
7.2.5 Simulation	103
7.3 Results	103
7.3.1 Conformational analysis	103
7.3.2 Room temperature cw EPR.....	104
7.3.3 NONA ₂ and NONA _{2,8} at X-band, in frozen solution.....	108
7.3.4 NONA ₂ and NONA _{2,8} at W-band, in frozen solution	109
7.4 Discussion.....	110
7.4.1 Origin of the narrow-line contribution.....	111
7.4.2 The relation between structure and J-coupling	111
7.4.3 The contribution of dipolar interaction	112
7.4.4 Summary and conclusions	112
Appendix A: supporting information on chapter 2	115
Appendix B: supporting information on chapter 6	117
List of abbreviations.....	123
Summary	125
Samenvatting	129
Publications	133
Curriculum Vitae	135
Acknowledgement	137

