

**Bridging the gap between
Natural & Artificial Light-Harvesting**

A structure-function investigation with MAS NMR

ISBN 978-90-9023697-1

Cover figure courtesy of Gert Oostergetel, University of Groningen

Bridging the gap between Natural & Artificial Light-Harvesting

A structure-function investigation with MAS NMR

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 woensdag 26 november 2008
klokke 10.00 uur

door

Swapna Ganapathy

geboren te Vancouver, Canada in 1980

Promotiecommissie

Promoter: Prof. dr. H. J. M. de Groot

Referent: Prof. dr. M. Baldus, Universiteit Utrecht

Overige Leden: Prof. dr. T. J. Aartsma
Prof. dr. J. Lugtenburg
Prof. dr. J. Brouwer

For my parents

Table of contents

Abbreviations	10
Amino acid abbreviations	11
Chapter 1	13
A general introduction	
1.1 Introduction	13
1.2 The LH2 complex.....	15
1.3 The chlorosomes	17
1.4 Pigment aggregates forming π -stacks: Artificial antenna	19
1.5 Solid-state NMR for the structural and structure-function investigation of biological and biomimetic systems.....	21
Chapter 2	27
Methodological background	
2.1 Introduction	27
2.2 Solid-state NMR techniques used	29
2.2.1 Magic angle spinning.....	29
2.2.2 Cross-polarization	32
2.2.3 2D homonuclear and heteronuclear correlation experiments.....	34
2.2.4 The CHHC experiment.....	35
Chapter 3	39
Probing secondary, tertiary, and quaternary structure along with protein-cofactor interactions for a helical transmembrane protein complex through ^1H spin diffusion with MAS NMR spectroscopy	
3.1 Introduction	40
3.2 Experimental section	41
3.2.1 Sample preparation	41
3.2.2 MAS-NMR measurements	41
3.3 Results and discussion.....	43
3.4 Conclusions	45

Chapter 4

49

Alternating *syn-anti* bacteriochlorophyll *d* forms concentric helical nanotubes in chlorosomes

4.1	Introduction.....	50
4.2	Experimental section.....	51
4.2.1	Sample preparation.....	51
4.2.2	NMR measurements.....	51
4.2.3	Cryo-EM measurements.....	52
4.2.4	Structure modeling and ring-current calculation.....	53
4.3	Results.....	55
4.3.1	Chemical shift assignment.....	55
4.3.2	Distance constraints.....	58
4.3.3	Aggregation and ring-current shifts.....	60
4.3.4	Cryo-EM data.....	62
4.4	Discussion.....	63
4.4.1	NMR and cryo-EM in tandem for the suprastructure..	63
4.4.2	Suprastructure heterogeneity in chlorosomes.....	68
4.4.3	The mechanism of self-assembly of BChl <i>d</i>	69
4.5	Conclusions.....	71

Chapter 5

75

Self-assembly of BChl *c* in chlorosomes of the green sulfur bacteria *C. tepidum*: a comparison between the *bchQR* mutant and the wild type

5.1	Introduction.....	76
5.2	Experimental section.....	77
5.3	Results and discussion.....	79
5.3.1	Chemical shift assignment.....	79
5.3.2	Distance constraints.....	85
5.3.3	Ring deformation.....	87
5.3.4	Spectral doubling.....	88
5.3.5	Structural assessment: Mutant vs. WT.....	90
5.4	Conclusions.....	91

Chapter 6	95
MAS NMR investigation of self-assembled zinc chlorin dyes for artificial light-harvesters	
6.1 Introduction	96
6.2 Experimental section	99
6.3 Results.....	100
6.4 Discussion	105
6.5 Conclusions	107
Chapter 7	111
General discussion and outlook	
7.1 Structural assessment.....	111
7.2 Functional relevance	113
7.3 Future experiments	113
Summary	117
Samenvatting	121
Publications	123
Curriculum vitae	125
Nawoord	127

Abbreviations

[E,E]	[8-ethyl, 12-ethyl]
[E,M]	[8-ethyl, 12-methyl]
[M,M]	[8-methyl, 12-methyl]
[Pr,E]	[8-propyl, 12-ethyl]
1D	one-dimensional
2D	two-dimensional
AFM	atomic force microscopy
BChl	bacteriochlorophyll
<i>bchQR</i>	<i>bchQ bchR</i>
<i>bchQRU</i>	<i>bchQ bchR bchU</i>
BLYP	Becke, Lee, Yang and Parr
<i>C.</i>	<i>Chlorobium</i>
<i>Cf.</i>	<i>Chloroflexus</i>
Chl	chlorophyll
COSY	correlation spectroscopy
CP	cross-polarization
DARR	dipolar-assisted rotational resonance
DFT	density functional theory
EM	electron microscopy
FMO	Fenna-Matthew-Olson
FSLG	frequency-switched Lee-Goldburg
GIAO	Gauge-Independent Atomic Orbital
HETCOR	heteronuclear correlation
HOPG	highly ordered pyrolytic graphite
HRMS	high resolution mass spectrometry
LH1	light-harvesting complex 1
LH2	light-harvesting complex 2
MAS	magic angle spinning
NICS	nucleus-independent chemical shift
NMR	nuclear magnetic resonance
NSD	normal-coordinate structural decomposition
OD	optical density
PDB	protein data bank
PDSD	proton driven spin diffusion
PMLG	phase-modulated Lee-Goldburg
ppm	parts per million
RC	reaction center

Abbreviations

<i>rf</i>	radio frequency
RFDR	radio frequency-driven dipolar recoupling
<i>Rps.</i>	<i>Rhodopseudomonas</i>
S/N	signal-to-noise
STM	scanning tunneling microscopy
THF	tetrahydrofuran
TPPM	two pulse-phase modulation
<i>wPMLG</i>	windowed phase-modulated Lee-Goldburg
WT	wild-type

Amino acid abbreviations

A	Alanine
R	Arginine
N	Asparagine
D	Aspartic acid (Aspartate)
C	Cysteine
Q	Glutamine
E	Glutamic acid (Glutamate)
G	Glycine
H	Histidine
I	Isoleucine
L	Leucine
K	Lysine
M	Methionine
F	Phenylalanine
P	Proline
S	Serine
T	Threonine
W	Tryptophan
Y	Tyrosine
V	Valine

