



Universiteit
Leiden
The Netherlands

Design and synthesis of paramagnetic probes for structural biology

Liu, W.

Citation

Liu, W. (2013, November 25). *Design and synthesis of paramagnetic probes for structural biology*. Retrieved from <https://hdl.handle.net/1887/22357>

Version: Not Applicable (or Unknown)

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

Downloaded from: <https://hdl.handle.net/1887/22357>

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

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/22357> holds various files of this Leiden University dissertation.

Author: Liu, Wei-Min

Title: Design and synthesis of paramagnetic probes for structural biology

Issue Date: 2013-11-25

Design and synthesis of paramagnetic probes for structural biology

Wei-Min Liu

Design and synthesis of paramagnetic probes for structural biology

Wei-Min Liu

Doctoral Thesis, Leiden University, 2013

ISBN number: 978-94-6203-470-9

© 2013, Wei-Min Liu

Printed by CPI-Wöhrmann Print Service – Zutphen

Design and synthesis of paramagnetic probes for structural biology

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 maandag 25 November 2013
klokke 15.00 uur

door

Wei-Min, Liu

geboren te Kaohsiung, Taiwan in 1983

Promotiecommissie

Promotor: Prof. Dr. M. Ubbink
Co-promotor: Dr. M. Overhand
Overige leden : Prof. Dr. J. Brouwer
Prof. Dr. L. Bouwman
Dr. M. Merkx (Technische Universiteit Eindhoven)
Prof. Dr. M. Zweckstetter (Georg-August-Universitat
Gottingen, Duitsland)

Cover Image: Model of CLaNP-7 attached to Cyt *c* N56C/L58C.

Cover designed by Ching-Ya, Huang

The investigations described in this thesis were performed at the Protein Chemistry department of the Leiden Institute of Chemistry, Leiden University, Leiden, the Netherlands.

Financial support for the research was provided by the Netherlands Organisation for Scientific research (NWO), grant 700.58.441.

'Lanthanoids: These elements perplex us in our researches, baffle us in our speculations, and haunt us in our very dreams. They stretch like an unknown sea before us; mocking, mystifying and murmuring strange revelations and possibilities.'- *Sir William Crookes, Royal Society of Chemistry, 1887*

To My Family

Contents:

List of abbreviations	1
Chapter I Introduction and thesis outline	3
Chapter II A pH sensitive, colorful, lanthanoid-chelating paramagnetic NMR probe.....	31
Chapter III The development of new methods for the attachment of caged lanthanoids NMR probes.....	51
Chapter IV CLaNP-5 derivatives: An approach to obtain multiple paramagnetic restraints sets from a single mutation site	83
Chapter V The application of spin labels	101
Chapter VI General discussion, conclusions and prospects.....	117
Reference List	123
Nederlandse Samenvatting	135
English Summary	139
Appendices	141
List of Publications	149
Curriculum vitae	150

List of abbreviations

<i>aq</i>	aqueous
AzF	<i>p</i> -azido phenylalanine
Boc	<i>tert</i> -butyloxycarbonyl
calcd.	Calculated
CLaNP	caged lanthanoid NMR probe
CuAAC	copper-catalyzed 1,3-dipolar azide-alkyne cycloaddition
Cyt <i>c</i>	cytochrome <i>c</i>
Cys	cysteine
δ	chemical shift
d	doublet
Da	Dalton
dd	double doublet
DCM	dichloromethane
DHP	3,4-dihydropyran
DIPEA	diisopropylethyl amine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate
DTPA	diethylene triamine pentaacetic acid
DTT	dithiothreitol
EDC	1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide
equiv.	molar equivalent
h	hour(s)
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-oxid hexafluorophosphate
His	histidine
HOBt	<i>N</i> -hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
Hz	Hertz
IR	infrared
<i>J</i>	coupling constant
K	Kelvin
LC/MS	liquid chromatography/mass spectrometry
M	molar
m	multiplet
<i>m</i> CPBA	meta-chloroperoxybenzoic acid
MeCN	acetonitrile
min	minute(s)
MsCl	methanesulfonylchloride
<i>m/z</i>	mass-to-charge ratio

NHS	<i>N</i> -hydroxysuccinimide
NMR	nuclear magnetic resonance
<i>p</i>	para
Paz	pseudoazurin
PCS	pseudocontact shift
ppm	parts per million
PPTs	pyridinium <i>p</i> -toluenesulfonate
PRE	paramagnetic relaxation enhancement
q	quartet
RT	room temperature
s	singlet
sat.	saturated
SDS-PA gel	sodium dodecyl sulphate-poly acrylamide gel electroforesis
Su	succinimidyl
t	triplet
TBTA	<i>tris</i> -(Benzyltriazolylmethyl)amine
THP	tetrahydropyranyl
HTFA	trifluoroacetic acid
TLC	thin layer chromatography