



Universiteit  
Leiden  
The Netherlands

## Structure-based insights into the repair of UV-damaged DNA

Meulenbroek, E.M.

### Citation

Meulenbroek, E. M. (2012, October 9). *Structure-based insights into the repair of UV-damaged DNA*. Retrieved from <https://hdl.handle.net/1887/19938>

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/1887/19938>

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

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/19938> holds various files of this Leiden University dissertation.

**Author:** Meulenbroek, Elisabeth Maria

**Title:** Structure-based insights into the repair of UV-damaged DNA

**Issue Date:** 2012-10-09

# **Structure-based insights into the repair of UV-damaged DNA**

---

Elisabeth M. Meulenbroek

Printed by: Gildeprint Drukkerijen, the Netherlands.

Cover Design: E.M.Meulenbroek

ISBN: 978-94-6108-339-5

# Structure-based insights into the repair of UV-damaged DNA

---

## 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 dinsdag 9 oktober 2012  
klokke 16.15 uur

door

**Elisabeth M. Meulenbroek**

geboren te Middelburg, Nederland  
in 1985

## PROMOTIECOMMISSIE

Promotoren:	Prof. dr. J. P. Abrahams	Universiteit Leiden
	Prof. dr. L.H.F. Mullenders	Leids Universitair Medisch Centrum
Copromotor:	Dr. N.S. Pannu	Universiteit Leiden
Leden:	Prof. dr. J. Brouwer	Universiteit Leiden
	Dr. N. Goosen	Universiteit Leiden
	Prof. dr. J. H. J. Hoeijmakers	Erasmus Medisch Centrum
	Prof. dr. T. K. Sixma	Nederlands Kanker Instituut, Erasmus Medisch Centrum

The work reported in this thesis is financed by the Netherlands Organization for Scientific Research (NWO) through a Toptalent grant (N° 021.002.024).

The research was carried out at the 'Leiden Institute of Chemistry' (LIC).

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

*aan mijn ouders  
Bernard en Nicolette*





# Contents

<b>1</b>	<b>Introduction</b>	<b>1</b>
1.1	DNA under attack . . . . .	1
1.2	Direct reversal . . . . .	2
1.3	Base excision repair . . . . .	4
1.4	Mismatch repair . . . . .	5
1.5	Double-strand break repair . . . . .	7
1.6	Nucleotide excision repair . . . . .	8
1.7	Transcription-coupled repair . . . . .	10
1.7.1	Discovery and mechanism of transcription-coupled repair . .	10
1.7.2	Cockayne syndrome proteins A and B . . . . .	11
1.7.3	Cockayne Syndrome . . . . .	16
1.8	UV damage endonuclease repair . . . . .	17
1.9	Contents of this thesis . . . . .	20
<b>2</b>	<b>Purification and crystallization of Cockayne Syndrome protein A</b>	<b>23</b>
2.1	Introduction . . . . .	24
2.2	Materials and Methods . . . . .	24
2.2.1	Cloning and overproduction . . . . .	24
2.2.2	Purification . . . . .	25
2.2.3	Crystallization . . . . .	26
2.2.4	X-ray diffraction analysis . . . . .	26
2.3	Results and discussion . . . . .	26
<b>3</b>	<b>Crystal structure of Cockayne syndrome protein A</b>	<b>31</b>
3.1	Introduction . . . . .	32
3.2	Materials and methods . . . . .	32
3.2.1	Protein overproduction, purification and crystallization . . . .	32
3.2.2	Data collection and processing . . . . .	33
3.2.3	Structure solution and refinement . . . . .	33
3.2.4	Superpositions . . . . .	34
3.2.5	Complementation assays . . . . .	35
3.2.6	Protein solubility assays . . . . .	35
3.3	Results . . . . .	35

3.3.1	Overall structure . . . . .	35
3.3.2	Interaction of CSA and DDB1 . . . . .	36
3.3.3	Substrate binding site of CSA . . . . .	38
3.3.4	Disease-causing mutations . . . . .	41
3.4	Discussion . . . . .	45
<b>4</b>	<b>Substrate-binding by Cockayne Syndrome protein A</b>	<b>47</b>
4.1	Introduction . . . . .	48
4.2	Material and methods . . . . .	48
4.2.1	Protein overproduction and purification . . . . .	48
4.2.2	Bandshift assay . . . . .	49
4.2.3	Construction of CSA mutants . . . . .	49
4.2.4	CSA interaction studies . . . . .	49
4.3	Results . . . . .	50
4.3.1	Verification of CSA's substrate binding site . . . . .	50
4.3.2	Determination of CSA's binding capacity to DNA . . . . .	50
4.3.3	CSB is a possible substrate for CSA . . . . .	53
4.4	Discussion . . . . .	54
<b>5</b>	<b>Involvement of a carboxylated lysine in UV damage endonuclease</b>	<b>55</b>
5.1	Introduction . . . . .	56
5.2	Materials and methods . . . . .	56
5.2.1	Proteins . . . . .	56
5.2.2	DNA substrates . . . . .	57
5.2.3	Incision assay . . . . .	57
5.2.4	Incision of supercoiled plasmid DNA . . . . .	57
5.2.5	Filter binding assay . . . . .	57
5.2.6	Mass-spectrometry . . . . .	58
5.2.7	Crystallization . . . . .	58
5.2.8	Data collection and processing . . . . .	58
5.2.9	Structure solution and refinement . . . . .	58
5.3	Results . . . . .	59
5.3.1	Identity of the modification . . . . .	59
5.3.2	Functional studies of Lys229 mutants . . . . .	62
5.3.3	Structural studies of the Lys229 mutants . . . . .	64
5.4	Discussion . . . . .	66
<b>6</b>	<b>Unravelling UVDE's uncanny ability to recognize and incise different types of damaged DNA</b>	<b>71</b>
6.1	Introduction . . . . .	72
6.2	Materials and methods . . . . .	72
6.2.1	Cloning . . . . .	72
6.2.2	Expression and purification . . . . .	73
6.2.3	DNA substrates . . . . .	73
6.2.4	Incision assays . . . . .	74

---

6.2.5	Bandshift assays . . . . .	74
6.2.6	Filter-binding assays . . . . .	74
6.2.7	Crystallization . . . . .	74
6.2.8	Data collection . . . . .	75
6.2.9	Structure solution and refinement . . . . .	75
6.3	Results . . . . .	76
6.3.1	Overall structure of <i>SacUVDE</i> with and without damaged DNA	76
6.3.2	Recognition of pyrimidine-dimer lesions by UVDE . . . . .	79
6.3.3	<i>SacUVDE</i> has a preference for 6-4 photoproduct over CPD damaged DNA . . . . .	81
6.3.4	Structure-based mutagenesis explains <i>SacUVDE</i> 's substrate specificity . . . . .	83
6.4	Discussion . . . . .	87
<b>7</b>	<b>Crystal structure of a heterodimeric, double-headed Kunitz-type serine protease inhibitor from potato.</b>	<b>91</b>
7.1	Introduction . . . . .	92
7.2	Methods . . . . .	92
7.3	Results and Discussion . . . . .	93
7.3.1	Overall structure . . . . .	93
7.3.2	Identification of reactive site loops . . . . .	97
7.3.3	Interfaces for aggregation . . . . .	99
	<b>Bibliography</b>	<b>101</b>
	<b>List of publications</b>	<b>115</b>
	<b>Summary</b>	<b>117</b>
	<b>Samenvatting</b>	<b>121</b>
	<b>Curriculum vitae</b>	<b>125</b>

