Modelling copper-containing proteins

Bosch, Marieke van den

MODELLING COPPER-CONTAINING PROTEINS

Proefschrift

ter verkrijging
van de graad van Doctor
aan de Universiteit Leiden,
op gezag van de Rector Magnificus
dr. D.D. Breimer, hoogleraar in de
faculteit der Wiskunde en Natuurwetenschappen
en die der Geneeskunde, volgens besluit
van het College voor Promoties te
verdedigen op 18 januari 2006
klokke 16.15 uur.

door

Marieke van den Bosch

geboren te Helmond in 1975

Promotiecommissie:

Promotor:	Prof. dr. G. W. Canters
Referent:	Prof. dr. A. E. Mark (University of Queensland, AUS)
Overige leden:	Prof. dr. H. J. C. Berendsen (Rijks Universiteit Groningen) Prof. dr. J. Brouwer Prof. dr. W. F. van Gunsteren (Eidgenössische Technische Hochschule Zürich, CH) Prof. dr. M. C. van Hemert

These investigations were supported by the Netherlands Research Council for Chemical Sciences (NWO-CW)

Voor mijn ouders

Omslag voorzijde

Leÿda, Batavorum Lugdunum, volgo Leÿden, concinna edificiorum et incolarum freque[n]tia pulcherrimum nitidissimumq[ue] opp[idum] ab Hispanis obsidione cinctum, ab Auriacis autem com[m]eatus invectione liberatum anno parte salutis MDLXXIIII.
vrij vertaald:
Leÿda, Lugdunum van Batavia, ook bekend als Leÿden, versierd door gebouwen en gevuld met bewoners, mooie en blinkende stad, bezet door de Spanjaarden en bevrijd door de Prins van Oranje in het jaar 1574.
[Braun en Hogenberg, 1576]

Omslag achterzijde, van boven naar beneden

Kaart van Leiden en omgeving, kopie uit de 19-de eeuw
[Sluyter, 1550]

Leiden
[Blaeu, 1649]

Beschrijving der Stad Leyden
[Orlers, 1780]

Stafkaart Leiden en omgeving
[Topografische dienst, 2005]

Met dank aan A. van Benthem, Regionaal Archief Leiden en Y. A. Meurs Antiquariaat

Printed by: Optima Grafische Communicatie, Rotterdam

CONTENTS

ABBREVIATIONS **8**

Chapter 1 INTRODUCTION **9**

1.2 Molecular Dynamics simulations *12*

1.3 Force field: interaction functions and parameterisation .. *13*

1.3.1 Covalent bond potential 14

1.3.2 Angle bend interaction 17

1.3.3 Dihedral angle interaction 19

1.3.4 Electrostatic interactions 20

1.3.5 Van der Waals interaction 22

1.4 Conclusions *22*

1.5 Scope of this thesis *23*

Chapter 2 MOLECULAR DYNAMICS SIMULATIONS OF TYPE-I CU-CONTAINING PROTEINS: DEVELOPMENT OF AN EMPIRICAL, NON-BONDED FORCE FIELD **25**

2.1 Introduction *27*

2.2 Methods *28*

2.2.1 Potential Energy Surface 28

2.2.2 Force field 30

2.2.3 Molecular Dynamics simulations 30

2.3 Results and discussion *32*

2.3.1 Force field development using a potential energy surface 32

2.3.2 Force field testing 40

2.4 Comparison of force fields *45*

2.4.1 Azurin 45

2.4.2 Plastocyanin 46

2.5 ***Concluding remarks*** *48*

Chapter 3 A TEST CASE: N42C/H117G AZURIN MUTANT **49**

3.1 ***Introduction*** *51*
3.1.1 *UV-visible Spectroscopy* 51
3.1.2 *Resonance Raman Spectroscopy* 51

3.2 ***Methods*** *52*

3.3 ***Results and discussion*** *55*
3.3.1 *Structure* 55
3.3.2 *Stability* 56
3.3.3 *Structure and stability of the Cu-site* 58
3.3.4 *Model structure* 59

3.4 ***Conclusions*** *61*

Chapter 4 DYNAMICS ANALYSIS OF THE HYDROPHOBIC CORE OF THE BLUE COPPER PROTEIN AZURIN **63**

4.1 ***Introduction*** *65*

4.2 ***Methods*** *70*

4.3 ***Results*** *71*
4.3.1 *Stability* 71
4.3.2 *Structure* 72
4.3.3 *Flexibility* 73
4.3.4 *Heterogeneity* 80
4.3.5 *Diffusion of water* 82

4.4 Discussion *85*
4.4.1 *Structure & Heterogeneity* 85
4.4.2 *Flexibility* 86
4.4.3 *Diffusion of water* 86

4.5 Conclusion *87*

Chapter 5 CALCULATION OF THE REDOX POTENTIAL OF THE PROTEIN AZURIN AND SOME MUTANTS **89**

5.1 Introduction *91*

5.2 Methods *95*

5.3 Results and Discussion *99*
5.3.1 *Overall structure of the protein* 99
5.3.2 *Free energy calculations* 100

5.3.3 Relative redox potentials of azurin mutants 109

Chapter 6 SIMULATION OF THE SUBSTRATE CAVITY DYNAMICS OF QUERCETINASE 111

6.1 Introduction *113*

6.2 Methods *117*

6.3 Results and discussion *124*

6.3.1 Protein stability and fluctuation 124
6.3.2 Loop stability and fluctuation 125
6.3.3 Proline 164 128
6.3.4 Driving force 129
6.3.5 Presence and behaviour of water molecules inside the cavity. 130

6.4 Conclusion *132*

Chapter 7 CONCLUDING REMARKS 135

7.1 Empirically derived, non-bonded force field *137*

7.2 Quantum-chemically derived, bonded force field *139*

REFERENCES 141

SUMMARY 149

SAMENVATTING 153

LIST OF PUBLICATIONS 157

CURRICULUM VITAE 158

NAWOORD 159

ABBREVIATIONS

2,3QD	2,3 quercetin dioxygenase
DFT	Density Functional Theory
EPR	Electron Paramagnetic Resonance
IR	Infra-Red
KMP	kaempferol
MD	Molecular Dynamics
MM	Molecular Mechanics
NMR	Nuclear Magnetic Resonance
PC	Plastocyanin
QM	Quantum Mechanics
RR	resonance Raman
RMS	root mean square
wt	wild type
UV-vis	ultraviolet and visible light
UFF	Universal Force Field
XRD	X-ray Diffraction