



Universiteit
Leiden
The Netherlands

A new method to reconstruct the structure from crystal images

Li, Y

Citation

Li, Y. (2017, May 3). *A new method to reconstruct the structure from crystal images*. Retrieved from <https://hdl.handle.net/1887/48877>

Version: Not Applicable (or Unknown)

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/48877>

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

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/48877> holds various files of this Leiden University dissertation

Author: Li, Y.

Title: A new method to reconstruct the structure from crystal images

Issue Date: 2017-05-03

A new method to reconstruct the structure from crystal images

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 woensdag 3 mei 2017
klokke 15:00 uur

door

Yao-Wang Li
geboren te Jiangxi, China
in 1983

Promotiecommissie

Promotores: prof.dr. Jan Pieter Abrahams
co-promotores: dr. Tim Grüne (Paul Scherrer Instituut)

Voorzitter: prof.dr. H.P. Spaink
Secretaris: prof.dr.ir. T.H. Oosterkamp
Overige leden: dr. N.S. Pannu
dr R.B.G. Ravelli (Maastricht Universiteit)
dr. Xiaodan Li (Paul Scherrer Instituut)
prof.dr. Henning Stahlberg (Universiteit Basel)
prof.dr. T.J. Aartsma

ISBN 978-94-6332-171-6

©2017 Yaowang Li

Contents

Contents	i
List of Tables	v
List of Figures	vii
1 Introduction	1
1.1 Biological macromolecules and cryo-EM	2
1.1.1 Biological macromolecules	2
1.1.2 Electron microscopy	5
1.1.3 Cryo-EM in structural biology	5
1.2 Methods in cryo-EM	8
1.2.1 Electron crystallography	8
1.2.2 SPA and ET	13
1.3 Principle of single particle analysis	15
1.3.1 Image acquisition	15
1.3.2 Image formation and CTF	17
1.3.3 Picking up particles	19
1.3.4 Multivariate statistics analysis	21
1.3.5 Alignment	23
1.3.6 Orientation determination and 3D reconstruction	24
1.3.7 Resolution determination	26
1.4 Principle of electron diffraction	28
1.5 The phase problem in 3D crystals	33

Contents

1.6	Outlines of this thesis	34
2	Imaging protein three-dimensional nano-crystals	37
2.1	Abstract	38
2.2	My contribution	38
2.3	Introduction	38
2.4	Materials and methods	41
2.4.1	Crystallization	41
2.4.2	Vitrification	41
2.4.3	Electron-microscopy data collection	41
2.5	Results	43
2.5.1	Structure of the Bragg spots	43
2.5.2	Centrosymmetry of the Fourier transform	46
2.5.3	Visualizing the lattice	50
2.6	Discussion and conclusions	53
3	Filter for processing image data	59
3.1	Abstract	60
3.2	My contribution	60
3.3	Introduction	60
3.4	Method	64
3.5	Results	67
3.6	Discussion	74
4	A new approach of estimating crystallographic phases	77
4.1	Abstract	78
4.2	Introduction	78
4.3	Materials and methods for lysozyme	81
4.3.1	Cryo-EM images and diffraction patterns	81
4.3.2	Forward projection	81
4.3.3	Comparison in Fourier space and real space	82
4.3.4	Reconstruction and validation	83
4.4	Materials and methods for peptide VQIVYK	86
4.4.1	Cryo-EM images and diffraction patterns	86

4.4.2	Contrast improvement	86
4.4.2.1	Lattice filter	86
4.4.3	The simulated 3D lattice and forward projection .	87
4.4.4	Comparison in Fourier space and real space . . .	87
5	Preliminary results of phasing crystallographic data	89
5.1	Abstract	90
5.2	Introduction	90
5.3	Results and discussion for lysozyme	91
5.3.1	Reference and experimental projections	91
5.3.2	Determination of freedom for the experimental projections	92
5.3.3	Map validation	94
5.4	Results and discussion for VQIVYK peptide	99
5.4.1	Contrast improvement using lattice filter	103
5.4.2	Comparison in Fourier space	104
5.4.3	Parallel alignment in real space and reconstruction	104
5.5	Conclusion	107
6	Discussion, prospects and summary	111
6.1	Discussion	112
6.1.1	Data acquisition for 3D protein crystals	113
6.1.1.1	Detectors in cryo-EM	113
6.1.1.2	Data collection in cryo-EM	115
6.1.2	Data processing for 3D protein crystals	116
6.1.3	The phase problem in 3D electron crystallography	118
6.2	Prospects	119
6.2.1	Detectors	120
6.2.2	Sample holder	120
6.2.3	Data processing	120
6.3	Summary	121
7	Samenvatting	125

Contents

Bash and IMAGIC scripts	131
References	137
Curriculum Vitae	157
Publications	159

List of Tables

1.1	Comparison of electron crystallography and SPA.	14
-----	---	----

List of Tables

List of Figures

1.1	Growth of gene sequences on the GenBank website (data obtained on 26 September 2016).	3
1.2	Growth of released structures per year by experimental method (obtained from the PDB website on 26 September 2016).	4
1.3	Layout of optical components in a basic TEM (Transmission electron microscopy on Wikipedia).	6
1.4	The procedure of electron crystallography: the steps are described in detail.	11
1.5	The procedure of SPA: the steps are described in detail.	16
1.6	Particles of worm hemoglobin picked from images by the author during the 6th Brazil School for Single Particle Cryo Electron Microscopy.	20
1.7	Averaged images of worm hemoglobin particles calculated by the author during the 6th Brazil School for Single Particle Cryo Electron Microscopy.	21
1.8	Flowchart of angular reconstitution and 3D reconstruction.	25
1.9	Interaction of electrons with atoms in matter.	29
1.10	The Laue equation.	30
1.11	Bragg's law.	31
1.12	The Ewald sphere.	32
2.1	Lysozyme nano-crystals in a crystallization drop imaged with a light microscope.	42

List of Figures

2.2	The shape of the crystal can be showed after calculating the local variance and its lattice contrast can be enhanced by a Wiener type filter.	44
2.3	Fourier transformation shows the lattice spots of crystal. .	45
2.4	Histogram of the maximum resolution observed in electron images of 200 different lysozyme three-dimensional nano-crystals.	46
2.5	Two defocused frames from raw data of a tomographic series of one of the crystals shows its thickness.	50
2.6	Electron diffraction pattern showing lunes (at 200 keV)..	52
2.7	The patches in the same domain can be averaged to enhance the contrast.	54
2.8	Examples of averaged images with high contrast.	55
3.1	The measured structure factor $F_m(\mathbf{h})$ is the sum of the unknown structure factor corresponding to the lattice signal $F_l(\mathbf{h})$ and the unknown structure factor corresponding to the noise $F_n(\mathbf{h})$	64
3.2	Plot of the rotational average of fig. 3.3.	65
3.3	Images of 100 nm thick 3D crystals, produced by a Titan Krios 300 kV FEG transmission EM, captured with a Falcon 2 camera (4096×4096 pixels) using an 0.5 sec exposure time and an illumination of 3 to 10 $e^-/\text{Å}^2$	68
3.4	Rotational average which is subtracted from the power spectrum during lattice filtering.	70
3.5	Unique half of the centro-symmetric lattice filter of the top lysozyme crystal image.	71
3.6	Unique half of the centro-symmetric lattice filter of the top lysozyme crystal image.	72
3.7	Results from the same area as in figure fig:original-processed and results.	73
4.1	The flowchart of reconstructing <i>map-3</i>	83
4.2	The flowchart of creating <i>map-2</i>	84

4.3	The flowchart of creating <i>map-1</i>	85
5.1	The CC value curves in Fourier space.	95
5.2	The experimental projections and reference projections were compared in Fourier space and real space.	96
5.3	The FSC curves between the four maps (three reconstructed maps and the original map).	100
5.4	The FSC curves of <i>map-4</i> with <i>map-0</i> and <i>map-1</i>	101
5.5	The two β distributions in the Euler angles.	101
5.6	The orthogonal views of the reconstructed maps and the original map.	102
5.7	Noise was suppressed by the lattice filter.	103
5.8	The experimental projections and reference projections were compared in Fourier space.	105
5.9	The CC value curves in Fourier space.	106
5.10	The FSC curve of the map reconstructed from the experimental projections for peptide VQIVYK.	107
5.11	The orthogonal views of the reconstructed map of the peptide.	108

List of Figures
