University of Groningen

Engineering biological nanopores for proteomics study

Huang, Kevin

DOI:
[10.33612/diss.102598418](https://doi.org/10.33612/diss.102598418)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Huang, K. (2019). *Engineering biological nanopores for proteomics study*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.102598418>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Engineering Biological Nanopores for Proteomics Study

Gang Huang

**university of
 groningen**

**faculty of science
and engineering**

The work described in this thesis was performed in the Chemical Biology Group of the Groningen Biomolecular Sciences and Biotechnology Institute at the University of Groningen.

The project was funded by the University of Groningen and European Research Council (ERC).

Printed by IPSKAMP printing

ISBN: 978-94-034-2184-1 (printed version)

ISBN: 978-94-034-2183-4 (digital version)

university of
 groningen

Engineering Biological Nanopores for Proteomics Study

PhD thesis

to obtain the degree of PhD at the
University of Groningen
on the authority of the
Rector Magnificus Prof. C. Wijmenga
and in accordance with
the decision by the College of Deans.

This thesis will be defended in public on

Thursday 28 November 2019 at 12.45 hours

by

Gang Huang

born on 5 December 1987
in Jiangxi, China

Supervisor
Prof. G. Maglia

Assessment Committee
Prof. D.J. Slotboom
Prof. M.D. Witte
Prof. C. Joo

Contents

Chapter 1 9

Introduction: Protein study with nanopores

1. Introduction.....	9
1.1. Proteomics.....	10
1.2. Single molecule nanopore technology	10
1.3. Biological Nanopores.....	11
1.4. Solid-state nanopores	13
2. Proteomics with nanopores	14
2.1. A nanopore peptide mass identifier.....	16
2.2. Protein sequencing by reading amino acids with nanopores	22
2.3. Folded proteins detection with nanopores.....	26
3. Outline of the thesis	32
4. Reference	36

Chapter 2 53

Electro-osmotic capture and ionic discrimination of peptide and protein biomarkers with FraC nanopores

1. Abstract	54
2. Introduction.....	55
3. Results	57
3.1. Protein capture with FraC nanopores	57
3.2. The charge of the constriction dictates the ion selectivity	59
3.3. The electro-osmotic flow promotes the entry of polypeptides into FraC	61
3.4. Biomarker detection with the WtFraC nanopore	62
3.5. Protein translocation might deform the transmembrane helices of FraC	62
3.6. Threshold potential and stretched polypeptides during translocation	64
3.7. Recognition of peptide and protein biomarkers	67

4. Discussion	68
5. Methods and materials	70
6. Supplementary information	76
7. References.....	99
Chapter 3	107
FraC nanopores with adjustable diameter identify the mass of opposite-charge peptides with 44 dalton resolution	
1. Abstract	108
2. Introduction.....	109
3. Results	111
3.1. Engineering the size of FraC Nanopores	111
3.2. Identification of single amino acid substitutions with type II FraC nanopores.....	116
3.3. A nanopore mass spectrometer for peptides	117
3.4. Peptide translocation across nanopores.....	118
4. Discussion	119
5. Methods and materials	122
6. Supplementary information	127
7. References.....	146
Chapter 4	151
Pleurotolysin Nanopores are Engineered to Capture and Recognize 80 kDa Folded Proteins	
1. Abstract	152
2. Introduction.....	153
3. Results	155
3.1. Structure and expression of pleurotolysin nanopores.....	155
3.2. Engineering the nanofluidic properties of PlyAB nanopores.	156
3.3. Protein capture with PlyAB nanopores.	158
3.4. Analysis of human plasma proteins with PlyAB-R nanopores....	160
3.5. Discrimination between protein blockades	164

4. Conclusion	165
5. Methods and Materials	165
6. Supplementary information	170
7. References.....	182
Summary.....	189
1. Conclusion	190
2. Future perspective	193
Samenvatting.....	195
1. Conclusie	196
2. Toekomstperspectief.....	199
Acknowledgements.....	Error! Bookmark not defined.