Review of the thesis

Integrated structural study of the FrpD protein from *Neisseria meningitidis*

Author: Ekaterina Sviridova

The main aim of the submitted thesis is detailed structural study of the iron-regulated protein FrpD and its complex with FrpC in order to understand mechanism of *Neisseria meningitidis* virulence.

The thesis is introduced by useful List of abbreviations and it consists of six parts. After a short introduction of thesis aims, the author briefly characterizes *Neisseria meningitidis* and related above mentioned proteins. This is followed by a chapter on the approaches of structural biology. It appeared later how useful was a combination of several complementary methods in addition to primary X-ray diffraction. Since this method requires crystals, the chapter begins with a description of the problem of protein crystallization and a survey of basic crystallization techniques. More attention is given to the X-ray crystallography, i.e. crystal symmetry, X-ray data collection and evaluation and methods for phase problem solution, in particular in macromolecular crystallography, and finally model building, refinement and validation. Three complementary methods – NMR, cryo-TEM and SAXS (Small-angle X-ray scattering) are described at the end of the part 2.3 that is on 24 pages, very compact and clear.

Next chapter – Materials and methods (9 pages) is devoted to detailed experimental specifications starting with purification of proteins. For structural solution of FrpD additional protein with selenium (SeMet FrpD) was prepared and for NMR experiment ^{15}N and ^{13}C isotope-labeled $FrpD_{43-271}$ was also purified.

Suitable crystals for X-ray experiment were grown by the sitting drop vapor diffusion method only for native $FrpD_{43-271}$ and for SeMet FrpD. Numerous attempts to prepare crystals of FrpC were finally not successful. The crystals could be measured at synchrotron BESSY in Berlin to very reasonable resolution and the crystal structure of the native protein could be solved by molecular replacement method. Since there were no crystals available of the FrpD-$FrpC_{1-414}$ complex, SAXS experiment, not requiring single crystals, was performed and could provide low-resolution protein structure. The binding interface in the complex was studied by NMR. Finally, comparative analysis of FrpD structure was performed in PDB. It indicated that FrpD fold did not contain the topology similar to other know proteins and that the structure found is really novel. The data were deposited in the PDF. The studies showed the presence of one monomer molecule of the protein in the asymmetric unit and that the structure has a bean-like shape. The shape obtained from the SAXS data was completely consistent with the X-ray diffraction study. By combination of several techniques the author proposed a model of FrpD/$FrpC_{1-414}$ complex localization and function activity.

The results are mainly described in part 4. Publications. They have been published in two papers. The first one from *Acta Crystallographica F* is completely devoted to the crystallization of membrane lipoprotein FrpD and its basic crystallographic characterization without structure solution and the second one for *Biomolecular NMR assignments* is basically on NMR studies and atomic assignments. The main third paper containing most of results is declared as prepared for submission to *Structure*.

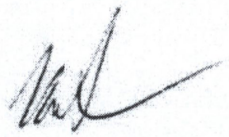
The results are summarized in part 5 in about three pages. I assume that part 6 – References containing 152 references is related to chapter 1-3 since all the papers in part 4 have their own section

References. The form of citations by reference to the author names was chosen and seems to be consistent.

There are only a few misprints in the thesis. English is not ideal but I think that it is quite acceptable. Perhaps also the structure of thesis leads to several repetitions of some findings. However, this should be avoided in conclusion. For example the sentence „The fold of FrpD resembles a compact bean-shaped structure slightly concaved from one side" is repeated in two subsequent paragraphs on page 113 bottom.

I recommend to the Faculty of Science at the University of South Bohemia in České Budějovice acceptance of the doctoral thesis of Ekaterina Sviridova, if successfully defended. There is no doubt about its quality and novelty as well as about significant contribution of the student to all individual parts of the research mentioned in the thesis. The thesis is well supported by two publications. However, I would prefer, if the thesis is submitted after acceptance of the third, the most substantial, manuscript (that is a part of the thesis) for publication.

In Prague 19. 9. 2016



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OPPONENT´s REPORT

on PhD. thesis of Ekaterina Sviridova

„Integrated structural study of the FrpD protein from Neisseria meningitidis"

The submitted thesis is devoted to a structural characterization of two iron regulated proteins FrpD and FrpC, which are involved into the virulence mechanism of bacteria *Neisseria meningitidis* which colonize nasopharynx and can cause serious diseases upon penetration into the bloodstream. The major goal was structural characterization of FrpD protein and its interaction with FrpC protein.

Ambitious goals have been reached and the crystal structures of native and SeMet forms of the unique protein FrpD have been determined at 2.3 and 1.4 Å resolution and the FrpD/FrpC interaction interface has been characterized by NMR spectroscopy and small angle scattering methods. Based on the structural information, the role of the two proteins in the process of bacterial adhesion to host cells has been proposed.

The thesis includes 2 publications in international CC journals and the third one has been submitted. The manuscript is of high research quality and I have no doubt about its publishing in CC journal. This is a very good outcome of a PhD. study. Since the papers went through a thorough refereeing process, it warrants a high level of the work. Without any doubt, the scientific results presented in the thesis are original, interesting, and of high quality.

I only have a small formal remarks relating to the manner of literature citations. Literature should be cited more accurate and attentive. As an example:

- based on the instructions at PDB database (e.g. p. 15), primary reference H.M. Berman, K. Henrick, H. Nakamura (2003) Announcing the worldwide Protein Data Bank, Nature Structural Biology 10, 980 should be cited. The correct address is www.wwpdb.org, not www.pdb.org.

- the textbooks should be cited more carefully, as an example of possible source of information or for review and where appropriate also primary citation should be placed. For instance, on page 26 at the Bragg's law description, the textbook of Rupp (2010) is cited. A primary citation is Bragg W.H., Bragg W.L. (1913) The reflexion of X-rays by crystals. Proc R. Soc. Lond. A. 88, 428–38. Similarly, Rupp (2010) is cited on page 27 at explanation of non-crystallographic symmetry, Rhodes (2006) at introducing the Miller indices, etc. By my means it should be clear from the context that the textbook or review are cited, not primary citation.

- on the page 28 the citations of molecular replacement programs are missing

- in the part 2.3.1.2 (pp. 19-22) crystallization techniques are described and papers from 1992 and 1999 years are cited. These techniques have been developed much earlier.

  - vapor diffusion method has been described in the papers Hampel A.E., Bock R.M. (1970) General procedure for crystallization of transfer ribonucleic acid. Biochemistry 9, 1873–1880 or Davies D.R., Segal D.M. (1971) Methods in Enzymology 22, 266–269.

  - microdialysis method has been introduced in 1968 (Zeppezauer M., Eklund H., Zeppezauer E.S. (1968) Arch. Biochem. Biophys. 126, 564–573)

  - free interface diffusion method has been introduced in 1972 (Salemme F.R. (1972) A free interface diffusion technique for the crystallization of proteins for X-ray crystallography. Arch. Biochem. Biophys. 151, 533).

- on the page 30, at SAD method description, the paper Dauter Z., Dauter M., Dodson E. (2002) Acta Cryst. D58, 494-506 should be cited rather than Dodson 2003, which is its continuation. The first structure solved by the SAD method was published even sooner, in Dauter Z., Dauter M., de La Fortelle E., Bricogne G., Sheldrick G.M. (1999) *J. Mol. Biol.* **289**, 83–92.

I would like to address the author with the questions listed below:

- The native and SeMet FrpD crystallized at different conditions. Did you test the crystallization of native protein using the conditions successful for obtaining SeMet crystals? Is it possible to understand from the structure why the two FrpD forms crystallized at different conditions and in different crystal forms? Did you use crystal seeding?

- As it is mentioned on page 76, "...FrpC is translated as an unfolded polypeptide.. and ...the presence of Ca^{2+} ions promotes the folding of self-processing module". Are there some evidences about the structure of $FrpC_{1-414}$ and $FrpC_{415-1829}$ fragments? Are they fully unstructured proteins? Remains the part representing self-processing module folded?

I would like to say, that the mentioned above remark about the dissertation cannot affect the overall very positive opinion about the submitted work. Author in her thesis has demonstrated her knowledge and overview in the field of structural biology and ability to work independently and creatively. I recommend to accept the dissertation and after its successful defense to grant **Ekaterina Sviridova** the scientific degree - "**philosophiae doctor**" (PhD).

RNDr. Lubica Urbanikova, CSc.

Bratislava, 20 September, 2016

Opponent's report on the dissertation
„Integrated structural study of the FrpD protein from Neisseria meningitidis"

Author: **Ekaterina Sviridova, MSc**

PhD thesis: **Integrated structural study of the FrpD protein from *Neisseria meningitidis***

Opponent: **RNDr. Jindřich Hašek, DrSc**

The PhD thesis presented by Ekaterina Sviridova tries to clarify the structure and function of the extracellular domain (22-271) of the FrpD protein and the fragment (1-414) of protein FrpC produced by bacteria Neisseria meningitides. Because the presence of the bacteria is common in population and in case of penetration into blood can be dangerous for life, it is well selected target for research.

The first part of thesis (pages 1-38) summarizes the current state of the topic. The chapter 3 (pages 41-49) describes the basics of preparation of samples for X-ray methods. Results of study are presented as a summary of three papers on pages 53-109. Pages 53-64 describe sample preparation and measurements on synchrotron sources of radiation in Berlin and Hamburg. Pages 65-71 describe NMR signals assignments. Pages 72-82 describe X-ray structure determination of extracellular domain of FrpD. Pages 83-87 summarize the determination of contact residues in complex FrpC-FrpD by NMR. Pages 87-94 describe the determination of protein envelope shapes by SAXS. Discussion on pages 94-103 and the conclusions at the end of the thesis on pages 113-116 summarize the results achieved.

The thesis brings new information on structure and function of the proteins FrpC, FRpD and its complex FrpC-FrpD. The author determined the structure of the extracellular domain of the protein FrpD by diffraction methods. The structure of extracellular domain of FrpD and its selenomethion variant were deposited into PODB under codes 5EDJ and 5EDF. NMR modęls of FrpD were detopited in the BMRB database under the code 18779. The SAXS models of the FrpD fragment and the complex FrpD-FrpC fragments were deposited into SASBDB.

Questions:

1. The FrpD, FrpC studies were studied by several people for many years in several closely cooperating laboratories. The author should clarify her participation on the works and the contribution of other co-authors of the related publications.
2. Also the negative results are worth to show. The author should describe the unsuccessful attempts to crystallize the complex of the two FrpC-FrpD fragments, and analyse the reasons of the problem. Proofs that the 1:1 molar mixtures of FrpC-FrpD fragments used for crystallization were really monodisperse, etc.
3. What was the experience to crystallize fragments of the FrpC (1-114) ?
4. SAXS measurements show the overall shape of the complex of FrpC-FrpD fragments. The author should show the complex envelope with the interface forming residues highlighted on surface to proof that the residues forming interface were assigned correctly. X-ray structure of FrpD fragment fitted in its correct position and? Eventually, to show the whole complex FrpC-FrpD complex modelled into the envelope.

Summary

The author of the submitted work focused on very important topic of understanding the bacterial processes with possible practical impacts in medical care and drug design. She published 6 conference proceedings, 3 publications in impacted journals. Manuscript of the key paper (Sviridova et al) was accepted for publication in well impacted journal.
E. Sviridova correctly used combination of qualitative and quantitative approaches showing modern and useful approach. The work as a whole satisfies basic requirements set down for PhD thesis. The author demonstrated her ability to focus on topical important subject and correctly addressed the scientific problems and methods of their solution.

Thesis of Ekaterina Sviridova fulfil the requirements laid by law and therefore I recommend her dissertation thesis for the defense procedure.

In Praha, 1. 9. 2016

RNDr. Jindřich Hašek, DrSc