Method development for the study of the structure and interactions of intrinsically unstructured proteins

KH 125597 project, FINAL REPORT

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In the recent years, it has become more evident that the structure-function paradigm of proteins, i.e., a well-ordered, folded structure is required for the function, needs reconsideration. Now it is clear that the intrinsically unstructured proteins (IUPs) or disordered protein regions play fundamental role in various processes of the living cell. Aberrant function of these proteins can be associated with cancer, methabolic or (neuro)degenerative diseases. There are limited experimental methods available for the structural studies of unstructured proteins because the use of atomic resolution techniques, such as X-ray crystallography or NMR is problematic. Circular dichroism spectroscopy is a widely used technique for the secondary structure estimation of proteins. However, the previous CD spectrum analysis algorithms were unreliable, especially for beta-structured and disordered proteins. We developed a new algorithm which provides more accurate and increased structural information from the CD spectra (Micsonai és mtsai. (2015) PNAS, 112, E3095). The method is useful for the study of protein aggregates and amyloids that are associated with several neurodegenerative diseases, such as Alzheimer's. In this project our major aim was to improve our method for accurate structure prediction of unstructured proteins.

In the first year, we have expressed and purified several intrinsically disordered proteins or protein segments, such as the disordered plant chaperone ERD14, its scrambled mutant, the transactivation domain of tumor suppressor protein p53TAD, different fragments of H3 lysine-methyltransferase (HKMT) (such as MLL4 and MLL2), disordered loop of Ezh2 (HKMT) and its mutant. Moreover, we constructed artificial peptide sequences with high propensity for disordered structure. We had these peptides synthesized, which expense was kindly covered by our French collaborator's budget (10k EUR) because it was beyond the limits of the budget of the present grant. The advantage of these peptides are the relatively short length (25 a.a.) which makes easier the in silico modelling of their structure and their experimental characterization by CD spectroscopy and other methods. These peptides have good solubility and expected to have low tendency to aggregate.

The disordered protein and peptide samples along with protein of highly twisted beta-structures were measured on a conventional spectropolarimeter and in the SOLEIL Synchrotron Facility (Gif sur Yvette, France). We applied for beamtime to SOLEIL and our further experiments were approved.

The structural studies by CD spectroscopy on the disordered MLL4 fragments were published in the International Journal of Molecular Sciences (Szabó et al., Int. J. Mol. Sci, 19 : 11 E3478 (2018), IF: 3.687). The last author of this paper is Ágnes Tantos, senior researcher of our KH project.

We carried out molecular dynamics simulations (using the GROMACS package) on the disordered peptides using a specific Amber forcefield that was optimized for disordered proteins, to provide structural ensembles to serve as structural references for the development of BeStSel on intrinsically disordered proteins. One microsecond simulations were performed which was by far sufficient to

reach a dynamic structural equilibrium. We collaborated in MD simulations using replica exchange method with Dr. Lucio Colombi Ciacchi and co-workers (University of Bremen, Germany). The work, studying the unfolding of an alpha-helix segment by forming a disordered structure of a protein upon adsorbed on silica surface has been just accepted in ACS Biomaterials Science & Engineering (Hildebrand et al., ACS Biomat. Sci. Eng. (2018) accepted (ms. ID: ab-2018-00819m.R1), impact factor: 4.432).

We started the optimization of the BeStSel method for IDPs and the preliminary results were very promising. Our results were also presented at the 2018 Biophysical Society Meeting in San Francisco.

We started the collaboration with the group of Örs Legeza in order to develop the methodology for ab initio calculations of the CD spectra from the atomic resolution 3D structures. Their quantum chemical DMRG-TCCSD method is under optimization to calculate the electric and magnetic transition dipole momentum of the molecular system which are the basis of the rotatory strength that will provide the CD signal.

We presented our results at the French-Hungarian Scientific Conference organized by the French Embassy at Budapest in September, 2018.

The BeStSel webserver was further improved and a new paper was published in Nucleic Acids Research on the new developments and case studies (Micsonai et al., Nucleic Acids Res. 46, W315-W322 (2018), impact factor: 11.561).

In the second year, we continued to collect the CD and SRCD spectra of disordered proteins to extend our reference database for the development of the BeStSel algorithm. We visited SOLEIL Synchrotron to carry out SRCD experiments in November, 2018 and in March, 2019. We collected SRCD spectra of synthesized and expressed peptides having mostly disordered structures under various experimental conditions. After normalization and checking the quality of the spectra, they have become part of the new CD spectrum reference set to further develop our BeStSel method for accurate structural estimation of intrinsically disordered proteins. To be paired with the SRCD spectra of the peptides, structural information were provided by molecular dynamic simulations. Analyzing the trajectories of simulations with different starting conformations, we calculated the average secondary structure content of the peptides. Moreover, our collaborator, Orsolya Tőke examined the structure of several peptides, measured in SOLEIL, by NMR spectroscopy which is complementary experimental technique and provided more detailed information compared to FTIR. We used the improved, extended reference set to optimize BeStSel. Another development we made is that we used a more sophisticated, neuronal network (NN) algorithm for accurate secondary structure determination. The optimization of the NN method is a long work and is still undergoing, however, the results are very promising. The BeStSel_NN version is proved to perform significantly better on almost all the secondary structure types. The RMSD of the estimation of the “Others” (disordered) component is improved from 0.057 to 0.036 and its correlation from 0.81 to 0.93 when compared to the original BeStSel algorithm. The estimation accuracy of the highly twisted antiparallel β-structure, which has a spectral contribution similar to the disordered structure, has been improved from 0.043 to 0.025 with correlation improved from 0.87 to 0.96. Besides our own experiments, we collected CD spectra of peptides and proteins from the literature. This meant heavy work on the literature. We had to inspect and evaluate the reliability and quality of the published data. After rigorous filtering, the high quality reliable data were added to our database and used as reference spectra or target spectra in the method development.

With Ágnes Tantos, we started to study the role of disordered segments in nucleic acid binding proteins and continued the work on the function of disordered plant chaperone ERD14. We carried out SRCD experiments on these samples in SOLEIL in November, 2019.

We continued the collaboration with the group of Örs Legeza in order to develop the methodology for ab initio calculations of the CD spectra from the atomic resolution 3D structures. Our goal is the optimization of their quantum chemical DMRG-TCCSD method for our molecular system to calculate the electric and magnetic transition dipole momentum which are the basis of the rotatory strength that will provide the CD signal. The theoretical calculation of the CD spectra from the atomic coordinates of proteins is still an unresolved issue and our goal is the make an advancement in this filed.

We were invited to the 2nd Workshop on Intrinsically Disordered Proteins in Core Data Resources, (March 13-14, 2019, Prague) to give a lecture and discuss the perspectives of using CD spectroscopy for the investigation and validation of the disorder in peptides and proteins and make it a standard method. We attended the CD2019 Conference on Chirality (June, 2019, Pisa, Italy) where we presented our newest results and József Kardos gave a “cutting edge invited lecture”. In October, 2019 we participated the International Symposium on Disordered Proteins, Protein Folding, and Disease-causing Aggregation (KRIBB, Daejeon, Korea), József Kardos and András Micsonai were invited speakers. In December 2019, we attended the “Towards a cure for amyloid diseases: a successful example of precision and translational medicine, conference in Pavia, Italy with 3 posters.

We continued our collaboration with Dr. Lucio Colombi Ciacchi and co-workers (University of Bremen, Germany) in MD simulations using replica exchange and calculation of the CD spectra (yet using our empirical method) of oligopeptides and published a new paper (Michaelis et al., J. Phys. Chem. B, 2019. IF: 2.857).

We carried out CD measurements and MD simulations to study the structural background of the function of inhibitory peptides in collaboration with Gábor Pál and his group and published the results in the Journal of Molecular Biology (Boros et al., J. Mol. Biol. 2019. IF: 5.067).

Our KH 125597 grant was extended for 6 more months thanks to OTKA and the National Research, Development and Innovation Fund. In this period, we successfully published a paper on the ERD14 intrinsically disordered plant chaperon. The paper is a result of a huge joint work of several labs, our work was the structural characterization of ERD14 by CD spectroscopy and to prove its chaperon function under stress conditions (high temperature) by inhibiting the denaturation of its binding partner proteins (Murvai et al., Cells, 2020, IF: 4.366).

We compared the structure and conformational stability of human and malaria parasite calmodulins by CD spectroscopy. The goal of the experiments were the investigation if the malaria parasite calmodulin could be a drug target. Because of its high similarity to the human calmodulin and because CaM have essential cellular functions, we anticipate significant side effects in case of targeting calmodulin in a treatment. The work was published in FASEB BioAdvances (Juhász et al., FASEB BioAdvances, 2020).

We published a book chapter in the Methods in Molecular Biology, which is a protocol paper on the experimental work with CD spectroscopy and on the analysis of the CD spetra by BeStSel (Micsonai et al., Methods Mol. Biol. 2020).

Our Nucleic Acids Research paper on the new developments of BeStSel and case studies (Micsonai et al., Nucleic Acids Res. 46, W315-W322 (2018), received around 200 citations in two years and become highly cited in the Web of Science (top 1%) and for several months it won the “Hot Paper” title (top 0.1 %). This achievement meets the goals of the KH excellence grant that was declared to support internationally known and highly cited research projects.

Our manuscript on the new BeStSel method that is improved on disordered proteins is under preparation.

Publications:

1. Micsonai A, Wien F, Bulyaki E, Kun J, Moussong E, Lee YH, Goto Y, Refregiers M, Kardos J. BeStSel: a web server for accurate protein secondary structure prediction and fold recognition from the circular dichroism spectra. NUCLEIC ACIDS RESEARCH 46:(W1) pp. W315-W322. (2018)

2. Szabó B, Murvai N, Abukhairan R, Schád É, Kardos J, Szeder B, Buday L, Tantos Á. Disordered Regions of Mixed Lineage Leukemia 4 (MLL4) Protein Are Capable of RNA Binding. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 19 : 11 Paper: E3478 (2018)

3. Adachi M, Noji M, So M, Sasahara K, Kardos J, Naiki H, Goto Y. Aggregation-phase diagrams of β2-microglobulin reveal temperature and salt effects on competitive formation of amyloids versus amorphous aggregates. Journal of Biological Chemistry, 293:(38) pp.14775-14785 (2018)

4. Hildebrand N, Michaelis M, Wurzler N, Li Z, Hirst JD, Micsonai A, Kardos J, Gil-Ley A, Bussi G, Köppen S, Piane MD, and Ciacchi LC. Atomistic details of chymotrypsin conformational changes upon adsorption on silica. ACS BIOMATERIALS SCIENCE & ENGINEERING, accepted, manuscript ID: ab-2018-00819m.R1

5. Michaelis M, Hildebrand N, Meißner RH, Wurzler N, Li Z, Hirst JD, Micsonai A, Kardos J, Delle Piane M, Colombi Ciacchi L. Impact of the Conformational Variability of Oligopeptides on the Computational Prediction of Their CD Spectra. JOURNAL OF PHYSICAL CHEMISTRY B 123: 6694-6704 (2019)

6. Eszter Boros, Fanni Sebák, Dávid Héja, Dávid Szakács, Katalin Zboray, Gitta Schlosser, András Micsonai, József Kardos, Andrea Bodor and Gábor Pál. Directed evolution of canonical loops and their swapping between unrelated serine proteinase inhibitors disprove the interscaffolding additivity model. JOURNAL OF MOLECULAR BIOLOGY 431: 557–575 (2019)

7. Murvai, Nikoletta ; Kalmar, Lajos ; Szalaine Agoston, Bianka ; Szabo, Beata ; Tantos, Agnes ; Csikos, Gyorgy ; Micsonai, András ; Kardos, József ; Vertommen, Didier ; Nguyen, Phuong N. et al. Interplay of Structural Disorder and Short Binding Elements in the Cellular Chaperone Function of Plant Dehydrin ERD14. CELLS 9 : 8 Paper: 1856 (2020)

8. Tünde Juhász, József Kardos, Zsolt Dürvanger, Veronika Harmat, Károly Liliom. Comparison of ligand binding and conformational stability of human calmodulin with its homolog from the malaria parasite Plasmodium falciparum. FASEB BIOADVANCES, DOI: 10.1096/fba.2020-00013 (2020)

9. András Micsonai, Éva Bulyáki, and József Kardos. BeStSel: From Secondary Structure Analysis to Protein Fold Prediction by Circular Dichroism Spectroscopy. ch 11. in Methods in Molecular Biology, vol. 2199, DOI: 10.1007/978-1-0716-0892-0_11, Springer Science+Business Media, LLC, part of Springer Nature (2020)

Conferences:

1. Micsonai A, Murvai N, Bulyáki É, Szabó B, Wien F, Lee YH, Réfrégiers M, Goto Y, Tompa P, Han KH, Tantos Á, Kardos J. Improved structural estimation of disordered proteins by CD spectroscopy: Method development and application. BPS18 62nd Annulal Biophysical Meeting of Biophysical Society, San Francisco, February 17-21, 2018.

2. Micsonai A, Wien F, Kun J, Vadászi H, Réfrégiers, Kardos J. Protein fold recognition by circular dichroism spectroscopy. BPS18 62nd Annulal Biophysical Meeting of Biophysical Society, San Francisco, February 17-21, 2018.

3. Kardos & András Micsonai, 2nd Workshop on Intrinsically Disordered Proteins in Core Data Resources, March 13-14, 2019, Prague

4. András Micsonai, Nikoletta Murvai, Éva Moussong, Beáta Szabó, Frank Wien, Young-Ho Lee, Matthieu Réfrégiers, Yuji Goto, Péter Tompa, Kyou-Hoon Han, Ágnes Tantos, and József Kardos. Structural estimation of globular, disordered and aggregated proteins by CD spectroscopy: method development and application. 3th. Ulm Meeting Biophysics of Amyloid Formation. Ulm, Germany, 19-21 February 2019.

5. József Kardos, Éva Bulyáki, Judit Kun, Moussong Éva, Frank Wien, Young-Ho Lee, Yuji Goto, Matthieu Réfrégiers, András Micsonai. Improved structural characterization of protein aggregates and amyloid fibrils by CD spectroscopy. 3th. Ulm Meeting Biophysics of Amyloid Formation. Ulm, Germany, 19-21 February 2019

6. András Micsonai, Frank Wien, Éva Moussong, Éva Bulyaki, Judit Kun, Beáta Szabó, Nikolett Murvai, Péter Tompa, Young-Ho Lee, Kyou-Hoon Han, Yuji Goto, Ágnes Tantos, Matthieu and József Kardos. New perspectives in the structural analysis of proteins by ECD spectroscopy. CD2019, 17th International Conference on Chiroptical Spectroscopy 23-27 June 2019, Pisa, Italy

7. András Micsonai, Frank Wien, Éva Moussong, Éva Bulyaki, Judit Kun, Beáta Szabó, Nikolett Murvai, Péter Tompa, Young-Ho Lee, Kyou-Hoon Han, Yuji Goto, Ágnes Tantos, Matthieu

Réfrégiers and József Kardos. BeStSel: a web server for improved protein secondary structure estimation and fold prediction from the ECD spectra. CD2019, 17th International Conference on Chiroptical Spectroscopy 23-27 June 2019, Pisa, Italy

8. József Kardos. New perspectives in CD spectroscopy: from secondary structure to the protein fold. An international cutting-edge network for the study of protein aggregation, Workshop, 19 July, 2019, Osaka University, Osaka, Japan

9. András Micsonai, Éva Moussong, Dominik Mátyás, Frank Wien, Young-Ho Lee, Yuji Goto, Péter Tompa, Kyou-Hoon Han, Ágnes Tantos, Matthieu Réfrégiers, and József Kardos. Secondary structure determination of amyloid fibrils and disordered proteins by CD spectroscopy. International Symposium on Disordered Proteins, Protein Folding, and Disease-causing Aggregation. KRIBB, Daejeon, Korea, 22-26 October 2019.

10. András Micsonai, Éva Moussong, Dominik Mátyás, Frank Wien, Young-Ho Lee, Yuji Goto, Matthieu Réfrégiers, and József Kardos. Protein fold prediction and experiment-based homology modelling by CD spectroscopy. International Symposium on Disordered Proteins, Protein Folding, and Disease-causing Aggregation. KRIBB, Daejeon, Korea, 22-26 October 2019.

11. Éva Bulyáki, Judit Kun, András Micsonai, Tamás Molnár, Alexandra Papp, Keiichi Yamaguchi Masatomo So, Young-Ho Lee, Mihály Józsi, Yuji Goto, József Kardos. Understanding the mechanism of amyloid formation of Asp76Asn variant β2-microglobulin. TOWARDS A CURE FOR AMYLOID DISEASES: A SUCCESSFUL EXAMPLE OF PRECISION AND TRANSLATIONAL MEDICINE, 16-19 DECEMBER 2019 PALAZZO VISTARINO – PAVIA, ITALY

12. Judit Kun, Éva Bulyáki, Henrietta Vadászi, András Micsonai, Tamás Molnár, Attila Kovács, Balázs Györffy, Vilmos Módos-Tóth, Frank Wien, Katalin Adrienna Kékesi, József Kardos. Investigating the Structural Diversity of Amyloid-β Peptide Aggregates by Circular Dichroism Spectroscopy. TOWARDS A CURE FOR AMYLOID DISEASES: A SUCCESSFUL EXAMPLE OF PRECISION AND TRANSLATIONAL MEDICINE, 16-19 DECEMBER 2019 PALAZZO VISTARINO – PAVIA, ITALY

13. András Micsonai, Frank Wien, Éva Moussong, Éva Bulyáki, Judit Kun, Young-Ho Lee, Yuji Goto, Matthieu Réfrégiers and József Kardos. BeStSel: a web server for improved structural characterization of protein aggregates and amyloid fibrils by CD spectroscopy. TOWARDS A CURE FOR AMYLOID DISEASES: A SUCCESSFUL EXAMPLE OF PRECISION AND TRANSLATIONAL MEDICINE, 16-19 DECEMBER 2019 PALAZZO VISTARINO – PAVIA, ITALY