Closing Report on the NKFI/OTKA grant K112716 (2015-2019) entitled

A new combined spectroscopic and molecular modelling approach to folding and assembly of membrane proteins

I. Overview of the project

Our long-term prime objective is to improve basic fold and then structure prediction of proteins in general and membrane proteins in particular because of the well-known challenges and shortcomings of classical structure biology especially on membrane proteins. Our proposed approach focuses on the following novelty elements: Combination of (*i*) environment-aware and statistically significant information from known structures and (*ii*) low resolution, but functionally relevant experimental data on the target proteins. These data then serve as constraints in structure prediction at a reduced resolution, via (*iii*) new fold generators inspired by the relevant biological folding process. To achieve this it requires new ways of analysing existing protein structures, modifying folding engines to satisfy all constraints, measurement of functionally relevant data and, if possible, studying the folding and un-folding of the target proteins. Certainly, it is a long way and it requires a lot of man-month efforts to develop such a new protein structure prediction platform, and the field is very competitive, but we made significant progress, despite lack of sufficient human resources. Indeed, the main reason for why we could not strictly follow our original work plan is that we found no suitable young researcher, physicists or chemists, to work on the theoretical tasks. (Only in the end of 2019 joined a new B.Sc student to the project, and in the beginning of 2020 joined a Japanese postdoc, both to work on the theory.) The unexpected problem with the human resources lead us to change focus: (*i*) We made more experiments and less theoretical work than planned. (*ii*) Making use of our expertise in membrane biophysics and spectroscopic techniques we helped other projects to an extent that was higher than in previous years and higher than planned.

Our main selected proteins were representatives of the two main types, alpha-helix bundles and beta-barrel, of trans-membrane proteins, namely the vacuolar proton-ATPase protein complex, an enzyme (and some of its subunits) and the FomA pore protein of the outer membrane of *E.coli*, respectively. Although not planned, we also included a soluble protein, lysozyme, to be studied because its folding is well known but its interaction with membranes is not known, although it could be biologically relevant. All the proteins are important in their own right not only from the point of view of protein folding and structure prediction. In the case of V-ATPase, after refining our yeast culturing and vacuole purification system, we had to prove that V-ATPase is functional in our preparations, to ensure the functional relevance of structural data to be gathered. Therefore, we made ATPase activity measurements, involving the development and refinement of a real-time kinetic system. We have also shown that this enzyme gives most of the ATPase activity in these membranes and it makes rotation, hence pumps protons. We published two strong papers from these experiments. Most recently, we made the first structural studies on intact native V-ATPase with electron paramagnetic resonance (EPR) spectroscopy of spin-labelled lipids. As concerns the beta-barrel FomA we studied its secondary structure and the kinetics of its un-folding and re-folding. These data will be important in designing assisted molecular dynamics algorithms for fold prediction.

On the theory side, we wrote a review on protein folding, with focus on the experimental and theoretical approaches. Based on our results, and in line with our overall spectroscopy-based

strategy, we also published a book chapter on how to use EPR spectroscopy of spin-labelled lipids to gain functionally relevant structural data on membrane proteins that serve as constraints in their modelling. We have set up a new computer cluster and made the first steps in the structure prediction of selected proteins of the *ductin* family, which subunit *c* of V-ATPase also belongs to. Witnessing the great success of most recent (past 1-2 years) prediction efforts based on machine learning and neuronal network approaches, since last December we are now heavily working on readapting our plans for a prediction pipeline to include these latest developments.

We are proud that, realising our promise, 6 new young researchers could participate in this project and 4 dissertations (2 B.Sc, 1 M.Sc, 1 ITC) were written from their works, and all of them received their respective degree with best marks. It should be also noted that the main techniques (for protein folding) used in this project were subjects of the teaching course (talk and laboratory practical demonstration) of the International Training Course of the Biological Research Centre.

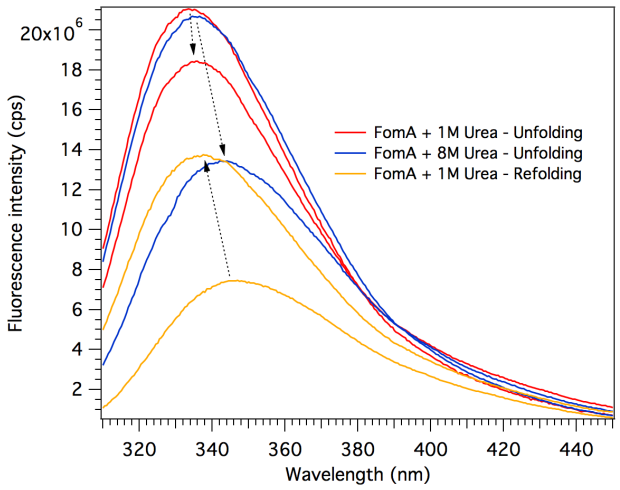
Below are detailed descriptions of our experimental and theoretical work followed by a short summary of why and how we served other projects with relevant expertise and methods, yielding additional publications. References to our new results are underlined. The experiments that have not yet been published are described in greater detail than the published ones.

II. Experimental structure-function studies on membrane proteins

Beta-barrel protein FomA

Pore proteins of the outer membrane of *E.coli* belong to an important class of trans-membrane structural forms: beta-barrels [Tamm et al., 2001, 2004]. The slow kinetics of their folding from solvent into membrane [Kleinschmidt and Tamm, 1996] makes them ideal subjects of folding studies [Pocanschi et al., 2006]. Although many of the membrane-spanning proteins possess a β-barrel structure, much less is known about this class of proteins than about trans-membrane helix bundle proteins. Studies have revealed the versatility and ubiquity of the β-barrel membrane proteins [Schulz, 2000]. Although they have many common structural features, beta-barrel proteins carry out diverse functions in diverse organisms. OmpA (outer membrane protein of *E. coli*) is a well-known member of the family, which is often used as a model in folding studies. Recently, Pocanschi et al. successfully performed equilibrium unfolding titrations with OmpA to determine the free energy of unfolding and the stability of the protein [Pocanschi et al., 2013]. We were working on FomA, which is much less characterised. It is the major outer membrane protein of *Fusobacterium nucleatum*. It is a voltage-dependent general diffusion porin possessing a trans-membrane β-barrel domain that is thought to consist of 14 β-strands.

FomA was purified by our parter (J.H. Kleinschmidt, Univ. of Kassel, Germany). It was expressed in form of cytoplasmic inclusion bodies in *E. coli*. The purification procedure yielded un-folded FomA, which was refolded in a refolding buffer (10mM borate buffer, 2mM EDTA, pH 10.0) containing LDAO detergent in 1000 fold molar excess. Refolded samples were pooled and concentrated. DSC measurements were performed using a VP-DSC calorimeter, with 0.5 mL sample and reference cells, from MicroCal, LLC (Northampton, MA). Samples were scanned between 25 and 110 °C, at a rate of 0.5 °C/min. Immediately after completion of the heating scan, a down-scan from 110 to 25 °C was performed at the same rate. Reversibility and repeatability of the unfolding process were checked by subsequent up- and down-scans. Since the protein contains



tryptophans, which are sensitive to the hydrophobicity of their environment, we used Trp fluorescence to follow structural changes of the protein upon unfolding. Fluorescence spectra were measured with our Fluorolog FL3-22 spectro-fluorometer (HORIBA Jobin-Yvon). The excitation wavelength was 295 nm, the bandwidths of the excitation and emission monochromators were 2.5 and 5 nm, respectively. The measurements were carried out using a thermostated cuvette holder and a computer-controlled water bath. For background, spectra of the refolding buffer without FomA were used in each case.

First we have proven with CD spectroscopy that the secondary structure of the folded FomA is indeed beta barrel. We could also identify the folded and unfolded form by gel-electrophoresis similarly how it was done with OmpA [Pocanschi et al., 2006].
Then our goal was to study the stability and unfolding of FomA with fluorescence spectroscopy, and to compare the results with our calorimetric data. Denaturing agents like urea and guanidine hydrochloride are often used to study protein stability, and the molecular mechanism and dynamics of protein folding and unfolding processes, so we have also investigated the effects of urea on the structure and stability of the protein. The (yet unpublished) **figure above** shows how the Trp peak shifts upon unfolding and refolding of FomA. Our main observations [Kóta et al., 2015, 2016; Kozák, 2017]: The thermal unfolding of FomA occurs around 85 °C as shown by both our calorimetric and spectroscopic data. The thermal unfolding of FomA is irreversible. The protein (similarly to OmpA) is very stable. Unfolding of FomA seems to be very slow even at a high urea concentration (6-7M), the unfolding is not completed after 17 days incubation at 40 °C. The urea-induced unfolding is reversible, FomA can be refolded by dilution of the urea-unfolded protein, and this process is much faster. These results serve as new data to take biological folding into account in structure prediction, but are best to be complemented with kinetic studies of the folding into lipid membranes. We are working on a new manuscript to publish the above results.

The vacuolar proton ATPase (V-ATPase)

Rotary enzymes are complex, highly challenging biomolecular machines whose biochemical working mechanism involves inter-subunit rotation. The true intrinsic rate of rotation of any rotary enzyme is not known in a native, unmodified state. V-ATPase hydrolyses ATP and pumps protons across biomembranes [Nishi and Forgac, 2002; Beyenbach and Wieczorek, 2006]. ATP hydrolysis and proton pumping are coupled via the rotary mechanism [Grabe, 2000; Imamura et al., 2003; Sun-Wada et al., 2003; Ferencz et al., 2013]. Because of its size and complexity [Finbow and Harrison, 1997; Stevens and Forgac, 1997], understanding the details of structure-function relationship of this membrane-bound molecular machinery is one of the major challenges in biophysics today [Cotter et al., 2015]. Subunit *c* of the Vo domain is very important for proton translocation [see, e.g., Nishi and Forgac, 2002]. It is of great interest under what conditions subunit *c* monomers assemble into a ring and whether the stator (subunit *a*) has an effect on this process. In a very well cited paper we have identified the ductins family [Holzenburg et al., 1993]. Some members of this family function

as passive proton channel, gap junction or neurotransmitter release pore in certain systems [Finbow and Harrison, 1997].

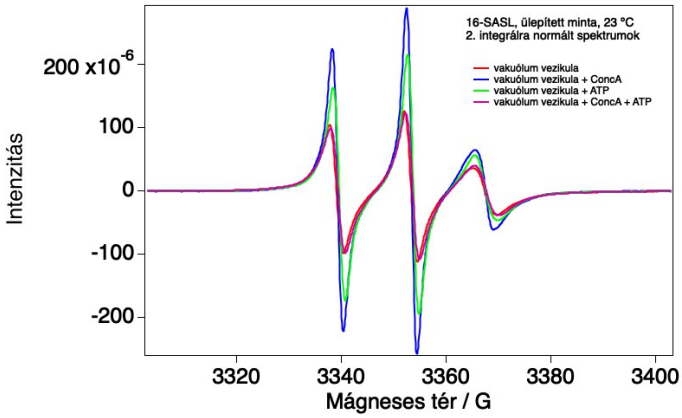
First, we aimed at structural studies on the whole native enzyme because we had a yeast vacuolar membrane vesicle system hosting high concentration of V-ATPase. We wanted to prove that the enzyme is working properly in these membranes (consumes ATP, makes rotation and consequently transfers proton – rotation without proton transfer is not possible due to the strong coupling mechanism), so that any new structural data will be functionally relevant.

We have optimised our spectrophotometric NADH-driven coupling enzyme system rendering real-time measurement of V-ATPase activity possible [Knódel, 2019]. This method, which is a further developed version of that of [Palmgren, 1990], provides more information with smaller error than the previous end-point assay, as it is based on spectrophotometric measurement of the NADH concentration used by a coupling enzyme system that re-synthesises ADP and inorganic phosphate, from ATPase activity, to ATP. It is much faster than the ATP hydrolysis by V-ATPase. We have developed optimal conditions to continuously monitor the effects of several changed measurement conditions. The calibration and optimisation of the system involved phosphate and protein assays, checking concentration of the proteins, substrates and the constituents of the buffer, pH, reaction time. An unexpected observation was that the effect of the potent and specific V-ATPase inhibitor depends on whether it is administered to working or standby V-ATPase. This observation demands further experiments before publication.

Unfortunately, NADH got electrolysed under voltages that we used to test the rotation in the enzyme (see below). For this reason, we had to test NADH analogues. Synthetic NADH analogues with a dihydropyridine structure also serve as the sources of two electrons and one proton, which can be transferred via three different reaction pathways: direct hydride transfer [Zhu et al. 1999; Zhao et al., 2000] electron transfer followed by hydrogen atom transfer [Cheng and Lu, 1997] and electron–proton–electron transfer. [Zhu et al., 2003] This oxidation mechanism was already investigated sixty years ago [Mauzerall and F. H. Westheimer; 1955] but despite considerable experimental and thermodynamic calculation efforts the characterisation of the free radical reactions has not been completed yet. Therefore, as a side project, we have carried out a detailed EPR study to investigate the free radical reactions of an NADH analogue [Sebők-Nagy, 2016a,b, 2018]. For the spectrum analysis, we have improved our algorithm for fitting EPR spectra of free radicals and spin labels in solution. Apart from using NADH (analogue) as a tool in kinetic studies of V-ATPase activity, its electron-proton-electron transfer reaction might lead to observation of a specific interaction with V-ATPase under certain conditions.

Next, we used the effect of an oscillating electric (AC) field on the biochemical activity of a rotary enzyme (for the first time), the vacuolar proton-ATPase (V-ATPase), to directly measure its mean rate of rotation in its native membrane environment, without any genetic, chemical or mechanical modification of the enzyme, also for the first time [Ferencz et al., 2017, 2017a]. The results suggest that a transmembrane AC field is able to synchronise the steps of ion-pumping in individual enzymes via a hold-and-release mechanism, which opens up the possibility of biotechnological exploitation and might serve as a tool in structure-function studies. Our approach is likely to work for other transmembrane ion-transporting assemblies, not only rotary enzymes, to determine intrinsic in situ rates of ion pumping. All co-authors of this paper, published in a Nature Group journal, were researchers of our Institute and with one exception also members of my group. Financial support was only form Hungarian grants. The on-line publication (https://

www.nature.com/articles/srep45309) contains animation videos about the rotary mechanism of the V-ATPase and effect of oscillating electric field on it. Some of the videos are also available on youtube (https://youtu.be/A2PdDLJWiMY , https://youtu.be/XbReI5Qi-30) and they had so far ~4900 views collectively. In addition, this paper was a cover story of the web page of the Hungarian Academy of Sciences, it also received the Qualitas Biologica prize (2nd position) from the Biological Research Centre.

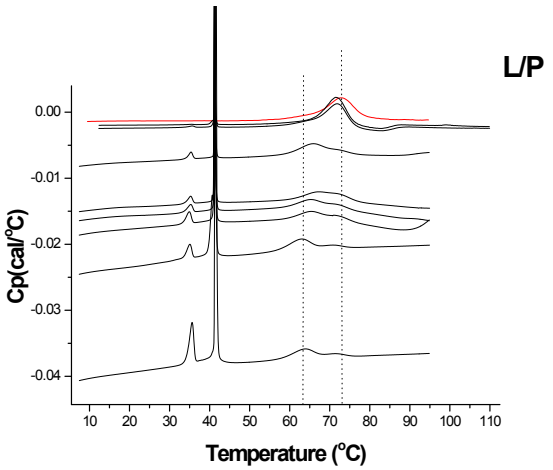


The above experiments prove that the V-ATPase is active in these vacuolar vesicle preparations: it consumes ATP, its rotor rotates hence and it pumps protons.
Most recently, also as a side project, we used non-steady waveforms such as noise or even music to which the enzyme showed great sensitivity. The manuscript has been submitted to Advanced Science (which has an impact factor of ~15). The coupling enzyme system will render future kinetic structure-function measurements possible using fluorescent and spin labels. Thanks to our results on V-ATPase, my group joined to the EU COST Action CM1306 "Understanding Movement and Mechanism in Molecular Machines", and I also presented a talk on spectroscopy-based structural biology [Páli, 2015] and on V-ATPase [Páli, 2018].

After proving the V-ATPase is functional in our preparations we started EPR experiments using spin-labelled lipids to gain functionally relevant structural information on the intra-membrane organisation of the whole enzyme. According to our first results the spectrum of the first-shell lipids is different in the case of a standby and a working engine. The **figure above** shows how the EPR spectrum of the 16-SASL spin label, that is sampling the centre of the bilayer, changes under different working conditions of the enzyme (unpublished result). If we can determine the concentration of the immobile, first-shell lipids it will give information about intramembranous surface of the enzyme, which serves as an additional constraint for structure prediction. This work is in progress. As a most promising achievement, our partner group (Gábor Juhász, Momentum Group) informed us in December that they could express subunit *c* of the Vo (membrane) domain of V-ATPase and there is a good chance that they can also express subunit *a* of Vo, and single point mutants of both proteins for spin-labelling work. These two subunits are among our single protein targets for structure prediction, folding and structure-function studies. As soon as we get these purified proteins we will start spin-label EPR studies on their folding and assembly in membranes.

Lysozyme

We continued previous (yet unpublished) work on a water soluble protein, lysozyme. The reason is that we have made the surprising observation that its thermal folding and unfolding is affected by lipid membrane and lysozyme affects lipid phase transitions. Lysozyme is an antibacterial enzyme that hydrolyses the cell wall of bacteria. Therefore its interaction with the model and biomembranes may very well be of strong biological and even medical relevance. Since the folding of lysozyme is well studied in water [see, e.g., Mallamace et al., 2018] our studies on lysozyme-membrane interaction could provide valuable data on the first steps of folding of membrane proteins from the



unfolded state in the aqueous phase into a lipid bilayer. The set of differential scanning calorimetry (DSC) thermograms on the **figure on the left** (with curves from top to bottom representing low to high lipid/protein ratio) shows that the enzyme and the lipid membrane mutually affect their phase transition (unpublished result). Recently, we carried out further DSC experiments and we made the following observations: the presence of lipids decreased the reversibility of thermal un-folding of lysozyme, and this effect was larger in the case of lipids with negatively charged head-group [Kertész, 2019].

III. Theoretical work

Reviews

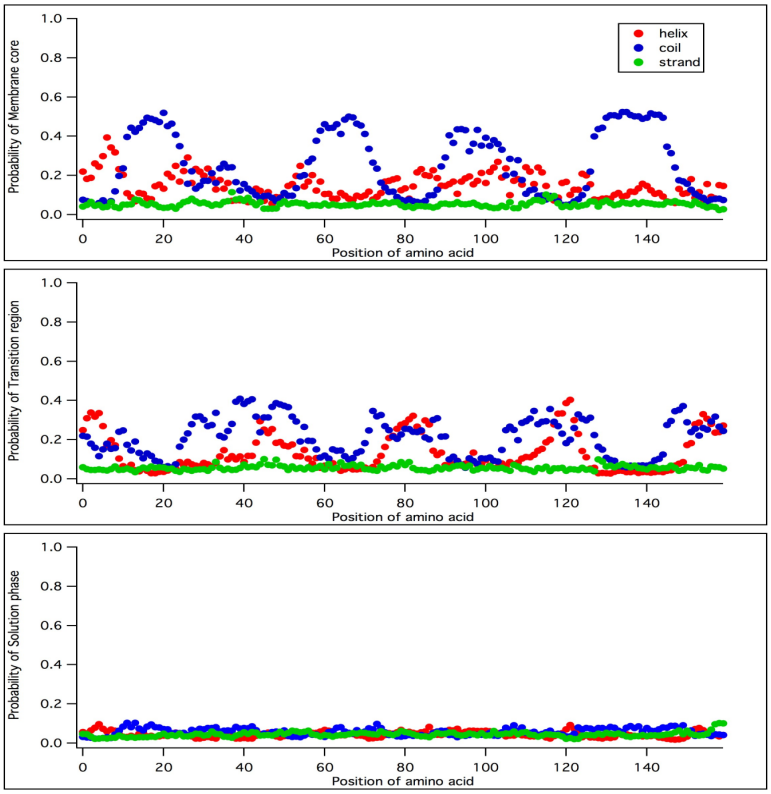
In agreement with our plans we studied literature of protein folding with special focus on experimental and theoretical approaches. Understanding the details of the molecular mechanisms responsible for protein folding is one of the demanding queries in molecular biology today. Studying protein folding is strongly linked with studying protein structure, i.e. structure biology, of proteins under different conditions. In this review we first describe the basic principles of protein folding and structure biology then muster experimental and theoretical approaches to the problem. This review was published in a BRC booklet [Páli et al., 2015], and we are writing another review on the state-of-the-art of knowledge- and constraint-based protein structure prediction methods. We have also contributed to an e-learning material of Szeged University on ATPases [Páli, 2015; Sebők-Nagy and Páli, 2015a,b].

Based on our previous and recent results [see, e.g., Kóta et al., 2002; Marsh and Páli, 2004; Páli et al., 2003 for some of our strongly cited papers in the field] we now published a book chapter on how to use EPR spectroscopy of spin-labelled lipids to gain functionally relevant structural data on membrane proteins that serve as constraints in their modelling [Páli and Kóta, 2019]. Spin-label EPR spectroscopy is the technique of choice to characterise the protein solvating lipid shell in its highly dynamic nature, because the EPR spectra of lipids that are spin-labeled close to the terminal methyl end of their acyl chains display two spectral components, those corresponding to lipids directly contacting the protein and those corresponding to lipids in the bulk fluid bilayer regions of the membrane. In our chapter, spin label EPR procedures are presented that allow determination of the stoichiometry of interaction of spin-labeled lipids with the intramembranous region of membrane proteins or polypeptides, as well as the association constant of the spin-labeled lipid with respect to the host lipid. Stoichiometry and selectivity are directly related to the structure of the intramembranous sections of membrane-associated proteins or polypeptides and can be used to study the state of assembly of such proteins in the membrane. In our chapter we focus on how to attach spin labels to model membranes and biomembranes and how to measure and analyse the two-component EPR spectra of spin-labeled lipids in phospholipid bilayers that contain proteins or polypeptides. We also present the reader with computational procedures for determining the molar

fraction of motionally restricted lipids. The interpretation of the data is discussed briefly, as well as other relevant and recent spin label EPR techniques for studying lipid–protein interactions, not only from the point of view of lipid chain dynamics.

Protein structure prediction pipelines

According to our original plan, we worked on modelling of subunit *c* of V-ATPase by computational methods, which implicate multiple sequence alignment and secondary structure prediction methods as first steps. A high-quality model of secondary structure and topology are needed for experimental structure-function studies, and can be a starting point for attempts to model the 3D structure before molecular dynamics or simulated annealing simulations. We studied both Rosetta [see, e.g., Simons et al., 1999; Kaufmann et al., 2010] and BCL:Fold [Karakas et al., 2012; Heinze et al., 2015] suite of software tools for protein structure prediction because they are the key part of a recent promising protein prediction pipeline [Fisher et al., 2016]. We have set up our own computer cluster (with 8 core units) and installed both Rosetta and BCL:Fold. Using the latter suite, we made the homology analysis of the *ductin* family, which subunit *c* of both V-ATPase and F-ATPase belong to [Holzenburg et al., 1993]. Ten sequences from the family were studied with a complex method by BCL::Align and BCL::JUFO, to serve more accurate data than earlier. We predicted the fully conserved regions and strongly and weakly similar regions as well as the most likely secondary structure forms for all the selected proteins by the combination of neural network based on the position specific scoring matrices generated by PSIBLAST during the homology search. The **figure on the right** shows a typical output of the analysis. These results have so far been presented as an ITC dissertation of the Biological Research Centre [Sahu, 2018].



In the meanwhile, we discovered that most recent prediction efforts based on deep machine learning and neuronal network approaches have demonstrated great success [Xu, 2019; Senior et al., 2020]. Therefore, instead of exclusively going on the Rosetta-BCL:Fold route, we started to work on our own structure prediction pipeline to be based on neuronal network and machine learning approach. Currently, we are evaluating which neural network and folding engine to use, and the best candidates are the X-PLOR-based CNS and Alphafold, mentioned in the references from 2019 and 2020. We plan to implement several novelty elements, taking into account: the biological and physical folding process, functional relevance of experimental and knowledge-based data, environment-awareness, inclusion of all possible structural constraints.

IV. Additional work

Our group has an internationally recognised expertise in membrane biophysics [Bashtovyy et al., 2003; Dér et al., 2007; Páli and Szalontai, 2013], lipid-protein interactions [Kóta et al., 2002; Marsh and Páli, 2004; Páli et al., 2003] and spin-labelling and spin-trapping EPR spectroscopy [Csonka et al., 1999; Páli et al., 1995, 1999], and we run well-maintained modern spectroscopic techniques, including unique ones such as the best equipped X-band CW/Pulsed EPR spectrometer in Central and East Europe [Sebők-Nagy and Páli, 2017, 2018], as well as biomembrane preparation protocols. As such, we are expected and are also always willing to help when other projects need our expertise. Certainly, such joint experiments take some time of our group members and research infrastructure but are nevertheless strongly relevant topically and/or methodologically to our main projects. In two published studies we helped with lipid liposome preparation techniques and fluorescence spectroscopy [Mészáros et al., 2018; Veszelka et al., 2017]. A third study [Dancs et al., 2017] and a review [Csonka et al., 2015] were also published, to which we contributed with EPR measurements of free radicals and with our experience in spin-trapping EPR spectroscopy, respectively. In 2019, we started a new collaboration with the László Vigh Group (Institute of Biochemistry BRC) on the interaction of chaperon Hsp27 protein with membranes, using similar techniques.

Results: papers, *in extenso* publications
(Team members who worked on this project are underlined.)

Csonka C, Páli T, Bencsik P, Görbe A, Ferdinandy P, Csont T: Measurement of NO in biological samples, BRITISH JOURNAL OF PHARMACOLOGY 172(6), 1620-1632, 2015. https://bpspubs.onlinelibrary.wiley.com/doi/epdf/10.1111/bph.12832

Dancs Á, May NV, Selmeczi K, Darula Z, Szorcsik A, Matyuska F, Páli T, Gajda T: Tuning the coordination properties of multi-histidine peptides by using a tripodal scaffold: solution chemical study and catechol oxidase mimicking, NEW JOURNAL OF CHEMISTRY 41(2), 808-823, 2017. https://pubs.rsc.org/en/content/articlelanding/2017/nj/c6nj03126a/unauth#!divAbstract

Ferencz CM, Petrovszki P, Dér A, Sebők-Nagy K, Kóta Z, Páli T: Oscillating Electric Field Measures the Rotation Rate in a Native Rotary Enzyme., SCIENTIFC REPORTS 7, 45309, 2017. https://www.nature.com/articles/srep45309

Kertész D: A lizozim fehérje lipid membránokkal való kölcsönhatásának biofizikai vizsgálata, B.Sc dissertation, Szegedi Tudományegyetem, Természettudományi és Informatikai Kar és Szegedi Biológiai Kutatóközpont, 2019.

Knódel P: A vakuoláris proton-atpáz aktivitásának valósidejű mérése élesztő vakuólum eredetű lipid vezikulákban, B.Sc dissertation, Szegedi Tudományegyetem, Mérnöki Kar és Szegedi Biológiai Kutatóközpont, 2019.

Kozák V: A FomA fehérje stabilitásának és gombolyodásának vizsgálata biofizikai módszerekkel, M.Sc diploma dissertation, Szegedi Tudományegyetem, Természettudományi és Informatikai Kar és Szegedi Biológiai Kutatóközpont, 2017.

Páli T: 3. Non-SLC transporters, 3.3 Pumps (ATP-ase), 3.3.2. V-type, In: Rakonczay, Z; Hegyi, P (szerk.) Transporter, channel, membrane diseases, Szegedi Tudományegyetem (2015) e-document, 2015. (on-line teaching material)

Páli T, Kóta Z: Studying Lipid–Protein Interactions with Electron Paramagnetic Resonance Spectroscopy of Spin-Labeled Lipids, In: Methods in Molecular Biology, HUMANA PRESS INC. (2019), 529-561, 2019.
https://www.amazon.com/Lipid-Protein-Interactions-Methods-Protocols-Molecular/dp/1493995111

Mészáros M, Porkoláb G, Kiss L, Pilbat AM, Kóta Z, Kupihar Z, Keri A, Galbacs G, Siklós L, Tóth A, Fülöp L, Csete M, Sípos A, Hulper P, Sípos P, Páli T, Rákhely G, Szabó-Révész P, Deli MA, Veszelka S: Niosomes decorated with dual ligands targeting brain endothelial transporters increase cargo penetration across the blood-brain barrier., EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 123, 228-240, 2018.
https://www.sciencedirect.com/science/article/pii/S0928098718303403?via%3Dihub

Páli T, Sebők-Nagy K. Kóta Z: Protein folding, In: Vágvölgyi Cs, Siklós L (szerk.), Selected Topics from Contemporary Experimental Biology, Volume 2. 288 p. Szeged: MTA Szegedi Biológiai Központ, 2015, 189-208, 2015.
http://www.brc.hu/file/documents/itc/BRC_2015_Vol_2.pdf

Sahu B: A molecular modelling approach to folding and assembly of membrane proteins, International Training Course diploma work, Biological Research Centre, 2018.

Sebők-Nagy K, Páli T: 3. Non-SLC transporters, 3.3 Pumps (ATP-ase), P-type, In: Rakonczay, Z; Hegyi, P (szerk.) Transporter, channel, membrane diseases, Szegedi Tudományegyetem (2015) e. document, 2015a. (on-line teaching material)

Sebők-Nagy K, Páli T: 3. Non-SLC transporters, 3.3 Pumps (ATP-ase), 3.3.3. F-type, In: Rakonczay, Z; Hegyi, P (szerk.) Transporter, channel, membrane diseases, Szegedi Tudományegyetem (2015) e. document, 2015b. (on-line teaching material)

Sebők-Nagy K, Rózsár D, Puskás LG, Balázs Á, Páli T: Electron paramagnetic resonance spectroscopic studies of the electron transfer reaction of Hantzsch ester and a pyrylium salt., RSC ADVANCES 8, 29924-29927, 2018.
https://pubs.rsc.org/en/content/articlehtml/2018/RA/C8RA05693E

Veszelka S, Mészáros M, Kiss L, Kóta Z, Páli T, Hoyk Z, Bozsó Z, Fülöp L, Tóth A, Rákhely G, Deli MA: Biotin and glutathione targeting of solid nanoparticles to cross human brain endothelial cells, CURRENT PHARMACEUTICAL DESIGN 23(28), 4198-4205, 2017.
http://publicatio.bibl.u-szeged.hu/12607/1/Veszelka_CPD_2017_proof_.pdf

Results: conference presentations
(Team members who worked on this project are underlined.)

Ferencz Cs-M, Petrovszki P, Dér A, Sebők-Nagy K, Kóta Z, Páli T: Natív forgó ATPáz működése oszcilláló elektromos térben, A Magyar Biofizikai Társaság XXVI. Kongresszusa, MTA Szegedi Biológiai Kutatóközpont, Szeged, August, 2017a (invited talk, T.P.).

Kóta Z, Talmon E, Kleinschmidt JH, Páli T: Stability and unfolding of FomA, a β-barrel membrane protein, studied by fluorescence spectroscopy, 16th European Conference on the Spectroscopy of Biological Molecules (ECSBM), Bochum, Germany, September 6-10, 2015, 2015 (poster).

Kóta Z, Talmon E, Kleinschmidt JH, Páli T: Stability and unfolding of FomA, a β-barrel membrane protein, studied by fluorescence spectroscopy, Straub Days 2016, Biological Research Centre, May 25-26, 2016, 2016 (poster).

Páli T: Functional structure biology of biomembranes and membrane proteins, Research Seminar at Institute of Integrative Biology, Life Sciences Building, University of Liverpool, July 6, 2015, 2015 (invited talk, T.P.).

Páli T: The Rotary Mechanism of the Vacuolar Proton-ATPase., Workshop on Autophagy in model organisms, Eötvös Loránd University, Budapest (Hungary), September 6-8, 2018 (invited talk, T.P.).

Sebők-Nagy K, Páli T: Az impulzus üzemmódú Fourier-transzformációs elektron paramágneses rezonancia spektroszkópia lehetőségei, A Magyar Biofizikai Társaság XXVI. Kongresszusa (MBFT), MTA SZBK, Szeged, Hungary, 22-25th August 2017 (poster).

Sebők-Nagy K, Páli T: Az impulzus üzemmódú Fourier-transzformációs elektron paramágneses rezonancia spektroszkópia lehetőségei., Straub Days 2018, Biological Research Centre, May 10-11, 2018 (poster).

Sebők-Nagy K, Rozsár D, Balázs A, Puskás LG, Páli T: Electron paramagnetic resonance studies of radical reactions of a NADH analogue, Straub Days 2016a, Biological Research Centre, May 25-26, 2016a (poster).

Sebők-Nagy K, Rozsár D, Balázs A, Puskás LG, Páli T: Electron paramagnetic resonance studies of radical reactions of a NADH analogue, Regional Biophysical Conference (RBC2016), Trieste, Italy, 25-28th August 2016b (poster).

References

Bashtovyy D, Bérczi A, Asard H, Páli T: Structure prediction for the di-heme cytochrome b 561 protein family, Protoplasma 221 (1-2), 31-40, 2003.

Beyenbach KW, Wieczorek H: The V-type H+-ATPase: molecular structure and function, physiological roles and regulation, J Exp Biol 209, 577-589, 2006.

Cheng J-P, Lu Y: J Phys Org Chem, 10, 577–584, 1997.

Cotter K, Stransky L, McGuire C, Forgac M: Recent Insights into the Structure, Regulation, and Function of the V-ATPases, Trends Biochem Sci 40, 611-622, 2015.

Csonka C, Szilvássy Z, Fülöp F, Páli T, Blasig IE, Tosaki A, Schulz R, et al.: Classic preconditioning decreases the harmful accumulation of nitric oxide during ischemia and reperfusion in rat hearts, Circulation 100 (22), 2260-2266, 1999.

Dér A, Kelemen L, Fabian L, Taneva SG, Fodor E, Pali T, Cupane A, et al.: Interfacial water structure controls protein conformation, The Journal of Physical Chemistry B 111 (19), 5344-5350, 2007.

Ferencz C, Petrovszki P, Kóta Z, Fodor-Ayaydin E, Haracska L, Bóta A, Varga Z, Dér A, Marsh D, Páli T: Estimating the rotation rate in the vacuolar proton-ATPase in native yeast vacuolar membranes. European Biophysics Journal 42, 147–158, 2013.

Finbow ME, Harrison MA: The vacuolar H+-ATPase: a universal proton pump of eukaryotes. Biochem J. 324: 697-712, 1997.

Fischer AW, Heinze S, Putnam DK, Li B, Pino JC, Xia Y, Lopez CF, Meiler J: CASP11–An Evaluation of a Modular BCL::Fold – Based Protein Structure Prediction Pipeline. Plos One 11(4): 1371, 2016.

Grabe M, Wang H, Oster G: The mechanochemistry of V-ATPase proton pumps. Biophysical Journal, 78(6), 2798-2813, 2000.

Heinze S, Putnam DK, Fischer AW, Kohlmann T, Weiner BE, Meiler J: CASP10-BCL::Fold efficiently samples topologies of large proteins. Proteins Struct Funct Bioinform 83, 547–563, 2015.

Holzenburg A, Jones PC, Franklin T, Páli T, Heimburg T, Marsh D, Findlay JBC, Finbow ME: Evidence for a Common Structure for a Class of Membrane Channels, European Journal of Biochemistry 213(1), 21-30, 1993.

Imamura H, Nakano M, Noji H, Muneyuki E, Ohkuma S, Yoshida M, Yokoyama K: Evidence for rotation of V1-ATPase. Proc Natl Acad Sci USA, 100(5), 2312-2315, 2003.

Karakaş M, Woetzel N, Staritzbichler R, Alexander N, Weiner BE, Meiler J.: BCL::Fold—De Novo Prediction of Complex and Large Protein Topologies by Assembly of Secondary Structure Elements, PLoS One 7, e49240, pmid:23173050, 2012.

Kaufmann KW, Lemmon GH, DeLuca SL, Sheehan JH, Meiler J: Practically useful: What the Rosetta protein modeling suite can do for you, Biochemistry 49(14), 2987–2998, 2010.

Kleinschmidt JH, Tamm LK: Folding Intermediates of a Beta-Barrel Membrane Protein. Kinetic Evidence for a Multi-Step Membrane Insertion Mechanism. Biochemistry, 35(40), 12293-13000, 1996.

Kóta Z, Horváth LI, Droppa M, Horváth G, Farkas T, Páli T: Protein assembly and heat stability in developing thylakoid membranes during greening, Proc Natl Acad Sci USA 99 (19), 12149-12154, 2002.

Mallamace D, Fazio E, Mallamace F, Corsaro C: The Role of Hydrogen Bonding in the Folding/ Unfolding Process of Hydrated Lysozyme: A Review of Recent NMR and FTIR Results., Int J Mol Sci 19, 2018.

Marsh D, Páli T: The protein–lipid interface: perspectives from magnetic resonance and crystal structures, Biochimica et Biophysica Acta (BBA)-Biomembranes 1666 (1-2), 118-141, 2004.

Mauzerall D, Westheimer FH: J Am Chem Soc, 77, 2261–2264, 1955.

Nishi T, Forgac M: The vacuolar (H+)-atpases - Nature's most versatile proton pumps. Nature Reviews Molecular Cell Biology 3(2), 94-103, 2002.

Palmgren MG: An H-ATPase Assay: Proton Pumping and ATPase Activity Determined Simultaneously in the Same Sample., Plant Physiol 94, 882-886, 1990.

Páli T, Szalontai B: Membrane proteins—at the forefront of biophysical research, European Biophysics Journal 42 (2-3), 81-83, 2013.

Páli T, Finbow ME, Holzenburg A, Findlay JBC, Marsh D: Lipid-protein interactions and assembly of the 16-kDa channel polypeptide from Nephrops norvegicus. Studies with spin-label electron spin resonance spectroscopy and electron microscopy, Biochemistry 34 (28), 9211-9218, 1995.

Páli T, Finbow ME, Marsh D: Membrane assembly of the 16-kDa proteolipid channel from Nephrops norvegicus studied by relaxation enhancements in spin-label ESR, Biochemistry 38 (43), 14311-14319, 1999.

Páli T, Garab G, Horváth LI, Kóta Z: Functional significance of the lipid-protein interface in photosynthetic membranes, Cellular and Molecular Life Sciences CMLS 60 (8), 1591-1606, 2003.

Pocanschi CL, Apell HJ, Puntervoll P, Hogh B, Jensen HB, Welte W, Kleinschmidt JH: The major outer membrane protein of fusobacterium nucleatum (FomA) folds and inserts into lipid bilayers via parallel folding pathways. Journal of Molecular Biology, 355(3), 548-561, 2006.

Pocanschi CL, Popot J-L, Kleinschmidt JH: Folding and stability of outer membrane protein A (OmpA) from Escherichia coli in an amphipathic polymer, amphipol A8-35, Eur Biophys J 42, 103-118, 2013.

Schulz GE: beta-Barrel membrane proteins, Curr Opin Struct Biol 10, 443-447, 2000.

Senior AW et al.: Improved protein structure prediction using potentials from deep learning, Nature 577, 706–710, 2020.

Simons KT, Bonneau R, Ruczinski I, Baker D: Ab initio protein structure prediction of CASP III targets using ROSETTA. Proteins Suppl 3, 171-6, 1999.

Stevens TH, Forgac M: Structure, function and regulation of the vacuolar (H+)-ATPase. Annu Rev Cell Dev Biol 13, 779-808, 1997.

Sun-Wada G-H, Wada Y, Futai M: Vacuolar H+ pumping ATPases in luminal acidic organelles and extracellular compartments: Common rotational mechanism and diverse physiological roles. Journal of Bioenergetics and Biomembranes 35(4), 347-358, 2003.

Tamm LK, Arora A, Kleinschmidt JH: Structure and assembly of beta-barrel membrane proteins. Journal of Biological Chemistry, 276(35), 32399-32402, 2001.

Tamm LK, Hong H, Liang B: Folding and assembly of beta-barrel membrane proteins. Biochimica et Biophysica Acta - Biomembranes, 1666(1-2), 250-263, 2004.

Xu J: Distance-based protein folding powered by deep learning, Proc Natl Acad Sci USA 116(34), 16856-16865, 2019.

Zhao B, Zhu X, Lu Y, Xia C-Z, Cheng J-P: Tetrahedron Lett, 41, 257–260, 2000.

Zhu X-Q, Liu Y-C, Cheng J-P: J Org Chem, 64, 8980–8981, 1999.

Zhu X-Q, Li H-R, Li Q, Ai T, Lu J-Y, Yang Y, Cheng J-P: Chem–Eur J, 9, 871–880, 2003.

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