WP1.4. Targeting Ameloblastin, an intrinsically disordered protein from enamel matrix (Vondrášek, Bouř)

Research aims

- To synthesize AMBN proteins in quantities enabling thorough experimental studies of its behavior
- To determine AMBN regions responsible for aggregation properties in shorter sequence context and utilize the sequence to build fusion constructs for consequential experimental characterization.
- To identify products of proteolytic cleavage by specific proteases and test their proliferation and differentiation properties both in in vitro and in vivo studies.
- To study the effect of phosphorylation on AMBN-derived peptides and compare their proliferation and differentiation properties with their non-phosphorylated counterparts.
- Map regulated genes and their products by advanced through-output methods including RNAseq and ChipSeq methods

Research plan and methodology

Understanding the AMBN function and AMBN aggregation properties. A combination of experimental methods including Dynamic Light Scattering, Small Angle X-ray Scattering, Electron Microscopy, spectroscopic and theoretical methods (molecular dynamics simulations) will be used to describe conformational behavior of the whole AMBN molecule or its products obtained by proteolysis techniques. In combination with Mass Spectroscopy methods, we will obtain a map of AMBN accessible regions which should be further scrutinized as the candidates for aggregation and protein-protein interactions.

Role of post-translational modifications on AMBN structure. Based on the experimentally proved effect of phosphorylation on the binding and structural properties of IDPs, we will also study in silico conformational behaviour of model synthetic peptides derived from AMBN upon this post translational modification and we will perform in vitro and in vivo screening of designed peptides and proteolytic products as probes for protein-protein interaction or cell signalling. Bioinformatics analysis of phophosite databases[1] should provide consensual properties of protein sequences recognized by phosphokinases and will help us to elucidate the mechanisms of their recognition by various protein partners. This should also include in vivo screening for potential AMBN receptor which will be performed in collaboration with the laboratories at the University of Oslo. For purification and analyses of synthetic peptides, we will make extensive use of the UHPLC chromatography apparatus which is a part of the proposed capital investment of this project.

Role of processed AMBN in gene regulation and cell signalling. We aim to utilize the RNAseq technique to look on changes in gene expression and different populations of RNA to include miRNA, tRNA and ribosomal profiling. This technique should point out the translational products and their properties studied by ChipSeq technology to determine effect on interaction with DNA with respect to specific binding sites of DNA interacting proteins.

Understanding the relation of VCD signal and fibril structure of AMBN and model systems. In the past, the general principles providing the link between protein structure and the VCD spectra have been established.[2] However, fibrillation often leads to an atypical enhancement of VCD signal,[3] which can be used as an indicator of fibril formation. To understand the relationship between the local- and long-range order of protein structure and the spectrum, we will use a combination of molecular dynamics and quantum chemical techniques. The spectra will be generated using the Cartesian transfer tensor procedure developed previously at the IOCB.[4] According to a preliminary hypothesis, the enhancement is connected to long-range phonon-like vibrations enabled by the regular structure, observed and explained previously for aggregates of nucleic acids.[5]

Increasing the detection limits of vibrational optical activity spectroscopy. Currently, VCD fibril measurements are difficult as the precipitated proteins are not stable and inhomogeneous samples

provide polarization artefacts in the spectra. We will therefore systematically explore and optimize the experimental conditions providing the highest signal to noise ratio, making the spectroscopy more suitable to protein characterization in everyday use. As an alternative to VCD, we will explore the more sensitive (but so far more difficult to interpret[6]) electronic circular dichroism spectroscopy and Raman optical activity, especially in connection with the possibility to measure circular polarized luminescence,[7] as preliminary data indicate extreme sensitivity also of the latter technique to fibril structure.

The project will be realized in a close collaboration with Pavel Majer medicinal chemistry team (protein synthesis and purification), J. Cvačka mass spectrometry department (structural analysis) and P. Hobza (computer sources and know-how) computational chemistry group. The project will extensively exploit the capacities of the computer cluster for molecular modelling and the results will serve also for collaborating experimental groups.

[1] Hornbeck PV, et al. *Nucleic Acids Res.* **2015**, 43:D512-20.
[2] Bouř, P.; Keiderling, T. A. *J. A. Chem. Soc.* **1993**, 115, 9602-9607. Chi, H., Welch, W. R. W., Kubelka, J., and Keiderling, T. A. *Biomacromolecules* **2013**, 14**,** 3880−3891. Measey, T., and Schweitzer-Stenner, R., *J. Am. Chem. Soc.* 2011, 133**,** 1066.
[3] Fulara, A., Lakhani, A., Wójcik, S., Nieznańska, H., Keiderling, T. A., and Dzwolak, W.. *J. Phys. Chem. B* **2011**, 115**,** 11010.
[4] Kessler, J.; Kapitán, J.; Bouř, P. *J. Phys. Chem. Lett.* **2015**, *6* (16), 3314-3319. Yamamoto, S.; Li, X.; Ruud, K.; Bouř, P. *J. Chem. Theory Comput.* **2012,** 8(3), 977-985.
[5] Andrushchenko, V.; Bouř, P. *J. Comput. Chem.* **2008,** *29*, 2693-2703
[6] Kaminský, J.; Kubelka, J.; Bouř, P. *J. Phys. Chem. A.* **2011**, 115, 1724-1742.
[7] Wu, T.; Kapitán, J.; Mašek, V.; Bouř, P. *Angew. Chem. Int. Ed.* **2015**, *49*, 14933-14936

Research schedule

2017-2018

- Structural characterization of AMBN and its derivatives by DLS and SAXS methods in combination with MD simulations, its aggregation and protein-protein interaction properties.

2017-2019

- Experimental characterization by means of VCD experiments of AMBN derived peptides and proteolytic products as well as fusion constructs containing aggregation prone region.

2019-2020

- Effect of phosphorylation on the behaviour of AMBN derived peptides by means of computational and NMR methods.

2017-2020

- Optimizing the VCD experiments on model systems, testing the computational models.

2020-2022

- In vitro and in vivo screening of designed peptides and proteolytic products as probes for protein protein interaction or cell signalling.

2020-2022

- RNAseq and ChipSeq methods to decipher transcriptome regulated by AMBN and other associated AMBN dependent proteins.

2021-2022

Extending the spectroscopic fibril detection to physiological conditions, explore the sensitivity to the environment and temperature.

Publications and patents

Publications (Jimp)

	Jimp
2017	0
2018	4
2019	4
2020	4
2021	5
2022	5
Total	22

Nucleic Acids Research
Chemistry a European Journal
Protein Science
Journal of Chemical Theory and Computation
ACS Chemical Biology
ChemBioChem
Structure
Journal of Physical Chemistry

Patents and patent applications

	Patents (granted)	International patent applications (filed)
2017	0	0
2018	0	0
2019	0	0
2020	0	1
2021	0	1
2022	1	0
Total	1	2

We expect IP protection in the following areas:

Peptides as probes for AMBN protein-protein interactions or cell signalling.

Cooperation with foreign institutions

- Prof. Havard Haugen, Prof. Petter Lyngstadaas and Prof. Janne Reseland, University of Oslo, Norway,
- Prof. Gary Drobny, University of Washington, Seattle,
- Prof. Timothy A. Keiderling, University of Illinois at Chicago,
- Prof. Jan Kubelka, University of Wyoming, USA,
- Prof. Kenneth Ruud from the Center of Theoretical and Computational chemistry, Tromsø in Norway,
- Prof. Shigeki Yamamoto from Osaka University, Japan