



Laboratory of Structural biology of signaling proteins is looking for PhD student

Laboratory website (Institute of Physiology/BIOCEV):

[Structural Biology of Signaling Proteins - Institute of Physiology AS CR, v.v.i. \(cas.cz\)](http://www.ifo.cas.cz)

[Structural biology of signaling proteins | Biocev](http://www.biocev.cz)

Project supervisor (email): RNDr. Veronika Obšilová, Ph.D., veronika.obsilova@fgu.cas.cz

PhD project: Regulatory mechanisms of C2-WW-HECT (Nedd4-like) E3 ubiquitin ligases and the role of protein-protein interactions in their regulation.

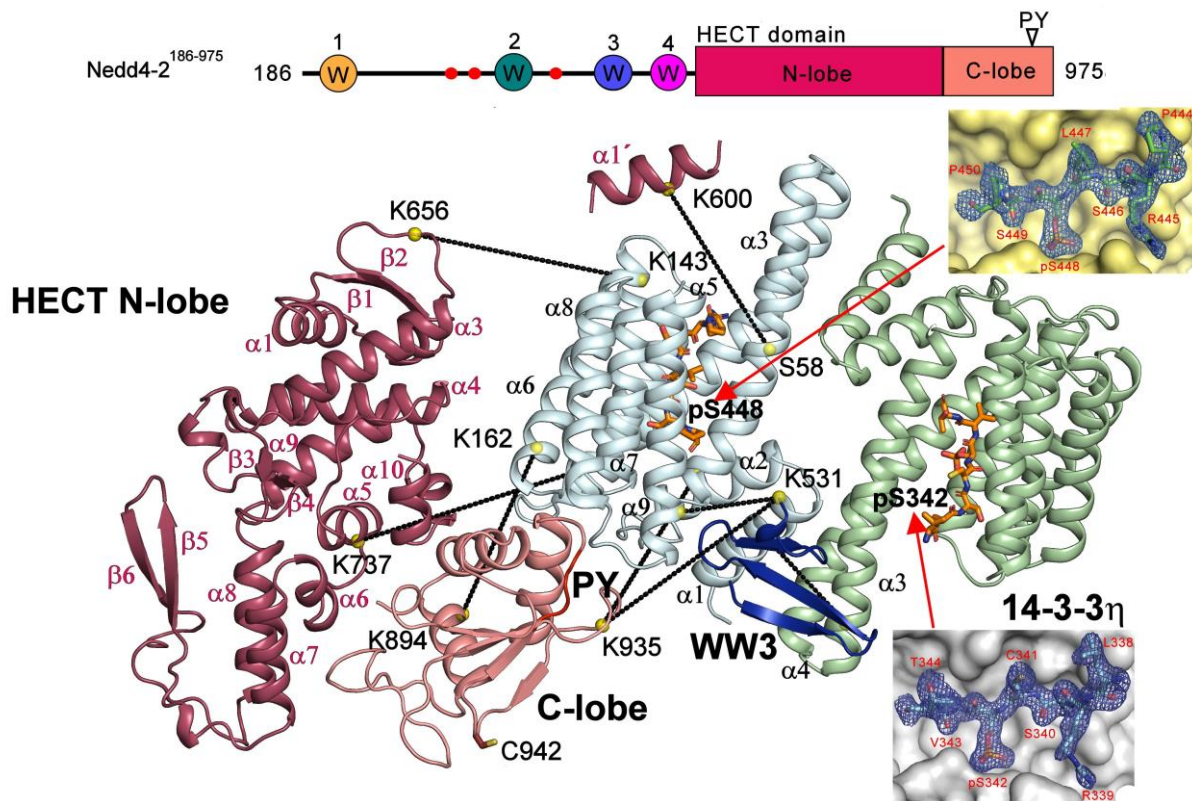
Nedd4-2 is a HECT E3 ligase that plays a critical role in pathophysiology by regulating multiple substrates, including the epithelial sodium channel (ENaC). Pathological consequences of Nedd4-2 dysregulation include respiratory distress, hypertension, electrolyte imbalance, and kidney disease. In addition to the not yet fully elucidated Ca^{2+} -dependent regulation of Nedd4-2, Nedd4-2 is regulated via phosphorylation and binding to various binding partners including scaffolding proteins 14-3-3. 14-3-3 proteins have the ability of binding the functionally different signal proteins, including kinases, phosphatases and transmembrane receptors by changing their function. The aim of this project is to elucidate the regulatory mechanism human (Smurf1, Nedd4L), yeast (Rsp5) HECT E3 ubiquitin ligases and to understand the role protein-protein interactions in their regulation. For this purpose, the structure of Nedd4L, Smurf1, Rsp5 complexes with different binding partners (Clc-5, 14-3-3 proteins, arrestins) will be studied using integrative structural biology methods, in particular cryo-EM, SAXS, H/D exchange coupled to MS and chemical crosslinking coupled to MS. The structure of these complexes will be solved by combining the results of experimental methods and methods of structural bioinformatics (if needed). The activity of E3 ligases will be measured using liposome-binding, pulse-chase and autoubiquitination assays. Combined, these approaches will allow us to elucidate regulatory mechanisms of Nedd4-like E3 ubiquitin ligases. This is a project in the field of structural biology and biophysical chemistry of proteins and is funded by grants from the research group. Cooperation with the Faculty of Science, Charles University in Prague.

We offer: A 4-years Ph.D. student position starting from September 2023 (possible to start earlier June/July), funding from the Czech Science Foundation (GA ČR), opportunity to gain expertise in preparation of recombinant proteins and methods of integrative structural biology, work in an international group of scientists, participation at international conferences and workshops, friendly, motivating environment, part-time contract for 3 years with the possibility of renewal, 5 weeks of vacation, subsidized lunch

Candidate's profile (requirements): Successful candidate should enjoy working in the field of structural biology/biophysics/physical chemistry, be curious and has the courage to try out new things. Required is M.Sc. degree with the defense until June 2022 (fields: Chemistry, Biochemistry, Molecular Biology or similar). The knowledge of molecular biology, protein expression and purification is a strong advantage. Good knowledge of English is required.

Five relevant publications of the research group:

- Joshi R, Pohl P, Strachotova D, Herman P, Obsil T, **Obsilova V*** (2022) Nedd4-2 binding to 14-3-3 modulates the accessibility of its catalytic site and WW domains. *Biophys. J.* **121**, 1299-1311.
- Pohl P, Joshi R, Petrvalska O, Obsil T, **Obsilova V.*** (2021) 14-3-3-protein regulates Nedd4-2 by modulating interactions between HECT and WW domains. *Commun. Biol.* **4**, 899.
- Kalabova D, Filandr F, Alblova M, Petrvalska O, Horvath M, Man P, Obsil T, **Obsilova V.*** (2020) 14-3-3 protein binding blocks the dimerization interface of caspase-2. *FEBS J.* **287**, 3494-3510.
- Hagenbuchner J.#, **Obsilova V.#**, Kaserer T., Kaiser N., Rass B., Psenakova K., Docekal V., Alblova M., Kohoutova K., Schuster D., Aneichyk T., Vesely J., Obexer P., Obsil T., Ausserlechner M.J. (2019) Modulating FOXO3 transcriptional activity by small, DBD-binding molecules. *eLife* **8**:e48876. doi: 10.7554/eLife.48876
- Alblova M., Smidova A., Docekal V., Vesely J., Herman P., **Obsilova V.***, Obsil T. (2017) Molecular basis of the 14-3-3 protein-dependent activation of yeast neutral trehalase Nth1. *Proc. Natl. Acad. Sci. U S A* **114**, E9811-E9820. doi: 10.1073/pnas.1714491114



Structural model of the complex between Nedd4-2 and 14-3-3 protein by integrative SAXS-based structural modeling with the crystal structures of the 14-3-3 binding sites of Nedd4-2 (Pohl et. al (2021) Communications Biology).