

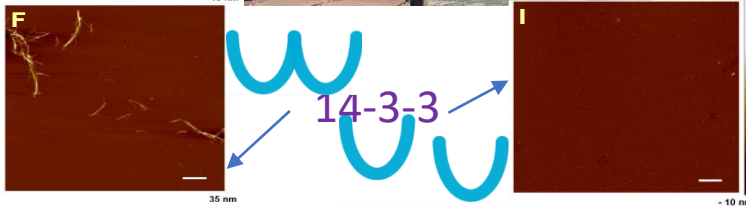
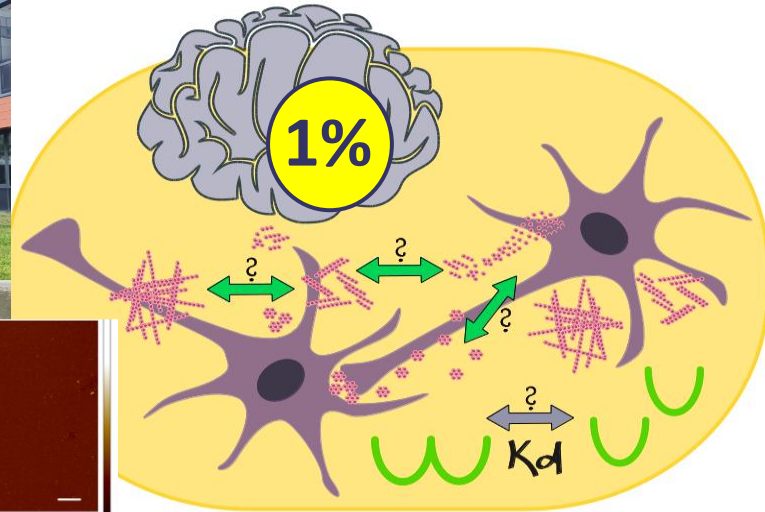
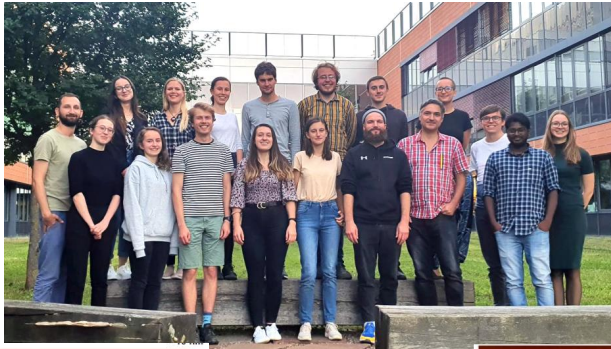
# Biophysical chemistry – tool for the understanding of the molecular mechanism of diseases

Jozef Hritz (jozef.hritz@ceitec.muni.cz)

Biophysical Chemistry, Institute of Chemistry, MU

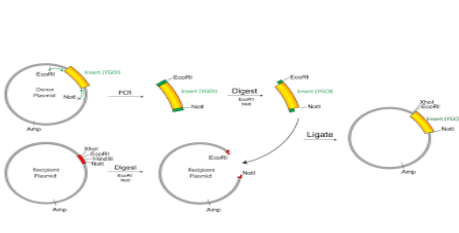
Structural Biology, CEITEC-MU

# JOZEF HRITZ team

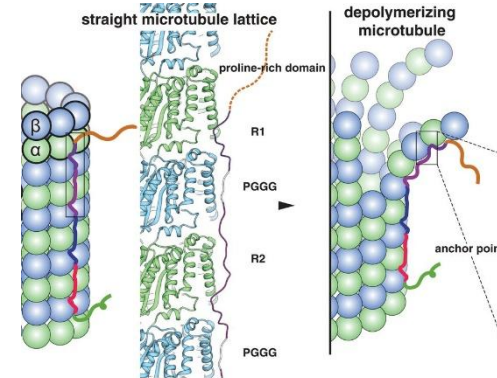
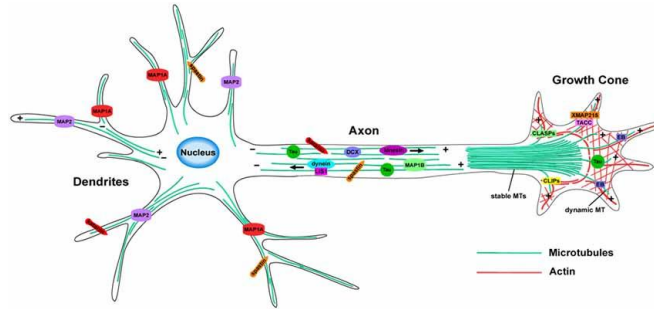


Research methodologies:

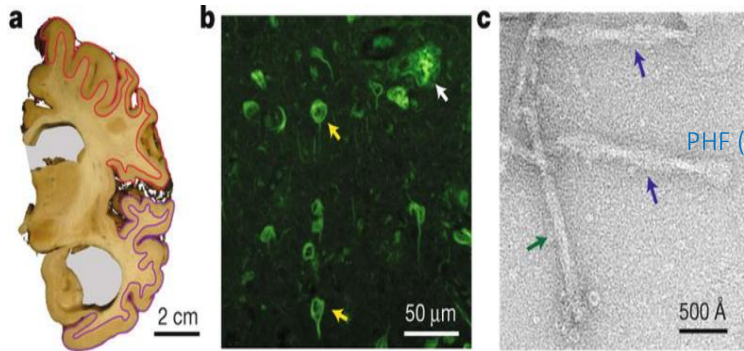
- Experimental: **protein expression and purification, structural and chemical biology** solution NMR, biophysical interac. methods, fluoresc. spectroscopy, cryoEM, MS AFM
- Computational: molecular dynamics. REMD, free energy calculations, NMR pa



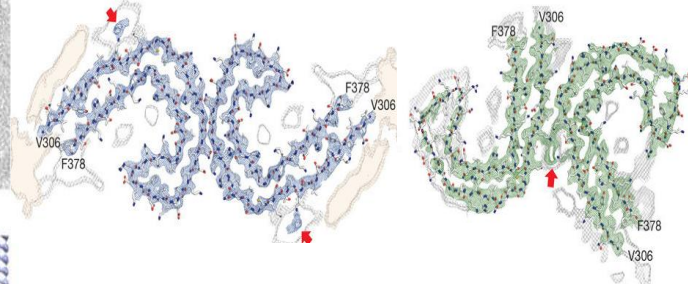
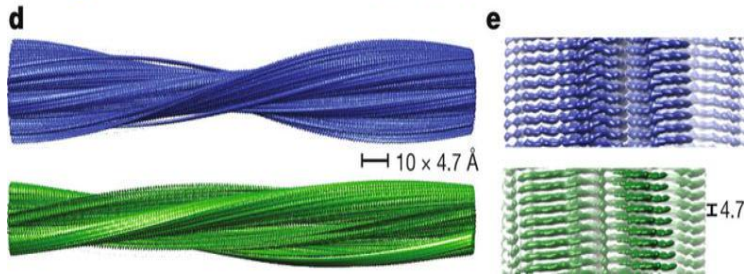
# Tau - Microtubule Associated Protein (MAP)



Binding of Tau to microtubules



PHF (paired helical filaments) SF (straight filaments)



Fitzpatrick A, Falcon B, He S, Murzin A, Murshudov G, Garringer H, Crowther A, Ghetti B, Goedert M, Scheres, S: Cryo-EM structures of Tau filaments from Alzheimer's disease brain, *Nature* **2017**, 547, 185-190  
 Shi, Y., Zhang, W., Yang, Y. *et al.* Structure-based classification of tauopathies. *Nature* **598**, 359–363 (2021).

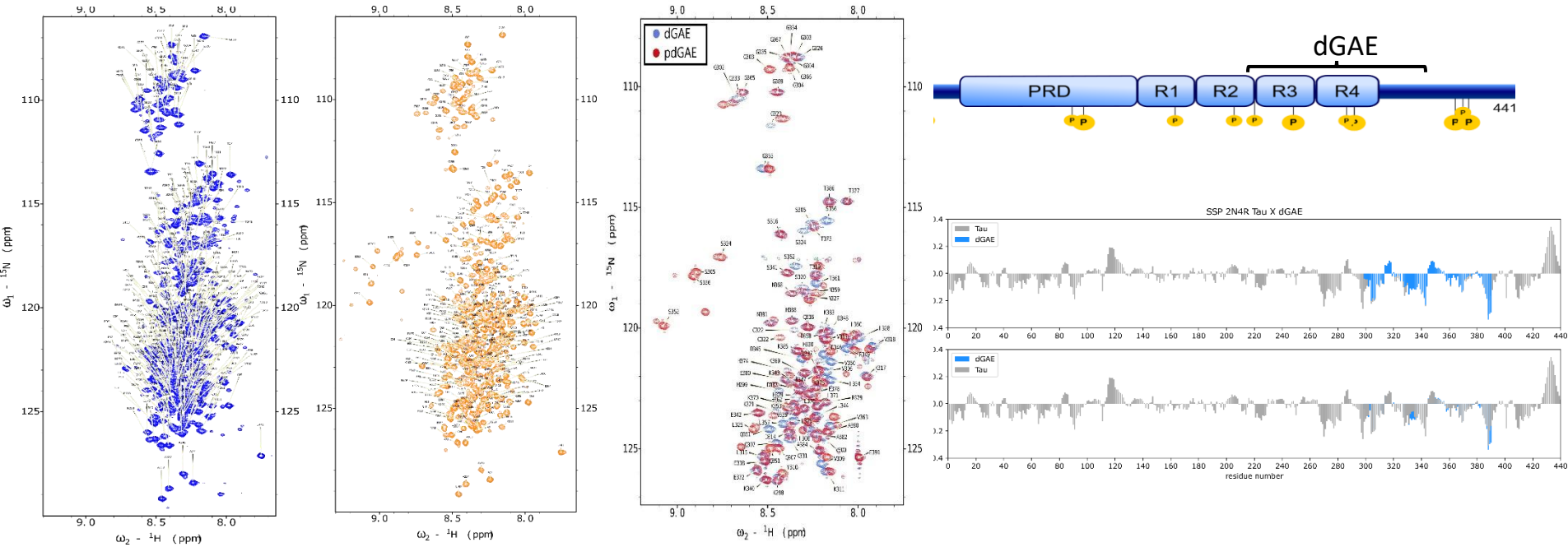
# Topic 1: Conformational changes within Tau or $\alpha$ -Syn protein – from physiological to the pathological forms



full length Tau,  
440/441 AA,

phosphorylated Tau  
433/441 AA

Characterization of selected Tau fragments, e.g. dGAE



- Crha R., Kozelekova A., Hofrova A., Ilkovicova L., Gasparik N., Kadeřavek P., Hritz J.\*: Hiding in plain sight: Complex interaction patterns between Tau and 14-3-3 $\zeta$  protein variants. *Int. J. Biol. Macromol.* 2024, 266, 130802 (<https://doi.org/10.1016/j.ijbiomac.2024.130802>)
- Jansen S., Narasimhan S., Fernandez P.C., Ilkovicova L., Kozelekova A., Králová K., Hritz J., Zidek L.\* Characterization of multiple binding sites on microtubule associated protein 2c recognized by dimeric and monomeric 14-3-3 $\zeta$  (accepted in FEBS J. 2025; <https://doi.org/10.1111/febs.17405>);
- Kitoka K, Lends A, Kučinskas G, Bula AL, Krasauskas L, Smirnovas V, Zilkova M; Kovacech B, Skrabana R, Hritz J, Jaudzems K\*: dGAE(297-391) tau fragment promotes formation of CTE-like full-length tau filaments, *Angew. Chem. Int. Ed.* 2024, e202407821
- Lasorsa A., Bera K., Malki I., Dupré E., Cantrelle F., Merzougui H., Sinnaeve D., Hanouille X., Hritz J.\*, Landrieu I.\*: Conformational impact of multiple phosphorylations within BIN1 SH3 domain binding site in the proline rich region of Tau protein. *Biochemistry* 2023, 62, 1631–1642





EU Excellence Hubs project (success rate:9.8%)

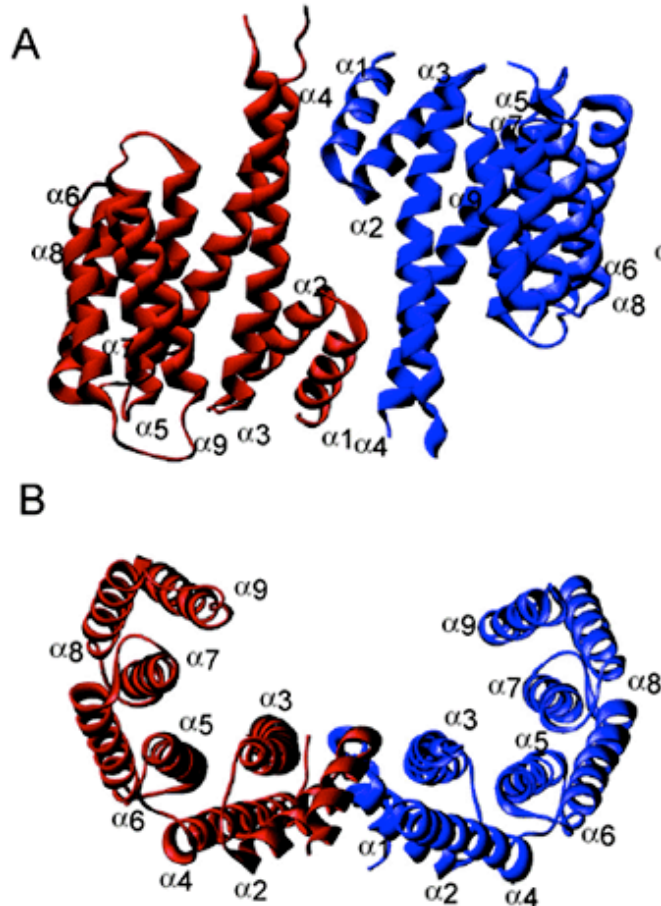


**Alzheimer's Disease Diagnostics Innovation and Translation to Clinical Practice in Central Europe**

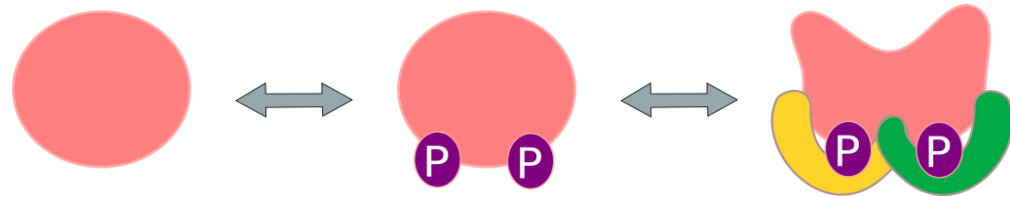
**ADDIT-CE : Consortium of 12 partners**  
**Main coordinator: Jozef Hritz**



# 14-3-3 protein family

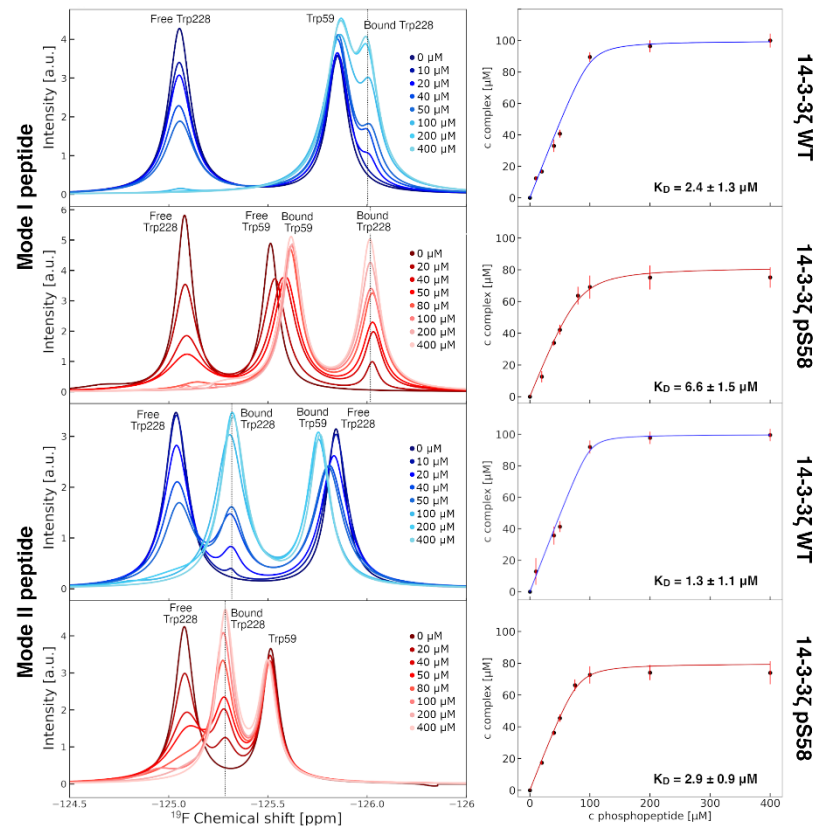
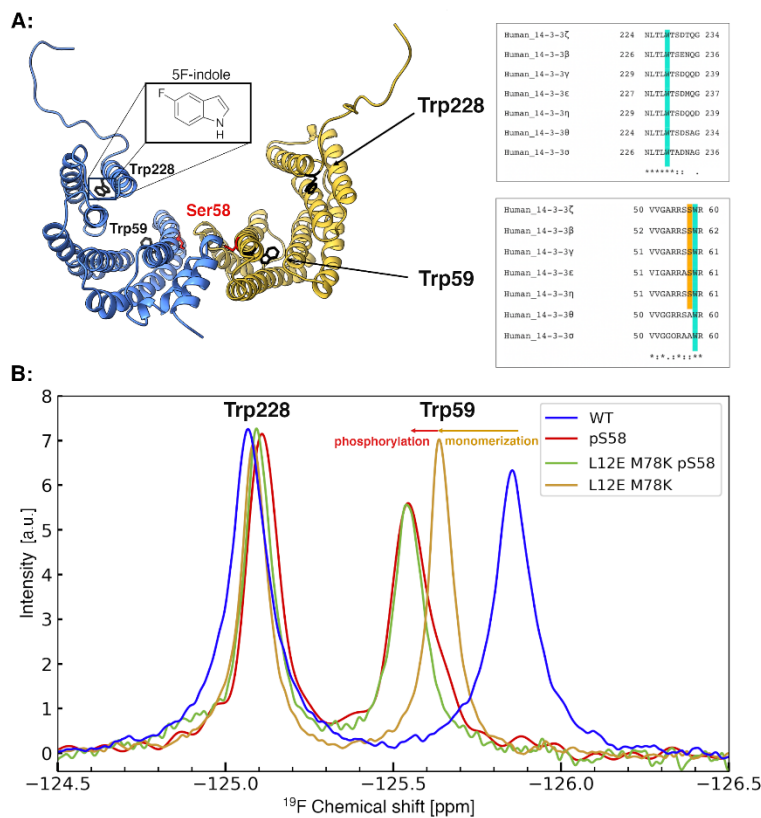


- ~28 kDa soluble acidic proteins
- Different isoforms:  $\beta$ ,  $\gamma$ ,  $\epsilon$ ,  $\zeta$ ,  $\eta$ ,  $\sigma$ ,  $\tau$
- They form homo and heterodimers
- 14-3-3 proteins make up 1% of all the soluble proteins in the brain
- More than 1200 known phosphorylated binding proteins



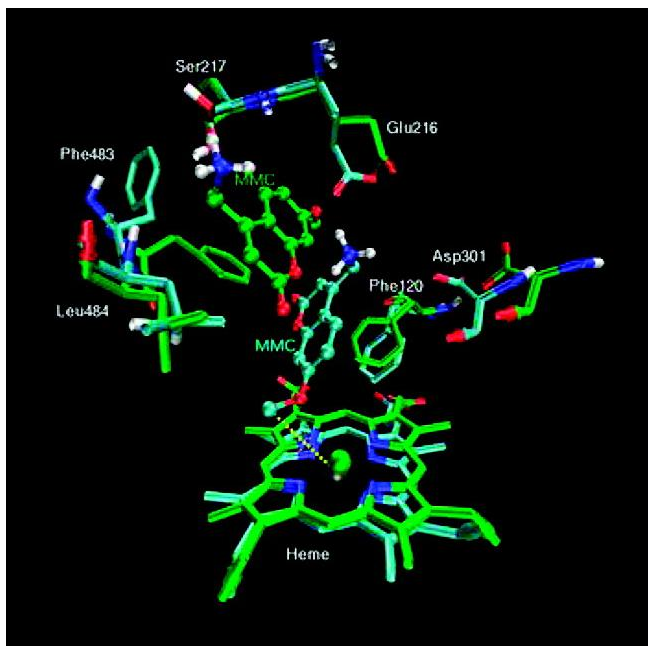
- Kozeleková A., Náplavová A., Brom T., Gašparik N., Šimek J., Houser J., Hritz J.\*: Phosphorylated and phosphomimicking variants may differ - a case study of 14-3-3 protein. *Front. Chem.* **2022**, 10:835733
- Trošanová Z., Lousa P., Kozeleková A., Brom T., Gašparik N., Tungli J., Weisová V., Zupa E., Zoldák G., Hritz J.\*: Quantitation of human 14-3- $\zeta$  dimerization and the effect of phosphorylation on dimer-monomer equilibrium. *J. Mol. Biol.* **2022**, 434, 167479

# Topic 2: $^{19}\text{F}$ NMR for characterizing dimerization, ligand binding and dynamics of 14-3-3 proteins



- Náplavová A., Kozeleková A., Crha R., Gronenborn A, Hritz J.\* Harnessing the power of  $^{19}\text{F}$  NMR for characterizing dimerization and ligand binding of 14-3-3 proteins. *Int. J. Biol. Macromol.* **2025** (accepted), <https://doi.org/10.1016/j.ijbiomac.2025.141253>
- Lousa P., Nedožralova H., Zupa E., Novacek J. Hritz J.\* Phosphorylation of the regulatory domain of human tyrosine hydroxylase 1 monitored using non-uniformly sampled NMR, *Biophys. Chem.* (**2017**), **223**, 25-29
- Hritz J.; Byeon I.-J.; Krzysiak T.; Martinez A.; Sklenář V.; Gronenborn A.M. Dissection of binding between a phosphorylated tyrosine hydroxylase peptide and 14-3-3ζ: a complex story elucidated by NMR. *Biophys. J.* **2014**, **107**, 2185-2194
- Nagy G., Oostenbrink C., Hritz J.\*: Exploring the Binding Pathways of the 14-3-3ζ Protein: Structural and Free-Energy Profiles Revealed by Hamiltonian Replica Exchange Molecular Dynamics with Distance Field Distance Restraints. *PLoS ONE* **2017**, **12**(7), e0180633

# Topic 3: Rational design of small-molecules inhibitors towards studied proteins. Computational simulations.

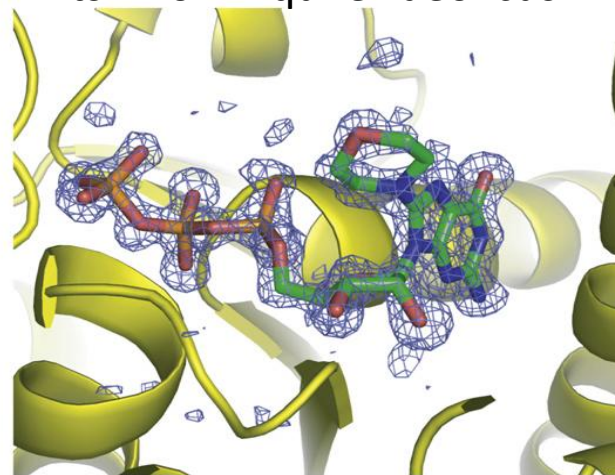


•Hritz, J.; de Ruiter, A.; Oostenbrink, C. Impact of plasticity and flexibility on docking results for Cytochrome P450 2D6: a combined approach of molecular dynamics and ligand docking. *J. Med. Chem.* **2008**, 51, 7469-7477

•Santos, R.; Hritz, J.; Oostenbrink, C. The role of water in molecular docking simulations of Cytochrome P450 2D6. *J. Chem. Inf. Model.* **2010**, 50, 146-154

•Byeon I-J., Ahn J., Mitra M., Byeon C-H., Hercik K., Hritz J., Charlton L., Levin J., Gronenborn A.M. NMR structure of human restriction factor APOBEC3A reveals substrate binding and enzyme specificity. *Nature Commun.* **2013**, 4, 1890

## FtsZ from *Aquifex aeolicus*



•Hritz, J.; Lapchen T.; Oostenbrink, C. Calculations of binding affinity between C8-substituted GTP analogs and the bacterial cell-division protein FtsZ. *Eur. Biophys. J.* **2010**, 39, 1573-1580

•Hritz, J.; Oostenbrink, C. Efficient free energy calculations for compounds with multiple stable conformations separated by high energy barriers. *J. Phys. Chem. B* **2009**, 113, 12711-12720

•Hritz J., Oostenbrink C. Hamiltonian replica exchange molecular dynamics using soft-core interactions. *J. Chem. Phys.* **2008**, 128, 144121

•Hritz J., Oostenbrink C. Optimization of Replica Exchange Molecular Dynamics by Fast Mimicking. *J. Chem. Phys.* **2007**, 127, 204104

•Zuzana Jandova; Zuzana Trosanova; Veronika Weisova; Chris Oostenbrink, Jozef Hritz\*: Free energy calculations on the stability of the 14-3-3ζ protein. *BBA - Proteins and Proteomics*, **2018**, 1866, 442-450.

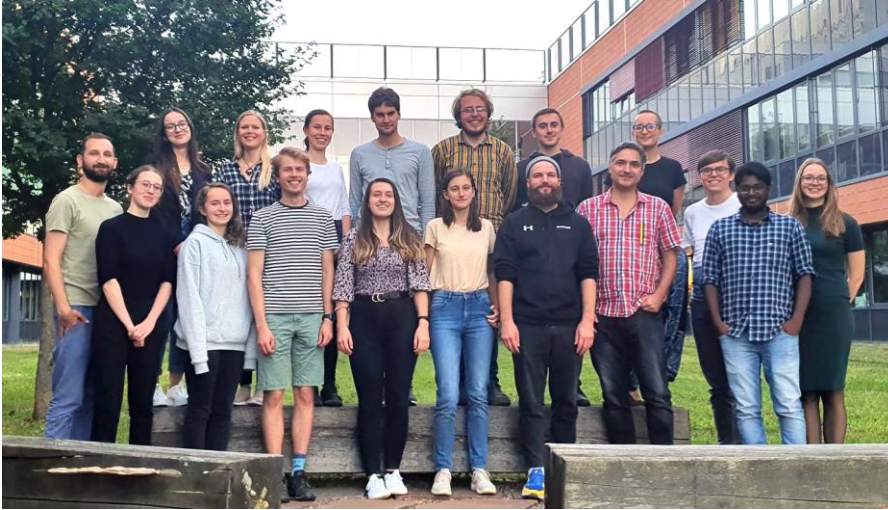


Didactic Topic 1 (kombinace fyzika/chemie): Vytvoření sbírky příkladů z biofyzikální chemie (základy i metody).

- Pro každou přednášku budou připraveny dvě vzorově vyřešené úlohy a pět neřešených úloh, u kterých bude uveden pouze číselný výsledek.

Didactic Topic 2 (kombinace biologie/chemie): Zpracování problematiky Alzheimerovy choroby populárně-pedagogickou formou na základě aktuálních poznatků.

# ACKNOWLEDGMENT



EVROPSKÁ UNIE  
Evropské strukturální a investiční fondy  
Operační program Výzkum, vývoj a vzdělávání

## Running grant projects with Dr. Jozef Hritz in role of PI:

- 2023 – 2026 **Horizont Europa: Excellence Hubs: HORIZON-WIDERA-2022-ACCESS-04 (ID: 101087124): Alzheimer's Disease Diagnostics Innovation and Translation to Clinical Practice in Central Europe**
- 2020 – 06/2025 **H2020-MSCA-RISE-2019 InterTau (ID: 873127): Integrative structural biology of pathological tau protein, an appealing therapeutic target for Alzheimer's disease**
- 2022 – 2024 **GAMU-Interdisciplinary Insight into CAIX structure and function and design of selective inhibitors as potential anti-cancer drugs**