1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

Interactions of the photoactive ligands with the biotechnological enzyme nitrile hydratase - research by theoretical molecular biophysics methods and quantum chemistry.

1.1. Project goals

- To use molecular modelling techniques like molecular dynamics QM/MM methods, quantum calculations, and protein-ligand docking to find how nitrile hydratase will interact with photoactive substrates and products of catalysis in the ground and excited states.
- To obtain knowledge if nitrile hydratase can catalyze two different progucts from the same substrate in different light conditions.
- To design mutational variants of nitrile hydratase which will catalyze or improve catalytic activity toward substrate in cis and trans form.

1.2. Outline

Nitrile hydratase (NHase) is an enzyme commonly used to convert toxic nitriles to amides. This enzyme owes its activity to the presence of a non-standard catalytic active site, which contains, among other things, two post-translationally modified cysteines and a cobalt or iron ion.

Optogenetics made it possible to control living organisms with light through significant genetic modifications. It turned out that without genetic modification, it is also possible to control organisms with a light using ligands with the azobenzene motif. Azobenzenes can change shape after illumination. In the ground state, they have an elongated (trans) form, which is altered by a photon absorption (usually UV light) switching to the excited state and adopting a cis conformation. The shape of the molecule changes strongly.

Many enzymes show specificity to the catalyzed substrates. However, some enzymes generally do not exhibit this specificity. Such an enzyme is nitrile hydratase (NHase) Naturally, it does not show selectivity of catalyzed substrates, but the author of this proposal has co-authored several publications where this enzyme was modified to catalyze only selected optical isomers of rac-mandelonitrile or catalyze to amide only one nitrile group in dinitriles.

In this project, PhD candidate will test the following hypothesis: "Control of catalytic activity is possible with light. It will be possible to obtain two different products only depending on the lighting conditions."

For this purpose, azobenzodinitriles will be used as catalysis substrates, and NHase as the enzyme. The PhD will be divided into 3 tasks:

T1) Study of the UV-Vis and IR spectra of azobenzodinitriles, azobenzodiamides and azobenzonitrileamides.

T2) Docking of azobenzodinintriles to NHase and determination of key amino acids in enzymatic catalysis".

T3) Molecular dynamics studies of the interaction of azobenzene substrate and possible products of catalysis with nitrile hydratase.

The research will be conducted in close collaboration with the group of Prof. Zhemin Zhou from School of Biotechnology, Jiangnan University, Wuxi, China. This experimental research group is working hard to solve the crystal structure of NHase, elucidate the mechanism of NHase selectivity, and develop more efficient, specific or universal variants of NHase enzyme.

1.3. Work plan

I. Mastering molecular dynamics QM/MM, Quantum chemistry and docking techniques

II. Obtaining and analysis of UV-Vis and IR spectra of azobenzodinitriles, azobenzodiamides and azobenzonitrileamides.

III. Docking of azobenzodinintriles to NHase and determination of key amino acids in enzymatic catalysis".

IV. Molecular dynamics studies of the interaction of azobenzene substrate and possible products of catalysis with WT nitrile hydratase and mutational variants of NHase.

1.4. Literature *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

1. **Peplowski, L.,** et al., Vibrational spectroscopy studies of methacrylic polymers containing heterocyclic azo dyes. Vibrational Spectroscopy, 2022. 120: p. 103377.
2. Ma, D., **Peplowski, L.,** et al., Insight into the broadened substrate scope of nitrile hydratase by static and dynamic structure analysis. Chemical Science, 2022. 13(28): p. 8417-8428.
3. Cheng, Z., **Peplowski, L.,** et al., Modulation of Nitrile Hydratase Regioselectivity towards Dinitriles by Tailoring the Substrate Binding Pocket Residues. ChemCatChem, 2018. 10(2): p. 449-458.
4. Cheng, Z., **Peplowski, L.,** et al., Identification of key residues modulating the stereoselectivity of nitrile hydratase toward rac-mandelonitrile by semi-rational engineering. Biotechnology and Bioengineering, 2018. 115(3): p. 524-535.
5. Slanska, M., et al., Azobenzene-Based Photoswitchable Substrates for Advanced Mechanistic Studies of Model Haloalkane Dehalogenase Enzyme Family. ACS Catal, 2024. 14(15): p. 11635-11645.
6. Quick, M., et al., Photoisomerization Dynamics and Pathways of trans- and cis-Azobenzene in Solution from Broadband Femtosecond Spectroscopies and Calculations. The Journal of Physical Chemistry B, 2014. 118(29): p. 8756-8771.

1.5. Required initial knowledge and skills of the PhD candidate

- Ability to work in Linux system
- Basic knowledge of programming in bash, C, Python
- Understanding of molecular biology, physics and chemistry
- Basic knowledge about biotechnology, biology.
- Analytical thinking
- Eager to learn

1.6. Expected development of the PhD candidate's knowledge and skills

- Better understanding of advanced modeling methods used in computer physics, chemistry and computational biophysics
- Practical knowledge of molecular modeling of enzymes and photoactive ligands using classical molecular dynamics methods
- Programming and Linux skills (bash, C, Python)
- "Fluency" in work in collaboration with international scientific groups