PHD PROJECT DESCRIPTION
(4000 characters max., including the aims and work plan to de published online)

Project title:

Studies of the activation process of the biotechnology enzyme nitrile hydratase - research by theoretical molecular biophysics methods.

1.1. Project goals

- To find mechanism of nitrile hydratase activation
- To use molecular modelling techniques like molecular dynamics QM/MM methods, protein-protein docking, protein-ligand docking, AI based scientific tools to find mechanism of nitrile hydratase activation
- To find mutational variants of activators with improved stability and activation rate towards selected nitrile hydratase.
- To obtain knowledge how to modify activators using semi-rational design of mutational variants

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. The formation of this catalytic center is made possible by the existence of metallochaperones that introduce a metal ion into the active center and most probably induce posttranslational changes in NHase. These chaperones are called NHase activators. Currently, several activator sequences are known, not always showing strong sequence similarity, but having a similar motif: HHH(E/D). Unfortunately, the key activation process is not known, as NHase activators show low expression and low stability.

The project will consist of constructing homology models of several variants of Co-NHase activators or using 3D structures developed by the experimental groups and then using such molecular modeling tools as ligand docking (metal ion) protein-protein docking, Molecular Dynamics and QM/MM molecular dynamics (also using artificial intelligence methods) to elucidate the process of metal ion insertion into the NHase and induction of posttranslational modifications.

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 activators, elucidate the activation mechanism, and develop more efficient, universal and stable variants of NHase activators.

1.3. Work plan

I. Mastering molecular dynamics, QM/MM, docking techniques and AI scientific tools.

II. To find mechanism of nitrile hydratase activation by its activators.

III. Design mutational variants of NHase activators with desirable properties.

IV. Finding general activator available to activate all Co-NHases.

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

[1] Y. Xia, L. Peplowski, et al., Discovery of the ATPase Activity of a Cobalt-Type Nitrile Hydratase Activator and Its Promoting Effect on Enzyme Maturation, Biochemistry 61(24) (2022) 2940.

[2] Y. Xia, L. Peplowski, et al., Metallochaperone function of the self-subunit swapping chaperone involved in the maturation of subunit-fused cobalt-type nitrile hydratase, Biotechnol Bioeng 116(3) (2019) 481.

[3] W. Sun, et al., Successful expression of the Bordetella petrii nitrile hydratase activator P14K and the unnecessary role of Ser115, BMC Biotechnol 16(1) (2016) 21.

[4] Y. Xia, et al., Construction of a subunit-fusion nitrile hydratase and discovery of an innovative metal ion transfer pattern, Sci Rep 6 (2016) 19183.

[5] S. Kataoka, et al., Functional expression of thiocyanate hydrolase is promoted by its activator protein, P15K, FEBS Lett 580(19) (2006) 4667.

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 and physics
- Basic knowledge about biotechnology, biology and chemistry.
- 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 and computational biophysics
- Practical knowledge of designing chaperons and enzymes properties
- Programming and Linux skills (bash, C, Python)
- "Fluency" in work in collaboration with international scientific groups