Computational approaches to identify small-molecule inhibitors for the N-domain of p97

S. Bothe, Würzburg/GER, P. Hänzelmann, Würzburg/GER,
H. Schindelin, Würzburg /GER, C. Sotriffer, Würzburg/GER

Sebastian Bothe, Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

The AAA ATPase p97 is an essential protein involved in numerous cellular processes and plays a key role in multiple aspects of protein homeostasis. Its functional diversity is mediated through the interaction with a large number of distinct cofactors. Due to its role in regulating a variety of physiological processes, p97 has emerged as a potential therapeutic target. Inhibitors of the cofactor binding would be a promising tool to understand the specific molecular and cellular functions of the different cofactors interacting with the N-domain of p97 [1].

Based on crystal structures of p97-cofactor complexes [2,3], different computational approaches were used to identify hot spot regions within the binding interface and to detect possible binding pockets for small molecules. Virtual screening protocols were developed, using structure-based pharmacophore models and docking studies. MD simulations and MM-GBSA calculations were carried out to select potential compounds for in vitro tests. MD simulations were also used to understand the influence of water molecules within the selected pockets, and the results were incorporated into the virtual screening protocols.

Selected compounds will be tested for their activity using fluorescence-polarization and biolayer-interferometry assays. In addition, the crystallisation conditions for the N-domain of p97 were improved and will form the basis for the crystallographic analysis of the identified compounds. Experimentally validated hits will be used as starting point for further optimization towards cofactor-specific inhibitors.

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[2] P. Hänzelmann, H. Schindelin: The Structural and Functional Basis of the p97/Valosin-containing Protein (VCP)-interacting Motif (VIM). *J Biol Chem*, **2011**, *286*, 38679–38690.

[3] L. Thi, M.Le, W.Kang et al.: Structural Details of Ufd1 Binding to p97 and Their Functional Implications in ER-Associated Degradation. *PLoS ONE*, **2016**, *11*, 1-15.