WP1.2. Targeting protein-protein interactions in leukemia (Řezáčová)

Research aims

- To validate druggability of selected protein-protein interaction targets using structural biology techniques.
- To construct an extended diverse fragment library using the input from recent screening campaigns.
- To develop lead molecules against the selected cancer protein-protein interactions using the fragment-based approach

Research plan and methodology

We will use a fragment-based approach (FBDD) alongside the standard methods of hit identification and elaboration against selected protein-protein interactions (PPIs). The FBDD proved to be instrumental for targeting so-called 'undruggable' PPIs. The success rate of traditional hit discovery and development, such as high-throughput screening (HTS) will be increased by FBDD in order to reveal novel directions for modifications of existing hits or to identify new molecules. This will yield PPI inhibitors that can be further elaborated into drugs or chemical tools for further validation of selected PPI targets that will lead to better understanding of the underlying biological processes. We will focus on relapsed acute lymphoblastic leukemia with cytosolic 5'-nucleotidase II (cN-II) mutations, MLL fusion-mediated acute leukemia and also novel protein-protein interaction targets identified through collaboration with KU Leuven. In this WP, we will extensively use new FPLC and preparative LC apparatuses, which are proposed capital investments of this project.

Alongside the expertise in all aspects of protein-protein and protein-small molecule structural characterization by the means of integrative structural biology, we have an established and functional pipeline for FBDD. It consists of an in-house fragment library formatted for screening either by ligand- or protein detected NMR experiments, orthogonal biophysical validation and structural determination of the protein small molecule complexes using X-ray crystallography or NMR spectroscopy.

We will screen the components of the selected protein-protein complexes against the optimized in-house fragment library using the ligand detected STD NMR experiments. The first round of screening will be carried out in mixtures of 5 fragments and the identified binders will be confirmed in the independent experiment as singletons. We will also utilize the information from the differential STD enhancements for ligand protons to identify the parts of the fragments responsible for binding. Next, we will rapidly characterize the fragment binding interface on the protein using protein detected NMR, that will allow for selection of compounds that bind at the targeted protein-protein interaction interface. The binding affinity of fragments will be independently corroborated using orthogonal biophysical methods, such as surface plasmon resonance, isothermal titration calorimetry or micro-scale thermophoresis.

The obtained data will be utilized for further hit elaboration based on the chemical structure as well as the detailed knowledge of their binding site. The second generation of fragments will be chosen from commercially available compounds derived from the original hits. We will compare their binding behaviour and suggest further modifications. This procedure will yield molecules with binding affinities sufficient for X-ray crystallography. Some protein targets, such as IBD domain of LEDGF/p75, are not amenable for crystallization and their complexes will be therefore structurally characterized by NMR. The medicinal chemistry development and testing of developed compounds in cell assays as well as animal models will be carried out by our collaborators at KU Leuven.

Research schedule

2017-2019

- Probing the druggability of cN-II.
- Structural characterization of MLL-LEGF/p75 interaction inhibitors identified by HTS using NMR spectroscopy and their development.
- Optimization of the fragment library.

2018-2022

- Fragment screening campaigns of selected targets (cN-II, MLL-LEDGF/p75, newly identified targets).
- Validation of newly identified protein-protein interactions as therapeutic targets.

2019-2022

- Validation of the identified fragment hits.
- Structural and biophysical characterization of the identified fragments.
- Fragment elaboration and inhibitor development

Publications and patents

Publications (Jimp)

	Jimp
2017	0
2018	2
2019	2
2020	2
2021	2
2022	2
Total	10

Journal of Biological Chemistry
Journal of Molecular Biology
Journal of Medicinal Chemistry
Plos one

Patents and patent applications

	Patents (granted)	International patent applications (filed)
2017	0	0
2018	0	0
2019	0	0
2020	0	0
2021	0	1
2022	1	0
Total	1	1

We expect IP protection in the following areas:
Compounds with antileukemic activity.

Cooperation with foreign institutions

- Prof. Zeger Debyser and Dr. Patrick Chaltin, KU Leuven, Belgium