WP1.9. Targeting protein-protein interactions of host-virus interface (Nencka, Bouřa)

Research aims

- Use fragment based inhibitor design against the well characterized UEV domain of the ESCRT system to develop compounds active against budding viruses that use the ESCRT system (HIV-1, Ebola).
- Characterize the structural determinants of human ACBD3 protein hijacking by several picornaviruses from *Enterovirus* and *Kobuvirus* genera.
- Structurally characterize PI4K hijacking by the HCV virus.
- Prepare fast, accurate, non-radioactive and sensitive assays for K_d determination of interactors of UEV domain of TSG101 protein and a method for the high throughput screening of the IOCB compound library for identification appropriate scaffolds fragments for fragment based drug design approach using microplate handling robot proposed within the project (in collaboration with J. Konvalinka group).
- Apply the fragment-based process mentioned in Aim 1 for the disruption of the interactions characterized in Aims 2 and 3

Research plan and methodology

We have already performed a first round of NMR-based fragment screening against the UEV domain using our in-house fragment library to test the feasibility of the project. The screen based on the STD (saturation transfer difference) NMR experiment yielded over 40 hits, which proved this part of the project to be highly feasible.

We will elaborate these fragments by two methodologies (Fig. 2). First, we will cluster the existing hits into groups that bind within the same area of the UEV domain and merge the hits into larger structures allowing interactions with larger segments of the interacting protein. Next, we will use fragment growing strategy directed by docking studies and enhanced synthetic exploration to elaborate these merged structures into a viable lead compounds. Here we will use the newly purchased flash chromatography apparatuses for the purification of synthesized compounds and their precursors (Nencka's group) and new FPLC and preparative LC apparatuses for biological applications (Bouřa's group), which are proposed capital investments of this project. The project will also extensively exploit the capacities of the computer cluster for molecular modelling and the results will serve also for collaborating experimental groups.

The identified lead structures will be subsequently tested both in protein-based competitive fluorescent and DIANA assay (novel screening assay based on qPCR being developed in J. Konvalinka's Group - see above WP2.5) assessing the direct binding affinity as well as in cell-based assays for determination of antiviral potency of the obtained compounds. The obtained PPI inhibitors will not only serve as potential antiviral compounds but they will be useful as tools for unveiling further details of ESCRT role in viral replication and participation of TSG101 in carcinogenesis.

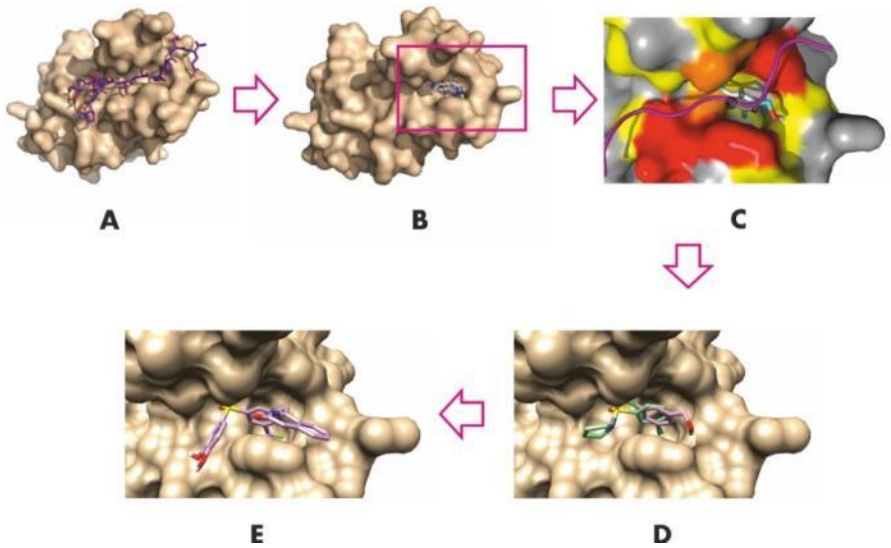

Fig. 2. The PPI inhibitor discovery process workflow: A) structure of the target PPI –UEV domain in a complex short peptide derived from HIV-1 p6^{Gag} protein; B) identification of ligand binding hot-spots of UEV domain and fragment screening by NMR or X-ray crystallography; C) molecular docking – STD experiment correlation data; D) merging of two fragment molecules; E) optimization of the final lead structure.

This workflow pattern will be subsequently applied to the discovery of inhibitors of other PPI essential for virus-host interaction, in particular to proteins responsible for the assembly of the replication complex of RNA viruses. Based on the results of our structural studies focused on picornaviruses from *Enterovirus* (poliovirus, enterovirus-71, coxsackievirus-B3, rhinovirus-14) and *Kobuvirus* (Aichi virus-1) genera, we will try to prepare a ligand disrupting the interactions between viral and host proteins involved in phosphorylation of host plasmatic membranes, e.g. 3A:ACDB3:PI4K.

The project will be realized in a close collaboration with P. Řezáčová's and J. Konvalinka's groups and J. Cvačka's mass spectrometry department using new high-resolution mass spectrometer, which is a part of the proposed capital investment of this project.

Research schedule

2017-2022

- Validation and structurally characterization of fragment hits against the UEV domain using NMR spectroscopy.
- Development of high throughput binding assays for fragment screening.
- Validation of the hits against the UEV domain in a fluorescence based competition assay and newly developed DIANA assay.
- Design and development of the fragments into high-affinity inhibitors based on advanced docking studies.
- Structural characterization of 3A:ACBD3 GOLD domain from variety of ss(+)RNA viruses.

2020-2022

- Structural analysis of the prepared UEV ligands.
- Optimization of the new UEV binders.
- Structural characterization of HCV NS5A protein in a complex with PI4K.
- Validation of the effectiveness and toxicity of the prepared compounds in cell-based assays.
- Advanced molecular modelling studies on potential ligands for disruption of other PPIs important for virus host interactions and assessment of the most promising targets.
  Preparation of the ligands for the selected most promising targets.

Publications and patents

Publications (Jimp)

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

Journal of Medicinal Chemistry
Journal of Biological Chemistry
Bioorganic & Medicinal Chemistry
Journal of Virology
EMBO Reports
PNAS
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: Novel ligand interfering with PPIs involved in pathogenesis of viruses.

Cooperation with foreign institutions

- Prof. Johan Neyts and Dr. Graciela Andrei form Rega Institute, KU Leuven, Belgium,
- Dr. Tamas Balla from National Institutes of Health (NIH), Bethesda, USA.