Timesheet – how to fill it

Please, note a new form of timesheet – there is a newly added column with a key activity code. This code has to be filled in all rows according to the following description:

- KA 1 = Implementation of the proposed research agenda of the project, i.e. research activity
- KA 2 = Upgrade and completion of the research infrastructure, i.e. preparation of the technical specification for the purpose of the public procurement, installation and testing of a new equipment
- KA 3 = Team building and its development, i.e. personnel activities of the research group leaders
- KA 4 = Internationalization of the research related to the project, i.e. business trips abroad, meetings and cooperation activities with the international partners, preparation of the international grant application, publications with international cooperation
- KA 5 = Project management, i.e. relevant only for the administrative positions (Jolana Hořejší, Lenka Štěrbová, Blanka Hajná)

Additional recommendation

- Two-word repetitive description of activity is not detailed enough.
- Fill the whole row with a description of activity
- Please, go into detail, where possible, linking the activity to the final outcome (e.g. publication) is more than welcome
- You cannot repeat one activity every day; put in at least three various activities each month. If you do just one activity in the month, describe the phases.
- Do not forget that the activity description ought to comply with your job description

You do not need to create detailed description from scratch. If possible, copy or rewrite the activities planned in feasibility study for your workpackage with an extension that cover particular job you have done. A clear, visible link to feasibility study is appreciated.

Example:

Preparation of small-protein scaffolds: recombinant expression or isolation of protein inhibitors – designing inhibition derivates
Preparation of small-protein scaffolds: recombinant expression or isolation of protein inhibitors – proteomic analysis
Preparation of small-protein scaffolds: recombinant expression or isolation of protein inhibitors – sample preparation for spectroscopic analysis
Preparation of small-protein scaffolds: recombinant expression or isolation of protein inhibitors – data evaluation

Activities from feasibility study for all WP:

5.2.2.1.4 WP1.1. Time plan (Mareš)

2018-2020/21

- Preparation of small-protein scaffolds: recombinant expression or isolation of protein inhibitors.
- Engineering of small-protein scaffolds using rational and combinatorial methods.

- Structural analysis of small-protein scaffolds: crystallization screening, crystallographic or NMR analysis of the inhibitor complexes.
- In silico analysis: computational analysis of 3D structures of the protein inhibitor complexes, prediction of mimetic inhibitors.
- Functional analysis of protein inhibitors: in vitro screening against peptidases and various substrates, in vivo screening in cell-based assay systems.

2020/21-2022

- Preparation and engineering of protein inhibitors and preparation of their complexes.
- In silico computational analysis of the complexes of the protein inhibitors and mimetic inhibitors.
- Synthesis of mimetic inhibitors.
- Functional analysis of protein inhibitors and mimetic inhibitors: in vitro screening against peptidases and various substrates, in vivo screening in cell-based assay systems.
- Optimization of functional properties of protein inhibitors and mimetic inhibitors: from designing to testing.
- Structural analysis of the complexes of optimized protein and mimetic inhibitors.

5.2.2.2.4 WP1.2. Time plan (Řezáčová)

2018-2019/20

- 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.

2018/19-2022

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

2019/20-2022

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

5.2.2.3.4 WP1.3. Time plan (Hobza)

2018-2018/19

- Running molecular dynamics (MD) of insulin receptor, CTSK and GlpG in complex with known ligands.
- Calculate at the SQM level the binding energy contributions, identify hotspots for the design.
- Identifying noncovalent interaction motifs and add the missing ones in the databases.

2019-2020/21

- Developing a database of important interaction motifs present at the protein-protein interfaces.
- Validation of SQM and MM methods.
- Deriving corrections for non-covalent interactions in SQM and MM methods.

2019/20-2022

- Running virtual screening of fragment libraries for binding to hotspots and verify hits experimentally.
- Design and prediction of new protein ligands for insulin receptor, CTSK and GlpG by joining or growing fragment-based approach.
- Iterative optimization of the design of new protein ligands after validation by syntheses and testing of the compounds.

5.2.2.4.4 WP1.4. Time plan (Vondrášek, Bouř)

2018-2019

- Structural characterization of AMBN and its derivatives by DLS and SAXS methods in combination with MD simulations, its aggregation and protein-protein interaction properties.

2018-2019/20

- Experimental characterization by means of VCD experiments of AMBN derived peptides and proteolytic products as well as fusion constructs containing aggregation prone region.

2020-2021

- Effect of phosphorylation on the behaviour of AMBN derived peptides by means of computational and NMR methods.

2018-2020/21

- Optimizing the VCD experiments on model systems, testing the computational models.

2020-2022

- In vitro and in vivo screening of designed peptides and proteolytic products as probes for protein protein interaction or cell signalling.

2020-2022

- RNAseq and ChipSeq methods to decipher transcriptome regulated by AMBN and other associated AMBN dependent proteins.

2021-2022

- Extending the spectroscopic fibril detection to physiological conditions, explore the sensitivity to the environment and temperature.

1.1.1.1.1 WP1.5. Time plan (Jiráček, Jahn, Konvalinka)

2018-2022

- Design and synthesis of series of azides and polyfunctional non-natural α-amino, β-amino, β,γ-diamino, α,β,γ-triamino acid derivatives, and oxidatively modified amino acids.
- Design and synthesis of peptides and peptidomimetics.
- Structural characterization of amino acid derivaties and peptidomimetics.
- Design and validation of high-throughput method for library testing.
- Design and solid phase synthesis of combinatorial libraries of scaffold-based compounds.

2019/20-2022

- Testing of biological properties of compounds.

- Structural characterization of active compounds.
- Molecular modelling of the best hits and comparison with the insulin structure bound to IR.
- Optimization of the active structures.
- Mapping or receptor binding sites with selectively labelled/modified compounds.

1.1.1.1.2 WP1.6. Time plan (Jiráček)

2018-2019

- Study of insulin interaction with small ligands in vitro and in silico.

2019-2022

- Investigation of the available permanent pancreatic cell lines, primary pancreatic cells and pancreatic tissues for their ability to produce, store and release insulin.
- Optimization of protocols for the isolation of intact and native insulin secretory granules.
- Mass spectrometry analysis of insulin secretory granule content.

2020-2022

- Structural analysis of insulin structures in isolated insulin secretory granules.

2021-2022

- Study of the effects of selected ligands on insulin structural storage forms and insulin release in model cell lines, primary cell cultures and tissues.

Chemical derivatization of the most interesting ligands and studying of their effects *in vitro* and *in vivo*.

1.1.1.1.3 WP1.7. Time plan (Lazar)

2018-2019/20

- Development of 2PPM into HT technique (microstructure optimization, software development).
- Development of mallet FPs.

2018-2022

- Development of 2PPM probes of interactions of GPCRs with other proteins, interactions of insulin and IGF-1 receptors with insulin mimetics
- Observations of receptor-other protein/peptide mimetics interactions.
- Testing of series of compounds for their interactions with receptors.

1.1.1.1.4 WP1.8. Time plan (Cígler, Bouř)

2018-2022

- Purification and isolation of representative ND samples.
- Surface modification of NDs.
- Large-scale preparation and isolation of the proteins and their isotope-labelled variants.
- Synthesis of model shorter peptides mimicking suggested binding domains.
- Acquiring spectral data on interaction of NDs with proteins.

2019-2022

- Ascertaining the structure of the ND surface.
- Multiple-spectroscopy strategies, analysis, theoretical binding model proposition.
- Suggestion of simple model peptide molecules.
- Synthesis of model peptides.

- Monitoring of the conformational behaviour during interaction with the surface.
- In vivo tests of novel compounds.

1.1.1.1.5 WP1.9. Time plan (Nencka, Bouřa)

2018-2022

- 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.

1.1.1.1.6 WP1.10. Time plan (Pichová, Weber, Cvačka)

Interactions of HBV core protein with cellular proteins

2018-2022

- Identification, verification and characterization of the host proteins interacting with HBc.
- Analysis of upregulation and downregulation of HBc-interacting partners on HBV replication.
- Characterization of HBc modifications and their influence on its epigenetic function in the HBV life cycle.
- Identification and verification of HBc-interaction domains.

2021-2022

- Testing the known HBc modulators and IOCB library of small molecular compounds.
- Analysis and optimization of hits.

Interactions of HBx with cellular proteins

2018-2022

- Verification and characterization (mutational analysis) of the HBx-host proteins interactions in cells
- Identification of the HBx minimal binding regions required for the interaction.
- Optimization of the screening procedures and initial testing of the IOCB library of small molecular compounds for potential inhibitors.

2019-2022

- Determination of the structural basis for the studied interactions by X-ray or NMR spectroscopy
- Screening for inhibitors of HBx-host protein interactions - identification of the leading compounds for further modifications and development.

HBV Precore maturation and involvement of cellular protein in precore localization

2018-2022

- Mapping the intracellular pathway of the HBe, looking for new interacting partners, validation of the interactions.

2019-2022

- Transcriptome sequencing and gene expression analysis.

2020-2022

- Structural characterization of protein-protein interactions between precore and individual cellular proteins, molecular modelling and design of potential inhibitors.

1.1.1.1.7 WP1.11. Time plan (Konvalinka)

2018 – 2019/20

- Final optimization of the AlphaScreen assay for high-throughput screening using microplate handling robot.
- Optimization of hits identified in preliminary screening in order to get improved competitors.
- High-throughput screening (using microplate handling robot) of the "IOCB library" containing various classes of organic compounds and derivatives prepared at the IOCB CAS, v.v.i. in order to identify other hits disrupting the interaction between polymerase subunits.

2020 – 2022

- X-ray structural and thermodynamic analyses of the most active compounds determined by the AlphaScreen assay.
- Validation of the most active compounds by *in vitro* infectivity testing and cell based assay for polymerase activity testing.
- Detailed thermodynamic analysis of the PB1-derived peptides binding to CPA and determination of the minimal peptide sequence responsible for efficient binding.
- Analysis of the resistance development against the most active inhibitor by the *in vitro* selection of virus performed by serial passage in the presence of increasing concentrations of tested compound.

1.1.1.1.8 WP2.1. Time plan (Jungwirth, Jahn, Lazar, Bouř)

2018

Targeting glycocalyx structure:

- Development of coarse-grained models for the main structural motives in the glycocalyx bulk region, *i.e.* glycosaminoglycan–glycosaminoglycan and glycosaminoglycan–albumin interaction.
- Development of hydrogels containing glycosaminoglycans as *in vitro* glycocalyx models.
- Calibration of 2PPM assay to explore relevant interactions in the glycocalyx such as the albumin–glycosaminoglycans interactions.

Targeting the cell plasma membrane and its interior:

- Study of the interaction of arginine rich peptides with model plasma membranes using molecular dynamics atomistic and coarse-grained resolution.
- Synthesis and characterization of foldamers constituted by non-natural amino acids containing guanidinium units and other functional groups.
- Synthesis of fluorescent probes of peptide/protein structure.

2019

Targeting glycocalyx structure:

- Development a fully functional model of the glycocalyx bulk region to be use in coarse-grained molecular dynamics simulation.
- Development of a coarse-grained models for the main structural motives of the glycocalyx membrane proximal region, *i.e.* CD44, Syndecan.
- Characterization of the hydrogels models of the glycocalyx upon the addition of foldamers specially designed to perturb the glucocalyx structure.

Targeting the cell plasma membrane and its interior:

- Study of the interaction of the arginine rich peptides with more complex membranes, e.g. membranes containing proteins.
- Performing ROA and VCD experiments with micelles and heterogeneous systems, aimed at elucidating the action of the self-penetration peptides action
- Using of 2PPM to monitor cell penetration by cationic peptides.

2020 -2022

Targeting glycocalyx structure:

- Development of a full coarse-grained model of the glycocalyx including bulk and membrane proximal region which should capture its main features.
- Usage of the model to explore possible targets to change its structure effectively.
- Application of the glycocalyx model to study its repercussion in drug delivery systems.
- Extension of the spectroscopic techniques such as ROA and VCD to the glycoprotein systems.
- Confrontation of computational prediction of protein dynamics in a heterogeneous environment with experimental results

Targeting the cell plasma membrane and its interior:

- Usage of arginine rich peptides and foldamers as drug delivery methods. We will explore this issue *in silico* and *in vitro*. The collection of spectroscopic techniques we aim to develop will be intensively use during this part of the project.
- Developing computational protocols allowing to account for the dynamics and environment in vibrational molecular spectra to study details of the peptides membrane interaction.

1.1.1.1.9 WP2.2. Time plan (Stříšovský)

2018-2020

- Preparation of recombinant full-length iRhom, NTD, IRHD, and ADAM17.
- Bicyclic phage display of NTD, IRHD and ADAM17 to identify their ligands.

2018-2022

- Structural characterization of full-length iRhom, NTD, and IRHD.
- Identification of iRhom interactors using BioID.
- Cell biological and structural characterization of iRhom interactors.
- Development of high-throughput assays for the disruptors of iRhom-client interactions.
- HTS, hit validation and development.
- Hit validation in cells and testing in mice.

1.1.1.1.10 WP2.3. Time plan (Bouřa, Nencka)

2018-2022

- Design and generation of recombinant non-structural 3A, 3BVpg, and 3Dpol proteins from several picornaviruses of the Enterovirus and Kobuvirus genera.
- Purification of human PI4KB and ACBD3 proteins, and fluorescent PI4P biosensor (SidC PI4P binding domain fused to mCherry).
- Design and synthesis of "super substrates" of phosphatidylinositol kinases and their evaluation in enzymatic assays. Crystallization of the "super substrates" with PI4Ks.
- Screening for antiviral activity of the synthesized compounds.
- Selection of suitable protein targets for design of novel "super substrate" analogues.

2019-2022

- Reconstitution of the formation of membranous webs in vitro using the biomimetic GUV system.
- Analysis of the membrane recruitment of the picornaviral 3Dpol enzymes.
- Design of a new generation of inhibitors targeting the inositol side of PI4K active center structural information from X-ray crystallography.

1.1.1.1.11 WP3.1. Time plan (Hocek)

2018-2022

- Design and synthesis of novel modified (d)NTPs bearing diverse functional groups
- Systematic study of polymerase incorporation of the modified nucleotides to DNA or RNA
- SELEX-based selection of modified aptamers against target proteins

2019-2022

- Systematic study of incorporation amino-acid specific as well as non-specific reactive groups to DNA or RNA and study of covalent cross-linking with DNA- or RNA-binding proteins
- Design and synthesis of inherently reactive aptamers against selected proteins
- Testing of cytostatic or antiviral activity of selected aptamers

1.1.1.1.12 WP3.2. Time plan (Pichová, Curtis, Hocek)

2018- 2019/20

- Expression and purification of the wild type and Cp Y132A mutant
- Optimization of conditions for the stable purification and storage of Cp dimers.
- Cloning, expression, and purification of C-terminally truncated Cp variants.
- Initial *in vitro* selection experiments.
- Motif optimization by random mutagenesis and *in vitro* selection.
- Identification of mutations that increase activity by high-throughput sequencing.
- Motif optimization by synthetic recombination and *in vitro* selection.
- Enrichment and qPCR analyses

2020-2022

- Investigating the effects of these DNA motifs on capsid formation.
- Determining the effects of these DNA motifs on HBV replication.

1.1.1.1.13 WP3.3. Time plan (Birkuš, Nencka)

2018-2022

- Design and synthesis of phosphonate/phosphinate analogs of CDNs
- Profiling of biological and biochemical activity of CDNs

- Crystallography of CDNs with STING
- Computational modelling of CDNs bound to STING

2018-2019

- Expression of recombinant STING, ENPP1 and bacterial and eukaryotic cyclic dinucleotide synthases
- Development of cell-based reporter assays to monitor activity of CDNs
- Enzymatic synthesis of CDNs from nucleoside triphosphate precursors

2019-2022

- Synthesis of prodrugs of one or two CDN leads
- S9 stability profiling of CDNs and their prodrugs, PK/PD of drug candidates in animal models and anti HBV activity in woodchuck model
- Design and synthesis of novel small-molecule agonists of STING based on virtual screening and fragment based hits.

1.1.1.1.14 WP3.4. Time plan (Konvalinka)

2018-2021

- Design and characterization of membrane anchored T7 RNA polymerase and production of stable regulatory Tet-off HEK293 cells expressing modified T7 RNA polymerase
- Design and characterization of adaptor protein
- Development of cell-based methodology, including abovementioned modified T7 RNA polymerase and adaptor protein, that enables production of cell-derived functionalized nanoparticles carrying mRNA
- Design of CRISPR/Cas9 for degradation of HBV cccDNA
- Large production of IVT-mRNA encoding specific CRISPR/Cas9
- Development of synthetic approach of targeted mRNA-nanoparticles production including large production of IVT-mRNA encoding CRISPR/Cas9 and its subsequent coating with liposome-based nanoparticles with specific targeting moiety

2019-2022

- Testing of biological and activity properties of our nanoparticles in tissue cultures.
- Optimization of the most efficient procedure for carrying CRISPR/Cas9 mRNA or DNA aptamers

1.1.1.1.15 WP3.5. Time plan (Vondrášek)

- 2017-2018 Bioinformatics analysis of structural and thermodynamic databases for ETS/HIF-1 transcription factors, MD simulations and calculations of binding affinities
- 2019-2020 Gibbs free energy change determination using ITC Microcalorimetry, Binding constant determination using Microscale Thermophoresis and VCD Spectroscopy
- 2017-2020 ECD Spectroscopy and structural studies using NMR spectroscopy
- 2020-2022 Total chemical synthesis of the low molecular molecules/ peptides and shifting the conformational state equilibria.