# D5.1 Mode of action or compound distribution report for compounds from selected projects from phase 1

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| Author(s) | Hanne Haslene-Hox (SINTEF), Eduardo Dominguez (USC), Stephanie Heinzlmeir (TUM), Jacek Kolanowski (IBCH-PAS), Marc Nazare (FVB-FMP), Tanja Miletic (EU-OS), Robert Harmel (EU-OS) and Philip Gribbon (ITMP) |
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# 1 Executive Summary

The overall objective of Work Package (WP) 5 is to provide trans-national access to chemical proteomics and compound disposition analyses as state-of-the-art methodologies to complement existing EU-OPENSCREEN (EU-OS) capabilities. EU-OPENSCREEN[1] is a European Research Infrastructure Consortium (ERIC) for Chemical Biology and early Drug Discovery with more than 20 affiliated high-throughput screening and chemistry facilities. It operates an open-access database and a central compound management facility which stores, quality-controls and manages the jointly-used EU-OPENSCREEN compound collection. Access to these methods would be of great benefit to all EU-OPENSCREEN users, from basic researchers to industrial drug discovery scientists, adding considerably to the impact of the Research Infrastructure's (RI) offering.

Target identification and advanced mass spectrometry imaging to elucidate the mechanism of action of lead compounds are not yet available within EU-OPENSCREEN. In WP5 we focused on establishing this new service category using the instrument of a transnational access call and identified six (6) suitable projects (first call organized in 2019,[2] second call in 2021[3]). Within this call, we have also taken the opportunity to widen the capabilities of EU-OPENSCREEN partner sites. Several projects were executed by two partners (one expert and one establishing this capability) who were able to share their technical knowledge and learn from one another.

Deliverable 5.1 "Mode of action or compound distribution report for compounds from selected projects from phase 1" reports on the strategy adopted to reveal the mode of action of the compound(s) of interest for three (3) user projects awarded for funding in 2019. Due to the multidisciplinary nature of chemical proteomics (e.g., chemical optimization of a probe with a linker followed by biological characterization), projects typically required access to at least two (2) partners facilities. Due to the COVID-19 pandemic[4] leading to restricted working conditions in 2020, the experimental work for two (2) of the selected projects was delayed and it is currently in progress (see Table 1).

# 2 Report on the deliverable

## 2.1 Overview

Chemical proteomics and Mass Spectrometry Imaging (MSI) tools have emerged as key technologies to advance our understanding of the behavior and the pharmacological effects of small molecule drugs within a cellular environment. Within the first EU-OPENSCREEN-DRIVE call, European and international researchers were given the opportunity to harness the expertise of our EU-OPENSCREEN-DRIVE partners in these areas (see Annex 1 for list of partners). Selected projects will aid the elucidation of underlying mechanisms of action of small molecule hit or tool compounds. To this end, applicants could either apply for access to MALDI MSI technologies for quantitative analysis of compound disposition in disease-relevant tissue or for access to chemoproteomic tools to identify interacting proteins partners.

[1] https://www.eu-openscreen.eu/about/about-eu-openscreen-eric.html
[2] https://drive.eu-openscreen.eu/drive-startseite/calls/small-molecule-screening-call-2019.html
[3] https://drive.eu-openscreen.eu/drive-startseite/calls/chemoproteomics-and-msi-call-2021.html
[4] https://www.who.int/emergencies/diseases/novel-coronavirus-2019

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Among received proposals (8), three (3) proposals from Germany and the United Kingdom with high scientific potential were selected following a rigorous external review process. The research fields covered by the proposals were diverse and characterized by broad scientific interests from mood disorders to immune oncology. Selected users gained access to state-of-the-art chemical synthesis facilities for the production of conjugated probe molecules to support affinity-based studies (FMP, IBCH-PAS) and advanced proteomics facilities for the prosecution of affinity-based analyses (SINTEF, TUM and USC).

All of the work undertaken towards this deliverable is contributing to the EU-OPENSCREEN-DRIVE main goal to ensure long-term sustainability of EU-OPENSCREEN operations by promoting measures for *i*) widening awareness of academia and industry for its services and data, *ii*) growing capacity and competence in its field across Europe, and *iii*) optimizing the management processes needed for a large, distributed infrastructure. Chemical proteomics and MSI pilot projects supported within WP5 will be used to evaluate and establish EU-OPENSCREEN capability to assess the mechanism of action of lead compounds.

## 2.2 Detailed report on the work carried out

As described above, five (5) WP5 members have provided experimental support to three (3) access projects (PID9452, PID9068 and PID8335) from the first phase of applications to the chemoproteomics call launched in May 2019. A summary of services provided is reported in Table 1 and the detailed work is described in sections 2.2.1 through 2.2.3. Due to the public nature of this deliverable, sensitive and projects specific information (e.g., compound structures, targets etc) are not disclosed.

*Table 1 summary of phase 1 transnational access projects to SINTEF, USC, TUM, FVB-FMP, and IBCH-PAS*

| Entry | Project | Research area | Service | Partner | Project status |
|---|---|---|---|---|---|
| 1 | PID8335 | Mood disorders (e.g., depression, obesity etc) | Advanced mass spectrometry-based proteomics | SINTEF, USC | completed |
| 2 | PID9452 | Inflammatory diseases | Synthesis, purification and spectroscopic characterization of probes | FVB-FMP | completed |
| | | | Advanced mass spectrometry-based proteomics | TUM, USC | in progress |
| 3 | PID9068 | Cancer | Synthesis, purification and spectroscopic characterization of probes | IBCH-PAS | in progress |
| | | | Activity based profiling | USC | in progress |

### 2.2.1 PID8335: Chemoproteomic analysis of FKBP51 inhibitors

**Abstract.** Researchers from the University of Darmstadt (Germany) collaborated with EU-OPENSCREEN partner sites SINTEF and USC (Table 1, entry 1) to generate a hypothesis about the mechanism of action of novel FKBP51 inhibitors, which have been developed by the user as potential antidepressants, analgesics and/ or anti-obesity drugs.[5] To this end, we aimed to identify possible protein-protein interactions that are specifically hindered by the selected inhibitor using

[5] *Nat Chem Biol* **11**, 33–37 (2015)

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affinity chromatography followed by proteomic analysis. In this approach a protein binding motif of FKBP51 is immobilized on a column and treated with cell lysates from two different cell lines to induce binding.

By elution of column in the presence or absence of specific binding ligands (inhibitor), the proteins that are displaced by the soluble small molecule ligands should be eluted specifically. Samples were then analyzed by mass spectrometry at two (2) partner sites. The resulting proteomic data was processed using in-house statistical analysis workflows and then semi-automatically curated on the basis of relevance of the protein function by user.

**Proteomics analyses**. Affinity chromatography was performed under a range of conditions, including *i)* different cell lysates; *ii)* different concentration of immobilized protein in column; *iii)* different elution ligands; *iv)* different ligand concentrations; and *v)* different elution buffers. All of these conditions impact proteomic results, with ligand/ protein motif concentration and presence of specifically eluting ligand resulting in the largest overall changes. Proteomic analyses, data processing and user support have been offered by both sites, SINTEF and USC to validate the results. This process involved extensive expertise sharing between the well-established sites and those which have more recently entered the field. Such knowledge sharing served to elevate the local capacities of the EU-OPENSCREEN sites, therefore supporting provision of chemoproteomics services in the future.

Proteomic analyses showed a clear difference between the proteins identified in samples with ligand-specific elution compared with samples with unspecific elution, both in terms of which at proteins are identified, and the number of identified proteins. A higher concentration of immobilized protein during affinity chromatography provided detection of a higher number of protein identifications. A complete list of selected proteins that are competitively displaced from binding to FKBP51 in the presence of inhibitor was provided to the user.

Overall, a good correlation between identified proteins was found between sample sets and proteomic sites (73% of proteins identified at both partner sites). There was a good correlation between abundantly identified proteins for both sites, although sample preparation and mass spectrometric analysis was performed differently. However, there were differences in what proteins were deemed unique and significantly up-regulated in specifically eluted samples. The present dataset can provide basis for such evaluation of new protein-protein interaction partner candidates for follow up-experiments.

**Conclusions.** In total, between 1779 and 2219 proteins were confidently identified for all performed proteomic analyses (across different sample sets and different sites). Samples with specific elution was compared with various wash fractions and mock column elutions. This evaluation resulted in lists of proteins enriched in specifically eluted samples from the four different proteomic experiments performed. Across all analyzed sample sets and sites, only 26 proteins were consistently reported as enriched in specific eluted samples in all experiments. Further work is needed to extract biological relevant protein-binding candidates from the extensive proteomic data that were provided. Although we expect a specific protein-protein-binding to be interrupted by the studied ligand, the number of proteins uniquely present or up-regulated in the specifically eluted samples is high. This can e.g. be due to the use of cell lysates rather than intact cells, with promiscuous proteins binding to both protein binding ligand in column as well as column stationary phase. A proteomic analysis is usually performed for generating hypotheses for further studies. The proteomic data produced in this project was curated towards biological function, cell compartment placement and literature, and led to candidate proteins that can be validated in e.g. Western blots, or other relevant methods. A refinement of interaction partners

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could also be identified by crosslinking coupled to mass spectrometry. However, this was out of scope for the current project and will be pursued by the user as part of future work.

### 2.2.2 PID9452: Traceless labelling of cannabinoid receptors

**Abstract**. Cannabinoid receptors CB1 and CB2 are an integral part of the endocannabinoid signaling system and have many regulatory functions in the human body related various inflammatory conditions, such as neuropathy, nephropathy, pruritus, osteoporosis, Alzheimer's disease and cancer.[6] This project between the user from the University of Nottingham (UK) and three EU-OPENSCREEN-DRIVE partners FVB-FMP, TUM and USC (Table 1, entry 2) aims at developing a traceless ligand-directed endogenous protein labeling method tailored for the CB2 receptor as non-genetic covalent modification strategy. The rationale is to covalently attach a fluorescent label selectively at the CB2 receptor without altering its biological/ pharmacological behavior. Thereby, detection and localization of the receptor, to follow their fate in real time under a variety of conditions and different ligands, will allow mechanistic studies of the receptor pharmacology. To this end, several ligand-fluorophore constructs bearing nitrobenzoxadiazole (NBD) and *N*-acyl-*N*-alkyl sulfonamide (LDNASA) warhead were successfully synthesized at FVB-FMP and evaluated for their affinity to the receptor at the user's site. In ongoing studies, the CB2 receptor membrane preparations will be labeled by these most suitable constructs and transferred to TUM and USC for proteomic analysis of the labeling site and quantification of labeling efficiency.

**Synthesis, purification and spectroscopic characterization of probes.** For the construction of the traceless ligand-directed endogenous protein labelling probe for the CB2 receptor we used an appropriate CB2 agonist functionalized at two attachment positions with a PEG linker of variable length prioritized by molecular modelling studies. For attachment of reactive warheads, FVB-FMP synthesized the NBD, LDNASA, *N*-sulfonyl pyridone (LDSP) and coumarin-based precursors. After probe assembly, LDSP and coumarine showed chemical instability and decomposed extensively within a short period of time. Therefore, these two warheads were excluded from the subsequent evaluation of the linker length. In support for the necessary control experiments versus the CB1 receptor subtype, the group at FVB-FMP also included a CB1 antagonist in the synthesis program and generated analogous CB1 traceless ligand-directed endogenous protein labelling probes using a similar synthetic route. The synthesis of the final probes consisted of 8–18 synthetic steps depending on the attachment point of the linker and end group functionalization of the LDNASA warhead (i.e. azide, alkyne or fluorophore). All intermediates and final compounds were characterized by LCMS, HRMS, 1H-NMR, 13C-NMR for their structural integrity. Overall, more than 25 probes were obtained in >95% purity and tested for affinity on CB2 and CB1, respectively with several of the showing promising binding affinity.

**CB2-modifying activity profiling of the compounds.** To assess if the probes show the expected activity (covalently attach a cargo fluorophore to the CB2 receptor), all constructs were profiled for specific binding to CB2 and CB1 receptors over-expressed in HEK293 cells using TR-FRET technique. Most of the compounds showed a comparable binding profile expected for the pharmacophore. To interrogate if the compounds bind covalently to the receptor they were displaced with a selective antagonist after an incubation period of 1–2 hours. If the fluorescence intensity could not be displaced, that was a strong indication that the compounds indeed covalently transferred onto the fluorescent "cargo" to the target receptors. The covalent nature of the fluorescent group attachment was further confirmed using SDS-PAGE electrophoresis and in

[6] *Nat. Rev. Drug Discov*. **3**, 771-784 (2004)

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gel fluorescence detection. As the protein is unfolded in the SDS-PAGE, the fluorescence could only be conferred to the protein band if the fluorescent group is covalently attached to the protein. Several compounds showed successful labelling of the CB2 receptor. The current direction of research is to incorporate brighter fluorescence groups for imaging applications, and to identify the nature and location of the covalent bond formed to identify the specific labelling site.

**Specialized chemical proteomics technologies for drug target deconvolution.** Labeling of CB2 in its biological background is performed by the user's group and samples will be sent to TUM for proteomic sample work up and measurement. Samples will be processed according to common proteomic sample processing protocols. Data acquisition will be focused towards measurement of CB2 by implementing an inclusion list for peptides derived from CB2. Data processing will include the introduced modification to identify the labeling site.

**Conclusion.** In this project more than 25 potential traceless ligand-directed endogenous protein labelling probes for the CB2 receptor as well for CB1 receptor as controls were developed and synthesized. The NBD and LDNASA warheads probed to be sufficiently chemically stable while LDSP and coumarine warheads showed inherent chemical lability and were not further pursued. Testing the NBD and LDNASA probes showed promising binding affinity and a strong indication for covalent binding to the receptor in initial experiments. In a subsequent refinement step rapid tetrazine-based labeling end groups as well as most versatile fluorophore systems will be introduced. A set of 10 selected probes is currently under processed with CB2 receptor membrane preparations and is being transferred to TUM and USC for proteomic analysis of the receptor labeling site and quantification of labeling efficiency.

### 2.2.3 PID9068: Synthesis, purification and spectroscopic characterization of probes

**Abstract**. Unlike normal cells, malignant cells possess supernumerary centrosomes which require clustering in two spindle poles for effective proliferation. Therefore, targeting this mechanism is of great potential for development of cancer cell-specific antiproliferative agents. The GF15 analogue of natural product griseofulvin suppressed tumor cell growth both in vitro and in vivo through inhibition of this centrosomal reorganization, possibly by altering microtubules.[7] In attempts to identify the cellular protein targets of GF15 researchers at the German Cancer-research center (DKFZ) in Heidelberg previously performed pull down experiments with biotinylated as well as photo-affinity probes but these efforts did not deliver proteins which could be confirmed as real targets. Also, identification of 35 gene candidates in clones with acquired GF resistance by whole genome sequencing failed to deliver novel regulators of centrosome reorganization. EU-OPENSCREEN partner sites IBCH-PAS and USC (Table 1, entry 3) decided to offer a new, unique design of the linker to use alternative mechanisms of protein capture. The new probe was envisioned to perform activity-based protein profiling in cellular lysates followed by enrichment of the labeled proteins by click chemistry. Recently, the synthetic feasibility of the probe was confirmed by the synthesis of the first linker-inhibitor construct. However, the efficiency of the synthetic route is currently under further optimization prior to the synthesis of additional potential probes.

**Synthesis, purification and spectroscopic characterization of probes.** Previously, the user has applied synthetic probes to pull down experiments, which included *i*) biotinylated probe and *ii*) photo-affinity probe. The former did not give specific proteins to be tested further while the latter identified some pulled-down proteins which, however, could not be independently validated. In the

[7] *Cancer Res*. **72**, 5374-5385 (2012)

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consequence, we have decided to offer a new, unique design of the linker to use alternative mechanisms of protein capture based on ligand-directed endogenous labelling (Figure 1a). The design is based on the presence of a reactive moiety (similarly to the photo-affinity design) which is not activated by light, but which is intrinsically susceptible to the nucleophilic attack by the amino acid residues on the surface of the protein outside the active site. Such probe is recruited to the target thanks to the presence of the inhibitor, which brings the labelling reactive moiety (yellow colour on Figure 1a) to the proximity of the protein surface. Subsequently, the nucleophile on protein surface reacts with the labelling reactive moiety forming a covalent bond between the part of the linker containing the click moiety. Part of the linker together with the inhibitor is eliminated and can diffuse away from the protein leaving however target protein labelled covalently with a part of the linker containing the click moiety. This pseudo-intra-molecular reaction between the probe recruited to the protein but bound only through a non-covalent interaction of the inhibitor with the target is significantly more efficient (usually 3-4 orders of magnitude bigger than when probe is not bound) ensuring high selectivity towards the target over non-specific binding. Unlike in the case of photo-affinity labelling, which allows only for covalently labelling targets to which probes are bound to in the moment of photoactivation, our design allows for binding targets over prolonged period of time and accumulation of the covalently labelled targets increasing the efficiency of target capture.

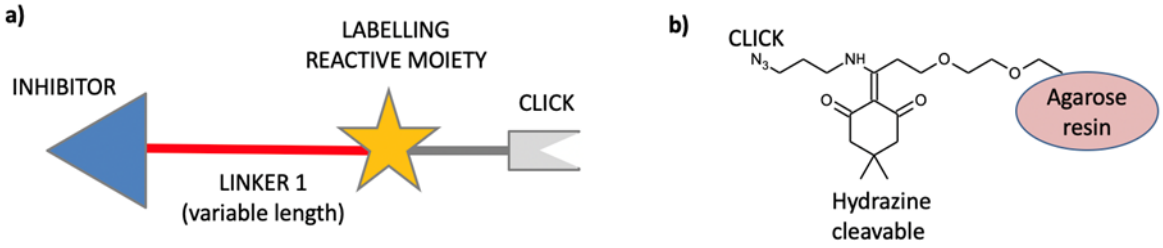


*Figure 1 a) schematic representation of the chemoproteomic probe with critical design features and b) example of the resin to be used for a pull-down experiment*

With this aim in view, candidate griseofulvin analogue and a place of linker attachment has been agreed on with the user according to its previous results including SAR. Hydroxylamine-like connection has been used to attach linkers to the active form of griseofulvin analogue according to the recommendations by the user. In addition, stability of this linker in a buffer was confirmed to ensure the stability of the probe in the future experiments. As labelling reaction requires a proximity of the labelling reactive moiety to the nucleophile on the surface of the protein, for the unknown targets it is critical to provide probes with variable linker lengths (red color on Figure 1a) to increase chances of capturing variable targets. Therefore, three (3) different linker lengths were considered with PEG character to improve association of the probe to the protein surface. Initial synthetic pathway has to be modified due to the formation of byproduct of one of the reactions. Final synthetic route, consisting of 10 steps have been explored and a formation of the probe was confirmed but it could not be isolated due to a low yields and quantities of the reaction. Therefore, the scale-up of the reaction is currently in progress. Additionally, alkyl linkers instead of PEG ones are developed, as their reactions are better known in the literature and therefore promise higher reaction efficiencies and easier access to the probes.

**Activity based profiling.** To narrow down the number of variables in the initial experiments, it was decided to carry out them firstly in cell lysates prepared by the user (to avoid additional off-effects

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of variability in cell membrane penetration, which is not known for these probes). To ensure compatibility with the probes designed, a theoretical protocol for a pull-down experiment has been prepared, using a resin with a click handle and a chemically cleavable linker to enable a release of the potentially bound proteins (Figure 1 b).

**Conclusions.** We have explored a whole synthetic path for a synthesis of these unique chemoproteomic probes enabling endogenous labelling with a single length linker, confirming the feasibility of the synthesis. However, significant challenges associated with low yields of two of the synthetic steps led to a need of increase in the scale of synthesis which is underway. Additionally, a replacement of the PEG linker with an alkyl linker, the reactions of which have been previously described in the literature, has begun. In the meantime, a most suitable biological matrix for the first target identification experiment (cell lysates) was proposed and a pull-down protocol has been theoretically developed, which is intended to be tested once first versions of the probes are delivered.

# 3 Next steps

In the upcoming months, synthesis of probes in project PID9068 and pull-down experiments together with proteomic analysis for PID9452 will be performed to finalize the respective projects. In the meantime, three (3) other projects have been accepted for the second round of TNA activities, including one (1) from industry and one (1) to perform Mass Spectrometry Imaging based studies. The expertise gained by EU-OPENSCREEN-DRIVE sites in working on the user projects in chemoproteomics equipped us with a better understanding of the demands and challenges associated with the recruitment as well as risks of carrying out these projects. All of these experiences will feed into a preparation of a guideline for introduction of a new category of EU-OPENSCREEN partner site associated with chemoproteomics capacities. Broadening of the service profile of EU-OPENSCREEN to include chemoproteomics investigations will enable the more complete follow up of phenotypic studies in particular. This should further enhance the attractiveness of EU-OPENSCREEN services to the user community and improve the overall impact of the research infrastructure.

# 4 Annexes

## 4.1 Annex 1

| EU-OPENSCREEN-DRIVE institute | Description of expertise offered |
|---|---|
| Institute of Bioorganic Chemistry Polish Academy of Sciences, Department of Molecular Probes and Prodrugs & Center for High Throughput Screening Studies (IBCH PAS) | IBCH PAS offers support for the synthesis of 2-4 probes to study target engagement and/or fluorescent labelling for one validated bioactive compound with identified activity-benign point of attachment on its scaffold. Probes will be composed of linkers with variable length connecting the ligand (derived by the user) with a specific tag (e.g. biotin for proteomics or fluorophore for imaging). Synthesis will be performed in a convergent manner with several linker-tag adducts being synthesized in parallel. The resulting probes are then attached through a stable moiety to the identified hit scaffold. IBCH PAS provides also advanced proteomic identification of the targets. |
| Helmholtz Centre for Infection Research, Department of Chemical Biology (HZI) | The department "Chemical Biology" at HZI aims at discovering new antibacterial and antiviral drugs, |

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| HZI HELMHOLTZ Centre for Infection Research | characterizing their functionality and optimizing their properties. HZI focuses on infection research and small molecules that can function as antimicrobial or antiviral agents, interfere with pathogenicity factors or stimulate the immune system.<br>Within this call, HZI offers expertise in the area of drug conjugates to synthesize functionalized chemical probes. HZI has ample experience in the synthesis of bacteria-targeting conjugates that combine a small molecule and an effector moiety (an antibiotic, a fluorophore, etc) through cleavable and non-cleavable linkers. Using these probes, HZI will support the affinity-based target identification using chemoproteomics. |
| Leibniz Research Institute for Molecular Pharmacology (FVB-FMP) | The medicinal chemistry group at the FMP offers chemical probe optimization (activity, selectivity, linker attachment exploration) and follow-up modification towards labeled chemical probes for target deconvolution, fluorescent labeling, and crosslinking. |
| SINTEF Trondheim, Department of Biotechnology and Nanomedicine (SIN) | SINTEF provides access to advanced mass spectrometry-based proteomics facilities for drug target interaction studies. This includes facilities for quantitative proteomics to support non-targeted and targeted screening of drug-protein interactions. Using robotic handling SINTEF offers high-throughput sample preparation (extraction, lysis, in solution digestion, FASP, SPE clean-up) for both targeted and shotgun proteomics analysis. For shotgun proteomics to support non-targeted screening of drug-protein interaction, QExactive-HF-X mass spectrometer is employed (at a Core facility). For targeted screening of identified interactions, LC-MS/MS (Agilent QQQ) analysis is offered for highly sensitive analysis of multiple peptides, and targeted screening of identified interactions in a high-throughput manner is offered using Rapidfire-MS/MS (Agilent). In targeted analysis, isotope-labelled peptide standards are employed.<br>Data processing and bioinformatics data interpretation pipelines are also offered including statistical analysis. Moreover, within this call SINTEF offers Mass Spectrometry Imaging (MSI) for compound disposition studies. MSI facilities at SINTEF include sample preparation set-ups (cryostat sectioning, ImagePrep for matrix and reagent deposition), and sample analysis using ultra-high-resolution mass spectrometry on a Bruker Solarix XR 12T FTICR. Analysis of resulting imaging data by multivariate statistical analysis tools is also provided (SCiLS software). The facilities offered by SINTEF using MSI support the determination of discrete tissue distribution of the parent compound, its metabolites, synthetic derivatives, as well as endogenous molecules linked to pharmacological and toxicological |

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| | mechanisms of the compound, and thus, investigations of i) the relative compound disposition of close structural analogues and ii) the target activation and thus possibly modes of action for compounds at their site of action. |
| Technical University of Munich, Chair of Proteomics and Bioanalytics (TUM) | TUM Proteomics offers a broad range of advanced mass spectrometry based proteomic methods and expertise on specialized chemical proteomics technologies for drug target deconvolution. Established chemical proteomics workflows include the generation and application of affinity matrices from small molecules as well as natural compound derivatives. Specialized affinity matrices enriching subproteomes (protein kinases, HDACs) are readily available at TUM and allow for selectivity profiling of small molecule kinase and HDAC inhibitors.<br>In addition, TUM infrastructure provides facilities for cellular thermal shift assays (CETSA) and iso-thermal dose response (ITDR) experiments. It also provides bioinformatics infrastructure (400 TB online data storage, about 20 server computers totaling 300 CPUs and about 1 TB memory, 6 GPUs) and operates ProteomicsDB, a powerful in-memory database for the real-time analysis of proteomic data. |
| Mannheim University of Applied Sciences, Center for Mass Spectrometry and Optical Spectroscopy (MUAS-CeMOS) | CeMOS combines expertise in development of measurement devices, in optics and spectroscopy and in mass spectrometry with substantial expertise in application development for the chemical, pharmaceutical, MedTech and Biotech industries. Within this call, CeMOS will provide access to Mass Spectrometry Imaging (MSI) for (quantitative) compound disposition studies, discovery and imaging of response markers and evaluation of toxicology findings. Focus areas include lipid/metabolite as well as infrared microscopy-guided MSI in fresh-frozen tissue sections, mainly in the therapy areas of oncology, neurodegeneration and metabolic diseases. MUAS offers state-of-the-art instrumentation for MSI sample preparation (HTX M5 and SunChrom sprayers) and analysis using high-resolution mass spectrometry on a Bruker solariX XR 7T FTICR and high-speed mass spectrometry on a Bruker rapifleX. Suitable data analysis tools are available (SCiLSlab and in-house bioinformatics).<br>Moreover, MUAS offers access to MALDI MS-based assays for drug discovery. Assay facilities include an Analytik Jena CyBio Felix pipetting robot for sample preparation and a high-speed Bruker rapifleX mass spectrometer for measurements. Assays are currently run in 384-well format and can be either biochemical assays with enzymes causing mass changes or cell-based assays where enzyme inhibition causes an accumulation of the substrate. |

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| USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA | BioFarma at USC aims at discovering new drugs by identifying chemical matter with pharmacological activity. USC focuses on drug discovery research and small molecules interfering with relevant mechanisms of disease.<br>USC offers expertise in chemoproteomics of functionalized chemical probes. USC has experience in the assay development, screening and target identification approaches. USC may support comprehensive biological characterization of probes for affinity-based target identification using chemoproteomics. |

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# 5 Abbreviations

CB1: Cannabinoid receptor type 1

CB2: Cannabinoid receptor type 2

COVID-19: Coronavirus disease 2019

ERIC: European Research Infrastructure Consortium

GF: Griseofulvin

HRMS: High-resolution mass spectrometry

LCMS: Liquid chromatography–mass spectrometry

LDNASA: *N*-acyl-*N*-alkyl sulfonamide

LDSP: *N*-sulfonyl pyridone

MALDI MSI: Matrix-assisted laser desorption/ionization mass spectrometry imaging

NBD: Nitrobenzoxadiazole

NMR: Nuclear Magnetic Resonance

PEG: Polyethylene glycol

PID: Project identification number

RI: Research Infrastructure

SDS-PAGE: Sodium dodecyl sulphate–polyacrylamide gel electrophoresis

TR-FRET: Time-resolved fluorescence resonance energy transfer

WP: Work-package

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