D4.2: Publication describing the performance of the fragment library in screening campaigns.

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1 Executive Summary

EU-OPENSCREEN-DRIVE (EU-OS-DRIVE) work-package (WP) 4 addresses the intention of EU-OPENSCREEN ERIC (EU-OS) to extend its capacities in the field of fragment-based drug discovery (FBDD) to complement its high-throughput screening (HTS) services, attract new user communities and foster its early drug discovery pipeline. Since August 2020, WP4 was actively involved in including chemical evolution of fragment hits into the EU-OS service portfolio and strengthening the collaboration with the structural biology community represented by iNEXT-Discovery and Instruct ERIC representatives. The assembly of the fragment library, procedures and rules on its usage have been part of the collaborative work in WP4. The main achievement concerns the implementation of fragment screening projects during the past two years, collection of the feedback on the library usage, and establishment of a medicinal chemistry consultation service. We demonstrate that, with fragments structures linked to the European Chemical Biology Library (ECBL), the step of going from fragment hit to small molecule hit is largely facilitated. A manuscript reporting the design of the European Fragment Screening Library, results of successful proof-of-concept fragment screening/ECBL follow up and statistics on the library performance has been prepared and it is currently under finalisation.

2 Introduction

EU-OPENSCREEN (EU-OS) is the European Research Infrastructure Consortium (ERIC) for Chemical Biology and early Drug Discovery, which was established in 2018 and offers access to state-of-the-art high-capacity screening and medicinal chemistry services throughout Europe for the development of small molecule tools and lead compounds. EU-OS services are currently provided by over thirty partner sites in ten member countries across Europe (CZ, DK, ES, FI, LV, NO, PL, PT, SE and DE as host country). It operates an open-access database, the European Chemical Biology Database (ECBD)[1], hosted in Prague at IMG, and a central compound management facility (CCMF) in Berlin, Germany which stores quality-controls and manages the jointly-used EU-OS compound collection.[2] The latter is comprised of about 100,000 commercially sourced compounds (European Chemical Biology Library (ECBL)), and a growing number of academically sourced compounds (European Academic Compound Library (EACL)), which are used in high-throughput screening (HTS) assays at screening partner sites.

In 2019, EU-OS-DRIVE started as a European Union HORIZON 2020 project to ensure long-term sustainability of EU-OS operations by promoting measures for i) widening awareness of academia and industry for its services and data, ii) growing capacity and competence in the field of chemical biology and early drug discovery across Europe, and iii) developing the management processes needed for a large, distributed infrastructure. In a dedicated WP “Extension of capacities 1: Fragment-based screening”, led by OSI, WP4 contributors worked at extending the scientific scope and technical capacity of the ERIC providing user access to fragment-screening and fragment-to-lead development support. Specifically, WP4 objectives were to i) extend EU-OS compound collections with a fragment collection to ensure scientific excellence; ii) extend EU-OS capabilities to include chemical evolution

[1] https://ecbd.eu/

[2] https://www.eu-openscreen.eu/services/compound-collection.html

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of fragment hits, iii) establish cooperation and exploit the complementarity between EU-OS and other life sciences RIs, especially Instruct-ERIC[3], which offers complementary expertise in structural biology.

Upon the end of the EU-OS-DRIVE project on 31.10.2023, all above objectives have been tackled and FBDD services are part of the EU-OS service portfolio as described in detail in deliverable 1.2 *"Handbook* and defined user workflows for new categories of partner sites"[4] (and available at EU-OS webpage).[5]

Fragment screening projects implemented during 2020-2023 in collaboration with Instruct ERIC and iNEXT-Discovery[6] sites generate the first comprehensive datasets to analyse the library performance. In this deliverable 4.2 "Publication describing the performance of the fragment library in screening campaigns" we report a non-confidential summary of results included in the publication entitled "*Design, Quality and Validation of the EU-OPENSCREEN Fragment Library Poised to a High-Throughput Screening Collection*" which is in preparation. Authors of this publication are researchers and collaborators from the following EU-OS-DRIVE and iNEXT-Discovery facilities:

- Research Group on Systems Pharmacology, Research Program on Biomedical Informatics (GRIB) at the Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain. – an EU-OS-DRIVE beneficiary.
- Center for Biomolecular Magnetic Resonance (BMRZ), Institute for Organic Chemistry and Chemical Biology, Goethe University Frankfurt (GUF), Frankfurt am Main, Germany – an iNEXT-Discovery beneficiary.
- Department of Biomedicine, University of Bergen (UiB), and Department of Chemistry, University of Bergen, Bergen, Norway – an EU-OS-DRIVE beneficiary and EU-OS partner site.
- Latvian Institute of Organic Synthesis (OSI), Riga, Latvia - an EU-OS-DRIVE beneficiary and EU-OS partner site.
- Fraunhofer Institute for Translational Medicine and Pharmacology (ITMP), Hamburg, Germany an EU-OS-DRIVE beneficiary and EU-OS partner site.
- EU-OPENSCREEN ERIC, Berlin, Germany – EU-OS-DRIVE coordinator.

[3] https://instruct-eric.org/

[4] https://drive.eu-openscreen.eu/fileadmin/user_upload/DRIVE_D1.2_Handbook-UserWorkflowsForNewCategoriesOfPartnerSites.pdf

[5] https://www.eu-openscreen.eu/index.php?id=240

[6] https://inext-discovery.eu/

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3 Report on the deliverable

The new European Fragment Screening Library assembled and acquired within WP4 is part of the current EU-OS ERIC compound collection[7] and it offers a unique value by being poised to the EU-OS high-throughput screening small molecules library (ECBL). The fragment library is stored at the CCMF at EU-OS central hub and fragment structures are made available in the ECBD.[8]

The publication is a comprehensive report of the overall work performed in WP4 going from the description of the computational design of the Fragment Library to the usage of the library in user projects with the highlight of a case example. By making the EU-OS fragment library available to the worldwide research community, we were able to collect valuable information on library performance in a variety of projects on multiple diverse targets that is serving to refine the contents of the fragment library. In sections 3.1-3.5 we report a summary of the work reported in the publication:

3.1 Fragment Library design

The fragment library was designed by EU-OS-DRIVE computational chemists from IMIM and Fraunhofer institutes with input from collaborators of the iNEXT-Discovery and Instruct ERIC sites, which provided expertise on large-scale fragment-based screening using diverse structure-based assessments including crystallography and NMR.

In June 2019, a workgroup comprising EU-OS-DRIVE WP4, iNEXT-Discovery and Instruct ERIC representatives met in Riga, Latvia at OSI facility to discuss and agree on a series of priority criteria to select the minimum number of candidate fragments that would represent the widest coverage of the ECBL. The following criteria were applied: i) fragments link to the ECBL; ii)) diversity of fragments; iii) inclusion of minifrags; iv) maximisation of number of vectors; v) size of similar fragment clusters; vi) inclusion of fluorinated compounds. Based on the design principles, selection of commercially available fragments was performed by Fraunhofer and IMIM. The selection methodology used is described in detail in the publication.

A total of 1056 compounds were acquired. 88 of those compounds are “minifrags” (ultra-low molecular weights fragments). As described by *H. Jhoti* and coworkers, these smaller fragments are shown to be largely applicable and a powerful tool for identifying energetically favourable interaction points on proteins.[9] The property profile of the final Fragment library is compliant with the Rule of three (Ro3) and fits well with the property profiles reported for some of the most recent fragment libraries developed.[10] After analysis of the most common functional groups and linkers in bioactive

[7] https://www.eu-openscreen.eu/services/compound-collection.html

[8] https://ecbd.eu/

[9] O’Reilly, M. *et al.* Drug Discov. Today 2019, 24, 1081–1086.

[10] Cox, O. B. *et al. Chem. Sci.* **2016**, *7*, 2322-2330; Wollenhaupt, J. *et al*. *Structure* **2020**, *28*, 694-706; Bührmann, M. *et al. J. Med. Chem.* **2023**, *66*, 6297-6314.

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molecules, we found that 4.2% of the fragment library contains the top-10 most common functional groups. The Fragments are stored and delivered to partners as 100 mM solutions in d_6-DMSO, while minifrags as 1000 mM solutions in d_6-DMSO.

Therefore, the main design concept is depicted in Figure 1, and it shows the possibility to quickly follow up fragment hits by searching the ECBL for small molecules containing the fragment substructure of one or more fragments. Moreover, users can benefit from the access to EU-OS chemistry partners to start in a timely and cost-effective manner their fragment-to-lead optimization projects.

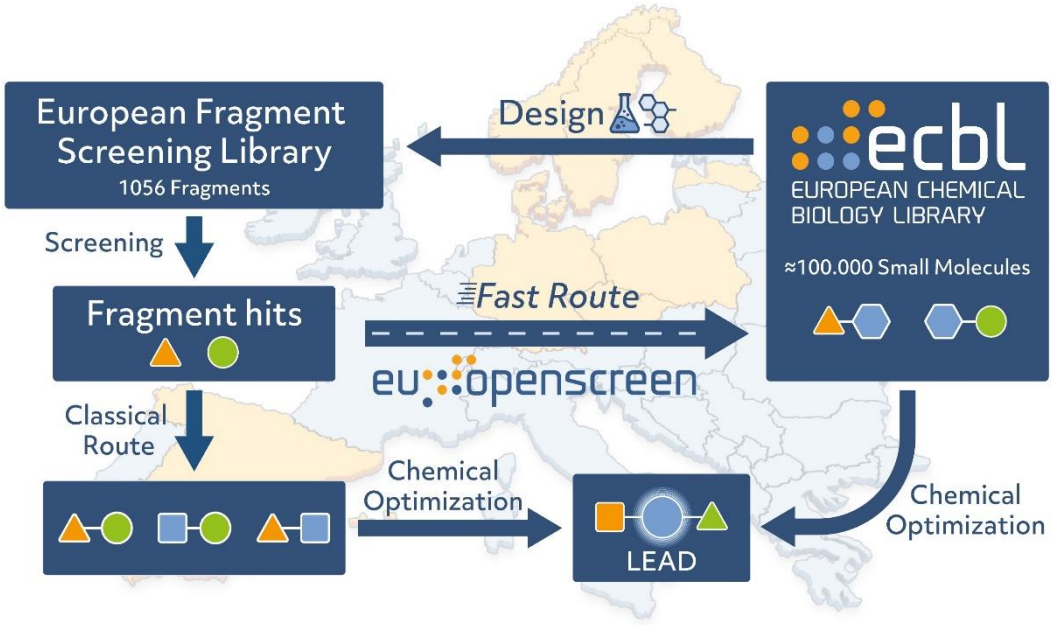

Figure 1: Graphical representation of the fragment library design concept and the fragment-to-lead optimization

3.2 Quality control of the fragment library

To assess the suitability of the fragment library for screening campaigns, quality control (QC) of the fragment library was performed by partners at GUF. They applied NMR methods and protocols for quality assessment which were previously utilized with success and benchmarked for fragment libraries.[11] Integrity and solubility of fragments in physiological buffer was assessed by ^{1}H-NMR spectroscopy of the individual fragments with a final concentration of 1 mM (or 10 mM for the minifrags). Detailed protocol is reported in the publication. 86.5% of fragments successfully passed the QC analysis, while 13.5% failed due to solubility issues and additional or missing signals in the spectra. Identified poorly soluble fragments and the fragments that failed the QC will be replaced by more stable and soluble fragments in a future improved version of the fragment library.

[11] Sreeramulu, S. *et al. J. Biolmol. NMR* **2020**, *74*, 555-563.

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3.3 Fragment library access

The fragment library is available to the research community, both from academia and industry, to be screened in user projects. Users are coming from both, EU-OS and Instruct ERIC user communities.

By joining forces EU-OS and Instruct ERIC partners offer a whole pipeline of services and resources to implement FBDD projects, from fragment hit identification and confirmation to follow-up support on fragment growth and optimization, including access to biochemical assays and chemoinformatics tools, which greatly accelerates chemical probe discovery and FBDD.

3.3.1 Case study

To proof the library design principle, we included in the publication a success story that highlights the usage of the fragment library and the quick follow up of small molecules from the ECBL.

Partners at UiB screened the fragment library using bio-layer interferometry (BLI) against a target for antibiotics to discover starting points for hit optimization. The case study identifies µM fragment hits, of which binding to the protein of interest was confirmed by X-ray crystallography. Subsequently, we rapidly explore the ECBL virtually screening of small molecules containing the fragment hit as a core substructure. 147 compounds from the ECBL were cherry picked by the CCMF team at EU-OS and tested using the BLI assay at UiB. Twelve compounds were progressed to a dose-response experiment, from which seven compounds had K_D values below 100 µM and showed selective binding to the desired target. Two of them had slightly more potent confirmed affinities than the original fragment hit and were shown to bind by co-crystallization.

3.3.2 Summary of screening campaigns using the fragment library

The fragment library is available to researchers for their fragment screening projects at several EU-OPENSCREEN and iNEXT-Discovery and/or Instruct ERIC partners.[12] The access to the fragment library is managed by the EU-OS ERIC which acts as a single point of contact for interested users. EU-OS central office monitors the screening projects using the fragment library, including those implemented at Instruct ERIC facilities. After the fragment screening, users can benefit from the access to EU-OS chemistry partners[13] to start fragment-to-lead optimisation project in a timely and cost-effective manner. Fragment screening data will made available to researchers using open access databases.

To date, 22 requests for screening the fragment library were processed by the EU-OS central office and eight projects are completed. Applications address several disease areas from infectious diseases, cancer, depression, obesity-induced diabetes or chronic pain among others. Up to five different techniques were employed in this set of eight screening projects: two opted for X-ray crystallography as primary screening technology, another three used BLI, and the remaining three applied Small-angle X-ray scattering (SAXS), NMR, and thermal shift assay (TSA). Detailed information on the average hit rate obtained in the screening campaigns and hit criteria used for each screening technology will be published as part of the manuscript in preparation. Looking at the low hit rate of one BLI project we

[12] See https://www.eu-openscreen.eu/index.php?id=240

[13] https://www.eu-openscreen.eu/services/medicinal-chemistry.html

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could confirm that the minimum presence of fragments containing carboxylic acids (three out 1056) in the fragment library can limit the performance of the library on specific targets.

Four out of eight fragment-based screening campaigns took advantage of the poised feature of European fragment screening library and ordered parent fragment-containing compounds present in the ECBL to the CCMF. Some of the users exploited the consultation service offered by EU-OS chemistry partners to get support for hit evolution and search of ECBL compound containing the desired fragment structure. Two out of four projects benefit from the “fast approach” and identified interesting binders after testing the ECBL small molecules in relevant assays proving the benefit of the library design.

4 Conclusions

WP4 integrated results from multiple sites and screening campaigns and actively monitored the library performance to improve the library in the future. The results are summarised in a publication in preparation which will be submitted in 2023. The usage of the library has been widely promoted during the last three years within the EU-OS-DRIVE, iNEXT-Discovery and Instruct ERIC consortia and to their communities. On the long-term EU-OS central office will continue collecting valuable information on library performance by monitoring the progress and outcome of screening campaigns using the fragment library.

5 Delivery and schedule

This deliverable was postponed to the end of the EU-OS-DRIVE projects to collect experimental data for the assessment of the library performance (screening campaigns and data analysis requires up to several months to be completed) and thereby maximizing the impact of the results.

6 Glossary

BLI: Bio-layer Interferometry

CCMF: Central Compound Management Facility

CIPF: Centro de Investigacion Principe Felipe

CSIC: CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS

DRC: Dose-response curves

d_6-DMSO: deuterated Dimethylsulfoxide

ECBD: European Chemical Biology Database

ECBL: European Chemical Biology Library

EU-OS: EU-OPENSCREEN ERIC

EU-OS-DRIVE: EU-OPENSCREEN-DRIVE

ERIC: European Research Infrastructure Consortium

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FBDD: Fragment-Based Drug Discovery

GRIB: Research Program on Biomedical Informatics

GUF: Goethe University Frankfurt

^{1}H-NMR: proton NMR spectroscopy

HTS: High-throughput Screening

IMG: Institute of Molecular Genetics of the Czech Academy of Sciences

IMIM: Hospital del Mar Medical Research Institute

ITMP: Institute for Translational Medicine and Pharmacology

K_D: Dissociation Constant

mM: Millimolar

µM: Micromolar

NMR: Nuclear Magnetic Resonance

OSI: Latvian Institute of Organic Synthesis

QC: quality control

RI: Research Infrastructure

Ro3: Rule of three Ro3

SAXS: Small-angle X-ray scattering

TSA: Thermal shift assay

UiB: University of Bergen

WP: Work-package

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