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Crystallographic fragment screening workflow at HZB

The only method utilized routinely to discover starting points for ligand development that at the same time delivers detailed 3D-information for downstream ligand optimization is macromolecular crystallography. Typically, small organic compounds, called fragments, are screened against an optimized crystal system of a target protein, and detected by electron density differences compared to the apo protein, i.e. the protein structure without fragments present. This technique, crystallographic fragment screening (CFS), is widely established now in academia and indispensable in the pharmaceutic industry. However, its routine use requires specialized workflows including suitable fragment libraries, dedicated beamlines able to handle large amounts of samples and largely automated software solutions for concise analysis of the results.

At Helmholtz-Zentrum Berlin, we provide the full workflow as well as practical guidance for our users conducting CFS campaigns. We prepared the 96-membered F2X-Entry Screen, an efficient and CFS-focused fragment library, as ready-to-use plates with dried-in compounds that can be used with or without DMSO present in the soaking experiment. In addition, special tools like the EasyAccess Frame, an evaporation protection device for crystallization plates, speed up the crystal handling. The HZB beamlines BL 14.1 and BL 14.2 are both equipped with state-of-the-art photon-counting detectors as well as with robotic sample changers, enabling enhanced throughput. Processing of the data is achieved via FragMAXapp, a user friendly, browser-controlled tool employed at several CFS facilities that manages in and output of many processing, refinement and hit identification software pipelines that are routinely used for CFS nowadays.

Taken together, HZB provides a workflow for routine crystallographic screening experiments via fragments. The intertwined workflow components and gathered expertise increase the chances for successful identification of fragments as starting points for the development of potent ligand molecules.

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