REPRESENTING FRAGMENT 3D-PHARMACOPHORE SPACE FOR EXHAUSTIVE SCREENING

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The key idea of fragment screening is that already a small selection of appropriate fragments (~10^3 cpds.) covers a much larger proportion of the overall chemical fragment space (~10^7 cpds.) than a typical high-throughput screening collection (10^5 – 10^6 cpds.) with respect to the drug-sized chemical space (~10^{63} cpds., *MW* < 500 Da). Moreover, in contrast to larger molecules, fragments may bypass strict steric requirements for the binding, leading to high hit rates up to 20%. For the same reason fragments often find a a well-suited anchor positions leading to low-affinity yet highly efficient binding and making them excellent starting points for subsequent ligand design, with the inherent potential to reconstruct the larger lead- or drug-sized chemical space.

Customarily, different biophysical pre-screening methods are applied, often serially, to limit the number of fragments to a selection manageable by routine X-ray crystallography. However, a comparative study with our 361-fragment library [1] confirmed a limited overlap amongst pre-screening methods [2] that also missed many of direct crystallographic screening hits [1]. In contrast, we strive for an appropriate library design and use state-of-the-art virtual screening to equally increase effectiveness and enrichment at no extra work or cost of materials.

An optimal generic fragment library for diverse targets aims at fully exploiting the above described potential. Generally, physicochemical requirements delineated by the rule of three (Ro3) are important for fragment binding. Beyond that, however, a fragment library will only meet the expectations if containing adequate molecules that bear diverse pharmacophores, are chemically evolvable and representative in order to yield high hit rates and facilitate fragment-to-lead evolution for generic targets, allowing to reconstructing the desired chemical space.

To complement our established in-house fragment library, which led to 70 (20%) crystallographic hits for endothiapepsin [3,4], we compiled a set of 1271 high-quality fragments suited for crystal soaking, immediate fragment-to-lead evolution, prepared for computer-aided subset selection and fragment-to-lead evolution.

Because ligand binding pockets differ regarding their 3D shape and arrangement of potential interaction sites, we designed our general-purpose 3D-diverse fragment library for crystallographic fragment screening. Thus, aiming at a representative coverage of chemical space, all sufficiently available and biophysically suitable fragments (> 250,000 cpds. adopting > $1.4 \cdot 10^6$ conformational and molecular states) were clustered in groups of 3D-similar compounds. To this end, we calculated > 10^{12} pairwise molecular similarities based on the 3D overlap of volume and interaction features (charges, hydrogen bond donors/acceptors, aromatic ring, etc.) using the ROCS (rapid overlay of chemical structures) method [5] and used these similarities for a hierarchical clustering with the SPARSEHC algorithm [6]. Selecting favourable (Ro3-criteria, solubility, PAINS, etc.) representative fragments from each cluster allowed covering the available chemical space with fragments that are particularly suited for crystallographic fragment screening. Moreover, this strategy provides readily available 3D pharmacophore analogues for scaffold hopping and synthetic building blocks as starting points for drug discovery projects.

In addition, together with Jena Bioscience, we develop a subset of our fragment libraries consisting of crystallographic hits validated on multiple targets, which will be made available in 96-well format as an easy entry point for crystallographic fragment screening.

These libraries are part of the Frag2Xtal service facility for crystallographic fragment screening soon available at the semi-automated crystallographic BL14.2 at the BESSY II storage ring of the Helmholtz-Zentrum Berlin [7].

References

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