Integrating HTS and fragment-based drug discovery

High-Throughput Screening (HTS) and Fragment Based Drug Discovery (FBDD) have been considered as alternative approaches to drug discovery for enzyme targets. HTS tends to be a rather quick way of identifying potent chemical matter with reasonable likelihood while FBDD has its strength in systematically probing the active site of enzyme targets with a solved crystal structure. By demonstrating synergies in the HTS and FBDD processes we like to reposition these techniques as complementary approaches. We review recent developments in concept and process integration both from in-house and recently published work.

By Dr John Barker, Dr Thomas Hesterkamp and Dr Mark Whittaker

High throughput screening has been the leading paradigm of small molecule drug discovery for the biopharmaceutical industry throughout the last two to three decades. Companies have invested in hardware (liquid handling, robotics and read-out technologies), process integration and large compound collections of frequently more than one million compounds. Depending on the size of the organisation and their research goals, approximately 10 to 30 HTS campaigns are run per HTS unit per year, delivering approximately, after medicinal chemistry hit-to-lead (H2L) optimisation, five to 15 programmes with viable lead series on an annual basis (this assumes that every second HTS generates viable lead series compounds after H2L). During the 1990s significant flaws associated with an HTS approach became apparent, in particular the propensity for such campaigns to yield hits which frequently had lipophilic, 'drug-unlike' properties. In response to this, desirable lead-like and drug-like criteria for screening compounds were developed that have served as benchmarks for library design, HTS hit selection and downstream medicinal chemistry activities. Different criteria for screening compound profiles have been proposed and these include Lipinski's rule-of-five for oral absorption and Oprea's and Rishton's lead-like and drug-like concepts. In addition, the emphasis on early high throughput ADME/Tox profiling has provided the impetus for the development and application of *in silico* filters and predictive models to aid the selection of screening compounds that are likely to be free of toxicophores and display desired ADME profiles.

The industry has responded to the challenge of ensuring that appropriate compounds are screened and today HTS compound collections follow more closely the ideal of screening lead-like compounds rather than drug-like or drug-unlike compounds (**Table 1**). In parallel to this development, it has become recognised in the field that target classes with a high degree of mechanistic or structural knowledge can be better addressed with more rationale

approaches. The use of target-focused compound collections (enriched with typical chemical scaffolds of the corresponding target class) has been spearheaded for the kinase and protease target families. For these, the use of structure-based drug design is often applied to aid the H2L process in a more informed manner than by simple SAR exploration around HTS hits. The lack of productivity of HTS for certain target families and the early successes with structure-based drug design have paved the way for fragment based drug discovery, which arguably started with the development of the SAR-by-NMR™ technology by the Abbott group in 1996.

Differences between HTS and FBDD

The key conceptual difference between FBDD and HTS driven lead discovery is that FBDD does not rely on potency as the key and sometimes sole driving force for hit compound selection and progression. FBDD rather focuses on the efficient fit of a hit compound to a binding site on the protein target. To identify a compound that achieves the most favourable fit, it is argued that the preferred strategy involves the screening of small chemical fragments of compounds that do not come with superfluous functionality to sterically impair the optimal fit with the target. If many of the heavy atoms (ie non-hydrogen atoms) of a fragment are directly engaged in the interaction with the target protein, then the fragment is said to be an efficient ligand or binder. Such a small and efficient ligand is a superb starting point for medicinal chemistry, irrespective of its absolute potency. Indeed fragment IC_{50}s are frequently in the 0.1 to 1.0mM K_i/K_d range, which is two to three orders of magnitude less potent than typical HTS hits. Furthermore, FBDD usually involves a strong element of structural characterisation of the target:ligand interaction, typically by x-ray crystallography or alternatively by solution based NMR experiments. The structure determination of the target:ligand interaction is followed by an extensive phase of analysis by molecular modelling in order to derive design ideas for subsequent compound optimisation.

Table 1: Comparison of literature criteria for drugs, leads and fragments

COMPOUND PROPERTY	RULE OF FIVE CRITERIA	LEADLIKE CRITERIA	RULE OF THREE CRITERIA	REDUCED COMPLEXITY CRITERIA
Molecular weight	≤ 500	≤ 460	≤ 300	≤ 350
ClogP	≤ 5	-4 to 4.2	≤ 3	≤ 2.2
No of H-bond donors	≤ 5	≤ 5	≤ 3	≤ 3
No of H-bond acceptors	≤ 10	≤ 9	ND	≤ 8
No of rotatable bonds	ND	≤ 10	(≤ 3)*	≤ 6
Polar surface area	ND	ND	(≤ 60A^2)*	ND
No of heavy atoms	ND	ND	ND	≤ 22
No of rings	ND	≤ 4	ND	ND
LogS	ND	≥ 5	ND	ND
3D Pharmacophoric patterns	ND	ND	ND	GaP

*GaP = Gridding and partitioning; ND = Not Defined; *Useful additional criteria for fragment selection*

Another important differentiator of HTS and FBDD is the extent of sampling of the respective chemical diversity space which is less comprehensively achieved by lead-like or drug-like compound collections compared to that achieved by collections of fragment-sized compounds. 10,000-20,000 fragments can readily offer a substantial sampling of the chemical diversity space of fragment-sized compounds while, in comparison, even the largest HTS decks of greater than a million compounds only sparsely samples the chemical diversity space for lead- and drug-like chemical matter. However, a key advantage of HTS-based drug discovery is that the greater potency of the hit molecules ensures that the discovery of inhibitors/modulators with cellular activity can be achieved with a reasonable probability of success in a rather short period of time.

Clearly then FBDD and HTS have different key strengths and we consider that these techniques are complementary rather than (mutually exclusive) alternatives. An organisation may decide to run a HTS campaign against a novel target to rapidly gain access to chemical matter for target modulation and validation in cells or even *in vivo*. Likewise, the same organisation may decide to run a FBDD project on a well exemplified and highly competitive target where the novelty of the chemical matter and predictability of success in generating lead series compounds are key prerogatives. Additionally, aside from these project specific tactical considerations, there may be technical aspects that predetermine the choice of approach (**Figure 1**): is there an assay available that suits the requirements for HTS? Is the target amenable to structural biology and has it been previously crystallised? Is the crystal structure of sufficient resolution to support structure-based drug design? Have high-capacity protein production routes been described that give access to sufficient starting material for crystallography and/or NMR?

Areas of overlap between HTS and FBDD

FBDD has fallen within the domain of direct biophysical screening techniques that have a high degree of sensitivity for detecting weak binders with a correspondingly low rate of false-positive hits. While the clear distinction between weak specific binding and non-specific binding can be blurred, direct biophysical screening techniques are information rich and allow the target:fragment interaction to be characterised in terms of binding affinity, binding stoichiometry and also binding site if HT crystallography or protein observed NMR are used.

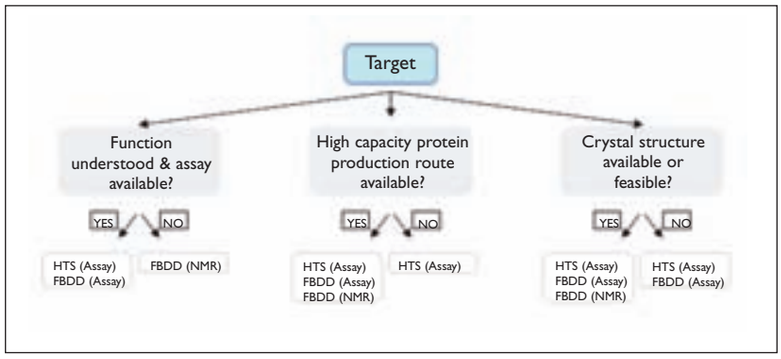

Figure 1

In concert with NMR, we are using a confocal fluorescence correlation spectroscopy approach for fragment screening. This technique was initially conceived for its application to HTS but since then has been adapted for effective fragment screening. This approach combines high-throughput with high-sensitivity and we are in a position to screen large compound files of more than one million compounds and complement that with fragment screening at an elevated compound concentration. Details of this screening platform are summarised in **Table 2**. Certain screening groups within the pharmaceutical industry have started to either flag the fragment-like compounds in their HTS screening decks for separate screening at higher concentration or to develop specific fragment screening sets (typically 5,000-10,000). These are routinely screened as part of HTS campaigns (in parallel to the main HTS screening collection), albeit at concentrations in the 0.1 to 0.5mM range. Eli Lilly and Co, Novartis, GSK, AstraZeneca, Roche and Abbott among others are either applying or seriously exploring the application of high concentration screening of fragment-like molecules by bioassays as part of their screening paradigm. The rationale behind this is to significantly increase the probability of success in the early hit discovery and subsequent H2L optimisation. Often, high concentration screening by bioassay may be accompanied in a complementary manner by well established approaches to FBDD undertaken by specialised biophysics and structural biology groups outside the core HTS screening units.

The benefits of a dual HTS and FBDD approach

We consider that there are at least three benefits in pursuing a dual HTS and FBDD approach and these are discussed in the following.

Table 2

Liquid handling	Piezo-electric pipettor/dispenser
Detection technology	FCS+*plus*, single-molecule detection
Assay formats	Catalytic and binding assay formats
Assay results	IC_{50}/K_i
Assay volumes	1µl/well to 30µl
Compound transfer	DMSO or DMSO-free
Compound concentration range	Up to 1.5mM
Size of libraries	250,000 (HTS); 20,000 (FBDD)
Secondary assay formats	NMR; LC/MS; Thermal Shift; Others

Firstly, there are target-specific, empirical factors and every organisation will have multiple examples for targets that have failed a traditional HTS approach. There are cases for instance in the area of aspartyl and cysteine proteases and in the protein:protein interaction field that have consistently failed to generate viable leads from HTS. Frequently this can be attributed to a general mismatch of lead-like or drug-like chemical matter and the relevant catalytic pockets (proteases) or interaction hot spots (protein:protein interaction). In fact, for proteases, the side chain pockets for the substrate specificity residues may be better addressed individually with fragment-sized compounds, with lead compounds being derived by subsequent fragment linking or merging. This is conceptually a better strategy than gambling on a HTS generated hit series that would satisfy the binding requirements of multiple sub-pockets at the same time. There are other targets outside the protease field that show a very good fit to fragment-sized molecules and have consequently benefited enormously from the application of FBDD: for example the iNOS, nNOS and eNOS family of enzymes, carbonic anhydrase, D-amino acid oxidase, certain kinases and ATPases (including JNK1, CDK2, Hsp90 and DNA gyrase), hematopoietic prostaglandin D2 synthase, some phosphodiesterases and many more. Not knowing the drugability of one's target at the outset, a dual-strategy can significantly de-risk the early discovery of chemical starting points without substantially affecting timelines and budget.

Secondly, fragments may represent minimum pharmacophores or discrete pharmacophoric elements and thereby enable various strategies for optimisation for enhanced binding including:

- Computational search approaches (such as in silico docking of available compounds structurally related to the fragment hits).
- Merging and/or linking of relevant fragments.
- Fragment growth.

Fragment hit data can be used in combination with HTS derived hit compounds to develop preliminary SAR that helps to prioritise the fragment/hit series over, for example, singleton hits. The availability of fragments and HTS derived hits from one chemical scaffold may be considered as an ideal but certainly not unrealistic situation where the transition from screening into structural biology and chemical optimisation can be particularly smooth. From a more technical perspective, the prioritisation of hit compounds for ligand structure determination by crystallography can benefit from a dual fragment/HTS approach, too, namely in the situation where the relationship of potency to aqueous solubility is unfavourable for the fragment but less so for the corresponding HTS hit. In our experience not every target and crystallographic system will lend itself to the determination of ligand structures of weak (ie fragment) binders and in these cases the more highly functionalised HTS hit may form a viable alternative for key structural studies.

Thirdly, a dual HTS and fragment strategy enables staged screening, a relatively new concept which is a resource-friendly combination of diverse (Stage 1) and subsequent diverse plus focused (Stage 2) screening, using the hit information derived from Stage 1 to augment the outcome of Stage 2 with the corresponding hit analogues. Novartis have coined the term 'virtual fragment

linking' whereby a collection of fragment molecules is first screened at high concentration and based on the results a Bayesian *in silico* model is developed that is then used to cherry pick thousands of compounds predicted to be active against the target from their HTS screening collection of millions of compounds. This is reported to enable successful focused screening at lower cost (fewer wells are screened) and higher hit rates.

We advocate the use of a fragment collection for an initial diverse screen to direct the selection of commercial analogues containing substructure features of the fragment hits for supplementation of the HTS compound file used in subsequent HTS. A benefit of this approach is a significant gain in SAR information within the timelines of a HTS project. This benefit arises from the fact that the hit expansion work can be efficiently dealt with on the screening robots, thereby saving resource and time. Additionally, there is a greater level of data consistency achieved if all IC_{50}s/K_is are derived from one seamless process. Researchers at Vertex, pioneers of fragment and structure-based drug design, reported on their in-house approach of pre-screening novel targets against fragments, using NMR. No understanding is required of target function, using a NMR binding assay protocol, and the question of reagent development and assay development is only addressed if the NMR screen indicates a high degree of drugability of the target. Thus, NMR is used as a pre-screen to assess the drugability prior to committing resource to a HTS project. Once again, HTS can be augmented with compounds that contain substructural feature of the fragment hits.

In conclusion, there are great opportunities for using FBDD, particularly high concentration bioassays, in conjunction with HTS not only for target families that are amenable to structure-based design approaches but also for membrane bound targets where screening of fragments enables rapid generation of SAR information and hypotheses for more efficient H2L. Given the power of this approach and the potential to shorten the early phase of the drug discovery process providing robust lead compound series we expect that this will become a standard approach to hit discovery within the pharmaceutical and biotech industries. DDW

Dr John Barker *is currently Group Leader of the Protein Crystallography team at Evotec, a company he joined four years ago to establish the structural biology group and assist in the development of a fragment screening platform. Prior to that, he was Director of Structural Biology for PanTherix, an antibacterial drug discovery company. Dr Barker gained his PhD in Chemistry at the University of Bristol before moving on to work in the field of protein crystallography, with a focus on structure-guided drug discovery.*

Dr Thomas Hesterkamp *is the VP Fragment Based Drug Discovery of Evotec and oversees the technology platform and its project application. Previously, he headed the Biochemical Assays group of Evotec's Hamburg operations where the Biology Services Division is located. Before joining Evotec in 2000 he spent three years with the Swiss pharmaceutical company Arpida as a Research Scientist in the field of drug discovery anti-infectives. Dr Hesterkamp graduated in Human Biology at the University of Marburg, Germany where he also obtained his PhD in 1997 with work on cellular protein folding.*

Dr Mark Whittaker *is Senior Vice-President Drug Discovery at Evotec where he manages a large drug discovery collaboration and the groups of computational chemistry, structural biology and IT application. Prior to joining Evotec in 2001, Mark spent 13 years at British Biotech Pharmaceuticals where he led a number of medicinal chemistry programmes and was latterly Director of Chemistry. At British Biotech, Mark contributed to the discovery and development of six compounds that have progressed into human clinical trials. Prior to his career at British Biotech, Mark carried out post-doctoral research at the University of Oxford and at York University, Toronto and obtained a DPhil in Chemistry from the University of York.*