J Comput Aided Mol Des (2009) 23:449–451
DOI 10.1007/s10822-009-9293-0

GUEST EDITORIAL

Challenges of fragment screening

Diane Joseph-McCarthy

Received: 19 May 2009 / Accepted: 10 June 2009 / Published online: 30 June 2009

Fragment-based screening for lead generation has seen tremendous growth and success in the last few years. Furthermore, the careful design of fragment libraries has ensured both that the coverage of chemical space is as great as possible and also that the included fragments have desirable physical properties. This particular effort has thus enhanced the advantages of a fragment-based approach. Other technological advances and applications have increased the speed of the process, resulting in more successful case studies being presented.

Nonetheless challenges still remain. Weak fragment hits are often overlooked in favor of more potent HTS hits that may have poorer physical properties and ligand efficiencies. The timelines necessary to realize success with fragment-based screens can be long relative to other lead generation approaches, since fragment hits need to be given sufficient consideration and may require more cycles for optimization. A robust system for crystallography, which can be difficult to develop, can also dramatically affect the final outcome of a fragment-based lead generation campaign by enabling the determination of high-resolution complex structures that can serve as starting points for structure-based design.

Active research is helping to address these challenges. Computational approaches that can aid in the optimization process either through growing or linking of fragments continue to be developed and can play a significant role in reducing the time required for improving the potency of an initial fragment lead. Methods aimed at exploiting fragment hits for uses other than scaffold generation are also being established to take full advantage of this information as well as any associated structural information available for a project. For example, fragment hits can be merged onto an HTS scaffold during the lead optimization process. In addition, for a given target, potential pharmacophores can be derived from fragment hits and later used for virtual screening of databases to enable scaffold hopping.

Fragment positioning methods such as GRID [1], MCSS [2–4], SPROUT [5], MUSIC [6], LUDI [7, 8], and Superstar [9] have been in use for over two decades now and are typically employed during the early stage of lead optimization. These methods determine energetically favorable binding site positions for various functional group types or chemical fragments based on molecular mechanics or knowledge-based potentials. "Hot spots" can be calculated for a wide range of functional groups in a given target binding site/region. Such target-derived pharmacophoric points can also be used to guide docking calculations to more finely sample the relevant regions of a binding site (e.g., [10, 11]) or to perform pharmacophore searches of large databases. Caveat [12] and HOOK [13] were among the first "fragment-linking" computational approaches developed in the early 1990s. Newer generation computational methods continue to be developed (e.g., Re-core [14], Allegrow (Boston De Novo Design, Boston, MA, 2009), Confirm [15], MED-SuMo [16], and pharmacophore modeling for scaffold replacement in MOE (Chemical Computing Group, Montreal, Canada, 2009)) and successful uses of these newer programs are being reported.

Increasingly, standard molecular docking programs are being utilized to screen large databases of small molecules to created target-focused fragment sets for experimental testing either in a high-concentration biochemical assay or by biophysical means such as NMR, Biacore, mass spectrometry, or X-ray crystallography. Focused fragment sets

D. Joseph-McCarthy (✉)
Infection Computational Sciences, AstraZeneca R&D Boston,
35 Gatehouse Drive, Waltham, MA 02451, USA
e-mail: diane.joseph-mccarthy@astrazeneca.com

often are screened along side generic fragment libraries in an attempt to increase hit rates. Chen and Shoichet [17] recently reported the use of molecular docking to identify several millimolar fragment hits eventually leading to the first micromolar noncovalent inhibitors of class A beta-lactamase. It has also been suggested that smaller fragment libraries can be screened in advance of an HTS campaign as an indicator of the druggability of the target binding site to prioritize targets for larger scale screening and further study [18].

A number of successful examples of using fragment-based approaches to develop a drug candidate or a new lead series in a pharmaceutical project have been reported (e.g., [19]). Howard et al. [20] used a novel fragment-linking approach to develop a thrombin inhibitor. A subset of an in-house library was screened virtually against several conformations of thrombin to select a thrombin-focused fragment library. Based on the docking results, 80 fragments were selected for screening by X-ray crystallography following the pyramid-screening paradigm employed at Astex [21]. An overlay of the binders identified by X-ray crystallography revealed a clear opportunity for fragment linking. S1 pocket-binders were linked to an S2–S4 pocket binder, resulting in compounds with dramatically increased potency and selectivity versus trypsin. Astex has also recently described the fragment-based identification of a clinical candidate for the treatment of cancer that is a potent Aurora kinase inhibitor [22]. Using a similar approach, Card et al. [23] identified a new family of phosphodiesterase (PDE) inhibitors. A library of 20,000 fragments was screened by a high-throughput scintillation proximity assay against a set of PDEs and over a hundred hits were confirmed by X-ray crystallography through co-crystallization. One of the low-affinity hits was selected as a possible scaffold and a close analog of that hit was also co-crystallized with the protein confirming the expected binding mode. A virtual combinatorial library around the new scaffold was docked into the binding site and scored using an MM-PBSA (Molecular Mechanics-Poisson Boltzmann Surface Area) method to include solvation effects. This approach ultimately led to the design of a relatively low molecular weight, high potency inhibitor. At AstraZeneca a novel series of cyclic amidine-based inhibitors of β-secretase were discovered via a fragment based approach [24, 25]. NMR affinity screening was used to identify the initial hits that were evolved into micromolar inhibitors using a combination of X-ray crystallography, molecular modeling, surface plasmon resonance and functional enzyme assays. Fragment-based lead generation has also been successfully applied across a number of therapeutic areas at AstraZeneca [26].

In this special issue of JCAMD devoted to fragments, the range of topics covered includes the development of fragment screening libraries by Blomberg and coworkers, a comparison of experiment and computational "hot spot" mapping by Landon et al., fragment docking using Glide by Kawatkar et al., the use of fragment positions to identify pharmacophore features for use in database searching by Loving et al., the determination of target druggability indices by Chen et al., as well as case studies of fragment-based lead generation. The diversity of topics covered reflects the increasing use of fragment-based lead generation in drug discovery, and that computational approaches to augment experimental fragment-binding information are continuing to be developed and to have significant impact on drug discovery projects.

References

1. Goodford P (1985) A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. J Med Chem 28:849–857
2. Miranker A, Karplus M (1991) Functionality maps of binding sites: a multiple copy simultaneous search method. Proteins 11:29–34
3. Evensen E, Joseph-McCarthy D, Karplus M (1997) MCSSv2. Harvard University, Cambridge
4. Evensen E et al (2007) Ligand design by a combinatorial approach based on modeling and experiment: application to HLA-DR4. J Comput Aided Mol Des 21:395–418
5. Gillet VJ et al (1995) SPROUT, HIPPO and CAESA: tools for de novo structure generation and estimation of synthetic accessibility. Perspect Drug Discov Des 3:34–50
6. Carlson HA et al (2000) Developing a dynamic pharmacophore model for HIV-1 integrase. J Med Chem 43(11):2100–2114
7. Bohm HJ (1992) Ludi—rule-based automatic design of new substituents for enzyme-inhibitor leads. J Comput Aided Mol Des 6(6):593–606
8. Bohm HJ (1994) On the use of Ludi to search the fine chemicals directory for ligands of proteins of known 3-dimensional structure. J Comput Aided Mol Des 8(5):623–632
9. Verdonk ML, Cole JC, Taylor R (1999) SuperStar: a knowledge-based approach for identifying interaction sites in proteins. J Mol Biol 289(4):1093–1108
10. Joseph-McCarthy D, Alvarez JC (2003) Automated generation of MCSS-derived pharmacophoric DOCK site points for searching multiconformation databases. Proteins 51:189–202
11. Joseph-McCarthy D et al (2003) Pharmacophore-based molecular docking to account for ligand flexibility. Proteins 51:172–188
12. Lauri G, Bartlett PA (1994) Caveat—a program to facilitate the design of organic-molecules. J Comput Aided Mol Des 8(1):51–66
13. Eisen MB et al (1994) HOOK: a program for finding novel molecular architectures that satisfy the chemical and steric requirements of a macromolecule binding sites. Proteins 19:199–221
14. Maass P et al (2007) Recore: a fast and versatile method for scaffold hopping based on small molecule crystal structure conformations. J Chem Inf Model 47:390–399
15. Thompson DC et al (2008) Confirm: connecting fragments in receptor molecules. J Comput Aided Mol Des 22:761–772
16. Moriaud F et al. (2009) Computational Fragment-Based Approach at PDB Scale by Protein Local Similarity. J Chem Inf Model ahead of print

17. Chen Y, Shoichet BK (2009) Molecular docking and ligand specificity in fragment-based inhibitor discovery. Nat Chem Biol 5:358–364
18. Hajduk PJ, Huth JR, Fesik SW (2005) Druggability indices for protein targets derived from NMR-based screening data. J Med Chem 48:2518–2525
19. Schneider G, Fechner U (2005) Computer-based de novo design of drug-like molecules. Nat Rev Drug Disc 4:649–663
20. Howard N et al (2006) Application of fragment screening and fragment linking to the discovery of novel thrombin inhibitors. J Med Chem 49:1346–1355
21. Hartshorn MJ et al (2005) Fragment-based lead discovery using X-ray crystallography. J Med Chem 48:403–413
22. Howard S et al (2009) Fragment-based discovery of the Pyrazol-4-yl Urea (AT9283), a multi targeted kinase inhibitor with potent aurora kinase activity. J Med Chem 52(2):379–388
23. Card GL et al (2005) A family of phosphodiesterase inhibitors discovered by cocrystallography and scaffold-based drug design. Nat Biotechnol 23:201–207
24. Edwards PD et al (2007) Application of fragment-based lead generation to the discovery of novel, cyclic amidine beta-secretase inhibitors with nanomolar potency, cellular activity, and high ligand efficiency. J Med Chem 50(24):5912–5925
25. Geschwindner S et al (2007) Discovery of a novel warhead against beta-secretase through fragment-based lead generation. J Med Chem 50(24):5903–5911
26. Albert JS et al (2007) An integrated approach to fragment-based lead generation: philosophy, strategy and case studies from AstraZeneca's drug discovery programmes. Curr Top Med Chem 7(16):1600–1629