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WARR'S PIECE

Fragment-based drug discovery: what really works. An interview with Sandy Farmer of Boehringer Ingelheim

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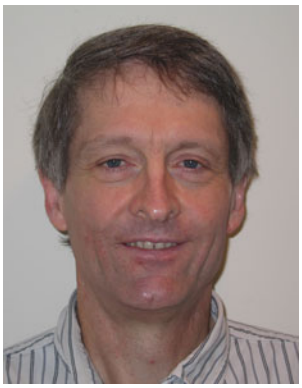

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Interview

WAW: Most of the people publishing in fragment-based drug discovery (FBDD) have a vested interest in advocating its use. What I want is an impartial view of how FBDD really fits in drug discovery in terms of timeliness, cost, and results. Where do you see fragment-based methods fitting in drug discovery?

SF: Boehringer Ingelheim (BI) pursues FBDD as an integrated drug-discovery effort, and not simply as another screening exercise to assess, for example, target druggability. In an integrated drug-discovery effort, the main difference between the fragment-based method and the traditional high-throughput screening (HTS) based method is the change in risk profile for the follow-on chemistry effort. In FBDD, the predictable chemistry risk is highest early on because fragments bind only weakly to the target and balancing their evolution is challenging, but in later stages of optimization, the chemistry risk has been markedly reduced, and is usually lower than with HTS-based methods.

The HTS-based method almost necessarily yields larger, more potent hits. The initial potency is such that high-throughput assays are usually quite sensitive to any changes introduced by chemical modifications, and the molecular size is such that the general binding mode to the target is relatively insensitive to those same modifications. Neither of these beneficial characteristics applies to FBDD. The challenge for FBDD is like having to locate a deep crater by peering across a flat landscape from very far away. HTS will usually put you close enough and perhaps high enough up to see one or more craters in the near distance; FBDD may place you too far away and too low on the horizon to see any at all. Applying structure-based

design methodologies to FBDD is like being given access to a satellite from which you can look down to locate not only any crater, but perhaps the deepest crater, no matter how far away it may initially be.

The chemistry effort on HTS hits starts with a small triage team to establish chemical tractability in one or more series, progresses to a larger team (4–8 chemists) in hit-to-lead (HTL), and then moves finally to a full-sized chemistry team in lead optimization. The chemistry to make the required analogs in a given series may not change dramatically or may only involve different coupling strategies to link various functional moieties. What's more, the preparation of such analogs is often amenable to parallel synthesis techniques. Design-enabling technologies such as crystallography and computational modeling, may not be ready in time to help with the selection of analogs. In most, if not all, pharmaceutical companies, the approval of an HTS campaign does not require that these technologies be enabled for the target or even, as in the case of crystallography, that a plan to do so be in place or organizationally supported.

In contrast, the chemistry effort on fragment-based screening (FBS) hits starts only after the design-enabling technologies are fully in place. FBS rarely benefits from parallel synthetic approaches early on, and should therefore be consistently resourced by a mid-sized team of synthetically skilled, structurally astute chemists with strong support from computational chemistry and structural biology. The pre-requisite for synthetic prowess arises because the initial hits are so small that analoging may require a number of different core chemistries and is not amenable to the more chemically simple linking approaches I mentioned before. The pre-requisite for structurally astute chemists comes into play because the traditional approaches and insights brought to bear by medicinal chemists in the progression of hits to leads are at best inefficient and at worst ineffective in FBDD. Teamwork among medicinal chemists, computational chemists and structural biologists is not just beneficial, it is mandatory. So, FBDD cries out for a different type of medicinal chemist.

WAW: What else is different?

The chemistry risk profile is yet another key difference between HTS-based and FBDD methods. Management expects a consistently progressive improvement in compound properties, such as potency, selectivity, solubility and other ADME-related ones. This is often easy to achieve in the early stages of HTS hit-to-lead due to a perhaps unfortunate focus on potency, but it becomes increasingly more difficult to achieve as the compounds mature to a point where potency is secondary yet they lie in a shallow "crater". By this time, any decision to terminate the project will be much more difficult because of the amount of effort already expended. This is a case of the momentum of commitment. In FBDD, however, the progression of hit to lead usually remains plateaued early on when, for example, 50 or more analogs may be made without any appreciable increase in potency, but with a significant amount of structural insight pointing to success being just around the corner. At that point, FBS begins to stand for "Faith-Based Synthesis". As a medicinal chemist, you now have to believe in that structural insight. And sure enough, when you least expect it, a fragment analog locks into the desired binding mode, SAR begins to develop with increased potency, and the neighboring opportunities for additional affinity line up.

In the end, the key to FBDD is not how well you can screen fragments: you could equally deconstruct a set of HTS hits into their corresponding fragments to determine the most efficient, irreducible binding element. The keys to FBDD are a medicinal chemist with the correct mindset, a chemistry team with the correct motivation, chemistry management with the correct expectations, and seamless integration of design-enabling technologies into the entire process. Having chemists in one department, and the enabling technologies in another rarely, if ever, produces the required seamless integration. Organizational structure and alignment are critically important to the success of FBDD.

WAW: Should FBDD be the primary approach or should it be used only when other methods fail?

SF: No! You cannot wait until another method fails. If you do that, you will lose the biologists' support before an alternative method can be tried. At BI, FBS is carried out in parallel with HTS, often with only slightly modified assay conditions for the high-concentration screening (HiCoS) portion. This parallel occurrence is facilitated because the HTS group also performs HiCoS on the generic and extended FBS libraries, usually involving a fluorescence polarization (FP) or robust catalytic assay. The generic FBS library contains about 2,000 fragments, and the extended one, an additional 6,000 or so. The Structural Biology group performs nuclear magnetic resonance (NMR) and surface plasmon resonance (SPR) screening on the generic FBS library, with the intent to apply both techniques to every suitable target. At the end of this multi-pronged FBS campaign, fragment hits are prioritized based upon the intersection of NMR, SPR and HiCoS hit sets.

WAW: Does FBDD have some other role, for example, testing the druggability of a new target?

SF: We will consider FBDD for all targets that are or can be structurally enabled, preferably by high-throughput crystallography techniques. Structural enablement is an absolute requirement, but the final decision to apply FBDD

to any such target is more complex. It also depends both on the geometric and evolutionary properties of the binding site and on the link between site occupancy and the desired functional effect. By evolutionary property, I mean whether the site is sufficiently unique in its pharmacophore profile to afford a foothold towards selectivity early on in the fragment-to-hit evolution process. For example, we currently avoid selecting kinases as FBDD targets because their binding pockets do not meet this evolutionary requirement.

What is definitely true at BI is that we do not use the screening part of FBDD as a standalone methodology simply to evaluate the druggability of a target. Fragments are used in a fully integrated drug-discovery methodology whose goal is to produce novel chemical matter suitable for progression to pre-clinical development: a lead candidate. It is fine to think of the first stages of FBDD as a more effective way to assess target druggability, but such an assessment should always be done with the *upfront intention* of generating a lead candidate.

At a conference about a year ago, colleagues from another company reported that early on, FBS had been used to prioritize genomics-derived targets for HTS campaigns. By restricting themselves to just FBS, they avoided the need for structural enablement of the target, but now that their company is dedicating chemists to the fragment hit-to-lead (FHTL) process, the goal for fragments has become exactly what ours is at BI: generation of a lead candidate. My take-home message from this is that it was a lack of chemistry commitment early on, and not the science or engineering behind fragment-based approaches, which dictated how fragments were leveraged in the drug-discovery space at this company.

We have found that FBDD has truly failed in only 2–3 targets out of over a dozen or so. Schering Plough even succeeded with BACE. We have also used FBDD to augment options in an advanced series with solubility or other ADME issues, but the requirement for such chemical options is far more stringent than that for developing an entirely new lead series. This use of FBDD has not been the most successful. For example, finding a suitable fragment to replace a problem moiety in one advanced series just led to a different problem later on, and by that time, traditional chemistry approaches had solved the original problem. So we see this particular use of FBDD as a niche application.

WAW: Should it be implemented for all projects or just select ones?

SF: As I said before, we will consider FBDD for all targets that are or can be structurally enabled. Note that structural enablement is an absolute must for us to proceed. As a next step, we evaluate the binding pocket. We do this computationally, based on existing structures or on high-confidence models. For protein–protein interactions, where the link between site occupancy and functional effect may not be deterministic, we look to develop an in vitro functional assay that can give us an early read on whether a weakly binding fragment is also a weakly, functionally inhibiting fragment. This assay does not need to be high-throughput, but should have the capacity to spot check the functional potency of fragments along the FHTL process. The absolute requirement for this assay is the ability to resolve functional IC_{50} values in the 20–200 μM range. In most cases, this assay simply shows when a binding event breaks up the interaction between the two protein partners involved in the protein–protein interaction.

Catalytic enzymes represent the classic example of FBDD targets. Many either are or can be structurally enabled with only reasonable efforts. The link between site occupancy and functional inhibition is both deterministic and highly amenable to modeling. Except in the case of kinases, selectivity is often afforded early on based on how the catalytic pocket has evolved to its particular function. We do not apply FBS to GPCR targets, although at least one other company has published some success along these lines. Even though X-ray structures of GPCRs are beginning to appear with greater rapidity, it is still true that each one is in itself at least a year-long effort. Bear in mind that for each FBDD project, we currently produce 30–50 co-structures before we have a lead candidate. That sort of throughput is simply not possible with GPCRs.

The other challenge with GPCRs, and this is mainly for agonists, is that the desired effect comes not just from any type of binding, but from a specific type of binding that induces the GPCR to undergo the required conformational change to drive the downstream agonistic events. FBDD can only identify binders; it cannot identify only this desired subset of binders unless sufficient structural guidance, and understanding, can be brought to bear in a triage step. Even if a cellular assay could be developed that would resolve functional EC_{50} values in the 20–200 μM range, the lack of sufficient structural guidance, and understanding, would dramatically increase the risk to any FHTL campaign.

We also struggle with nuclear hormone receptors (NHRs) as FBDD targets because these proteins are usually not structurally stable in the absence of a bound, high-affinity ligand, and the presence of such a ligand complicates the ability to detect weakly binding fragments to that now occluded binding site. Another complication is that these proteins enjoy a complex modulatory behavior through their interactions with various co-activator and co-repressor peptides. So, there is no simple way to identify the appropriate system in which to evaluate fragment binding, no simple way to measure weak binding events, and no simple way to link site occupancy with functional

inhibition. Taken together, these considerations dramatically increase the risk for an FHTL campaign against NHRs.

WAW: Large companies have talked about harnessing the complementary nature of FBDD and HTS, and so-called "high concentration screening" or "reduced complexity screening"? Is this a valid compromise?

SF: We believe it works. In 2007, we prosecuted MMP-13 by FBDD. We ran a virtual screen against our compound deck using a pharmacophore model constructed to identify fragment binders that engaged the S1'* region. The top 100 virtual, fragment-like hits were put through the HTS catalytic assay, but not at the standard HTS concentration. Instead, these virtual, fragment-like hits were tested at 100 μM. This was the key step. Another key to success was that the fragment-like nature of the hits, and their physicochemical properties, led to adequate aqueous solubility. We identified several real fragment-like hits, two of which yielded co-structures, as it happened, with unexpected, but useful, binding modes. One of the these hits was eventually evolved (between July 2007 and April 2008) to a sub-nM, highly selective compound that was tested in disease-relevant animal models.

This exercise established that our HTS compound pool does itself contain fragments that can support a successful FBDD campaign. The question then became: "How do we more systematically and reliably exploit this potential?" We cannot test all fragment-like compounds in this pool by our standard, multi-pronged FBS approach, since we estimate that there are around 75,000 such compounds. The throughput of the NMR and SPR assays is too low, the amount of protein required in a HiCoS FP assay is too high, and even the most robust catalytic assay may produce too many false positives, given the expected distribution of analytical purity within this large set of fragments. We could use virtual screening, but the results from MMP-13 suggest that while this approach can work, it did so in that case more by good fortune than by pure design.

So, our approach has been to select from this pool of 75,000 fragment-like compounds the ones that are the most diverse structurally and geometrically, are of sufficient quality (based on computational filters and subsequent experimental verification), and offer reasonable chemical starting points for evolution. The selection process led to the extended FBS library of about 6,000 additional fragments that I mentioned earlier. We could prosecute a fragment library of that size by our multi-pronged FBS approach and then use the resulting hits to mine for related fragments in the larger available pool of 75,000.

WAW: What is the cost relative to other methods?

SF: What is the cost of not finding a progressible hit to a validated target? And the cost of FBDD is small compared to the cost of identifying and validating that target, and insignificant compared to the downstream development costs later on. FBDD must be viewed as an investment opportunity, not a manufacturing process. And the business decisions surrounding FBDD should factor that in. FBDD is more about the opportunity cost (of not doing it) than the "run" cost (of doing it).

WAW: Are the results uniformly well received and implemented by drug discovery teams?

SF: Insuring that FBDD results are implemented by a drug-discovery team still requires that there are dedicated resources to bring the fragment hit to the point where it is undeniably a viable lead-candidate series. At BI, chemistry, structural biology, protein chemistry, biophysics, computational chemistry and compound profiling work as a highly integrated and balanced team to accomplish this goal. Because all of these disciplines are contained within the same, local department at BI, it is organizationally straightforward to focus these resources on achieving success in an FBDD campaign.

But a viable lead-candidate series does not guarantee acceptance by the full project team. Even project leaders who may be intimately aware of FBDD successes at BI can become caught back up in the push for project milestones, with its almost unavoidable focus on "driving forward the best series at all costs". Because management expects consistent progress and because potency is one of the easiest parameters to optimize, "best" during HTL has historically meant "most potent" because of the more limited chemistry resourcing. Over the past several years, BI has started to balance potency better against other key parameters during HTL by consistently incorporating many more ADME data very early on. That philosophy is a natural fit to FBDD, and is helping to drive acceptance of FBDD lead candidates by project teams.

WAW: What are the timescales of getting a lead?

SF: Some HTS-based projects go slowly and some go quickly. There are often issues with the production or purchase of suitable reagents, with the development of a suitable assay that is HTS compatible, and with the introduction of requisite counter-screens or triage methods, especially when the hit set is littered with many false positives or may be intrinsically highly promiscuous. HTS-based projects can go quickly, but examples usually fall into two categories. In the first there is no promising chemical matter in the hit set, and the project is stopped. In

the second, there is promising chemical matter in the hit set, at least from the perspective of having multiple and druglike series, initial SAR within each, and favorable experience (in past projects) for at least some of these series. In the first case, the question remains as to whether promising chemical matter could have been identified, but was not because of limitations in the chosen reagents, assay design or compound pool. In the second case, significant effort is still required, typically 6–9 months with on average 4–8 chemists, to assess the promise fully, and that assessment is by no means guaranteed to be positive, which brings you back to the question of whether better chemical matter could have been identified.

The situation is quite different with FBDD. Since upfront structural enablement is required, reagent concerns rarely exist. Given that a multiplicity of orthogonal assays (NMR, SPR, FP, catalytic) are employed to identify the initial fragment hit set, assay concerns are minimized, and prioritizing what to follow up on structurally is usually straightforward. A fragment deck is of higher quality and more druglike compared to the HTS compound deck. A fragment deck is recognized to cover significantly more chemical space, largely because of combinatorial considerations surrounding the achievable chemical diversity per irreducible binding element across the entire binding region presented by a protein target. Fragment assays are developed to be 10–50 fold more sensitive in resolving meaningful binding or inhibition compared to traditional HTS assays. All in all, a target for which FBDD yields no progressible fragment hit is most likely a target for which suitable chemical matter cannot be found. The confidence in this conclusion allows you to consider other options for prosecuting this target more aggressively, such as the use of protein therapeutics (new biological entities) to modulate the function of that target. It is worth noting that this analysis highlights why some companies advocate using FBDD methodologies to prioritize druggability of targets. While we at BI clearly recognize this use case for FBDD, we do not believe that it should be the primary goal for FBDD activities, if for no other reason than this lets the project teams off the hook to follow up on any promising fragment hits that are found. There are other reasons too.

WAW: What is the quality of hits relative to those from conventional screening?

SF: The quality of any initial fragment hit is on average higher, but more importantly, easier to recognize. The higher quality comes about because of the highly druglike nature of fragments (for example excellent solubility), and their small size which guarantees any detected binding or inhibition to be ligand efficient. In addition, our parallel assay approach to FBS enables a straightforward and reasonably deterministic process for hit prioritization. Fragment hits are by definition small and must therefore be efficient (and not always weak) binders. Their size does not allow the chemists to just swap out different moieties, as is often the the case for the larger, more modular HTS hits, so there is a clear, progressive benchmark expectation as these fragment hits are either modified or elaborated chemically.

In the end, the quality of a fragment hit lies less in the fragment itself, and more in how its properties and needs force a reshaping of the HTL process that will yield a more predictable end product. In this FHTL process, the fragment scaffold itself is often first optimized, not necessarily for potency, but for binding complementarity, alignment of chemical trajectories with binding-site opportunities, chemical novelty (most fragments are commercially available with no intrinsic IP), metabolic liabilities (yes, even initial fragments are checked for metabolic stability), and synthetic tractability. Chemically elaborating a fragment is more akin to sculpting than to building. There is an intense focus on establishing value for any added functionality, on preserving the overall appearance and properties of the elaborated fragment, and on globally re-optimizing after each elaborative step to insure that all aspects of the molecule are well aligned.

As a perhaps curious aside, note that HTS hits could avail themselves of this FHTL process if only such hits were first "deconstructed" into combinations of irreducible fragment elements. Those fragment elements could then be subjected to the FBS process, and the resulting fragment hits pushed through the FHTL process. We only need to insure that the respective HTS target is fully structurally enabled to support "reconstruction" during FHTL. In short, I would argue that it is the FHTL process that increases the ultimate quality of the lead candidate, and that this process could be put in play for HTS as well as FBS hits.

WAW: Are the derived series of molecules in any way superior to those from conventional screening?

SF: The concept of series does not really exist *per se* in FBDD, at least not until mid-way through the FHTL process when sufficient chemical elaboration and the presence of similarly behaving analogs have created a more "series-like" look and feel. Another point to keep in mind is that one fragment can be easily evolved into a number of different "series", depending upon core modifications, observed binding modes and elaboration choices. But back to your original question, yes, the derived series from FBDD are *more likely* to be superior to those from HTS, although that outcome arises mainly from key, and perhaps necessary, differences between the follow-on FHTL and conventional HTL processes.

WAW: What is the cost of entry?

SF: The cost of entry for HTS starts with a sufficiently large and diverse compound deck. To amass such a deck

takes time and money, which smaller companies may not have (especially time). HTS also requires highly specialized and expensive equipment, but the same can be said of FBDD when you think of NMR and crystallography. Amassing a fragment library, while much less expensive, also takes time. It took us at BI a year to have a minimally sized fragment library, and a full 5 years to have a properly sized one. Only now, almost 7 years later, are we hitting full stride. While FBDD can be substantially outsourced to companies that will perform NMR and SPR fragment screening, as well as crystallography follow-up, the same can also be said of HTS, and the outsourcing network required to support FBDD is more complicated. Few companies offer all the desired forms of fragment screening (for example NMR, SPR, FP and catalytic assays) as well as the high-throughput crystallography follow-on work. But in the end, it comes down to the compound deck: 1,000 well-chosen fragments provide a powerful starting point for FBDD. It would take at least a 100-fold more compounds, and of equal diversity, to achieve the same coverage by HTS. In the end, FBDD will always have a lower barrier to entry than HTS for a small company wanting to get into the drug-discovery space.

WAW: Are all the big companies doing FBDD?

SF: No. A significant number do currently, for example, AstraZeneca, Abbott and GSK. BMS does not, and I don't believe that Pfizer has enjoyed much success with FBDD. I also believe that J&J has discontinued their efforts in this area. But I think it is fair to say that all big pharmaceutical companies have at one time or another attempted or at least dabbled in FBDD. In most cases, the difference between success and failure has little to do with the process and supporting technologies (they work!), but rather much more to do with the organizational structure to support FBDD and the organizational mindset to accept the different risk profile and resource model behind FBDD.

WAW: How much experience and expertise is required?

SF: A lot! You need expertise in chemical informatics (to select the fragments), high-quality protein production (to supply the downstream screens and structural work), fluorescence spectroscopy, NMR spectroscopy, SPR, protein crystallography, structure-based drug design with its arsenal of supporting computational tools (for example, de novo design, ligand docking, and conformational analysis), synthetic chemistry, and the experience born of successes, or a boatload of faith instead. Even with skill sets in all of these areas and with sufficient experience, those first few iterations in elaborating upon an initial fragment hit are nothing short of art. The resulting compounds have to look right (in their binding mode), and they have to measure up right (in their assay data), but they also have to feel right. It's that "feel right" component which over-metricized departments miss. Successful FBDD still requires a strong gut feeling.

WAW: What is the best result that you have seen?

SF: We had a target with two HTS series. The FBS campaign did not get started until late in the game, and we did not have all methods enabled when we needed them. To speed up the process, we pushed any fragment into crystallography that hit in both the NMR and catalytic screens: first come, first serve as it were. A simple, commercially available compound was identified early on as a fragment hit, and its binding mode indicated that it could replace what was previously believed to be a required chemical motif for this target. This simple fragment hit was pushed through the FHTL process, and I would emphasize the word "pushed" because there was some resistance from the project team and management since we already had a "front-runner series from HTS". That project moved to lead optimization in late 2010, on the back of this fragment-based series. The best compound in this series, and there are quite a number, will most likely enter pre-development less than 8 months later; and the HTS series, while not forgotten, are well back in the hunt. Something similar happened with MMP-13, but in the end, there was no pre-development achievement for any FBDD series. Simply put, I have become a convert to FBDD. Back in the late 90s, I was introduced to the potential of FBDD, but I also saw how organizational constraints and a lack of cross-functional management support could basically kill any real potential for success. To the benefit of FBDD, BI has demonstrated a start-up company mindset with large company resources, and has been equally fortunate to have had the right management for the right people with the right ideas at the right time.

WAW: Is there a future for all the little companies [1] that have sprung up on the fringes of FBDD?

SF: About 10% of them will succeed. We have collaborated with a number of them. Plexxikon has succeeded by focusing on kinases with a hybrid approach between FBS and HTS, and with an almost relentless focus on ADME properties early on. In all cases, I believe that the key to success for such companies is to identify or construct some technology platform. It could even be as simple as to combine several existing technologies in a new way that affords a clear competitive advantage in at least one target-class area for those members whose therapeutic validation is high but whose druggability is still challenging.

WAW: I was looking for an objective viewpoint and you have certainly given me a very different set of answers from the usual ones. It is clear that you too are an enthusiast but I did appreciate hearing your comments on the cultural and managerial aspects of FBDD. Thank you so much for talking to me.

Reference

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