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Integrating biophysics with HTS-driven drug discovery projects

Rutger H.A. Folmer

Dept of Structure & Biophysics, Discovery Sciences, AstraZeneca R&D, Mölndal SE-431 83, Sweden

Over the past decade biophysics has become an established discipline in HTS hit triaging, owing to its high fidelity in detecting protein–ligand interactions. Many pharma companies are using biophysical techniques to filter HTS output for false positives, as will be discussed in this review. Moreover, I will demonstrate how the earlier application of biophysics, already at the HTS assay development stage, is potentially even more impactful. Two key areas here are early mode-of-action studies and ensuring that the HTS assay and subsequent cascade are fit for purpose. Top-level results from 20 in-house projects are shown to underpin the impact of these studies.

Introduction

The advent of biophysics in drug discovery started in the mid 1990s when Fesik and co-workers described how NMR could be used to screen very small ligands (fragments) for binding to target proteins [1]. These fragments could be developed, using structure-based design, to more-potent ligands in a process that they called SAR by NMR but that we now refer to as fragment-based lead generation (or fragment-based drug discovery) [2]. Various NMR techniques emerged during the late 1990s and early 2000s that can be used reliably to detect the weak binding that is typical for fragment-sized ligands (150–250 Da) [3]. The strength of many of these techniques is that, while detecting comparatively weak binding, they display very low false-positive and false-negative rates. In the early 2000s we and others started to realize that these high-fidelity NMR experiments could be used to profile binding of any set of compounds, and not just to screen fragment libraries [4,5]. In some cases, the experimental set up needed to be adapted, in particular if the compounds to be tested were expected to bind potently (low μM or better). But the outcome was the same: NMR could give a definite answer as to whether or not the tested compound bound to the protein (under the experimental conditions). This led to the first application of NMR in hit profiling, namely looking at compounds that behaved erratically in biochemical assays. A typical case would be compounds that came up as actives in one assay but did not show activity in another. NMR was trusted to identify which compounds were the true actives, by assessing whether they interacted with (bound to) the protein target. We started using the term target-engagement studies for these NMR hit-profiling activities, which turned out to be a very useful expression that project members could easily relate to.

This usage of NMR quickly became successful but at AstraZeneca (and probably elsewhere) project engagement was often largely on an ad hoc basis (trouble-shooting mode). However, as the impact of these profiling activities became increasingly apparent, there came a drive to do this more consistently in drug discovery projects. Indeed, several groups, from academia and industry, have since described how they applied NMR binding studies as a hit validation approach promptly after a HTS. For recent case studies see [6–8]. In addition, NMR has found applications in pharmacokinetic profiling and as an assay to assess compound promiscuity [9–11].

During recent years, several other biophysical techniques have matured and have been established in drug discovery as affinity-based screening and characterization tools, notably surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). This has been driven by hardware developments, such as the higher sensitivity and throughput of SPR and ITC instruments, and by the desire to address two major shortcomings of NMR, namely high reagent consumption and low throughput. Other

E-mail address: rutger.folmer@astrazeneca.com.

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novel label-free techniques are resonant waveguide grating [12], back-scattering interferometry [13] and microscale thermophoresis [14]. Differential scanning fluorimetry (also termed: thermal shift assay) has revived interest in recent years [15]. The technological pros and cons of these biophysical methods in drug discovery and the criteria on which to choose the best available method have been reviewed elsewhere [16–20], and will not therefore be the focus of the current review. Most pharma biomolecular NMR groups are now referred to as biophysics groups and, although NMR is still a core technology in many places, SPR and ITC have been almost universally added to the toolkit of high-fidelity technologies for target-engagement studies. Driven by the success and impact that these studies have had, biophysics is well recognized as an important discipline in support of drug discovery.

Here, I will describe how AstraZeneca has integrated biophysics in drug discovery projects that rely primarily on HTS for hit generation. The importance of post-HTS triaging will be highlighted, with focus on the timing of the biophysics activities; but, moreover, I will show why and how the company is using biophysics consistently before the HTS screen (i.e. during assay development). Having profiled hits from dozens of HTS projects during the past ten years, AstraZeneca has become convinced that there is much impact to be made by aligning biophysics resources very early to HTS projects, to assist in developing a robust, fit-for-purpose screening assay.

Biophysics post-screen: hit profiling

The first reports of integrating biophysics with HTS go back to the early 2000s [4], and both academic and industrial groups have published in this area [21]. What the reported case studies have in common is that a large majority of HTS actives did not display target engagement in the biophysics experiments, and these presumed actives were subsequently dismissed as false positives. For example, Hajduk and Burns show five projects where more than 98% of HTS actives tested did not show target protein binding in their NMR experiments [4] across four different assay types (enzyme, coupled enzyme, whole cell and fluorescence polarization). Jahnke and Widmer also describe several examples where HTS hits (some already advanced into chemistry programs) were found not to demonstrate target engagement but were false positives through various different mechanisms [5].

This highlights an important point, namely that these target engagement studies should be performed early on in the hit evaluation process. If only 2% of actives are genuine, and have the true potential to be developed into a lead compound, one cannot afford to wait with the biophysics until the HTS output has been triaged down to a handful of hit series, using typical criteria as potency, synthetic feasibility or physicochemical properties. For an excellent review on hit triaging see [22]. Preferably, biophysics is performed immediately when the dose–response data are available, and the compounds have been grouped into hit clusters (Fig. 1). NMR and SPR will usually have sufficient throughput to profile one or two representative compounds from each cluster, which is seldom more than 100. In particular, if the assay is sensitive to mechanisms that lead to false positives, filtering HTS output based on potency before doing biophysical characterization can be detrimental for the success of the hit-finding campaign. One can easily be drawn to potent compounds that later turn out to be oxidizing or covalently modifying or aggregating the protein. And once these compound clusters receive frontrunner status genuine binders could have dropped off the lists.

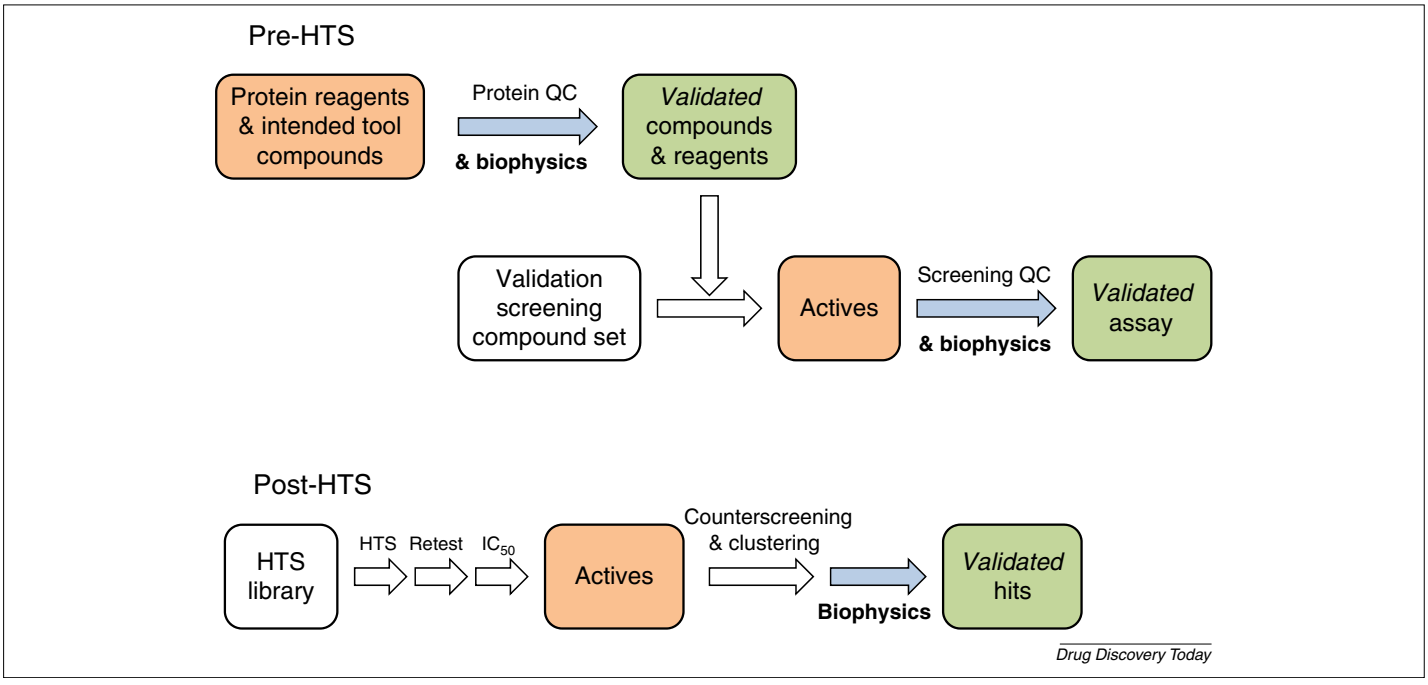

FIGURE 1

Integration of biophysics in HTS-driven hit-finding projects. Blue arrows indicate biophysical target-engagement studies. Orange and green boxes indicate raw and validated tools and results, respectively.

Experience shows it is often difficult to get renewed interest in those discarded, less potent compounds when the project makes the disheartening discovery that the top series turn out to be false positives.

Evenäs *et al.* describe an elegant case study from our laboratories where NMR was used as a counterscreen assay early in the HTS evaluation process [23]. In short, 2500 HTS hits with IC_{50} <10 μM were identified in a screen against xanthine oxidase (XO) and clustered according to 2D fingerprints and Tanimoto index. Representatives from these clusters were then profiled in an NMR-based binding assay. This showed that many clusters consisted of suicide inhibitors or redox active compounds. In the end, five clusters and one singleton displayed unambiguous reversible binding without oxidation of the compounds. This enabled the project team to put all downstream resources (chemistry, modeling, screening, DMPK) on compounds with the desired mode of action, which led to the development of a series of potent inhibitors of XO. Worth mentioning is that this type of hit profiling is also very impactful in a due diligence context. That is, ensuring that compounds that are being considered for in-licensing undergo biophysical target-engagement studies. A classical example in the literature where this would have been essential is the sirtuin story [24], but this is by no means an exception. We have seen several cases – during due diligence processes – where external molecules turned out not bind to the target of interest (data not shown). Of course, we have had projects where the majority of compounds identified in the primary screen that also passed the high-throughput counter assay(s) nicely showed target engagement in the biophysical experiment. Here, the value of the biophysical work is in providing confidence that the assay is robust, and that the emerging structure–activity relations will be trustworthy. In addition, it can help to understand the relation between IC_{50} in the assay and the K_D of the interaction, provide mode-of-action information from competition experiments or give initial insights in the kinetics of compound binding.

Finally, standard biophysical experiments (NMR, SPR, ITC) typically use protein from a recombinant source, and often with only a single domain being expressed. It is worthwhile reflecting on the relevance of such a biophysical hit validation when the HTS assay had a cellular readout. *A priori*, one cannot exclude that a HTS hit has a genuine and productive in-cell interaction with the target protein, which is simply not possible when the protein is taken out of its cellular context. This is particularly true for proteins that undergo significant structural changes upon complexing with their partner proteins (intrinsically disordered proteins being the extreme case), but this is not always known beforehand. So, does this compromise the use of biophysics downstream of a cellular HTS assay? Probably not, but more care should be taken in interpreting and communicating the results. Reference compounds are particularly useful in these situations. If they behave as anticipated in the biophysical assay (binding to the protein at the expected site), one would expect HTS compounds with similar modes of action to respond similarly in the biophysics experiment. If there are discrepancies that cannot be explained, one should of course be careful not to dismiss proper assay hits. Recent developments in in-cell NMR [25] and in-cell thermal shift assays [26] will help in answering these questions.

Biophysics pre-screen: assay development

The XO case study is a good example of the impact that post-HTS biophysics can have on drug discovery projects, which is all about ensuring that we focus all downstream chemistry efforts on *bona fide* compounds. Unfortunately, we have also seen many cases where, after the target-engagement studies, there were no valid compounds left. We have seen this across a variety of protein targets and disease areas, but it is most pronounced in projects where the intrinsic ligandability of the target was low, while the assay used was prone to artifacts – ligandability is the potential for a protein to bind ligands with high affinity [27], an aspect of druggability, which, in turn, describes the feasibility of finding a molecule that can modulate the target in a disease context. True binders to proteins with low ligandability will, almost by definition, have comparatively low affinity. The false positives can then display IC_{50}s that are one or even two orders of magnitude 'better' than the true actives, burying these true hits at the bottom of the list, or even down into the noise of the assay. The first five rows in Table 1 illustrate projects where this has happened. In those projects, the biophysics data have ultimately led to closure of the campaign. Although this is a depressing result, it is still an impactful use of biophysics that saves the company several hundred thousand US dollars per project by preventing downstream resource on compounds that are bound to fail six or 12 months down the road. We have come to realize that, in these projects, we could have had much more impact if we had done some hit profiling before the HTS. If we had demonstrated that the assay predominantly produced false positives, there could have been scope for the assay to be configured into a more robust format before running the full HTS. The last column in Table 1 summarizes our findings in these projects.

Reagents

In the past few years we have ensured alignment of biophysics early to HTS projects, basically from the start of assay development. Typical early investigations focus on the available tool compounds. In most cases, target engagement of these compounds can be confirmed, and they will be used as tools for biochemical, cellular or biophysical assay development. But we have come across cases where the biophysics experiment showed that the proposed tool compounds did not act as expected or intended, or that the protein batch used did not show the expected behavior. This can be as trivial as unexpected co-purification of a ligand or as startling as a literature compound not showing target engagement. Examples of such early profiling and mode-of-action studies are compiled in Table 2.

Worth highlighting from Table 2 is the example of the transcription factor. A compound was reported in the literature to bind to and inhibit the dephosphorylated form of the protein. This was based on mass spectrometer studies and, initially, we could reproduce these experiments in-house. However, in our NMR and SPR experiments we could not demonstrate target engagement for the compound. This prompted us to do more mass spectrometry, which eventually led to the finding that the compound binds to (and probably inhibits) the phosphatase that was used to dephosphorylate the transcription factor, and not to the transcription factor itself. This obviously disqualified the compound as a tool compound, and we prevented it from troubling biochemical

TABLE 1

Hit validation post-HTS.

	Assay type	Biophysical profiling	#cpds profiled	Issue observed	Impact of the profiling activity	Findings and/or scope for additional impact
Serine protease	Quenched FRET using peptide substrate	NMR, then SPR and ITC	30 representative HTS hits	Enzyme activity affected by metal ions such as Zn^{2+}. Conditions at which enzyme was found to be most active (30 μM Zn^{2+}) were chosen for HTS. This resulted in an output consisting mainly of Zn-chelators	Nonprogressable hits were quickly identified. No genuine inhibitors remained and the project was closed	Pre-HTS biophysical profiling would probably have captured issue with Zn^{2+} and flagged Zn-chelation as a problem. Different assay conditions should have been chosen for the HTS
Microtubule-associated protein	Monitoring fibril formation using thioflavin T binding	NMR	14	None of the hits displayed target engagement in NMR studies. Subsequent NMR data showed that compounds sped up consumption of DTT and glutathione. All hits were probably active because of redox behavior	No genuine inhibitors could be identified and project was stopped	Pre-HTS biophysical profiling would have identified redox problems, which would have reshaped the HTS assay (preferred) or resulted in a more rigorous secondary screening
Phosphatase	*p*-Nitrophenyl phosphate enzymatic assay	NMR	132	The NMR studies showed that none of the HTS-hits interacted with the protein with an affinity comparable to the enzymatic IC_{50}. Further analysis showed that a majority of the HTS-hits oxidized the reducing agents glutathione (used in the enzymatic assay) as well as DTT	With no viable hits the project was closed	Pre-HTS biophysical profiling would probably have captured the redox issues and enabled more-thorough assay development
Aspartyl protease	Secretion of substrate from cellular system	NMR, SPR, ITC, Tm	65	All biophysical assays showed a large drop-off from high potency in the cellular assay to weak affinities in the biophysical binding studies	None of the hit series were selected for further chemistry	Pre-HTS biophysical profiling would have identified and explained the drop-off. Would have prevented the HTS from being run with these conditions or setup
Oxidase	Cell-based assay monitoring inhibition of peroxide release by fluorescent dye	NMR	28	Majority of hits did not bind reversibly to the protein. Subsequently, it was shown that most had redox activity	None of the redox compounds were progressed chemically	Pre-HTS biophysical profiling would have identified redox problems, which would have reshaped the HTS assay (preferred) or resulted in a more rigorous secondary screening
Serine protease	Substrate-based chromogenic assay	NMR and SPR, then ITC	128	HTS hits did not show the expected affinity in Biacore and NMR studies and the active form of the protein failed to show any binding at all. Subsequent ITC studies revealed that the HTS hits targeted a latent form of the protein, not the active as was anticipated	Project could focus on desirable hits during hit evaluation	A limited number of ITC experiments during assay development would have informed upfront what kind of inhibitors could be expected from the HTS. Awareness of novel MOA could have been exploited through different assay design
Decarboxylase	Coupled enzymatic assay detecting NADH	NMR, ITC, SPR	183 in NMR. 104 were confirmed and further studied	Drop-off between potencies in *in vitro* enzymatic assay and cell-based assay, protein stability issues, protein behaving differently with different reducing agents and protein concentrations. ITC showed 20–70-fold lower K_d than the IC_{50} values from the enzymatic assay. Kinetic analysis pointed toward uncompetitive or mixed inhibition	A lot of time was spent during HE to understand the issues. Once solved, the HE was better informed, and compounds with the right profile could be progressed	Biophysical techniques correlated very well (NMR, SPR, ITC) but discrepancy with enzyme assay could have informed earlier about the importance of protein concentration on inhibitor potency by using tool compounds. Would have saved valuable time later in the project, and resulted in quicker and cleaner hit evaluation
Cysteine protease	Quenched FRET using peptide substrate	NMR	25 representative HTS hits	Only one single noncovalent compound could be verified by NMR. Although efforts were made to develop this compound the project ultimately failed to deliver any hit series despite >2 years of effort	Project could focus on the one compound that showed target engagement	Pre-HTS biophysical profiling would have shown that the rate of genuine binders would be extremely low. This should have changed the assay strategy

Each row represents a protein target that we supported with biophysical studies between 2009 and 2012. The protein class is denoted in the left-most column. These were projects where a HTS was run, and at some time during the hit evaluation process we became involved in profiling the output from that screen (or parts of it). The HTS assay technology is listed under 'assay type', and the biophysical technique used is in the column to the right of that.
Abbreviations: FRET, fluorescence resonance energy transfer; HE, Hit Evaluation; ITC, isothermal titration calorimetry; MOA, mode of action; SPR, surface plasmon resonance.

TABLE 2

Compound validation and mode-of-action studies.

	Biophysical technique	Compounds profiled	Observation	Action or impact
Reductase	ITC	A competitor molecule intended as tool compound	Competitor compound was believed to bind competitively with cofactor. ITC showed instead that compound bound most tightly when cofactor was present	Project lead-generation focus shifted from looking for cofactor competitors to substrate competitors
Transcription factor	SPR	Literature compounds	No target engagement could be demonstrated for these compounds in SPR. Subsequent MS experiments showed that the compounds instead bound to a phosphatase (which was used for dephosphorylation of the target)	False compounds were prevented from entering the assay development phase as tool compounds
Phosphatase	SPR, NMR	Intended tool compounds identified in biochemical assay	All compounds showed super-stoichiometric binding in SPR. Several compounds confirmed by NMR	Several more compounds were tested and, after validation with SPR and NMR, ultimately a useful tool compound was found
Dehydrogenase	ITC, SPR	A few tool compounds	Known cofactor-competitive compounds did not show binding. It was found that the protein had been unexpectedly purified with cofactor	This prevented a problematic protein batch being used during assay development and possibly HTS
Hydrolase	ITC, SPR	Literature compounds	Several tool compounds were found not to bind to the protein, whereas an ADP-ribose analog showed good agreement between SPR, ITC and assay IC_{50}	The ADP-ribose analog could be used as a validated reference compound, and less trustworthy compounds could be avoided
Helicase	SPR	Analog of a literature compound	Binding could be confirmed	Provided the first validated tool compound to the project
Bromodomain	NMR	Thermal shift assay hits	Profiled compounds aggregated the protein construct, which appeared to be their mode of action	This led to design a new and more stable construct, which was subsequently used in the project (and which showed that the thermofluor hits were non-binders)

Each row represents a project (protein target listed in first column) that we supported with mode-of-action studies on selected compounds. These were performed between 2011 and 2014, and typically the experiments were done during assay development for HTS. The last two columns show what was observed in the biophysical experiment, and how that impacted the project.

Abbreviations: ITC, isothermal titration calorimetry; MS, mass spectrometry; SPR, surface plasmon resonance.

assay development. It goes without saying that the earlier we discover that the reagents for assay development require attention the better. I should stress that such an early biophysics activity can be a relatively minor effort. Often a one-off ITC or NMR experiment is sufficient, and these are rather generic assay formats that are rapidly set up. Sometimes we configure an SPR assay but that same assay will later be used for hit validation, so we are only shifting the SPR assay development to an earlier phase in the project.

Assay conditions

HTS assay development at AstraZeneca typically involves a step where a small (about 7000) number of compounds is screened, the so-called validation set, which broadly represents the diversity of the larger deck (nearly 2,000,000 in size). The purpose of this prescreen is to assess assay reproducibility, technology artifact rate, false-positive and/or -negative rates, uncover possible plate patterns and give an indication of hit rate. Even with a high hit rate of 1%, this screen will yield no more than 70 hits, a number easily profiled with NMR or SPR. We always aim therefore to characterize the output from this mini-screen using a biophysical assay. Here, the goal is not to focus on the genuine binders, as we do post-HTS, but rather to understand why false positives occur. So, whereas we are addressing compound-specific issues in our post-HTS hit profiling (aggregation, solubility, redox, nonspecific or high stoichiometry, wrong compound, etc.), we are looking for assay-specific issues when looking at the mini-screen output pre-HTS. For example, a high number of redox actives among these prescreen hits could point to the need for a better control of the reducing conditions; or, if all hits looked like metal chelators, we should further investigate the role of Zn^{2+} in the assay. Alternatively, more generally, if the vast majority of the hits in the prescreen are false positives, one should at least evaluate whether one has the proper counter assays in place to secure a successful hit evaluation phase. All projects listed in Table 1 that failed or had trouble post-HTS would have had a good chance of being rescued if we had had our practice in place at that time to scrutinize the output of a mini-screen biophysically pre-HTS.

Table 3 illustrates a few (more recent) projects where we did run NMR or SPR profiling on the output from such a validation screen. As one can read in the last column, these activities were generally impactful. In two cases, it was decided not to run a HTS and in a third case the team went back and designed a new primary screening assay. In another two cases the biophysical analysis informed which assay of the available alternatives would be most likely to be successful, and finally there is one example where the

TABLE 3

Hit validation pre-HTS.

	Assay type	Biophysical technique	cpds profiled	Observation	Action or impact
Protein–protein interaction	HTRF using partner protein peptide	NMR, SPR	177 hits from a random 7700 screening set	Only 2 of the 177 hits showed genuine target engagement	It was decided not to run a HTS with this format (instead fragment-based discovery was chosen), resulting in a significant cost saving
Protease	Enzymatic using fluorogenic substrate	NMR	63 hits from 10,000 validation set	Very large proportion of hits turned out to be redox active	Screening cascade was redesigned to encompass a new redox assay directly after HTS
MAPEG-family synthase	HTRF and RapidFire (LCMS)	NMR	53 hits from 10,000 validation set	NMR could confirm 90% of RapidFire actives, but only 40% of the HTRF assay	RapidFire was chosen as the assay to go into HTS
Serine protease	Four different options were evaluated; chromogenic, coupled peptide fluorescent readout, direct peptide MS readout and fibrin clot assay	SPR, NMR	183 from 10,000 validation set	Three of four assays only picked up known and undesired motifs (e.g. benzamidines). The fourth assay found compounds binding competitively to an exo site	It was decided to pursue the assay finding the exo site binders, because this gave best opportunity for novel chemistry
Nuclear hormone receptor	FRET	NMR	183 hits from 10,000 pre-screen	NMR could only confirm 10% of the hits, so large number of false positives. Also, hit rate was low	Low rate in combination with false positives will require careful evaluation. Although there was confidence that this could work, it felt that likelihood of success was too low and an HTS was never run
Kinase	ADP-Glo and cell-based	SPR	~200 from 10,000 validation set	Assay picked up far too many reactive compounds (looking for non-ATP competitive)	Team decided not to run the HTS in either of these two formats

Projects are listed where we performed biophysical profiling on the output of a small validation screen before the HTS. These studies were run between 2011 and 2014. The column denoted 'assay type' shows the HTS assay that was used for this pre-screen, and to the right of that is listed the biophysical method(s) used for the profiling. The last two columns show what was observed in the biophysical experiment, and what impact that had on the course of the project.

Abbreviations: FRET, fluorescence resonance energy transfer; HTRF, homogenous time-resolved fluorescence; MS, mass spectrometry; SPR, surface plasmon resonance.

HTS progressed as planned but an extra counter assay was added to the screening cascade.

The past couple of years have seen an upswing in the generation of kinetic and thermodynamic data, and we have been applying biophysics to that effect as well. I believe the impact of those parameters is subtle and not fully understood at present [28,29], and this is outside the scope of this overview. However, there is one aspect related to assay development that deserves mentioning here, and that is to what extent the on-rate of compounds will affect the assay readout if too short incubation times are used. Phrased differently: is there a risk that the assay will miss compounds that display slow kinetics (slow association)? In a screening context, the answer is almost certainly no – because the on-rate is concentration-dependent and we are typically working with rather high (10 μM) concentrations in HTS. Assuming first-order binding kinetics (and no transport limitations, etc.) in a Langmuir binding isotherm model, at a compound concentration of 10 μM, even an active with an unreasonably slow on-rate of 100 M^{-1} s^{-1} will require no more than 15 min to achieve >50% binding. But of course, when in doubt, this can be addressed experimentally by running the assay in dose–response at two different incubation times on a small library subset, or on a tool compound with a known slow on-rate (as determined by SPR for example).

Lead generation approach

The first entry in Table 2 is a very good example of how timely biophysical studies can influence the entire lead-generation strategy of a project. In this particular case, the project originally aimed to target the cofactor site (NAD) in the reductase. This was based on a public compound reported to be competitive with NAD. Our in-house ITC studies, however, showed very clearly that this compound preferentially bound to the NADH-bound form of the enzyme. This was obviously at odds with the compound binding to the cofactor site, and the project team decided to change focus from cofactor competitors to substrate competitors. Likewise, more-conventional enzymology can play an influential part during the early phase of projects when it comes to defining the desired mechanism to approach the disease target [30,31].

Organizational aspects

The challenges with the activities described above are seldom of experimental nature. We use mostly established biophysical

approaches and, by combining results from more than one technique, we are confident that we arrive at the right conclusions. The key to making impact with biophysics in a HTS project is almost exclusively around planning, processes and team interactions. An obvious prerequisite is to engage biophysics very early with the project. We assign a biophysicist to a hit-finding team immediately after the target-selection decision. This scientist will make sure dedicated SPR (tagged) constructs are ordered, investigate the need for a target-definition compound [32], define the need for isotope-labeled protein for NMR and will find biophysics a place in the screening cascade. At this stage, the project team is multidisciplinary, with members from cell and protein science, assay development, screening, chemistry and structural biology. This provides the biophysicist with an excellent platform to make this early engagement. In our experience, it has been particularly valuable to have an NMR or SPR assay explicitly present in the screening cascade. This will ensure that, post-HTS, all compound clusters are profiled for *in vitro* target engagement, before any further triaging is done. In turn, this will help with resisting the temptation to zoom in on interesting looking chemical series and maybe even defining front-runners before the biophysical validation is completed.

Whereas it is straightforward from an organizational perspective to engage with biophysics pre-HTS to profile tool compounds and support assay development, it can be challenging to profile the output from the mini-screen that is run very close in time to the HTS. At this stage, a HTS screening slot has often been scheduled, and people are working to defined dates. It would therefore require good planning and communication between the biophysicist, assay development and screening scientists to make sure this important profiling step is done in time to have an impact. Arguably, this is more relevant for certain assays than for others. For example a kinase ADP-Glo assay is rather well-established and standard HTS quality criteria will go a long way to ensure a reliable screening output. But as the industry is moving more toward novel and frequently less tractable targets (often protein–protein interactions, e.g. E3 ligases) we are dealing increasingly often with less conventional assays. The observations listed in Table 1 should leave no doubt that spending an extra week to profile the output from a mini-screen is time well spent. If the result is satisfying we have only lost a week; if the result is troubling we would take a step back and consider our options – maybe do more assay development. Maybe we would still run the HTS with the planned assay but change the downstream cascade.

Assessing impact: cost avoidance

In drug discovery, niche technologies are often scrutinized for their value to the business, and one will inevitably be asked to put a figure on the impact of biophysics in drug discovery. After all, these are disciplines with significant capital investments, as well as appreciable running costs. To quantify the impact of applying biophysics early during assay development, it is useful to think in terms of cost avoidance. In a back-of-the-envelope calculation, we could consider two scenarios:

Case 1

HTS output resulting in only false series. This would cost four full-time employees (FTEs) over 12 months [reagent production, assay development, HTS itself, downstream hit evaluation work, compound (re)synthesis, computational chemistry, biophysics and the overhead of running the project].

Case 2

HTS where 50% of hit series are false. This results in an estimated 30% less chance of reaching the next milestone (lead optimization) and costs 30% of six FTEs over 18 months.

When we were not applying biophysics as early and consistently as we do today, we would typically observe on ten HTS: one 'case 1′ and three 'case 2′. Today, we should be able to avoid these altogether. Assuming a modest FTE rate of US$150,000, this amounts to a total cost avoidance of US$1.8 million per ten HTS, in working time only. Obviously, these are rough estimates, and different rates might be more appropriate for other organizations. But the take home message is very clear: rigorous application of biophysics during assay development is excellent value for money.

This is not different in a non-profit setting. The importance of well-validated assays and thoroughly profiled hit compounds is equally high in academic drug discovery. Arguably even more so, because the natural larger flux of personnel will make it more challenging to build experience around specialized or demanding assays, or to recognize a promiscuous compound from one project to the next one. Any academic hit-finding exercise should therefore also liaise with a biophysical group as early in the project as possible.

Concluding remarks

We have seen biophysics in relation to HTS projects evolve at our company from a trouble-shooting activity in the early 2000s to being systematically involved in hit evaluation in the late 2000s, and now also being applied consistently during assay development. This has been a logical development, considering the observations we have made throughout the years and the impact biophysics has had. We and other pharma companies are moving to operational models with fewer but more highly validated disease targets. The importance of working with well-validated compounds and assays will therefore only increase. Target engagement studies with biophysics should therefore not be restricted to post-HTS activities but should also be an integral part of any gene-to-assay activity.

Conflicts of interest

The author declares no competing interests.

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