

High throughput screening (hts) assays: uses and formats



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The increasing demands placed upon the pharmaceutical industry to produce a rapid turnaround of new drugs is a driving factor in the automation of the processes at the initial screening stage of drug discovery. This has led to the development of numerous high throughput screening (HTS) assays, with the increasing miniaturization of the whole process (1). An explosion in genomic and proteomic studies in recent decades has led to the generation of large numbers of functional protein molecules. The physiological function of these proteins has yet to be elucidated, but many could be important future drug targets, such as receptors or enzymes involved in disease pathogenesis (2). These 'orphan receptors' can be studied by high throughput screens of small molecules, which may be potential ligands. These chemicals can be sourced from existing drugs, pharmaceutical company chemical compound libraries or from natural products, such as plants or animals (figure 1; 3). Chemical libraries are now vast, since the advent of combinatorial chemistry, which produces novel compounds by high throughput methods. These mixtures can then be assessed for biological activity against a specific target molecule (most commonly a protein), either as a mixed chemical pool, or in parallel. A positive/active interaction, or 'hit', can then be further explored.

Numerous assay detection formats that are suitable for automation have been developed to detect such receptor - ligand and enzyme - substrate interactions, to allow the potential drug molecule to be further explored. Each assay has advantages and problems and the most commonly applied techniques are discussed in this review. As research progresses these processes become modified to overcome problems created by the

progressive automation and miniaturization of the assays. Use of computation to analyse the interactions and extract more information from them is also increasing (4). Recent advances in the literature suggest that future development of HTS is likely to result in ultra-HTS assay formats, which may be within closed systems such as glass capillaries, or on silicon wafer chips.

References

1. Fonseca MH & List B (2004) *Curr Opin Chem Biol* , 8, 319-326.
2. Gilchrist A (2004) *Expert Opin Ther Targets*, 8, 495-8.
3. Bleicher KH, Bohm HJ, Muller K, Alanine AI (2003) *Nat Rev Drug Disc* 2, 369-378.
4. Kato R, Nakano H, Konishi H, Kato K, Koga Y et al. (2005) *J Mol Biol*, 351, 683-92.

Techniques in molecular biology, chemistry and their associated branches are advancing at a rapid rate. This has enabled the mechanisms underlying many diseases to be explored at the molecular level. The ever-increasing sophistication of proteomic and genomic research procedures are producing an explosion in the number of possible drug targets. Until the development of high throughput screening (HTS) assays, the time taken to evaluate the potential bioactivity and usefulness of compounds to act on target molecules and become drugs to act to ameliorate symptoms or even cure or prevent a disease from occurring, was a rate-limiting factor. Since the automation of a number of suitable assays for HTS the trend has led to the number of compounds available for testing against targets becoming the limiting factor.

This has spurred the growth of combinatorial chemistry, to such an extent that many consider it to be a branch of chemistry in itself.

HTS can be defined as an automated method of conducting a large number of *in vitro* assays on a small scale (Patrick 2005). Most commonly, 96-well plates of 0.1 ml are used for a number of bioassays to detect the biological activity of compounds which have the potential to be developed as drugs. These may interact with the target, as a ligand-receptor interaction, or may involve inhibition of an enzyme or interaction with a nucleic acid macromolecule. The reaction produces a detectable output change, which can be detected and/or measured. Thousands of chemicals can easily and quickly be screened this way, and only active compounds taken to the next stage of testing to find out if it has the potential to become marketed as a drug. There is increasing pressure on drug companies to produce new drugs to keep pace with developments in medical research, as well as an increasingly demanding public and share holders. HTS technology is a crucial to meeting these demands, and continues to be developed to produce faster, cheaper and more efficient ways of screening compounds during the initial stages of the drug discovery process.

ROLE OF HIGH THROUGHPUT SCREENING IN DRUG SCREENING

Molecular biology techniques are allowing us to understand more about the mechanisms of disease, thus providing biomacromolecular targets for potential drugs to interact with. Such targets include receptors, enzymes and nucleic acids and may require inhibition (enzymes) or agonistic/antagonist receptor ligand binding to produce the desired pharmaceutical effect.

In addition, studies using proteomic and genomic techniques are revealing more and more 'orphan receptors'; these are proteins (predominately), lipids and nucleic acids (and to some extent carbohydrates) that are now known to be produced by the body, but their messenger and function is unknown. Using these as targets against which to screen compounds will help to elucidate their function, and more importantly, may turn out to be drug - target interaction sites which will be beneficial in disease. As the function of these targets is unknown, there are no lead compounds that could be used as a starting point for exploration, so HTS is particularly beneficial for screening vast numbers of compounds in the hope that at least one will interact with the mystery target.

The number of potential drugs to be screened is vast; pharmaceutical companies have libraries of 0.5-2 million synthetic compounds (King 2002) that have not made it through screening to become marketed drugs. There are also commercially available libraries of compounds, such as the Chemical Abstract Service (CAS) registry file, which contains 39 million compound (Abraham 2003). Intermediates in synthetic processes to make another drug should also be screened, as they may have the desired pharmaceutical properties. Isoniazid is an intermediate and has now been developed into an effective anti-tuberculosis drug. Existing drugs are also worth screening, as their biological activity may stretch beyond that for which they are intended. For example, cyclosporin A was isolated from soil and had been intended for use as an antibiotic, until its immune-suppressive properties were observed and for which it is now sold. The body's own endogenous chemical messengers, such as morphine, which has similar activity to released

endorphins, could also be screened as they may provide a lead compound that can be modified to enhance activity.

Combinatorial synthetic processes can also be used to generate vast numbers of novel compounds, which is crucial to prevent the availability of new compounds being a limiting factor in drug development, as HTS is able to screen them so quickly (Carell et al. 1995). It is common for combinatorial synthesis to produce mixtures of compounds, which can be tested as a chemical pool or batch by HTS for biological activity against a specific target macromolecule. This means that thousands of compounds can be screened in a very short time and only pools containing biologically active constituents screened further. This usually involves deconvolution processes (such as micromanipulation, recursive deconvolution or sequential release from resin beads used for the synthetic process) to identify exactly which component(s) of the pool is/are active, so that they can be isolated and screened further for drug potential (Wilson-Lingardo et al. 1996). It is now being superseded by production of new compounds in parallel, with a single component in each well. Potential drug molecules can also be derived from natural sources, such as plant extracts, but these are less abundant as isolation and purification take time. They are often novel, complex molecules and can produce unexpected interactions. An example is artemisinin, an effective antimalarial drug developed from extracts from a Chinese plant; it has a highly unstable trioxane ring (Ploypradith 2004).

There are so many compounds to be tested against a large range of potential drug targets that high throughput methods are essential to test the

numerable combinations of drug and target interaction to find those that are biologically compatible.

HIGH THROUGHPUT SCREENING ASSAYS

In theory any assay that can be performed on the laboratory bench could be automated and scaled up to be used for HTS. In practice, however, some assays are intrinsically more suitable than others. High throughput screens require the automation of the entire process. This is best achieved if there are as few steps as possible in the assay; ideally the test should be able to be performed in a single well, with addition of the test sample the last key step. Obviously, it is important that the reaction between a target and biologically active compound must be readily detectable. It should ideally be detectable with high sensitivity while the products are still mixed together in the well, rather than needing further steps to separate or purify components of the reaction mixture. This can be difficult to achieve with automation and will increase the time taken per test, so becoming less efficient and cost effective.

Assays used for chemical screening can be cell-free or cell-based (Silverman et al. 1998). Cell-free assays use solutions of relatively pure protein targets, such as receptors or enzyme substrates, which minimizes the number of steps required. It also allows for easy detection of biological activity in the wells of reaction mixture. Cell-based assays have the advantage that they are a closer representation of the normal physiology of the chemical environment inside a cell. Receptor-ligand interactions and enzyme inhibition reactions are more likely to be indicative of what will happen *in vivo*

(Silverman et al. 1998), especially if ligand-gated ion channels are involved. Cell assays also allow specific processes to be studied and the output can be measured. Indirect effects of small molecule/protein binding which trigger secondary messenger systems, such as calcium ions or cAMP, can also be observed in their biological context. Cells can be manipulated to express target molecules on the surface, so that they are available to bind to novel ligands, which may be tagged for detection. Cell assays can also provide additional information about cytotoxicity and bioavailability of a potential drug. Mammalian cells are expensive and can be difficult to culture in automated HT systems, but yeasts can provide a suitable alternative. Microorganisms such as yeasts are easy to propagate and have been demonstrated to have some homologous chemical processes, or can be easily genetically modified to express human processes accurately (Klein & Geary 1997).

HTS ASSAYS AND DETECTION FORMATS

- Fluorescence

Fluorescence occurs when a fluorophore molecule absorbs a high-energy photon (often in the ultraviolet range) and emits a lower energy photon, which is typically in the visible range of the spectrum. There are many naturally occurring substances which have this intrinsic property, such as luciferin in fire-flies. There are a number of fluorescence - based assays available for use in HTS, to detect whether an interaction has occurred between target and potential drug molecule during random screening. Fluorescence assays are generally sensitive, versatile, stable, safe and easy

to perform, which gives them a great advantage in automated systems for HTS. They have the disadvantage that quenchers can be present in the sample which dampen the light emission. There may also be background autofluorescence from free reagents in the reaction mixture (Grepin & Pernelle 2000).

Many of the assays have developed protocols that take these problems into account.

Energy transfer formats:

Homogeneous time resolved fluorescence (HTRF) uses the ion of the rare earth metal lanthanide (Eu^{3+}), bound to cryptate as a donor molecule. Following laser excitation (at a wavelength of 337nm), energy is transferred from this complex to an allophycocyanin (APC) acceptor molecule. This results in emission of light of 665nm, over a long period (milliseconds), which is recorded in a time resolved fashion so that any background fluorescence from free APC or media is not recorded. Peak emission of light occurs at 620nm for unbound Eu-cryptate, so the ratio of 620: 665nm emissions can be used to quantify biological complexes in solution (see Figure 1). This technique can be widely applied to screening programs and has already been developed into a 1, 536 well plate HTS, with plans to expand this to become ultra HTS (uHTS) and for use in cell-based assays.

Figure 1: HTRF schematic explanation

(from: Grepin & Pernelle 2000)

Fluorescence resonance energy transfer (FRET) is a slightly different form of detection, using the principle that excitation energy can be transferred between two fluorophore molecules. These can be different types of green fluorescent protein (GFP) or other bioluminescent molecules, such as luciferase (Hu et al. 2005).

from: Becker et al. 2004

Fluorescence polarization (FP)

FP can be used as the basis for homogeneous HTS assays for enzymatic and ligand-receptor binding interactions. The principle behind this detection assay is that when polarized light hits a small molecule that is binding to a larger (target) molecule, there will be rotational diffusion of the light beam. This change induced by binding can be detected by measuring the light emitted in orthogonal and normal planes of the polarized light. There is no interference from absorptive compounds in complex mixtures, as can occur with other fluorescence based techniques, and FP is quick and easy. Because of this it is used widely in high-throughput screening systems. Kim and colleagues (2004) developed a FP assay for the molecular chaperone Hsp90 (heat-shock protein), which is believed to have a role in cancer. They validated the assay for a high throughput format using molecules known to bind Hsp90, such as geldanamycin. The assay can now be used to screen for novel inhibitors of Hsp90, which may lead to a cancer drug being developed. Stricher and others (2005) have developed a high throughput FP assay for the CD4 binding site of HIV-1 glycoproteins, such as gp120, which are crucial targets to protect against HIV infection. Their assay used a 384-well plate

and CD4M33, a mimic of host cell receptor antigen CD4, found on T helper lymphocytes.

Some studies indicate that FP assay technology can also be developed as part of a HT structure-activity (SAR) study. Newman & Josiah (2004) showed that FP is sufficiently sensitive to differentiate between high-affinity small molecule inhibitors interacting with the target and low-affinity ones, with Src kinase activity as a model.

FP can also be used in cell-based assays, in conjunction with confocal microscopy (Heilker et al. 2005), as it shows high sensitivity even at minute volumes of reaction mixture, down to femtolitres. This type of assay can be described as fluorescence intensity distribution analysis (FIDA) and measures the absolute concentration of both bound and unbound ligand, thus providing the data with its own internal control. FIDA has been used to explore ligands which bind to G-protein coupled receptors (GPCR), which are widespread throughout the body and involved in signal transduction for numerous cell processes. They are therefore important therapeutic pharmaceutical targets, and can be studied in association with membrane fragments from cells over-expressing GPCR or associated with virus-like particles.

Fluorescence correlation spectroscopy (FCS)

FCS uses a two-photon excitation to measure the relative fluorescence of different molecules within a homogeneous mixture, from which the amount of each can be calculated. The technique can be applied to measure the relative amounts of ligand bound versus unbound receptor molecules, or <https://assignbuster.com/high-throughput-screening-hts-assays-uses-and-formats/>

cleaved versus intact enzyme substrates. FCS can be conducted using minute reaction volumes, less than 1 femtolitre (fl) is adequate for this sensitive, fast assay, which can study interactions of single molecules (Sterrer & Henco 1997). FCS can also be used to study ligand-receptor interactions in live cells (Pramanik 2004), which allows reactions to be assessed, and to some extent the properties of the interaction quantified, in their biological context. The use of such live cell assays in a high throughput format will provide a wealth of information not observable in chemical solutions alone.

Many applications of FCS are conducted in conjunction with confocal microscopy, which allows interaction kinetics to be examined on a molecular level, by the changes in diffusion patterns of the excitation. Confocal microscopy uses a high numerical aperture lens to focus the laser, to provide excitation and produces minimal background excitation, which allows such minute quantities to be studied. It can be used to detect spatial and temporal interactions in live cells, increasing the amount of information that can be obtained and used for drug development (such as interactions with other cell components or pH effects within the cell; Zemanova et al. 2003)

The dual-colour cross- correlation spectroscopy method of FCS uses two different, spectrally separated, fluorophore molecules, which are attached to each of, for example, a receptor and possible ligand, or potential substrate to be cleaved. The two colours will be observed to fluoresce together if an interaction occurs, or separately if a substrate has been cleaved and the kinetics of this can then be assessed (Kettling et al. 1998). This can be demonstrated using, a DNA strand that has a red fluorophore molecule
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attached to one end and a green one to the opposing end. The strand is cleaved by restriction endonuclease enzyme *ecoRI*, which is detected by spectroscopy as a decrease in the quantity of DNA molecules with fluorescence at both ends. The method was shown to be suitable for this type of enzyme kinetics study, by accurate detection of catalytic activity down to an enzyme concentration of 1 pM (pico molar) and proper description of the reaction by the Michaelis-Menten equation. This method, dual-colour FCCS, therefore has great potential for HTS of enzyme and ligand binding reactions.

Biomolecular fluorescence/reporters

There are numerous molecules produced by plants and animals naturally that produce fluorescence, or what is sometimes called bioluminescence. Some of these have been adopted as research tools, such as green fluorescent protein (GFP), which produces green light at 509nm following excitation by blue light (Arun et al. 2005). The gene that encodes for this fluorescent protein has been elucidated and is now commonly inserted into the genomes of genetically modified microorganisms and cell lines. It is then expressed under the control of desired promoters, often as a fusion protein. In this way patterns of gene expression can be observed and changes in transcription detected. HTS for new drugs use this technology to detect changes in transcription that occur via secondary messenger systems following receptor-ligand binding in a live cell. For example, Changsen and others have validated a GFP microplate assay, using an acetamidase promoter associated with the *gfp* gene, to screen for antituberculosis drugs (2003), and found it to be suitable for HTS for novel drugs.

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GFP reporter technology requires a detection system and most of those described for detecting fluorescence from synthetic fluorophores can also be applied, such as FRET (Zhang 2004), FCS and confocal microscopy.

FITC (fluorescein - 5- isothiocyanate)

FITC can be bound to other molecules as a marker and the fluorescence measured robotically in HTS systems. For example, FITC bound to heparin sulphate (HS) has been used to screen for heparanase inhibitors in a HT assay: 384-well microtitre plates are used, which are coated with fibroblast growth factor (FGF). This captures the FITC-HS, and labelled fragments are only released into the media when cleavage by heparanase has occurred. This is quantitatively measured by robotic detection of the amount of fluorescence in solution. Heparanase is believed to have roles in inflammation, tumour angiogenesis and metastasis, so is an important drug target in the treatment of cancer (Huang et al. 2004).

- Chemiluminescence

Some assays using chemiluminescence have been adapted to use HT formats. These rely on chemical reactions to produce light emission as a side-product, which can be detected. One such HTS uses coupled reactions involving the enzymes acetylcholinesterase, choline oxidase and horse-radish peroxidase, in 96 and 384-well plate formats, to screen for novel acetylcholinesterase inhibitors to become new drugs to treat Alzheimer's Disease (Andreani et al. 2005).

- Scintillation proximity assay (SPA)

Scintillation proximity assays are used for quantitatively studying binding reactions. The receptor/target is bound to a surface such as a plastic bead. The ligand is labelled with radioactive isotope (typically H^3 or I^{125}) and emit electrons with a short range of about 10um. A scintillation counter under the surface to which the target is bound detects the ligand only when it is bound. When it is free in solution the media absorbs the electrons and they are not counted. This allows binding interactions to be quantified whilst at equilibrium. Zheng and others (2004) have used SPA as part of a HTS and have identified several novel inositol monophosphatase inhibitors, which may be developed as drugs for bipolar disorder.

- Mass spectrometry

Mass spectrometry is currently a popular option for HTS, as it is sensitive, selective and easily automated. It allows the activity, molecular weight (most drug-like molecules are 150-400 Da), elemental composition and structural features of a test compound to be analysed. This wealth of information is of great use for exploring the molecular interactions between target and potential drug compounds. A very high throughput can be achieved using flow injection analysis, which does not require any sample preparation. Solutions of the samples are sprayed, using electrospray or APCI (atmospheric pressure chemical ionization), which ionises the molecules in the sample, prior to analysis by the mass spectrometer. Sometimes tandem mass spectrometers are employed, to glean more structural information and elemental composition. The advantage of these techniques are that as well as being very high precision they can be conducted on the original sample, without the need to separate the compound out from a mixture.

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For LC-MS (liquid chromatography - mass spectrometry), semipreparative HPLC (High Performance Liquid Chromatography) is often used before the HTS to verify the structure and purity of each compound to be tested, especially those from a combinatorial library. Improving purity in this way facilitates more accurate observation of any biological interactions that occur between the target and the test compound, as well as easing interpretation of structural information about the test molecule or changes induced by the interaction (Abraham 2003). HPLC is easily automated and involves detection of a UV signal above a threshold level, which triggers collection of the fraction. Several fractions may be obtained from one sample, or the computers controlling sample collection can be programmed to detect only at peaks of desired molecular ions, following ionisation by a suitable technique, such as electrospray or MALDI (matrix-associated laser desorption/ionisation), which can be used as a gentle way of ionising more fragile molecules (Hillenkamp et al. 1991). LC-MS can be slower than other approaches but is sometimes necessary. Further developments to speed up the automated process include parallel LC-MS, in which multiple HPLC columns are interfaced to a single mass spectrometer (Kenseth & Coldiron 2004), and fast HPLC.

- NMR

NMR is a useful technique for exploring the 3-dimensional structure of biomacromolecules, in a concentrated solution. It is limited by the small size of molecule amenable to this technique; typically below 30kDa, so is more useful for small drug-like molecules than the molecular target they interact with. Structure-activity relationships (SAR) can be studied by observing <https://assignbuster.com/high-throughput-screening-hts-assays-uses-and-formats/>

alterations in a protein's NMR spectrum. This not only indicates that ligand binding has occurred, but can give an indication of the location of the binding site (Shuker et al. 1996).

- X-ray crystallography

X-ray crystallography enables the 3-dimensional structure of protein molecules to be studied, with resolution to the atomic level. The technique requires the molecule to be studied in its crystalline form, which is not a problem for the majority of biomacromolecules that are drug targets. Protein crystallization technology has also had to adapt to high throughput methods, so as not to become a bottleneck. Some fully automated systems can now produce as many as 2, 500 to 140, 000 crystallization experiments a day (Kuhn et al. 2002).

Studying the 3-D structure of the target often produces clues to the type of ligand that will bind, which speeds up the time taken to find lead compounds in drug discovery. An example of this is the development of antiretroviral drugs used to treat AIDS (acquired immunodeficiency syndrome), such as amprenavir ('Agenerase'), which followed from the study of the structure of the drug target, HIV (human immunodeficiency virus) viral protease. Another drug developed from such structure based studies is zanamivir ('Relenza'); a flu treatment based on the crystal structure of the surface glycoprotein, neuraminidase, which is crucial for viral infectivity (Varghese 1998). This is likely to be an important weapon in the fight against an influenza pandemic.

In X-ray crystallography, the macromolecular 3-D crystal is bombarded with X-rays, by a rotating-anode X-ray generator or a synchrotron, and the

diffraction pattern produced is detected. Multiple measurements of diffracted waves generate much data, which can be analysed using calculations, such as Fourier synthesis and a structure revealed (Blundell et al. 2002).

Advances in the structure determination process have aided the resolution of structures, for example, multiple-wavelength anomalous dispersion (MAD), in which selenomethionine is incorporated into proteins that are overexpressed by genetically modified micro-organisms, which simulates isomorphous replacement and allows the phases to be calculated (Hendrickson et al. 1990).

Low-affinity binding reactions between ligand and target may have important properties and provide leads that would be missed by other HTS methods.

Development of high throughput X-ray crystallography, by increased automation at all stages of the procedure, has led to its growing use in lead discovery as well as its more traditional role in lead optimisation (Abola et al. 2000). This enables the technique to screen compound libraries, including those from combinatorial synthesis. Crystallographic screening for novel ligands in this way has already had some success; for example, a new class of urokinase inhibitors have been discovered, for treating cancer (Nienaber et al. 2000).

Co-crystallization of receptor-ligand complexes allows the interaction between the molecules to be studied and conformational changes in the target, upon binding, to be discovered. This approach is known to be used by several industrial laboratories and has the capacity to compare the interactions of 'hit' ligands in the generation of a lead series. It also

decreases the time taken to explore hits, which is a crucial factor for the pharmaceutical industry (Abraham 2003). Another way of achieving crystallized receptor-ligand interactions is to soak the ligand, often as molecular fragments dissolved in DMSO (dimethyl sulphoxide), into the receptor protein crystal (Nienaber et al. 2000), and observe changes in electron densities indicative of interaction.

Structure based drug design *in silico*

Three-dimensional structures can also be used in computer modelling programs to predict which ligands might bond/interact with targets or receptors, as an initial stage of drug design. This is truly a high throughput method as computing power allows the rate at which ligand-receptor interactions can be virtually screened to be incomparable to even the fastest high throughput methods involving physical experimentation. This is often termed virtual ligand screening (VLS), or *in silico* screening (Klebe 2000). Perrakis and his colleagues (1999) combined automated protein model building with iterative structure refinement, using ARP (automatic pattern recognition), which has been crucial for structure based drug design (SBDD). Diffraction data is fed directly into the computer program and a protein crystallization model produced automatically.

Various programs have been developed to assess docking of virtual ligands into known target receptor sites and scoring of their suitability and fit, determined by energy. Some algorithms seem to have some bias towards certain chemical families; this can be reduced by using multiple docking algorithms simultaneously (Charifson et al. 1999).

Software programmers and chemical modellers must remember to take into account the natural properties of protein molecules, as they are more flexible and accommodating of small changes than the rigidity suggested by traditional computer programs, although some programs now attempt to recreate this (Schapira et al 2000).

Optimal results for structure based drug design are likely to be achieved by combining virtual and experimental methods, such as ' SAR-NMR' technology advocated by Shuker, Fesik and colleagues (1996).

- Microarrays

DNA microarrays have been constructed following the sequencing of the human genome, using cDNA to study thousands of genes. From this has stemmed the growth of proteomics and protein biochips, as these are the functional molecules encoded by the genome. Protein arrays/biochips consist of immobilized proteins, which can be used, in the drug development context, to study ligand-receptor interactions (Lueking et al. 2005).

Interactions of known pharmaceutical chemicals with proteins can also be explored. For example, Leflunomide (an isoxazole derivative) has been shown to have anti-inflammatory properties *in vivo*. Analysis of protein interactions using an array revealed that it was not only interacting with the suspected mitochondrial enzyme, but a number of other proteins in the cell, such as pyruvate kinase (Mangold et al. 1999). As the majority of drug targets are proteins, and many of the drugs themselves proteins too, protein arrays are likely to become more popular, as well as higher throughput.

CONCLUSION

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CHOOSING THE BEST HTS ASSAY:

The literature reveals numerous modifications and validated systems of all the possible assays that are suitable for adaptation to high throughput screening in drug discovery. Many of the traditional weak points of each assay have therefore been addressed in this way, making critici