Expert Opinion

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Advances in nuclear magnetic resonance for drug discovery

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Background: Drug discovery is a complex and unpredictable endeavor with a high failure rate. Current trends in the pharmaceutical industry have exacerbated these challenges and are contributing to the dramatic decline in productivity observed over the last decade. The industrialization of science by forcing the drug discovery process to adhere to assembly-line protocols is imposing unnecessary restrictions, such as short project timelines. Recent advances in NMR are responding to these self-imposed limitations and are providing opportunities to increase the success rate of drug discovery. *Objective/method*: A review of recent advancements in NMR technology that have the potential of significantly impacting and benefiting the drug discovery process is presented. These include fast NMR data collection protocols and high-throughput protein structure determination, rapid protein–ligand co-structure determination, lead discovery using fragment-based NMR affinity screens, NMR metabolomics to monitor *in vivo* efficacy and toxicity for lead compounds, and the identification of new therapeutic targets through the functional annotation of proteins by functional annotation screening technology using NMR. *Conclusion*: NMR is a critical component of the drug discovery process, where the versatility of the technique enables it to continually expand and evolve its role. NMR is expected to maintain this growth over the next decade with advancements in automation, speed of structure calculation, in-cell imaging techniques and the expansion of NMR amenable targets.

Keywords: drug discovery, fragment-based screening, metabolomics, NMR, structural biology

1. Introduction

A troubling decline (**Figure 1**) has been observed in the productivity and creativity of the pharmaceutical industry over the last decade [1-5]. This is a complex issue and a number of factors are contributing to the observed reduction in new drugs approved by the FDA. One primary cause is the fact that the drug discovery process is a fundamentally challenging endeavor with a high failure rate and price tag. Estimates indicate that only 1 new chemical entity (NCE) out of 25 NCEs identified from active research projects will become a marketable drug. The corresponding success rate of clinical trials is only 11% [6,7]. The development of the average drug costs > $800 million dollars [8], where current industrial trends to minimize these costs are contributing to the decline in new drugs [5].

Because of these high costs, pharmaceutical companies tend to focus their research efforts in therapeutic areas with potential profits exceeding a billion dollars a year [9]. These tend to be chronic diseases that require potentially life-time treatments for a significant percentage of the population, such as cardiovascular, oncology and CNS disorders. The end result is a concentrated effort in a very few research areas [10] leading to a high-level of competition, a limited number of new

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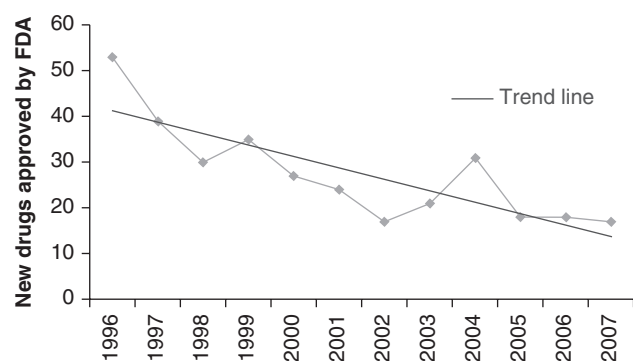
Figure 1. The number of new drugs approved by the FDA on a yearly basis. The straight line highlights the negative trend in drug approval rates.

therapies and a number of redundant drugs [11] serving the same market [12]. Similar efforts to minimize costs also results in a negative impact on the discovery of new drugs.

The expanding trend of applying traditional business practices to the drug discovery process is misguided and has the unintended consequence of diminishing output and eliminating originality [2,3,13,14]. Current methods being applied to reduce cost and increase efficiency include outsourcing [15,16], metrics [17,18] and limiting the duration of a research project [19]. This is despite the obvious fact that drug discovery is a complex, highly interdisciplinary process [20] and inherently unpredictable [21,22]. Treating each component as simply an independent step in an assembly line protocol [23] clearly undermines the need for routine interactions and the exchange of ideas between each essential discipline [24]. In drug discovery, the whole is clearly greater than the sum of the parts where critical breakthroughs and insights come from the cross-fertilization of ideas and information between separate research groups. Outsourcing and metrics isolate these groups and place a high priority on inconsequential book-keeping: the number of compounds synthesized, assays run, spectra collected and structures solved that creates the illusion of accomplishments [2]. Furthermore, placing artificial time-limits on research projects simply creates an endless cycle of identifying new therapeutic targets, generating chemical leads and rapidly abandoning a project when the typical challenges with bioavailability, stability, toxicity and efficacy are encountered. This focus on project lifetime evolves from the current drug discovery process that is based on high-throughput screening (HTS) and structure-based drug design [25,26].

HTS is an efficient and effective approach for identifying high-affinity binders to protein targets, but as recent reports have indicated it has not increased the number of successful drugs [27]. HTS chemical leads are not routinely translated into new drugs because a preponderance of inhibitors have undesirable modes of action [28,29]. Misleading compounds that lack a confirmed correlation between functional activity

and a direct binding interaction with the protein target routinely emerge from HTS [30]. These false leads are a significant factor in the delay or derailment of drug discovery projects and may contribute to clinical failures as well [28-32]. At the onset of a drug discovery program, it is clearly desirable to identify lead compounds that interact with the therapeutic target in a biologically relevant mechanism. In this manner, promising chemical leads can be quickly prioritized for more detailed evaluations, with an anticipated increase in the development of new drugs.

In an era where time is a critical consideration in the success of a drug discovery project, NMR has become an integral component of the process [33-35]. The diversity of NMR enables the technique to be applied at multiple stages along the drug discovery pathway and addresses some of these self-imposed challenges that are hindering progress in discovering new therapeutics [36]. NMR is being used for lead discovery and optimization, evaluating *in vivo* selectivity and efficacy, analyzing drug toxicity profiles and identifying new drug discovery targets. Recent advances in NMR technology enable NMR to rapidly determine protein and protein–ligand structures, to efficiently screen fragment-based libraries for identifying biological relevant ligand interactions and new therapeutic targets, and to monitor changes in the metabolome from biofluids and cell lysates for exploring *in vivo* drug activity. This review discusses these recent advancements of NMR for drug discovery.

2. Rapid protein structures

A high-resolution protein structure is a key requirement to evaluate the biological relevance of potential chemical leads identified from HTS. Validating that a compound binds specifically to a functional region of the protein structure dramatically increases the likelihood of the compound being evolved into a drug. Due to the increasingly limited time available for a research project to produce an NCE, obtaining a rapid protein structure during the early stages of the drug discovery project is essential. Despite the prevalent use of X-ray crystallography in the pharmaceutical industry for determining protein structures, NMR and X-ray should be viewed as complimentary techniques with limited redundancies [37,38]. Recent statistical analysis indicates that ~ 20 – 40% of protein structures determined from structural genomics may be amenable to analysis by NMR, where a preponderance of protein structures were determined by NMR only or X-ray only. More importantly, NMR had an equal success rate for both prokaryote and eukaryote proteins [39], which is an important consideration for drug discovery.

The application of NMR has also been unnecessarily curtailed because of the general upper weight limitation of ≤ 25 kDa. Nevertheless, the average domain size for eukaryotic proteins is ~ 150 residues or ~ 17 kDa, which is within the molecular weight (MW) range for NMR. Additionally, advancements in NMR methodology has significantly

expanded this upper limit, where the NMR analysis of large MW complexes are becoming common place: 900 kDa GroEL–GroES complex, 300 kDa cylindrical protease ClpP [40], 95 kDa homotrimeric complex of the acyltransferase protein [41], 82.4 kDa malate synthase [42,43], 69 kDa α1-proteinase inhibitor Pittsburgh-trypsin covalent complex [44], 45.3 kDa catalytic domain of human BACE-1 [45], 44 kDa nucleotide-binding domain [46], 486 kDa TET2 aminopeptidase protein [47] and 441 residue τ protein [48], among others. Obtaining these results requires advanced labeling and NMR techniques that includes deuterium labeling [49], selective residue labeling [50], selective methyl labeling [51] and TROSY-based experiments [52]. This requires a robust expression system (e.g., *Escherichia coli* or a cell-free system) to obtain milligram quantities of various labeled protein for multiple NMR samples [53-56]. Also, it is important to note that these studies did not yield high-resolution solution structures of the indicated large MW proteins. Generally, specific insights regarding the structure and dynamics of the proteins or complexes related to its biological function were obtained. This requires using existing X-ray structures. Chemical shift perturbations, residual dipolar coupling constants (RDCs) [57] and/or ^{13}C-labeled methyl probes are typically used to model protein–protein complexes (from existing NMR or X-ray structures of each monomer), identify protein–ligand interactions or monitor the dynamics of domain or ligand interactions. Low-resolution structures may also be obtained from a minimal nuclear Overhauser effect (NOE) data set [58,59] combined with other structural constraints such as RDCs [57] and pseudocontact shifts [60].

Similarly, significant improvements have been made to increase the throughput of NMR structure determination. This has occurred, in part, due to the NIH Protein Structure Initiative that has provided the incentive to develop the infrastructure and technology for high-throughput structure determination [61]. First, robotic systems have been developed for the automated production of protein samples for both NMR and X-ray [62]. Second, advancements in NMR pulse sequences and protocols for rapid data collection have shortened the time required to collect a complete set of NMR spectra for a protein structure. These methods include automated projection spectroscopy [63], G-matrix Fourier transform NMR [64,65], high-resolution iterative frequency identification [66], projection-reconstruction NMR [67] and reduced-dimensionality NMR [68,69]. The primary goal of these methods is to reduce the dimensionality of traditional multi-dimensional triple resonance experiments, and thus shorten the acquisition time, by joint sampling of multiple chemical shifts in a single indirect dimension. Generally, a series of low-dimensional NMR spectra are collected that are either linearly combined or used to reconstruct the desired higher-dimensionality spectrum. A general concern with reduced dimensionality experiments is the potential loss of information or resolution by projecting *n* indirect dimensions into a 2D spectrum. The encoding of multiple chemical

shifts within a single resonance results in peak multiplicity with distinct phasing. As the size of the protein increases, peak overlap and the canceling of anti-phase peaks becomes an issue. Of course, reduced dimensionality experiments have been successfully applied to proteins with an MW up to 20 kDa [64].

An alternative approach to reduce the acquisition time of multidimensional NMR experiments for protein structure determination is the application of sparse data collection or non-uniform sampling [70-72]. The major factor that contributes to the long (~ 1 – 3 days) acquisition times for typical triple resonance experiments is the requirement of collecting a uniform series of data points to properly represent the free induction decay (FID) along each indirect dimension of the multidimensional experiment. Even for a 3D experiment with modest resolution ($512 \times 128 \times 32$ complex points), the complete matrix of FIDs expands exponentially. The uniform sampling of each FID is necessary for the discrete fast Fourier transformation algorithm to correctly convert the NMR data from the time-domain to the frequency domain for further analysis. But, acquiring the complete FID matrix overly defines the system and unnecessarily wastes instrument time and prolongs data collection. A simple solution is to randomly sample the FID matrix, usually at a > 30% sampling rate, with a bias towards the early time points that have a higher signal-to-noise [72]. This generally results in a two to threefold reduction in acquisition time along each indirect dimension. The incomplete FID matrix is then processed using non-fast Fourier transformation methods: filter diagonalization [73], maximum entropy techniques [70,74], multidimensional decomposition [75,76] or nonlinear FT techniques [77-79]. The use of non-uniform sampling does introduce artifacts in the NMR spectra [71,80], which includes ridges, aliasing artifacts, baseline artifacts and folding artifacts that may make it difficult to detect weak signals. The signal:artifact ratio increases as the square-root of the number of data points and artifacts are minimized using a random sampling scheme [72].

In addition to reducing the absolute number of FIDs that are collected, the overall experimental time can also be shortened by decreasing the time required to collect each individual FID [81]. The SOFAST-heteronuclear multiple quantum coherence (HMQC) [82] pulse scheme permits the collection of a 2D ^{1}H-^{15}N HMQC spectrum in a few seconds by dramatically reducing the recycle time and allowing for high repetition rates. Band-selective excitation short-transient-NMR applies the same general concept within standard triple-resonance pulse sequences [81]. Rapid repetition rates are obtained by ensuring efficient T_1 relaxation during the significantly shortened recycle delay (50 – 150 ms) relative to typical recycle delays of 0.75 – 1 s. A further decrease in experimental times could be gained by combining the band-selective excitation short-transient-NMR technique with non-uniform sampling or reduced dimensionality methods.

Third, the development of software for the semi-automated assignment of NMR spectra and the calculation of protein structures has greatly reduced the time required to generate an NMR protein structure. A number of software packages exist for the semi-automated assignment of protein NMR spectra (readers are referred to [83] for a detailed review). These include: AutoAssign [84], BATCH [85], GARANT [86], DYNASSIGN [87], GANA [88], MATCH [89] and PISTACHIO [90], among others. Basically, theses programs implement a set of rules that mimic an expert's approach to the analysis of spin-systems from a standard set of triple-resonance experiments [91]. The spin-systems are then joined by two or more overlapping $C\alpha_i$, $C\alpha H_i$, C'_i, $C\beta_i$ and $C\alpha_{i-1}$, $C\alpha H_{i-1}$, C'_{i-1} and $C\beta_{i-1}$ chemical shifts. The primary challenge to the approach is due to incomplete data sets (missing peaks), noisy spectra (false peaks) and degeneracy (overlapping peaks). An expert deals with these problems through an iterative visual inspection of the NMR spectra. Conversely, an automated approach is either limited to peak lists or attempts to differentiate between a real peak and noise in the original spectra. Thus, the various software packages apply distinct approaches including exhaustive search algorithms, genetic algorithm, graph theory, heuristic algorithms neural networks, simulated annealing, system energy function or Monte Carlo algorithms in an attempt to overcome incomplete and noisy data. In general, these approaches tend to correctly assign backbone residues 70 – 100% of the time.

There is also a variety of software packages used to automate the calculation of a protein structure from NMR data (see [92] for a detailed review). This includes: AutoStructure [61,93], ARIA [94], CLOUDS [95], CYANA [96], PASD [97], FLYA [98] and Rosetta [99], to name a few. One primary difference between these programs is the choice of the molecular mechanics/dynamics engine, XPLOR/CNS [100] or DYANA [101]. XPLOR/CNS refines the protein structure in distance space and tends to be significantly slower, but more accurate than DYANA that refines in torsion angle space. Again, the automated approach to refining a protein structure is basically achieved by incorporating rules to simulate the manual analysis of NMR structural data by an expert along with well-defined structural characteristics. For example, secondary structure elements α-helices, β-strands and turns have expected patterns of NOEs. Similarly, there are distinct regions of allowable backbone and side-chain dihedral angles. Additionally, long-range contact or distance maps are generally self-consistent. The unambiguous assignment of a long-range NOE between non-sequential residues increases the likelihood that other NOEs between the same two residues or sequential neighbors of the two residues are observed.

Again, a primary challenge to the automated analysis of NMR spectra to generate a protein structure is incomplete and noisy data. Furthermore, the success of a structure determination is predicated on the completeness of the chemical shift assignments, because these assignments are required to correctly annotate the structural NOEs. It has been estimated that 90% completeness of chemical shift assignments are required for an accurate protein structure [102,103]. Structure determination is an iterative process, where an initial structure is calculated based on a subset of unambiguous NOEs and other readily available structural constraints (torsion angles, chemical shifts, coupling constants, RDC constants and so on). A consistency with the initial protein structure is then used to filter ambiguous NOEs. The success of the protocol is thus highly dependent on obtaining a correct fold for the initial structure, where either a significant lack of chemical shift assignments or unambiguous NOEs will result in failure [104]. To address these problems, ambiguous NOE methods [94,96,97], assignment-free methods [95], RDC-based methods [105,106] and chemical shift-based methods (CS-ROSETTA) [107] are actively being developed. Protein structures determined solely by chemical shift information is particularly promising and exciting, because the chemical shift information is rapidly obtained and eliminates the need for additional NMR experiments to measure NOEs, coupling constants and RDCs. Protein structures calculated using CS-ROSETTA tend to yield a backbone root-mean squared deviation (rmsd) ≤ 2Å relative to original NMR structures using complete NOE data sets. CS-ROSETTA is currently limited to proteins < 130 residues. Importantly, assessments of protein structures emerging from structural genomics centers, which primarily utilize automated techniques, indicate a higher structure quality relative to NMR structures solved by traditional techniques [39,108].

The availability of automated software and rapid NMR data collection schemes have resulted in a significant improvement in the throughput of NMR structure determination. This is illustrated by the > 300 protein NMR structures generated as part of the NIH Protein Structure Initiative and by a recent example (**Figure 2**) of eight NMR structures that required, on average, 1 – 2 weeks for completing each structure. The high-throughput generation of protein NMR structures is still generally restricted to proteins with an MW < 30 kDa. But, expanding this limit beyond 50 kDa in the near future appears promising, especially because obtaining backbone chemical shift assignments have been demonstrated for a number of large MW proteins [42,45,46]. Obtaining a structure for high MW proteins may be achieved by expanding the CS-ROSETTA methodology to include additional structural constraints such as RDCs, pseudocontact shifts [60], minimal NOE constraints [59] and chemical crosslinking data [109].

3. Rapid protein–ligand complex structures

Obtaining a rapid protein structure provides an important foundation for a drug discovery project. It enables the validation of chemical leads through the determination of a protein–ligand co-structure. Confirmation that the compound binds in a biologically relevant region of the protein or induces a conformational change that affects the biological
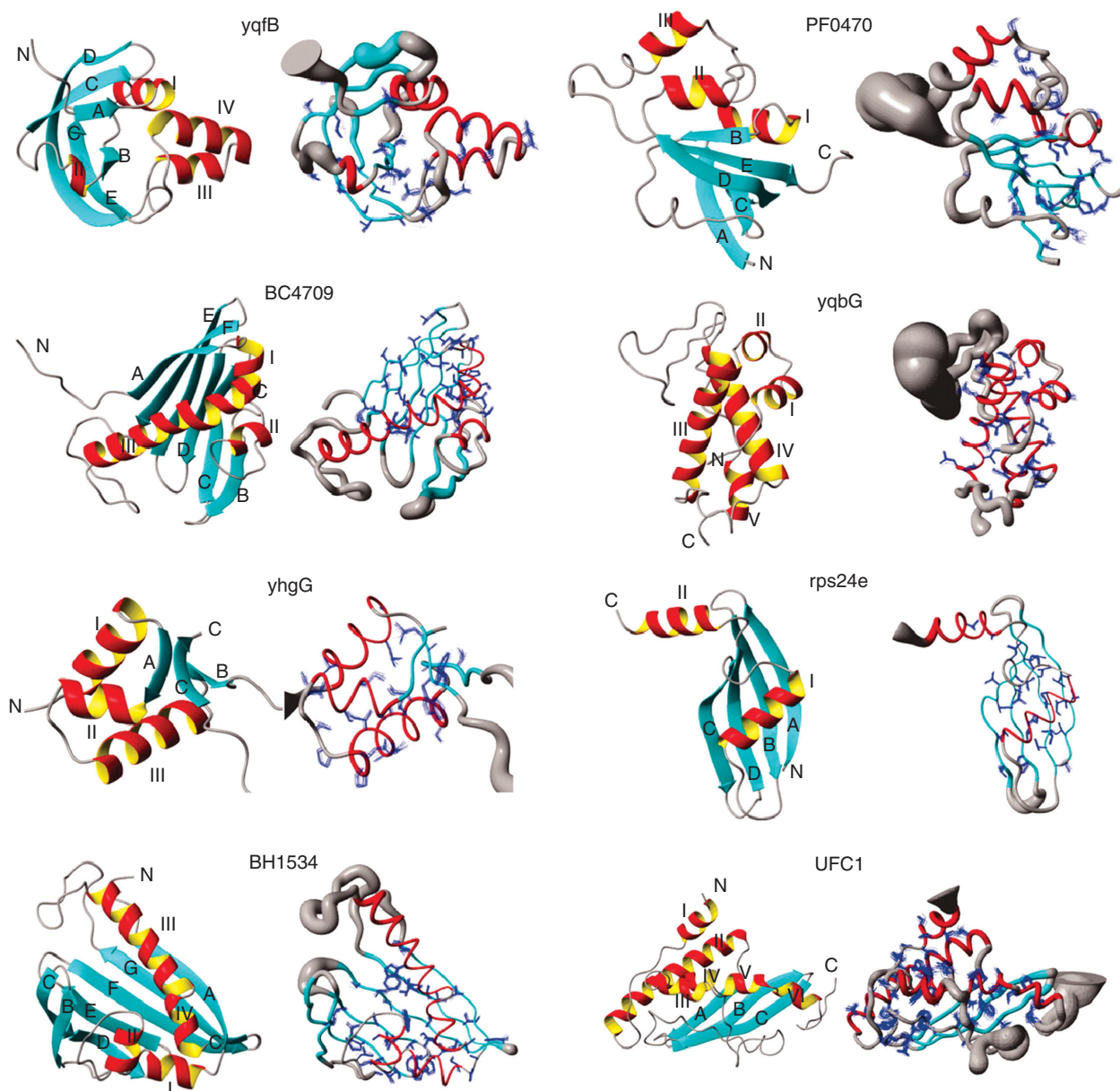
Figure 2. High-quality NMR solution structures of Northeast Structural Genomics consortium target proteins. A methodology for semiautomated data analysis was used to solve the eight NMR protein structures. NMR data collection was accomplished in ~ 1 – 9 days per structure and ~ 1 – 2 weeks of an expert's time was required for semiautomated data analysis and structure calculation. For each structure, a ribbon drawing is shown on the left and a 'sausage' representation of the backbone is shown on the right where the thickness reflects the precision achieved for the determination of the polypeptide backbone conformation.
Reprinted with permission from [64], Copyright 2005 by the National Academy of Sciences of the United States of America.

activity of the protein infers a viable lead compound. Again, obtaining this information rapidly is critical to the success of the drug discovery program especially because an iterative series of structures is required to optimize the chemical lead and a limited amount of time is available before the project is terminated. A number of recent techniques have been developed that enable NMR to rapidly determine a structure for a protein–ligand complex.

NMR chemical shift perturbations (CSPs) are routinely used to identify ligand bindings sites based on a clustering of residues on the proteins surface that incur CSPs. Generally, 2D ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC), 2D ^{1}H-^{15}N heteronuclear multiple quantum coherence (HMQC), or 2D ^{1}H-^{13}N HSQC/HMQC spectra are rapidly acquired in minutes (FHSQC [110] or SOFAST-HMQC [82]) for both the free protein and the protein–ligand complex. An overlay of

the two spectra readily identifies the residues that incur CSPs, which are then simply mapped onto the protein's surface. Thus, a ligand-binding site is obtained extremely rapidly and the process is automatable with the addition of robotic sample-changers or flow-probes to an NMR spectrometer [111]. It was recently demonstrated that a high-quality protein–ligand complex model can be obtained rapidly by combining traditional *in situ* ligand docking software with experimental NMR CSPs [112]. Specifically, the CSP identified ligand-binding site is used to define a 3D search-grid for AutoDock 4, which is the highest-cited ligand docking software package [113,114]. AutoDock searches the 3D grid using a Lamarckian genetic algorithm to explore different translational, rotational and torsional orientations for the ligand and estimates a free energy of binding. AutoDock is optimized for ligand-binding energetics and includes numerous terms related to dispersion/ repulsion, directional hydrogen bonding, electrostatics, desolvation and conformational energy. Typically, 120 different ligand conformations are calculated within the 3D searchgrid, where the program AutoDockFilter (ADF) is used to select the best conformers based on a consistency with the experimental CSPs. ADF uses an NMR energy function based on the magnitude of the CSPs and the proximity of the ligand to amino-acid residues with a CSP. Simply, the ligand is expected to be closer to amino-acid residues that incurred large CSPs. On average, the approach takes ~ 40 min to obtain a protein–ligand co-structure, where the resulting structure exhibits an average rmsd of 1.17 ± 0.74 Å relative to the original X-ray structure. **Figure 3A** illustrates the high similarity between the CSP-guided docking of thymidine 3′,5′-bisphosphate to *Staphylococcus aureus* nuclease relative to the original X-ray structure (Protein database-ID: 1SNC) [115].

Similarly, the program HADDOCK takes advantage of this relationship between CSPs and ligand binding to generate biomolecular complexes (protein–protein, protein–DNA, protein–RNA) [116]. HADDOCK utilizes ambiguous interaction constraints [117] to refine a biomolecular complex using a three stage refinement: i) a rigid body docking and energy minimization; ii) a semirigid simulated annealing in torsional angle space; and iii) a final refinement with explicit solvent. Basically, HADDOCK defines an ambiguous intermolecular distance (≤ 3Å) between all sets of residues that incur a CSP from molecule A to molecule B in the complex. HADDOCK has recently been applied to the docking of low MW compounds to proteins [64]. A clear advantage of the HADDOCK approach is the fact that the resulting co-structure is a result of a direct refinement against the experimental CSPs. But, HADDOCK uses XPLOR/CNS [100], which is optimized for protein refinement and dynamics, making it challenging to properly parameterize each new compound that is docked. Also, HADDOCK applies a more robust, multistage minimization and dynamics protocol resulting in a significantly longer computational time (~ 2 days) compared to AutoDock/ ADF. **Figure 3B** illustrates the application of HADDOCK for the determination of the structure of human arylamine

N-acetyltransferase (NAT) complexed with *p*-aminobenzoic acid (PABA) [118]. The clear advantages of the CSP approaches to determining a protein–ligand model is the speed, ease and relative simplicity of the techniques. But, these modeled structures should not be viewed as a replacement for traditional high-resolution protein–ligand complexes obtained by either NMR or X-ray crystallography for drug discovery. Instead, these CSP modeled structures provide a rapid approach to evaluate potential drug discovery targets before pursuing the more time-consuming and resource intensive experimental structures. Importantly, the CSP methods are restricted to proteins that do not undergo significant conformational changes upon ligand-binding. This is especially valid if only the apo-structure of the protein is available for modeling, because either a static version of the protein structure or a structure with limited side-chain mobility is used for ligand docking.

In addition to AutoDock/ADF and HADDOCK, other NMR approaches have been described to shorten the timeframe to determine a protein–ligand co-structure. These methods are, in general, significantly slower, more complex to execute (requiring a variety of experiments and/or labeled samples) or limited in scope (requiring multiple known binders or unique site-specific labeling). But, the relative resolutions of some of these structures are increased compared to CSP-based structures. Again, these techniques are typically limited to protein structures that do not undergo significant conformational changes upon ligand binding because a static protein structure is used to model the protein–ligand complex. These alternative methods include NOE-based protein–ligand models [119,120], paramagnetic shifts [121], differential CSPs [122], SOS-NMR [123], NMR-SOLVE [124] and NMR-DOC [125]. The NOE-based protein–ligand model utilizes a refined NMR apo-protein structure and a minimal NMR data set to assign intermolecular NOEs. Recent techniques minimize the need to re-assign the protein NMR spectra in the complex [126]. These NOEs are simply added to the complete set of structural constraints used to solve the apo-structure in order to solve the complex structure. The differential chemical shift approach uses a series of structurally similar ligands known to bind the protein. The differences in structure and CSPs are expected to correlate, which then identifies each ligand's orientation within the similar binding pocket. The NMR-DOC approach is essentially identical, but simplifies the protein NMR spectra by using a series of residue-specific ^{13}C-methly labeled protein samples. The NMR-SOLVE method is also very similar; it uses a reference ligand (natural cofactor) to identify chemical shifts associated with the ligand-binding site. This information is then used to map the location and orientation of novel compounds.

The paramagnetic shifts and SOS-NMR methods also utilize selective labeling to identify the structure of the protein–ligand complex. The SOS-NMR method uses a series of residue specific labeling to identify which residues
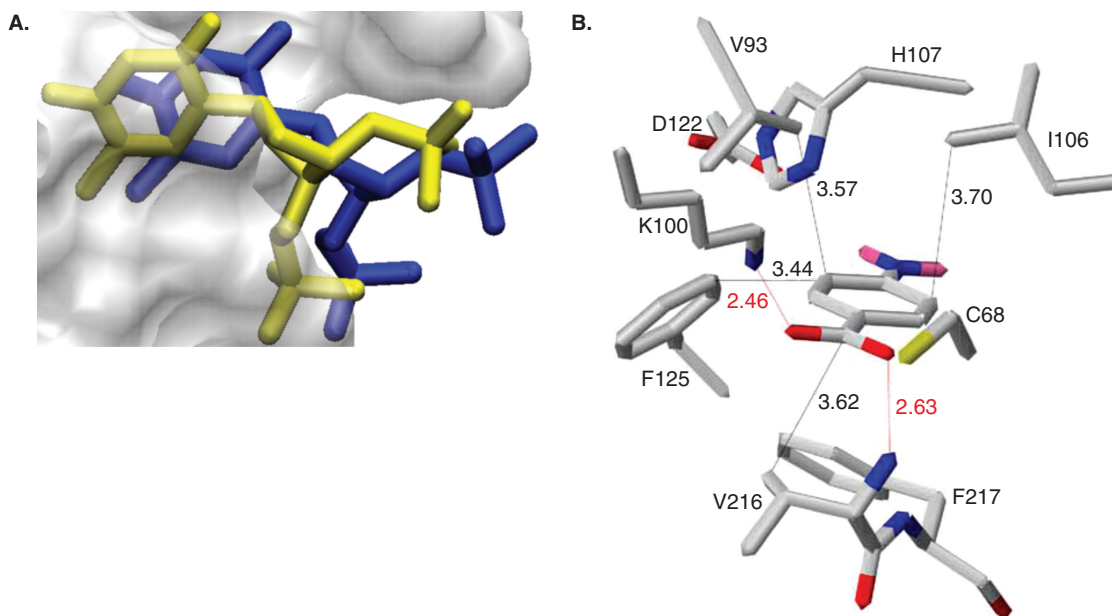
Figure 3. Examples of rapid protein-ligand structure determination methods. A. Superposition of the CSP-guided docking with ADF filtering conformers (blue) with the original X-ray structures (yellow) for Staphylococcus aureus nuclease complexed to thymidine 3',5'-bisphosphate (PDB-ID: 1SNC). **B.** Expanded view of the HADDOCK model of human NAT1 bound to its substrate PABA. Carbon, nitrogen, oxygen, sulfur, and hydrogen atoms are colored white, blue, red, yellow, and pink, respectively. The lengths of several hydrophobic contacts are provided and the hydrogen bonds to the carboxylic acid group of PABA are indicated in red.
Figure 3A was reprinted with permission from [112], Copyright 2008 by American Chemical Society. **Figure 3B** was reprinted with permission from [118], Copyright 2006 by Elsevier Ltd.
ADF: Autodockfilter; CDA: Chenodeoxycholic acid; cL-BABP: Chicken liver bile acid binding protein; CSP: Chemical shift perturbation; PDB: Protein database.

are proximal to the ligand similar in concept to CSPs. Every residue type except one is deuterium labeled. If a saturation transfer difference is still observed, the unlabeled residue is proximal to the ligand. The process is repeated for each relevant amino acid. The paramagnetic shifts approach requires a site-specific lanthanide label proximal to the ligand-binding site. The lanthanide-binding site is identified from an X-ray crystal structure and $\Delta\chi$ tensors are determined from chemical shift changes in 2D ^{1}H-^{15}N HSQC spectra for the protein complexed to paramagnetic and diamagnetic lanthanides. Pseudocontact shifts are then measured for the ligand in the presence of the protein–lanthanide complex from 1D ^{1}H and ^{13}C NMR spectra. The pseudocontact shifts are used to position the ^{1}H and ^{13}C nuclei from the ligand relative to the $\Delta\chi$ tensors to dock the ligand.

4. Fragment-based ligand screens

Traditional HTS is routinely used in the pharmaceutical industry to screen hundreds of thousands to millions of compounds against a therapeutic protein target [26]. But, HTS has an extremely high failure rate. Only 0.1 – 0.5% of the compounds in the screening library will be 'active' in a particular screen [127]. A significant percentage of the best 'hits', with the highest observed inhibition, are false positives [30].

These compounds generate an HTS response through a number of undesirable mechanisms, such as: protein aggregation, protein denaturation, protein precipitation, micelle formation, chemical modification of the protein, nonspecific binding, promiscuous binders or interfering with other reagents of the assay [28-31,128]. These problematic chemical leads inevitably result in a significant amount of wasted resources and time.

Intrinsically linked to the success of HTS is the composition of the screening library. Obviously, the quality of 'hits' identified from an HTS assay is dependent on the quality of compounds present in the library [32]. The accumulation of corporate chemical libraries occurs over years if not decades where the composition of the library is inherently biased to previous drug discovery projects. The analysis of typical large, random chemical libraries compared to the collection of known drugs identified a number of problems associated with corporate libraries while identifying a set of structural properties or Lipinski's 'rule of 5' that are exhibited by known drugs [129,130]. Lipinski's 'rule of 5' is a general predictor for compound solubility and permeability that is related to bioactivity and bioavailability [131]. In contrast, compounds in chemical libraries have a tendency to trend toward higher MW, higher lipophilicity and/or higher H-bond properties resulting in either poor solubility or permeability.

An outcome of these studies has been an extensive effort to design or reconfigure chemical libraries based on the Lipinski's 'rule of 5' or similar predictors of 'drugability' [32,132-135]. Concurrent with drug-like features are additional approaches to build in structural diversity in the design of chemical libraries [134,136-140]. But, estimates indicate that ~ 10^{10} – 10^{50} compounds would be needed to properly cover structural space [141]. Regardless of the size of the chemical library, the number of compounds that can be practically screened represents an infinitesimally small fraction of the total number of potential compounds (10^{60}) [142]. Concurrent with any effort to increase the size of the library will be a proportional increase in the total number of troublesome false leads. From a practical perspective, it is not possible to follow-up all the hits identified from an HTS assay. Because false positives tend to over-populate the best inhibitors identified from an HTS assay, more effort is spent chasing after false leads [143]. Another practical consideration is that the integrity of individual compounds used in the HTS screen may be suspect because of issues associated with long-term storage of compounds in conditions amenable to HTS [144,145]. Given these challenges, the decline in the productivity of the pharmaceutical industry has been attributed, in part, to the heavy reliance of HTS in the drug discovery process [146]. Fragment-based ligand screens provide an alternative to HTS and address a number of these problems [147]. NMR is the primary method being used to screen fragment-based libraries [148].

4.1 Library design

Chemical libraries used for fragment-based screening are significantly different from the typical library used in a traditional HTS assay. This is because the goals are fundamentally different. In an HTS assay, the aim is to identify 3 – 5 chemical classes with the best binding affinity ($K_d \leq \mu M$) to the therapeutic target. These compounds go through an iterative series of chemical modifications to maximize binding affinity. Unfortunately, this process generally results in an increase in hydrophobicity and MW and a decrease in solubility [149]. Both factors are detrimental for evolving the lead compound into a drug. Critical absorption, distribution, metabolism and excretion (ADME) and pharmacokinetic [150,151] issues are typically dealt with at a later point in the drug discovery process [152,153]. Conversely, the goal of a fragment-based screen is to identify a number of weak binders ($K_d \sim \mu M$ to mM) that bind in separate but proximal sub-regions of a functionally relevant ligand-binding site. In effect, the ligand-binding site is chemically mapped with low MW compound fragments (≤ 200 – 300 Da) [154,155]. Chemical linking and optimization of the individual fragments generally results in a substantial improvement in binding affinity, where K_d in the nM range are commonly observed [156]. Importantly, an additional goal of the fragment-based approach is to maintain drug-like ADME and pharmacokinetic characteristics. This is a fundamental

consideration in the design of the fragment-based screening library.

A number of reviews have discussed in detail the underlying theory in the construction of a fragment-based chemical library [154,155,157-159]. Basically, the objective is to identify low MW compounds (≤ 200 – 300 Da) that correspond to fragments of known drugs, adhere to the Lipinski 'rule of 5' and exhibit high aqueous solubility. A comparable approach is using a fragment-based library composed of biologically active compounds [160]. The relatively small libraries are usually composed of hundreds to thousands of compounds. Maintaining a chemical library of low MW compounds has a number of advantages that address common problems associated with HTS. First, it maximizes ligand efficiency, the number of nonhydrogen atoms relative to free energy of binding [161]. The estimated maximum affinity per atom is ~ 1.5 kcal mol^{-1}, which is approached by known drugs and fragments. Conversely, typical HTS leads exhibit a ligand efficiency of ~ 0.3 kcal mol^{-1} [159]. Second, fragment-based libraries have a 10 – 1000 times higher hit rate relative to traditional HTS corporate libraries [154]. Third, fragment-based libraries more efficiently cover structural space. This occurs because the number of low MW compounds is significantly reduced. Instead of a potential 10^{60} compounds that a HTS library is compared against, there are only ~ 14 million compounds with MWs below 160 Da [162]. Thus, even a few thousand compounds represent a significant percentage of the possible number of compounds for a fragment-based library, which cannot be reasonably approached with higher MW HTS libraries. Additionally, because fragments are commonly linked to generate chemical leads, a fragment-based screen is actually examining a combinatorial number of compounds relative to the actual size of the library that is experimentally tested [163]. Fourth, smaller chemical libraries are significantly easier to handle, store, replenish and maintain quality control. Finally and most importantly, fragment-based libraries generate better-quality leads with a higher success rate [164]. This results from the smaller size of the fragments and the associated 'drug-like' features. The smaller structures permit a greater flexibility to 'grow' the fragments to improve binding affinities before inducing detrimental physiochemical characteristics that is common with HTS leads.

4.2 NMR ligand screening methodologies

High-throughput NMR (HTS-NMR) ligand affinity screens cannot compete with the efficiency of traditional HTS that routinely assay millions of compounds in a few weeks [165]. Nevertheless, HTS-NMR is perfectly amenable to screening a fragment-based library composed of hundreds to thousands of compounds [148,164,166]. The value of NMR for HTS is its unique capability of providing direct evidence for binding between the ligand and protein target while subsequently being able to identify the binding site and determining a co-structure of the protein–ligand complex [91,167-170]. An important advantage of NMR is the versatility of the

Table 1. Description of different NMR-based screens.

| Screening technique	Method of detecting ligand binding	Labeled protein?	Protein–ligand co-structure?	Limited by protein MW?	Ref.
3-FABS	Chemical shift changes, requires fluorinated ligands	No	No	No	[252]
Affinity NMR	Change in translational diffusion	No	No	No	[174]
AIDA-NMR	Line-broadening change (T_2) due to protein–protein complex formation, labeled protein or Trp reporter in ligand binding site	Yes/No	Yes/No	Yes	[253,254]
FAXS	Line-broadening change (T_2) due to ligand competition, requires fluorinated ligands	No	No	No	[255]
MS/NMR	Retention on size-exclusion column and chemical shift changes	Yes	Yes	Yes	[256]
Multi-step NMR	Line-broadening change (T_2) and chemical shift changes	Yes	Yes	Yes	[237]
NOE pumping	Transfer NOEs	No	No	No	[183]
RAMPED-UP NMR	Chemical shift changes, screening multiple proteins	Yes	No	Yes	[257]
SAR by NMR	Chemical shift changes	Yes	Yes	Yes	[258]
SLAPSTIC	Line-broadening change (T_2) due to protein spin label	Yes	No	No	[259]
STD NMR	STD from protein	No	No	No	[181]
WaterLOGSY	STD from solvent	No	No	No	[182]

MW: Molecular weight; MS: Mass spectrometry; NOE: Nuclear overhauser effect; SAR: Structure–activity relationship; STD: Saturation transfer difference.

technique to monitor protein–ligand interactions through a variety of physical measurements. Observation of a binding event may occur through changes in line-width and/or peak intensity (T_1 and T_2 relaxation changes) [171,172], change in the measured diffusion coefficient for the ligand [173-175], CSPs for either the ligand or protein [176-178], induced transferred NOE for the ligand [179,180], a saturation transfer difference between either the protein or bulk solvent to the ligand [181,182], or the appearance of new NOEs and/or intermolecular NOEs between the ligand and protein [167,183]. Correspondingly, a variety of different NMR-based screens have been described (**Table 1**). The various HTS-NMR methods have a range of distinct advantages that include: i) eliminating a need for isotopically labeled proteins; ii) reducing the amount of protein required for the screen; iii) increasing the throughput of the assay; iv) identifying the ligand-binding site; v) determining a protein–ligand co-structure; and vi) eliminating the protein MW limitation. NMR fragment-based screens have resulted in a number of chemical leads that

have advanced to clinical studies [164]. A recent example is the discovery of novel β-secretase inhibitors [184]. Cyclic amidine with an isocytosine core fragments were identified from an NMR screen as a lead hit. The further optimization of these fragments is illustrated in **Figure 4A**, which led to a unique dihydroisocytosine inhibitor with a cellular activity of 470 nM (**Figure 4B**).

5. Metabolomics

The discovery of a compound with a high-affinity to a therapeutic target does not necessarily lead to a marketable drug. Chemical leads routinely fail in preclinical and clinical trials because of problems with bioavailability, efficacy and toxicity [6,7]. Typically, *in vivo* assays and animal studies attempt to identify these problems prior to proceeding with clinical trials. But, animal studies also have significant practical limitations [185-187]. They are fundamentally low-throughput, requiring kilogram quantities of the lead compound and
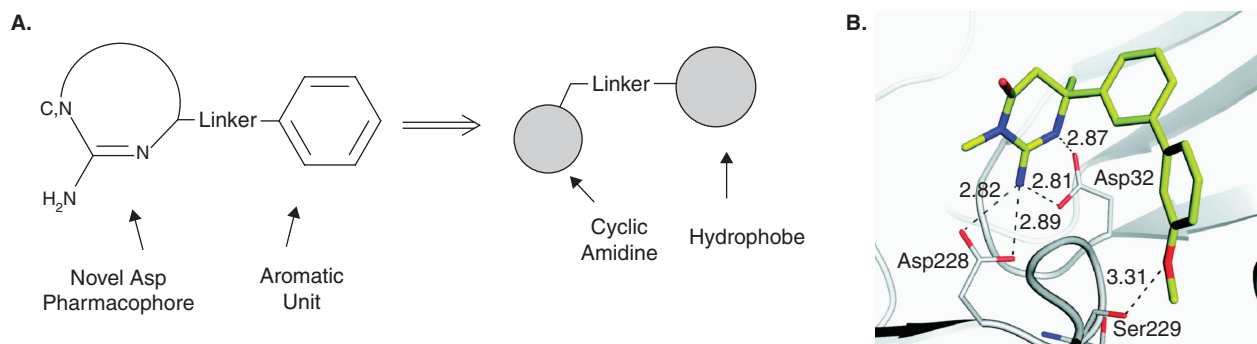
Figure 4. A. General fragment-based scheme using a novel cyclic amidine-based aspartyl protease pharmacophore to generate BACE-1 inhibitors. B. BACE-1 inhibitor complex.
Reprinted with permission from [184], Copyright 2007 by American Chemical Society.
BACE-1: β-Secretase.

weeks to months to complete a single study. Furthermore, there is typically little structure–activity relationship or feedback when a negative result is encountered, because such a small number of compounds are routinely evaluated. NMR-based metabolomics is providing drug-discovery with a filter to identify *in vivo* activity, selectivity and toxicity problems before proceeding with animal trials. Furthermore, NMR metabolomics techniques can be used as part of an animal study to identify and evaluate the mechanisms of drug induced toxicity. Similarly, mass spectrometry is also routinely used to monitor the metabolome [188,189] and is complimentary to NMR [190]. Mass spectrometry is significantly more sensitive than NMR, but requires the inclusion of separation techniques because of the low MW diversity of metabolites and is limited to detecting metabolites that readily ionize. Conversely, NMR typically only observes the most abundant metabolites, but requires minimal sample preparation and can readily identify each metabolite from the multiple distinct peaks in its NMR spectrum. Correspondingly, a number of techniques have been proposed that combine NMR and mass spectrometry data for the analysis of metabolomic samples [190-195]. The application of NMR metabolomics in drug discovery has been recently reviewed [196-198] and is only briefly summarized here.

Basically, a 1D ^{1}H NMR spectrum of a lysed cell captures its metabolic state. The metabolome directly measures the biological activity of the proteome because relative concentrations of various metabolites are dependent on enzymatic activity. Peak intensities in an NMR spectrum reflect both the abundance and the presence of specific metabolites and the corresponding activity of an enzyme. Environmental stress and drug activity will perturb protein activity resulting in changes in the metabolome that can be monitored by NMR. Principal component analysis is a well-established statistical technique that is commonly used to analyze 1D ^{1}H NMR spectra to monitor these perturbations in the metabolome [199]. The multivariate NMR spectrum is converted to a single point in principal component-space

that is usually represented by a 2D scores plot. Similar NMR spectra will cluster together in a 2D scores plot. The differential NMR metabolomics approach [200,201] uses comparative clustering patterns in a 2D scores plot to monitor the *in vivo* activity and selectivity of a chemical lead. Simply, four different cell cultures are prepared with 10 replicates each for a total of 40 NMR samples. The four cultures correspond to: i) wild-type cells; ii) wild-type cells treated with the chemical lead; iii) mutant cells where the protein target of the chemical lead has been inactivated; and iv) mutant cells treated with the chemical lead. The metabolome for the wild-type cells and mutant cells will be different because of the inactivated protein resulting in distinct clustering in a 2D scores plot. The relative clustering of the wild-type cells and mutant cells treated with the chemical lead will determine the compound's *in vivo* activity and selectivity. Specifically, if the wild-type cells treated with the chemical lead cluster together with the mutant cells with and without the addition of the chemical lead, this would then indicate that the chemical lead is active and selective *in vivo*. Inhibiting the cellular activity of the therapeutic target either chemically or genetically results in the same change in the metabolome. Different clustering patterns occur in the 2D scores plot if the chemical lead is inactive, inhibits multiple proteins or inhibits a different protein from the expected protein target [201]. Any of these outcomes would clearly indicate a problem, suggesting that other compounds with a positive response from the differential NMR metabolomics method should be prioritized for follow-up animal studies. The method has been successfully applied to demonstrate the selective activity of 8-azaxanthine against urate oxidase in *Aspergillus nidulans* (**Figure 5A**) and the promiscuous activity of D-cycloserine in *Mycobacterium smegmatis* (**Figure 5B**).

The differential NMR metabolomics method is limited to disease states that can be modeled by a cell system. Also, the technique requires generating a viable mutant cell line where the activity of the target protein has been eliminated. This
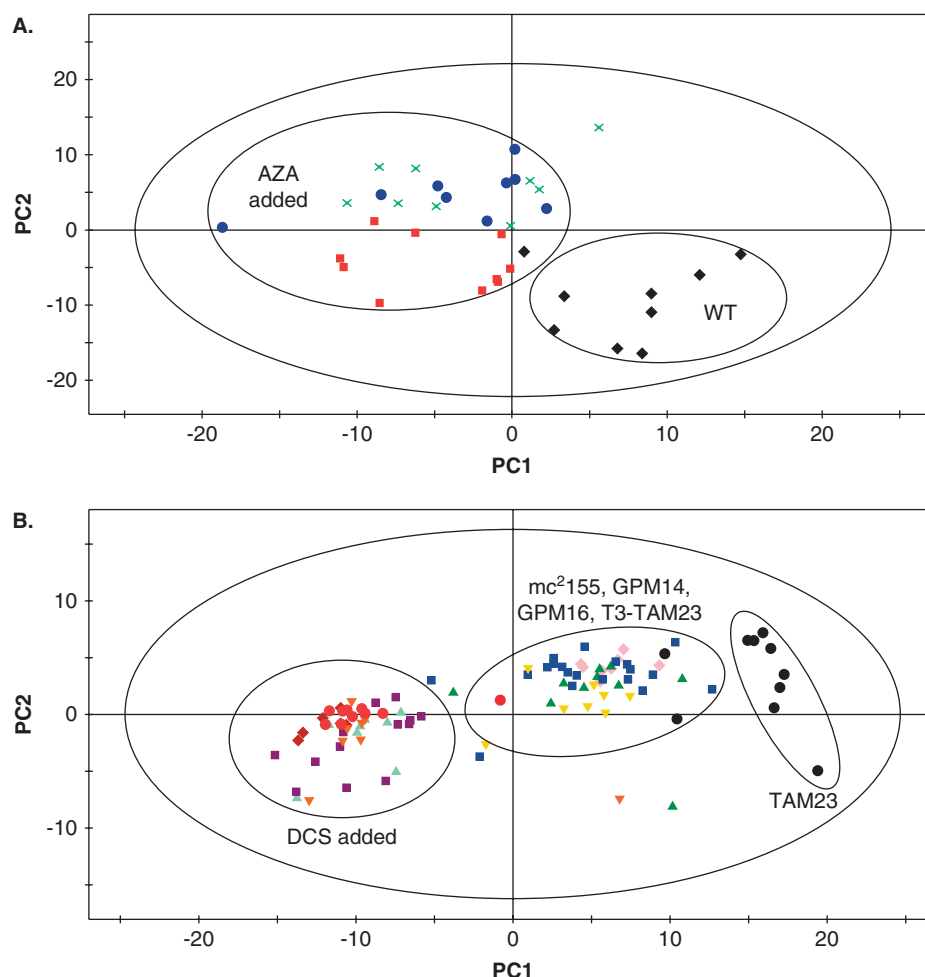
Figure 5. Illustration of the differential NMR metabolomics method. A. Analysis of the *in vivo* activity of AZA in Aspergillus nidulans targeting urate oxidase. The PCA scores plot comparisons of *A. nidulans* inactive urate oxidase mutant (*uaZ14*) (x), wild-type with AZA (✕), *uaZ14* mutant with AZA (▪) and wild-type cells (●). Results clearly demonstrate the selective activity (see Figure 3B in [201]) of AZA. **B.** Analysis of the *in vivo* activity of DCS in mycobacteria targeting alanine racemase. PCA scores plot comparisons of wild-type (mc²155) (♦), inactive D-alanine racemase mutant (TAM23) (▪), DCS resistant mutants (GPM14 (●), GPM16 (♦)), restored D-alanine racemase activity mutant (TAM23 pTAMU3) (▲) mc²155 with DCS (▼), and TAM23 with DCS (▪), GPM14 with DCS (●), GPM16 with DCS (♦), and TAM23 pTAMU3 with DCS (▲). The results clearly demonstrate the active, non-selective inhibition of DCS (see Figure 3C in [201]). The secondary target of DCS is predicted to be D-alanine-D-alanine ligase.

Figure 5A was reprinted with permission from [201], Copyright 2006 by American Chemical Society. **Figure 5B** was reprinted with permission from [200], Copyright 2006 by American Chemical Society.

AZA: 8-Azaxanthine; DCS: D-cycloserine; PCA: Principal component analysis.

may be challenging if the protein is critical or essential to the cell's viability. In these cases, a number of alternate strategies can be used instead of simply generating a knock-out mutant. These include: a mutant protein with only diminished activity, supplementing the cell media or growth conditions to compensate for the inactive protein, treating the cells with sub-lethal dosages of siRNA or a known inhibitor of the target protein. The differential NMR metabolomics method is also predicated on the assumption that inactivating the target protein will perturb the metabolome in a manner that is detectable by NMR. It is plausible that eliminating the biological activity of a particular protein will not disturb the cellular metabolome or it may affect the

relative flux of metabolites in a concentration range that is too low to be detectable by NMR.

NMR metabolomics can also be used to monitor body fluids (blood, urine, saliva, and so on) to identify drug induced toxicity in animal trials. This can be achieved by the identification of metabolites known to be associated with toxicity, such as trimethylamine N-oxide and renal dysfunction or lipids and phospholipidosis [202-205]. Alternatively, a comparative analysis with compounds known to induce toxicity with other compounds with no known side effect can be used to generate a predictive model for unknown compounds based on the correlated changes in the metabolome and NMR spectrum [202,206]. Again, the

method assumes that the metabolites associated with a toxic event are in a concentration range observable by NMR and present in the body fluid being examined. Thus, a lack of a change in the metabolite composition and concentration in a body fluid would only eliminate toxicities previously demonstrated to be observed by NMR for compounds with known toxic side effects. Additionally, the metabolome is not completely defined and a reference spectrum for every observable metabolite is not currently available. Therefore, identifying every metabolite that is perturbed by treatment with a drug candidate may not be readily obtained. Nevertheless, there is an ongoing effort to catalog the NMR observable metabolites and there are a number of NMR metabolomic databases currently available [207-209].

NMR-based metabolomics have two important contributions to the current drug discovery protocol. First, the differential NMR metabolomics method could serve as a filter before proceeding with *in vivo* animal trials with lead candidates. Only compounds that proved to be active and selective for the desired protein target would continue on for animal testing. Other compounds would be eliminated or proceed through further rounds of chemical optimization. Changes in the structure that do not affect binding affinity, but are correlated with preferred activity in the differential NMR metabolomics screen could be pursued. Second, NMR-based metabolomics would augment animal studies. Initially, *in vivo* animal models are primarily focused on efficacy. The efficiency of these assays would be greatly improved by simultaneously analyzing animal biofluids by NMR for toxicity. Animal-based studies are typically time intensive assays requiring weeks to complete and usually result in sacrificing the animals. Furthermore, a detailed autopsy may be required to analyze the efficacy of the drug and identify any potential toxicity issues. NMR-based metabolomics may effectively identify a toxicity problem early enough in the study to stop the test, saving valuable time and resources. Alternatively, the lack of any observable toxicity problem by NMR in combination with an overall positive outcome for a particular lead candidate in an animal assay would support proceeding with NCE and clinical trials.

6. Functional annotations and new therapeutic targets

To date, 916 genomes have been completely sequenced with an additional 3454 in progress resulting in ~ 6.5 million protein sequences [210-214]. Sequence similarity techniques may provide functional information for, at most, 50% of these proteins [215-217]. Valuable therapeutic targets are inevitably hidden within these vast numbers of unannotated proteins, providing an indispensable wealth of information for developing novel drugs [218-220]. Assigning a function is an essential first step for determining the potential utility of an unannotated protein in treating human disease; but this is an extremely challenging and time-consuming endeavor.

Drug discovery cannot simply focus on the crucial or essential proteins because non-essential proteins play important roles in antibiotic resistance, cancer and tumor differentiation and metastasis, and the long-term development of Alzheimer, cardiovascular disease, diabetes and Parkinson disease. NMR is also making a significant contribution to the functional assignment of these unannotated proteins to identify new drug discovery targets.

The functional annotation screening technology using NMR (FAST-NMR) [221,222] combines the NMR methodology described above in a single assay to assign a function through similarities in functional epitopes or ligand-binding sites. This is based on the premise that amino-acid residues associated with active-sites are evolutionary-stable relative to the remainder of the protein's sequence [223-225]. Essential to this understanding is the knowledge that a protein's active-site has been optimized by nature to interact with a unique and specific set of targets. Protein surfaces are exquisitely selective and only bind ligands at very specific locations [226-229]. Binding promiscuity is inherently detrimental to the overall biological process, which is evident by the high specificity of interactions that have been well-documented in numerous metabolic and signaling pathways [230-232]. This understanding is also an essential aspect of drug discovery and supports the observed rationale that high-affinity and selective compounds targeting a specific protein can be developed and used therapeutically [233-236].

FAST-NMR combines fragment-based ligand affinity screens with rapid determination of protein–ligand co-structures and bioinformatics. Specifically, a chemical library containing only biologically active compounds (cofactors, drugs, metabolites, inhibitors, substrates and so on) [160] are screened by NMR using a tiered approach [237]. Potential ligands are rapidly identified by a 1D ^{1}H line-broadening experiment. These hits are then confirmed by CSPs in a 2D ^{1}H-^{15}N HSQC spectrum, where the CSPs cluster together to identify a specific ligand-binding site. The CSPs are then used to quickly determine a protein–ligand co-structure [112] that provides the input for the CPASS (comparison of protein active-site structures) software and database [238]. CPASS compares the experimentally determined ligand-defined-binding site against a database of ~ 35,000 unique ligand-binding sites identified from the Protein database. A similarity in the sequence and structural characteristics of the ligand-defined-binding site from the unannotated protein with a ligand-binding site for a protein of known function is then used to leverage a functional assignment.

FAST-NMR and CPASS were used to assign a function to hypothetical proteins SAV1430 from *S. aureus* [222] and PA1324 from *Pseudomonas aeruginosa* [239]. This analysis suggests SAV1430 is similar to an SH2 domain and may function as part of a multi-protein complex within the [Fe-S] cluster assembly network. SAV1430 may exhibit activity comparable to NifU or may regulate NifU activity. PA1324 may be involved in the binding and transport of

sugars or polysaccharides as part of the peptidoglycan matrix required for biofilm formation. FAST-NMR has also been used to suggest an evolutionary relationship between the bacterial type III secretion system and eukaryotic apoptosis [240]. This is based, in part, on the similarity in ligand-binding sites between PrgI (forms the needle complex in the type III secretion system) and Bcl-xL (involved in eukaryotic apoptosis).

7. Expert opinion

The quantity of new drugs emerging from the pharmaceutical industry has declined dramatically. A number of self-inflicted factors have contributed to this decrease in productivity that has resulted from a need to decrease cost and, ironically, from a need to increase efficiency. Traditional business practices that include outsourcing, metrics and short timelines have been implemented into the drug discovery process. Scientific discovery is effectively being forced to adhere to an assembly-line protocol [23], isolating disciplines, diminishing creativity and negatively affecting morale [5]. This is an antithesis to a successful drug discovery effort, which is fundamentally interdisciplinary, complex and unpredictable. Resolving these serious problems will require a major paradigm shift in the culture of the pharmaceutical industry; nevertheless, evolving drug discovery technologies are providing avenues to improve the success rate [164] within these self-imposed limitations.

In this context, recent developments in NMR are greatly expanding its value to the drug discovery process. Specifically, NMR is a valuable and complimentary technique to X-ray crystallography for the high-throughput structure determinations of protein and protein–ligand complexes. It has been recently demonstrated that a significant percentage (20 – 40%) of protein structures are only solvable by NMR. Also, rapid (30 – 45 min) protein–ligand co-structures are routinely obtainable by NMR as part of an iterative drug design program. This speed is essential given the short timelines imposed on a drug discovery project. Additionally, fragment-based NMR affinity screens are providing a more efficient and productive alternative to traditional HTS for lead generation. A fundamental advantage of NMR affinity screens is the direct knowledge of the chemical lead's mechanism of action. This occurs because an NMR affinity screen identifies a specific binding interaction between the ligand and protein target, where the ligand-binding site can be correlated with the functionally relevant regions of the protein. Furthermore, NMR-based metabolomics can assist in the most challenging and problematic stage of the drug discovery process, turning a high-affinity ligand into a drug. This requires verification of *in vivo* efficacy with a corresponding lack of toxic side effects and acceptable ADME profiles. Animal models are the primary source of this information, but are extremely costly and time consuming, significantly limiting the number of leads that can be practically evaluated.

Differential NMR metabolomics provides a simple, cheap and fast approach to filter-out problematic compounds and prioritize leads that demonstrate cellular activity and specificity while confirming the *in vivo* target(s) of the compound. Similarly, NMR metabolomics can be incorporated as part of the animal trials to monitor body fluids and ascertain possible toxic side effects of chemical leads. NMR is also being used to functionally annotate the proteome and identify novel drug discovery targets.

Despite the obvious important contributions of NMR to the drug discovery process, the pharmaceutical industry has reduced or completely eliminated biomolecular NMR groups over the last number of years. Again, this short-sighted decision has been primarily driven by cost and mergers, by the incorrect perspective that NMR is a redundant, but costlier and limited version of X-ray crystallography, and by the large financial investment in HTS robotics along with the perspective that weak-binding fragments are not viable drug leads. The pharmaceutical industry, like other businesses, is cyclical and responds to short-term needs instead of long-term goals. Thus, I believe the reduction in biomolecular NMR has bottomed out and this trend will begin to reverse. This will be driven, in part, by the growing acceptance and success of fragment-based ligand screens. Furthermore, the achievements from structural genomics and the NIH Protein Structure Initiative along with the new NMR metabolomics technologies will further stimulate a renewed interest in biomolecular NMR by the pharmaceutical industry.

Finally, our basic understanding of the mechanism for generating a protein–ligand complex is undergoing a dramatic shift from the current induced-fit model or two-state model [241]. The recent application of relaxation dispersion NMR spectroscopy [242,243] and RDCs [244] has supported the selected-fit model of ligand binding, where protein structures exist as an ensemble of conformations that includes sampling a higher-energy ligand bound form in the apo-state. The role of both protein and ligand dynamics [245-247] has been recognized as important factors in drug discovery, but has been rarely utilized [248]. Clearly, structure-focused drug discovery efforts will greatly benefit from the inclusion of a detailed NMR analysis [249] of the flexibility–function relationship. The growing recognition of the role dynamics plays in ligand binding is also expected to contribute to a revitalized interest and an improved appreciation by the pharmaceutical industry of the vital role for NMR in drug discovery.

A reversal in the decade-long decrease in the productivity of the pharmaceutical industry will obviously require more than the just the simple adoption of these recent advances in NMR. A major philosophical shift in the current approach to drug discovery is necessary. One potential avenue is for the pharmaceutical industry to move beyond the target-focused approach to drug discovery [1] and to adopt system biology techniques [250]. Basically, chemical genetics [251] is used to identify desirable phenotypic responses in cell-based disease

models followed by the identification of the molecular target of the chemical probes. Once the target is identified and verified, traditional drug optimization techniques would be applied in an iterative fashion using positive feedback from the original cell-based assays. NMR-based metabolomics would play an integral component of chemical genetics as an approach to classifying and differentiating between different cellular phenotypes. NMR ligand affinity screens and NMR structural biology techniques would continue to play important roles in the drug optimization stage of the process. Thus, the diversity and flexibility of NMR will enable the technique to continually expand its contribution to drug discovery as the pharmaceutical industry responds to business pressures and progresses beyond current drug discovery paradigms.

Declaration of interest

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