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PERSPECTIVE

Perspectives of biomolecular NMR in drug discovery: the blessing and curse of versatility

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Abstract The versatility of NMR and its broad applicability to several stages in the drug discovery process is well known and generally considered one of the major strengths of NMR (Pellecchia et al., Nature Rev Drug Discov 1:211–219, 2002; Stockman and Dalvit, Prog Nucl Magn Reson Spectrosc 41:187–231, 2002; Lepre et al., Comb Chem High throughput screen 5:583–590, 2002; Wyss et al., Curr Opin Drug Discov Devel 5:630–647, 2002; Jahnke and Widmer, Cell Mol Life Sci 61:580–599, 2004; Huth et al., Methods Enzymol 394:549–571, 2005b; Klages et al., Mol Biosyst 2:318–332, 2006; Takeuchi and Wagner, Curr Opin Struct Biol 16:109–117, 2006; Zartler and Shapiro, Curr Pharm Des 12:3963–3972, 2006). Indeed, NMR is the only biophysical technique which can detect and quantify molecular interactions, and at the same time provide detailed structural information with atomic level resolution. NMR should therefore be ideally suited and widely requested as a tool for drug discovery research, and numerous examples of drug discovery projects which have substantially benefited from NMR contributions or were even driven by NMR have been described in the literature. However, not all pharmaceutical companies have rigorously implemented NMR as integral tool of their research processes. Some companies invest with limited resources, and others do not use biomolecular NMR at all. This discrepancy in assessing the value of a technology is striking, and calls for clarification—under which circumstances can NMR provide added value to the drug discovery process? What kind of contributions can NMR make, and how is it implemented and integrated for maximum impact? This perspectives article suggests key areas of impact for NMR, and a model of integrating NMR with other technologies to realize synergies and maximize their value for drug discovery.

Keywords Fragment-based screening · Protein structure determination · Protein–ligand complexes · Hit validation · Integration

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The advent of SAR-by-NMR, or more generally of fragment-based screening, opened a new field for NMR and profoundly changed the face of NMR in drug discovery research (Shuker et al. 1996; Jahnke and Erlanson 2006; Hajduk and Greer 2007; Hubbard et al. 2007). NMR, traditionally seen primarily as a tool for structure determination with utility in lead optimization, suddenly gained a role in lead identification (Pellecchia et al. 2002; Stockman and Dalvit 2002; Lepre et al. 2002; Wyss et al. 2002; Huth et al. 2005b; Zartler and Shapiro 2006). Fragment-based screening has been around for more than a decade, and it is legitimate to ask for its track record and how it has shaped drug discovery research. If success is defined by the discovery of high-affinity protein ligands and potent inhibitors, a rather impressive list of successes is documented in the literature (Hajduk and Greer 2007). But even if compound progress into late preclinical or clinical stages is taken as a measure of success, there are several reported examples, the most remarkable being arguably the discovery and development of a subnanomolar ligand for Bcl-xL (Oltersdorf et al. 2005). In addition to these published cases, the results of many more successful fragment-based screening projects are still hidden in the early pipelines of pharmaceutical companies.

These achievements are remarkable, but do they make NMR a competitive technology for drug discovery? Other techniques have evolved as well during the past decade, and there is a constant race amongst technologies for their share in the drug discovery market. For example, mass spectrometry, calorimetric or thermal denaturation techniques and methods that screen ligand binding using immobilized protein, e.g. by using surface plasmon resonance, have developed into sensitive and quantitative (although not always very robust) detection techniques with medium or high throughput (Lundqvist 2005). With competitive techniques developing very fast, NMR would be obsolete within a few years if it did not progress as well. So, what else can NMR offer besides the detection and quantification of protein–ligand interactions? The distinguishing feature to other binding assays is its atomic resolution, which allows structural (and dynamic) characterization of intermolecular interactions (Takeuchi and Wagner 2006). It is this feature, for which NMR was traditionally recognized, which seemingly has become somewhat forgotten with the enthusiasm about NMR as a screening technique. It is now time for the pendulum to swing back, and take advantage of the unique feature of NMR to provide quantitative and robust binding information in addition to structural characterization. For example, for the identification of allosteric binding pockets and ligands binding to these allosteric pockets, NMR is an ideal technique since the NMR binding assay does not a priori require any functional modulation, and ligand binding sites are easily mapped if isotope labeled protein and resonance assignments are available.

In order for NMR (like any other technique) to provide useful structural information for the drug discovery process, its timelines must fit into the drug discovery cycle, i.e. modelers and chemists must have the required information at the time when new compounds are designed or synthesized. Ideally, a protein–ligand complex should be characterized within 1 or 2 weeks. This does not necessarily need a high-resolution structure determination, but may simply be a crude model of the complex based on experimental data and molecular modeling. Unfortunately, currently available methods for NMR-based structure determination rarely match these timelines. Although some methods have been proposed for the rapid determination of a low-resolution complex structure (e.g. INPHARMA (Sanchez-Pedregal et al. 2005), SOS-NMR (Hajduk et al. 2004), inter-ligand NOEs (Becattini and Pellecchia 2006; Vazquez et al. 2007), spin labels (Jahnke 2002)), the most popular methods still rely on protein–ligand NOEs, which currently require resonance assignments and significant time. Moreover, synergies with other techniques such as crystallography have not been fully realized. A situation commonly encountered in a drug discovery project is the availability of one or several crystal structures of the protein target with different ligands, but the inability to crystallize the protein with other ligands. It should be straightforward to use the crystal structure with ligand 1 to assign most resonances and NOEs in the protein with ligand 2. Some software is available that does part of this job, but none of these programs is well integrated and generally accepted.

Speaking of general acceptance: There is a lack of broadly accepted and commonly used software in the NMR community—each group seems to have their own favorite, possibly home-made, software. This is closely linked to the lack of a consensus set of standard experimental procedures for tasks that might by now be considered "routine", such as structure determination of small soluble proteins, or screening of a library of low molecular weight compounds for their ability to bind to a given protein. In terms of consensus procedures and software, crystallography is far ahead by the development and general use of software developed through the CCP4 initiative (http://www.ccp4.ac.uk/) decades ago. This common software unifies the community and enables rapid development and progress with optimized software. The NMR community would benefit tremendously from a similar approach, and this has been recognized and realized by the CCPN initiative (see http://www.ccpn.ac.uk)—it is to be hoped that the software of this initiative will be as successful and widely accepted and applied as CCP4.

The versatility of biomolecular NMR, which is extremely valuable for the tailor-made solution of particular problems in drug discovery, is at the same time actually an obstacle for the rapid development of the field. The fact that particular tasks, such as protein–ligand structure determination by NOEs, can be tackled by NMR spectroscopy with diverse methods and small variations thereof, all of which lead to slightly different experimental data and slightly different requirements for processing and analysis, makes it difficult to define the best path for results. In crystallography, the path to the experimental diffraction pattern and from there to the electron density map and structural model is in general much more straightforward. The versatility of NMR, its ability to tackle a variety of problems with a variety of approaches, makes it difficult to find a consensus on "best practices", and consequently on "best software", for particular tasks.

Not less importantly for the status of NMR in pharmaceutical industry, the versatility of NMR and its broad applicability are confusing to the non-experts. NMR seems to be able to do almost anything from structure determination, dynamics characterization, and detection and characterization of protein–ligand interactions, including in-cell spectroscopy, mega-Dalton proteins and membrane proteins, let alone other areas such as metabonomics or

solid-state NMR. But then for all of these abilities, there are some "if's" and exceptions, some special requirements and pitfalls. Actually, many methods are still under development or have only been applied to model proteins rather than daily-life drug targets. Not properly communicating these limitations, and thereby overselling NMR leads to unmanaged expectations and subsequent disappointment, which is not doing good deeds to the field. In addition, the wide applicability makes the perception of NMR fuzzy and unclear. X-ray crystallography, on the other hand, has a sharp profile and a clear mission: It solves structures, period. The ability of NMR to contribute to various steps and to multiple tasks of the drug discovery process is the true power of NMR, but it can be seen as a weakness if not correctly applied and communicated.

Figure 1 attempts to describe the key areas for impact of NMR in pharmaceutical research, and presents a model of how NMR can be integrated in the drug discovery process. NMR is certainly not a stand-alone method, but develops its full power only in combination with other biophysical or biochemical techniques, to which it should be closely linked. Besides in the two areas discussed above, fragment-based screening and structural information on protein–ligand complexes, NMR can have significant impact in hit validation and triaging of hit lists from HTS, in silico screening, or other hit finding methods (Jahnke and Widmer 2004; Dalvit et al. 2006; Huth et al. 2005a). By integrating NMR as a filter to validate molecular interactions between the hit and its proposed target, it can be ensured that chemists touch only true ligands instead of the many false positives that often result from HTS campaigns. Characterization by NMR of protein or ligand dynamics is not mentioned in Fig. 1, since the track record of impact on drug discovery is presently very small in spite of its great potential.

In a competitive environment, the true value of NMR—as well as of any other technique—is revealed by the added value in the drug discovery process: Does it make drug discovery research more productive, reliable, and efficient? Impact is required, and it remains to the spectroscopist to ensure that such impact is generated. The spectroscopist has to produce the results that are required to bring a drug discovery project forward, and to communicate them in a way to ensure their optimal use. If chemists, modelers or biologists do not make use of the NMR data, the spectroscopist produced the wrong type of data—no excuses accepted. As described above, areas of impact can be lead finding by fragment-based screening, which leads to highly visible contributions, or structural information on protein–ligand complexes, which helps in lead optimization. But high impact can also be achieved in hit validation, and triaging of hit lists from high-throughput screening. This type of contribution is not highly visible, but it takes full advantage of the nature of NMR as a robust, label-free, solution-state detection method, and by cleaning hit lists of false positives or by identifying valid ligands in the lower parts of HTS hit lists, it has saved many chemists from working on the wrong compounds, and directing them to the true ligands. Such impact is not readily quantifiable and does not lead to a clinical candidate discovered "by NMR", but it renders NMR an indispensable tool for pharmaceutical research.

I am convinced that the successful application of NMR in drug industry depends on good communication skills of the NMR spectroscopist and on its close integration with other drug discovery techniques, and that it has to strike a sound balance between hit validation, fragment-based screening, and structural characterization. Drug candidates can also be identified without NMR, but a functional NMR

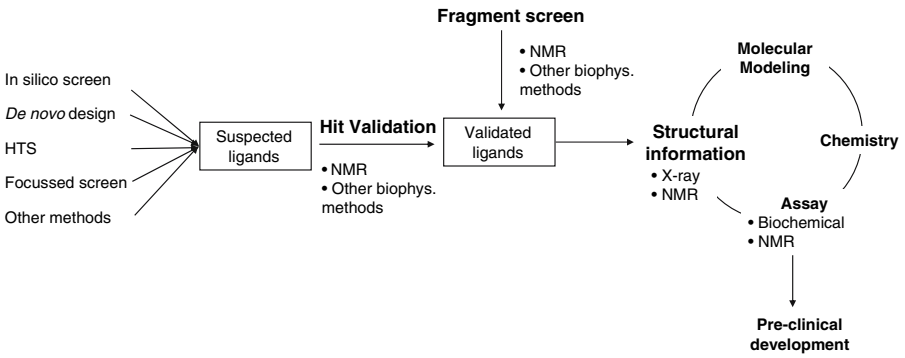

Fig. 1 A model for integration of biomolecular NMR in the drug discovery process. Other flowcharts have been published previously (Coles et al. 2003; Klages et al. 2006). The main areas of impact for NMR are hit validation, fragment screening and structural information on protein–ligand complexes (Jahnke and Widmer 2004). The extent to which this model is applicable depends on the target protein: Hit validation or fragment screening by ligand observation can be performed with proteins of any molecular size without isotope label, if milligram quantities of purified protein can be produced. Further characterization and structural information generally requires isotopically labeled protein and puts a limit on protein size

facility makes the drug discovery process faster, more reliable and more efficient.

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References

Becattini B, Pellecchia M (2006) SAR by ILOEs: an NMR-based approach to reverse chemical genetics. Chemistry 12:2658–2662

Coles M, Heller M, Kessler H (2003) NMR-based screening technologies. Drug Discov Today 8:803–810

Dalvit C, Caronni D, Mongelli N, Veronesi M, Vulpetti A (2006) NMR-based quality control approach for the identification of false positives and false negatives in high throughput screening. Curr Drug Discov Technol 3:115–124

Hajduk PJ, Greer J (2007) A decade of fragment-based drug design: strategic advances and lessons learned. Nat Rev Drug Discov 6:211–219

Hajduk PJ, Mack JC, Olejniczak ET, Park C, Dandliker PJ, Beutel BA (2004) SOS-NMR: a saturation transfer NMR-based method for determining the structures of protein–ligand complexes. J Am Chem Soc 126:2390–2398

Hubbard RE, Chen I, Davis B (2007) Informatics and modeling challenges in fragment-based drug discovery. Curr Opin Drug Discov Devel 10:289–297

Huth JR, Mendoza R, Olejniczak ET, Johnson RW, Cothron DA, Liu Y, Lerner CG, Chen J, Hajduk PJ (2005a) ALARM NMR: a rapid and robust experimental method to detect reactive false positives in biochemical screens. J Am Chem Soc 127:217–224

Huth JR, Sun C, Sauer DR, Hajduk PJ (2005b) Utilization of NMR-derived fragment leads in drug design. Methods Enzymol 394:549–571

Jahnke W (2002) Spin labels as a tool to identify and characterize protein–ligand interactions by NMR spectroscopy. Chembiochem 3:167–173

Jahnke W, Erlanson DA, (eds) (2006) Fragment-based approaches in drug discovery. Methods and principles in medicinal chemistry. Wiley-VCH

Jahnke W, Widmer H (2004) Protein NMR in biomedical research. Cell Mol Life Sci 61:580–599

Klages J, Coles M, Kessler H (2006) NMR-based screening: a powerful tool in fragment-based drug discovery. Mol Biosyst 2:318–332

Lepre CA, Peng J, Fejzo J, Abdul-Manan N, Pocas J, Jacobs M, Xie X, Moore JM (2002) Applications of SHAPES screening in drug discovery. Comb Chem High throughput screen 5:583–590

Lundqvist T (2005) The devil is still in the details—driving early drug discovery forward with biophysical experimental methods. Curr Opin Drug Discov Devel 8:513–519

Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW, Rosenberg SH (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 435: 677–681

Pellecchia M, Sem DS, Wüthrich K (2002) NMR in drug discovery. Nature Rev Drug Discov 1:211–219

Sanchez-Pedregal VM, Reese M, Meiler J, Blommers MJ, Griesinger C, Carlomagno T (2005) The INPHARMA method: protein-mediated interligand NOEs for pharmacophore mapping. Angew Chem Int Ed 44:4172–4175

Shuker SB, Hajduk PJ, Meadows RP, Fesik SW (1996) Discovering high-affinity ligands for proteins: SAR by NMR. Science (Washington, DC) 274:1531–1534

Stockman BJ, Dalvit C (2002) NMR screening techniques in drug discovery and drug design. Prog Nucl Magn Reson Spectrosc 41:187–231

Takeuchi K, Wagner G (2006) NMR studies of protein interactions. Curr Opin Struct Biol 16:109–117

Vazquez J, Tautz L, Ryan JJ, Vuori K, Mustelin T, Pellecchia M (2007) Development of molecular probes for second-site screening and design of protein tyrosine phosphatase inhibitors. J Med Chem 50:2137–2143

Wyss DF, McCoy MA, Senior MM (2002) NMR-based approaches for lead discovery. Curr Opin Drug Discov Devel 5:630–647

Zartler ER, Shapiro MJ (2006) Protein NMR-based screening in drug discovery. Curr Pharm Des 12:3963–3972