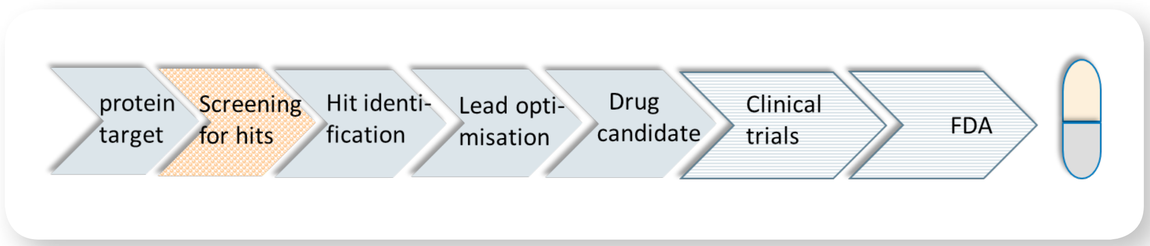
Tools for Fragment Based Drug Discovery

Introduction

NMR has established itself as a indispensable tool in fragment based drug discovery. It is used from fragment verification and quantification, through solubility testing to actual target-ligand binding studies. The recent increased interest in fluorine containing molecules lead to the introduction of optimized probes that allow highest sensitivity measurements of ^{19}F.



Initial Fragment Verification, Quantification and Solubility Testing

Compound libraries with tens of thousands of compounds with customized compound selection are commercially available for Fragment Based Drug Discovery. Before engaging in actual screening high resolution NMR offers ideal methods to verify the structure of the fragments, quantify the concentration of each fragment and to determine the solubility of these in aqueous solutions. Structure verification and quantification is typically done in a small volume of the original stock solution of the ligand or fragment. The 1mm TXI room temperature probe or the 1.7 mm Micro-Cryoprobe are the ideal tools to measure between 5 or 35 microliters of a library stock solution. Our dedicated software tool for this analysis is CMC-q, but also CMC-assist and the new FUSION-SV can perform the tasks of structure verification and compound quantification. Before screening fragments are diluted in aqueous solutions and NMR measurements and analysis with CMC-q are again used to determine the solubility.

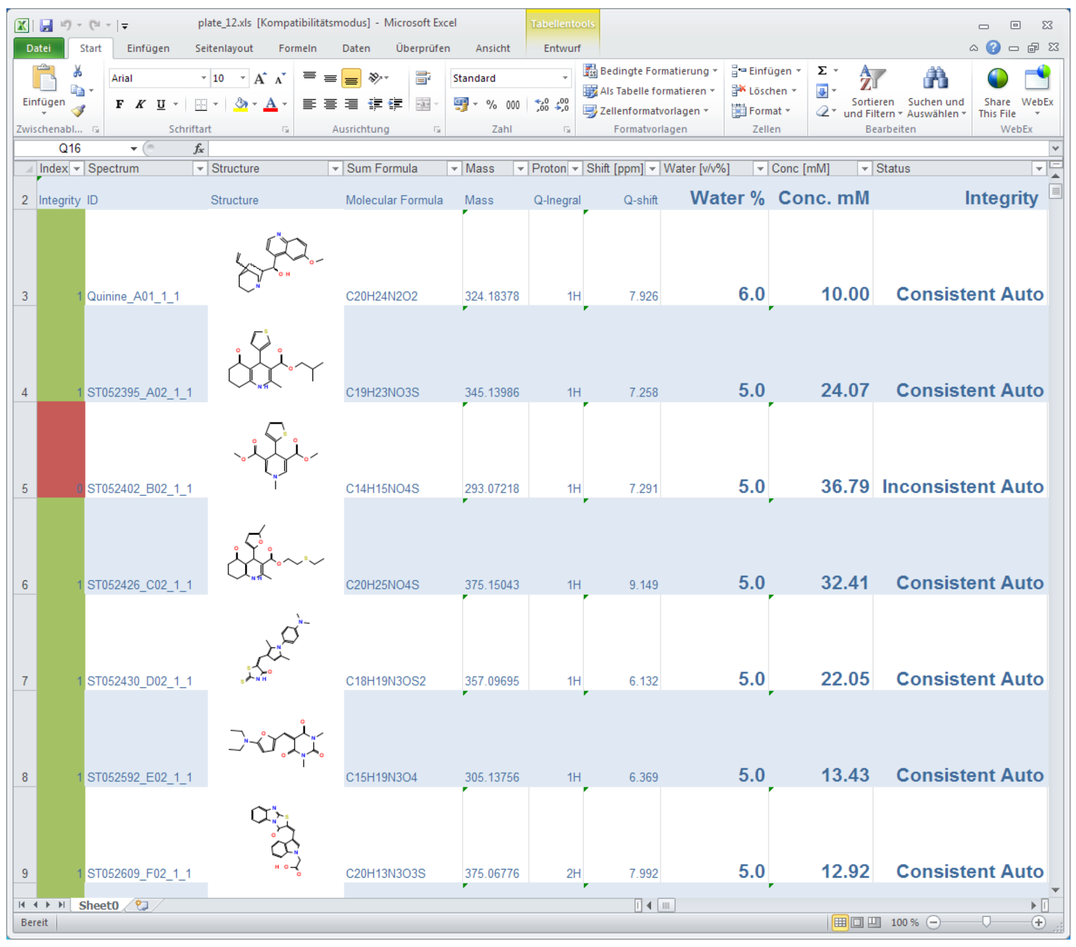


Fig.1 CMC-q provides a consistency check of structure and spectrum. The concentration of the fragment and the water content in the sample are also determined.

Literature: STD: M. Mayer & B. Meyer, Angew. Chem. Int. Ed. 38, 1784-1788 (1999) Waterlogsy: Dalvit et al, J. Biomol. NMR, 18, 2000, pp 65-68 19F Methods: Claudio Dalvit , et. Al J. Am. Chem. Soc., 2003, 125 (25), pp 7696–7703
John B. Jordan et.al. J. Med. Chem., 2012, 55 (2), pp 678–687

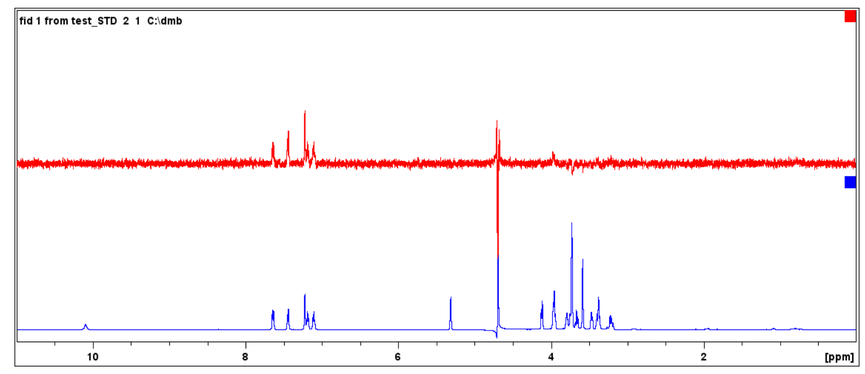


Fig. 2 STD Saturation Transfer Difference is on of the standard experiments for ligand observed drug screening NMR experiments. Shown here Tryptophan binding to human serum albumin.

Applications:

Proton Based Screening Methods

The proton based screening methods can be divided into two classes. One of these are the ligand observed experiments such as STD, Waterlogsy, and some diffusion based methods. And on the other side are the target observed methods such as SAR by NMR and other chemical shift disturbance experiments. Typical samples consist of 6 – 10 fragments. To minimize the use of proteins and ligands these experiments are often performed in 1.7 mm CryoProbes or in 3mm tubes in 5mm Probes. The SampleJet sample changer with cooling option keeps the solutions stable during extended runs.

Fluorine Based Screening Methods

Fluorine-19 NMR has become the tool of choice for drug discovery with ^{19}F containing fragments or molecules. The large chemical shift range and the strong chemical shift anisotropy make it very simple to measure mixtures of up to 30 fragments in a single sample. Fluorine markers are also used on the targets as an indicator of ligand target binding. These target based methods usually involve modified proteins with either fluorinated amino acids or modifications with fluorine containing compounds. With the HFCN quadruple nucleus CryoProbe we provide the most sensitive tool for these applications.

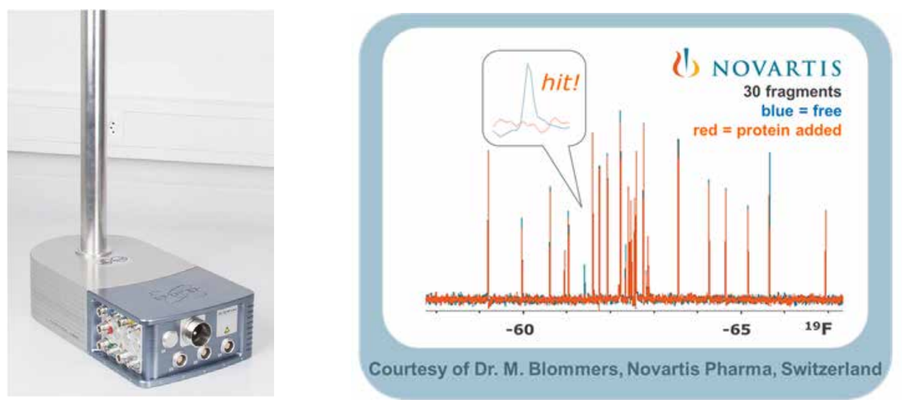


Fig. 3 Left: HFCN QXI cryoprobe. Right: Typical result of ^{19}F CPMG experiment. The linewidth of the binding ligand increases dramatically, making the line practially disappear form the spectrum.

Summary

FBDD options and tools:

- Highest sensitivity with CryoProbes, micro-CryoProbes and micro-volume probes.
- Comprehensive set of experiments for FBDD.
- Software tools for structure verification and ligand quantification.
- SampleJet with sample cooling for long term stability of protein solutions.