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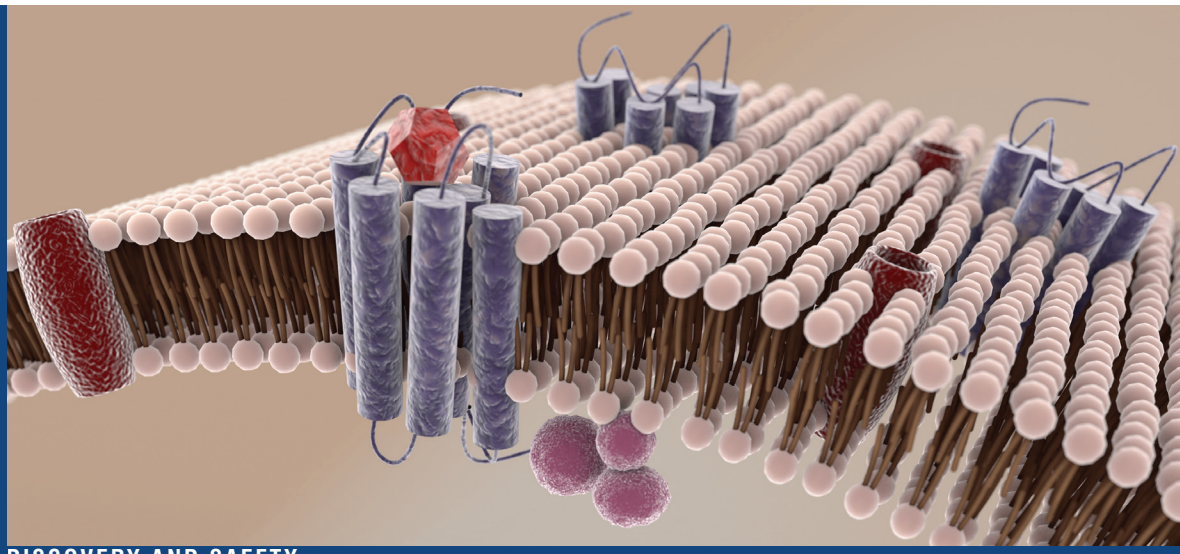
Summary

Charles River Laboratories' innovative Capture Compound® Mass Spectrometry (CCMS) platform allows for the identification and characterization of small molecule-protein interactions to de-risk a molecule.



DISCOVERY AND SAFETY

Capture Compound® Mass Spectrometry: Selectivity Profiling of Bioactive Compounds in Support of Drug Safety

Charles River Laboratories' innovative Capture Compound® Mass Spectrometry (CCMS) platform allows for the identification and characterization of small molecule-protein interactions. CCMS is a powerful analytical tool that can be used to understand both the on-[1] and off-target[2] protein binding interactions of a small molecule. The success of the CCMS technology platform lies in the proprietary chemistry that permits construction of tri-functional Capture Compounds®. These small synthetic probes interrogate native proteins, including lipophilic membrane proteins, enabling the isolation and identification of target proteins directly from a relevant biological matrix, such as cultured cells and *ex vivo* tissue samples.

Why CCMS in Drug Safety?

During the lead optimization phase, the timely assessment of the off-target liabilities of a lead molecule[3] or even the cause of an unexpected toxic event *in vitro* or *in vivo*[2] can de-risk a molecule. Elucidation of targets mediating toxicity can allow off-target interactions to be designed out in back-up molecules. With CCMS, both toxic pharmacological interactions and drug-protein adducts can be detected.

Information regarding compound selectivity is essential to the drug discovery and development process. With CCMS, screening the entire proteome of a biological sample provides an unbiased approach versus typical testing against a limited panel of candidate off-target proteins. High sensitivity means that potentially relevant but low abundance proteins can be detected.

This data can be important in two main areas:

1. Correlating generated data with nonclinical and/or clinical toxicity phenotypes can potentially lead to the discovery of toxicity mechanisms and open the opportunity to screen for compounds without toxicity-related off-target interactions[2]. Species differences in off-target toxicity can also be investigated.
2. Off-target profiles can influence the lead selection process. This data can be used during drug development to provide information regarding target understanding and secondary pharmacodynamics for IND/CTA submission in line with regulatory guidelines for first time in man[5].

Utilizing CCMS as part of a drug safety program to aid selection of lead compounds with minimized off-target effects could save time and money and generate safer drugs.

The Capture Compound®

A schematic representation of a Capture Compound® is shown in Figure 1. The tri-functional compound uses a three step process; bind, capture and isolate. The **selectivity function (bind)** mediates a reversible affinity interaction with target proteins, subsequent UV irradiation causes photo-activation of the **reactivity function (capture)** to generate a covalent bond with target proteins. The **sorting function (isolate)** enables the isolation of the complex directly out of the complex biological sample. The covalent interaction between target proteins and the Capture Compound® is one key advantage of this platform, allowing for stringent wash steps during the isolation step resulting in very low background signal from non-covalently captured proteins.

The CCMS Experiment

The experimental strategy for on- and off-target profiling using CCMS is outlined in Figure 2. A panel of Capture Compounds® is synthesized with the selectivity function in a position compliant with on-target SAR and in alternative orientations to allow for a comprehensive interaction profile to be generated. Each Capture Compound® is incubated with a biological sample whereby affinity driven binding of the selectivity function to interacting proteins and subsequent covalent capture via photo-irradiation occurs. Competition experiments are performed in parallel, such that incubation of the sample with both the Capture Compound® and an excess of the free ligand allows specifically interacting proteins to be determined. Captured proteins are isolated from the matrix, prior to proteolytic digestion analysis by high resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS). Interrogation of the MS data reveals specific binding proteins for each Capture Compound® orientation, identifying both on- and off-target interactions. The capture process inherently enriches the proteins of interest, so that it may be possible to detect low potency interactions and less abundant proteins that would otherwise go unidentified with a standard proteomic analysis. Without the enrichment process, these potentially adverse interactions would not be identified.

CCMS Case Study – Mechanism of Tolcapone Toxicity

The CCMS technology has been used to determine on- and off- target interactions of the catechol-O-methyl transferase (COMT) inhibitor tolcapone[2] in a human liver cancer cell line (HepG2). Utilizing different orientations of the selectivity function within the Capture Compound® allowed a comprehensive interaction profile to be generated, revealing both on- and off-target binding proteins (Figure 3). Differential profiles of tolcapone which causes liver toxicity and entacopone that does not were elucidated and highlighted 3-hydroxyisobutyrly-CoA hydrolase (HIBCH) as a candidate target mediating toxicity. Medicinal chemistry was then initiated focusing on molecules without HIBCH activity resulting in 'tolcapone-like' molecules with reduced toxicity profiles that could lead the way to the development of improved COMT inhibitors.

CCMS Applications

The CCMS platform offers a robust route for target deconvolution, predicting toxic liabilities, binding site identification or determining the on-target selectivity of a small molecule (Figure 4). The CCMS technology at Charles River Laboratories can be applied from target ID throughout discovery to candidate selection and IND submission, thus reducing risk and cost for our clients.

CCMS is a powerful analytical tool, compatible with cell lysate, live cell applications, and tissue samples which can be deployed by Charles River Laboratories to support the drug discovery and development journey.

References

1 Fischer, JJ et al. Chemical Proteomics – Methods in Molecular Biology, 795 (2012): 135-47

2 Von Kleist, L et al. Journal of Medicinal Chemistry, 59(10), (2016): 4664-4675

3 Dambach, DM et al. Chemical Research in Toxicology 29(4), (2016): 452-472

4 Fischer, JJ et al. Toxicological Sciences 113(1), (2010): 243-253

5 European Medicines Agency, EMEA/CHMP/SWP/28367/07 Rev. 1 20 July 2017

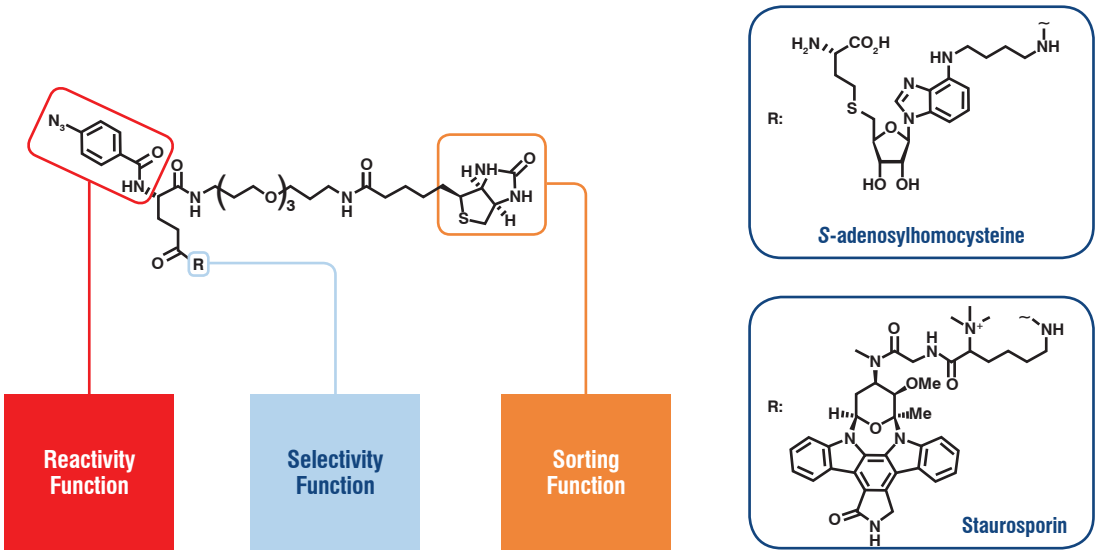


Figure 1: Capture Compounds® are small, tri-functional molecules, consisting of a reactivity function, a sorting function and a variable selectivity function.

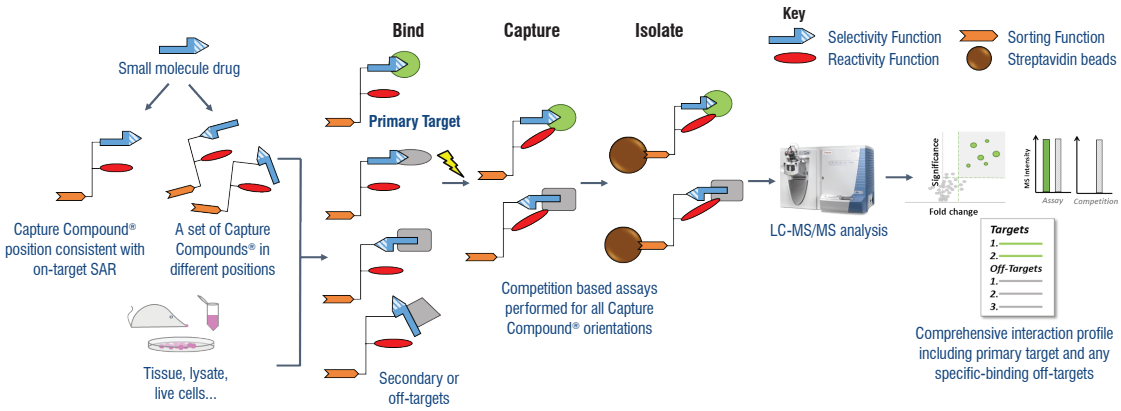


Figure 2: Experimental strategy for on- and off-target profiling using CCMS. A panel of Capture Compounds® with different orientations is used to build a comprehensive interaction profile of the small molecule drug compound.

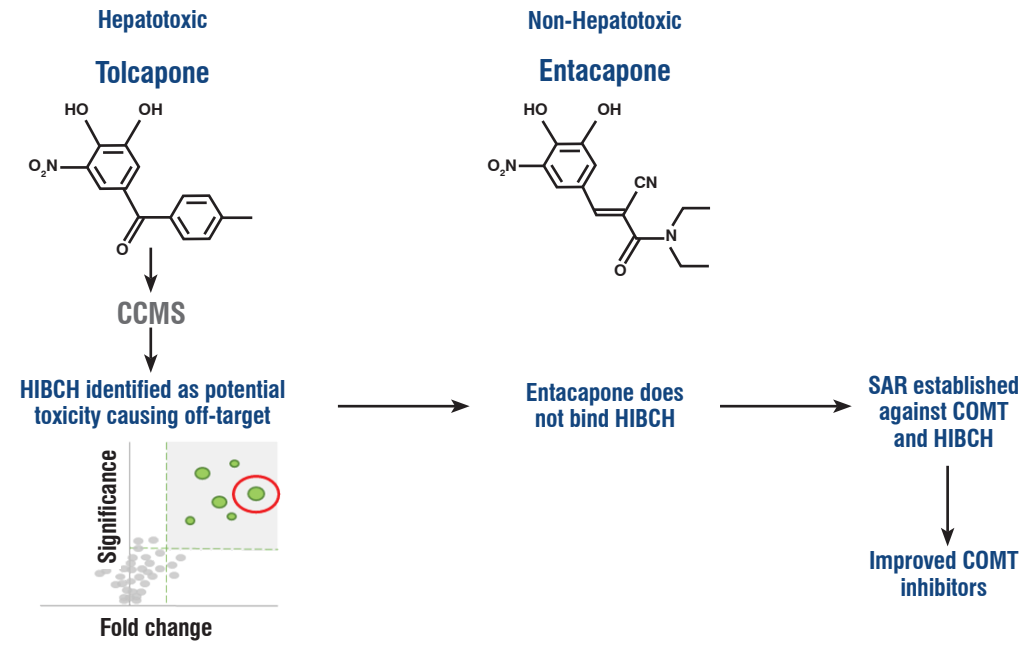


Figure 3: The use of CCMS to determine and avoid toxicity liabilities of COMT inhibitors

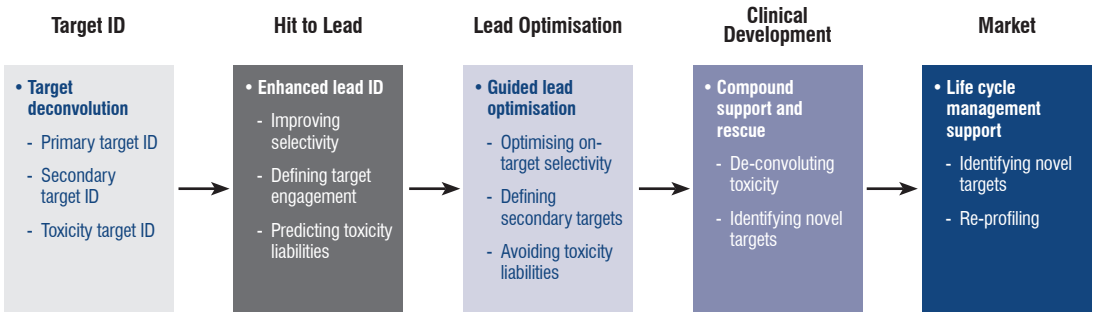


Figure 4: CCMS is a powerful chemoproteomic tool for profiling protein interactions of small molecules.