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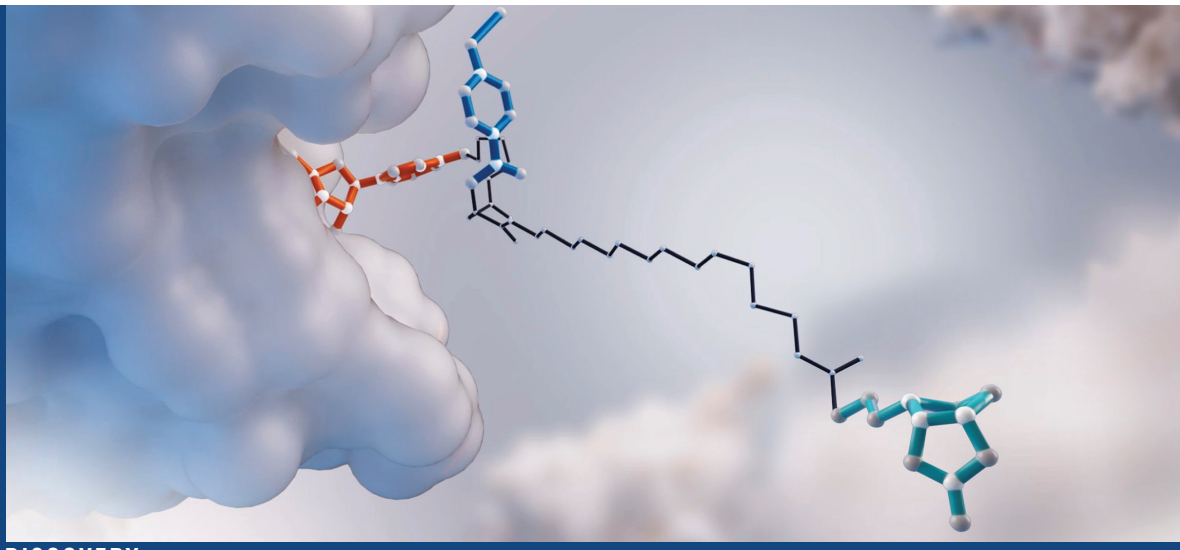
Summary

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



DISCOVERY

Capture Compound® Mass Spectrometry: Target Deconvolution of Bioactive Compounds

Charles River Laboratories' innovative Capture Compound® Mass Spectrometry (CCMS) platform allows for the identification and characterisation 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 complex biological sample. Capture Compounds® efficiently enable complexity reduction of the proteome to a subset of proteins, based on the protein's affinity for the selectivity group in the molecule. Thus, CCMS encompasses discovery, isolation and profiling of functional protein families within a variety of biological samples.

Molecular target identification, also known as target de-convolution, is an essential part of the drug discovery process[3] for understanding a compound's mechanism of action and the subsequent optimisation of the compound[4] against the newly found protein target. The ability to rapidly identify the protein(s) affected in a phenotypic screen can accelerate a project forward to a rapid go/no-go decision. Equally, during the lead optimisation phase, the timely assessment of the off-target liabilities of a lead molecule[5] or even the cause of an unexpected toxic event *in vivo*[6] can de-risk your molecule.

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. Traditional "affinity pull-down" approaches used to probe small molecule-protein interactions are limited in their ability to analyze lipophilic/membrane-associated proteins, such as GPCRs, or to investigate low-affinity interactions due to relying on weak interactions between ligand and protein. This covalent interaction between the target protein 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 unspecific protein binding. This ultimately leads to an enhanced signal-to-noise ratio during the data deconvolution steps. Crucially, CCMS offers improved detection limits over traditional biological approaches (western blotting or whole proteome analysis by LC-MS/MS) through direct enrichment of target proteins. CCMS is compatible with protein samples from a variety of different sources (e.g. cell culture, serum, tissues, microorganism and plants), both in lysate and live-cell formats.

During CCMS, the primary experimental approach for target deconvolution is via a "competition" experiment, outlined in Figure 2. Capture Compounds® are incubated with a biological sample whereby a reversible, affinity driven interaction between the selectivity function and the target proteins occurs. For the competition sample an excess of the free ligand is added. Subsequent photo-activation of the reactivity function causes covalent binding to the proteins; in the competition sample only non-specific proteins will be covalently captured. The sorting function enables isolation of the captured proteins from the matrix, which then undergo proteolytic digestion and are analysed by high resolution LC-MS/MS. Interrogation of the MS data enables identification of specific binding proteins.

The CCMS platform provides the possibility to easily integrate internal controls in the experiment thus extending the potential applications of the Capture Compound® technology. For example, the functional selectivity of the capture event can be directly monitored through the addition of competitors in a control reaction. During the CCMS process the equilibrium between the selectivity function and interacting protein is essentially frozen in the UV cross-linking step. This feature can be used to obtain quantitative information about K_d values between the ligand and the affinity selected proteins. Furthermore, the combination of Capture Compound® technology and Charles River Laboratories' extensive suite of bioinformatic software tools can yield valuable information on the binding site of a small molecule on a target protein.

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. The CCMS technology at Charles River Laboratories can be applied from target ID and hit-to-lead through to lead optimisation and beyond, 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 your drug discovery journey.

References

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[3] Schenone, M et al. Nature chemical biology 9(4), (2013): 232–240

[4] Hughes, JP et al. British Journal of Pharmacology 162(6), (2011): 1239–1249

[5] Dambach, DM et al. Chemical *Research in Toxicology 29*(4), (2016): 452-472

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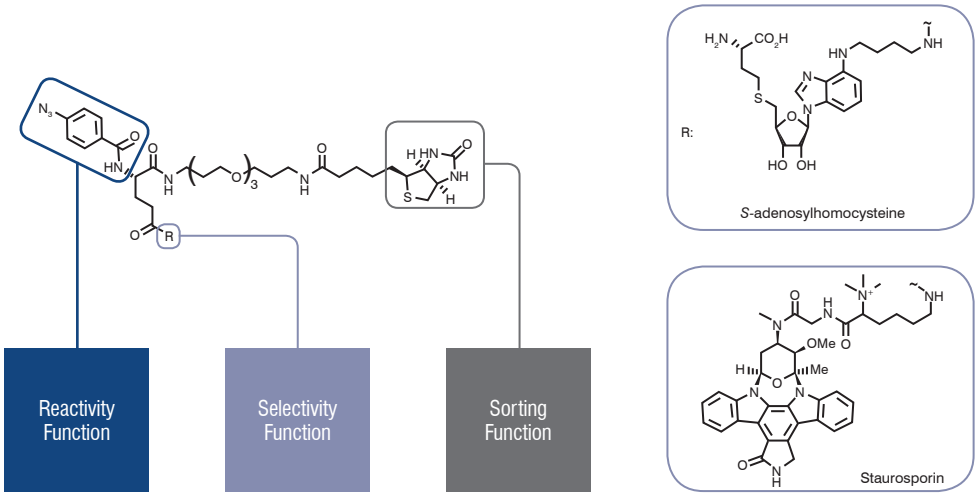

Figure 1: Capture Compounds® are small, tri-functional molecules, consisting of a reactivity function, a sorting function and a variable selectivity function.

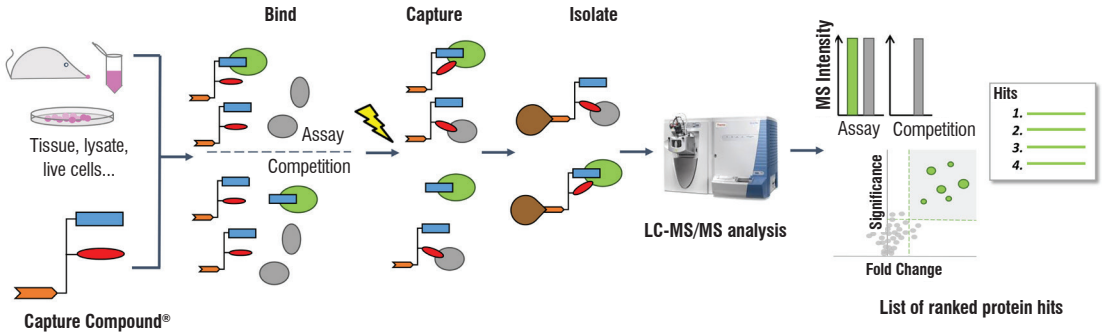

Figure 2: The principle of a CCMS experiment is a competition assay. The client will recieve a ranked list of potential binding partners for their small molecule compound.

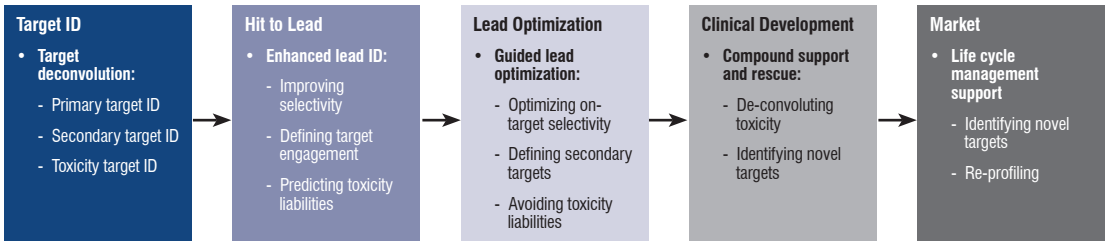

Figure 3: CCMS is a powerful chemoproteomic tool for profiling protein interactions of small molecules.