Capture Compound Mass Spectrometry: Target De-Convolution and Other Drug-Protein Interactions

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1 CCMS Technology: An Introduction

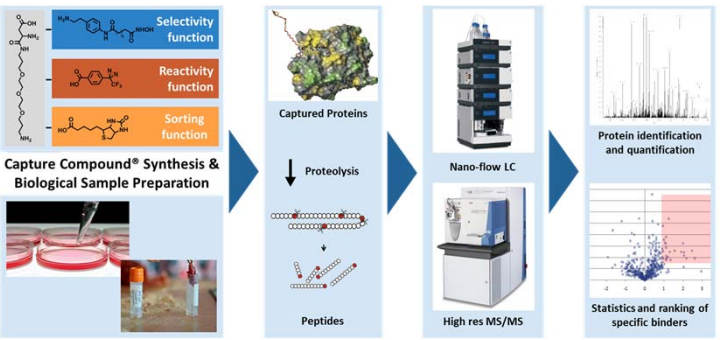
Capture Compound Mass Spectrometry (CCMS) is an unbiased, proteome-wide approach for the identification of specific-binding protein targets for small molecules of interest. The technology combines medicinal chemistry and *in vitro* pharmacology, coupled to high resolution proteomics mass spectrometry to isolate and identify target proteins that are responsible for the observed biological response.

Capture Compounds® are unique tri-functional small molecule probes designed to interrogate proteins in their native environments. The distinct molecular architecture of the Capture Compounds® enables a three-stage process of binding, capture and isolation, which offers significant advantages over other chemoproteomic techniques, such as affinity pull-down and photoaffinity labelling[1,2]. These advantages include:

Greater sensitivity
- Target proteins are covalently-captured, meaning even very weak or low-affinity binding interactions can be interrogated

Superior cross-linking yields
- The design of the Capture Compounds® enables the reactivity function to sample a radius around the binding pocket for favourable cross-linking sites, giving yields of up to 50%

Wider application
- Capture Compounds® engage their targets in homogenous phase, meaning no solid support is required during the binding and capture steps. This enables CCMS to interrogate protein targets that are largely intractable by other chemoproteomic techniques, including transmembrane (e.g. GPCRs)[3] and even intracellular targets from living cells



The leading technology for identifying protein targets of small molecules, CCMS can be used to investigate any protein target class in an unmatched variety of biological matrices (cell and tissue lysates; membrane suspensions; live cells; primary cells; human post mortem tissue).

With a diverse range of applications, CCMS technology can be used in:
- De-convolution of novel targets mediating therapeutic effects of small molecules identified in phenotypic screens
- Identification of novel targets of known compounds with unexplained activities (drug repurposing, for example)
- De-convolution of off-target (or undesired) activities of key compounds to shed light on mechanisms causing toxicity

2 Case Study: Target De-Convolution by CCMS Identifies Alternative MoA in Phenotypic Screen

2.1 Capture Compound® Synthesis

CCMS was used in the target de-convolution of two hit series from a phenotypic screen. The two series displayed the same phenotype, despite being structurally unrelated.

- Two Capture Compounds® were synthesised from each series
- Surrogate Capture Compounds® were prepared to confirm that phenotypic activity was retained with the linker and probe in-place
- Capture Compounds® were confirmed as suitable for CCMS in an induced photo-degradation assay

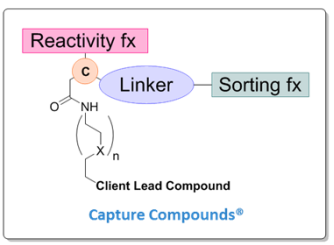


Figure 1. Generic structure of Capture Compounds®

2.2 Optimisation of CCMS Parameters

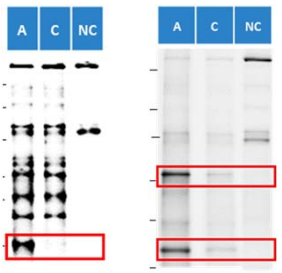


Figure 2. Western blots of Series 1 (left) and Series 2 (right) capture experiments showing clear differences between assay (A) and competition (C); NC = No CC

Initial capture experiments were conducted by western blot in HepG2 lysates, optimising parameters for specific protein capture, including:

- Capture Compound®/competitor concentrations
- Irradiation and incubation time
- Protein input and buffers/detergents
- Lysis conditions

Both sets of Capture Compounds® were used with the entire unfractionated lysate and with a membrane enriched fraction.

2.3 Full Scale CCMS Experiment: Determination of Specific Binding Protein Partners

- Samples were assessed label-free by LC-MS/MS on a Quadrupole-Orbitrap™ mass spectrometer
- Output files were analysed using the MaxQuant quantitative proteomics software package

A protein was considered a specific binder if the data for either Capture Compound® presented:
- An average assay sample two-fold higher than both competition and NoCC control ($\log_2$[assay/competition] > 1)
- Significant difference between assay/competition and assay/NoCC control (unpaired T-test, $P < 0.05$)

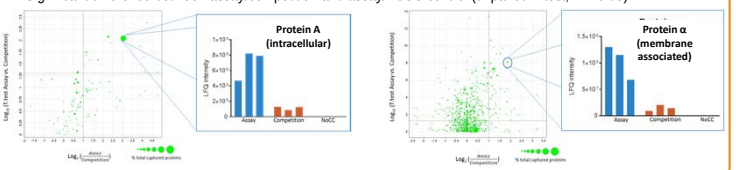


Figure 3. Volcano plots of Series 1 (left) and Series 2 (right) showing specificity and statistical significance of captured proteins

2.4 Target De-Convolution by CCMS: Data Output

- Lists of the top-ranking specific protein binders for each series
- Subcellular location of target proteins
- Divergent profiles indicating that the two series are likely operating by different mechanisms of action

Series 1			Series 2		
Rank	Protein ID	Score	Rank	Protein ID	Score
1	Protein A	32.6	1	Protein α	12.4
2	Protein B	4.3	2	Protein β	3.9
3	Protein C	3.9	3	Protein γ	2.9
4	Protein D	3.9	4	Protein δ	2.8
5	Protein E	2.1	5	Protein ε	2.8

Figure 4. Top-ranking specific binders for each series identified by CCMS

CCMS provided a ranked list of the most specific binding protein targets for each series, scored using a combination of specificity, statistical significance and % total protein captured

3 CCMS: Supporting Projects Across Discovery and Development

CCMS is a powerful chemoproteomic tool for profiling protein interactions of small molecules, which can be used to support projects at a variety of stages throughout drug discovery and development.

- *Capture Compounds® offer several advantages over traditional chemoproteomic techniques*
- *Live cell CCMS enables the capture of native transmembrane (GPCRs) and intracellular protein targets*
- *CCMS can probe the potential mechanisms causing toxicity by identifying specific binding off-targets*

References:
1 – Fischer et al (2010). Comprehensive Identification of Staurosporine-Binding Kinases in the Hepatocyte Cell Line HepG2 using Capture Compound Mass Spectrometry (CCMS). Journal of Proteome Research 9: 806-817. DOI: 10.1021/pr9007333; 2 – Fischer et al (2011). SAHA Capture Compound – A novel tool for the profiling of histone deacetylases and the identification of additional vorinostat binders. Proteomics 11:4096-4104. DOI 10.1002/pmic.201000717; 3 – Blex et al (2017). Targeting G Protein-Coupled Receptors by Capture Compound Mass Spectrometry: A Case Study with Sertindole. ChemBioChem 18. DOI: 10.1002/cbic.201700152