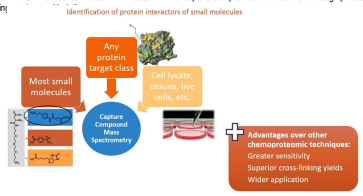
Capture Compound Mass Spectrometry®: on- and off-target deconvolution

T. Ladduwahetty, S. Almond*, E. Bush*, A. Cridland*, S. Dowler*, G. Hardman*, D. Kenny*, I. Linney*, N. Macabuag*, D. Mitchell*, P. Mitchell*
Charles River Laboratories, Chesterford Research Park, Saffron Walden, UK
Tammy.Ladduwahetty@crl.com
* CCMS team at Charles River; Sarah.Almond@crl.com

charles river

1 CCMS INTRODUCTION

- Capture Compound Mass Spectrometry (CCMS) is an unbiased, proteome-wide approach for the identification of specific on- and off-target binding protein targets for small molecules of interest.
- Capture Compounds® (CCs) are unique tri-functional probes designed to interrogate proteins in their native environments. The distinct molecular architecture of the Capture Compounds® enables a three-stage process of binding



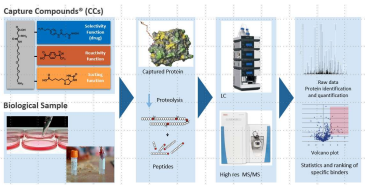
2 CCMS is a chemoproteomic tool for profiling protein interactions of small molecules

- Unbiased screening of the entire proteome compared with traditional selectivity screening

- *CCMS can support projects at a variety of stages throughout drug discovery and development.*

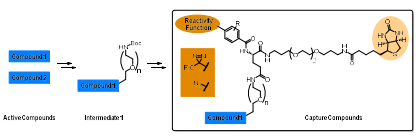
3 THE CCMS EXPERIMENT

- A panel of CCs are synthesized with the selectivity function in a position compliant with on-target SAR and in alternative orientations to allow generation of a full interaction profile
- CCs are incubated with a biological sample whereby binding of the selectivity function to interacting proteins occurs before covalent capture via photo-irradiation
- Competition experiments include incubation of the sample with both the CC and an excess of the free ligand to allow specifically interacting proteins to be determined
- Sample analysis by high-resolution LC-MS/MS reveals specific binding partners

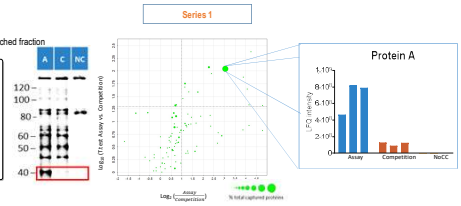


4 CASE STUDY: CCMS IDENTIFIES ON TARGET MECHANISM FROM PHENOTYPIC SCREEN

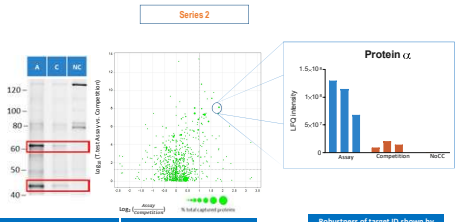
- Client showed that two structurally distinct series displaying the same phenotype
  - Structural diversity between the two series suggested alternative MoA possible
- A precursor molecule from each series synthesised
  - Each retained activity in the phenotypic assay
- Two Capture Compounds® were synthesised from each series to attempt target deconvolution
- The Capture Compounds® were used with the entire unfractionated lysate and with a membrane enriched fraction



	Phenotypic assay EC_{50} [µM]
Compound 1	<0.01
Intermediate 1	<0.01
Compound 2	0.22
Intermediate 2	1.29



One band significantly modulated by Series 1 CC & competition | Volcano plot shows one principal intracellular target | Robustness of target ID shown by analysis of individual samples



Two distinct bands modulated by Series 2 CC & competition | Volcano plot shows one principal membrane associated target | Robustness of target ID shown by analysis of individual samples

Series 1	
Rank	Protein ID
1	Protein A
2	Protein B
3	Protein C
4	Protein D
5	Protein E

Series 2	
Rank	Protein ID
1	Protein α
2	Protein β
3	Protein γ
4	Protein δ
5	Protein ε

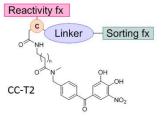
Lysate-based CCMS was successfully used to:

- Deconvolute both intracellular and membrane associated targets
- Identify different MoA from two series of compounds displaying the same phenotype
- Determine the subcellular location of the target protein in each case
- Provide a ranked list of specific-binding partners, including off-targets, for each series
- Literature searches provided evidence that the principal targets are associated with the relevant indication

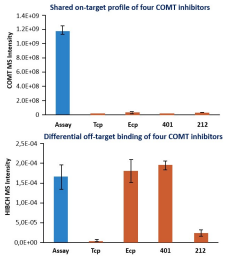
Principal intracellular and membrane bound protein targets deconvoluted using CCMS

5 CASE STUDY: CCMS IDENTIFIES OFF-TARGET MECHANISM OF TOLCAPONE TOXICITY

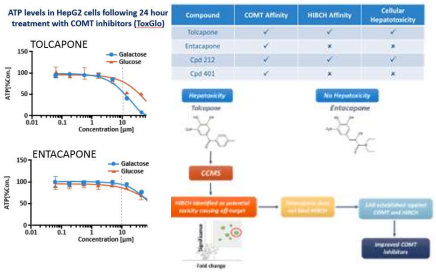
- CCMS was used to determine on- and off-target interactions of the catechol-O-methyl transferase (COMT) inhibitor tolcapone in a human liver cancer cell line (HepG2)
- Aim of study: determine hepatotoxicity causing off-target for tolcapone and compare with entacapone which has no toxicity



Inhibitors and CCs	IC_{50} [nM], rec. human COMT
Tolcapone	127
Tolcapone CC - T1	>6,000
Tolcapone CC - T2	448
Entacapone	386
Cmpd 212	179
Cmpd 401	54



- Differential binding profiles were seen against on- and off target proteins. These binding profiles correlated with cellular hepatotoxicity



Compound	COMT Affinity	HIBCH Affinity	Cellular Hepatotoxicity
Tolcapone	✓	✓	✓
Entacapone	✓	×	×
Cpd 212	✓	✓	✓
Cpd 401	✓	×	×

Toxicity causing off-target identified using CCMS

CCMS® is a powerful addition to drug discovery capabilities at Charles River