caprotec
capture protein technology

Functional isolation of staurosporine binding protein kinases using CCMS technology

Capture Compound mass spectrometry (CCMS) is an innovative technology to reduce biological sample complexity by selective isolation of targeted protein or enzyme families. Small synthetic molecules (Capture Compounds™) are used to interrogate native proteins. Using staurosporine as a selectivity function for a kinase-specific Capture Compound™ enables efficient complexity reduction of the proteome and allows discovery, isolation and profiling of functional kinases in biological samples. Kinase-specific Capture Compounds™ are available as ready-to-use caproKits™ for proteomic research.

In the last few years proteomics has become a very important aspect in modern molecular and cellular biology and in the whole of life sciences. Especially the emerging field of systems biology but also the development of new drug candidates benefit from results generated by proteomic approaches[1]. Caprotec bioanalytics' kinase-specific Capture Compound™ enables efficient reduction of proteome complexity for such studies.

The proteome is a highly complex mixture of diverse proteins and peptides varying in concentrations and states. To understand the time-dependent interplay of the different pathways, and the structure and function of all involved proteins, is a challenging task. Post-translational protein modifications (phosphorylation, glycosylation and others) constitute an additional layer of complexity and are important factors in the development of certain diseases[2,3].

Investigation of kinases is of outstanding interest as these enzymes have key roles in cell development, signaling and metabolism. Mutations and deregulation of kinases are causative for many human diseases, which has placed kinases in the focus of biomarker discovery and drug development[4]. The selectivity profiling of different kinase inhibitors against large panels of kinases has provided a broad data basis for assessing the utility of certain inhibitors to address subsets of the kinome. As an example, staurosporine is a prototypical ATP-competitive kinase inhibitor that interacts with up to 253 human protein kinases[5]. Its broadband affinity makes staurosporine an attractive candidate for probing large sets of kinases in biological samples. Therefore, staurosporine is an ideal selectivity function for a kinase-specific Capture Compound™.

Erik Dülsner, Jenny J Fischer, Christian Jurinke & Hubert Köster

caprotec bioanalytics GmbH, Berlin, Germany. Correspondence should be addressed to E.D. (erik.duelsner@caprotec.com) or C.J. (christian.jurinke@caprotec.com).

Capture Compounds™ are tri-functional molecules that allow a reversible affinity interaction between their selectivity function and specific target proteins, even lipophilic membrane proteins. Subsequently, a reactivity function forms a covalent bond with the

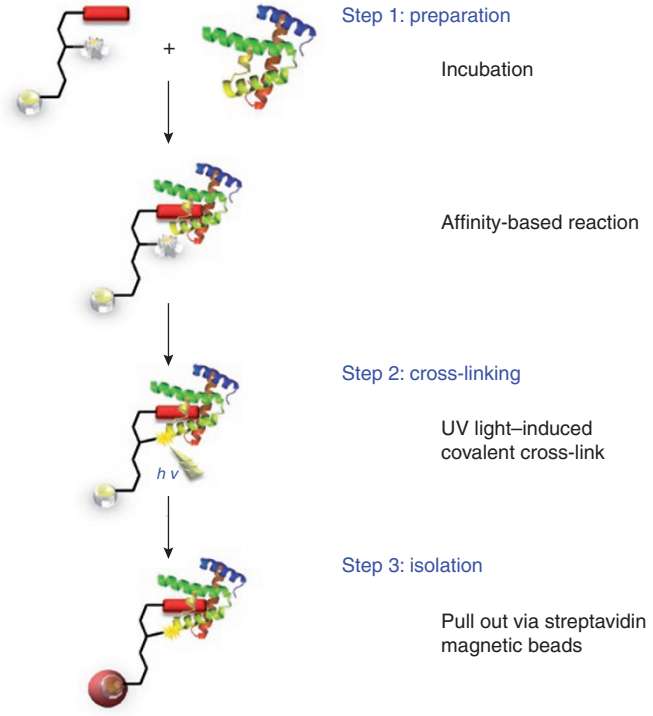

Figure 1 | Capture Compounds bind proteins through reversible affinity interactions. A covalent bond between Capture Compound and target protein is then generated by photo-cross-linking. Streptavidin-biotin interactions are used to isolate captured proteins for western blot or mass spectrometry analysis, respectively.

APPLICATION NOTES

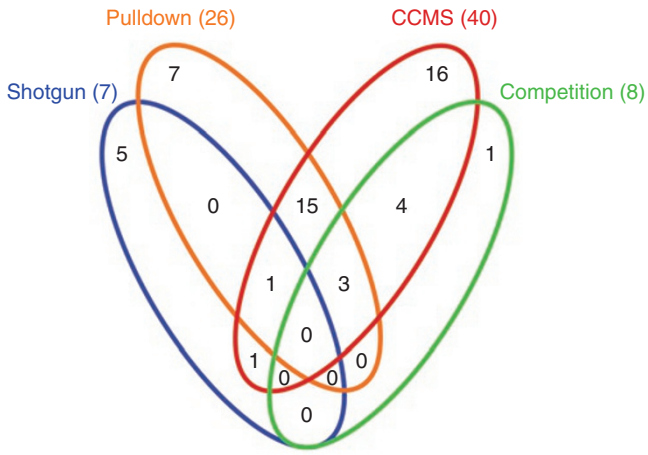

Figure 2 | CCMS yields more captured kinases in HepG2 cell lysate than shotgun and pulldown assays. Competition with free staurosporine was used as a control.

interacting proteins and a sorting function enables isolation directly from cell lysates. The isolated proteins can be analyzed and characterized via gel electrophoresis or directly by mass spectrometry (**Fig. 1**).

To characterize the performance of the Stauro caproKit™, we used it to isolate and identify staurosporine-binding proteins in HepG2 cell lysate samples and compared this process to pulldown methods, which lack a covalent cross-link, and to shotgun analysis without any pre-enrichment. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis of the experiments revealed that, in a single run, Capture Compound mass spectrometry (CCMS) identified 40 different kinases, whereas the pulldown approach yielded 26 and the shotgun approach only 7 kinases, each from 500 µg of protein lysate. The results and the overlap between the identified kinases are depicted in **Figure 2**.

In comparison to the widely used pulldown assays, CCMS uses a covalent cross-link to the targeted protein, which allows the analysis of low-affinity interactions and the use of stringent wash procedures because material loss is minimized during washing steps.

The Stauro caproKit™ enables a particularly easy, sensitive and effective means of profiling kinases from biological samples. In addition, the technology allows identification of kinases from different functional classes. **Table 1** provides a functional classification of kinases isolated from the HepG2 cell lysate.

Table 1 | Identified kinases in HepG2 cell lysates

Family	Captured kinases
Serine-threonine kinases	25
Small-molecule kinases	7
Tyrosine-protein kinases	5
Dual-specificity kinases	3

The Stauro caproKit™ is a highly effective tool for the isolation of staurosporine-binding kinases from complex protein mixtures. The specificity of the assay is demonstrated in competition experiments; control reagents to perform these reactions are included in each caproKit™.

In conclusion, CCMS allows the affinity enrichment of interacting proteins with a considerably higher efficiency than pulldown assays. This advantage results from a covalent cross-link between Capture Compounds™ and the targeted protein. With its high reproducibility and efficiency, the CCMS technology is poised to play an important role in analyzing the structure and function of cellular proteins.

Capture Compounds™ are available as caproKits™ from caprotec bioanalytics. In addition to the Stauro caproKit™, Capture Compounds™ are available with cyclic GMP (cGMP), cAMP and *S*-adenosyl-L-homocysteine (SAH) selectivity functions to target cyclic nucleotide monophosphate binding proteins, methyltransferases and other *S*-adenosyl methionine (SAM)-binding proteins, and customized Capture Compounds™ can be created for specific projects—for example, characterizing drug candidates.

1. Köster, H. *et al.* Capture compound mass spectrometry: a technology for the investigation of small molecule protein interactions. *Assay Drug Dev. Technol.* **5**, 381–390 (2007).
2. Gygi, S.P., Rochon, Y., Franza, B.R. & Aebersold, R. Correlation between protein and mRNA abundance in yeast. *Mol. Cell. Biol.* **19**, 1720–1730 (1999).
3. Ideker, T. *et al.* Integrated genomic and proteomic analyses of systematically perturbed metabolic network. *Science* **292**, 929–934 (2001).
4. Cohen, P. Protein kinases—the major drug targets of the twenty-first century? *Nat. Rev. Drug Discov.* **1**, 309–315 (2002).
5. Karaman, M.W. *et al.* A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **26**, 127–132 (2008).