

CCMS technology enables improved proteomic analysis through functional isolation of sub-proteomes

Capture Compound Mass Spectrometry (CCMS) enables gel-free LC-MS/MS-based analysis of small molecule - protein interactions. The method is based on complexity reduction of biological samples via novel Capture Compounds™. These small synthetic probes interrogate native proteins, even lipophilic membrane proteins, for affinity interactions and are used to isolate, discover or profile specific protein families in complex biological samples.

In the last few years proteomics has become a very important approach in modern molecular and cellular biology and the whole area 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.

The proteome is a highly complex mixture of diverse proteins and peptides varying in concentrations and conditions. To understand the time-dependent interplay of the different pathways and the structure and function of all involved proteins is a challenging task. Additionally, post-translational protein modifications (e.g., phosphorylation, glycosylation) and differences in the level of protein expression are important factors in the development of certain diseases [1, 2] and constitute an additional layer of complexity.

Different methods are available to analyze complex protein mixtures [3, 4]. The most common approaches include two-dimensional gel

electrophoresis [5], affinity chromatography [6], and mass spectrometry. These methods are widely accepted and routinely applied in many laboratories as useful tools to analyze the structure and function of cellular proteins. However, they also have inherent limitations in the analysis of complex samples: the resolution capacity for high and low abundant proteins, the capability to analyze lipophilic or very large proteins or to investigate low-affinity small molecule protein interactions. These limitations have created a need for methods to reduce the complexity of biological samples prior to analysis.

One approach to accomplish this task is the removal of high-abundant proteins. However, this approach also introduces new and unwanted bias by itself [7, 8]. Another approach to reduce the complexity of biological samples is based on selective interactions with affinity reagents. Here, a significant drawback of most methods is the lack of covalent interactions between ligand and protein, leading to the loss of many weak or low

affinity interactions during the purification process.

At caprotec bioanalytics we have developed an innovative technology to significantly reduce the complexity of biological samples through selective and functional isolation of targeted protein or enzyme families via small, multifunctional

the isolation of the complex directly out of the sample.

The complexity reduction is accomplished in three simple steps:

In the **first step**, Capture Compounds are incubated with a biological sample. In this phase a

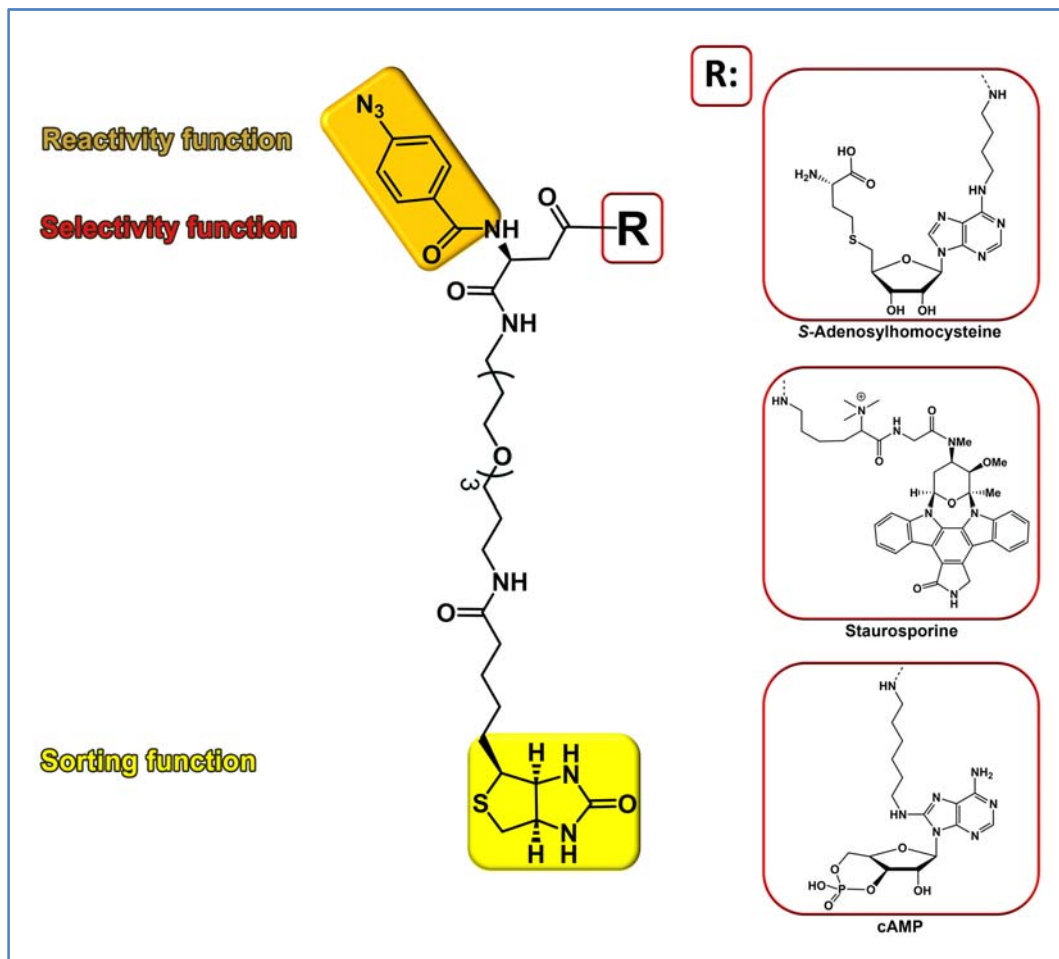


Figure 1 The core of Capture Compound Mass Spectrometry is a small, tri-functional molecule, the Capture Compound which consists of a sorting function, reactivity function and a variable selectivity function.

molecules, called Capture Compounds™ [9]. A schematic representation of a Capture Compound is shown in Figure 1.

Capture Compounds mediate a reversible affinity interaction between their specific selectivity function and target proteins. Subsequently, a reactivity function forms a covalent bond with the interacting proteins and a sorting function enables

reversible, affinity driven interaction between the selectivity function and the target proteins occurs.

In the **second step**, the caproBox™ is used to photo-activate the reactivity function via UV light for a covalent attachment to the proteins.

In the **third step**, biotin is used as sorting function to isolate captured proteins from the mixture through Streptavidin coated magnetic beads. The

isolated proteins can be analyzed and directly identified and characterized by mass spectrometry (Figure 2).

Important advantages of CCMS are the ability to use protein samples from different sources and origins (e.g. cell culture, bacteria, plants or tissues) and the possibility to omit the depletion of albumin or other highly abundant proteins. Furthermore, the assay is not dependent on substrate conversion so that target molecules do

not need to be active enzymes.

Another important advantage of CCMS is that capture reactions are performed in a simple, homogeneous protocol which can be easily performed within a working day. The researcher follows a robust “one-pot” reaction that is amenable to automation and up-scaling with off-the-shelf liquid handling devices. CCMS processes complement and improves the detection limits of traditional biological approaches (e.g. western

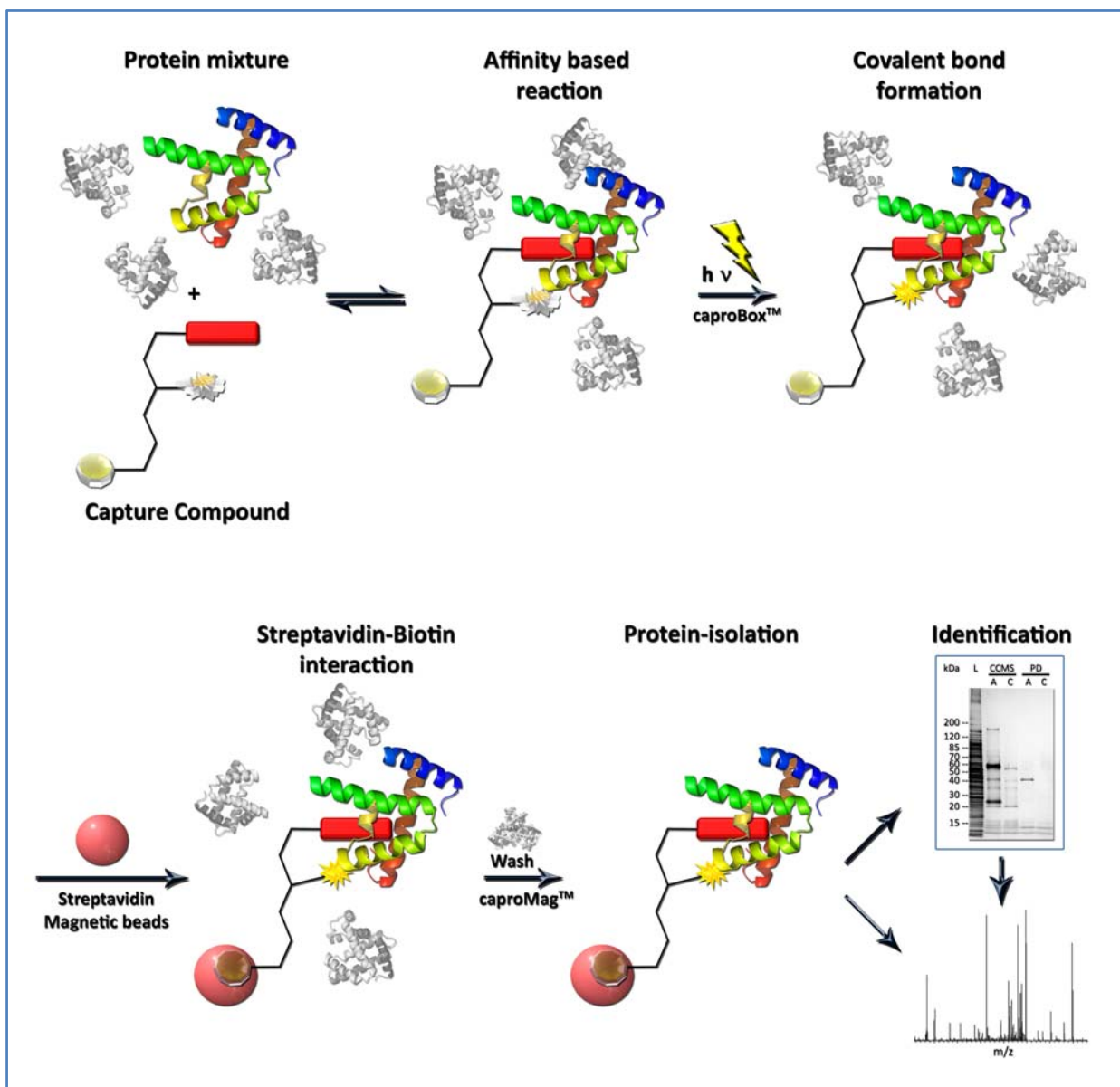


Figure 2 Capture Compounds bind proteins through reversible affinity interaction. A covalent bond between Capture Compound and target protein is generated by photo cross-linking. Streptavidin-Biotin interactions are used to isolate captured proteins for Western blot or MS analysis, respectively. L: *E coli* lysate; PD: Pull-down; A: Assay; C: Control using SAH as competitor.

blotting, mass spectrometry) through the direct enrichment of functional sub-proteomes and, additionally, a generated covalent bond between the targeted proteins and the Capture Compounds by photo cross-linking.

Finally, CCMS provides the possibility to easily integrate internal controls in the experiment. 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 practically 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.

This novel platform technology based on multifunctional small molecules enables the isolation of functional sub-proteomes, the identification and gel-free analysis of individual proteins and post-translational modifications based on mass spectrometry, and, in addition, the comparison of expression levels between population subsets.

Capture Compounds enable an efficient complexity reduction of the proteome to a subset of proteins based on their affinity to the selectivity group. Thus, CCMS allows discovering, isolating and profiling members of functional protein families within a variety of biological samples.

References

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Ordering information

Capture Compounds are available as ready-to-use caproKits™ for profiling of: cAMP/cGMP binding proteins, methyltransferases/HDAC, GDP/GTP binding proteins, and protein kinases.

Item Nr.	Description
1-1020-050	Stauro caproKit™ 50 reactions
1-1020-010	Stauro caproKit™ 10 reactions
1-1030-050	C8-cAMP caproKit™ 50 reactions
1-1030-010	C8-cAMP caproKit™ 10 reactions
1-1031-050	C2-cAMP caproKit™ 50 reactions
1-1031-010	C2-cAMP caproKit™ 10 reactions
1-1032-050	N ⁶ -cAMP caproKit™ 50 reactions
1-1032-010	N ⁶ -cAMP caproKit™ 10 reactions
1-1035-000	cAMP caproKit™ complete
1-1040-050	N ² -cGMP caproKit™ 50 reactions
1-1040-010	N ² -cGMP caproKit™ 10 reactions
1-1050-050	GDP caproKit™ 50 reactions
1-1050-010	GDP caproKit™ 10 reactions
1-1070-050	SAHA caproKit™ 50 reactions
1-1070-010	SAHA caproKit™ 10 reactions
1-1080-050	Dasatinib caproKit™ 50 reactions
1-1080-010	Dasatinib caproKit™ 10 reactions
1-1090-050	SAH caproKit™ 50 reactions
1-1090-010	SAH caproKit™ 10 reactions
1-1100-060	Biotin Capping Kit 60 reactions
1-8050-010	CCMS Starter Kit
1-8050-050	CCMS Starter Kit XL

The caproKit includes the respective Capture Compound, all buffers, reaction vials, protein positive control, competitor, and Streptavidin magnetic beads. CCMS Starter Kit includes caproBox™, caproMag™, Biotin Capping Kit, and three 10 reactions caproKits™ of choice. CCMS Starter Kit XL includes caproBox™, caproMag™, Biotin Capping Kit, and two 50 reactions caproKits™ of choice.

Products & Services

CCMS technology is made available as ready to use caproKit reagents and services.

For more information please visit www.caprotec.com

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