

## SAH Capture Compound™, a powerful analytical tool for the profiling of methyltransferases

Capture Compound Mass Spectrometry (CCMS) is an innovative technology to reduce the complexity of biological samples through selective isolation of targeted protein or enzyme families. Small synthetic molecules (Capture Compounds™) interrogate native proteins, even lipophilic membrane proteins. CCMS technology enables an efficient complexity reduction of the proteome and allows discovering, isolating and profiling members of functional protein families within a variety of biological samples.

### Introduction

S-Adenosyl-L-methionine (SAM) is the second most abundant metabolite in eukaryotic organisms (1, 2). It is one of the donors of methyl groups in enzymatic reactions to methylate small molecules, nucleic acids, and proteins. With respect to small molecules, methylation is one step in the catabolism of important transmitter substances such as histamine or dopamine. Regarding DNA, methylation is the basis for the genomic imprinting, the epigenetic control of gene expression that is brought about by eukaryotic DNA methyltransferases that can methylate cytosine bases within the CpG sequence of DNA (prokaryotic DNA methyltransferases can methylate adenine or cytosine within various recognition sequences). Also, RNA is subject to methylation, and this is one of the key posttranscriptional modifications of, e.g., ribosomal RNA. Methylation occurs also on proteins, e.g., in the form of mono-, di-, or trimethylation of lysine residues of histones, thus constitutes a component of the “histone code” that is considered crucial for the epigenetic control of gene expression. Matching

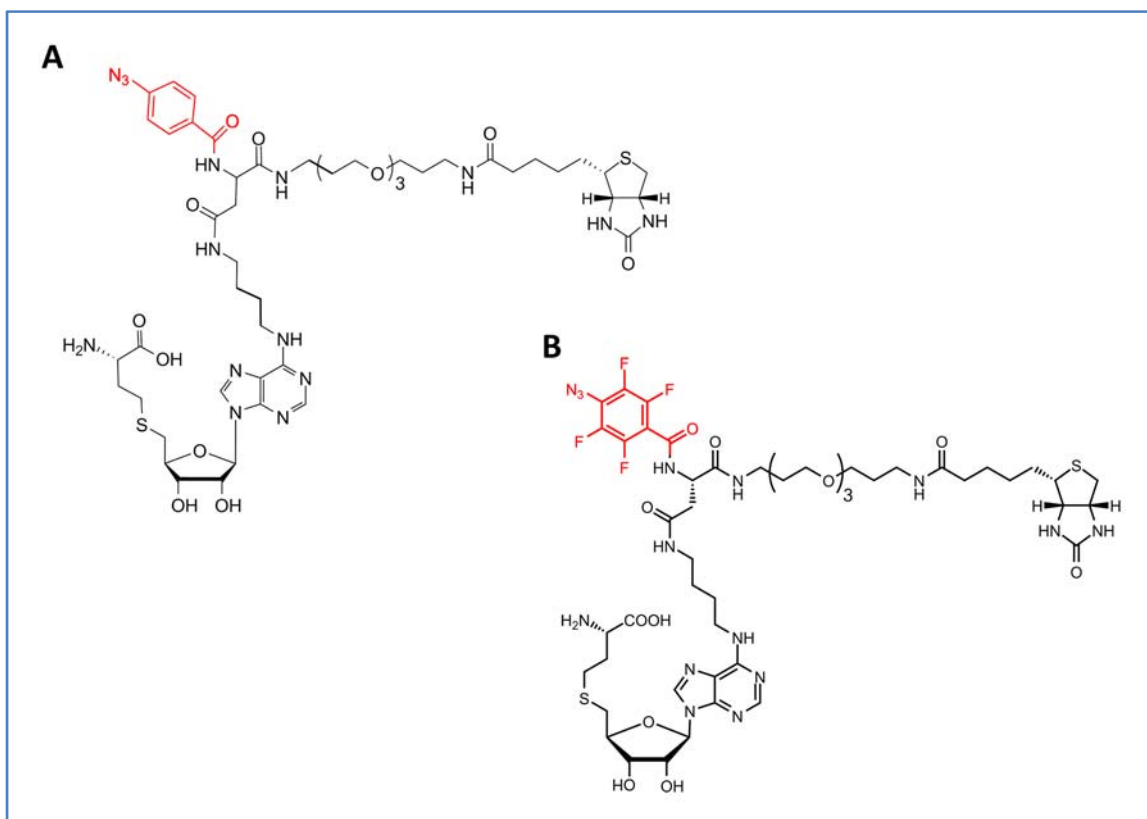
the diverse methylation events, there is a diverse range of methyltransferases that catalyse the methylation reactions. Given the crucial role of methylation reactions in diverse physiological scenarios, the profiling of methyltransferases can be expected to become of similar importance in functional proteomics as the profiling of kinases. The majority of known methyltransferases contains a Rossmann-fold-like motif and is product-inhibited by S-adenosyl-L-homocysteine (SAH, de-methylated SAM). Analytical tools for their profiling, however, have not been available. Recently a Capture Compound™ with SAH as selectivity function has been introduced to fill this technological gap. The Capture Compound™ is suitable for the direct isolation and identification of a wide range of methyltransferases from complex biological samples. This Capture Compound™ has now been improved with respect to its performance in the “OffBead” (3) configuration. This was achieved by a switch of the reactivity function, from a phenyl azide to a tetrafluorophenyl azide. In this application note, data is presented on the benefits of the new SAH-

CC, with respect to increased cross-link yield and OffBead capturing of methyltransferases.

## Materials

The SAH caproKit™ consists of the SAH Capture Compound™, SAH competitor solution, streptavidin magnetic beads, 5x concentrated capture buffer, 5x concentrated wash buffer 1, and purified MTAql as the positive control. In the new version of the SAH caproKit™, the SAH Capture Compound™ with the phenyl azide reactivity function is replaced by the corresponding Capture Compound™ with the tetrafluorophenyl azide reactivity function (Figure 1).

experiments, wash and isolate the magnetic beads. Irradiation of the samples for photo cross-linking was performed using the caproBox™, and the beads were isolated using the caproMag™. As sources of complex protein mixtures, *E.coli* DH5α lysate and human hepatocyte-derived HepG2 cell lysate were used. *E.coli* DH5α lysate was prepared from cells grown to the early logarithmic phase, and, subsequent to harvesting by centrifugation, resuspended in lysis buffer (6.7 mM MES, 6.7 mM NaOAc, 6.7 mM HEPES, 1.0 mM EDTA, 10.0 mM 2-mercaptoethanol, 200 mM NaCl, 0.2 mM PMSF, 10 % glycerol, pH 7.5), and sonicated. The lysate was cleared of debris by centrifugation and concentrated to a final concentration of 30 mg/ml. The lysate was used in the assay at a final concentration of 5 mg/ml. As a source of mammalian proteins,



**Figure 1** SAH Capture Compound™ with the phenyl azide reactivity function (A) will be replaced by the corresponding Capture Compound with the tetrafluorophenyl azide reactivity function (B)

Assays were prepared in PCR tube strips for volumes up to 200 µl (Thermo Fisher, cat. No. AB-1114) as reaction vessels to conduct the capturing

HepG2 lysate at a stock concentration of 7 mg protein/ml was used. HepG2 cells were lysed by French Press in lysis buffer (6.7 mM MES, 6.7 mM NaOAc, 6.7 mM HEPES, pH 7.5, supplemented to 200 mM NaCl, 10 mM β-mercaptoethanol, 1 mM EDTA, and protease inhibitor cocktail). Debris was removed by centrifugation at 30,000 x g for 60 min at 4 °C. The supernatant filtrated through a 0.2 µm

filter, and was then dialysed against lysis buffer for the removal of small molecules. After dialysis, centrifugation at 4000 x g was carried out to remove precipitated proteins during dialysis.

## Capture experiment

The capture experiments were carried out in the OffBead configuration as described in the CCMS guideline [downloadable at \[www.caprotec.com/support/downloads\]\(http://www.caprotec.com/support/downloads\)](#), and further detailed in the specific guideline for the SAH caproKit™. In comparison to the phenyl azide-containing SAH-CC, the new SAH-CC with the tetrafluorophenyl azide reactivity function requires a shorter irradiation time of 4 instead of 10 min. In brief, the assays were prepared as follows: First, water was pipetted into PCR tubes followed by 5x capture buffer, and the tubes were vortexed thoroughly. Then, Cell lysate was added to a final concentration of 3 mg/ml and carefully mixed. The Capture Compound™ and, for competition experiments, the SAH Competitor was added to the sample and incubated for 10 min at 0-4 °C. The lids were removed from the tubes and the samples were placed in a pre-cooled caproBox™. The samples were irradiated for 4 or 10 min at 2-4 °C depending on their respective reactivity function. Following irradiation, competitor solution as described in the Guideline was added to the samples and the lids are re-fitted onto the PCR tubes. After mixing and 10 min incubation of the samples at 4 °C, 5x wash buffer 1 was added, and the samples were mixed thoroughly. Finally, streptavidin magnetic beads were added and the samples were rotated for 30 min at 4 °C. Capture Compound-protein conjugates collected by the beads were washed and isolated using the caproMag™ as described in the Guideline ([www.caprotec.com/support/downloads](http://www.caprotec.com/support/downloads)). Additionally, to assess the performance of the new SAH-CC in the OnBead configuration, OnBead experiments were performed as described in the guideline and samples were then either incubated with 2x concentrated sample buffer for SDS-PAGE and boiled for 5 min or subjected to OnBead tryptic digestion as described in the respective guideline [www.caprotec.com/support/downloads](http://www.caprotec.com/support/downloads).

## Mass spectrometry

LC-MS/MS experiments were performed using a nanoflow HPLC system (Proxeon) coupled on-line to an ESI-LTQ-Orbitrap system. From the LC column, peptides were eluted during an 90-min linear gradient from 5 % ACN/0.1 % FA to 40 % ACN/0.1 % FA followed by additional 2 min to 100 % ACN/0.1 % FA and remaining at 100 % for another 8 min with a controlled flow rate of 300 nl/min. MS/MS fragmentation was performed in a data-dependent mode using one survey MS scan followed by four MS/MS scans per second. Proteins were identified by automated database searching against the human UniProtKB/Swiss-Prot database using SEQUEST and Tandem X! as implemented in the software scaffold 2.0. Specific search parameters were 5 ppm precursor tolerance, 1 amu fragment ions tolerance, and full trypsin specificity allowing for up to two missed cleavages. Phosphorylation at serine, threonine, and tyrosine, oxidation of methionines, deamination at asparagines and glutamine, acetylation at lysine and serine, formylation at lysine, and methylation at arginine, lysine, serine, threonine and asparagine were allowed as variable modifications. No fixed modifications were used in database search.

## Results

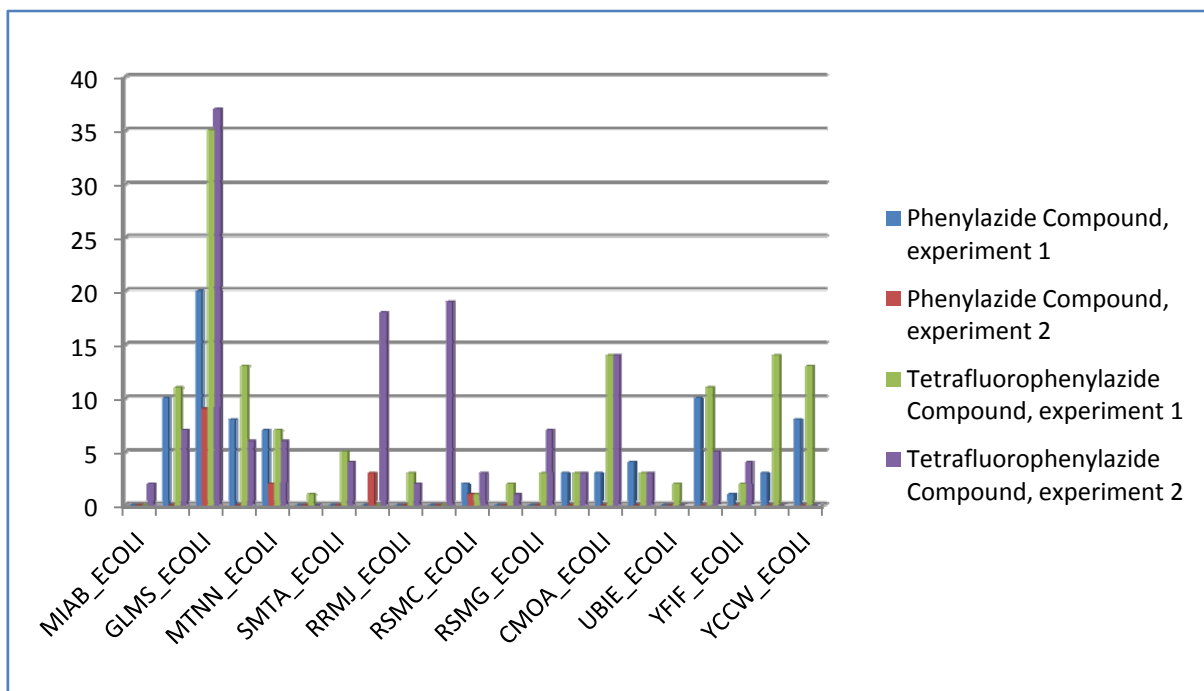
Capturing SAH-binding proteins from *E.coli* lysate in the OffBead configuration was significantly more effective with the new S-Adenosyl-L-homocysteine Capture Compound™ (SAH-CC) that carried a tetrafluorophenyl azide reactivity function. This SAH-CC captured all of the methyltransferases captured by the old SAH-CC, but more robustly as deduced from the number of identified peptides in the LC-MS/MS analysis. Furthermore, the new SAH-CC allowed the isolation and identification of additional SAH-binding proteins (Figure 2). This is particularly striking in the case of the tRNA methyltransferase CMOA (Swiss-Prot Acc. P76290) that was detected in the LC-MS/MS analysis of the peptide mixtures from proteins captured in the OffBead configuration with 14 unique peptides in duplicate experiments when

the tetrafluorophenylazide Capture Compound™ was used, but only once with 3 unique peptides when the phenylazide Capture Compound™ was used. However, in the OnBead configuration, both new and old SAH-CC performed equally well (Figure 3). This can be seen for example in the case of CMOA. The numbers of identified unique peptides were 8/7 peptides with the phenylazide Capture Compound compared to 15/9 with the tetrafluorophenylazide Capture Compound, demonstrating a similar outcome.

Taken together, these results suggests that the new SAH-CC is a substantially improved tool for the isolation of methyltransferases, combining the established strength of the old SAH-CC with additional features, making the OffBead configuration with this CC much more powerful. This

was confirmed further through capture experiments in HepG2 cell lysate. Again, in the OffBead configuration, methyltransferases were identified more robustly with the new SAH-CC than with the old SAH-CC (Figure 4). Several of the human methyltransferases were only identified in the experiments with the tetrafluorophenylazide Capture Compound™, such as the putative RNA methyltransferase FTSJD2 (Uniprot/SwissProt Acc. Q8N1G2) or the ribosomal RNA methyltransferase NOP2 (Uniprot/SwissProt Acc. P46087).

This performance makes the SAH-CC more efficient and sensitive, as well as improves methyltransferase coverage from complex biological samples. The new SAH-CC is an even more powerful analytical tool for the profiling of methyltransferases from complex biological mixtures.



**Figure 2** Performance of old and new SAH Capture Compounds™ in capturing methyltransferases and other SAM-binding proteins from *E.coli* lysate in the OffBead configuration. The new SAH-CC clearly outperforms the old SAH-CC.

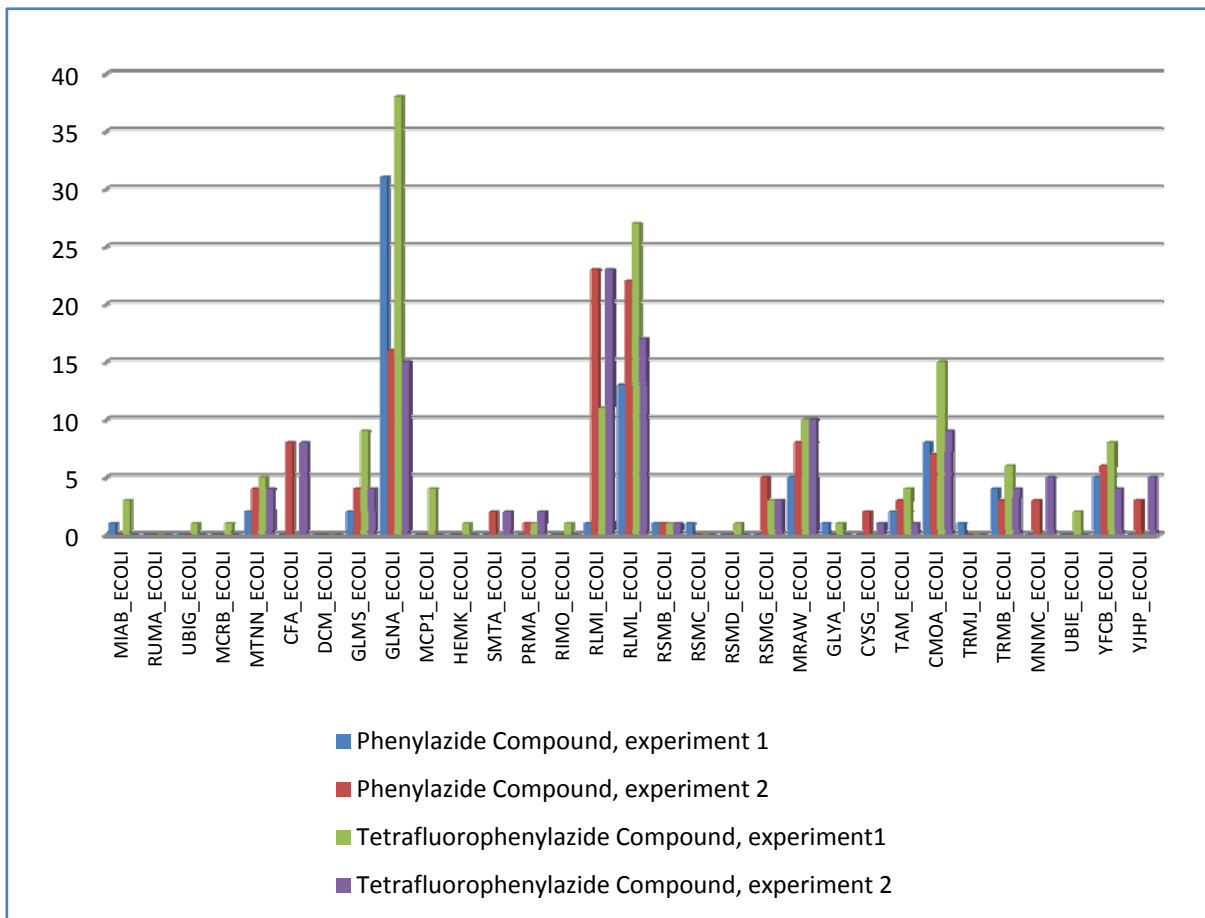


Figure 3 Performance of old and new SAH Capture Compounds™ in capturing methyltransferases and other SAM-binding proteins from *E.coli* lysate in the OnBead configuration. The new SAH-CC performs at least equally well as compared to the old SAH-CC.

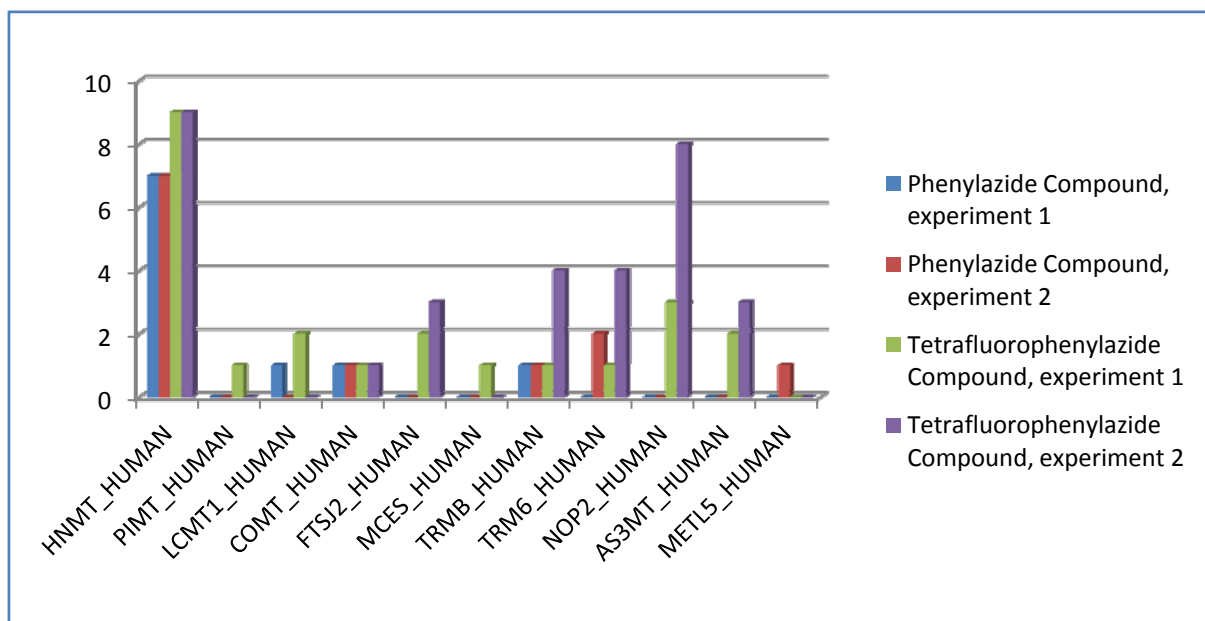


Figure 4 Performance of old and new SAH Capture Compounds™ in capturing methyltransferases from human-derived HepG2 cell lysate in the OffBead configuration. The new SAH-CC outperforms the old SAH-CC.

**References:**

- 1) Lu SC (2000) S-Adenosylmethionine. Int J Biochem Cell Biol 32:391–3952
- 2) Cantoni GL (1975) Biological methylation: selected aspects. Annu Rev Biochem 44:435–451
- 3) Dalhoff C, Hüben M, Lenz T, Poot P, Nordhoff E, Köster H, and Weinhold E. (2010). Synthesis of S-adenosyl-L-homocysteine capture compounds for selective photoinduced isolation of methyltransferases. Chembiochem 11, 256-265

**Downloads:**

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**Ordering information:**

Item Nr.	Description
1-1090-050	SAH caproKit™ 50 reactions
1-1090-010	SAH caproKit™ 10 reactions

The caproKit includes the SAH specific Capture Compound, all buffers, protein controls, SAH competitor, and Streptavidin magnetic beads.

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