

# Functionality Directed Reduction of Proteome Complexity

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## Introduction

Isolation and identification of proteins from biological samples is a challenging task. Separation by physical properties like molecular weight or charge reduces complexity but lacks functional selectivity. Affinity chromatography and chip based technologies achieve functional separation but are limited by the inherent properties of stationary phases. Here we describe a novel chemical probe strategy for selective complexity reduction in solution. The method is exemplified using S-adenosyl-L-methionine (SAM) derivatives as probes to selectively isolate methyltransferases (MTases).

## Methods

Our method, called Capture Compound Mass Spectrometry (CCMS) is based on small, tri-functional molecules (Capture Compounds, Fig 1). They comprise a selectivity function for specific, reversible binding to targeted proteins, an UV-activated reactivity function for covalent attachment to proteins and biotin as sorting function for magnetic bead-based isolation of the captured proteins (1).

S-adenosyl-L-homocysteine (SAH) has a similar  $K_D$  as SAM and was used as selectivity function, due to its higher stability. Capture Compound scaffold (CC) was attached to N6 of SAH, as many MTases tolerate modifications at this position (2).

As model system, we used purified MTases and bacterial lysates. Captured proteins were analyzed by SDS-PAGE and MALDI-TOF MS.

## Results

First, we demonstrated that the selectivity function is necessary to form a covalent bond with proteins. To confirm selective capture, an excess of free SAH as competitor was added in a control experiment. Results are exemplified with the DNA MTase M.TaqI in Figure 2A. The SAH Capture Compound (CCSAH) does not capture M.TaqI in the presence of free SAH. Identical results were obtained with M.HhaI and Trn1 (data not shown).

To demonstrate selectivity of complexity reduction, *E.coli* lysate was incubated with CCSAH and CC, respectively. The results (Fig 2B) show that treatment with CCSAH leads to significant complexity reduction but also that CC alone generates background signals. To investigate further, tryptic fragments of individual proteins were analyzed with MALDI-MS/MS (data not shown). This revealed that background consists mostly out of biotin-binding proteins (e.g. acetyl-CoA-carboxylase) while specific signals are mainly SAH-binding proteins (e.g. 5'-methylthioadenosine nucleosidase and trans-aconitate 2-methyltransferase).

The results show the viability of CCMS to isolate functional subproteomes from complex samples.

## Innovative aspects

- Novel technology platform for isolation of protein families based on functionality
- Selectivity function is presented to the protein in its native state in solution.
- No interaction with a solid phase is required for the capture step, potential to isolate and analyze hydrophobic proteins

## References

- (1) H. Köster et al, (2007) Capture Compound Mass Spectrometry: A Technology for the Investigation of Small Molecule Protein Interactions; Assay and Drug Development Tech. (5); 381-390.
- (2) G. Pljevaljcic, F. Schmidt, E. Weinhold, (2004) Sequence-specific methyltransferase-induced labeling of DNA (SMILING DNA); ChemBioChem (5); 265-269.

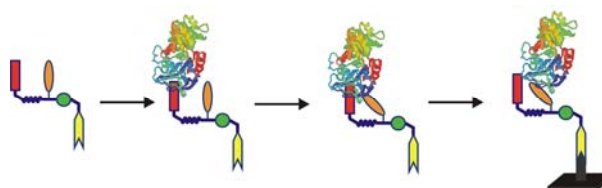


Figure 1. Schematic representation of a Capture Compound. In this study, the selectivity function was SAH (red), the sorting function was biotin (yellow) and the reactivity function was an aromatic azide (orange). The green circle represents an adaptable linker.

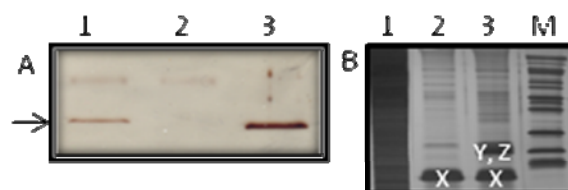


Figure 2. A): SDS-PAGE of M.TaqI after incubation with CCSAH and photoactivation (lane 1) after incubation with CCSAH, free SAH and photoactivation (lane 2) and pure M.TaqI (lane 3). B): SDS-PAGE of *E.coli* lysate (lane 1), after treatment with CC (Lane 2) after treatment with CCSAH (lane 3). M: marker; X: acetyl-CoA-carboxylase; Y: 5'-methylthioadenosine nucleosidase; Z: trans-aconitate 2-methyltransferase