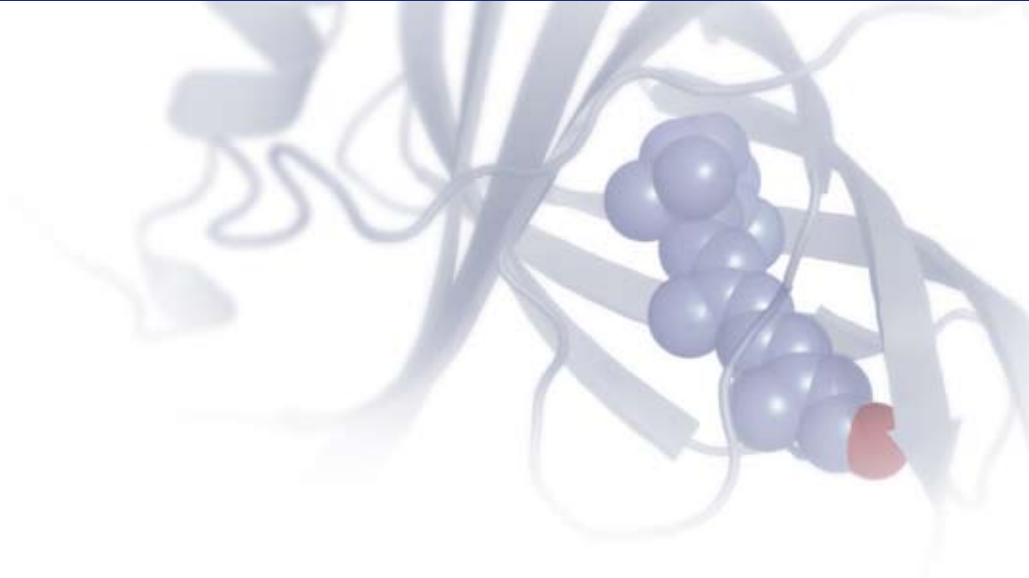


Improved Proteomics through functional Isolation of Proteins



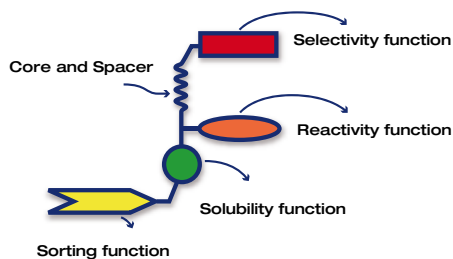
Discovery - Profiling - Identification

Improved Proteomics through functional Isolation of Proteins

The proteome is a highly complex mixture of diverse proteins and peptides in varying concentrations and conditions. The qualitative and quantitative analysis of this mixture is a challenging task even with today's advanced separation and analysis capabilities. caprotec is pioneering an innovative and proprietary technology to reduce the complexity of biological samples through selective isolation of targeted protein- or enzyme families.

Capture Compound Mass Spectrometry (CCMS)[™] is using small synthetic molecules (Capture Compounds) to interrogate native proteins, even lipophilic membrane proteins, in virtually any biological sample.

Capture Compounds allow a reversible affinity interaction with proteins through a selectivity function. Subsequently, a reactivity function forms a covalent bond with interacting proteins and a sorting function enables their isolation directly from cell lysates.

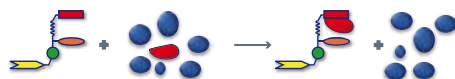


The core of Capture Compound Mass Spectrometry (CCMS) is a small, multifunctional molecule, the Capture Compound.

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.

A typical CCMS experiment can be divided into three phases:

PHASE 1 Sample Preparation



Capture Compounds bind proteins through reversible affinity interaction

Protein samples from different sources and origins can be the starting point of a CCMS experiment. Material from cell culture, bacteria, plants or tissues can be investigated and in all these cases, CCMS offers a universal solution to address a selected sub-population of the proteome. Contrary to many other technologies, depletion of albumin or other highly abundant proteins is not necessary during the preparation step. Also, target molecules do not need to be active enzymes since the assay is not dependent on substrate conversion - the capture event is strictly based on reversible affinity interaction.

PHASE 2

Protein Isolation



Photocrosslinking generates a covalent bond between capture compound and target protein

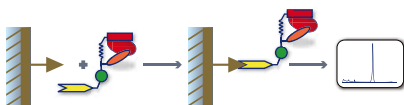
The isolation of interacting target proteins is accomplished in two steps: First, the reactivity function is covalently attached to the protein. Second, the sorting function (biotin) is used to isolate captured proteins from the mixture.

The first step is performed through photocrosslinking the reactivity function to the protein. The functional selectivity of the capture event can be directly monitored through control experiments in the presence of competitors which can replace the selectivity function.

The second step uses streptavidin-coated magnetic particles to isolate the captured proteins from the mixture. Subsequently, the proteins can be analyzed further by gel electrophoresis and mass spectrometry. Isolated proteins can also be subjected to enzymatic digest for characterization and identification.

PHASE 3

Protein Identification and Characterization

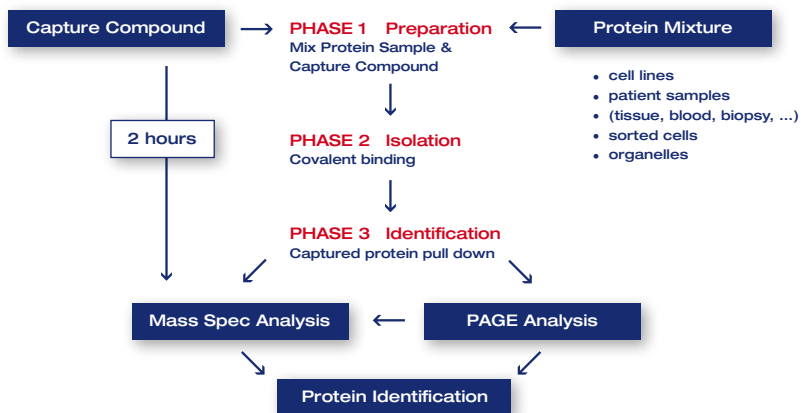


Streptavidin-Biotin interactions are used to pull down captured proteins for MS analysis

Because of this flexibility, CCMS experiments can provide a wide range of information. For example, post-translational modifications of the captured proteins can be determined via mass spectrometric analysis.

The CCMS process allows determining K_d values between the ligand and the affinity selected proteins which can be achieved by performing capture experiments with increasing Capture Compound to protein ratios, thereby shifting the equilibrium towards weaker ligand protein interactions. As a result of this experiment relative K_d values can be determined simply from the MS data.

An additional advantage of CCMS technology is that capture reactions are performed in a simple, homogeneous protocol that is amenable to automation and up-scaling with off the shelf liquid handling devices and CCMS experiments can be performed within a working day.



Advantages of CCMS:

- **QUALITY CONTROL:** Capture Compounds are small molecules, thus their integrity can be unambiguously assessed by analytical methods. A coupling of ligands or proteins to a solid support is not necessary
- **LIGAND ACCESSIBILITY:** The Capture Compound protein interaction is taking place in a homogeneous solution, no solid phase interactions are required during the critical affinity interaction to capture protein targets, no bias with regards to protein size is created
- **SOLUBILITY:** Capture Compounds can be modified to react in hydrophilic and lipophilic environments, allowing to investigate a wide range of target proteins, including membrane proteins
- **DYNAMIC RANGE:** Weak and strong interacting proteins can be identified by varying the ratio of Capture Compound to protein. Stringent wash conditions can be employed even with low affinity binders due to the covalent photocrosslinking



Products & Services

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

For more information please visit www.caprotec.com or contact us:

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Phone: + 49 30 6392 4004

Products and Services are for Research use only.

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