

Introduction

The analysis of protein interactions by mass spectrometry is typically labor intensive because of the tendency of non-covalent interactions to dissociate during analysis. CovalX has developed a method for the easy and fast analysis of protein interactions using High-Mass MALDI mass spectrometry. The first step of the analysis is to stabilize the non-covalent interactions of interest using dedicated cross-linking reagents and protocols. After stabilization, the samples are ready for direct analysis by High-Mass MALDI ToF mass spectrometry.

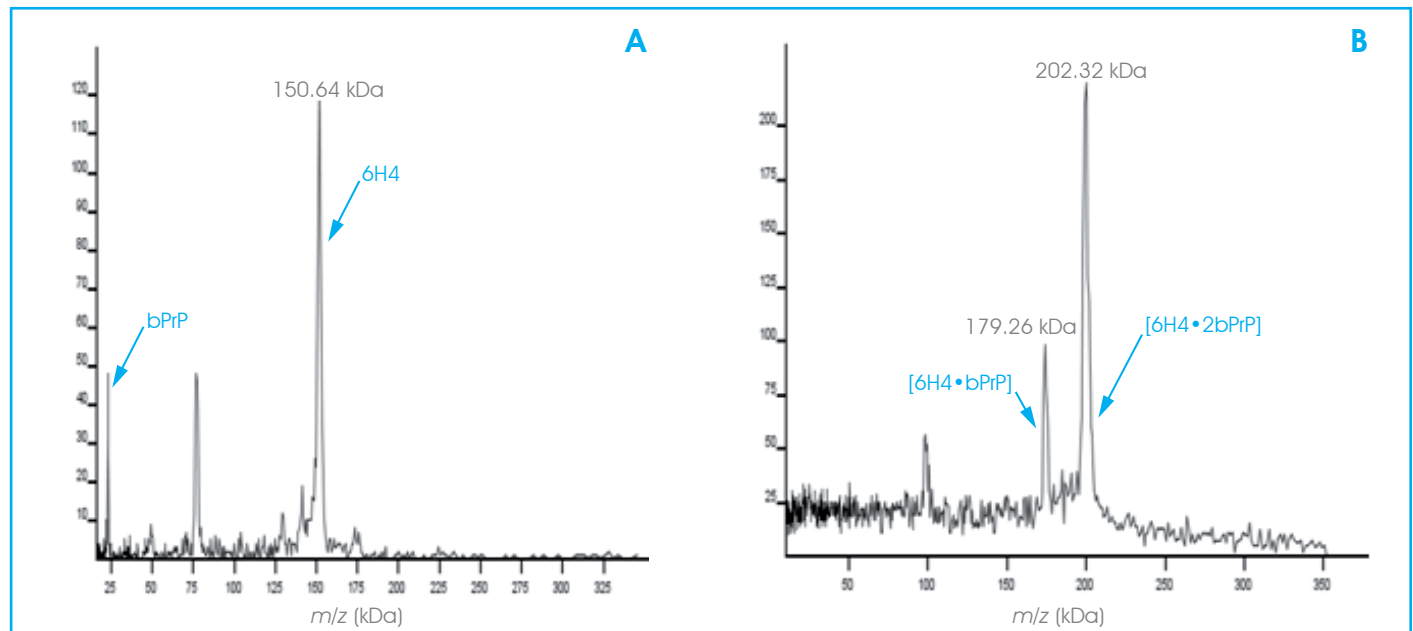
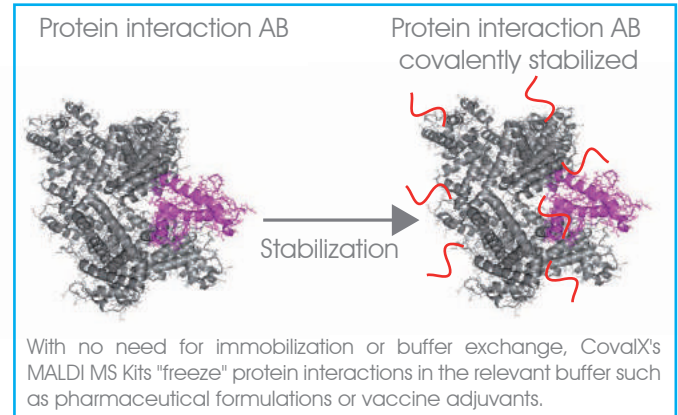


Figure 1. High-Mass MALDI ToF mass spectra of the protein complex formed between the bovine prion protein (bPrP, 2 μ M) and a monoclonal antibody anti-bPrP (6H4, 1 μ M).

A. High-Mass MALDI ToF mass spectrum of the immuno-complex before cross-linking.

B. Spectrum obtained after cross-linking using the K200 MALDI MS Analysis Kit. After cross-linking, the immuno-complexes [6H4•bPrP] and [6H4•2bPrP] are clearly detected with $m=179.26$ kDa and 202.32 kDa, respectively.



K200 MALDI MS Analysis Kit

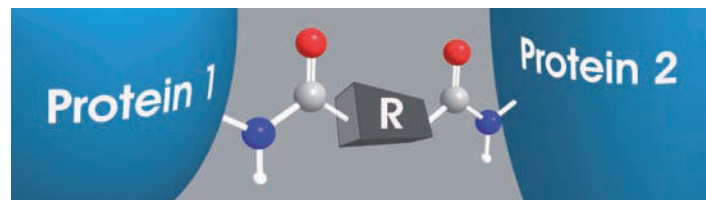
MALDI MS Analysis Kits and Reagents for Protein Complex Analysis by High-Mass MALDI MS

- Dedicated cross-linking reagents with superior efficiency and kinetic properties for stabilizing specific non-covalent interactions
- Prepare directly in relevant buffer: Pharmaceutical formulations, vaccine adjuvant, zwitterionic detergent, glycerol containing buffer, nonionic surfactant
- No need for dilution or buffer exchange
- For soluble or membrane protein interactions
- Fast: with minimal sample preparation

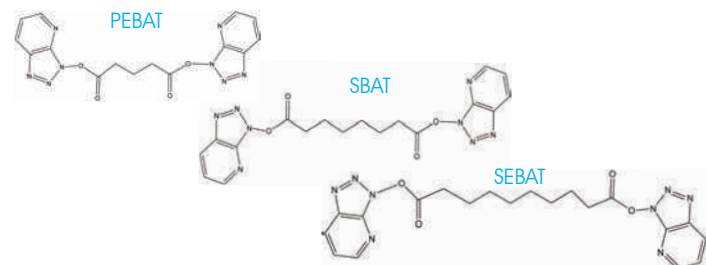
Cross-linking cocktails to increase efficiency

To analyze intact protein complexes by High-Mass MALDI ToF mass spectrometry it is crucial to specifically stabilize the complexes with highly efficient cross-linking reagents. CovalX has developed dedicated reagents and buffers to prepare non-covalent complexes for High-Mass MALDI analysis. To increase cross-linking efficiency, CovalX reagents contain cocktails of cross-linkers offering different spacer lengths which are able to covalently bind specific protein complexes with maximal efficiency. The specificity, higher efficiency and faster kinetics of CovalX cross-linking reagents¹ enables stabilization of covalent protein complexes with outstanding sensitivity, even in contaminated or unpurified samples. The cross-linking reagents have been developed for fast reaction in any relevant sample matrix including pharmaceutical formulations or vaccine adjuvants.

¹Bich et al., Anal. Chem. (2010) 82, 172-179.



CovalX Proprietary Cross-linking Reagents



CovalX's dedicated cross-linking reagents developed for High-Mass MALDI analysis. These reagents have 1-hydroxy-7-azabenzotriazole groups as reactive groups with different spacer lengths ranging from 8.8 to 13.2 Å.

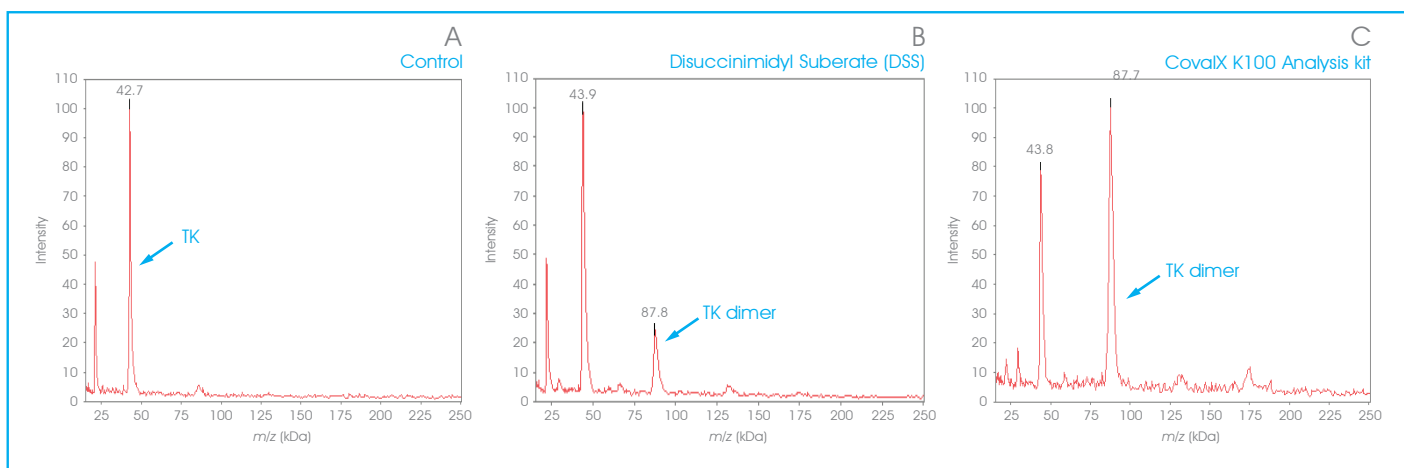


Figure 2. High-Mass MALDI ToF mass spectra of the protein complex Thymidine Kinase (TK).

A. TK (1 μM) analysed without cross-linking. Only the monomer is detected.

B. TK analysed after cross-linking with disuccinimidyl suberate (0.1 mg/ml, 30 minutes incubation time). TK dimer is detected with m=87.8 kDa.

C. Same analysis with CovalX K100 analysis reagent (0.1 mg/ml, 30 minutes incubation time), demonstrating increased detection of the dimer at m=87.7kDa.

MALDI MS Analysis Kits and Reagents. Order online at www.covalx.com

MALDI MS Analysis Kit for Protein interactions	Mass Range	Part No
K50 MALDI MS Analysis Kit	0-50 kDa	W2010k50
K100 MALDI MS Analysis Kit	20-100 kDa	W2010k100
K200MALDI MS Analysis Kit	100-500 kDa	W2010k200
R50 Stabilizer Reagent	0-50 kDa	W2010R50
R100 Stabilizer Reagent	20-100 kDa	W2010R100
R200 Stabilizer Reagent	100-500 kDa	W2010R200