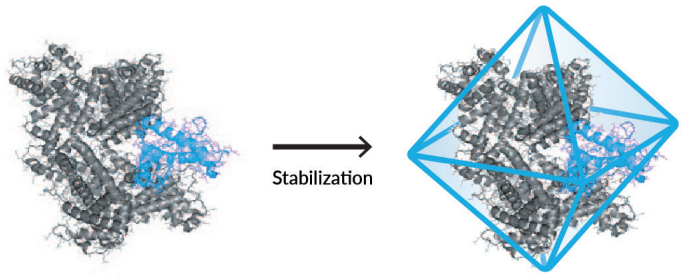
Cross-linking Chemistry for High-Mass MALDI ToF Mass Spectrometry

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.



With no need for immobilization or buffer exchange, CovalX's MALDI MS Kits "freeze" protein interactions in the relevant buffer such as pharmaceutical formulations or vaccine adjuvants. After stabilization, the samples are ready for direct analysis by High-Mass MALDI ToF mass spectrometry.

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

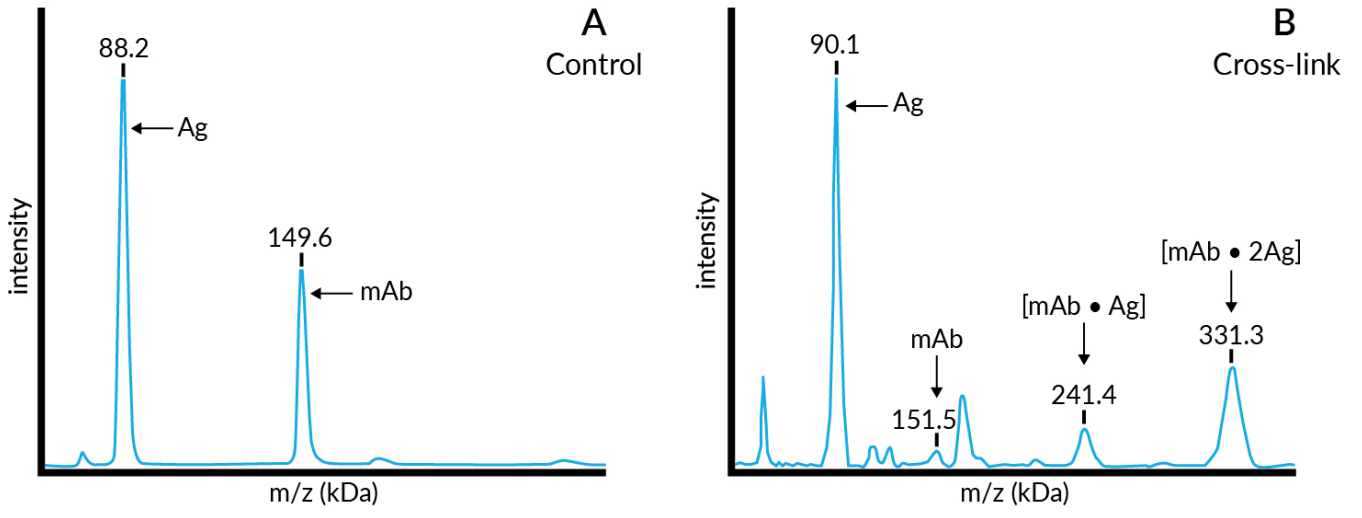


Figure 1. High-Mass MALDI ToF mass spectra of the protein complex formed between the target antigen protein (Ag, 2 µM) and a mono-clonal antibody (mAb, 0.3 µM).
A. High-Mass MALDI ToF mass spectrum of the immuno-complex before cross-linking.
B. Spectrum obtained after cross-linking using the K200 MALDI MS Analysis Kit. After cross-linking, the immuno-complexes [mAb•Ag] and [mAb•2Ag] are clearly detected with MH+=241.4 kDa and 331.3 kDa, respectively.
These measurements were performed using an HM5 High-Mass MALDI MS system (CovalX)

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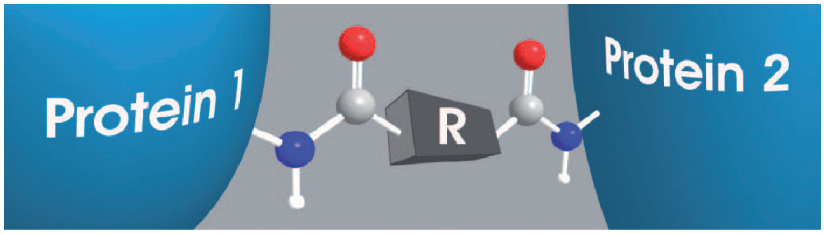
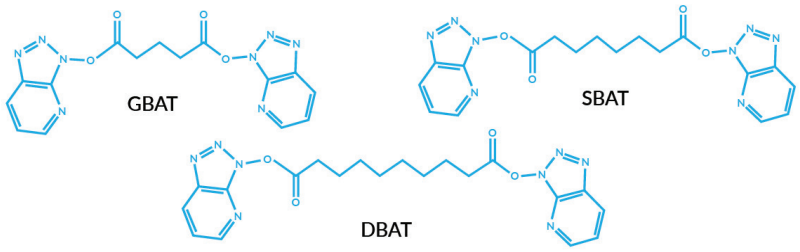
Cross-linking cocktails to increase efficiency

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 reagents enables stabilization of non-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.





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.

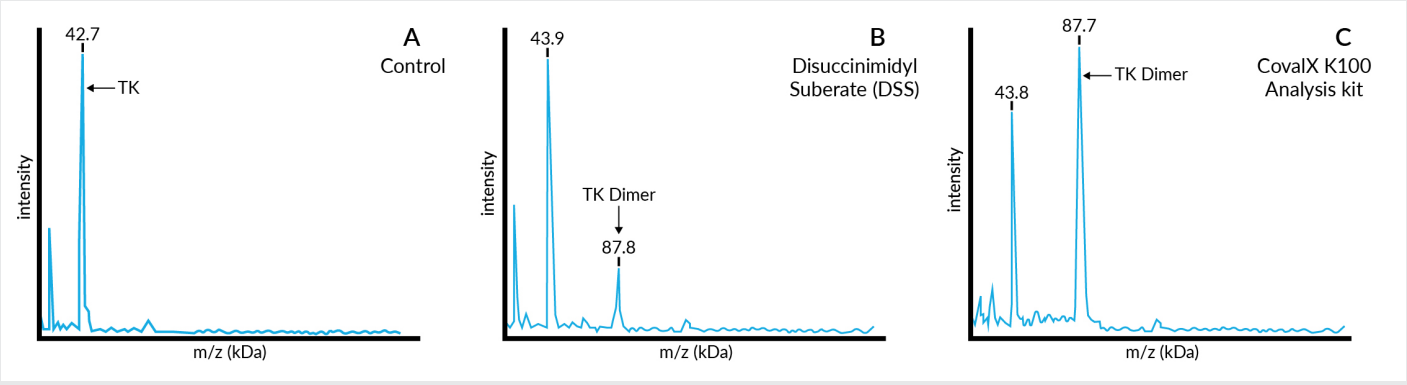


Figure 2. High-Mass MALDI ToF mass spectra of the protein complex Thymidine Kinase (TK)
A. Control: TK (1µM) analyzed without cross-linking. Only the monomer is detected.
B. Disuccinimidyl Suberate (DSS): TK analyzed after cross-linking with disuccinimidyl suberate (0.1 mg/ml, 30 minutes incubation time). TK dimer is detected with MH+=87.8 kDa.
C. CovalX K100 Analysis Kit: Same analysis with CovalX K100 analysis reagent (0.1 mg/ml, 30 minutes incubation time). Demonstrating increased detection of the dimer at MH+=87.7kDa.
These measurements were performed using an HM5 High-Mass MALDI MS system (CovalX)

MALDI MS Analysis Kits and Reagents	Mass Range	Part No.
K50 MALDI MS Analysis Kit	0-50 kDa	W2010k50
K100 MALDI MS Analysis Kit	20-100 kDa	W2010k100
K200 MALDI MS Analysis Kit	100-1000 kDa	W2010k200
R50 Stabilizer Reagent	0-50 kDa	W2010R50
R100 Stabilizer Reagent	20-100 kDa	W2010R100
R200 Stabilizer Reagent	100-1000 kDa	W2010R200

CovalX
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