A Study of Non-covalent Protein-protein Complexes Under Native Condition by Matrix-assisted Laser Desorption/Ionization

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Major References of MALDI to Study Non-covalent Complexes

F. Hillenkamp, 1990, Strepavidin, a tetramer, nicotinic acid in 10% ethanol in water

F. Hillenkamp, 1995, Omp F porin, a trimer, feruic acid in THF

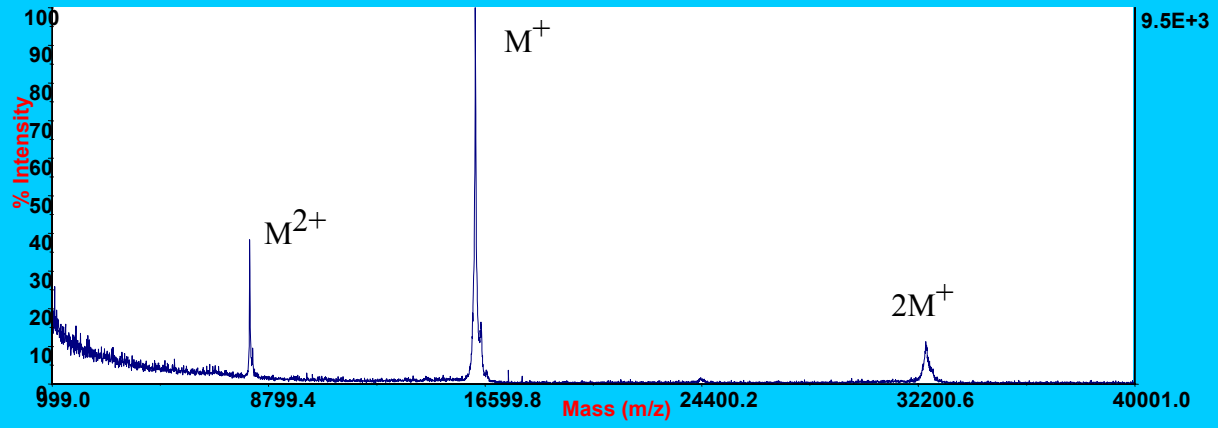
A. S. Woods and R. J. Cotter, 1995, enzyme and substrate complexes, sinapinic acid in ethanol-1M ammonium citrate at near pH 7.

M. Pryzbylski et al, 1996, RNAse S consisting of S-protein and S-peptide using ATT with 10 mM ammonium acetate at pH5.5 .

F. Hillenkamp, 1997, Strepavidin, a tetramer, using DHAP in THF, ethanol, THAP in THF and ACN-TFA.

Problems

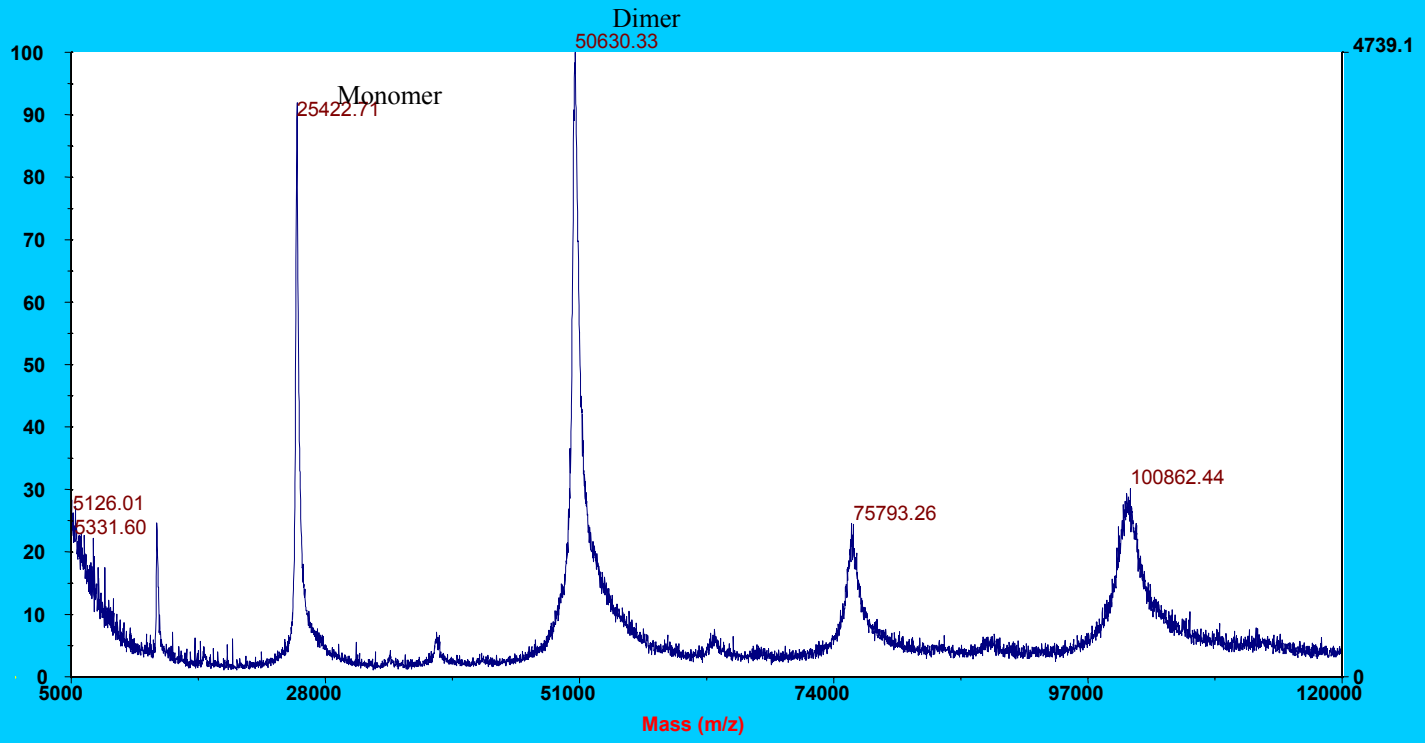
- The solution-phase acidity of these matrix substances and some typical additives (e.g the hydrophobicity of the organic (co)solvents and TFA) leads to dissociation of the noncovalent assemblies in most cases.
- It has been observed occasionally that an abundant signal of intact subunit assemblies could be obtained for the first laser shot on a not-yet-irradiated sample spot exclusively.
- Not very much is known about the gas-phase stability of molecular ions from noncovalent biomolecular complexes formed under MALDI-MS conditions.
- A further complication in MALDI-MS is the fact that nonspecific, noncovalent homo- and heteroligomeric aggregates, called cluster ions, are frequently observed.



Possible Solutions

- Eliminate the organic co-solvent and TFA.
- Other new neutral matrices; use acidic matrices, then adjust the pH to neutral with base such as ammonium hydroxide.
- Compounds tested: 4-bromo-2,6-dimethylaniline, 2-pyridine carboxylic acid, 2-pyrazine-carboxylic acid, 2-qunaldic acid, 2-isoqunolinecarboxylic acid, and other compounds.
- Sinapinic acid with ammonium citrate adjusted to pH 7 with ammonium hydroxide was found to be effective matrix for the detection of non-covalent protein complexes.

Oligomeric States of Proteins



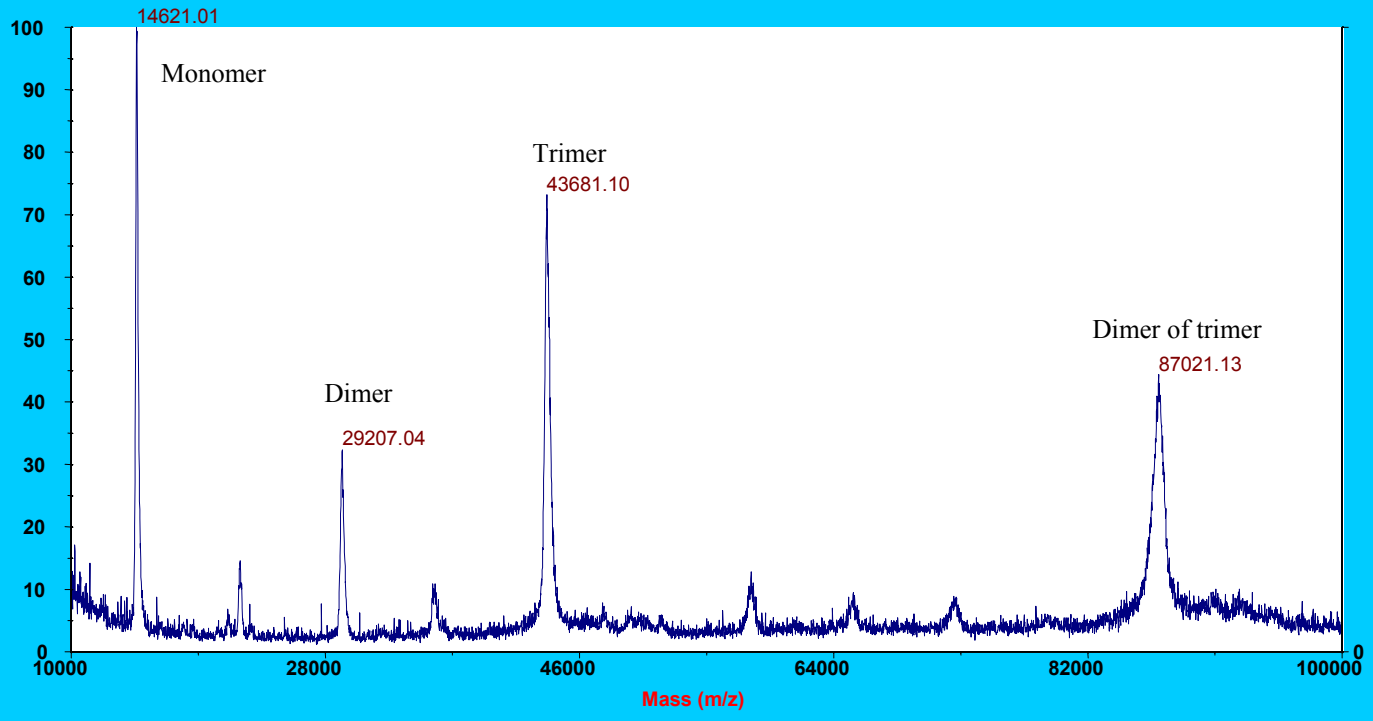
MALDI Spectrum of PhzD

A Protein Trimer HI0719

Protein HI0719 belongs to a family of proteins are widely distributed in bacteria, archaea, plants and eukaryote.

HI0719 is a hometrimer by light scattering measurement and was proved as a trimer by solution NMR study.

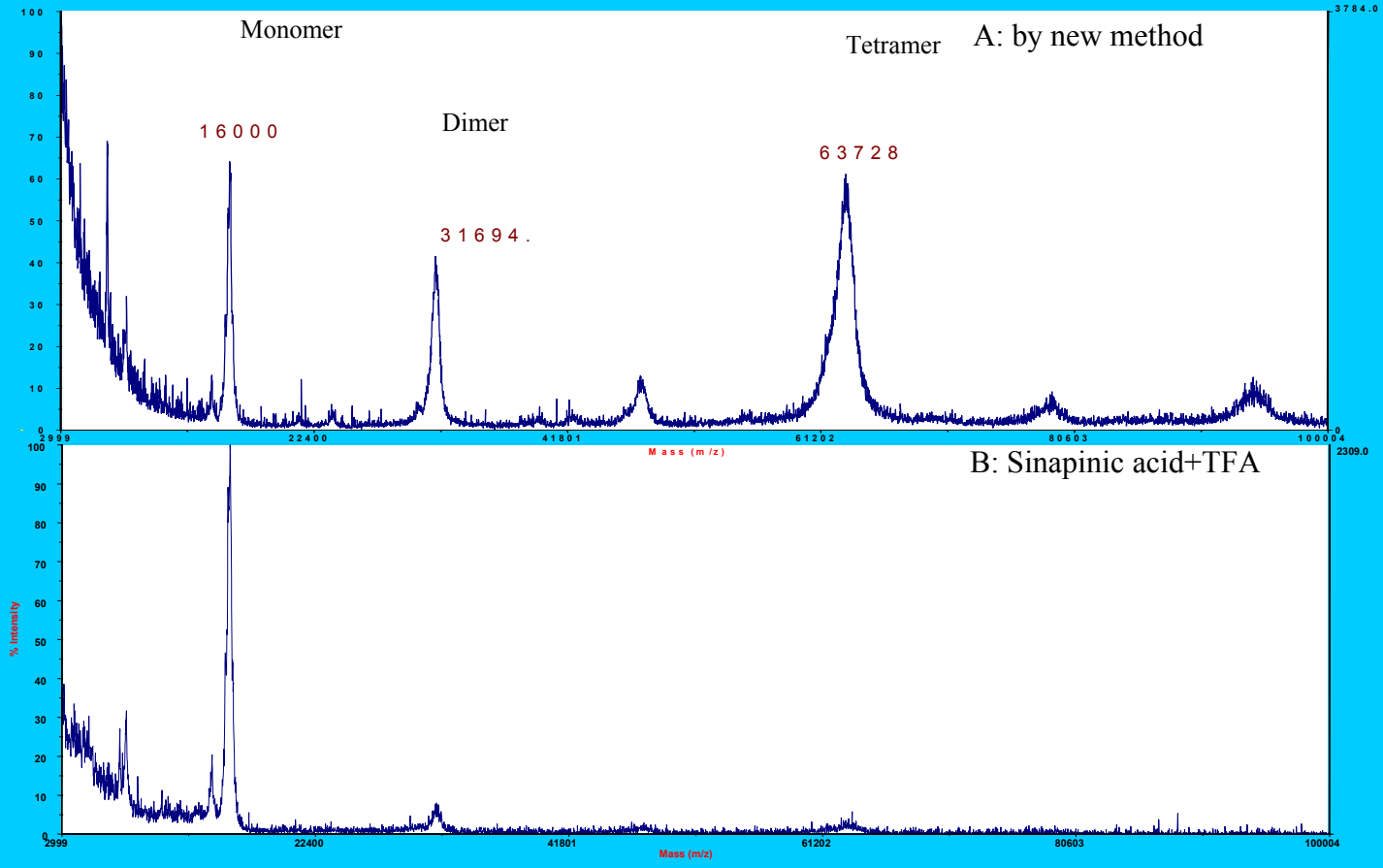
This protein is known as a trimer in solid state by X-ray crystallography.



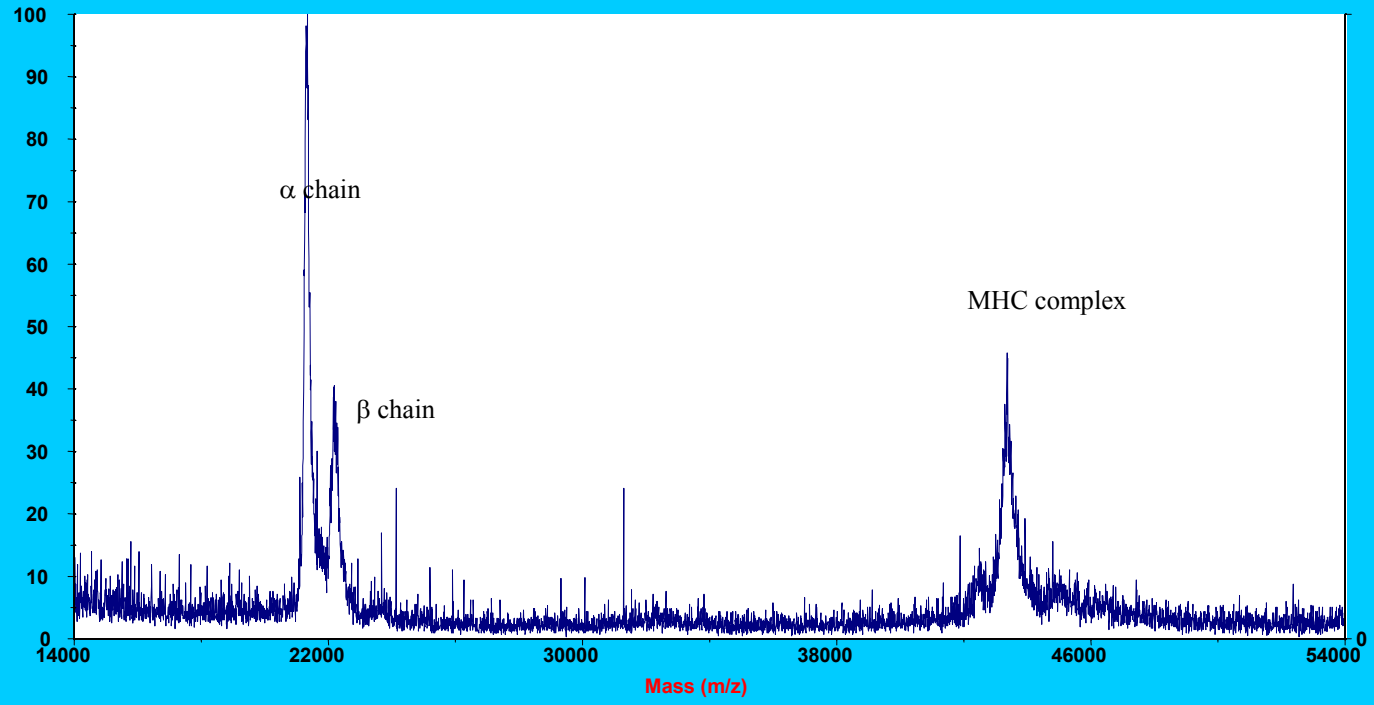
MALDI Spectrum of Protein HI0719

Comparison of New Method with the Traditional Method

Avidin is a glycoprotein found in egg white whose active form is a tetramer composed of identical subunits. The tetramer has been detected by MALDI.

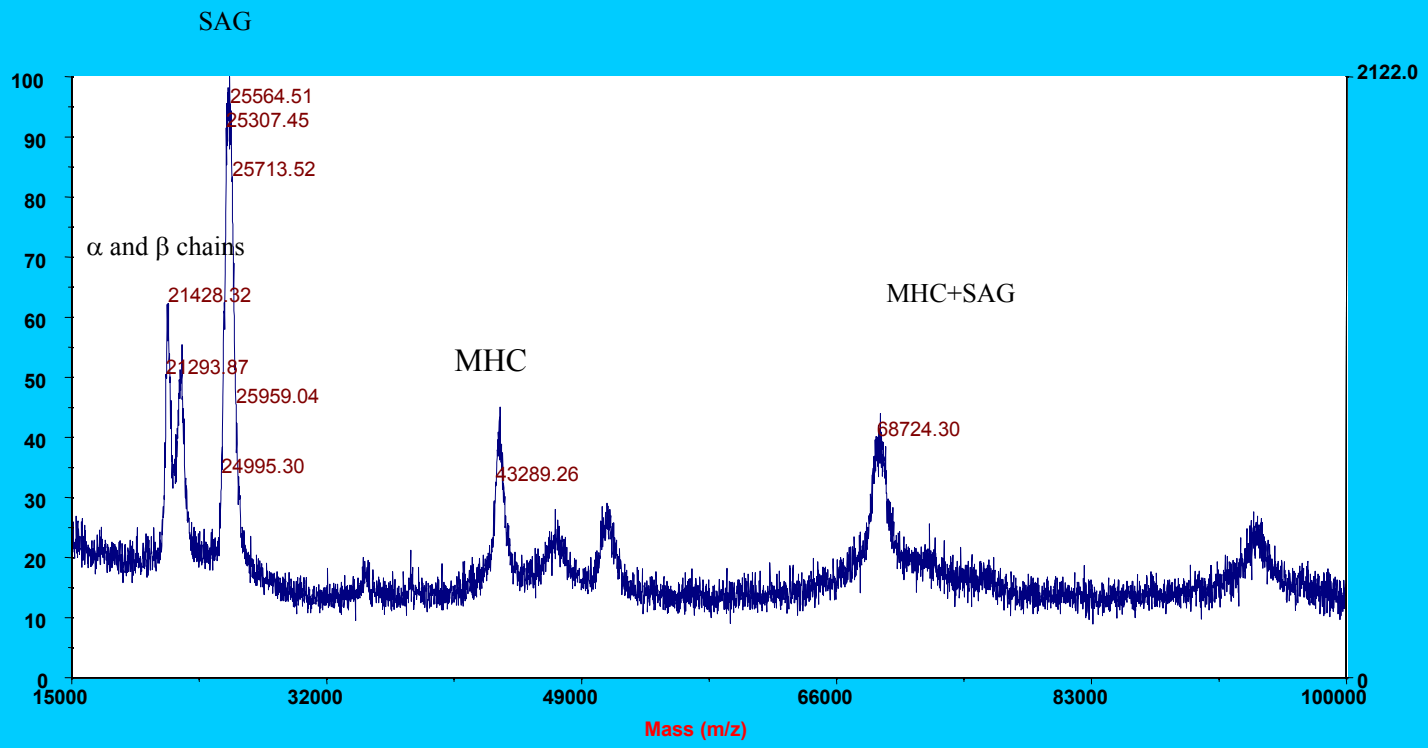


Class II Major Histocompatibility Complex

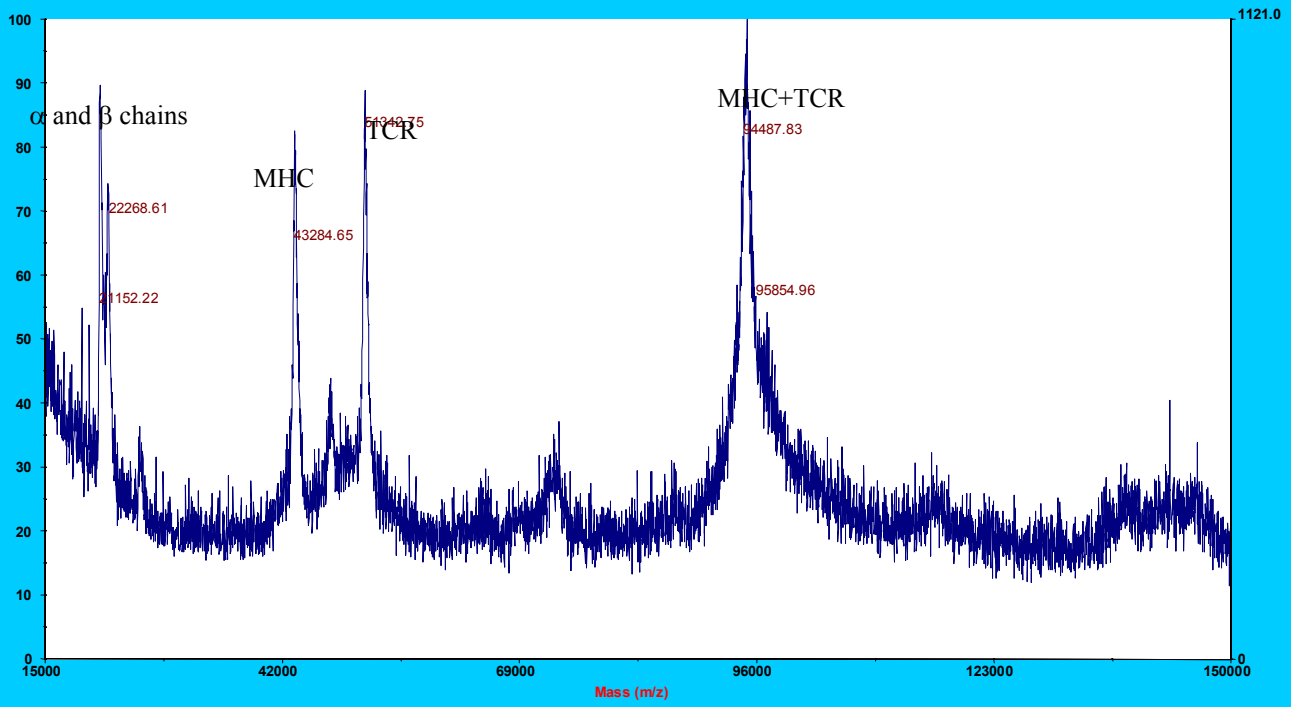


MALDI Spectrum of a Class II Major Histocompatibility Complex

The Complex of Superantigen (SAG) with Peptide-bound Major Histocompatibility (MHC) Molecule

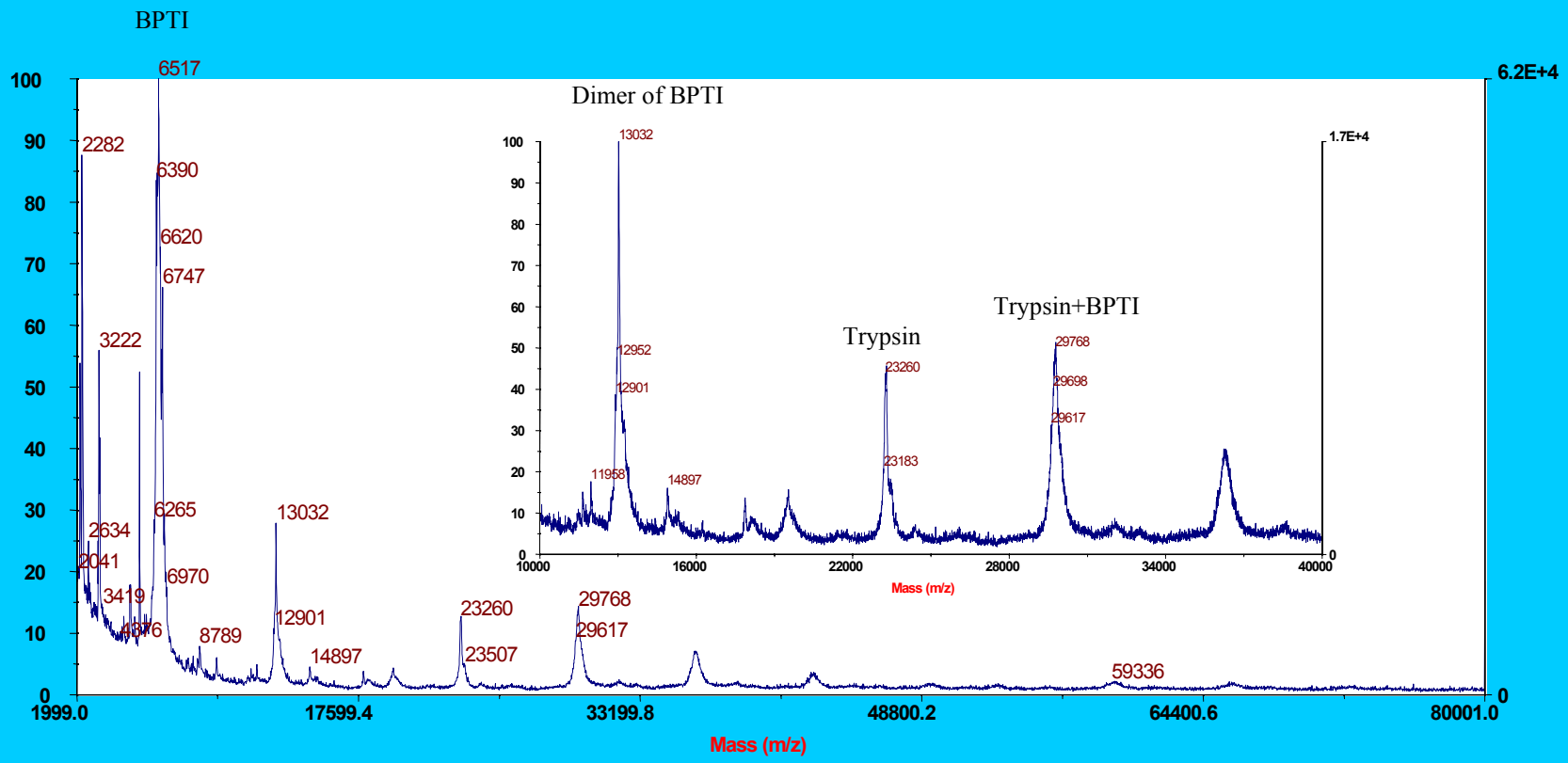


The Complex of Peptide-linked T Cell Receptor (TCR) and MHC Molecule



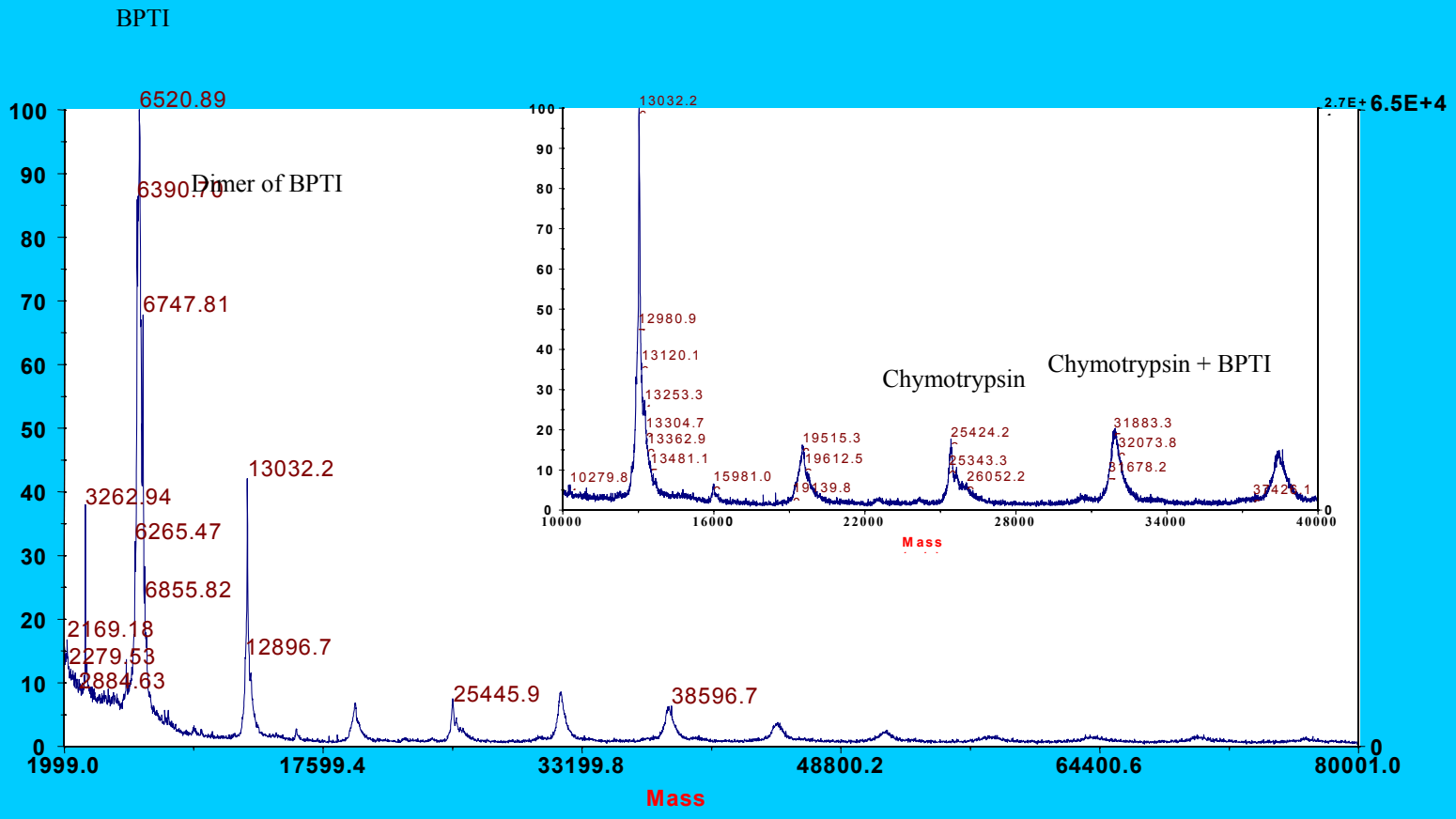
MALDI Spectrum of the Complex of Peptide-linked T Cell Receptor (TCR) and MHC Molecule

Detection of Enzyme Inhibitor Complexes



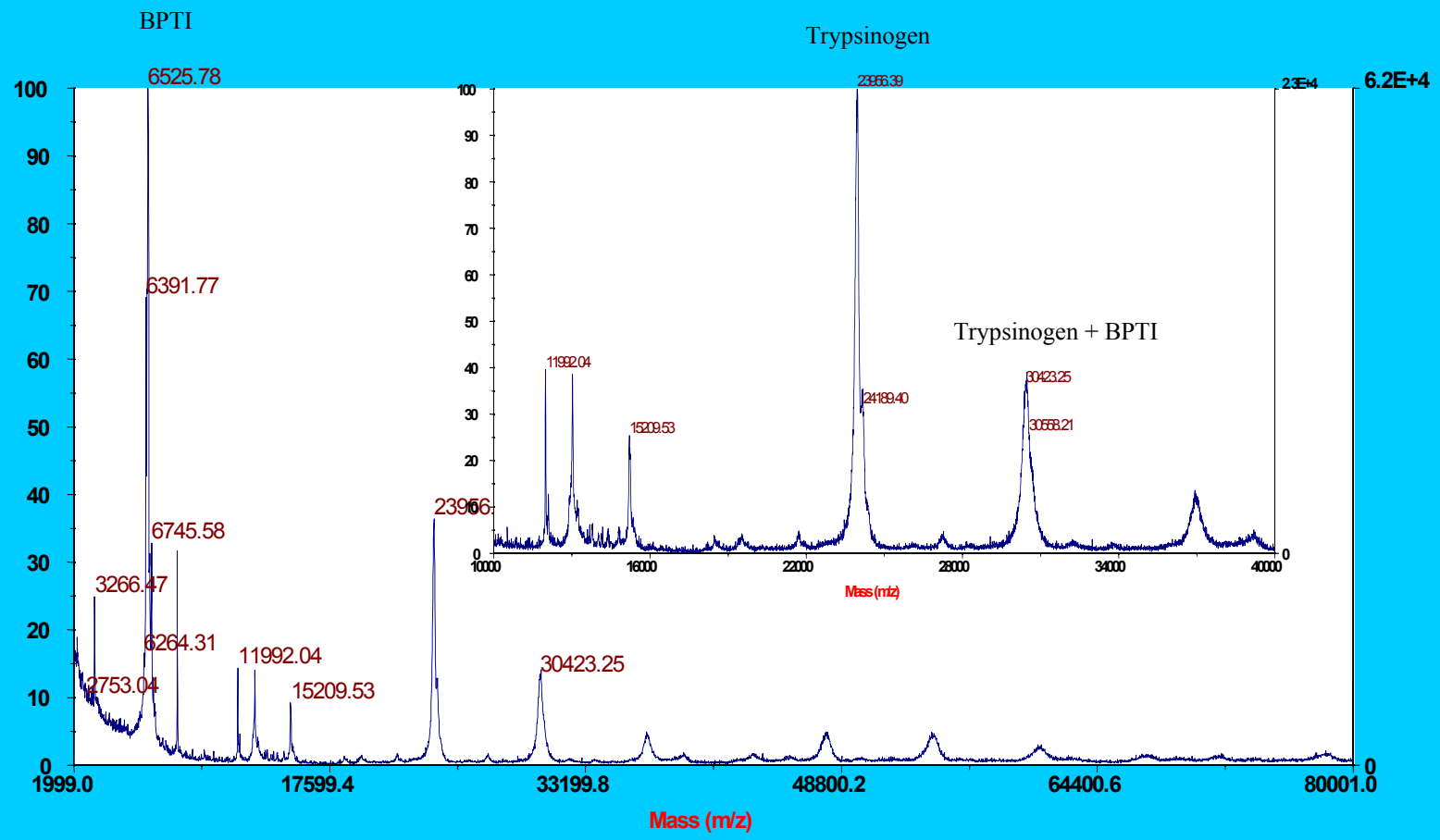
MALDI Spectra of Trypsin (6.3 uM) with Bovine Pancreatic Trypsin Inhibitor (BPTI) (25 uM)

Complex of Chymotrypsin with BPTI



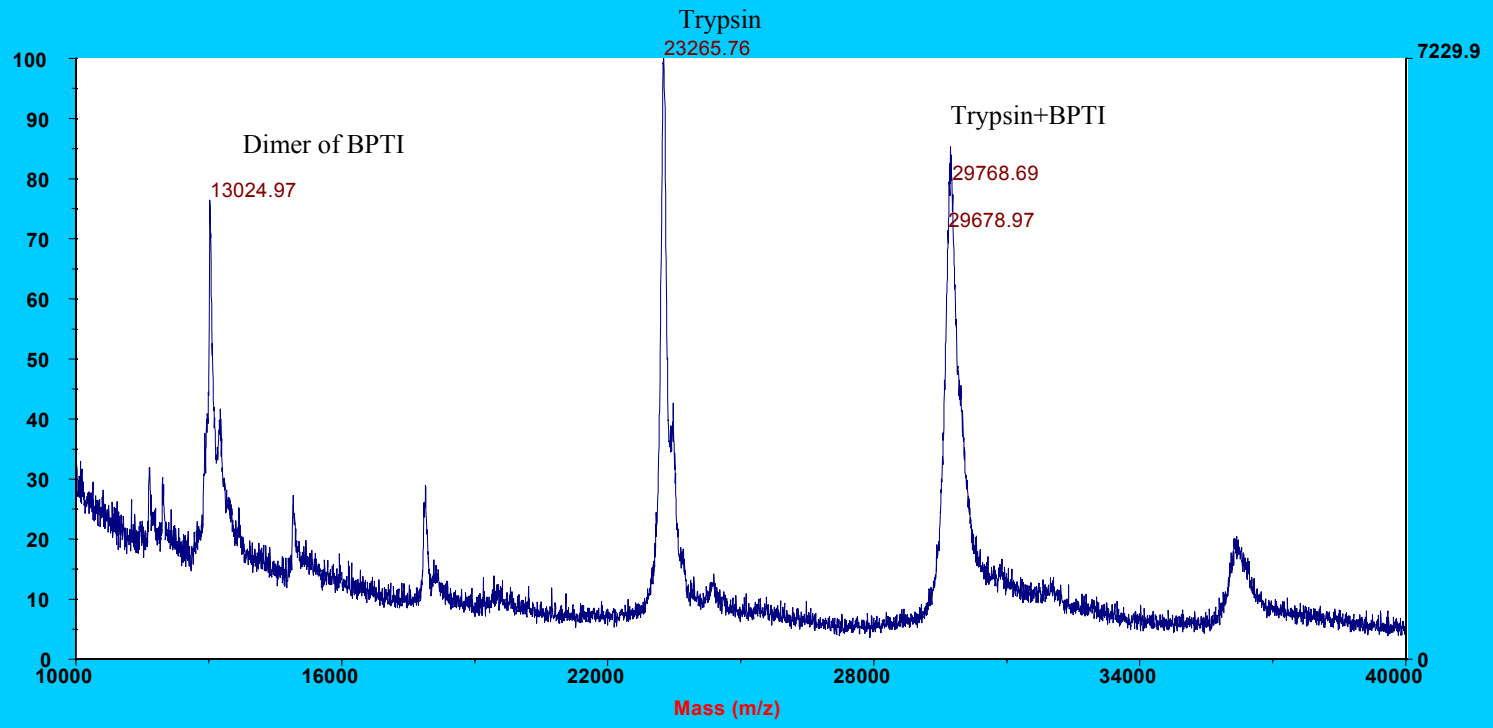
MALDI Spectrum of Chymotrypsin (6.3 uM) With BPTI (25 uM)

Complex of Trypsinogen with BPTI



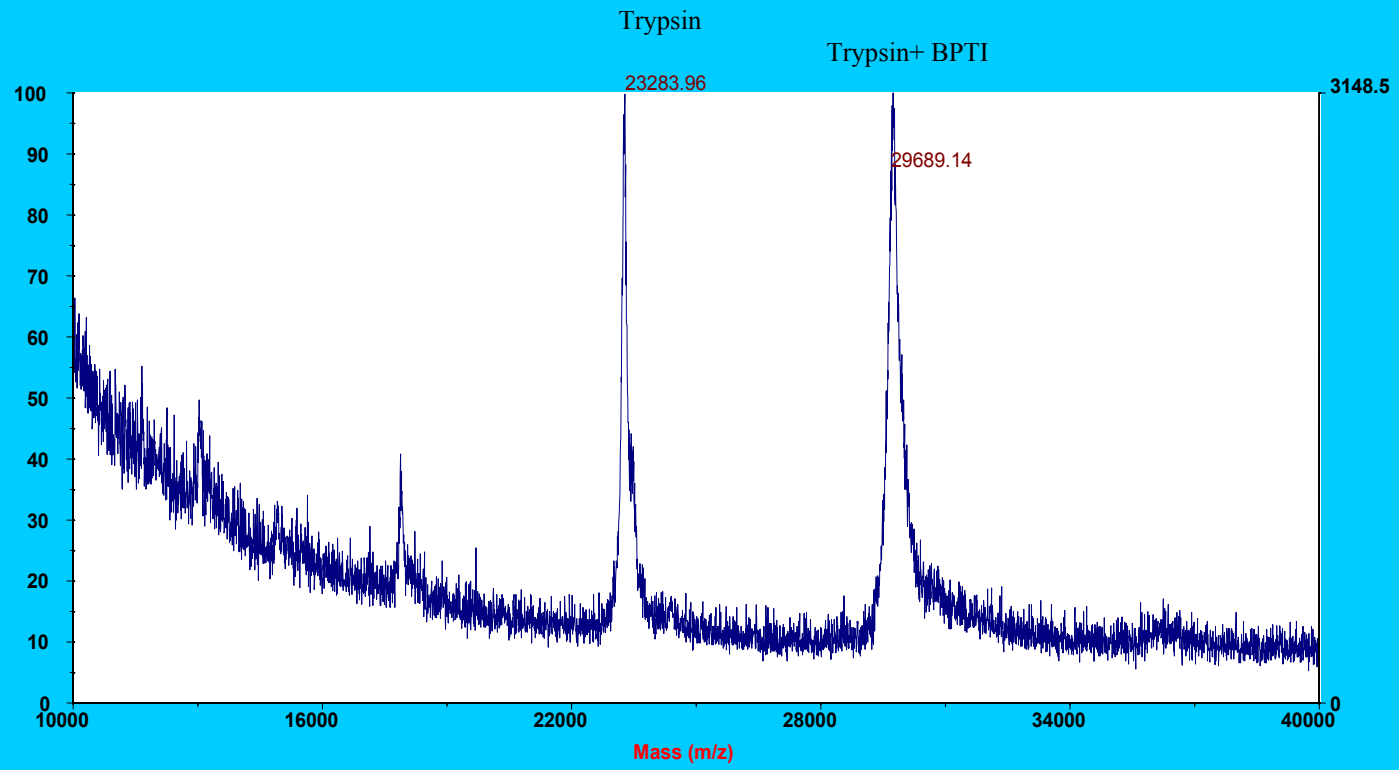
MALDI Spectrum of Trypsinogen (6 uM) With BPTI (25 uM)

Dilution Study of Trypsin with Bovine Pancreatic Trypsin Inhibitor (BPTI) Complex



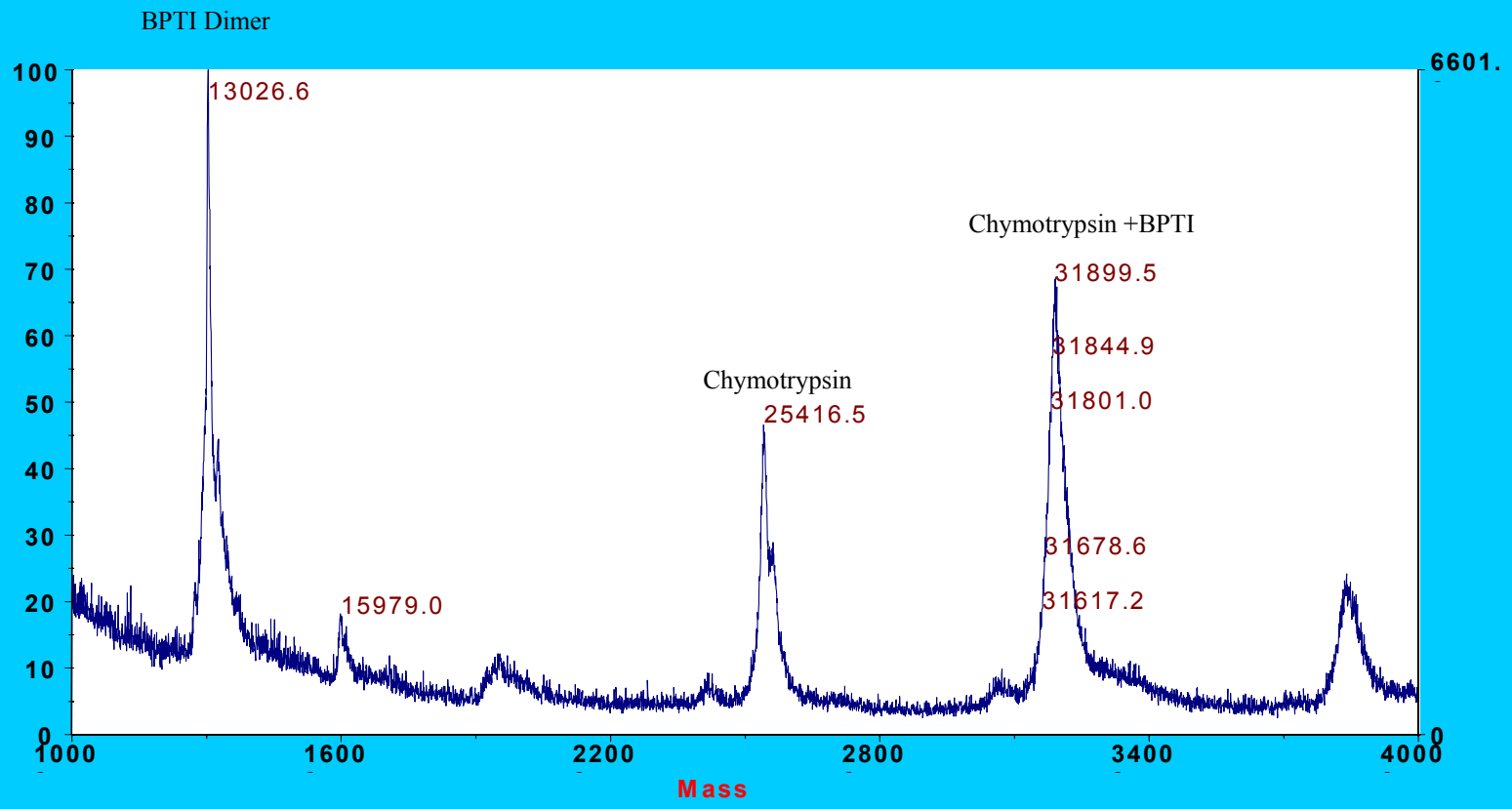
MALDI Spectra of Trypsin (2.4 uM) with Bovine Pancreatic Trypsin Inhibitor (BPTI) (10 uM)

Further Dilution Study of Trypsin with Bovine Pancreatic Trypsin Inhibitor (BPTI) Complex



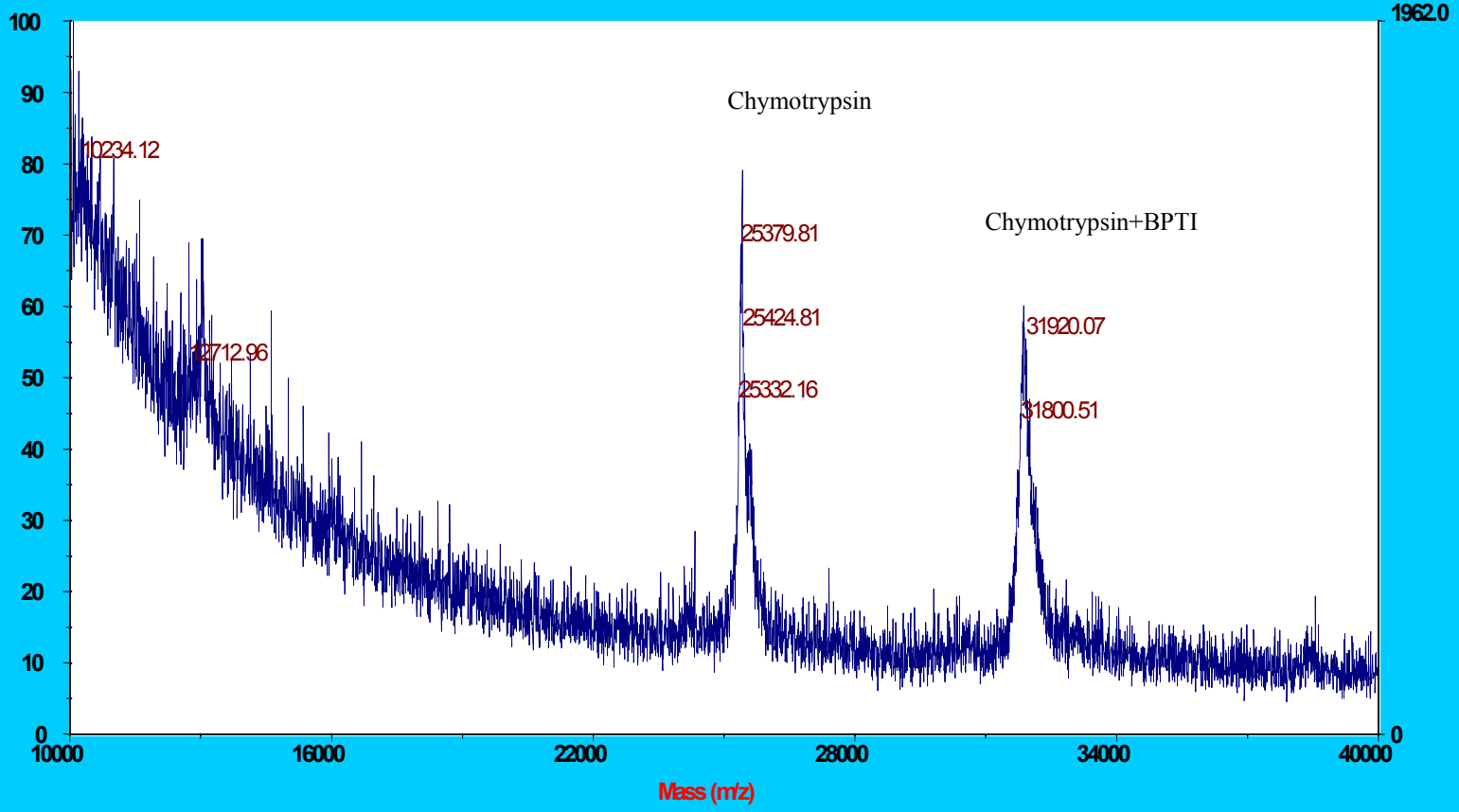
MALDI Spectrum of Trypsin (0.63 uM) with Bovine Pancreatic Trypsin Inhibitor (BPTI) (2.5 uM)

Dilution Study of Complex of Chymotrypsin with BPTI



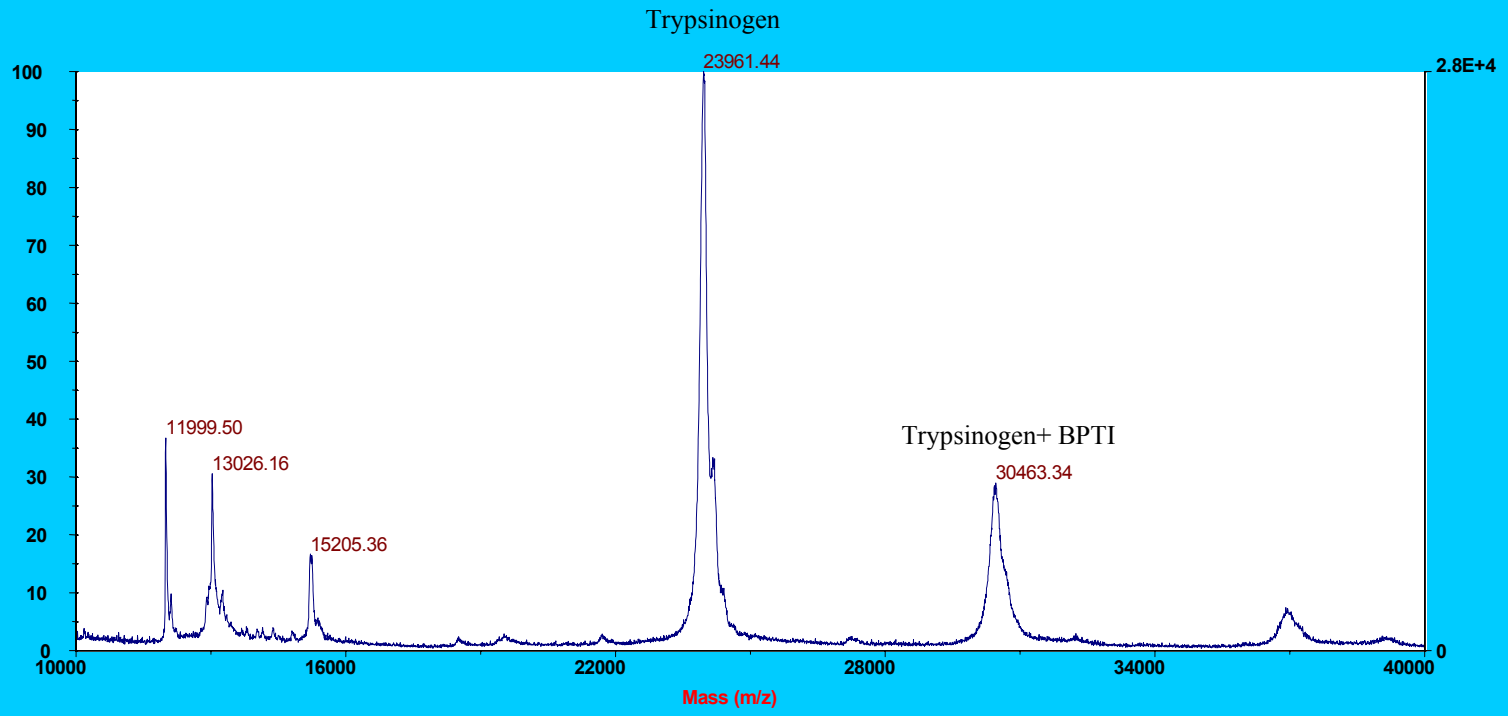
MALDI Spectrum of Chymotrypsin (2.5 uM) With BPTI (10 uM)

Further Dilution Study of Complex of Chymotrypsin with BPTI



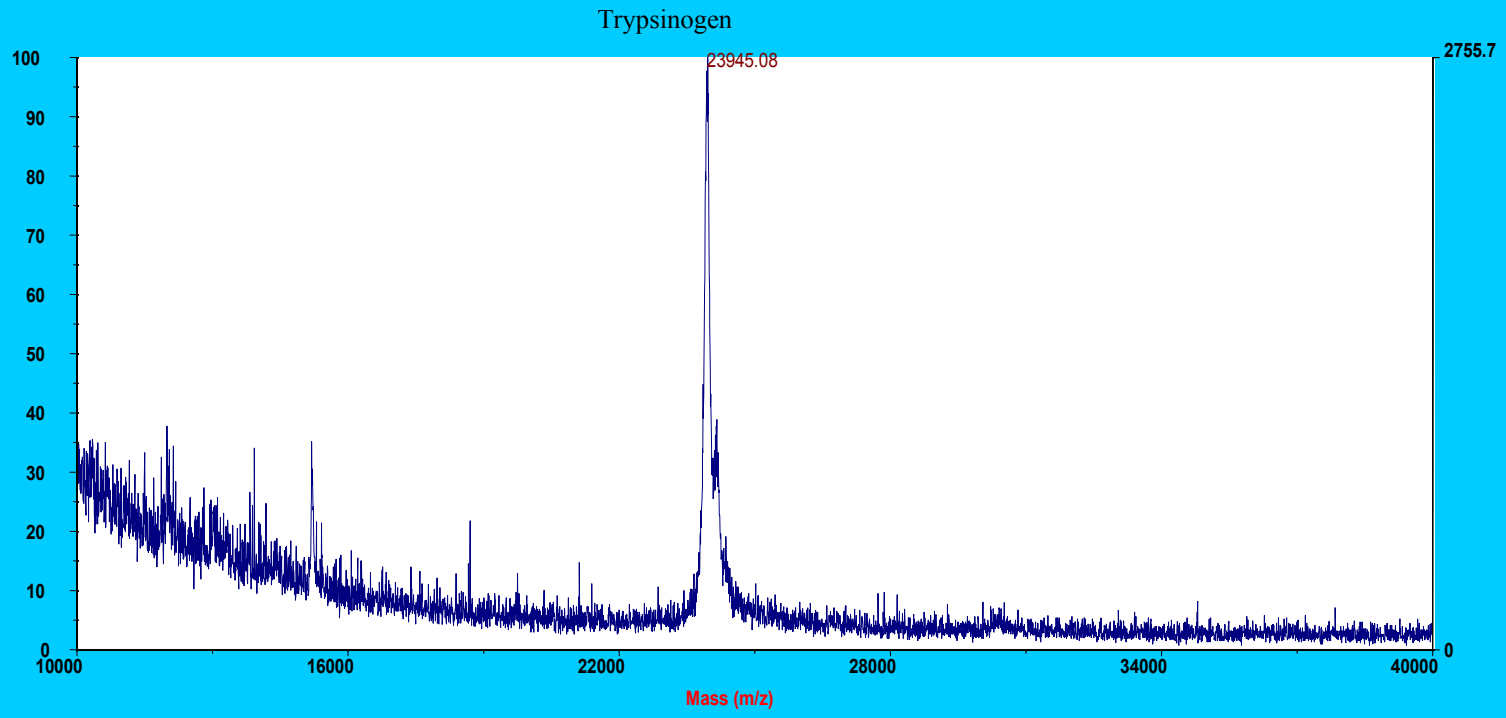
MALDI Spectra of Chymotrypsin (0.63uM) With BPTI (2.5 uM)

Dilution Study of Complex of Trypsinogen with BPTI



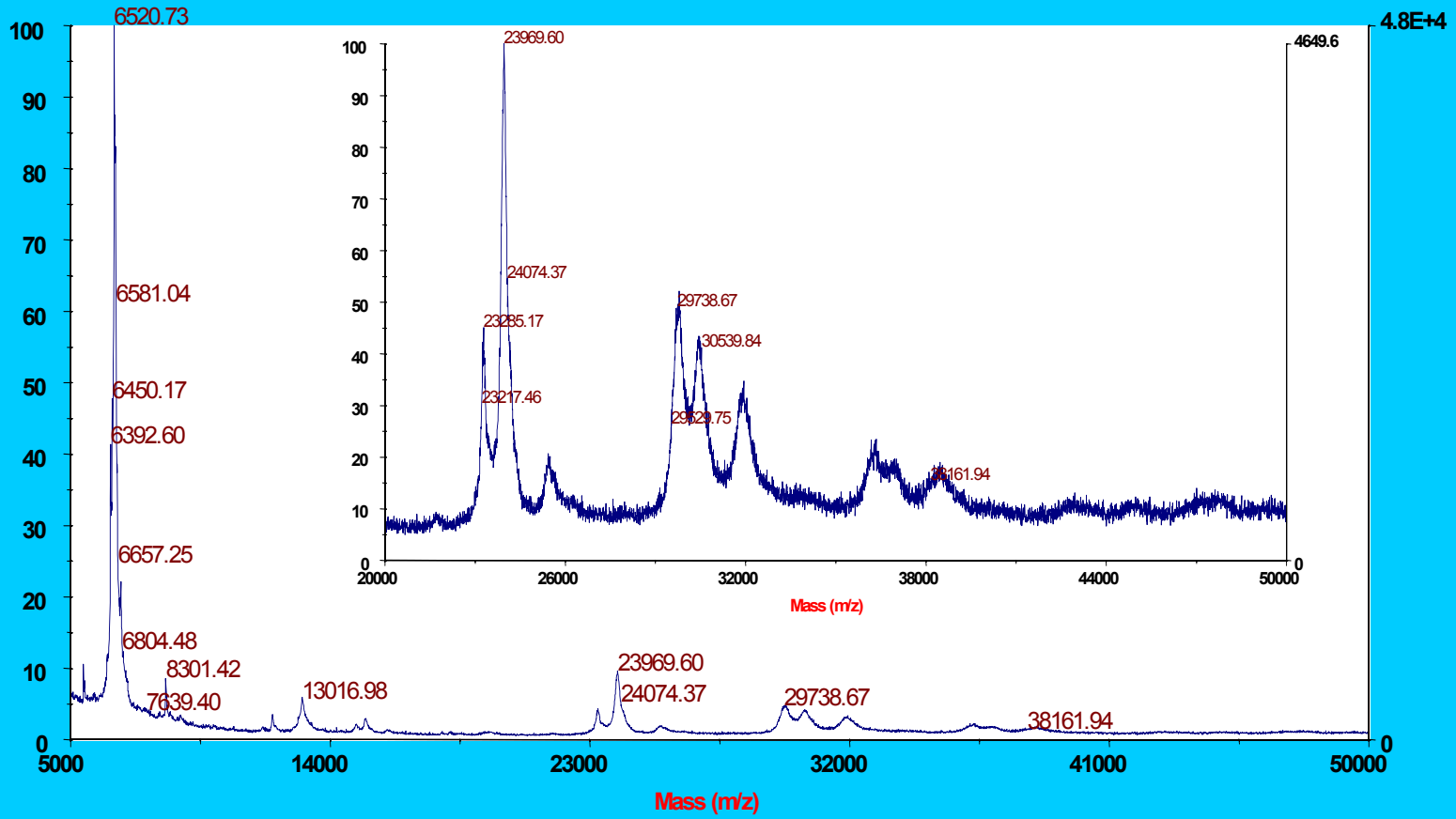
MALDI Spectrum of Trypsinogen (2.5uM) With BPTI (10 uM)

Further Dilution Study of Complex of Trypsinogen with BPTI



MALDI Spectra of Trypsinogen (0.63 uM) With BPTI (2.5 uM)

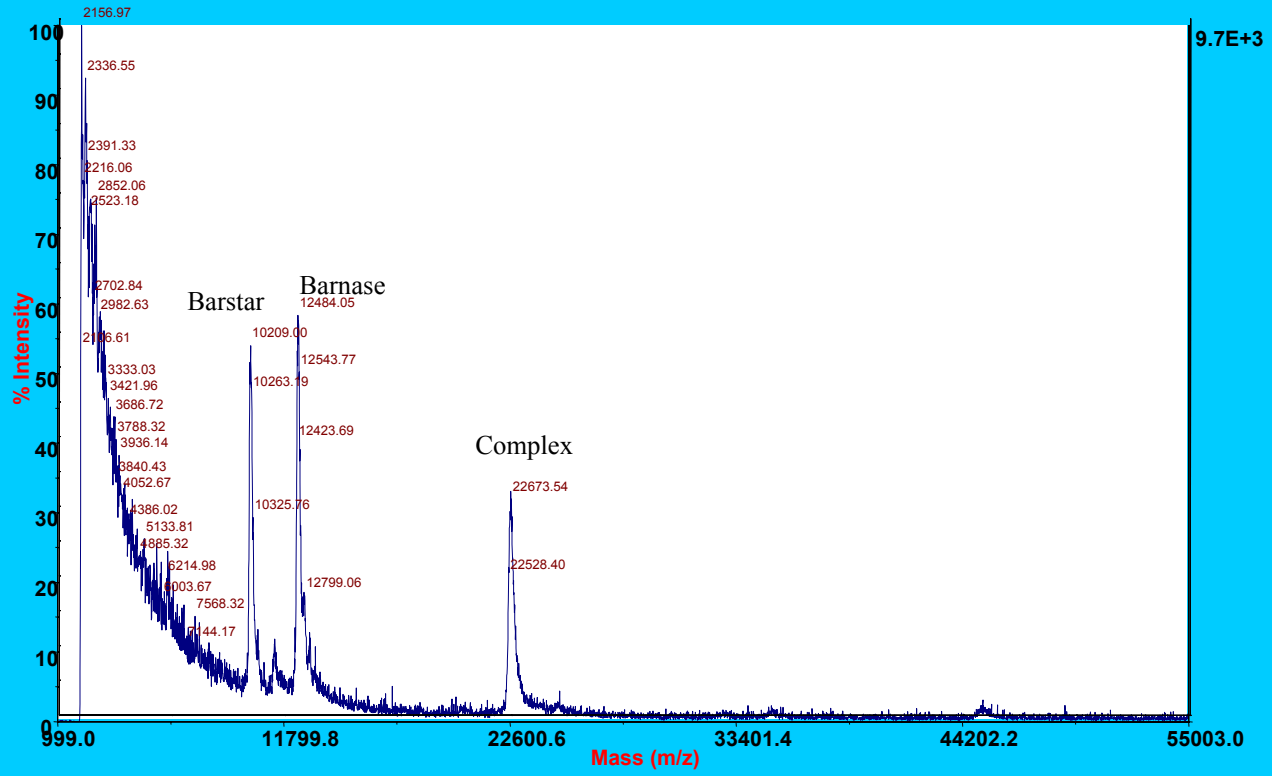
Competitive Binding Study



MALDI Spectra of Complexes of Trypsin, Chromotrypsin and Trypsinogen in about Equal Molar Ratio with BPTI.

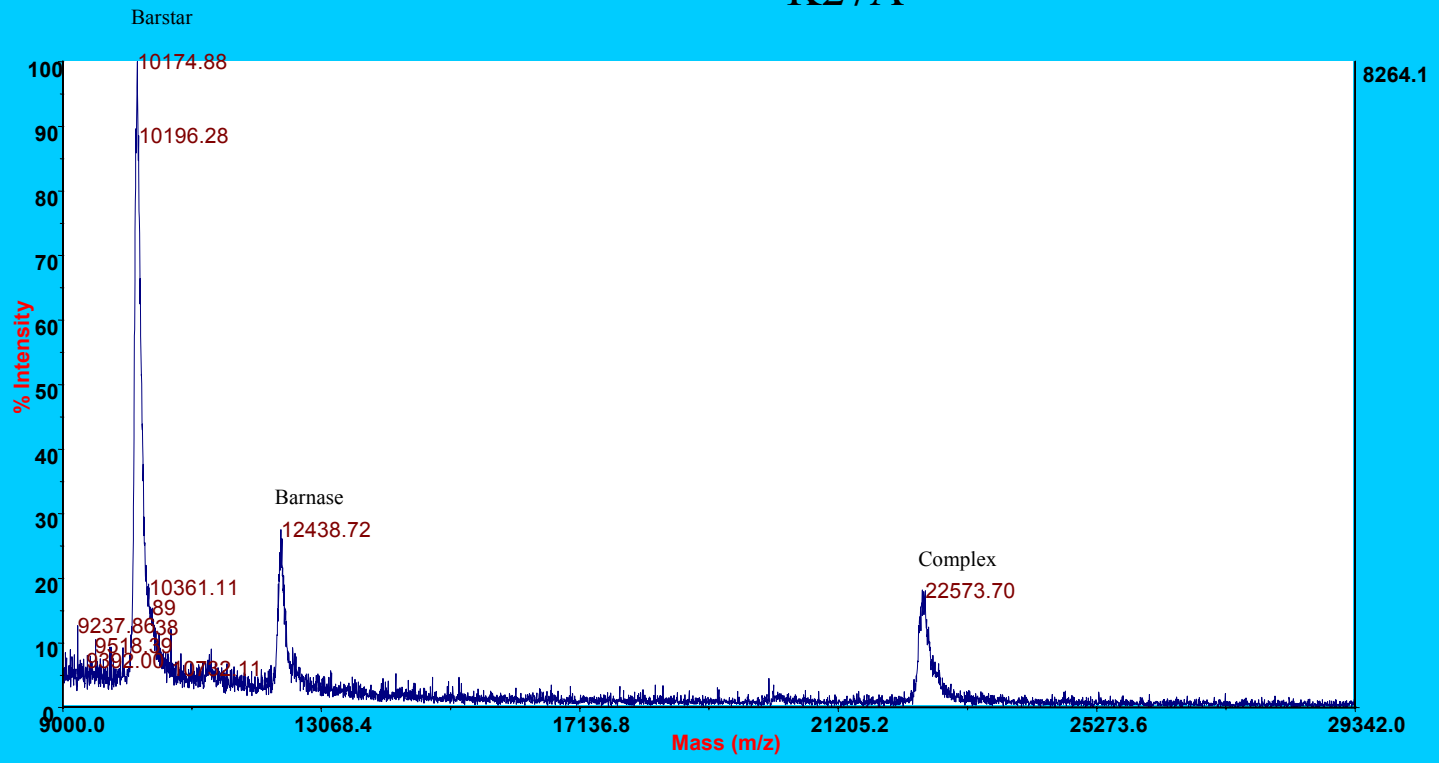
Complex of Barnase and Barstar

Barnase is a 110-residue extraceullular protein found in bacillus amyloliquefaciens. It is a ribonuclease whose potentially lethal functions within the cell are inhibited by barstar, a 90-residue polypeptide.

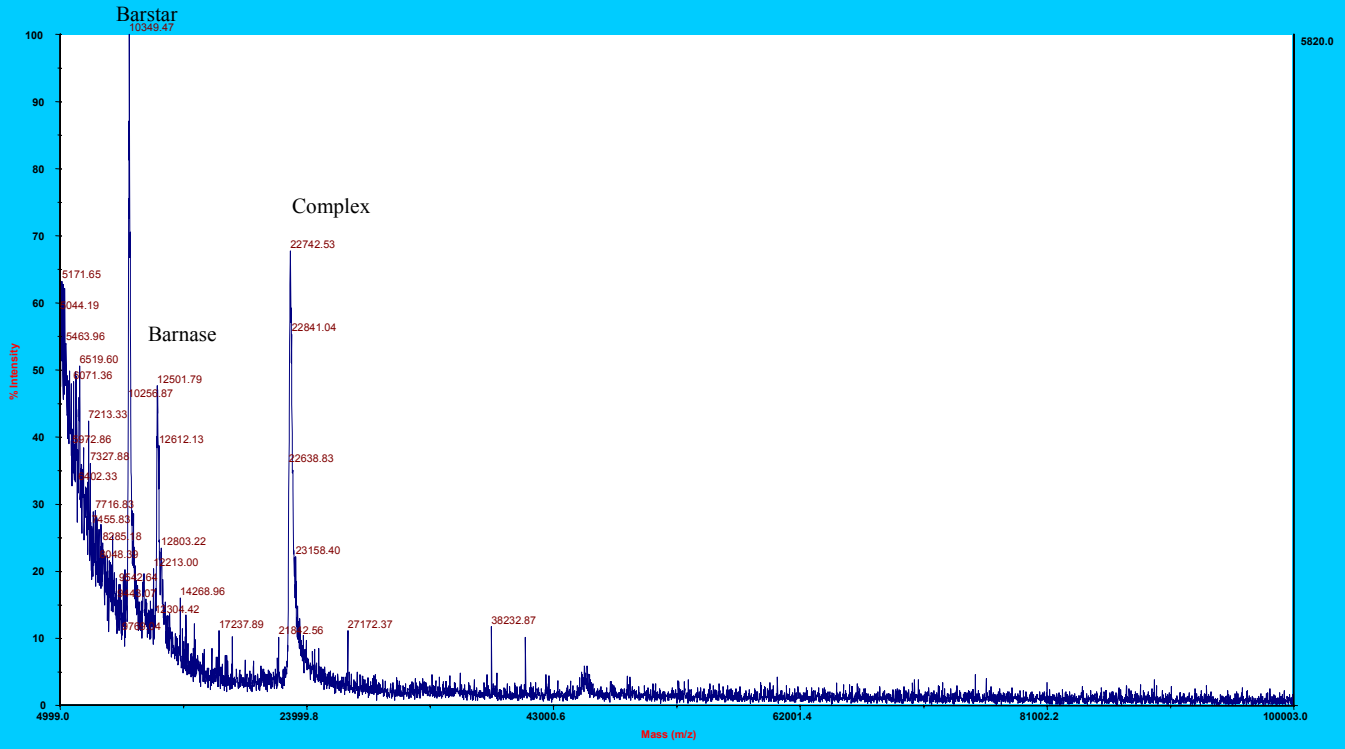


Complex of Barnase and Barstar Mutants A

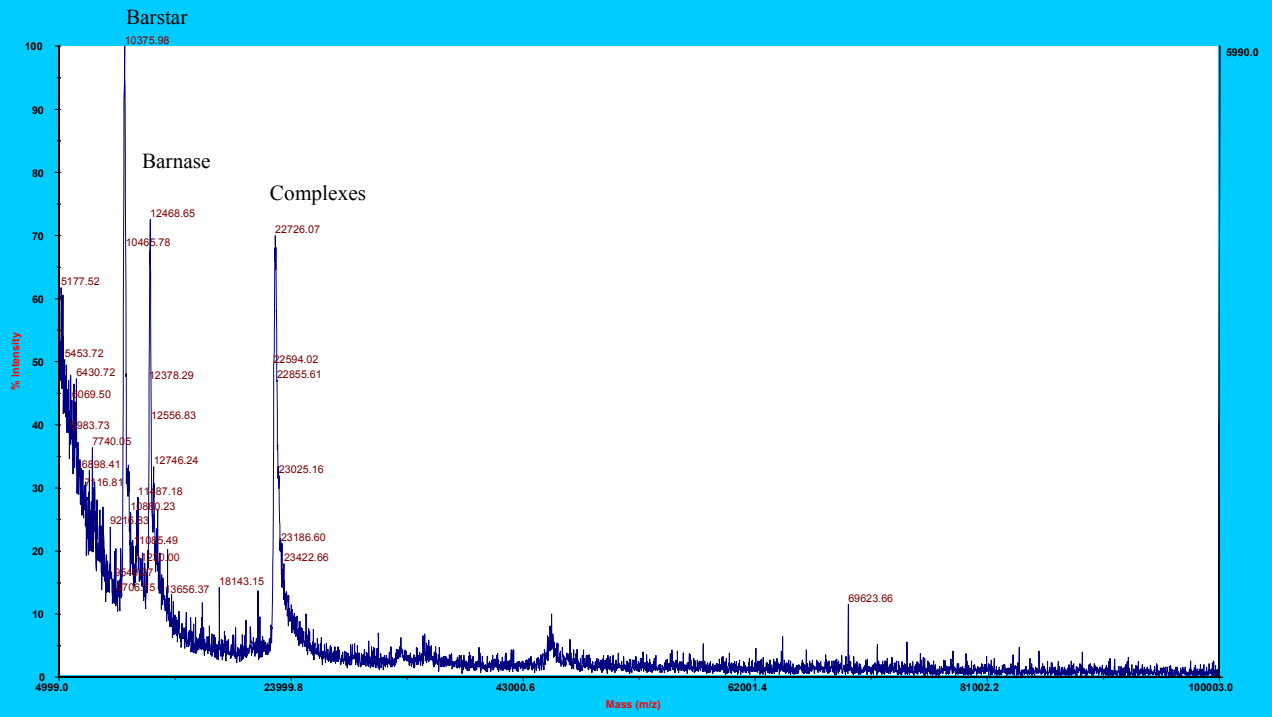
Barstar	Barnase
Y29A	H102A
Y29F	R59A
D39A	K27A



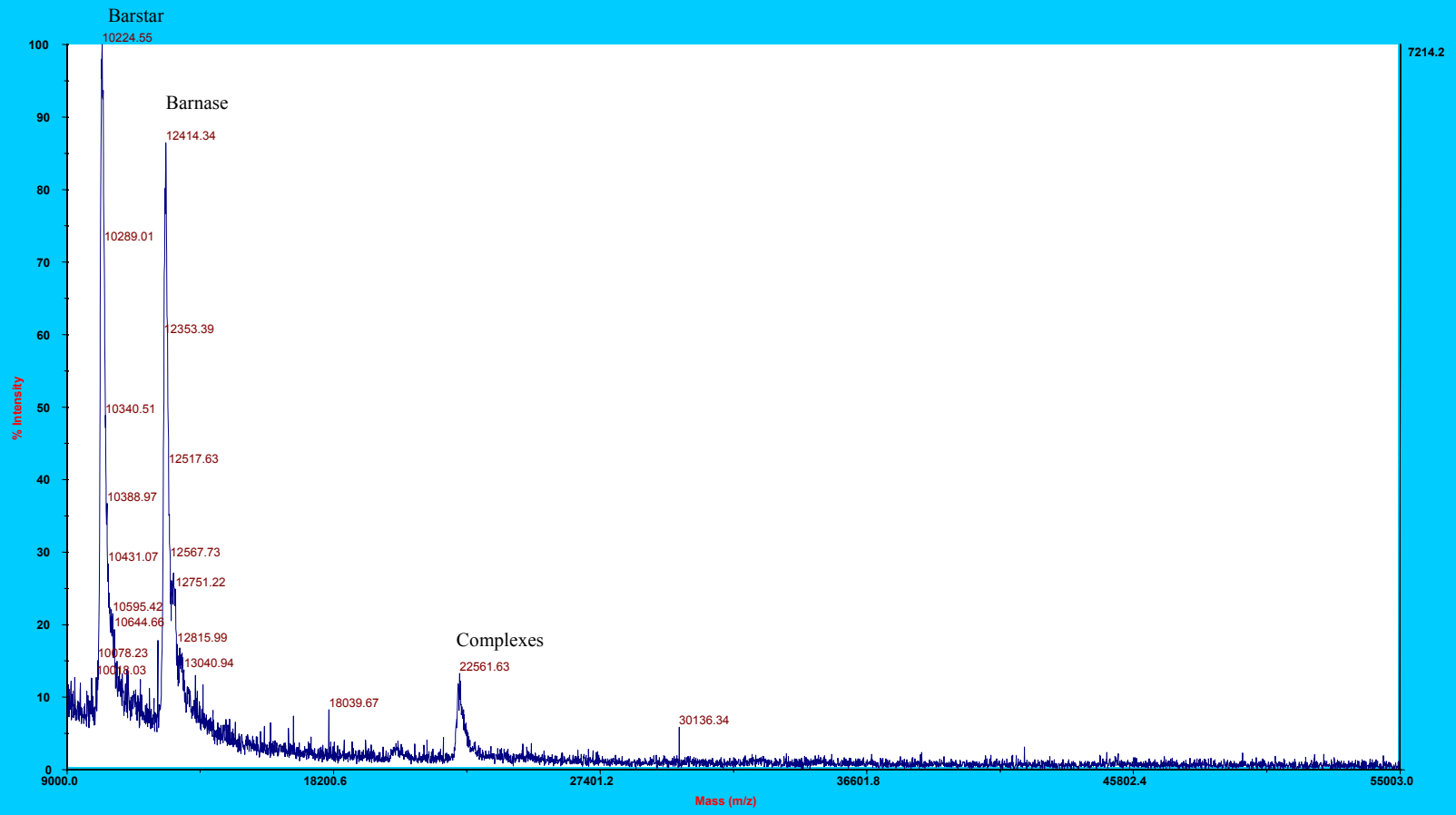
Complex of Barnase and Barstar Mutants B



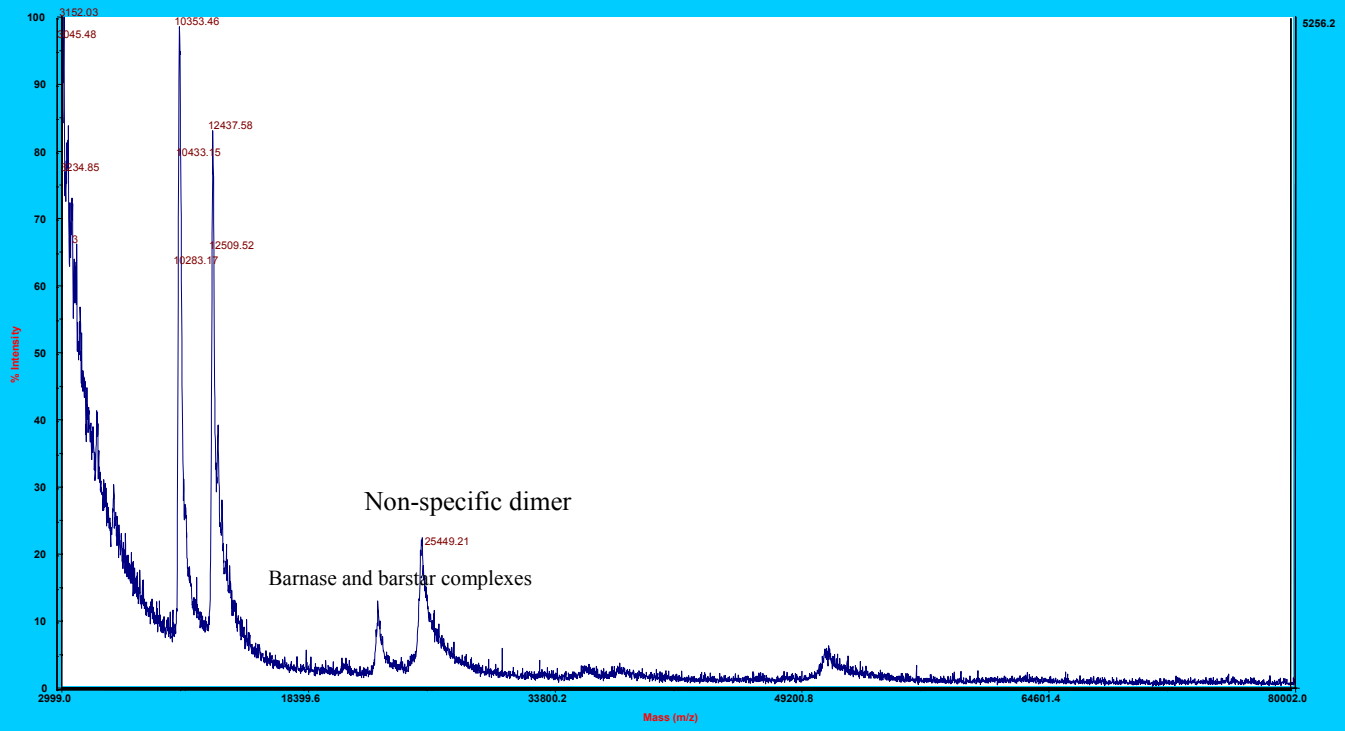
Complex of Barnase and Barstar Mutants C



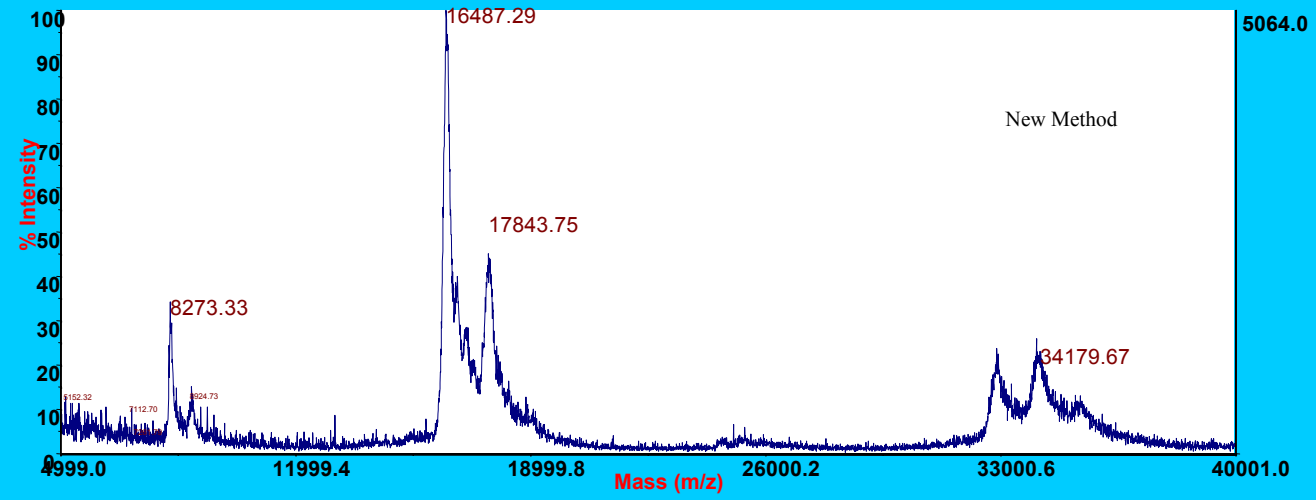
Complex of Barnase and Barstar Mutants D

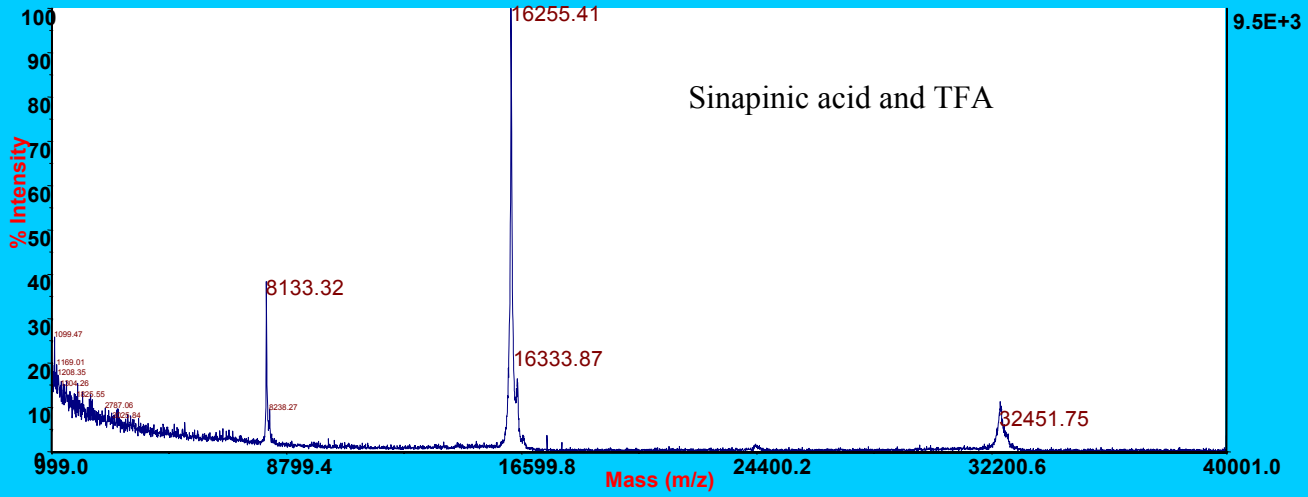


Complex of Barnase and Barstar Mutants E



A Complexes of PAL Protein and Peptidoglycan





Conclusions

- Protein-protein complexes were detected by MALDI using a new sample preparation method.
- The new method uses aqueous solution at physiological pH, which can be broadly used to study protein-protein complexes.
- Good correlation was observed between the gas phase complexes detected by MALDI and their known associations in solution by other methods.
- The results suggest that MALDI can be used to study protein quaternary structures.

Acknowledgements:

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