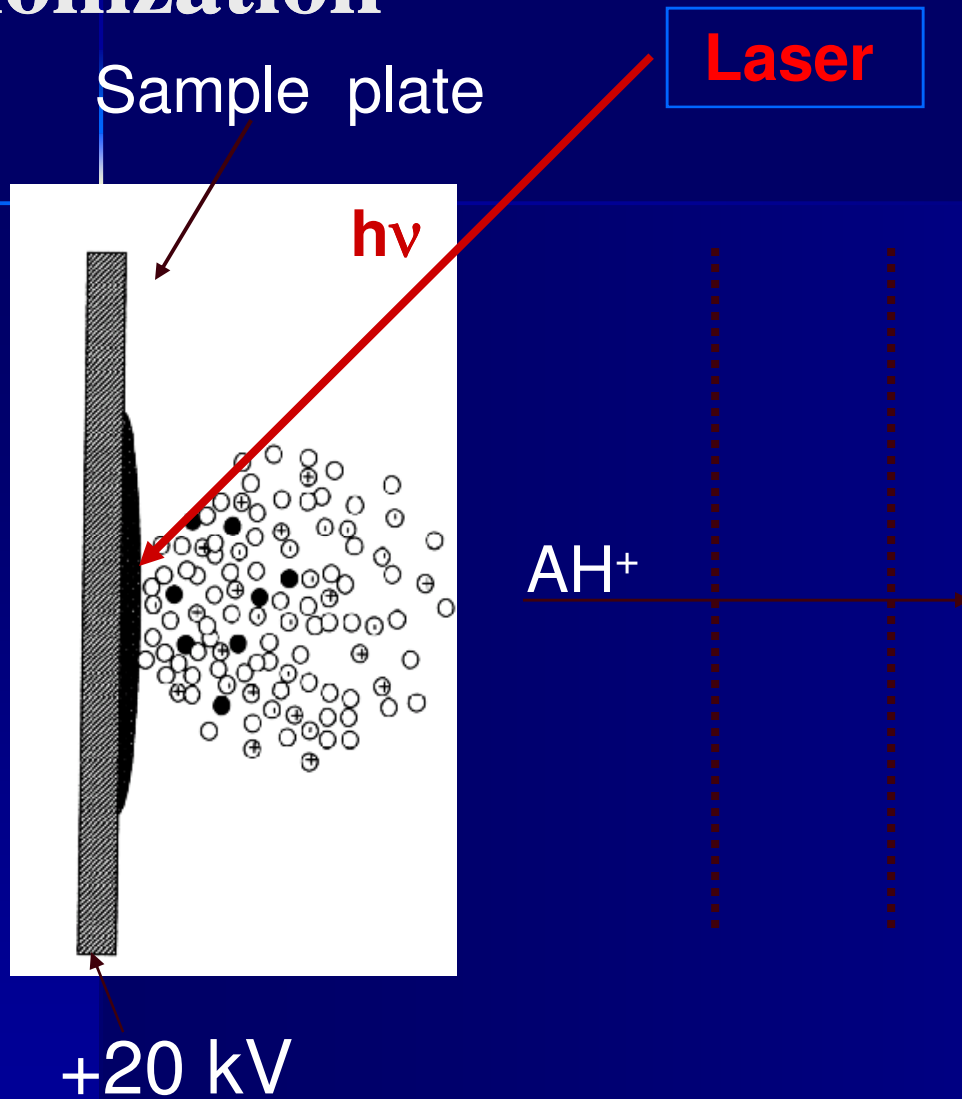


# Matrix-Assisted Laser Desorption/Ionization (MALDI)

# MALDI: Matrix Assisted Laser Desorption Ionization



1. Sample (A) is mixed with excess matrix (M) and dried on a MALDI plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules are ionized by proton transfer from matrix:  
 $MH^+ + A \rightarrow M + AH^+$ .

# MALDI

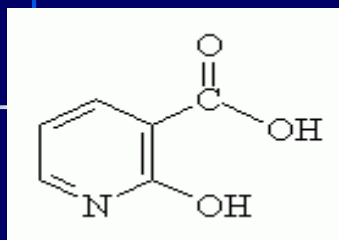
## Matrix-Assisted Laser Desorption/Ionization:

1. Soft ionization — analyze intact biomolecules and synthetic polymers
2. Broad mass range — analyze a wide variety of biomolecules
3. Simple mixtures are okay
4. Relatively tolerant of buffers and salts
5. Fast data acquisition
6. Easy to use and maintain, no water or gas hook ups required
7. *High sensitivity, superior mass resolution and accuracy*

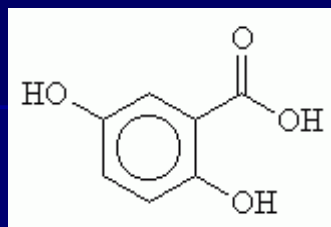
# Functions that the matrix must perform:

1. Disperse the analyte
2. Reduce/eliminate interaction with the sample surface
3. Absorb the laser light
4. Disintegrate/dissociate at low energy
5. Desorb the analyte from the sample surface
6. Ionize the analyte
7. Be able to embed and isolate analytes (e.g., by co-crystallization)
8. Be soluble in solvents compatible with analyte
9. Be vacuum stable

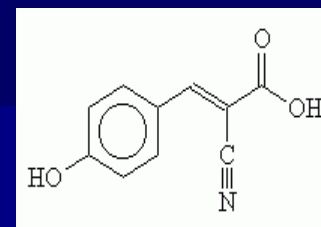
# UV matrices



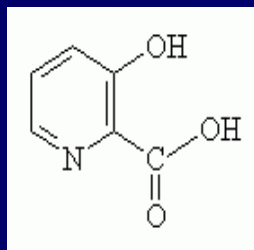
Nicotinic acid



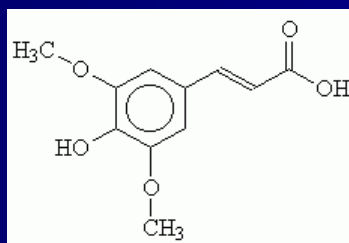
DHB  
gentisic acid



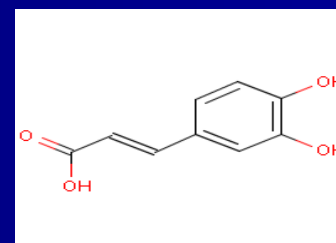
CHCA  
( $\alpha$ -cyano-4-hydroxycinnamic acid)



3-hydroxypicolinic acid



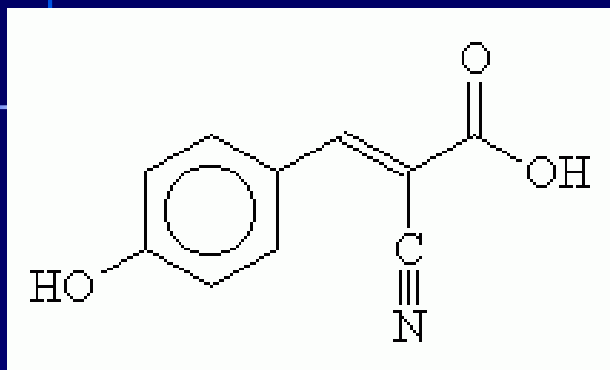
Sinapinic acid  
(3,5-dimethoxy-4-hydroxycinnamic acid)



Caffeic acid

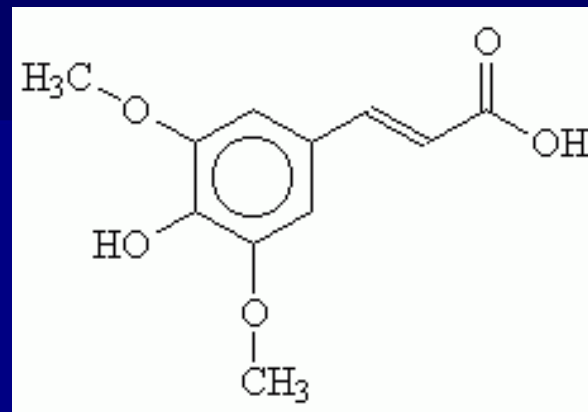
(3,4-dihydroxy cinnamic acid)

## Alpha-cyano-4-hydroxycinnamic acid (CHCA)



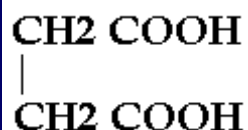
- very efficient at ionizing proteins (intense signals)
- produces large fragments particularly in the 500-5000 range where tryptic digests appear (Hence use for <10,000 Da proteins)
- Peptide mass fingerprinting

## Sinapinic acid (SA) (3,5-dimethoxy-4-hydroxycinnamic acid)

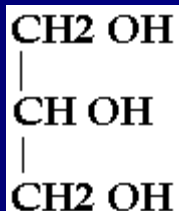


- not strongly ionizing so fewer multiply charged ions detected
- high affinity for proteins
- very non-selective
- best for hydrophobic proteins
- best for crude biological extracts

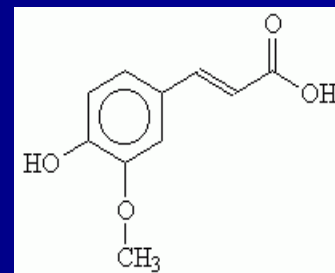
# IR Matrices



Succinic acid

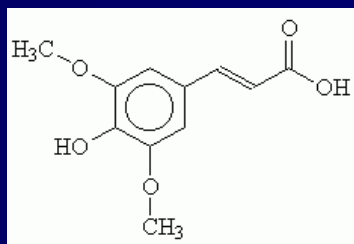


Glycerol



Ferulic acid

(4-hydroxy-3-methoxycinnamic acid)



Sinapinic acid

Water (ice)

# Methods for Sample Deposition

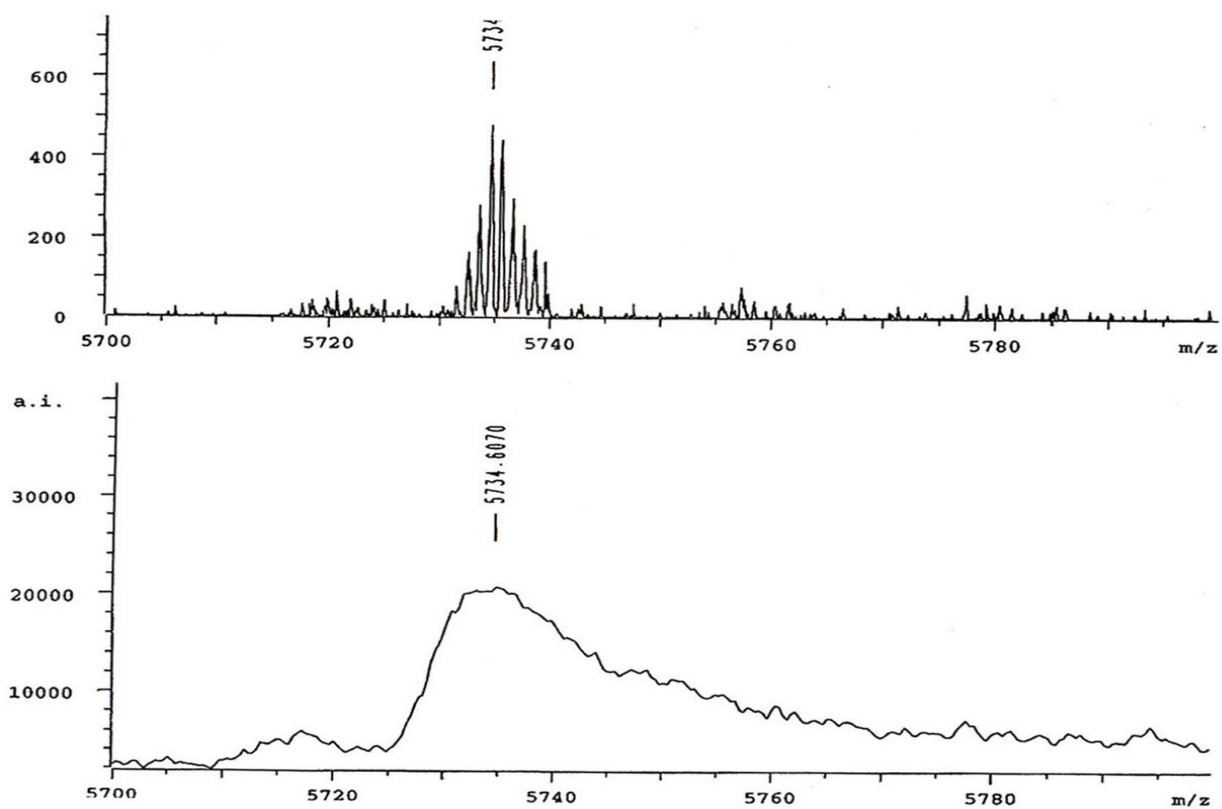
Sample must be mixed with matrix solution in a ratio of 1: 10,000 on the sample plate



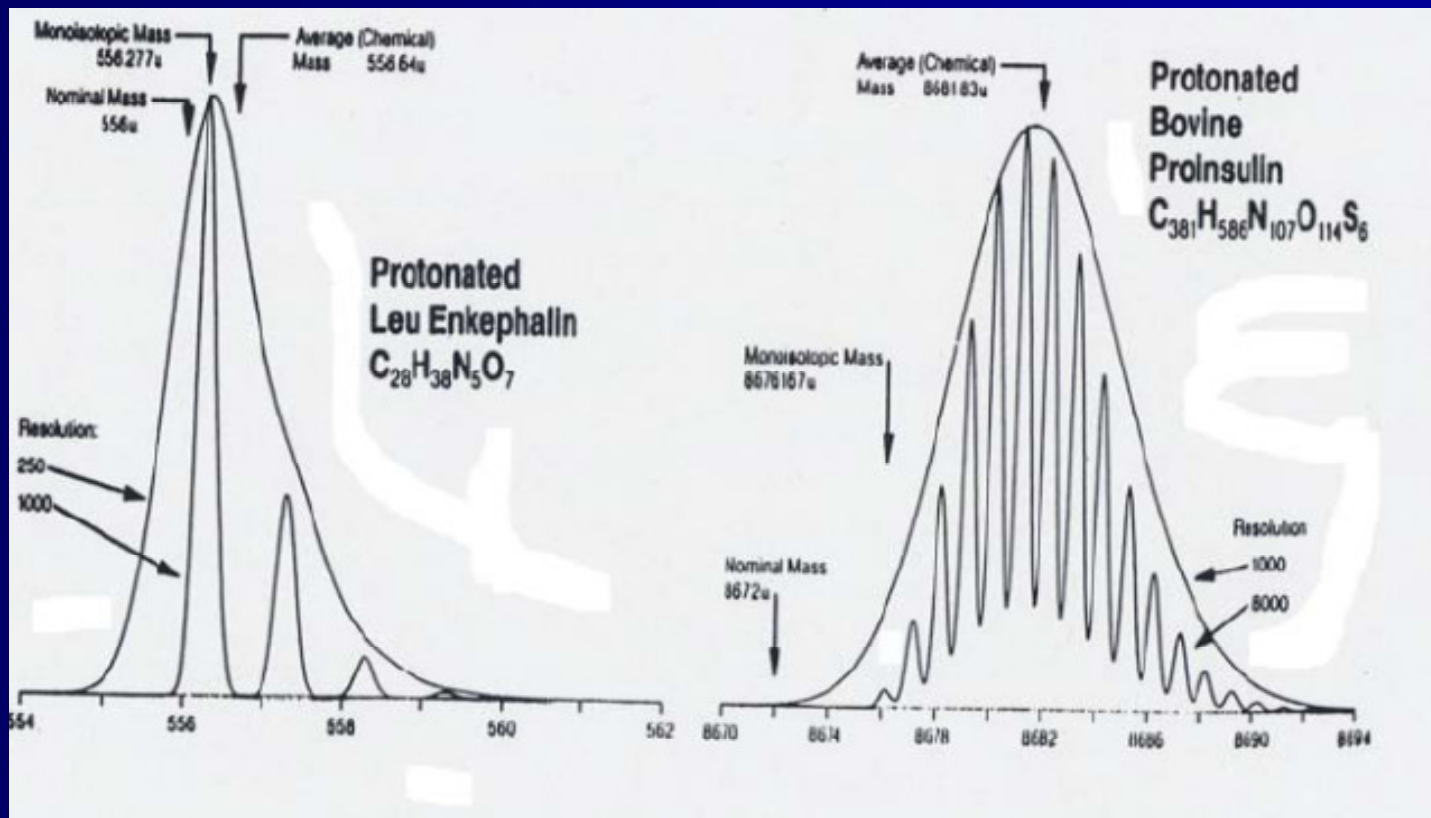
# +ve and -ve Ionization in MALDI

- In positive ionization mode the protonated molecular ions  $[M+H]^+$  are usually the dominant species, although they can be accompanied by salt adducts, a trace of the doubly charged molecular ion at approximately half the  $m/z$  value, and/or a trace of a dimeric species at approximately twice the  $m/z$  value. Positive ionization is used in general for protein and peptide analyses.
- In negative ionization mode the deprotonated molecular ions  $[M-H]^-$  are usually the most abundant species, accompanied by some salt adducts and possibly traces of dimeric or doubly charged materials. Negative ionization can be used for the analysis of oligonucleotides and oligosaccharides.

# Lin vs Ref of Insulin



# What mass are we looking at in MALDI ?



# Benefits and Limitations of LIN TOF

## Advantages

1. Extremely High Mass Range (>10<sup>6</sup>Da)
2. Fast Scanning

## Disadvantages

1. Low Resolution (4000)
2. Low Accuracy (>200ppm)
3. MS/MS not possible

# Benefits and Limitations of REF TOF

## Advantages

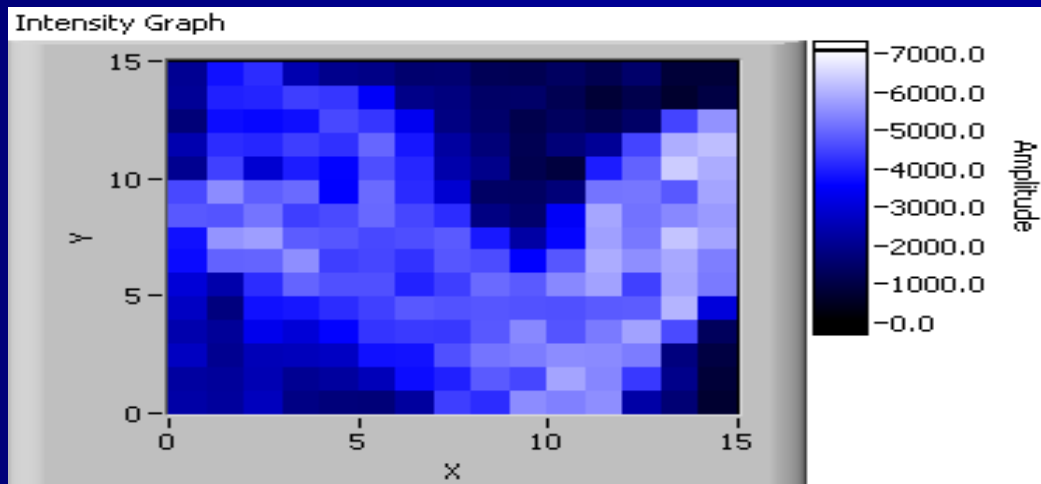
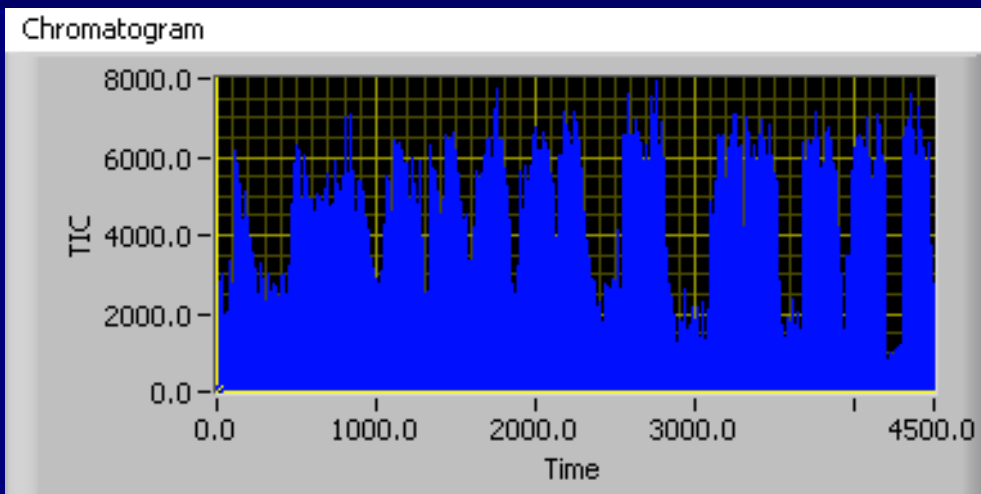
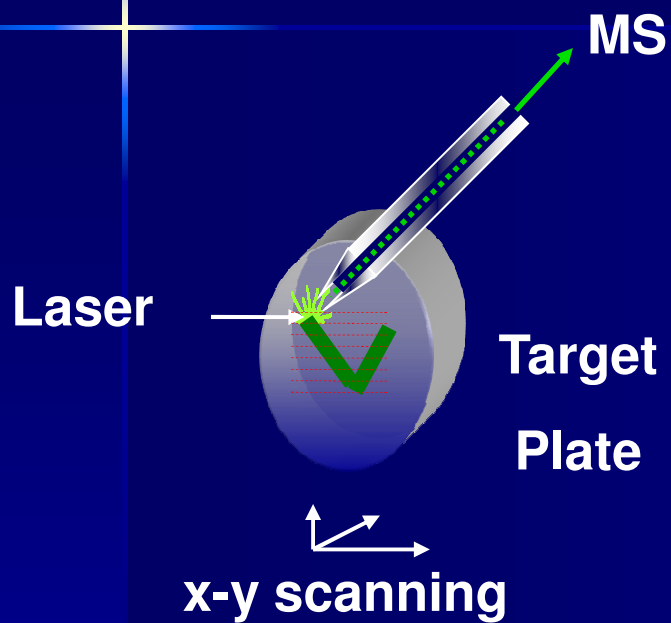
- High Resolution (>20,000 in some models)
- High Accuracy (<5ppm)
- 10,000 Mass Range
- Fast Scanning

## Disadvantages

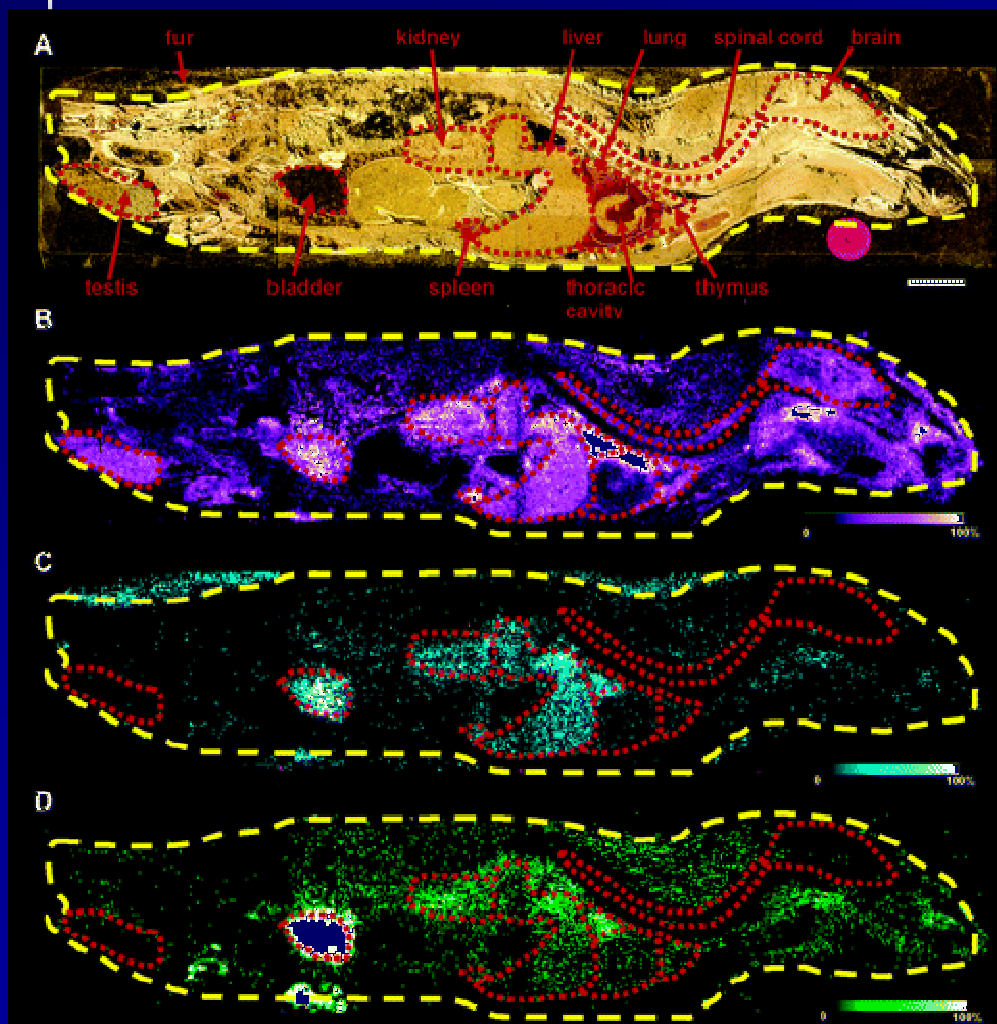
- Low Resolution for MS/MS (PSD)

# MALDI-MS Imaging

# MALDI-MS Imaging



# MALDI MS – Animal Tissue Section Imaging



Detection of drug and metabolite distribution at 2 h postdose in a whole rat sagittal tissue section by a single IMS analysis. Optical image of a 2 h post OLZ dosed rat tissue section across four gold MALDI target plates (A). Organs outlined in red. Pink dot used as time point label. MS/MS ion image of OLZ ( $m/z$  256) (B). MS/MS ion image of *N*-desmethyl metabolite ( $m/z$  256) (C). MS/MS ion image of 2-hydroxymethyl metabolite ( $m/z$  272) (D). Bar, 1 cm.

Khatib-Shahidi *et al. Anal. Chem.*, **78** (18), 6448 -6456, 2006.



# MALDI Applications

- Accurate Mass determination
- Post Translational Modification
- Peptide Mass Fingerprinting
- Disulphide Bond Assignment
- Proteomics