Exploring the molecular organization in solvent-free MALDI samples by solid-state NMR to study

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Background: solid-state techniques to study MALDI

❖ Many studies focused on sample morphology

- optical microscopy

SJ Doktycz *et al*. *Rapid Commun. Mass Spectrom*. 1991, 5, 145
LM Preston *et al*. *Biol. Mass Spectrom.* 1993, 22, 744

- scanning electron microscopy

A Westman *et al*. *J. Mass Spectrom*. 1995, 30, 206
Al Gusev *et al*. *Anal. Chem*. 1995, 67, 1034
V Horneffer *et al*. *Int. J. Mass Spectrom.* 2003,226, 117

- confocal laser scanning microscopy

V Horneffer *et al*. *Anal. Chem.* 2001, 73, 1016
V Horneffer *et al*. *J. Am. Soc. Mass Spectrom*. 2006, 17, 1599

- Raman spectrometry

TWD Chan *et al*. *Org. Mass Spectrom.* 1992, 27, 188

- mass spectrometry imaging

SD Hanton *et al*. *J. Am. Soc. Mass Spectrom*. 1999, 10, 104
RW Garden *et al*. *Anal. Chem*. 2000, 72, 30
SD Hanton *et al*. *J. Am. Soc. Mass Spectrom.* 2004, 15, 168

- X-Ray diffraction

K Strupat *et al*. *J. Am. Soc. Mass Spectrom.* 1997, 169, 43

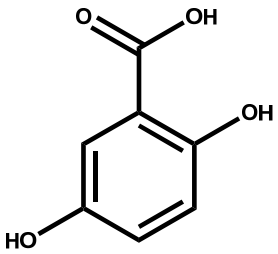
❖ Solid-state NMR: structural information at the atomic level

- chemical shifts are sensitive to local changes in the structure
- explores local nucleus environment: no need for long distance order
- access to many different nuclei ↔ complementary information

H Pizzala, C Barrere, M Mazarin, F Ziarelli, L Charles *J. Am. Soc. Mass Spectrom.* 2009, 20, 1906

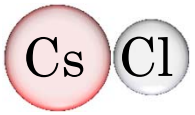
Solid-state NMR for MALDI

❖ **A model system to evaluate the potential of solid-state NMR for MALDI**



matrix

analyte



salt

❖ **Optimized component concentrations to address sensitivity issues**

matrix / analyte / salt : 50 / 1 / 10

❖ **A solid-state protocol for sample preparation**

- solvent-free MALDI

R Skelton *et al*. *Anal. Chem.* 2000, 72, 1707
S Trimpin *et al*. *Rapid Commun. Mass Spectrom.* 2001, 15, 1364

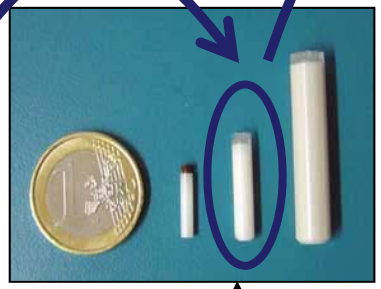
- the vortex method

SD Hanton *et al*. *J. Am. Soc. Mass Spectrom.* 2005, 16, 90

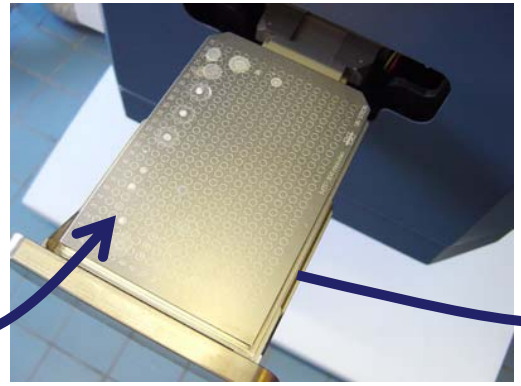
Experimental flow chart

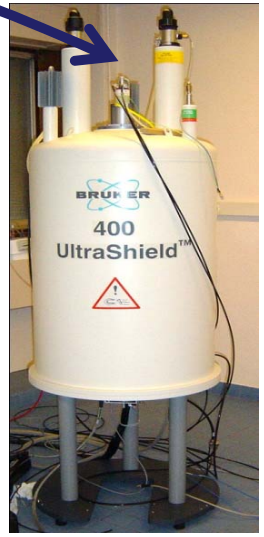
Matrix
Salt
Analyte

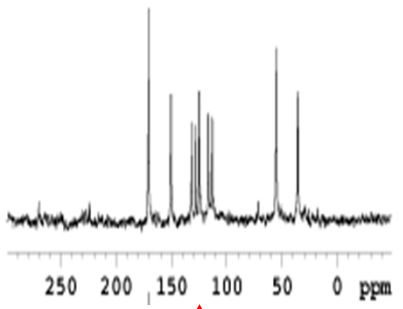
40-50 mg



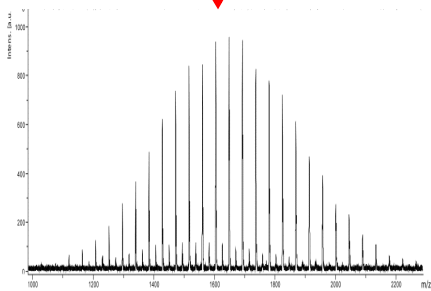
Rotor





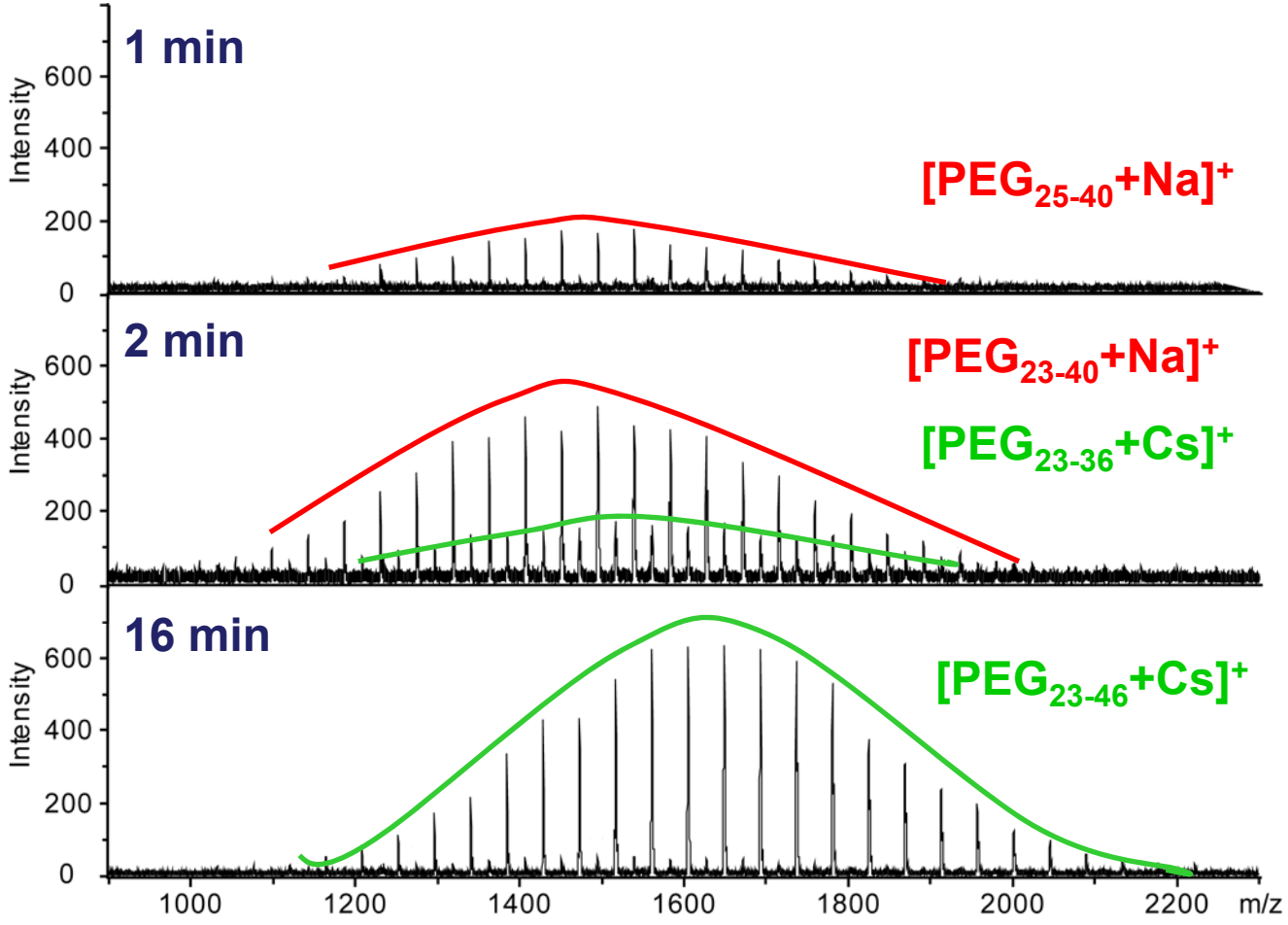


correlations?



Changes in sample preparation

Influence of grinding time

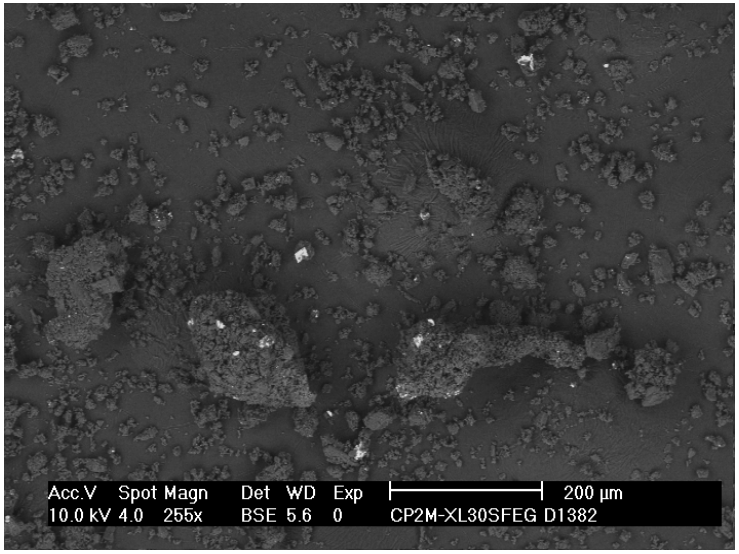


Y Major, H Pizzala, F Ziarelli, T Phan, G Mollica, L Charles. *Anal. Meth.* 2012, *in press.* DOI: 10.1039/C2AY25708D

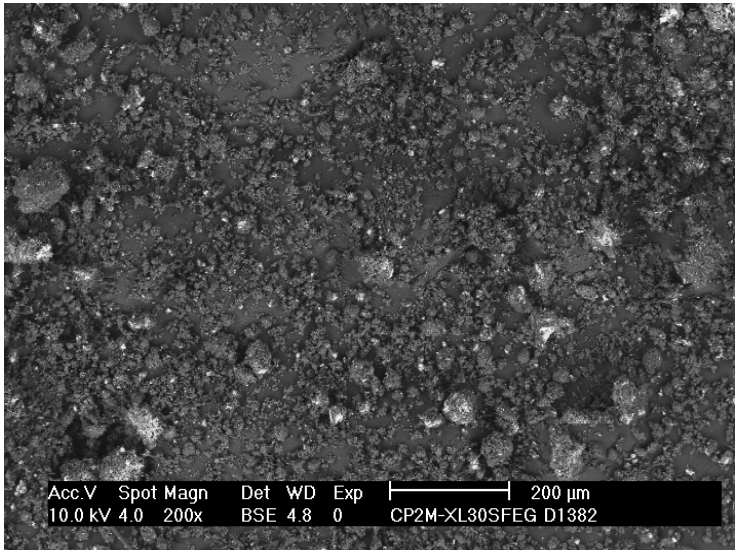
Effect of grinding time on particle size

Scanning electron microscopy

2,5-DHB/PEG/CsCl ground for 2 min



2,5-DHB/PEG/CsCl ground for16 min

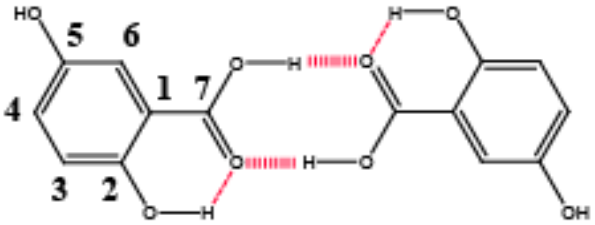


Particle size reduction → increase of surface area

V Horneffer, M Gluckmann, B Kruger, M Karas, K Strupat, F Hillenkamp *Int. J. Mass Spectrom.* 2006, 249, 426

^{13}C solid-state NMR analysis of the matrix

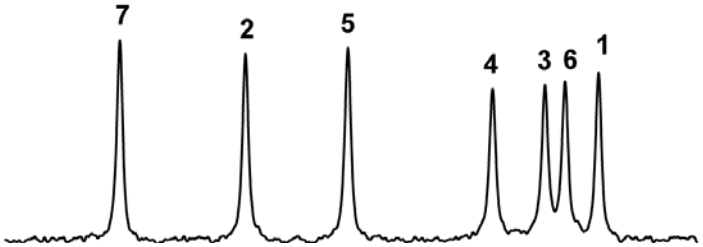
Magic angle spinning (MAS) → increase of resolution; Cross polarization (CP) → sensitivity improvement



sample

interpretation

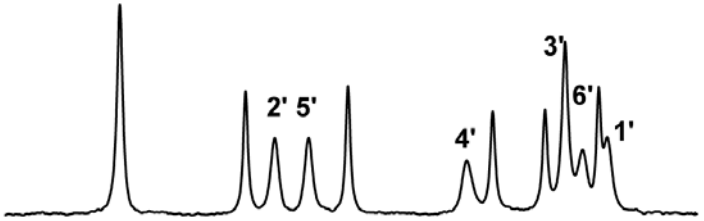
2,5-DHB only ground for 2 or 16 min



matrix dimer
Phase I

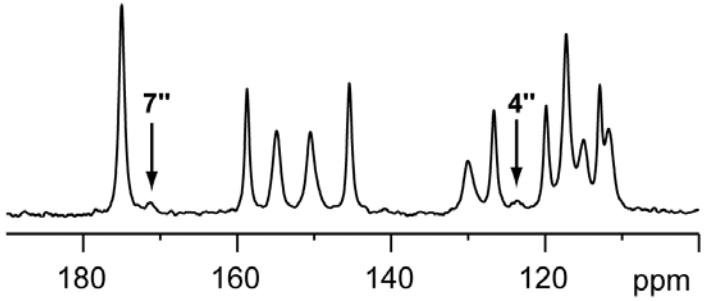
M Haisa *et al*. *Acta Crystallogr.* 1982, 38, 1480
MS Adam *et al*. *New J. Chem.* 2010, 34, 85

2,5-DHB/PEG/CsCl ground for 2 min



matrix/PEG complex
Phase II

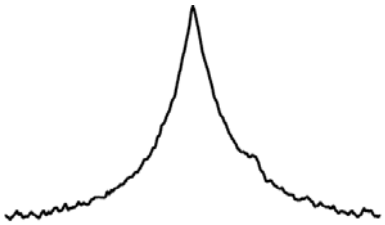
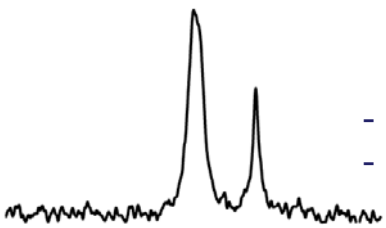
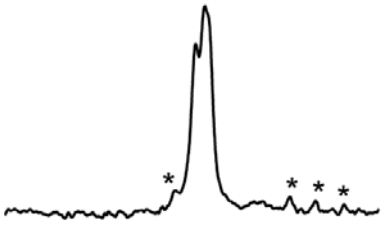
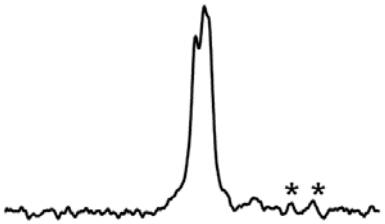
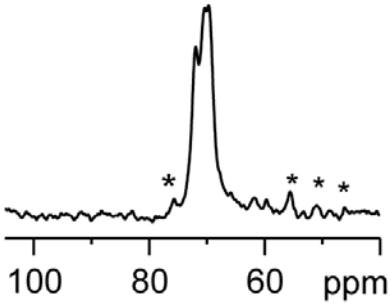
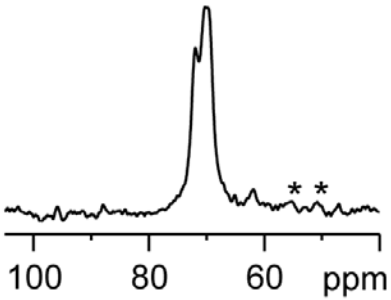
2,5-DHB/PEG/CsCl ground for 16 min



matrix/Cs complex
Phase III

^{13}C solid-state NMR analysis of the PEG analyte

Short contact time → PEG crystalline phase; Long contact time → PEG amorphous phase

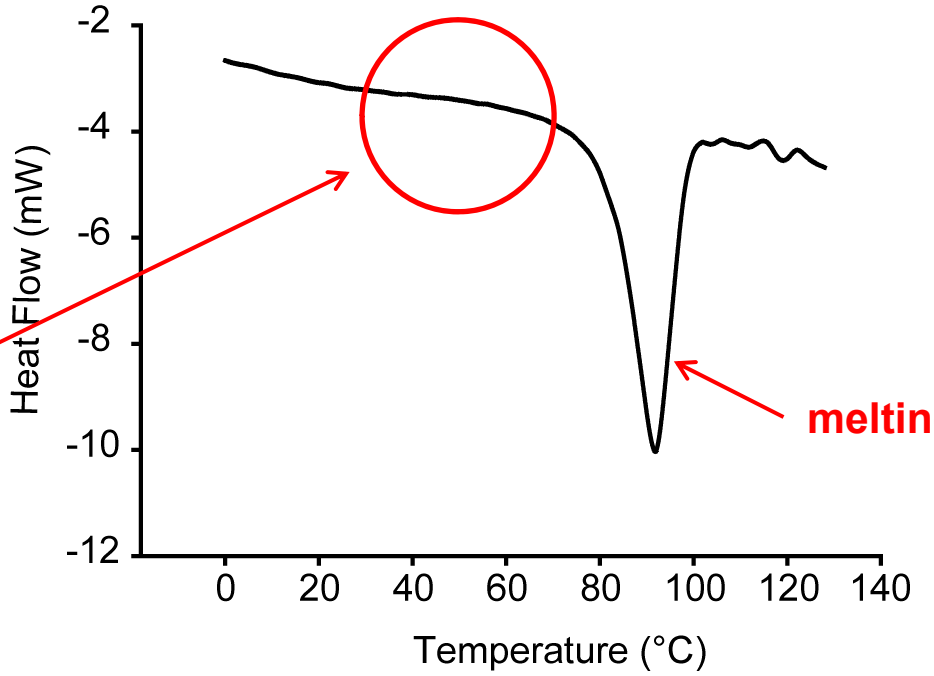
sample	Contact time: 0.1 ms	Contact time: 2.0 ms	*interpretation*
PEG only ground for 2 or 16 min			- large peak: crystalline PEG - narrow peaks: amorphous PEG
2,5-DHB/PEG/CsCl ground for 2 min			**Phase II** **matrix/PEG complex**
2,5-DHB/PEG/CsCl ground for 16 min			**Phase II** **matrix/PEG complex**

* *spinning band*

J Spevacek *et al*. *Macromolecules* 1998, 31, 3612
DJ Harris *et al*. *Macromolecules* 2000, 33, 3375

Differential Scanning Calorimetry

2,5-DHB/PEG/CsCl
ground for 16 min



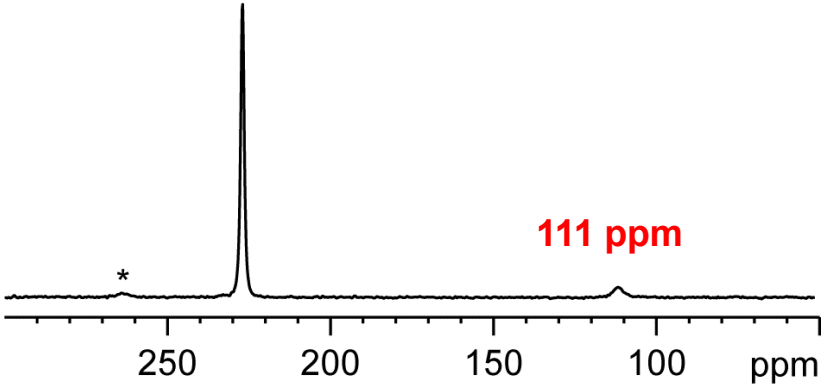
no peak at 44 °C for crystalline PEG

melting peak @ 90-100 °C

a complex between PEG and hydroxybenzene compounds
Phase II

J Spevacek *et al*. *Macromolecules* 1998, 31, 3612
DJ Harris *et al*. *Macromolecules* 2000, 33, 3375

^{133}Cs solid-state NMR analysis of the salt

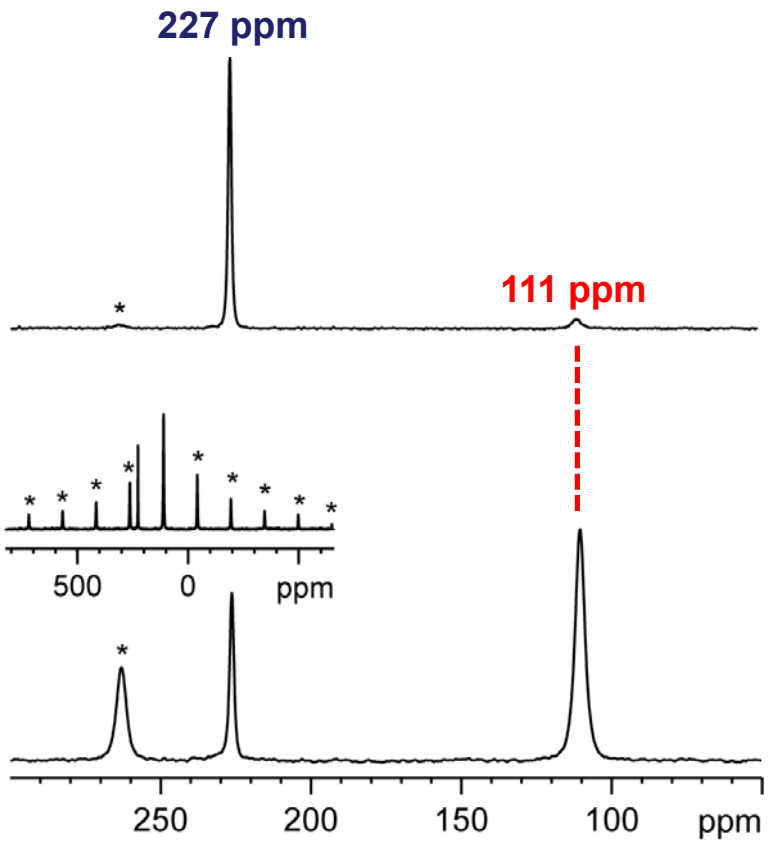
sample		*interpretation*
CsCl only ground for 2 or 16 min		**Cs**
2,5-DHB/PEG/CsCl ground for 2 min		**Cs**
2,5-DHB/PEG/CsCl ground for 16 min		**Phase III matrix/Cs complex**

** spinning band*

^{133}Cs solid-state NMR analysis of the salt

2,5-DHB/PEG/CsCl
(50:1:10)

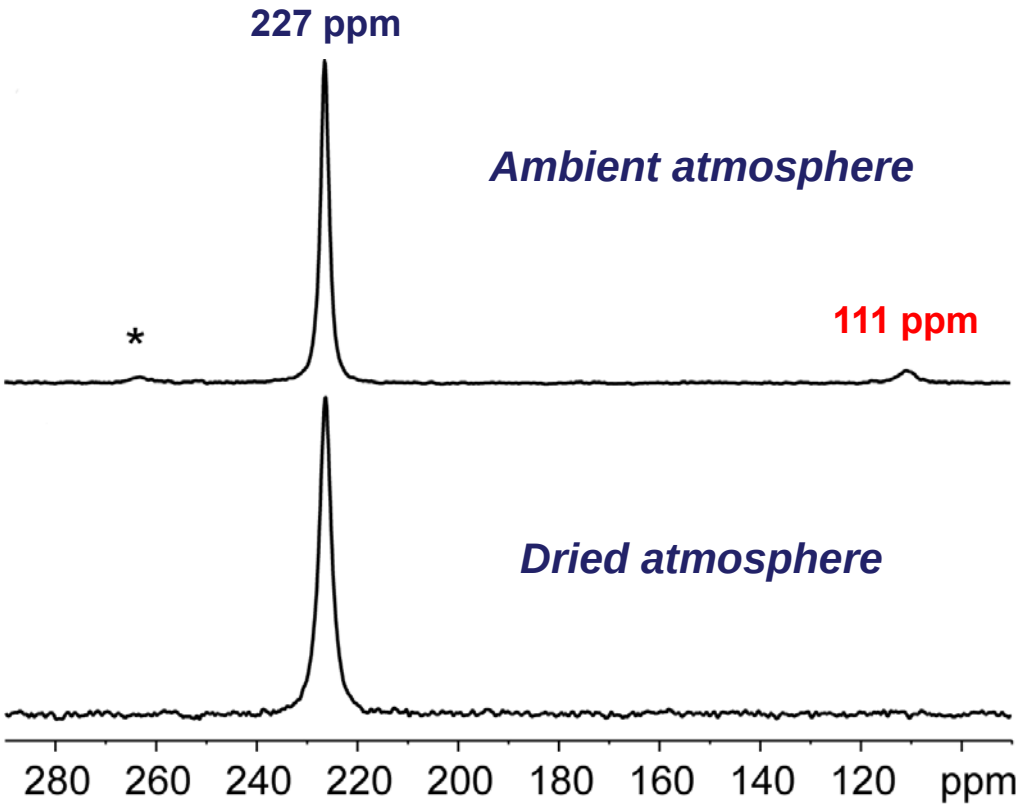
2,5-DHB/CsCl
(1:1)

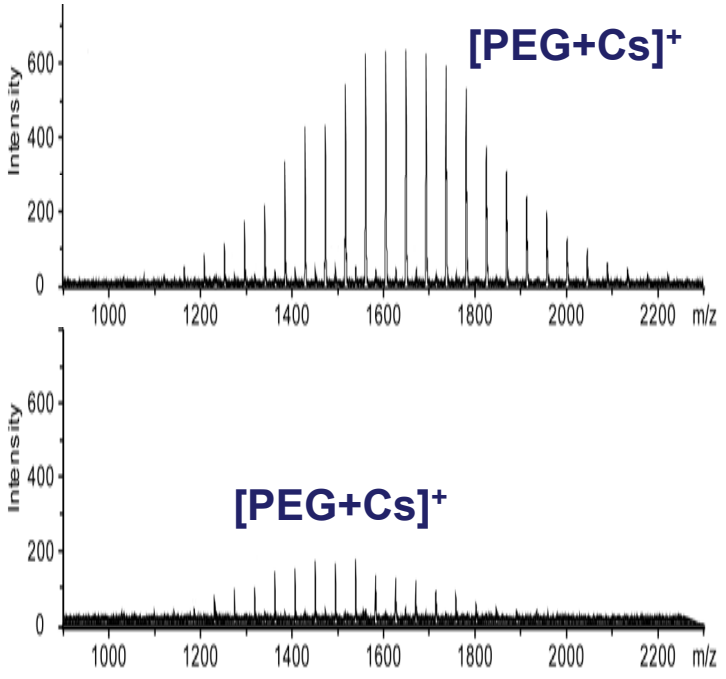


A very extended spinning sideband envelope for the 111 ppm signal reflects a significant loss of symmetry for Cs in the new environment of phase III

Influence of atmospheric water

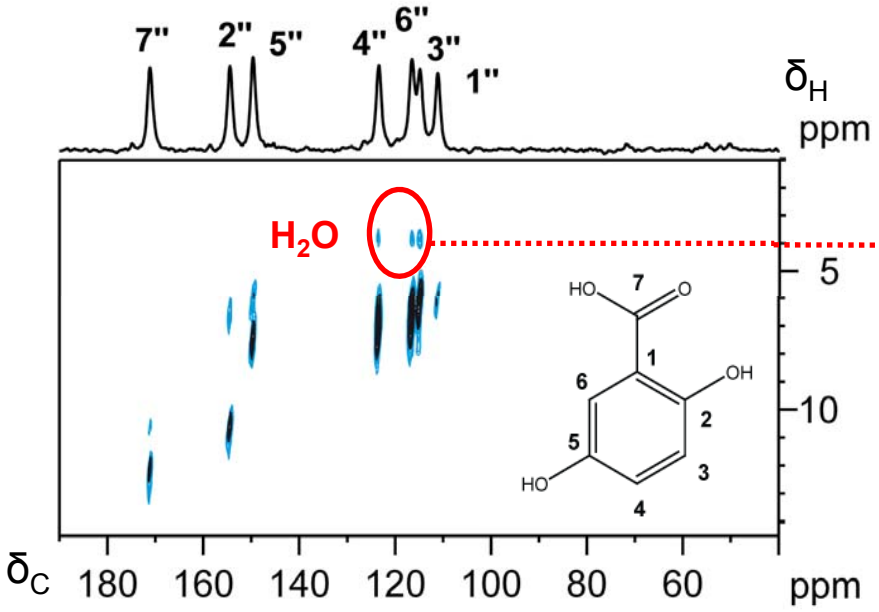
2,5-DHB/PEG/CsCl
ground for 16 min



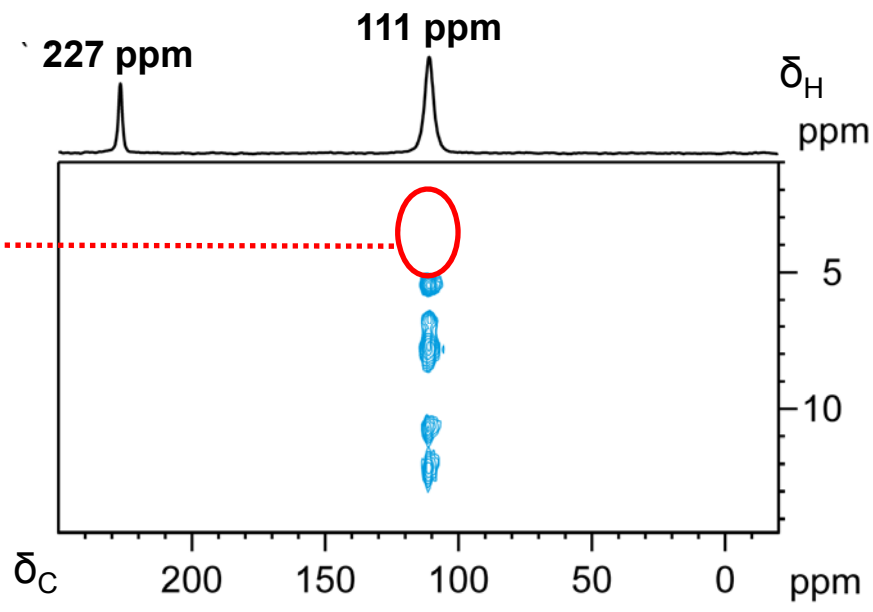


2D NMR dipolar correlation experiments

- **1 sample:** 2,5-DHB/CsCl (1:1) ground for 16 min ↔ **Phase III**
- **2 experiments:** ➢ ***short distance interactions: < 3 Å***
  ➢ ***long distance interactions: < 10 Å***



^{1}H-^{13}C HETCOR



^{1}H-^{133}Cs HETCOR

Water molecules are components of Phase III

Y Major, H Pizzala, F Ziarelli, T Phan, G Mollica, L Charles. *Anal. Meth.* 2012, *in press.* DOI: 10.1039/C2AY25708D

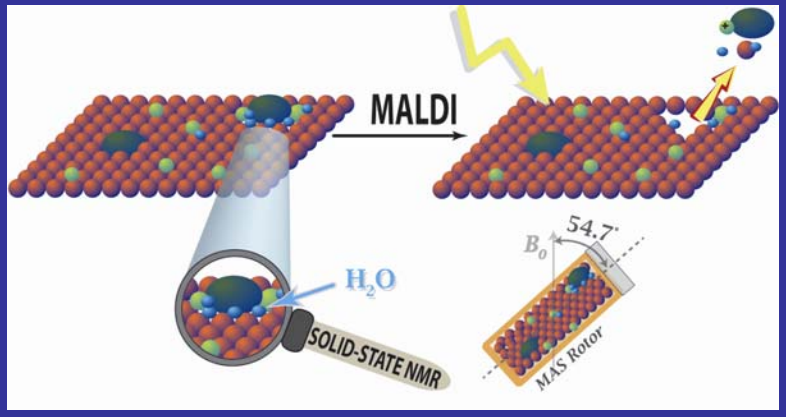
Conclusions

Solid-state NMR provides essential information to understand the molecular origin of changes observed in MALDI mass spectra as a function of experimental conditions used for sample preparation.

Next experiments

- **the influence of the atmospheric environment could be rationalized**
- **solvent-based preparation**

Solid-state NMR for MALDI



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