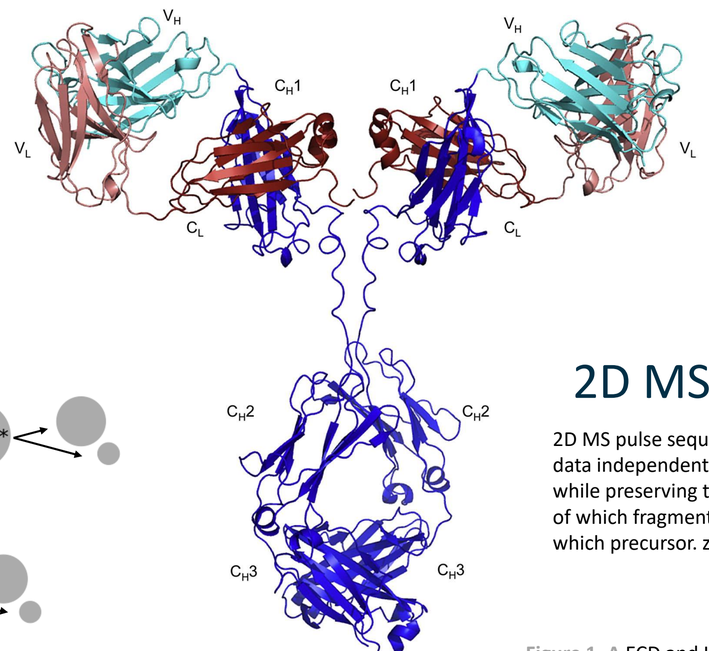
2D FT-ICR MS/MS analysis of IgG1

Johanna Paris[1], Tomos E. Morgan[1], Yuko P. Y. Lam[1], Christopher A. Wootton[1], Mark André Delsuc[2], Mark P. Barrow[1], John O'Hara[3], and Peter B. O'Connor[1]

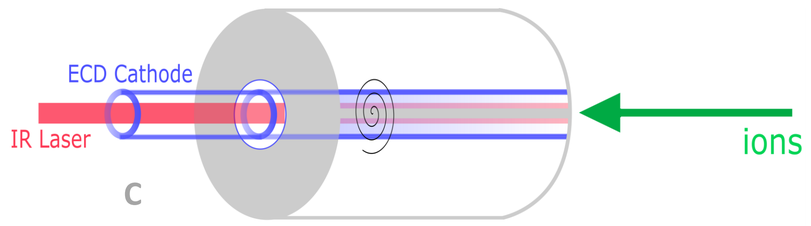
1. ICR lab, University of Warwick, CV4 7AL, Coventry, UK 2. 3IGBMC, Illkirch-Graffenstaden, France 3. UCB, SL1 3WE, Slough, UK

Antibody (IgG1)

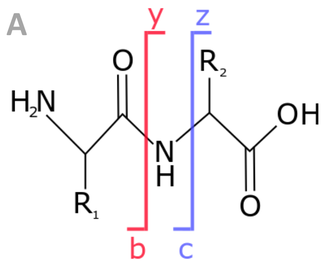
Recombinant monoclonal antibodies and derivatives are widely used as therapeutic drugs. They are susceptible to post-translational modifications that could occur during the manufacturing process and storage, resulting in product-related impurities. PTMs can change the efficacy, toxicity, or the clearance of the antibody; therefore they need to be well monitored.



12T FT-ICR MS/MS

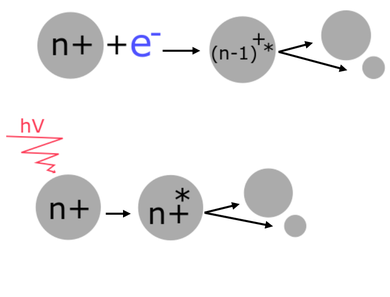


ECD and IRMPD fragmentations



Electron Capture Dissociation
Ions capture a free electron. The liberation of the electric potential energy result in fragmentation (c and z).

Infrared Multiphoton Dissociation
Ions are heated by a CO2 laser. The sequential absorption of photons leads to dissociation. Fragments follow the lowest energy pathway (b and y).



2D MS

2D MS pulse sequence allows data independent acquisition while preserving the informatior of which fragment derives from which precursor. z

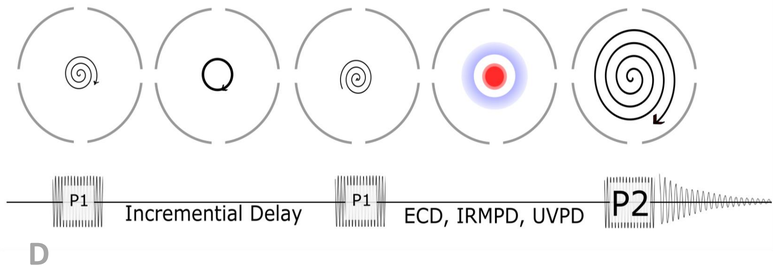
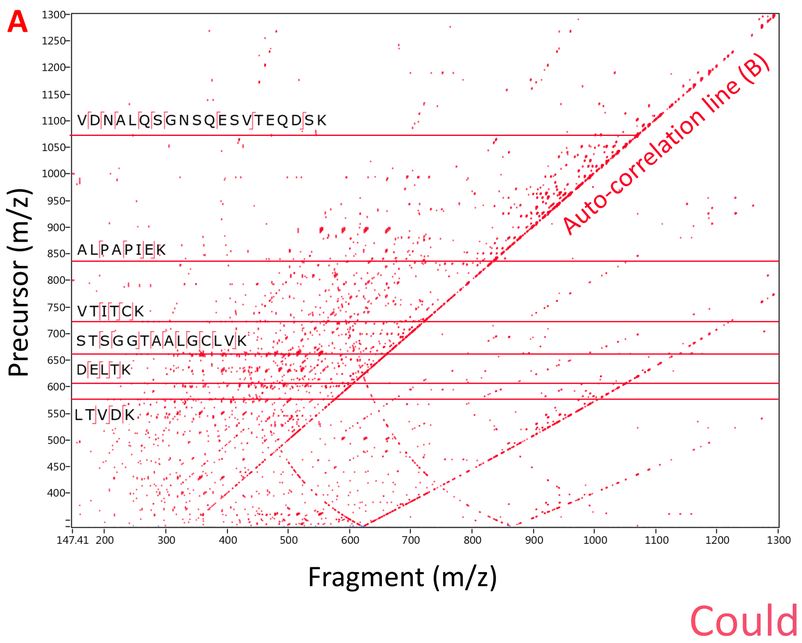


Figure 1. A ECD and IRMPD fragmentation techniques **B** Antibody structure (IgG1) Rouet et Al, 2014 **C** ICR cell design **D** 2D MS Pulse sequence

How can 2DMS improve the analysis of the tryptic digest of IgG1?



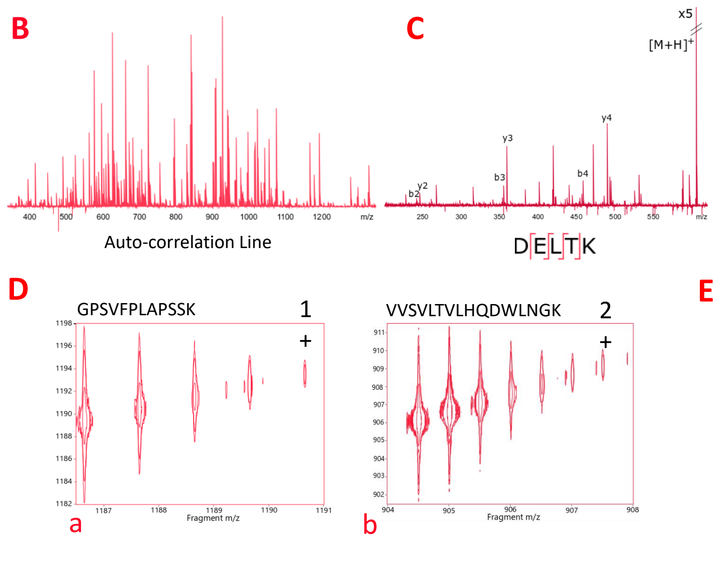


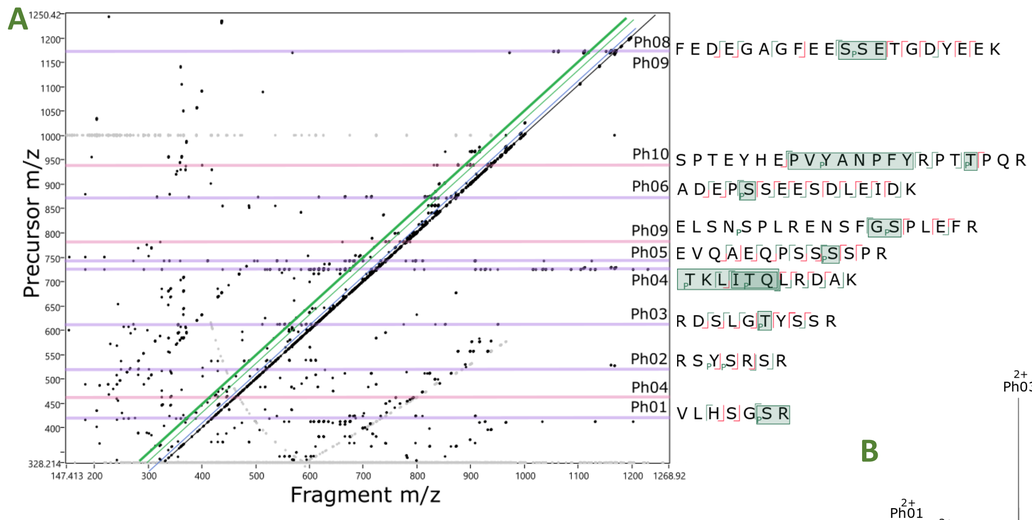
Figure 2. A 2D FT-ICR spectra IRMPD of the IgG1 tryptic digest, 8192 lines, 1 MW (20 bit) **B** Auto-correlation line (showing precursors) **C** 2D extracted precursor line, DELTK peptide. **D** Zooms in the 2D spectra (showing isotope resolution) **a** 1+ charge precursor **b** 2+ charge precursor. **E** Results. Found peptides in Purple. The sequence coverage is 71%. The cleavage coverage is 34%. The cleavage coverage on the found peptides is 48%.

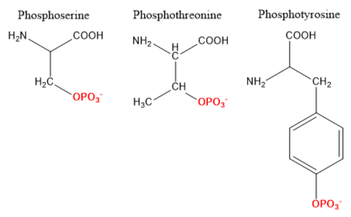
E

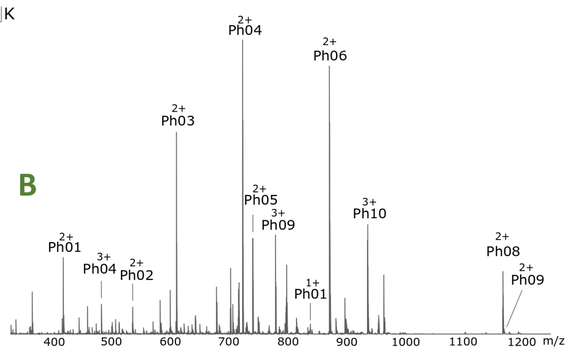
N-term
1 D I Q M T Q S P S S L S A S V G D R V T I T **C** K A 25
26 S Q N V R T V V A W Y Q Q K P G K A P K T L I Y L 50
51 A S N R H T G V P S R F S G S G S G T D F T L T I 75
76 S S L Q P E D F A T Y F **C** L Q H W S Y P L T F G Q 100
101 G T K V E I K R T V A A P S V F I F P P S D E Q L 125
126 K S G T A S V V **C** L L N N F Y P R E A K V Q W K V 150
151 D N A L Q S G N S Q E S V T E Q D S K D S T Y S L 175
176 S S T L T L S K A D Y E K H K V Y A **C** E V T H Q G 200
201 L S S P V T K S F N R G E **C** 214
C-term

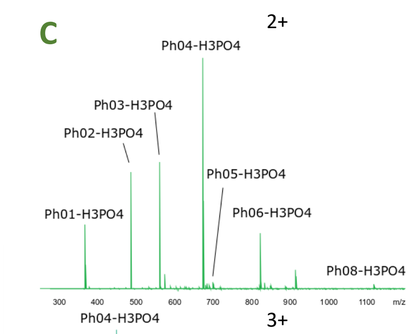
N-term
1 E V Q L V E S G G G L V Q P G G S L R L S **C** A A 25
26 S G F A F S T Y D M S W V R Q A P G K G L E W V A T 50
51 I S S G G S Y T Y Y L D S V K G R F T I S R D S S 75
76 K N T L Y L Q M N S L R A E D T A V Y Y **C** A P T T 100
101 V V P F A Y W G Q G T L V T V S S A S T K G P S V 125
126 F P L A P S S K S T S G G T A A L G **C** L V K D Y F 150
151 P E P V T V S W N S G A L T S G V H T F P A V L Q 175
176 S S G L Y S L S S V V T V P S S S L G T Q T Y I **C** 200
201 N V N H K P S N T K V D K K V E P K S **C** D K T H T 225
226 **C** P P **C** P A P E L L G G P S V F L F P P K P K D T 250
251 L M I S R T P E V T **C** V V V D V S H E D P E V K F 275
276 N W Y V D G V E V H N A K T K P R E E Q Y **N** S T Y 300
301 R V V S V L T V L H Q D W L N G K E Y K **C** K V S N 325
326 K A L P A P I E K T I S K A K G Q P R E P Q V Y T 350
351 L P P S R D E L T K N Q V S L T **C** L V K G F Y P S 375
376 D I A V E W E S N G Q P E N N Y K T T P P V L D S 400
401 D G S F F L Y S K L T V D K S R W Q Q G N V F S **C** 425
426 S V M H E A L H N H Y T Q K S L S L S P G K 447
C-term

Could 2DMS be a tool for Proteomics and PTM investigation?









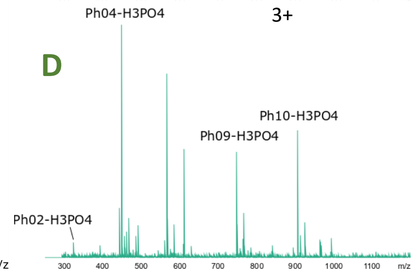
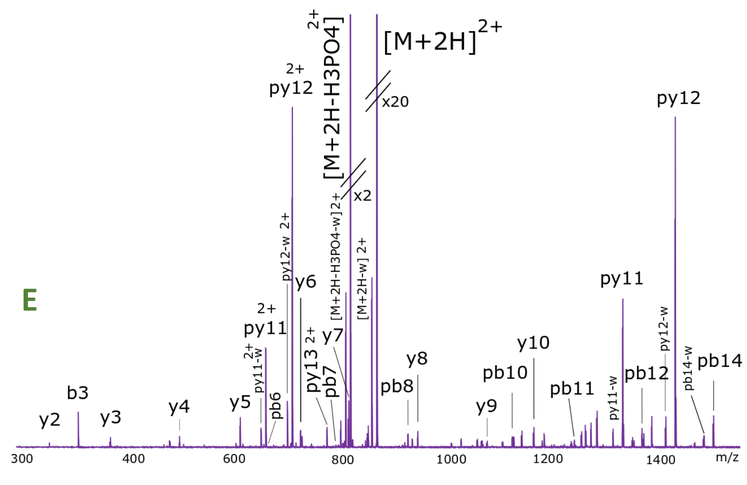


Figure 3. A 2D FT-ICR spectra IRMPD of the Phosphomix, 8192 lines, 1 MW (20 bit) **B** Auto-correlation line (showing precursor) **C** Phosphate Neutral loss Line 2+ **D** Phosphate Neutral loss Line 3+. The neutral loss lines allow the quick identification of the phosphopeptides. **E** 2D extracted precursor line, ADEP$_p$SSEESDLEIDK peptide.

The P-O stretch of the phosphate (9.6-11µm) is in resonance with the CO2 laser of the IRMPD (10.6µm). IRMPD has the ability to selectively photodissociate phosphorylated peptide, producing a phosphate neutral loss. 2D MS allows the extraction of the neutral losses lines and therefore, the detection of the phosphopeptides.



Could 2DMS be used for Top Down Analysis?

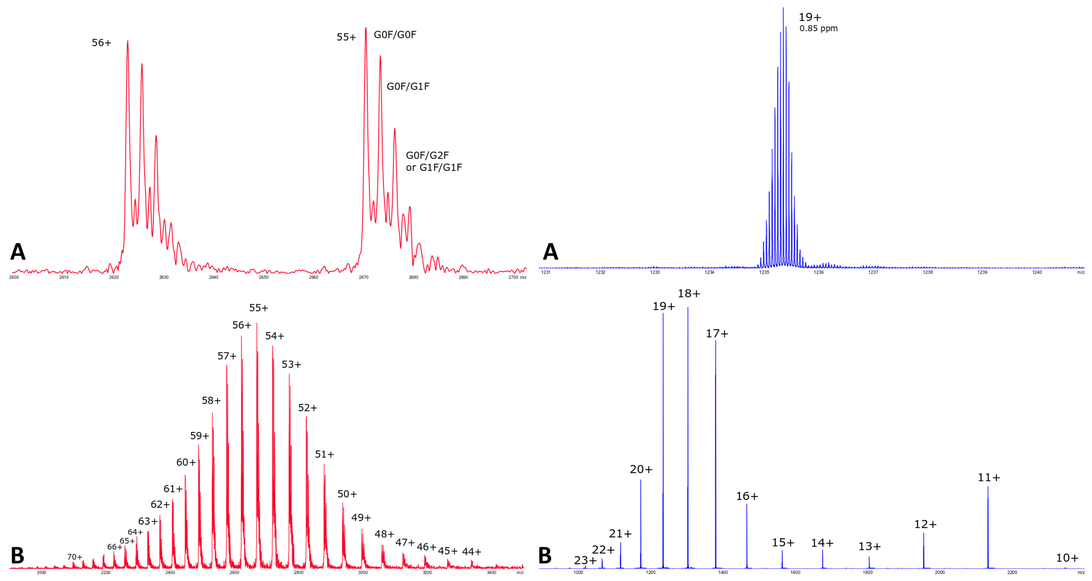


Figure 4. FullMS of IgG1 (150kDa), 50:50 ACN:Water +0.1%FA, 1µM, 147-6000m/z, Q1 1200m/z, 1000 scans, 262k.
A Details **B** Full Spectra

Figure 5. FullMS of the light Chain (25kDa), 1:3 ACN:Water +0.1%FA, 2µM, 147-4000m/z, Q1 1000m/z, 50 scans, 8M.
A Details **B** Full Spectra

MS/2D MS

The intact protein is fragmented in the quadrupole by ETD or CAD and then the fragments are analysed by 2DMS by ExD or IRMPD.

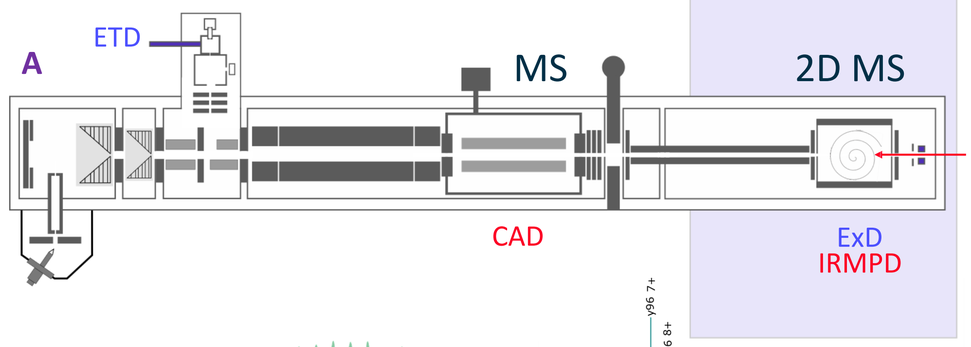
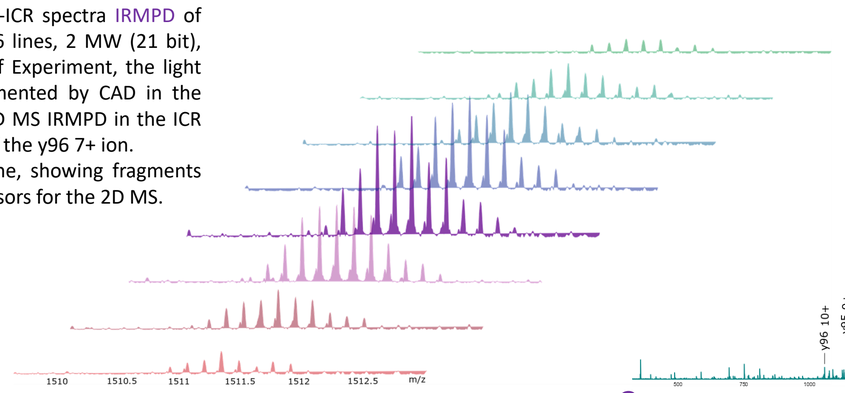
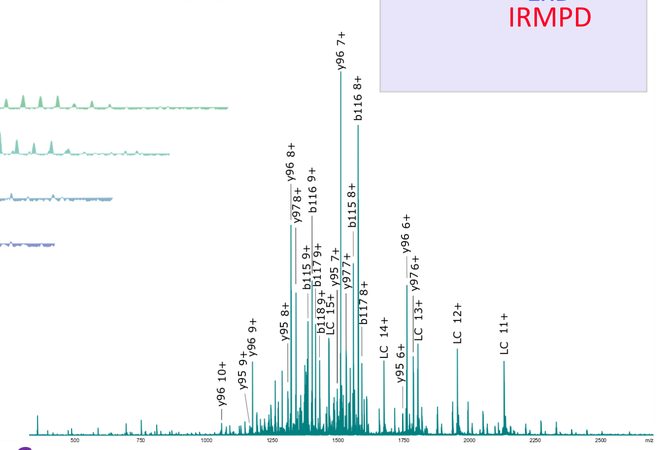


Figure 6. 2D CAD FT-ICR spectra IRMPD of the Light Chain, 4096 lines, 2 MW (21 bit), CAD 25V **A** Design of Experiment, the light chain was first fragmented by CAD in the front end, then by 2D MS IRMPD in the ICR cell. **B** Modulation of the y96 7+ ion.
C Auto-correlation line, showing fragments from the CAD, precursors for the 2D MS.





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Conclusion

- 2D MS is a useful tool for the analysis of bottom up mixtures, and could permit to identify and locate PTMs.
- With 2D MS and IRMPD, it is possible to selectively analyse phosphopeptides.
- MS/2D MS offers a new approach to top down proteomics.
- The 2D MS technique offers an alternative to MS/MS with a different set of limitations.

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