Facile determination of phosphorylation sites in peptides using two-dimensional mass spectrometry

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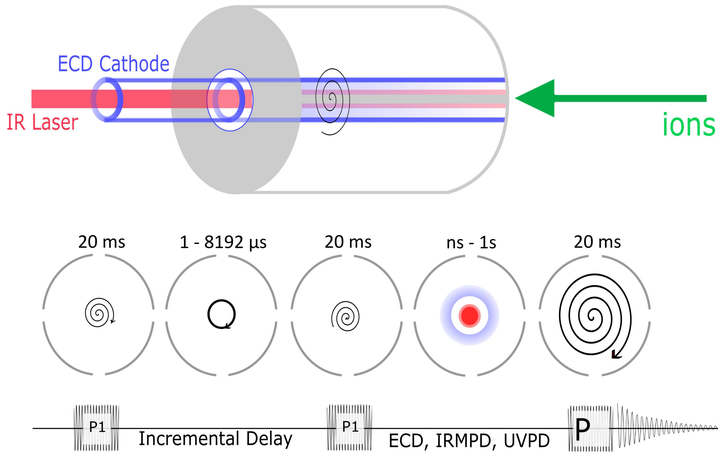
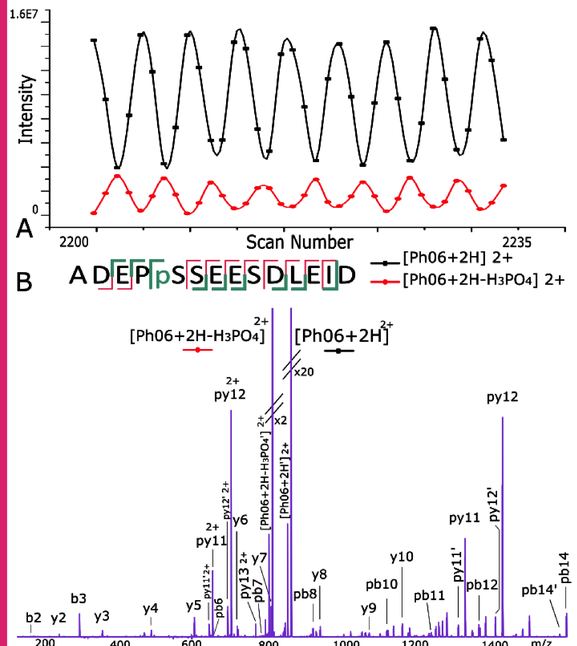
The two-Dimensional Approach



Schema of the ICR cell[1,2], 2DMS sequence pulse[3]; in red IRMPD laser (CO_2, continuous wave, 25W), in blue ECD electrons, P excitation pulse.

2DMS enables the data independent analysis of a mixture of phosphopeptides without quadrupole isolation or prior separation. A different sequence pulse in the ICR cell allows the modulation in space of the precursors depending of their m/z. Spatially resolved in-cell fragmentation transforms the modulation of space in modulation of intensities of fragments and precursors, at the same frequency but out of phase, allowing identification of which fragment is from which precursor.

Fragmentation of Precursors



Phosphopeptide Ph06 A. Modulation of intensities of precursor and phosphate loss fragment. B. Extracted 2DMS fragment line. ‘: water, p: fragment with phosphate, green (bold): b/y fragment with phosphate, red: b/y fragment without a phosphate group.

InfraRed Multiphoton Dissociation is a slow heating method where the molecule absorbs photons until it breaks.[4,5] The IR radiation is resonant with vibrational mode of the phosphate group. Phosphorylated peptides fragments at a broader range of photon energy, and in IRMPD (10.6 µm) at a lower threshold than their unphosphorylated counterpart.[6,7,8]

2DMS fragments simultaneously all precursors, by computing the modulation of intensities, each fragment is assigned to a precursor, and it is possible to extract for each precursor, its fragment line.

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[1] Amster, I. J. *J. Mass Spectrom.* **1996,** *31* (12), 1325-1337. [2] Tsybin, Y. O.; Witt, M.; Baykut, G.; Kjeldsen, F.; Håkansson, P., *Rapid Commun. Mass Spectrom.* **2003,** *17* (15), 1759-1768. [3] Pfändler, P.; Bodenhausen, G.; Rapin, J.; Houriet, R.; Gäumann, T., *Chem. Phys. Lett.* **1987,** *138* (2-3), 195-200. [4] Little, D. P.; Speir, J. P.; Senko, M. W.; O'Connor, P. B.; McLafferty, F. W. *Anal. Chem.* **1994**, *66*, 2809-2815. [5] Talebpour, A.; Bandrauk, A.; Yang, J.; Chin, S. *Chem. Phys. Lett.* **1999**, *313*, 789-794. [6]Correia, C. F.; Balaj, P. O.; Scuderi, D.; Maitre, P.; Ohanessian, G. *J. Am. Chem. Soc.* **2008**, *130*, 3359-3370. [7] Flora, J. W.; Muddiman, *Anal Chem.* **2001**, 73 (14), 3305-3311. [8] Flora, J. W.; Muddiman, *JACS* **2002**, 124 (23), 6546-6547. [9] van Agthoven, M. A.; Kilgour, D. P.; Lynch, A. M.; Barrow, M. P.; Morgan, T. E.; Wootton, C. A.; Chiron, L.; Delsuc, M.-A.; O'Connor, P. B. *J. Am. Soc. Mass. Spectrom.* **2019**, *30*, 2594-2607.

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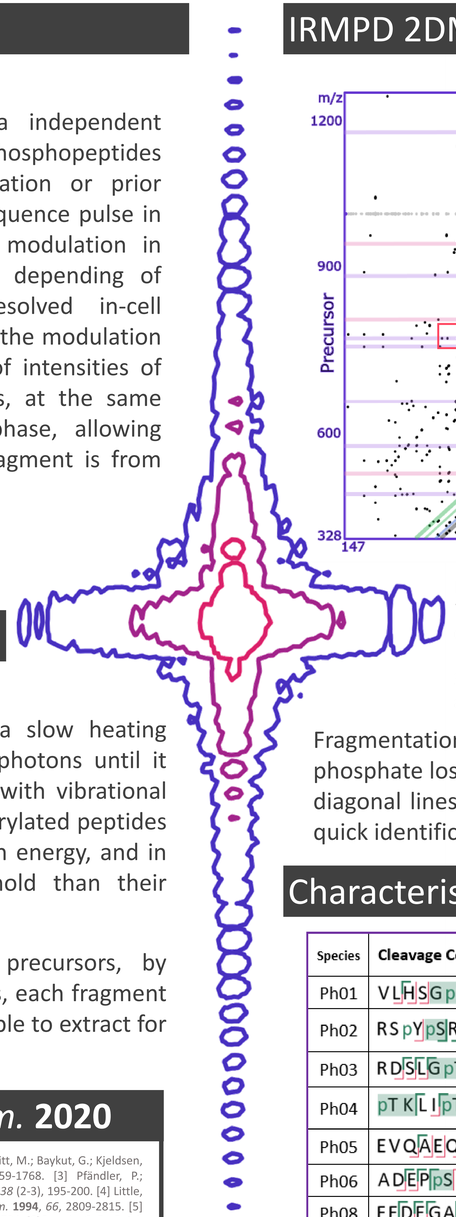
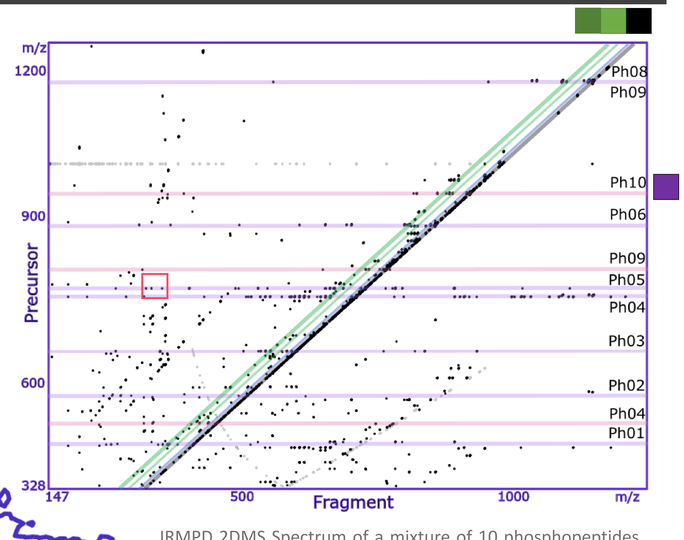


IRMPD 2DMS Spectrum



IRMPD 2DMS Spectrum of a mixture of 10 phosphopeptides. The sample was sprayed by direct infusion with a pulled glass capillary into a 12 T FT-ICR Solarix. 8192 scans of 1MW (16-bit) data points were acquired over a mass range of *m/z* 328-3000 on the vertical axis, and *m/z* 148-3000 on the horizontal axis. In purple: 2+ species, pink: 3+ species.

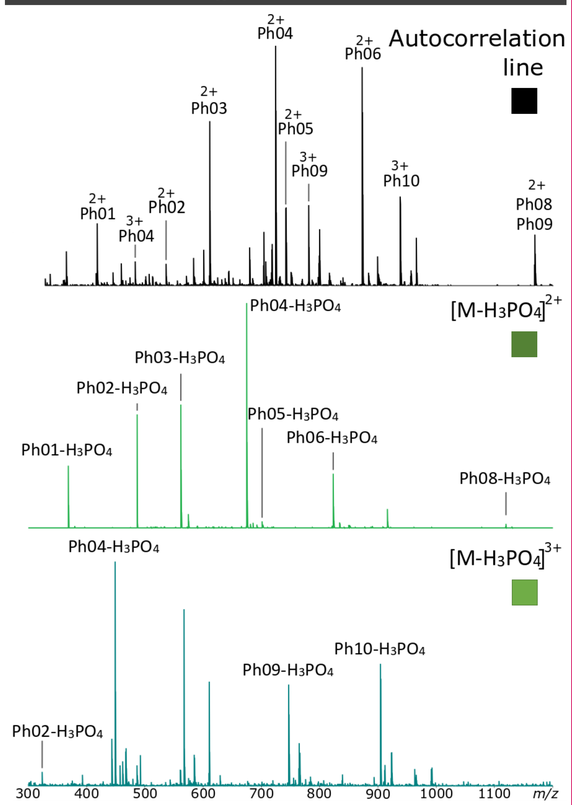
Fragmentation of phosphopeptide by IRMPD produces a phosphate loss fragment. With 2DMS, it is possible to extract diagonal lines and therefore neutral loss lines. It allows the quick identification of all phosphopeptides in the mixture.

Characterisation of Phosphopeptides

Species	Cleavage Coverage		Phosphate sites & comments
Ph01	VLHSGpSR	50%	GSR
Ph02	RSpYpSRSR	50%	S. Loss of the phosphate at Y in b3, pb4 and pb5
Ph03	RDSLGpTYSSR	67%	GT
Ph04	pTKLIpTQLRDAK	70%	TK & TQ Loss of phosphate at b4
Ph05	EVQAEQPSSpSSPR	75%	S
Ph06	ADEPpSSEESDLEID	92%	S
Ph08	FEDEGAGFEESpSETGDYEEK	63%	SSE
Ph09	ELSNpSPLRENSFGpSPLEFR	22%	ELSNSPLRENDF & GS
Ph10	SPTEYHEPVpYANPFYRPTpTPQR	24%	PVYANPFY & T

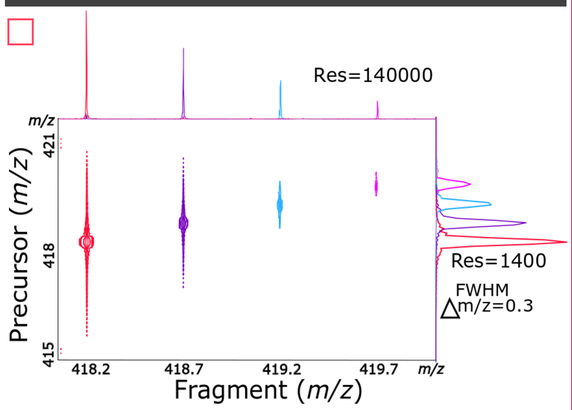
IRMPD 2DMS allows the simultaneous fragmentation of precursors in a complex mixture, the quick identification of phosphopeptides and the localisation of phosphorylation sites.

Extracted diagonal lines



Extracted diagonals lines of 2DMS. Auto-correlation line showing all detected precursors (similar to a 1D MS spectrum). Extracted 2+ and 3+ Phosphate Neutral (H_3PO_4) loss line showing all phosphopeptides.

2DMS Resolution



Zoom of $[Ph01+2H]^{2+}$. The resolution is around 140 000x1400 at 420 *m/z*.