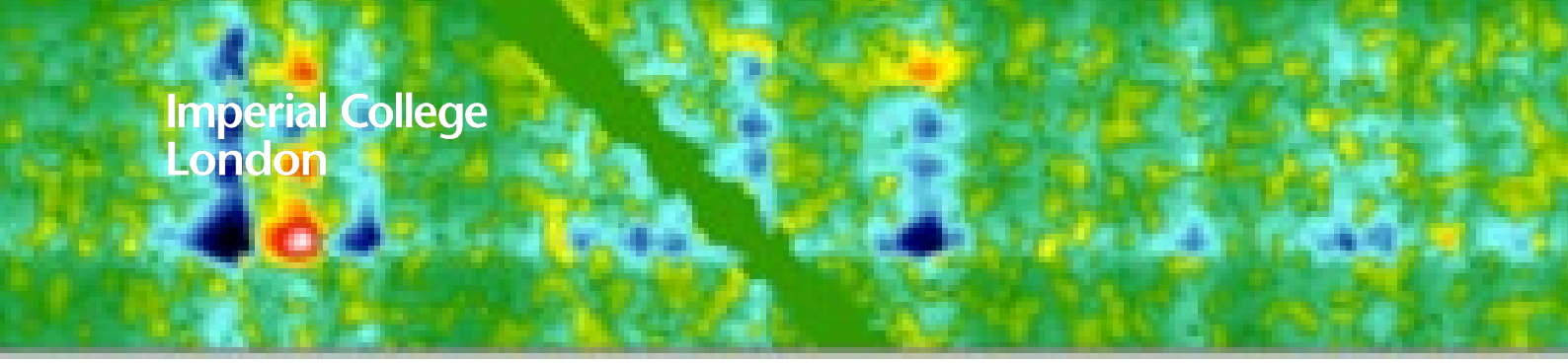

Partial Covariance 2D Mass Spectrometry

A software package to uncover "hidden" structurally relevant signals that are below the ad hoc intensity thresholds in conventional MS.

Proposed use

Partial covariance 2D mass spectrometry is the first of its kind and has the potential to revolutionise not only the field of proteomics, but biomolecular mass spectrometry in general. It is not limited to a specific apparatus (e.g. ion trap or TOF), activation technique (e.g. CID, ECD/ETD, etc.) or biomolecular structure (e.g. peptide, protein, oligonucleotide, etc.).

The 2D MS technology produces spectral signatures that are more specific than 1D MS spectra can be, even at theoretical infinite mass precision and resolution (see Figure 2) and is well suited for structurally challenging molecules such as isomers. The invention is fully compatible with top-down experiments as shown in Figure 3 by using the pC-2DMS software to extract sequence identities of co-fragmented proteins from a single 'chimera' 2D map. The fragment-fragment connectivity data uniquely provided by the pC-2DMS are expected to strongly enhance the capability of de novo sequencing in MS.

Problem addressed

1D mass spectrometry (MS) often fails in assigning experimental proteomic MS and tandem MS (MS/MS) data to correct peptide sequences despite its remarkable success in identification and quantification of proteins. Some high biological importance problems, such as identifying mixtures of combinatorially modified peptides, cannot be solved by the state of the art 1D MS.

The partial covariance 2D mass spectrometry (pC-2DMS) for the first time gives access to an entirely new type of structure-specific information: the connectivity between biomolecular fragments detected in tandem MS through covariance correlations between structurally linked pairs. Knowing this connectivity gives a vital advantage for solving the peptide sequence puzzle by piecing the fragments together.

Technology overview

The principle of the covariance 2D mass spectrometry is shown in Figure 1. The 2D MS approach provides direct experimental access to fragment-fragment correlations thereby revealing how these fragments originated, even in the case of very low abundance structural signals.

Using our pC-2DMS analysis software suite, biomolecular sequences are identified by matching the experimentally measured fragment-fragment correlations to 'theoretical' pairs of fragments, generated by computer simulation based on protein databases and fragmentation rules. This targeted sequence-specific fragment connectivity strategy can therefore reduce ambiguity in peptide identification dramatically.

Intellectual property information

Applied patents: EP3513338A2, US20190206509A1 and GB201803940D0

Benefits

- Solves structural problems which cannot be solved by the standard 1D MS as a matter of principle. Demonstrated in Figure 2.
- Extracts sequence from highly congested spectra of intractable complexity for 1D MS/MS. Demonstrated in Figure 3.
- Uncovers "hidden" structurally relevant signals that are below the ad hoc intensity thresholds and have to be disregarded in conventional MS.
- Implemented as a software to offer a standalone measurement-to-sequence identification package. Fully compatible with commercial MS instrumentation software.
- Does not require any hardware modifications and can simply be used on a standard mass spectrometer. The technique has been demonstrated using a linear ion trap mass spectrometer.

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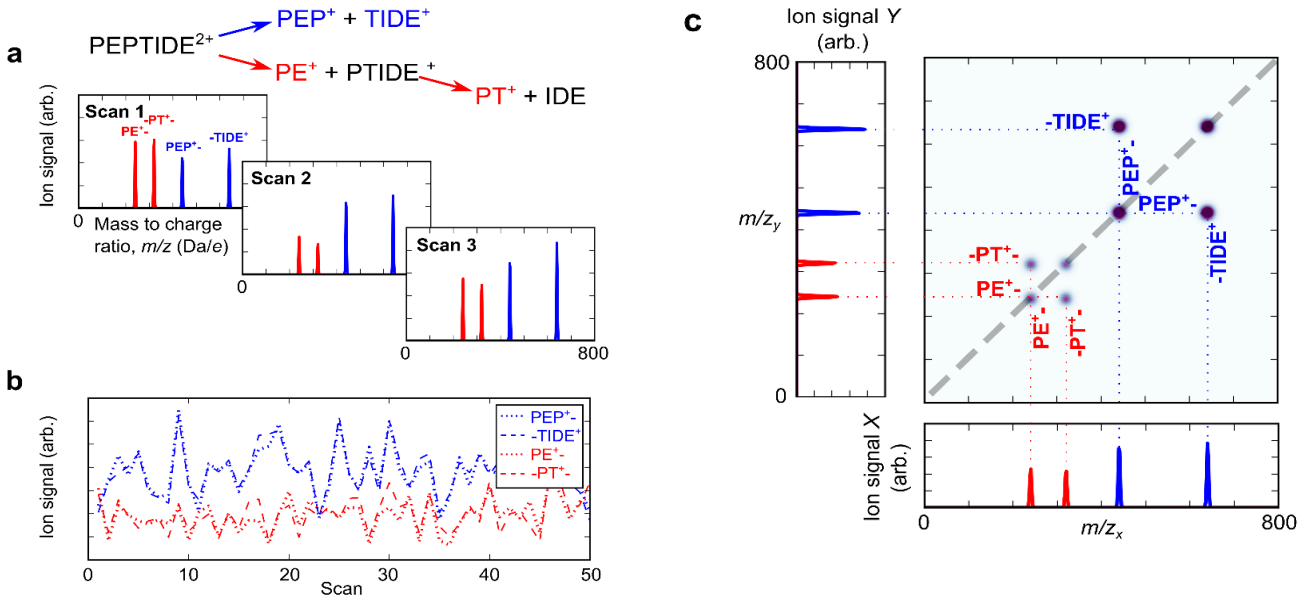

Figure 1 | The principle of covariance 2D mass spectrometry. (a) A computer records fragment mass spectra ("scans") of a biomolecular ion "PEPTIDE^{2+}", which dissociates along two pathways: blue and red. (b) Due to the statistical nature of the dissociation processes, the ion signal of each kind of fragment fluctuates randomly from scan to scan, but these signals are positively correlated for fragments formed in the same (blue) or consecutive (red) dissociation processes. (c) Calculating the covariance between all pairs of signals gives us a map of fragment origins – effectively the technique automatically reassembles the fragments in a pairwise manner. The fragment connectivity is used for spectrum-to-structure assignments, in addition to the fragment m/z ratio and the relative abundance available from 1D MS.

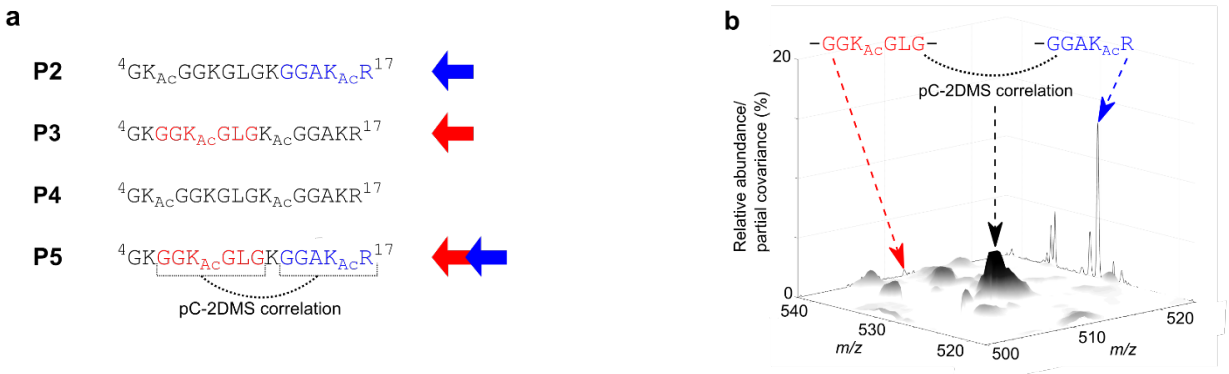

Figure 2 | Distinguishing between combinatorially modified histone peptides. (a) In an arbitrary mixture of fragmented diacetylated isomers P2-P5, the Lys-8, Lys-16 diacetylated peptide P5 has no unique 1D marker fragments, enabling the detection of this isomer in the mixture by 1D MS. For example, each of the highlighted red and blue fragments of P5 can be generated by more than one of the co-fragmented isomers. However, the connectivity between these fragments revealed by pC-2DMS is unique to P5 and represents just one of many isomer-specific marker ion correlations of the peptide. (b) 3D view of a region of the experimental 2D MS map of a mixture of triply protonated P2–P5 exhibiting the unique correlation of P5. The corresponding segments of the averaged 1D spectrum, featuring the individual non-unique 1D fragments, are plotted against the back walls of the map.

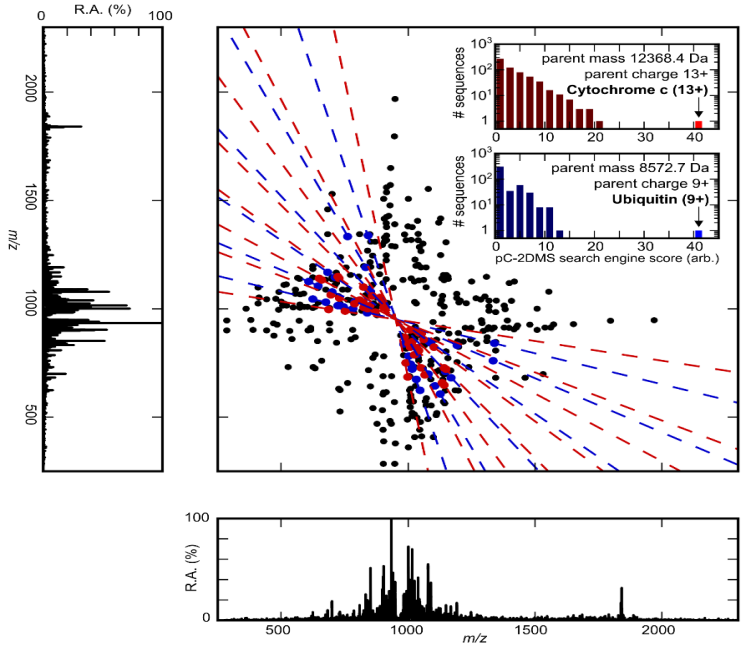

Figure 3 | Top down pC-2DMS. The co-fragmentation of intact proteins cytochrome c and ubiquitin produces an intractably complex 1D MS/MS spectrum. Thanks to the 2D nature of pC-2DMS, our computer vision algorithm straightforwardly pulls out two individual sets of connected fragment signals from the pC-2DMS map (blue and red). These are individually fed to the sequence identification software, which confidently identifies the two co-fragmented protein sequences. This measurement was performed using a linear ion trap MS.

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