

# Two-Dimensional Partial Covariance Mass Spectrometry for Macromolecular Decompositions

Marina Edelson-Averbukh<sup>1</sup>, Vitali Averbukh<sup>1</sup>, Taran Driver<sup>1</sup>, Rüdiger Pipkorn<sup>2</sup>, Serguei Patchkovskii<sup>3</sup>, Jon Marangos<sup>1</sup>, Lezsek Frasinski<sup>1</sup>

*1. Department of Physics, Imperial College London, Prince Consort Rd. London SW7 2AZ UK*

*2. German Cancer Research Centre, Department of Translational Immunology, INF 580, 69120 Heidelberg, Germany*

*3. Max Born Institute for Nonlinear Optics and Short Pulse Spectroscopy, Max-Born-Straße 2A, 12489 Berlin, Germany*

**Abstract:** The new spectroscopic technique of self-correcting partial covariance allows us for the first time to uncover peptide and protein fragment-fragment correlations leading to the biomolecular sequence reconstruction of unparalleled fidelity.

Two-dimensional covariance mapping spectroscopy was originally developed as a tool for the study of mechanisms of radiation-induced fragmentation of di- and tri-atomic molecules [1]. Successful applications of covariance mapping have so far been restricted to low mass systems of up to around 50 daltons. The main problem arising in applications of covariance mapping stems from the overwhelming spurious signals of no physical significance induced by fluctuations of experimental parameters. Partial covariance mapping attempts to tackle this problem by taking into account the independently measured parameter fluctuations. However, for macromolecules this becomes unfeasible because of their complex methods of gas-phase delivery and activation that result in a rich manifold of fluctuating experimental parameters, precluding identification of the true physical correlations.

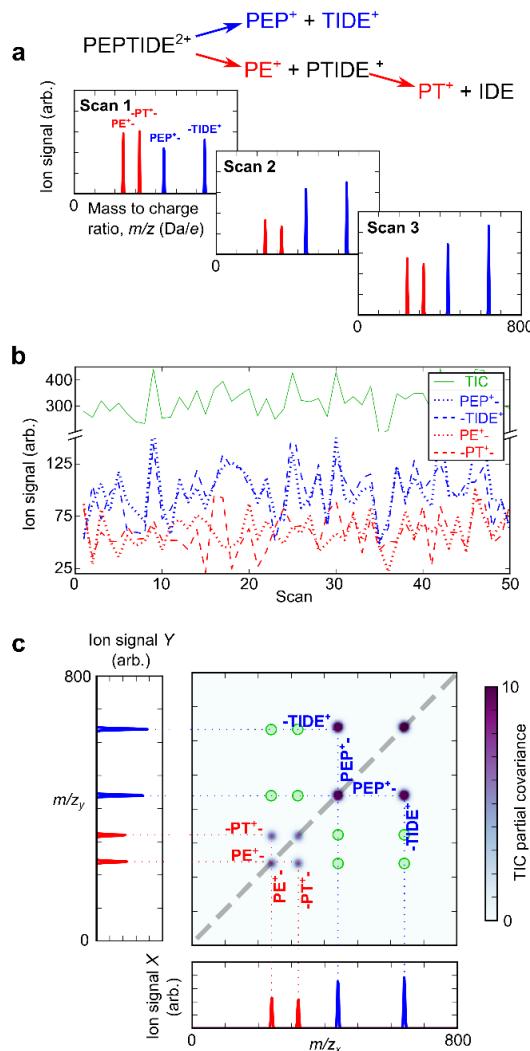
We have recently pioneered the self-correcting partial-covariance spectroscopy (Fig. 1) which eliminates the need for continuous monitoring of multiple fluctuating experimental parameters enabling covariance mapping of macromolecular decompositions [2]. The self-correcting partial covariance spectroscopy removes the spurious correlations using a single parameter extracted from the spectrum itself – the total ion count (TIC):

$$pCov(X, Y, TIC) = Cov(X, Y) - \\ Cov(X, TIC)Cov(Y, TIC) / Cov(TIC, TIC).$$

The TIC-based self-correcting partial covariance spectroscopy has been shown to be false-positive-free. It leads to successful mapping of the true physical fragment-fragment correlations for decompositions of molecules as large as  $10^3$ - $10^4$  Da. This opens the opportunity for mechanistic studies of macromolecular decompositions using covariance mapping. We demonstrate that the self-correcting partial covariance also has an unparalleled analytical capability enabling the high-fidelity reconstruction of

## References

- [1] L. J. Frasinski, K. Codling, and P. A. Hatherly, *Science* **246**, 1029 (1989).
- [2] T. Driver *et al.*, *Phys. Rev. X* **10**, 041004 (2020).



**Fig. 1** Schematic representation of the TIC-based self-correcting partial covariance spectroscopy.

a biomolecular sequence within two-dimensional partial-covariance mass spectrometry (2D-PC-MS).