Supporting Information

Increasing analytical separation and duty cycle with non-linear analytical scan functions in TIMS-FT-ICR MS

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EXPERIMENTAL SECTION

Materials and Reagents

A Tuning Mix calibration standard (G24221A) was obtained from Agilent Technologies (Santa Clara, CA) and used as received. Peptides DFTPAELR and TTILQSTGK were added at 10 μM to an MCF-7 digest at 2.68 μg/gl and 10% of 0.1% methanol:formic acid.

TIMS analysis

The concept behind TIMS is the use of an electric field to hold ions stationary against a moving gas, so that the drag force is compensated by the electric field and ion packages are separated across the TIMS analyzer axis based on their mobility; during mobility separation, a quadrupolar field confines the ions in the radial direction to increase trapping efficiency[29-31]. The mobility, K, of an ion in a TIMS cell is described by:

$$K = \frac{v_g}{E} \cong \frac{A}{(V_{elution} - V_{out})} \quad (1)$$

where v_g, E, $V_{elution}$ and V_{out} are the velocity of the gas, applied electric field, elution voltage and tunnel out voltage, respectively. TIMS separation was performed using nitrogen as a bath gas at *ca.* 300 K, P_1 = 2.2-2.47 and P_2 = 0.9 mbar, and a constant V_{out} = 50 V and RF (840 kHz and 240-260 Vpp) in all electrodes of the entrance funnel, mobility separating section and exit funnel. Details regarding the construction and gating of the TIMS has been described previously[35]. Mobility spectra were calibrated using a Tuning Mix calibration standard (Tunemix, G2421A, Agilent Technologies, Santa Clara, CA) with the *m/z* 622 K_0=1.008, *m/z* 922 K_0=0.826, *m/z* 1222 K_0=0.711 , *m/z* 1522 K_0=0.632 $cm^2 V^{-1}s^{-1}$ values. Mobilities were correlated with CCS (Ω) using the equation:

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{z}{(k_B T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_b} \right]^{1/2} \frac{1}{K} \frac{760}{P} \frac{T}{273.15} \frac{1}{N^*} \quad (2)$$

where z is the charge of the ion, k_B is the Boltzmann constant, N^* is the number density and m_I and m_b refer to the masses of the ion and bath gas, respectively.[47] Under these conditions, the experimental TIMS resolving power for Tuning Mix (*m/z* 622-1522) was ~100-300 as determined by equation 3

$$R = \frac{\Omega}{\Delta\Omega} \qquad (3)$$

where Ω is the center of the CCS peak and ΔΩ is the full width at half maximum of the fit gaussian peak. The this work the TIMS duty cycle is defined as the amount of TIMS ramp time that is sampled by the FT-ICR MS. This was calculated using equation 4 by dividing the width of the gate in time by the total TIMS time.

$$\text{Duty Cycle (\%)} = \frac{\text{Gate width}}{\text{Total TIMS time}} * 100 \quad (4)$$

TIMS-FT-ICR MS analysis

Samples analyzed in positive ion mode with a custom pulled quartz capillary nanoESI source coupled to a custom-built TIMS – FT-ICR MS instrument based on the 7T Solarix FT-ICR MS spectrometer (Bruker Daltonics Inc., MA). Ions from the nanoESI were introduced to the TIMS-FT-ICR MS via a 0.6 mm inner diameter, single-bore resistive glass capillary tube, allowing the nebulizer to be maintained at ground potential, while the ends of the capillary can be independently biased. Typical nanoESI operating conditions were 900-1400 V at the capillary entrance while the capillary was maintained at ground. FT-ICR MS ion optics were optimized as follows: -900 V endcap source capillary voltage, 180 V endcap TIMS capillary voltage, 5kHz 100 Vpp segmented hexapole, 2kHz 1200 Vpp collision cell, and 4kHz 350 Vpp ion guide transfer line. The TIMS-FT-ICR MS experiments were acquired in chromatography mode where each scan was a single 1-8 Megaword (0.5-4 second) transient, which was zero-filled to 2-16Megaword, processed using a sine-squared apodization followed by fast-Fourier transform (FFT) in magnitude mode with an experimental resolving power of 50,000-550,000 at *m/z* 400.

Data analysis

TIMS-FT-ICR MS spectra were internally mass calibrated using the Tuning Mix standard in Data Analysis (Bruker Daltonics v 4.2). Ion chromatogram traces were exported to OriginPro 2016 (Originlab Co., MA) for each of the tuning mixture masses and a linear calibration from steps to $1/K_0$ was performed. Ion chromatograms for peptides DFTPAELR and TTILQSTGK were extracted with a *m/z* tolerance of ±0.01. Spectra were exported to Biotools (Bruker Daltonics v 3.2) and ProSight Lite (Northwestern University, v1.3) for sequencing processing, matching tolerance was set to 1 ppm.

Table S1. Summary of experimental modes of operation

Mode of operation	Length of ramp	Resolving power	Analytical Scan rate	Experiment length	Duty Cycle	*a priori* knowledge
Linear TIMS	100 ms 100 ms 200 ms 200 ms	70-115 (1 ms gate) 80-120 (0.5 ms gate) 92-140 (1 ms gate) 130-160 (0.5 ms gate)	2 V/s 2 V/s 1 V/s 1 V/s	12 min 30 min	0.5%	No
Non-linear Targeted TIMS	7.5 ms	110-140 200-300	0.66 V/s 0.26 V/s	5 min	0.85%	Yes
Non-linear Stepping function	9 ms 12 ms 15 ms	40-90 95-130 110-160	1 V/s 0.5 V/s 0.3 V/s	11 min 20 min 45 min	4%	No

Figure S1. Labeled IRMPD spectra of peptides (a) DFTPAELR and (b) TTILQSTGK peptides