Supplementary Information

High-performance miniature linear time-of-flight mass spectrometry as an advantageous tool in high mass-to-charge range

Ko-Keng Chang,[a,b] Yi-Hong Cai,[a,c] Chih-Hao Hsiao,[a] Cheng-Chih Hsu,[b] Yi-Sheng Wang*[a]

[a] Genomics Research Center, Academia Sinica, Taipei 115, Taiwan.
[b] Department of Chemistry, National Taiwan University, Taipei 106, Taiwan.
[c] Department of Chemistry, National Taiwan Normal University, Taipei 116, Taiwan.

Contents:

Calculation procedure to find optimal parameters

To determine suitable instrument dimensions and other experimental parameters, the previously developed comprehensive calculation method is employed.[1-3] It involved the optimization of the length of extraction (s_0) and acceleration (d) regions, the electric fields therein, and extraction delay (τ). The most essential part in the calculation is the flight-time spread of two ions, or the $f(\tau)$ (see Equation 2, Ref. 1), in order to calculate precise mass resolving power (MRP). The calculation proceeds through several steps based on the presumptions and starting conditions below:

- The average initial velocity of the desorbed ions is approximately 522 m/s, and they have a Maxwell-Boltzmann distribution. The boundary velocities at the full width at half maximum (FWHM) are approximately 167 and 878 m/s.
- Define voltage range of every electrode to be calculated. Practical considerations include the voltage rating of HV feedthroughs, the dielectric property of the spacers between electrodes, vacuum condition, and voltage range of HV components. In this work, the bias voltage of the sample electrode is mainly kept at 15 kV, and the extraction voltage is set to 2 – 6 kV.
- Define range of s_0 and d to be calculated. Practical considerations include space limitation and field homogeneity. In this work, both s_0 and d are set to 3 – 10 mm.

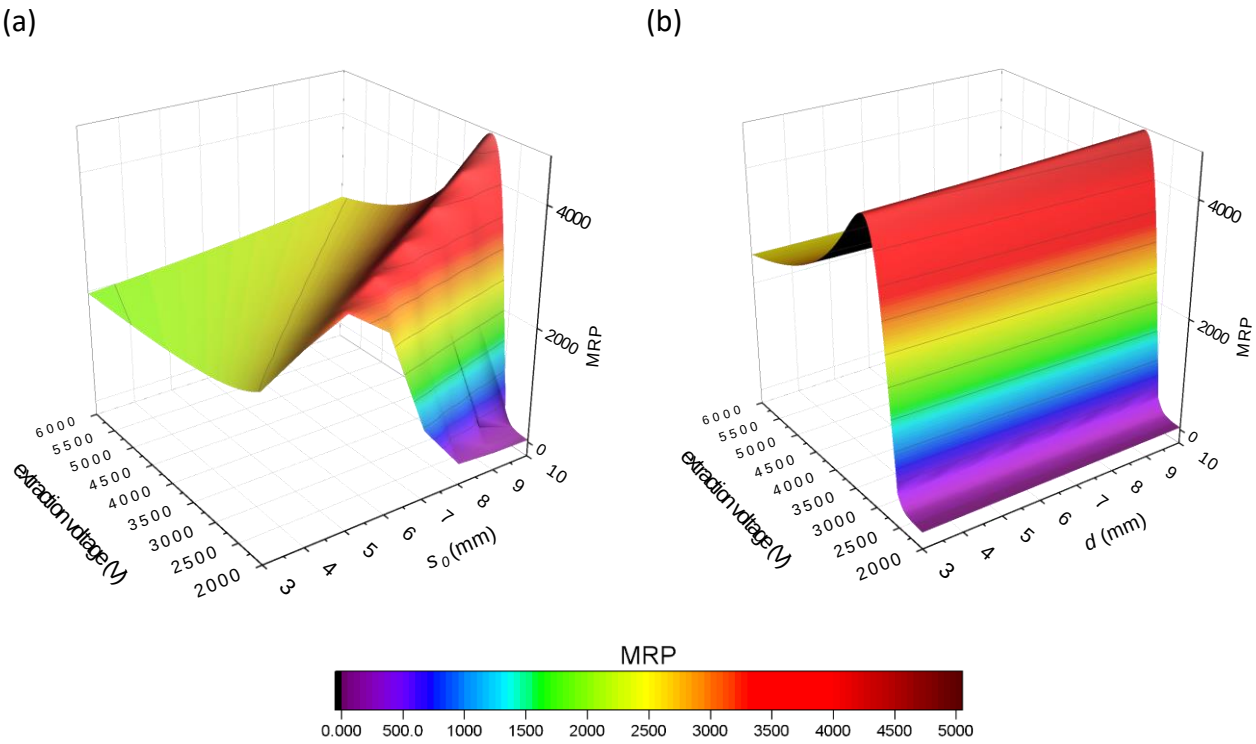
The calculation includes two parts. Part I consists of steps to determine s_0 and d to be developed. Once s_0 and d are determined, the suitable value of other parameters used during measurements is calculated in Part II.

Part I: Optimization of instrument dimensions: s_0 and d

1. Select the *m/z* (or an *m/z* range) of interest for calculation. The results discussed herein use cytochrome C *(m/z* 12,360) as an example.
2. Determine the total instrument length to be used. This can be determined arbitrarily. It is chosen to be 45 cm in this work, as described in the Experimental Section of the main text.
3. Select a set of s_0 and d.
4. Select an extraction voltage.
5. Solve $f(\tau)$ that gives the minimum flight-time spread, or $f(\tau) \approx 0$.
6. Apply the τ to calculate the flight time of all ions within the FWHM of the Maxwell-Boltzmann velocity distribution.
7. Calculate the resultant peak width and MRP.
8. Repeat Steps 3-7 until all combinations have been calculated.

9. Plot 3D topological spaces showing the correlation between (a) MRP, s_0, and extraction voltage, as shown in Figure S1a, and (b) MRP, d, and extraction voltage, as shown in Figure S1b.
10. Select an appropriate set of s_0 and d that provides the required MRP. In this work, both s_0 and d are chosen to be 10 mm.

Figure S1 The 3D topological space showing the correlation between MRP and other parameters for ions of *m/z* 12,360. (a) The correlation between MRP, extraction region length, and extraction voltage; (b) the correlation between MRP, acceleration region length, and extraction voltage.



Part II: Optimization of instrument parameters: extraction voltage and τ

1. Select the *m/z* to be measured.
2. Repeat Steps 4 – 8 in Part I with the selected s_0 and d.
3. Find the extraction voltage and τ that give required MRP. The required MRP in this work is above 2,000.

Figure S2 Comparison of the observations and the simulated spectra of C60/C70, angiotensin I, and cytochrome C. The simulated spectra are calculated based on the method reported by Loos and co-workers.[4] The simulated MRP of every group of peaks is indicated.

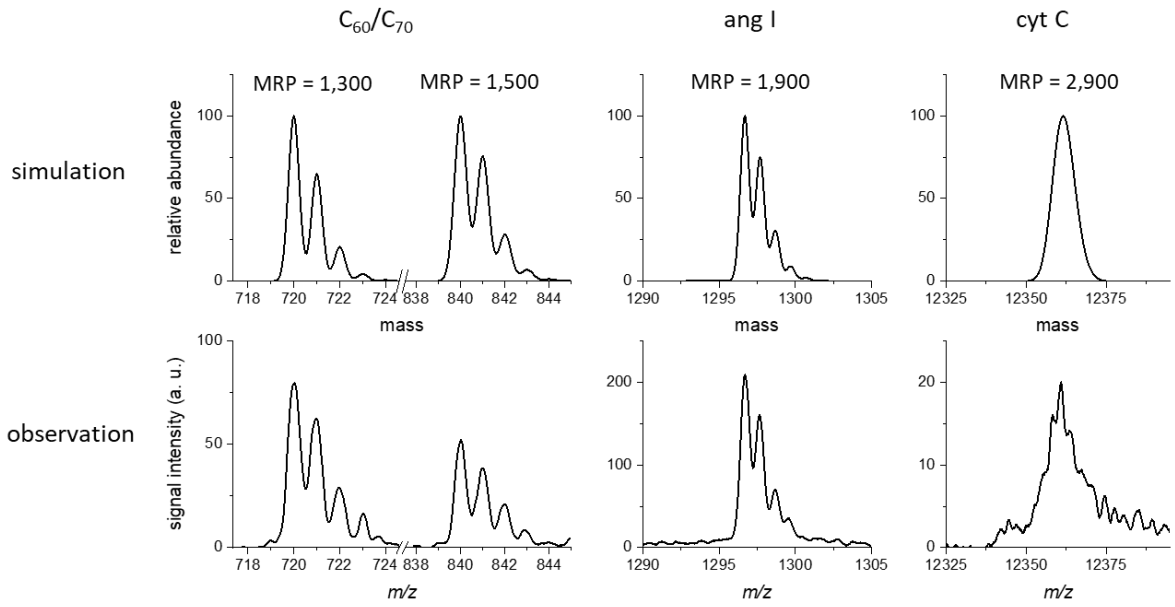
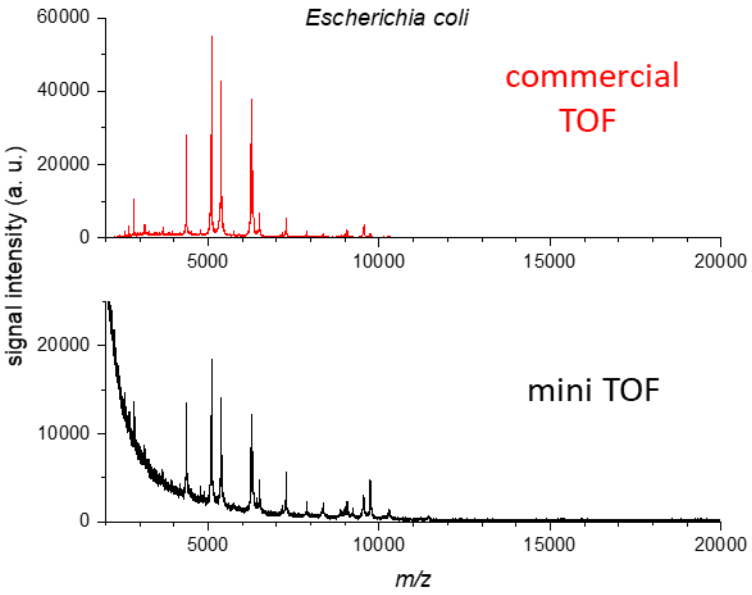
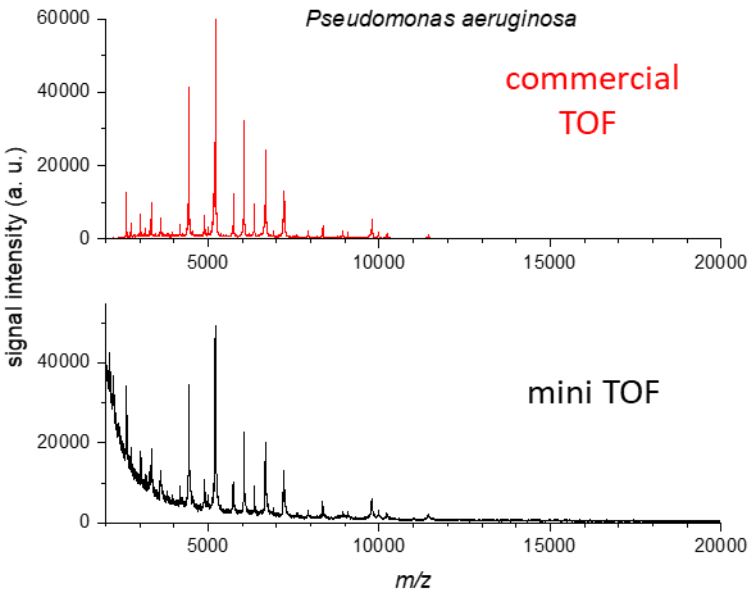


Figure S3 Mass spectra of proteins extracted from bacteria obtained using the Bruker Ultraflex II and the miniature instrument developed in this work. (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa*, (c) *Staphylococcus aureus*, (d) *Klebsiella pneumoniae*, (e) *Enterococcus faecium*.

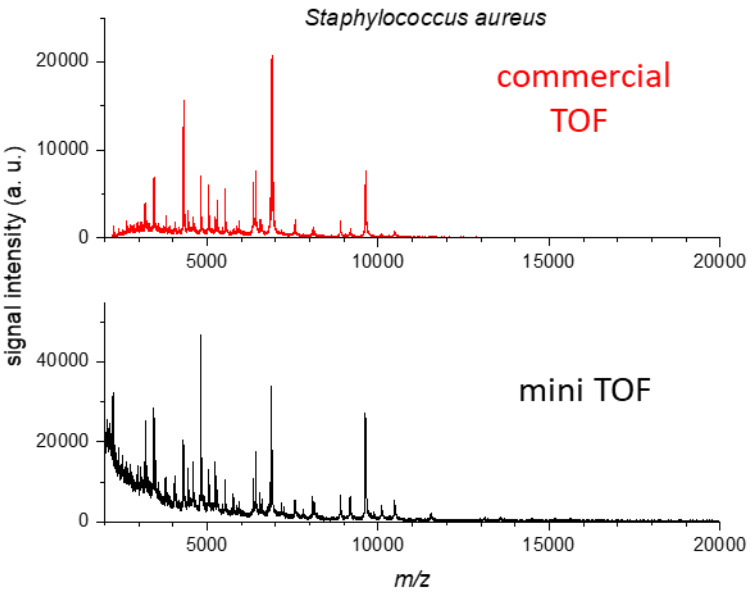
(a)



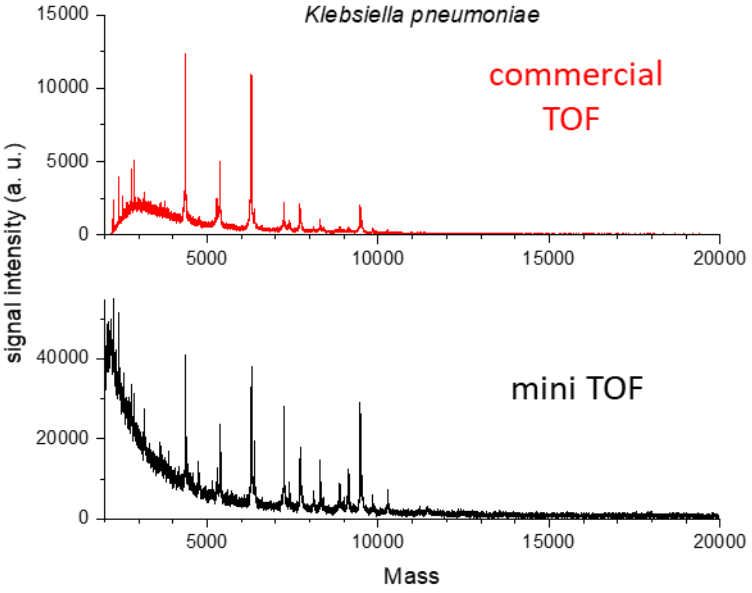
(b)



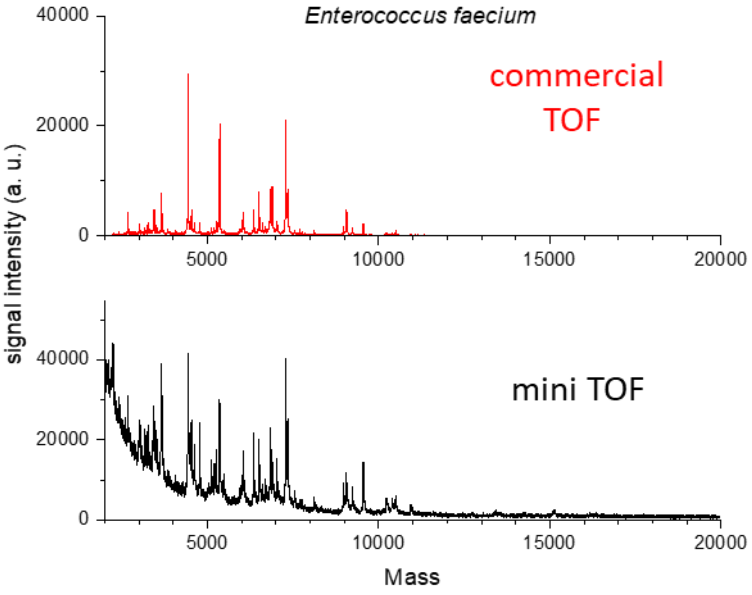
(c)


Staphylococcus aureus
commercial
TOF
mini TOF
signal intensity (a. u.)
m/z

(d)


Klebsiella pneumoniae
commercial
TOF
mini TOF
signal intensity (a. u.)
Mass

(e)


Enterococcus faecium
commercial
TOF
mini TOF
signal intensity (a. u.)
Mass

References

1. Y.-H. Cai, C.-H. Lin and Y.-S. Wang, *Int. J. Mass spectrom.*, 2022, **471**, 116756.
2. Y.-H. Cai and Y.-S. Wang, *J. Mass Spectrom.*, 2018, **53**, 361-368.
3. Y.-H. Cai, Y.-H. Lai and Y.-S. Wang, *J. Am. Soc. Mass. Spectrom.*, 2015, **26**, 1722-1731.
4. M. Loos, C. Gerber, F. Corona, J. Hollender and H. Singer, *Anal. Chem.*, 2015, **87**, 5738-5744.