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Experimental Studies of a Novel Multi-turn Time-of-Flight Mass Spectrometer and Its Applications for High Mass Molecules

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Overview

A novel Multi-turn Time-of-Flight mass spectrometer has been developed for biopharma and life science. High mass resolving power of over 150k has been demonstrated for biomolecules including reduced monoclonal antibody.

1. Introduction

The needs for high resolving power and sensitivity analyses of high mass molecules has been increasing in many fields such as biopharmaceutical and material industries. Time-of-Flight mass spectrometer (TOF MS) is a suitable solution to meet these needs. A novel multi-turn ion optical system has been proposed, which has 3D rotational reference orbit and thereby achieves nearly 50 m flight path with compact size [1]. Based on the proposed ion optical system, a new TOF mass spectrometer has been developed. Here, we show the details of the instrument and the results of the performance evaluations for high mass biomolecules.

2. Methods

2-1. Ion Optics

We have been developing a TOF MS with a novel multi-turn ion optical system. The multi-turn section consists of rotationally symmetric sector electrodes, in which ions fly along polar orbit (Figure 1). Ions are injected at optimized position off the central axis of electrodes, thereby the polar orbit rotate in the longitudinal direction. Eventually ions fly along an open-loop multi-turn orbit without any overtaking among different speed ions (Figure 2). The ion optics were designed using 3D ion trajectory simulations. The electrode geometry and voltages are optimized to achieve both spatial and time focusing for each lap. In order to achieve high mass accuracy, the multi-turn section is composed only static field that does not require voltage switching. Before injecting into the multi-turn section, ions are temporarily accumulated and compressed by using an ion trap, thus high sensitivity is realized.

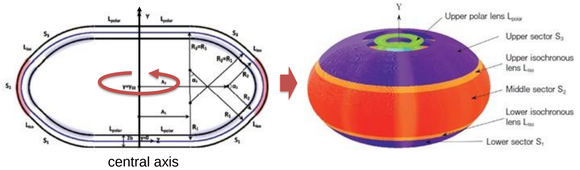


Figure 1 Cross section and 3D view of sector electrodes.

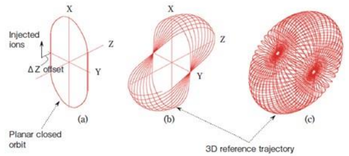


Figure 2 A example of planar closed orbit, and 3D reference trajectory.

Although the size is compact with a diameter of about 500 mm and a height of about 240 mm, a long flight path of nearly 50 m was realized. A simulation was performed to study its performance, which showed a TOF peak with high mass resolving power of over 150k (fwhm) (Figure 3).

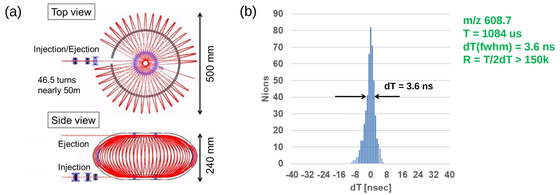


Figure 3 (a) Simulated ion trajectories and (b) TOF spectrum.

2-2. Construction of the multi-turn TOF MS system

We have constructed the multi-turn TOF MS system adopting a new ion optical design described above (Figure 4 (a)). Electro-Spray Ionization (ESI) is used as an ion source. Before injecting into the multi-turn section, ions are temporarily accumulated and compressed by using a linear ion trap (LIT), thus high sensitivity is realized (Figure 4 (b)). MS/MS-analysis can also be performed by the Hybrid-MS configuration, with Qq in front of the LIT.

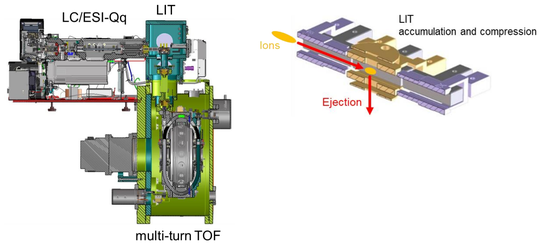


Figure 4 (a) Overview of multi-turn TOF MS system and (b) configuration of linear ion trap

3. Result

3-1. Evaluation of the mass resolving power

As a test of the system, mass resolving power has been evaluated by using several samples. First, mass spectrum of Angiotensin II (1,046 Da) was observed and high resolving power of over 150k (fwhm) was attained (Figure 5 (a)). Next, we analyzed Cytochrome c (12,384 Da), and thanks to the high resolving power, isotope peaks could be clearly separated (Figure 5 (b)).

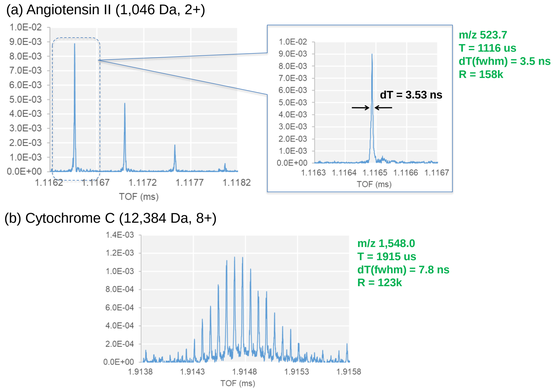
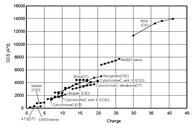


Figure 5 TOF spectrum of (a) Angiotensin II and (b) Cytochrome C

3-2. Improve of the sensitivity for high-mass molecules

As for higher molecular weights, such as myoglobin (~17,600 Da), isotope peaks were not separated due to insufficient sensitivity. Generally, the higher molecular mass and/or charge state become, the larger collisional cross sections (CCS) of molecules become (Figure 6) [2], [3]. In the case of large molecular samples, decrease in sensitivity due to the collision with residual gas becomes a problem, especially for multi-turn TOF MS that have long flight paths. In order to solve this, the pressure in the multi-turn section was decreased by enhancing the vacuum exhaust system.



sample	CCS (Å²)
Angiotensin II (1,046 Da, 2+)	~300
Cytochrome C (12 kDa, 8+)	~1,800
Myoglobin (18 kDa,+16)	~4,000
IgG1 Monoclonal Antibody(150 kDa,+22)	~7,000

Figure 6 the relationship between charge states and CCS for typical samples

Thanks to the improvement of the ion transmission for high-mass molecules, the sensitivity for was improved while keeping mass resolving power of 150 k (Figure 7). Subunits of monoclonal antibodies (Light Chain, ~23,000 Da) could also be detected with isotope peak separation (Figure 8).

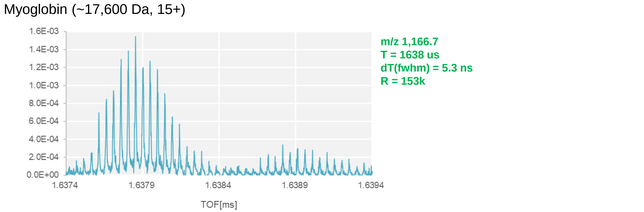


Figure 7 TOF spectrum of Myoglobin after decreasing the pressure in the multi-turn section

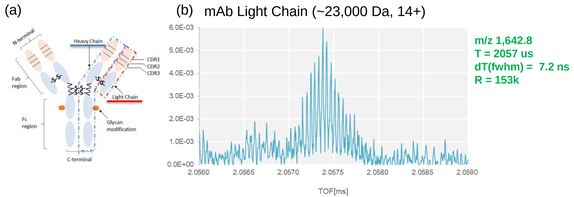


Figure 8 (a) Typical mAb structure and (b) TOF spectrum of mAb LC

4. Conclusions

- A novel multi-turn time-of-flight mass spectrometer was constructed.
- The sensitivity for high-mass molecules was improved while keeping high resolving power by decreasing the pressure in the multi-turn section and increasing injection efficiency of ions into the multi-turn part.
- Thanks to high resolving power over 100k, isotope peaks of high-mass molecules could be clearly separated.

References

[1] 63rd ASMS Conference on Mass Spectrometry (St. Louis, Missouri, 2015) MP-092.
[2] J Am Soc Mass Spectrom. Rev. 4 (1993) 619-623
[3] Anal. Chem. 2018, 90, 15, 8865–8872