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Supporting Information

Highly sensitive and specific detection of histamine via the formation of self-assembled magic number cluster with thymine by mass spectrometry

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1. The dissociation process of $[T_{17} + HIM + 2H]^{2+}$ and its identification

The dissociation process of the cluster $[T_{17} + HIM + 2H]^{2+}$ at m/z 1127 have been studied by varying the CID collision energy. Fig. S1(a) shows the relative abundances of at *m/z* 1127, *m/z* 812 and *m/z* 749 in the MS/MS spectra of $[T_{17} + HIM + 2H]^{2+}$ at different collision energy. The characteristic fragment ion $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812 was produced by the loss of a thymine pentamer and it appeared when the collision energy was above 8% and became the base peak when the collision energy was at 9.5%. In addition, the fragment ion $[T_{11} + HIM + 2H]^{2+}$ at *m/z* 749 was emerging increasingly when the collision energy rose from 14% to 16% (see Fig. S1(b)), and then its relative abundance remained steadily with the increase of collision energy. The above CID experimental results show that the fragmentation process of $[T_{17} + HIM + 2H]^{2+}$ is unique and the characteristic fragment ion $[T_{12} + HIM + 2H]^{2+}$ is remarkably stable.

Multistage MS experiments have also been performed to identify the cluster $[T_{17} + HIM + 2H]^{2+}$ (see Fig. S1(c-e)). In the MS/MS/MS spectrum of $[T_{17} + HIM + 2H]^{2+}$, shown in Fig. S1(c), the parent ion $[T_{17} + HIM + 2H]^{2+}$ at 812 underwent an loss of single unit (thymine) and produced its fragment $[T_{11} + HIM + 2H]^{2+}$ at 749, which indicate that these clusters are doubly charged. The sequential loss of single unit also occurred in the MS^4 and MS^5 spectra, which suggest that these clusters are non-covalent bonded. It is reasonable that there is no characteristic fragment ion for HIM in the MS/MS and MS^n spectra of $[T_{17} + HIM + 2H]^{2+}$, which can be supported by the CID experiment of $[T_{17} + HIM(D4) + 2H]^{2+}$ at m/z 1129 induced by the isotope standard HIM (D4). As is shown in Fig. S1(f), its CID fragment process also displayed the loss of a pentamer of thymine, producing the characteristic fragment ion $[T_{12} + HIM(D4) + 2H]^{2+}$ at *m/z* 814.

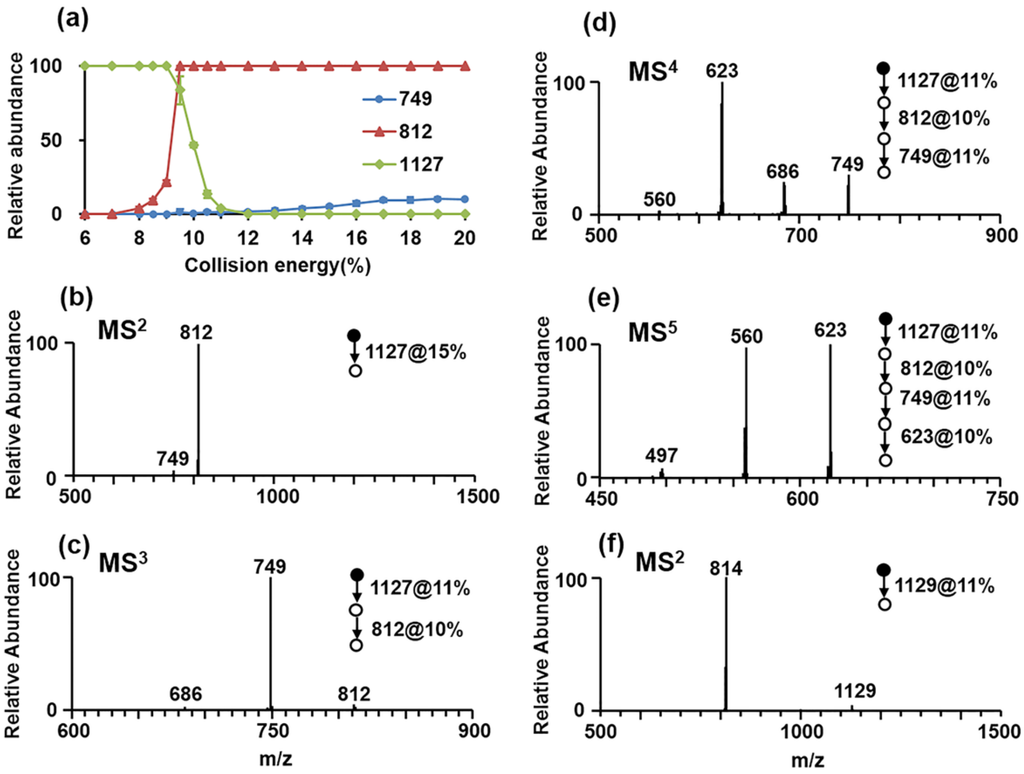

Fig. S1 (a) Dissociation profile of $[T_{17} + HIM + 2H]^{2+}$ at m/z 1127. The collision energy was in percentage of the maximum 'tickling' voltage, the relative abundances of parent ion at 1127 and fragment ions at 749 and 812 were expressed; MS/MS spectrum (b) and Multistage MS spectra (c - e) of $[T_{17} + HIM + 2H]^{2+}$; MS/MS spectrum of $[T_{17} + HIM (D4) + 2H]^{2+}$ at m/z 1129.

2. The optimization of the MS instrumental parameters

The abundances of the clusters in the ESI-MS spectra are influenced by several instrumental parameters.[1] To minimize dissociation of the non-covalent clusters during desolvation, the relative abundance of $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127 was optimized by selectively tuning the instrumental parameters including tube lens offset potential, capillary temperature and capillary voltage. Among these instrumental parameters, the tube lens offset potential was the most critical one. The result showed that the relative signal intensity of $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127 was firstly increased and then significantly decreased as the tube lens offset potential was increased from -100 V to 100 V. In this process, other clusters of thymine induced by protonated histamine, e.g. $[T_8 + HIM + 2H]^{2+}$ at *m/z* 560, $[T_9 + HIM + 2H]^{2+}$ at *m/z* 623, $[T_{11} + HIM + 2H]^{2+}$ at *m/z* 749 and $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812 were emerging increasingly. We calculated the fraction of the signal intensity of the cluster ion at m/z 1127 with respect to all the clusters of thymine induced by protonated histamine. A plot of the fraction of the cluster ion at m/z 1127 as the function of the tube lens offset potential is shown in Fig. S2 (a). The optimal tube lens offset potential was about -70 V. The lowest fraction of the cluster ion at m/z 1127 was obtained when the tube lens offset potentials were in the range between - 20 V and + 10 V. The resulting MS spectra are illustrated in Fig. S2 (b) and (c) in which the tube lens offset potentials were at -70 V and 0 V, respectively. The capillary temperature and the capillary voltage could also influence the relative ion abundances (shown in Fig. S3). They were optimized at 150 °C and 3.0 V, respectively, which were used for all the experiments.

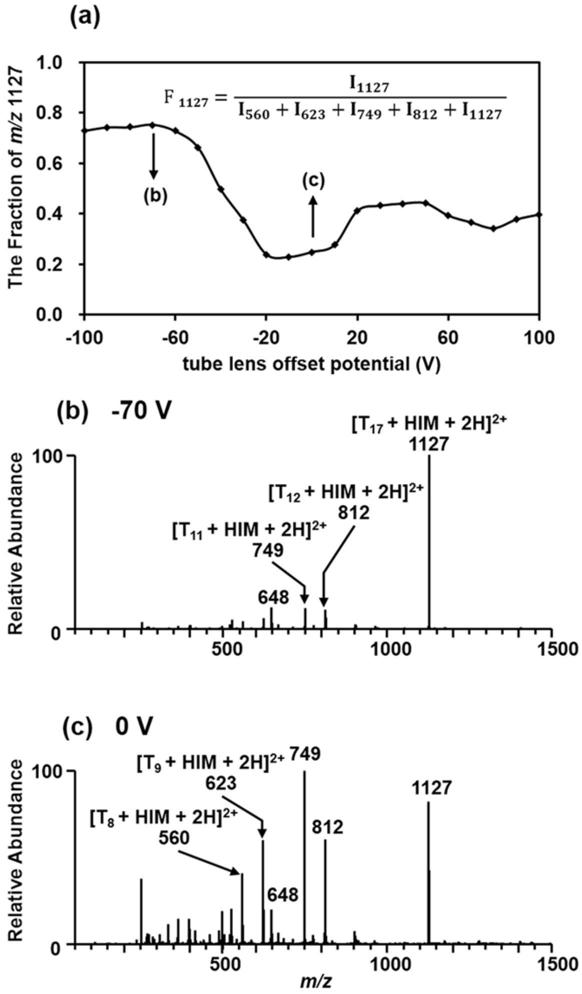

Fig. S2 (a) The fraction of $[T_{17} + HIM + 2H]^{2+}$ at m/z 1127 (in all the forms of clusters of thymine induced by protonated histamine) as a function of the tube lens offset potential (V) for a solution containing 1.0×10^{-5} mol/L histamine (HIM) and 3.0×10^{-4} mol/L thymine (T). The ESI-MS spectra were obtained at tube lens offset potential of (b) -70V and (c) 0V.

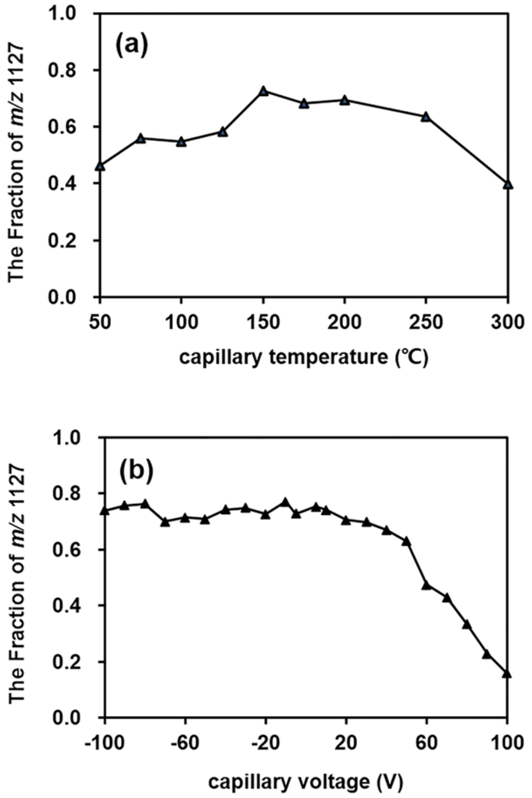

Fig. S3 The fraction of $[T_{17} + HIM + 2H]^{2+}$ at m/z 1127 (in all the forms of clusters of thymine induced by protonated histamine) as a function of (a) capillary voltage (V) and (b) capillary temperature (℃) for a solution containing 1.0×10^{-5} mol/L histamine (HIM) and 3.0×10^{-4} mol/L thymine (T). The tube lens offset potential was kept as -70 V; the capillary voltage was set as 3.0 V when investigating the effect of capillary temperature; and the capillary temperature was set as 150 ℃ when investigating the effect of capillary voltage.

3. The magic number clusters of thymine induced by protonated phenethylamine, dopamine, tryptamine and imidazole-4-acetic acid

The ESI-MS spectra of thymine (T) mixed with phenethylamine (PEA), dopamine (DOPA), tryptamine (TRY), Imidazole-4-acetic acid (IAA) are shown in Fig. S4. Protonated PEA, DOPA, TRY can induce thymine to form the pentameric clusters, corresponding to $[T_5 + PEA + H]^+$ at *m/z* 752, $[T_5 + DOPA + H]^+$ at *m/z* 784, $[T_5 + TRY + H]^+$ at *m/z* 791, respectively, and dimeric clusters of thymine, corresponding to $[T_2 + PEA + H]^+$ at *m/z* 374, $[T_2 + DOPA + H]^+$ at *m/z* 406 and $[T_2 + TRY + H]^+$ at *m/z* 413. The protonated ions $[DOPA + H]^+$ at *m/z* 154, and $[TRY + H]^+$ at *m/z* 161 were also observed. In the solution of IAA and thymine, the hexameric cluster of thymine $[T_6 + IAA + H]^+$ at *m/z* 882 and trimeric cluster $[T_3 + IAA + H]^+$ at *m/z* 504 were observed. The existence of the cluster $[T_{14} + 2Na]^{2+}$ at *m/z* 905 may be due to the adventitious Na^+ present in the sample. The clusters induced by ammonium ion, e.g. $[T_4 + NH_4]^+$ at *m/z* 522, $[T_5 + NH_4]^+$ at *m/z* 648 and $[T_{15} + 2NH_4]^{2+}$ at *m/z* 963, have been previously reported owing to the presence of about 0.02% ammonium ion in the thymine sample. [2, 3]

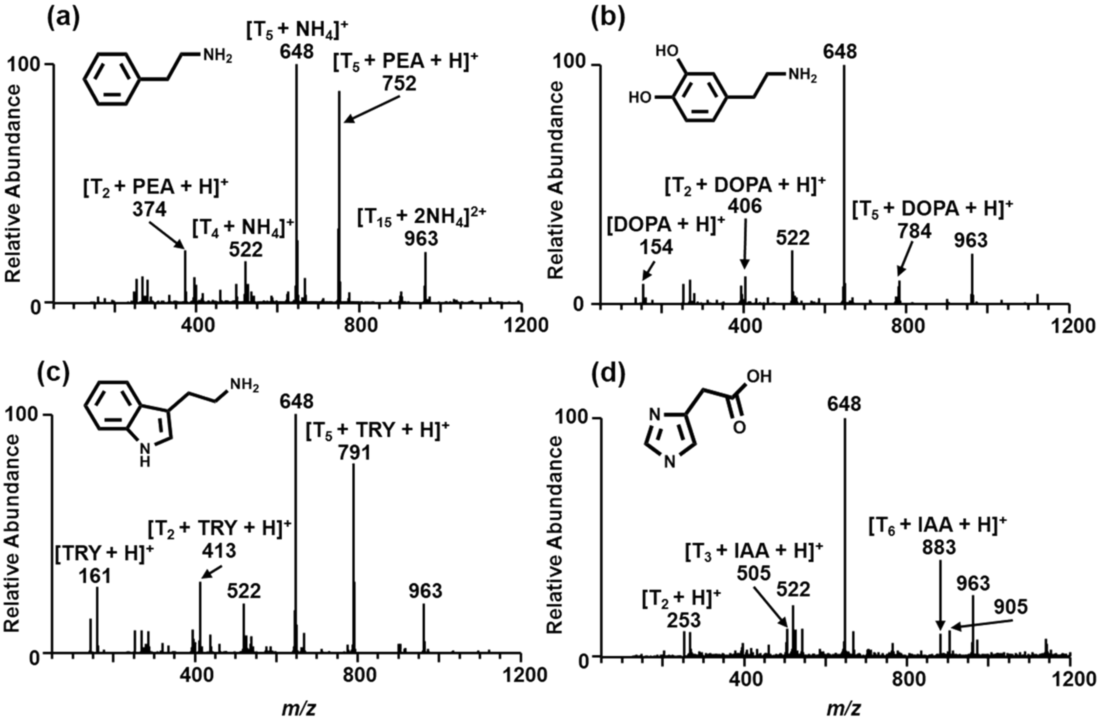

Fig. S4 ESI mass spectra of 3.0×10^{-4} mol/L thymine (T) and 1.0×10^{-5} mol/L (a) phenethylamine (PEA); (b) dopamine (DOPA); (c) tryptamine (TRY); (d) imidazole-4-acetic acid (IAA).

4. The magic number clusters of thymine induced by imidazolium ion

In the presence of imidazole (IM), the ESI-MS spectrum of thymine (T) solution shows the formation of magic number clusters $[T_6 + IM + H]^+$ at *m/z* 825 and $[T_{18} + 2IM + 2H]^{2+}$ at *m/z* 1202 (see Fig. S5). The cluster $[T_4 + Na]^+$ at *m/z* 527, $[T_5 + K]^+$ at *m/z* 668 and $[T_{14} + Na_2]^{2+}$ at *m/z* 905 were also observed, which may be formed due to the adventitious Na^+ and K^+ present in the sample. Clearly, the imidazolium ion (protonated imidazole) can induce thymine to form magic number clusters, which are very different from the pentameric clusters induced by the alkali metal ions or ammonium.[2-5] The hexameric thymine cluster $[T_6 + IM + H]^+$ might be a unique feature of imidazolium induced thymine clusters. The great abundance of $[T_{18} + 2IM + 2H]^{2+}$ in the mass spectrum suggests that it has unusual stability presumably due to a unique structure, which might consist of three stacked hexamers of thymine with two “sandwiched” imidazolium ions. The further research on the formation of the magic number clusters of thymine induced by imidazolium ion and the related structural studies are being carried out in our group. The data presented here at least suggests the inevitable role of the imidazole group of histamine (HIM) in forming the magic number clusters of thymine induced by protonated histamine, e.g. $[T_{17} + HIM + 2H]^{2+}$, which is reported in the main document.

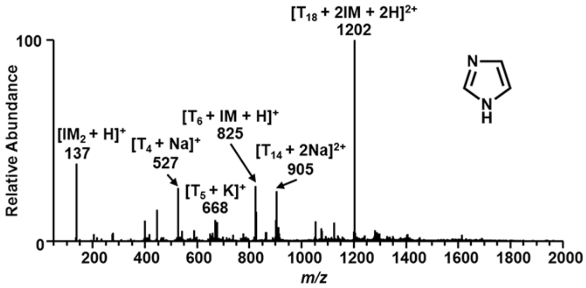

Fig. S5 ESI mass spectrum of 10^{-3} mol/L thymine (T) and 10^{-3} mol/L imidazole (IM).

5. Salt effect and acidification effect on the signal intensity of $[T_{17} + HIM + 2H]^{2+}$

The effect of salt was investigated using solutions containing histamine (1.0 × 10^{-5} M) thymine (3.0 × 10^{-4} M) and various amounts of NaCl (0, 1.0 × 10^{-6} , 1.0 × 10^{-5} , 1.0 × 10^{-4} , 2.0 × 10^{-4} , 4.0 × 10^{-4} , 6.0 × 10^{-4} and 1.0 × 10^{-3} M). Increasing the salt concentration from 0 to 1 × 10^{-3} M led to an overall decrease of the signal intensity of $[T_{17} + HIM + 2H]^{2+}$ at *m/z* 1127, especially when the salt concentration was above 10^{-4} M (shown in Fig. S6). This is mainly due to the competition between protonated histamine and Na^+, both of which can induce thymine to form clusters. Besides Na^+, other alkaline metal ions such as K^+ can also induce thymine to form clusters. Therefore, dilution or desalination is essential before the analysis of real samples by using this approach.

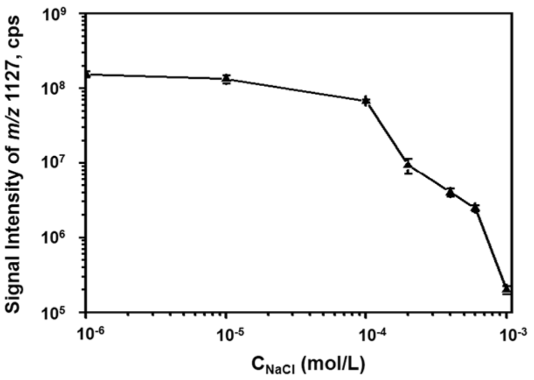

Fig. S6. The effect of adding NaCl on the signal intensity of the cluster $[T_{17} + HIM + 2H]^{2+}$ at m/z 1127 generated from a solution containing 1.0 × 10^{-5} mol/L histamine (HIM) and 3.0 × 10^{-4} mol/L thymine (T). Each data point represents the average of 4 measurements. The error bar represents one standard deviation of the data.

The acidification effect was investigated by adding increasing amount of formic acid (1 ‰, 1 % and 2 %) into the mixture of 3.0 × 10^{-4} mol/L thymine and 1.0 × 10^{-5} mol/L histamine (The pH values were 3.82, 2.78 and 2.64, respectively). The addition of formic acid decreased the signal intensity of $[T_{17} + HIM + 2H]^{2+}$ (see Fig. S7), because an extreme acidic condition makes it favorable for the formation of the protonated monomer instead of the clusters for thymine.[2] In this way, our studies on the thymine clusters were mostly undertaken from solutions near neutral conditions.

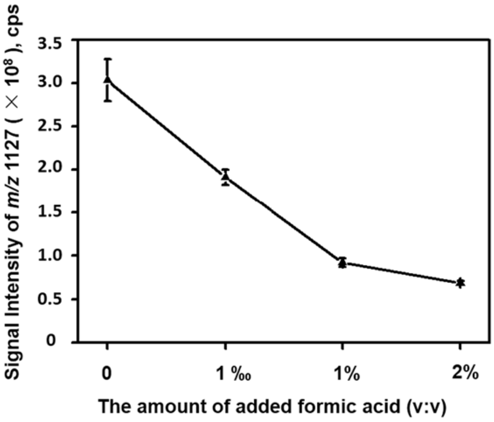

Fig. S7. The acidification effect of formic acid on the signal intensity of the cluster $[T_{17} + HIM + 2H]^{2+}$ at m/z 1127 generated from a solution containing 1.0 × 10^{-5} mol/L histamine (HIM) and 3.0 × 10^{-4} mol/L thymine (T).

6. The isotope distribution of $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812

The theoretical isotope distribution of $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812 was calculated by using Isotope Viewer version 1.0 (Thermo Electron Corp., Waltham, MA, USA) (see Fig. S8). The calculation parameters were as the following. The output style was profile. The charge state was set to 2 +. The resolution was 0.05 Dalton.

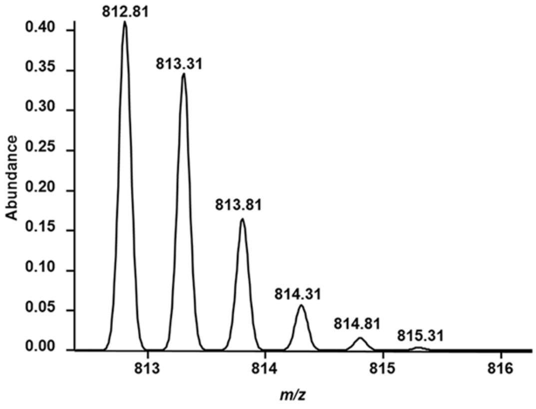

Fig. S8 The calculated isotope distribution of $[T_{12} + HIM + 2H]^{2+}$ at *m/z* 812.

7. References

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