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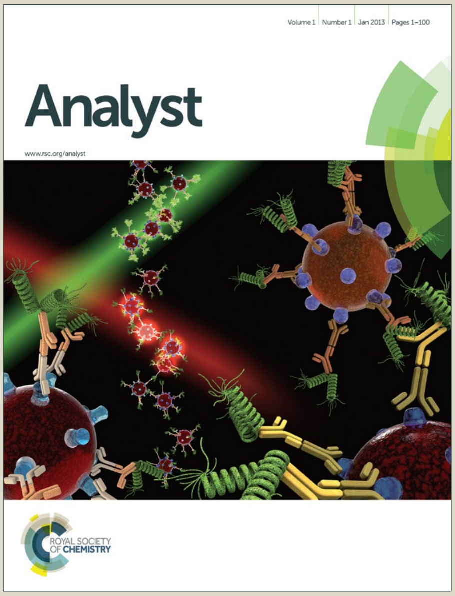

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Isomer Differentiation through Supramolecular Self-assembly in Microdroplets of Milliseconds Life-time

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Supramolecular recognition of thymine (or its analogs) with various central cations can form magic number clusters. Dual nano-ESI *via* theta tip emitters were used to on-line synthesize clusters. Even thermodynamically unstable clusters can be detected by MS thanks to the very short life-time (~ms) of generated microdroplets. By recording characteristic cluster distributions, isomers can be clearly differentiated in a novel "bottom-up" way. Theoretical calculations were performed to explain MS results.

Supramolecular recognition exists widely in nature and facilitates intra-communications in living cells.[1-3] Self-assembled building blocks can be constructed in a bottom-up way into complex materials, such as nanohelices[4] and hydrogels[5, 6]. Thus the study of self-assembled building blocks at the initial formation stage is important, which will assist rational design and synthesis of nano-structures[7, 8], as well as offer a deeper insight into supramolecular recognition processes[9, 10].

Magic number cluster is a typical kind of small-scale self-assembly. Previously, Qiu *et al.*[11] studied the thymine (or its analogs) quintet induced by alkali metal ions by electrospray ionization mass spectrometry (ESI-MS). Later, Qin *et al.*[12] applied reactive desorption electrospray ionization (DESI) MS to study the ammonium cation induced uracil (and its analogs) quintets. They demonstrated that such self-assembly process can happen in milliseconds. Recently, the groups of Williams[13, 14] and Mcluckey[15, 16] have introduced theta capillary tips as dual nanospray emitters into the mass spectrometry community. The protein charge state distributions could be on-line manipulated, and several reactions have been perform by this novel ionization method. It was evidenced that two reagents from each of the two barrels mixed at the tip end (Taylor cone) and the life-time of the microdroplets generated subsequently could be from milli to micro-seconds level[13], depending on the diameter of the tip ends. Intrigued by their pioneering work, we try to on-line synthesize the thymine (or its analogs) based self-assembled clusters by using theta capillary tips (Fig 1). Isomer differentiation has long been a fundamental topic in mass spectrometry, and several methods have been proposed to differentiate isomers by primary MS (e.g. EI-MS[17]) or tandem MS (e.g. kinetic methods[18]). Previous methods differentiate isomers mainly by recording their different gas phase fragment patterns, which can be described as "top-down strategies". In this work, we will show through inducing different kinds of clusters, isomers can be differentiated directly in primary MS. Surprisingly, the host isomers being induced by the same guest molecule can also be differentiated. Such a "bottom-up strategy" to differentiate isomers is novel and unique. Meantime, the reasonable structures of the on-line synthesized self-assemblies are proposed and studied by theoretical calculations. The calculated data are consistent with MS experiments.

The experimental setup (adapted from literature[13-15]) was schematically shown in Fig 1. Briefly, the guest molecule solution (10 ppb) was injected in one barrel, and the other barrel was infused by the host molecule solution (1 ppm). All solutions were prepared in MeOH/water (1:1 v/v). The electrode was inserted in the barrel in which the host solution was infused (previous results evidenced the electrode insertion to either of the two barrels led to identical mass spectra[15]). Because the diameters of our theta tip ends are about 1 micrometer (Fig S1), based on literature[13], the life-times of the generated microdroplets are in the milliseconds time range. The primary mass spectra of the on-line synthesized clusters were recorded and by comparing the different distributions of various clusters, the isomers can be clearly differentiated.

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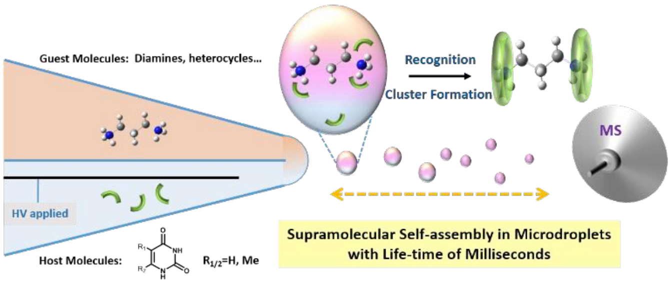

Fig 1 Schematic illustration of the supramolecular self-assembly process. Host-guest interactions were initiated within the Taylor cone at the theta capillary tip end. During the fast desolvation process of the microdroplets (in milliseconds), supramolecular recognition of host-guest molecules led to specific clusters formation. By recording the mass spectra, the information of the cluster distributions can be obtained and isomers can be clearly differentiated.

We chose 1,3-propanediamine (1,3-PDA) and 1,2-propanediamine (1,2-PDA) as our first proof-of-concept example to demonstrate that the formation of thymine clusters is highly structure-specific. Isomers of 1,3-PDA and 1,2-PDA cannot be differentiated by ESI-MS due to their same molecular weight. Even via ESI-MS/MS, 1,3-PDA and 1,2-PDA still cannot be differentiated, because they have the same fragmentation pathway and the relative intensities of the fragment ions were similar (Fig 2a and Fig 2b). However, by using the dual nanospray of theta tips and thymine was applied as the host molecule, we can clearly differentiate the two diamines. As shown in Fig 2c and 2d, 1,3-PDA (di-protonated, similarly hereinafter) can self-assemble to *16*-mer (m/z 1046, denoted as $+T_{16}^{2+}$ in the figure, the "*n*-mer" nomenclature was used in the text for convenience and clarity. "*n*-mer" means the cluster contains *n* host molecules. One central cation is involved unless otherwise specified) with thymine, while 1,2-PDA (di-protonated, similarly hereinafter) can not. This clear difference can help to rapidly differentiate them without further effort to run tandem MS. Both of the two diamines can form *7*-mer, *10*-mer, *11*-mer and *12*-mer clusters, respectively, but with different relative intensities. For example, 1,2-PDA has a high tendency to form *11*-mer, while the relative signal corresponding to the *11*-mer of 1,3-PDA is much weaker.

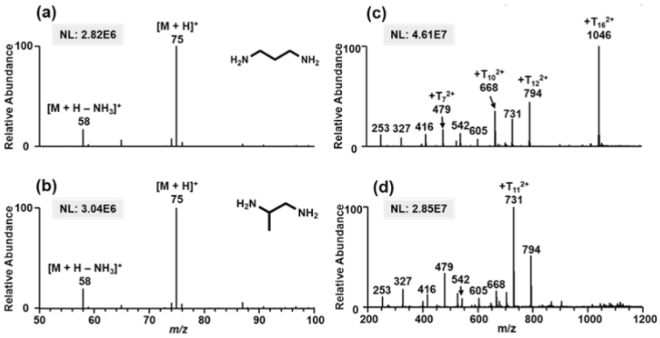

Fig 2 The ESI-MS/MS spectra of 10 ppb (a) 1,3-PDA; (b) 1,2-PDA. Dual nano-ESI MS of 10 ppb guest molecule in one barrel of a theta tip, and 1 ppm thymine as the host molecule in the other barrel. (c) 1,3-PDA as the guest molecule; (d) 1,2-PDA as the guest molecule. The absolute signal intensities are shown as insets.

To explain the different behaviours in the clusters formation, we did theoretical calculations to study the relative stabilities of the clusters formed by thymine with 1,3-PDA and 1,2-PDA, respectively. The possible structures were proposed according to previously established model[11, 12, 19, and 20], in which 5 or 6 thymine molecules arranged into circles *via* double complementary hydrogen bonds, and the circles entrapped the nitrogen charge centres. Schalley *et al.*[21] has used molecular dynamics to calculate model structures of organometallic clusters formed in ESI-MS, and we applied similar theoretical methods in our study. All structures discussed in this paper were optimized and converged to 0.1 kJ/mol. *11*-mers of 1,3-PDA and 1,2-PDA, respectively, were shown in Fig 3a. From the calculated Gibbs free energy, we can know that the *11*-mer of 1,2-PDA is more stable than that of 1,3-PDA (11.0 kJ/mol lower in energy). Optimized distance between two adjacent thymine circles is about 3.0 Å in alkali metal cations induced thymine clusters[19]. The distance between the two nitrogen charge centres in 1,3-PDA is calculated to be 5.1 Å, while that of 1,2-PDA is 3.4 Å. Due to the closer distance of the two charge centres in 1,2-PDA, the two adjacent thymine circles would be closer and have stronger π-π interactions, thus the *11*-mer of 1,2-PDA is more stable than that of 1,3-PDA. The computational results are consistent with mass spectra in Fig 2c and 2d, in which the *11*-mer signal of 1,2-PDA is higher than that of 1,3-PDA. Similarly, the structures of thymine *16*-mers of the two diamines were calculated and shown in Fig 3b. The *16*-mer of 1,3-PDA is more stable than that of 1,2-PDA. As mentioned above, optimized distance of two adjacent thymine circles is about 3.0 Å[19], while the two nitrogen charge centres in 1,2-PDA is calculated to be 3.4 Å, so it is hardly possible for it to "hold" 3 consecutive thymine circles. While the two nitrogen charge centres in 1,3-PDA is 5.1 Å, so it is possible for this cation to stabilized three consecutive thymine circles. The results from theoretical calculations are consistent with the mass spectra in Fig 2c and 2 d, in which the signal corresponding to *16*-mer of 1,3-PDA is significant while that of 1,2-PDA is absent.

Nitrogen-containing heterocycles are important and fundamental molecules in organic chemistry. Unfortunately, the differentiation of imidazole and pyrazole, two commonly encountered aromatic heterocycles, by EI-MS is nearly impossible, because they generated almost identical MS spectra (Fig S2). While by formation of clusters with uracil (an analog of thymine), these two compounds can be clearly differentiated, as shown in Fig 4a and 4b. Imidazole can induce *6*-mer, *11*-mer, *12*-mer and *18*-mer clusters with uracil. While pyrazole can only induce clusters of *6*-mer and *19*-mer (note that the MS signals of these two clusters are very weak in Fig 4b). Theoretical calculations were performed on *6*-mer uracil clusters induced by imidazole and pyrazole, respectively, and the results (Fig S3) are consistent with MS results. 1,2,4-triazole and 1,2,3-triazole are also hardly differentiable by EI-MS (Fig S2). However, following the same protocol, by comparing the different ability to induce supramolecular uracil clusters, these two triazoles can also be clearly differentiated, as shown in Fig 4c and 4d. 1,2,4-triazole can form *6*-mer, *11*-mer, *17*-mer and *18*-mer with uracil. While 1,2,3-triazole can form *18*-mer and *19*-mer with uracil, and the signal intensities of these two clusters were very weak (Fig 4d). We noticed that N-CH=N moiety is crucial in the *6*-mer cluster formation (discussions of Fig S3). This structure-function relationship again demonstrated the formation of uracil (or its analogs) clusters was very structure-specific. In certain cases, isomers can be better differentiated by our method than by EI-MS.

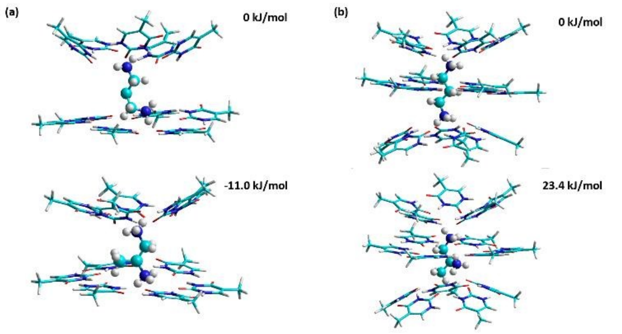

Fig 3 (a) Theoretically optimized structure of thymine 11-mer clusters of 1,3-propanediamine (up) and 1,2-propanediamine (down). (b) Theoretically optimized structure of thymine 16-mer clusters of 1,3-propanediamine (up) and 1,2-propanediamine (down). Calculated Gibbs free energy were shown accordingly.

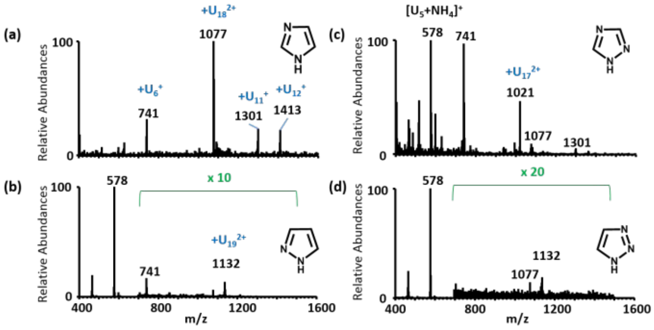

Fig 4 NanoESI MS done with 10 ppb guest molecule in one barrel of a theta tip, and 1 ppm uracil as the host molecule in the other barrel. (a) Imidazole, (b) Pyrazole, (c) 1,2,4-triazole, and (d) 1,2,3-triazole as the guest molecule, respectively. Note that in this figure, the two positively charged clusters contain two central cations.

Previous works by our group[11, 12, 19, and 20] have demonstrated that alkali metal ions or primary amines induced thymine (or analogs) were thermodynamically stable and can be detected by ESI-MS or DESI-MS. Tertiary amines were not able to induce such clusters, probably due to the cavity in thymine (or analogs) circle was too small to entrap the charged moiety in tertiary amine. Such steric effect may cause such clusters thermodynamically unstable. However, if dual nanoESI via theta emitter was applied, due to the very short life-time of the microdroplets, tertiary amines induced clusters may be kinetically “trapped” and thus detected by MS. N,N,N’,N’-tetramethyl-1,2-ethylenediamine (TMEDA) was used as the guest molecule to induce thymine (T) and 6-methyl uracil (6MU) clusters. TMEDA can induce thymine to form *12*-mer, *17*-mer and *18*-mer clusters in dual nano-ESI *via* theta tip emitter (Fig 5a). If TMEDA and thymine were pre-mixed and analysed *via* single-barrel nano-ESI method, no clusters induced by TMEDA can be obtained (Fig S4a). TMEDA can induce 6MU to form *12*-mer and *18*-mer clusters in dual nano-ESI *via* theta tip emitter (Fig 5b), but also cannot induce 6MU clusters *via* single-barrel nano-ESI method (Fig S4b). Note that the dual nano-ESI MS spectra (Fig 5) corresponding to thymine and 6MU, respectively, are quite different. Although the MS signals of *12*-mers (m/z 815) were significant and became base peaks, the relative intensities of their *17*-mers (m/z 1130) and *18*-mers (m/z 1193) differed greatly. This example demonstrated that besides differentiating guest molecules, by properly choosing the same guest molecule, different host molecules can also be differentiated by our method. This case also evidenced through *in situ* mixing and generating microdroplets of milliseconds life-time, thermodynamically unstable clusters can be kinetically trapped and detected by MS.

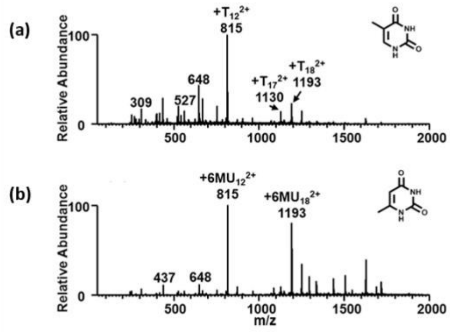

Fig 5 Dual nano-ESI MS of 10 ppb N,N,N’,N’-tetramethyl-1,2-ethylenediamine (TMEDA) as the guest molecule in one barrel of a theta tip, and 1 ppm (a) thymine and (b) 6-methyluracil (6MU), respectively, as the host molecule in the other barrel.

Conclusions

In conclusion, a state-of-the-art MS technique, dual nanoESI *via* theta tip emitters, was applied to study the different self-assembly behaviour of isomers. Thermodynamically unstable clusters can be kinetically trapped in microdroplets of milliseconds life-time. This special feature of dual nano-ESI have made clusters not obtainable by single-barrel nano-ESI detectable *via* MS. Isomers of very similar structure, even can hardly be differentiated by EI-MS, can be clearly differentiated in primary MS. By choosing proper reagent as guest component, the differentiation of host molecules can also be accomplished. The present work demonstrated dual nanoESI-MS *via* theta tips can be a powerful tool not only for analyses, but also for studying supramolecular interactions in rapidly desolvating conditions (quasi-gas phase). Our results here may intrigue and assist future design and synthesis of thymine (or its analogs) based self-assembly materials, following the successful and pioneering example of G-quartet.[5, 6]

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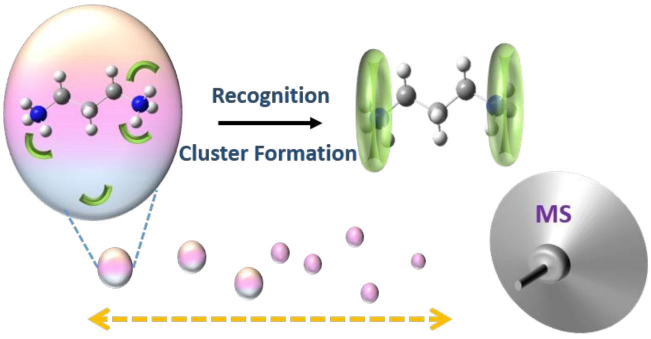

Supramolecular Self-assembly in Microdroplets with Life-time of Milliseconds