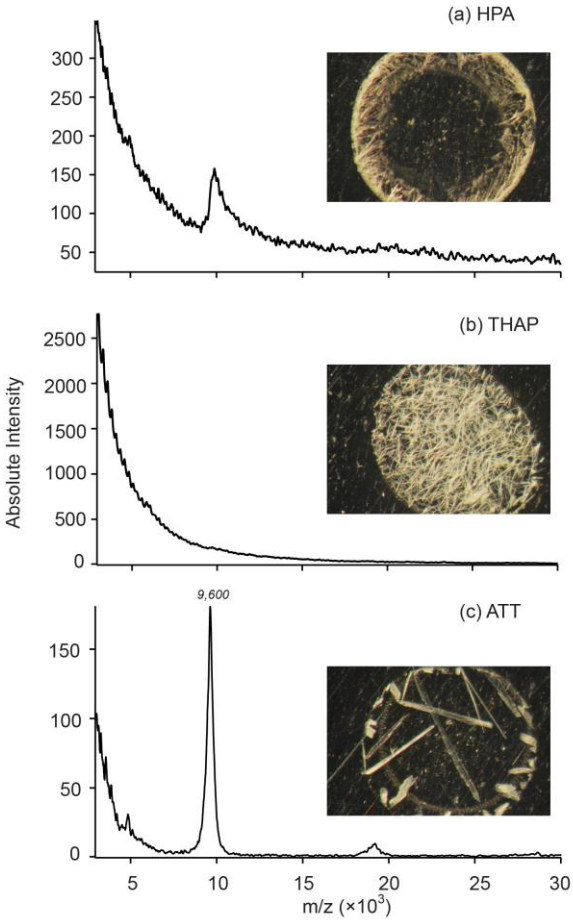
Supporting Information

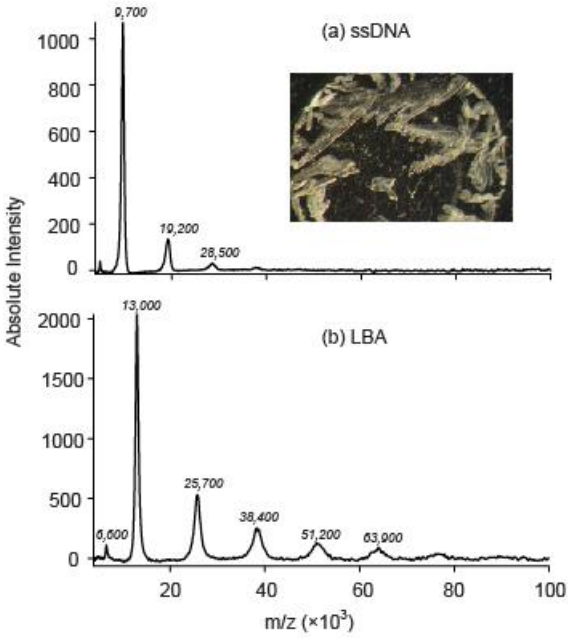
Analyzing Aptamers with MALDI-MS. Because aptamers are single-stranded DNAs, a single stranded control DNA (ssDNA, TCT-GAC-CTT-TGA-CCT-ACT-GAC-CTT-TGA-CCT-CT, theoretical average MW= 9652.3 Da) was investigated first with high mass MALDI-MS with different matrixes in the positive ion detection mode. Supporting Figure 1 shows mass spectra of 500-fmol ssDNA via dried dropled sample preparation with three different matrixes, including HPA, THAP and ATT.[1] Only a weak signal corresponding to the ssDNA was detected when HPA was used (Supporting Figure 1(a)). In the microscope image, a clear aggregation of high amounts of matrix or analyte crystals was observed in a ring around the edge of the droplet. Although very homogeneous crystals were noticed in the image wih THAP as the matrix, we hardly observe any signal corresponding to ssDNA (Supporting Figure 1(b)). ATT with ammonium citrate as the comatrix provides a much better signal of ssDNA as observed in Supporting Figure 1(c). It is well known the presence of alkali metal ions (Na^+ and K^+), which will adduct to the negatively charged phosphate backbone of oligonucleotides, would result in peak broadening and loss of sensitivity. Analyzing ssDNA directly, without any further purification, might limit the sensitivity here.

To enhance the detection of the ssDNA in high-mass MALDI-MS we also optimized the sample preparation. Supporting Figure 2(a) showed the mass spectra of the same amount of the ssDNA as used in Supporting Figure 1 by using modified sandwich method. The signal corresponding to the ssDNA monomer was improved dramatically. Besides the dominant monomeric ssDNA species, the non-specific aggregates, including the ssDNA dimer and

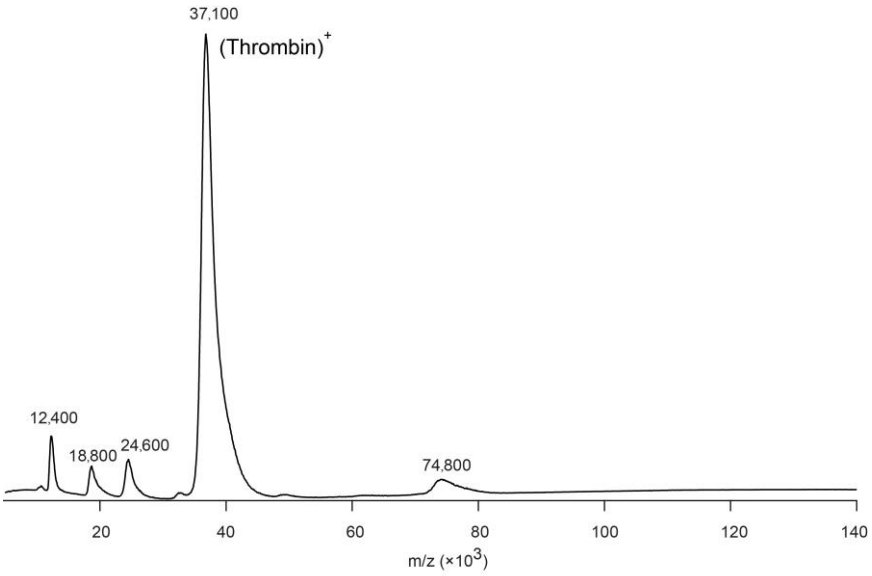
the ssDNA trimer that could be the result of clustering occurring in the MALDI plume, and also the doubly charged species were also detected. A much more homogeneous crystallization pattern, which was observed when modified sandwich sample preparation applied, could explain the signal enhancement. In Supporting Figure 2(b), we recorded the lysozyme binding aptamer (LBA, ATC-TAC-GAA-TTC-ATC-AGG-GCT-AAA-GAG-TGC-AGA-GTT-ACT-TAG, theoretical average MW= 12985.5 Da). A major peak at *m/z* 13,000 was observed and assigned to the monomer of LBA. The peaks of the LBA homodimer (m/z 25,700), the homotrimer (m/z 38,400), the homotetramer (m/z 51,200), and the homopentamer (m/z 63,900) were observed at low intensity, and attributed to the non-specific complexes. The doubly charged LBA was also detected at *m/z* 6,600.



Supporting Figure 1 High-mass MALDI mass spectra of ssDNA with different matrixes (a) HPA, (b) THAP and (c) ATT by using dried droplet preparation.



Supporting Figure 2. High-mass MALDI mass spectra of (a) ssDNA and (b) LBA by using the modified sandwich method.



Supporting Figure 3. High-mass MALDI mass spectra of thrombin interacting with TBA29 at a thrombin-to-TBA29 molar ratio of 1:1 with ATT (0.1% TFA) as matrix.

References

1 R. Sudha and R. Zenobi, *Helv. Chim. Acta*, 2002, **85**, 3136-3142.