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Application News

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MALDI-TOF Mass Spectrometry

Analysis of Peptide Nucleic Acids (PNAs) using the MALDI-8020 benchtop linear MALDI-TOF mass spectrometer

Matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is gaining popularity due to the ability of MALDI-MS to quickly provide information on accurate molecular mass using a simple sample preparation workflow. The MALDI-TOF MS can be used to confirm and guide synthesis of PNA oligomers, either in QC or R&D laboratories. Here, we demonstrate the analysis of three PNA samples using MALDI-8020 benchtop linear MALDI-TOF mass spectrometer.

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Peptide Nucleic Acids (PNAs) are synthetic mimicking analogues of DNA oligomers where the nucleobases (A/G/C/T) are retained but the backbone of deoxyribose and phosphate blocks are replaced by peptide-like structures (Fig. 1). These structures are N-(2-amino-ethyl) glycyl units held together by peptide bonds which are neutral and achiral in nature, unlike the negatively charged sugar-phosphate backbone of DNA.

The charge state difference enhances binding of the PNA ligand to complementary DNA/RNA sequences, which is key to the importance of PNAs, conferring stronger hybridisation affinity to DNA/RNA compared to native DNA. This property facilitates the application of PNAs in techniques such as single nucleotide polymorphism (SNP) detection, fluorescent in-situ hybridisation (FISH) and molecular biosensors, in the molecular diagnostic and research sectors.

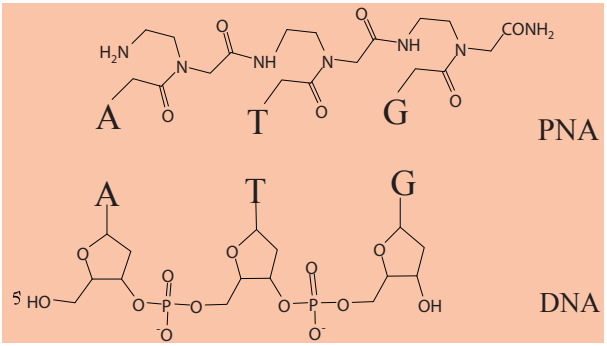

Fig. 1 Comparison of PNA and DNA illustrating differences in the backbone structure

In these applications, the improved biological stability and sequence specificity of the PNAs is utilised as highly selective molecular probes, capable of distinguishing between closely matched but dissimilar sequences found in single point mutations and related inherited clinical disorders. PNAs also have potential application as antisense drugs for HIV-1 treatment.

Fig. 2 MALDI-8020 benchtop linear MALDI-TOF instrument

Samples and method

PNA samples were obtained by kind donation from DestiNA Genomica S.L., Spain. The sample solutions (4-15 µM, in 0.1 % (aq.) TFA) were deposited with 1 volume of MALDI matrix, either alpha-cyano-4-hydroxycinnamic acid (CHCA, 5 mg/mL), or sinapinic acid (20 mg/mL) in 1:1 acetonitrile/ 0.1 % (aq.) TFA) and allowed to dry on a stainless steel MALDI target. Data acquisition was performed by benchtop MALDI-TOF MS (MALDI-8020, Fig. 2) using parameters listed in Table 1.

Table 1 MALDI-TOF MS data acquisition parameters

Tuning	linear
Polarity	positive
Mass range	100-12000 Da
Laser rep. rate	200 Hz
Accumulation rate (shots/profile)	50
Profiles	200
Sampling method	Dither

Results

Fig. 3 shows the MALDI-MS spectra of some PNA samples. The base peak has a detected monoisotopic mass of *m/z* 4510.9 and the inset shows the well-resolved isotopic peaks of the PNA species, demonstrating the excellent linear mode resolving power of the instrument (>8000 FWHM in this example, Fig. 3 upper). The spectra for 2 other higher mass PNA samples are also shown (Fig. 3 lower).

References
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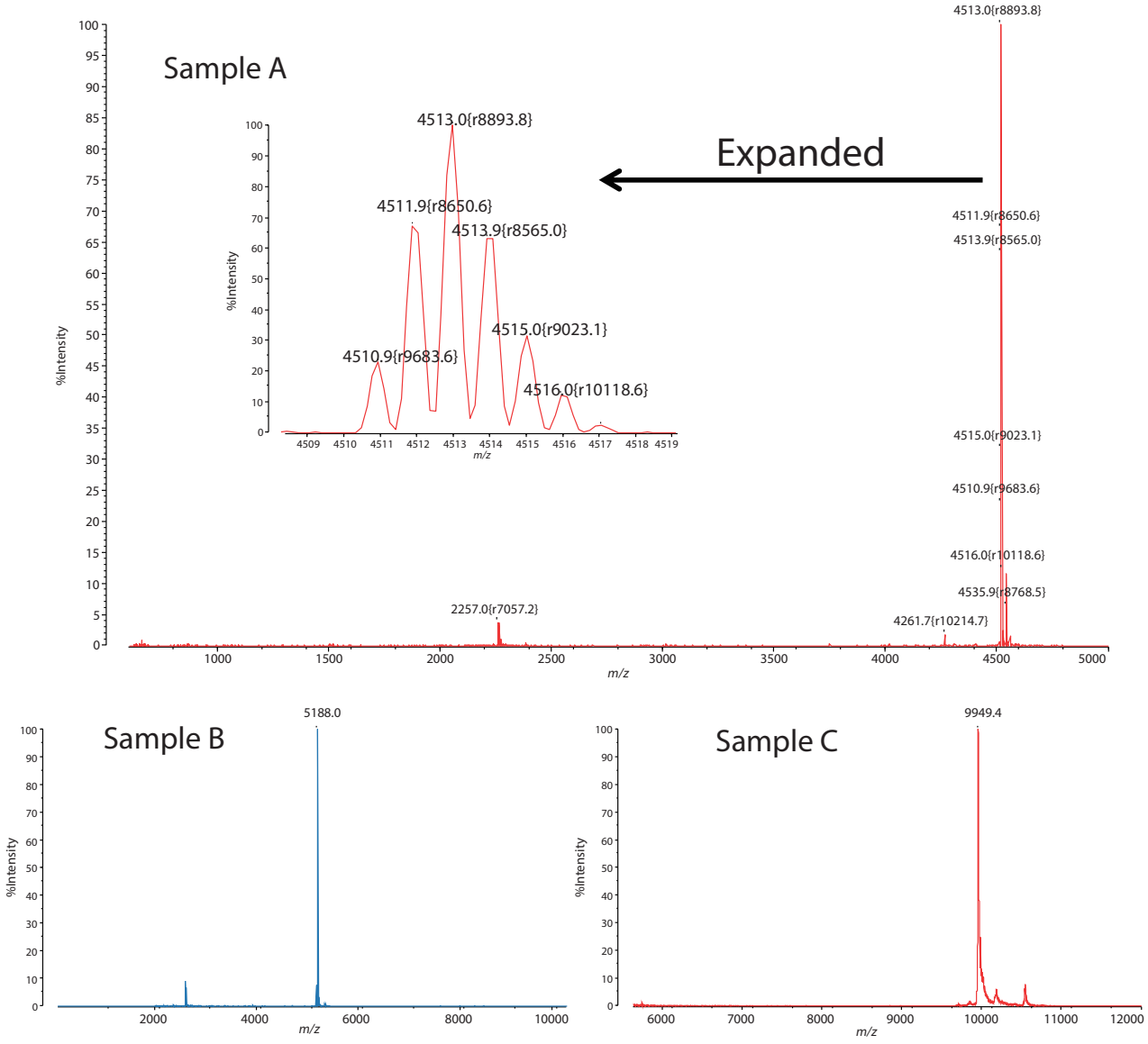

Fig. 3 Examples of PNA analysis using benchtop MALDI-TOF MS:
Sample A (2 pmol in CHCA) shows excellent resolving power (>8000 demonstrated) in linear mode.
Sample B (5.5 pmol) and sample C (7.5 pmol), both in sinapinic acid, demonstrating average mass detection in the higher mass range.

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