Oligonucleotide analysis by Linear MALDI-TOF mass spectrometry

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Introduction

Matrix assisted laser desorption/ionization (MALDI) mass spectrometry is a powerful tool for performing oligonucleotide (ON) analyses. MALDI-TOF mass spectrometry is routinely applied to the analysis of ON for the investigation of naturally occurring anomalies like single nucleotide polymorphisms (SNPs), alternative splicing, methylation and for purposes of quality control in synthetic production. Despite its popularity, MALDI analysis of ON is more particular to analytical conditions in comparison to MALDI analysis of proteins and peptides. In working with synthetic, purified, single stranded DNA samples, even subtle differences in analytical formulation, spotting procedure and instrument acquisition parameters can have a profound impact on analytical success.

Methods

Sample matrix preparation

Normal preparation (pH ~ 4.0)

3-hydroxypicolinic acid (3-HPA)

-**Stock solution** at 50mg/mL in 1:1 (acetonitrile:H2O)

diammonium citrate

-**Stock solution** at 50mg/mL in H_2O

Working matrix solution

combine 3-HPA and diammonium Citrate stock in 9:1 ratio.

9 parts HPA : 1 part diammonium Citrate

Acidic preparation (pH ~ 3.0)

3-hydroxypicolinic acid (3-HPA)

-**Stock solution** at 50mg/mL in 1:1 (acetonitrile:H_2O 0.1% TFA)

diammonium Citrate

-**Stock solution** at 50mg/mL in H_2O

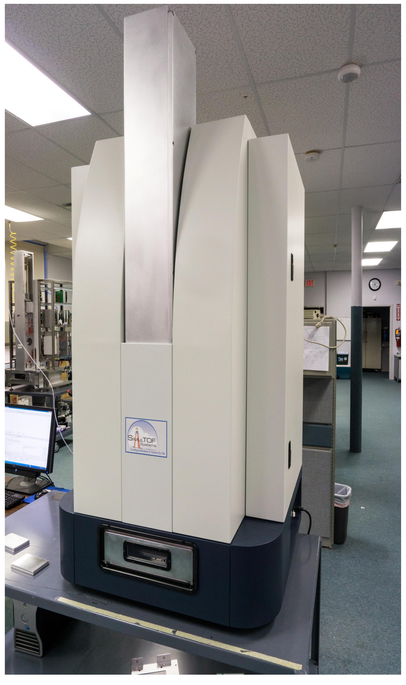
Working-matrix solution

combine 3-HPA and diammonium Citrate stock in 9:1 ratio.

9 parts HPA : 1 part diammonium Citrate

Sample deposition

1) Mix working matrix solution with sample 1:1 to give ~ 0.1 – 1 pmol / uL [sample]
2) Spot matrix on sample plate and allow to dry then ***overlay*** sample on matrix crystals



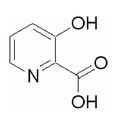
-The **SimulTOF 100 MALDI-TOF** mass spectrometer is a bench-top linear system that is well suited for oligonucleotide, intact protein, and peptide analyses.

-Using high energy ion acceleration and post acceleration in the detector, the SimulTOF 100 provides sensitivity and dynamic range for detection for ON, protein and peptides

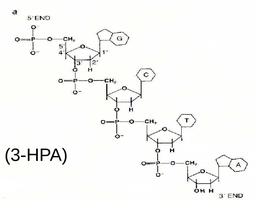
-**5000 Hz** laser and **5mm / sec** scan speed allows for routine high throughput analysis

-Aquisition parameters for current analysis
-laser *f* = 3000 hz
-scan speed = 3 mm / sec
-laser power = 18 µ joule
-Signal average = 500 shots / spectrum

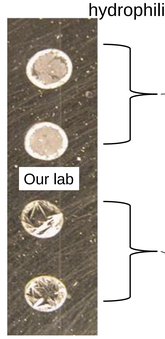
Results



3-hydroxypicolinic acid (3-HPA)

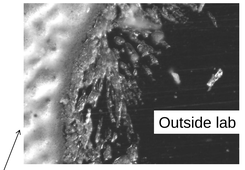


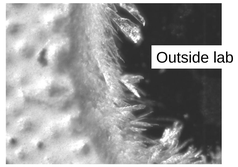
Oligonucleotide structure has both hydrophilic and hydrophobic character

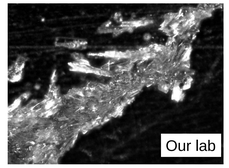


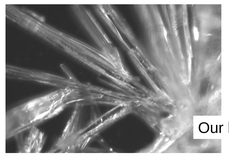
pH ~ 4.0

pH ~ 3.0







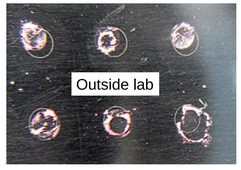


Effects of sample preparation pH

HPA matrix at pH 4 (normal preparation) tends to crystallize into rings with matrix and sample depositing on around the perimeter of the spot

HPA matrix with pH 3 (acidic preparation) crystallized into large shards with **marked drop-off in signal intensity**

Subtle change in pH has a dramatic effect on crystal morphology. This change in morphology may impact ON incorporation and ionization? 3-HPA has pKa of ~4, therefore lower pH will keep the molecule protonated. Results are confirmed with preps from other labs.



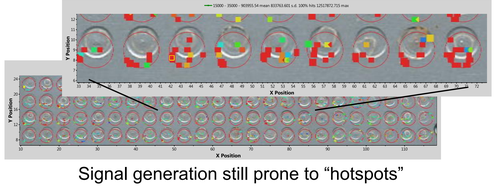
Other observations

- Drying time similar for both preparations ~4-5min
- Sample deposition method (co-mingled or overlay) had no effect on results

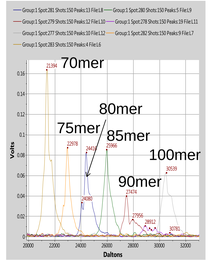
Sample analysis

-With proper sample preparation SimulTOF 100 is easily capable of detecting ON with 100s of bases

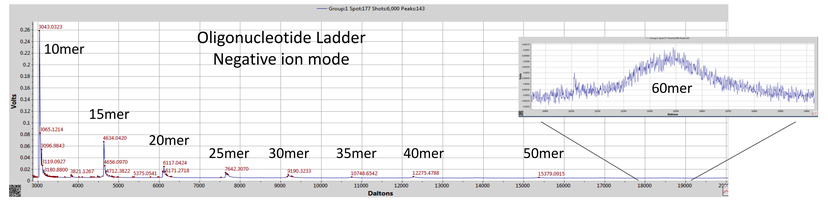
-Regardless, the sample preparation is still prone to having signal generate from discreet locations in the sample (hotspots)

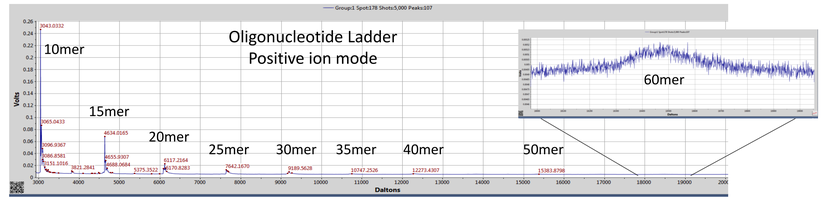


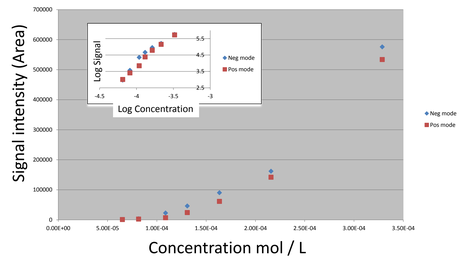
Signal generation still prone to “hotspots”



With proper sample preparation analysis of ON spanning a large mass range is possible







Sample Acquisition

-despite the “hotspot” phenomenon thorough interrogation of sample produces reasonably quantitative signal across an order of magnitude in mass range and sample concentration

-Results are consistent for both positive and negative modes of instrument operation

Conclusions

-Success of ON analysis by MALDI mass spectrometry is highly dependent on proper sample preparation

-Subtle changes in 3-HPA matrix preparation formulation can have drastic impact on crystal morphology

-Subtle changes in ON sample formulation (pH) can have a drastic impact on signal intensity

-Analyses using 3-HPA matrix are prone to “hotspots” in sample signal generation

-Adequate sample interrogation can still produce quantitative signal intensity over a large range in sample mass and concentration

-Both the quality and quantity of signal are similar in positive and negative modes of instrument operation

References

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2)S. Sauer; “The essence of DNA sample preparation for MALDI mass spectrometry.” J. Biochem. Biophys. Methods. 2007; (70): 311-318

3)S. Sauer, D. Lechner, I. G. Gut; *Mass Spec and Genomic Analysis*. Kluwer Academic Publishers, Norwell, MA (2001):49-55