

BMG/PHR 744
02-02-11 class

Nucleic acid and nucleotide mass spectrometry

Stephen Barnes, PhD

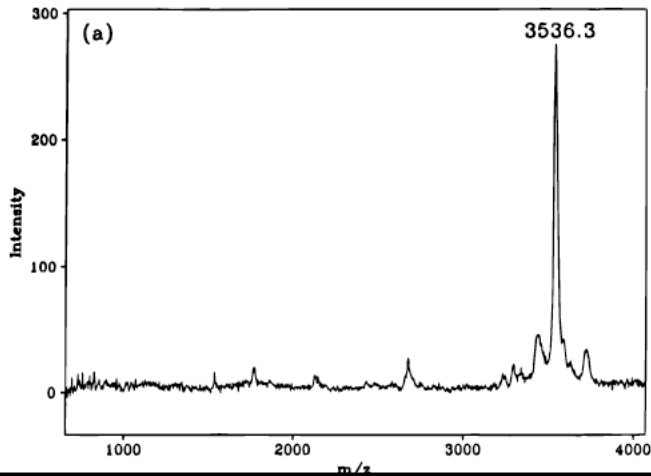
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Synopsis

- **MALDI**
 - Sample prep
 - UV laser
 - SNPs, multiplexing
 - IR laser (large DNA/RNA)
- **ESI**
 - Bases
 - Nucleosides
 - Nucleotides

UV-MALDI TOF of nucleic acids

- Limited in the number of residues (up to 70 nt)



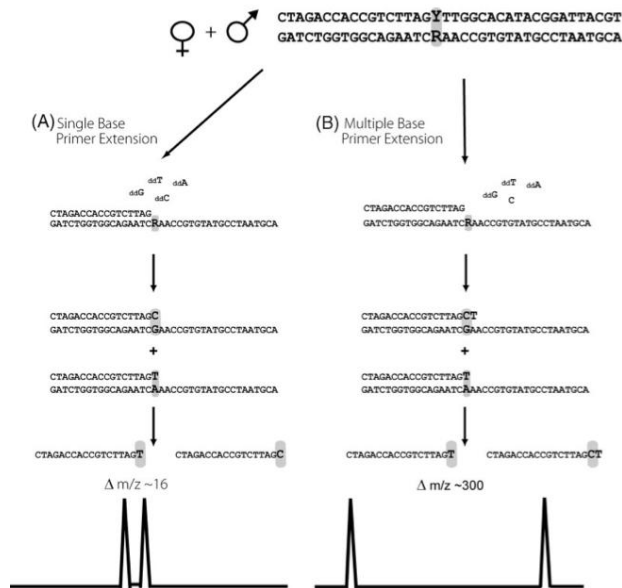
12 nt Antisense Oligo DNA

Ferulic acid as the matrix

254 nm laser

Keough et al., 1996

Single nucleotide polymorphisms (SNPs)



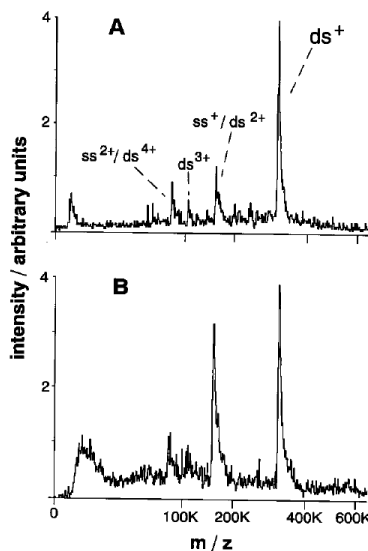
Infra-red MALDI-TOF of DNA

516 nt PCR product

Pptd with EtOH from 2 M ammonium acetate

IR laser at 2.4 μm

Sample in glycerol



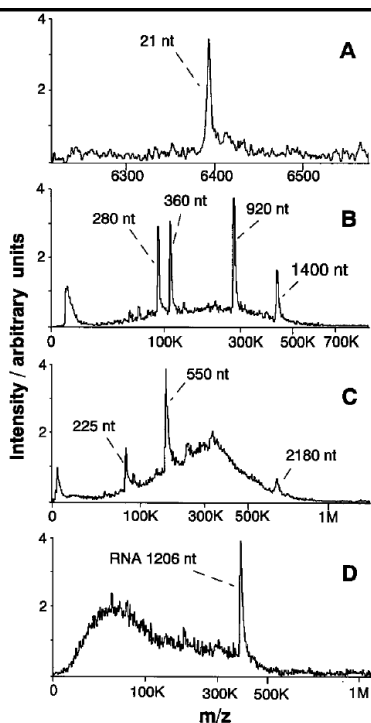
300 fmol, 1 shot

300 amol, 30 shots

Berkenkamp et al., Science 1998

IR MALDI of Nucleic acids

oligo



Pptd with EtOH from 2 M ammonium acetate

IR laser at 2.4 μm

Sample in glycerol

Berkenkamp et al., Science 1998

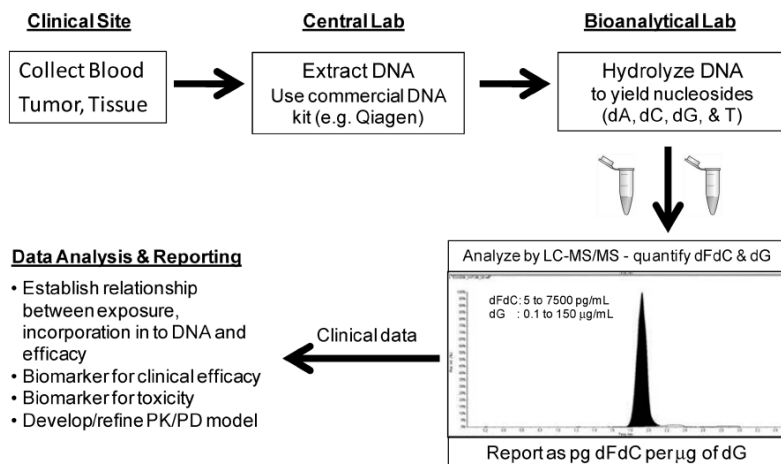
Fundamentals of nucleic acids analysis by ESI-mass spectrometry

- **Analysis as bases, nucleosides**
 - No different from other small molecules
 - Bases are sufficiently hydrophobic to be analyzed by reverse-phase LC-MS
 - The addition of ribose or deoxyribose not a problem
 - Detected in the positive ion mode
- **Nucleotides are more difficult**
 - The phosphate group reduces net charge
 - Na⁺ salts cause multiple ion states

LC-MS analysis of nucleosides

- **There are nucleoside drugs**
 - e.g., gemcitabine (2,2'-difluorocytidine)
 - Xu et al., J. Chromatogr B (2004)
 - LC-MS – negative ESI – m/z 262
 - Honeywell et al., J Chromatogr B (2007)
 - LC-MRM-MS – positive APCI – m/z 264/112
 - Bowen et al., J Chromatogr B (2009)
 - uPLC-MRM-MS – dansyl derivatives – 1.5 min

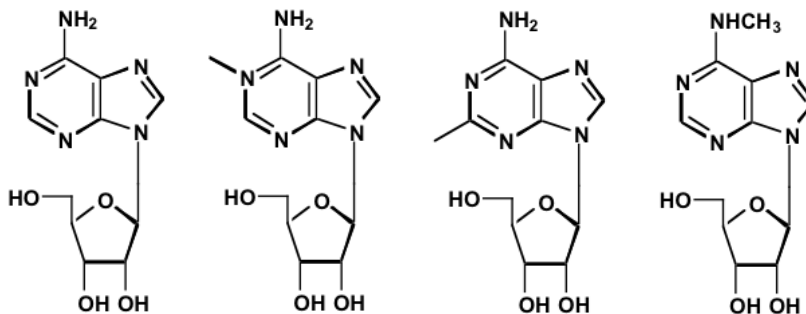
Measuring gemcitabine in DNA



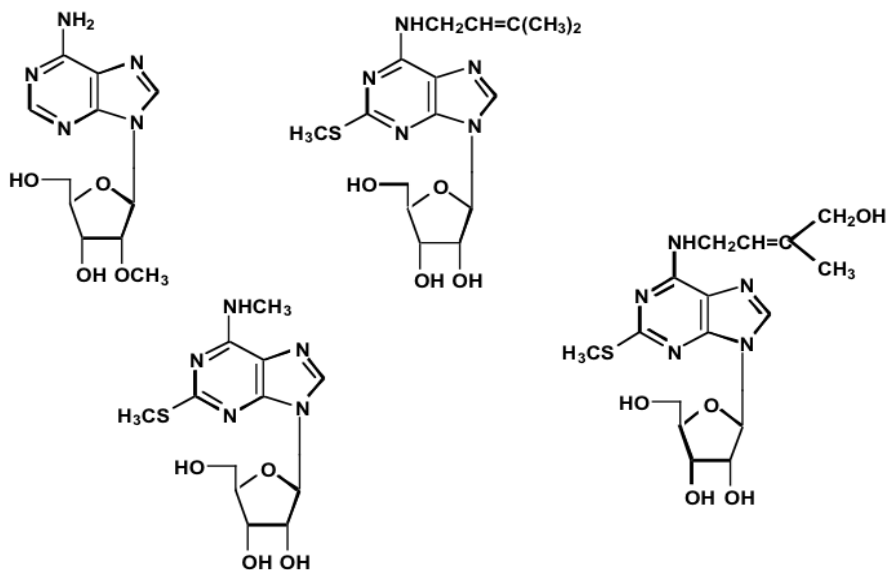
Wickremsinhe et al., Anal Chem 2010

Nucleosides

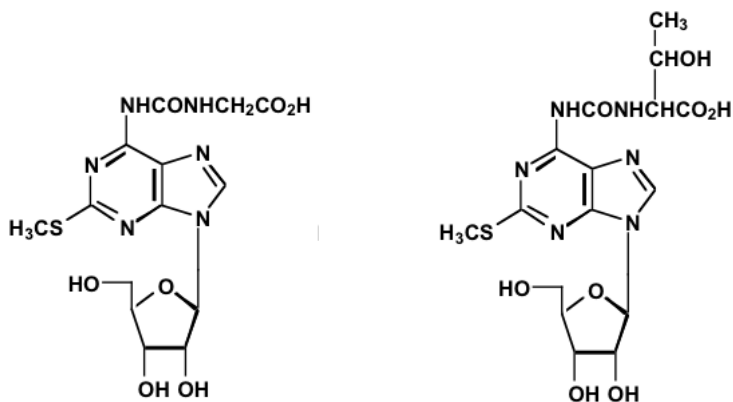
- Nucleosides are not just adenosine, cytosine, guanosine or uridine
 - “Different strokes for different folks”



More hydrophobic nucleosides



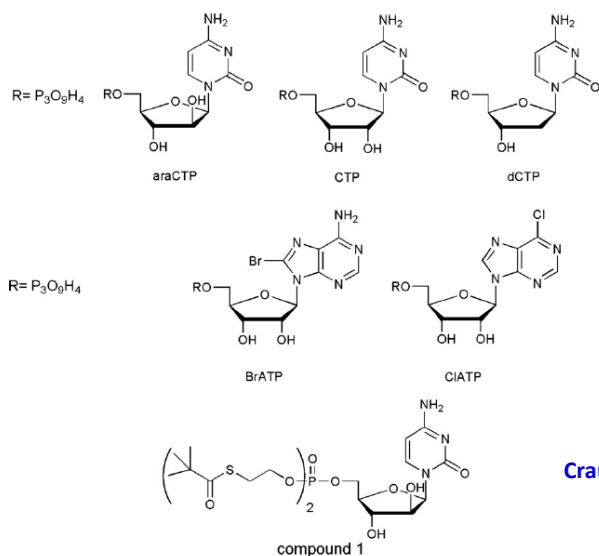
Hydrophilic nucleosides



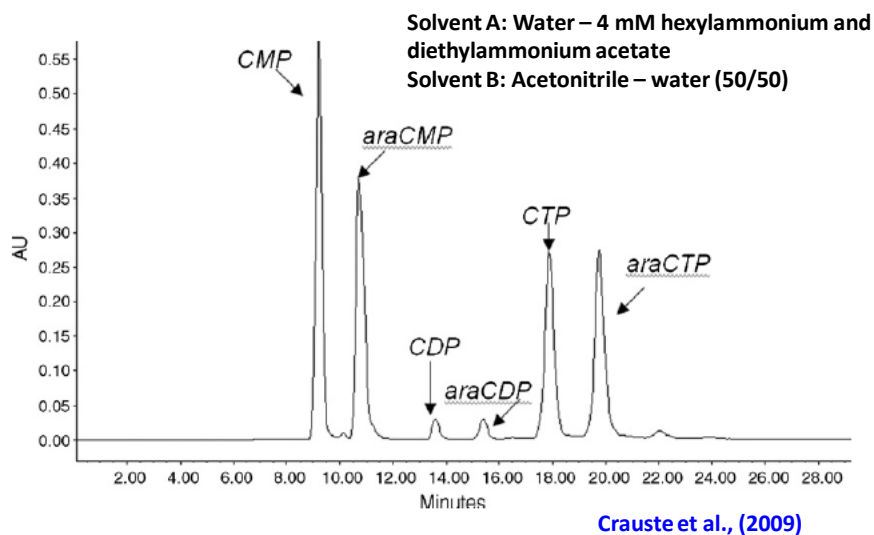
Nucleotides

- Important to convert them to ammonium, not Na^+ salts
 - DNA/RNA reprecipitated from 2 M ammonium acetate by alcohol at -20°C
- Nucleotides can be analyzed by LC-MS using a hydrophobic counter ion
 - E.g., tetrabutyl ammonium acetate (TBAA)
- Both NH_4^+ and TBAA salts dissociate in the ESI interface

CTP and ATP derivatives



LC-UV of NTPs



Sample prep for NTPs from cells

Washed cells extracted with 60% aqueous methanol and kept at -20°C overnight



Extract centrifuged at 13,200 xg for 3 minutes at RT



NTPs eluted with methanol-water-ammonia (24:5:1)

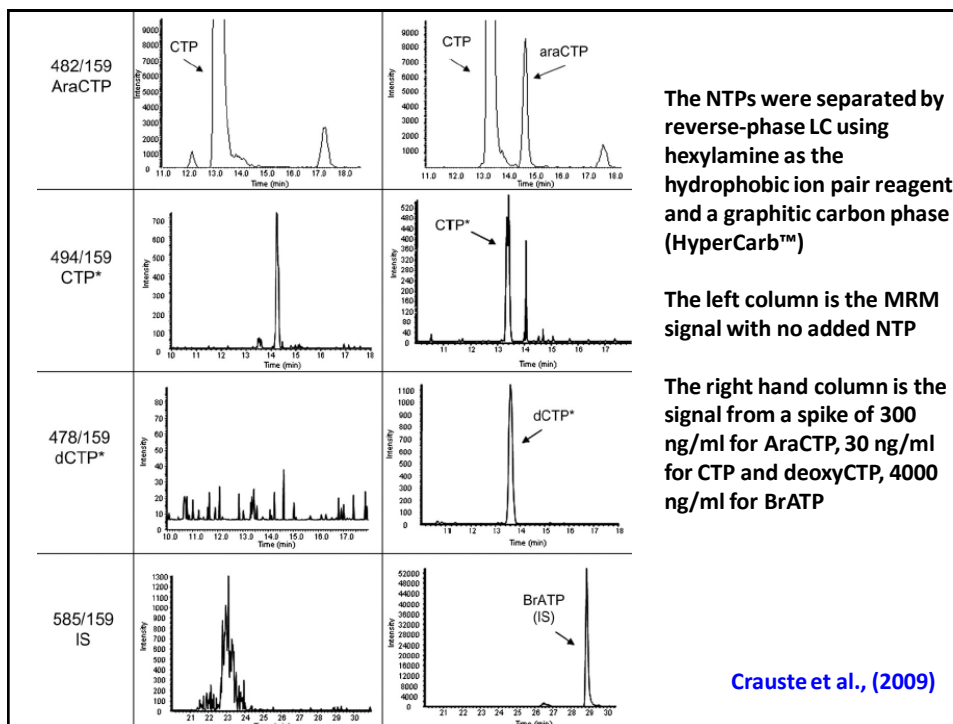


Supernatant diluted in 60% aqueous methanol and passed over a weak anion exchange column.

Evaporated to dryness and reconstituted in LC starting buffer.

Column washed with 50 mM ammonium acetate, pH 4.5

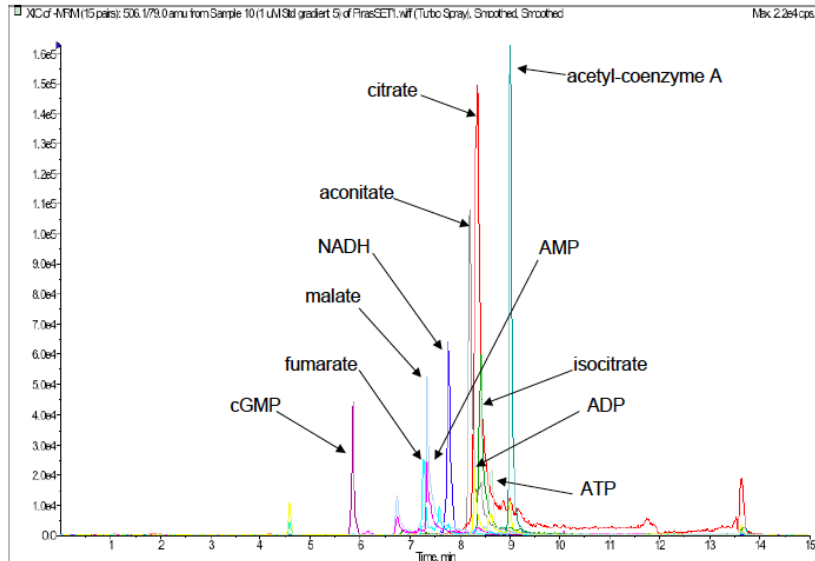
Crauste et al., (2009)



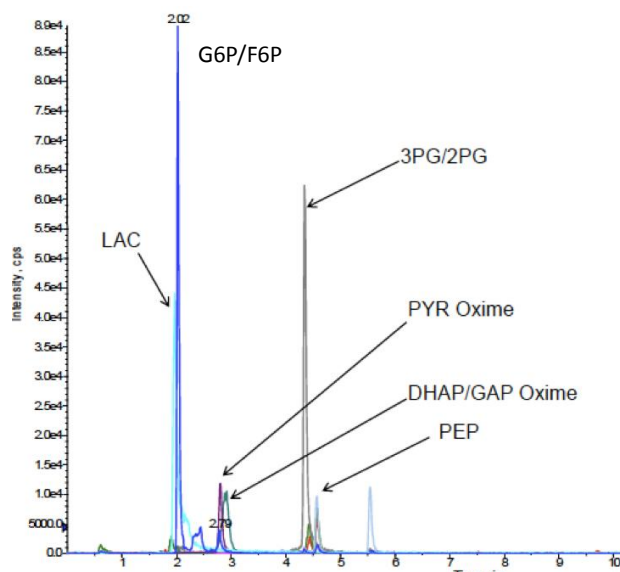
High energy intermediates

- Hexose and triose phosphates as well as Krebs cycle intermediates
 - These are very hydrophilic compounds
- Do not separate well on a reverse-phase column
 - Good separation in tributylammonium and better in tetrabutylammonium acetate
- “Naked” molecular ions

Ion-pair LC-MRM-MS of energy intermediates



Glycolytic intermediates by LC-MS



Suggested reading (ESI)

- Crauste C, Lefebvre I, Hovaneissian M, Puy JY, Roy B, Peyrottes S, Cohen S, Guitton J, Dumontet C, Perigaud C. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009;877:1417-1425.
- Cantara WA, Crain PF, Rozenski J, McCloskey JA, Harris KA, Zhang X, Vendeix FA, Fabris D, Agris PF. *Nucleic Acids Res.* 2011;39(Database issue):D195-201.
- Wickremsinhe ER, Lutzke BS, Jones BR, Schultz GA, Freeman AB, Pratt SE, Bones AM, Ackermann BL. *Anal Chem.* 2010;82:6576-6583.
- Xu Y, Keith B, Grem JL. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2004;802:263-270.
- Honeywell R, Laan AC, van Groeningen CJ, Strocchi E, Ruiten R, Giaccone G, Peters GJ. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007;847:142-152.
- Bowen C, Wang S, Licea-Perez H. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009;877:2123-9.

Suggested reading (MALDI)

- Tost J, Gut IG. *J Mass Spectrom* 2006; 41:981-995.
- Berkenkamp S, Kirpekar F, Hillenkamp F. *Science.* 1998;281:260-262.
- Mengel-Jørgensen J, Sanchez JJ, Børsting C, Kirpekar F, Morling N. *Anal Chem.* 2004;76:6039-6045.
- Keough T, Shaffer JD, Lacey MP, Riley TA, Marvin WB, Scurria MA, Hasselfield JA, Hesselberth EP. *Anal Chem.* 1996;68:3405-3412.