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All posters, sessions, breaks and receptions will be in the Long/Bird/Indian Ballroom

Speakers – please arrive ½ hour before your session begins to load your presentation.

Friday, January 22

6:00 – 7:00 pm **Registration**, *Outside of the Long/Bird/Indian Ballroom*

7:00 – 8:00 pm
Opening Lecture
Session Chair, Patrick Limbach, *University of Cincinnati*
Long/Bird/Indian Ballroom

7:15 – 8:15 pm **Opening Lecture: Juan Alfonzo**, *The Ohio State University*
"RNA Editing or Sequence Mistake? Dial 1-800-mss-spec"

8:15 – 10:00 pm **Reception/Informal Poster Session,** *setup all posters*

Saturday, January 23

8:00 – 9:00 am **Continental Breakfast**, *Long/Bird/Indian Ballroom*

9:00 – 10:30 am
Fundamental Mass Spectrometry Approaches pt I
Session Chair: Jennifer Brodbelt, *University of Texas*
Long/Bird/Indian Ballroom

9:00 – 9:30 am **Franz Hillenkamp**, *University of Muenster*
"MALDI-MS of Nucleic Acids: Problems, Promise and Performance"

9:30 – 10:00 am **Edwin de Pauw**, *Universite de Liege*
"On the Role of H+ and H° in Oligonucleotides Reactivity: H/D Exchange and ISD"

10:00 – 10:30 am **Patrick Limbach**, *University of Cincinnati*
"Quantitative Identification of Modified Nucleic Acids"

10:30 – 11:00 am **Coffee Break**

11:00 am – 12:30 pm
Fundamental Mass Spectrometry Approaches pt II
Session Chair: Jennifer Brodbelt, *University of Texas*
Long/Bird/Indian Ballroom

11:00 – 11:30 am **Michael L Gross**, *Washington Univ*
"Mass-Spectrometry-Based Methods and Outcomes for Exploring DNA Photodamage and DNA/Protein Interactions"

11:30 – 12:00 pm **Henning Urlaub**, *Max-Planck-Institute*
"Detection and Sequencing of Protein-RNA Conjugates by Mass Spectrometry"

12:00 – 12:30 pm **Edward Dudley**, *University of Swansea*
"Mass Spectrometric Analysis of Urinary Modified Nucleosides"

Saturday is continued on the next page

Saturday, January 23

7:30 – 9:00 pm
Emerging Methods for Nucleic Acids Analysis
Session Chair: Yinsheng Wang, *UC Riverside*
Long/Bird/Indian Ballroom

7:30 – 8:00 pm	**Scott McLuckey**, *Purdue University* "Ion/Ion, Ion/Molecule, and Unimolecular Reactions of Multiply-Charged RNA and LNA Oligomers"
8:00 – 8:30 pm	**Christiane Honisch**, *Sequenom* "Nucleic Acid Analysis by MALDI-TOF MS – Standardized Protocols, Software Solutions and Applications"
8:30 – 9:00 pm	**Daniele Fabris**, *Univ Maryland, Baltimore County* "MS-based Approaches for the Elucidation of Nucleic Acids Higher Order Structure"
9:00 – 10:30 pm	**Poster Session & Reception**, *odd-number posters will be presented*

Sunday, January 24

8:00 – 9:00 am **Continental Breakfast**, *Banyan Breezeway*

9:00 – 10:30 am
Pharmaceutical and Clinical Applications of Nucleic Acids Mass Spectrometry, pt I
Session Chair: Edward Dudley, *University of Swansea*
Long/Bird/Indian Ballroom

9:00 – 9:30 am **Herbert Oberacher**, *Innsbruck Medical University*
"Mass Spectrometry of Nucleic Acids as a Tool in Pharmaceutical Research"

9:30 – 10:00 am **Linlin Zhao**, *University of Connecticut*
"High Throughput Genotoxicity Screening Using Magnetic Bioreactors with LC-MS/MS"

10:00 – 10:30 am **Julie Farand***, Merck*
"Sequence Determination of Highly Modified Oligonucleotides"

10:30 – 11:00 am **Coffee Break,** *Long/Bird/Indian Ballroom*

11:00am – 12:30 pm
Pharmaceutical and Clinical Applications of Nucleic Acids Mass Spectrometry, pt II
Session Chair: Edward Dudley, *University of Swansea*
Long/Bird/Indian Ballroom

11:00 – 11:30 am **Yinsheng Wang**, *UC Riverside*
"LC-MS/MS for Interrogating the Replication of DNA Adducts in Cells"

11:30 am– 12:00 pm **Natalia Tretyakova**, *University of Minnesota*
"Mass Spectrometry of DNA-Protein Cross-links"

12:00 – 12:30 pm **Steven Hofstadler,** *Ibis Biosciences*
"High Throughput Mass Spectrometric Analysis of Amplified Nucleic Acids: Applications in Pathogen Detection and Human Forensics"

12:30 – 2:00 pm **Group Lunch**

Sunday is continued on the next page

Sunday, January 24

7:30 – 9:00 pm
Emerging Technologies for Analysis of Nucleic Acids
Session Chair: Kristina Hakansson, *Univ Michigan*
Long/Bird/Indian Ballroom

7:30 – 8:00 pm	**Jennifer Brodbelt**, *University of Texas* "Photodissociation of DNA Complexes and DNA Adducts"
8:00 – 8:30 pm	**Anders Geissing**, *University of Southern Denmark* "A Chip-based nano-LC Ion-Trap ESI-MS^n platform for Mass Spectrometry of Nucleic Acids"
8:30 – 9:00 pm	**Kristina Hakansson**, *Univ Michigan* "Gas-phase Ion-Electron Reactions and Hydrogen Exchange for Mapping Nucleic Acid Higher Order Structure"
9:00 – 10:30 pm	**Poster Session & Reception**, *even-number posters will be presented*

Monday, January 25

7:00 – 9:00 am	**Continental Breakfast**, *Long/Bird/Indian Ballroom* *Remove all posters by 11:30 am*

9:00 – 11:15 am
Structural Biology of Nucleic Acids & Protein Complexes
Session Chair: Daniele Fabris, *Univ Maryland, Baltimore Count*
Long/Bird/Indian Ballroom

9:00 – 9:30 am	**Carol Robinson**, *University of Cambridge* "RNA - A Structural Biology Perspective"
9:30 – 10:00 am	**Peter Stockley**, *University of Leeds* "Defining a Virus Assembly Pathway by Mass Spectrometry"
10:00 – 10:30 am	**Stuart Le Grice,** *National Cancer Institute* "Examining HIV Proteins, Nucleic Acids and Nucleoprotein Complexes by Mass Spectrometry"
10:30 – 11:00 am	**Sarah Woodson**, *Johns Hopkins University* "RNA Folding During Ribosome Assembly"
11:00 – 11:15 am	**Closing Remarks** Dan Fabris

Posters

Odd-numbered posters will be presented on Saturday night. Even-numbered posters will be presented on Sunday night

1 **Mass Spectrometry Based Detection of Pseudouridines in RNA;** Balasubrahmanyam Addepalli[1], Patrick A. Limbach[1], Pavanapuresan Vaidyanathan[2,] Arun Malhotra[2]; [1]*Department of Chemistry, Rieveschl Laboratories for Mass Spectrometry, University of Cincinnati, Cincinnati, OH;* [2]*Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL*

2 **Investigation of Structure and Dynamics of HIV-1 5'-UTR by ESI-FTICR;** Sonnet Davis, Kevin Turner, Daniele Fabris; *University of Maryland Baltimore County Baltimore, MD*

3 **Direct Purification and Analysis of CRISPR RNA using Denaturing RNA Chromatography and ESI MS;** Sakharam P. Waghmare, Ambrosius P. Snijders and Mark J. Dickman; *Chelsi Institute, Dept of Chemical and Process Engineering, University of Sheffield, Sheffield, UK.*

4 **Ion Spectroscopy of Nucleic Acids;** V. Gabelica[1], F. Rosu[1], L. Joly[1], A. Giuliani[2], L. Nahon[2], G. Gregoire[3], D. Scuderi[4], J. Lemaire[4], P. Maitre[4], R. Antoine[5], P. Dugourd[5] and E. de Pauw[1] ;[1]*Mass Spectrometry Laboratory, University of Liège, Belgium,* [2]*Synchrotron Soleil, Gif-sur-Yvette, France,* [3]*Laboratoire de Physique des Lasers, Université Paris 13, France,* [4]*Laboratoire de Chimie Physique, Université de Paris Sud, France,* [5]*Laboratoire de Spectrométrie Ionique et Moléculaire, Université Lyon 1, France*

5 **Top-down Sequence Confirmation of q-PCR Oligonucleotides;** Alex Herrault; Travis S. Smith; *Biosearch Technologies, Inc., Novato CA*

6 **G-Quadruplex Formation Pathways Studied by Mass Spectrometry and Traveling Wave Ion Mobility Spectrometry;** L. Joly, F. Rosu, E. De Pauw and V. Gabelica; *Mass Spectrometry Laboratory, University of Liège, Belgium*

7 **Identification of New Photolytic and Photocatalytic Transformation Products of Antibiotic Trimethoprim in Aqueous Solutions by Combination NanoESI-QqTOF-MS-MS and LC-MS-MS;** Sotirios N. Katsikis[1], Anastasios Economou[1], Theodoros Roumeliotis[2] ,Spiros D. Garbis[2], Helen V. Botitsi[3], Despina F. Tsipi[3; 1]*University of Athens, Athens, Greece,* [2]*Foundation for Biomedical Research of the Academy of Athens, (B.R.F.A.A)., Athens, Greece,* [3]*General Chemical State Laboratory, Athens , Greece*

8 **Determination of Dye-Labeling Distribution in $tRNA^{Phe}$ and $tRNA^{Arg}$ using MALDI Mass Spectrometry and Thin Layer Chromatography;** Jaskiran Kaur, Barry S. Cooperman; *The Department of Chemistry, University of Pennsylvania , Philadelphia, PA*

9 **Shifting Charge State Distributions and Reducing the Presence of Metal Counter Ions in Oligonucleotides via Vapor Introduction into the Interface of a QqTOF;** Anastasia Kharlamova, Boone M. Prentice, Teng-Yi Huang, Scott A. McLuckey; *Department of Chemistry, Purdue University, West Lafayette, Indiana*

10 **Novel Complex Modifications in $tRNA^{Lys(UUU)}$ from *Trypanosoma brucei* Revealed by Mass Spectrometry;** *Jesper Schak Krog[1], Yaiza Español[2], Lluís Ribas de Pouplana[2] & Finn Kirpekar[1];* [1]Department of Biochem. and Mol. Biol., University of Southern Denmark, Odense M, Denmark, [2]**Institute for Research in Biomedicine,** Parc Científic de Barcelona, Barcelona, Spain.

11 **Agmatidine is the Modified Cytidine in the Anticodon of $tRNA^{Ile}$ in Archaea Base-Pairs with Adenosine but Not with Guanosine;** Kady Krivos[3], Susan P. Russell[3], Colette M. Castleberry[3], Debrabrata Mandal[1], Caroline Köhrer[1], Dan Su[2], Paul Blum[4], Patrick A. Limbach[3], Dieter Söll[2], Uttam L. RajBhandary[1]; *[1]Massachusetts Institute of Technology, Department of Biology, Cambridge, MA, USA, [2]Yale University, Departments of Molecular Biophysics and Biochemistry, New Haven, CT, USA [3]University of Cincinnati, Department of Chemistry, Cincinnati, OH, USA,[4]University of Nebraska, George Beadle Center for Genetics, Lincoln, NE, USA*

12 **Integrated Approach to GMP Quality Control of Oligonucleotides by LC-MS;** C. Rentel, H. Gaus, D. Capaldi; *Isis Pharmaceuticals*

13 **Ribosome Assembly Defects Identified by Mass Spectrometry;** Rebecca Rohlfs[1], Romel Dator[1], Steven Gregory[2], Al Dahlberg[2], and Patrick A Limbach[1];[1]*Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry, University of Cincinnati, Cincinnati, OH, [2]Department of Biology, Brown University, Providence, RI*

14 **Functional Characterization of the YmcB and YqeV tRNA Methylthiotransferases of *Bacillus subtilis;*** Susan P. Russell[3], Brian P. Anton[1,2], Patrick A. Limbach[3], Jason Vertrees, Simon Kasif[4,5], Elisabeth A. Raleigh[1], and Richard J. Roberts[1]*; *[1]New England Biolabs, Ipswich, MA, [2]Bioinformatics Program, Boston University, Boston, MA, [3]Department of Chemistry, University of Cincinnati, Cincinnati, OH, [4]Department of Biomedical Engineering and [5]Center for Advanced Genomic Technology, Boston University, Boston, MA*

15 **Enzymatic Syntheses and Biophysical Applications of Stable Isotopic Labeled Nucleotides and Their Analogs;** Thomas P. Shields, Lincoln G. Scott; *Cassia, LLC, San Diego, CA*

16 **193 nm Dissociation of Deprotonated DNA Oligonucleotides;** Suncerae I. Smith, Jennifer S. Brodbelt; *Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, TX*

17 **Interloop Photocrosslinking of Human Telomeric G-quadruplexes;** Dian Su, John-Stephen Taylor, Michael Gross; *Washington University in St. Louis*

18 **Mass Spectrometry of DNA-protein Cross-Links;** Erin Michaelson-Richie, Rachel Loeber, Colin Campbell, Natalia Tretyakova*; University of Minnesota, Minneapolis, MN*

19 **Interplay of Oxidative DNA Damage and DNA-Protein Complexes;** Ting Wang, Nicholas J. Amato, Rehana Zaidi, Timothy C. Mueser, Amanda C. Bryant-Friedrich; *University of Toledo, Toledo, OH*

20 **Direct Probing of Tertiary Structure of RNA Rev-Response Element by Solution X-ray Scattering and Mass Spectrometry 3D;** Yi Wang[1], Xiaobing Zuo[2], Alberto Berton[3], Timothy Garrett Jr[3], Dan Fabris[3], Yun-Xing Wang[2], Stuart F.J. Le Grice[1]; *[1]RT Biochemistry Section, DRP, NCI; [2]Structural Biophysics Laboratory, NCI; [3]Department of Chemistry and Biochemistry, University of Baltimore County, Baltimore*

21 **Gas-Phase Approaches for Investigating Nucleic Acids Higher Order Structures;** Joshua A. Wilhide[1], Shelley N. Jackson[2], Amina S. Woods[2], Daniele Fabris[1]; *[1]University of Maryland Baltimore County, Baltimore, MD, [2]National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD*

22 **High Throughput Genotoxicity Screening Using Magnetic Bioreactors with LC-MS/MS;** Linlin Zhao[1], James F. Rusling[1,2,3]; [1]*Department of Chemistry, University of Connecticut, Storrs, Connecticut, [2]Department of Cell Biology, University of Connecticut Health Center, Farmington, Connecticut, U. S. A., [3]School of Chemistry, National Univ. of Ireland at Galway, Ireland*

Poster 1

Mass Spectrometry Based Detection of Pseudouridines in RNA

Balasubrahmanyam Addepalli[1], Patrick A. Limbach[1], Pavanapuresan Vaidyanathan[2], Arun Malhotra[2];
[1]*Department of Chemistry, Rieveschl Laboratories for Mass Spectrometry, University of Cincinnati, Cincinnati, OH;* [2]*Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL*

The goal of this project is development of suitable mass spectrometry based method for accurate detection, quantification, and sequence placement of pseudouridine (ψ, an isomer of uridine) in a given RNA. The current methods of pseudouridine detection depend on either chemical (CMCT: N-cyclohexyl-N'-β-(4-methylmorpholinum) ethyl carbodiimide □-tosylate or acrylonitrile) derivatization or non-derivatization approaches (1). In the chemical approach, the pseudouridine specific chemical derivatives are detected by analysis of reverse transcriptase mediated radiolabeled primer extension products on a sequencing gel (2). Alternatively, the derivatized RNA, after subjecting to nuclease treatment, is analyzed by MALDI-MS (matrix- assisted laser desorption/ionization mass spectrometry analysis) where mass shifts due to chemical derivatization allows differentiation of pseudouridine from uridine (3-4). The non-derivative approach depends on the characteristic dissociation pattern of pseudouridine-containing oligonucleotides (referred to as selected reaction monitoring) by liquid chromatography coupled with electrospray ionization and tandem mass spectrometry (LC-ESI-MS/MS) (5). Using this non-derivative approach, here, we report the simultaneous detection of three pseudouridines (a process referred to as multiple reaction monitoring) in the helix 69 specific RNase T1 oligo (ψAACm^3ψAψAACG) of 23S rRNA. Further, we also report the development of hybrid method for detection and sequence placement of pseudouridine. In this method, the oligonucleotide mixture such as RNaseT1 digestion products is initially derivatized by CMCT. The carbodiimide labeled pseudouridines are subsequently detected by LC-ESI-MS/MS analysis. The possibility of quantification of pseudouridine at a given site and utility of this approach in finding modifications at novel sites will be discussed.

References:

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3. Durairaj A, Limbach PA. (2008) *Anal Chim Acta*. 612:173-181.
4. Mengel JJ, Kirpekar F. (2002) *Nucleic Acids Res.* 30:e135.
5. Pomerantz SC, McCloskey JA. 2005 Anal Chem 77:4687.

Poster 2

Investigation of Structure and Dynamics of HIV-1 5'-UTR by ESI-FTICR

Sonnet Davis, Kevin Turner, Daniele Fabris; *University of Maryland Baltimore County Baltimore, MD*

Our lab is interested in the development of approaches based on electrospray ionization and Fourier transform ion cyclotron resonance (ESI-FTICR) mass spectrometry for the investigation of structure/function relationships in large RNA molecules of viral origin. The ability of ESI to preserve relatively weak non-covalent complexes and the high resolution afforded by FTICR make their combination a unique platform for studying nucleic-acid assemblies. We have taken advantage of its favorable characteristics to investigate the structure and dynamics of the 5'-untranslated region (5'-UTR) of the genome of HIV-1. This non-coding sequence has been proposed to assume alternative conformations that function as a riboswitch regulating the different genomic and mRNA activities performed by the viral RNA.[1-4]

The 3D structure of a key section of 5'-UTR called Psi-RNA was recently elucidated by following an approach that combines classical monofunctional probes, bifunctional crosslinkers, and high-resolution MS detection, collectively known as MS3D.[4] This structure has revealed a tertiary GNRA loop-receptor interaction between the AUG (nt334-352) and SL1 (nt243-277) domains, in which both sequences are folded into full-fledged stemloop structures. At the same time, other probing experiments have suggested that AUG may be involved in a long-range pairing interaction with the U5 (nt105-125) region of 5'-UTR, thus losing its stemloop morphology.[1,3] Finally, the results of electrophoretic mobility shift assays (EMSA) and folding-prediction algorithms have lead to the proposal that U5 may in turn interact with SL1 by base-pairing with its palindromic loop, which is responsible for initiating genome dimerization. Taken together, the three interactions define putative conformers in which key structures are exposed or eliminated, thus providing a possible mechanism for turning on or off the respective functions.

We have applied ESI-FTICR to explore the stability of the alternative SL1:AUG, U5:AUG, and SL1:U5 interactions by employing synthetic oligonucleotides that recreate the pertinent regions of 5'-UTR. Competitive binding experiments have been carried out to assess the partitioning of the individual components among the three possible complexes. The assays were completed both in the absence and presence of the viral chaperone protein nucleocapsid p7 (NC).

Experiments using preformed U5:AUG complex have shown the detectable formation of the alternative SL1:AUG complex upon addition of SL1, consistent with the competitive formation of the GNRA loop-receptor interaction. This interaction, however, was readily eliminated by the addition of NC, which shifted the equilibrium completely toward U5:AUG formation. These observations are consistent with the greater stability expected for U5:AUG on the basis of the relatively large number of base pairing interactions, than for SL1:AUG that can only count on a few H-bonds and stacking interactions. The fact that U5:AUG represents the more thermodynamically favorable complex indicates that its corresponding 5'-UTR conformer should be the predominant form present during viral events requiring suppression of protein expression, which is accomplished by masking the AUG start codon. Alternatively, when protein expression is required, the AUG region may fold back in its discrete stemloop conformation that is translation competent. The fact that NC is clearly capable of enhancing the formation of the thermodynamic U5:AUG product supports its possible role as switch actuator, thus providing a mechanism for turning off its own expression.

Following similar strategies, we are now investigating the significance of the alternative SL1:U5 interaction, which could serve to switch on/off genome dimerization by masking the SL1 palindromic loop during specific phases of viral development. At the same time, we are employing ESI-FTICR to assess the effects of archetypical nucleic acids ligands on the stability of the alternative complexes, which

could yield valuable information for the possible development of new therapeutic strategies.

Acknowledgements: This research was funded by the National Institutes of Health (2R01GM064328) and the National Science Foundation (CHE-0439067). In addition, S. D. received student support provided by an NIGMS Initiative for Minority Student Development Grant (R25-GM55036) and Procter and Gamble.

References:

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2. J. C. Paillart, et al. *J Biol Chem*, 2004, 279, 48397-403.
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4. E. T. Yu, et al. *Proc Natl Acad Sci U S A*, 2008, 105, 12248-53.

Poster 3

Direct Purification and Analysis of CRISPR RNA using Denaturing RNA Chromatography and ESI MS

Sakharam P. Waghmare, Ambrosius P. Snijders and Mark J. Dickman

Chelsi Institute, Dept of Chemical and Process Engineering, University of Sheffield, Sheffield, UK.

Prokaryotes acquire virus resistance by integrating short fragments of viral nucleic acid into clusters of regularly interspaced short palindromic repeats (CRISPRs). CRISPRs are used by CRISPR-associated (Cas) proteins from the host to mediate an antiviral response that counteracts infection. After transcription of the CRISPR, a complex of Cas proteins termed Cascade, cleaves a CRISPR RNA (crRNA) precursor in each repeat and retains the cleavage products containing the virus-derived sequence. Assisted by the helicase Cas3, these mature crRNAs then serve as small guide RNAs that enable Cascade to interfere with virus proliferation. We have previously demonstrated that the generation of mature guide RNAs by the crRNA endonuclease subunit of Cascade is a specific requirement for antiviral defense [1].

We have used electrospray ionisation mass spectrometry (ESI MS) to further characterise the crRNA processing events performed by the Cascade-crRNA ribonucleoprotein complex. crRNA was directly purified from the Cascade-crRNA complex using denaturing RNA chromatography. Using this approach a significant reduction of metal ion adduct formation of the crRNA was observed during ultra high resolution time of flight MS (maXis, Bruker Daltonics). The ESI MS spectra of the purified mature crRNA is shown in Figure 1, with an observed molecular weight of 19660.803 Da. These results are consistent with a processed 61nt RNA fragment with a single CasE cleavage site present in each repeat given the size of the repeat (29 nucleotides) and spacer (32 nucleotides). The purified mature crRNA was also analysed using ESI MS/MS analysis following RNase T1 and A digestion. A number of oligoribonucleotides generated from the RNase T1 and A digests were assigned to the mature crRNA sequence. The intact MW analysis of the crRNA predicted the presence of a 5'-hydroxyl and 2'-3'-cyclic phosphate termini. Further evidence of the presence of the 2'-3'-cyclic phosphate termini was obtained following acid treatment of the purified crRNA. A corresponding mass shift (18 Da) is observed, demonstrating the conversion of the 2'-3'-cyclic phosphate to the 3' (or 2')-phosphate. The analysis of the crRNA using mass spectrometry has enabled further insight into crRNA processing by the Cascade-crRNA ribonucleoprotein complex.

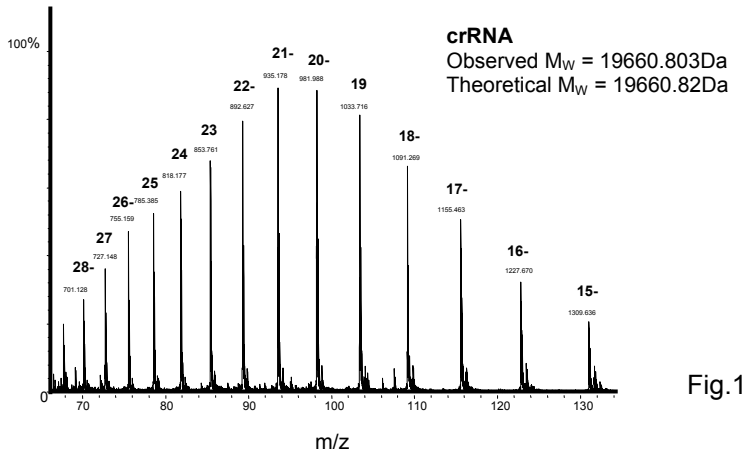


Fig.1

1) Brouns SJ, et al., Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science*. 2008 321 (5891) 960-4

Poster 4

Ion Spectroscopy of Nucleic Acids

V. Gabelica[1], F. Rosu[1], L. Joly[1], A. Giuliani[2], L. Nahon[2], G. Gregoire[3], D. Scuderi[4], J. Lemaire[4], P. Maitre[4], R. Antoine[5], P. Dugourd[5] and E. de Pauw[1] ;*[1]Mass Spectrometry Laboratory, University of Liège, Belgium, [2]Synchrotron Soleil, Gif-sur-Yvette, France, [3]Laboratoire de Physique des Lasers, Université Paris 13, France, [4]Laboratoire de Chimie Physique, Université de Paris Sud, France, [5]Laboratoire de Spectrométrie Ionique et Moléculaire, Université Lyon 1, France*

Electrospray mass spectrometry (ESI-MS) can be used to transfer large biomolecular complexes, including nucleic acids, from the solution to the gas phase. However, a longstanding question is whether the gas-phase multiply charged ions produced by ESI-MS keep a folded conformation in the absence of solvent. Our objective is to use IR and UV ion spectroscopy to probe the folding of nucleic acids ions produced by electrospray.

On the contrary to spectroscopy in solution which is an absorption spectroscopy, ion spectroscopy is an action spectroscopy: the absorption of IR and UV light by the ions must result into an action (e.g., fragmentation) that can be detected by the mass spectrometer. The ion cloud is irradiated with a laser, and the efficiency of the action as a function of the wavelength gives the action spectrum of the ion. Therefore, before discussing the nucleic acids IR and UV action spectra, we will also discuss about the nature of the action upon IR and UV irradiation.

The IR action spectra were recorded using the tunable free electron laser at the CLIO Facility in Orsay, France, which is coupled to a quadrupole ion trap. In the wavenumber range 900-1800 cm^{-1}, the action of the IR laser irradiation is vibrational excitation of the nucleic acids by multiple photon absorption, resulting in fragmentation pathways very similar to those observed by CID. When all fragments are taken into account, the action spectrum therefore reflects the absorption spectrum. In the region [1500-1800 cm^{-1}], we find an interesting signature of hydrogen bonding by the bases: NH_2 bending modes are blue-shifted, and C=O stretching modes are red-shifted upon hydrogen bond formation. Moreover, for cytosine-rich sequences, we find a signature of the formation of C–H^+–C base pairs in negative ions. This signature was used to probe the formation of intramolecular i-motif structures in the C-rich strand of telomeric DNA.

The UV action spectra were recorded using a OPO laser tunable in the range 220-300 nm coupled to a quadrupole ion trap, or the synchrotron radiation coupled to a linear ion trap to explore the VUV region (below 220 nm). Surprisingly, the action of UV light on nucleic acid anions is not fragmentation, but electron detachment. The action itself is therefore intriguing, and our current understanding of the electron photodétachment mechanism will be discussed. Briefly, the proposed mechanism is fast electron detachment from electronically excited states. However, we currently don't know whether every populated excited state has the same tendency to electron detachment, so the UV action spectrum may reflect the absorption spectrum of only those state that lead to photodetachment. We will present recent results comparing the action spectra of single strands as a function of the base composition and as a function of the charge. We will also present the comparison between single strand and duplex or quadruplex structures, and discuss the potential of UV spectroscopy to probe nucleic acid structure in the gas phase.

Poster 5

Top-down Sequence Confirmation of q-PCR Oligonucleotides

Alex Herrault; Travis S. Smith; *Biosearch Technologies, Inc., Novato CA*

Microbore LC combined with Q-TOF mass spectrometry enables top-down sequence confirmation of 18- to 55-mer dye/quencher modified oligonucleotides used in q-PCR reactions. The market for quantitative PCR (qPCR) is rapidly expanding, with applications beyond pathogen detection, drug development, biotechnology, and food safety. Of particular interest is the use of oligonucletides as Analyte Specific Reagents (ASRs) in detection of nucleic acid sequences associated with disease. Consequently, the need to better characterize the dual labeled probes and primer sets used as ASRs is increasing. The most accurate intact mass measurements used to qualify ASRs will only at best, confirm the empirical formula of an oligonucleotide, but will not confirm the actual primary sequence. The possibility of sequence defects is particularly acute in modern complex oligonucleotide probe synthesis, such as in dual labeled, internally modified probes**,** which have three or more non-native modifications. For this reason, a fast, precise analytical method that can detect differences in such oligonucleotide sequences is needed.

Two syntheses of eight disparate oligonucleotide primers (18 to 28 nucelotides) and two syntheses of five disparate fluorescence quenched probes, (44 to 56 nucleotides, each with different dye/quencher combinations) were fingerprinted in triplicate by LC-MS/MS and stored as controls. The primer sequences were synthesized with double base inversions at the 3'- and 5'- ends, as well as with two base inversions in the middle of the sequence. Additional fluorescence quenched probe sequences were synthesized with two base inversions at the 3'-end, two base inversions in the middle of the priming region, two base inversions in the stem-loop structure, as well as inversions in the position of the spacer and dye/quencher. All of these sequence inversions were fingerprinted in triplicate by LC-MS/MS.

Fingerprinting on Q-TOF MS was executed using the quadropole in non-resolving mode, thus transmitting all multiply charged ions to the collision cell. All fragmentation spectra were smoothed, background-subtracted, centered and stored in a spectral library. Replicate control sequences, as well as sequence inversions were compared by peak intensity and mass position. Match scores for replicate controls were averaged and the standard deviation calculated to establish the precision of this method, and the sensitivity to lot-to-lot variation. Sequence inversion scores were compared to controls to determine the sensitivity to changes in primary sequence.

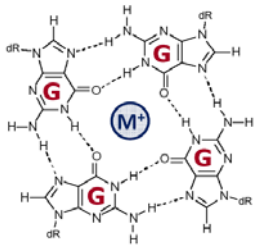
The top-down strategy was sensitive to all sequence inversions tested, on all compounds tested, with scores at least two standard deviations outside of the control mean. Compared to most enzymatic sequence confirmation strategies, this easily automated method represents an advance in speed, precision, and effectiveness against unintended modifications.

Poster 6

G-Quadruplex Formation Pathways Studied by Mass Spectrometry and Traveling Wave Ion Mobility Spectrometry

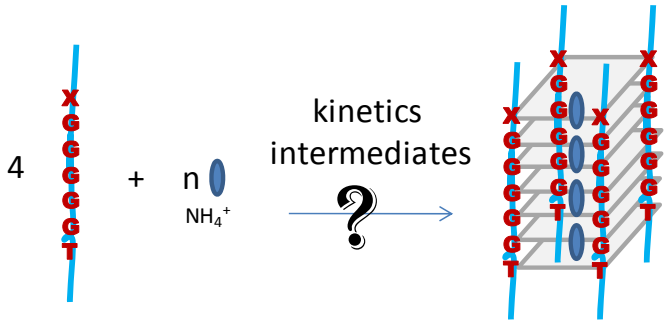
L. Joly, F. Rosu, E. De Pauw and V. Gabelica
Mass Spectrometry Laboratory, University of Liège, Belgium

DNA is well known to form the double helix structure. However a particular arrangement of DNA that can also be observed in guanine-rich sequences of telomeres and promoters is the G-quadruplex structure. The quadruplex folding corresponds to the stacking of G-quartet and is stabilized by Hoogsten hydrogen bonds and by cation incorporation between each G-quartet



Hydrogen bonding motif in a G-quartet

We investigated the formation kinetics of tetramolecular G-quadruplex structures by mass spectrometry and ion mobility spectrometry. The chosen DNA sequences differ from one base: TG_5T, AG_5T, G_6T, CG_5T. The influence of the first base substitution has been studied.



Electrospray mass spectrometry (negative ion mode, Q-TOF, Waters) allows to follow as a function of time the strand stoichiometry and the number of ammonium ions incorporated in each complex. Moreover, we use our recently developed method to estimate the relative response factors for the different species*. Monomers, dimers, trimers, quadruplexes but also pentamers and octamers are detected. The presence of octamer depends on the first base in DNA sequence.

The number of incorporated cations is an indicator of number of consecutive G-quartets**. Moreover, ion mobility spectrometry (IMS, SYNAPT, Waters) allows to know the conformation of intermediates and ultimate formed complexes. This technique is based on the spreading speed of the complexes in the drift tube and it used to obtain the experimental collision cross section. The intermediates were studied by IMS, and we found that the intermediates lacking one inner cation had a more extended conformation than the perfectly formed quadruplex. The octamer structure will also be discussed.

* Gabelica, V.; Rosu, F.; DePauw E. *Anal. Chem.*, 2009, **81**, 6708-6715
** Gros, J.; Rosu, F.; Amrane, S.; DeCian, A.; Gabelica, V.; Lacroix, L.; Mergny, J.L. *Nucleic Acids Res.*, 2007, **35**, 3064-3075

Poster 7

Identification of New Photolytic and Photocatalytic Transformation Products of Antibiotic Trimethoprim in Aqueous Solutions by Combination NanoESI-QqTOF-MS-MS and LC-MS-MS

Sotirios N. Katsikis[1], Anastasios Economou[1], Theodoros Roumeliotis[2] ,Spiros D. Garbis[2], Helen V. Botitsi[3], Despina F. Tsipi[3]; *[1]University of Athens, Athens, Greece, [2]Foundation for Biomedical Research of the Academy of Athens, (B.R.F.A.A)., Athens, Greece, [3]General Chemical State Laboratory, Athens , Greece*

The occurrence of pharmaceutical residues in the different environmental compartments has raised concern because they can disturb the natural ecosystems leading to a serious threat to human health as they are biologically active compounds with limited biodegrability. Residues of the antifungal compound trimethoprim have been detected in the wastewater effluent samples, which indicated that there were not completely eliminated in the sewage treatment plants. The present work is focused on the degradation of trimethoprim ($C_{14}H_{18}N_4O_3$) by means of photolysis and heterogeneous photocatalysis, under near visible/UV irradiation. Identification of its photoproducts was performed by nano-ESI high resolution quadrupole-time-of-flight (QqTOF) tandem mass spectrometry.

The degradation of pharmaceutical trimethoprim ($C_{14}H_{18}N_4O_3$) was studied under near visible/ UV irradiation in the presence and absence of a photocatalyst. After 6 h irradiation a number of photolysis products were identified by nESI-QqTOF-MS without LC separation. Several degradation products not present in a blank control were also observed after a few minutes irradiation of trimethoprim in TiO_2 aqueous suspension. Structure elucidation of photo species was based on the accurate mass measurements on both precursor and product ions obtained under positive ion mode. The product ion mass spectra of trimethoprim $[M+H]^+$ 291.1457 m/z was examined to obtain useful information for the interpretation of the product ion spectra of the unknown degradation products. The main transformation products identified were photooxidation and photoreduction derivatives of the target compounds. The results of nESI-QqTOF-MS were also confirmed by using LC separation on a triple quadrupole tandem mass spectrometer in the MRM mode. The mechanisms of photolysis and photocatalysis followed by the trimethoprim under near visible/UV irradiation, proposing different photoproducts resulting from the photodegradation of the target compound.

Poster 8

Determination of Dye-Labeling Distribution in $tRNA^{Phe}$ and $tRNA^{Arg}$ using MALDI Mass Spectrometry and Thin Layer Chromatography

Jaskiran Kaur, Barry S. Cooperman; *The Department of Chemistry, University of Pennsylvania , Philadelphia, PA*

We are using tRNAs labeled with Cy3 or Cy5 within the D-loop [1] to carry out single molecule FRET (smFRET) experiments [2] that monitor tRNA:tRNA and tRNA:ribosome interactions during the elongation cycle of protein synthesis. Such labeled tRNAs are produced via reaction at dihydroU (D) positions following $NaBH_4$ reduction [3]. tRNAs typically contain 1 – 3 D residues within the D-loop. Here we report a methodology to determine and quantify the sites of dye labeling within tRNA that assist our understanding of the smFRET results. Our approach is based on a combination of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry and thin-layer chromatography (tlc) analyses of RNase fragments to determine and quantify the sites of dye labeling. As one example, we report results obtained with $tRNA^{Phe}$ (E.Coli) which has D residues at positions 16 and 20. The samples analyzed have overall labeling stoichiometries of approximately 1.0 dye molecule/tRNA. MALDI analysis of unlabeled tRNA digested with RNase A shows peaks corresponding to the trinucleotides AGD^{16} and GGD^{20}. New peaks, corresponding to AGD^{16}-Cy3 and GGD^{20}-Cy3, as verified using MALDI-tof-tof are seen in the RNase A digest of Cy3-labeled $tRNA^{Phe}$ indicating at least partial labeling at both D^{16} and D^{20}. We quantified the labeled fragments by separating AGD^{16}-Cy3 and GGD^{20}-Cy3 by tlc, and determined their relative amounts to be approximately 1:1 by measuring the dye absorbance in the resolved nucleotides. This method of analysis is now being applied to other labeled tRNAs, using other RNAses (such as T1) as needed.

An unexpected result of the MALDI analysis has been the demonstration that dye substitution proceeds with retention of the ureidopropanol moiety that is the product of $NaBH_4$ reduction of D, rather than by replacement of this moiety as has been described in the literature [3].

Acknowledgement: This work is supported by NIH grants GM-071014 and GM-080376 and NIST grant 70NANB7H7011.

1. **Synthesis and functional activity of tRNAs labeled with fluorescent hydrazides in the D-loop.** Pan, D. Qin, H. and Cooperman, B. S. *RNA (2009),15:346–354.*

2. **Translation at the Single-Molecule Level**. Marshall, R. A., Echeverr´ıa Aitken, C., Dorywalska, M. and Puglisi, J. D. *Annu. Rev. Biochem. (2008),77:177-203.*

3. **Incorporation of amines or hydrazines into tRNA replacing wybutine or dihydrouracil** Wintermeyer, W., Schleich, H.G., Zachau, H.G. *Methods Enzymol.* (1979) 59:110-121.

Poster 9

Shifting Charge State Distributions and Reducing the Presence of Metal Counter Ions in Oligonucleotides via Vapor Introduction into the Interface of a QqTOF

Anastasia Kharlamova, Boone M. Prentice, Teng-Yi Huang, Scott A. McLuckey; *Department of Chemistry, Purdue University, West Lafayette, Indiana*

With ESI, standard oligonucleotide samples typically produce a distribution of molecular ions, varying in the number and type of metal counter ions present, causing a decrease in the signal-to-noise ratio. Several methods have previously been used to remove these adducts such as the use of chromatography, ethanol or ammonium acetate precipitation, use of cation exchange resin, or the addition of triethylammonium salts or piperidine to the solution. However, these methods are either time consuming or require the oligonucleotide solution to be altered. A new procedure has been developed in which chemical vapors are introduced into the interface of a QqTOF instrument. Acidic or basic vapors can be introduced to shift the charge state distribution of samples subjected to either positive or negative nanoelectrospray ionization. This process occurs in the nanoelectrosprayed droplet and enables one to change the pH of the droplet without changing the pH of the sample solution. The introduction of some acidic vapors also results in the reduction of the number of salt adducts observed in DNA, RNA and LNA samples. This enables the observation of $[M\text{-}nH]^{n-}$ and results in an overall cleaner spectrum. Different reagents have been studied to determine which cause a greater shift in the charge state distribution via proton transfer and which elicit the greatest decrease in the presence of metal counter ions. This new optimized procedure and its benefits in oligonucleotide analysis will be presented.

Poster 10

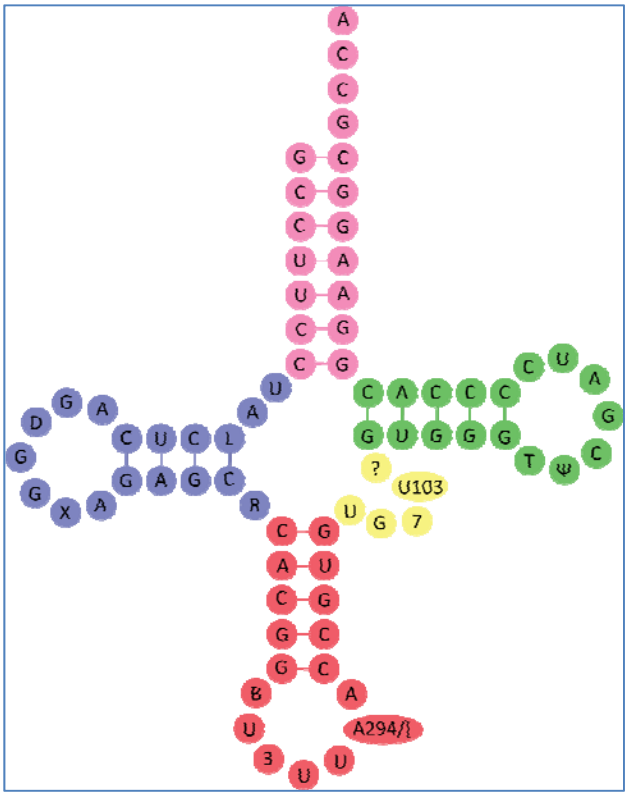
Novel Complex Modifications in tRNA$^{Lys(UUU)}$ from *Trypanosoma brucei* Revealed by Mass Spectrometry

Jesper Schak Krog[1], Yaiza Español[2], Lluís Ribas de Pouplana[2] & Finn Kirpekar[1]; [1]Department of Biochem. and Mol. Biol., University of Southern Denmark, Odense M, Denmark, [2]Institute for Research in Biomedicine**,** Parc Científic de Barcelona, Barcelona, Spain.

Transfer RNAs (tRNAs) are heavily altered on the route from the primary transcript to the final biologically active molecule. The alterations include trimming of the termini, removal/insertion of nucleotides and specific modifications of individual nucleosides, which vary from mass-neutral isomerisation to bulky additions that are larger than the original nucleoside. The nucleoside modifications are involved in numerous cellular processes, such as interaction between mRNA codon and tRNA anticodon, amino acid charging of tRNA and subcellular localisation.

As part of a project on the relation between tRNA$^{Lys(UUU)}$ modification and subcellular localisation, we have investigated the modification status of the cytoplasmic version of this tRNA. Since modifications in tRNA are abundant, we have used several approaches relying on enzymatic digestion followed by liquid chromatography, mass spectrometry, mass spectrometric sequencing and MS^n for a thorough localisation and identification of modified nucleosides. The main procedures were as follows:

- The primary screen was done by digestion with nucleotide-specific RNases (RNase A or RNase T1) followed by MALDI TOF analysis, which gave a sequence coverage of 95%.
- This was followed by sequencing on a MALDI QTOF instrument to locate modified nucleosides in the primary sequence.
- The modifications were identified at the nucleoside level by MS^n (up to MS^5) on an ion trap mass spectrometer and by chromatographic retention time; we employed primarily reverse phase chromatography to separate the nucleosides.
- Comparison with known tRNA modifications in other systems



We identified 12 modified positions in the tRNA, of which one showed a heterogeneous modification pattern. Two of the modifications have not been described previously, but MS^n data gave good indications of structural features.

L: 2-methylguanosine
D: Dihydrouridine
X: 3-(3-amino-3-carboxypropyl) uridine
R: N^2,N^2-dimethylguanosine
B: 2'-*O*-methylcytidine
3: 5-methoxycarbonylmethyl-2-thiouridine
A294: Novel adenosine modification of 294 Da
[: 2-methylthio-N^6-threonyl carbamoyladenosine
7: 7-methylguanosine
U103: Novel uridine modification of 103 Da
?: 5-methylcytidine
T: 5-methyluridine
Ψ: Pseudouridine

Thanks to Anders Giessing for a lot of help with the LC-MS^n.

Poster 11

Agmatidine is the Modified Cytidine in the Anticodon of $tRNA^{Ile}$ in Archaea Base-Pairs with Adenosine but Not with Guanosine

Kady Krivos[3], Susan P. Russell[3], Colette M. Castleberry[3], Debrabrata Mandal[1], Caroline Köhrer[1], Dan Su[2], Paul Blum[4], Patrick A. Limbach[3], Dieter Söll[2], Uttam L. RajBhandary[1]; *[1]Massachusetts Institute of Technology, Department of Biology, Cambridge, MA, USA, [2]Yale University, Departments of Molecular Biophysics and Biochemistry, New Haven, CT, USA [3]University of Cincinnati, Department of Chemistry, Cincinnati, OH, USA,[4]University of Nebraska, George Beadle Center for Genetics, Lincoln, NE, USA*

We have identified the modified cytidine which is present in the anticodon wobble position of the AUA-decoding $tRNA^{Ile}$ and which base pairs with adenosine instead of guanosine. This modification is essential in allowing $tRNA^{Ile}$ to read the isoleucine AUA codon. We have purified the two $tRNA^{Ile}$ species of *Haloarcula marismortui* to homogeneity and have shown that the minor of the two tRNA acceptors binds to the codon AUA, but not to AUC or AUG, on the ribosome. Gel electrophoretic analysis of partial alkali digest of the tRNA indicates that the modified cytidine carries at least one if not two positive charges. Tandem mass spectrometry sequencing of the RNase T1 and pancreatic RNase fragments shows that the modified cytidine is present in the anticodon wobble position and that the glycosidic bond is unusually labile. The modification adds 112 mass units to the molecular mass of cytidine. Accurate mass LC-MS nucleoside analysis confirms the presence of a modified nucleoside with an elemental composition of $C_{14}H_{25}O_4N_7$ for the nucleoside and $C_9H_{17}N_7$ for the base, demonstrating the absence of a C2-oxo group in the modified cytidine. MS/MS of the modified base strongly suggests that in the modified cytidine, agmatine (decarboxylated arginine) is linked to the C2 through a secondary amino bond. We are, therefore, proposing to name this modified cytidine agmatidine. Agmatidine is similar to lysidine in that it is also derived from an amino acid.

Agmatidine is also present in $tRNA^{Ile}$ purified from *Methanococcus maripaludis* and in total tRNA isolated from *Sulfolobus solfataricus*. Thus, it may be present in the AUA-decoding $tRNA^{Ile}$ of all euryarchaea and crenarchaea. The structure of agmatidine implies a pathway for its biosynthesis, similar to that of lysidine, through adenylation of the 2-oxo group of cytidine followed by nucleophilic attack on the activated carbon by the primary amino group of agmatine.

It is interesting that agmatine, a neuromodulator, shown to be essential for its role in polyamine biosynthesis in the archaeon *Thermococcus kodakaraensis* may also be essential in euryarchaea and crenarchaea for modification of the anticodon cytidine in the $tRNA^{Ile}$ required for decoding the codon AUA.

Poster 12

Integrated Approach to GMP Quality Control of Oligonucleotides by LC-MS

C. Rentel, H. Gaus, D. Capaldi; *Isis Pharmaceuticals*

Ion-pair LC-MS Method for Quality Control of Oligonucleotides

Oligonucleotide drugs contain structural very similar impurities which are difficult or impossible to detect by traditional chromatographic techniques. We have developed an ion-pair HPLC–ESI-MS method to determine assay and impurity profile of oligonucleotides that is suitable for use in the QC laboratory. Amounts of chromatographically resolved impurities are estimated by their UV response, whereas co-eluting impurities, similar in length and MS response, are quantified using their mass spectrometric signal. To increase MS sensitivity and facilitate data analysis, an ion-pair buffer system that enables MS detection of more than 70% of oligonucleotide molecules in a single charge state was developed. The non-linear response of the mass spectrometer is described by a second-order polynomial calibration curve, which serves to correct for reduced responses of components due to ion suppression. The method has been shown to be accurate, precise and robust. The limit of quantification is 0.2%. Stress studies have demonstrated the stability indicating nature of the method.

Full Impurity Profiling of Nucleoside Phosphoramidites by LC-MS

Nucleoside phosphoramidites are key starting materials for the synthesis of oligonucleotide drug substances. Control of critical phosphoramidite impurities, i.e. impurities causing modifications to the targeted oligonucleotide, is vital to ensuring drug substance quality. The linear nature of solid phase synthesis amplifies the impact of these phosphoramidite impurities, which means that analytical methods capable of detecting and identifying impurities at low levels are required. As HPLC methods do not allow unambiguous distinction of critical and non-critical impurities, and MS sensitivity for these compounds was found to be fairly low on quadruple and time-of-flight systems, we have developed an HPLC-ion trap MS method for identification and quantitation of impurities in phosphoramidites. Fragmentation patterns of 2′-deoxy and 2′-*O*-(2-methoxyethyl) nucleoside phosphoramidites and more than 300 impurities have been established. These data facilitate identification of known impurities as well as rapid structural elucidation and classification of unknown species. Qualification and validation data indicate the method is suitable for controlling the quality of nucleoside phosphoramidites intended for use in the GMP manufacture of therapeutic oligonucleotides.

Poster 13

Ribosome Assembly Defects Identified by Mass Spectrometry

Rebecca Rohlfs[1], Romel Dator[1], Steven Gregory[2], Al Dahlberg[2], and Patrick A Limbach[1]

[1]*Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry, University of Cincinnati, Cincinnati, OH,* [2]*Department of Biology, Brown University, Providence, RI*

The goal of this research is to examine ribosome assembly defects using mass spectrometry-based methods. The aim of this project is to use mass spectrometry to identify the post-transcriptional RNA modifications and post-translational ribosomal protein modifications that are unique or absent from improperly folded ribosome assembly subunits.

Escherichia coli is being used as the model system. Two strains of *E. coli* (K-12 and SK5665) are grown in the presence and absence of erythromycin at 25 ^{o}C and 37 ^{o}C. Subunits are isolated by use of sucrose fractionation. Post-transcriptional modifications to RNA are identified using liquid chromatography with ultraviolet absorption (LC-UV) and mass spectrometry (LC-MS). Ribosomal proteins are identified, and any post-translational modifications confirmed, using matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS).

When erythromycin sensitive cells are cultured in the presence of erythromycin, the 50S subunit is unable to properly form, and an intermediate 50S particle is formed which sediments between 30S and 50S. The assembly particle is composed of both 5S and 23S rRNA and an undetermined number of large subunit ribosomal proteins. In wild-type cells, this mis-folded particle is degraded by the RNase E complex. *E. coli* strain SK5665 cells contain a temperature-sensitive RNase E phenotype, and RNase E can be inactivated by culturing at 25 ^{o}C, thus allowing this mis-folded 50S intermediate to be accumulated and isolated by sucrose fractionation. Currently, we have been characterizing the rRNAs and ribosomal proteins present in this mis-folded 50S intermediate and comparing their identity and modification status to the rRNAs and ribosomal proteins found in properly assembled 50S subunits. These data will provide insight into differences at the primary structure level between ribosomal subunits that can correctly fold with those that do not fold correctly.

Poster 14

Functional Characterization of the YmcB and YqeV tRNA Methylthiotransferases of *Bacillus subtilis*

Susan P. Russell[3], Brian P. Anton[1,2], Patrick A. Limbach[3], Jason Vertrees, Simon Kasif[4,5], Elisabeth A. Raleigh[1], and Richard J. Roberts[1]*; *[1]New England Biolabs, Ipswich, MA, [2]Bioinformatics Program, Boston University, Boston, MA, [3]Department of Chemistry, University of Cincinnati, Cincinnati, OH, [4]Department of Biomedical Engineering and [5]Center for Advanced Genomic Technology, Boston University, Boston, MA*

The many post-transcriptional modifications found in transfer RNA play a central role in the structure and function of tRNA. These modifications, formed postranscriptionally by specific modifying enzymes, are frequently found in the anticodon wobble position, position 34, and in position 37, 3' adjacent to the wobble position in tRNA. The modifications present at position 37 in *Escherichia coli* and *Bacillus subtilis* are N^6-isopentenyladenosine (i^6A), 2-methylthio-N^6-isopentenyladenosine (ms^2i^6A), N^6-threonylcarbamoyladenosine (t^6A) and 2-methylthio-N^6-threonylcarbamoyl adenosine (ms^2t^6A). The modifying enzymes responsible for these tRNA modifications are methylthiotransferases (MTTases) and are a closely related family of proteins that perform both radical-*S*-adenosylmethionine (SAM) mediated sulfur insertion and SAM-dependent methylation to modify nucleic acid or protein targets with a methyl thioether group ($–SCH_3$). Members of two of the four known subgroups of MTTases have been characterized, typified by MiaB, which modifies N^6-isopentenyladenosine (i^6A) to 2-methylthio-N^6-isopentenyladenosine (ms^2i^6A) in tRNA, and RimO, which modifies a specific aspartate residue in ribosomal protein S12. In this work, we have characterized the two MTTases encoded by *Bacillus subtilis* 168 and find that, consistent with bioinformatic predictions, *ymcB* is required for ms^2i^6A formation (MiaB activity), and *yqeV* is required for modification of N^6-threonylcarbamoyladenosine (t^6A) to 2-methylthio-N^6-threonylcarbamoyladenosine (ms^2t^6A) in tRNA. We performed domain-swapping experiments between YmcB and YqeV to narrow down the protein domain(s) responsible for distinguishing i^6A from t^6A and found that the C-terminal TRAM domain, putatively involved with RNA binding, is likely not involved with this discrimination. An LC/MS based approach was used for the analysis of the mutant strains constructed.

Poster 15

Enzymatic Syntheses and Biophysical Applications of Stable Isotopic Labeled Nucleotides and Their Analogs

Thomas P. Shields, Lincoln G. Scott; *Cassia, LLC, San Diego, CA*

Mass spectrometry (MS) is but one powerful tool for the study of oligonucleotide structure. The routine production of stable isotopic labeled nucleotides remains critical to current MS methodology, allowing structural biology, pharmacology, and clinical research to be made possible. Cassia, LLC is a small biotechnology research and development company specializing in custom syntheses of stable isotopic labeled nucleotides for both biomedical and biophysical research. The *in vitro* recombination of enzymes from a variety of biosyntheses pathways and organisms, provides the tools necessary to execute robust and universal syntheses of nucleotides from very inexpensive precursors. These nucleotides have been employed in a number of MS-based, as well as, nuclear magnetic resonance (NMR) spectroscopy applications. A variety of syntheses and applications will be presented, as well as, new directions being explored.

Poster 16

193 nm Dissociation of Deprotonated DNA Oligonucleotides

Suncerae I. Smith, Jennifer S. Brodbelt; *Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas, USA*

Ultraviolet Photodissociation (PD) has emerged as an alternative ion activation method to collision induced dissociation (CID) that entails exposing an ion population to ultraviolet photons. Pioneering work by McLafferty *et al.* used 193 nm photons to irradiate multiply charged dT_{30} ions, producing electron photodetachment in addition to *w* and *a* ions (which have the same masses as d and z ions in palindromic sequences such as dT_{30}).[1] More recently, Gabelica et al. has explored the electron photodetachment of anionic DNA at 260 nm.[2,3] Minimal fragmentation is observed upon irradiation at 260 nm, with almost complete electron photodetachment. CID of the charge reduced radical ions predominantly produces *w*, *d*, *a*, and *z* ion series compared to the *w* and *a-B* ions observed in CID. We have evaluated the use of UVPD (193 nm) and tandem UVPD/CID method for characterization of DNA.

In all cases, UVPD at 193 nm of multiply charged DNA oligonucleotides primarily induces electron photodetachment after one 5-ns laser pulse. An array of low abundance sequence ions consisting of all ion types, *a*, *a-B*, *b*, *c*, *d*, *w*, *x*, *y*, and *z* ions are observed, with *w*, *d*, and *a-B* ions most dominant. UVPD is more dependent on the charge state of the precursor than is CID, with a significant decrease in the number of product ions observed for precursors in the highest or lowest charge states.

Ejection of one electron is a one-photon process while ejection of two electrons and backbone sequence ions are the result of a multi-photon absorption process. The extent of electron photodetachment is strongly sequence dependent, following the trend: $dA_6 > dG_6 > dC_6 > dT_6$. This trend is different from that observed upon electron photodetachment at 260 nm, $dG_6 > dA_6 > dC_6 > dT_6$, suggesting factors other than base ionization potentials (G < A < C < T) are responsible for electron photodetachment at 193 nm.

Subsequent CID of the radical anions (UV-CID) produced by electron photodetachment results in a different set of product ions from either CID alone or UVPD alone. The sequence ions observed include *w*, *a*, *d*, *z*, in addition to the occasional *a-B* ion. Base loss ions not present in UVPD spectra are observed to a small extent in the UV-CID spectra; internal ions observed in both CID and UVPD are not present in UV-CID spectra. *w* and *d* ions are the dominant series observed. Although fewer types of ions are observed with UV-CID compared to UVPD, the number of total ions and sequence coverage are higher with UV-CID compared to UVPD.

1. Guan, Z. et al. *Int. J. Mass Spectrom. Ion Processes.* **1996**, *157-158*, 357.
2. Gabelica, V. et al. *Anal. Chem.* **2006**, *78*, 6564.
3. Gabelica, V. et al. *J. Am. Chem. Soc.* **2007**, *129*, 4706.

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Poster 17

Interloop Photocrosslinking of Human Telomeric G-quadruplexes

Dian Su, John-Stephen Taylor, Michael Gross; *Washington University in St. Louis*

The unusual structural forms of telomere DNA, which protect the ends of chromosomes during replication, may render it vulnerable to unprecedented photodamage, possibly involving nonadjacent bases that are made proximate by the folding. The G-quadruplex for the human telomere sequence consisting of a repeating d(TTAGGG) is one unusual form. To investigate whether human telomeric G-quadruplexes are susceptible to photodamage by forming the common cyclobutane pyridine dimers (CPDs), we selected to study a G-quadruplex sequence Tel22, d[AGGG(TTAGGG)$_3$], by utilizing the combined approach of HPLC correlation, nuclease P1 digestion and tandem MS assay. Tel22 forms a basket structure in the presence of Na+ and may form multiple equilibrating structures in the presence of K^+ with hybrid-type structures predominating. UVB irradiation of d[AGGG(TTAGGG)$_3$] in the presence of Na^+ results mainly in a *cis,syn* thymine dimer between two adjacent T's in a TTA loop. Irradiation in the presence of K^+, however, produces, in addition to the adjacent dimers, a large amount of specific *anti* thymine dimers formed between either T in loop 1 and the central T in loop 3. Interloop-specific *anti* thymine dimers are incompatible with hybrid-type structures but could arise from a chair or basket-type structure (Figure 1). If these unique nonadjacent *anti* thymine dimer photoproducts also form *in vivo*, they would constitute a previously unrecognized type of DNA photodamage that may interfere with telomere replication and present a unique challenge to DNA repair. Furthermore, these unusual anti photoproducts may be used to establish the presence of G-quadruplex or quadruplex-like structures *in vivo*. [We thank NIH Grants CA40463 and 2P41RR000954) for support of this research.]

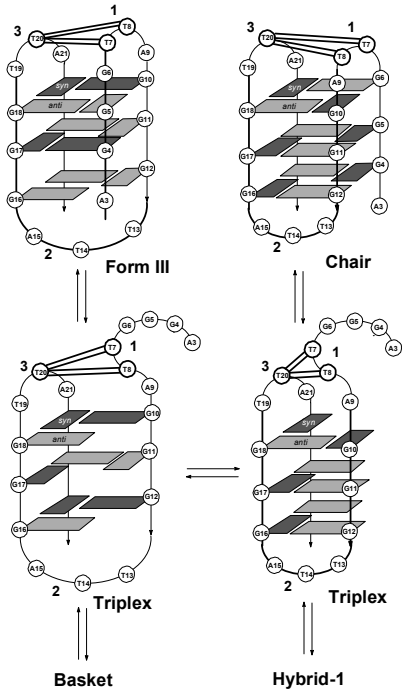


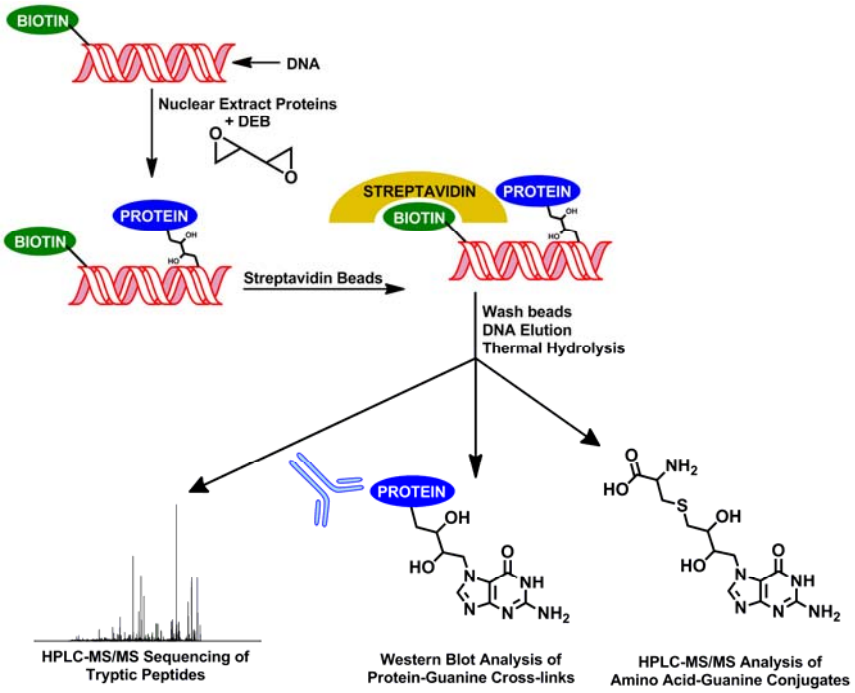
Fig. 1. Photodimer sites mapped onto chair and Form III G-quadruplex forms of Tel22 and onto triplex intermediates possibly involved in equilibrating between these and other structures. Solid double lines indicate sites of observed *anti* thymine photodimer formation between loops 1 and 3.

Poster 18

Mass Spectrometry of DNA-protein Cross-Links

Erin Michaelson-Richie, Rachel Loeber, Colin Campbell, Natalia Tretyakova; *University of Minnesota, Minneapolis, MN*

Many common DNA alkylating agents currently used in cancer therapy, e.g. platinum compounds and antitumor nitrogen mustards, are *bis*-electrophiles capable of cross-linking cellular molecules. While DNA-DNA cross-linking by such compounds is well characterized, the corresponding DNA-protein conjugates (DPCs) have not been identified. The purpose of the present work was to identify nuclear proteins which become cross-linked to DNA in the presence of antitumor drugs. A combination of affinity capture and mass spectrometric analysis was used to analyze DNA-protein cross-links present in cell free protein extracts and HT1080 human fibrosarcoma cells subjected to treatment with cytotoxic concentrations of *cis*-platinum (II) diamine dichloride (cisplatin), diepoxybutane, or mechlorethamine. Following isolation of DNA-protein cross-links, the identities of the cross-linked proteins were established using mass spectrometry-based proteomics. Drug treatment resulted in the formation of DPCs with nuclear proteins involved in chromatin regulation, DNA replication and repair, cell cycle control, transcriptional regulation, and cell architecture. Mass spectrometry of amino acid-nucleobase conjugates found in total proteolytic digests revealed that mechlorethamine-induced DPCs are formed *via* alkylation of the N7 position of guanine in duplex DNA and cysteine thiols within the proteins to give *N*-[2-[*S*-cysteinyl]ethyl]-*N*-[2-(guan-7-yl)ethyl]methylamine lesions, while cisplatin treatment induced guanine-lysine and guanine-arginine conjugates. The results described herein suggest that cellular exposure to common antitumor drugs leads to cross-linking of chromosomal DNA to a large spectrum of nuclear proteins, potentially contributing to the cytotoxic and mutagenic effects of these drugs.



Poster 19

Interplay of Oxidative DNA Damage and DNA-Protein Complexes

Ting Wang, Nicholas J. Amato, Rehana Zaidi, Timothy C. Mueser, Amanda C. Bryant-Friedrich;
University of Toledo, Toledo, OH

During the process of replication, local regions of the DNA helix are unwound to form replication forks, which serve as templates for DNA synthesis. Single-stranded binding proteins (SSBs), bind to the unwound DNA, serving to stabilize and protect the single-stranded regions of the replication fork, to promote DNA polymerase activity. The mechanism of protection provided by SSBs against damage, in particular oxidative damage, is unclear. Herein, we have investigated the impact of SSBs on DNA lesions induced by oxidative stress. A C3'-thymidinyl radical precursor that efficiently generates sugar centered nucleoside radicals upon photolysis at $\geq$ 320 nm was successfully incorporated into various DNA architectures which model those found in DNA replication. These architectures, containing the sugar radical precursor demonstrate the same binding affinity to SSBs as their unmodified analogs. Modified DNA substrates were photolyzed with and without SSBs, and analyzed with HPLC, gel electrophoresis, and mass spectrometry. Our results showed that binding to SSBs does influence the outcome of oxidative damage to DNA. For example, strand-break yields are decreased when DNA is in complex with SSBs. Our finding suggests that, in addition to preventing damage, SSBs redirect the outcome of oxidative damage if it occurs.

Poster 20

Direct Probing of Tertiary Structure of RNA Rev-Response Element by Solution X-ray Scattering and Mass Spectrometry 3D

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Functional RNA motifs are typically too large for resolution by NMR and/or are refractory to crystallization. Our goal is to develop efficient and reliable biochemical and biophysical methodologies to complement more established techniques for high-resolution RNA structure determination, and apply them to solve the secondary and tertiary structures of essential human retroviral RNAs. HIV-1 Rev-response element (RRE) is a structural element located in the Env region that interacts with the viral accessory protein Rev to facilitate the nuclear export of viral RNA. A trans-dominant negative variant of Rev, RevM10, disrupts viral RNA export and inhibits replication. Two silent G->A mutations within the HIV-1 RRE (RRE61) confer RevM10 resistance. Secondary structures of Rev protein binding domains in RRE wild type and mutant RRE 61 were shown to be unchanged previously. Therefore we propose that the tertiary structural difference of RRE Wild type and mutant RRE 61 could be essential for RevM10 resistance. To investigate the tertiary structures of RRE wild type and mutant RRE61, a combination of biophysical and biochemical methods was developed. Small-angel X-ray scattering (SAXS) data contain information about the overall shape of RNAs and can be utilized to define the interface of sub domains. Furthermore, the tertiary interaction in RNAs can also be analyzed precisely by Mass Spectrometric three-dimensional (MS3D) approach. Electrospray ionization (ESI) Fourier Transform Mass Spectrometry (FTMS) combined with a crosslink method generates information about the spatial relationship of contiguous domains in folded structures. The tertiary structures of RRE and RevM10 resistance mutant RRE 61 were investigated using SAXS and MS3D.

Poster 21

Gas-Phase Approaches for Investigating Nucleic Acids Higher Order Structures

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As part of our long-standing interest in the investigation of nucleic acid relevant to retroviral pathogenesis, we have explored MS-based approaches that take full advantage of what solution and gas-phase methodologies have to offer for 3D structure elucidation. Supported by bottom up and top down analysis, the implementation of structural probing in solution has provided valid spatial constraints that have enabled the generation of all-atom 3D models of large RNA substrates. However, the observation that hydrogen bonding and base stacking are preserved in the gas phase has prompted the investigation of top-down strategies for the identification of base paring interactions responsible for the secondary and tertiary structures of nucleic acids.

Based on earlier observations that weak non-covalent interactions, such as hydrogen bonds, are preserved during activation by non-ergodic methods, we have explored the effects of electron capture dissociation (ECD) on the fragmentation of constructs capable of folding into well-defined secondary structures. The fact that only single-stranded nucleotides were observed to undergo fragmentation was interpreted with the possibility that backbone cleavage may occur over the entire span of the construct sequence, but its effects may not be evident in double-stranded regions, where mutual base-pairing may prevent the dissociation of cleaved products into discrete fragment ions. This hypothesis was confirmed when the same constructs were analyzed using infrared multi-photon dissociation (IRMPD), which represents a finely tunable ergodic method. When sufficient energy was imparted to the system, backbone fragmentation was observed covering the entire precursor ion sequence. This outcome clearly indicates that the weak base-pairing interactions are not preserved by this ergodic dissociation process. We are currently exploring the combined application of these activation techniques to identify the boundaries between single- and double-stranded regions of progressively larger RNA constructs, which would lead to the efficient determination of their secondary structures.

If gas-phase dissociation strategies can provide detailed information about the molecular interactions defining nucleic acid 3D structures, ion mobility spectrometry (IMS) has the potential for providing very valuable information about their global fold. According to this technique, the travel of ions through a gas-filled drift tube is affected by several parameters, including the cross section determined by the respective 3D structure. For this reason, we have tested the ability of IMS to clearly discriminate between different RNA folds by using oligonucleotides of identical base composition, but different sequences, which can either remain unstructured, or fold into a stable stemloop hairpin. The experiments have clearly shown that IMS is capable of resolving the isobaric species according to their drift time. In particular, the unstructured construct displayed a higher velocity than the structured hairpin, consistent with a compact conformation assumed by the former in solvent-free environment, versus an elongated shape bestowed to the latter by an intact double-stranded stem. We are currently exploring the application of IMS to the structural analysis of larger, more complex RNA constructs.

In conclusion, the results provided by the top-down and IMS approaches clearly indicate that their concerted application can provide complementary information about the 3D structure of nucleic acids in the gas-phase. Given the ability of soft ionization techniques to preserve base pairing and stacking interactions, these techniques have a great potential for extending the applicability of mass spectrometry to the structural elucidation of biologically relevant nucleic acids.

Poster 22

High Throughput Genotoxicity Screening Using Magnetic Bioreactors with LC-MS/MS

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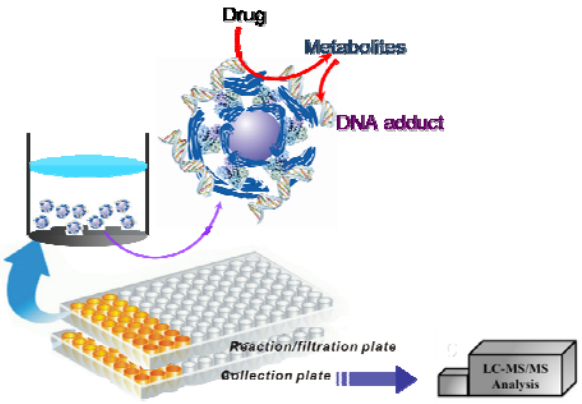

Damage of cellular DNA can have profound biological consequences, such as inhibiting cell division, triggering cell death, or causing mutations if not properly repaired. Formation of nucleic acid base adducts represents a major class of DNA damage, which results from exposure to xenobiotics through various sources, such as environmental pollutants and drugs, and endogenous nucleophilic compounds as well. DNA adducts has proven to be an accurate and reliable biomarker for predicting carcinogenicity of chemical compounds. Tandem mass spectrometry is an excellent method for high sensitivity detection of DNA adducts, and provides detailed structural information that could be used by synthetic chemists to tune out genotoxicity while retaining desired therapeutic activity.

We recently developed bioreactors with thin DNA-metabolic enzyme films on silica nanoparticles that can be used for assembly for rapid screening of potentially genotoxic drugs and pollutants molecules. Fast reaction rates are facilitated by efficient enzyme utilization and high local concentrations of DNA and metabolites in the films. The novelty of using magnetic particle coated with DNA/microsome/cytosol films enables fast and easy separation during the film preparation compared with previously used silica particles. In addition, the embedded DNA film on the particle avoids time-consuming and labor intensive DNA precipitation and isolation compared to conventional sample preparation. Important metabolic enzymes from microsomal and cytosolic sources in the film allow *in vitro* bioactivation of compounds to generate reactive metabolites that are trapped by DNA in the film as DNA adducts. The magnetic bioreactors coupled with a 96-well plate allow investigations of numerous metabolic reactions under various conditions, followed by DNA hydrolysis without transferring the samples. Then an automated LC with an on-line sample cleaning system together with tandem MS is used to quantitatively measure the rate of formation of individual nucleobase adducts and their structures. Herein, we present the use of this high throughput system for the detailed structural analysis and the relative DNA adduct formation rate of several known toxicants using LC-tandem MS. Relative DNA adduct formation rates of a series of compounds correlated with carcinogenic potency metrics, such as TD_{50} values. Taken together, magnetic nanoparticle bioreactors coupled with LC-MS for DNA adduct analysis is a promising new technology for genotoxicity screening.