Press Release: The Nobel Prize in Chemistry 2002
9 October 2002
The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry for 2002

"for the development of methods for identification and structure analyses of biological macromolecules"

with one half jointly to

John B. Fenn
Virginia Commonwealth University, Richmond, USA, and

Koichi Tanaka
Shimadzu Corp., Kyoto, Japan

"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

and the other half to

Kurt Wüthrich
Swiss Federal Institute of Technology (ETH), Zürich, Switzerland and The Scripps Research Institute, La Jolla, USA

"for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution".
The Nobel Prize in Chemistry for 2002 is to be shared between scientists working on two very important methods of chemical analysis applied to biological macromolecules: mass spectrometry (MS) and nuclear magnetic resonance (NMR). Laureates John B. Fenn, Koichi Tanaka (MS) and Kurt Wüthrich (NMR) have pioneered the successful application of their techniques to biological macromolecules. Biological macromolecules are the main actors in the makeup of life whether expressed in prospering diversity or in threatening disease. To understand biology and medicine at molecular level where the identity, functional characteristic structural architecture and specific interactions of biomolecules are the basis of life, we need to visualize the activity and interplay of large macromolecules such as proteins. To study, or analyse, the protein molecules, principles for their separation and determination of their individual characteristics had to be developed. Two of the most important chemical techniques used today for the analysis of biomolecules are mass spectrometry (MS) and nuclear magnetic resonance (NMR), the subjects of this year’s Nobel Prize award.
Nobel Laureates – to do with mass spectrometry

Thomson 1906
Frederick Soddy 1921
Aston 1922
Smalley, Kroto, Curl 1996
“I feel sure that there are many basic problems in chemistry which could be solved with far greater ease with this than with any other method. The method is surprisingly sensitive – more so even than the method of spectrum analysis, requiring an infinitesimal amount of material, and does not require this to be specially purified.”

Joseph John Thomson, 1913
The Rays of Positive Electricity and their Applications to Chemical Analysis
New advances in ion production

Electrospray Ionization

Laser Desorption Ionization

“The method is surprisingly sensitive ….requiring an infinitesimal amount of material, and does not require this to be specially purified…”
Soft Ionization Methods

PDMS, FABMS, SIMS

Figure 32.2 The FAB or LSIMS sample insertion probe, inserted into the ion source of a magnetic mass spectrometer. The sample is bombarded by either Xe^0 atoms or Cs^+ ions.
Figure 32.3 FAB bombardment and sputtering processes viewed at the molecular level.

Field ionization, variations..
Electrospray Ionization for Mass Spectrometry of Large Biomolecules

John B. Fenn, Matthias Mann, Chin Kai Meng, Shek Fu Wong, Craig M. Whitehouse

Science 246 (1989) 64
Figure 1. Schematic diagram of electrospay ion source.

Fig. 1. Sketch of the ion desolvation process. Small, charged droplets produced by the electrospray evaporate, generating a high electric field at the droplet surface. Analyte molecules that were dissolved in the droplet can attach to charges and be lifted into the gas phase by this field.
Fig. 2. Schematic diagram of an ES-MS apparatus. For details of operation, see text.
Fig. 3. (A) Typical electrospray spectra for nonvolatile small molecules. A mixture of quaternary ammonium and phosphonium halides was dissolved at concentrations from 2 to 10 ppm in 50:50 methanol:water. (B) Electrospray spectrum for the peptide gramicidin S ($M_r = 1141.5$).
Fig. 7. Application of the "deconvolution algorithm" to a measured spectrum of cytochrome c. The insert shows the deconvoluted peak resulting from the transformation. Intensities of the measured and deconvoluted spectra are to the same scale.
Fig. 8. Electrospray mass spectra for large molecules obtained by other investigators: (A) for a synthetic oligonucleotide (21); (B) for a large protein, bovine albumin dimer (molecular weight, 133,000) (23). [Adapted from (21) with permission of the authors and the editor of Rapid Communications in Mass Spectrometry]
Fig. 9. Electrospray mass spectra obtained by coupling an ES source to a Fourier transform mass spectrometer (24): (A) cytochrome c (equine); calculated average molecular weight, 12,360.1; measured molecular weight, 12,356.9 ± 0.9; (B) myoglobin (equine skeletal muscle); calculated average molecular weight, 16,950.7; measured molecular weight, 16,947.5 ± 1.4.
Direct sequencing with femtomole!

**FIG. 1** Testing and sensitivity of the new procedure using BSA. 

**a.** Peptide ion spectrum of BSA at 800 fmol total material loaded on a gel. Peptide peaks are labelled according to their identification by tandem mass spectrometry. 

**b.** Fragmentation of the selected doubly charged peptide ion at mass-to-charge m/z 740.5. Fragmentation of tryptic peptides at the amide bonds predominantly produces ion series containing the C terminus (designated Y1, Y2, and so on, see ref. 14). A continuous Y ion series could be assigned to the dominant peaks in the spectrum yielding the sequence Lxx-Gly-Glu-Tyr-Gly-Phe-Gln-Asn-Ala-Lxx-Lxx-Val-Arg, where Lxx is either Leu or Ile, which corresponds to a tryptic peptide of BSA. 

**c.** The same ion as in b obtained in a separate experiment in which 80 fmol BSA was loaded onto a gel that was silver stained.

**METHODS.** BSA was quantified by amino-acid analysis. Acrylamide gels were prepared using standard protocols and stained with Coomassie blue. In gel reduction, acetylation and trypsin digestion were similar to published procedures. After washing with 100 mM NH4HCO3 and acetonitrile, gel pieces were swollen in the digestion buffer containing 50 mM NH4HCO3, 5 mM CaCl2 and 0.5% TFA (Boehringer Mannheim, sequencing grade) at 4 °C. After 45 min, the supernatant was aspirated and replaced with 5–10 μl of the same buffer without TFA to keep gel pieces wet during enzymatic cleavage (37 °C, overnight). Peptides were extracted by three changes of 5% formic acid and acetonitrile and dried down. Approximately 100 nL of POROS R2 sorbent (Perseptive Biosystems) was placed in the tip of a pulled GC 100F-10 (CEI, Panger bourne) capillary. Note that the resin is not packed, and that no frit or other micro LC assembling is necessary. A new capillary and a new portion of resin are used for each analysis to avoid cross-contaminations even at the femtomole level. Dried peptide mixture was dissolved in 10 μl 5% formic acid, loaded onto the pre-equilibrated capillary, washed and eluted with 60% methanol in 5% formic acid into the spraying capillary. The elution volume is 10-fold larger than the resin volume, resulting in good peptide recovery. Nano-electrospray was performed on an API III (Perkin-Elmer Sciex, Ontario, Canada) mass spectrometer as described. For precusor ion selection, quadrupole 1 was set to transmit a mass window of 2 Da. Stepping size for the tandem mass spectra was 0.2 Da, and resolution was set so that fragment masses could be assigned to better than 1 Da.
Figure 33.2 Schematic of a typical ESI source.
Protein and Polymer Analyses up to m/z 100 000
By Laser Ionization Time-of-flight Mass Spectrometry

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SPONSOR REFEREE: T. Matsuo, Osaka University, Osaka, Japan

Hitherto $^{252}$Cf plasma desorption mass spectrometry (PDMS) has been used to study peptides and proteins in the molecular weight range from 1 kDa to 35 kDa. 1, 2 Fast atom bombardment mass spectrometry (FABMS) and secondary ion mass spectrometry (SIMS) have been applied to the analyses of proteins and polymers molecules. 3, 4 On the other hand, in the area of laser desorption time-of-flight (TOF) mass spectrometry (MS), though there have been many papers on analyses of organic compounds, the molecular weight of these compounds has been relatively low. 5

For the purpose of investigating the mass spectrometry of high-mass molecular organic compounds, we developed a laser ionization TOF mass spectrometer. To assess the utility of this spectrometer for high masses, we evaluated and tried the mass spectrometer on various organic compounds. 6, 7 This spectrometer was able to obtain useful spectra up to m/z 100000. Typical spectra of proteins and polymers with molecular weights up to 25 kDa are shown in this paper.
Figure 1. Construction of the laser ionization TOF mass spectrometer.
Figure 4. Laser ionization mass spectrum of poly(propylene glycol); average mol. wt. 4kDa (PPEG4K).

Figure 5. Laser ionization mass spectrum of poly(ethylene glycol); average mol. wt. ca 20kDa (PEG20K).
Applications

Biology: Sequencing of proteins with femtomole material, rapid screening, drug discovery, proteomics


Disease management: Identification of biomolecules.

Food and life: Pesticides and chemicals in femtogram quantities, quality control of foodstuff.

Mass spectrometry of ceramics!
Figure 34.1 The mass spectrum of cascade polymer 48-cascade-benzene[3-1,3,5][5-ethynyl-1,3-phenylene]:5-ethynyl-1,3-di(tert-butyl)benzene, containing 94 phenylacetylene monomer units (D-94) analyzed by UV MALDI-TOF. The measured mass accuracy is 40 ppm using the PEG as an internal mass standard. The spectral resolution of the molecular ion is 1100.
Figure 34.2 The mass spectrum of a substituted fullerene obtained with UV MALDI-FTMS. The mass measurement accuracy was found to agree with that of the proposed structure to within 3.7 ppm, externally calibrated, and the spectral resolution is 10,000.
Figure 34.4  A high-resolution UV MALDI-FTMS spectrum of the protein standard bovine insulin. The lower-intensity distribution corresponds to the mass (M+H)$^+$ – 17 Da. The average resolution of the molecular ion peak is 60,000 and the average mass accuracy is 70 ppm, externally calibrated.
MALDI spectrum of polystyrene 145000 distribution

MALDI spectrum of polystyrene 295000 distribution
Immunoglobin (IgG)
MALDI-TOF based mutation detection using tagged in vitro synthesized peptides  Alex M. Garvin et al., Nature Biotechnology 18, 95 - 97 (2000)
Following Cancer Progression by MALDI Mass Spectrometry

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Figure 1. Protein profiles obtained by MALDI MS after tissue blotting on polyethylene comparing the ventral lobes of a) a normal (CD1 strain) and b) cancerous (12T-10 transgenic line) mouse prostate. The signals labeled a through e have been found unique to the cancer.

Fig. 3. The negative-ion MALDI mass spectra for Greengold under poor (a) and well (b) co-crystallized conditions [26]. The inset (a) shows an expanded view of the main band, plotted in Au-atom units. The arrow in (b) marks the tentative assignment of the total molecular weight.
Figure 1. (a) Laser desorption ionization mass spectra from the original, as-prepared, cluster compounds (R = C6). (b) LDI-MS of the mixture after heating in neat dodecanethiol for 18 h and (c) the LDI-MS of the final separated cluster compound that produces ions in the 29 kDa range.
Figure 1 Differential extraction of parasite-infected red blood cells (RBCs) and flow chart of MS analysis. a, Giemsa staining of purified trophozoites and schizonts (left panel; asexual stage parasites), and gametocytes (right panel; sexual stage parasites). b, Extraction procedure for infected RBCs using freeze–thaw lysis and centrifugation, yielding a soluble and insoluble (pellet) fraction. c, The soluble and insoluble fraction from $5 \times 10^7$ gametocytes were separated on a 10% SDS gel and stained with Coomassie blue (left panel) or processed for western blotting and developed with a rabbit polyclonal antibody to Pf48/45. The arrowhead indicates Pf48/45. The right hand panel shows the insoluble fraction of $2 \times 10^7$ gametocytes. d, Mass spectrometric analysis of one of the gel slices shown in c. The top panel shows the summed ion current of all peptides eluting at a particular time. The middle panel shows a typical mass spectrum obtained from the eluting peptides. The bottom panel shows a fragmentation spectrum of the peptide with a mass of 515.82 indicated in the middle panel and magnification (inset) of one of the fragments. Database searching with this fragmentation information results in identification of peptide LFG/VGSSIPK from Pf48/45.
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