DNA Introduction into Living Cells by Water Droplet Impact with an Electrospray Process

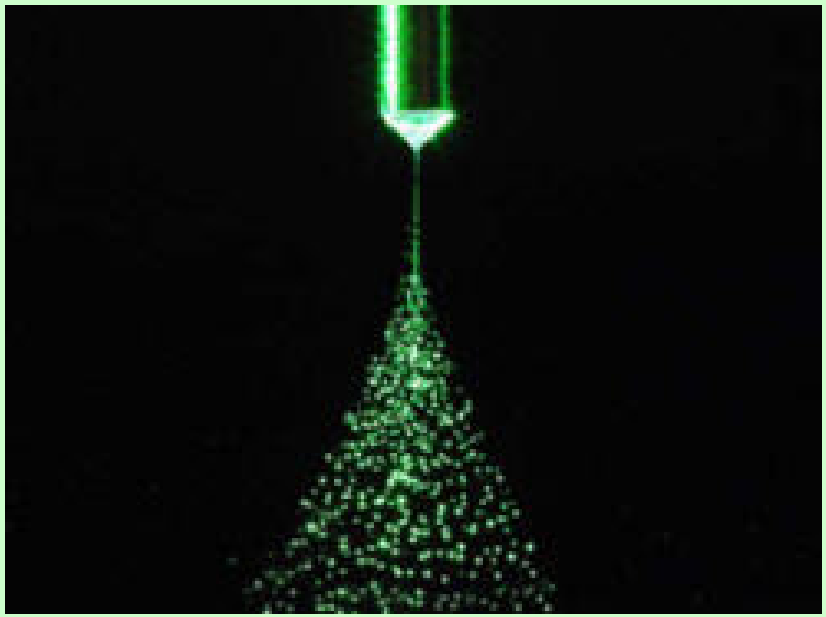
K. Ikemoto
Mitsubishi Gas Chemical Company, Japan

Prof. T. Sakai *et al.*
Graduate School of Science and Engineering
Saitama University, Japan

Zenobi et al.
ETH Zurich

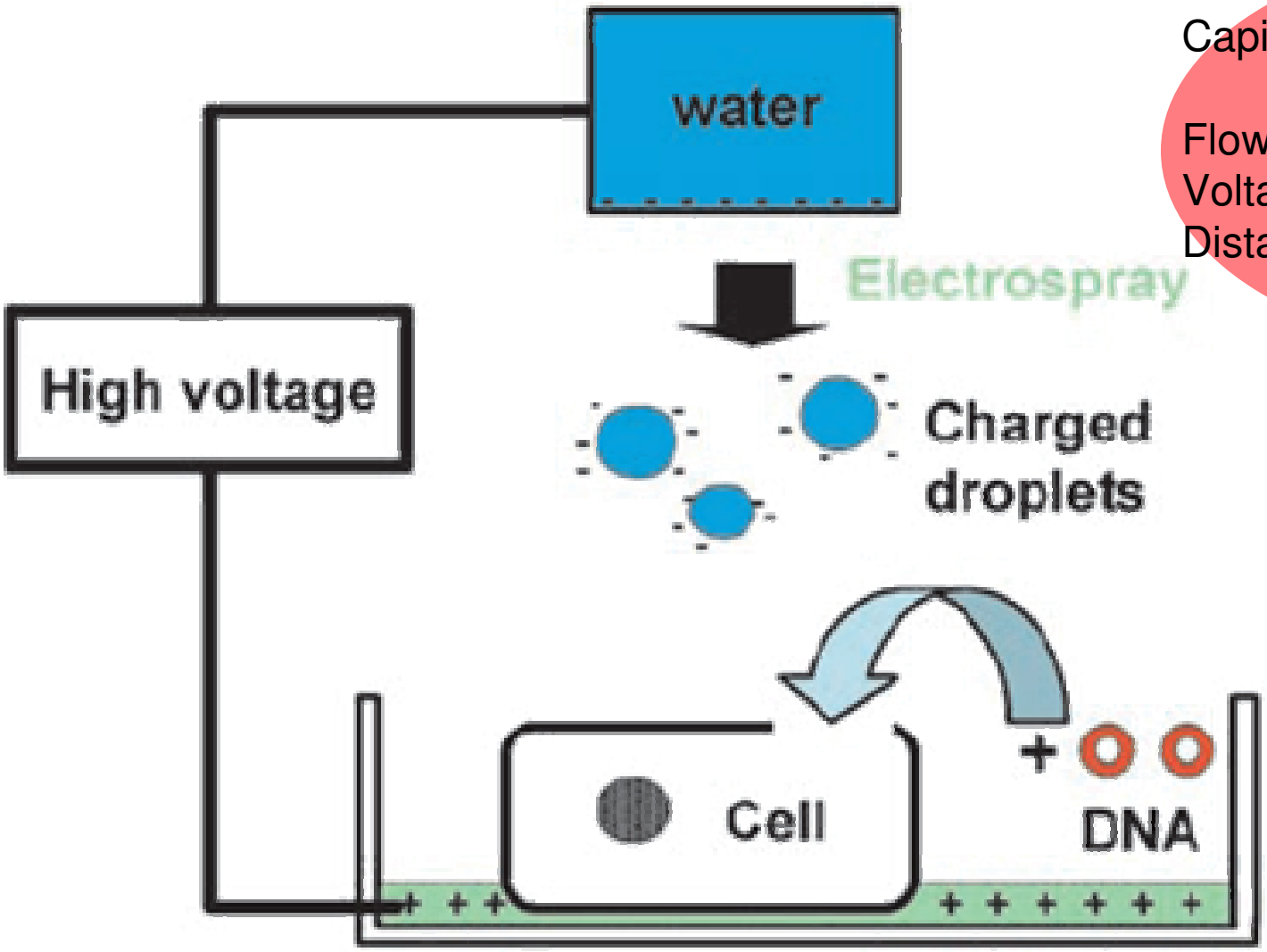
Angew. Chem. Int. Ed. 2008, 47, 1429

Introduction



Highly charged droplet at the capillary tip splits into micro- or nanoscale droplets

- Cationic polymer
- Lipofection
- Gene Gun
- Electroporation



Capillary : 0.1 mm ID,
40 mm
Flow : 100-200 microL/min
Voltage : 7 – 18 kV
Distance : 2 cm

Diagram of the electrospray equipment for gene delivery

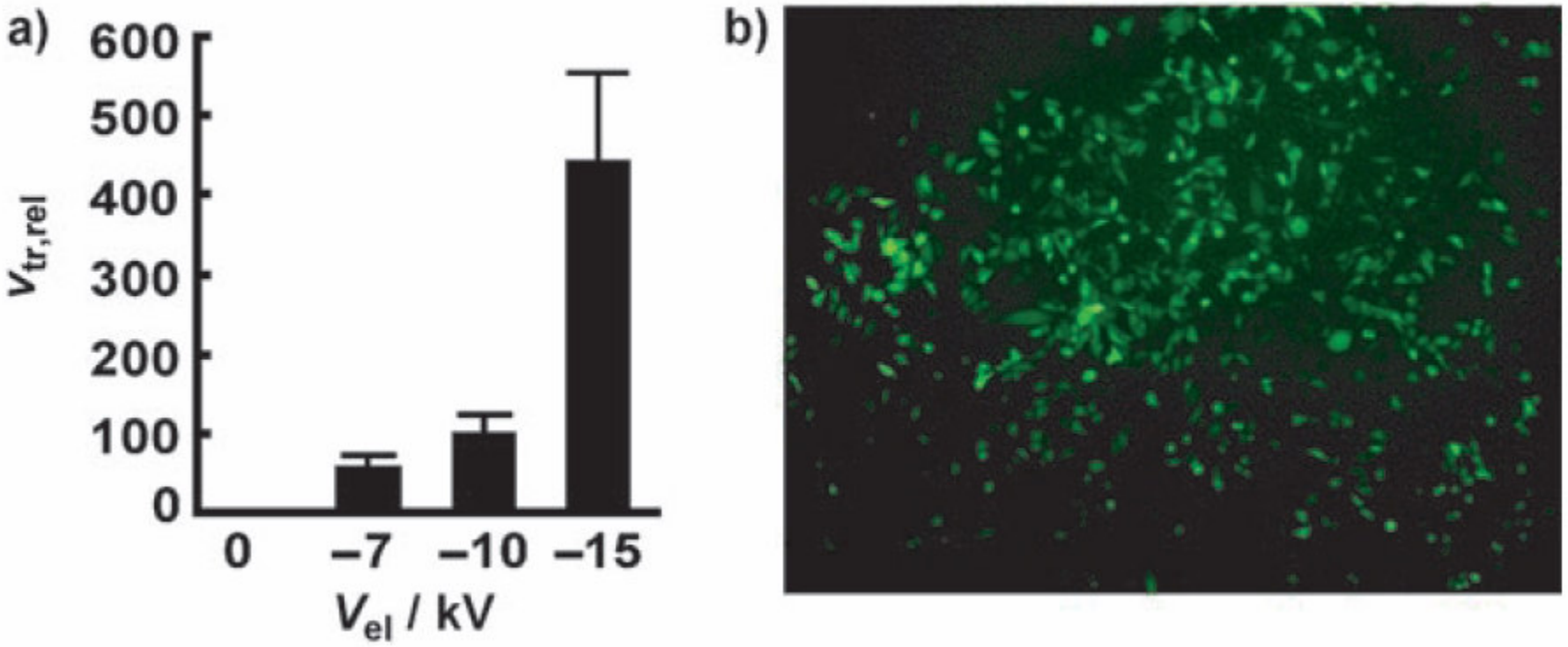
Model cells:

Adhesive Chinese hamster ovary (CHO) cells

HeLa cells

CHO cells were cultured in minimum essential medium (a-MEM, Gibco, USA) supplemented with 10% fetal bovine serum. Cells were plated in a 35-mm culture dish at 37 C under 5% CO_2 in air. Three days later, the cells were used for electrospraying. The culture medium was removed from the dish and an aqueous solution (100 microL) of plasmid vector pEGFP-N1, as green fluorescence protein (GFP) encoding DNA, was added to the dish.

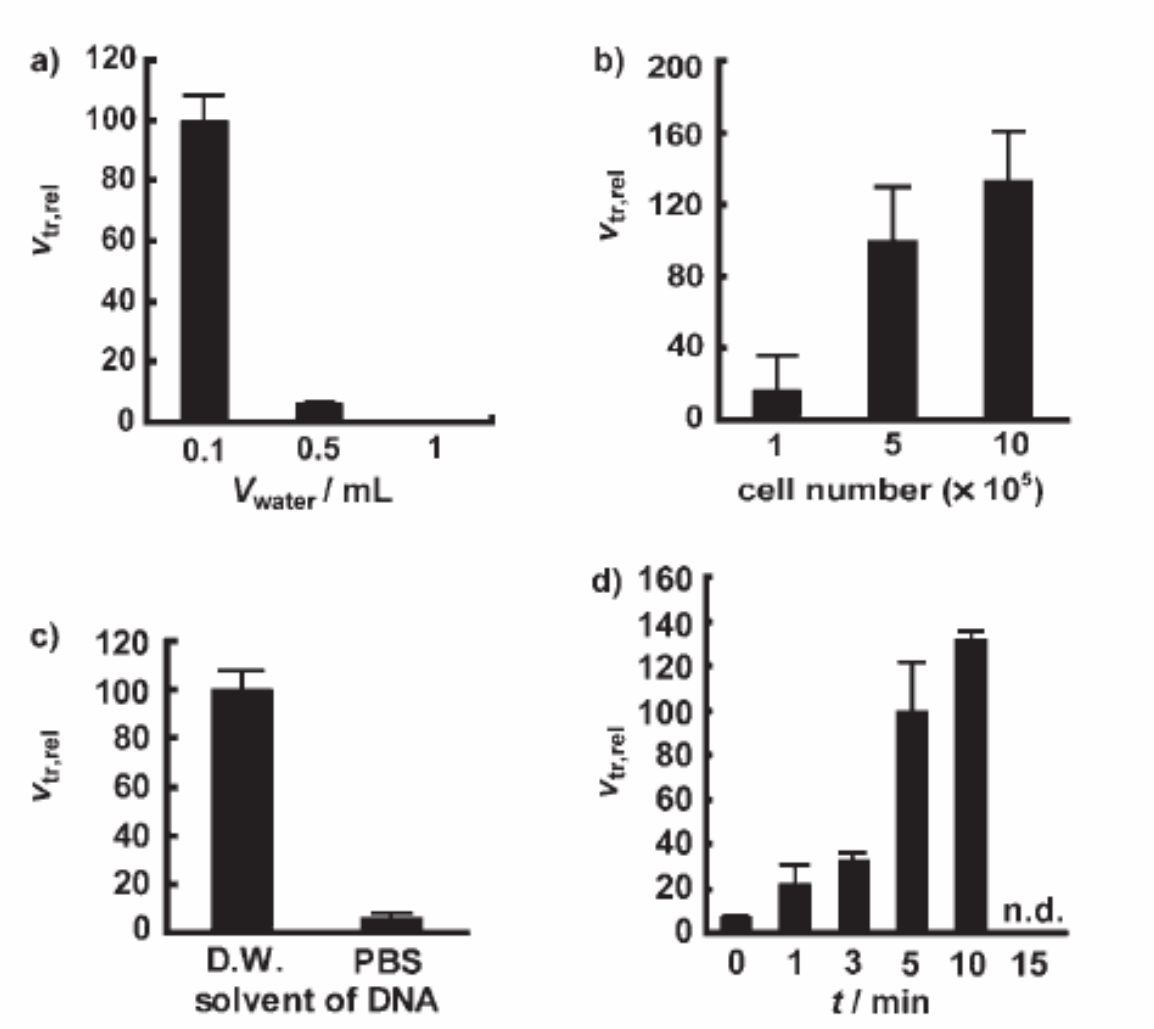
Water was electrosprayed onto the cells from a height of 2 cm at 10 kV, and culture medium was directly added to the dish. After 24 h of cultivation, GFP-positive cells were counted with a hemocytometer under a fluorescence microscope.



a) Relative value of GFP transfection rate (vtr,rel) to electrospray voltage (V_{el}). The transfection rate increased with electrospraying in a voltage-dependent manner.
b) GFP expression in CHO cells induced by electrospraying.

Transfection rate ~ 1.6 %

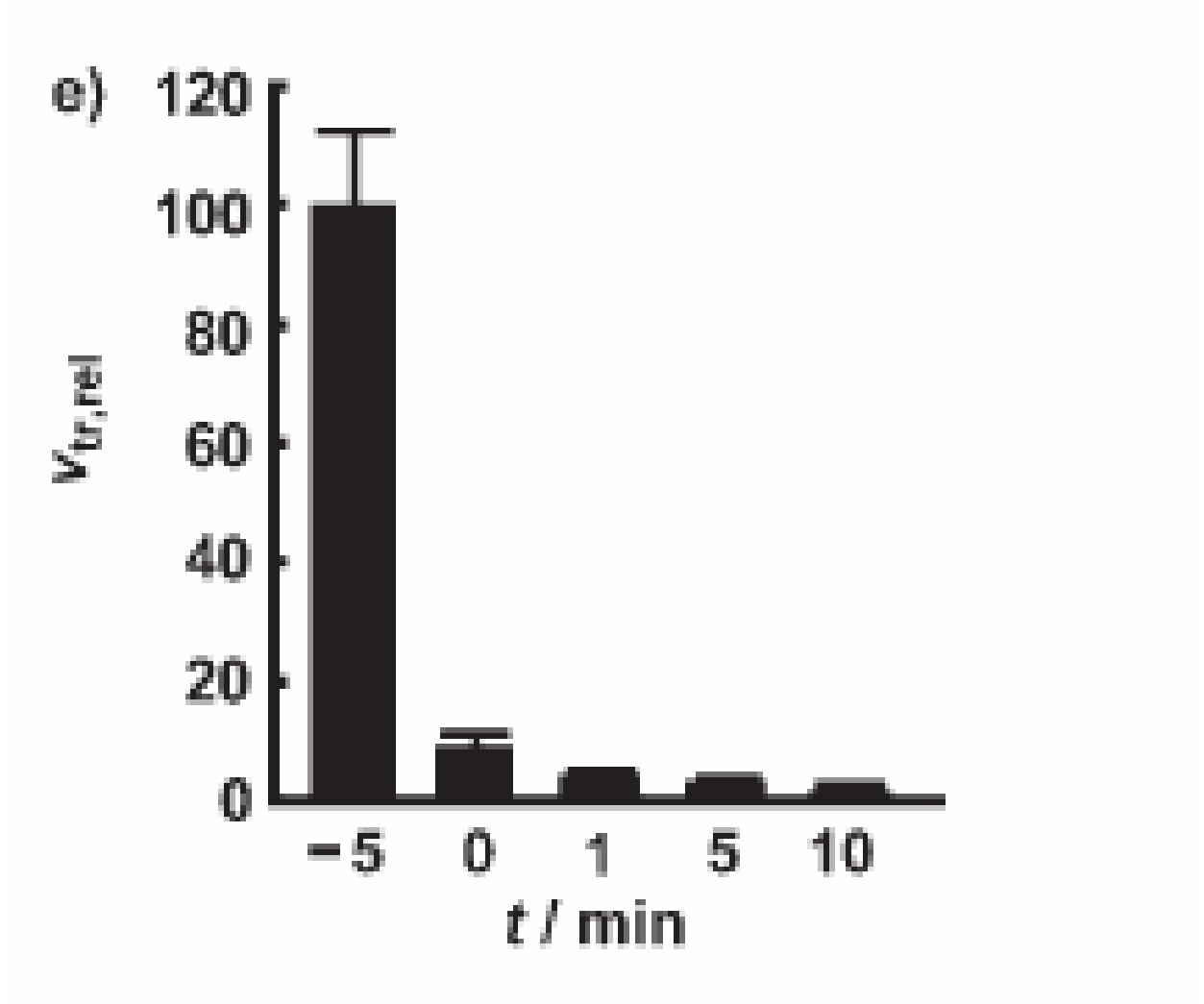
Parameters



a) Effect of solution volume covering CHO cells on transfection rate $v_{tr,rel}$.

b) Effect of number of culture cells on transfection efficiency.

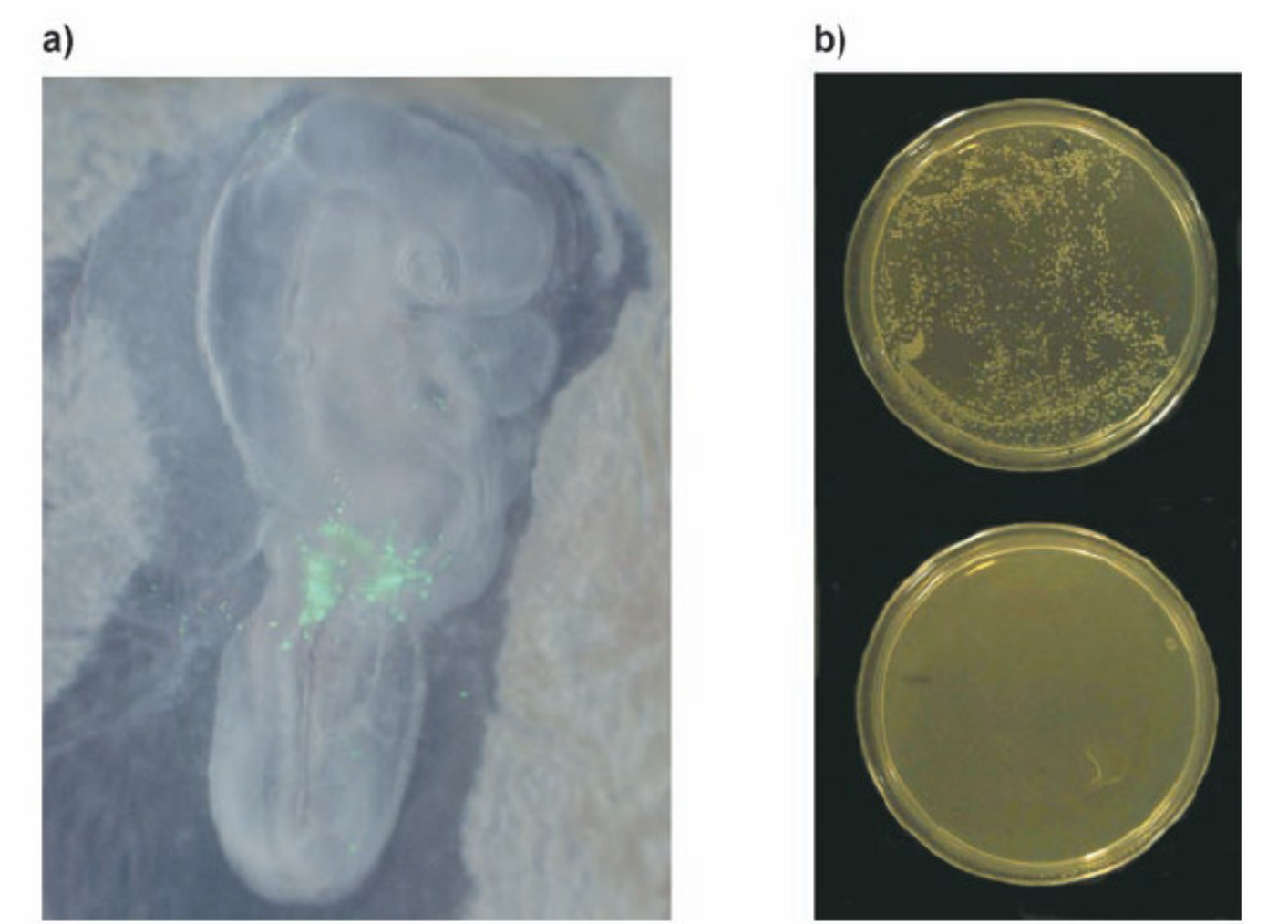
c,d) Swollen state of CHO cells and transfection efficiency.



Relationship between transfection rate and timing of DNA addition

White Leghorn fertile eggs were incubated at 38 C. After 1.5 days, an embryo was cultured. The embryo was placed in an agarose dish, and GFP plasmid (4.3 microg/mL, 1 microL) with 2% Fast Green was put on the target region of the embryo.
The PBS was electrosprayed onto this target region from a height of 10 cm for 20 s at 10 kV with a flow rate of 120 microL/min. After electrospraying, the PBS was removed from the agarose dish and the embryo was incubated overnight.

E. coli (K12 strain) solution was plated on a 1.5%agar/Luria–Bertani (LB) dish and incubated at 25 C for 2 days. Then the plasmid pUC19 (10 microg) in Tris/ethylenediaminetetraacetic acid (TE) buffer (100 mL) was spread on the *E. coli* lawn, and water (100 microL) was electrosprayed at 7 kV onto the colonies at a flow rate of 100 mLmin1. The colonies were collected in a 2-mL tube and washed with LB medium.
The E. coli was seeded on a 1.5%agarose dish with ampicillin



a) Region-specific GFP expression in a chicken embryo, which was localized in the E2.5 embryo.
b) Electrospray transformation in E. coli.
Upper plate: 10 kV impress; many ampicillin-resistant colonies were found. Lower plate: 0 V impress.

Conclusions

- The physical-force-dominant method can be applied to many kinds of cells and tissues.
- The equipment is simple and portable, and can be downsized.
- Cytotoxic reagents are not necessary, and this may lead to a high survival rate of cells. With refinement of the configuration of the spray tubes or scanning of the whole surface of the dish, expansion of the exposed area or homogeneous electrospraying of the aerosol is expected.

Neutral Desorption Sampling of Living Objects for Rapid Analysis by Extractive Electrospray Ionization Mass Spectrometry

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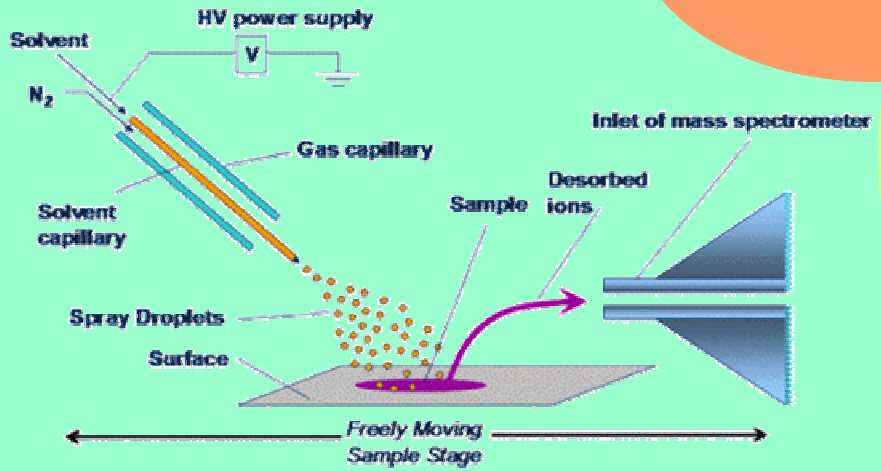
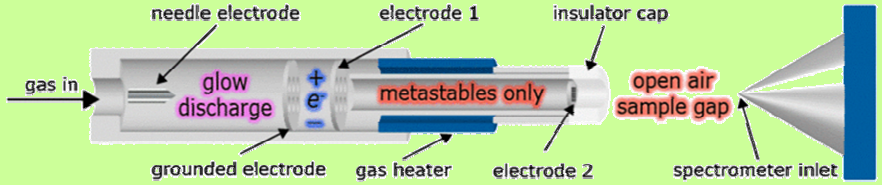
Angew. Chem. Int. Ed. 2007, 46, 7591

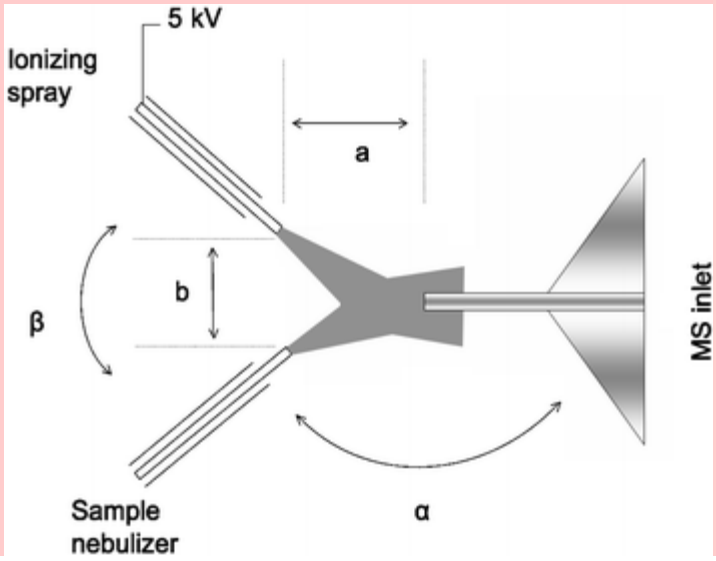
ESI MS



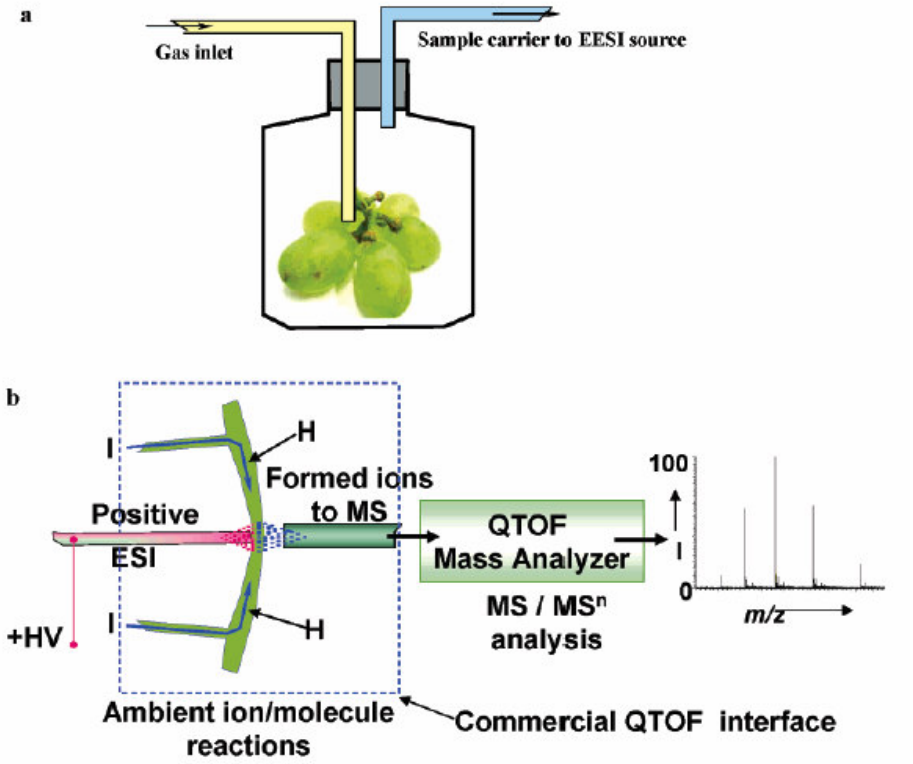
DESI



DART



EESI


a
Gas inlet
Sample carrier to EESI source
b
H
Formed ions
to MS
Positive
ESI
QTOF
Mass Analyzer
MS / MSⁿ
analysis
100
I
0
m/z
+HV
H
Ambient ion/molecule
reactions
Commercial QTOF interface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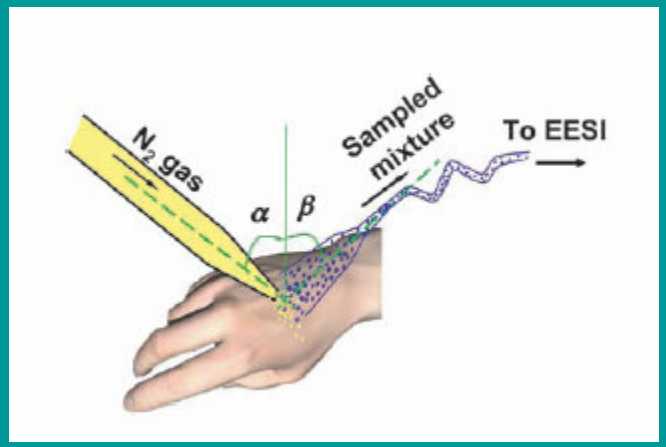
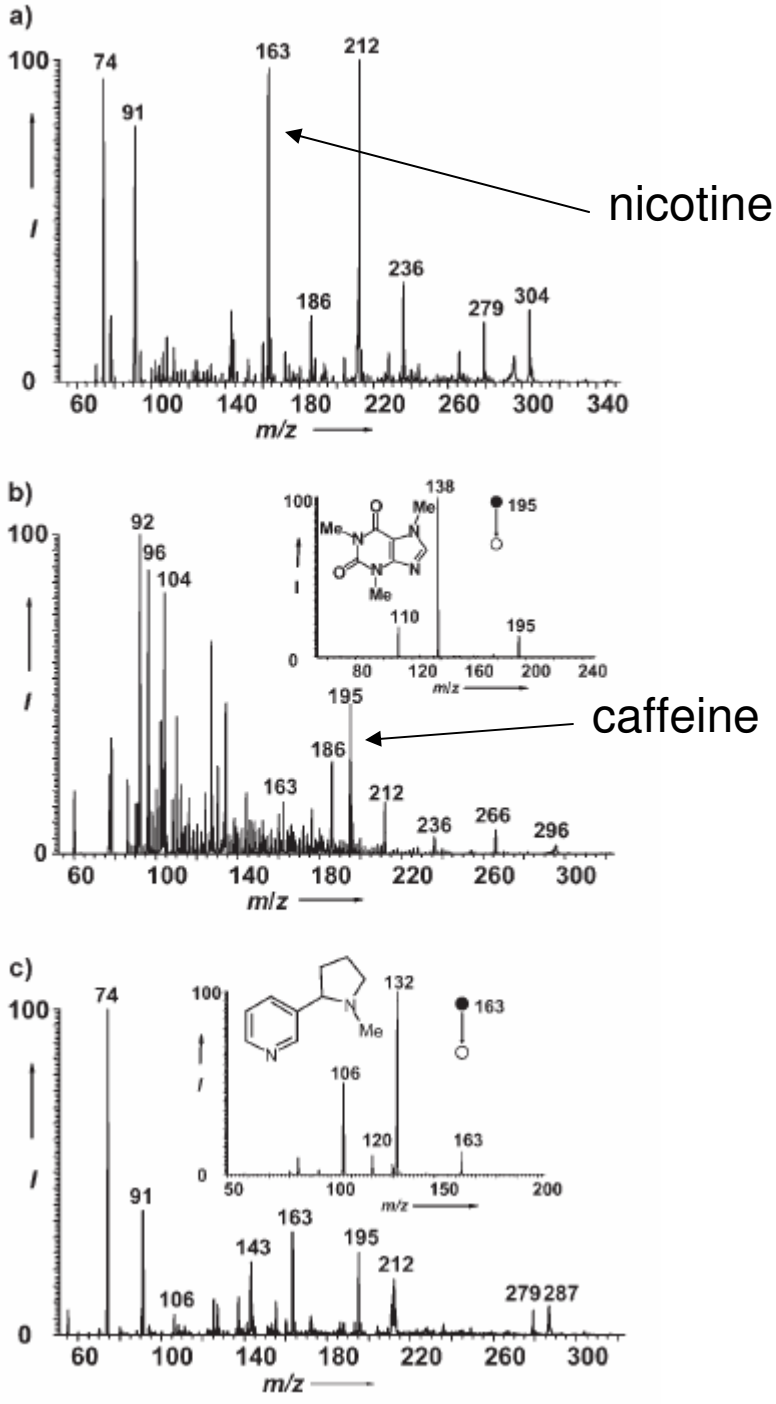

N2 gas
α
β
Sampled
mixture
To EESI

Analytes were sampled from a biological surface by dry nitrogen gas (1–3 L/min) to form an aerosol gas phase mixture, which was then transported through a teflon tube (5 mm inside diameter, 1.2 m in length)to reach the EESI source, where the molecular analytes were ionized by ESI spraying of aqueous acetic acid solution (10 vol%) for mass analysis. To facilitate the neutral desorption sampling process, a sharp jet (i.d. 1 mm) was formed at the end of the gas line so that a high gas speed (about 10 m/s)was created. A V-shaped collecting tube (i.d. 10 mm) was mounted in front of the teflon tube so that more aerosol could be collected. The angles between the axes of the desorption gas flow and the collecting tube were adjusted. The distance between the gas jet tip and the surface was 2–10 mm.

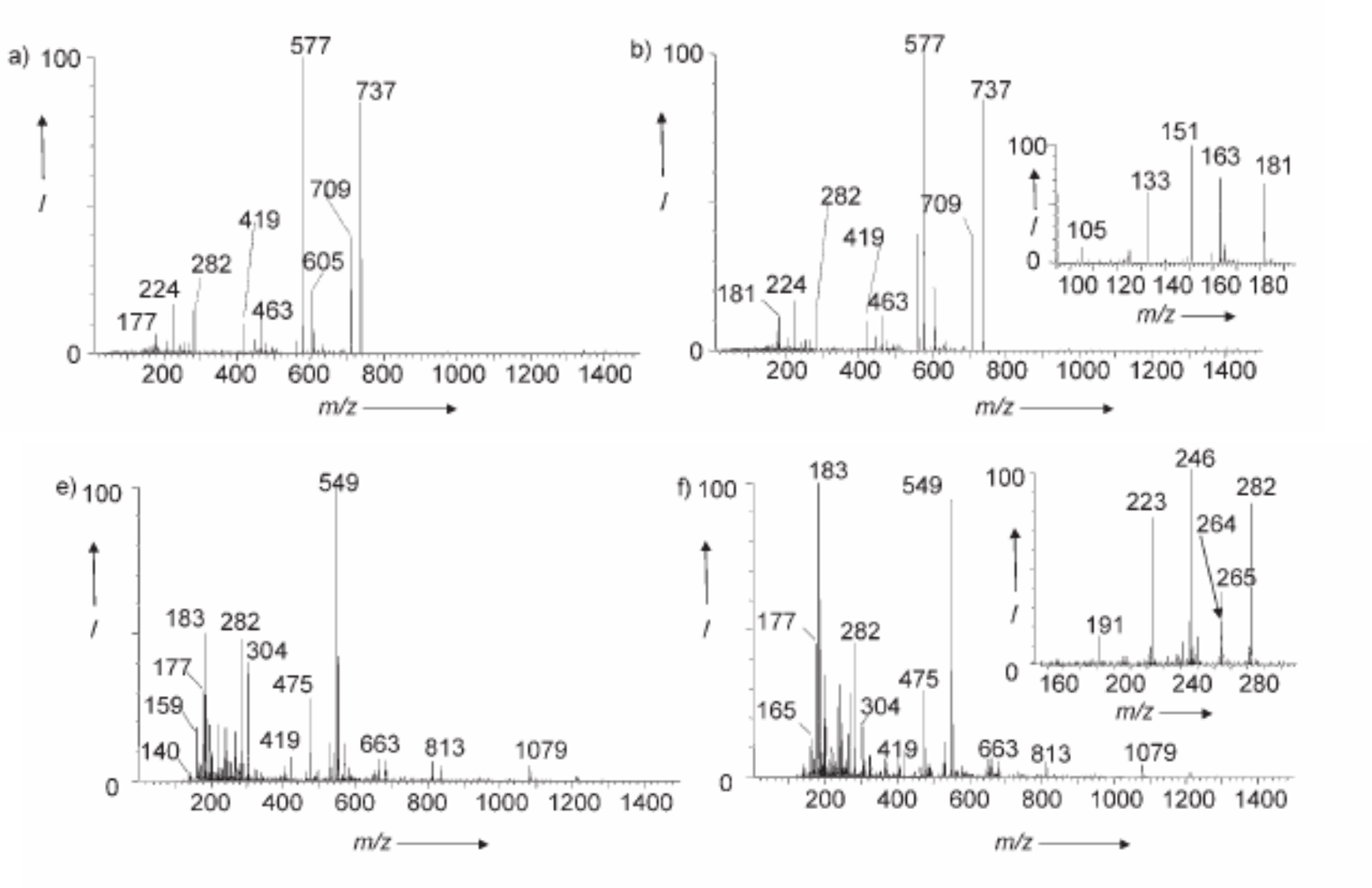
Neutral analytes can be transported over a distance longer than 1.2 m.

This feature is of practical relevance for multiple applications; especially in cases where the sample is not accessible because of extreme environmental conditions

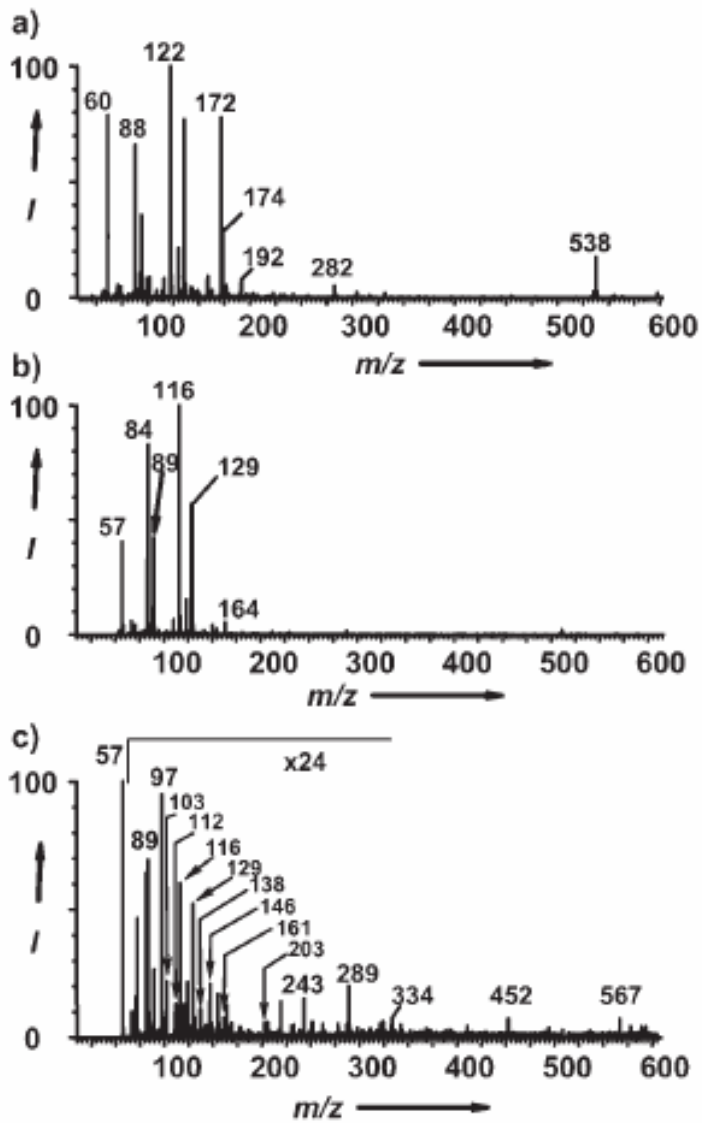


a) Hand skin before coffee consumption;
b) Same human hand skin area 30 min after coffee consumption
c) Same human hand skin area 60 min after coffee consumption.

Insets: (b) and (c) show CID spectra of caffeine and nicotine, respectively.



Breath fingerprints, obtained by EESI-QTOF-MS of an individual Asian (left) and European (right) (a, b) before and (e, f) after drinking beer (3 bottle).



EESI MS spectra of
fish meat at different stages:

a) frozen fish not exposed to room temperature;
b) frozen fish after exposure to room temperature (22 8C) for one day
c) frozen fish after exposure to room temperature for two days.

Conclusion

The sampling technique implemented in front of a commercial QTOF mass spectrometer for direct analysis of various biological samples without any chemical contamination or sample pretreatment.

Thank you…

- The impact of electrospray
- water research and cluster research
- Sampling of bulk ice