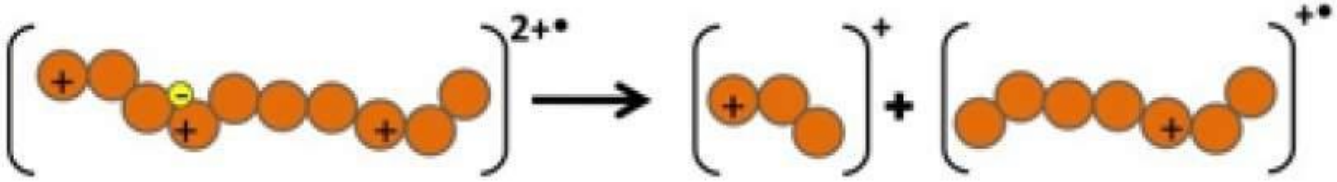
Instrumental Technique

Electron Transfer Dissociation (ETD)



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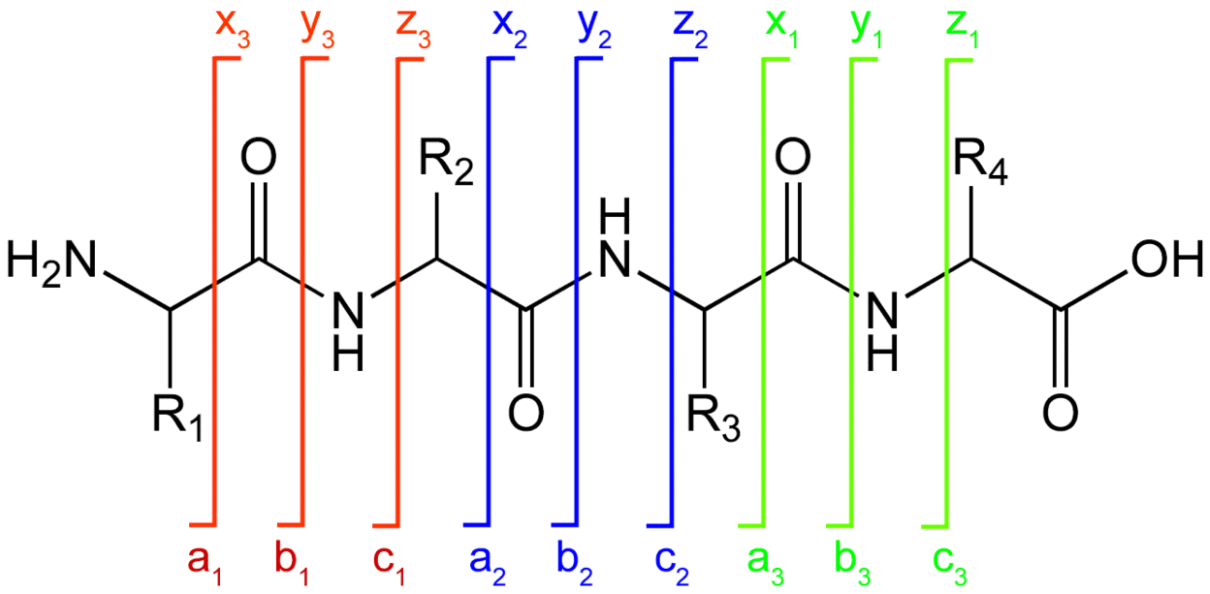
Electron-transfer dissociation (**ETD**) is a method of fragmenting multiply-charged gaseous macromolecules in a mass spectrometer between the stages of tandem mass spectrometry (MS/MS).

ETD induces fragmentation of large, multiply-charged cations by transferring electrons to them. ETD is used extensively with polymers and biological molecules such as proteins and peptides for sequence analysis.



History

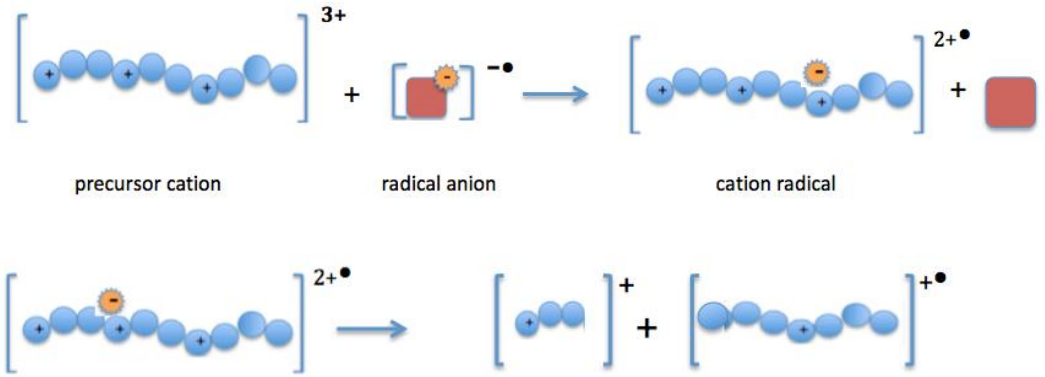
- Electron-capture dissociation (ECD) was developed in 1998 to fragment large proteins for mass spectrometric analysis. Because ECD requires a large amount of near-thermal electrons (<0.2eV), originally it was used exclusively with Fourier transform ion cyclotron resonance mass spectrometry (FTICR).

- Less costly options such as quadrupole time-of-flight (Q-TOF), quadrupole ion trap (QIT) and linear quadrupole ion trap (QLT) instruments used the more energy-intensive collision-induced dissociation method (CID), resulting in random fragmentation of peptides and proteins.

- In 2004 Syka et al. announced the creation of ETD, a dissociation method similar to ECD, but using a low-cost, widely available commercial spectrometer.

- The first ETD experiments were run on a QLT mass spectrometer with an electrospray ionization (ESI) source.

Principle of operation



In ETD, an electron is transferred to the positively-charged protein or peptide, causing fragmentation along the peptide backbone.

1. Radical anion preparation

In the original ETD experiments anthracene ($C_{14}H_{10}$) was used to generate reactive radical anions through negative chemical ionization. Several polycyclic aromatic hydrocarbon molecules have been used in subsequent experiments, with fluoranthene currently the preferred reagent. Fluoranthene has only about 40% efficiency in electron transfer, however, so other molecules with low electron affinity are being sought.

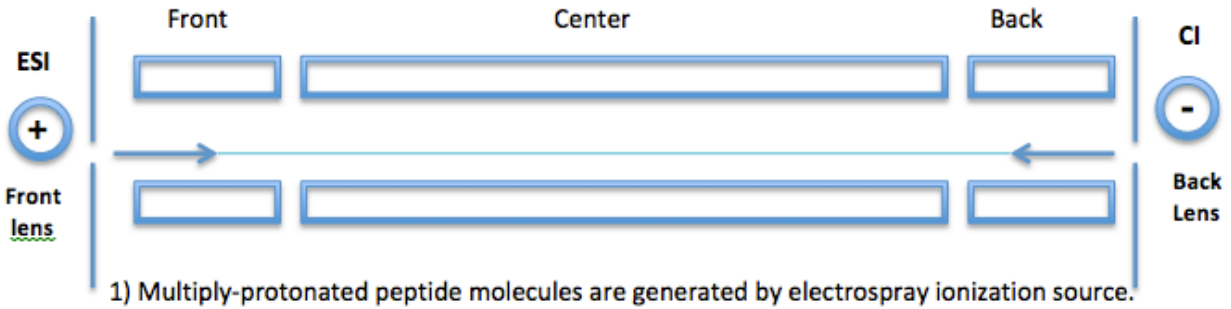
2. Injection and fragmentation

When the precursor cations (proteins or peptides) and radical anions are combined in the ion trap an electron is transferred to the multiply-charged cation.

3. Mass analysis

Instrumentation

Schematic of LTQ mass spectrometer modified for ETD



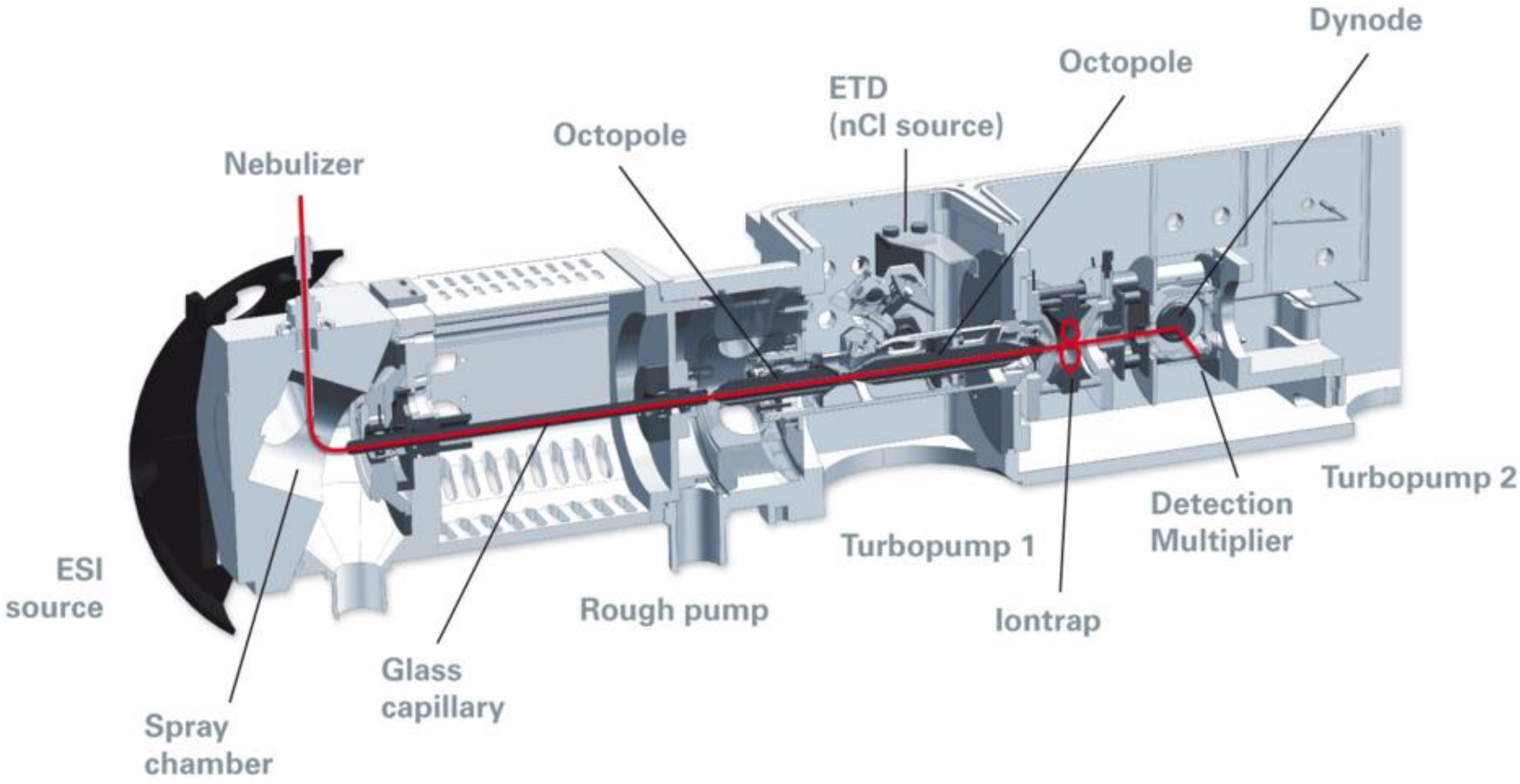
1) Multiply-protonated peptide molecules are generated by electrospray ionization source.
2) DC offset moves precursor ions to front of the linear trap.
3) Negatively charged reagent ions are injected into the center section of the linear trap.
4) Secondary RF voltage is applied to the end plates to allow negative and positive ions to mix.

- The first ETD experiments at the University of Virginia utilized a radio frequency quadrupole linear ion trap (LQT) modified with a chemical ionization (CI) source at the back side of the instrument.

- Having a negative CI source at the back of the instrument interfered with the high-resolution analyzer in LQT-Orbitrap and quadrupole time-of-flight (QTOF), so alternate ionization methods for the radical anions have been introduced.

- In 2006 a group at Purdue University led by Scott McLuckey used a quadrupole/time-of-flight (QqTOF) tandem mass spectrometer with pulsed nano-ESI/atmospheric pressure chemical ionization (APCI) dual ionization source using **radical anions of 1,3-dinitrobenzene as the electron donor**. Later a lab at the University of Wisconsin adapted a hybrid quadrupole linear ion trap-orbitrap mass spectrometer to use ETD.

- As ETD is increasingly popular for protein and peptide structure analysis, implementation on easily available ion-trap mass spectrometers coupled with high resolution mass analyzers continues to evolve.



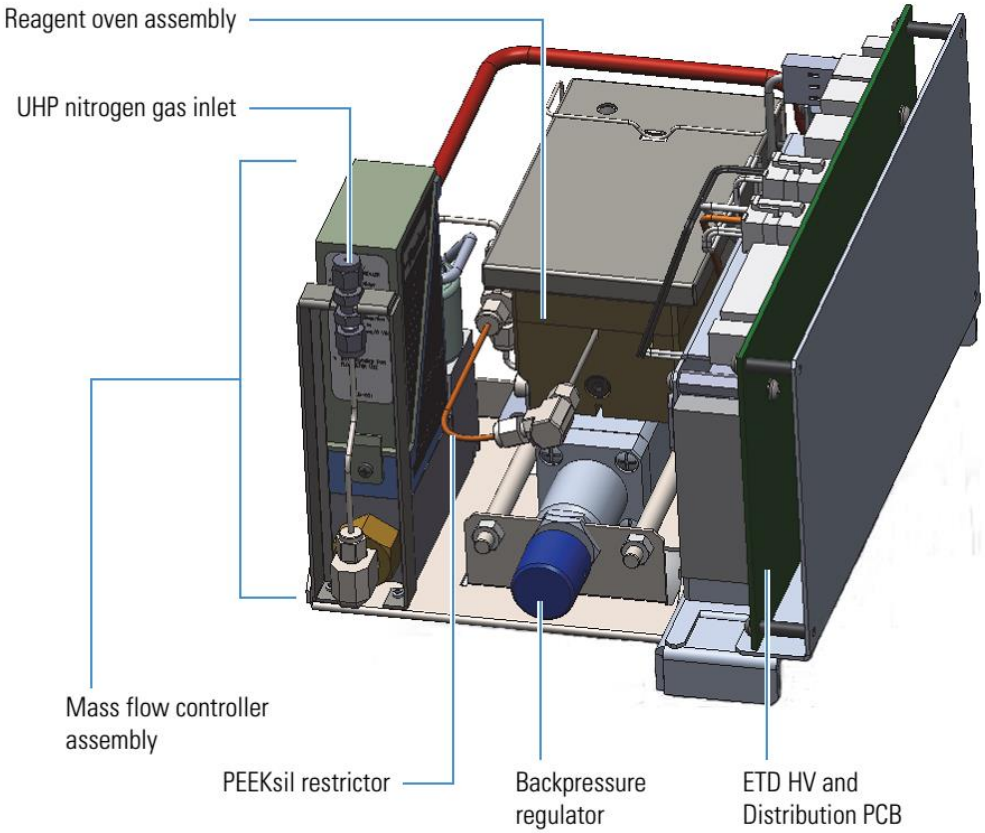
Ion trap mass spectrometer with ETD (schematic diagram)

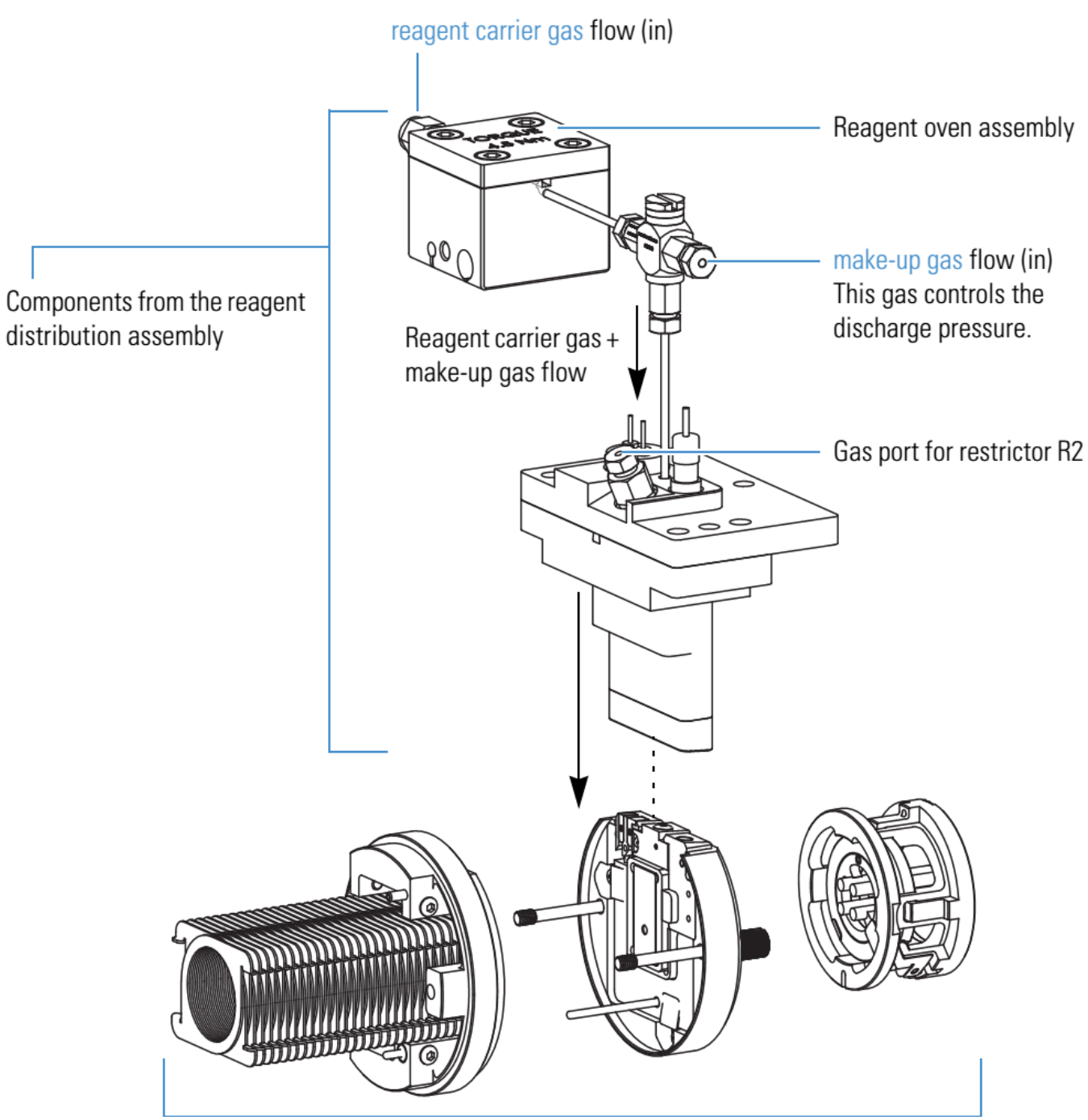
Electronic Assemblies

ETD Source PCB — Controls the reagent distribution assembly.

ETD HV and Distribution PCB — Distributes the signals from the ETD Source PCB to various components, including the heaters, the mass flow controller (MFC). This PCB also powers the high voltage (HV) power supply unit, and contains a pulser circuit that regulates the output current for the HV power supply unit.

ETD RF PCB —Generates the RF voltage applied to the LIT front and center lenses during the charge sign independent trapping, which is necessary to enable the ETD reaction.




reagent carrier gas flow (in)
Reagent oven assembly
make-up gas flow (in)
This gas controls the discharge pressure.
Components from the reagent distribution assembly
Reagent carrier gas + make-up gas flow
Gas port for restrictor R2

Applications

- **Proteomics**

ETD is widely used in the analysis of protein and large peptides. Important post translational modifications including phosphorylation, glycosylation and disulfide linkages are all analysed using ETD.

- **Polymer chemistry**

Although MS-based analyses of polymers have largely been performed using single-stage MS, tandem MS has also been used to characterize polymer components. CID is the most common method of dissociation used, but ETD has been used as a complementary method. Unique bond cleavages resulting from ETD supply valuable diagnostic information.


THANK
YOU !