



EU FT-ICR MS

**Sample preparation
FT-ICR MS sample analysis
Top down of proteins
Data interpretation**

**Petr Novák, Zdeněk Kukačka, Petr Man,
Jan Fiala & Tereza Kadavá**





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Protein desalting under denaturing conditions

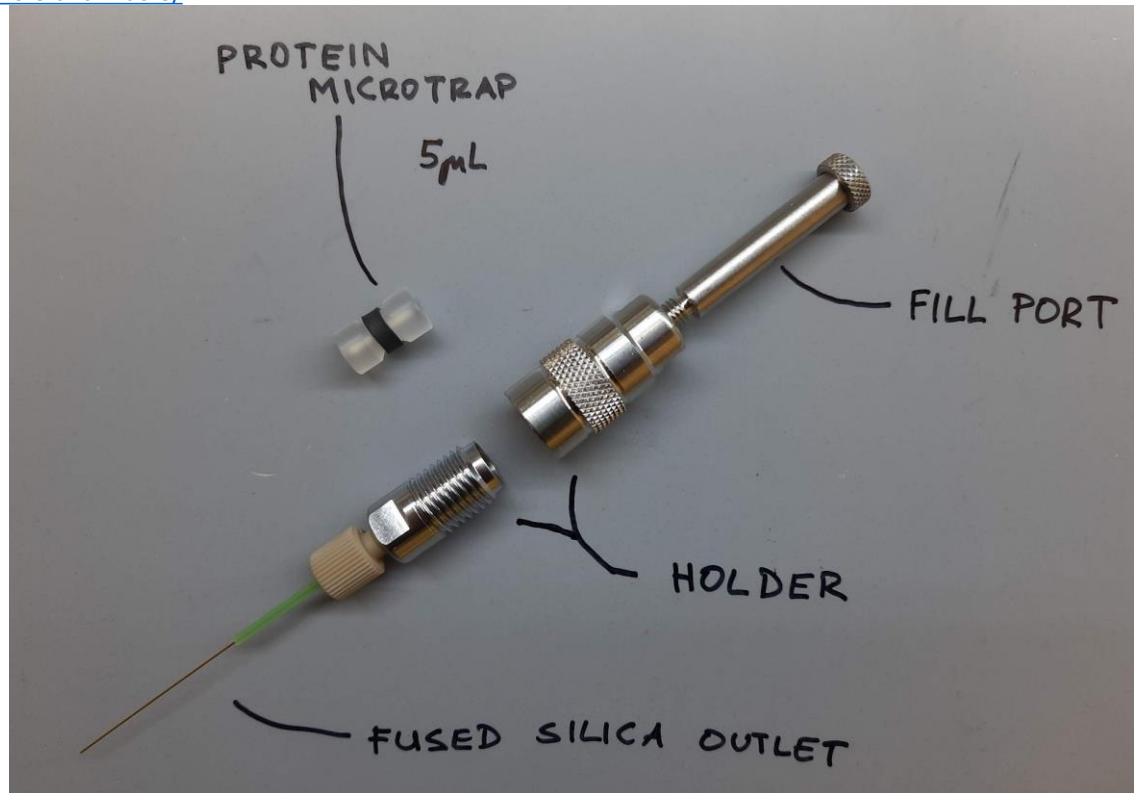
Protein MicroTrap

<https://www.optimizetech.com/opti-trap-cartridges/>

Manual trap holder

<https://www.optimizetech.com/opti-trap-manual-holder-kit/>

<https://www.optimizetech.com/opti-trap-loop-trap-holder-kit-micro-and-macro/>



Protein desalting under denaturing conditions

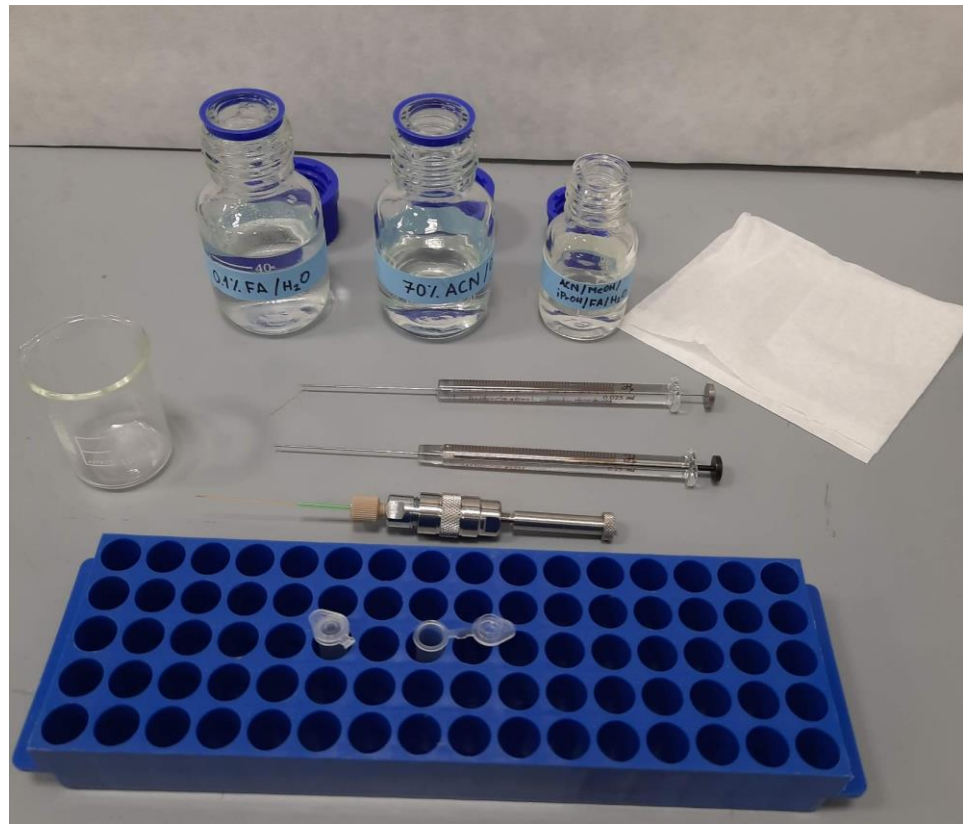
- Solvents
- A 0.4 % formic acid in water
 - B 70% acetonitrile / 0.4% formic acid
 - C water / MeOH / iPrOH / ACN / FA

Hamilton (gas tight) syringes – 100 and 25uL

Kim Wipe

Protein solution

Microtube (Eppendorf)



Protein desalting under denaturing conditions

Solvents	A	0.4 % formic acid in water
	B	70% acetonitrile / 0.4% formic acid
	C	water / MeOH / iPrOH / ACN / FA

1. Clean trap with solvent C
2. Wash with solvent B and equilibrate to A
3. Dilute sample with A
4. Slowly load onto the trap
5. Desalt with A
6. Elute with B into a clean tube
7. Go to 1.



Native Mb desalting – Zeba Spin columns

150mM Ammonium Acetate, pH set to 7.4

Zeba Spin columns (7K MWCO, 75uL)

Protein sample

Pipettes and tips

Collection and wash tubes
(Eppendorf tube 2 and 1.5mL)



Native Mb desalting – Zeba Spin columns



Remove storage buffer by centrifugation.

Making nESI tips

Laser puller P-2000 (Sutter Instruments)

<https://www.sutter.com/MICROPIPETTE/p-2000.html>

Quartz glass capillaries – Q-100-70-7.5

<https://www.sutter.com/MICROPIPETTE/glass.html>

Petri dish for nESI tips

Plastic (silicon covered) forceps



Making nESI tips



Loading nESI tip

Long gel loader tips (20uL) + pipette (5-10uL)

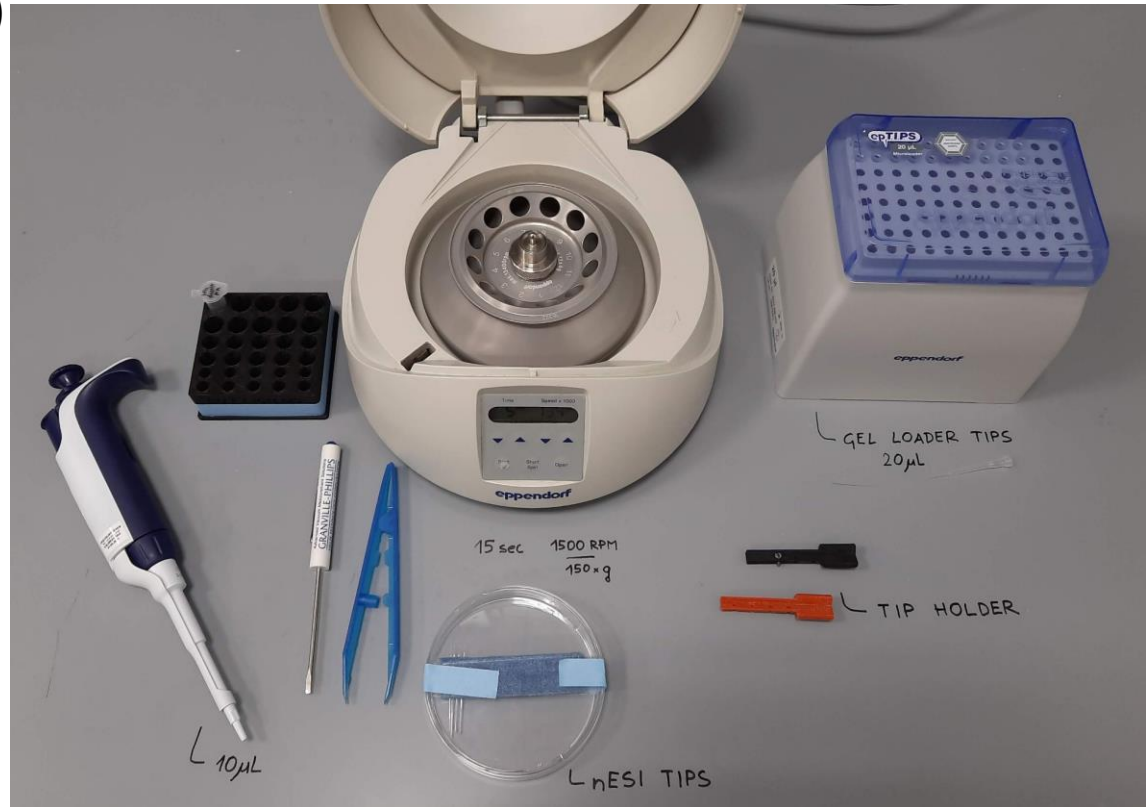
nESI tips and 3D printed nESI tip holders

Desalted protein solution (1-10uM)

Desktop centrifuge set to
15sec
150 x g

Plastic (silicon covered) forceps

Small screw-driver

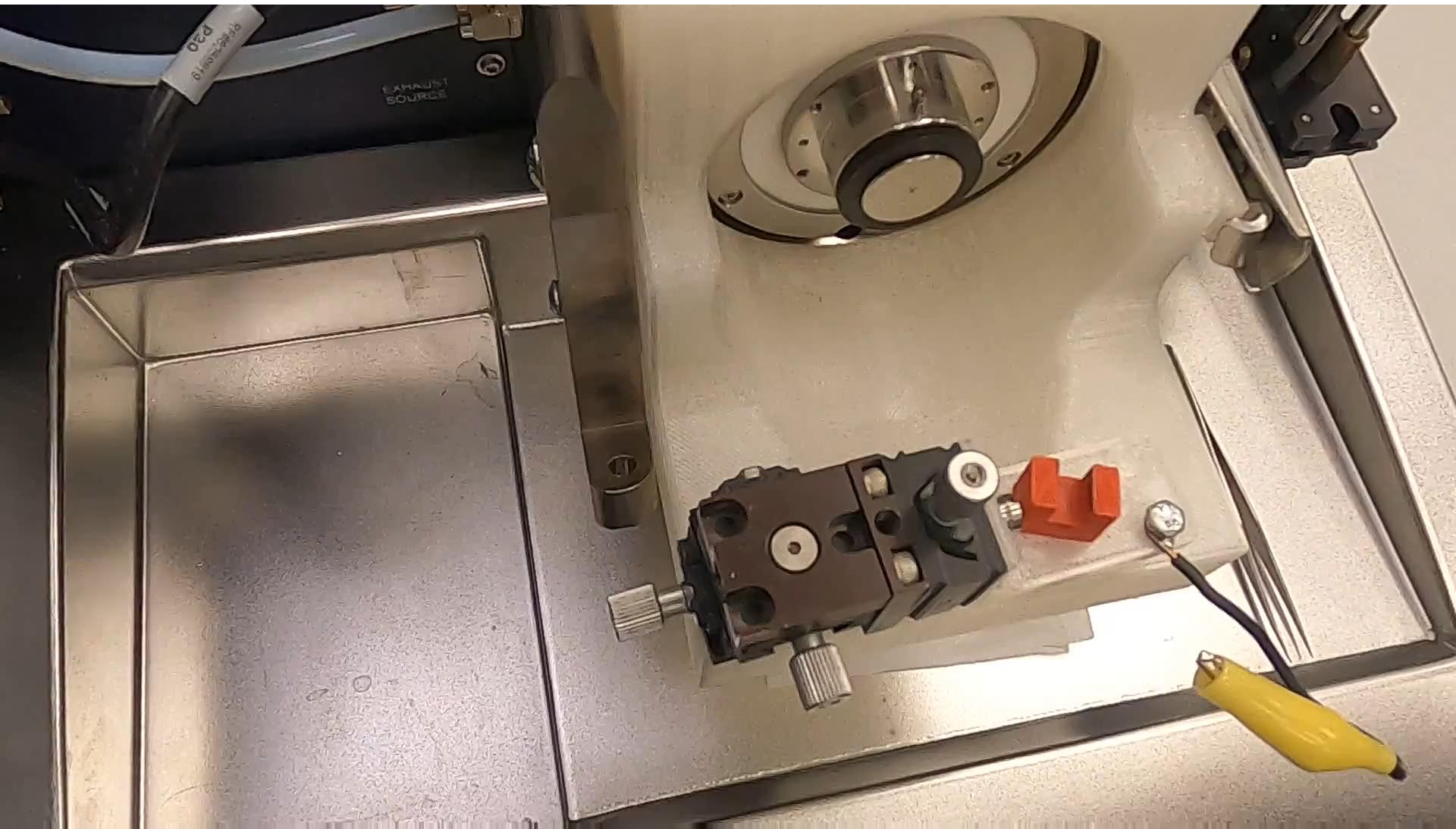


Loading nESI tip

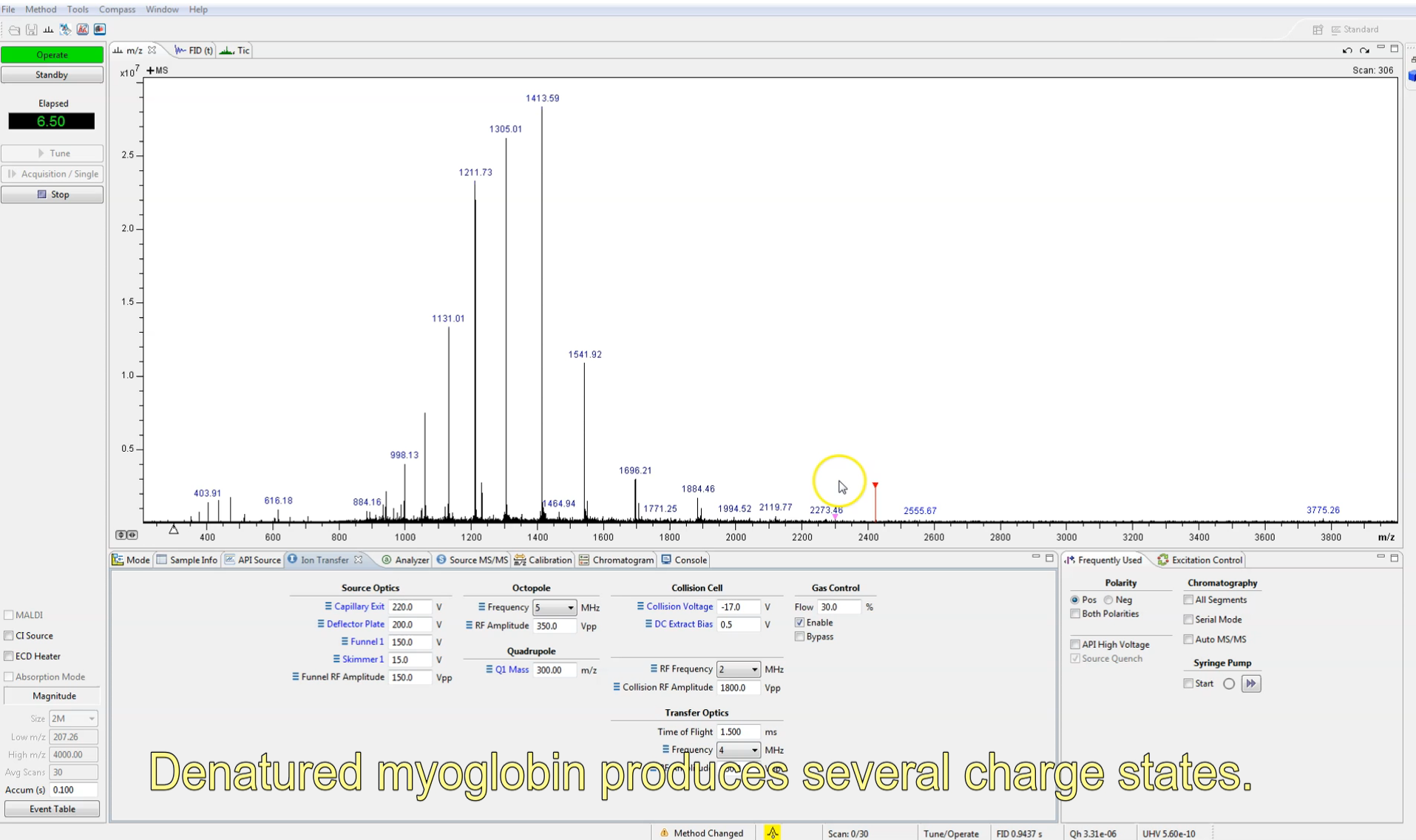


Place a nanoESI tip into a holder.

nESI setup

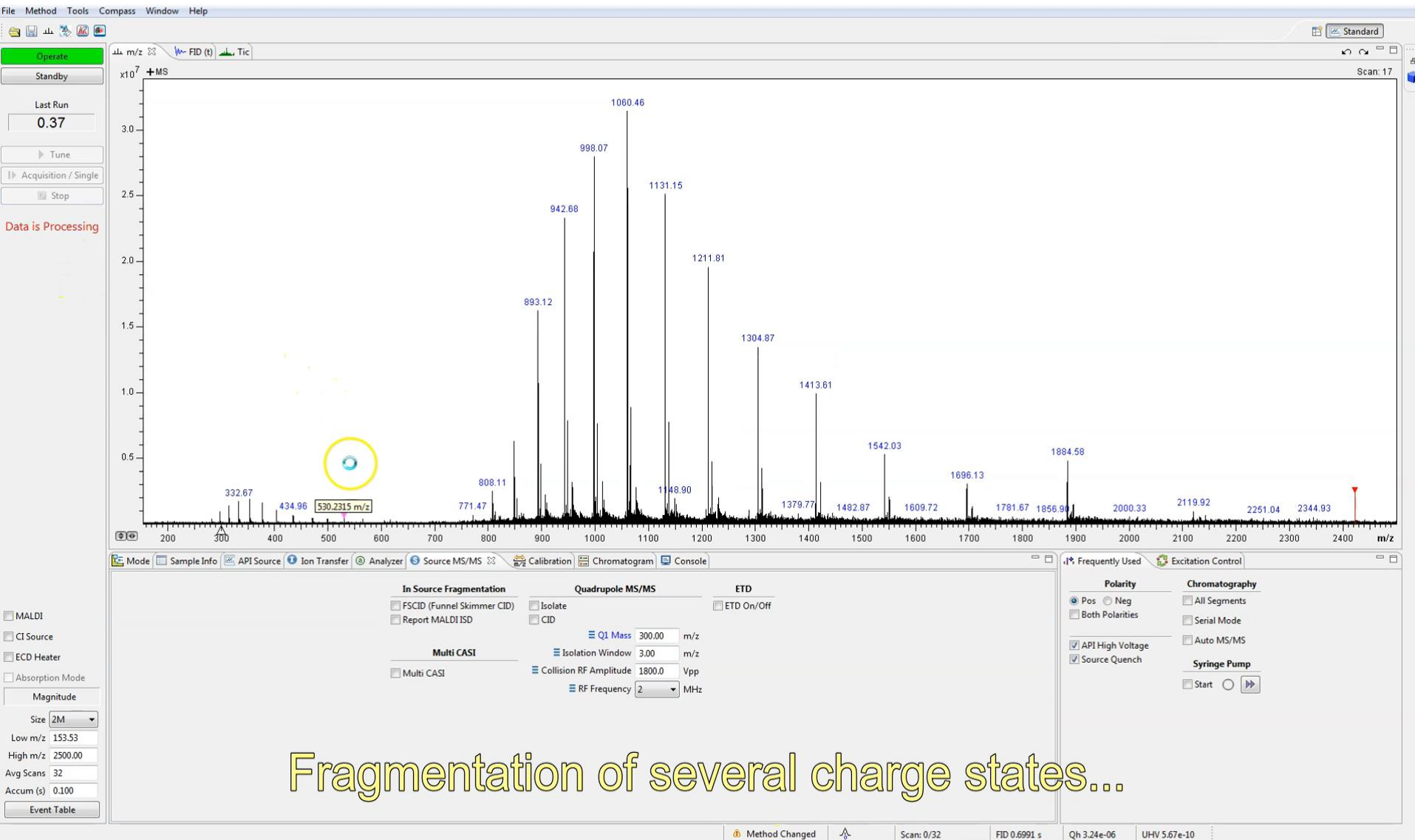


MS analysis - direct infusion - under denaturing conditions



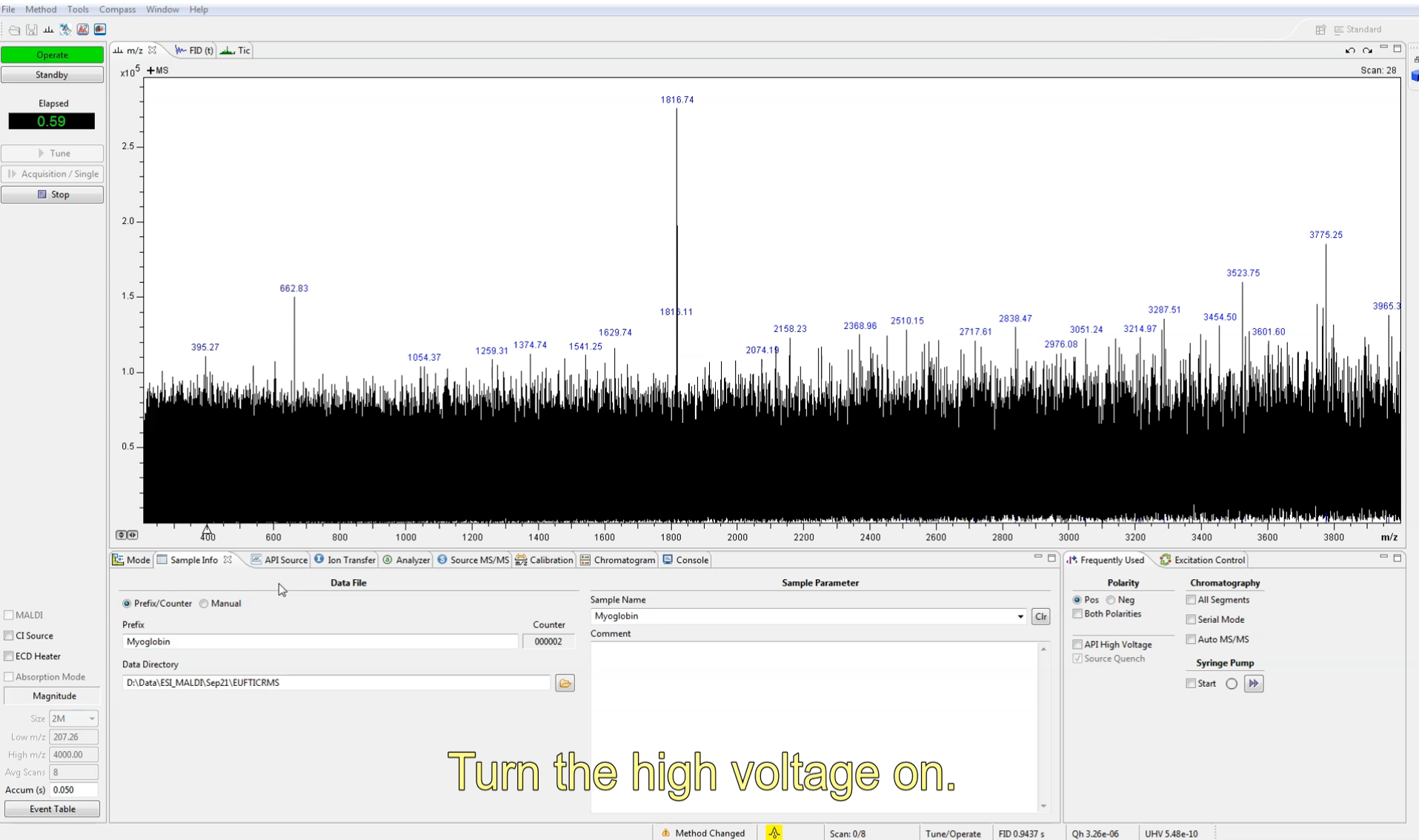
Denatured myoglobin produces several charge states.

MS analysis - multiCASI

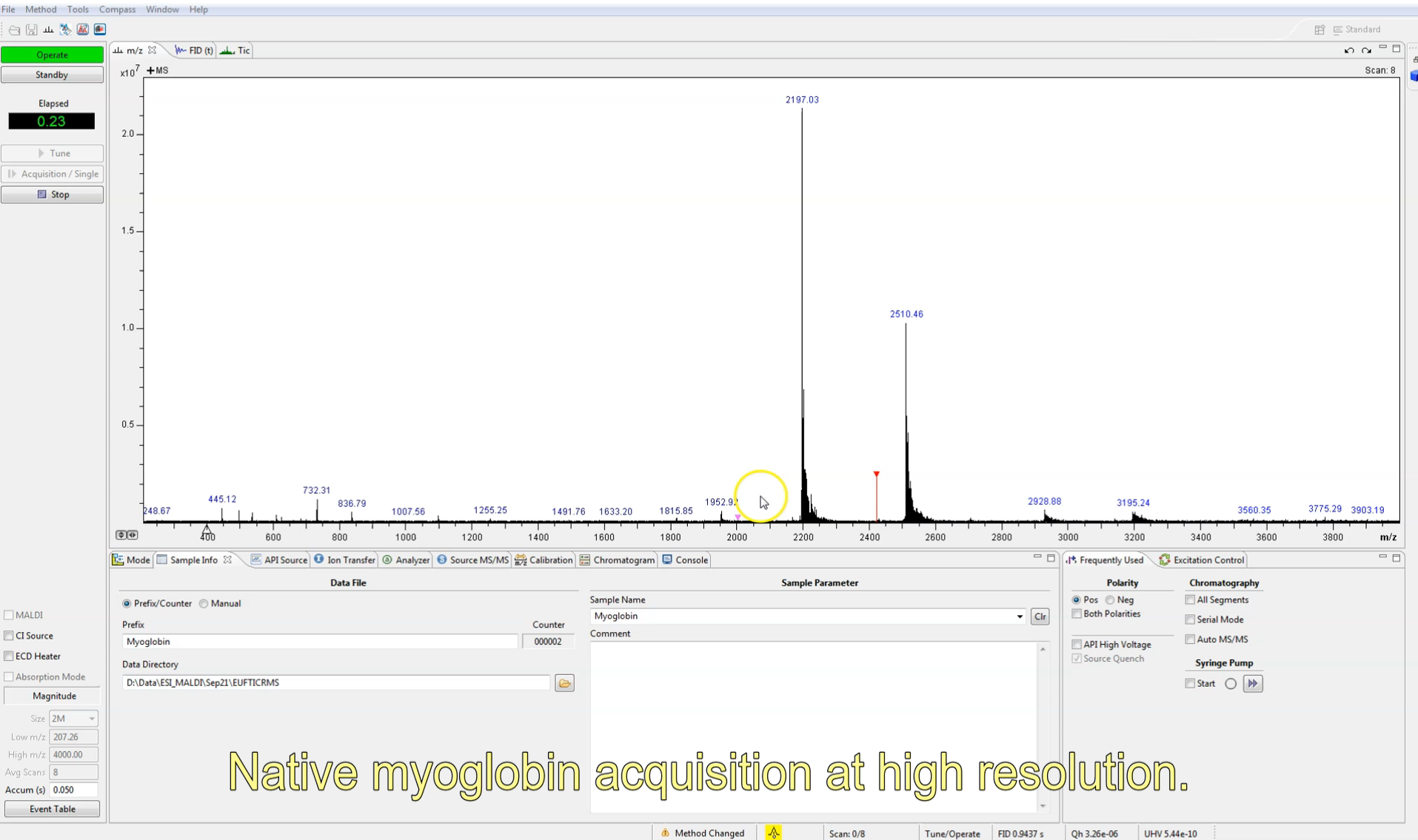


Fragmentation of several charge states...

MS analysis – native Mb (and its activation)



MS analysis – native Mb (resolution)



Native myoglobin acquisition at high resolution.



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