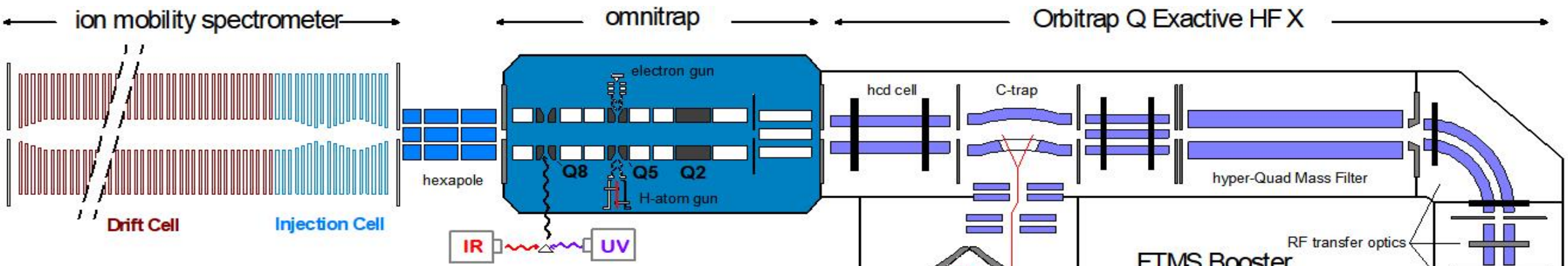
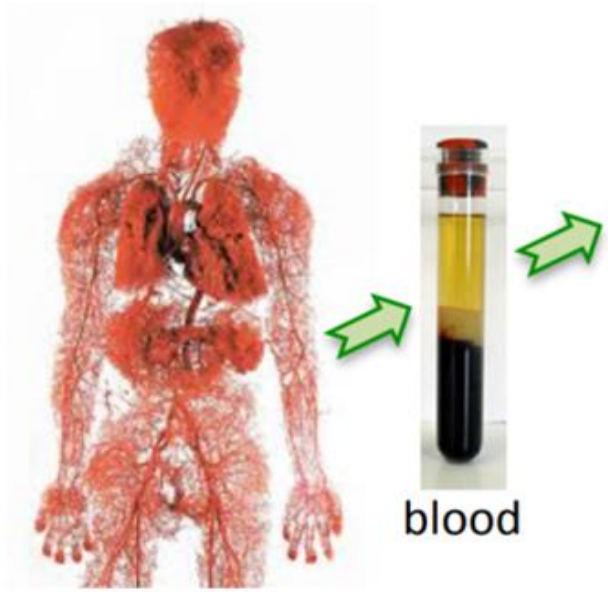
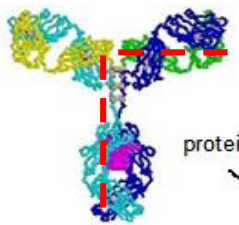
# WP5 Development of pI-Trap-ESI Combination

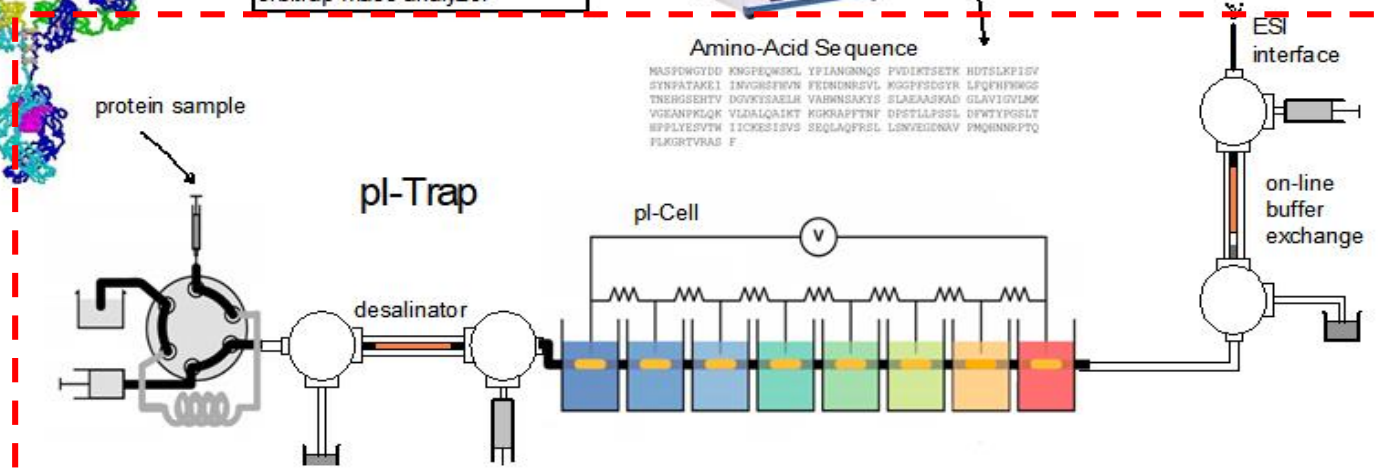
14.45-15.15

# Background



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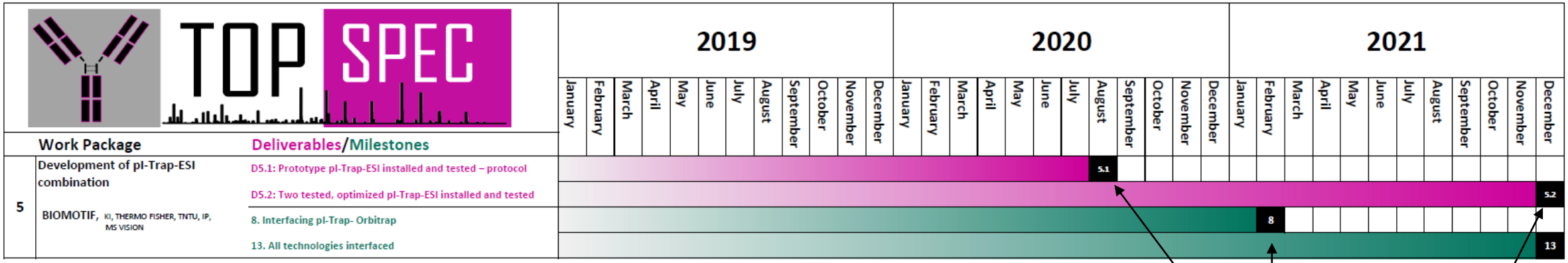



| Deliverable | Deliverable Title | Status | Completion |
|---|---|---|---|
| D5.1 | Prototype pI-Trap ESI Installed and Tested Protocol | Ongoing | 2020-08-31 |
| D5.2 | Two tested, optimized pI-Trap-ESI Installed and tested | Ongoing | 2021-12-31 |

| Milestone | Deliverable Title | Status | Completion |
|---|---|---|---|
| 8 | Interfacing pI-Trap-Orbitrap | Ongoing | 2021-02-28 |
| 13 | All technologies Interfaced | Ongoing | 2021-12-31 |

Delivery: D5.1 Prototype pI-Trap-ESI Installed and Tested - Protocol

Action: Development of a Integrated Buffer Exchanger ESI-Interface

# Challenges

**Normal IEF pI-Trap**

Ampholytes, Urea, glycerol

pH gradient

→ Detrimental for ESI MS

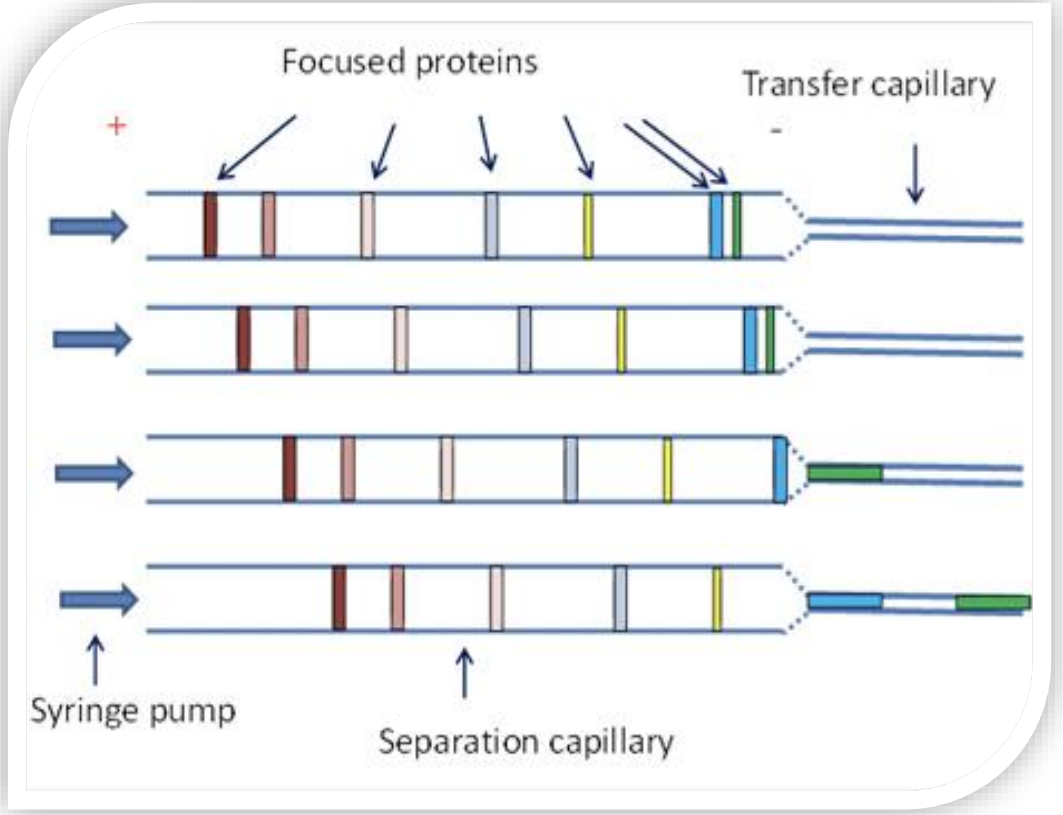
↓

- High background
- Spray stability

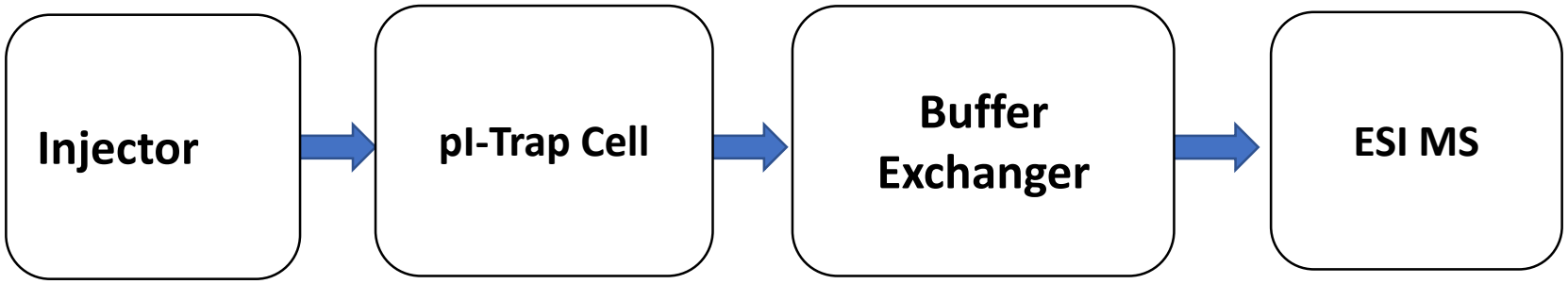
# pI-Trap: High-Capacity Isoelectric Focusing

Steadby-state separation based on the electrophoretical migration of polypeptides in channel containing a pH gradient.

Basic Requirements



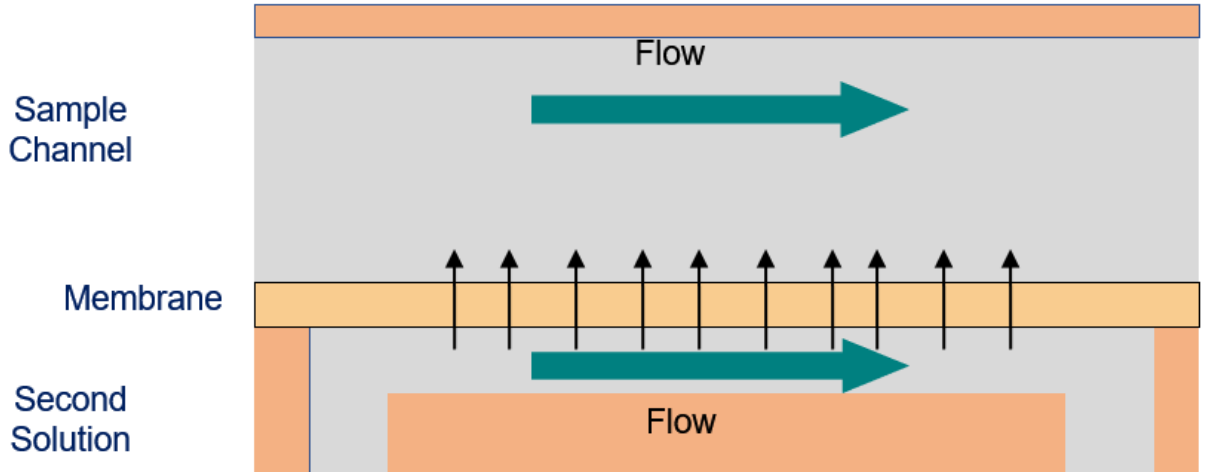
# pI-Trap based Isoelectric focusing combined with ESI MS (Orbitrap Elite)




Workflow of fractionation system

# Development of Interface pI-Trap ESI MS

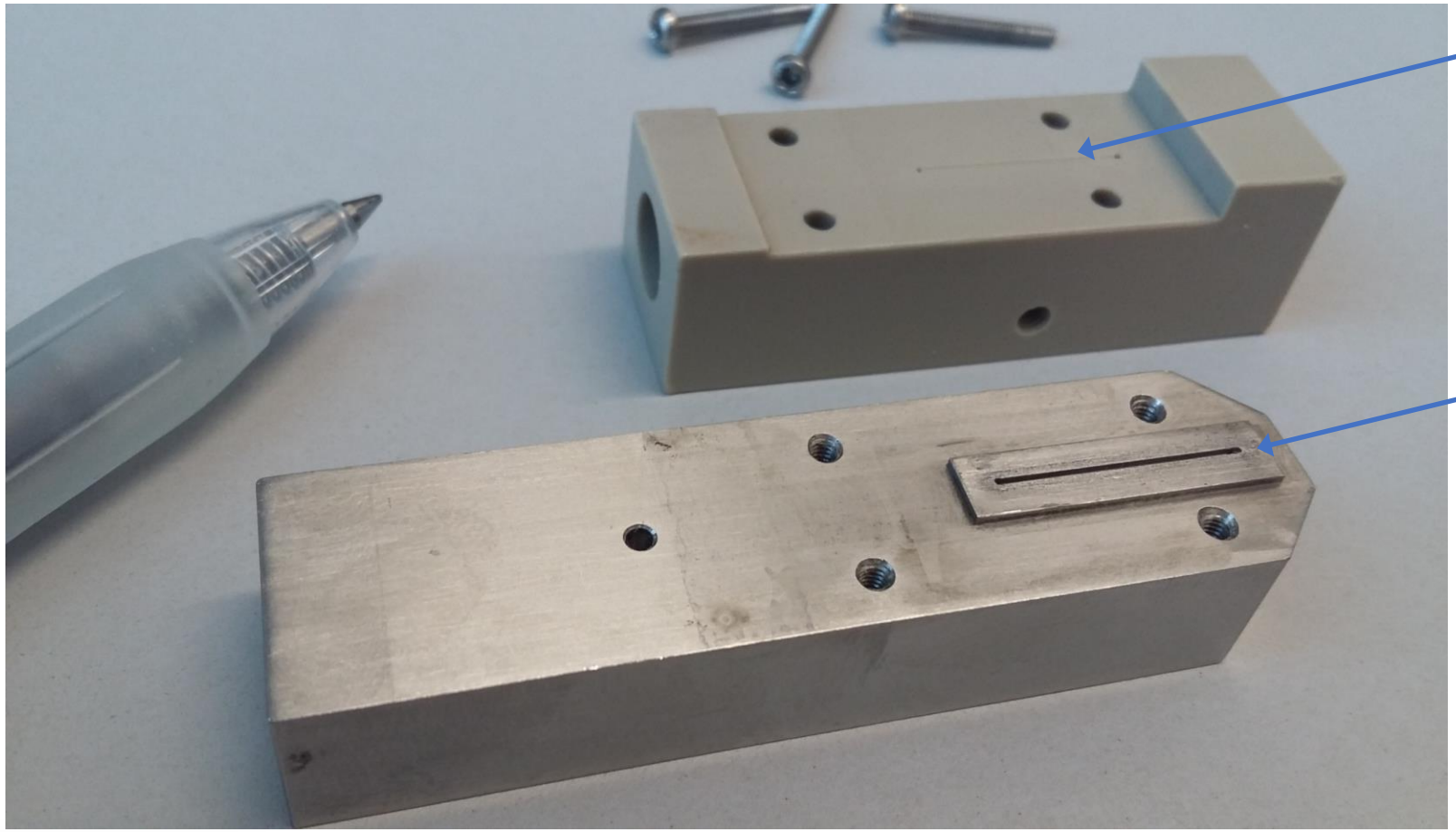
## Buffer exchanger




Acidify Eluent of the pI-Trap

Remove Contaminats

# Integrated Electrospray and Buffer exchanger



Sample Channel

100 µm x 400 µm x 1.6 cm

Cleaning Channel

400 µm x 400 µm x 1.6 cm

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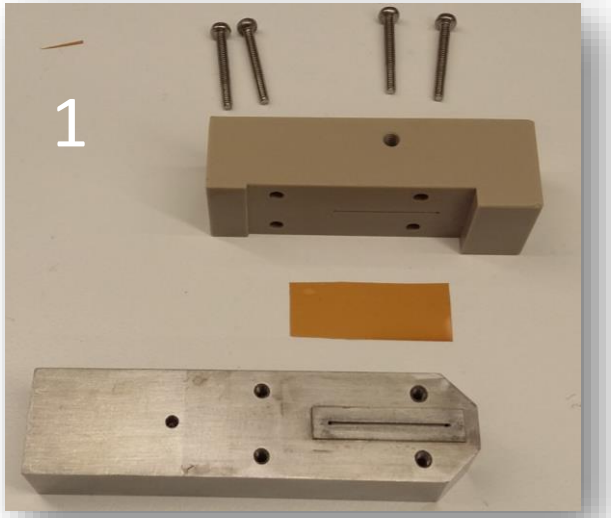
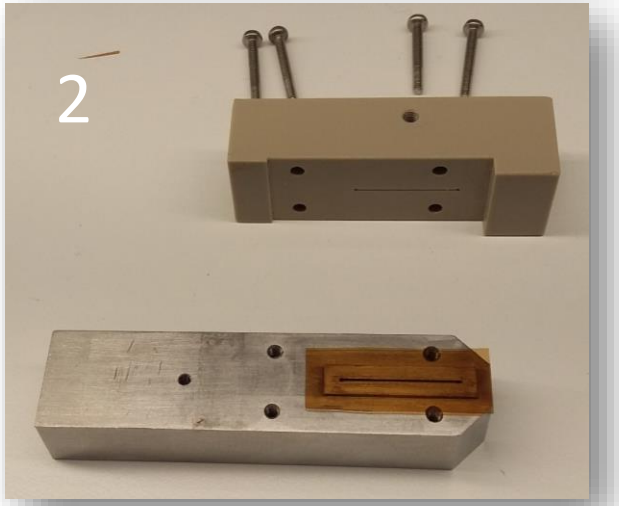
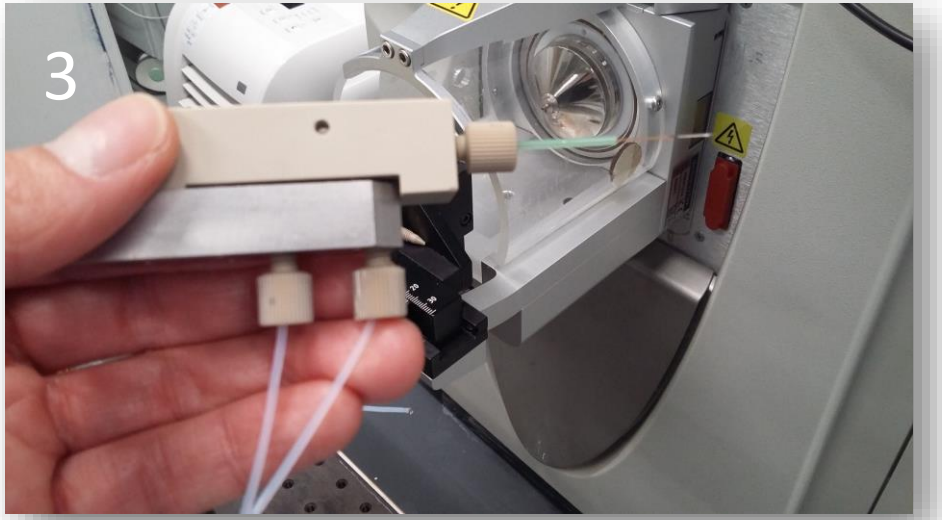
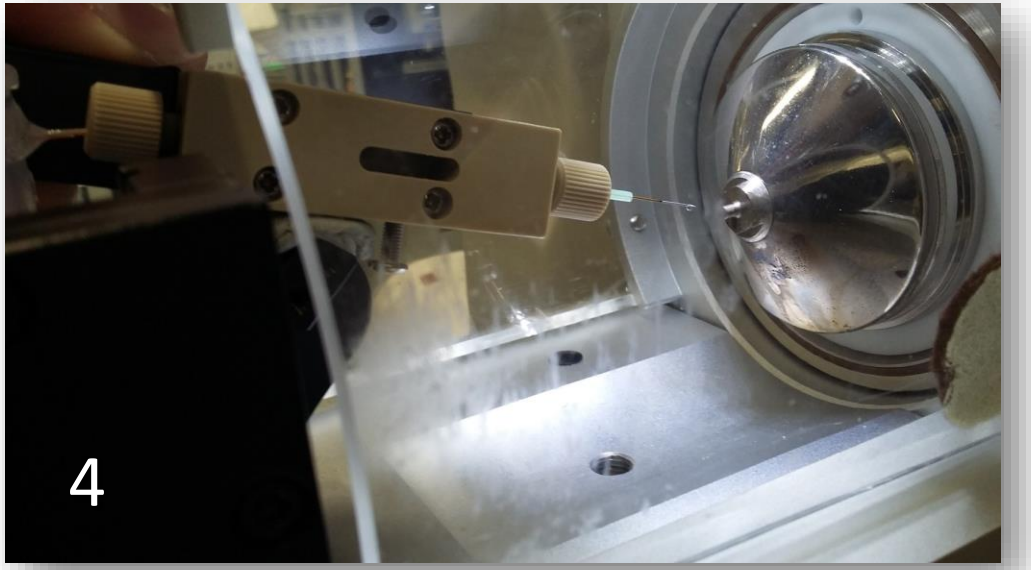
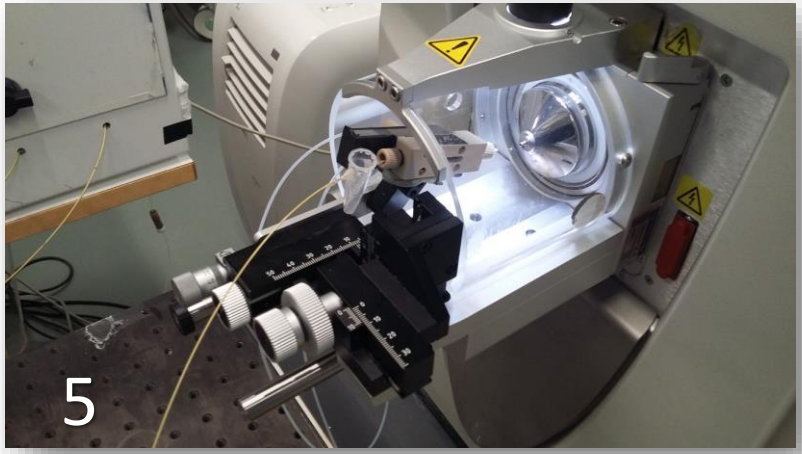
# Assembly of the Integrated Electrospray and Buffer Exchanger



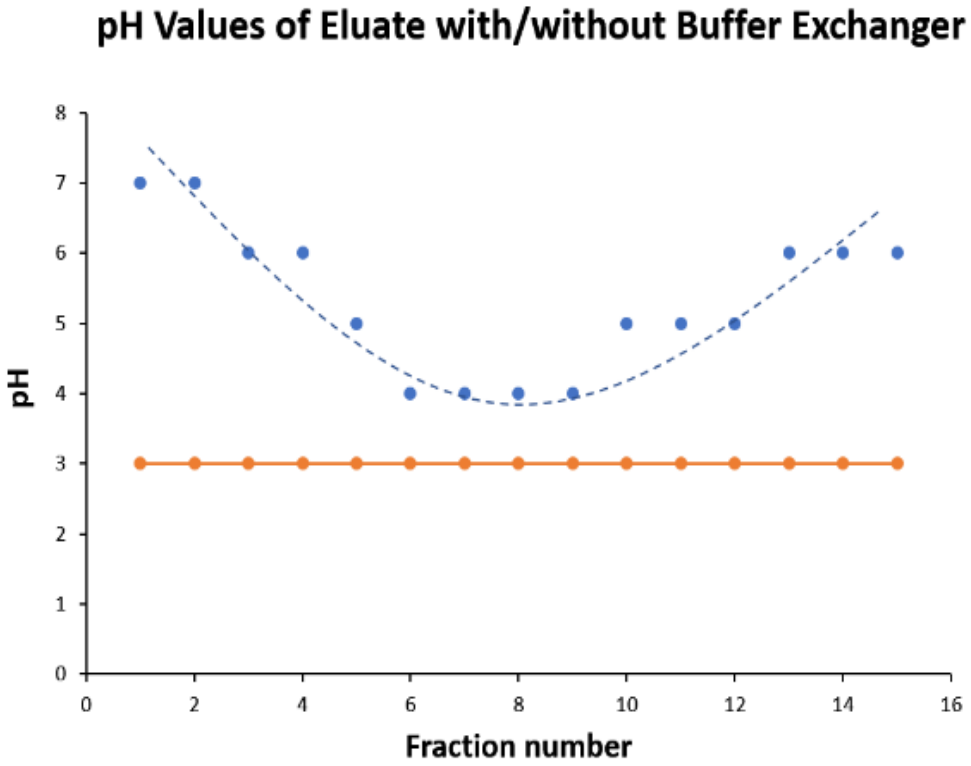



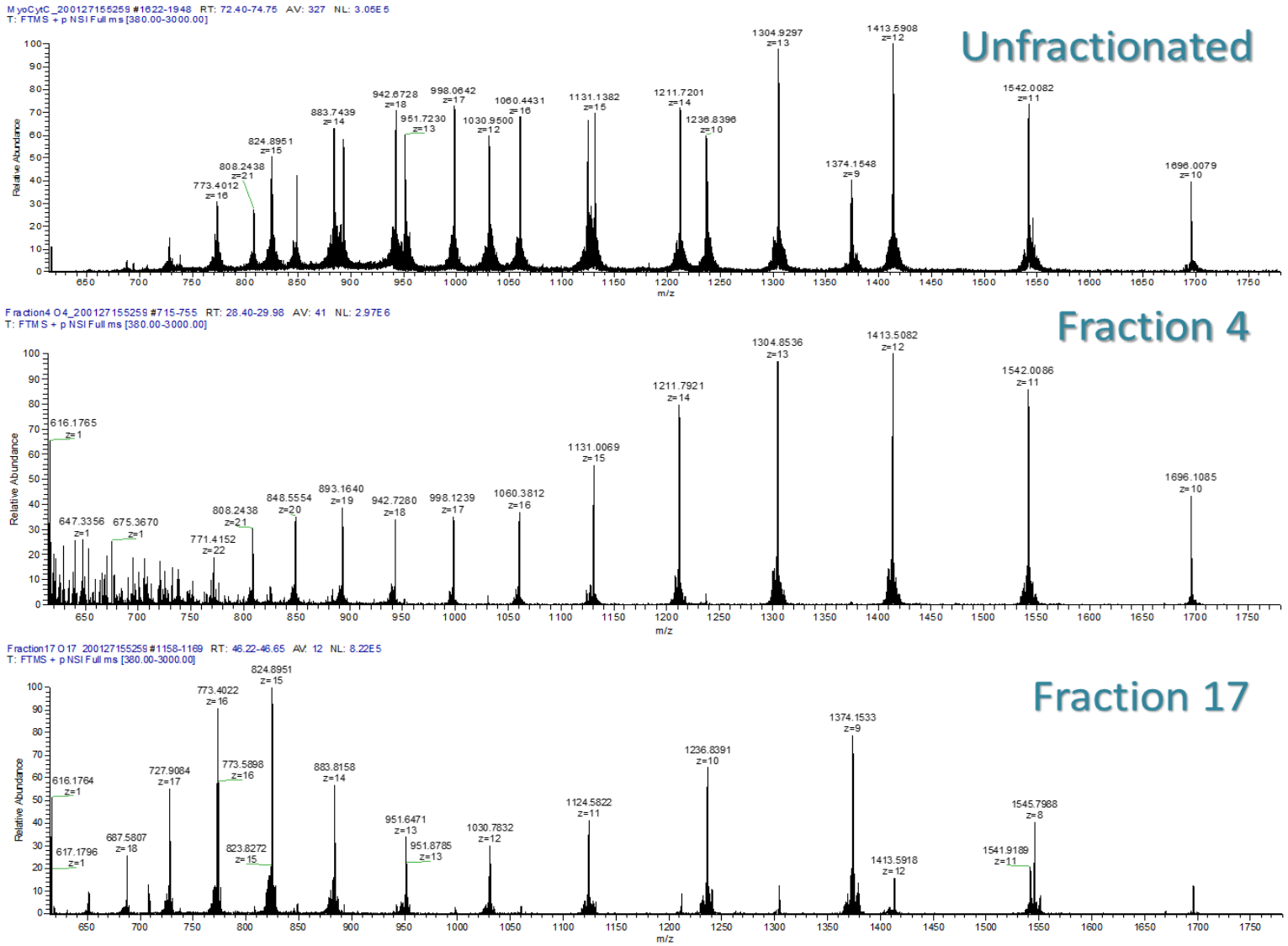



# Buffer Exchanger – On-Line Acidification




Plot: pH versus fractions eluted from the pI-trap cell without (blue) and with (orange) the buffer exchanger. An ampholyte with a pI range between 3-6 was used in these experiments.

# Data from the Integrated Electrospray Buffer Exchanger Device



Delivery: D5.2 Two-Tested, Optimized pI-Trap ESI Installed and Tested

Action: Design and Manufacture of a pI-Trap-ESI Instrument

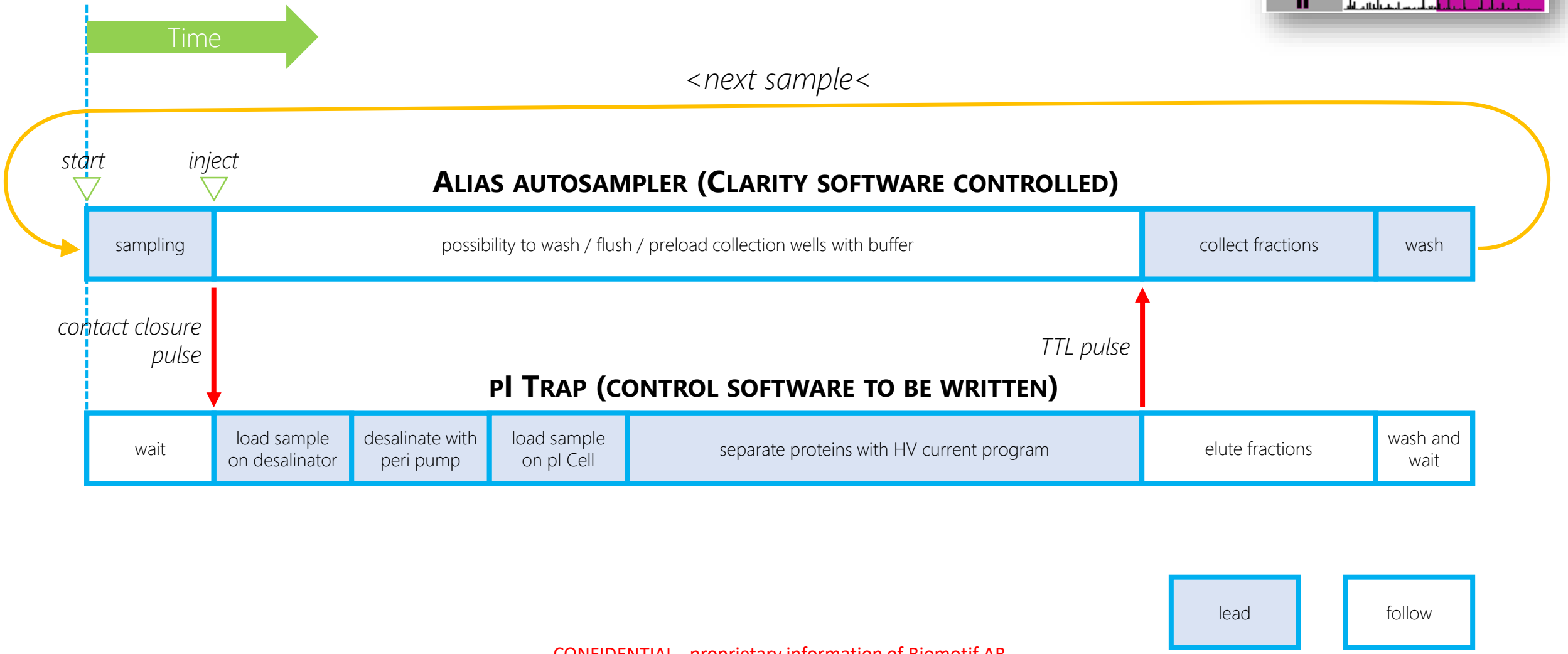
# Action: Design and Manufacture of a pI-Trap-ESI Instrument

- Instrument Specifications - Hardware & Software: **Completed**
- BOM list: **Completed**
- Mechanical Drawings: **Completed**
- Manufacture of the Mechanical Parts: Expected April 2020
- Software Development:
  - Software requirements: **Completed**
  - Electronics & Hardware Requirement gathering for Software implementation. **Completed**
  - Software and Electronic Design. **Completed**
  - Design and Manufacture of a dedicated Printed Circuit Board (PCB): **Completed**
  - Implementation or coding. Expected February 2020
- Testing. Expected April 2020
- Installation and Troubleshooting. Expected July 2020
- Assembly and off-line testing of the pI-Trap: Expected July 2020
- Prototype installed for pI-Trap-ESI: Delivery by August 2020

# Inside the pI Trap

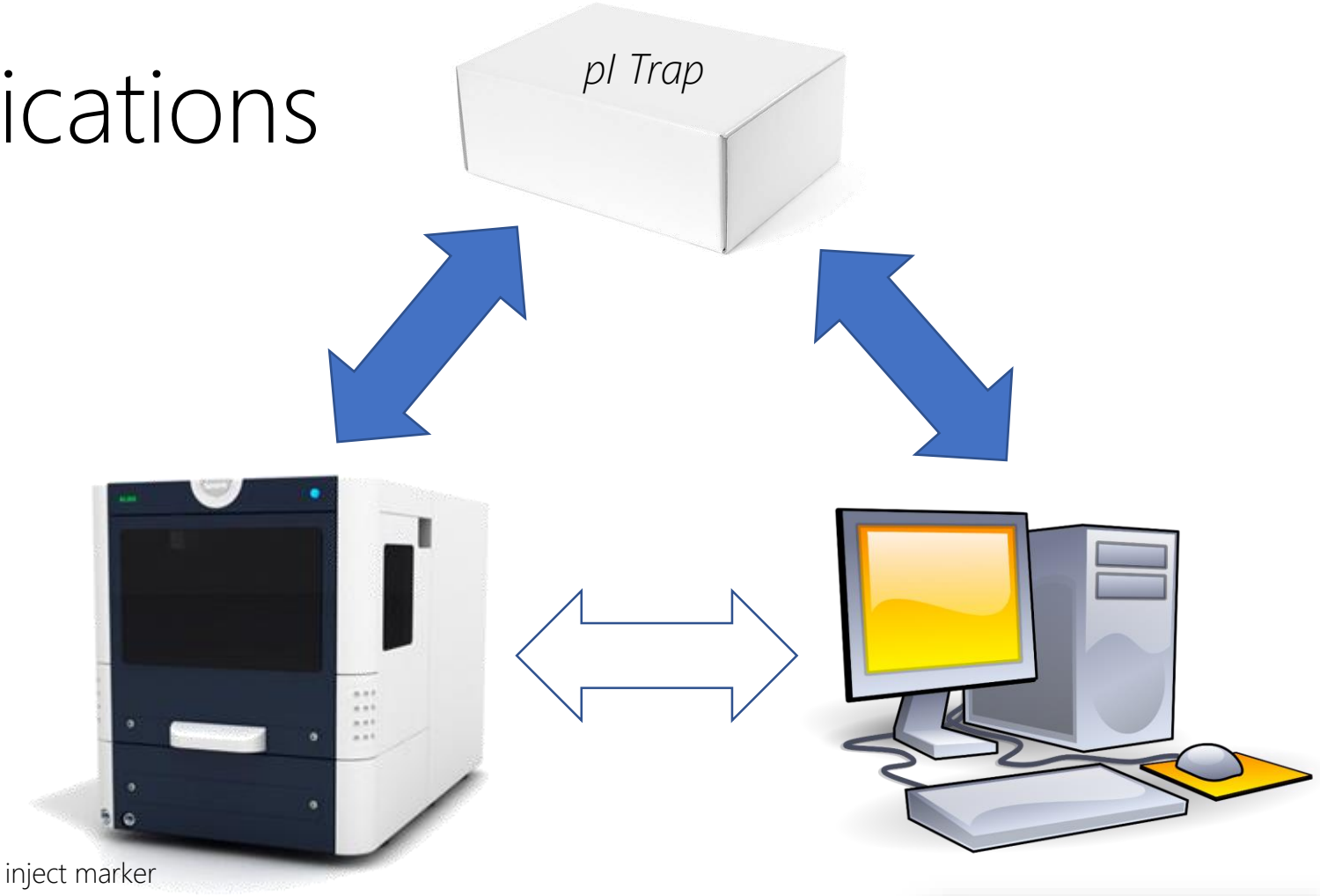
- Syringe pump:
  Tecan Cavro Centris (part no. 30063058)
  RS232 or RS485 control
- 2 high voltage power supplies:
  Spellman MPS series (MPS3P10/24/VCC/DCC2 and MPS3N10/24/VCC/DCC2)
  one for posivite and one for negative mode
  on/off by relay, DAC reference control
- Peristaltic pump
  Williamson series 100 micro (part no 100.035.024.016/2)
  on/off by relay
- HV relays
  Cynergy3 (DAT72410FU)
  one for posivite and one for negative mode
  actuated by LV relays?
- Safety interlocks
  Generic microswitches

# Analytical sequence



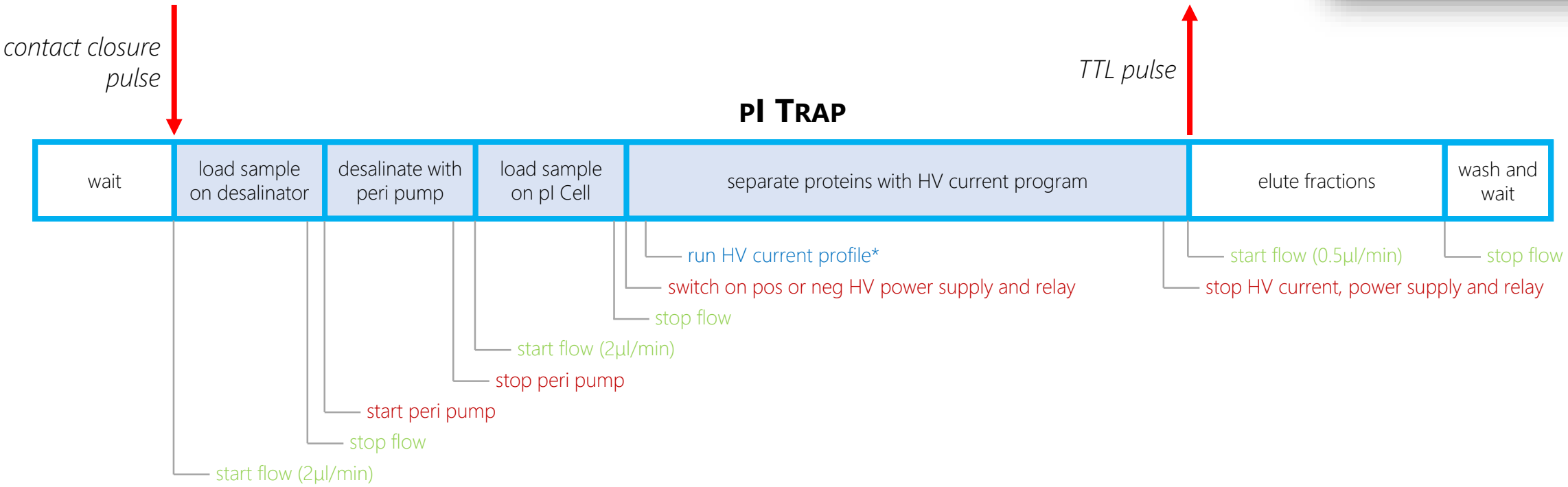
# System communications

- Between PC, Alias and pI Trap
- PC: control of analytical sequence
- Alias: limited in- and outputs, cannot relay communications




| | |
|---|---|
| Outputs | Single auxiliary output which can be programmed as inject marker (default), alarm, etc.<br>Contact closure, Vmax = 28 Vdc/Vac, Imax = 0.25A |
| Inputs | 2 programmable TTL inputs.<br>Free programmable as next injection (default), freeze or stop command |

# pI Trap sequence details




A pre-loaded method runs from receipt of a contact closure start pulse (inject marker from Alias autosampler). At the end of a run the pI trap waits for the next start pulse and the method is repeated. This cycle simply continues until the last sample is finished.

Legend:

RS485 or RS232
relay control
DAC control

*Typical current profile:
1. 50 µA during 30 sec
2. 100 µA for 30 sec
3. 150 µA for 4 min
4. 200 µA for 10 min
5. 210 µA for 20min
6. turn off voltage and collect fractions

# Conclusions 1

- Successful connection of pI-Trap with ESI MS achieved for the analysis of narrow pI fractions.
- Stable spray
- pH gradient generated during the IEF solved by a membrane-base buffer exchanger during mobilization
- An Integrated Buffer Exchanger ESI-Interface have been developed

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# Conclusions 2

- The pI-Trap Instruments is being built and no mayors “unknow critical problems” events are expected.
- Delivery by summer 2020

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# Next Steps

- Optimize the separation for the NISTmAb
- Reduce dead volumes & Optimize Protocols
- Test Instrument & Feedback to the developers
- Built a housing for the Buffer Exchanger ESI Interface
- Write SOP

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