



QTOF Data Acquisition – What to Know Before You Hit “Go”

Society of Forensic Toxicologists (SOFT) Annual Meeting – Monday October 30, 2023
Workshop 3: QTOF 101: A Guide to Successful Development and Validation

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INTRODUCTION

- **Center for Forensic Science Research & Education**

- Associate Director
 - Toxicology & Chemistry
- Program Manager
 - NPS Discovery

- **Thomas Jefferson University**

- Assistant Program Director
 - MS in Forensic Toxicology
- Faculty / Lecturer



DISCLOSURES

- I have no conflicts of interest to disclose.
- I am a scientist and employee of FRFF / CFSRE, a 501(c)(3) non-profit research and educational facility.
- CFSRE's NPS Discovery program is funded in part by the National Institute of Justice (NIJ), Office of Justice Programs (OJP), U.S. Department of Justice (DOJ).
 - Award Number: 15PNIJ-22-GG-04434-MUMU
 - The opinions, findings, conclusions and/or recommendations expressed in this publication are those of the author(s) and do not necessarily represent the official position or policies of the U.S. Department of Justice.



NIJ | *National Institute
of Justice*

STRENGTHEN SCIENCE. ADVANCE JUSTICE.



INTRODUCTION



THE CFSRE & OUR LAB

- The Center for Forensic Science Research and Education (CFSRE)
 - 501(c)(3) non-profit research and educational facility
 - Surveillance & Casework



Waters Xevo® G2-S LC-QTOF-MS



Sciex X500R LC-TOF-MS



Sciex TripleTOF® 5600+ LC-TOF-MS



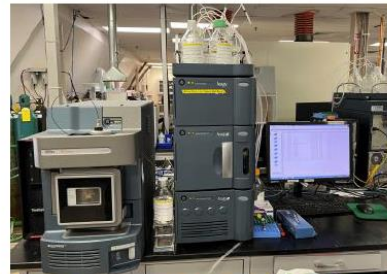
Agilent 6495 LC-QQQ-MS



Agilent 6430 LC-QQQ-MS



Waters TQS LC-QQQ-MS



Waters TQD LC-QQQ-MS



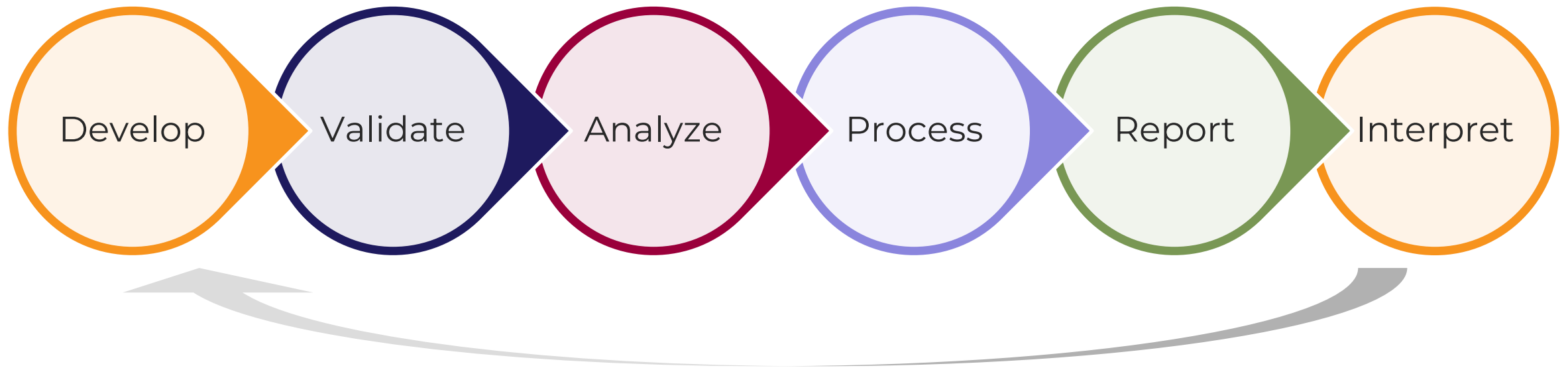
Agilent 5975 GC-MS



Agilent 5975 GC-MS

WORDS OF WISDOM

- “The decisions you make during method development will affect analysis, data processing, result reporting, and ultimately interpretation.”





QTOF HARDWARE



QTOF HARDWARE

▪ Source

- ESI, APCI, etc.
- Pos. vs. Neg.
- Set points

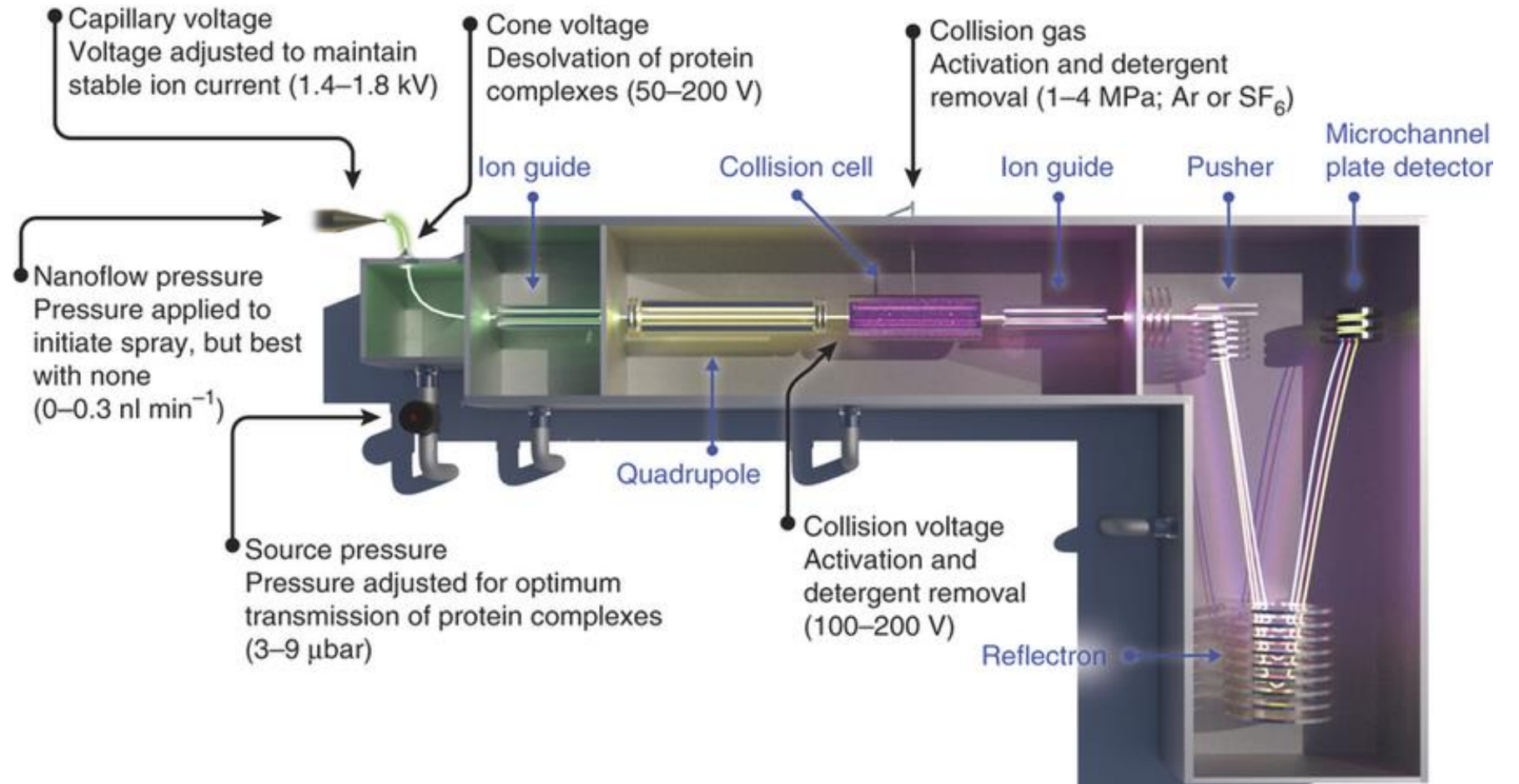
▪ Ion Guides

▪ Quadrupole

▪ Collision Cell

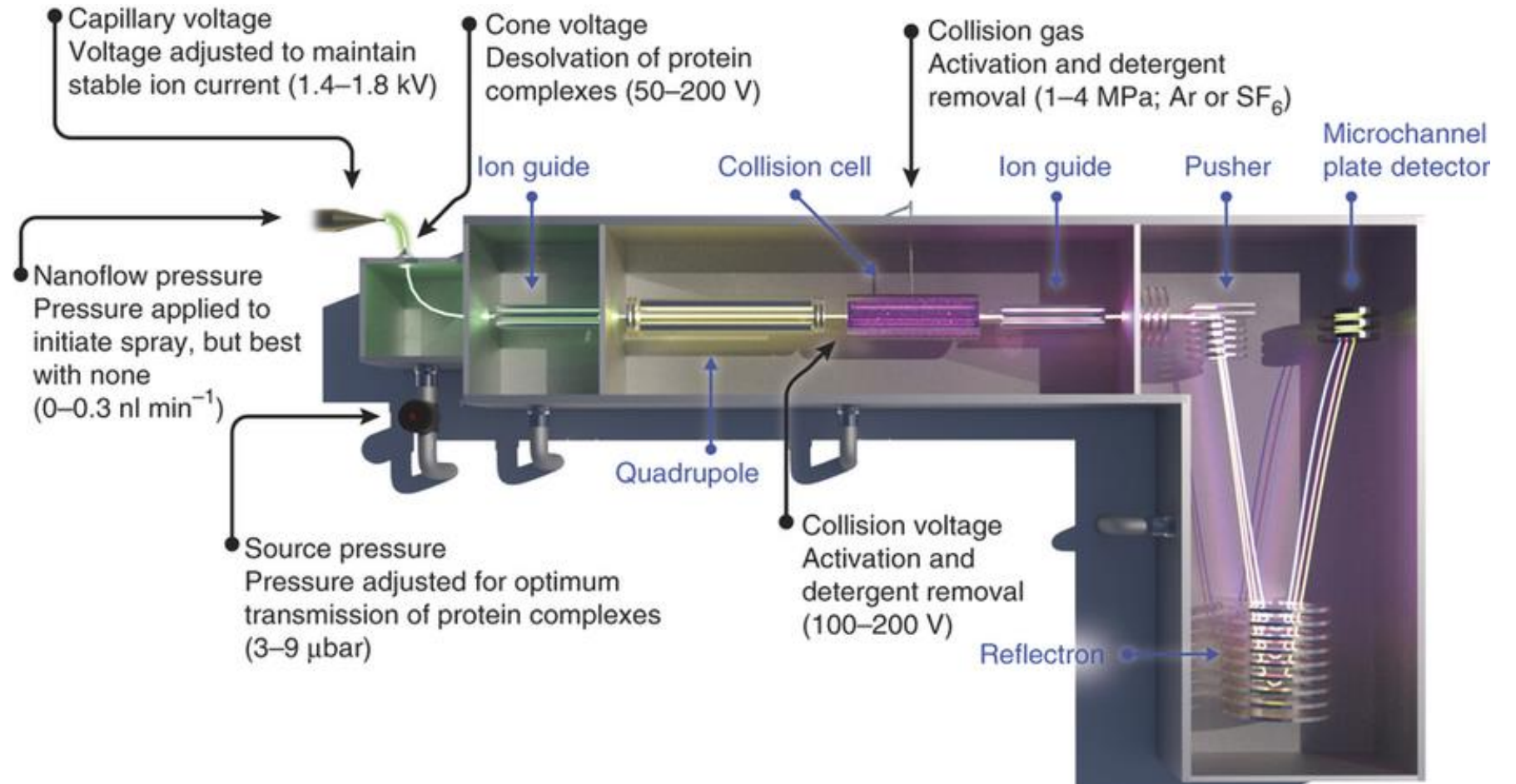
▪ TOF Analyzer

▪ Detector



QTOF HARDWARE

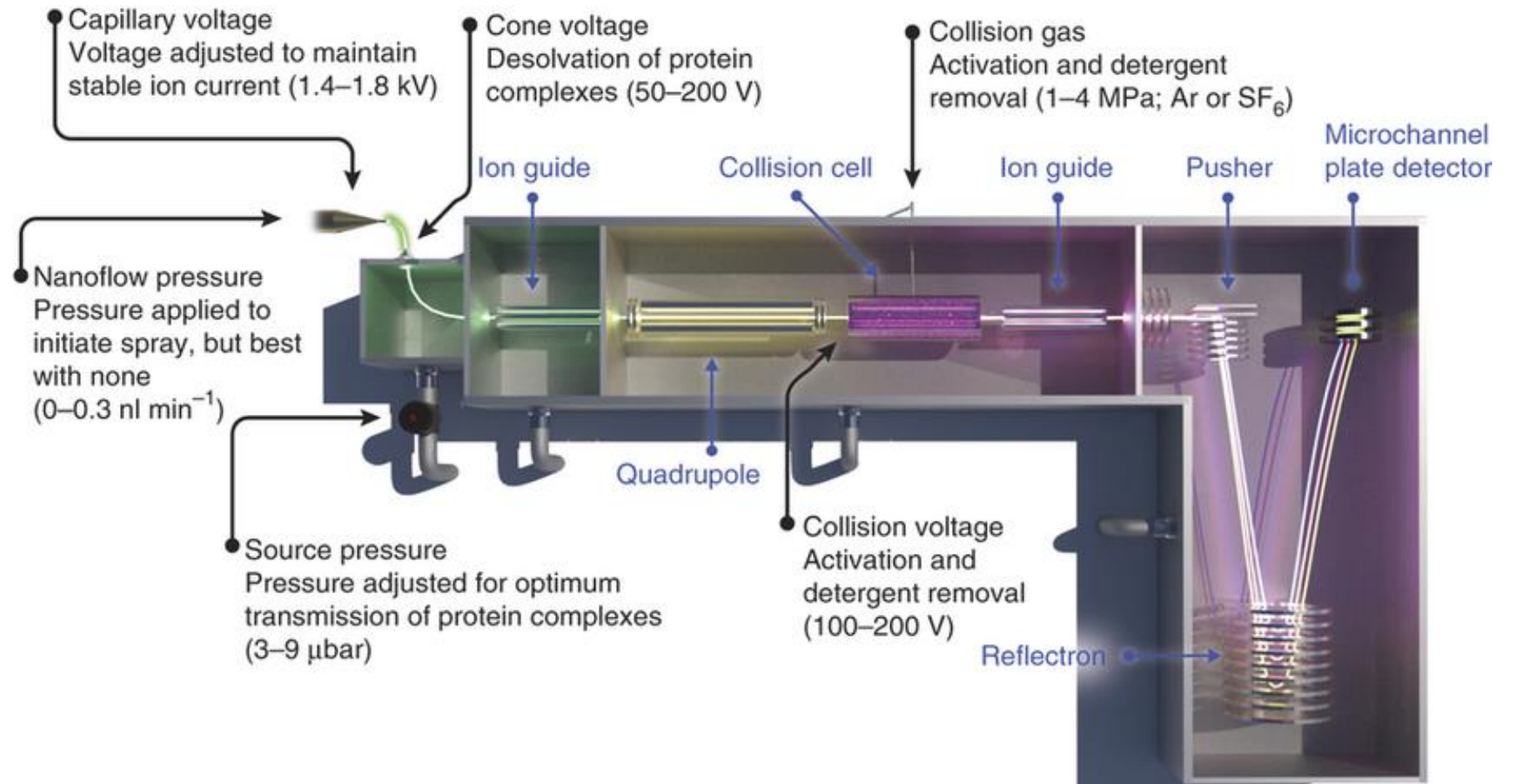
- Source
- **Ion Guides**
 - Options vary
 - Low impact
- Quadrupole
- Collision Cell
- TOF Analyzer
- Detector



Source: <https://www.nature.com/articles/nprot.2013.024>

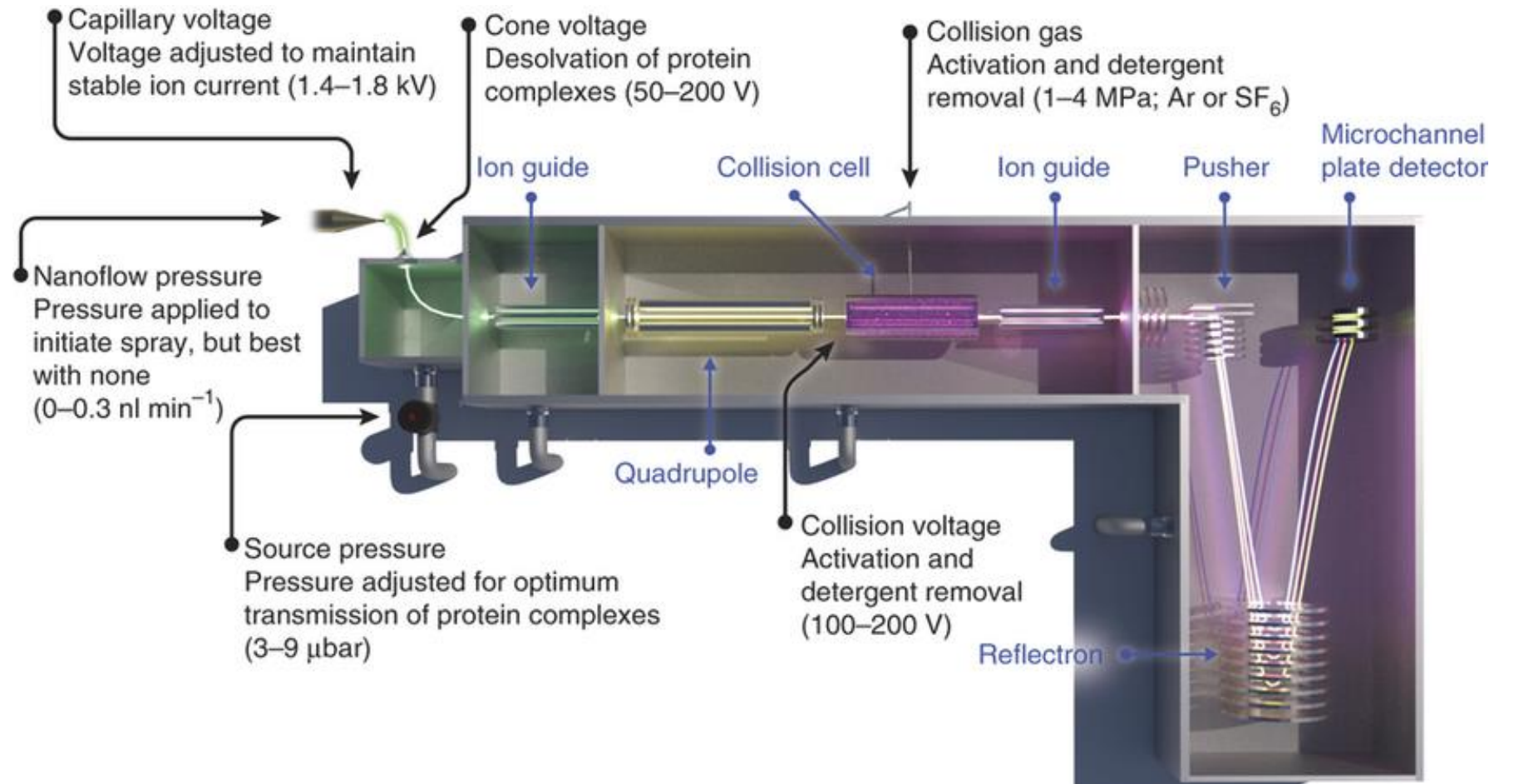
QTOF HARDWARE

- Source
- Ion Guides
- **Quadrupole**
 - On vs. Off
 - Isolation
 - Function
- Collision Cell
- TOF Analyzer
- Detector



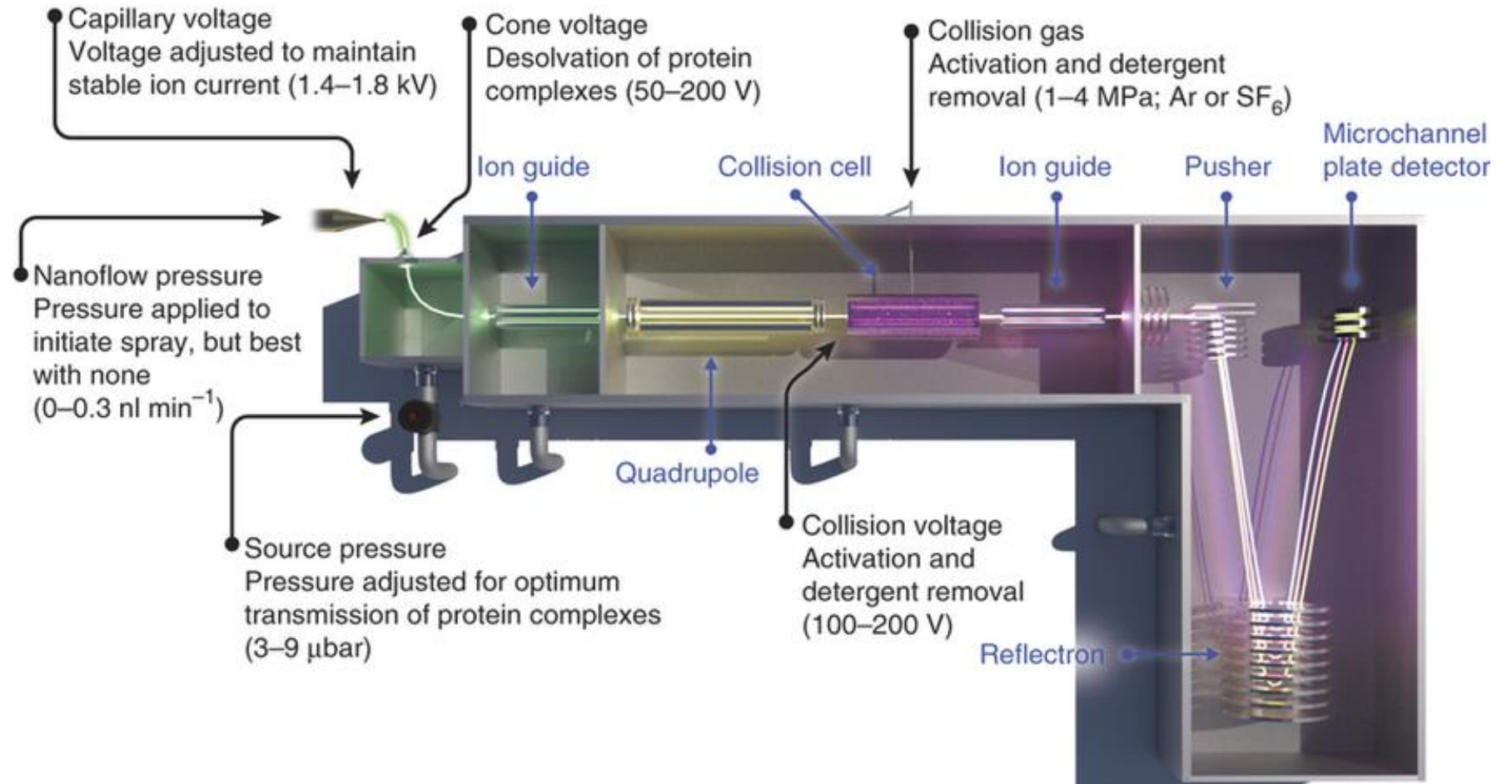
QTOF HARDWARE

- Source
- Ion Guides
- Quadrupole
- **Collision Cell**
 - On vs. Off
 - Collision Energy
 - Functions
- TOF Analyzer
- Detector



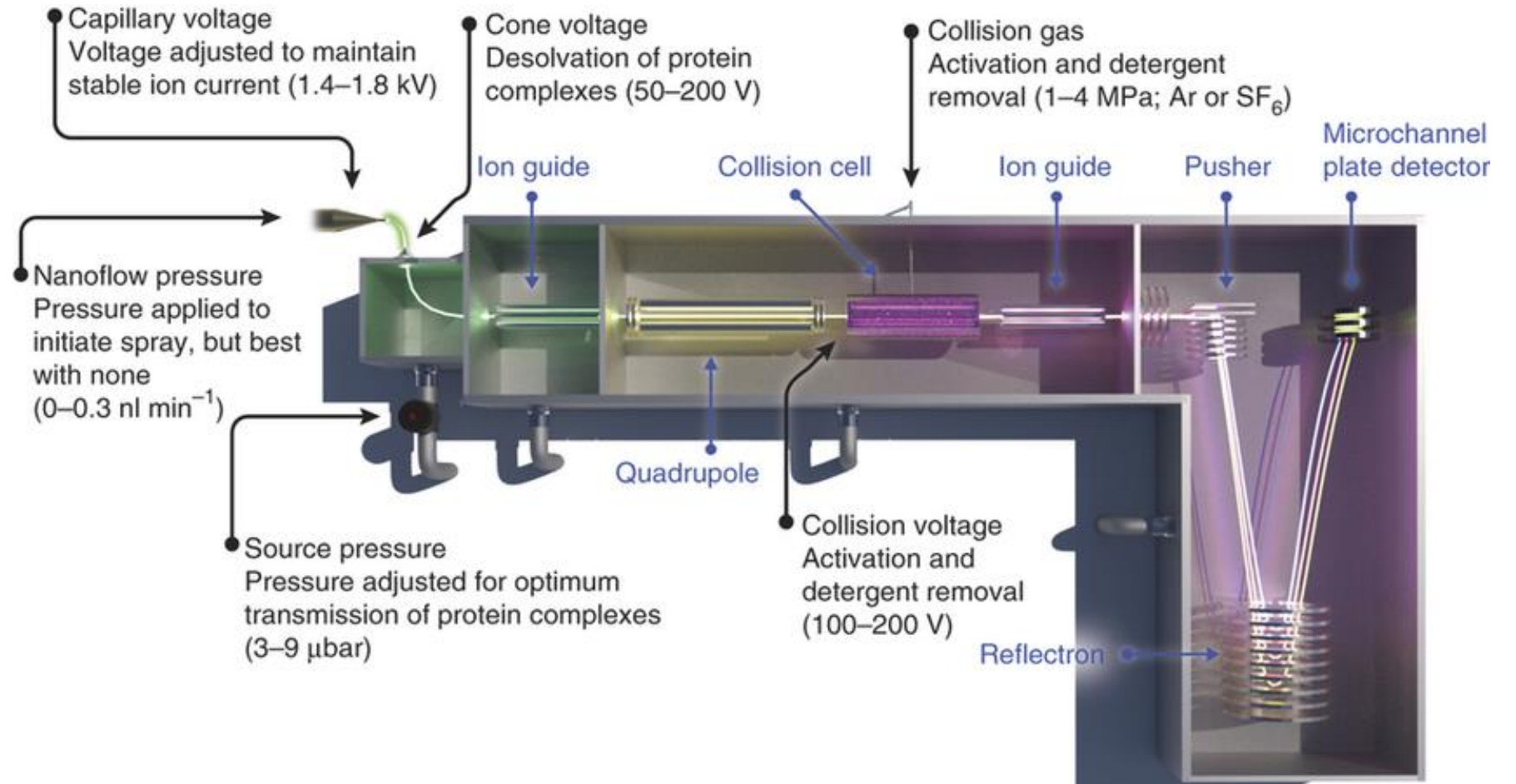
QTOF HARDWARE

- Source
- Ion Guides
- Quadrupole
- Collision Cell
- **TOF Analyzer**
 - “V” vs. “N” vs. “W”
 - Low impact
- Detector



QTOF HARDWARE

- Source
- Ion Guides
- Quadrupole
- Collision Cell
- TOF Analyzer
- **Detector**
 - Low impact



Source: <https://www.nature.com/articles/nprot.2013.024>



ACQUISITION MODES



TARGETED VS. NON-TARGETED

- **Definitions:**

- Targeted: Directed at a particular group or activity [or analyte]
- Non-Targeted: Not directed at a particular group or activity [or analyte]

- **What are the benefits?**

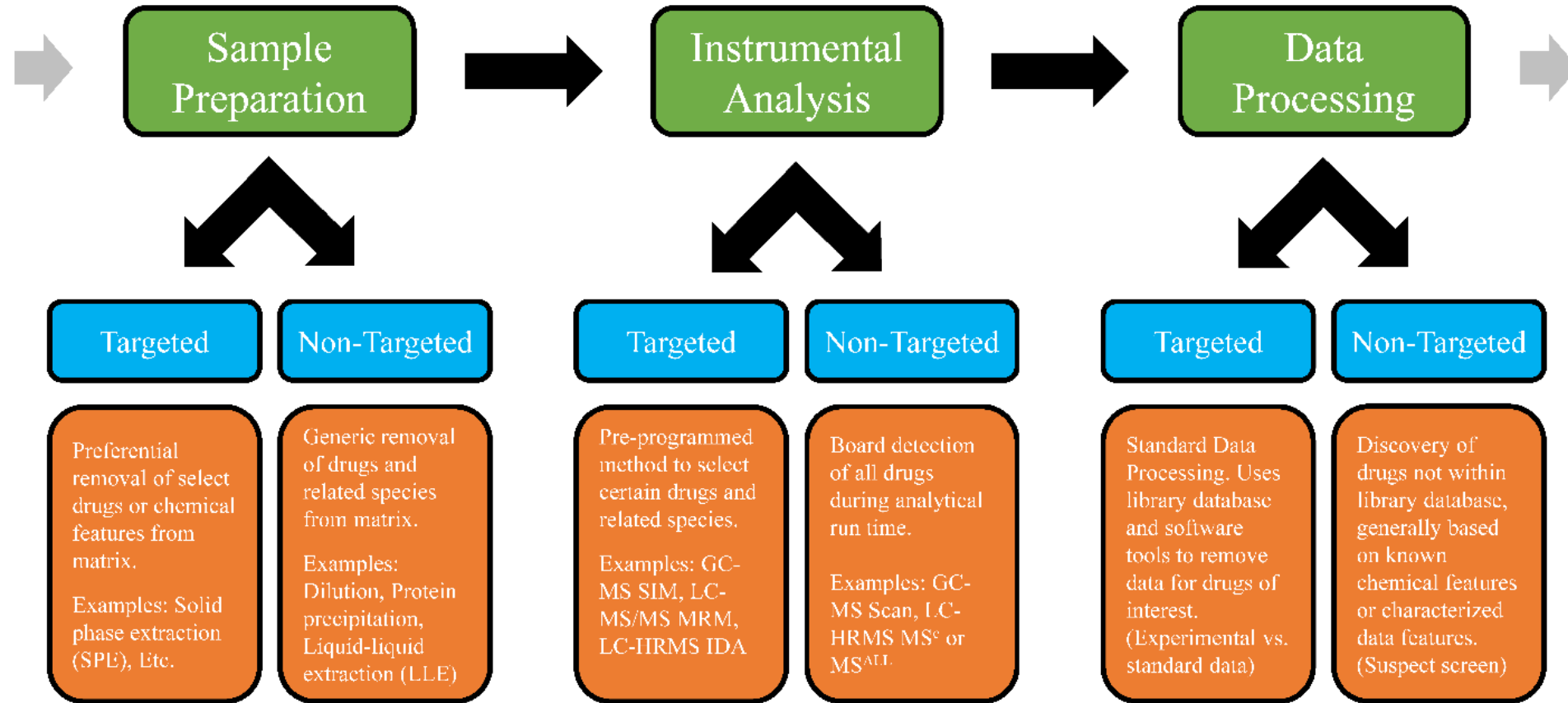
- Targeted: Specific, Direct, Intended, Focused, Aimed, Straightforward
- Non-Targeted: Non-specific, Comprehensive, Indirect, Broad, Complicated

- **How do these two fit into forensic practice?**

- Does non-targeted sound like the opposite of what we know in the forensic world???
- Non-targeted ends up being more research related (can help in certain case scenarios)



TARGETED VS. NON-TARGETED



TARGETED VS. NON-TARGETED



TARGETED ACQUISITION MODES

- Data Dependent Acquisition (DDA)
- Information Dependent Acquisition (IDA)
- MS/MS acquisition dependent on “sample”
- “If x → then y” (x = mass, intensity, etc.)
- *Multiple reaction monitoring (MRM^{HR})*

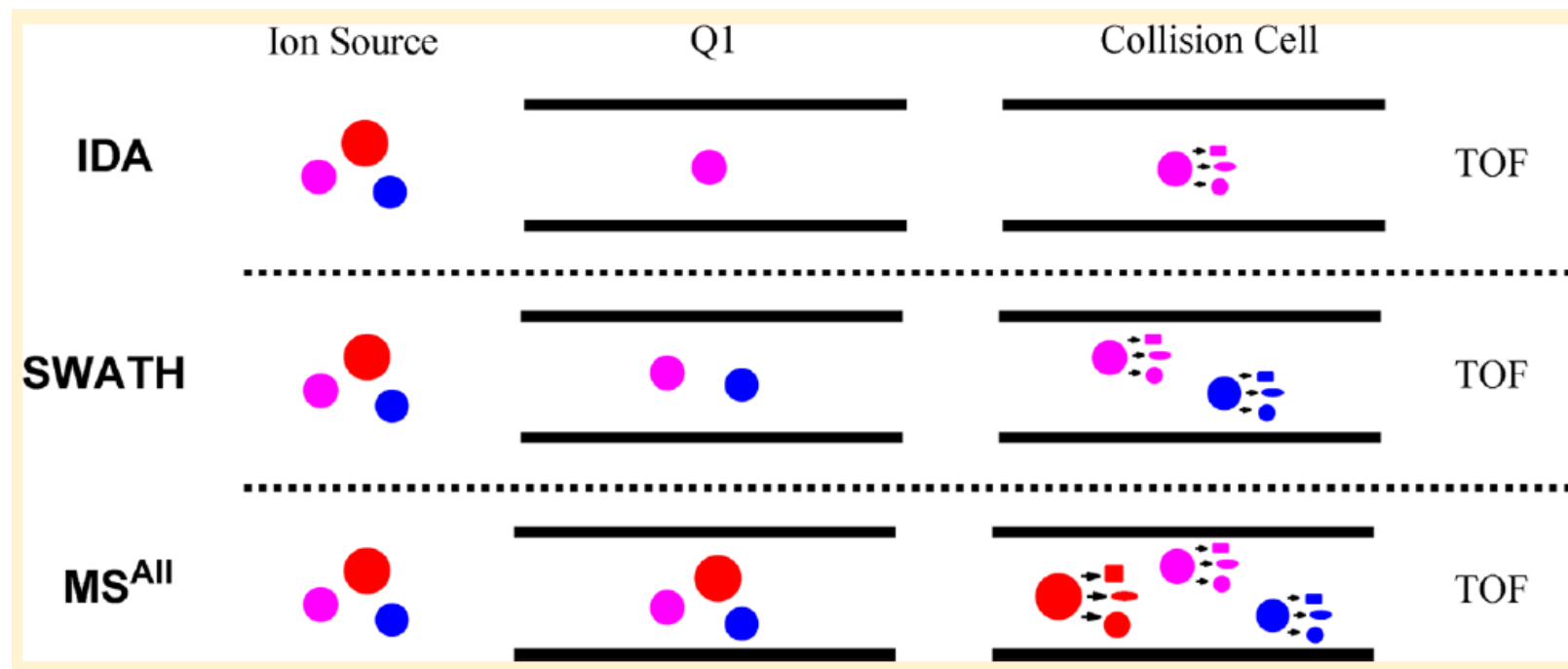
NON-TARGETED ACQUISITION MODES

- Data Independent Acquisition (DIA)
 - Generic, comprehensive, cycling acquisition
- MS^e (or MS^{ALL})
 - E.g., Waters, Agilent All Ions MS/MS
- MS/MS^{ALL}
 - E.g., Sciex SWATH Acquisition

Arguably the most important piece to the puzzle !!!

ACQUISITION MODES

- **Quadrupole**
 - “Ion Guide” or Mass Filter
- **Collision Cell**
 - Fragmentation
- **Time-of-Flight**
 - Accurate Mass
 - TOF MS & MS/MS

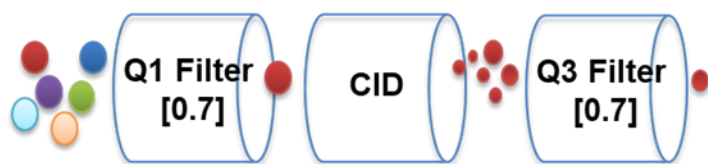


Source: <https://pubs.acs.org/doi/10.1021/ac403385y>

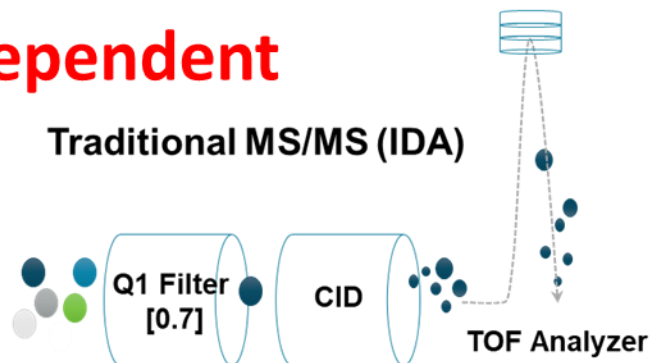
ACQUISITION MODES

Targeted / Data Dependent

Traditional "Triple Quad"



Traditional MS/MS (IDA)

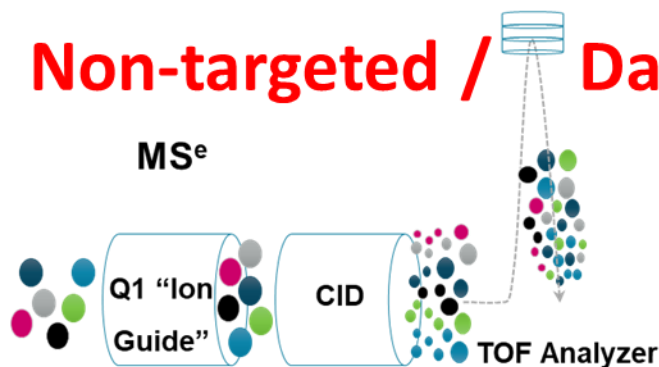


Non-targeted / Data Independent

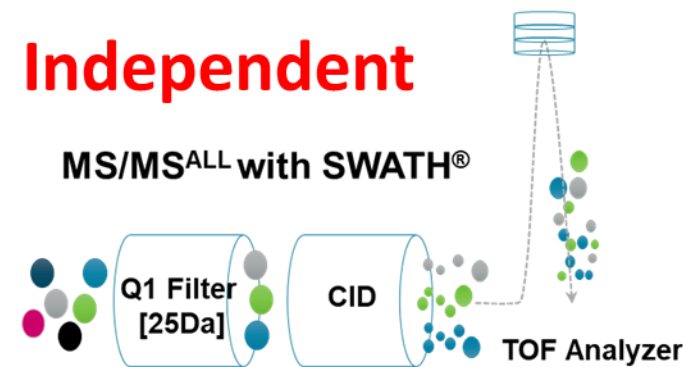
"Single Quad" / Scan



MS^e

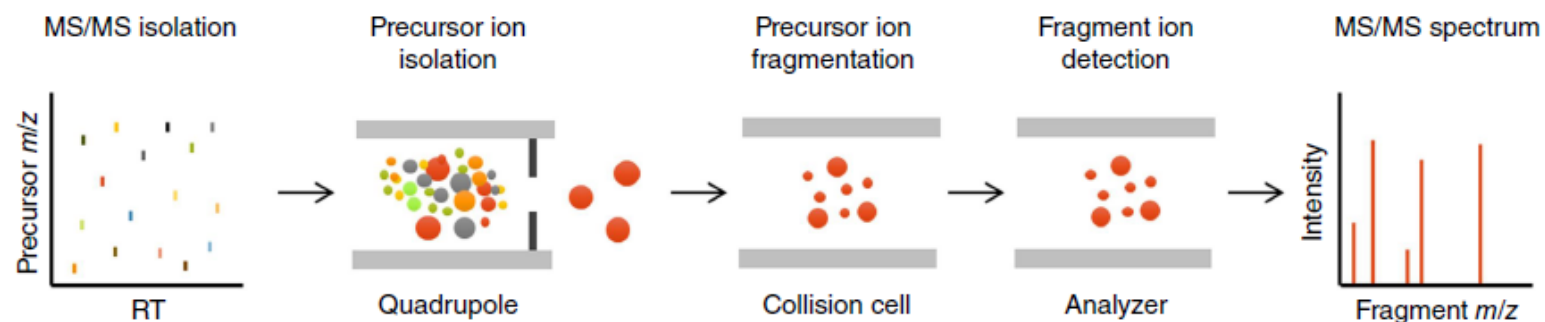


MS/MS^{ALL} with SWATH®

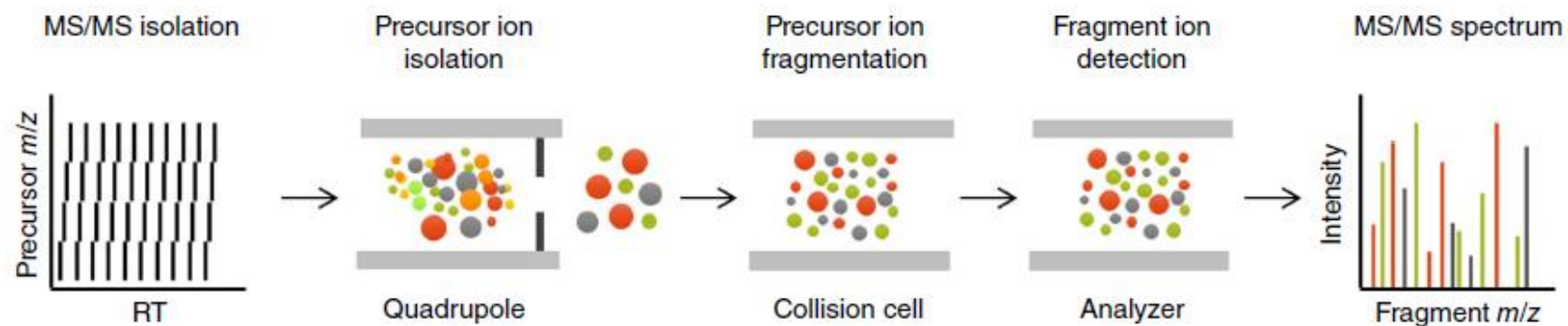


ACQUISITION MODES

(a) Data-dependent acquisition (DDA)



(c) Data-independent acquisition (DIA)





CFSRE EXPERIENCES WITH QTOF



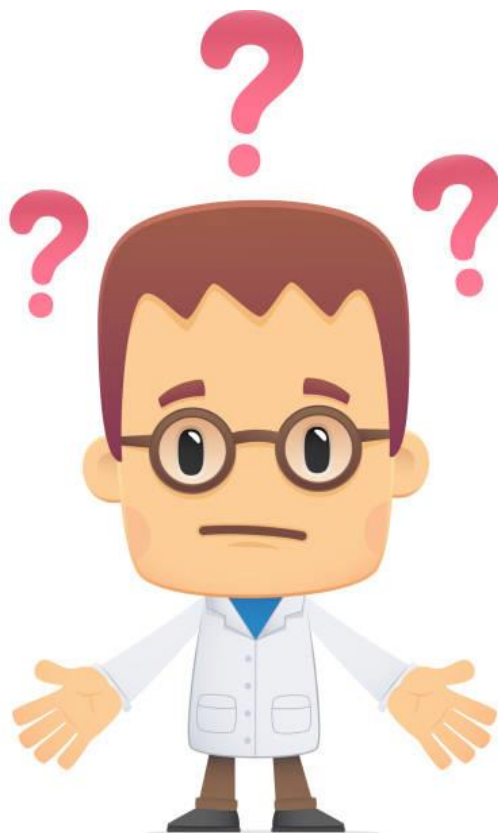
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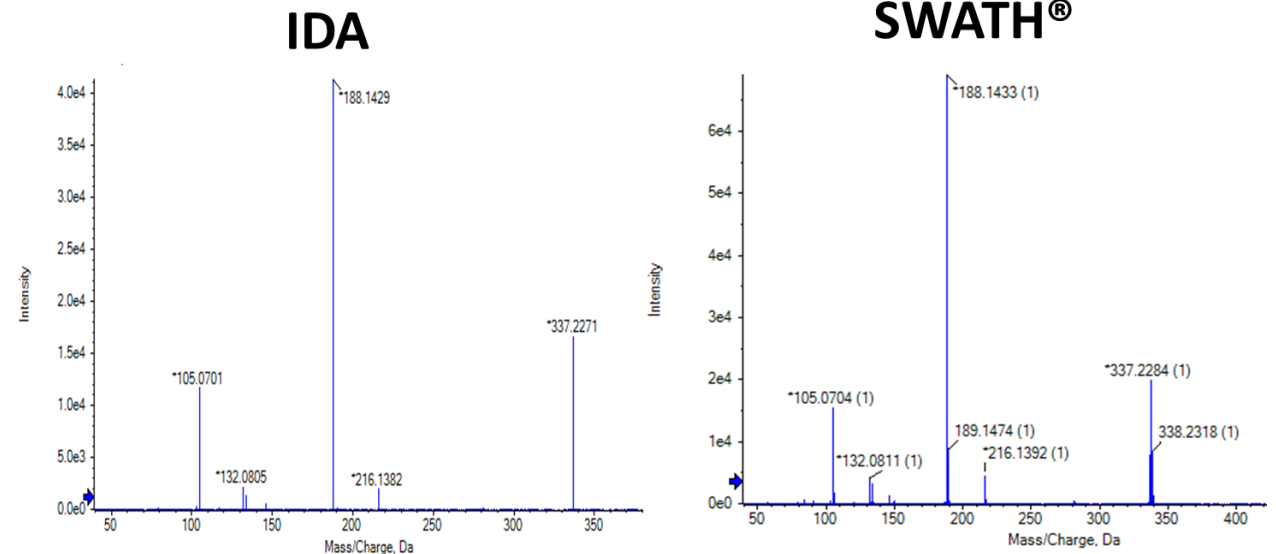
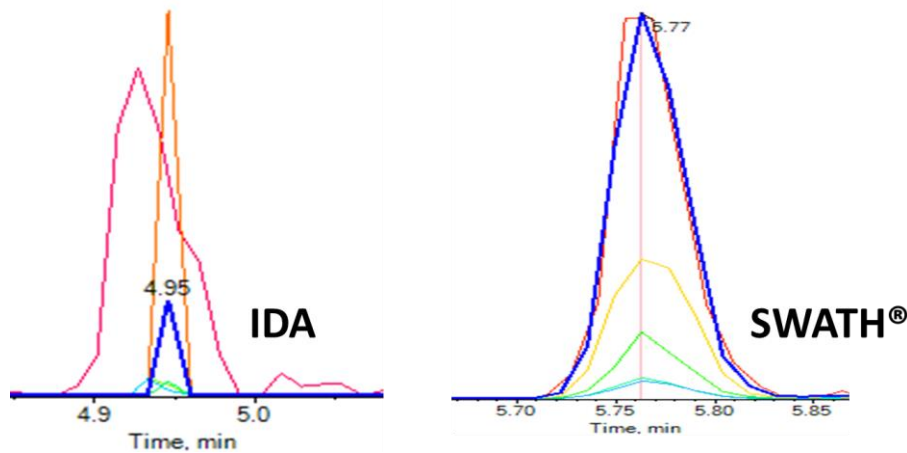
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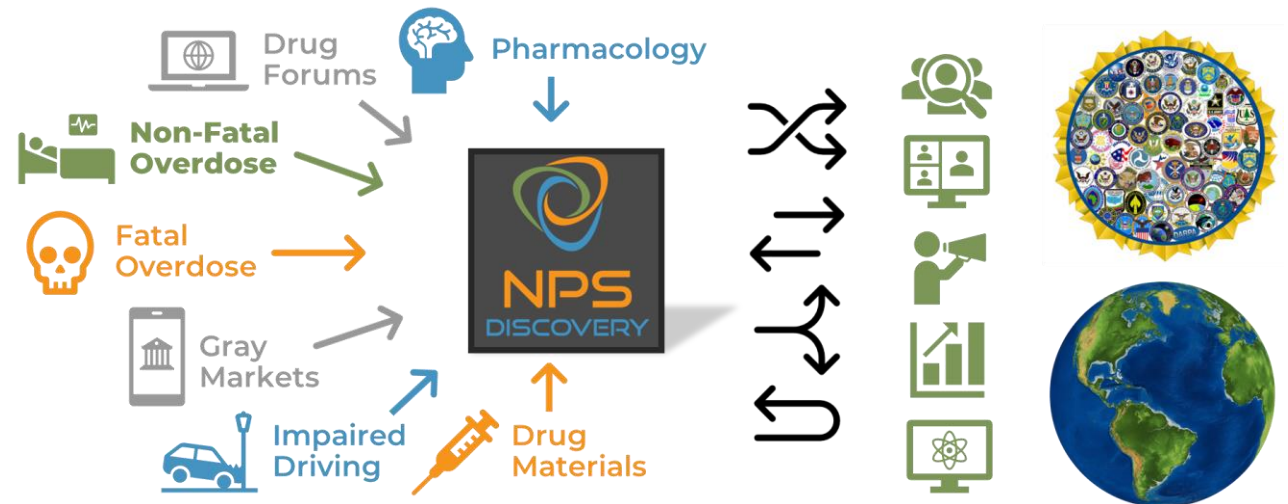
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- **Side-by-side comparison – what are the impacts on data processing and drug identification?**



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- **Selected non-targeted SWATH method (paired with non-targeted LC separation)**
 - Primarily targeted data processing, but with secondary options for non-targeted (non-routine)



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- Employment of method for 8+ years – surveillance, forensic casework, research, and more
- **Remember – what works/worked for the CFSRE (or another lab) may not work for your lab**



FINAL COMMENTS



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- Expert understanding of QTOF acquisition modes is important
- Instruments, hardware, and software vary by vendor
 - MS^e vs. MS^{ALL} vs. MS/MS^{ALL}
- There is no “one-size-fits” all approach
- Don’t worry – there are many resources available!



ACKNOWLEDGEMENTS

- **CFSRE Team**

- Barry Logan
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- Brianna Stang
- Alexis Quinter
- Max Denn
- Many others!

- **NMS Labs**

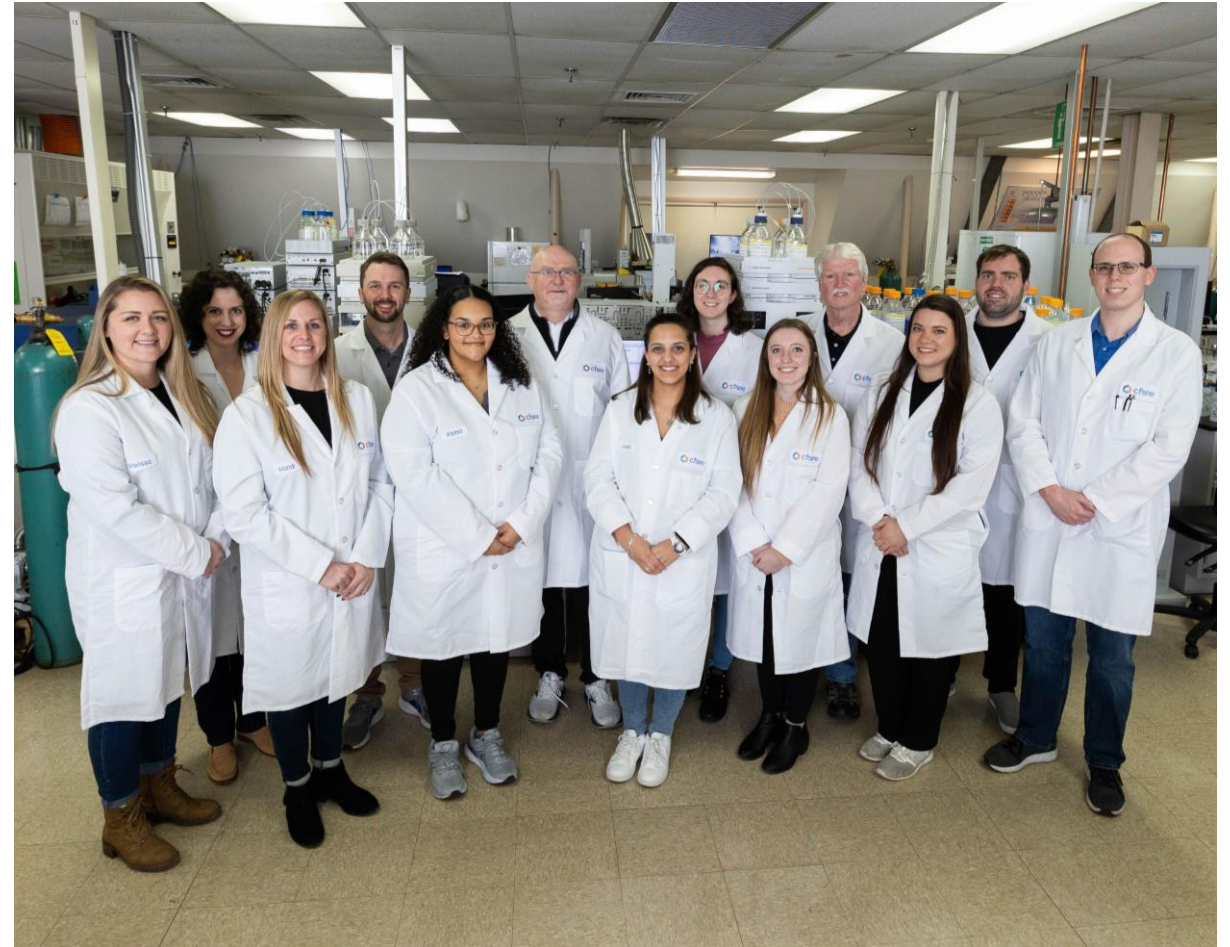
- Donna Papsun

- **Funding Agencies**

- NIJ

- **Collaborators & Partners**

- Forensic
- Clinical
- Medical Examiners
- Coroners
- Crime Labs
- Etc.





THANK YOU! **QUESTIONS?**



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