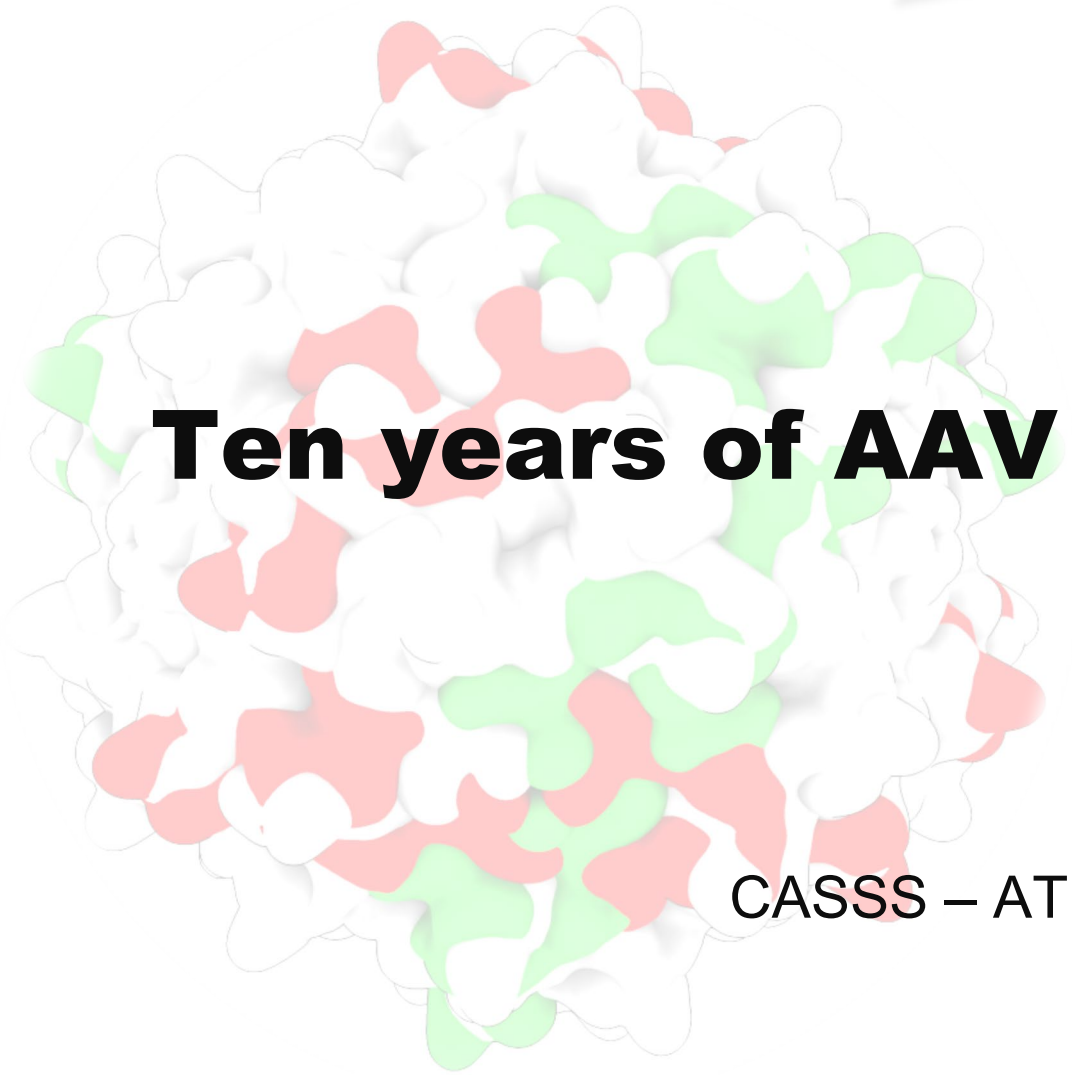
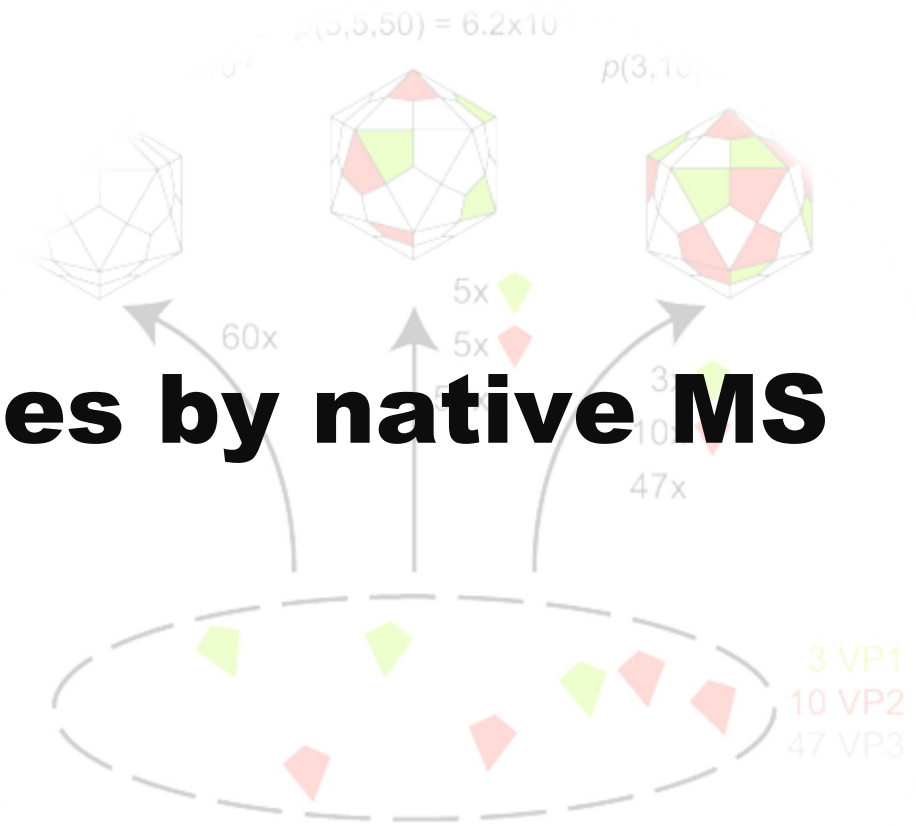
Utrecht University

BioMS

netherlands proteomics centre



Ten years of AAV analyses by native MS



CASSS – AT Europe – Joost Snijder

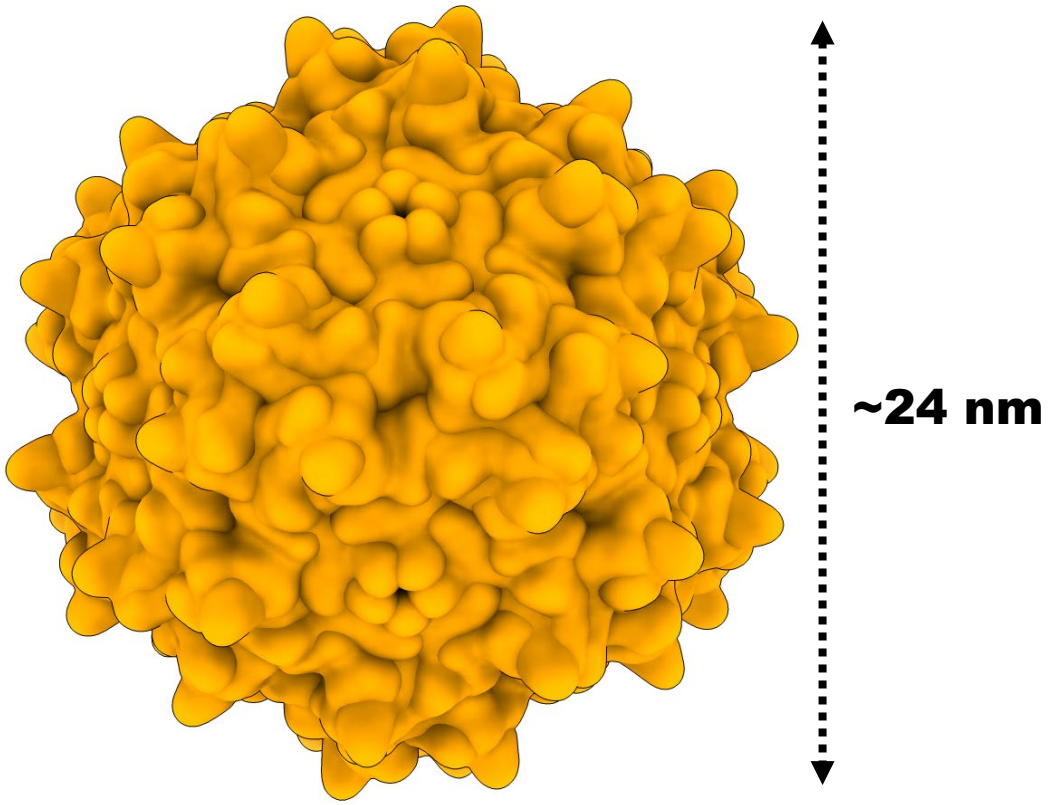
Adeno Associated Viruses as tools for gene delivery

- Parvoviruses
- ssDNA genome (~4 kb)
- Serotypes with wide range of tissue tropism

- Gene delivery in cell culture and animal models
- Vector for CRISPR-Cas9 gene editing

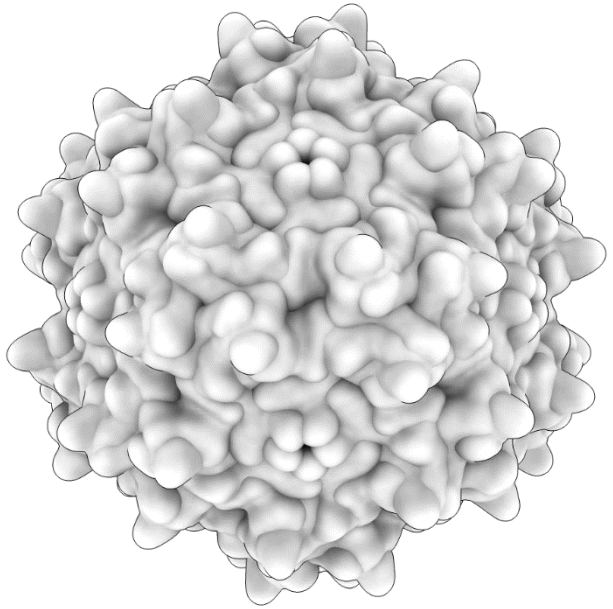
- Approved gene therapies
  - Zolgensma
  - Glybera
  - Luxturna
  - …

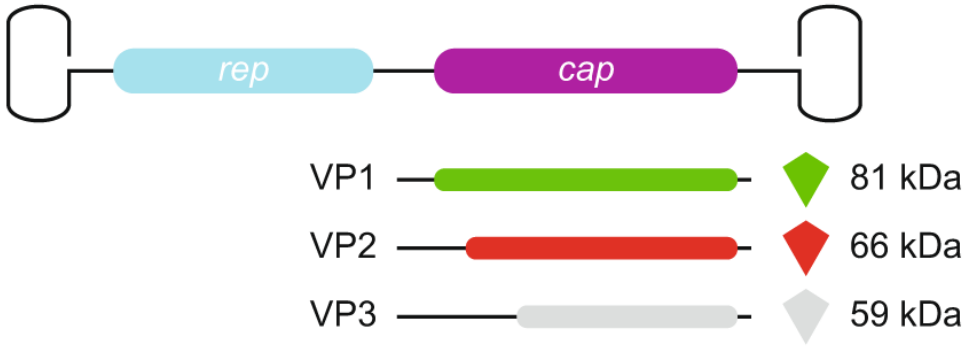
- Engineered AAV capsids with tuned tropism

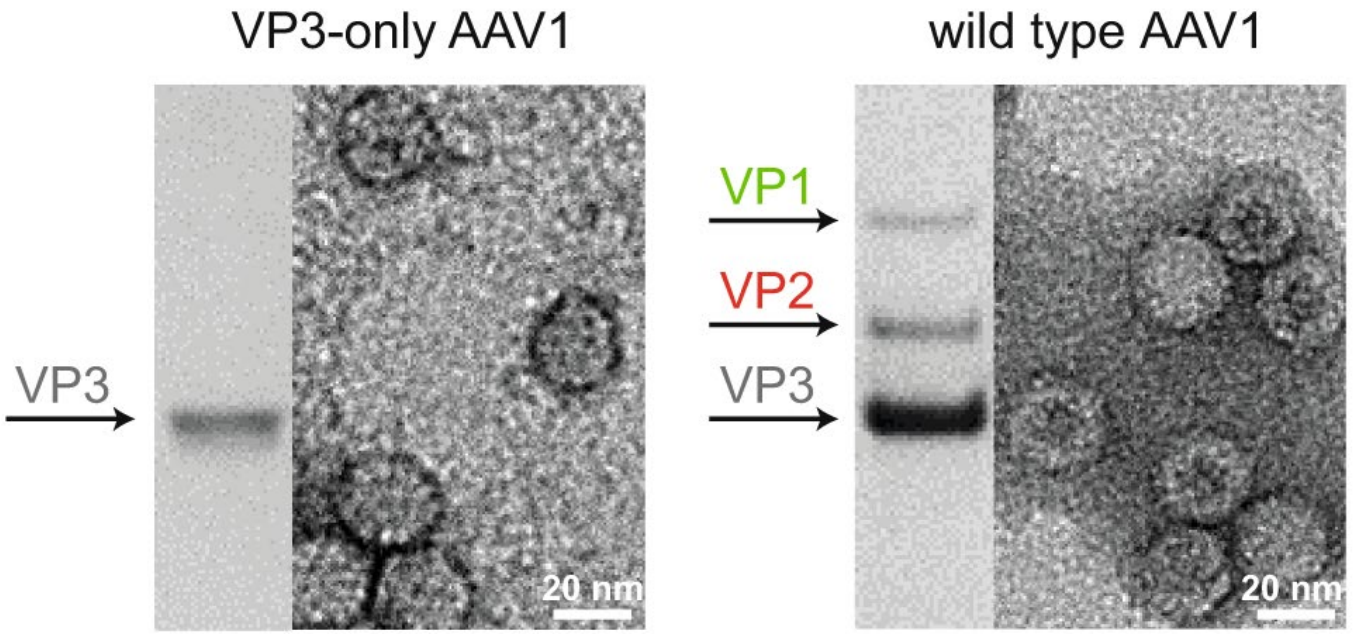


AAV capsids consist of a mixture of 3 *cap* isoforms

~24 nm
T=1
60 copies



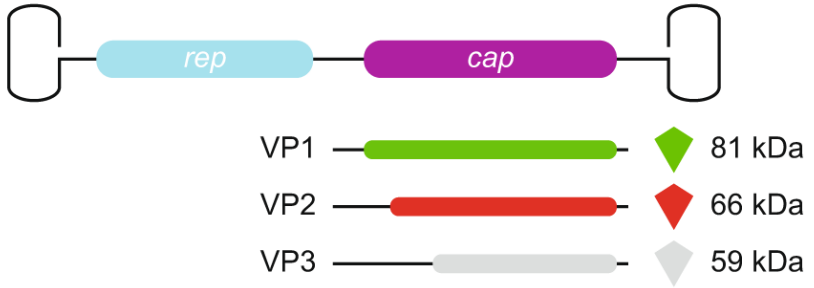


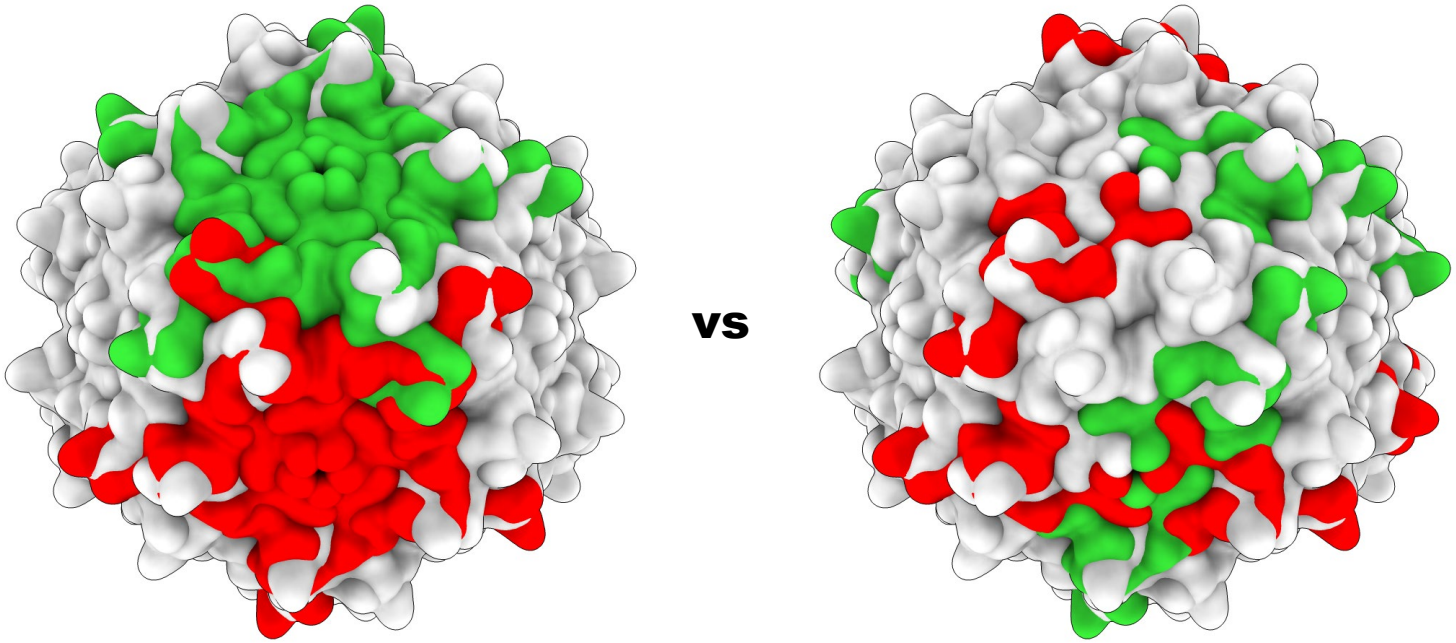


"1:1:10 ratio of VP1:VP2:VP3"

Woerner et al. 2021, NCOMMS

How are the VP capsid proteins arranged?



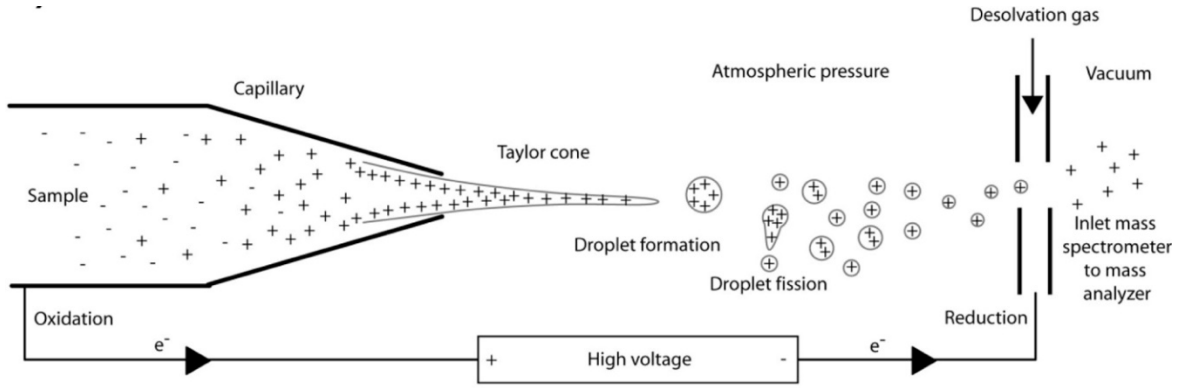


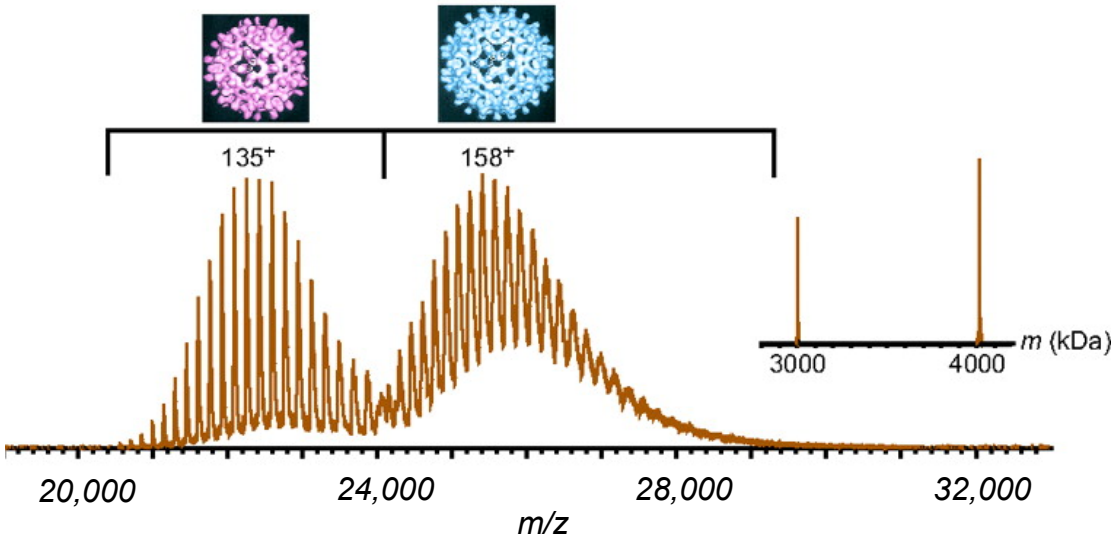
total # of possible stoichiometries is 1891!

Woerner et al. 2021, NCOMMS

Native MS as a tool to study virus assembly

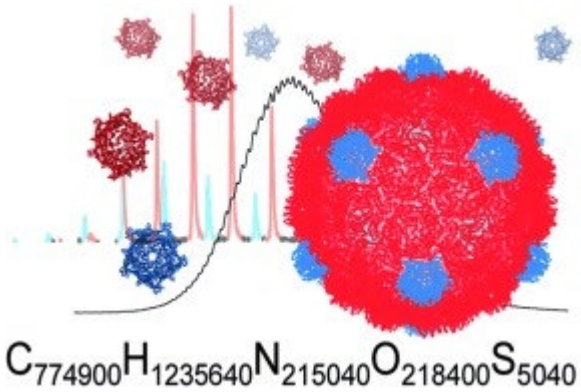
- VP1/VP2 unique regions are not resolved in Xtal/cryoEM reconstructions
  - The 1891 possible stoichiometries have unique masses!
- Mass analysis of very large protein complexes under native conditions
- nanoESI for gentle transfer to gas phase
- High mass accuracy and resolving power to study composition
- Can we determine the composition of the AAV capsids?

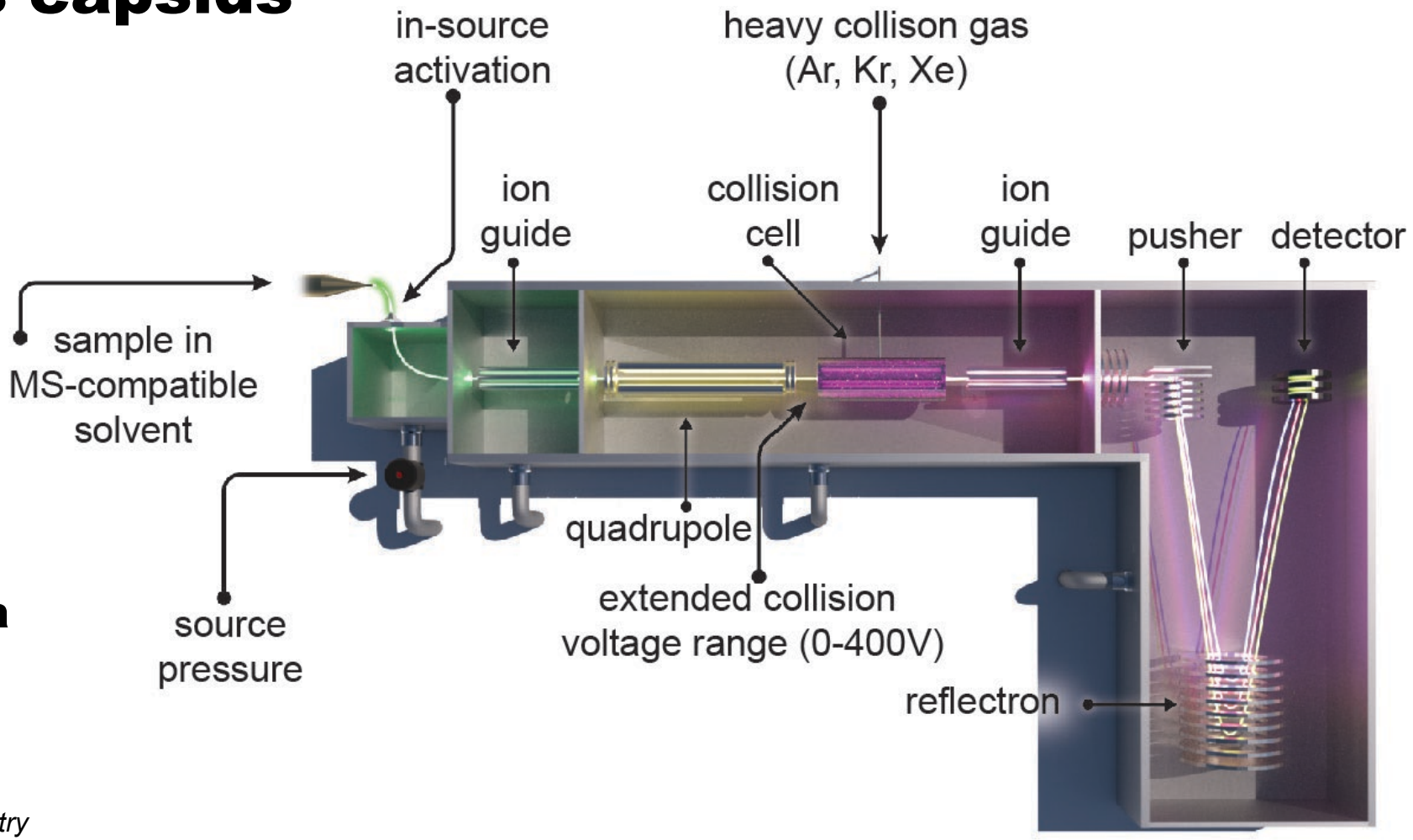




Uetrecht et al. 2008, PNAS

Modified QTOF platform enables native MS of megadalton virus capsids

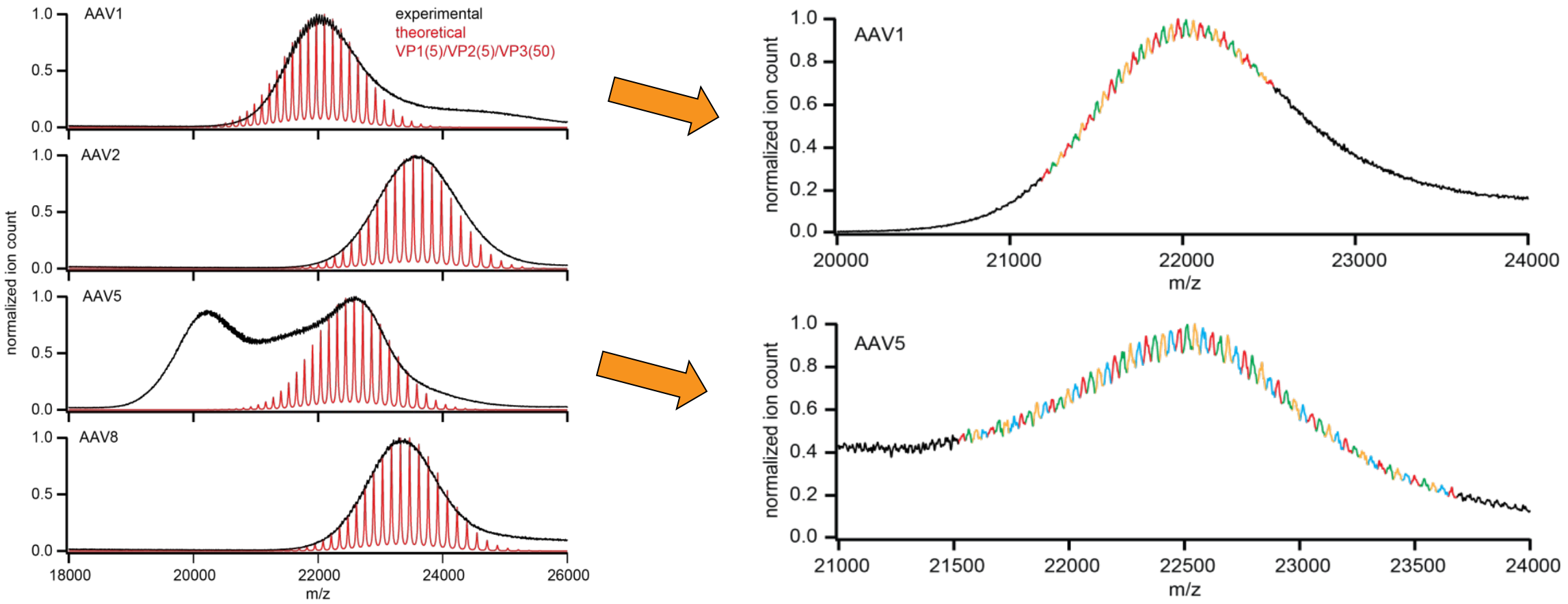




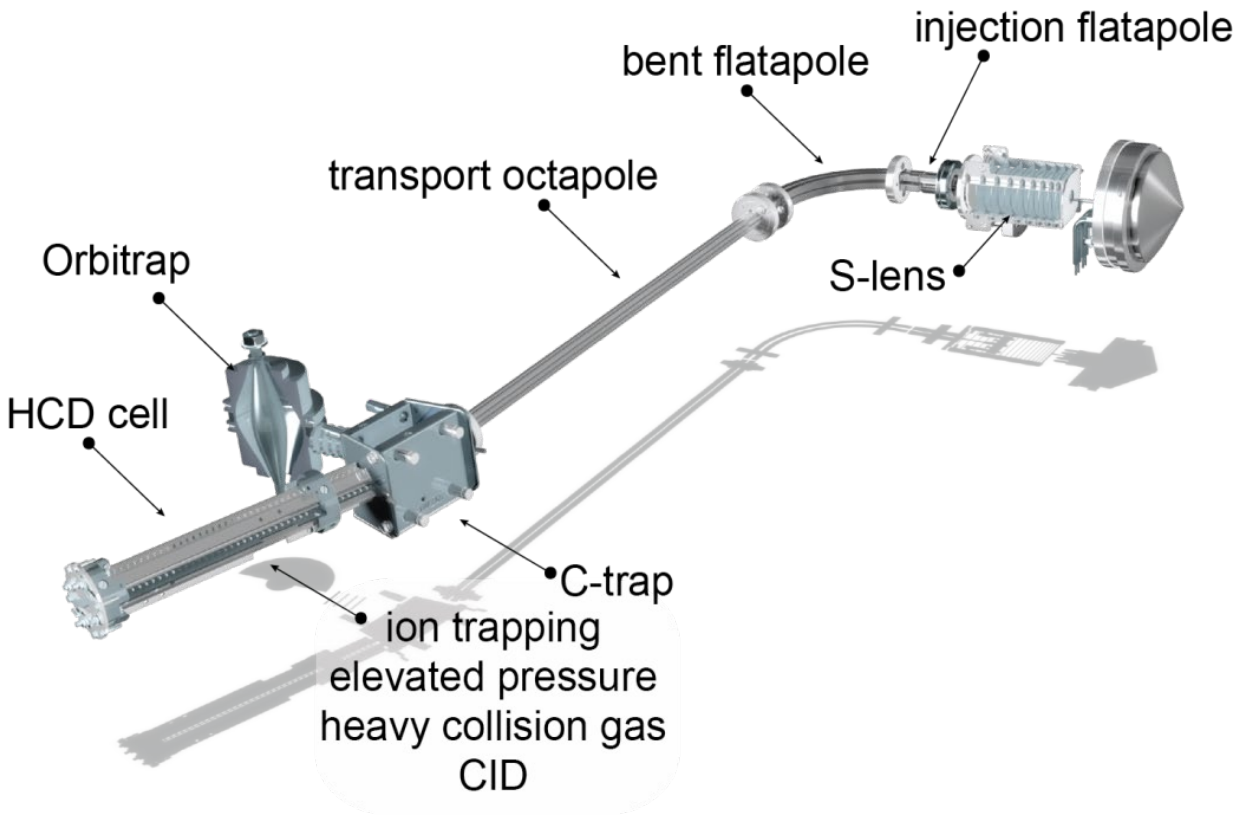
Capsids up to ~20 MDa
Precision ~1-10 kDa

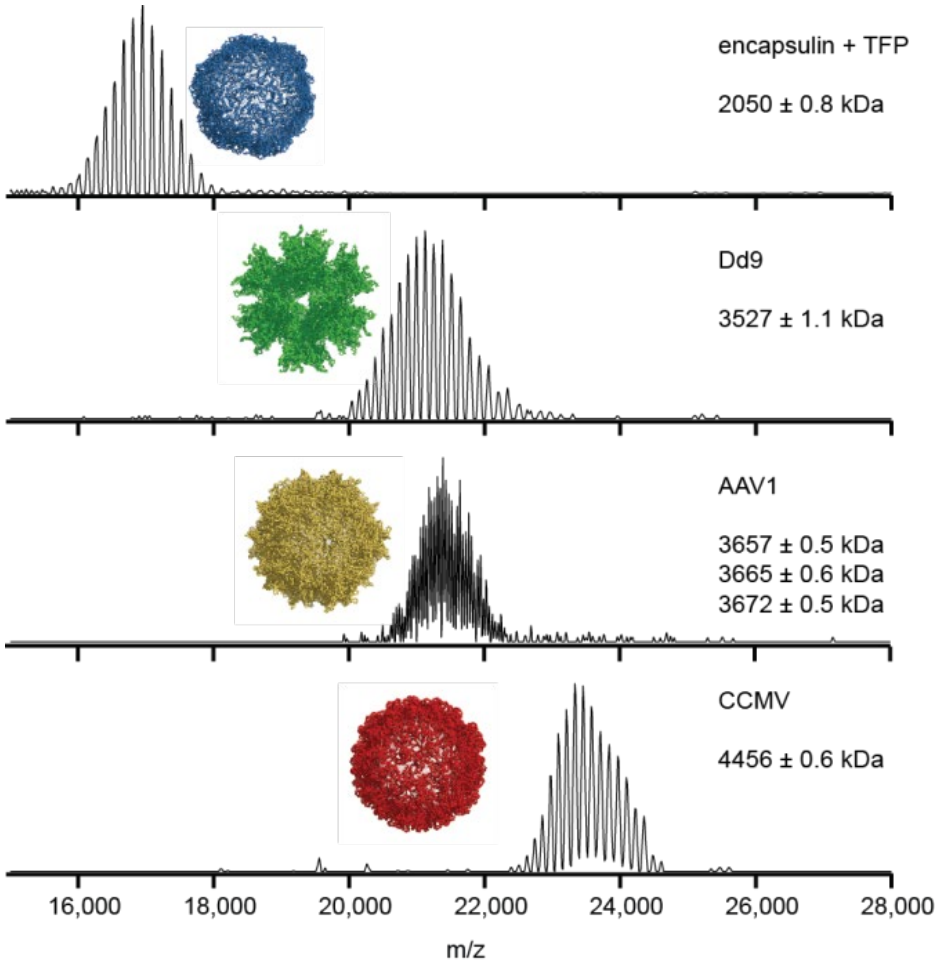
Snijder et al. 2012, Angewandte Chemie
Snijder et al. 2014, Annual Review of Analytical Chemistry

AAV capsids are heterogeneous



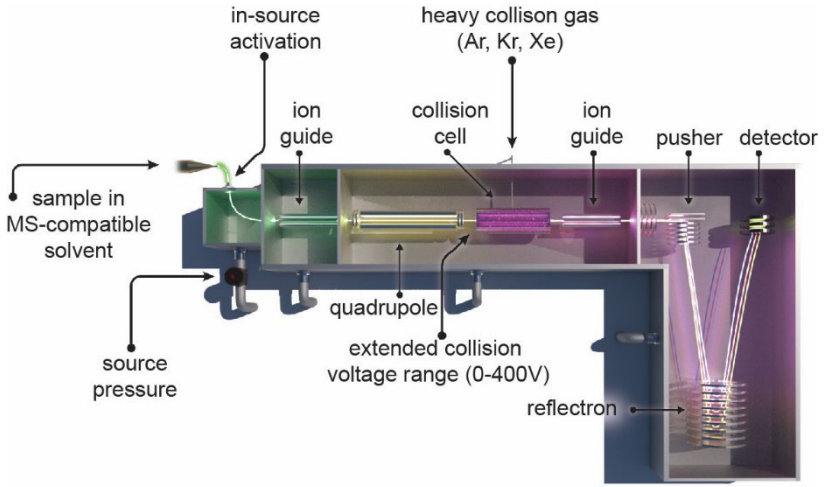
A new Orbitrap-based platform for native MS in the MDa range

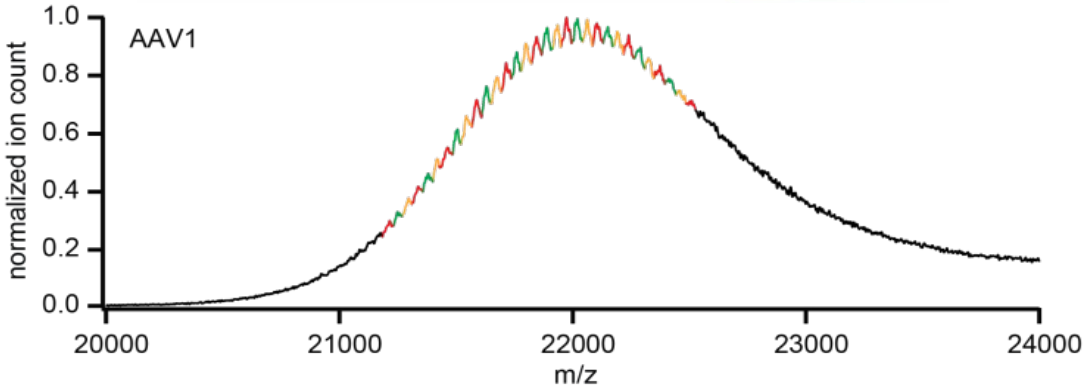


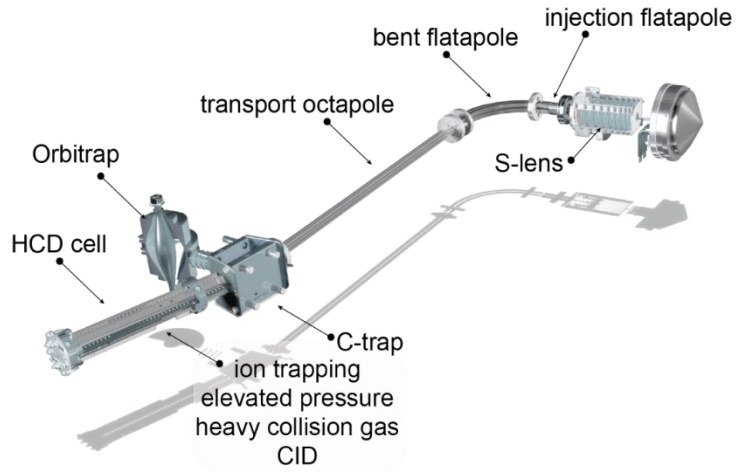


Snijder et al. 2014, JACS

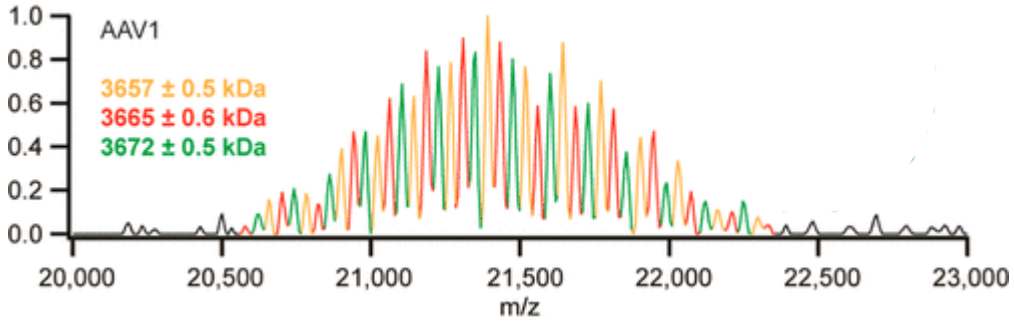
Improved resolution on the Orbitrap platform: what can we learn about AAV assembly?





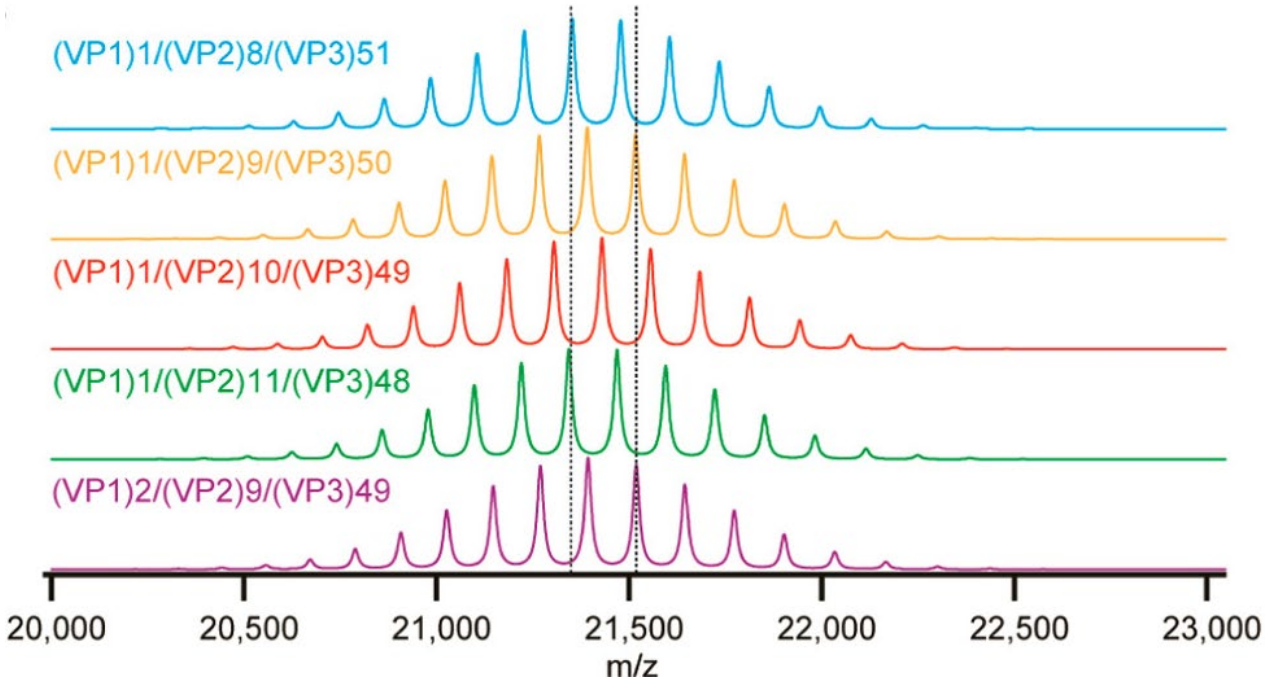


Snijder et al. 2014, JACS



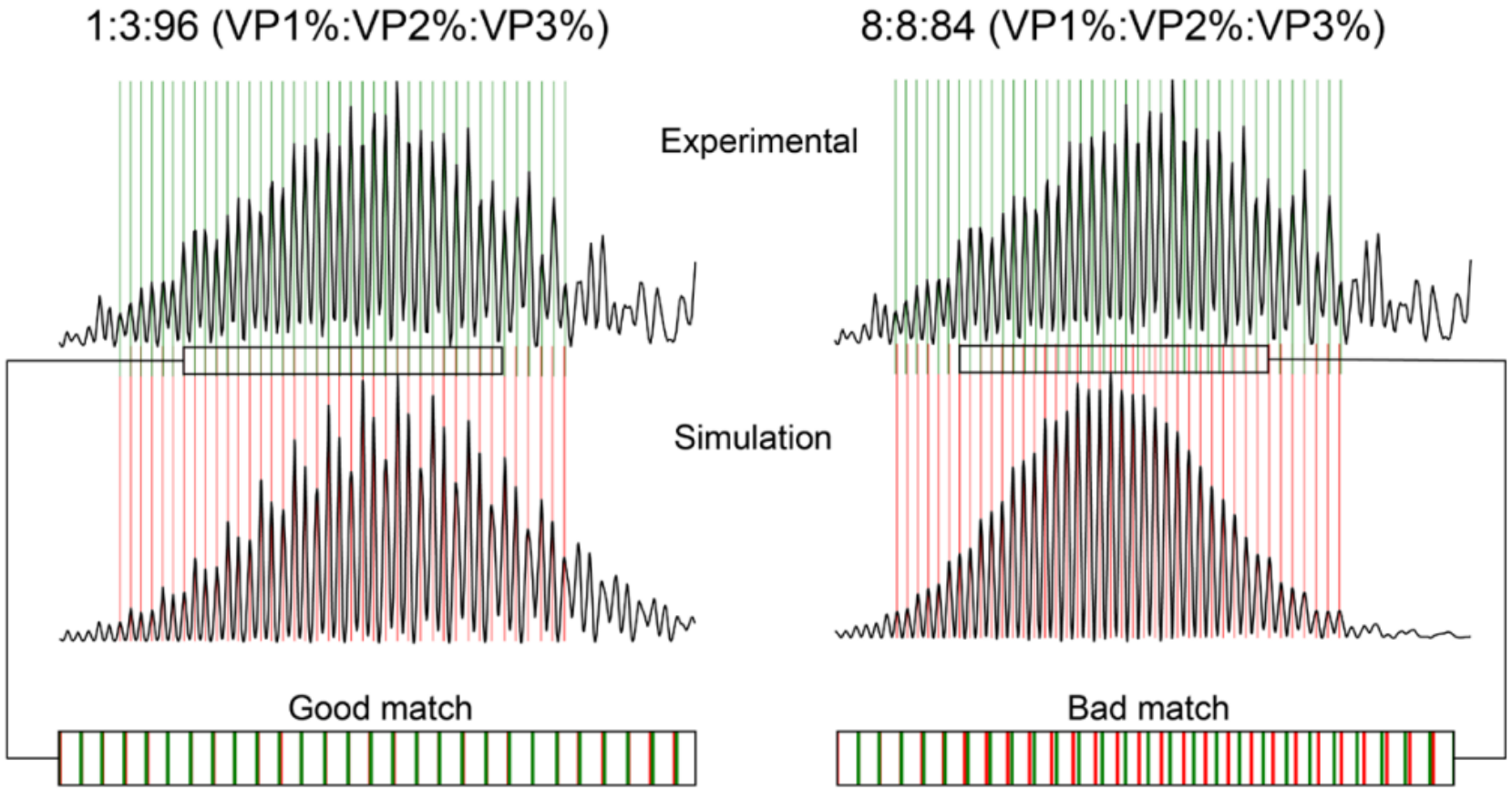
AAV spectra are shaped by interference patterns

- AAV capsids are heterogeneous
- Coincidentally, signals appear at m/z where:
  - The masses of +3*VP2 overlap
  - The masses of +1*VP1 overlap
- At the current resolution no more than 3 series of peaks are resolved
- The convoluted peak positions shift according to the ratio of VP1:VP2:VP3



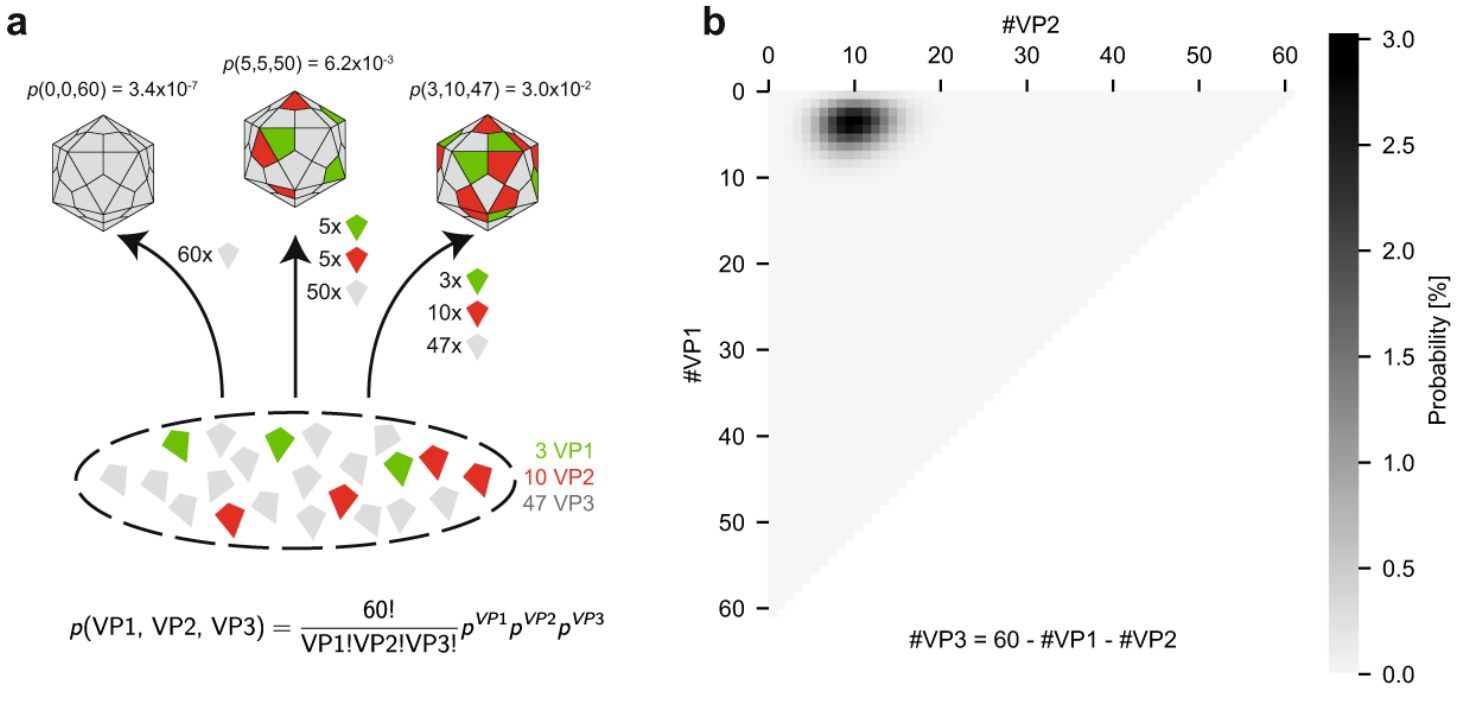
Snijder et al. 2014, JACS

The interference patterns in AAV spectra shift with changing VP ratios



Woerner et al. 2021, NCOMMS

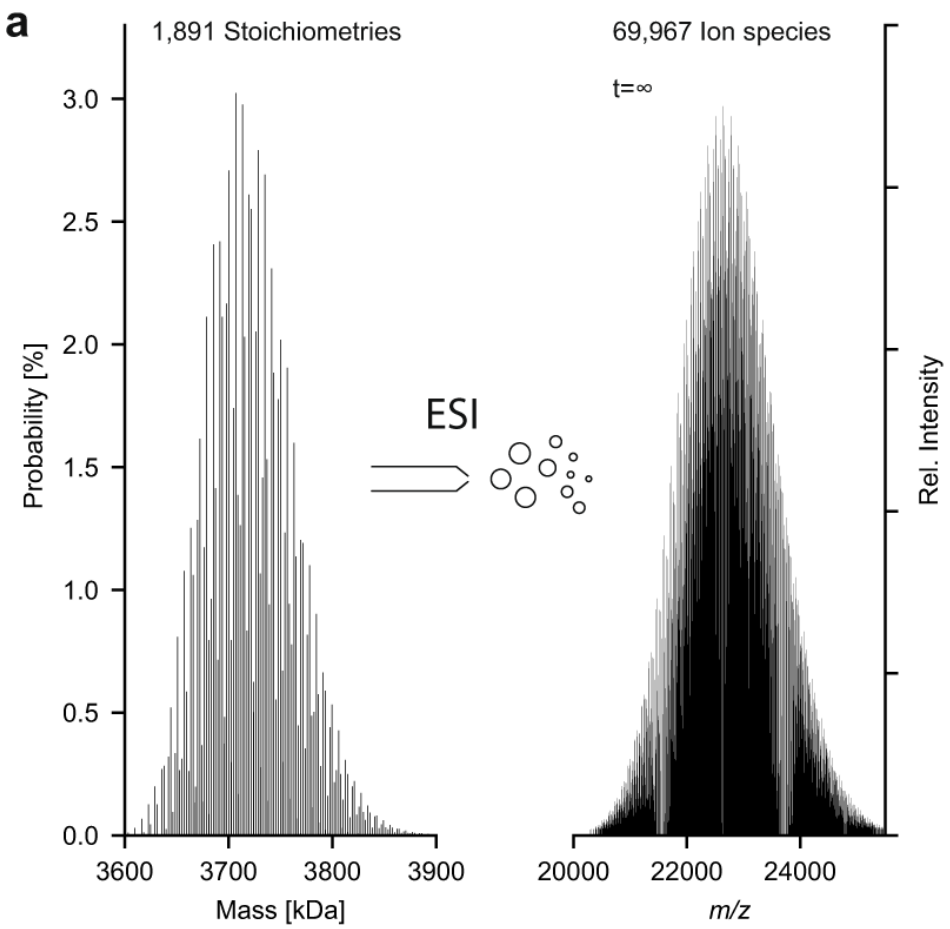
AAV capsids assemble stochastically from a mixed pool of VP1/VP2/VP3

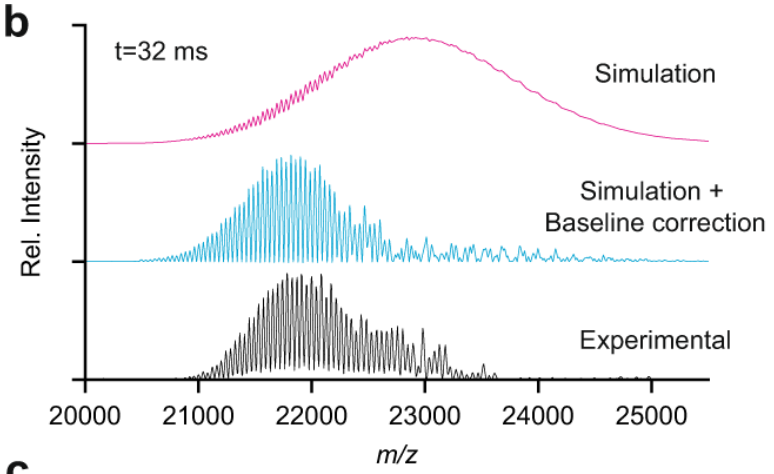


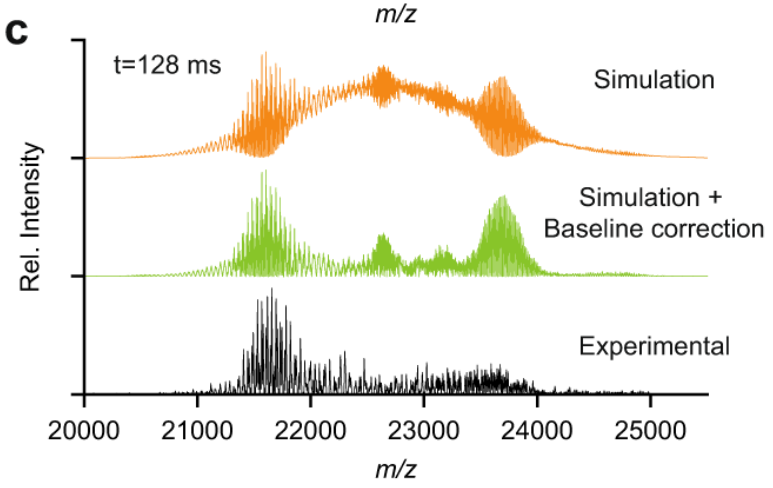
AAV9:
6% VP1 / 16% VP2 / 78% VP3 (from LC-MS)

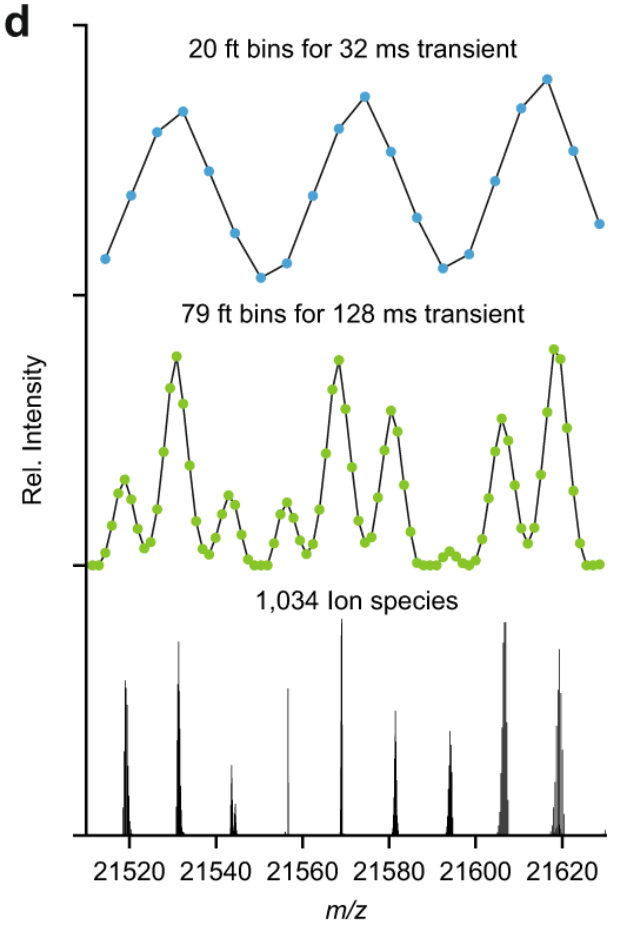
Woerner et al. 2021, NCOMMS

Stochastic assembly model fits experimental spectra of AAV capsids



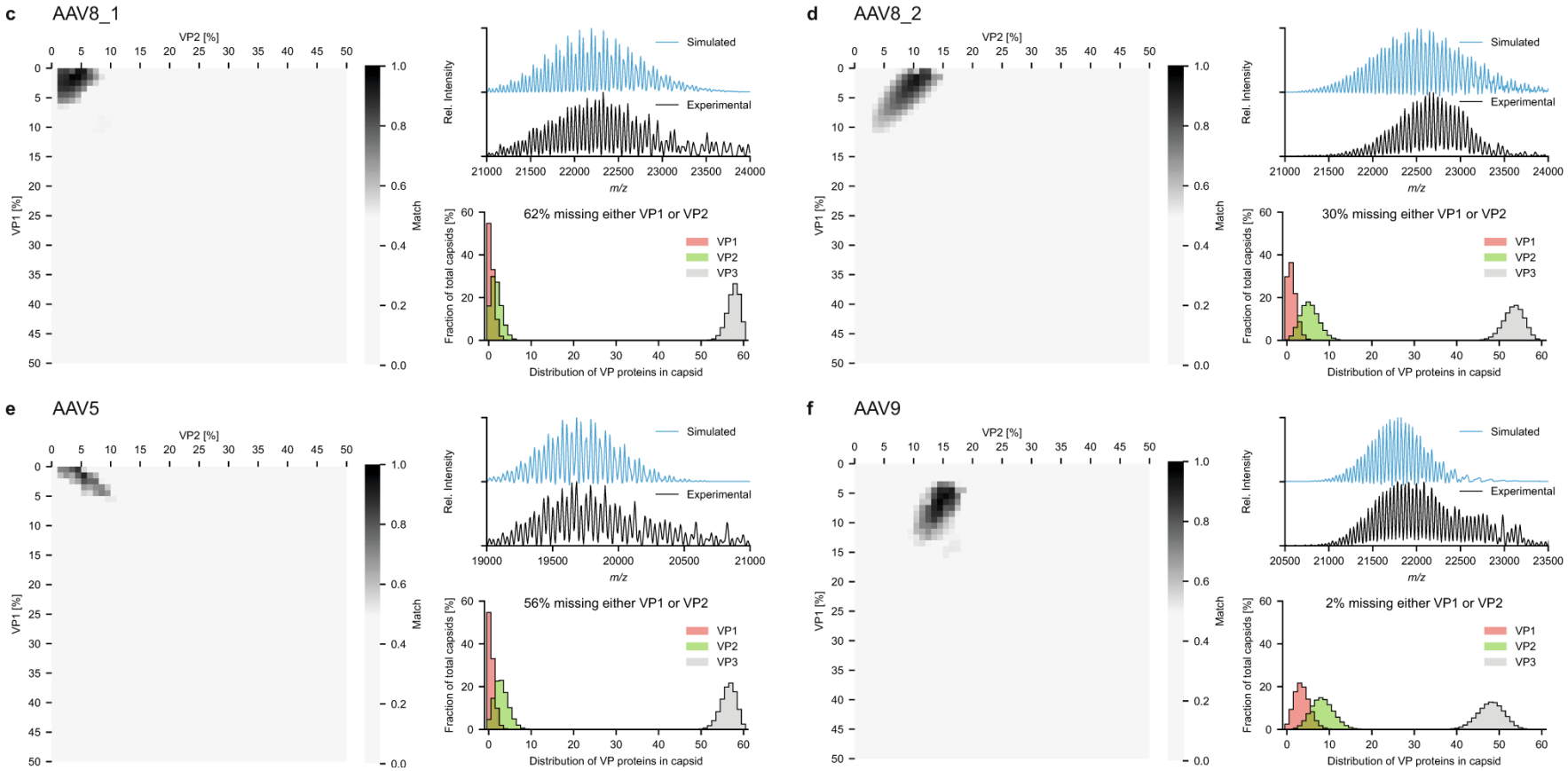






Woerner et al. 2021, NCOMMS

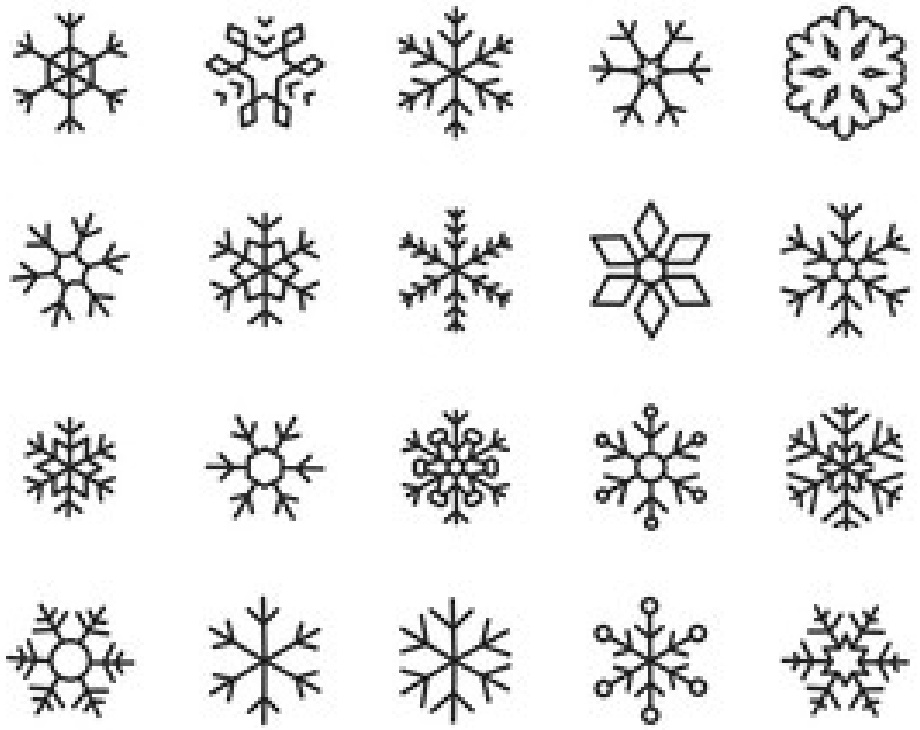
AAV preps may contain large subpopulations without a single copy of VP1 or VP2



Woerner et al. 2021, NCOMMS

AAV capsids assemble stochastically from a mixed pool of VP1/VP2/VP3

- AAV capsids are a mixture of 1891 unique stoichiometries of VP1/VP2/VP3
- Even the single-most abundant VP ratio represents less than 2.5% of the total capsid population
- For a given stoichiometry there are 60!/(VP1!VP2!VP3!) unique configurations
  - Less than 60-fold redundancy (icosahedral symmetry)
  - ~10^{12} unique configurations of 5:5:50 ratio
- Probability of an AAV capsid with an exact composition+configuration is ~10^{-14}

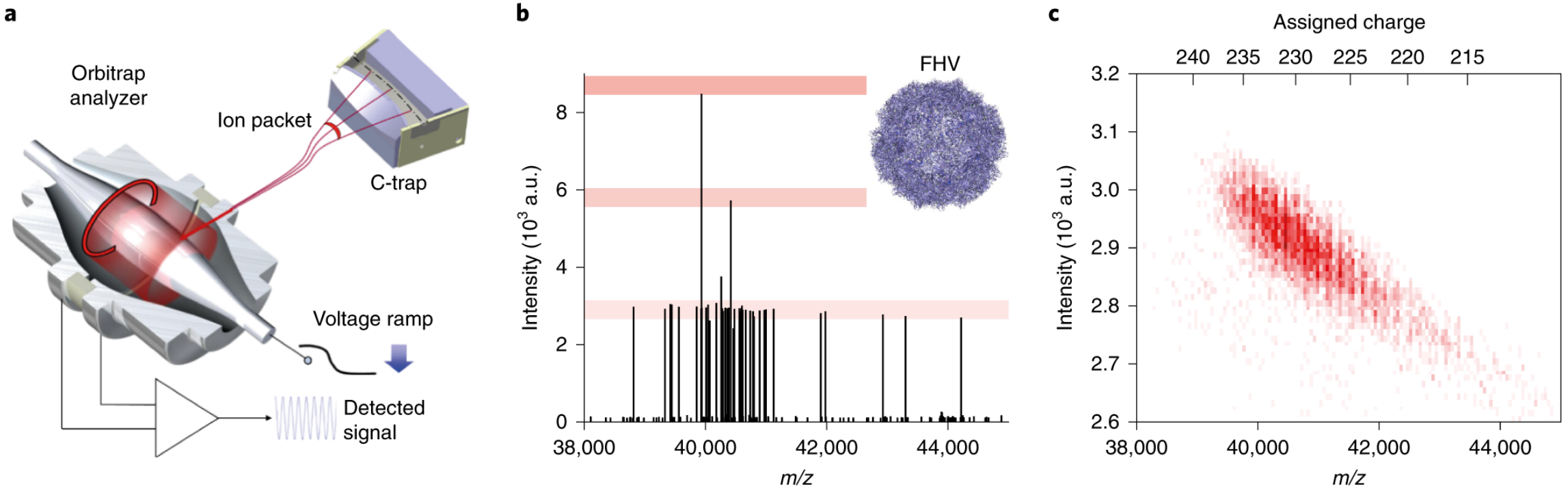


Utrecht University

BioMS

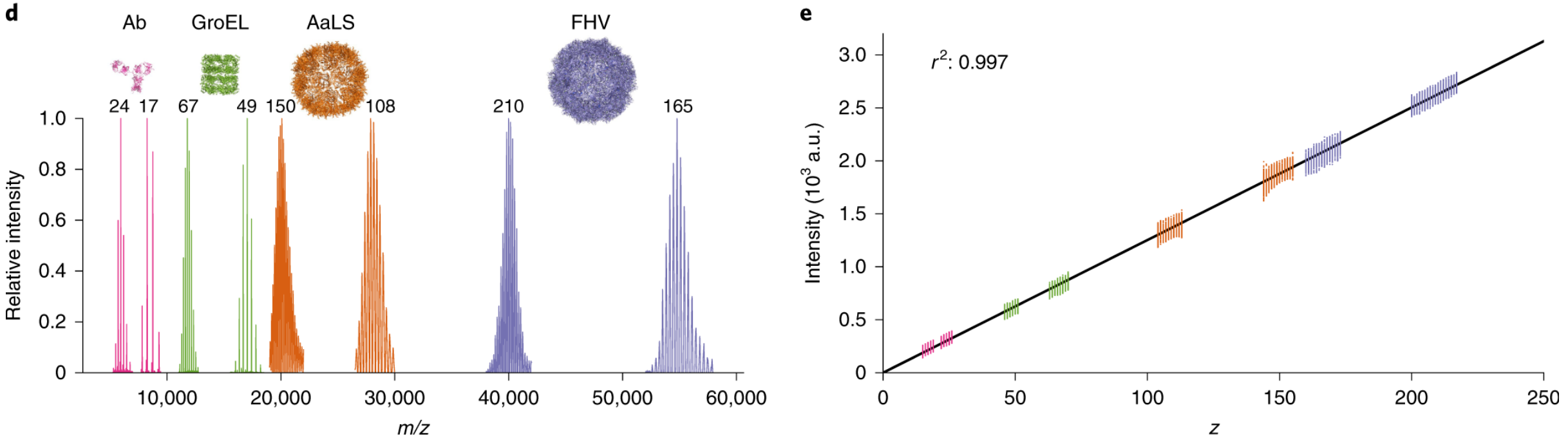
… but what about the genome filled particles?

The Orbitrap can detect individual ions at unique frequencies



Woerner et al. 2020, Nature Methods

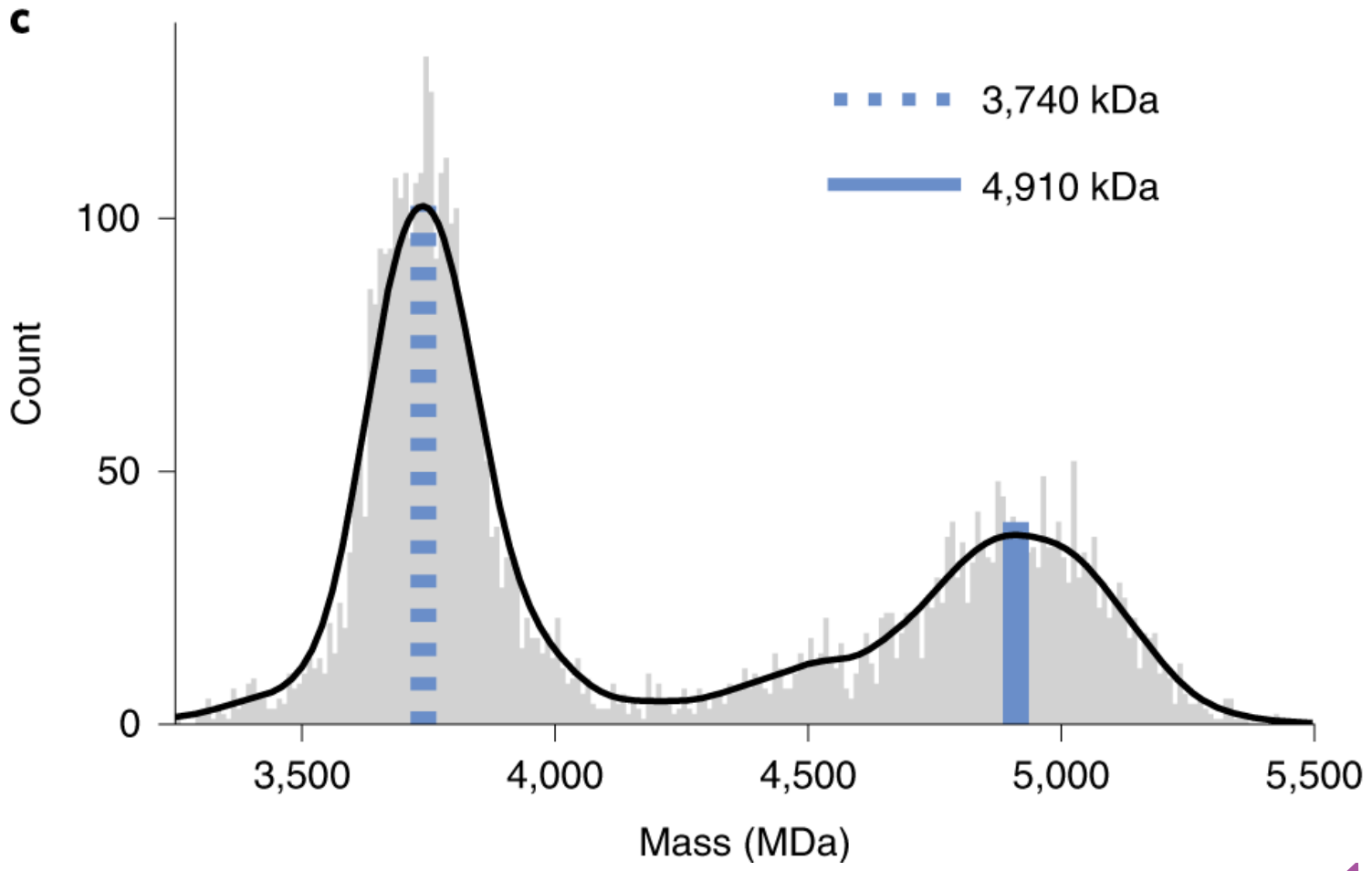
The intensity of individual ions scales linearly with charge state: CD-MS



Woerner et al. 2020, Nature Methods

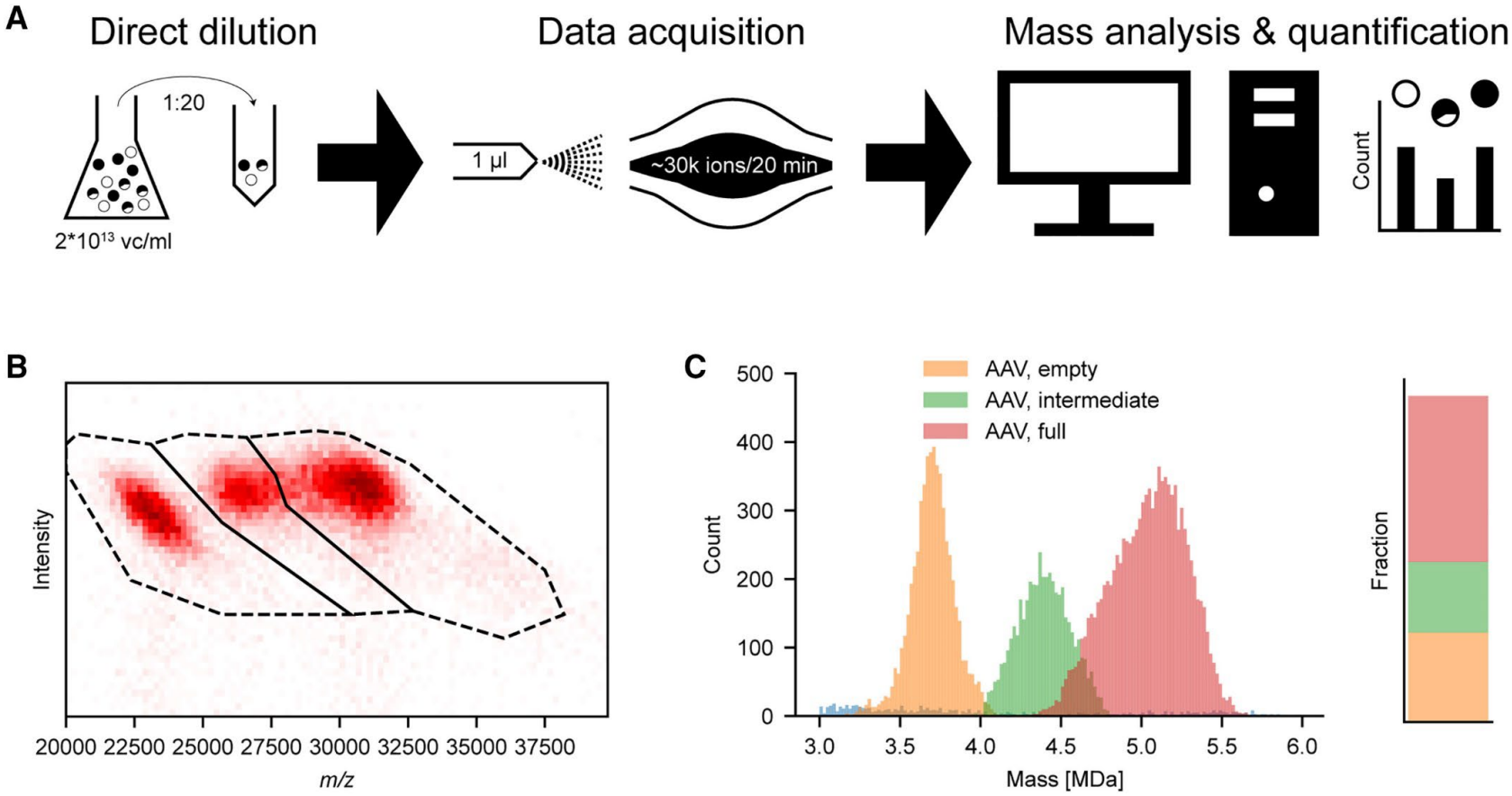
Single particle CD-MS of AAV resolves empty, full, and aberrant capsid products

- Single ion detection of AAV capsids
- Resolving empty and full
- Detecting aberrant side products
- Quantitative sampling of subpopulations



Woerner et al. 2020, Nature Methods

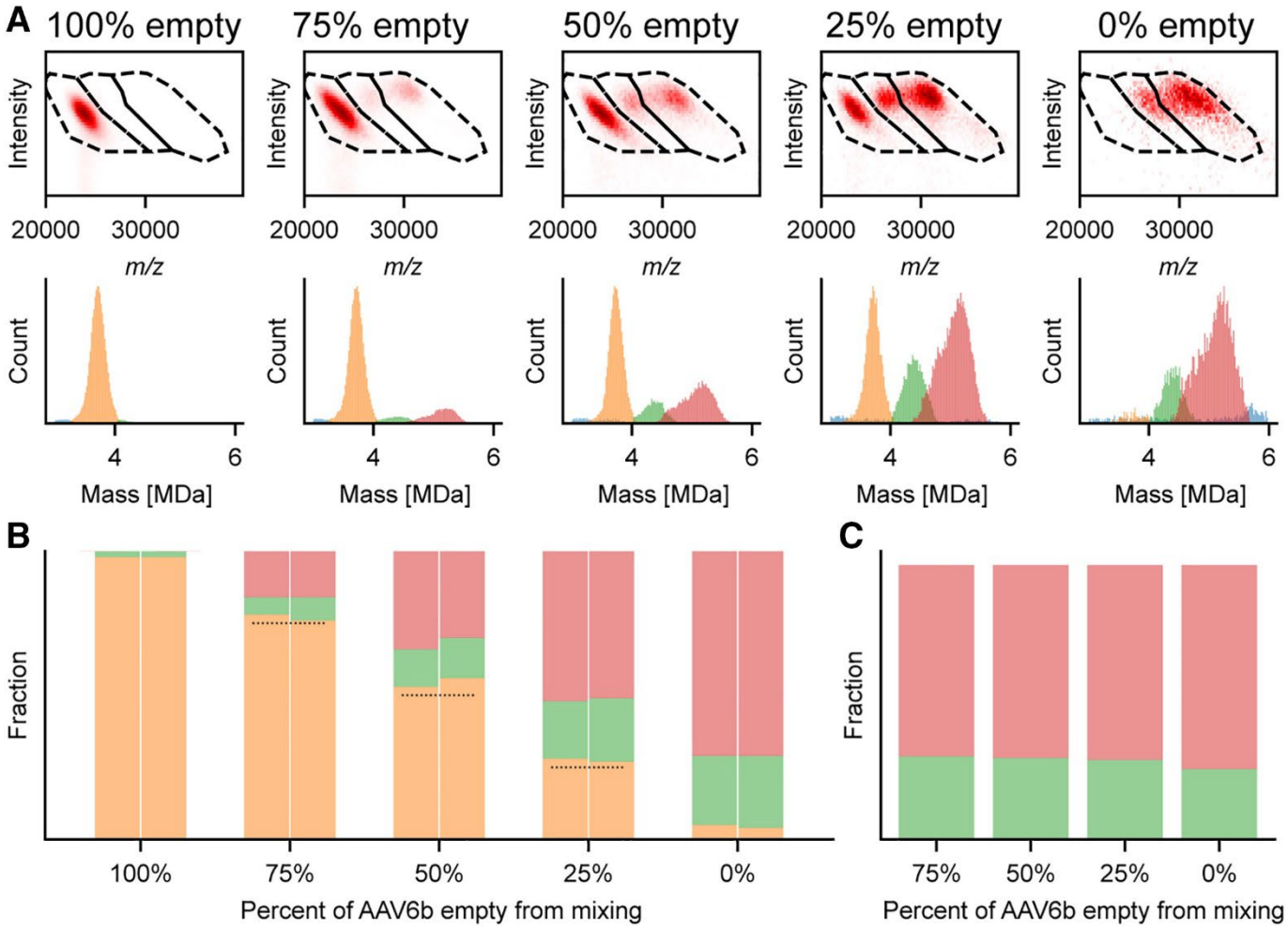
Using CD-MS for characterization and quality control of AAV



Woerner et al. 2021, Mol Therapy – Methods & Clinical development

CD-MS can quantitatively monitor subpopulations of AAV capsids

- Monitoring AAV packaging
- Evaluating the quantitative accuracy
- Empty capsids are mixed with ‘full’ capsids
- Subpopulations resolved in CD-MS
- Quantitatively accurate



Woerner et al. 2021, Mol Therapy – Methods & Clinical development

Ten years of AAV analysis by native MS

- AAV capsids are heterogeneous
- Particles assemble stochastically from a mixed pool of VP1/VP2/VP3
- VP ratios can be accurately determined from native MS spectra
- New Orbitrap based platforms for native MS enable high resolution analysis of AAV
- Orbitrap based CD-MS enables accurate monitoring of empty:full ratios, and detection of aberrant side products

Acknowledgements

- BioMS and Proteomics group UU
  - Tobias Woerner
  - Albert Heck
- University of Florida
  - Antonette Bennett
  - Mavis Agbandje-McKenna
- Pfizer
  - Thomas Powers
  - Olga Friese
- ThermoFisher Scientific
  - Konstantin Aizikov
  - Kyle Fort
  - Eugen Damoc
  - Eduard Denisov
  - Alexander Makarov