



# Pittcon 2022 Wallace H. Coulter Lecture

## New Horizons in Mass Spectrometry

- I. Sizing and Counting Particles by Native Mass Spectrometry
- II. How unique is your immune response?  
Monitoring personalized antibody repertoires
- III. Science meets art

Albert J.R. Heck

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Utrecht University  
[www.hecklab.com](http://www.hecklab.com)

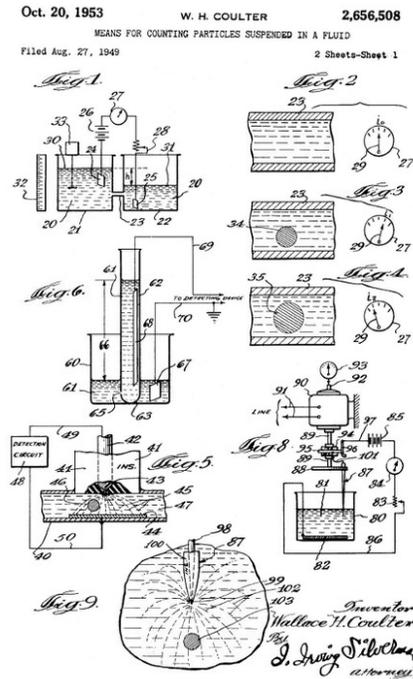


 @hecklab

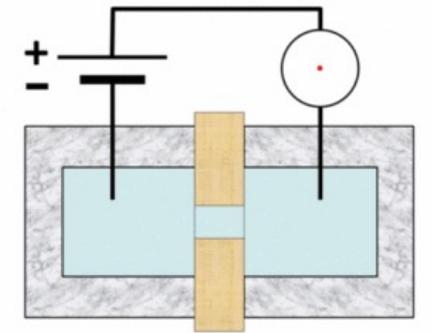
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A **Coulter counter** counts and sizes particles suspended in electrolytes

Sizing and counting particles in the gas-phase



Wallace H. Coulter

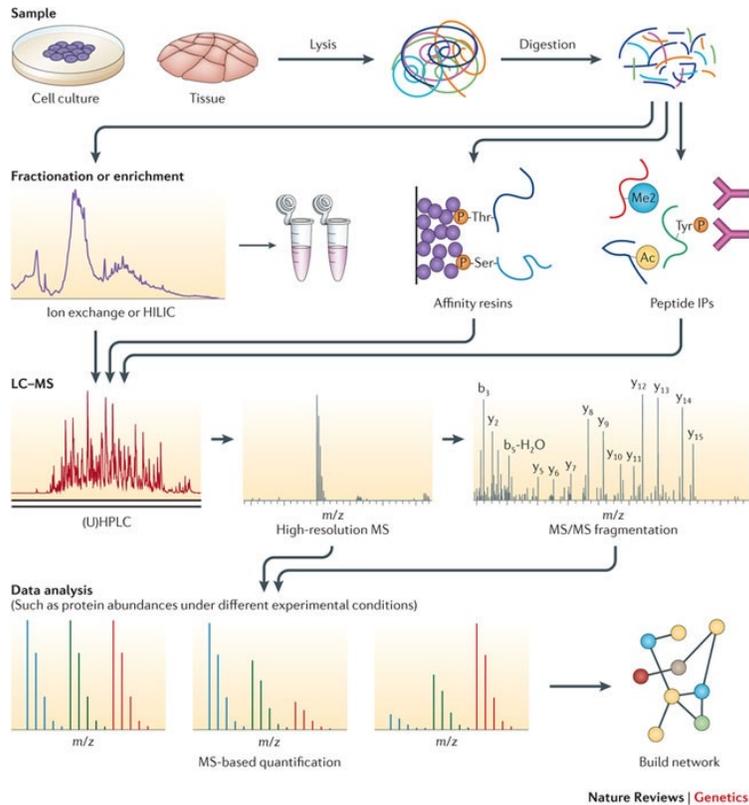


## Coulter principle

Particles pulled through an orifice, concurrent with an electric current, produce a change in impedance proportional to the volume of the particle. This pulse in impedance originates from the displacement of electrolyte caused by the particle.

The principle has found success in the medical industry, particularly in hematology, cell counting, particle sizing. It can be applied to count and size the various cells that make up whole blood.

# Many flavours of Biomolecular Mass Spectrometry

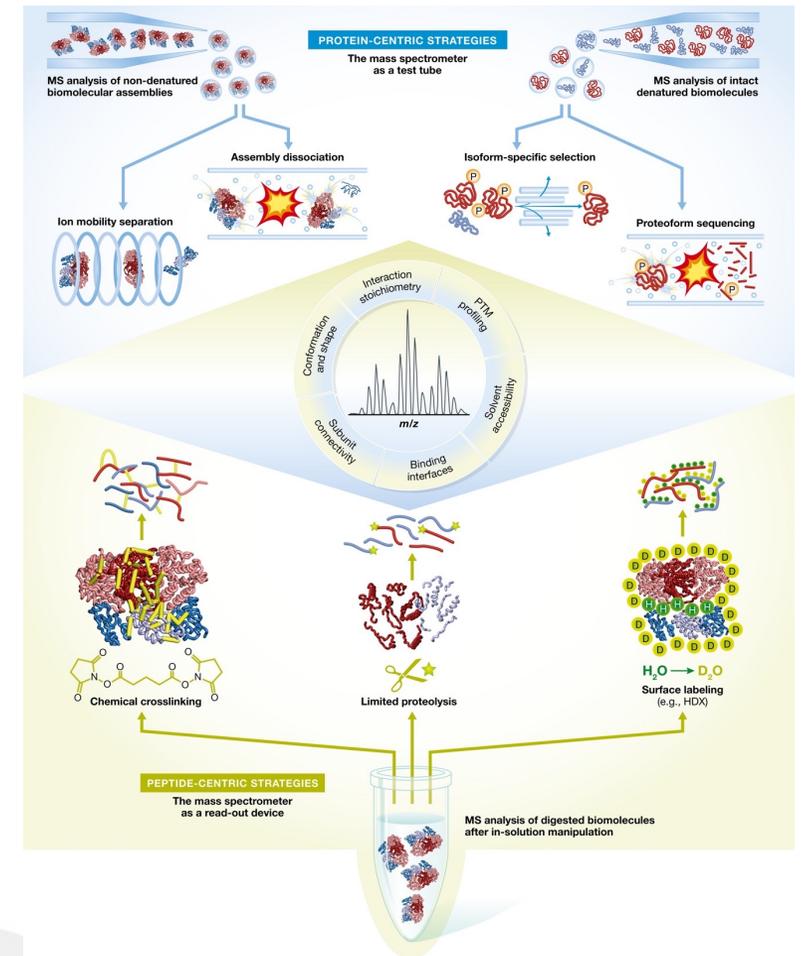


Peptide-centric  
Protein-centric  
Protein-complexes

Pure proteins  
Purified assemblies  
Cells  
Tissues  
Organisms



Many different  
MS-technologies



Next-generation proteomics: towards an integrative view of proteome dynamics  
Nat Rev Genet (2013) 14:35-48.  
doi: 10.1038/nrg3356

The diverse and expanding role of mass spectrometry in structural and molecular biology  
EMBO Journal (2016) 35:2634-2657  
[doi.org/10.15252/emboj.201694818](https://doi.org/10.15252/emboj.201694818)

# Native or denaturing mass spectrometry ?

## Native Mass Spectrometry: What is in the Name?

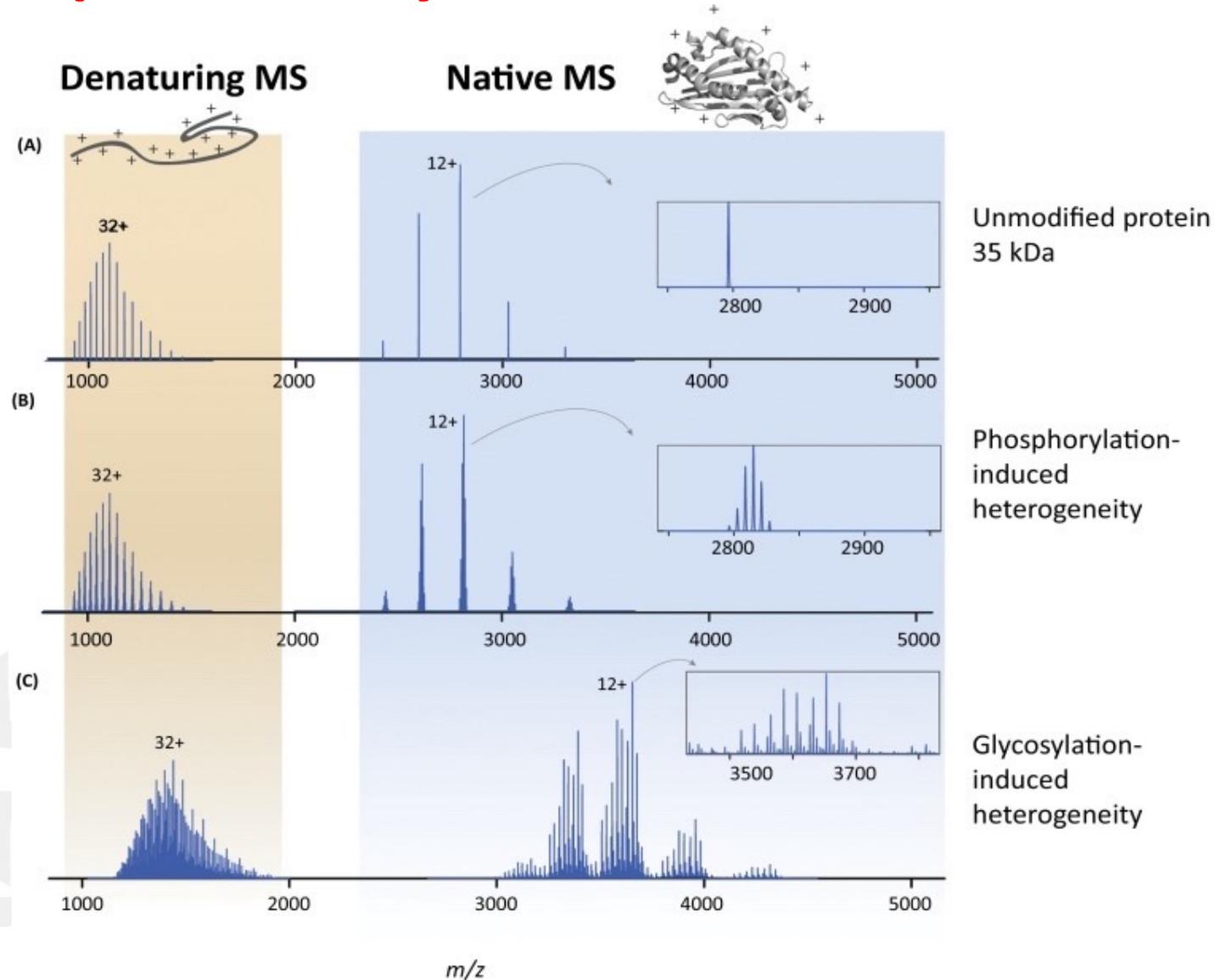
Leney & Heck

*JASMS* 28 (2017) 5-13

## High-Resolution Native Mass Spectrometry

Den Boer, Tamara & Heck

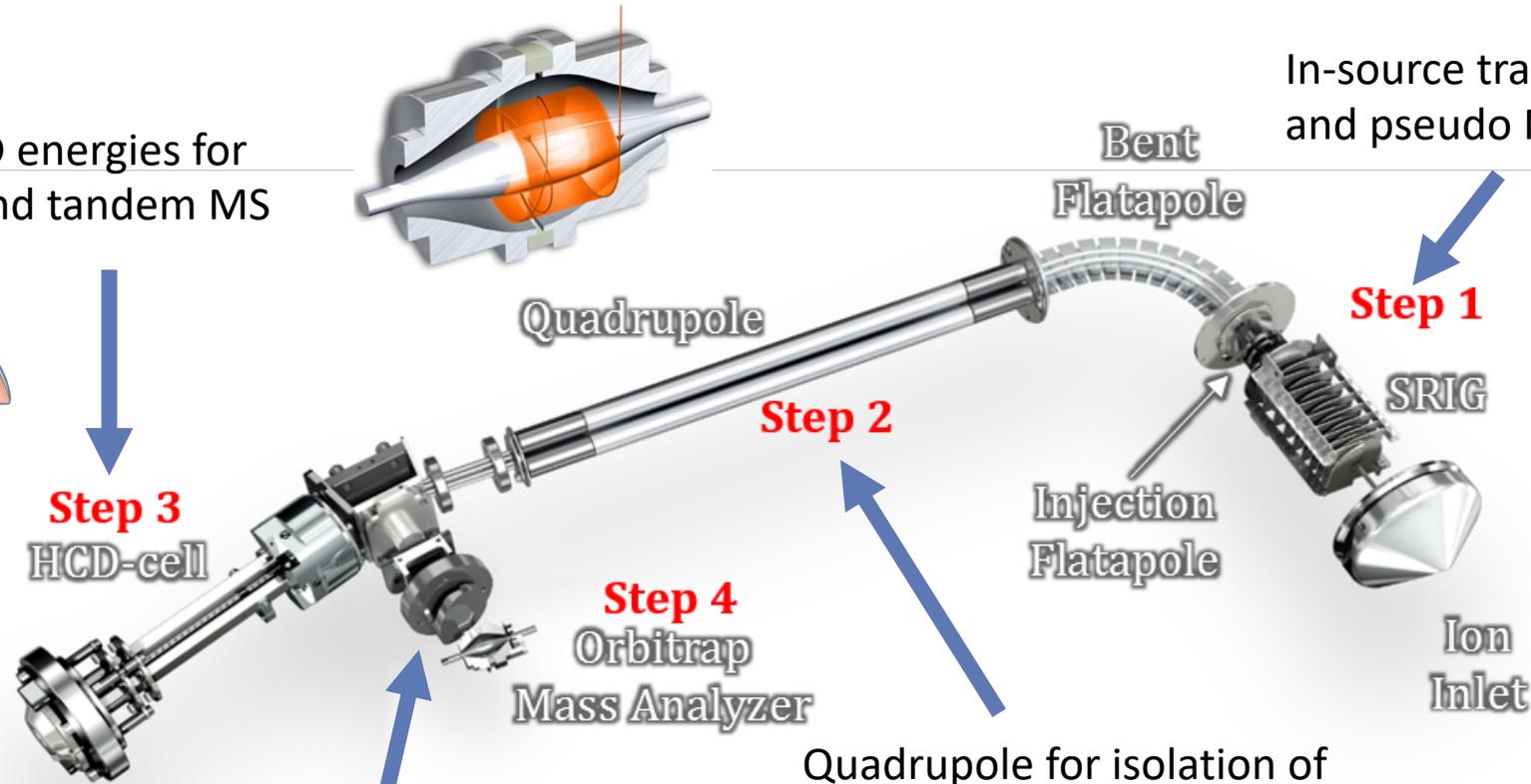
*Chem Rev* x (2022) y



# New Detectors for Native MS: Orbitrap Technology

*Our game changer*

Extended HCD energies for desolvation and tandem MS



In-source trapping for desolvation and pseudo MS<sup>3</sup> experiments

**Step 3**  
HCD-cell

Improved transport of high  $m/z$  ions from the C-trap to the Orbitrap

**Step 4**  
Orbitrap  
Mass Analyzer

**Step 2**

Quadrupole for isolation of ions up to 40,000  $m/z$

**Step 1**

Improved focusing of high mass ions throughout the instrument; EMR (2012) UHMR (2017)



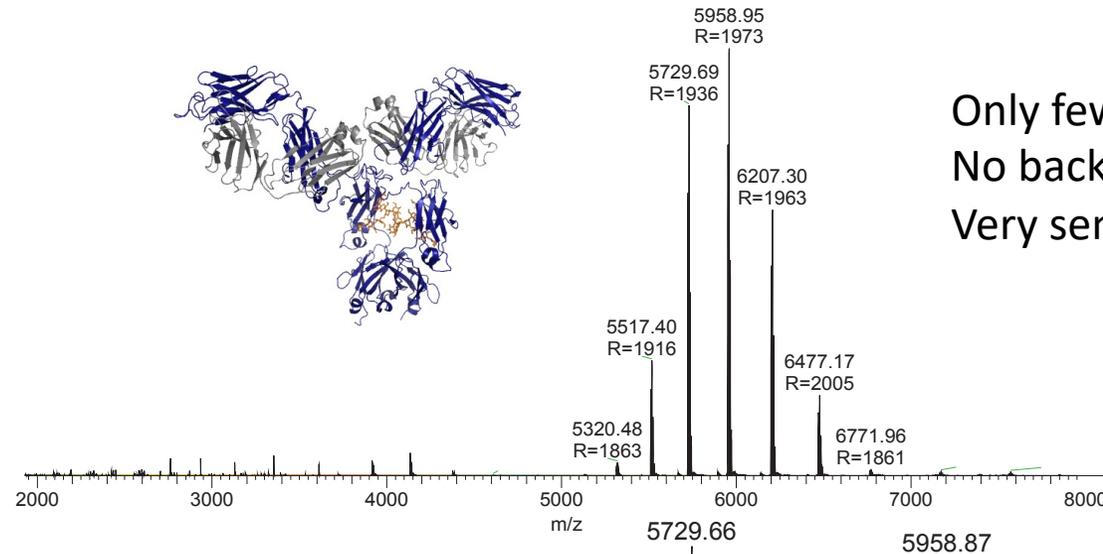
# Exploring an Orbitrap Mass Analyzer for Native Mass Spectrometry

- *Orbitrap appears to be extremely powerful for the analysis of protein assemblies*
- The impact of the high mass resolving power at very high sensitivity is tremendous; it opens up avenues to measure dynamic protein-protein interactions, and (non)covalent binding of small molecules to protein assemblies, and *very large protein assemblies*
- Wide-ranging applications include the analysis of post-translational modifications, e.g. phosphorylation, *glycosylation on intact proteins* and protein assemblies, co-factor binding to native enzymes, nucleotide binding to DNA repair enzymes, and lipid binding to ATPase systems and other membrane proteins



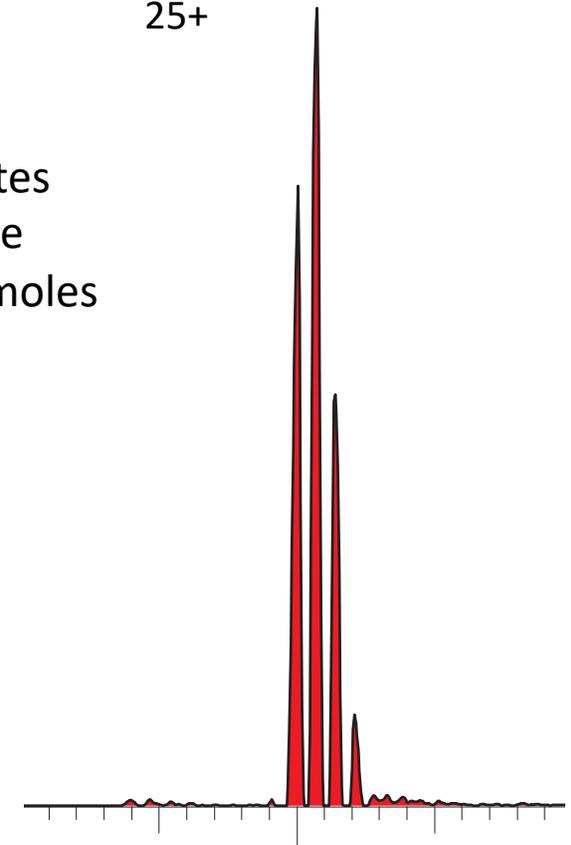
Rose *et al.* **Nature Methods** 2012  
Rosati *et al.* **Angew Chemie** 2012  
Waterbeemd *et al.* **Nature Methods** 2017  
Fort *et al.* **Analyst** 2017

# Glycosylated antibodies on Orbitrap EMR



Only few charge states  
No background noise  
Very sensitive, attomoles

25+



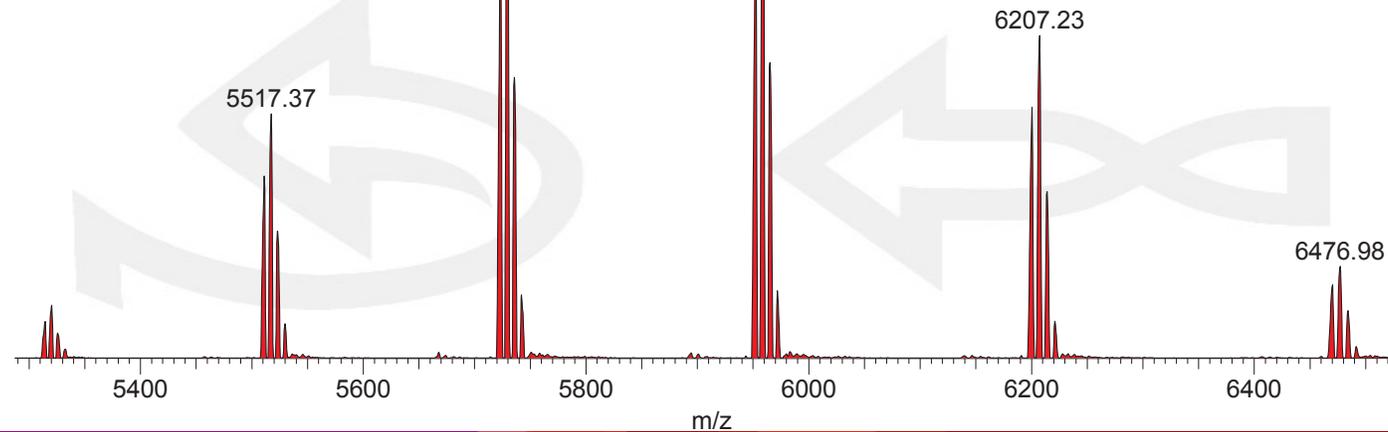
## High-Resolution Native Mass Spectrometry

Sem Tamara, Maurits A. den Boer, and Albert J. R. Heck\*

✓ Cite this: *Chem. Rev.* 2021, XXXX, XXX, XXX-XXX  
Publication Date: August 20, 2021  
<https://doi.org/10.1021/acs.chemrev.1c00212>

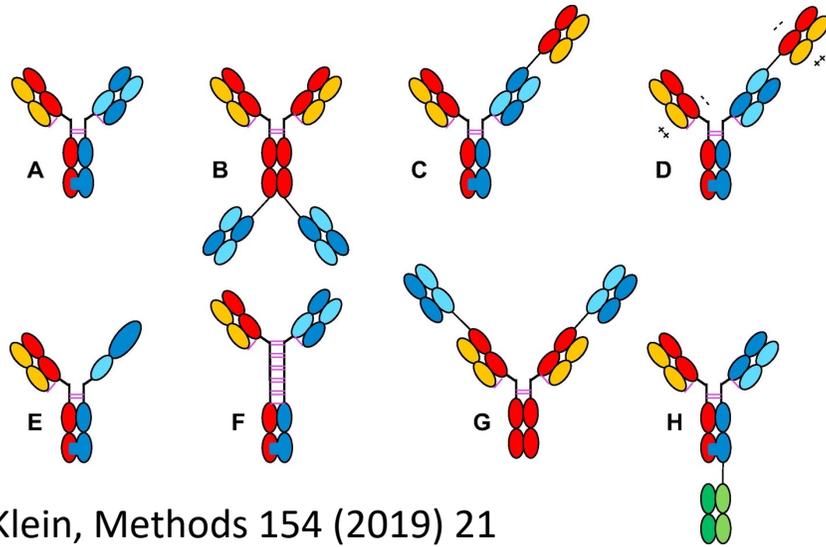
Article Views  
**6316**

Altmetric  
**24**



# Antibody-formats become more and more complex

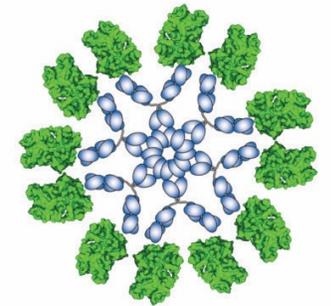
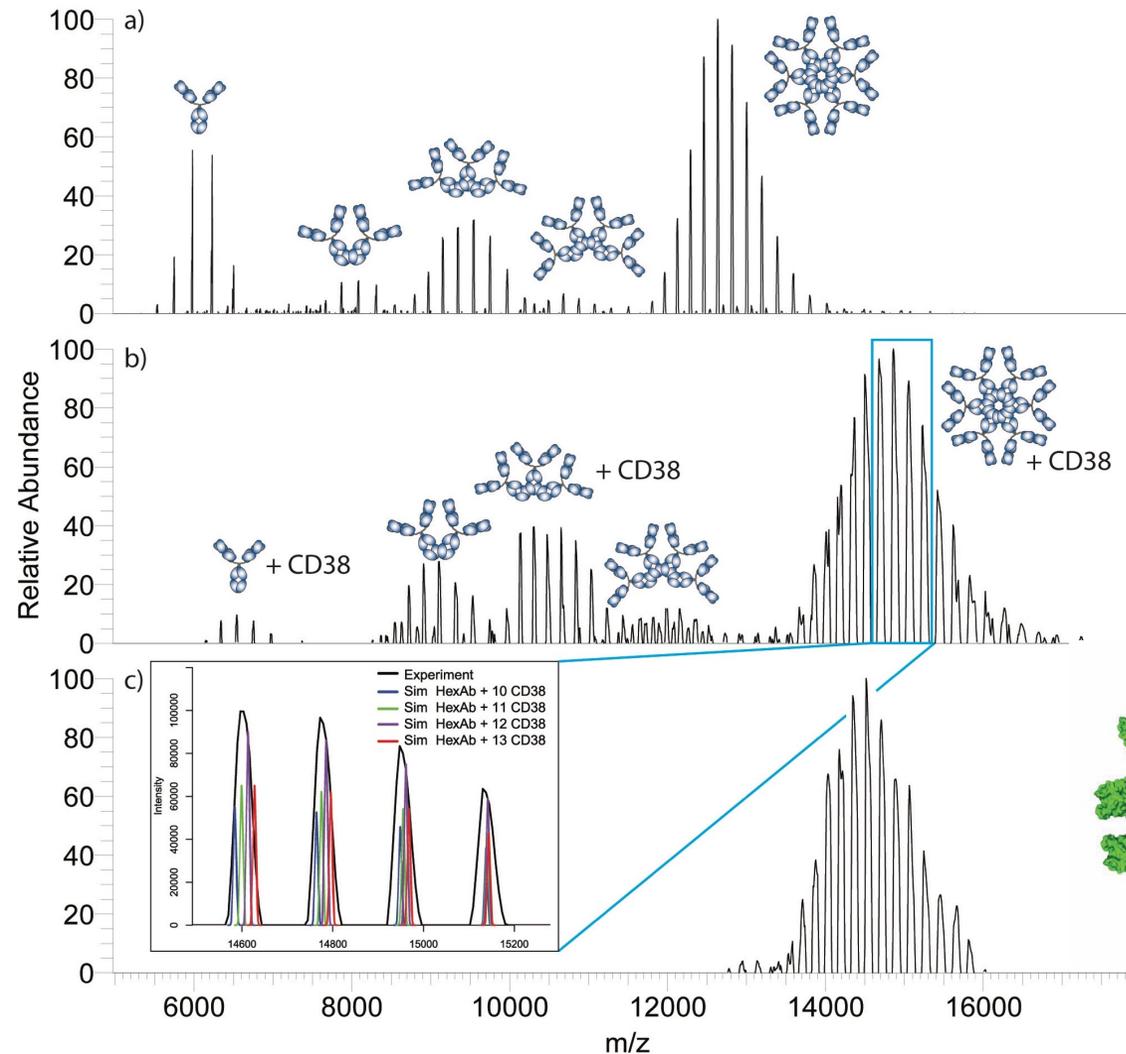
Dyachenko, Anal Chem (2015) 87:6095-102



Klein, Methods 154 (2019) 21

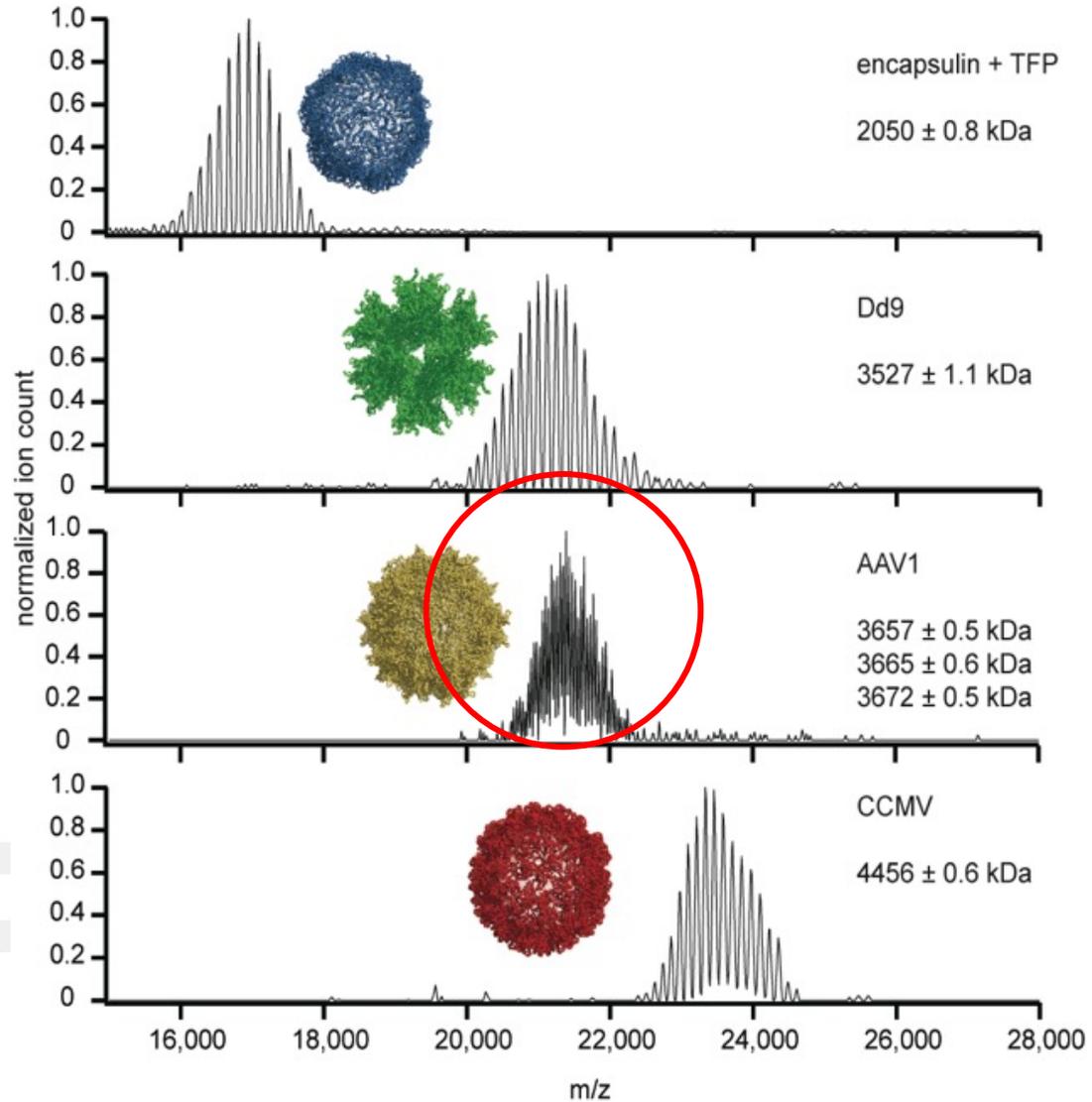
These new formats are more complex to make and analyze

Their size but also mass heterogeneity will ever increase



IgG1-RGY-antigen complexes  $(\text{IgG})_6(\text{CD38})_{12}$ , molecular weight 1.26 MDa

# Orbitrap performance for virus analysis



Defining the stoichiometry and cargo load of viral nanoparticles by Orbitrap mass spectrometry. Snijder J, *et al.* **JACS** 136 (2014) 7295-9.



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*Sizing and counting AAV particles by high-resolution native mass spectrometry and single molecule charge detection mass spectrometry*

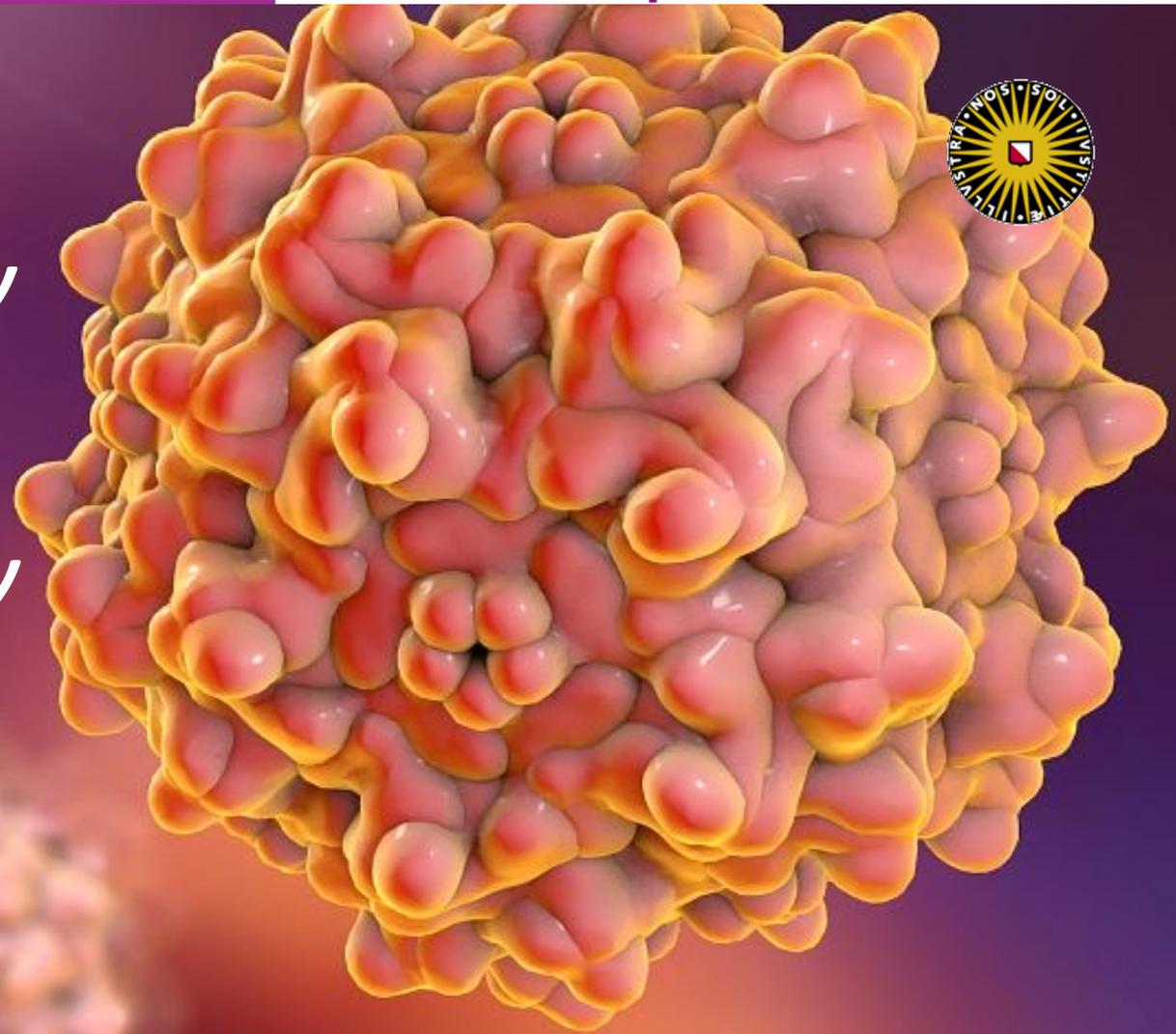
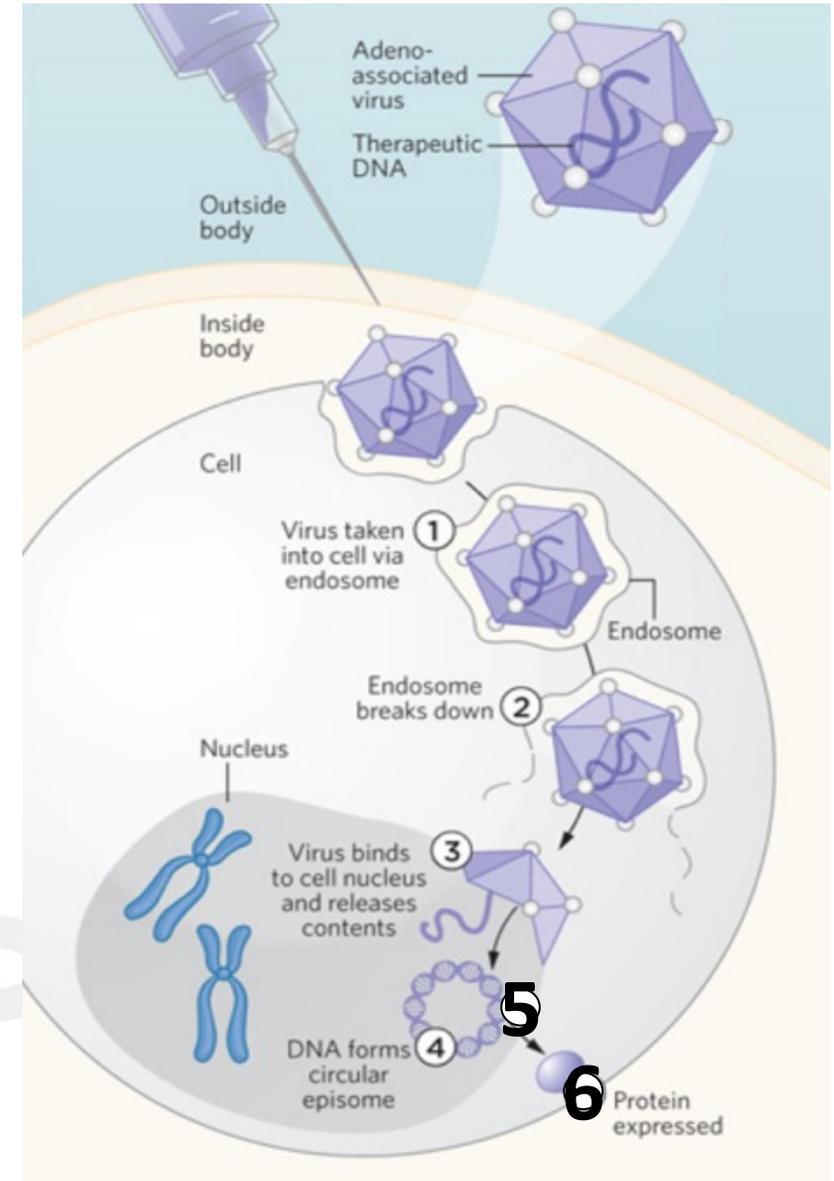


Image credit

# AAV Gene Therapy

AAVs deliver genes without integrating them into the genome

1. Virus is taken into the cell via the endosome
2. The endosome breaks down
3. Therapeutic DNA enters cell nucleus as a double-stranded molecule ready for transcription
4. The resulting transcript leaves the nucleus and travels to the ribosome for translation (protein synthesis)



\$2 million

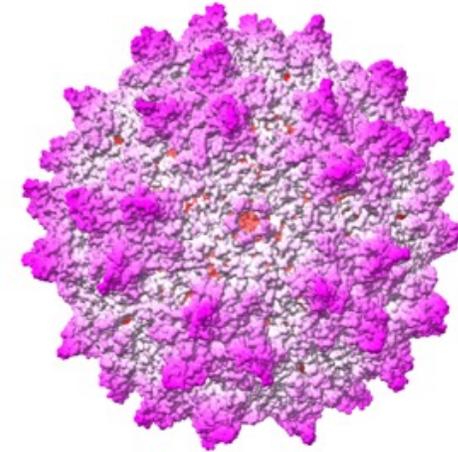
# Structural Properties of AAV

The AAV capsid is composed of 60 capsid protein subunits, VP1, VP2, and VP3, arranged in an icosahedral symmetry

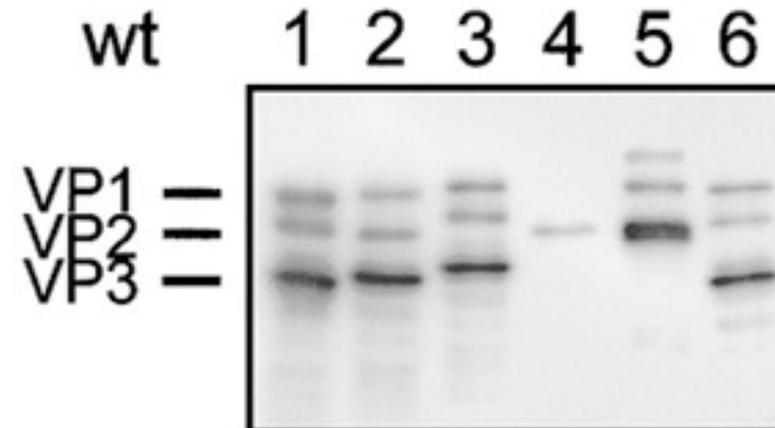
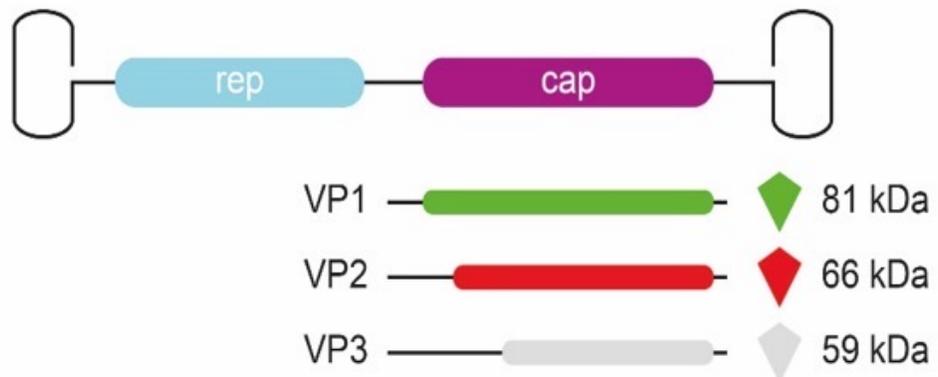
The ratio is serotype dependent and batch dependent but estimated to be 5:5:50.

Vp1 and Vp2 are essential for viral entry

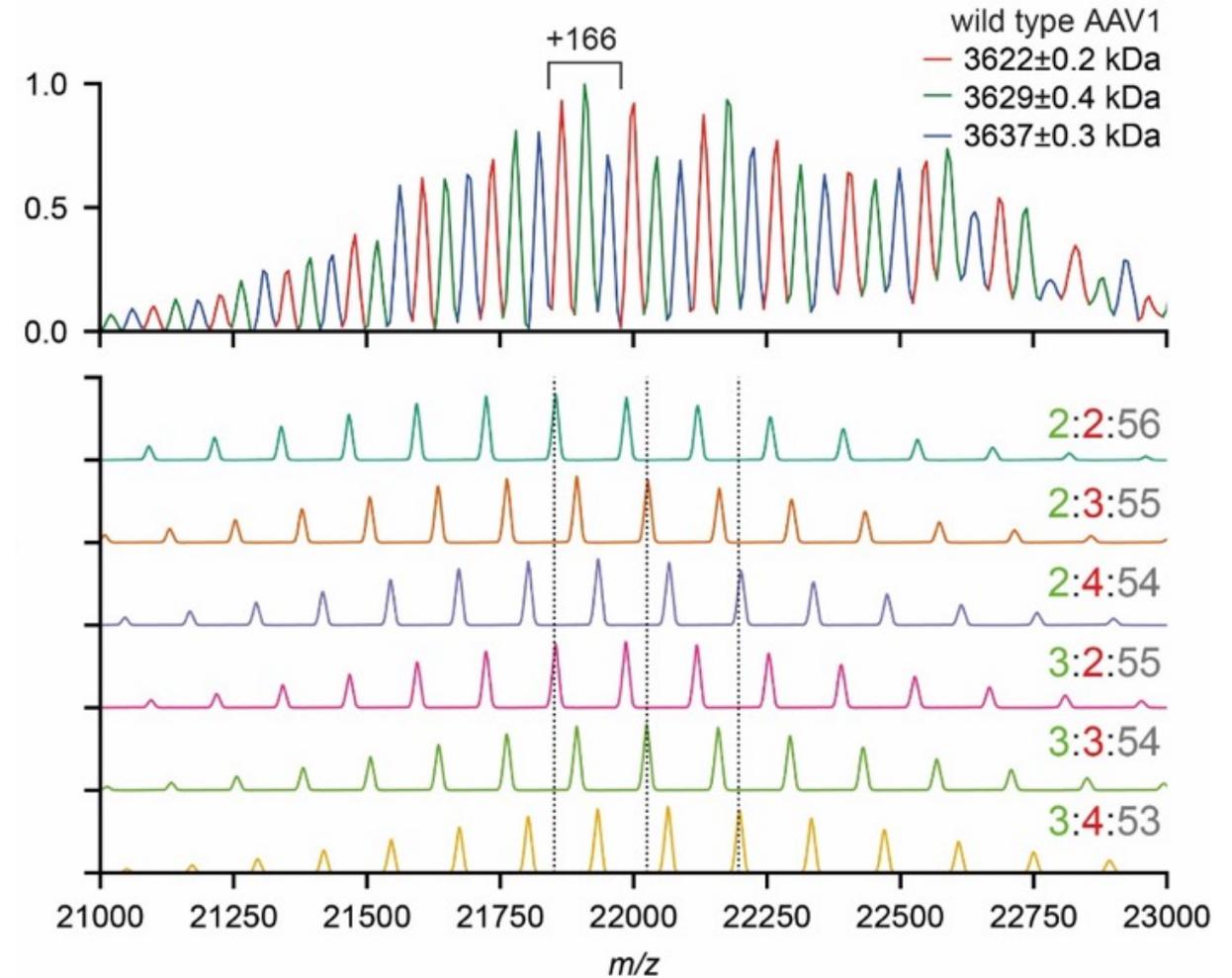
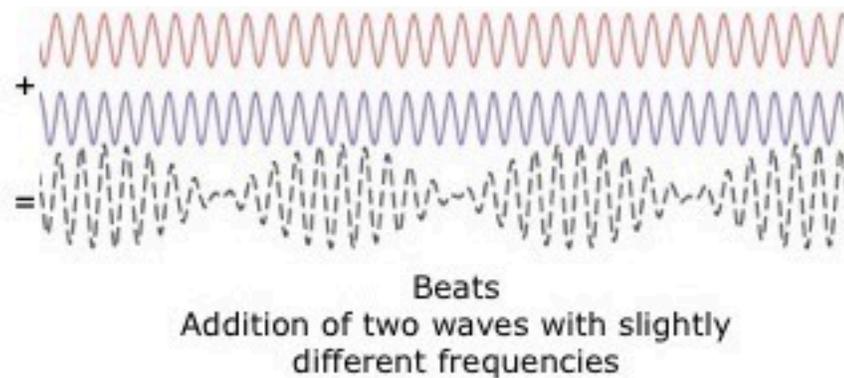
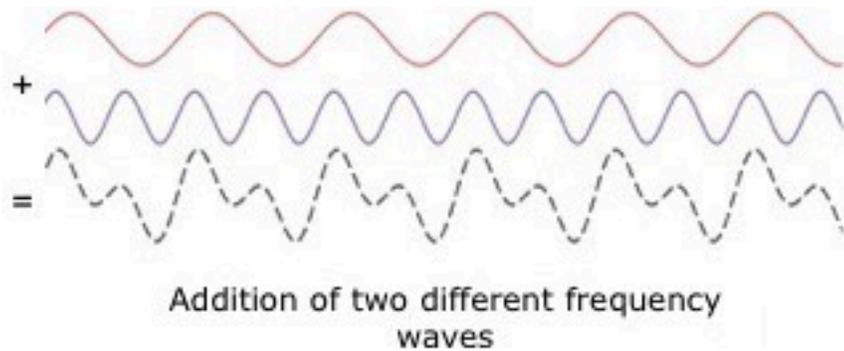
But what is the exact composition of the virus?



r=11nm



# AAV Adeno Associated virus assembly

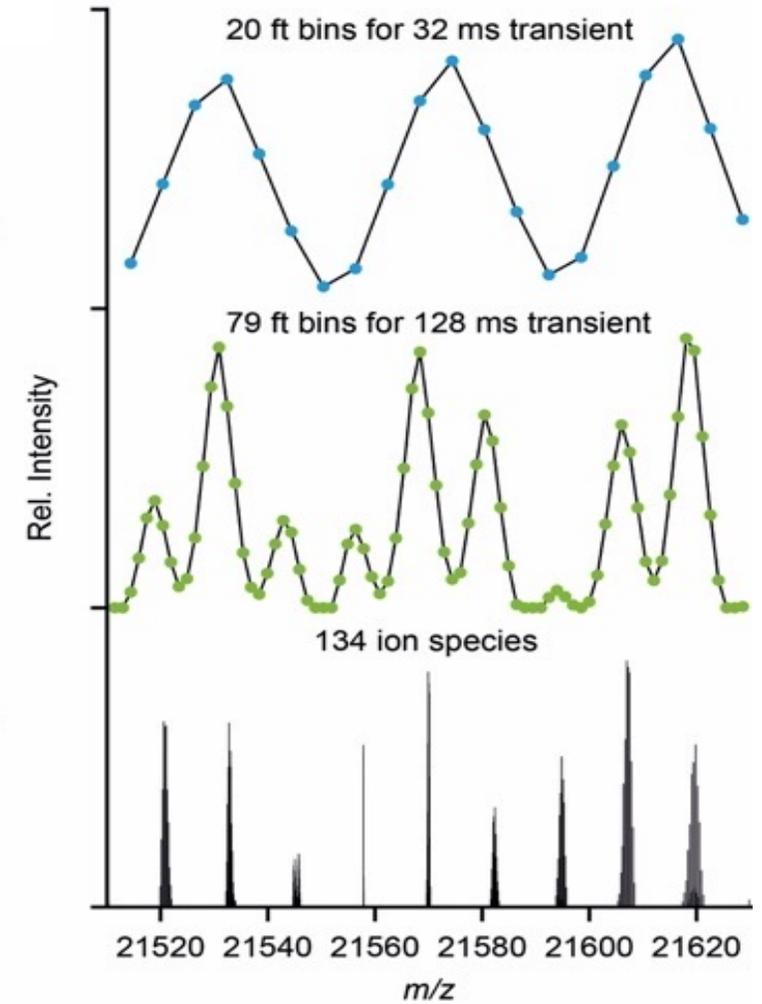
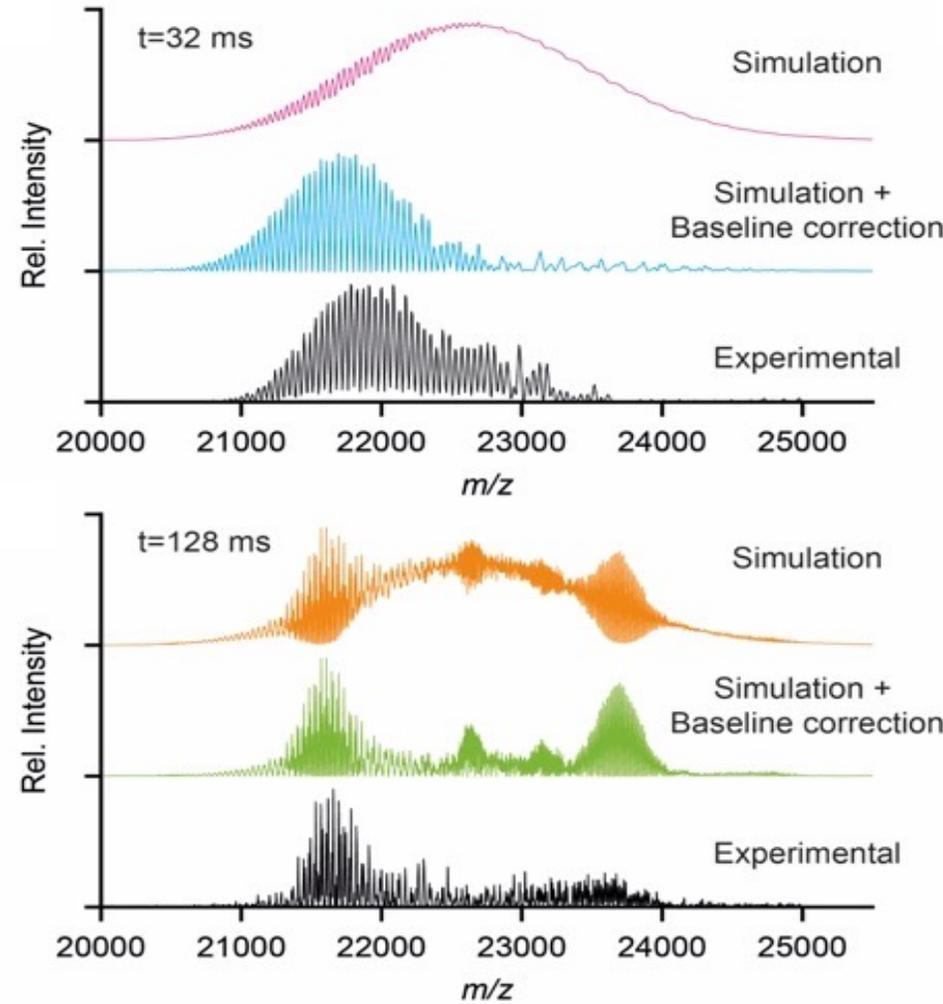
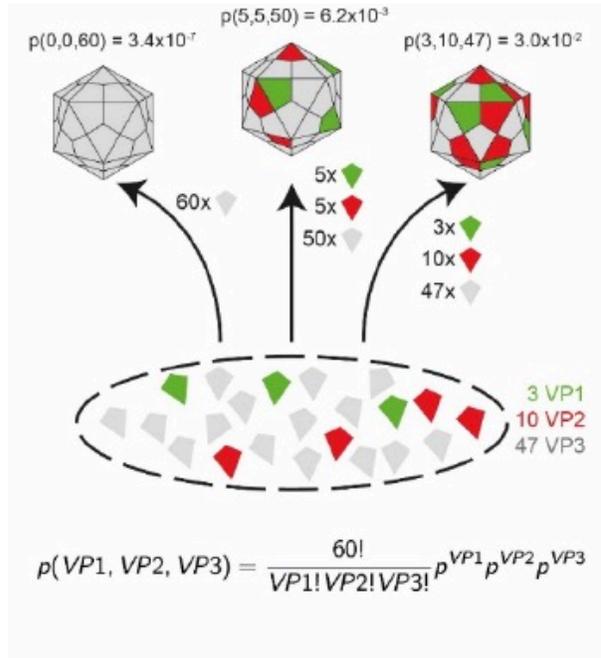


Slightly different masses, slightly different frequencies; interference

AAV capsid assembly is stochastic, no particle is the same

Wörner T, *et al. Nature Comm* (2021)

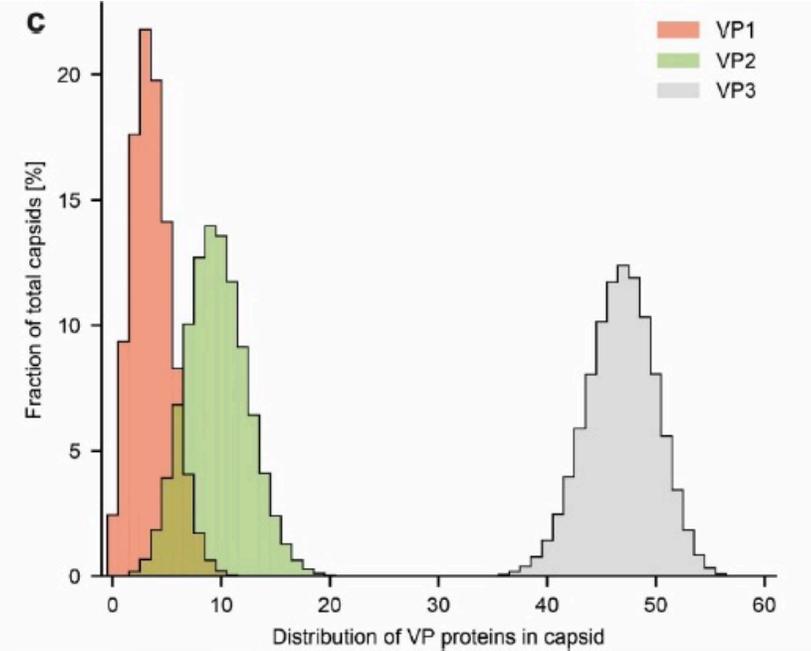
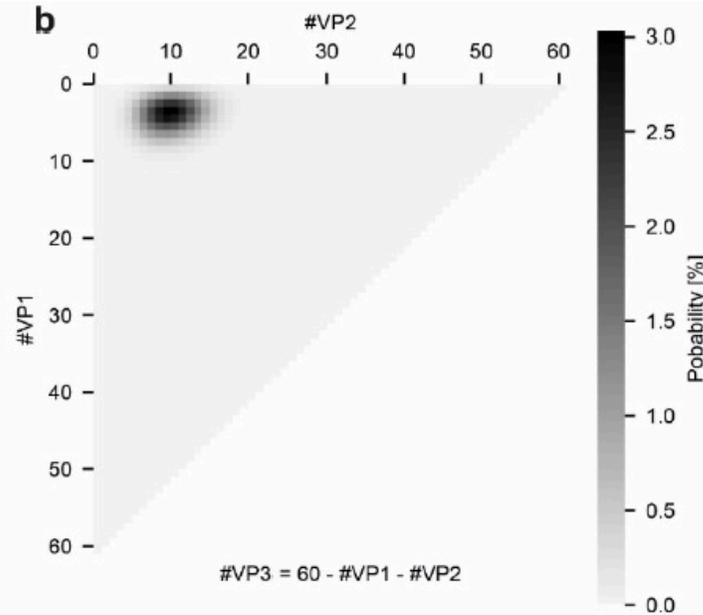
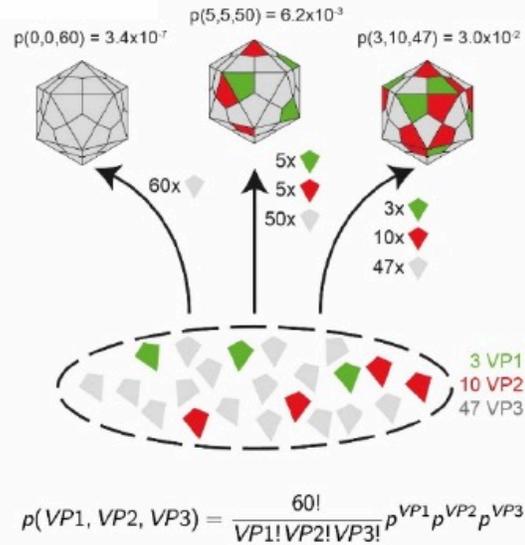
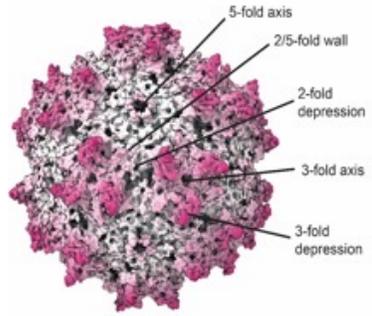
# AAV Adeno Associated virus assembly



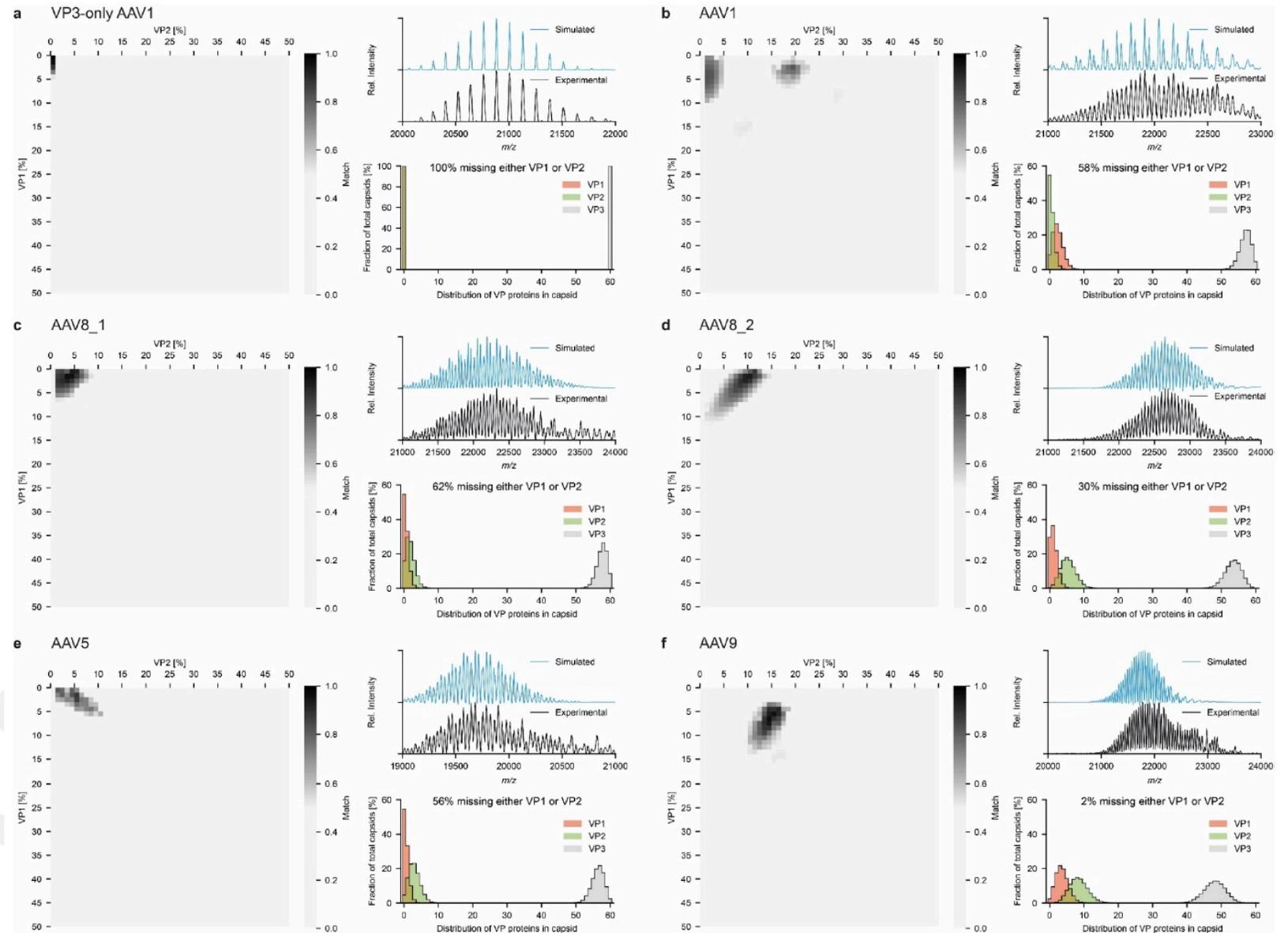
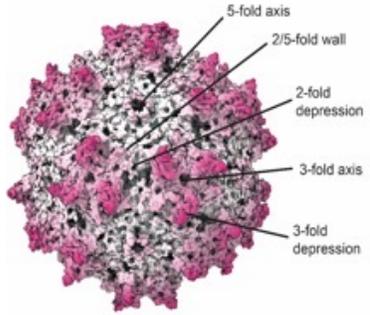
Simulations with right particle distributions match experimental spectra

AAV capsid assembly is stochastic, no particle is the same

# AAV Adeno Associated virus assembly stochastic model



# AAV Adeno Associated virus assembly of different serotypes



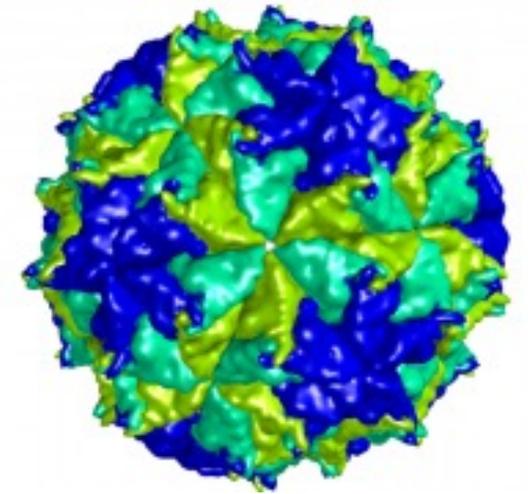
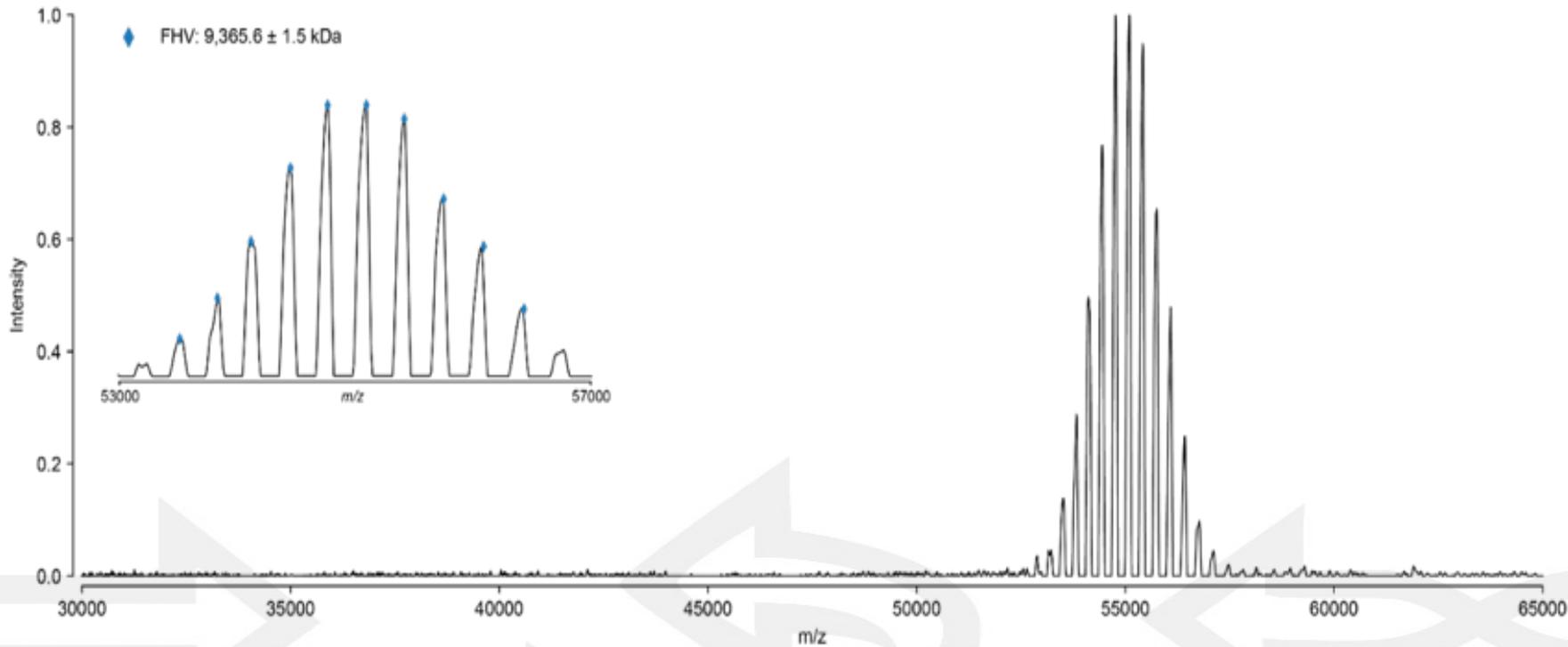
# Mass determination of Flock house virus

Mw = 9 365 600 +/- 1500 Da

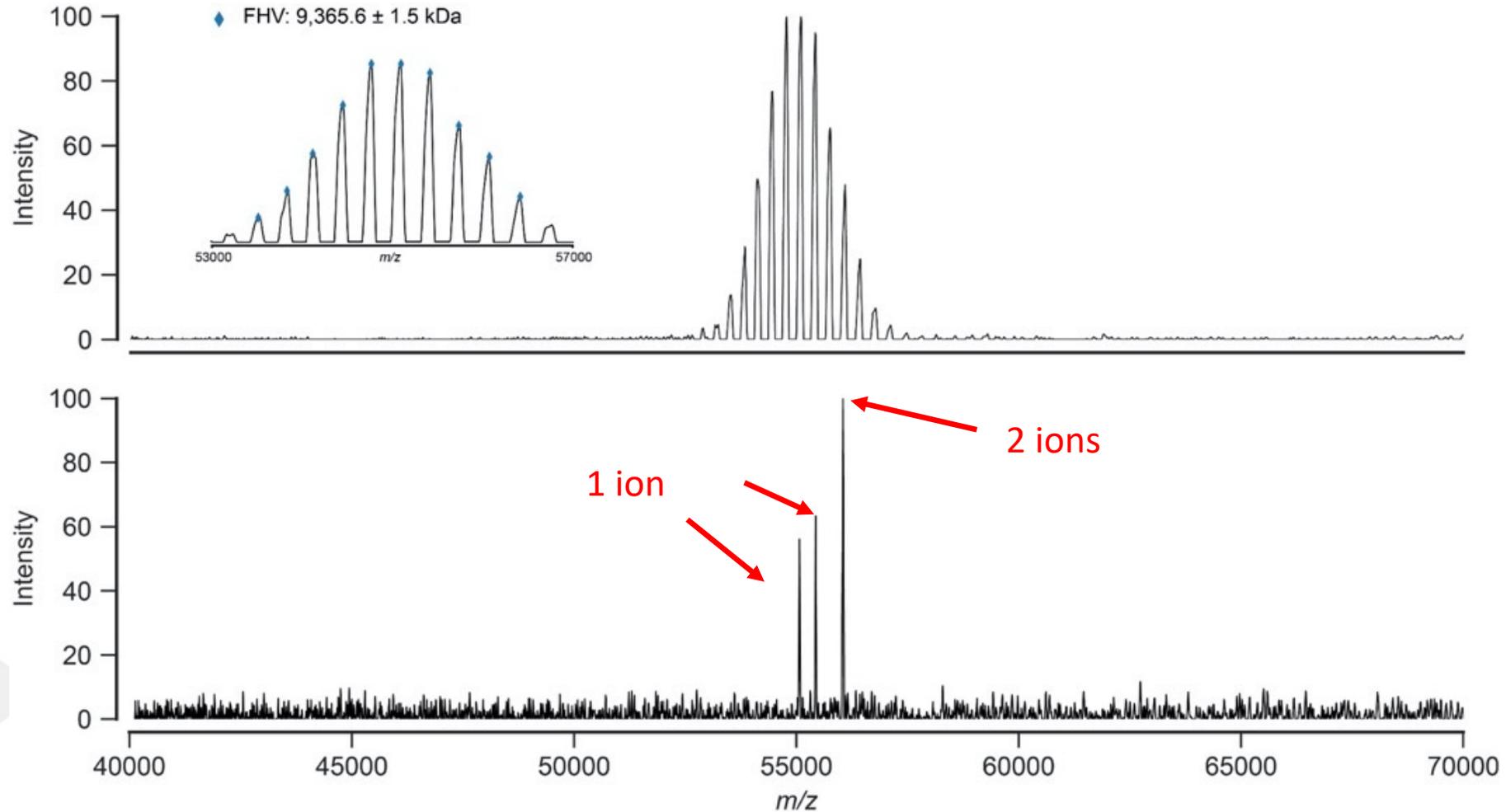
cs 170

**Flock House virus**

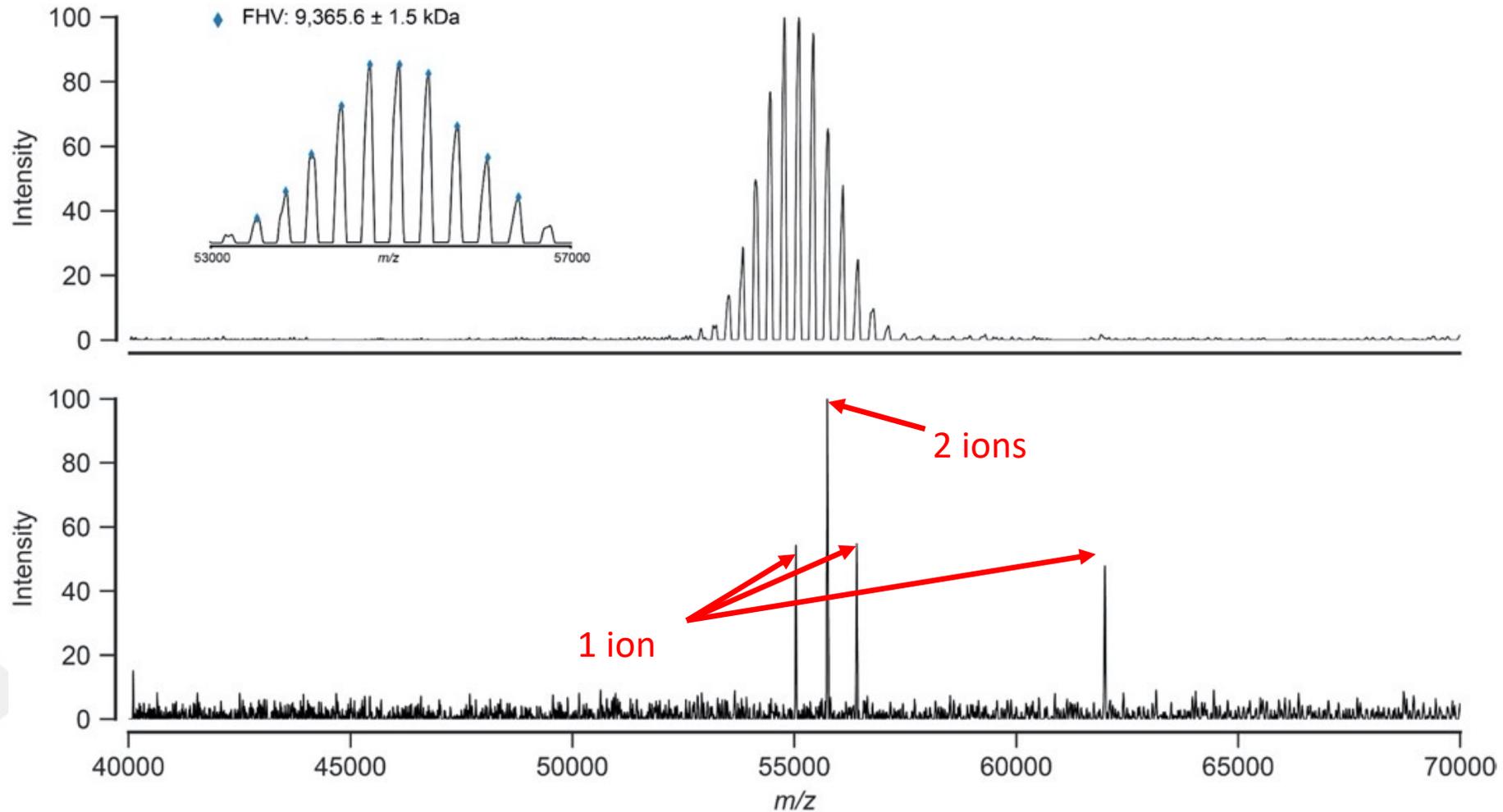
180 ×	capsid protein beta	7,056.1 kDa
180 ×	peptide gamma	791.3 kDa
1 ×	RNA1	1,000.6 kDa
1 ×	RNA2	449.6 kDa
240 ×	Ca <sup>2+</sup>	9.6 kDa
		+
Full virion		9,307.2 kDa



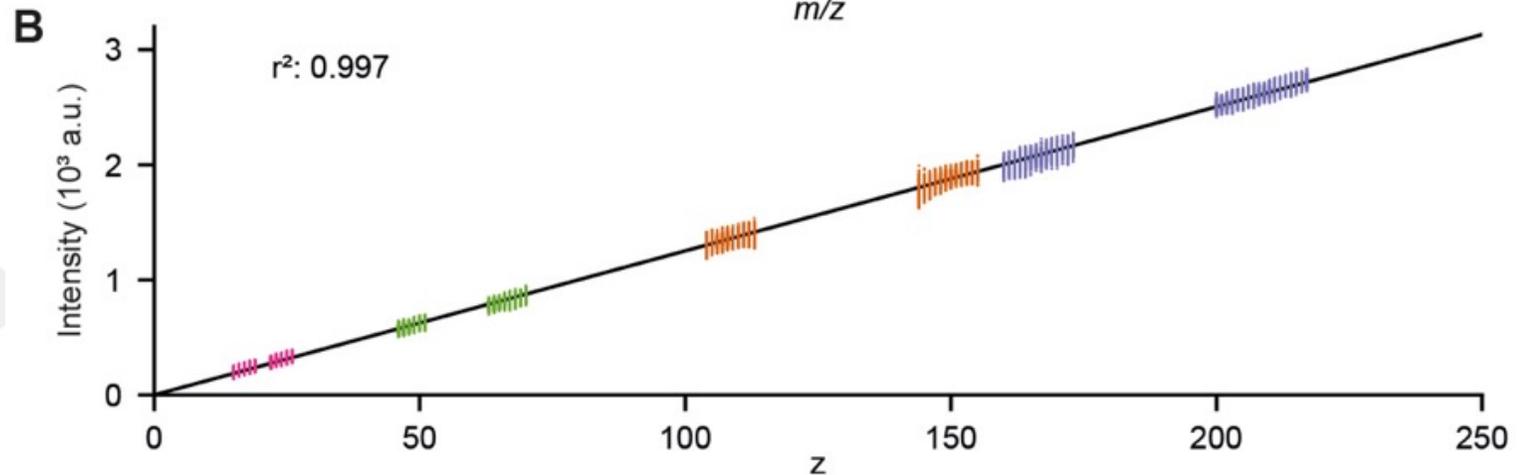
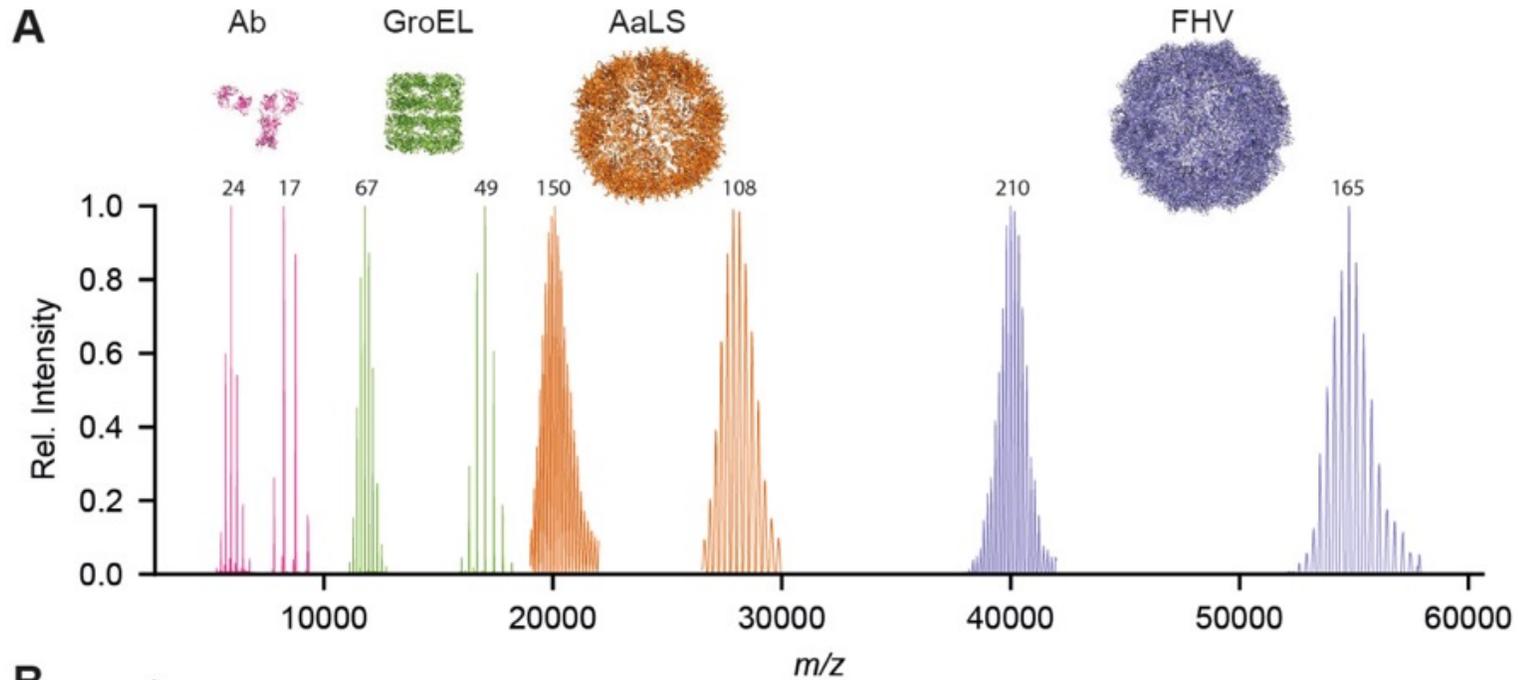
# Single ion detection of Flock house virus (FHV)



# Single ion detection of Flock house virus (FHV)



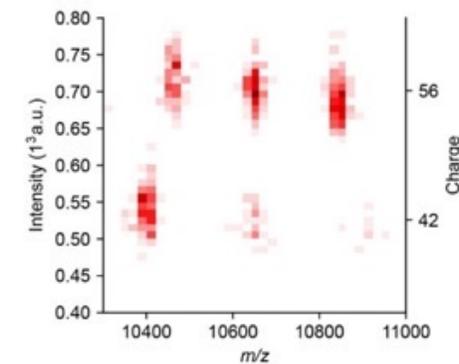
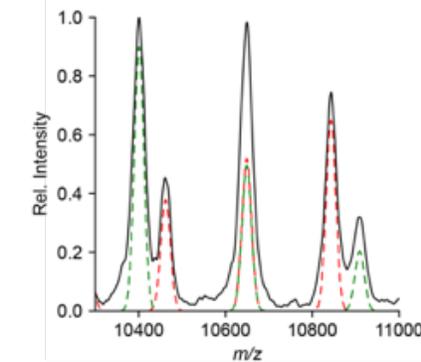
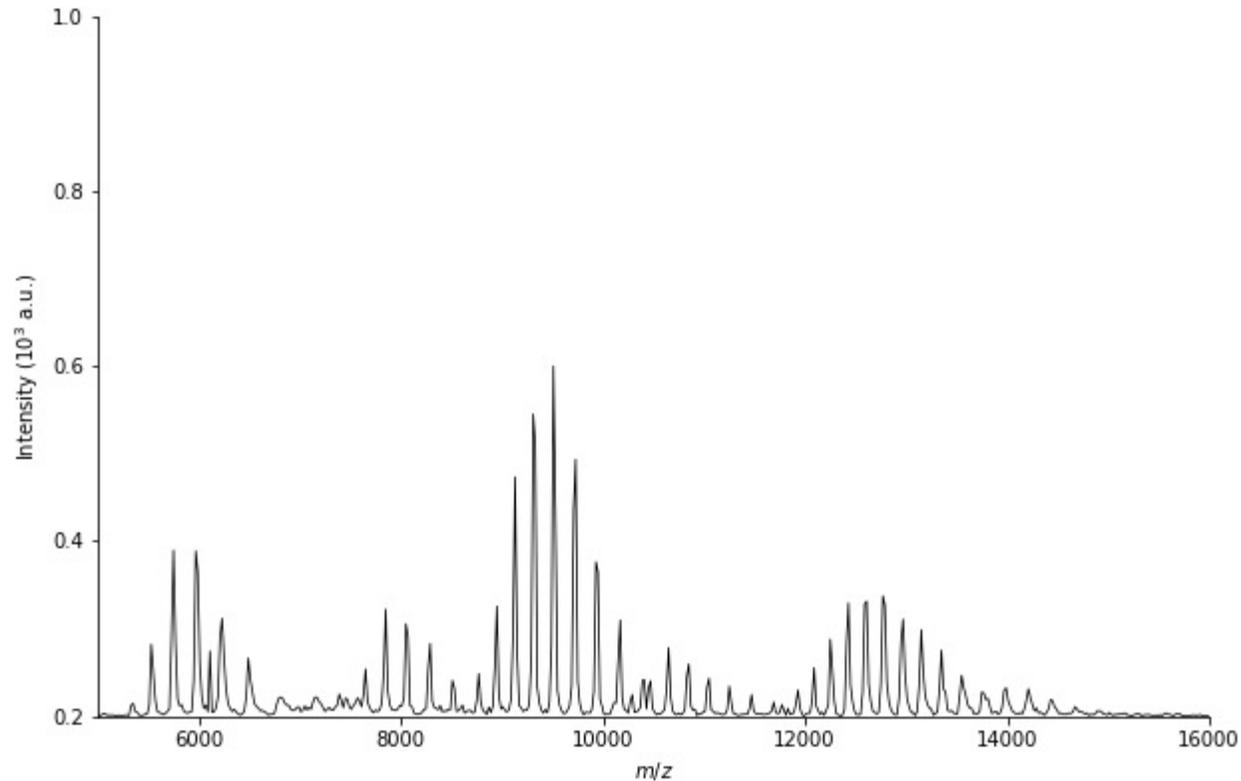
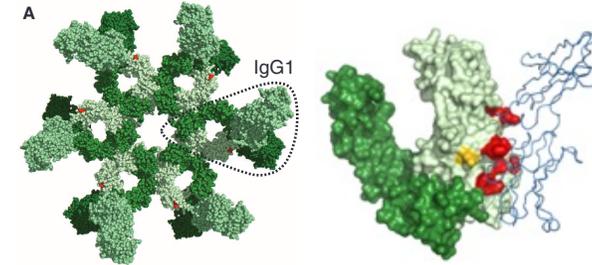
# Single ion charge detection mass spectrometry



*Single ion intensities scale linearly with charge state*

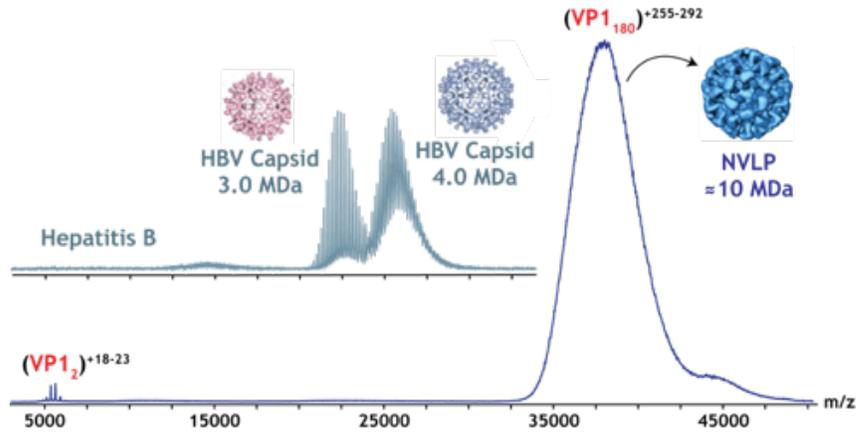
# Single ion charge detection mass spectrometry

Resolving in the intensity dimension



Resolving a complex mixture of **IgG** oligomers using single ion intensities

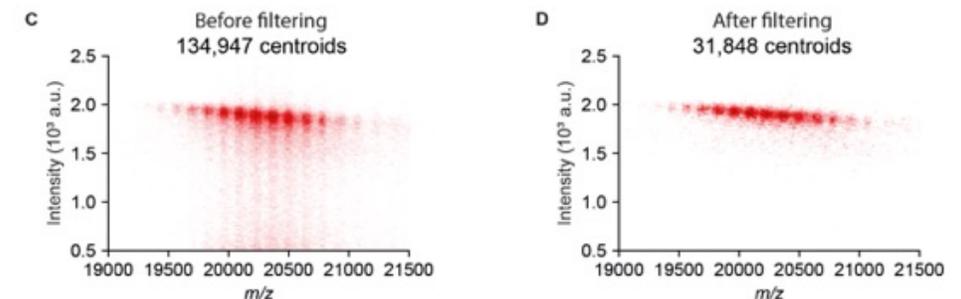
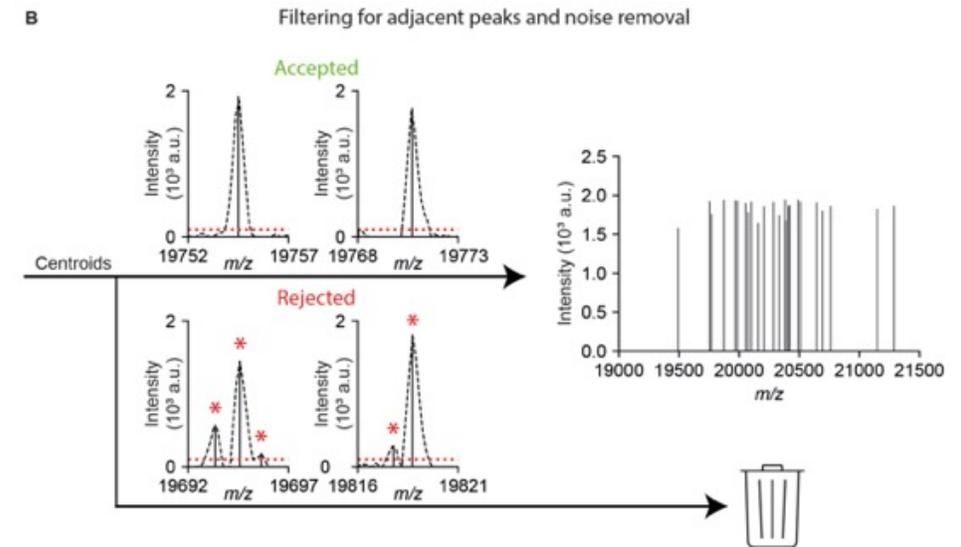
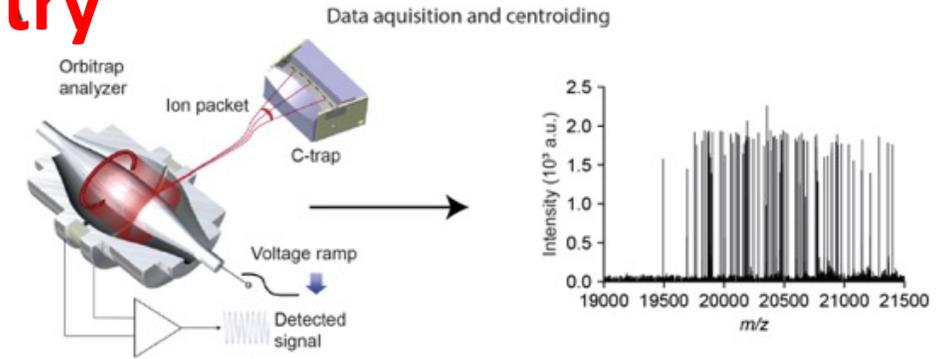
# Single ion charge detection mass spectrometry



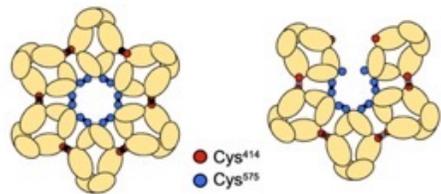
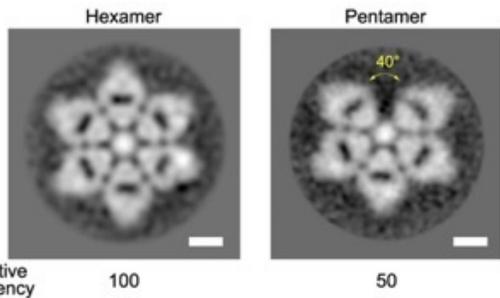
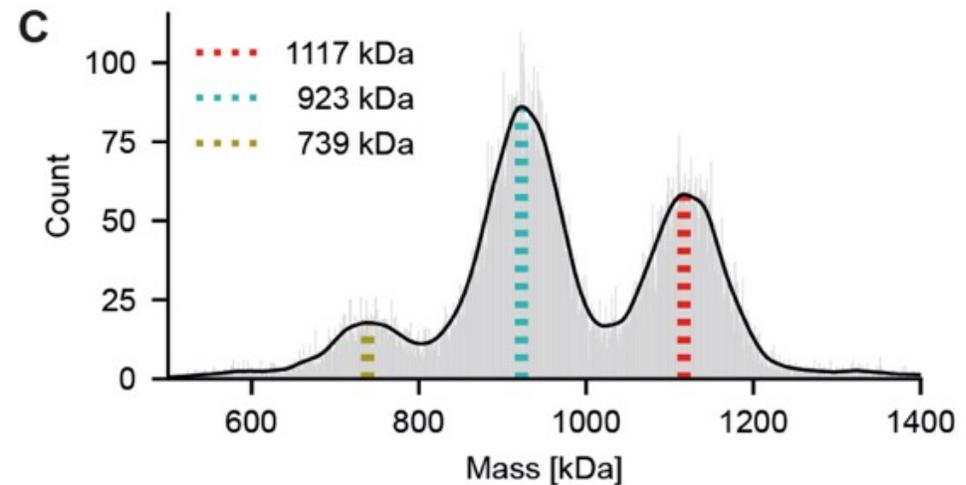
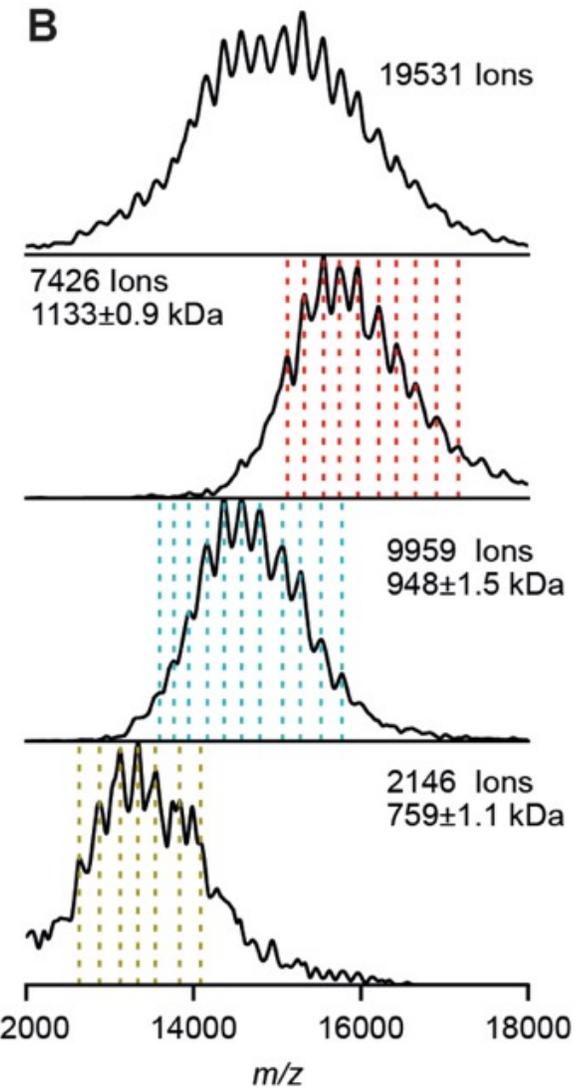
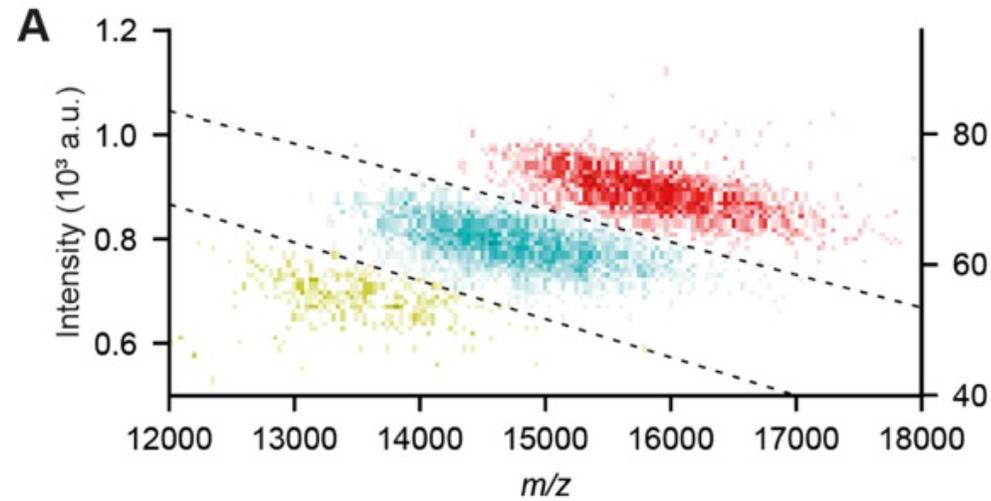
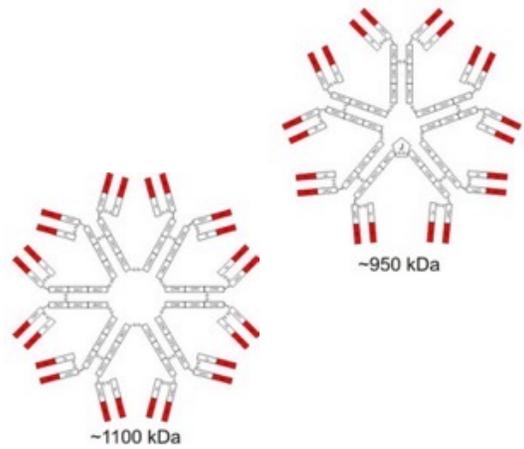
When charge states not resolved,  
hard to determine Mw

General workflow for single particle pre-processing

Wörner T, et al. *Nature Methods* (2021)



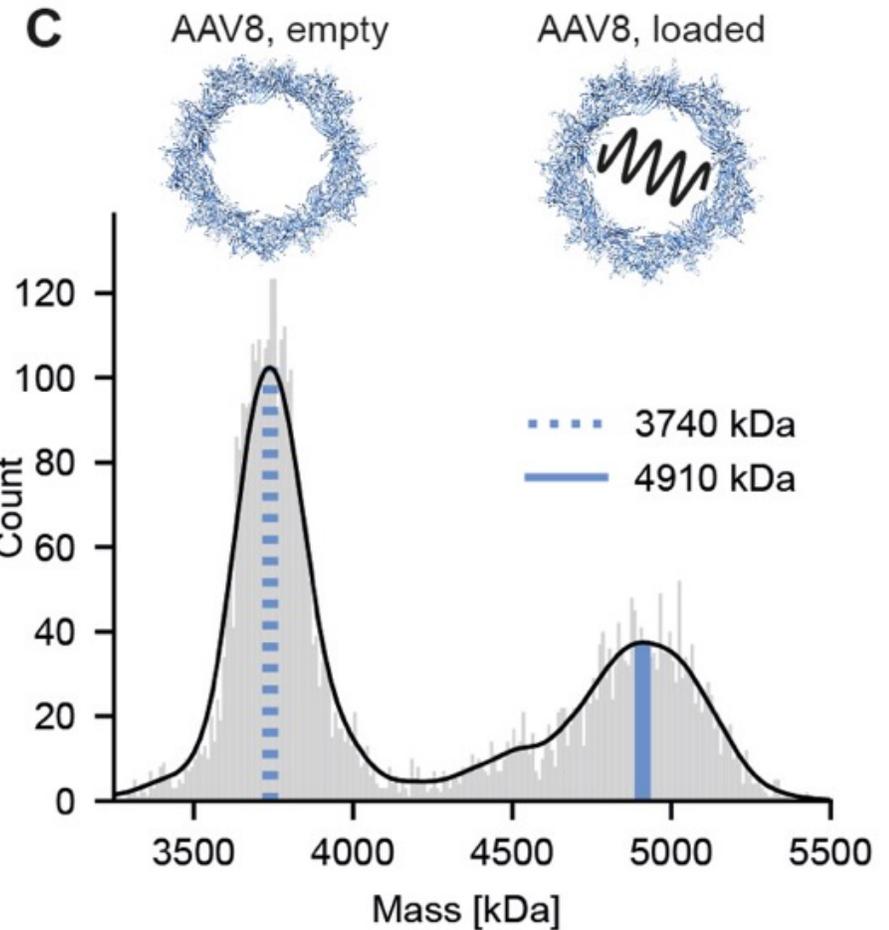
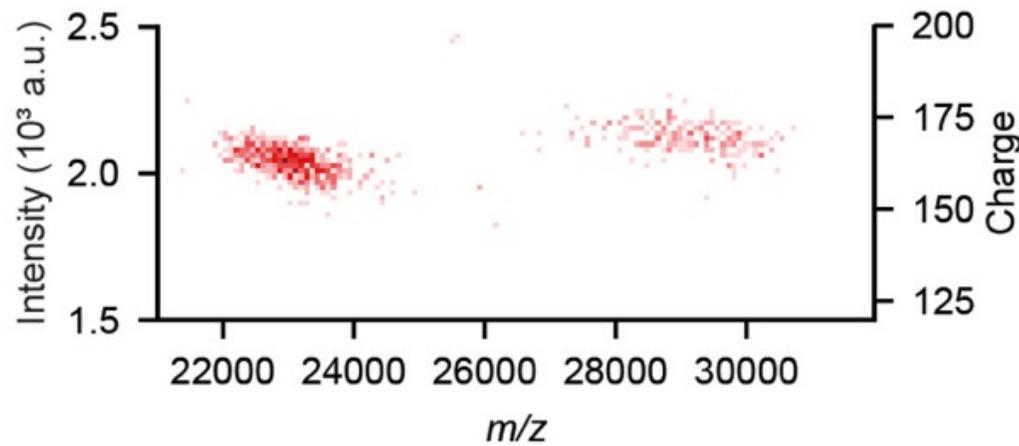
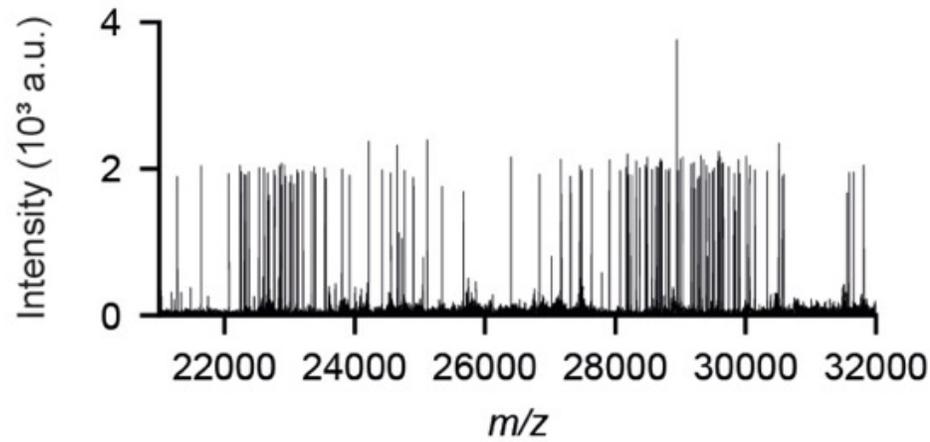
# Single ion charge detection mass spectrometry



Science Advances 4 (2018)  
DOI: 10.1126/sciadv.aau1199

Resolving a complex mixture of **IgM** oligomers using single ion intensities

# Single ion charge detection mass spectrometry

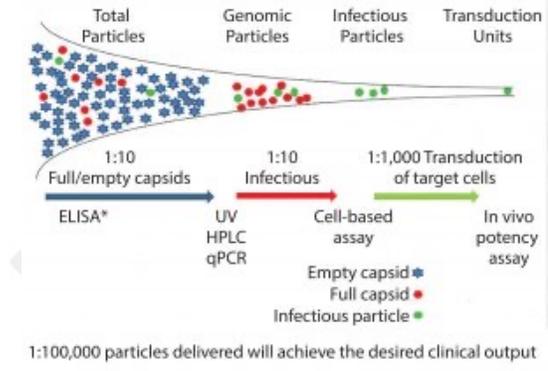
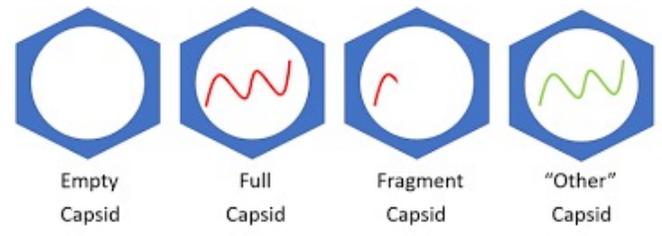
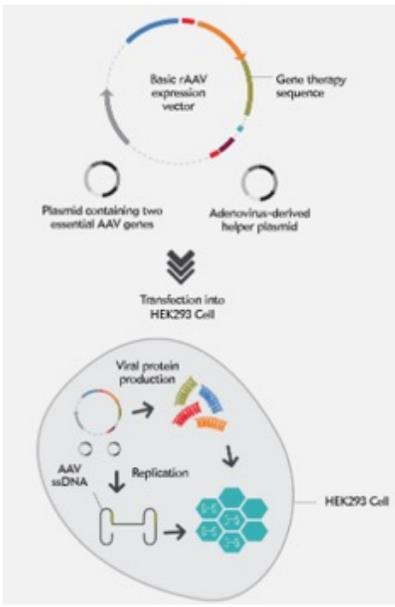


\$2 million

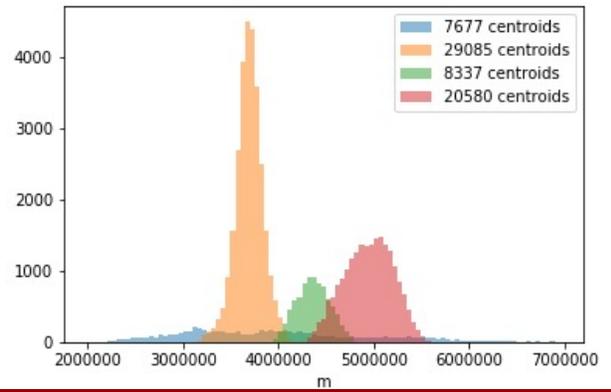
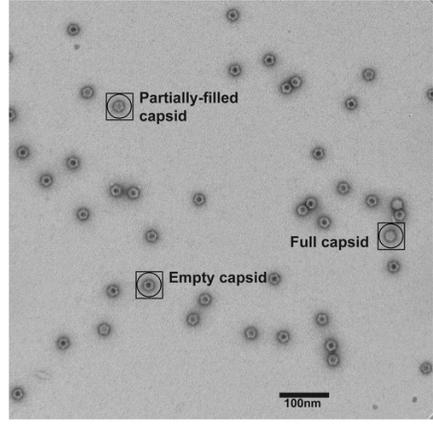
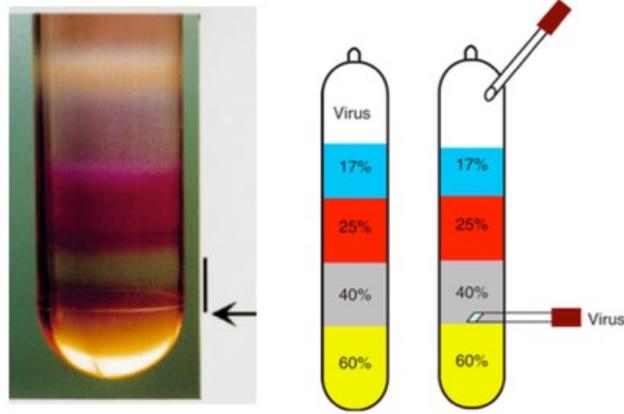
Single particle CD-MS of co-occurring empty and genome loaded AAV particles

# Production of AAV gene delivery particles

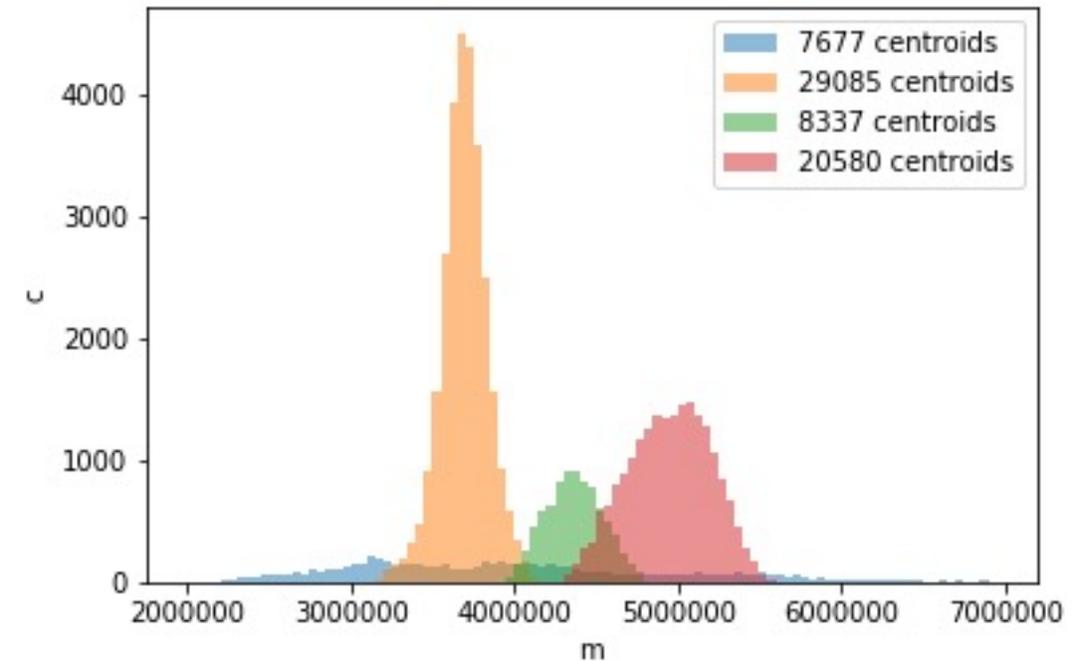
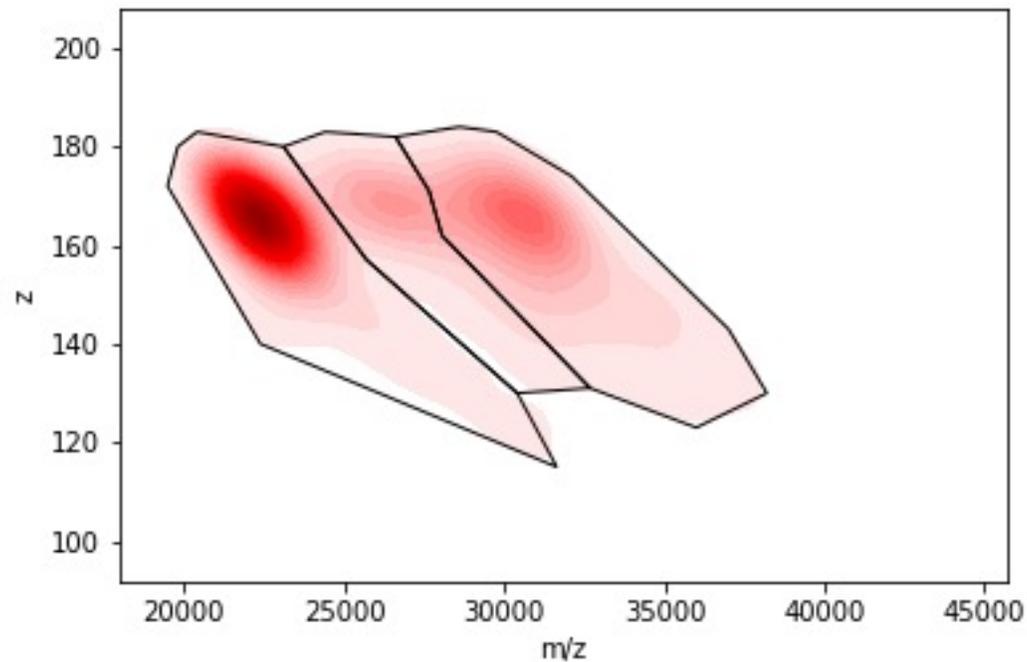
Is rather difficult



Sucrose gradient  
EM  
CD-MS



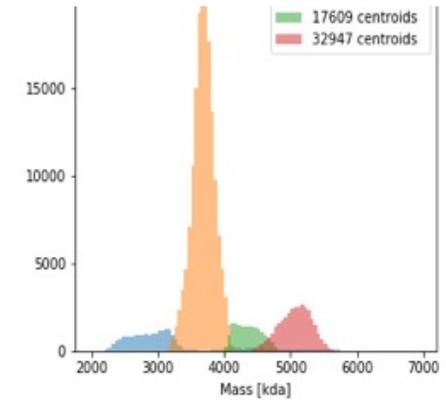
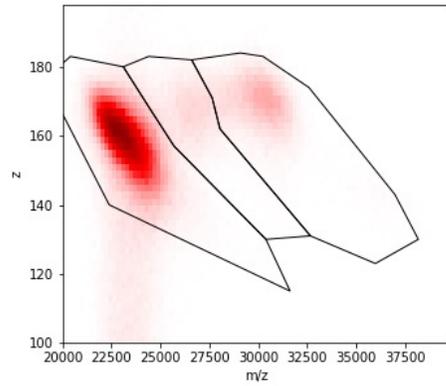
# Quantitative CD-MS analysis of AAV gene delivery particles



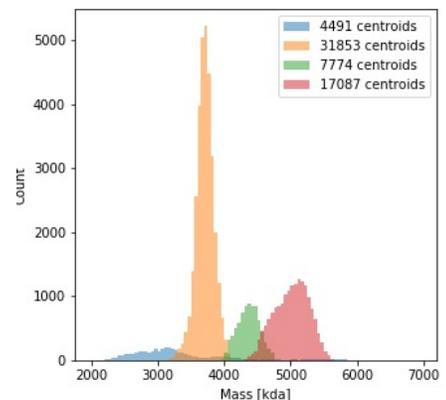
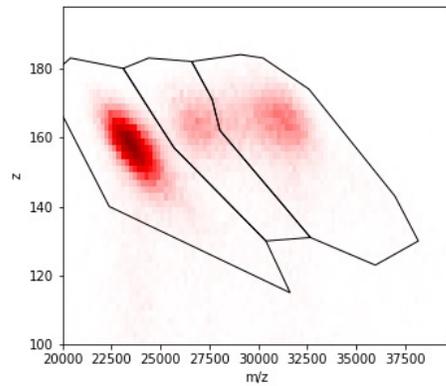
Data selection/clustering in 2D  
Can be done manually or automated

# Quantitative CD-MS analysis of AAV gene delivery particles

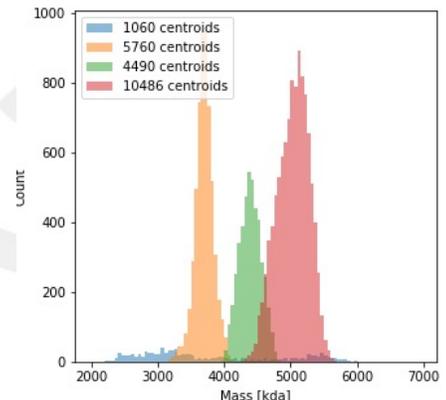
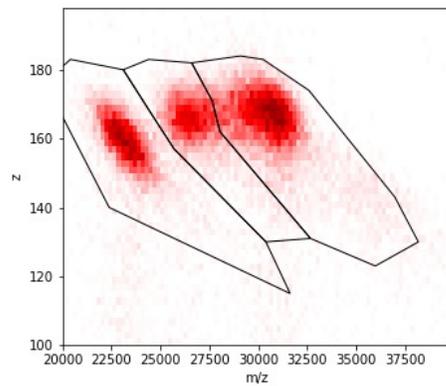
25% Full



50% Full



75% Full



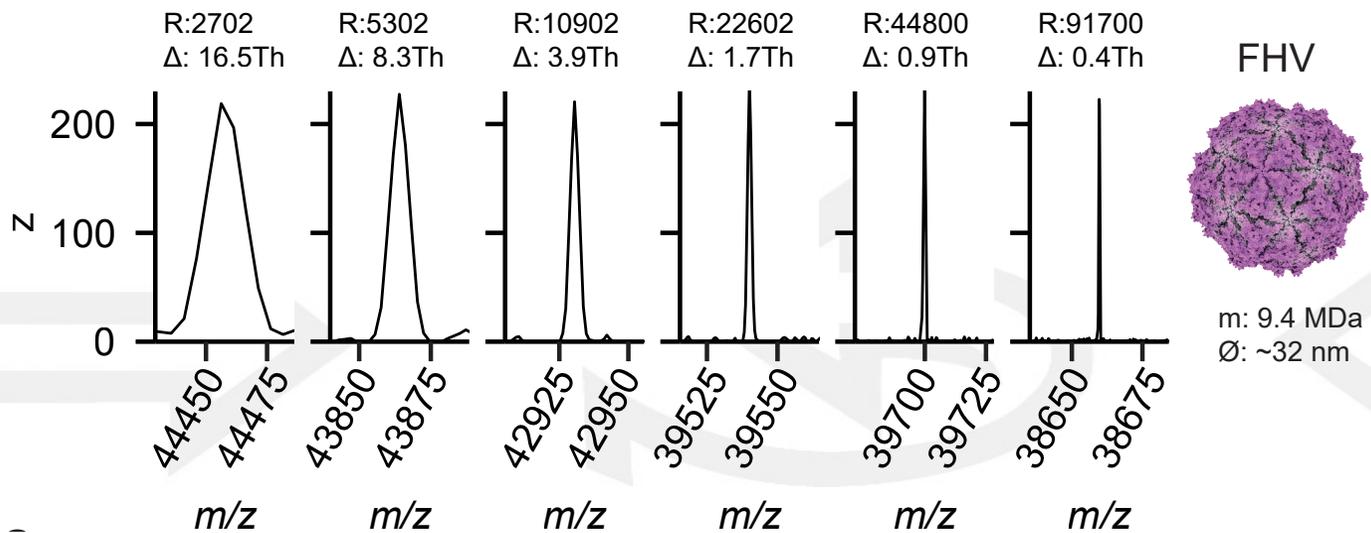
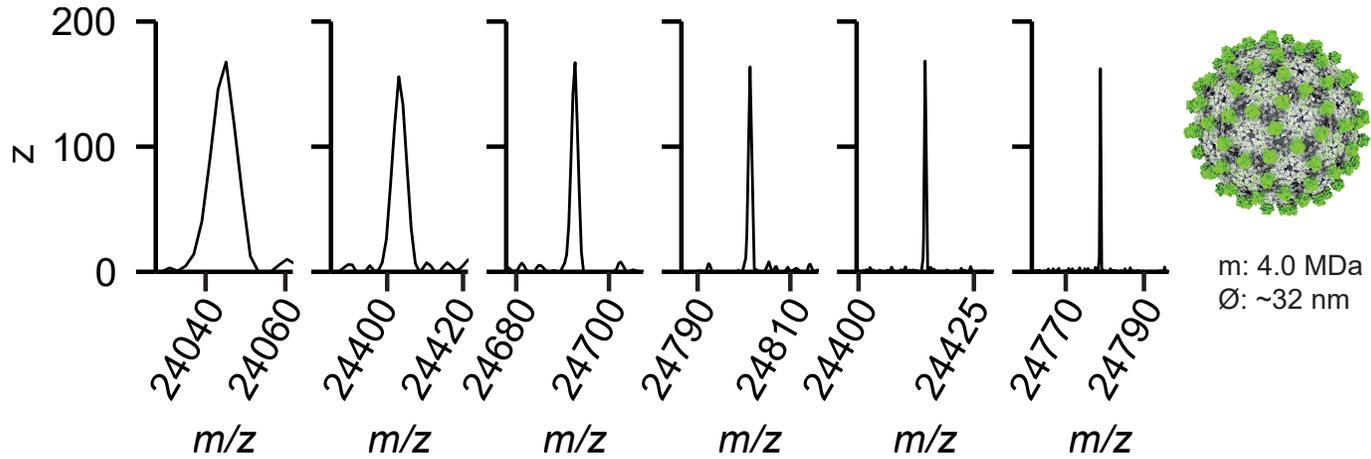
How accurate/reproducible is the measure?

Sample	
25% full	23±1.2
50% full	45±2.8
75% full	71.5±0.7

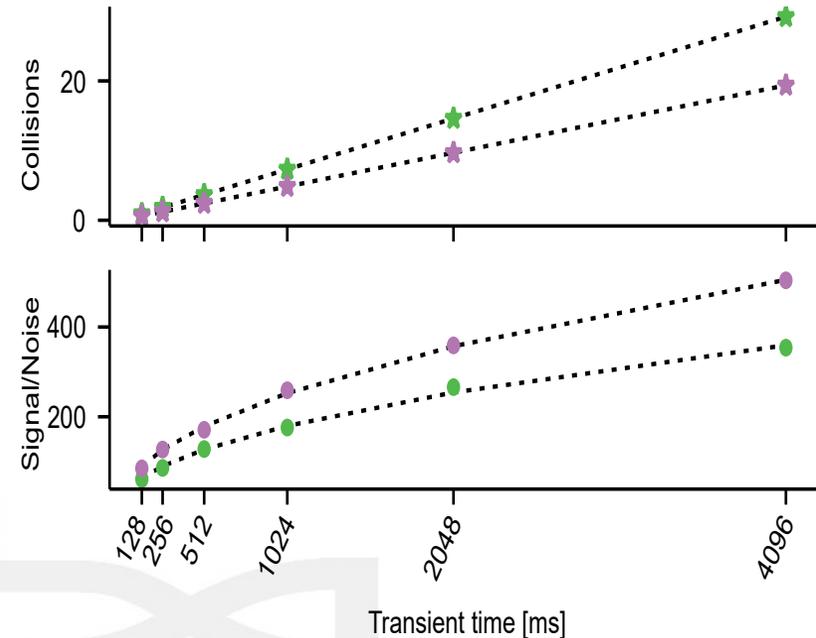
Empty: 3,702 kDa  
 Half-full: 4,393 kDa (691 kDa, 2.28 kb genome)  
 Full: 5,103 kDa (1,401 kDa, 4.62 kb genome)

Sample	Full	Half-full	Empty
25% full	15.0	8.0	77
50% full	30.9	14.1	55
75% full	50.1	21.4	28.5

# Single particle analysis enables super-resolution

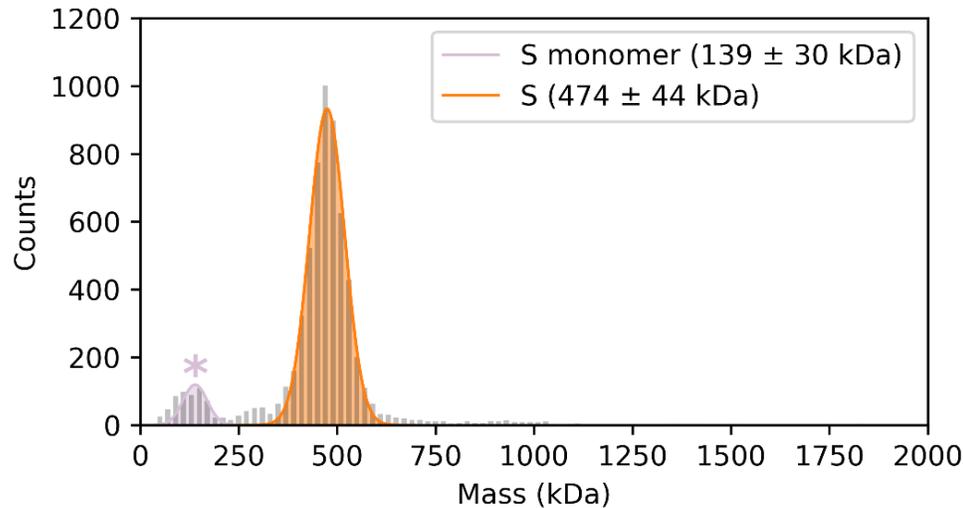


Long transients, better S/N  
 High mass ions very stable  
 No isotopes  
 Super-resolution

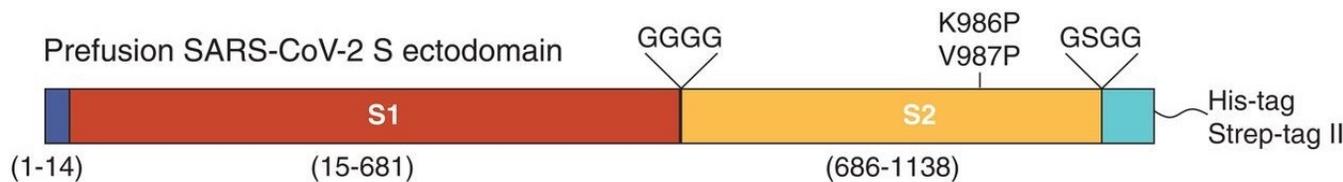
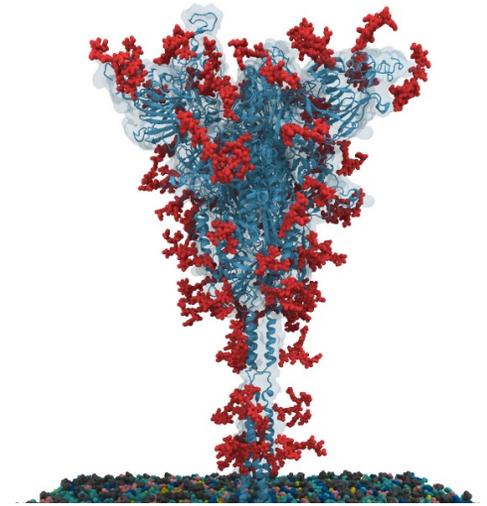
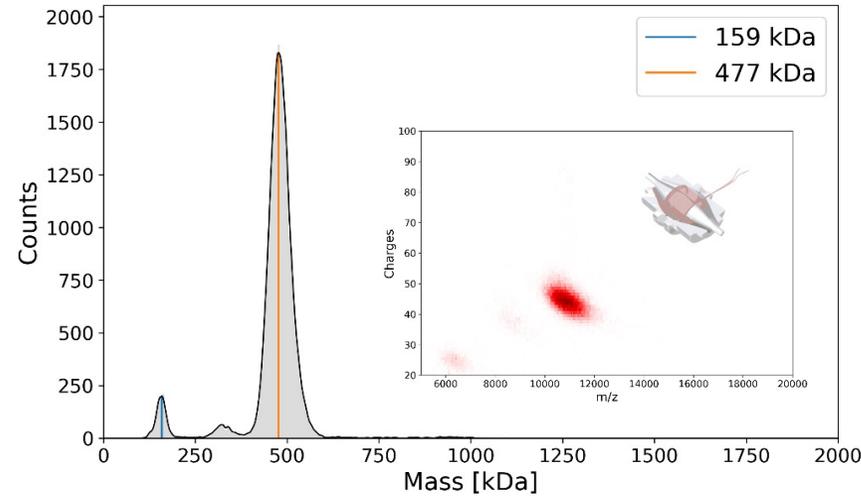


# Mass analysis of the S-trimer by Single Particle Methods

Mass Photometry



Orbitrap CD-MS



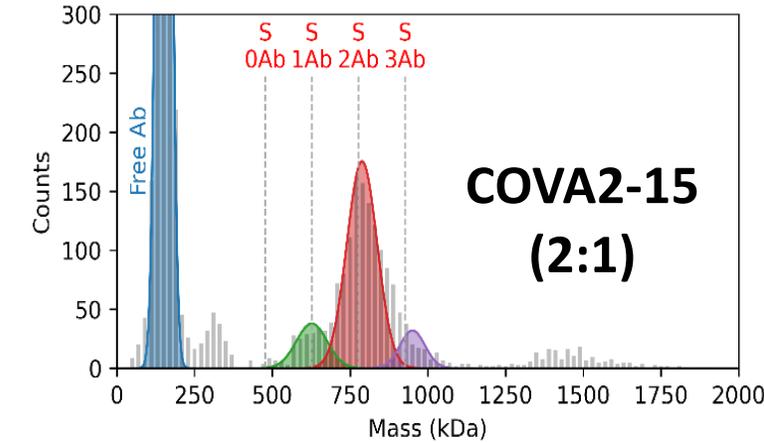
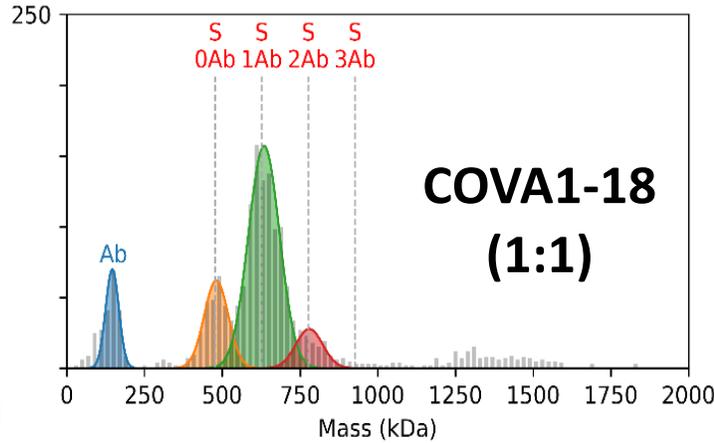
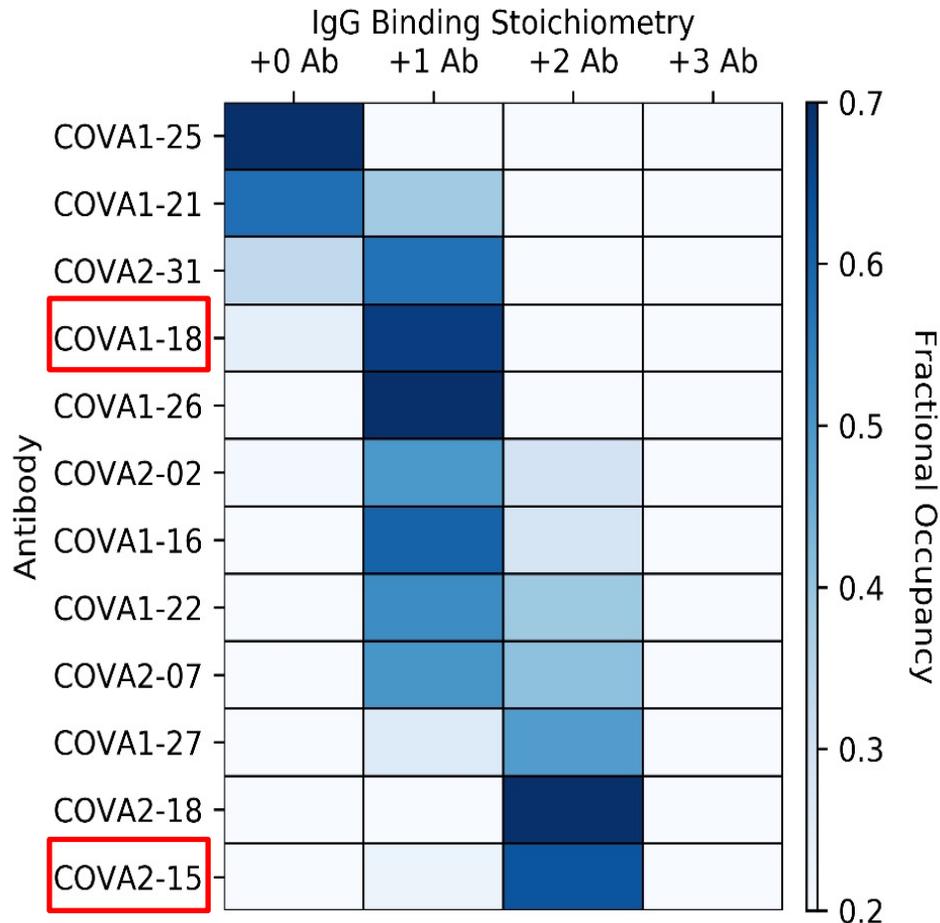
~30% of the S-trimer mass are glycans!

Sequence MW = 385 kDa  
Measured Mass = 475 kDa

Yin... Heck, *ACS Cent. Sci.* 2021.  
Brouwer... van Gils, *Science.* 2020.

# Binding of monoclonal anti-SARS-CoV-2 IgGs to S

COVA2-15 and COVA1-18



**Identical neutralization potencies ( $IC_{50} \sim 0.008 \mu\text{g/mL}$ ) yet very different stoichiometries**

Potential as Biotherapeutics

ARTICLE

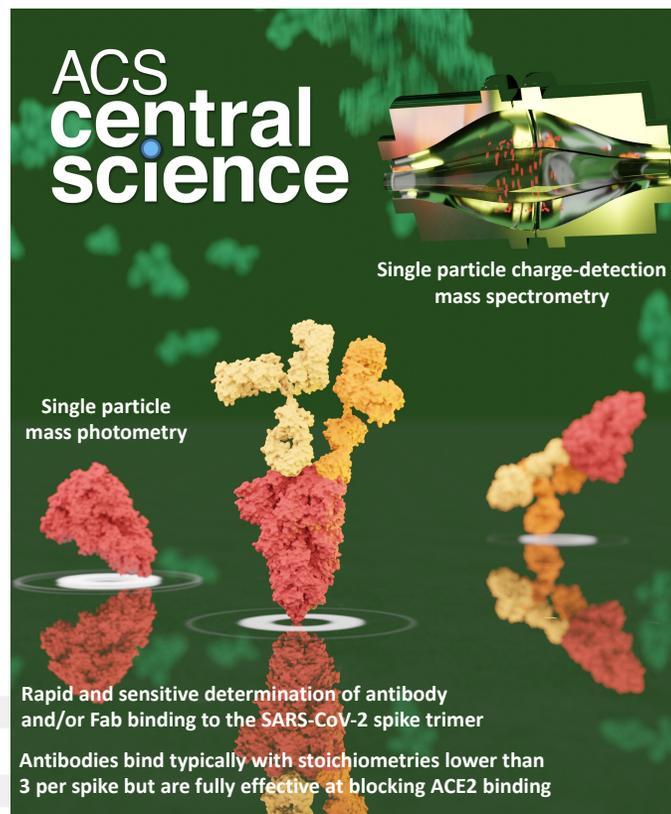
<https://doi.org/10.1038/s41467-021-26354-0>

OPEN

COVA1-18 neutralizing antibody protects against SARS-CoV-2 in three preclinical models

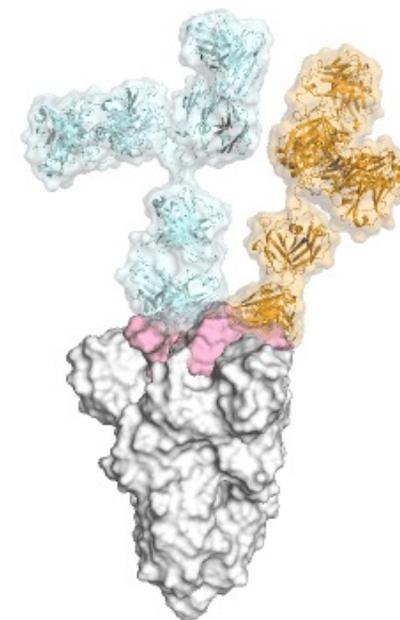
# Weighing in on SARS-CoV-2 Spike / Antibody Interactions

Sizing and counting Sars-Cov-2 spike trimer particles bound to antibodies



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<http://pubs.acs.org/journal/acscii>



Research Article

## Probing Affinity, Avidity, Anticooperativity, and Competition in Antibody and Receptor Binding to the SARS-CoV-2 Spike by Single Particle Mass Analyses

Victor Yin,<sup>▽</sup> Szu-Hsueh Lai,<sup>▽</sup> Tom G. Caniels, Philip J. M. Brouwer, Mitch Brinkkemper, Yoann Aldon, Hejun Liu, Meng Yuan, Ian A. Wilson, Rogier W. Sanders, Marit J. van Gils, and Albert J. R. Heck\*

Cite This: *ACS Cent. Sci.* 2021, 7, 1863–1873

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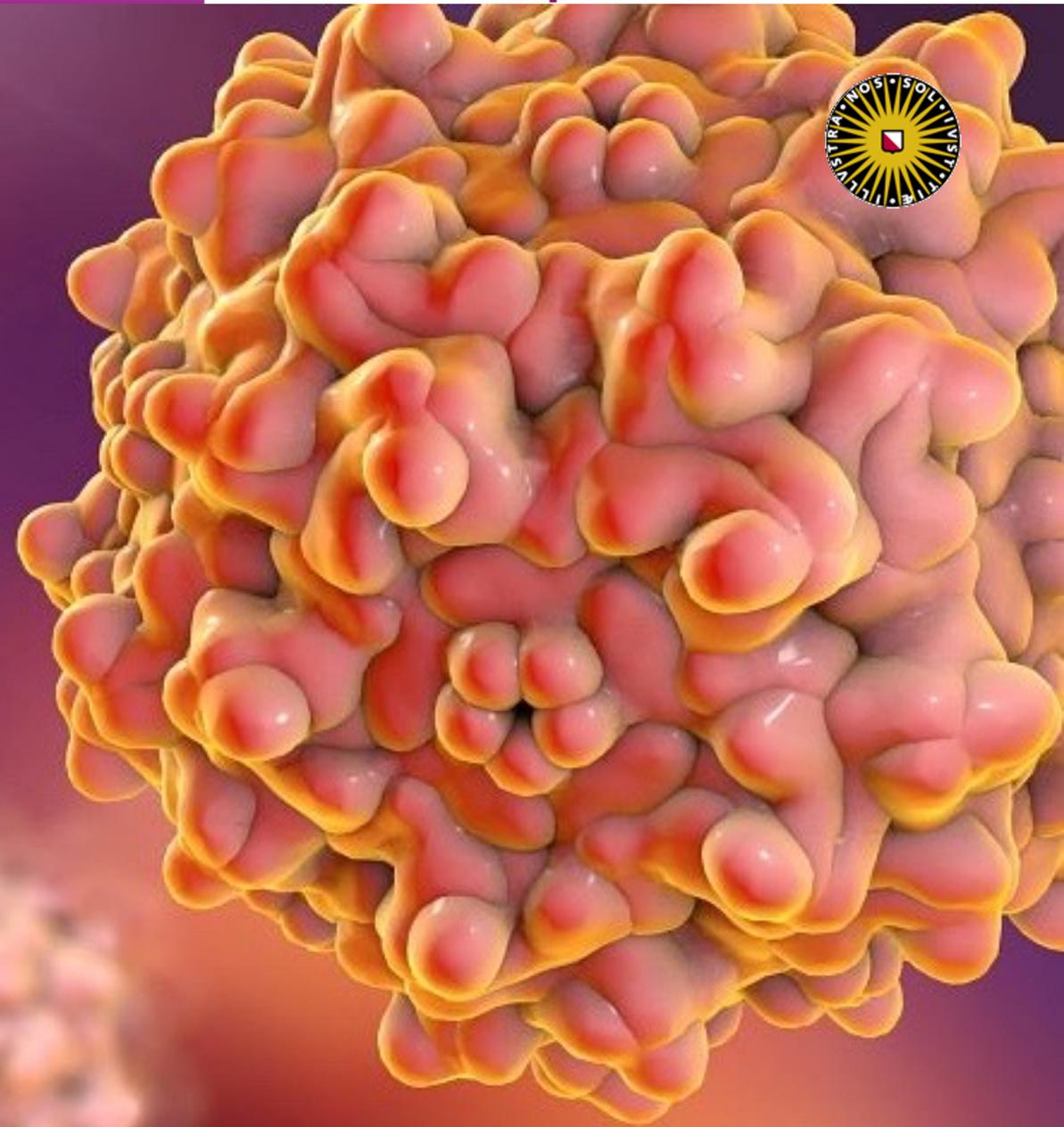


Next generation biopharmaceuticals pose new analytical challenges

High-resolution native mass spectrometry provides in-depth structural details on such protein assemblies

Single-particle charge detection mass spectrometry provides unique sensitivity

Viruses, Vaccines, but also highly decorated glycoproteins





# Pittcon 2022 Wallace H. Coulter Lecture

## New Horizons in Mass Spectrometry

- I. Sizing and Counting Particles by Native Mass Spectrometry
- II. How unique is your immune response?**  
Monitoring personalized antibody repertoires
- III. Science meets art

Albert J.R. Heck

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Utrecht University  
[www.hecklab.com](http://www.hecklab.com)

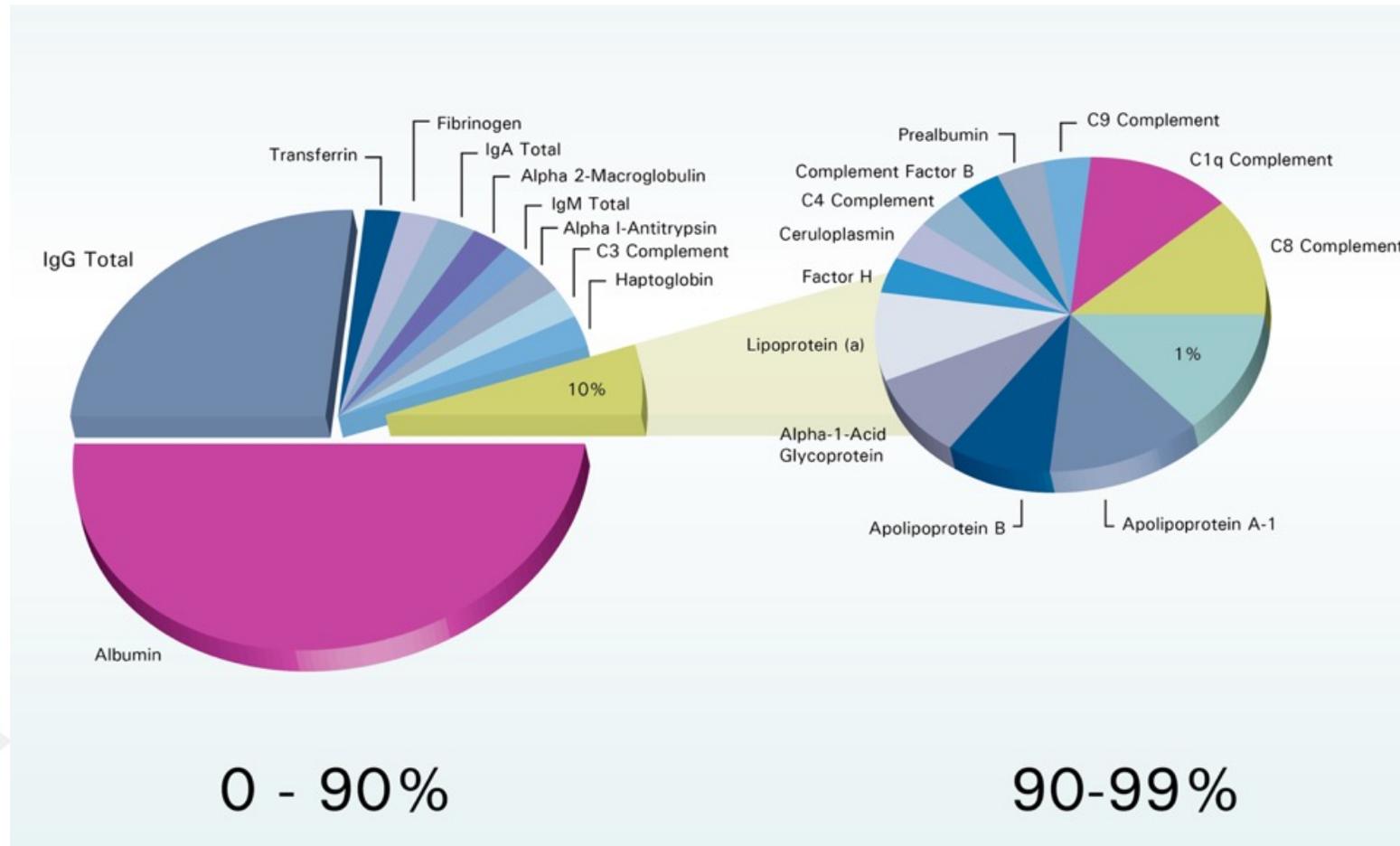




**A Human Source of Antibodies**

# The Human Plasma Proteome and IgG1 repertoire

A direct view at the individuals' plasma antibody repertoire by mass spectrometry

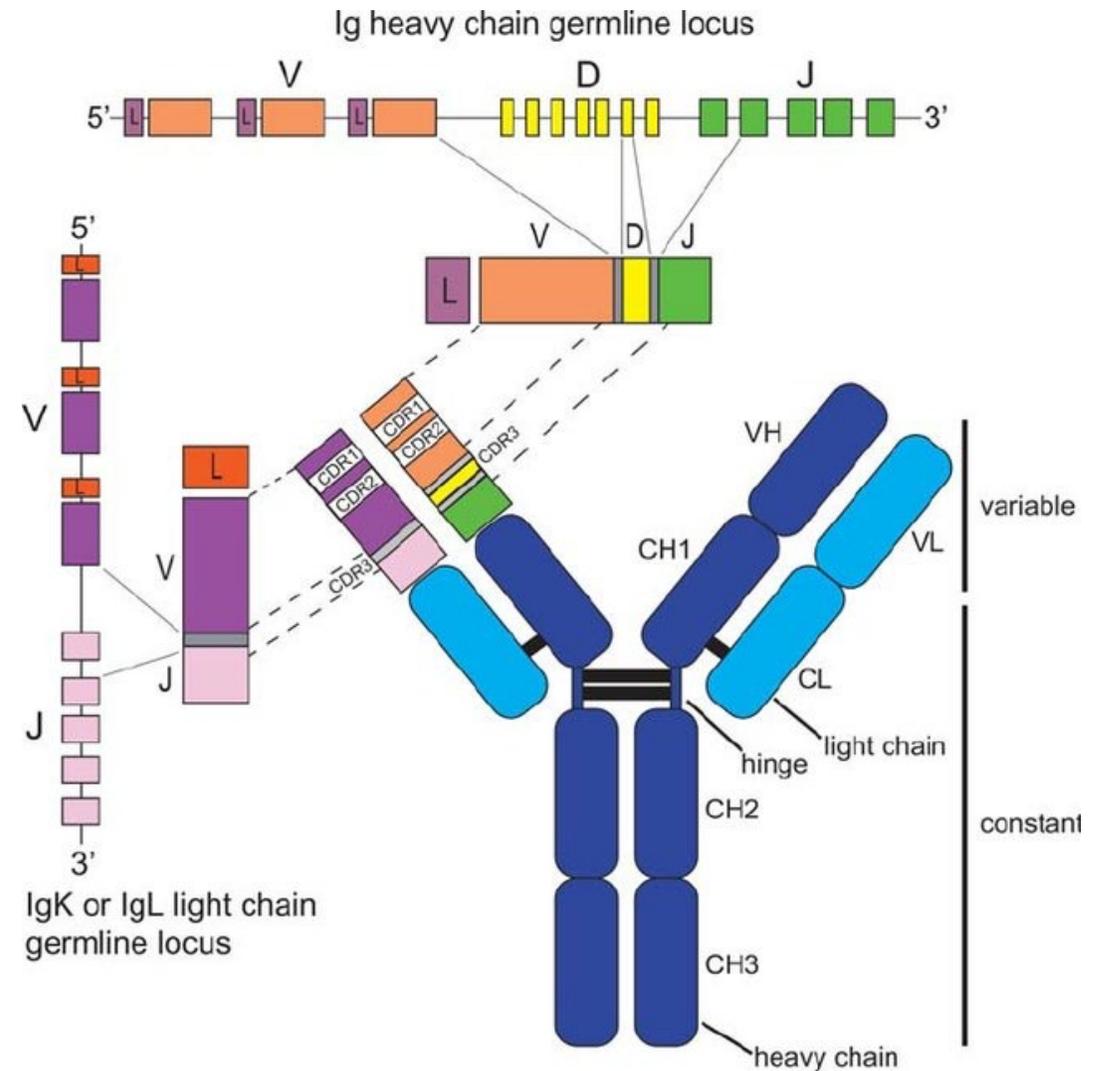


Major part of the proteins in plasma are IgGs. Are they representing a unique profile for individuals?

# The Human Plasma IgG1 repertoire

The human immune system creates billions of different antibodies using a limited number of genes by rearranging DNA segments during B cell development

Further mutations can also increase genetic variation in antibodies

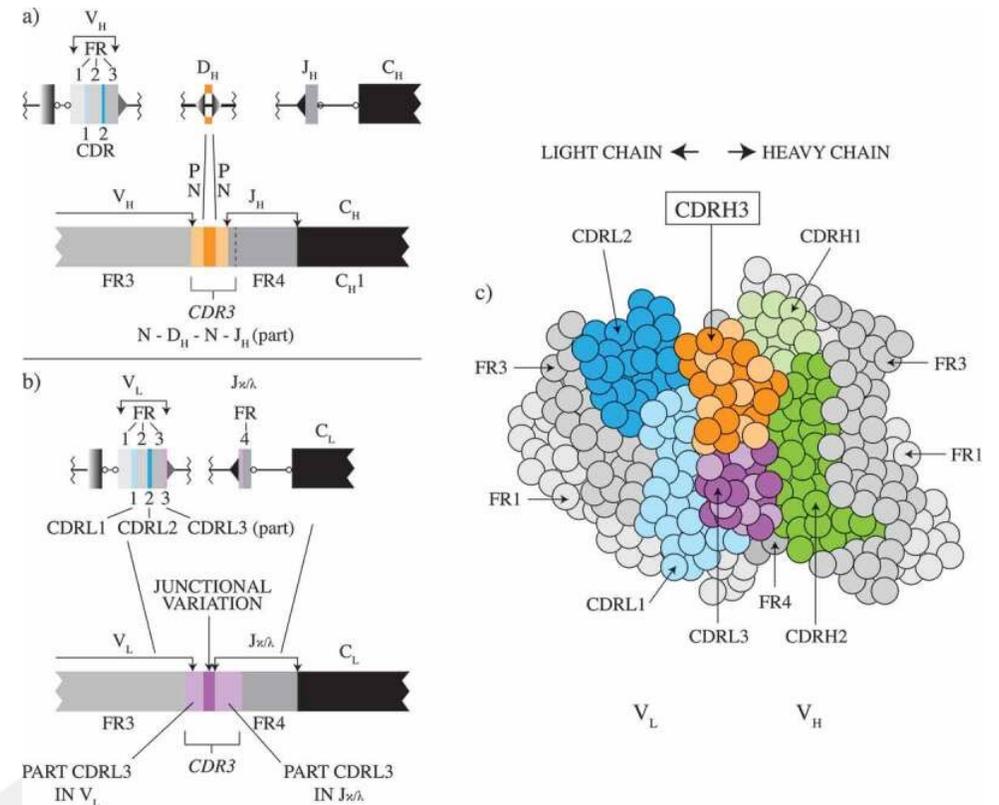


# The Human Plasma IgG1 repertoire

The human antibody repertoire size  
 $\sim 10^{15}$  members for the naïve repertoire  
 $\sim 10^{18}$  based on combinatorial calculations

$\sim 10^{13}$  total number of cells in the body  
 $\sim 10^{11}$  total number of B cells in the body  
 $\sim 10^9$  circulating peripheral naïve mature B-cells

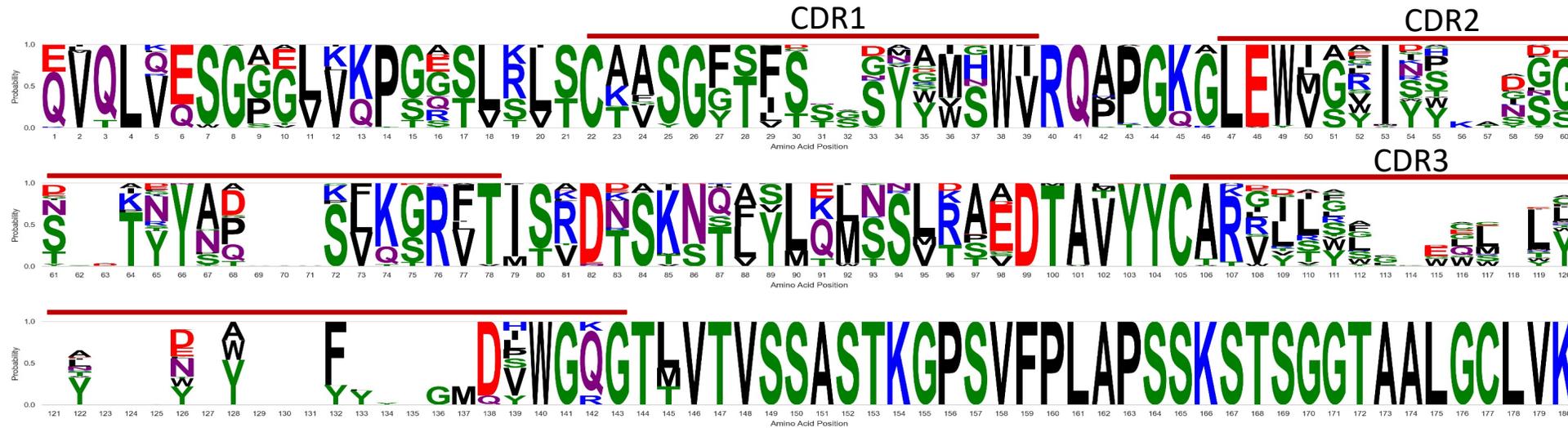
But how many different antibodies are really present in plasma at a given moment?



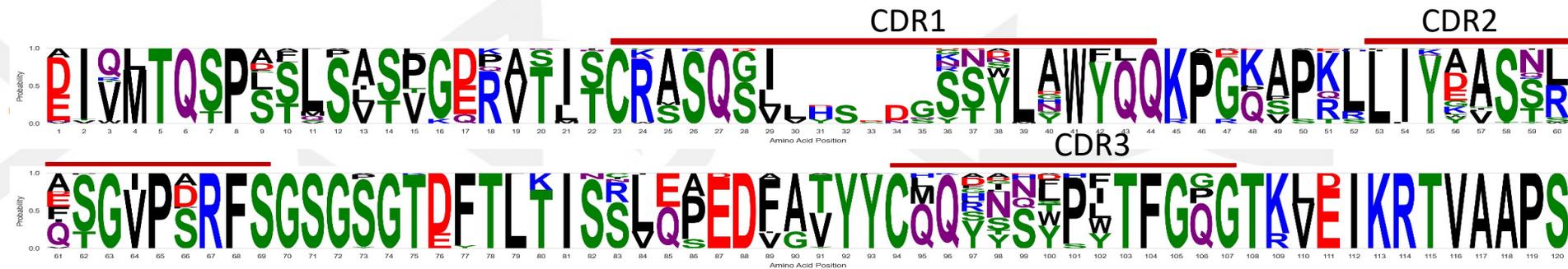
Understanding the human antibody repertoire  
 A R Rees *MAbs* 12 (2020) 1729683

# How variable are IgG1 molecules ?

Immunoglobulin G1 heavy chain consensus based on IMGT

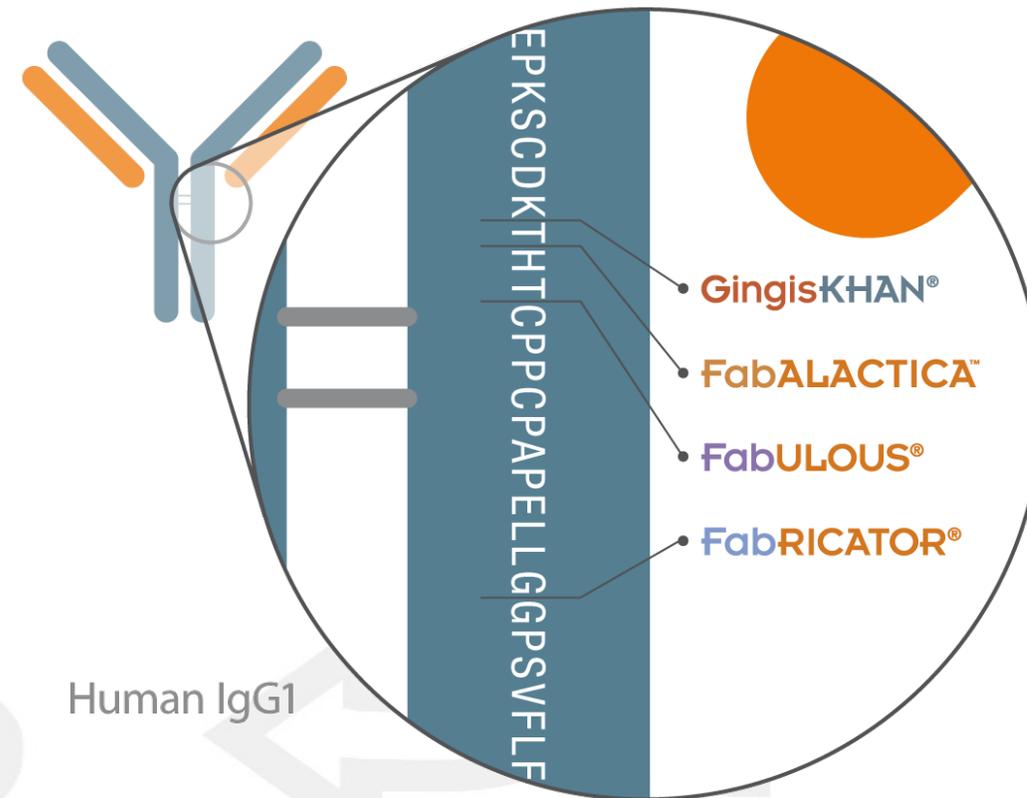


■ Immunoglobulin kappa light chain (IGK) based on IMGT

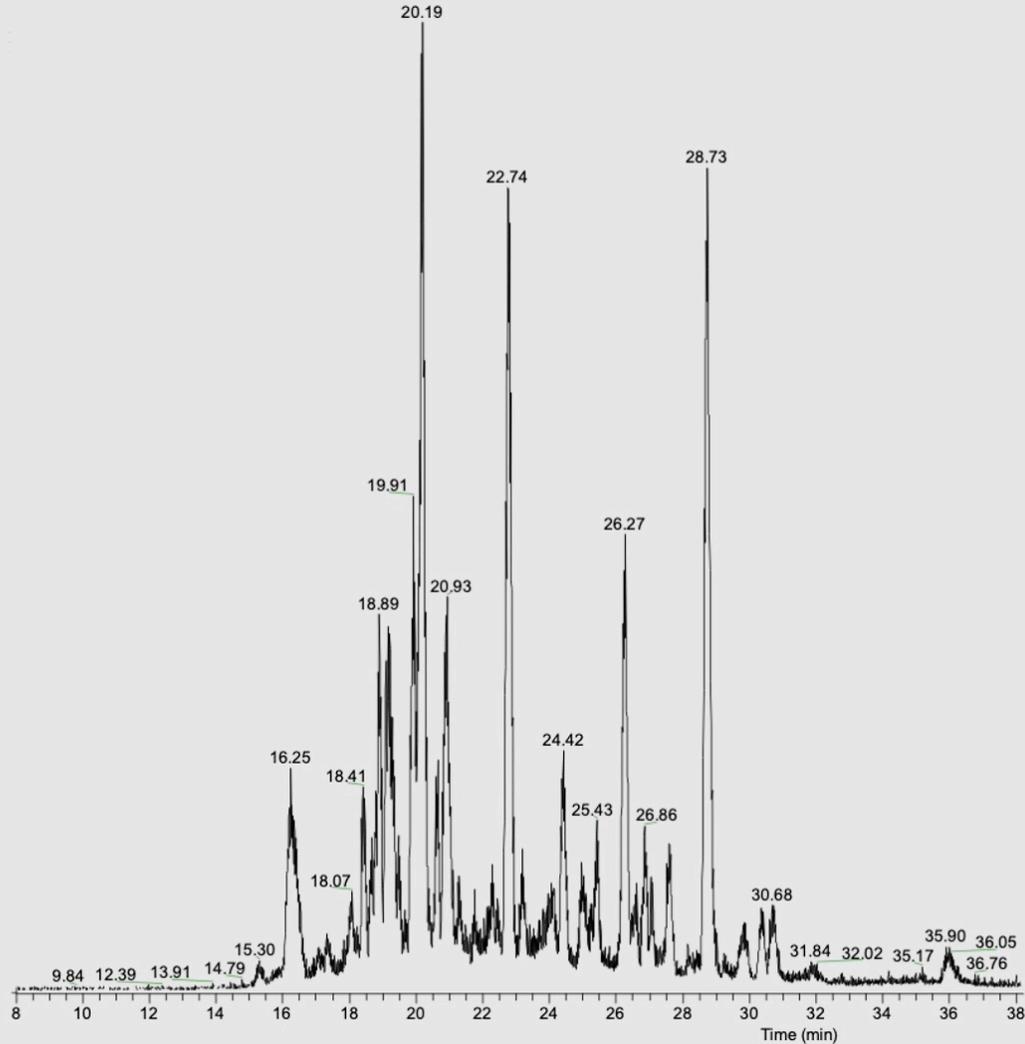


# Capture and Analysis of the Human Plasma IgG1 repertoire

- Capture IgG
- Use bacterial protease cleaving above hinge
- Collect Fab
- Perform intact mass LC-MS
- Deconvolute mass spectra



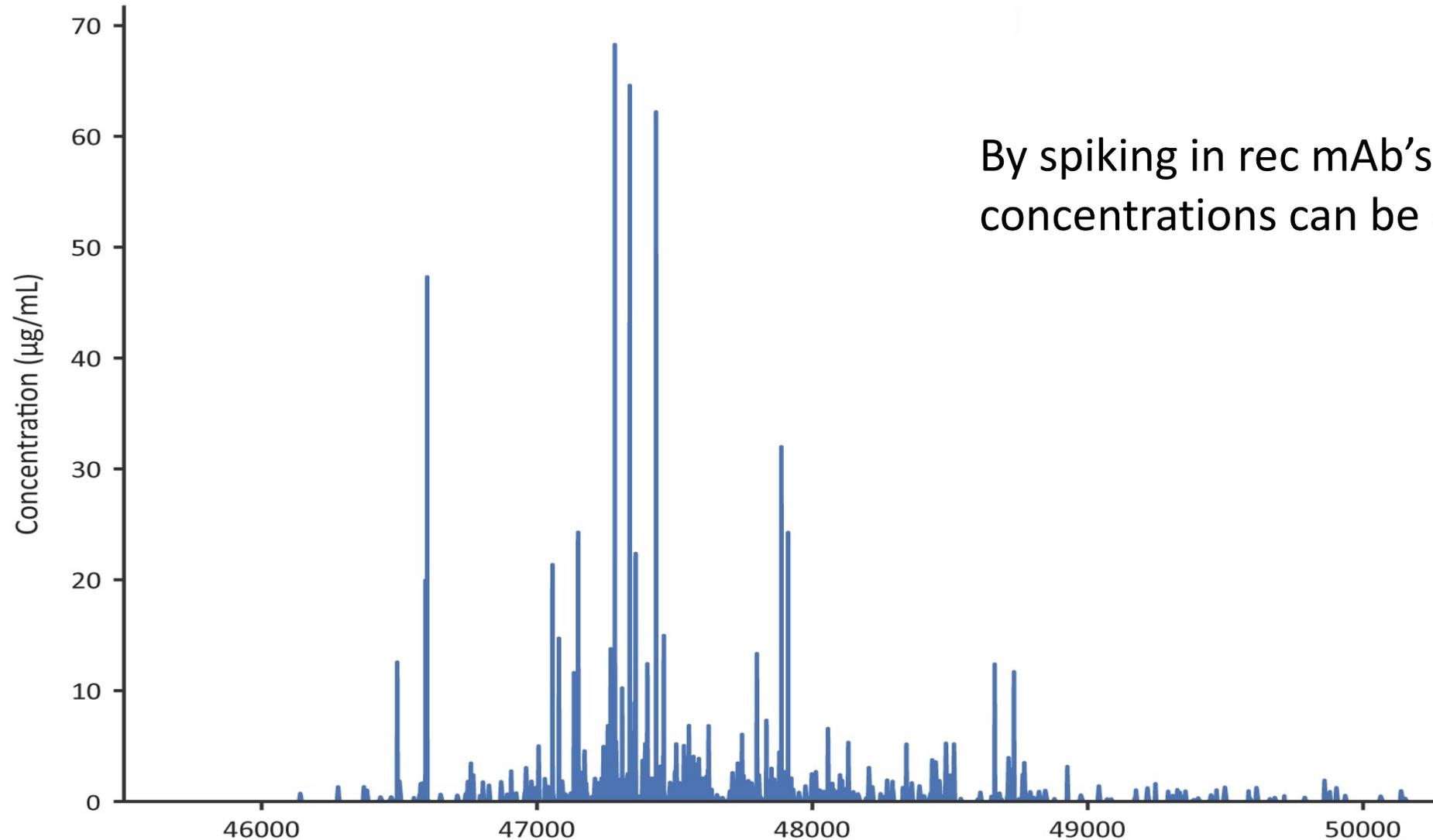
# A Human Plasma IgG1 repertoire



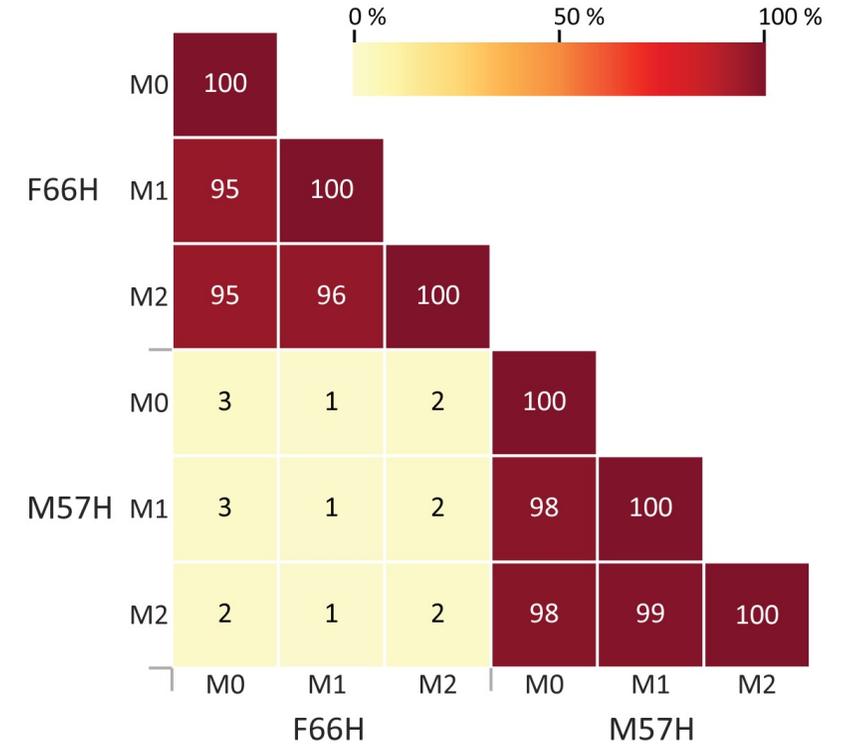
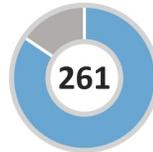
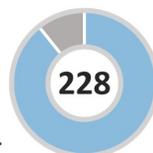
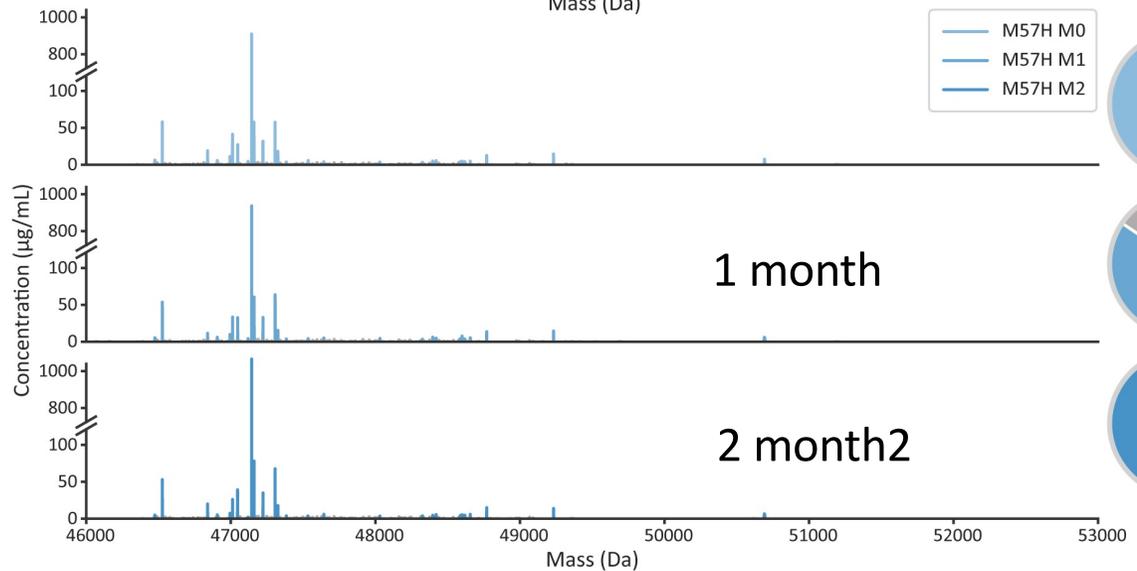
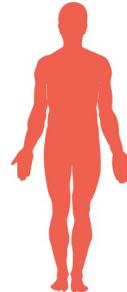
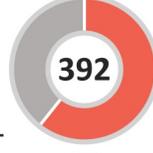
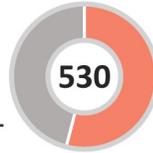
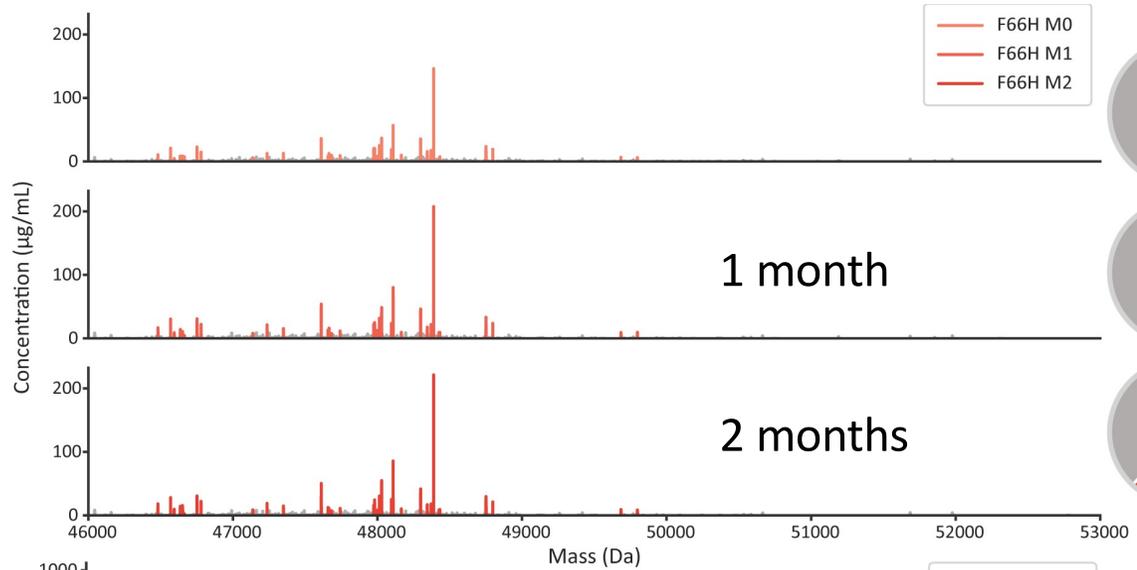
Each clone can be typed by  $^{RT}Clone_{Mw}$

The IgG1 clonal repertoire is simple

# A Human Plasma IgG1 repertoire

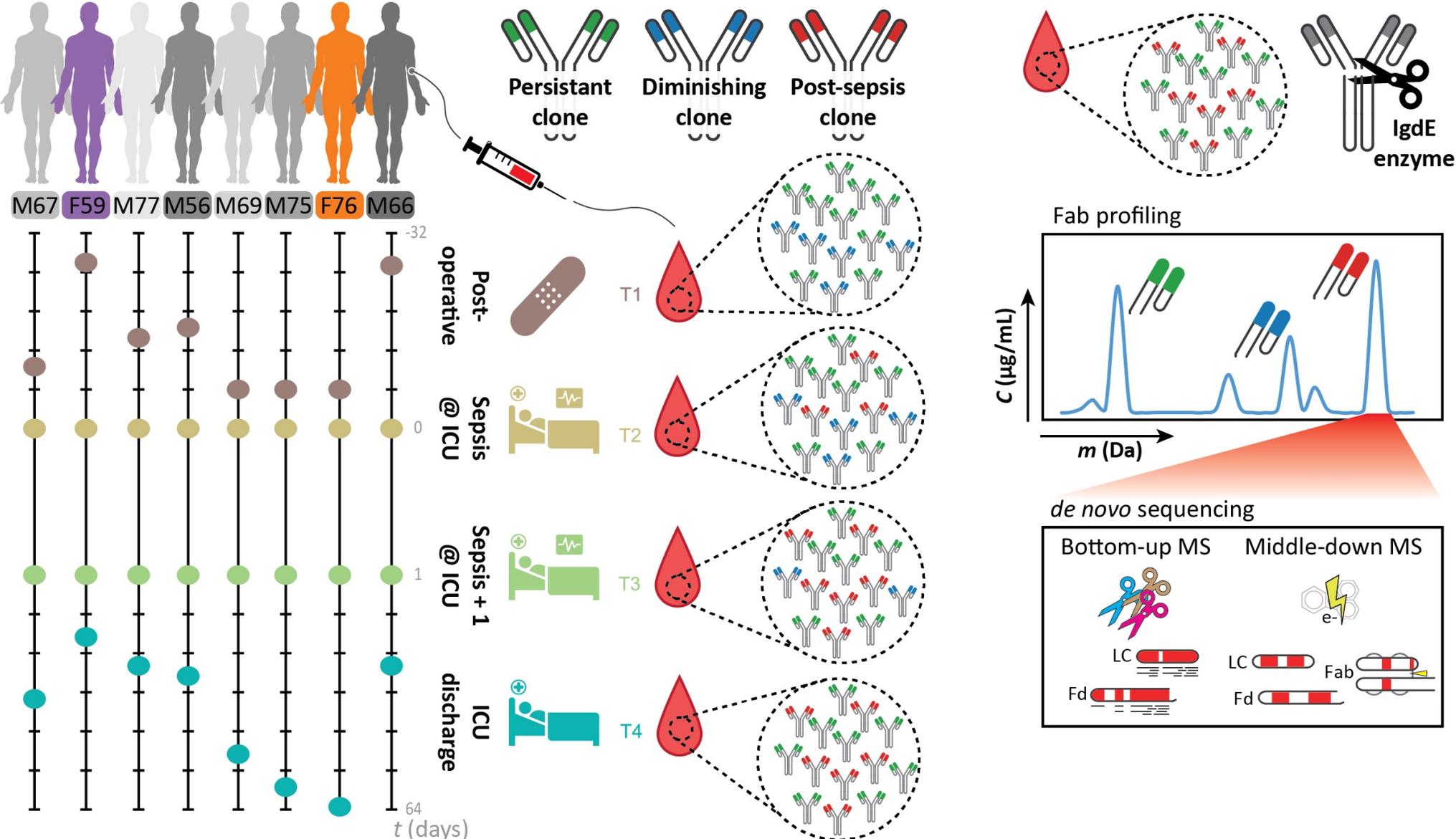


# The Human Plasma IgG1 repertoire; unique healthy donors?

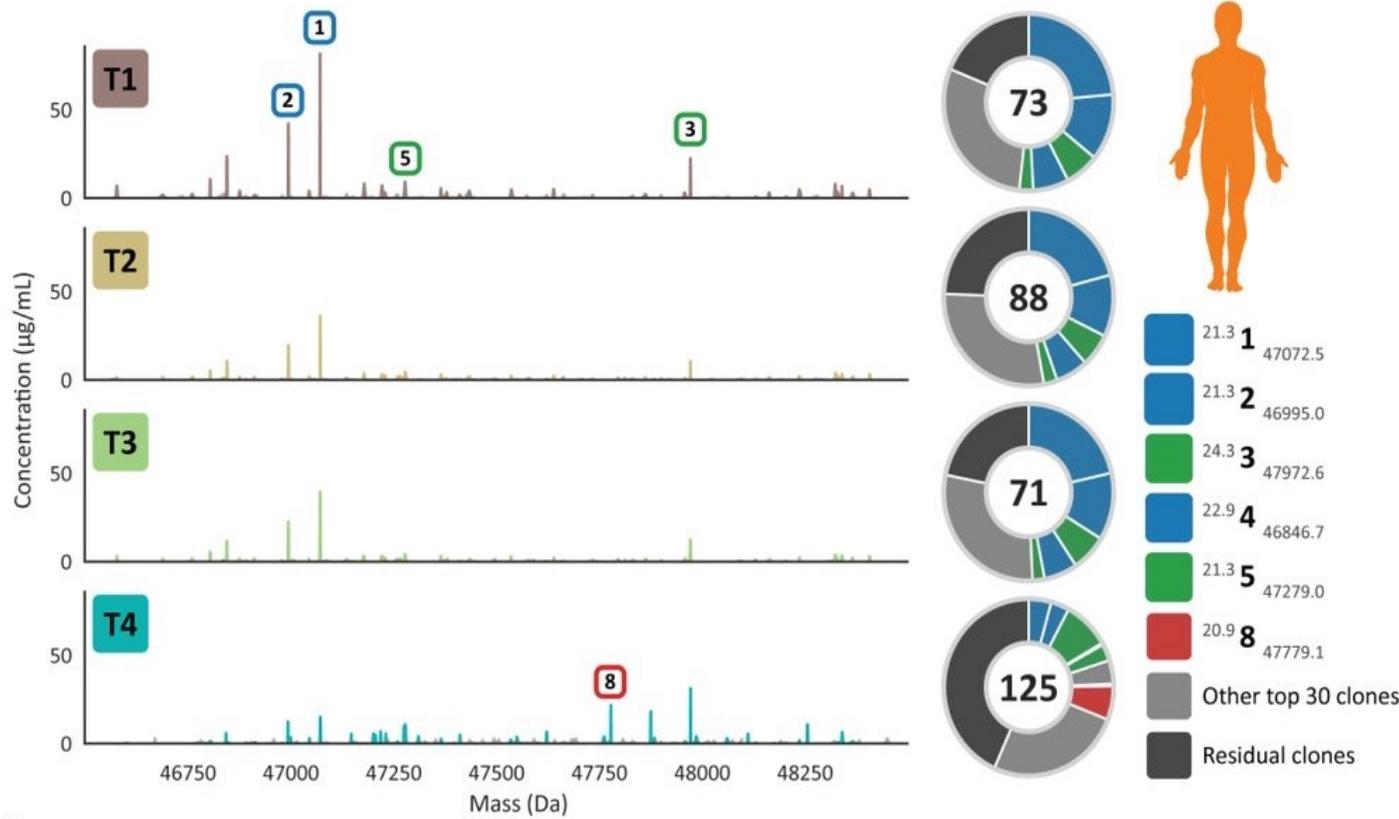


Each IgG1 clonal repertoire is personalized

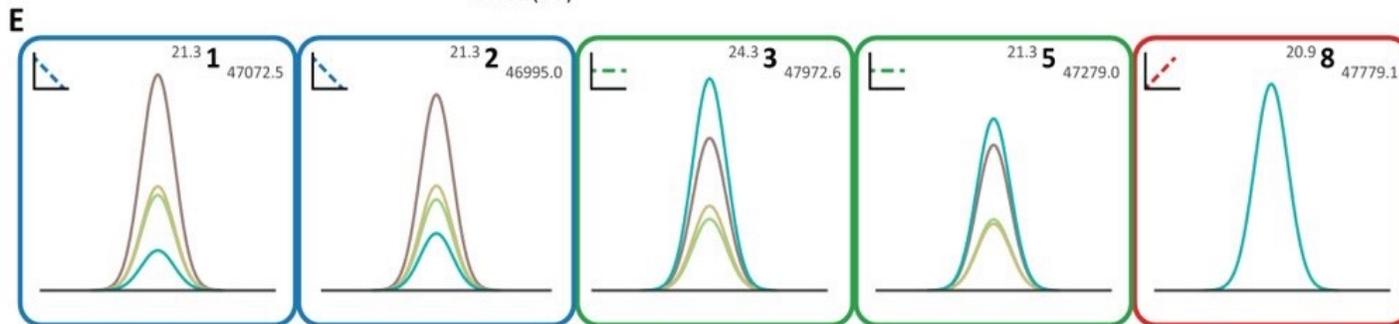
# The Human Plasma IgG1 repertoire in 8 patients with sepsis



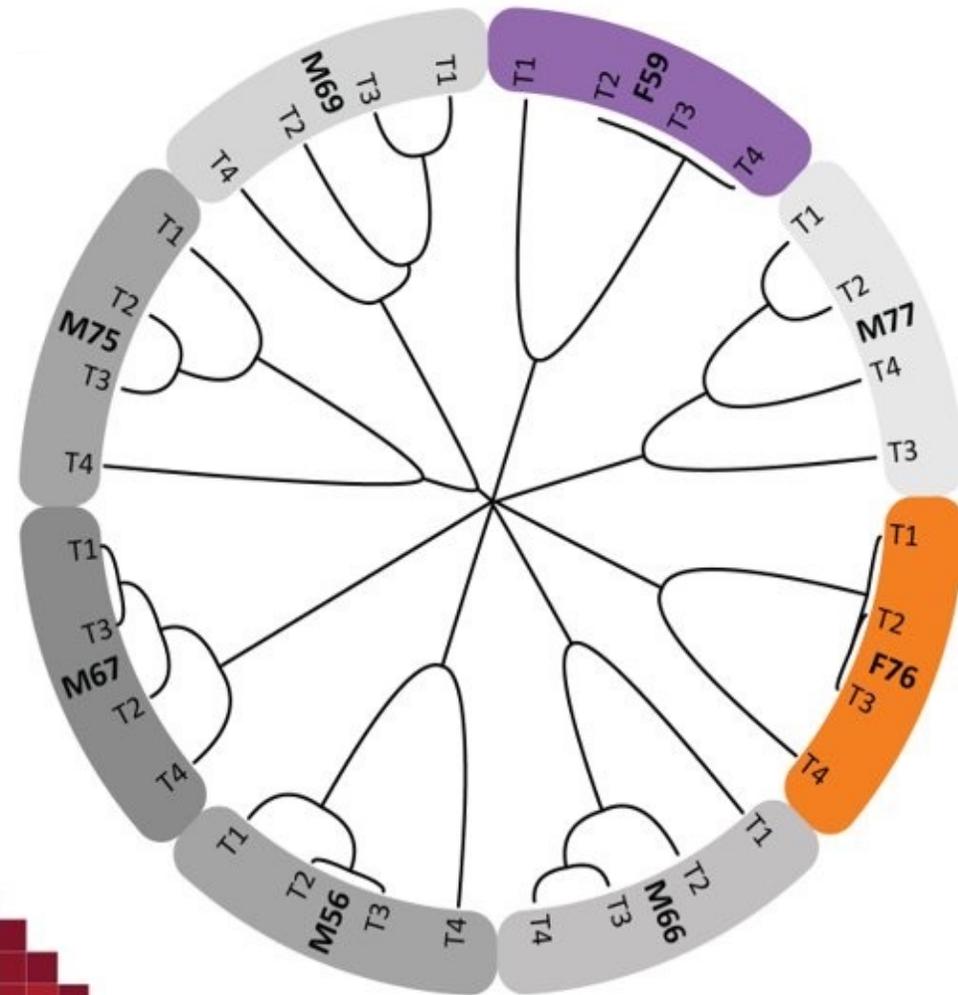
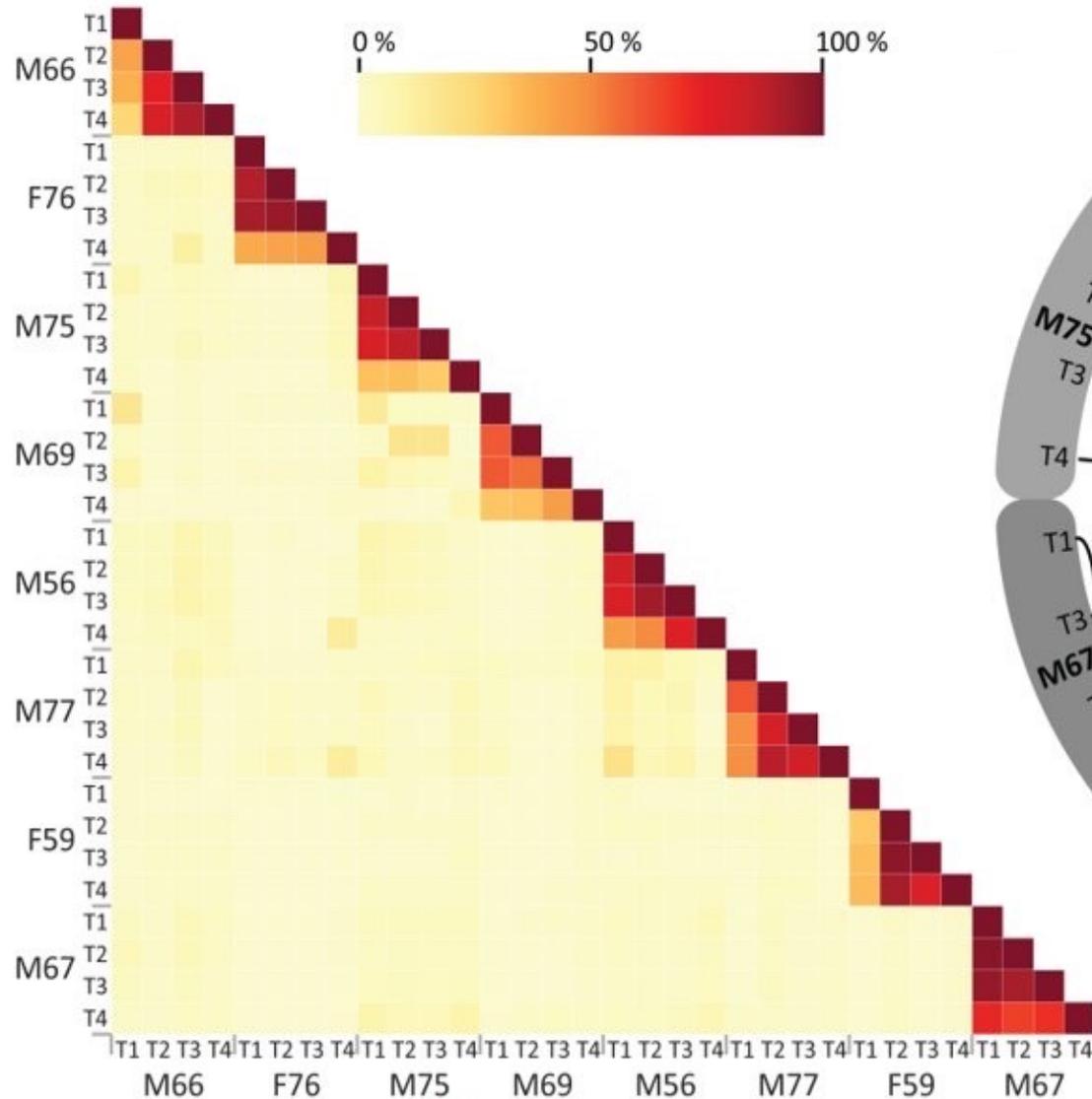
# The Human Plasma IgG1 repertoire is simple but unique



Dominant IgG clones display distinct dynamics

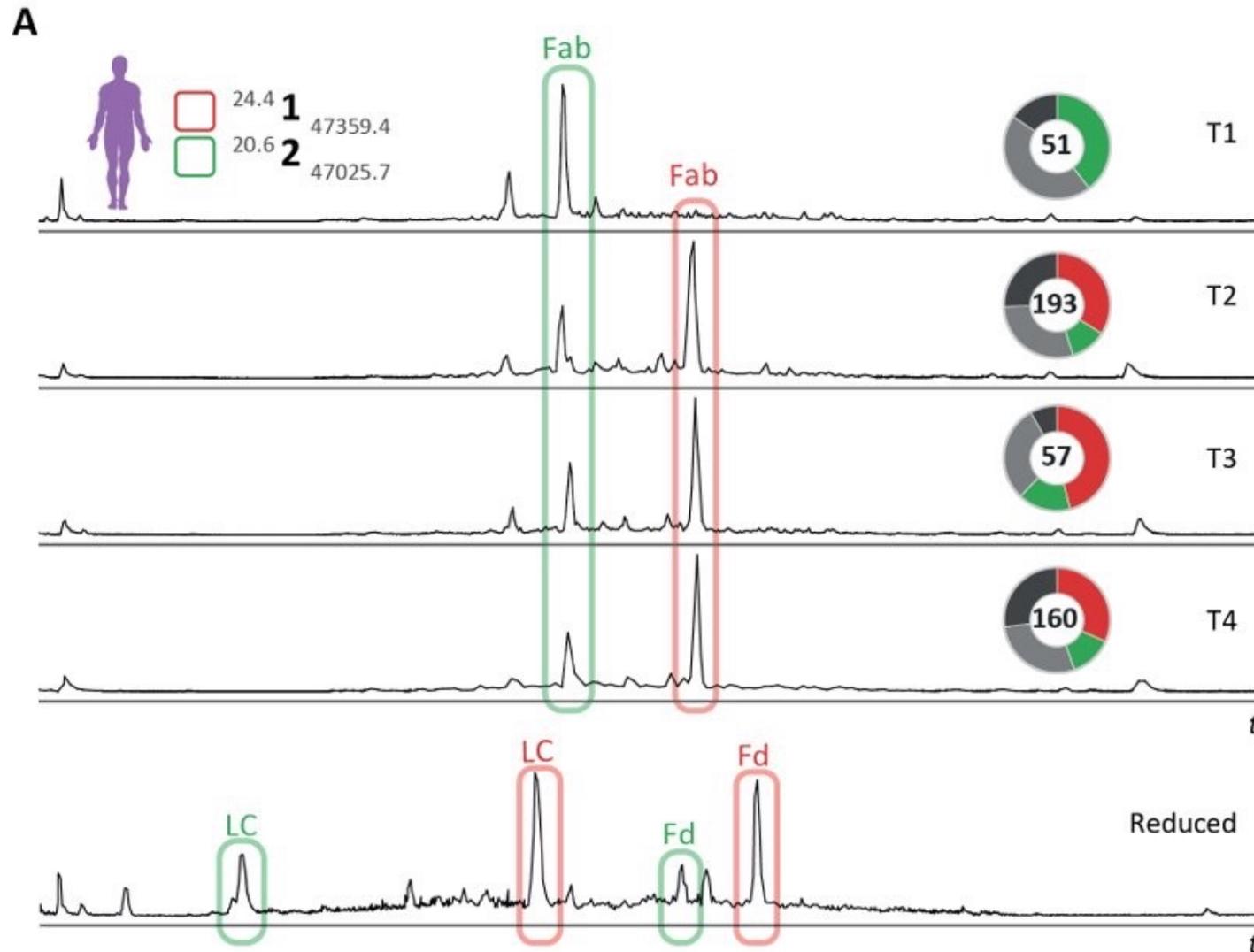


# The Human Plasma IgG1 repertoire is simple but unique



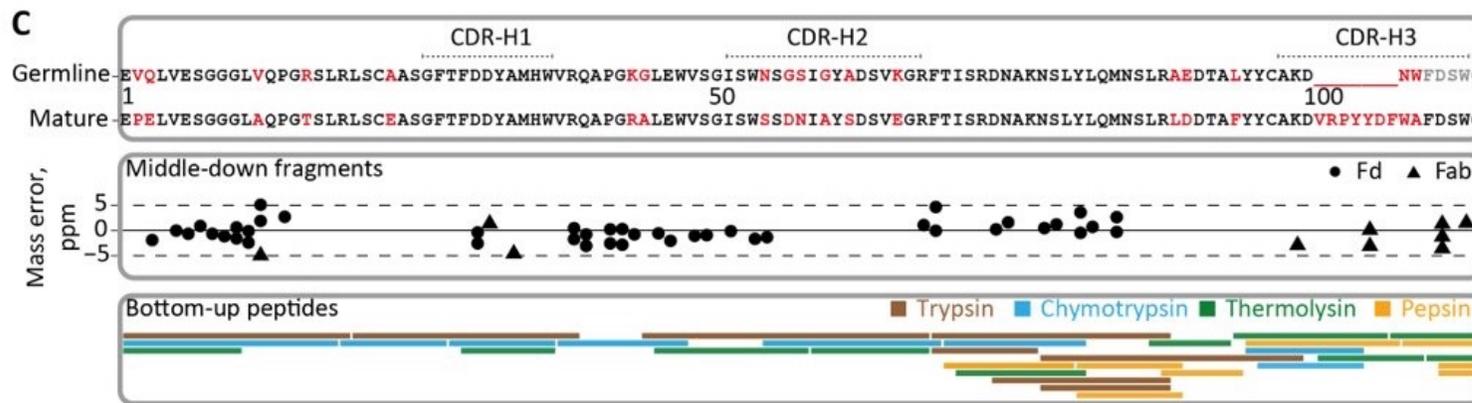
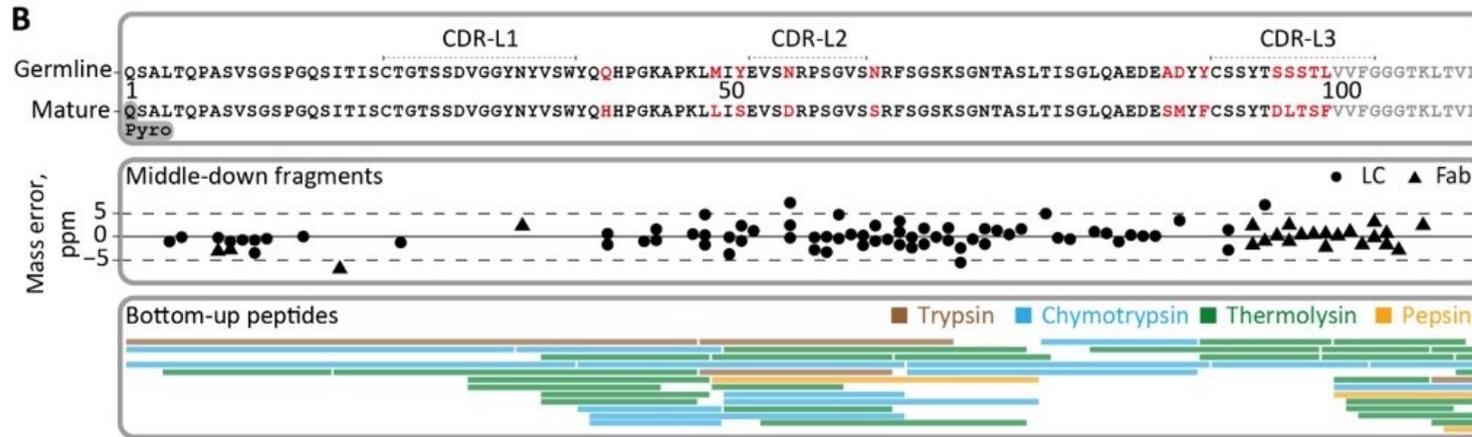
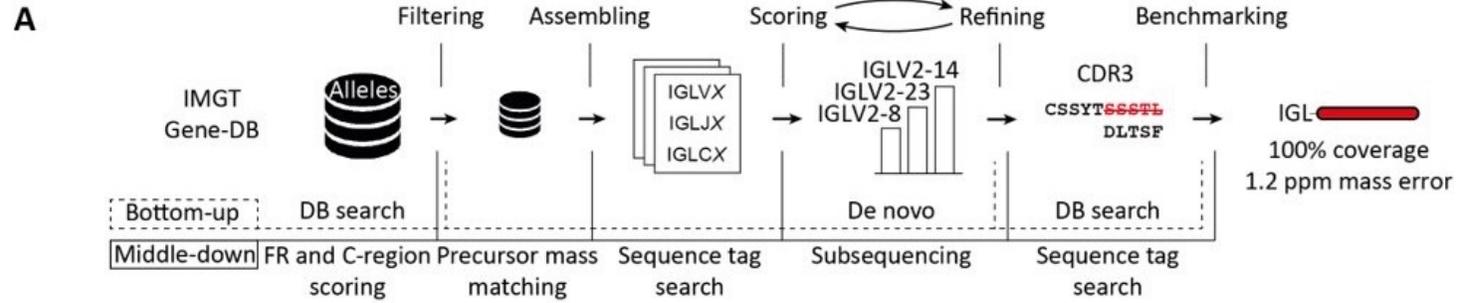
Each donor is unique

# The Human Plasma IgG1 repertoire; a unique donor?



Can we obtain the sequence of the plasma clone  $^{24.4} 1_{47359.5}$

# Fab, Lc and Fd middle-down sequencing by LC-ETD

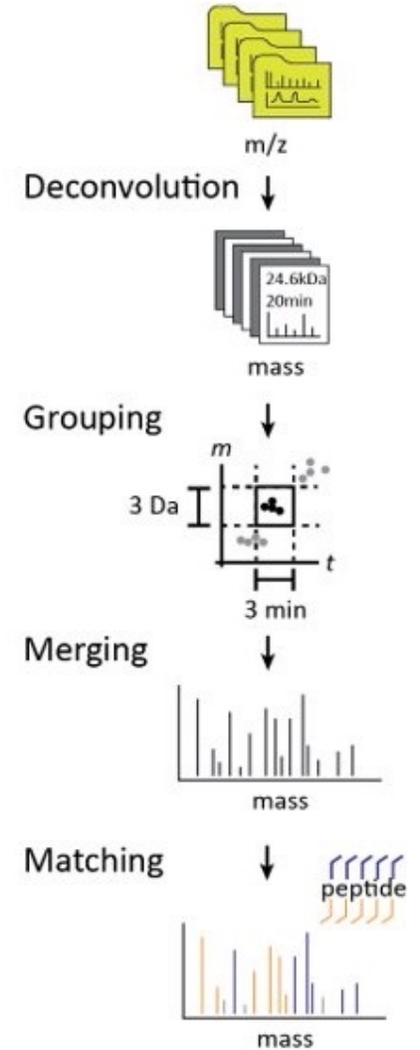
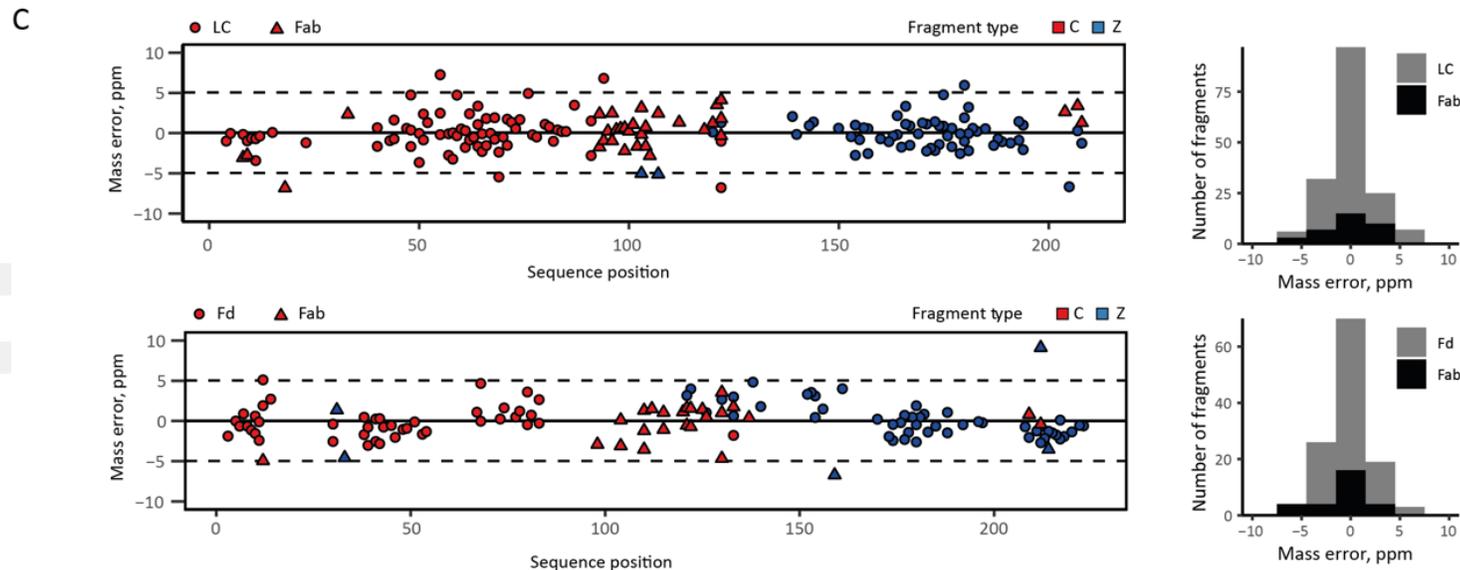
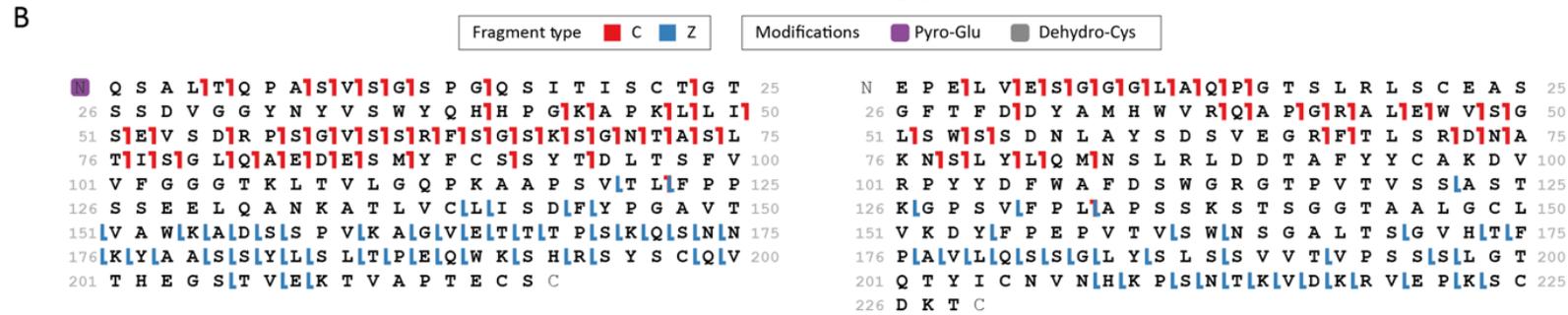
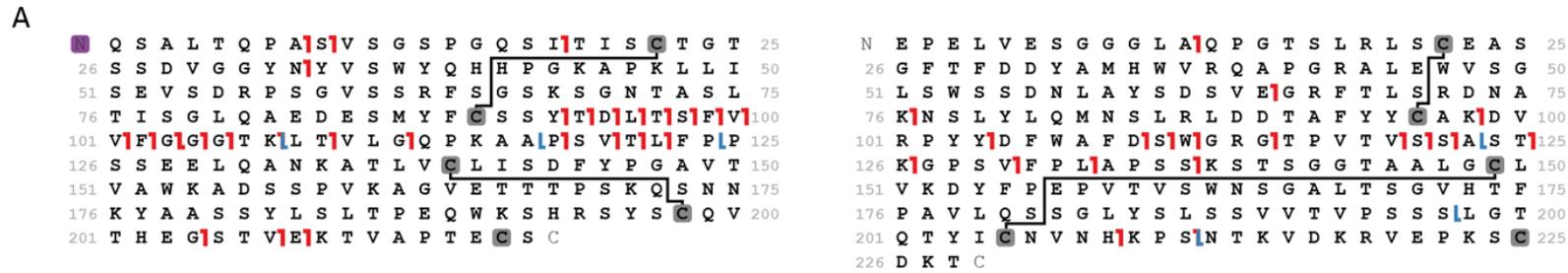


*De novo* sequencing of plasma IgG clone

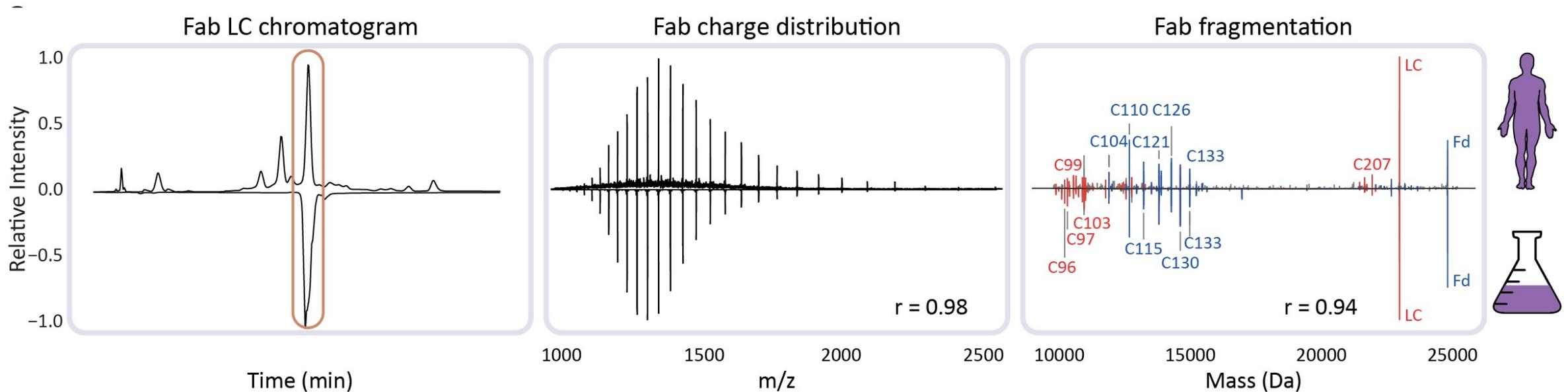
- Fab protein centric approach
- LC and Fd fragment centric approach
- Peptide centric approach
  - Four proteases



# Fab, Lc and Fd middle-down sequencing by LC-ETD



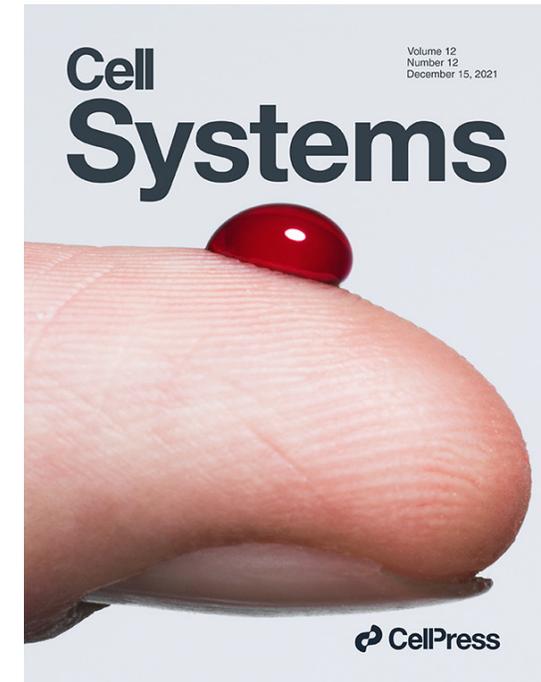
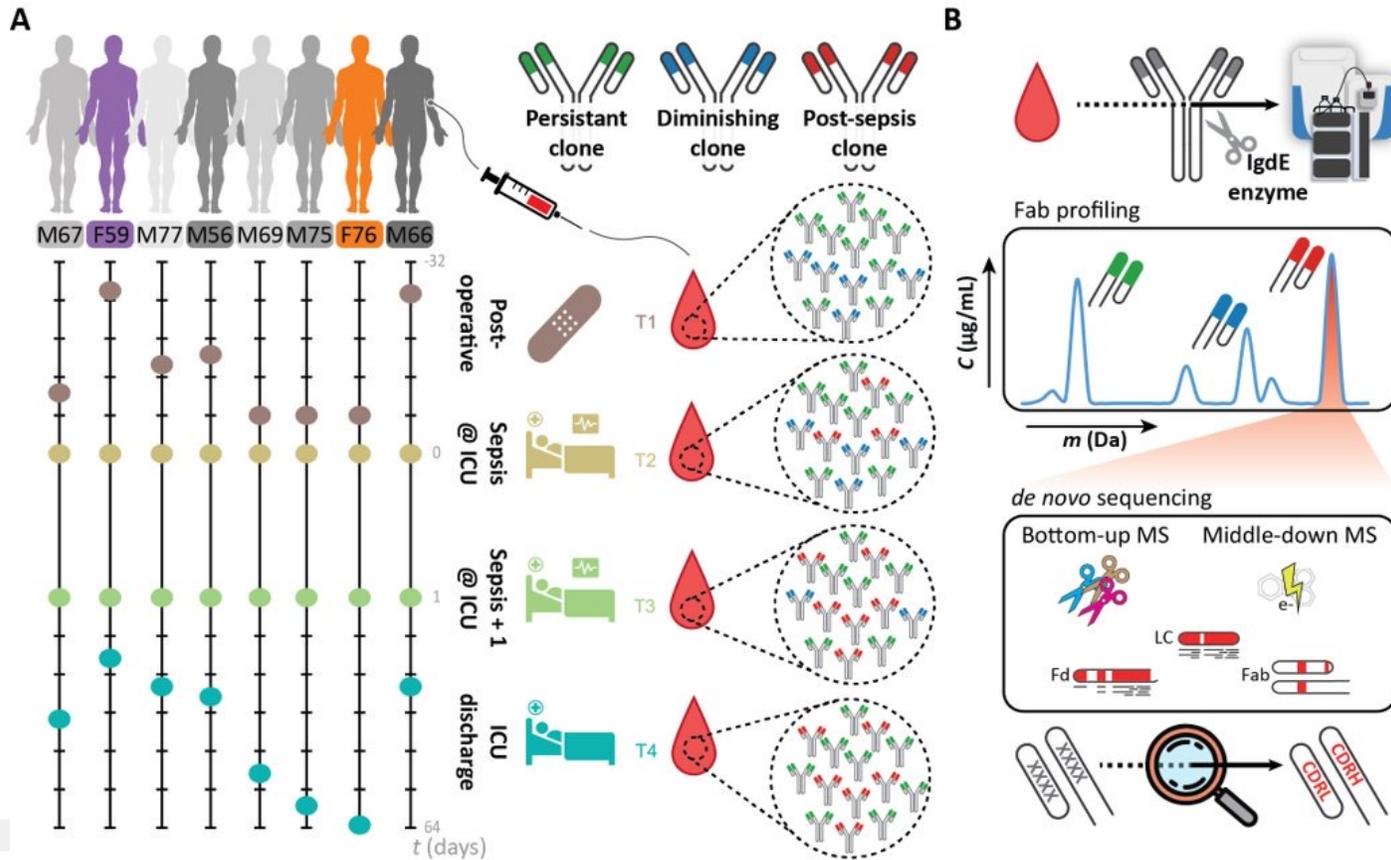
# Is the sequence right?



Validation of key sequencing evidence for donor F59's clone  $^{24.4}1_{47359.4}$  through comparison to a recombinantly produced antibody.



# The Human Plasma IgG1 repertoire

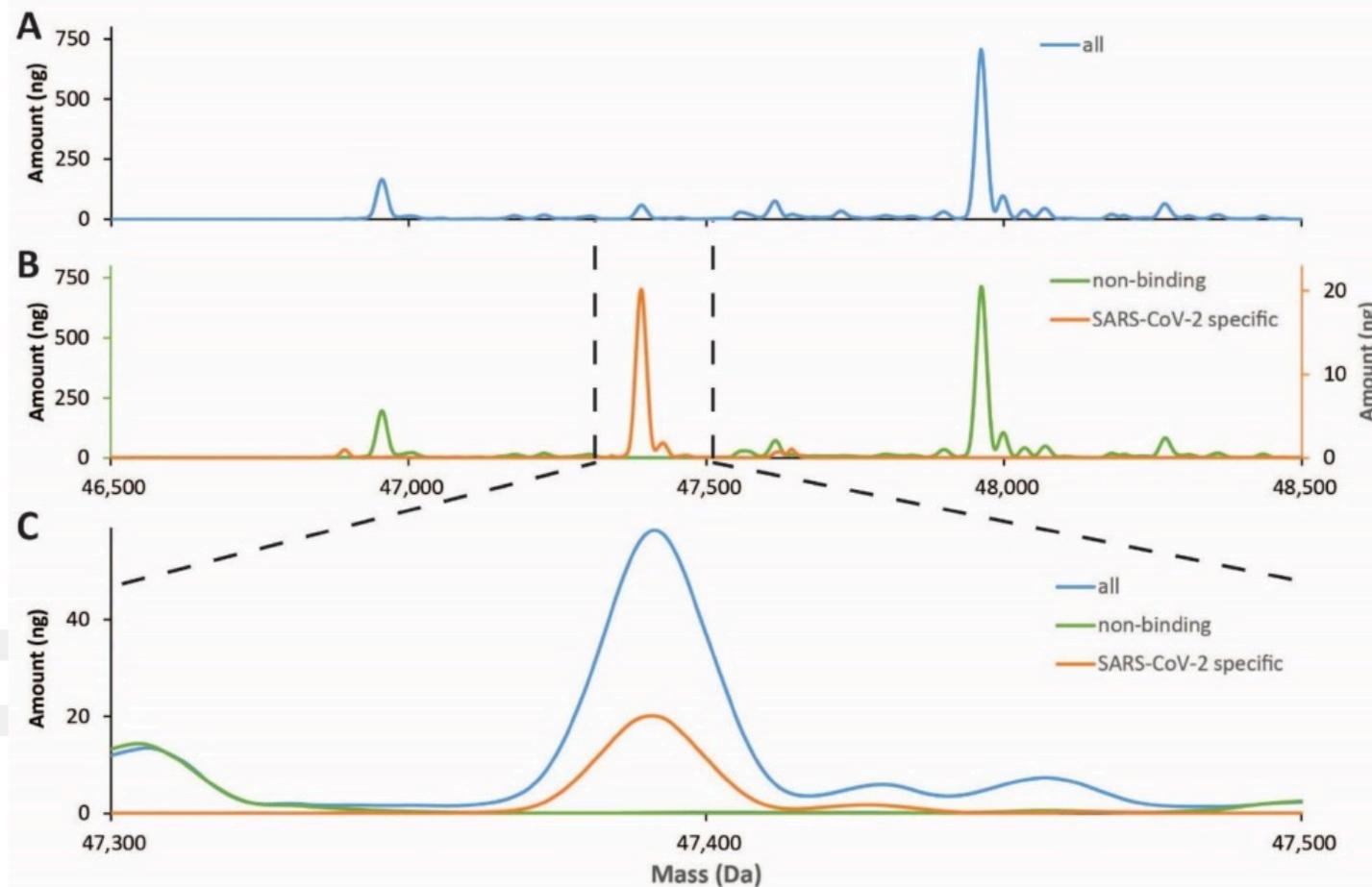


2021, 12, 1131-1143

On the cover: A drop of blood sits atop an index finger with its distinctive fingerprint

# COVID-specific IgGs in the Human Plasma repertoire

With a complementary method, using the spike protein from SARS-CoV-2 to enrich for antibodies from milk, analyzing their Fab fragments subsequently by LC-MS, we were able to demonstrate the presence of a few high abundant SARS-CoV-2 antigen-specific antibodies in milk of COVID-19 recovered women (Figure 4).

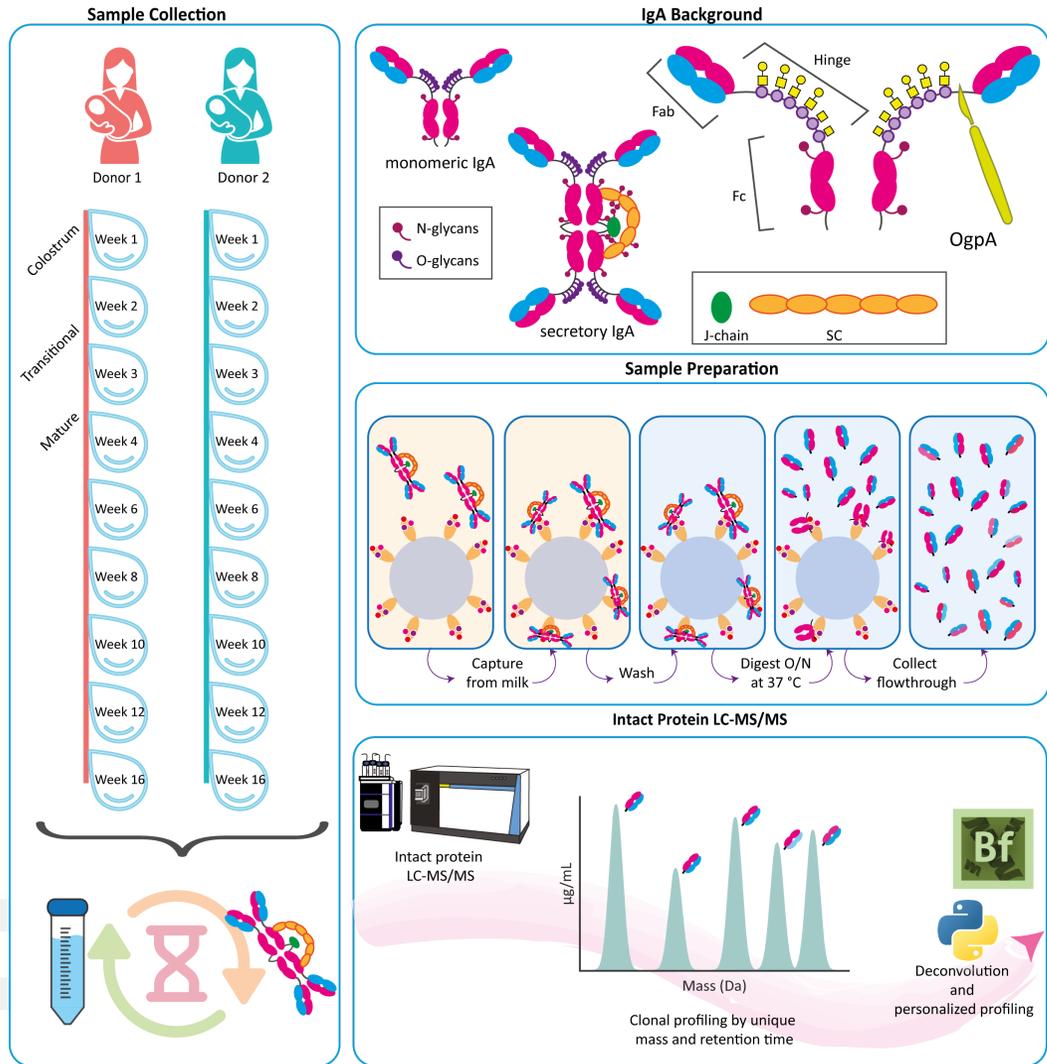


Article

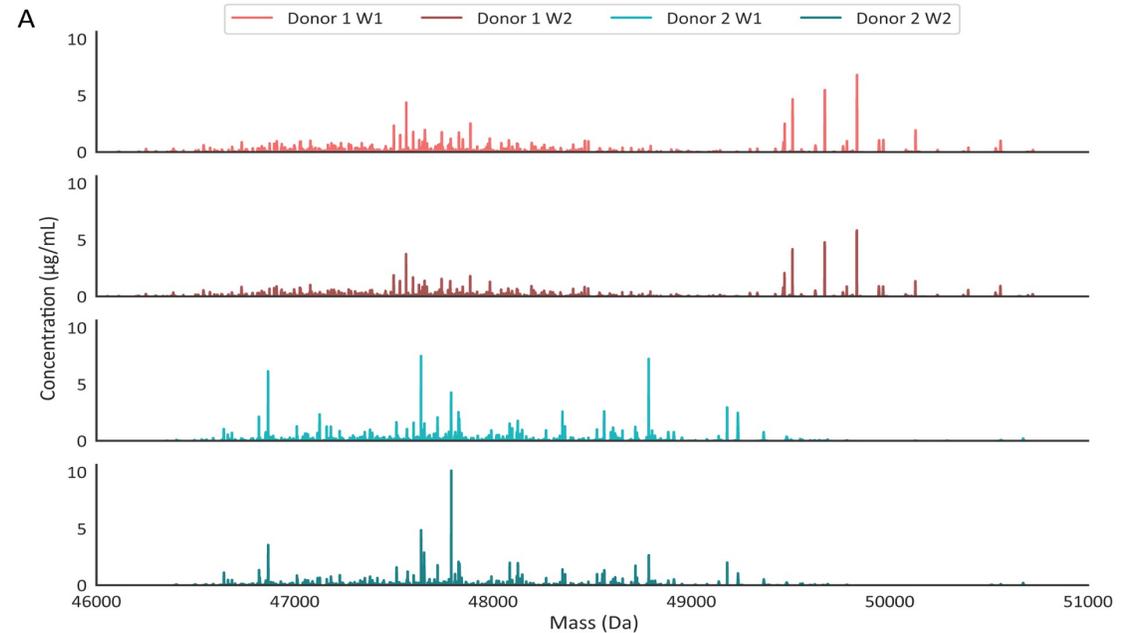
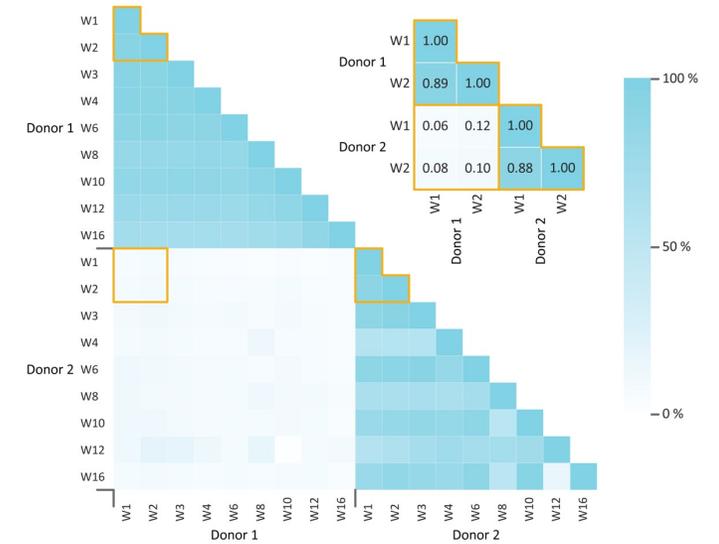
## Human Milk from Previously COVID-19-Infected Mothers: The Effect of Pasteurization on Specific Antibodies and Neutralization Capacity

Britt J. van Keulen <sup>1,†</sup>, Michelle Romijn <sup>1,†</sup>, Albert Bondt <sup>2,3,†</sup>, Kelly A. Dingess <sup>2,3,†</sup>, Eva Kontopodi <sup>1,4,†</sup>, Karlijn van der Straten <sup>5</sup>, Maurits A. den Boer <sup>2,3</sup>, Judith A. Burger <sup>5</sup>, Meliawati Poniman <sup>5</sup>, Berend J. Bosch <sup>6</sup>, Philip J. M. Brouwer <sup>5</sup>, Christianne J. M. de Groot <sup>7</sup>, Max Hoek <sup>2</sup>, Wentao Li <sup>6</sup>, Dasja Pajkrt <sup>1</sup>, Rogier W. Sanders <sup>5,8</sup>, Anne Schoonderwoerd <sup>1</sup>, Sem Tamara <sup>2,3</sup>, Rian A. H. Timmermans <sup>9</sup>, Gestur Vidarsson <sup>10</sup>, Koert J. Stittelaar <sup>11</sup>, Theo T. Rispsens <sup>12</sup>, Kasper A. Hettinga <sup>4</sup>, Marit J. van Gils <sup>5</sup>, Albert J. R. Heck <sup>2,3</sup> and Johannes B. van Goudoever <sup>1,†</sup>

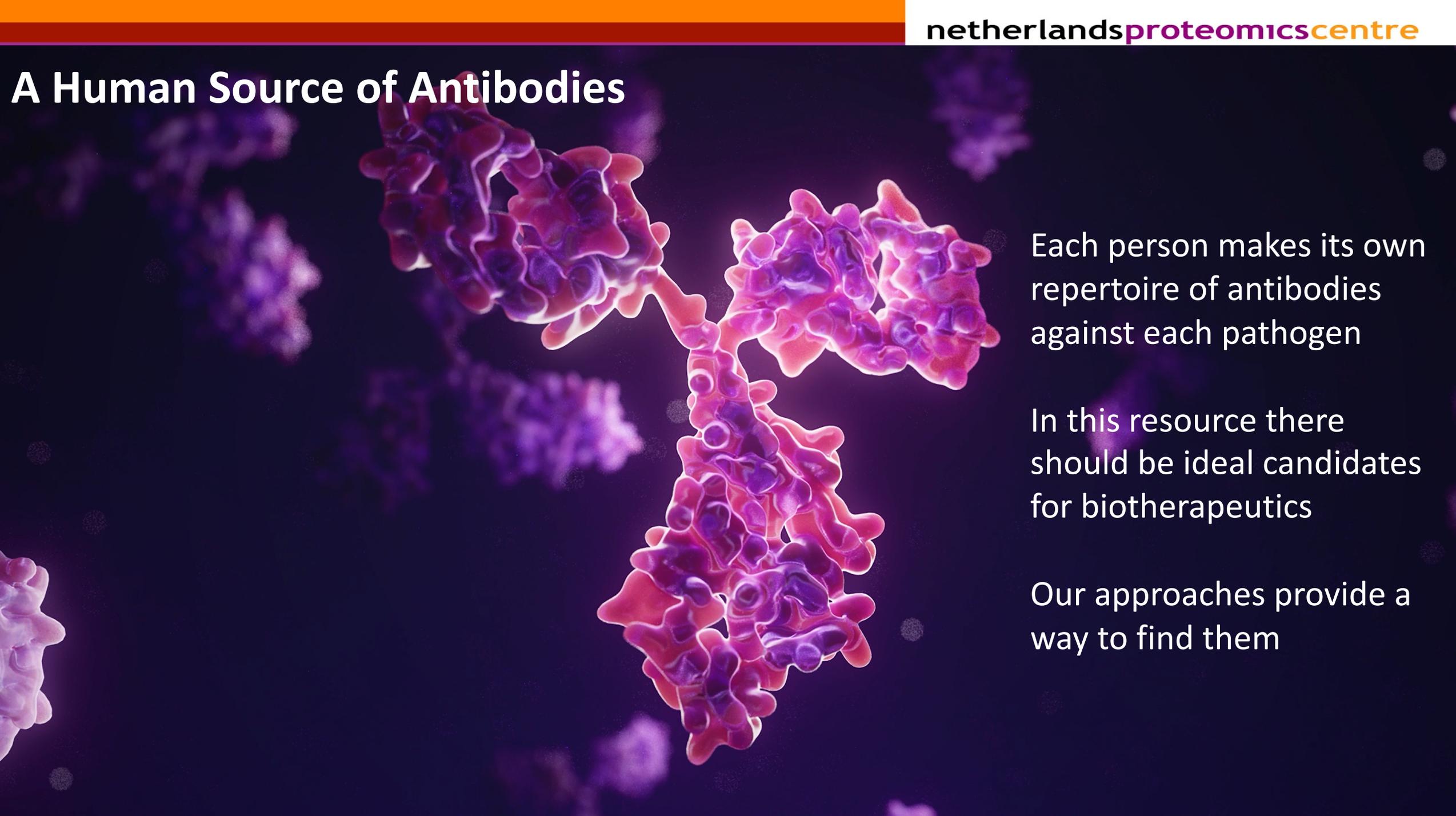
# IgA1 repertoires in human milk



Also the human milk IgA1 repertoire is simple but unique



# A Human Source of Antibodies



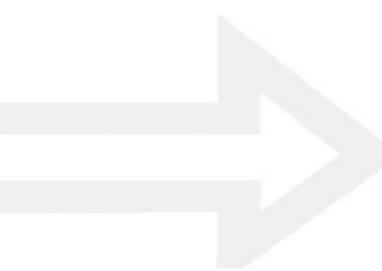
Each person makes its own repertoire of antibodies against each pathogen

In this resource there should be ideal candidates for biotherapeutics

Our approaches provide a way to find them

# Acknowledgements

Thank you





# Pittcon 2022 Wallace H. Coulter Lecture

## New Horizons in Mass Spectrometry

- I. Sizing and Counting Particles by Native Mass Spectrometry
- II. How unique is your immune response?  
Monitoring personalized antibody repertoires
- III. Science meets art**

Albert J.R. Heck

*Science Faculty of Utrecht University The Netherlands*

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 @hecklab

# A Human Source of Antibodies

Science meets art

Each person makes its own repertoire of antibodies against each pathogen  
In this vast resource there should be ideal candidates for biotherapeutics  
Our approaches may provide a way to find them

