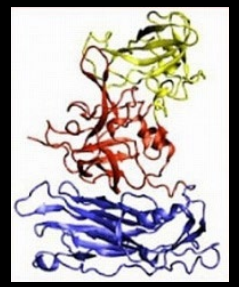
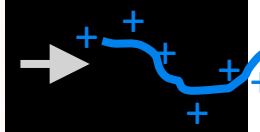
Flying viruses – mass spectrometry meets X-rays

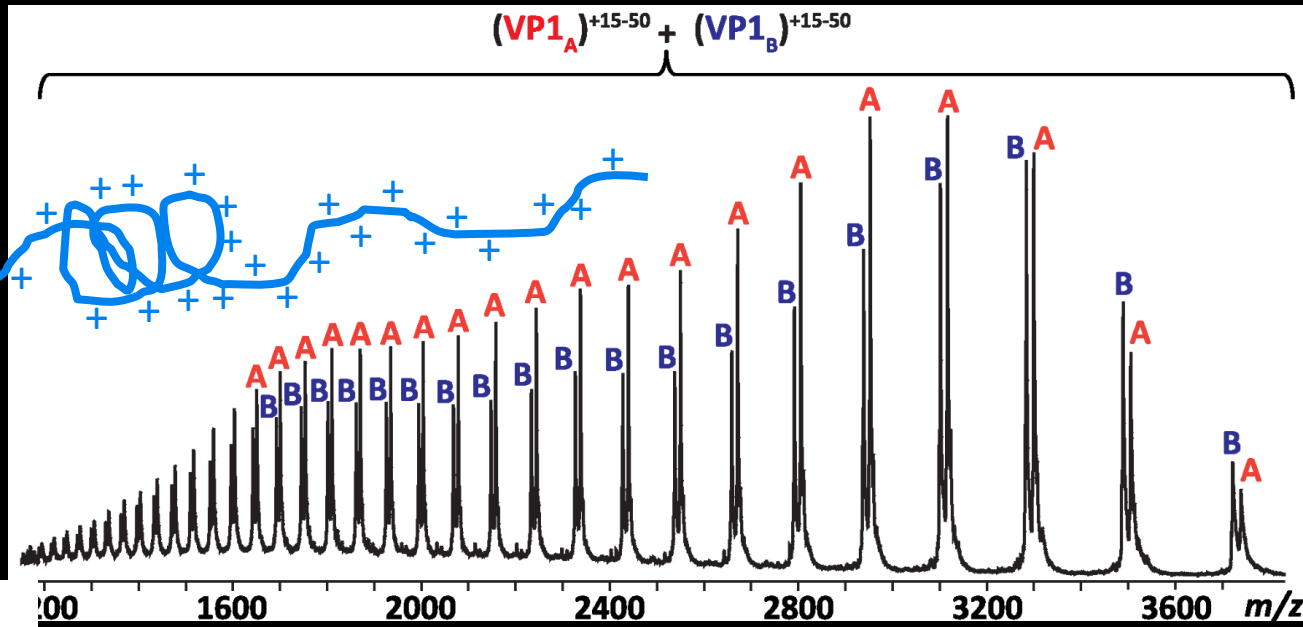
Charlotte Uetrecht

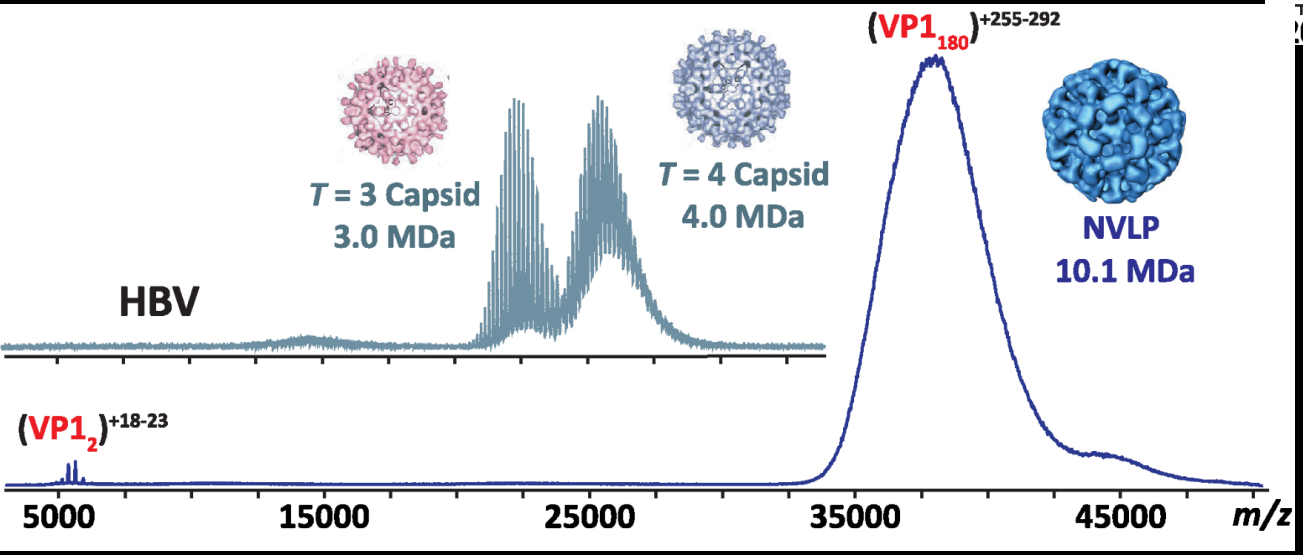
Native MS – up to viruses

- Denatured VP1









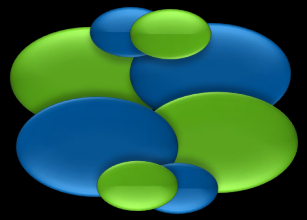
- Buffered solution

→ Intact VLP

Shoemaker *et al.*, *Mol Cell Proteomics* **2010**

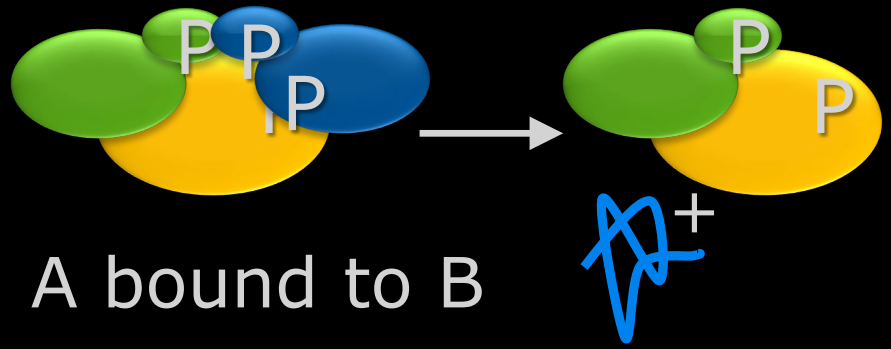
Information from native MS

Stoichiometry



M = xA+yB

Topology



A bound to B

Shape/
Conformational change

Amount A or B

Information from native MS

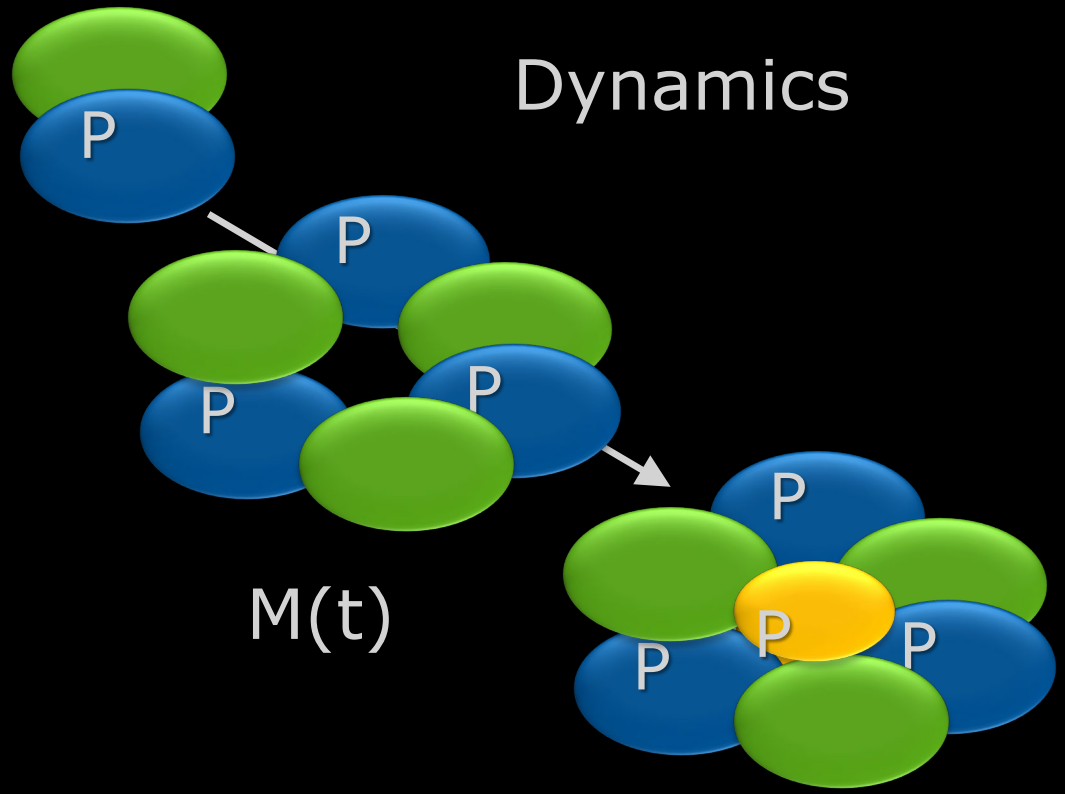
Universität Siegen

CSSB Centre for Structural Systems Biology

Binding

[M]/[ML]

Dynamics



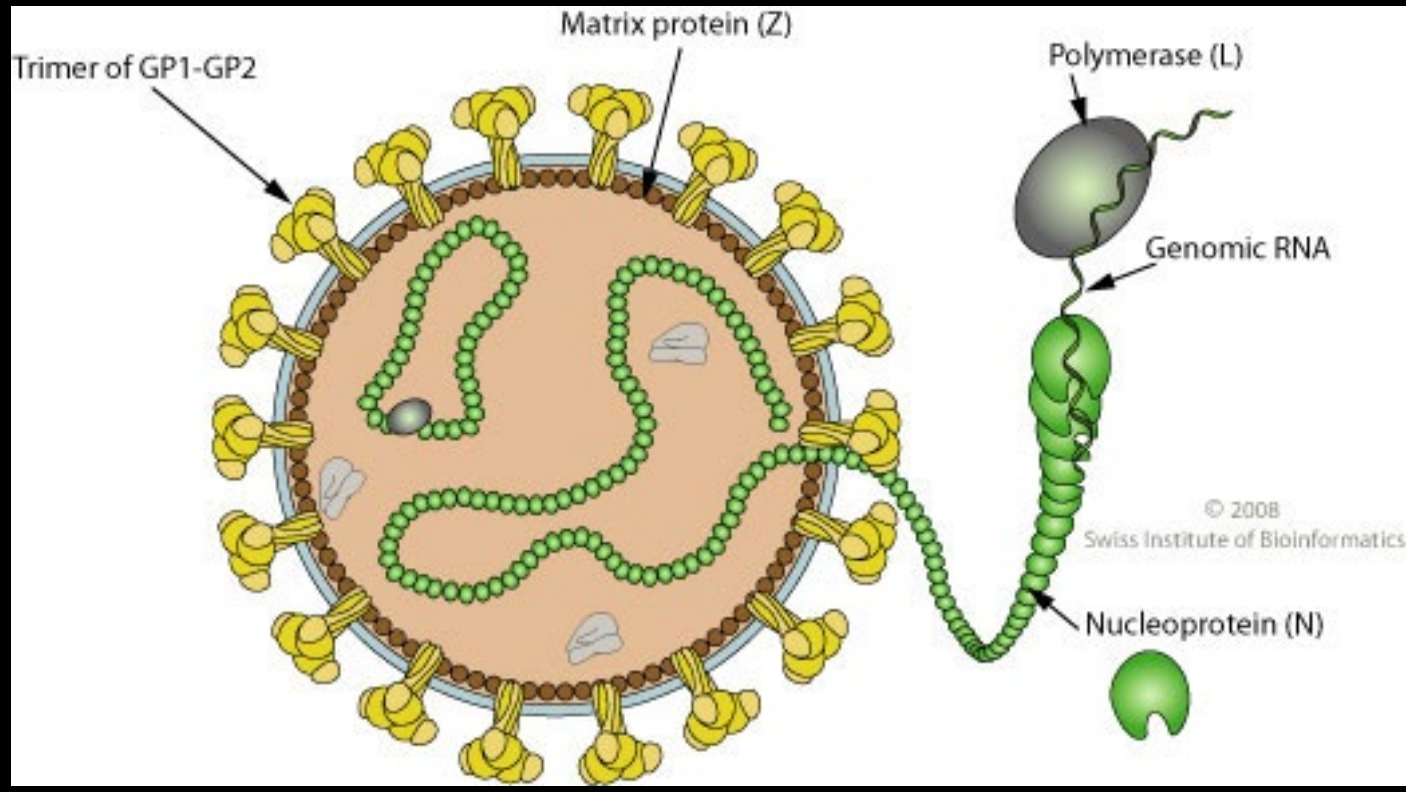
M(t)

Lassa Virus

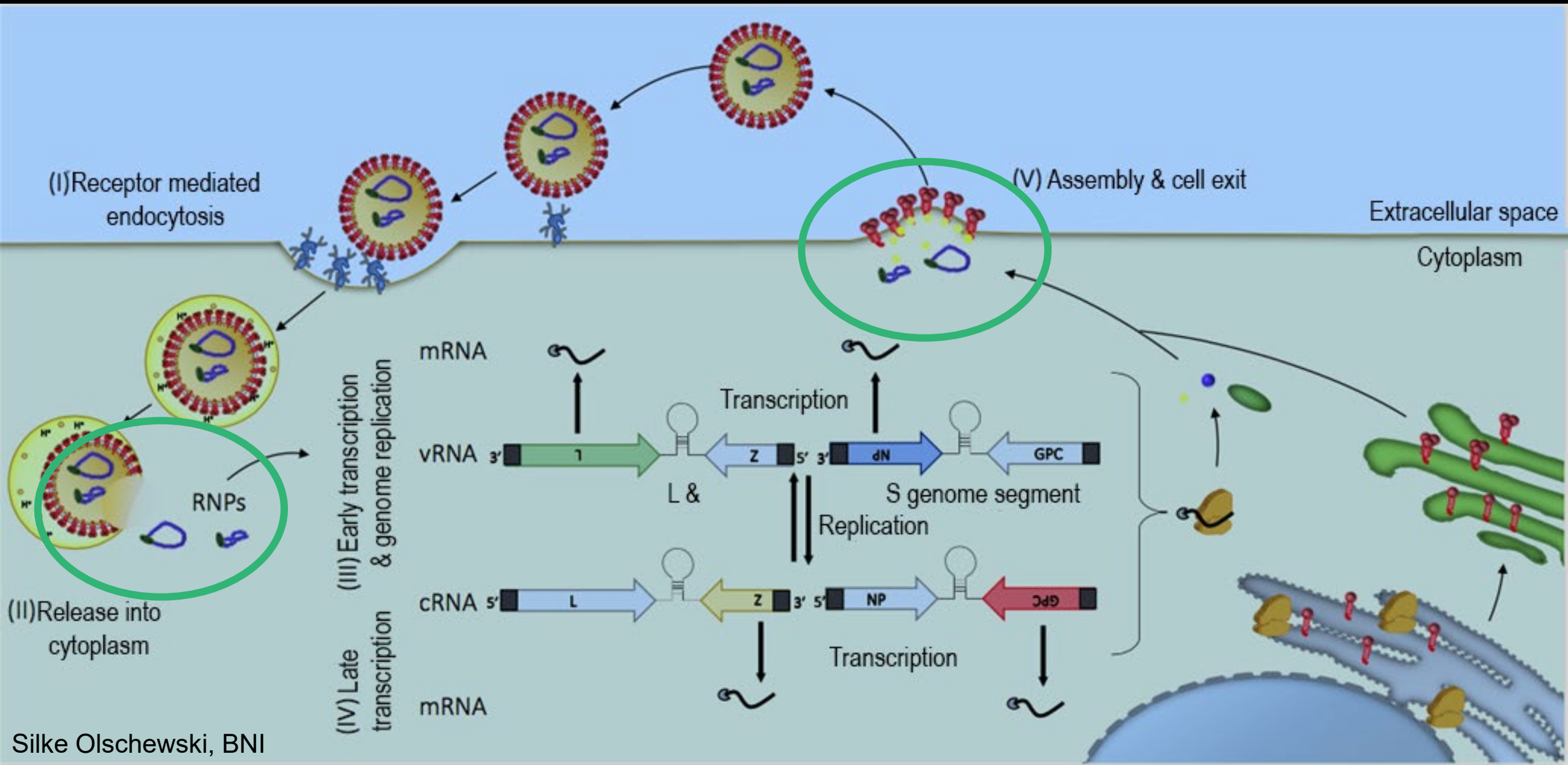
- *Bunyavirales, Arenaviridae*
- Endemic in West Africa, reservoir: rodents

→Lassa hemorrhagic fever in humans (~5000 fatalities / a)

- - ssRNA, L & S segment
- 4 proteins
  - (pre)GP
  - L (253 kDa)
  - NP (63 kDa)
  - Z (11 kDa)



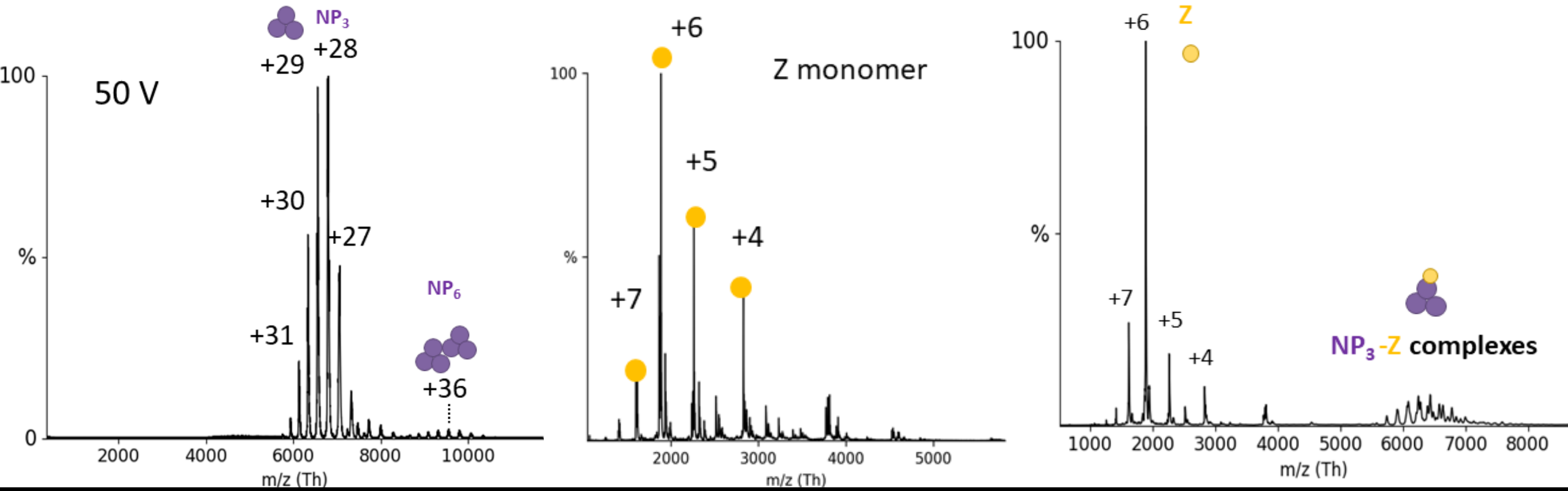
Lassa Virus



Silke Olschewski, BNI

NP & Z

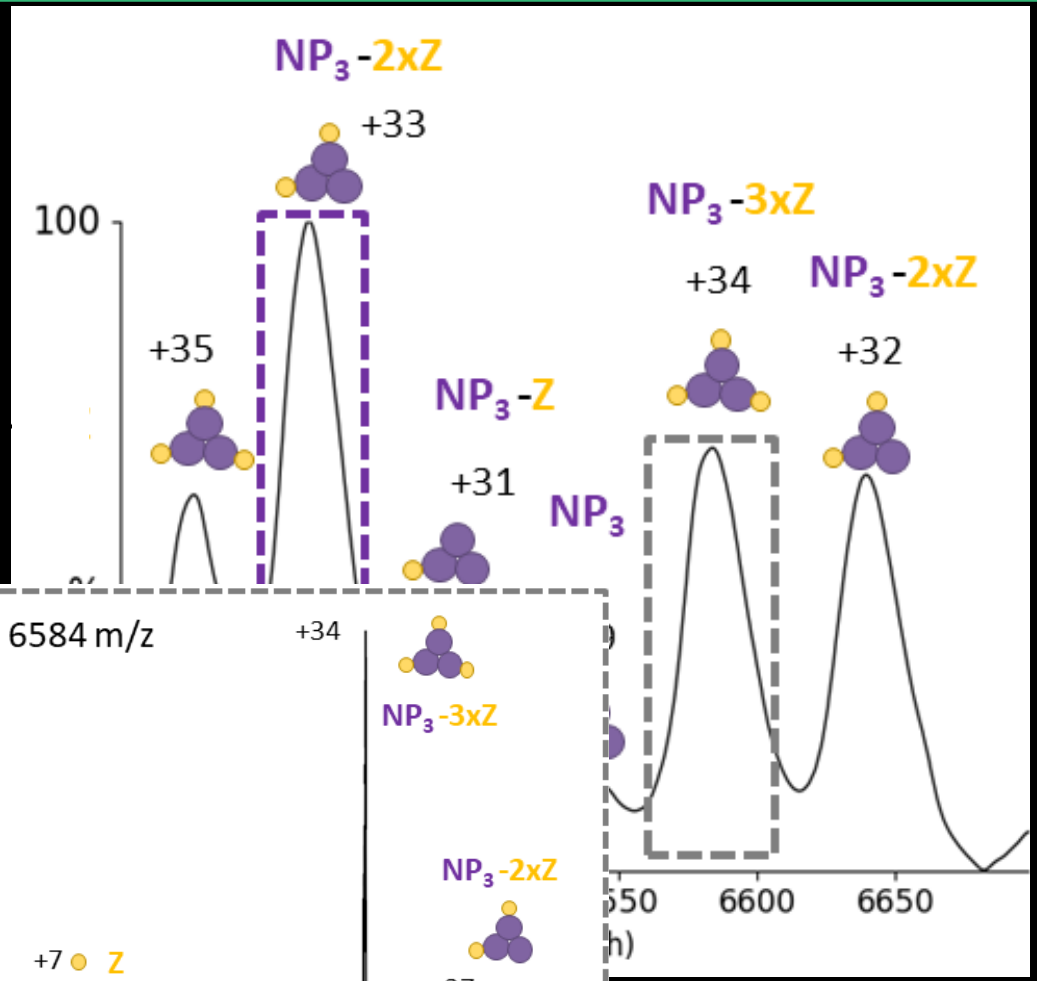
- Trimeric NP
- Monomeric Z

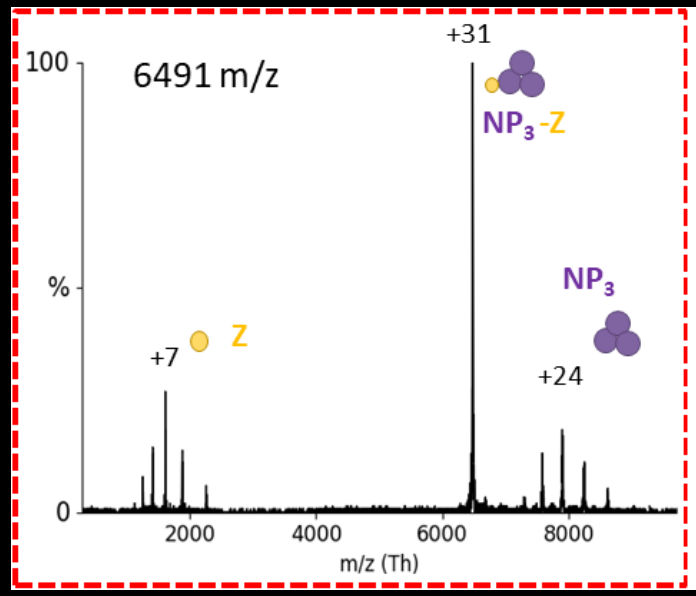


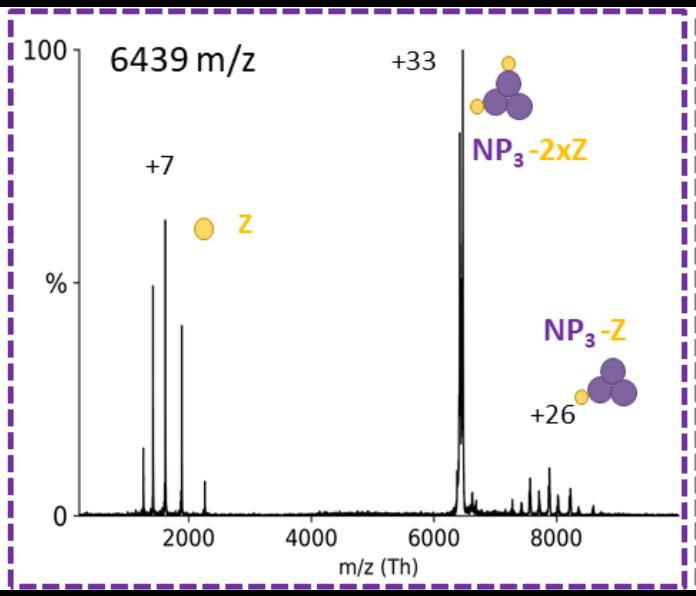
Sänger *et al. biorxiv* **2023** *J Am Chem Soc* accepted

NP & Z

- Up to 3 Z per NP_3
- K_D ~ 110 ± 10 µM





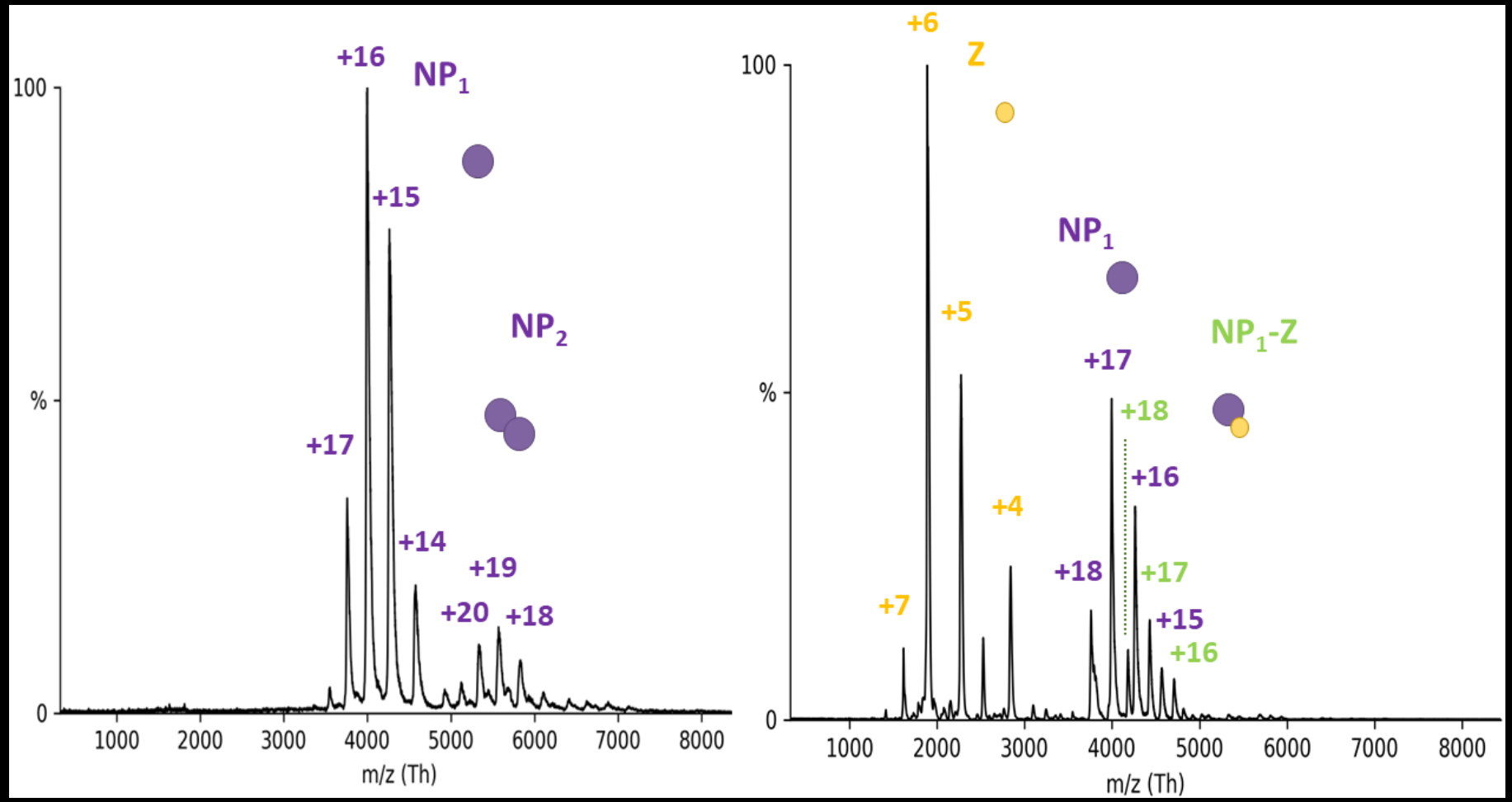


Sänger *et al. biorxiv* **2023** *J Am Chem Soc* accepted

NP & Z

- R52A NP trimerization mutant

→K_D ~ 33 ± 2 µM



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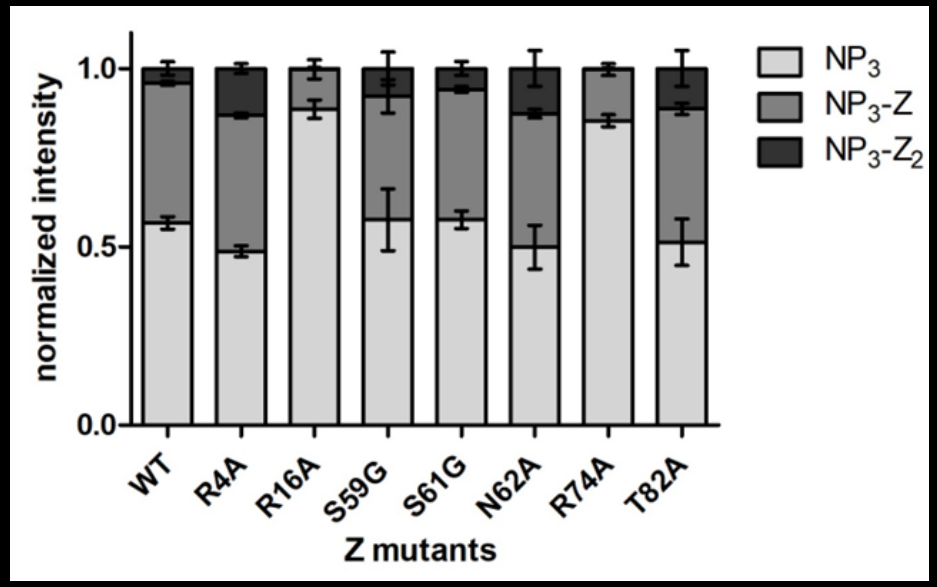
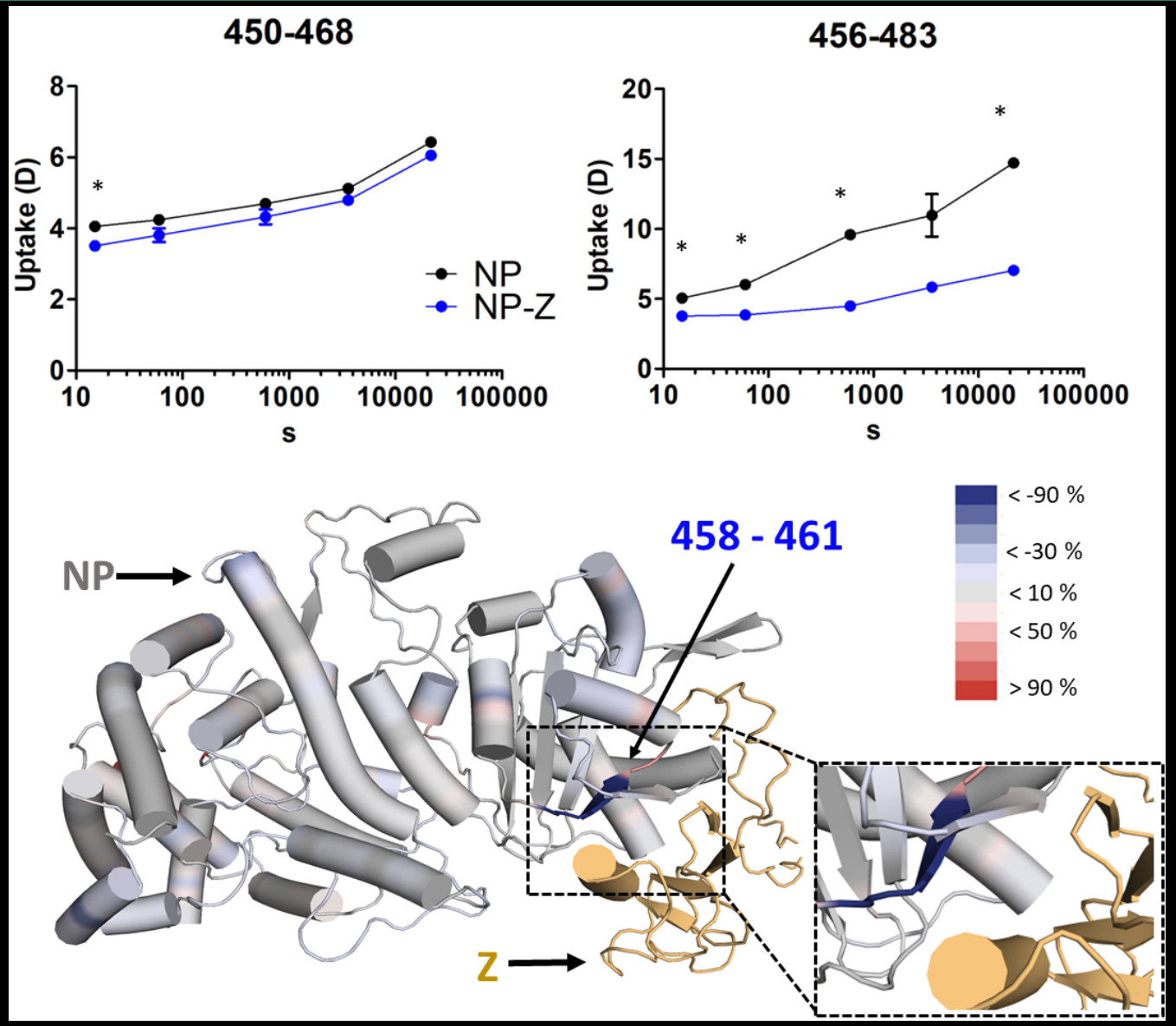
NP & Z

- AlphaPulldown
- Z via NP C-terminal domain
- HDX-MS NP_3 vs. NP_3-Z
- Z binding mutants

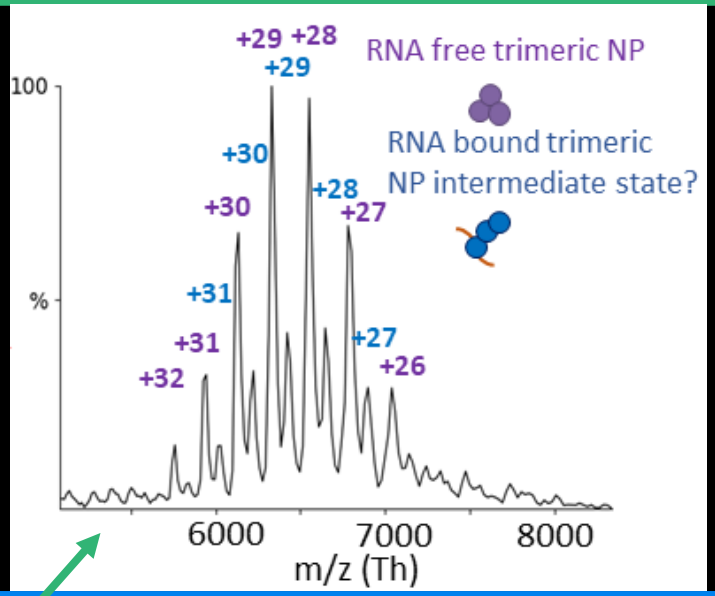




Sänger *et al. biorxiv* **2023** *J Am Chem Soc* accepted

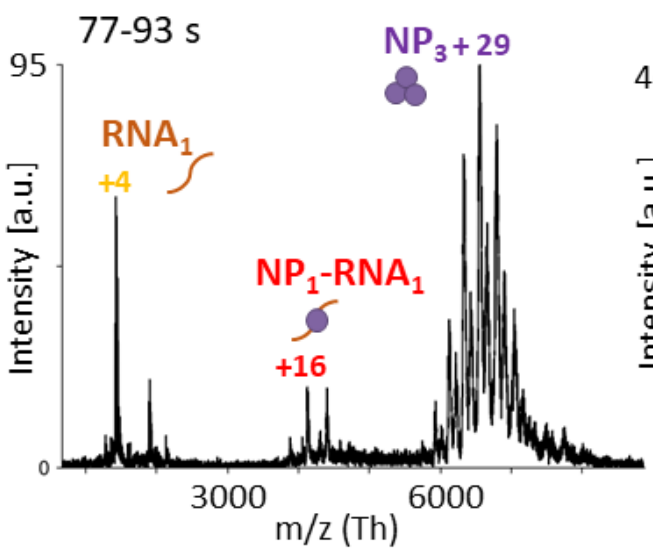
NP & RNA

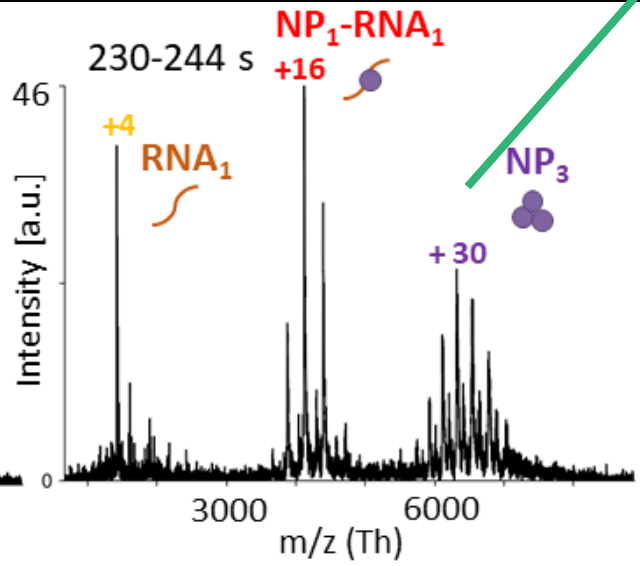
- 9 nt RNA dissociates NP_3
- NP_3-RNA as intermediate

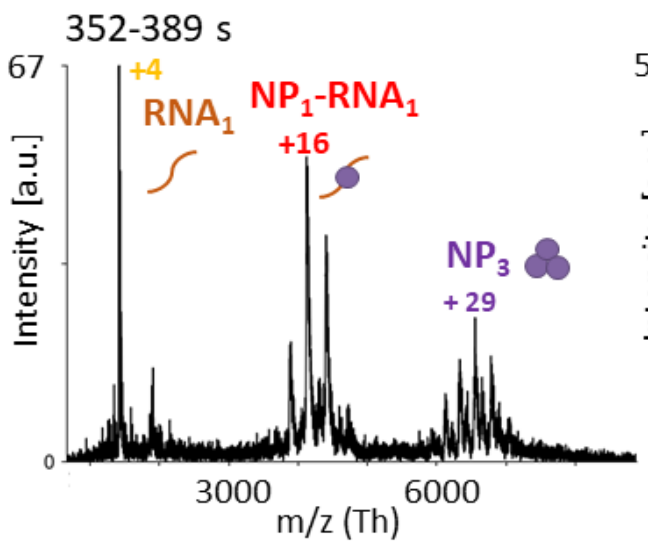


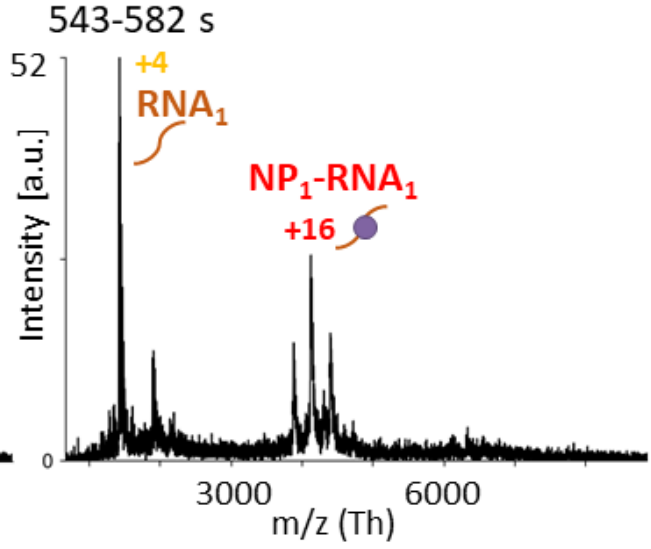
Start

End









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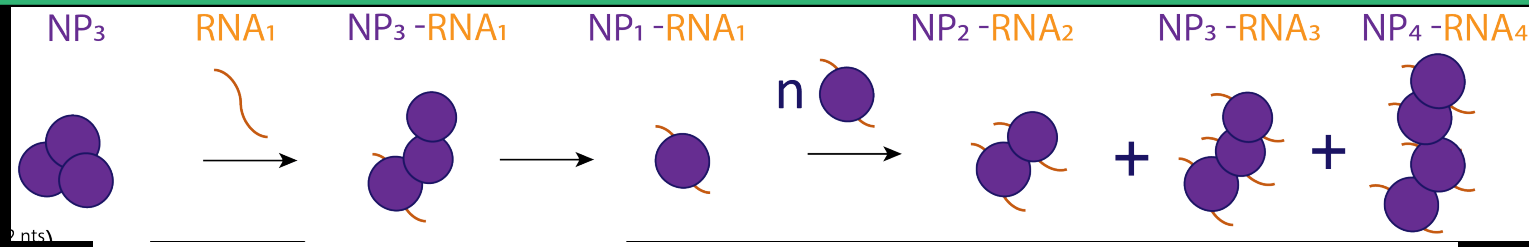
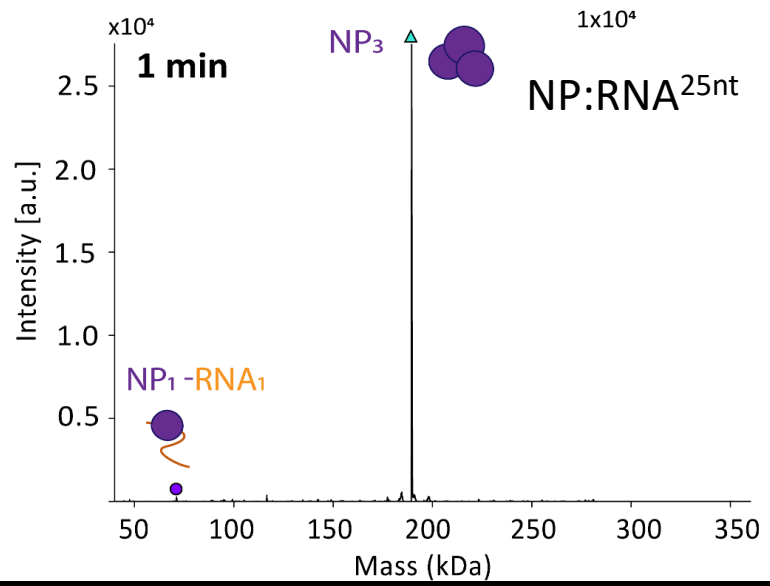
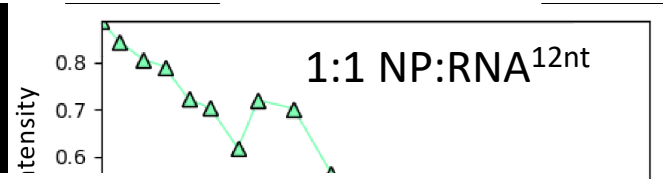
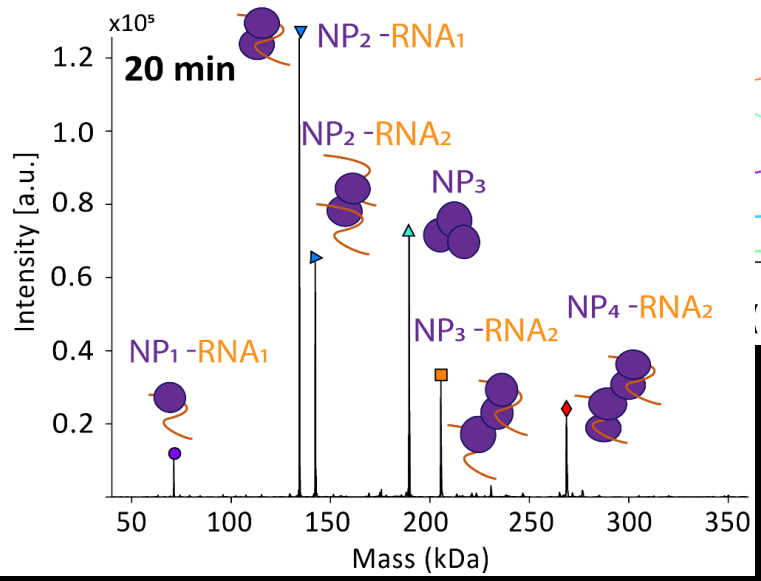
NP & RNA

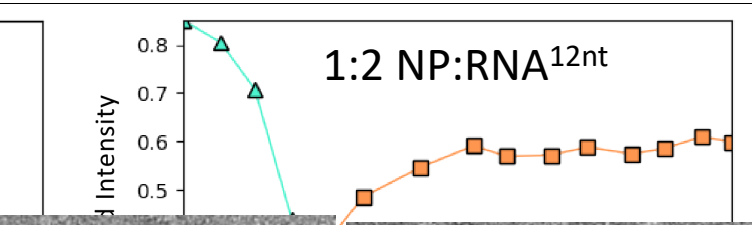
- RNP assembly
  - ≥18 nt → 2 NP / RNA

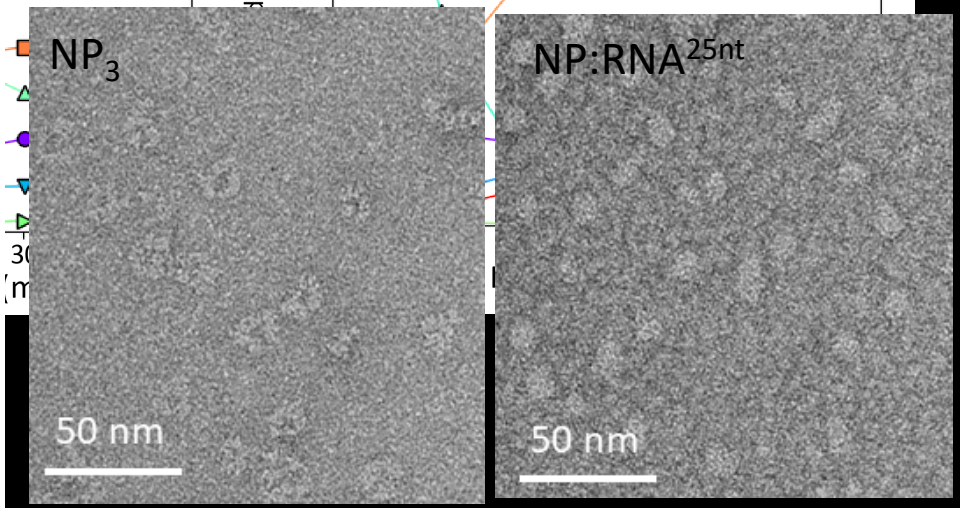








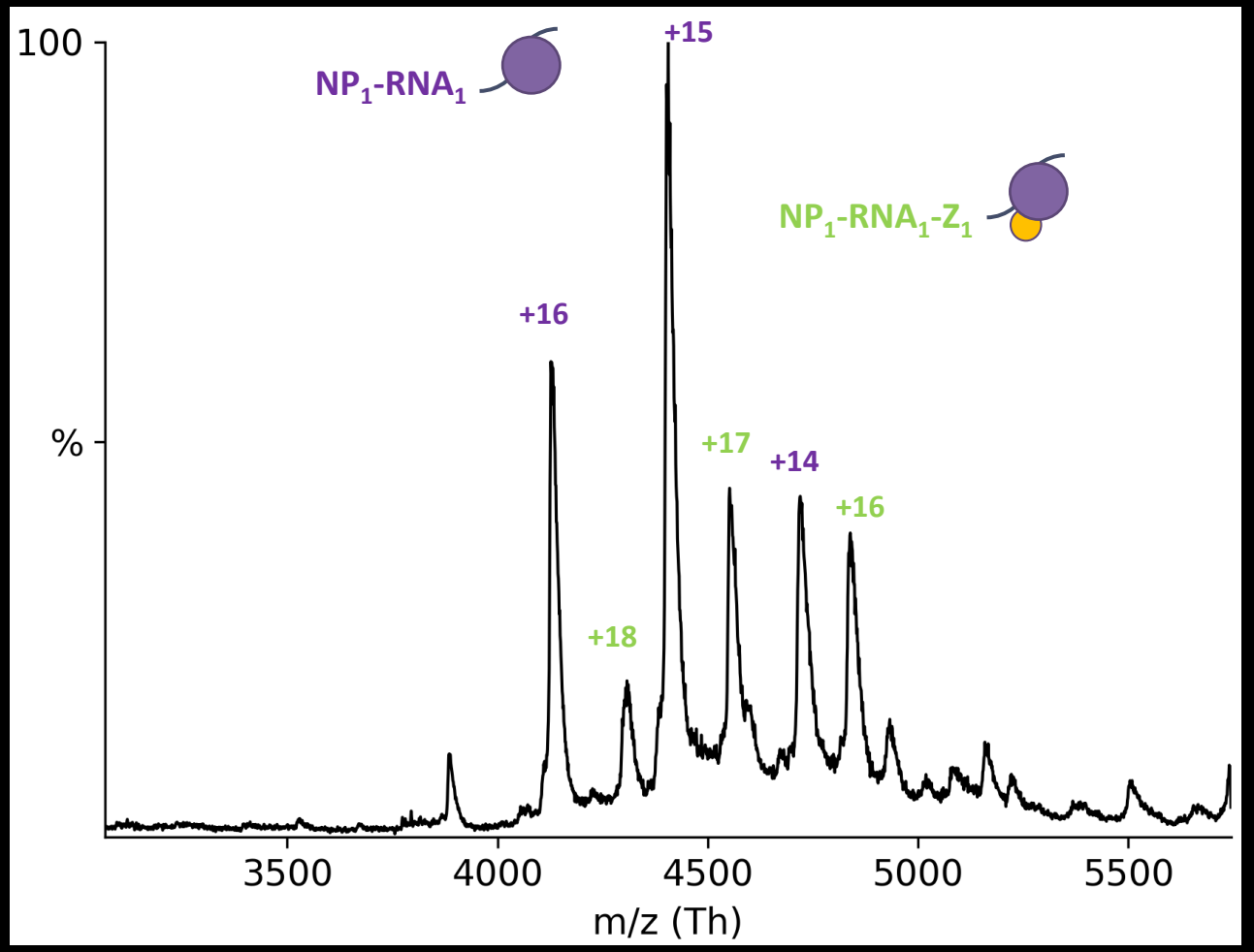




Sänger *et al. biorxiv* **2023** *J Am Chem Soc* accepted

NP & RNA & Z

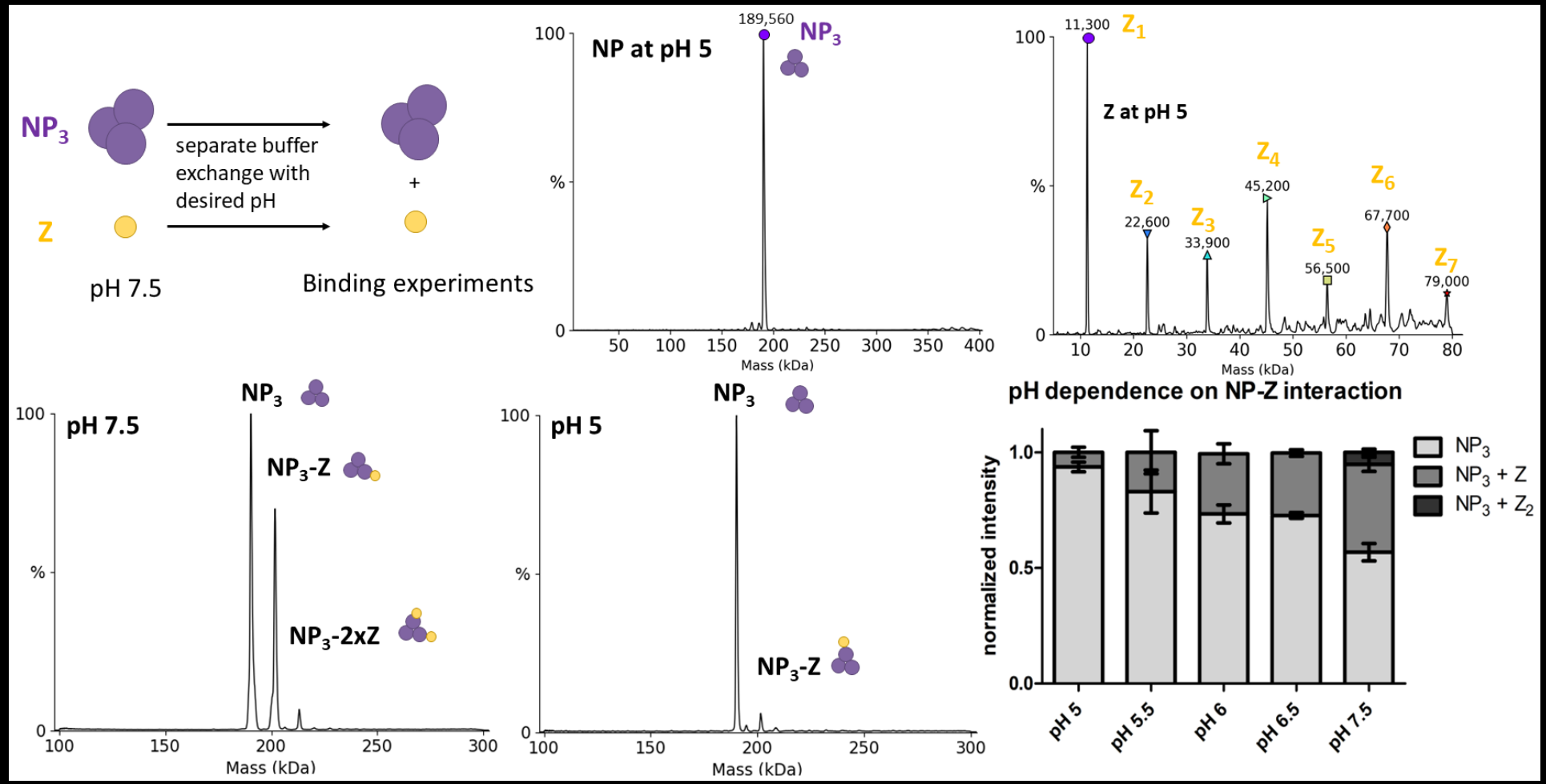
- RNA & Z bind simultaneously



Sänger *et al. biorxiv* **2023** *J Am Chem Soc* accepted

NP & RNA & Z

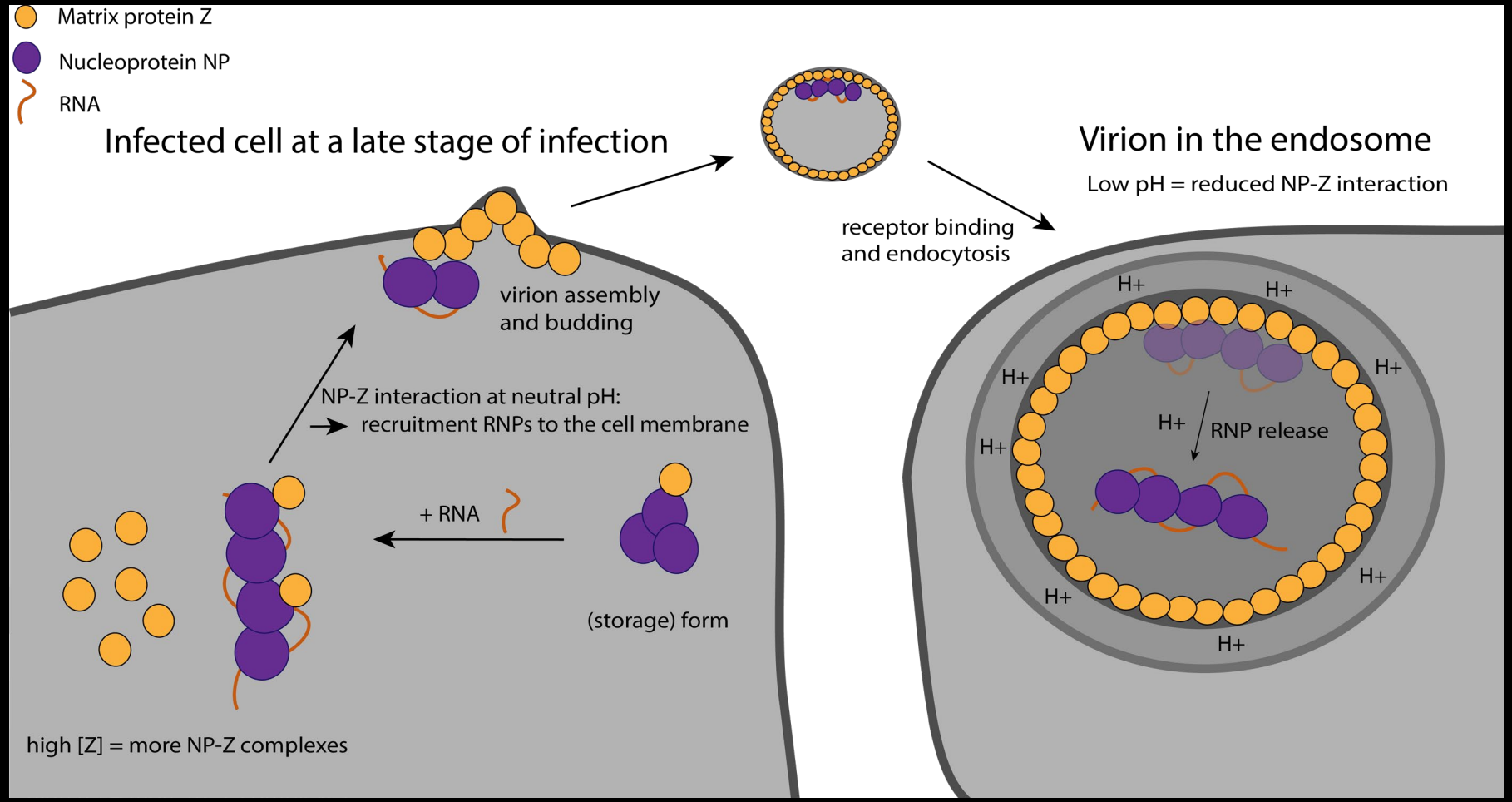
- Z oligomerization & dissociation at low pH



Sänger *et al. biorxiv* **2023** *J Am Chem Soc* accepted

Model

- RNP assembly, recruitment and uncoating

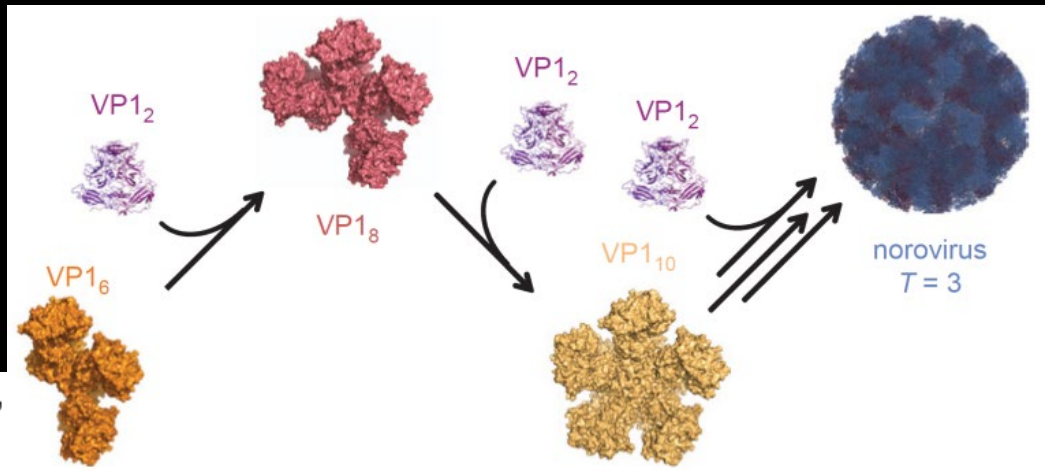


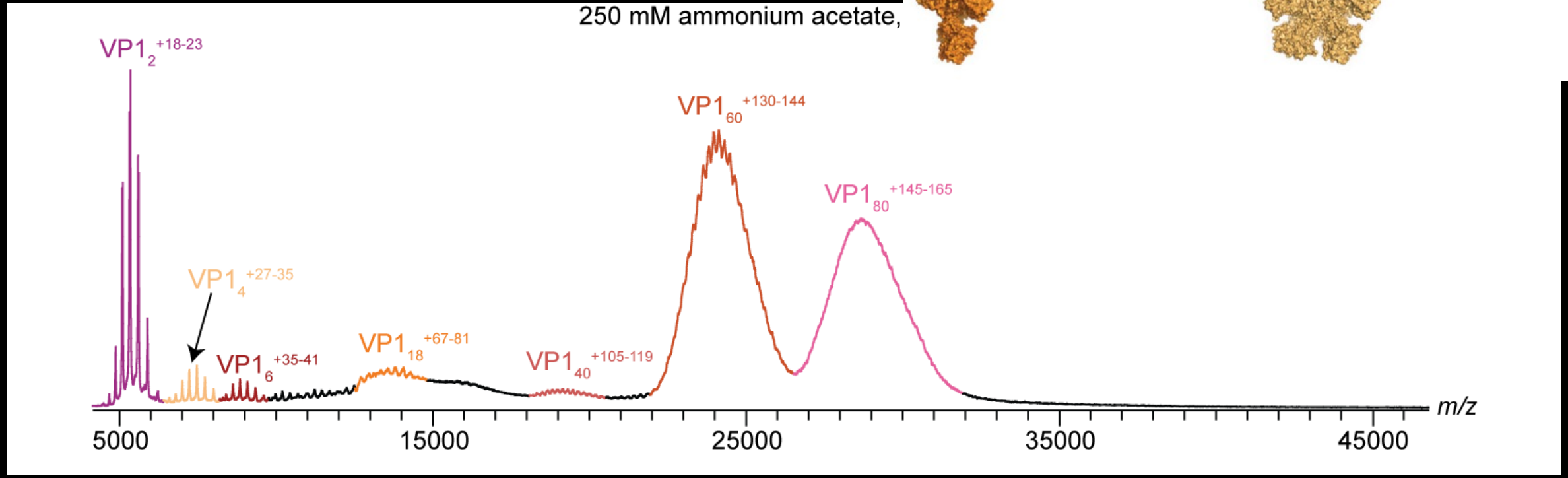
Sänger *et al. biorxiv* **2023** *J Am Chem Soc* accepted

Structure of assembly intermediates?

- Assembly model from ion mobility data
- Nucleation – fine-tuned process
- Low abundant intermediates

→no detailed structures

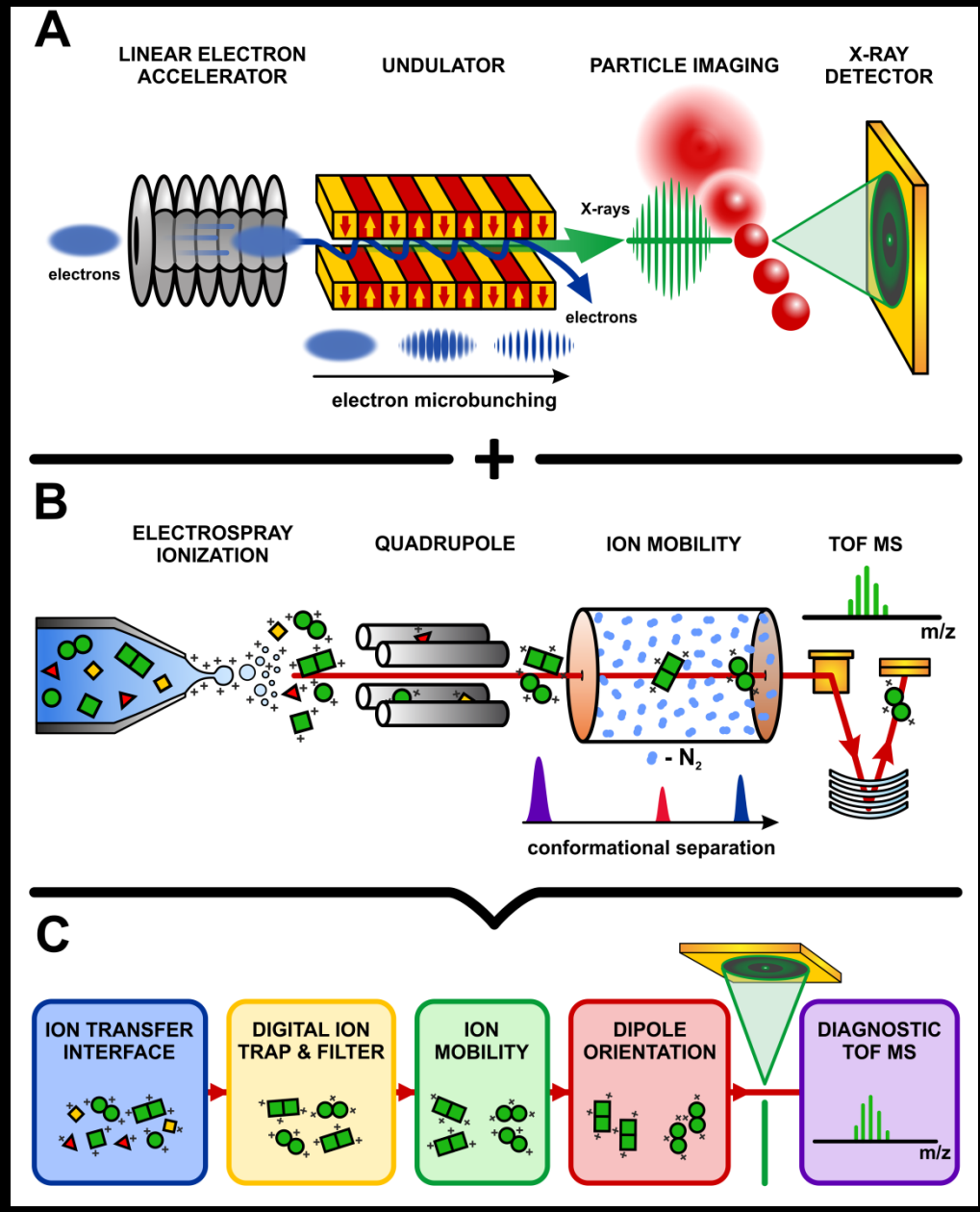




Shoemaker *et al.*, *Mol Cell Proteomics* **2010**, Uetrecht et al., *Nat Chem* **2011**

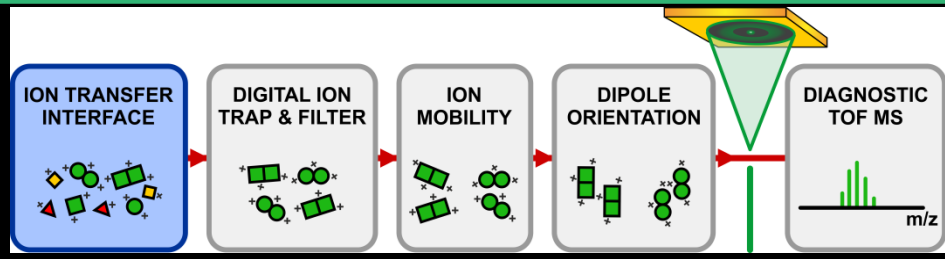
Why use native MS at European XFEL?

- *Imaging before destruction*
  - Potential for transient / low abundant states - sorting problem
  - Successful scattering down to GroEL (SQS, F Maia et al)
  - Use EuXFEL for SPI
- Native MS for selectivity and low background
  - Gas phase structural integrity (Esser et al PNAS 2022)
- *Imaging on the fly*

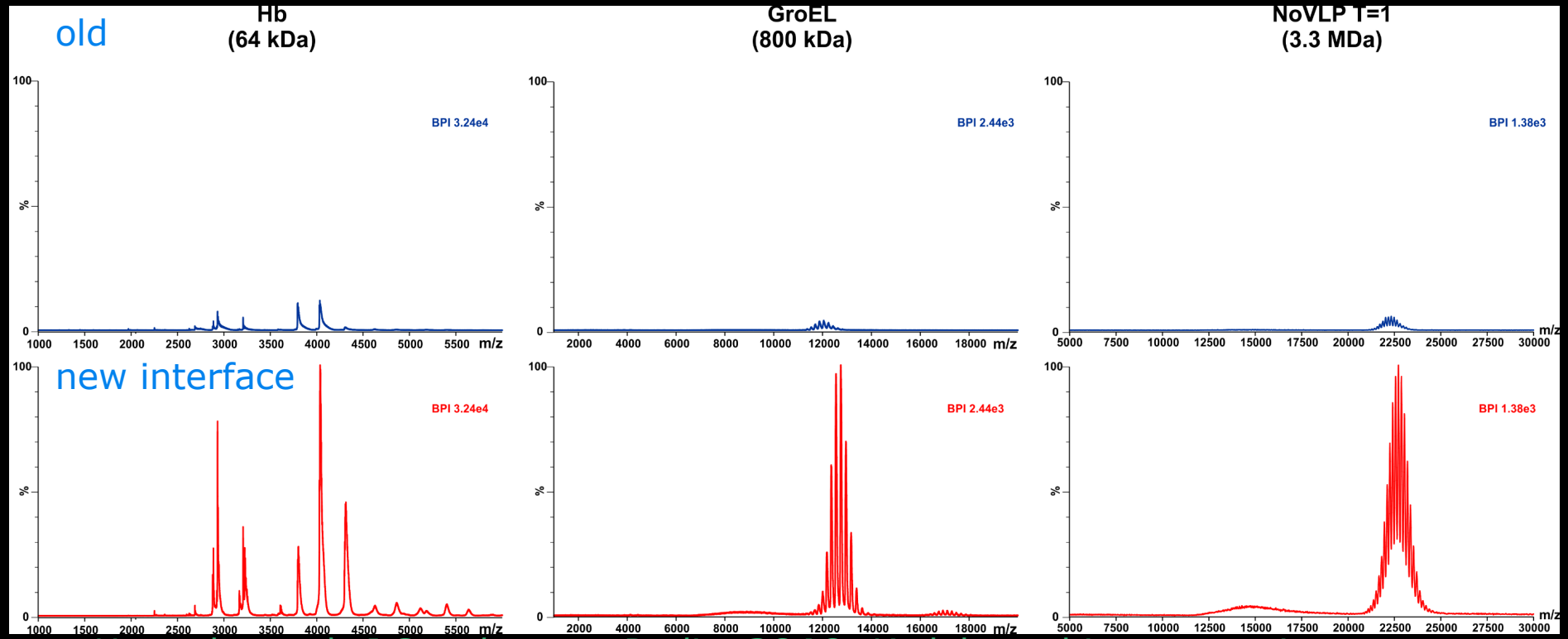


 Kadek et al., *Drug Discov Today Tech* **2021**

Why use native MS at European XFEL?

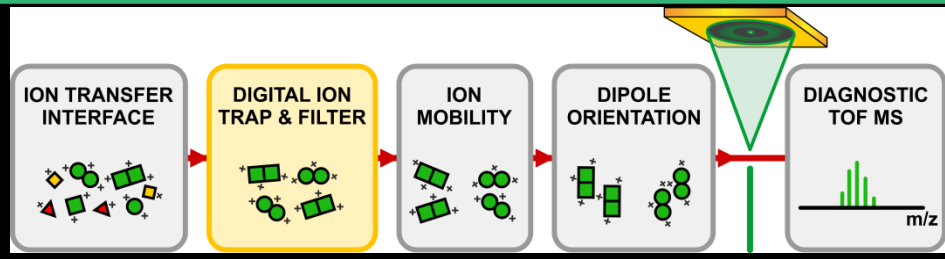


- nanoESI
  - low background & sample consumption
  - 10,000 patterns in 16 min with 1 µm focus
  - Aerolens (Fasmatech)

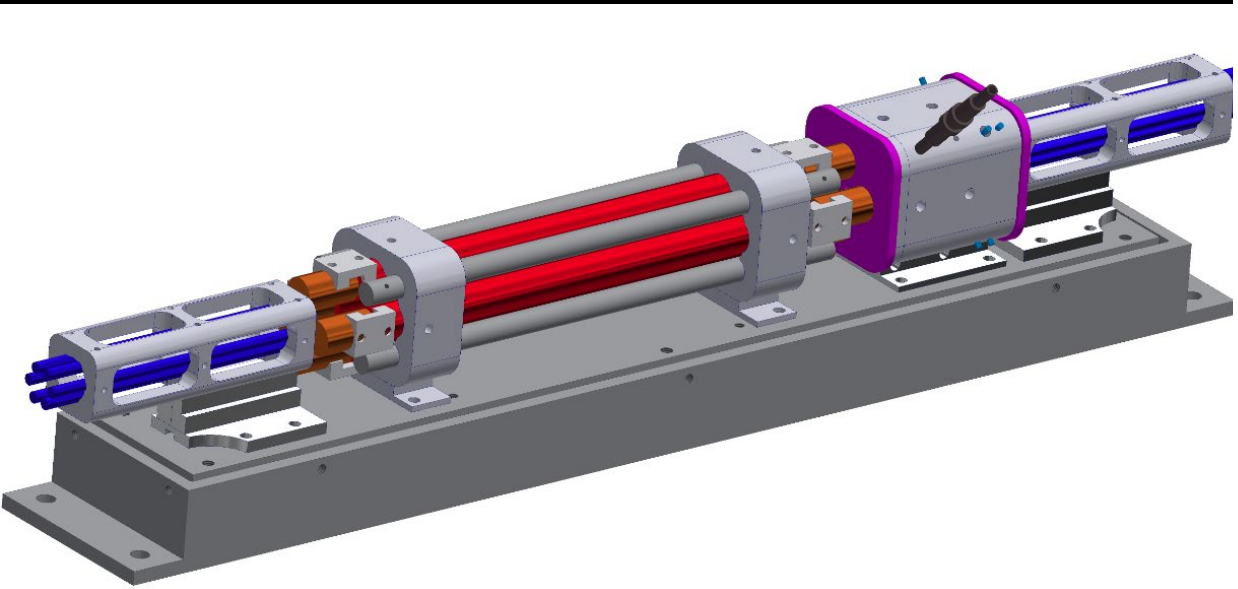


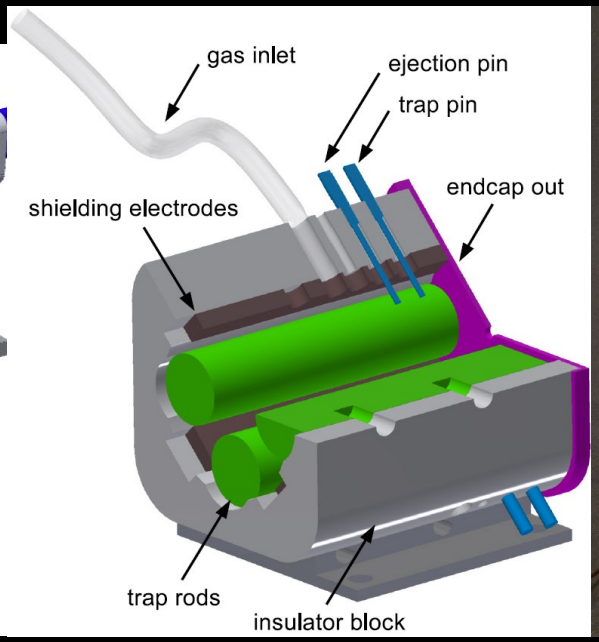
Uetrecht et al. *J Synchrotron Radiat* **2019;** Kadek et al in preparation

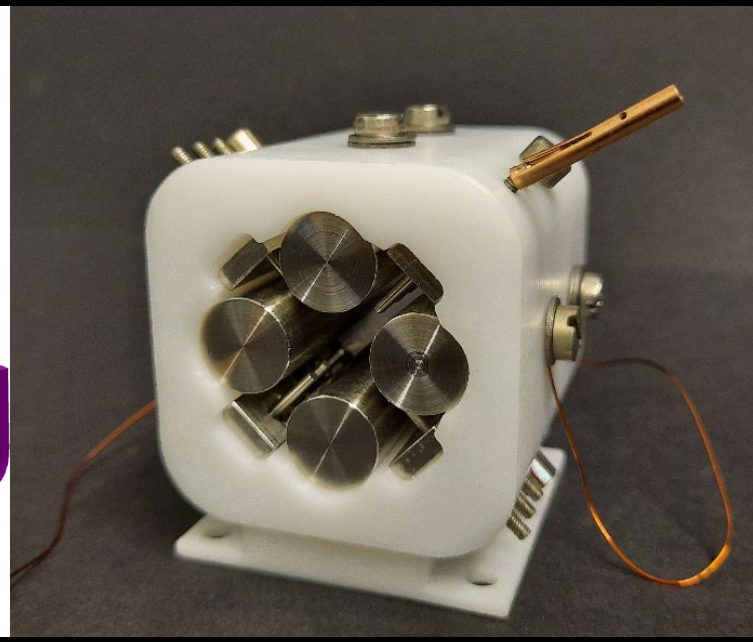
Why use native MS at European XFEL?



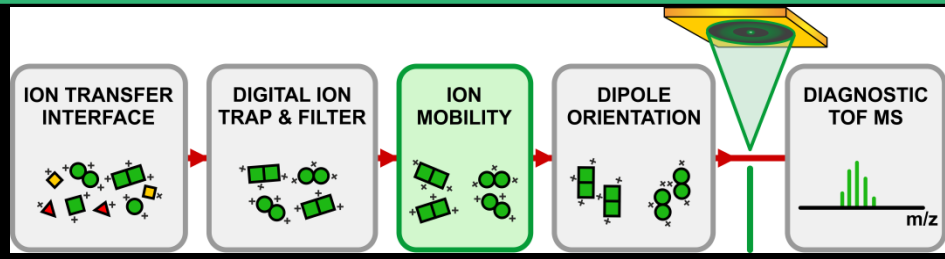
- Digital quadrupole & trap (Greifswald)
  - Mass selection → purify low abundant species
  - Time particle release
  - Increase ion density
  - Trapping capacity for 100 ms
  - No structural damage



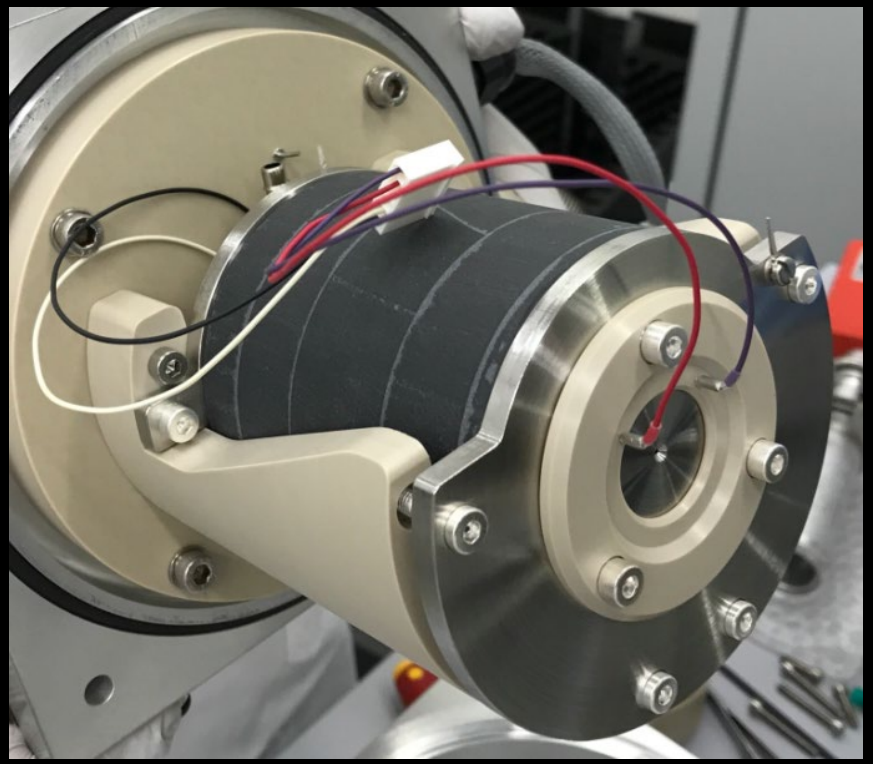


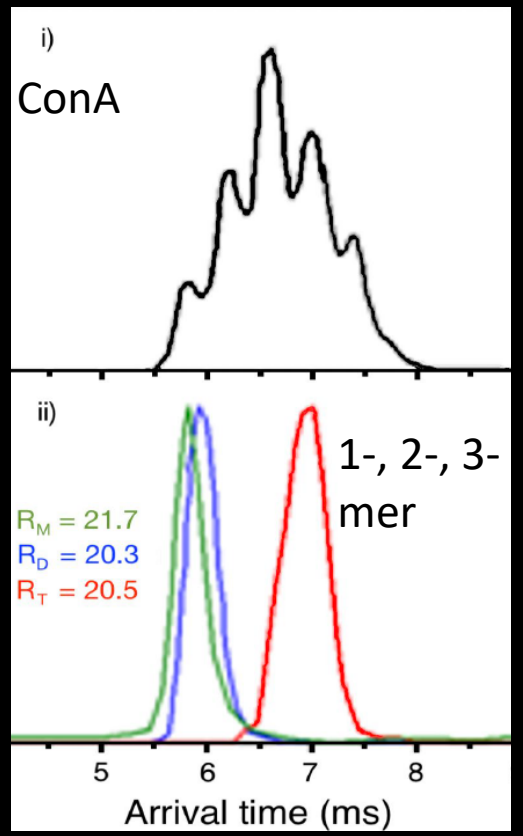


Why use native MS at European XFEL?



- Ion mobility separation (Manchester/ MS Vision)
  - Conformational separation

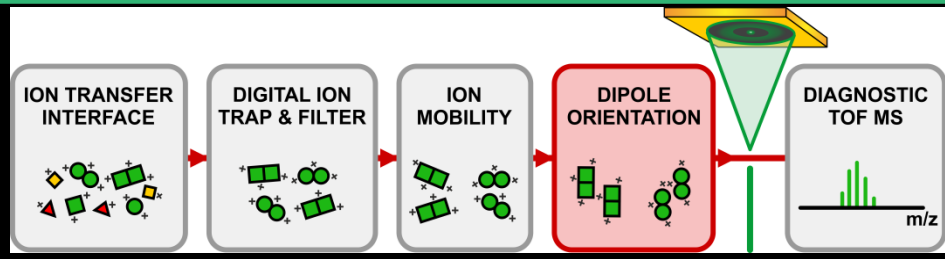


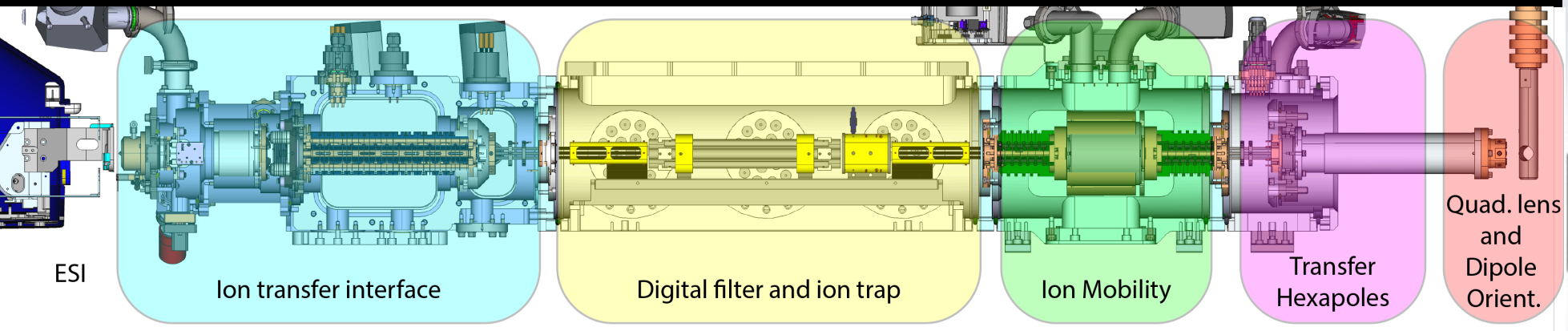


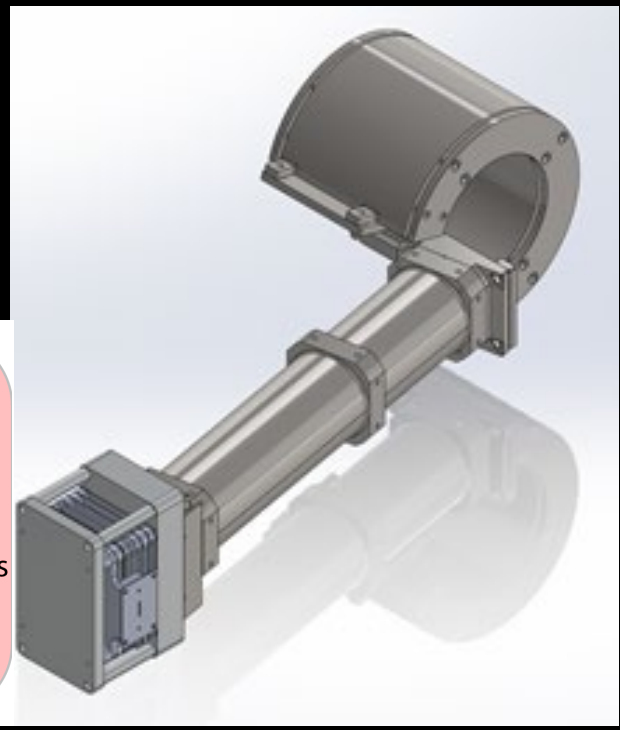
Unpublished

Why use native MS at European XFEL?

- Dipole orientation (Uppsala, Fasmatech)
  - Simplify pattern assembly
  - Use patterns with scarce / missing data
- ToF – online diagnostics (MS Vision, John Hoyes)
  - Sample quality & influx
  - Proper selection







Marklund et al *J Phys Chem Letters* **2017**; Kierspel et al *Anal Bioanal Chem* **2023**; unpublished

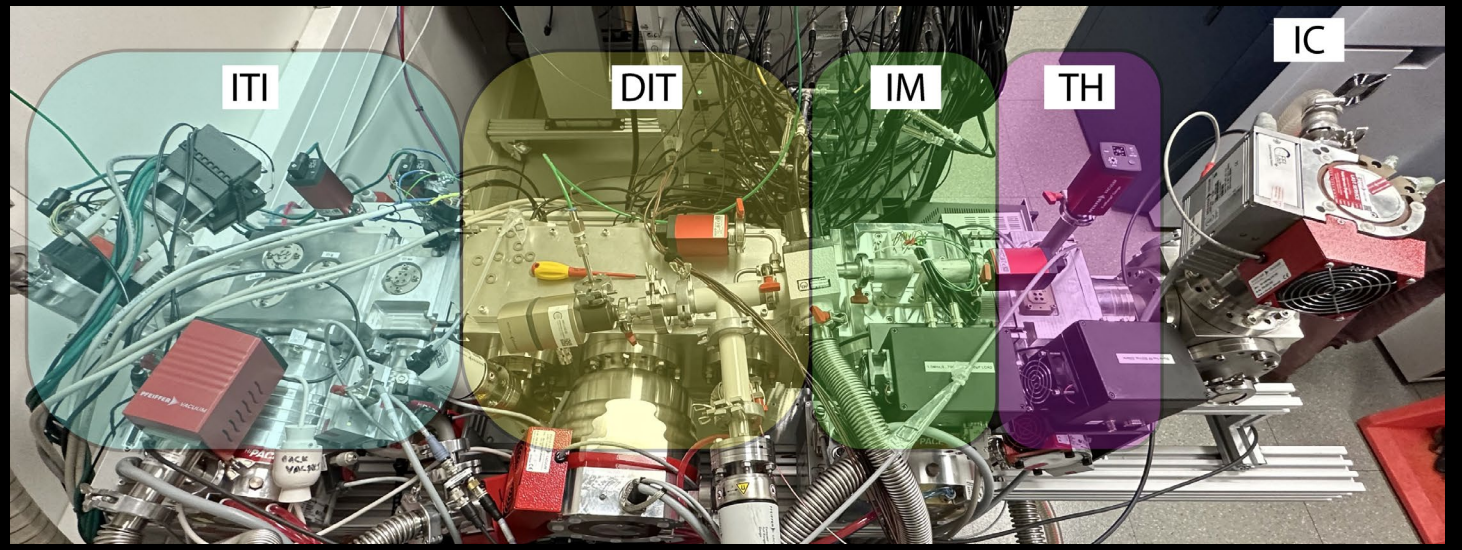
Why use native MS at European XFEL?

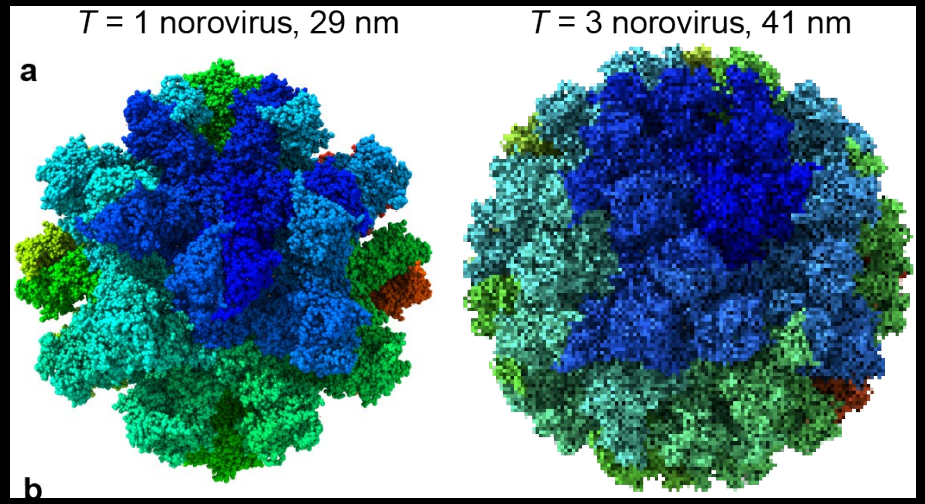
Universität Siegen

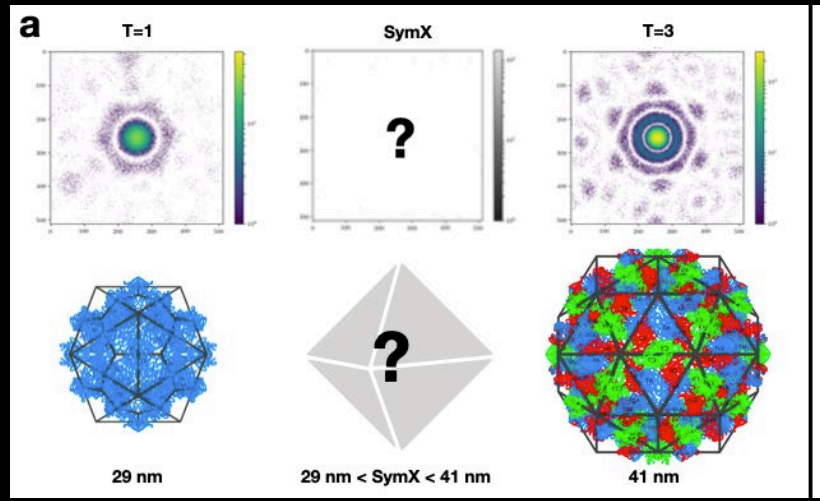
CSSB Centre for Structural Systems Biology

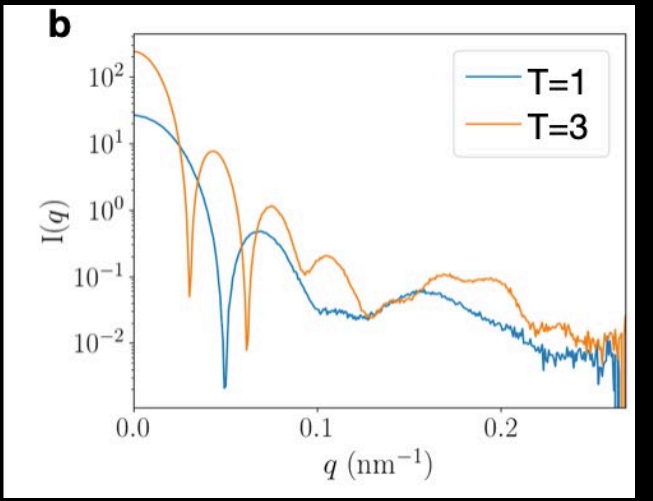
→Proof-of-principle on norovirus

→Identify symmetry





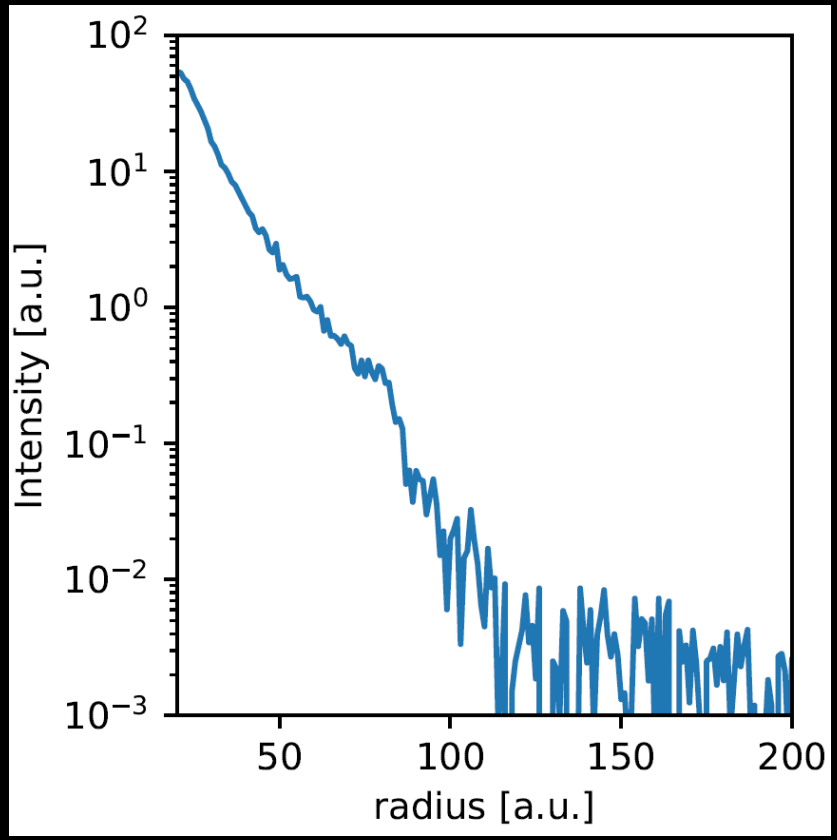


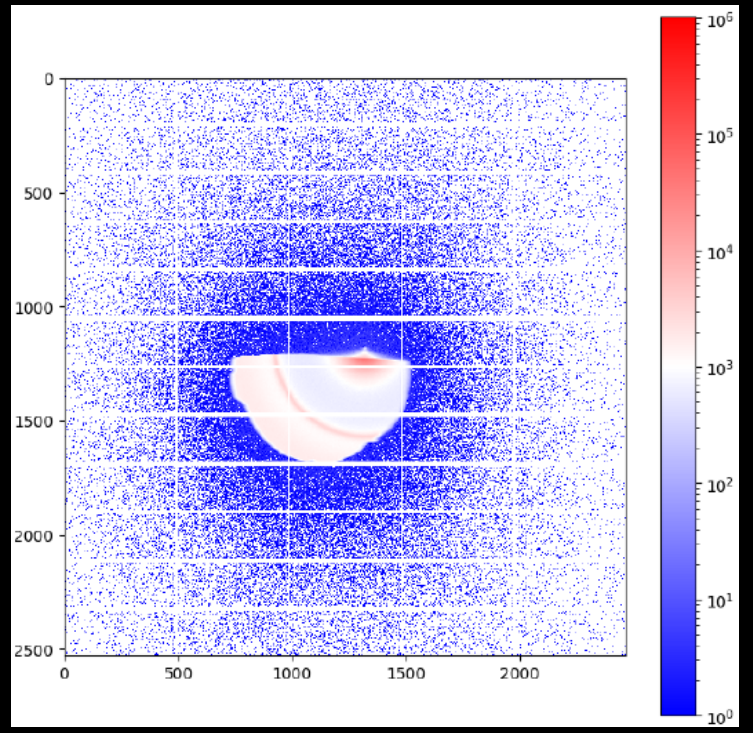


Kierspel et al *Anal Bioanal Chem* **2023**; unpublished

Gas phase SAXS @ P12

- Nov 2022 – dominated by beamline background
- Nov 2023 – signal after background reduction
  - 20 nm Au nanoparticles, 10 min acquisition
- Next stop proteins ☺
  - Heterogenous samples
  - Time resolved

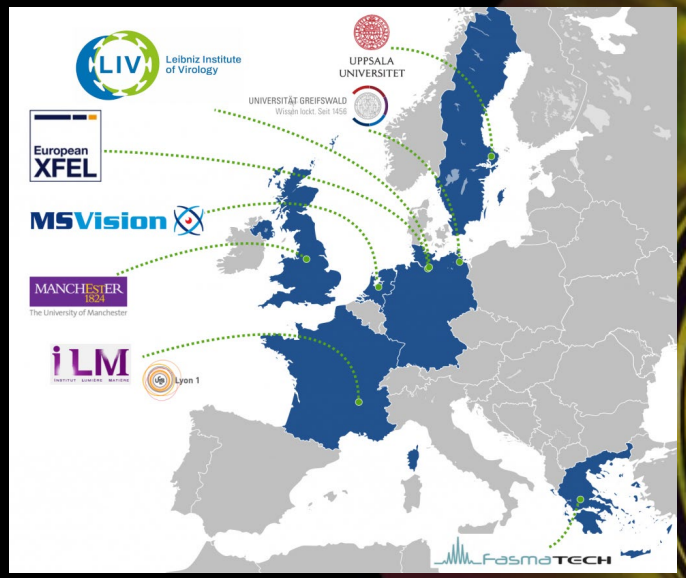




Silver behenate powder diffraction

Acknowledgements

- DoVS: Alan, Jocky, Kira, Lennart, Thomas, Tomislav, Yinfei …
- BNI/CSSB: M Rosenthal; EMBL/CSSB: J Kosinski; EMBL Hamburg: C Blanchet; MS SPIDOC (ms-spidoc.eu); …



- Funding: FET OPEN MS SPIDOC, ERC-StG SPOCk'S MS, BMG Rapid Response, EIC Pathfinder ARIADNE VIBE & VIRUSong, RÅC SAXFELS, BMBF VirMScan, RTG 2771 Humans & Microbes, RTG 2887 VISION, MSCA DN SPI*Doc's*

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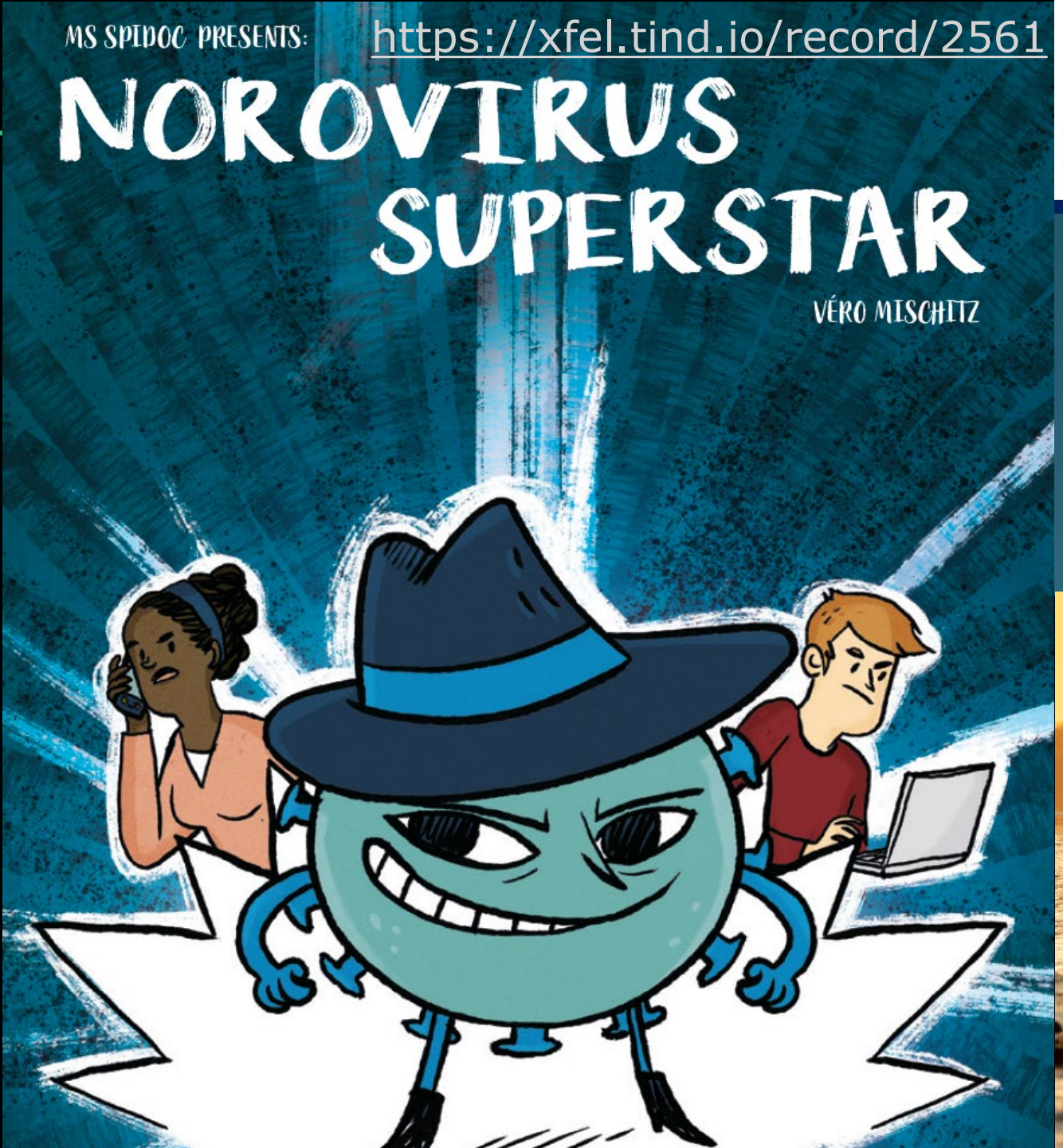

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