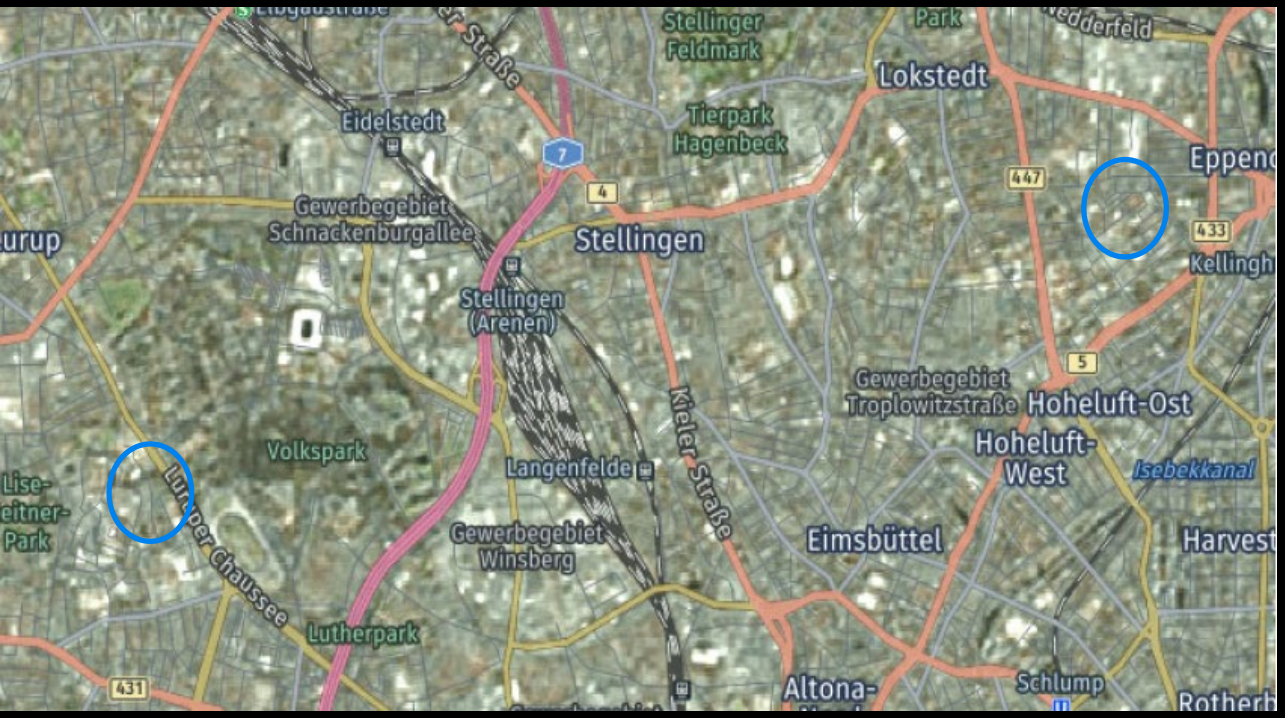
Heinrich Pette Institute, Leibniz Institute for Experimental Virology
and European XFEL GmbH

Charlotte Uetrecht

Flying viruses - from biophysical to structural characterisation

HPI – member of Leibniz association

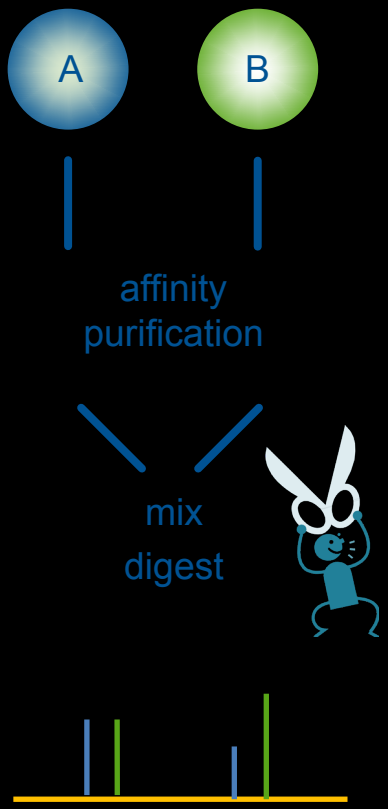
- Belonging to Leibniz association
- Focus on human pathogenic viruses



MS in structural biology

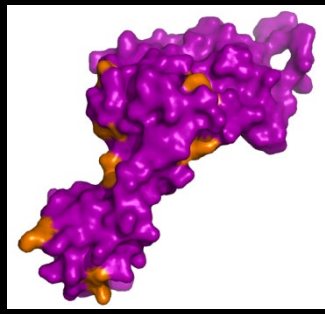
Protein identification

- Bottom up or top down proteomics
- Post-translational modifications
- New binding partners



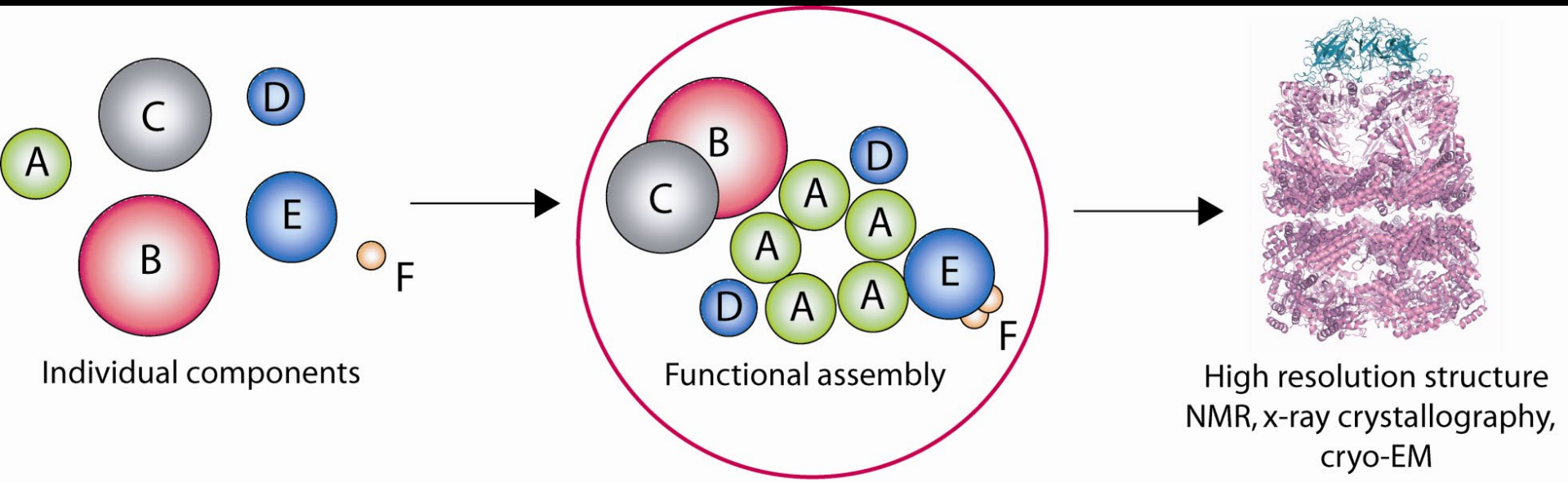
Chemical modification

- Surface labelling
- Cross-linking
- Hydrogen/ deuterium exchange
- Binding interfaces
- Conformation and folding



MS in structural biology

- Higher order assemblies as functional form
- Native MS: complex dynamics at low resolution



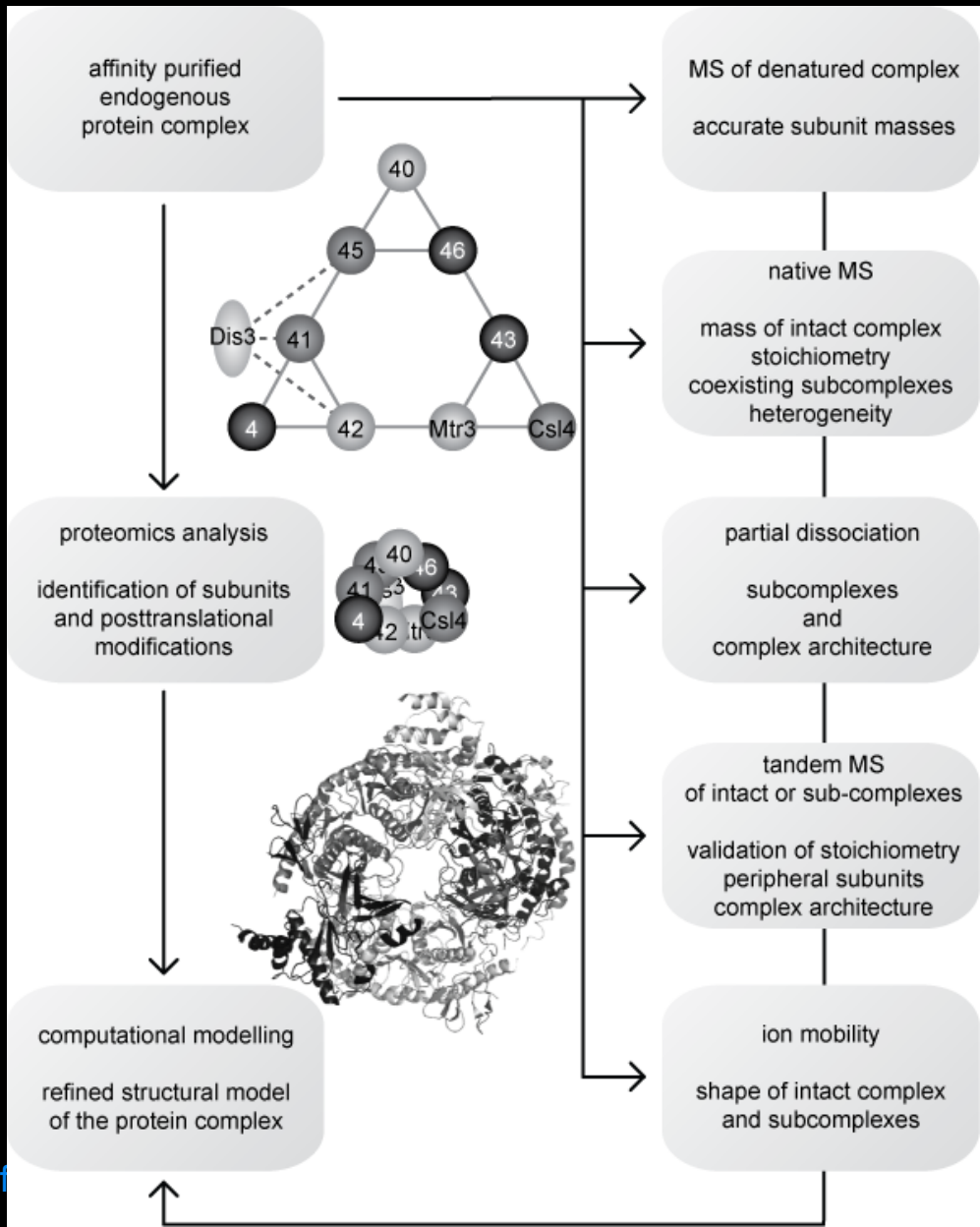
Native mass spectrometry

large complexes (>1 MDa)
selectivity, sensitivity
complex dynamics and stability

Native MS workflow

- Affinity purification
- *in vitro* assembly
- (Whole cell top-down)

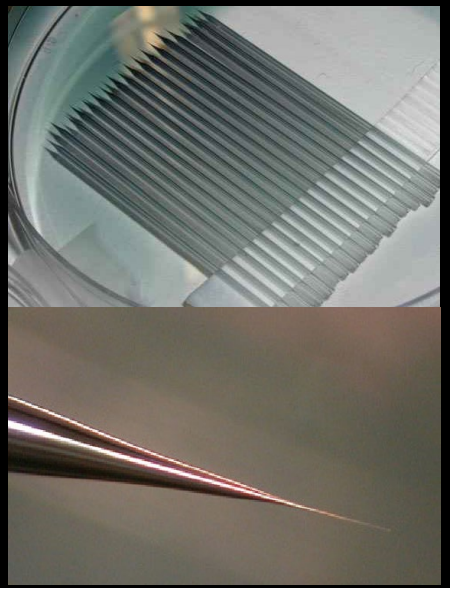
- Global structure
  - Stoichiometry
  - Topology
  - Shape
  - Dynamics
  - Binding affinity

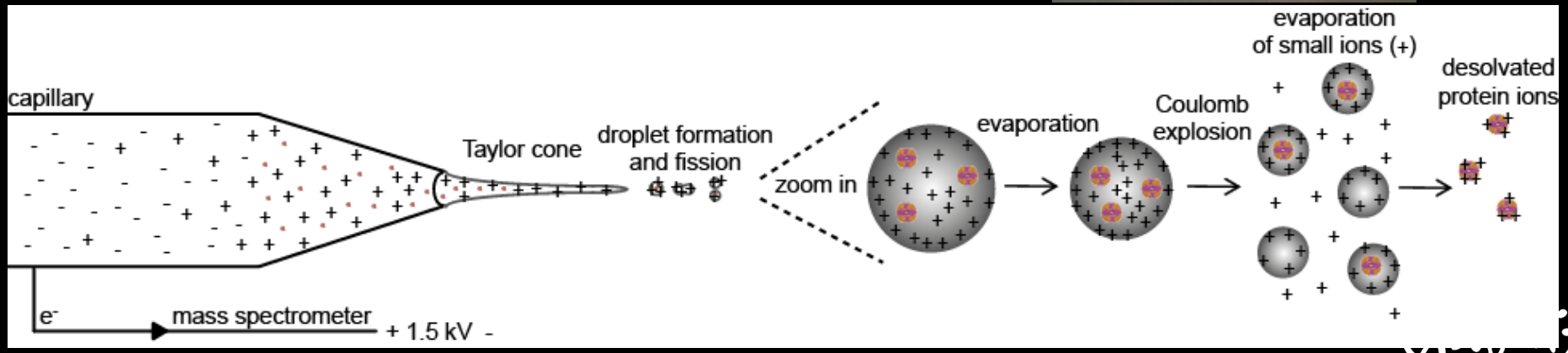


Nano-ESI ToF

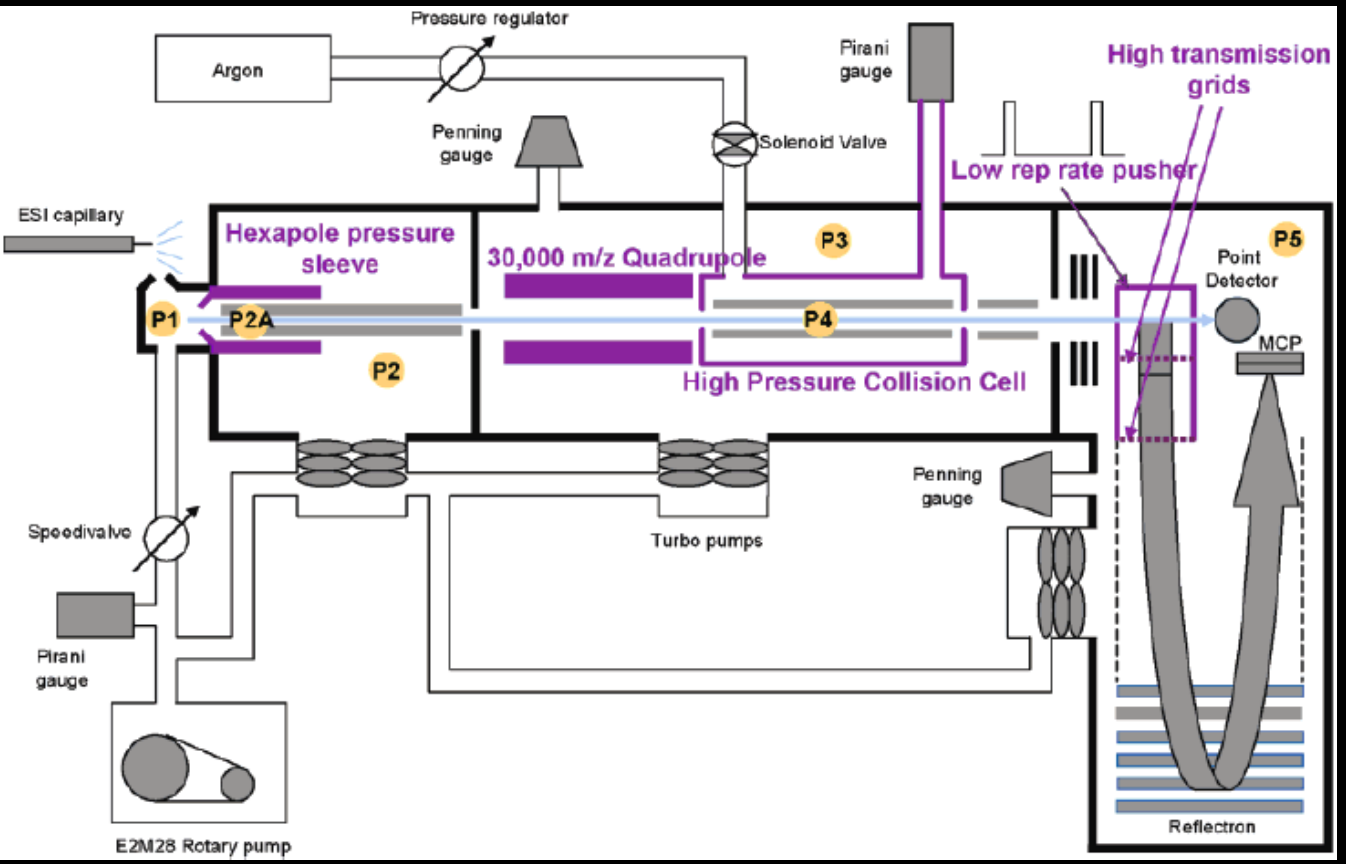
- Gentle like MALDI
- Sample in solution
- Home-made capillaries
- Positive or negative ion mode

- “unlimited” mass range in time of flight





Q-ToF



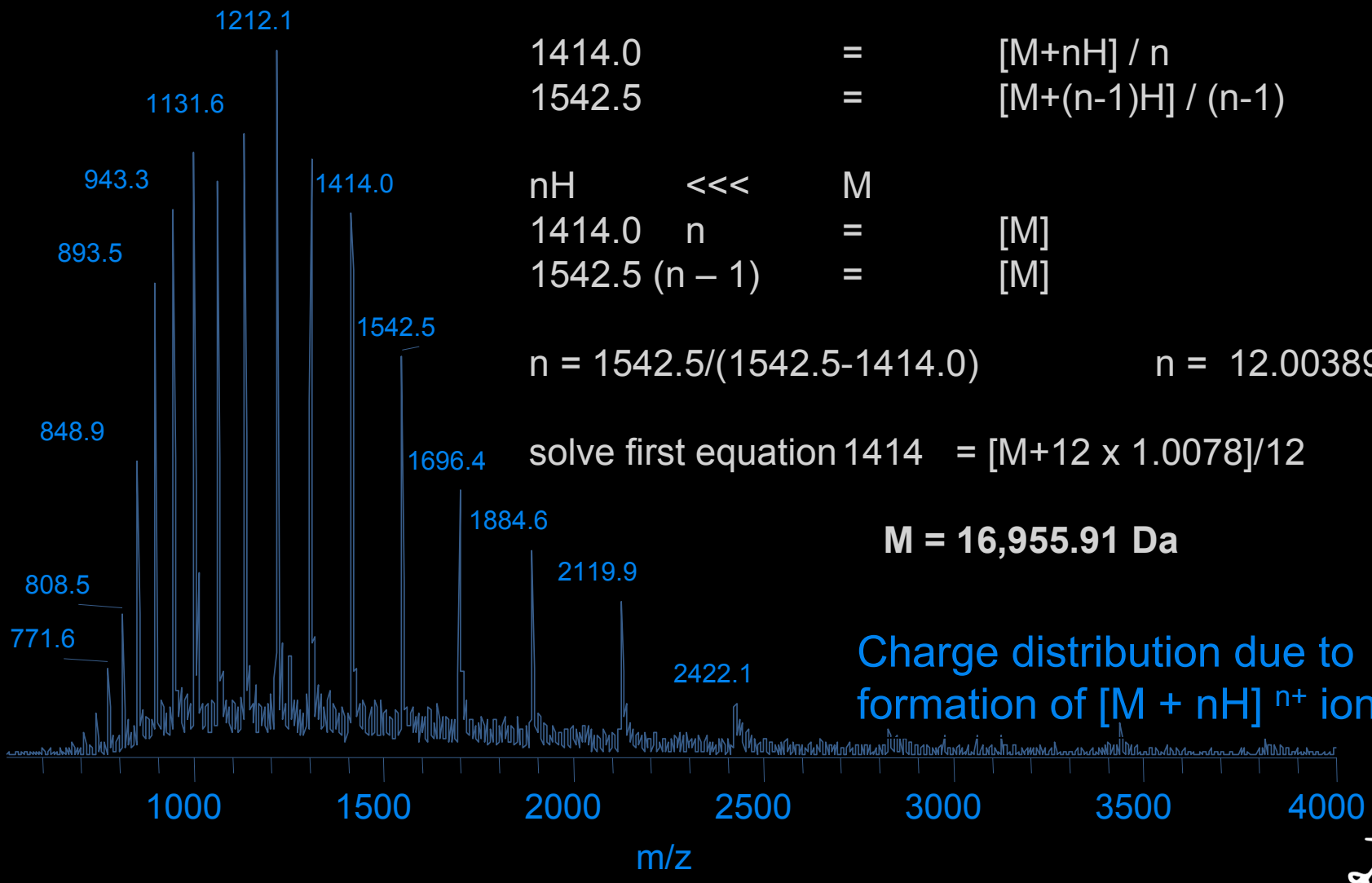
1. Hexapole pressure sleeve
2. Low frequency ion selecting quadrupole
3. High pressure collision cell
4. High transmission grids
5. Low repetition pusher in the TOF

P1: 10 mbar P2A: 8 10^{-3} mbar P2: 4 10^{-3} mbar
P3: 6 10^{-4} mbar P4: 2 10^{-2} mbar P5: 2 10^{-6} mbar

R.H. van den Heuvel *et al., Anal Chem* **2006**

Mass determination

Two adjacent *m/z* peaks with $\Delta z = 1$: *eg.* 1414 and 1542.5



1414.0 = [M+nH] / n
1542.5 = [M+(n-1)H] / (n-1)

nH <<< M
1414.0 n = [M]
1542.5 (n – 1) = [M]

n = 1542.5/(1542.5-1414.0) n = 12.00389 = 12

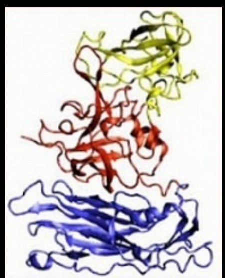
solve first equation 1414 = [M+12 x 1.0078]/12

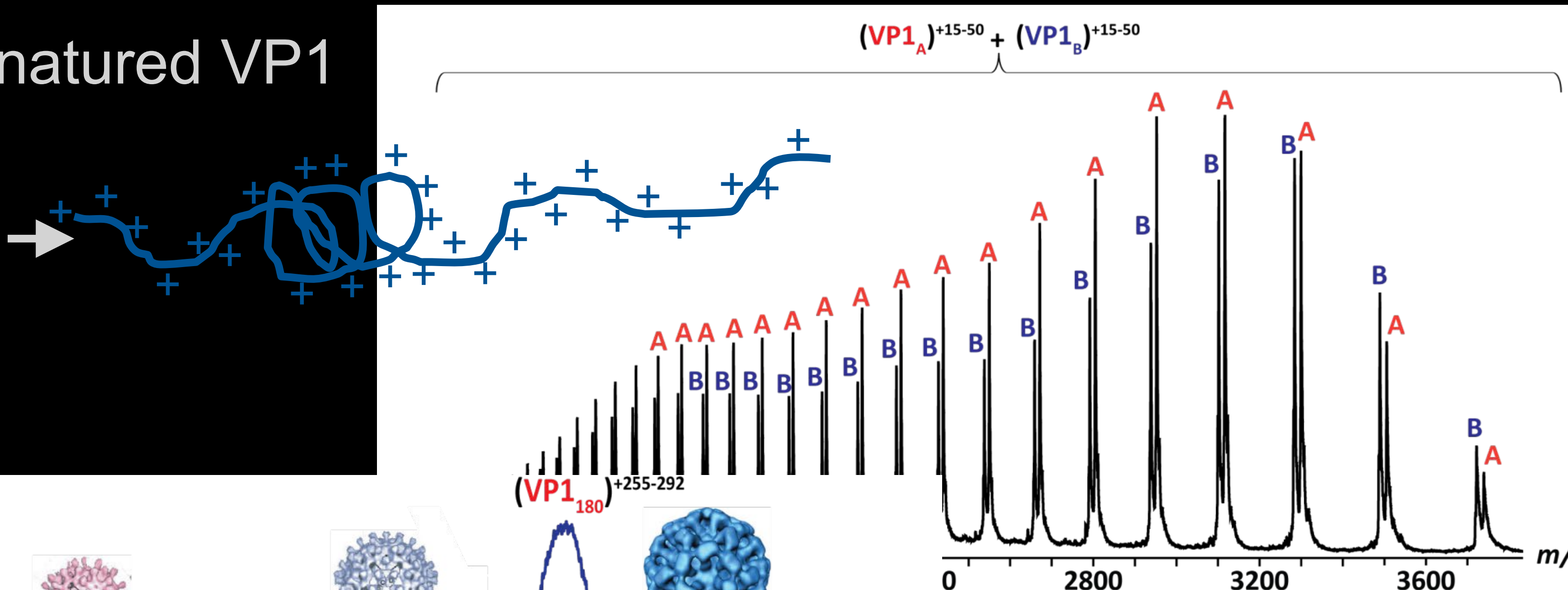
M = 16,955.91 Da

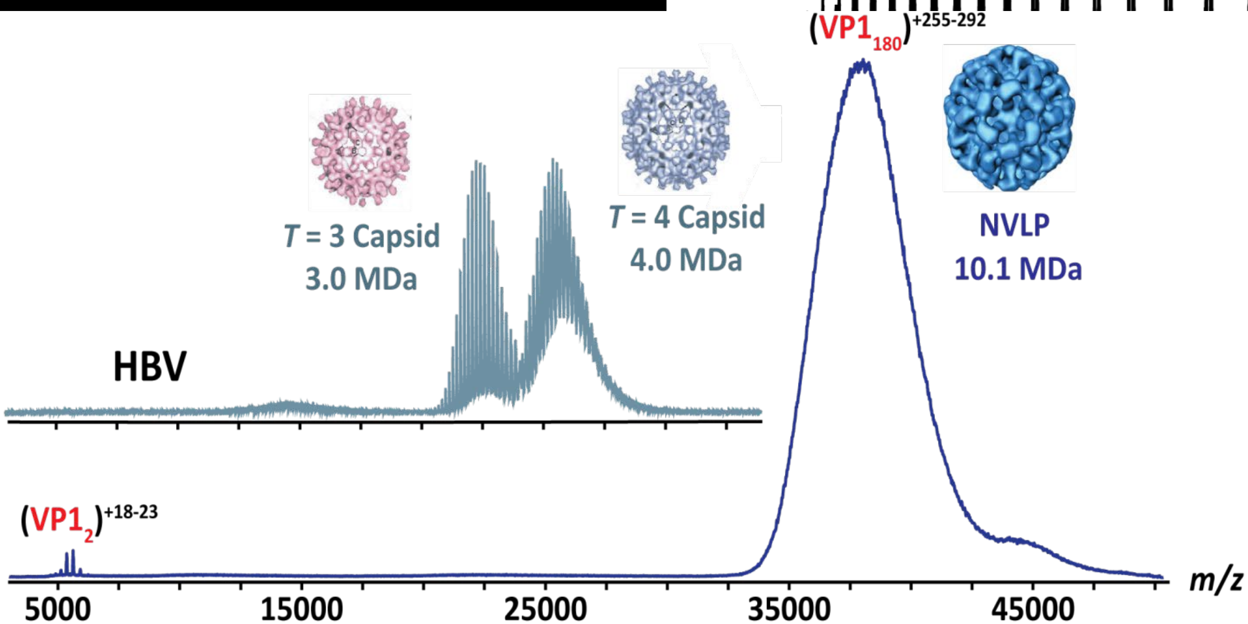
Charge distribution due to formation of $[M + nH]^{n+}$ ions

Native MS - up to viruses

- Denatured VP1







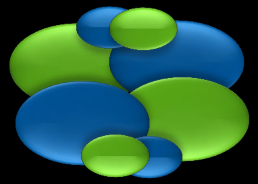
- Buffered solution
→ Intact VLP

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

Leibniz Association

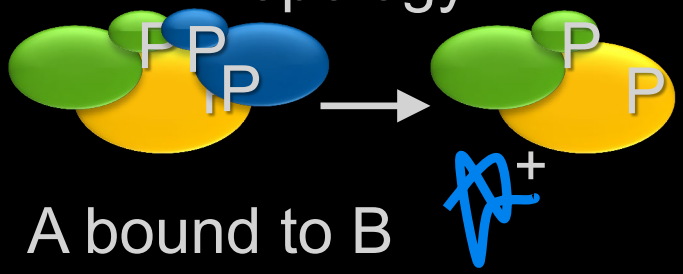
Information from native MS

Stoichiometry



M = xA+yB

Topology

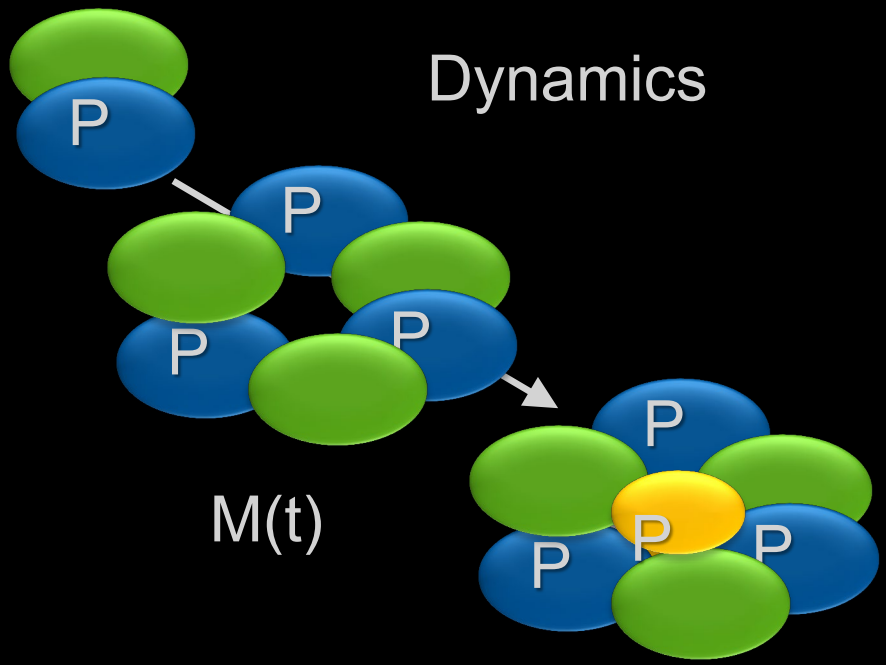


A bound to B

Shape/
Conformational change

Amount A or B

Information from native MS



Dynamics

M(t)

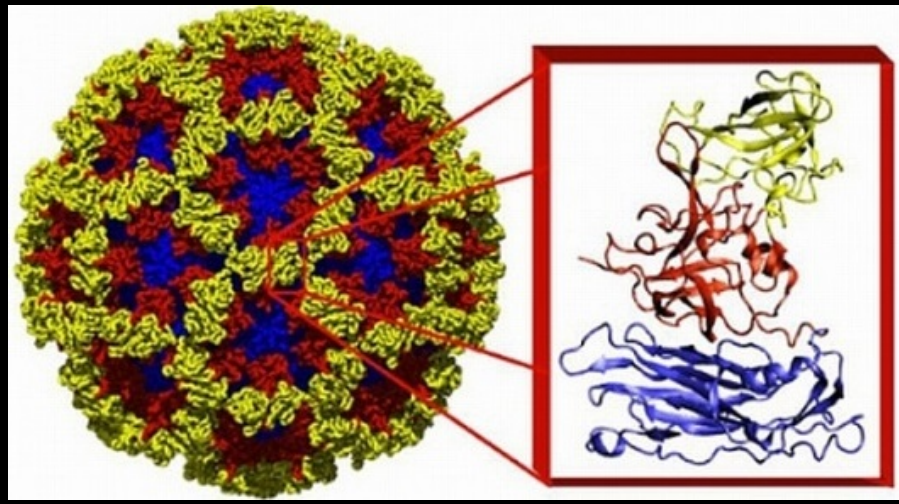
Binding

[M]/[ML]

Norovirus

- *Caliciviridae*
- Main cause of viral gastroenteritis
- Highly contagious

- +ssRNA
- Non-enveloped
- *T* = 3 capsid



- VP1: 530 aa, ~56 kDa
- Shell and protruding domain
- Glycans as attachment factor

B.V.V. Prasad *et al.*, *Science* **1999**

Viral “lifecycle”

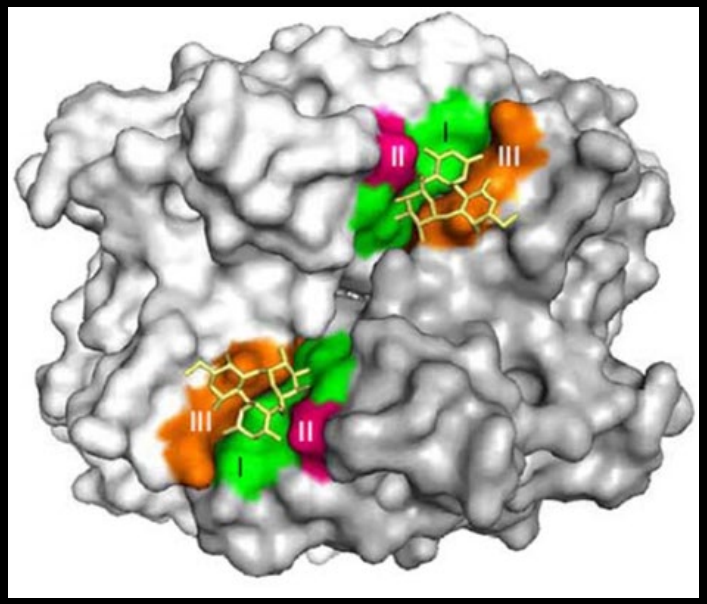
van den Berg *et al.* Circ Res ***2003***

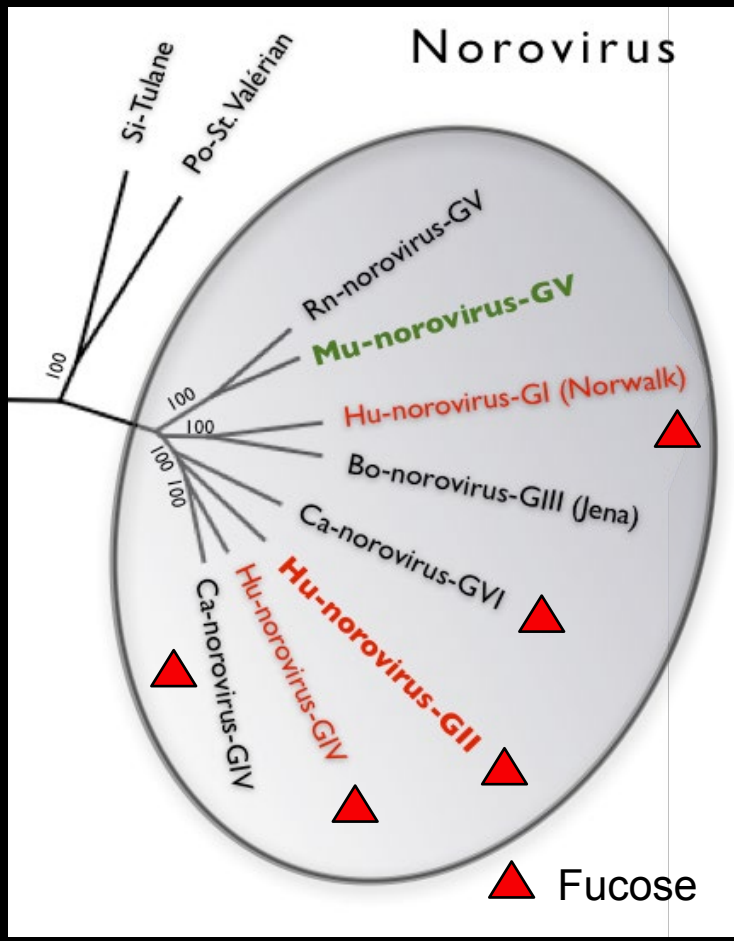
C. Uetrecht & A. Heck, *Angew Chem* **2011**

Norovirus

- Binding studied with P domain
- Fucose as attachment factor

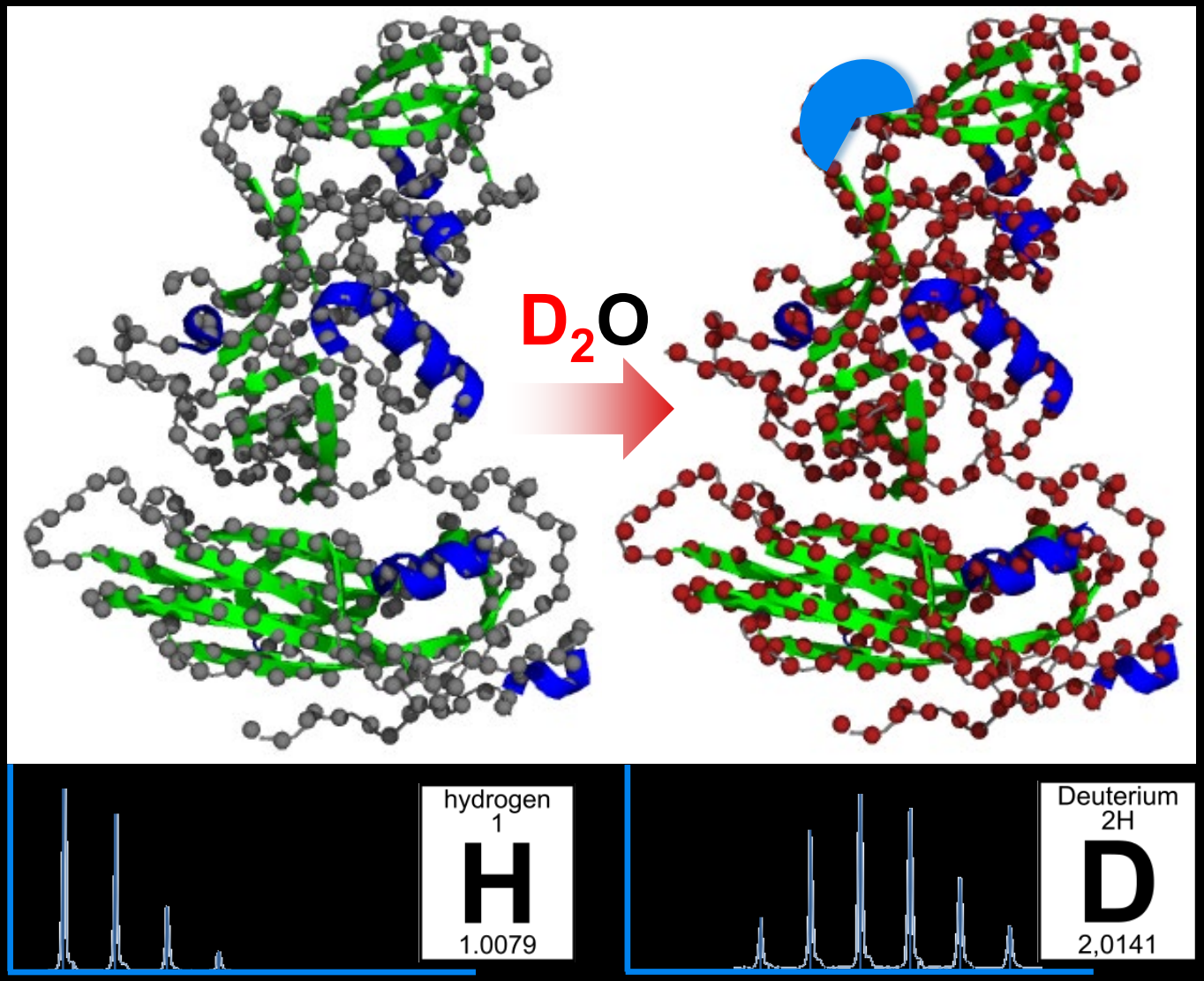
- Effects on structural dynamics?



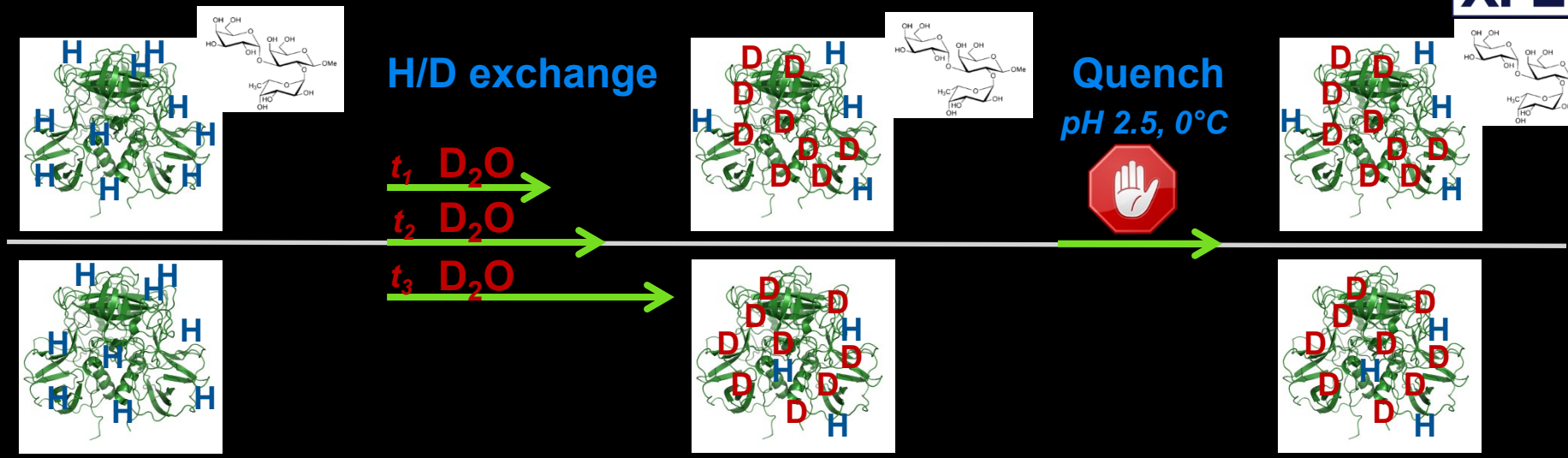


Tan *et al.*, *Plos One* **2009**, Goodfellow & Taube **2015**

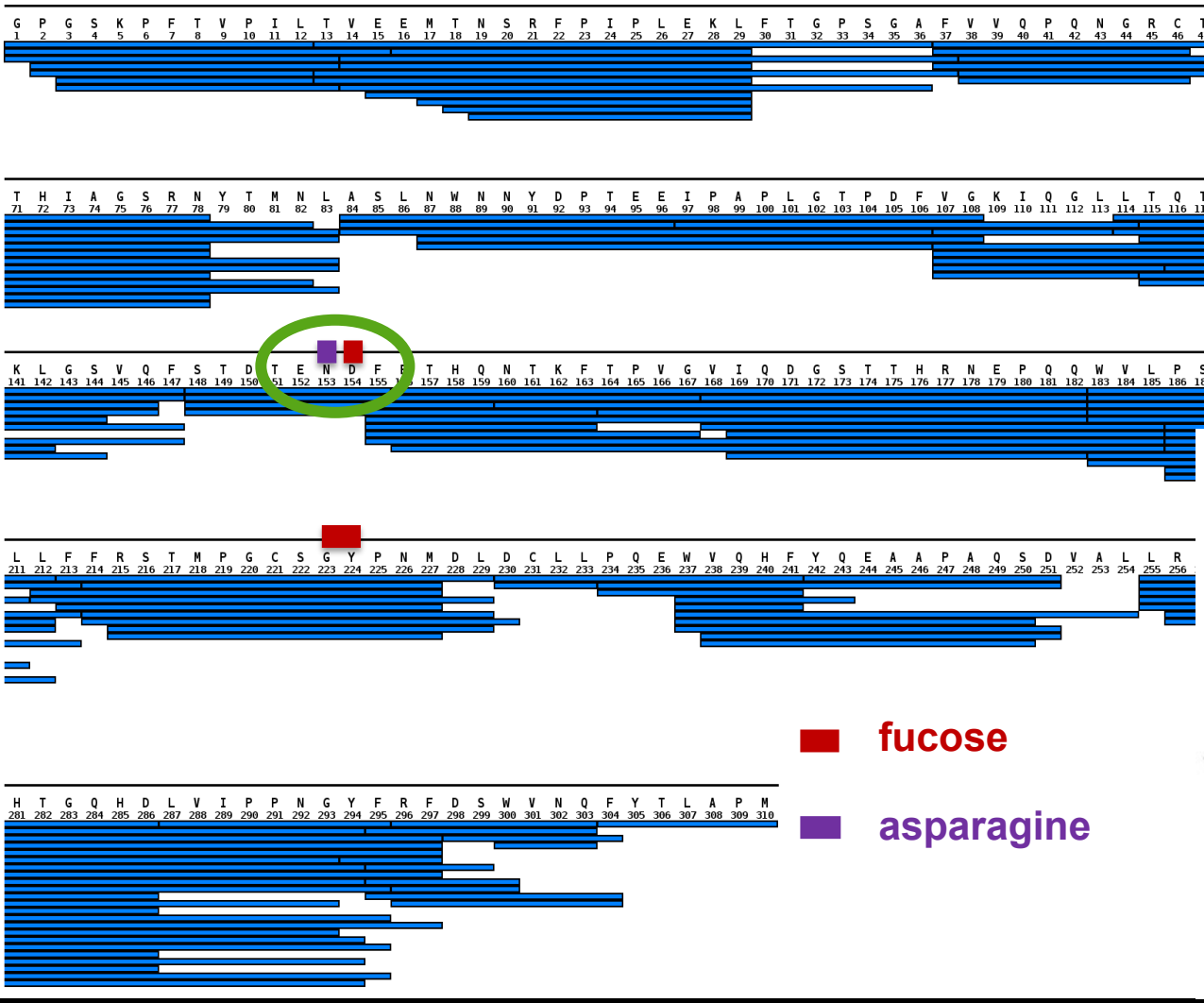
Hydrogen/deuterium exchange

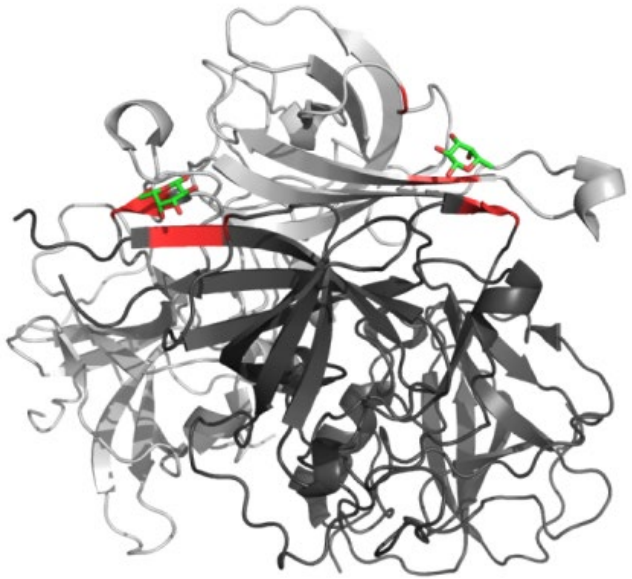


Hydrogen/deuterium exchange



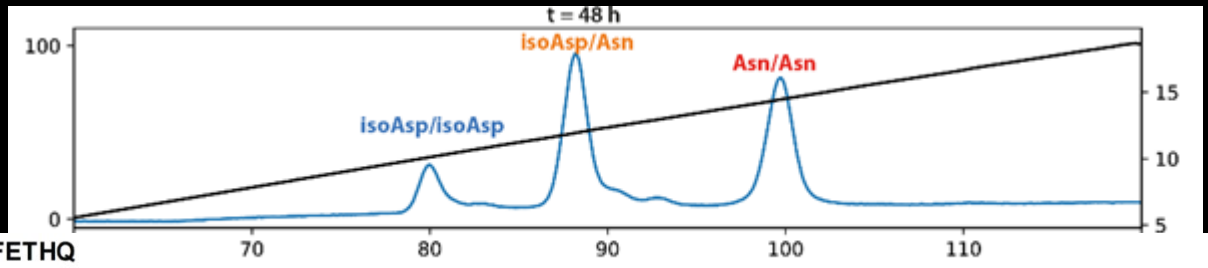
Peptide mapping





A. Mallagaray *et al*,. Nat Commun, 2019

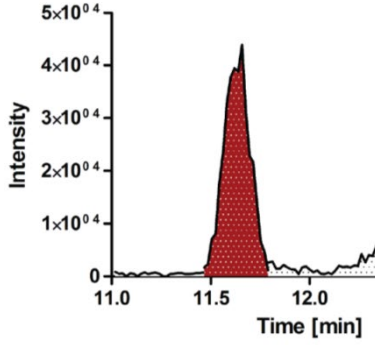
Asparagine deamidation

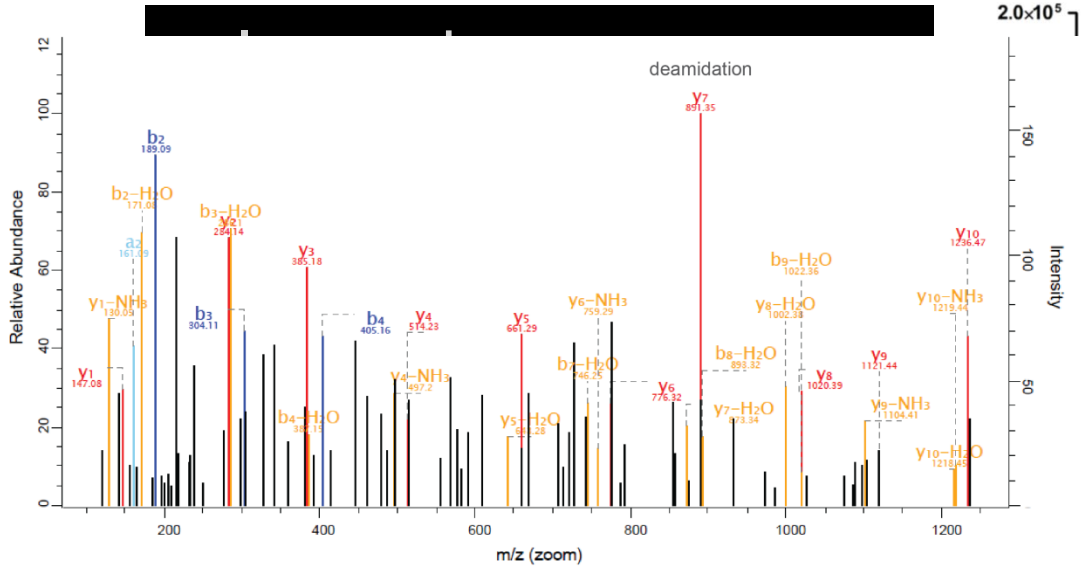


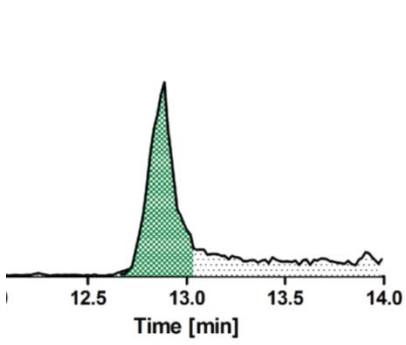
STDTEDDFETHQ

STDTENDFETHQ

Elution volume in ml

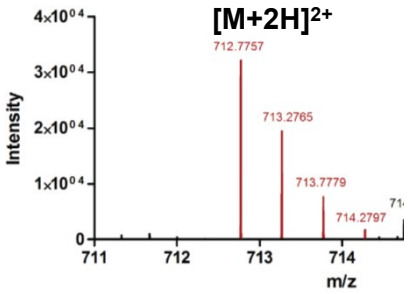


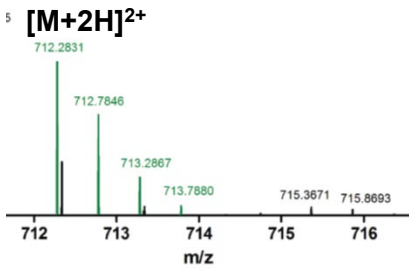


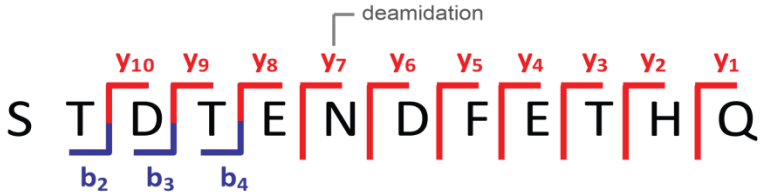


STDTEDDFETH
712.7760

STDTENDFETHQ
712.2840

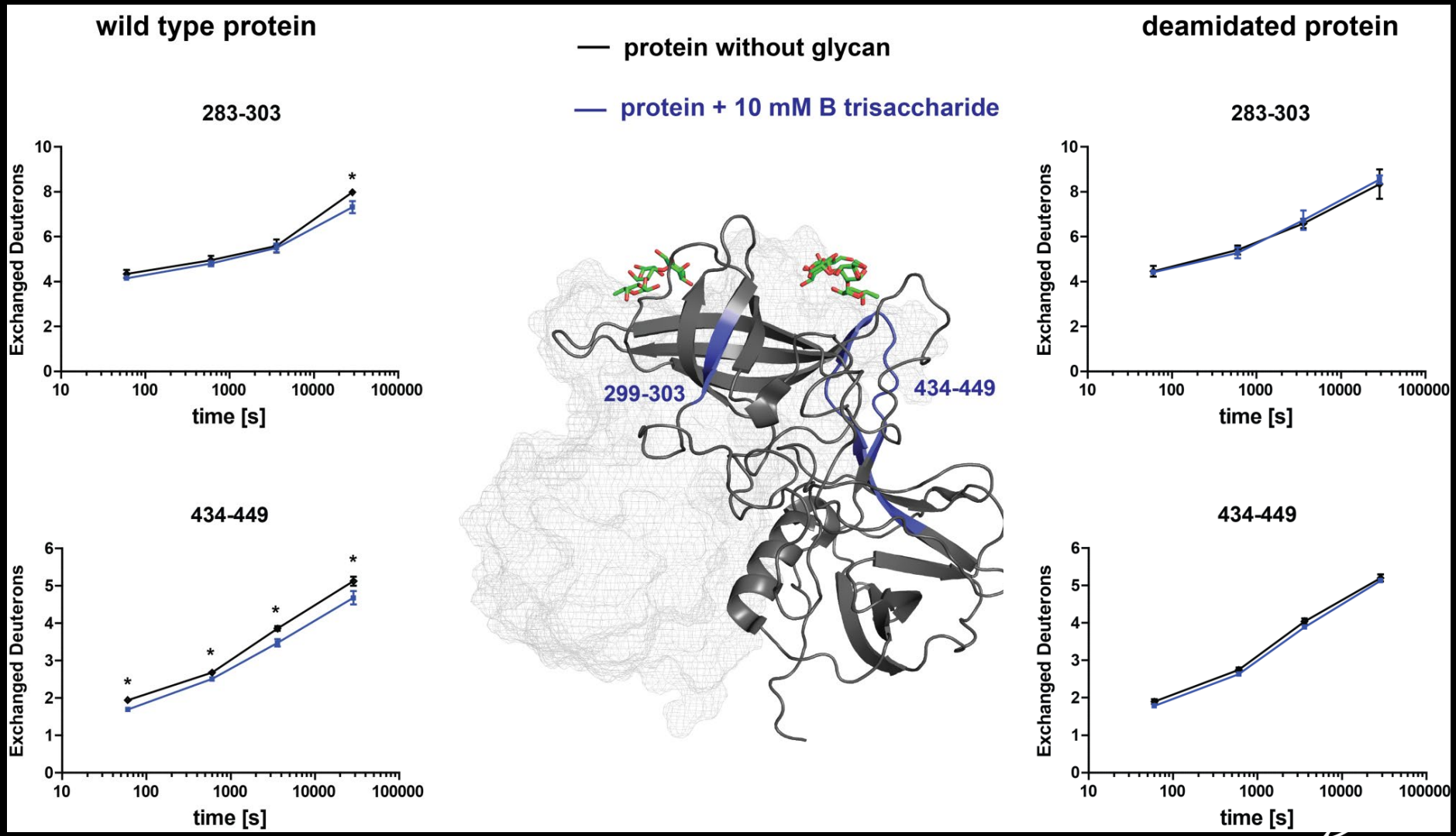






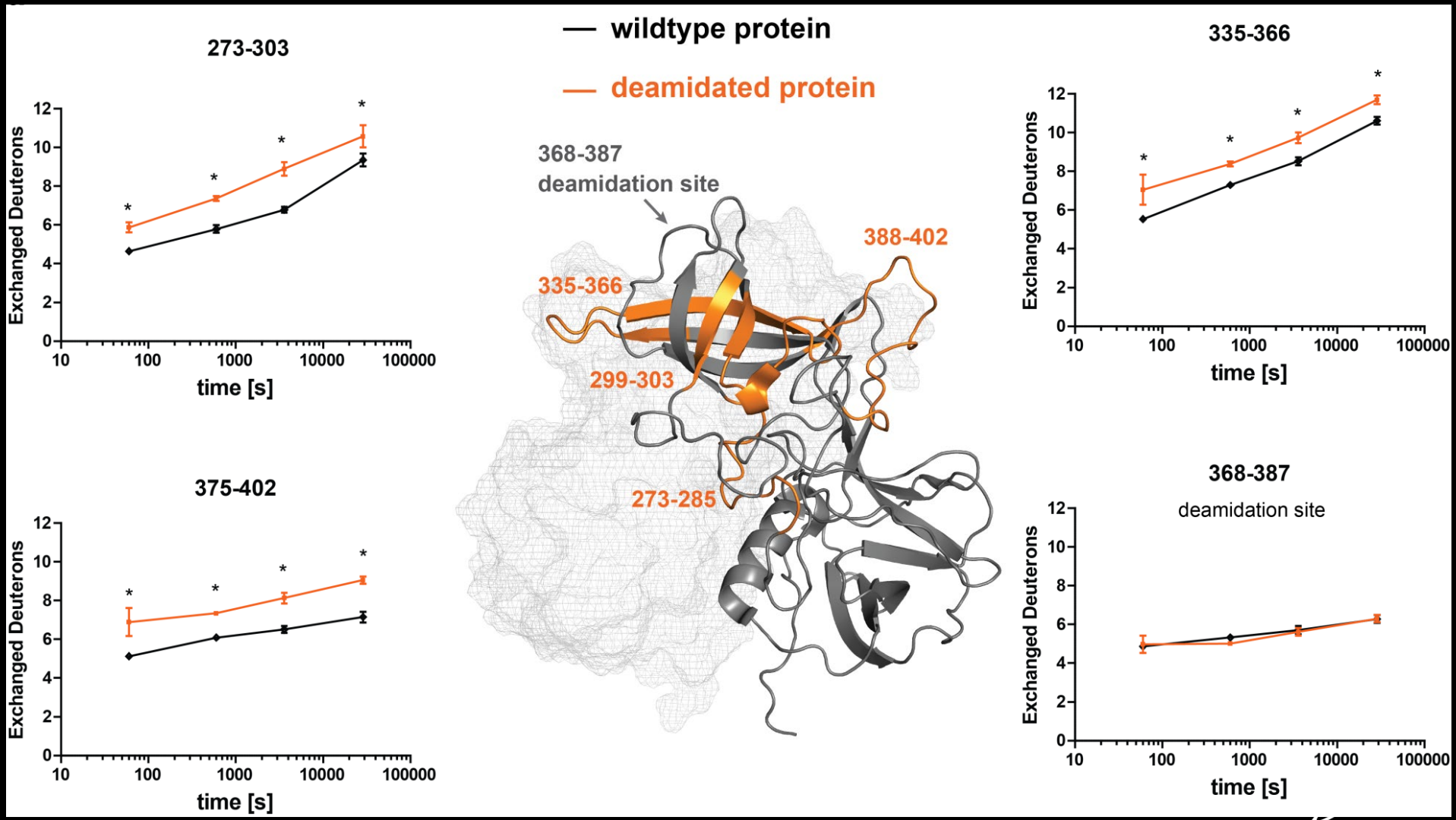
A. Mallagaray *et al.*, Nat Commun, 2019

Glycan binding to wildtype



A. Mallagaray *et al,*. Nat Commun, 2019

Higher flexibility abrogates binding



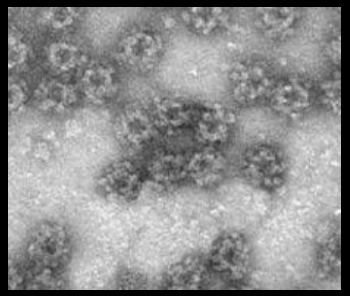
A. Mallagaray *et al*,. Nat Commun, 2019

Role of deamidation?

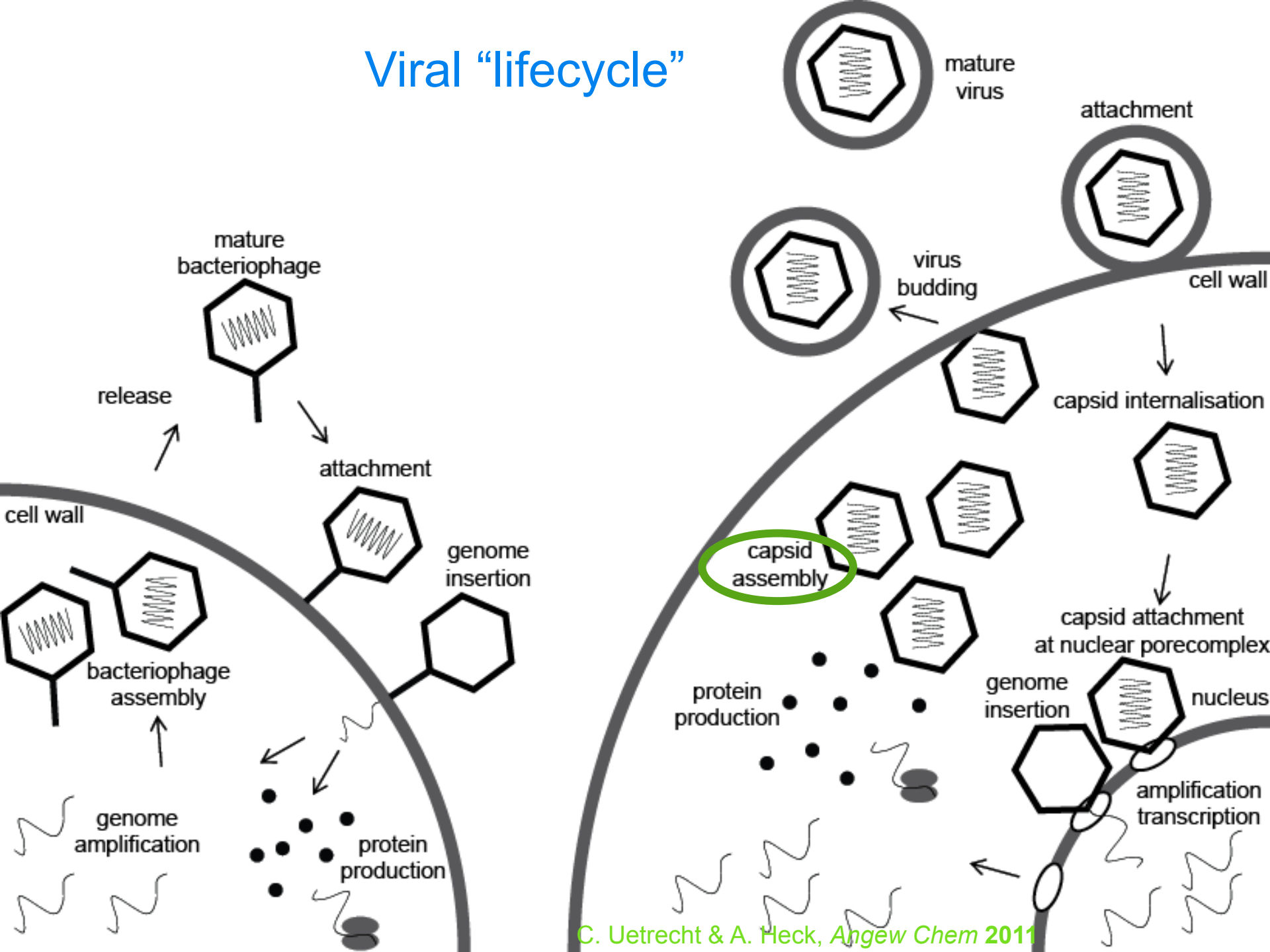
- 60% of all GII.4 with Asn
- $t_{1/2}$ = 1.5-2 d @ 37°C
- Potential role in infection

P-dimer strain	sequence	37°C	5°C
GII.4 Saga	STDTE**N**D	☹	☺
GII.4 MI001	STDTS**N**D	☹	☺
GII.10 Vietnam	STWETQD	☺	☺
GII.17 Kawasaki	LRISDNDD	☺	☺

- Also on VLP level
  - 60% Asn373 deamidated after t = 9 m @ 5°C



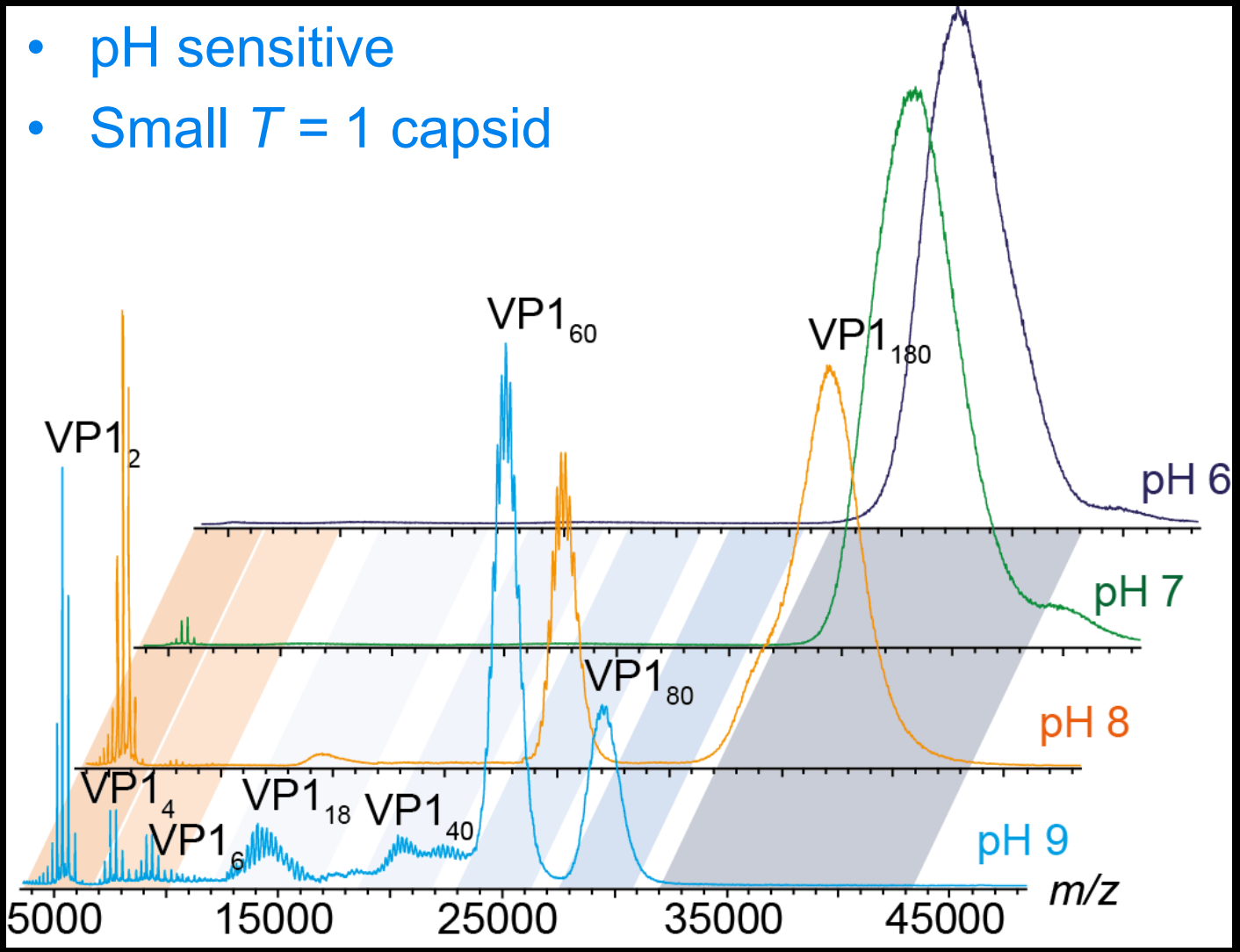
Viral “lifecycle”



C. Uetrecht & A. Heck, *Angew Chem* **2011**

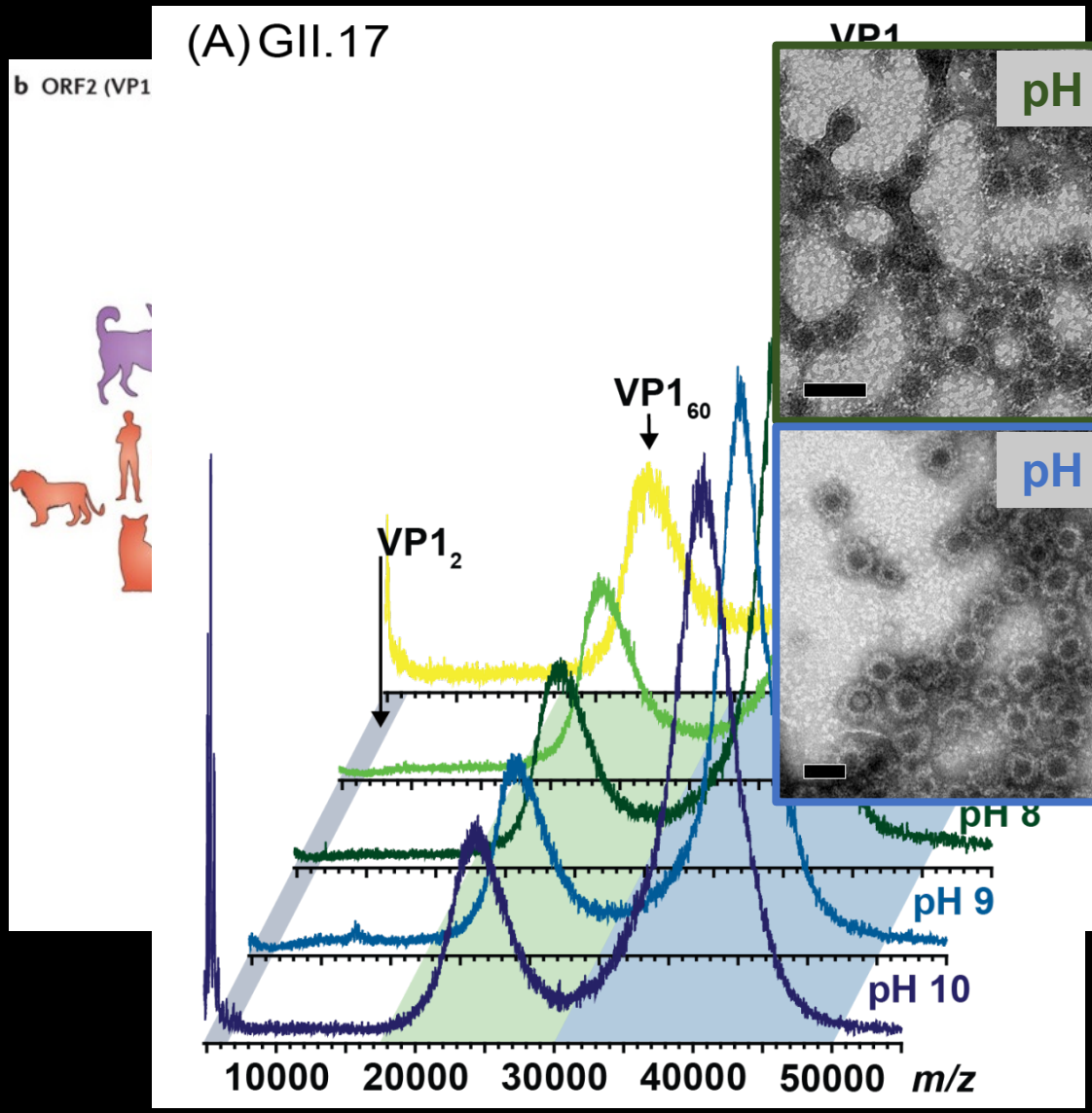
GI.1 Norwalk norovirus capsid stability

- pH sensitive
- Small *T* = 1 capsid



Shoemaker et al, Mol Cell Proteomics 2010; Uetrecht & Heck Angew Chem Int Ed Engl 2011

Is stability a conserved feature?

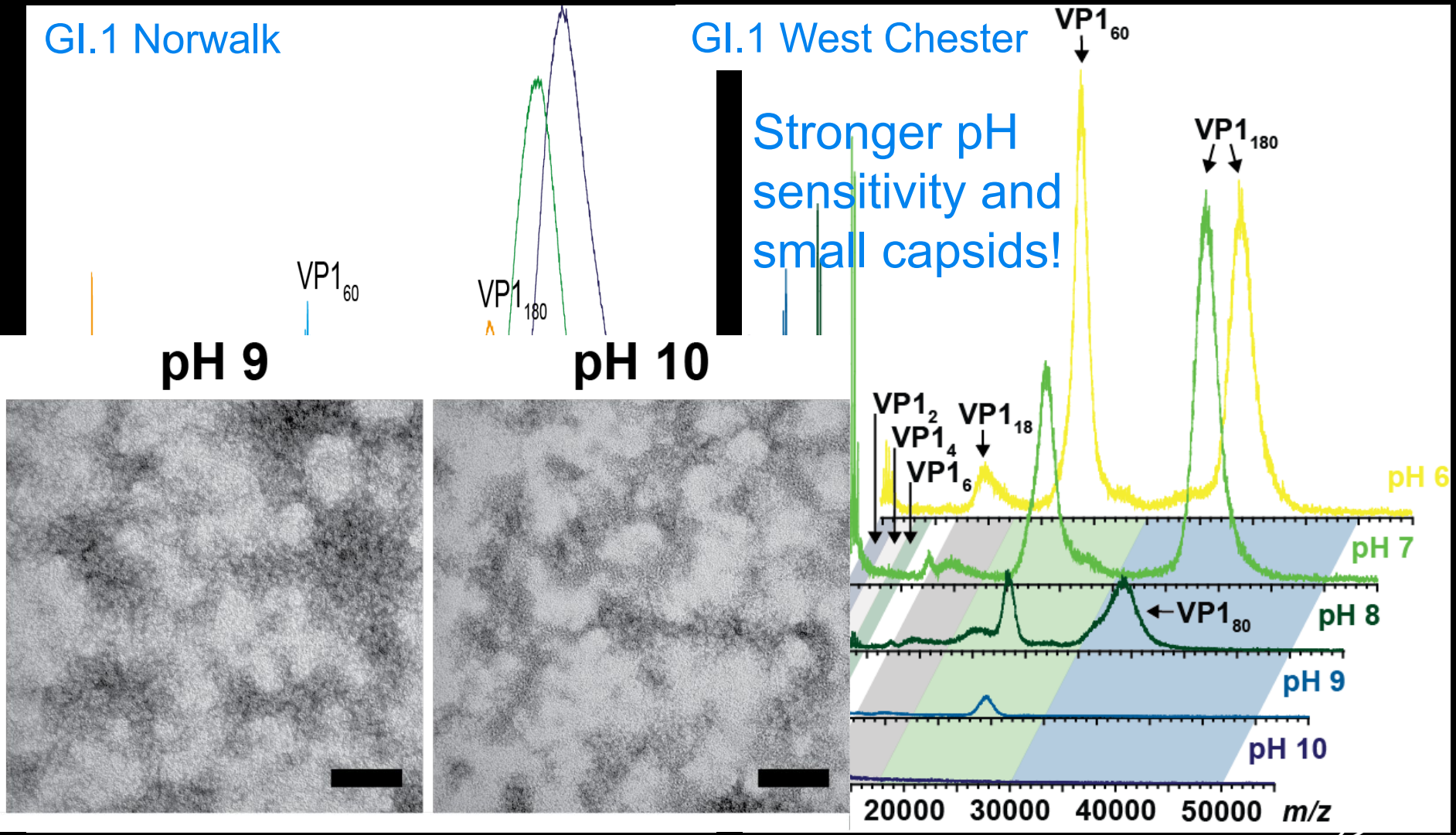


bar = 50 nm

No pH sensitivity!

Pogan et al, J Phys Condens Matter 2018

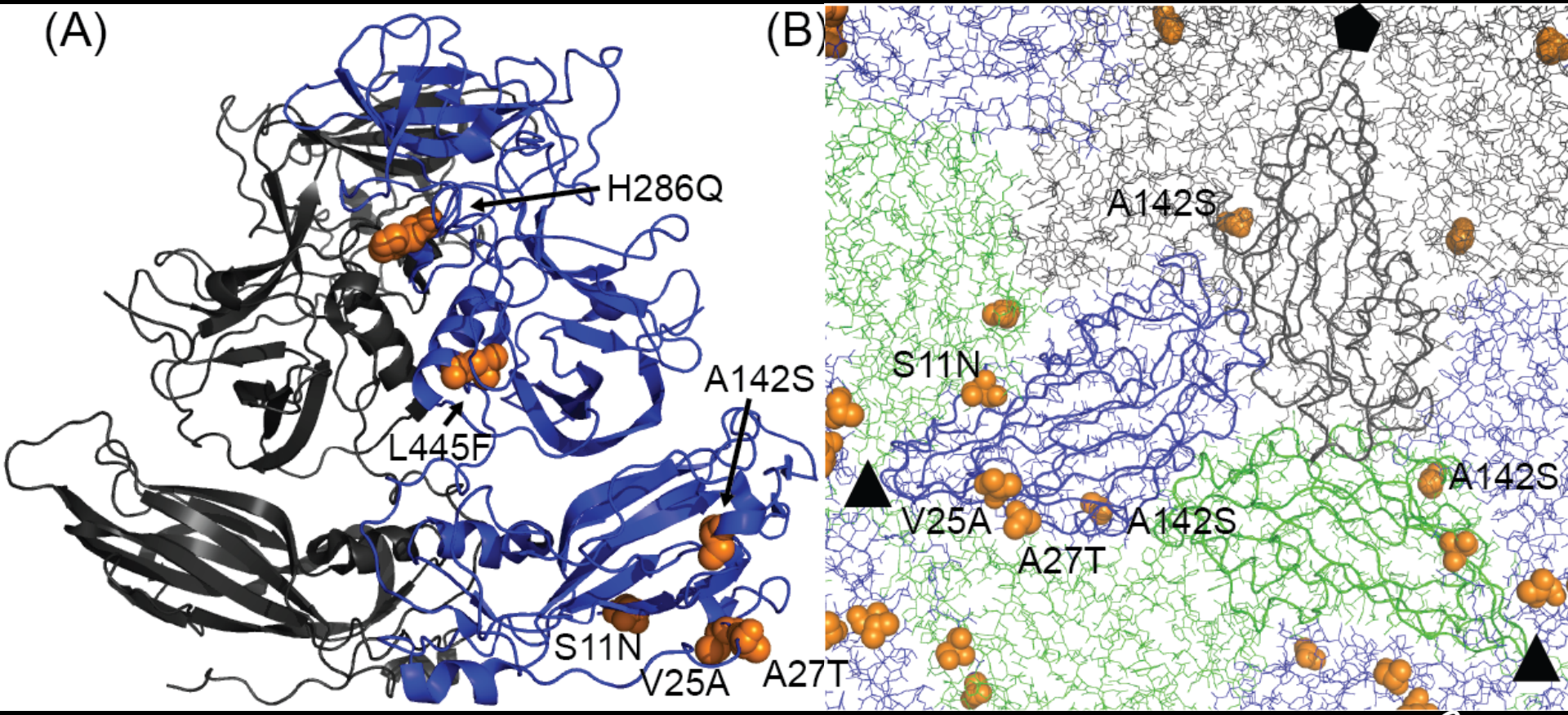
And closely related GI.1 isolates?



Shoemaker et al, Mol Cell Proteomics 2010; Pogan et al, J Phys Condens Matter 2018

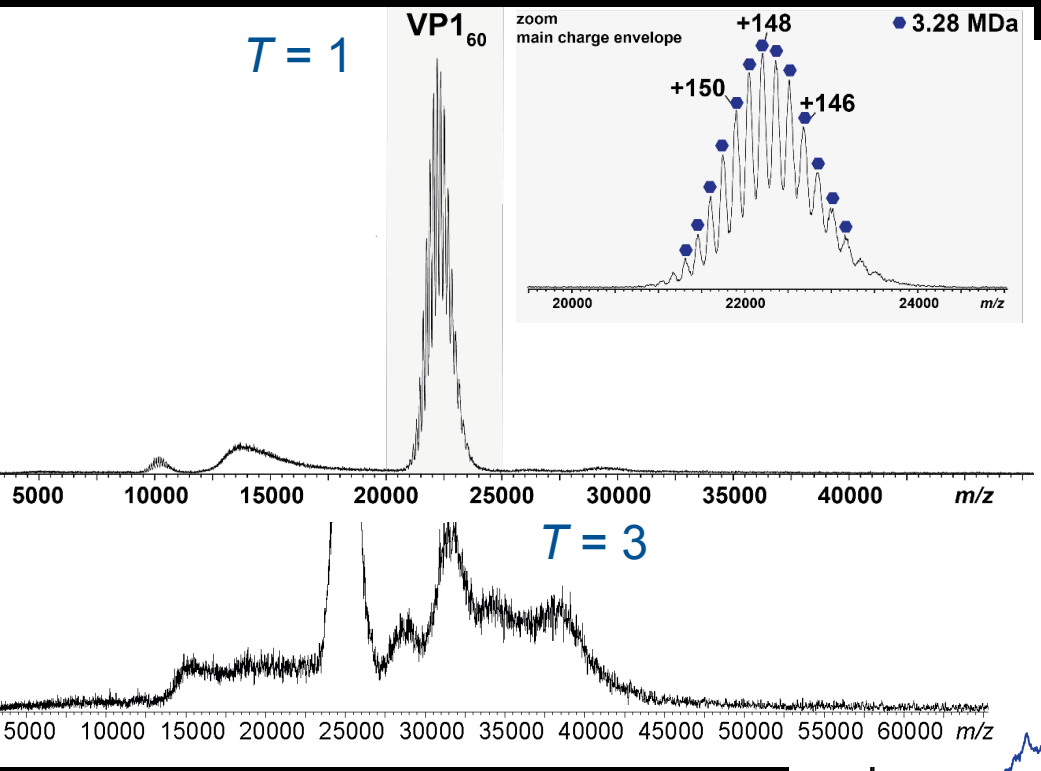
GI.1 Norwalk vs West Chester

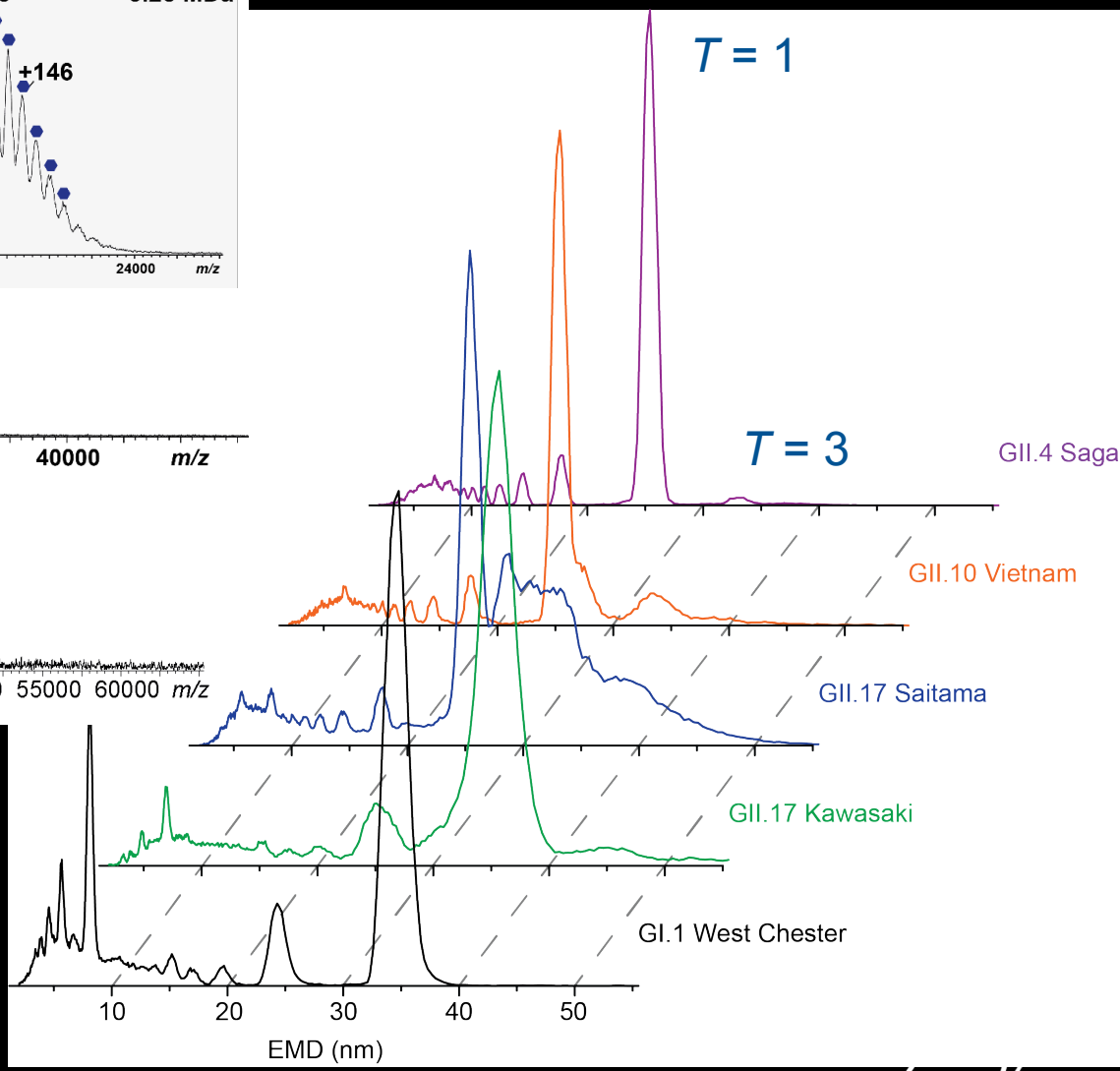
- 13 aa substitutions: 7 conservative, 6 different



Pogan et al, J Phys Condens Matter 2018

Extension to other VLPs

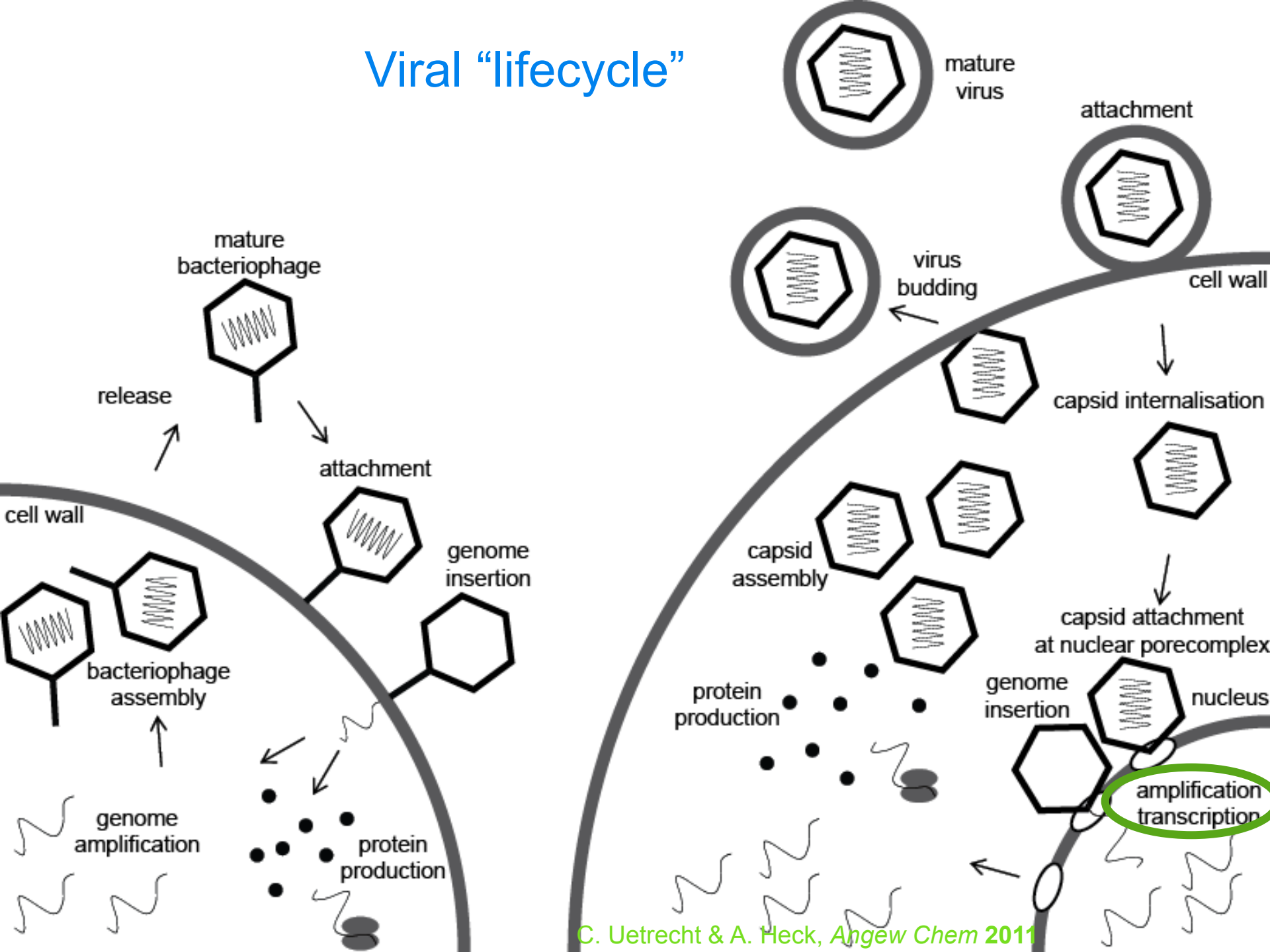




- Saga and Vietnam resolved in MS

→ Hint why mainly *T*=1

Viral “lifecycle”



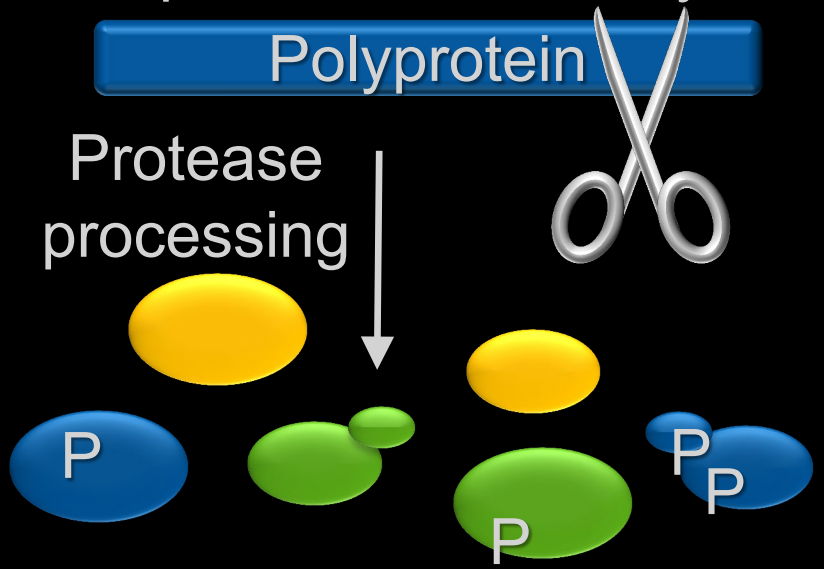
C. Uetrecht & A. Heck, *Angew Chem* **2011**

Coronaviral replication/transcription complexes

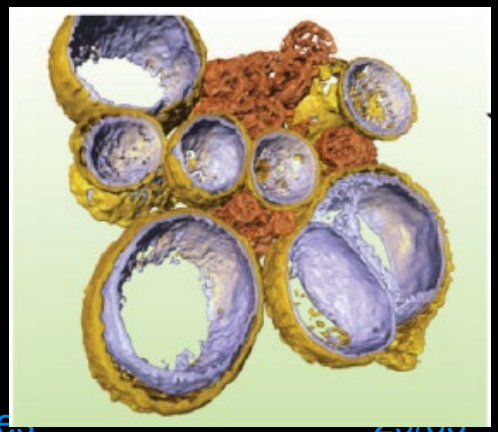
Coronaviruses

- Mild cold to severe zoonoses (SARS, MERS)

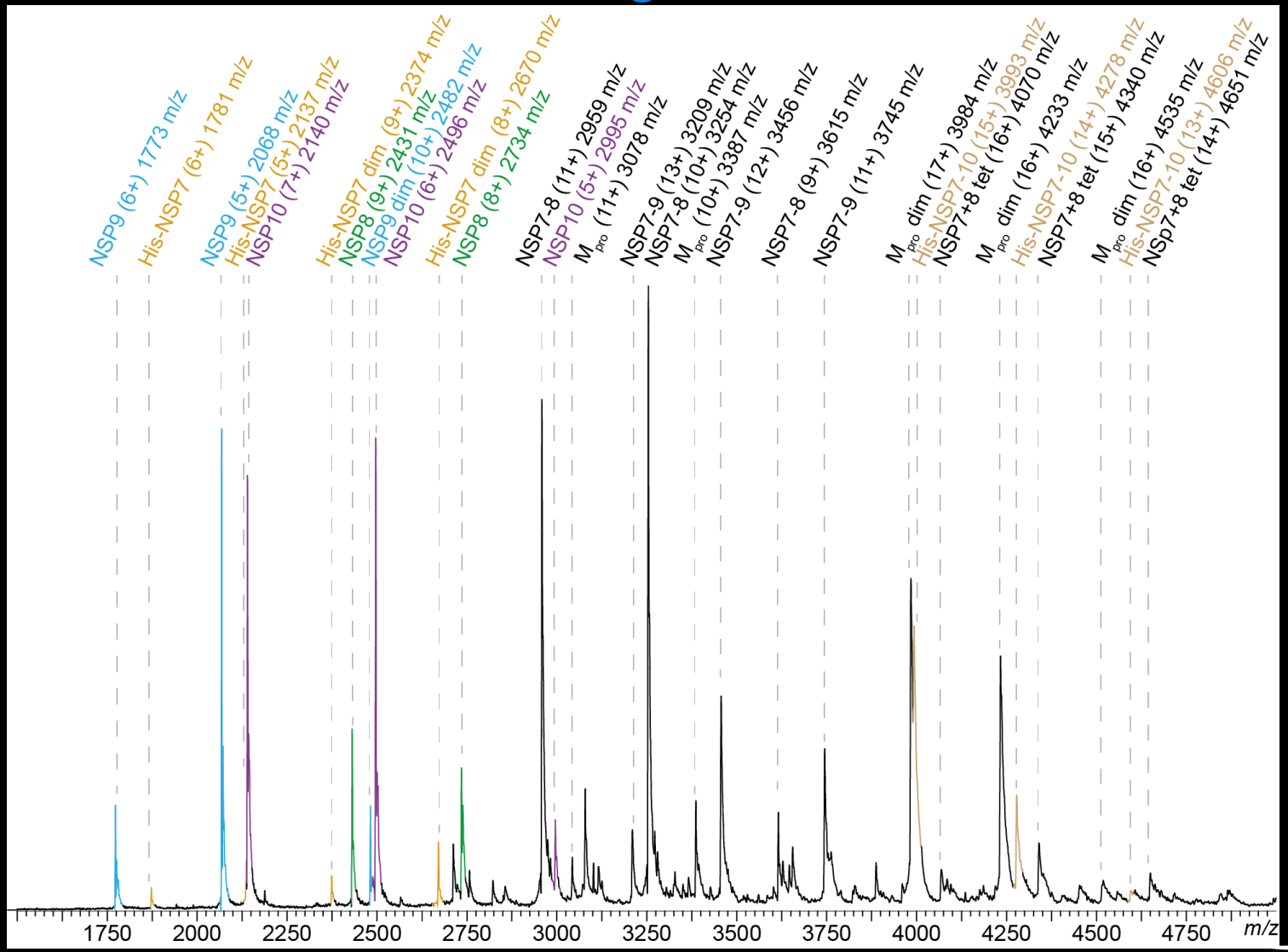
Replication machinery



16 non-structural proteins

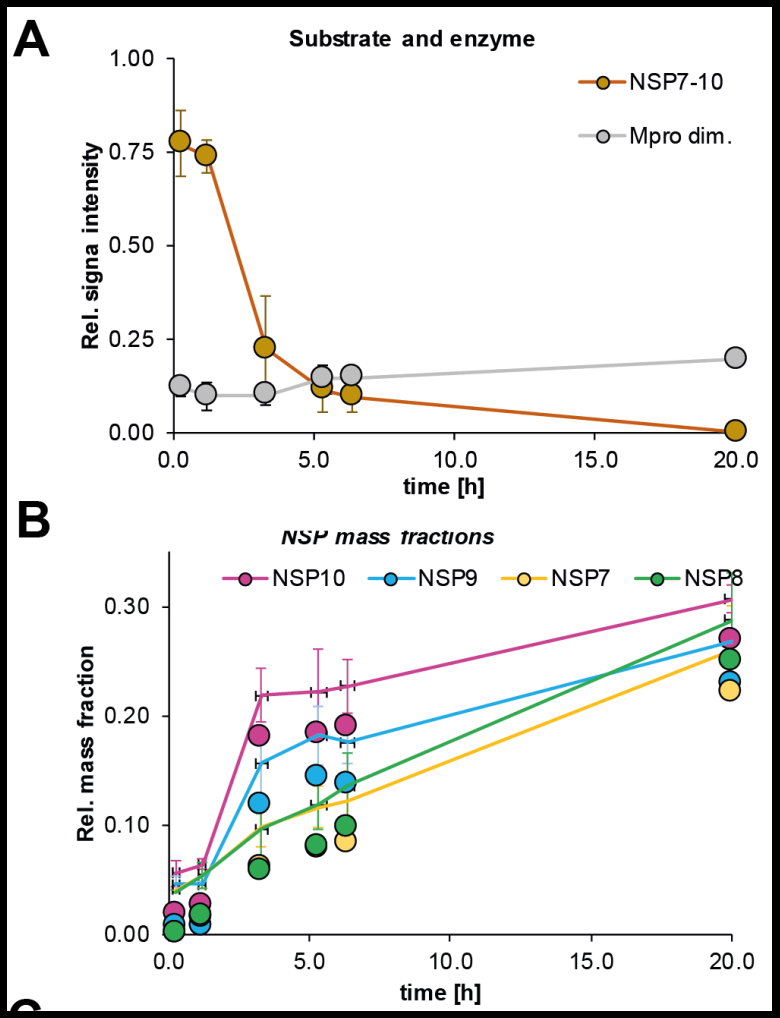


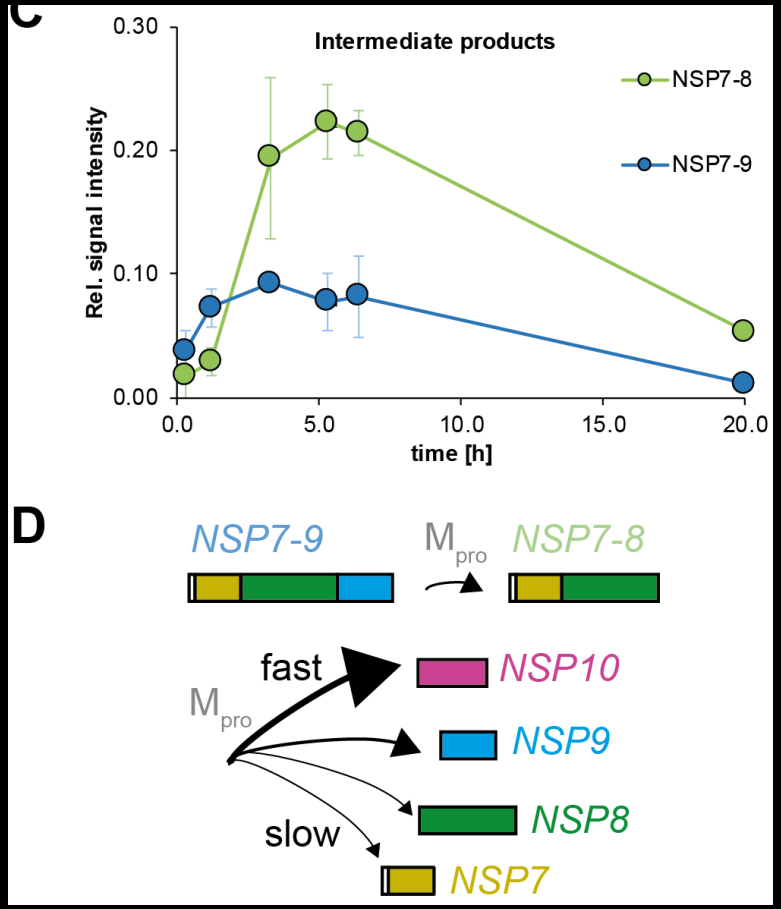
Processing SARS nsp7-10 regulatory region



Krichel et al., in preparation

Determining processing order

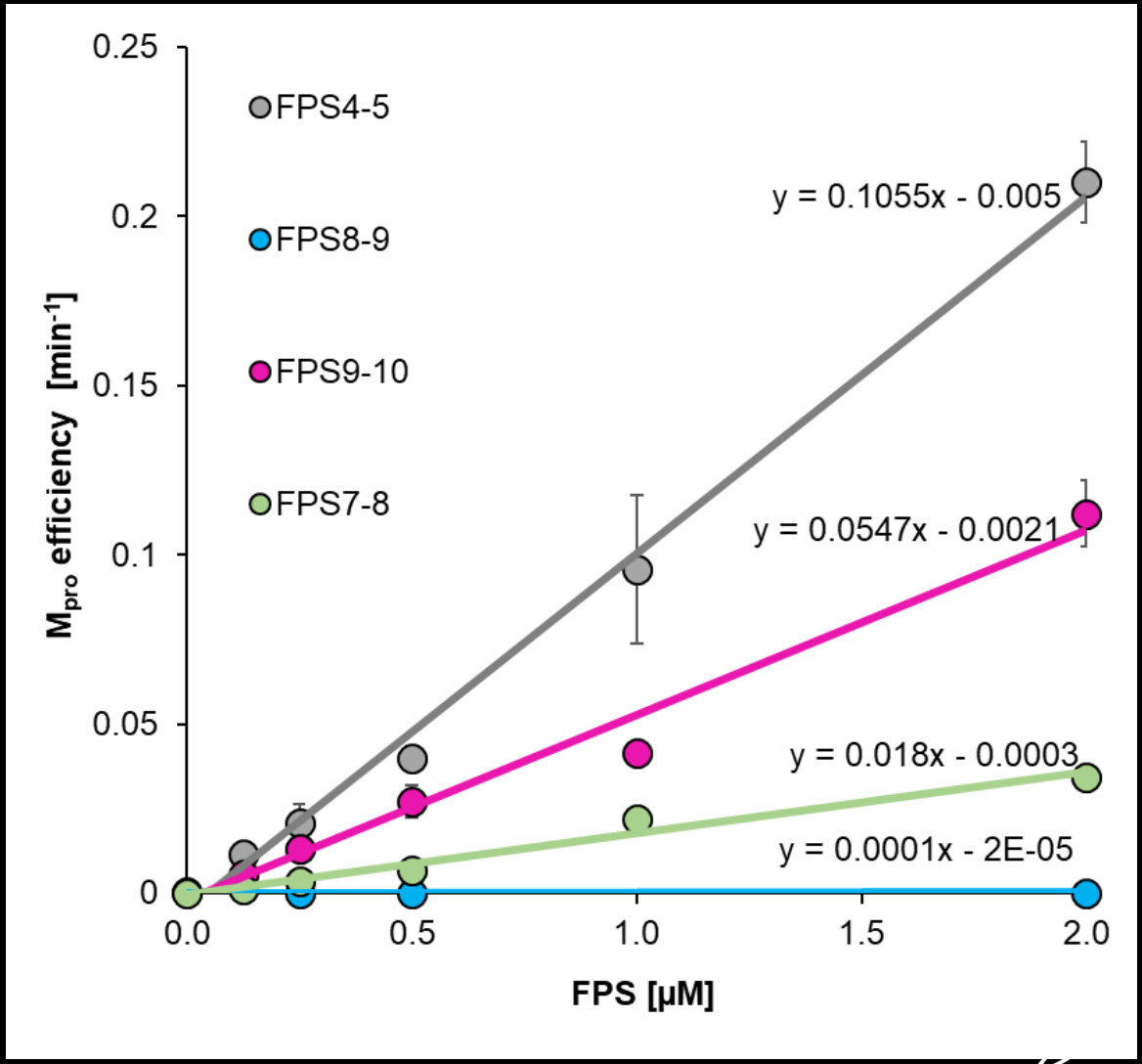




Krichel et al., in preparation

FRET peptide assay

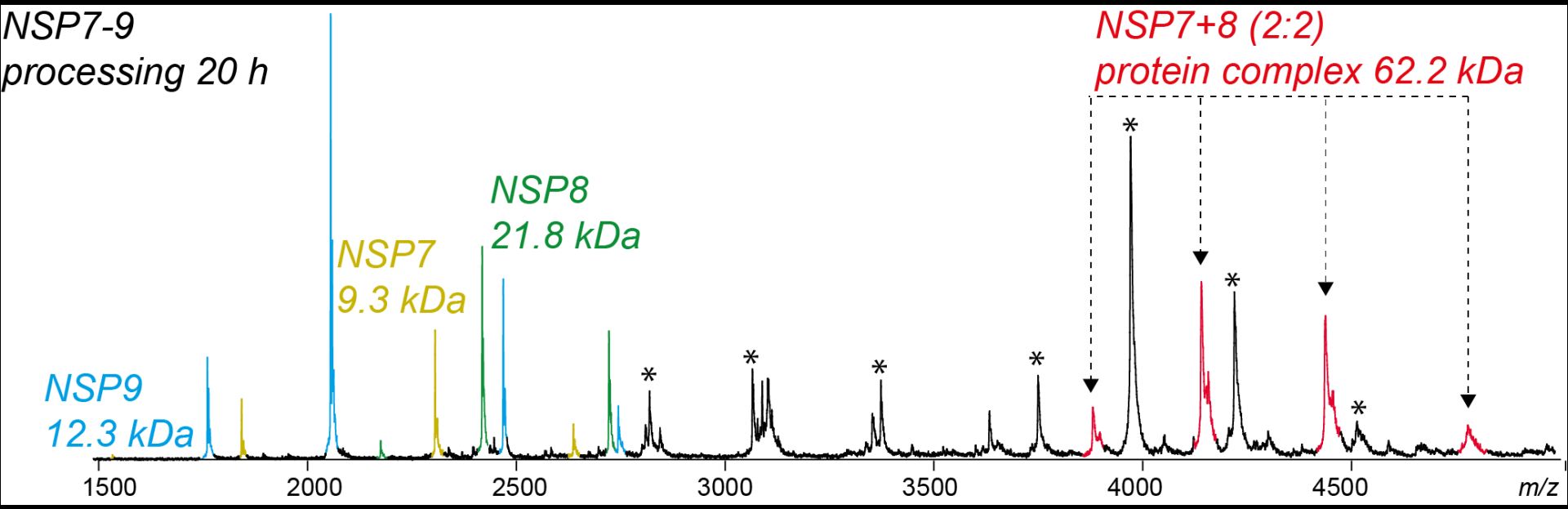
- Peptides are processed differently!
- Structural context is relevant



Krichel et al., in preparation

Nsp7+8 complexes

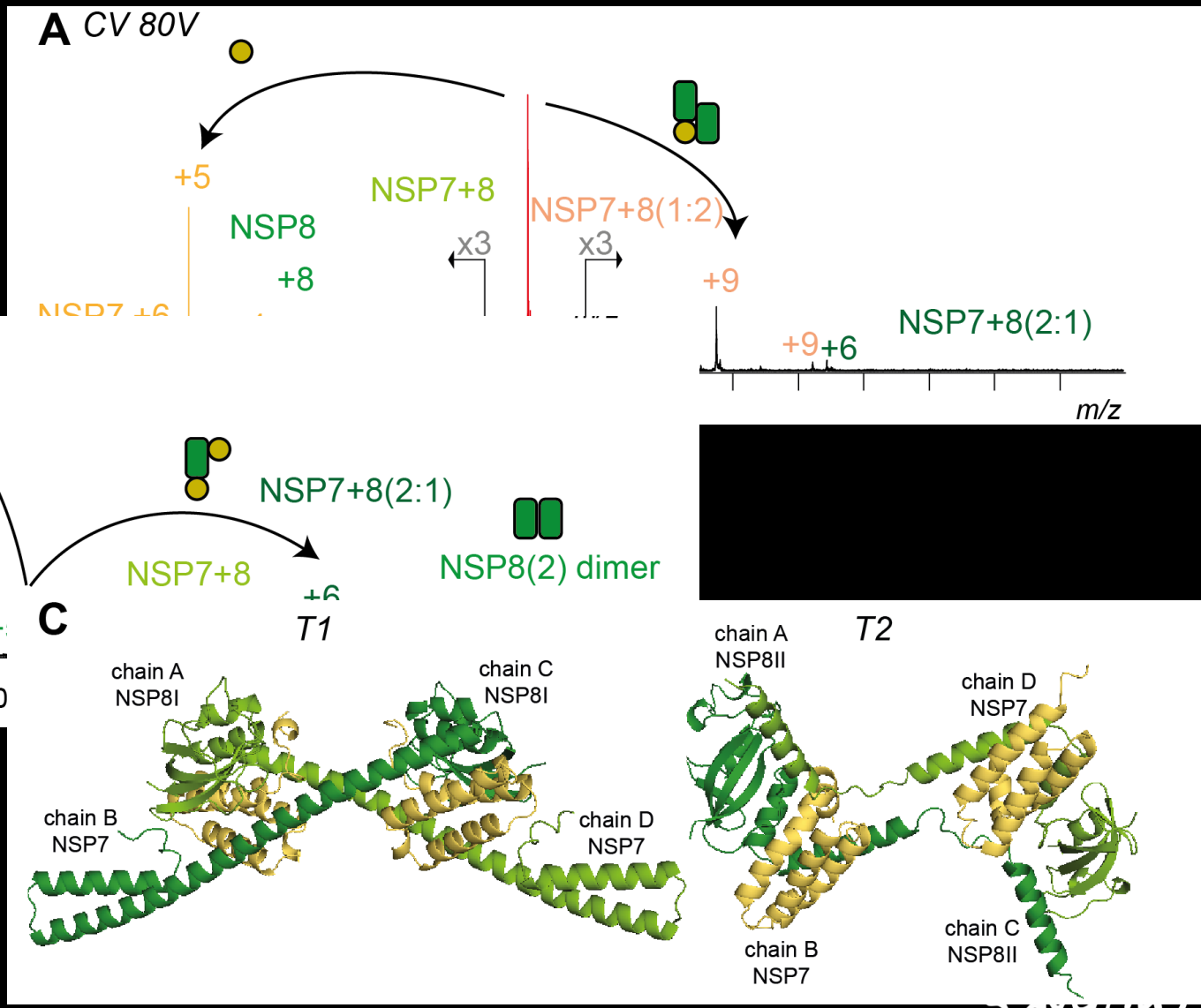
- Nsp7+8 heterotetramers formed



Krichel et al., in preparation

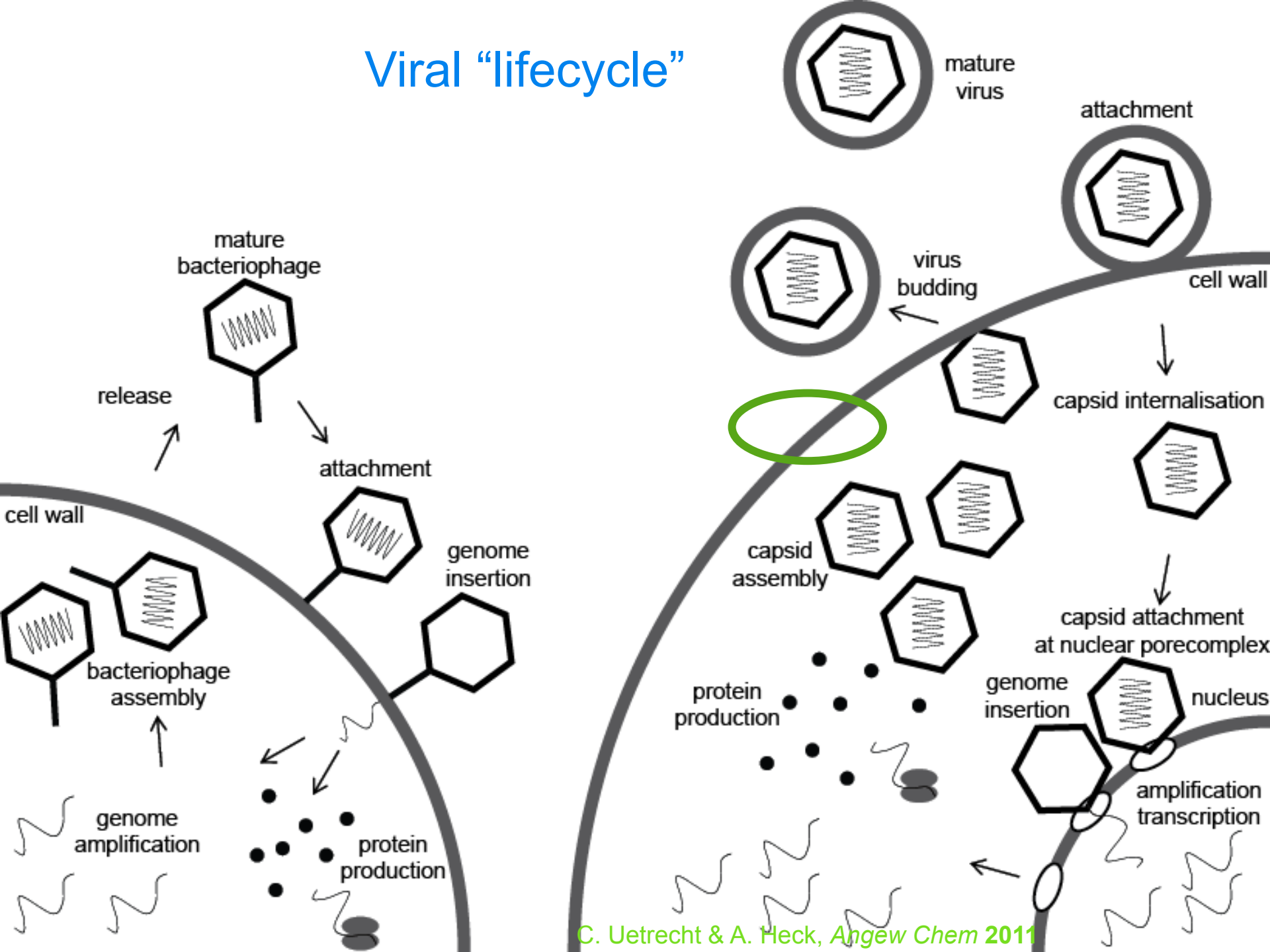
Nsp7+8 complexes in CID

- Nsp8 dimer forms core



- Antiparallel arrangement of heterodimers

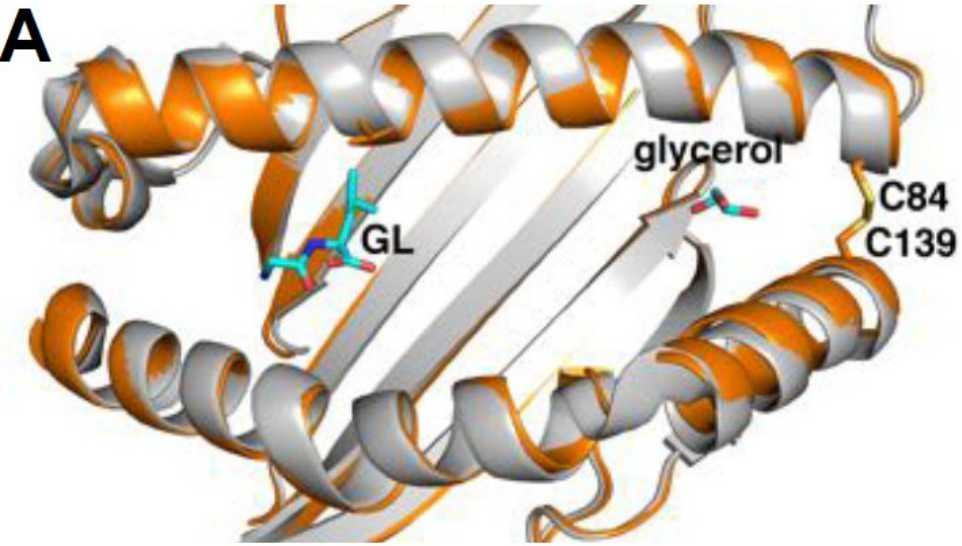
Viral “lifecycle”

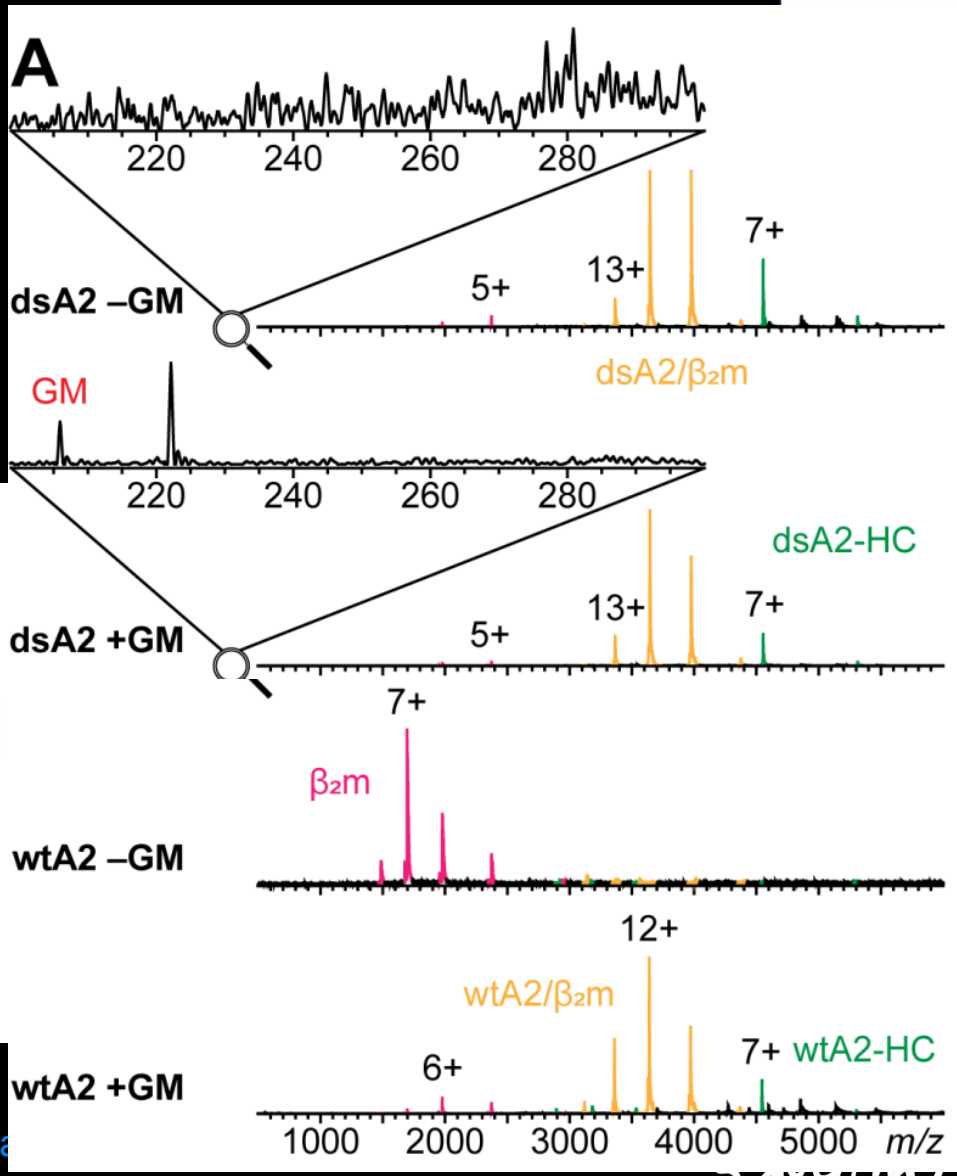


C. Uetrecht & A. Heck, *Angew Chem* **2011**

Antigen presentation

- Soluble MHC construct folds with dipeptide
- Disulfide stabilized MHC

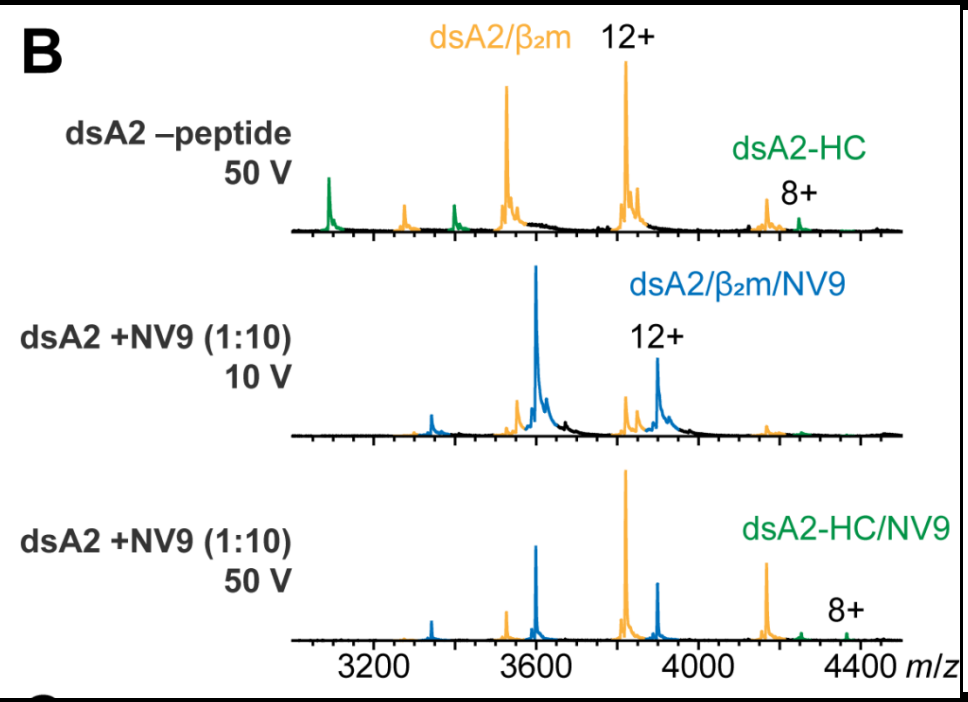


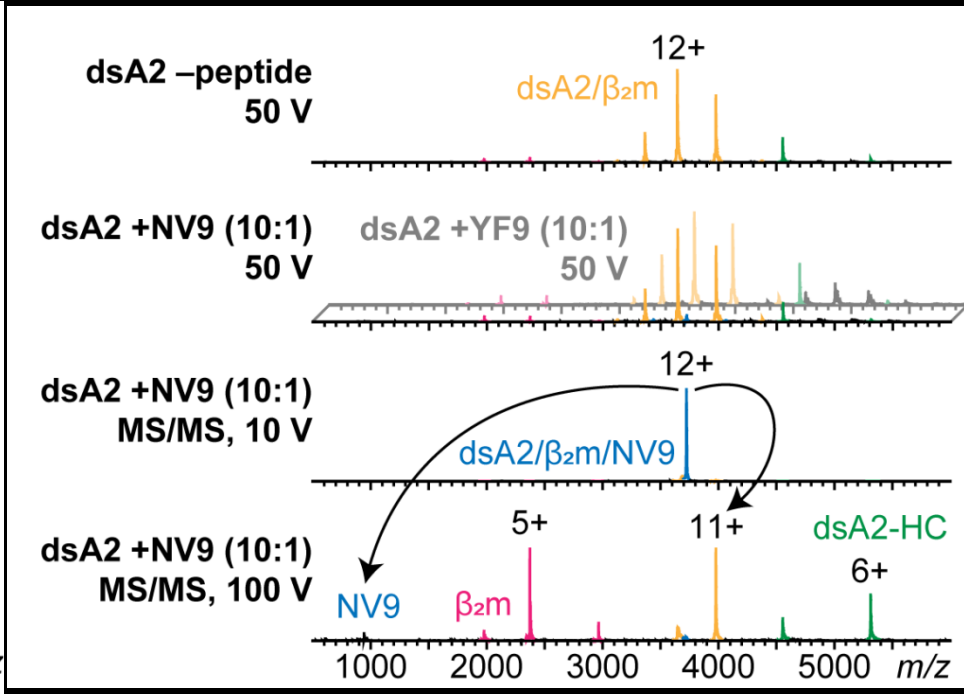


Anjanappa et a

Antigen presentation

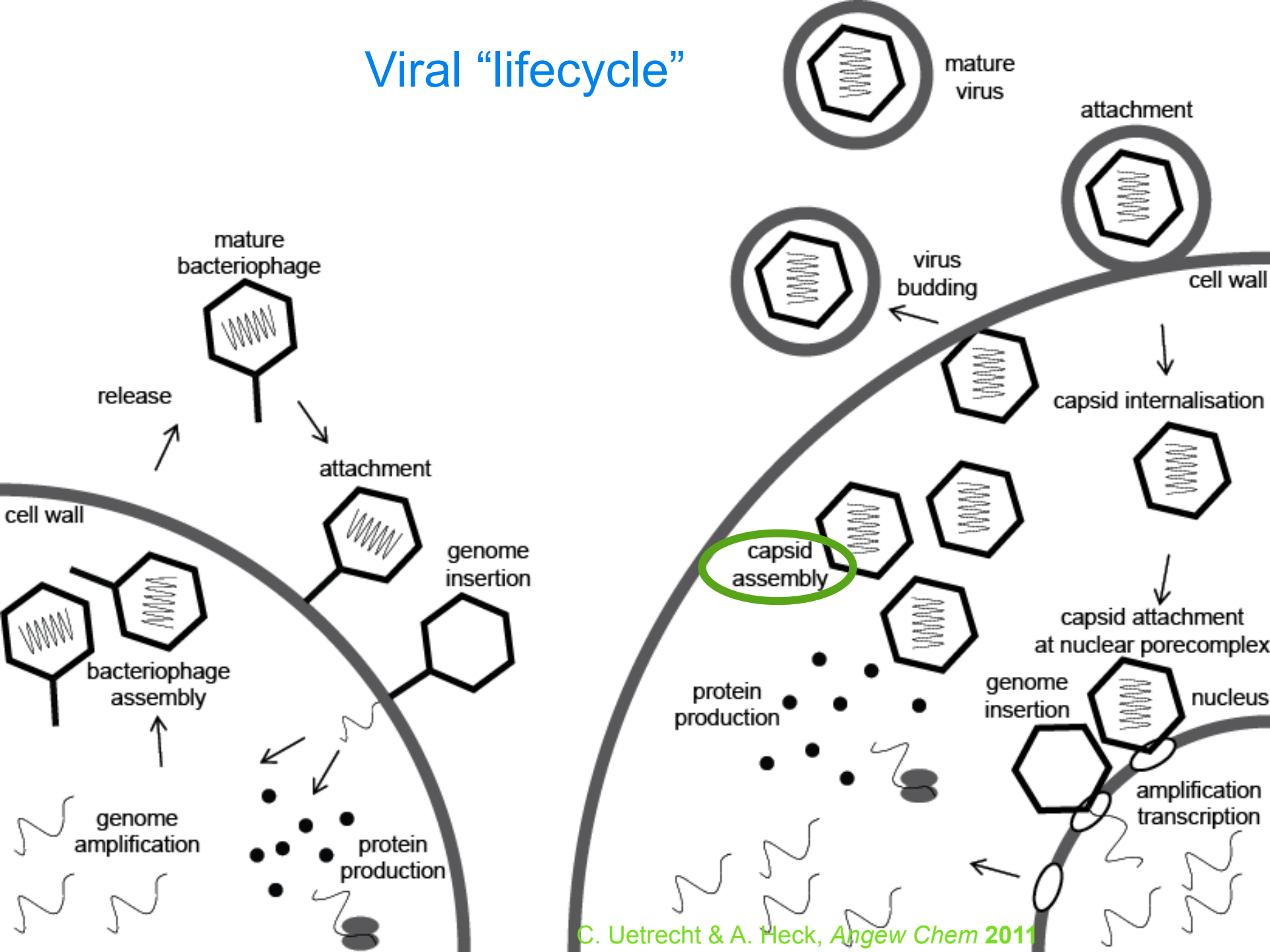
- Study peptide binding
- For high affinity even at substoichiometric ratio
- Potential screening tool





Anjanappa et al., in revision

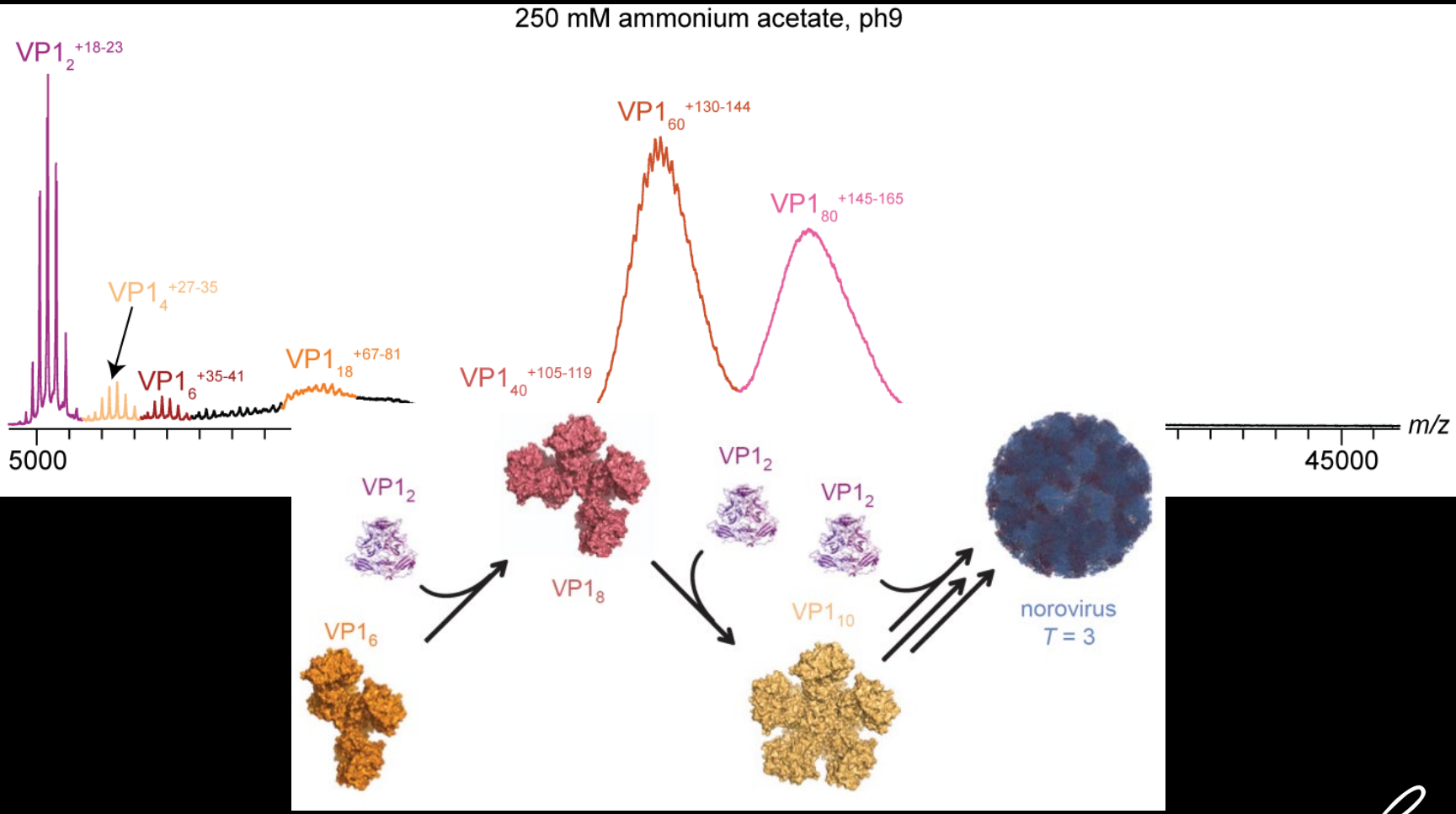
Viral “lifecycle”



C. Uetrecht & A. Heck, *Angew Chem* **2011**

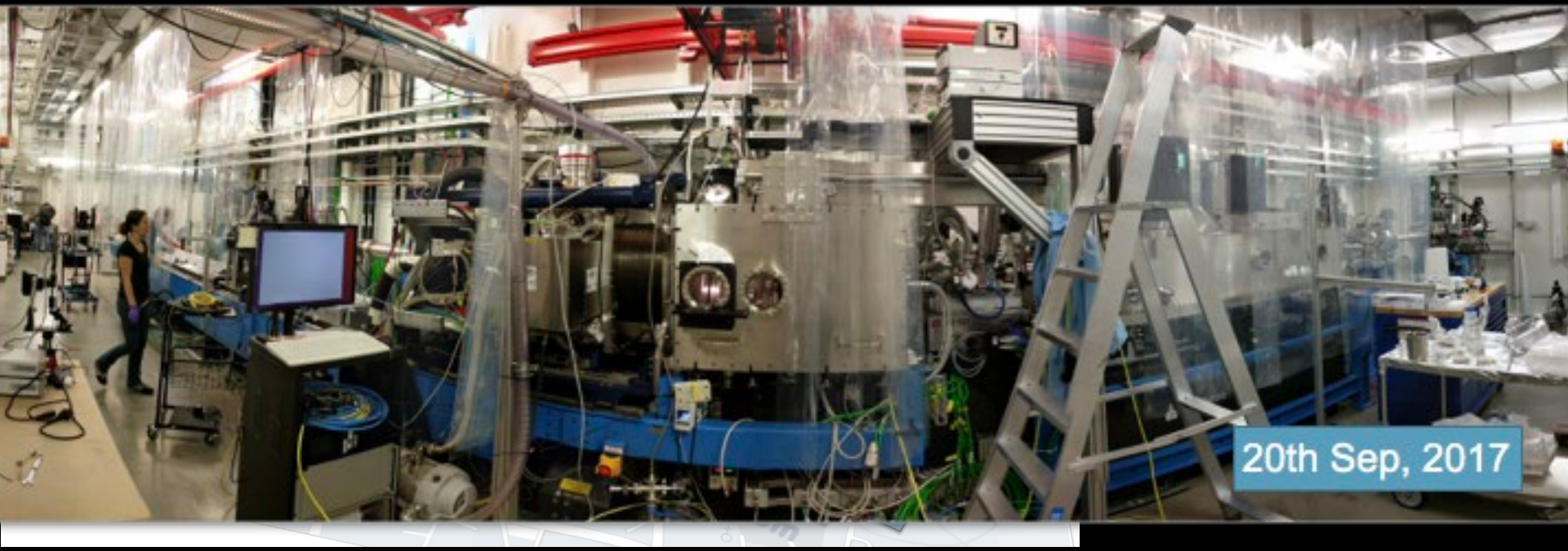
Structure of assembly intermediates?

- Assembly model based on ion mobility data



G.Shoemaker et al., MCP 2010; C. Uetrecht et al., Nat Chem 2011

The European XFEL

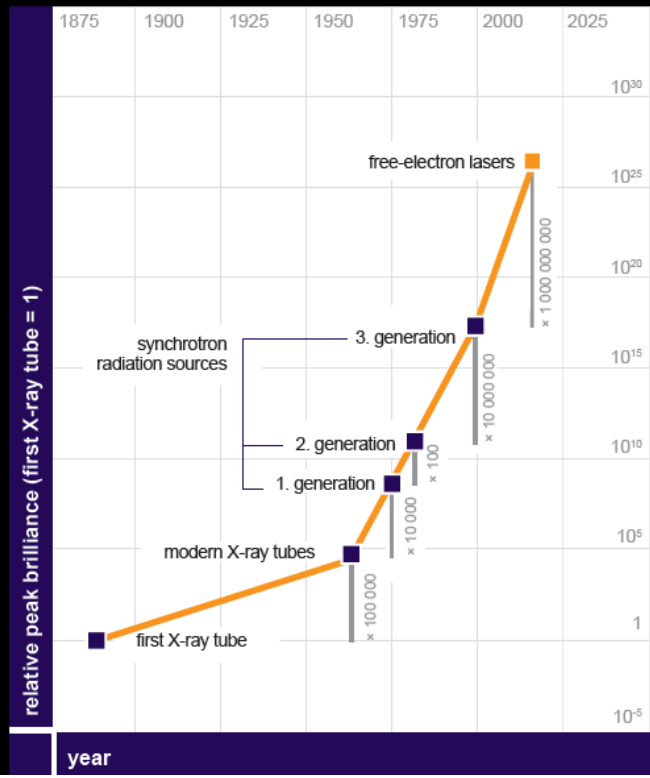


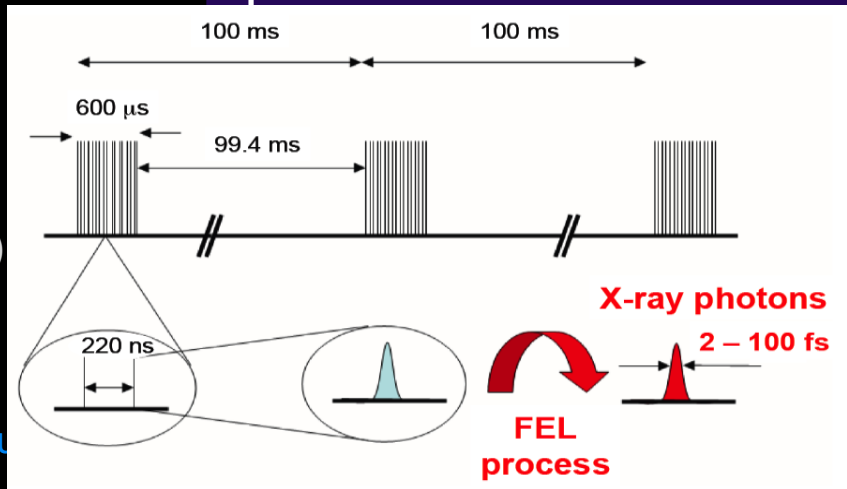
- X-ray free-electron laser (XFEL)
- 3.4 km long, linear accelerating
- In operation since Sep 2017
- User labs, 6 instruments

Properties

- Femtosecond X-ray pulses
- Peak brilliance >> synchrotron
- Mostly coherent
- 27,000 pulses/s at European XFEL

- Higher brilliance and repetition rate than other XFELs:
  - Soft X-rays: FLASH (2005)
    FERMI (2011)
  - Hard X-rays: LCLS (2009)
    SACLA (2011)
    PAL-XFEL (2017)
    European XFEL (2017)

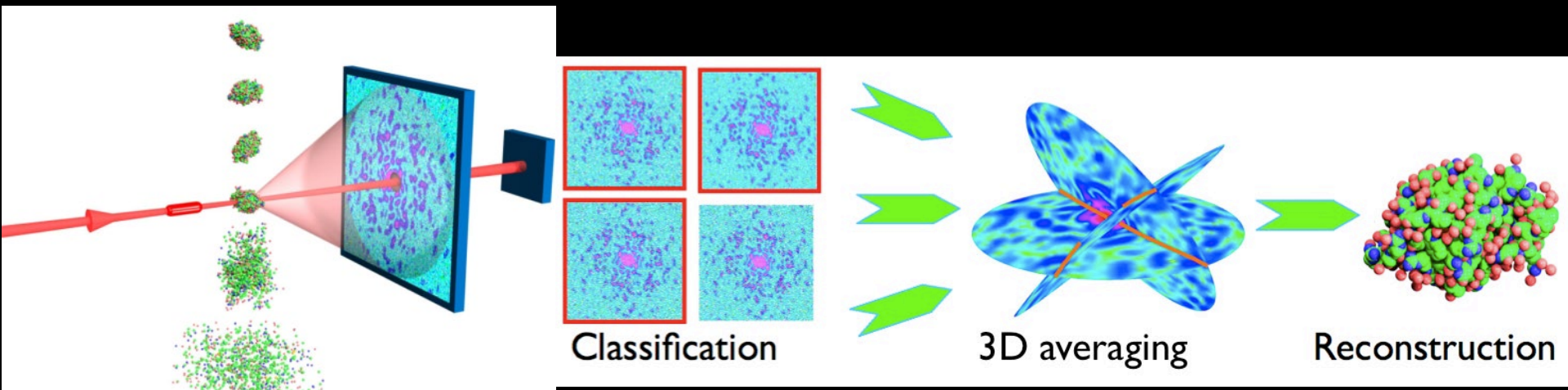




Why use European XFEL for biology?

Why use European XFEL for biology?

- Single particles require high intensity – plasma explosion
  - Short fs pulses outrun destruction
  - Many images from different particles
  - Direct diffraction – no separate phasing

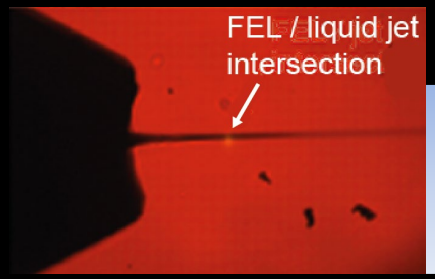


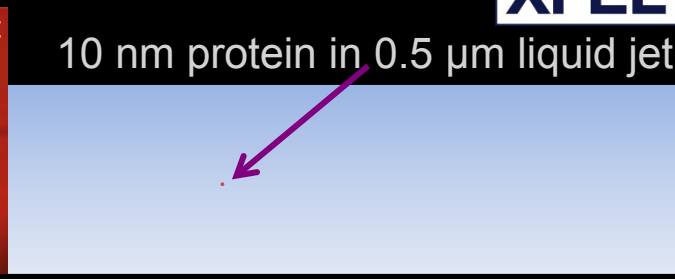
Scientific case: R. Neutze *et al.*, *Nature* **2000**

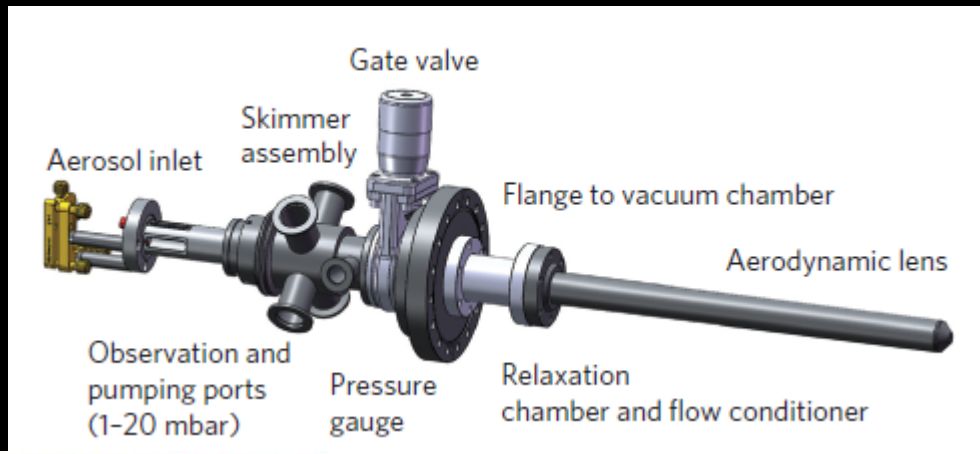
Current injection systems

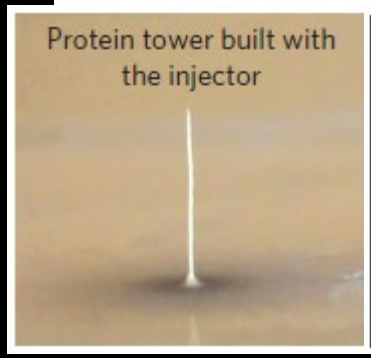
- High background
- **Liquid** – water column
- **Aerosol** – $V_{1\ \mu m\ droplet}$ = 106 x $V_{10\ nm\ object}$
- Pulsing/Sorting difficult
- *In silico* classification/ alignment
  - $t_{analysis}$ >> $t_{acquisition}$
  - Similar for EM

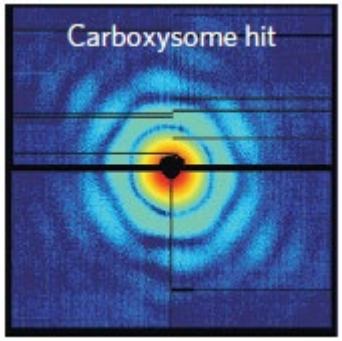
→ Sample delivery critical

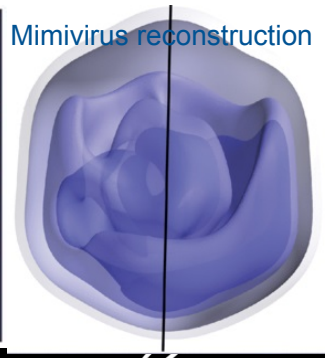








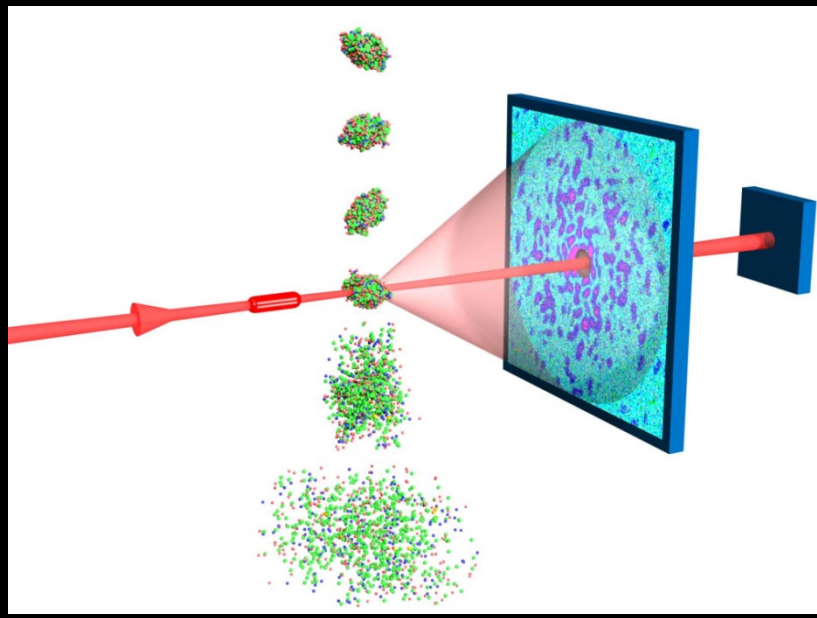




M. Hantke *et al.*, *Nat Photonics* **2014**; T. Ekeberg *et al.*, *Phys Rev Lett* **2015**

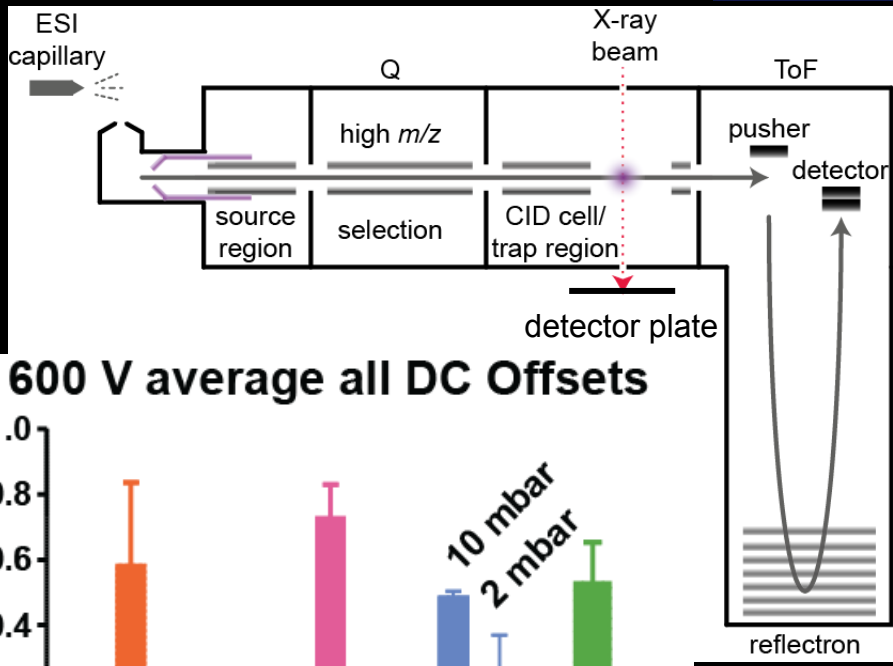
The “ideal” sample delivery system

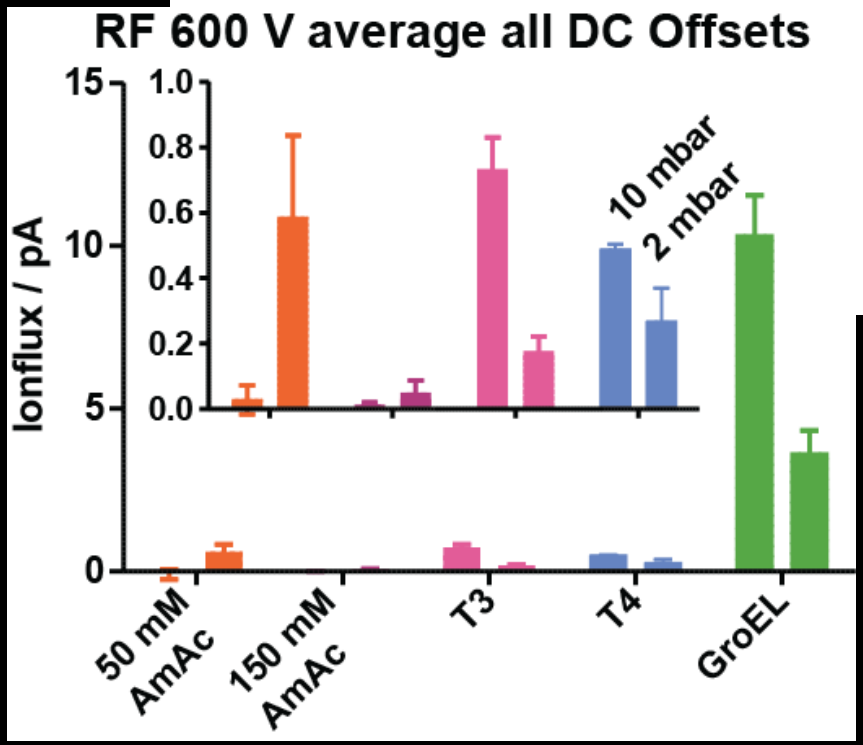
- Low sample consumption
  - Timed particle release
- Natural environment
- No background gas or liquid
- Select species from a mixture
  - Pre-sorting
  - Speed up data analysis (also an issue in cryo-EM)



Why use native MS at European XFEL?

- nanoESI
  - low background & sample consumption
  - 10,000 patterns in 16 min with 1 µm focus
  - No buffer background at high source pressure

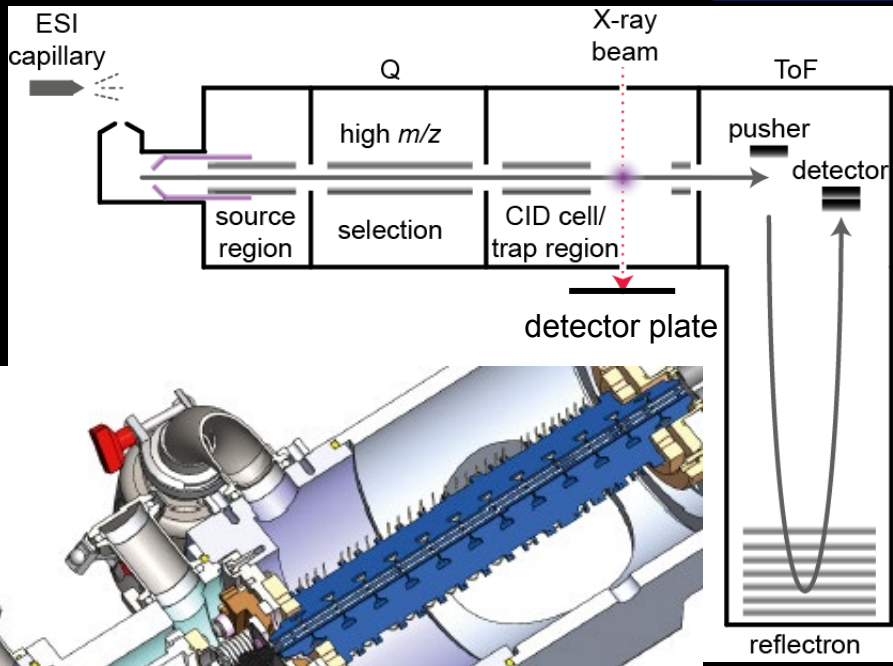


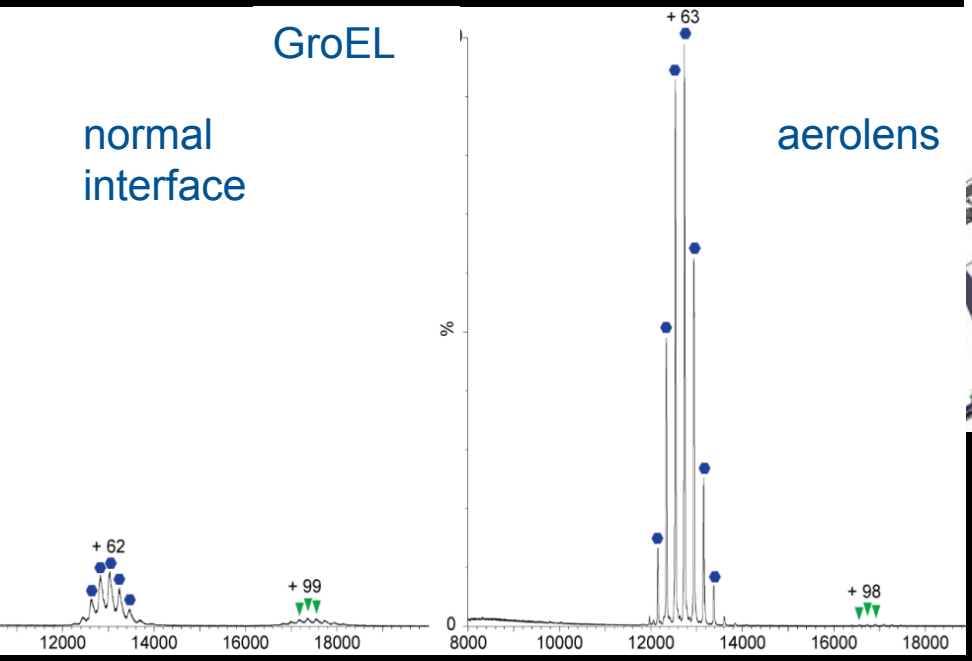


J.Schulz *et al., SPIE Proceedings* **2013;** Uetrecht et al. *J Synchrotron Radiat* **2019**

Why use native MS at European XFEL?

- nanoESI
  - Aerolens (Fasmatech)
    - Higher flux
    - Low abundant intermediates

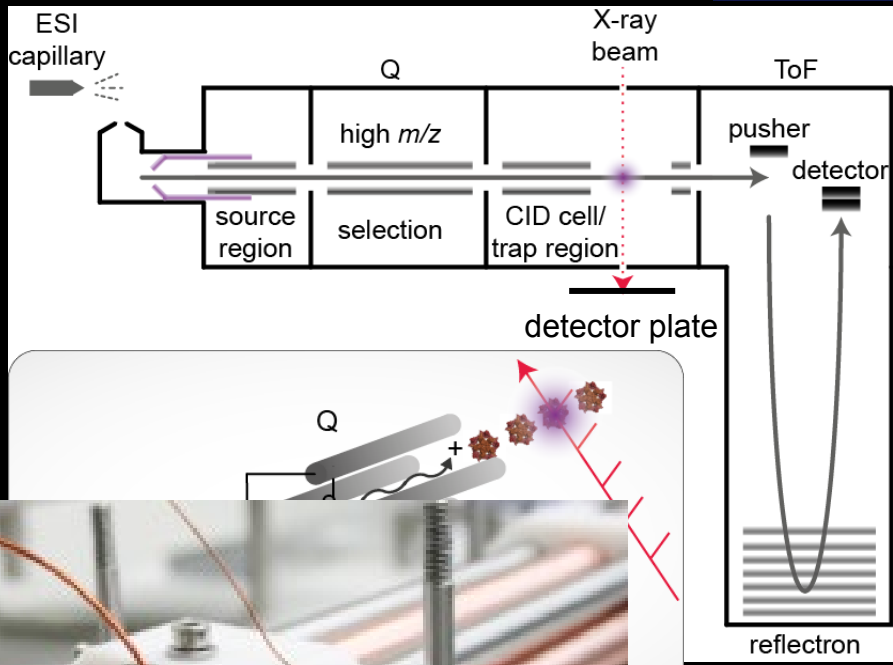


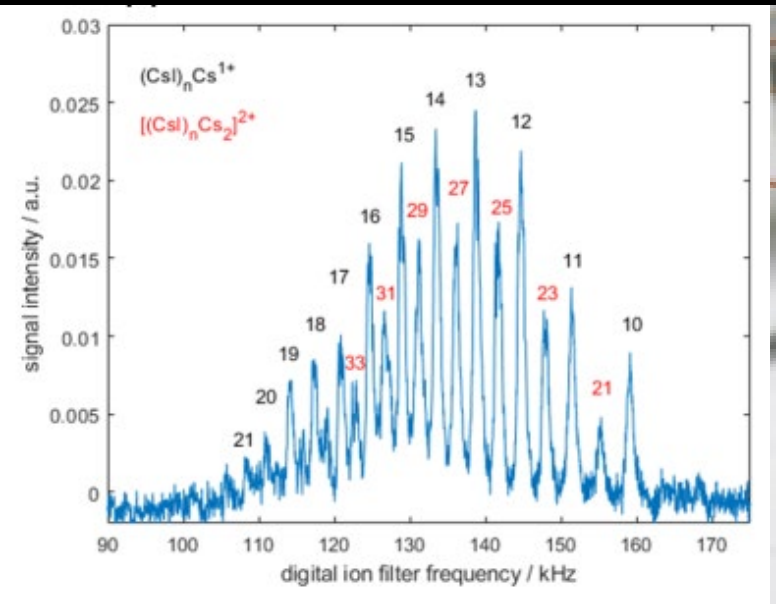


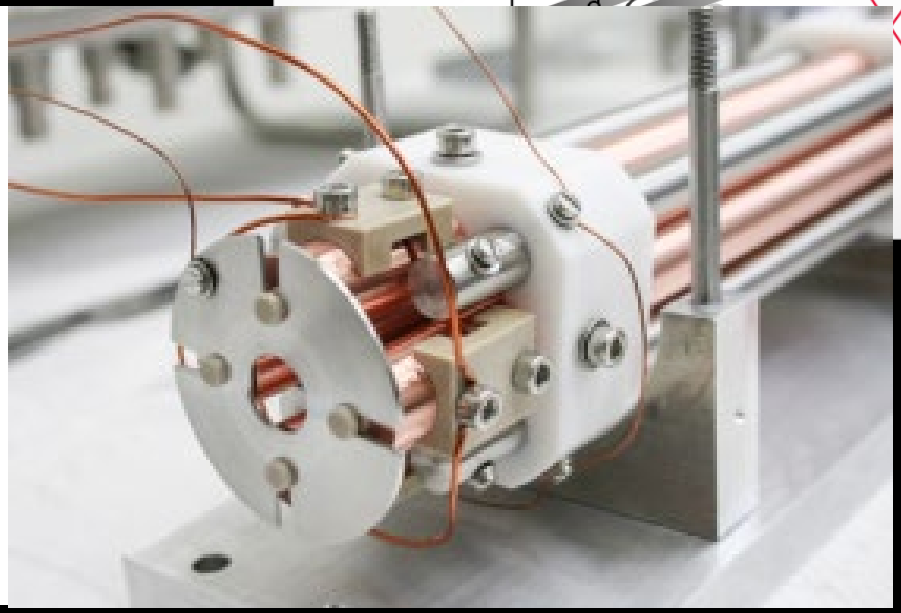
Unpublished & J.Schulz *et al., SPIE Proceedings* **2013**

Why use native MS at European XFEL?

- Quadrupole
  - Mass selection
  - Purify low abundant species
  - Digitally driven (Greifswald)



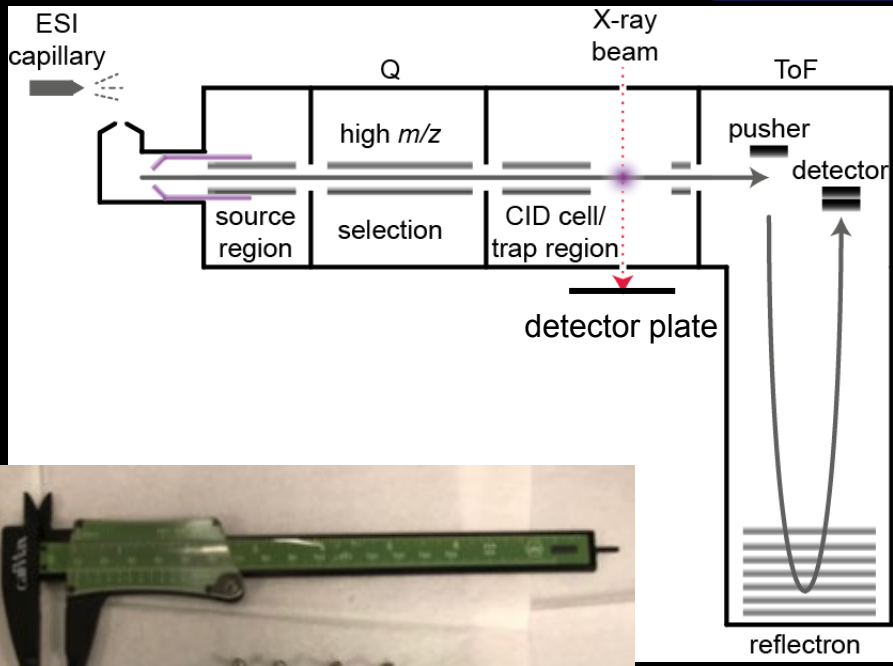


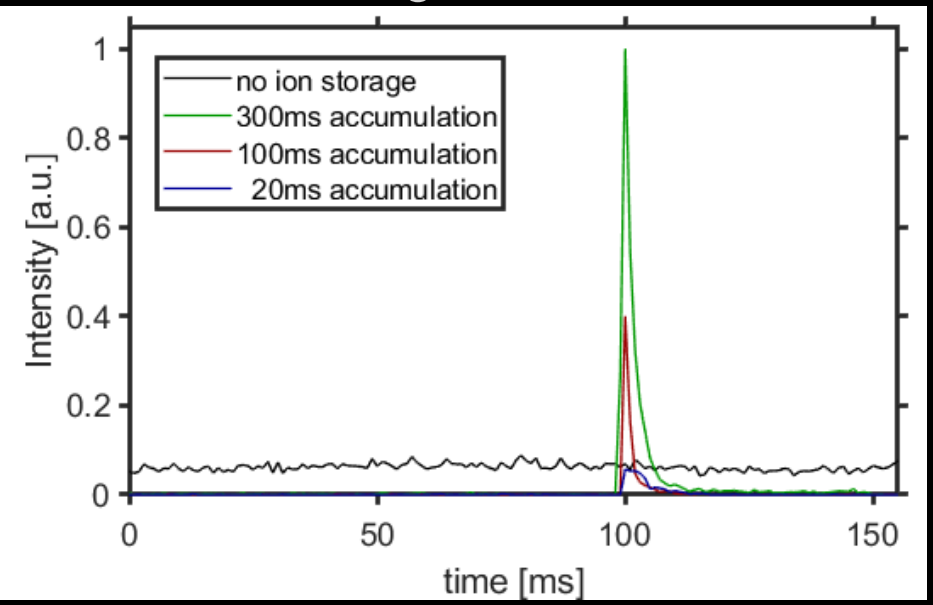


Unpublished & J.Schulz *et al.*, *SPIE Proceedings* **2013**

Why use native MS at European XFEL?

- Trap (Greifswald)
  - Time particle release with FEL
  - Increase ion density
  - Trapping capacity sufficient for 100 ms
  - No indication of structural damage

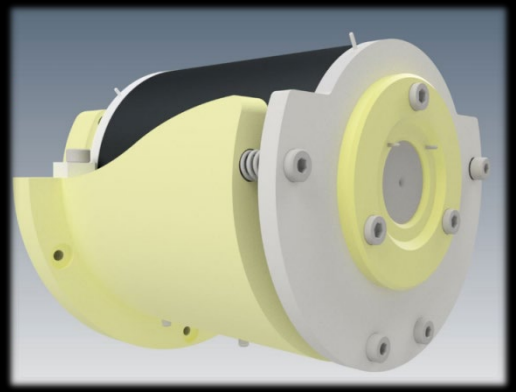


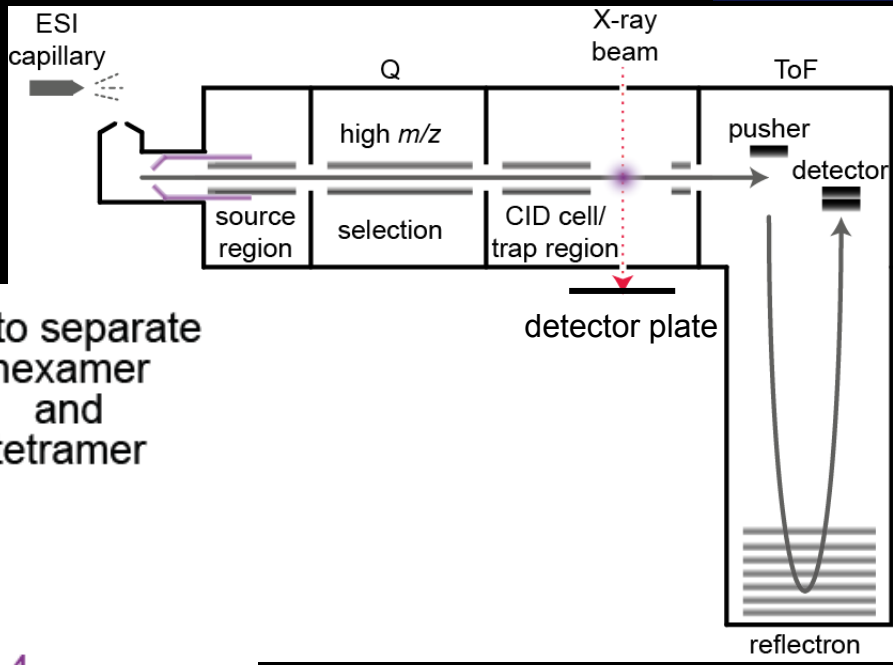


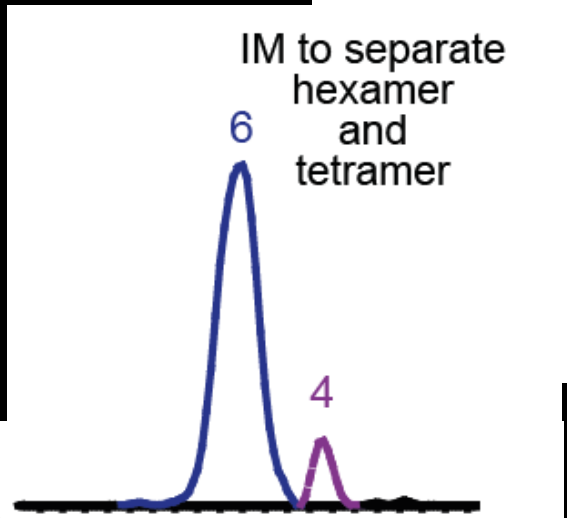
Unpublished & J.Schulz *et al., SPIE Proceedings* **2013**

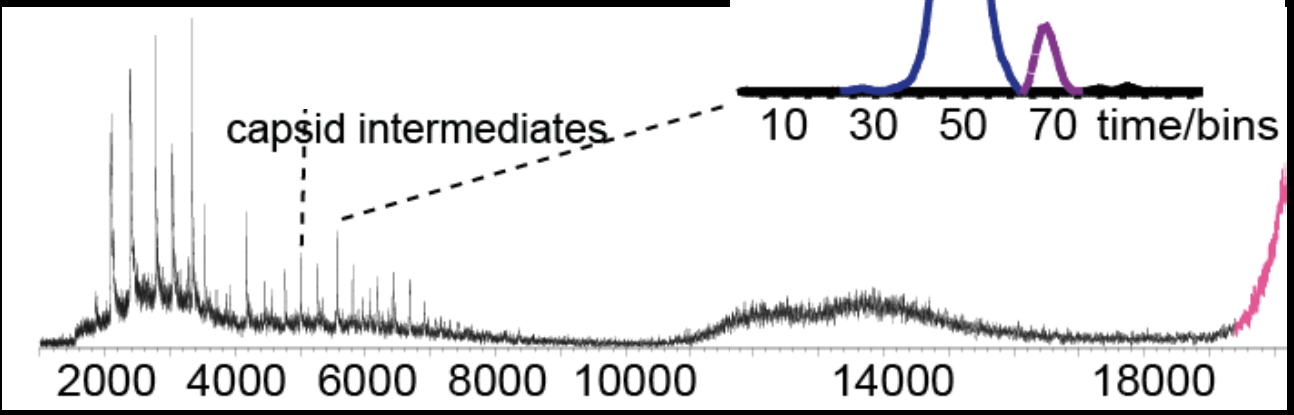
Why use native MS at European XFEL?

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







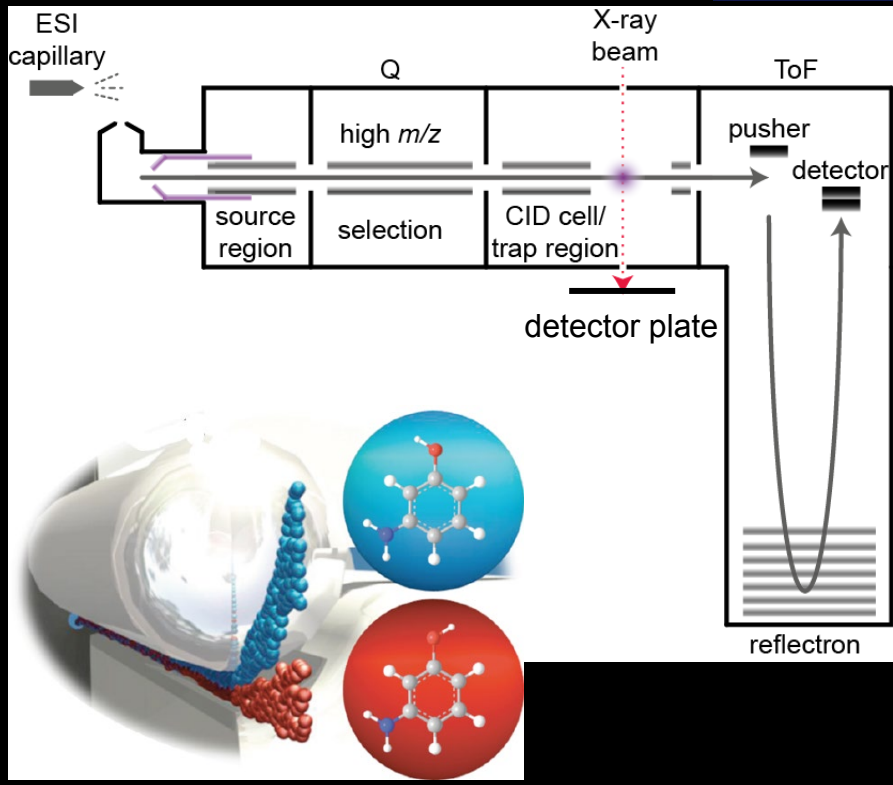


J.Schulz *et al.*, *SPIE Proceedings* **2013**

Why use native MS at European XFEL?

- (Dipole orientation)
- ToF – online diagnostics
  - Sample quality
  - Sample influx
  - Proper selection

- Current status
  - Testing all components
  - → experiments at FLASH I/II, PETRA III
  - → Assemble prototype
  - → Proof-of-principle on norovirus capsids in 2020



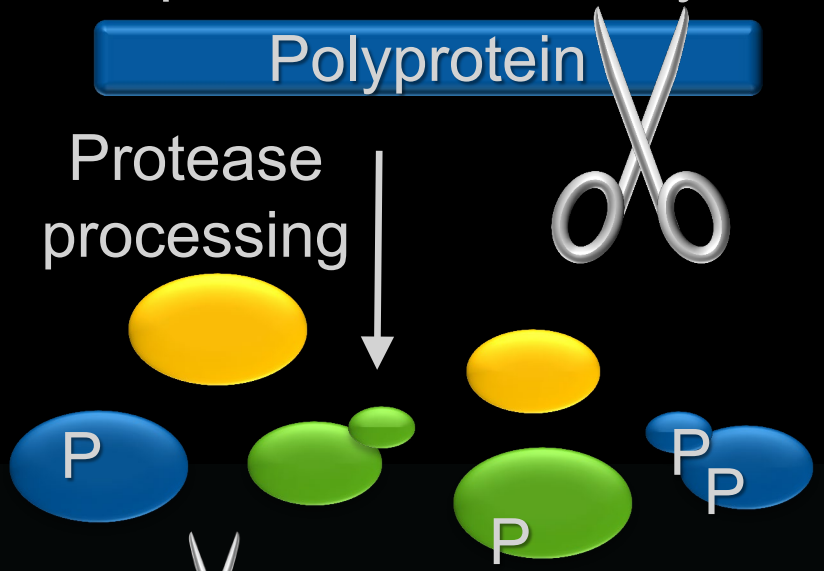
J.Schulz *et al.*, *SPIE Proceedings* **2013**

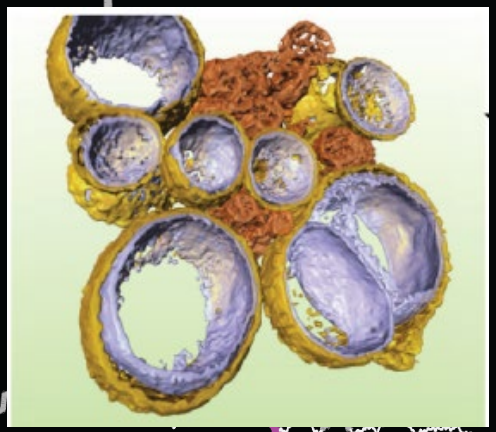
What about small complexes?

Coronaviruses

- Mild cold to severe zoonoses (SARS, MERS)

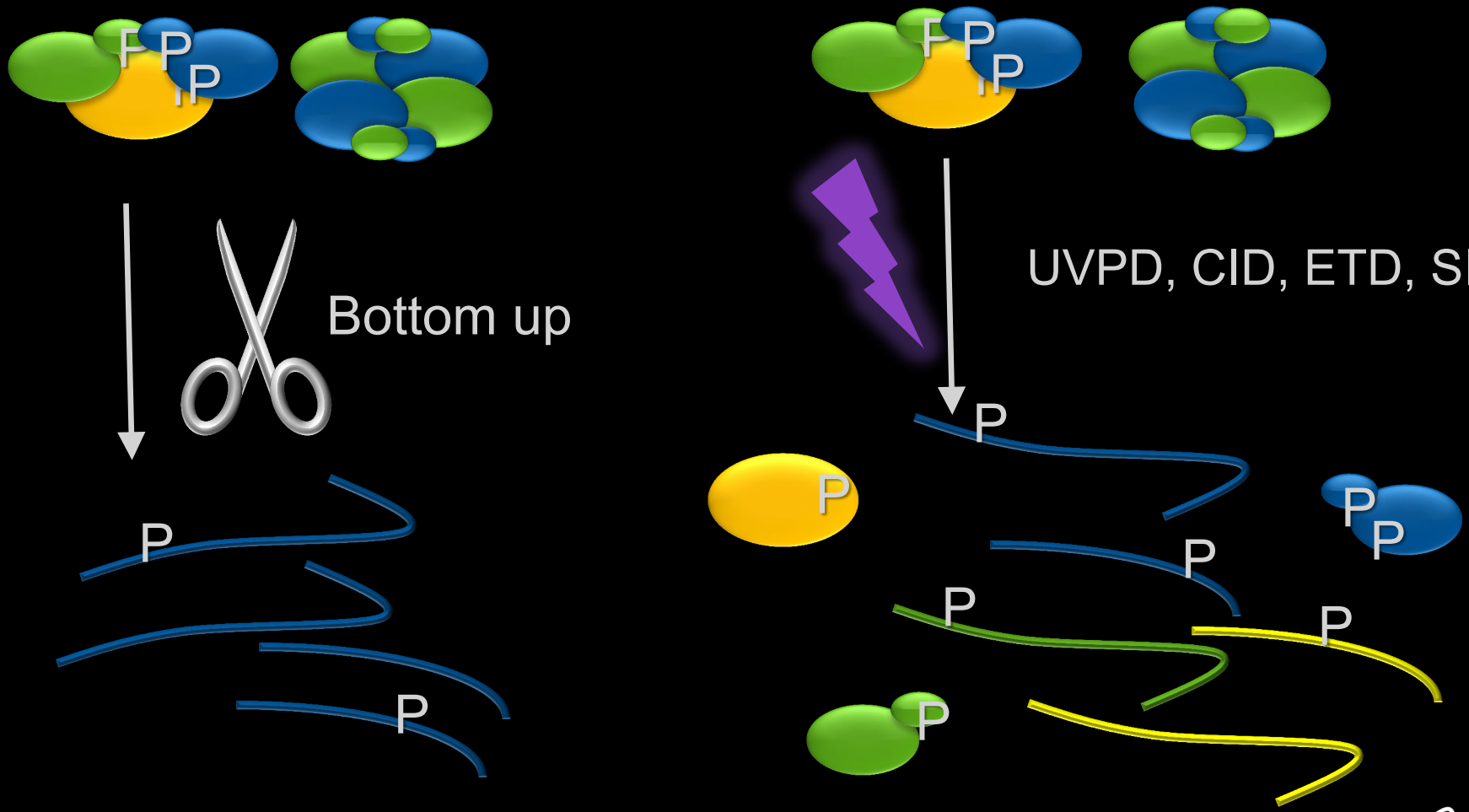
Replication machinery



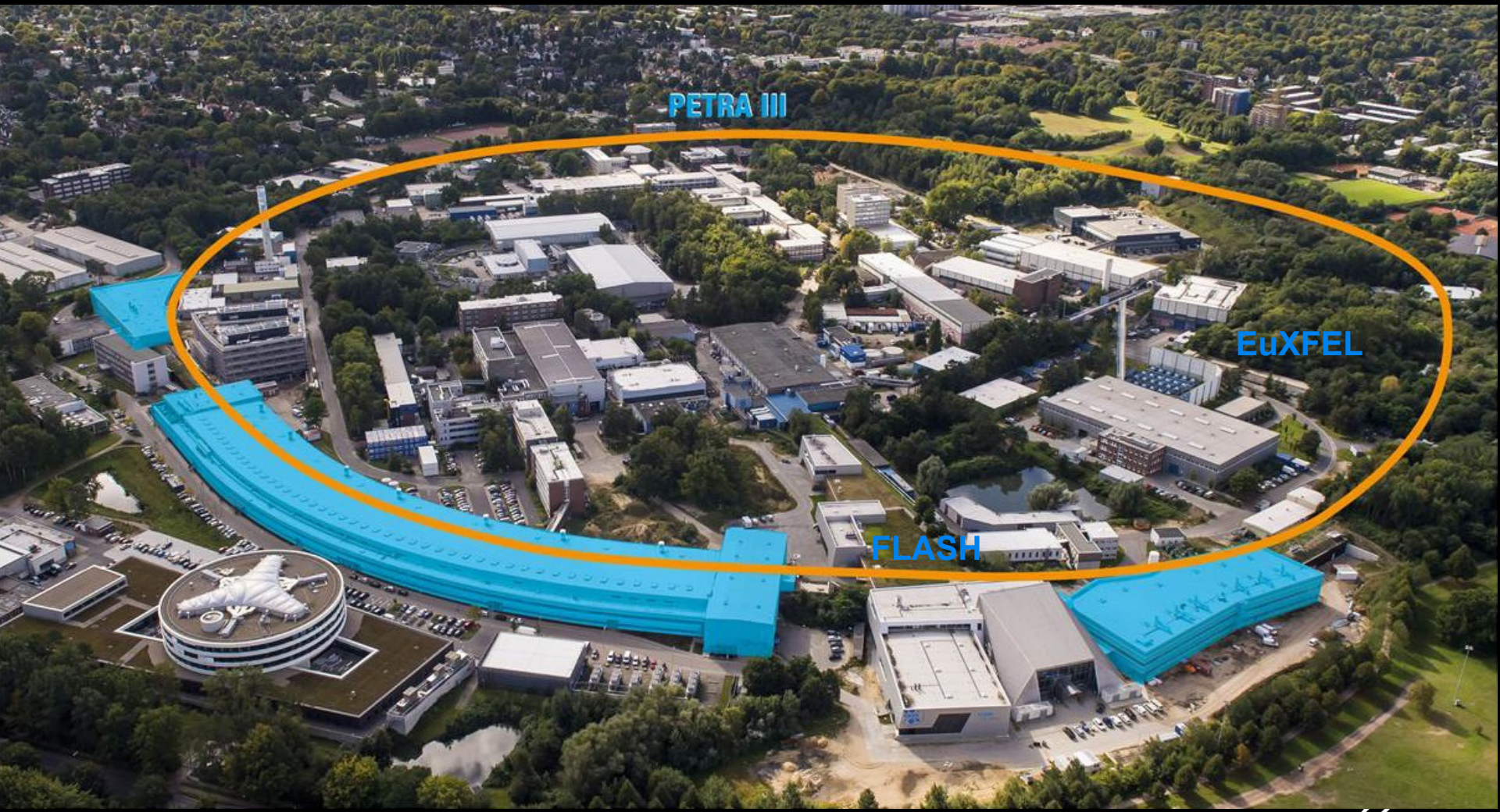


Why native top down MS?

Identification, modifications



X-rays for top-down?

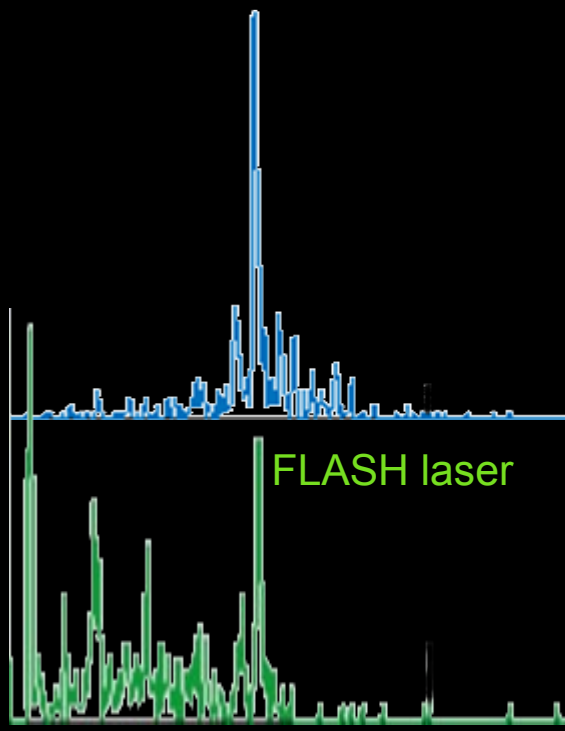


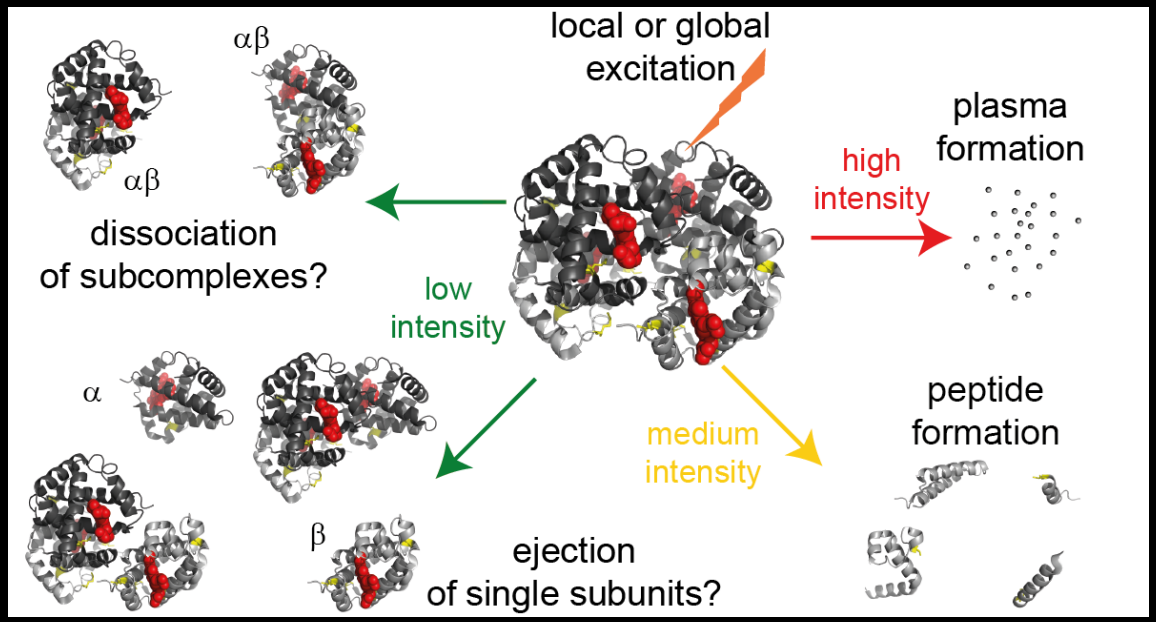
Soft X-rays for protein fragmentation?

- Soft X-rays - no structural resolution
- Instantaneous multiphoton absorption

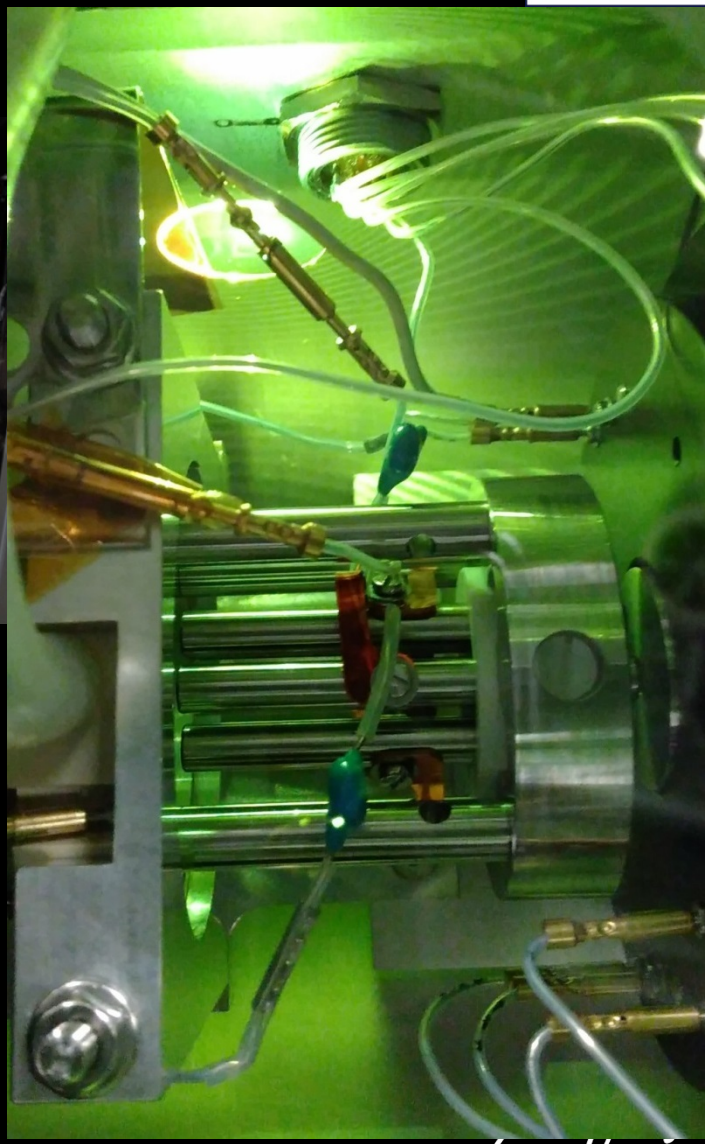
→ Structural information?

→ Native top-down MS?

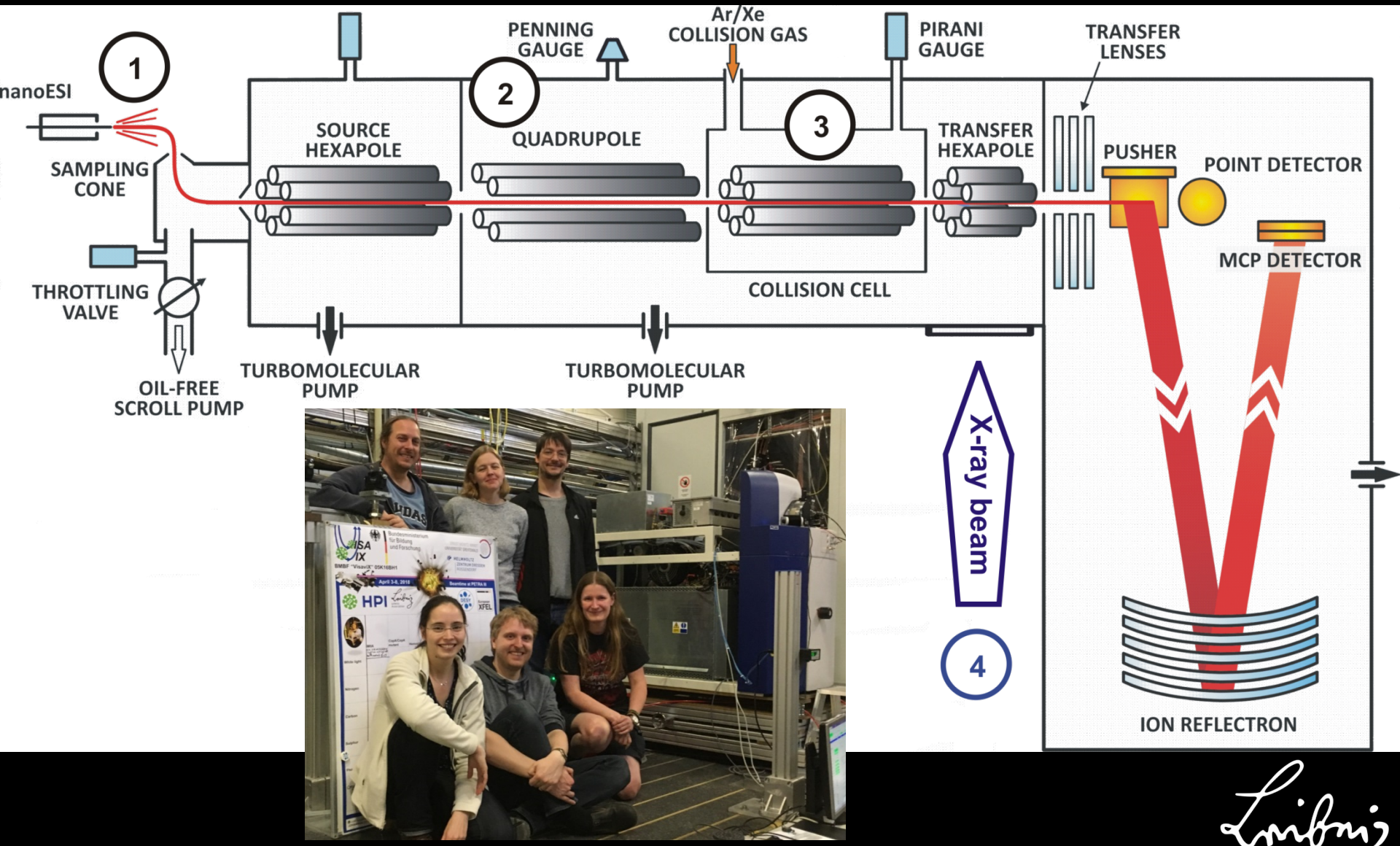




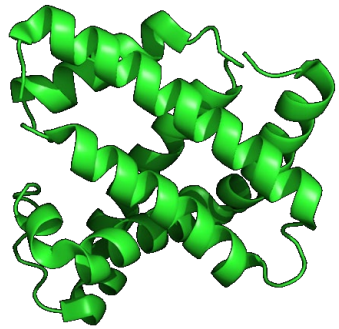
Our QToF @ PETRA III P04

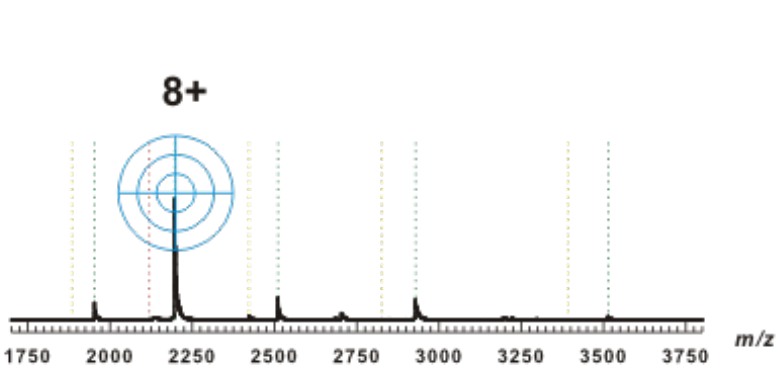


Our setup

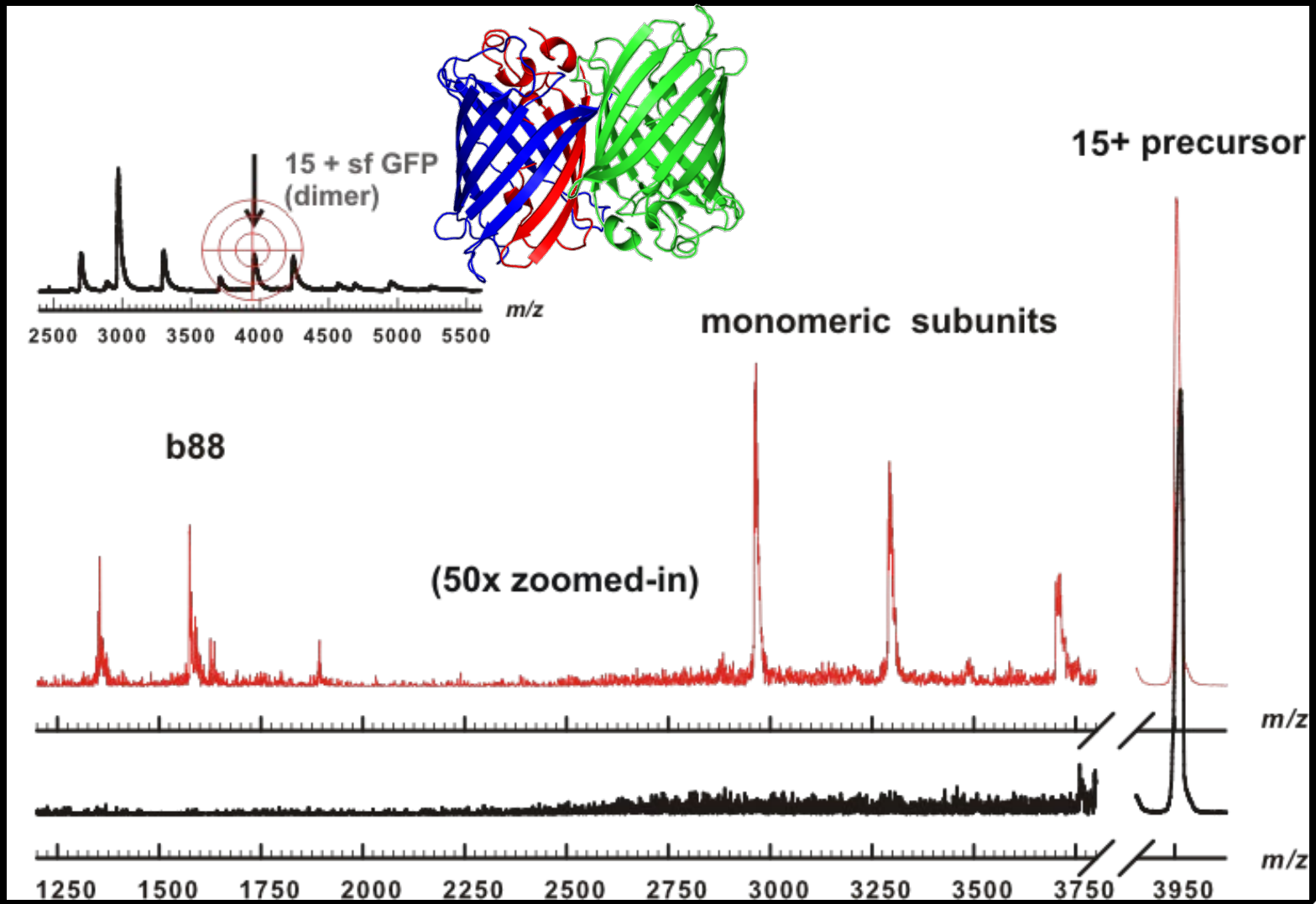

1
nanoESI
SAMPLING CONE
THROTTLING VALVE
OIL-FREE SCROLL PUMP
SOURCE HEXAPOLE
TURBOMOLECULAR PUMP
2
PENNING GAUGE
QUADRUPOLE
TURBOMOLECULAR PUMP
Ar/Xe COLLISION GAS
3
COLLISION CELL
PIRANI GAUGE
TRANSFER HEXAPOLE
TRANSFER LENSES
PUSHER
POINT DETECTOR
MCP DETECTOR
X-ray beam
4
ION REFLECTRON

Backbone fragmentation in myoglobin

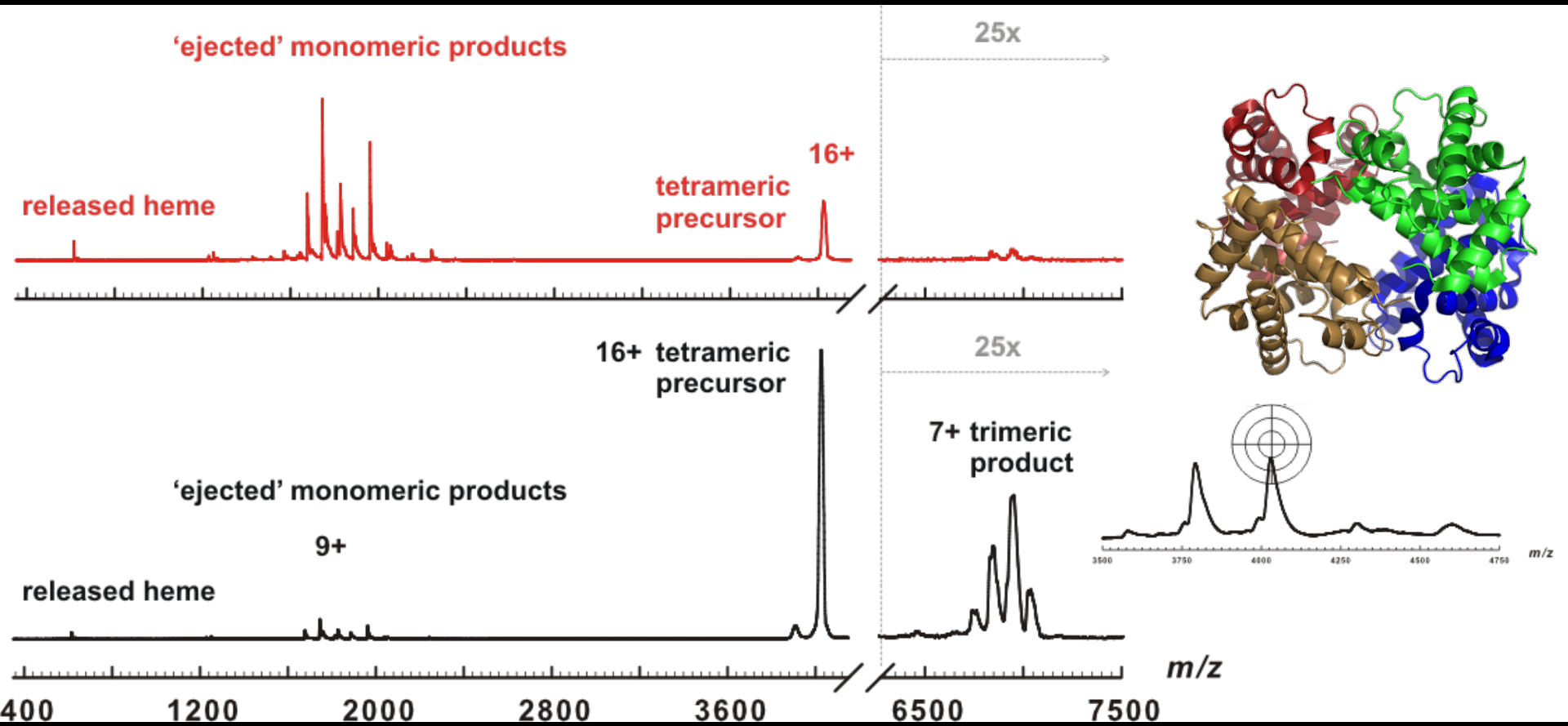




Fragmentation/dissociation of sfGFP

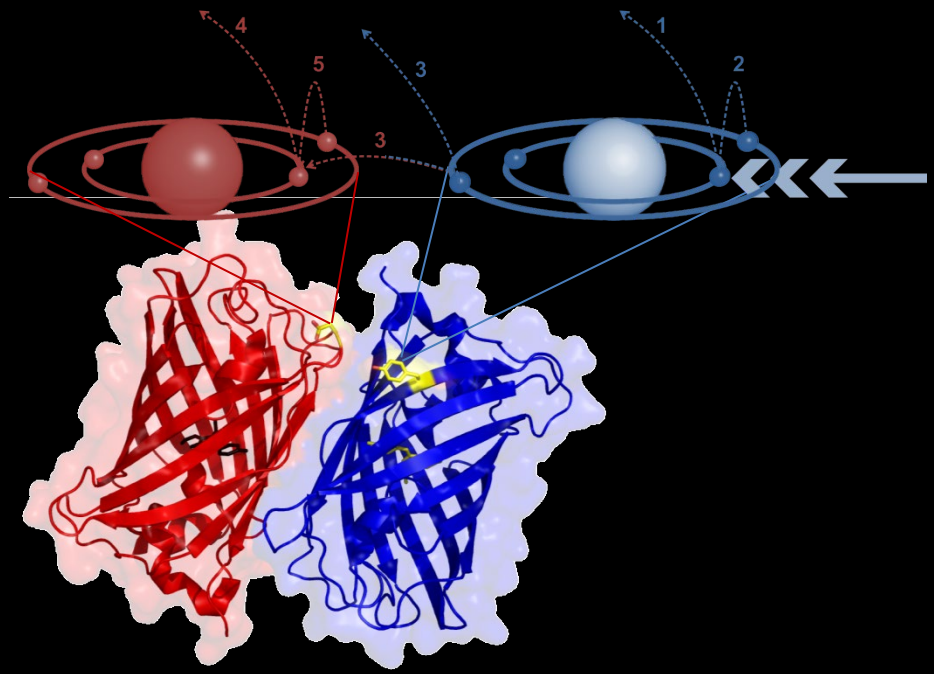


Dissociation of hemoglobin

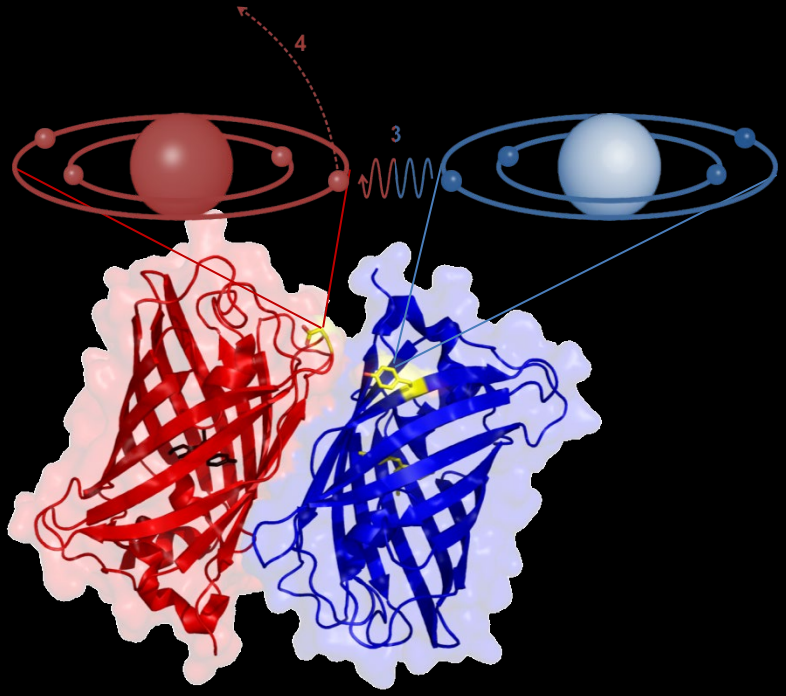


Possible mechanisms

- Auger emission & cascade
- More efficient in large systems



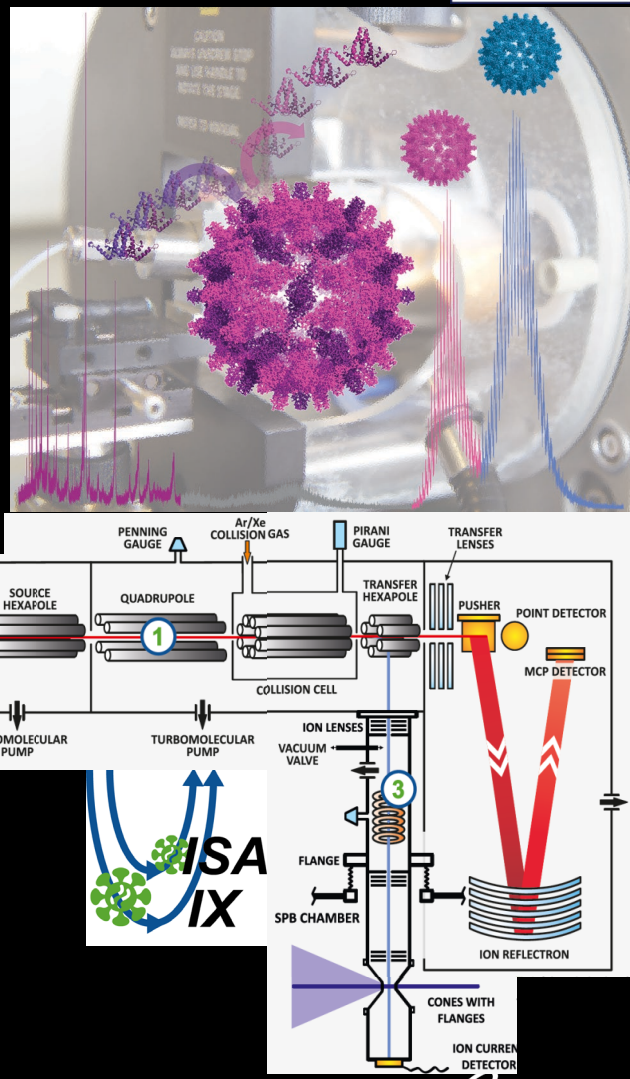
- Auger emission & intermolecular Coulomb decay



Summary

- Viruses
  - Isolate specific dynamics/assembly
  - Single deamidation abrogates glycan binding
  - Polyprotein processing and complexation
  - Peptide binding to MHC

- X-rays beyond crystallography
  - Go well with gas phase ions!
  - Intermediates at high resolution?
  - New fragmentation technique?



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