



# Determining detergent dependence of Cytolysin A oligomeric state through native mass spectrometry

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## Introduction

Pore-forming toxins (PFTs) are a class of membrane proteins. PFTs have bionanotechnological applications, such as nanopore sensors or drug delivery aids<sup>1</sup>. In detergents, PFTs transition from water-soluble monomers into transmembrane oligomeric pore complexes<sup>2</sup>. **Here, we studied how native oligomeric states of an  $\alpha$ -PFT varied in different detergent environments using native mass spectrometry (MS).**

**Cytolysin A (ClyA)**  
 $\alpha$ -PFT

34.5 kDa monomer  
Pathogenic strains of *Escherichia coli*



Monomer  
PDB: 1QOY



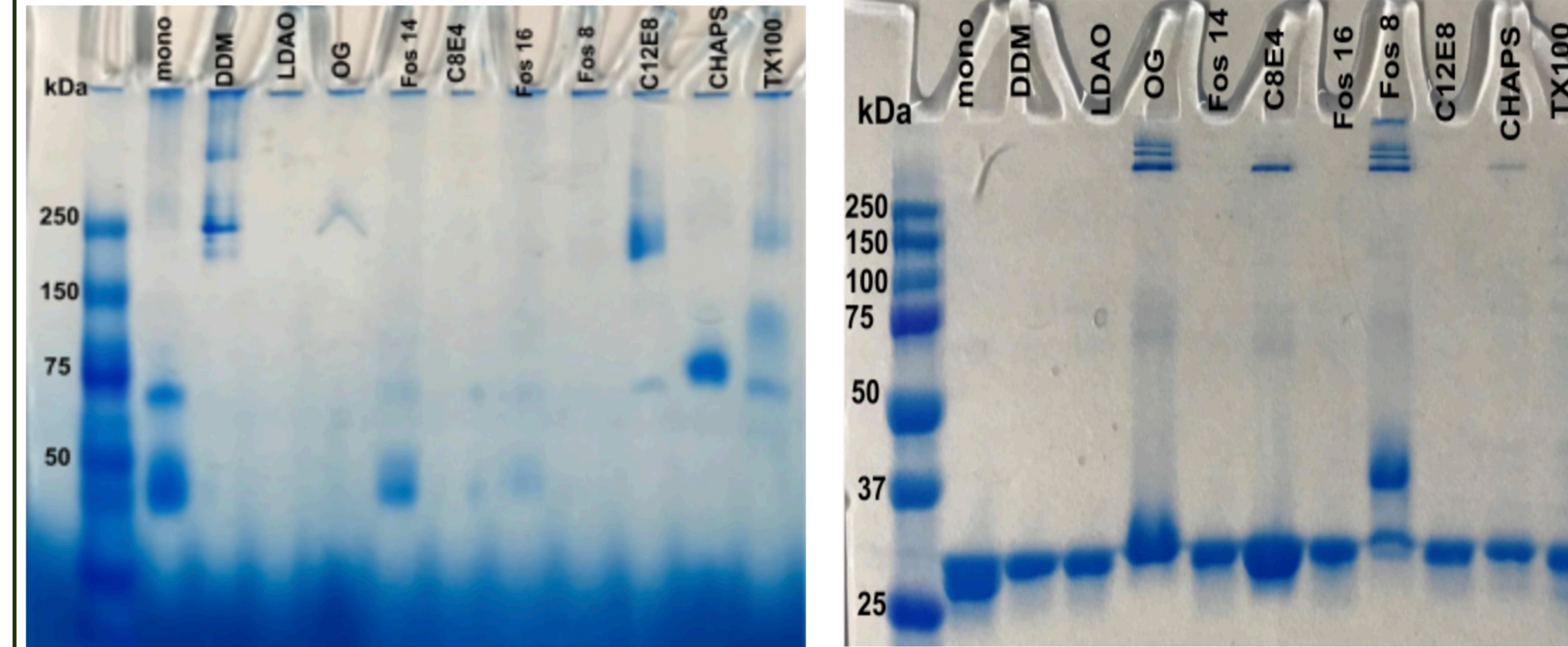
Detergent or Lipid Addition



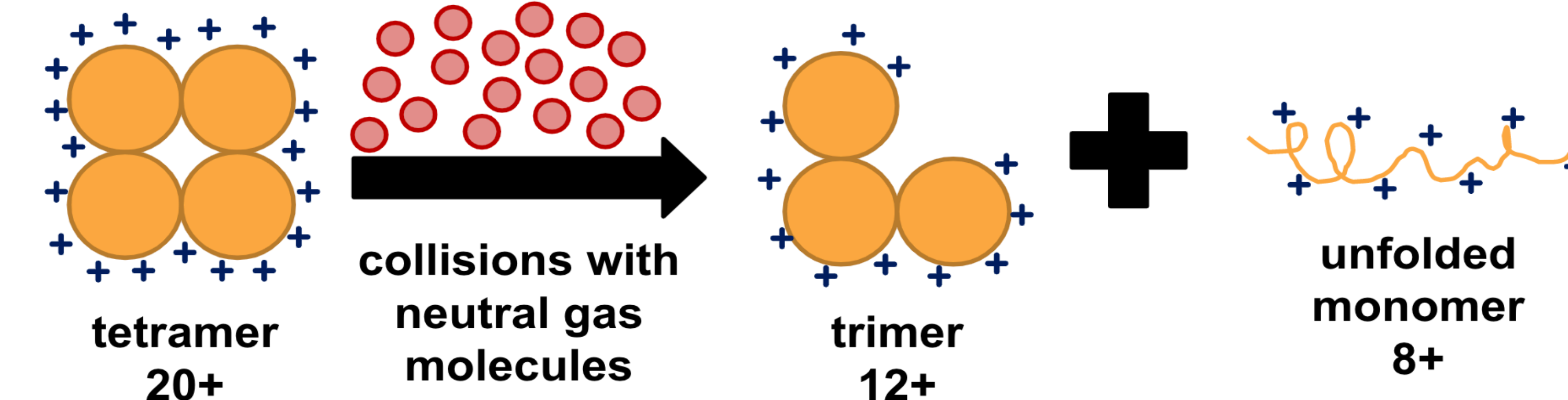
Dodecamer  
PDB: 2WCD

## Results

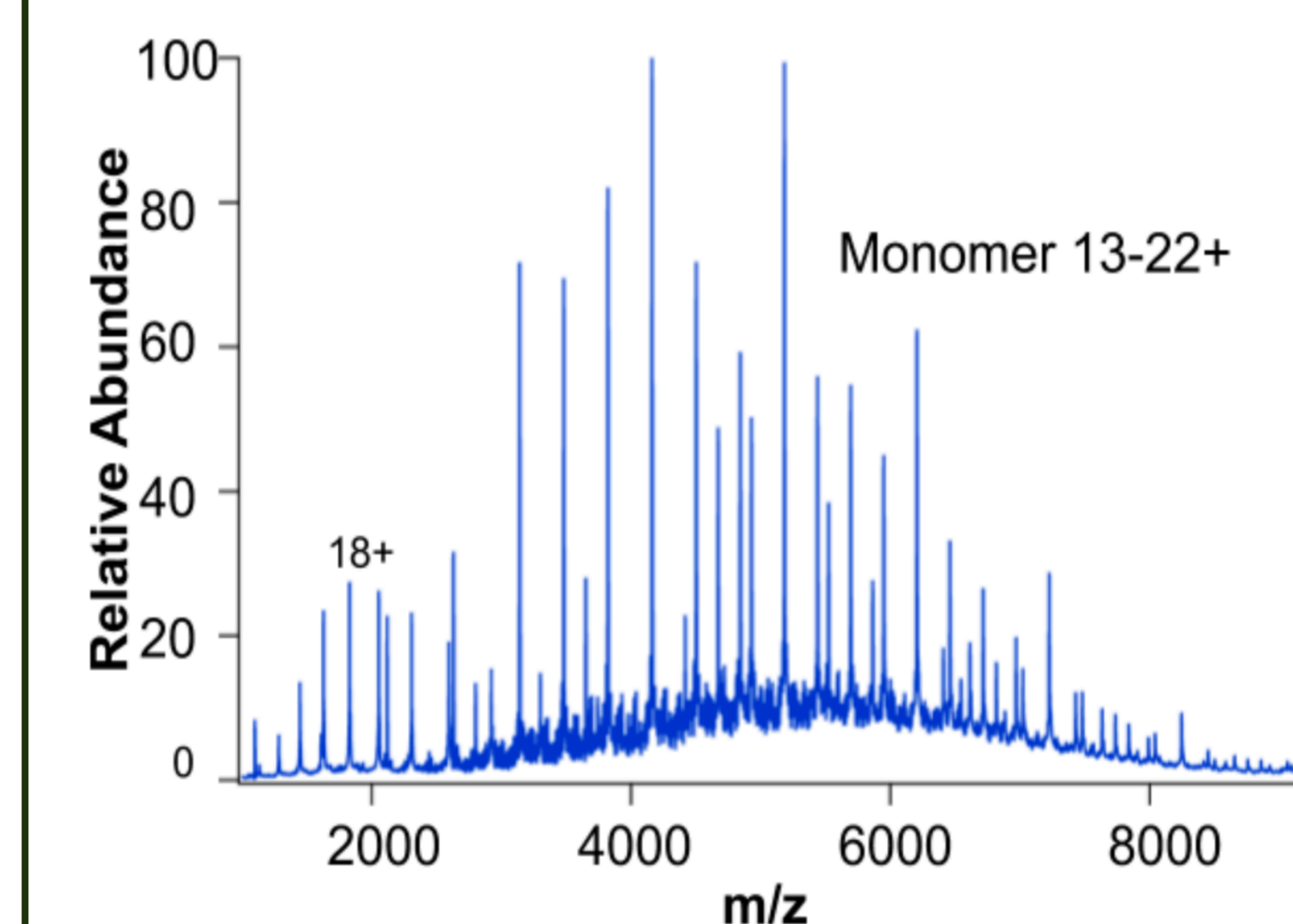
**ClyA detergent samples were assessed with BN-PAGE (to identify oligomers) and SDS-PAGE (to identify aggregates), with DDM, C<sub>12</sub>E<sub>8</sub>, and Triton X-100 selected for further analysis.**



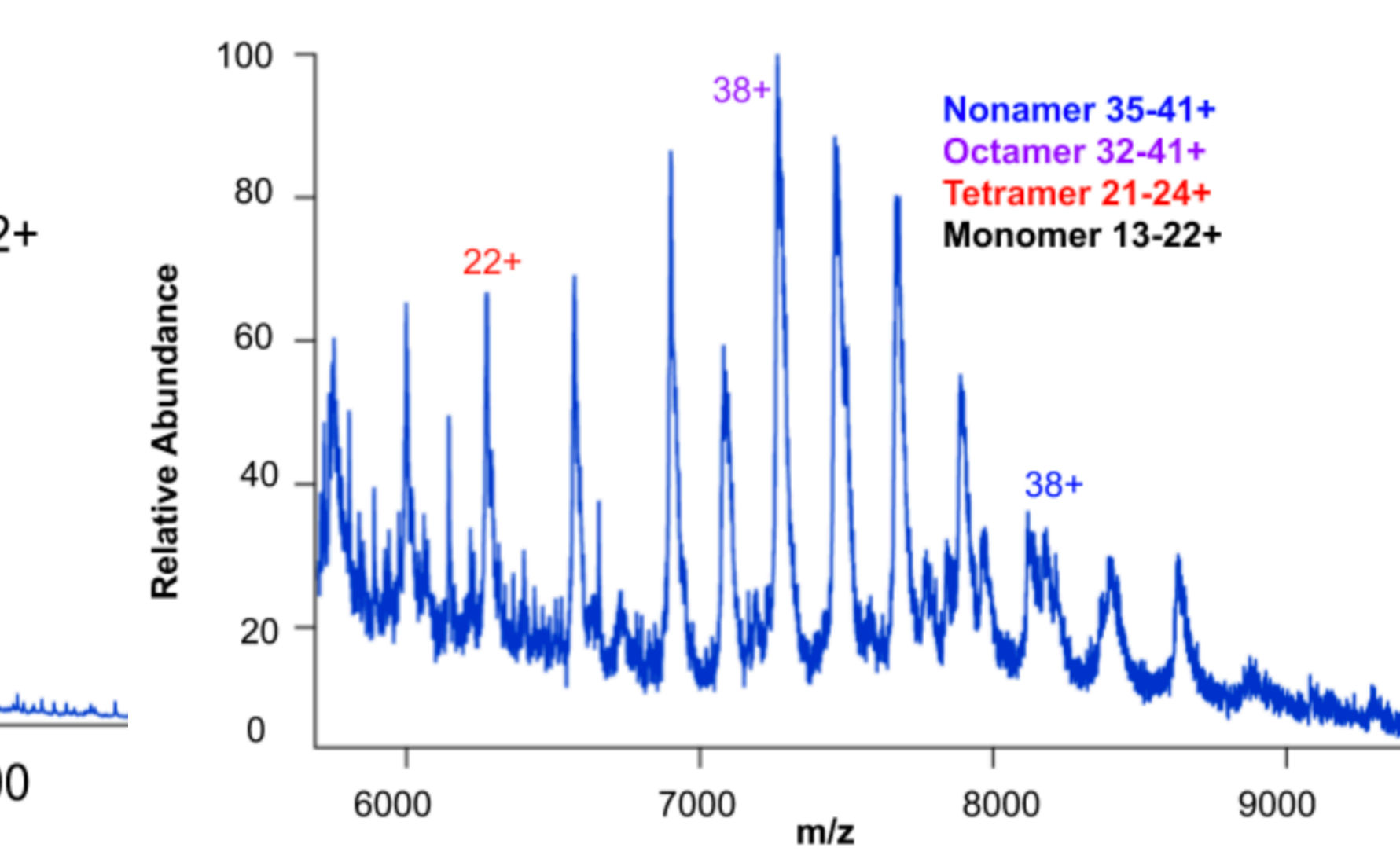
**Collision-induced dissociation (CID) enables identification of oligomeric state of intact ClyA detergent complexes<sup>4</sup>.**



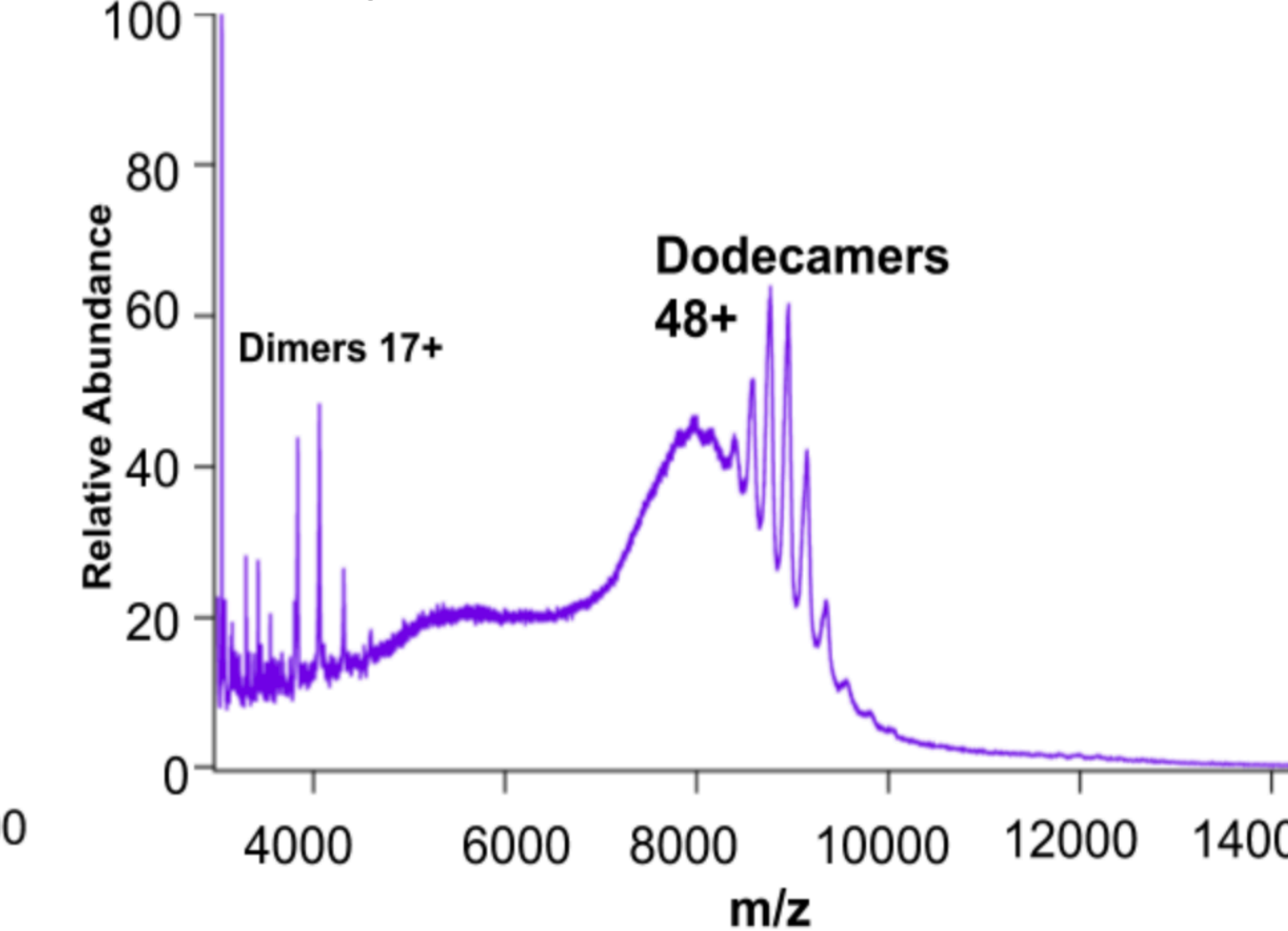
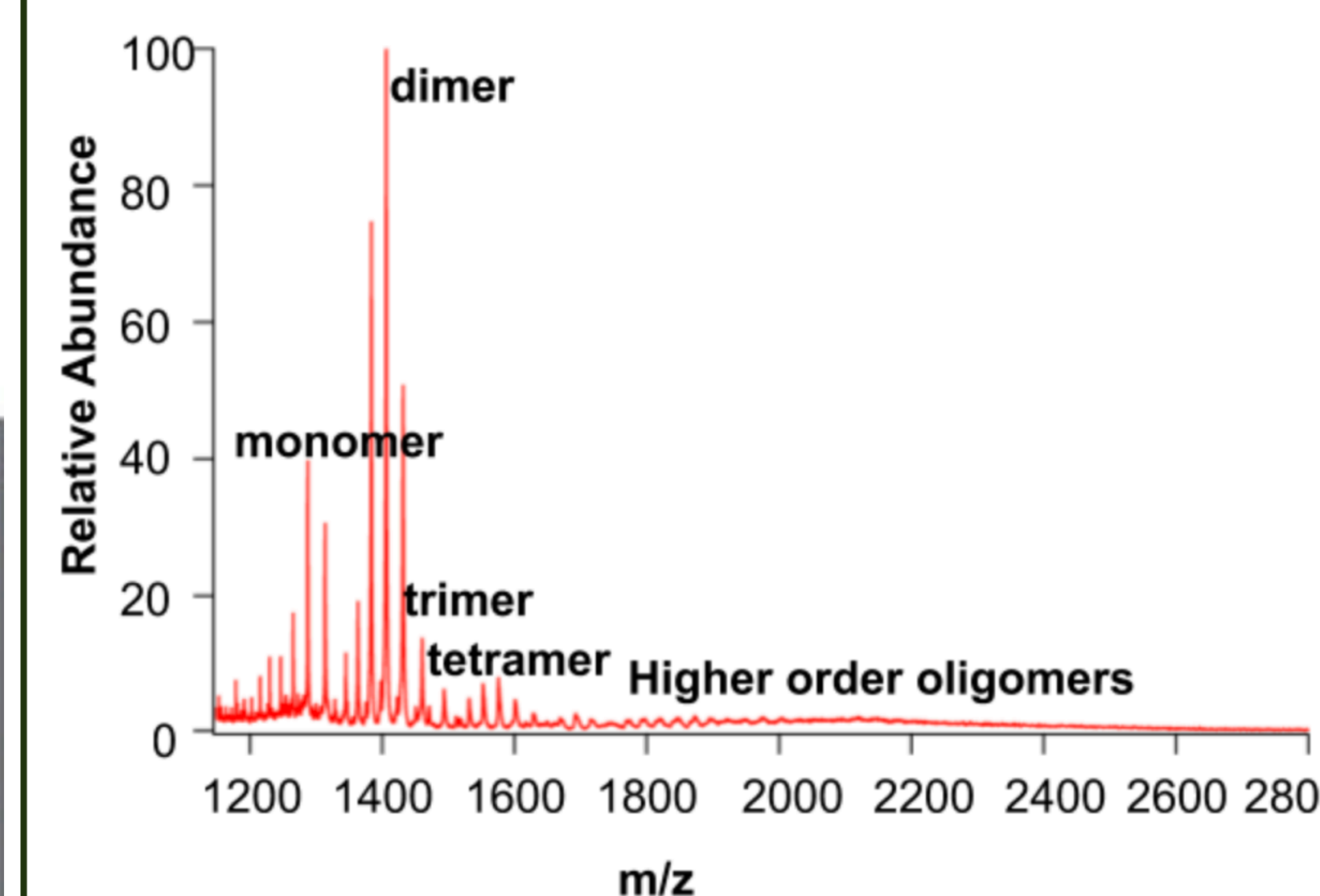
**ClyA forms up to decamers in DDM.**



lower charge than for native-like



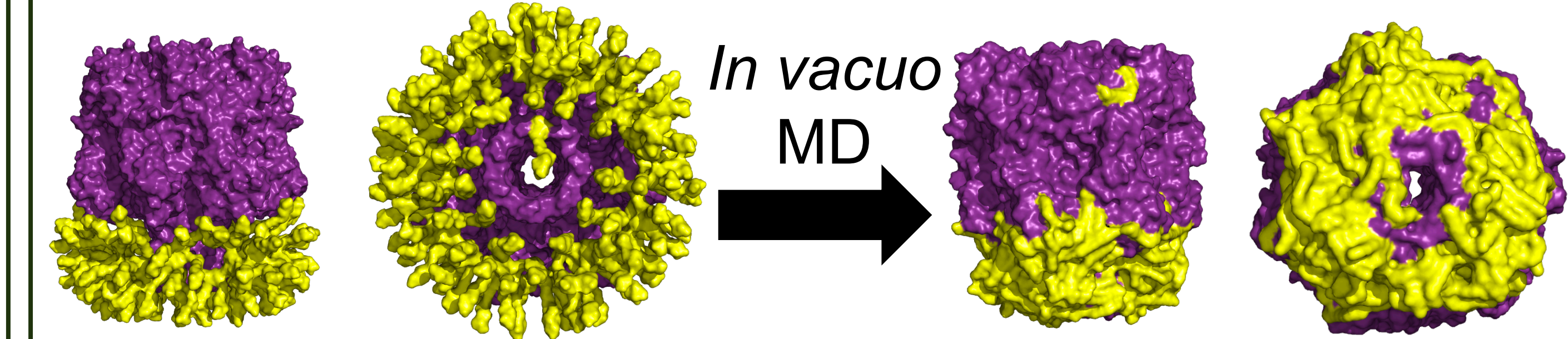
**ClyA forms up to dodecamers in C<sub>12</sub>E<sub>8</sub>.**



**ClyA also formed many oligomers in Triton X-100. The detergent OG caused ClyA to aggregate and crash out of solution, while Fos-14 heavily adducted to only monomers.**

## Ongoing Work

Model structures of varying ClyA oligomeric states are being inserted into different detergent micelles, and *in vacuo* molecular dynamics simulations<sup>5</sup> are being performed to predict the stability of the complexes.

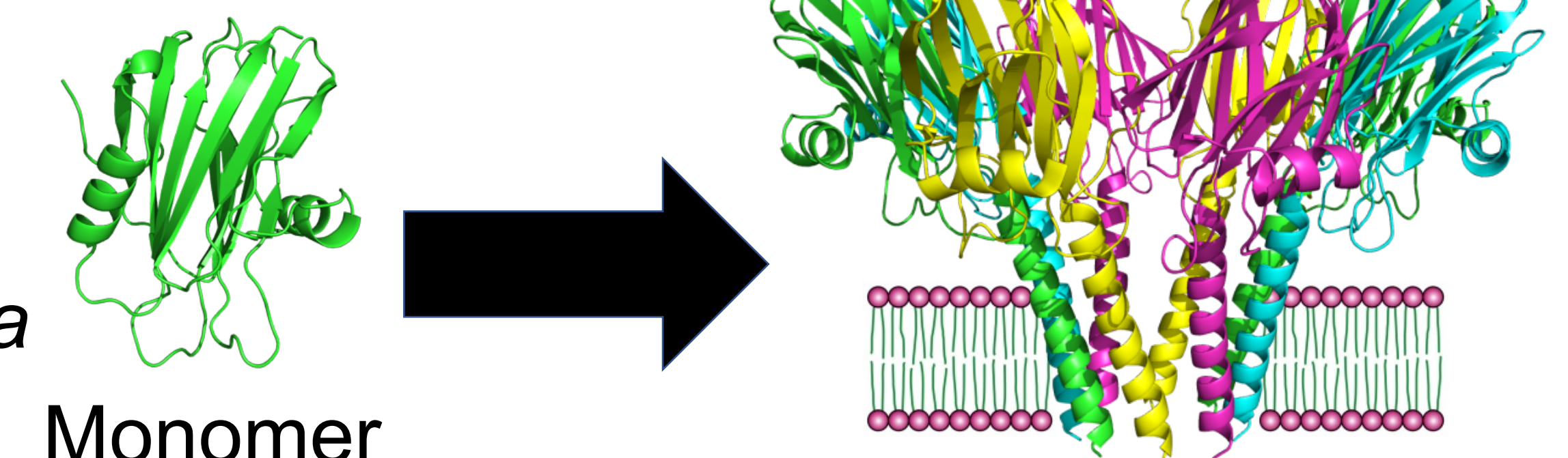


AHL (a  $\beta$ -PFT, PDB: 7AHL, purple) inserted into a Fos-14 micelle (yellow) before and after *in vacuo* MD

## Conclusions & Future Directions

**The oligomeric state(s) of ClyA pore complexes varies in different detergents.** These findings provide insight for PFT applications in nanotechnology through manipulation of desired pore size. We are continuing to investigate the relationship between detergents and oligomeric state using MD simulations, as well as by repeating this methods for another  $\alpha$ -PFT, fragaceatoxin C (FraC).

**FraC**  
*Actinia fragacea*



Monomer  
PDB: 3VWI

Octamer, PDB: 4TSY

## Methods

ClyA affinity & SEC purification

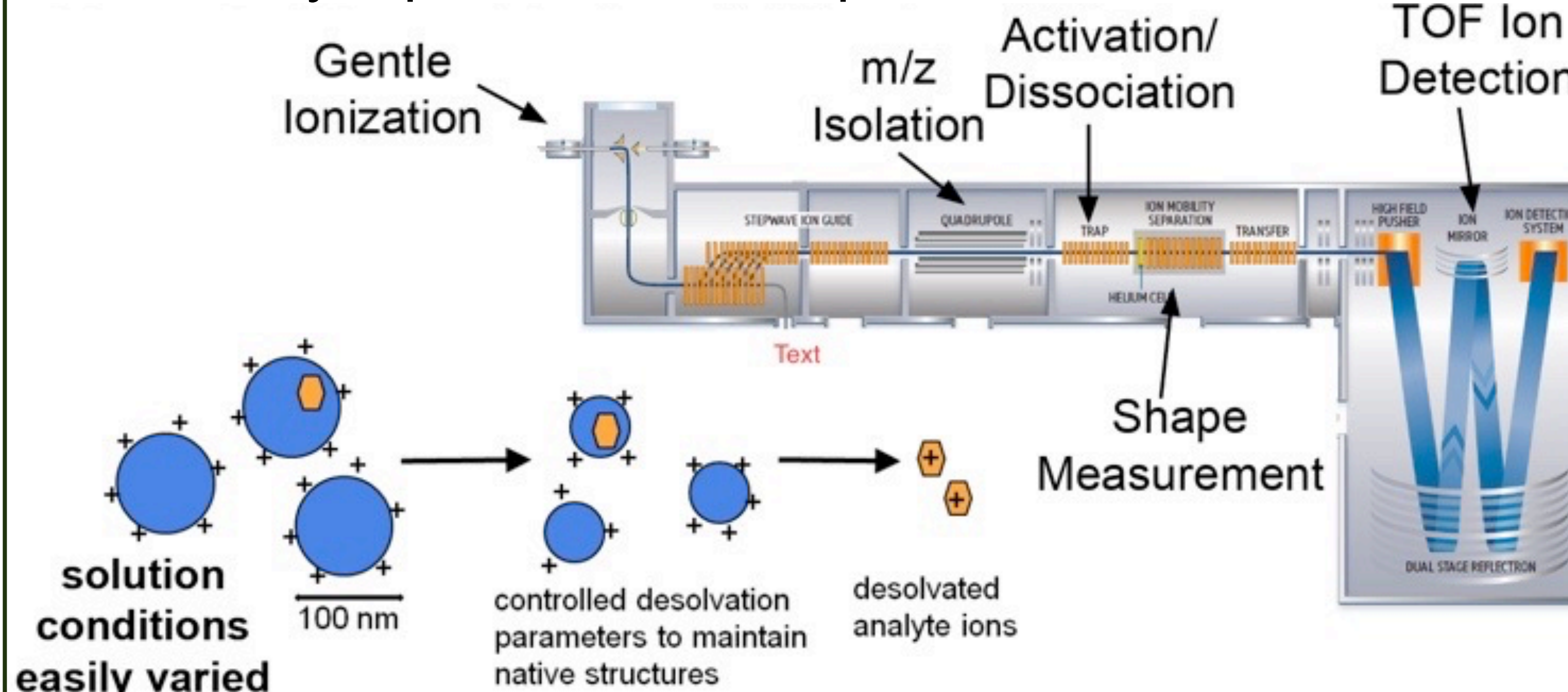
Incubate monomers in different detergents

Screening: Blue Native PAGE and SDS PAGE

Identify oligomers using native mass spectrometry

**Native mass spectrometry enables preservation of noncovalent complexes, and detergent (above the CMC) protects membrane complexes from solution into vacuum<sup>3</sup>.**

Waters Synapt G2-Si mass spectrometer



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**References.** (1) Lu, B.; Stokes, C.; Fahie, M.; Chen, M.; Golovchenko, J.A.; Vestergaard Hau, L. *Biophys. J.* **2018**, *115*, 801-8. (2) Dal Peraro, M.; van der Goot, F.G. *Nat. Rev. Microbiol.* **2016**, *14*, 77-92. (3) Barrera, N.P.; Di Bartolo, N.; Booth, P.J.; Robinson, C.V. *Science* **2008**, *321*, 243-6. (4) Benesch, J.L.P.; Aquilina, J.A.; Ruotolo, B.T.; Sobott, F.; Robinson, C.V. *Chem. Biol.* **2006**, *13*, 597-605. (5) Rolland, A.D.; Prell, J.S. *TRAC Trends Anal. Chem.* **2019**, *116*, 282-291.