Variable Temperature Native Mass Spectrometry for Studying Thermodynamics of Protein-Ligand Interactions.

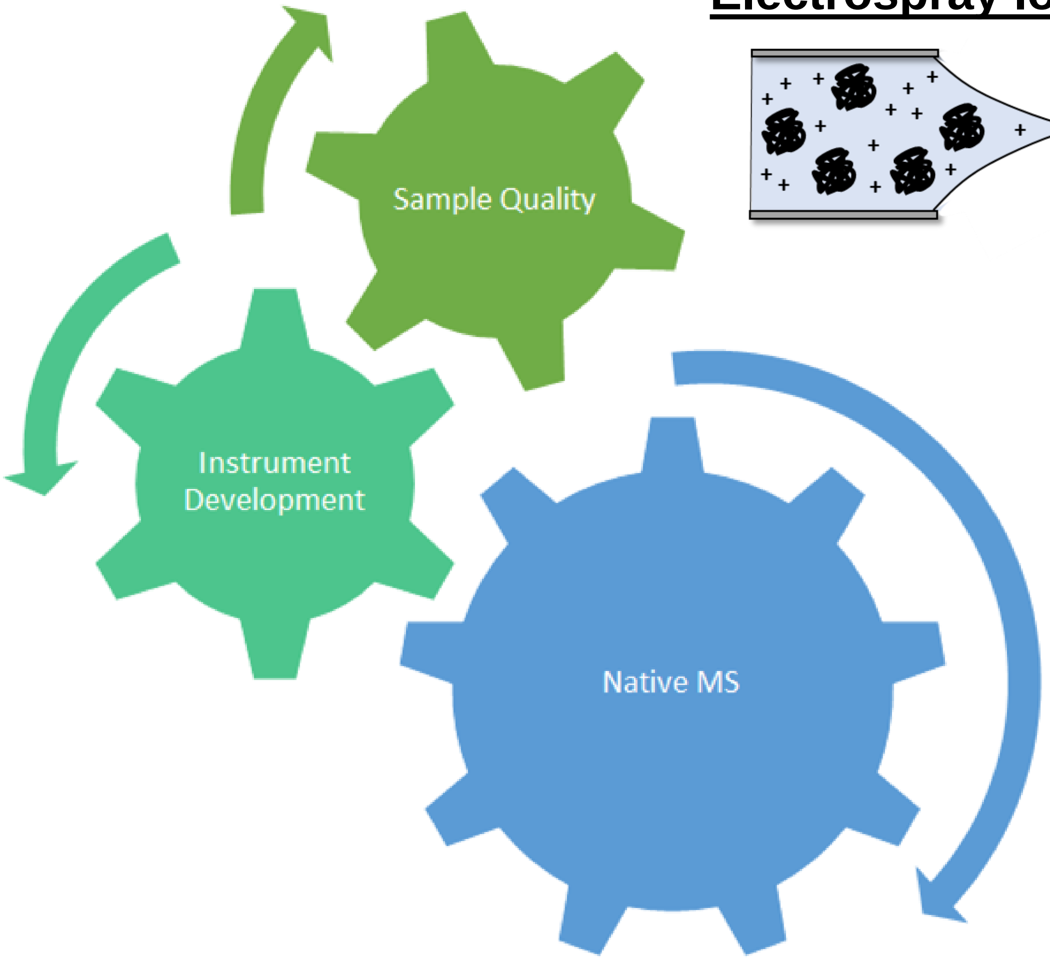
Thomas E Walker III

David H Russell Research Group

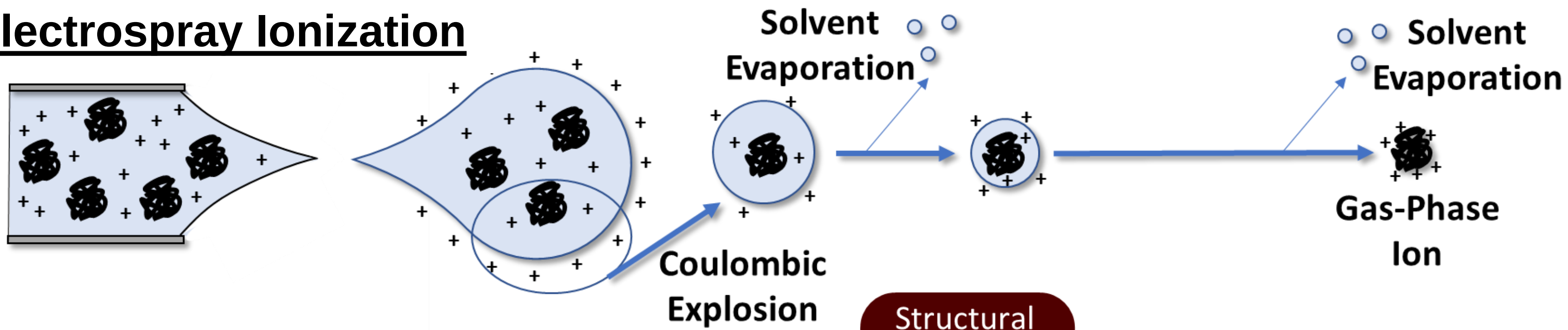
Introduction

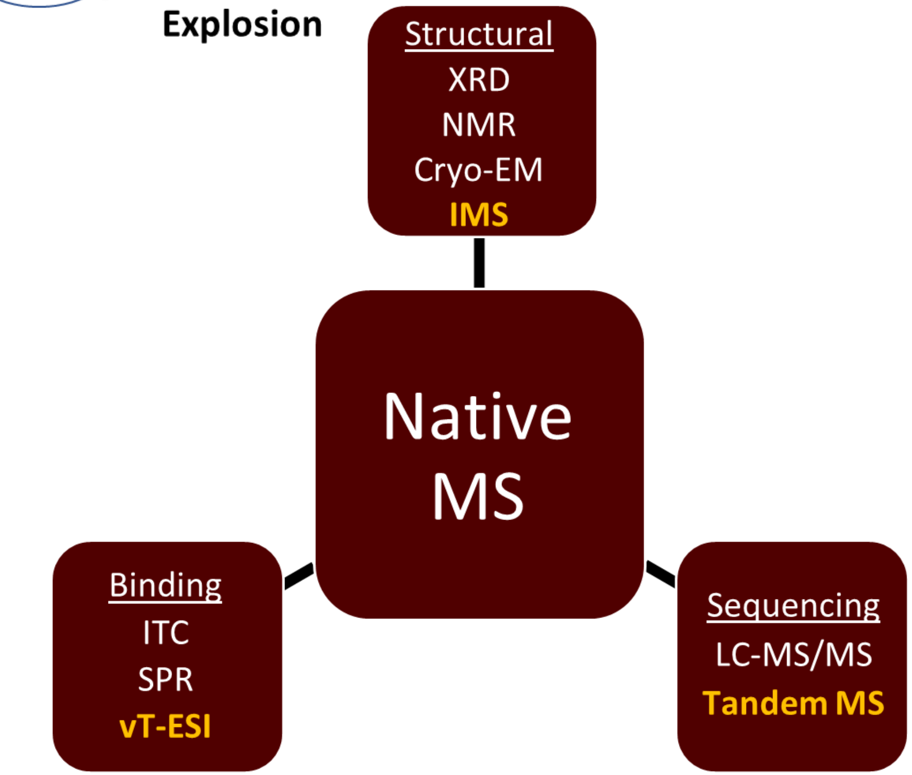
- Native MS
- Variable Temperature ESI
  - Design
  - Implementation
- HSP60 Chaperonin GroEL
  - Structure and Function
- Ligand Binding Thermodynamics with GroEL-ATP
  - Enthalpy-Entropy Compensation
  - Difficulties with Hydrolysis
  - Advantages of MS-guided Thermodynamic Measurements

Native Mass Spectrometry



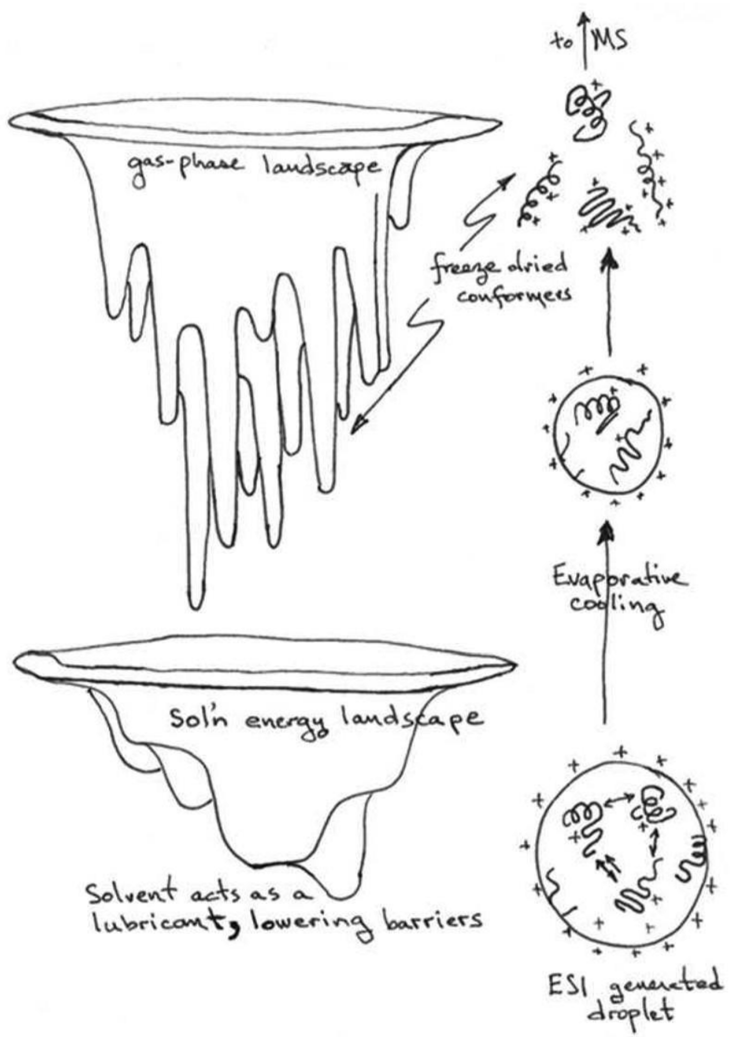
Electrospray Ionization

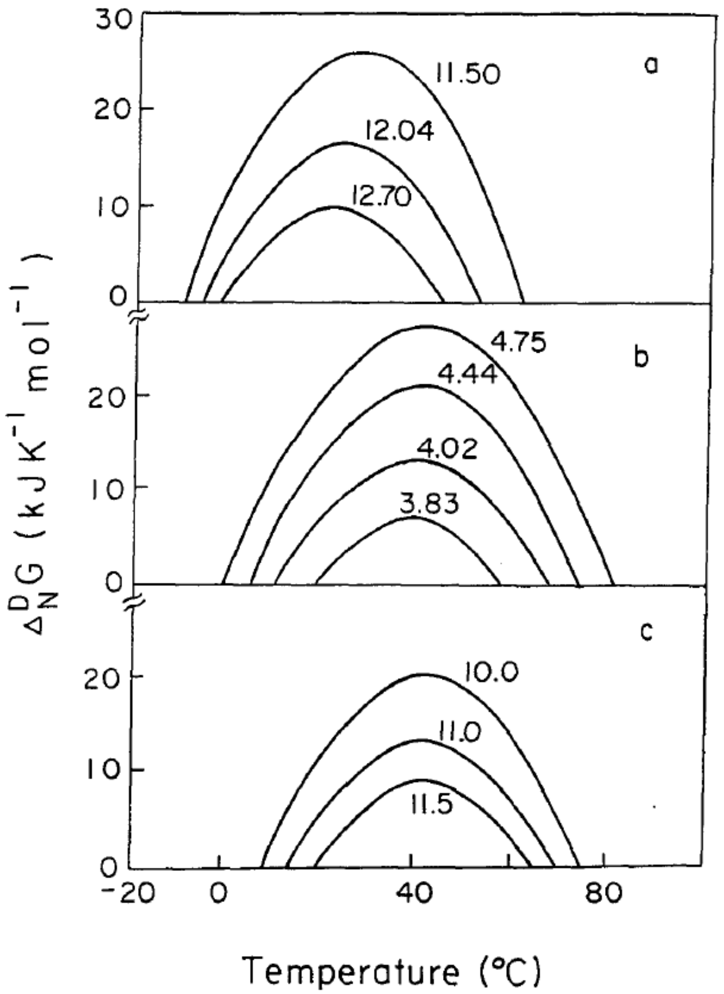




Leney, A.C, Heck, A.J.R; J Am Soc Mass Spectrom. 2017; 28(1): 5–13
Clemmer, D.E, Russell, D.H., Williams, E.R.;*Acc.Chem.Res.* **2017**, 50, 556−560

The Gibb’s Free Energy Landscape

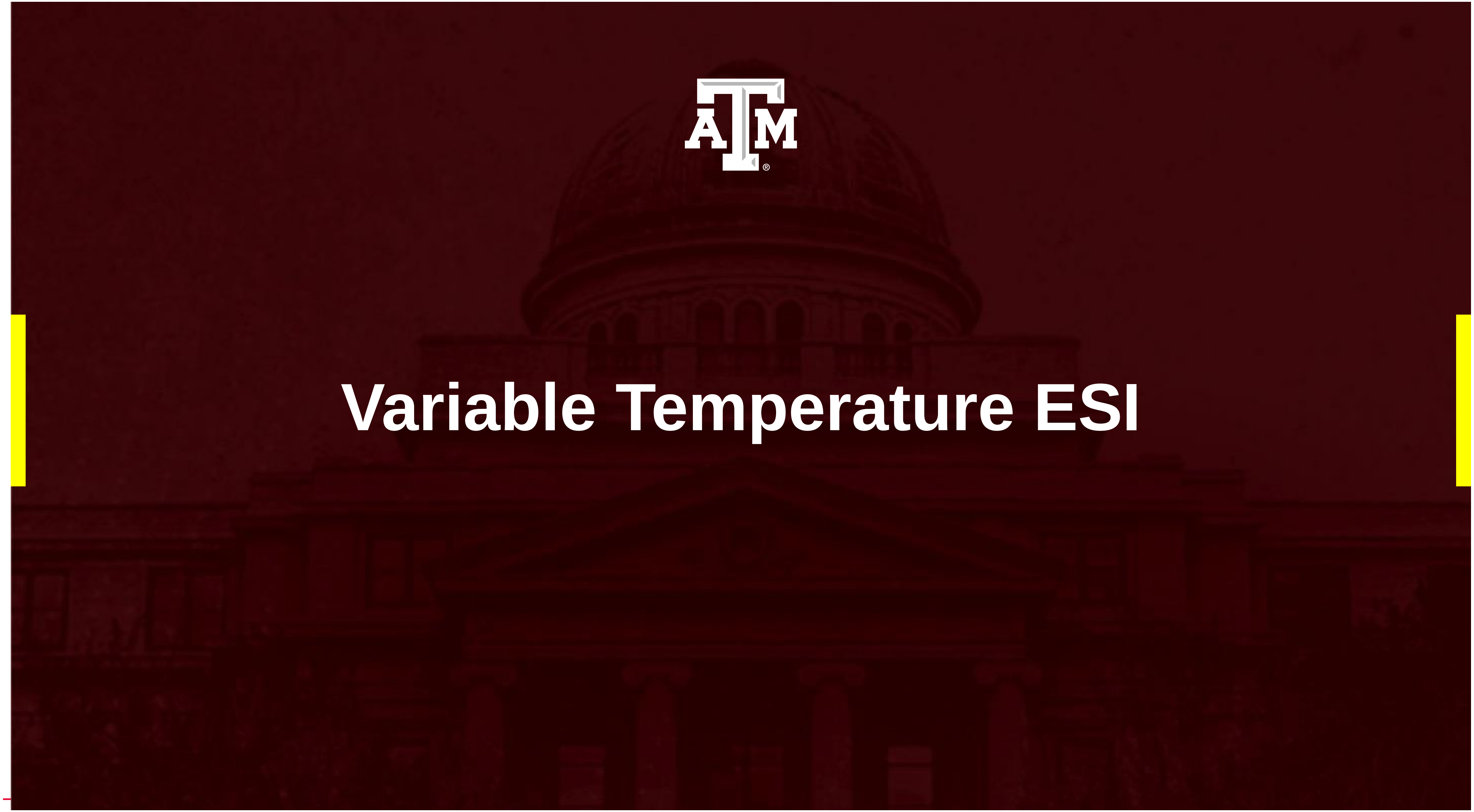




The Gibbs energy function denatured and native states for metmyoglobin in glycine (a) and acetate (b) buffers and in the glycine buffer in the presence of 2 M urea (c) at various pH conditions (indicated on the curves).

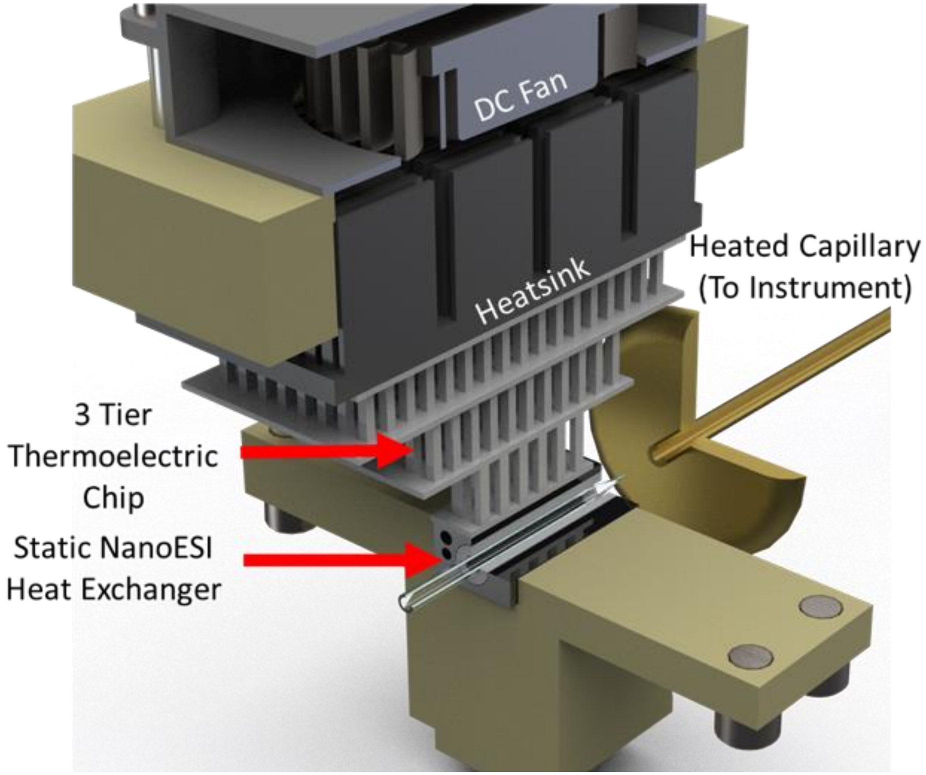
Clemmer *et al. Acc. Chem. Res.* 2017, 50, 3, 556–560

Peter L. Privalov (1990), Critical Reviews in Biochemistry and Molecular Biology, 25:4, 281-306

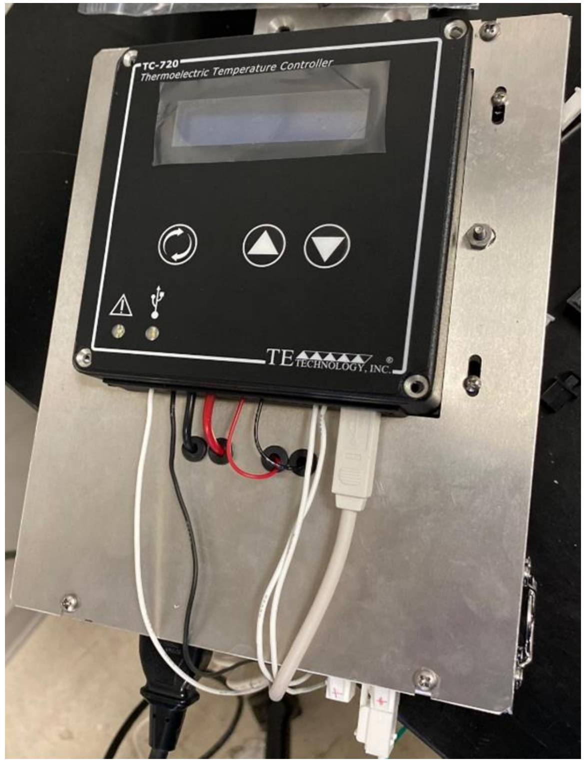


Variable Temperature ESI

VT-ESI Device



Solidworks Rendering of vT-ESI Device



Custom vT-ESI Power Supply

VT-ESI Device

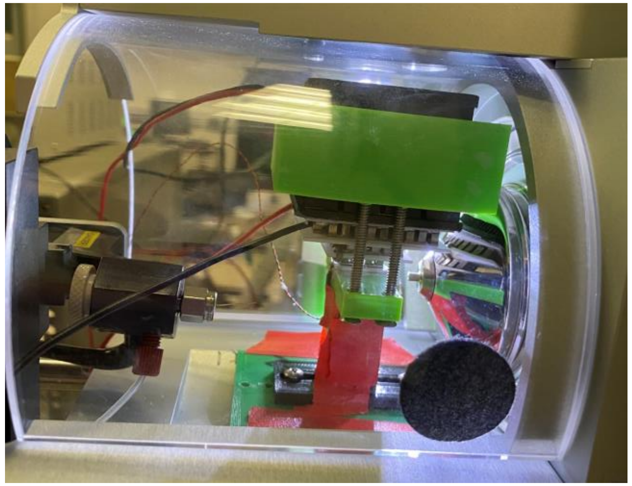
- Amendable to Static Spray ESI
- 3-tier P-Chip
- Quick temperature response (<1 min)
- Can be 3D Printed or Machined

Custom vT-ESI Power Supply

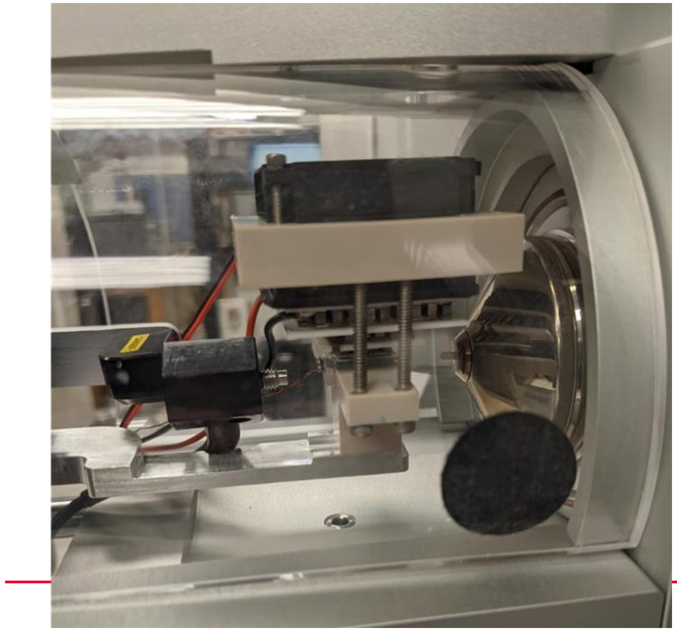
- Safe and portable
- USB Interface
- Can heat and cool

McCabe, et al. *Anal. Chem.* **2021**, 93, 18, 6924–6931

Implementation of vT-ESI on Thermo UHMR

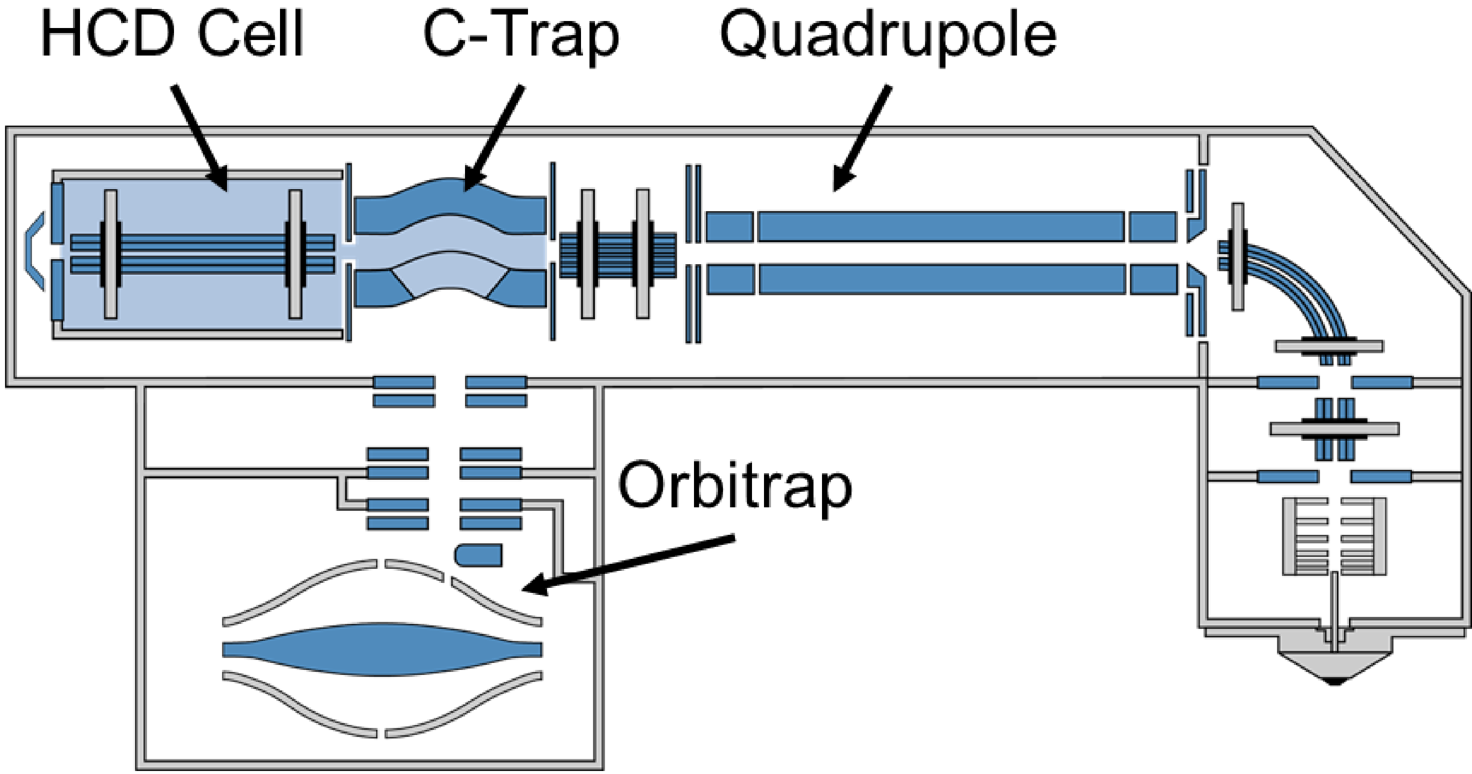


3D Printed assembly for quick prototyping

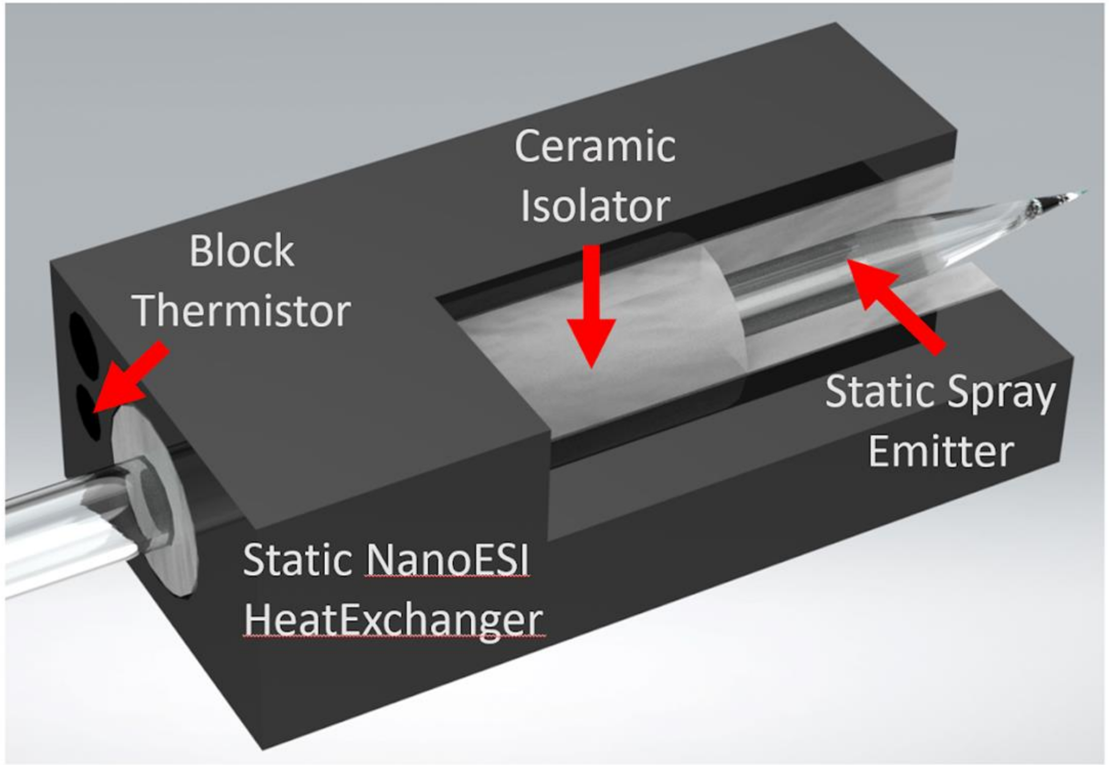


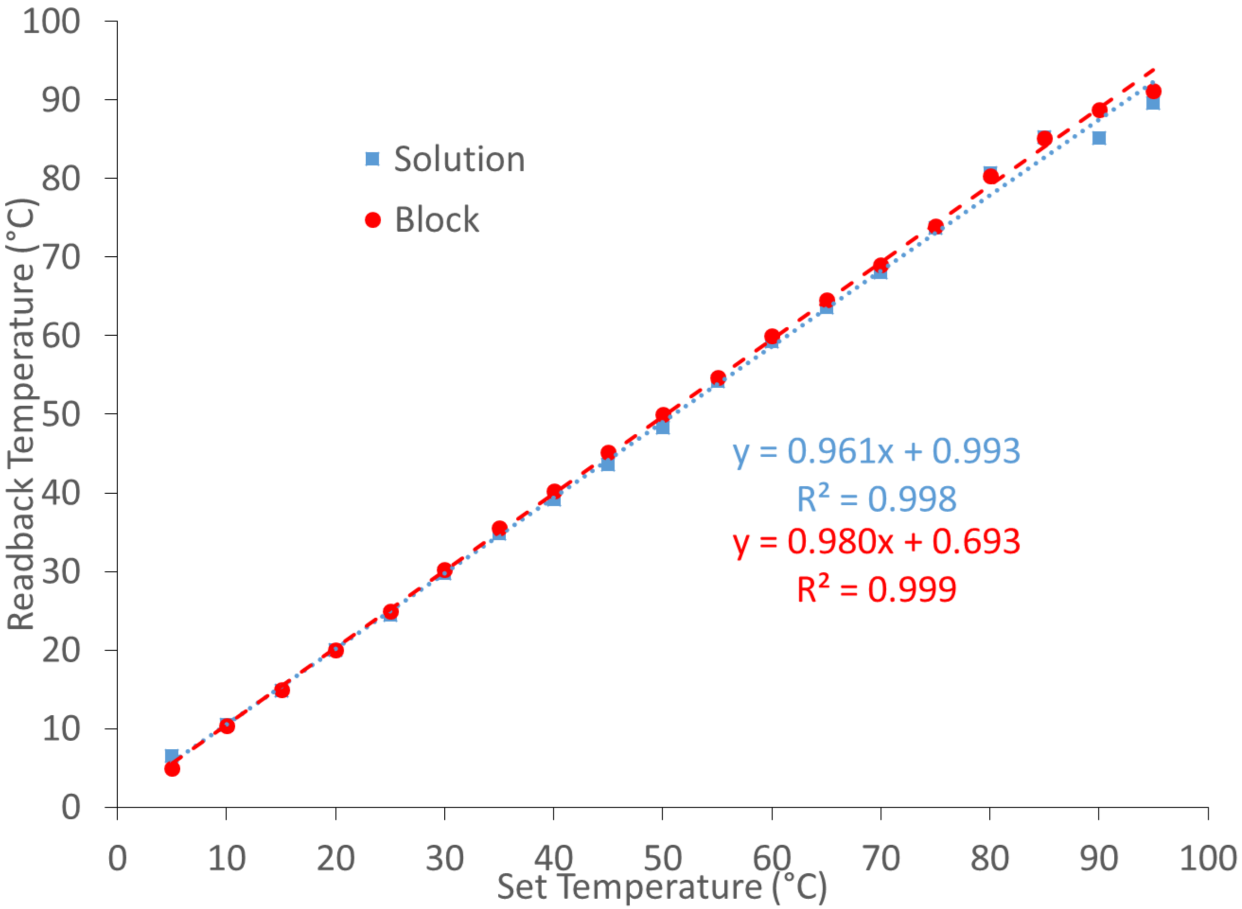
Finished Product

Thermo UHMR



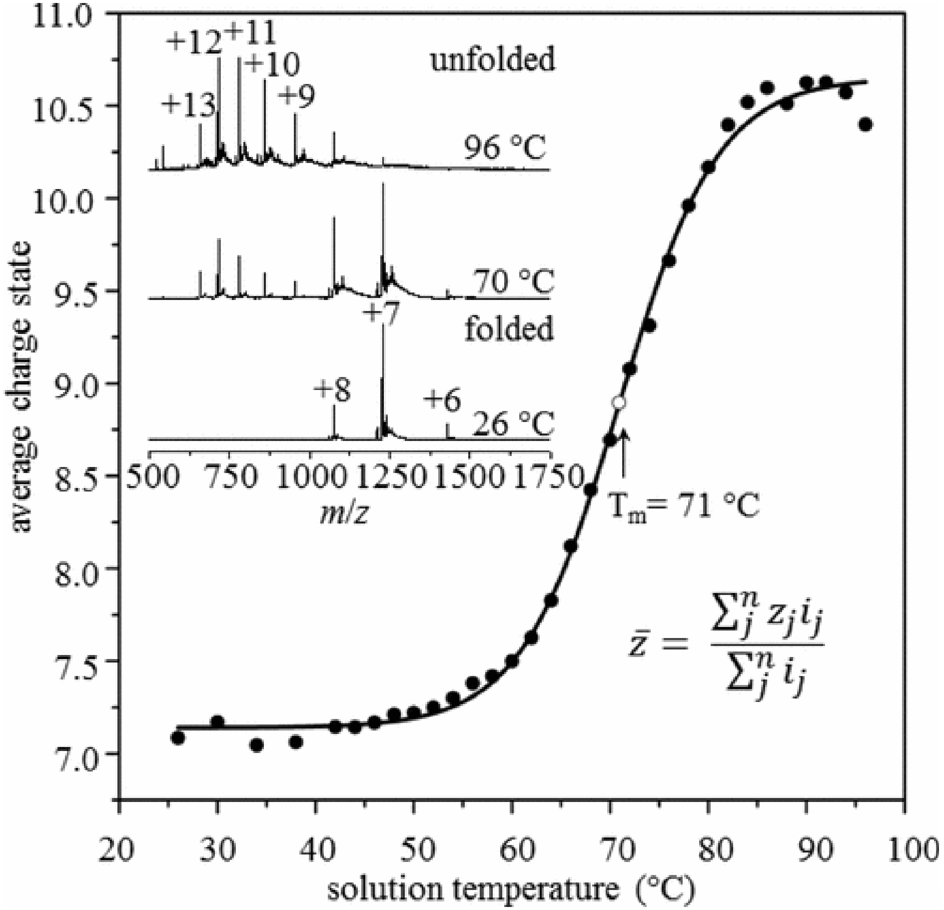
Calibration





McCabe, et al. *Anal. Chem.* **2021**, 93, 18, 6924–6931

Comparison to Earlier vT-ESI Experiments



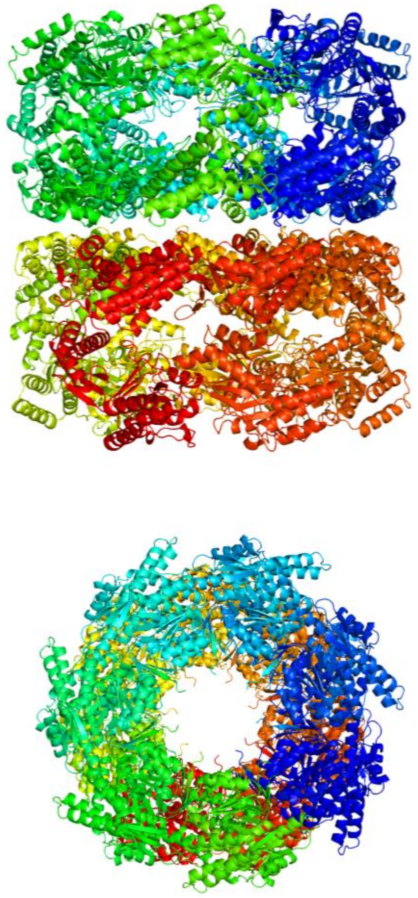
El-Baba,T.J.; *et al. J. Am. Chem. Soc.* **2017**, 139, 18, 6306–6309

McCabe, et al. *Anal. Chem.* **2021**, 93, 18, 6924–6931

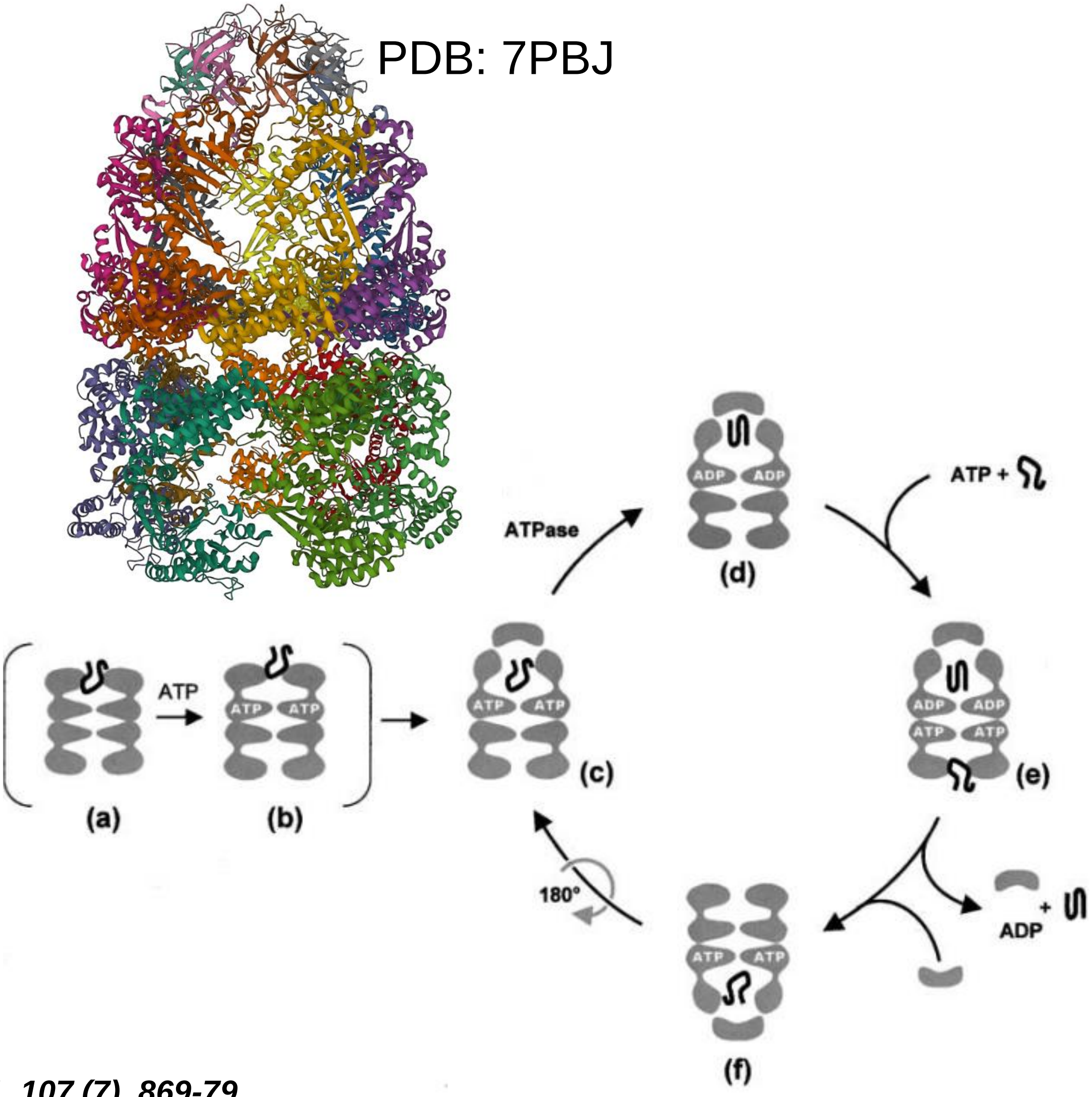
HSP60 Chaperonin GroEL

HSP60 Chaperonin GroEL

PDB: 1KP8



PDB: 7PBJ



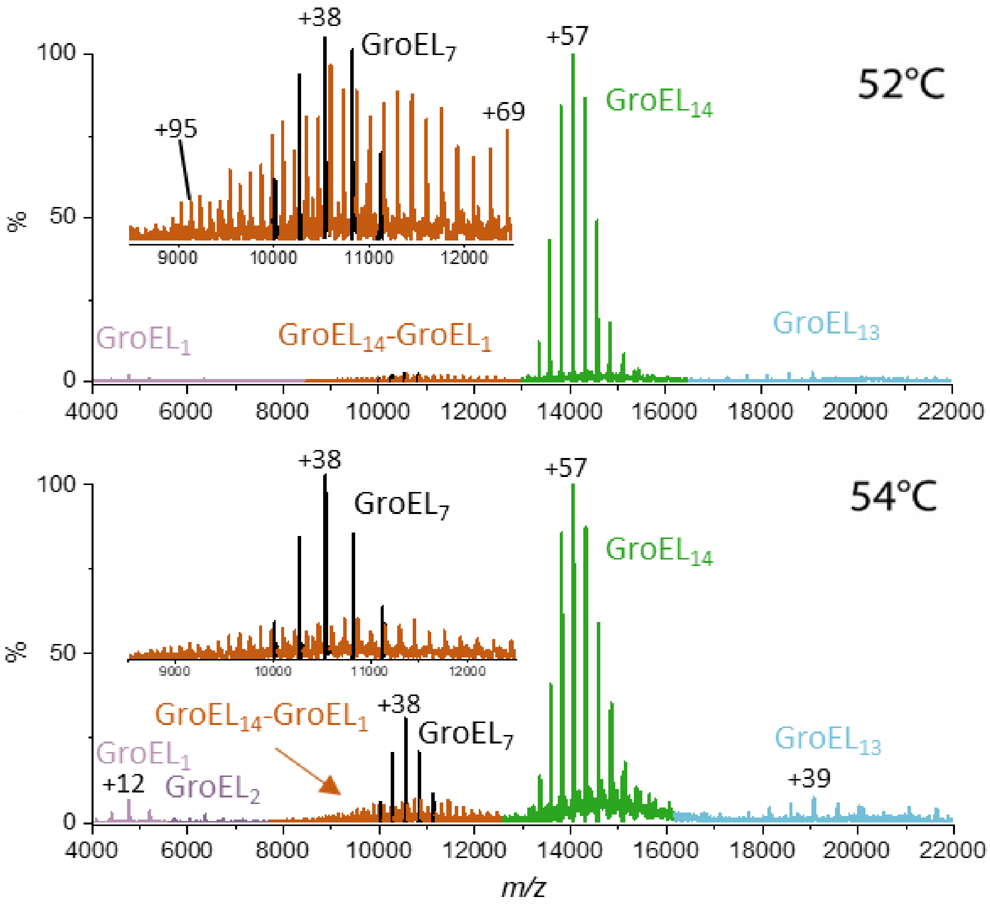
GroEL

- 801 kDa tetradecamer complex
- Composed of 2 stacked heptameric rings
- Primary function is to fold proteins to their native state
- Utilizes ATP hydrolysis
- Can bind 14 Mg-ATP molecules
- Operates in conjunction with co-chaperonin GroES

Ranson, N. A., et al. *Cell* ***2001, 107 (7), 869-79.***

Kudryavtseva, S. S., et al. *Sci Rep* ***2021, 11 (1), 18241.***

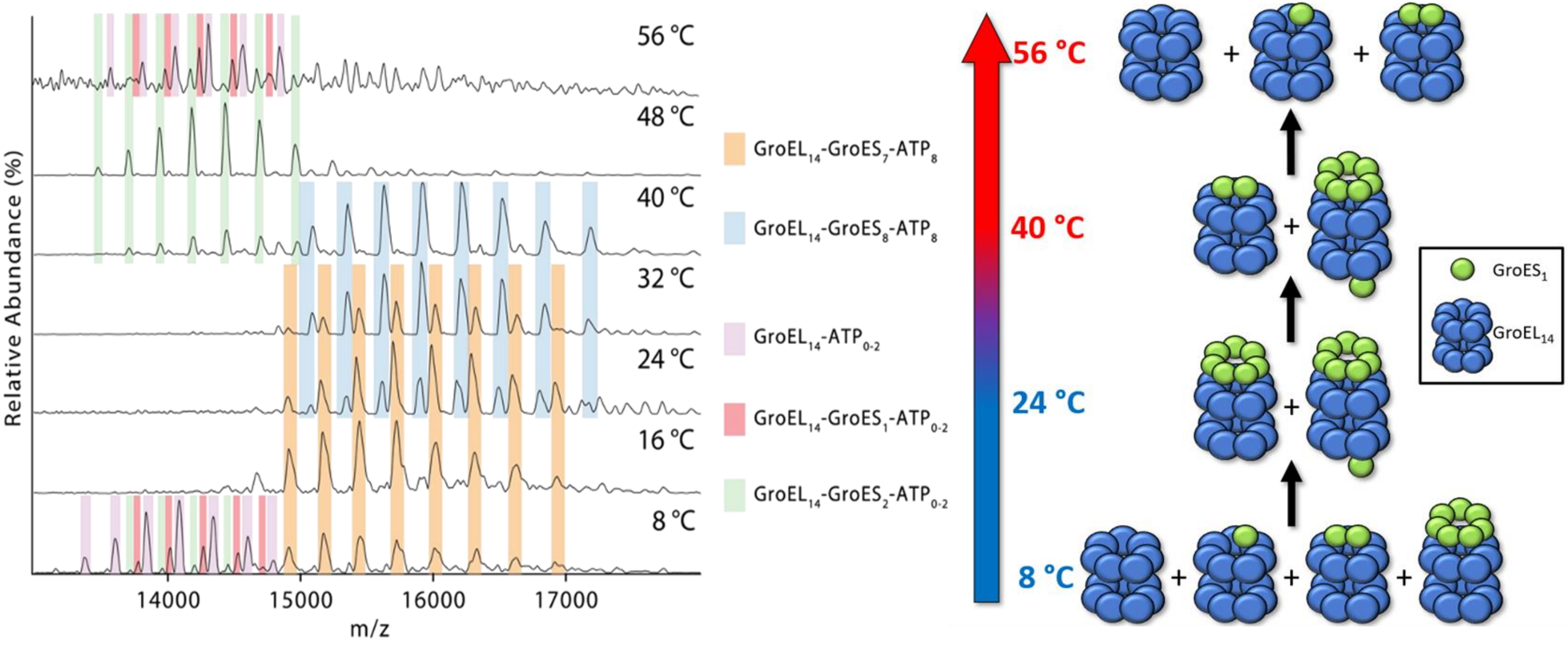
Temperature Dependent Degradation



- GroEL remains stable until ~50 °C
- GroEL$_{14}$ becomes unstable, forming monomers and GroEL$_{13}$
- High charge GroEL$_{15}$ species becomes abundant
  - Potential self preservation mechanism

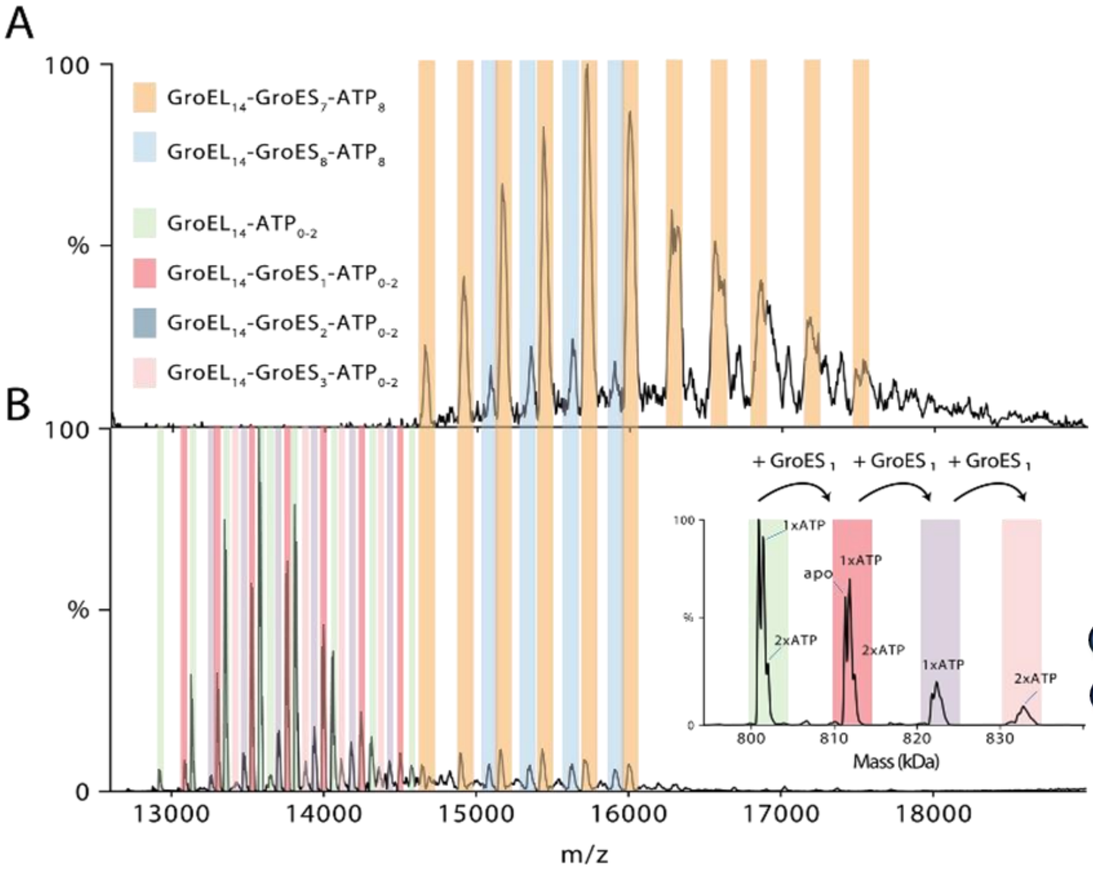
Walker, T. E., et al. *J Am Chem Soc* ***2022, 144 (6), 2667-2678.***

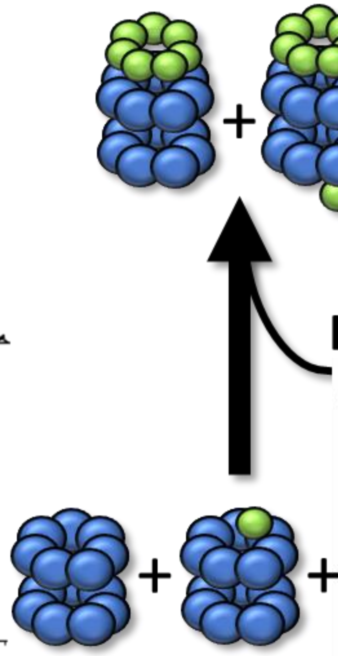
Temperature Dependance of GroEL-GroES Reaction



Walker, T. E., et al. *J Am Chem Soc* ***2022, 144 (6), 2667-2678.***

Mg^{2+} Dependance of ATP Binding

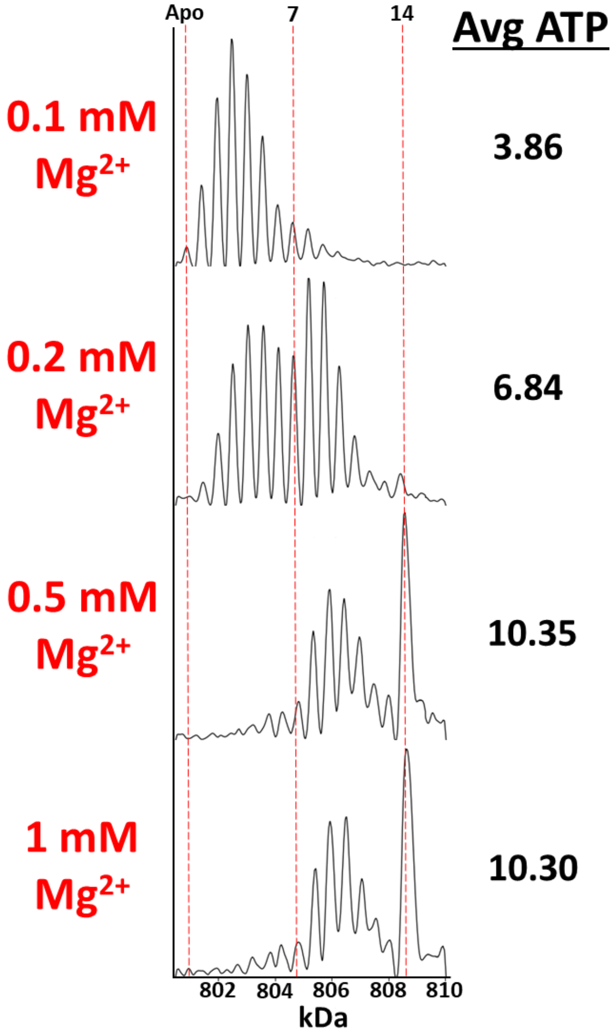




Solution F:
500 µM Mg^{2+}

Increased Mg^{2+} Concentration

Solution G:
25 µM Mg^{2+}

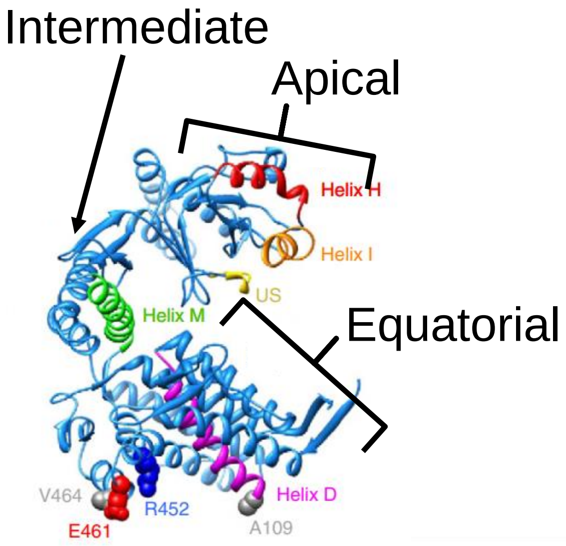


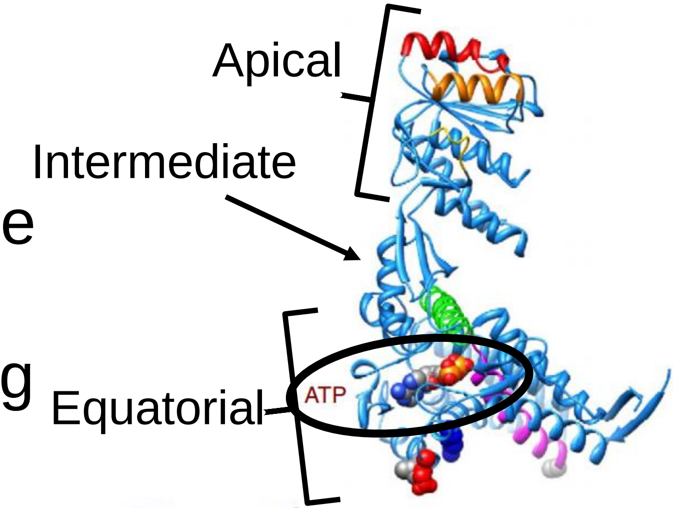
Walker, T. E., et al. *J Am Chem Soc* ***2022, 144 (6), 2667-2678.***

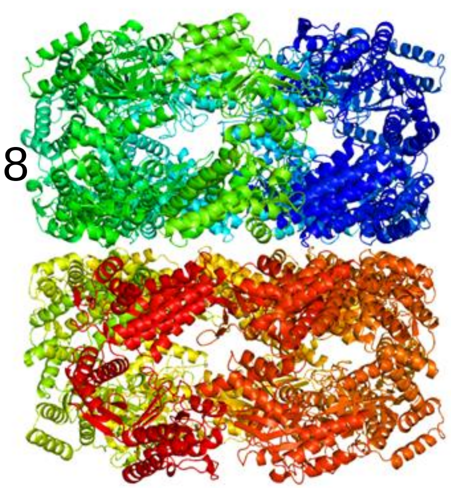
GroEL-ATP$_n$ Thermodynamic Measurements

Allosteric Transitions of GroEL

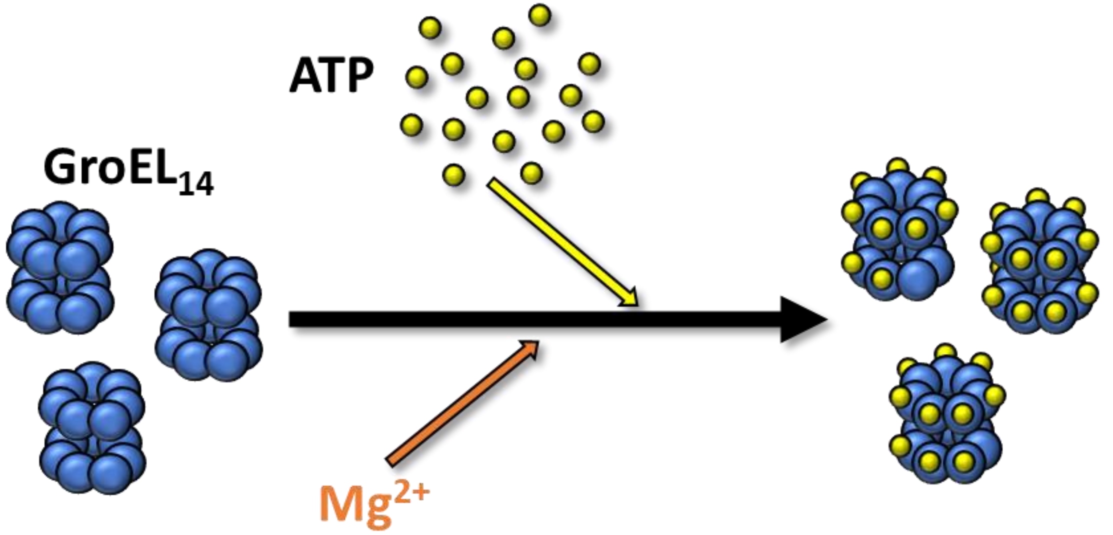
- ATP acts as an allosteric ligand enabling docking of co-chaperonin GroES
- ATP binding has been shown have positive cooperativity for intra-ring binding
- Negative cooperativity for inter-ring binding

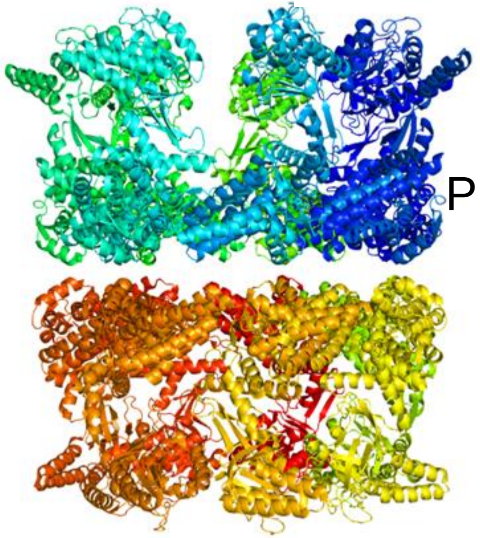






PDB: 1KP8



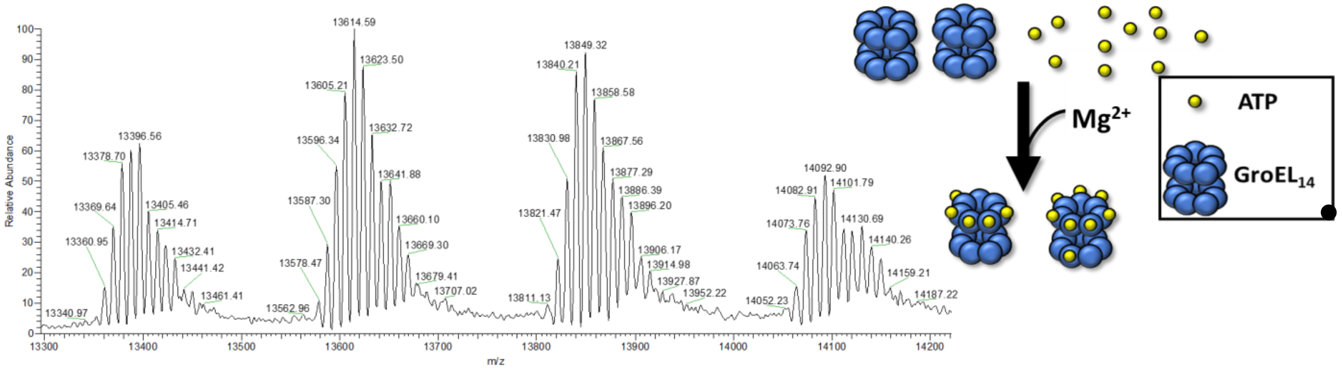


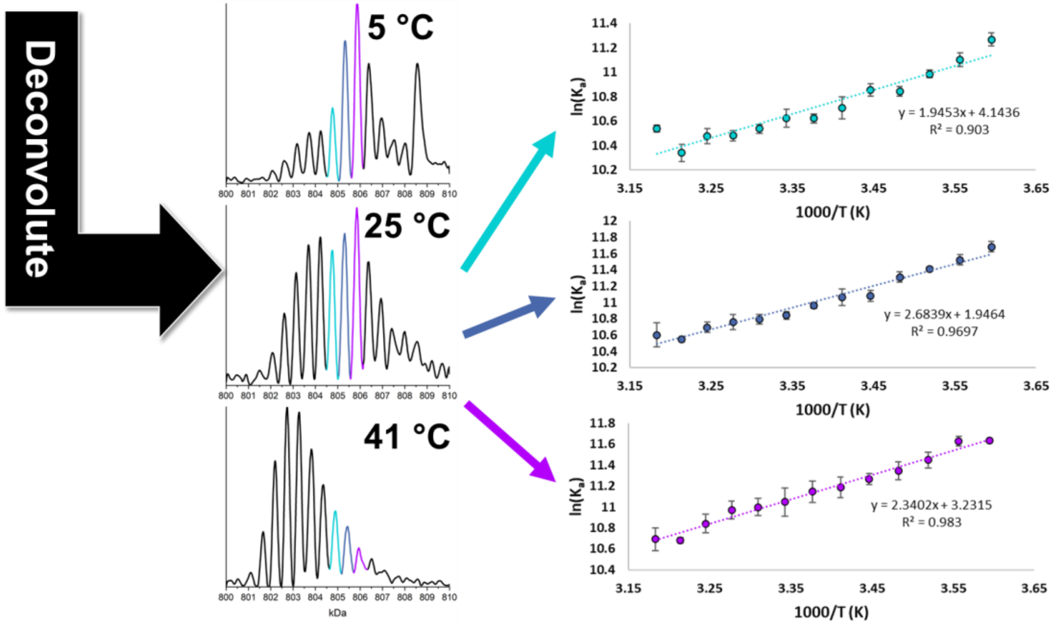
PDB: 4AB3

Saibil, H. R., et al. *J Mol Biol* ***2013, 425 (9), 1476-87.***

Yifrach, O., et al. *Biochemistry* ***1995, 34 (16), 5303-5308.***

Van’t Hoff Analysis for MS



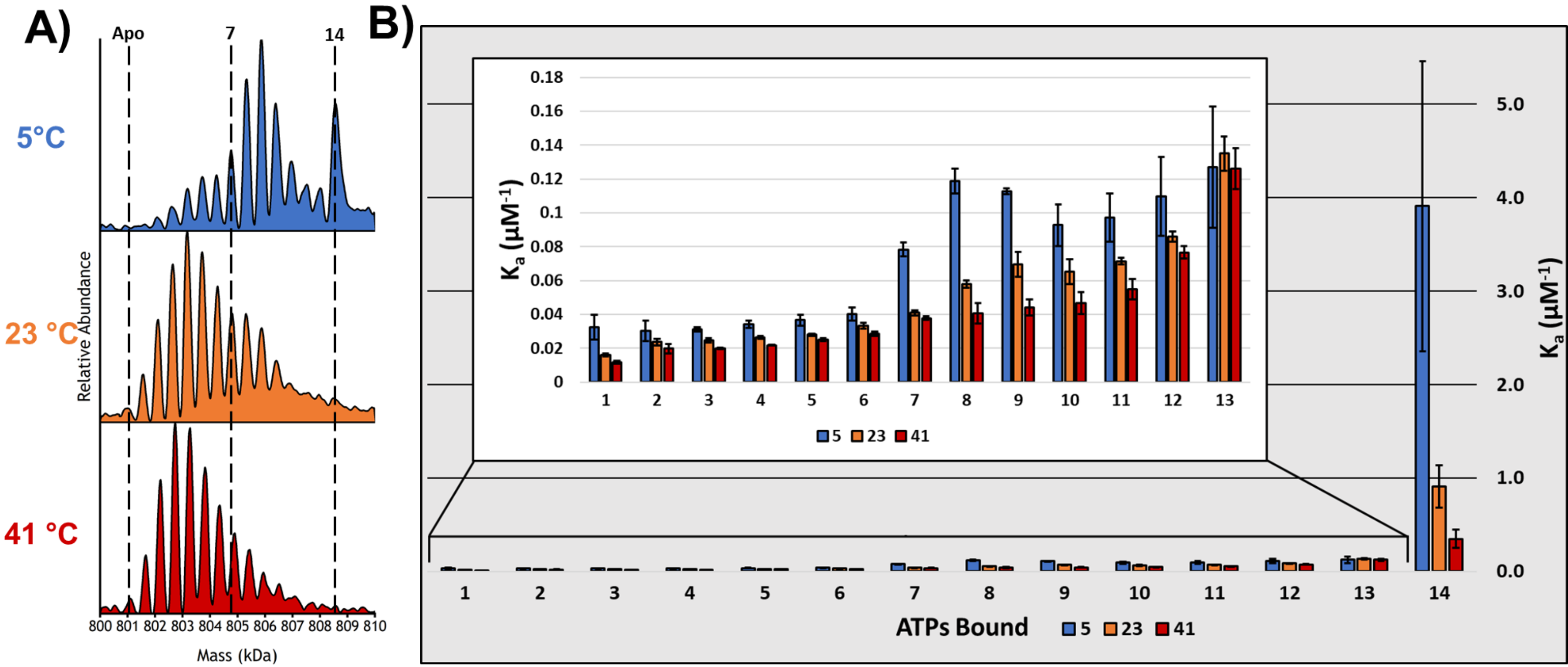


- For the Van’t Hoff plots we use the association constant K_a (the reciprocal of K_d).
- We use 1000/T for the X-axis rather than 1/T
  - Helps to avoid small decimal numbers
- $K_a = \frac{[PL]}{[P][L]_{free}} = \frac{1}{K_d}$
  - [P] = concentration of protein
  - [L] = concentration of free ligand
  - [PL] = concentration of ligated protein
- For MS: $K_a = \frac{I_{PL_i}}{I_{PL_{i-1}} \cdot [L]_{free}}$
  - I = Relative Intensity

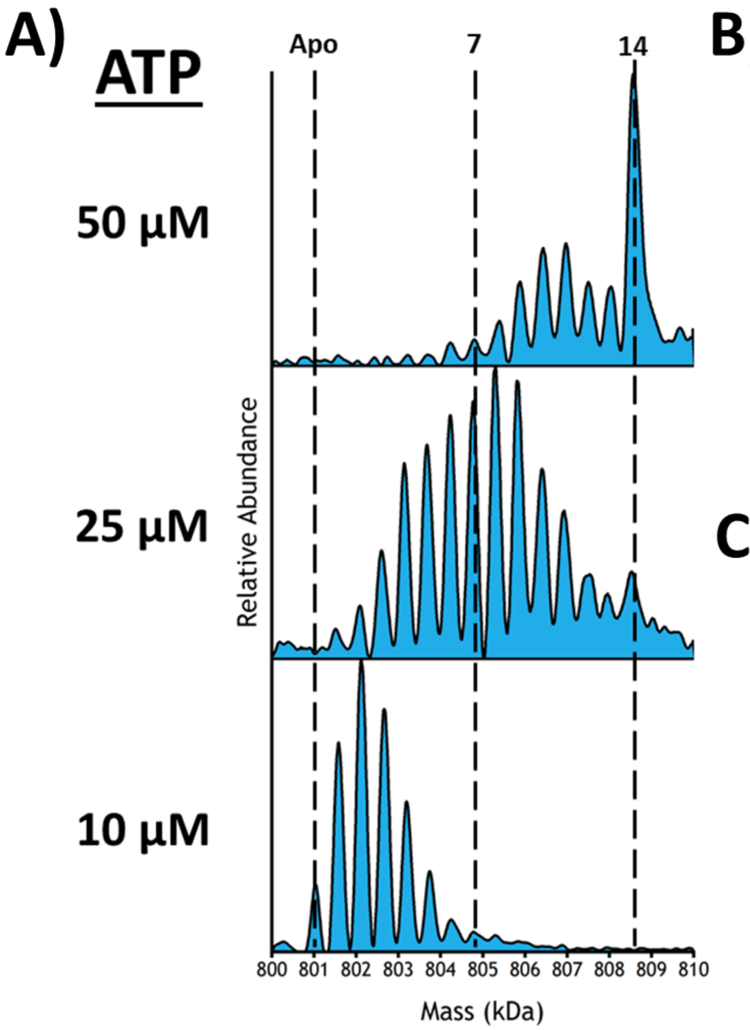
El-Baba, T. J., et al. *J Am Soc Mass Spectrom* ***2019, 30 (1), 77-84.***
Cong, X., et al. *J Am Chem Soc* ***2016, 138 (13), 4346-9.***

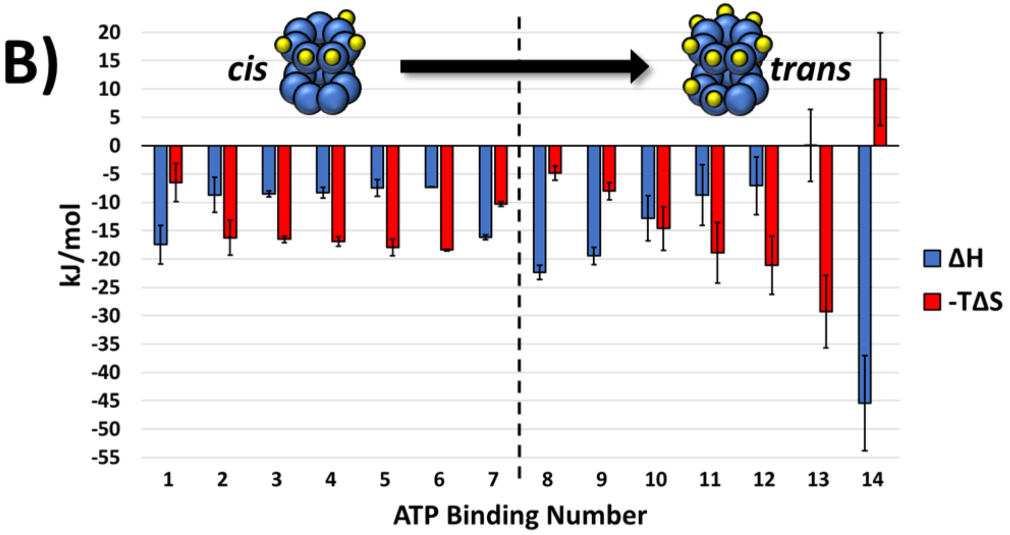
Van’t Hoff Analysis for MS

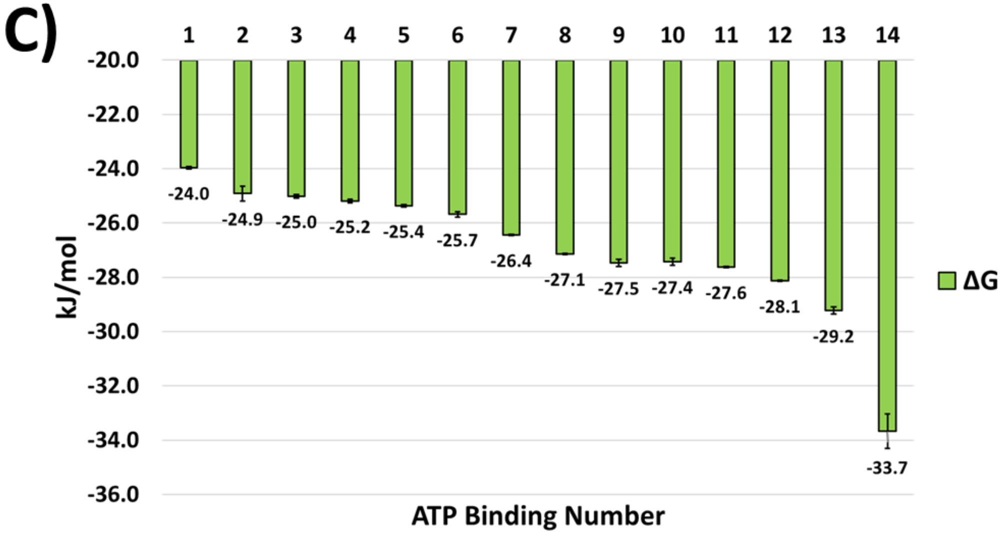
- Temperature dependent ATP binding
- Low temperatures promote disproportionate GroEL-ATP$_{14}$
- Loss of ATP binding at higher solution temperatures
- Solution conditions: 200 mM EDDA



GroEL-ATP Binding in EDDA



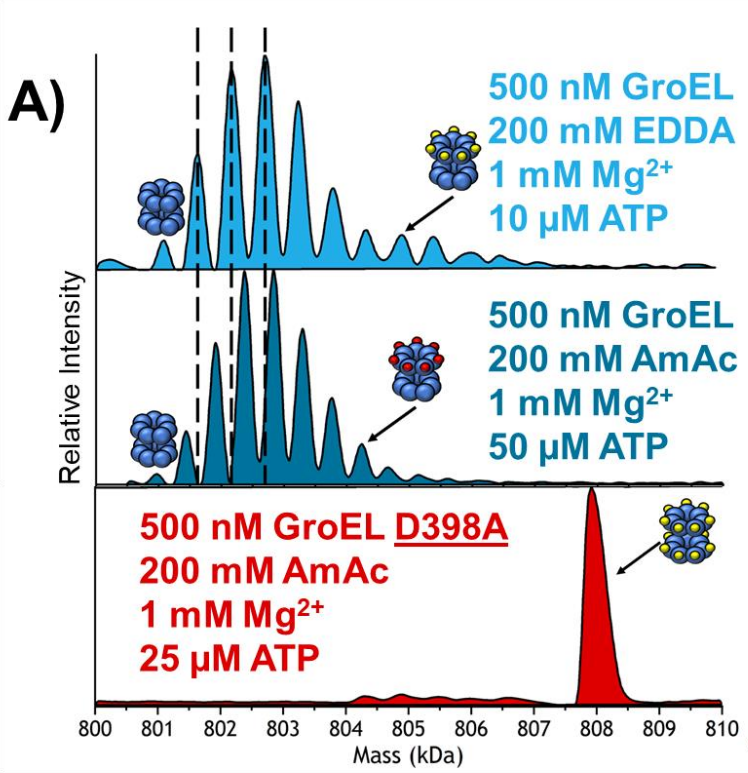




- Ethylenediamine diacetate (EDDA)
  - 200 mM
- Enthalpy-entropy compensation holds ΔG values relatively constant
- Marked difference in *cis* vs *trans* ring binding reactions.
  - Potentially a sign of negative inter-ring cooperativity

Issues with ATP Binding in AmAc

- Ammonium Acetate (AmAc) showed a much different pattern of nucleotide binding than EDDA.
- Mass shift was ~450 Da rather than 530 Da
  - Mg-ADP!
- Hydrolysis in the presence of NH_4^+ ions
- Hydrolysis deficient mutant (D398A) was capable of binding 14 ATP molecules with elevated level of positive cooperativity



Todd, M. J., et al. *Biochemistry* **1993, 32 (33), 8560-8567.**
Viitanen, P. V., et al. *Biochemistry* **1990, 29 (24), 5665-71.**

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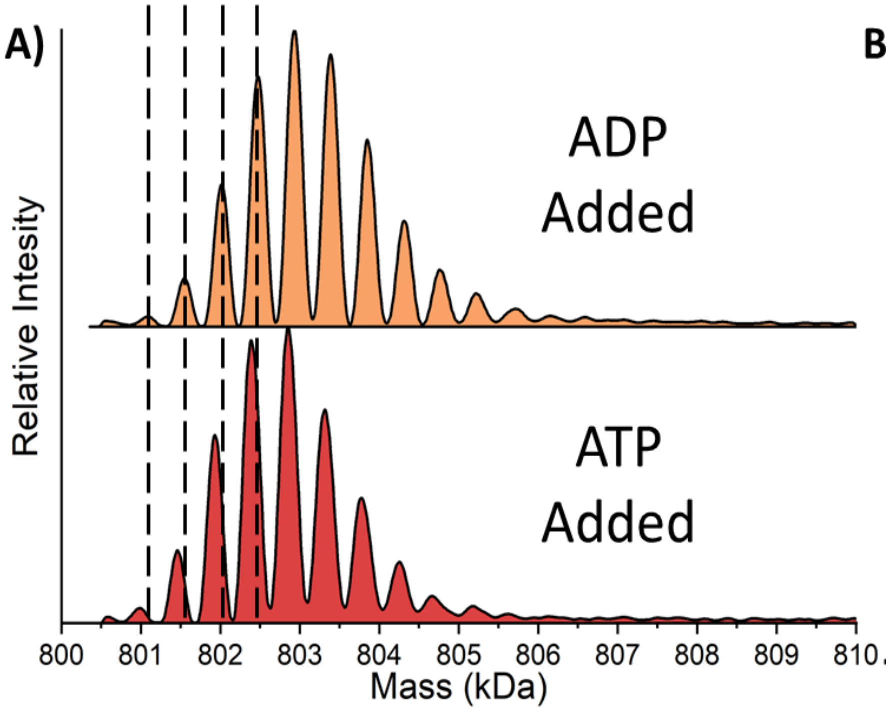
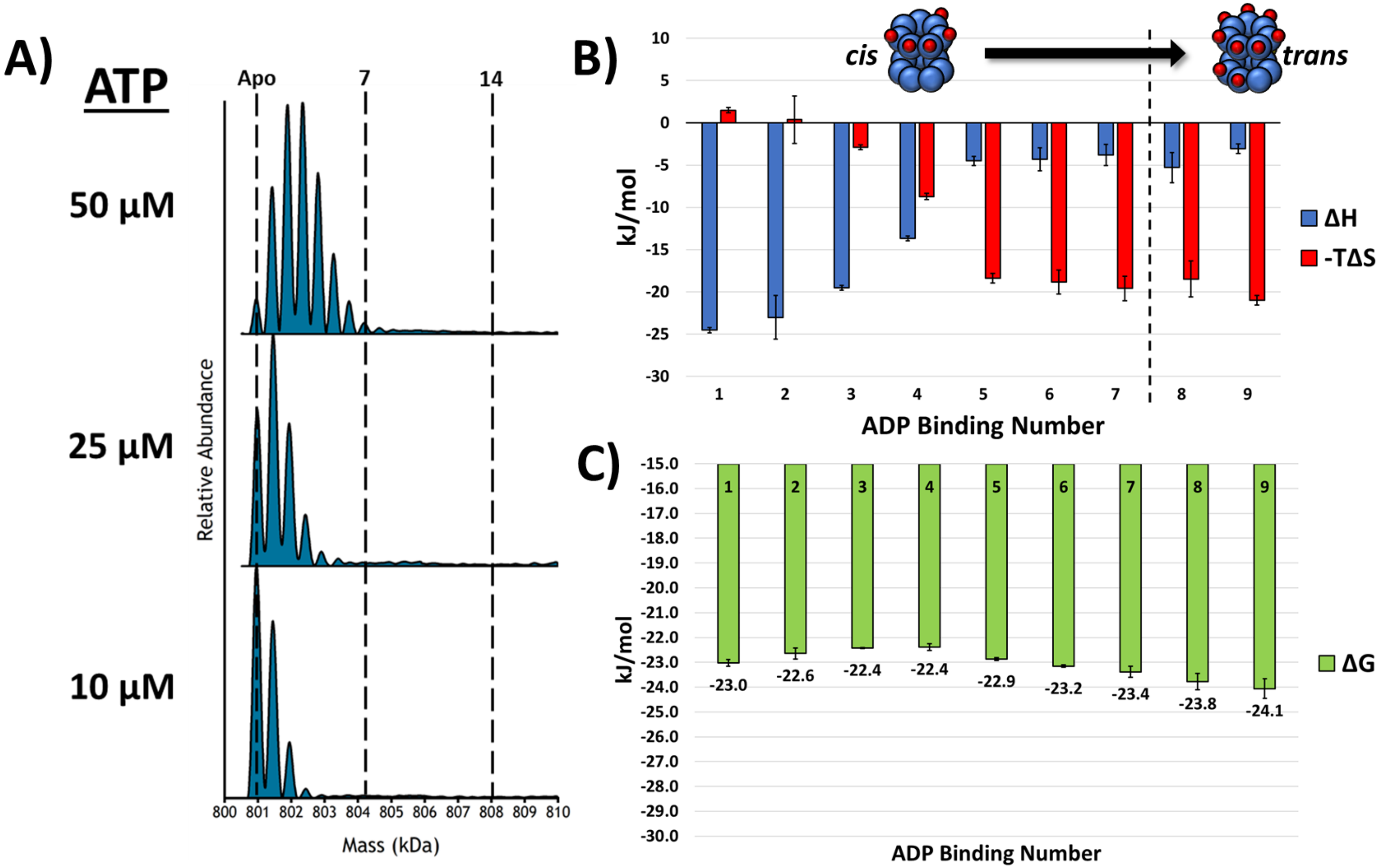


B)

	ATP Added		ADP Added	
	Mass (Da)	ΔMass (Da)	Mass (Da)	ΔMass (Da)
Apo	800969		801086	
1	801439	470	801545	459
2	801908	469	802012	467
3	802364	456	802472	460
4	802830	466	802932	460
5	803295	465	803386	454
6	803754	459	803846	460
7	804233	479	804309	463
8	804648	415	804757	448
9	805148	500	805213	456
10	805603	455	805709	496
11			806143	434
12			806590	447
Average		**463.4**		**458.7**

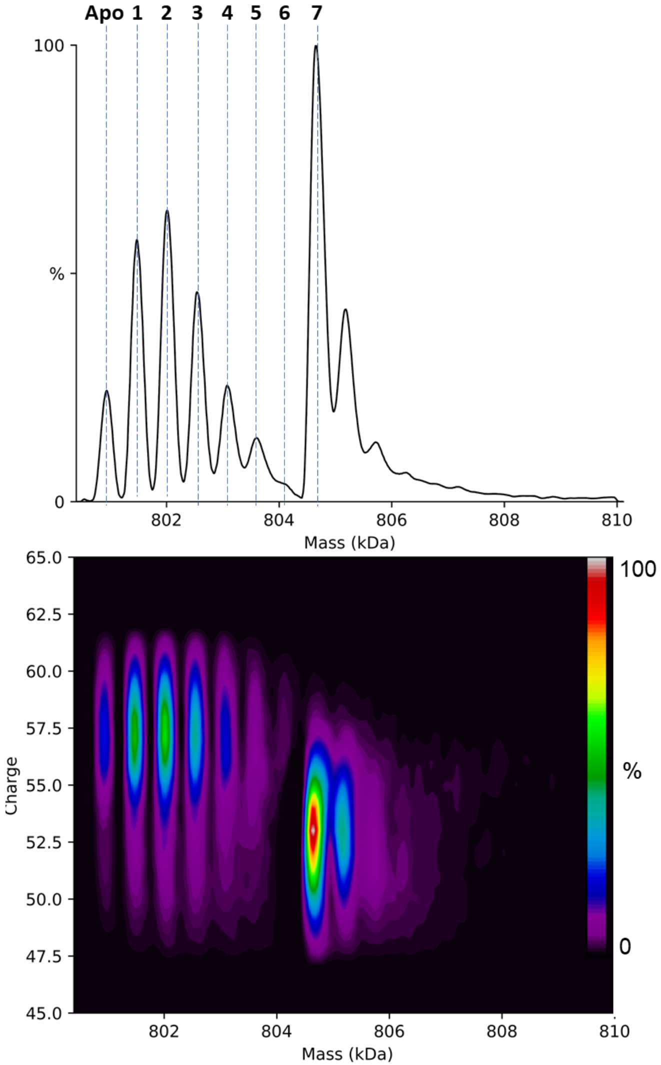
Todd, M. J., et al. *Biochemistry* ***1993, 32 (33), 8560-8567.***
Viitanen, P. V., et al. *Biochemistry* ***1990, 29 (24), 5665-71.***

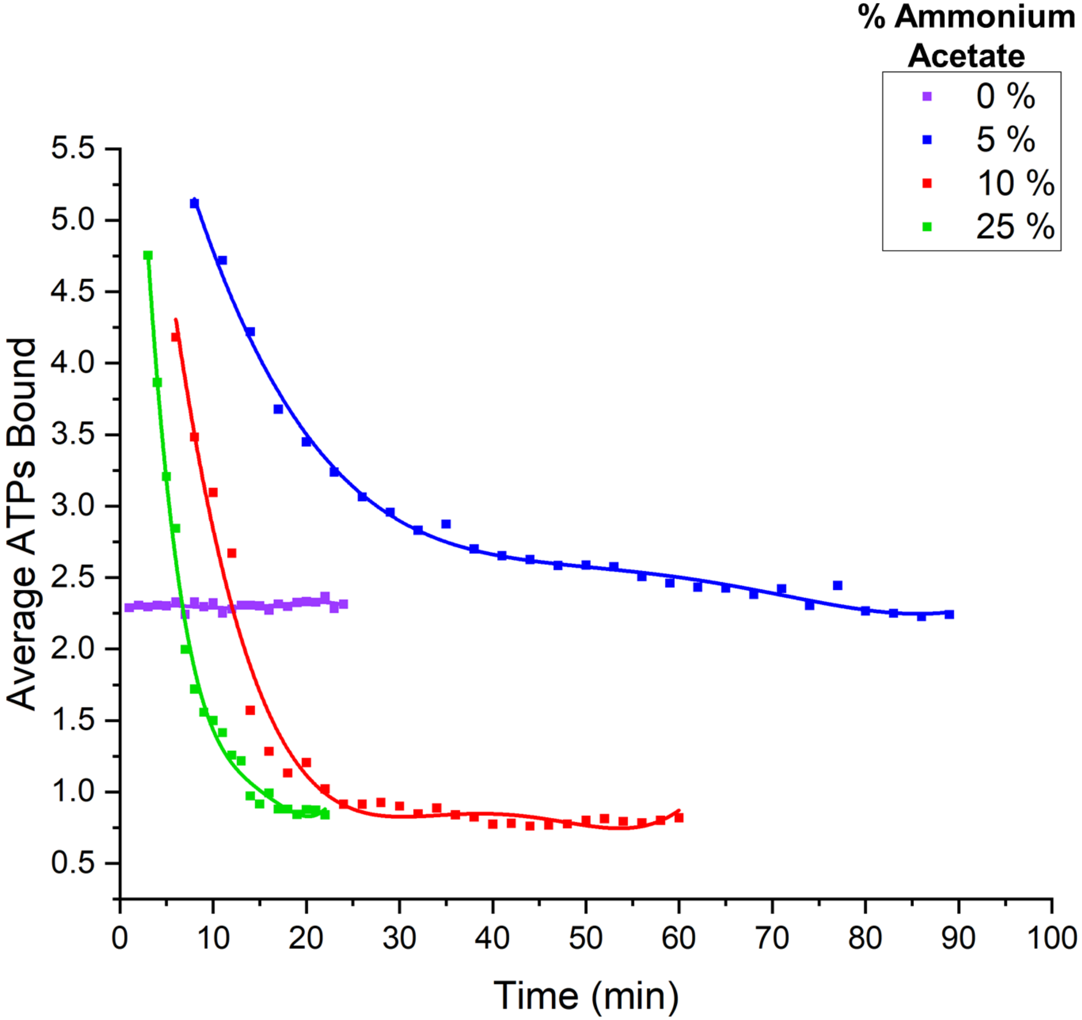
GroEL-ADP Binding in AmAc



- Binding of ADP is overall weaker than ATP in EDDA.
  - Higher ΔG values
- Enthalpy-entropy compensation (EEC) is more present
- No observed cooperativity for ADP binding
- Entropy becomes dominant after GroEL-ATP_4 reaction

ATPase Activity Dependent on Monovalent Cations





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Viitanen, P. V., et al. *Biochemistry* ***1990, 29 (24), 5665-71.***

Conclusions

- Native MS is quickly becoming an advantageous approach to structural biology and biochemistry.
- VT-ESI MS enables the examination of individual binding reactions for multidentate binding systems.
  - Determination of binding entropy and enthalpy
- Disadvantages:
  - Limited to “MS-friendly” buffer conditions
  - Current methods are not high-throughput

Acknowledgments

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Dr. Christopher Mallis
Dr. Jixing Lyu

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Russell Group Members

Texas A&M Chemistry Machine Shop

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Karl Yeager

Gordon Anderson

GAA Custom Electronics, LLC

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P41 Resource in Native MS-Guided Structural Biology