# Tuning the Higher Order Structure of ADCs by Traversing the Formulation Design Space

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**Principal Investigator, GSK**
**CASSS HOS**
**September 12, 2024**

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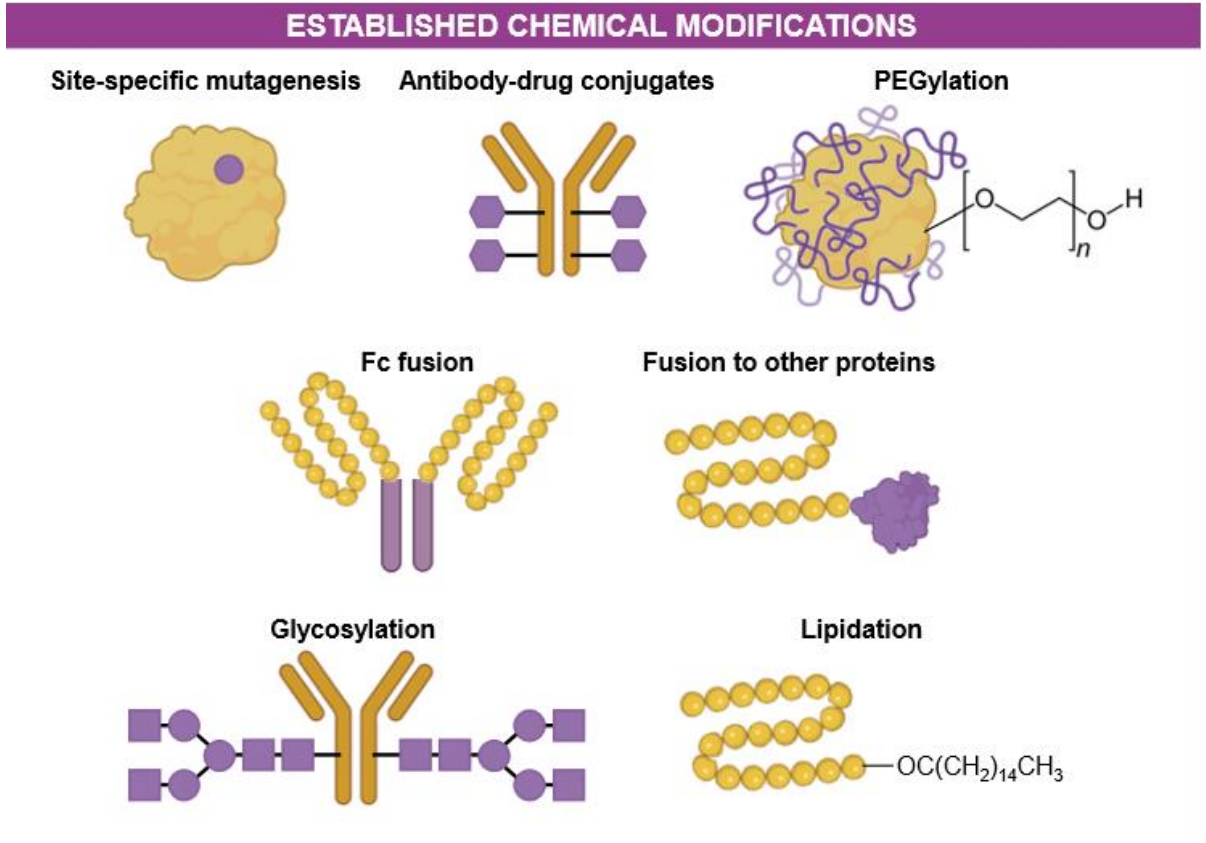
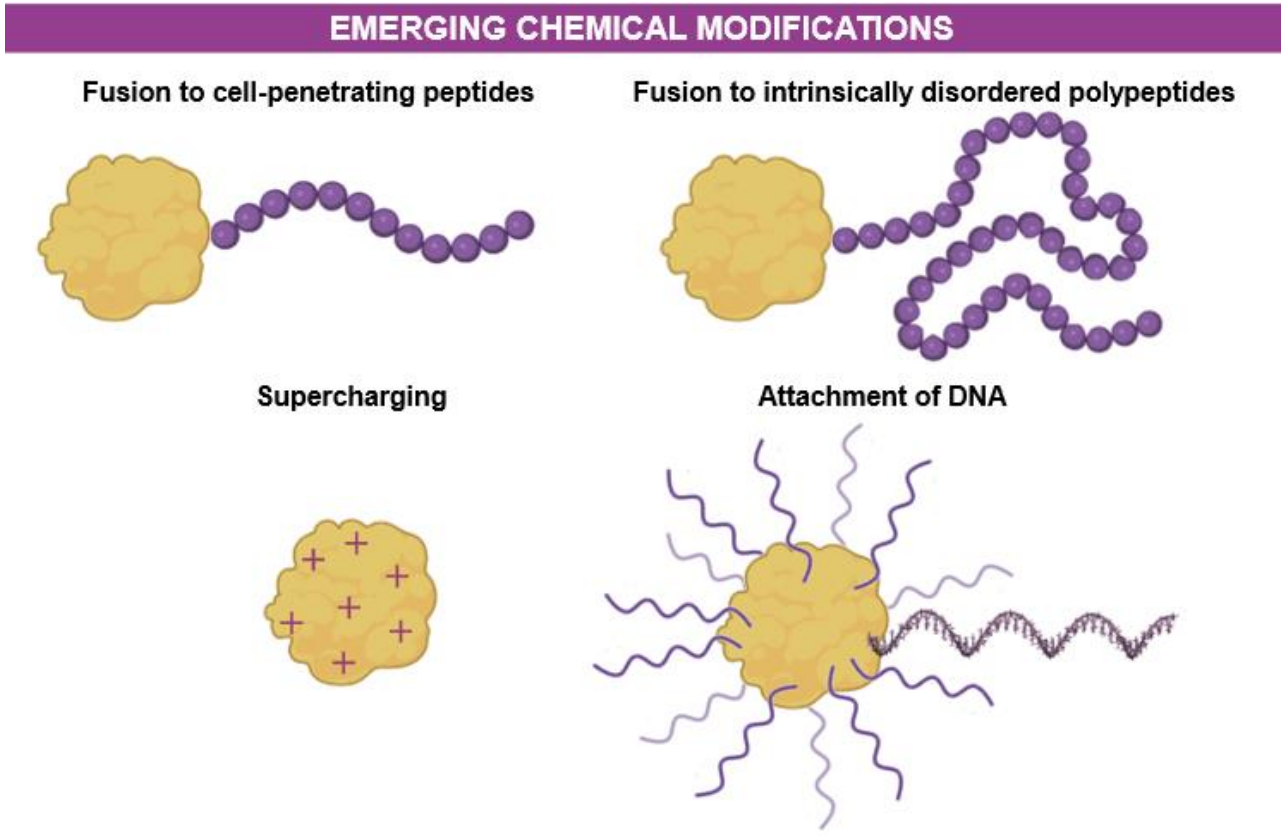
# Advances in the Chemical Design of Protein-Based Therapeutics have Led to Important Breakthroughs in Medicine





Golan, D.E. *et al. Nat. Rev. Drug. Discov.* **2008**, *7*, 21.

Ebrahimi, S.B., Samanta, D., *Nat. Comm.* **2023**, *14*, 2411.

Stemmer W., *et al. Nat. Biotech.* **2009**, *27*, 1186.

Liu D.R., *et al. J. Am. Chem. Soc.* **2007**, *129*, 10110.

Ebrahimi, S.B., Samanta, D., Mirkin C.A., *et al. Nat. Protoc.* **2022**, *17*, 327.

Samanta, D., Ebrahimi, S.B., Mirkin C.A., *et al. J. Am. Chem. Soc.* **2020**, *142*, 13350.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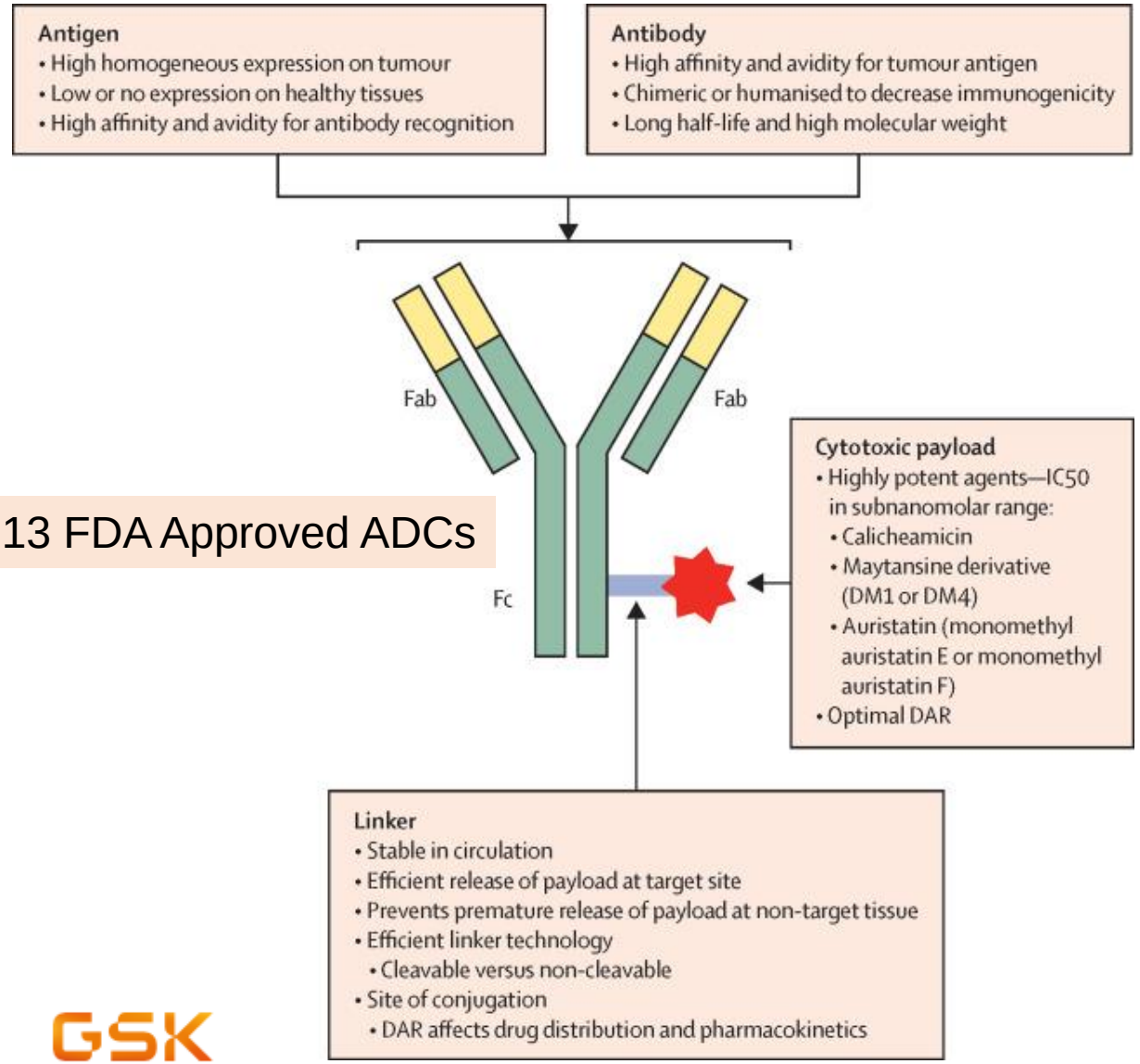
# Antibody-Drug Conjugates (ADCs) are an Important Class of Medicine




13 FDA Approved ADCs

REPORT

## Selective Destruction of Target Cells by Diphtheria Toxin Conjugated to Antibody Directed against Antigens on the Cells

FREDERICK L. MOOLTEN AND SIDNEY R. COOPERBAND

*SCIENCE* • 3 Jul 1970 • Vol 169,Issue 3940 • pp.68-70 • DOI: 10.1126/science.169.3940.68

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### Abstract

Monkey-kidney cells bearing new surface antigens induced by infection with mumps virus were lysed selectively by diphtheria toxin conjugated to antibody against mumps antigens.

What are the challenges associated with ADC development?

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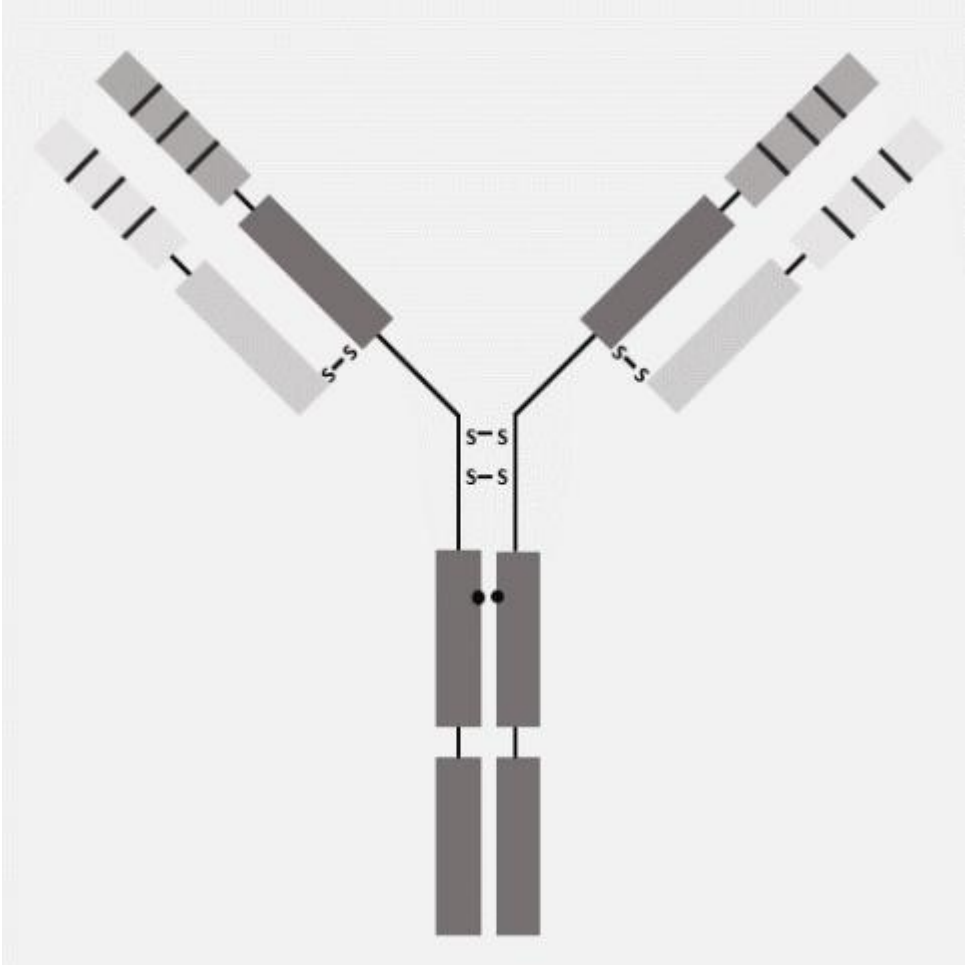
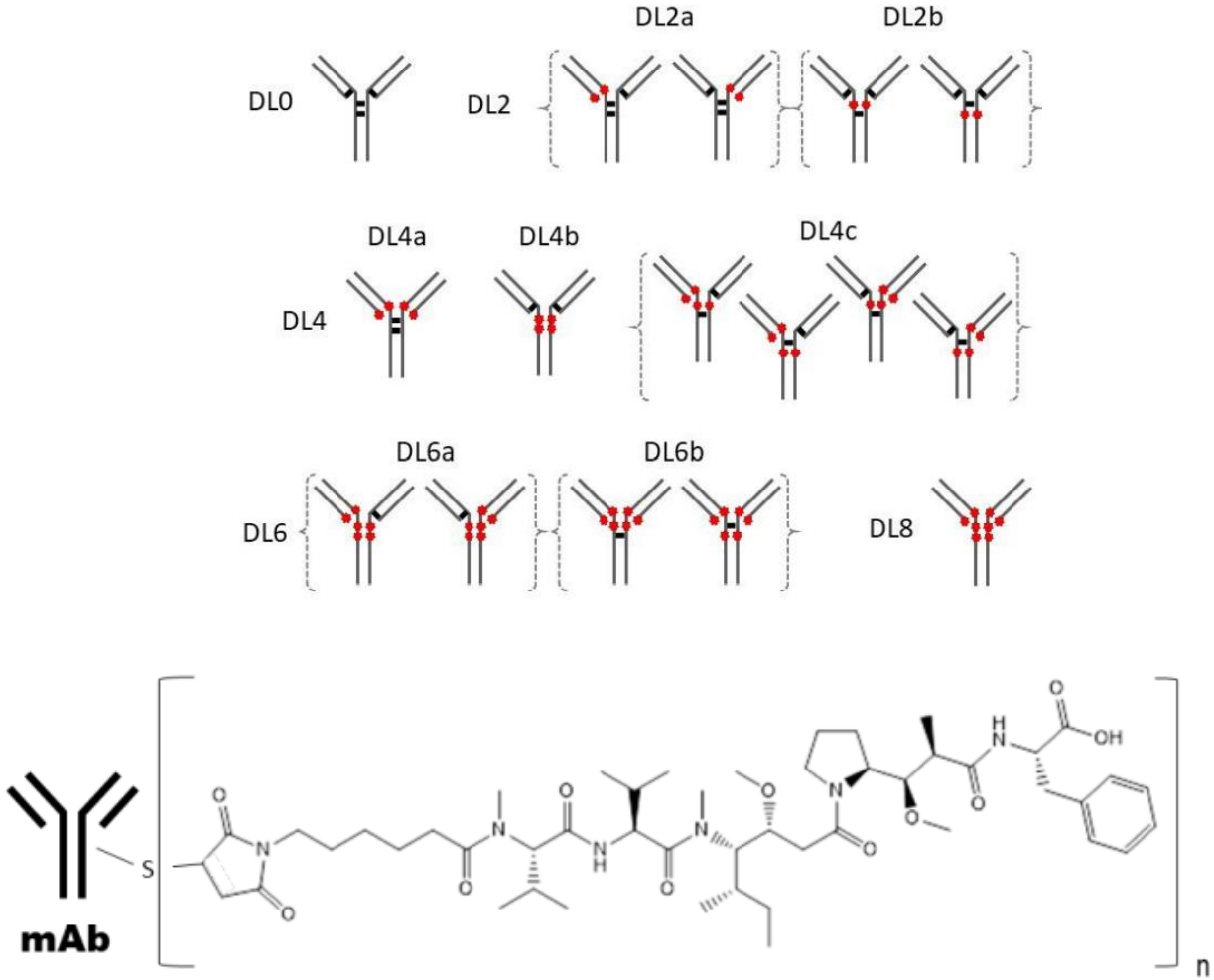
# Structural Considerations for an IgG1-based ADC

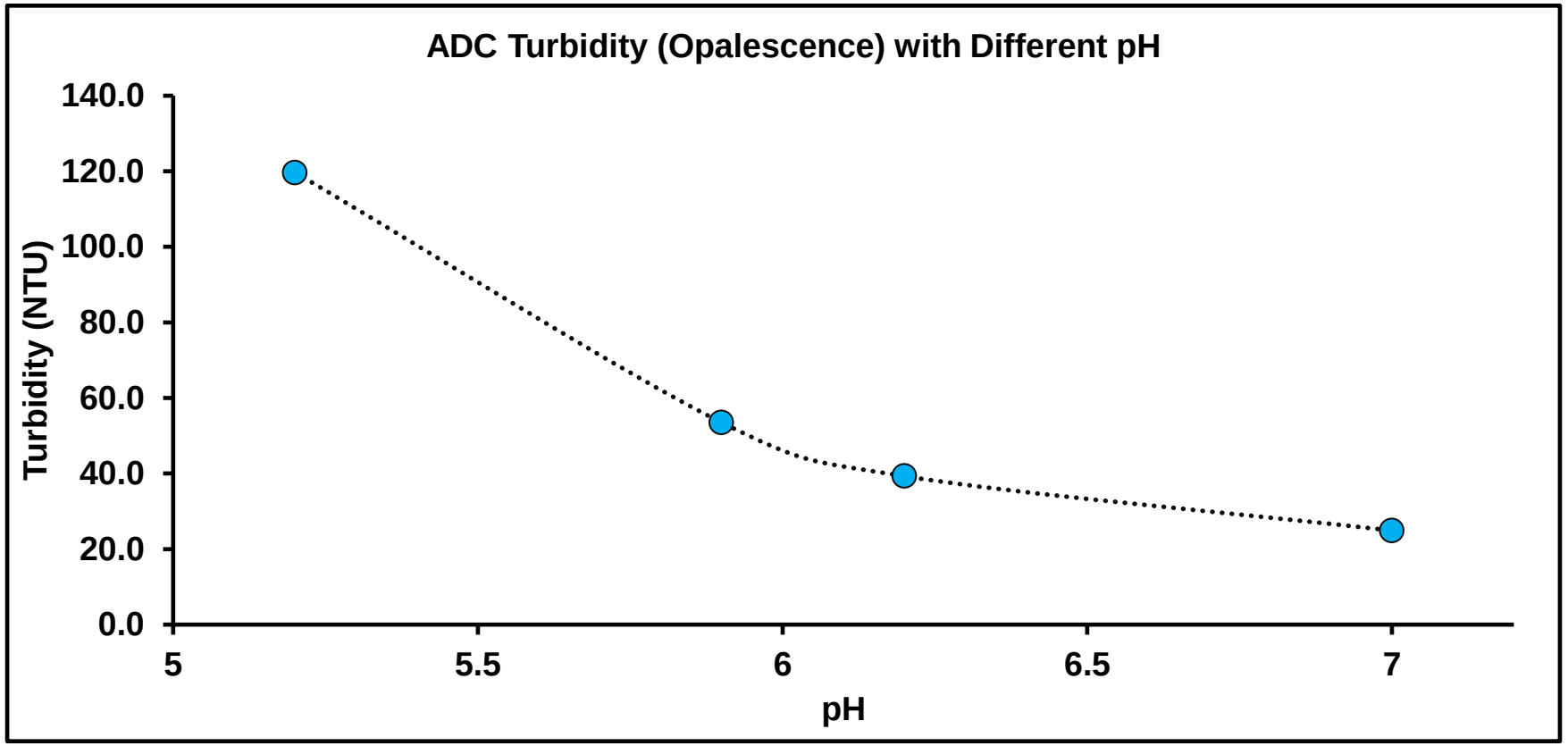



Unconjugated (Naked) mAb




ADC

# The ADC Exhibits High Turbidity at Low pH




**25 mM sodium citrate, 154 mM NaCl, 75.6 g/L trehalose dihydrate, 0.05 mM EDTA disodium dihydrate, 0.02% (w/v) polysorbate 80 (PS80)**

Turbidities observed were as high as 120 NTU at pH 5.2. This high turbidity is observed at a pH far from the pI (~8.7) of the ADC

Question to ask:

What is the mechanistic reason for this behavior?

High opalescence (turbidity) can signal the presence of reversible or irreversible aggregates that impact therapeutic efficacy or elicit *in vivo* toxicity.

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## Understanding and modulating opalescence and viscosity in a monoclonal antibody formulation

**Branden A Salinas**[1,3], **Hasige A Sathish**[1,3,4], **Steven M Bishop**[4], **Nick Harn**[4], **John F Carpenter**[2,3], and **Theodore W Randolph**[1,3]

[1] Department of Chemical and Biological Engineering, University of Colorado, Boulder, Colorado

[2] University of Colorado Health Sciences Center, Denver, Colorado

[3] Center for Pharmaceutical Biotechnology, University of Colorado

[4] MedImmune, LLC. Gaithersburg, MD 20878

molecular pharmaceutics

Article

pubs.acs.org/molecularpharmaceutics

## Liquid–Liquid Phase Separation in a Dual Variable Domain Immunoglobulin Protein Solution: Effect of Formulation Factors and Protein–Protein Interactions

Ashlesha S. Raut* and Devendra S. Kalonia*

ELSEVIER

Journal of Chromatog

Volume 1488, 10 March 2017, P

## Liquid-liquid phase separat … turbidity and pressure during low pH elution process in Protein A chromatography

Haibin Luo [a], Nacole Lee [b], Xiangyang Wang [c], Yuling Li [a], Albert Schmelzer [d], Alan K. Hunter [a], Timothy Pabst [a], William K. Wang [a]

Even though not yet systematically summarized, it has been shown in several studies that LLPS often occurs at conditions close to the pI [1–6,30]. To our knowledge, up to now just two studies have been published in which LLPS in antibody solutions was also observed more than one pH unit distant from pI at low ionic strength and in the absence of polyethylene glycol [3,31]. In general, LLPS has been shown to be more pronounced at lower temperature (upper critical solution temperature: UCST phase behavior) and lower ionic strength [2–5,7,8,14]. A strong correlation between LLPS and opalescence of antibody

## …ffects on Opalescence of a …ration MAb

…lesta

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## Opalescent Appearance of an IgG1 Antibody at High Concentrations and Its Relationship to Noncovalent Association

Muppalla Sukumar, Brandon L. Doyle, Jessica L. Combs & Allen H. Pekar

*Pharmaceutical Research* **21**, 1087–1093 (2004) | Cite this article



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# A Library of Formulations was Screened Towards Mechanistically Understanding ADC Behavior

| Structure | pH | [NaCl] mM | [Trehalose] g/L | [PS80] (w/v) % | Antibody Concentration (mg/mL) | Buffering Agents |
|---|---|---|---|---|---|---|
| ADC | 5.2, 5.9, 6.2, 7.0 | 0, 50, 100, 154 | 75.6, 189 | 0, 0.02, 0.1 | 80, 55, 27.5, 13.75, 6.88, 3.44, 1.72 | 25 mM Citrate 25 mM Histidine |

**Naked mAb also formulated at several conditions for comparison to the ADC**

**Taken together, this allows for investigating the impact of formulation conditions on the properties of the ADC**

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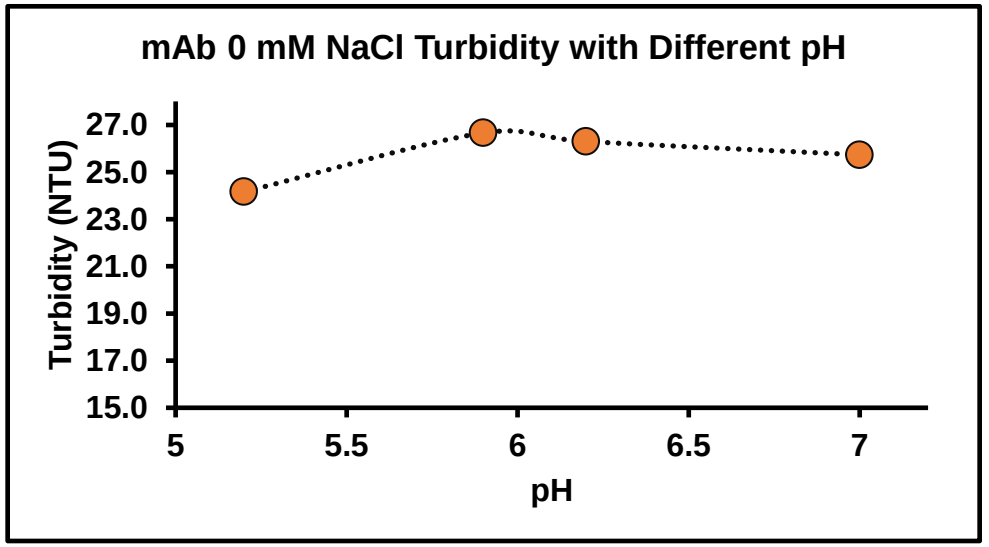
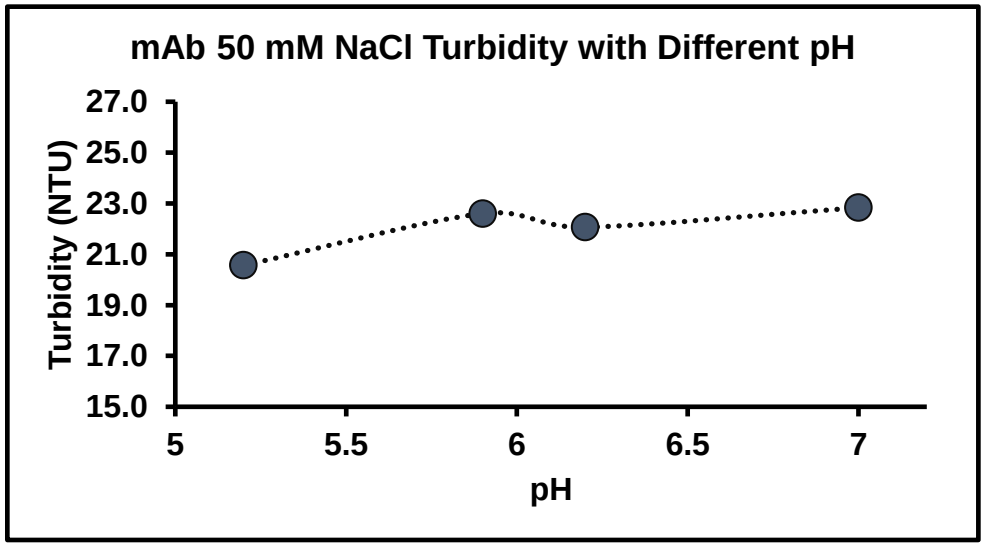
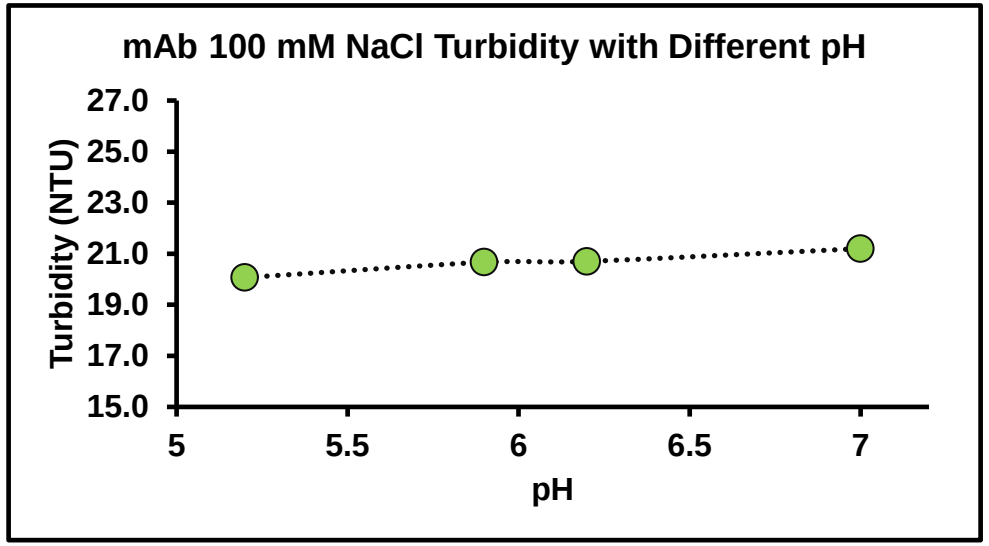
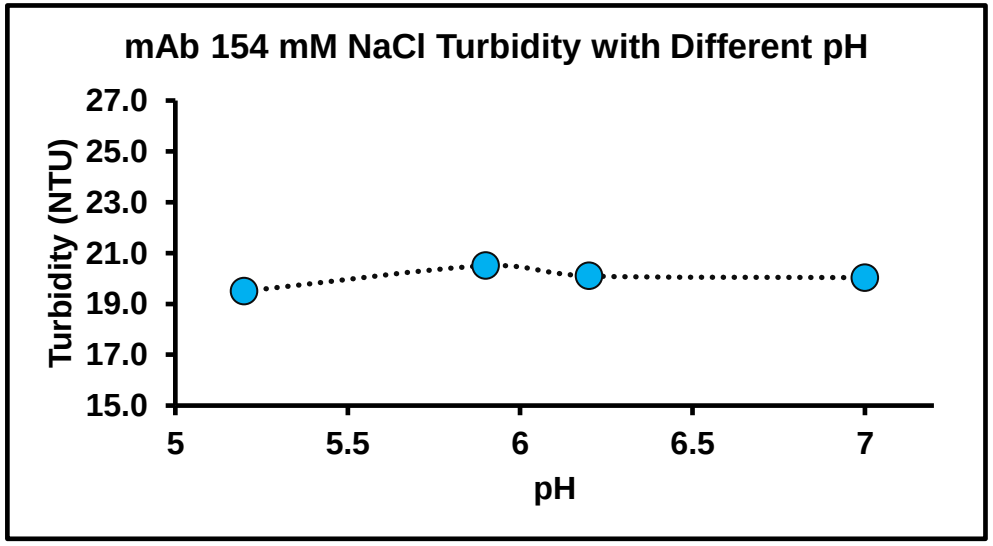
***This ADC is conventionally formulated in 25 mM sodium citrate, 75.6 g/L trehalose dihydrate, 0.05 mM EDTA disodium dihydrate, 0.02% (w/v) polysorbate 80 (PS80)**

# mAb Turbidity Does not Change with Varying pH at Constant NaCl Concentration



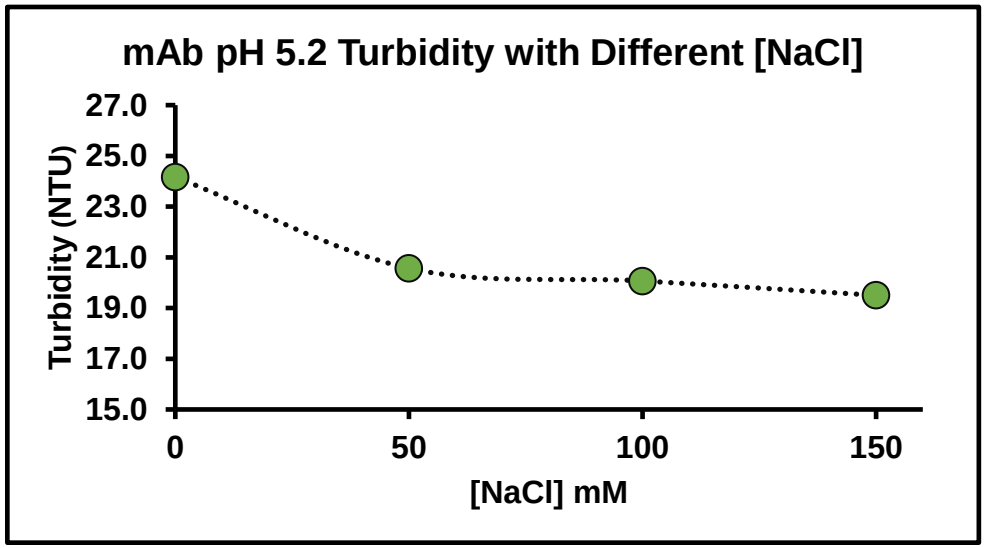



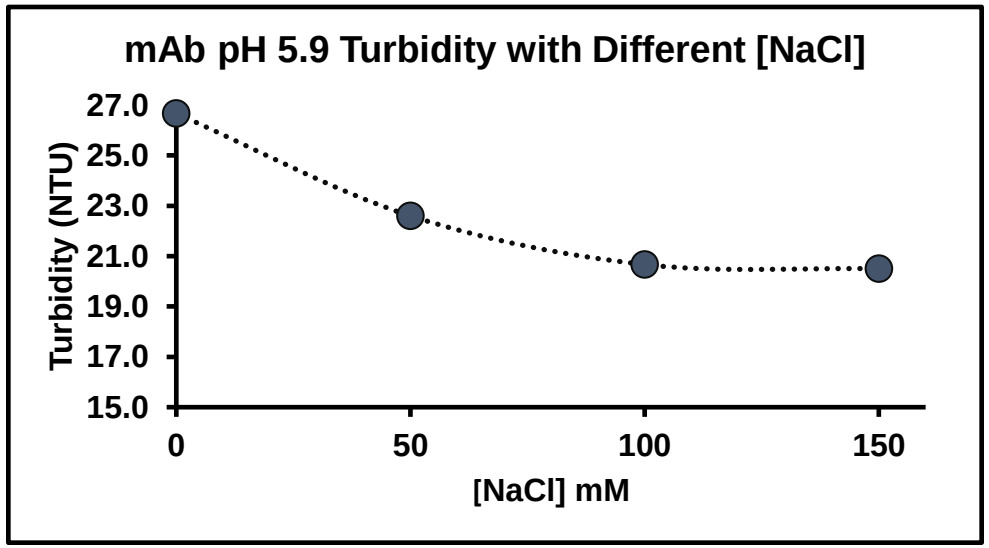



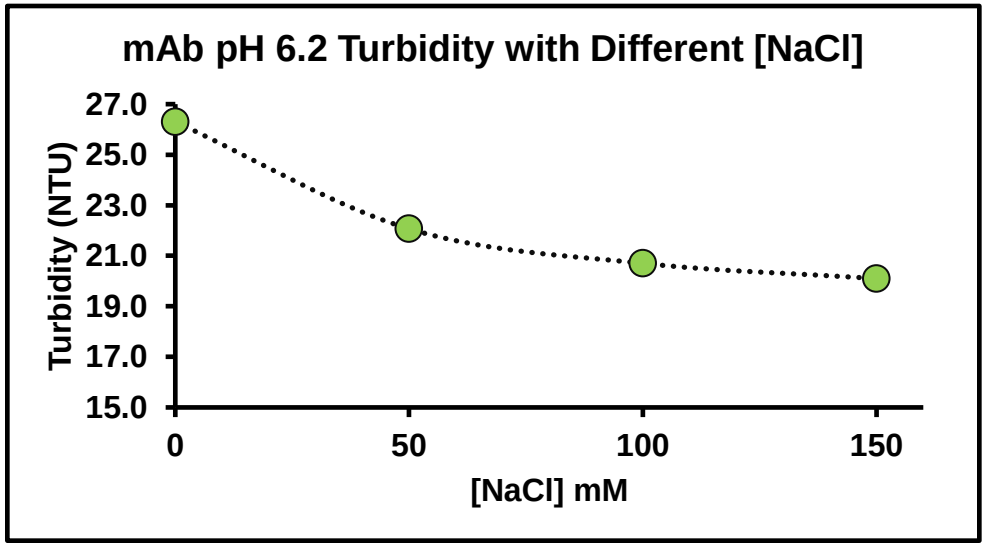



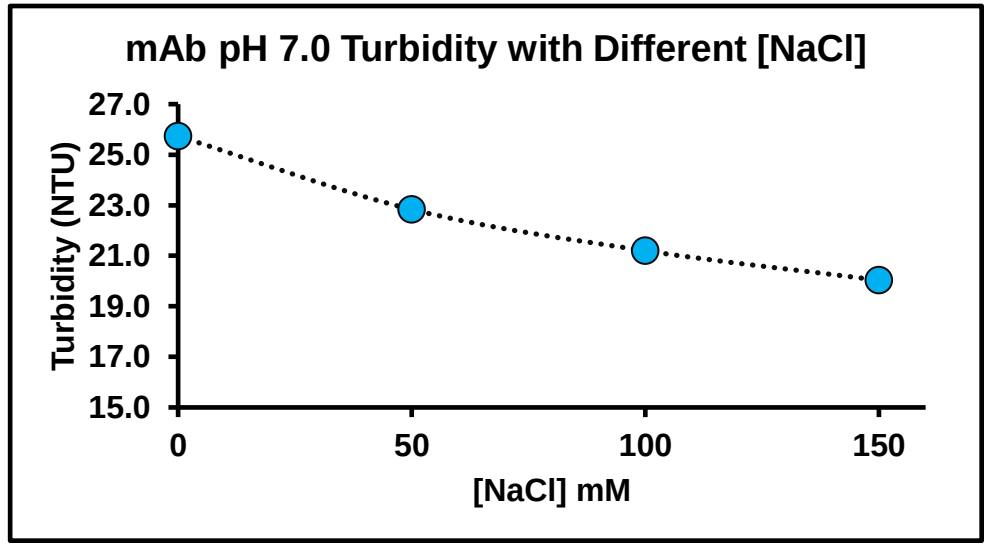

*All formulations 55 mg/mL Protein

# mAb Turbidity Decreases with Increasing NaCl Concentration at Constant pH

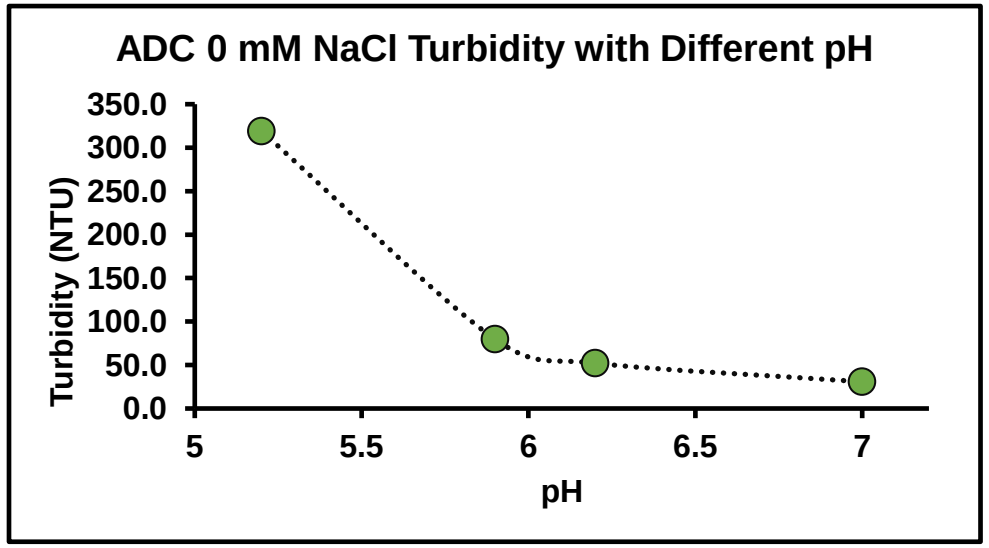


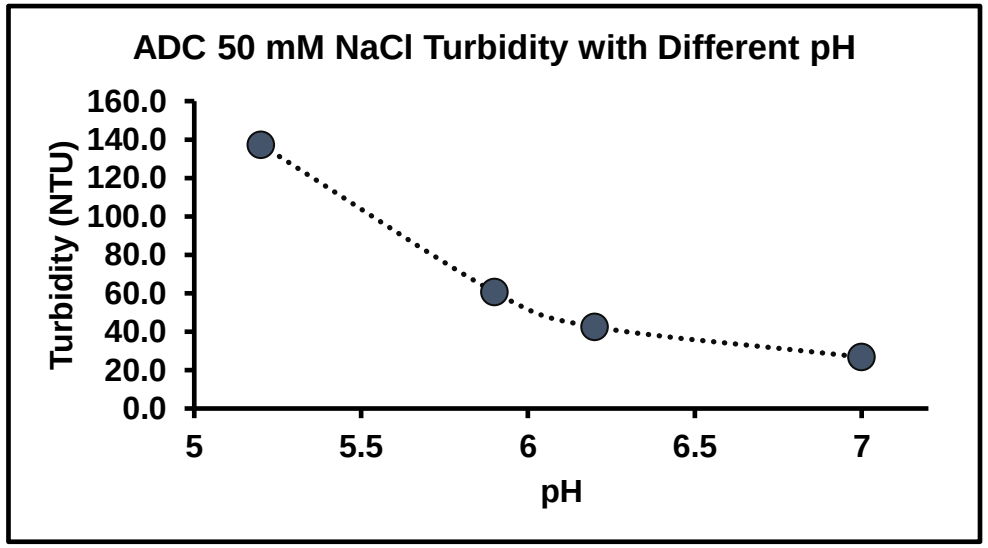



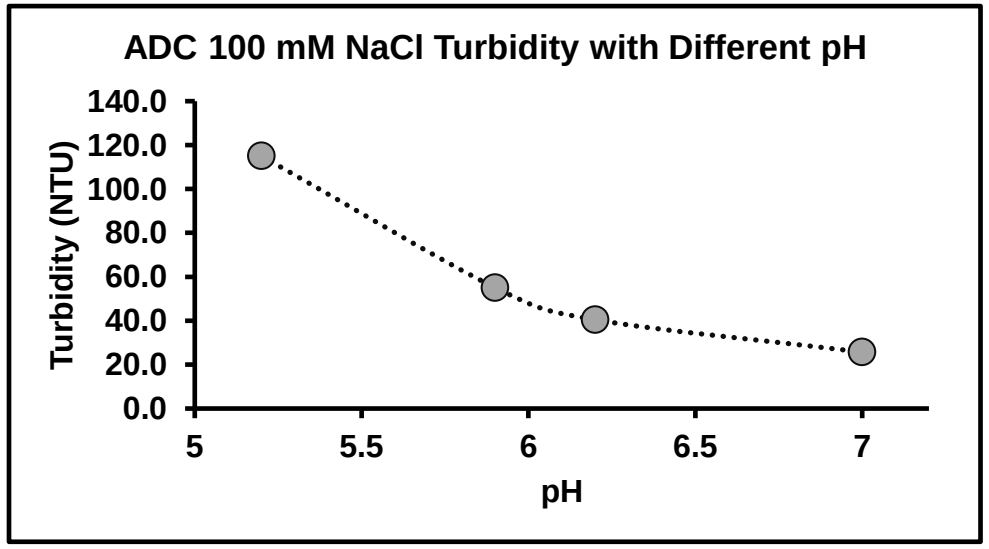



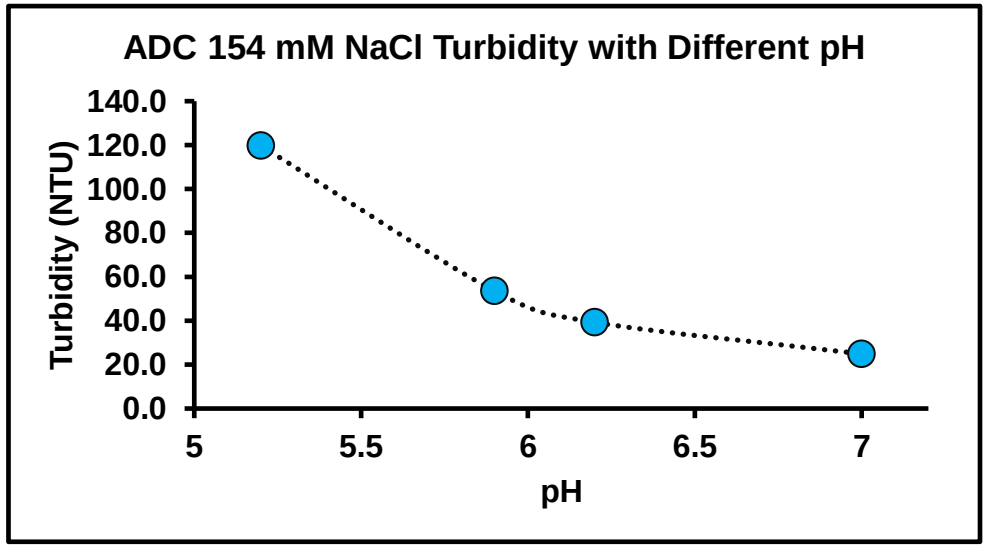



# ADC Turbidity Decreases with Increasing pH at Constant NaCl Concentration

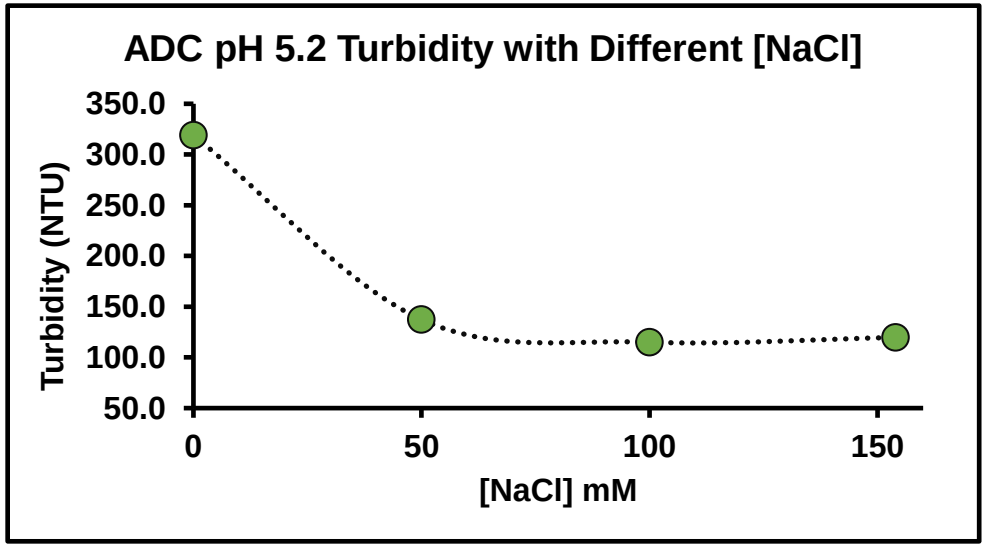


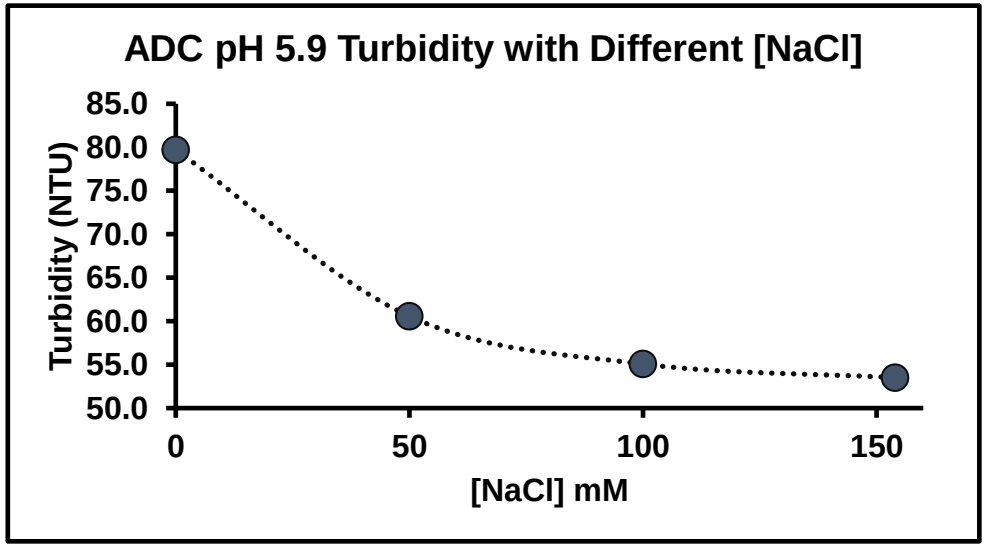



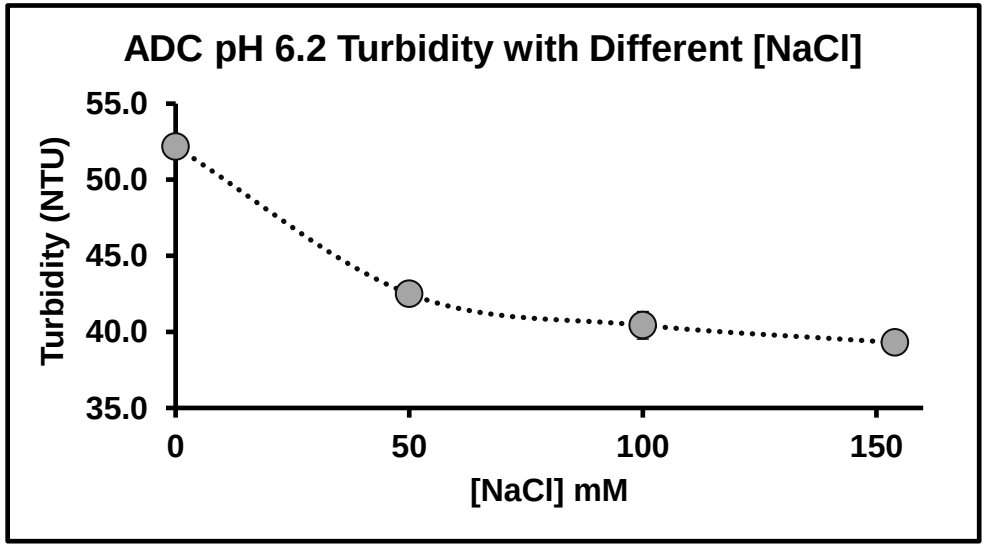



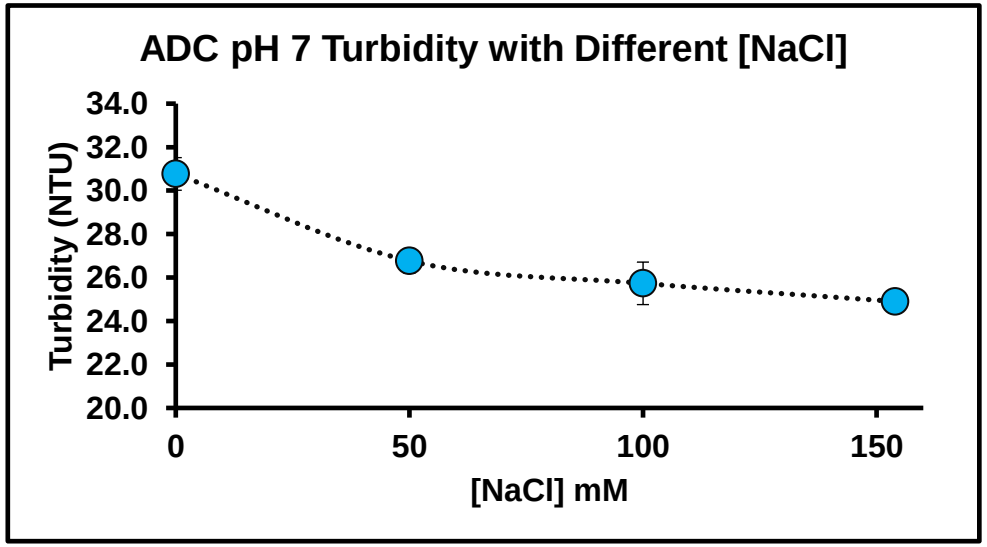



# ADC Turbidity Decreases with Increasing [NaCl] at Constant pH

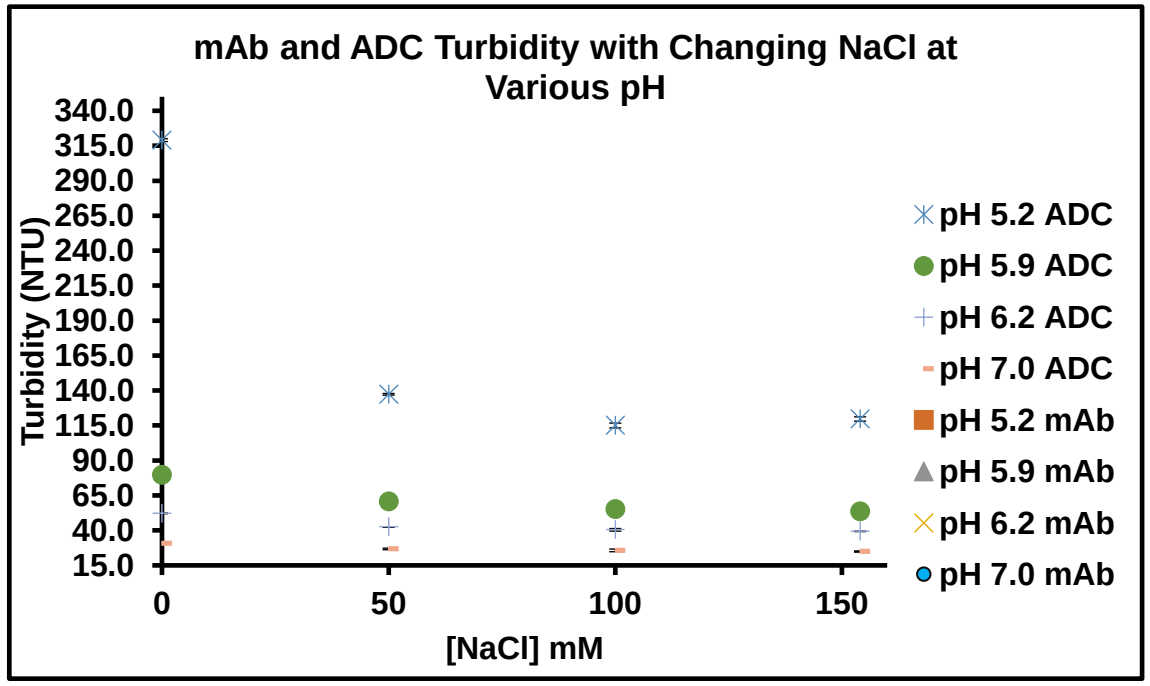


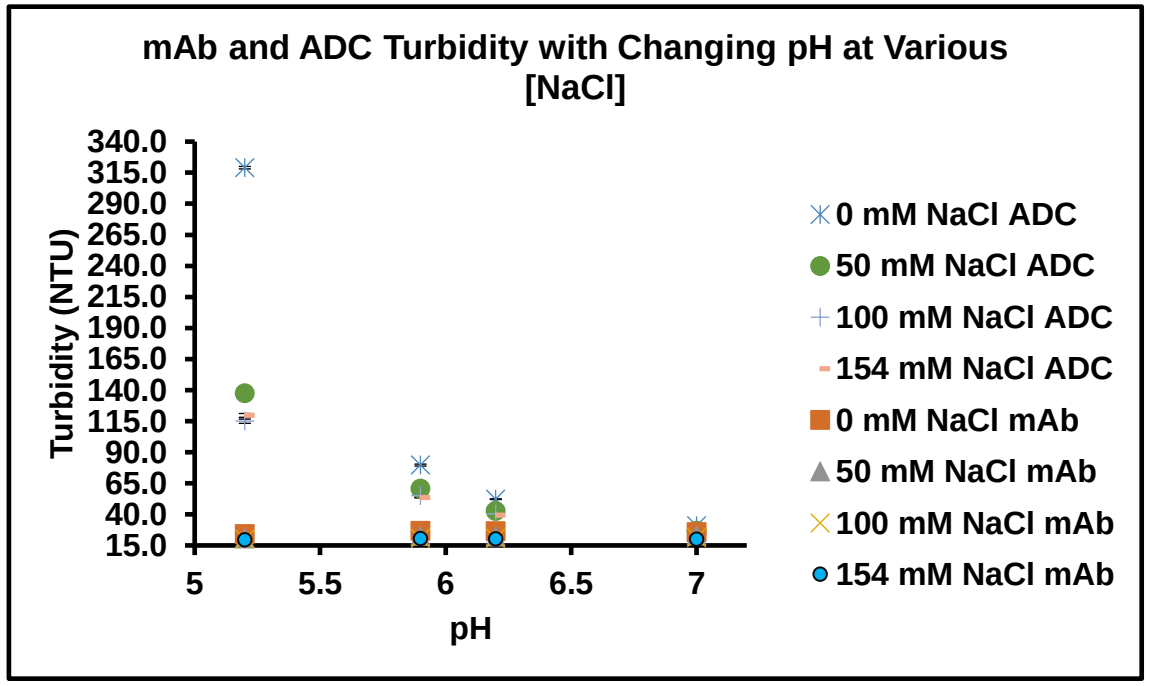



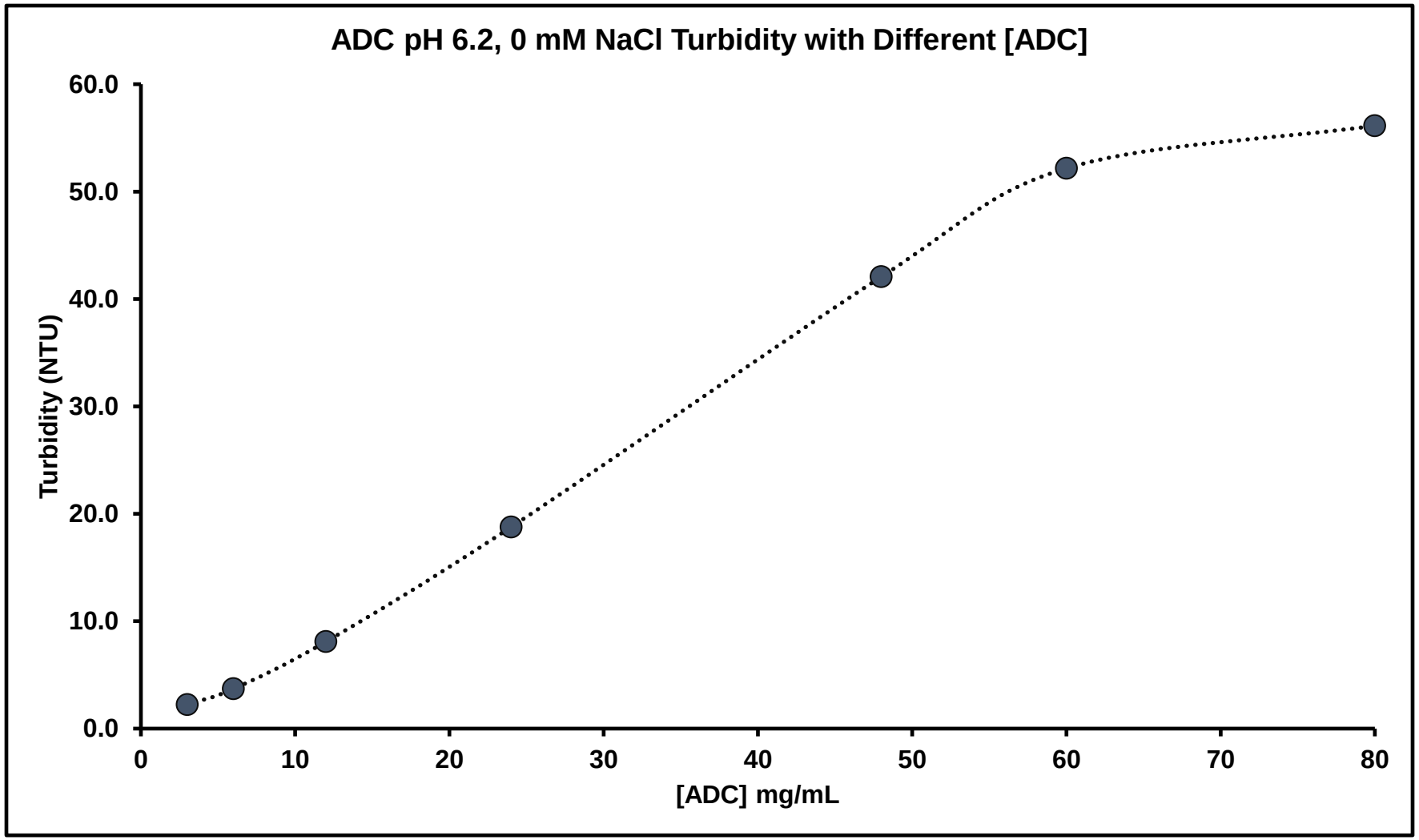



# ADC Turbidity is Substantially Higher than mAb Turbidity



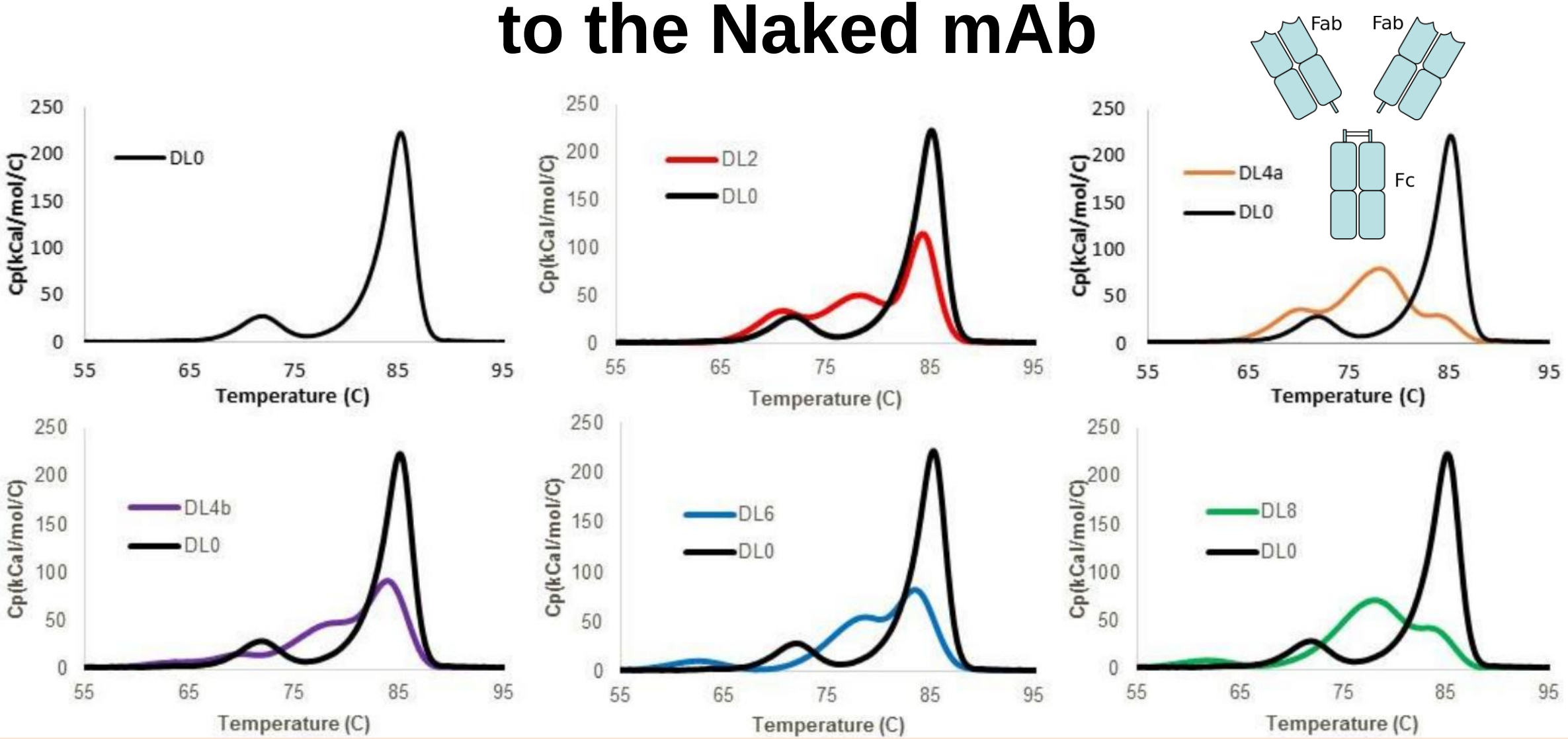



# ADC Turbidity Decreases with Decreasing ADC Concentration, with Non-Linear Behavior Observed In Certain Concentration Regimes




**Non-Linear Behavior Suggests the Presence of Protein-Protein Interactions**

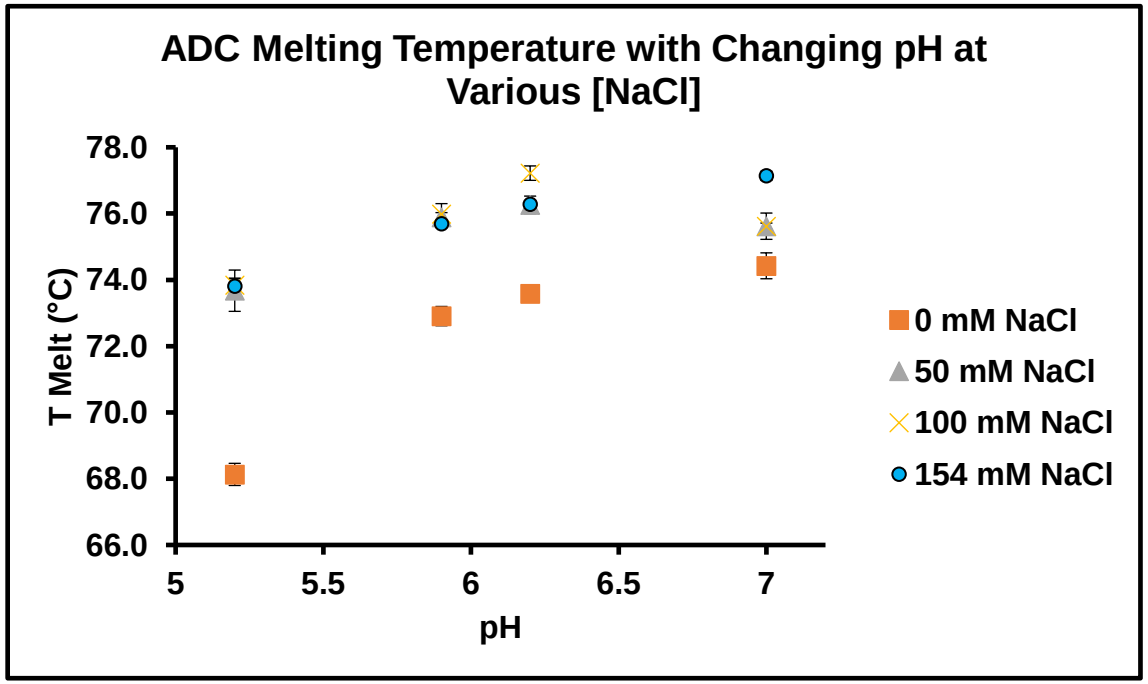
# ADC Melting Temperature Decreases Compared to the Naked mAb

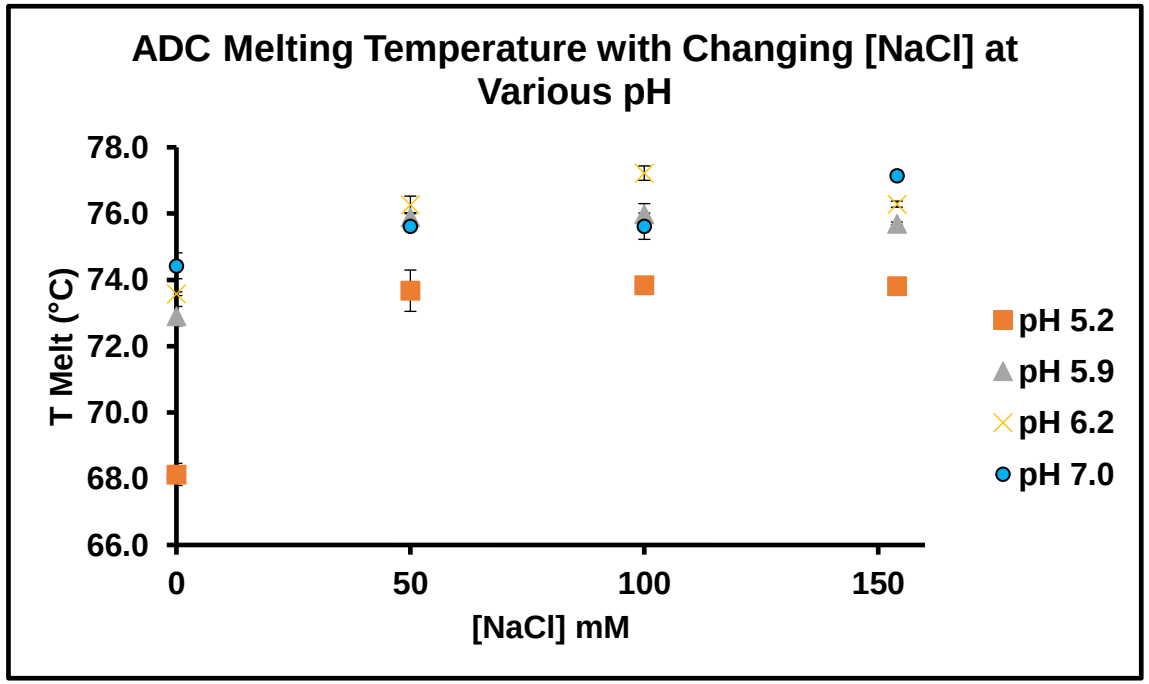



Labels denote drug loading (i.e. DL0 corresponds to a drug loading of 0)

The modification of cysteine residues results in an additional melting transition corresponding to unfolding of the drug-conjugated Fab domain

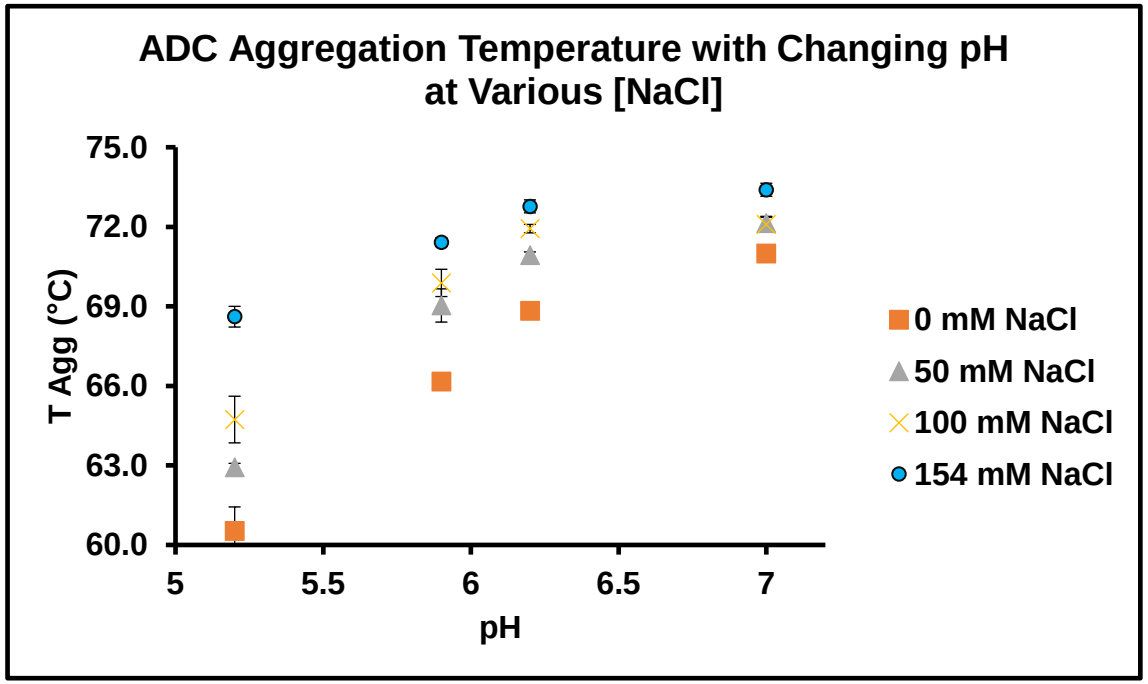
# ADC Melting Temperature can be Screened in High-Throughput Across Several Conditions

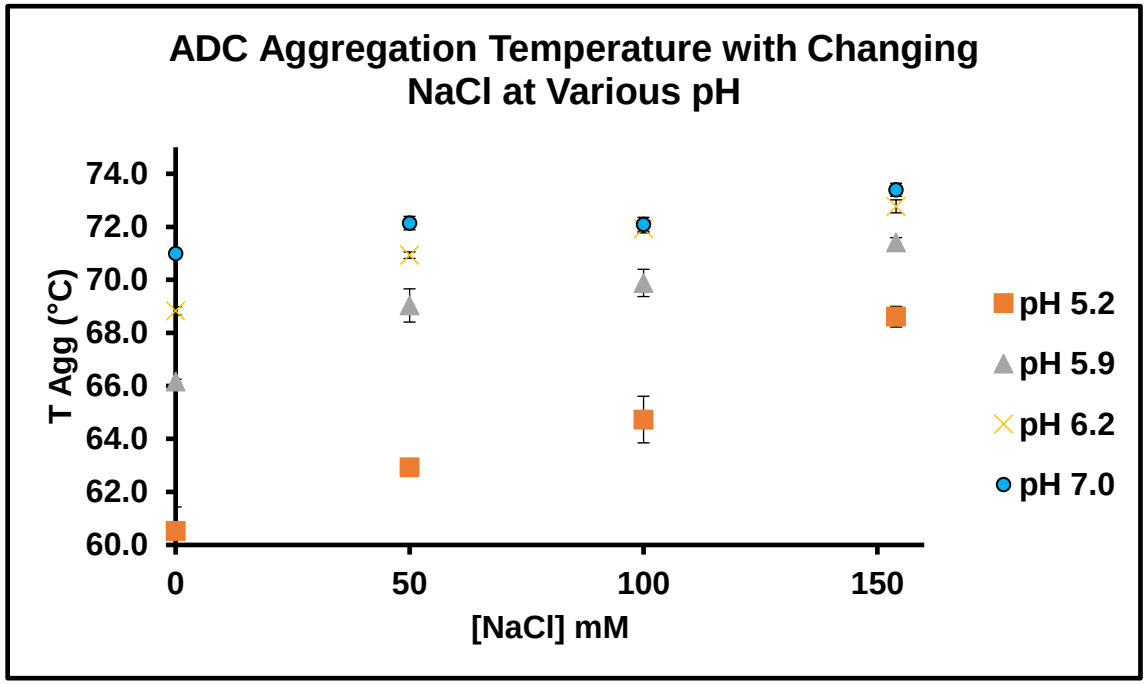



**ADC Melting Temperature Changes by ~6°C Across the Formulation Conditions**
**Conditions that Led to More Turbidity are those that have Lower Melting Temperatures**

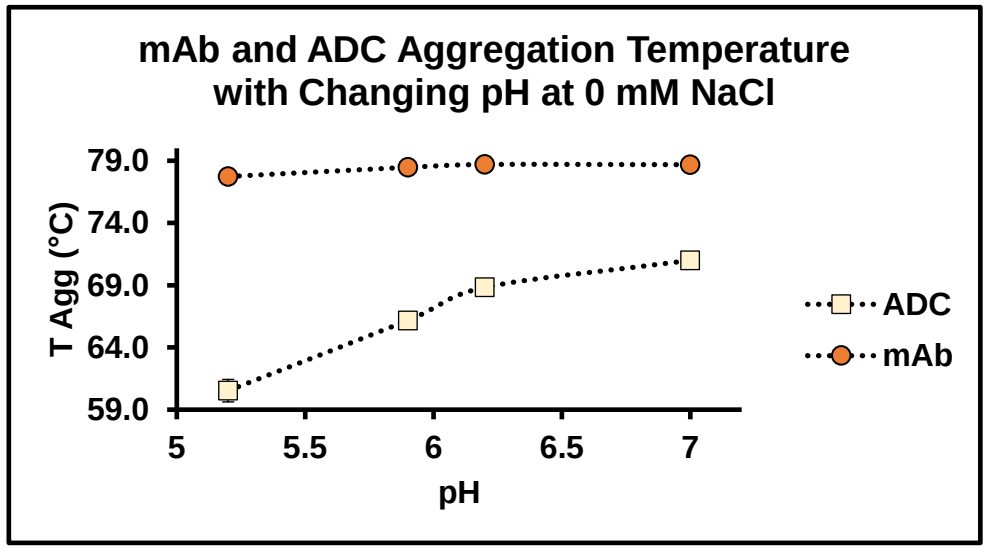
# ADC Aggregation Temperature can be Screened in High-Throughput Across Several Conditions

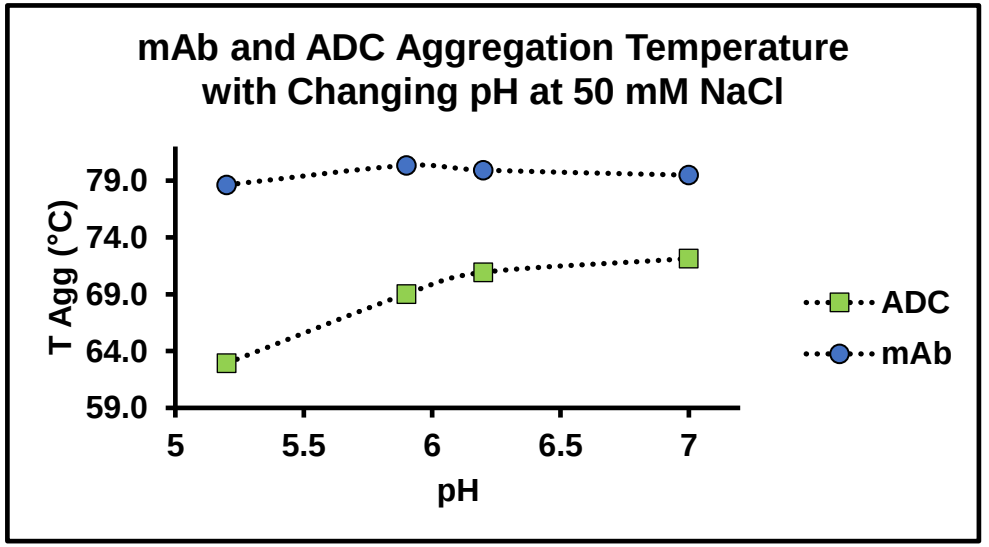


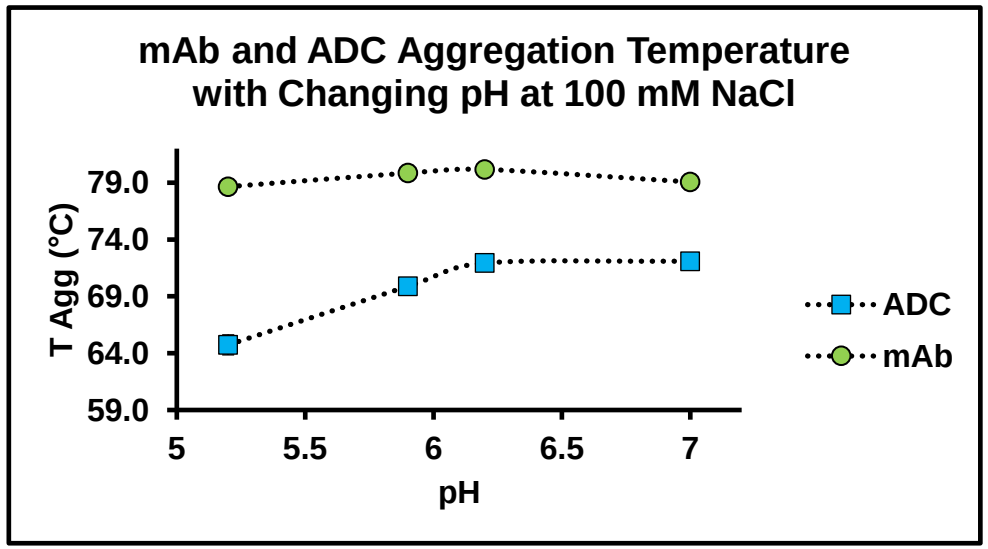



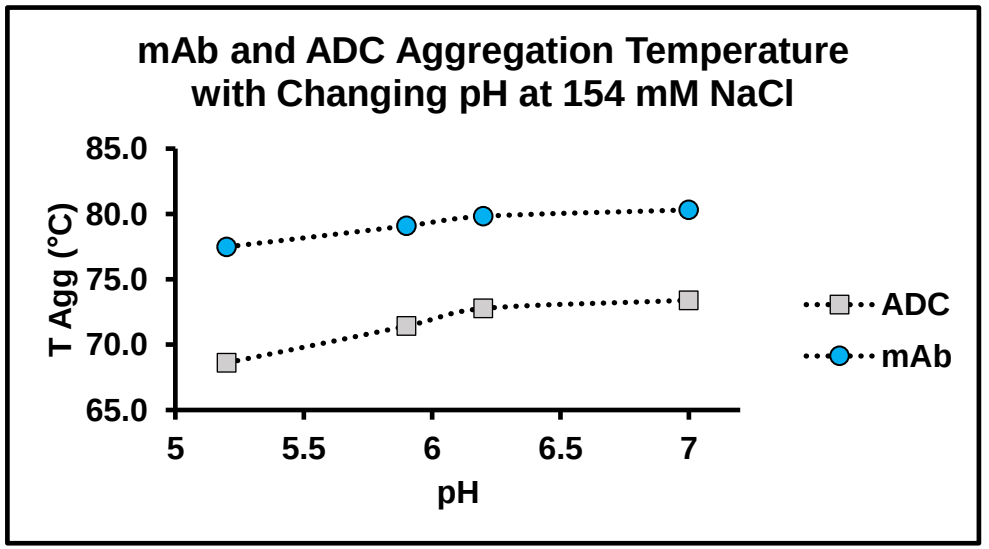

**ADC Aggregation Temperature Changes by ~10°C Across the Formulation Conditions**
**Conditions that Led to More Turbidity are those that have Lower Aggregation Temperatures**

# ADC and mAb Aggregation Temperature Comparison with Changing pH



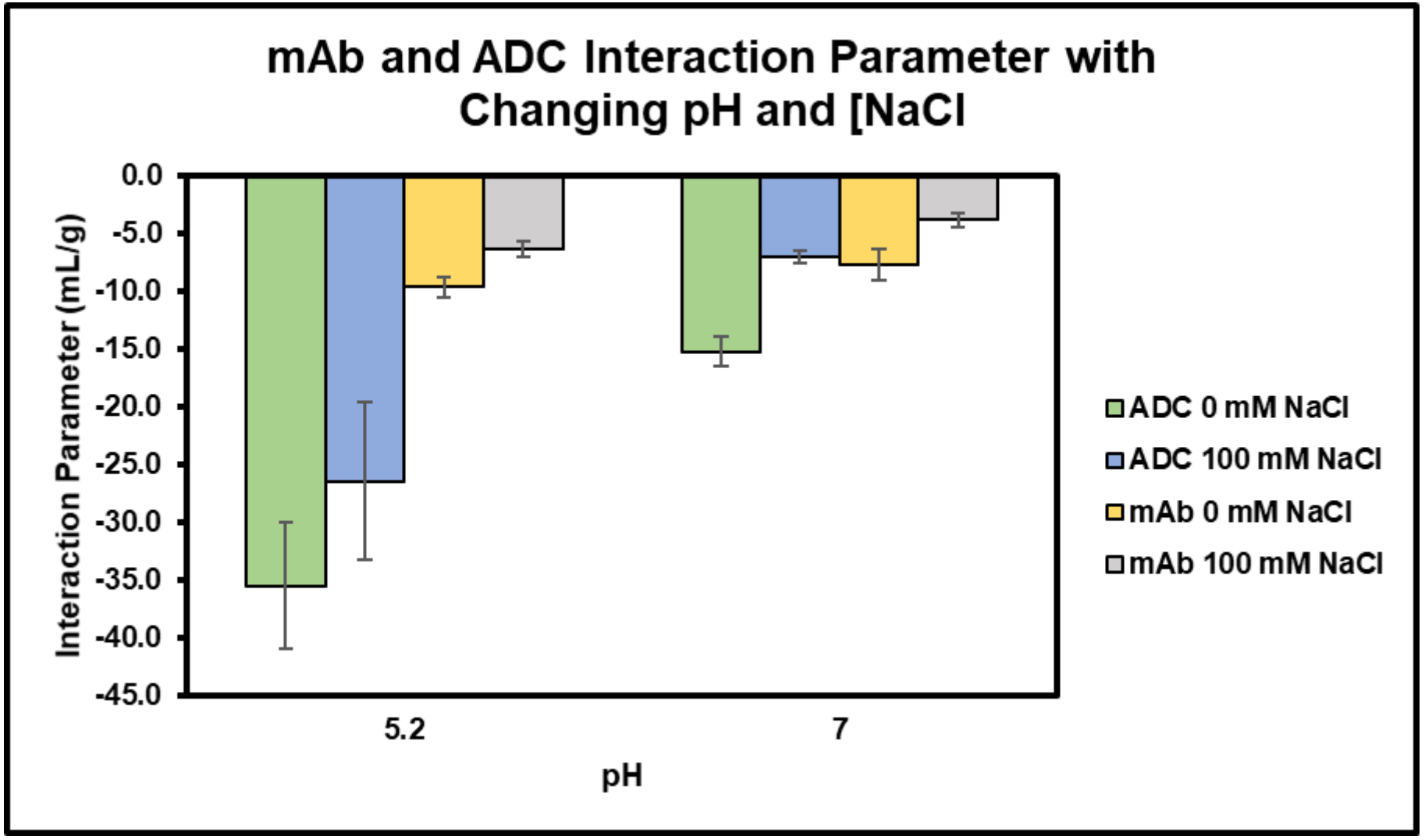




**ADC Exhibits Lower Aggregation Temperature than the Naked mAb**

# ADC Exhibits More Negative Interaction Parameter than mAb




**ADC Exhibits a Larger Degree of Protein-Protein Interactions Compared to the Naked mAb (i.e. lowering of colloidal stability upon drug conjugation)**

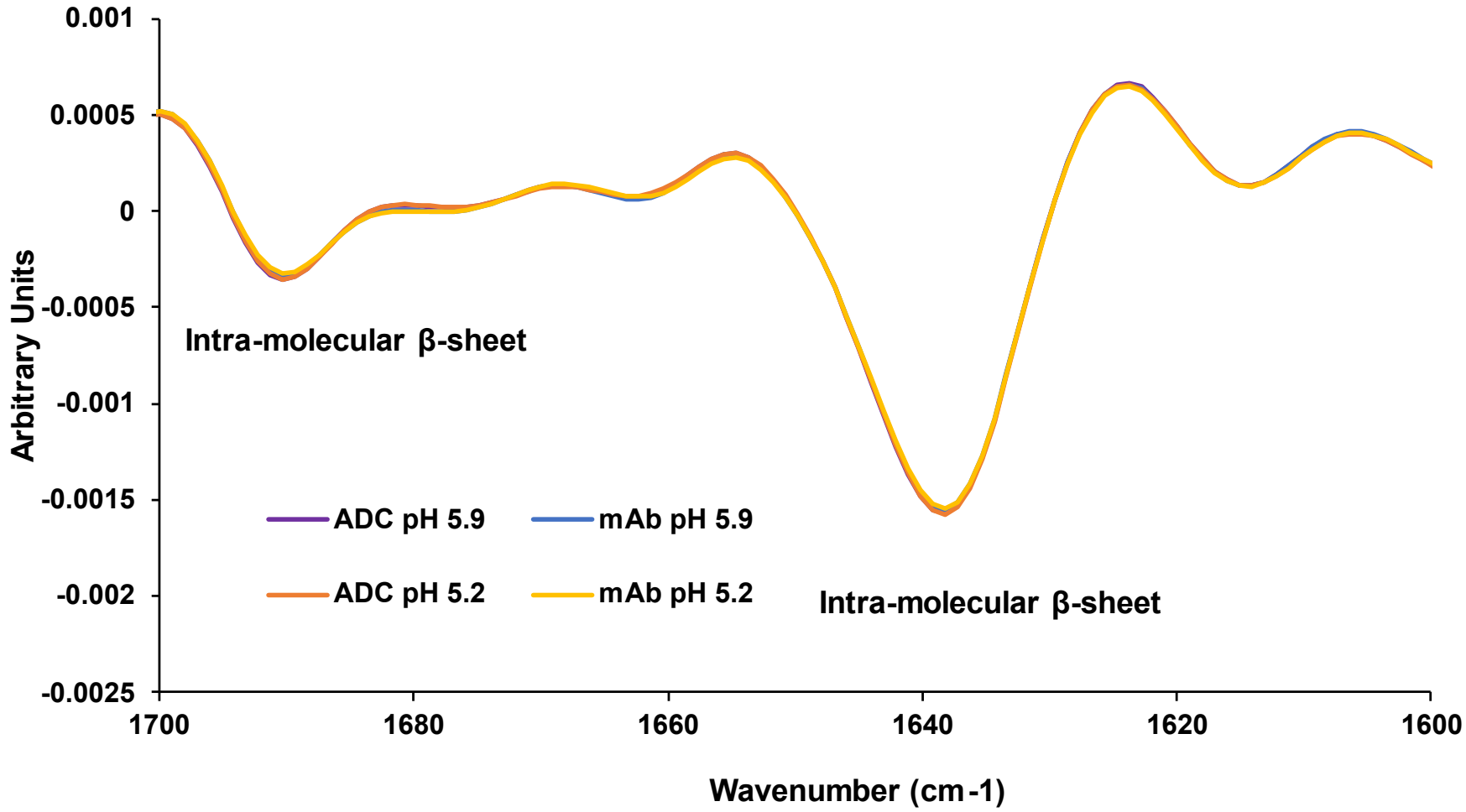
# Naked mAb Shows no Change in Amino Acid Modifications Across the Different Formulations

| Sequence | Amino Acid Position (Number/Chain) | Modified Amino Acid | Modification | pH 5.2, 0 mM NaCl | pH 5.2, 100 mM NaCl | pH 7.0, 0 mM NaCl | pH 7.0, 100 mM NaCl |
|---|---|---|---|---|---|---|---|
| ASGGTFSNYWMHWVR | 31/ Heavy Chain | N | Deamidation | 0.1 | 0.1 | 0.1 | 0.1 |
| CKVSNK | 329/ Heavy Chain | N | Deamidation | 0.5 | 0.4 | 0.4 | 0.6 |
| GAIYDGYDVLDNWGQG TLVTVSSASTK | 103/ Heavy Chain | D | Isomerization | 3.1 | 3.2 | 3.4 | 3.4 |
| SLSLSPGK | 451/ Heavy Chain | K | C-terminal Lysine Cleavage | 94.8 | 93.8 | 95.4 | 95.9 |
| ASGGTFSNYWMHWVR | 34/ Heavy Chain | M | Oxidation | 0.2 | 0.2 | 0.2 | 0.3 |
| DTLMISR | 256/ Heavy Chain | M | Oxidation | 2.2 | 2.6 | 2.5 | 2.5 |
| QVQLVQSGAEVK | 1/ Heavy Chain | Q | N-Terminal Pyroglutamylation | 100 | 99.9 | 99.9 | 99.9 |

# ADC Shows no Change in Amino Acid Modifications Across the Different Formulations

| Sequence | Amino Acid Position (Number/Chain) | Modified Amino Acid | Modification | pH 5.2, 0 mM NaCl | pH 5.2, 100 mM NaCl | pH 7.0, 0 mM NaCl | pH 7.0, 100 mM NaCl |
|---|---|---|---|---|---|---|---|
| ASGGTFSNYWMHWVR | 31/ Heavy Chain | N | Deamidation | 0 | 0.1 | 0.1 | 0.1 |
| CKVSNK | 329/ Heavy Chain | N | Deamidation | 0.9 | 0.4 | 0.5 | 0.8 |
| GAIYDGYDVLDNWGQG TLVTVSSASTK | 103/ Heavy Chain | D | Isomerization | 4 | 3.4 | 3.5 | 4.8 |
| SLSLSPGK | 451/ Heavy Chain | K | C-terminal Lysine Cleavage | 95.4 | 95.2 | 95.2 | 95.3 |
| ASGGTFSNYWMHWVR | 34/ Heavy Chain | M | Oxidation | 0.8 | 1 | 1 | 1.5 |
| DTLMISR | 256/ Heavy Chain | M | Oxidation | 3.6 | 3.5 | 3.1 | 3 |
| QVQLVQSGAEVK | 1/ Heavy Chain | Q | N-Terminal Pyroglutamylation | 100 | 100 | 100 | 100 |

# No Change in Secondary Structure is Observed Between the Naked mAb and ADC

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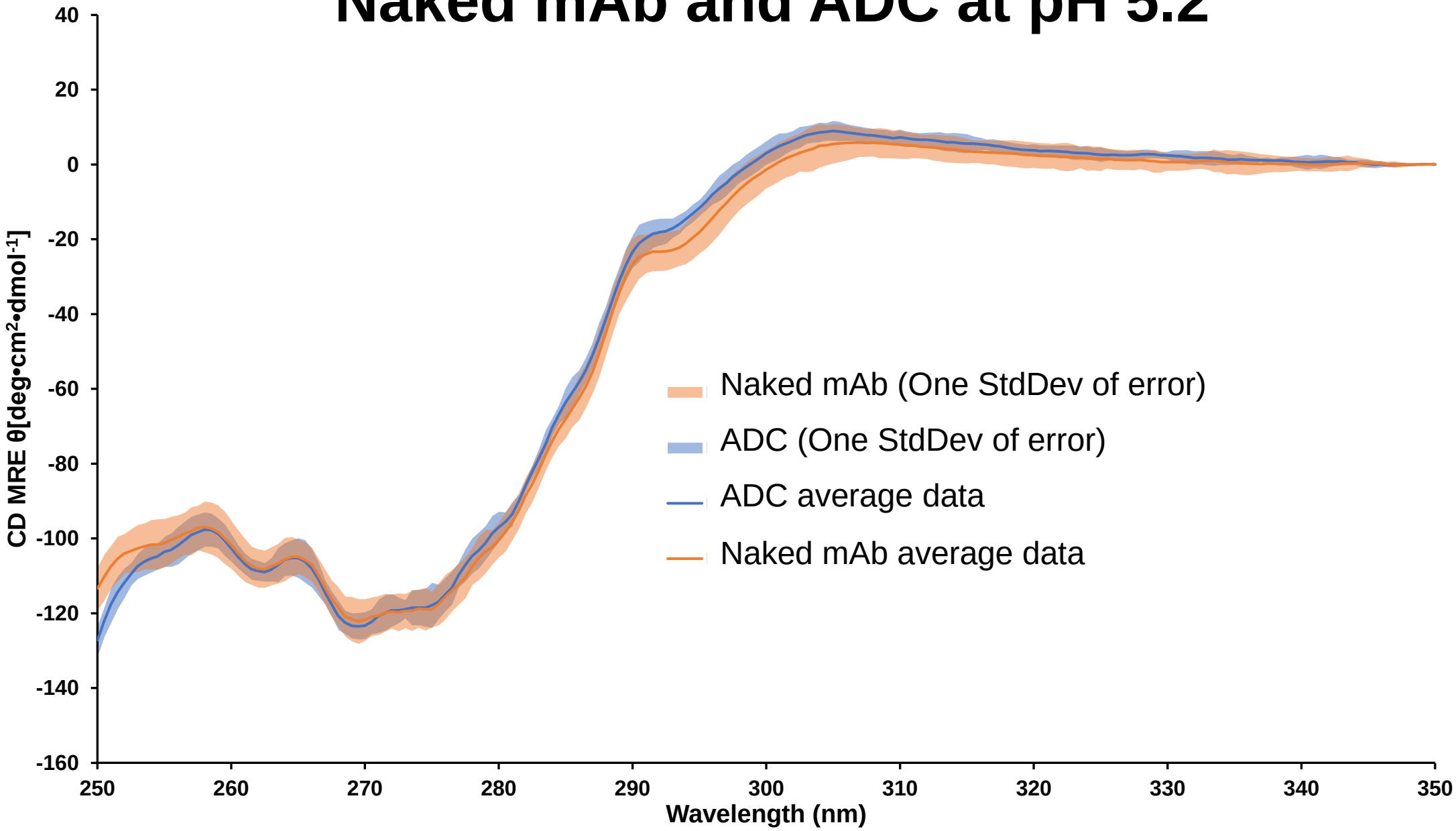
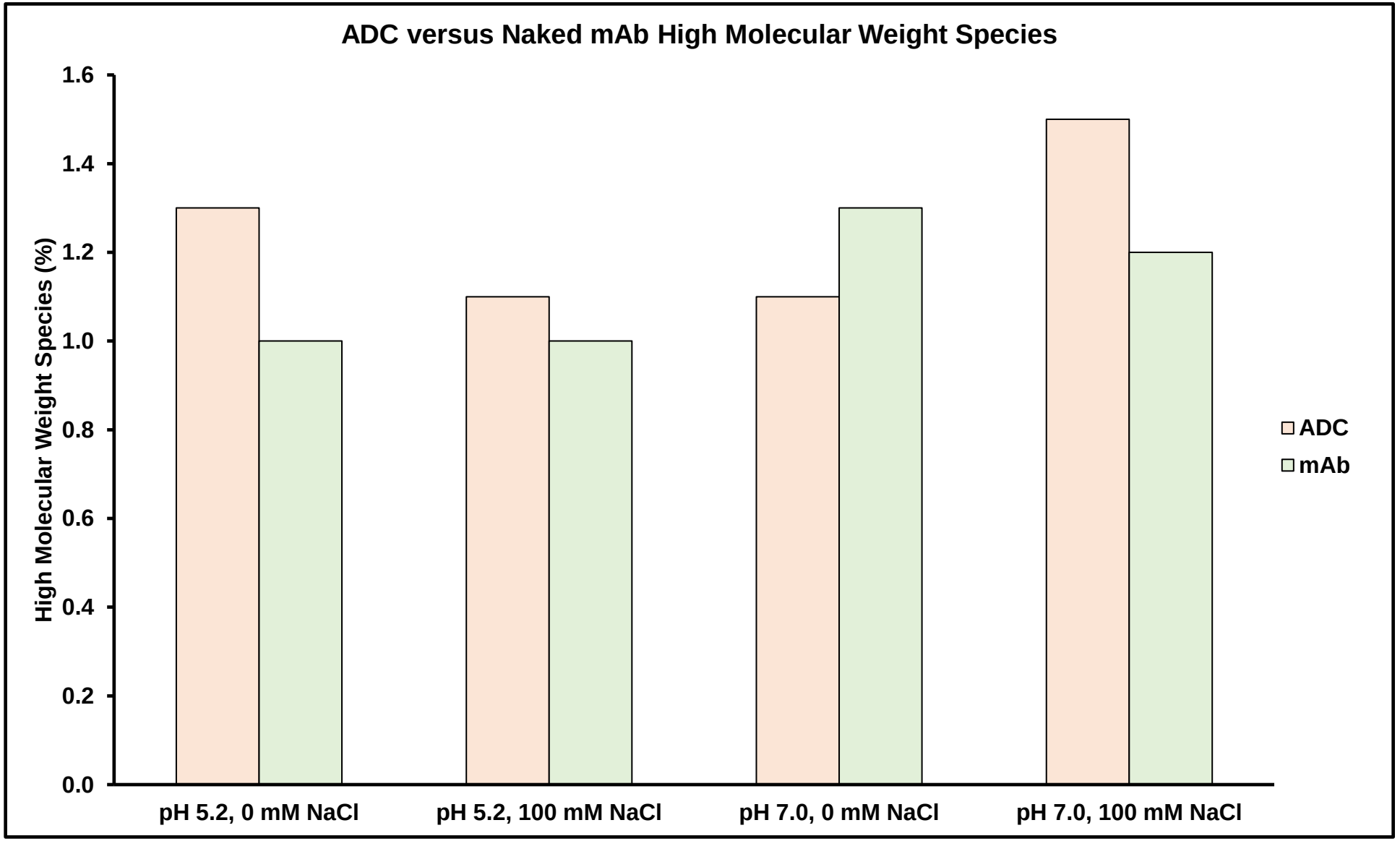
# No Change in Tertiary Structure is Observed Between the Naked mAb and ADC at pH 5.2



# ADC versus Naked mAb Shows No Difference in High Molecular Weight Species

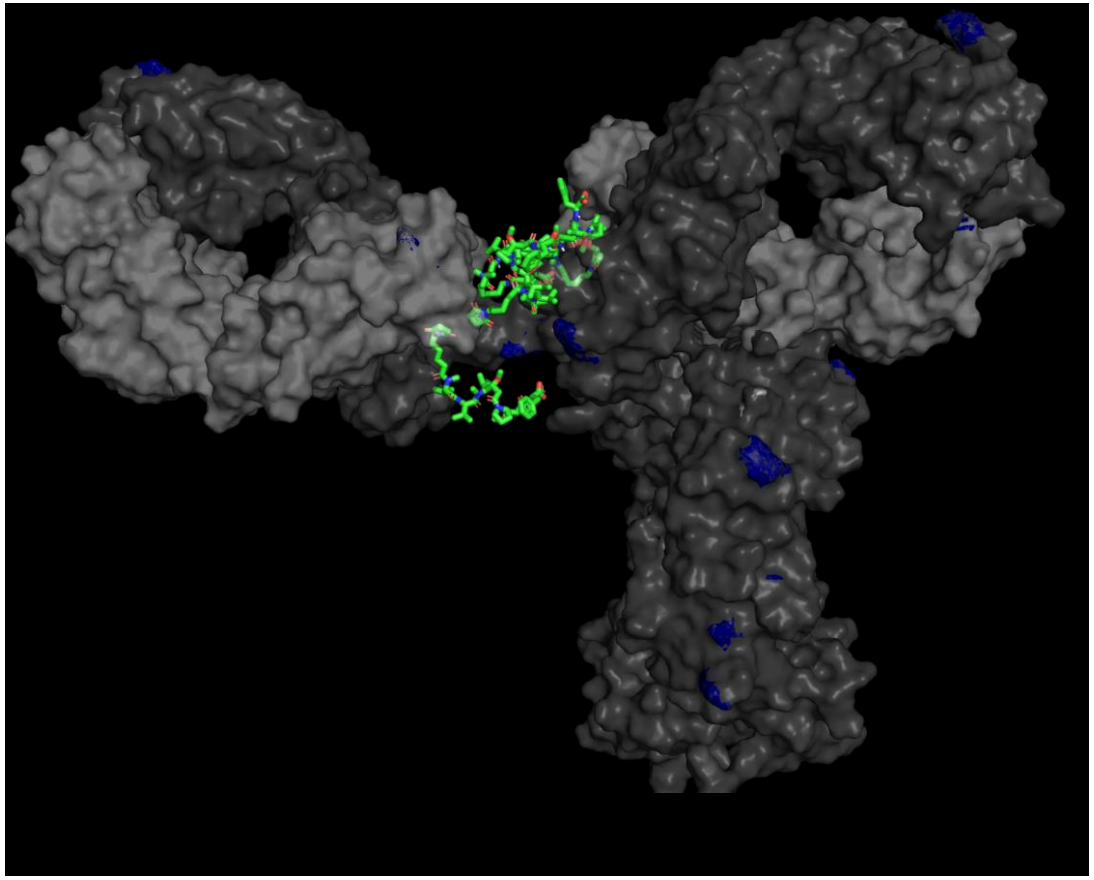


# Experimental Conclusions

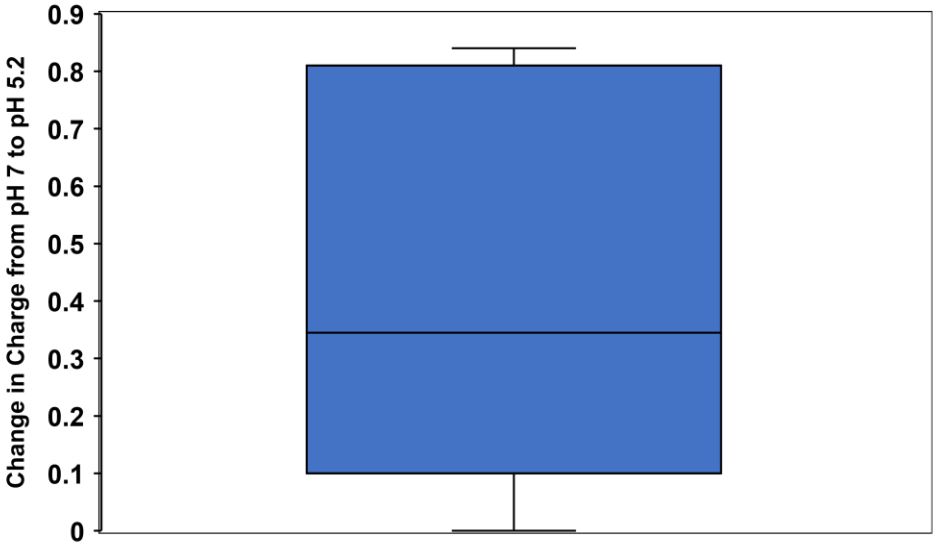
1. **The highest turbidity is observed at a pH far away from the pI of the ADC (electrostatic contribution)**
2. **Drug conjugation changes the stability of the ADC in comparison to the naked mAb**
3. **The ADC exhibits a larger degree of attractive protein-protein interactions in comparison to the naked mAb**
4. **Lowering pH and [NaCl] increases the magnitude of attractive interactions**
5. **The presence of aggregation arising from factors such as differences in oxidation or deamidation profiles between the different structures is ruled out**
6. **Experiments show the absence of significant unfolding in any formulation, confirming that the main secondary structure component of the antibody is intra-molecular β-sheet as expected for IgG1-based molecules**
7. **No change in tertiary structure is observed for the protein in any formulation tested**
8. **No change is observed in levels of soluble irreversible aggregates (<~0.1 μM) between the various formulations**

**Therefore, we hypothesized that the changing solution conditions lead to an increase in reversible electrostatic interactions between proteins with largely preserved secondary and tertiary structure**

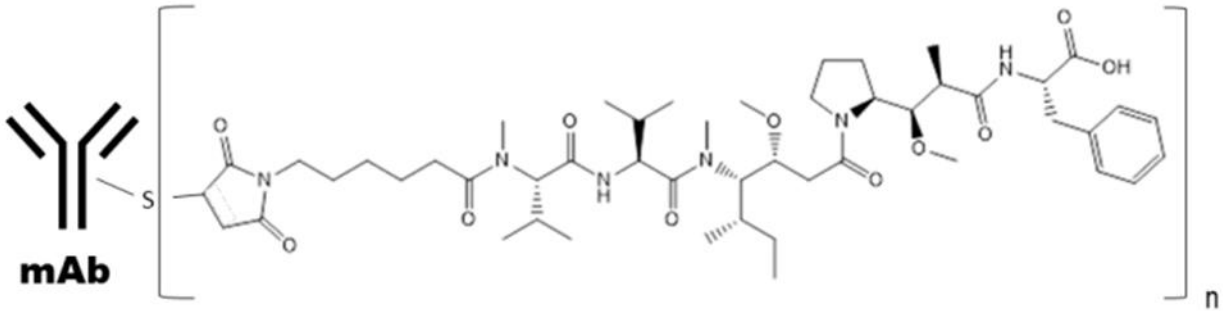
# Computational modelling of the ADC Reveals Significant Increase in Histidine Charge from pH 7 to pH 5.2



- Surfaces of the heavy chains and light chains of the ADC are shown in gray and white, respectively;
- Solvent accessible histidine residues defined as predicted solvent exposure ≥ 10% are shown in blue surface;
- The payload is shown in stick representation




Significant increase in histidine charge from pH 7.0 to pH 5.2



# Conclusions

1. **Experimental and computational techniques can be used in concert to understand the mechanism for ADC behavior in solution**

2. **The studies presented suggest the presence of intermolecular electrostatic interactions between ADC molecules, which drive the high turbidity observed**

3. **Traversing a library of different solution conditions enables the finding of “hits” that lead to more “well behaved” ADC formulations**

**Importantly, the studies presented here constitute a set of general screens that can be conducted across a wide scope of ADCs to enhance their stability**

GSK

# Acknowledgements

Xuan Hong

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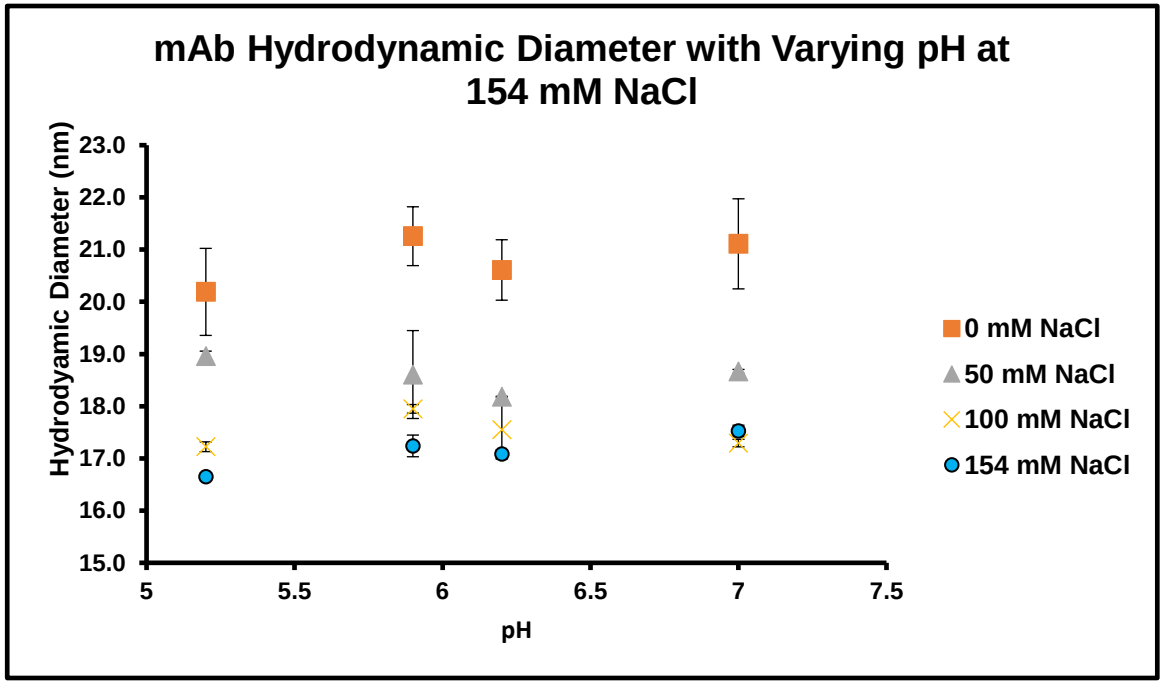
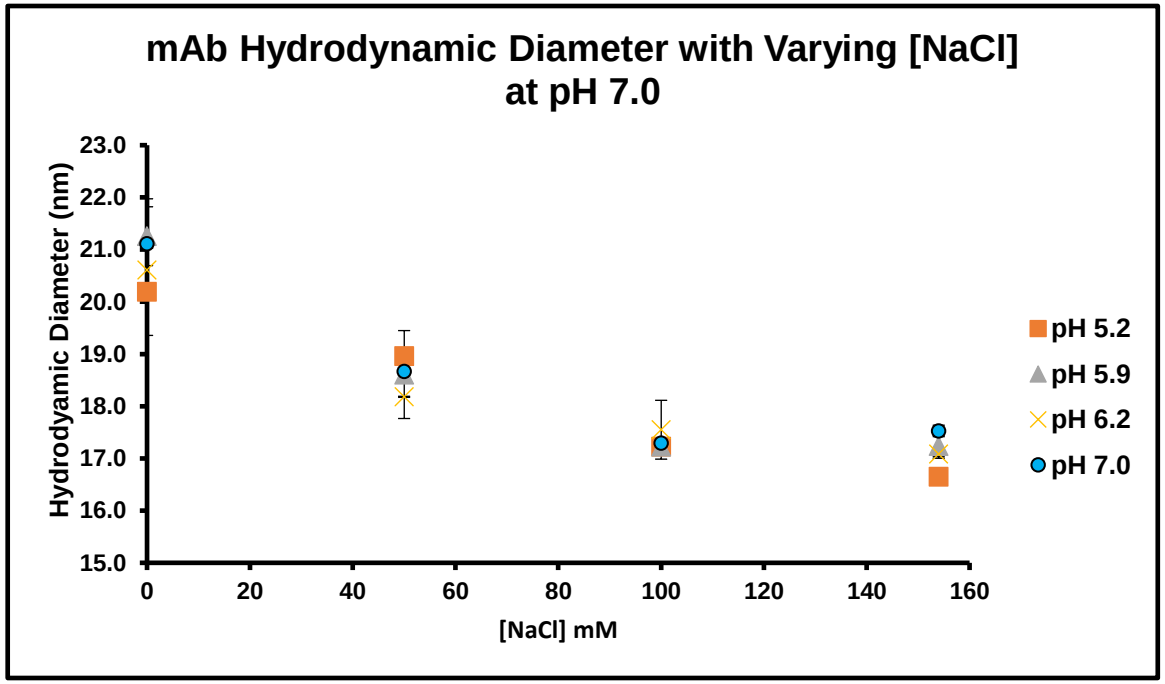
Dany Doucet

Renuka Thirumangalathu

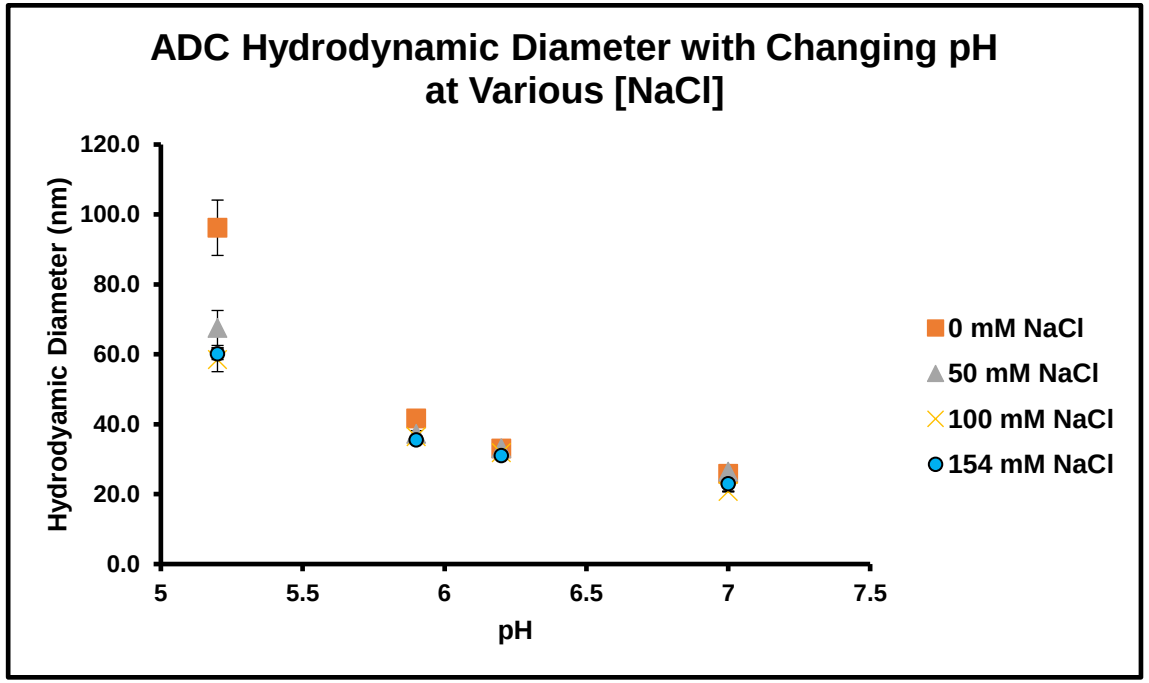
# Back-up Slides

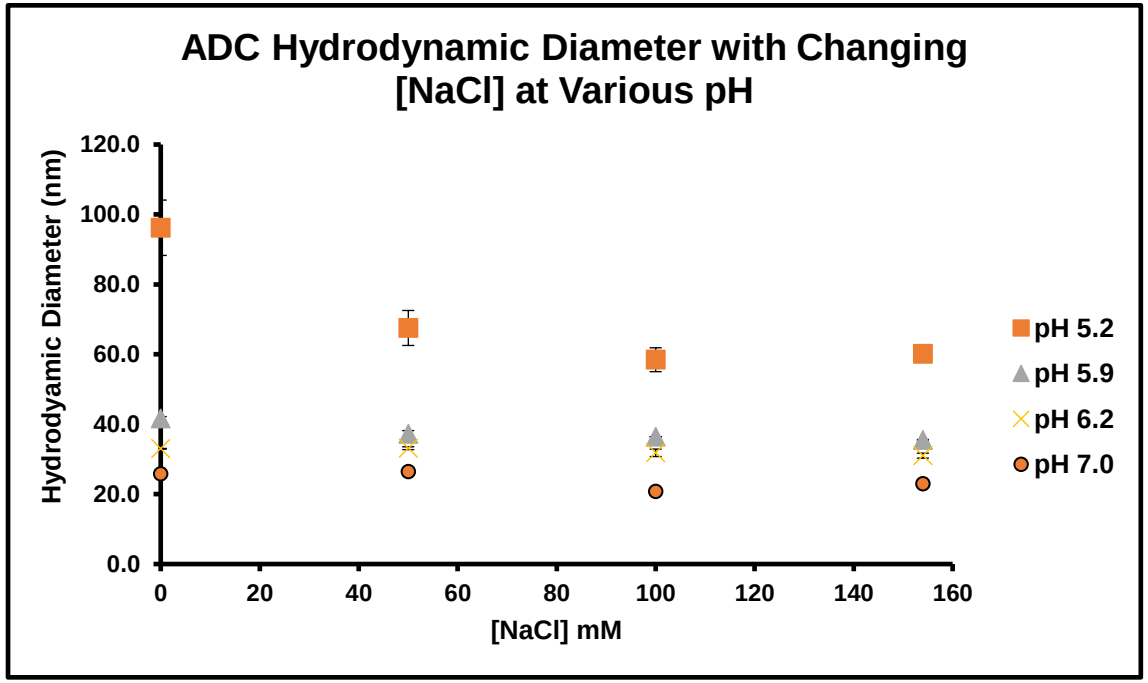
# Overlay of mAb Z-average Diameter



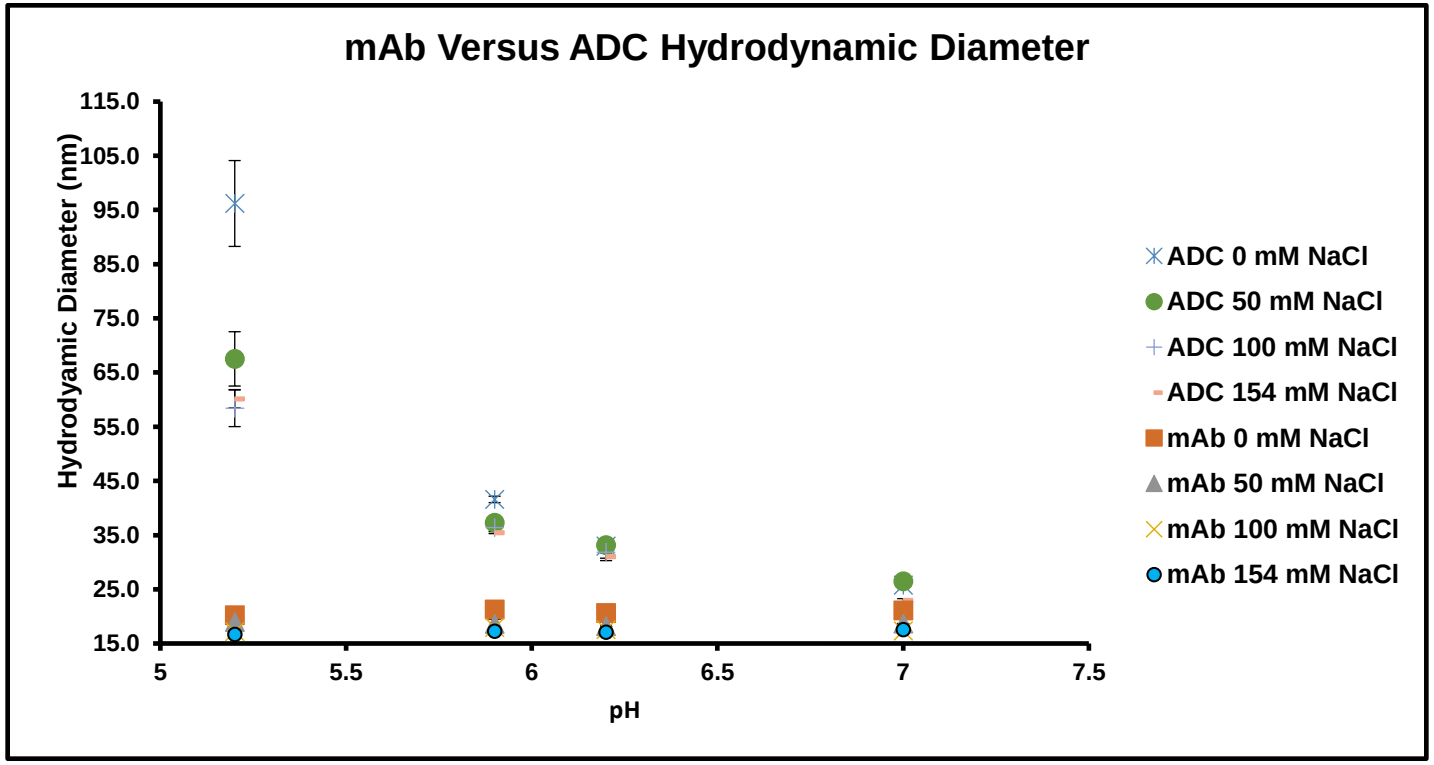
# Overlay of ADC Z-average Diameter



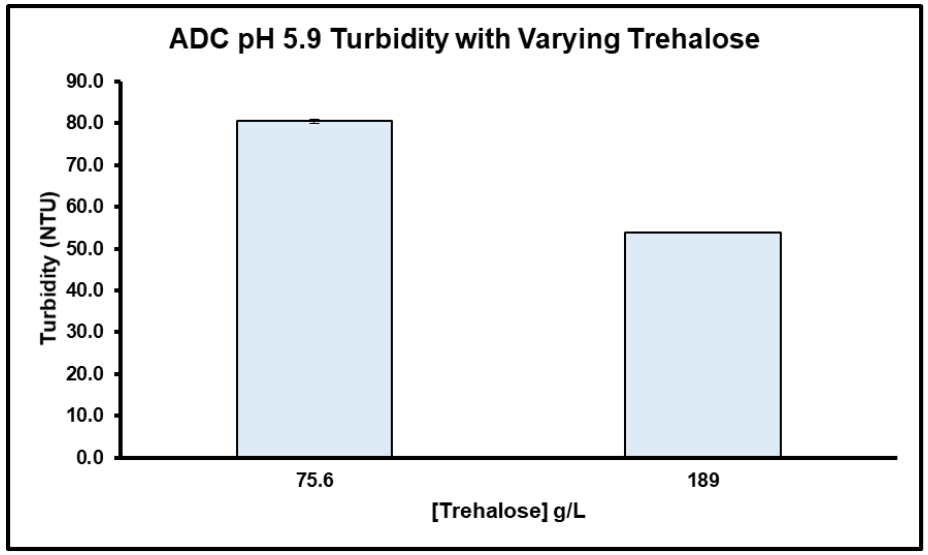

**Conditions with Increased Turbidity are those that Show Increased Diameter**

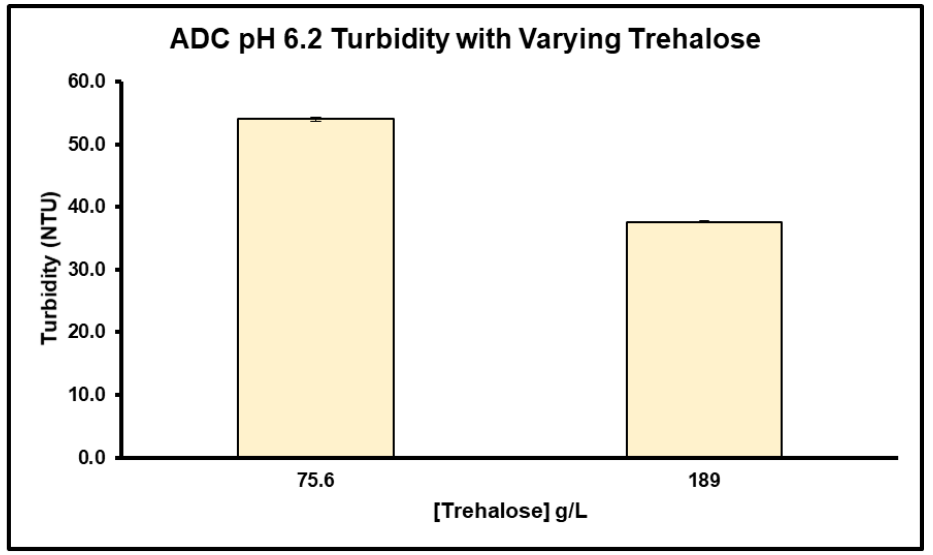
# Overlay of ADC and mAb Z-average Diameter

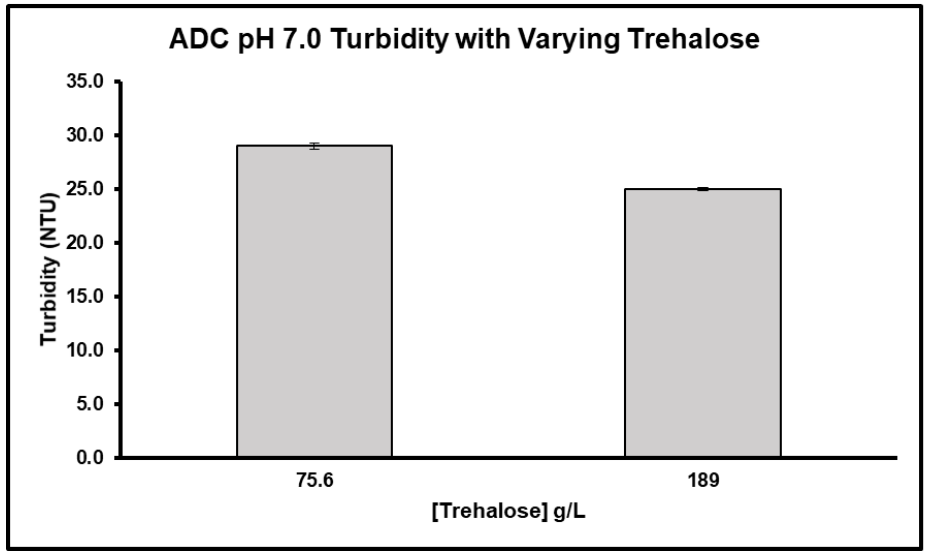


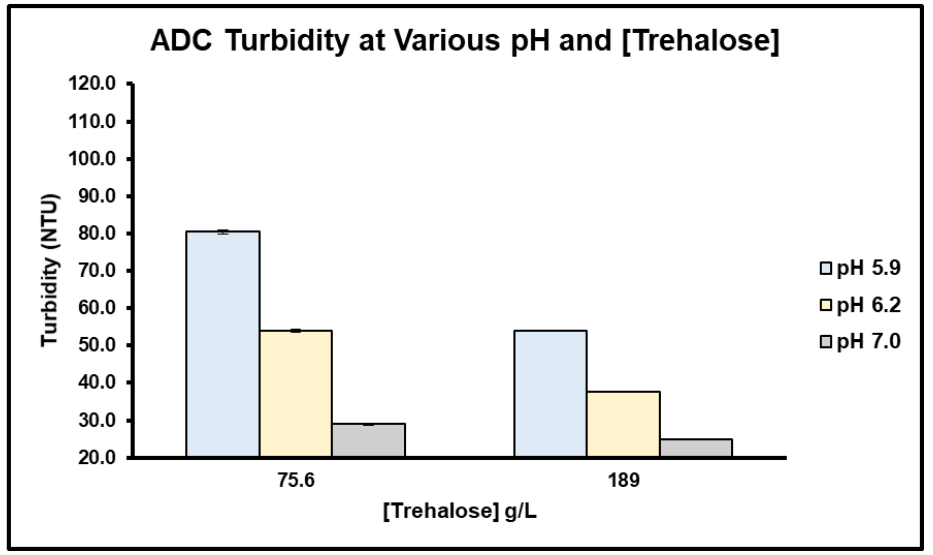

**ADC Exhibits Larger Z-average Diameter Compared to Naked mAb**

# Turbidity Studies with Varying Trehalose Concentration

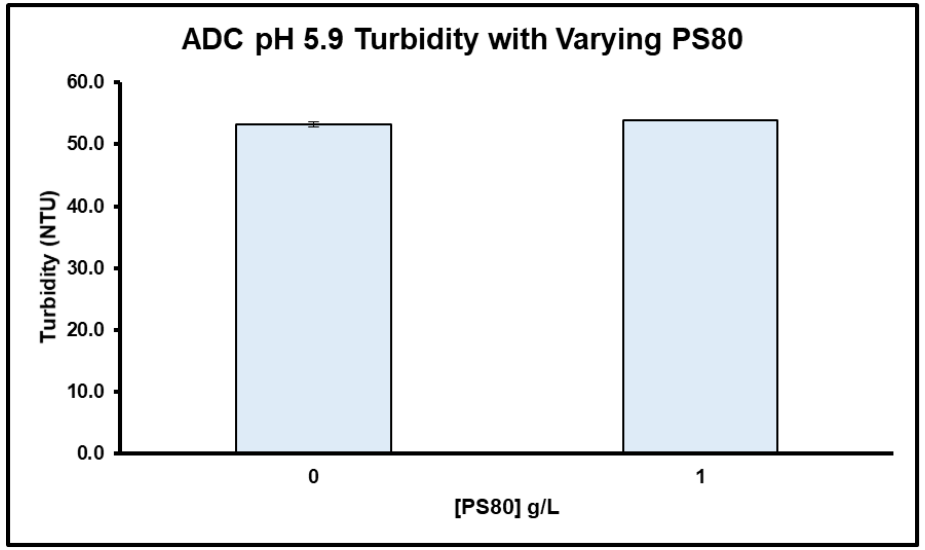


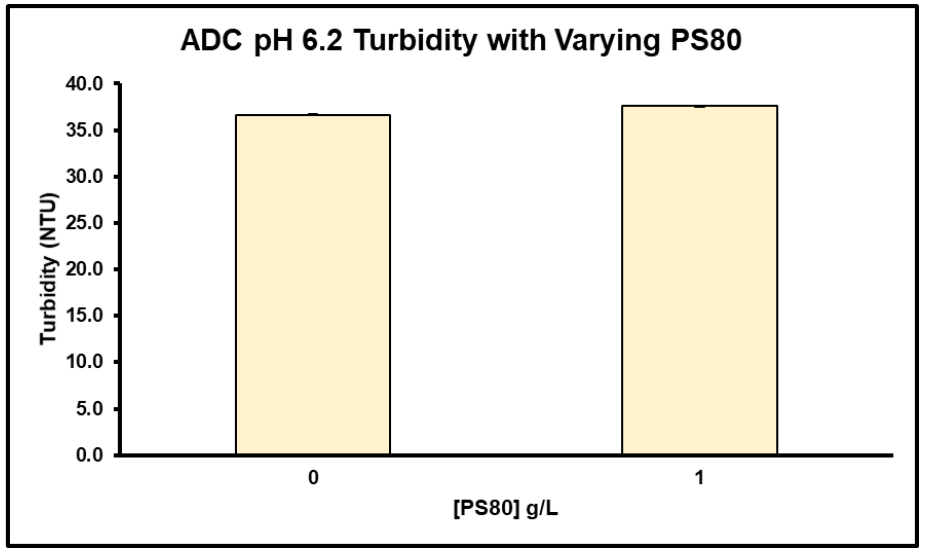



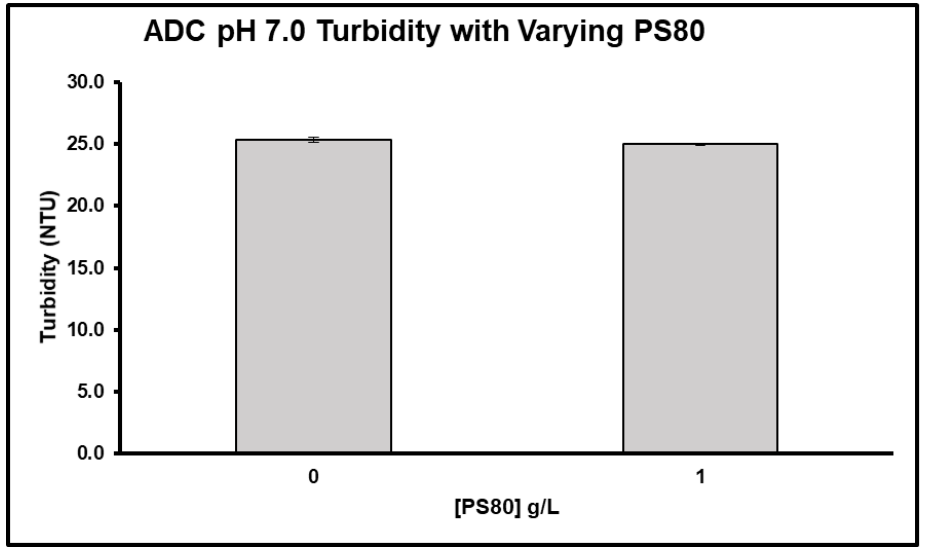



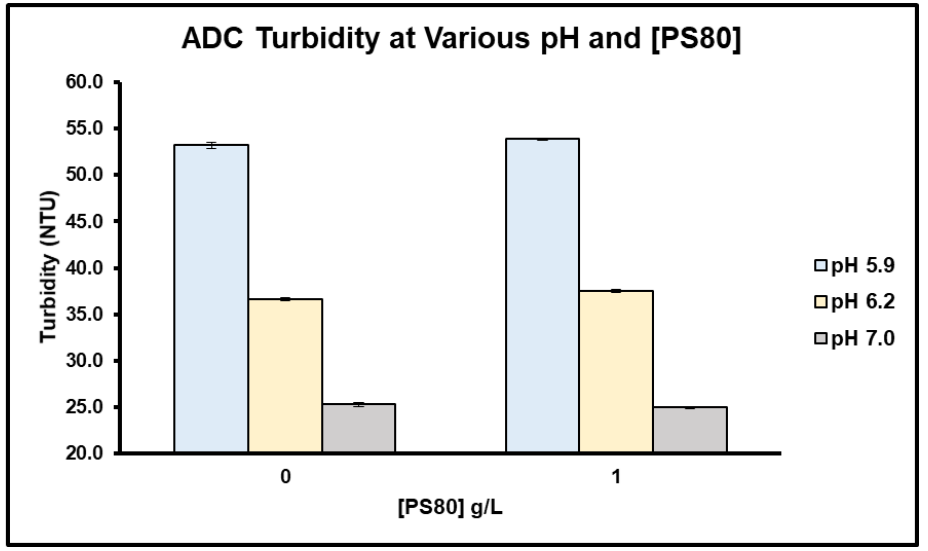



# Turbidity Studies with Varying PS80 Concentration

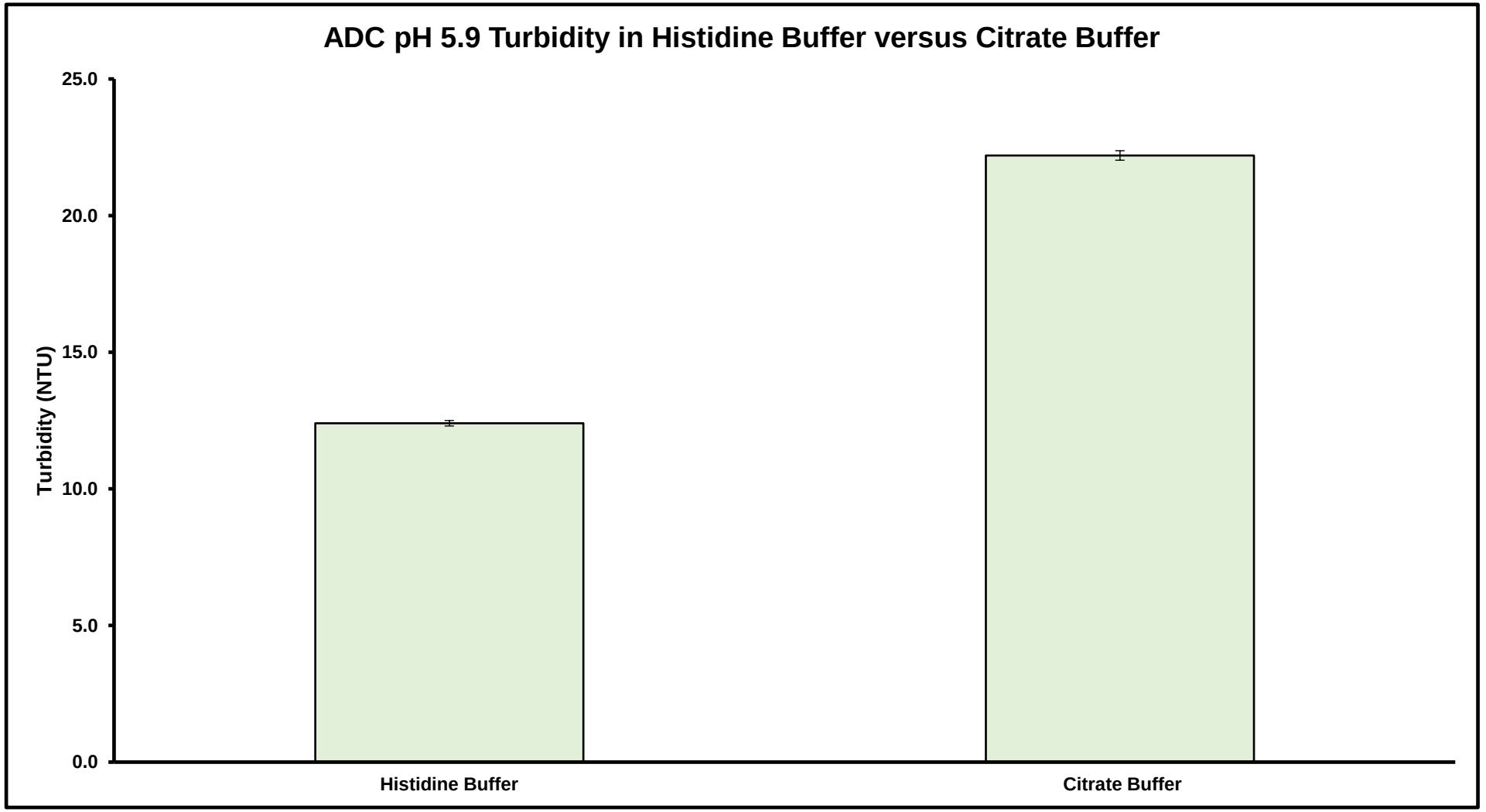


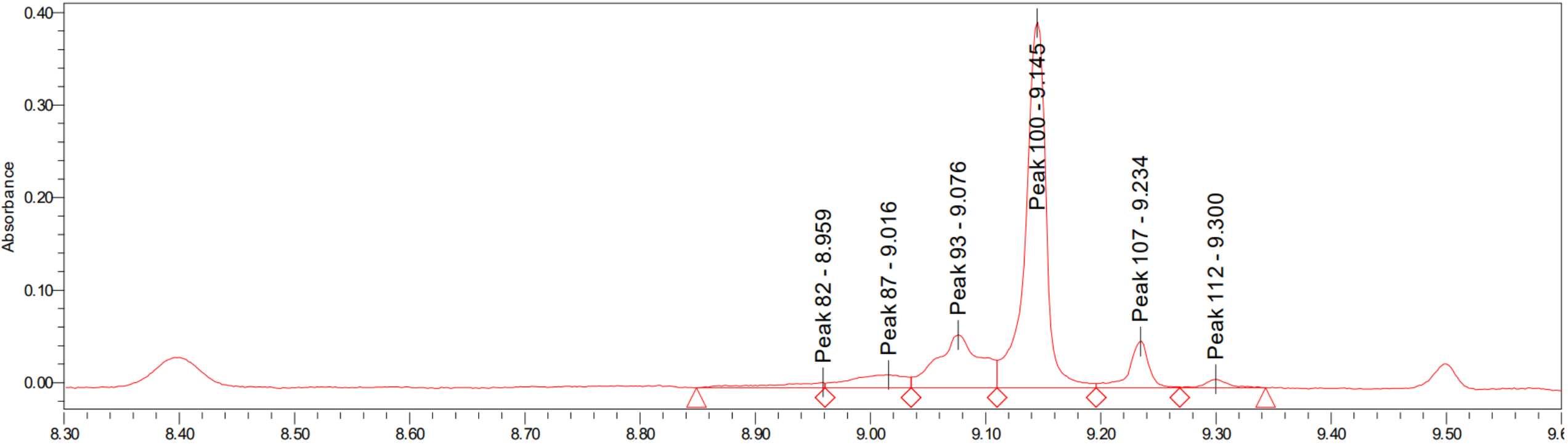



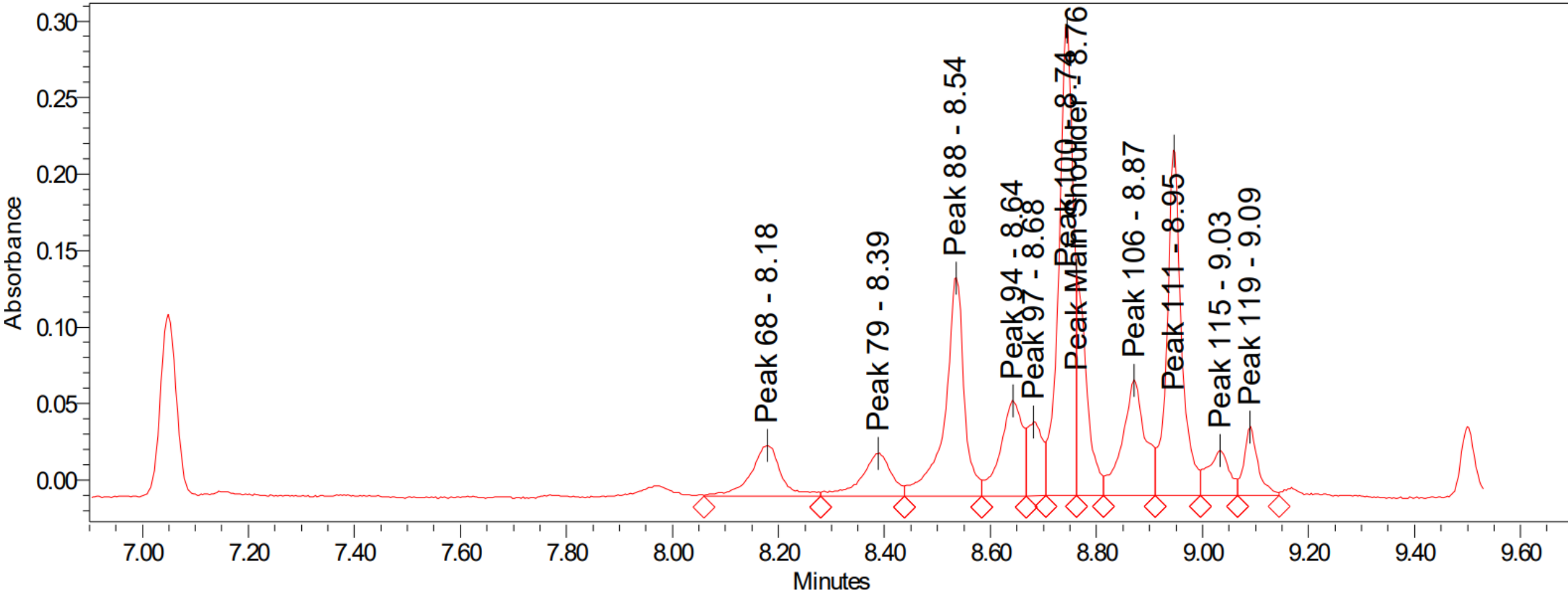
# Turbidity Studies with Changing Buffer



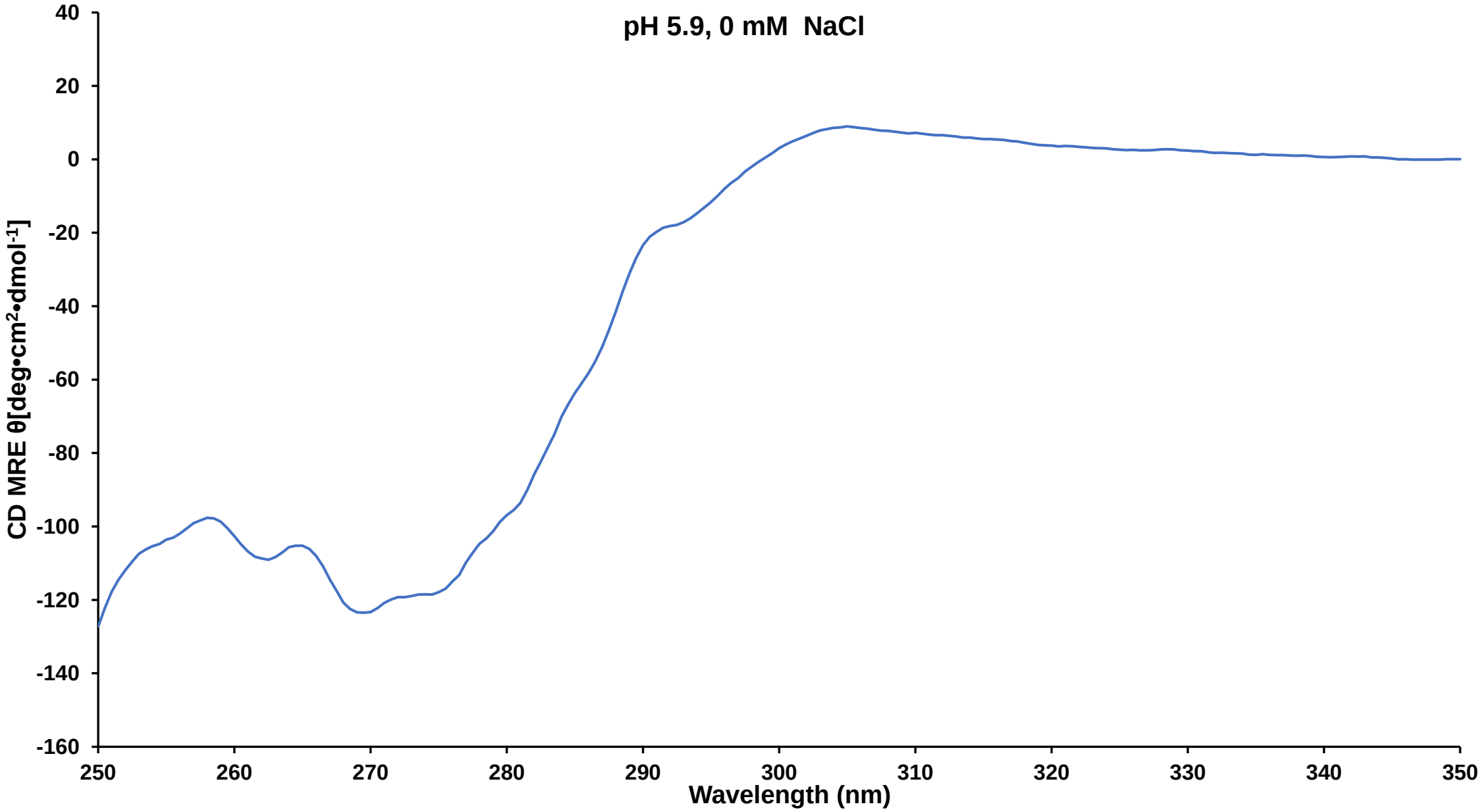
# cIEF Results- mAb



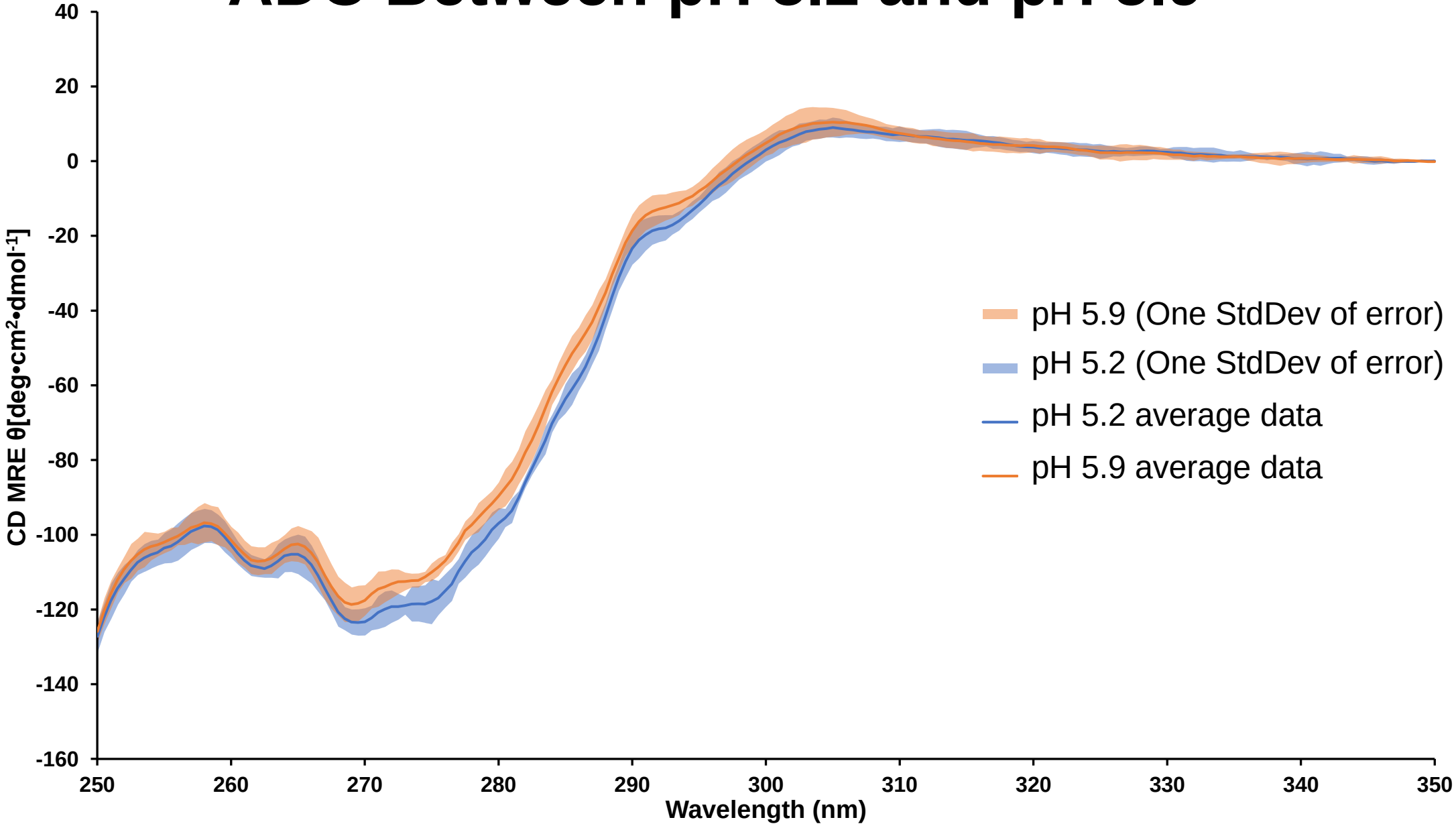
# cIEF Results- ADC



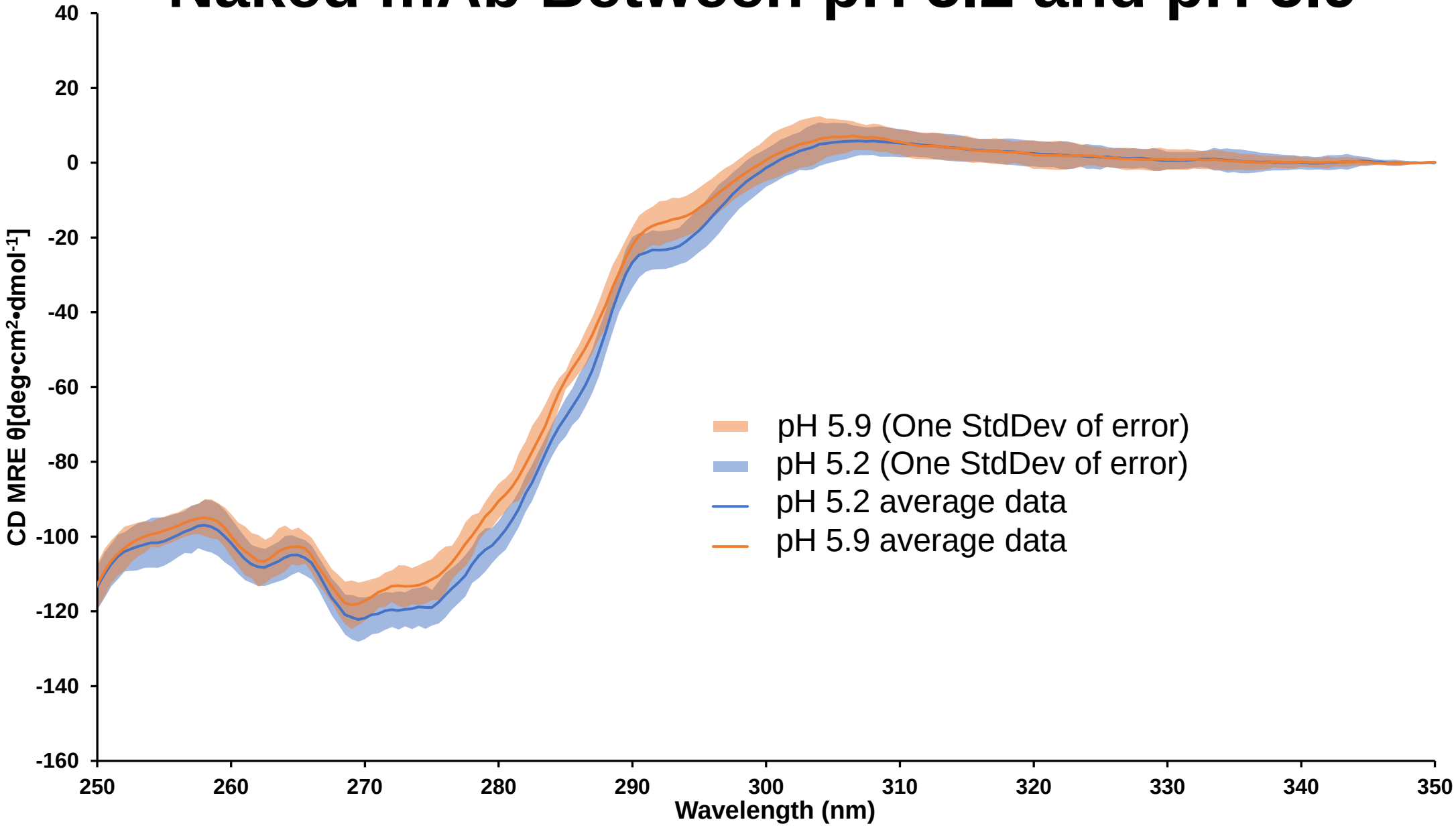
# CD experiments show ADC spectral characteristics expected of an IgG1 molecule



# CD Shows Subtle Differences in Tertiary Structure of ADC Between pH 5.2 and pH 5.9



# CD Shows Subtle Differences in Tertiary Structure of Naked mAb Between pH 5.2 and pH 5.9



# CD Shows Subtle Differences in Tertiary Structure Between Naked mAb and ADC at pH 5.9