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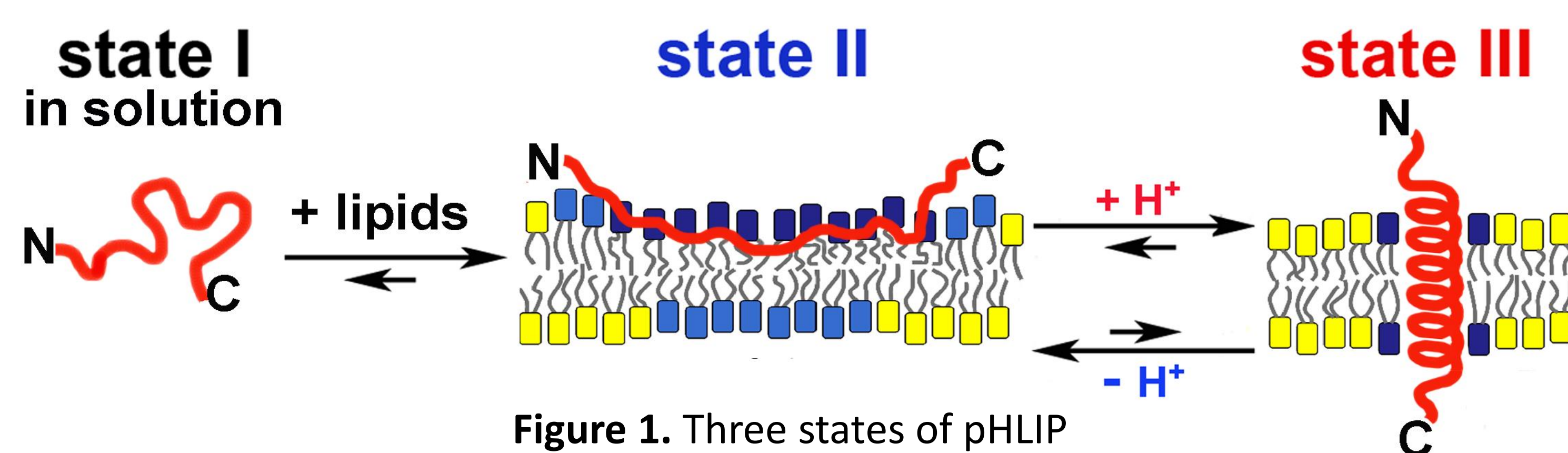
Biophysical Characterization of Membrane Active pHILIP Peptides with Higher Insertion pH

Thomas McPartlon, Jerel Gonzales, Vladslav Nazarenko, Lan Yao, Ming An. Department of Chemistry

Introduction

pH-low-insertion-peptide (pHLIP) can insert into a membrane at low pH to form a stable transmembrane α -helix. This pH-sensitivity can aid in delivery of drugs to cancer tumors based on the physiological pH difference between healthy tissue (pHe of 7.1-7.5) and tumors (pHe of 6.5-7.0). WT-pHLIP has been shown to exist in 3 states with the following sequence¹:

NH₂-GGEQNPIYWARYA**D**WLFTTPLLLL**D**LALLV**D**ADEGT-CO₂H



pHLIP is believed to insert through a series of protonation's which include these four Aspartic Acid residues show in the figure below².

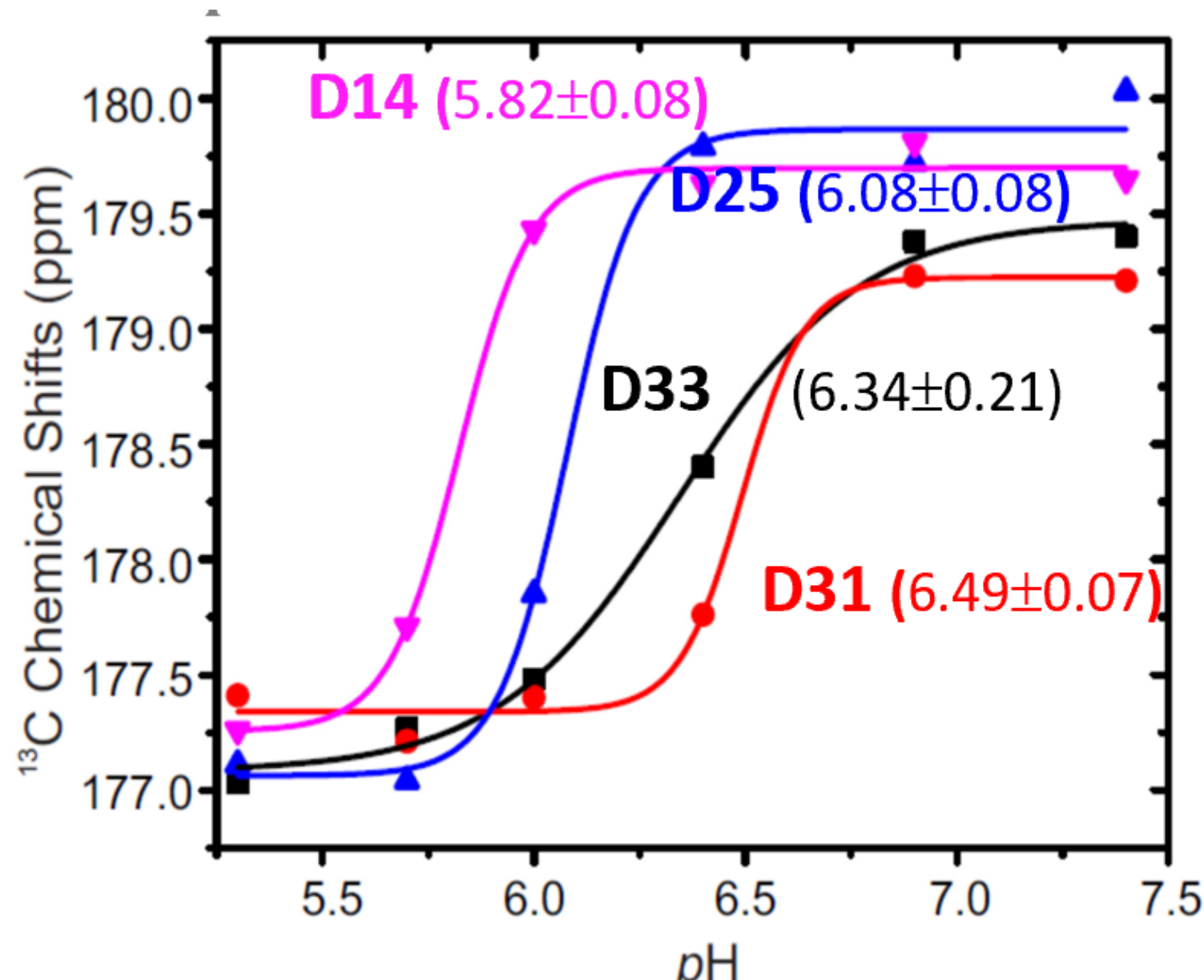


Figure 2. pKa values of pHLIP in POPC

Swapping these 4 groups with Glutamic Acid in a stepwise manner beginning with D31E and ending with D31E/D33E/D25E/D33E, the pH of insertion should continue to increase due to Glutamic Acid having a higher pKa than Aspartic Acid.

Methods

Two types of experiments were carried out. The first was Tryptophan Fluorescence which tracks the two tryptophan residues on pHLIP, located at the 9 and 15 positions. Since tryptophan fluorescence is sensitive to the environment, by monitoring the fluorescence, the states of pHLIP in membrane can be followed. The basic idea of this assay is that an increase of fluorescence intensity and a blue-shift of maximum wavelength should be observed when the peptide is moving into the hydrophobic membrane.

The second type of experiment was Circular Dichroism (CD) spectra analysis which tells structural conformation of the peptide at the 3 different states, confirming pH-dependent conformational change as wild type pHLIP. Characteristics such as the α -helix structure have distinct shapes in CD.

Results

Figure 3A-E. Plot of Trp fluorescence λ_{max} blue-shift for wild type and the four variants of pHLIP

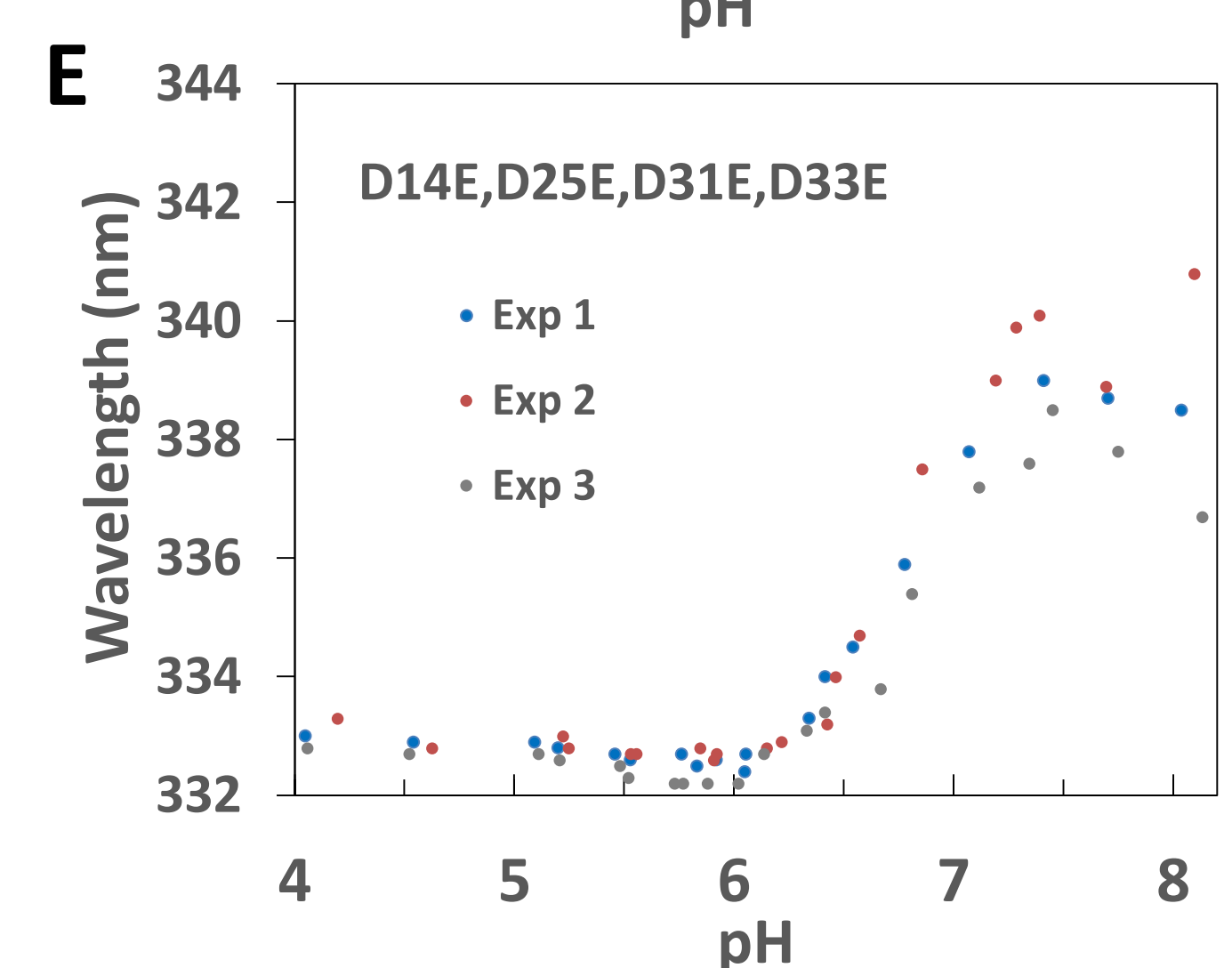
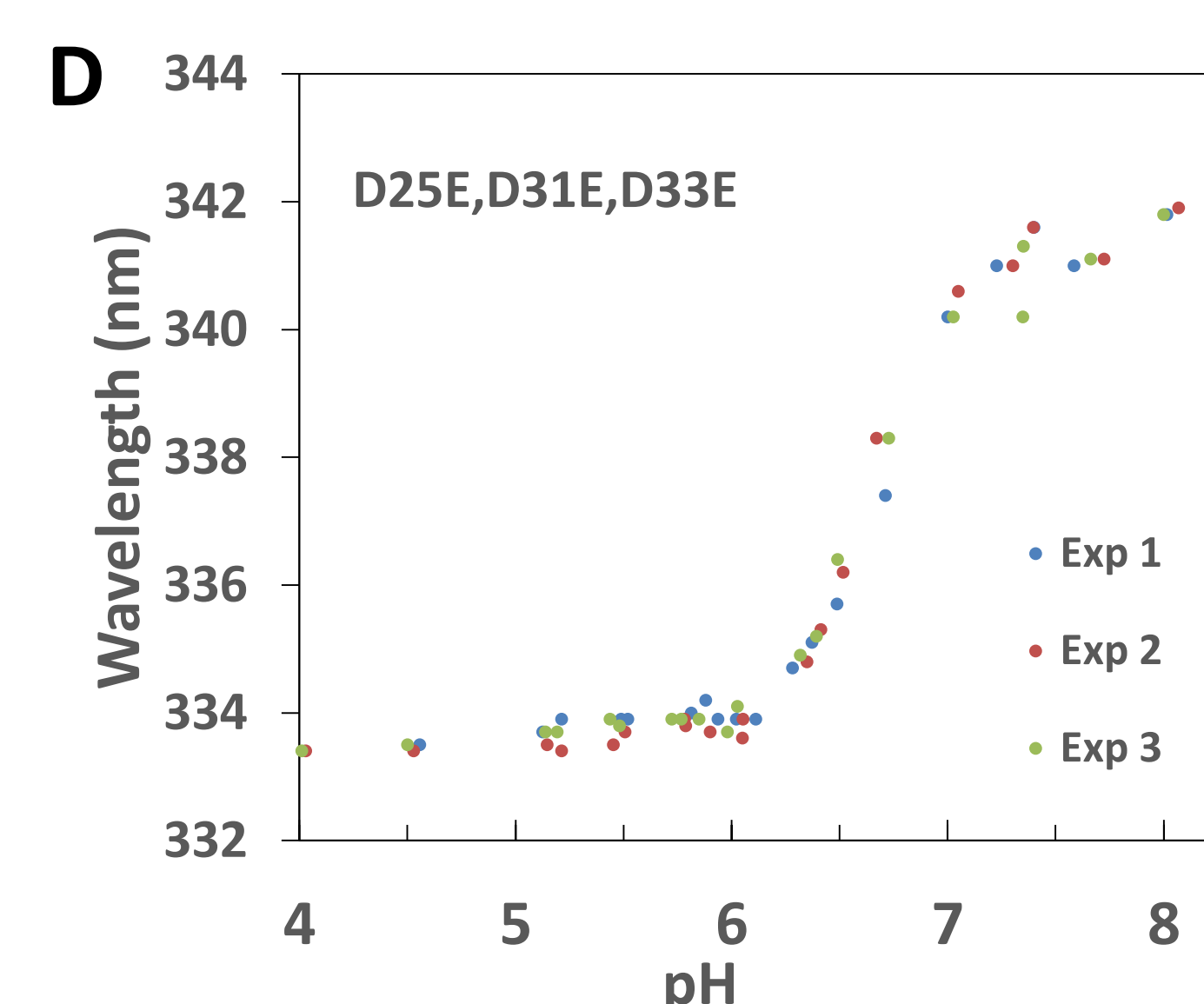
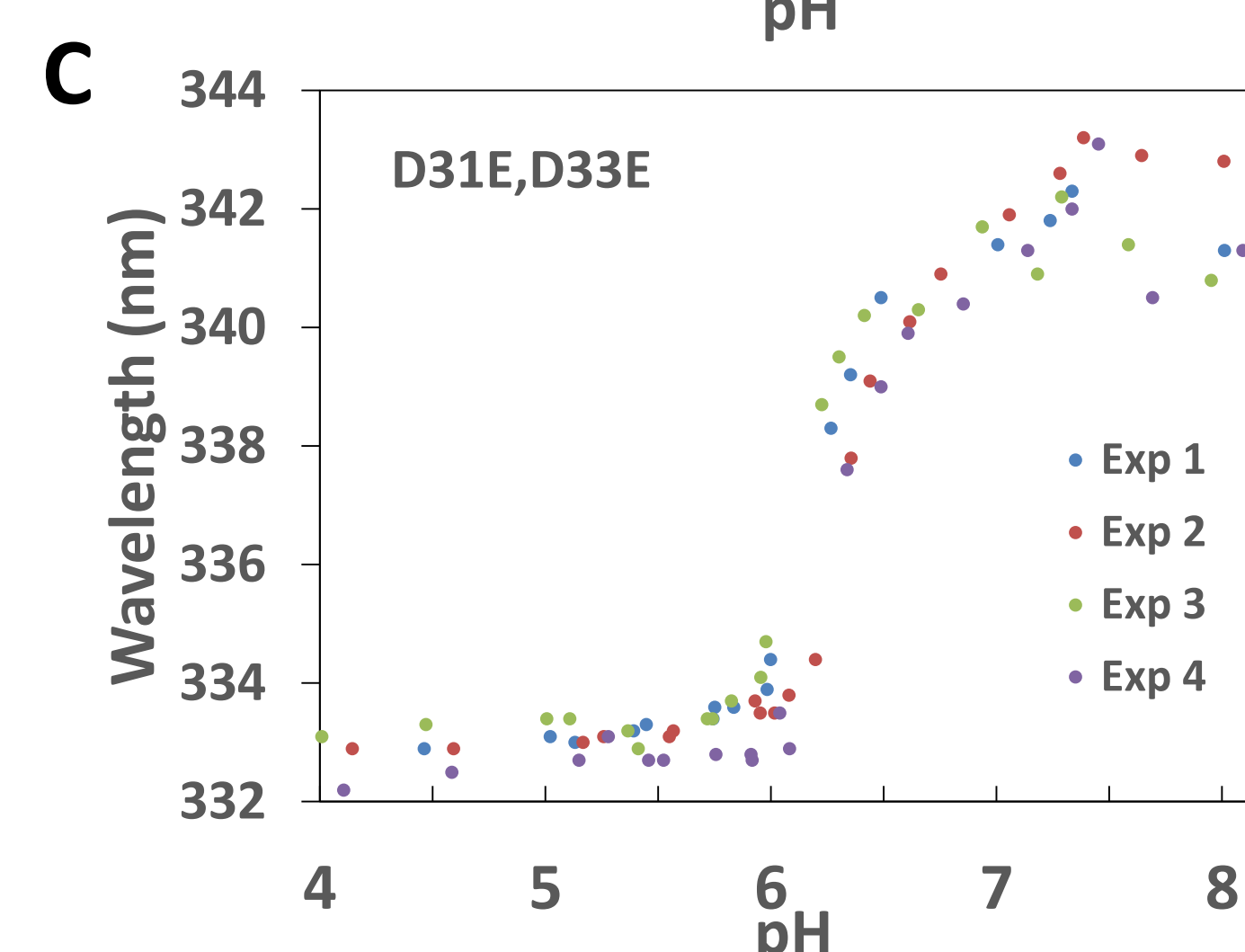
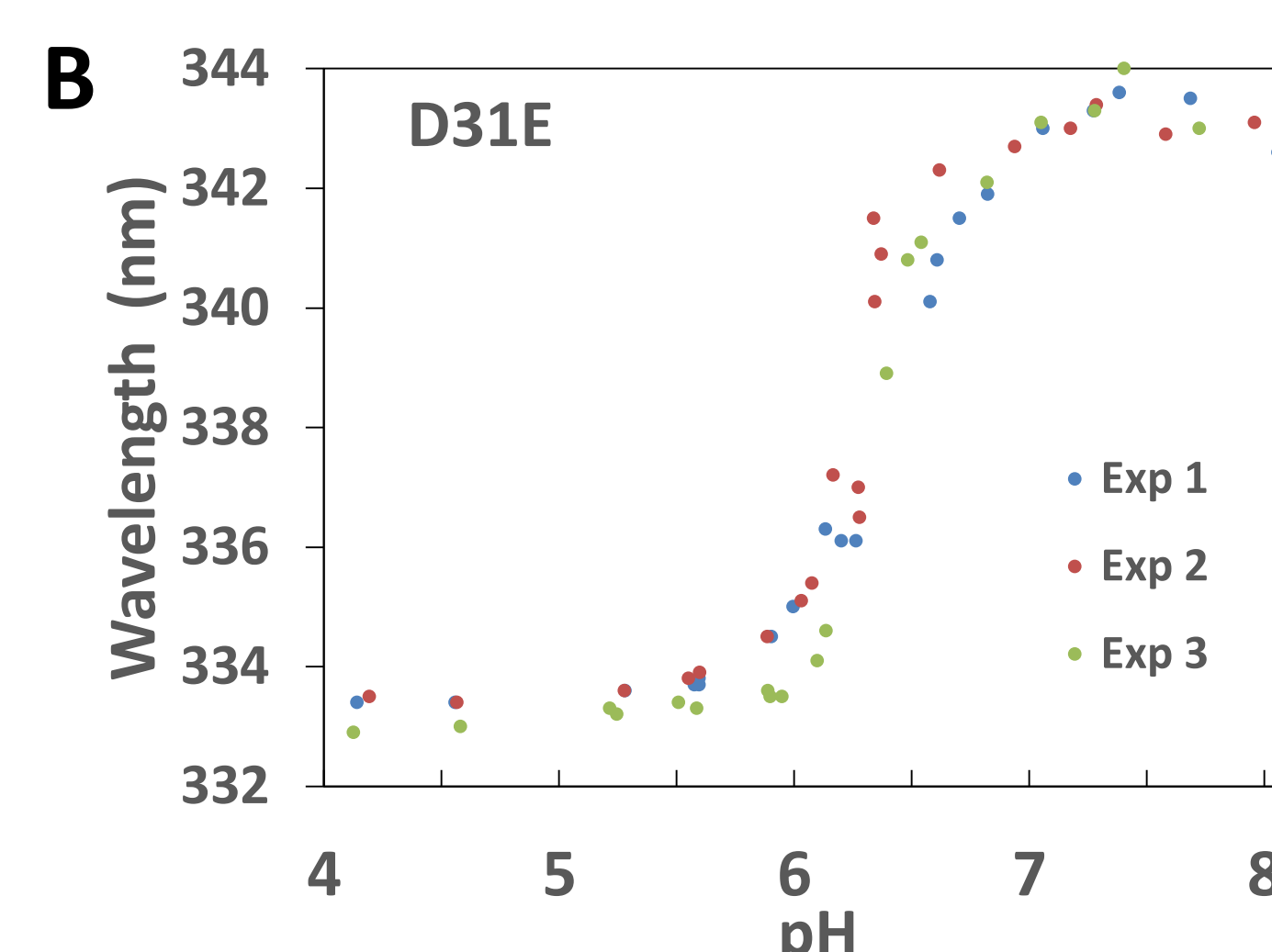
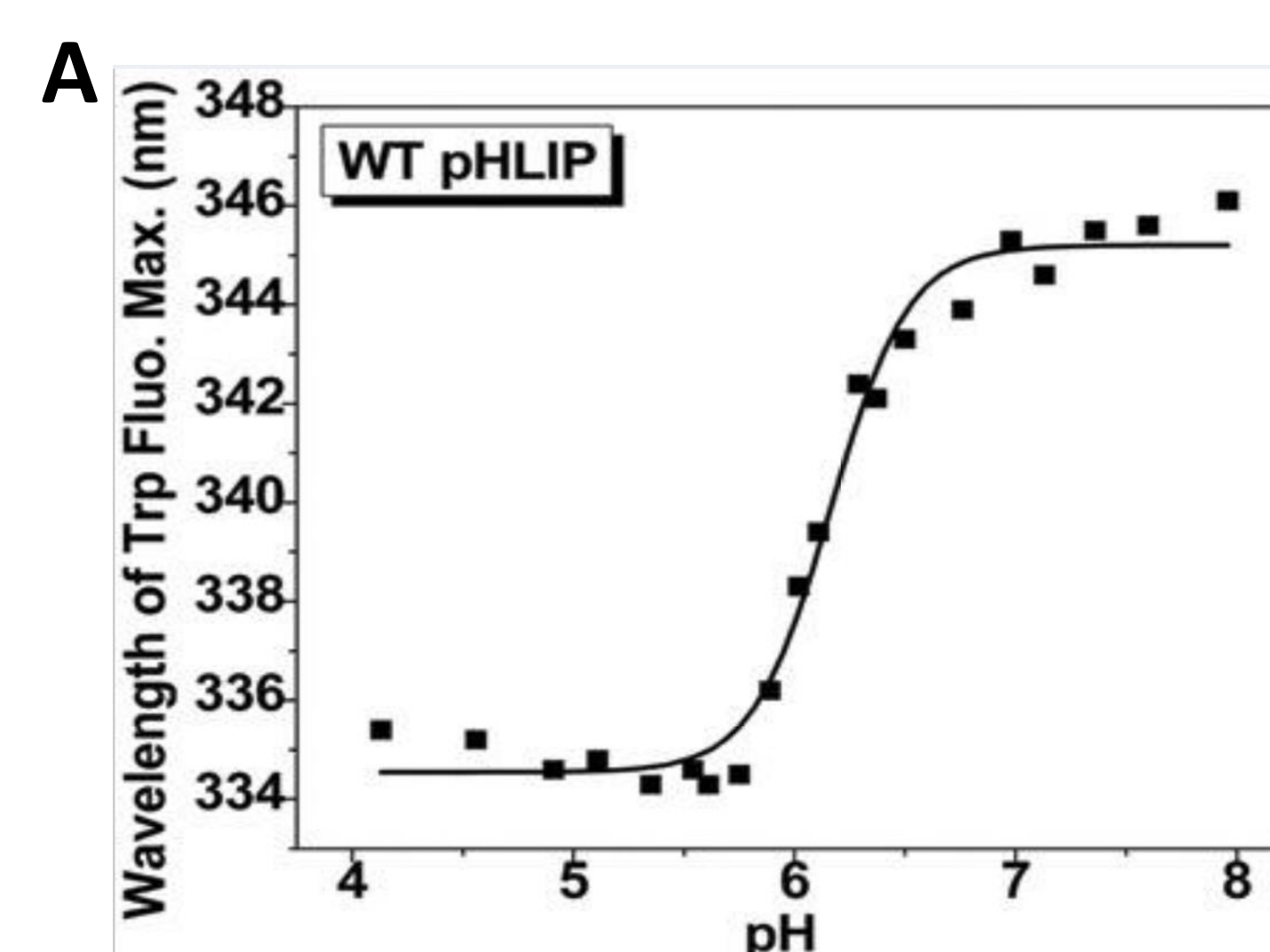
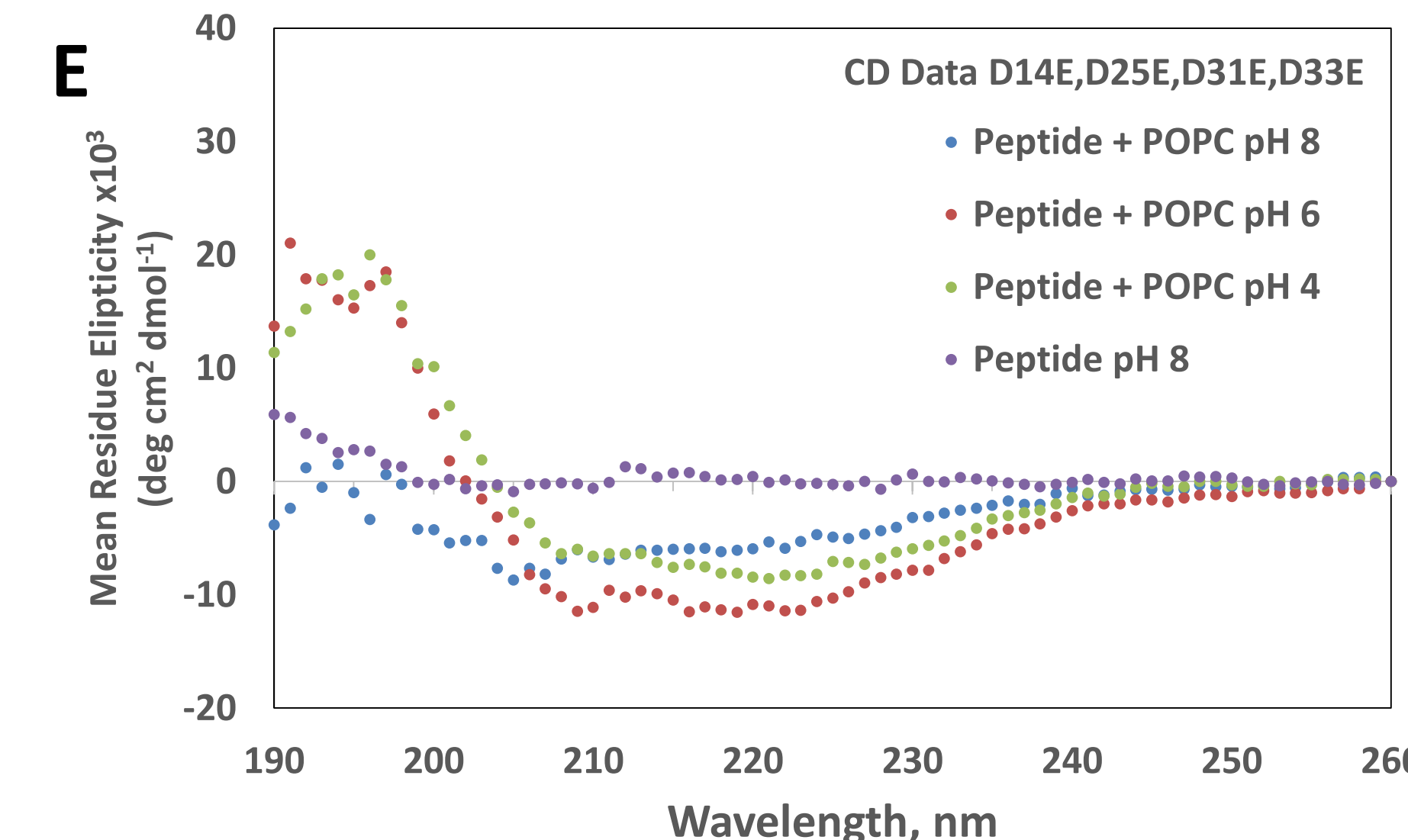
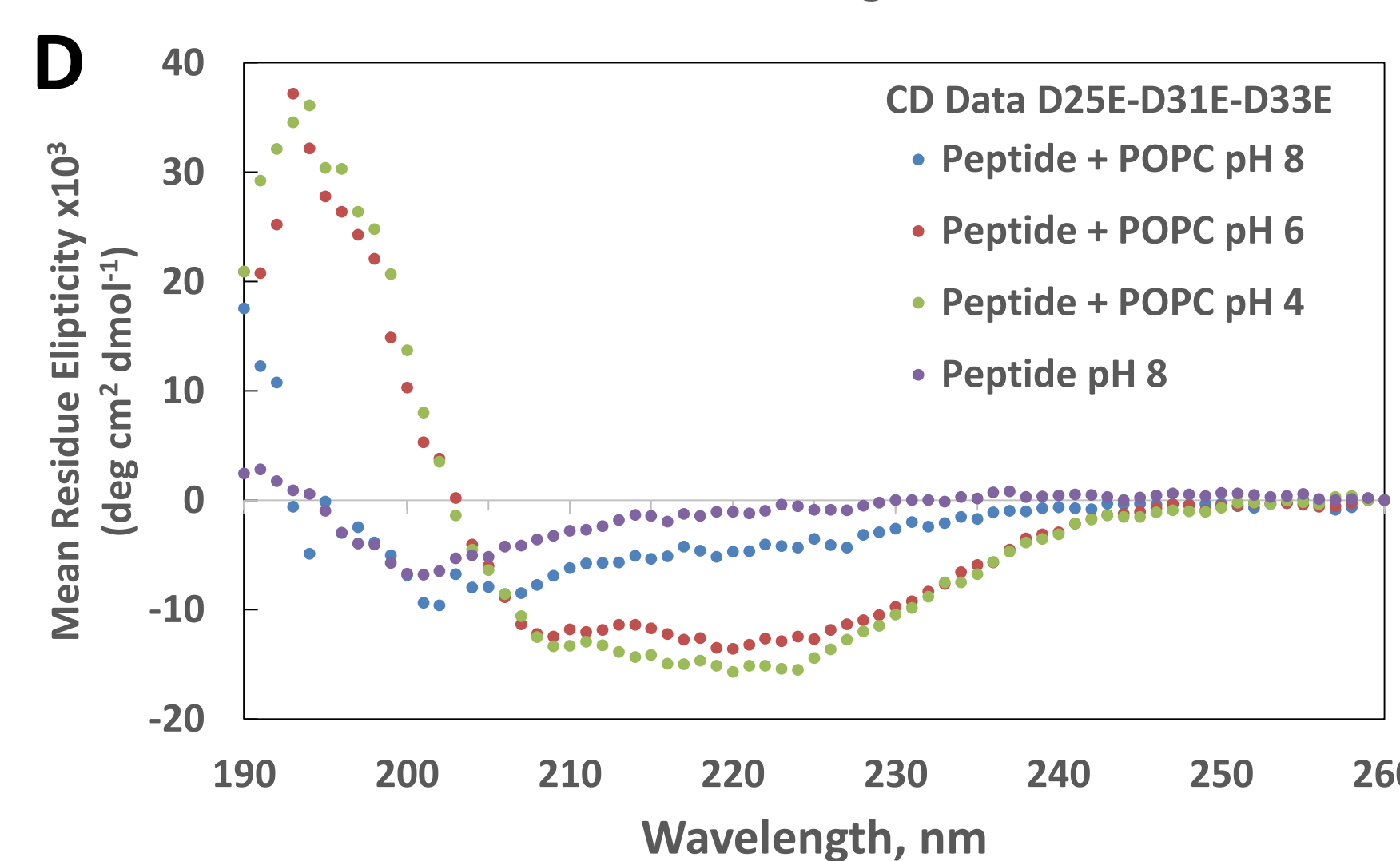
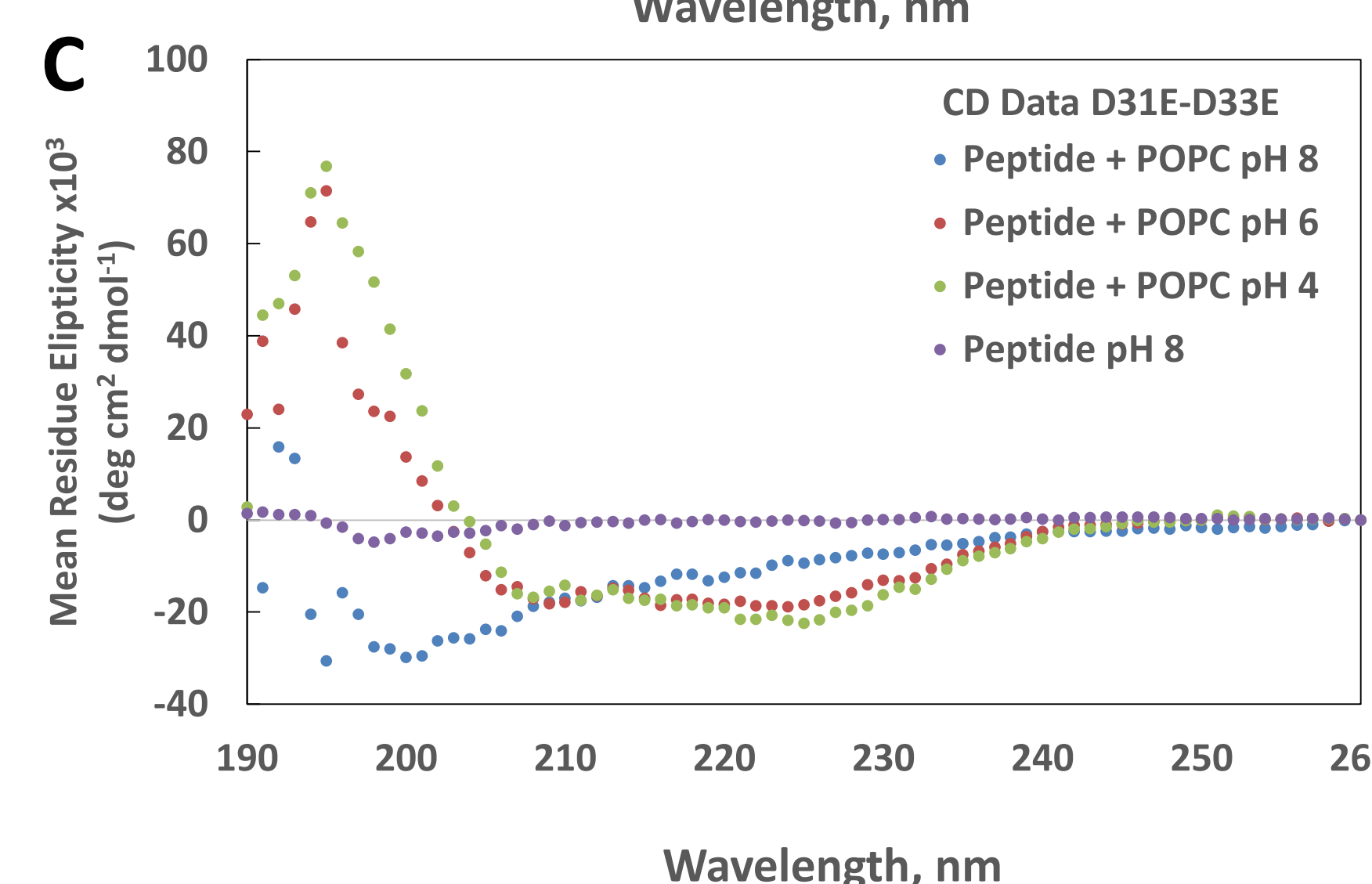
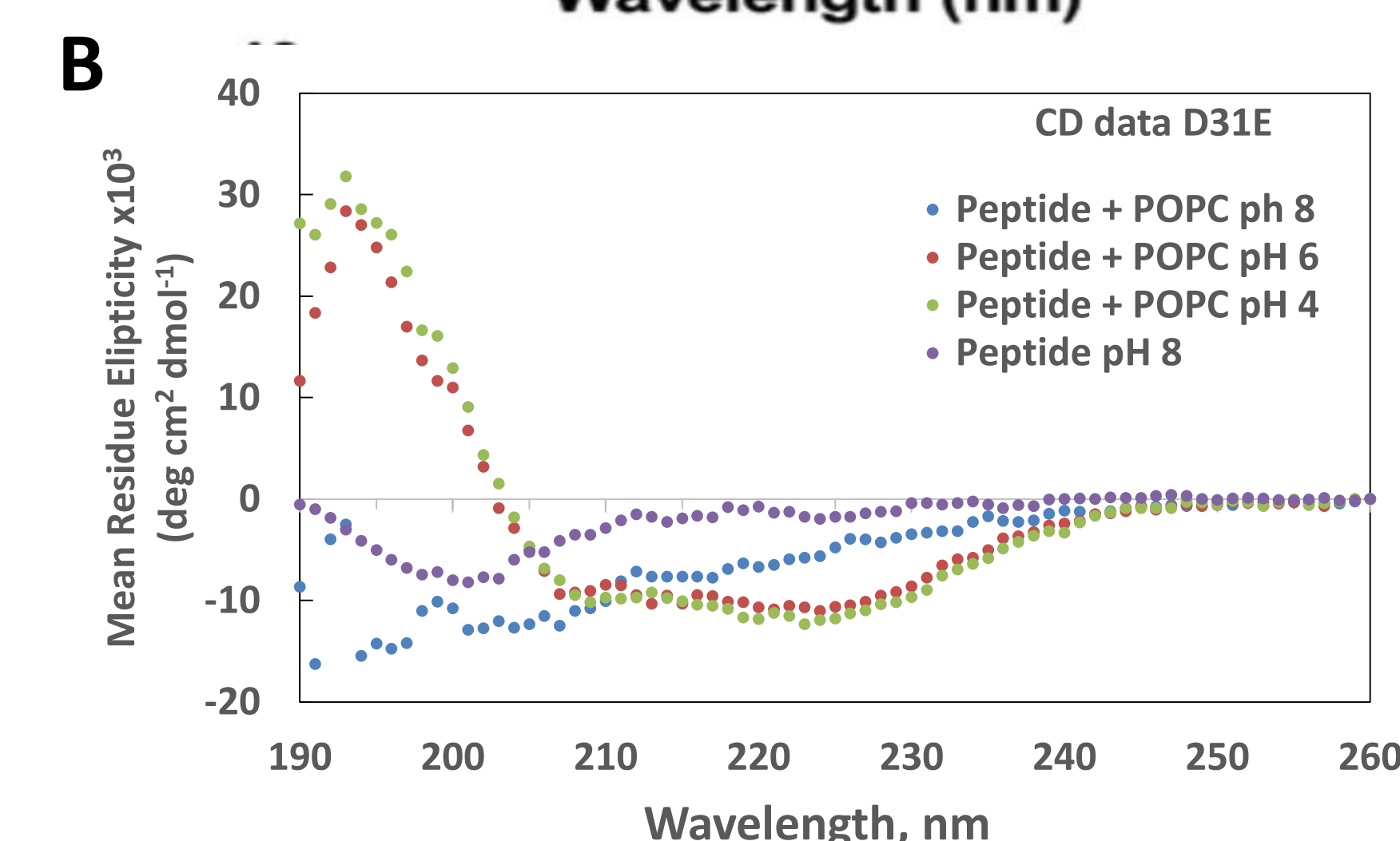
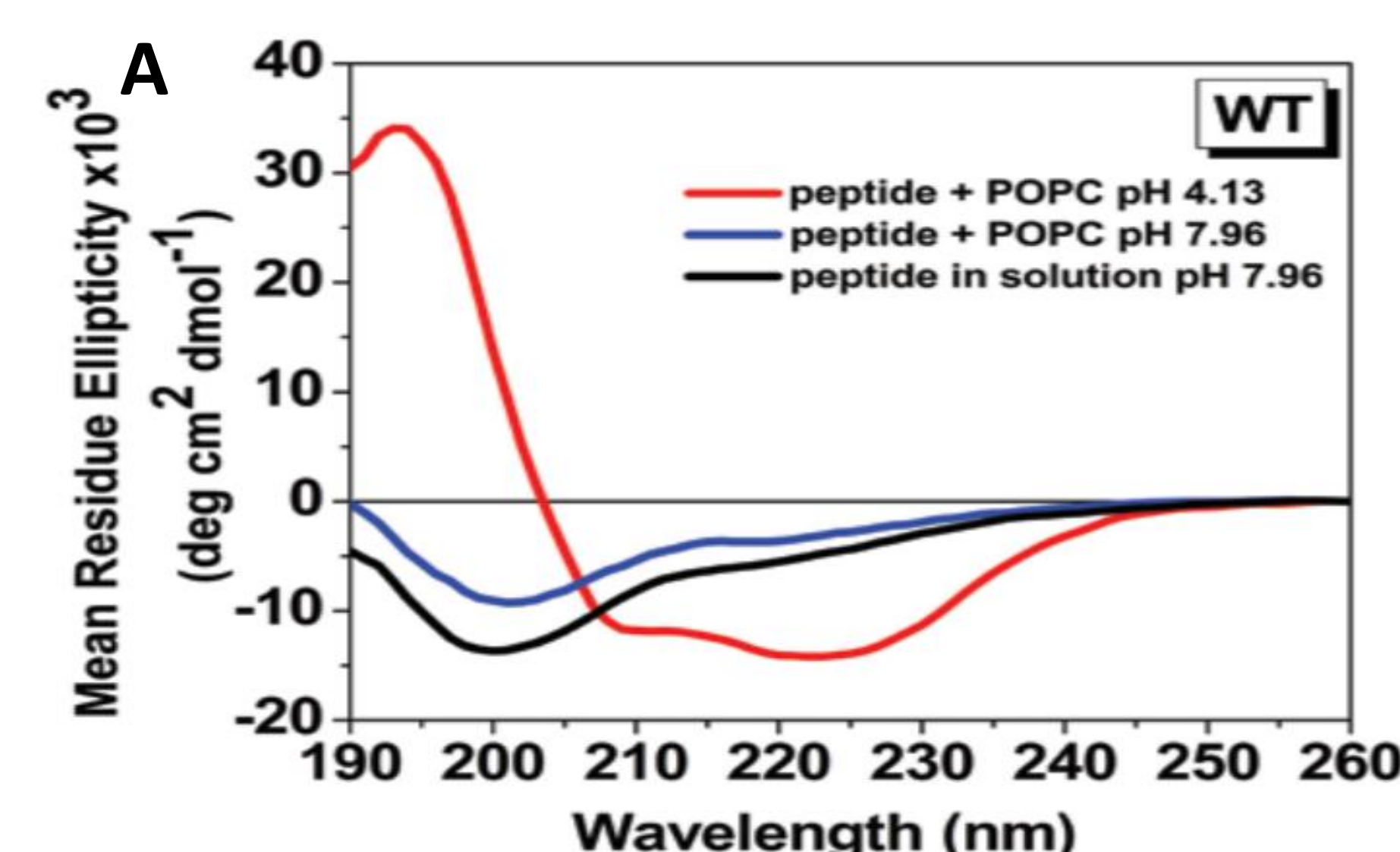


Figure 4A-E. Circular Dichroism plots of wild type and the four variants of pHLIP



Conclusions

- The results showed that the 4 variant, D31E, D33E, D25E, D14E completed insertion across the membrane at the highest pH. The value was approx. 6.4; while wild type pHLIP completed insertion at pH 5.8.
- D31E, D33E, D25E was the next best with insertion completed at approx. pH 6.3.
- D31E, D33E and D33E completed insertion at pH values of 6.1.
- Each of the four pHLIP variants existed in three states confirming they behaved like wild type pHLIP.
- Future studies will include testing the D31E, D33E, D25E, D14E variant in cells to measure effectiveness and selectivity in delivering cargo in a pH dependent manner.

Acknowledgements

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