

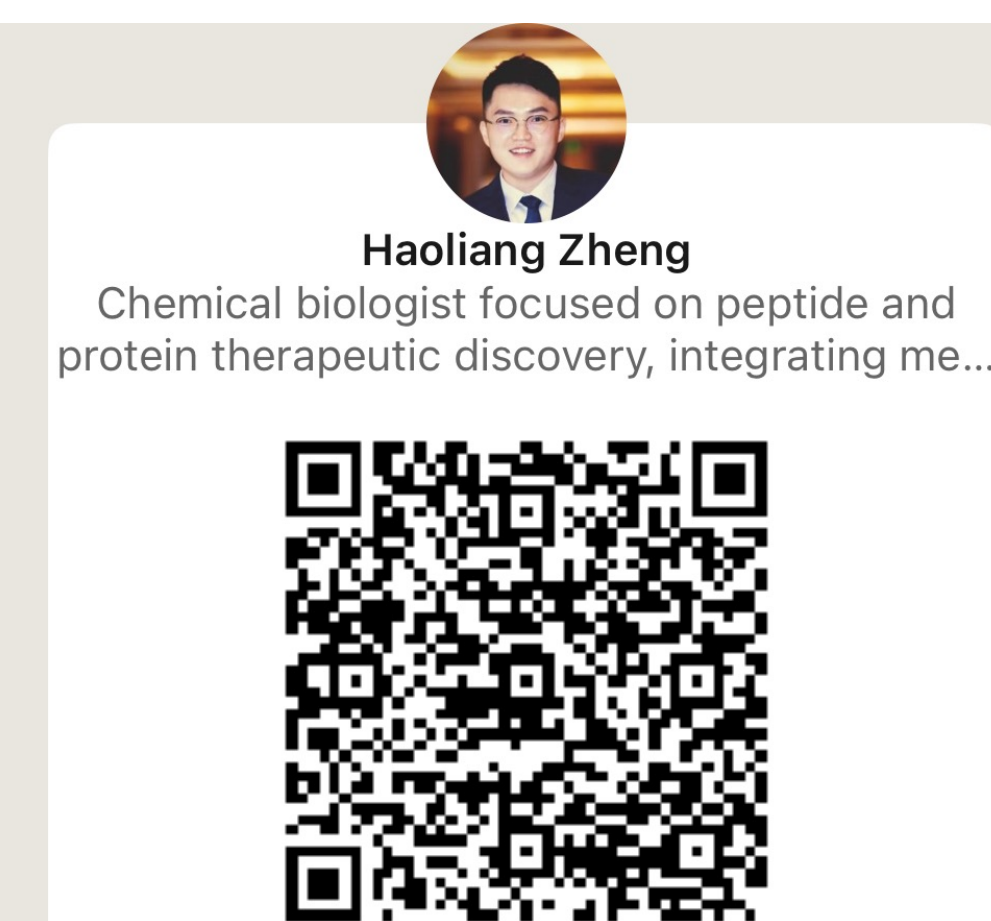


Engineering Protein Backbones to Control Structure and Aggregation

Haoliang Zheng and Prof. Robert W. Newberry

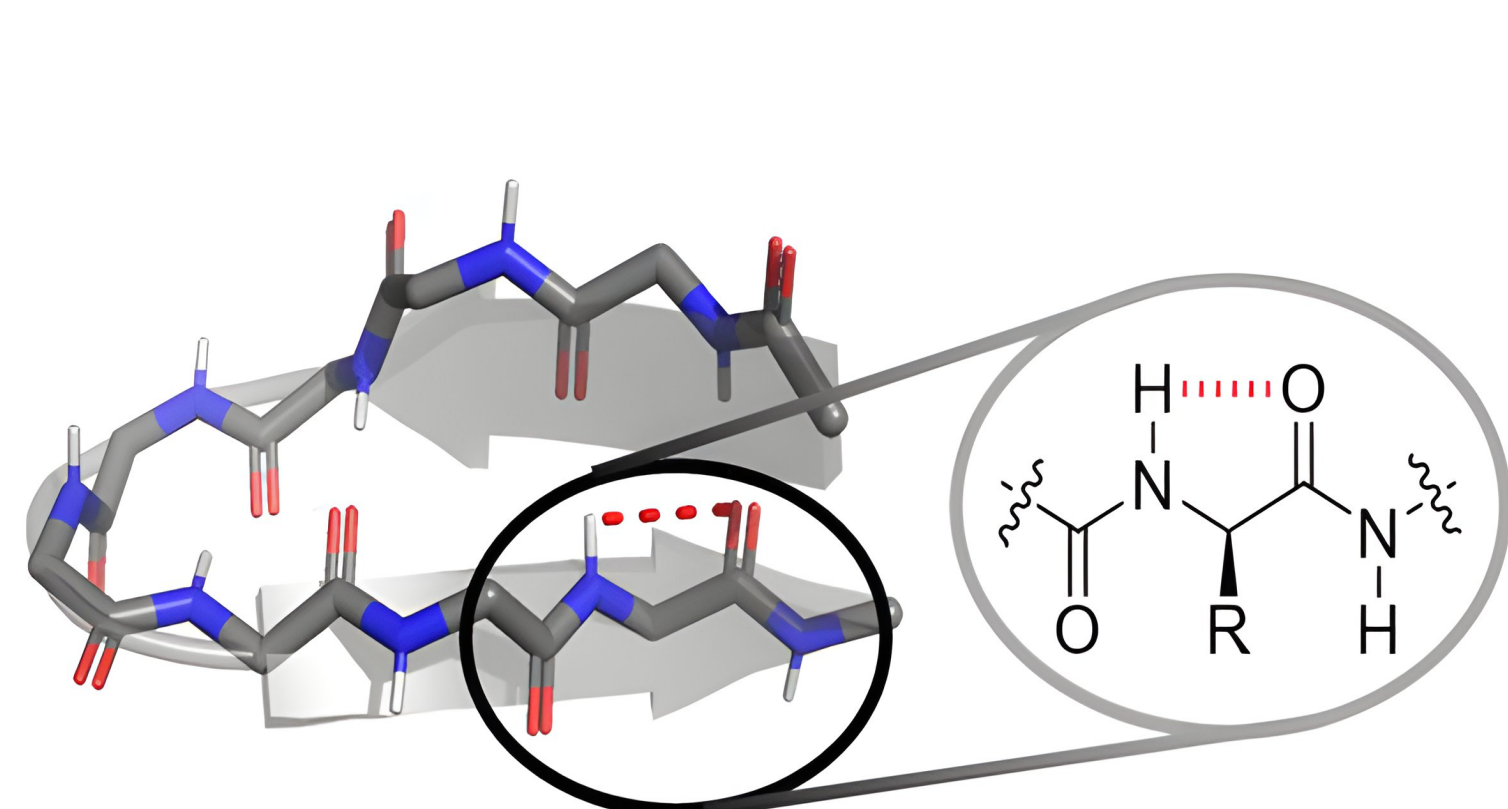
Department of Chemistry, The University of Texas at Austin

Email: haoliangzheng15@gmail.com

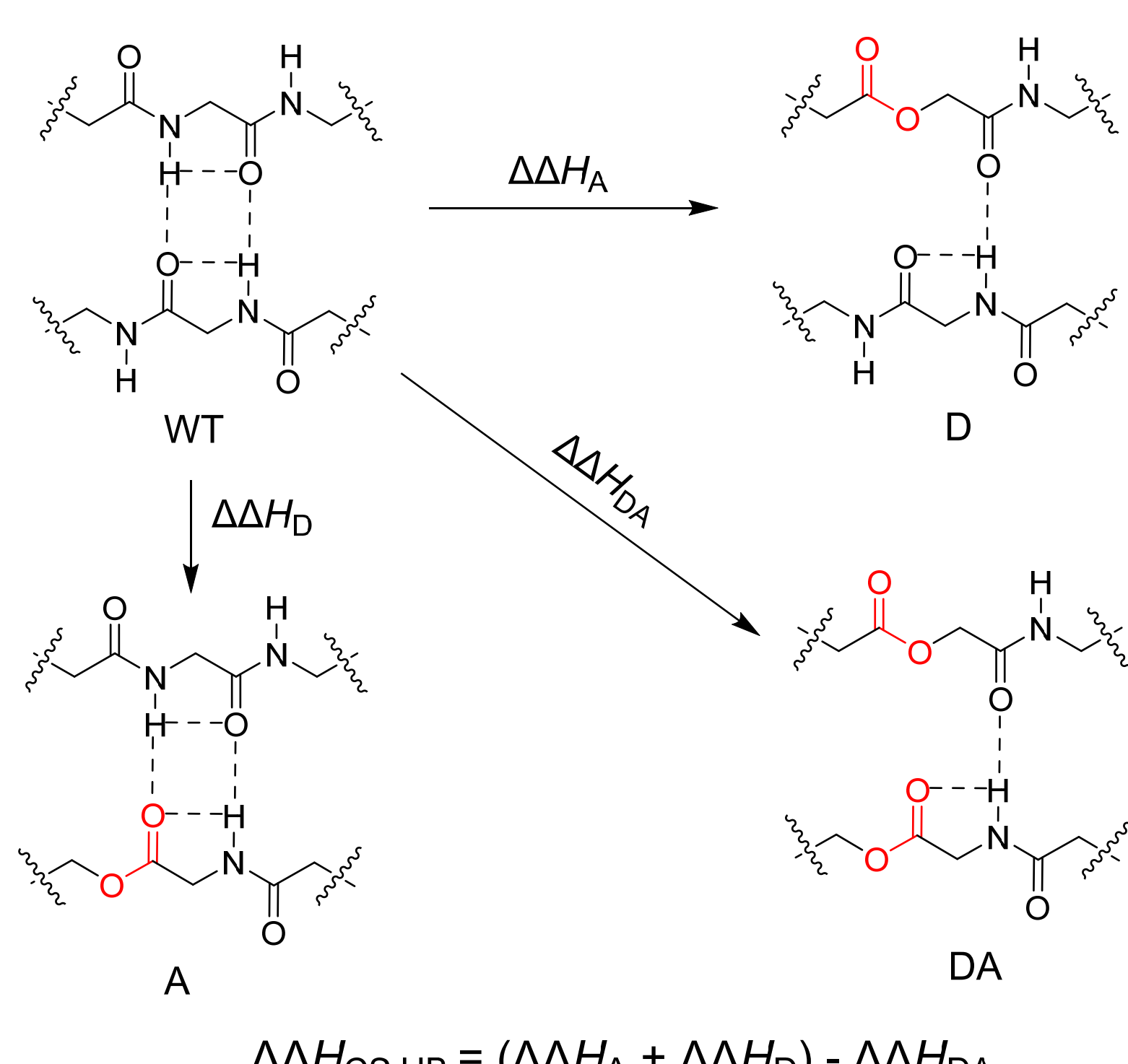


Quantifying and controlling C5 hydrogen bonds to guide protein structure and folding

C5 hydrogen bonds (~5% of residues in folded proteins) contribute to β -sheet stability and amyloid aggregation, but their intrinsic energetic contribution remains unresolved.

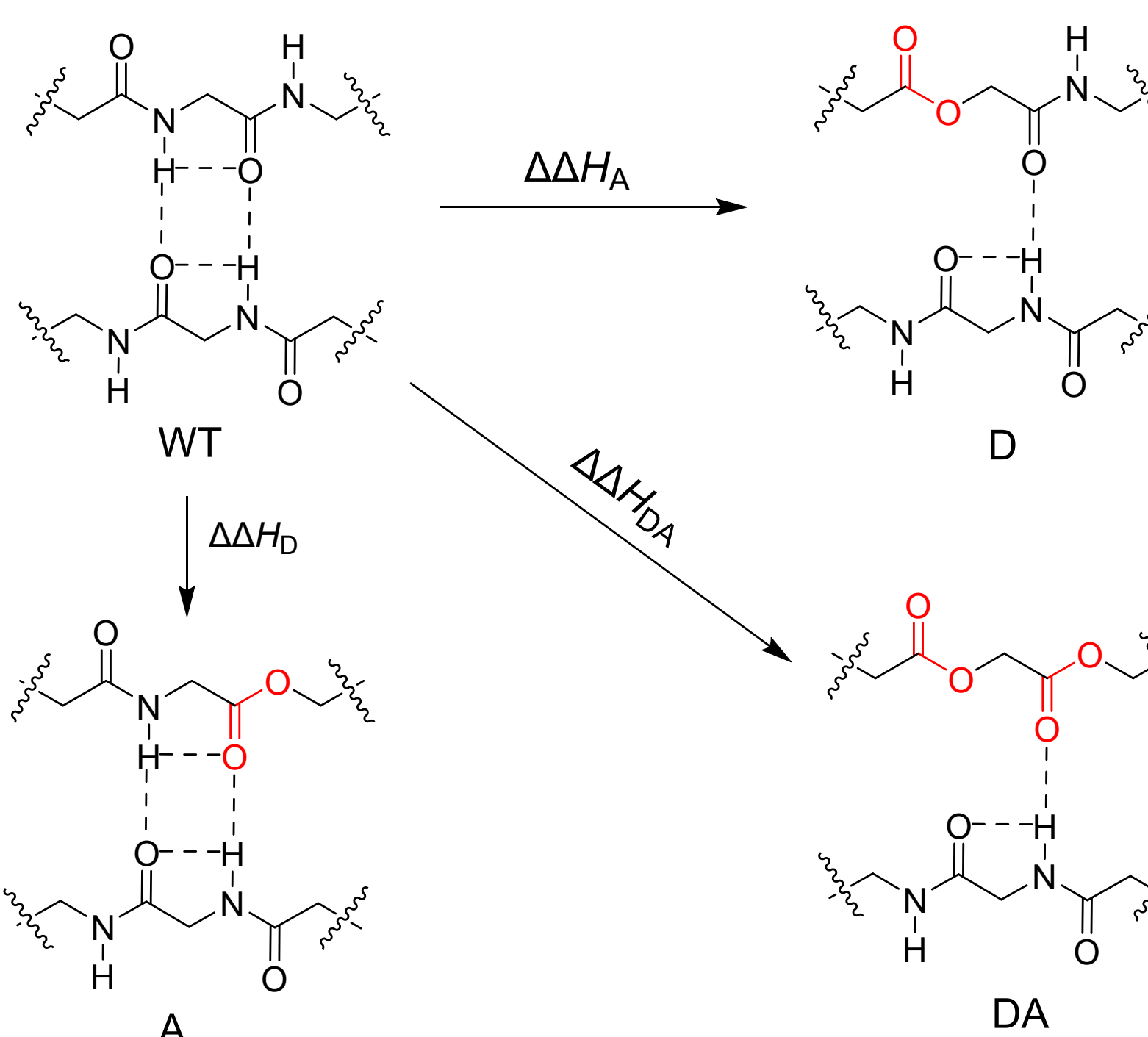


Cross-Strand hydrogen bonds

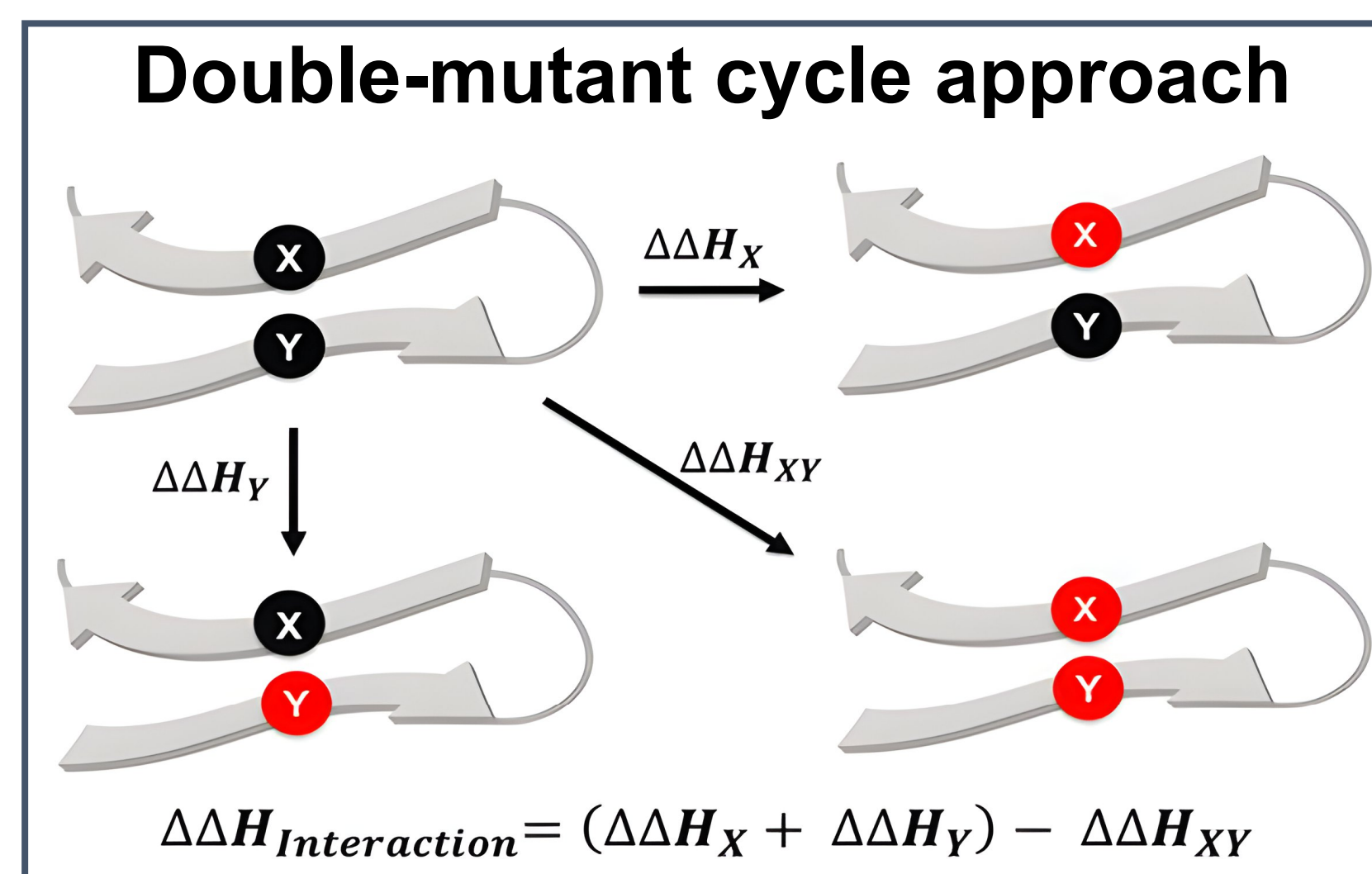


$$\Delta\Delta H_{CS\text{ HB}} = (\Delta\Delta H_A + \Delta\Delta H_D) - \Delta\Delta H_{DA}$$

C5 hydrogen bonds



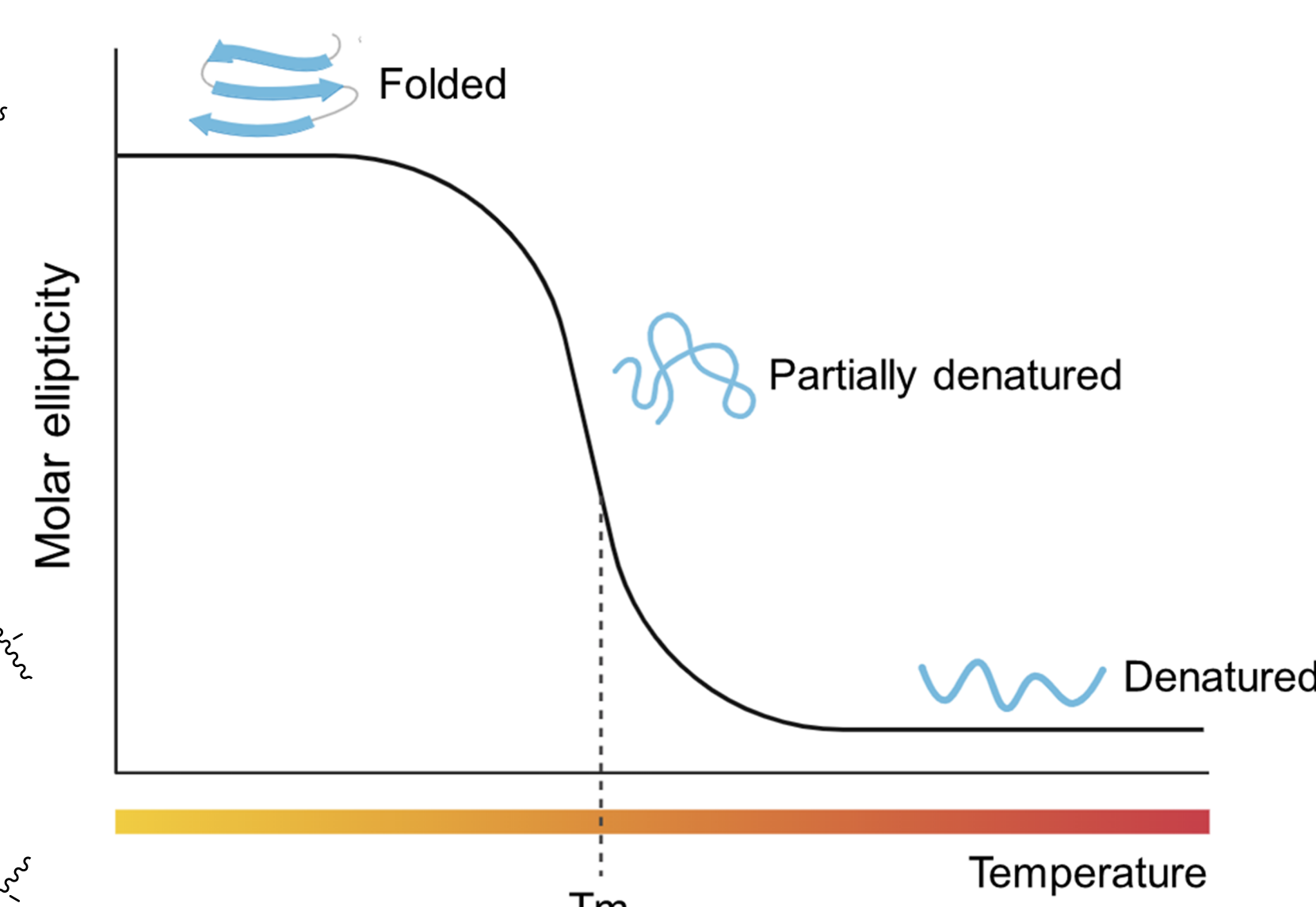
$$\Delta\Delta H_{C5\text{ HB}} = (\Delta\Delta H_A + \Delta\Delta H_D) - \Delta\Delta H_{DA}$$



TrpZip peptide:
1.32 $\pm$ 0.06 kcal/mol
0.89 $\pm$ 0.03 kcal/mol

Pin1 WW peptide:
2.79 $\pm$ 0.09 kcal/mol

✓ Consistent with the cross-strand hydrogen bond energy references.



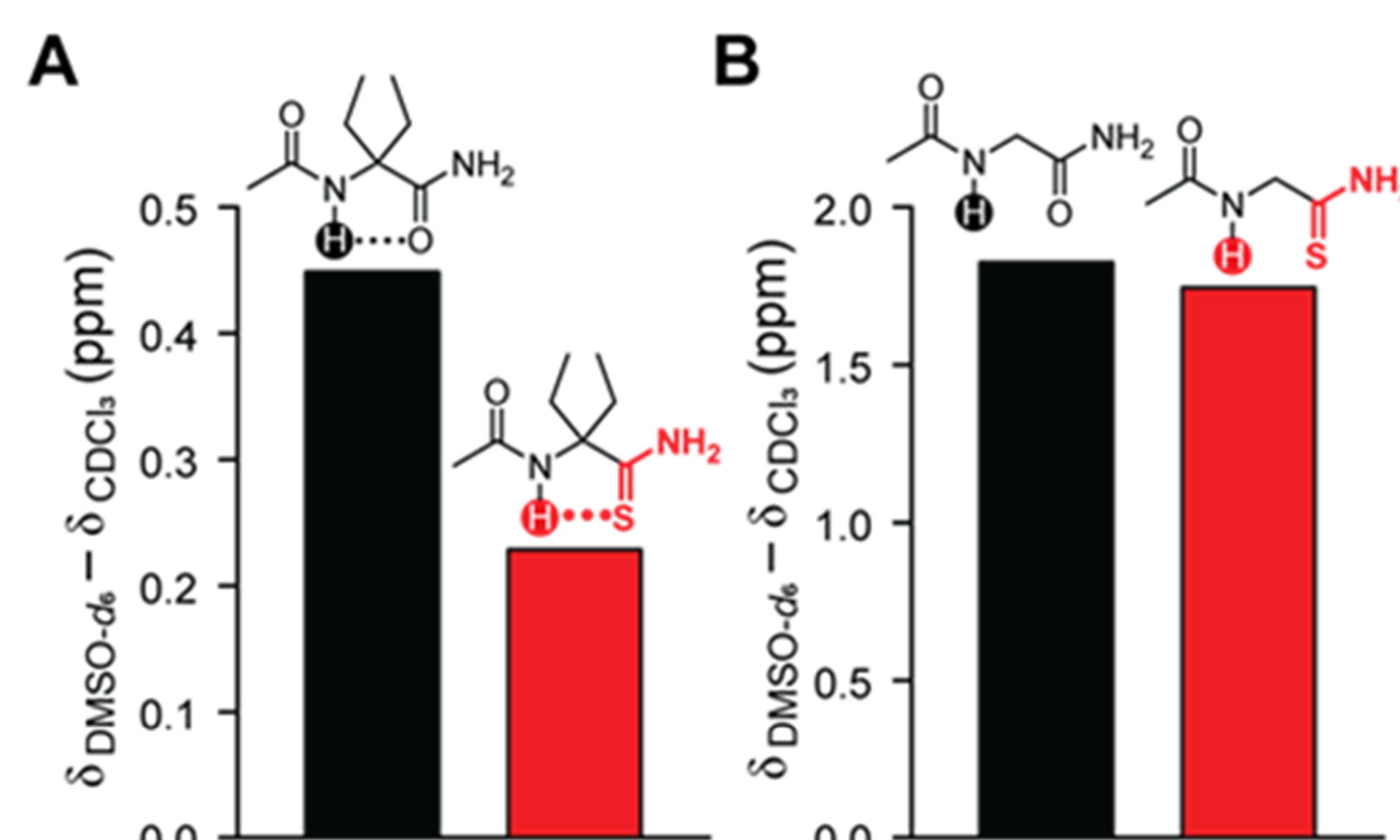
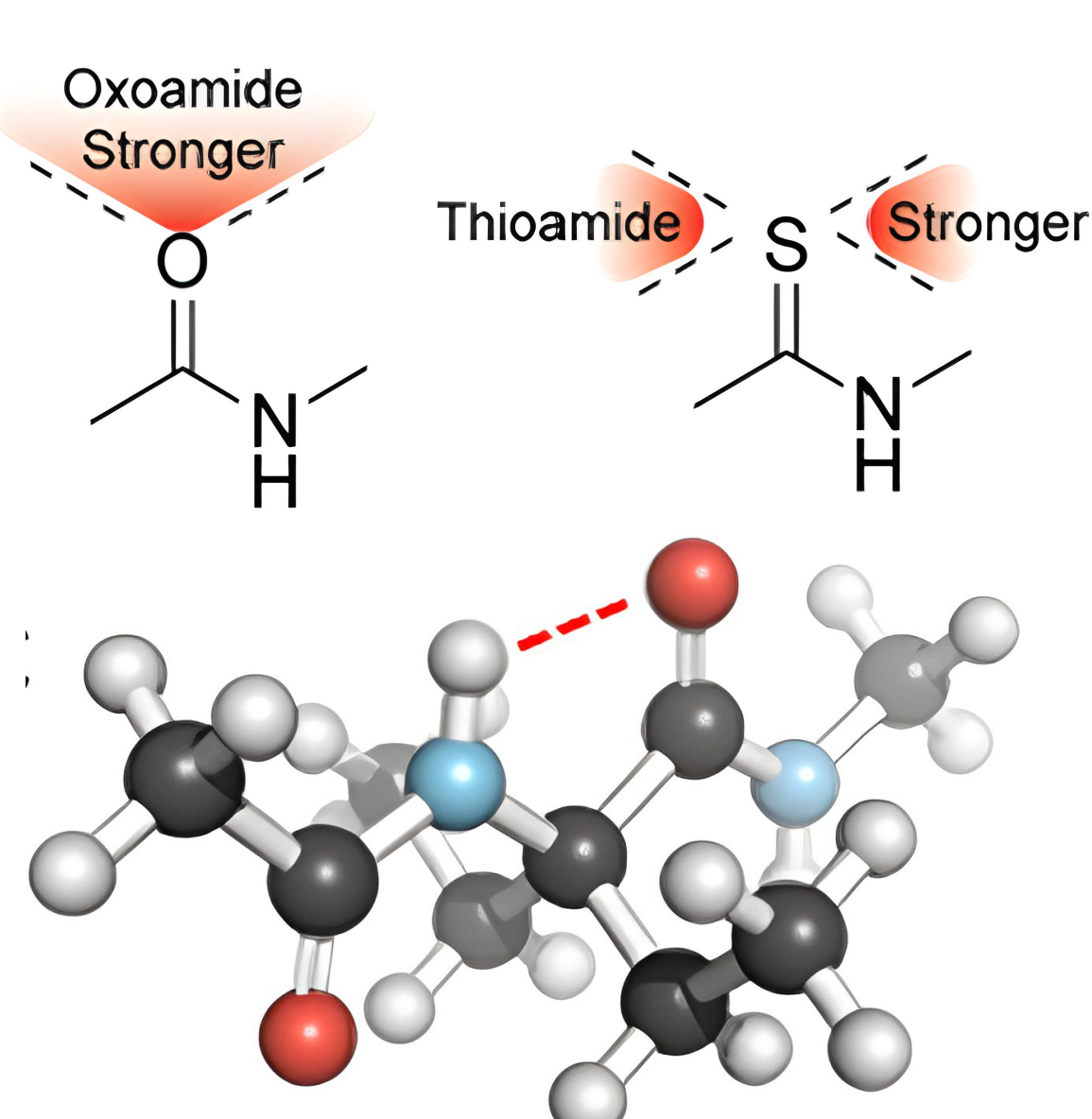
Pin1 WW peptide:
0.18 $\pm$ 0.07 kcal/mol

✓ Consistent with computational predictions. (0.25 kcal/mol)

✓ Double-mutant cycle approach can be applied to explore different interactions on the protein backbone.

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Thioamides are typically used as strong hydrogen bond donors and weak acceptors in proteins, but computational predictions and our analysis suggest they can be strong C5 hydrogen bond acceptors due to their electron density distribution.



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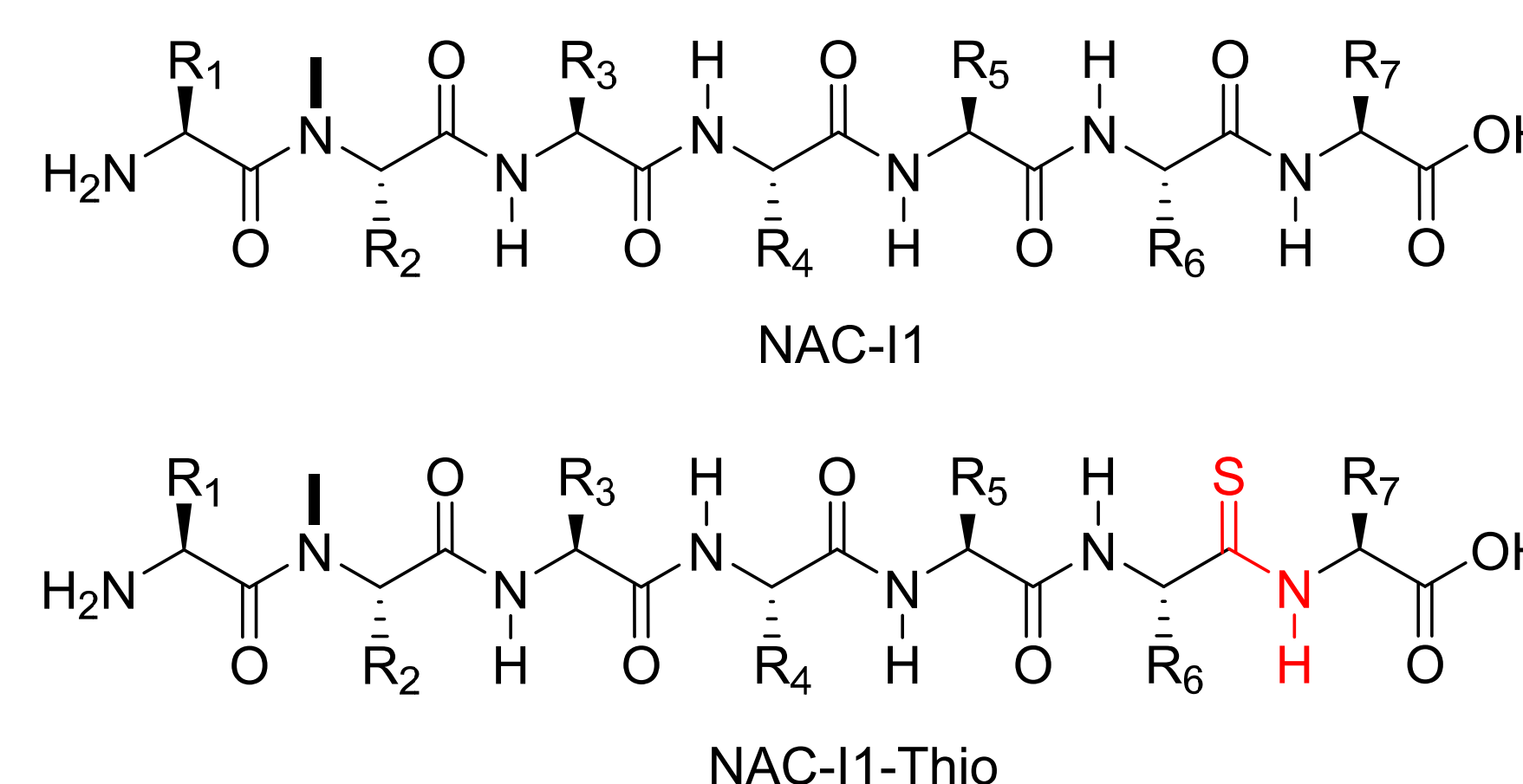
Future Directions:

1. Double-mutant cycle approach can be applied to probe additional backbone noncovalent interactions, such as C–H $\cdots$ O hydrogen bonds and chalcogen bonds.
2. Thioamide substitution and α -alkylation hold significant potential for modulating protein–protein interactions and for the design of peptide/protein therapeutics.

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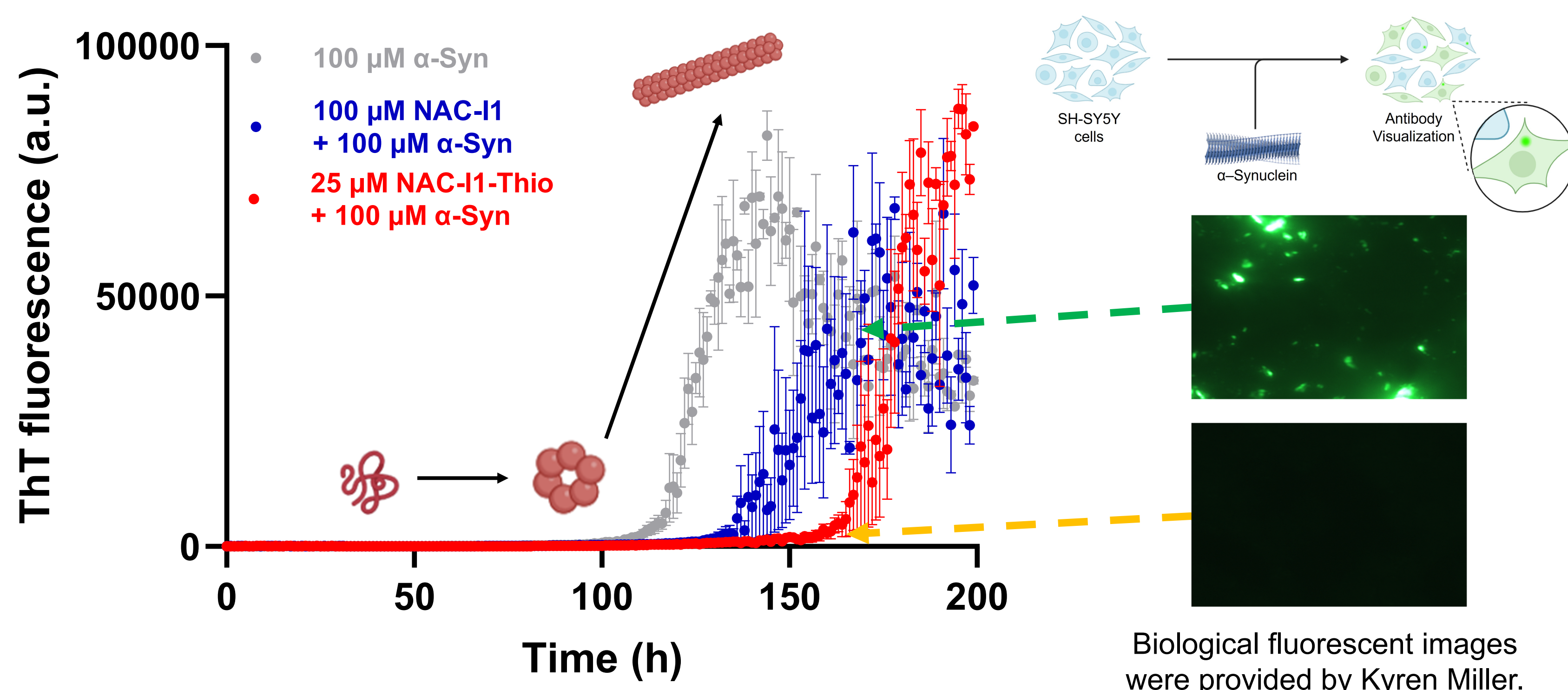
Modulating protein aggregation through backbone modification

α -Synuclein is an intrinsically disordered neuronal protein implicated in Parkinson's disease via amyloid aggregation. We designed a NAC-derived peptide inhibitor by combining **thioamides** with **N-methylation** to more effectively suppress aggregation.



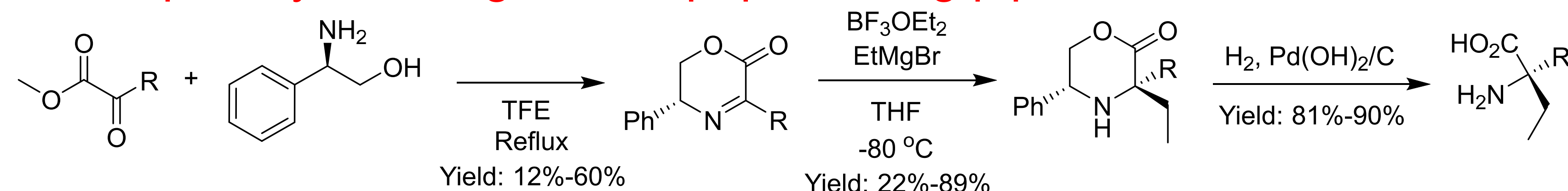
- Thioamides enhance binding to α -synuclein aggregates through stronger hydrogen-bond donation.
- Weak hydrogen-bond acceptance, together with N-methylation, synergistically inhibits subsequent aggregation.

Significantly, the thioamide inhibitor slows down aggregation at a much lower concentration than the target protein. Most capping agents require concentrations 10–100 times higher than that of the target protein to achieve a similar effect.

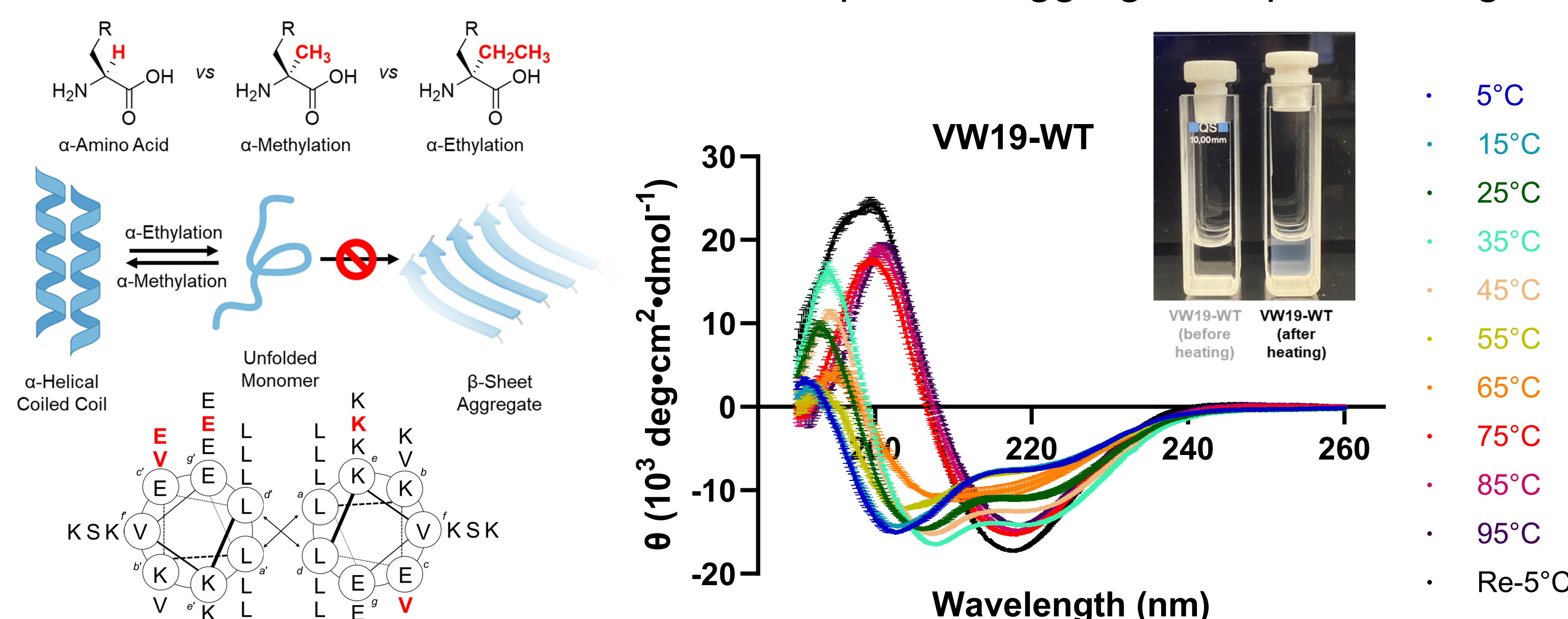


Biological fluorescent images were provided by Kyren Miller.

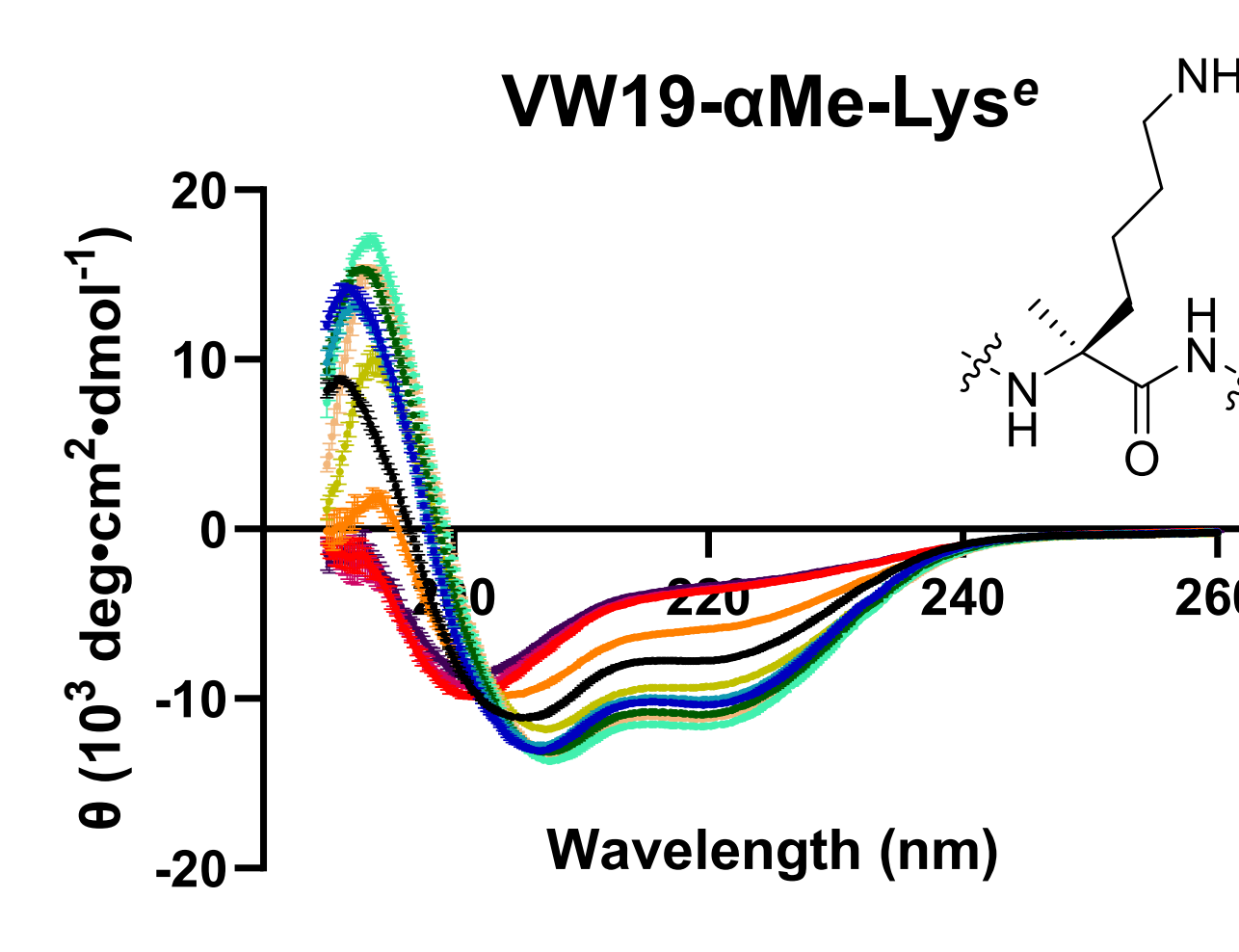
α -Methylation and α -Ethylation were used to tune backbone dihedral angles and alter secondary-structure equilibria, thereby potentially modulating aggregation of amyloidogenic peptides. **α -Ethylated amino acids can help mitigate peptide aggregation, addressing a key developability challenge in the peptide drug pipeline.**



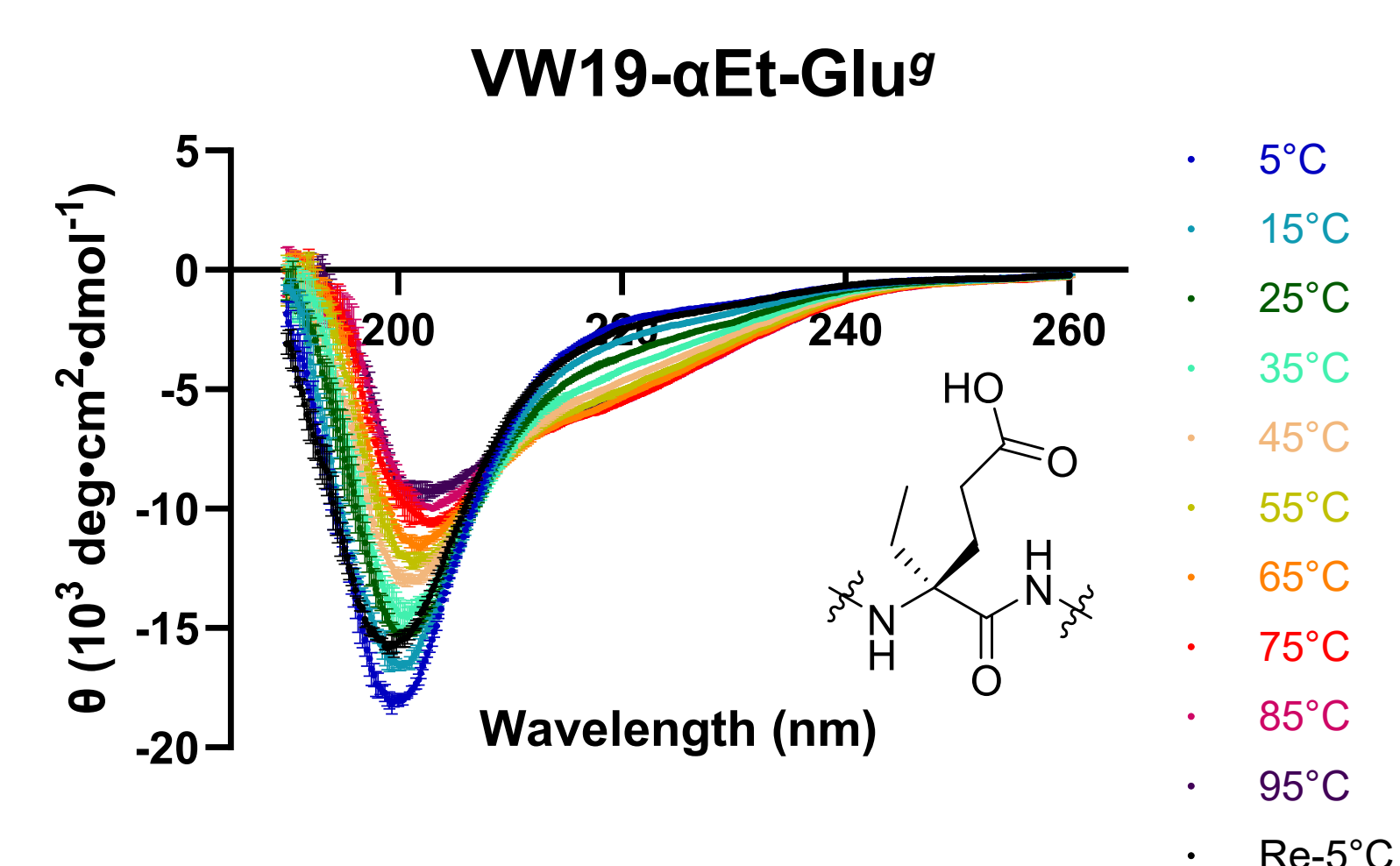
VW19, an amyloidogenic peptide, undergoes temperature-dependent transitions from a random coil to an α -helix and forms β -sheet aggregates upon heating.



Single-site incorporation of an α -methylated amino acid preorganizes backbone dihedral angles, promoting a stable α -helical conformation.



Single-site incorporation of an α -ethylated amino acid can completely disrupt protein secondary structure and aggregation.



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