



# Reconstructing the Parallel Folding Pathways of Intercalating Motifs by Thermo Hysteresis

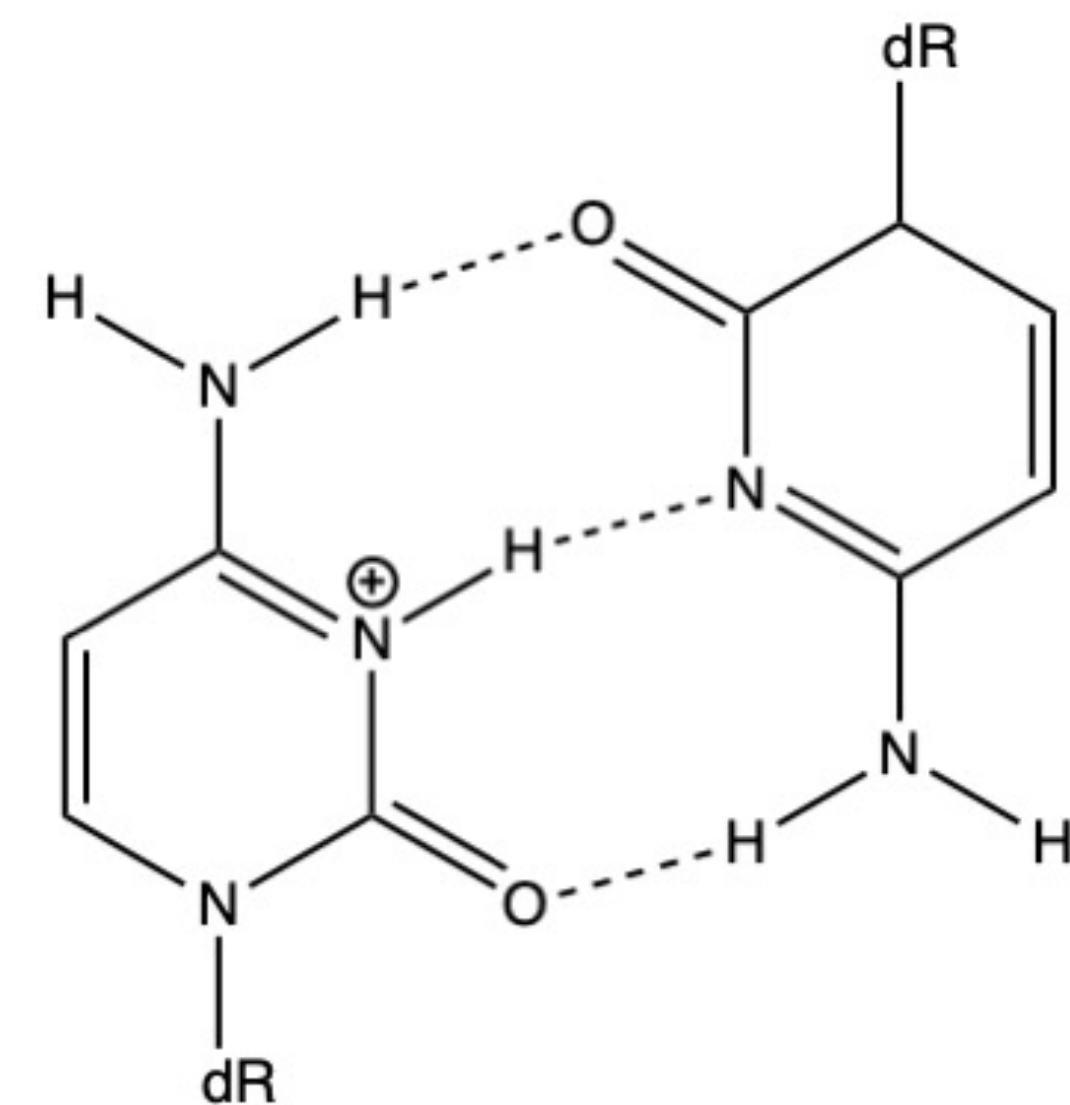
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## Introduction

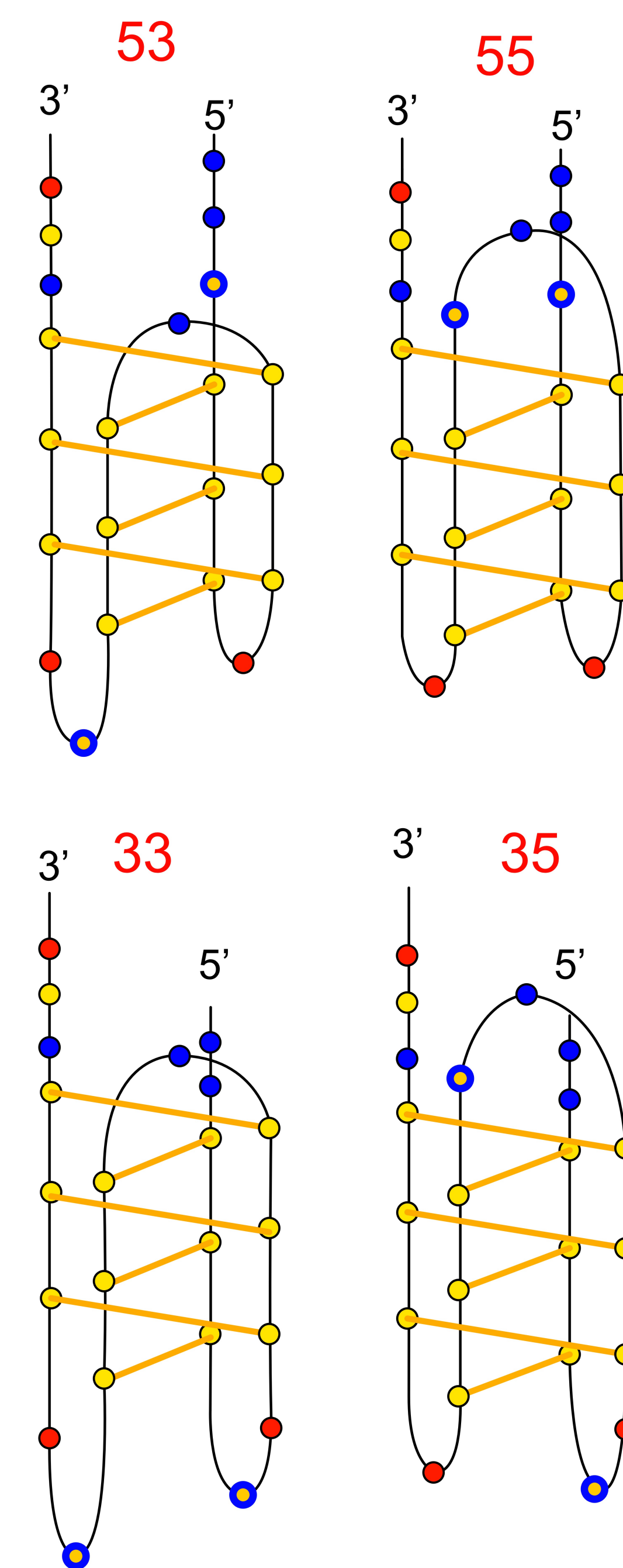
Intercalating motifs (i-motifs) are four-stranded, cytosine-rich nucleic acid structures that regulate many biological processes such as transcription. The i-motifs are structurally heterogeneous, which exist as interconvertible conformers. The coexistence of multiple folded conformations suggests that there may be multiple folding pathways. We aim to use a method that developed by our lab called thermal hysteresis that quantitatively measures the rates of individual pathways for the i-motif and applied it to the i-motif found in the human c-myc promoter region.

## The c-myc intercalating motif

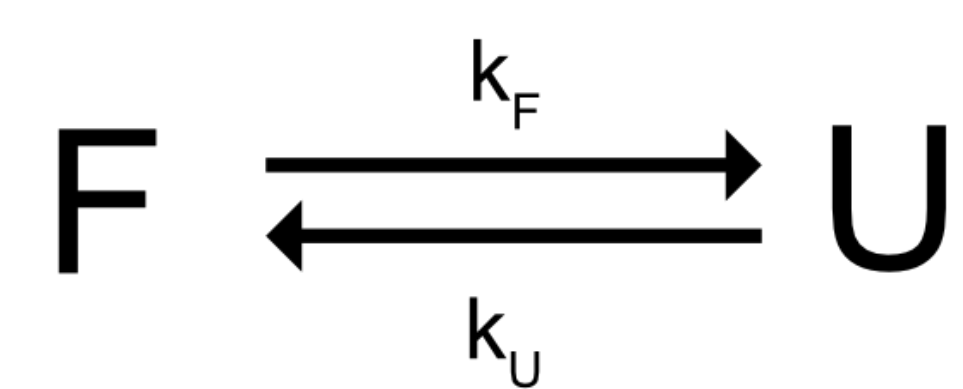
- The c-myc oncogene is one of the most upregulated oncogenes in cancer, the i-motif and G-quadruplex regulate c-myc expression<sup>(1)</sup>
- Protonated cytosine making hydrogen bonds with cytosine to stabilize the i-motif structure
- The wildtype c-myc i-motif could adopt multiple isomers
- Selectively mutating cytosine residue to thymine traps species in certain conformation



- Yellow: Cytosine
- Blue: Thymine
- Red: Adenine
- C → T mutation
- Orange Bonds: hydrogen bonds



## Two-State Model

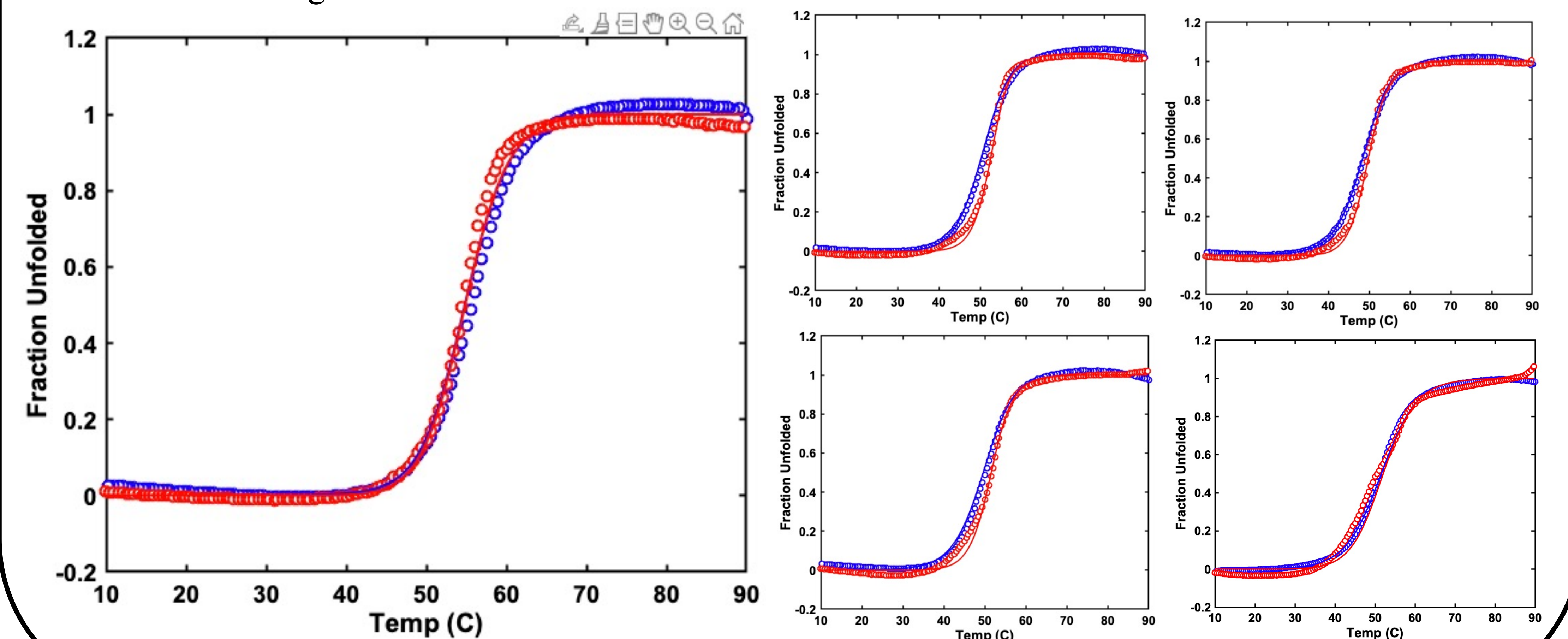


- The fully trapped i-motif sequence can be modelled as two states: the folded state and unfolded state
- Using the MATLAB, we can find the kinetic parameters for the fully trapped i-motif sequence

Fully trapped	$k_F$	$k_U$	$E_F$	$E_U$
55	1.58	$-5.1 \times 10^{-3}$	6.19	332.70
53	1.16	$1.51 \times 10^{-2}$	61.8	359.49
35	1.22	$1.27 \times 10^{-2}$	30.4	310.13
33	2.44	$6.66 \times 10^{-2}$	69.5	273.39

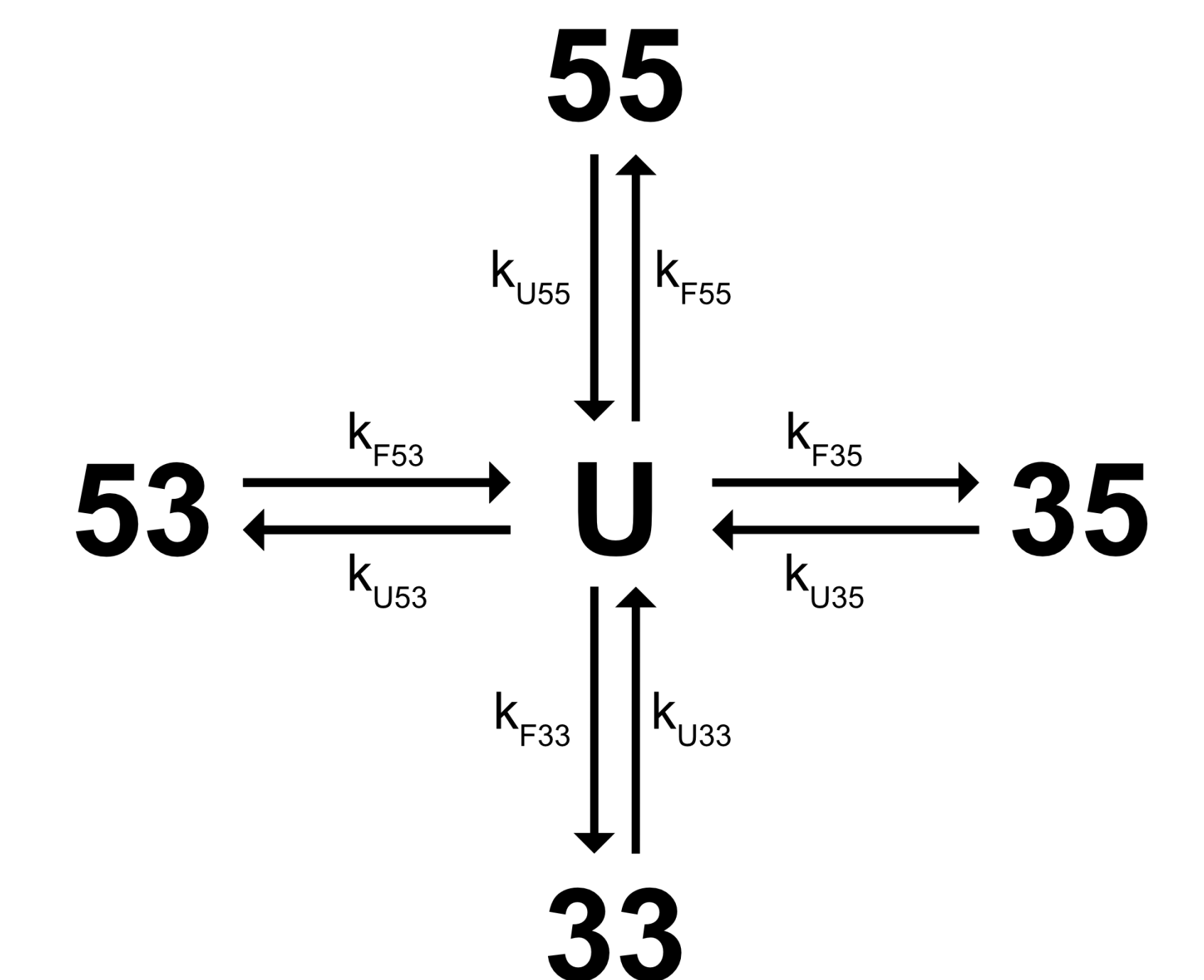
## Result

Thermal hysteresis is using rapid heating and cooling to push folding and assembly processes slightly out of equilibrium<sup>(2)</sup>  
The absorbance of UV light at 260 nm was monitored to follow the folding of the intercalating motifs



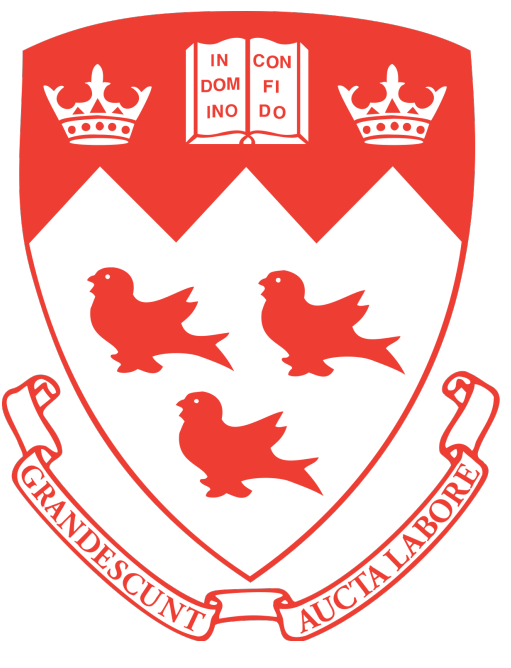
## Future Work

- For the thermal hysteresis experiment, we want to apply multiple scan rates in the future
- To develop a global model, which accurately represents the folding dynamics of the wild type i-motif.
- Understanding the i-motif folding pathway could help with the development of i-motif specific targeting drugs that might treat the cancer



## References

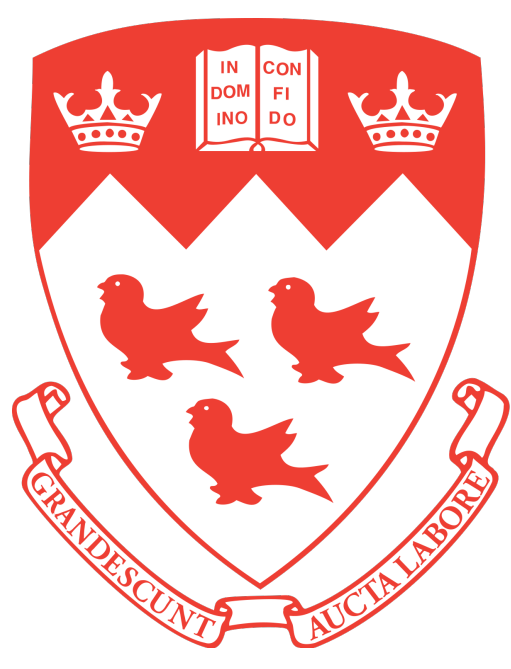
- Sun, D., and Hurley, L.H. The importance of negative superhelicity in inducing the formation of G-quadruplex and i-motif structures in the c-Myc promoter: implications for drug targeting and control of gene expression. *Journal of medicinal chemistry* 52 (2009), 2863-2874
- Mergny, Jean-Louis, and Laurent Lacroix. "Analysis of thermal melting curves." *Oligonucleotides* 13.6 (2003): 515-537.



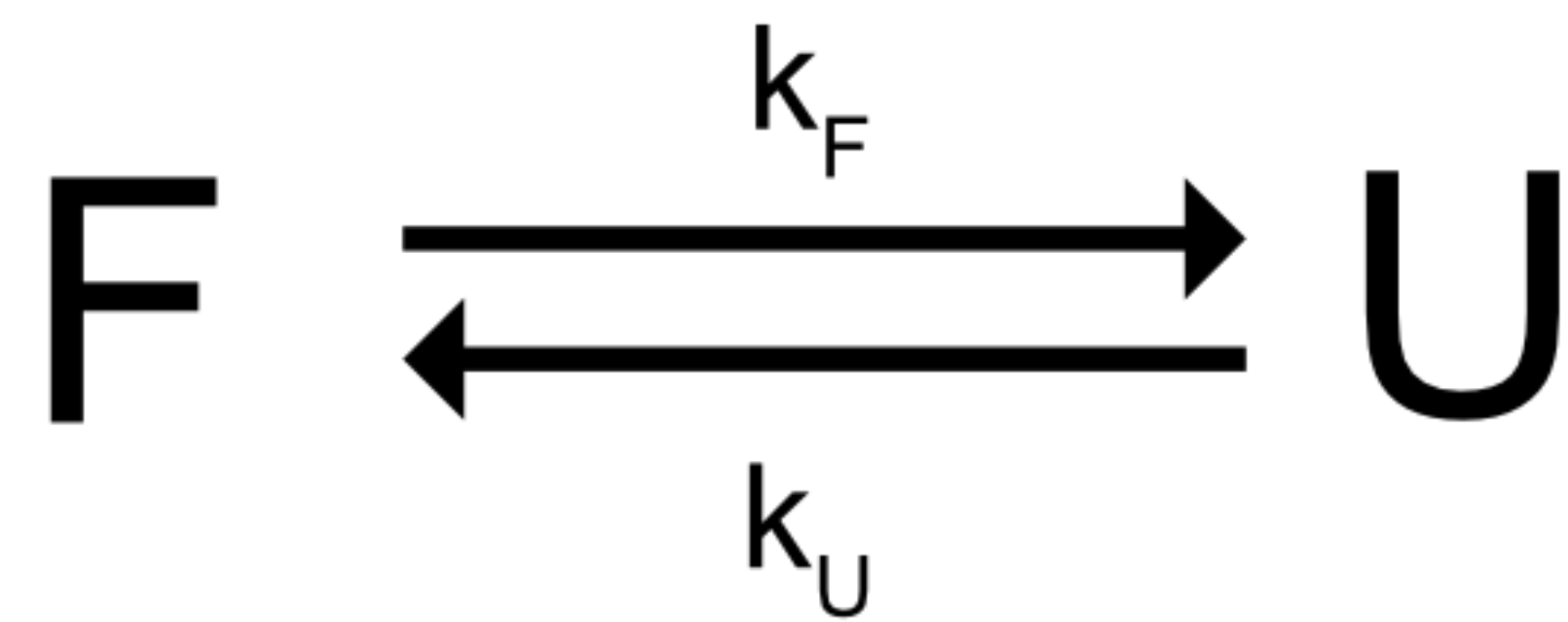
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# Two-State Model



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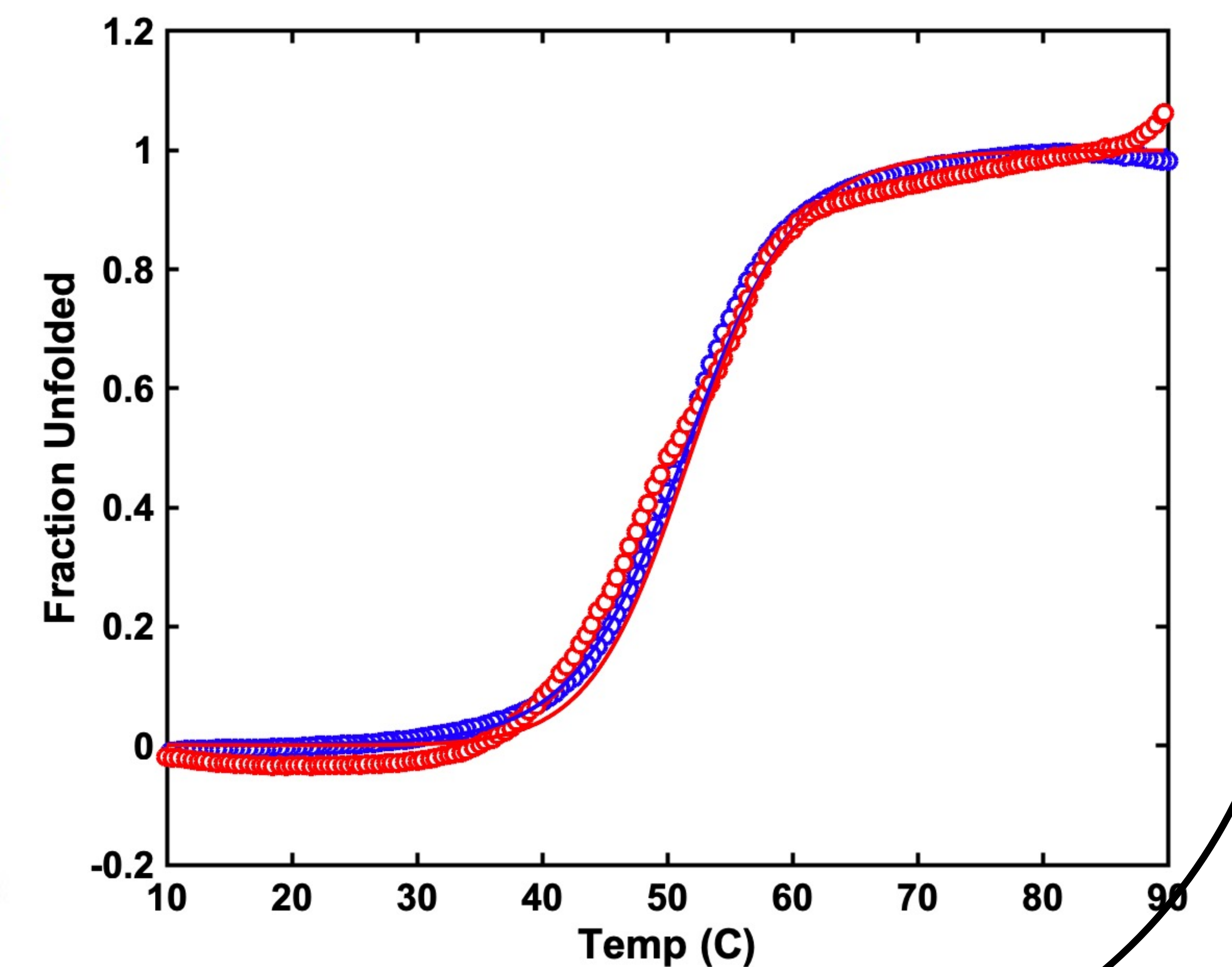
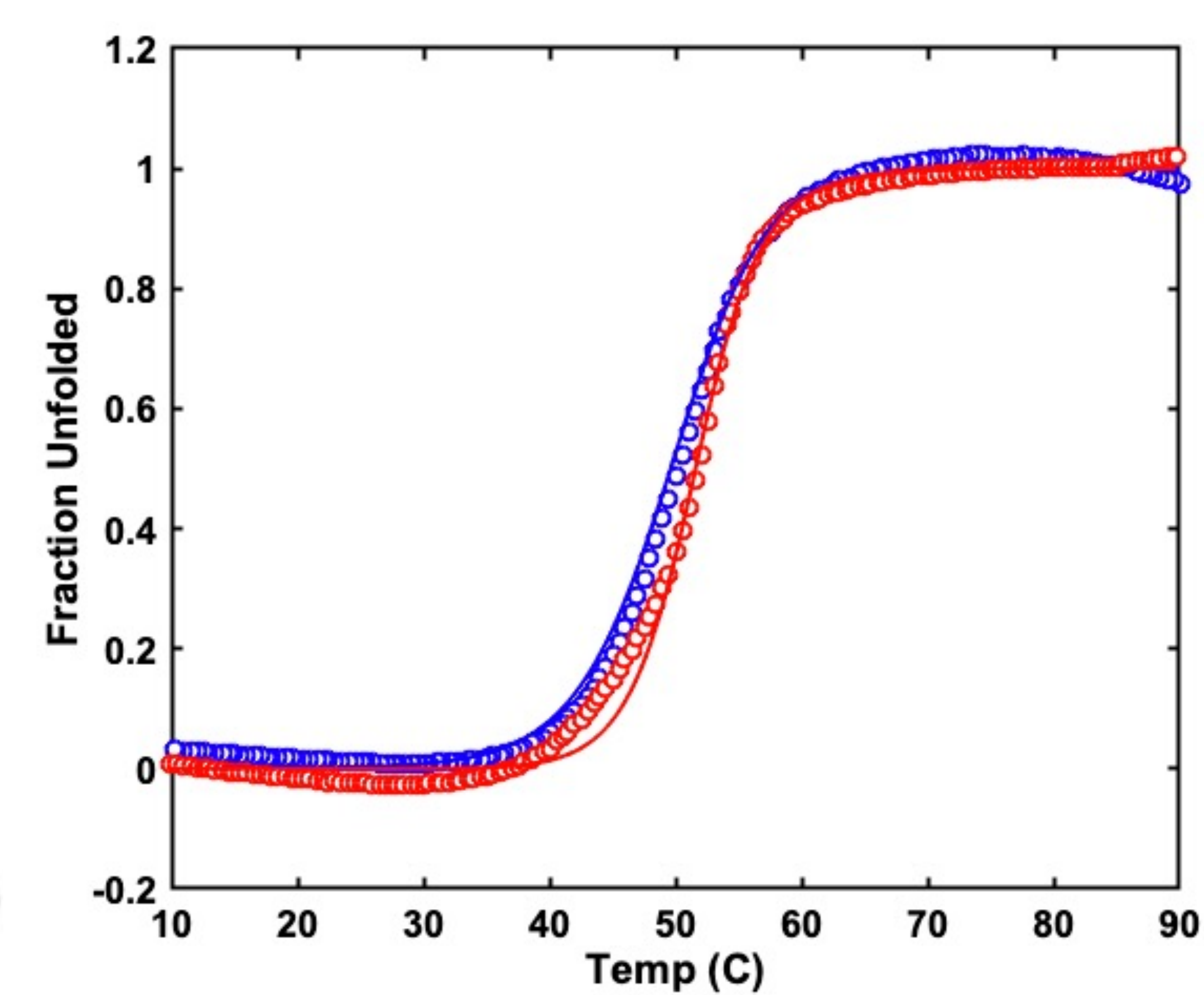
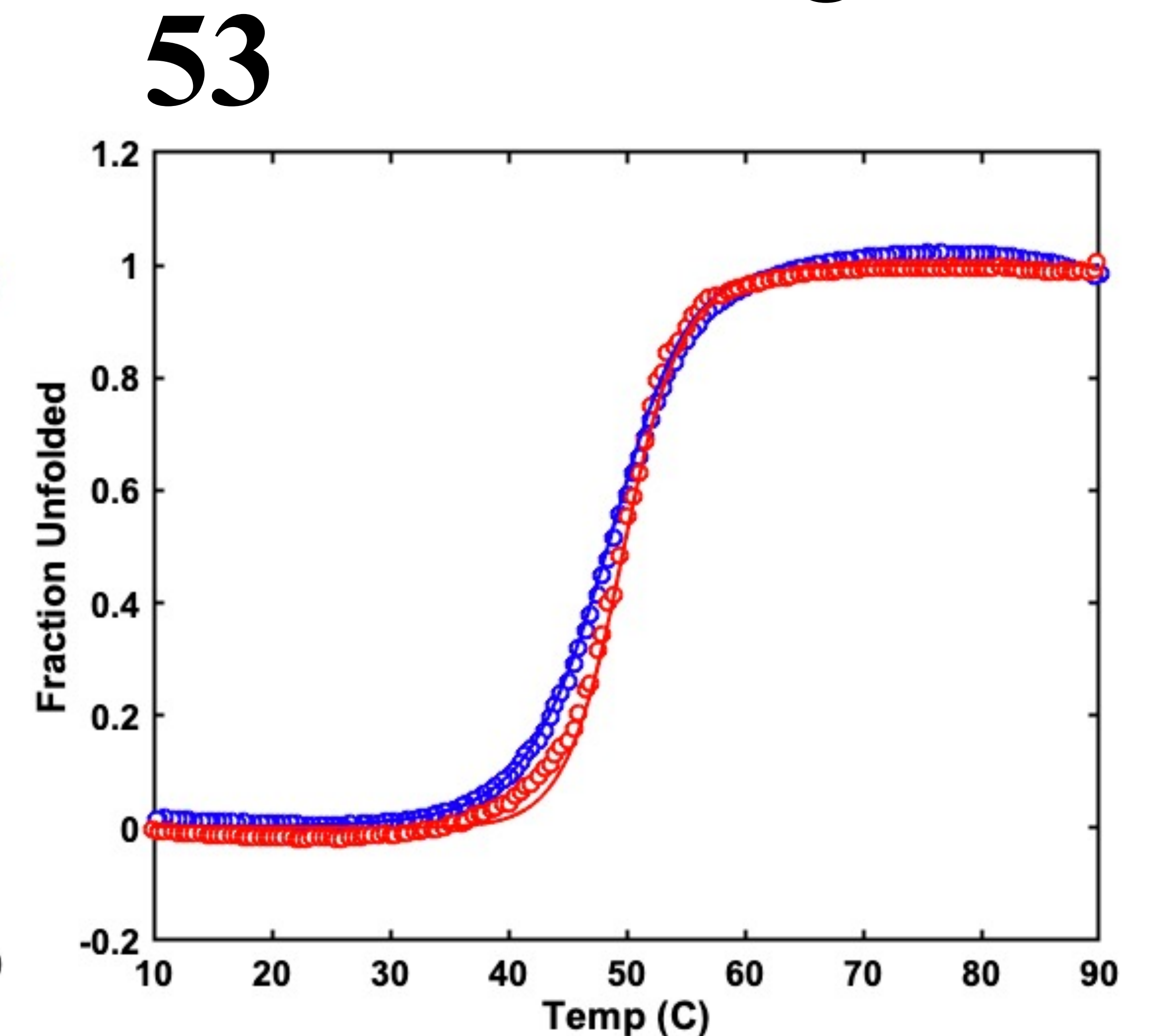
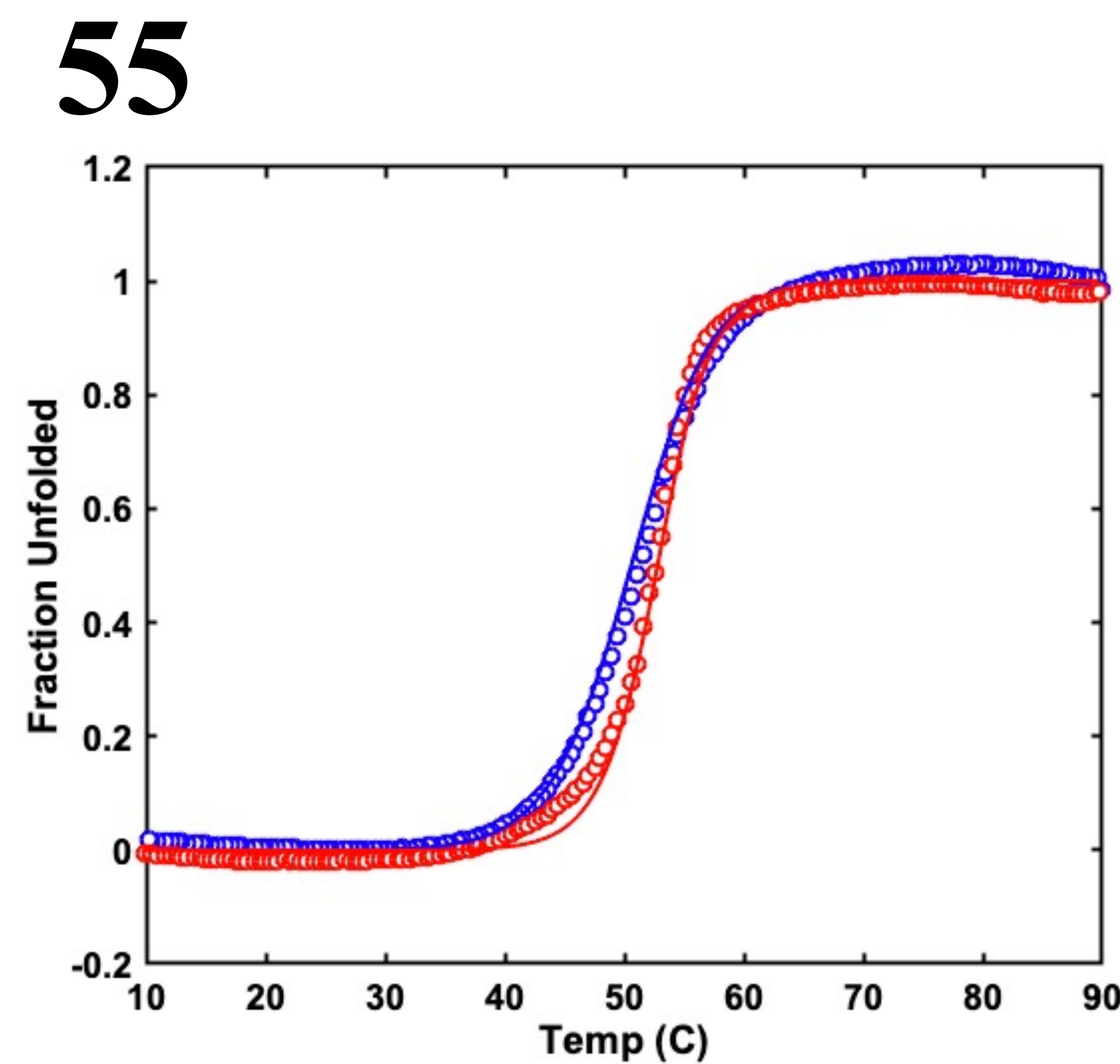
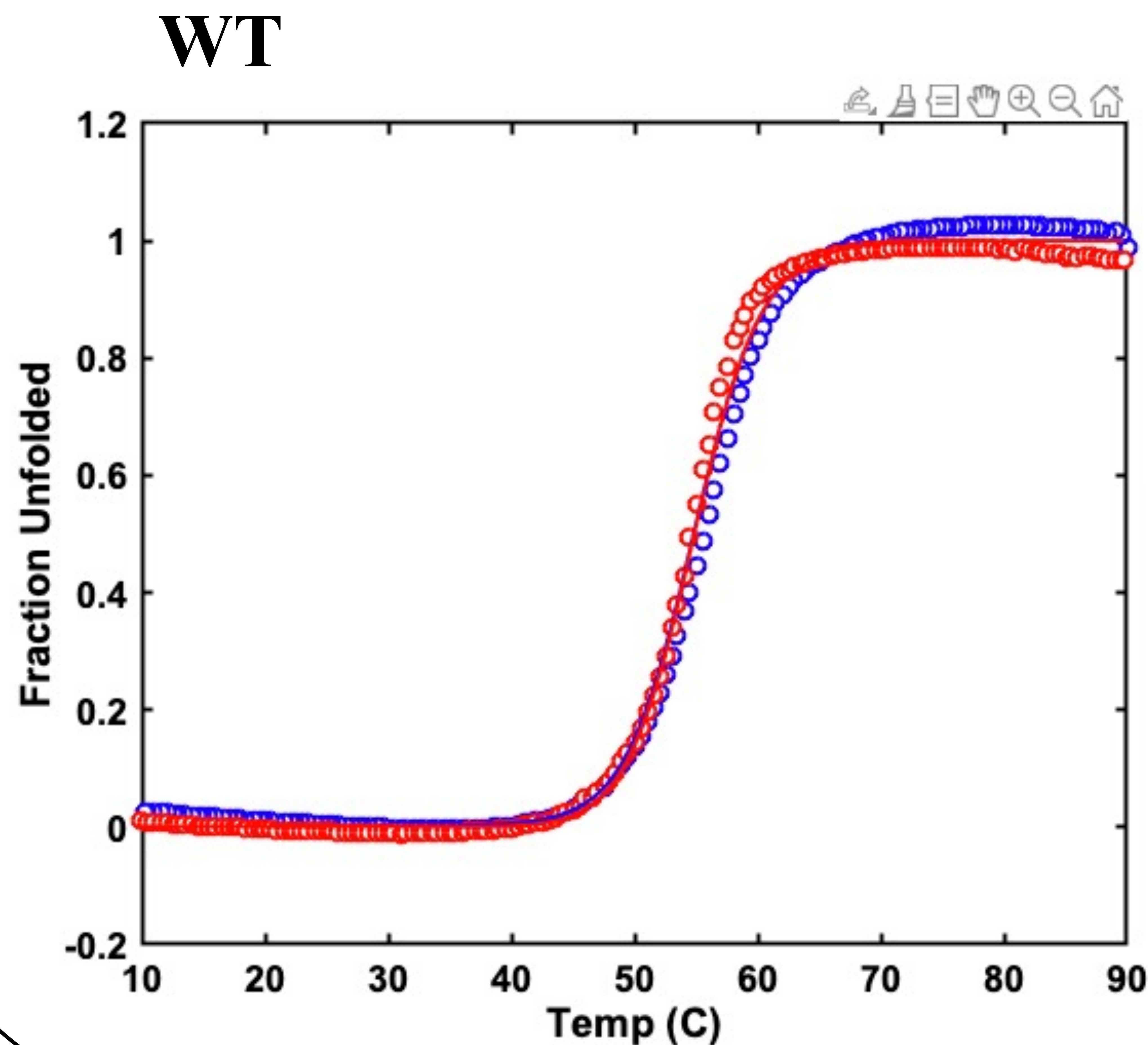
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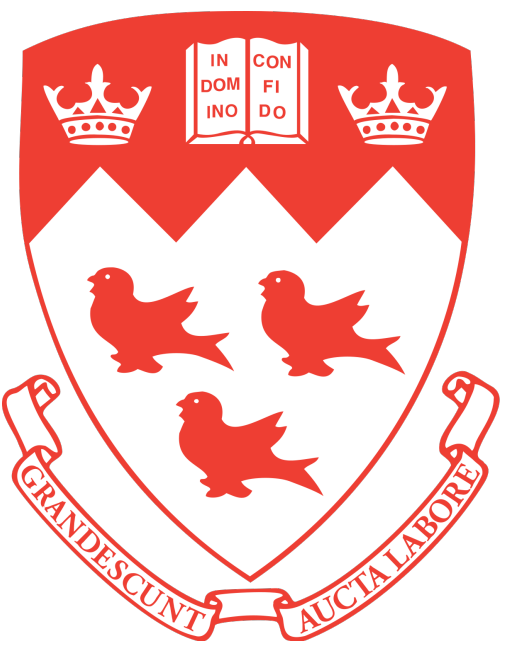


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