THE UNIVERSITY OF ALABAMA IN HUNTSVILLE | COLLEGE OF SCIENCE

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Developing and testing the thermofluor stability assay for protein crystallization

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Background

Protein stability is deeply important in biochemistry and life.

- Stability allows proteins to perform their biological functions.
- A stable protein maintains its native three-dimensional structure found in the body, while an unstable protein unfolds, exposing its inner hydrophobic region.
- To determine a protein's structure through crystallography, the protein should be in its native state.

The Thermofluor method is an inexpensive and accessible high-throughput method of screening for stability conditions.

- Uses only a RT-PCR instrument and hydrophobic fluorescent dye.
- The higher the fluorescence, the more unfolded the protein is at that temperature.
- The stability is quantified by melting temperature (Tm), the temperature at the midpoint of the melt curve.

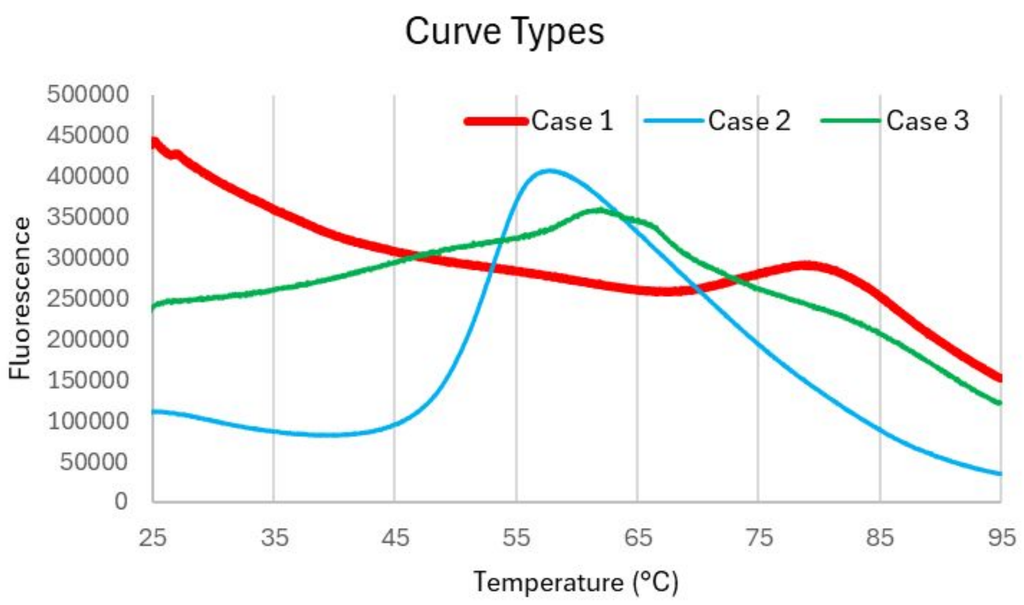

Figure 1. The most common melt curve cases are shown in the figure. **(1)** The protein has begun to unfold at room temperature. **(2)** The protein unfolded in accordance with a well defined sigmoid. **(3)** The melt curve is not well defined and cannot be accurately interpreted.

Objectives

- Implementation of the Thermofluor method in the lab.
- Demonstration of the effectiveness of the method by comparing stability conditions for the proteins beta-lactoglobulin A and B.

References

1. Thermofluor-based high-throughput stability optimization of proteins for structural studies. (Ulrika B. Ericsson et al.)
2. Optimization of protein buffer cocktails using Thermofluor (Linda Reinhard et al.)
3. Relative Structural Stabilities of β-Lactoglobulins A and B As Determined by Proteolytic Susceptibility and Differential Scanning Calorimetry (Xiaolin L. Huang et al.)

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Materials and Methods

Procedure

- The conditions shown in figure 2 were put into a 96 well PCR plate. Each plate contained 3 replicates.
- Each of the wells was filled with 16.5 µL of buffer condition, 2 µL of protein, and 1.5 µL of dye.
- Three trials were performed for both proteins for a total of 9 replicates per condition.

Final assay conditions

- Dye: 4X concentration of Sypro Orange.
- Protein: 10 µM concentration of beta-lactoglobulin.

Results

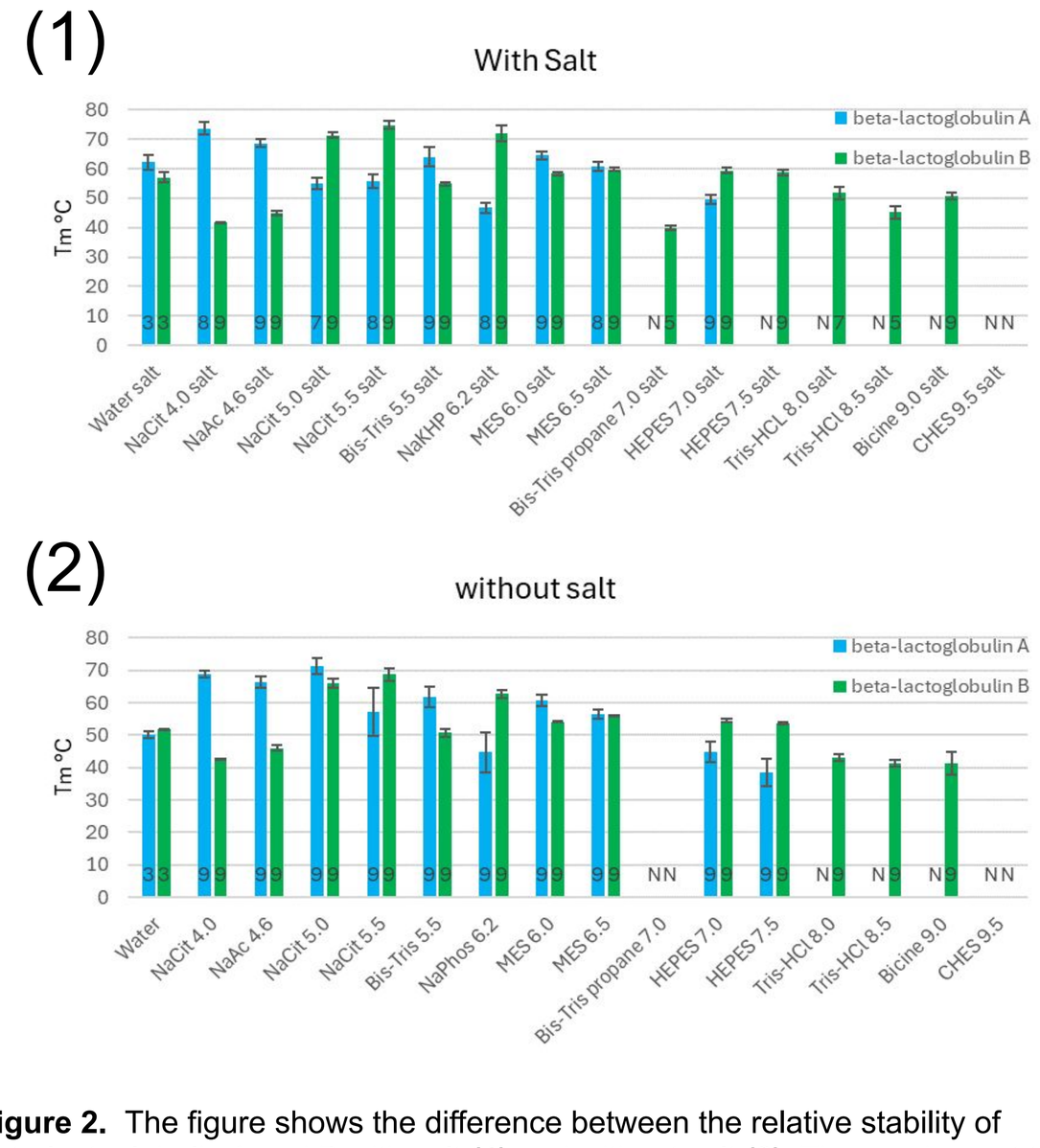

Figure 2. The figure shows the difference between the relative stability of beta-lactoglobulin A and B with salt **(1)** and without salt **(2)**. The numbers at the base of each bar represent the number of replicates in the dataset. Conditions with less than 9 replicates in the dataset are due to uninterpretable curves or curves that unfold at room temperature (case 1 and 3 from figure 1).

Conclusions and Future Plans

- The thermofluor method is relatively easy to set up and shows valuable information about protein stability.
- Beta-lactoglobulin A and B have different stability trends despite differing by only two amino acids.
- The proteins typically have contrasting stability conditions.
- The data obtained is currently being used in crystallization trials to compare how high and low stability conditions derived from Thermofluor affect these proteins during crystallization.