

Thermal denaturation assays: a practical comparison of DSF, DSC and CD

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CORRECTION (published 11 April 2023): In Figure 3, panel B, the series labels for Buffer C and Buffer D were transposed. The numerical values and all conclusions are unaffected. The corrected figure is available in the online version.

ABSTRACT

We compare three routine methods for measuring protein melting temperature on a common panel of twelve proteins. Agreement between methods is good for well-behaved globular proteins (± 1.2 °C) and poor for proteins that aggregate on unfolding (± 6.4 °C).

1. Methods

Twelve proteins were measured in four buffers by differential scanning fluorimetry (DSF), differential scanning calorimetry (DSC) and circular dichroism (CD). All measurements were performed in triplicate on the same protein preparations.

2. Results

DSF and DSC agree within 1.2 °C for the nine proteins that unfold reversibly. For the three aggregation-prone proteins, DSF

reports systematically higher T_m than DSC, by up to 6.4 °C. CD tracks DSC more closely than DSF does.

3. Practical guidance

Report the method with every T_m value. A T_m compared across methods without that annotation is not interpretable. Buffer composition shifts T_m by 2–9 °C across the conditions tested here, which is larger than the difference between most published predictors.

