Validation of Tycho NT.6 precision and repeatability

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Introduction and Results

Tycho™ NT.6 tells you so much about the quality of your protein — presence, purity, concentration, functionality and similarity — in a single experiment. It does this all by examining your protein's structural integrity or foldedness in a label-free way. The system measures the fluorescence of instrinsic tryptophan and tyrosine residues detected at both 350 nm and 330 nm as a specific defined temperature ramp is applied. Tycho generates thermal unfolding profiles and identifies the inflection temperature (T_i) that represent unfolding transition(s) or discrete changes in a protein's structural integrity. It records these unfolding profiles so you can study your protein in real-time or use the data as a reference to compare and validate the quality of your sample to any future batches.

In this study we demonstrate the outstanding repeatability and intermediate precision of measurements with Tycho for both raw data and analyzed results. A reference protein, streptavidin at a concentration of 1 mg/mL, was measured on 13 different Tycho systems over a period of two weeks. Each run consisted of all six capillaries filled with 10 µL streptavidin, and for each system, 3 replicate runs were performed. In total, 234 capillaries were analyzed.

We use the following definitions for repeatability and intermediate precision:

Repeatability describes how similar results are when always obtained by the same operator on the same measuring system in the same lab. It is also called intra-assay precision.

Intermediate precision is also called inter-assay precision or occasionally within-lab reproducibility. It describes how similar results are when obtained within the same laboratory, by different operators, or across measurement systems.

Exemplary data is shown in Figure 1. Calculated results were compared using the T_i, or the point on the curve which represents unfolding of the sample, which can also serve to assess the precision of the temperature control. Raw data was compared using two parameters: first, the initial ratio, which is well-suited for this purpose as it is sensitive towards misalignments of optics and sample tray.

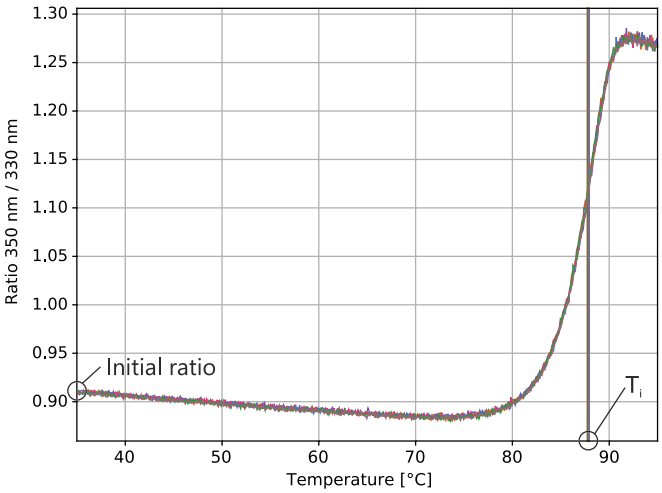

Figure 1: The figure shows exemplary data from one Tycho run. Two of the parameters used in this study, the initial ratio and the T_i, are labeled. The unfolding profiles of all six capillaries overlap perfectly, as do the vertical lines indicating the T_i. In this particular example run, the average initial ratio across all six capillaries was 0.910 ± 0.001 and the average T_i across all six capillaries was 87.83 °C ± 0.08 °C.

Good repeatability data on the initial ratio measurement across the six capillaries also suggests correct alignment and adjustment of the system. The second raw data parameter tested was sample brightness, which is the combined brightness signal at 330 nm and 350 nm at 35 °C. Both raw data parameters need to be precise in order for Tycho to perform well in day-to-day applications: the initial ratio can be used as an indicator of % unfolded sample, while sample brightness can be used to assess protein concentration.

Tables 1 and 2 list measured values (where applicable) along with standard deviations (SD) and relative standard deviations (RSD) for repeatability and intermediate precision. The RSD is also known as coefficient of variation (CV). The determined RSD is useful since it is dimensionless and independent of the magnitude of the measured values, which means it allows to compare different types of systems even with completely different output parameters.

Conclusions

Taken together, these results demonstrate that Tycho achieves excellent repeatability (intra-assay precision) and intermediate precision (inter-assay precision), making it an extremely reliable and an easy-to-validate system for every lab working with proteins.

Repeatability

	mean intra-system SD	mean intra-system RSD (CV)
T_i	0.1005 ± 0.0236	0.11 % ± 0.03 %
Initial ratio	0.0007 ± 0.0002	0.08 % ± 0.02 %
Sample brightness	13.4 ± 5.3	1.30 % ± 0.52 %

Table 1: Repeatability (intra-assay precision) was calculated by determining the variations (SD/RSD) for each of the 13 systems, then calculating average within-system variations across all 13 systems.

Intermediate precision

	mean	SD	RSD (CV)
T_i	87.57 °C	0.21 °C	0.24%
Initial ratio	0.903	0.007	0.73%
Sample brightness	1037.7	29.0	2.79%

Table 2: Intermediate precision (inter-assay precision) was directly determined across all 234 capillaries run.

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