Monitoring the impact of storage-dependent denaturation on protein quality using Tycho NT.6

Mariam Mohamadi, Nuška Tschammer and Dennis Breitsprecher
NanoTemper Technologies GmbH, Floessergasse 4, 81369 Munich

Introduction

Expression and purification are critical steps in protein discovery and characterization workflows. Equally important in the workflow is the optimization of storage conditions that will ensure protein stability. Unfortunately, researchers spend little effort evaluating the effects of storage on the quality of samples prepared in-house or of those obtained through commercial vendors, which can be attributed in part to the lack of quick and simple quality check methods.

The Tycho™ NT.6 system enables quick assessment of the effect of storage conditions on protein integrity.

Tycho NT.6 records precise thermal unfolding profiles of proteins in as little as three minutes, so researchers can quickly detect the presence of unfolded protein in their samples before it is used in an assay and negatively impacts the outcome. In this technical note, we show how such a storage-dependent increase in the amount of unfolded protein directly affects the quality of biophysical assays, highlighting the importance of rigorous quality checks.

Results

In these experiments, p38α kinase was selected as a test protein and subjected to a series of storage-related stresses. One aliquot of the protein was repeatedly frozen at -80°C and then thawed. A second aliquot was incubated for a prolonged period at temperatures above its inflection temperature (T_i) ~of 55 °C, resulting in fully unfolded protein. Fully unfolded sample generated by thermal treatment was mixed with native p38α to create a preparation that represented 50% unfolded protein. The three sample preparations were analyzed against a reference sample of freshly prepared material using the Tycho NT.6. The generated unfolding profiles showed significant differences when compared to the reference sample (Figure 1A). Repeated freeze-thaw cycles resulted in an increase in the initial ratio and thereby a decrease in Δ ratio, indicating an increase in unfolded protein (Table 1). As expected, the fully denatured sample showed the greatest increased initial ratio and lacked an unfolding transition, confirming denaturation. The sample containing 50% of unfolded protein showed an unfolding transition with a reduced Δ ratio value. Interestingly, the T_is of the two partially denatured samples (freeze-thaw and 50% denatured) were very similar to the T_i of the reference sample, suggesting that a fraction of the protein population is denatured, while the remaining material is still correctly folded.

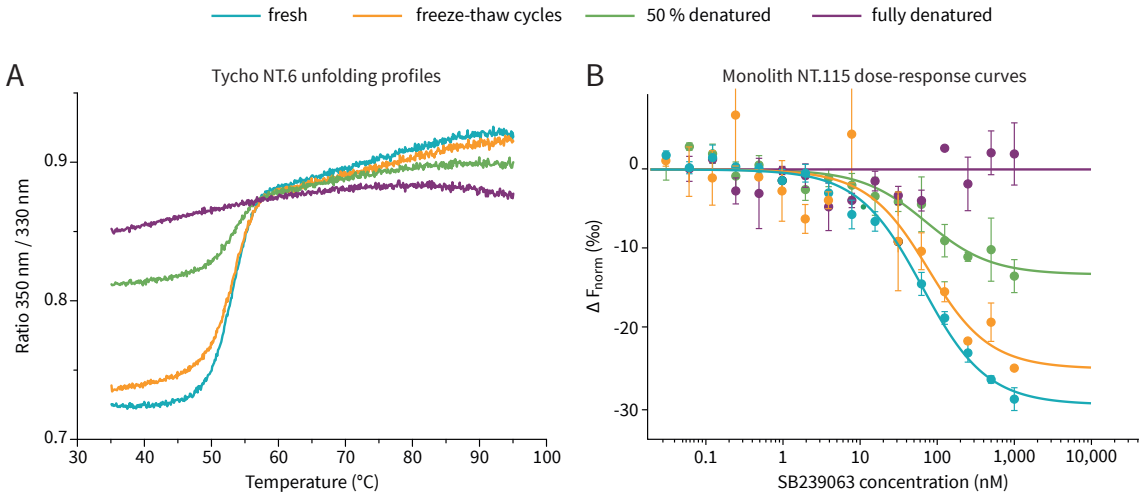

Figure 1: Effect of storage-dependent denaturation on p38α kinase thermal unfolding profile and functionality. A) The thermal unfolding profiles of p38α kinase were generated for freshly prepared protein (teal colored line), after 6 freeze-thaw cycles (orange-colored line), after full thermal denaturation (purple-colored line), and of a mix containing 50% fresh protein and 50% thermally denatured protein (green-colored line). B) Dose-response curves demonstrating the interaction between the small molecule inhibitor SB239063 to each of the four protein preparations described in A determined using Microscale Thermophoresis (MST). Experiments were performed on the Monolith NT.115 system.

The effect of storage-related stress on the outcome of biophysical experiments was then evaluated by measuring the interaction of the p38α samples with the small molecule inhibitor SB239063 by MicroScale Thermophoresis (MST) (Figure 1B). Results of the MST analysis confirmed that the presence of unfolded protein in the sample directly influences assay outcome. The MST binding amplitude decreased with the presence of increasing amounts of unfolded protein and the overall assay quality was lower, as indicated by greatly reduced signal-to-noise values (Table 1). Fully denatured p38α did not show an interaction.

	T_i (°C)	Initial Ratio	Δ Ratio	MST Kd (nM)	MST amplitude (F_{norm} (‰))	MST signal-to-noise
Fresh	53.1	0.725	0.193	59	28.5	25.7
Freeze-thaw cycles	53.2	0.739	0.176	64	21.6	7.4
50% denatured	53.5	0.812	0.088	68	12.8	9.5
Fully denatured	n.a.	0.852	0.025	n.a.	0	n.a.

Table 1: Summary of Tycho NT.6 and Monolith analysis of p38α kinase under different storage conditions

Conclusions

We show that the Tycho NT.6 system can be used to quickly evaluate different storage conditions, such as freezing strategies, to provide a better insight on the quality of the proteins being tested. The results demonstrate that poor sample quality and the presence of denatured protein can directly influence assay performance and the experimental outcome. The Tycho NT.6 system is well-suited to swiftly validate protein quality in as short as 3 minutes, prior to performing further biophysical analysis which can be time-consuming and costly to run.