Validation of protein complex functionality with Tycho NT.6

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Abstract

Macromolecular protein complexes are known to have key regulatory roles that are linked to most biological processes. Maximizing expression, efficient purification and deciphering the functional role of complexes and their components are main objectives for researchers working with proteins. Of equal importance but often overlooked is monitoring the quality of a protein preparation particularly as it relates to the protein's functionality. By having more insights into the quality of a protein sample, researchers can be more succesful and efficient in their experimental setup and planning. The Tycho NT.6 system quickly analyzes protein samples and provides detailed information on their folded state. The system analyzes low microliter amounts of material collected directly from a column purification without having to dilute or dialyze the sample. Functionality of the material can be easily tested and results generated in three minutes, removing the guesswork out of analyzing protein complexes.

Introduction

The low-hanging fruits have all been picked! The proteins that are the focus of today's research have quite little in common with the well-behaved globular proteins that were studied in the 20th century. Transmembrane proteins, large macromolecular complexes and unconventional antibody variants are a primary focus of researchers as they represent important classes of proteins that have key biological roles. Purification and storage of these varied types of proteins can be challenging and often require researchers to routinely prepare fresh material

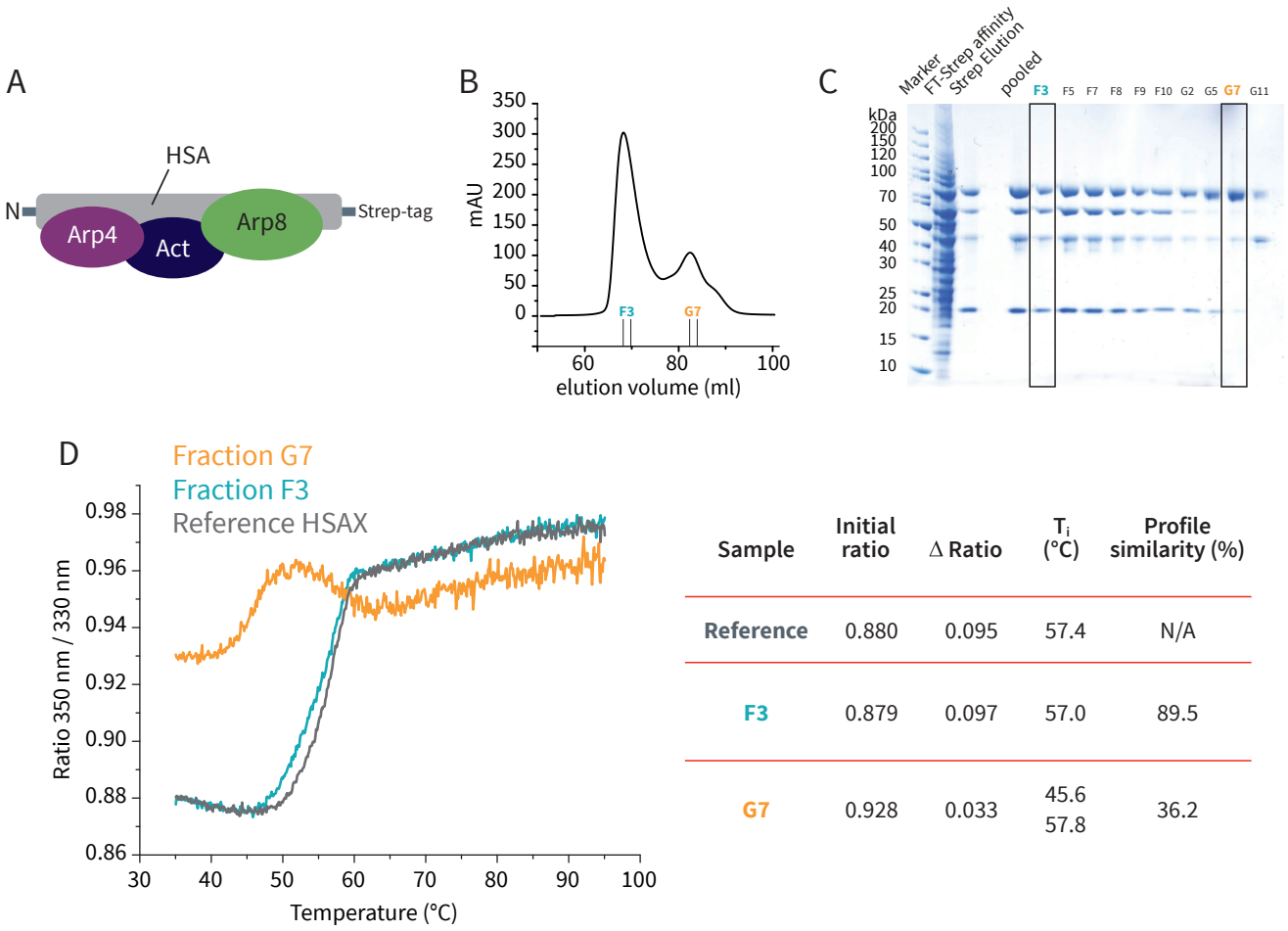
every time for new experiments. While standard operating procedures and protocols can be created to provide consistency and confidence in the sample preparations, it is important to rigorously test the quality and functionality of samples before committing valuable time and expense to performing more detailed characterizations. Quality tests are often overlooked as they can be time-consuming and require separate workflows which have to be performed in parallel. Functionality of a protein (or lack thereof) is typically evaluated long after the protein purification procedure is completed, or not at all, potentially wasting researchers' precious time and translating into inconclusive or non-reproducible results.

Tycho NT.6 is a simple label-free platform that provides quick protein quality validation.

It can be introduced with ease at virtually every step of the protein purification workflow after cell lysis and initial purification to final production. Tycho NT.6 generates thermal unfolding profiles of protein samples, which are instrumental to evaluate the quality and conformational integrity of proteins. In addition, Tycho NT.6 can monitor and detect binding events by ligand-induced changes in the unfolding profiles. Thus, the quality, stability and functionality of a protein can be addressed within a quick, three-minute experiment.

Results

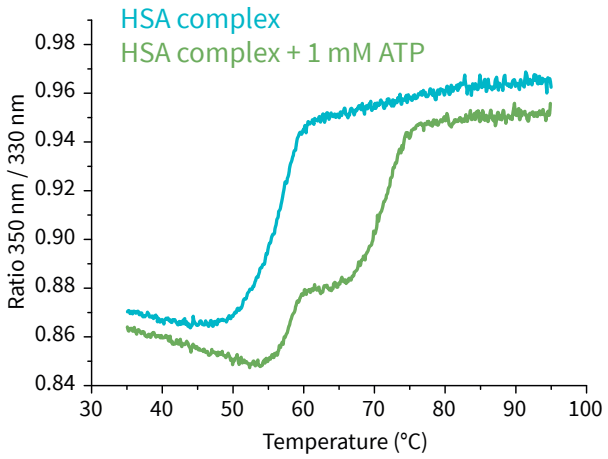
In these experiments, we demonstrate the utility of Tycho NT.6 for monitoring protein quality during a purification workflow. Thermal unfolding profiling and thermal shift functionality tests were used throughout the purification of an INO80 chromatin remodeler sub-complex (HSAX), consisting of the INO80 HSA domain ($INO80^{HSA}$, from hereon referred to as HSA), actin, and two actin-related proteins, Arp4 and Arp8 (Figure 1A)[1,2]. After initial purification using a C-terminal Strep-tag, the complex was further purified using size-exclusion chromatography (SEC) with a S200 SEC column. The elution profile showed two clearly distinct elution peaks (Figure 1B). After running SDS-PAGE analysis, peak F3 was shown to contain all 4 proteins of the complex, indicated by 4 different bands with the correct molecular weight. In contrast, fraction G7 mainly contained one protein, likely Arp8 which has the highest molecular weight (Figure 1C). Analysis of the main peak fractions with the Tycho NT.6 and comparison of the obtained unfolding profiles with a reference sample of previously purified HSAX unambiguously identified fraction F3 as the fraction containing properly assembled HSAX (Figure 1D). These experiments were performed much faster and efficiently when compared to traditional SDS-PAGE. Fraction G7 showed a distinct unfolding profile different from either fraction F3 or the reference, corroborating that this sample does not contain the intact HSAX complex.



Sample	Initial ratio	Δ Ratio	T_i (°C)	Profile similarity (%)
Reference	0.880	0.095	57.4	N/A
F3	0.879	0.097	57.0	89.5
G7	0.928	0.033	45.6 57.8	36.2

Figure 1: HSAX quality verification during purification using Tycho NT.6. A) Schematic representation of the HSAX complex. Act = actin, Arp = actin related protein, HSA = helicase–SANT–associated. B) Elution profile of HSAX after size-exclusion chromatography with fractions F3 and G7 indicated. C) SDS-PAGE of fractions from size-exclusion chromatography. D) Unfolding profiles of fractions G7 and F3 compared to a reference sample of purified HSAX. Measurement results are summarized in the table. T_i = inflection temperature, the temperature at which an unfolding transition occurs and is detected by the system.

The functionality of the HSAX complex was additionally tested in the same experiment by adding ATP as a ligand (Figure 2). The actin, Arp4 and Arp8 subunits of HSAX are known to bind to ATP.[3,4] The presence of ATP induced marked changes in the unfolding profile of HSAX. The previously observed single unfolding transition changed into two transitions with two clearly distinguishable, higher inflection temperatures, likely representing the different stabilizing effects of ATP on actin and the Arp proteins.



Sample	Initial ratio	Δ Ratio	T_i (°C)	Profile similarity (%)
HSAX	0.880	0.095	57.4	38.0
HSAX + ATP	0.873	0.088	58.0 71.1	

Figure 2: Functional validation of purified HSAX complex by thermal shift analysis. HSAX in presence and absence of 1 mM ATP was monitored using the Tycho NT.6. In the presence of 1 mM ATP, the unfolding transitions of the HSAX complex are different than for the complex alone. A summary of the results is shown in the table. Initial ratio is the initial value (at 35 °C) of the ratio of measurements collected at 350 and 330 nm. Δ Ratio: Difference between the ratio at the beginning and at the end of the thermal profile. T_i: Inflection temperature of the unfolding transition in the 350 nm / 330 nm ratio signal. Profile similarity, is an index which quantifies the sameness of the unfolding profiles of two or more samples.

Conclusions

Tycho NT.6 is a powerful technology for researchers to perform effortless, accurate and robust quality checks on the folding state and functionality of their protein samples during purification.

Generating results in three minutes, researchers can make faster and more educated decisions in their purification workflow.

Binding interactions with the respective ligands can be quickly tested before investing time, effort and expenses in other methodologies.

References

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