Quick protein binding analysis by label-free thermal shift analysis on the Tycho NT.6

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

Introduction

The lock-and-key model first postulated by the famous chemist Emil Fischer proposes that enzymes and their substrates must fit together like locks and keys in order to fulfill their physiological functions. Even though it has long become clear that biological systems are nowhere near as rigid as their metallic namesakes, the principle of molecules interacting together three-dimensionally is the basis for almost all biological processes. Of course this is not only true for enzymes, but for all types of protein interactions. Only a protein that is correctly folded and structurally intact can do its job properly. For researchers working with proteins, it is therefore fundamentally important to be able to analyze both protein structure and function.

The Tycho™ NT.6 system offers a rapid, simple and straightforward way to check protein functionality and, as a consequence, its quality.

To determine whether a protein is functional and able to bind its interaction partner or ligand, Tycho NT.6 can examine protein-ligand interactions by performing a label-free thermal shift analysis. Suitable ligands can be varied and diverse, ranging from ions, sugars and small molecules to nucleotides and lipids. By recording and comparing highly precise unfolding profiles, ligand-induced

changes in the protein's stability as monitored by fluorescence properties can be derived to conclude binding. Thus, the Tycho NT.6 system allows for evaluating protein functionality within minutes, and can be integrated in purification workflows or assay development routines with ease.

Results

In this study, the unfolding profiles of a series of proteins were examined in the absence and presence of ligands known to bind to their target proteins. Figures 1A and 1B show the binding of p38α kinase to Mg^{2+} ions and a small molecule inhibitor, respectively. Both interactions induce a shift of the unfolding transition to higher inflection temperatures (T_i), indicating that an interaction is occurring. In the case of the small molecule inhibitor (SB203580) binding also changes the ratio of the folded state (initial ratio), which is likely caused by effects of the small molecule on tryptophan fluorescence emission.

In Figure 2, the interaction between maltose-binding protein (MBP) and maltose is shown. The presence of maltose triggers a shift in the inflection temperature from 56.3 to 58.5 °C. Also observed is a change in the initial ratio value measured which can likely be attributed to the marked structural changes of the protein upon binding of maltose.

In the final example shown in Figure 3, the interaction between lysozyme and the bacterial cell wall component 3'NAG was analyzed. Besides a shift of the unfolding transition by 1.8 °C, the initial ratio of lysozyme in the presence of 3'NAG is much lower than in its absence. Reports suggest that binding of the ligand covers several otherwise exposed tryptophan residues and likely results in a shift of the tryptophan fluorescence towards lower wavelengths which translates into a lower initial ratio.[1]

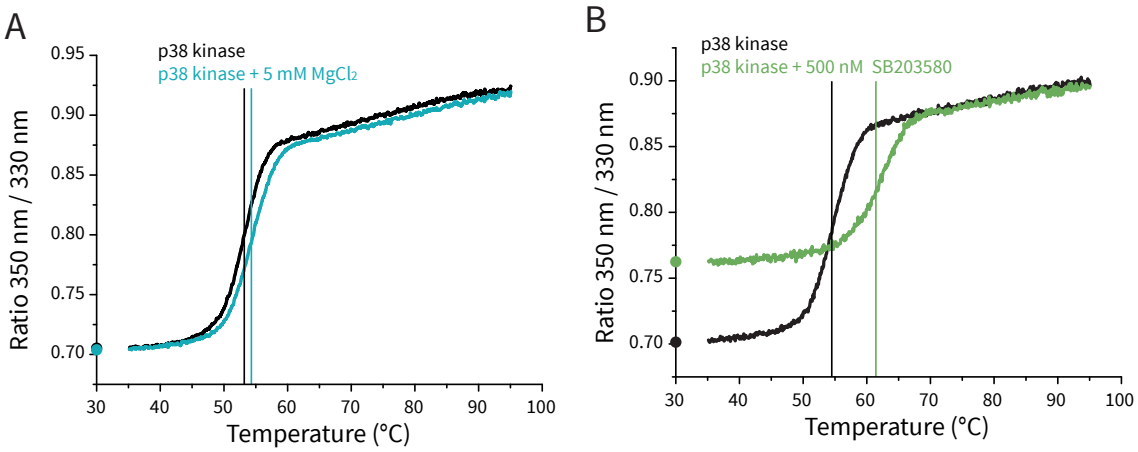

Figure 1: Monitoring p38 kinase binding activity using a label-free thermal shift analysis. A) Interaction of 4 µM p38α kinase with 5 mM Mg2+ ions. B) Interaction of 4 µM p38α kinase with 500 nM SB203580. Vertical lines indicate inflection temperatures (T_i), colored circles on the y-axis indicate initial ratio values.

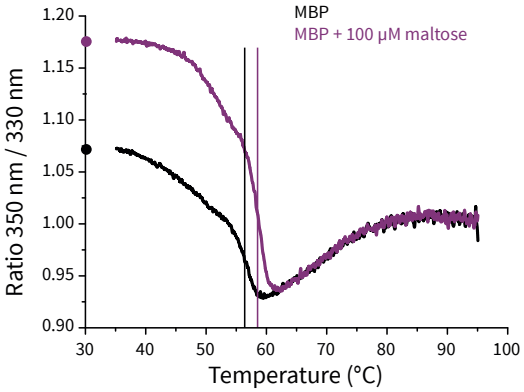

Figure 2: Label-free thermal shift analysis of. 10 µM maltose-binding protein (MBP) interacting with 100 µM maltose using Tycho NT.6. Dramatic differences in the initial ratio values are likely due to the conformational changes of MBP upon binding of maltose. Vertical lines indicate inflection temperatures (T_i), colored circles at the y-axis indicate initial ratio values.

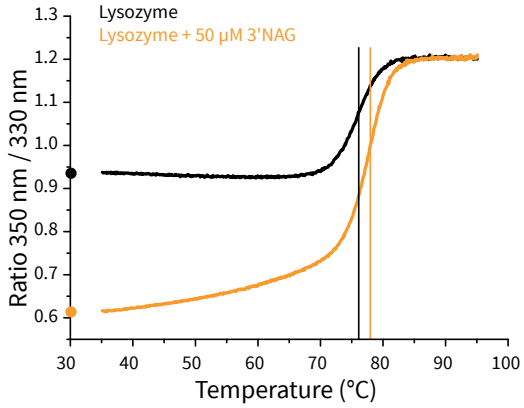

Figure 3: Testing the interaction of bacterial cell wall complex (NAG) with lysozyme using the Tycho NT.6. 5 µM lysozyme was incubated with 50 µM 3'NAG and analyzed. Vertical lines indicate inflection temperatures (T_i), colored circles at the y-axis indicate initial ratio values.

Conclusions

These examples illustrate that the Tycho NT.6 system can provide a fast and reliable yes/no binding checks utilizing label-free thermal shift assays. In addition, interactions detected on the system can serve as an initial test or predictor of functionality. Proteins interacting with their ligands as expected will likely have appropriate functionality in further downstream analysis to be run. Experiments on the Tycho NT.6 are fast to perform, easy to carry out, utilize microliter amounts of sample and serve as a standard quality validation tool for scientists doing protein research.

Reference

1. Telmer, P.G. and B.H. Shilton, Insights into the conformational equilibria of maltose-binding protein by analysis of high affinity mutants. J Biol Chem, 278(36): p. 34555-67 (2003).