Better optimization of biosensor assay development with Tycho™ NT.6

Dennis Breitsprecher and Nuska Tschammer
NanoTemper Technologies GmbH, Floessergasse 4, 81369 Munich

Abstract

Surface plasmon resonance (SPR) is an optical methodology used to detect and quantify molecular interactions. Although SPR is widely used and considered by many researchers to be the gold standard for quantification of protein interactions, assay optimization can be technically challenging, time-consuming and costly. The Tycho NT.6 system quickly analyzes different conditions typically tested when optimizing an SPR assay. It utilizes very small amounts of sample and provides dramatic time and cost savings compared to standard SPR assay development procedures. Tycho NT.6 is simple to use and enables researchers to make better educated decisions in developing and optimizing their binding interaction assays.

Introduction

Surface plasmon resonance (SPR) and other biosensor-based analytical methods are standard tools used in academic and pharmaceutical research laboratories for the quantification of protein interactions. Typically, one binding partner – the ligand – is covalently immobilized on the surface of a biosensor chip, while the other binding partner – the analyte – is added in solution to measure binding kinetics. Covalent immobilization of proteins to the biosensor surface is often problematic, since acidic, salt-free buffers are required for optimal coupling using lysine chemistry. These harsh conditions can negatively influence protein integrity and function.

Therefore, optimizing immobilization conditions often represents an early bottleneck in SPR assay development since it can damage the ligand during coupling, resulting in a non-functional biosensor surface and even a wasted biosensor chip.

Procedures such as “pH-scouting”, or “pre-concentration” are used to identify immobilization conditions which trigger sufficient surface attachment of the ligand (Figure 1). In these experiments, different immobilization buffers with varying, low pH-values containing the ligand are injected into the flow cell of the biosensor, and the accumulation of ligand on the sensor surface is monitored over time. This procedure typically takes 1-2 hours to prepare and perform, and identifies immobilization conditions without providing information on the functionality of the immobilized protein. If pH treatment conditions used for immobilization result in unfolding of the protein (partial or complete), this could render it inactive. This is only revealed after performing subsequent binding experiments and getting negative or questionable results. Ultimately, this results in discarding the biosensor chip and repeating optimization experiments. Although standard procedures for immobilization are well-established for certain types of proteins, the general process is time-consuming and bears the risk of irreversibly damaging the ligand on the chip surface, thereby wasting costly consumables.

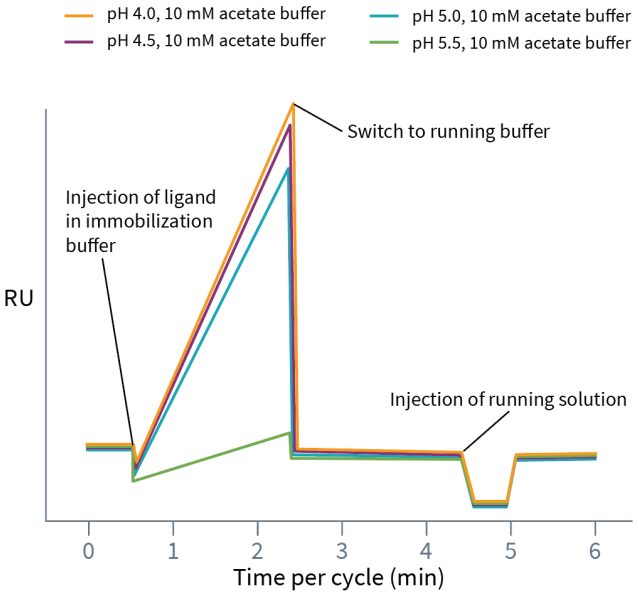

Figure 1 Optimizing ligand immobilization to a SPR biosensor chip by testing different buffer conditions.

Lower pH buffer conditions provide better conditions for immobilization of ligand to the SPR biosensor chip as determined by pH scouting experiments. The larger the resonance units (RU) value measured upon injection of a ligand solution, the better accumulation of the ligand on the chip surface due to electrostatic interactions. Buffers ranging in pH from 4.0 to 5.5 were tested.In this example, pH 4.0 – pH 5.0 are in principle suited for coupling of the ligand since they trigger a sufficient accumulation. In contrast, pH 5.5 is not suited for immobilization.

Here, we demonstrate how a quick thermal stability screening of immobilization conditions for SPR assays can dramatically accelerate the assay development procedure.

By using Tycho NT.6, different buffers can be tested within minutes to unambiguously identify conditions under which the ligand protein is stable or when it unfolds, saving hours of experimentation testing a potentially unfolded or inactive protein sample and preventing the unnecessary costs and waste of biosensor chips.

Results

To demonstrate the versatility and applicability of Tycho NT.6 to improve SPR assay development, two different classes of proteins were selected. One protein was a kinase, which are popular drug targets and used in small molecule screenings. The other protein was a monoclonal IgG antibody (mAb), which are of interest as therapeutic molecules. The two proteins were resuspended in phosphate buffered saline (PBS) and tested in four standard immobilization buffers containing 10 mM acetate with pH values between 4.0 and 5.5. The kinase showed marked effects of the immobilization buffer on protein conformation (Figure 2A). The PBS sample used as a reference of properly folded protein showed a low initial ratio and a clear unfolding event that is temperature dependent, indicated by the inflection of the unfolding profile curve. At low pH (4.0 and 4.5), the kinase was entirely unfolded already at the start of the experiment, suggested by the very high initial ratio as well as the absence of an unfolding inflection. At pH 5.0, the initial ratio was intermediate, showing that the kinase was partially unfolded, and an unfolding event was visible, but shifted to much lower temperatures compared to the PBS sample, signifying a destabilization of the kinase. Only pH 5.5 showed favourable conditions: the initial ratio was similar to the PBS sample, suggesting that the kinase was properly folded. These results suggest that pH 5.5 acetate buffer is the only buffer to be considered for immobilization tests for this kinase.

In contrast to the kinase, the mAb showed no major effect of the immobilization buffer on conformational stability as indicated by similar initial fluorescence ratios of all samples.

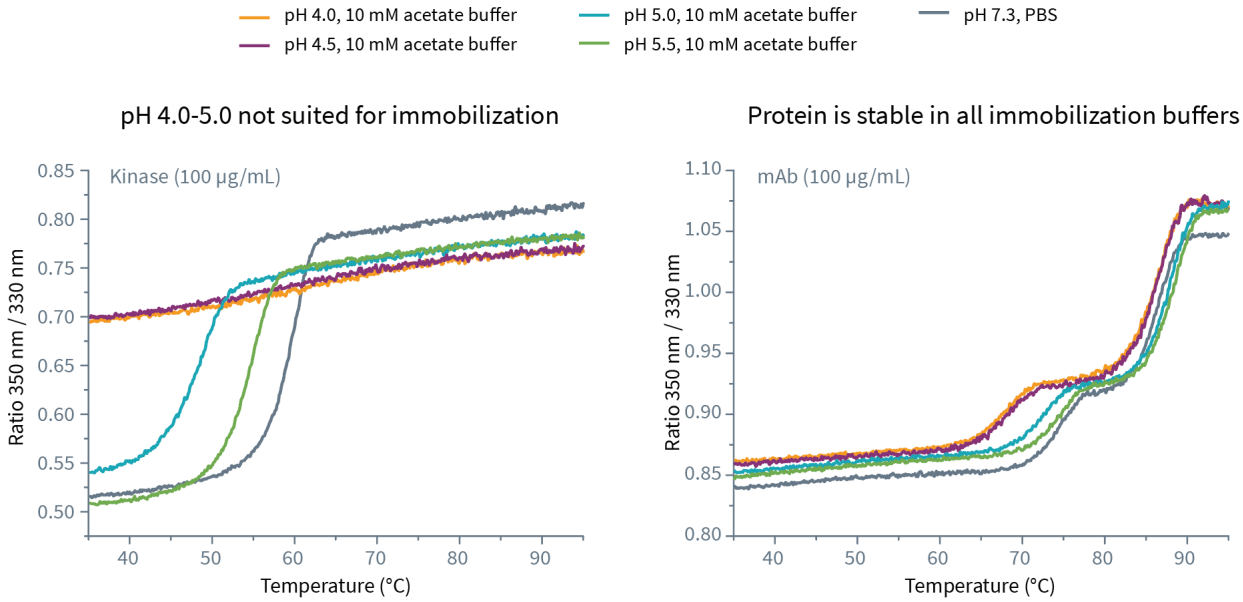

Figure 2 Quick evaluation of immobilization buffer conditions with Tycho NT.6. Two proteins were tested in standard SPR immobilization buffers and in PBS as a reference. A kinase (left) is fully denatured at pH values 4.0 and 4.5, indicated by the high initial fluorescence ratio and the absence of an unfolding event. The protein is partially denatured at pH 5.0, indicated by the increased initial ratio and the shifted unfolding event when compared to the reference sample. The best option for an immobilization buffer to be tested in SPR which guarantees the structural integrity of the kinase is the buffer with pH 5.5. In contrast, a mAb (right) is stable in all tested immobilization buffers.

Discussion

In summary, the effect of immobilization buffers on different types of proteins typically analyzed in SPR assays is quickly and easily assessed using the Tycho NT.6. Conditions that can impact the folded state of the protein can be identified with the system, which provides insight into SPR assay development and testing. Experimental runs on the Tycho NT.6 take three minutes, requires minimal sample handling and use less than 10 µL of sample material. This translates into obtaining results faster (~10x) than using conventional pH scouting experiments. Tycho NT.6 uses single-use capillaries to run experiments and affords significant cost savings (~60-fold less) compared to a standard biosensor chip.

Methodology	Measurement Time (minutes)	Protein Required Per Test	Experimental Cost
Tycho NT.6	3	0.5-1 µg	2€ / 2.40USD
SPR*	38	5-10 µg	125€ / 147USD

***Biacore CM5-Chip (standard)**

Table 1. Time, sample and cost-savings using the Tycho NT.6 over standard SPR assay development.

The Tycho NT.6 provides significant time and cost savings as well as using very low amounts of material to enable researchers to make better decisions in optimizing biosensor assay development and testing.