# Improving biosensor assay development by determining sample quality with Tycho NT.6

Surface plasmon resonance (SPR) is an optical methodology widely used to detect and quantify molecular interactions. SPR is considered the gold standard for quantification of protein interactions, but SPR assay optimization can be technically challenging, time-consuming and costly. Tycho™ NT.6 quickly analyzes different conditions typically tested during optimization of an SPR assay. The Tycho™ NT.6 system is simple to use and enables researchers to make better-educated decisions in developing and optimizing their binding-interaction assays.



SPR and other biosensor-based analytical methods are standard tools used in academic and pharmaceutical research laboratories for the quantification of protein interactions. Although considered the gold standard by many scientists, the method can be technically challenging, time-consuming and costly to perform. The Tycho NT.6 system quickly monitors protein quality at any stage of a protein-characterization workflow and enables researchers to better understand the integrity or conformation of the protein sample they are working with before they commit to more complex experiments that analyze protein interactions.

SPR and similar biosensor-based methods typically require one binding partner, the ligand, to be covalently immobilized on the surface of a biosensor chip. The other binding partner, the analyte, is presented in solution, and the binding kinetics are measured. Covalent immobilization of proteins to the biosensor surface is often problematic, because acidic, salt-free buffers are required for optimal coupling by lysine chemistry. These harsh conditions can negatively influence protein integrity and function. Therefore, optimization of immobilization conditions often represents an early bottleneck in SPR assay development, as it can damage the ligand during coupling, resulting in a nonfunctional biosensor surface and even a wasted biosensor chip.

Procedures such as 'pH scouting' and 'pre-concentration' are used to identify immobilization conditions that can trigger sufficient surface attachment of the ligand (**Fig. 1**). In these experiments, different ligand-containing immobilization buffers with varying, low pH values 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 one to two hours to both prepare and perform and identifies immobilization conditions without providing information on the functionality or integrity of the immobilized protein. If the pH treatment conditions used for immobilization result in unfolding of the protein (either partial or complete), the protein can be rendered inactive. This will be revealed only after negative or questionable results are obtained in subsequent binding experiments. Ultimately, the researcher will have to discard the biosensor chip and repeat the optimization experiments. Although standard procedures for immobilization are well established for certain types of proteins, the

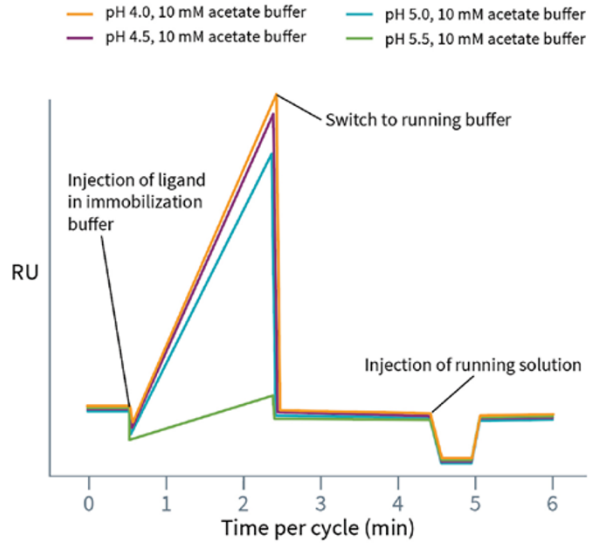


**Figure 1 |** Lower-pH buffer conditions are better for immobilization of ligand to the SPR biosensor chip, as determined by pH scouting experiments. The greater the resonance value (measured in resonance units (RU)) after injection of a ligand solution, the better the accumulation of the ligand on the chip surface owing to electrostatic interactions. Buffers ranging in pH from 4.0 to 5.5 were tested. This example demonstrates that pH 4.0–5.0 buffers are in principle suited for coupling of the ligand, as they trigger sufficient accumulation. In contrast, the pH 5.5 buffer is not suited for immobilization.

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general process is time-consuming and bears the risk of irreversibly damaging the ligand on the chip surface, thereby wasting costly consumables.

Here we demonstrate how swift, label-free, thermal-stability screening of immobilization conditions for SPR assays can dramatically accelerate and efficiently improve the SPR-assay-development procedure.

With Tycho, different buffers can be tested within three minutes to unambiguously identify conditions under which the ligand is stable or unfolds, which can save hours of experimentation spent testing a potentially unfolded or inactive protein sample and prevent the unnecessary costs and waste of biosensor chips.

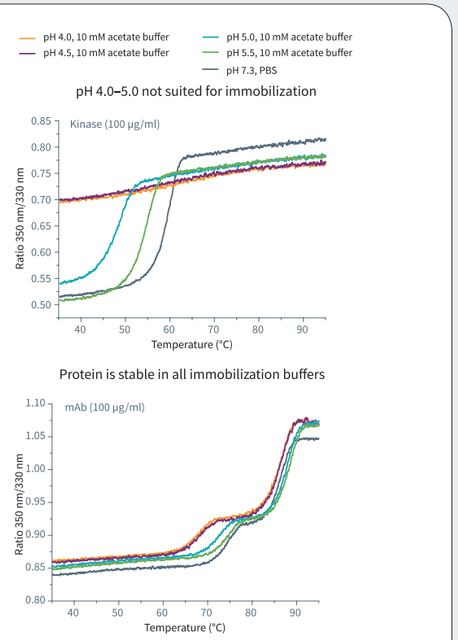


**Figure 2 |** Quick evaluation of immobilization buffer conditions with Tycho NT.6. Two proteins were tested in standard SPR immobilization buffers, or in PBS as a control. The kinase (top) was fully denatured at pH 4.0 and 4.5, as indicated by the high initial fluorescence ratio and the absence of an unfolding event. The protein was partially denatured at pH 5.0, as indicated by the increased initial ratio and the shifted unfolding event compared with that of the control sample. The preferable option for an SPR immobilization buffer that guarantees the structural integrity of the kinase is the pH 5.5 buffer. In contrast, the mAb (bottom) was stable in all tested immobilization buffers.

## Quick screening of buffer and pH conditions with Tycho

To demonstrate the versatility and applicability of Tycho in improving SPR assay development, we selected two different classes of proteins. One protein was a kinase, a type of enzyme that is a popular drug target and is used in small-molecule screenings. The other protein was an IgG monoclonal antibody (mAb); mAbs 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 (**Fig. 2**). The PBS sample used as a reference for a properly folded protein showed a low initial ratio and a clear unfolding event that was temperature dependent, indicated by the inflection of the unfolding profile curve. At low pH (4.0 and 4.5), the kinase was entirely unfolded at the start of the experiment, as indicated by the very high initial ratio and the absence of an unfolding inflection. At pH 5.0, the initial ratio was intermediate, which suggested that the kinase was partially unfolded, and an unfolding event was visible but shifted to much lower temperatures compared with those for the PBS sample, signifying destabilization of the kinase. Only pH 5.5 appeared to be a favorable condition: the initial ratio was similar to that for the PBS sample, which suggested that the kinase was properly folded. These results indicate that pH 5.5 acetate buffer is the only buffer that should be considered for immobilization tests for this kinase.

In contrast to the kinase, the mAb example protein showed no major effect of the immobilization buffer on conformational stability, as indicated by the similar initial fluorescence ratios of all samples (**Fig. 2**).

**Table 1** | Time and sample-consumption savings with the Tycho NT.6 compared with a standard SPR biosensor assay

| Methodology | Measurement time (min) | Amount of protein required per test (µg) |
|---|---|---|
| Tycho NT.6 | 3 | 0.5–1 |
| SPR biosensor | 38 | 5–10 |

The Tycho NT.6 provides substantial savings in time and costs and uses very low amounts of material to enable researchers to make better decisions in optimizing biosensor assay development and testing.

In summary, the effect of immobilization buffers on different types of proteins typically analyzed in SPR assays is quickly and easily assessed with the Tycho NT.6 system. Conditions that can affect the folded state or structural integrity of the protein being studied can be identified with the system, which provides insight into SPR assay development and testing. Experimental runs on the Tycho take three minutes, require minimal sample handling and use less than 10 µl of sample material. This allows researchers to obtain results faster (~10×) than in conventional pH scouting experiments (**Table 1**). Tycho uses single-use capillaries for experiments, thereby potentially providing cost savings and easier sample handling than in experiments using a standard biosensor chip.





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