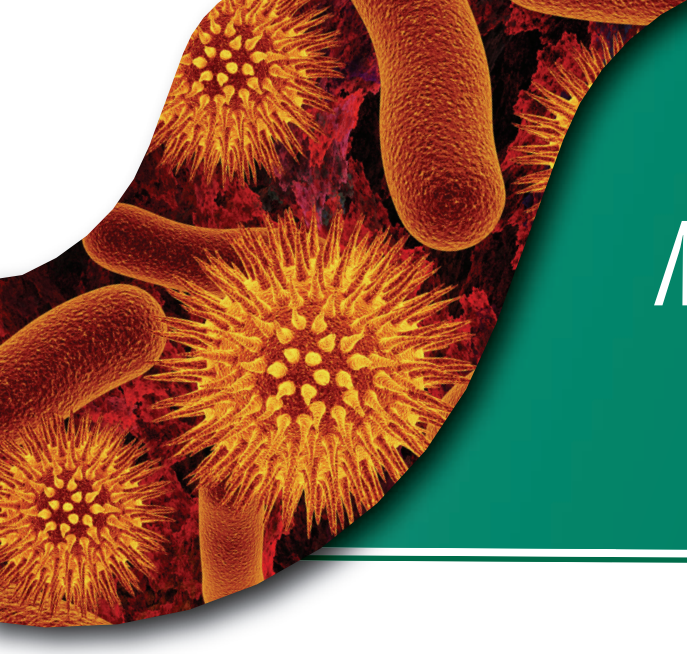



# Real-Time Binding Studies Using Viable Cells

Mihaela Stumbaum and Uwe Reusch PhD

The binding of proteins to their target molecules is of interest to anyone studying biological processes, from cellular function and organism development, through to human disease and drug discovery. Several approaches exist to investigate and measure protein binding, including ELISA, Western blot, isothermal titration calorimetry, KinExa, Surface Plasmon Resonance (SPR) and flow cytometry.

While each approach has its advantages and disadvantages, many suffer from technical limitations that impact on the biological insight they can provide. For example, many traditional methods involve labelling a protein of interest with radioisotopes or fluorescent tags, so that binding events can be tracked and measured. However, such labels may inhibit normal protein-target interactions in vitro, calling into question how well these data represent normal interactions in vivo. Also, for many of these methods, the reaction being investigated must be allowed to reach equilibrium before reliable binding kinetics can be ascertained. This means that experiments can be lengthy to perform and that the temporal resolution provided may not be fine enough for many applications.

To circumvent this argument, many methods have been developed that do not require the use of labels. For example, SPR utilises an optical approach to detect binding events, without the need for any labelling, by measuring changes in refractive index. As SPR uses light to detect binding events, it also requires that the sample mixture passing over the sensor be relatively simple and clean. For this reason, SPR can only be used with purified receptors and artificial membrane fractions, whereas deeper biological insight would be provided by being able to work with intact, viable cells.

## Real-time binding analysis using viable cells

sam® biosensors developed by SAW Instruments now offer a different approach, using surface acoustic waves (SAWs) instead of light to detect binding events. The phase and amplitude of the acoustic waves are sensitive to changes in mass loading and liquid viscosity respectively at the surface of the sensor chip, allowing biomolecular interactions to be detected with a high level of sensitivity.

This method also provides real-time data, rather than relying on equilibrium-based models, and can be used for the study of viable cells, vesicles, antibodies, enzymes and even low molecular weight (LMW) compounds (<200 Da in size), without the need for direct labelling. Molecular conformational changes, such as those that occur when a LMW ligand binds to its target site, can also be detected.

Recently, researchers at SAW Instruments and Affimed Therapeutics collaborated to investigate the interaction between a therapeutic (bispecific) antibody fragment and its tumour cell surface target *(Figure 1)*. By using the SAW approach, it was possible to carry out this study using viable cells, rather than purified receptors or membrane fragments.


*Figure 1. The sam®X biosensor for performing kinetic binding analysis (left). TandAb (bispecific) antibodies bind to tumour cell antigens, mediating effector and target cell interaction (right).*

## Methods

In order to study the binding of an Affimed TandAb (bispecific antibody) ligand to its target antigen, a sam biosensor system and sam short-chain CM Dextran (2D surface) sensor chips were used. These chips utilise state-of-the-art microfluidics, thereby allowing one chip to house multiple sensor elements. The TandAb ligand was incubated in 10 mM acetate buffer (pH 5) and immobilised on the surface of the sam sensor chip. Freshly cultured tumour cells in suspension, positive for the antigen of interest, were injected directly from the autosampler at a concentration of 10,000 cells per mL in phosphate buffered saline solution (PBS, pH 7.4). This allowed them to be captured on the immobilised ligands via the fluidic system. Different concentrations of TandAb ligand were injected and multiple experiments conducted, so that a concentration analysis could be performed and an accurate affinity constant ($K_D$) calculated based on a 1:1 binding model.

## Results

Using the sam biosensor, it was possible to assay ligand-target binding in real-time, on viable captured cells *(Figure 2)*. When the cells were injected into the system (grey bars), a phase change was detected, indicating that capture was taking place. The analysis in *Figure 2A* shows the binding curve observed when using the bispecific antibody detection ligand at a concentration of 15 nM. However, in order to calculate an accurate $K_D$ for the ligand-target interaction, the analysis was extended to include the use of TandAB ligands at a range of concentrations between 5 and 40 nM *(Figure 3)*.

Using the data generated by the concentration analysis, the association rate constant ($k_{on}$) and dissociation rate constant ($k_{off}$) for the ligand-target interaction were determined *(Figure 4)*. These data were then used to calculate the $K_D$ value for the on-cell interaction, which in this case was $K_D$ = 2 nM. This result was independently confirmed using laborious flow cytometric analysis, which is limited in that it uses labels and can only resolve steady-state $K_D$ values.

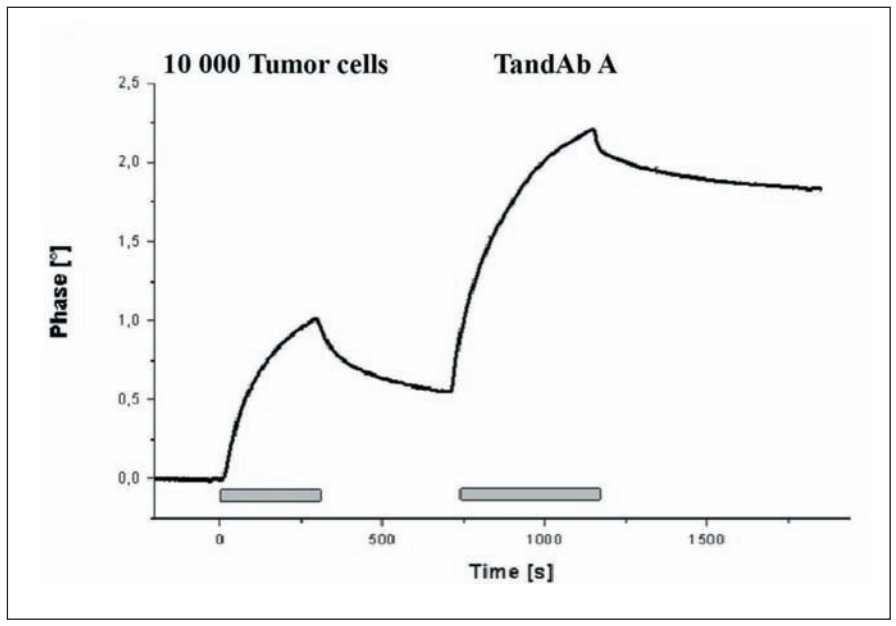


*Figure 2. Binding curve of 15 nM TandAb to antigen positive tumour cells (10 000 cells/mL).*

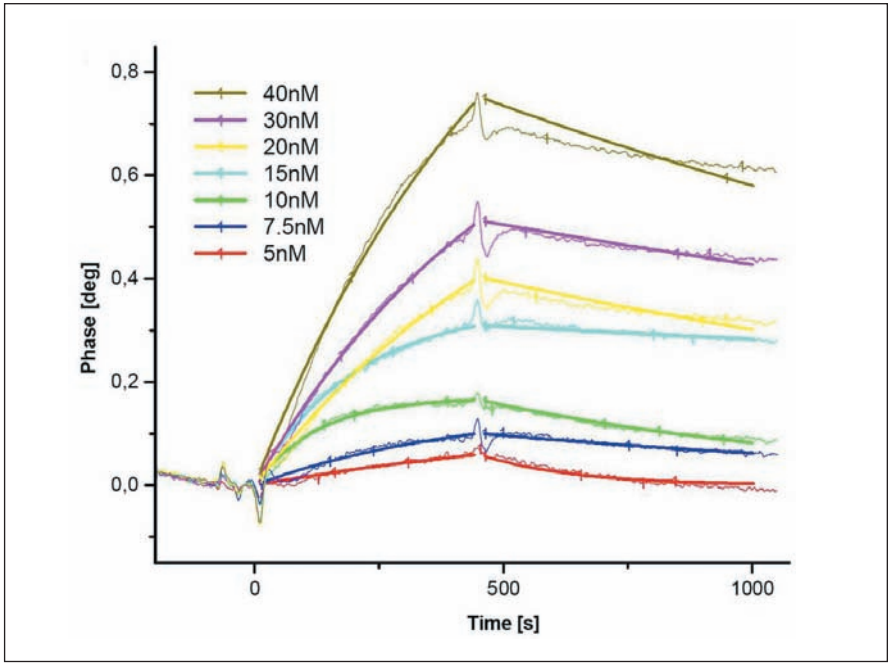


*Figure 3. Overlay plot illustrating TandAb binding data at different ligand concentrations, with fits based on a 1:1 binding model.*

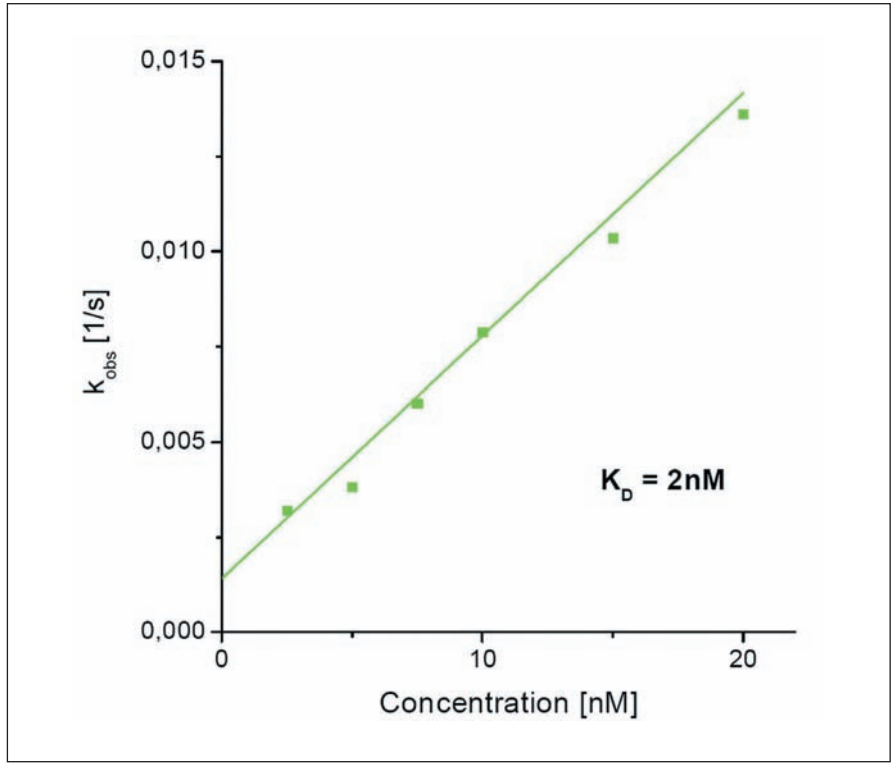


*Figure 4. Evaluation of TandAb antibody binding to antigen positive tumour cells using FitMaster data analysis software (Origin 8.5-based), part of the sam biosensor workstation.*

## Conclusions

The data presented here illustrate that the use of acoustic waves is a powerful tool for carrying out label-free kinetic binding analysis using viable mammalian cells. The results obtained during this study have also been confirmed using more traditional methods, suggesting that the system is robust and accurate enough for widespread adoption. Similar findings have also been produced by other customer projects conducted by Saw Instruments, illustrating that sam biosensors can be used to accurately measure on-cell affinity constants, over a large spread of concentrations, using viable cells.

The rapid speed (minutes rather than hours) of the assay means that freshly cultured, un-fixed cells can be analysed before any internalisation of surface antigens can take place. Such experiments open up a brand new range of applications for researchers interested in molecular interactions, many of which have previously been difficult or impossible to undertake using traditional techniques. These include the use of living cells for investigating target molecule accessibility, bispecific antibody functionality and receptor co-localisation.

Such studies can be conducted using cell suspensions, such as in the capture assay described, or adherent cells, which can be cultured and grown directly on the sensor chip surfaces. Whatever the application, the benefits offered by using acoustic waves make them an ideal solution for investigating molecular interactions using living cells.

## About the authors:

Mihaela Stumbaum is an Applications Scientist at Saw Instruments, Bonn, Germany, and Uwe Reusch PhD is Head of Cell Culture at Affimed Therapeutics AG, Heidelberg, Germany. For more information please contact Ian Taylor PhD, Head of Marketing and Sales for SAW Instruments (taylor@saw-instruments.com) +44 (0) 7554 446 185.