GE Healthcare
Life Sciences

A year of interaction with Biacore™ X100

Protein interaction research

Real-time monitoring of binding events using surface plasmon resonance (SPR) gives a deep understanding of the dynamics of molecular interactions. Analysis using Biacore systems allows for straightforward and rapid data generation, which leads to reliable conclusions and enables the design of new innovative studies. Biacore systems, used extensively in academic research worldwide, are cited in more than 12 000 peer-reviewed scientific publications.

Biacore X100 system

Biacore X100 (Fig 1) is an automated and versatile system for comprehensive, label-free analysis and characterization of biomolecular interactions in real-time. The high sensitivity and flexible analysis opportunities of Biacore X100 enable highly accurate kinetic and mechanistic characterization, affinity determination, concentration measurements, and detection of weak and transient binding events. The versatility of the Biacore X100 system makes it highly suitable for many different assay formats and the study of a wide variety of biomolecules including small molecules, proteins, DNA, and cells. Interactions can be measured in buffer solutions or in crude environments such as serum. These features enable Biacore X100 to support scientists in most areas of life science research, such as disease research, immunology, virology, and neuroscience.

Fig 1. Biacore X100 is an integrated system for rapid and reliable generation of molecular interaction data.

Extensive support from assay development to data interpretation

Biacore X100 integrates all the components needed to provide reliable molecular interaction data from day one. Automated instrumentation collects high quality data from small amounts of sample while minimizing the hands-on time. The software is workflow-oriented and provides a structured yet flexible framework for assay development and data interpretation. A broad range of consumables is available for various assay alternatives. Biacore sensor chips and kits for ligand capturing are supported by pre-programmed workflows in the Biacore X100 software and flexibility is provided through a number of software wizards (Fig 2). The system software helps you build expertise as you work, with an integrated support functionality that provides assay tips and guidelines. In addition, all Biacore X100 users have access to e-learning tools and an extensive methodology knowledge database on our Web site (www.gelifesciences.com/biacore).

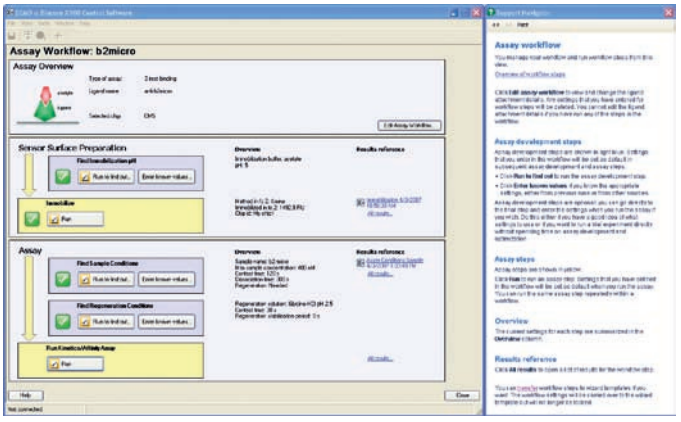


Fig 2. Guided workflows and software wizards provide support from assay development to data interpretation, promoting the development of your expertise and reducing the time to result.

Biacore X100 during a year of interaction research

In this paper we showcase a few examples of how an academic research group has used Biacore X100 in their research during a period of one year in order to:

- Investigate inhibition of viral RNA-protein interactions
- Confirm and characterize protein-protein interactions involved in cellular signaling
- Explore the interaction kinetics of different neural calcium sensor proteins
- Compare interactions of monoclonal antibodies with different antigen variants

Investigation of the inhibition of viral RNA-protein interactions by allosteric ligands

Several ways of treating hepatitis C virus (HCV) infections are currently explored. This study investigated the mechanisms involved in blocking RNA synthesis by the viral polymerase using substances inhibiting this process. The interaction between the HCV polymerase and a single-stranded RNA strand was assessed in the presence of two allosteric inhibitors (filibuvir and VX-222) using Biacore X100 biosensor analysis. Biotinylated RNA was captured on a CM5 sensor chip via immobilized streptavidin to a level of 200-240 RU. The HCV polymerase was injected with and without inhibitor and the interaction was monitored. The presence of either filibuvir or VX-222 clearly decreased the maximum signal level (Fig 3). This is interpreted as the inhibitors interfering with, but not completely blocking, the polymerase-RNA interaction. The observation was independent of injection order.

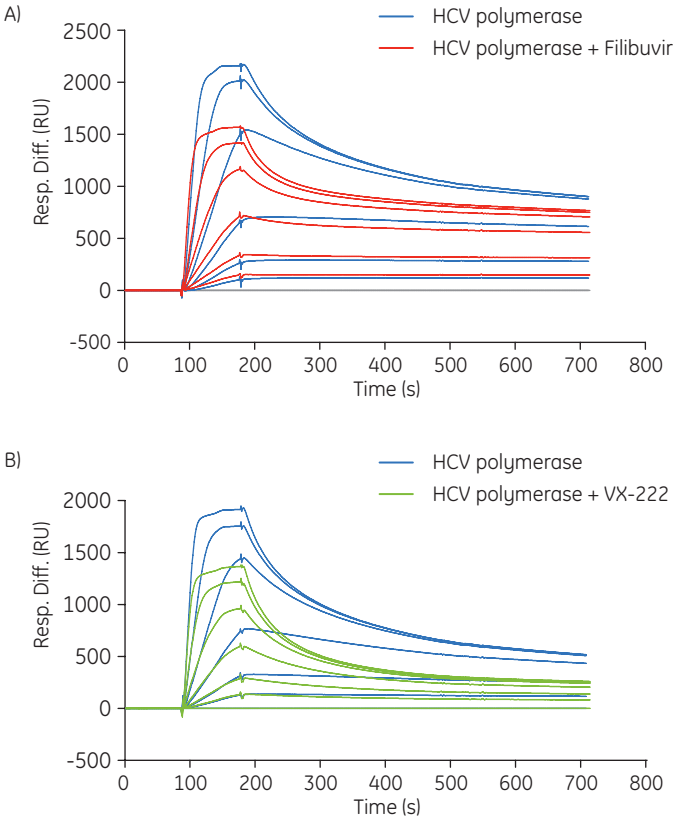


Fig 3. Sensorgrams representing the interaction between HCV polymerase and immobilized RNA in the absence and presence of inhibitor. (A) 240 RU of RNA was captured via streptavidin and HCV polymerase with 300 nM filibuvir was first injected, followed by HCV polymerase separately. (B) 200 RU of RNA was captured via streptavidin and HCV polymerase with 900 nM VX-222 was first injected, followed by HCV polymerase separately. The injection order did not influence the results.

The curves were not well described by any basic interaction model, suggesting that the interaction is complex. It was also noted that when injecting only the polymerase, the interaction signal was higher than the predicted maximum response if a 1:1 binding interaction is assumed. This can be explained by multiple enzymes binding to the same RNA strand, or by aggregation or oligomerization of the HCV polymerase, all of which could contribute to a complex interaction mechanism. To validate the results, the interaction set-up was reversed with the HCV polymerase immobilized on a CM5 surface (~2600 RU) and injection of a concentration series of non-biotinylated RNA, first without and then with added inhibitor (300 nM of VX-222 or filibuvir) (Fig 4). The maximum signal was again observed to be reduced by the presence of either of the inhibitors. This experiment confirms that both filibuvir and VX-222 interfered with the ability of the HCV polymerase to interact with RNA, with VX-222 having a slightly larger effect.

This study demonstrates that these two allosteric inhibitors, currently in clinical trials, interact with the HCV polymerase and thereby interfere with its ability to bind its substrate RNA. These insights will have important consequences for the future selection and optimization of new allosteric HCV polymerase inhibitors.

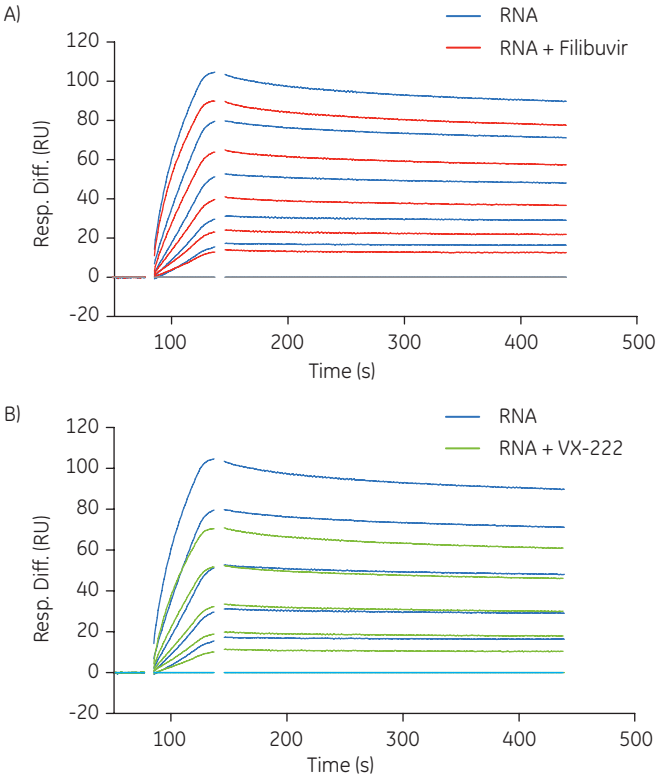


Fig 4. Sensorgrams representing the interaction between immobilized HCV polymerase and RNA in the absence and presence of inhibitor. (A) RNA in the absence and presence of 300 nM filibuvir. (B) RNA in the absence and presence of 300 nM VX-222. RNA concentrations were 8, 4, 2, 1, and 0.5 µg/ml. Sensorgrams are blank and reference subtracted.

Confirmation and characterization of protein-protein interactions involved in cellular signaling

Tumor growth and differentiation depends to a large extent on cell-to cell signaling pathways mediated by excreted ligand proteins from proliferating cancer cells. Through a combination of proximity ligation analysis (PLA) and mass spectrometry (MS) analysis, new and important candidate interactions between different receptors and ligand proteins involved in tumor development have been identified. Since the identification of interactions using PLA reflects the detection of a signal raised due to proteins in the vicinity of each other rather than a verification of an actual interaction, Biacore X100 was used to confirm and validate the potential interaction partners. A panel of different kinetic and affinity experiments was set up in order to complement the data acquired by the PLA studies. Standard amine coupling on a CM5 sensor chip was used in all analyses. The matrix of the experimental setup is shown in Table 1 and corresponding sensorgrams in Figure 5.

Table 1. Combinatorial matrix of the SPR analysis

Immobilized	Analyte	Figure
Protein X	Protein Y	5A
Protein X	Pro-peptide of protein Y	5B
Pro-peptide of protein Y	Protein Y	5C

Protein Y is involved in several processes in the body. It is synthesized as a precursor protein (pro-protein Y) which undergoes post-translational modifications and intracellular processing before it is secreted from the cell in its mature form. Protein X is known to be up-regulated in certain types of cancer. Protein Y has been used in attempts to block the function of protein X. However this gave rise to serious side effects in the clinic.

Biosensor studies show a clear interaction between proteins X and Y (Fig 5A). This interaction could not be described by a simple 1:1 binding interaction model, but by a 2-state model, indicating a possible conformational change upon interaction. If this is the case, needs to be further investigated. As controls, it was demonstrated that the pro-peptide of protein Y interacted only weakly with protein X (Fig 5B), and with the mature form of protein Y (Fig 5C). These experiments confirm the interaction between protein Y and protein X, originally observed by PLA. They also localize the binding site for protein X to the domain corresponding to mature protein Y. The combined data demonstrates how Biacore X100 can be used to confirm and characterize protein interactions hypothesized with other technologies.

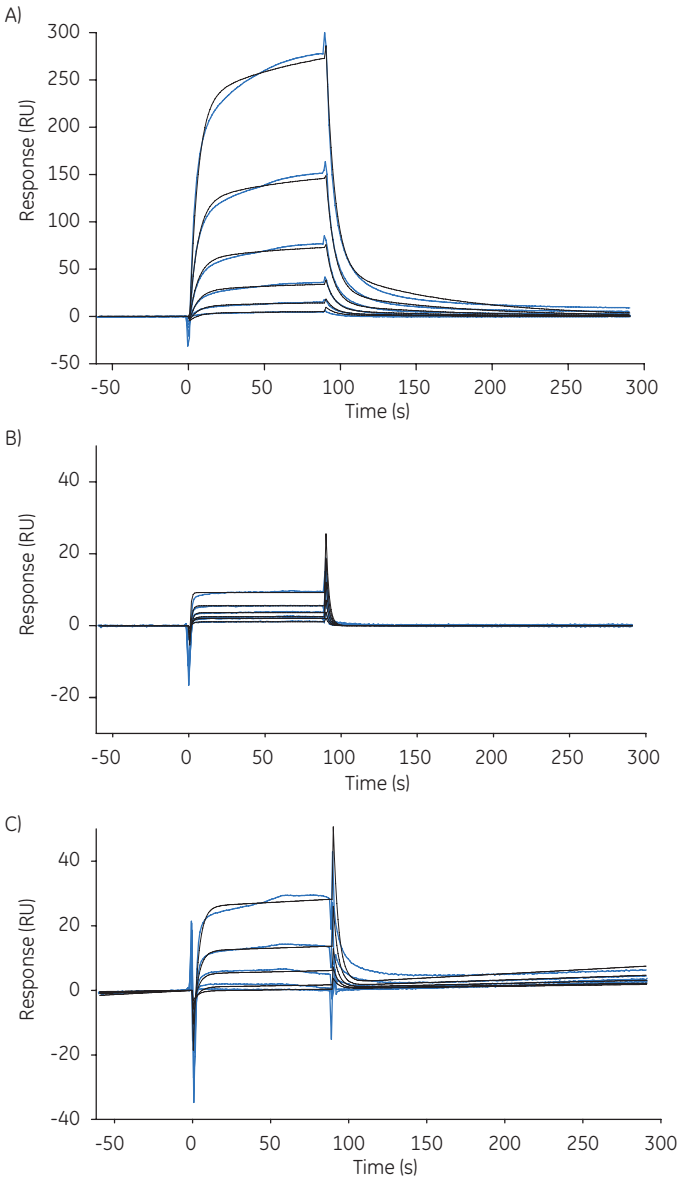


Fig 5. Sensorgrams showing kinetic analysis between the investigated cell-signaling proteins. (A) X and Y, (B) X and the pro-peptide of protein Y, and (C) Y and its pro-peptide.

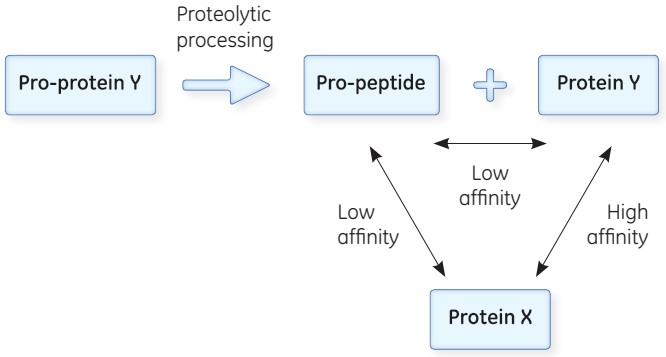


Fig 6. Schematic illustration of studied interactions between protein X, protein Y and the pro-peptide of protein Y.

Exploration of the mechanisms and kinetics of neural calcium sensor proteins interacting with a scaffolding protein

This study by Seeger *et al* (1) was undertaken in order to investigate the kinetic and mechanistic details of the interactions between calmodulin and caldendrin with A-kinase anchoring protein 79 (AKAP79) and the influence of calcium ions on these interactions. AKAP79 was immobilized on a Biacore CM5 sensor chip at surface densities of 5000-8000 RU. The interaction kinetics of calmodulin with AKAP79 could be well described as a reversible 1-step interaction. The binding between caldendrin and AKAP79 was more complicated to address.

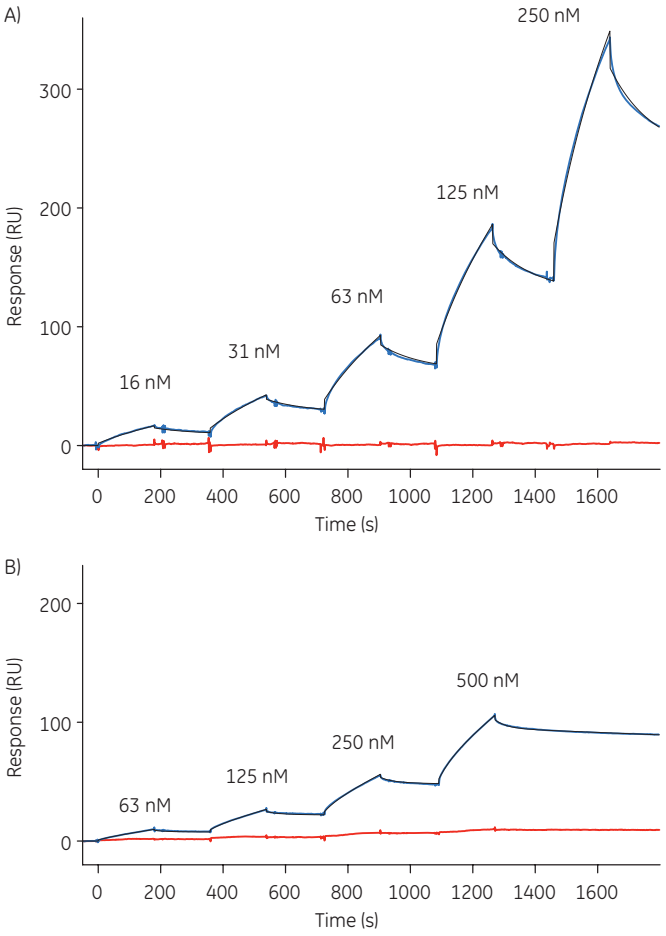


Fig 7. Sensorgrams showing singel cycle kinetic analysis of AKAP79 interactions with A) caldendrin with calcium B) caldendrin without calcium. Red curves show a negative control analyzed under the same conditions. Overlaid black lines represent theoretical best fit sensorgrams using a 2-state interaction model.

No suitable regeneration condition for the interaction with caldendrin could be established with a calcium free buffer. Therefore a single-cycle kinetics approach proved to be highly suitable, as this type of assay does not necessarily require a regeneration step (Fig 7). Using this approach it was determined that the binding of caldendrin to AKAP79 also showed calcium dependency, but because the binding was reduced up to 10-fold it was not dependent to the same extent as calmodulin. When the kinetics of the interaction were analyzed it became clear that this interaction could not be described as a 1:1 biding event, but data could be fitted to a conformational change model. A series of control experiments verified that the binding most likely was a 2-state interaction involving a conformational change of the proteins during the binding event (1). Competition experiments were performed to investigate caldendrin binding to AKAP79 in the presence of calmodulin (Fig 8). The results suggest that calmodulin effectively blocks caldendrin binding to the anchoring protein.

The present study confirms that caldendrin and calmodulin interact with AKAP79 at overlapping binding sites but exhibit different interaction characteristics, with caldendrin being able to form a stable complex even in the absence of calcium.

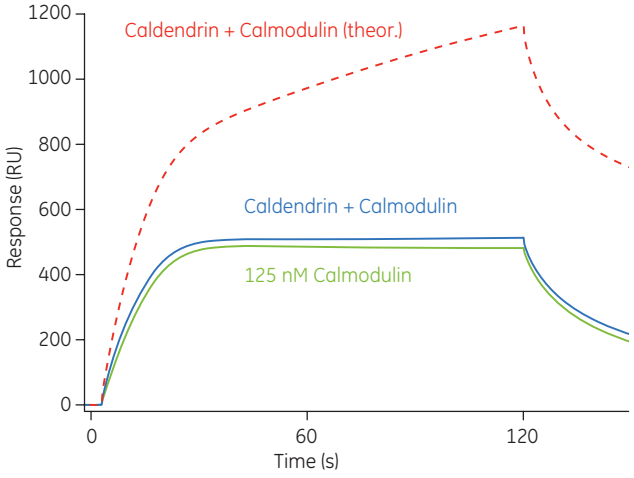


Fig 8. Competition analysis between calmodulin and caldendrin binding to AKAP79. Calmodulin was injected in the presence (red) and absence of caldendrin (blue). The red dashed curve is a simulation of the theoretical signal under non-competitive conditions. Experimental analysis was performed using a Biacore S51 system.

Comparison of interactions between a monoclonal antibody and three variants of an antigen

The characterization and mapping of antigen-antibody interactions is of major importance for the understanding and selection of candidate epitopes and monoclonal antibodies. These studies are normally based on analysis of binding specificity and affinity. With Biacore X100 both these features as well as binding kinetics can be analyzed using single-cycle kinetics. In this study the interaction of a monoclonal antibody was investigated against three variant forms of an antigen (AgVar1, AgVar2, and AgVar3) in order to distinguish any selection preferences of this antibody. For the purpose of this study the antibody was immobilized using standard amine coupling on a CM5 sensor chip.

The data from the Biacore X100 experiment showed that the monoclonal antibody had a selection preference to only one of the variant antigens (AgVar2) and no binding to either of the other variant antigens (Fig 9).

The interaction between AgVar2 and the antibody showed signs of being heterogeneous. The significance of this is yet unknown, but it is clear that the antibody recognizes epitopes on AgVar2 that are not present on the other antigen variants analyzed in this study.

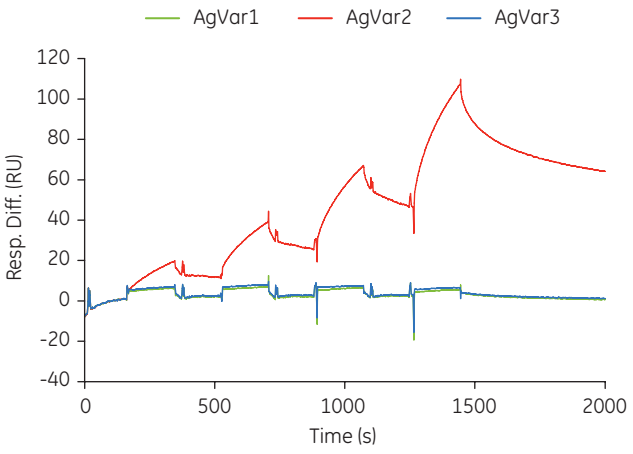


Fig 9. Single-cycle kinetics analysis of the interaction between the monoclonal antibody and AgVar1, AgVar2, and AgVar3. Data was analyzed using a heterogeneous ligand interaction model.

Summary

Biacore X100 is a comprehensive solution for biochemistry, molecular biology, and other research areas involved in the study of molecular interactions. The system contains the key functionalities needed for day-to-day molecular interaction research with the purpose of understanding protein function and biological mechanisms.

Biacore X100 offers versatility and flexibility, in combination with an application-tailored software, enabling the system to be used for a broad range of applications, including structure-function studies, pathway analysis, drug target identification and validation, and assay development.

Acknowledgements

Data provided courtesy of Professor Helena Danielson Department of Chemistry-BMC, Uppsala University, Uppsala, Sweden.

References

1. Seeger, C. *et al*. Kinetic and mechanistic differences in the interactions between caldendrin and calmodulin with AKAP79 suggest different roles in synaptic function. *J Mol Recognit*. **10**, 495-503 (2012).

Ordering information

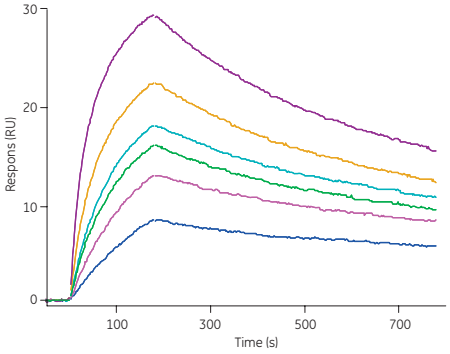
Product	Code number
Biacore X100 Processing Unit	BR-1100-73
Biacore X100 Plus package	BR-1007-98
Sensor chip CM5	BR-1003-99

Binding strength and kinetics with Biacore systems

Biacore systems monitor molecular interactions in real-time using surface plasmon resonance (SPR). Without the need for labels, Biacore assays provide information on affinity, kinetics, and specificity of molecular interactions. The active concentration of biomolecules can also be determined.

One of the interacting molecules is immobilized onto a sensor surface, while the other molecule flows over the sensor surface in solution. Any interaction between the two is detected in real-time via changes in mass concentration close to the sensor surface and binding data is presented in a sensorgram where SPR responses in resonance units (RU) are plotted versus time.

The formation and dissociation of complexes are followed during the course of an interaction, with the binding kinetics (k_a, k_d) revealed by the shape of the binding curve.



More information can be found at **www.gelifesciences.com/biacore**

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