

Label Free Cell Based Biosensors - Bridging the Gap between Biosensors and Cell Based Assays.

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Abstract

As the cost and risk involved in developing new pharmaceuticals continue to increase and tougher regulatory requirements are put in place, the need for technologies that accelerate the process of drug discovery becomes more urgent. In this article, we describe four instances of the use of Attana biosensors to obtain data that increases our understanding of molecular interactions and enhances our ability to select drug candidates based on physiologically relevant information:

- Characterization and selection in human sera and crude samples
- Impact of experimental conditions on binding characteristics
- Effect of off-target and label-molecules on binding profile
- Characterization of analyte aggregation

Introduction

The rise of biologics as therapeutic agents has resulted in the need for technologies that can be used to elucidate their properties. Here we present four methods based on Quartz Crystal Microbalance (QCM) technology developed by Attana is capable of characterizing molecular interactions in human sera and directly on cell surfaces. This approach can contribute with two important pieces of the puzzle to achieve a more successful drug development process, Fig. 1.

Attana's technology is a combination of advanced micro fluidics and a stable and robust technology found in computers and cell phones, Quartz Crystal Microbalance (QCM). QCM is based on the piezoelectric effect, *i.e.* that an electric potential can make a quartz crystal vibrate. The vibration is dependent on the mass of the quartz crystal and molecules bound to the quartz surface. Molecules that bind to the quartz crystal increase the mass and cause a change in the vibration frequency. Measuring this frequency shift provides detailed characterization of the interactions, Fig. 2.

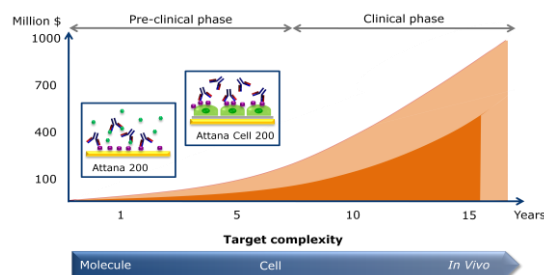


Fig.1. Time, cost and phases when developing new drugs displaying the increased complexity going from 1:1 molecular interaction in laboratories, via cell based assays, to in-vivo and clinical studies. Schematically depicted is the characterization of molecular interactions in human sera and on cell surface providing biologically relevant information, which can contribute to increased success and faster development at a lower cost and risk.

The combination of QCM and advanced fluidics enables characterization in human sera and on cell surfaces. Biologically relevant effects that incorporate the impact of sera components and the influence of cellular environment on the binding characteristics between a receptor and a drug candidate can be obtained.

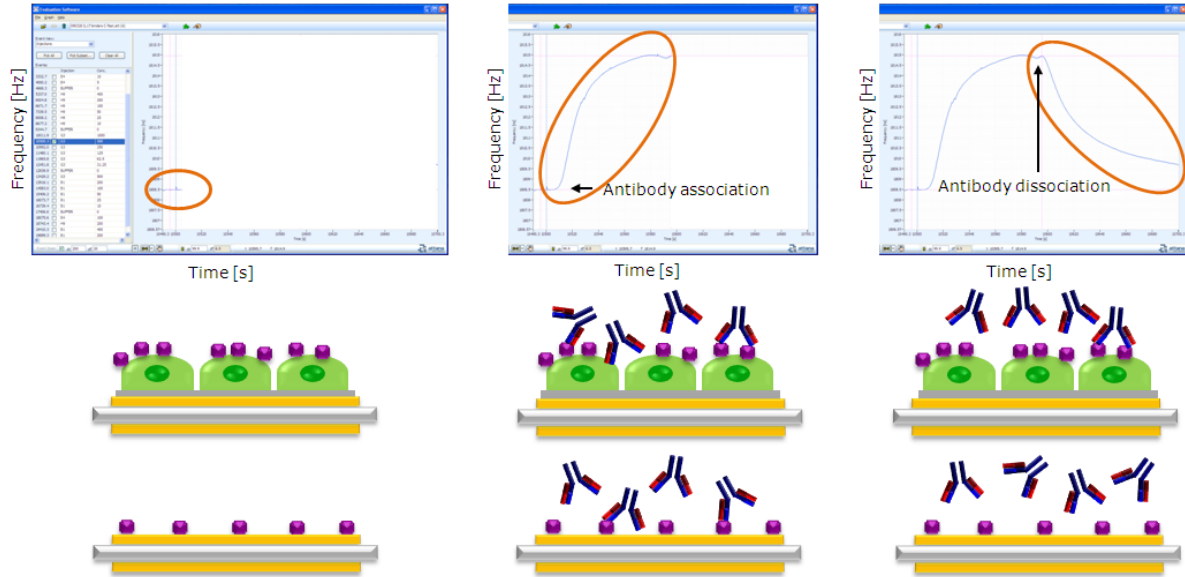


Fig. 2: Schematics of Quartz Crystal Microbalance (QCM) technology enabling cell based biosensors. Molecules that bind to the quartz crystal increase the mass and cause a change in the vibration frequency. Measuring this frequency shift provides detailed characterization of the interactions, revealing association and dissociation rate constants, affinity, concentration, specificity etc. The difference between traditional biochemical/biophysical biosensors on synthetic surfaces and the cell based biosensor is illustrated, where the latter consider natural binding events such as avidity, rebinding, low accessibility and off-target interactions compared to traditional biosensor that is only measuring a 1:1 interaction.

Characterization and selection in human sera and crude samples

Characterization directly in human sera or crude samples has several advantages.

Characterization of antibodies expressed in hybridomas usually requires purification. This includes several time consuming steps and may cause errors from e.g. dilution. The same is valid for characterization in human sera and additional information about sera effects on the binding can be obtained by direct experiments. Inhibiting effects from the sera components can result in exclusion of a candidate antibody even though excellent binding characteristics are observed with purified sample. The opposite effect can also be observed; in many cases protein and antibody binding/therapeutic characteristics are improved in the presence of sera as a result of sera components stabilizing/modulating the binding. Fig. 3 elucidates this influence of sera concentration on affinity and binding rate constants. Normalized binding constants are plotted vs.

sera concentration in the graph. The dissociation rate and affinity are affected one order of magnitude when the sera concentration increases from 0% to 10%.

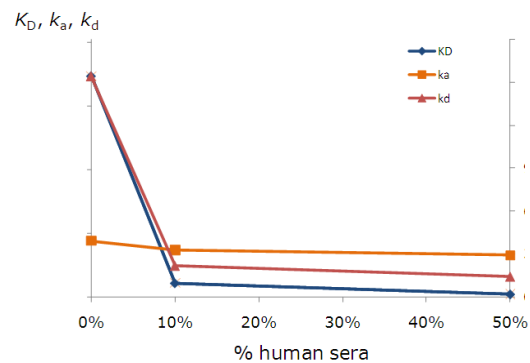


Fig. 3: Affinity, association and dissociation rates vs. sera concentration. An increase in affinity by one order of magnitude is observed with sera concentration increasing from 0 to 10%.

Impact of experimental conditions on binding characteristics

In a traditional biochemical/biophysical biosensor, receptors are chemically isolated and immobilized on a synthetic sensor surface. The sensor surface is designed to enable detailed characterization of molecule to molecule interaction and eliminate all other parameters that might affect the binding characteristics. The receptors are freely accessible, well separated and free from influence by other biological elements. This enables reproducible and detailed information on the one to one interaction between two molecules at the cost of biological relevance. To avoid these limitations, human cells can be used as sensor surface, resulting in the receptors being in a more natural environment. The benefit of this approach is depicted in Fig 4. Dr. Miriam Dwek's group at the University of Westminster, London, are developing tools for early detection of metastatic cancer, targeting the cell surface marker MD1. A-431 and MDA-MB 468 cancer

cells are seeded on the sensor surface and compared to experiments with MD1 receptors immobilized on a synthetic surface.

The impact of experimental conditions on the binding characteristics is clear. The dissociation rate is 100 times slower on the cell surface compared to the synthetic surface.

The cell based experiments suggest that the candidates are good whereas the synthetic experiments rule them out. Which answer is correct?

The cell based approach is in good agreement with other cell based and functional studies. The cell based approach considers the natural clustering of receptors not possible on synthetic surfaces. Receptor clustering on the cell surface causes avidity and rebinding of the candidate molecule to the receptors. Consequently the real dissociation rate and affinity are in the range required of a suitable candidate.

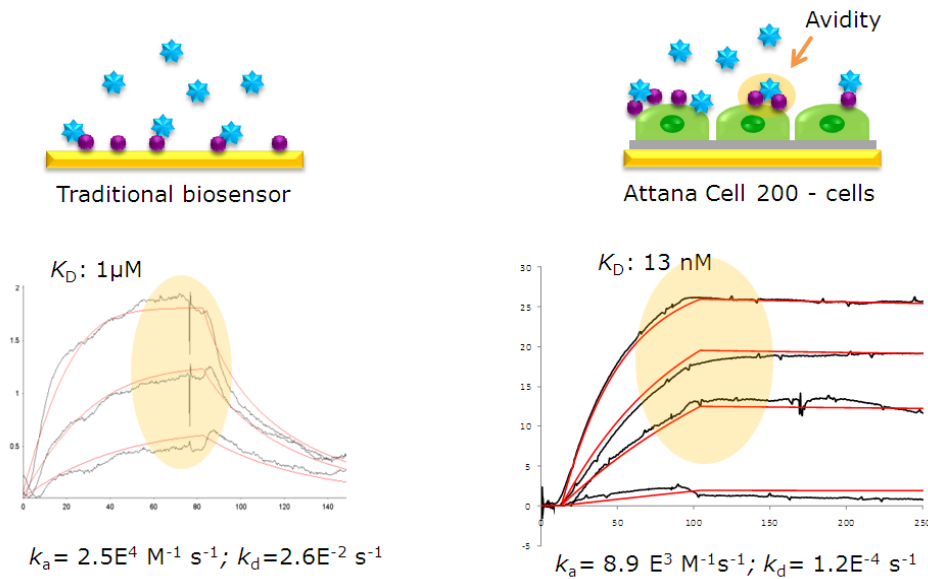


Fig. 4: Characterization of molecular interactions on synthetic sensor surface and directly on cell surface. The difference in dissociation rate and affinity is more than 100 fold and the data from the cell based sensor is in good agreement with other cell based assays and functional assays.

Off-target and label-molecule effect on binding profile

One approach to new therapeutic agent development is the use of variants of approved drugs. Figure 5 depicts an example of this approach by Dr. Sture Lindegren's group at Sahlgrenska University Hospital, Gothenburg. The antibody Herceptin® against HER2 receptor was labeled with the radioisotope ²¹³Bi. When characterizing the original and labeled antibodies with a traditional synthetic biosensor the binding characteristics were similar between the two cases, Fig. 5 A & B. However, when performed in animal studies the labeled antibody showed poor results compared to the unlabeled antibody. The reason for this is explained when characterizing the interaction on the cell based biosensor. The binding characteristics are different for the original

antibody on the cell surface, (5C) compared to the synthetic surface (5A), revealing a 10 fold difference in affinity. However, the conjugated antibody also showed off-target interactions with the cell membrane, highlighted in Fig 5D. The binding characteristics show a biphasic interaction with association followed by an initial fast dissociation and then a second slower dissociation. The larger association and the fast initial dissociation are only observed with the radio-labeled antibody, reflecting an off-target interaction. From control experiments it can be concluded that the labeling introduced an off-target non specific interaction with the cell membrane corroborating the observations from the animal studies.

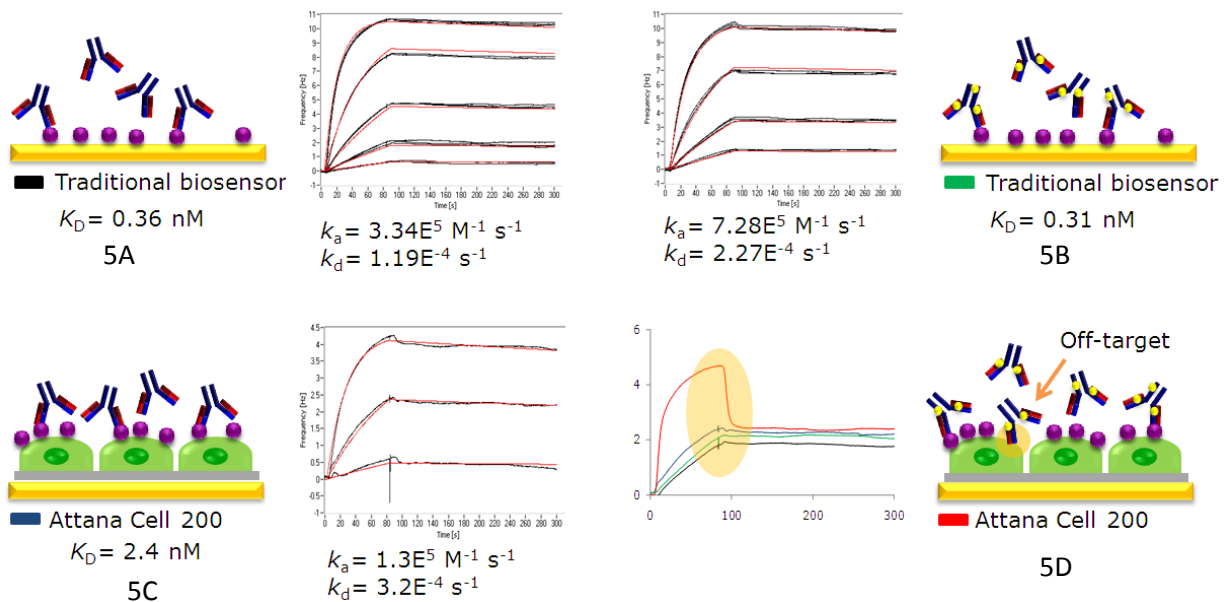


Fig 5: Comparison between synthetic and cell based biosensors. A: Synthetic surface and original antibody. B: Synthetic surface and conjugated antibody. C: Cell surface and original antibody. D: Cell surface and conjugated antibody and normalized curves from A-C.

Efficient characterization of aggregation

Addressing aggregation is necessary in several different processes. Aggregation can be a consequence of a disease, the effect of a drug, or result from inefficiencies of manufacturing processes. In collaboration with Jesus Zurdo and his group at Lonza UK, Attana has developed assays to characterize aggregation of biologics. These assays are efficient tools for improving antibody development to avoid aggregation or to study the impact of small molecules on protein aggregation.

Fig. 6 illustrates three straightforward assays to characterize the aggregation state. The first assay, “Off-rate”, considers the dissociation rate. The more aggregated the molecules the

slower the dissociation, due to avidity and rebinding. In the second assay, “Secondary antibody”, the sample molecule is captured on the surface. More aggregation gives more epitopes available for a secondary antibody to bind and thus a higher signal is obtained. The third assay, “Capturing” captures sample on the surface until saturation is reached. The more aggregation, the higher the signal. All three assays display good respective agreement with Lonza’s Oligomer Detection Assay (ODA) technology. The benefits of the assays presented here are the ease of use and fast and high throughput.

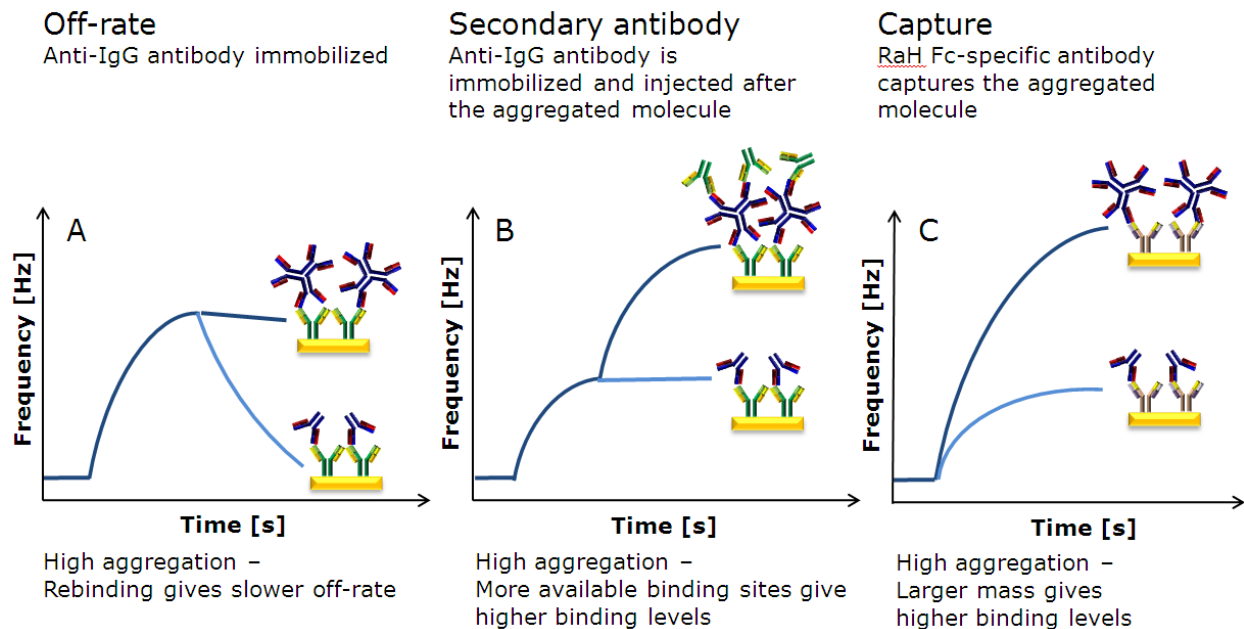


Fig. 6 Characterization of protein and antibody aggregation. A: Off-rate (dissociation rate) characterization, B: Secondary antibody binding. C: Saturation capturing

Conclusion

This paper highlights the power and simplicity of cell-based assays conducted with Attana biosensors for characterization and selection of drug candidates. The following applications were described:

- Characterization of interactions in human sera and crude samples
- Impact of experimental conditions on binding characteristics
- Effect of off-target binding and labeling on binding profile
- Characterization of aggregation