



# Early detection of clinical failure

## OBJECTIVE

Evaluate newly developed therapeutic antibodies in a biologically relevant context (cells) in order to detect failing candidates at an earlier stage.

## CONCLUSIONS

- Global screening of newly developed antibodies.
- Real-time screening of drug candidates in a synthetic and in a cellular context together provides highly informative and biologically relevant data.
- Early detection of failing drugs helps preventing late failure of drug candidates.

## BACKGROUND

The development of new pharmaceutical compounds is a long and expensive process, with highest cost reached when clinical trials start. It is important to avoid late failures and hence there is a need for highly informative techniques in preclinical stages. Here we demonstrate the ability of the Attana Cell™ 200 to provide a complete and highly relevant analysis of drug candidates' binding properties by characterizing the interaction both with purified target and in a biological context (cells).

## ATTANA CELL™ 200 BIOSENSOR

The Attana Cell 200 is a dual channel, label-free, temperature controlled, continuous flow system for manual (Attana Cell 200) or automated (Attana Cell A200) analysis of molecular interactions with cells.

The Attana Cell 200 system is characterized by the ability to study molecular interactions with cells grown directly on the sensor surface. Even higher biological relevance is achieved through features such as continuous flow, physiological temperatures and label-free detection. High data quality is achieved by direct measurements in real-time, avoiding disturbances caused by secondary detection.

The QCM core technology enables the study of biomolecules of varying species such as proteins, nucleic acids, carbohydrates, lipids and lectins and also binding moieties of vastly different sizes, ranging from peptides to cells.

## METHOD

**Preparation of a Her-2 coated surface:** Her-2 was immobilized on an Attana LNB carboxyl chip using amine coupling.

**Evaluation of Simple kinetics:** The binding properties of Herceptin® and modified Herceptin® were studied at a flow rate of 25 µl/min using the Attana Cell 200. Herceptin® and its modified versions (X1, X2, X3) were injected at different concentrations over the Her-2 surface. Regeneration of the surface was performed in between each binding event by injecting glycine 10 mM at pH 1.5; the association and dissociation phases of the interactions were recorded using the Attester Software. Kinetic parameters were then determined by fitting a 1:1 model using the Evaluation Kinetics software.

**Preparation of a MPT-1 cell sensor chip:** Cancer cells (SKOV-3) overexpressing Her-2 were seeded onto the Attana MPT-1 cell sensor surface to an appropriate density (40000 cells per sensing area). Cells were subsequently fixed with 3.7 % formaldehyde and inserted into the Attana Cell 200 biosensor.

**Evaluation of Complex kinetics:** Herceptin and modified Herceptin (X1, X2, X3) were injected over the MPT-1 cell sensor surface at a flow rate of 25 µl/min. The association and dissociation phases were subsequently recorded using the Attester Software. The kinetics parameters of Herceptin interacting with cells were determined by monitoring the interaction occurring at various concentrations. Removal of bound antibodies was performed using glycine 10 mM pH 1.0 supplemented with 0.5 mM NaCl after each binding event. Binding constants and overall complex affinity were subsequently determined by fitting a 1:1 model using the Evaluation kinetics software. The dynamic evaluation of the binding properties of modified Herceptin (X1, X2, X3) on cancer cells was achieved by comparing their binding profiles (association, dissociation) to the one obtained with the original Herceptin at the same concentration.

| Attana Cell 200           |                     |                     |                    |                     |                    |
|---------------------------|---------------------|---------------------|--------------------|---------------------|--------------------|
|                           | Simple Kinetics     |                     |                    | Complex Kinetics    |                    |
|                           | Herceptin           | Herceptin X1        | Herceptin X2       | Herceptin X3        | Herceptin          |
| $k_a$ ( $M^{-1} s^{-1}$ ) | 3.34E <sup>5</sup>  | 7.28E <sup>5</sup>  | 4E <sup>5</sup>    | 4.1E <sup>5</sup>   | 1.3E <sup>5</sup>  |
| $k_d$ ( $s^{-1}$ )        | 1.19E <sup>-4</sup> | 2.27E <sup>-4</sup> | 1.7E <sup>-4</sup> | 1.65E <sup>-4</sup> | 3.2E <sup>-4</sup> |
| $K_D$ (nM)                | 0.36                | 0.31                | 0.4                | 0.4                 | 2.4                |
| Reported Affinity (nM)    | 0.16                | N/A                 | N/A                | N/A                 | 5                  |

Table 1: Kinetic parameters of Herceptin and its conjugated variants X1, X2 and X3

Balancing **Power** and **Simplicity**  
in Molecular Interaction Studies

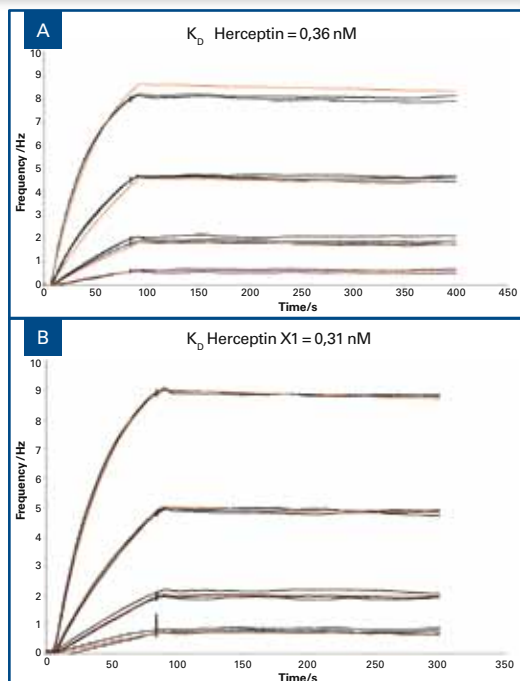


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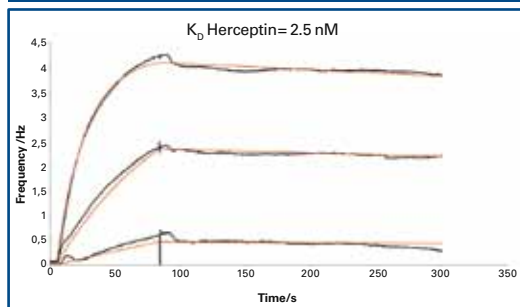
## RESULTS

**Simple kinetics:** An evaluation of the kinetic parameters of Herceptin and several modified versions of Herceptin (X1, X2, X3) was performed using a traditional biosensor approach. The binding of the above-mentioned drug candidates towards immobilized targets (Her-2) was monitored in real time as depicted in Figure 1 for Herceptin and one variant (X1) used as an example. The results from these experiments reveal that the modifications of Herceptin do not significantly affect neither the dynamics of the binding ( $k_a$  from  $3.3\text{-}7.3\text{E}^5 \text{ M}^{-1}\text{s}^{-1}$ ,  $k_d$  from  $1.19\text{-}2.27\text{E}^{-4} \text{ s}^{-1}$ ) nor the overall affinity ( $K_D$  from  $0.31\text{-}0.4 \text{ nM}$ ) (Table1).

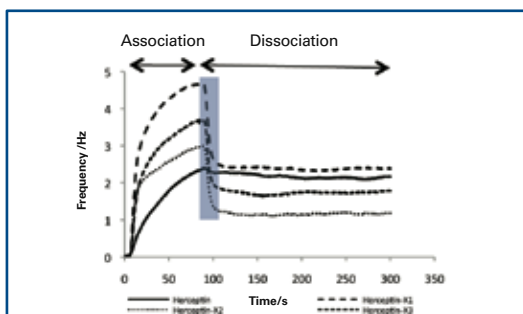
**Complex kinetics:** In an attempt to further understand the binding dynamics of drug candidates and generate information about off-target interactions that are likely to arise from drug modifications, a cell-based biosensor assay was performed. Cancer cells overexpressing Her-2 were immobilized on the sensor surface. The interaction of Herceptin with SKOV-3 cells was monitored at different concentrations in order for a complex kinetic evaluation to be performed as shown in Figure 2. The affinity extracted from the real-time measurements ( $K_D=2.4 \text{ nM}$ ) correlate with those previously reported ( $K_D=5 \text{ nM}$ ; product specification of Herceptin) and performed using end point assay (Table 1). Figure 3 presents the real-time measurement of the binding of Herceptin (solid black line) along with three variants (X1, X2, X3, dotted and dashed lines): the results reveal a significant contribution of off-target interactions for the three variants (seen as a fast dissociation, immediately after the association phase) inherent with drug modification, which was not detected using simple kinetics. This underlines the importance of using a cell-biosensor to monitor potential interfering binding events.



**Figure 1.** Kinetic evaluation of Herceptin (A) and Herceptin X1 (B) using a traditional biosensor approach (Her-2 immobilized on the sensor surface). Experimental binding curves (dark solid lines) were overlaid with theoretical binding curves (red lines) using the Evaluation kinetics software in order to determine the binding constants and affinities of the various candidates.



**Figure 2:** Kinetic evaluation of Herceptin using a cell biosensor. Cancer cells were immobilized onto the Attana MPT-1 sensor surface prior to measurement of Herceptin binding. Experimental data (black) and fitted curves from a 1:1 model (red) are shown.



**Figure 3:** Real-time evaluation of the interactions between the four candidates (Herceptin and modified Herceptin X1, X2, X3) and cancer cells. Herceptin is shown as a dark solid line and the three conjugated-Herceptin as dotted and dashed black lines. A second, unwanted binding event, measured as a fast dissociation immediately after association (grey box), occurred with Herceptin X1, X2 and X3, suggesting non-specific interactions.

| Attana Materials                 | Item Code                                       |
|----------------------------------|---|
| Amine Coupling Kit               | 3501-3001                                       |
| Attana® LNB Carboxyl Sensor Chip | 3623-3033 (pack of 3)<br>3623-3103 (pack of 10) |
| Attana Cell™ 200                 | 3745-3001                                       |
| Attana MPT-1 Cell sensor chip    | 3621-3103                                       |
| Attaché 2.0 Software suite       | 3470-3001                                       |

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