Talking Sense

A combined biochemical and cell-based biosensor is being developed to enable detailed interaction characterisation between biopharmaceutical molecules and target receptors on cell surfaces

Regardless of whether the function of new therapeutic candidate molecules is agonistic, antagonistic, inhibiting or toxic, they need to bind to their target receptor. It is therefore crucial to characterise these binding interactions when developing new therapeutics.

Detailed interaction characterisation between biopharmaceutical molecules – for example, antibodies – and target receptors on cell surfaces are enabled by Quartz Crystal Microbalance (QCM) biosensor technology. This combines the power of experimental details from label-free biochemical assays with the strength of biologically relevant information from cell-based assays.

The resulting combination provides cell-based information on kinetic rate constants and affinity for the interactions, as well as revealing off-target interaction between the antibody and cell membrane, and therapeutic accessibility of the receptors. The latter is a measure of how easy it is for a receptor on the cell surface to catch an antibody circulated by body liquids.

Early Insights

In developing new life sciences products – such as pharmaceuticals or vaccines – there is an ever-growing need to obtain detailed understanding of their function. The earlier these insights are generated, the more focus can be given to the most promising projects.

For all products which function inside the body, in vivo experiments are necessary to ensure the desired function is reached without side-effects being too high, as well as to meet regulatory requirements.

Pre-In Vivo Knowledge

Clinical trials on humans provide the true results but are often complicated to interpret, whereas experiments on animals can be more tailored to look at particular functions on a detailed level. Computer simulations have more or less 100 per cent control of the experimental data obtained. However, the drawback is that the more control there is over experimental data, the less control gained over the biologically relevant information. This is similar to Heisenberg’s uncertainty principle in physics: the more you know about a particle’s position, the less you know about its moment of inertia, and vice versa.

With the vision to characterise molecular interactions exactly as they occur in vivo in the human body, a technology has been developed – based on QCM and combined with new inventions and knowhow – to permit cell-based assays with similar experimental control and details as provided by biochemical assays.

This enables detailed interaction characterisation between biomolecules and receptors still intact on the cell surface, thereby providing cell-based information on kinetic rate constants and affinity for the interactions, as well as revealing off-target interaction between the antibody and cell membrane, and therapeutic accessibility of the receptors.
QCM Technology

QCM technology was invented in 1959 by Sauerbrey. It is based on the piezoelectric effect, providing a direct coupling between electric potential and mechanical forces. In practice, this forms a very sensitive microbalance. A quartz crystal is sandwiched between two electrodes and, when an electric potential is applied over the crystal, the mechanical force will be deformed. If an alternating potential is applied, the crystal starts to vibrate. At a given frequency, resonance occurs and this forms the base of the QCM technology. The resonance frequency is dependent on the mass of the total system.

When a molecule attaches to the surface, the system becomes heavier and the resonance frequency decreases, creating a very sensitive microbalance. Under certain conditions, the change of resonance frequency is linearly proportional to the mass change, in line with the Sauerbrey equation:

$$\Delta M = -\Delta f * k$$

$\Delta M$ represents the mass change, $\Delta f$ the frequency shift and $k$ a constant, including the area of the electrode area, the density and shear wave velocity of quartz.

Originally, the technology required a vacuum for high-precision applications, but the frequent use of quartz crystals in wrist watches, games, and computer and mobile phone applications has significantly improved the knowledge and quality of quartz crystals, enabling applications in relatively harsher environments, such as in liquid.

Biosensor Development

The development of the cell-based biosensor described here started as a PhD project within corrosion science. QCM and infrared spectroscopy were combined into one experimental set-up to enable mechanistic understanding on a molecular level of the oxidation processes on metal surfaces under real atmospheric conditions. Much effort was spent on modifying this QCM technology to enable the desired experiments. As a consequence, ideas were created about the usefulness of QCM in cell-based assays within life sciences.

This led to the development of a biosensor instrument. The electric field immobilisation was implemented in collaboration with Professor Jörnvall’s group at the Karolinska Institute (1), and high-specificity surfaces alongside sensor technology were then considered in collaboration with research groups at KTH, Linköping University, Uppsala University and Linnaeus University. Development of the technology has been performed in close collaboration with several partners, including Professor Dwek’s group at the University of Westminster (2) and Professor Bahibou at the European Membrane Institute.

The label-free biosensor can be used in biochemical-, crude-, sera- and cell-based assays (see Figure 1). The basic mode of operation is the same for all assays, being a continuous flow system allowing for full binding profiling by characterising the kinetics of a binding event and thus revealing kinetic rate constants, affinity, off-target interactions.
integrations and accessibility of receptors. This allows an understanding of the frequently observed difference between affinity in biochemical and cell-based assays (3,4).

Biochemical and Crude Assays

The new biosensor technology is used in a variety of fields – for example, surface chemistry, development of in vitro diagnostics, vaccines and therapeutic antibodies – to determine affinity and kinetic rates constants, concentration determination and epitope binning (5-8).

The system is designed to handle crude samples such as hybridomas, lysates or similar. Direct selection of the best antibodies without purification can be performed by off-rate screening. This is independent of the concentration, and thus avoids false positives expressed at high concentrations and removes the risk of missing good candidates expressed at a low concentration (see Figure 2).

Sera Assays

A specific case of crude samples is screening in sera. The positive effect of this screening is twofold. Firstly, molecules behave differently in sera compared to in buffer, in a similar way to the difference between the behaviour in buffer and deionised water. The implication of this is that the affinity is different and can change to be below or above a given cut-off value for the affinity. Secondly, fast off-target interactions between sera components with the receptor or antibody can occur.

These fast off-target interactions are usually not observed with endpoint methods, but are indirectly detected in in vivo experiments as there is lower efficacy than expected. This is because the off-target interaction has an inhibiting effect on the binding (see Figure 3).

Cell-Based Assays

Generally, the affinity differs when determined by biochemical or cell-based assays. The affinities determined biochemically and cell-based for different interactions are given in Table 1 (3,4). The quote between the affinities determined by the different methods varies from 0.02 to 46 – a factor of 2,300.

In the cell-based biosensor, the cells are either adherently grown on the sensor chip or captured from suspension. As a result, the receptor remains in a natural environment in the cell membrane, and conserves the clustering and accessibility effects of receptors. When performing interaction characterisation, the clustering and accessibility, as well as interactions with the cell membrane or other species present such as carbohydrates, affects the binding dynamics (see Figure 1, page 49).
In Figure 3, a flow cytometry study was conducted to illustrate the influence of incubation time on binding. Here, receptor accessibility was compared between biochemical and cell-based assays. The biosensor allows a bridge between the biochemical and cell-based assay by providing the full dynamics of the interaction. The receptor accessibility is lower on cells than in biochemical assays, resulting in a slower association rate, while the clustering of receptors on cells contributes to rebinding and multiple interactions, leading to a slower dissociation rate.

This significantly affects the interaction profile. Figure 4 shows the interaction of Herceptin with three HER2 expressing cell lines. The interaction profiles are different for each cell line. In all these cases, the association rate is lower compared to the purified biochemical assays. The difference in association rate is a measure of the receptor accessibility, which is not provided by traditional cell-based methods. Traditional cell-based assays have long incubation times in order to detect a binding event. From a therapeutic time perspective, this is an infinity of time to obtain binding. Thus, the time of binding is omitted as an important factor in the binding event.

By doing this, the transport mechanism of the antibody to the receptor is changed to provide an unnaturally long binding time. In Figure 3, a flow cytometry experiment is presented where the influence of incubation time on binding is depicted. 30 seconds, where little binding has occurred, is much longer than the residence time the antibody would have on the cell surface in vivo.

The continuous flow cell-based biosensor provides a method in measuring the binding with cells under in vivo-like conditions and thereby enables the accessibility studies indicated in Figures 3 and 4.

### Conclusion

The combined biochemical and cell-based biosensor enables detailed interaction characterisation between biopharmaceutical molecules, such as antibodies, and target receptors on cell surfaces. This results in cell-based information on affinity and kinetic rate constants considering accessibility and clustering of receptors for the interactions, as well as revealing off-target interaction between the antibody and cell membrane and therapeutic accessibility of the receptors.

### Table 1: Affinity for the same interactions determined by biochemical and cell-based assays. The ratio between the two methods illustrates the impact of the surrounding molecular environment on the affinity.

<table>
<thead>
<tr>
<th>System</th>
<th>Biochemical affinity</th>
<th>Cell-based affinity</th>
<th>Affinity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERB</td>
<td>50</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>ERB-HER2</td>
<td>30</td>
<td>4.5</td>
<td>7</td>
</tr>
<tr>
<td>ERB-HR2</td>
<td>7</td>
<td>1</td>
<td>7</td>
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<tr>
<td>Herceptin</td>
<td>0.1</td>
<td>5</td>
<td>0.02</td>
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<tr>
<td>ZnSO4/GC</td>
<td>92</td>
<td>2.1</td>
<td>43</td>
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<tr>
<td>ZnSO4/IgG</td>
<td>194</td>
<td>2.8</td>
<td>37</td>
</tr>
<tr>
<td>ZnSO4/CaCl4</td>
<td>169</td>
<td>3.7</td>
<td>46</td>
</tr>
</tbody>
</table>

### References

Dr Teodor Aastrup is co-founder and CEO of Attana AB. During his leadership, Attana has gone from the idea of characterising molecular interactions exactly as they occur in the body, to supplying the life sciences industry with label-free cell-based biosensors. Teodor gained his PhD in Corrosion Science from the Royal Institute of Technology in 1999 and worked in the automotive industry prior to founding Attana. He is also CEO and founder of the management consulting company TVAA AB, and a member of the Business Executives Council of the Royal Swedish Academy of Engineering Science. Email: teodor.aastrup@attana.com