Beyond Measuring Affinity:

Biacore to take Center Stage in Orthogonal Analytics for Comparability Exercises and Quality Tasks of Biologics.

Biacore QC Workshop
Christian Maasch, Xendo
Managing Consultant/Team Lead Drug Development and RA Biologics

Bringing scientists together...

Vienna, Nov 23rd, 2017
Confucius (Chinese philosopher) said …

Failure Is Not an Option -- It's a Requirement

The route/way becomes the destination

... this is also true for the development of using Biacore ...
Retrospective Look of “Working w Biacore”

Christian’s personal time line

1998 started working w Biacore at MPI Berlin

2000 purchased first “own” Biacore

2003 first Biacore paper published

1998 started working w Biacore at MPI Berlin

2003 first Biacore paper published

2009 Director of analytical department

2010 presented using Biacore as QC/ potency assay

2013 first Biacore paper about PK and Biomarker analysis published

2011 presented using biophysical assay as stand-alone batch release assay

2011 DiPIA Boston presented concept of bioanalytics by Biacore

2017

• Finally we are here to meet and talk about regulatory compliant QC/ potency assay and applications beyond
• Thank you for the chance to be part of this Biacore-family for more than nearly two decades and to spread these (once) “weird ideas” for using Biacore “beyond measuring affinity”.

2000 purchased first “own” Biacore

1998 started working w Biacore at MPI Berlin

GE BIACORE™ ANALYTICAL QC SYMPOSIUM
Lessons learnt by using Biacore for more than 15 years …
The Protagonist and What It Measures: Biacore

Biacore/ SPR

Measures:
- Binding rates constants $k_a$ and $k_d$ of an analyte to a sensor chip immobilized target

Data output:
- Binding association rate constant $k_a$
- Binding dissociation rate constant $k_d$
- Binding affinity ($K_D$)
- Stoichiometry or number of binding sites ($n$)
- Enthalpy $\Delta H$
- Entropy $\Delta S$
- Concentration ($c$) of unknown analyte

Information-rich data, but…

Know and understand your assay; do the right controls!
Use of Biacore Methods in Drug Discovery at NOXXON

- Hit identification/ binding kinetics and affinity

- Lead optimization or screening

- Thermodynamics/ transition-state

- Selectivity/ specificity

- Mode-of-Action (MoA) and structure-function relationship

- Biacore substantially supported the discovery of the “Spiegelmer” therapeutics
Proven Value of the Biacore Technology for Drug Discovery

Accepted technology (see below recent publication of 2 articles back-to-back on April 22, 2015)

.... but we think there is much more potential....
Positive Experience with Biacore ...

Well developed biophysical/ Biacore assays ...

...are robust and reproducible

...have a high precision and accuracy

Can we transfer our positive experiences to preclinical and clinical R&D?
..., but what I also learned ...
Paul Belcher on Linkedin/Twitter: “... I see Senorgrams everywhere”
“... following on from my blog posts about data fitting and when to believe the data or not - I saw this data in a publication this week. Discussion...3...2...1 and GO”
“It is always worth to use both kinetic and steady state fittings as a self-QC. And 900 binging RU without saturation.... scary.” Shuo W.

“I suspect those aren’t rate constants, but rather the author is referring to equilibrium constants KA and KD. That would agree with the numbers anyway. Still does not appear to be 0.5 pM interaction.” Michael M.

“I'm going to guess at some of the other numbers generated by the fit: Rmax <2 RU. RI up to 1000 RU. Even if this was a steady-state fit, they still haven't approached saturation closely enough to generate plausible KD values.” Eric R.

“... we might have to define a new unit.....10e-24 is Yoctomolar” Paul B.

“Association constant is 10e12 M... what a fast binding! Is it super glue?” Secil F.

“The numbers given are obviously not the rate constants ka and kd but the equilibrium association and dissociation constants, as Ka=1/Kd. Still the dimensionality of Ka should be M^-1, not M. Otherwise with on and off rates being too fast to be measured telling true response from bulk RI change is tricky if, as in this case, the system is far from saturation. Showing the response vs. concentration plot is the least the referee should have asked for.” Andrei Z.
BBP – “Best Biacore Practice”

- Know and understand your assay; do the right controls
- Describe your assay setup and evaluation/kinetic model with respect to the MoA of your analyte (show the fit!)
- Critical evaluate your data; potentially look for a “Biacore sparring partner” to discuss results
- Learn “reading your sensorgram data” without software
Reading, “Dissection” and Interpretation of Sensorgram Data

... start “reading sensorgrams” instead of “blind trusting” the software ...

**Baseline:**
- Slope? Drift?
- Memory effect?
- Chip integrity?
- Delay in injection? IFC or flow to slow?

**Association Phase:**
- Saturation?
- Rmax used for fitting; calculation of ka?
- Curvature sufficient?
- Biphasic association (fast/slow) -> Non-specific binding?
- Low signal for mAb@500 nM, must be saturated at lower concentrations when KD=1.63 nM
- Surface activity and control?
- Initial on-rate: is it concave? MTL?

**Regeneration:**
- Efficient?
- Monitor chip decay! Or memory effect

**Dissociation Phase**
- Off-rates of differ!
- NSB or bivalent or heterogeneous ligand?

**Evaluation:**
- What model?, fit and goodness of fit not shown!
- Why KD ~1.63 nM, but there is no saturation at higher concentrations
- No standard dev (but, 3 digits for rate constants ;))

**Assay setup:**
- Concentration range tested feasible for estimated affinity?

\[ K_D = 1.63 \text{ nM} \]
Towards Validation of Biacore Assays
Lessons Learned by ELISA Assays, Cell-based Potency Assays and HPLC Analysis

- Standard Curve/ “Internal” Reference Material
- Calibrators/QC sample analysed in duplicates/triplicates to monitor assay performance
- LOD/ LLOQ/ ULOQ
- Evaluation of sample of unknown concentration: standard curve was calculated by fitting a 4-parameter logistic function (4-PL-function) to the raw data of the calibrator and was used to calculate the concentration of the unknown samples thereof.
- Evaluation of relative “binding potency” of known sample by dose-response curve: e.g. by parallel-line analysis/ IC$_{50}$

ELISA and cell-based assays are steady-state analysis: How to transfer to a Biacore analysis?
Biacore Evaluation: Steady-State vs. Kinetics – do they fit?

- In an **ideal world** and under optimal assay conditions steady state analysis and kinetics fit well.
- In a **real world** parameters like e.g. non-specific binding, heterogeneous binding, assay limitations, bad data fitting, ... may influence that steady-state analysis does NOT fit to kinetic data.

K\(_D\) steady state: 0.87µM
K\(_D\) kinetics: 0.91µM

*Awful Fit, Chi2 = 740
K\(_D\) ~ 46.6 nM (kinetics) vs 481 nM (steady-state)*
From Report Points to Standard Curve

Assay Set up and Development: Report Points and Standard Curve

Report points
- Standardized and “easy-to-evaluate/transfer” data to analysis software, e.g. Prism, PLA 3.0 or SoftMax
- Fit data to a non-linear 4/5-parameter fit

DETERMINE STANDARD CURVE
- Define fitting parameters and confidence intervals for assays to “pass”
Introducing QC Samples/Calibrators to Your Biacore assay

- Role of QC samples is that they represent the matrix of the samples with known amounts of the analyte; QC samples are processed in the same manner as study samples
- Used during method validation to demonstrate e.g. accuracy, precision and stability
- Subsequently used during the conduct of the study to provide batch-level quality control

Biacore Assay Acceptance Criteria:

1) A minimum of 75 % of all calibration values in the working range (between the LLOQ and ULOQ) have to show an accuracy of 80-120 %, except at the LLOQ and ULOQ where limits of 75-125 % are acceptable.
2) At least 4 out of 6 QC samples at three concentrations have to show an accuracy of 80-120 % and a precision of ≤20 %. At least one QC sample per concentration needs to meet this criterion. If this criterion is not fulfilled, the assay is invalid and the samples must be re-analysed.
Example for Competitive Biacore Assay: Monitor Assay Performance by Bracketing

Sequential analysis of samples

Initial 3 injections

2 x standard curve

Concentration analyte [nM]

Response units [RU]
**Additional Information by Real-time Measurement: Kinetics to bring Value and Confidence to your Assay**

KNOW YOUR ANALYTE AND ASSAY!
- Measure $k_a$ and $k_d$ and define acceptance criteria for your analyte.

Qualitative and quantitative data in a sensorgram
- $k_a$ [1/ M*s]: a function of the concentration or/and activity/integrity of the analyte
- $k_d$ [1/s]: reflects the binding mode of action (MoA)/identity of the analyte
EXAMPLE:
Biacore as Surrogate Potency Assay

Read-out report points
- $P_1$, $P_2$ – slope, baseline stability
- $P_3$ – RU after injection time vs concentration ($R_{eq}$?!)  
- $P_4$, $P_5$ – regeneration efficiency

Read-out binding kinetics
- $k_a$ – association rate constant
- $k_{d(1)}$ – early dissociation rate constant
- $k_{d(2)}$ – late dissociation rate constant

Read-out data fitting
- Apply global fitting model !!!
- $RI$ – bulk or unspecific binding contribution
- Chi2 – parameter for “goodness of fit”, $ruol \leq 1(-5)$% of $R_{max}$
- Adjust $k_t$ value if appropriate

Intra assay controls/assay performance
- 6 QC samples (4 in linear range)
- Accuracy of 80-120 % and a precision of $\leq 20$ %.

Biacore fingerprint:
- sensorgram comparison tool;
  Sum of evidence for quality of data

12.5nM – 6.25 – 3.13 – 1.56 – 0.78 – 0.39 – 0 nM

double injection of 12.5nM

response units [RU]

time [s]
Biacore Assay KPIs
Establish ranges (e.g. CI) - pass or fail?

KPIs = **Key Performance Indicators** (kinetic analysis)

- Know AND understand how your assay setup/parameters (and their correlation) affect the performance of the assay (DoE/QbD approaches)
- Establish reliable ranges and statistics for the definition of acceptance criteria

* Relative “binding potency”: $KD_{\text{rel}} = \text{mean KD samples} / \text{KD internal standard}$
Further Biacore KPIs

- Acceptance criteria for baseline, blanks/matrices, non-functional analyte controls...
- Acceptance criteria regeneration efficiency
- Is curvature for appreciate number of concentrations established?
- $R_{\text{max}}$ consistency vs injected concentrations -> monitor NBS contribution
- Double injections -> chip integrity
- monitored $R_{\text{max}}$, $R_{\text{eq}}$ (local) -> validity of kinetic data
- ...

Giordana Bruni is going to talk about that topic and share her experience
Example: Clinical IMP Batch Release

Cell-based Assay

- Relative potency = 76.25%

Biophysical: Biacore

- Relative binding "potency" = 78.49% ± 4.867 (SD)
  - CI_{95}% of mean = 75.40 – 81.58%

Comparable results between biophysical and cell-based assay format
Why a Biophysical Assay?

➢ Technically less challenging
   - No batch-to-batch variation as in animal bioassays
   - No limitations of handling cells in cell-based bioassays

➢ Higher precision and sensitivity
   - Not subject to inherent variability of bioassays (e.g. passage number)

➢ Assay-to-assay consistency of critical reagents
   - Reagents may be frozen and used over a long period of time

Combines robustness with high sensitivity, precision, accuracy and low sample consumption
Regulatory Perspective: Biacore Beyond Lead Generation?

What's essentially new?

- Section III on 'Analytical Methods Development'
  Emphasis on getting the method right at the beginning with respect to specificity, linearity, limits of detection (LOD) and quantitation limits (LOQ), range, accuracy, and precision and method robustness

Encourages to find (new) analytical methods with best analytical performance characteristics
According to ICH Guideline Q6B, a biological assay to measure the biological activity of the product may be replaced by biophysical tests only in those instances where:

- sufficient mode-of-action (MoA) information about the drug...
- and relevant correlation to biologic activity can be demonstrated...
- and there exists a well-established manufacturing history...
Correlation of Binding Affinity to Biologic Activity?

Short reminder / tutorial – binding and thermodynamics belong together
van’t Hoff analysis:
\[ \Delta G = -RT \ln(K_a) = \Delta H - T \Delta S \]
\[ \ln(K_a) = \left( \frac{-\Delta H}{R} \right) \left( \frac{1}{T} \right) + \frac{\Delta S}{R} \]

Temperature matters ! – measure at 37°C !

Excellent correlation of binding affinity and biological activity/ potency for oligonucleotide-based scaffolds
Long-term Experience of Using a Biophysical (Surrogate) Potency Assay

- Competitive binding assay, low intra- and inter-assay variations, higher precision and accuracy than cell-based assay and linear correlation with biological potency assay

... used since 2010 as stand-alone surrogate potency assay for batch release
Further tasks using Biacore in CMC
Biacore Assays used to Support CMC for …

- QC/ batch release
- IPC = In process control/ QbD support (e.g. AMBR studies = rapidly screen and optimize cell culture process parameters) -> identification of CQAs/ comparability study from process performance to product quality attributes
- Formulation development/Stability tests (e.g. total vs active DS)
- Comparability studies
- Surrogate potency assay (clinical batch release)
- Formulation development/Stability tests
- QC/ re-analysis/ in-process controls
Biosimilars … coming back to Confucius

The route/way becomes the destination
... this is especially true for the development of Biosimilars

Biological/ Innovator drug

- New process
- Establish process and analytics
- Establish ranges/ QbD
- Identify CQAs

QTPP Biosimilar

- GOAL: Innovator
- QTPP in mind !!!

QTPP = Quality Target Product Profile

Innovator drug challenge:
QTPP not known/available
The CMC Challenge of a Biological

The very nature of a biologic means

- It is practically impossible for two different manufacturers to produce two identical biopharmaceuticals if identical host expression systems, processes and equivalent technologies are not used
- This has to be demonstrated in an extensive comparability program for biosimilars

 Biosimilars do have a few Developments in their Favor

- Technological advances to characterize biologic molecules and the analytics used to evaluate and prove biosimilarity.
- Improvements are being made in the manufacturing techniques used to produce biosimilars.

Are we opening Pandora´s Box by applying new processes and technologies?
Structure Function Relationship – Orthogonal Approaches

• Understanding the structure-function relationship of a biotech product is vital for its successful development.

• Awareness of this is important, and by selecting the right assays at the right time, it is possible to reduce significantly the costs and risks of the R&D process.

• By applying orthogonal methods and approaches for characterization, information regarding the structural, binding interactions and functional properties of the biological product are linked – providing a greater understanding and interpretation of the determined characteristics.
Additional Tasks for Biacore in Analytics

- Affinity and Binding kinetics
- Concentration/Purity (total vs active)
- Surrogate Potency Assay
- Structure-Function relationship
- Immunogenicity
- Receptor binding assays
- IPCs/ process development/CQA
- Off-Target binding
- (Accelerated) stability studies
- Identity/Integrity
- Stability
- Formulation
Orthogonal Use of Binding Analysis (SPR) for Structure-Function Relationship

**Target binding:** Affinity, rate constants and selectivity, specificity by SPR

- **Advantage:** High accuracy, precision and sensitivity, low inter- and intra-assay variations

**Purity:** HPLC/SEC data linked to total and active DS/DP

- **Advantage:** Linking the information of total to active DS/DP and refer to potency

**Identity/integrity:** LC-MS, Disulphide bridge, DSC analysis linked to SPR rate constants as “fingerprint” and prediction of MoA

- **Advantage:** Confidence in structure-function relationship

**Glycosylation:** Abundance of a glycosylation species was linked to effector receptor binding as determined by SPR

- **Advantage:** Bridging glycosylation pattern and effector function by descriptive (binding) MoA
### Potency:
Cell-based assay linked to SPR assay

### Advantage:
Only One Cell-Based Assay acceptable to demonstrate bioactivity

### Effector function:
Binding of FcγRs linked to cellular cytotoxicity (ADCC)

### Advantage:
Linking effector function as determined by cell-based assays to descriptive (binding) MoA

### FcRn Binding:
Used as total sum of evidence for identify/integrity and linked to FcRn binding assays as prediction of pharmacokinetic properties

### Stability:
see SPR analytics above

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Stuart Knowling (BioOutsource) and Mike Willcox (Eurofins) have already shared their experience and insights
Additional Information from Sensorgrams linked to other Analytics

CONFIDENCE IN DATA AND "PIVOTAL" EVIDENCE OF ANALYTICAL SIMILARITY by best practise analytics and orthogonal approaches

AGENCIES are happy

REDUCTION OF ANALYTICAL EFFORTS driving the reduction of costs and risks

SPONSORs are happy
Where Do We Go? –
The Challenge of Crude Sample Analysis

Pharmacokinetics
What is the half-life $t_{1/2}$ of a drug in the blood?

Pharmacodynamics/
Biomarkers
How long is the drug effective?

Bio-distribution/ADME
Where is the drug distributed in the body and where is it metabolized or cleared?

Immunogenicity
Does the drug induced anti-drug antibodies (ADAs)?
The Problem of Plasma Binding and Cut-Point Analysis

- Binding does not equal functional/biological significance
- Unspecific binding of biological matrices reduces the assay’s detection limit
- Unspecific binding to various surfaces differs

Rat plasma binding to dextran Biacore surface

From: Biacore Immunogenicity Package Manual
The Real Challenge: Individual Cut-points!

Unspecific binding of sera from healthy volunteers to an immobilized chemokine

- Unspecific binding of 250 individual sera from healthy volunteers differs dramatically
- Definition of a cut-point is challenging in terms of the assay sensitivity (more likely it is impossible)
- The only way out: pre-dose vs treated normalization of data
- What if there is no pre-dose sample available?

- Unspecific binding was reduced to a negligible level at NOXXON Pharma by optimized buffer conditions and the use of unspecific competitors (e.g. soluble dextran)
- Methods established for the analysis of plasma, serum, urine and issue homogenates

Reducing unspecific binding is mandatory for further assay development
Example: Compound (Scaffold) Pharmocokinetic/ Pharmacodynamic In Rats

Compound (Spiegelmer): L-nucleic acid linked to PEG

Blood sampling

Organ Dissection

Biacore analysis
- Plasma pharmacokinetic
- Tissue distribution
- Target/ Biomarker plasma levels

0’ 10’ 1h 3h 8h 24h 48h 96h
## Overview of Assay Performances for Bioanalytics by Biacore

<table>
<thead>
<tr>
<th>Assay</th>
<th>L-Nucleic Acid Qualification</th>
<th>PEG Qualification</th>
<th>Biomarker Quantification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay type</strong></td>
<td>Direct binding Biacore assay</td>
<td>Competitive Biacore Assay</td>
<td>Competitive Biacore Assay</td>
</tr>
<tr>
<td><strong>Matrices</strong></td>
<td>Plasma, liver, urine</td>
<td>Plasma, liver, urine</td>
<td>Plasma</td>
</tr>
<tr>
<td><strong>Sample preparation</strong></td>
<td>None; simple dilution</td>
<td>None; simple dilution</td>
<td></td>
</tr>
<tr>
<td><strong>On-target effects</strong></td>
<td>No influence; up to 1µM tested</td>
<td>No influence; up to 1µM tested</td>
<td>No influence; up to 1µM tested</td>
</tr>
<tr>
<td><strong>Biological matrices</strong></td>
<td>No influence; (up to 50%)</td>
<td>No influence; (up to 30%)</td>
<td>No influence; (up to 50%)</td>
</tr>
<tr>
<td><strong>In-use stability of chip</strong></td>
<td>&gt; 500 analysis cycles</td>
<td>&gt; 350 analysis cycles</td>
<td>&gt; 310 analysis cycles</td>
</tr>
<tr>
<td><strong>LLOQ</strong></td>
<td>≤ 0.2 nM</td>
<td>≤ 1.95 nM</td>
<td>0.2 nM</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>%CV &lt; 3</td>
<td>%CV &lt; 8</td>
<td>% CV &lt; 7</td>
</tr>
<tr>
<td><strong>% Accuracy</strong></td>
<td>98 -103%</td>
<td>93 -110%</td>
<td>97 -104%</td>
</tr>
<tr>
<td><strong>Dilution linearity</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>% Recovery</strong></td>
<td>&gt; 98%</td>
<td>&gt; 96%</td>
<td>&gt;93%</td>
</tr>
<tr>
<td><strong>Time per sample</strong></td>
<td>9 min</td>
<td>7 min</td>
<td>7 min</td>
</tr>
</tbody>
</table>
Questions Answered by Biacore Bio-Analytics

**Plasma pharmacokinetics**

- **Spiegelmer plasma conc. [nM]**
  - quantified by oligonucleotide
  - quantified by PEG moiety

**Target/Biomarker pharmacodynamics**

- **Biomarker in plasma [nM] ± SD**
  - release rate = 40.81 ± 2.916 nM/hour
  - C_{max} = 890.1 ± 273.8 nM at 48 hours

**Tissue distribution**

- **liver**
  - % of total dose ± SD
- **kidney**
  - % of total dose ± SD

**Renal clearance**

- **% of total dose ± SD**
  - 24h
  - 96h

**Bound-free Analysis**

- **plasma concentration [nM]**
- **% active of control**

How long is the compound in the blood?

How is the compound cleared from the body?

What is the efficacy of the compound?

When do I have to dose again for the best effect?
Biacore/ biophysical assays have the potential to support the lead identification and pharmaceutical development of biologics and other scaffolds from hit to clinical trials.

Biophysical analyses with their high accuracy and precision - compared to biological assays - increasingly gain importance as surrogate potency, QC and CMC assays.

The most critical issue to generate highly sensitive Biacore data with biological samples was overcome by intensive buffer optimization.

For therapeutic scaffolds Biacore could be used for a variety of analytical comparability and QC tasks.
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...and thank you for your attention

I still love to see great new ideas, data and also weird results
Beyond Measuring Affinity: Biacore to take center stage in orthogonal analytics for comparability exercises and quality tasks of biologics.

Christian Maasch, Xendo, Berlin, Germany

Understanding the structure-function relationship of a biotech product is vital for its successful development. Awareness of this is important, and by selecting the right assays at the right time, it is possible to reduce significantly the costs and risks of the R&D process. The diversity of therapeutic biologics and their complexity not only poses a challenge for robust manufacturing, but also comprehensive characterization of these molecules.

Surface Plasmon Resonance (SPR)-based methods (Biacore) have shown to combine robustness with high sensitivity, precision, and accuracy and substantially assisted the lead identification and characterization of biological and chemical compounds over the last decade. By recent SPR method developments, these assays gained increasing importance and acceptance to support pharmaceutical QC, CMC, bioanalytics and comparability studies.

By applying orthogonal methods and approaches for characterization, information regarding the structural, binding interactions and functional properties of the biological product are linked – providing a greater understanding and interpretation of the determined characteristics.

Herein we show examples and insights on how SPR with information-rich data could support the analysis of biologics in terms of Critical Quality Attributes (CQAs) and drug release testing, as well as for comparability exercises for the development of biosimilars.

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