Biosensor Technologies: SPR, BLI and DNA Nanolevers 2017

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MRC Laboratory of Molecular Biology

- Biophysics has a range of instruments based on biosensors
- Biosensors detect the interaction between a macromolecule attached to a solid chip surface (ligand) and a macromolecule in solution (analyte)
- Different physical changes at the surface can be used to monitor the interaction
Range of Experiments

- **Is there an interaction?**
  - binding partners

- **Equilibrium analysis**
  - determination of dissociation constant ($K_d$)
  - mutational analysis

- **Kinetic rate analysis**
  - determination of the on- and off-rate constants ($k_a$ and $k_d$)
  - to understand the dynamics of the system

- **Size analysis?**
  - determination of the diameter of the interacting partner

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What is the relevance of binding kinetics?

\[ A + B \xrightarrow{k_a} AB \xrightarrow{k_d} AB \]

- The cell is a dynamic system
- On-rates are concentration dependent
  - The level of binding is not just related to the affinity
- Off-rates are independent of concentration
  - Related to half-life
- The same affinity can be resolved into different on and off rates for different interactions - dissect different mechanisms

\[ K_d = \frac{k_d}{k_a} \]
Biosensors

Format: Biacore-Surface Plasmon Resonance (SPR)

- Dextran CM5
- Gold 50 nm for SPR
- Glass support stability

4 channels in series or singly
Format: Octet - Bio-Layer Interferometry (BLI)

- Optical layer
- Biocompatible matrix
- Immobilized molecules

Format: switchSense- DNA-nanolevers

- Nanolever with fluorescent dye and functionalized complementary DNA
- Detection spot with $10^4 - 10^6$ nanolevers per 0.01 mm²
- 6 microelectrodes in 1 flow channel
- Biochip with 4 flow channels, each with multiple microelectrodes in series
Detection: SPR
Sample is loaded onto chip

Incident polarised light
Optical detection unit

Sensor surface
Gold film

Sample
Flow channel

Reflected light

Reflected Light
Intensity

Angle of
reflection

SPR Angle

Mass change
Time

Reflected Light
Intensity

SPR Angle

Mass change
Time
Detection: SPR

SPR single reaches a maximum

Incident polarised light → Optical detection unit → Reflected light → Sensor surface → Gold film → Flow channel

Sample

Reflected Light

IntENSITY

SPR Angle

Mass change → Association → Time

Detection: SPR

Buffer is flowed across chip

Incident polarised light → Optical detection unit → Reflected light → Sensor surface → Gold film → Flow channel

Buffer

Reflected Light

Intensity

SPR Angle

Mass change → Angle of reflection → Time
Detection: SPR

SPR signal drops to baseline level

- Incident polarised light
- Optical detection unit
- Reflected light
- Sensor surface
- Gold film
- Flow channel

![Reflective Light Intensity vs SPR Angle](image)

Distance between the two reflecting surfaces = ℓ

Intensity \( I = f(\lambda, t) \)

Detection: BLI

Distance between the two reflecting surfaces = ℓ

Intensity \( I = f(\lambda, t) \)
Detection: BLI

Sensors

Detection: switchSense
switchSense: Time-resolved Switching Dynamics Measurement

f ~ 10 kHz

Scientific Reports 5:12066 (2015)
Analytical Chemistry 87:4538 (2015)
Nature Commun. 4:2099 (2013)
Bioanal. Rev. 4 (2) 97-114 (2012)
JACS 132:7935 (2010)

switchSENSE draws complementary information from three measurement modes

Molecular Dynamics

Basic Principle
Friction coefficient change

Application
Binding Kinetics/Affinity
Protein Diameter/Conformational Change
Melting & Thermodynamics
Multimers & Aggregation

Proximity Sensing

Basic Principle
Dye proximity change

Application
Binding Kinetics/Affinity
Melting & Thermodynamics

Molecular Ruler

Basic Principle
Dye position change

Application
Nuclease/Polymerase Activity
Aggregation/Interlinking
DNA modification

biotin
+ anti-biotin Fab
**Biosensor Kinetic Data**

![Graph showing association and dissociation kinetics with time (s) and signal levels.]

**SPR:** Resonance units: $1000$ RU = 1 ng of bound protein/mm$^2$ of chip surface area

$1$ RU = $1 \times 10^{-4}$

**BLI:** nm: wavelength shift and not size!

**SwitchSense:** rate of fluorescence recovery - dynamics of switching

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**Comparison of Biosensors**

<table>
<thead>
<tr>
<th></th>
<th><strong>SPR</strong></th>
<th><strong>BLI</strong></th>
<th><strong>SwitchSense</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Types of experiment</td>
<td>Affinity/Kinetics</td>
<td>Affinity/Kinetics</td>
<td>Affinity/Kinetics/<em>Sizing/DNA enzyme kinetics/conformation</em></td>
</tr>
<tr>
<td>Dynamic range</td>
<td>No limit?</td>
<td>&gt;150 Da</td>
<td>?</td>
</tr>
<tr>
<td>Affinities</td>
<td>pM to mM</td>
<td>10 pM – 1 mM</td>
<td>50 fM – 1 mM</td>
</tr>
<tr>
<td>Association rates</td>
<td>$10^3$ – $10^7$ M$^{-1}$s$^{-1}$</td>
<td>$10^2$ – $10^7$ M$^{-1}$s$^{-1}$</td>
<td>$10^3$ – $10^8$ M$^{-1}$s$^{-1}$</td>
</tr>
<tr>
<td>Dissociation rates</td>
<td>$10^{-5}$ – 1 s$^{-1}$</td>
<td>$10^{-6}$ – 10$^{-1}$ s$^{-1}$</td>
<td>$10^{-6}$ – 1 s$^{-1}$</td>
</tr>
<tr>
<td>Temperature control</td>
<td>4-45 °C</td>
<td>Ambient to 40 °C</td>
<td>8-75 °C</td>
</tr>
<tr>
<td>Throughput</td>
<td>96 well plate</td>
<td>96 or 384 plate</td>
<td>96 well plate</td>
</tr>
<tr>
<td>Sizing accuracy</td>
<td>n.a.</td>
<td>n.a.</td>
<td>0.1 nm</td>
</tr>
</tbody>
</table>
Comparison of Biosensors: Issues

- **Cost**: Capital cost and cost per experiment (chips vs sensors)
- **Artifacts of immobilisation**
  "God made the bulk; surfaces were invented by the devil" Wolfgang Pauli
- **Non-specific binding (care with controls)**
- **Buffers**: Biacore sensitive to RI changes, switchSense is salt sensitive
- **Real limits to detection**: response rate and total assay time
  - fast rates or very slow off-rates may not be accurately detected
  - switchSense can measure very long dissociations
- **Fitting issues**:
  - Some high-affinity interactions with slow dissociation rates can be difficult to analyse

Biosensor Workflow

- **Sample Preparation**
- **Ligand Attachment**
- **Capture**
- **Couple**
- **Test Binding**
- **Kinetic or affinity Binding**
- **Controls**
- **Fit Data**
Sample preparation: Buffers

- Buffers ensure filtered and degassed (Biacore 2000)
- Compatible with system (salt affects switchSENSE switching)
- Avoid Tris-HCl if coupling via amides
- Detergent? DMSO?
- For SPR: match buffers for running buffer and analyte to minimise effect of refractive index changes

Sample preparation: ligand and analyte

- Protein aggregates removed: spin, SEC
- Concentrations accurate (UV, AAA)
- Ligand vs analyte? Ideally, compare binding interaction with either attached:
  - Economic, Specificity, Aggregation or Valency issues
**Sample preparation: how much ligand for SPR?**

For kinetics, immobilise a low amount of ligand \((R_{\text{max}} = 100 - 200 \text{RU})\). For equilibrium, immobilise enough to get a good response.

\[
R_L = R_{\text{max}} \frac{M_{\text{wt}_L}}{n \cdot M_{\text{wt}_A}}
\]

- \(R_L\) is the amount of ligand bound, \(R_{\text{max}}\) is the maximum response, \(n\) is the stoichiometry, \(M_{\text{wt}}\) is the molecular weight of analyte \(A\) and ligand \(L\).

**Sample preparation: how much ligand for BLI?**

- **BLI**
  - Trial dilution series from 100 - 200 nM ligand
  - Plate-based run in parallel
  - Use analyte concentration 80% for max response
  - Aim for up to 1 nm over 5 min

**Sensors**

- Vary [ligand] vs constant analyte
Sample preparation: how much ligand for switchSense?

- **SwitchSense**
  - 100-500 nM DNA-coupled ligand
  - If avidity an issue need lower level
  - can vary by competition or reducing lever density by electrical desorption
  - use different density chips:

![SwitchSense diagram]

**SPR/BLI: Ligand Attachment**

**Capturing Methods**
- Streptavidin for Biotin
- Specific Ab
- Anti-GST for GST
- NTA- for His-tagged proteins
- Protein A for IgG

**Couple Methods**
- Amine coupling
- Thiol coupling

Advantages/Disadvantages?
SPR Immobilisation -CHIPS with Everything

- CM5 - the standard chip
- C1- flat carboxymethylated surface - multivalent or very large analytes
- CM3- shortened dextran matrix for large analytes
- CM4- low degree of carboxylation for high non-specific binders
- CM7- LMW analytes- denser x 3 immobilisation

- SA- biotinylated ligands
- L1- lipid membrane components
- HPA- hydrophobic for lipid membranes
- NTA- his-tagged proteins
- Protein A: Fc region of antibodies
- Protein L: wide range of antibody fragments

- Sensor Chip PEG: alternative to dextran based surfaces, flat surface good for very large or multivalent binding partners

Octet Immobilisation
Sensors with Everything

<table>
<thead>
<tr>
<th>Biosensor</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-Specific Capture</td>
<td></td>
</tr>
<tr>
<td>• Anti-Human IgG Fc Capture (AHC)</td>
<td>Human IgG Fc region, kinetic analysis</td>
</tr>
<tr>
<td>• Anti-Human IgG Fc Capture (AHQ)</td>
<td>Human IgG Fc region, quantitation</td>
</tr>
<tr>
<td>• Anti-Mouse Fc Capture (AMC)</td>
<td>Mouse IgG1, 2a &amp; 2b Fc regions, kinetic analysis</td>
</tr>
<tr>
<td>• Anti-Mouse Fc Capture (AMQ)</td>
<td>Mouse IgG1, 2a &amp; 2b Fc regions, quantitation</td>
</tr>
<tr>
<td>• Anti-Human Fab-CH1 (FAB)</td>
<td>Fab-CH1 domains of human IgG</td>
</tr>
<tr>
<td>• Protein A (ProA)</td>
<td>Quantitation of various species IgG</td>
</tr>
<tr>
<td>• Protein G (ProG)</td>
<td>Quantitation of various species IgG</td>
</tr>
<tr>
<td>• Protein L (ProL)</td>
<td>Quantitation of IgG via kappa light chain</td>
</tr>
</tbody>
</table>

| Affinity Tag Capture        |                                                       |
| • Streptavidin (SA)         | Biotinylated ligands                                 |
| • Super Streptavidin (SSA)  | Biotinylated ligands (high-density surface)          |
| • Anti-FLAG (FLG)           | FLAG-tagged recombinant proteins                     |
| • Anti-GST (GST)            | GST-tagged recombinant proteins                      |
| • Anti-Penta HIS (HIS)      | HIS-tagged recombinant proteins                      |
| • Anti-Penta HIS 2nd Gen (HIS2) | HIS-tagged recombinant proteins                  |
| • Ni-NTA (NTA)              | HIS-tagged recombinant proteins                      |

| Immobilization              |                                                       |
| • Amine Reactive 2nd Gen (AR2G) | Covalent coupling to reactive amine groups           |
| • Aminopropylsilane (APS)    | Adsorption to hydrophobic moieties                  |
Immobilisation - Chemistry

Amine Coupling

Thiol Coupling

Alternatives e.g. via maleimide

pH Scouting for amine coupling

Sample Preparation → Ligand Attachment → Capture → Couple → Test Binding → Kinetic or affinity Binding → Fit Data

Controls → pH scouting → Regenerate scouting
**pH Scouting for amine coupling**

- Look for linear traces
- Calculate potential $R_L$ vs injection time $vs$ pH

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**SPR Amine Coupling**

Prime  |  Attach  |  Block

- EDC/NHS solution
- LIGAND solution at pH < pI
- Ethanolamine solution

Response [kRU] vs Time [s]
SPR Immobilisation - Tagged Proteins

Sensor Chip NTA
- Pre-immobilized NTA
- Nickel chelation
- Capture of his-tagged protein
- Binding of analyte

Sensor Chip SA
- Stepavidin coated surface
- Binding of biontinylated ligand
- Binding of analyte

SPR Immobilisation – Membrane Applications

Sensor Chip HPA
- Hydrophobic surface
- Coating with user defined liposomes

Sensor Chip L1
- Immobilisation of liposomes
- Binding of analyte to receptor
switchSense Ligand Attachment

Functionalization of the complementary DNA

To link a ligand to the complementary DNA strand, there are various coupling methods:
- Chemical:
  - Amine reactive group
  - Thiol reactive group
  - Click Chemistry

- Capture tags:
  - Tris-NTA, Biotin, Streptavidin, Digoxigenin,
  - Protein A, Protein G, GST-binding, GFP-binding

switchSense Swings for all

- **Multi-purpose Biochips (MPC) 48 and 96 bp**
  - Binding Kinetics
  - Binding Affinity
  - Protein Diameter
  - Conformational Change
  - Melting & Thermodynamics
  - Multimers & Aggregation

- **Enzymatic Biochips**
  - Nuclease & Polymerase Activity
  - 54 or 80 bp
  - 3’ of fluorophor strand attached to chip surface

- **Bifunctional Biochips**
  - Bispecific Binders & Avidity (7 mm or 14 mm)
Controls and regeneration

Sample Preparation → Ligand Attachment → Capture → Test Binding → Kinetic or affinity Binding → Fit Data

Controls → pH scouting → Regenerate scouting

**SPR: Controls Reference Channel**

- Channels in series for reference subtraction e.g. Fc2 (ligand)-Fc1 (control) subtraction or Fc4-Fc3, or (Fc4, Fc3, Fc2)-Fc1
- Treat as active ligand bound channel
- Immobilize a non-interacting control biomolecule that mimics physical properties of ligand e.g. a mutant that doesn't bind, a random DNA sequence

![Graphs showing RU vs. time for Fc1 (blank), Fc2 (ligand), and Fc2-Fc1](attachment:image.png)
Anti-GST immobilised on Fc1 and Fc2 Chip

Raw data:
FC1 ligand GST
FC2 ligand GST-fusion

Reference corrected
Buffer corrected
and fitted

GST
GST-fusion

Analyte

Regeneration solution

Zero concentration (buffer) injection

SPR: Controls Reference Channel

One chip is used for many binding experiments
Therefore need to remove any residual analyte by regenerating the surface:

Poor removal of analyte
Higher baseline

Response Injection of regeneration solution

Regenerating scouting:
salt (1M NaCl)
acid (pH 2.5)
alkali (pH 11)
detergent

Can avoid problems using single cycle kinetics on T200
Or use BLI sensors in parallel
SPR Multiple vs Single Cycle Kinetics

<table>
<thead>
<tr>
<th>Sample</th>
<th>$k_a$ ($1/\text{M/s}$)</th>
<th>$k_d$ ($1/s$)</th>
<th>$K_D$ (M)</th>
<th>$R_{max}$1 (RU)</th>
<th>$R_{max}$2 (RU)</th>
<th>$\chi^2$ (RU²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCK</td>
<td>1.10E+06</td>
<td>0.002957</td>
<td>2.69E-09</td>
<td>5.17E+06</td>
<td>6.22E-10</td>
<td>34.72</td>
</tr>
<tr>
<td>SCK</td>
<td>7.46E+05</td>
<td>0.004182</td>
<td>5.61E-09</td>
<td>5.90E+06</td>
<td>4.02E-10</td>
<td>38.44</td>
</tr>
</tbody>
</table>

Controls and regeneration

Sample Preparation → Ligand Attachment → Controls

Ligand Attachment → Capture → Mass Transport

Controls → pH scouting → Capture

Capture → Couple → Test Binding

Test Binding → Kinetic or affinity Binding → Fit Data
SPR Mass transport problems

- During interactions analyte has to diffuse into or out of the dextran layer
- Mass transport problems occur when analyte is consumed faster than the flow can replenish it during association
- During dissociation, if analyte isn’t removed by flow then it can rebind.
- $k_m$ dependent on cube-root of flow rate

Overcome mass transport:
- Lowest amount of immobilized ligand
- Higher flow rates (e.g. 30 µl/min)
- Fit data using model that includes mass transport variable (caution: may be difficult to fit, don’t invoke unless there is a mass transport effect)
BLI Controls

Plate-based

In series: use zero concentration of analyte and no ligand
In parallel: use another row of sensors but don’t load ligand and repeat cycle

Naman B. Shah and Thomas M. Duncan J Vis Exp. 2014; (84): 51383.

SwitchSense controls

Binding to surface may prevent nanolevers from lying down flat on surface

Compare non-specific binding to nanolever with control spots hybridized with control NL-A strand
SwitchSense DRX² controls

- Each detection spot, a mixture of red & green nanolevers
- 2 nanolevers, each with a different sequence & fluorescent dye

Affinity and Kinetics

- At least five concentrations for kinetics, more for affinity
- Adequate concentration range (above and below Kd)
- Concentration in duplicate or triplicate
- Include one or two zero-concentration
Signal shape depends on kinetics

- All 4 analytes have the same affinity $K_d = 10 \text{nM} = 10^{-8} \text{M}$
- The binding kinetic constants vary by 4 orders of magnitude

\[ K_d = \frac{k_d}{k_a} \]

<table>
<thead>
<tr>
<th>$k_a$ (M$^{-1}$s$^{-1}$)</th>
<th>$k_d$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^6$</td>
<td>$10^{-2}$</td>
</tr>
<tr>
<td>$10^5$</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>$10^4$</td>
<td>$10^{-4}$</td>
</tr>
<tr>
<td>$10^3$</td>
<td>$10^{-5}$</td>
</tr>
</tbody>
</table>

- Analytes with slow off-rates occupy the target for a longer time
- Observed on-rate is also concentration dependent

Dynamic Space: Small Molecule Screening

\[ K_d = \frac{k_{off}}{k_{on}} \]

Kinetics determine Sensogram analysis

Steady-state
Fast on, fast off
Analyze Req vs [Analyte]

Kinetics
Slow on, slow off
Equilibrium not reached,
Analyze kinetics

Now to Fit

Before the analysis
- Subtract reference binding
- Double reference by subtracting zero analyte concentration
  (buffer control)
Determining kinetics and $K_d$ from SPR

BiaEval  T200 Evaluation  Prism

See Data Fitting lecture for more information

Determining kinetics and $K_d$ from switch Sense

switch Analyse

Local

Global
Determining size from switchSense

Plot $R_{eq}$ vs [Analyte]

$R_{eq} = \frac{C \cdot K_a \cdot R_{max}}{C \cdot K_a + 1}$

where $C$ is the [Analyte], $K_a$ is association constant $(1/K_d)$
Determining kinetics and $K_d$

Which model?

- **Drift**: linear component due to instrument or non-specificity
- **Bivalent**: analyte or ligand has two binding sites
- **Heterogenous ligand**: parallel reactions
- **Conformational Change**: two step reaction

$k_a$: Association rate constant $(M^{-1} s^{-1})$  
$k_d$: Dissociation rate constant $(s^{-1})$  
$[Analyte]$  
$K_d = \frac{k_d}{k_a}$
Determining kinetics and $K_d$

$$A + B \xrightleftharpoons{K_d}{k_a} AB$$

$K_d = \frac{k_d}{k_a}$

$R_t = R_0 \exp(-k_d t)$

Dependent on both $k_a$ + $k_d$
Determining kinetics and $K_d$

A + B $\xrightarrow{k_a \, k_d} AB$

$K_d = \frac{k_d}{k_a}$

$R_t = R_o \exp(-k_d t)$

$R_t = \left(\frac{C \cdot k_a \cdot R_{\text{max}}}{C \cdot k_a + k_d}\right) \times \left(1 - \exp\left(-\left(C \cdot k_a + k_d\right) \cdot t\right)\right)$

where $C$ is concentration of analyte and $R_{\text{max}}$ is the maximum response.

What if $k_d$ is wrong?
Global analysis of interactions can lead to errors in analysis

\[ A + B \xrightleftharpoons[k_d]{k_a} AB \]

\[ K_d = \frac{k_d}{k_a} \]

Global fit gives poor fitting for \( k_d \) very low value

Estimated \( K_d \) approximately 30 nM

Kinetic analysis of interaction can overcome problems in global analysis

\[ R_t = R_I + R_{max}(1-exp(k_{obs}t)) \]

\[ k_{obs} = k_a[\text{Analyte}] + k_d \]
Kinetic analysis of interaction can overcome problems in global analysis

\[ k_{\text{obs}} = k_a [\text{protein}] + k_d \]

\[
\begin{array}{ccc}
    k_a & k_d & K_d \\
    (M^{-1} \text{s}^{-1}) & (s^{-1}) & (\mu M) \\
    \hline
    1.5 \times 10^3 & 2.8 \times 10^{-3} & 1.8 
\end{array}
\]

\[ K_d = k_d / k_a \]

\[ K_d = 2.2 \ \mu M \text{ by ITC} \]

Tidow et al. (2006)

The Analysis

- Make a choice of evaluation model
- Try the simplest first
- Is the fit acceptable?
  - access by residuals, standard error of parameters, \( \text{Chi}^2 \)
- Don’t invoke a complicated model just because the fit looks better
- Are the results relevant?
SPR Project

APC/C
E3 Ring–cullin ligase
20 subunits

Cdh1

UbcH10-Ub
(E2 ligase)

How does activation of APC/C affect the affinity for the E2 UbcH10?

Attach biotin-UbcH10 to SA Chip and flow across apo- and ternary APC/C

SPR Workflow

Immobilisation UbcH10-biotin on Streptavidin Chip

Test Interaction with APC/C

High background binding to chip. Look at non-specific binding stability with different additives e.g. NSB, Tween-20, BSA, NaCl
SPR Workflow

Kinetics Wizard

Raw Sensograms

Processed data

Fit data

Compare Results

Kinetics

Equilibrium

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Analyte</th>
<th>$k_{\text{off}}$ (s$^{-1}$)</th>
<th>$k_{\text{on}}$ (M$^{-1}$ s$^{-1}$)</th>
<th>$K_{\text{eq}}$ (nM)</th>
<th>$K_{\text{kin}}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UbcH10</td>
<td>Ternary</td>
<td>0.42 ± 0.02</td>
<td>1.78 ± 0.2 $\times 10^6$</td>
<td>215</td>
<td>236 ± 29</td>
</tr>
<tr>
<td></td>
<td>Apo</td>
<td>0.75</td>
<td>1.1 ± 0.1 $\times 10^6$</td>
<td>703</td>
<td>682 ± 83</td>
</tr>
</tbody>
</table>

Chang et al. (2014) Nature
• Very small changes in mass/RU relative to control surface
• Need T200
• 96 or 384 well-format
• Up to three proteins in parallel
• Take care of DMSO – use solvent correction

SPR Ligand Screening

• Take care of baselines – positive and negative binding controls
• Ideal to have binding controls e.g. another protein
• Workflow built into evaluation software

(data from Javier Garcia-Nafria)
BLI in action:
BRC4 peptide vs Rad 51

Sensor Tray SA

Ligand Control

Sample tray

Raw Data

Ligand

Biotinylated-BRC4 peptide

Control

Loading Equilibration Association

Rad51

Data from Joe Maman, Dept of Biochemistry, University of Cambridge

BLI in action:
BRC4 peptide vs Rad 51

Corrected Data

Fit Data

Results

residuals

$K_d = \frac{k_{diss}}{k_{on}} = 43 \text{nM}$

Data from Joe Maman, Dept of Biochemistry, University of Cambridge
switchSense in action

1  →  2
Functionalization of the switchSENSE biochip

2  →  3
Interaction measurement

3  →  1
Wash away the ligand-cNL analyte complex

With switchSENSE the Detection Limit of Anti-EPO Antibody is < 800 fM within 20 Minutes
switchSENSE provides one Workflow for Polymerase DNA/RNA Interaction Measurements

Different primer/template combinations use modular sequence exchange

Polymerase Characterization with switchSENSE
switchSENSE Measures the Polymerase Activity in Basepairs per Second & Determines the Michaelis Constant $K_M$

Michaelis Constant $K_M = 0.58 \, \mu M$

Further Information?

SPR
http://www.biacore.com/
http://www.sprpages.nl/

switchSense
http://www.dynamic-biosensors.com/switchsense/

Octet
http://www.fortebio.com/octet-platform.html

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