Advanced Biophysical Analysis of Mono- and Bispecific Antibody Formats with the switchSENSE® Biosensor Platform

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High-affinity and bispecific antibody formats are challenging analytics for interaction analysis systems. The apparent binding kinetics crucially depend on how the target molecules are presented on the sensor surface. In order to emulate the presentation of heterogeneous antigens on a cell surface with a biosensor platform, it is necessary to functionalize the sensor with different antigens at a defined stoichiometry. Further it is crucial to control the spatial arrangement of those antigens relative to each other, which has not been feasible up to now. Here, we introduce the DNA-templated assembly of different ligands on a switchSENSE sensor and demonstrate the quantitative measurement of binding cooperativity, i.e., affinity effects. The influence of different ligand arrangements on the binding kinetics, in particular, the off-rate, is discussed for antibody formats. We show how the ligand-to-ligand distance can be controlled with sub-nanometer precision using bifunctional DNA scaffolds, i.e., nanolevers with adjustable arm-lengths. We believe the introduced workflows will be highly instrumental in the discovery and selection of bispecific biotherapeutics.

The switchSENSE® Principle | Electro-Switchable DNA Nanolevers

DNA nanolevers are electrically actuated at high-frequency on microelectrodes, while their orientation is monitored by time-resolved single photon counting. The binding of analyte molecules slows the switching dynamics in a characteristic way, providing an unprecedented level of information about the target.

Control over Steric Configurations

<table>
<thead>
<tr>
<th>Phase</th>
<th>Potential mean.</th>
<th>affinity</th>
<th>Cause</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass-transport limitation</td>
<td>Ligand density too high</td>
<td>Apparent kD &lt; real kD</td>
<td>Disassociation</td>
<td>Rebinding</td>
</tr>
<tr>
<td>Ligand density too low</td>
<td>Flow rate too low</td>
<td>Apparent kD &lt; real kD</td>
<td></td>
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</tbody>
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Bispecific Functionalization of Sensor Surface

Immobilization of Two Different Antigens with DRX² Instrument

- On-spot reference
- Green and red fluorescence

More advanced assay
- Detection of color-coded binding signals from individual antigens
- Analyzing bivalent binder

Biochip Surface Density Variation

- Adjustable avidity effects (interlinking)
- Adjustable & Restorable Antigen Density via Invisibility Cloaking

switchSENSE provides the control over steric configurations of antigens and a homogenous surface density

Density Variation | IgG binding to an TNFα-modified surface

- Fast dissociation (kD)
- Slow dissociation (kD)
- Anti-TNFα antibody with pM affinity and sub-pM avidity

Complex binding situations → bi-phasic dissociation:
- Antibodies bound via one antigen binding site to ligand feature fast dissociation (fast kD)
- Antibodies bound to two ligand feature slow dissociation rate (slow kD)

By interlinking both effects: slow and fast dissociation exists in parallel

Monovalent vs. Bivalent Binding – Affinity vs. Avidity

Adjusting the nanolever density → distinction between monovalent and bivalent binding (Affinity vs. Avidity)

Amplitudes reflect respective contributions of different dissociating species to the overall dissociation curve

- Monovalent phase
  - kD = 1.55 E-4 s⁻¹
  - kD = 0.12 E-4 s⁻¹

Dissociation curves fitted with a global, double-exponential model:

The thinner the ligand density the more dominant becomes the monovalent phase.

Bivalent Interaction – anti-TNFα – FcyRI

- Use of bifunctional nanolevers allows to study the contribution of a weak and strong binding site to the overall affinity.

Assay Orientation & Solution Titration | FcyRI – IgG binding

- Kinetics
- Equilibrium Titration (Langmuir)

- Affinity influenced by assay orientation and coupling strategy
- 10-fold weaker affinity of immobilized FcyRI (overcome by in solution competition assay)
- Complex formation of IgG and FcyRI in solution: surface-bound ligands determine free fraction of IgG, yielding "true" dissociation constant – a factor 10 lower than expected affinity of FcyRI matching Ki, from kinetic measurements of immobilized IgG