Procedure

Working at low ligand attachment levels in Biacore T200

This guideline provides recommendations for working with low surface densities in Biacore™ T200. The procedure is valid for amine coupling of ligands for kinetic analysis of antibodies in solution with minimized avidity effects. The avidity effects disappear below an R_{max} of approximately 5 RU.

Working procedure

1. Attach the ligand. Flow rate 5 µL/min. Activate the surface with EDC/NHS for 7 min. Use ligand concentration 1 µg/mL and contact time 30 s. Deactivate with ethanolamine for 7 min.
2. The attachment level may not always be detectable when working at low attachment levels. Therefore, check the surface density by injecting a high concentration of the analyte. Use the highest concentration from the concentration series optimized during assay development. Use flow rate 30 µL/min and a contact time reaching steady state if possible, e.g. 120 s.
   - If R_{max} is 2–5 RU, continue and run the kinetic/affinity assay.
   - If R_{max} is lower than 2 RU, the attached level is too low and the ligand has to be attached using a higher concentration, e.g. 2–10 µg/mL, and/or a longer contact time, 60–120 s. Check the attached level by injecting the highest concentration of the analyte.
   - If R_{max} is 6 RU or higher, the attached level is too high and the ligand may be attached to a lower level using a shorter EDC/NHS activation time. Check the attached level by injecting the highest concentration of the analyte.

$$R_L = (MW_L/MW_A) \times (R_{max}/S_m)$$

R_L (RU) = Attachment level
R_{max} (RU) = Maximum binding response
MW_A (Da) = Molecular weight of analyte
MW_L (Da) = Molecular weight of ligand
S_m = Stoichiometric ratio (number of binding sites per ligand)

3. Run the kinetic/affinity assay. Use flow rate 30 µL/min. Contact time 120 s and dissociation time 600 s are recommended as a start point when working with antibodies as analytes.

Important considerations

- When working with low surface densities the ligand attachment must be optimized for each model system, since the response depends on the molecular weight and activity of both the ligand and the analyte, and the kinetic properties.
- The activity of the ligand should always be checked with an analyte injection, even if the ligand attachment level is detectable.

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