Procedure

Aldehyde coupling of ligand to Biacore sensor chips

This guideline provides recommendations for immobilization of ligands by aldehyde coupling to Biacore™ sensor chips. Aldehyde coupling is suitable for carboxyl-derivatized sensor chips and Series S sensor chips of the following series: Sensor Chip CM3, Sensor Chip CM4, Sensor Chip CM5, Sensor Chip CM7, and Sensor Chip C1.

Oxidizing the ligand

Required solutions and materials

Required Solutions and materials are listed in Table 1. Amersham™ MicroSpin™ G-25 and Amersham NAP™-5 columns are available from Cytiva.

Table 1. Solutions and materials required for oxidation of ligands with metaperiodate

Buffer A	100 mM sodium acetate, pH 5.5
Buffer B	10 mM sodium acetate buffer, pH 4.0
Sodium metaperiodate	50 mM freshly prepared sodium metaperiodate ($NaIO_4$) in buffer A
Desalting column	Columns of Amersham MicroSpin or Amersham NAP-5 type (or other desalting methods)

Procedure

Follow the steps below to modify a protein ligand by metaperiodate (*see* Fig 1).

1. Prepare a cold solution of the ligand to be oxidized in Buffer A at 1 mg/mL. Store on ice.
2. For oxidation of sialic acid use a metaperiodate concentration of 1 mM (i.e., dilute 1:50 volume to the 50 mM metaperiodate ligand solution). For *cis*-diols (sugar residues) use a metaperiodate concentration of approximately 10 mM (i.e., dilute the ligand solution 1:5).
3. Incubate for 20 min on ice.
4. Stop the reaction by desalting the mixture on a desalting column eluted with buffer B. Store the oxidized ligand in the refrigerator.

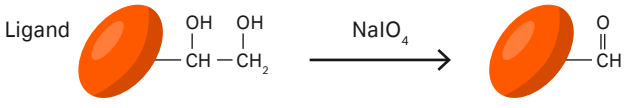

Fig 1. Ligands containing *cis*-diols are oxidized by sodium metaperiodate to introduce aldehyde groups, which enable immobilization by aldehyde coupling.

Important considerations for ligand modifications

- The degree of oxidation is most easily estimated by testing immobilization to a carbohydrazide-activated sensor chip surface.
- Unoxidized ligand is preferably used as negative control.
- If the results indicate insufficient oxidation, increase the time of oxidation, the metaperiodate concentration, or the oxidation temperature.

Immobilizing the ligand

Required solutions

Required solutions are listed in Table 2. EDC, NHS, and ethanolamine are included in Amine Coupling Kit from Cytiva.

Table 2. Solutions required for immobilization of ligands by aldehyde coupling

EDC	0.4 M of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide in water
NHS	0.1 M of N-hydroxysuccinimide in Milli-Q™ water
Carbohydrazide[1]	5 mM carbohydrazide in Milli-Q water
Ethanolamine	1 M ethanolamine-HCl, pH 8.5
Ligand	Typically 20–50 µg/mL in immobilization buffer
Cyanoborohydride	0.1 M sodium cyanoborohydride in 0.1 M sodium acetate, pH 4.0

[1] Some protocols and methods mention the use of hydrazine for introduction of hydrazide groups as an alternative to carbohydrazide. However, hydrazine is a very toxic molecule and the use of this reagent is no longer recommended in Biacore systems.

Suggested immobilization procedure

Follow the steps below to immobilize a protein ligand by aldehyde coupling (*see* Fig 2). Perform the immobilization on the active surface. Use low flow rates for activation, introduction of hydrazide groups, deactivation, and immobilization, for example, 5–10 µL/min.

1. Activate the surface by injecting a mixture of EDC/NHS (1:1) for 3 min.
2. Introduce hydrazide groups by injecting carbohydrazide for 6–7 min.
3. Deactivate excess reactive groups by injecting ethanolamine for 6–7 min.
4. Immobilize ligand by injecting the ligand solution for 6–7 min.
   - For detailed information on buffer and pH scouting refer to the Biacore Sensor Surface Handbook.
5. Stabilize the linkage by injecting cyanoborohydride for 20 min. Use a very low flow rate such as 2 µL/min.

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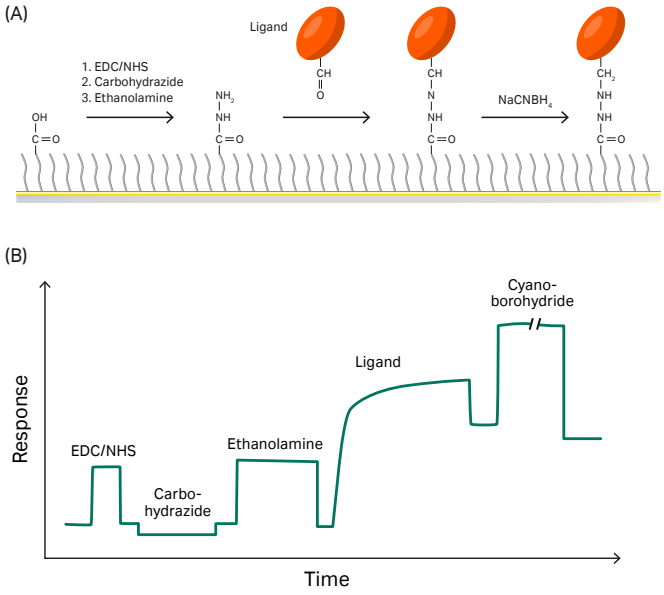

Fig 2. (A) The chemistry behind immobilization of ligands by aldehyde coupling. (B) A typical sensorgram of a ligand immobilization using aldehyde coupling.

Important considerations for ligand coupling

- Adjust immobilization levels by varying ligand concentration and contact time.
- Use a low flow rate to reduce ligand consumption.
- Recommended flow rates and contact times for optimal immobilization may vary between different Biacore systems.

Ordering information

Product	Product code
Amersham NAP-5 Columns	17085301
Amersham MicroSpin G-25 Columns	27532501
Amine Coupling Kit, type 2 (for Biacore 4000)	BR100633
Amine Coupling Kit (for all other Biacore systems)	BR100050

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