Procedure

Surface thiol coupling of ligand to Biacore sensor chips

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

Required solutions

Required solutions are listed in Table 1. All reagents for thiol coupling are available in the Thiol Coupling Kit from Cytiva. PDEA is also available separately from Cytiva.

Table 1. Solutions required for immobilization of ligands by surface thiol coupling

EDC	0.4 M of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in Milli-Q™ water
NHS	0.1 M of N-hydroxysuccinimide in Milli-Q water
Cystamine	40 mM cystamine dihydrochloride in 0.15 M sodium borate, pH 8.5
DTE	0.1 M dithioerythritol or dithiothreitol in 0.15 M sodium borate, pH 8.5
Ligand	Typically 20–50 µg/mL in immobilization buffer
PDEA/NaCl	20 mM of 2-(2-pyridinyldithio)ethaneamine and 1 M NaCl in 0.1 M sodium acetate, pH 4.0
MES buffer	0.1 M of 2-(4-morpholino)ethanesulfonic acid, pH 5.0

Modifying the ligand

Follow the steps below to modify a protein ligand with PDEA (*see* Fig 1). Volumes can be scaled down if required.

1. Prepare a ligand solution of 1 mg/mL in 0.5 mL of MES buffer.
2. Add 0.25 mL of 15 mg/mL PDEA in MES buffer (final PDEA concentration in the mixture, 22 mM).
3. Add 25 µL of 0.4 M EDC (final EDC concentration, 13 mM).
4. Mix and incubate for 10 min at 25°C or 1 h on ice.
5. Remove the excess reagents on a buffer exchange device equilibrated with a suitable buffer.

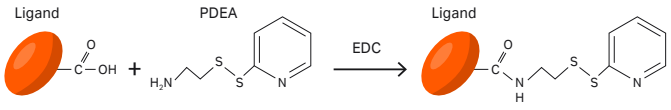

Fig 1. The chemistry behind modification of a protein ligand with PDEA.

Determining the degree of modification

The degree of modification with PDEA can be determined approximately by reduction of the disulfide bond and spectrophotometric estimation of the thiopyridone released (absorbance maximum 343 nm).

1. Measure the absorbance of the modified protein at 280 nm (A_{280}) and 343 nm (A_{343}).
2. To 1 mL of protein solution, add 50 µL of 100 mM DTE in water. Mix and allow reaction for a few minutes at room temperature.
3. Measure the absorbance again at 343 nm ($A2_{343}$). Calculate the degree of modification as follows:

$$C_{TP} = \frac{A2_{343} - A1_{343}}{\varepsilon_{343,TP} \times l} \qquad A_{280,PROT} = A_{280} - (C_{TP} \times \varepsilon_{280,TP} \times l)$$

$$C_{PROT} = \frac{A_{280,PROT}}{\varepsilon_{280,PROT} \times l} \qquad \text{Modification} = \frac{C_{TP}}{C_{PROT}}$$

C_{TP} = Molar concentration of thiopyridone

$\varepsilon_{343,TP}$ = Molar extinction coefficient of thiopyridone at 343 nm ($8.08 \times 10^3\ M^{-1}\ cm^{-1}$)

l = Path length of the spectrophotometer cell

$A_{280,PROT}$ = Contribution of the protein to A_{280}

$\varepsilon_{280,TP}$ = Molar extinction coefficient of thiopyridone at 280 nm ($5.18 \times 10^3\ M^{-1}\ cm^{-1}$)

$\varepsilon_{280,PROT}$ = Molar extinction coefficient of the protein at 280 nm

Important considerations

- Under these conditions, PDEA reacts with carboxyl groups on the ligand. In addition to introduce a reactive disulfide into the ligand, the isoelectric point is raised, which can be an advantage in immobilization of acidic proteins.
- Aim for an average modification level of 1 substitution/protein molecule. The modification level can be controlled by adjusting the PDEA concentration, incubation time, and temperature.

Immobilizing the ligand

Follow the steps below to immobilize a protein ligand by surface thiol coupling (*see* Fig 2). Perform the immobilization on the active surface. Use low flow rates of between 5 and 10 µL/min, set by the immobilization wizard or Biacore methods.

1. Activate the surface by injecting a mixture of EDC/NHS (1:1) for 2 min.
2. Introduce disulfide groups by injecting cystamine for 3 min.
3. Reduce disulfide groups by injecting DTE for 3 min.
4. Immobilize ligand by injecting the ligand solution for 6 to 7 min.
5. Deactivate excess reactive groups by injecting PDEA/NaCl for 4 min.

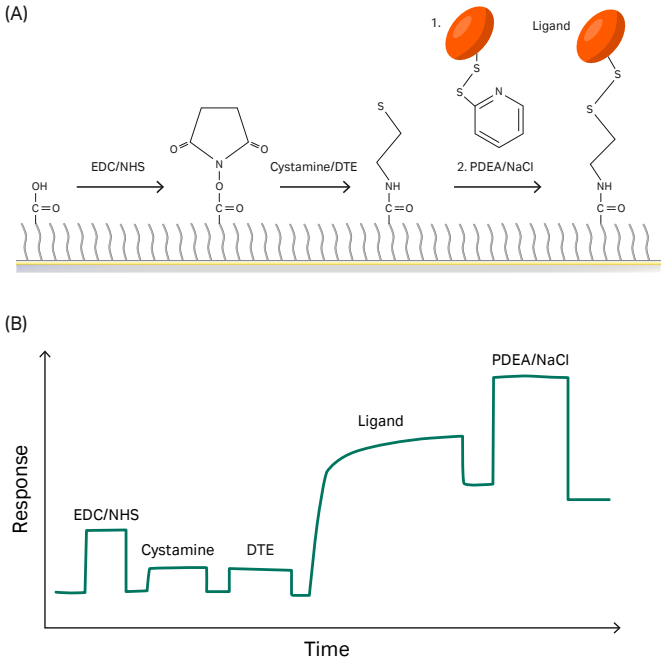

Fig 2. (A) The chemistry behind immobilization of ligands with surface thiol coupling. (B) A typical sensorgram of a ligand immobilization using surface thiol coupling.

Important considerations

- 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
- Running buffers can never be supplemented with a reducing agent (e.g., TCEP), since reducing agents will reduce PDEA prior to coupling. The coupling chemistry will then not work.

Ordering information

Product	Product code
Thiol Coupling Kit	BR100557
PDEA Thiol Coupling Reagent	BR100058

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