Procedure

GFP V_HH capture surface for Biacore assays

The GFP V_HH/nanobody is a single domain antibody derived from alpaca that selectively binds to green fluorescent protein (GFP)-tagged proteins. This nanobody is used for specific and stable capture of green fluorescent protein (GFP)-tagged proteins in Biacore™ assays (*see* Fig 1). GFP V_HH also recognizes proteins tagged with enhanced GFP (eGFP) and yellow fluorescent protein derivatives (YFP, eYFP, and Venus).

The GFP V_HH capture surface allows for easy and effective isolation and directed immobilization of fluorescent-tagged proteins from complex biological samples like prokaryotic or eukaryotic cell extracts. Immobilization of these proteins enables binding studies and kinetic analysis using Biacore systems.

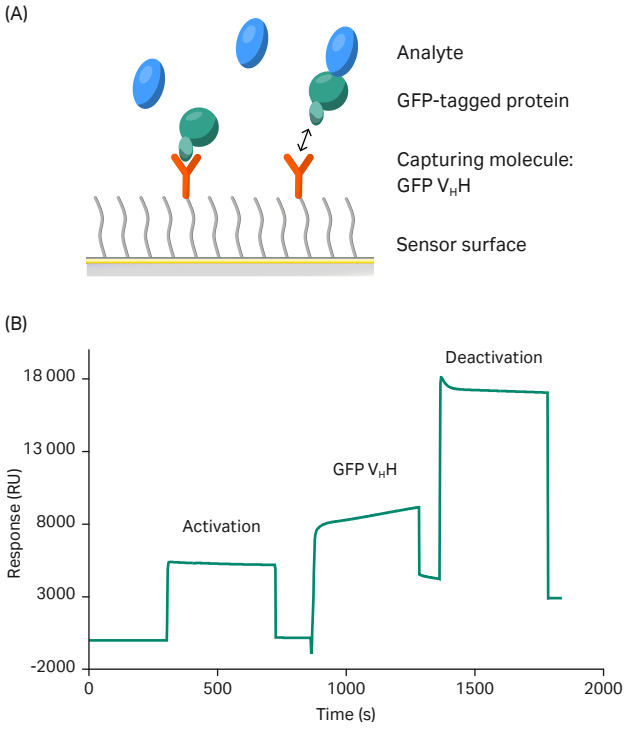

Fig 1. (A) The principle behind immobilization of ligands using GFP V_HH in a Biacore capture assay format. (B) A typical sensorgram of GFP V_HH immobilization by amine coupling.

Procedure

This procedure describes how to attach ligand, run the interaction analysis, and regenerate a GFP V_HH surface.

Attachment of GFP V_HH

GFP V_HH is attached on both active and reference surfaces of the following Biacore sensor chips: Sensor Chip CM5 (alternatively Sensor Chip CM4, Sensor Chip CM3, or Sensor Chip C1) and Series S sensor chips of the same series.

The conditions shown below should allow an attachment level of ~ 3000 RU on a Sensor Chip CM5 surface, which is close to saturation.

- Coupling chemistry: amine coupling
- Dilute GFP V_HH to 50 µg/mL in 10 mM acetate pH 5.5 immobilization buffer
- Activation time: 7 min, ligand contact time: 7 min, deactivation time: 7 min
- Flow rate: 10 µL/min

Capture of GFP-tagged protein

You can calculate how much of the GFP-tagged protein (ligand) you need to capture on the sensor surface to reach a theoretical R_{max} (maximum binding capacity for analyte) of 50–100 RU with your respective analyte using the following equation:

$$R_L = \frac{\text{ligand MW}}{\text{analyte MW}} \times \frac{R_{max}}{S_m}$$

R_L = Attachment or capture level
S_m = Stoichiometric ratio
R_{max} (RU) = Maximal binding response

1. Test optimal conditions to reach the respective capture level for your GFP-tagged protein, for example, in a manual run addressing the active flow cell only.
2. Start with a picomolar (pM) dilution of the GFP-tagged protein, inject at 10 µL/min for 60 s.
3. If the concentration of the GFP-tagged protein in the extract is not known, set up a series of 10-fold dilutions in running buffer. Start testing with the lowest concentration.

Investigate binding of analyte to GFP-tagged protein

1. Capture the desired amount of GFP-tagged protein on the active flow cell.
2. Inject dilutions of the analyte over both reference (GFP V_HH) and active (GFP-tagged protein captured by GFP V_HH) flow cells.
3. Start with a pM dilution of analyte in running buffer.
4. Set up a series of 10-fold dilutions in running buffer. Start testing with the lowest concentration.
5. Control reference flow cell for potential nonspecific binding of analyte to GFP V_HH.

Remove GFP-tagged protein from the GFP V_HH surface

Applying the conditions below regenerates the GFP V_HH surface and removes bound GFP-tagged protein. Apply regeneration over reference and active flow cells:

- Regeneration solution: 10 mM glycine pH 1.7
- Contact time: 2 × 30 s
- Flow rate: 30 µL/min

References

1. Della Pia, E.A. and Martinez, K.L. Single domain antibodies as a powerful tool for high quality surface plasmon resonance studies. PloS ONE **10(3)** (2015): e0124303. https://doi.org/10.1371/journal.pone.0124303

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